Electronic Acknowledgement Receipt

EFS ID:	20710107
Application Number:	14169927
International Application Number:	
Confirmation Number:	2069
Title of Invention:	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
First Named Inventor/Applicant Name:	Daniel MUETH
Customer Number:	13155
Filer:	Jean Ceceille Edwards./Kenneth Sowers
Filer Authorized By:	Jean Ceceille Edwards.
Attorney Docket Number:	81527.0018
Receipt Date:	17-NOV-2014
Filing Date:	31-JAN-2014
Time Stamp:	13:57:19
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1340
RAM confirmation Number	185
Deposit Account	505497
Authorized User	NEILS, PAUL F.
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Electronic Patent Application Fee Transmittal

Application Number:	14169927
Filing Date:	31-Jan-2014
Title of Invention:	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
First Named Inventor/Applicant Name:	Daniel MUETH
Filer:	Jean Ceceille Edwards./Kenneth Sowers
Attorney Docket Number:	81527.0018

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Petition fee- 37 CFR 1.17(h) (Group III)	1464	1	140	140
Request for Continued Examination	1801	1	1200	1200
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Adjustment date: 12/30/2014 SDIRETA1
11/18/2014 INTEFSW 00000185 14169927
02 FC:1801 -1200.00 OP

Refund Ref: 0030154690
12/30/2014

Credit Card Refund Total: $1200.00
Am Exp..: XXXXXXXXXXX1009

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/169,927	01/13/2015	8933395	81527.0018	2069

13155 7590 12/23/2014

Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

PREMIUM GENETICS (UK) LTD., Nantwich, UNITED KINGDOM, Assignee (with 37 CFR 1.172 Interest);
Daniel MUETH, Chicago, IL;
Joseph PLEWA, Park Ridge, IL;
Jessica SHIREMAN, Kansas City, MO;
Amy ANDERSON, Palatine, IL;
Lewis GRUBER, Chicago, IL;
Neil ROSENBAUM, Chicago, IL;

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/169,927	01/31/2014	Daniel MUETH	81527.0018	2069

13155 7590 12/18/2014

Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

EXAMINER	
NGUYEN, KIET TUAN	

ART UNIT	PAPER NUMBER
2881	

NOTIFICATION DATE	DELIVERY MODE
12/18/2014	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipgeneral@edwardsneils.com

Notice of Allowability	Application No. 14/169,927	Applicant(s) MUETH ET AL.	
	Examiner KIET T. NGUYEN	Art Unit 2881	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the request for consideration of IDS*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *73-86*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 11/17/14
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☐ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/KIET T NGUYEN/
Primary Examiner, Art Unit 2881

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	14169927
Filing Date	2014-01-31
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	KIET TUAN NGUYEN
Attorney Docket Number	81527.0018

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button. Add

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2000-512541	JP	A	2000-09-26	University of Washington	WO 97/47390	☒
	2	2001-504936	JP	A	2001-04-10	University of Washington	WO 97/39338	☒
	3	2002-503334	JP	A	2002-01-29	Technical University of Denmark	WO 98/10267	☒

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14169927
	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	2881
	Examiner Name	KIET TUAN NGUYEN
	Attorney Docket Number	81527.0018

	4	H11-508182	JP	A	1999-07-21	University of Washington	WO 97/00442	☒
	5	H05-26799	JP	A	1993-02-02	Nippon Steel Corporation	Abstract	☒
	6	97/39338	WO	A1	1997-10-23	University of Washington		☒
	7	98/10267	WO	A1	1998-03-12	Technical University of Denmark		☒
	8	97/00442	WO	A1	1997-01-03	University of Washington		☒
	9	02/087792	WO	A1	2002-11-07	Genoptix, Inc.		☒
	10	03/062867	WO	A1	2003-07-31	Genoptix, Inc.		☒
	11	97/47390	WO	A1	1997-12-18	University of Washington		☒

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	TAKAYAMA et al., "Patterning Cells and Their Environments Using Multiple Laminar Fluid Flows in Capillary Networks." Proceedings of National Academy of Sciences,USA 96 (1999)	☒

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14169927
	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	2881
	Examiner Name	KIET TUAN NGUYEN
	Attorney Docket Number	81527.0018

	2	Final Notice of Reasons for Rejection, issued by Japanese Patent Office on October 28, 2014 in related Japanese Patent Application No. 2011-256171	☒

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature	/Kiet Tuan Nguyen/	Date Considered	12/04/2014

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	14169927
Filing Date	2014-01-31
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	KIET TUAN NGUYEN
Attorney Docket Number	81527.0018

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Jean C. Edwards/	Date (YYYY-MM-DD)	2014-11-17
Name/Print	Jean C. Edwards, Esq.	Registration Number	41728

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

PATENT WITHDRAWAL NOTICE

DATE WITHDRAWN	WITHDRAWAL NUMBER
11/18/2014	27345

The following application has been **WITHDRAWN** from the

12/2/2014 issue.

SERIAL NO.	PATENT NUMBER
14169927	8901481

TITLE

MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

NAME AND ADDRESS

DANIEL MUETH
Chicago, IL

REASON FOR WITHDRAWAL

Auto-petition to withdraw - Granted
-

APPROVED

/Kimberly Terrell/, Manager

Patent Publication Branch
Office of Data Management

FORM PTO-302 -- (REV. 05-2009)

Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

PTO/SB/30EFS (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	14169927	Filing Date	2014-01-31	Docket Number (if applicable)	81527.0018	Art Unit	2881
First Named Inventor	Daniel MUETH			Examiner Name	KIET TUAN NGUYEN		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☐ Amendment/Reply
- ☒ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☐ Other _____

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____ (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

☒ **The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.**
The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 505497

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

☐ Applicant Signature

PTO/SB/30EFS (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Signature of Registered U.S. Patent Practitioner			
Signature	/Jean C. Edwards/	Date (YYYY-MM-DD)	2014-11-17
Name	Jean C. Edwards, Esq.	Registration Number	41728

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 July 2003 (31.07.2003)

PCT

(10) International Publication Number
WO 03/062867 A2

(51) International Patent Classification[7]: G02B

(21) International Application Number: PCT/US03/00340

(22) International Filing Date: 6 January 2003 (06.01.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/053,507 17 January 2002 (17.01.2002) US

(71) Applicant *(for all designated States except US)*: **GENOPTIX, INC.** [US/US]; 3398 Carmel Mountain Road, San Diego, CA 92121 (US).

(72) Inventor; and

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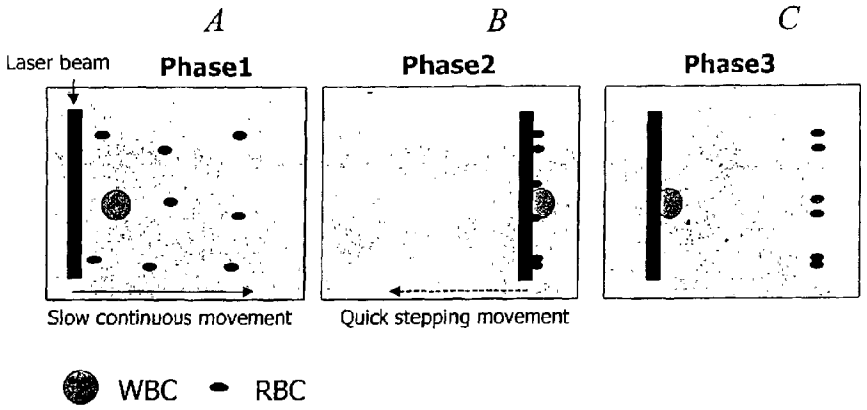
(54) **Title:** METHODS AND APPARATUS FOR GENERATING AND UTILIZING LINEAR MOVING OPTICAL GRADIENTS



(57) **Abstract:** Apparatus and methods are provided for interacting light with particles, including but not limited to biological matter such as cells, in unique and highly useful ways. Optophoresis consists of subjecting particles to various optical forces, especially optical gradient forces, and more particularly moving optical gradient forces, so as to obtain useful results. In one implementation, a population of particles, comprising two or more differing particles, e.g., red blood cells and white blood cells, are illuminated by a line of light which is moved slowly relative to the particle population. The particles are moved with the line until the population is aligned. Next, the line of particles is subject to relative motion of light relative to the particles, such as by rapidly moving the line of illumination relative to the physical position of the particles. By moving the line away from the particles at a rate great enough that certain particles remain behind, effective separation, characterization and/or identification of the particles may be made. Optionally, the direction of the low initial scan is in a direction opposition to the more rapid scan after the particles have been aligned.

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METHODS AND APPARATUS FOR GENERATING AND UTILIZING LINEAR MOVING OPTICAL GRADIENTS

Field of the Invention

[0001] This invention relates to methods and apparatus for the selection, identification, characterization, and/or sorting of materials utilizing at least optical or photonic forces. More particularly, the inventions find utility in biological systems, generally considered to be the use of optical forces for interaction with bioparticles having an optical dielectric constant.

Related Applications

[0002] This application is a continuation-in-part of Application Serial No. 09/993,377, filed November 14, 2001, entitled "Methods and Apparatus for Generating and Utilizing a Moving Optical Gradient", which is a continuation-in-part of Application Serial No. 09/845,245, filed April 27, 2001, entitled "Methods and Apparatus for Use of Optical Forces for Identification, Characterization and/or Sorting of Particles", which is related to Application Serial No. 09/843,902, filed on April 27, 2001, entitled "System and Method for Separating Micro-Particles", with named inventor Osman Kibar, which claims priority from provisional Application Serial No. 60/248,451, entitled "Method and Apparatus for Sorting Cells or Particles", filed November 13, 2000. Those applications are incorporated herein by reference as if fully set forth herein.

Background of the Invention

[0003] Separation and characterization of particles has a wide variety of applications ranging from industrial applications, to biological applications, to environmental applications. For example, in the field of biology, the separation of cells has numerous applications in medicine and biotechnology. Historically, sorting technologies focused on gross physical characteristics, such as particle size or density, or to utilize some affinity interaction, such as receptor-ligand interactions or reactions with immunologic targets.

[0004] Electromagnetic response properties of materials have been utilized for particle sorting and characterization. For example, dielectrophoretic separators utilize non-uniform DC or AC electric fields for separation of particles. See, e.g., U.S. Patent No. 5,814,200, Pethig et al., entitled "Apparatus for Separating By Dielectrophoresis". The

application of dielectrophoresis to cell sorting has been attempted. In Becker (with Gascoyne) et al., PNAS USA, Vol. 92, pp. 860-864, Jan. 1995, Cell Biology, in the article entitled "Separation of Human Breast Cancer Cells from Blood by Differential Dielectric Affinity", the authors reported that the dielectric properties of diseased cells differed sufficiently to enable separation of the cancer cells from normal blood cells. The system balanced hydrodynamic and dielectrophoretic forces acting on cells within a dielectric affinity column containing a microelectrode array. More sophisticated separation systems have been implemented. See, e.g., Cheng, et al., U. S. Patent No. 6,071,394, "Channel-Less Separation of Bioparticles on a Bioelectronic Chip by Dielectrophoresis". Yet others have attempted to use electrostatic forces for separation of particles. See, e.g., Judy et al., U.S. Patent No. 4,440,638, entitled "Surface Field-Effect Device for Manipulation of Charged Species", and Washizu "Electrostatic Manipulation of Biological Objects", Journal of Electrostatics, Vol. 25, No. 1, June 1990, pp. 109-103.

[0005] Light has been used to sort and trap particles. One of the earliest workers in the field was Arthur Ashkin at Bell Laboratories, who used a laser for manipulating transparent, μm-size latex beads. Ashkin's U.S. Patent No. 3,808,550 entitled "Apparatuses for Trapping and Accelerating Neutral Particles" disclosed systems for trapping or containing particles through radiation pressure. Lasers generating coherent optical radiation were the preferred source of optical pressure. The use of optical radiation to trap small particles grew within the Ashkin Bell Labs group to the point that ultimately the Nobel Prize was awarded to researchers from that lab, including Steven Chu. See, e.g., Chu, S., "Laser Trapping of Neutral Particles", Sci. Am., p. 71 (Feb. 1992), Chu, S., "Laser Manipulation of Atoms and Particles", Science 253, pp. 861-866 (1991).

[0006] Generally, the interaction of a focused beam of light with dielectric particles or matter falls into the broad categories of a gradient force and a scattering force. The gradient force tends to pull materials with higher relative dielectric constants toward the areas of highest intensity in the focused beam of light. The scattering force is the result of momentum transfer from the beam of light to the material, and is generally in the same direction as the beam. The use of light to trap particles is also sometimes referred to as an optical tweezer arrangement. Generally, utilizing the Rayleigh approximation, the force of trapping is given by the following equation:

$$F_g = 2\pi \cdot r^3 \frac{\sqrt{\varepsilon_B}}{c}\left(\frac{\varepsilon - \varepsilon_B}{\varepsilon + 2\varepsilon_B}\right)(\nabla I)$$

where F_g is the optical gradient force on the particle in the direction toward the higher intensity, r is the radius of the particle, ε_B is the dielectric constant of the background medium, ε is the dielectric constant of the particle, I is the light intensity in watts per square centimeter and ∇ is the spatial derivative. Fig. 1 shows a drawing of a particle in an optical tweezer. The optical tweezer consists of a highly focused beam directed to the particle.

[0007] As shown in Fig. 1, the focused beam 12 first converges on the particle 10 and then diverges. The intensity pattern 14 relates to the cross-section of the intensity of the beam in the horizontal dimension, and the intensity pattern 16 is the cross-section of intensity in the vertical dimension. As can be seen from the equation, the trapping force is a function of the gradient of the intensity of the light. Thus, the force is greater where the light intensity changes most rapidly, and contrarily, is at a minimum where the light intensity is uniform.

[0008] Early stable optical traps levitated particles with a vertical laser beam, balancing the upward scattering force against the downward gravitational force. The gradient force of the light served to keep the particle on the optical axis. See, e.g., Ashkin, "Optical Levitation by Radiation Pressure", Appl. Phys. Lett., 19(6), pp. 283-285 (1971). In 1986, Ashkin disclosed a trap based upon a highly focused laser beam, as opposed to light propagating along an axis. The highly focused beam results in a small point in space having an extremely high intensity. The extreme focusing causes a large gradient force to pull the dielectric particle toward that point. Under certain conditions, the gradient force overcomes the scattering force, which would otherwise push the particle in the direction of the light out of the focal point. Typically, to realize such a high level of focusing, the laser beam is directed through a high numerical aperture microscope objective. This arrangement serves to enhance the relative contribution from the high numerical aperture illumination but decreases the effect of the scattering force.

[0009] In 1987, Ashkin reported an experimental demonstration of optical trapping and manipulation of biological materials with a single beam gradient force optical trap system. Ashkin, et al., "Optical Trapping and Manipulation of Viruses and Bacteria", Science, 20 March, 1987, Vol. 235, No. 4795, pp. 1517-1520. In U.S. Patent No. 4,893,886, Ashkin et

al., entitled "Non-Destructive Optical Trap for Biological Particles and Method of Doing Same", reported successful trapping of biological particles in a single beam gradient force optical trap utilizing an infrared light source. The use of an infrared laser emitting coherent light in substantially infrared range of wavelengths, there stated to be 0.8 μm to 1.8 μm, was said to permit the biological materials to exhibit normal motility in continued reproductivity even after trapping for several life cycles in a laser power of 160 mW. The term "opticution" has become known in the art to refer to optic radiation killing biological materials.

[0010] The use of light to investigate biological materials has been utilized by a number of researchers. Internal cell manipulation in plant cells has been demonstrated. Ashkin, et al., PNAS USA, Vol. 86, 7914-7918 (1989). See also, the summary article by Ashkin, A., "Optical Trapping and Manipulation of Neutral Particles Using Lasers", PNAS USA, Vol. 94, pp. 4853-4860, May 1997, Physics. Various mechanical and force measurements have been made including the measurement of torsional compliance of bacterial flagella by twisting a bacterium about a tethered flagellum. Block, S., et al., Nature (London), 338, pp. 514-518 (1989). Micromanipulation of particles has been demonstrated. For example, the use of optical tweezers in combination with a microbeam technique of pulsed laser cutting, sometimes also referred to as laser scissors or scalpel, for cutting moving cells and organelles was demonstrated. Seeger, et al., Cytometry, 12, pp. 497-504 (1991). Optical tweezers and scissors have been used in all-optical in vitro fertilization. Tadir, Y., Human Reproduction, 6, pp. 1011-1016 (1991). Various techniques have included the use of "handles" wherein a structure is attached to a biological material to aid in the trapping. See, e.g., Block, Nature (London), 348, pp. 348-352 (1990).

[0011] Various measurements have been made of biological systems utilizing optical trapping and interferometric position monitoring with subnanometer resolution. Svoboda, Nature (London), 365, pp. 721-727 (1993). Yet others have proposed feedback based systems in which a tweezer trap is utilized. Molloy, et al., Biophys. J., 68, pp. 2985-3055 (1995).

[0012] A number of workers have sought to distort or stretch biological materials. Ashkin in Nature (London), 330 pp. 769-771 (1987), utilized optical tweezers to distort the shape of red blood cells. Multiple optical tweezers have been utilized to form an assay to measure the shape recovery time of red blood cells. Bronkhorst, Biophys. J., 69, pp. 1666-

1673 (1995). Kas, et al., has proposed an "optical stretcher" in U.S. Patent No. 6,067,859 which suggests the use of a tunable laser to trap and deform cells between two counter-propagating beams generated by a laser. The system is utilized to detect single malignant cancer cells. Yet another assay proposed colliding two cells or particles under controlled conditions, termed the OPTCOL for optical collision. See, e.g., Mammer, Chem & Biol., 3, pp. 757,763 (1996).

[0013] Yet others have proposed utilizing optical forces to measure a property of an object. See, e.g., Guanming, Lai et al., "Determination of Spring Constant of Laser-Trapped Particle by Self-Mining Interferometry", Proc. of SPIE, 3921, pp. 197-204 (2000). Yet others have utilized the optical trapping force balanced against a fluidic drag force as a method to calibrate the force of an optical trap. These systems utilize the high degree of dependence on the drag force, particularly Stokes drag force.

[0014] Yet others have utilized light intensity patterns for positioning materials. In U.S. Patent No. 5,245,466, Burnes et al., entitled "Optical Matter", arrays of extended crystalline and non-crystalline structures are created using light beams coupled to microscopic polarizable matter. The polarizable matter adopts the pattern of an applied, patterned light intensity distribution. See also, "Matter Rides on Ripples of Lights", reporting on the Burns work in New Scientist, 18 Nov., 1989, No. 1691. Yet others have proposed methods for depositing atoms on a substrate utilizing a standing wave optical pattern. The system may be utilized to produce an array of structures by translating the standing wave pattern. See, Celotta et al., U.S. Patent No. 5,360,764, entitled "Method of Fabricating Laser Controlled Nanolithography".

[0015] Yet others have attempted to cause motion of particles by utilizing light. With a technique termed by its authors as "photophoresis", Brian Space, et al., utilized a polarized beam to induce rotary motion in molecules to induce translation of the molecules, the desired goal being to form a concentration gradient of the molecules. The technique preferably utilizes propeller shaped molecules, such that the induced rotary motion of the molecules results in translation.

[0016] Various attempts have been made to form microfluidic systems, put to various purposes, such as sample preparation and sorting applications. See, e.g., Ramsey, U.S. Patent No. 6,033,546, entitled "Apparatus and Method for Performing Microfluidic

Manipulations for Chemical Analysis and Synthesis". Numerous companies, such as Aclara and Caliper, are attempting to form micro-systems comprising a 'lab on a chip'.

[0017] Others have attempted to combine microfabricated devices with optical systems. In "A Microfabricated Device for Sizing and Sorting DNA Molecules", Chou, et al., PNAS USA, Vol. 96, pp. 11-13, Jan. 1999, Applied Physical Sciences, Biophysics, a microfabricated device is described for sizing and sorting microscopic objects based upon a measurement of fluorescent properties. The paper describes a system for determining the length of DNA by measuring the fluorescent properties, including the amount of intercalated fluorescent dye within the DNA. In "A Microfabricated Fluorescence-Activated Cells Sorter", Nature Biotechnology, Vol. 17, Nov. 1999, pp. 1109-1111, a "T" microfabricated structure was used for cell sorting. The system utilized a detection window upstream of the "T" intersection and based upon the detected property, would sort particles within the system. A forward sorting system switched fluid flow based upon a detected event. In a reverse sorting mode, the fluid flow was set to route all particles to a waste collection, but upon detection of a collectible event, reversed the fluid flow until the particle was detected a second time, after which the particle was collected. Certain of these systems are described in Quake et al., PCT Publication WO 99/61888, entitled "Microfabricated Cell Sorter".

[0018] Yet others have attempted to characterize biological systems based upon measuring various properties, including electromagnetic radiation related properties. Various efforts to explore dielectric properties of materials, especially biological materials, in the microwave range have been made. See, e.g., Larson et al., U.S. Patent No. 4,247,815, entitled "Method and Apparatus for Physiologic Facsimile Imaging of Biologic Targets Based on Complex Permittivity Measurements Using Remote Microwave Interrogation", and PCT Publication WO 99/39190, named inventor Hefti, entitled "Method and Apparatus for Detecting Molecular Binding Events".

[0019] Despite the substantial effort made in the art, no comprehensive, effective, sensitive and reliable system has been achieved.

Summary of the Invention

[0020] The methods and apparatus of this relate generally to the use of light energy to obtain information from, or to apply forces to, particles. The particles may be of any form which have a dielectric constant. The use of light for these beneficial purposes is the field

of optophoresis. A particle, such as a cell, will have a Optophoretic constant or signature which is indicative of a state, or permits the selection, sorting, characterization or unique interaction with the particle. In the biological regime, the particles may include cells, organelles, proteins, or any component down to the atomic level. The techniques also apply in the non-biological realm, including when applied to all inorganic matter, metals, semiconductors, insulators, polymers and other inorganic matter.

[0021] Considering the biological realm, the cell represents the true point of integration for all genomic information. Accessing and deciphering this information is important to the diagnosis and treatment of disease. Existing technologies cannot efficiently and comprehensively address the enormous complexity of this information. By unlocking the fundamental properties of the cell itself, the methods and apparatus described herein create new parameters for cellular characterization, cellular analysis and cell-based assays.

[0022] This technology represents a practical approach to probing the inner workings of a particle, such as a living cell, preferably without any dyes, labels or other markers. The "Optophoretic Constant" of a cell uniquely reflects the physiological state of the cell at the exact moment in which it is being analyzed, and permits investigation of the inner workings of cells. These techniques allow simple and efficient gathering of a wide spectrum of information, from screening new drugs, to studying the expression of novel genes, to creating new diagnostic products, and even to monitoring cancer patients. This technology permits the simultaneous analysis and isolation of specific cells based on this unique optophoretic parameter. Stated otherwise, this technology is capable of simultaneously analyzing and isolating specific particles, e.g. cells, based on their differences at the atomic level. Used alone or in combination with modern molecular techniques, the technology provides a useful way to link the intricate mechanisms involving the living cell's overall activity with uniquely identifiable parameters.

[0023] In one embodiment, apparatus and methods are provided for identification, characterization and/or sorting of particles by first, providing a population of particles, illuminating the particles with an intensity profile which moves relative to the particles so as to organize the particle population in physical space, most preferably into an arrangement of a line, followed by subsequent movement of the illumination relative to the now physically organized particle population, to effect physical separation of particles having differing optophoretic properties. In one implementation, a population of particles,

comprising two or more differing particles, e.g., red blood cells and white blood cells, are illuminated by a line of light which is moved slowly relative to the particle population. The particles are moved with the line until the population is aligned. Next, the line of particles is subject to relative motion of light relative to the particles, such as by rapidly moving the line of illumination relative to the physical position of the particles. By moving the line away from the particles at a rate great enough that certain particles remain behind, effective separation, characterization and/or identification of the particles may be made. Optionally, the direction of the low initial scan is in a direction opposition to the more rapid scan after the particles have been aligned.

[0024] In yet another aspect, the invention is a method for the characterization of a particle by the steps of observing a first physical position of a particle, optically illuminating the particle to subject it to an optical force, observing the second physical position of the particle, and characterizing the particle based at least in part upon reaction of the particle to the optical force. The characterization may be that the particle, e.g., a cell, has a certain disease state based upon the detected optophoretic constant or signature.

[0025] While characterization may be done with or without physical separation of multiple particles, a method for separating particles may consist of, first, subjecting particles to optical gradient force, second, moving the particle, and third, separating desired particle from other particles. The particle may be separate from the others by further optical forces, by fluidic forces, by electromagnetic forces or any other force sufficient to cause the required separation. Separation may include segregation and sorting of particles.

[0026] In yet another aspect, the invention includes a method for analyzing particles by electrokinetically moving the particles, and subjecting the particles to optical forces for sorting. The electrokinetic forces may include, for example, eletroosmosis, electrophoresis and dielectrophoresis.

[0027] In addition to the use of the dielectric aspects of the particle for characterization and sorting, certain of the inventive methods may be used to determine the dielectric constant of a particle. One method consists of subjecting the particle to an optical gradient force in a plurality of media having different dielectric constants, monitoring the motion of the particle when subject to the optical gradient force in the various media, and

determining the dielectric constant of the particle based upon the relative amount of motion in the various media.

[0028] Yet other methods permit the sorting of particles according to their size. One method includes the steps of subjecting the particles to a optical fringe pattern, moving the fringes relative to the particles, wherein the improvement comprises selecting the period of the fringes to have a differential effect on differently sized particles. An allied method sorts or otherwise separates particles based upon the particles flexibility when subject to a optical force. One set of exemplary steps includes: subjecting the particles to an optical pattern having fringes, the fringe spacing being less than the size of the particle in an uncompressed state, moving the fringes relative to the medium containing the particles, and whereby particles having relatively higher flexibility are separated from those with relatively lower flexibility.

[0029] In addition to the use of optical gradient forces, the systems and methods may use, either alone or in combination with other forces, the optical scattering force. One method for separation in an optophoresis set up consists of providing one or more particles, subjecting the particles to light so as to cause a scattering force on the particles, and separating the particles based upon the reaction to at least the scattering force.

[0030] Various techniques are described for enhancing the sensitivity and discrimination of the system. For example, a sensitive arrangement may be provided by separating the particles in a medium having a dielectric constant chosen to enhance the sensitivity of the discrimination between the particles, and changing the medium to one having a dielectric constant which causes faster separation between the particles. One option for enhancing the sensitivity is to choose the dielectric constant of the medium to be close to the dielectric constant of the particles.

[0031] Accordingly, it is an object of this invention to provide a method of identification, characterization, selection and/or sorting of materials having an optical dielectric constant.

[0032] It is yet a further object of this invention to provide a system for sorting or identifying particles without labeling or otherwise modifying the particle.

[0033] It is yet another object of this invention to provide a system in which uncharged or neutral particles may be sorted or otherwise characterized.

[0034] Yet another object of this invention is to provide a system in which particles may be manipulated remotely, thereby reducing the contamination to the system under study.

[0035] It is yet another object of this invention to provide a system for characterizing, moving and/or sorting particles that may be used in conjunction with other forces, without interference between the optical forces and the other forces.

Brief Description of the Drawings

[0036] Fig. 1 is a graphical depiction of optical intensity patterns for a prior art optical tweezer system, showing both the focus beam, a particle and the cross-section of intensity of the beam.

[0037] Fig. 2 is a cross-sectional drawing of the optical system for interfering two beams utilizing a variable path length by moving a mirror.

[0038] Fig. 3 is a schematic diagram of a system utilizing interference between two beams where the path length is varied utilizing a phase modulator.

[0039] Fig. 4 is a cross-sectional drawing of an optical system utilizing an interferometer where the path length is adjustable via a phase modulator, and Fig. 4A is a side view of an alternate optical arrangement utilizing counterpropagating beams for particle levitation.

[0040] Fig. 5 is a cross-sectional drawing of an optical system including an interferometer and a phase modulator for changing the optical path length, and includes a photograph of a wave pattern generated by the system.

[0041] Fig. 6 is a cross-sectional drawing of an optical system utilizing separate illumination and imaging systems.

[0042] Fig. 7 is a depiction of an optical system interfacing with a fluidic system.

[0043] Fig. 8 is a cross-sectional drawing of an optical system utilizing a moving scanning system.

[0044] Figs. 9A and 9B are cross-sectional drawings of an optical system including a mask based generation of intensity pattern.

[0045] Fig. 10 is a side view of an array of illumination sources, illuminating a substrate or support.

[0046] Figs. 11A, 11B and 11C show graphs of intensity, forces and potential energy, respectively, as a function of position in one exemplary embodiment of the invention.

[0047] Fig. 12A shows two particles at first positions and a superimposed optical pattern.

[0048] Fig. 12B shows the particles at second positions after illumination by the optical pattern.

[0049] Fig. 12C shows the trapping of particle B in an optical trap.

[0050] Figs. 13A, 13B and 13C show graphs of the potential energy as a function of distance for the technique for separating particles.

[0051] Figs. 14A and 14B show graphical depictions of particle sorting from a one-dimensional particle source, in Fig. 14A showing the particle flow and in Fig. 14B showing particles transported in a fluid flow.

[0052] Fig. 15 is a plan view drawing of a "T" channel sorting structure.

[0053] Fig. 16 is a plan view of an "H" sorting structure.

[0054] Fig. 17 is a plan view of a "Y" shaped sorting structure.

[0055] Fig. 18 is a plan view of a "X" channel sorting structure.

[0056] Fig. 19 is a perspective view of a two-dimensional sorting structure.

[0057] Fig. 20 is a plan view of a multi-dimensional sorting structure.

[0058] Fig. 21 is a side view of a multi-dimensional sorting structure including a reflective surface for generation of the optical gradient pattern.

[0059] Fig. 22 is a side view of a sorting structure including a capture structure.

[0060] Fig. 23 is a plan view of a microfluidic system including a recycle path.

[0061] Fig. 24 is a plan view of a particle analysis system utilizing particle deformability as a factor in the selection or characterization.

[0062] Fig. 25 is a plan view of a sorting or characterization system utilizing the particle size relative to the optical gradient periodicity as a factor.

[0063] Fig. 26 is a system for separation of particles utilizing the scattering force of light for separation.

[0064] Fig. 27A is a perspective drawing of a scattering force switch.

[0065] Fig. 27B is a plan, side view of a scattering force switch.

[0066] Fig. 27C is a plan, side view of a scattering force switch with the beam on.

[0067] Fig. 28 is a schematic drawing of a system for determining the dielectric constant of particles in various fluidic media of varying dielectric constant.

[0068] Fig. 29 is a cross-sectional drawing of particles and a light intensity profile for separating particles in a dielectric medium.

[0069] Fig. 30 is a perspective view of a optical tweezer array.

[0070] Fig. 31 is a graph of molar extinction coefficient as a function of wavelength for hemoglobin-O_2 absorption spectrum.

[0071] Fig. 32 shows time lapse photographs of an experiment separating particles by size with a moving optical gradient field.

[0072] Fig. 33 shows time lapse photographs of an experiment separating particles by surface functionalization.

[0073] Fig. 34 shows a Before, After and Difference photograph of particles subject to a moving optical gradient field.

[0074] Fig. 35 is a graph of percent of cells measured in an experiment versus escape velocity, for a variety of cell types.

[0075] Fig. 36 shows photographs of sorting of two cell types in a microchannel device. 1 shows a red blood cell and a white blood cell successively entering the moving optical gradient field. 2 shows that white blood cell has been translated down by the action of the moving optical gradient field while the red blood cell has escaped translation. 3 and 4 show that the red blood cell and white blood cell continue to flow into separate channels, completing the sorting.

[0076] Figs. 37A, B and C show the steps in a scanning method comprising a first scanning of a particle population in phase one (Fig. 37A) , a movement of illumination relative to the aligned particle population in phase two (Fig. 37B), and separation of particles in phase three (Fig. 37C).

[0077] Fig. 38 shows a sequence of graphs of light intensity and particle position for the technique shown in Figs. 37A, B and C.

[0078] Fig. 39A shows a cross-sectional view of components for use in a line scanning system, and Fig. 39B shows a top view of the operational space.

[0079] Fig. 40A shows a cross-sectional view of a defractive optical set up to generate one or more lines of illumination. Fig. 40B shows a top view of the arrangement in Fig. 40A. Fig. 40C shows a scanning mirror arrangement to generate one or more lines of illumination. Fig. 40D shows a top view of the illumination space.

[0080] Fig. 41 shows a top view of a sectioned sample field.

[0081] Fig. 42 shows a top view of a sample field having multiple lines of illumination.

[0082] Figs. 43A, B and C are images of the effective separation of white blood cells and red blood cells, corresponding to the phases shown in Figs. 37A, B and C.

Detailed Description of the Invention

Definitions

[0083] The following definitions are provided for an understanding of the invention disclosed herein.

[0084] "Dielectric constant" is defined to be that property which determines the electrostatic energy stored per unit volume for unit potential gradient. (See, e.g., the New IEEE Standard Dictionary Of Electrical And Electronics Terms, ©1993).

[0085] The "optical dielectric constant" is the dielectric constant of a particle or thing at optical wavelengths. Generally, the optical wavelength range is from 150 Å to 30,000 Å.

[0086] An "optical gradient field" is an optical pattern having a variation in one or more parameters including intensity, wavelength or frequency, phase, polarization or other parameters relating to the optical energy. When generated by an interferometer, an optical gradient field or pattern may also be called an optical fringe field or fringe pattern, or variants thereof.

[0087] A "moving optical gradient field" is an optical gradient field that moves in space and/or time relative to other components of the system, e.g., particles or objects to be identified, characterized, selected and/or sorted, the medium, typically a fluidic medium, in contact with the particles, and/or any containment or support structure.

[0088] An "optical scattering force" is that force applied to a particle or thing caused by a momentum transfer from photons to material irradiated with optical energy.

[0089] An "optical gradient force" is one which causes a particle or object to be subject to a force based upon a difference in dielectric constant between the particle and the medium in which it is located.

[0090] "Optophoresis" or "Optophoretic" generally relates to the use of photonic or light energy to obtain information about or spatially move or otherwise usefully interact with a particle.

[0091] "Optophoretic constant" or "optophoretic signature" or "optophoretic fingerprint" refer to the parameter or parameters which distinguish or characterize particles for optical selection, identification, characterization or sorting.

[0092] An "optical tweezer" is a light based system having a highly focused beam to a point in space of sufficiently high intensity that the gradient force tends to pull a dielectric particle toward the point of highest intensity, typically with the gradient force being sufficiently strong to overcome the scattering force. Most typically, the laser beam is directed through a microscope objective with a high numerical aperture, with the beam

having a diffraction limited spot size of approximately the wavelength of the light, 5,000 to 20,000 Å, though more typically 10,000 Å. Generally, an optical tweezer has a beam width in the focal plane of 2 μm or less, and typically about 1 μm.

[0093] "Separation" of two objects is the relative spatial distancing over time of a particle from some other reference point or thing.

[0094] "Sorting" involves the separation of two or more particles in a meaningful way.

DESCRIPTION OF EXEMPLARY APPARATUS

Optical components -- Generation of moving optical gradient field.

[0095] Figs. 2 - 10 describe various systems for generation of optical patterns, sometimes termed fringe patterns or optical fringe patterns, including, but not limited to, a moving optical gradient field pattern. These exemplary embodiments are intended to be illustrative, and not limiting, as other apparatus may be utilized to generate the optical fields and forces to achieve the desirable results of these inventions.

[0096] The points raised in discussions of specific embodiments may be considered to be generally applicable to descriptions of the other embodiments, even if not expressly stated to be applicable.

[0097] The light source for use with systems has certain generally desirable properties. As to wavelength, the wavelength will generally be chosen based upon one or more considerations. In certain applications, it may be desirable to avoid damage to biological materials, such as cells. By choosing wavelengths in ranges where the absorption by cellular components, mostly water, are minimized, the deleterious effects of heating may be minimized. Wavelengths in the range from approximately 0.3 μm to approximately 1.8 μm, and more preferably, from substantially 0.8 to substantially 1.8 μm, aid in reducing biological damage. However, even for biological applications, a laser having a wavelength generally considered to be damaging to biological materials may be used, such as where the illumination is for a short period of time where deleterious absorption of energy does not occur. In yet other applications, it may be desirable to choose a wavelength based upon a property of the particle or object under consideration. For example, it may be desirable to choose the wavelength to be at or near an absorption band in order to increase (or decrease) the force applied against a particle having a particular attribute. Yet another consideration for wavelength choice may be compatibility with

existing technology, or a wavelength naturally generated by a source. One example would be the choice of the wavelength at 1.55 μm. Numerous devices in the 1.55 μm wavelength region exist commercially and are used extensively for telecommunications applications.

[0098] Generally, the light sources will be coherent light sources. Most typically, the coherent light source will consist of a laser. However, non-coherent sources may be utilized, provided the system can generate the forces required to achieve the desired results. Various laser modes may be utilized, such as the Laguerre-Gaussian mode of the laser. Furthermore, if there is more than one light source in the system, these sources can be coherent or incoherent with respect to each other.

[0099] The spot size or periodicity of the intensity pattern is preferably chosen to optimize the effective results of the illumination. In many applications, it is desirable to have a substantially uniform gradient over the particle, e.g., cell, to be interrogated such that the dielectric properties of the entire particle (cell) contribute to the resulting force. Broadly, the range varies from substantially 1 to substantially 8 times the size (diameter or average size) of the particle or object, more preferably, the range is from substantially 2 to substantially 4 times the size. Various methods and systems known to those skilled in the art may be utilized to achieve the desired spot size or periodicity, e.g., using a defocused beam or a collimated beam having the desired size. The typical characterization of the radius of the spot is the $1/e^2$ radius of the beam intensity. For many applications, including cellular applications, the beam size will be on the order of 10 microns, though sometimes as small as five microns, and in even certain other occasions, as small as two microns. In certain applications, it is desirable to have the periodicity of the illumination in the range from substantially 1 to substantially 2 times the size (diameter or average size) of the particle or object. For many biological applications, a periodicity of from substantially 5 μm to 25 μm, and more preferably from 10 μm to 20 μm. Certain applications may utilize smaller sizes, e.g., for bacteria, or larger sizes, e.g., for larger particles. In yet other applications, it may be desired to utilize a spot size smaller than the particle or object, such as where interrogation of a sub-cellular region is desired.

[00100] The examples of systems for generating intensity patterns, described below, as well as other systems for generating intensity patterns useful for the subject inventions include various optical components, as well as a control system to generate the desired pattern, intensity profile or other gradient, such as a moving optical field gradient.

Various optical systems may be adapted for use in the systems of the invention, so as to effectively carry out the methods and achieve the results described herein. Exemplary systems which may be adapted in whole or in part include: Young's slits, Michelson interferometer, Mach-Zender interferometer, Haidinger circular fringe systems, Fresnel mirror interferometer, plane-parallel plate interferometer, Fabry-Perot interferometer and any other system for generating an optical gradient intensity pattern or fringe pattern.

[00101] Turning now to a detailed description of exemplary systems for use with the subject inventions. Fig. 2 shows an optical component description of a system 20 generally configured to generate a moving optical gradient field pattern to provide a force on one or more particles provided to the system 20. The optical forces may then be used for characterization, identification, selection and/or sorting of the particles. A light source 22, preferably a laser, generates a first beam 24 directed toward beam splitter 26. Beam splitter 26 may be of any mode or type known to the art, such as a prism beam splitter, consistent with the goals and objects of this invention. A first transmitted beam 28 passes through the beam splitter 26. A first reflected beam 30 reflects from the beam splitter 26 to a reflective surface 32, typically a mirror, to generate a second reflected beam 34. The first transmitted beam 28 and second reflected beam 34 interfere and generate an intensity pattern 38, generally being located at the operative portion of the slide or support 36 where the light would interact with the particle or object of interest. The optical pattern 38 moves relative to other objects, e.g., the particles, the substrate, and/or the fluidic medium containing the particles, by virtue of a change in the optical path length between the first transmitted beam 28 and the combination of the first reflected beam 30 and second reflected beam 34. Mirror 32 is movable, by actuator 40. One example of an actuator 40 could comprise a motor and screw system to move mirror 32. Numerous alternative structures for moving mirror 32 are known to the art, e.g., piezoelectric systems, oscillating mirror systems and the like.

[00102] Fig. 3 shows a two-beam interference based system. A source of coherent light, such as laser 52, generates a first beam 54 directed to a beam splitter 56. A first reflected beam 58 is directed toward the sample plate 70 and a first transmitted beam 60 is directed to a modulator, such as a phase modulator 62. The phase modulator 62 may be of any type known to those skilled in the art. Phase modulator 62 is under control of the control system 64 and results in modulated beam output 66 which is directed to a mirror 74. The

modulated beam 66 reflects from mirror 74 to generate the second reflected beam 68 which is directed to the sample plate 70. The first reflected beam 54 and second reflected beam 68 generate a pattern 72 at the operative interface with the sample plate 70. The control system 64 is connected to the phase modulator 62 so as to cause the pattern 72 to move relative to the objects within the system 50, such as the sample plate 70.

[00103] Fig. 4 shows an optical component diagram of an interferometer system 80. A light source, such as laser 82, generates a first light beam 84 directed to beam splitter 86. An interferometer composed of the first mirror 88 and second mirror 90 generate an output beam 100 having the desired beam properties, including the desired gradient properties. The first beam 84 passes through beam splitter 86 to generate a first transmitted beam 94 directed to first mirror 88. The reflected beam retraces path 94 to the beam splitter 86. The first reflected beam 96 passes through phase modulator 92 to generate first modulated beam 98 directed to the second mirror 90. The reflected beam from second mirror 90 retraces the path 98 through the phase modulator 92 and beam 96 to the beam splitter 86. The beam 100 is output from the interferometer section of the system 80 and directed toward the microscope objective 104.

[00104] The objective 104 is directed toward the sample plate 106. Optionally, a mirror 108, most preferably a planar mirror, may be disposed beneath the sample plate 106. The mirror 108 is oriented so as to provide reflected light onto the sample plate 106 bearing or containing the particles or objects under analysis or action of the system 80. The scattering force caused by the beam 102 as initially illuminates the sample plate 106 may be counteracted, in whole or in part, by directing the reflected radiation from mirror 108 back toward the sample. As discussed more in the section relating to surface effects, below, the reflected light and the upward scattering force reduce the overall effects of the scattering forces, such that the gradient forces may be more effectively utilized.

[00105] Fig. 4 includes an optional imaging system. The light 102 from the objective 104 is reflected by the beam splitter 120 generating third reflected beam 110 which is directed toward imaging optics 112. The optics 112 image the light on a detector 114, such as a charge couple device (CCD) detector. The output of the detector 114 may be provided to an imaging system 116. The imaging system 116 may optionally include a display, such as a monitor (CRT, flat panel display, plasma display, liquid crystal display, or other displays known to those skilled in the art). The imaging system 116 may

optionally include image enhancement software and image analysis software, recording capability (to tape, to optical memory, or to any other form of memory known to those skilled in the art).

[00106] A control system 118 controls the modulator 92 so as to generate the desired optical force pattern within the system 80. Optionally, the imaging system 116 may be coupled to the control system 118. A feedback system may be created whereby the action of the particles on the sample plate 106 may be imaged through the system 116 and then utilized in the control system analysis to control the operation of the overall system 80.

[00107] Fig. 5 shows a interferometer based system 120. A light source, such as laser 122, generates a first beam 124 directed toward an optional spatial filter 126. The spatial filter 126 would typically include lenses 128 and a spatial filter aperture 130. The aperture typically is round. The spatial filters serves to collimate the laser beam and to produce a smooth intensity profile across the wavefront of the laser beam. The interferometer 140 includes first mirror 146 and second mirror 144, as well a beam splitter 142. The phase modulator 148 is disposed within one of the two arms of the interferometer 140.

[00108] As shown in Fig. 5, a mirror 132 is optionally disposed to reflect the light from the source 122 to the interferometer 140. As will be appreciated by those skilled in the art, optical systems may include any number or manner of components designed to transfer or direct light throughout the system. One such example is the planar mirror 132 which merely serves to direct the radiation from one major component, e.g., the spatial filter, to another major component, e.g., the interferometer 140. In addition to mirrors, other common transfer components may include fiber optics, lenses, beam splitters, diffusers, prisms, filters, and shaped mirrors.

[00109] Beam 150 exits the interferometer 140 and is directed toward objective 152 and imaged at or near the sample plate 154. As shown, a dichroic mirror 170 serves to reflect the light 150, but to also permit passage of light from source 168, such as a fiber providing radiation from a source through the dichroic mirror 170 and objective 152 to illuminate the operative regions of the sample plate 154.

[00110] Optionally, a detection system may be disposed to image the operative portions of the sample plate 154. As shown, objective 156 is disposed beneath the sample plate 154, with the output radiation being transferred via mirror 158 to an imaging apparatus 164, such as a charge couple device (CCD). Optionally, an infrared filter 160 may be

disposed within the optical path in order to select the desired wavelengths for detection. The output of the detector 164 is provided to an imaging system 166. As described in connection with other figures, the imaging system 166 may include image enhancement and image analysis software and provide various modes of display to be user. Optionally, the imaging system 166 is coupled to the control system 172 such as when used for feedback.

[00111] Fig. 6 shows an optical system having illumination of a sample plate 194 from the top side and imaging from the bottom side. A laser 180 generates a first beam 182 which optionally passes through a spatial filter 184. The spatial filter as shown includes lens 184 and aperture 188. The output of the spatial filter 184 passes through the objective 192 and is imaged onto the sample plate 194. The sample plate 194 and material supported on it may be imaged via an objective 196. An optional mirror 198 directs radiation to an optional filter 200 through an imaging lens 202 onto the detector 204. The detector 204 is coupled to an imaging system 206. Preferably, the imaging system 206 provides information to a control system 208 which controls various optical components of the system.

[00112] Fig. 7 shows an optical system interfacing a sample plate which includes bounded structures. The system 210 includes a sample plate 212 which optionally includes microfluidic channels. Alternatively, the sample plate 212 may support a separate structure containing the microfluidic channels. As one exemplary structure formed from the microfluidic channels, a "T" sorting arrangement is shown for a simple, though useful, example. An input reservoir 216 connects to a first channel 218 which terminates in a T at intersection 220. A first output channel 222 couples to a first output reservoir 224. A second output channel 226 couples to a second output chamber 228. As shown, the input chamber is coupled to ground and the first output chamber 224 and second output chamber 228 are connected to -V. The fluidic channel structures are discussed in more detail, below.

[00113] The microscope objective 232 serves to both provide the optical radiation to the sample plate 222 as well as to provide the imaging of the system. A light source 238, such as a laser, or more particularly, a laser diode, generates light which may be imaged by optics 240. A dichroic beam splitter 236 directs the radiation to the microscope objective 232. As shown, the objective has a magnification power of 100. For the biological

applications, a magnification range of from 1 to 200 is desired, and more preferably, from 10 to 100. The objective 232 has a 1.25 numerical aperture. The preferable range of numerical apertures for the lenses is from 0.1 to 1.50, and more preferably from 0.4 to 1.25. The output from the objective 232 passes through the beam splitter 236, reflects from optional mirror 242 through optics (e.g., lens) 244, through the optional filter 246 to the imaging device 280. The imaging device, shown as a CCD, is connected to the imaging system 282. The output of the imaging system 282 is optionally coupled to the control system 284. As shown, the control system 284 controls both the translation stage 232 connected to the sample plate 212, as well as to the light source 238.

[00114] Fig. 8 shows a system for generating an intensity pattern within the scanned area 260. An input beam 262, such as from a coherent light source, such as a laser, is directed toward the system. A first oscillating component 264, such as a galvanometer or resonant scanner, intercepts the input beam 262 and provides a first degree of motion to the beam. The beam is directed to a polygonal mirror 268 which contains multiple faces 270. As the polygonal mirror 268 rotates around axis 272, the light is swept across the scanner area 260. Lens 274 are provided as required to appropriately image the light into the scanned area 260. Optionally, a mask or other pattern 276 may be disposed within the optical pathway so as to provide for the variation of the optical forces within the scanned area 260. Any of a wide variety of techniques for generating either the oscillatory motion or the scanning via the polygonal mirror are known to those skilled in the art.

[00115] Fig. 9 shows a system utilizing masks to generate an optical force pattern. A source 280, such as a laser, generates a beam 282 directed to toward a mask 284. Optionally, a phase modulator 290 may be disposed between the source 280 and the mask 284. Optionally, the mask 284 may be moved, such as by actuator 286, which may be a motor, piezoelectric driven system, microelectromechanical (MEMs), or other driving structures known to those skilled in the art. The optical mask 284 creates a desired light intensity pattern adjacent the sample plate 288. The optical mask 284 may modulate any or all of the components of the light passing there through, include, but not limited to, intensity, phase and polarization. The mask 284 may be a holographic mask which, if used, may not necessarily require coherent light. Other forms of masks, such as spatial light modulators may be utilized to generate variations in optical parameters.

[00116] Yet another mirror arrangement consists of utilizing a micromirror arrangement. One such micromirror structure consists of an array of mirrors, such as utilized in the Texas Instrument Digital Micromirror product.

[00117] Fig. 10 shows an alternate system for illumination in which multiple sources 290 are directed toward the sample plate or surface 294. Each source 290 is controlled by control system 296, with the various outputs 292 from the sources 290 illuminating the surface of the support 294.

[00118] Arrays of sources 290 may be fabricated in many ways. One preferable structure is a vertical cavity surface emitting laser (VCSEL) array. VCSEL arrays are known to those skilled in the art and serve to generate optical patterns with control of the various lasers comprising the VCSELs. Similarly, laser diode bars provide an array of sources. Alternatively, separate light sources may be coupled, such as through fiber optic coupling, to a region directed toward the surface 294.

[00119] The imaging system may serve function beyond the mirror imaging of the system. In addition to monitoring the intensity, size and shape of the optical fringes, it may be used for purposes such as calibration.

OPTICAL FORCES

[00120] The apparatus and methods of the instant inventions utilize, at least in part, forces on particles caused by light. In certain embodiments, a light pattern is moved relative to another physical structure, the particle or object, the medium containing the particle or object and/or the structure supporting the particle or object and the medium. Often times, a moving optical pattern, such as moving optical gradient field moves relative to the particles. By moving the light relative to particles, typically through a medium having some degree of viscosity, particles are separated or otherwise characterized based at least in part upon the optical force asserted against the particle. While most of the description describes the light moving relative to other structures, it will be appreciated that the relative motion may be achieved otherwise, such as by holding the light pattern stationary and moving the subject particle, medium and/or support structure relative to the optical pattern.

[00121] Figs. 11A, 11B and 11C depict, respectively, the optical intensity profile, the corresponding optical force on a particle or cell and the corresponding potential energy of the particle in the optical intensity profile as a function of distance (x). Fig. 11A shows

the intensity profile generated and applied against one or more particles. As shown, the intensity varies in a undulating or oscillating manner. The intensity, as shown, shows a uniform periodicity and symmetric waves. However, the intensity variations may be symmetric or asymmetric, or of any desired shape. The period may be fixed or may be variable. Fig. 11B shows the absolute value of the force as a function of position. The force is the spatial derivative of the intensity. Fig. 11C shows the potential energy as a function of position. The potential energy is the integrated force through a distance.

[00122] The profiles of Figs. 11A -11C are shown to be generally sinusoidal. Generally, such a pattern would result from interference fringes. Differing profiles (of intensity, force and potential energy) may be desired. For example, it may be desirable to have a system where the potential energy well is relatively flat at the bottom and has steeper sides, or is asymmetric in its form.

[00123] Figs. 12A and 12B show two particles, labeled "A" and "B". in Fig. 12A, the particles are shown being illuminated by a two-dimensional intensity pattern 300. Fig. 12B shows the position of particles A and B at a later moment of time, after the intensity pattern has moved to position 302. In this example, the optical force has caused particle B to move relative to its prior position. Since the effect of the optical pattern 300 on particle A was less than on particle B, the relative positions of particles A and B are different in Fig. 12B as compared to Fig. 12A.

[00124] In one implementation of the system, the position of particles A and B in Fig. 12A would be determined. The system would then be illuminated with the desired gradient field, preferably a moving optical gradient field, and the system then imaged at a later point in time, such as shown in Fig. 12B. The absence of motion, or the presence of motion (amount of motion, direction of motion, speed of motion, etc.) may be utilized to characterize, or analyze the particle or particles. In certain applications, it may be sufficient to determine the response of a single particle to a particular optical pattern. Thus, information may be derived about the particle merely from the fact that the particle moved, or moved in a particular way or by a particular amount. That information may be obtained irrespective of the presence or absence of other particles. In yet other applications, it is desirable to separate two or more particles. In that case, by comparing the position of the particles relative to each other such as in Fig. 12A versus 12B, information regarding the particle may be obtained. Having determined which particle is

the desired particle, assume for purposes of discussion to be particle B, the particle may then be separated from the other particles. As shown in Fig. 12C, an optical tweezer intensity profile 304 may be used to capture and remove particle B. Alternatively, as will be discussed in connection with Figs. 14 - 19, the selected particle may be removed by other means, such as by fluidic means.

[00125] By utilizing a property of the particle, such as the optical dielectric constant, the light forces serve to identify, select, characterize and/or sort particles having differences in those attributes. Exposure of one or more particles to the optical force may provide information regarding the status of that particle. No separation of that particle from any other particle or structure may be required. In yet other applications, the application of the optical force causes a separation of particles based upon characteristics, such that the separation between the particles may result in yet further separation. The modes of further separation may be of any various forms, such as fluidic separation, mechanical separation, such as through the use of mechanical devices or other capture structures, or optically, such as through the use of an optical tweezer as shown in Fig. 12C, by application of a moving optical gradient, or by any other mode of removing or separating the particle, e.g., electromagnetic, fluidic or mechanical.

[00126] Figs. 13A, 13B and 13C show potential energy as a function of distance for one exemplary mode of operation. The figures show particle 1 and particle 2 displaced in the x dimension relative to one another. The physical positioning of the two particles would typically be in the same plane, e.g., the same vertical plane. The figures show the potential energy of the particle. In Fig. 13A, particle 1 310 is subject to light intensity pattern creating the potential energy profile 314. Particle 2 312 is subject to the same light intensity pattern but is subject to the second potential energy profile 316. The second potential energy profile 316 is different from the first potential energy profile 314 because the dielectric constants are different between particle 1 310 and particle 2 312. In Fig. 5A, the light intensity pattern is moving toward the right. As the potential energy profiles 314, 316 move to the right, the particles 310, 312 experience different forces. Particle 1 310 will experience a smaller force as compared to particle 2 312, as depicted by the size of the arrows adjacent the particles. The force experienced by the particles is proportional to the spatial derivative of the potential energy. Thus, particle 2 312 being on a relatively "steeper" portion of the potential energy "wave" would be subject to a larger force. In

Fig. 5A, the translation speed of the potential energy waves may be set to be larger than the speed at which particle 1 310 may move forward through the medium in which it is located. In that event, particle 1 310 may be subject to a force toward the left, Fig. 13A showing an arrow depicting the possible backward or retrograde motion of particle 1 310. The potential energy wells have a minimum 318 into which the particles would settle, absent motion or translation of the potential energy patterns 314, 316.

[00127] Fig. 13B shows particle 1 310 and particle 2 312 subject to the first potential energy 314 and second potential energy 316, respectively. As the potential energy patterns 314, 316 translate to the right, the particles 310, 312 are subject to a force to the right, though in different amounts as depicted by the relative size of the arrows. Fig. 13C shows the potential energy profiles 314, 316 after the potential energy profiles of Fig. 13B have been moved so as to place the potential energy maximum between particle 1 310 and particle 2 312. By "jerking" the intensity profiles 314, 316 forward quickly, particle 1 310 is then located on the "backside" of the potential energy "wave", and would be subject to a force to the left. The path of motion is then shown by the dashed arrow from particle 1 310. In contrast, particle 2 312 remains on the "front side" of the potential energy wave 316 and is subject to a force to the right. The effect of this arrangement is to cause further physical separation between particle 1 310 and particle 2 314. The potential energy profiles 314, 316 must be moved forward quickly enough such that the potential energy maximum is located between the particles to be separated, as well as to insure that the particle on the "backside" of the potential energy wave is caused to move away from the particle on the "front side" of the wave.

[00128] Figs. 37A, B and C show a time series depiction of a technique for the identification, characterization and/or sorting of particles. In Fig. 37A, a population of particles is subject to a beam of light, preferably a line of light shown as the laser beam in Fig. 37A. The direction of illumination is into the plane of the population of particles. The line of light is moved relative to the particle population to physically organize the particle population. Optionally, the beam is moved at a speed which is sufficiently slow as to permit capture of all desired particles and to move the particles to the desired location within the system. Fig. 37B shows phase two in which the line of light is moved relative to the now physically arranged line of particles. Optionally, the relative direction of the light relative to the particles in phase one is in one direction, and in phase two, in an

opposite direction. In phase two, the line of light is moved relative to the particles in a relatively quick, stepping movement. The speed of movement is at least great enough to effect the desired separation of particles. Those particles which are subject to a greater force are selectively moved from the physical position of the arranged particles in phase two. Fig. 37C shows the illumination of the white blood cell particle (shown as the larger particle in the shading) being effectively separated from the red blood cells (shown as the relatively smaller dark ellipses).

[00129] Fig. 38 is a time series graph of the intensity and its position relative to the population of particles. Beam position 1 shows the intensity profile within a few seconds after the beam is turned on. It has sometimes been observed that the particles are slightly offset from the intensity maximum. Beam position 2 depicts the stepping movement referred to in phase two (Fig. 37B). As can be seen, the white blood cell is subjected to a larger gradient force with the result being that it is physically moved more at the ending moment of beam position 2 than is the red blood cell. Beam position 3 depicts yet a subsequent step movement where again the white blood cell is subject to a larger gradient force resulting in its movement to the right. As the beam position continues to move to the right, the distance between the intensity peak and the particles remaining behind, e.g., the red blood cells, grows greater, and accordingly, the gradient force felt by the particles diminishes.

[00130] Fig. 39A shows a cross-sectional arrangement for generating a single line for use in this technique. A laser is directed through a cylindrical lens toward the system. Focusing optics maybe utilized as are described elsewhere herein, and are well known to those skilled in the art. An imaging system, such as the CCD imaging system depicted captures the information from the system. The light pattern may be moved relative to the particles, or alternately, the particles may be moved relative to the light by translating the stage. Preferably, the line of illumination has a relatively uniform intensity, which may be achieved, for example, by modifying the curvature of the lens.

[00131] Figs. 40A and 40B show a cross-section of a alternate arrangement to generate one or more lines of light. Defractive optics receive an incident beam, which when focused through the optics generate one or more lines of light within the sample region. Figs. 40C and D show yet another alternate arrangement for generating one or more lines. A scanning mirror system, such as those utilizing two scanning mirrors generally

oscillating around an access running through the plane of the mirror, where the axis are non-colinear, they result in a generation of one or more lines. Generally, one of the mirrors moves at a substantially higher rate than the other mirror. Alternates to the multiple scanning mirror system may be utilized, such as an acoustic/optic device for generating the desired intensity patterns.

[00132] Fig. 41 shows a top view of a sectioned sample field. The sample field as shown has been sectioned into 16 sub-regions, arranged as a 4 x 4 array. The various sections may be separately interrogated. Generally, commercially available optics may be utilized to generate lines having a size of about 200 microns x 15 microns. While not limited to the specifics stated here, the width of the line is typically on the order of the size of the cell or particle to be interrogated. By utilizing the sectioned sample field of Fig. 41, a relatively shorter line may be utilized, with the result that the line is more linear.

[00133] Fig. 42 shows a top view of a multiple line separation system. Six lines are shown having a timeline separation. Generally, the line separation is chosen such that the presence of the nearest neighbor line has an insubstantial effect on the neighboring particles.

[00134] The apparatus and methods of these inventions utilize optical forces, either alone or in combination with additional forces, to characterize, identify, select and/or sort material based upon different properties or attributes of the particles. The optical profiles may be static, though vary with position, or dynamic. When dynamic, both the gradient fields as well as the scattering forces may be made to move relative to the particle, medium containing the particle, the support structure containing the particle and the medium. When using a moving optical gradient field, the motion may be at a constant velocity (speed and direction), or may vary in a linear or non-linear manner.

[00135] The optical forces may be used in conjunction with other forces. Generally, the optical forces do not interfere or conflict with the other forces. The additional forces may be magnetic forces, such as static magnetic forces as generated by a permanent magnet, or dynamic magnetic forces. Additional electric forces may be static, such as electrostatic forces, or may be dynamic, such as when subject to alternating electric fields. The various frequency ranges of alternating electromagnetic fields are generally termed as follows: DC is frequencies much less than 1 Hz, audio frequencies are from 1 Hz to 50 kHz, radio frequencies are from 50 kHz to 2 GHz, microwave frequencies are from 1 GHz to 200

GHz, infrared (IR) is from 20 GHz to 400 THz, visible is from 400 THz to 800 THz, ultraviolet (UV) is from 800THz to 50 PHz, x-ray is from 5PHz to 20 EHz and gamma rays are from 5 EHz and higher (see, e.g., Physics Vade Mecum). .) The frequency ranges overlap, and the boundaries are sometimes defined slightly differently, but the ranges are always substantially the same. Dielectrophoretic forces are generated by alternating fields generally being in the single Hz to 10 MHz range. For the sake of completeness, we note that dielectrophoretic forces are more electrostatic in nature, whereas optophoretic forces are electromagnetic in nature (that is, comparing the frequency ranges is not meant to imply that they differ only in their frequency.) Gravitational forces may be used in conjunction with optical forces. By configuring the orientation of the apparatus, the forces of gravity may be used to affect the actions of the particle. For example, a channel may be disposed in a vertical direction so as to provide a downward force on a particle, such as where an optical force in the upward direction has been generated. The force of gravity takes into consideration the buoyancy of the particle. When a channel is disposed in the horizontal direction, other forces, e.g., frictional forces, may be present. Fluidic forces (or Fluidics) may be advantageously utilized with optical forces. By utilizing an optical force to effect initial particle separation, a fluidic force may be utilized as the mechanism for further separating the particles. As yet another additional force, other optical forces may be applied against the particle. Any or all of the aforementioned additional forces may be used singly or in combination. Additionally, the forces may be utilized serially or may be applied simultaneously.

[00136] Figs. 14A and 14B show sorting of particles or objects from a one-dimensional source. As shown in Fig. 14A, particles 320 progress in a generally downward direction from a source in the direction of the arrow labeled particle flow. At junction 322, and possibly additionally before the junction 322, the particles are subject to an optical separation force. Those particles having a different response property, such as a different dielectric constant, may be separated from the line of particles resulting in the separated particles 326. Those particles which are not separated continue on as the particles 324. Fig. 14B shows optical cell sorting from a one-dimensional source. Cells 330 move in a fluid flow in a direction from top to bottom as shown by the arrow. The cells 330 are subject to an optical force in the region of junction 332. Selected cells 336 are deviated from the path of the original fluid flow. The remaining particles 334 continue on in the

same direction as the original fluid flow. It will be appreciated that the term "selected" or "non-selected" or similar terminology as used herein is meant to be illustrative, and not intended to be limiting.

[00137] The techniques of this invention may be utilized in a non-guided, i.e., homogeneous, environment, or in a guided environment. A guided environment may optionally include structures such as channels, including microchannels, reservoirs, switches, disposal regions or other vesicles. The surfaces of the systems may be uniform, or may be heterogeneous.

[00138] Fig. 15 shows a plan view of a guided structure including channels. An input channel 340 receives particles 342 contained within a medium. An optical force is applied in region 344. The optical force would preferably be a moving optical gradient field. As the particles 342 move through the field 344, certain particles would be subject to a force causing them to move to the right in the channel as shown as particles 346, yet other particles 348 would move to the left of the T channel. By selection of the speed, orientation, periodicity, intensity and other parameters of the optical force gradient, the particles may be effectively separated.

[00139] The channels may be formed in a substrate or built upon some support or substrate. Generally, the depth of the channel would be on the order of from substantially 1 to substantially 2 diameters of the particle. For many biological cell sorting or characterization applications, the depth would be on the order of 10 to 20 μm. The width of the channels generally would be on the order of from substantially 2 to substantially 8 diameters of the particle, to allow for at least one optical gradient maximum with a width of the order of the particle diameter up to four or more optical gradient maxima with a width of the order of the particle diameter. For many biological cell sorting or characterization applications, the width would be of the order of 20 to 160 micrometers. The channels may have varying shapes, such as a rectangular channel structure with vertical walls, a V-shaped structure with intersecting non-planar walls, a curved structure, such as a semicircular or elliptical shaped channel. The channels, or the substrate or base when the channel was formed within it, may be made of various materials. For example, polymers, such as silicon elastomers (e.g., PDMS), gels (e.g., Agarose gels) and plastics (e.g., TMMA) may be utilized: glass, and silica are other materials. For certain applications, it may be desirable to have the support material be optically transparent. The

surfaces may be charged or uncharged. The surface should have properties which are compatible with the materials to be placed in contact therewith. For example, surfaces having biological compatibility should be used for biological arrays or other operations.

[00140] Various forms of motive force may be used to cause the particles, typically included within a fluid, to move within the system. Electroosmotic forces may be utilized. As known in the art, various coatings of the walls or channels may be utilized to enhance or suppress the electroosmotic effect. Electrophoresis may be used to transport materials through the system. Pumping systems may be utilized such as where a pressure differential is impressed across the inlet and outlet of the system. Capillary action may be utilized to cause materials to move through the system. Gravity feeding may be utilized. Finally, mechanical systems such as rotors, micropumps, centrifugation may be utilized.

[00141] Fig. 16 shows an "H" channel structure for sorting of particles. The H-shaped structure has two inlets and two outlets. The inlet 350 receives both fluid and the subject particles 352 to be sorted. Fluid is input in the second input arm of the H channel. The main or connecting channel 356 receives the fluid flow from both inputs. In the connecting channel 356, the particles 354 will flow through the connecting channel and be subject to the optical sorting force 358. At that stage, the particles are then separated based upon the differentiating parameter, such as the particle's dielectric constant. The particles being moved from the primary stream move as particles 360 to one output. The particles 362 which are not diverted by action of the optical force 358 continue to the left hand outlet 364. Laminar flow within the system will cause the particles 354 to move through the main channel 356, and if the channel width is large enough, will tend to cause the particles 354 to flow relatively closer to the wall nearer the input. The sorting process then consists of diverting the particle from the laminar flow adjacent the left wall to the laminar flow which will divert to the right hand output.

[00142] Fig. 17 shows a wide channel structure for particle separation. Input 370 receives the particles 372 in a fluidic medium. The particles are subject to an optical sorting force 374, whereupon the diverted particles 378 flow toward outlet 382 and particles 376 flow toward outlet 380.

[00143] Fig. 18 shows an X-channel structure for sorting. Input 390 receives particles 392 in a fluidic medium. Second input 394 received fluid. The particles 392 are then

subject to an optical sorting force 396. Diverted particles 402 flow to exit 404. Particles 398 flow to exit 400.

[00144] Fig. 19 is a perspective drawing of a two-dimensional sorting system. The source inflow of cells 410 intersect with an optical sorting force along line 412. The sorting force 412 results in an outflow of target cells 414 in one-dimension, typically in one plane, and an outflow of non-target cells 416 in another plane. The plane of outflow of targets cells 414 is non-coplanar with the plane of outflow of non-target cells 416.

[00145] Fig. 20 shows an arrangement comprising a three-dimensional cell sorting arrangement. A volume 420, most preferably a substantially three-dimensional volume, though possibly a volume of lower effective dimensionality, contains particles 422. An optical force gradient 428 is generated within the volume 420 to effect particle sorting. One embodiment for generating the optical field gradient 428 is to interfere first beam 424 with a second beam 426. The first beam 424 and second beam 426 interfere and generate the force pattern 428. As shown, a first particle 430 is subject to a force in a direction from bottom to top, whereas a second particle 432 is subject to a force from top to bottom. Alternately, the optical pattern 428 may cause forces on particles 430, 432 in the same direction, but with differing amounts of force.

[00146] Fig. 21 shows an embodiment having multiple degrees of freedom, preferably three degrees of freedom. The volume 440 contains particles 442 which are disposed adjacent a surface, near the inwardly disposed surface of mirror 450. An optical gradient force 444 is generated which causes selected ones of the particles 446 at the surface to be moved into the volume 440 such as particle 446. The optical force gradient 444 may be generated by shining an optical beam 448 onto a mirror 450, which causes interference between the beam 448 and its reflected beam.

[00147] Fig. 22 shows a multi-dimensional system in which a volume 450 is utilized to separate particles. First particles 452 are disposed adjacent the surface of the slide 454. A light intensity pattern 456 causes displacement of selected particles. Those displaced particles may then be attached to a sticky or adhesive mat 460 and comprises particles 458.

[00148] Fig. 23 shows a plan view of a complex channel based system for sorting, characterization or classification. An input 470 leads through channel 472 to a first optical sorting region 474. The sorting at a given channel is as described, before. The output of

the sorting results in a first set of particles 478 and a second set of particles 476. The first set of particles 478 flows to the second optical sorting region 480. As before, the particles are sorted into first particles 484 and second particles 482. A next optical sorting region 486 results in the output of sorted particles, the first output 488 and second output 490 then leading to further collection, counting or analysis. In one aspect, the complex system may include one or more recycle or feedback tabs 490. As shown, the output from the optical force region 492 includes output 7 but also a recycle path 494 leading to the input 496 coupling to the channel 472. Such a recycle system might be used in an enrichment system.

[00149] The systems described herein, and especially a more complex system, may include various additional structures and functionalities. For example, sensors, such as cell sensors, may be located adjacent various channels, e.g., channel 742. Various types of sensors are known to those skilled in the art, including capacitive sensors, optical sensors and electrical sensors. Complex systems may further include various holding vessels or vesicles, being used for source materials or collection materials, or as an intermediate holding reservoir. Complex systems may further include amplification systems. For example, a PCR amplification system may be utilized within the system. Other linear or exponential biological amplification methods known to those skilled in the art may be integrated. Complex systems may further include assays or other detection schemes. Counters may be integrated within the system. For example, a counter may be disposed adjacent an output to tally the number of particles or cells flowing through the output. The systems of the instant invention are useable with microelectromechanical (MEMs) technology. MEMs systems provide for microsized electrical and mechanical devices, such as for actuation of switches, pumps or other electrical or mechanical devices. The system may optionally include various containment structures, such as flow cells or cover slips over microchannels.

[00150] A computerized workstation may include a miniaturized sample station with active fluidics, an optical platform containing a laser (e.g., a near infrared laser for biological applications) and necessary system hardware for data analysis and interpretation. The system may include real-time analysis and testing under full computer control.

[00151] The inventions herein may be used alone, or with other methods of cell separation. Current methods for cell separation and analysis include flow cytometry, density gradients, antibody panning, magnetic activated cell sorting ("MACS™"), microscopy, dielectrophoresis and various physiological and biochemical assays. MACS separations work only with small cell populations and do not achieve the purity of flow cytometry. Flow cytometry, otherwise known as Fluorescent Activated Cell Sorting ("FACS™") requires labeling.

[00152] In yet another aspect, the systems of the present invention may optionally include sample preparation steps and structure for performing them. For example, sample preparation may include a preliminary step of obtaining uniform size, e.g., radius, particles for subsequent optical sorting.

[00153] The systems may optionally include disposable components. For example, the channel structures described may be formed in separable, disposable plates. The disposable component would be adapted for use in a larger system that would typically include control electronics, optical components and the control system. The fluidic system may be included in part in the disposable component, as well as in the non-disposable system components.

[00154] Fig. 24 shows a system for optical sorting based upon a physical parameter of the object, such as deformability. An optical gradient 500 may illuminate particles 502, 504. Particle 504 is more deformable than particle 502. As a result, given the periodicity of the optical force pattern 500, the deformable particle 504 may be subject to a relatively larger force, and move more under the optical field 500. Preferably, the optical field 500 is a moving optical gradient field. Alternatively, the particles 502, 504 may be subject to the optical force 500, and the structure of the particles 502, 504 monitored. In that way, by observing the deformability of the particles, relative to the light pattern 500, the particles may be identified, classified or otherwise sorted.

[00155] Fig. 25 shows a method for sorting particles based upon size. An optical intensity pattern 510 illuminates larger particle 512 and smaller particle 514. The differently sized particles 512, 514 are subject to different forces. Where, for example, larger particle 512 spans two or more intensity peaks of the optical gradient 510, the particle may have no net force applied to it. In contrast, the smaller particle 514 which has a size smaller than the period of the optical intensity pattern 510 may be subject to a

relatively larger force. By selection of the period of the optical pattern 510 relative to the size of particles to be sorted, the system may effectively sort based upon size. In one method, a set of particles may be subject to an increasing period of the light intensity, such that smaller particles are removed first, followed by the relatively larger particles at a later time. In this way, particles may be effectively sorted by size.

Methods for Reducing or Modifying Forces

[00156] The system and methods may include various techniques for reducing or otherwise modifying forces. Certain forces may be desirable in certain applications, but undesirable in other applications. By selecting the technique to reduce or minimize the undesired forces, the desired forces may more efficiently, sensitively and specifically sort or identify the desired particles or conditions. Brownian motion of particles may be an undesired condition for certain applications. Cooling of the system may result in a reduced amount of Brownian motion. The system itself may be cooled, or the fluidic medium may be cooled.

[00157] Yet another force which may be undesired in certain applications is friction or other form of sticking force. If surface effects are to be minimized, various techniques may be utilized. For example, a counterpropagating beam arrangement may be utilized to capture particles and to remove them from contact with undesired surfaces. Alternatively, the particles may be levitated, such as through the use of reflected light (see, e.g., Fig. 4, mirror 108). Fig. 4A shows an alternative arrangement for particle levitation. Opposing forces of two counter-propagating optical beams can be used to levitate a particle to reduce surface friction drag.

[00158] Yet other techniques exist for addressing friction, stiction, electrostatic and other surface interactions which may interfere with the mobility of cells and/or particles. For example, surfaces may be treated, such as through the use of covalent or non-covalent chemistries, which may moderate the frictional and/or adhesion forces. Surfaces may be pretreated to provide better starting surfaces. Such pretreatments may include plasma etching and cleaning, solvent washes and pH washes, either singly or in combination. Surfaces may also be functionalized with agents which inhibit or minimize frictional and adhesive forces. Single or multi-step, multi-layer chemistries may be utilized. By way of example, a fluorosilane may be used in a single layer arrangement which renders the surface hydrophobic. A two-step, two-layer chemistry may be, for example,

aminopropylsilane followed by carboxy-PEG. Teflon formal coating reagents such as CYTOP™ or Parylene™ can also be used. Certain coatings may have the additional benefit of reducing surface irregularities. Functional groups may, in certain cases, be introduced into the substrate itself. For example, a polymeric substrate may include functional monomers. Further, surfaces may be derivitized to provide a surface which is responsive to other triggers. For example, a derivatized surface may be responsive to external forces, such as an electric field. Alternatively, surfaces may be derivatized such that they selectively bind via affinity or other interactions.

[00159] Yet another technique for reducing surface interactions is to utilize a biphasic medium where the cells or particles are kept at the interface. Such aqueous polymer solutions, such as PEG-dextran partition into two phases. If the cells partitioned preferentially into one of the layers, then under an optical gradient the cells would be effectively floating at the interface.

Methods for Enhancing or Changing the Dielectric Constant

[00160] Optionally, the particles to be subject to the apparatus and methods of these inventions may be either labeled or unlabeled. If labeled, the label would typically be one which changes or contributes to the dielectric constant of the particle or new particle (i.e., the initial particle and the label will act as one new particle). For example, a gold label or a diamond label would effectively change most typical dielectric constants of particles.

[00161] Yet other systems may include an expressible change in dielectric constant. For example, a genetic sequence may exist, or be modified to contain, an expressible protein or other material which when expressed changes the dielectric constant of the cell or system. Another way to tune the dielectric constant of the medium is to have a single medium in a fluidic chamber where the dielectric constant can be changed by changing the temperature, applying an electric field, applying an optical field , etc. Other examples would be to dope the medium with a highly birefringent molecule such as a water-soluble liquid crystal, nanoparticles, quantum dots, etc. In the case of birefringent molecules, the index of refraction that the optical beam will see can be altered by changing the amplitude and direction of an electric field.

Methods for Increasing Sensitivity

[00162] Maximizing the force on a particle for a given intensity gradient suggests that the difference in dielectric constant between the particle and medium should be

maximized. However, when sensitivity is required in an application, the medium should be selected such that the dielectric constant of the medium is close to the dielectric constant of the particle or particles to be sorted. By way of example, if the particle population to be sorted has dielectric constants ranging from 1.25 to 1.3, it would be desirable to choose a dielectric constant which is close to (or even within) that range. For cells, a typical range of dielectric constants would be from 1.8 to 2.1. By close, a dielectric constant within 10% or, more particularly, within 5%, would be advantageous. While the absolute value of the magnitude of the force on the particle population may be less than in the case where the dielectric constant differs markedly from the dielectric constant of the medium, the difference in resulting motion of the particles may be larger when the dielectric constant of the medium is close to the range of dielectric constants of the particles in the population. While utilizing the increased sensitivity of this technique at the outset, once the separation begins, the force may be increased by changing the dielectric constant of the medium to a more substantial difference from the dielectric constants of the particle or particle collection. As indicated, it is possible to choose the dielectric constant of the medium to be within the range of dielectric constants of the particle population. In that instance, particles having a dielectric constant above the dielectric constant of the medium will feel a force in one direction, whereas those particles having a dielectric constant less than the dielectric constant of the medium will feel a force moving in the opposite direction.

Scattering Force Systems

[00163] It is possible to utilize the scattering force, either alone or in combination with the optical gradient force, such as supplied by a moving optical field gradient, for separation of particles. Fig. 26 shows the before and after depiction of a system including a laser 520 and a lens 522 which collimates the optical beam. A capillary 524 receives the illumination, preferably along its axis. A set of particles, first particles 526 and second particles 528, are illuminated by the light beam and are subject to different scattering forces depending upon their different scattering properties. Because of the different forces, first particles 526' move a shorter distance than second particles 528', as shown in the second drawing. In this way, optical forces, particularly optical scattering forces, may be utilized to separate particles.

[00164] Figs. 27A, 27B and 27C depict a scattering force switch. A first input 530 couples via a channel to a first output 536. The second input 532 couples to a second output 538 via a channel. The two channels overlap by providing a fluidic connection between them. In operation, a particle entering in input 1 530 may be switched by a scattering force switch 540 by deviating the particle from the channel coupled to input 1 530 to the channel containing output 2 538. Scattering force switches may be used in conjunction with the optical gradient force systems, especially the moving optical gradient force systems described herein.

Static Systems

[00165] Fig. 28 shows a system for the measurement of dielectric constants of particles. A particle 558 having a dielectric constant may be subject to different media having different dielectric constants. As shown, a first vessel 550, a second vessel 552, and so on through an end vessel 554 contain a medium having different dielectric constants ε_1 ε_2, ... ε_n, respectively. By illuminating the particle 558 with an optical gradient force 556, and observing the motion, the dielectric constant of the particle may be determined. If the dielectric constant of the medium is equal to the dielectric constant of the particle then no force is imposed by the optical illumination 556. In contrast, if there is a difference between the dielectric constant of the particle and the dielectric constant of the medium, an optical force will be imposed on the particle by the optical illumination 556. Different dielectric constant media may be supplied as shown in Fig. 28, namely, where a plurality a vessels 550, 552 . . . 554 are provided. Alternately, a particle may be subject to a varying dielectric constant over time, such as through use of a titration system. In on implementation, the titration may be accomplished in a tube containing the particle by varying the dielectric constant of the fluid over time, such as by mixing fluids having different dielectric constants, preferably at the inlet to the tube, or by providing a varying dielectric constant profile, such as a step profile. Additionally, the dielectric constant of a particle may be approximated by interpolation, such as where two or more data points are obtained regarding the force on the particle in different media, and then the expected dielectric constant in which no force is present may be determined.

[00166] Fig. 29 shows a static system in which separation may occur. A light pattern 560 illuminates first particle 562 and second particle 564. If the dielectric constant of the first particle 562 is less than the dielectric constant of the medium, then the particle moves

toward an area of lower intensity. In contrast, if the second particle 564 has a dielectric constant which is greater than the dielectric constant of the medium, the particle will move toward the region of higher intensity. As a result, the first particle 562 and second particle 564 are subject to forces in opposite directions. Given the proximity shown, they would move away from one another.

[00167] Fig. 30 shows a system for the use of a plurality of optical tweezers, preferably in an array, such as to move materials. A substrate 570 may contain one or more sites 572 on which materials may be placed. The materials may comprise particles, cells, or any other material to be selected or moved. An optical tweezer array may selectively move materials, such as those shown as light circles 576, and move those materials to yet another portion of the substrate 570, such as array 574. Alternatively, the optical tweezer array may illuminate the entire array 572, and then selectively move the materials as to which the optical tweezer array provides sufficient force to cause separation of the particles 576, 578 from the array 572 on the substrate 570. For example, the particles may have attachment mechanisms, such as complimentary nucleic acids, which selectively bind them to the substrate 570.

[00168] Fig. 31 shows a graph of molar extinction coefficient as a function of wavelength for hemoglobin-O_2 absorption. For certain sorting applications, it may be desirable to select a wavelength for illumination which is at or near a peak of absorption. For example, it may be desirable to choose a wavelength at the 500,000 molar extinction coefficient peak. Alternatively, it may be desirable to choose a secondary peak, e.g., the peak at substantially 560 nm or at substantially 585 nm.

[00169] The first setup is a moving fringe workstation for optophoresis experiments. A high power, 2.5 watt, Nd-YAG laser (A) is the near IR, 1064 nm wavelength, light source. The fringe pattern is produced by directing the collimated laser beam from the mirror (1) through the Michelson interferometer formed by the prism beam splitter (2) and the carefully aligned mirrors (3). A variable phase retarder (4) causes the fringe pattern to continuously move. This fringe pattern is directed by the periscope (5) through the telescope (5a) and (5b) to size the pattern to fill the back focal plane of the microscope objective, and then is directed by the dichroic beam splitter (6) through a 20× microscope objective (7) to produce an image of the moving fringe pattern in the fluidic chamber holding the sample to be sorted. A second, 60× microscope objective (8) images the flow

cell onto a CCD camera to provide visualization of the sorting experiments. A fiber-optic illuminator (9) provides illumination, through the dichroic beam splitter (6), for the sample in the fluidic chamber. The fluidic chamber is positioned between the two microscope objectives by means of an XYZ-translation stage.

[00170] It will be appreciated by those skilled in the art that there are any number of additional or different components which may be included. For example, additional mirrors or other optical routing components may be used to 'steer' the beam where required. Various optical components for expanding or collimating the beam may be used, as needed. In the set-up implementing Fig. 5, the laser used additional mirrors to steer the laser beam into the spatial filter, which that produced a well collimated Gaussian beam that is then guided to the Michelson interferometer.

[00171] The second setup is a workstation for measuring and comparing the dielectric properties of cells and particles at near IR optical frequencies, using a 600 mW, ultra-low noise Nd-YAG laser (B) as a light source. The remainder of the optical setup is similar to the moving fringe workstation, except there is no interferometer to produce moving fringes. Instead a single, partially focused illumination spot is imaged within the fluidic chamber. The interaction of cells with this illumination field provides a measurement of the dielectric constant of the cells at near IR optical frequencies.

Exemplary Applications

High Throughput Biology

[00172] The methods and apparatus herein permit a robust cell analysis system suitable for use in high throughput biology in pharmaceutical and life sciences research. This system may be manufactured using higher performance, lower cost optical devices in the system. A fully integrated high throughput biology, cell analysis workstation is suitable for use in drug discovery, drug discovery, toxicology and life science research.

These systems may utilize advanced optical technologies to revolutionize the drug discovery process and cellular characterization, separation and analysis by integrating optophoresis technology into devices for the rapid identification, selection and sorting of specific cells based on their innate properties, including their innate optical dielectric properties. In addition, since the technology is based on the recognition of such innate properties, labels are not required, greatly simplifying and accelerating the testing process. The lasers employed are preferably in the biologically-compatible infrared wavelengths,

allowing precise cell characterization and manipulation with little or no effect on the cell itself. The technology is suited to the post-genomics era, where the interaction of the cell's molecular design/make-up (DNA, RNA and proteins) and the specific cellular changes (growth, differentiation, tissue formation and death) are of critical importance to the basic understanding of health and disease.

[00173] The Optophoresis technology changes the nature of cell-based assays. Applications would include all methods of cellular characterization and sorting. The technology also offers diverse applications in the areas of molecular and cellular physiology. Optophoresis technology addresses fundamental properties of the cell itself, including its optical dielectric properties. The optophoretic properties of the cell change from cell type to cell type, and in response to external stimuli. These properties are reflective of the overall physiologic status of the cell. Active cells have dielectric properties that are different from resting cells of the same type. Cancer cells have different optophoretic properties than their normal counterparts. These cellular properties can also be used effectively in drug discovery and pharmaceutical research, since nearly all drugs are targeted ultimately to have direct effects on cells themselves. In other words, drugs designed to effect specific molecular targets will ultimately manifest their effects on cellular properties as they change the net dielectric charge of the cell. Therefore, rapid screening of cells for drug activity or toxicity is an application of the technology, and may be referred to as High Throughput Biology. Other main applications include drug discovery and pharmaceutical research.

[00174] The Human Genome Project and other associated genome programs will provide enormous demand for improved drug development and screening technologies. Sophisticated cellular approaches will be needed for cost-effective and functional screening of new drug targets. Likewise, information from the genome projects will create demand for improved methods of tissue and organ engineering, each requiring access to well characterized cellular materials. Moreover, optical technology from the information and telecommunications industry will provide the system hardware for improved optical cell selection and sorting. The price/performance ratios for high powered near infrared and infrared lasers originally developed for telecommunications applications continue to improve significantly. In addition, solid-state diode lasers may be used having a variety of new wavelengths, with typically much higher power output than older versions. Vertical

Cavity Surface Emitting Lasers ("VCSELs") provide arrays of diode lasers at very reasonable costs with increasing power output.

[00175] A computerized Workstation may be composed of a miniaturized sample station with active fluidics, an optical platform containing a near infrared laser and necessary system hardware for data analysis and interpretation. The system includes real-time analysis and testing under full computer control. Principal applications of the technology include cell characterization and selection, particularly for identifying and selecting distinct cells from complex backgrounds.

[00176] Importantly, unlabelled, physiologically normal, intact test cells will be employed in the system. The sample is quickly analyzed, with the cells classified and sorted by the optical field, thereby allowing characterization of drug response and identify toxicity or other measures of drug efficacy. Characterizing the cellular optophoretic properties uniquely associated with various drug testing outcomes and disease states is a part of this invention. Identification of these novel parameters constitutes useful information.

[00177] An integrated system may, in various aspects, permit: the identification, selection and separation of cells without the use of labels and without damaging the cells; perform complex cell analysis and separation tasks with ease and efficiency; observe cells in real time as they are being tested and manipulated; establish custom cell sorting protocols for later use; isolate rare cells from complex backgrounds; purify and enrich rare cells (e.g. stem cells, fragile cells, tumor cells); more easily link cell phenotype to genotype; study cell-cell interactions under precise and optical control; and control sample processing and analysis from start to finish.

[00178] The technology offers a unique and valuable approach to building cellular arrays that could miniaturize current assays, increase throughput and decrease unit costs. Single cell (or small groups of cells) based assays will allow miniaturization, and could allow more detailed study of cell function and their response to drugs and other stimuli. This would permit cellular arrays or cell chips to perform parallel high-throughput processing of single cell assays. It could also permit the standardization of cell chip fabrication, yielding a more efficient method for creation of cell chips applicable to a variety of different cells types.

[00179] Mammalian cell culture is one of the key areas in both research (e.g., discovery of new cell-produced compounds and creation of new cell lines capable of producing specific proteins) and development (e.g., developing monoclonal cell lines capable of producing highly specific proteins for further research and testing). Mammalian cell culture is also a key technology for the production of new biopharmaceuticals on a commercial scale.

[00180] Once researchers have identified drug targets, compounds or vaccines, mammalian cell culture is an important technology for the production of quantities necessary for further research and development. There are currently more than 70 approved biotechnology medicines and more than 350 such compounds in testing, targeting more than 200 diseases.

[00181] Optical cell characterization, sorting and analysis technologies could be useful in selecting and separating lines of mammalian cells according to whether they produce a new protein or biopharmaceutical compound and according to the yield of the protein or compound. Cell yield is a key factor in determining the size of the plant a manufacturer must build to produce commercial quantities of a new biotechnology drug.

[00182] We turn now to more specific discussions of applications. First, we address separation applications, and second, address monitoring applications.

SEPARATION APPLICATIONS

[00183] White cells from red cells. White blood cells are the constituents of blood which are responsible for the immune response as compared with red cells which transport oxygen through the body. White cells need to be removed from red cells prior to transfusion for better tolerance and to decrease infection risks. It is also often important to remove red cells in order to obtain enriched populations of white cells for analysis or manipulation. Optophoresis can allow the separation of these two distinct cell populations from one another for use in applications where a single population is required.

[00184] Reticulocytes from mature red blood cells. Reticulocytes, which are immature red blood cells normally found at very low levels can be indicators of disease states when they are found at increased levels. This application would use optophoresis for the separation and enumeration of the levels of reticulocytes from whole blood.

[00185] Clinical Care Applications, e.g., Fetal stem cells from maternal circulation. The Clinical Care applications include cell-based treatments and clinical diagnostics. The

successful isolation of fetal cells from maternal blood represents a source of fetal DNA obtainable in a non-invasive manner. A number of investigators worldwide have now demonstrated that fetal cells are present in the maternal circulation and can be retrieved for genetic analysis. The major current challenges in fetal cell isolation include selection of the target fetal cell type, selection and isolation of the cells and the means of genetic analysis once the cells are isolated. Using a maternal blood sample, the system can identify the rare fetal cells circulating within the mother's blood and to permit the diagnosis of genetic disorders that account for up to 95% of prenatal genetic abnormalities, e.g., Down's Syndrome. Cell-based treatments refer to procedures similar to diagnostic procedures, but for which the clinical purpose is somewhat broader. During pregnancy, a small number of fetal cells enter the maternal circulation. By purifying these cells using optophoresis prenatal diagnosis of a variety of genetic abnormalities would be possible from a single maternal blood sample.

[00186] Clinical Care Applications, e.g., Stem Cell Isolation. The purpose of stem cell isolation is to purify stem cells from stem cell grafts for transplantation, i.e., to remove T-cells in allogeneic grafts (where the donor and the recipient are not the same person) and cancer cells in autologous grafts (where the donor and the recipient are the same person). Currently stem cell technologies suffer from several drawbacks. For example, the recovery efficiency of stem cells obtained using currently available systems is on the order of 65 – 70%. In addition, current methods do not offer the 100% purity which is beneficial in transplant procedures.

[00187] Tumor cells from blood. Minimal Residual Disease (MRD) Testing The National Cancer Institute (NCI) estimates that approximately 8.4 million Americans alive today have a history of cancer, and that over 1.2 million new cancer cases were diagnosed in 2000. The NCI also estimates that since 1990 approximately 13 million new cancer cases were diagnosed, excluding noninvasive and squamous cell skin cancers. Optophoresis technology addresses some of the key unmet needs for better cancer screening, including: accurate, reproducible and standardized techniques that can detect, quantify and characterize disseminated cancer cells; highly specific and sensitive immunocytological techniques; faster speed of cell sorting; and techniques that can characterize and isolate viable cancer cells for further analysis.

[00188] Cancer cells may be found in low numbers circulating in the blood of patients with various forms of that disease, particularly when metastasis has occurred. The presence of tumor cells in the blood can be used for a diagnosis of cancer, or to follow the success or failure of various treatment protocols. Such tumor cells are extremely rare, so a means of enrichment from blood such as optophoresis would need to be employed in order to have enough cells to detect for accurate diagnosis. Another application for optophoresis in this regard would be to remove tumor cells from blood or stem cell products prior to them being used to perform an autologous transplant for a cancer patient.

[00189] Fetal stem cells from cord blood. The umbilical cord from a newborn generally contains blood which is rich in stem cells. The cord blood material is usually discarded at birth; however, there are both academic and private concerns who are banking cord blood so that such discarded material can be used for either autologous or allogenic stem cell replacement. Enrichment of the cord blood stem cells by optophoresis would allow for a smaller amount of material to be stored, which could be more easily given back to the patient or another host.

[00190] Adult stem cells from liver, neural tissue, bone marrow, and the Like. It is becoming increasingly clear that many mature tissues have small subpopulations of immortal stem cells which may be manipulated ex vivo and then can be reintroduced into a patient in order to repopulate a damaged tissue. Optophoresis can be used to purify these extremely rare adult stem cells so that they may be used for cell therapy applications.

[00191] Islet cells from pancreas. It has been proposed that for persons with diabetes resulting from lack of insulin production, the insulin producing beta islet cells from a healthy pancreas could be transplanted to restore that function to the diabetic person. These cells make up only a small fraction of the total donor pancreas. Optophoresis provides a method to enrich the islet cells and would be useful for preparation of this specific type of cell for transplantation.

[00192] Activated B or T cells. During an immune response either T or B white cell subsets which target a specific antigen become active. These specific activated cells may be required as separate components for use in ex vivo expansion to then be applied as immunotherapy products or to be gotten rid of, since activated B or T cells can cause unwanted immune reactions in a patient such as organ rejection, or autoimmune diseases such as lupus or rheumatoid arthritis. Optophoresis provides a method to obtain activated

cells either to enrich and give back to a patient or to discard cells which are causing pathological destruction.

[00193] Dendritic cells. Dendritic cells are a subset of white blood cells which are critical to establishing a T-cell mediated immune response. Biotech and pharmaceutical companies are working on ways to harvest dendritic cells and use them ex vivo in conjunction with the appropriate antigen to produce a specific activated T cell response. Optophoresis would allow isolation of large numbers of dendritic cells for such work.

[00194] HPRT- cells. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) is an enzyme which exits in many cells of the blood and is involved in the nucleoside scavenging pathway. Persons who have high mutation rates due to either endogenous genetic mutations or exogenous exposure to mutagens can be screened for HPRT lacking cells (HPRT-) which indicate a mutation has occurred in this gene. Optophoresis following screening by compounds which go through the HPRT system can be used to easily select HPRT minus cells and quantitate their numbers.

[00195] Viable or mobile sperm cells. Approximately 12% of couples are unable to initiate a pregnancy without some form of assistance or therapy. In about 30% these cases, the male appears to be singularly responsible. In an additional 20% of cases, both male and female factors can be identified. Thus, a male factor is partly responsible for difficulties in conception in roughly 50% of cases. The number of women aged 15-44 with impaired ability to have children is well over 6 million. Semen analysis is currently performed using a variety of tests and is based on a number of parameters including count, volume, pH, viscosity, motility and morphology. At present, semen analysis is a subjective and manual process. The results of semen analysis do not always clearly indicate if the male is contributing to the couple's infertility. Gradient centrifugation to isolate motile sperm is an inefficient process (10 to 20% recovery rate). Sperm selection is accomplished using either gradient centrifugation to isolate motile sperm used in In Utero Insemination (IUI) and In Vitro Fertilization (IVF) or visual inspection and selection to isolate morphologically correct sperm used in IVF and Intracytoplasmic Sperm Injection (ICSI). Each year in the U.S., 600,000 males seek medical assistance for infertility.

[00196] One of the reasons for male infertility is the lack of high enough percentages of viable and/or mobile sperm cells. Viable and/or mobile sperm cells can be selected using

optophoresis and by enriching their numbers, higher rates of fertilization can be achieved. This application could also be used to select X from Y bearing sperm and vice versa, which would then be used selectively to induce pregnancies in animal applications where one sex of animal is vastly preferred for economic reasons (dairy cows need to be female, while it is preferable for meat producing cattle to be male for example).

[00197] Liposomes loaded with various compounds. A recent mode of therapeutic delivery of pharmaceutical products is to use liposomes as the delivery vehicle. It should be possible using optophoresis to separate liposomes with different levels of drug in them and to enrich for those liposomes in which the drugs are most concentrated.

[00198] Tissue Engineering, e.g., Cartilage precursors from fat cells. Tissue engineering involves the use of living cells to develop biological substitutes for tissue replacements which can be used in place of traditional synthetic implants. Loss of human tissue or organ function is a devastating problem for a patient and family. The goal of tissue engineering is to design and grow new tissue outside the body that could then be transplanted into the body.

[00199] A recent report has demonstrated that cells found in human adipose tissue can be used ex vivo to generate cartilage which can be used as a transplant material to repair damage in human joints. Optophoresis can be used to purify the cartilage forming cells from the other cells in adipose tissue for ex vivo expansion and eventual tissue engineering therapy.

[00200] Nanomanipulation of small numbers of cells. Recent miniaturization of many lab processes have resulted in many lab analyses being put onto smaller and smaller platforms, evolving towards a "lab-on-a-chip" approach. While manipulation of biomolecules in solution has become routine in such environments, manipulation of small numbers of cells in microchannel and other nano-devices has not been widely achieved. Optophoresis will allow cells to be moved in microchannels and directed into the region with the appropriate processes on the chip.

[00201] Cellular organelles; mitochondria, nucleus, ER, microsomes. The internal constituents of a cell consists of the cytoplasm and many organelles such as the mitochondria, nucleus, etc. Changes in the numbers or physical features of these organelles can be used to monitor changes in the physiology of the cell itself.

Optophoresis can allow cells to be selected and enriched which have particular types, morphologies or numbers of a particular organelle.

[00202] Cow reticulocytes for BSE assays. It has been reported that a cellular component of the reticulocyte, EDRF, is found at elevated levels in the reticulocytes of cows infected with BSE (bovine spongiform encephalopathy). Reticulocytes are generally found at low levels in the blood and therefore the use of optophoresis would allow their enrichment and would increase the accuracy of diagnostic tests based on the quantitation of the EDRF mRNA or protein.

MONITORING

[00203] Growing/dividing cells vs. resting cells. Cells may be stimulated to grow by various growth factors or growth conditions. Most assays which exist for cell growth require the addition of external labeling reagents and/or significant time in culture before cell growth can be demonstrated. By using optophoresis, cells which have begun to divide will be identified, providing a rapid method for calculating how much of a given cell population is in the growth phase. Cells in different parts of the cell cycle should have different optical properties and these may be used to either sort cells based on where in the cycle they are as well as to determine what fraction of the total cell population is in each stage of the cell cycle.

[00204] Apoptotic cells. Cells which are undergoing programmed cell death or apoptosis can be used to identify specific drugs or other phenomenon which lead to this event. Optophoresis can be used to identify which cells are undergoing apoptosis and this knowledge can be used to screen novel molecules or cell conditions or interactions which promote apoptosis.

[00205] Cells with membrane channels open; change in membrane potentials. The outer membrane of many types of cells contain channels which facilitate the passage of ions and small molecules into and out of the cell. Movement of such molecules can lead to further changes in the cell such as changes in electrical potential, changes in levels of second messengers, etc. Knowledge of these changes can be useful in drug screening for compounds which modulate membrane channel activity. Optophoresis can be used to indicate when membrane channels are being perturbed by exogenous compounds.

[00206] Live vs. dead cells. Many applications exist which require the identification and quantitation of live versus dead cells. By using optophoresis dead cells can be identified and counted.

[00207] Virally infected cells. There are many diagnostic applications where it is important to measure cells which contain virus, including ones for CMV, HIV, etc. Optophoresis can be used to differentiate cells which contain virus from cells which do not.

[00208] Cells with abnormal nucleus or elevated DNA content. One of the hallmarks of a tumor cell is that it will contain either excess DNA, resulting in an abnormal size and/or shape to it's nucleus. By using optophoresis tuned to the nuclear content of a cell populations with abnormal amounts of DNA and/or nuclear structure may be identified and this information can be used as a diagnostic or prognostic indicator for cancer patients.

[00209] Cells decorated with antibodies. A large selection of commercially available antibodies exists which have specificities to cellular markers which define unique proteins and/or types of cells. Many diagnostic applications rely on the characterization of cell types by identifying what antibodies bind to their surface. Optophoresis can be used to detect when a cell has a specific antibody bound to it.

[00210] Cells with bound ligands, peptides, growth factors. Many compounds and proteins bind to receptors on the surface of specific cell types. Such ligands may then cause changes inside the cell. Many drug screens look for such interactions. Optophoresis provides a means to monitor binding of exogenous large and small molecules to the outside of the cell, as well as measurement of physiological changes inside the cell as a result of compound binding.

[00211] Bacteria for viability after antibiotic exposure. Microorganisms are often tested for sensitivity to a spectrum of antibiotics in order to determine the appropriate therapy to pursue to kill an infectious organism. Optophoresis can be used to monitor bacterial cells for viability and for cessation of growth following antibiotic exposure.

[00212] Drug screening on the NCI 60 panel. A panel of 60 tumor cell lines has been established by the National Cancer Institute as a screening tool to determine compounds which may have properties favorable to use as chemotherapeutic agents. It should be possible to use optophoresis to array all 60 lines and then to challenge them with known and novel chemicals and to monitor the cell lines for response to the chemicals.

[00213] Cells for cytoskeletal changes. The cytoskeleton is a complex of structural proteins which keeps the internal structure of the cell intact. Many drugs such as taxol, vincristine, etc... as well as other external stimuli such as temperature are known to cause the cytoskeleton to be disrupted and breakdown . Optophoresis provides a means to monitor populations of cells for perturbations in the cytoskeleton.

[00214] Beads with compounds bound to them, to measure interactions with the cell surface or with other beads. The interactions of microspheres with cells or other compounds has been used in a number of in vitro diagnostic applications. Compounds may be attached to beads and the interactions of the beads with cells or with beads with other compounds on them can be monitored by optophoresis.

[00215] Progenitor cell/colony forming assays. Progenitors are cells of a given tissue which can give rise to large numbers of more mature cells of that same tissue. A typical assay for measuring progenitor cells is to allow these cells to remain in culture and to count how many colonies of the appropriate mature cell type they form in a given time. This type of assay is slow and cumbersome sometimes taking weeks to perform. By using optophoresis to monitor the growth of a single cell, progenitor proliferation can be measured on a nano-scale and results should be obtained within a much shorter length of time.

[00216] Dose limiting toxicity screening. Almost all compounds are toxic at some level, and the specific levels of toxicity of compounds are identified by measuring at what concentration they kill living cells and organisms. By monitoring living cells with optophoresis as the dose of a compound is slowly increased, the level at which optical properties indicative of cell damage and/or death can be ascertained.

[00217] Monitor lipid composition/membrane fluidity in cells. The membranes of all cells are composed of lipids which must maintain both the proper degree of membrane fluidity at the same time that they maintain basic cell membrane integrity. Optophoresis should be able to measure the fluidity of the membrane and to provide information on compounds and conditions which can change membrane fluidity, causing membranes to be either more or less fluid.

[00218] Measure clotting/platelet aggregation. Components found in the blood such as platelets and clotting proteins are needed to facilitate blood clot formation under the appropriate circumstances. Clotting is often monitored in order to measure disease states

or to assess basic blood physiology. Optophoresis can provide information on platelet aggregation and clot formation.

[00219] Certain of the data reported herein were generated with the following setup. Optical gradient fields were generated using a Michelson interferometer and either a 150 mW, 812 nm laser (812 system) or a 2.5 W, 1064 nm laser (1064 system). The 812 system used a 100X (1.25 NA) oil immersion lens to focus the fringe pattern and to visualize the sample. The 1064 system used a 20X objective to focus the fringes and a 60X objective to visualize the sample. In general the sample cell was a coated microscope slide and/or coverslip that was sealed with Vaseline. Coverslip spacers controlled the height of the cell at approximately 150 micrometers

[00220] Coating Of Surfaces; Rain-X™, Agarose, CYTOP, Fluorosilane Scattering forces tend to push the particles or cells against the surface of the sample cell. Therefore, a number of surface coatings were evaluated to minimize nonspecific adhesion and frictional forces. Hydrophobic/hydrophilic and covalent/noncovalent surface treatments were evaluated.

[00221] Covalent/Hydrophobic Glass slides and coverslips were treated with perfluoro-octyltrichlorosilane (Aldrich, Milwaukee, WI) using solution or vapor deposition. Solution deposition was as follows: a 2-5% silane solution in ethanol, incubate 30 minutes at room temperature, rinse 3 times in ethanol and air dry. Vapor deposition involved applying equal volumes of silane and water in separate microcentrifuge tubes and sealing in a vacuum chamber with the substrate to be treated. Heat to 50°C, 15 hrs.

[00222] Noncovalent/Hydrophobic -- A commercial water repellent containing polysiloxanes, Rain-X, was applied according to the manufacturer's instructions.

[00223] A liquid Teflon, CYTOP (CTL-107M, Wilmington, Delaware) was spun coated using a microfuge. The CYTOP was diluted to 10% in fluorooctane (v/v) and 50 microliters was pipetted and spun for 5 seconds. This was repeated a second time and then air dried.

[00224] Noncovalent/Hydrophilic -- Agarose hydrogel coatings were prepared as follows: melt 2% agarose in water, pipette 100 microliters to the substrate, spin for 5 seconds, bake at 37°C for 30 minutes.

[00225] All of the coatings were effective when working with particles. The CYTOP was more effective at preventing adhesion when working with biological cells.

[00226] Separation By Size -- Polystyrene particles (Bangs Labs, Fishers, IN) of different sizes (1, 3 and 5 micrometer diameter) were separated using moving optical gradient fields. Three and five micrometer diameter particles were diluted 1/500 in distilled water and ten microliters was pipetted onto a Rain-X coated slide. The 812 system was used to generate a spot size of 25-30 micrometers consisting of 4-5 fringe periods and moving at 15 micrometers/second.

[00227] Fig. 32 shows a sorting sequence at 1-second intervals with 3 and 5 micrometer polystyrene particles. The smaller, 3 micrometer diameter, particle was readily moved by the gradient fields whereas the larger, 5 micrometer diameter., particle was unaffected. The larger particle was not moved because it spanned multiple fringes so gradient forces were effectively cancelled. Similar results were obtained with 1 and 3 micrometer diameter particles.

[00228] Separation By Refractive Index --Polystyrene, polymethylmethacrylate and silica particles of similar size (~5 micrometer diameter, Bangs Labs) and refractive indexes of 1.59, 1.49 and 1.37, respectively, were sorted by moving optical gradient fields. Observed escape velocities for polystyrene, PMMA and silica were 44, 47 and 32 micrometers/second, respectively. Briefly, a particle is aligned in the fringe and the fringes are moved at increasing speed until the particle slips. This results in a semi-quantitative measurement of the total forces experienced by the particle, i.e. photonic, hydrodynamic and frictional. It will be appreciated by those skilled in the art that the absolute value of the escape velocity will differ depending upon system conditions, e.g., laser power. The numerical results provided herein are meant to provide measured data for the system actually used, and are not to be considered a limitation on the values which might exist in a different system.

[00229] Particles were diluted 1/500 in distilled water (n = 1.33). The 812 system was used to generate a gradient field with a fringe period of 10 micrometers. Polystyrene and PMMA particles were sorted from silica particles by moving the gradient field at a threshold value of approximately 40 micrometers/second.

[00230] Separation By Surface Functionalization and Doping -- Polystyrene particles (~6 micrometer diameter) colored with blue or pink dye were purchased from Polysciences, Inc. The pink particles also had carboxyl groups on the particle surface. The particles were diluted 1/500 in distilled water and 10 microliters was pipetted onto a

Rain-X coated slide. The 812 system was used to generate a moving optical gradient field with a fringe period of approximately 12 micrometers. In the fringes, the pink particle moved preferentially.

[00231] Fig. 33 shows the actual movement of the particles.

[00232] In another experiment, 1 micrometer latex beads labeled with biotin were used to determine changes in escape velocity when different ligands were attached. The biotin labeled beads were diluted 1/100 in PBS buffer. A 50 ul aliquot was incubated with an excess of streptavidin or 10 nanometer colloidal gold-streptavidin conjugate for 10 minutes. The beads were pelleted by centrifugation and resuspended in PBS buffer. Measured escape velocities, using the 1064 system, were 5.3, 4.3 and 3.6 micrometers/second for biotin labeled beads, beads with streptavidin and beads with streptavidin-colloidal gold, respectively.

[00233] Separation By Wavelength Resonance (812 vs. 1064 nm) -- The above experiment with colored polystyrene particles was repeated using the 1064 system and the results were reversed. The blue particle was preferentially moved. Similar results were obtained when the 1064 system was set at 150 mW rather than 2.5 W. This suggests that wavelength tuning could enhance the discrimination process.

[00234] Separation By Index Matching --Silica and polystyrene particles (3 and 5 micrometer diameter, respectively) were diluted 1/500 in hydrophilic silicone (dimethylsiloxane-ethylene oxide block copolymer, Gelest, Inc., Tullytown, PA). The refractive index of the medium (n = 1.44) was intermediate between the silica (n = 1.37) and polystyrene (n = 1.59) particles. The particle size was not important in this experiment.

[00235] Using the 1064 system, the gradient force was focused into a diffuse spot approx. 15 micrometers in diameter. More generally, for all of the systems and applications described herein, a defocused beam, such as a defocused laser beam may be utilized. Preferably, the beam is defocused such that the spot or beam size is on the order of magnitude of the size of the particle. For cells, the size would be approximately 10 to 20 microns. The polystyene particle moved towards the gradient field while the silica particle moved away from it. This demonstrated that the suspending medium could be changed to optimize separation.

Separation Red Blood Cells vs. Retic

[00236] A reticulocyte control (Retic-Chex) was obtained from Streck Labs. A sample containing 6% reticulocytes was stained for 15 minutes with New Methylene Blue for 15 minutes, a nucleic acid stain that differentially stains the reticulocytes versus the unnucleated red blood cells. The sample was diluted 1/200 in PBS and mounted on a fluorosilane coated slide The 812 system was used to generate optical gradient fields. The fringe period was adjusted to 15 micrometers and was moved at 15 micrometers/second. The reticulocytes were preferentially moved relative to red blood cells.

Separation of White Blood Cells vs. Red Blood Cells

[00237] A whole blood control (Para12 Plus) was obtained from Streck Labs. The sample was stained for 15 minutes with New Methylene Blue, a nucleic acid stain that differentially stains the nucleated white blood cells versus the unnucleated red blood cells. The sample was diluted 1/200 in PBS and mounted on a fluorosilane coated slide. The 812 system was used to generate optical gradient fields. The fringe period was adjusted to 15 micrometers and was moved at 22 micrometers/second. The white blood cells were moved by the fringes while the red blood cells were not.

Separation of Leukemia vs. Red Blood Cells

[00238] One milliliter of the leukemia cell line U937 suspension was pelleted and resuspended in 100 microliters PBS containing 1% BSA. Equal volumes of U937 and a 1/200 dilution of red blood cells were mixed together and 10 microliters was placed on a CYTOP coated slide. Separate measurements with moving fringe fields showed that the escape velocity for U937 cells was significantly higher than the escape velocity for red blood cells, 60 and 23 micrometers/second, respectively. The 1064 system was used to generate optical gradient fields with a fringe period of approximately 30 micrometers and moving at 45 micrometers/second, an intermediate fringe velocity. As expected the U937 cells move with the fringes and the red blood cells do not. In one embodiment, the moving fringe may be reduced to a single peak. Preferably, the peak is in the form of a line. In operation, a slow sweep (i.e., at less than the escape velocity of the population of particles) is made across the region to be interrogated. This causes the particles to line up. Next, the fringe is moved quickly (i.e., at a speed greater than the escape velocity of at least some of the particle in the population), preferably in the direction opposite the slow sweep. This causes the selective separation of those particles having a higher escape

velocity from those having a lower escape velocity. Optionally, the remaining line of particles may then be again interrogated at an intermediate fringe velocity. While this technique has general applicability to all of the applications and systems described herein, it has been successfully implemented for the separation of U937 cells from red blood cells. Figs. 43A, 43B and 43C show the separation of white blood cells (the larger cells) from red blood cells. The images in Figs. 43A, B and C correspond to the phases 1, 2 and 3 depicted in Figs. 37A, B and C.

Sorting of Red Blood Cells vs. Polystyrene Particles in Microchannels

[00239] Glass microchannels with an "H" configuration (see Fig. 16) were used to demonstrate sorting of red blood cells and 6 micrometer polystyrene particles. The channels were purchased from Agilent (DNA 500 LabChip) and were 40 micrometers wide and 10 micrometers deep. Unwanted or unused channels and reservoir ports were blocked by backfilling with Norland 61 optical adhesive followed by UV and thermal curing. The channels were primed with ethanol, followed by water and finally by PBS buffer with 1% BSA. The inlet reservoirs were built up about 1 mm higher than the outlet reservoirs. Flow rates were controlled by a combination of pressure and electrokinetic forces. A Keithley 236 power supply was used to apply an electric field between 5 and 10 V/cm.

[00240] A 1/200 mixture of red blood cells and particles in PBS buffer, 1% BSA was added to an inlet reservoir and an equal volume of PBS buffer, 1% BSA was added to the other inlet reservoir. The gradient field was positioned in the crossbar of the "H" near the downstream junction. The 1064 system was fitted with a cylindrical lens to increase the aspect ratio of the gradient field. The resultant gradient field was approximately 40 micrometers wide by 80 micrometers long with a fringe period of 12 ums and moving at 30 micrometers/second.

[00241] In the absence of or with a nonmoving optical gradient field, the cells and particles remain in the top half of the "H" channel and exit via the upper outlet. In the presence of a moving optical gradient field, the particles are diverted to the lower outlet arm and are sorted from the red blood cells.

[00242] The flow rate was adjusted to approximately 80 micrometers/second. The sorting process was digitally recorded and subsequently analyzed. Out of 132 possible

sorting events (121 red blood cells and 11 particles), 2 red blood cells and no particles were mis-sorted. The sort rate was approximately 2/second.

Sorting of Red Blood Cells vs. White Blood Cells in Microchannels

[00243] Fig. 36 shows photographs of sorting of two cell types in a microchannel device. 1 shows a red blood cell and a white blood cell successively entering the moving optical gradient field. 2 shows that white blood cell has been translated down by the action of the moving optical gradient field while the red blood cell has escaped translation. 3 and 4 show that the red blood cell and white blood cell continue to flow into separate channels, completing the sorting.

Gradient Force Manipulation of Liposomes

[00244] Fluorescently labeled liposomes, approximately 0.2 micrometers in diameter, were obtained from a B-D Qtest Strep kit. Ten microliters was placed in a Rain-X coated slide and the 1064 system was used to generate an optical gradient field. A 15 mW 532 nm diode laser was also focused through the objective to visualize the liposome fluorescence. When a standing gradient field was projected onto the sample, fluorescence was more intense in this area. This suggests that the liposomes were moving towards the gradient field.

Differential Motion Imaging

[00245] Polystyrene and silica particles were diluted in distilled water. As shown in the photographs of Fig. 34, a "before" image was captured using a CCD camera and Image Pro Express software. A moving optical gradient field generated by the 1064 system was scanned over the particles. Another image (an "After" image) was captured and the "before" image was subtracted. The resultant image (labeled "Difference") clearly identifies that the polystyrene particle had moved.

Escape Velocities of Different Cell Types

[00246] Escape velocities were measured using a gradient field generated by the 1064 system on CYTOP coated coverslips.

Cell Type	Escape Velocity (um/sec.)
Red Blood Cell	5.6 +/- 0.4
White Blood Cell	11.0 +/- 1.8
Chicken Blood (Retic. Model)	7.3 +/- 1.4
K562 Cells, No Taxol Treatment	10.0 +/- 0.7

K562 Cells, 26 Hr. Taxol Treatment	8.2 +/- 0.4

K562 Cells: Chronic myelogenous leukemia, lymphoblast

[00247] Fig. 35 shows a graph of percent of cells measured as a function of escape velocity (μm/second).

Separation of Treated and Untreated Leukemia Cells

[00248] PMA was dissolved in ethanol at a concentration of 5mg/mL. 3 mls of U937 cells grown in RPMI 1640 media with supplements were removed from the culture flask and 1 ml was placed into each of three eppendorf tubes. Cells from the first tube were pelleted for 4 minutes at 10,000 rpm and resuspended in 250uL PBS/1%BSA buffer for escape velocity measurements. PMA was added to the remaining two tubes of U937 cells to a final concentration of 5ug/mL. These tubes were vortexed and placed in a 37°C water bath for either one hour or six hours. At the end of the time point, the tube was removed, cells were pelleted and then resuspended as described above and escape velocity measurements taken. The cells treated for 6 hours had a significantly higher escape velocity as compared to the untreated cells.

[00249] While preferred embodiments and methods have been shown and described, it will be apparent to one of ordinary skill in the art that numerous alterations may be made without departing from the spirit or scope of the invention. Therefore, the invention is not limited except in accordance with the following claims.

We Claim:

1. A method for separating cells or particles utilizing a moving beam of light comprising the steps of:

providing a population of cells or particles,

illuminating the population of cells or particles with a line of light and moving the line of light relative to the population of cells or particles so as to physically organize the population of cells or particles in a line, and

moving the line of light relative to the physically organized population of cells or particles at a speed to effectively separate at least a portion of the physically organized population of cells or particles in a line.

2. The method of claim 1 wherein the step of moving the line of light relative to the population of cells or particles so as to physically organize the population of cells or particles in a line is performed at a uniform speed.

3. The method of claim 1 wherein the direction of motion of the line of light used to physically organize the population of cells or particles in a line is opposite to the direction of motion of the line of light used to effectively separate at least a portion of the physically organized population of cells or particles in a line.

4. The method of claim 1 wherein the population of cells or particles are located within a sample field sectioned into sub-regions.

5. The method of claim 4 wherein the sample field is an n x n array.

6. The method of claim 1 wherein the movement of the line of light used to effectively separate at least a portion of the physically organized population of cells or particles in a line is a stepping movement.

7. The method of claim 4 wherein the stepping movement moves less than ¼ of the sample field length.

8. The method of claim 1 wherein the relative motion of the line of light relative to the population of cells or particles results from movement of the population of cells or particles relative to a stationary line of light.

9. The method of claim 1 wherein the relative motion of the line of light relative to the population of cells or particles results from movement of the line of light relative to a stationary population of cells or particles.

10. The method of claim 1 wherein the relative motion of the population of cells or particles and the line of light during the separation step is greater than the escape velocity of at least some of the cells or particles within the population.

11. The method of claim 1 wherein the relative motion of the population of cells or particles and the line of light during the separation step is less than the escape velocity of at least some of the cells or particles within the population.

12. The method of claim 1 wherein the method is used to separate maternal blood cells from fetal blood cells.

13. The method of claim 1 wherein the method is used to separate red blood cells from white blood cells.

14. The method of claim 1 wherein the method is used to separate reticulocytes from mature red blood cells.

15. The method of claim 1 wherein the method is used to separate out stem cells.

16. The method of claim 1 wherein the method is used to separate out tumor cells from blood.

17. The method of claim 1 wherein the population of cells includes sperm cells.

18. The method of claim 1, wherein the line of light has a width on the order of the size of at least a portion of the cells or particles contained within the population.

19. A device for separating cells or particles utilizing a moving beam of light comprising:

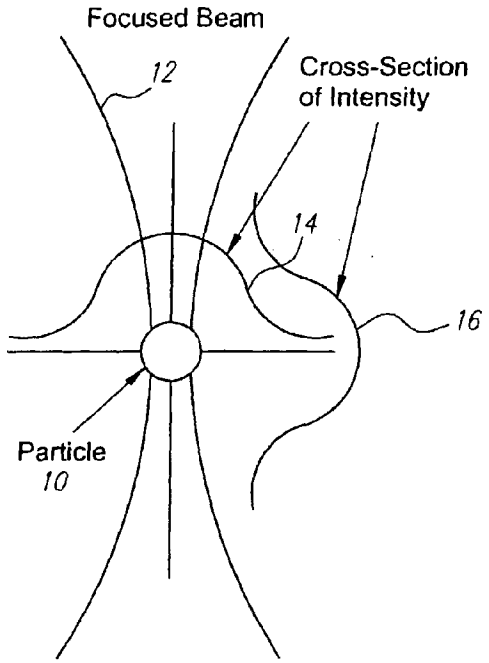
a stage containing a sample field adapted to hold a population of cells or particles;

a source of illumination disposed so as to direct a line of light onto the sample field;

means for moving the line of light relative to the sample field; and

an imaging system disposed so as to capture images of the sample field.

5



$$F_{\nabla} = 2\pi \cdot r^3 \frac{\sqrt{\varepsilon_B}}{c} \left(\frac{\varepsilon - \varepsilon_B}{\varepsilon + 2\varepsilon_B} \right) (\nabla \cdot I)$$

F_{∇} = Optical force on particle towards higher intensity

r = Radius of particle

ε_B = Dielectric constant of backround medium

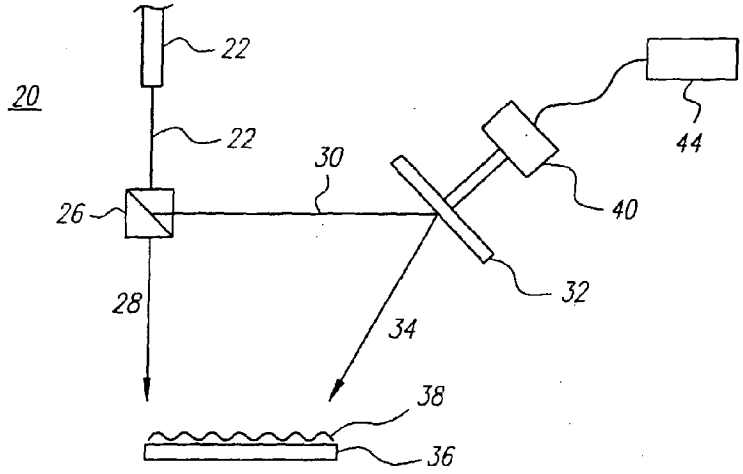
ε = Dielectric constant of particle

I = Light intensity (W/cm^2)

∇ = Spatial derivative

FIG. 1

FIG. 2



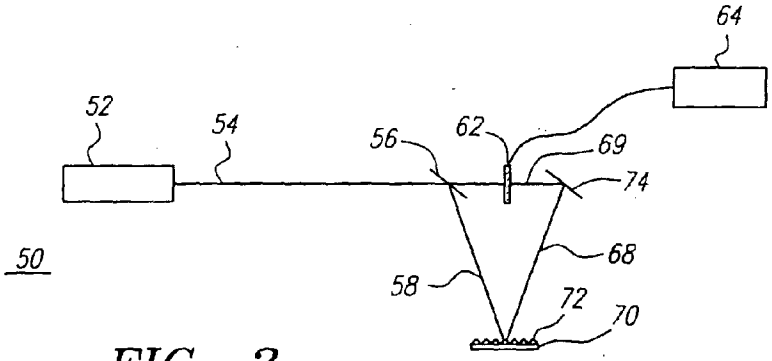


FIG. 3

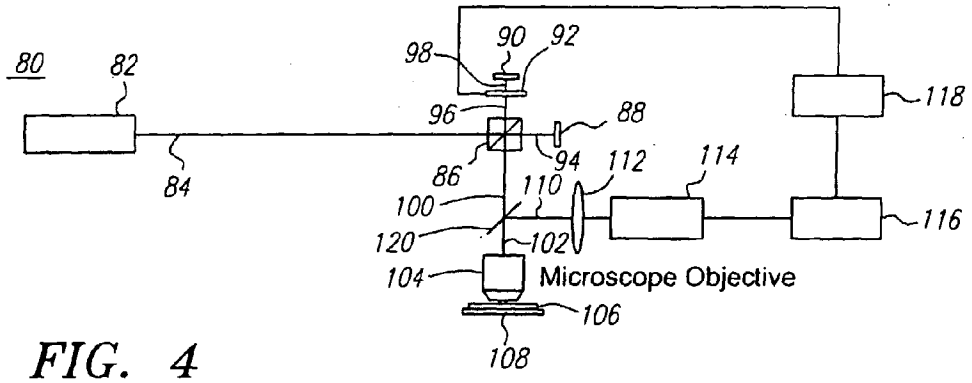


FIG. 4

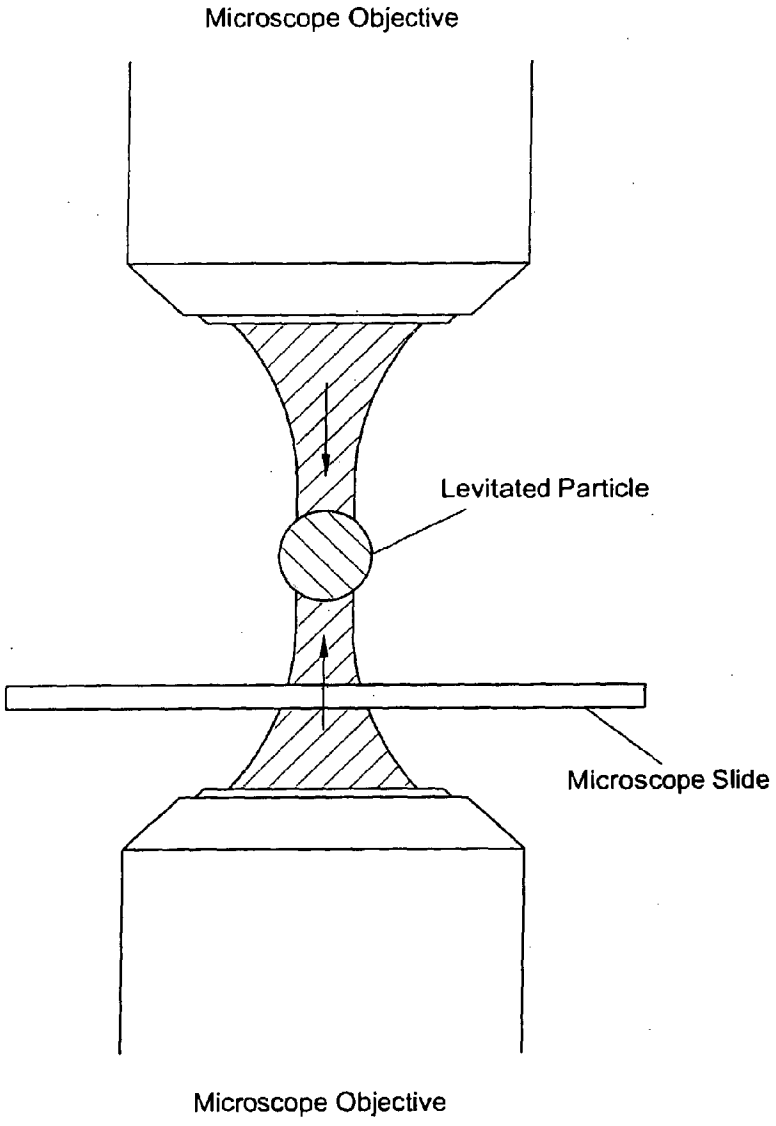

Microscope Objective
Levitated Particle
Microscope Slide
Microscope Objective

FIG. 4A

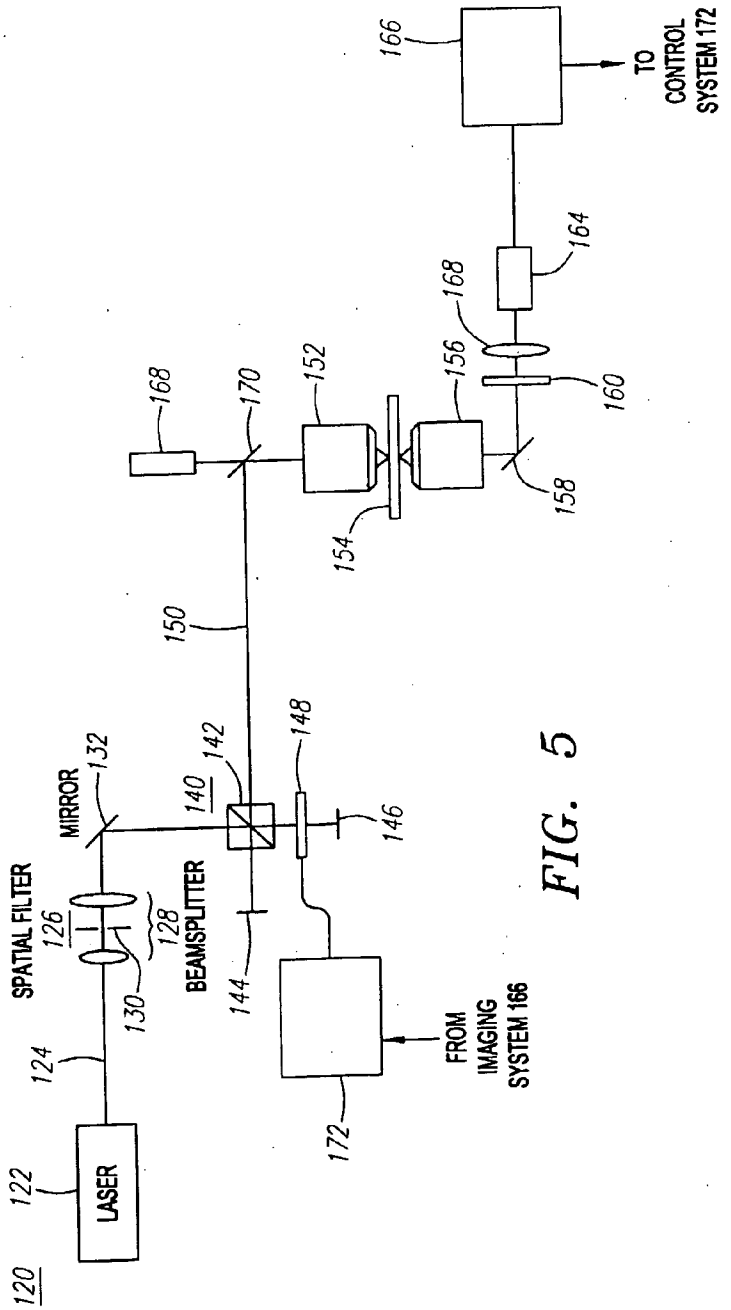

120
122
LASER
124
SPATIAL FILTER
126
128
130
MIRROR
132
140
142
BEAMSPLITTER
144
146
148
150
152
154
156
158
160
164
166
168
170
172
TO CONTROL SYSTEM 172
FROM IMAGING SYSTEM 166

FIG. 5

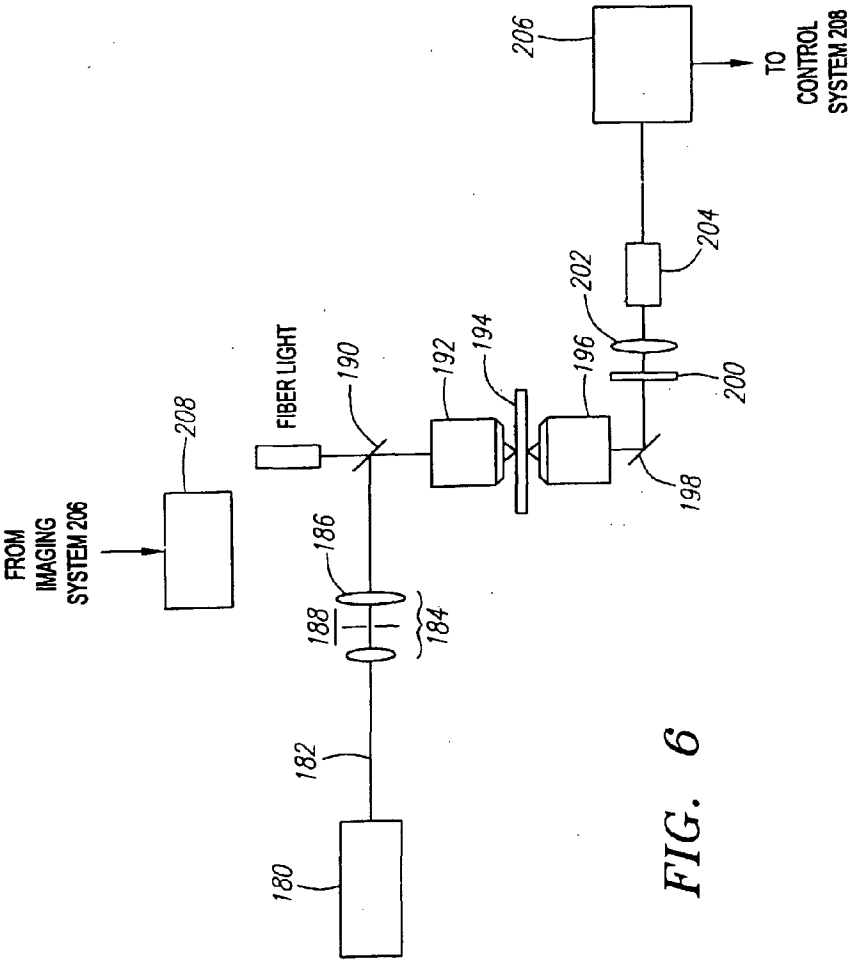

206
TO CONTROL SYSTEM 208
204
202
196
200
198
194
192
190
FIBER LIGHT
208
FROM IMAGING SYSTEM 206
186
188
184
182
180

FIG. 6

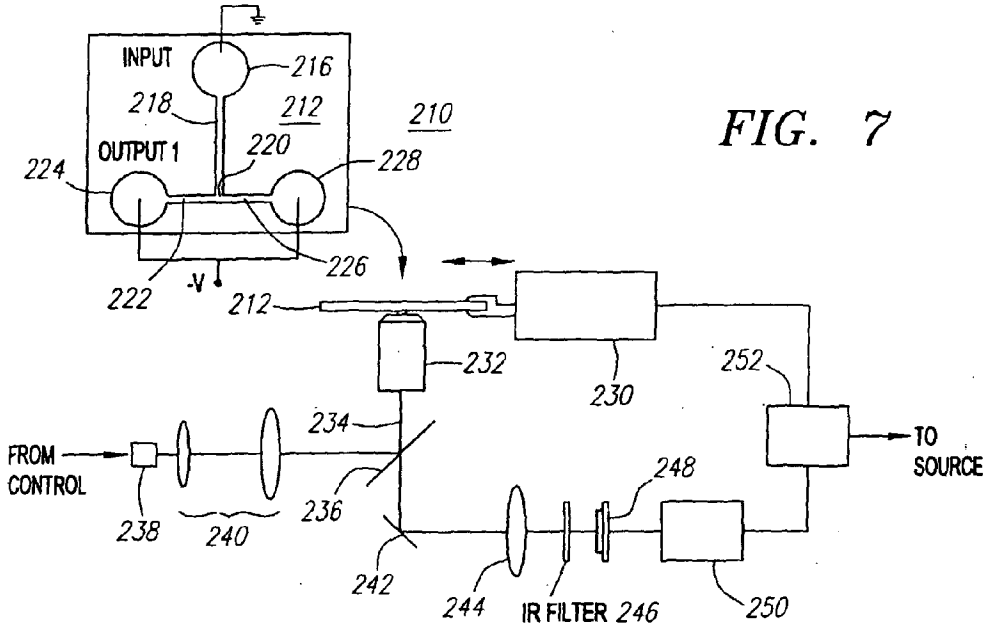

INPUT
216
218
212
210
OUTPUT 1
220
228
224
226
222
-V
212
232
230
252
234
TO SOURCE
FROM CONTROL
248
238
240
236
242
244
IR FILTER 246
250

FIG. 7

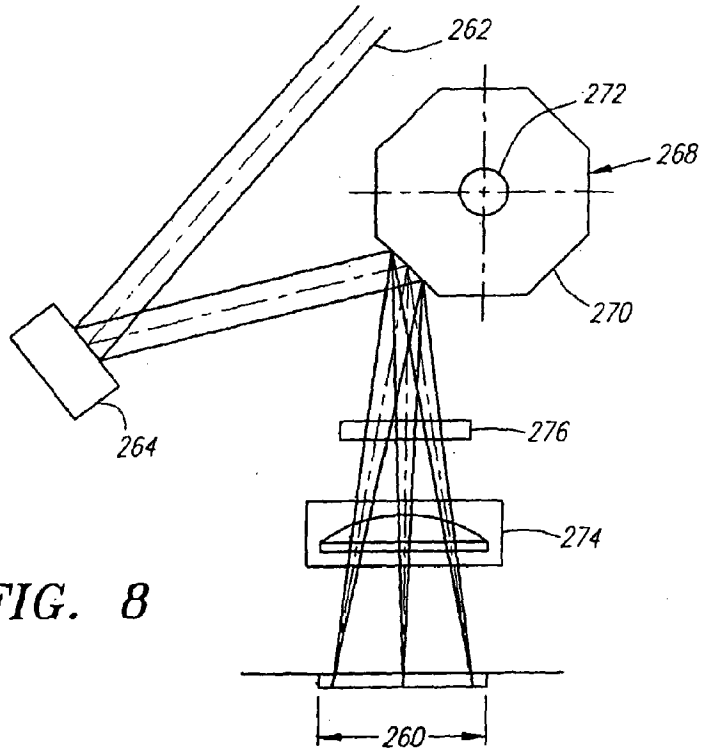

262
272
268
270
264
276
274
260

FIG. 8

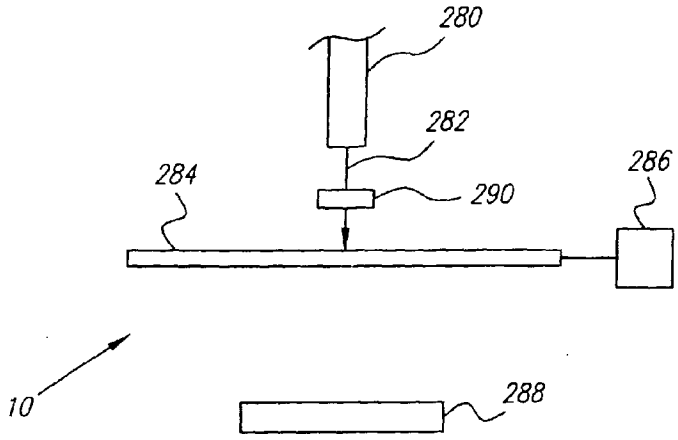


FIG. 9A

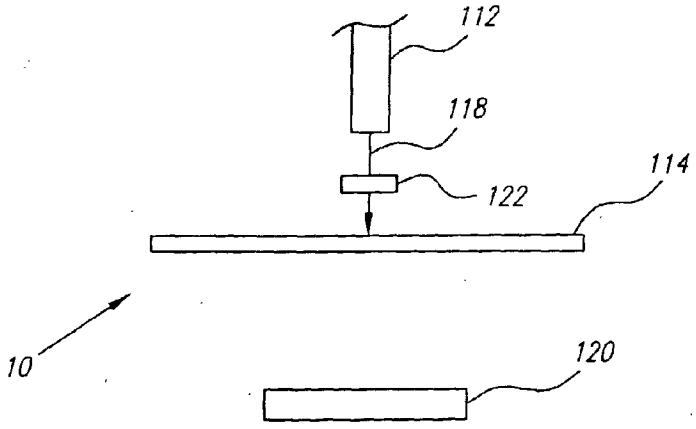


FIG. 9B

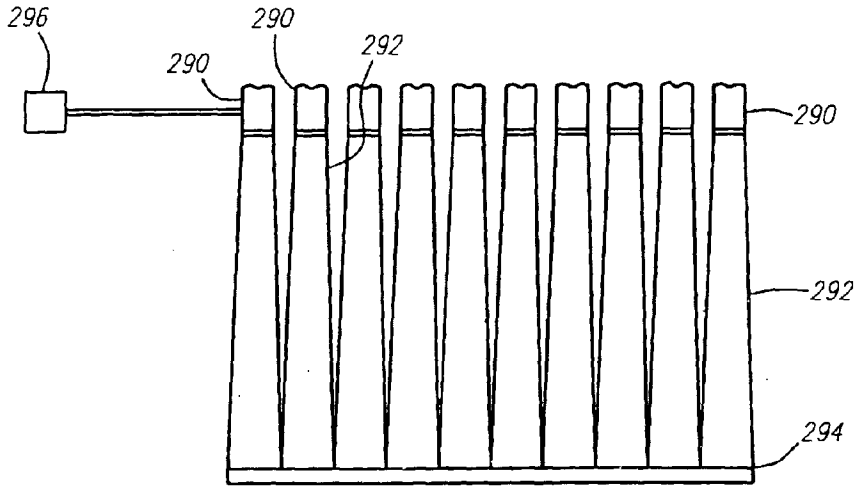


FIG. 10

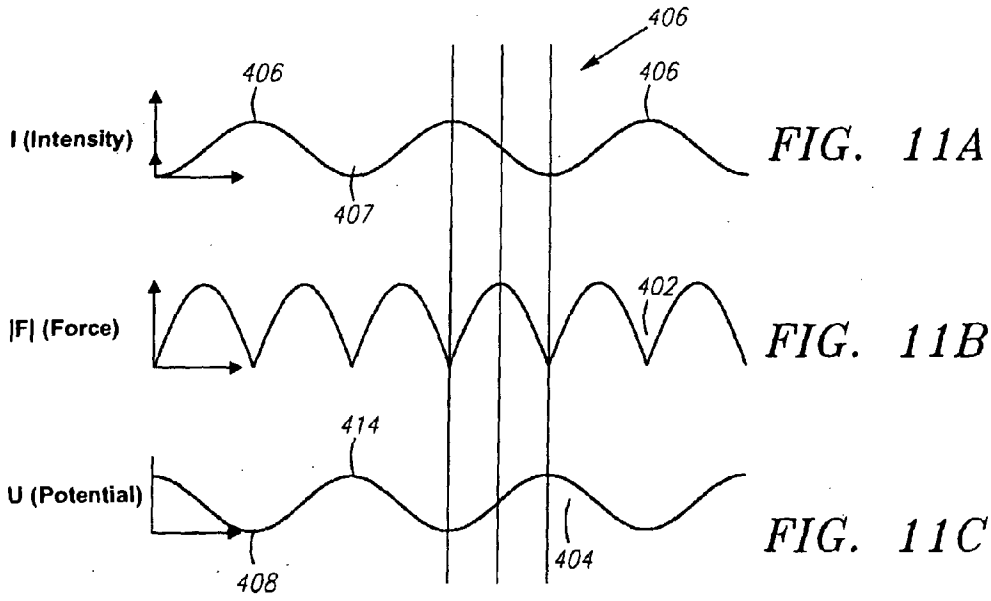


FIG. 11A

FIG. 11B

FIG. 11C

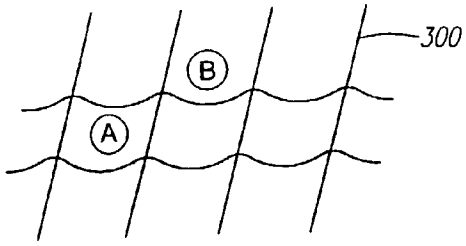


FIG. 12A

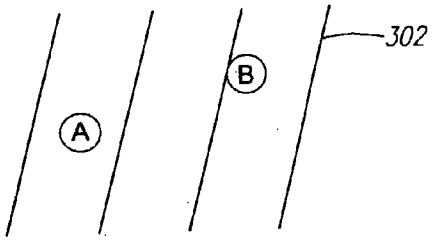


FIG. 12B

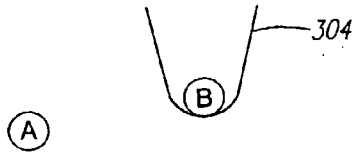


FIG. 12C

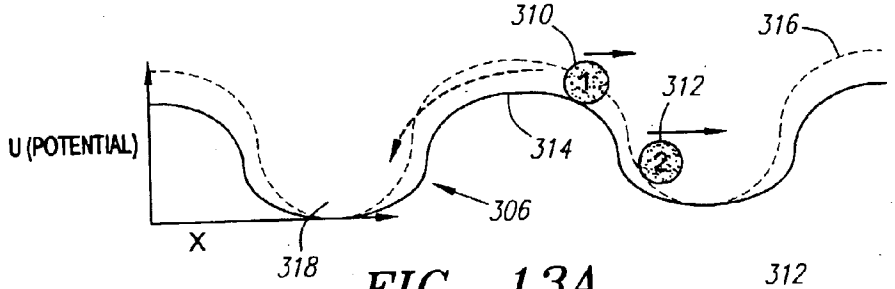


FIG. 13A

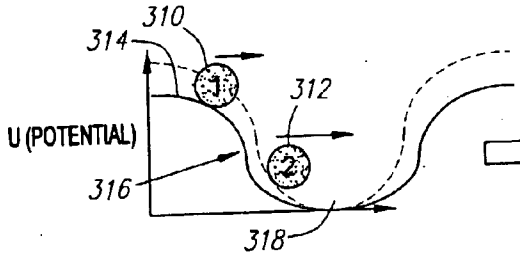


FIG. 13B

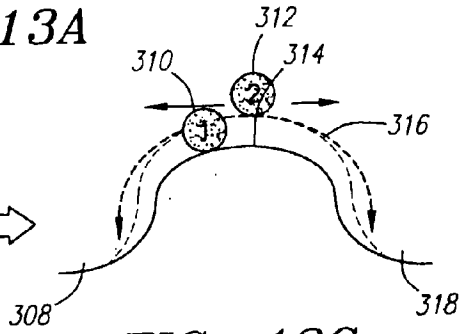


FIG. 13C

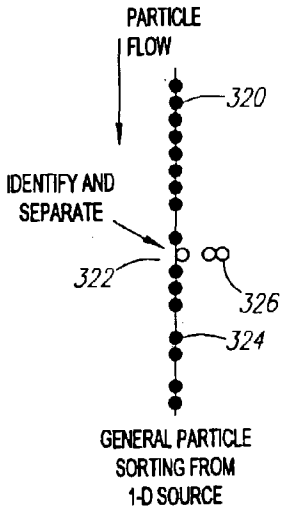


FIG. 14A

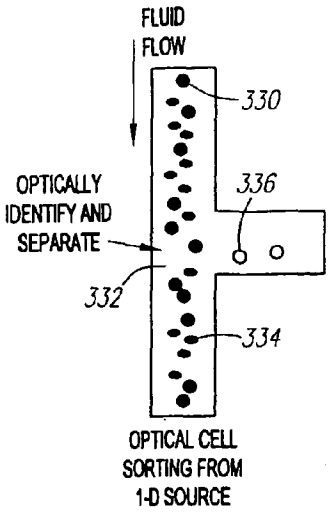


FIG. 14B

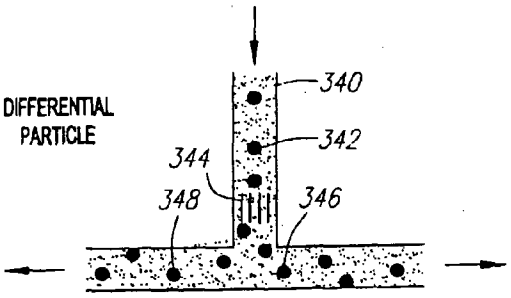


FIG. 15

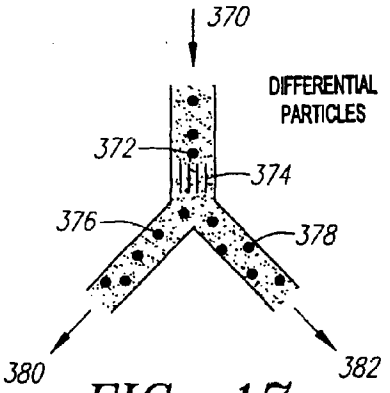


FIG. 17

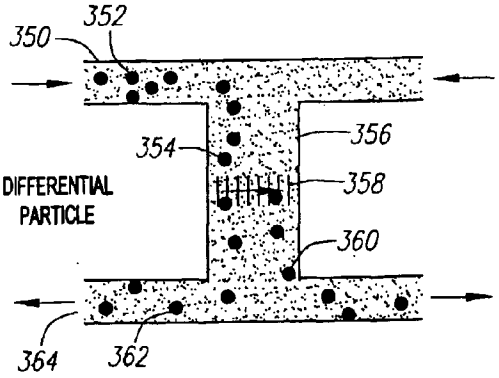


FIG. 16

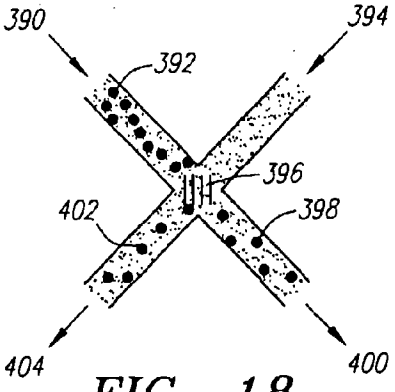


FIG. 18

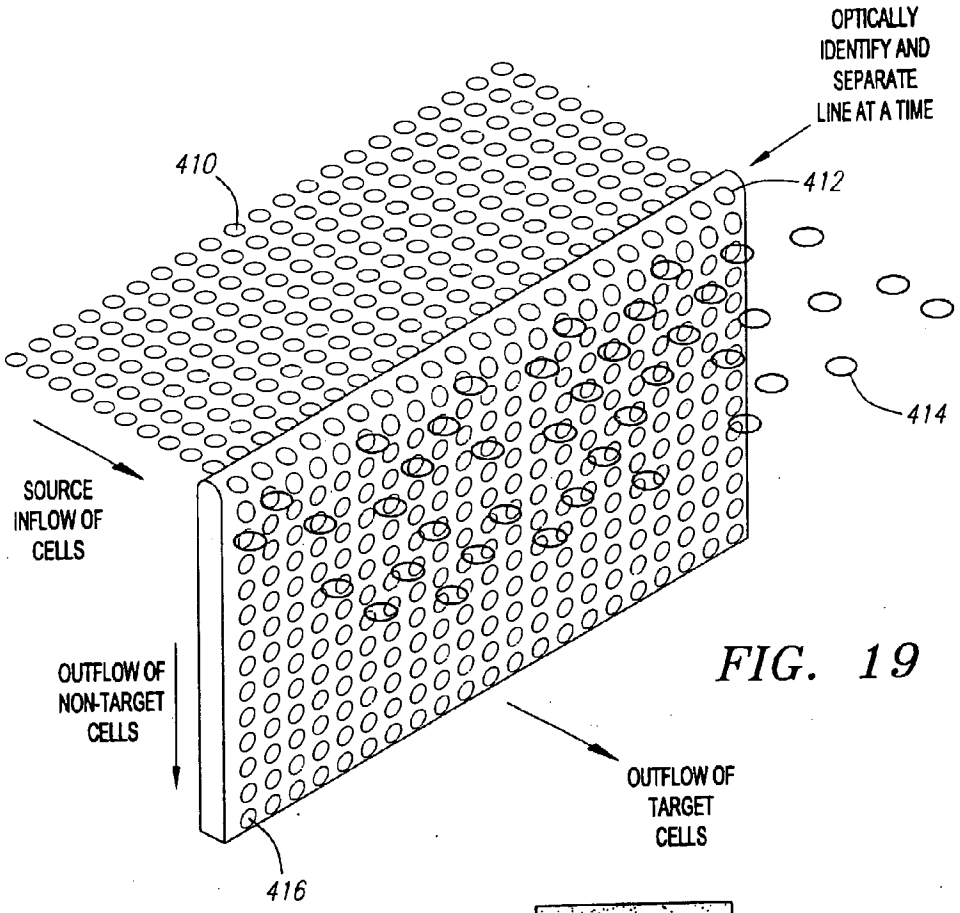


FIG. 19

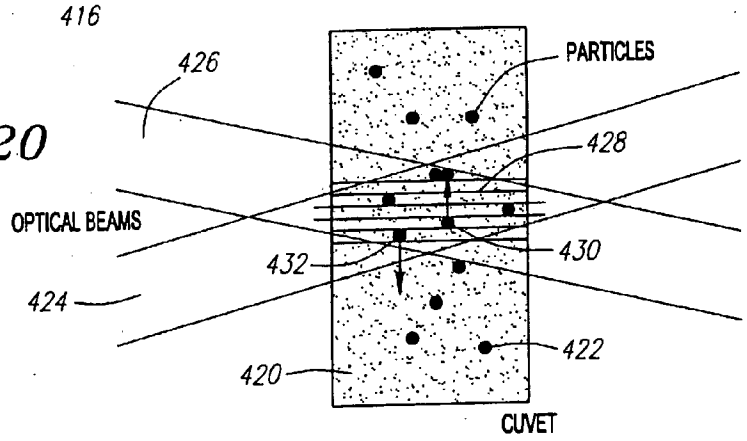


FIG. 20

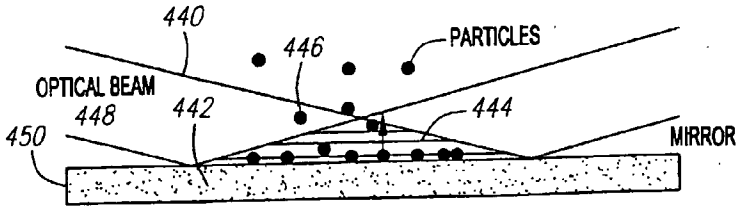


FIG. 21

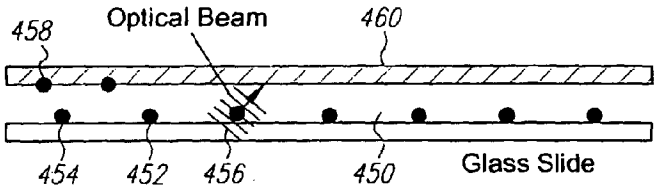


FIG. 22

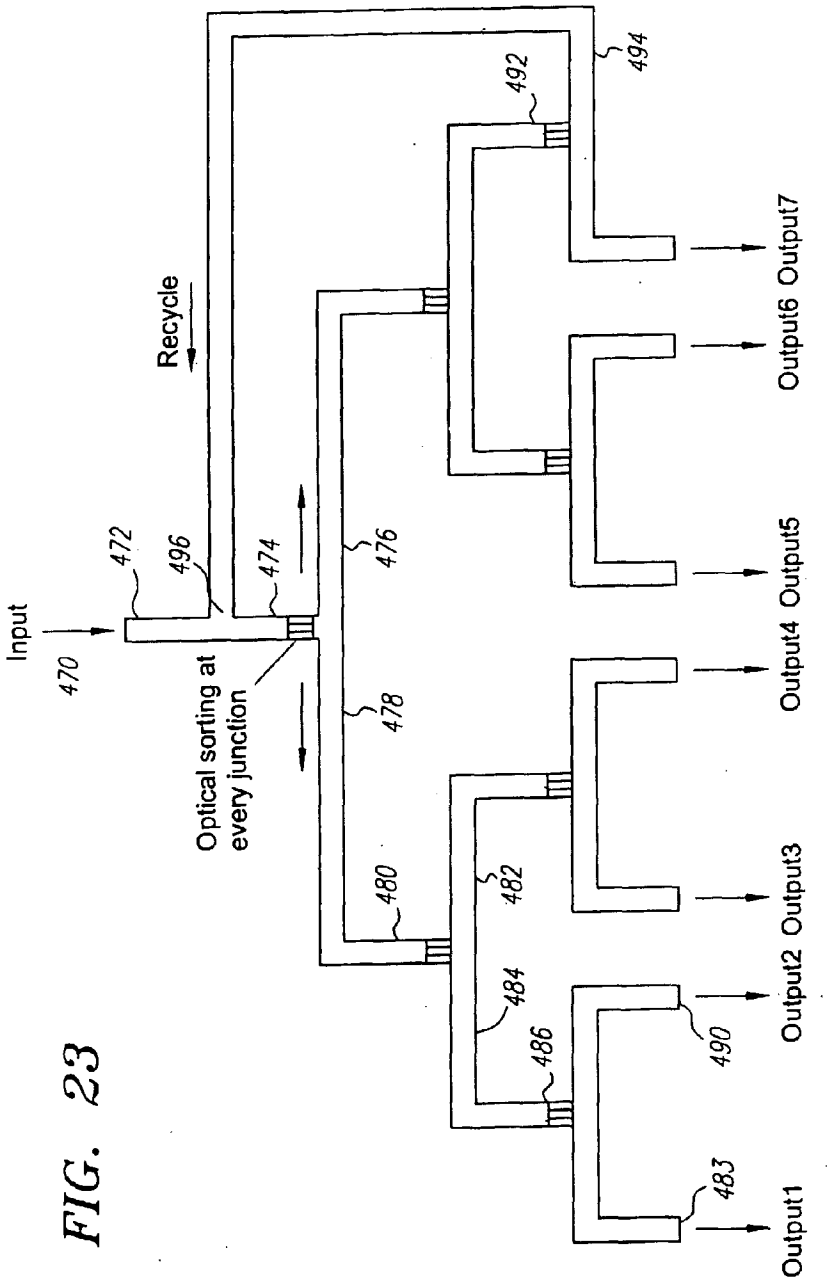

Input
470
472
496
474
476
478
480
482
484
486
483
490
492
494
Recycle
Optical sorting at every junction
Output1
Output2
Output3
Output4
Output5
Output6
Output7

FIG. 23

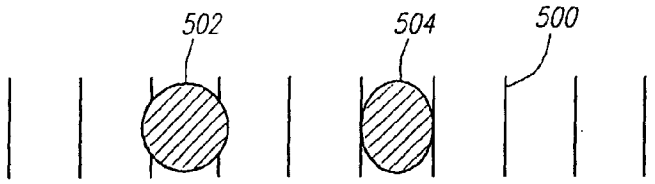


FIG. 24

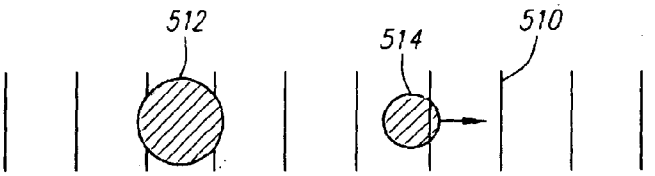


FIG. 25

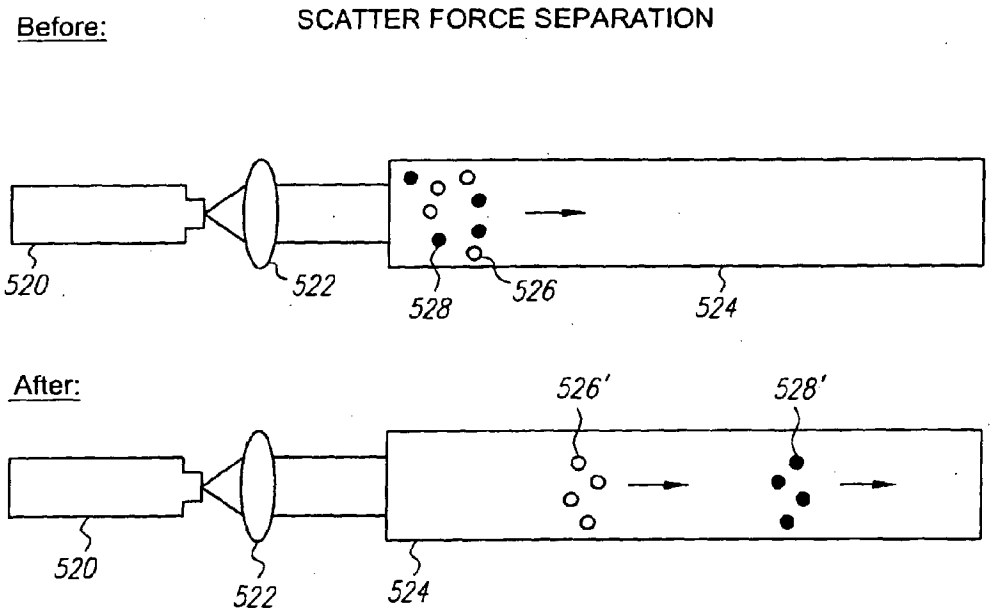
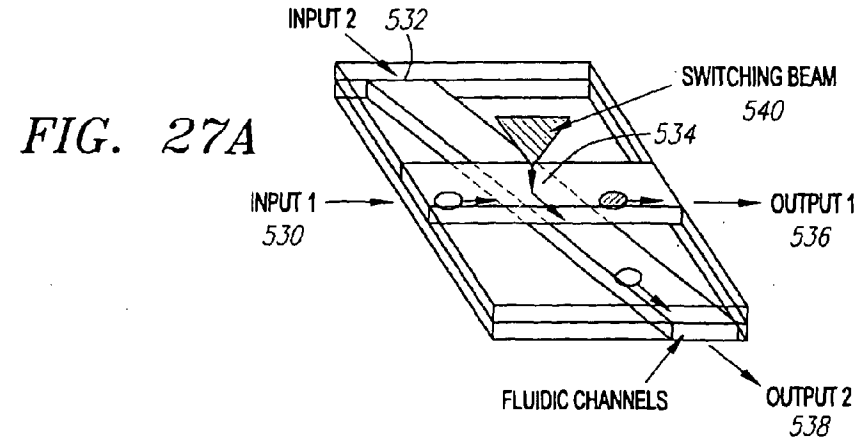


FIG. 26

FIG. 27A



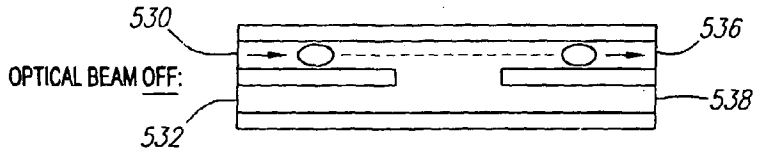


FIG. 27B

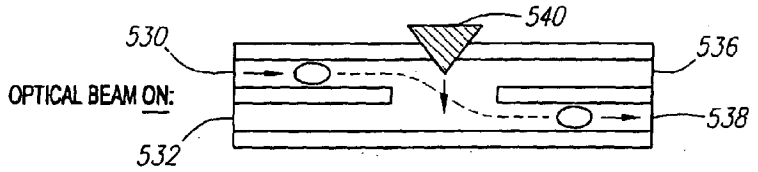


FIG. 27C

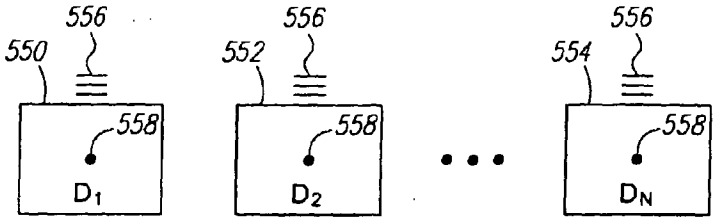
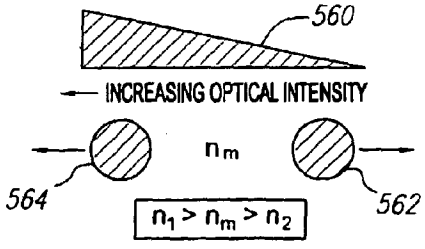


FIG. 28

FIG. 29



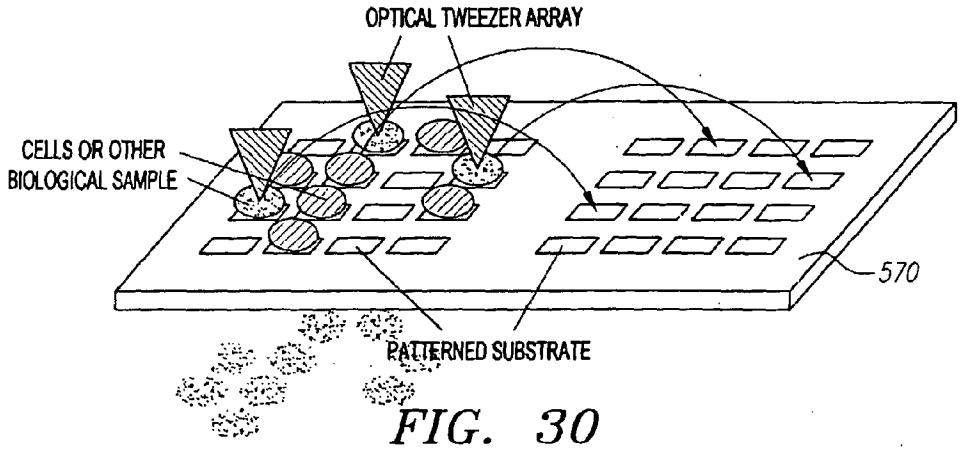

OPTICAL TWEEZER ARRAY
CELLS OR OTHER
BIOLOGICAL SAMPLE
570
PATTERNED SUBSTRATE

FIG. 30

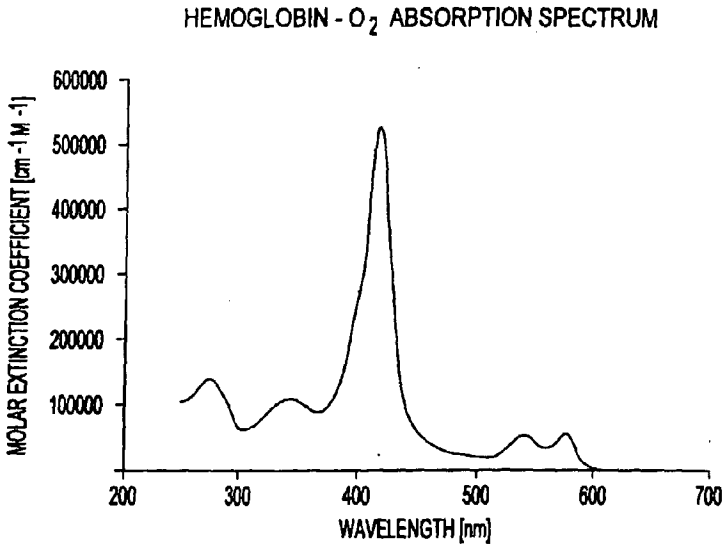

HEMOGLOBIN - O_2 ABSORPTION SPECTRUM
MOLAR EXTINCTION COEFFICIENT [cm-1M-1]
600000
500000
400000
300000
200000
100000
200
300
400
500
600
700
WAVELENGTH [nm]

FIG. 31

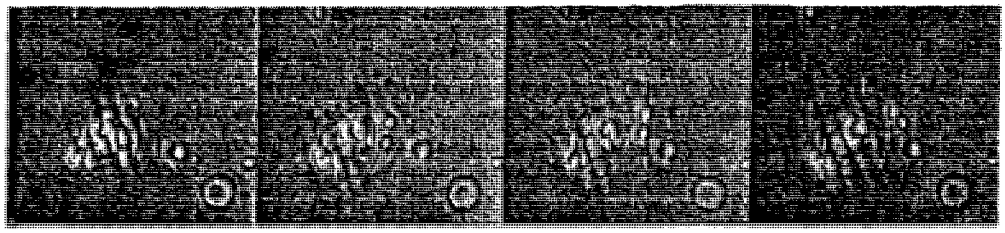


FIG. 32

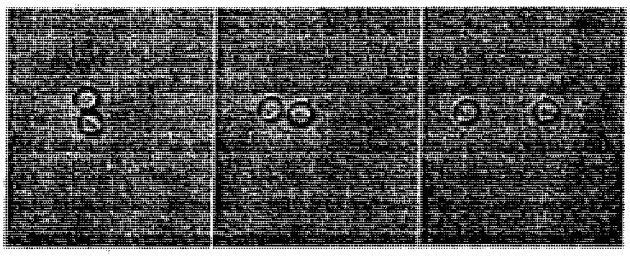
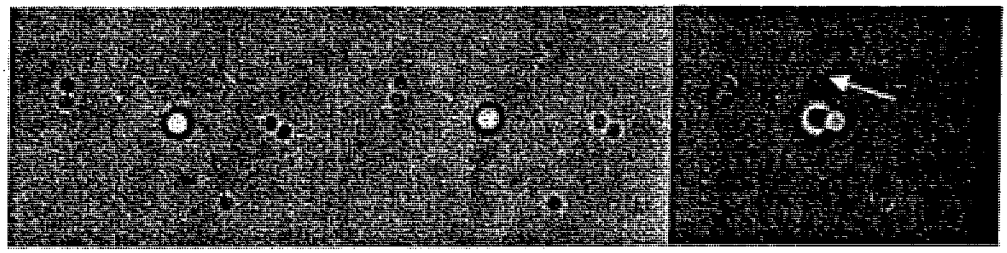


FIG. 33



Before After Difference

FIG. 34

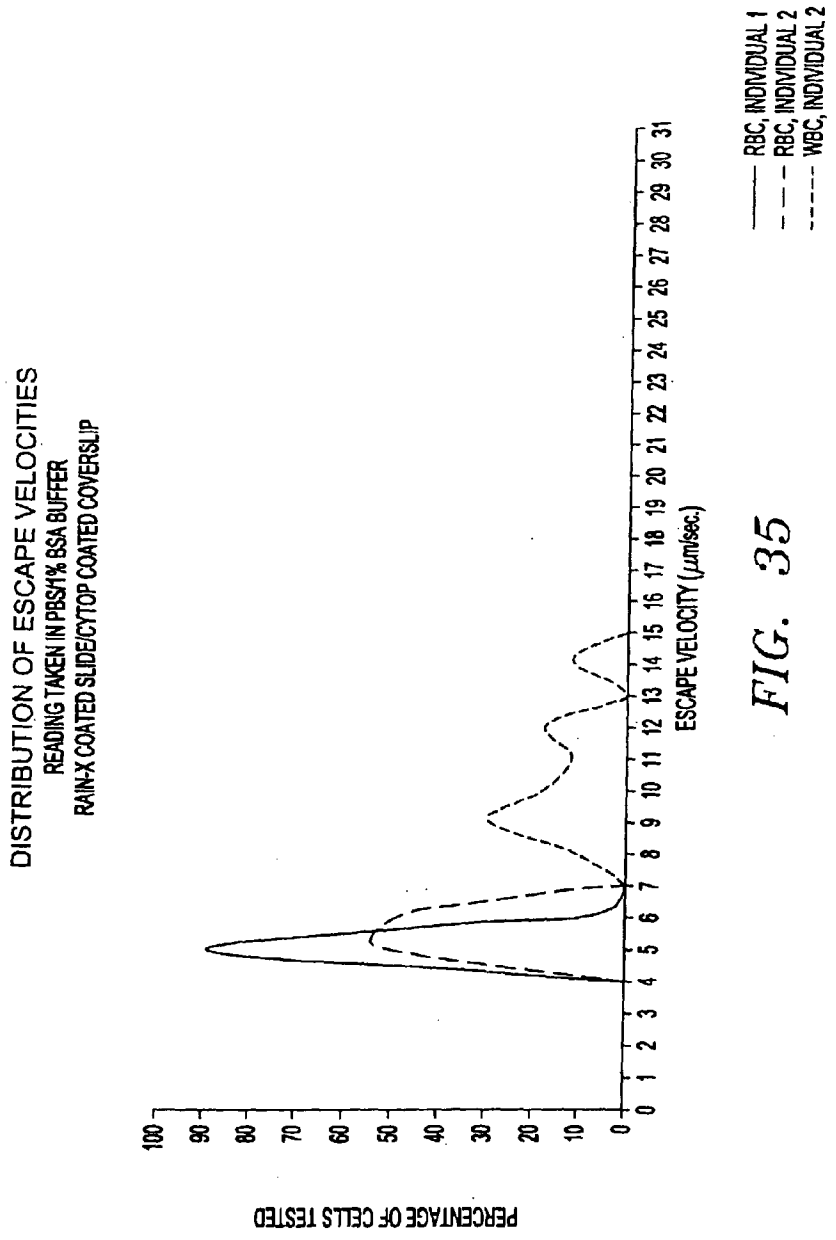

DISTRIBUTION OF ESCAPE VELOCITIES
READING TAKEN IN PBS/1% BSA BUFFER
RAIN-X COATED SLIDE/CYTOP COATED COVERSLIP
PERCENTAGE OF CELLS TESTED
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
ESCAPE VELOCITY (μm/sec.)
RBC, INDIVIDUAL 1
RBC, INDIVIDUAL 2
WBC, INDIVIDUAL 2

FIG. 35

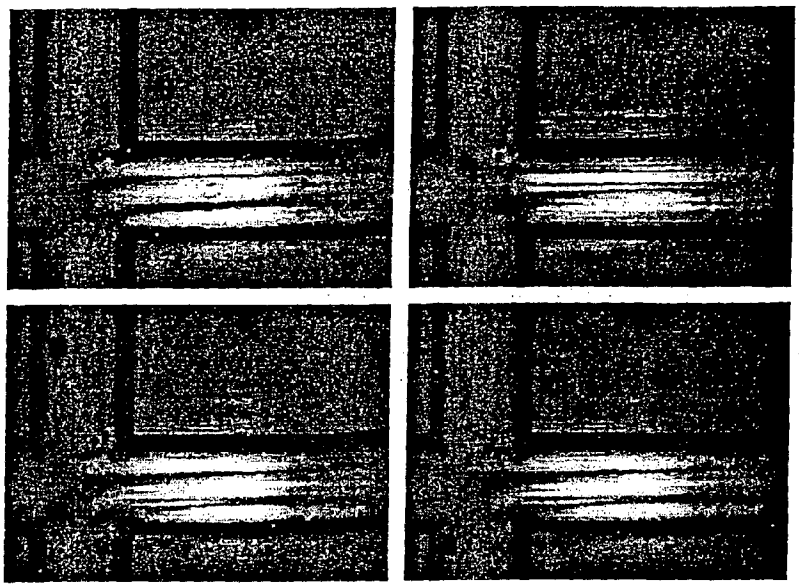
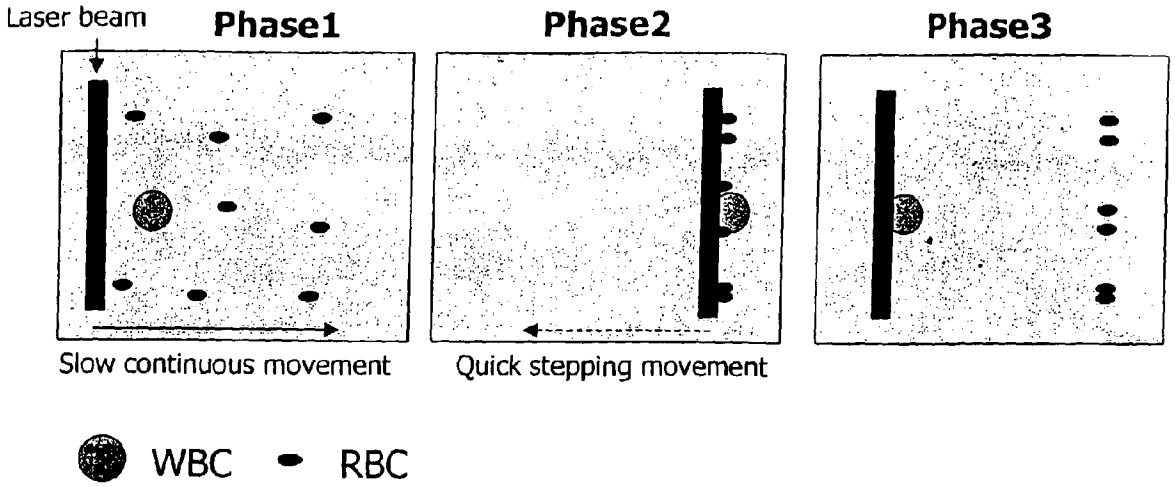


FIG. 36

FIG. 37A *FIG. 37B* *FIG. 37C*



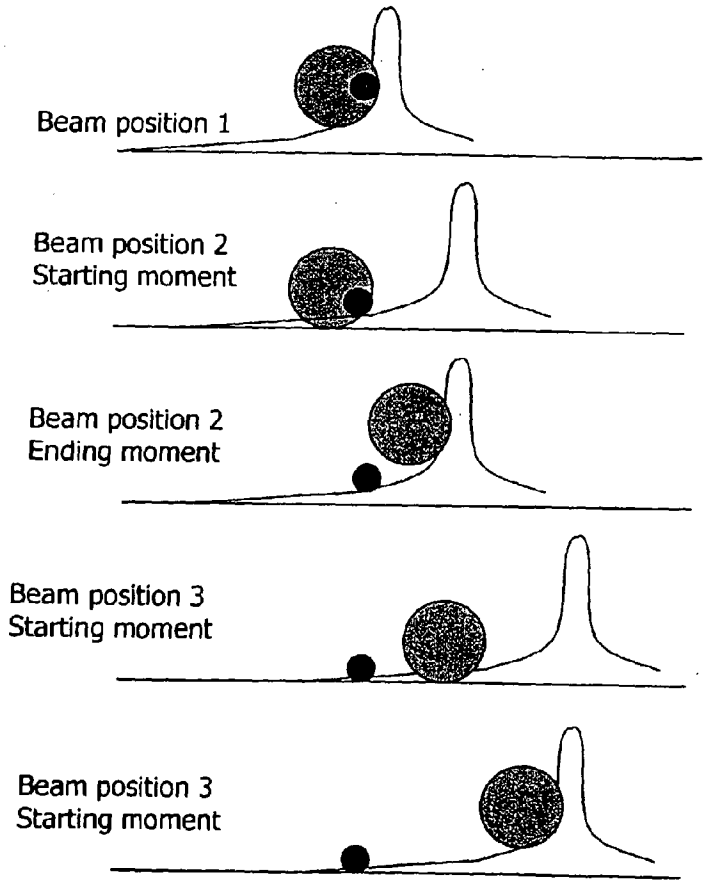


FIG. 38

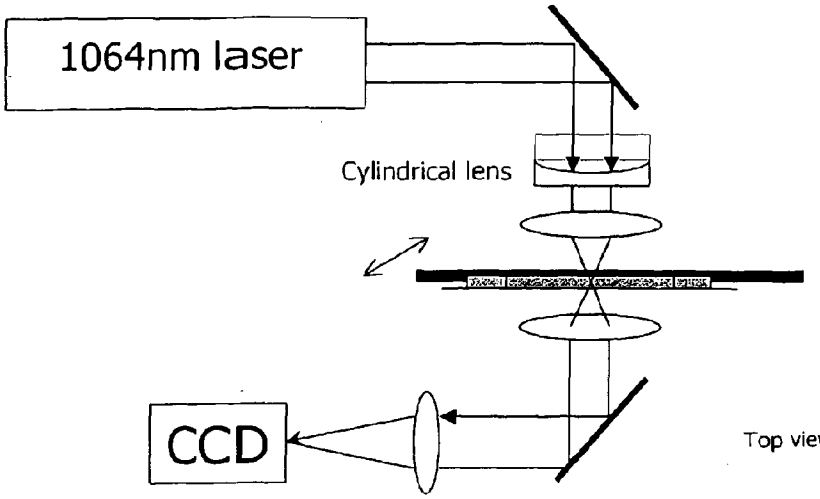


FIG. 39A

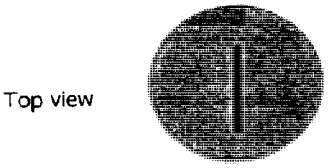


FIG. 39B

Using diffractive optics

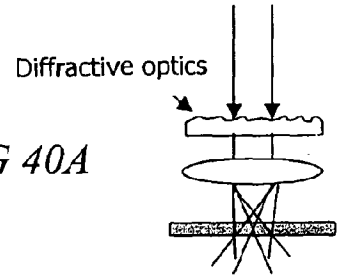


FIG 40A

FIG. 40B

Using fast scanning

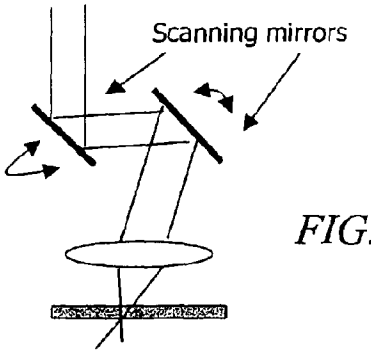


FIG. 40C

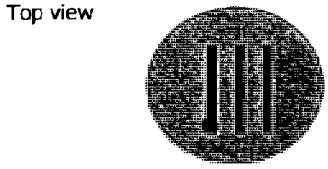


FIG. 40D

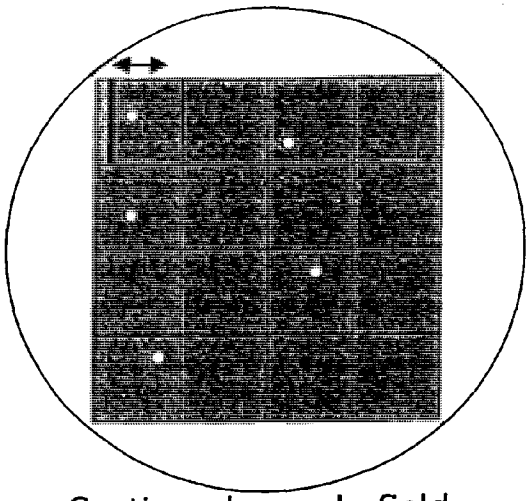

Sectioned sample field

FIG. 41

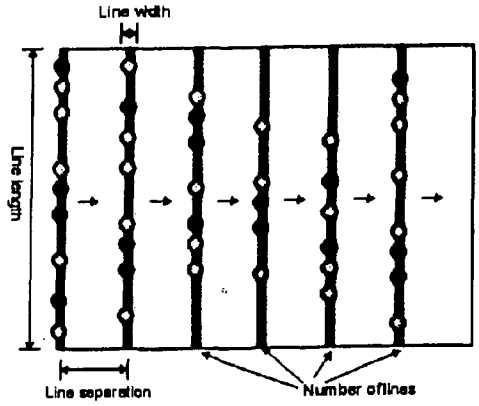

Line width
Line length
Line separation
Number of lines

FIG. 42

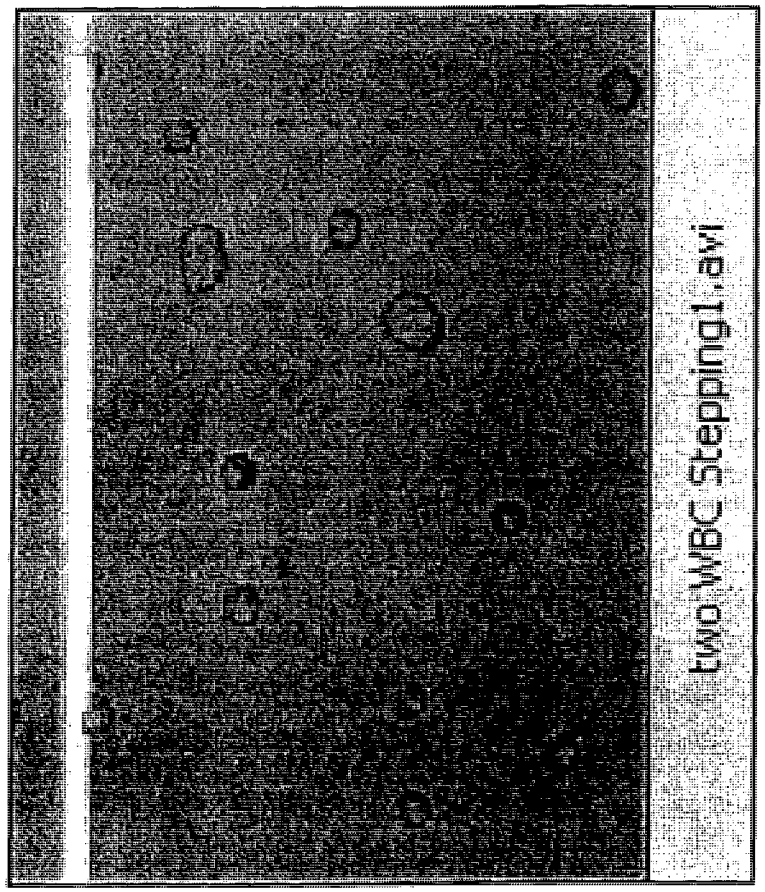


FIG. 43A

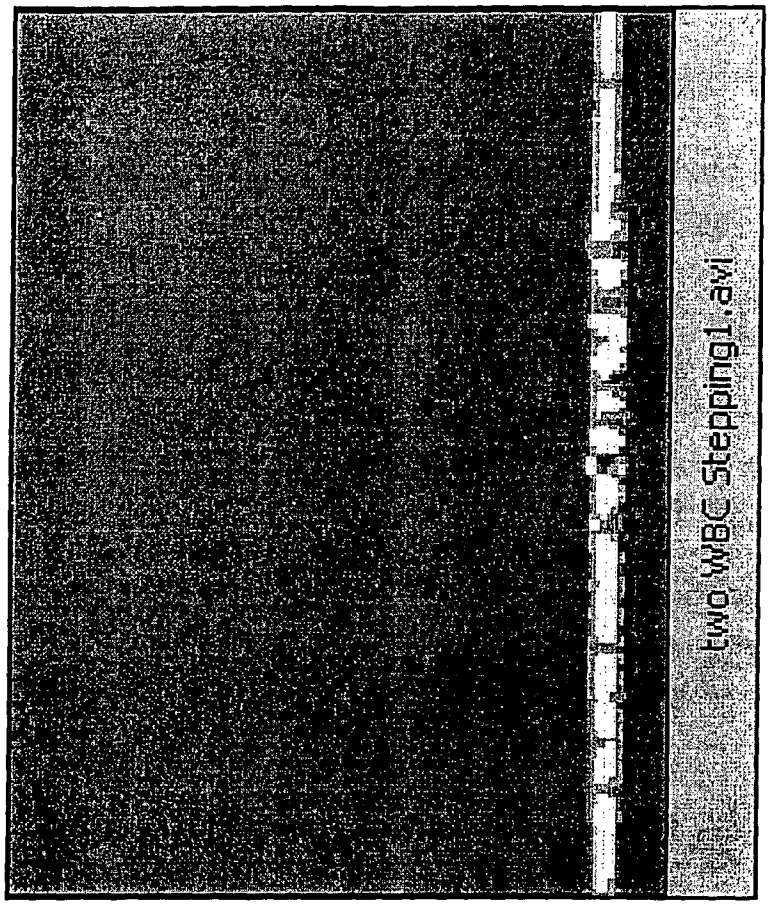


FIG. 43B

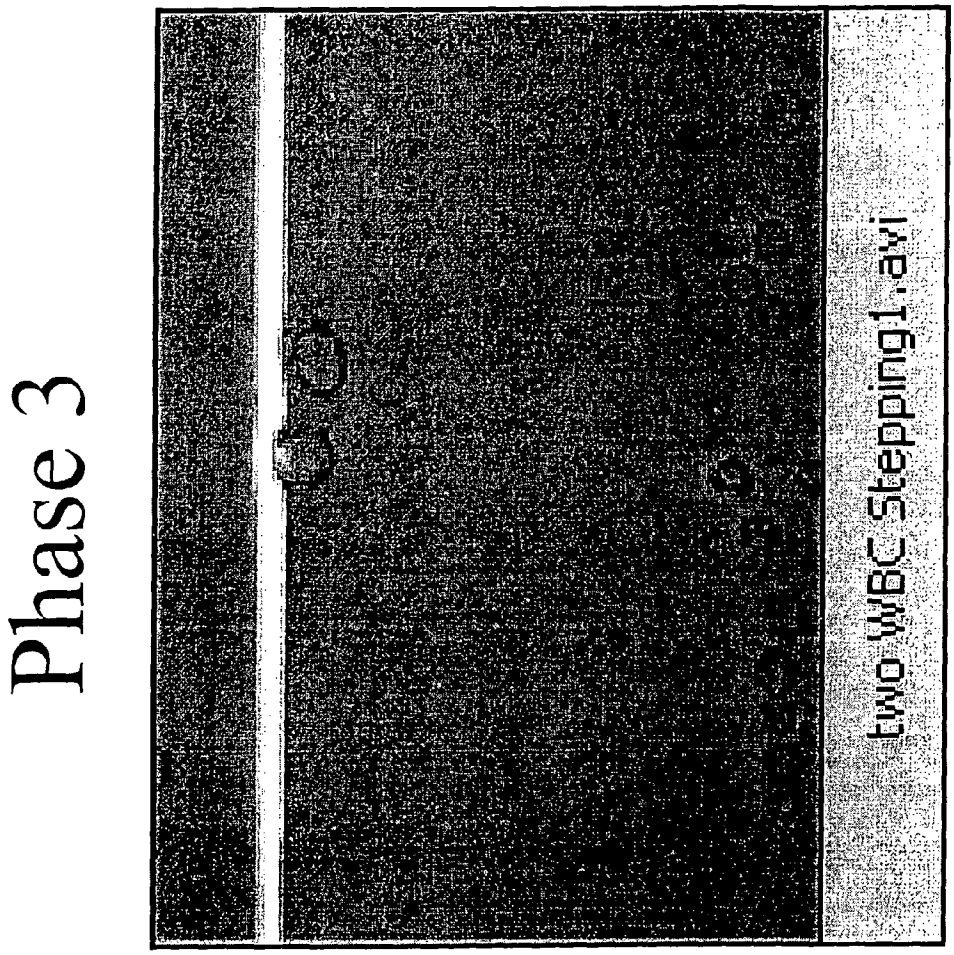


FIG. 43C

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(54) Title: METHODS AND APPARATUS FOR GENERATING AND UTILIZING LINEAR MOVING OPTICAL GRADIENTS

(57) Abstract: Apparatus and methods are provided for interacting light with particles, including but not limited to biological matter such as cells, in unique and highly useful ways. Optophoresis consists of subjecting particles to various optical forces, especially optical gradient forces, and more particularly moving optical gradient forces, so as to obtain useful results. In one implementation, a population of particles, comprising two or more differing particles, e.g., red blood cells and white blood cells, are illuminated by a line of light which is moved slowly relative to the particle population. The particles are moved with the line until the population is aligned. Next, the line of particles is subject to relative motion of light relative to the particles, such as by rapidly moving the line of illumination relative to the physical position of the particles. By moving the line away from the particles at a rate great enough that certain particles remain behind, effective separation, characterization and/or identification of the particles may be made. Optionally, the direction of the low initial scan is in a direction opposition to the more rapid scan after the particles have been aligned.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/00340

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,752,606 A (WILSON et al) 19 May 1998 (19.05.1998), abstract, column 1 lines 14-18, column 4 lines 14-34.	1-18
Y	US 5,170,890 A (WILSON et al) 15 December 1992 (15.12.1992), claims 1-16, especially claim 16.	1-18
Y	US 4,939,081 A (FIGDOR et al) 03 July 1990 (03.07.1990), abstract.	19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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INTERNATIONAL SEARCH REPORT

PCT/US03/00340

Continuation of B. FIELDS SEARCHED Item 3:
searched west: 435/6.icls. and beam and separat$ and cell$; (method$) near3 (separat$) near5 (cell$ or particl$) same (beam$0 near3 (light$)

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WO 02/087792 A1

(54) Title: METHODS AND APPARATUS FOR USE OF OPTICAL FORCES FOR IDENTIFICATION, CHARACTERIZATION AND/OR SORTING OF PARTICLES

(57) Abstract: Apparatus and methods are provided for interacting light with particles, including but not limited to biological matter such as cells in unique and highly useful ways. Optophoresis consists of subjecting particles to various optical forces, especially optical gradient forces, and more particularly moving optical gradient forces, so as to obtain useful results. In biology, this technology represents a practical approach to probing the inner workings of a living cell, preferably without any dyes, labels or other markers. In one aspect, a particle may be characterized by determining its optophoretic constant or signature. For example, a diseased cell has a different optophoretic constant from a healthy cell, thereby providing information, or the basis for sorting. In the event of physical sorting, various forces may be used for separation, including fluidic forces, such as through the use of laminar flow, or optical forces, or mechanical forces, such as through adhesion. Various techniques for measuring the dielectric constant of particles are provided.

METHODS AND APPARATUS FOR USE OF OPTICAL FORCES FOR IDENTIFICATION, CHARACTERIZATION AND/OR SORTING OF PARTICLES

Field of the Invention

[0001] This invention relates to methods and apparatus for the selection, identification, characterization, and/or sorting of materials utilizing at least optical or photonic forces. More particularly, the inventions find utility in biological systems, generally considered to be the use of optical forces for interaction with bioparticles having an optical dielectric constant.

Related Applications

[0002] This application is a continuation-in-part of Application Serial No. 09/845,245, filed April 27, 2001, entitled "Methods and Apparatus for Use of Optical Forces for Identification, Characterization and/or Sorting of Particles", which is related to Application Serial No. 09/843,902, filed on April 27, 2001, entitled "System and Method for Separating Micro-Particles", with named inventor Osman Kibar, which claims priority from provisional Application Serial No. 60/248,451, entitled "Method and Apparatus for Sorting Cells or Particles", filed November 13, 2000. Those applications are incorporated herein by reference as if fully set forth herein.

Background of the Invention

[0003] Separation and characterization of particles has a wide variety of applications ranging from industrial applications, to biological applications, to environmental applications. For example, in the field of biology, the separation of cells has numerous applications in medicine and biotechnology. Historically, sorting technologies focused on gross physical characteristics, such as particle size or density, or to utilize some affinity interaction, such as receptor-ligand interactions or reactions with immunologic targets.

[0004] Electromagnetic response properties of materials have been utilized for particle sorting and characterization. For example, dielectrophoretic separators utilize non-uniform DC or AC electric fields for separation of particles. See, e.g., U.S. Patent No. 5,814,200, Pethig et al., entitled "Apparatus for Separating By Dielectrophoresis". The application of dielectrophoresis to cell sorting has been attempted. In Becker (with Gascoyne) et al., PNAS USA, Vol. 92, pp. 860-864, Jan. 1995, Cell Biology, in the article

entitled "Separation of Human Breast Cancer Cells from Blood by Differential Dielectric Affinity", the authors reported that the dielectric properties of diseased cells differed sufficiently to enable separation of the cancer cells from normal blood cells. The system balanced hydrodynamic and dielectrophoretic forces acting on cells within a dielectric affinity column containing a microelectrode array. More sophisticated separation systems have been implemented. See, e.g., Cheng, et al., U. S. Patent No. 6,071,394, "Channel-Less Separation of Bioparticles on a Bioelectronic Chip by Dielectrophoresis". Yet others have attempted to use electrostatic forces for separation of particles. See, e.g., Judy et al., U.S. Patent No. 4,440,638, entitled "Surface Field-Effect Device for Manipulation of Charged Species", and Washizu "Electrostatic Manipulation of Biological Objects", Journal of Electrostatics, Vol. 25, No. 1, June 1990, pp. 109-103.

[0005] Light has been used to sort and trap particles. One of the earliest workers in the field was Arthur Ashkin at Bell Laboratories, who used a laser for manipulating transparent, μm-size latex beads. Ashkin's U.S. Patent No. 3,808,550 entitled "Apparatuses for Trapping and Accelerating Neutral Particles" disclosed systems for trapping or containing particles through radiation pressure. Lasers generating coherent optical radiation were the preferred source of optical pressure. The use of optical radiation to trap small particles grew within the Ashkin Bell Labs group to the point that ultimately the Nobel Prize was awarded to researchers from that lab, including Steven Chu. See, e.g., Chu, S., "Laser Trapping of Neutral Particles", Sci. Am., p. 71 (Feb. 1992), Chu, S., "Laser Manipulation of Atoms and Particles", Science 253, pp. 861-866 (1991).

[0006] Generally, the interaction of a focused beam of light with dielectric particles or matter falls into the broad categories of a gradient force and a scattering force. The gradient force tends to pull materials with higher relative dielectric constants toward the areas of highest intensity in the focused beam of light. The scattering force is the result of momentum transfer from the beam of light to the material, and is generally in the same direction as the beam. The use of light to trap particles is also sometimes referred to as an optical tweezer arrangement. Generally, utilizing the Rayleigh approximation, the force of trapping is given by the following equation:

$$F_g = 2\pi \cdot r^3 \frac{\sqrt{\varepsilon_B}}{c}\left(\frac{\varepsilon - \varepsilon_B}{\varepsilon + 2\varepsilon_B}\right)(\nabla \cdot I)$$

where F_g is the optical gradient force on the particle in the direction toward the higher intensity, r is the radius of the particle, ε_B is the dielectric constant of the background medium, ε is the dielectric constant of the particle, I is the light intensity in watts per square centimeter and ∇ is the spatial derivative. Fig. 1 shows a drawing of a particle in an optical tweezer. The optical tweezer consists of a highly focused beam directed to the particle.

[0007] As shown in Fig. 1, the focused beam 12 first converges on the particle 10 and then diverges. The intensity pattern 14 relates to the cross-section of the intensity of the beam in the horizontal dimension, and the intensity pattern 16 is the cross-section of intensity in the vertical dimension. As can be seen from the equation, the trapping force is a function of the gradient of the intensity of the light. Thus, the force is greater where the light intensity changes most rapidly, and contrarily, is at a minimum where the light intensity is uniform.

[0008] Early stable optical traps levitated particles with a vertical laser beam, balancing the upward scattering force against the downward gravitational force. The gradient force of the light served to keep the particle on the optical axis. See, e.g., Ashkin, "Optical Levitation by Radiation Pressure", Appl. Phys. Lett., 19(6), pp. 283-285 (1971). In 1986, Ashkin disclosed a trap based upon a highly focused laser beam, as opposed to light propagating along an axis. The highly focused beam results in a small point in space having an extremely high intensity. The extreme focusing causes a large gradient force to pull the dielectric particle toward that point. Under certain conditions, the gradient force overcomes the scattering force, which would otherwise push the particle in the direction of the light out of the focal point. Typically, to realize such a high level of focusing, the laser beam is directed through a high numerical aperture microscope objective. This arrangement serves to enhance the relative contribution from the high numerical aperture illumination but decreases the effect of the scattering force.

[0009] In 1987, Ashkin reported an experimental demonstration of optical trapping and manipulation of biological materials with a single beam gradient force optical trap system. Ashkin, et al., "Optical Trapping and Manipulation of Viruses and Bacteria", Science, 20 March, 1987, Vol. 235, No. 4795, pp. 1517-1520. In U.S. Patent No. 4,893,886, Ashkin et al., entitled "Non-Destructive Optical Trap for Biological Particles and Method of Doing Same", reported successful trapping of biological particles in a single beam gradient force optical trap utilizing an infrared light source. The use of an infrared laser emitting

coherent light in substantially infrared range of wavelengths, there stated to be 0.8 μm to 1.8 μm, was said to permit the biological materials to exhibit normal motility in continued reproductivity even after trapping for several life cycles in a laser power of 160 mW. The term "opticution" has become known in the art to refer to optic radiation killing biological materials.

[0010] The use of light to investigate biological materials has been utilized by a number of researchers. Internal cell manipulation in plant cells has been demonstrated. Ashkin, et al., PNAS USA, Vol. 86, 7914-7918 (1989). See also, the summary article by Ashkin, A., "Optical Trapping and Manipulation of Neutral Particles Using Lasers", PNAS USA, Vol. 94, pp. 4853-4860, May 1997, Physics. Various mechanical and force measurements have been made including the measurement of torsional compliance of bacterial flagella by twisting a bacterium about a tethered flagellum. Block, S., et al., Nature (London), 338, pp. 514-518 (1989). Micromanipulation of particles has been demonstrated. For example, the use of optical tweezers in combination with a microbeam technique of pulsed laser cutting, sometimes also referred to as laser scissors or scalpel, for cutting moving cells and organelles was demonstrated. Seeger, et al., Cytometry, 12, pp. 497-504 (1991). Optical tweezers and scissors have been used in all-optical in vitro fertilization. Tadir, Y., Human Reproduction, 6, pp. 1011-1016 (1991). Various techniques have included the use of "handles" wherein a structure is attached to a biological material to aid in the trapping. See, e.g., Block, Nature (London), 348, pp. 348-352 (1990).

[0011] Various measurements have been made of biological systems utilizing optical trapping and interferometric position monitoring with subnanometer resolution. Svoboda, Nature (London), 365, pp. 721-727 (1993). Yet others have proposed feedback based systems in which a tweezer trap is utilized. Molloy, et al., Biophys. J., 68, pp. 2985-3055 (1995).

[0012] A number of workers have sought to distort or stretch biological materials. Ashkin in Nature (London), 330 pp. 769-771 (1987), utilized optical tweezers to distort the shape of red blood cells. Multiple optical tweezers have been utilized to form an assay to measure the shape recovery time of red blood cells. Bronkhorst, Biophys. J., 69, pp. 1666-1673 (1995). Kas, et al., has proposed an "optical stretcher" in U.S. Patent No. 6,067,859 which suggests the use of a tunable laser to trap and deform cells between two counter-propagating beams generated by a laser. The system is utilized to detect single malignant cancer cells. Yet another assay proposed colliding two cells or particles under controlled

conditions, termed the OPTCOL for optical collision. See, e.g., Mammer, Chem & Biol., 3, pp. 757,763 (1996).

[0013] Yet others have proposed utilizing optical forces to measure a property of an object. See, e.g., Guanming, Lai et al., "Determination of Spring Constant of Laser-Trapped Particle by Self-Mining Interferometry", Proc. of SPIE, 3921, pp. 197-204 (2000). Yet others have utilized the optical trapping force balanced against a fluidic drag force as a method to calibrate the force of an optical trap. These systems utilize the high degree of dependence on the drag force, particularly Stokes drag force.

[0014] Yet others have utilized light intensity patterns for positioning materials. In U.S. Patent No. 5,245,466, Burnes et al., entitled "Optical Matter", arrays of extended crystalline and non-crystalline structures are created using light beams coupled to microscopic polarizable matter. The polarizable matter adopts the pattern of an applied, patterned light intensity distribution. See also, "Matter Rides on Ripples of Lights", reporting on the Burns work in New Scientist, 18 Nov., 1989, No. 1691. Yet others have proposed methods for depositing atoms on a substrate utilizing a standing wave optical pattern. The system may be utilized to produce an array of structures by translating the standing wave pattern. See, Celotta et al., U.S. Patent No. 5,360,764, entitled "Method of Fabricating Laser Controlled Nanolithography".

[0015] Yet others have attempted to cause motion of particles by utilizing light. With a technique termed by its authors as "photophoresis", Brian Space, et al., utilized a polarized beam to induce rotary motion in molecules to induce translation of the molecules, the desired goal being to form a concentration gradient of the molecules. The technique preferably utilizes propeller shaped molecules, such that the induced rotary motion of the molecules results in translation.

[0016] Various attempts have been made to form microfluidic systems, put to various purposes, such as sample preparation and sorting applications. See, e.g., Ramsey, U.S. Patent No. 6,033,546, entitled "Apparatus and Method for Performing Microfluidic Manipulations for Chemical Analysis and Synthesis". Numerous companies, such as Aclara and Caliper, are attempting to form micro-systems comprising a 'lab on a chip'.

[0017] Others have attempted to combine microfabricated devices with optical systems. In "A Microfabricated Device for Sizing and Sorting DNA Molecules", Chou, et al., PNAS USA, Vol. 96, pp. 11-13, Jan. 1999, Applied Physical Sciences, Biophysics, a microfabricated device is described for sizing and sorting microscopic objects based upon

a measurement of fluorescent properties. The paper describes a system for determining the length of DNA by measuring the fluorescent properties, including the amount of intercalated fluorescent dye within the DNA. In "A Microfabricated Fluorescence-Activated Cells Sorter", Nature Biotechnology, Vol. 17, Nov. 1999, pp. 1109-1111, a "T" microfabricated structure was used for cell sorting. The system utilized a detection window upstream of the "T" intersection and based upon the detected property, would sort particles within the system. A forward sorting system switched fluid flow based upon a detected event. In a reverse sorting mode, the fluid flow was set to route all particles to a waste collection, but upon detection of a collectible event, reversed the fluid flow until the particle was detected a second time, after which the particle was collected. Certain of these systems are described in Quake et al., PCT Publication WO 99/61888, entitled "Microfabricated Cell Sorter".

[0018] Yet others have attempted to characterize biological systems based upon measuring various properties, including electromagnetic radiation related properties. Various efforts to explore dielectric properties of materials, especially biological materials, in the microwave range have been made. See, e.g., Larson et al., U.S. Patent No. 4,247,815, entitled "Method and Apparatus for Physiologic Facsimile Imaging of Biologic Targets Based on Complex Permittivity Measurements Using Remote Microwave Interrogation", and PCT Publication WO 99/39190, named inventor Hefti, entitled "Method and Apparatus for Detecting Molecular Binding Events".

[0019] Despite the substantial effort made in the art, no comprehensive, effective, sensitive and reliable system has been achieved.

Summary of the Invention

[0020] The methods and apparatus of this relate generally to the use of light energy to obtain information from, or to apply forces to, particles. The particles may be of any form which have a dielectric constant. The use of light for these beneficial purposes is the field of optophoresis. A particle, such as a cell, will have a Optophoretic constant or signature which is indicative of a state, or permits the selection, sorting, characterization or unique interaction with the particle. In the biological regime, the particles may include cells, organelles, proteins, or any component down to the atomic level. The techniques also apply in the non-biological realm, including when applied to all inorganic matter, metals, semiconductors, insulators, polymers and other inorganic matter.

[0021] Considering the biological realm, the cell represents the true point of integration for all genomic information. Accessing and deciphering this information is important to the diagnosis and treatment of disease. Existing technologies cannot efficiently and comprehensively address the enormous complexity of this information. By unlocking the fundamental properties of the cell itself, the methods and apparatus described herein create new parameters for cellular characterization, cellular analysis and cell-based assays.

[0022] This technology represents a practical approach to probing the inner workings of a particle, such as a living cell, preferably without any dyes, labels or other markers. The "Optophoretic Constant" of a cell uniquely reflects the physiological state of the cell at the exact moment in which it is being analyzed, and permits investigation of the inner workings of cells. These techniques allow simple and efficient gathering of a wide spectrum of information, from screening new drugs, to studying the expression of novel genes, to creating new diagnostic products, and even to monitoring cancer patients. This technology permits the simultaneous analysis and isolation of specific cells based on this unique optophoretic parameter. Stated otherwise, this technology is capable of simultaneously analyzing and isolating specific particles, e.g. cells, based on their differences at the atomic level. Used alone or in combination with modern molecular techniques, the technology provides a useful way to link the intricate mechanisms involving the living cell's overall activity with uniquely identifiable parameters.

[0023] In one aspect, the invention is a method for the characterization of a particle by the steps of observing a first physical position of a particle, optically illuminating the particle to subject it to an optical force, observing the second physical position of the particle, and characterizing the particle based at least in part upon reaction of the particle to the optical force. The characterization may be that the particle, e.g., a cell, has a certain disease state based upon the detected optophoretic constant or signature.

[0024] While characterization may be done with or without physical separation of multiple particles, a method for separating particles may consist of, first, subjecting particles to optical gradient force, second, moving the particle, and third, separating desired particle from other particles. The particle may be separate from the others by further optical forces, by fluidic forces, by electromagnetic forces or any other force sufficient to cause the required separation. Separation may include segregation and sorting of particles.

[0025] In yet another aspect, the invention includes a method for analyzing particles by electrokinetically moving the particles, and subjecting the particles to optical forces for sorting. The electrokinetic forces may include, for example, eletroosmosis, electrophoresis and dielectrophoresis.

[0026] In addition to the use of the dielectric aspects of the particle for characterization and sorting, certain of the inventive methods may be used to determine the dielectric constant of a particle. One method consists of subjecting the particle to an optical gradient force in a plurality of media having different dielectric constants, monitoring the motion of the particle when subject to the optical gradient force in the various media, and determining the dielectric constant of the particle based upon the relative amount of motion in the various media.

[0027] Yet other methods permit the sorting of particles according to their size. One method includes the steps of subjecting the particles to a optical fringe pattern, moving the fringes relative to the particles, wherein the improvement comprises selecting the period of the fringes to have a differential effect on differently sized particles. An allied method sorts or otherwise separates particles based upon the particles flexibility when subject to a optical force. One set of exemplary steps includes: subjecting the particles to an optical pattern having fringes, the fringe spacing being less than the size of the particle in an uncompressed state, moving the fringes relative to the medium containing the particles, and whereby particles having relatively higher flexibility are separated from those with relatively lower flexibility.

[0028] In addition to the use of optical gradient forces, the systems and methods may use, either alone or in combination with other forces, the optical scattering force. One method for separation in an optophoresis set up consists of providing one or more particles, subjecting the particles to light so as to cause a scattering force on the particles, and separating the particles based upon the reaction to at least the scattering force.

[0029] Various techniques are described for enhancing the sensitivity and discrimination of the system. For example, a sensitive arrangement may be provided by separating the particles in a medium having a dielectric constant chosen to enhance the sensitivity of the discrimination between the particles, and changing the medium to one having a dielectric constant which causes faster separation between the particles. One option for enhancing the sensitivity is to choose the dielectric constant of the medium to be close to the dielectric constant of the particles.

[0030] Accordingly, it is an object of this invention to provide a method of identification, characterization, selection and/or sorting of materials having an optical dielectric constant.

[0031] It is yet a further object of this invention to provide a system for sorting or identifying particles without labeling or otherwise modifying the particle.

[0032] It is yet another object of this invention to provide a system in which uncharged or neutral particles may be sorted or otherwise characterized.

[0033] Yet another object of this invention is to provide a system in which particles may be manipulated remotely, thereby reducing the contamination to the system under study.

[0034] It is yet another object of this invention to provide a system for characterizing, moving and/or sorting particles that may be used in conjunction with other forces, without interference between the optical forces and the other forces.

Brief Description of the Drawings

[0035] Fig. 1 is a graphical depiction of optical intensity patterns for a prior art optical tweezer system, showing both the focus beam, a particle and the cross-section of intensity of the beam.

[0036] Fig. 2 is a cross-sectional drawing of the optical system for interfering two beams utilizing a variable path length by moving a mirror.

[0037] Fig. 3 is a schematic diagram of a system utilizing interference between two beams where the path length is varied utilizing a phase modulator.

[0038] Fig. 4 is a cross-sectional drawing of an optical system utilizing an interferometer where the path length is adjustable via a phase modulator, and Fig. 4A is a side view of an alternate optical arrangement utilizing counterpropagating beams for particle levitation.

[0039] Fig. 5 is a cross-sectional drawing of an optical system including an interferometer and a phase modulator for changing the optical path length, and includes a photograph of a wave pattern generated by the system.

[0040] Fig. 6 is a cross-sectional drawing of an optical system utilizing separate illumination and imaging systems.

[0041] Fig. 7 is a depiction of an optical system interfacing with a fluidic system.

[0042] Fig. 8 is a cross-sectional drawing of an optical system utilizing a moving scanning system.

[0043] Figs. 9A and 9B are cross-sectional drawings of an optical system including a mask based generation of intensity pattern.

[0044] Fig. 10 is a side view of an array of illumination sources, illuminating a substrate or support.

[0045] Figs. 11A, 11B and 11C show graphs of intensity, forces and potential energy, respectively, as a function of position in one exemplary embodiment of the invention.

[0046] Fig. 12A shows two particles at first positions and a superimposed optical pattern.

[0047] Fig. 12B shows the particles at second positions after illumination by the optical pattern.

[0048] Fig. 12C shows the trapping of particle B in an optical trap.

[0049] Figs. 13A, 13B and 13C show graphs of the potential energy as a function of distance for the technique for separating particles.

[0050] Figs. 14A and 14B show graphical depictions of particle sorting from a one-dimensional particle source, in Fig. 14A showing the particle flow and in Fig. 14B showing particles transported in a fluid flow.

[0051] Fig. 15 is a plan view drawing of a "T" channel sorting structure.

[0052] Fig. 16 is a plan view of an "H" sorting structure.

[0053] Fig. 17 is a plan view of a "Y" shaped sorting structure.

[0054] Fig. 18 is a plan view of a "X" channel sorting structure.

[0055] Fig. 19 is a perspective view of a two-dimensional sorting structure.

[0056] Fig. 20 is a plan view of a multi-dimensional sorting structure.

[0057] Fig. 21 is a side view of a multi-dimensional sorting structure including a reflective surface for generation of the optical gradient pattern.

[0058] Fig. 22 is a side view of a sorting structure including a capture structure.

[0059] Fig. 23 is a plan view of a microfluidic system including a recycle path.

[0060] Fig. 24 is a plan view of a particle analysis system utilizing particle deformability as a factor in the selection or characterization.

[0061] Fig. 25 is a plan view of a sorting or characterization system utilizing the particle size relative to the optical gradient periodicity as a factor.

[0062] Fig. 26 is a system for separation of particles utilizing the scattering force of light for separation.

[0063] Fig. 27A is a perspective drawing of a scattering force switch.

[0064] Fig. 27B is a plan, side view of a scattering force switch.

[0065] Fig. 27C is a plan, side view of a scattering force switch with the beam on.

[0066] Fig. 28 is a schematic drawing of a system for determining the dielectric constant of particles in various fluidic media of varying dielectric constant.

[0067] Fig. 29 is a cross-sectional drawing of particles and a light intensity profile for separating particles in a dielectric medium.

[0068] Fig. 30 is a perspective view of a optical tweezer array.

[0069] Fig. 31 is a graph of molar extinction coefficient as a function of wavelength for hemoglobin-O_2 absorption spectrum.

[0070] Fig. 32 shows time lapse photographs of an experiment separating particles by size with a moving optical gradient field.

[0071] Fig. 33 shows time lapse photographs of an experiment separating particles by surface functionalization.

[0072] Fig. 34 shows a Before, After and Difference photograph of particles subject to a moving optical gradient field.

[0073] Fig. 35 is a graph of percent of cells measured in an experiment versus escape velocity, for a variety of cell types.

[0074] Fig. 36 shows photographs of sorting of two cell types in a microchannel device. 1 shows a red blood cell and a white blood cell successively entering the moving optical gradient field. 2 shows that white blood cell has been translated down by the action of the moving optical gradient field while the red blood cell has escaped translation. 3 and 4 show that the red blood cell and white blood cell continue to flow into separate channels, completing the sorting.

Detailed Description of the Invention

Definitions

[0075] The following definitions are provided for an understanding of the invention disclosed herein.

[0076] "Dielectric constant" is defined to be that property which determines the electrostatic energy stored per unit volume for unit potential gradient. (See, e.g., the New IEEE Standard Dictionary Of Electrical And Electronics Terms, ©1993).

[0077] The "optical dielectric constant" is the dielectric constant of a particle or thing at optical wavelengths. Generally, the optical wavelength range is from 150 Å to 30,000 Å.

[0078] An "optical gradient field" is an optical pattern having a variation in one or more parameters including intensity, wavelength or frequency, phase, polarization or other

parameters relating to the optical energy. When generated by an interferometer, an optical gradient field or pattern may also be called an optical fringe field or fringe pattern, or variants thereof.

[0079] A "moving optical gradient field" is an optical gradient field that moves in space and/or time relative to other components of the system, e.g., particles or objects to be identified, characterized, selected and/or sorted, the medium, typically a fluidic medium, in contact with the particles, and/or any containment or support structure.

[0080] An "optical scattering force" is that force applied to a particle or thing caused by a momentum transfer from photons to material irradiated with optical energy.

[0081] An "optical gradient force" is one which causes a particle or object to be subject to a force based upon a difference in dielectric constant between the particle and the medium in which it is located.

[0082] "Optophoresis" or "Optophoretic" generally relates to the use of photonic or light energy to obtain information about or spatially move or otherwise usefully interact with a particle.

[0083] "Optophoretic constant" or "optophoretic signature" or "optophoretic fingerprint" refer to the parameter or parameters which distinguish or characterize particles for optical selection, identification, characterization or sorting.

[0084] An "optical tweezer" is a light based system having a highly focused beam to a point in space of sufficiently high intensity that the gradient force tends to pull a dielectric particle toward the point of highest intensity, typically with the gradient force being sufficiently strong to overcome the scattering force. Most typically, the laser beam is directed through a microscope objective with a high numerical aperture, with the beam having a diffraction limited spot size of approximately the wavelength of the light, 5,000 to 20,000 Å, though more typically 10,000 Å. Generally, an optical tweezer has a beam width in the focal plane of 2 μm or less, and typically about 1 μm.

[0085] "Separation" of two objects is the relative spatial distancing over time of a particle from some other reference point or thing.

[0086] "Sorting" involves the separation of two or more particles in a meaningful way.

DESCRIPTION OF EXEMPLARY APPARATUS

Optical components -- Generation of moving optical gradient field.

[0087] Figs. 2 - 10 describe various systems for generation of optical patterns, sometimes termed fringe patterns or optical fringe patterns, including, but not limited to, a moving optical gradient field pattern. These exemplary embodiments are intended to be illustrative, and not limiting, as other apparatus may be utilized to generate the optical fields and forces to achieve the desirable results of these inventions.

[0088] The points raised in discussions of specific embodiments may be considered to be generally applicable to descriptions of the other embodiments, even if not expressly stated to be applicable.

[0089] The light source for use with systems has certain generally desirable properties. As to wavelength, the wavelength will generally be chosen based upon one or more considerations. In certain applications, it may be desirable to avoid damage to biological materials, such as cells. By choosing wavelengths in ranges where the absorption by cellular components, mostly water, are minimized, the deleterious effects of heating may be minimized. Wavelengths in the range from approximately 0.3 μm to approximately 1.8 μm, and more preferably, from substantially 0.8 to substantially 1.8 μm, aid in reducing biological damage. However, even for biological applications, a laser having a wavelength generally considered to be damaging to biological materials may be used, such as where the illumination is for a short period of time where deleterious absorption of energy does not occur. In yet other applications, it may be desirable to choose a wavelength based upon a property of the particle or object under consideration. For example, it may be desirable to choose the wavelength to be at or near an absorption band in order to increase (or decrease) the force applied against a particle having a particular attribute. Yet another consideration for wavelength choice may be compatibility with existing technology, or a wavelength naturally generated by a source. One example would be the choice of the wavelength at 1.55 μm. Numerous devices in the 1.55 μm wavelength region exist commercially and are used extensively for telecommunications applications.

[0090] Generally, the light sources will be coherent light sources. Most typically, the coherent light source will consist of a laser. However, non-coherent sources may be utilized, provided the system can generate the forces required to achieve the desired results. Various laser modes may be utilized, such as the Laguerre-Gaussian mode of the

laser. Furthermore, if there is more than one light source in the system, these sources can be coherent or incoherent with respect to each other.

[0091] The spot size or periodicity of the intensity pattern is preferably chosen to optimize the effective results of the illumination. In many applications, it is desirable to have a substantially uniform gradient over the particle, e.g., cell, to be interrogated such that the dielectric properties of the entire particle (cell) contribute to the resulting force. Broadly, the range varies from substantially 1 to substantially 8 times the size (diameter or average size) of the particle or object, more preferably, the range is from substantially 2 to substantially 4 times the size. Various methods and systems known to those skilled in the art may be utilized to achieve the desired spot size or periodicity, e.g., using a defocused beam or a collimated beam having the desired size. The typical characterization of the radius of the spot is the $1/e^2$ radius of the beam intensity. For many applications, including cellular applications, the beam size will be on the order of 10 microns, though sometimes as small as five microns, and in even certain other occasions, as small as two microns. In certain applications, it is desirable to have the periodicity of the illumination in the range from substantially 1 to substantially 2 times the size (diameter or average size) of the particle or object. For many biological applications, a periodicity of from substantially 5 μm to 25 μm, and more preferably from 10 μm to 20 μm. Certain applications may utilize smaller sizes, e.g., for bacteria, or larger sizes, e.g., for larger particles. In yet other applications, it may be desired to utilize a spot size smaller than the particle or object, such as where interrogation of a sub-cellular region is desired.

[0092] The examples of systems for generating intensity patterns, described below, as well as other systems for generating intensity patterns useful for the subject inventions include various optical components, as well as a control system to generate the desired pattern, intensity profile or other gradient, such as a moving optical field gradient. Various optical systems may be adapted for use in the systems of the invention, so as to effectively carry out the methods and achieve the results described herein. Exemplary systems which may be adapted in whole or in part include: Young's slits, Michelson interferometer, Mach-Zender interferometer, Haidinger circular fringe systems, Fresnel mirror interferometer, plane-parallel plate interferometer, Fabry-Perot interferometer and any other system for generating an optical gradient intensity pattern or fringe pattern.

[0093] Turning now to a detailed description of exemplary systems for use with the subject inventions. Fig. 2 shows an optical component description of a system 20 generally

configured to generate a moving optical gradient field pattern to provide a force on one or more particles provided to the system 20. The optical forces may then be used for characterization, identification, selection and/or sorting of the particles. A light source 22, preferably a laser, generates a first beam 24 directed toward beam splitter 26. Beam splitter 26 may be of any mode or type known to the art, such as a prism beam splitter, consistent with the goals and objects of this invention. A first transmitted beam 28 passes through the beam splitter 26. A first reflected beam 30 reflects from the beam splitter 26 to a reflective surface 32, typically a mirror, to generate a second reflected beam 34. The first transmitted beam 28 and second reflected beam 34 interfere and generate an intensity pattern 38, generally being located at the operative portion of the slide or support 36 where the light would interact with the particle or object of interest. The optical pattern 38 moves relative to other objects, e.g., the particles, the substrate, and/or the fluidic medium containing the particles, by virtue of a change in the optical path length between the first transmitted beam 28 and the combination of the first reflected beam 30 and second reflected beam 34. Mirror 32 is movable, by actuator 40. One example of an actuator 40 could comprise a motor and screw system to move mirror 32. Numerous alternative structures for moving mirror 32 are known to the art, e.g., piezoelectric systems, oscillating mirror systems and the like.

[0094] Fig. 3 shows a two-beam interference based system. A source of coherent light, such as laser 52, generates a first beam 54 directed to a beam splitter 56. A first reflected beam 58 is directed toward the sample plate 70 and a first transmitted beam 60 is directed to a modulator, such as a phase modulator 62. The phase modulator 62 may be of any type known to those skilled in the art. Phase modulator 62 is under control of the control system 64 and results in modulated beam output 66 which is directed to a mirror 74. The modulated beam 66 reflects from mirror 74 to generate the second reflected beam 68 which is directed to the sample plate 70. The first reflected beam 54 and second reflected beam 68 generate a pattern 72 at the operative interface with the sample plate 70. The control system 64 is connected to the phase modulator 62 so as to cause the pattern 72 to move relative to the objects within the system 50, such as the sample plate 70.

[0095] Fig. 4 shows an optical component diagram of an interferometer system 80. A light source, such as laser 82, generates a first light beam 84 directed to beam splitter 86. An interferometer composed of the first mirror 88 and second mirror 90 generate an output beam 100 having the desired beam properties, including the desired gradient properties.

The first beam 84 passes through beam splitter 86 to generate a first transmitted beam 94 directed to first mirror 88. The reflected beam retraces path 94 to the beam splitter 86. The first reflected beam 96 passes through phase modulator 92 to generate first modulated beam 98 directed to the second mirror 90. The reflected beam from second mirror 90 retraces the path 98 through the phase modulator 92 and beam 96 to the beam splitter 86. The beam 100 is output from the interferometer section of the system 80 and directed toward the microscope objective 104.

[0096] The objective 104 is directed toward the sample plate 106. Optionally, a mirror 108, most preferably a planar mirror, may be disposed beneath the sample plate 106. The mirror 108 is oriented so as to provide reflected light onto the sample plate 106 bearing or containing the particles or objects under analysis or action of the system 80. The scattering force caused by the beam 102 as initially illuminates the sample plate 106 may be counteracted, in whole or in part, by directing the reflected radiation from mirror 108 back toward the sample. As discussed more in the section relating to surface effects, below, the reflected light and the upward scattering force reduce the overall effects of the scattering forces, such that the gradient forces may be more effectively utilized.

[0097] Fig. 4 includes an optional imaging system. The light 102 from the objective 104 is reflected by the beam splitter 120 generating third reflected beam 110 which is directed toward imaging optics 112. The optics 112 image the light on a detector 114, such as a charge couple device (CCD) detector. The output of the detector 114 may be provided to an imaging system 116. The imaging system 116 may optionally include a display, such as a monitor (CRT, flat panel display, plasma display, liquid crystal display, or other displays known to those skilled in the art). The imaging system 116 may optionally include image enhancement software and image analysis software, recording capability (to tape, to optical memory, or to any other form of memory known to those skilled in the art).

[0098] A control system 118 controls the modulator 92 so as to generate the desired optical force pattern within the system 80. Optionally, the imaging system 116 may be coupled to the control system 118. A feedback system may be created whereby the action of the particles on the sample plate 106 may be imaged through the system 116 and then utilized in the control system analysis to control the operation of the overall system 80.

[0099] Fig. 5 shows a interferometer based system 120. A light source, such as laser 122, generates a first beam 124 directed toward an optional spatial filter 126. The spatial filter 126 would typically include lenses 128 and a spatial filter aperture 130. The aperture

typically is round. The spatial filters serves to collimate the laser beam and to produce a smooth intensity profile across the wavefront of the laser beam. The interferometer 140 includes first mirror 146 and second mirror 144, as well a beam splitter 142. The phase modulator 148 is disposed within one of the two arms of the interferometer 140.

[00100] As shown in Fig. 5, a mirror 132 is optionally disposed to reflect the light from the source 122 to the interferometer 140. As will be appreciated by those skilled in the art, optical systems may include any number or manner of components designed to transfer or direct light throughout the system. One such example is the planar mirror 132 which merely serves to direct the radiation from one major component, e.g., the spatial filter, to another major component, e.g., the interferometer 140. In addition to mirrors, other common transfer components may include fiber optics, lenses, beam splitters, diffusers, prisms, filters, and shaped mirrors.

[00101] Beam 150 exits the interferometer 140 and is directed toward objective 152 and imaged at or near the sample plate 154. As shown, a dichroic mirror 170 serves to reflect the light 150, but to also permit passage of light from source 168, such as a fiber providing radiation from a source through the dichroic mirror 170 and objective 152 to illuminate the operative regions of the sample plate 154.

[00102] Optionally, a detection system may be disposed to image the operative portions of the sample plate 154. As shown, objective 156 is disposed beneath the sample plate 154, with the output radiation being transferred via mirror 158 to an imaging apparatus 164, such as a charge couple device (CCD). Optionally, an infrared filter 160 may be disposed within the optical path in order to select the desired wavelengths for detection. The output of the detector 164 is provided to an imaging system 166. As described in connection with other figures, the imaging system 166 may include image enhancement and image analysis software and provide various modes of display to be user. Optionally, the imaging system 166 is coupled to the control system 172 such as when used for feedback.

[00103] Fig. 6 shows an optical system having illumination of a sample plate 194 from the top side and imaging from the bottom side. A laser 180 generates a first beam 182 which optionally passes through a spatial filter 184. The spatial filter as shown includes lens 184 and aperture 188. The output of the spatial filter 184 passes through the objective 192 and is imaged onto the sample plate 194. The sample plate 194 and material supported on it may be imaged via an objective 196. An optional mirror 198 directs

radiation to an optional filter 200 through an imaging lens 202 onto the detector 204. The detector 204 is coupled to an imaging system 206. Preferably, the imaging system 206 provides information to a control system 208 which controls various optical components of the system.

[00104] Fig. 7 shows an optical system interfacing a sample plate which includes bounded structures. The system 210 includes a sample plate 212 which optionally includes microfluidic channels. Alternatively, the sample plate 212 may support a separate structure containing the microfluidic channels. As one exemplary structure formed from the microfluidic channels, a "T" sorting arrangement is shown for a simple, though useful, example. An input reservoir 216 connects to a first channel 218 which terminates in a T at intersection 220. A first output channel 222 couples to a first output reservoir 224. A second output channel 226 couples to a second output chamber 228. As shown, the input chamber is coupled to ground and the first output chamber 224 and second output chamber 228 are connected to -V. The fluidic channel structures are discussed in more detail, below.

[00105] The microscope objective 232 serves to both provide the optical radiation to the sample plate 222 as well as to provide the imaging of the system. A light source 238, such as a laser, or more particularly, a laser diode, generates light which may be imaged by optics 240. A dichroic beam splitter 236 directs the radiation to the microscope objective 232. As shown, the objective has a magnification power of 100. For the biological applications, a magnification range of from 1 to 200 is desired, and more preferably, from 10 to 100. The objective 232 has a 1.25 numerical aperture. The preferable range of numerical apertures for the lenses is from 0.1 to 1.50, and more preferably from 0.4 to 1.25. The output from the objective 232 passes through the beam splitter 236, reflects from optional mirror 242 through optics (e.g., lens) 244, through the optional filter 246 to the imaging device 280. The imaging device, shown as a CCD, is connected to the imaging system 282. The output of the imaging system 282 is optionally coupled to the control system 284. As shown, the control system 284 controls both the translation stage 232 connected to the sample plate 212, as well as to the light source 238.

[00106] Fig. 8 shows a system for generating an intensity pattern within the scanned area 260. An input beam 262, such as from a coherent light source, such as a laser, is directed toward the system. A first oscillating component 264, such as a galvanometer or resonant scanner, intercepts the input beam 262 and provides a first degree of motion to

the beam. The beam is directed to a polygonal mirror 268 which contains multiple faces 270. As the polygonal mirror 268 rotates around axis 272, the light is swept across the scanner area 260. Lens 274 are provided as required to appropriately image the light into the scanned area 260. Optionally, a mask or other pattern 276 may be disposed within the optical pathway so as to provide for the variation of the optical forces within the scanned area 260. Any of a wide variety of techniques for generating either the oscillatory motion or the scanning via the polygonal mirror are known to those skilled in the art.

[00107] Fig. 9 shows a system utilizing masks to generate an optical force pattern. A source 280, such as a laser, generates a beam 282 directed to toward a mask 284. Optionally, a phase modulator 290 may be disposed between the source 280 and the mask 284. Optionally, the mask 284 may be moved, such as by actuator 286, which may be a motor, piezoelectric driven system, microelectromechanical (MEMs), or other driving structures known to those skilled in the art. The optical mask 284 creates a desired light intensity pattern adjacent the sample plate 288. The optical mask 284 may modulate any or all of the components of the light passing there through, include, but not limited to, intensity, phase and polarization. The mask 284 may be a holographic mask which, if used, may not necessarily require coherent light. Other forms of masks, such as spatial light modulators may be utilized to generate variations in optical parameters.

[00108] Yet another mirror arrangement consists of utilizing a micromirror arrangement. One such micromirror structure consists of an array of mirrors, such as utilized in the Texas Instrument Digital Micromirror product.

[00109] Fig. 10 shows an alternate system for illumination in which multiple sources 290 are directed toward the sample plate or surface 294. Each source 290 is controlled by control system 296, with the various outputs 292 from the sources 290 illuminating the surface of the support 294.

[00110] Arrays of sources 290 may be fabricated in many ways. One preferable structure is a vertical cavity surface emitting laser (VCSEL) array. VCSEL arrays are known to those skilled in the art and serve to generate optical patterns with control of the various lasers comprising the VCSELs. Similarly, laser diode bars provide an array of sources. Alternatively, separate light sources may be coupled, such as through fiber optic coupling, to a region directed toward the surface 294.

[00111] The imaging system may serve function beyond the mirror imaging of the system. In addition to monitoring the intensity, size and shape of the optical fringes, it may be used for purposes such as calibration.

OPTICAL FORCES

[00112] The apparatus and methods of the instant inventions utilize, at least in part, forces on particles caused by light. In certain embodiments, a light pattern is moved relative to another physical structure, the particle or object, the medium containing the particle or object and/or the structure supporting the particle or object and the medium. Often times, a moving optical pattern, such as moving optical gradient field moves relative to the particles. By moving the light relative to particles, typically through a medium having some degree of viscosity, particles are separated or otherwise characterized based at least in part upon the optical force asserted against the particle. While most of the description describes the light moving relative to other structures, it will be appreciated that the relative motion may be achieved otherwise, such as by holding the light pattern stationary and moving the subject particle, medium and/or support structure relative to the optical pattern.

[00113] Figs. 11A, 11B and 11C depict, respectively, the optical intensity profile, the corresponding optical force on a particle or cell and the corresponding potential energy of the particle in the optical intensity profile as a function of distance (x). Fig. 11A shows the intensity profile generated and applied against one or more particles. As shown, the intensity varies in a undulating or oscillating manner. The intensity, as shown, shows a uniform periodicity and symmetric waves. However, the intensity variations may be symmetric or asymmetric, or of any desired shape. The period may be fixed or may be variable. Fig. 11B shows the absolute value of the force as a function of position. The force is the spatial derivative of the intensity. Fig. 11C shows the potential energy as a function of position. The potential energy is the integrated force through a distance.

[00114] The profiles of Figs. 11A -11C are shown to be generally sinusoidal. Generally, such a pattern would result from interference fringes. Differing profiles (of intensity, force and potential energy) may be desired. For example, it may be desirable to have a system where the potential energy well is relatively flat at the bottom and has steeper sides, or is asymmetric in its form.

[00115] Figs. 12A and 12B show two particles, labeled "A" and "B". in Fig. 12A, the particles are shown being illuminated by a two-dimensional intensity pattern 300. Fig.

12B shows the position of particles A and B at a later moment of time, after the intensity pattern has moved to position 302. In this example, the optical force has caused particle B to move relative to its prior position. Since the effect of the optical pattern 300 on particle A was less than on particle B, the relative positions of particles A and B are different in Fig. 12B as compared to Fig. 12A.

[00116] In one implementation of the system, the position of particles A and B in Fig. 12A would be determined. The system would then be illuminated with the desired gradient field, preferably a moving optical gradient field, and the system then imaged at a later point in time, such as shown in Fig. 12B. The absence of motion, or the presence of motion (amount of motion, direction of motion, speed of motion, etc.) may be utilized to characterize, or analyze the particle or particles. In certain applications, it may be sufficient to determine the response of a single particle to a particular optical pattern. Thus, information may be derived about the particle merely from the fact that the particle moved, or moved in a particular way or by a particular amount. That information may be obtained irrespective of the presence or absence of other particles. In yet other applications, it is desirable to separate two or more particles. In that case, by comparing the position of the particles relative to each other such as in Fig. 12A versus 12B, information regarding the particle may be obtained. Having determined which particle is the desired particle, assume for purposes of discussion to be particle B, the particle may then be separated from the other particles. As shown in Fig. 12C, an optical tweezer intensity profile 304 may be used to capture and remove particle B. Alternatively, as will be discussed in connection with Figs. 14 - 19, the selected particle may be removed by other means, such as by fluidic means.

[00117] By utilizing a property of the particle, such as the optical dielectric constant, the light forces serve to identify, select, characterize and/or sort particles having differences in those attributes. Exposure of one or more particles to the optical force may provide information regarding the status of that particle. No separation of that particle from any other particle or structure may be required. In yet other applications, the application of the optical force causes a separation of particles based upon characteristics, such that the separation between the particles may result in yet further separation. The modes of further separation may be of any various forms, such as fluidic separation, mechanical separation, such as through the use of mechanical devices or other capture structures, or optically, such as through the use of an optical tweezer as shown in Fig. 12C, by application of a

moving optical gradient, or by any other mode of removing or separating the particle, e.g., electromagnetic, fluidic or mechanical.

[00118] Figs. 13A, 13B and 13C show potential energy as a function of distance for one exemplary mode of operation. The figures show particle 1 and particle 2 displaced in the x dimension relative to one another. The physical positioning of the two particles would typically be in the same plane, e.g., the same vertical plane. The figures show the potential energy of the particle. In Fig. 13A, particle 1 310 is subject to light intensity pattern creating the potential energy profile 314. Particle 2 312 is subject to the same light intensity pattern but is subject to the second potential energy profile 316. The second potential energy profile 316 is different from the first potential energy profile 314 because the dielectric constants are different between particle 1 310 and particle 2 312. In Fig. 5A, the light intensity pattern is moving toward the right. As the potential energy profiles 314, 316 move to the right, the particles 310, 312 experience different forces. Particle 1 310 will experience a smaller force as compared to particle 2 312, as depicted by the size of the arrows adjacent the particles. The force experienced by the particles is proportional to the spatial derivative of the potential energy. Thus, particle 2 312 being on a relatively "steeper" portion of the potential energy "wave" would be subject to a larger force. In Fig. 5A, the translation speed of the potential energy waves may be set to be larger than the speed at which particle 1 310 may move forward through the medium in which it is located. In that event, particle 1 310 may be subject to a force toward the left, Fig. 13A showing an arrow depicting the possible backward or retrograde motion of particle 1 310. The potential energy wells have a minimum 318 into which the particles would settle, absent motion or translation of the potential energy patterns 314, 316.

[00119] Fig. 13B shows particle 1 310 and particle 2 312 subject to the first potential energy 314 and second potential energy 316, respectively. As the potential energy patterns 314, 316 translate to the right, the particles 310, 312 are subject to a force to the right, though in different amounts as depicted by the relative size of the arrows. Fig. 13C shows the potential energy profiles 314, 316 after the potential energy profiles of Fig. 13B have been moved so as to place the potential energy maximum between particle 1 310 and particle 2 312. By "jerking" the intensity profiles 314, 316 forward quickly, particle 1 310 is then located on the "backside" of the potential energy "wave", and would be subject to a force to the left. The path of motion is then shown by the dashed arrow from particle 1 310. In contrast, particle 2 312 remains on the "front side" of the potential energy wave

316 and is subject to a force to the right. The effect of this arrangement is to cause further physical separation between particle 1 310 and particle 2 314. The potential energy profiles 314, 316 must be moved forward quickly enough such that the potential energy maximum is located between the particles to be separated, as well as to insure that the particle on the "backside" of the potential energy wave is caused to move away from the particle on the "front side" of the wave.

[00120] The apparatus and methods of these inventions utilize optical forces, either alone or in combination with additional forces, to characterize, identify, select and/or sort material based upon different properties or attributes of the particles. The optical profiles may be static, though vary with position, or dynamic. When dynamic, both the gradient fields as well as the scattering forces may be made to move relative to the particle, medium containing the particle, the support structure containing the particle and the medium. When using a moving optical gradient field, the motion may be at a constant velocity (speed and direction), or may vary in a linear or non-linear manner.

[00121] The optical forces may be used in conjunction with other forces. Generally, the optical forces do not interfere or conflict with the other forces. The additional forces may be magnetic forces, such as static magnetic forces as generated by a permanent magnet, or dynamic magnetic forces. Additional electric forces may be static, such as electrostatic forces, or may be dynamic, such as when subject to alternating electric fields. The various frequency ranges of alternating electromagnetic fields are generally termed as follows: DC is frequencies much less than 1 Hz, audio frequencies are from 1 Hz to 50 kHz, radio frequencies are from 50 kHz to 2 GHz, microwave frequencies are from 1 GHz to 200 GHz, infrared (IR) is from 20 GHz to 400 THz, visible is from 400 THz to 800 THz, ultraviolet (UV) is from 800THz to 50 PHz, x-ray is from 5PHz to 20 EHz and gamma rays are from 5 EHz and higher (see, e.g., *Physics Vade Mecum*). .) The frequency ranges overlap, and the boundaries are sometimes defined slightly differently, but the ranges are always substantially the same. Dielectrophoretic forces are generated by alternating fields generally being in the single Hz to 10 MHz range. For the sake of completeness, we note that dielectrophoretic forces are more electrostatic in nature, whereas optophoretic forces are electromagnetic in nature (that is, comparing the frequency ranges is not meant to imply that they differ only in their frequency.) Gravitational forces may be used in conjunction with optical forces. By configuring the orientation of the apparatus, the forces of gravity may be used to affect the actions of the particle. For example, a channel may be

disposed in a vertical direction so as to provide a downward force on a particle, such as where an optical force in the upward direction has been generated. The force of gravity takes into consideration the buoyancy of the particle. When a channel is disposed in the horizontal direction, other forces, e.g., frictional forces, may be present. Fluidic forces (or Fluidics) may be advantageously utilized with optical forces. By utilizing an optical force to effect initial particle separation, a fluidic force may be utilized as the mechanism for further separating the particles. As yet another additional force, other optical forces may be applied against the particle. Any or all of the aforementioned additional forces may be used singly or in combination. Additionally, the forces may be utilized serially or may be applied simultaneously.

[00122] Figs. 14A and 14B show sorting of particles or objects from a one-dimensional source. As shown in Fig. 14A, particles 320 progress in a generally downward direction from a source in the direction of the arrow labeled particle flow. At junction 322, and possibly additionally before the junction 322, the particles are subject to an optical separation force. Those particles having a different response property, such as a different dielectric constant, may be separated from the line of particles resulting in the separated particles 326. Those particles which are not separated continue on as the particles 324. Fig. 14B shows optical cell sorting from a one-dimensional source. Cells 330 move in a fluid flow in a direction from top to bottom as shown by the arrow. The cells 330 are subject to an optical force in the region of junction 332. Selected cells 336 are deviated from the path of the original fluid flow. The remaining particles 334 continue on in the same direction as the original fluid flow. It will be appreciated that the term "selected" or "non-selected" or similar terminology as used herein is meant to be illustrative, and not intended to be limiting.

[00123] The techniques of this invention may be utilized in a non-guided, i.e., homogeneous, environment, or in a guided environment. A guided environment may optionally include structures such as channels, including microchannels, reservoirs, switches, disposal regions or other vesicles. The surfaces of the systems may be uniform, or may be heterogeneous.

[00124] Fig. 15 shows a plan view of a guided structure including channels. An input channel 340 receives particles 342 contained within a medium. An optical force is applied in region 344. The optical force would preferably be a moving optical gradient field. As the particles 342 move through the field 344, certain particles would be subject to a force

causing them to move to the right in the channel as shown as particles 346, yet other particles 348 would move to the left of the T channel. By selection of the speed, orientation, periodicity, intensity and other parameters of the optical force gradient, the particles may be effectively separated.

[00125] The channels may be formed in a substrate or built upon some support or substrate. Generally, the depth of the channel would be on the order of from substantially 1 to substantially 2 diameters of the particle. For many biological cell sorting or characterization applications, the depth would be on the order of 10 to 20 μm. The width of the channels generally would be on the order of from substantially 2 to substantially 8 diameters of the particle, to allow for at least one optical gradient maximum with a width of the order of the particle diameter up to four or more optical gradient maxima with a width of the order of the particle diameter. For many biological cell sorting or characterization applications, the width would be of the order of 20 to 160 micrometers. The channels may have varying shapes, such as a rectangular channel structure with vertical walls, a V-shaped structure with intersecting non-planar walls, a curved structure, such as a semicircular or elliptical shaped channel. The channels, or the substrate or base when the channel was formed within it, may be made of various materials. For example, polymers, such as silicon elastomers (e.g., PDMS), gels (e.g., Agarose gels) and plastics (e.g., TMMA) may be utilized: glass, and silica are other materials. For certain applications, it may be desirable to have the support material be optically transparent. The surfaces may be charged or uncharged. The surface should have properties which are compatible with the materials to be placed in contact therewith. For example, surfaces having biological compatibility should be used for biological arrays or other operations.

[00126] Various forms of motive force may be used to cause the particles, typically included within a fluid, to move within the system. Electroosmotic forces may be utilized. As known in the art, various coatings of the walls or channels may be utilized to enhance or suppress the electroosmotic effect. Electrophoresis may be used to transport materials through the system. Pumping systems may be utilized such as where a pressure differential is impressed across the inlet and outlet of the system. Capillary action may be utilized to cause materials to move through the system. Gravity feeding may be utilized. Finally, mechanical systems such as rotors, micropumps, centrifugation may be utilized.

[00127] Fig. 16 shows an "H" channel structure for sorting of particles. The H-shaped structure has two inlets and two outlets. The inlet 350 receives both fluid and the subject

particles 352 to be sorted. Fluid is input in the second input arm of the H channel. The main or connecting channel 356 receives the fluid flow from both inputs. In the connecting channel 356, the particles 354 will flow through the connecting channel and be subject to the optical sorting force 358. At that stage, the particles are then separated based upon the differentiating parameter, such as the particle's dielectric constant. The particles being moved from the primary stream move as particles 360 to one output. The particles 362 which are not diverted by action of the optical force 358 continue to the left hand outlet 364. Laminar flow within the system will cause the particles 354 to move through the main channel 356, and if the channel width is large enough, will tend to cause the particles 354 to flow relatively closer to the wall nearer the input. The sorting process then consists of diverting the particle from the laminar flow adjacent the left wall to the laminar flow which will divert to the right hand output.

[00128] Fig. 17 shows a wide channel structure for particle separation. Input 370 receives the particles 372 in a fluidic medium. The particles are subject to an optical sorting force 374, whereupon the diverted particles 378 flow toward outlet 382 and particles 376 flow toward outlet 380.

[00129] Fig. 18 shows an X-channel structure for sorting. Input 390 receives particles 392 in a fluidic medium. Second input 394 received fluid. The particles 392 are then subject to an optical sorting force 396. Diverted particles 402 flow to exit 404. Particles 398 flow to exit 400.

[00130] Fig. 19 is a perspective drawing of a two-dimensional sorting system. The source inflow of cells 410 intersect with an optical sorting force along line 412. The sorting force 412 results in an outflow of target cells 414 in one-dimension, typically in one plane, and an outflow of non-target cells 416 in another plane. The plane of outflow of targets cells 414 is non-coplanar with the plane of outflow of non-target cells 416.

[00131] Fig. 20 shows an arrangement comprising a three-dimensional cell sorting arrangement. A volume 420, most preferably a substantially three-dimensional volume, though possibly a volume of lower effective dimensionality, contains particles 422. An optical force gradient 428 is generated within the volume 420 to effect particle sorting. One embodiment for generating the optical field gradient 428 is to interfere first beam 424 with a second beam 426. The first beam 424 and second beam 426 interfere and generate the force pattern 428. As shown, a first particle 430 is subject to a force in a direction from bottom to top, whereas a second particle 432 is subject to a force from top to bottom.

Alternately, the optical pattern 428 may cause forces on particles 430, 432 in the same direction, but with differing amounts of force.

[00132] Fig. 21 shows an embodiment having multiple degrees of freedom, preferably three degrees of freedom. The volume 440 contains particles 442 which are disposed adjacent a surface, near the inwardly disposed surface of mirror 450. An optical gradient force 444 is generated which causes selected ones of the particles 446 at the surface to be moved into the volume 440 such as particle 446. The optical force gradient 444 may be generated by shining an optical beam 448 onto a mirror 450, which causes interference between the beam 448 and its reflected beam.

[00133] Fig. 22 shows a multi-dimensional system in which a volume 450 is utilized to separate particles. First particles 452 are disposed adjacent the surface of the slide 454. A light intensity pattern 456 causes displacement of selected particles. Those displaced particles may then be attached to a sticky or adhesive mat 460 and comprises particles 458.

[00134] Fig. 23 shows a plan view of a complex channel based system for sorting, characterization or classification. An input 470 leads through channel 472 to a first optical sorting region 474. The sorting at a given channel is as described, before. The output of the sorting results in a first set of particles 478 and a second set of particles 476. The first set of particles 478 flows to the second optical sorting region 480. As before, the particles are sorted into first particles 484 and second particles 482. A next optical sorting region 486 results in the output of sorted particles, the first output 488 and second output 490 then leading to further collection, counting or analysis. In one aspect, the complex system may include one or more recycle or feedback tabs 490. As shown, the output from the optical force region 492 includes output 7 but also a recycle path 494 leading to the input 496 coupling to the channel 472. Such a recycle system might be used in an enrichment system.

[00135] The systems described herein, and especially a more complex system, may include various additional structures and functionalities. For example, sensors, such as cell sensors, may be located adjacent various channels, e.g., channel 742. Various types of sensors are known to those skilled in the art, including capacitive sensors, optical sensors and electrical sensors. Complex systems may further include various holding vessels or vesicles, being used for source materials or collection materials, or as an intermediate holding reservoir. Complex systems may further include amplification systems. For

example, a PCR amplification system may be utilized within the system. Other linear or exponential biological amplification methods known to those skilled in the art may be integrated. Complex systems may further include assays or other detection schemes. Counters may be integrated within the system. For example, a counter may be disposed adjacent an output to tally the number of particles or cells flowing through the output. The systems of the instant invention are useable with microelectromechanical (MEMs) technology. MEMs systems provide for microsized electrical and mechanical devices, such as for actuation of switches, pumps or other electrical or mechanical devices. The system may optionally include various containment structures, such as flow cells or cover slips over microchannels.

[00136] A computerized workstation may include a miniaturized sample station with active fluidics, an optical platform containing a laser (e.g., a near infrared laser for biological applications) and necessary system hardware for data analysis and interpretation. The system may include real-time analysis and testing under full computer control.

[00137] The inventions herein may be used alone, or with other methods of cell separation. Current methods for cell separation and analysis include flow cytometry, density gradients, antibody panning, magnetic activated cell sorting ("MACS™"), microscopy, dielectrophoresis and various physiological and biochemical assays. MACS separations work only with small cell populations and do not achieve the purity of flow cytometry. Flow cytometry, otherwise known as Fluorescent Activated Cell Sorting ("FACS™") requires labeling.

[00138] In yet another aspect, the systems of the present invention may optionally include sample preparation steps and structure for performing them. For example, sample preparation may include a preliminary step of obtaining uniform size, e.g., radius, particles for subsequent optical sorting.

[00139] The systems may optionally include disposable components. For example, the channel structures described may be formed in separable, disposable plates. The disposable component would be adapted for use in a larger system that would typically include control electronics, optical components and the control system. The fluidic system may be included in part in the disposable component, as well as in the non-disposable system components.

[00140] Fig. 24 shows a system for optical sorting based upon a physical parameter of the object, such as deformability. An optical gradient 500 may illuminate particles 502, 504. Particle 504 is more deformable than particle 502. As a result, given the periodicity of the optical force pattern 500, the deformable particle 504 may be subject to a relatively larger force, and move more under the optical field 500. Preferably, the optical field 500 is a moving optical gradient field. Alternatively, the particles 502, 504 may be subject to the optical force 500, and the structure of the particles 502, 504 monitored. In that way, by observing the deformability of the particles, relative to the light pattern 500, the particles may be identified, classified or otherwise sorted.

[00141] Fig. 25 shows a method for sorting particles based upon size. An optical intensity pattern 510 illuminates larger particle 512 and smaller particle 514. The differently sized particles 512, 514 are subject to different forces. Where, for example, larger particle 512 spans two or more intensity peaks of the optical gradient 510, the particle may have no net force applied to it. In contrast, the smaller particle 514 which has a size smaller than the period of the optical intensity pattern 510 may be subject to a relatively larger force. By selection of the period of the optical pattern 510 relative to the size of particles to be sorted, the system may effectively sort based upon size. In one method, a set of particles may be subject to an increasing period of the light intensity, such that smaller particles are removed first, followed by the relatively larger particles at a later time. In this way, particles may be effectively sorted by size.

Methods for Reducing or Modifying Forces

[00142] The system and methods may include various techniques for reducing or otherwise modifying forces. Certain forces may be desirable in certain applications, but undesirable in other applications. By selecting the technique to reduce or minimize the undesired forces, the desired forces may more efficiently, sensitively and specifically sort or identify the desired particles or conditions. Brownian motion of particles may be an undesired condition for certain applications. Cooling of the system may result in a reduced amount of Brownian motion. The system itself may be cooled, or the fluidic medium may be cooled.

[00143] Yet another force which may be undesired in certain applications is friction or other form of sticking force. If surface effects are to be minimized, various techniques may be utilized. For example, a counterpropagating beam arrangement may be utilized to capture particles and to remove them from contact with undesired surfaces. Alternatively,

the particles may be levitated, such as through the use of reflected light (see, e.g., Fig. 4, mirror 108). Fig. 4A shows an alternative arrangement for particle levitation. Opposing forces of two counter-propagating optical beams can be used to levitate a particle to reduce surface friction drag.

[00144] Yet other techniques exist for addressing friction, stiction, electrostatic and other surface interactions which may interfere with the mobility of cells and/or particles. For example, surfaces may be treated, such as through the use of covalent or non-covalent chemistries, which may moderate the frictional and/or adhesion forces. Surfaces may be pretreated to provide better starting surfaces. Such pretreatments may include plasma etching and cleaning, solvent washes and pH washes, either singly or in combination. Surfaces may also be functionalized with agents which inhibit or minimize frictional and adhesive forces. Single or multi-step, multi-layer chemistries may be utilized. By way of example, a fluorosilane may be used in a single layer arrangement which renders the surface hydrophobic. A two-step, two-layer chemistry may be, for example, aminopropylsilane followed by carboxy-PEG. Teflon formal coating reagents such as CYTOP™ or Parylene™ can also be used. Certain coatings may have the additional benefit of reducing surface irregularities. Functional groups may, in certain cases, be introduced into the substrate itself. For example, a polymeric substrate may include functional monomers. Further, surfaces may be derivitized to provide a surface which is responsive to other triggers. For example, a derivatized surface may be responsive to external forces, such as an electric field. Alternatively, surfaces may be derivatized such that they selectively bind via affinity or other interactions.

[00145] Yet another technique for reducing surface interactions is to utilize a biphasic medium where the cells or particles are kept at the interface. Such aqueous polymer solutions, such as PEG-dextran partition into two phases. If the cells partitioned preferentially into one of the layers, then under an optical gradient the cells would be effectively floating at the interface.

Methods for Enhancing or Changing the Dielectric Constant

[00146] Optionally, the particles to be subject to the apparatus and methods of these inventions may be either labeled or unlabeled. If labeled, the label would typically be one which changes or contributes to the dielectric constant of the particle or new particle (i.e., the initial particle and the label will act as one new particle). For example, a gold label or a diamond label would effectively change most typical dielectric constants of particles.

[00147] Yet other systems may include an expressible change in dielectric constant. For example, a genetic sequence may exist, or be modified to contain, an expressible protein or other material which when expressed changes the dielectric constant of the cell or system. Another way to tune the dielectric constant of the medium is to have a single medium in a fluidic chamber where the dielectric constant can be changed by changing the temperature, applying an electric field, applying an optical field , etc. Other examples would be to dope the medium with a highly birefringent molecule such as a water-soluble liquid crystal, nanoparticles, quantum dots, etc. In the case of birefringent molecules, the index of refraction that the optical beam will see can be altered by changing the amplitude and direction of an electric field.

Methods for Increasing Sensitivity

[00148] Maximizing the force on a particle for a given intensity gradient suggests that the difference in dielectric constant between the particle and medium should be maximized. However, when sensitivity is required in an application, the medium should be selected such that the dielectric constant of the medium is close to the dielectric constant of the particle or particles to be sorted. By way of example, if the particle population to be sorted has dielectric constants ranging from 1.25 to 1.3, it would be desirable to choose a dielectric constant which is close to (or even within) that range. For cells, a typical range of dielectric constants would be from 1.8 to 2.1. By close, a dielectric constant within 10% or, more particularly, within 5%, would be advantageous. While the absolute value of the magnitude of the force on the particle population may be less than in the case where the dielectric constant differs markedly from the dielectric constant of the medium, the difference in resulting motion of the particles may be larger when the dielectric constant of the medium is close to the range of dielectric constants of the particles in the population. While utilizing the increased sensitivity of this technique at the outset, once the separation begins, the force may be increased by changing the dielectric constant of the medium to a more substantial difference from the dielectric constants of the particle or particle collection. As indicated, it is possible to choose the dielectric constant of the medium to be within the range of dielectric constants of the particle population. In that instance, particles having a dielectric constant above the dielectric constant of the medium will feel a force in one direction, whereas those particles having a dielectric constant less than the dielectric constant of the medium will feel a force moving in the opposite direction.

Scattering Force Systems

[00149] It is possible to utilize the scattering force, either alone or in combination with the optical gradient force, such as supplied by a moving optical field gradient, for separation of particles. Fig. 26 shows the before and after depiction of a system including a laser 520 and a lens 522 which collimates the optical beam. A capillary 524 receives the illumination, preferably along its axis. A set of particles, first particles 526 and second particles 528, are illuminated by the light beam and are subject to different scattering forces depending upon their different scattering properties. Because of the different forces, first particles 526' move a shorter distance than second particles 528', as shown in the second drawing. In this way, optical forces, particularly optical scattering forces, may be utilized to separate particles.

[00150] Figs. 27A, 27B and 27C depict a scattering force switch. A first input 530 couples via a channel to a first output 536. The second input 532 couples to a second output 538 via a channel. The two channels overlap by providing a fluidic connection between them. In operation, a particle entering in input 1 530 may be switched by a scattering force switch 540 by deviating the particle from the channel coupled to input 1 530 to the channel containing output 2 538. Scattering force switches may be used in conjunction with the optical gradient force systems, especially the moving optical gradient force systems described herein.

Static Systems

[00151] Fig. 28 shows a system for the measurement of dielectric constants of particles. A particle 558 having a dielectric constant may be subject to different media having different dielectric constants. As shown, a first vessel 550, a second vessel 552, and so on through an end vessel 554 contain a medium having different dielectric constants ε_1 ε_2, ... ε_n, respectively. By illuminating the particle 558 with an optical gradient force 556, and observing the motion, the dielectric constant of the particle may be determined. If the dielectric constant of the medium is equal to the dielectric constant of the particle then no force is imposed by the optical illumination 556. In contrast, if there is a difference between the dielectric constant of the particle and the dielectric constant of the medium, an optical force will be imposed on the particle by the optical illumination 556. Different dielectric constant media may be supplied as shown in Fig. 28, namely, where a plurality a vessels 550, 552 . . . 554 are provided. Alternately, a particle may be subject to a varying dielectric constant over time, such as through use of a titration system. In on

implementation, the titration may be accomplished in a tube containing the particle by varying the dielectric constant of the fluid over time, such as by mixing fluids having different dielectric constants, preferably at the inlet to the tube, or by providing a varying dielectric constant profile, such as a step profile. Additionally, the dielectric constant of a particle may be approximated by interpolation, such as where two or more data points are obtained regarding the force on the particle in different media, and then the expected dielectric constant in which no force is present may be determined.

[00152] Fig. 29 shows a static system in which separation may occur. A light pattern 560 illuminates first particle 562 and second particle 564. If the dielectric constant of the first particle 562 is less than the dielectric constant of the medium, then the particle moves toward an area of lower intensity. In contrast, if the second particle 564 has a dielectric constant which is greater than the dielectric constant of the medium, the particle will move toward the region of higher intensity. As a result, the first particle 562 and second particle 564 are subject to forces in opposite directions. Given the proximity shown, they would move away from one another.

[00153] Fig. 30 shows a system for the use of a plurality of optical tweezers, preferably in an array, such as to move materials. A substrate 570 may contain one or more sites 572 on which materials may be placed. The materials may comprise particles, cells, or any other material to be selected or moved. An optical tweezer array may selectively move materials, such as those shown as light circles 576, and move those materials to yet another portion of the substrate 570, such as array 574. Alternatively, the optical tweezer array may illuminate the entire array 572, and then selectively move the materials as to which the optical tweezer array provides sufficient force to cause separation of the particles 576, 578 from the array 572 on the substrate 570. For example, the particles may have attachment mechanisms, such as complimentary nucleic acids, which selectively bind them to the substrate 570.

[00154] Fig. 31 shows a graph of molar extinction coefficient as a function of wavelength for hemoglobin-O_2 absorption. For certain sorting applications, it may be desirable to select a wavelength for illumination which is at or near a peak of absorption. For example, it may be desirable to choose a wavelength at the 500,000 molar extinction coefficient peak. Alternatively, it may be desirable to choose a secondary peak, e.g., the peak at substantially 560 nm or at substantially 585 nm.

[00155] The first setup is a moving fringe workstation for optophoresis experiments. A high power, 2.5 watt, Nd-YAG laser (A) is the near IR, 1064 nm wavelength, light source. The fringe pattern is produced by directing the collimated laser beam from the mirror (1) through the Michelson interferometer formed by the prism beam splitter (2) and the carefully aligned mirrors (3). A variable phase retarder (4) causes the fringe pattern to continuously move. This fringe pattern is directed by the periscope (5) through the telescope (5a) and (5b) to size the pattern to fill the back focal plane of the microscope objective, and then is directed by the dichroic beam splitter (6) through a 20× microscope objective (7) to produce an image of the moving fringe pattern in the fluidic chamber holding the sample to be sorted. A second, 60× microscope objective (8) images the flow cell onto a CCD camera to provide visualization of the sorting experiments. A fiber-optic illuminator (9) provides illumination, through the dichroic beam splitter (6), for the sample in the fluidic chamber. The fluidic chamber is positioned between the two microscope objectives by means of an XYZ-translation stage.

[00156] It will be appreciated by those skilled in the art that there are any number of additional or different components which may be included. For example, additional mirrors or other optical routing components may be used to 'steer' the beam where required. Various optical components for expanding or collimating the beam may be used, as needed. In the set-up implementing Fig. 5, the laser used additional mirrors to steer the laser beam into the spatial filter, which that produced a well collimated Gaussian beam that is then guided to the Michelson interferometer.

[00157] The second setup is a workstation for measuring and comparing the dielectric properties of cells and particles at near IR optical frequencies, using a 600 mW, ultra-low noise Nd-YAG laser (B) as a light source. The remainder of the optical setup is similar to the moving fringe workstation, except there is no interferometer to produce moving fringes. Instead a single, partially focused illumination spot is imaged within the fluidic chamber. The interaction of cells with this illumination field provides a measurement of the dielectric constant of the cells at near IR optical frequencies.

Exemplary Applications

High Throughput Biology

[00158] The methods and apparatus herein permit a robust cell analysis system suitable for use in high throughput biology in pharmaceutical and life sciences research. This system may be manufactured using higher performance, lower cost optical devices in the

system. A fully integrated high throughput biology, cell analysis workstation is suitable for use in drug discovery, drug discovery, toxicology and life science research.

These systems may utilize advanced optical technologies to revolutionize the drug discovery process and cellular characterization, separation and analysis by integrating optophoresis technology into devices for the rapid identification, selection and sorting of specific cells based on their innate properties, including their innate optical dielectric properties. In addition, since the technology is based on the recognition of such innate properties, labels are not required, greatly simplifying and accelerating the testing process. The lasers employed are preferably in the biologically-compatible infrared wavelengths, allowing precise cell characterization and manipulation with little or no effect on the cell itself. The technology is suited to the post-genomics era, where the interaction of the cell's molecular design/make-up (DNA, RNA and proteins) and the specific cellular changes (growth, differentiation, tissue formation and death) are of critical importance to the basic understanding of health and disease.

[00159] The Optophoresis technology changes the nature of cell-based assays. Applications would include all methods of cellular characterization and sorting. The technology also offers diverse applications in the areas of molecular and cellular physiology. Optophoresis technology addresses fundamental properties of the cell itself, including its optical dielectric properties. The optophoretic properties of the cell change from cell type to cell type, and in response to external stimuli. These properties are reflective of the overall physiologic status of the cell. Active cells have dielectric properties that are different from resting cells of the same type. Cancer cells have different optophoretic properties than their normal counterparts. These cellular properties can also be used effectively in drug discovery and pharmaceutical research, since nearly all drugs are targeted ultimately to have direct effects on cells themselves. In other words, drugs designed to effect specific molecular targets will ultimately manifest their effects on cellular properties as they change the net dielectric charge of the cell. Therefore, rapid screening of cells for drug activity or toxicity is an application of the technology, and may be referred to as High Throughput Biology. Other main applications include drug discovery and pharmaceutical research.

[00160] The Human Genome Project and other associated genome programs will provide enormous demand for improved drug development and screening technologies. Sophisticated cellular approaches will be needed for cost-effective and functional

screening of new drug targets. Likewise, information from the genome projects will create demand for improved methods of tissue and organ engineering, each requiring access to well characterized cellular materials. Moreover, optical technology from the information and telecommunications industry will provide the system hardware for improved optical cell selection and sorting. The price/performance ratios for high powered near infrared and infrared lasers originally developed for telecommunications applications continue to improve significantly. In addition, solid-state diode lasers may be used having a variety of new wavelengths, with typically much higher power output than older versions. Vertical Cavity Surface Emitting Lasers ("VCSELs") provide arrays of diode lasers at very reasonable costs with increasing power output.

[00161] A computerized Workstation may be composed of a miniaturized sample station with active fluidics, an optical platform containing a near infrared laser and necessary system hardware for data analysis and interpretation. The system includes real-time analysis and testing under full computer control. Principal applications of the technology include cell characterization and selection, particularly for identifying and selecting distinct cells from complex backgrounds.

[00162] Importantly, unlabelled, physiologically normal, intact test cells will be employed in the system. The sample is quickly analyzed, with the cells classified and sorted by the optical field, thereby allowing characterization of drug response and identify toxicity or other measures of drug efficacy. Characterizing the cellular optophoretic properties uniquely associated with various drug testing outcomes and disease states is a part of this invention. Identification of these novel parameters constitutes useful information.

[00163] An integrated system may, in various aspects, permit: the identification, selection and separation of cells without the use of labels and without damaging the cells; perform complex cell analysis and separation tasks with ease and efficiency; observe cells in real time as they are being tested and manipulated; establish custom cell sorting protocols for later use; isolate rare cells from complex backgrounds; purify and enrich rare cells (e.g. stem cells, fragile cells, tumor cells); more easily link cell phenotype to genotype; study cell-cell interactions under precise and optical control; and control sample processing and analysis from start to finish.

[00164] The technology offers a unique and valuable approach to building cellular arrays that could miniaturize current assays, increase throughput and decrease unit costs.

Single cell (or small groups of cells) based assays will allow miniaturization, and could allow more detailed study of cell function and their response to drugs and other stimuli. This would permit cellular arrays or cell chips to perform parallel high-throughput processing of single cell assays. It could also permit the standardization of cell chip fabrication, yielding a more efficient method for creation of cell chips applicable to a variety of different cells types.

[00165] Mammalian cell culture is one of the key areas in both research (e.g., discovery of new cell-produced compounds and creation of new cell lines capable of producing specific proteins) and development (e.g., developing monoclonal cell lines capable of producing highly specific proteins for further research and testing). Mammalian cell culture is also a key technology for the production of new biopharmaceuticals on a commercial scale.

[00166] Once researchers have identified drug targets, compounds or vaccines, mammalian cell culture is an important technology for the production of quantities necessary for further research and development. There are currently more than 70 approved biotechnology medicines and more than 350 such compounds in testing, targeting more than 200 diseases.

[00167] Optical cell characterization, sorting and analysis technologies could be useful in selecting and separating lines of mammalian cells according to whether they produce a new protein or biopharmaceutical compound and according to the yield of the protein or compound. Cell yield is a key factor in determining the size of the plant a manufacturer must build to produce commercial quantities of a new biotechnology drug.

[00168] We turn now to more specific discussions of applications. First, we address separation applications, and second, address monitoring applications.

SEPARATION APPLICATIONS

[00169] **White cells from red cells.** White blood cells are the constituents of blood which are responsible for the immune response as compared with red cells which transport oxygen through the body. White cells need to be removed from red cells prior to transfusion for better tolerance and to decrease infection risks. It is also often important to remove red cells in order to obtain enriched populations of white cells for analysis or manipulation. Optophoresis can allow the separation of these two distinct cell populations from one another for use in applications where a single population is required.

[00170] Reticulocytes from mature red blood cells. Reticulocytes, which are immature red blood cells normally found at very low levels can be indicators of disease states when they are found at increased levels. This application would use optophoresis for the separation and enumeration of the levels of reticulocytes from whole blood.

[00171] Clinical Care Applications, e.g., Fetal stem cells from maternal circulation. The Clinical Care applications include cell-based treatments and clinical diagnostics. The successful isolation of fetal cells from maternal blood represents a source of fetal DNA obtainable in a non-invasive manner. A number of investigators worldwide have now demonstrated that fetal cells are present in the maternal circulation and can be retrieved for genetic analysis. The major current challenges in fetal cell isolation include selection of the target fetal cell type, selection and isolation of the cells and the means of genetic analysis once the cells are isolated. Using a maternal blood sample, the system can identify the rare fetal cells circulating within the mother's blood and to permit the diagnosis of genetic disorders that account for up to 95% of prenatal genetic abnormalities, e.g., Down's Syndrome. Cell-based treatments refer to procedures similar to diagnostic procedures, but for which the clinical purpose is somewhat broader. During pregnancy, a small number of fetal cells enter the maternal circulation. By purifying these cells using optophoresis prenatal diagnosis of a variety of genetic abnormalities would be possible from a single maternal blood sample.

[00172] Clinical Care Applications, e.g., Stem Cell Isolation. The purpose of stem cell isolation is to purify stem cells from stem cell grafts for transplantation, i.e., to remove T-cells in allogeneic grafts (where the donor and the recipient are not the same person) and cancer cells in autologous grafts (where the donor and the recipient are the same person). Currently stem cell technologies suffer from several drawbacks. For example, the recovery efficiency of stem cells obtained using currently available systems is on the order of 65 – 70%. In addition, current methods do not offer the 100% purity which is beneficial in transplant procedures.

[00173] Tumor cells from blood. Minimal Residual Disease (MRD) Testing The National Cancer Institute (NCI) estimates that approximately 8.4 million Americans alive today have a history of cancer, and that over 1.2 million new cancer cases were diagnosed in 2000. The NCI also estimates that since 1990 approximately 13 million new cancer cases were diagnosed, excluding noninvasive and squamous cell skin cancers. Optophoresis technology addresses some of the key unmet needs for better cancer

screening, including: accurate, reproducible and standardized techniques that can detect, quantify and characterize disseminated cancer cells; highly specific and sensitive immunocytological techniques; faster speed of cell sorting; and techniques that can characterize and isolate viable cancer cells for further analysis.

[00174] Cancer cells may be found in low numbers circulating in the blood of patients with various forms of that disease, particularly when metastasis has occurred. The presence of tumor cells in the blood can be used for a diagnosis of cancer, or to follow the success or failure of various treatment protocols. Such tumor cells are extremely rare, so a means of enrichment from blood such as optophoresis would need to be employed in order to have enough cells to detect for accurate diagnosis. Another application for optophoresis in this regard would be to remove tumor cells from blood or stem cell products prior to them being used to perform an autologous transplant for a cancer patient.

[00175] **Fetal stem cells from cord blood.** The umbilical cord from a newborn generally contains blood which is rich in stem cells. The cord blood material is usually discarded at birth; however, there are both academic and private concerns who are banking cord blood so that such discarded material can be used for either autologous or allogenic stem cell replacement. Enrichment of the cord blood stem cells by optophoresis would allow for a smaller amount of material to be stored, which could be more easily given back to the patient or another host.

[00176] **Adult stem cells from liver, neural tissue, bone marrow, and the Like.** It is becoming increasingly clear that many mature tissues have small subpopulations of immortal stem cells which may be manipulated ex vivo and then can be reintroduced into a patient in order to repopulate a damaged tissue. Optophoresis can be used to purify these extremely rare adult stem cells so that they may be used for cell therapy applications.

[00177] **Islet cells from pancreas.** It has been proposed that for persons with diabetes resulting from lack of insulin production, the insulin producing beta islet cells from a healthy pancreas could be transplanted to restore that function to the diabetic person. These cells make up only a small fraction of the total donor pancreas. Optophoresis provides a method to enrich the islet cells and would be useful for preparation of this specific type of cell for transplantation.

[00178] **Activated B or T cells.** During an immune response either T or B white cell subsets which target a specific antigen become active. These specific activated cells may be required as separate components for use in *ex vivo* expansion to then be applied as

immunotherapy products or to be gotten rid of, since activated B or T cells can cause unwanted immune reactions in a patient such as organ rejection, or autoimmune diseases such as lupus or rheumatoid arthritis. Optophoresis provides a method to obtain activated cells either to enrich and give back to a patient or to discard cells which are causing pathological destruction.

[00179] Dendritic cells. Dendritic cells are a subset of white blood cells which are critical to establishing a T-cell mediated immune response. Biotech and pharmaceutical companies are working on ways to harvest dendritic cells and use them ex vivo in conjunction with the appropriate antigen to produce a specific activated T cell response. Optophoresis would allow isolation of large numbers of dendritic cells for such work.

[00180] HPRT- cells. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) is an enzyme which exits in many cells of the blood and is involved in the nucleoside scavenging pathway. Persons who have high mutation rates due to either endogenous genetic mutations or exogenous exposure to mutagens can be screened for HPRT lacking cells (HPRT-) which indicate a mutation has occurred in this gene. Optophoresis following screening by compounds which go through the HPRT system can be used to easily select HPRT minus cells and quantitate their numbers.

[00181] Viable or mobile sperm cells. Approximately 12% of couples are unable to initiate a pregnancy without some form of assistance or therapy. In about 30% these cases, the male appears to be singularly responsible. In an additional 20% of cases, both male and female factors can be identified. Thus, a male factor is partly responsible for difficulties in conception in roughly 50% of cases. The number of women aged 15-44 with impaired ability to have children is well over 6 million. Semen analysis is currently performed using a variety of tests and is based on a number of parameters including count, volume, pH, viscosity, motility and morphology. At present, semen analysis is a subjective and manual process. The results of semen analysis do not always clearly indicate if the male is contributing to the couple's infertility. Gradient centrifugation to isolate motile sperm is an inefficient process (10 to 20% recovery rate). Sperm selection is accomplished using either gradient centrifugation to isolate motile sperm used in *In Utero* Insemination (IUI) and *In Vitro* Fertilization (IVF) or visual inspection and selection to isolate morphologically correct sperm used in IVF and Intracytoplasmic Sperm Injection (ICSI). Each year in the U.S., 600,000 males seek medical assistance for infertility.

[00182] One of the reasons for male infertility is the lack of high enough percentages of viable and/or mobile sperm cells. Viable and/or mobile sperm cells can be selected using optophoresis and by enriching their numbers, higher rates of fertilization can be achieved. This application could also be used to select X from Y bearing sperm and vice versa, which would then be used selectively to induce pregnancies in animal applications where one sex of animal is vastly preferred for economic reasons (dairy cows need to be female, while it is preferable for meat producing cattle to be male for example).

[00183] **Liposomes loaded with various compounds**. A recent mode of therapeutic delivery of pharmaceutical products is to use liposomes as the delivery vehicle. It should be possible using optophoresis to separate liposomes with different levels of drug in them and to enrich for those liposomes in which the drugs are most concentrated.

[00184] **Tissue Engineering, e.g., Cartilage precursors from fat cells**. Tissue engineering involves the use of living cells to develop biological substitutes for tissue replacements which can be used in place of traditional synthetic implants. Loss of human tissue or organ function is a devastating problem for a patient and family. The goal of tissue engineering is to design and grow new tissue outside the body that could then be transplanted into the body.

[00185] A recent report has demonstrated that cells found in human adipose tissue can be used ex vivo to generate cartilage which can be used as a transplant material to repair damage in human joints. Optophoresis can be used to purify the cartilage forming cells from the other cells in adipose tissue for ex vivo expansion and eventual tissue engineering therapy.

[00186] **Nanomanipulation of small numbers of cells**. Recent miniaturization of many lab processes have resulted in many lab analyses being put onto smaller and smaller platforms, evolving towards a "lab-on-a-chip" approach. While manipulation of biomolecules in solution has become routine in such environments, manipulation of small numbers of cells in microchannel and other nano-devices has not been widely achieved. Optophoresis will allow cells to be moved in microchannels and directed into the region with the appropriate processes on the chip.

[00187] **Cellular organelles; mitochondria, nucleus, ER, microsomes**. The internal constituents of a cell consists of the cytoplasm and many organelles such as the mitochondria, nucleus, etc. Changes in the numbers or physical features of these organelles can be used to monitor changes in the physiology of the cell itself.

Optophoresis can allow cells to be selected and enriched which have particular types, morphologies or numbers of a particular organelle.

[00188] Cow reticulocytes for BSE assays. It has been reported that a cellular component of the reticulocyte, EDRF, is found at elevated levels in the reticulocytes of cows infected with BSE (bovine spongiform encephalopathy). Reticulocytes are generally found at low levels in the blood and therefore the use of optophoresis would allow their enrichment and would increase the accuracy of diagnostic tests based on the quantitation of the EDRF mRNA or protein.

MONITORING

[00189] Growing/dividing cells vs. resting cells. Cells may be stimulated to grow by various growth factors or growth conditions. Most assays which exist for cell growth require the addition of external labeling reagents and/or significant time in culture before cell growth can be demonstrated. By using optophoresis, cells which have begun to divide will be identified, providing a rapid method for calculating how much of a given cell population is in the growth phase. Cells in different parts of the cell cycle should have different optical properties and these may be used to either sort cells based on where in the cycle they are as well as to determine what fraction of the total cell population is in each stage of the cell cycle.

[00190] Apoptotic cells. Cells which are undergoing programmed cell death or apoptosis can be used to identify specific drugs or other phenomenon which lead to this event. Optophoresis can be used to identify which cells are undergoing apoptosis and this knowledge can be used to screen novel molecules or cell conditions or interactions which promote apoptosis.

[00191] Cells with membrane channels open; change in membrane potentials. The outer membrane of many types of cells contain channels which facilitate the passage of ions and small molecules into and out of the cell. Movement of such molecules can lead to further changes in the cell such as changes in electrical potential, changes in levels of second messengers, etc. Knowledge of these changes can be useful in drug screening for compounds which modulate membrane channel activity. Optophoresis can be used to indicate when membrane channels are being perturbed by exogenous compounds.

[00192] Live vs. dead cells. Many applications exist which require the identification and quantitation of live versus dead cells. By using optophoresis dead cells can be identified and counted.

[00193] Virally infected cells. There are many diagnostic applications where it is important to measure cells which contain virus, including ones for CMV, HIV, etc. Optophoresis can be used to differentiate cells which contain virus from cells which do not.

[00194] Cells with abnormal nucleus or elevated DNA content. One of the hallmarks of a tumor cell is that it will contain either excess DNA, resulting in an abnormal size and/or shape to it's nucleus. By using optophoresis tuned to the nuclear content of a cell populations with abnormal amounts of DNA and/or nuclear structure may be identified and this information can be used as a diagnostic or prognostic indicator for cancer patients.

[00195] Cells decorated with antibodies. A large selection of commercially available antibodies exists which have specificities to cellular markers which define unique proteins and/or types of cells. Many diagnostic applications rely on the characterization of cell types by identifying what antibodies bind to their surface. Optophoresis can be used to detect when a cell has a specific antibody bound to it.

[00196] Cells with bound ligands, peptides, growth factors. Many compounds and proteins bind to receptors on the surface of specific cell types. Such ligands may then cause changes inside the cell. Many drug screens look for such interactions. Optophoresis provides a means to monitor binding of exogenous large and small molecules to the outside of the cell, as well as measurement of physiological changes inside the cell as a result of compound binding.

[00197] Bacteria for viability after antibiotic exposure. Microorganisms are often tested for sensitivity to a spectrum of antibiotics in order to determine the appropriate therapy to pursue to kill an infectious organism. Optophoresis can be used to monitor bacterial cells for viability and for cessation of growth following antibiotic exposure.

[00198] Drug screening on the NCI 60 panel. A panel of 60 tumor cell lines has been established by the National Cancer Institute as a screening tool to determine compounds which may have properties favorable to use as chemotherapeutic agents. It should be possible to use optophoresis to array all 60 lines and then to challenge them with known and novel chemicals and to monitor the cell lines for response to the chemicals.

[00199] Cells for cytoskeletal changes. The cytoskeleton is a complex of structural proteins which keeps the internal structure of the cell intact. Many drugs such as taxol, vincristine, etc... as well as other external stimuli such as temperature are known to cause the cytoskeleton to be disrupted and breakdown . Optophoresis provides a means to monitor populations of cells for perturbations in the cytoskeleton.

[00200] Beads with compounds bound to them, to measure interactions with the cell surface or with other beads. The interactions of microspheres with cells or other compounds has been used in a number of in vitro diagnostic applications. Compounds may be attached to beads and the interactions of the beads with cells or with beads with other compounds on them can be monitored by optophoresis.

[00201] Progenitor cell/colony forming assays. Progenitors are cells of a given tissue which can give rise to large numbers of more mature cells of that same tissue. A typical assay for measuring progenitor cells is to allow these cells to remain in culture and to count how many colonies of the appropriate mature cell type they form in a given time. This type of assay is slow and cumbersome sometimes taking weeks to perform. By using optophoresis to monitor the growth of a single cell, progenitor proliferation can be measured on a nano-scale and results should be obtained within a much shorter length of time.

[00202] Dose limiting toxicity screening. Almost all compounds are toxic at some level, and the specific levels of toxicity of compounds are identified by measuring at what concentration they kill living cells and organisms. By monitoring living cells with optophoresis as the dose of a compound is slowly increased, the level at which optical properties indicative of cell damage and/or death can be ascertained.

[00203] Monitor lipid composition/membrane fluidity in cells. The membranes of all cells are composed of lipids which must maintain both the proper degree of membrane fluidity at the same time that they maintain basic cell membrane integrity. Optophoresis should be able to measure the fluidity of the membrane and to provide information on compounds and conditions which can change membrane fluidity, causing membranes to be either more or less fluid.

[00204] Measure clotting/platelet aggregation. Components found in the blood such as platelets and clotting proteins are needed to facilitate blood clot formation under the appropriate circumstances. Clotting is often monitored in order to measure disease states

or to assess basic blood physiology. Optophoresis can provide information on platelet aggregation and clot formation.

[00205] Certain of the data reported herein were generated with the following setup. Optical gradient fields were generated using a Michelson interferometer and either a 150 mW, 812 nm laser (812 system) or a 2.5 W, 1064 nm laser (1064 system). The 812 system used a 100X (1.25 NA) oil immersion lens to focus the fringe pattern and to visualize the sample. The 1064 system used a 20X objective to focus the fringes and a 60X objective to visualize the sample. In general the sample cell was a coated microscope slide and/or coverslip that was sealed with Vaseline. Coverslip spacers controlled the height of the cell at approximately 150 micrometers

[00206] **Coating Of Surfaces; Rain-X™, Agarose, CYTOP, Fluorosilane** Scattering forces tend to push the particles or cells against the surface of the sample cell. Therefore, a number of surface coatings were evaluated to minimize nonspecific adhesion and frictional forces. Hydrophobic/hydrophilic and covalent/noncovalent surface treatments were evaluated.

[00207] **Covalent/Hydrophobic** Glass slides and coverslips were treated with perfluoro-octyltrichlorosilane (Aldrich, Milwaukee, WI) using solution or vapor deposition. Solution deposition was as follows: a 2-5% silane solution in ethanol, incubate 30 minutes at room temperature, rinse 3 times in ethanol and air dry. Vapor deposition involved applying equal volumes of silane and water in separate microcentrifuge tubes and sealing in a vacuum chamber with the substrate to be treated. Heat to 50°C, 15 hrs.

[00208] **Noncovalent/Hydrophobic** -- A commercial water repellent containing polysiloxanes, Rain-X, was applied according to the manufacturer's instructions.

[00209] A liquid Teflon, CYTOP (CTL-107M, Wilmington, Delaware) was spun coated using a microfuge. The CYTOP was diluted to 10% in fluorooctane (v/v) and 50 microliters was pipetted and spun for 5 seconds. This was repeated a second time and then air dried.

[00210] **Noncovalent/Hydrophilic** -- Agarose hydrogel coatings were prepared as follows: melt 2% agarose in water, pipette 100 microliters to the substrate, spin for 5 seconds, bake at 37°C for 30 minutes.

[00211] All of the coatings were effective when working with particles. The CYTOP was more effective at preventing adhesion when working with biological cells.

[00212] Separation By Size -- Polystyrene particles (Bangs Labs, Fishers, IN) of different sizes (1, 3 and 5 micrometer diameter) were separated using moving optical gradient fields. Three and five micrometer diameter particles were diluted 1/500 in distilled water and ten microliters was pipetted onto a Rain-X coated slide. The 812 system was used to generate a spot size of 25-30 micrometers consisting of 4-5 fringe periods and moving at 15 micrometers/second.

[00213] Fig. 32 shows a sorting sequence at 1-second intervals with 3 and 5 micrometer polystyrene particles. The smaller, 3 micrometer diameter, particle was readily moved by the gradient fields whereas the larger, 5 micrometer diameter., particle was unaffected. The larger particle was not moved because it spanned multiple fringes so gradient forces were effectively cancelled. Similar results were obtained with 1 and 3 micrometer diameter particles.

[00214] Separation By Refractive Index --Polystyrene, polymethylmethacrylate and silica particles of similar size (~5 micrometer diameter, Bangs Labs) and refractive indexes of 1.59, 1.49 and 1.37, respectively, were sorted by moving optical gradient fields. Observed escape velocities for polystyrene, PMMA and silica were 44, 47 and 32 micrometers/second, respectively. Briefly, a particle is aligned in the fringe and the fringes are moved at increasing speed until the particle slips. This results in a semi-quantitative measurement of the total forces experienced by the particle, i.e. photonic, hydrodynamic and frictional. It will be appreciated by those skilled in the art that the absolute value of the escape velocity will differ depending upon system conditions, e.g., laser power. The numerical results provided herein are meant to provide measured data for the system actually used, and are not to be considered a limitation on the values which might exist in a different system.

[00215] Particles were diluted 1/500 in distilled water (n = 1.33). The 812 system was used to generate a gradient field with a fringe period of 10 micrometers. Polystyrene and PMMA particles were sorted from silica particles by moving the gradient field at a threshold value of approximately 40 micrometers/second.

[00216] Separation By Surface Functionalization and Doping -- Polystyrene particles (~6 micrometer diameter) colored with blue or pink dye were purchased from Polysciences, Inc. The pink particles also had carboxyl groups on the particle surface. The particles were diluted 1/500 in distilled water and 10 microliters was pipetted onto a Rain-X coated slide. The 812 system was used to generate a moving optical gradient field

with a fringe period of approximately 12 micrometers. In the fringes, the pink particle moved preferentially.

[00217] Fig. 33 shows the actual movement of the particles.

[00218] In another experiment, 1 micrometer latex beads labeled with biotin were used to determine changes in escape velocity when different ligands were attached. The biotin labeled beads were diluted 1/100 in PBS buffer. A 50 ul aliquot was incubated with an excess of streptavidin or 10 nanometer colloidal gold-streptavidin conjugate for 10 minutes. The beads were pelleted by centrifugation and resuspended in PBS buffer. Measured escape velocities, using the 1064 system, were 5.3, 4.3 and 3.6 micrometers/second for biotin labeled beads, beads with streptavidin and beads with streptavidin-colloidal gold, respectively.

[00219] Separation By Wavelength Resonance (812 vs. 1064 nm) -- The above experiment with colored polystyrene particles was repeated using the 1064 system and the results were reversed. The blue particle was preferentially moved. Similar results were obtained when the 1064 system was set at 150 mW rather than 2.5 W. This suggests that wavelength tuning could enhance the discrimination process.

[00220] Separation By Index Matching --Silica and polystyrene particles (3 and 5 micrometer diameter, respectively) were diluted 1/500 in hydrophilic silicone (dimethylsiloxane-ethylene oxide block copolymer, Gelest, Inc., Tullytown, PA). The refractive index of the medium (n = 1.44) was intermediate between the silica (n = 1.37) and polystyrene (n = 1.59) particles. The particle size was not important in this experiment.

[00221] Using the 1064 system, the gradient force was focused into a diffuse spot approx. 15 micrometers in diameter. More generally, for all of the systems and applications described herein, a defocused beam, such as a defocused laser beam may be utilized. Preferably, the beam is defocused such that the spot or beam size is on the order of magnitude of the size of the particle. For cells, the size would be approximately 10 to 20 microns. The polystyene particle moved towards the gradient field while the silica particle moved away from it. This demonstrated that the suspending medium could be changed to optimize separation.

Separation Red Blood Cells vs. Retic

[00222] A reticulocyte control (Retic-Chex) was obtained from Streck Labs. A sample containing 6% reticulocytes was stained for 15 minutes with New Methylene Blue for 15

minutes, a nucleic acid stain that differentially stains the reticulocytes versus the unnucleated red blood cells. The sample was diluted 1/200 in PBS and mounted on a fluorosilane coated slide The 812 system was used to generate optical gradient fields. The fringe period was adjusted to 15 micrometers and was moved at 15 micrometers/second. The reticulocytes were preferentially moved relative to red blood cells.

Separation of White Blood Cells vs. Red Blood Cells

[00223] A whole blood control (Para12 Plus) was obtained from Streck Labs. The sample was stained for 15 minutes with New Methylene Blue, a nucleic acid stain that differentially stains the nucleated white blood cells versus the unnucleated red blood cells. The sample was diluted 1/200 in PBS and mounted on a fluorosilane coated slide. The 812 system was used to generate optical gradient fields. The fringe period was adjusted to 15 micrometers and was moved at 22 micrometers/second. The white blood cells were moved by the fringes while the red blood cells were not.

Separation of Leukemia vs. Red Blood Cells

[00224] One milliliter of the leukemia cell line U937 suspension was pelleted and resuspended in 100 microliters PBS containing 1% BSA. Equal volumes of U937 and a 1/200 dilution of red blood cells were mixed together and 10 microliters was placed on a CYTOP coated slide. Separate measurements with moving fringe fields showed that the escape velocity for U937 cells was significantly higher than the escape velocity for red blood cells, 60 and 23 micrometers/second, respectively. The 1064 system was used to generate optical gradient fields with a fringe period of approximately 30 micrometers and moving at 45 micrometers/second, an intermediate fringe velocity. As expected the U937 cells move with the fringes and the red blood cells do not. In one embodiment, the moving fringe may be reduced to a single peak. Preferably, the peak is in the form of a line. In operation, a slow sweep (i.e., at less than the escape velocity of the population of particles) is made across the region to be interrogated. This causes the particles to line up. Next, the fringe is moved quickly (i.e., at a speed greater than the escape velocity of at least some of the particle in the population), preferably in the direction opposite the slow sweep. This causes the selective separation of those particles having a higher escape velocity from those having a lower escape velocity. Optionally, the remaining line of particles may then be again interrogated at an intermediate fringe velocity. While this

technique has general applicability to all of the applications and systems described herein, it has been successfully implemented for the separation of U937 cells from red blood cells.

Sorting of Red Blood Cells vs. Polystyrene Particles in Microchannels

[00225] Glass microchannels with an "H" configuration (see Fig. 16) were used to demonstrate sorting of red blood cells and 6 micrometer polystyrene particles. The channels were purchased from Agilent (DNA 500 LabChip) and were 40 micrometers wide and 10 micrometers deep. Unwanted or unused channels and reservoir ports were blocked by backfilling with Norland 61 optical adhesive followed by UV and thermal curing. The channels were primed with ethanol, followed by water and finally by PBS buffer with 1% BSA. The inlet reservoirs were built up about 1 mm higher than the outlet reservoirs. Flow rates were controlled by a combination of pressure and electrokinetic forces. A Keithley 236 power supply was used to apply an electric field between 5 and 10 V/cm.

[00226] A 1/200 mixture of red blood cells and particles in PBS buffer, 1% BSA was added to an inlet reservoir and an equal volume of PBS buffer, 1% BSA was added to the other inlet reservoir. The gradient field was positioned in the crossbar of the "H" near the downstream junction. The 1064 system was fitted with a cylindrical lens to increase the aspect ratio of the gradient field. The resultant gradient field was approximately 40 micrometers wide by 80 micrometers long with a fringe period of 12 ums and moving at 30 micrometers/second.

[00227] In the absence of or with a nonmoving optical gradient field, the cells and particles remain in the top half of the "H" channel and exit via the upper outlet. In the presence of a moving optical gradient field, the particles are diverted to the lower outlet arm and are sorted from the red blood cells.

[00228] The flow rate was adjusted to approximately 80 micrometers/second. The sorting process was digitally recorded and subsequently analyzed. Out of 132 possible sorting events (121 red blood cells and 11 particles), 2 red blood cells and no particles were mis-sorted. The sort rate was approximately 2/second.

Sorting of Red Blood Cells vs. White Blood Cells in Microchannels

[00229] Fig. 36 shows photographs of sorting of two cell types in a microchannel device. 1 shows a red blood cell and a white blood cell successively entering the moving optical gradient field. 2 shows that white blood cell has been translated down by the action of the moving optical gradient field while the red blood cell has escaped translation.

3 and 4 show that the red blood cell and white blood cell continue to flow into separate channels, completing the sorting.

Gradient Force Manipulation of Liposomes

[00230] Fluorescently labeled liposomes, approximately 0.2 micrometers in diameter, were obtained from a B-D Qtest Strep kit. Ten microliters was placed in a Rain-X coated slide and the 1064 system was used to generate an optical gradient field. A 15 mW 532 nm diode laser was also focused through the objective to visualize the liposome fluorescence. When a standing gradient field was projected onto the sample, fluorescence was more intense in this area. This suggests that the liposomes were moving towards the gradient field.

Differential Motion Imaging

[00231] Polystyrene and silica particles were diluted in distilled water. As shown in the photographs of Fig. 34, a "before" image was captured using a CCD camera and Image Pro Express software. A moving optical gradient field generated by the 1064 system was scanned over the particles. Another image (an "After" image) was captured and the "before" image was subtracted. The resultant image (labeled "Difference") clearly identifies that the polystyrene particle had moved.

Escape Velocities of Different Cell Types

[00232] Escape velocities were measured using a gradient field generated by the 1064 system on CYTOP coated coverslips.

Cell Type	Escape Velocity (um/sec.)
Red Blood Cell	5.6 +/- 0.4
White Blood Cell	11.0 +/- 1.8
Chicken Blood (Retic. Model)	7.3 +/- 1.4
K562 Cells, No Taxol Treatment	10.0 +/- 0.7
K562 Cells, 26 Hr. Taxol Treatment	8.2 +/- 0.4

K562 Cells: Chronic myelogenous leukemia, lymphoblast

[00233] Fig. 35 shows a graph of percent of cells measured as a function of escape velocity (μm/second).

Separation of Treated and Untreated Leukemia Cells

[00234] PMA was dissolved in ethanol at a concentration of 5mg/mL. 3 mls of U937 cells grown in RPMI 1640 media with supplements were removed from the culture flask and 1 ml was placed into each of three eppendorf tubes. Cells from the first tube were pelleted for 4 minutes at 10,000 rpm and resuspended in 250uL PBS/1%BSA buffer for escape velocity measurements. PMA was added to the remaining two tubes of U937 cells to a final concentration of 5ug/mL. These tubes were vortexed and placed in a 37°C water bath for either one hour or six hours. At the end of the time point, the tube was removed, cells were pelleted and then resuspended as described above and escape velocity measurements taken. The cells treated for 6 hours had a significantly higher escape velocity as compared to the untreated cells.

[00235] While preferred embodiments and methods have been shown and described, it will be apparent to one of ordinary skill in the art that numerous alterations may be made without departing from the spirit or scope of the invention. Therefore, the invention is not limited except in accordance with the following claims.

We Claim:

1. A method for the characterization of a particle comprising the steps of:

observing a first physical position of a particle,

optically illuminating the particle to subject it to an optical force,

observing the second physical position of the particle, and

characterizing the particle based at least in part upon reaction of the particle to the optical force.

2. The method of claim 1 wherein the optical illumination includes an optical gradient field.

3. The method of claim 2 wherein the optical gradient field is a moving optical gradient field.

4. The method of claim 1 wherein the optical illumination includes an optical scattering force field.

5. The method of claim 1 wherein the optical illumination includes a moving optical gradient force field and another force.

6. The method of claim 1 wherein the first position and second position are different.

7. The method of claim 1 wherein the positions are the same.

8. The method of claim 7 wherein the characterization includes non-movement as indicative of the state.

9. The method of claim 7 wherein the characterization includes a non-positional parameter.

10. The method of claim 9 wherein the non-positional parameter is rotation of the particle.

11. The method of claim 6 wherein the characterization involves a comparison of the first position and the second position.

12. The method of claim 11 wherein the amount of difference of movement indicates a characterization state.

13. The method of claim 11 wherein the direction of movement is indicative of a characterization state.

14. The method of claim 1 wherein the characterization utilizes the optophoretic constant of the particle.

15. The method of claim 1 wherein the characterization utilizes the optophoretic signature of the particle.

16. A method for separating particles comprising the steps of:
subjecting particles to optical gradient force,
analyzing, based at least in part on the relative motion of the particles, and
separating desired particle from other particles.

17. The method of claim 16 wherein the separation is fluidic.

18. The method of claim 16 wherein the separation is mechanical.

19. The method of claim 18 wherein the mechanical separation utilizes a capture structure.

20. The method of claim 16 wherein the separation is optical.

21. The method of claim 20 wherein the optical separation uses an optical tweezer.

22. The method of claim 20 wherein the optical separation uses an optical gradient force.

23. The method of claim 20 wherein the optical separation uses an optical scattering force.

24. A method for separating particles comprising the steps of:

providing one or more particles,

subjecting particles to light so as to cause a scattering force on the particles, and

separating the particles based upon the reaction to at least the scattering force.

25. A method for separating particles comprising the steps of:

determining first positions of two or more particles,

subjecting the particles to an optical gradient force to effect relative motion of the particles,

determining second positions of the particles, and

selectively removing a subset of the particles based upon a force other than the gradient force.

26. A method for analyzing particles comprising the steps of:

electrokinetically moving the particles, and

subjecting the particles to optical forces for analysis.

27. The method of claim 26 wherein the optical force is an optical gradient force.

28. The method of claim 26 wherein the optical force is a moving optical gradient field.

29. The method of claim 26 wherein the optical force is an optical scattering force.

30. The method of claim 26 wherein the electrokinetic force is an electrophoretic force.

31. The method of claim 26 wherein the electrokinetic force is a dielectrophoretic force.

32. The method of claim 26 wherein the electrokinetic force is an electroosmotic force.

33. The method of claim 26 wherein the particles are moved along a surface.

34. The method of claim 33 wherein the surface is a planar surface.

35. The method of claim 34 wherein the planar surface includes electrodes.

36. The method of claim 35 wherein the electrodes are arranged in an array.

37. The method of claim 33 wherein the surface includes a channel.

38. The method of claim 26 wherein the movement occurs through a tube.

39. A method for determining the dielectric constant of a particle comprising the steps of:

subjecting the particle to an optical gradient field in a plurality of media having different dielectric constants,

monitoring the motion of the particle when subject to the optical gradient force in the various media, and

determining the dielectric constant of the particle based upon the relative motion in the various media.

40. The method of claim 39 wherein the media are in different vessels.

41. The method of claim 39 wherein the media gradient is in one vessel.

42. The method of claim 41 wherein the vessel is a tube.

43. A method for separating particles in a medium, the particles having differing dielectric constants, comprising the steps of:

providing a medium having a dielectric constant between the dielectric constants of the particles,

subjecting the particles in the media to an optical gradient field, and

separating the particles.

44. The method of claim 43 wherein the optical gradient field comprises an expanding optical gradient field.

45. The method of claim 44 wherein the expanding optical gradient field constitutes an expanding area of illumination.

46. The method of claim 43 wherein the illumination has a constant intensity.

47. The method of claim 43 wherein the optical gradient field comprises a moving optical gradient field.

48. The method of claim 47 wherein the moving optical gradient field includes a jerk motion.

49. A method for reducing forces between a particle and a surface in a system for optically moving particles, comprising the steps of:

providing particles adjacent a first surface,

subjecting the particles to a first light intensity pattern to effect sorting of the particles, and

subjecting the particles to a second force in an amount and direction to reduce the interaction between the particle and the surface.

50. The method of claim 49 wherein the second force causes levitation of the particles.

51. The method of claim 50 wherein the second force is electrostatic.

52. The method of claim 50 wherein the second force is dielectrophoretic.

53. The method of claim 50 wherein the second force is optical.

54. The method of claim 53 wherein the optical force is generated by a counterpropagating beam.

55. A method for generating a moving optical gradient comprising the steps of:
providing an array of sources,
creating a moving optical gradient by selective operation of the sources,
optically shaping the output of the sources, and
illuminating a media containing a plurality of particles.

56. The method of claim 55 wherein the array of sources comprises a VCSEL array.

57. The method of claim 55 wherein the array of sources comprises a diode bar.

58. The method of claim 55 wherein the array of sources comprises an array of optical fibers.

59. The method of claim 55 wherein the optical shaping utilizes lenses.

60. A method for interacting an optical gradient field in three dimensions with a particle, comprising the steps of:
interfering two beams to generate a plurality of planar fronts,
providing a plurality of particles in a medium, and

moving the planar fronts relative to the particles,

whereby the particles are separated at least in part based upon the dielectric constant of the particles.

61. The method of claim 60 wherein the interfering of the two beams utilizes two separate beams.

62. The method of claim 60 wherein the interfering of the two beams utilizes a single source to generate the two beams.

63. The method of claim 62 wherein the two beams comprise a direct and reflected beam.

64. The method of claim 63 wherein the reflected beam is reflected at an oblique angle to the mirror.

65. The method of claim 60 wherein the medium has a dielectric constant which is less than the dielectric constant of the particle.

66. The method of claim 60 wherein the planar front moves in a direction perpendicular to the interference planes.

67. The method of claim 60 wherein the planar fronts move through a volume.

68. The method of claim 60 wherein the particles and media are contained in a sample volume.

69. The method of claim 68 wherein the sample volume is a three dimensional volume.

70. A method for separating a population of particles according to size comprising the steps of:

subjecting the particles to an optical gradient pattern having a defined spatial periodicity,

moving the gradient relative to the particles,

wherein the improvement comprises selecting the spatial periodicity of the gradient to have a differential effect on differently sized particles.

71. The method of claim 70 wherein certain of the particles are smaller than the spatial periodicity of the gradient and certain of the particles are larger than the period.

72. The method of claim 71 wherein the larger particles are larger than the spatial periodicity of the gradient.

73. The method of claim 70 further including the step of varying the osmotic properties of the medium to change the size of the particles.

74. A method for separating particles comprising the steps of:

flowing the particles within a first constrained path, the first constrained path having an input and an output, and a sorting region, the sorting region coupling to a second constrained path, the second constrained path including an output,

illuminating the sorting region with a moving optical gradient,

characterized in that certain of the particles flow in a laminar manner between the first inlet and the output of the first constrained path, and

selected particles are diverted from the first constrained path to the second constrained path under the force of the moving optical gradient.

75. The method of claim 74 wherein the constrained path is a channel.

76. The method of claim 74 wherein the constrained path is a plane.

77. An apparatus for collecting optically sorted particles comprising:

a first surface adapted to support a plurality of particles,

an optical illumination system for subjecting the particles to a moving gradient force to cause the separation of the particles from the first surface, and

a second adhesive surface for adhering the separated particles to the second surface.

78. The apparatus of claim 77 wherein the adhesive surface has a specific affinity.

79. The apparatus of claim 77 wherein the adhesive surface has a non-specific affinity.

80. The apparatus of claim 77 wherein the first surface is planar.

81. The apparatus of claim 77 wherein the first surface is parallel to the second surface.

82. A method for analysis or separation of a plurality of particles:
selecting a wavelength for illumination based upon an analysis of absorption spectra,
illuminating the particles with the selected wavelength,
considering response of particles to multiple wavelengths,
selecting wavelengths based on desired parameter,
illuminating population to obtain optimized differential motion.

83. The method of claim 82 wherein the step of selecting the wavelength includes use of the spectral response.

84. The method of claim 83 where the selection of the wavelength is adjacent to a region comprising a local absorption maximum in the absorption spectra.

85. The method of claim 82 wherein the step of selecting the wavelength includes the use of empirical data.

86. A method for separating particles having different dielectric constants comprising the steps of:

separating the particles in a medium having a dielectric constant chosen to enhance the sensitivity of the discrimination between the particles, and

changing the medium to one having a dielectric constant which causes faster separation between the particles.

87. The method for separating particles of claim 86 wherein the sensitivity is enhanced by utilizing a medium having a dielectric constant which is closer to one species of particle than the other.

88. The method for separating particles of claim 86 wherein the sensitivity is enhanced by utilizing a medium where the difference in dielectric constant between the medium and the first particle is substantially the same as the difference in dielectric constants between the particles.

89. A device for optically sorting particles comprising:

an inlet for receiving a fluidic media including the particles,

a first fluidic path in communication with the inlet, the path including a first sorting region, the first sorting region including at least two outlets,

an illumination system for providing an optical moving gradient at the first sorting region,

a second fluidic path connected to at least one of the outlets from the first sorting region,

a second sorting region coupled to the second fluidic path, the region having at least two outlets, and

a second optical moving gradient for illuminating the second sorting region, to sort particles between the at least two outlets of the second sorting region.

90. The device of claim 89 wherein a feedback loop is provided.

91. The device of claim 89 wherein the first junction comprises a T junction.

92. The device of claim 89 wherein the first junction comprises a H junction.

93. The device of claim 89 wherein the first junction comprises a X junction.

94. The device of claim 89 wherein the first junction comprises a Y junction.

95. The device of claim 89 wherein the second output of the first sorting region is connected to a third sorting region.

96. The device of claim 95 wherein the third sorting region is illuminated by an optical moving gradient.

97. The device of claim 89 wherein the second optical moving gradient is formed by the same illumination system which generates the first optical moving gradient.

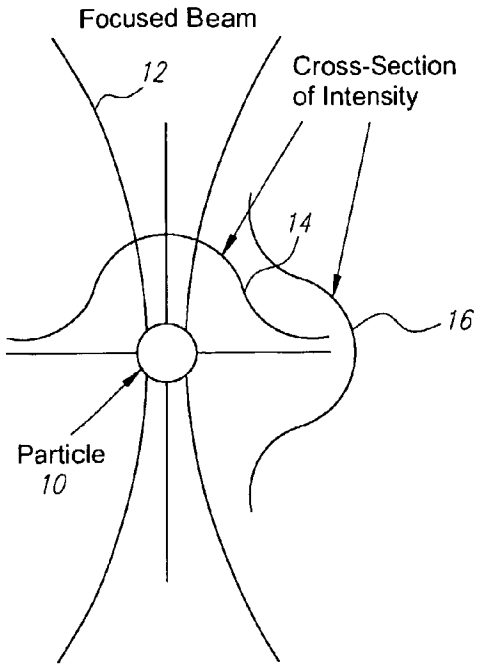
98. The device of claim 89 wherein the second optical moving gradient is formed by a different illumination system than the one which generates the first optical moving gradient.

99. The device of claim 89 further including a pumping system.

100. The device of claim 89 further including a plurality of reservoirs to hold sorted particles.

101. The device of claim 89 further including a sensor disposed to detect the movement of particles.

102. The device of claim 89 wherein the illumination system includes a laser.



$$F_{\nabla} = 2\pi \cdot r^3 \frac{\sqrt{\varepsilon_B}}{c} \left(\frac{\varepsilon - \varepsilon_B}{\varepsilon + 2\varepsilon_B} \right) (\nabla \cdot I)$$

F_{∇} = Optical force on particle towards higher intensity

r = Radius of particle

ε_B = Dielectric constant of backround medium

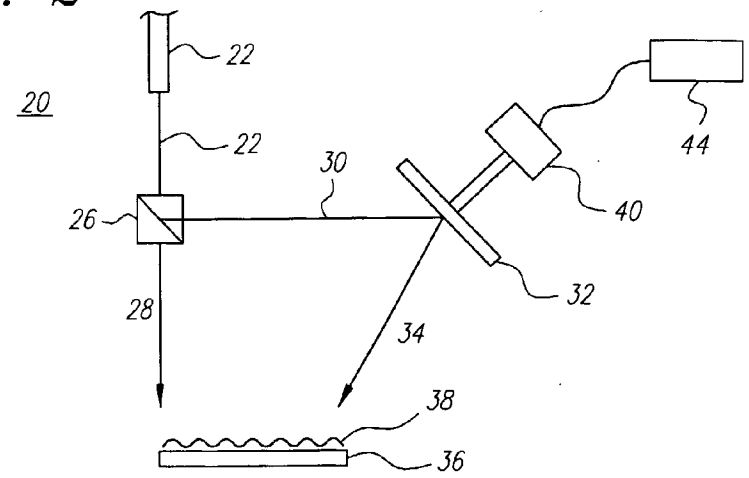
ε = Dielectric constant of particle

I = Light intensity (W/cm^2)

∇ = Spatial derivative

FIG. 1

FIG. 2



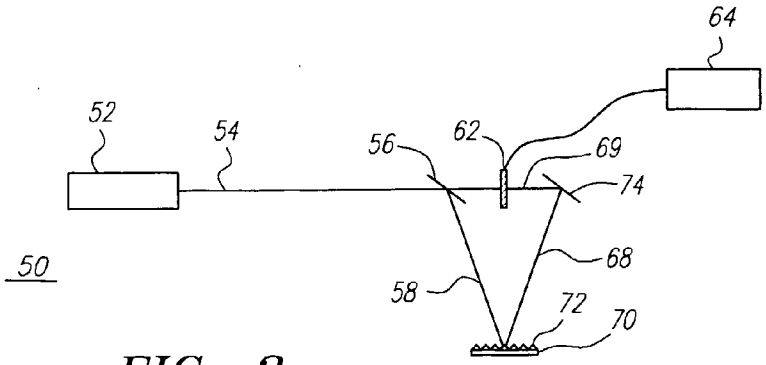


FIG. 3

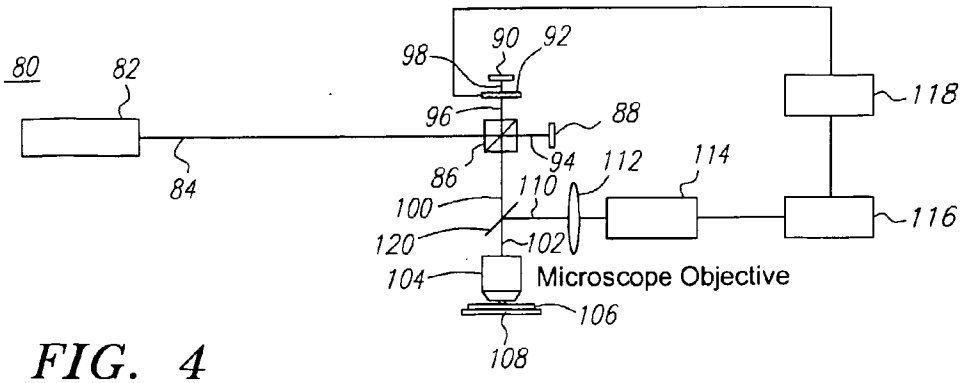


FIG. 4

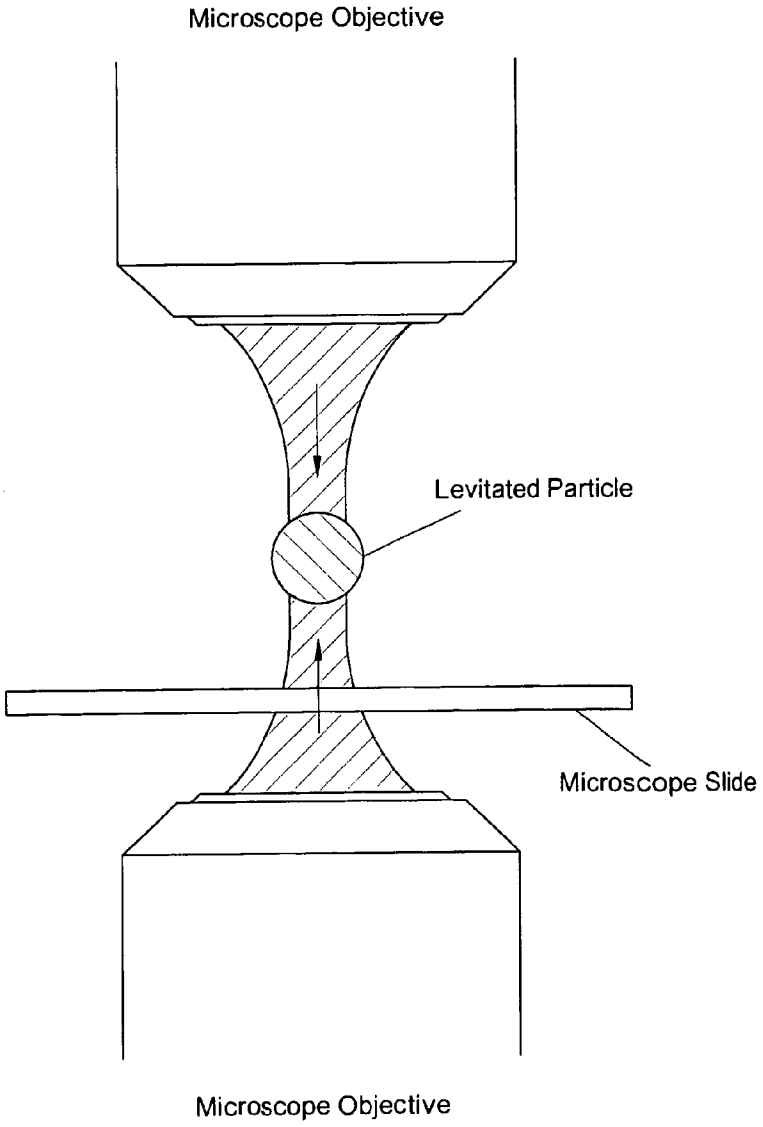

Microscope Objective
Levitated Particle
Microscope Slide
Microscope Objective

FIG. 4A

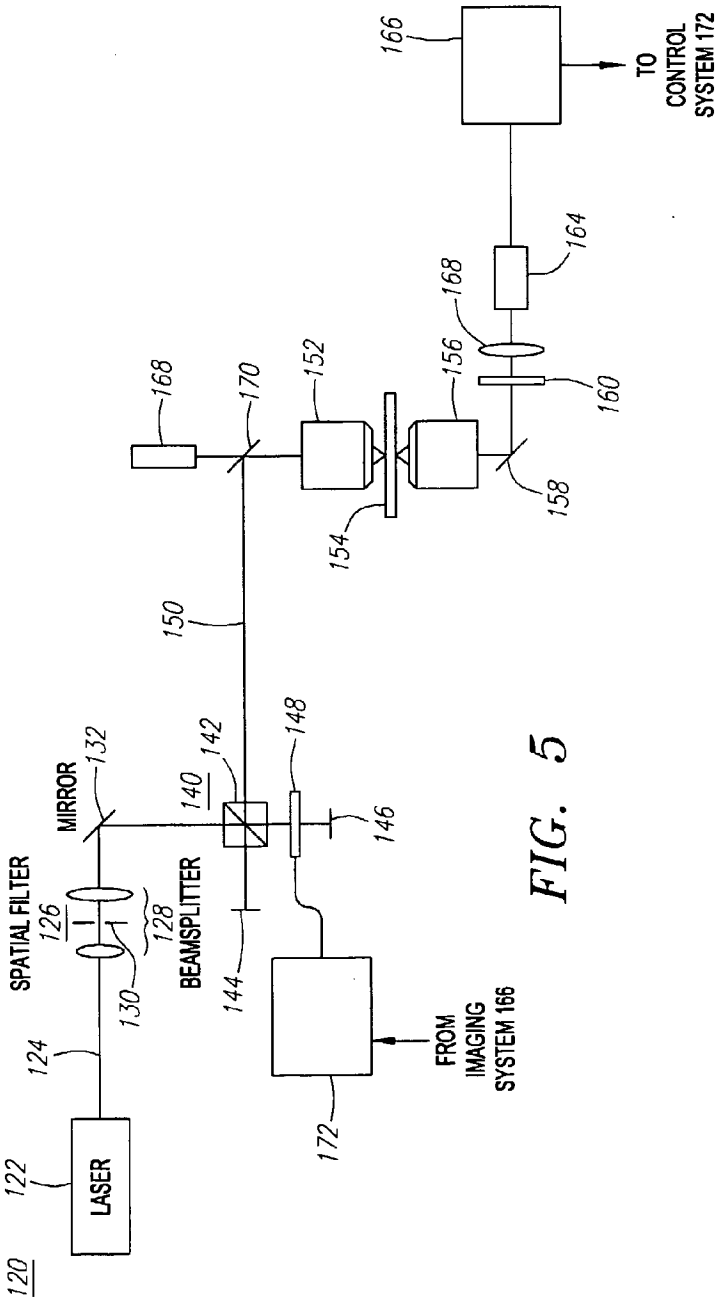

120
122
LASER
124
SPATIAL FILTER
126
128
130
MIRROR
132
140
142
BEAMSPLITTER
144
146
148
150
152
154
156
158
160
164
166
168
170
172
TO CONTROL SYSTEM 172
FROM IMAGING SYSTEM 166

FIG. 5

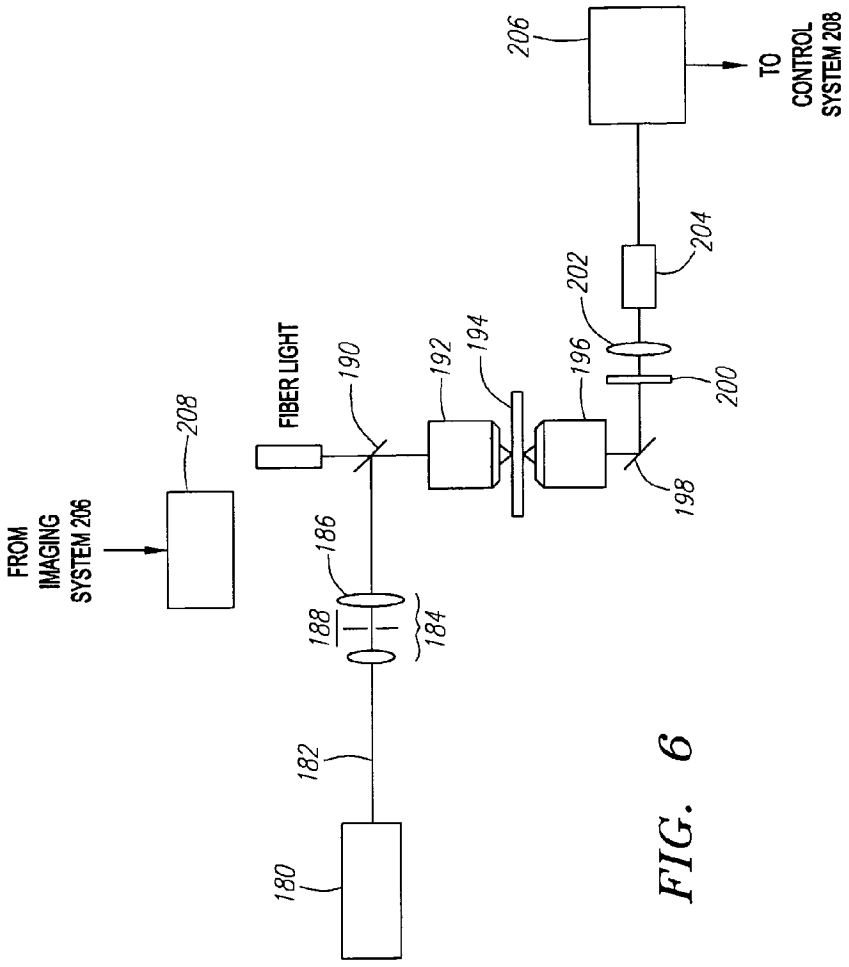

206
TO CONTROL SYSTEM 208
204
202
196
200
198
194
192
190
FIBER LIGHT
208
FROM IMAGING SYSTEM 206
186
188
184
182
180

FIG. 6

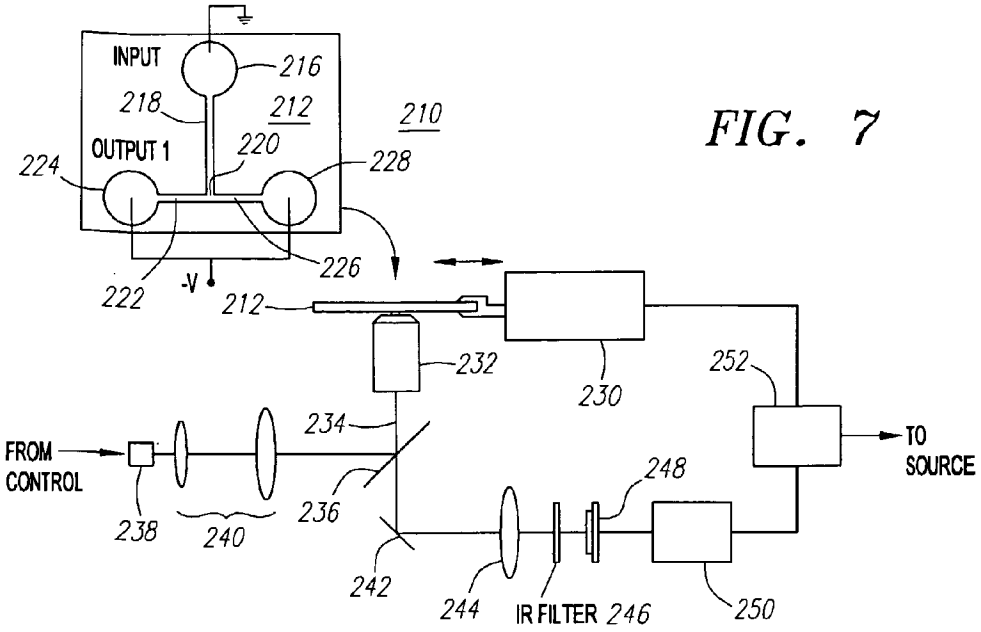

INPUT
216
218
212
210
OUTPUT 1
220
228
224
226
222
-V
212
232
230
252
234
FROM CONTROL
TO SOURCE
248
238
240
236
242
244
IR FILTER 246
250

FIG. 7

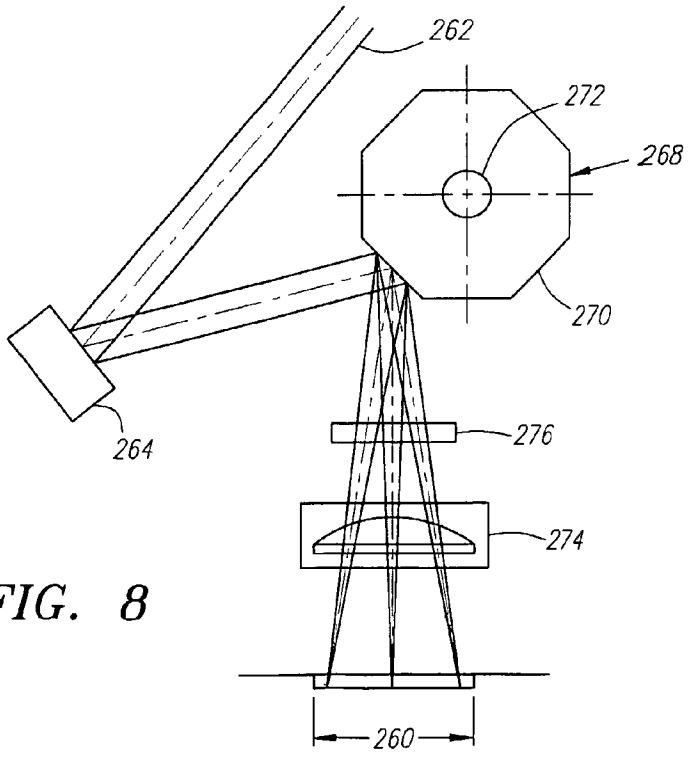

262
272
268
270
264
276
274
260

FIG. 8

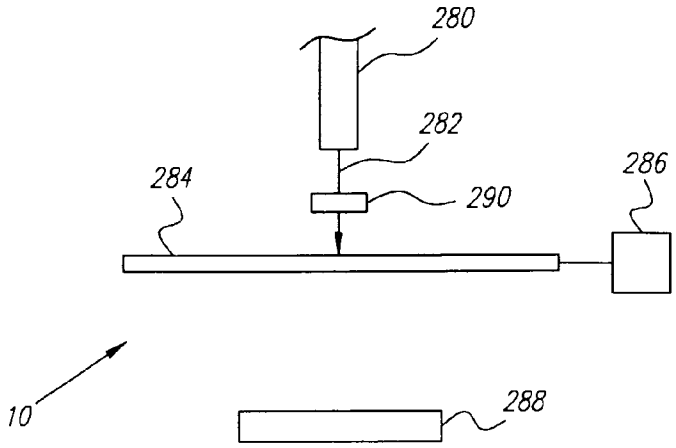


FIG. 9A

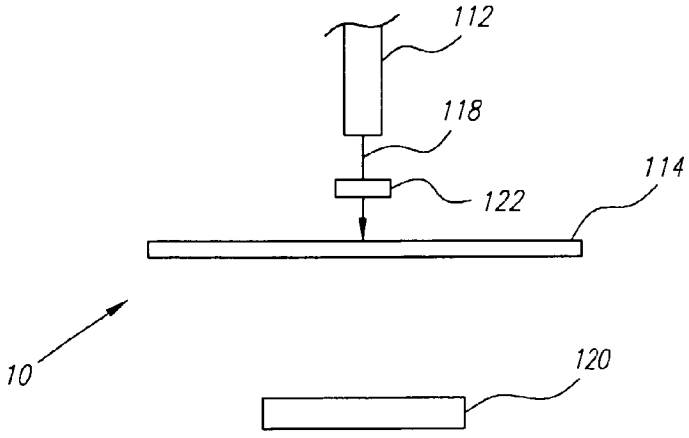


FIG. 9B

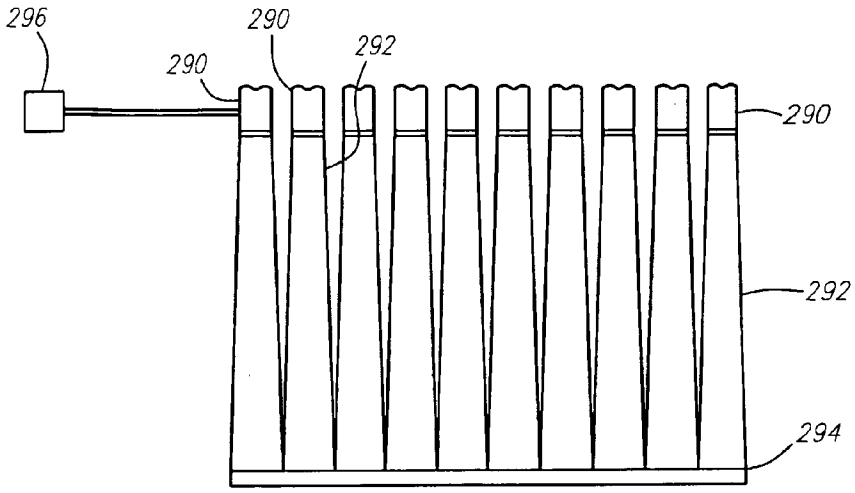


FIG. 10

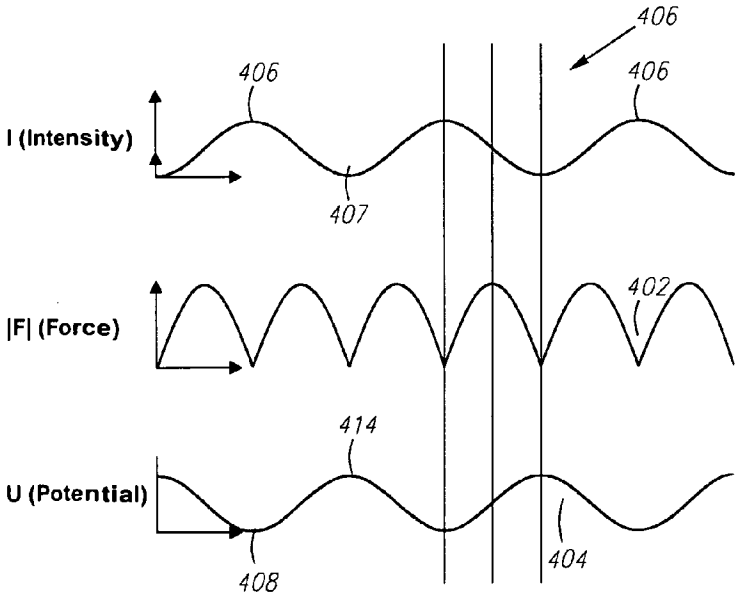


FIG. 11A

FIG. 11B

FIG. 11C

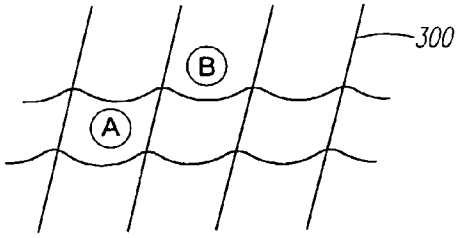


FIG. 12A

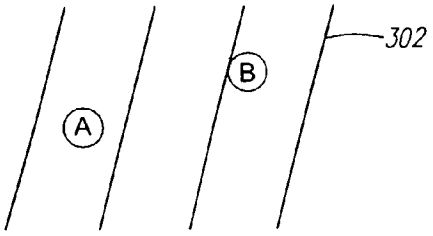


FIG. 12B

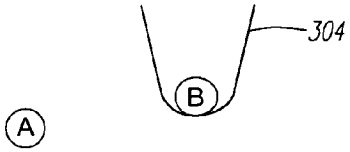


FIG. 12C

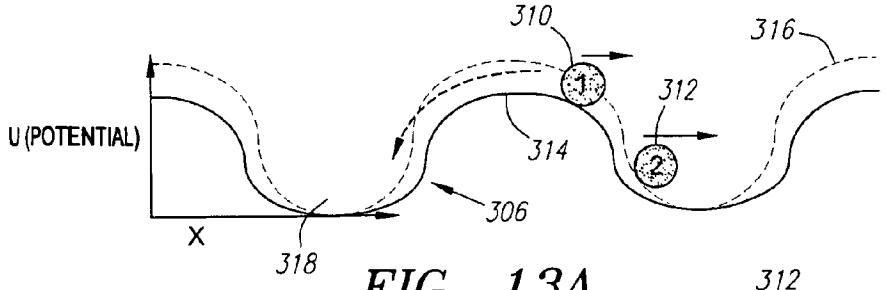


FIG. 13A

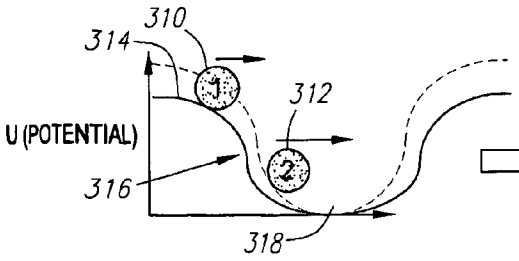


FIG. 13B

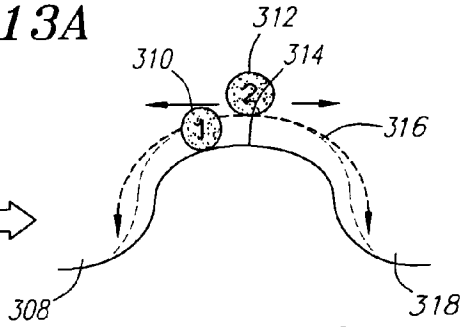


FIG. 13C

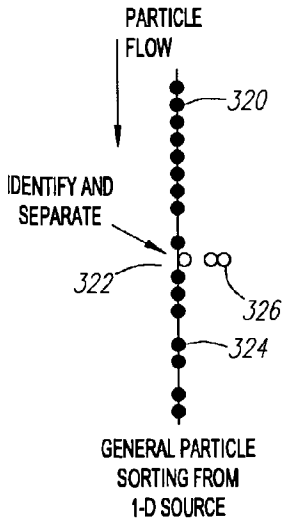


FIG. 14A

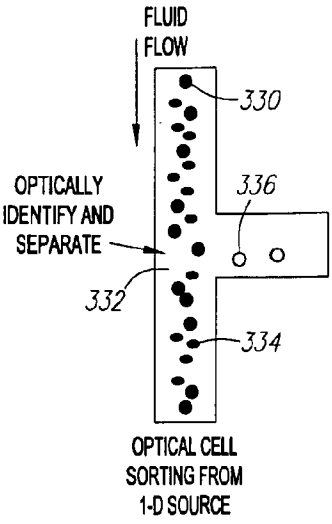


FIG. 14B

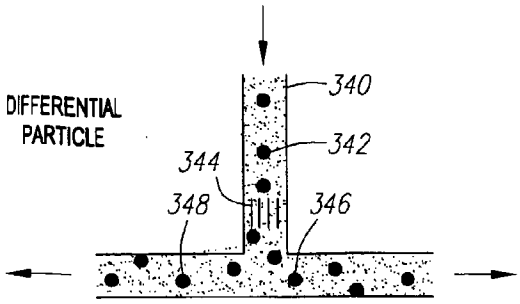


FIG. 15

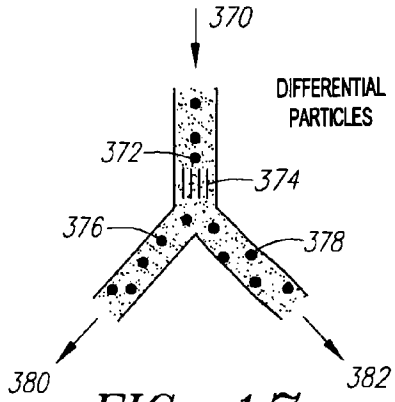


FIG. 17

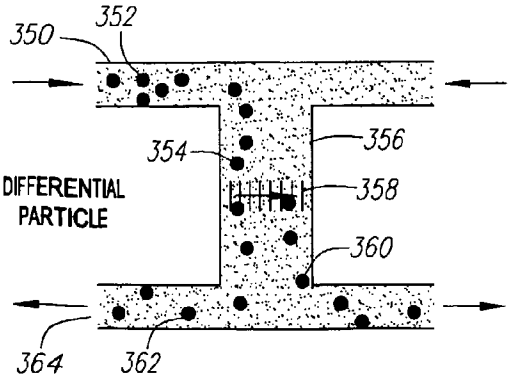


FIG. 16

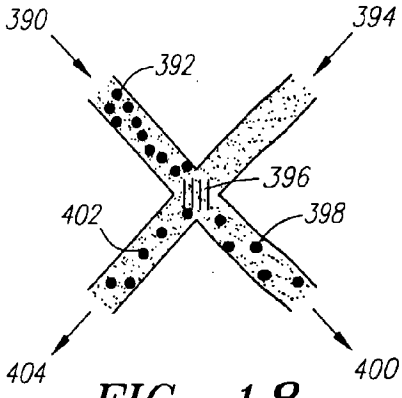


FIG. 18

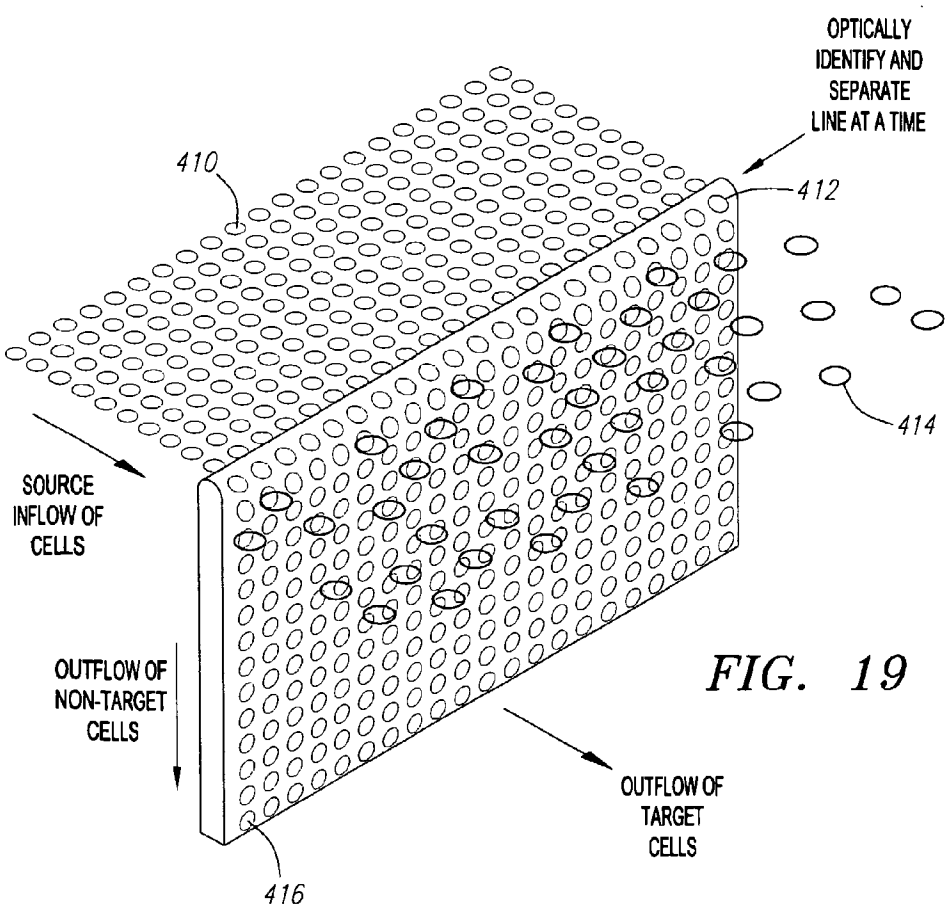


FIG. 19

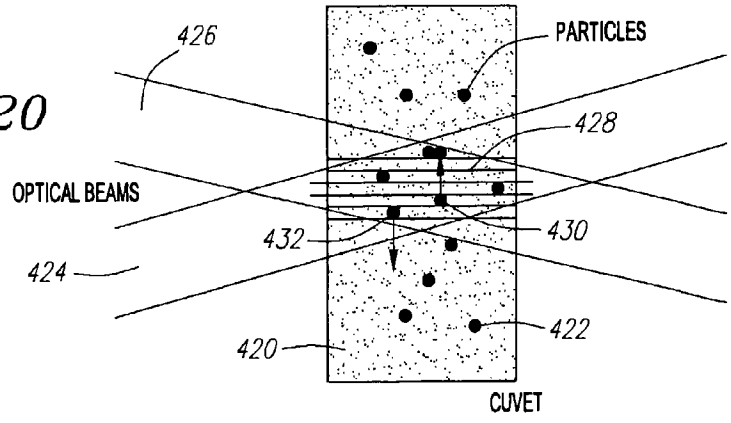


FIG. 20

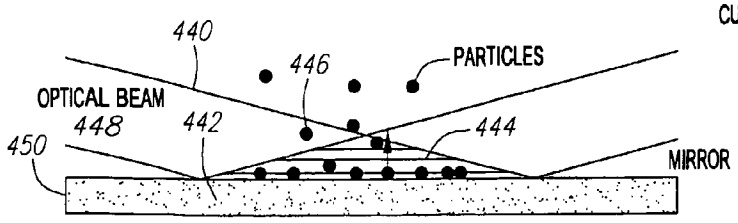


FIG. 21

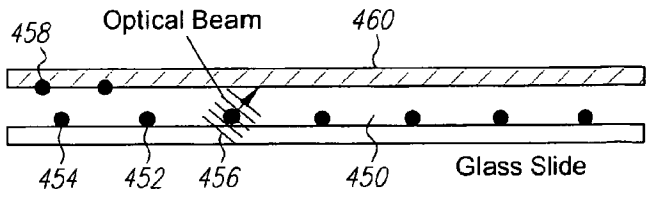


FIG. 22

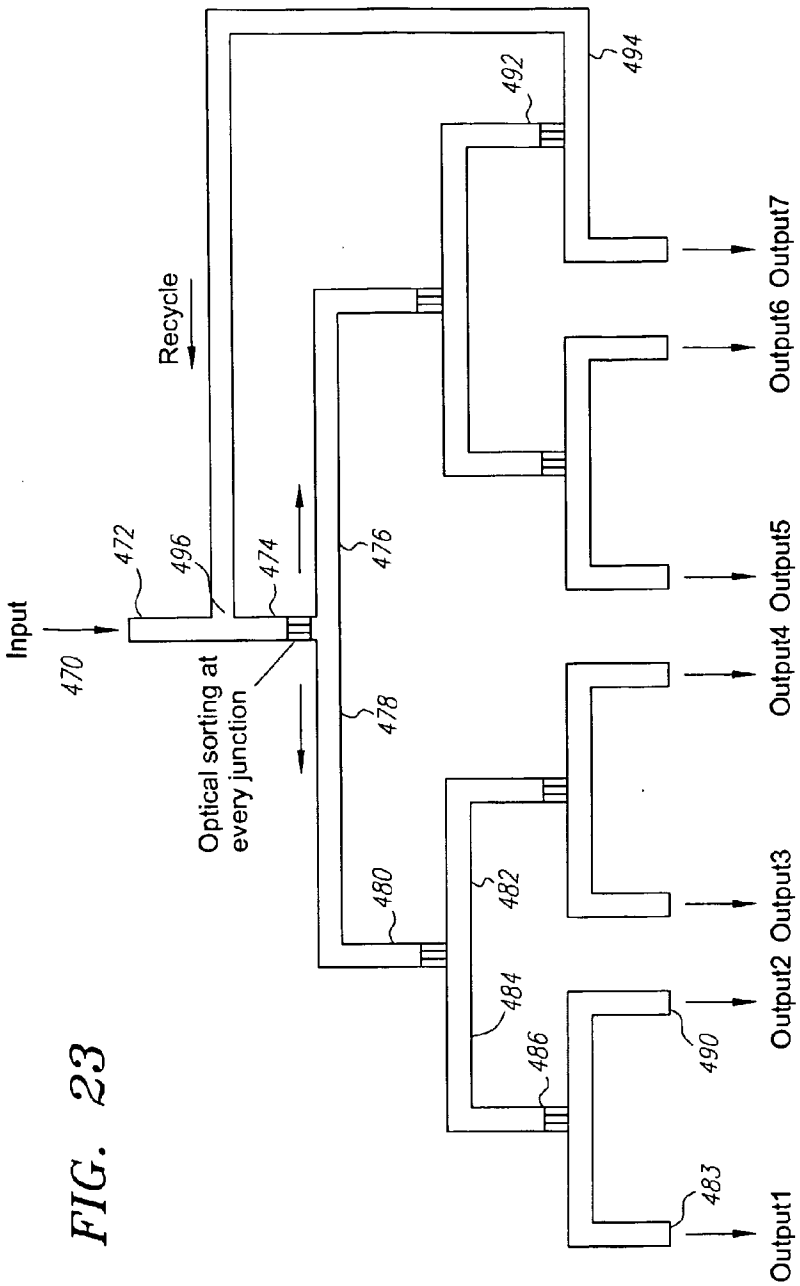

Input
470
472
496
Recycle
474
476
478
Optical sorting at every junction
480
482
484
486
483
490
492
494
Output1
Output2
Output3
Output4
Output5
Output6
Output7

FIG. 23

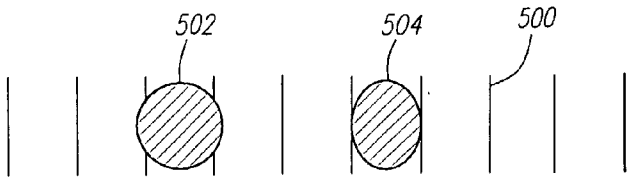


FIG. 24

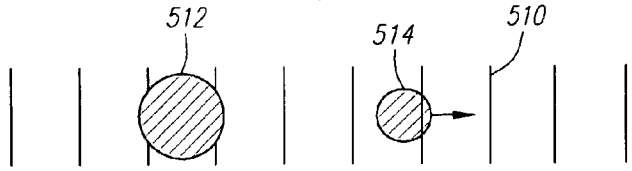


FIG. 25

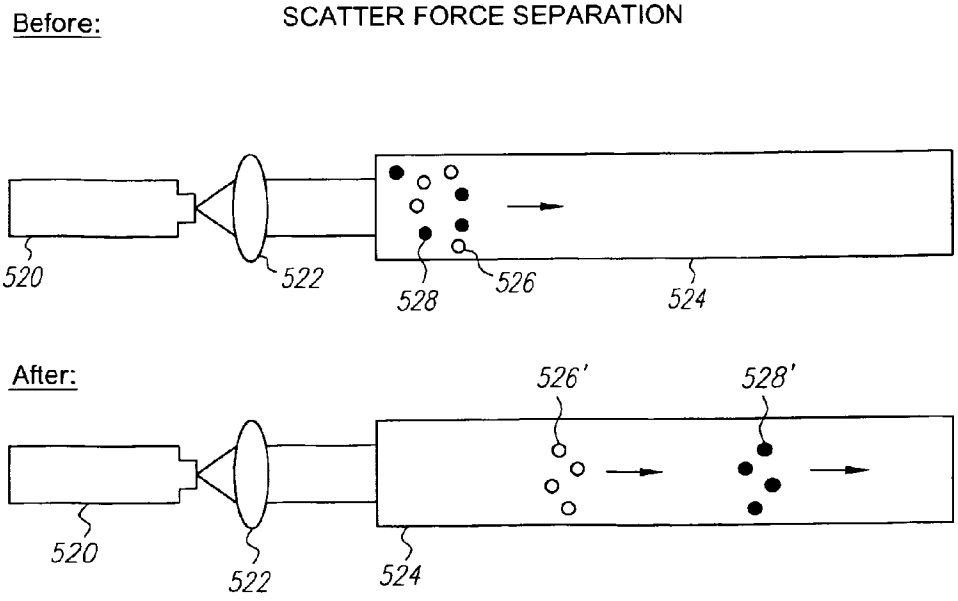
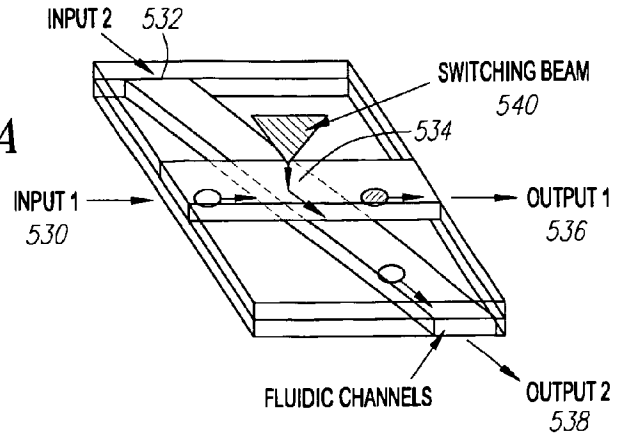


FIG. 26

FIG. 27A



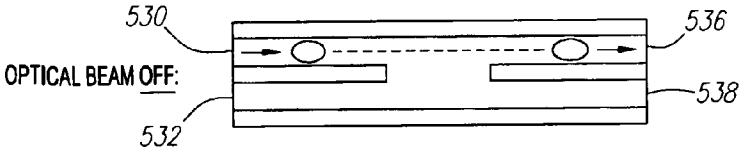


FIG. 27B

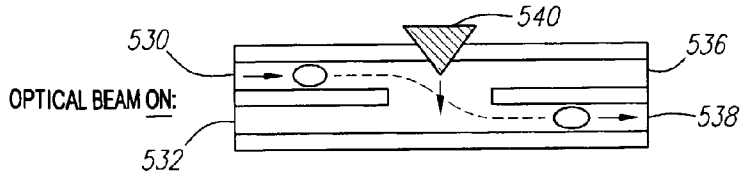


FIG. 27C

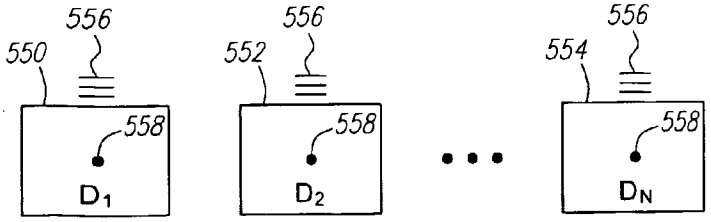


FIG. 28

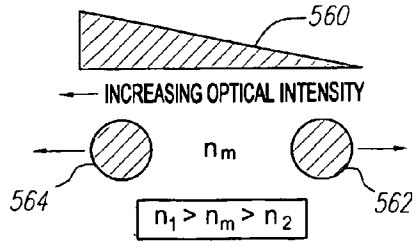


FIG. 29

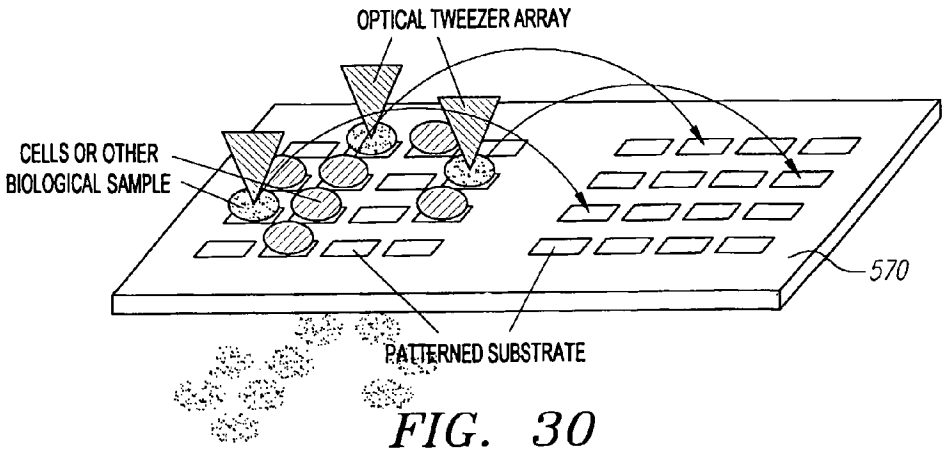

OPTICAL TWEEZER ARRAY
CELLS OR OTHER
BIOLOGICAL SAMPLE
570
PATTERNED SUBSTRATE

FIG. 30

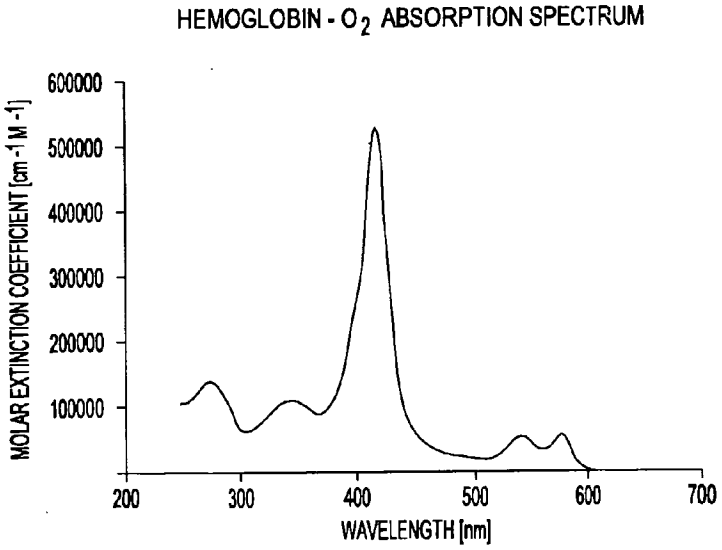

HEMOGLOBIN - O_2 ABSORPTION SPECTRUM
MOLAR EXTINCTION COEFFICIENT [cm -1 M -1]
600000
500000
400000
300000
200000
100000
200
300
400
500
600
700
WAVELENGTH [nm]

FIG. 31

FIG. 32

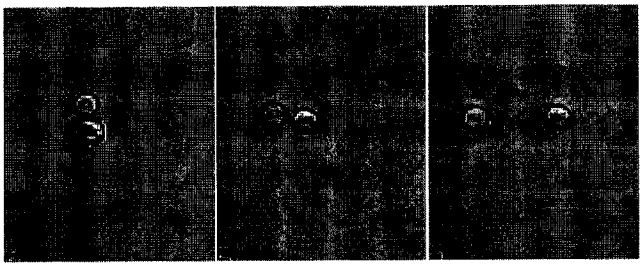


FIG. 33

Before After Difference

FIG. 34

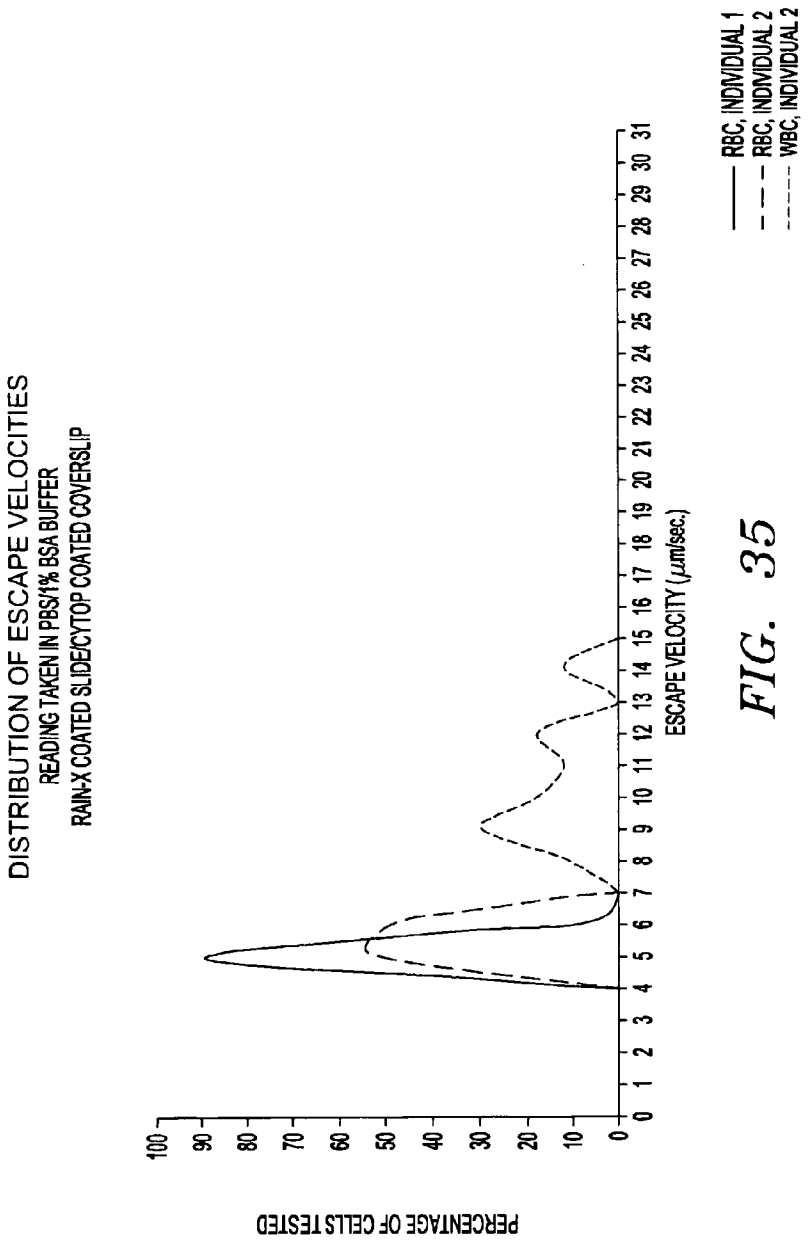

DISTRIBUTION OF ESCAPE VELOCITIES
READING TAKEN IN PBS/1% BSA BUFFER
RAIN-X COATED SLIDE/CYTOP COATED COVERSLIP
PERCENTAGE OF CELLS TESTED
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
ESCAPE VELOCITY (μm/sec.)
RBC, INDIVIDUAL 1
RBC, INDIVIDUAL 2
WBC, INDIVIDUAL 2

FIG. 35

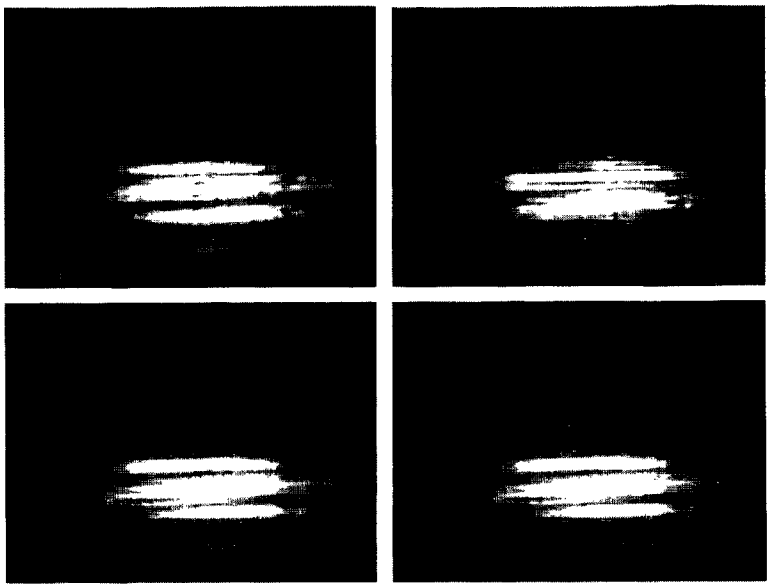


FIG. 36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/51001

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :B07C 5/02
US CL :436/161, 164, 165, 177, 178; 422/82.05; 209/11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/161, 164, 165, 177, 178; 422/82.05; 209/11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, CAPLUS
search terms: optophoresis, optical phoresis, optical gradient force, optical scattering force

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,170,890 A (WILSON et al) 15 December 1992, entire document.	1-102
A	US 5,950,071 A (HAMMOND et al) 07 September 1999, entire document.	1-102
A	US 6,280,960 A (CARR) 28 August 2001, entire document.	1-102
A	WO 01/05514 A1 (LOCK et al) 25 January 2001, entire document.	1-102
A	WO 00/23825 A3 (RENN et al) 27 April 2000, entire document.	1-102

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 20 JUNE 2002	Date of mailing of the international search report 18-07-2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JEFFREY R. SNAY DEBORAH THOMAS PARALEGAL SPECIALIST Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/51001

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SASAKI, K. et al, "Pattern formation and flow control of fine particles by laser-scanning micromanipulation" Optics Letters, Vol. 16, No. 19, October 1991, pages 1463-1465.	1-102
A	ASHKIN, A. "Acceleration and Trapping of Particles by Radiation Pressure" Physical Review Letters, Vol. 24, No. 1, January 1970, pages 156-159.	1-102
A	MISAWA, H. et al, "Spatial Pattern Formation, Size Selection, and Directional Flow of Polymer Latex Particles by Laser Trapping Technique" Chemistry Letters, 1991, pages 469-472.	1-102
A	MASTON, T.G. et al, "Optical Measurements of Frequency-Dependent Linear Viscoelastic Moduli of Complex Fluids" Physical Review Letters, Vol. 74, No. 7, February 1995, pages 1250-1253.	1-102
A	LAI, G. et al, "Determination of Spring Constant of Laser-Trapped Particle by Self-Mixing Interferometry" Proceedings of SPIE, Vol. 3921 (2000), pages 197-204.	1-102
A	DUFRESNE, E.R. et al, "Optical Tweezer Arrays and Optical Substrates Created with Diffractive Optics" Review of Scientific Instruments, Vol. 69, No. 5, May 1998, pages 1974-1977.	1-102

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

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7 November 2002 (07.11.2002)

PCT

(10) International Publication Number
WO 02/087792 A1

(51) International Patent Classification[7]: B07C 5/02

(21) International Application Number: PCT/US01/51001

(22) International Filing Date:
9 November 2001 (09.11.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/845,245 27 April 2001 (27.04.2001) US

(71) Applicant *(for all designated States except US)*: **GENOPTIX, INC.** [US/US]; 3398 Carmel Mountain Road, San Diego, CA 92121 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WANG, Mark, M.** [US/US]; 8090 Regents Road #302, San Diego, CA 92122 (US). **TU, Eugene** [US/US]; 3527 Lark Street, San Diego, CA 92103 (US). **PESTANA, Luis, M.** [US/US]; 4123 Avati Drive, San Diego, CA 92117 (US). **SENYEI, Anfrew, E.** [US/US]; 1547 eL Camino Del Teatro, La Jolla, CA 92037 (US). **O'CONNELL, James, P.** [US/US]; 682 Hoska Drive, Del Mar, CA 92067 (US). **NOVA, Tina, S.** [US/US]; 18299 Lago Vista, Rancho Santa Fe, CA 92067 (US). **LYKSTAD, Kristie, L.** [US/US]; 18622 Caminito Cantilena, #309, San Diego, CA 92128 (US). **HALL, Jeffrey, M.** [US/US]; 7226 Columbine Drive, Carlsbad, CA 92009 (US). **BUTLER, William, F.** [US/US]; 8519 Sugarman Drive, La Jolla, CA 92037 (US).

(74) Agents: **MURPHY, David, B.** et al.; O'Melveny & Myers LLP, 114 Pacifica, Suite 100, Irvine, CA 92618-3318 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(48) Date of publication of this corrected version:
20 November 2003

(15) Information about Correction:
see PCT Gazette No. 47/2003 of 20 November 2003, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/087792 A1

(54) Title: METHODS AND APPARATUS FOR USE OF OPTICAL FORCES FOR IDENTIFICATION, CHARACTERIZATION AND/OR SORTING OF PARTICLES

(57) Abstract: Apparatus and methods are provided for interacting light with particles, including but not limited to biological matter such as cells in unique and highly useful ways. Optophoresis consists of subjecting particles to various optical forces, especially optical gradient forces, and more particularly moving optical gradient forces, so as to obtain useful results. In biology, this technology represents a practical approach to probing the inner workings of a living cell, preferably without any dyes, labels or other markers. In one aspect, a particle may be characterized by determining its optophoretic constant or signature. For example, a diseased cell has a different optophoretic constant from a healthy cell, thereby providing information, or the basis for sorting. In the event of physical sorting, various forces may be used for separation, including fluidic forces, such as through the use of laminar flow, or optical forces, or mechanical forces, such as through adhesion. Various techniques for measuring the dielectric constant of particles are provided.

Doc Code: PET.AUTO Document Description: Petition automatically granted by EFS-Web	PTO/SB/140 U.S. Patent and Trademark Office Department of Commerce

Electronic Petition Request	**PETITION TO WITHDRAW AN APPLICATION FROM ISSUE AFTER PAYMENT OF THE ISSUE FEE UNDER 37 CFR 1.313(c)**
Application Number	14169927
Filing Date	31-Jan-2014
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	KIET NGUYEN
Attorney Docket Number	81527.0018
Title	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

An application may be withdrawn from issue for further action upon petition by the applicant. To request that the Office withdraw an application from issue, applicant must file a petition under this section including the fee set forth in § 1.17(h) and a showing of good and sufficient reasons why withdrawal of the application from issue is necessary.

APPLICANT HEREBY PETITIONS TO WITHDRAW THIS APPLICATION FROM ISSUE UNDER 37 CFR 1.313(c).

A grantable petition requires the following items:
(1) Petition fee; and
(2) One of the following reasons:
(a) Unpatentability of one or more claims, which must be accompanied by an unequivocal statement that one or more claims are unpatentable, an amendment to such claim or claims, and an explanation as to how the amendment causes such claim or claims to be patentable;
(b) Consideration of a request for continued examination in compliance with § 1.114 (for a utility or plant application only); or
(c) Express abandonment of the application. Such express abandonment may be in favor of a continuing application, but not a CPA under 37 CFR 1.53(d).

Petition Fee

◯	Small Entity
◯	Micro Entity
◉	Regular Undiscounted

Reason for withdrawal from issue

◯ One or more claims are unpatentable ◉ Consideration of a request for continued examination (RCE) (List of Required Documents and Fees) ◯ Applicant hereby expressly abandons the instant application (any attorney/agent signing for this reason must have power of attorney pursuant to 37 CFR 1.32(b)).
RCE request,submission, and fee. ☐ I certify, in accordance with 37 CFR 1.4(d)(4) that : The RCE request ,submission, and fee have already been filed in the above-identified application on ☒ Are attached.
THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES I certify, in accordance with 37 CFR 1.4(d)(4) that I am: ◉ An attorney or agent registered to practice before the Patent and Trademark Office who has been given power of attorney in this application. ◯ An attorney or agent registered to practice before the Patent and Trademark Office, acting in a representative capacity. ◯ A sole inventor ◯ A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application ◯ A joint inventor; all of whom are signing this e-petition

Signature	/Jean C. Edwards/
Name	Jean C. Edwards
Registration Number	41728

Electronic Patent Application Fee Transmittal

Application Number:	14169927
Filing Date:	31-Jan-2014
Title of Invention:	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
First Named Inventor/Applicant Name:	Daniel MUETH
Filer:	Jean Ceceille Edwards./Kenneth Sowers
Attorney Docket Number:	81527.0018

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Petition fee- 37 CFR 1.17(h) (Group III)	1464	1	140	140
Request for Continued Examination	1801	1	1200	1200
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1340**

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Decision Date : November 17, 2014

In re Application of :

Daniel MUETH

DECISION ON PETITION

UNDER CFR 1.313(c)(2)

Application No : 14169927

Filed : 31-Jan-2014

Attorney Docket No : 81527.0018

This is an electronic decision on the petition under 37 CFR 1.313(c)(2), filed November 17, 2014, to withdraw the above-identified application from issue after payment of the issue fee.

The petition is **GRANTED.**

The above-identified application is withdrawn from issue for consideration of a submission under 37 CFR 1.114 (request for continued examination). See 37 CFR 1.313(c)(2).

Petitioner is advised that the issue fee paid in this application cannot be refunded. If, however, this application is again allowed, petitioner may request that it be applied towards the issue fee required by the new Notice of Allowance.

Telephone inquiries concerning this decision should be directed to the Patent Electronic Business Center (EBC) at 866-217-9197.

This application file is being referred to Technology Center AU 2881 for processing of the request for continuing examination under 37 CFR 1.114 .

Office of Petitions

Electronic Acknowledgement Receipt

EFS ID:	20710107
Application Number:	14169927
International Application Number:	
Confirmation Number:	2069
Title of Invention:	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
First Named Inventor/Applicant Name:	Daniel MUETH
Customer Number:	13155
Filer:	Jean Ceceille Edwards./Kenneth Sowers
Filer Authorized By:	Jean Ceceille Edwards.
Attorney Docket Number:	81527.0018
Receipt Date:	17-NOV-2014
Filing Date:	31-JAN-2014
Time Stamp:	13:57:19
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1340
RAM confirmation Number	185
Deposit Account	505497
Authorized User	NEILS, PAUL F

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	2014-11-17-81527-0018_RCE-QPIDS-Petition_TTL.pdf	22270 2965bab3c692def450cd17f0a2b6d58f19084d61	no	1
Warnings:					
Information:					
2	Quick Path Information Disclosure Statement	2014-11-17-81527-0018_Request-QIPDS.pdf	438398 91c7d5d06ea53d179d40216c22804be48d585cb2	no	1
Warnings:					
Information:					
3	Quick Path Information Disclosure Statement	2014-11-17-81527-0018_QPIDS.pdf	27723 27c61daa372cdf55a2baa7099fe9418d5e90bec0	no	3
Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	2014-11-17-81527-0018_SB08A-QPIDS.pdf	613060 a4811195cc44fe6130c25f1873168fdef6c4215c	no	5
Warnings:					
Information:					
A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
5	Other Reference-Patent/App/Search documents	2014-11-17-81527-0018_JP-Office-Action.pdf	365446 79ca140178f6cb7388b07ea6171b06a28f925369	no	8
Warnings:					
Information:					
6	Non Patent Literature	2014-11-17-81527-0018_Takayama-et-al.pdf	578193 3449fcfc492788c4f8443c98d3c057ccf5332230	no	5
Warnings:					
Information:					
7	Foreign Reference	2014-11-17-81527-0018_WO9747390.pdf	716997 c1f503d2e3f2579ec2bdf2ba4d7bd7ae307363a9	no	57
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Information:					
8	Foreign Reference	2014-11-17-81527-0018_JPH05-26799.pdf	295540 2ffed79315518246bfcca49d447364179b2fa12e	no	5
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Warnings:					
Information:					
10	Foreign Reference	2014-11-17-81527-0018_WO9810267.pdf	971025 13df4d356f314aeee2df9adcaa014590751eeb99	no	62
Warnings:					
Information:					
11	Foreign Reference	2014-11-17-81527-0018_JPH11-508182.pdf	1868289 3a1c1b12b536a25312c7405117e44218aea348df	no	63
Warnings:					
Information:					
12	Foreign Reference	2014-11-17-81527-0018_JP2001-504936.pdf	1442085 db7d67a7144d403cc89232f047c7b92f3ef621bf	no	57
Warnings:					
Information:					
13	Foreign Reference	2014-11-17-81527-0018-JP2002-503334.pdf	1037883 58105502129cac2693902d998d4ef0d04c0044a2	no	68
Warnings:					
Information:					
14	Foreign Reference	2014-11-17-81527-0018_JP2000-512541.pdf	1772270 4193e6e5cf8e02e281315dfd2bc3ee4b5b4bc806	no	78
Warnings:					
Information:					
15	Foreign Reference	2014-11-17-81527-0018_WO9739338.pdf	656546 7046fe0779d7e4651c3f04f1991c73c14a8a25c1	no	54
Warnings:					
Information:					
16	Request for Continued Examination (RCE)	2014-11-17-81527-0018_RCE-QPIDS.pdf	697799 8434943453705a5763a7bf6aec2a0eccd586eff9	no	3
Warnings:					

Information:					
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Information:					
18	Foreign Reference	2014-11-17-81527-0018_WO2002-087792.pdf	10593461 c082536cb7fa3bc35b45840afe3f744605b8273c	no	85
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19	Petition automatically granted by EFS	petition-request.pdf	31564 b6b48e54dc5130897cefd83927988304c39d9989	no	2
Warnings:					
Information:					
20	Fee Worksheet (SB06)	fee-info.pdf	32412 bdbf0e92ea7af06494a5660dbce9a9e04622d3ff	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	33596526	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Daniel MUETH et al.	Attorney Docket No.: 81527.0018
Application No.: 14/169,927	Group Art Unit: 2881
Filed: January 31, 2014	Examiner: KIET TUAN NGUYEN
Customer No.: 13155	Confirmation No.: 2069

For: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION (as amended)

TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Please find enclosed:

- Request for Continued Examination
- Petition to Withdraw an Application from Issue after Payment of the Issue Fee Under 37 C.F.R. 1.313(c)
- Certification and Request for Consideration of an Information Disclosure Statement Filed after Payment of the Issue Fee Under the QPIDS Pilot Program
- Information Disclosure Statement along with form PTO/SB/08A, Japanese Office Action, and cited foreign references
- Fee payment of $1,520.00 (RCE Fee ($1,200.00), IDS Fee ($180.00), and Petition Fee ($140.00))

Please charge any deficiencies or credit any overpayments to Deposit Account No. 50-5497. In the event the credit card payment submitted herewith fails to process, please charge the filing fees to deposit account No. 50-5497.

Respectfully submitted,

/Jean C. Edwards/
Jean C. Edwards
Registration No. 41,728

13155)
EDWARDS NEILS PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, Virginia 20191
Telephone: 703-466-0150
Facsimile: 703-537-8149
Date: November 17, 2014

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	14169927
Filing Date	2014-01-31
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	KIET TUAN NGUYEN
Attorney Docket Number	81527.0018

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	1	2000-512541	JP	A	2000-09-26	University of Washington	WO 97/47390	☒
	2	2001-504936	JP	A	2001-04-10	University of Washington	WO 97/39338	☒
	3	2002-503334	JP	A	2002-01-29	Technical University of Denmark	WO 98/10267	☒

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14169927
	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	2881
	Examiner Name	KIET TUAN NGUYEN
	Attorney Docket Number	81527.0018

	4	H11-508182	JP	A	1999-07-21	University of Washington	WO 97/00442	☒
	5	H05-26799	JP	A	1993-02-02	Nippon Steel Corporation	Abstract	☒
	6	97/39338	WO	A1	1997-10-23	University of Washington		☒
	7	98/10267	WO	A1	1998-03-12	Technical University of Denmark		☒
	8	97/00442	WO	A1	1997-01-03	University of Washington		☒
	9	02/087792	WO	A1	2002-11-07	Genoptix, Inc.		☒
	10	03/062867	WO	A1	2003-07-31	Genoptix, Inc.		☒
	11	97/47390	WO	A1	1997-12-18	University of Washington		☒

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NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	TAKAYAMA et al., "Patterning Cells and Their Environments Using Multiple Laminar Fluid Flows in Capillary Networks." Proceedings of National Academy of Sciences,USA 96 (1999)	☒

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14169927
	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	2881
	Examiner Name	KIET TUAN NGUYEN
	Attorney Docket Number	81527.0018

	2	Final Notice of Reasons for Rejection, issued by Japanese Patent Office on October 28, 2014 in related Japanese Patent Application No. 2011-256171	☒

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	14169927
Filing Date	2014-01-31
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	KIET TUAN NGUYEN
Attorney Docket Number	81527.0018

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Jean C. Edwards/	Date (YYYY-MM-DD)	2014-11-17
Name/Print	Jean C. Edwards, Esq.	Registration Number	41728

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

RYUKA
with Free Vision

Organized Translation of "Final Notice of Reasons for Rejection"

Patent Application Number: 2011-256171
Agent for Applicant: RYUKA IP Law Firm
Date of Delivery: October 28, 2014

The present application should be rejected for the following reasons. The applicant may present an argument refuting the reasons for rejection within three months from the date of delivery of this notice.

REASON FOR FINALITY

This notice was necessitated by the amendment made in response to a previous Notice of Reasons for Rejection.

REASONS

1. Comparisons with the Prior Art (Art. 29)

Claim	Ground*	Cited Reference	Examiner's Remarks
1-40 and 42-45	Y	1	Cited References 1 and 2 disclose an apparatus for separating a liquid component containing particles into constituent components, wherein the apparatus includes an inlet for a plurality of flows including a first flow and a second flow and a flow path through which the flows travel in contact with each other. The apparatus also includes a separating region to separate a constituent component such as the particles using distinguishing and removing means provided in the flow path. Cited References 1 and 2 also disclose that the particles may be cells, the distinguishing and removing means may be diffusion, an electric field, an optical property, a magnetic property, a temperature gradient, a pressure gradient, a concentration gradient and the like. Cited Reference 3 discloses that, in a flow path through which first, second and third flows merge and flow from first, second and third inlets, a non-turbulent interface may be formed between the individual flows. Cited Reference 3 also discloses that, even if a larger number of flows merge and flow into the single flow path from a larger number of inlets, a non-turbulent interface may be still formed between the individual flows. Cited References 4 and 5 disclose an apparatus for exposing a liquid mixture containing a plurality of types of particles, such as a plurality of types of cells, to an optical force in a flow path to separate at least one type of particles. The apparatus is a computerized work

			station including a plurality of channels into which the liquid mixture or a solution to be separated is injected, a flow path through which the liquid mixture and the solution to be separated flow in a parallel direction, a channel for collecting a liquid containing the particles separated from the liquid mixture, optical detecting means, a control system to generate a desired optical force pattern in the system, and analysis software. Cited Reference 4 and 5 also disclose that the computerized work station includes a sample station, an optical platform such as a laser and system hardware and that the system performs real-time analysis and tests under sufficient computer control. Cited Reference 6 discloses an apparatus for applying an optical force to a flow system containing different types of particles or cells to distinguish and separate their particle sizes, wherein non-desired particles are destroyed by increasing the optical output of the laser beam applied to the particles. The inventions disclosed in Cited References 1-6 are all directed to a method for processing a liquid containing a plurality of components within a flow path and separating at least one component. The person skilled in the art would have easily appreciated that, in the apparatus for separating at least one component from a liquid containing a mixture of particles disclosed in Cited References 1-3, the computer to select at least one component, which is included in the apparatus disclosed in Cited References 4 and 5, is used, that the optical detecting means, which is also disclosed in Cited References 4 and 5, is provided, and that the means for destroying non-desired particles using laser is provided in accordance with the disclosure of Cited Reference 6. In addition, the person skilled in the art would have easily appreciated that the flow velocities of the respective liquids are controlled by the pumps connected to the respective channels and that the detection is performed using substances such as tags designed for particular particles. Furthermore, no special effects are produced by the present invention that does not have any mechanism for applying an external stress to at least one component to select the component from a liquid mixture. For the reasons stated above, the inventions as set forth in Claims 1-40 and 42-45 would have been easily made by the person skilled in the art based on the inventions disclosed in Cited References 1-6 and the common technical knowledge prior to the priority date of the present application.
46-58	Y	1-8	Cited Reference 7 discloses an apparatus for detecting or separating a to-be-analyzed particle within a sample liquid using laminar flow in a flow path, wherein the to-be-analyzed particle flows through a V groove in a detecting unit and flows in a single line at the bottom of the V groove.

			Cited Reference 8 discloses an apparatus for extracting a desired particle from a liquid containing the particle, wherein, in an extraction channel in which a plurality of channels merge so that laminar flow constituted by a plurality of flows is formed, the flow path is smaller in width than the flow at the inlet and outlet thereof and thus constricted. In addition, Cited Reference 1 also discloses an apparatus including a flow path that is shaped as disclosed in Cited Reference 8. It is known to configure an apparatus such that, when a particle within a liquid, which is a sample, is examined and selected in a flow path, the width of the flow of the sample is constricted to facilitate the examination and selection of the particle as disclosed in Cited References 7 and 8. Considering this, the person skilled in the art would have easily appreciated that the flow path in the detecting unit is constricted so that the cells form a line in the method of selecting cells disclosed in Cited References 1-5. In addition, there are no special difficulties in, in such an apparatus, using the laser to destroy specific particles as disclosed in Cited Reference 6 and incorporating the computer that performs any control to select specific cells as disclosed in Cited References 4 and 5. Accordingly, the present invention does not produce special effects. For the reasons stated above, the inventions as set forth in Claims 46-58 would have been easily made by the person skilled in the art based on the inventions disclosed in Cited References 1-8 and the common technical knowledge prior to the priority date of the present application.

* Y: The invention would have been Easily Made (Art. 29.2).

List of Cited References

1. Japanese Patent Application Publication No. 11-508182
2. Japanese Patent Application Publication No. 2002-503334
3. Proceedings of the National Academy of Sciences of USA, 1999, Vol.96, pp.5545-5548
4. International Publication No. 2003/062867
5. International Publication No. 2002/087792
6. Japanese Patent Application Publication No. 5-26799
7. Japanese Patent Application Publication No. 2001-504936
8. Japanese Patent Application Publication No. 2000-512541

2. Enablement (Art. 36(4))

A person skilled in the art cannot easily work the inventions of claims since the specification does not disclose the following points in detail.

Claim	Examiner's Remarks
1-40 and 42-58	The specification discloses a method of guiding movement of particles between layers of laminar flow using a holographic optical trap to separate a desired particle, but does not describe the use of other mechanisms to separate a desired particle. Claim 1 does not specify any mechanism that acts on a desired component to move the desired component from the first flow to the plurality of additional flows. At the time of the filing of the present application, it is commonly known that a particular particle does not selectively and actively move between a plurality of flows flowing through a flow path at a constant flow velocity. Considering this, some external stress is necessary to move only a desired particle from a liquid mixture containing a plurality of particles between flows that constitute laminar flow. However, the apparatus as set forth in Claim 1, which has no mechanism configured to use force acting on a particular particle, is not capable of separating a desired particle. The same rejection applies to Claims 2-40 and 42-58. For the reasons stated above, the specification of the present application is neither clear nor sufficient to enable the person skilled in the art to work the inventions as set forth in Claims 1-40 and 42-58.

3. Claim Clarity (Art. 36(6)(ii))

The technical meaning of the following wording, phrases or statements in the Claims are unclear. Claims 14 and 46-58 are rejected as being indefinite.

Claims 14 and 46-58 are rejected as being indefinite.

Claim	Examiner's Remarks
14	The terms "flow plate" and "flat sorter" are not the terms widely used in the relating technical field. These terms are not clearly defined in the specification and thus indefinite. The same terms appear in Claim 55.
46	Referring to the recitation "said sample fluid is in a contiguous relationship ... with said buffer fluids," it is not clear how the sample fluid is related to the buffer fluids. Thus, Claim 46 is rejected as being indefinite.
46	Referring to the recitation "two orthogonal directions," it is not clear what are indicated by the term "two." Thus, Claim 46 is rejected as being indefinite.
46	Referring to the recitation "constricted in two orthogonal directions," it is not clear in what directions the sample fluid is constricted and how the sample fluid is constricted. Thus, Claim 46 is rejected as being indefinite.

ALLOWABLE CLAIMS

No reason has been found for rejecting the following claims:

Claim 41

When new reasons for rejection are found, the applicant will be notified thereof.

整理番号　S0-0012-4
発送番号　562790
発送日　　平成26年10月28日

拒絶理由通知書

特許出願の番号	特願２０１１－２５６１７１
起案日	平成２６年１０月２４日
特許庁審査官	伊達　利奈　　３９６０　４８００
特許出願人代理人	誠華国際特許業務法人　様
適用条文	第２９条第２項、第３６条

<<<<　　最　　後　　>>>>

この出願は、次の理由によって拒絶をすべきものです。これについて意見がありましたら、この通知書の発送の日から３か月以内に意見書を提出してください。

理　由

１．この出願の下記の請求項に係る発明は、その出願前に日本国内又は外国において、頒布された下記の刊行物に記載された発明又は電気通信回線を通じて公衆に利用可能となった発明に基いて、その出願前にその発明の属する技術の分野における通常の知識を有する者が容易に発明をすることができたものであるから、特許法第２９条第２項の規定により特許を受けることができない。

２．この出願は、発明の詳細な説明の記載が下記の点で、特許法第３６条第４項第１号に規定する要件を満たしていない。

３．この出願は、特許請求の範囲の記載が下記の点で、特許法第３６条第６項第２号に規定する要件を満たしていない。

記　　（引用文献等については引用文献等一覧参照）

・理由　　1
・請求項　　１－４０、４２－４５
・引用文献　　1
・備考
引用文献１及び２には、粒子を含む液体成分を構成成分に分離するための装置であって、第１及び第２の流れである複数の流れの注入口を備え、これらの流れが接触して流れる流路を備え、流路に設けられた差別除去手段によって粒子等の構成成分が分離される分離領域を有する装置が記載されている。当該装置において、粒子は細胞であってよいこと、差別除去手段は拡散、電場、光学的性質、磁性、温度勾配、圧力勾配、濃度勾配等であってよいことが記載されている。
引用文献３には、第１、第２及び第３の注入口から第１、第２及び第３の流れが合流して流れる流路において、各流れの間には非乱流状の界面が形成されることが記載されており、さらに多くの注入口から１つの流路に合流した流れであっても、各流れの間に非乱流状の界面を形成して流れることが記載されている。
引用文献４及び５には、複数種類の細胞等の複数種類の粒子を含む液体混合物を流路中で光学的力にさらすことによって少なくとも１つの粒子を分離する装置であって、液体混合物又は分離のための溶液を注入する複数のチャネル、液体混合物と分離のための溶液が平行して流れる流路、液体混合物から分離された粒子を含む液体を回収するチャネル、光学的検出手段、所望の光学的力パターンをシステム内に生成するための制御システム、解析ソフトウェアを含むコンピューター化されたワークステーションである装置が記載されている。そして、コンピューター化されたワークステーションは、サンプルステーション、レーザー等の光学プラットフォーム及びシステムハードウエアを含み、システムは十分なコンピューターの制御のもとにリアルタイムでの分析及びテストを行うことが記載されている。
引用文献６には、異なる粒子又は細胞を含む流れ系に光学的力を加えて粒径判別と分離を行う装置であって、所望でない粒子に対してはレーザビームの光出力を上げて照射して当該粒子を破壊する方法が記載されている。

引用文献１－６に記載されている発明は、いずれも複数成分を含む液体を流路中で処理して少なくとも１つの成分を分離する方法に係るものである。
引用文献１－３に記載されている粒子の混合物を含む液体から少なくとも１つの成分を分離する装置において、引用文献４及び５に記載されている装置に備えられている少なくとも１つの成分を選択する制御を行うコンピュータを用いること、及び、光学的検出手段を設けること、並びに、引用文献６の記載に基づいて所望でない粒子をレーザによって破壊する手段を設けることは、当業者が容易に想到することである。
そして、各液体の流速を各チャネルにつながるポンプによって制御すること、特定の粒子に対するタグ等の物質を用いて検出を行うことは、当業者であれば容易になし得ることである。
また、液体混合物から少なくとも１つの成分を選択するために、当該成分に外的応力を作用させる何らかの機構を備えていない本願発明によって、格別な効果がもたらされるとは認められない。
よって、請求項１－４０及び４２－４５に係る発明は、引用文献１－６に記載された発明及び本願優先日前の技術常識に基づいて、当業者が容易に発明をすることができたものである。

・理由　　１
・請求項　　４６－５８
・引用文献　　１－８
・備考
引用文献７には、試料液体中の分析物粒子を、流路中の層流を用いて検出又は分離する装置であって、検出部では分析物粒子はV溝を流れ、V溝の底に一列で流れることが記載されている。
引用文献８には、粒子を含む液体から所望の粒子を抽出するための装置であって、複数のチャネルが合流して複数の流れの層流を形成する抽出チャネルにおいて、流路が注入口及び流出口における流れの幅より狭く圧縮されている装置が記載されている。
そして、引用文献１にも引用文献８に記載されている形状の流路を備える装置が記載されている。
引用文献７及び８に記載されているように、試料である液体中の粒子を流路中で検査及び選択する場合には、試料の流れ幅を圧縮して各粒子について検査及び選択がしやすいように装置を構成することは周知であった。
そうすると、引用文献１－５に記載されている細胞を選択する方法において、検出部における流路を圧縮して細胞を整列させることは、当業者が容易に想到することである。
そして、そのような装置において、引用文献６に記載されている特定の粒子を破壊するレーザを使用することや、引用文献４及び５に記載されている特定の細胞を選択する何らかの制御を行うコンピュータを備えることに、特段の困難性は認められない。
本願発明が格別な効果を奏するものとは認められない。
よって、請求項４６－５８に係る発明は、引用文献１－８に記載された発明及び本願優先日前の技術常識に基づいて、当業者が容易に発明をすることができたものである。

・理由　　２
・請求項　　１－４０、４２－５８
明細書において、層流間の粒子の移行をホログラフィック光トラップを用いて誘導して、所望の粒子の分離を行う方法が記載されているが、それ以外の機構を用いて所望の粒子の分離を行ったことについては説明されていない。
一方、請求項１においては、第１の流れから複数の追加の流れへと所望の成分を移行させるために当該成分に作用する機構は何ら特定されていない。
一定の流速で流路中を流れている複数の流れの間で、特定の粒子が選択的かつ能動的に流れ間を移行することはないというのが、本願出願時の技術常識であったといえる。
そうすると、複数の粒子を含む液体混合物から所望の粒子だけを、層流として流れている別の流れに移行させるためには何らかの外的応力が必要であり、特定の粒子に働く力を使用する機構を有していない請求項１に記載の装置によって、所望の粒子の分離が可能であるとは認められない。請求項２－４０及び４２－５８についても同様である。
よって、この出願の発明の詳細な説明は、当業者が請求項１－４０及び４２－５８に係る発明を実施することができる程度に明確かつ十分に記載されたものでない。

・理由　　３
・請求項　　１４、４６－５８

（１）請求項１４に記載されている「流動板」及び「フラットソーター」は、当該技術分野において広く用いられている用語ではなく、各々どのようなものであるのかについて明細書に明確な定義がなされていないため不明確である。
　請求項５５にも同様の記載が含まれている。

（２）請求項４６における「前記試料液は、・・・前記複数の緩衝液と連続的に関連し」という記載は、どのように関連していることを意味しているのかが不明であるため、請求項４６に係る発明が不明確となっている。

（３）請求項４６における「２つの直交する方向」という記載の「２つ」とは、何と何であるのかが不明であるため、請求項４６に係る発明が不明確となっている。

（４）請求項４６における「２つの直交する方向へ収縮する」とは、どのような方向に収縮するのか、「２つの直交する方向に収縮する」とはどのように収縮した状態を意味しているのかが不明であるため、請求項４６に係る発明を明確に捉えることができない。

最後の拒絶理由通知とする理由

　この拒絶理由通知は、最初の拒絶理由通知に対する応答時の補正によって通知することが必要になった拒絶理由のみを通知するものである。

＜拒絶の理由を発見しない請求項＞
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（３）特許請求の範囲について補正をする際には、特許法第１７条の２第４項に違反する補正とならないよう、注意されたい。

引　用　文　献　等　一　覧

１．特表平１１－５０８１８２号公報
２．特表２００２－５０３３３４号公報
３．Proceedings of the National Academy of Sciences of USA, 1999, Vol.96, pp.5545-5548
４．国際公開第２００３／０６２８６７号
５．国際公開第２００２／０８７７９２号
６．特開平５－２６７９９号公報
７．特表２００１－５０４９３６号公報
８．特表２０００－５１２５４１号公報

先行技術文献調査結果の記録等

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　　Ｃ１２Ｍ　３／００－３／１０
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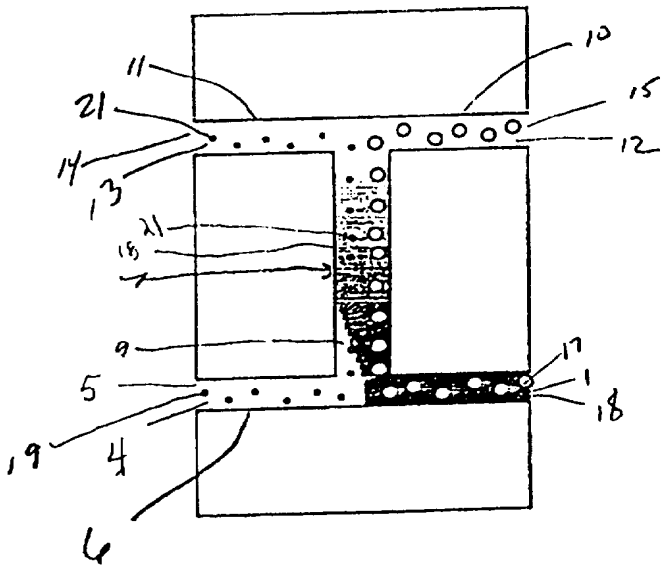
(54) Title: ABSORPTION-ENHANCED DIFFERENTIAL EXTRACTION DEVICE

(57) **Abstract**

This invention provides an extraction device and method for extracting desired particles from a sample stream (2) containing the desired particles. The device comprises a sample stream inlet (1); an extraction stream inlet (5); an extraction channel (7) in fluid communication with the sample stream inlet (1) and the extraction stream inlet (5) for receiving a sample stream (2) from the sample stream inlet (1) in adjacent laminar flow with an extraction stream (4) from the extraction stream inlet (5); a sequestering material within the extraction channel (7) for capturing desired particles (18) in the extraction stream (9); a by-product stream outlet (15) in fluid communication with the extraction channel (7) for receiving a by-product stream (12) comprising at least a portion of the sample stream (2) from which desired particles (18) have been extracted; and a product outlet (14) in fluid communication with the extraction channel (7) for receiving a product comprising the sequestering material and at least a portion of the desired particles (18).



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ABSORPTION-ENHANCED DIFFERENTIAL EXTRACTION DEVICE

This invention was made with government support under Army research contract DAMD17-94-J-4460 awarded by the U.S. Army. The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a utility patent application taking priority from provisional patent application 60/019,904 filed June 14, 1996, which is incorporated herein in its entirety by reference.

FIELD OF THE INVENTION

This invention relates generally to extraction systems and methods for separating analytes from streams containing other constituents by differential transport principles such as diffusion and applied fields, providing an improved method involving the use of absorbents or adsorbents in the extraction stream. The devices and methods of this invention can be used for diagnostic and therapeutic/treatment purposes.

BACKGROUND OF THE INVENTION

Field flow fractionation devices involve particle size separation using a single inlet stream. See, e.g., Giddings, J.C., U.S. Patent 3,449,938, June 17, 1969, "Method for Separating and Detecting Fluid Materials"; Giddings, J.C., U.S. Patent 4,147,621, April 3, 1979, "Method and Apparatus for Field-Flow Fractionation"; Giddings, J.C., U.S. Patent 4,214,981, July 29, 1980, "Steric Field-Flow Fractionation"; Giddings, J.C. et al., U.S. Patent 4,250,026, February 10, 1981, "Continuous Steric FFF Device for The Size Separation of Particles"; Giddings, J.C. et al. (1983), "Outlet Stream Splitting for Sample Concentration in Field-Flow Fractionation," Separation Science and Technology **18**:293-306; Giddings, J.C. (1985), "Optimized Field-Flow Fractionation System Based on Dual Stream Splitters," Anal. Chem. **57**:945-947; Giddings, J.C., U.S. Patent 4,830,756, May 16, 1989, "High Speed Separation of Ultra-High Molecular Weight Polymers by Hyperlayer Field-Flow Fractionation"; Giddings, J.C., U.S. Patent 4,141,651, August 25, 1992, "Pinched Channel Inlet System for Reduced Relaxation Effects and Stopless Flow Injection in Field-Flow Fractionation"; Giddings, J.C., U.S. Patent 5,156,039,

October 20, 1992, "Procedure for Determining the Size and Size Distribution of Particles Using Sedimentation Field-Flow Fractionation"; Giddings, J.C., U.S. Patent 5,193,688, March 16, 1993, "Method and Apparatus for Hydrodynamic Relaxation and Sample Concentration in Field-Flow Fraction Using Permeable Wall Elements"; Caldwell, K.D. et al., U.S. Patent 5,240,618, August 31, 1993, "Electrical Field-Flow Fractionation Using Redox Couple Added to Carrier Fluid"; Giddings, J.C. (1993), "Field-Flow Fractionation: Analysis of Macromolecular, Colloidal and Particulate Materials," Science **260**:1456-1465; Wada, Y. et al., U.S. Patent 5,465,849, November 14, 1995, "Column and Method for Separating Particles in Accordance with Their Magnetic Susceptibility"; Yve, V. et al. (1994), "Miniature Field-Flow Fractionation Systems for Analysis of Blood Cells," Clin. Chem. **40**:1810-1814; Afromowitz, M.A. and Samaras, J.E. (1989), "Pinch Field Flow Fractionation Using Flow Injection Techniques," Separation Science and Technology **24(5 and 6)**:325-339.

Thin-channel split flow fractionation (SPLITT) technology also provides particle separation in a separation cell having a thin channel. A field force is exerted in a direction perpendicular to the flow direction. Particles travel from a particle-containing stream across a transport stream to a particle-free stream. The device for operating the process is generally fabricated from glass plates with teflon sheets used as spacers to form the channels. The channel depth can therefore be no smaller than the spacers, which are generally about 100 to 120 μm thick. See, e.g., Giddings, J.C., U.S. Patent 4,737,268, April 12, 1988, "Thin Channel Split Flow Continuous Equilibrium Process and Apparatus for Particle Fractionation"; Giddings, J.C., U.S. Patent 4,894,146, January 16, 1990, "Thin Channel Split Flow Process and Apparatus for Particle Fractionation"; Giddings, J.C., U.S. Patent 5,093,426, August 13, 1991, "Process for Continuous Particle and Polymer Separation in Split-Flow Thin Cells Using Flow-Dependent Lift Forces"; Williams, P.S. et al. (1992), "Continuous SPLITT Fractionation Based on a Diffusion Mechanism," Ind. Eng. Chem. Res. **31**:2172-2181; and Levin, S. and Tawil, G. (1993), "Analytical SPLITT Fractionation in the Diffusion Mode Operating as a Dialysis-like System Devoid of Membrane. Application to Drug-Carrying Liposomes," Anal. Chem. **65**:2254-2261.

The object of this invention is to provide an improved extraction system utilizing differential transport principles in which the analyte can be extracted, detected and quantified. A further object of this invention is to provide an improved extraction system for purification and treatment of fluids, including bodily fluids such as blood.

All publications, patents and patent applications referred to herein are incorporated in their entirety by reference.

SUMMARY OF THE INVENTION

Differential extraction devices as described above allow desired particles to move from a sample stream into an extraction stream running in parallel laminar flow with the extraction stream. A simple embodiment of such systems uses a concentration gradient across the streams so that desired particles diffuse from the sample stream into the extraction stream. Other gradients and forces can also be used, e.g., magnetic, electrical, gravitational, dielectrical, sedimentation, shear, centrifugal force, temperature, pressure, and cross-flow gradients.

An improvement in the above processes provided herein is the addition of a sequestering material to the extraction stream.

The invention provides an extraction device for extracting desired particles from a sample stream containing said desired particles, said device comprising:

a. a sample stream inlet;

b. an extraction stream inlet;

c. an extraction channel in fluid communication with said sample stream inlet and said extraction stream inlet for receiving a sample stream from said sample stream inlet in adjacent laminar flow with an extraction stream from said extraction stream inlet;

d. a sequestering material within said extraction channel for capturing desired particles in said extraction stream;

e. a by-product stream outlet in fluid communication with said extraction channel for receiving a by-product stream comprising at least a portion of said sample stream from which desired particles have been extracted;

f. a product outlet in fluid communication with said extraction channel for receiving a product comprising said sequestering material and at least a portion of said desired particles.

A sequestering material is a material which captures, e.g., by adsorbing, binding or sticking to the desired particles, or by absorbing them. Enzymes, antibodies, antigens and other ligands for desired particles are known to the art and are useful sequestering materials for this invention. Any ligand known to the art for a desired particle may be used as a sequestering material. Such ligands may be added to the extraction stream "as-is" or may be immobilized on substrates such as polymeric beads, high molecular weight polymers, or other materials known to the art. "High molecular weight polymers" refers to those polymers of sufficient molecular weight that they do not substantially diffuse into the sample stream during their transit through the device. Examples of high molecular weight polymers include but are not limited to high molecular weight dextrans, high molecular weight polypeptides, and high molecular weight nucleic acids. The sequestering material may also be an absorbent material such as activated charcoal, or porous polymers. Absorbents or adsorbents may be either specific to a particular particle type, such as an antibody, or nonspecific, such as activated charcoal.

The sequestering material is preferably substantially non-diffusing, i.e., should diffuse sufficiently slowly that it does not cross from the extraction stream into the sample stream to any significant degree, so that it does not become detectable in the by-product stream, or does not interfere with analysis of analytes in the by-product stream.

The sequestering material captures the desired particles by preventing them from exiting the device with the exiting by-product stream. The desired particles may be loosely bound to the sequestering material, so long as the sequestering material retains the particles long enough to prevent them from exiting with the by-product stream. The desired particles may be reversibly bound to or captured by the sequestering material so that they can be removed for further analysis or to allow re-use of the sequestering material.

Differential extraction devices fabricated on the microscale provide numerous advantages over the larger devices discussed above. Such microfabricated devices are described in application serial no. 08\663,916 filed June 14, 1996, which is specifically incorporated herein in its entirety by reference along with all references incorporated therein by reference. Definitions of terms used in that application as applied to the microscale structures disclosed therein are applicable herein to macroscale structures as well as microscale structures. "Macroscale structures" are defined herein as structures larger than microscale structures but still small enough to permit laminar flow.

The desired particles in this invention may be analytes or they may be substances that interfere with analytes. They may also be particles desired to be recovered and used for another purpose, or toxins, such as poisons or metabolites in a patient's blood. For example, this invention can be used to detoxify blood, e.g., remove toxic metals from blood, or to detoxify other bodily fluids, e.g., to perform hemodialysis. This invention can be used in waste-water treatment, e.g., to remove impurities from water. Alternatively, this invention can be used to remove a drug or other product produced by microorganisms, e.g., bacterial cells, in a fermentation reactor without damaging the microorganisms. Such treatments can be performed in a continuous fashion.

This invention also provides a method for extraction of at least a portion of desired particles from a sample stream comprising said desired particles, comprising the steps of:

a. introducing the sample stream into the sample stream inlet of an extraction device as described above;

b. introducing an extraction stream into the extraction channel of said extraction device; and

c. introducing into said extraction channel a sequestering material for capturing the desired particles such that the desired particles are captured by the sequestering material, and such that said extraction stream, comprising said sequestering material and at least a portion of said desired particle, exits said device as a product stream, and such that said sample stream from which desired particles have been extracted, exits said device as a by-product stream.

The device and method of the present invention provide a means for performing affinity chromatography. As is understood by those in the art, affinity chromatography refers to a method of purifying or isolating desired substances and generally involves covalently attaching a specific ligand to an insoluble inert support. In affinity chromatography the ligand must have a high affinity for the desired substance, so that on passage in solution down a column the desired substance is preferentially retained by the ligand.

The present invention provides a device and method for performing affinity chromatography, however with at least one particular advantage. The extraction of a desired substance (particles) can be performed in a continuous fashion. The sample streams and extraction streams of the present invention can be run continuously through the device. Art-known affinity chromatography involves multiple steps, e.g., loading the ligand onto the inert material, flushing the column, loading the sample, rinsing, then rinsing again to release the desired substance, with product loss occurring at each step usually. In the device of the present invention, for example, a virus can be extracted from whole blood by 1) introducing an extraction stream comprising an antibody to the virus immobilized on beads and a sample stream of whole blood into the device and 2)

after transfer of the virus particles to the extraction stream, releasing them by changing the pH of the solution. The beads can be chosen, for example, so that they will fall to the bottom of the channel or they can be magnetic and therefore pulled to one side of the channel with a magnet.

The sequestering material can be present in the extraction stream prior to the extraction stream's being introduced into the extraction channel. Alternatively, the sequestering material can be added to the extraction stream by suspending or dissolving the sequestering material in a liquid which is introduced into the extraction stream, which is already in the extraction channel, via the extraction stream inlet.

The extraction system of this invention in simplest concept is illustrated by a diffusion extraction device comprising microchannels in the shape of an "H". A mixture of particles suspended in a sample stream enters the extraction channel (the crossbar of the "H") from one of the arms, e.g., the top left, and an extraction stream (a dilution stream) enters from the bottom left. The two streams flow together in the extraction channel; however, due to the small size of the channels, the flow is laminar and the streams do not mix. The sample stream exits as by-product stream at the upper right and the extraction stream exits as product stream from the lower right. While the streams are in adjacent laminar flow in the extraction channel, particles having a greater diffusion coefficient (smaller particles such as albumin, sugars and small ions) have time to diffuse into the extraction stream, while the larger particles (e.g., blood cells) remain in the sample stream. Particles in the exiting extraction stream (now called the product stream) may be analyzed without interference from the larger particles.

In this patent application, the flow direction of a channel is called its length (L). The channel dimension in the direction of particle transport at right angles to the length (L) is called its depth (d). The third channel dimension at right angles to both the length and depth is called its width (w). The depth (d) is therefore perpendicular to the plane of interface of the sample and extraction streams. Table 1 lists other abbreviations used herein.

Table 1

V	Volume
V_{ss}	Sample stream flow rate (m^3/s)
V_{es}	Extraction stream flow rate (m^3s)
V_{ps}	Product stream flow rate (m^3s)
V_{bps}	By-product stream flow rate (m^3s)
V_{ind}	Indicator dye stream flow rate (m^3s)
V_{ds}	Detection stream flow rate (m^3s)
$C_{i,ss}$	Sample stream constituent i concentration (kg/kg)
$C_{i,es}$	Extraction stream constituent i concentration (kg/kg)
$C_{i,bps}$	By-product stream constituent i concentration (kg/kg)
$C_{i,ps}$	Product stream constituent i concentration (kg/kg)
$C_{dye,ind}$	Indicator stream dye concentration (kg/kg)
$C_{i,ds}$	Detector stream constituent i concentration (kg/kg)
d	Diffusion direction extraction channel depth (m)
w	Extraction channel width (m)
L	Extraction channel length (m)
$a_{\%}$	Percentage deviation from equilibrium concentration
$L_{a\%}$	Device length required to achieve $a_{\%}$ (m)
z_s	Interface streamline location between sample and extraction streams at the extraction channel entrance (m)
z_p	Interface streamline location between the by-product and product streams (m)
P	Absolute pressure within the fluid stream (Pa)
Δp	Differential pressure between the entrance and exit of the extraction channel (Pa)
D_i	Binary diffusion coefficient of constituent i (m^2/s)
μ	Fluid viscosity (Pa · s)
ρ	Fluid density (kg/m^3)
ξ	Equilibrium normalized constituent concentration for an infinite length extraction channel (dimensionless)
$\bar{c}$	Normalized constituent concentration (dimensionless)
x	Channel length coordinate direction (flow direction)
y	Channel width coordinate direction
Z	Diffusion direction coordinate
$\bar{x}, \bar{z}$	Non-dimensional normalized variables (dimensionless)
w/d	Aspect ratio
D	Diffusion coefficient
Re	Reynolds number
T	Temperature
u	Axial velocity
V	Average velocity

The length of the extraction channel and the extraction channel flow velocity are key parameters determining the amount of time the particles have to diffuse into the extraction stream. The sequestering material provides for increased diffusion of the desired particles by decreasing the effective concentration of the desired particles in the extraction stream. That is, the sequestering material effects a shift in the equilibrium (in a positive direction) of diffusion of the desired particles into the extraction stream.

The particles in the case described above are differentially transported from the sample stream to the extraction stream using diffusion as the transport mechanism. Other means for effecting differential transport of the desired particles can also be used. The term "differential transport" means that a portion of the desired particles are transported from the sample stream into the extraction stream to the substantial exclusion of the undesired particles. For example, magnetic, electrical or other forces can be applied across the extraction stream, temperature gradients can be used, or absorbent or adsorbent materials such as antibodies can be added to the extraction stream to capture the desired particles.

The sample stream and extraction stream inlets and the byproduct stream and product stream outlets may comprise channels, reservoirs, ports, or other containers. The sample stream inlet is designed to receive a sample stream containing "desired particles," e.g., particles it is desired to extract so that their presence may be detected. The sample stream also includes other particles which are not extracted, termed "undesired particles" herein. These undesired particles include particles which might interfere with the detection of the desired particles. In a preferred embodiment, the sample stream comprises whole blood. The desired particles may be albumin or other blood plasma components, and the undesired particles may be blood cells. The device is especially useful for obtaining cell-free plasma components from whole blood. Other fluids for which the present invention is useful include solutions or suspensions of DNA fragments of different lengths, proteins of varying sizes, or heterogeneous chemical reaction mixtures. Sample streams useful in the practice of this invention include fermentation broths, raw sewage, liquefied food samples, soil samples and biological fluids such as sputum, urine, and cerebral spinal fluid.

The term "particles" refers to molecules; cells; macromolecules such as proteins, nucleic acids and complex carbohydrates; small molecules comprised of one to several atoms; and ions. The particles may be suspended or dissolved in the stream. The term "stream" refers to a carrier fluid such as water or other liquid, air or other gas, containing desired and/or undesired particles. The term "particles" as used herein does not include the molecules of the carrier stream.

The term "extraction" refers to the transfer of at least a portion, i.e., a detectable portion, of desired particles from the sample stream to the extraction stream, to the substantial exclusion of undesired particles. It is recognized that undesired particles may be transported into the extraction stream, particularly those that diffuse faster than the desired particles; however, the presence of such undesired particles will be minimized such that they do not interfere with detection or subsequent processing of the streams containing the desired particles. The transfer of undesired particles from the sample stream to the extraction stream can be minimized by pre-loading the extraction stream with such undesired particles. Pre-loading the extraction stream with undesired particles may be preferable in embodiments wherein the by-product stream is of interest, e.g., for further use or analysis. For example, if blood is to be returned to a patient's body, the extraction stream preferably contains the appropriate concentrations of electrolytes, as will be understood by those of skill in the art. The sequestering material increases the efficiency of separation of the desired particles from the sample by decreasing the effective concentration of the desired particles in the extraction stream.

The term "extraction efficiency" refers to the percentage of desired particles in the sample which are transferred to the extraction stream and exit in the product stream. Extraction efficiency can be increased by using a sequestering material.

The term "laminar flow" of two streams means stable, side-by-side, non-recirculating, flow of two streams without mixing. There are no zones of recirculation, and turbulence is negligible. As is known to the art, the Reynolds number of a flow is the ratio of inertial forces to viscous forces. For flow through a duct, the Reynolds number is calculated using the equation $Re = \rho d(\bar{V}/\mu)$ where Re is the Reynolds number,

ρ is the mass density of the fluid, d is a typical cross-sectional dimension of the duct depending on the shape of the duct, $\bar{V}$ is the mean velocity over the duct cross-section and μ is the viscosity.

As the Reynolds number is reduced, flow patterns depend more on viscous effects and less on inertial effects. Below a certain Reynolds number (based on lumen size for a system of channels with bends and lumen size changes), inertial effects are insufficient to cause phenomena indicative of their significant presence such as laminar recirculation zones and turbulent flow. Therefore, non-turbulent, laminar non-recirculating flow occurs in the extraction devices discussed herein. In such devices minimal dispersive mixing occurs as a result of the viscous flow velocity profiles present within any laminar viscous flow. This allows two laminar non-recirculating fluid streams to flow down an extraction channel for the purpose of desired particle extraction from one stream to the other.

The streams may be separated at the end of the conduit at any arbitrary location by precise regulation of the exit flow rate of the outlets, something which is not possible at higher Reynolds numbers not satisfying the non-recirculating and non-turbulent criteria.

The extraction stream inlet is designed to receive an extraction stream capable of accepting desired particles when in laminar flow contact with the sample stream. The extraction stream can be any fluid capable of accepting particles being transported from the sample stream. The extraction stream contains sequestering material which binds desired particles which have been transported from the sample stream to the extraction stream. Preferred extraction streams are water and isotonic solutions such as physiological saline. Other useful extraction streams comprise organic solvents such as acetone, isopropyl alcohol, supercritical carbon dioxide or ethanol. Air and other gases may also be used as sample and extraction stream carriers.

The by-product stream comprises at least a portion of said sample stream from which desired particles have been extracted, and may or may not, as discussed below, include a fraction of the extraction stream into which desired particles have been

conveyed from the sample stream. The sequestering material effects greater extraction of the desired particles from the sample, thereby yielding a more pure by-product stream. If an excess of sequestering material is used and it has a high binding constant for the desired particles, then essentially all of the desired particles in the sample stream can be extracted from the sample stream upon treatment of the sample just once, depending on flow rate and extraction channel length. Without the sequestering material and assuming equal flow rates of sample and extraction fluids, the equilibrium concentration of the desired particles is 50% in the extraction stream. That is, at most, only 50% of the desired particles diffuse into the extraction stream. Therefore, without the sequestering material the sample has to be treated at least five times to remove 97% of the desired particles from the sample.

The by-product stream outlet is designed to conduct the by-product stream (composed of the sample stream and perhaps a portion of the extraction stream) that is removed from the extraction channel to disposal, recycle, or other system component, for further processing.

The product stream comprises at least a portion of said desired particles and the sequestering material. The product stream outlet, which as stated above, may comprise a product stream channel, is designed to conduct the product stream containing a detectable quantity of desired particles to a detection or further processing area or system component. A sufficient quantity of the extraction stream must be present in the product stream, comprising a sufficient quantity of desired particles, such that the presence of the desired particles is detectable in the product stream by means known to the art.

The product stream may be conducted to a reservoir chamber, or other device where it may be further treated, e.g., by separating the sequestering material from the desired particles, mixing, separating, analyzing, heating or otherwise processing, for example as disclosed in Wilding, P., et al. U.S. Patent 5,304,487 issued April 19, 1994, incorporated herein by reference. The by-product stream may also be conducted to a reservoir chamber or other container or apparatus for further treatment.

The devices of this invention may be "microfabricated," which refers to devices capable of being fabricated on silicon wafers readily available to those practicing the art of silicon microfabrication and having the feature sizes and geometries producible by such methods as LIGA, thermoplastic micropattern transfer, resin based microcasting, micromolding in capillaries (MIMIC), wet isotropic and anisotropic etching, laser assisted chemical etching (LACE), and reactive ion etching (RIE), or other techniques known within the art of microfabrication. In the case of silicon microfabrication, larger wafers will accommodate a plurality of the devices of this invention in a plurality of configurations. A few standard wafer sizes are 3", 4", 6", and 8". Application of the principles presented herein using new and emerging microfabrication methods is within the scope and intent of the claims hereof.

In a preferred embodiment, called the "H-filter device" herein, the inlet and outlet channels are between about 2 to 3 times the maximum-sized stream particulate diameter and about 100 micrometers in width and between about 2 to 3 times the diameter of the maximum-sized particles and less than about 100 micrometers in depth, and the extraction channel is between about 2 to 3 times the diameter of the maximum-sized particles and about 2/3 the wafer thickness in width, between about 2 to 3 times the diameter of the maximum-sized particles and less than about 100 micrometers in depth, and between about 4 and about 10 times the diameter of the maximum-sized particles and less than or equal to 5 mm long.

In a second embodiment in which the particle transport direction is rotated 90 degrees from that of the "H-filter device" design, called the "flat extraction device" or "flat filter device" herein, the inlet channels have a width equal to the extraction channel width at the entrance to the extraction channel of preferably between 2 and 3 particle diameters and about 500 micrometers, and the extraction channel is preferably between about 2 and 3 times the diameter of maximum-sized particles and less than or equal to 5 mm in width, between about 2 and 3 times the diameter of the maximum-sized particles and less than about 100 micrometers in depth, and at least about 4 times the diameter of the maximum-sized particles in length.

The extraction channel receives the inflow of the sample and extraction streams from the sample and extraction stream inlets and conducts these streams in adjacent laminar flow for a distance sufficient to allow extraction of the desired particles into the extraction stream. The length of the extraction channel can be increased by forming it in a convoluted geometry, e.g., serpentine (set of "hairpin" turns) or coiled, as are the flow channels disclosed in Weigl et al., U.S. Patent Application 08/829,679, filed March 31, 1997 and PCT Application No. PCT/US97/05245, filed March 31, 1997, which are incorporated herein by reference.

The width and depth of the extraction stream channel and product outlet channels must be large enough to allow passage of the desired particles, the sequestering material, and any complex of the desired particles with the sequestering material.

If the width dimension is in the wafer thickness direction, as it is in the H-filter device embodiment, then for the silicon microfabricated embodiments of the microscale extraction devices of the present invention, the widths of the sample, extraction, product, and by-product channels, inlets and outlets are less than the silicon wafer thickness, i.e., about 300 micrometers. Alternatively, if the device is made from other materials, preferably moldable materials such as plastic, or in the "flat extraction device" embodiment, then there is no theoretical maximum limit to the width. Widths up to 0.5 meter, 1 meter, and even greater are contemplated. The width has no theoretical maximum limit provided that one can control the delivery of fluids (sample stream and extraction stream) into the device, e.g., the flow rate of each fluid can be controlled across the width of the channel. The dimensions of the extraction channel are chosen to maintain laminar flow and uniform flow rate, e.g., no turbulence or build up of particles on channel walls.

If the depth dimension is in the wafer thickness direction, as it is in the "flat filter" embodiment, then for silicon microfabricated embodiments of the microscale extraction devices of the present invention, the depth of the sample, extraction, product, and by-product channels, inlets and exits is less than the silicon wafer thickness, i.e., about 300 micrometers. Preferably, for microfabricated devices, the depth, particularly

of the extraction channel, is less than about 200 micrometers, and more preferably less than about 100 micrometers.

Some fields known to the art which may be used for differential transport of the particles in the devices of this invention are those produced by:

- Sedimentation
- Electrical potential
- Temperature gradients
- Cross Flow
- Dielectrical gradients
- Shear forces
- Magnetic forces
- Concentration gradients

Means for producing such fields are known to the art.

Because of the small size of the diffusion direction (depth) of the channels described herein, differential transport of desired particles by diffusion or other means occurs extremely rapidly, e.g., within less than about 300 seconds, and if desired, less than about one second. The presence of the sequestering material in the extraction stream provides for increased desired particle transport by lowering the effective concentration of the desired particles in the extraction stream, maximizing the effective concentration difference between the sample stream and the extraction stream. This maximizes the net transfer along the depth (diffusion dimension) of the extraction channel, thus providing rapid separation of desired particles from the sample.

The sample and extraction streams may have different properties, e.g., viscosities, densities, surface energies, homogeneities, chemical compositions and the like, which may affect the differential transport rates. System parameters may need to be adjusted and optimized to take account of these differing properties, as will be apparent to, and can be done without undue experimentation by, those skilled in the art.

The sample and extraction streams are kept in contact in the extraction channel for a period of time sufficient to allow at least an analyzable quantity, and preferably a major portion, of desired particles to be transported into the extraction stream. The flow rate of the product stream from the device may be between about 0.001 picoliter/sec and about 10 ml/sec or more in devices with large widths, e.g., greater than about 50 μm. For example, an optimal flow rate for the product stream can be about 200 nanoliters/sec. As is known in the art, even the very small amounts of analytes present in such small product streams may be detected by spectroscopic and other means.

The average flow velocity, $\bar{V}$, is is chosen to fit the following relationship:

$$\bar{V} < f\frac{DL}{d^2}$$

where f is a time factor (proportionality constant) related to how long the two streams must be in contact with each other in order for a certain percentage of desired particles to be transferred from the sample stream to the extraction stream.

The volumetric flow rate (Q) per unit width (w) is thus limited to be less than f(DL)/d: $Q = \bar{V}wd$, $Q/w = \bar{V}d$

$$\frac{Q}{w} < f\frac{DL}{d}$$

It may be convenient for calculation purposes to choose f = 1, and calculate the maximum flow rate per unit width based thereon. For example, for biotin (with diffusion coefficient, D = 500 μm^2/sec in a channel of length (L) = 1 cm and depth (diffusion dimension) (d) = 10 μm, the maximum flow rate per unit width is approximately 500 picoliters/sec per μm of width.

From the above the following relationship can be derived:

$$d^2/D = 2t$$

which means that a molecule will diffuse across distance d (the depth of the channel) in an average time of 2t.

A "major portion" of the desired particles is more than 50% of said particles present in the sample stream.

The sequestering material enhances the efficiency of the extraction process, allowing for greater than 50% extraction (the maximum extraction obtained with equal volumes of the sample and extraction stream, but without sequestering material or other differential transport forces, e.g., magnetic and electircal fields). Preferably, the sequestering material allows for extraction of greater than about 50% to about 80% of the desired particles. More preferably, the sequestering material allows for extraction of about 75% to about 95% of the desired particles. Most preferably, the sequestering material allows for extraction of about 85% to about 100% of the desired particles.

Successful operation of the invention described herein requires precise control of volume flow rates on three of the four channels of the device (i.e., sample, extraction, product, and by-product streams). The fourth channel need not and should not be regulated, as leaving this channel unregulated will allow the device to accommodate unpredictable changes in volume of the sample because of ΔV of mixing of the sample and extraction streams. Means for achieving precisely regulated flow rates are known to the art.

To aid in controlling the size of particles being transported to the product stream in a diffusion-based extraction system of this invention, and reduce the appearance of larger particles in the product stream, a fluid barrier may be created in the extraction channel. Such a fluid barrier is present when the extraction stream is present in sufficient volume to cause a portion of the extraction stream to flow through the by-product exit with the exiting by-product stream, as illustrated in Figure 3. Smaller particles diffusing into the extraction stream must cross the width of this fluid barrier before being able to exit with the product stream. Such fluid barriers formed on a larger scale are discussed

in Williams P. S., et al. (1992), "Continuous SPLITT Fractionation Based on a Diffusion Mechanism," Ind. Eng. Chem. Res. 2172-2181, incorporated herein by reference.

By controlling the flow rate of the sample and extraction streams, the ratio of volume from each that enters the extraction channel can be controlled. The volume ratio of the sample stream and the extraction stream can also be set by the geometry of the outlet and inlet channels for a fixed delivery pressure on the sample and extraction streams. The volume flow rate of the product and by-product streams may also be controlled by manipulating the product and by-product stream pressures or by using arbitrary port (inlet) pressures and altering the flow resistance of the inlets. Whatever the control mode, the inlet and outlet channels must satisfy the criteria for minimum channel dimensions based on the size of the particulate to be processed as described herein. If the volume of the extraction stream entering the extraction channel is greater than the volume of the sample stream, and the two exit streams are identical, a fluid barrier is formed. If the volume flow rate of the product stream is too small to accommodate the entire volume flow of the extraction stream then a fluid barrier will also be formed.

Extraction devices of this invention may comprise means for controlling the volume of extraction stream in the extraction channel with respect to the volume of the sample stream, which means include a product stream outlet smaller than required to allow the entire extraction stream to exit coupled with a by-product stream outlet large enough to handle the excess extraction stream. Extraction devices of this invention may comprise multiple product stream outlets so that product streams comprising different types of desired particles may be recovered.

The devices of this invention may be utilized as a sample pretreatment system for an analytical system including sensing means for detecting desired particles in the product stream. Such means include means for mixing the product stream with an indicator stream which interacts with the desired particles so as to allow them to be detected by sensing means known to the art, including optical means, such as optical spectroscopic equipment, and other means such as absorption spectroscopic equipment or means for detecting fluorescence, chemical indicators which change color or other properties when

exposed to the desired particles of analyte, immunological means, electrical means, e.g., electrodes inserted into the device, electrochemical means, radioactive means, or virtually any microanalytical technique known to the art including magnetic resonance equipment or other means known to the art to detect the presence of analyte particles such as ions, molecules, polymers, viruses, DNA sequences, antigens, microorganisms, or other factors. Preferably, optical or fluorescent means are used, and antibodies, DNA sequences and the like are attached to fluorescent markers. Indicators and microfabricated mixing means, as well as detection and sensing means are described in U.S. Application Serial No. 08/625,808 incorporated herein by reference.

In one embodiment of this invention the differential extraction device described above is integrated into an analytical system comprising means for further processing the product and/or by-product streams, such as diffusion-based mixing devices for mixing the product stream with an indicator substance (e.g., as described in U.S. Application Serial No. 08/625,808 incorporated herein by reference), and detection chambers wherein the presence of desired analyte particles may be detected. These additional processing means are preferably incorporated with the differential extraction device in a "lab-on-a-chip", fabricated on a standard silicon wafer. The system may comprise quantitation means for determining the concentration of the analyte particles (desired or undesired particles) in the product and/or by-product stream and/or determining the concentration of the analyte particles in the sample stream. Such means include spectroscopic equipment, potentiometric, amperometric, and dielectric relaxation equipment. Concentration determinations can be made by calculation or calibration by means known to the art and disclosed herein.

In another embodiment of this invention used for purification of fluids, e.g., waste-water treatment, hemodialysis, blood detoxification, large volumes, e.g., about 10 ml/sec of sample stream may be treated. In this embodiment, preferably the width of the extraction channel is large, e.g., up to about one meter, although as discussed above, there in no fixed theoretical maximum for the channel width. For treatment of large fluid volumes, devices of this invention, including microfabricated devices, may be connected in parallel, and optionally also in series.

It may be preferable to pre-treat the device, i.e., pre-coat the internal walls of the device to enhance performance, as will be illustrated in the Examples below. The walls can be coated with the sequestering material to be used, before the device is used to effect separation of the desired particles. Without wishing to be bound to any particular theory, it is believed that pre-coating the walls with the sequestering material prevents further adherence of sequestering material to the walls when the sample and sequestering material are later introduced into the device. Alternatively, the internal walls of the device can be pre-coated to effect surface passivation with hydrophilic coating materials, which are commercially available and include, but are not limited to, albumins (e.g., bovine serum albumin, lact albumin and human serum albumin), and art-known silanizing reagents, preferably polyethyleneglycol silanes.

As will be appreciated by those skilled in the art, numerous substitutions may be made for the components and steps disclosed herein, and the invention is not limited to specific embodiments discussed.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a microchannel configuration showing laminar flow of two input streams having a low Reynolds number.

Figure 2 illustrates a microchannel configuration showing the diffusion of smaller particles from a sample stream into an extraction stream.

Figure 3 illustrates a microchannel configuration showing the formation of a fluid barrier between a sample stream and an extraction stream.

Figure 4 is a diagram of the inlet and outlet interface streamline in the extraction channel showing the flow rates of the sample, extraction, product and by-product streams.

Figure 5 illustrates an extraction device without sequestering material.

Figure 6 illustrates an embodiment (the H-filter embodiment) of the extraction device of this invention which includes sequestering material.

Figure 7, comprising Figures 7A-7D, illustrates diffusion in the extraction device of Figure 5 as time progresses.

Figure 8, comprising Figures 8A-8D, illustrates diffusion in the extraction device of Figure 6 as time progresses.

Figure 9 shows the flow direction (L) and the diffusion/transport direction (depth) of the device of this invention.

Figure 10 shows one embodiment of this invention in which a plurality of the extraction devices are connected in parallel.

Figure 11 shows one embodiment of this invention in which a plurality of the extraction devices are connected in series.

Figure 12 shows a perspective view of microfabricated flat diffusion extraction device with the diffusion direction rotated 90° from the "H" design shown in Figures 1-3, 5 and 6.

Figure 13 shows a plan view of the microfabricated flat diffusion extraction system design of Figure 12.

Figure 14 is a graph showing the fraction of urea remaining after treatment versus time as a function of various numbers of flat filters connected in parallel.

Figure 15 is a graph showing the fraction of urea remaining after treatment versus time as a function of total blood volume in liters.

Figure 16 is a graph showing the fraction of urea remaining after treatment versus time as a function of extraction efficiency.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Diffusion of small molecules occurs rapidly over typical microfabricated dimensions. The relationship between the size of a particle, r, the diffusion coefficient, D, and temperature, T, was discovered by Einstein and for the simplest case, spherical particles, this can be written as:

$$D = \frac{k_b T}{6\pi\mu r}.$$

The characteristic distance, l, which a particle with diffusion coefficient D will diffuse in time, t, is

$$l = \sqrt{Dt}.$$

Table 2 gives some typical diffusion coefficients and characteristic times.

Table 2:

Some typical values for different sized particles and molecules. The characteristic time to diffuse 10 μm is given.

Particle	D(20°C)	t
0.5 μm sphere	5 x 10^{-9} cm^2/sec	200 sec
Protein (hemoglobin)	7 x 10^{-7} cm^2/sec	1 sec
Small Molecule (fluorescein)	5 x 10^{-6} cm^2/sec	0.2 sec

As shown in Figure 1, in microchannels of small enough dimensions, inertial effects are negligible, such that a sample stream **2** entering a sample stream inlet **1** can flow from a sample stream channel **3** into an extraction channel **7** without mixing with an extraction stream **4** entering an extraction stream inlet **5** and flowing from an extraction stream inlet channel **6** into extraction channel **7**. The two streams in the extraction channel **7** form a laminar sample stream **8** and a laminar extraction stream **9**.

In Figure 2, the arrows at the upper left show the direction of flow in sample stream channel **3** of sample stream **2** entering sample stream inlet **1**, and the arrows at the lower left show the direction of flow in extraction stream inlet channel **6** of extraction stream **4** entering extraction stream inlet **5**. Sample stream **2** contains larger ("undesired") particles **17** and smaller ("desired") particles **18** (shown by cross-hatching). The sample stream **2** and extraction stream **4** come together in laminar flow in extraction channel **7** to form laminar sample stream **8** and laminar extraction stream **9** and the smaller desired particles **18** begin to diffuse from laminar sample stream **8** into laminar extraction stream **9** to form laminar product stream **16** which contains diffused smaller desired particles **18**. The laminar sample stream **8** flows into by-product outlet channel **10** to form by-product stream **12**, and leaves the channel through by-product outlet **15**. The laminar extraction stream **9** receives smaller desired particles **18** diffused from laminar sample stream **8** and becomes laminar product stream **16** which, in product outlet channel **11**, becomes product stream **13** and leaves the channel through product outlet **14**.

In Figure 3, the direction of the arrow at the upper left shows the direction of flow in sample stream channel **3** of sample stream **2** entering through sample stream inlet **1**. The direction of the arrow at the lower left shows the direction of flow in extraction stream inlet channel **6** of extraction stream **4** entering through extraction stream inlet **5**. Extraction stream **4** is indicated by cross-hatching. The upper arrow in extraction channel **7** shows the direction of flow of laminar sample stream **8** and the lower arrow in extraction channel **7** shows direction of flow of laminar extraction stream **9**. When the volume of extraction stream **4** is greater than the amount which can exit through product outlet channel **11** and product outlet **14**, part of laminar extraction stream **9** exits through by-product outlet channel **10** and by-product outlet **15** as excess extraction stream **22**.

This excess extraction stream **22** is in laminar flow in extraction channel **7** and forms fluid barrier **20**. Smaller desired particles **18** (not shown in Figure 3; see Figure 2) in the sample stream **2** diffuse from laminar sample stream **8** through fluid barrier **20** into laminar extraction stream **9** to form product stream **16** (not shown in Figure 3; see Figure 2).

A simple calculation shows that few particles or molecules with diffusion coefficients smaller than $D = w_{fb}^2\bar{V}/L$ will be found in the exiting product stream, where w_{fb} is the width of the fluid barrier, $\bar{V}$ is the average flow velocity of the laminar sample stream and L is the length of the extraction channel. Particles or molecules with diffusion coefficients larger than $D = w^2\bar{V}/L$, where w is the width of the extraction channel, will be in the exiting product stream in the same concentration as in the by-product stream.

Means for injecting feed liquid into the device are provided, as when the device of this invention is used as part of an analytical system. Such means include standard syringes (fixed volumes per unit time) and tubes (fixed pressure). Means for removing fluid from the product exit may also be provided, including receptacles for the fluid, inducing flow by capillary attraction, pressure, gravity, and other means known to the art as described above. Such receptacles may be part of an analytical or other device for further processing the product stream.

Figure 4 shows the extraction channel **7** with laminar extraction stream **9** moving at a velocity V_{es}, and laminar sample stream **8** moving at a velocity V_{ss}, and having a stream height, (diffusion direction coordinate) Z_s defining the interface streamline location (dotted line) between the laminar sample stream **8** and the laminar extraction stream **9** near the entrance of the extraction channel **7**. The combined height of both streams, and thus the depth of the extraction channel **7**, is shown as *d*. The curved line indicates the shape of the velocity profile. As the streams move along the length of the extraction channel **7**, laminar sample stream **8** becomes by-product stream **12** moving with a velocity V_{bps} and having a stream height (diffusion direction coordinate) Z_p defining the interface streamline location (dotted line) between the by-product stream **12** and the

product stream **13**. Laminar extraction stream **9** becomes product stream **13** moving with a velocity V_{ps}.

Several steps commonly performed in the chemical assay of a fluid mixture are: (1) precise mixture dilution; (2) extraction of a specific constituent; (3) precise mixing of indicator reagents or test probes (e.g., fluorescently tagged polymer beads); and (4) non-invasive detection of the indicator or probe (e.g., absorbance or fluorescence spectroscopy).

Figure 5 illustrates an extraction device without sequestering material. A sample stream **2** containing smaller, desired particles **18** and larger, undesired particles **17** is introduced/injected via sample stream inlet **1**. An extraction stream **4**, e.g., an aqueous buffered solution, is introduced/injected via extraction stream inlet **5**. The two streams flow in laminar fashion through extraction channel **7**, during which time smaller particles **18** from the sample stream diffuse across the extraction channel into the extraction stream **4**, as a result of a concentration gradient. Other gradients can be used, e.g., magnetic, electrical, and centrifugal force. Product stream **13**, containing at least some smaller particles **18**, exits product outlet **14**. By-product stream **12** containing larger particles **17** and smaller particles **18**, both from the sample stream, exits by-product outlet **15**. If equal flow rates and volumes of the sample stream and extraction stream are used, and if the extraction channel is long enough to allow for complete equilibration to occur, then at most 50% of the small particles in the sample stream will have diffused into the extraction stream and exit the product outlet. With such devices not containing sequestering material, diffusion of small particles into the extraction stream can be increased by injecting a smaller amount of (lower flow rate for) the sample stream than the extraction stream. However, the increase in diffusion is proportional to the ratio of the extraction stream volume/flow rate to that of the sample. Hence the increased efficiency in diffusion as a result of increasing the ratio of the extraction stream volume/flow rate to that of the sample is counteracted by the decreased amount of sample (or flow rate thereof) which can be injected each time.

A concentration profile of the smaller, desired particles **18** diffusing in the device of Figure 5 is illustrated in Figure 7 where particle transport occurs by diffusion. The curved line **23** shows concentration versus position of diffusing particles. Time progress from 7A, to 7B, to 7C and equilibration has occurred at illustration 7D. In 7D an equal concentration of small particles is in the two streams (left and right). The concentration in each stream (on both sides, left and right) is 50% of the concentration in the starting sample stream.

With a device not containing sequestering material, the product stream would have to be run through such a device multiple times to achieve a greater than 50% removal of desired particles from the sample stream. For example, a sample would have to be run through such a device 5 times to extract about 97% of the desired small particles, assuming total equilibration were achieved on each run.

The present invention provides increased extraction efficiency by employing a sequestering material in the extraction channel. The sequestering material lowers the effective concentration of the desired particle in the extraction stream, thereby allowing for more rapid and complete diffusion of the desired particles into the extraction stream.

Figure 6 illustrates an embodiment of the present invention. A sample stream containing smaller desired particles **18** shown by dark, shaded areas, and larger undesired particles **17** shown by white circles, is introduced/injected via sample stream inlet **1**. An extraction stream **4**, containing sequestering material **19**, is introduced/injected via extraction stream inlet **5**. The two streams flow in laminar fashion through extraction channel **7**, during which time smaller desired particles **18** (shown by shaded areas) from the sample stream diffuse across the extraction channel into the extraction stream **4**, as a result of a concentration gradient, and bind to the sequestering material, forming a complex **21** of sequestering material bound to desired particles. Additionally, other gradients can be used, e.g., magnetic, electrical, and centrifugal force. Product stream **13**, containing at least some smaller desired particles **18**, some of which are bound to the sequestering material thereby forming complexes **21**, exits product outlet **14**. By-product

stream **12** containing larger particles **17** and possibly some smaller particles **18**, both from the sample stream, exits by-product outlet **15**.

The binding constant and amount of the sequestering material in the extraction stream determine the concentration of free desired particles in the extraction stream. Diffusion of desired particles from the sample stream is proportional to the concentration gradient. A sequestering material with a high binding constant for the desired particle provides an effective concentration (or activity) essentially equal to zero, if the binding sites of the sequestering material are in excess compared to the desired particle. Thus, the desired particles continue to diffuse into the extraction stream until the sequestering material is saturated. Only after saturation of the sequestering material does the free concentration of the desired particle begin to equilibrate in the two streams. If an excess of binding sites of the sequestering material is used (compared to the amount of desired particles in the sample), then essentially all of the desired particles are extracted from the sample into the extraction stream.

Both the amount of and the binding constant of the sequestering material for the desired particles affect the efficiency of extraction. The higher the binding constant, the more efficient the extraction will be. In some cases, it may be preferable for the binding to be reversible, e.g., in cases in which it is desirable after extraction of the desired particle to analyze it absent the sequestering material. Preferably the binding constant of the sequestering material is at least 10^{-1}M or 10^{-2}M, which is in the range of binding constants for sequestering material with non-specific binding, e.g., activated charcoal. For sequestering material which is specific to a particular type of particle, binding constants of about 10^{-6}M to about 10^{-8}M can be preferable; many antibodies bind antigens with binding constants in this range. Essentially irreversible binding occurs at binding constants in the range of 10^{-14}M to 10^{-15}M, the latter being the binding constant of biotin to avidin. Those of ordinary skill in the art recognize that even "irreversible" binding can be reversed, e.g., by varying the temperature, pH, and solvent type of the reaction system. Such a reversal of binding (dissociation) is preferable after extraction of the desired particle in cases in which one wants to analyze the desired particles absent the sequestering material.

A concentration profile of the smaller desired particles **18** diffusing in the device of the Figure 6 is illustrated in Figure 8. The curved line **23** shows concentration versus position of diffusing particles. Time progress from 8A, to 8B, to 8C, to 8D. In Figure 8A the desired particles **18** are in the sample stream on the right side of the device, and the sequestering material **19** is in the extraction stream on the left side of the device. Figure 8B shows that some of the desired particles **18** have diffused across the channel and bound to the sequestering material **19**, forming a complex **21**. Figure 8C shows that more of the desired particles **18** have diffused across the channel and bound to the sequestering material **19**, forming more of the complex **21**. Figure 8D shows that more of the desired particles **18** have diffused across the channel and bound to the sequestering material **19**, forming more of complex **21**. The free concentration of desired particles **18** in the extraction stream (left side) is kept extremely low, effectively zero in this case where tight binding occurs between the sequestering material and the desired particles **18**. With an excess of sequestering material and a high binding constant, essentially all of the desired particles **18** can be extracted from the sample into the extraction stream.

The dimensions of the device are chosen so that laminar flow is maintained in the extraction channel. As noted above, and referring to Figure 9, the flow direction of a channel is called its length (L). The length can be between about 1 centimeter (cm) and about 5 centimeters. The channel dimension in the direction of particle transport (diffusion across extraction channel) at right angles to the length (L) is called its depth (d). The depth is preferably less than about 100 micrometers, and more preferably about 20 micrometers to about 50 micrometers. The third channel dimension at right angles to both the length and depth is called its width (w). In Figure 9 the width dimension is not shown because it is orthogonal to the plane of the paper. The width can be up to about one meter or greater, including widths of 500 micrometers, 1 mm, 5 cm, and one half meter. The width is large enough to allow passage of the any particles in the streams, including sequestering material. A large width allows for large volumes to be processed in the device. The width can be quite large, e.g., one meter or larger, as long as the diffusion direction (depth) is small enough to maintain laminar flow, and the length is long enough to allow effective diffusion to occur.

Large sample volumes can be processed by the device of this invention by one of at least two configurations. The first, mentioned above, is to use a large width in the device, so that the device holds a large volume of fluid. Second, a plurality of, i.e., two or more, devices can be joined in parallel so that a portion of the sample is processed in each device at the same time. Figure 10 illustrates a configuration with several extraction devices in parallel, the sample stream inlet **1** of each device in fluid connection via sample connector **27** with a sample manifold line **24**, and the extraction stream inlet **5** of each device in fluid connection via extraction connector **26** with an extraction manifold line **25**. By-product stream exits via by-product outlet line **28**, and product stream exits via product outlet line **29**. All of the by-product outlet lines **28** can be connected and flow into one reservoir. All of the product outlet lines **29** can be connected and flow into another, single reservoir. In Figure 10 the devices are illustrated with a width much larger than the depth and length. This relatively large width is optional and can be employed in addition to, or instead of, connecting several devices in parallel, to increase the sample volume processed per unit of time.

A plurality of devices can be connected in series to improve particle separation, i.e., the product stream exiting being in fluid connection with the sample stream inlet of another device, as shown in Figure 11. In Figure 11, each extraction device is labeled **100**. The by-product stream **12** exits each device and the product stream **13** becomes the sample stream for the next device in series.

Figure 12 shows a perspective view and Figure 13 shows a plan view of a further embodiment of the invention, a "flat extraction device," in which the diffusion direction in extraction channel **7** is rotated 90° from the embodiments shown in Figures 1-3, 5 and 6. This embodiment provides the advantage that the volume of material which can be processed is no longer limited by the width of the extraction channel **7**.

The flat extraction device of Figures 12 and 13 is made by etching a silicon substrate **34** to provide sample stream inlet groove **35**, extraction stream inlet groove **36**, product stream exit groove **37**, and by-product stream exit groove **38**, as well as extraction channel **7**. A glass cover **33** serves to enclose extraction channel **7**. In Figure

12, the arrows shown pointing downward into sample stream inlet **1** indicate the flow of sample stream **1**. Similarly, the arrows pointing down into extraction stream inlet **5** indicate the flow of extraction stream **4**. The arrows pointing up from product outlet **14** indicate the flow of product stream **16**, and the arrows pointing up from by-product outlet **15** indicate the flow of by-product stream **12**. The length of extraction channel **7** is shown as *L* and the width of the channels is indicated by the dark arrow as *w*. The depth of the extraction channel **7** is shown as *d*. A coupling manifold **32** shown in Figure 13 with openings extends the depth of sample stream inlet groove **35** to form sample stream channel **3** and sample stream inlet **1**, extends the depth of extraction stream inlet groove **36** to form extraction stream channel **6** and extraction stream inlet **5**, extends the depth of product stream exit groove **37** to form product outlet channel **11** and product outlet **14**, and extending the depth of by-product stream exit groove **38** to form by-product outlet channel **10** and by-product exit **15**.

In the flat extraction device shown in Figure 13 operating by diffusion (concentration gradient) a sample stream **2** shown by the arrow in the upper left enters sample stream inlet **1** and flows in sample stream channel **3**. Extraction stream **4** is indicated by an arrow entering extraction stream inlet **5**, and flows in extraction stream inlet channel **6**. Sample stream **2** flows as a laminar sample stream **8** in extraction channel **7** beneath laminar extraction stream **9**. Laminar sample stream **8** is in contact with laminar extraction stream **9** in extraction channel **7** for a length *L*. Smaller ("desired") particles **18** from laminar sample stream **8** indicated by the stippling in laminar extraction stream **9** flow into product outlet channel **11** as product stream **13** which exits at product outlet **14** as shown by the upward-pointing arrow. By-product stream **12** is the continuation of laminar sample stream **8** past product stream **13**. By-product stream **12** contains both the larger ("undesired") particles and a portion of the smaller ("desired") particles which have not diffused into product stream **13**. By-product stream **12** flows through by-product outlet channel **10** out through by-product outlet **15**.

As noted above, the devices of this invention can be used for hemodialysis. The following discussion points out considerations to be made when designing the devices of

this invention and provides an example of removing urea from blood and various details of such a procedure.

When the extraction device of this invention (either H-filter or flat filter embodiment) is used as a blood dialyzer, blood is supplied to the device via a shunt. The flow rate within the device, F_F, is determined by the diffusion rate (preferably enhanced by the use of a sequestering material) of the desired particles of interest, as well as the geometry of the device itself. The extraction efficiency of the device depends, in part, on the time that the particles have to diffuse, which determines the maximum overall device flow rate.

The diffusion coefficient of urea is 11.8 X 10^{-6} cm^2/s. First, consider the situation where diffusion is not absorption enhanced, i.e., no sequestering material is employed. The following variables used in this example, along with relevant assumptions are:

M_U: Total mass of urea in the body. Generation and excretion will not be considered mathematically in this application, but their affects will be discussed. It is assumed that this value is known (it is easily measurably in dialysis patients).

V_B: Total blood volume (including the volume in the shunt at any moment). This value is typically 5-6 L for adults and 2-3 L for children.

E: Extraction efficiency of the device. Without sequestering material, this is 0.5 since the final output streams are in equilibrium. The device is designed to allow equilibration (i.e., low enough flow rate so that contact time is high enough for the given geometry).

We are interested in, then, how M_U varies in time. The value of M_u is harder to determine because although we know that any pass through the device removes half of the incident urea, the overall amount in the body is constantly decreasing. From this statement, or from the fact that we must fundamentally be solving Fick's Second Law of Diffusion, we expect the solution to be an exponential decay. One final, crucial

assumption is that the blood which returns to the body becomes well-mixed with the rest of the blood. Due to the forceful action of the heart, this is a valid assumption.

The general equation for M_U is:

$$M_U = M_{U(o)}e^{-kt}$$

Taking the derivative with respect to time yields:

$$\frac{dM_U}{dt} = -kM_{u(o)}e^{-kt}$$

Consider this derivative at time = 0:

$$\frac{dM_U}{dt}\Big|_{t=0} = -kM_{U(o)}$$

As is typical with this type of problem, k is the ratio between the initial mass and the initial rate of action. Since the initial mass is known, k can be determined by finding an expression for the initial rate. The flow rate in the device, F_F, represents the rate at which volume from the body is treated in the device. Multiplying by the initial concentration of urea gives the initial treatment rate (initial rate of extraction of desired particles) in the correct units (mass/time). Remembering the extraction efficiency and that this rate must have a negative sign since it represents removal, the initial rate can be written as:

$$\frac{dM_{U(o)}}{dt}\Big|_{t=0} = -\left(\frac{M_{U(o)}}{V_B}\right)F_F E$$

where the term in parentheses is the initial concentration. Substituting back into the initial condition equation allows solution for k:

$$k = \left(\frac{F_F}{V_B}\right)E$$

And substituting back into the mass equation yields:

$$M_U = M_{U(o)} e^{-\left[\left(\frac{F_F}{V_B}\right)E\right]t}$$

An increase in the coefficient of t means a faster decay, which implies faster removal from the system. Increasing the flow rate in the device, F_F, quickens removal of urea since the blood can be treated faster. Increasing the extraction efficiency also quickens removal of urea because the same volume passing through the device is more thoroughly cleansed, i.e., more urea is extracted. Both of these predictions are consistent with the equation. Increasing the total blood volume slows removal since the urea would be more dilute and more volume would have to be treated to achieve the same removal. This prediction is also consistent with the equation.

Considering the example above wherein no sequestering material is employed, the equilibrium condition specifically for urea imposes some limitations on design. Preferably, the diffusion dimension of the device, d, is as small as possible. This serves to reduce diffusion times and increase the upper limit of flow rate. This dimension, however, may be limited by the possible clogging of the channel by red cells (which have a diameter of approximately 8 μm), and generally is at least about 100 μm in cases in which the sample is whole blood. The average distance a diffusing molecule must travel for equilibrium is, then, half of this value, or 50 μm. Consider the equation of Brownian motion:

$$\frac{(\Delta x)^2}{\Delta t} = 2D$$

where D is the diffusion coefficient, which is 11.8 X 10^{-6} cm^2/s for urea. Solving for average diffusion time yields a value of $\Delta t = 1.06$ s. This is the lower limit of contact time for the two streams. For the purposes of the following calculation, we chose a length of the device, L, to be 10 mm. In this case, the fluid must travel 10 mm in no less than 1.06 seconds, yielding a maximum average velocity:

$$\bar{V}=\frac{L}{\Delta t} = \frac{10mm}{1.06s} = 9.434\frac{mm}{s}$$

Flow rate is the product of average velocity and cross-sectional area. Because the amount of time the blood needs to be in contact with the extraction stream is to be determined, only the half of the channel which is introducing blood is considered.

A fundamental difference between the H-filter embodiment and the flat filter embodiment is the width dimension, w. In the H-filter, the width is preferably about 50 μm if the substrate is silicon. In the flat filter, as discussed above, the width is theoretically limitless, and a width of about 1 meter is contemplated.

The contact time necessary for hemodialysis without sequestering material, as well as the number of devices needed in parallel in order to decrease the necessary contact time, in both the H-filter embodiment and flat filter embodiment are compared below. Then the effects of various extraction efficiencies are provided for comparison.

In an H-filter embodiment wherein w = 50 μm, the flow rate is:

$$F_F = \bar{V}\cdot w\cdot\frac{d}{2} = (9.434\frac{mm}{s})\left(\frac{1x10^3\mu m}{mm}\right)(50\mu m)\left(\frac{100\mu m}{2}\right) = 2.358x10^7\frac{\mu m^3}{s}$$

Converting to standard units yields a flow rate of 2.358 X 10^{-5} ml/s. This value can be substituted into the mass-removal equation above. A slight rearrangement of that equation is useful:

$$\frac{M_U}{M_{U(o)}} = e^{-\left[\left(\frac{F_F}{V_B}\right)E\right]t}$$

The left-hand-side now represents the fraction remaining (FR, current mass divided by initial mass). A target fraction can be chosen and the necessary contact time calculated. Generally, the target for such an exponential process is 99% completion, which corresponds to 0.01 FR. Using the above value of F_F, and E = 0.5 and V_B = 5L for a

typical adult, the time is 1.953 X 10^9 seconds, or 61.9 years. Alternatively, a target time of 4 hours (an estimate based upon typical hemodialysis session length) can be chosen and the necessary F_F can be calculated. Multiple H-filters can be connected in parallel. Dividing by our single-device flow rate determines the number of H-filters required. A contact time of 4-hours requires an F_F of 3.198 ml/s. Dividing by our single-device rate of 2.358 X 10^{-5} ml/s predicts the requirement of over 130,000 H-filters in parallel. This illustrates the advantage of using a sequestering material in an H-filter extraction device to increase extraction efficiency.

For comparison, consider a flat filter width of 1 meter, which effectively scales the H-filter by a factor of 20,000 (without the use of sequestering material). This increases the single-device flow rate, F_F, to 0.4716 ml/s, or 28.30 ml/min, or 1.7 L/hr. The 5 liters is not considered a "serial" volume, so the 1.7 liters cannot simply be divided into 5 to determine treatment time. We must revert to the mass equation, and the target FR of 0.01. Using the flat filter flow rate, E = 0.5 and a blood volume of 5 liters, the necessary contact time (treatment time) is 97,650 seconds or 27.1 hours. The use of several flat filters in parallel can decrease the necessary contact time.
A target time of 4 hours yields a necessary flow rate of 3.198 ml/s (the same as in the H-filter case). This predicts the need for 7 flat filters in parallel. Alternatively, a single flat filter with a width of 7 meters is considered. However, this seems less preferable from a design standpoint. The effect of multiple flat filters is parallel (with no sequestering material) is illustrated in Figure 14. The fraction of urea remaining decreases as the number of flat filters in parallel increases.

Total blood volume, V_B, is an important factor in the mass removal equation. Patient blood volume can range realistically from 1 liter to 6 liters, ranging from infants to children to adults. The effect of total blood volume when using 7 flat filters in parallel (with no sequestering material) is illustrated in Figure 15. The fraction of urea remaining decreases as the total blood volume decreases.

Figure 16 illustrates the effect of increasing extraction efficiency when using 7 flat filters in parallel. The fraction of urea remaining decreases as the extraction efficiency of the sequestering material increases.

In the flat filter embodiment, it may be preferable to choose an extraction channel length greater than 10 mm. A preferred embodiment is one in which the length is 50 cm and the width is 50 cm. This makes the filter a square shape comparable in size to current hemodialysis machines. Moreover, it increases the maximum possible flow rate by a factor of 25, which decreases the 0.01 fraction time from 27.1 hours to 1.1 hours. The possibility of shearing of cells at high flow rates must be considered.

When considering extraction efficiencies less than 1.0, a commonly suggested notion is to place multiple flat filters in series, rather than parallel. Consider a case wherein E = 0.5. Two flat filters in parallel increases E to 0.75 because only one quarter of the original urea would remain. When considering the decrease in the time constant, this is an improvement factor of 1.5. However, if the same two devices are connected in parallel (each with E = 0.5), the improvement factor is 2.0 since the flow rate is doubling. Thus, it is preferable to connect multiple devices in parallel rather than in series in order to increase extraction efficiency.

Numerous embodiments besides those mentioned herein will be readily apparent to those skilled in the art and fall within the range and scope of this invention. All references cited in this specification are incorporated in their entirety by reference herein. The following examples illustrate the invention, but are in no way intended to limit the invention.

EXAMPLES

Example 1

An extraction device was prepared by etching a silicon wafer using techniques known in the art (Brody and Yager, *Solid State Sensor and Actuator Workshop* Hilton Head, SC June 2-6, 1996). The channel length was approximately 100 μm, the channel

depth (diffusion dimension) was approximately 15 μm, and the channel width was approximately 10 μm. FITC (fluorescein) labeled biotin (Sigma Chemical #B8889) (0.5 μg/ml) in distilled water was conducted into the sample stream inlet. Rhodamine labeled avidin (Sigma Chemical #A3026) (160 μg/ml) in distilled water was conducted into the extraction stream inlet. The flow rate through the extraction channel was approximately 100 picoliters/sec. It is known to those in the art that approximately 1 mg of avidin binds 10-15 μg of biotin. The rhodamine labeled avidin was observed moving at a rate substantially less than the flow rate, and it was determined that it was adhering to the walls of the channel, inlets and outlets.

To counteract this adhering of the avidin to the walls, the amount of avidin needed to coat the walls of the device uniformly with a monolayer of avidin was calculated. The device had a volume/surface area ratio of about 10 μm. A 1 mg/ml solution of avidin was calculated to be the minimum amount needed to cover the walls of a device with a volume/surface area ratio of about 10 μm, assuming that all of the avidin would be adsorbed by the walls. (The internal volume of the device was calculated to be 15 picoliters, and thus this is the volume of solution needed to fill the device and coat the walls.) A solution of avidin (0.17 mg/ml) (which was 1/6 the concentration of a 1 mg/ml solution) in distilled water was introduced into the device in 6 aliquots. The avidin coated front was moving at a rate approximately 1/6 the average flow rate, thereby indicating that avidin was adhering to the walls.

Example 2

The device with its walls substantially completely and uniformly coated with avidin (prepared in Example 1) was used in Example 2. A solution (10 μL) of streptavidin immobilized on 1 μm iron oxide particles (Sigma Chemical #S2415) in distilled water was introduced into the extraction stream inlet. A solution of biotin (Sigma Chemical #B8889) (10 ng/ml) in distilled water was conducted into the sample stream inlet. It was apparent to the naked eye that biotin became concentrated in the extraction stream. However, the fluorescence was low because the number of molecules of streptavidin immobilized on the iron oxide was small, necessitating a low biotin concentration in order to maintain a stoichiometric excess of streptavidin. Additionally,

streptavidin partially quenches the fluorescent marker (FITC), making fluorescent measurements more difficult.

To improve fluorescent measurements, the device is pre-loaded with a polyethyleneglycol silane to achieve surface passivation. A streptavidin with a longer arm prior to its binding site for biotin helps prevent quenching of fluoroscein.

CLAIMS

1. An extraction device for extracting desired particles from a sample stream containing said desired particles, said device comprising:

   a. a sample stream inlet;

   b. an extraction stream inlet;

   c. an extraction channel in fluid communication with said sample stream inlet and said extraction stream inlet for receiving a sample stream from said sample stream inlet in adjacent laminar flow with an extraction stream from said extraction stream inlet;

   d. a sequestering material within said extraction channel for capturing desired particles in said extraction stream;

   e. a by-product stream outlet in fluid communication with said extraction channel for receiving a by-product stream comprising at least a portion of said sample stream from which desired particles have been extracted; and

   f. a product outlet in fluid communication with said extraction channel for receiving a product comprising said sequestering material and at least a portion of said desired particles.

2. A microfabricated device of claim 1.

3. A device of claim 1 wherein said sequestering material is a material capable of adsorbing desired particles.

4. A device of claim 1 wherein said sequestering material is a material capable of absorbing desired particles.

5. A device of claim 1 wherein said sequestering material is effectively non-diffusing in said device.

6. A device of claim 1 wherein said sequestering material is capable of interacting with said desired particles so as to enable detection of said desired particles.

7. A device of claim 1 wherein said sequestering material is capable of releasing said desired particles to enable their analysis after extraction from said sample stream.

8. A device of claim 1 wherein said product contains greater than 50% of said desired particles in said sample stream.

9. A device of claim 1 wherein said product contains greater than 75% of said desired particles in said sample stream.

10. A device of claim 1 wherein substantially all of said desired particles are removed from said sample stream.

11. A device of claim 1 having a width of between about 2 micrometers and one meter.

12. A device of claim 1 having a width between about 5 micrometers and 5 centimeters.

13. A device of claim 1 having a width between about 10 micrometers and 100 micrometers.

14. An analytic system comprising a device of claim 1 in combination with means for detecting the presence of said desired particles captured by said sequestering material.

15. An analytic system comprising a device of claim 1 in combination with means for detecting the presence of an analyte in said by-product stream from which desired particles have been removed.

16. A system comprising a device of claim 1 in combination with means for removing said sequestering material with said captured desired particles from said product stream.

17. An analytic system comprising a device of claim 14 in combination with means for detecting the presence of an analyte in said product stream from which desired particles and sequestering material have been removed.

18. An apparatus containing a plurality of devices of claim 1 connected in parallel.

19. A method for extraction of at least a portion of desired particles from a sample stream comprising said desired particles, said method comprising the steps of:

a. introducing said sample stream into the sample stream inlet of an extraction device of claim 1;

b. introducing an extraction stream into the extraction channel of said extraction device; and

c. introducing into said extraction channel a sequestering material for capturing the desired particles such that the desired particles are captured by the sequestering material, and said extraction stream, comprising said sequestering material and at least a portion of said desired particle, exits said said device as a product stream, and said sample stream from which desired particles have been extracted, exits said device as a by-product stream.

20. The method of claim 19 wherein said sample stream is blood and said desired particles are toxin particles.

21. The method of claim 19 wherein said desired particles are particles of a substance which interferes with analysis of said sample stream.

22. The method of claim 19 wherein said sequestering material comprises polymeric beads.

23. The method of claim 19 wherein said sequestering material comprises an enzyme which binds to said desired particles.

24. The method of claim 19 wherein said sequestering material is an absorbent material.

25. The method of claim 19 wherein said sequestering material is a substantially non-diffusing high molecular weight polymer.

26. The method of claim 19 wherein before performing steps a, b, and c, the device is pre-coated with a hydrophilic material by injecting the hydrophilic material into the sample stream inlet and extraction stream inlet.

27. The method of claim 19 wherein a major portion of said desired particles are extracted.

Fig. 1

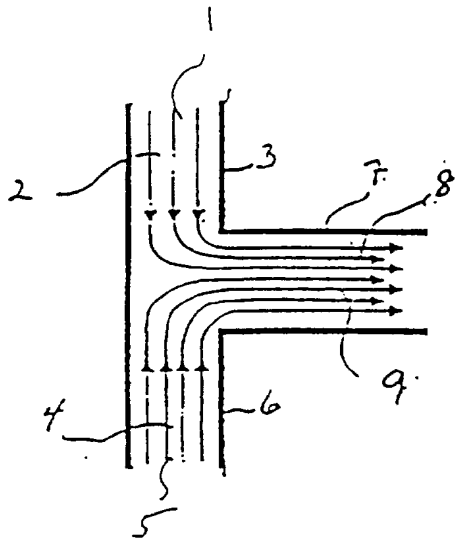


Fig. 2

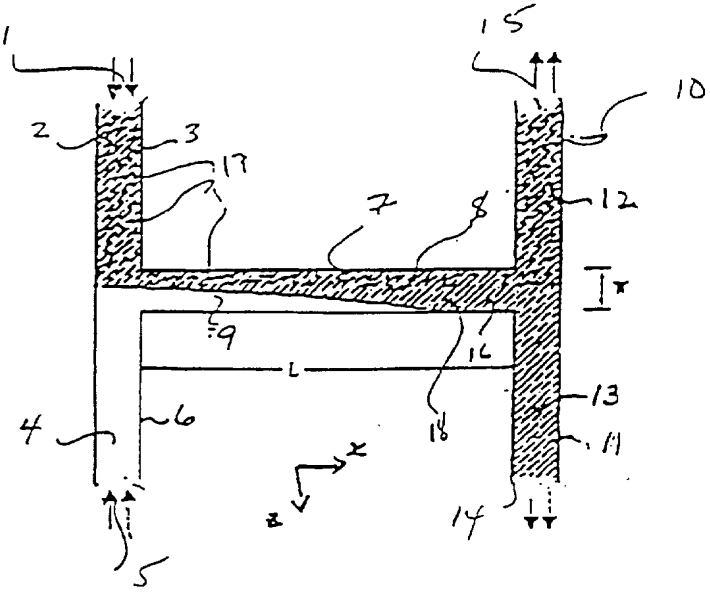


Fig. 3

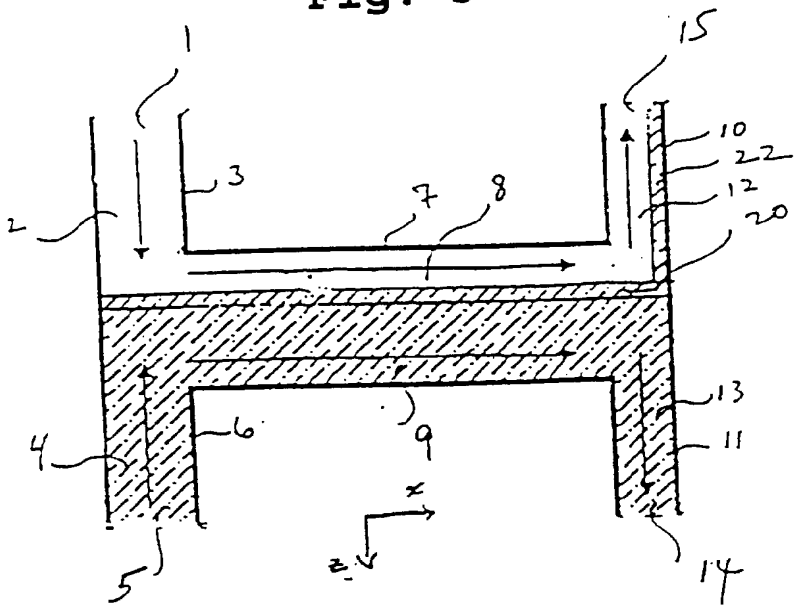
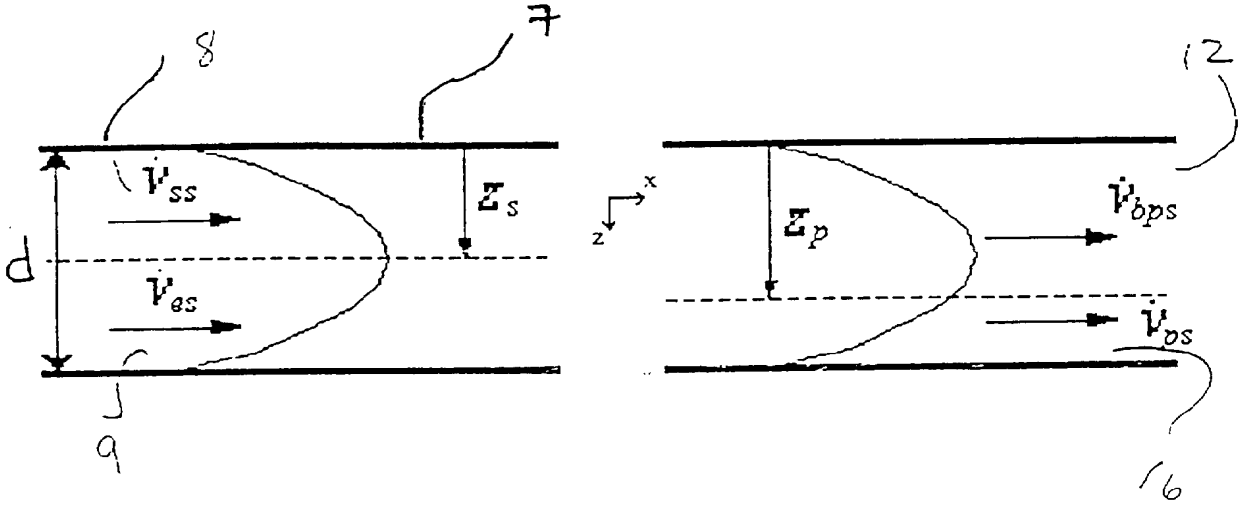


Fig. 4



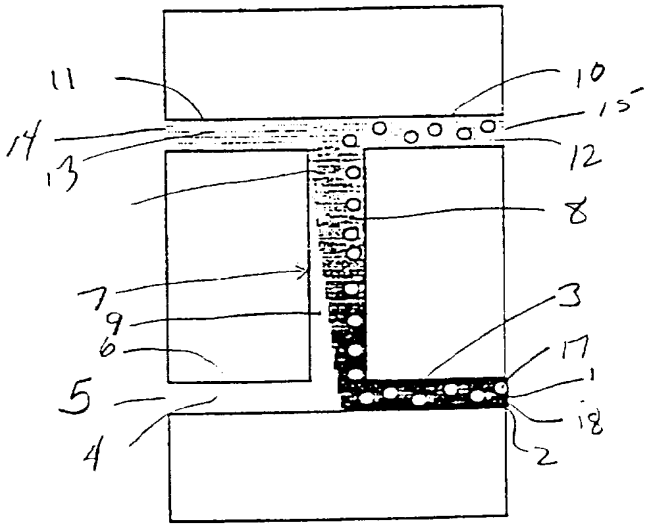

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Fig. 5

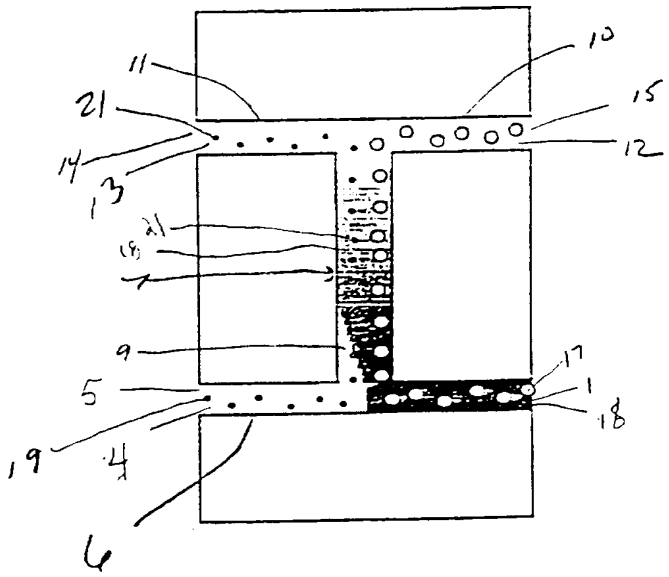
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Fig. 6

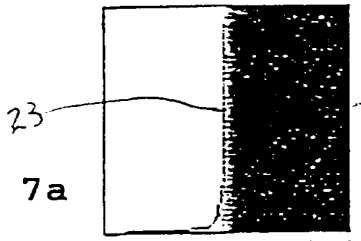


Fig. 7a

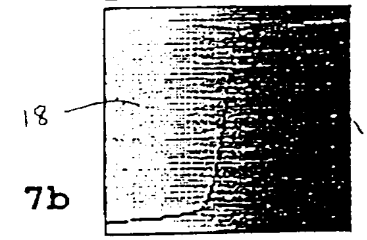


Fig. 7b

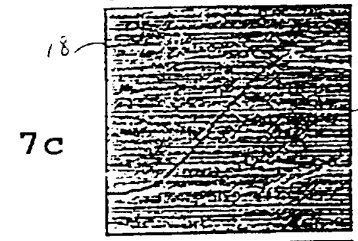


Fig. 7c

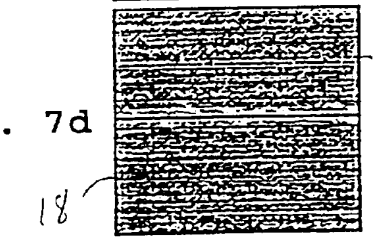


Fig. 7d

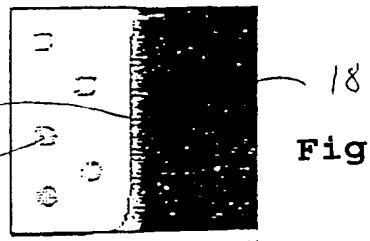


Fig. 8a

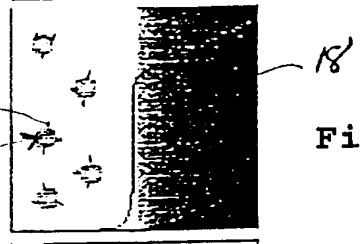


Fig. 8b

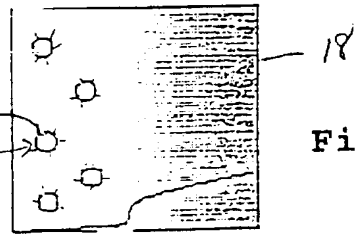


Fig. 8c

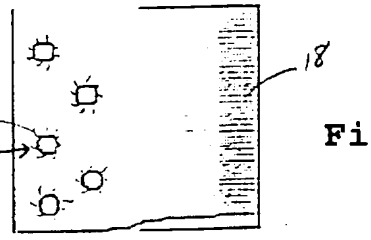


Fig. 8d

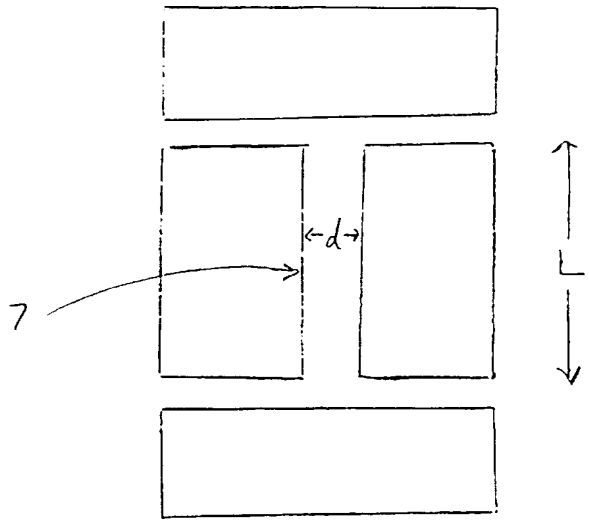


Fig. 9

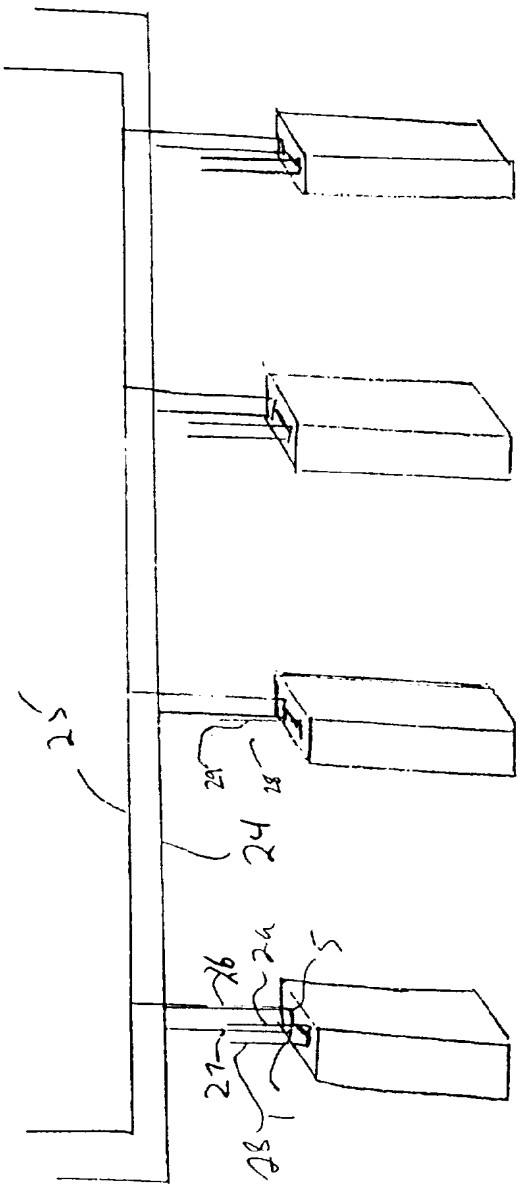


Fig. 10

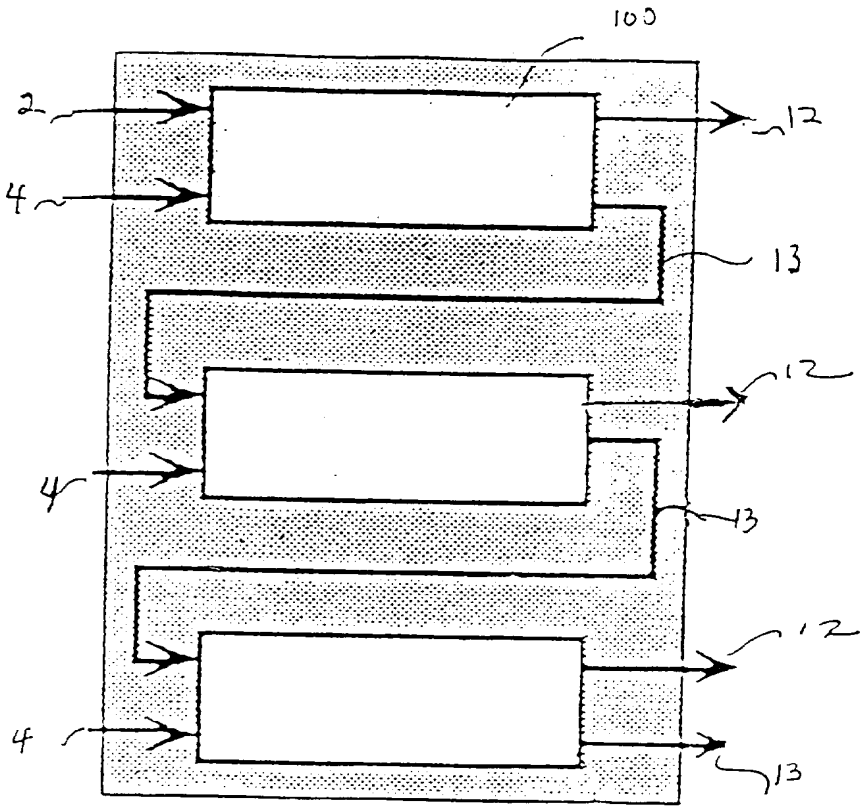


Fig. 11

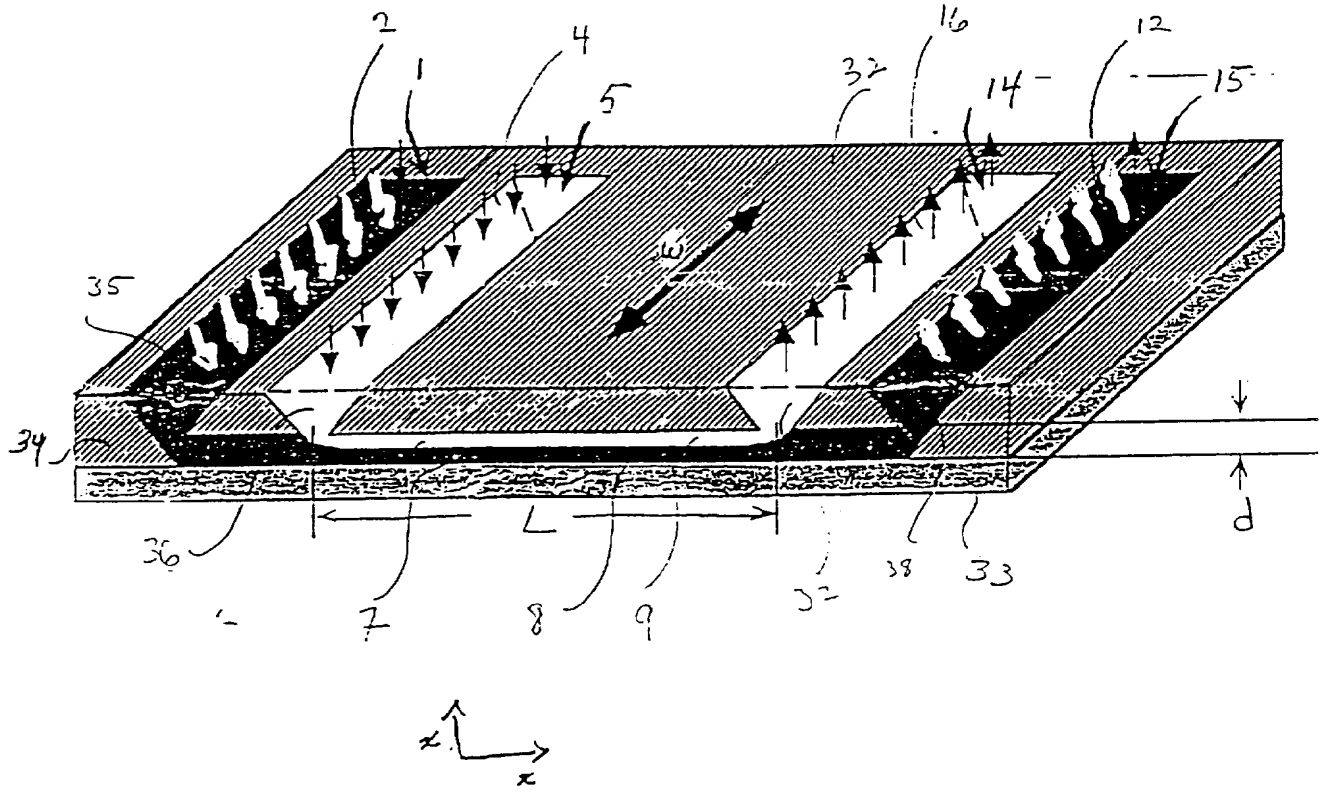


Fig. 12

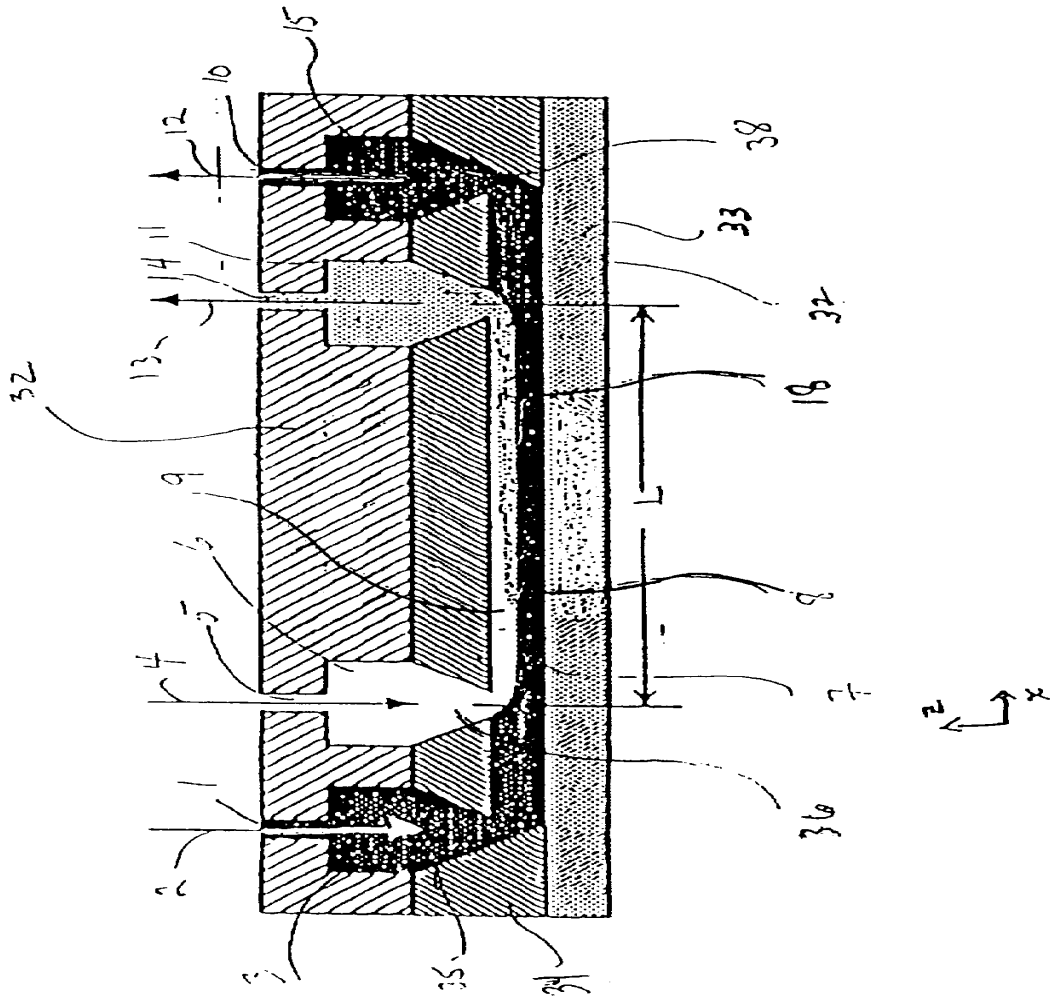


Fig. 13

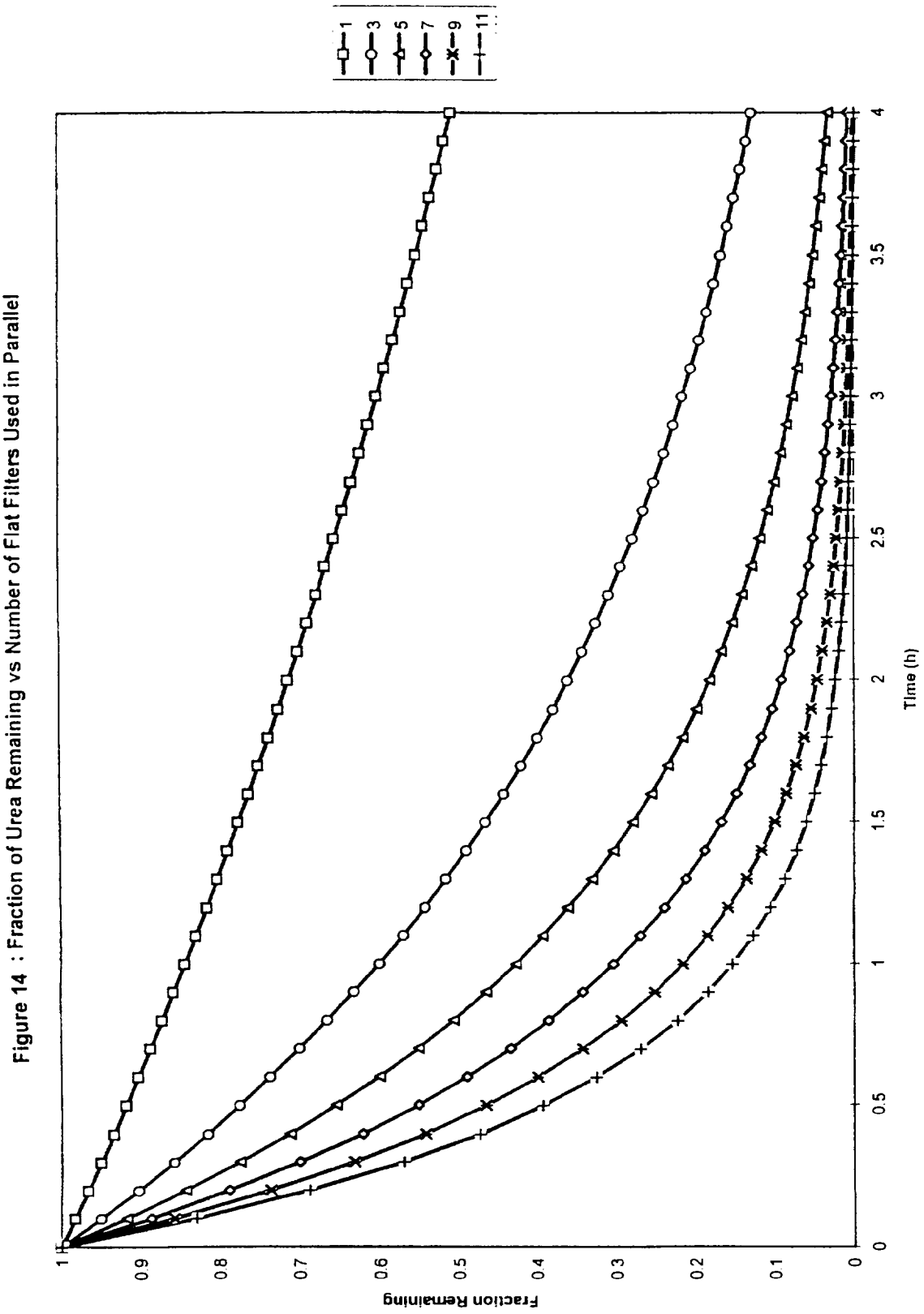


Figure 14 : Fraction of Urea Remaining vs Number of Flat Filters Used in Parallel

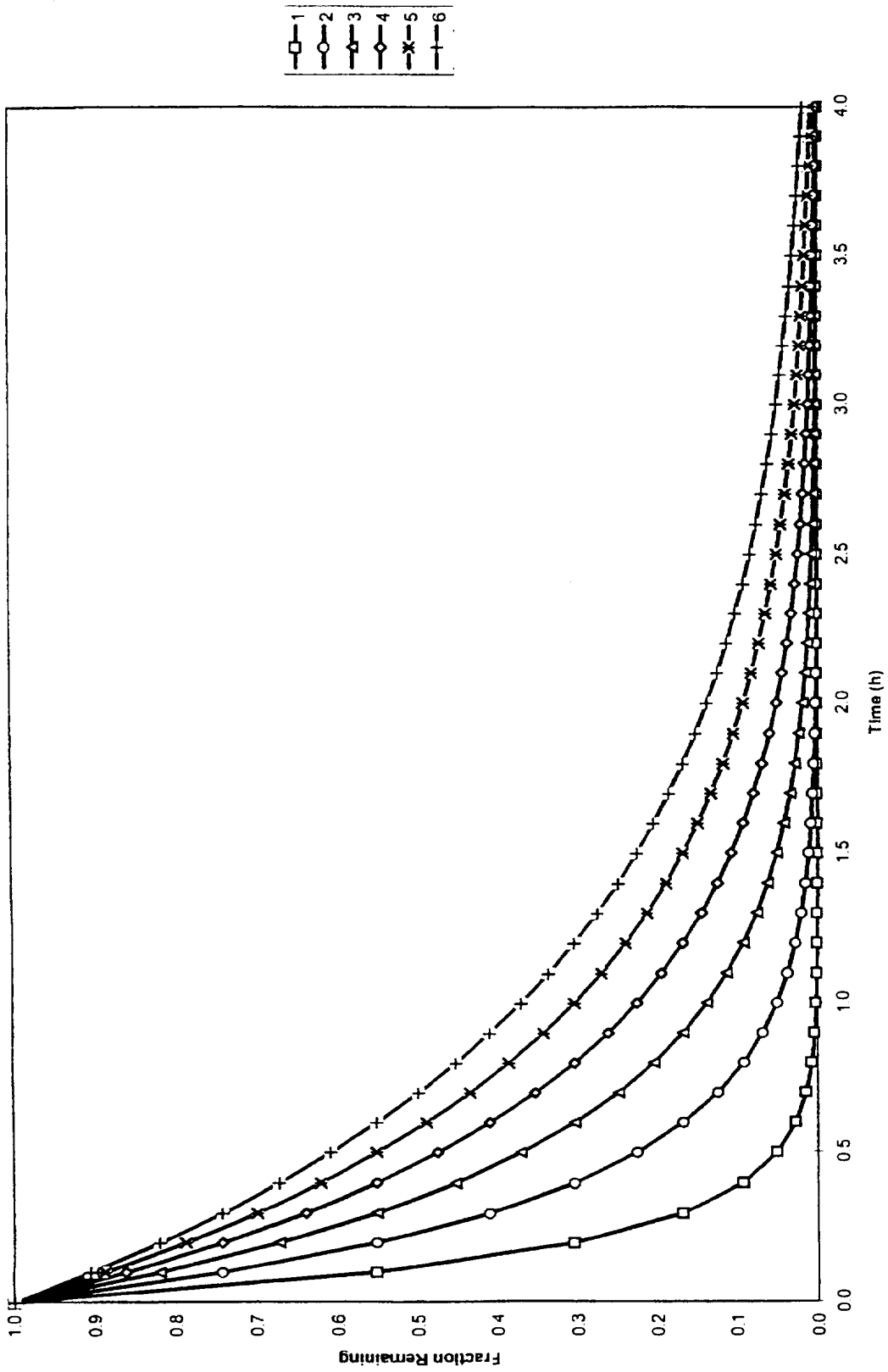


Figure 15 : Fraction of Urea Remaining vs Total Blood Volume (liters)

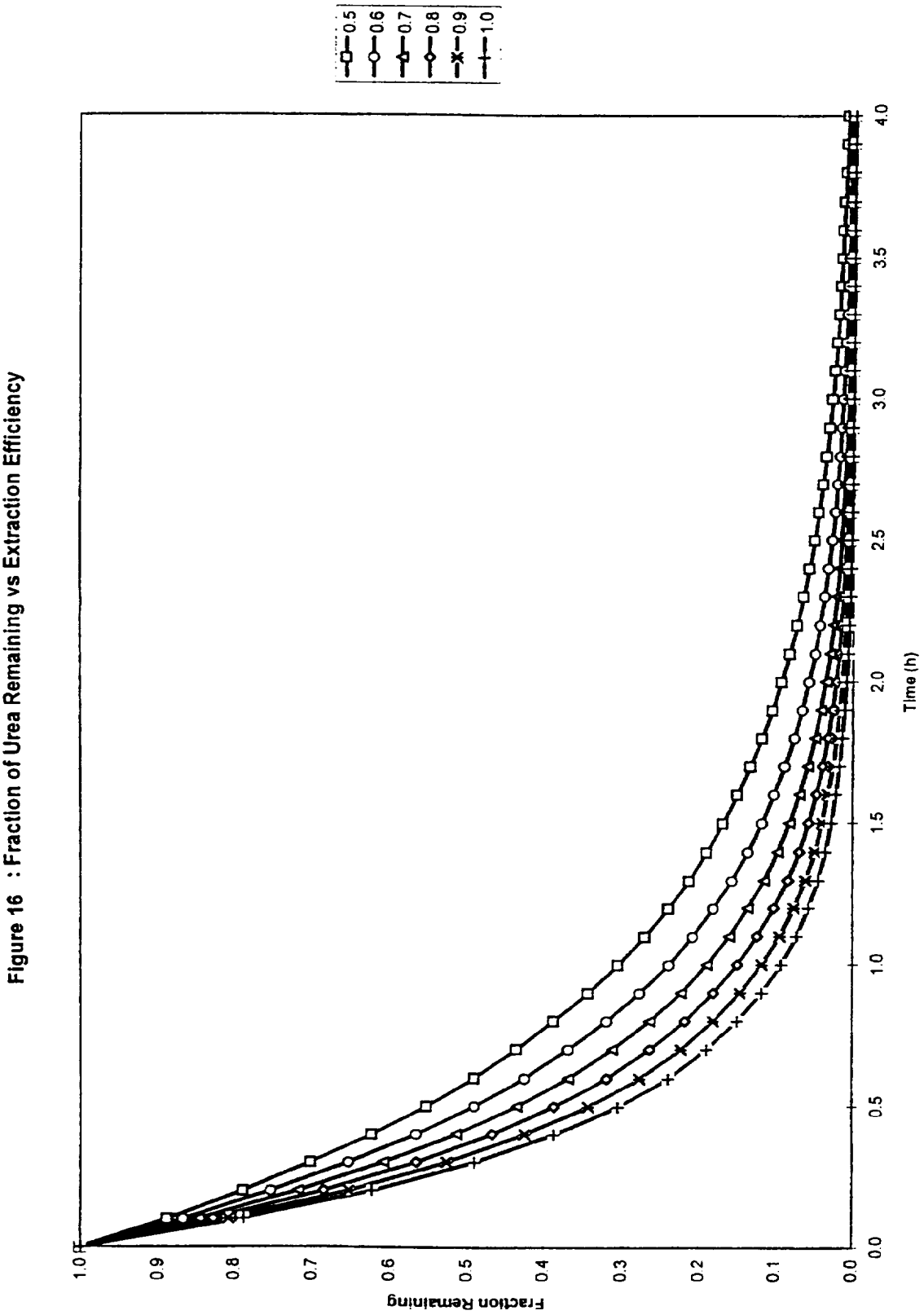


Figure 16 : Fraction of Urea Remaining vs Extraction Efficiency

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/10307

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :B03B 5/28
US CL :209/155
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 209/2, 12.1, 18, 155, 208, 209, 210, 458; 210/656, 659, 723, 726, 198.2, 198.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,193,688 A (GIDDINGS) 16 March 1993	
A	US 5,039,426 A (GIDDINGS) 13 August 1991	
A	US 4,894,146 A (GIDDINGS) 16 January 1990	
A	US 4,737,268 A (GIDDINGS) 12 April 1988	
A	US 4,830,756 A (GIDDINGS) 16 May 1989	
A	US 3,449,938 A (GIDDINGS) 17 June 1969	

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 SEPTEMBER 1997	Date of mailing of the international search report 03 OCT 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID H BOLLINGER Telephone No. (703) 308-1113

Form PCT/ISA/210 (second sheet)(July 1992)*

Espacenet

Bibliographic data: JPH0526799 (A) — 1993-02-02

METHOD FOR SEPARATING PARTICLE

Inventor(s): HORI MITSUHIRO ± (HORI MITSUHIRO)

Applicant(s): NIPPON STEEL CORP ± (NIPPON STEEL CORP)

Classification: **- international:** ***C12M1/10; C12N1/02; G01N15/02; G01N15/14;*** (IPC1-7): C12M1/10; C12N1/02; G01N15/02; G01N15/14

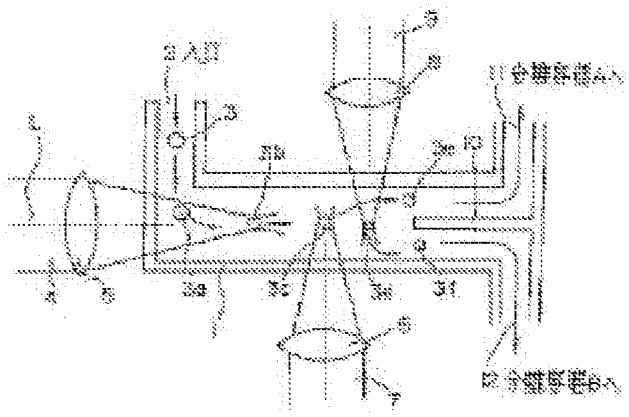
- cooperative: G01N2015/149

Application number: JP19910203230 19910719

Priority number(s): JP19910203230 19910719

Abstract of JPH0526799 (A)

PURPOSE:To perform judging particle size and separation without touching in a flow system including different particles and cells, etc., by using a focused laser beam. CONSTITUTION:Particles 3 are placed in a row on the optical axis of a laser beam 4 by the laser beam 4 and a condenser lens 5 and moved. By measuring the scattered light intensity at the beam waist 3b, the particle size is judged. If the particle size is the desired one, a laser beam 7 or a laser beam 9 is turned on at the flowing particle position of 3c or 3d to change the flow direction of the particle 3 by taking advantage of light pressure. The particle 3 is thus deflected to the direction 11 of a separation vessel A or the direction 12 of a separation vessel B. Furthermore, if the particle size is not the desired one, the light power of the laser beam 7 or 9 is raised and the particle 3 is destroyed by irradiation.



(19)日本国特許庁（ＪＰ）　(12) **公開特許公報（Ａ）**　(11)特許出願公開番号

特開平5－26799

(43)公開日　平成５年(1993)２月２日

(51)Int.Cl.[5]		識別記号	庁内整理番号	ＦＩ	技術表示箇所
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Ｃ１２Ｍ	1/10	Ｚ	2104－4Ｂ		
Ｃ１２Ｎ	1/02		7236－4Ｂ		
Ｇ０１Ｎ	15/02	Ｆ	7005－2Ｊ		
	15/14	Ａ	7005－2Ｊ		

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(21)出願番号　特願平3－203230

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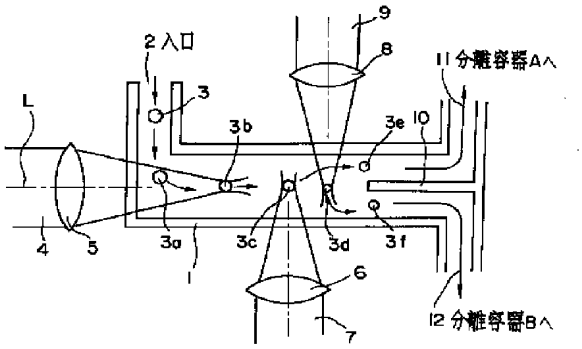
(74)代理人　弁理士　半田　昌男

(54)【発明の名称】　微粒子の分離方法

(57)【要約】

【目的】　異なる微粒子あるいは細胞等を含む流れ系において、集光されたレーザビームを用いて粒径判別と分離を非接触にて行う。

【構成】　微粒子３はレーザビーム４と集光レンズ５によってレーザビーム４の光軸上に一列に並べられ、移動する。ビームウエスト３ｂで散乱光強度を計測することにより粒径を判別し、所望の粒径の場合には、下流の位置３ｃあるいは３ｄに流れてきたときにレーザビーム７もしくはレーザビーム９をどちらかを点灯し、光の圧力によって微粒子３の流れの方向を変え、分離容器Ａの方向１１あるいは分離容器Ｂの方向１２へ微粒子３を振り分け、分離する。さらに、所望の粒径でない場合にはレーザビーム７あるいは９の光出力を上げて照射し、微粒子３を破壊する分離方法。



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【特許請求の範囲】

【請求項１】　異なる微粒子あるいは細胞（以下、細胞等も含めて微粒子と称す。）を含む溶液が一定流量で流れている流れ系において、集光したレーザビームを照射し、微粒子をレーザの出射方向の光軸上へ一列に並ぶように移動させ、ビームウエスト付近で散乱光強度を計測することにより、流れてきた個々の微粒子の粒径の違いを判別し、その結果、所望の粒径をもった微粒子である場合には、前記微粒子が分離容器近くに流れてきた時に、別の集光されたレーザビームを前記微粒子に照射することにより、前記微粒子を特定の分離容器の方向に流れ込ませ、一方、所望の粒径でない場合にはさらに別の集光されたレーザビームを照射し、微粒子を破壊・分解し、異なる微粒子から同種のあるいは必要とする微粒子を分離することを特徴とする微粒子の分離方法。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、非接触で細胞、高分子等の微粒子を分離、選別する方法に関するものである。

【０００２】

【従来の技術】従来の微粒子の分離においては、フローサイトメトリーによる方法がある。この方法は、微粒子を含む溶液をノズルから振動させながら押し出すことによって液滴を飛ばし、この液滴にレーザ光を照射し液滴の蛍光、散乱光強度を計測することによって微粒子の大きさあるいは種類を判別し、この情報をもとに必要とする微粒子を含む液滴には帯電させ、分離容器前の高電圧の印加された電極板間を通過するとき液滴の飛ぶ方向が変わり、所定の分離容器に入り、分離するものである。

【０００３】

【発明が解決しようとする課題】前記、従来の方法に述べたようなフローサイトメトリーによる微粒子の分離においては、微粒子を含む液滴が、ノズルから高速に押し出され、高速で分離容器にはいるため、壊れ易い微粒子（たとえば、植物細胞のプロトプラスト等）の場合は、損傷を受ける可能性がある（文献：鷲津ら、応用物理、第５８巻　第3号Ｐ３８３（１９８９））。また前記手法では、微粒子が含まれる液滴が外気にふれるため完全な非接触で微粒子を分離することはできない。それゆえ、微粒子を壊さずに、非接触で分離する方法が望まれていた。

【０００４】また、非接触で分離するためには、ガラス等で覆われたセル内を分離する微粒子を含んだ溶液を流している状態で微粒子の粒径や質量等を識別し、分離できることが望ましい。

【０００５】ガラスで覆われたセル内を流れている微粒子を分離するためには、微粒子を押して微粒子の流れの方向を変えて分けることが考えられ、押す手段としては、水流あるいは空気圧や光の圧力が上げられる。光の圧力によって微粒子を押す現象については、文献：Ａ，

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Ａｓｈｋｉｎ，Ｐｈｙｓ．Ｒｅｖ．Ｌｅｔｔ，，２４，１５６（１９７０）に報告されており、光が微粒子内を屈折して通過する際の運動量変化によって生ずるものである。微粒子を押す手段として、水流の場合には渦の発生による流れの乱れの問題があり、また空気圧の場合には、泡の発生及びその混入の影響があるため、光を用いて分離することが望ましい。

【０００６】

【課題を解決するための手段】上記のような課題を解決するため、一定流量で流れている流れ系において、集光したレーザビームを照射し、微粒子をレーザの出射方向の光軸上へ一列に並ぶように移動させ、ビームウエスト付近で散乱光強度を計測することにより、流れてきた個々の微粒子の流径の違いを判別し、その結果、所望の粒系をもった微粒子である場合には、前記微粒子が分離容器近くに流れてきた時に、別の集光されたレーザビームを前記微粒子に照射することにより、前記微粒子を特定の分離容器の方向に流れ込ませ、一方、所望の粒系でない場合にはさらに別の集光されたレーザビームを照射し、微粒子を破壊・分解し、異なる微粒子から同種あるいは必要とする微粒子を分離する方法を提供する。

【０００７】

【実施例】以下に本発明の一実施例を図１を参照して説明する。本実施例の分離方法では、図１に示すような上下面及び側面すべてガラス製の流路形状をもったセル１に対し、入口２より粒径等の異なる微粒子３を一定流量あるいは一定流速で流し込み、微粒子が位置３ａに来た時、左側からレーザビーム４がレンズ５で集光され、セル１のガラス側面より微粒子３に照射され、レーザビーム４の光軸Ｌ方向でかつビームウエスト３ｂ方向へ、一定流量の流速に集光されたレーザビームによる光の圧力によって微粒子３は、光軸Ｌ上に引き寄せられて一列状になってビームウエスト方向へ移動する。

【０００８】また、ビームウエスト３ｂ付近では、微粒子３はミー散乱を生じ、ミー散乱光強度は、微粒子３の粒径等できまるものであるため、レーザビーム４の光軸Ｌに垂直な方向に設置したフォトダイオード等で、前記ミー散乱光強度を計測し、微粒子３の粒径の情報を得る。必要とする粒径を持った微粒子の場合には、微粒子３がビームウエスト３ｂ位置より下流のレーザビーム４の光軸上にある位置３ｃへ流れて来たときに、別のレーザビーム７を集光レンズ６で集光したビームを流れの方向と垂直な方向より照射する。前記、集光されたレーザビームによる光の圧力によって微粒子３の流れる方向は変わり、位置３ｅの方向へ流れる。セル１においては仕切り板１０があるため、微粒子３は、分離容器Ａ，１１の方向に流れ込む。また、前記計測して得た粒径情報から必要とする微粒子でない場合には、レーザビーム７と集光レンズ６による集光ビームを照射させずに、位置３ｃよりも下流のレーザビーム４の光軸上にある位置３ｄ

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に微粒子３が達したとき、レーザビーム７と集光レンズ６による集光ビームよりも光出力の大きいレーザビーム９と集光レンズ８による集光ビームを照射して微粒子３を、破壊することができる。細胞等の場合には焼き殺された状態となる。

【０００９】以上のように本方法ではレーザビームの光出力によって、微粒子の流れの方向を変えたり、微粒子を粉々に破壊分解することが可能であり、レーザビーム７とレーザビーム９がともに光出力が可変であれば、位置３ｃにおいても微粒子３を破壊することができる。

【００１０】また、レーザビーム９と集光レンズ８で微粒子３を位置３ｄから位置３ｆ方向に流し、分離容器Ｂ，１２方向へ流れ込ませ分離することができる。このようにセル１の場合には、不要な微粒子は破壊できるため、必要な２種類の粒径の微粒子に分けることができる。図１では、分離容器Ａ、Ｂの２方向に流路が分かれたが、流路は分けずに一方向とし、レーザビーム７と集光レンズ６あるいはレーザビーム９と集光レンズ８のどちらか一方からなる系にて、所望の粒径の場合には、レーザビームを照射せずに、微粒子を流し、所望の粒径の場合には、レーザビームと集光レンズにより、集光させたレーザビームで微粒子を破壊する分離方法も考えられる。

【００１１】このような流れ系において集光された光の圧力を用いた分離方法は、微粒子３が浮遊した状態で分離するため壊れ易い細胞等でも、損傷なく分離することができる。

【００１２】細胞を光の圧力で押す際の光出力は、細胞の光の波長に対する吸収特性を考慮し細胞を死滅させない範囲の出力を与える必要があり、約数ｍＷ～数十Ｗである。この場合にはレーザビームには、高効率、長寿命、安定である半導体レーザを用いてもかまわない。波長７９０ｎｍ，光出力２２ｍＷの半導体レーザを用いた場合では、レンズ５，６，８は、ＮＡ（開口数）＝０．４程度以上で集光すれば、粒径数μサイズの細胞あるいはポリスチレンラテックス粒子を光軸上に並ばせることができ、かつ光の圧力で押し、本方法で分離することができた。また、粒径数μサイズの細胞あるいはポリスチレンラテックス粒子では、数百ｍＷの光出力を前記ＮＡ＝０．４程度以上のレンズで集光、照射することによって死滅し、分子、原子のオーダーまで破壊できる。

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【００１３】

【発明の効果】以上説明したように本発明によれば、壊れ易い微粒子等でも溶液に浮遊した状態で分離するため壊れにくい。また、微粒子を含んだ流れ系はガラス越しの集光されたレーザビームを用いて分離するため、完全な非接触で微粒子を分離することが可能である。さらに不要な微粒子を完全に分子、原子のオーダーまで破壊できる。

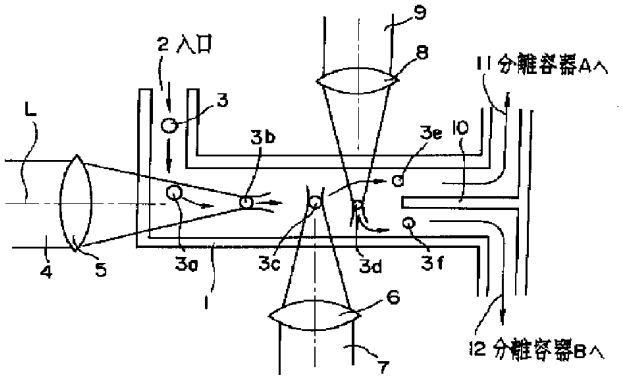
【図面の簡単な説明】

【図１】微粒子を分離するための模式図である。

【符号の説明】

１　微粒子を分離するためのセル
２　セルの入口
３　微粒子
４，７，９　レーザビーム
５，６，８　集光レンズ
１０　仕切り板
１１　分離容器Ａへの流れの方向を示す矢印
１２　分離容器Ｂへの流れの方向を示す矢印

【図１】



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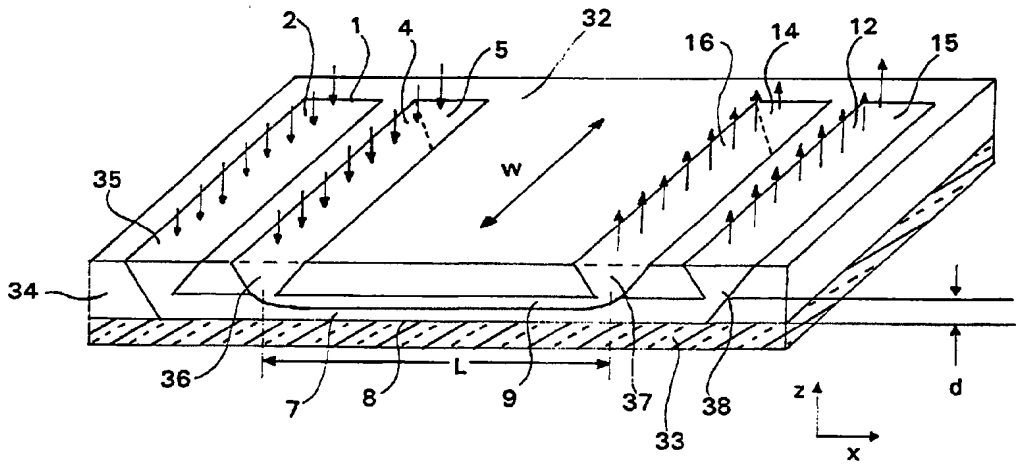

(57) Abstract

This invention provides a microfabricated extraction system and methods for extracting desired particles from a sample stream containing desired and undesired particles. The sample stream is placed in laminar flow contact with an extraction stream under conditions in which inertial effects are negligible. The contact between the two streams is maintained for a sufficient period of time to allow differential transport of the desired particles from the sample stream into the extraction stream. In a preferred embodiment the differential transport mechanism is diffusion. The extraction system of this invention coupled to a microfabricated diffusion-based mixing device and/or sensing means allows picoliter quantities of fluid to be processed or analyzed on devices no larger than silicon wafers.

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MICROFABRICATED DIFFERENTIAL EXTRACTION DEVICE AND METHOD

This invention was made with government support under Army research contract DAMD17-94-J-4460 awarded by the U.S. Army. The government has certain rights in the invention.

Field of the Invention

This invention relates generally to microfabricated extraction systems and methods for separating analytes from streams containing other constituents by differential transport principles such as diffusion and applied fields. The invention is useful, for example, for processing blood to separate a stream containing smaller particles such as albumin molecules from a stream containing cells.

Background of the Invention

Chemical analysis of biological samples is constrained by sample size. Withdrawing a few milliliters of blood from an adult may have little effect, but repeating this procedure every hour or even withdrawing this amount once from an infant can significantly alter the health of the subject. For these reasons, a miniaturized blood analysis system would be useful. Furthermore, while many sophisticated tests that have great

importance for critical care can be performed in major hospital laboratories, a substantial impact could be made on the practice of emergency medicine if some key tests could be performed on the patient at the site of injury. For some assays it is vital to make measurements in the absence of red blood cells, so some form of separation of cells from plasma is required.

Diffusion is a process which can easily be neglected at large scales, but rapidly becomes important at the microscale. The average time t for a molecule to diffuse across a distance d is $t = d^2/D$ where D is the diffusion coefficient of the molecule. For a protein or other large molecule, diffusion is relatively slow at the macroscale (e.g. hemoglobin with D equal to 7×10^{-7} cm^2/s in water at room temperature takes about 10^6 seconds (ten days) to diffuse across a one centimeter pipe, but about one second to diffuse across a 10 μm channel).

Using tools developed by the semiconductor industry to miniaturize electronics, it is possible to fabricate intricate fluid systems with channel sizes as small as a micron. These devices can be mass-produced inexpensively and are expected to soon be in widespread use for simple analytical tests. See, e.g., Ramsey, J.M. et al. (1995), "Microfabricated chemical measurement systems," Nature Medicine **1**:1093-1096; and Harrison, D.J. et al (1993), "Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip," Science **261**:895-897.

Miniaturization of analytic instruments is not a simple matter of reducing their size. At small scales different effects become important, rendering some processes inefficient and others useless. It is difficult to replicate smaller versions of some devices because of material or process limitations. For these reasons it is necessary to develop new methods for performing common laboratory tasks on the microscale.

Devices made by micromachining planar substrates have been made and used for chemical separation, analysis, and sensing. See, e.g., Manz, A. et al. (1994), "Electroosmotic pumping and electrophoretic separations for miniaturized chemical analysis system," J. Micromech. Microeng. **4**:257-265.

Field flow fractionation devices involve particle size separation using a single inlet stream. See, e.g. Giddings, J.C., U.S. Patent 3,449,938, June 17, 1969, "Method for Separating and Detecting Fluid Materials"; Giddings, J.C., U.S. Patent 4,147,621, April 3, 1979, "Method and Apparatus for Flow Field-Flow Fractionation"; Giddings, J.C., U.S. Patent 4,214,981, July 29, 1980, "Stearic Field-Flow Fractionation"; Giddings, J.C. et al., U.S. Patent 4,250,026, February 10, 1981, "Continuous Stearic FFF Device for The Size Separation of Particles"; Giddings, J.C. et al. (1983), "Outlet Stream Splitting for Sample Concentration in Field-Flow Fractionation," Separation Science and Technology **18**:293-306; Giddings, J.C. (1985), "Optimized Field-Flow Fractionation system Based on Dual Stream Splitters," Anal. Chem. **57**:945-947; Giddings, J.C., U.S. Patent 4,830,756, May 16, 1989, "High Speed Separation of Ultra-High Molecular Weight Polymers by Hyperlayer Field-Flow Fractionation"; Giddings, J.C., U.S. Patent 4,141,651, August 25, 1992, "Pinched Channel Inlet System for Reduced Relaxation Effects and Stopless Flow Injection in Field-Flow Fractionation"; Giddings, J.C., U.S. Patent 5,156,039, October 20, 1992, "Procedure for Determining the Size and Size Distribution of Particles Using Sedimentation Field-Flow Fractionation"; Giddings, J.C., U.S. Patent 5,193,688, March 16, 1993, "Method and Apparatus for Hydrodynamic Relaxation and Sample Concentration in Field-Flow Fraction Using Permeable Wall Elements"; Caldwell, K.D. et al., U.S. Patent 5,240,618, August 31, 1993, "Electrical Field-Flow Fractionation Using Redox Couple Added to Carrier Fluid"; Giddings, J.C. (1993), "Field-Flow Fractionation: Analysis of Macromolecular, Colloidal and Particulate Materials," Science **260**:1456-1465; Wada, Y. et al., U.S. Patent 5,465,849, November 14, 1995, "Column and Method for Separating Particles in Accordance with Their Magnetic

Susceptibility"; Yue, V. et al. (1994), "Miniature Field-Flow Fractionation Systems for Analysis of Blood Cells," Clin. Chem. **40**:1810-1814; Afromowitz, M.A. and Samaras, J.E. (1989), "Pinch Field Flow Fractionation Using Flow Injection Techniques," Separation Science and Technology **24**(5 and 6):325-339.

Thin-channel split flow fractionation (SPLITT) technology also provides particle separation in a separation cell having a thin channel. A field force is exerted in a direction perpendicular to the flow direction. Particles diffuse or are otherwise transported from a particle-containing stream across a transport stream to a particle-free stream. The device for operating the process is generally fabricated from glass plates with teflon sheets used as spacers to form the channels. The channel depth can therefore be no smaller than the spacers, which are generally about 100 to 120 μm thick. See, e.g., Giddings, J.C., U.S. Patent 4,737,268, April 12, 1988, "Thin Channel Split Flow Continuous Equilibrium Process and Apparatus for Particle Fractionation"; Giddings, J.C., U.S. Patent 4,894,146, January 16, 1990, "Thin Channel Split Flow Process and Apparatus for Particle Fractionation"; Giddings, J.C., U.S. Patent 5,093,426, August 13, 1991, "Process for Continuous Particle and Polymer Separation in Split-Flow Thin Cells Using Flow-Dependent Lift Forces"; Williams, P.S. et al. (1992), "Continuous SPLITT Fractionation Based on a Diffusion Mechanism," Ind. Eng. Chem. Res. **31**:2172-2181; and Levin, S. and Tawil, G. (1993), "Analytical SPLITT Fractionation in the Diffusion Mode Operating as a Dialysis-like System Devoid of Membrane. Application to Drug-Carrying Liposomes," Anal. Chem. **65**:2254-2261.

The object of this invention is to provide a microfabricated extraction system utilizing differential transport principles in which an analyte can be extracted, detected and quantified.

The advantages, as disclosed herein, of diffusion separation devices on the microscale, e.g., having channel depths no greater than about 100 μm, do not appear to have been recognized in the

prior art. See, e.g., Kittilsand, G. and Stemme, G. (1990), Sensors and Actuators A21-A23:904-907, and Wilding, P. et al. (1994), J. Clin. Chem. **40**:43-47.

All publications, patents and patent applications referred to herein are hereby incorporated by reference.

Summary of the Invention

This invention provides an extraction method and device distinguished from conventional filtration techniques and devices in possessing advantages of size, production economy, integrability with micro chemical analysis systems, low power consumption, and which may be operated in either a sample-to-sample or continuous processing mode. The device is particularly well suited to integration with microfabricated chemical analysis systems in which, for example, a preferred embodiment provides a microfabricated extraction device or system capable of providing a diluted plasma product having a volume ranging from picoliters to nanoliters starting from samples as small as a microliter of whole blood, with a comparable extraction stream volume.

The extraction system is useful as an element in an integrated system of microfluidic and detection elements (such as optical detectors) for tests of medical interest on blood, and also has applications in many other areas of analytical chemistry. In a preferred embodiment useful for blood analysis, the device allows for the extraction of plasma constituents from whole blood, thereby producing a cell-free fluid stream for subsequent analysis.

The microfabricated extraction system of this invention in simplest concept is illustrated by a diffusion extraction device comprising microchannels in the shape of an "H". A mixture of particles suspended in a sample stream enters the extraction channel (the crossbar of the "H") from one of the arms, e.g. the top left, and an extraction stream (a dilution stream) enters

from the bottom left. The two streams flow together in the extraction channel; however, due to the small size of the channels, the flow is laminar and the streams do not mix. The sample stream exits as by-product stream at the upper right and the extraction stream exits as product stream from the lower right. While the streams are in parallel laminar flow in the extraction channel, particles having a greater diffusion coefficient (smaller particles such as albumin, sugars and small ions) have time to diffuse into the extraction stream, while the larger particles (e.g. blood cells) remain in the sample stream. Particles in the exiting extraction stream (now called the product stream) may be analyzed without interference from the larger particles.

In this patent application, the flow direction of a channel is called its length (L). The channel dimension in the direction of particle transport at right angles to the length (L) is called its depth (d). The third channel dimension at right angles to both the length and depth is called its width (w). The depth (d) is therefore perpendicular to the plane of interface of the sample and extraction streams. Table 1 lists other abbreviations used herein.

Table 1

V	Volume
$\dot{V}_{ss}$	Sample stream flow rate (m^3/s)
$\dot{V}_{es}$	Extraction stream flow rate (m^3s)
$\dot{V}_{ps}$	Product stream flow rate (m^3s)
$\dot{V}_{bps}$	By-product stream flow rate (m^3s)
$\dot{V}_{ind}$	Indicator dye stream flow rate (m^3s)
$\dot{V}_{ds}$	Detection stream flow rate (m^3s)
$C_{i,ss}$	Sample stream constituent i concentration (kg/kg)
$C_{i,es}$	Extraction stream constituent i concentration (kg/kg)
$C_{i,bps}$	By-product stream constituent i concentration (kg/kg)
$C_{i,ps}$	Product stream constituent i concentration (kg/kg)
$C_{dye,ind}$	Indicator stream dye concentration (kg/kg)
$C_{i,ds}$	Detector stream constituent i concentration (kg/kg)
d	Diffusion direction extraction channel depth (m)

w	Extraction channel width (m)
L	Extraction channel length (m)
$a_\%$	Percentage deviation from equilibrium concentration
$L_{a\%}$	Device length required to achieve $a_\%$ (m)
z_s	Interface streamline location between sample and extraction streams at the extraction channel entrance (m)
z_p	Interface streamline location between the by-product and product streams (m)
P	Absolute pressure within the fluid stream (Pa)
Δp	Differential pressure between the entrance and exit of the extraction channel (Pa)
D_i	Binary diffusion coefficient of constituent i (m^2/s)
μ	Fluid viscosity (Pa · s)
ρ	Fluid density (kg/m^3)
ξ	Equilibrium normalized constituent concentration for an infinite length extraction channel (dimensionless)
$\hat{c}$	Normalized constituent concentration (dimensionless)
x	Channel length coordinate direction (flow direction)
y	Channel width coordinate direction
z	Diffusion direction coordinate
$\hat{x},\hat{z}$	Non-dimensional normalized variables (dimensionless)
w/d	Aspect ratio
D	Diffusion coefficient
Re	Reynolds number
T	Temperature
u	Axial velocity

The length of the extraction channel and the extraction channel flow velocity are key parameters determining the amount of time the particles have to diffuse into the extraction stream. The particles in the case described above are differentially transported from the sample stream to the extraction stream using diffusion as the transport mechanism. Other means for effecting differential transport of the desired particles can also be used. The term "differential transport" means that a portion of the desired particles are transported from the sample stream into the extraction stream to the substantial exclusion of the undesired particles. For example, magnetic, electrical or other forces can be applied across the extraction stream, temperature gradients can be used, or absorbent or adsorbent materials such as

antibodies can be added to the extraction stream to capture the desired particles.

One preferred embodiment entails the incorporation in the extraction stream of an adsorbent material such as a receptor with specificity for the desired ligand particles, onto an effectively non-diffusing substrate, such as plastic beads or high molecular weight polymers. Another preferred embodiment utilizes an effectively non-diffusing absorbent particulate material with specificity for the desired particles. Such materials are considered "effectively non-diffusing" when they do not diffuse into the sample stream, or do not diffuse into the sample stream in quantities large enough to interfere with detection of the undesired particles in the by-product stream. In the absorbent embodiment, desired particles are absorbed within the effectively non-diffusing absorbing particulate material, whereas in the adsorbent embodiment, the desired particles attach to the surface of the effectively non-diffusing substrate plastic beads or to ligands attached thereto. Numerous suitable ligands for desired particles in the adsorbent/absorbent embodiment are known to the art, and specific teachings relative to these techniques are disclosed in co-pending provisional application serial no. 60/019904 [Attorney Docket No. 35-96P filed concurrently herewith.]

The microfabricated device of this invention for extracting desired particles from a sample stream containing said particles comprises:

a. a sample stream inlet;

b. an extraction stream inlet;

c. an extraction channel having an aspect ratio (channel width to depth) less than 50 in fluid communication with said sample stream inlet and said extraction stream inlet for receiving a sample stream from said sample stream inlet in

parallel laminar flow with an extraction stream from said extraction stream inlet;

d. a by-product stream outlet in fluid communication with said extraction channel for receiving a by-product stream comprising at least a portion of said sample stream from which desired particles have been extracted;

e. a product stream outlet in fluid communication with said extraction channel for receiving a product stream comprising at least a portion of said extraction stream and comprising desired particles extracted from said sample stream.

The sample stream and extraction stream inlets and the by-product stream and product stream outlets may comprise channels, reservoirs, ports, or other containers. The sample stream inlet is designed to receive a sample stream containing "desired particles," i.e. particles it is desired to extract so that their presence may be detected. The sample stream also includes other particles which are not extracted, termed "undesired particles" herein. These undesired particles include particles which might interfere with the detection of the desired particles. In a preferred embodiment, the sample stream comprises whole blood. The desired particles may be albumin or other blood plasma components, and the undesired particles are blood cells. The device is especially useful for obtaining cell-free plasma from whole blood. Other fluids for which the present invention is useful include solutions or suspensions of DNA fragments of different lengths, or proteins of varying sizes. Sample streams useful in the practice of this invention include fermentation broths, raw sewage, liquefied food samples, soil samples and biological fluids such as sputum, urine, and cerebral spinal fluid.

The term "particles" refers to molecules, cells, large molecules such as proteins, small molecules comprised of one or several atoms, and ions. The particles may be suspended or

dissolved in the stream. The term "stream" refers to a carrier fluid such as water or other liquid, air or other gas, containing desired and/or undesired particles. The term "particles" as used herein does not include the molecules of the carrier stream.

The term "extraction" refers to the separation of at least a portion, i.e. a detectable portion, of desired particles from the sample stream to the substantial exclusion of undesired particles. It is recognized that very small amounts of undesired particles may be transported into the extraction stream; however, the presence of such undesired particles will be minimized such that they do not interfere with detection or subsequent processing of the streams containing the desired particles.

The term "laminar flow" of two streams means stable, side-by-side, non-recirculating, flow of two streams without mixing. There are no zones of recirculation, and turbulence is negligible. As is known to the art, the Reynolds number of a flow is the ratio of inertial forces to viscous forces. For flow through a duct, the Reynolds number is calculated using the equation $Re = \rho d(v/\mu)$ where Re is the Reynolds number, ρ is the mass density of the fluid, d is a typical cross-sectional dimension of the duct depending on the shape of the duct, v is the mean velocity over the duct cross-section and μ is the viscosity.

As the Reynolds number is reduced, flow patterns depend more on viscous effects and less on inertial effects. Below a certain Reynolds number (based on lumen size for a system of channels with bends and lumen size changes), inertial effects are insufficient to cause phenomena indicative of their significant presence such as laminar recirculation zones and turbulent flow. Therefore, non-turbulent, laminar non-recirculating flow occurs in the extraction devices discussed herein. In such devices minimal dispersive mixing occurs as a result of the viscous flow velocity profiles present within any laminar viscous flow. This allows two laminar non-recirculating fluid streams to flow down

an extraction channel for the purpose of desired particle extraction from one stream to the other.

The streams may be separated at the end of the conduit at any arbitrary location by precise regulation of the exit flow rate of the outlets, something which is not possible at higher Reynolds numbers not satisfying the non-recirculating and non-turbulent criteria.

The extraction stream inlet is designed to receive an extraction stream capable of accepting desired particles when in laminar flow contact with the sample stream. The extraction stream can be any fluid capable of accepting particles being transported from the sample stream. Preferred extraction streams are water and isotonic solutions such as physiological saline. Other useful extractant streams comprise organic solvents such as acetone, isopropyl alcohol, supercritical carbon dioxide or ethanol. Air and other gases may also be used as sample and extraction streams.

The by-product stream comprises the sample stream from which a portion of the desired particles have been extracted and may or may not, as discussed below, be comprised of a fraction of the extraction stream into which desired particles have been conveyed from the sample stream.

The by-product stream outlet is designed to conduct the by-product stream (composed of the sample stream and perhaps a portion of the extraction stream) that is removed from the extraction channel to disposal, recycle, or other system component, for further processing.

The product stream comprises at least a portion of the extraction stream into which desired particles have been extracted. The product stream outlet, which as stated above, may comprise a product stream channel, is designed to conduct the product stream containing a detectable quantity of desired

particles to a detection or further processing area or system component. A sufficient quantity of the extraction stream must be present in the product stream, comprising a sufficient quantity of desired particles, such that the presence of the desired particles is detectable in the product stream by means known to the art.

The product stream may be conducted to a reservoir chamber, or other device where it may be further treated, e.g. by mixing, separating, analyzing, heating or otherwise processing, for example as disclosed in Wilding, P., et al. U.S. Patent 5,304,487 issued April 19, 1994, incorporated herein by reference.

The term "microfabricated" refers to devices capable of being fabricated on silicon wafers readily available to those practicing the art of silicon microfabrication and having the feature sizes and geometries producible by such methods as LIGA, thermoplastic micropattern transfer, resin based microcasting, micromolding in capillaries (MIMIC), wet isotropic and anisotropic etching, laser assisted chemical etching (LACE), and reactive ion etching (RIE), or other techniques known within the art of microfabrication. In the case of silicon microfabrication, larger wafers will accommodate a plurality of the devices of this invention in a plurality of configurations. A few standard wafer sizes are 3", 4", 6", and 8". Application of the principles presented herein using new and emerging microfabrication methods is within the scope and intent of the claims hereof.

The sample stream inlet and the extraction stream inlet need only be sized large enough to conduct the sample and extraction streams into parallel laminar flow, e.g., may comprise channels less than or equal to about 5 mm in length, less than about 100 micrometers in depth and less than or equal to 5 mm in width. The by-product exit and product outlets may similarly be minimal in size, comprising channels with dimensions as stated above for the sample, or extraction stream inlet. These inlets and outlets

may be as long, deep and wide as required by the system of which they are a part, however, they preferably have a volume less than about 2.5 microliters to accommodate small sample sizes.

The extraction channel receives the inflow of the sample and extraction streams from the sample and extraction stream inlets and conducts these streams in parallel laminar flow for a distance sufficient to allow extraction of the desired particles into the extraction stream.

The width and depth of the sample stream inlet channel, extraction channel and by-product exit must be large enough to allow passage of the undesired particles, preferably anywhere between about 2 or 3 times the diameter of the undesired particles in the sample stream and less than or equal to about 5 mm. Particle sizes range from one or a few Å for small organic and inorganic molecules and ions to about 0.01 micrometers in depth for proteins, to about 0.1-1 micrometers for flexible long-chained molecules, to about 8 micrometers for red blood cells, to about 15 micrometers for most white blood cells, and up to about 25 micrometers for some white blood cells. The extraction channel must additionally be large enough to allow passage of particles used in the extraction stream such as adsorbent or absorbent particles, and is preferably between about 2 or 3 times the diameter of such particles and less than or equal to 5 mm. The extraction channel is most preferably less than 100 micrometers in order to achieve particle transport in a reasonable period of time.

The width and depth of the extraction stream channel and product outlet channels must be large enough to allow passage of the desired particles, and any other particles associated with them, such as adsorbent or absorbent particles, and is preferably between about 2 or 3 times the diameter of any absorbent or adsorbent particles present in the extraction and by-product streams and less than or equal to 5 mm.

If the width dimension is in the wafer thickness direction, then for the silicon microfabricated embodiments of the microscale extraction devices of the present invention, the width of the sample, extraction, product, and by-product channels, inlets and outlets is less than the silicon wafer thickness, i.e. about 300 micrometers.

If the depth dimension is in the wafer thickness direction then for the silicon microfabricated embodiments of the microscale extraction devices of the present invention, the depth of the sample, extraction, product, and by-product channels, inlets and exits is less than the silicon wafer thickness, i.e. about 300 micrometers. Preferably the depth, particularly of the extraction channel, is less than about 200 micrometers, and more preferably less than about 100 micrometers.

In a preferred embodiment, in the "H" design, the inlet and outlet channels are between about 2 to 3 times the maximum-sized stream particulate diameter and about 100 micrometers in width and between about 2 to 3 times the diameter of the maximum-sized particles and less than about 100 micrometers in depth, and the extraction channel is between about 2 to 3 times the diameter of the maximum-sized particles and about 2/3 the wafer thickness in width, between about 2 to 3 times the diameter of the maximum-sized particles and less than about 100 micrometers in depth, and between about 4 and about 10 times the diameter of the maximum-sized particles and less than or equal to 5 mm long.

In a second embodiment in which the particle transport direction is rotated 90 degrees from that of the "H" design, called the "flat extraction device" herein, the inlet channels have a width equal to the extraction channel width at the entrance to the extraction channel of preferably between 2 and 3 particle diameters and about 500 micrometers, and the extraction channel is preferably between about 2 and 3 times the diameter of maximum-sized particles and less than or equal to 5

mm in width, between about 2 and 3 times the diameter of the maximum-sized particles and less than about 100 micrometers in depth, and between about 4 and about 10 times the diameter of the maximum-sized particles and less than or equal to 5 mm long.

The term "aspect ratio" as used herein refers to the ratio of the width to the depth of a channel.

The extraction channels of this invention have an aspect ratio less than 50. The aspect ratio may be less than 25 or any number from less than 1 to 49. Microfabricated devices of this invention which can be manufactured with extraction channels having aspect ratios less than 50 and having depths less than 100 micrometers have numerous advantages over similar constructions with larger aspect ratios and larger extraction channel depths. Motive forces on particles capable of effecting differential transport of desired particles within the extraction channel are the result of local field gradients. Ultra-small transport distances enable differential transport of desired particles faster than undesired particles in short periods of time, allowing for significant minimization of the size needed for the device at moderate extraction channel flow rates. In addition lower flow rates can be used.

Devices within the size range described above yield distinctive advantages when evaluated in the following performance categories: (a) power consumption to achieve objective, (b) size of device required to achieve the objective, and (c) integrability of devices in a plurality of systems for management and processing of very small fluid volumes in a batch (sample to sample) mode.

Some fields known to the art which may be used for differential transport of the particles in the devices of this invention are those produced by:

° Sedimentation

- Electrical energy
- Temperature gradients
- Cross Flow
- Dielectrical gradients
- Shear forces
- Magnetic forces
- Concentration gradients

Means for producing such fields are known to the art in connection with mesoscale and macroscale devices.

Because of the small sizes of the channels described herein, differential transport of desired particles by diffusion or other means occurs extremely rapidly, e.g. within less than about 300 seconds, and if desired, less than about one second. Devices according to this invention can be fabricated which will detect the presence or determine the concentration of desired or undesired particles in the product and/or by-product streams where these particles occur in less than five minutes, or if desired in less than four minutes, or less than three minutes, or less than two minutes, or less than one minute, or less than ten seconds, or less than one second.

In the microfabricated devices of this invention in comparison to the larger-scale devices of the prior art having channel depths greater than 100 micrometers, samples of much smaller size, e.g. about 1 mL, and down to about 1 picoliter, may be treated, whereas in larger devices, very small samples could be absorbed onto the channel walls. In addition, low Reynolds numbers for the flow are achieved, allowing for laminar flow and minimizing or totally eliminating turbulence which would interfere with differential extraction of desired particles.

A portion of the desired particles in the sample stream (having larger diffusion coefficients than the undesired particles, or being more susceptible than the undesired particles to transport into the extraction stream when differential

transport means are applied to the system) is transported to the product stream. When the extraction is diffusion-based, some of the smaller particles will always remain in the sample stream; however, the percentage of desired particles transported to the product stream can be increased by increasing the time of contact of the sample and extraction streams, e.g. by increasing the length of the extraction channel or reducing the flow velocity. For simple diffusion systems, the process may be timed such that the two streams are in contact up to the point where the concentration of smaller particles in both streams is almost equal.

The sample and extraction streams may have different properties e.g. viscosities, densities, surface energies, diffusion coefficients, homogeneities, chemical compositions and the like, which may affect the differential transport rates. System parameters may need to be adjusted and optimized to take account of these differing properties, as will be apparent to those skilled in the art.

The sample and extraction streams are kept in contact in the extraction channel for a period of time sufficient to allow an analyzable quantity of desired particles to be transported into the extraction stream. The amount of product recovered from the device may be between about 0.001 picoliter/sec and about 50 microliters/sec or more. For example, illustrated herein is an optimal flow rate for the product stream of about 200 nanoliters/sec. As is known in the art, even the very small amounts of analytes present in such small product streams may be detected by spectroscopic and other means.

Successful operation of the invention described herein requires precise control of volume flow rates on three of the four channels of the device (i.e. sample, extraction, product, and by-product streams). The fourth channel need not and should not be regulated, as leaving this channel unregulated will allow the device to accommodate unpredictable changes in volume of the

sample because of ΔV of mixing of the sample and extraction streams. Means for achieving precisely regulated flow rates are known to the art.

To aid in controlling the size of particles being transported to the product stream in a diffusion-based extraction system of this invention, and reduce the appearance of larger particles in the product stream, a fluid barrier may be created in the extraction channel. Such a fluid barrier is present when the extraction stream is present in sufficient volume to cause a portion of the extraction stream to flow through the by-product exit with the exiting by-product stream, as illustrated in Figure 3. Smaller particles diffusing into the extraction stream must cross the width of this fluid barrier before being able to exit with the product stream. Such fluid barriers formed on a larger scale are discussed in Williams P. S., et al. (1992), "Continuous SPLITT Fractionation Based on a Diffusion Mechanism," Ind. Eng. Chem. Res. 2172-2181, incorporated herein by reference.

By controlling the pressure of the sample and extraction streams, the ratio of volume from each that enters the extraction channel can be controlled. The volume ratio of the sample stream and the extraction stream can also be set by the geometry of the outlet and inlet channels for a fixed delivery pressure on the sample and extraction streams. The volume flow rate of the product and by-product streams may also be controlled by manipulating the product and by-product stream pressures or by using arbitrary port (inlet) pressures and altering the flow resistance of the inlets. Whatever the control mode, the inlet and outlet channels must satisfy the criteria for minimum channel dimensions based on the size of the particulate to be processed as described above. If the volume of the extraction stream entering the extraction channel is greater than the volume of the sample stream, and the two exit streams are identical, a fluid barrier is formed. If the volume flow rate of the product stream is too small to accommodate the entire volume flow of the extraction stream then a fluid barrier will also be formed.

Extraction devices of this invention may comprise means for controlling the volume of extraction stream in the extraction channel with respect to the volume of the sample stream, which means include a product stream outlet smaller than required to allow the entire extraction stream to exit coupled with a by-product stream outlet large enough to handle the excess extraction stream. Extraction devices of this invention may comprise multiple product stream outlets so that product streams comprising different types of desired particles may be recovered.

The devices of this invention may be utilized as a sample pretreatment system for an analytical system including sensing means for detecting desired particles in the product stream. Such means include means for mixing the product stream with an indicator stream which interacts with the desired particles so as to allow them to be detected by sensing means known to the art, including optical means, such as optical spectroscopic equipment, and other means such as absorption spectroscopic equipment or means for detecting fluorescence, chemical indicators which change color or other properties when exposed to the desired particles of analyte, immunological means, electrical means, e.g. electrodes inserted into the device, electrochemical means, radioactive means, or virtually any microanalytical technique known to the art including magnetic resonance equipment or other means known to the art to detect the presence of analyte particles such as ions, molecules, polymers, viruses, DNA sequences, antigens, microorganisms, or other factors. Preferably, optical or fluorescent means are used, and antibodies, DNA sequences and the like are attached to fluorescent markers. Indicators and microfabricated mixing means, as well as detection and sensing means are described, e.g in copending application serial no. 08/625,808 incorporated herein by reference.

In a preferred embodiment of this invention the differential extraction device described above is integrated into an analytical system comprising means for further processing the

product and/or by-product streams, such as diffusion-based mixing devices for mixing the product stream with an indicator substance (e.g. as described in copending application serial no. 08/625,808 incorporated herein by reference), and detection chambers wherein the presence of desired analyte particles may be detected. These additional processing means are preferably incorporated with the differential extraction device in a "lab-on-a-chip", fabricated on a standard silicon wafer. In a preferred embodiment, the system comprises quantitation means for determining the concentration of the analyte particles (desired or undesired particles) in the product and/or by-product stream and/or determining the concentration of the analyte particles in the sample stream. Such means include spectroscopic equipment, potentiometric, amperometric, and dielectric relaxation equipment. Concentration determinations can be made by calculation or calibration by means known to the art and disclosed herein.

The differential extraction devices of this invention are used in a method for extraction of at least a portion of desired particles from a sample stream comprising said desired particles and also containing undesired particles, comprising:

a. introducing said sample stream into the sample stream inlet of a microfabricated extraction device as described above;

b. introducing an extraction stream into the extraction channel of said extraction device; and

c. withdrawing a product stream comprising desired particles from the product stream outlet of said device.

The method is performed in either batch or continuous mode operation. In batch mode, sample sizes may be as small as about one picoliter, preferably no more than about 250 microliters and more preferably are no more than about 50 microliters, although sample sizes of up to 1 mL or 10 mL or greater are also

contemplated. The method is completed in a time period from less than 1 second to no more than about 5 minutes, although, again, the device can be fabricated to allow batch processing times of 10, 30, or 45 seconds, or 1, 2, 3 or 4 minutes, or less.

The batch method includes a start-up transition period wherein the fluid (which may be a gas) present within the extraction device is displaced by the extraction and sample streams as they enter the extraction channel until such time as the sample and extraction streams exist in a nearly equilibrium mass transport state.

An extraction period follows during which time the sample and extraction streams are in contact in the extraction channel for a period of time sufficient to allow sufficient desired particles to be differentially transported into the extraction stream for analysis or further processing.

A shut-down device flush period then may be required during which a cleansing fluid such as water (or soap solution) or air or sequential combinations of water (or soap solution) and air is cycled through the device to remove both desired and undesired particles which may have been retained on the surface of the device.

The batch method of this invention which involves processing of one single, discrete sample at a time, may include recycle of the by-product stream into the sample stream inlet and repetition of the process to increase the amount of desired particles removed from the original sample. In this embodiment a sample of the undesired particles is generated which may be useful for subsequent analysis. The processes of this invention can be repeated until the desired particles have been substantially completely extracted from the sample stream.

In the continuous mode of this invention, the process may be continued for periods greater than 5 minutes. Multiple

devices of this invention can be arranged in series for the continuous mode so that the by-product stream from each device becomes the incoming sample stream to the next. This continuous application of the described devices produces as a result a series of finely regulated dilutions of the desired particles as well as a substantially clean stream of undesired particles upon exit from the last device of the series. In such an embodiment, the clean undesired particle by-product stream may also be routed to detection elements of the type mentioned above or to particulate sorting devices, counters, or sizing elements, such as a Si microfabricated flow cytometer, e.g. a silicon-based V-groove flow cytometer as described in U.S. Patent Application No. 08/534,515 filed September 27, 1995; and 08/621,170 filed March 20, 1996, incorporated herein by reference, or for further use. For example, in continuous mode, the devices of this invention may be used for dialysis, and the clear plasma stream recycled to a patient's body.

Brief Description of the Drawings

Figure 1 shows a microchannel configuration showing laminar flow of two input streams having a low Reynolds number.

Figure 2 shows a microchannel configuration illustrating the diffusion of smaller particles from a sample stream into an extraction stream.

Figure 3 shows a microchannel configuration illustrating the formation of a fluid barrier between a sample stream and an extraction stream.

Figure 4 shows a microchannel configuration (not to scale) illustrating an embodiment of this invention having multiple product channels to separate different sized particles. Black circles represent the largest particle sizes. Diagonal lines running from upper left to lower right represent medium sized particles, and diagonal lines running from lower left to upper right represent the smallest sized particles.

Figure 5 shows a perspective view of microfabricated flat diffusion extraction system design with the diffusion direction rotated 90° from the "H" design shown in Figures 1-4.

Figure 6 shows a plan view of the microfabricated flat diffusion extraction system design of Figure 5.

Figure 7 is a diagram of the entrance and outlet interface streamline in the extraction channel showing the flow rates of the sample, extraction, product and by-product streams.

Figure 8 illustrates the "lab-on-a-chip" concept of this invention for assay of constituents present in a particulate or cell-laden sample stream.

Figure 9 illustrates optimization of extraction channel length, channel depth and product stream flow rates for a diffusion extraction system microfabricated on a 4 mm wide silicon chip for extracting albumin from a carrier fluid having the viscosity of water.

Figure 10 illustrates optimization of pressure differential, channel depth and product stream flow rates for a diffusion extraction system microfabricated on a 4 mm wide silicon chip for extracting albumin from a carrier fluid having the viscosity of water.

Figure 11 illustrates the velocity profiles of two homogenous, immiscible fluids behaving as Newtonian fluids but having differing viscosities. The dotted line shows a fluid having the viscosity of water. The solid line shows a fluid having a viscosity three times that of water.

Figure 12 illustrates a comparison between a two-viscosity model of a diffusion-based extraction system of this invention using the fluids of Figure 11, and a model assuming the same interface location but with no differences in diffusivity or viscosity in the two fluids.

Detailed Description of the Preferred Embodiments

Diffusion of small molecules occurs rapidly over typical microfabricated dimensions. The relationship between the size of a particle, Re, the diffusion coefficient, D, and temperature, T, is due to Einstein and for the simplest case, spherical particles, this can be written as:

$$D = \frac{k_b T}{6\pi\mu Re}. \quad (1)$$

The characteristic distance, l, which a particle with diffusion coefficient D will diffuse in time, t, is

$$l = \sqrt{Dt}. \quad (2)$$

Table 2 gives some typical diffusion coefficients and characteristic times.

Table 2:

Some typical values for different sized particles and molecules. The characteristic time to diffuse 10 μm is given.

Particle	D(20°C)	t
0.5 μm sphere	5 x 10^{-9} cm^2/sec	200 sec
Protein (hemoglobin)	7 x 10^{-7} cm^2/sec	1 sec
Small Molecule (fluorescein)	5 x 10^{-6} cm^2/sec	0.2 sec

As shown in Figure 1, in microchannels of small enough dimensions, inertial effects are negligible, such that a sample stream **2** entering a sample stream inlet **1** can flow from a sample stream channel **3** into an extraction channel **7** without mixing with an extraction stream **4** entering an extraction stream inlet **5** and flowing from an extraction stream inlet channel **6** into extraction channel **7**. The two streams in the extraction channel **7** form a laminar sample stream **8** and a laminar extraction stream **9**.

In Figure 2, the arrows at the upper left show the direction of flow in sample stream channel **3** of sample stream **2** entering sample stream inlet **1**, and the arrows at the lower left show the direction of flow in extraction stream inlet channel **6** of extraction stream **4** entering extraction stream inlet **5**. Sample stream **2** contains larger ("undesired") particles **17** and smaller ("desired") particles **18** such as dye particles (shown by cross-hatching). The sample stream **2** and extraction stream **4** come together in laminar flow in extraction channel **7** to form laminar sample stream **8** and laminar extraction stream **9** and the smaller desired particles **18** begin to diffuse from laminar sample stream **8** into laminar extraction stream **9** to form laminar product stream **16** which contains diffused smaller desired particles **18**. The laminar sample stream **8** flows into by-product outlet channel **10** to form by-product stream **12**, and leaves the channel through by-product outlet **15**. The laminar extraction stream **9** receives smaller desired particles **18** diffused from laminar sample stream **8** and becomes laminar product stream **16** which in product outlet channel **11** becomes product stream **13** and leaves the channel through product outlet **14**.

In Figure 3, the direction of the arrow at the upper left shows the direction of flow in sample stream channel **3** of sample stream **2** entering through sample stream inlet **1**. The direction of the arrow at the lower left shows the direction of flow in extraction stream inlet channel **6** of extraction stream **4** entering through extraction stream inlet **5**. Extraction stream **4** is indicated by cross-hatching. The upper arrow in extraction channel **7** shows the direction of flow of laminar sample stream **8** and the lower arrow in extraction channel **7** shows direction of flow of laminar extraction stream **9**. When the volume of extraction stream **4** is greater than the amount which can exit through product outlet channel **11** and product outlet **14**, part of laminar extraction stream **9** exits through by-product outlet channel **10** and by-product outlet **15** as excess extraction stream **22**. This excess extraction stream **22** is in laminar flow in extraction channel **7** and forms fluid barrier **20**. Smaller desired

particles **18** (not shown in Figure 3; see Figure 2) in the sample stream **2** diffuse from laminar sample stream **8** through fluid barrier **20** into laminar extraction stream **9** to form product stream **16** (not shown in Figure 3; see Figure 2).

In Figure 4 another embodiment of the invention is shown. A sample stream **2** containing large particles (black dots), medium-sized particles (diagonal lines from upper left to lower right), and small particles (diagonal lines from lower left to upper right) enters sample stream inlet **1**. An extraction stream **4** enters extraction stream inlet **5** and flows to meet sample stream **2** in extraction channel **7**. Small particles with larger diffusion coefficients which diffuse most rapidly exit first product outlet **23** in first exiting product stream **25** flowing through first product outlet channel **24** which is placed closest to the sample stream inlet **1**. Medium-sized particles with medium-range diffusion coefficients exit along with small particles through second product outlet **26** in second exiting product stream **28** through second product outlet channel **27** placed further from sample stream inlet **1** than first product outlet channel **24** so as to allow more time for medium-sized particles to diffuse into the extraction stream. Large particles which have smaller diffusion coefficients and which diffuse more slowly exit third product outlet **29** in third exiting product stream **31** through third product outlet channel **30**, along with small and medium-sized particles. The by-product stream **12** in feed exit channel **10** exiting through by-product outlet **15** also contains particles of all three sizes.

Figure 5 shows a perspective view and Figure 6 shows a plan view of a further embodiment of the invention, a "flat extraction device," in which the diffusion direction in extraction channel **7** is rotated 90° from the embodiments shown in Figures 1-4. This embodiment provides the advantage that the volume of material which can be processed is no longer limited by the depth of the extraction channel 7.

The flat extraction device of Figures 5 and 6 is made by etching a silicon substrate **34** to provide sample stream inlet groove **35**, extraction stream inlet groove **36**, product stream exit groove **37**, and by-product stream exit groove **38**, as well as extraction channel **7**. A glass cover **33** serves to enclose extraction channel **7**. In Figure 5, the arrows shown pointing downward into sample stream inlet **1** indicate the flow of sample stream **1**. Similarly, the arrows pointing down into extraction stream inlet **5** indicate the flow of extraction stream **4**. The arrows pointing up from product outlet **14** indicate the flow of product stream **16**, and the arrows pointing up from by-product outlet **15** indicate the flow of by-product stream **12**. The length of extraction channel **7** is shown as *L* and the width of the channels is indicated by the dark arrow as *w*. The depth of the extraction channel **7** is shown as *d*. A coupling manifold **32** shown in Figure 6 with openings extends the depth of sample stream inlet groove **35** to form sample stream channel **3** and sample stream inlet **1**, extends the depth of extraction stream inlet groove **36** to form extraction stream channel **6** and extraction stream inlet **5**, extends the depth of product stream exit groove **37** to form product outlet channel **11** and product outlet **14**, and extends the depth of by-product stream exit groove **38** to form by-product outlet channel **10** and by-product exit **15**.

In the flat extraction system design shown in Figure 6 operating by diffusion (concentration gradient) a sample stream **2** shown by the arrow in the upper left enters sample stream inlet **1** and flows in sample stream channel **3**. Extraction stream **4** is indicated by an arrow entering extraction stream inlet **5**, and flows in extraction stream inlet channel **6**. Sample stream **2** flows as a laminar sample stream **8** in extraction channel **7** beneath laminar extraction stream **9**. Laminar sample stream **8** is in contact with laminar extraction stream **9** in extraction channel **7** for a length *L*. Smaller ("desired") particles from laminar sample stream **8** indicated by the stippling in laminar extraction stream **9** flow into product outlet channel **11** as product stream **13** which exits at product outlet **14** as shown by the upward-

pointing arrow. By-product stream **12** is the continuation of laminar sample stream **8** past product stream **13** which contains both the larger ("undesired") particles and a portion of the smaller ("desired") particles which have not diffused into product stream **13**. By-product stream **12** flows through by-product outlet channel **10** out through by-product outlet **15**.

By adjusting the configuration of the channels in accordance with the principles discussed herein to provide an appropriate channel length, flow velocity and contact time between the sample stream and the extraction stream, the size of the particles remaining in the sample stream and diffusing into the product stream can be controlled. The contact time required can be calculated as a function of the diffusion coefficient of the particle D (which generally varies as the linear size of a particle), and the distance d over which the particle must diffuse by $t = d^2/D$. Particles or molecules that have diffusion coefficients larger than D will be in the exiting product stream, and particles or molecules having a diffusion coefficient substantially smaller than D will not. If the diffusion coefficient of the larger particles being separated is about ten times smaller than D, the product should be almost entirely free of the large particles.

A simple calculation shows that few particles or molecules with diffusion coefficients smaller than $D = w_{fb}^2 v/L$ will be found in the exiting product stream, where w_{fb} is the width of the fluid barrier, v is the flow velocity of the laminar sample stream and L is the length of the extraction channel. Particles or molecules with diffusion coefficients larger than $D = w^2 v/L$, where w is the width of the extraction channel, will be in the exiting product stream in the same concentration as in the by-product stream.

Means for injecting feed liquid into the device are provided, as when the device of this invention is used as part of an analytical system. Such means include standard syringes

and tubes. Means for removing fluid from the product exit may also be provided, including receptacles for the fluid, inducing flow by capillary attraction, pressure, gravity, and other means known to the art as described above. Such receptacles may be part of an analytical or other device for further processing the product stream.

Figure 7 shows the extraction channel **7** with laminar extraction stream **9** moving at a velocity $\dot{V}_{es}$, and laminar sample stream **8** moving at a velocity $\dot{V}_{ss}$, and having a stream height, (diffusion direction coordinate) Z_s defining the interface streamline location (dotted line) between the laminar sample stream **8** and the laminar extraction stream **9** near the entrance of the extraction channel **7**. The combined height of both streams, and thus the depth of the extraction channel **7**, is shown as *d*. The curved line indicates the shape of the velocity profile. As the streams move along the length of the extraction channel **7**, laminar sample stream **8** becomes by-product stream **12** moving with a velocity $\dot{V}_{bps}$ and having a stream height (diffusion direction coordinate) Z_p defining the interface streamline location (dotted line) between the by-product stream **12** and the product stream **13**. Laminar extraction stream **9** becomes product stream **16** moving with a velocity $\dot{V}_{ps}$.

Several steps commonly performed in the chemical assay of a fluid mixture are: (1) precise mixture dilution; (2) extraction of a specific constituent; (3) precise mixing of indicator reagents or test probes (e.g. fluorescently tagged polymer beads); and (4) non-invasive detection of the indicator or probe (e.g. absorbance or fluorescence spectroscopy).

The extraction devices of this invention may be integrated into total analytical systems such as the microfabricated "lab-on-a-chip" illustrated in Figure 8.

Figure 8 shows a diffusion-based extraction device 100 of this invention fabricated on a single silicon wafer. A sample

stream **2** having a sample stream flow rate $\dot{V}_{ss}$ and a sample stream constituent i concentration $C_{i,ss}$ flows into the diffusion-based extraction device along with an extraction stream **4** having an extraction stream flow rate $\dot{V}_{es}$. By-product stream **12** having a by-product stream flow rate $\dot{V}_{bps}$ and a by-product constituent i concentration $C_{i,bps}$ is removed from the system. Product stream **13** having a product stream flow rate $\dot{V}_{ps}$ and a product stream constituent i concentration $C_{i,ps}$ flows to a diffusion-based mixing device **43** microfabricated onto the same chip. An indicator dye stream **39** having an indicator dye stream flow rate $\dot{V}_{ind}$ and an indicator stream dye concentration $C_{dye,ind}$ also flows into the diffusion-based mixing device **43**. Detector stream **40** exits diffusion-based mixing device **43** and flows into detection chamber **44** and optical detection means **41** are actuated to detect a signal, preferably a fluorescence signal **42** while detector stream **40** is in the detection chamber **44**. Detector stream **40** then exits detection chamber **44** at a detector stream flow rate $\dot{V}_{ds}$, a detector stream constituent i concentration $C_{i,ds}$ and an indicator dye concentration $C_{dye,ind}$.

The detection strategy presented in Fig. 8 requires constituent extraction from the particulate laden sample, fluorescent indicator mixing with the diluted analyte, and fluorescent optical detection. Critical to the precise operation of the inference technique is the precise regulation of all stream flow rates in the system. Using a calibration between fluorescence intensity and constituent concentration and information precisely defining the constituent extraction and indicator mixing dilution ratios, the concentration of constituent in the original sample stream is estimated. The complete system also includes data reduction, pressure regulation and waste collection. Precise flow control in integrated total analytical systems may in part be achieved using on-chip micro-pumps (Gravesen, P. et al. (1993), "Microfluidics - a review," J. Micromechanics and Microengineering **3(4)**:168-182; Elwenspoek, M. et al. (1994), "Towards integrated microliquid handling systems," J. Micromechanics and Microengineering **4(4)**:227-245;

and Forster, F.K. et al. (1995), "Design, Fabrication and Testing of Fixed-Valve Micro-Pumps," ASME International Mechanical Engineering Congress & Exposition, San Francisco, ASME).

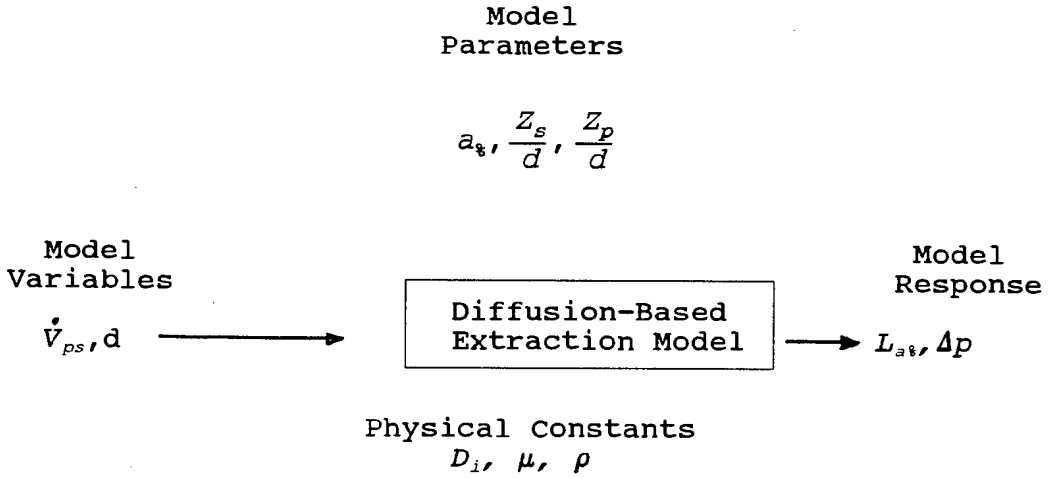
In both the "H" design for the extraction system, e.g. Figure 2 as described in the Example, and the flat extraction system of Figure 5 and 6, the diffusing constituents migrate into the extraction stream **4** and tend toward an approximate uniform concentration throughout the extraction channel **7**. The sample, extraction, and by-product flow rates are externally regulated, thereby fixing the product stream flow rate. In the design of Figure 2, fabricated as described in the Example hereof, the channel dimension in the diffusion direction (d), is less than 100 μm in the Example, and the aspect ratio, defined as the channel dimension normal to the diffusion and flow directions (w) divided by the channel depth (d), is less than 1. In the flat diffusion extraction system of Figures 5 and 6, the aspect ratio w/d, where d again is less than about 100 μm, is greater than 1, but still much less than 50.

The distance required for the constituent being extracted to achieve a concentration throughout the microchannel cross section that is within a fixed percentage of the equilibrium concentration is defined as the equilibration length. The constituent concentration within the microchannel is calculated using a 1-D analytical diffusion model. The equilibration length is used to construct a family of process space design curves specific to the extracted constituent. The optimization objective function is specified to identify the design which maximizes the volume flow rate of product stream within constraints imposed by a system microfabricated on a silicon chip.

The methodology is applied to the design of an optimal device for the extraction of albumin (a protein constituent present in human blood) from a carrier sample stream with viscosity approximately that of water. Whole blood typically has

a red blood cell (RBC) content of 40-50% by volume, the RBCs having ellipsoidal shape and 8 μm major axis dimension, and white blood cells having nominal diameters of approximately 15-25 μm. In this discussion, the analysis is simplified by considering a single viscosity, single diffusivity process model. Considerations relating to multiple viscosity cases are presented hereinafter. The device presented here is specified for a 1% equilibration length (within 1% of the equilibrium concentration of albumin for an infinite length device). This process sensitivity information provides design requirements for upstream and downstream fluidic components and is essential for integration of the device into a "lab on a chip" chemical analysis system.

A process model is defined by its parameters, physical constants, independent variables, dependent variables, and by the equations used to model the process. The extraction process examined in this paper is illustrated below:



Physical constants cannot be altered with either the design of the device or through its control. There are three physical constants identified above: binary constituent diffusivity, D_i; viscosity, μ; and density, ρ. The constant parameters are the desired percentage to complete constituent equilibration, $a_{\%}$, the normalized sample-extraction streamline interface position, z_s/d, and the normalized by-product-product streamline interface

position, z_p/d. The variable model parameters are the product stream flow rate, $\dot{V}_{ps}$, and the diffusion direction channel depth, d. Under this definition the model outputs are the channel length required to achieve $a_{\%}$, $L_{a\%}$, and the pressure differential across the extraction channel in the direction of flow, Δp.

A 2-D flow and constituent transport model of the extraction process is presented. The discussion begins by stating the general 3-D transport problem. Simplifying assumptions are then defined for the 2-D approximations and are applied. Solutions to the resulting descriptive modeling equation and associated boundary conditions are then presented for the inviscid flow case and for a numerical solution to the viscous flow case.

General 3-D Mass Transport Model Equation. The general equation describing the transport of a constituent by both diffusive and convective transport is given as (Cussler, E.L. (1984), Diffusion, Mass Transfer in Fluid Systems, Cambridge, Cambridge University Press):

$$\frac{\partial c_i}{\partial t} + v_x \frac{\partial c_i}{\partial x} + v_y \frac{\partial c_i}{\partial y} + v_z \frac{\partial c_i}{\partial z} = D_i \left[\frac{\partial^2 c_i}{\partial x^2} + \frac{\partial^2 c_i}{\partial y^2} + \frac{\partial^2 c_i}{\partial z^2} \right] + r_i \qquad (3)$$

where: c_i is the concentration of the i^{th} constituent; D_i is the binary diffusion coefficient for the i^{th} constituent; v_x, v_y, and v_z are the velocity vector components; and r_i is the rate of production of the i^{th} constituent via chemical reactions in the mixture.

2-D Steady Flow Approximation. The mathematical relations representing the modeling assumptions used in this discussion are presented in Equation 4.

$$\frac{\partial c_i}{\partial t} = 0, \qquad 4(a)$$

$$v_y = v_z = 0, \qquad 4(b)$$

$$\frac{\partial^2 c_i}{\partial x^2} = \frac{\partial^2 c_i}{\partial y^2} = 0, \qquad 4(c)$$

$$r_i = 0 \qquad 4(d)$$

Equation 4(a) represents the steady state device operation assumption. The extraction device is intended for dynamic operation but steady state operation is used to target a final configuration design configuration. Flow occurs in a single coordinate direction as reflected in Equation 4(b). Equation 4(c) is justified using two arguments: (1) the spatial scale for diffusion is an order of magnitude smaller in the diffusion extraction direction (*z - coordinate*) than in the channel flow direction (*x - coordinate*) (the time required for diffusion over a distance ℓ is proportional to ℓ^2/D); (2) diffusion in the channel width direction (*y - coordinate*) will tend to flatten the concentration profile in the case of viscous flow and cause the solution to more closely approximate diffusion in the invisid flow case with identical mean flow velocities. Equation 4(d) is justified in this discussion because there are no chemical equilibrium kinetics reflecting the change of species in the flow stream for the assays of interest considered here. This is not always the case. Application of Equation 4 to Equation 3 yields the simplified relation,

$$\frac{\partial c_i}{\partial x} = \frac{D_i}{v_x}\frac{\partial^2 c_i}{\partial z^2}. \qquad (5)$$

Non-dimensional Form. Equation 5 can be normalized with respect to the sample stream constituent concentration and the diffusion channel depth by defining the following non-dimensional change of variables,

$$\tilde{c}_i = \frac{c_i}{c_{i,0}}, \; \tilde{x} = \frac{x}{d}, \; \tilde{z} = \frac{z}{d}, \qquad (6)$$

where: $c_{0,i}$ is the concentration of constituent i in the sample stream, and d is the channel depth. Substitution of Equation 6 into Equation 5 yields

$$\frac{\partial \tilde{c}_i}{\partial \tilde{x}} = \left[\frac{D_i}{v_x d}\right]\frac{\partial^2 \tilde{c}_i}{\partial \tilde{z}^2}. \qquad (7)$$

The bracketed term in Equation 7 is the inverse of the Peclet number. The Peclet number provides a useful gauge of the relative significance of convective mass transport to diffusion mass transport and is defined as

$$Pe = \frac{v_x d}{D_i} \propto \frac{convective\ transport}{diffusion\ transport}. \qquad (8)$$

The concentration is therefore a function of normalized position and the Peclet number, $\tilde{c}_i(\tilde{x}, \tilde{z}, Pe)$.

Steady Flow Entrance Boundary Condition. The position of the streamline separating the sample and extraction streams at the inlet of the extraction device is z_s. The boundary condition at the extraction channel inlet, $\tilde{x} = 0$, is unity. The extraction stream normalized concentration is zero,

$$\tilde{c}_i(0, \tilde{z}) = \begin{cases} 1, 0 < \tilde{z} < \frac{z_s}{h} \\ 0, \frac{z_s}{h} < \tilde{z} < 1 \end{cases} \qquad (9)$$

Infinite Length Channel Far Field Boundary Condition. The far field boundary condition is defined by postulating an infinitely long extraction channel. For such a channel all diffusing constituents must equilibrate across the channel cross-section. Therefore,

$$\tilde{c}_i(\infty, \tilde{z}) = \xi \qquad (10)$$

where: ξ is the equilibrium normalized concentration. The normalized equilibrium concentration is given as

$$\xi = \frac{\dot{V}_{ss}}{\dot{V}_{ss} + \dot{V}_{es}}. \qquad (11)$$

Impermeable Channel Wall Boundary Conditions. During steady state operation of the device adsorption of constituents on the device surfaces is assumed to have equilibrated and therefore the mass flux across a control surface defined by the device boundaries is zero. Therefrom from Fick's law the concentration gradient at the boundary must be zero,

$$\frac{\partial \tilde{c}_i(\tilde{x},0)}{\partial \tilde{z}} = \frac{\partial \tilde{c}_i(\tilde{x},1)}{\partial \tilde{z}} = 0. \qquad (12)$$

Inviscid Flow (Plug Flow). If inviscid flow is assumed the velocity across the channel in the z-direction will be constant. With this modeling approximation the location of the streamline interface between the sample and extraction steams is given as

$$\frac{z_s}{d} = \xi. \qquad (13)$$

The solution to Equation 7 subject to the boundary conditions given by Equation 9, Equation 10, and Equation 12 and the streamline interface location (Equation 9) was derived and is given as

$$\tilde{c}_i(\tilde{x},\tilde{z}) = \xi + \sum_{n=1}^{\infty} \frac{2}{\pi(2n-1)} \sin[(2n-1)\pi\xi]x \exp\left[-(2n-1)^2\pi^2\left(\frac{D_i}{v_x d}\right)\tilde{x}\right]\cos[(2n-1)\pi\tilde{z}]. \qquad (14)$$

Equation 14 was derived using the method of separation of variables. See Folland, G.B. (1992) Fourier Analysis and its Applications, Pacific Grove, Wadsworth & Brooks/Cole Advanced Books and Software, for a detailed presentation of this method and its applications to physical systems.

Viscous Flow - Single Viscosity Fluid. The location of the streamline separating the sample and extraction stream for a viscous flow velocity profile is achieved using conservation of mass. The velocity profile for a single viscosity fluid stream is given as

$$v_x(z) = -\frac{d^2}{2\mu}\frac{dP}{dz}\left[\left(\frac{z}{d}\right) - \left(\frac{z}{d}\right)^2\right]. \qquad (15)$$

The total volume flow in a channel of depth, d, and width, w, is equal to the sum of the sample and extraction stream flow rates. In terms of the velocity profile this net channel flow rate is given as

$$\dot{V}_{ss} + \dot{V}_{es} = w \int_{z=0}^{z=d} v_x(z)\, dz = -\frac{d^3 b}{12\mu} \frac{dP}{dz} \qquad (16)$$

The volume flow rate in the sample stream portion of the extraction channel is given as

$$\dot{V}_{ss} = w \int_{z=0}^{z=z_s} v_x(z)\, dz \qquad (17)$$

where z_s is the location of the equilibrium streamline separating the sample and extraction streams. For a viscous flow profile the total sample stream volume flow must reside in the region $0<z<z_s$. Equation 17 may be solved using equations 16 and 15 to yield the cubic relation

$$2\left(\frac{z_s}{d}\right)^3 - 2\left(\frac{z_s}{d}\right)^2 + \xi = 0. \qquad (18)$$

Any convenient root search technique may be applied to determine the position of the separation streamline separating the sample and extraction streams, z_s.

To examine the error associated with assuming inviscid flow a 2-D numerical model was written and used to analyze the flow profile of the "optimal" design suggested by the inviscid flow model. In the numerical simulation model the equation solved is given as

$$\frac{\partial^2 \tilde{c}_i}{\partial \tilde{z}^2} = \left[\frac{v_x(\tilde{z})\, d}{D_i}\right] \frac{\partial \tilde{c}_i}{\partial \tilde{x}}, \qquad (19)$$

where the Peclet number is now a function of position within the flow channel due to the viscous flow velocity profile. A

centered finite difference in $\tilde{z}$ and upstream difference in $\tilde{x}$ was used to solve the above equation numerically. For $\tilde{z}_s = z_s d = 0.5$ a 20% reduction in the required extraction channel length was observed for identical net channel flow rates. Therefore, using the inviscid assumption to generate design curves should give a conservative calculation of the size of the device required for extraction.

Optimization Objective Function. The goal of this design optimization problem was to maximize the volume flow rate of product stream per unit filter channel breadth, w. The function describing this design object is given as

$$maxF(d, L_{a_i}) = \dot{V}_{ps}(d, L_{a_i}) . \qquad (20)$$

where: d is the channel depth, and L_{a_i} is the a_i equilibration length. Equation 20 describes the design objective and insures maximum device throughput. In other applications competing design objects may also be considered using a *multiobjective* design objective function where the *competing* design objectives are ordered using *subjective weights* to form the composite multiobjective function. On the microscale, in specific applications, it would be advantageous to maximize the ratio of volume flow rate to unit device volume while simultaneously minimizing the surface area to unit device volume (or equivalently maximizing the volume flow rate to unit surface area) of the micro-fluidic device. These ratios are primarily a function of diffusion direction depth which would directly couple into any device design. In addition, it may also be required that the silicon real estate required to realize the device be simultaneously minimized. For each design objective that must be simultaneously optimized, an additional subjective weight is required. Selection of the appropriate weights will vary from one design configuration to another.

Design Constraints. Because the silicon wafers used to produce the micro-fluidic devices are of finite size, there is

a practical limit to the maximum allowable filter length. The $a_{\%}$ equilibration length, L_a, must be less than the maximum practical filter length, L_{max}, or

$$L_{a=1\%} < L_{max}. \quad (21)$$

Similarly, the channel must be sufficiently deep such that any particulate present in sample and extraction streams will not violate the extraction stream simply due to geometric confinement in the channel, $d > d_{min}$. Further, the channel must not be so deep that the strength of the silicon wafer is excessively compromised, $d < d_{max}$. Combining these two constraints yields the single constraint equation

$$d_{min} < d < d_{max}. \quad (22)$$

Finally, the maximum time allowable to complete a set of extraction and subsequent analysis operations will determine a minimum acceptable product stream flow rate for the device. That is,

$$\dot{V}_{ps} > \dot{V}_{ps,min}. \quad (23)$$

Figures 9 and 10 present the process space for a family of diffusion extraction devices designed for $a_{\%} = 1\%$.

Figure 9 illustrates the design space for a 4 mm wide parallel flow diffusion extraction device to extract albumin from whole blood to achieve a 1% equilibration length, calculated assuming a flow ratio of 1:1 for the sample and extraction stream, and a fluid viscosity of 10^{-3} [Pas] and a fluid density of 10^3 [kg/m^3]. The diffusion coefficient for albumin in the saline solution used in this study is $D_{albumin} = 7 \cdot 10^{-11}$ [m^2/s].

The physical constants are $D_i = 7 \cdot 10^{-11}\ m^2/s$ (albumin), $\mu = 10^{-3}\ Pa/s$ (water), and $p = 10^3 kg/m^3$ (water). These properties are unvarying for a dilute aqueous solution of albumin. The

constants would only change if one were to consider another chemical assay. The parameters chosen as fixed for this design optimization are: a = 1%; z_s/d = 0.5; and w = 4mm. These values were chosen as representative for this application and could be varied to achieve specific objectives. For instance, the channel width could be increased to increase the total flow throughput.

In Figure 9, Area A, illustrates the constrained parameters for the process, with the large black dot at the upper right of this area at a channel length of 40 mm, a channel depth 50 μm, and a product stream flow rate (V_{ps}) of about 0.23 μl/s illustrating the most optimal design. Area B, requiring channel lengths greater than 40 mm, is outside the optimal design because these channel lengths exceed the 40 mm width of the silicon chip ($L > L_{max}$). Area C where the required channel depth is greater than 100 μm, is outside the optimal design range because the channel depth exceeds that allowable for efficient diffusion ($d > d_{max}$). Area D, with channel depth less than 50 μm, is outside the optimal design range because the channel is too shallow to pass common cellular constituents ($d < d_{min}$). Area E, where the product stream flow rate is 0 to about 0.10 μl/s is outside the optimal design range because the product flow rate is too small ($Q_{product} < Q_{product.min}$).

Figure 10 shows the optimal design parameters for conditions as specified in Figure 9 with respect to the pressure differential across the extraction channel in the direction of flow. Area A, as defined with respect to flow rate and channel depth as described for Figure 9, is the optimal design area. The large black dot at the upper right of this area again illustrates the most optimal design at a pressure differential of 0.5 kPa.

Equilibration length ($L_{a=1\%}$) is shown to be a linear function of $\dot{V}_{ps}$ at a given channel depth (d). Equation 14 shows the exponential decay of concentration with $\bar{x}$. Since the diffusivity is a constant for the given constituent of interest, v_x, and d control the rate of exponential decay. The factor $1/Pe = D_i/v_x d$ acts like a time constant. If as d is reduced and v_x is increased to compensate with same $1/Pe$ resulting, then the $L_{a\%=1\%}$ will remain unchanged. As $\dot{V}_{ps}$ increases linearly at a given d, v_x increases proportionately and $L_{a=1\%}$ increases linearly due to the linear reduction in $1/Pe$. Convection is becoming more important relative to diffusion and a longer channel length is required to reach equilibrium.

To maximize flow rate at a given equilibrium length, one would be driven to the upper right hand corner of the constrained process space and operate at a small channel depth (Figure 9) and high pressure differential (Figure 10). To minimize area requirements, design to operate in the lower left of Figure 10 at much lower pressure differentials. One should reduce d as far as possible as long as surface effects can be avoided.

In the following discussion, it is assumed that the two fluids being considered have differing viscosities and are homogeneous, immiscible fluids behaving as Newtonian fluids. To model the two-viscosity case and obtain design parameters and results, three separate steps are required. In what follows, the sample stream is identified as region 2 and the extraction stream is identified as region 1. The ratio of absolute viscosity in region 1 to that in region 2 is m, and location of the fluid interface from mid-channel in the direcion of region 1 as a fraction of the half-channel width is α. Here the height of the extraction channel is taken as 2ω. The first step is to calculate the velocity profile across both streams in terms of

m and α. The second step is to use the velocity profiles to determine the numerical values of α and the ratio of mean velocity of each stream given a volume flow ratio $\dot{V}_{es}/\dot{V}_{ss} \equiv F$. The third step is to solve the diffusion equations based on the location of the inerface, the mean velocites in each stream, and the diffusion coefficent of the particles of interest in each stream.

To accomplish the first step, the Navier-Stokes equations are solved for one-dimensional two-phase fully-developed steady flow of a Newtonian fluid in a rectangular duct to determine the axial velocity profile $u(z)$. The equations in that case reduce to (White, F.M. (1994) Fluid Mechanics):

$$\nabla p + \mu \nabla^2 u = 0. \qquad (24)$$

The resulting velocity profile non-dimensionalized by $\omega^2 \Delta p/\mu_1 L$ and with $z = z/\omega$ measured from mid-channel into region 1 is given by

$$\tilde{u}_1(\tilde{z}) = \frac{1}{2}\left(-\tilde{z}^2 + \frac{\tilde{z}(\alpha^2 m - \alpha^2 + 1 - m) - \alpha^2 m + 2m + \alpha^2 - \alpha + m\alpha}{m + m\alpha - \alpha + 1}\right) \quad \alpha < \tilde{z} < 1 \qquad (25)$$

and

$$\tilde{u}_2(\tilde{z}) = \frac{1}{2}\left(-m\tilde{z}^2 + \frac{m\tilde{z}(\alpha^2 m - \alpha^2 + 1 - m) + m(\alpha^2 m - \alpha^2 - \alpha + m\alpha + 2)}{m + m\alpha - \alpha + 1}\right) \quad -1 < \tilde{z} < \alpha. \qquad (26)$$

The second step is to calculate the numerical value of α for a particular value of F by solving for α in the equation

$$F = \frac{\int_{\alpha}^{1} \overline{u_1} d\tilde{z}}{\int_{-1}^{\alpha} \overline{u_2} d\tilde{z}}, \qquad (27)$$

and then with that value of α calculate the ratio of mean flows in each region from

$$\frac{\overline{U_1}(1 - \alpha)}{\overline{U_2}(1 + \alpha)} = F \quad . \tag{28}$$

The last step is to solve the diffusion equation (7) in each region subjected to the boundary conditions given by Eqs. (9), (10), and (12) with two additional interface conditions that require continuity of concentration and conservation of mass of the diffusing species at the interface. Now taking $\tilde{z}$ to be measured from the interface into region 1, those conditions are

$$\tilde{c}_{i1}(\tilde{x}, 0+) = \tilde{c}_{i2}(\tilde{x}, 0-) \quad , \tag{29}$$

and

$$D_1 \frac{\partial \tilde{c}_{i1}(\tilde{x}, 0+)}{\partial \tilde{z}} = D_2 \frac{\partial \tilde{c}_{i2}(\tilde{x}, 0+)}{\partial \tilde{z}} \quad . \tag{30}$$

The resulting equation for the mass concentration throughout the channel is given by

$$\tilde{c}_i(\tilde{x}, \tilde{z}) = \xi + \sum_{n-1}^{\infty} K_n f_n(\tilde{z}) \exp(-\lambda^2 \tilde{x}/Pe_1) \tag{31}$$

where $\tilde{x} = x/\omega$, the eigenfunctions $f_n(\tilde{z})$ are given by

$$f_n(\tilde{z}) = \begin{cases} cosk\,\lambda_n \beta_2 \cos\lambda_n(\tilde{z} - \beta_1) & 0 < \tilde{z} < \beta_1 \\ \cos\lambda_n \beta_1 cosk\lambda_n(\tilde{z} + \beta_2) & -\beta_2 < \tilde{z} < 0 \end{cases} \quad , \tag{32}$$

the eigenvalues λ_n are solutions of the characteristic equation

$$\tan\lambda_n(\beta_1) + \sigma \tan k\lambda_n(\beta_2) = 0 \quad , \tag{33}$$

the constants K_n are given by

$$K_n = 4\frac{-\xi\cos k\lambda_n\beta_2\sin\lambda_n\beta_1 + (1-\xi)\sigma\cos\lambda_n\beta_1 sink\lambda_n\beta_2}{\cos^2 k\lambda_n\beta_2(sin2\lambda_n\beta_1 + 2\lambda_n\beta_1) + \sigma\cos^2\lambda_n\beta_1(sin2k\lambda_n\beta_2 + 2k\lambda_n\beta_2)} \quad (34)$$

with $\beta_1 = 1 - \alpha$, $\beta_2 = 1 + \alpha$, $k = \sqrt{Pe_2/Pe_1}$, and $\sigma = k(D_2/D_1)$.

As an example of the use of the art described above for streams of different viscosity, consider the extraction stream (1) to be water and the sample stream (2) to be a fluid having three times the viscosity of water. Also consider the ratio of volume flow rates to be equal, F=1. Also assume $m \simeq 1/3$, and $D_2/D_1 \simeq 1/2$. From the equations above $\alpha = 0.0960$, $\bar{U}_1/\bar{U}_2 = 1.21$, and the velocity profile across the channel is shown in Figure 11. In Figure 12 a comparison is shown between the two-viscosity model of these fluids and one assuming the same interface location, but with no difference in viscosity or diffusivity in each stream. The comparisons in the concentration across the height of the channel are made near the upstream end of the extraction channel (x/w/Pe1 = 0.01) and also relatively far downstream (x/w/Pe1 = 1.0). The two-viscosity calculations are shown as solid lines, and the simpler one-viscosity calculations are shown as dashed lines. Note particularly at the downstream location there is a significant difference between the curves. These results demonstrate the importance of the art as described above for the design and quantitative use of the differential extraction device when used with fluids of different viscosity in each stream.

Example

In a preferred process for making a device of this invention, a 1 μm thick wet thermal oxide is grown in a 3" silicon wafer. This oxide is photolithographically patterned with the flow channels and etched to a depth of 60 nm. The wafer is recoated with photoresist and patterned with the through-hole

connections. The oxide is completely removed from this pattern. EDP etching is done to etch completely through the wafer (approximately 400 μm). An oxide etch is performed to uniformly remove 400 nm of oxide from the wafer. The flow channels are etched into the silicon approximately 10 μm deep. Finally the wafer is anodically bonded to a 3" disk of Pyrex glass.

The following example demonstrates the use of diffusion based extraction to separate diffusing constituents from a particle laden sample stream using micron sized devices microfabricated in silicon. See Figure 2. Fluorescein dye was extracted from a sample stream containing 0.5 μm fluorescent polystyrene spheres and fluorescein dye. Operation was demonstrated with zero contamination of the extraction stream by fluorescent spheres. The device had a total extraction channel fluid volume of approximately 1 femtoliter. The example demonstrates that separation is possible at the femtoliter scale given appropriate attention to precise flow stream regulation. Further, it demonstrates that efficient separation is possible in extraction channels with aspect ratios much less than 50 and in channels with diffusion direction dimension much less than 100 μm. The extraction device with $w/d << 50$, $d < 100$ μm demonstrated the effectiveness of a micro-fluidic system fabricated using silicon microfabrication technology and the essential attributes of ultra-low Reynolds number flow.

A two mask level process was needed to fabricate the device. The first level defined connection ports, which were etched completely through the wafer to the rear side of the silicon. The second level defined the fluid transport channels.

Four-inch chrome masks were made to our specifications by Photo Sciences, Inc. (Torrance, CA) and 3" wafers ({100}, n-type) with 500 nm of SiO_2 grown on them.

Wafers were cleaned in a Piranha bath (H_2SO_4 and H_2O_2) (2:1) before processing. A primer (HMDS spun on at 3000 rpm) was used

to enhance photoresist adhesion. About one μm of AZ-1370-SF (Hoechst) photoresist was deposited by spin coating (3000 rpm), and this was followed by a soft bake (30 min at 90°C).

A contact aligner was used to align and expose wafers. Exposure time was varied to yield best results. No post-exposure bake was done. Wafers were developed in AZ-351 (diluted 4:1) (Hoechst) for one minute, and rinsed in DI water. Blue tack tape (Semiconductor Equipment Corporation, Moorpark, CA) was applied to the backsides of the wafers to protect the oxide from the oxide etch.

The wafers were immersed in a buffered oxide etch (BOE, 10:1 HF (49%) and NH_4F (10%)) for eleven minutes to completely etch away the unprotected oxide. The blue tack tape was removed by hand, and the photoresist was removed in an acetone rinse.

Silicon etching was done in a mixture of ethylene-diamine, pyro-catechol, and water (EPW F-etch) set up in a reflux boiling flask. This etch attacks the {100} planes of silicon at a rate of about 100 μm an hour. Fluid attachment ports were etched in the first step. Flow channels between fluid ports and the filter region were etched in the second step. The barrier was etched in the final step.

After final processing the wafers were once again cleaned in a Piranha bath and rinsed in DI water. They were then diced into individual devices.

We used anodic bonding (Wallis, G. and Pomerantz, D.I. (1969), J. Appl. Physics **40**:3946-3949) to attach Pyrex glass to the silicon devices. We obtained 1" square pieces of Pyrex glass (100 μm thickness) from Esco Products Inc. (Oak Ridge, NJ). First, the silicon and Pyrex glass were immersed in a solution of H_2O, NH_4OH, and H_2O (1:4:6) heated to 50°C. This process removes any organic matter on the surfaces and also makes the surfaces hydrophilic. After 20 minutes in this solution, the

silicon and Pyrex were rinsed with DI water and dried. Anodic bonding was done at 400°C with 400 V applied between the glass and the silicon.

Fluid connections were made to ports on the back side of the wafer. A glass tube (1/8" inner diameter, about 3 cm long) was epoxied around the fluid ports. The flow was driven by a pressure difference between the entrance ports and the exit port. This pressure difference, less than 3 cm of H_2O, is enough to induce a flow velocity of greater than 100 μm per second.

Observations were made on a Zeiss ICM-405 inverted microscope and recorded with a Dage silicon intensified target camera. First, the device was wet with isopropyl alcohol and any trapped air bubbles were removed by applying approximately 70 kPa of pressure. Then a mixture of water, carboxyfluoroscein (Molecular Probes), and 0.5 μm diameter fluorescent balls (Duke Scientific) was introduced into one of the fluid entrance ports. Pure water was introduced at the other entrance port. All the 0.5 μm spheres flowed to the exit channel for the sample stream. The dye diffused throughout the extraction channel and some flows out with the product stream.

The invention has been illustrated with specific embodiments; however, as will be appreciated by those skilled in the art, various substitutions can be made for the specific elements and process steps disclosed herein. The invention is limited only by the scope of the appended claims.

Claims

1. A microfabricated extraction device for extracting desired particles from a sample stream containing said desired particles comprising:

a. a sample stream inlet;

b. an extraction stream inlet;

c. an extraction channel having an aspect ratio (w/d) less than 50 in fluid communication with said sample stream inlet and said extraction stream inlet for receiving a sample stream from said sample stream inlet in parallel laminar flow with an extraction stream from said extraction stream inlet;

d. a by-product stream outlet in fluid communication with said extraction channel for receiving a by-product stream comprising at least a portion of said sample stream from which desired particles have been extracted;

e. a product stream outlet in fluid communication with said extraction channel for receiving a product stream comprising at least a portion of said extraction stream and comprising desired particles extracted from said sample stream.

2. The device of claim 1 wherein said extraction channel has an aspect ratio less than about 25.

3. The device of claim 1 wherein said extraction channel has an aspect ratio less than about 1.

4. A microfabricated extraction device for extracting desired particles from a sample stream containing said desired particles comprising:

a. a sample stream inlet;

b. an extraction stream inlet;

c. an extraction channel having a depth less than about 100 micrometers in fluid communication with said sample stream inlet and said extraction stream inlet for receiving a sample stream from said sample stream inlet in parallel laminar flow with an extraction stream from said extraction stream inlet;

d. a by-product stream outlet in fluid communication with said extraction channel for receiving a by-product stream comprising at least a portion of said sample stream from which desired particles have been extracted;

e. a product stream outlet in fluid communication with said extraction channel for receiving a product stream comprising at least a portion of said extraction stream and comprising desired particles extracted from said sample stream.

5. The device of claim 1 fabricated of materials comprising a silicon wafer.

6. The device of claim 1 also comprising means for effecting differential transport of said desired particles from said sample stream into said extraction stream.

7. The device of claim 5 wherein said means for effecting differential transport are means for producing fields selected from the group consisting of magnetic, electrical,

dielectrical, sedimentation, shear, centrifugal force, temperature, pressure, and concentration gradients.

8. The device of claim 6 wherein said means for effecting a concentration gradient field comprises an effectively non-diffusing absorbent or adsorbent material selective for said desired particles in said extraction stream.

9. The device of claim 1 comprising a plurality of product stream outlets.

10. A microfabricated analytical system comprising a device of claim 1 in combination with means for detecting said desired particles in said product stream.

11. A microfabricated analytical system comprising a device of claim 1 wherein desired particles are extracted from a sample stream containing desired and undesired particles, which system comprises means for detecting said undesired particles in said product stream.

12. The analytical system of claim 10 wherein said means for detecting said desired particles comprise optical sensing means.

13. The analytical system of claim 11 wherein said means for detecting said undesired particles comprise optical sensing means.

14. The analytical system of claim 10 comprising means for mixing said product stream with an indicator substance capable of interacting with said desired particles so as to enable their detection.

15. An analytical system microfabricated on a silicon wafer comprising:

a. extraction means for extracting desired particles of an analyte comprising:

(1) a sample stream inlet;

(2) an extraction stream inlet;

(3) an extraction channel having an aspect ratio (w/d) less than 50 in fluid communication with said sample stream inlet and said extraction stream inlet for receiving a sample stream from said sample stream inlet in parallel laminar flow with an extraction stream from said extraction stream inlet;

(4) a by-product stream outlet in fluid communication with said extraction channel for receiving a by-product stream comprising at least a portion of said sample stream from which at least a portion of said desired analyte particles have been extracted;

(5) a product stream outlet in fluid communication with said extraction channel for receiving a product stream comprising at least a portion of said extraction stream and comprising desired analyte particles extracted from said sample stream;

b. a diffusion-based mixing device for mixing said product stream with an indicator substance capable of interacting with said desired analyte particles so as to enable their detection;

c. a detection chamber wherein the presence of said desired analyte particles may be detected.

16. The analytical system of claim 15 in combination with sensing means whereby the presence of said desired analyte particles in said detection chamber may be detected.

17. The analytical system of claim 16 in combination with quantitation means whereby the concentration of said desired analyte particles in said detection chamber may be measured.

18. A method for extraction of at least a portion of desired particles from a sample stream comprising said desired particles and also containing undesired particles, comprising:

a. introducing said sample stream into the sample stream inlet of a microfabricated extraction device of claim 1;

b. introducing an extraction stream into the extraction channel of said extraction device;

c. withdrawing a product stream comprising desired particles from the product stream outlet of said device.

19. A method for extraction of at least a portion of desired particles from a sample stream comprising said desired particles and also containing undesired particles, comprising:

a. introducing said sample stream into the sample stream inlet of a microfabricated extraction device of claim 2;

b. introducing an extraction stream into the extraction channel of said extraction device;

c. withdrawing a product stream comprising desired particles from the product stream outlet of said device.

20. The method of claim 18 conducted as a continuous process.

21. The method of claim 18 conducted as a batch process.

22. The method of claim 18 wherein a field selected from the group consisting of magnetic, electrical, dielectrical, sedimentation, shear, centrifugal force, temperature gradient, pressure gradient, and concentration gradient fields is produced across said extraction channel to aid in differential transport of desired particles into said extraction stream in said extraction channel.

23. The method of claim 22 wherein said field is a concentration gradient field and said differential transport is effected by diffusion.

24. The method of claim 18 wherein the sample and extraction stream have different properties.

25. The method of claim 22 wherein said sample fluid volume is at least about 1 picoliter.

26. The method of claim 22 wherein said sample fluid volume is between about 1 nanoliter and about 10 microliters.

27. The method of claim 22 wherein said sample fluid volume is between about 1 microliter and about 1 microliter.

28. The method of claim 18 also comprising detecting the presence of said desired particles or said undesired particles in said product stream.

29. The method of claim 18 also comprising determining the concentration of said desired particles or said undesired particles in said product stream.

30. The method of claim 18 also comprising determining the concentration of said desired particles in said sample stream.

31. The method of claim 18 wherein the presence and/or concentration of said desired and/or undesired particles in said product stream is determined in less than about 1 second after said sample stream is introduced into said sample stream inlet.

32. The method of claim 18 wherein the presence and/or concentration of said desired and/or undesired particles in said product stream is determined between about 1 second and about 5 minutes after said sample stream is introduced into said sample stream inlet.

33. The method of claim 18 wherein the presence and/or concentration of said desired and/or undesired particles in said product stream is determined within about 1 to about 4 minutes after said sample stream is introduced into said sample stream inlet.

34. The method of claim 18 wherein said device is flushed to remove both desired and undesired particles after said product stream has been withdrawn, and said extraction method is repeated.

35. The method of claim 18 comprising withdrawing a by-product stream and repeating said method by introducing said by-product stream as sample stream into said sample stream inlet.

36. The method of claim 18 comprising detecting the concentration of a blood component wherein the sample stream comprises whole blood, and particles of a cleaned blood component are extracted into the product stream.

36. The method of claim 18 comprising withdrawing a by-product stream and introducing said by-product stream into a microfabricated flow cytometer.

37. The method of claim 18 comprising withdrawing a product stream and introducing said product stream into a microfabricated flow cytometer.

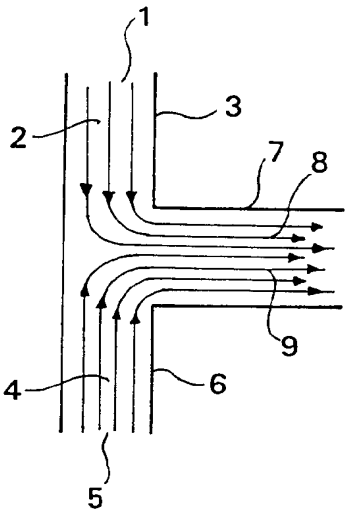


FIGURE 1

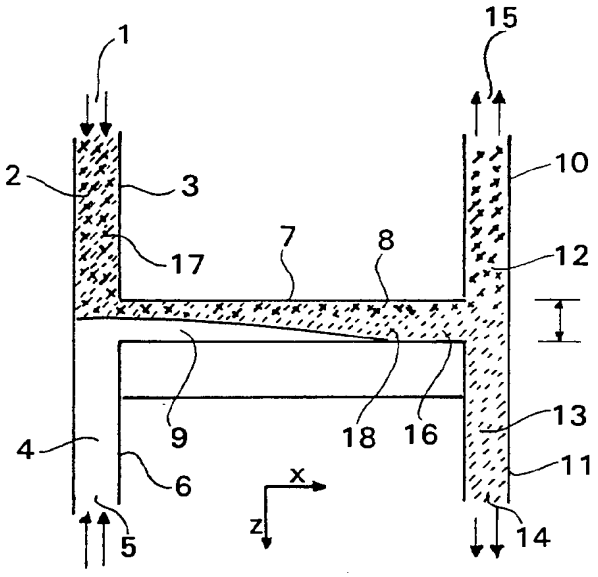


FIGURE 2

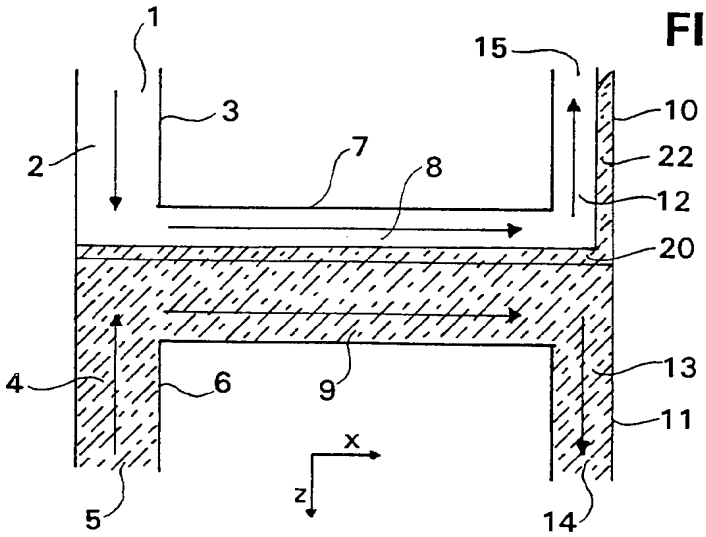


FIGURE 3

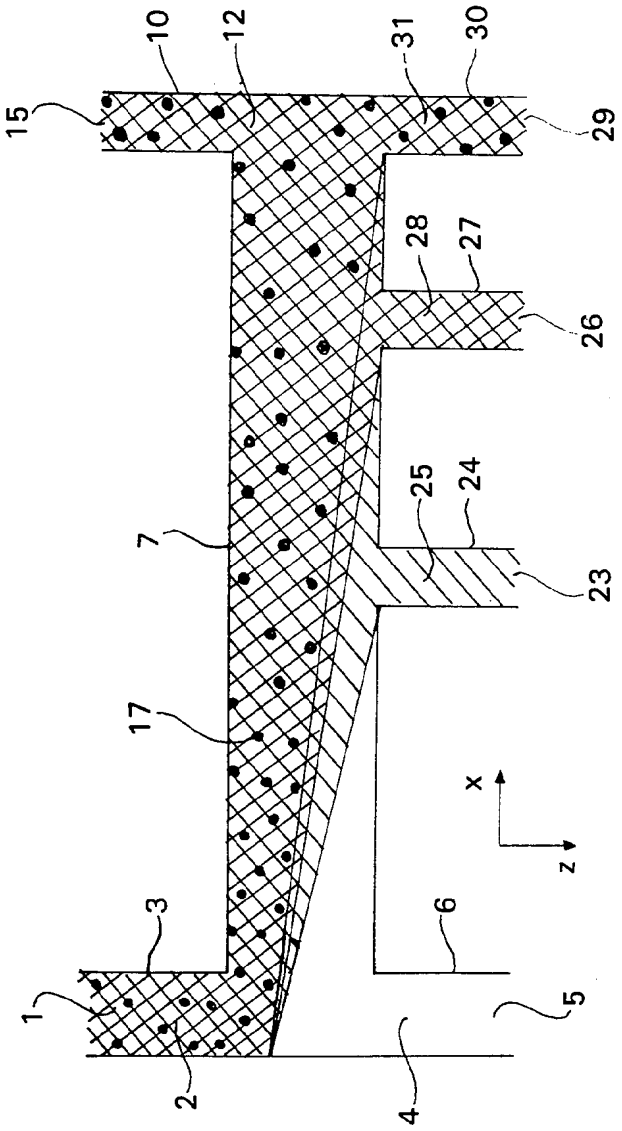


FIGURE 4

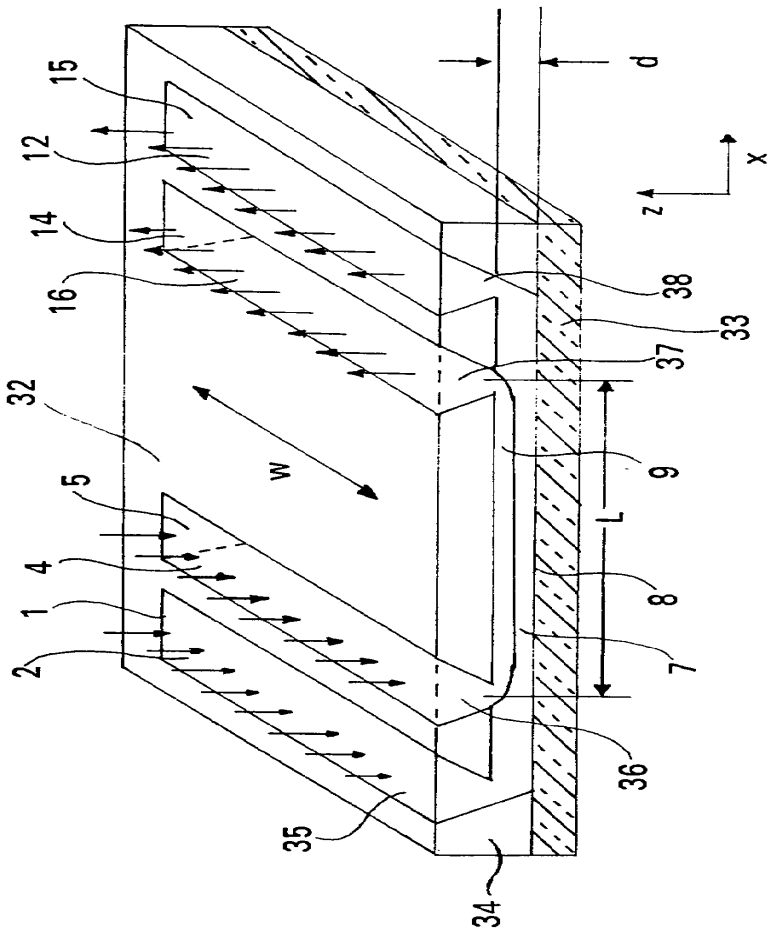
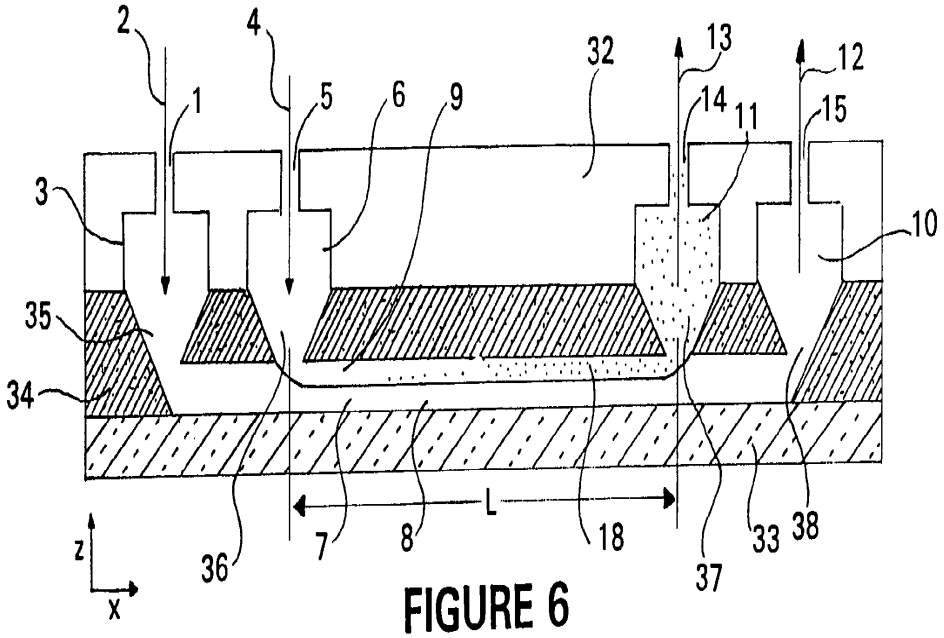


FIGURE 5



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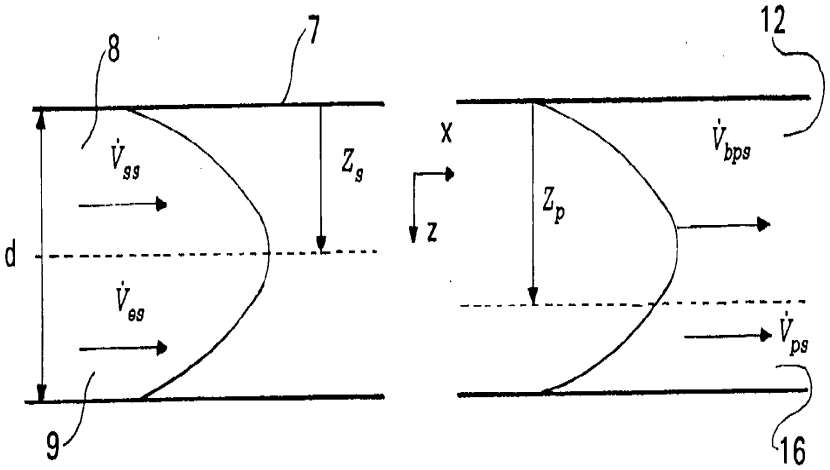


FIGURE 7

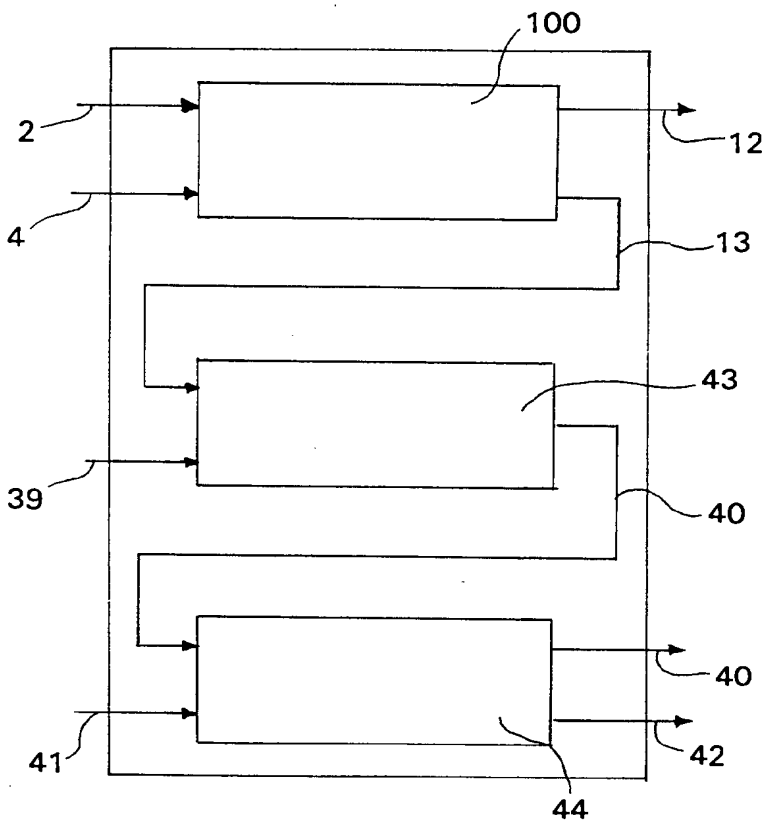
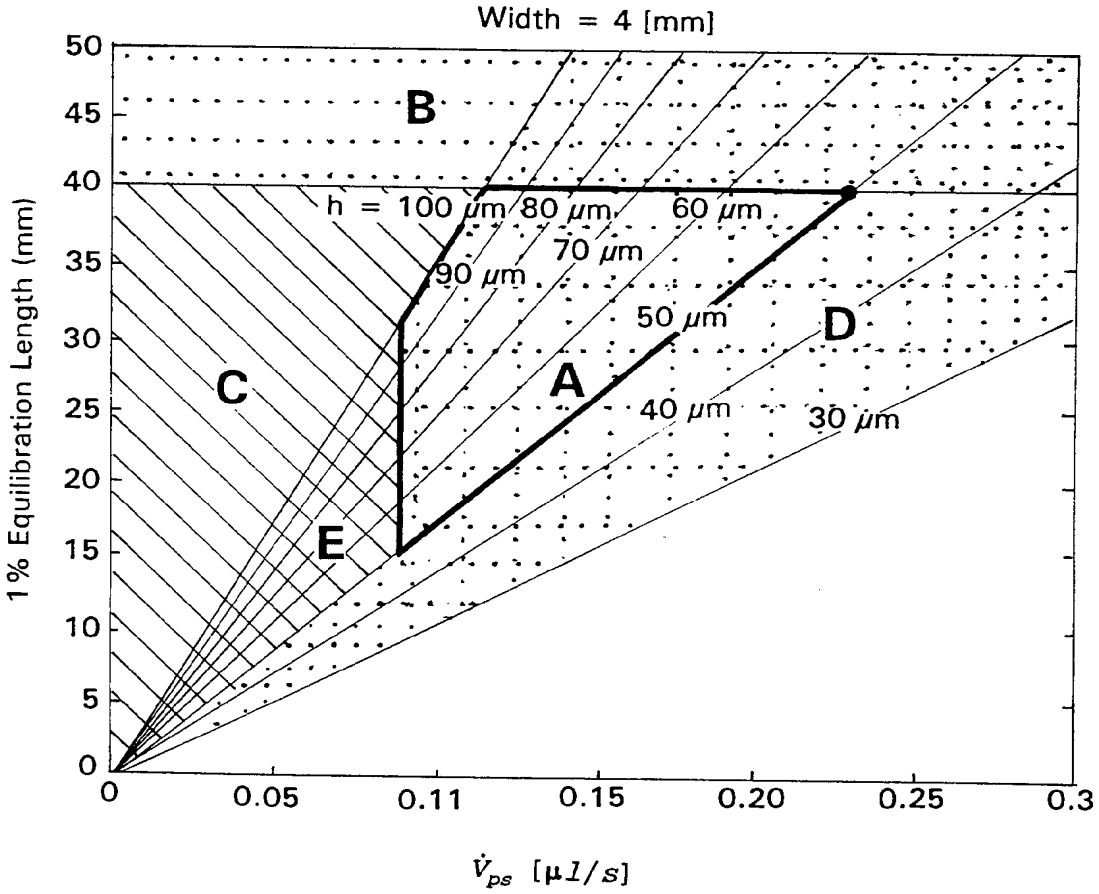


FIGURE 8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B
C
D
E
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70 μm
60 μm
50 μm
40 μm
30 μm
0
5
10
15
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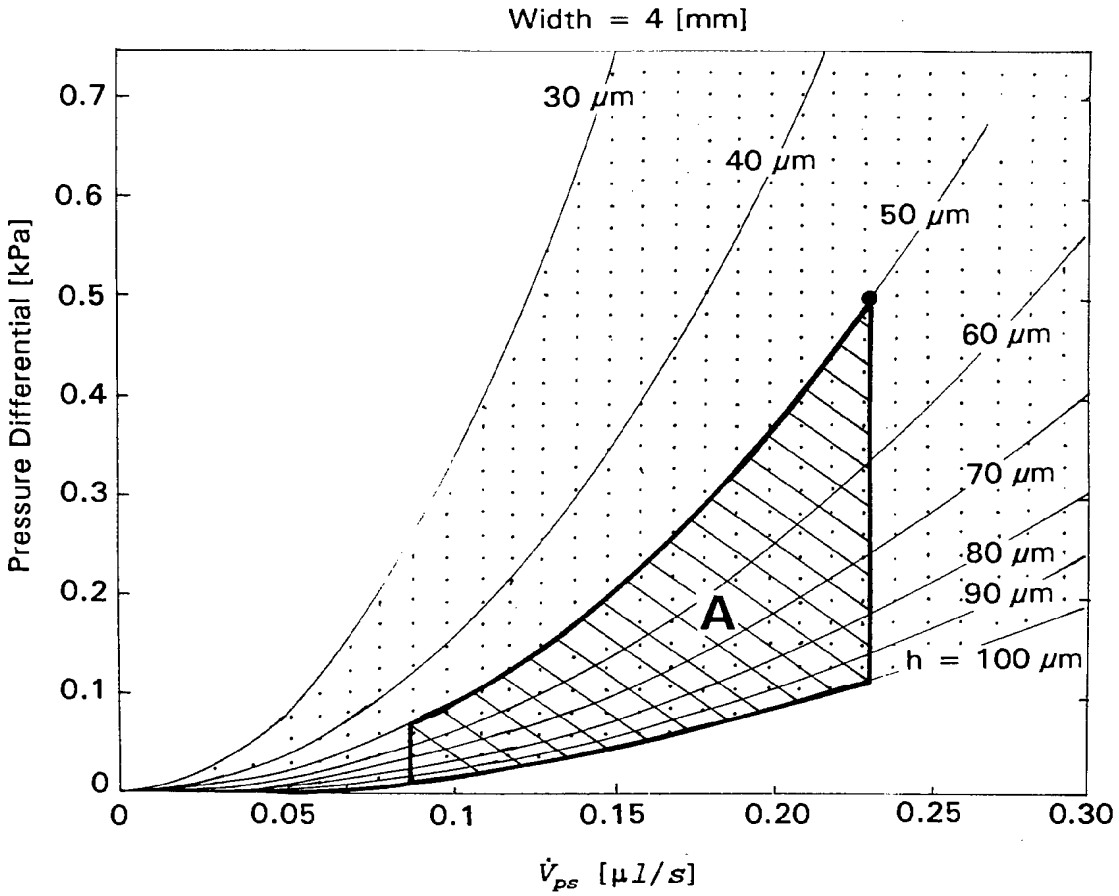
0.15
0.20
0.25
0.3

FIGURE 9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70 μm
80 μm
90 μm
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0.30
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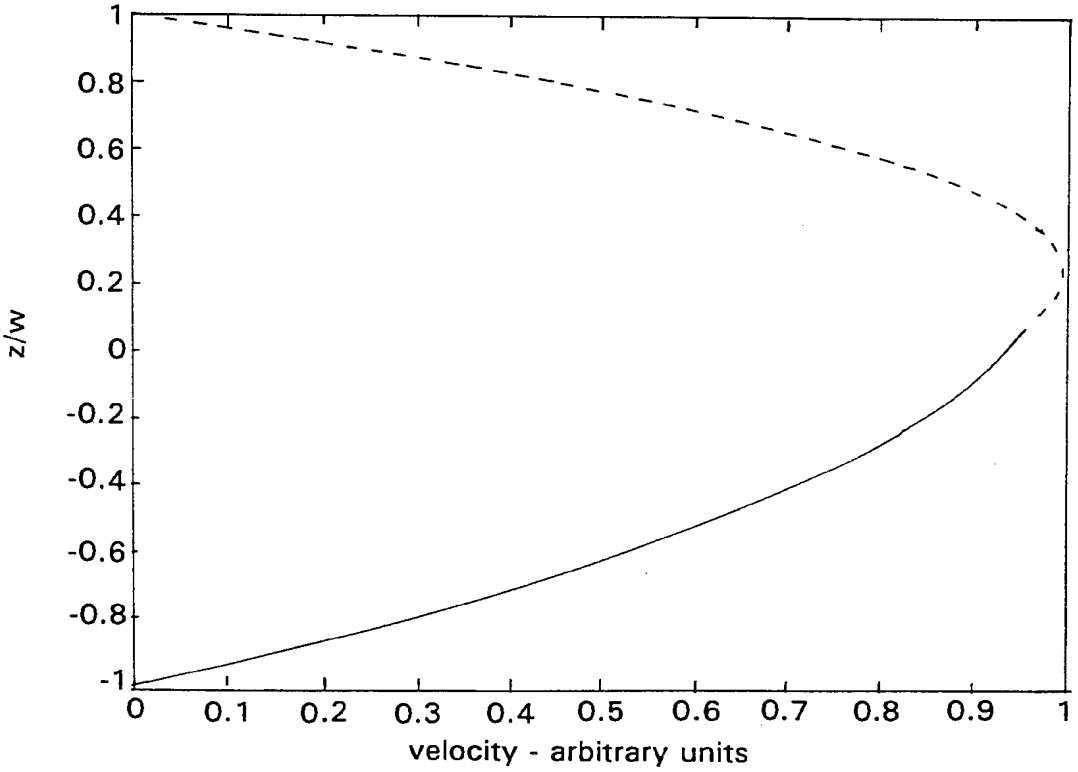
FIGURE 10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-0.8
-1
z/w
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0.8
0.9
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velocity - arbitrary units

FIGURE 11

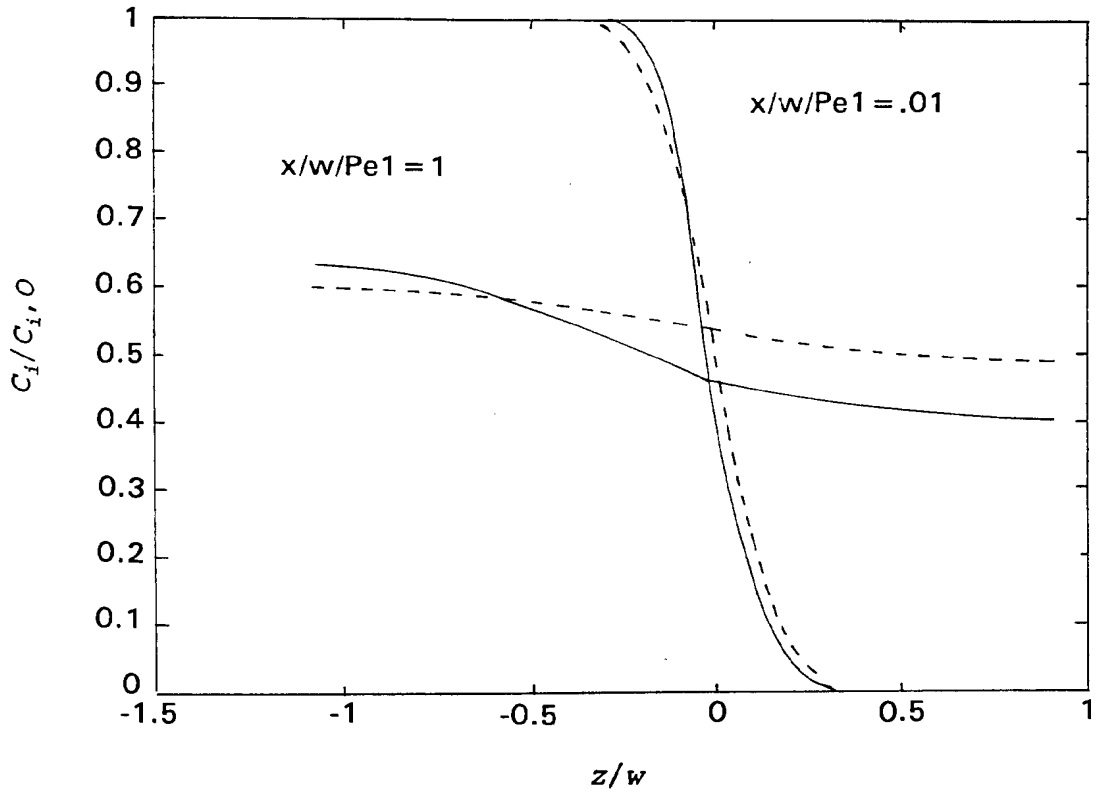

FIGURE 12

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 96/10308

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N30/00 G01N15/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANALYTICAL CHEMISTRY, vol. 59, no. 2, 15 January 1987, COLUMBUS US, pages 344-350, XP002018195 S.R. SPRINGSTON ET AL: "continuous particle fractionation based on gravitational sedimentation in split-flow thin cells"	1-3,18, 19
Y	see abstract see figures 1,2 ---	15-17
X	US 4 737 268 A (GIDDINGS) 12 April 1988 see column 6, line 54-58 see column 9, line 57 - column 10, line 19 ---	4
A	US 5 039 426 A (GIDDINGS) 13 August 1991 see column 11, line 19-41; figure 3 ---	1-4
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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3

Date of the actual completion of the international search	Date of mailing of the international search report
25 November 1996	04. 12. 96

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

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PCT/US 96/10308

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF MIRCHOMECHANICS AND MICROENGINEERING, vol. 4, 1994, pages 227-245, XP000601275 M. ELWENSPOEK: "towards integrated microliquid handling systems" cited in the application	1-4
Y	see page 232; figure 8	15-17

A	JOURNAL OF CLINICAL CHEMISTRY, vol. 40, no. 1, 1994, pages 43-47, XP000601246 P. WILDING ET AL.: "manipulation and flow of biological fluids in straight channels micromachined in silicon" cited in the application see page 46, column 2, paragraph 2	4

A	JOURNAL OF MIRCHOMECHANICS AND MICROENGINEERING, vol. 3, 1993, pages 168-182, XP000601274 P. GRAVENSEN ET AL.: "microfluidics - a review" cited in the application	

A	JOURNAL OF MIRCHOMECHANICS AND MICROENGINEERING, vol. 4, 1994, pages 257-265, XP000601273 A. MANZ ET AL.: "electroosmotic pumping and alectrophoretic separations for miniaturised chemical analysis systems" cited in the application	

3

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INTERNATIONAL SEARCH REPORT

information on patent family members

Intern al Application No
PCT/US 96/10308

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4737268	12-04-88	NONE	
US-A-5039426	13-08-91	NONE	

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(74) Agent: PLOUGMANN, VINGTOFT & PARTNERS A/S; Sankt Annae Plads 11, P.O. Box 3007, DK-1021 Copenhagen K (DK).

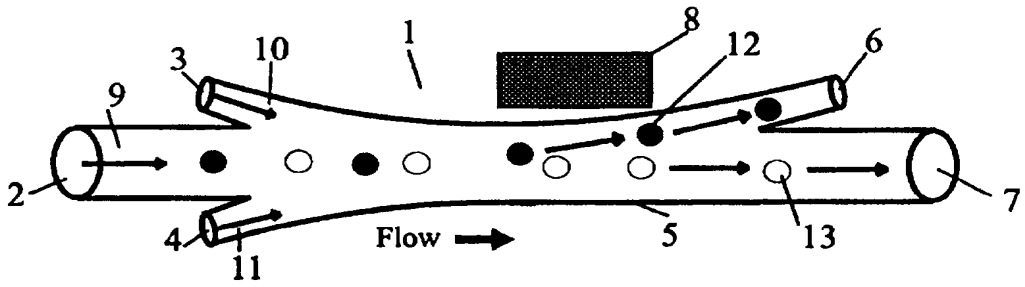
(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, ES, FI, FI (Utility model), GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: A MICRO FLOW SYSTEM FOR PARTICLE SEPARATION AND ANALYSIS



(57) Abstract

A micro flow system is provided for separating particles, comprising a microfabricated member having a flow channel (5) defined therein for guiding a flow of a fluid containing the particles through the flow channel, first inlet means (2) positioned at one end of the flow channel for entering the fluid into the flow channel, first outlet means (7) positioned at the other end of the flow channel for discharging the fluid from the flow channel, the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross section of the flow channel, the member being positioned in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field. Further, a micro flow system is provided for analysing components of a fluid comprising a microfabricated member having a flow channel defined therein for guiding a flow of a fluid through the flow channel, first inlet means for entering particles into the flow channel, first outlet means for discharging of fluid from the flow channel and a plurality of assay sites located in the flow channel and comprising immobilised reagents whereby the fluid may be analyzed for a plurality of components while residing in the flow channel.

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A MICRO FLOW SYSTEM FOR PARTICLE SEPARATION AND ANALYSIS

FIELD OF THE INVENTION

The present invention relates to methods and apparatuses for detection, separation, sorting, and analysis of particles, such as cells, cell organelles, beads, molecules, such as Deoxyribonucleic acid (DNA), proteins, etc. in a fluid. In particular, the invention relates to particle separation by using different forces such as magnetic, electrophoretic, hydrodynamic and/or gravitational forces, e.g. for utilisation in flow cytometry, light microscopy, electrophoretic separation, magnetophoresis, etc.

BACKGROUND OF THE INVENTION

Flow cytometry is a well known technique that is used for high throughput measurements of optical and/or electrical characteristics of microscopic biological samples. Flow cytometry instruments analyse and isolate cells and organelles with particular physical, biochemical, and immunological properties.

Traditionally, cell sorting by flow cytometry (fluorescence activated cell sorting) has been the method of choice for isolation of specific cell populations by surface markers. However, cell sorting by flow cytometry suffers from several drawbacks, especially high dilution of desired cell sample, low speed and sterility problems. Furthermore, the equipment is very costly with high operation and maintenance cost, making the technique available only to a limited number of laboratories.

During the last few years, isolation of cells by antibody-coupled magnetic beads and carriers has been developed into a reliable tool for the isolation and characterisation of cell populations. Immunomagnetic cell separation, e.g. as commercially introduced by Dynal A/S and Miltenyi Biotec, has become an established method for cell analysis in clinical diagnostics. Due to the relatively low prize, this method is attractive in flow cytometry, especially in sorting of rare cellular events. For example, sorting of fetal cells contained in maternal blood sample provides a non-invasive alternative to prenatal diagnostic procedures, such as amniocentesis of chorionic villus sampling. Another rare event scenario is the detection of low concentration of cancer cells which has an important role in diagnosis of minimal residual disease and evaluation of appropriate therapies. Another medical application for cell sorting systems is the diagnosis of bacterial and viral diseases.

Although this method offers relatively inexpensive approach to sort rare cellular event, it adds considerable time onto the overall rare event detection and it does not offer the multiparameter

analysis readily available with flow cytometry techniques. Existing techniques for magnetic separation are suffering from the low purity of the sorted cell fraction and the low recovery rate of the sorted cells. In most systems several washing steps have to be implemented into the separation procedure which then causes cell losses. Additionally small subpopulation of labelled cells cannot be directly isolated by existing magnetic separation techniques.

A good overview about fluorescence activated cell sorting procedures and magnetic activated cell sorting is given in Melamed et. al., "Flow Cytometry and Sorting, (Ed. Melamed et. al., Wiley & Sons Inc., 1990).

SUMMARY OF THE INVENTION

Advances in microfabrication and microfluidic technologies continue to fuel further investigation into the miniaturisation of bioanalytical instruments and biochemical assays in general. The present invention relates to development of a low cost non-invasive diagnostic test method and devices for carrying out such tests that include measuring, monitoring, sorting and analysing samples containing particles, such as organic cells, microbeads, cell organells and macromolecules such as DNA. The present invention provides a cheap, fast and reliable method and devices for handling, sorting and analysis of such particles.

Separation may be performed according to various physical properties, such as fluorescent properties or other optical properties, magnetic properties, density, electrical properties, etc. According to an important aspect of the invention, particle separation is performed by aligning the particles in one row of particles in a micro flow channel so that particles can be treated individually.

Thus, it is an object of the present invention to provide a micro flow system and a method of particle separation having an improved efficiency of particle separation compared to the prior art.

It is another object of the present invention to provide a micro flow system and a method for particle separation in which cell lysis is minimised.

It is yet another object of the present invention to provide an improved method for preparation of fluids containing particles for separation and analysis of the particles.

It is a still further object of the present invention to provide a micro flow system and a method for simultaneous separation of particles into a plurality of groups of particles.

It is a still further object of the present invention to provide a micro flow system including facilities for pre-treatment and/or post-treatment of a sample.

It is a still further objective of the invention is develop a system for separation and analysis of fetal cells in whole maternal blood samples using an integrated automated micro flow system. The system is designed by downscaling and combining different methods for handling, manipulation and analysis of biochemical samples. Thus, prenatal diagnostics by analysis of fetal cells separated from a whole maternal blood sample is an area, which can benefit from advances in miniaturisation.

It is another objective of the invention is develop a system for separation and analysis of cancer cells from a sample containing cancer cells and healthy cells using an integrated automated micro flow system. The system is also designed by downscaling and combining different methods for handling, manipulation and analysis of biochemical samples. Thus, cancer diagnostics by analysis of cancer cells separated from healthy cells is also an area which can benefit from advances in miniaturisation.

According to a first aspect of the invention the above and other objects are fulfilled by a micro flow system for separating particles, comprising a member having

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

first inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

first outlet means positioned at the other end of the flow channel for discharging the fluid from the flow channel,

the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross-section of the flow channel,

the member being positioned in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field.

According to a second aspect of the invention the above and other objects are fulfilled by a method of separating particles, comprising the steps of

guiding a flow of a fluid containing the particles through a flow channel in such a way that one particle at the time passes a cross-section of the flow channel,

positioning the flow channel in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field and thereby separated from the fluid.

According to a third aspect of the invention the above and other objects are fulfilled by a micro flow system for separating particles, comprising a member having

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

first inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

first and second outlet means positioned at the other end of the flow channel for discharging of fluid from the flow channel,

the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross-section of the flow channel,

monitoring means positioned at the flow channel for monitoring parameters of a particle residing within a measurement volume within the flow channel and providing an output signal corresponding to a monitored parameter,

control means for controlling passage of fluid through the first and the second outlet means, respectively, in response to the output signal of the monitoring means whereby particles may be separated according to values of a parameter monitored by the monitoring means.

According to a fourth aspect of the invention the above and other objects are fulfilled by a method of separating particles, comprising the steps of

guiding a flow of a fluid containing the particles through a flow channel in such a way that one particle at the time passes a cross-section of the flow channel, the flow channel having first and second outlet means for discharging of fluid from the flow channel,

monitoring parameters of a particle residing within a measurement volume within the flow channel and

controlling passage of fluid through the first and the second outlet means, respectively, in response to a monitored parameter value whereby particles may be separated according to values of a monitored parameter.

According to a preferred embodiment of the invention, a method of separating fetal cells from maternal cells, comprising the steps of selective magnetically staining of fetal cells in a fluid containing fetal and maternal cells, guiding a flow of the fluid containing the fetal cells through a flow channel in such a way that one fetal cell at the time passes a cross-section of the flow channel, positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained fetal cells residing in the flow channel are deflected in the direction of the magnetic field.

Further a method is provided for separating cancer cells from other cells, comprising the steps of selective magnetically staining of cancer cells in a fluid containing cancer and other cells, guiding a flow of the fluid containing the cancer cells through a flow channel in such a way that one cancer cell at the time passes a cross-section of the flow channel, positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained cancer cells residing in the flow channel are deflected in the direction of the magnetic field.

The particles to be separated from other particles in a fluid and/or to be separated from the fluid containing the particles may comprise living cells, chromosomes, organelles, beads, biomolecules, such as Deoxyribonucleic acid (DNA), proteins, etc.

Preferably, the flow through the flow channel is a laminar flow so that flow of particles are predictable and easy to control, e.g. with a flow of guiding buffers.

When the flow is laminar, the stream of particles can be positioned as desired within the flow channel, e.g. by controlling flow velocities of the fluid containing particles at the particle inlet of the member and flow velocities of guiding buffers at corresponding inlets.

Preferably, the flow channel is small for the flow through the channel to have a low Reynolds number, e.g. in the range of 0.01-500, such as 0.05-50, preferably 0.1-25. Thereby, inertial effects, which causes turbulence and secondary flows are negligible, viscous effects dominate the

dynamics, and mixing is caused only by diffusion. Flow of the sample, which is the fluid containing particles and guiding buffers can be laminated in guided layers through the channel and displacement of particles in the channel is only caused by the external force applied. The Reynolds number referred to is based on the hydraulic diameter of the flow channel, the flow velocity in the axial direction and the fluid density and viscosity, $Re=\rho Dh/\mu$ where the hydraulic diameter Dh is defined as four times the cross-sectional area divided by the wetted perimeter.

The illustrated flow channels of the micro flow system have a width ranging from 0.1 to 0.55 mm, preferably ranging from 0.1 to 0.4 mm, in particular ranging from 0.1 to 0.2 mm, and a depth ranging from 0.04 to 0.2 mm, preferably ranging from 0.04 to 0.1. With respect to the lowest cross-sectional area of the flow channel, it is preferred that this area is in the range of 0.004 to 0.11 mm^2, in particular in the range of 0.004 to 0.02 mm^2.

It is believed that any length of the flow channel within the range of 0.1 to 20 mm, preferably 1.0 to 3.5 mm, would lead to satisfactory results.

Preferably, the system is operating with total volumetric flow rates of 0.1 up to 200 µl/min, which gives a flow velocity of 15 mm/min up to 180 mm/min. The average residence time of a particle inside the flow channel, which corresponds to a separation time ranging from 0.1 to 6 sec. The residence time of the sample is defined by the total volumetric flow rate of the system.

The micro flow system may comprise flow rate adjustment means for adjustment of the time the particles reside in the flow channel.

Preferably, the fluid channel is sized so that for efficient separation, particles are displaced 10 - 30 µm in the flow channel. Thereby, the particle may only be exposed to a field for a very short period of time and thus, continuous separation of particles may be facilitated.

In order to collect the particles, which are deflected in the flow channel, the micro flow system may further comprise second outlet means for discharging particles having been deflected in the flow channel.

The micro flow system may comprise second inlet means for entering a first guiding buffer into the flow channel together with the fluid containing particles. When the flow is laminar, the two fluids flow through the flow channel in parallel abutting each other along a small area extending along a longitudinal axis of the flow channel whereby the cross-section and the path through the flow channel of the flow of the fluid containing particles may be controlled by the first guiding buffer flow. Further, particles in the fluid containing particles may be deflected into the guiding

buffer fluid when the two fluids pass the field essentially perpendicular to the longitudinal axis of the flow channel. Furthermore, two (or even more) outlets may be provided at the down stream end of the flow channel for discharging the guiding buffer now containing separated particles and fluid substantially without particles susceptible to the field essentially perpendicular the flow channel, correspondingly.

The micro flow system may further comprise third inlet means for entering a second guiding buffer for improved control of the path of particle flow through the flow channel. By adjustment of the flow velocities of the guiding buffers and the fluid containing particles, the flow within the flow channel of fluid containing particles may be controlled to flow within an essentially cylindrical shaped domain with a longitudinal axis extending substantially parallel to a longitudinal axis of the flow channel and further the position within the flow channel and the diameter of the flow cylinder may be controlled by corresponding adjustments of the volumetric ratio between the flow rate of the fluid containing particles and the flow rate of the guiding buffers.

It is possible to control the cross-sectional area of the domain containing the sample to be a little larger than the cross-sectional area of the particles by adjusting the volumetric flow rates of the sample and of the one or two guiding buffers in such a way that the particles contained in the sample are aligned in a single row of particles. This is a very important feature since it enables individual treatment of each particle and it leads to a sensitive method of sorting particles according to their susceptibility to a field. A sample flow layer thickness less than 1 μm may be achieved.

Preferably, the channel depth is small enough, e.g. below 50 μm, to allow observation of the particles flowing through the channel by a microscope. In an important embodiment of the present invention, the micro flow system comprises a cover, e.g. a transparent or translucent cover, for covering the flow channel. When the cover is transparent or translucent, it will be possible to observe events in the flow channel, e.g. passage of a stained or coloured particle or cell.

The member with the flow channel may be produced from any suitable material, such as silicon, polymers, such as Plexiglas, Teflon, etc., glass, ceramics, metals, such as copper, alumna, stainless steel, etc., etc.

The channel may be provided in the member by any suitable manufacturing process, such as milling, etching, etc.

In a preferred embodiment of the invention, the member is a silicon chip manufactured utilising photolithography and the channel is etched into the silicon chip.

The field may be a magnetic field, an electric field, a gravity field, etc., and any combination of such fields.

A magnetic field may be generated by permanent magnets, such as rare earth magnets, such as samarium-germanium magnets, a mixture of ferromagnetic powder and epoxy, etc., etc., electromagnets, e.g., in silicon integrated electromagnets, etc. The magnets are preferably positioned adjacent to the flow channel so that the magnetic field is substantially perpendicular to a longitudinal axis of the flow channel.

In a preferred embodiment of the invention, the magnets are positioned in and glued to rectangular slots that are etched into a silicon chip. The slots are located adjacent to the separation flow channel. In the example shown in Fig. 1, a permanent magnet or an electromagnet can be received by slots in the micro flow system. The slots are, e.g., 0.5 mm wide, 0.5 mm long and 0.2 mm deep. For generation of a magnetic field, a solid magnetic block, i.e. rare earth magnet can be glued into the slot. Alternatively, a mixture of ferromagnetic powder and epoxy can be injected into the slots to produce a high magnetic field gradient.

The strength of the magnetic field inside the micro flow system may be adjustable. If an electromagnet is used for generation of the magnetic field, the magnitude of the field may be varied by varying the amplitude of the voltage input to the electromagnet. If a permanent magnet generated the magnetic field, the magnitude of the field may be varied by varying the distance between the magnet and the flow channel of the micro flow system.

As already mentioned, the net displacement of a particle in the micro flow system depends on the force applied to it by the field. This can be utilised for separation of a first group of particles of various types in a fluid into a plurality of set of particles; each set comprising a specific type of particles. A micro flow system with e.g. five separation outlets may be used to separate a fluid containing particles into five sets of particles, each set comprising particles that are influenced by the field with a force of a specific magnitude, in the following denoted particles with a specific F-value. Particles with a low F-value are only deflected by a small amount by the field and are discharged from the flow channel through a corresponding outlet port. Particle deflection is increased with increasing F-values whereby such particles are discharged from the flow channel through the corresponding other outlets.

The particles to be separated from other particles in a fluid and/or to be separated from the fluid containing the particles may be magnetically stained to facilitate separation in a magnetic field.

In the present context, the term staining is to be understood in a broad sense. The term is intended to cover any way of marking a particle thereby facilitating detection of the particle. For example a cell may be stained with a fluorescent substance, such as acridin orange, methylene blue, etc, facilitating detection of the stained particles by a fluorescence detector, or, a particle is said to be magnetically stained when it is coupled to a magnetic microbead. The microbead may for example carry a monoclonal or polyclonal antibody on its surface for coupling to an antigene of a cell to be separated utilizing a magnetic field.

In the case where particles have to be detected in a flow channel by optical means, such particles are preferably stained with a chromophoric reagent, or, a fluorescent probe.

An electric field may be generated by electrodes, such as metal electrodes, such as gold electrodes, etc. The electrode may be positioned inside the flow channel, e.g. to introduce electrophoretic forces, e.g. for separation of charged molecules in the fluid, or outside the flow channel e.g. to introduce dielectrophoretic forces, e.g. for separation of particles contained in the flow according to the susceptibility of the particles to the field. Preferably, the electrodes are positioned in such a way that the electric field is essentially perpendicular to a longitudinal axis of the flow channel.

The electric field may be a high frequency field, e.g. a 5 MHz field generated by electrodes positioned inside the flow channel. Living cells positioned in an electric field will be polarized and will be influenced by the field and thus, an alternating field may be used to separate living cells from other particles.

The field generated across the flow channel may be utilised for immobilisation of particles whereby particles may be held in substantially fixed positions within the flow channel for a specific period, e.g. as outlined in Fig. 6, allowing chemical reactions with the particles to take place and/or kinetic measurements on the particles to be performed and/or to bring the particles into contact with different chemical substances or for separating the particles from the sample. The particles may undergo a washing step before removal or reduction of the field redisperses them.

According to a fifth aspect of the invention the above and other objects are fulfilled by a micro flow system for separating particles, comprising a member having

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

field generating means positioned proximate to the other end of the flow channel for generating a field substantially along a longitudinal axis of the flow channel whereby the particles are drawn by the field along the channel and distributed according to their susceptibility to the field and their mobility.

For example, means for generating a magnetic field may be situated at the closed end of a micro flow channel, which at the other end has at least one inlet for entering a sample containing magnetic labelled macromolecules, i.e. ribonucleic acid or proteins. The sample is entered into the channel where the particles are drawn by the magnetic field along the channel and, as by electrophoresis, the particles will be distributed according to their susceptibility to the magnetic field and their mobility. The generated magnetic field is removed after a predetermined time interval and the distribution of particles can then be observed.

According to another embodiment of the invention, the flow through the sort outlet is not continuous but only allowed by a controlling means, e.g. a valve, when a particle with the desired characteristics is detected by a detection means. The particles are sorted using hydrodynamic forces in the sense that the flow is diverged from the ordinary outlet to the sort outlet only when it contains a particle that fulfils certain criteria. The concentration of sorted particles in the flow out of the sort outlet will consequently be high. This is especially an advantage for sample flow with rare occurrence of particles that are searched for. The detection means can be e.g. optical detection means or magnetic detection means e.g. a Hall sensor or means for detecting e.g. electrical or other properties of the particles. The detection means can in an alternative embodiment be used for counting of particles with the desired characteristics as a separate function or in connection with any of the other embodiments described herein.

In yet another embodiment, the field strength is adjustable, e.g. by adjusting the voltage supplied to an electromagnet or to a set of electrodes or by adjusting the distance from a permanent magnet to the flow channel. Particles are in a first operation mode entrapped inside the flow channel by the field at high intensity while at the same time the sort outlet is closed. In a second operation mode, the field is reduced and the sort outlet is open in such a way that the entrapped particles are redispersed and moved out of the sort outlet. Particles that are rare in the sample can by switching between these two operational modes be sorted out in a highly concentrated form. An example of this embodiment is outlined in Fig. 6.

In a further interesting embodiment, the micro flow system according to the invention involves facilities for performing pre-treatment and/or post-treatment of the fluid comprising the particles. These possibilities are outlined in Figs. 5(f), 7 and 10. As an example, the particles may be treated with a reagent before entering the flow channel, e.g. undergo magnetic or chromophoric staining. Post-treatment may comprise means for collecting or concentrating the deflected particles or means for contacting the deflected particles with one or more reagent(s).

By one possible combination of the pre-treatment and the post-treatment facilities, cells may undergo magnetic staining before entering the flow channel, and after separation the staining may be removed by treatment of the stained cells with a suitable reagent.

It is an important advantage of the present invention that a micro flow system is provided that operates continuously with no requirement for operator intervention.

It is another advantage of the present invention that separation may be performed in one step.

It is still another advantage of the present invention that the particles can be separated in a continuous flow without substantially interfering with the flow itself and that separated particles may be collected at corresponding separated outlets of the flow channel without having to interrupt the flow in the flow channel.

It is another important advantage of the invention that the particles contained in the sample by the adjustment of the flow rate of one or more guiding buffers can be lined up in one row such that the particles can be analysed and sorted individually. This results in a sorting system with the highest sensitivity to the susceptibility of the single particle to the field applied to the sorting channel and a sorting system with the highest resolution of the detection means of the characteristics exhibited by the particles.

It is yet another advantage of the present invention that the micro flow system is easily integrated into other continuous flow systems, such as flow cytometers, flow injection analysis systems, etc.

It is a further advantage of the present invention that particles may be separated into a plurality of groups of particles, e.g. different subpopulations of cells, based on different susceptibility to the field generated across the flow channel of the different groups of particles. This may be obtained by using a multiple outlet micro flow system as outlined in Fig. 5(c).

It is a still further advantage of the present invention that the micro flow system allows observation of particles in the flow channel using a microscope.

It is a still further advantage of the invention that a closed system is provided allowing biohazardous samples, such as samples containing pathogens, to be entered into the system without contaminating the laboratory environment and without causing hazard for operators working with pathogen biomaterials.

It is a still further advantage of the invention that a system with a low shear stress in the flow is provided allowing a gentle treatment of biological samples, e.g. fragile living cells, especially when two guiding buffers are introduced in the channel.

It is a still further advantage of the invention that a high concentration of the sorted particles can be obtained even from samples with rare occurrence of particles that are searched and sorted for.

According to an important aspect of the invention, a new system for immunomagnetic cell separation and manipulation is provided that utilises a silicon based micro fabricated flow chip. The system combines the advantage of flow cytometry and immunomagnetic separation technique. The flow chip will be an important component of a portable micro system for cell sorting and analysis. The flow chip is designed for rapid immunomagnetic cell separation nearly without any pressure drop. Its simple and cheap fabrication and versatile sorting and detection properties offer an alternative to conventional cell separation systems.

It is an advantage of the invention that a micro flow system is provided that is cheap, easy to operate, versatile, simple and portable and that offers the possibility of automation.

A miniaturised flow channel system is provided that utilises the advantageous fluid behaviour in micro systems. The invented system operates continuously. Instead of holding back the magnetisable particles in the separation unit, the particles are deflected into the direction of the magnetic field while passing it continuously. By splitting the fluid flow into two or more outlets, the deflection of the particles can be used for separation of particles into different sets of particles, each of which leaves the flow channel through a specific outlet.

The continuous separation system (CSS) allows efficient enrichment as well as depletion of labelled sample contents of interest. The CSS is designed to fit under a microscope allowing parallel detection of the optical properties of the sample and the control of separation of particles.

An advantage of the geometry of the invented separation flow channel is that a magnetised or electrically charged particle has to be moved only over a distance of 10 - 30 μm to be separated from the fluid containing particles.

Furthermore, the invention enables isolation of multiple cell or particle subpopulations from a single sample at the same time. The magnitude and direction of the force F on a magnetisable particle, e.g. a magnetically labelled cell, is dependent on the magnitude of the magnetic field and the number of magnetic moments inducible on a labelled cell.

F = N*S *μB* grad B

where S is the number of Bohr magnetons (μB) per particle and N is the number of particles per cell.

Beads with small S are moving a less distance in lateral direction in relation to the flow through the flow channel than beads with a higher S value. This can be used to separate subpopulation of cells labelled with different magnetisable beads: By splitting the flow channel in various outlet channels cells can be distinguish and separated due to their individual F values.

The drag force on a spherical particle can be found from the particle Reynolds number, based on particle diameter, particle velocity relative to the fluid and fluid viscosity and density. In a flow with a Reynolds number less than 100, the drag force D on the particle can be found from a modified version of Stokes law

$$D = 3\pi\mu \, Ud \left(1 + \tfrac{3}{16} Re\right)^{1}$$

where μ denotes the viscosity of the liquid, U is the relative velocity of the particle and d is the diameter. The numerical value of the parenthesis on the right hand side of the above formula is close to unity for Reynolds numbers less than one why it in that case can be omitted. The magnitude of the drag force on the particles, the force applied to the particle by the field, the distance the particle needs to be moved and the time available for the separation are all important aspects to be considered when a separation process and the device for carrying it out is designed.

An example is given for separation by gravitational means. The effective gravitational force G defined as the gravitational force minus the buoyancy force is found as

$$G = (\rho_{particle} - \rho_{liquid}) g \frac{\pi}{6} d^3$$

where g is the gravitational constant. For simplicity, a Reynolds number for the particle of less than one is assumed why the drag force D is given in a simple form. These two forces, D and G, are equal when the maximum velocity, the settling velocity U_∞ has been reached. This velocity is found as

$$U_\infty = \frac{(\rho_{particle} - \rho_{liquid}) g d^2}{18\mu}$$

The velocity to a given time t can be found as

$$U(t) = U_\infty (1 - e^{-t\frac{g}{U_\infty}})$$

For a particle submerged in water with a diameter of 30 μ m and a density of 1.2 times the density of water the settling velocity is 9×10^{-5} m/s. The particle will reach 90 % of this velocity after 2.1×10^{-5} seconds why the transient phase can be neglected. It will take the particle 0.33 seconds to travel a distance of 30 μ m, which makes the method reasonable to employ for separation purposes.

While instrumentation in chemistry and biochemistry has become more automated in recent years, the preparation of samples remains a highly laboratory intensive task. The demand is increasing for high throughput, easier to use cost effective analytical devices and assays. Creating this opportunity is e.g. the world-wide effort to sequence the Human Genome, resulting in the development of new DNA diagnostics and therapeutics. Another important trend is the minimisation of health care costs and hospital admissions by testing patients and monitoring therapeutic use in less expensive environments, the so-called point-of-care analysis.

Micro flow devices containing arrays of nucleic acid hybridisation sites, known as genosensors, are being developed for a variety of uses in genomic analysis. A great deal of the overall genosensor development effort involves optimisation of experimental conditions in the actual use of genosensors.

Another embodiment of the invention is dealing with a low-tech form of genosensor and immunosensor technology, involving arrays of oligonucleotides on a microchip, which can be used to define optimal operating conditions and to develop applications of hybridisation arrays in genome mapping and sequencing. The genosensor array is placed in a micro flow channel system allowing an operation in a flow-through mode. Thus several steps of microliquid handling, e.g. washing and staining steps, reagent addition, can be integrated as an automated routine

procedure. Additionally, micro flow devices containing arrays of antibody/antigen sites, known as immunosensors, can be designed in the same way. The system could be used for combinatorial screening (high-throughput screening) and pharmacokinetic studies.

According to a sixth aspect of the invention the above and other objects are fulfilled by a micro flow system for analysing components of a fluid, comprising a member having a flow channel defined therein for guiding a flow of a fluid through the flow channel, first inlet means for entering particles into the flow channel, first outlet means for discharging of fluid from the flow channel and a plurality of assay sites located in the flow channel and comprising immobilised reagents whereby the fluid may be analyzed for a plurality of components while residing in the flow channel.

The system may further comprise field generating means positioned proximate to at least some of the assay sites for generation of a field proximate to the corresponding assay site whereby reagents residing in the flow channel and being susceptible to the field when generated at a selected assay site are attracted to and immobilised at the selected assay site, or, are rejected from the selected assay site.

In an embodiment of the invention, the member comprises a plurality of flow channels arranged in parallel or in series and each of which has assay sites whereby the fluid containing particles is brought into contact with a large number of assay sites.

According to a seventh aspect of the invention, a method of analysing components of a fluid is provided, comprising the steps of entering a fluid containing the particles into a flow channel and allowing the fluid to flow in the channel, the channel having a plurality of assay sites, each of which comprises immobilised reagents whereby the fluid can be analyzed for a plurality of components while residing in the channel.

According to a eighth aspect of the invention, a method of forming assay sites comprising immobilised reagents in a flow channel is provided, the method comprising the steps of

preparing selected surfaces of the assay sites for immobilisation of selected reagents,

dispensing a selected reagent proximate to a corresponding selected assay site, and

generating a field proximate to the selected site whereby the reagent is attracted towards and brought into contact with the surface of the selected assay site by the field generated and is immobilised upon contact with the surface.

Thus, the micro flow system of the previous section with a flow channel with assay sites may further comprise field generating means positioned proximate to at least some of the areas adapted to comprise immobilised reagents, each field generating means generating a field proximate to the corresponding area whereby reagents entering the flow channel and being susceptible to the field generated at the area are attracted to and immobilised at the area or are rejected from the area. Alternatively, the width of the channel of the micro flow system can be extended to accommodate a two-dimensional grid of areas to comprise immobilised reagents with fields generating means positioned proximate to at least some of these areas. In another embodiment the micro flow system for analysing a sample with a large number of reagents simultaneously may consist of an array comprising a number of parallel channels each with a plurality of areas adapted to comprise immobilised reagents located in the flow channels and further comprising field generating means to generate a field proximate to the areas whereby reagents being susceptible to the field are immobilised at the area. The field generating means may be e.g. permanent magnets, electrodes or electromagnets.

The devices with assay sites enable rapid manipulation, detection, and analysis of macromolecules, particles and cells in biologic or chemical samples in that a plurality of tests can be performed on the same microchip. According to the invention, micro flow systems and molecular biology are combined.

BRIEF DESCRIPTION OF THE DRAWINGS

Exemplary embodiments of the invention will now be described with reference to the accompanying drawings in which

Fig. 1 illustrates the operation of particle separation according to the present invention,

Fig. 2 shows a cross-sectional view of a separation flow channel according to the present invention. (a) shows the main embodiment and (b) shows a cross-sectional view of a separation flow channel for gravitational separation,

Fig. 3 shows a micro flow system with electrodes as field generating means,

Fig. 4 shows a flow diagram of a magnetic particle separation apparatus according to the present invention,

Fig. 5 shows flow diagrams of various embodiments of the present invention. (a)-(d) show embodiments with various numbers of inlets and outlets, and (e) shows an embodiment with an enlarged separation chamber, and (f) shows an embodiment with an enlarged chamber for collecting separated particles,

Fig. 6 illustrates entrapment of magnetic particles in a flow channel,

Fig. 7 shows a flow diagram for optical detection and hydrodynamic separation using a blocking valve,

Fig. 8 shows a flow diagram for optical detection and hydrodynamic separation using syringe pumps,

Fig. 9 shows a flow diagram of two flow channels coupled in parallel (a) and in sequence (b) and (c),

Fig. 10 illustrates the principle of introducing a pre-treatment facility in the member comprising the micro flow system, here further combined with a post-treatment facility or a hydrodynamic separation facility,

Fig. 11 shows a flow channel for magnetophoresis,

Fig. 12 shows a flow channel having a serial array of assay sites equipped with electrodes to immobilise probes,

Fig. 13 shows a flow channel having a serial array of assay sites equipped with magnets to immobilise probes,

Fig. 14 shows a flow channel having a two-dimensional array of assay sites equipped with magnets to immobilise probes,

Fig. 15(a) and (b) shows two devices each comprising a parallel array of micro flow channels each of which contains an assay site,

Fig. 16 illustrates the preparation of a micro flow system,

Fig. 17 shows diagrams from the magnetic separation described in Example 3, and

Fig. 18 is a flow chart illustrating a process for separating fetal cells from a maternal blood sample by combining different separation methods as described in Example 4.

DETAILED DESCRIPTION OF THE DRAWINGS

According to a preferred embodiment of the invention, magnetically stained particles, e.g. cells labelled immunologically with magnetic particles, such as antibody-coupled magnetic beads, are separated from non-magnetic particles, i.e. non-labelled cells, by exposing the particles to a magnetic field generated with a permanent or an electromagnet. Positive or negative selection methods may be employed. By positive cell separation, cells of a specific cell type are separated and isolated from a heterogeneous mixture of cells.

Fig. 1 illustrates the principle of the separation method according to the invention. A micro flow system 1 is shown having three inlet and two outlet ports. The sample 9 containing particles enters the separation flow channel 5 through a central inlet port 2 and is continuously guided through the separation flow channel 5 of the micro flow system 1 by two guiding buffers 10 and 11, each of which enters the separation flow channel through inlet ports 3 and 4, respectively. A field generating means comprising a magnet 8 is located adjacent to the flow channel 5 and generates a magnetic field across the flow channel 5. When the sample 9 containing particles passes the magnetic field, magnetically stained particles 12 are drawn into the guiding buffer 10 and leave the flow channel 5 together with the guiding buffer 10 through the sort outlet 6 while

non-labelled cells 13 which are not influenced by the magnetic force remain in the fluid 9 leaving the flow channel 5 through the waste outlet 7.

Due to the small channel dimensions, the flow is laminar with negligible influence of inertial forces. Mixing of the sample flow and the guiding buffers is not detectable since the contact area is small and the contact time is reduced to a subsecond range. The thickness of the sample flow can be precisely adjusted by variation of the flow rate of the two guiding buffers. This enables the adjustment and optimisation of the magnetic micro flow system for various cell types and sizes. The volume flow of the sample and the two guiding buffers are adjusted so that the particles in the sample are lined up into a single stream of particles.

The magnetic field in the micro flow channel operates as an extremely sensitive filter for magnetic particles, e.g. cells. Cells labelled with superparamagnetic beads (e.g. MACS, Dynal) are magnetised and attracted by the magnetic field whereby the flow of magnetised particles is deflected into the sort outlet. The short residence time of the fluids in the flow channel and the low Reynolds numbers of the flow in the flow channel minimise the influence of gravity compared to the influence of the magnetic force.

Fig. 2 shows a cross-sectional view of two variants of the micro flow system 1 manufactured utilising semiconductor technology. The micro flow system may be manufactured in any suitable material such as polymers, glass, semiconductors, such as silicium, germanium, gallium arsenate, etc., etc.

The first micro flow system (a) shown is a 3-layer sandwich. The central layer 14 is a silicon wafer having a flow channel 5 etched into it. The silicon wafer 14 is covered with a transparent plate 15, such as a glass plate, having a thickness of, e.g., 0.16 mm. Fluids inside the flow channel 5 may be observed through the glass plate 15, e.g. utilising a microscope 16 (detection means). The fluid inlet 2 and outlet 7 are connected to tubings 17, 18, e.g. fused silica capillary or Teflon tubings, for entering fluids into or discharging fluids from the flow channel 5. Buffer inlets 3 and 4 and the outlet 6 for the separated particles are not shown. The bottom plate 19, e.g. made of plastic, facilitates mounting of the tubings 17, 18.

A modified version (b) of the micro channel system for separation was designed with gravitation as the force field, thus sorting particles due to their density and/or diffusion constant, the latter mainly being controlled by the shape and size of the particles. The system is during operation positioned with the flow plane substantially perpendicular to the direction of the force of gravity. As illustrated in Fig. 2(b), this embodiment of a micro flow system 1 has a sample inlet port 2 and an outlet port 7 located above the micro channel 5 and a buffer inlet port 3 and an outlet port 6

located below the micro channel 5. The sample containing particles 9 enters the separation flow channel through inlet port 2, and a guiding buffer 10 enters the separation flow channel 5 through inlet port 3. In this way, two laminated layers of fluid extending along the horizontal plane are created continuously flowing through the separation flow channel 5 of the micro flow system 1. Particles move from the particle containing layer to the guiding buffer layer by sedimentation. When the sample containing particles 9 passes the flow channel 5, particles with certain density and size properties are drawn into the guiding buffer 10 by the gravitational force and leave the flow channel 5 together with the guiding buffer 10 through the outlet port 6 while particles which are less susceptible to the gravitational field remain in the sample 9 leaving the flow channel 5 through the waste outlet 7. The vertical displacement of a specific particle in the sample is given by its density and diffusion constant and the contact time of the sample layer with the guiding buffer layer. The contact time is defined by the total flow rate of the fluids passing through the micro systems 1 and the length of the micro channel 5. The system can be adjusted such that a desirable or appropriate specimen can be withdrawn and separated from the sample flow due to their density and/or diffusion properties by adjusting the volumetric flow rates of the guiding buffer and particle containing sample.

Alternatively, the micro flow system may comprise two further inlet ports for entering a second and a third guiding buffer into the micro channel 5, where the two further inlet ports are positioned above the micro channel, one on each side of the sample inlet port 2. The flow rates of the sample and the second and third guiding buffers may be adjusted so that the particles contained in the sample are lined up in a single line.

Characteristic features of an exemplary embodiment of a micro flow system according to the invention, e.g. as shown in Figs. 1 and 2, is shown in Table 1.

Table 1 Characteristics, micro flow system

Manufacturing method	Material: Silicium Oxide, SiO_2
	Photo-lithography
	Wet-chemical etching
Flow Channel	
Cross sectional area	0.1 - 0.55 mm width x 0.04 - 0.2 mm depth
Length	1.0 - 200 mm
Total flow rate [µl/min]	1 - 200
Flow velocity [mm/min]	15 - 180
Reynolds number	0.1 - 20
Separation time	0.1 sec - 6.0 sec [2 µl/min]

Magnet

Permanent Magnet

Rare Earth Samarium-Germanium 0.5 x 0.5 x 0.2 mm

Electromagnet

Holding Magnet 25 mm 12 V D.C. RS

Fig. 3 shows a micro flow system 1 utilising electrodes 20, 21 to generate an electric field across the flow channel 5. The electrodes 20, 21 may introduce dielectrophoretic or electrophoretic forces utilised for particle separation. For electrophoretic separation to take place, gold electrodes may be positioned at the inside of the walls of the flow channel 5. By applying a voltage across the electrodes, an electrical field is generated substantially perpendicular to a longitudinal axis of the flow channel. The electrical field cause deflection of charged particles or molecules in the flow channel 5 whereby electrically charged particles can be deflected away from the sample containing particles flowing in the micro flow channel and into a guiding buffer also flowing in the flow channel and abutting the sample containing particles in the micro flow channel.

Fig. 4 shows a micro flow apparatus 22 including a micro flow system 1 as shown in Figs. 1 and 2. The micro flow system 1 has two inlets 2, 3 and two outlets 6,7, two syringe pumps 23, 24, two 3-way control valves 25,26 and capillary tubings 27, 28. The capillary tubings 27, 28 are used for interconnecting the two syringe pumps 23, 24 with the inlets 3, 2, respectively, of the micro flow system 1.

Conventional syringe pumps with means, e.g. stepping-motors (not shown), to move the pistons at a predetermined speed have been utilised for generating a continuous flow of the guiding buffer through the inlet tube 27 and a continuous flow of the sample through the inlet tube 28. The system can be operated in a first loading mode where the two 3-way control valves 25, 26 open for flow between the syringe pumps 23, 24 and the buffer reservoir 29 and the sample reservoir 30, respectively, and the syringe pumps 23, 24 are loaded with buffer and sample from the reservoirs 29, 30, respectively. In a consecutive second operational mode the two 3-way valves 25, 26 open for flow between the syringe pumps 23, 24 and the capillary tubing 27 to the buffer inlet 3 and the capillary tubing 28 to the sample inlet 2 of the micro flow system 1, respectively. The syringe pumps are in this second operational mode controlled to generate a predetermined volumetric flow rate through the micro flow system 1.

Fig. 5 illustrates various micro flow systems 31, 32, 33, 34, 35, and 36 having flow channels of different geometries, illustrating different embodiments of the invention. Micro flow systems with two or three inlet ports and two, three or five outlet ports, respectively, are shown in Figs. 5(a)-

(d). The system shown in 5(a) with inlet ports for sample and two guiding buffers, respectively, and sort outlet port and waste outlet port is similar to the system shown in Fig. 1. Figs. 5(b) and (c) show systems with multiple outlet ports, three and five, respectively, whereto particles can be sorted and leave the flow channel through according to their susceptibility to the applied field. A simple system with two inlet and two outlet ports are shown in Fig. 5(d) similar to the one in Fig. 2(b) that is used for gravitational sorting. A micro flow system with a separation channel equipped with a magnet where the width of the separation channel is enlarged before the bifurcation in a sort outlet and a waste outlet is shown in Fig. 5(e). According to the behaviour of liquids in a flow channel, the size of the cross-sectional area occupied by the sample flow is proportional to the width of the separation channel. According to this, the transversal distance between two particles A and B is increased proportional to the increase of the width of the separation channel. A larger distance between particles, which are to be separated, yields a higher selectivity of the mechanical separation. Fig. 5(f) shows a micro flow system where the width of the outlet channel 6 is increased to form a chamber where the sorted particles are collected for further processing or analysis, e.g. detection, staining, destaining or cultivation.

Fig. 6 illustrates a system in which particles are entrapped inside the micro flow channel 5 for a desired period using the electromagnet-equipped apparatus. In this case, the magnetic field is adjusted so that magnetic particles 12 are drawn to the inner wall of the micro flow channel 5 close to the electromagnet 8. Upon removal of the current to the electromagnet 8 the particles 12 are redispersed and are rapidly moved to the sorting outlet port 6. This 2-step sorting procedure is an alternative to the continuous sorting procedure that is particularly useful in sorting of extremely rare events where dilution of the sorted cell fraction could be a problem. The sorting outlet port 6 may be closed when the current to the electromagnet 8 is turned on and is open when the current to the electromagnet 8 is turned off. The figure shows magnetic particles 12 in the process of being withdrawn from a continuous sample flow 9. The magnetic particles 12 are attracted by the magnetic field and withdrawn from the sample flow 9 by precipitation at the inner wall of the micro flow channel 5 proximate to the electromagnet 8. When the current supplied to the electromagnet 8 is turned off, the magnetic particles 12 are released into the flow again. The separation flow channel may not have a sort outlet, instead a buffer may enter the micro flow channel 5 after the sample and the entrapped particles may be released by removing the current supplied to the electromagnet 8.

Fig. 7 illustrates another embodiment of the present invention for separation of particles fulfilling certain criteria from the sample 9 by hydrodynamic force. The apparatus comprises a 2-way valve 40 and a micro flow system having a separation flow channel 5 with three inlets 2, 3, 4, two outlets 6, 7 and a collecting chamber 37. The sample 9 containing particles enters the separation flow channel 5 through a central inlet port 2 and is continuously guided through the separation

flow channel 5 of the micro flow system by two guiding buffers 10 and 11, each of which enters the separation flow channel 5 through inlet ports 3 and 4, correspondingly. The sample 9 is monitored utilising a microscope objective 16. The apparatus has control means 38 for controlling the two-way valve 40. The control means comprises monitoring means having an optical detection means, e.g. a photomultiplier system (PMT), a CCD camera/chip or a photo diode, optically connected to the microscope objective 16. The objective 16 is focused on the measuring volume, which is located inside the flow channel 5. The size of the measuring volume is defined by a pinhole or slit 39 positioned in front of the optical detector and by the magnification of the objective 16. The 2-way valve 40, e.g. a piezoelectric drop-on-demand ink-jet printing valve, is connecting the collecting chamber 37 to the sort outlet 6. The flow restriction of the waste outlet channel 7 is much higher than the flow restriction of the sort outlet channel. This can be achieved by attaching a flow restrictor (not shown) to the waste outlet channel 7. Thus, if the 2-way valve 40 is open the sample 9 containing particles is deflected towards the sort outlet 6. The collecting chamber 37 is used to collect and capture the sorted particles for post-analysis. Other particles continue to flow out through the waste outlet 7.

Particles are physically separated using hydrodynamic forces according to optical measurements on each particle. The photomultiplier (PMT) signal generated when a particle resides in the measuring volume is transmitted to a pulse-height analyser also comprised within the control means 38. A selection circuit provides an activating signal whenever a specific particle exhibits photometric properties of a predetermined type. If the PMT signal for a specific particle indicates that the particle is of a specific type an actuation pulse is produced. The valve 40 opens at the actuation pulse, causing the liquid containing the specific particle to flow through the sort outlet 6 and to be captured inside the collecting chamber 37. The duration of the actuation pulse is made sufficiently long for the desired particle to be transported into the collecting chamber 37. For light excitation several sources can be used, e.g. laser, tungsten lamp, photo diode. For bundling of the light, a fibre optic cable, a photo lens, an objective or a light microscope can be used. Various optical detection methods, e.g. fluorescence, absorbency, can be used.

The micro flow system may be positioned on a movable table so that the micro flow system may be moved into selected positions relative to the microscope whereby an appropriate volume of the micro flow channel may be moved into the measurement volume of the apparatus.

During or after sorting, the captured sample can be analysed again, using e.g. a microscope. When the valve 40 is closed, particles are entrapped inside the collecting chamber 37 and can be observed for a desired period. A desired liquid or reagent for washing, cultivation or staining of particles or cells may be entered into the collecting chamber 37. After the separation process, the

particles may be withdrawn by flushing the collecting chamber 37 with an appropriate buffer entering the micro flow system through one of the inlets 2, 3, 4.

The sorting apparatus was designed to achieve a minimal dilution of the separated sample fraction. Hydrodynamic separation of particles can be performed due to the optical, electrical, magnetic and/or other properties of the particle-containing sample.

An example of an optical and mechanical arrangement of the apparatus based on fluorescence detection is illustrated schematically in Fig. 7. The sample 9, e.g. particle suspension, is guided through the separation channel 5 by two guiding buffers 10, 11 so that the particles contained in the sample 9 are lined up in a single stream flowing in a plane perpendicular to the optical axis of the objective 16. The flow is illuminated with a mercury arc lamp passing excitation filters for e.g. fluorescein measurement. A dicroic mirror reflects the excitation light to the sorting chip via e.g. a 20x microscope objective 16. The fluorescence light emission is collected by the same objective 16 passing a dicroic mirror. Behind the mirror, a slit 39 works as field stop limiting the detection area to a small stripe. Each particle passing the objective 16 is generating a short signal from the photomultiplier that is optically connected to the objective 16. The photomultiplier signal is amplified and transmitted to a peak detector.

The actuation frequency of the valve 40 used in this device is 1500 Hz which corresponds to a minimal actuation time of 0.6 msec.

Fig. 8 illustrates an alternative embodiment of the separation apparatus shown in Fig. 7 with a separation flow channel 5 having three inlets 2, 3, 4 and two outlets 6, 7. The sample containing particles enters the separation flow channel 5 through the centre inlet port 2 and two guiding buffers enters the channel 5 through the other two inlet ports 3, 4, respectively. Flow speed adjustment means comprising stepper motor driven syringe pumps 41, 42 are connected to the two outlet ports 6, 7, respectively. The syringe pumps 41, 42 suck the sample and buffer via inlet 2, 3 and 4, respectively, through the separation flow channel 5. The cells are monitored at the optical axis of the microscope objective 16 and flow to the separation junction. The guiding buffers and the sample containing unselected cells flow out into the waste outlet syringe pump 42. If a specific cell has optical properties causing an actuation pulse, the stepper motor of the pump 41 at the sort outlet is actuated and the stepper motor of the pump 42 at the waste outlet is stopped causing the liquids to flow to the sort outlet 6. The period the pump 41 at the sort outlet is switched on, respectively the pump 42 at the waste outlet is switched off is made sufficiently long to ensure that the desired cell has entered into the collecting chamber 37. When one of or both syringe pumps 41, 42 after some operation time need to be emptied, the 3-way valves 43, 44 are switched from their normal operation position where they open for flow from the separation flow

channel 5 to the syringe pumps 41, 42, respectively, into a position where the 3-way valves 43, 44 open for flow between the syringe pumps 41, 42 and a waste container (not shown) and where the stepper motors driving the syringe pumps 41, 42 are operated in the reverse direction of the normal operation direction to empty the syringe pumps 41, 42.

Fig. 9(a) shows two flow channels 45, 46 operating in parallel. The sample containing particles enters the flow channels 45, 46 through inlet ports 47, 48, respectively. The guiding buffer enters the flow channels through the inlet ports 49, 50, respectively. In the flow channels 45, 46, particles susceptible to the magnetic field generated by magnets 51, 52, respectively, are deflected from the sample containing particles into the corresponding guiding buffer and flow thereafter through the sort outlet 53. The remaining part of the sample leave the flow channels 45, 46 through the waste outlets 54, 55, respectively. Separation is increased by using a plurality of flow channels coupled in parallel.

Fig. 9(b) and (c) shows examples of combinations of micro flow systems for magnetic, hydrodynamic or gravitational separation. In Fig. 9(b), particles are first separated from a sample in a magnetic separation channel, where after the sorted particles are subjected to a hydrodynamic separation due to the optical properties of the particles. Thus, it is possible to analyse and separate particles from a sample based on both optical and magnetic properties of the particles or to another combination of properties or characteristics. In Fig. 9(c), two magnetic separation channels are coupled in series in order to obtain a highly purified product.

Fig. 10 illustrates examples of micro flow systems having means for automated labelling of particles with fluorescence or magnetic probes. The system may be combined with post-treatment means for removal of the probes or for other treatment of the sorted particles. The system contains a micro flow system containing channels 56, 57 for addition of liquids to the sample, e.g. reagents for cell lysis or staining, a channel 58 for incubation and cultivation or storage of the sample for further processing and a separation channel 5. A sample is introduced into the micro flow system via an inlet 2 and one or more reagents can be added continuously to the sample, which is transported into the incubation channel 58. A simple micro flow structure was constructed for sample pre-treatment. Preferably, the flow rates are managed by computer-controlled syringe pumps. The incubation period between mixing and analysis of the sample is given by the volumetric flow rate of the syringe pumps and the cross-sectional area and length of the incubation channel.

Fig. 11 shows a micro flow system for magnetic separation of macromolecules, i.e. ribonucleic acid or proteins from a sample. Magnetic beads labelled with a fluorescence dye and a probe, specific for i.e. DNA are added to the sample which is then incubated. This sample is entered via inlet

port 2 into the separation chamber 5 and the particles are drawn by the field generated by the magnet 8 along the separation channel 5 due to their mobility. After a defined period, the magnetic field is removed and the fluorescence banding can be observed under a microscope. By running standards of known size, it is possible to calibrate the system and to separate particles of e.g. DNA due to their size and shape, similar to electrophoresis.

Fig. 12(a) with details in Fig. 12(b) shows a serial sensor array. A micro flow channel 5 has a plurality of assay sites, each equipped with field generating means 59 that may be individually turned on and off. The flow channel 5 shown has rectangular electrodes 59 positioned in small grooves at the bottom wall of the flow channel 5. A voltage can be applied selectively to each electrode 59. Various probes, receptors, indicators, etc. may be attracted to and immobilised at selected electrodes 59 by applying a voltage to the selected electrodes 59 while a fluid containing the corresponding probes, receptors, indicators, etc. resides in the flow channel 5. Preparation of the multiple assay sites may be accomplished by sequentially loading each assay site with a specific probe. Voltage is applied to one or more specific electrodes in the micro flow channel 5, and a fluid containing a specific probe, reagent or indicator, etc. is entered into the micro flow channel 5 where the probes etc. will be attracted to the electrodes to 59 which the voltage is applied. Subsequently, the voltage is turned off. Then, a voltage is applied to the next electrode 59 and the next fluid containing a specific probe etc. is entered into the micro flow channel 5. Thus, various assay sites each containing a specific probe, reagent, or indicator can be created. Antibodies, fluorescence molecules, DNA, RNA and protein dyes are examples of probes.

As an alternative to the electrodes 59, magnetic force can be selectively applied to the assay sites with an array of electromagnets positioned at or near the surface of the micro flow channel 5 to immobilise probes etc. that have magnetic properties to desired assay sites. Alternatively, a photoactivation process can be used for covalent coupling of molecules or particles to the surface of the channel 5 at the assay site.

One example of a probe is DNA, which has an overall negative charge, drawn to the electrode 59 surface by a positive bias, another example is DNA-coated magnetic particles that are drawn to the surface of micro flow channel 5 by magnetic means.

By modification or coating of the surface of the micro flow 5 channel and/or the electrodes 59 or magnets, specific chemical and mechanical properties can be created. To increase the binding forces of the probe, the surface may be coated with a specific layer or matrix, e.g. a polymer such as urethane or a reactive chemical group. When the current to the selected electrode or electromagnet is switched off the probe remains on the surface e.g. by absorption. Thus, an encapsulation or immobilization of the molecule is achieved.

Thus, a field generated at selected assay sites across the flow channel 5 may be utilized for immobilization of particles, such as biomolecules, whereby these may be held in substantially fixed positions within the flow channel permanently or for a specific period of time allowing chemical reactions between the particles and an entered reagent to take place and/or kinetic measurements on the particles to be performed and/or the particles to be brought into contact with different chemical substances. For analysis of the reactions in the micro flow system, optical detection means, e.g. a microscope, may be used.

It is an important advantage of the device that a number of assays can be performed in a single device. During operation of the device, various processing steps, such as e.g. washing steps, and reagent addition, etc., may be performed.

Fig. 13 shows a micro flow device with a flow channel 5 and with a serial array of assay sites and permanent magnets 8 positioned on a separate cartridge 60. A second cartridge 61 has a flow channel 5 with an inlet 2 and an outlet 7. The cartridge 60 carrying the magnets 8 can be positioned exactly below the second cartridge 61 so the magnets 8 are accurately positioned below the assay sites in the flow channel 5 as shown in the figure below cartridge 61.

Probes to be immobilised at a specific assay site utilising a magnetic field, as described in Fig. 12 or Fig. 13, may be positioned at the desired assay site by a method comprising the steps of positioning a defined volume of the liquid containing the magnetic probe or reagent using e.g. inkjet based dispenser technology, within a specific volume of the flow channel 5 right over one of the permanent magnets 8 for immobilization of the magnetic probe or reagent in an assay site at the surface of the flow channel 5. The method may be repeated for various probes to be immobilised at various assay sites, respectively. After the immobilization, the cartridge 61 containing the flow channel 5 is covered by a transparent cartridge 15, e.g. a glass plate, allowing the assay site array with the probes inside the micro flow channel 5 to be observed. An analysis with the assay site array is performed by introducing a sample through inlet 2 into the micro flow channel 5 where it passes the array of assay sites and leaves the micro flow channel 5 through outlet 7. An objective 16, optically connected to an optical detector, e.g. a fluorescence microscope, may be focused on the array in the micro flow channel 5 to monitor the chemical reactions at the assay sites.

Fig. 14 shows another embodiment of the invention comprising a cartridge 62 with a micro flow channel containing assay sites 63 arranged in a two-dimensional array, a cartridge 64 with permanent magnets 8 and a transparent cartridge (not shown) to cover the cartridge 62 with the micro flow channel. The assay sites 63 are formed as small grooves at the surface of the bottom

wall of the micro flow channel. The dimensions of the cartridges 62, 64 and the position of the assay sites 63 and the magnets 8 are the same, so if cartridge 62 is placed over cartridge 64 as shown in Fig. 14(b), the magnets 8 are located under the assay sites 63.

The embodiments shown in Fig. 12, 13 and 14 may be utilised for hybridisation of DNA as described below. A magnetic carrier including a DNA probe may be immobilised at a specific assay site as described previously. In this way, an array of assay sites is created in a micro flow channel wherein each assay site contains a different DNA probe. Thereafter, a sample containing target molecules is entered into the micro flow channel preferably until the sample has filled the micro flow channel. After the target molecule has been hybridised to a DNA of a specific assay site, a solution of reporter probes, e.g. fluorescence probes, is entered into the flow channel where it binds on the assay site carrying the hybridised DNA. By using a fluorescence detector, e.g. a photomultiplier, focused on the different assay sites the reaction at each assay site can be monitored. By removing the magnetic field on a specific assay site the magnetic material including the DNA probe can be removed, so the process can be reversed. Thus a renewable array of assay sites can be created and wash processes can be implemented in the operation of the array of assay sites.

Fig. 15(a) shows a device according to the invention for performing a multiple assay analysis in a micro flow system by using a parallel array of assay sites. The system comprises an array of parallel micro flow channels 65 each of which contains one assay site with a specific probe immobilised using e.g. an electrical or magnetic field or by photoactivation as described previously. For example, a cartridge containing a permanent magnet (not shown) can be positioned below the parallel micro flow channels 65 in whereby magnetic probes can be immobilized in the micro flow system using dispensing technology. In this way, a plurality of assay sites may be created in the parallel flow channel 65 array allowing a simultaneous analysis of a sample with a plurality of probes or reagents defined by the number of parallel micro flow channels 65.

The micro flow system consists of two parallel flow channels 66, 67 which are connected via a number of parallel micro flow channels 65 each containing an assay site. An injection flow channel 66 has an inlet 2 and is connected to an outlet 68 via a blocking valve 69, and the waste flow channel 67 is connected to a waste outlet 70 via a blocking valve 71. By blocking one of the two outlets 68, 70 with the blocking valves 69, 71, respectively, it is possible to guide the injected flow through the array of channels 65 containing assay sites or through the injection channel outlet 68, respectively. The flow through all channels 65 containing assay sites is merged into the waste channel 67 and is leaving the system via the waste outlet 70. During passage of the

channels 65 containing the assay sites, the sample comes into contact with sensing probes, which are immobilized at the assay sites. Chemical reactions may be detected as described for Fig. 12.

In Fig. 15(b) an alternative embodiment of an array of parallel channels 65 containing assay sites is shown. The micro flow system has three inlet ports 2, 3, 4 to enter different liquids into the micro flow system. By connecting outlet port 68 to a flow restrictor, only one blocking valve 71 is needed to operate the system. If the blocking valve 71 between the channel array 65 and the waste outlet 70 is closed, the channel array 65 is blocked and the flow from inlet 2, 3, 4 will pass the injection channel 66 and leave the micro flow system via outlet 68. When the blocking valve 71 is open, the liquid introduced in the injection channel 66 will flow into the sensor channel array 65 because of the higher flow restriction at outlet 68 compared to waste outlet 70.

Fig. 16 shows a micro flow system manufactured as a 3-layer sandwich. The central layer is a silicon wafer having a flow channel etched into it. The silicon wafer is covered with a transparent plate, such as a glass plate, having a thickness of, e.g., 0.16 mm. Fluids inside the flow channel may be monitored through the glass plate, e.g. utilising a microscope or other optical detection means. The fluid inlet and outlet are connected to tubings, e.g. fused silica capillary or Teflon tubings, for entering fluids into or discharging fluids from the flow channel. Buffer inlets and the outlet for the sorted particles are not shown. The bottom plate, e.g. made of plastic, facilitates mounting of the tubings.

Figs. 16(1) to (5) illustrates the following description of the manufacturing and preparation of a micro flow system. A separation flow channel was designed to fit into a system comprising a bonded silicon/glass sandwich. The micro channels were etched into a silicon wafer covered with a boron glass plate having a thickness of 0.2 mm allowing monitoring of the micro channels, using i.e. a microscope. The separation flow channel was fabricated on a 4", 350μm, <100> silicon wafer. A 1.5μm layer of SiO_2 was applied to the surface of the silicon wafer and was patterned with a mask containing the channel layout. A 2.6μm layer of photoresist was spun on top of the SiO_2 and patterned with a mask defining intermediate holes. The two-step mask consisting of a SiO_2 mask and a photoresist mask was used for etching a two level structure with vertical walls by reactive ion etching (RIE) in a SF_6:O_2 plasma. The holes were initially etched to a depth of 22μm and then etched deeper together with the channels, which were etched to depths in the range from 40μm to 100μm. A layer of 1.8μm SiO_2 was patterned with a mask for inlets and outlets on the back of the silicon wafer. The etching was carried out in KOH at 80°C and was stopped when all the intermediate holes were clearly visible from the back. Finally, a glass wafer was anodically bonded to the silicon wafer. The micro channels were designed for volumetric flow rates of 0.1 to 200 μl/min with a mean flow speed of maximum 100 (mm/min).

The separation flow channel may be provided with one or two permanent or electromagnets in three different ways:

(a) Rare earth Samarium-Cobalt block magnets of 1.0 x 1.0 x 0.5 mm (Goudsmit, Netherlands) may be glued with silicon rubber into the opening slot of the separation flow channel.

(b) Rare earth (Sr) magnetic powder (Tropag, Hamburg, Germany) can be mixed with epoxy 1:1 (v/v) and this magnetic paste may be glued into the opening slot of the separation flow channel yielding a thick film magnetic layer of 1.0 x1.0 x 0.5 mm.

(c) Ferrite steel wool may be glued with silicon rubber into the opening slot of the separation flow channel. A high magnetic field gradient can then be induced inside the opening slots by applying an external magnetic field, e.g. by an electromagnet (Goudsmit, Netherlands) positioned proximate to the separation flow channel.

EXAMPLE 1

A micro flow system with a layout as sketched in Fig. 5(d) with two inlets and two outlets has been tested utilising it for separation of various magnetisable particles. The test conditions are listed below.

Particle concentration	10^7 particles/ml
Total flow rate	25 µl/min
Length flow chip	3.5 mm
Channel width	250 µm
Channel depth	60 µm
Separation time	2.4 sec
Desired particle deflection:	10 µm

The separation efficiency (enrichment rate) E and depletion rate 1/E are defined by

$$E = \frac{\dfrac{\%\ positive\ particles\ after\ separation}{\%\ negative\ particles\ after\ separation}}{\dfrac{\%\ positive\ particles\ before\ separation}{\%\ negative\ particles\ before\ separation}}$$

For separation of various paramagnetic standard beads of different sizes and paramagnetic field strength, the results are shown in the Table 2.

Table 2 Separation efficiencies

Paramagnetic Bead	Size µm	Separation Efficiency [%][1] A)	B)	C)
Polysciences				
25 % iron-oxide	1-10	>99	>99	95
50 % iron-oxide	1-10	>99	>99	96.5
Paesel + Lorei				
Magnetic Affinity	0.5-1.5	>99	>99	97.5
Boehringer				
Streptavidin Magnetic	1	90.5	88.7	89.5
Dynal				
Magnetic Mass Dyal M-450	1-10	98.0	>99	96.5

[1] total flow rates: A)= 10 µl/min, B)= 50 µl/min, C)= 100 µl/min

EXAMPLE 2

Further, the micro flow system used in Example 1 has also been tested by utilising it for separation of Human T-lymphocytes (JURKAT cells). Magnetically stained and unstained JURKAT cells were used to form a heterogeneous cell sample. For magnetic staining of the cells, a CD4-magnetic surface marker from Miltenyi Biotech was used. JURKAT cells were suspended in 1% PBS/BSA to a concentration of 10^7/ml. Biotin-conjugated CD4 magnetic microbeads were added at 2 µl stock/10^7 cells following the manufacturer instruction.

The magnetically stained cells (10^7 cells/ml) flowed through the microchip for 10 min. and fluids were collected at the two outlets. Three experiments at different flow rates (5, 25, 50µl/min) were performed. The same experiments were performed using magnetically unstained cells.

An aliquot of the collected samples was analysed under a microscope and the particles were counted using a Neubauer microscopy chamber. For each experiment 1 µl sample was analysed:

Run	flow rate [µl/min]	cells [%] at Sort outlet
Negative (unstained cells)		
	5	<0.1
	25	<0.1
	50	<0.1
Control[1]		
	5	n.n.
	25	n.n.
	50	n.n.
Positive (stained cells)		
	5	95.5
	25	92.8
	50	80.5
Control[1]		
	5	n.n.
	25	n.n
	50	n.n.

[1] using the micro flow system without an integrated magnet

EXAMPLE 3

The system employed for separation of magnetisable particles from a sample is shown in Fig. 4. It comprises two syringe infusion pumps (Harvard Apparatus, Southnatik, Az) that provides constant flow rates of 0.1 to 100 µl/min using a 0.5 ml micro syringe (Hamilton, Bonaduz, Switzerland), a separation flow channel of silicon for the separation of the magnetisable particles, and a collecting unit for collecting of the sorted particles. Two 3-way microvalves (Lee, Parameter AB, Sweden) were integrated into the apparatus for sterile solution handling. All components were interconnected with fused silica capillaries (340 µm id., Supelco, U.S.A.). The SFC was placed under an inverted microscope (Axiovert 100, Zeiss, Germany) for visualisation of the separation procedure. All micro channels and tubing were deactivated by silanisation as described in Blankenstein, G. Scampavia L, Branebjerg J, Larsen UD, Ruzicka J (1996): Flow switch for analyte injection and cell/particle sorting in Analytical Methods and Instrumentation, µTAS '96 conference, 17-22 November 1996, Basel. A FACScan with 488 nm argon laser excitation and collection of forward and side scatter and fluorescence of fluorescein were used (Becton Dickinson,

Mountain View, CA) for all experiments. Results were collected and analysed using the FACScan research software (Becton Dickinson).

Results on the use of a separation flow channel equipped with a permanent magnet optimised for Dynal beads are shown in Fig. 17. A bead suspension of 1-5 x 10^8 particles/ml containing a mixture of non labelled magnetic Dynal particles (d: 4.5 µm, M-450) and fluorescence calibration beads (d: 3.2 µm, Dako A/S, Glostrup, Denmark) have been separated. About 1 ml of the non-magnetic, non-deflected fraction was collected at the waste outlet and analysed by flow cytometry (B). To enumerate the positive and negative fractions, two windows were set for the statistic evaluation. Before separation, the sample contained 38.3 % fluorescence particles and 55.8 % magnetic particles, respectively (a). After sorting by the described system almost all magnetic particles were found in the sorted fraction collected from the sort outlet (b) and non-magnetic particles were found in the negative fraction (c) collected from the waste outlet, respectively. Under optimised conditions, an enrichment rate of 350 was achievable.

EXAMPLE 4

This example concerns enrichment of fetal cells in a sample for magnetic activated cell sorting. A combination of the embodiment of the invention as shown in Figs. 7 and 10 (upper), optical cytometry, and Figs. 4 and 10 (lower), magnetic cell separation, provides a powerful apparatus for efficient enrichment of fetal cells in a sample.

The process for increasing the concentration of fetal cell in maternal blood samples involves the following steps (see Fig. 18): (i) A first selection step for removal of the majority of the maternal blood cells based upon their volume, size and density; (ii) A second sorting step for isolation of the fetal blood cells from the remaining maternal blood cells based on immuno-fluorescent separation using a device as described in Fig. 7 and/or based on immuno-magnetic separation using a device as described in Fig. 4. In the examples shown in Fig. 9(b), the magnetic blood sample is first separated in a magnetic separation chamber, followed by a separation due to optical properties of the sample, or two magnetic separations are performed one after the other, see Fig. 9(c), in order to obtain a highly purified product.

An example of sorting of particles of very low concentration from a sample of maternal blood in a non-invasive prenatal screening test is presented in the following paragraph.

Nucleated red blood cells are found in maternal blood in a concentration of 10 to 1000 per ml of all nucleated cells. Bianchi has shown (D.W. Bianchi, Journal of Pediatrics, 1995, 127, 6, p. 847-856) that it is possible to use such cells for genetic screening in prenatal diagnosis. The cell surface

marker CD71+ for example, is an appropriate marker to select such cells from maternal blood. Test results demonstrates that magnetic activated cell sorting is powerful enrichment system for sorting and isolating fetal nucleated blood cells from maternal blood. For this the magnetic activated cell micro technology as described in this invention is used. Fetal cells are distinguished and separated from maternal blood by the use of a specific surface marker (CD71) which is only present on the cell membrane of fetal nucleated blood cells. By selectively attaching a magnetic antibody probe to CD71, a magnetic probe is attached substantially exclusively to fetal cells.

EXAMPLE 5

This example concerns depletion of magnetically labelled CD45 positive cells (maternal leukocytes) from a maternal blood sample spiked with cord blood. A flow chip described in Fig. 1 was used in a system as described in Fig. 4. In this experiment a 1:3 spike (fetal/maternal, v/v) was used to demonstrate the performance of the magnetic separation. Heparin was used as an anti-coagulant. The nucleated cells were labelled with CD45 coated magnetic 0.1 μ micro particles (Immunicom, U.S.A.), using a monoclonal antibody against CD45 as the first layer. The cell suspension was collected at both outlets 6 and 7 (see Fig. 1). For testing the sorting efficiency, parts of both the collected fractions were analysed on microscope slides. The results showed that most of the cells, more than 95%, collected at the sort outlet 6 were CD45 positive.

EXAMPLE 6

Fluorescence activated cell sorting using the device described in Fig. 7. First results have shown an enrichment factor of more than 300, which indicates that the employed device is a powerful tool for enrichment of rare cellular events.

EXAMPLE 7

An example is given for the embodiment of the invention as described in Fig. 12 for the use of multiple sensor array technology for sensing of a group of analytes in one step. For this purpose, the biosensing components such as antigens or antibodies can be loaded into a specific assay of the flow channel and immobilised there.

Magnetic particles carrying an antigen probe are immobilised on the surface of the micro flow channel by magnetic means. The immobilisation of each probe is exactly specified to a site by switching on a specific electro-magnet.

After loading the surface with different groups of antigen probes, the test solution is guided through the flow channel 5 of the microchip. If the sample contains an antibody, which is complementary to one of the different antibodies, it will bind to that specific site where this antibody is immobilised. In a third step, the sample solution has to be removed, and a liquid containing a secondary antibody against the FC region of the first antibody is guided through the micro flow channel. The secondary antibody is coupled to a fluorescence dye allowing the identification of a specific assay site whereto the antibodies has been binded. The device can be used for rapid screening of blood samples, e.g. for identification of bacteria or virus in blood samples having a micro flow channel with virus/bacteria specific antigen probes.

CLAIMS

1. A micro flow system for separating particles, comprising a member having

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

first inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

first outlet means positioned at the other end of the flow channel for discharging the fluid from the flow channel,

the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross-section of the flow channel,

the member being positioned in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field.

2. A micro flow system according to claim 1, wherein the member further comprises field generating means positioned proximate to the flow channel for generating a field substantially perpendicular to a longitudinal axis of the flow channel.

3. A micro flow system according to claim 1 or 2, further comprising monitoring means positioned at the flow channel for monitoring parameters of a particle residing within a measurement volume within the flow channel and providing an output signal corresponding to a monitored parameter.

4. A micro flow system according to claim 3, wherein the monitoring means comprise optical detection means for monitoring optical parameters of a particle residing within a measurement volume within the flow channel and providing an output signal corresponding to an optical parameter.

5. A micro flow system according to claim 3 or 4, wherein the monitoring means comprise a Hall sensor for measurement of a magnetic parameter of a magnetic particle within a specific volume of the flow channel.

6. A micro flow system according to any of claims 3-5, further comprising field generating control means for controlling the strength of the field generated by the field generating means in response to the output signal of the monitoring means whereby particles are separated according to values of a parameter monitored by the monitoring means.

7. A micro flow system according to any of the preceding claims, wherein the Reynolds number of the flow of the fluid containing the particles through the channel is in the range of 0.01-500, preferably in the range of 0.05-50, in particular in the range of 0.1-25.

8. A micro flow system according to any of claims 1-6, wherein the lowest cross-sectional area of the flow channel is in the range of 0.004-0.11 mm^2.

9. A micro flow system according to any of the preceding claims, further comprising second outlet means for discharging particles having been deflected in the flow channel.

10. A micro flow system according to any of the preceding claims, wherein the field generating means comprises a magnet.

11. A micro flow system accoding to claim 10, wherein the field generating means further comprise ferrite members positioned at the flow channel for focussing of a magnetic field.

12. A micro flow system according to any of the preceding claims, wherein the field generating means comprises an electrode.

13. A micro flow system according to any of the preceding claims, wherein positions in relation to the flow channel of the field generating means are adjustable for adjustment of the strength of the field across the flow channel.

14. A micro flow system according to any of the preceding claims, further comprising flow speed adjustment means for adjustment of the time the particles reside in the flow channel.

15. A micro flow system according to any of the preceding claims, further comprising a cover for covering the flow channel.

16. A micro flow system according to claim 15, wherein the cover is a transparent or translucent cover allowing optical monitoring of the flow channel.

17. A micro flow system according to any of the preceding claims, further comprising second inlet means for entering a first guiding buffer, the cross-section and the path through the flow channel of the flow of the fluid containing particles being controlled by the first guiding buffer flow.

18. A micro flow system according to claim 17, further comprising third inlet means for entering a second guiding buffer, the cross-section and the path through the flow channel of the flow of the fluid containing particles being controlled by the first and second guiding buffer flows that surround the flow of the fluid containing particles.

19. A micro flow system according to claim 18, wherein the width and the position of the flow of fluid containing particles is controlled by adjustment of the volumetric ratio between the fluid flow rate and the flow rate of the guiding buffers.

20. A micro flow system according to any of the preceding claims, wherein the deflected particles comprise living cells.

21. A micro flow system according to any of the preceding claims, wherein the deflected particles comprise beads, microspheres, chromosomes, organelles, biomolecules, or proteins.

22. A micro flow system according to any of the preceding claims, wherein the deflected particles have been magnetically, chromophorically, or fluorescently stained.

23. A micro flow system according to any of the preceding claims, comprising a plurality of outlets for discharging of particles separated according to their different susceptibility to the field across the flow channel.

24. A micro flow system according to any of the preceding claims, wherein the member further comprises pre-treatment and/or post-treatment facilities.

25. A micro flow system according to claim 24, wherein the pre-treatment facilities comprise incubation means for preparing or pre-reacting the fluid comprising the particles.

26. A micro flow system according to claim 24 or 25, wherein the pre-treatment facilities comprise means for magnetic, fluorescent, or chromophoric staining.

27. A micro flow system according to claim 24, wherein the post-treatment facilities comprise means for collecting or concentrating the deflected particles.

28. A micro flow system according to claim 24, wherein the post-treatment facilities comprise means for bringing the deflected particles into contact with one or more reagent(s).

29. A micro flow system for separating particles, comprising a member having

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

first inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

first and second outlet means positioned at the other end of the flow channel for discharging of fluid from the flow channel,

the flow of the fluid containing the particles being controlled in such a way that one particle at the time passes a cross-section of the flow channel,

monitoring means positioned at the flow channel for monitoring parameters of a particle residing within a measurement volume within the flow channel and providing an output signal corresponding to a monitored parameter,

control means for controlling passage of fluid through the first and the second outlet means, respectively, in response to the output signal of the monitoring means whereby particles may be separated according to values of a parameter monitored by the monitoring means.

30. A micro flow system for separating particles, comprising a member having

a flow channel defined therein for guiding a flow of a fluid containing the particles through the flow channel,

inlet means positioned at one end of the flow channel for entering the fluid into the flow channel,

field generating means positioned proximate to the other end of the flow channel for generating a field substantially along a longitudinal axis of the flow channel whereby the particles are drawn by the field along the channel and distributed according to their susceptibility to the field and their mobility.

31. A micro flow system for analysing components of a fluid, comprising a member having a flow channel defined therein for guiding a flow of a fluid through the flow channel, first inlet means for entering particles into the flow channel, first outlet means for discharging of fluid from the flow channel and a plurality of assay sites located in the flow channel and comprising immobilised reagents whereby the fluid may be analyzed for a plurality of components while residing in the flow channel.

32. A micro flow system according to claim 31, further comprising field generating means positioned proximate to at least some of the assay sites for generation of a field proximate to the corresponding assay site whereby reagents residing in the flow channel and being susceptible to the field when generated at a selected assay site are attracted to and immobilised at the selected assay site, or, are rejected from the selected assay site.

33. A micro flow system according to claim 31 or 32, wherein the member comprises a plurality of flow channels arranged in parallel or in series and each of which has assay sites whereby the fluid containing particles is brought into contact with a large number of assay sites.

34. A method of separating particles, comprising the steps of

guiding a flow of a fluid containing the particles through a flow channel in such a way that one particle at the time passes a cross-section of the flow channel,

positioning the flow channel in a field that is substantially perpendicular to a longitudinal axis of the flow channel so that particles residing in the flow channel and being susceptible to the field across the flow channel are deflected in the direction of the field and thereby separated from the fluid.

35. A method of separating fetal cells from maternal cells, comprising the steps of

selective magnetically staining of fetal cells in a fluid containing fetal and maternal cells,

guiding a flow of the fluid containing the fetal cells through a flow channel in such a way that one fetal cell at the time passes a cross-section of the flow channel,

positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained fetal cells residing in the flow channel are deflected in the direction of the magnetic field.

36. A method of separating cancer cells from other cells, comprising the steps of

selective magnetically staining of cancer cells in a fluid containing cancer and other cells,

guiding a flow of the fluid containing the cancer cells through a flow channel in such a way that one cancer cell at the time passes a cross-section of the flow channel,

positioning the flow channel in a magnetic field that is substantially perpendicular to a longitudinal axis of the flow channel so that magnetically stained cancer cells residing in the flow channel are deflected in the direction of the magnetic field.

37. A method of separating particles, comprising the steps of

guiding a flow of a fluid containing the particles through a flow channel in such a way that one particle at the time passes a cross-section of the flow channel, the flow channel having first and second outlet means for discharging of fluid from the flow channel,

monitoring parameters of a particle residing within a measurement volume within the flow channel and

controlling passage of fluid through the first and the second outlet means, respectively, in response to a monitored parameter value whereby particles may be separated according to values of a monitored parameter.

38. A method of analysing components of a fluid, comprising the steps of entering a fluid containing the particles into a flow channel and allowing the fluid to flow in the channel, the channel having a plurality of assay sites, each of which comprises immobilised reagents whereby the fluid can be analyzed for a plurality of components while residing in the channel.

39. A method of forming assay sites comprising immobilised reagents in a flow channel, comprising the steps of

preparing selected surfaces of the assay sites for immobilisation of selected reagents,

dispensing a selected reagent proximate to a corresponding selected assay site, and

generating a field proximate to the selected site whereby the reagent is attracted towards and brought into contact with the surface of the selected assay site by the field generated and is immobilised upon contact with the surface.

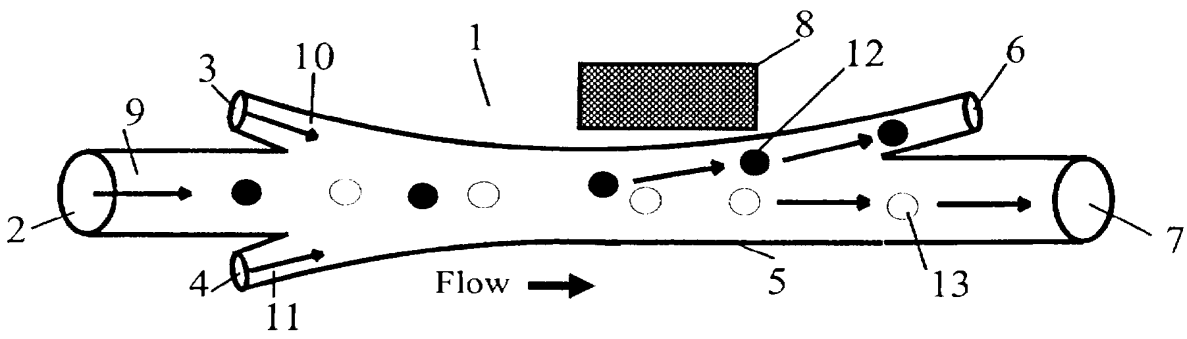


Fig. 1

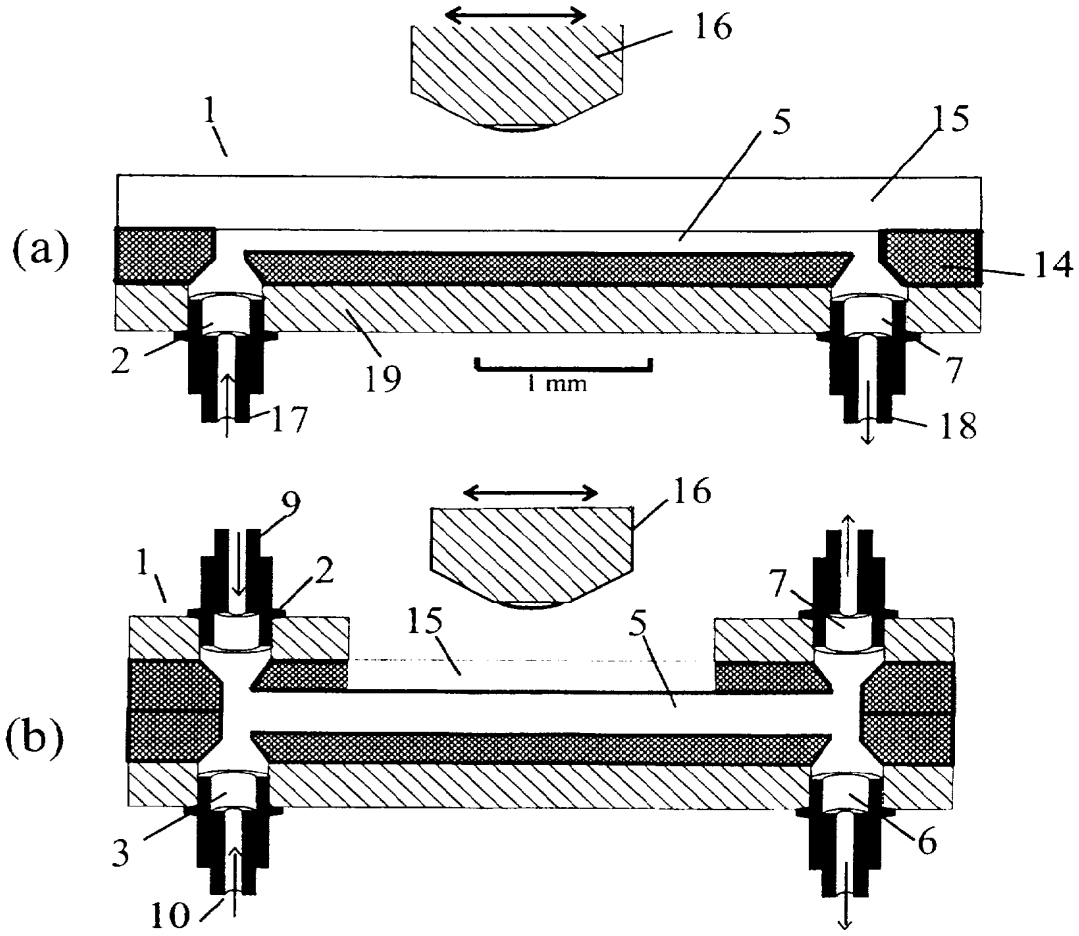


Fig. 2

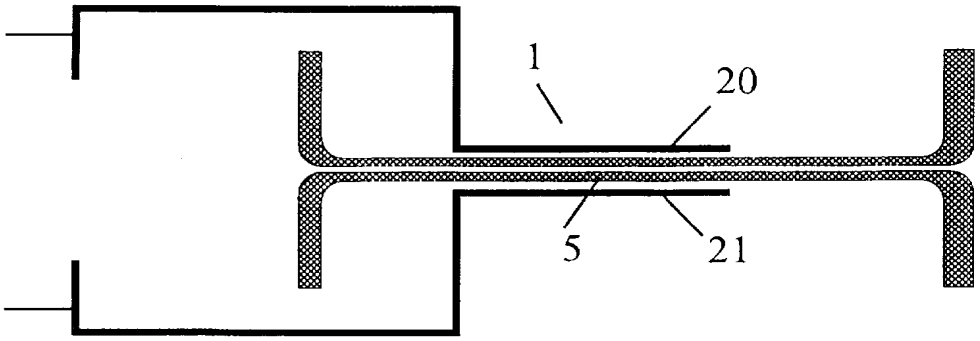
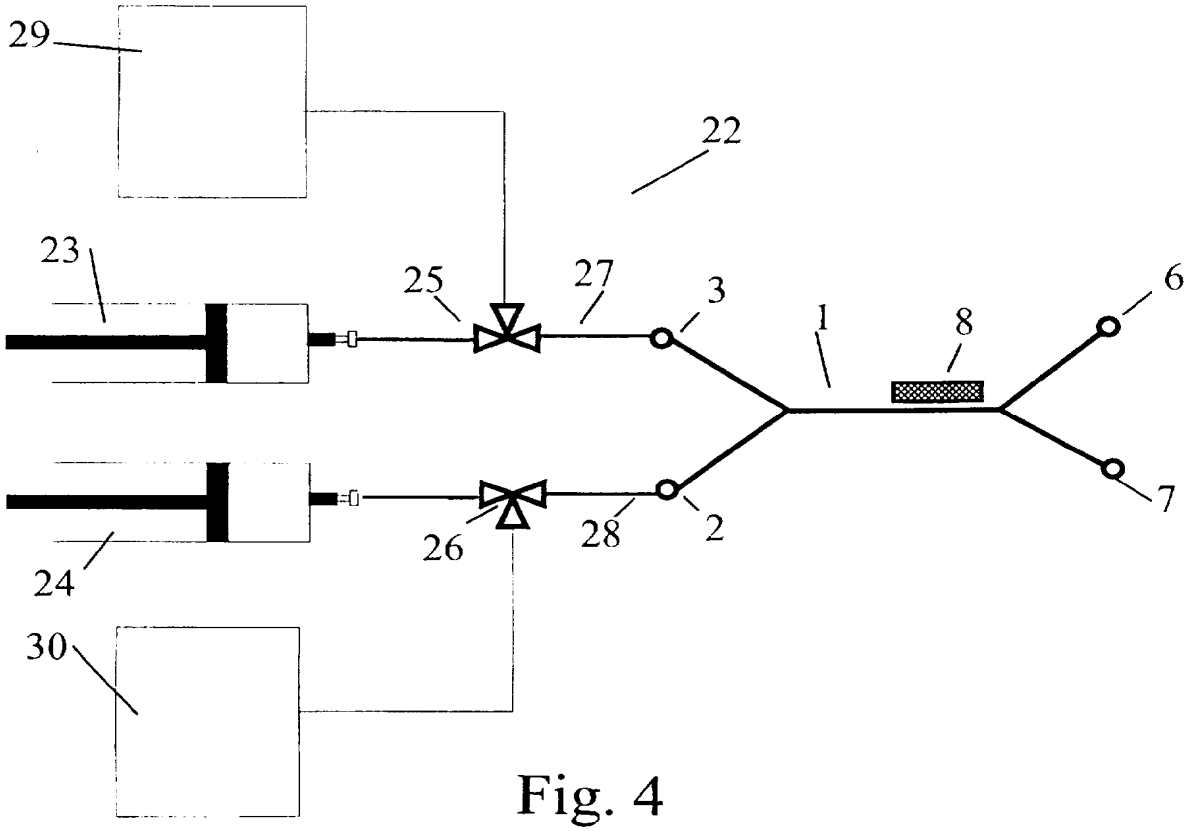


Fig. 3



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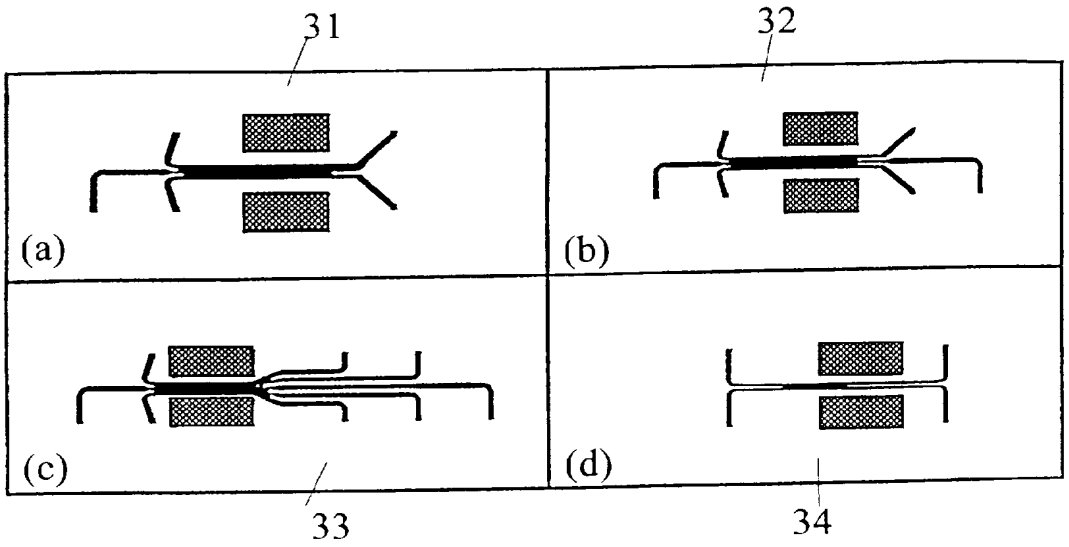
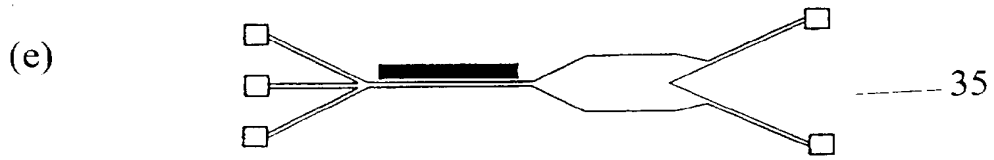




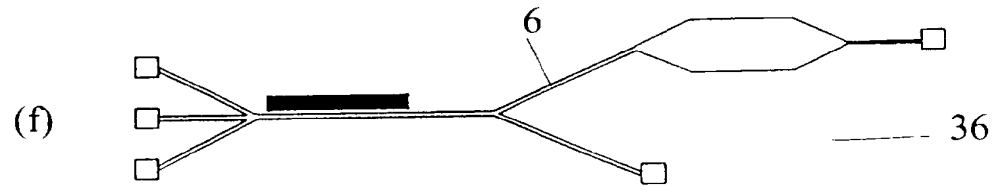
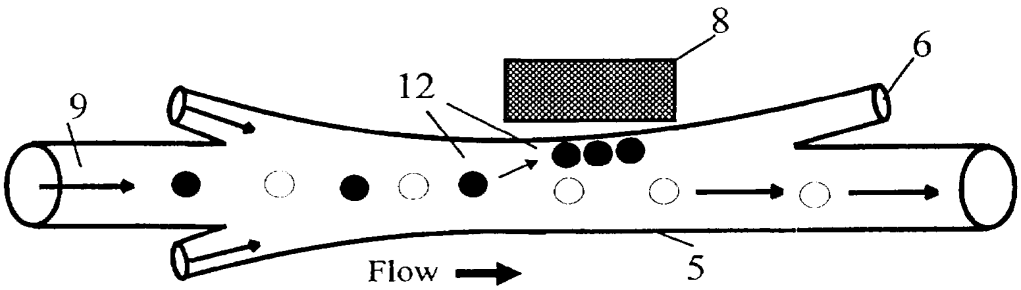


Fig. 5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6
12
9
Flow
5

Fig. 6

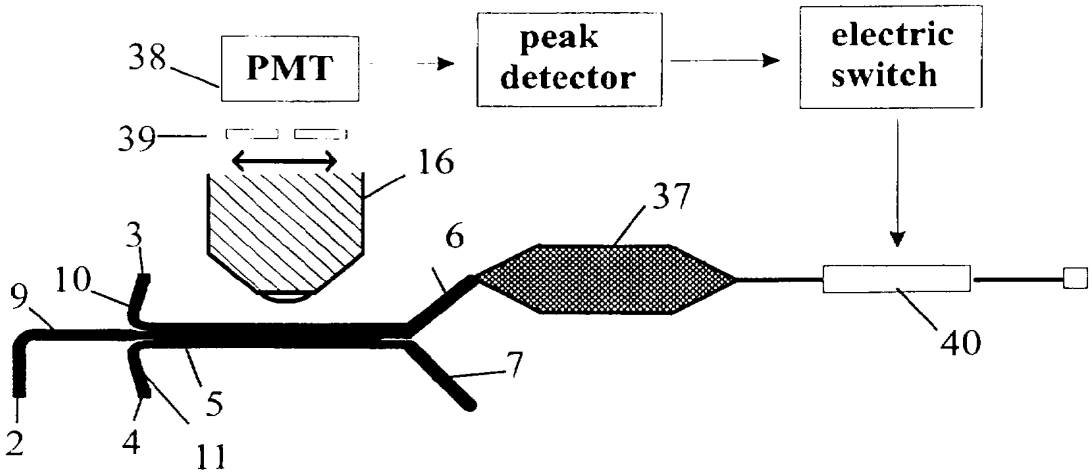
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Fig. 7

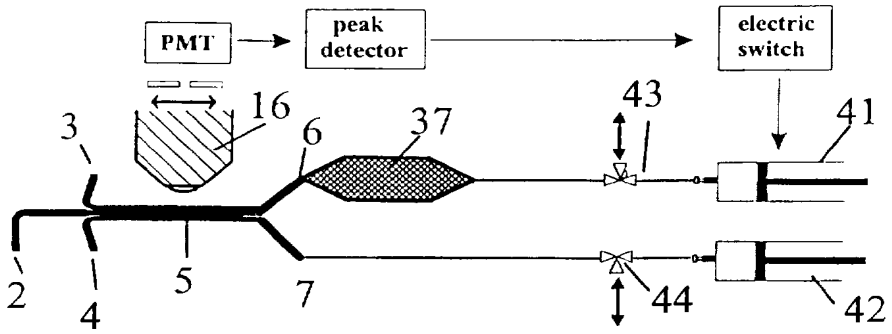
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Fig. 8

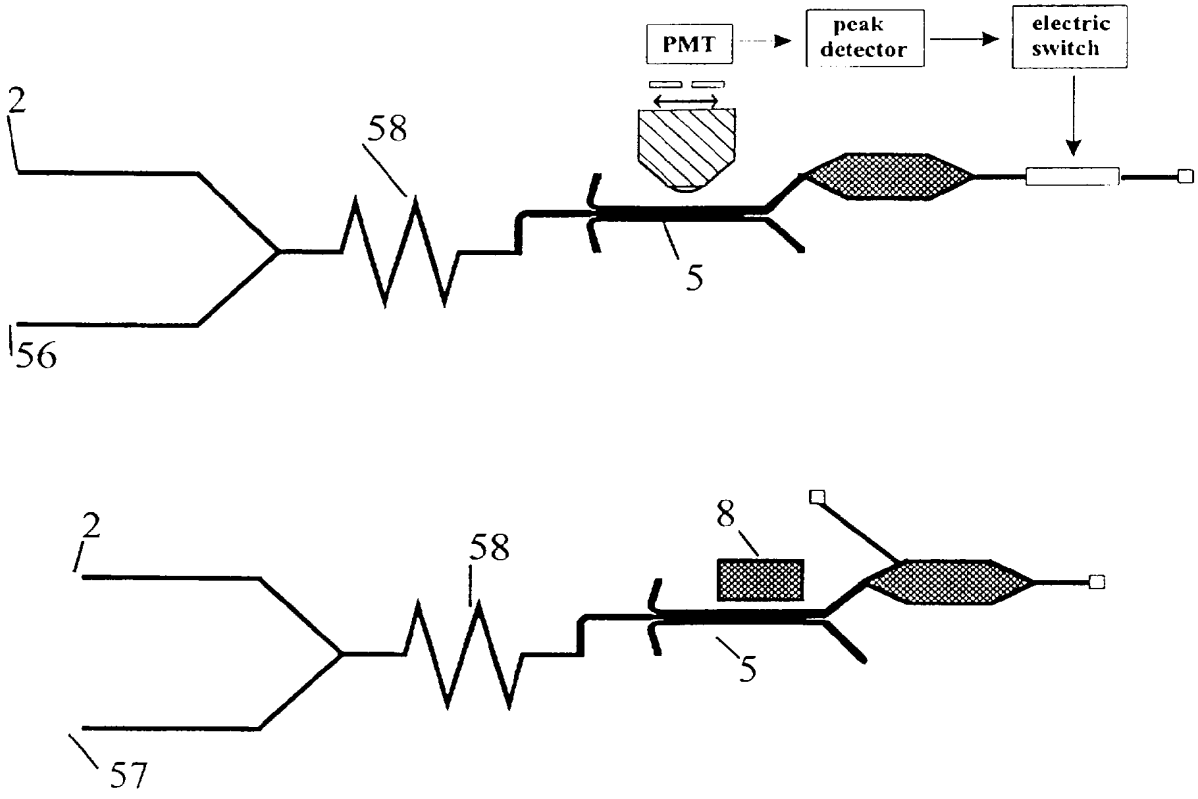
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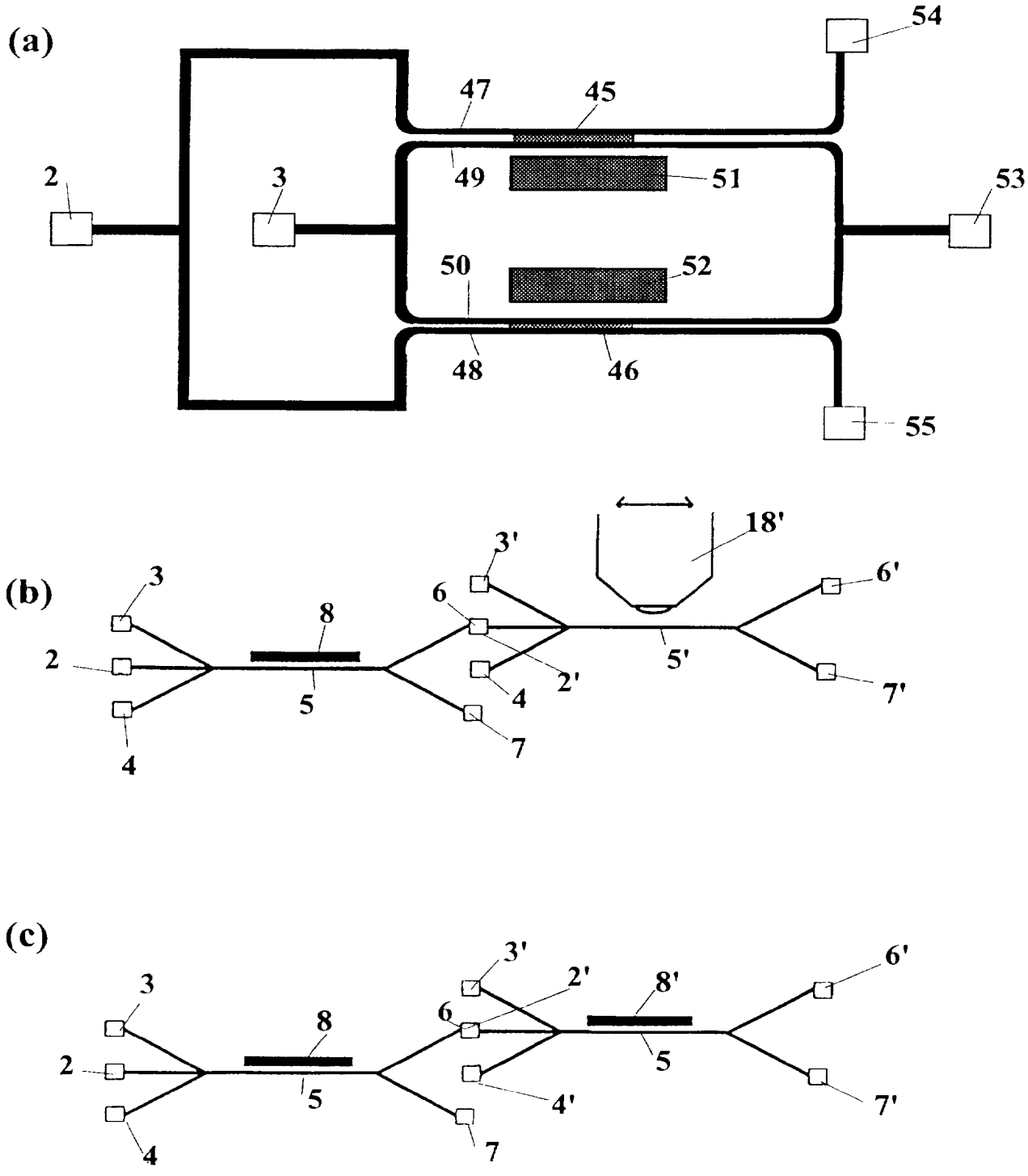
Fig. 10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53
50
52
48
46
55
(b)
18'
3'
3
6'
6
8
2
5'
2'
4
7'
5
4
7
(c)
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6'
3
2'
8'
6
8
2
5
4'
7'
5
4
7

Fig. 9

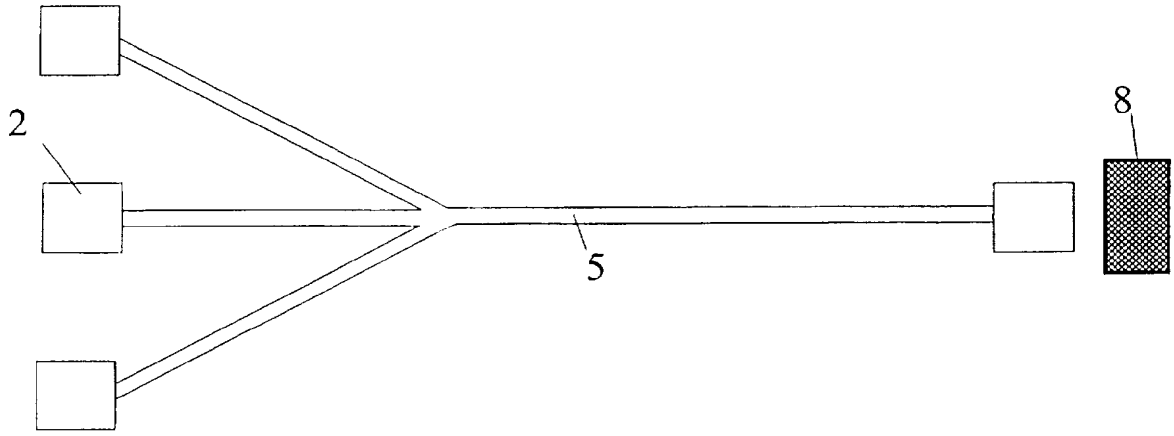


Fig. 11

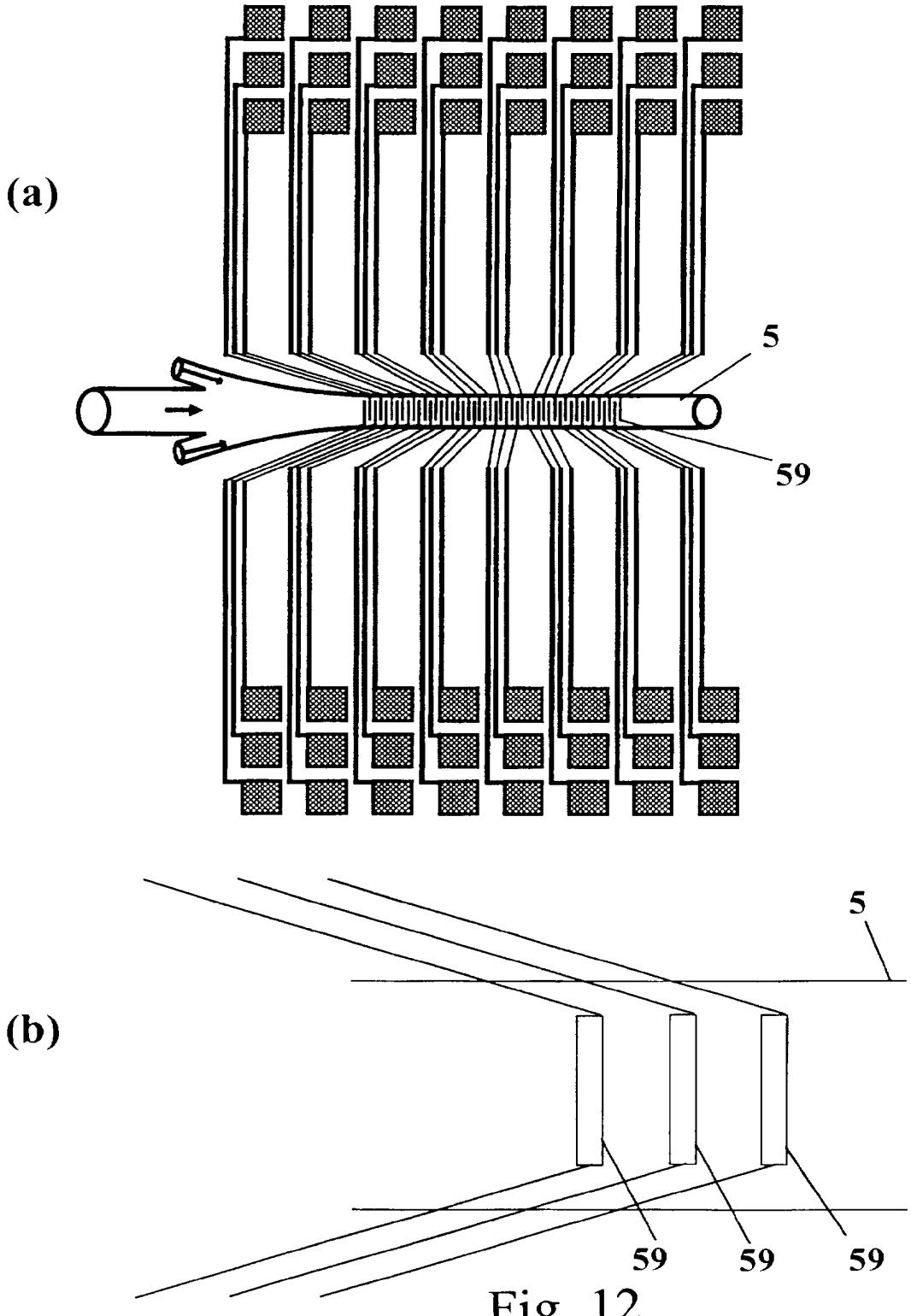
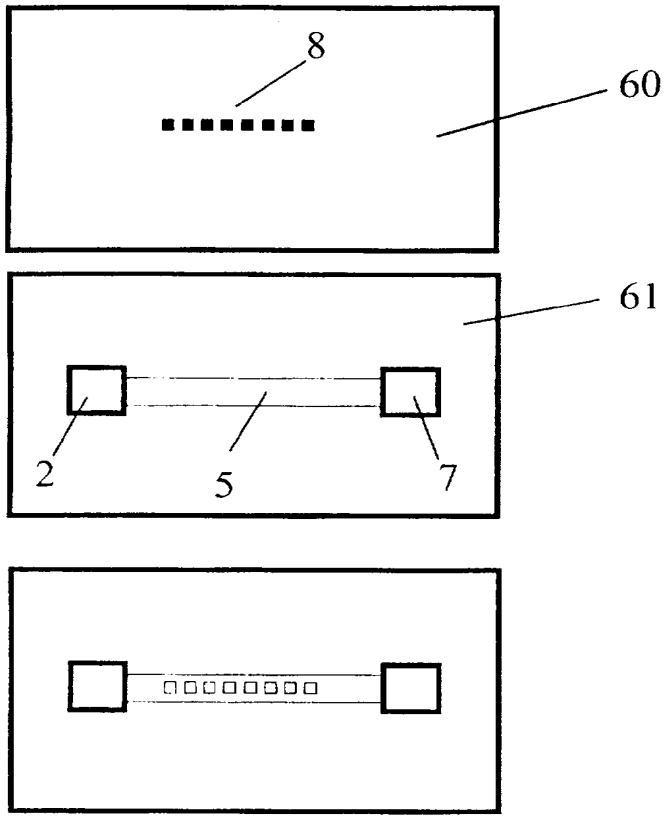


Fig. 12



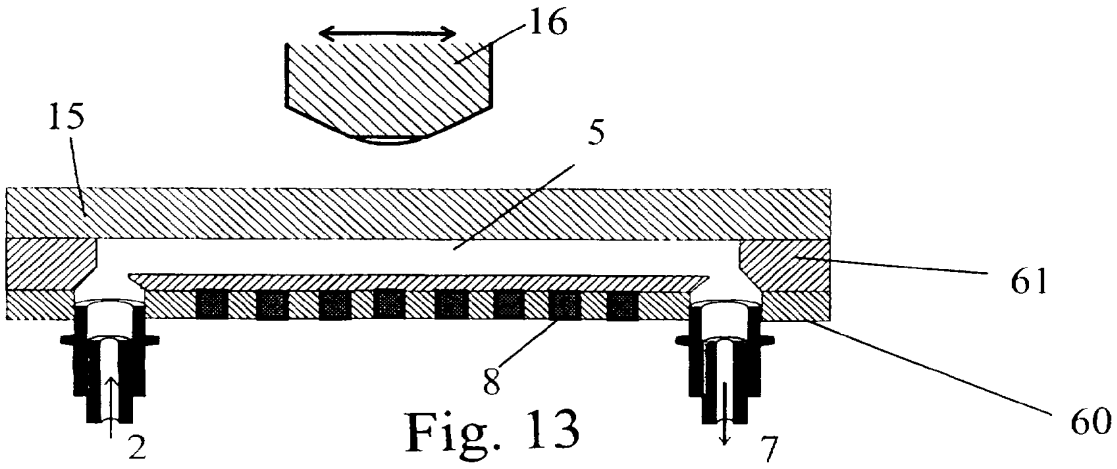


Fig. 13

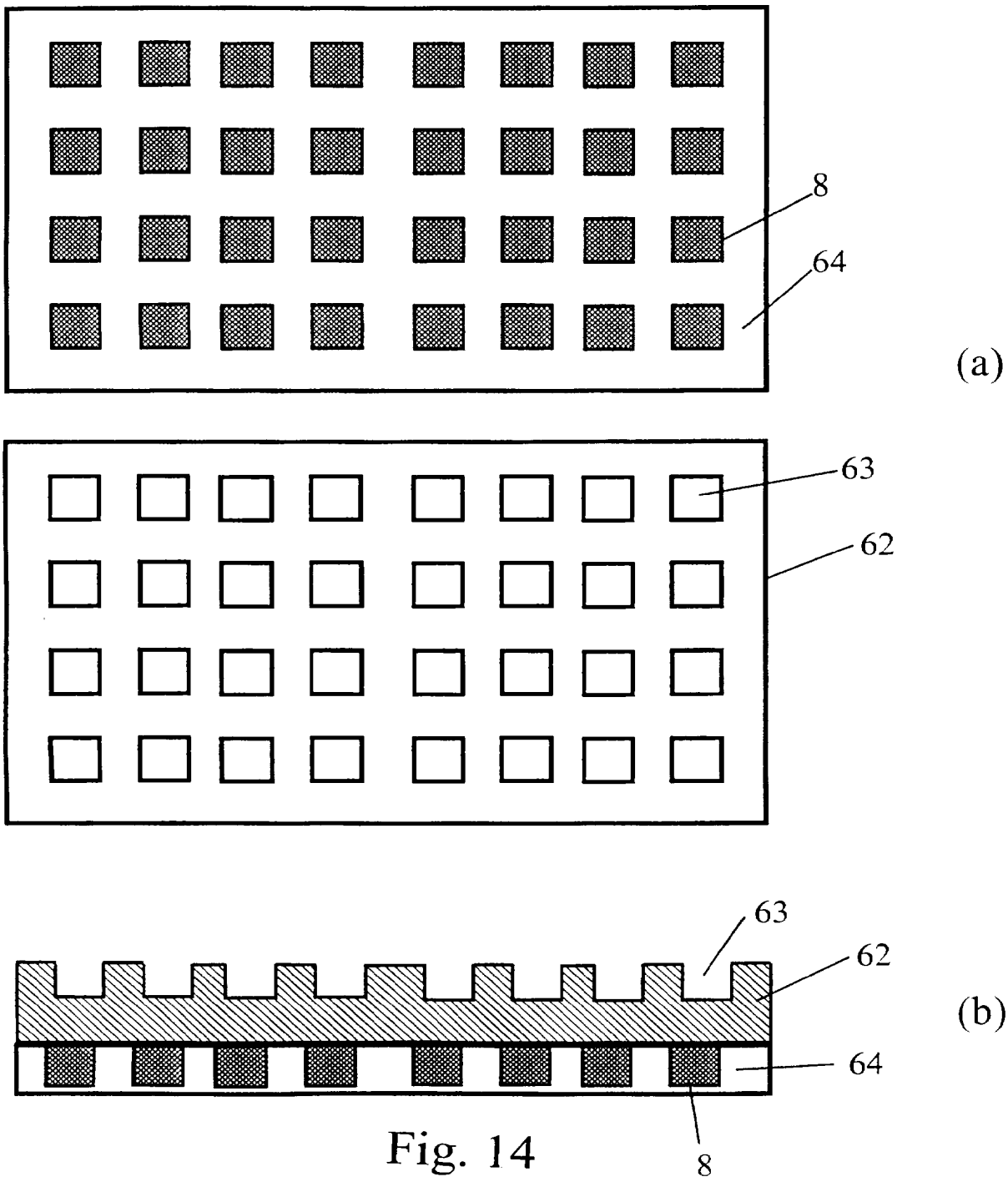


Fig. 14

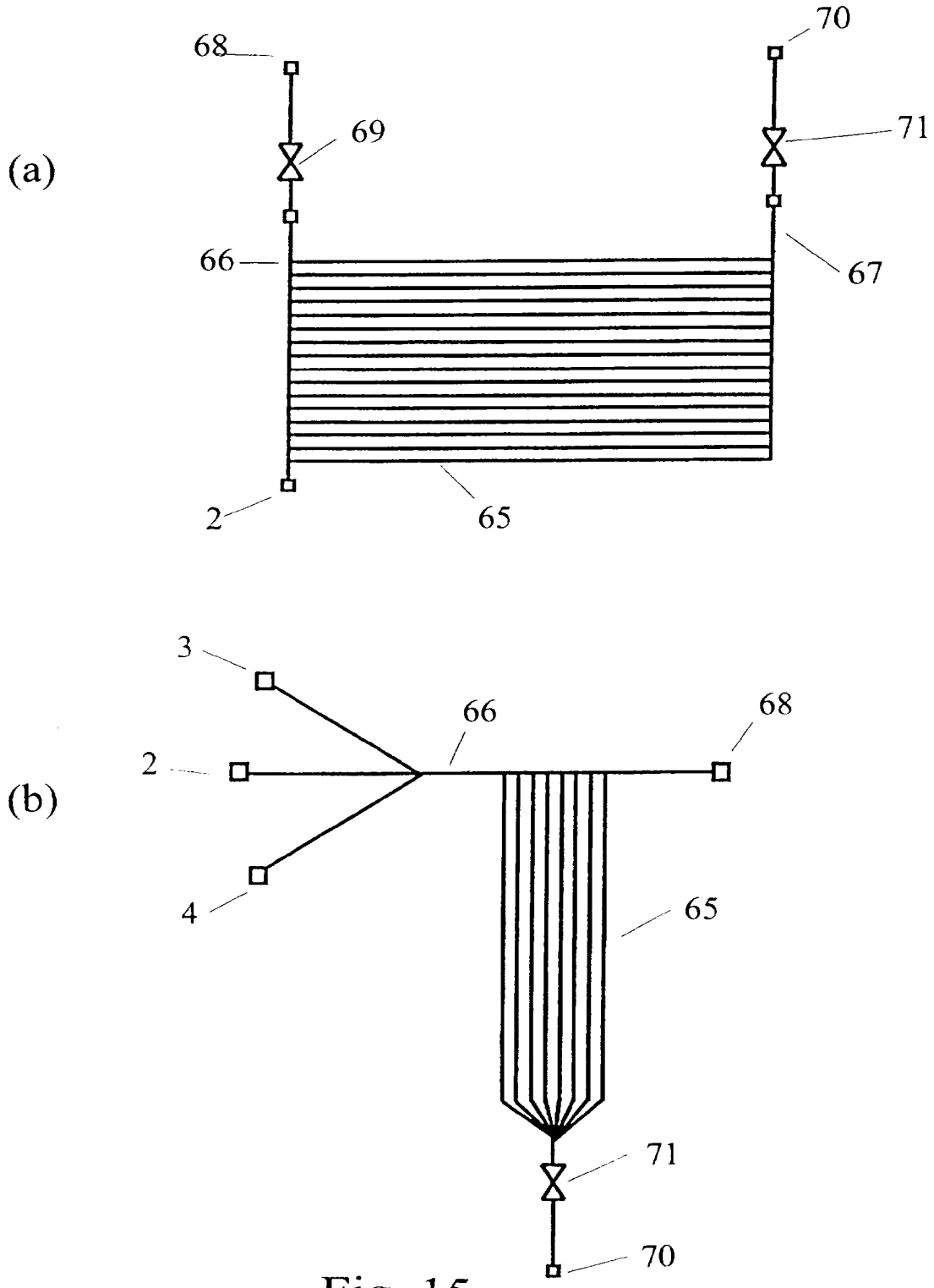


Fig. 15

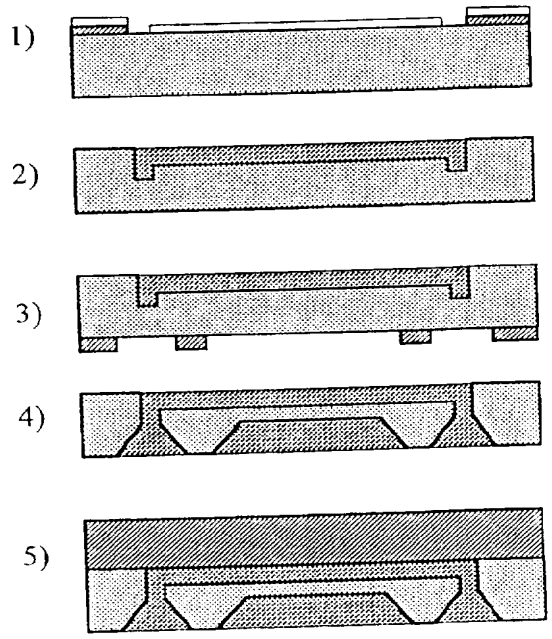
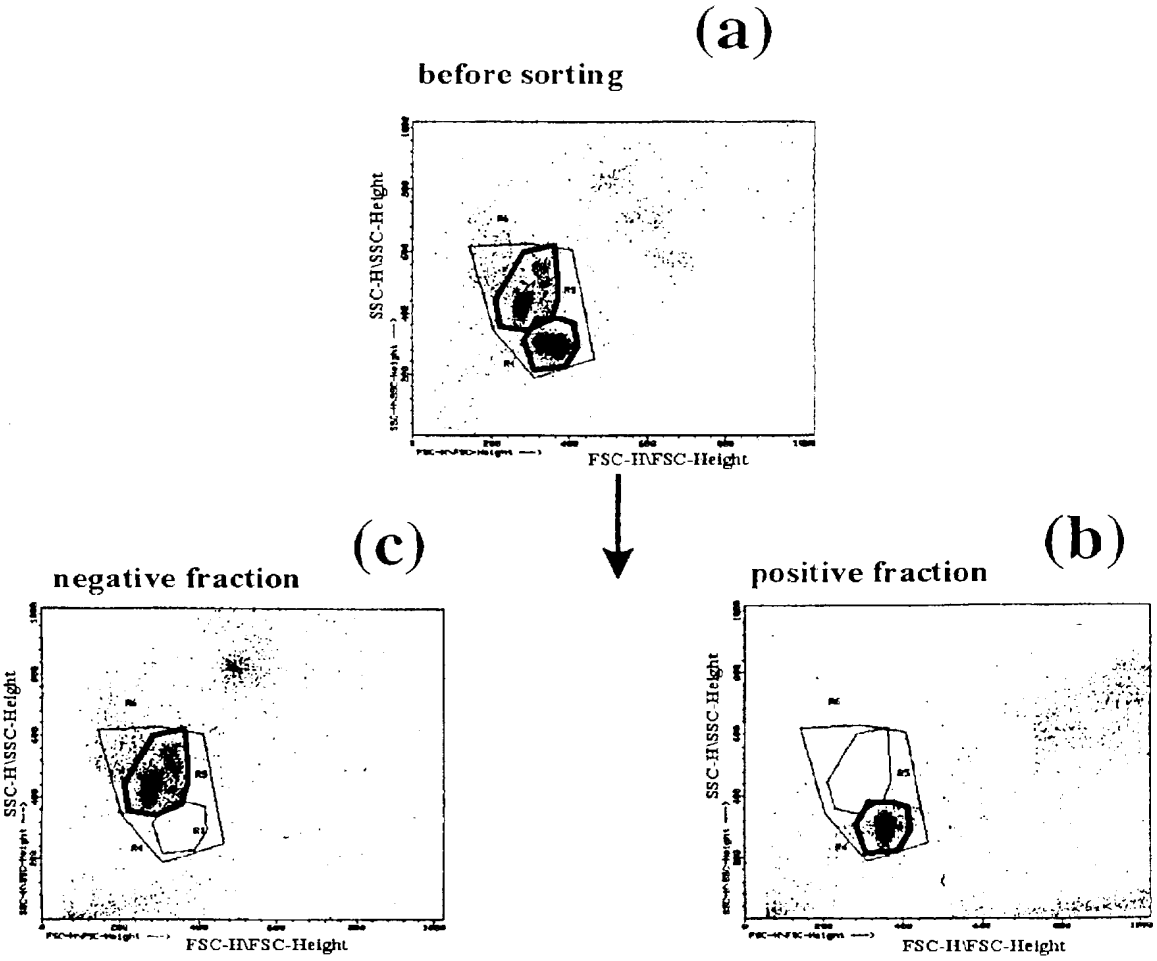


Fig. 16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before sorting
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FSC-H\FSC-Height
(c)
negative fraction
SSC-H\SSC-Height
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(b)
positive fraction
SSC-H\SSC-Height
FSC-H\FSC-Height

Fig. 17

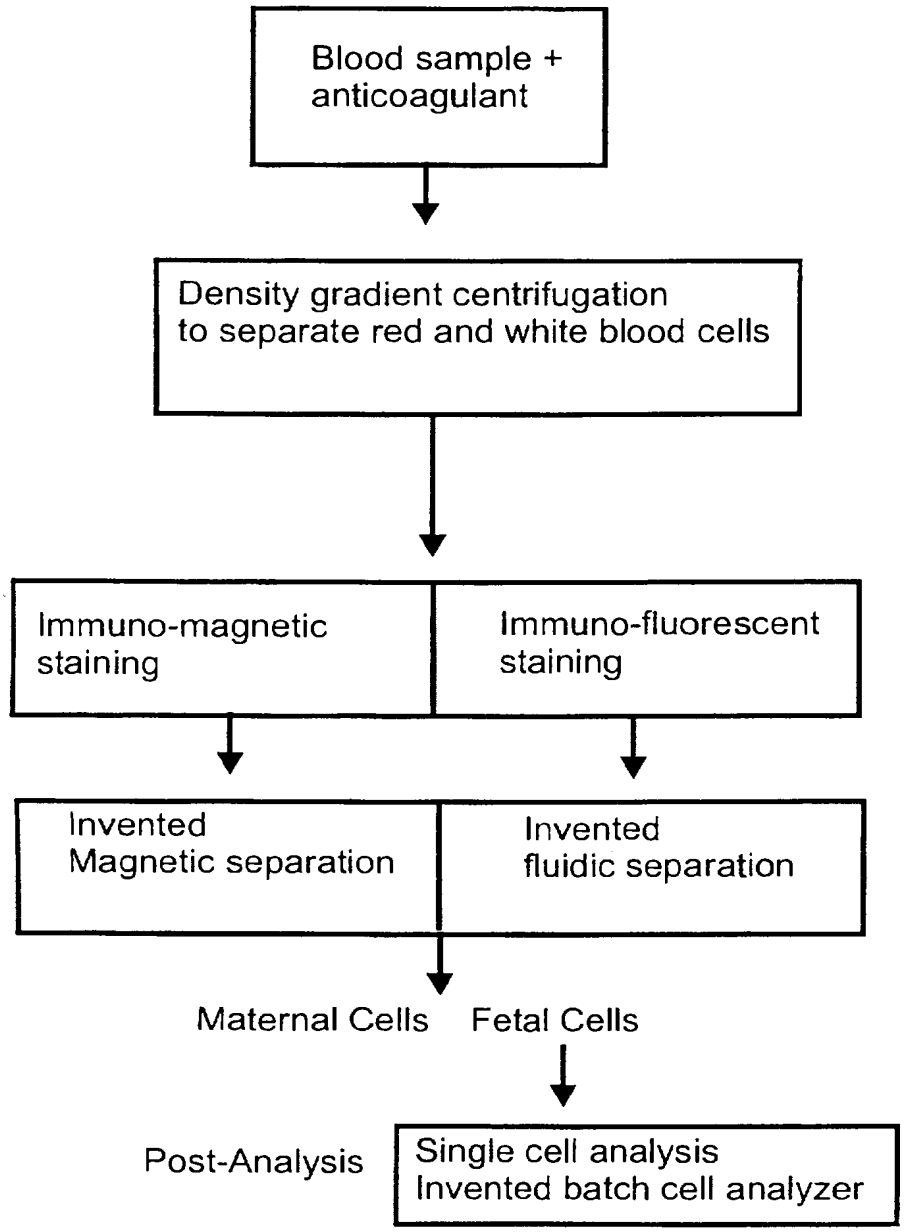
Blood sample + anticoagulant
Density gradient centrifugation to separate red and white blood cells
Immuno-magnetic staining
Immuno-fluorescent staining
Invented Magnetic separation
Invented fluidic separation
Maternal Cells
Fetal Cells
Post-Analysis
Single cell analysis Invented batch cell analyzer

Fig. 18

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/DK 97/00368

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N15/14 G01N33/543 B03C1/035

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N B03C B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AHN C H ET AL: "A FULLY INTEGRATED MICROMACHINED MAGNETIC PARTICLE MANIPULATOR AND SEPARATOR" PROCEEDING OF THE WORKSHOP ON MICRO ELECTRO MECHANICAL SYSTEMS (MEM, OISO, JAN. 25 - 28, 1994, no. WORKSHOP 7, 25 January 1994, INSTITUTE OF ELECTRICAL AND ELECTRONICS ENGINEERS, pages 91-96, XP000528399 see the whole document --- -/--	1-4,9, 10,12, 17, 20-27, 29,30, 34,35

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 1997	-7. 01. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Brison, O

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/DK 97/00368

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3 827 555 A (KAMENTSKY L ET AL) 6 August 1974 see the whole document	1-4,9, 10,12, 17, 20-27, 29,30,34
X	---	37
Y	US 4 279 345 A (ALLRED JOHN C) 21 July 1981 see the whole document	12
Y	---	35
	WO 96 09409 A (MILTENYI BIOTECH INC) 28 March 1996 see page 5, line 10 - page 8, line 20	
Y	---	36
	US 4 910 148 A (SORENSEN OTTO ET AL) 20 March 1990 see the whole document	
Y	---	36
	US 3 560 754 A (KAMENTSKY LOUIS A) 2 February 1971 see the whole document	
X	---	38
	WO 94 15193 A (SIENNA BIOTECH INC) 7 July 1994 see abstract	
X	---	39
	WO 93 22058 A (UNIV PENNSYLVANIA) 11 November 1993 see page 11, line 1-14	
A	---	1
	US 5 053 344 A (ZBOROWSKI MACIEJ ET AL) 1 October 1991 see abstract	
A	---	1
	US 5 483 469 A (VAN DEN ENGH GER ET AL) 9 January 1996 see the whole document	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/DK 97/00368

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 3827555 A	06-08-74	US 3984307 A	05-10-76
US 4279345 A	21-07-81	NONE	
WO 9609409 A	28-03-96	AU 3593595 A	09-04-96
		CA 2200294 A	28-03-96
		EP 0778899 A	18-06-97
US 4910148 A	20-03-90	NONE	
US 3560754 A	02-02-71	BE 689813 A	02-05-67
		CH 446769 A	
		DE 1598634 A	15-04-71
		FR 1501326 A	29-01-68
		GB 1101063 A	
		NL 6615120 A	18-05-67
		SE 333264 B	08-03-71
WO 9415193 A	07-07-94	AU 670489 B	18-07-96
		AU 5873494 A	19-07-94
		CA 2151060 A	07-07-94
		CN 1092866 A	28-09-94
		EP 0676045 A	11-10-95
		JP 8505231 T	04-06-96
		US 5589401 A	31-12-96
WO 9322058 A	11-11-93	US 5304487 A	19-04-94
		US 5296375 A	22-03-94
		AT 155711 T	15-08-97
		AT 140025 T	15-07-96
		AT 140880 T	15-08-96
		AU 677780 B	08-05-97
		AU 4222393 A	29-11-93
		AU 680195 B	24-07-97
		AU 4222593 A	29-11-93
		AU 677781 B	08-05-97
		AU 4222693 A	29-11-93
		AU 4222793 A	29-11-93
		AU 677197 B	17-04-97
		AU 4223593 A	29-11-93

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/DK 97/00368

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9322058 A		CA 2134474 A	11-11-93
		CA 2134475 A	11-11-93
		CA 2134476 A	11-11-93
		CA 2134478 A	11-11-93
		DE 69303483 D	08-08-96
		DE 69303483 T	06-02-97
		DE 69303898 D	05-09-96
		DE 69303898 T	20-02-97
		DE 69312483 D	04-09-97
		EP 0637996 A	15-02-95
		EP 0637997 A	15-02-95
		EP 0639223 A	22-02-95
		EP 0637998 A	15-02-95
		EP 0637999 A	15-02-95
		HK 16897 A	13-02-97
		JP 7506430 T	13-07-95
		JP 7506431 T	13-07-95
		JP 7506256 T	13-07-95
		JP 7506257 T	13-07-95
		JP 7506258 T	13-07-95
		WO 9322053 A	11-11-93
		WO 9322054 A	11-11-93
		WO 9322421 A	11-11-93
		WO 9322055 A	11-11-93
		US 5635358 A	03-06-97
		US 5427946 A	27-06-95
		US 5498392 A	12-03-96
		US 5587128 A	24-12-96
		US 5486335 A	23-01-96
		US 5637469 A	10-06-97
US 5053344 A	01-10-91	NONE	
US 5483469 A	09-01-96	NONE	

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(54)【発明の名称】　微細製造差動抽出デバイスおよびその方法

(57)【要約】
本発明は、所望の粒子および所望でない粒子を含有する試料ストリームから所望の粒子を抽出するための、微細製造された抽出システムおよび方法を、提供する。試料ストリームを、慣性の効果が無視できる条件下において、抽出ストリームとの層流フロー接触下におく。２つのストリーム間の接触は、所望の粒子が試料ストリームから抽出ストリームへと差動輸送されることを可能にするのに十分な時間、維持される。好適な実施態様において、差動輸送メカニズムは拡散である。本発明の抽出システムを微細製造された拡散ベースの混合デバイスおよび／または感知手段と結合することにより、ピコリットル量の流体を、シリコンウェハの大きさだけのデバイス上において、処理あるいは分析することが可能になる。

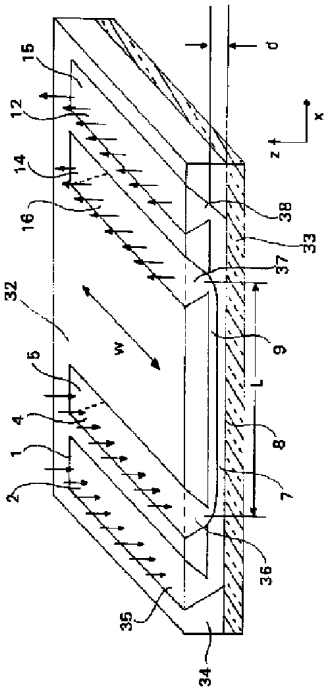

FIGURE 5

【特許請求の範囲】

１．所望の粒子を、該所望の粒子を含有する試料ストリームから抽出するための、微細製造された抽出デバイスであって、

ａ．試料ストリーム入口と；

ｂ．抽出ストリーム入口と；

ｃ．該試料ストリーム入口および該抽出ストリーム入口と流体的に連通しており、該抽出ストリーム入口からの抽出ストリームとの平行層流フローとして、該試料ストリーム入口から試料ストリームを受ける、50未満のアスペクト比（w/d）を有する抽出チャネルと；

ｄ．該抽出チャネルと流体的に連通しており、所望の粒子が抽出された後の該試料ストリームの少なくとも一部を有する副産物ストリームを受ける、副産物ストリーム出口と；

ｅ．該抽出チャネルと流体的に連通しており、該抽出ストリームの少なくとも一部および該試料ストリームから抽出された所望の粒子を有する生産物ストリームを受ける、生産物ストリーム出口と；

を有するデバイス。

２．前記抽出チャネルが約25未満のアスペクト比を有する、請求項１に記載のデバイス。

３．前記抽出チャネルが約１未満のアスペクト比を有する、請求項１に記載のデバイス。

４．所望の粒子を、該所望の粒子を含有する試料ストリームから抽出するための、微細製造された抽出デバイスであって、

ａ．試料ストリーム入口と；

ｂ．抽出ストリーム入口と；

ｃ．該試料ストリーム入口および該抽出ストリーム入口と流体的に連通しており、該抽出ストリーム入口からの抽出ストリームとの平行層流フローとして、該試料ストリーム入口から試料ストリームを受ける、約100ミクロン未満の深さを有する抽出チャネルと；

ｄ．該抽出チャネルと流体的に連通しており、所望の粒子が抽出された後の該試料ストリームの少なくとも一部を有する副産物ストリームを受ける、副産物ストリーム出口と；

ｅ．該抽出チャネルと流体的に連通しており、該抽出ストリームの少なくとも一部および該試料ストリームから抽出された所望の粒子を有する生産物ストリームを受ける、生産物ストリーム出口と；

を有するデバイス。

５．シリコンウェハを含む材料で製造された、請求項１に記載のデバイス。

６．前記所望の粒子の、前記試料ストリームから前記抽出ストリームへの差動輸送を実現するための手段をさらに有する、請求項１に記載のデバイス。

７．前記差動輸送を実現するための手段が、磁力、電気力、誘電、沈殿、せん断、遠心力、温度、圧力、および濃度勾配からなる群より選択されるフィールドを生成する手段である、請求項５に記載のデバイス。

８．濃度勾配フィールドを実現するための前記手段が、前記抽出ストリーム中の前記所望の粒子に対して選択性を有し、実効的に非拡散である(effectively non-diffusing)、吸収材または吸着材を含む、請求項６に記載のデバイス。

９．複数の生産物ストリーム出口を有する、請求項１に記載のデバイス。

１０．請求項１に記載のデバイスならびに、前記生産物ストリーム中において前記所望の粒子を検出するための手段と組み合わせる、微細製造された分析システム。

１１．請求項１に記載のデバイスを有する微細製造された分析システムであって、所望の粒子は、所望の粒子および所望でない粒子を含有する試料ストリームから抽出され、前記生産物ストリーム中の該所望でない粒子を検出するための手段を有する、システム。

１２．前記所望の粒子を検出するための前記手段が、光学的感知手段を有している、請求項１０に記載の分析システム。

１３．前記所望でない粒子を検出するための手段が、光学的感知手段を有している、請求項１１に記載の分析システム。

１４．前記生産物ストリームを、検出を可能にするために前記所望の粒子と相互作用し得るインジケータ物質と混合するための手段を有する、請求項１０に記載の分析システム。

１５．シリコンウェハ上に微細製造された分析システムであって、

ａ．分析物の所望の粒子を抽出するための抽出手段であって、

(1) 試料ストリーム入口と；

(2) 抽出ストリーム入口と；

(3) 該試料ストリーム入口および該抽出ストリーム入口と流体的に連通しており、該抽出ストリーム入口からの抽出ストリームとの平行層流フローとして、該試料ストリーム入口から試料ストリームを受ける、50未満のアスペクト比（w/d）を有する抽出チャネルと；

(4) 該抽出チャネルと流体的に連通しており、該所望の分析物粒子の少なくとも一部が抽出された後の該試料ストリームの少なくとも一部を有する副産物ストリームを受ける、副産物ストリーム出口と；

(5) 該抽出チャネルと流体的に連通しており、該抽出ストリームの少なくとも一部および該試料ストリームから抽出された所望の分析物粒子を有する生産物ストリームを受ける、生産物ストリーム出口と；

とを有する手段と；

ｂ．該生産物ストリームを、検出を可能にするために前記所望の粒子と相互作用し得るインジケータ物質と混合するための拡散ベースの混合デバイスと；

ｃ．前記所望の分析物粒子の存在を検出し得る検出チャンバと；

を有するシステム。

１６．感知手段と組み合わせることによって、前記検出チャンバ中における前記所望の分析物粒子の存在を検出し得る、請求項１５に記載の分析システム。

１７．定量化手段と組み合わせることによって、前記検出チャンバ中における前記所望の分析物粒子の濃度を測定し得る、請求項１６に記載の分析システム。

１８．所望の粒子の少なくとも一部を、該所望の粒子および所望でない粒子を含有する試料ストリームから抽出するための方法であって、

ａ．該試料ストリームを、請求項１に記載の微細製造された抽出デバイスの前記試料ストリーム入口中に導入する工程と；

ｂ．抽出ストリームを該抽出デバイスの前記抽出チャネル中に導入する工程と；

ｃ．所望の粒子を有する生産物ストリームを、該デバイスの前記生産物ストリーム出口から引き抜く工程と；

を包含する方法。

１９．所望の粒子の少なくとも一部を、該所望の粒子および所望でない粒子を含有する試料ストリームから抽出するための方法であって、

ａ．該試料ストリームを、請求項２に記載の微細製造された抽出デバイスの前記試料ストリーム入口中に導入する工程と；

ｂ．抽出ストリームを該抽出デバイスの前記抽出チャネル中に導入する工程と；

ｃ．所望の粒子を有する生産物ストリームを、該デバイスの前記生産物ストリーム出口から引き抜く工程と；

を包含する方法。

２０．連続的なプロセスとして行われる、請求項１８に記載の方法。

２１．バッチプロセスとして行われる、請求項１８に記載の方法。

２２．磁力、電気力、誘電、沈殿、せん断、遠心力、温度勾配、圧力勾配、および濃度勾配フィールドからなる群より選択されるフィールドを前記抽出チャネルに横切って生成することにより、所望の粒子の、前記抽出チャネル中の前記抽出ストリーム中への差動輸送を助ける、請求項１８に記載の方法。

２３．前記フィールドが濃度勾配フィールドであり、前記差動輸送は拡散によって起こる、請求項２２に記載の方法。

２４．前記試料および抽出ストリームが異なる特性を有する、請求項１８に記載の方法。

２５．前記試料流体体積が少なくとも約１ピコリットルである、請求項２２に記載の方法。

２６．前記試料流体体積が約１ナノリットルと約１０マイクロリットルとの間である、請求項２２に記載の方法。

２７．前記試料流体体積が約１マイクロリットルと約１マイクロリットルとの間である、請求項２２に記載の方法。

２８．前記生産物ストリーム中における、前記所望の粒子または前記所望でない粒子の存在を検出する工程をさらに包含する、請求項１８に記載の方法。

２９．前記生産物ストリーム中における、前記所望の粒子または前記所望でない粒子の濃度を決定する工程をさらに包含する、請求項１８に記載の方法。

３０．前記試料ストリーム中における、前記所望の粒子の濃度を決定する工程をさらに包含する、請求項１８に記載の方法。

３１．前記生産物ストリーム中における、前記所望の粒子および／または前記所望でない粒子のおよび／または濃度を、前記試料ストリームを前記試料ストリーム入口中に導入してから約１秒未満後に決定する、請求項１８に記載の方法。

３２．前記生産物ストリーム中における、前記所望の粒子および／または前記所望でない粒子の存在および／または濃度を、前記試料ストリームを前記試料ストリーム入口中に導入してから約１秒後から約５分後の間に決定する、請求項１８に記載の方法。

３３．前記生産物ストリーム中における、前記所望の粒子および／または前記所望でない粒子の存在および／または濃度を、前記試料ストリームを前記試料ストリーム入口中に導入してから約１分後から約４分後以内の間に決定する、請求項１８に記載の方法。

３４．前記生産物ストリームが引き抜かれた後に、前記デバイスを洗い流すことによって所望の粒子および所望でない粒子の両方を除去し、前記抽出方法を繰り返す、請求項１８に記載の方法。

３５．副産物ストリームを引き抜き、該副産物ストリームを試料ストリームとして前記試料ストリーム入口中に導入することによって前記方法を繰り返すことを包含する、請求項１８に記載の方法。

３６．血液成分の濃度を検出することを包含する請求項１８に記載の方法であって、前記試料ストリームは全血を含み、清浄された血液成分の粒子が前記生産物ストリーム中へと抽出される、方法。

３７．副産物ストリームを引き抜く工程と、該副産物ストリームを微細製造されたフローサイトメータ中に導入する工程とを包含する、請求項１８に記載の方法。

３８．生産物ストリームを引き抜く工程と、該生産物ストリームを微細製造されたフローサイトメータ中に導入する工程とを包含する、請求項１８に記載の方法。

【発明の詳細な説明】

微細製造差動抽出デバイスおよびその方法

本発明は、米国陸軍によって授与された陸軍研究契約DAMD17-94-J-4460の下に、政府の援助を得てなされたものである。米国政府は、この発明において特定の権利を有する。

発明の分野

本発明は、一般的に、微細製造抽出システムと、拡散および印加された電場（applied field）などの差動輸送原理によって、他の要素を含有するストリームから分析物を分離する方法に関する。本発明は、例えば、血液を処理し、それによって、アルブミン分子などの比較的小さな粒子を含有するストリームを、細胞を含有するストリームから分離するのに有用である。

発明の背景

生物学的試料の化学分析は、試料のサイズに制約を受ける。成人からの２、３ミリリットルの血液の回収は、ほとんど影響がないかもしれないが、この手順を１時間毎に繰り返す、またはこの量を乳児から一度回収することは、被験体の健康を顕著に変化させ得る。これらの理由から、小型化血液分析システムは有用であろう。さらに、重大な看護にとって大きな重要性を有する高度な検査の多くは、主に病院の研究室で行われ得るが、もし、幾つかの重要な検査を怪我をしたフィールド所で患者に行うことができれば、救急医療の実施にかなりの影響を与え得る。特定のアッセイに関しては、赤血球の非存在下で測定を行うことが不可欠であり、その結果、血漿から細胞を分離する何らかの形態が必要とされる。

拡散は、大きなスケールでは容易に無視され得るが、微細スケールでは、急速に重要となるプロセスである。分子が、距離ｄにわたって拡散する平均時間ｔは、$２ｔ＝ｄ^{２}／Ｄ$である（Ｄは、分子の拡散係数である）。タンパク質または他の大きな分子に関しては、マクロスケールでは、拡散は比較的ゆっくりである（例

えば、Ｄが室温の水中で$７×１０^{-７}ｃｍ^{２}／$秒であるヘモグロビンは、１センチメートルのパイプにわたって拡散するのに約$１０^{６}$秒（１０日間）かかるが、１

０μｍチャネルにわたって拡散するのには約１秒かかる）。

半導体業界によって、電子デバイスを小型化するために開発された道具を用いて、１ミクロン程のチャネルサイズを有する複雑な流体システムを製造することが可能である。これらのデバイスは、安価に大量生産され得るが、すぐに、単純な分析試験に広く使用されることが予期される。例えば、Ramsey、J.M.らによる”Microfabricated chemical measurement systems”、Nature Medicine 1:1093～1096；およびHarrison、D.J.ら（1993）、”Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip”、Science 261:895～897を参照。

分析器具の小型化は、サイズの縮小という単純な問題ではない。小さなスケールでは、異なる影響が重要となり、それによって、非能率的となるプロセスもあれば、無用となるプロセスもある。材料またはプロセスの制限故に、あるデバイスの小型のバージョンを複製することは困難である。これらの理由から、微細スケールで一般的な実験作業を行うための新しい方法の開発が必要である。

微細機械加工平面基板によって製造されたデバイスは、化学的分離、分析、および検出のために製造され、用いられてきた。例えば、Manz、A.ら、（1994）、”Electroosmotic pumping and electrophoretic separations for miniaturized chemical analysis system”、J.Micromech. Microeng. 4:257～265を参照。

フィールドフローフラクショネーションデバイスは、単一入口ストリームを用いた粒子サイズ分離を伴う。例えば、Giddings、J.C.、米国特許第3 449 938号、1969年６月17日、”Method for Separating and Detecting Fluid Materials”；Giddings、J.C.、米国特許第4 147 621号、1979年４月３日、”Method and Apparatus for Flow Field-Flow Fractionation”；Giddings、J.C.、米国特許第4 214 981号、1980年７月29日、”Steric Field-Flow Fractionation”；Giddings、J.C.ら、米国特許第4 250 026号、1981年２月10日、”Continuous Steric FFF Device for The Size Separation of Particles”；Giddings、J.C.ら、（1983）、”Outlet Stream Splitting for Sample Concentration in Field-Flow Fractionation”、Separation Science and Technology 18:293～306; Giddi

ngs、J.C.、（1985）、”Optimized Field-Flow Fractionation System Based on Dual Stream Splitters”、Anal. Chem. 57:945～947; Giddings、J.C.、米国特許第4 830 756号、1989年５月16日、”High Speed Separation of Ultra-High Molecular Weight Polymers by Hyperlayer Field-Flow Fractionation”；Giddings、J.C.、米国特許第4 141 651号、1992年８月25日、”Pinched Channel Inlet System for Reduced Relaxation Effects and Stopless Flow Injection in Field-Flow Fractionation”；Giddings、J.C.、米国特許第5 156、039号、1992年10月20日、”Procedure for Determining the Size and Size Distribution of Particles Using Sedimentation Field-Flow Fractionation”；Giddings、J.C.、米国特許第5 193 688号、1993年３月16日、”Method and Apparatus for Hydrodynamic Relaxation and Sample Concentration in Field-Flow Fraction Using Permeable Wall Elements”；Caldwell、K.D.ら、米国特許第5 240 618号、1993年８月31日、”Electrical Field-Flow Fractionation Using Redox Couple Added to Carrier Fluid”；Giddings、J.C.、（1993）、”Field Flow Fractionation: Analysis of Macromolecular、Colloidal and Particulate Materials”、Science 260:1456～1465；Wada、Y.ら、米国特許第5 465 849号、1995年11月14日、”Column and Method for Separating Particles in Accordance with Their Magnetic Susceptibility”；Yue、V.ら、（1994）、”Miniature Field-Flow Fractionation Systems for Analysis of Blood Cells”、Clin. Chem. 40:1810～1814；Afromowitz、M.A.およびSamaras、J.E.（1989）、”Pinch Field Flow Fractionation Using Flow Injection Techniques”、Separation Science and Technology 24（５および６）:325～339を参照。

薄いチャネル分割フロー分別（SPLITT）技術によりまた、薄いチャネルを有する分離セルにおいて粒子分離が提供される。フィールドの力は、フロー方向に垂直な方向に働く。粒子は、粒子含有ストリームから輸送ストリームを横切り、粒子を含有しないストリームへと拡散する、または輸送される。このプロセスを操作するデバイスは、一般的に、チャネルを形成するためにスペーサーとして用いられるテフロンシートを用いてガラス板から製造される。従って、チャネルの深

さは、スペーサーの深さより小さいことはなく、その深さは、一般的に、約100～120μｍの厚みである。例えば、Giddings、J.C.、米国特許第4 737 268号、1988年４月12日、”Thin Channel Split Flow Continuous Equilibrium Process and Apparatus for Particle Fractionation”；Giddings、J.C.、米国特許第4 894 146号、1990年１月16日、”Thin Channel Split Flow Process and Apparatus for Particle Fractionation”；Giddings、J.C.、米国特許第5 093 426号、1991年８月13日、”Process for Continuous Particle and Polymer Separation in Split-Flow Thin Cells Using Flow-Dependent Lift Forces”；Williams、P.S.ら、（1992）、”Continuous SPLITT Fractionation Based on a Diffusion Mechanism”、Ind. Eng. Chem. Res. 31:2172～2181；およびLevin、S.およびTawil、G.（1993）、”Analytical SPLITT Fractionation in the Diffusion Mode Operating as a Dialysis-like System Devoid of Membrane. Application to Drug-Carrying Liposomes”、Anal. Chem. 65:2254～2261を参照。

本発明の目的は、分析物が抽出され、検出され、定量され得る差動輸送原理を利用する、微細製造抽出システムを提供することである。

本明細書中に記載されるように、微細スケールでの拡散分離デバイス、例えば、約100μｍを越えないチャネル深さを有するデバイスの利点は、先行技術においては認識されていたとは思われない。例えば、Kittilsand、G.およびStemme、G.（1990）、Sensors and Actuators A21-A23:904～907、およびWilding、P.ら、（1994）、J. Clin. Chem. 40:43～47を参照。

本明細書中で参照される全ての刊行物、特許、および特許出願は、本明細書によって参考として援用される。

発明の要旨

本発明は、サイズの加工利点、生産経済性、微細化学分析システムとの集積化性、低電力消費の点で従来の沪過技術およびデバイスとは異なり、試料対試料または連続的処理モードで動作し得る抽出方法およびデバイスを提供する。このデバイスは、微細製造化学分析システムとの集積化に特によく適しており、この微細製造化学分析システムにおいては、例えば、好適な実施形態によって、同等の

抽出ストリーム量を有する１マイクロリットルの全血ほどの少量の試料から生じる、ピコリットルからナノリットルの範囲の量を有する希釈された血漿生産物を提供可能な微細製造抽出デバイスまたはシステムが提供される。

抽出システムは、医療対象の血液検査のための、微細流体素子および検出素子（例えば、光学検出器など）の集積化システムにおける素子として有用であり、分析化学の他の多くの分野に適用を有する。血液分析に有用な好適な実施形態においては、このデバイスは、全血から血漿要素を抽出することを可能にし、それによって、後続の分析にために、細胞を含有しない流体ストリームが生成される。

最も単純な概念での本発明の微細製造抽出システムは、「Ｈ」形状の微細チャネルを含有する拡散抽出デバイスによって例示される。試料ストリームに懸濁された粒子の混合物は、Ｈの腕の一方、例えば、上部左から抽出チャネル（「Ｈ」の横線）に入り、抽出ストリーム（希釈ストリーム）は、下部左から入る。２つのストリームは、抽出チャネルで合流するが、チャネルの小さなサイズのために、流れは層状で、それらのストリームが混ざることはない。試料ストリームは、上部右で副産物ストリームとして流出し、抽出ストリームは、下部右から生産物ストリームとして流出する。これらのストリームは、抽出チャネルにおいて平行の層流であるが、より大きな拡散係数を有する粒子（アルブミン、砂糖、および小さいイオン等のより小さな粒子）は、抽出ストリームへと拡散する時間を要するが、より大きな粒子（例えば、血球）は、試料ストリームにとどまる。出ていく抽出ストリーム（この時点で生産物ストリームと呼ばれる）中の粒子は、より大きな粒子からの妨害を受けずに分析され得る。

この特許出願においては、チャネルのフロー方向は、長さ（Ｌ）と呼ばれる。長さ（Ｌ）に対して直角な粒子輸送の方向におけるチャネル次元は、深さ（ｄ）と呼ばれる。長さおよび深さの両方に対して直角な第３のチャネル次元は、幅（ｗ）と呼ばれる。従って、深さ（ｄ）は、試料ストリームと抽出ストリームとの界面に対して垂直である。表１に、本明細書中に用いられる他の略語を記載する。

表１

V	容量
$\dot{V}_{ss}$	試料ストリーム流速（m^3／ｓ）
$\dot{V}_{es}$	抽出ストリーム流速（m^3 ｓ）
$\dot{V}_{ps}$	生産物ストリーム流速（m^3 ｓ）
$\dot{V}_{bps}$	副産物ストリーム流速（m^3 ｓ）
$\dot{V}_{ind}$	インジケータ色素ストリーム流速（m^3 ｓ）
$\dot{V}_{ds}$	検出ストリーム流速（m^3 ｓ）
$C_{i,ss}$	試料ストリーム要素ｉ濃度（ｋｇ／ｋｇ）
$C_{i,es}$	抽出ストリーム要素ｉ濃度（ｋｇ／ｋｇ）
$C_{i,bps}$	副産物ストリーム要素ｉ濃度（ｋｇ／ｋｇ）
$C_{i,ps}$	生産物ストリーム要素ｉ濃度（ｋｇ／ｋｇ）
$C_{dye,ind}$	インジケータストリーム色素濃度（ｋｇ／ｋｇ）
$C_{i,ds}$	検出ストリーム要素ｉ濃度（ｋｇ／ｋｇ）
d	拡散方向抽出チャネル深さ（ｍ）
w	抽出チャネル幅（ｍ）
L	抽出チャネル長さ（ｍ）
$a_{\%}$	平衡濃度からの偏差率％
$L_{a\%}$	$a_{\%}$の達成に必要なデバイス長さ（ｍ）
Z_s	抽出チャネル入口での試料ストリームと抽出ストリームとの間の界面ストリームライン　位置（ｍ）
Z_p	副産物ストリームと生産物ストリームとの間の界面ストリームライン位置（ｍ）
P	流体ストリーム内の絶対圧（Ｐａ）
ΔP	抽出チャネルの入口と出口との間の圧力差（Ｐａ）
D_i	要素ｉの２進拡散係数（m^2／ｓ）
μ	流体粘度（Ｐａ×ｓ）

ρ　流体密度（kg／m^3）

ξ　無限長さの抽出チャネルに対する平衡正規化要素濃度（無次元）

c　正規化要素濃度（無次元）

x　チャネル長さ座標方向（フロー方向）

y　チャネル幅座標方向

z　拡散方向座標

$\tilde{X}$，$\tilde{Z}$　無次元正規化変数（無次元）

w／d　アスペクト比

D　拡散係数

Re　レイノルズ数

T　温度

u　軸方向速度

抽出チャネルの長さおよび抽出チャネルのフロー速度は、抽出ストリームへと拡散するのに粒子が要する時間量を決定するための主要パラメータである。上記の場合における粒子は、試料ストリームから抽出ストリームへと輸送メカニズムとして拡散を用いることにより差動的に輸送される。所望の粒子の差動輸送をもたらす他の手段もまた用いられ得る。「差動輸送」という用語は、所望の粒子の一部が、試料ストリームから抽出ストリームへと、所望でない粒子を実質的に除外して輸送されることを意味する。例えば、磁気的、電気的、または他の力が、抽出ストリームに加えられ得、温度勾配が用いられ得、または、抗体などの吸収材料または吸着材料が抽出ストリームに加えられ、それによって所望の粒子が捕らえられ得る。

１つの好適な実施形態は、抽出ストリームにおける、所望のリガンド粒子に対して特異性を有するレセプタなどの吸着材料の、プラスチックビーズまたは高分子量ポリマー等の効率的非拡散基質上への取り込みを伴う。別の好適な実施形態は、所望の粒子に対して特異性を有する効率的非拡散吸収粒子材料を利用する。このような材料は、試料ストリームに拡散しない、または、副産物ストリームにおいて所望でない粒子の検出を妨害するのに十分な量で試料ストリームに拡散しない場合に「効率的非拡散」であると考えられる。この吸収剤の実施形態におい

ては、所望の粒子は、効率的非拡散吸収粒子材料内に吸収されるが、吸着剤の実施形態においては、所望の粒子は、効率的非拡散基質プラスチックビーズの表面またはそれに付着したリガンドに付着する。吸着剤／吸収剤の実施形態における、所望の粒子に適する多数のリガンドは、当該分野で公知であり、これらの技術に関連する特定の教示は、同時係属仮出願番号第60/019904号［本出願と同時に提出された代理人事件番号35-96P］に開示されている。

所望の粒子を、該所望の粒子を含有する試料ストリームから抽出するための、本発明の微細製造デバイスは、以下を備える：

ａ．試料ストリーム入口と；

ｂ．抽出ストリーム入口と；

ｃ．該試料ストリーム入口および該抽出ストリーム入口と流体的に連通しており、該抽出ストリーム入口からの抽出ストリームとの平行層流フローとして、該試料ストリーム入口から試料ストリームを受ける、50未満のアスペクト比（w/d）を有する抽出チャネルと；

ｄ．該抽出チャネルと流体的に連通しており、所望の粒子が抽出された後の該試料ストリームの少なくとも一部を有する副産物ストリームを受ける、副産物ストリーム出口と；

ｅ．該抽出チャネルと流体的に連通しており、該抽出ストリームの少なくとも一部および該試料ストリームから抽出された所望の粒子を有する生産物ストリームを受ける、生産物ストリーム出口。

試料ストリーム入口と抽出ストリーム入口、および副産物ストリーム出口と生産物ストリーム出口は、チャネル、リザーバ、ポートまたは他の容器を備え得る。試料ストリーム入口は、「所望の粒子」、すなわち、存在が検出され得るように抽出することが望ましい粒子を含有する試料ストリームを受け取るように設計されている。試料ストリームは、抽出されない他の粒子（本明細書中においては「所望でない粒子」と呼ばれる）も含有する。これらの所望でない粒子は、所望の粒子の検出を妨害し得る粒子を含有する。好適な実施形態においては、試料ストリームは全血を含む。所望の粒子は、アルブミンまたは他の血漿要素であり得

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所望でない粒子は血球である。このデバイスは、全血から無細胞血漿を得るのに特に有用である。本発明が有用である他の流体は、異なる長さのＤＮＡフラグメントの溶液または懸濁液、あるいは、種々のサイズのタンパク質を含有する。本発明の実施に有用な試料ストリームは、発酵培養液、未処理汚水、液化食品試料、土壌試料、および痰、尿、および大脳脊髄液などの生体液を含む。

「粒子」という用語は、分子、細胞、タンパク質などの大分子、１つまたは数個の原子から構成される小分子、およびイオンを意味する。これらの粒子は、ストリームに懸濁または溶解され得る。「ストリーム」という用語は、所望の粒子および／または所望でない粒子を含有する、水または他の液体、空気または他の気体などのキャリア流体を意味する。本明細書中で用いられる「粒子」という用語は、キャリアストリームの分子を含まない。

「抽出」という用語は、所望の粒子の少なくとも１部分、すなわち、検出可能な部分を、所望でない粒子を実質的に除外して、試料ストリームから分離することを意味する。ごく微量の所望でない粒子が、抽出ストリームに輸送され得るが、このような所望でない粒子の存在は、所望の粒子を含有するストリームの検出または後続の処理を妨害しないように最小限にされる。

２つのストリームの「層流」という用語は、混じることのない、２つのストリームの安定し、平行の、再循環しないフローを意味する。再循環ゾーンは存在せず、乱れは無視し得る。当該分野に公知であるように、フローのレイノルズ数は、粘性力に対する慣性力の比率である。管を通るフローに関しては、レイノルズ数は、式Ｒｅ＝ρｄ（Ｖ／μ）を用いて計算される（Ｒｅは、レイノルズ数で、ρは、流体の質量密度、ｄは、管の形状に応じた管の典型的な横断面寸法、ｖは、管の横断面にわたる平均速度、およびμは、粘度である）。

レイノルズ数が減少するにつれ、フローパターンの粘性の影響への依存性が高まり、慣性の影響への依存性が低下する。特定のレイノルズ数（屈曲を有するチャネルシステムの管腔サイズおよび管腔サイズ変化に基づく）より低い場合、慣性の影響は、層流再循環ゾーンおよび乱流などの、慣性の影響の顕著な存在を示す現象を引き起こすには不十分である。従って、乱れのない、層状非再循環フローが、本発明書中に記載される抽出デバイスにおいて発生する。このようなデバ

イスにおいては、最小限の分散的混合が、任意の層流粘性フロー内に存在する粘性フロー速度プロファイルの結果生じる。これにより、２つの層流非再循環流体ストリームが、１方のストリームから他方のストリームへの所望の粒子抽出のために抽出チャネルを流れ落ちることが可能となる。

これらのストリームは、任意の位置にある導管の端で、出口の流出速度の正確な調節によって分離され得、これは、非再循環および不乱基準を満たしていない高レイノルズ数においては不可能なことである。

抽出ストリーム入口は、試料ストリームと接触している層流にある場合、所望の粒子を受け取り得る抽出ストリームを受け取るように設計されている。抽出ストリームは、試料ストリームから輸送される粒子を受け取り得るいかなる流体であり得る。好適な抽出ストリームは、水および生理食塩水などの等張液である。他の有用な抽出溶媒ストリームは、アセトン、イソプロピルアルコール、超臨界二酸化炭素またはエタノールなどの有機溶媒を含む。空気および他の気体も、試料および抽出ストリームとして用いられ得る。

副産物ストリームは、試料ストリームを含有し、この試料ストリームから所望の粒子の一部が抽出され、以下に記載のように、所望の粒子が試料ストリームから運ばれて行った抽出ストリームの一画分から構成され得る、または構成され得ない。

副産物ストリーム出口は、抽出チャネルから取り除かれる副産物ストリーム（試料ストリームおよびおそらくは抽出ストリームの１部から成る）を、処分、リサイクル、またはさらなる処理のために他のシステムコンポーネントに導く。

生産物ストリームは、所望の粒子が抽出されていった抽出ストリームの少なくとも一部を含む。上記のように生産物ストリームチャネルを含有し得る生産物ストリーム出口は、検出可能な量の所望の粒子を含有する生産物ストリームを、検出またはさらなる処理領域またはシステムコンポーネントへと導く。十分な量の所望の粒子を含む、十分な量の抽出ストリームが、所望の粒子の存在が当該分野に公知の手段によって生産物ストリームにおいて検出可能なように生産物ストリームに存在していなければならない。

生産物ストリームは、リザーバチャンバ、または、例えば参考として本明細書

に援用される、1994年４月19日に発行された、Wilding、P.らによる米国特許第5 304 487号に開示されるように、例えば混合、分離、分析、加熱、または他の処理によって生産物ストリームがさらに処理され得る他のデバイスに導かれ得る。

「微細製造」という用語は、シリコン微細製造の当業者にとって容易に入手可能なシリコンウェハ上に製造されることが可能で、LIGA、熱可塑性微細パターン転写、樹脂ベースの微細鋳造、毛細血管における微細造型（MIMIC）、等方性および異方性ウェットエッチング、レーザ補助化学エッチング（LACE）、および反応イオンエッチング（RIE）、または微細製造の分野内で公知の他の技術などの方法によって製造可能な特徴のあるサイズおよび形状を有するデバイスを意味する。シリコン微細製造の場合、比較的大きなウェハが、複数の構成において、本発明の複数のデバイスを収容する。若干の標準ウェハサイズは、３”、４”、６”、および８”である。新規で新生の微細製造方法を用いた、本明細書中に提示される原理の適用は、本明細書の請求項の範囲および目的の内にはいる。

試料ストリーム入口および抽出ストリーム入口は、試料ストリームおよび抽出ストリームを平行する層流へと導くのに十分な大きさに作られることのみが必要とされ、例えば、長さは約５mm以下で、深さは約100マイクロメータを下回り、幅は５mm以下であるチャネルを含み得る。副産物流出口および生産物出口は、同様に最小のサイズとなり得、上記のような試料または抽出ストリーム入口の寸法を有するチャネルを含み得る。これらの入口および出口は、それらが属するシステムによって必要とされる長さ、深さ、および幅となり得るが、好ましくは、小さな試料サイズに適応するためには、約2.5マイクロリットルを下回る容量を有する。

抽出チャネルは、試料および抽出ストリーム入口から、試料および抽出ストリームの流入を受け取り、所望の粒子を抽出ストリームへと抽出することが可能となるのに十分な距離にわたって、これらのストリームを平行する層流へと導く。

試料ストリーム入口チャネル、抽出チャネルおよび副産物流出口の幅および深さは、所望でない粒子の通過が可能となるのに十分な大きさでなければならず、好ましくは、試料ストリームの所望でない粒子の直径の約２倍～３倍の間であり、約５mm以下である。粒子サイズは、小さな有機および無機分子、そしてイオン

に

関しては１または数Å、タンパク質に関しては深さ約0.01マイクロメーター、可撓性のある長鎖分子に関しては約0.1～１マイクロメーター、赤血球に関しては約８マイクロメーター、ほとんどの白血球に関しては約15マイクロメーター、そしていくらかの白血球に関しては、最大約25マイクロメーターに及ぶ。抽出チャネルは、追加的に、吸着または吸収粒子などの、抽出ストリームにおいて用いられる粒子の通過が可能となるのに十分な大きさでなければならず、好ましくは、このような粒子の直径の約２倍～３倍の間であり、５mm以下である。抽出チャネルは、最も好ましくは、適切な期間の内に粒子輸送を達成するために、100マイクロメーターを下回る。

抽出ストリームチャネルおよび生産物出口チャネルの幅および深さは、所望の粒子およびこれらに関連する他のいかなる粒子（吸着または吸収粒子）の通過が可能となるのに十分な大きさでなければならず、好ましくは、抽出および副産物ストリームに存在するいかなる吸収または吸着粒子の直径の約２～３倍の間であり、５mm以下である。

幅寸法が、ウェハの厚み方向である場合には、本発明の微細スケール抽出デバイスのシリコン微細製造実施形態に関しては、試料、抽出、生産物、および副産物それぞれのチャネル、入口、および出口の幅は、シリコンウェハの厚み、すなわち約300マイクロメーターを下回る。

深さ寸法が、ウェハの厚み方向である場合には、本発明の微細スケール抽出デバイスのシリコン微細製造実施形態に関しては、試料、抽出、生産物、および副産物それぞれのチャネル、入口、および流出口の深さは、シリコンウェハの厚み、すなわち約300マイクロメーターを下回る。好ましくは、深さ、特に抽出チャネルの深さは、約200マイクロメーターを下回り、より好ましくは、約100マイクロメーターを下回る。

「Ｈ」設計の好適な一実施形態においては、入口および出口チャネルは、幅は、最大寸法のストリーム粒子の直径の約２倍～３倍の間であり、約100マイクロメーター、そして、深さは、最大寸法の粒子の直径の約２倍～３倍の間であり、

約100マイクロメーターを下回る。抽出チャネルは、幅は、最大寸法の粒子の直径の約２倍～３倍の間であり、ウェハの厚みの約２／３で、深さは、最大寸法の粒

子の直径の約２倍～３倍の間であり、約100マイクロメーターを下回り、長さは、最大寸法の粒子の約４倍と約１０倍との間であり、５㎜以下である。

本明細書中において「平面抽出デバイス」と呼ばれる、粒子の輸送方向が「Ｈ」設計の粒子輸送方向から９０度回転した第２の実施形態においては、入口チャネルは、好ましくは、２倍～３倍の間の粒子直径であり、約500マイクロメーターである抽出チャネルへの入口で、抽出チャネル幅と等しい幅を有し、抽出チャネルは、好ましくは、幅は、最大寸法の粒子の直径の約２倍と３倍との間であり、５㎜以下で、深さは、最大寸法の粒子の直径の約２倍と３倍との間であり、約100マイクロメーターを下回り、長さは、最大寸法の粒子の直径の約４倍と１０倍との間であり、５㎜以下である。

本明細書中で用いられる「アスペクト比」という用語は、チャネルの深さに対する幅の比を意味する。

本発明の抽出チャネルは、50を下回るアスペクト比を有する。このアスペクト比は、２５を下回る、または１より少ない数から４９までのいかなる数でもよい。５０を下回るアスペクト比を有し、100マイクロメーターを下回る深さを有する抽出チャネルを有して製造され得る本発明の微細製造デバイスは、より大きなアスペクト比を有し、より大きな抽出チャネル深さを有する類似の構造に対して多数の利点を有する。抽出チャネル内で所望の粒子の差動輸送をもたらし得る、粒子に対する輸送力は、局所フィールド勾配（local field gradient）の結果である。超短輸送距離により、短い時間の間に、所望の粒子の差動輸送が所望でない粒子よりも速く行われることが可能となり、それによって、中庸の抽出チャネル流速でデバイスに必要とされるサイズの大幅な小型化が可能となる。さらに、より低い流速が用いられ得る。

上述のサイズ範囲内にあるデバイスにより、以下の性能カテゴリーにおいて評価された場合、特有の利点が生じる。（ａ）目的を達成するための電力消費、（

ｂ）目的の達成に必要とされるデバイスのサイズ、および（ｃ）バッチ（試料対試料）モードでの微流体容量の管理および処理のための複数のシステムにおけるデバイスの集積化性。

本発明のデバイスにおいて粒子の差動輸送に用いられ得る、当該分野に公知の

フィールドの幾つかは：

・沈殿

・電気エネルギー

・温度勾配

・クロスフロー

・誘電性勾配

・せん断力

・磁力

・濃度勾配

によって生成されるフィールドである。

このようなフィールドを生成する手段は、メゾスケールまたはマクロスケールのデバイスに関連して当該分野に公知である。

本明細書中に記載されるチャネルの小さなサイズの理由により、拡散または他の手段による所望の粒子の差動輸送が、極端に急速、例えば、約300秒以内に生じ、もし所望であれば、約１秒より速く生じる。生産物および／または副産物ストリームにおいて所望または所望でない粒子の存在を検出する、またはそれらの粒子の濃度を決定する、本発明によるデバイスが製造され得、生産物および／または副産物ストリームにおいては、これらの粒子は、５分以内に生じ、もし所望であれば、４分以内、３分以内、２分以内、１分以内、１０秒以内、または１秒以内に生じる。

本発明の微細製造デバイスでは、100マイクロメーターを越えるチャネル深さを有する従来技術のより大きなスケールのデバイスと比較して、かなり小型サイズの試料、例えば、約１mL、そして下は約１ピコリットルまでの試料が処理され得、その一方で、より大型のデバイスにおいては、微小な試料がチャネル壁に吸

収され得る。さらに、フローに対する低レイノルズ数が達成され、それによって、層流が可能となり、所望の粒子の差動抽出を妨害する乱れが最小限に抑えられる、または完全に排除される。

試料ストリームにおける所望の粒子（所望でない粒子よりも大きい拡散係数を有する、または、差動輸送手段がシステムに適用されたときに、所望でない粒子に比べて、抽出ストリームに輸送されやすい）の一部は、生産物ストリームに輸送される。抽出が拡散に基づく場合、比較的小さな粒子のいくらかは、試料ストリームに常にとどまるが、生産物ストリームに輸送される所望の粒子の割合は、試料および抽出ストリームの接触時間を増やすこと、例えば、抽出チャネルの長さを増す、または流速（flow velocity）を減少させることにより、上昇され得る。単純な拡散システムに関しては、比較的小さな粒子の濃度が両方のストリームでほぼ等しい地点まで２つのストリームが接触するように、このプロセスは調節され得る。

試料および抽出ストリームは、異なる特性、例えば、粘度、密度、表面エネルギー、拡散係数、均一性、および化学組成などを有し得、これらの特性は、差動輸送速度に影響を与え得る。当業者に明白であるように、システムパラメータは、これらの異なる特性を考慮に入れるために調整され、最適化される必要があり得る。

試料および抽出ストリームは、分析可能な量の所望の粒子が抽出ストリームに輸送されることが可能となるのに十分な時間、抽出チャネルにおいて接触を保つ。デバイスから回収された生産物量は、約0.001ピコリットル／秒と約50マイクロリットル／秒以上との間であり得る。例えば、本明細書に示されるのは、約200ナノリットル／秒の生産物ストリームに対する最適流速である。当該分野で公知のように、このような小さな生産物ストリームに存在する非常に微量の分析物でさえ、分光学的および他の手段によって検出され得る。

本発明書中に記載される発明の好都合な操作は、デバイスの４つのチャネル（すなわち、試料、抽出、生産物、および副産物ストリーム）の内の３つに対する、容量流速の正確な制御を必要とする。第４のチャネルは、このチャネルを無調

節にしておくことにより、試料および抽出ストリームの混合のΔＶから生じる、試料の予測不可能な容量変化にデバイスが適応することが可能となるので、調節される必要はなく、調節されるべきではない。正確に調節された流速を達成するための手段は、当該分野に公知である。

本発明の拡散ベース抽出システムにおいて、生産物ストリームに輸送される粒子のサイズの制御を助けるため、および生産物ストリームにおいて比較的大きな粒子の出現を減少させるために、流体障壁が、抽出チャネルにおいて生成され得る。このような流体障壁は、図３に示されるように、抽出ストリームの１部が、流出する副産物ストリームと共に副産物流出口を通って流れることが引き起こされるのに十分な量で抽出ストリームが存在するときに、存在する。抽出ストリームに拡散する比較的小さな粒子は、生産物ストリームと共に流出することができる前に、この流体障壁の幅を横断しなければならない。より大きなスケールで形成されたこのような流体障壁は、参考として本明細書中に援用されている、Williams P. S.ら、（1992）、”Continuous SPLITT Fractionation Based on a Diffusion Mechanism” Ind. Eng. Chem. Res. 2172～2181に論じられている。

試料および抽出ストリームの圧力を制御することにより、抽出チャネルに入る各々のストリームからの容量比が制御され得る。試料ストリームおよび抽出ストリームの容量比は、試料および抽出ストリームに関して固定された送達圧力に対して出口および入口チャネルの形状によっても設定され得る。生産物および副産物ストリームの容量流速は、生産物および副産物ストリーム圧を操作する、または、任意のポート（入口）圧力を用い、入口のフロー抵抗を変化させることによっても制御され得る。どのような制御モードであっても、入口および出口チャネルは、上記のように処理される粒子のサイズに基づく最小チャネル寸法の基準を満たしていなければならない。抽出チャネルに入いる抽出ストリームの容量は、試料ストリームの容量よりも大きく、これら２つの流出ストリームは、同一で、流体障壁が形成される。生産物ストリームの容量流速が、抽出ストリームの全容量フローに適応するには小さすぎる場合にもまた、流体障壁が形成される。

本発明の抽出デバイスは、試料ストリームの容量に対して、抽出チャネルにお

ける抽出ストリーム容量を制御する手段を含み得、この手段は、過剰抽出ストリームを扱うのに十分な大きさの副産物ストリーム出口に連結した、全抽出ストリームの流出が可能となるのに必要とされる大きさより小さい生産物ストリーム出口を含む。本発明の抽出デバイスは、複数の生産物ストリーム出口を含み得、それによって、異なるタイプの所望の粒子を含有する生産物ストリームが回収され得る。

本発明のデバイスは、生産物ストリームにおいて所望の粒子を検出する検出手段含む分析システムに対する試料前処理システムとして利用され得る。このような手段は、生産物ストリームを、当該分野に公知の検出手段によって所望の粒子が検出されることが可能となるように所望の粒子と相互作用するインジケータストリームと混合させる手段を含む。当該分野に公知の検出手段は、光学分光デバイスなどの光学手段および吸収分光デバイスなどの他の手段、または、蛍光検出手段、分析物の所望の粒子に曝されるときに色または他の特性を変化させる化学的インジケータ、免疫手段、例えばデバイスに挿入された電極などの電気的手段、電気化学的手段、放射性手段、または磁気共鳴デバイスを含む、当該分野に公知の、実質的にいかなる微量分析技術、または、イオン、分子、ポリマー、ウイルス、ＤＮＡ配列、抗原、微生物、または他の因子などの分析物粒子の存在を検出するための、当該分野に公知の他の手段を含む。好ましくは、光学または蛍光手段が用いられ、抗体およびＤＮＡ配列などが、蛍光標識に付着される。インジケータおよび微細製造混合手段、ならびに検出および検知手段が、例えば、本明細書中に参考として援用される同時係属出願番号08/625 808に記載されている。

本発明の好適な実施形態においては、上述のような差動抽出デバイスが、生産物ストリームとインジケータ物質とを混合する拡散ベース混合デバイス（例えば、本明細書中に参考として援用される同時係属出願番号08/625 808に記載されるようなもの）、および所望の分析物粒子の存在が検出され得る検出チャンバなどの、生産物および／または副産物ストリームのさらなる処理のための手段を含む分析システムへと集積化される。これらの追加的処理手段は、好ましくは、標準シリコンウェハ上に製造される、「チップ上実験室」の差動抽出デバイスに組み

込まれる。好適な実施形態においては、このシステムは、生産物および／または副産物ストリームにおいて分析物粒子（所望または所望でない粒子）の濃度を決定する、および／または試料ストリームにおいて分析物粒子の濃度を決定する定量化手段を含む。このような手段は、分光デバイス、電位差測定、電流測定、および誘電緩和デバイスを含む。濃度決定は、当該分野に公知の手段および本明細書に開示されている手段による計算または較正によってなされ得る。

本発明の差動抽出デバイスは、所望の粒子を含み、かつ所望でない粒子も含む試料ストリームから所望の粒子の少なくとも１部を抽出する方法に用いられ、

ａ．試料ストリームを上述のような微細製造抽出デバイスの試料ストリーム入口に導入することと、

ｂ．抽出ストリームを抽出デバイスの抽出チャネルに導入することと、

ｃ．デバイスの生産物ストリーム出口から所望の粒子を含有する生産物ストリームを引き抜くことと、

を包含する。

この方法は、バッチまたは連続的モード動作で行われる。バッチモードにおいては、１mLまで、または10mL以上の試料サイズも意図されるが、試料サイズは、約１ピコリットル程であり得、好適には、約250マイクロリットルを越えず、より好適には、約50マイクロリットルを越えない。このデバイスは、10、30、または45秒、あるいは、１、２、３または４分、あるいはそれ以下のバッチ処理時間が可能となるように製造され得るが、この方法は、１秒より短い時間から約５分までの時間で完了される。

バッチ方法は、抽出デバイス内に存在する流体（気体であり得る）が、抽出および試料ストリームが流入するにつれ、試料および抽出ストリームがほぼ均衡な大量輸送状態で存在するようになるまで、抽出および試料ストリームによって置換される、起動移行期を含む。

試料および抽出ストリームが抽出チャネルにおいて接触している抽出期間が、十分な量の所望の粒子が、分析またはさらなる処理のために、抽出ストリームへと差動的に輸送されることが可能となるのに十分な期間続く。

水（または石鹸液）、空気、または、水（または石鹸液）および空気の連続的併用などの洗浄流体が、デバイスの表面上に保持され得た所望および所望でない粒子の両方を取り除くためにデバイス中を通って循環される、デバイス洗浄停止期間が、次に、必要とされ得る。

一度に単一の分離試料の処理を伴う本発明のバッチ方法は、試料ストリーム入口への副産物ストリームの再循環、および、最初の試料から取り除かれた所望の粒子量を増加させるためのプロセスの反復を含み得る。この実施形態においては、所望でない粒子の試料が生成され、この試料は、後続の分析に有用であり得る。本発明のこれらのプロセスは、所望の粒子が、実質的に試料ストリームから完全

に抽出されるまで反復され得る。

本発明の連続性モードにおいては、プロセスが、５分を越える期間の間続けられ得る。本発明の複数のデバイスが、連続性モードのために連続して構成され得、それによって、各デバイスからの副産物ストリームが、次のデバイスに対して入ってくる試料ストリームとなる。記載されたデバイスのこの連続性適用が、緻密に調節された一連の所望の粒子の希釈、および一連のデバイスの最後のデバイスから流出する際の、所望でない粒子の実質的にきれいなストリームの結果生じる。このような実施形態においては、きれいな、所望でない粒子の副産物ストリームが、上記のタイプの検出素子に経路づけられる、または、Si微細製造フローサイトメータ（例えば、本明細書中に参考として援用される、1995年９月27日に出願された米国特許出願第08/534 515号および1996年３月20日に出願された米国特許出願第08/621 170号に記載されるような、シリコンベースのＶ形溝のフローサイトメータ）などの粒子分類デバイス、カウンター、または寸法測定素子に経路づけられる、またはさらなる使用のために経路づけられ得る。例えば、連続性モードにおいては、本発明のデバイスは、透析に用いられ得、きれいな血漿ストリームが患者の体に再循環される。

図面の簡単な説明

図１は、低レイノルズ数を有する２つの入力ストリームの層流を示す微細チャ

ネル構成を示す。

図２は、試料ストリームから抽出ストリームへの比較的小さな粒子の拡散を例示する微細チャネル構成を示す。

図３は、試料ストリームと抽出ストリームとの間の流体障壁の形成を例示する微細チャネル構成を示す。

図４は、異なるサイズの粒子を分離するための複数の生産物チャネルを有する本発明の１つの実施形態を示す微細チャネル構成（一定の比例に拡大していない）を示す。黒い丸は、最大粒子サイズを表す。上部左から下部右に走る対角線は、中間のサイズの粒子を表し、下部左から上部右に走る対角線は、最小サイズの粒子を表す。

図５は、図１～４に示される「Ｈ」設計から90°回転した拡散方向を有する、微細製造平坦拡散抽出システムの斜視図を示す。

図６は、図５の微細製造平坦拡散抽出システム設計の平面図を示す。

図７は、試料、抽出、生産物、および副産物ストリームの流速を示す、抽出チャネルにおける流入口と出口との界面ストリームラインの図である。

図８は、粒子または細胞含有試料ストリームに存在する要素のアッセイに対する、本発明の「チップ上実験室」概念を示す。

図９は、水の粘度を有するキャリア流体からアルブミンを抽出するための、４mm幅のシリコンチップ上に微細製造された拡散抽出システムに対する、抽出チャネル長さ、チャネル深さおよび生産物ストリーム流速の最適化を示す。

図10は、水の粘度を有するキャリア流体からアルブミンを抽出するための、４mm幅のシリコンチップ上に微細製造された拡散抽出システムに対する、圧力差、チャネル深さおよび生産物ストリーム流速の最適化を示す。

図11は、ニュートン流体として作用するが、異なる粘度を有する、同質の、混合不可能な２つの流体の速度プロファイルを示す。点線は、水の粘度を有する流体を示す。実線は、水の粘度の３倍の粘度を有する流体を示す。

図12は、図11の流体を用いた本発明の拡散ベース抽出システムの２つの粘度のモデルと、同じ界面位置を想定するが、２つの流体において、拡散率または粘度

に差異を有さないモデルとの比較を示す。

好適な実施態様の詳細な説明

典型的な微細製造次元上において、小さな分子の拡散は急速に起こる。粒子サイズr_a、拡散係数D、および温度Tの間の関係は、アインシュタインの式に帰せられ、最も単純な場合である球体粒子の場合、これは、

$$D = \frac{k_b T}{6\pi\mu r_a}. \qquad (1)$$

と記述され得る。拡散係数Dを有する粒子が時間tの間に拡散する特徴的な距離lは、

$$l = \sqrt{Dt}. \qquad (2)$$

である。表2に、いくつかの典型的な拡散係数および特徴的な時間を挙げる。

表2：

異なるサイズを有する粒子および分子の典型的な値のいくつかである。10μmを拡散するための特徴的な時間を示す。

粒子	D(20°C)	t
0.5 μm 球体	5×10^{-9} cm²/秒	200 秒
タンパク質（ヘモグロビン）	7×10^{-7} cm²/秒	1 秒
小さな分子（フルオレセイン）	5×10^{-6} cm²/秒	0.2 秒

図1に示すように、十分小さい次元の微細チャネルにおいて慣性の効果は無視することができるため、試料ストリーム入口1を通って入る試料ストリーム2は、抽出ストリーム入口5に入り抽出ストリーム入口チャネル6から抽出チャネル7に流れる抽出ストリーム4と混ざることなしに、試料ストリームチャネル3から抽出チャネル7中へ流れ込むことができる。抽出チャネル7内の2つのストリームは、層流試料ストリーム8および層流抽出ストリーム9を形成する。

図2において、左上の矢印の方向は、試料ストリーム入口1に入った試料ストリーム2の、試料ストリームチャネル3中におけるフローの方向を示し、左下の矢印は、抽出ストリーム入口5に入った抽出ストリーム4の、抽出ストリーム入

口チャネル６中におけるフローの方向を示す。試料ストリーム２は、大きな（「所望でない」）粒子１７および、小さな（「所望の」）粒子１８（斜線で示す）を含有している。試料ストリーム２および抽出ストリーム４は、合わさって抽出チャネル７内の層流となり、層流試料ストリーム８および層流抽出ストリーム９を形成し、小さい所望の粒子１８は、層流試料ストリーム８から層流抽出ストリーム９へ拡散し始め、拡散した小さい所望の粒子１８を含有する層流生産物

ストリーム１６(laminar product stream)を形成する。層流試料ストリーム８は副産物出口チャネル１０に流れ込んで副産物ストリーム１２を形成し、副産物出口１５を通ってチャネルから離れる。層流抽出ストリーム９は、層流試料ストリーム８から拡散してきた小さな所望の粒子１８を受け取り、生産物ストリーム１６となる。生産物ストリーム１６は、生産物出口チャネル１１中において副産物ストリーム１２となり、生産物１４を通ってチャネルを離れる。

図３において、左上の矢印の方向は、試料ストリーム入口１を通って入る試料ストリーム２の試料ストリームチャネル３中におけるフローの方向を示す。左下の矢印は、抽出ストリーム入口５に入った抽出ストリーム４の、抽出ストリーム入口チャネル６中におけるフローの方向を示す。抽出ストリーム４を斜線で示す。抽出チャネル７中の上側の矢印は、層流試料ストリーム８のフローの方向を示し、抽出チャネル７中の下側の矢印は、層流抽出ストリーム９のフローの方向を示す。抽出ストリーム４の体積が、生産物出口チャネル１１および生産物出口１４を通って出ることが可能な量よりも大きいとき、層流抽出ストリーム９の一部は、副産物出口チャネル１０および副産物出口１５を通って、余剰抽出ストリーム２２として出る。この余剰抽出ストリーム２２は、抽出チャネル７内の層流フロー中にあり、流体障壁２０を形成する。試料ストリーム２中の小さな所望の粒子１８（図３には図示せず；図２を参照）は、流体障壁２０を通って、層流試料ストリーム８から層流抽出ストリーム９中に拡散し、生産物ストリーム１６を形成する（図３には図示せず；図２を参照）。

図４に、本発明の別の実施態様を示す。大きな粒子（黒点）、中間サイズの粒子（左上から右下へ向かう斜線）、および小さい粒子（左下から右上へ向かう斜

線）を含有する試料ストリーム２が、試料ストリーム入口１に入る。抽出ストリーム４が、抽出ストリーム入口５に入り、流れて、抽出チャネル７中において試料ストリーム２と出会う。大きな拡散係数を有し最も急速に拡散する小さい粒子は、試料ストリーム入口１に最も近く位置する第１の生産物出口チャネル２４中を流れる第１の流出(exiting)生産物ストリーム２５となって、第１の生産物出口２３から出る。中間サイズの粒子が抽出ストリーム中に拡散するためにはより多くの時間がかかるために、中間範囲の拡散係数を有する中間サイズの粒子は、

小さい粒子とともに、第１の生産物出口チャネル２４よりも試料ストリーム入口１から遠く位置する第２の生産物出口チャネル２７中を通る第２の流出生産物ストリーム２８となって、第２の生産物出口２６を通って出る。より小さい拡散係数を有し最も遅く拡散する大きな粒子は、小さい粒子および中間サイズの粒子とともに、第３の生産物出口チャネル３０中を通る第３の流出生産物ストリーム３１となって、第３の生産物出口２９を通って出る。また、副産物出口１５を通って出る供給流出チャネル１０中の副産物ストリーム１２は、３つのサイズ全ての粒子を含有している。

図１～４に示した実施態様に対して抽出チャネル７中の拡散方向が９０°回転した、本発明の更なる実施態様である「平坦抽出デバイス」の、図５は斜視図、図６は平面図をそれぞれ示す。この実施態様は、処理可能な物質の体積が抽出チャネル７の幅によって制限されなくなるという利点を提供する。

図５および６の平坦抽出デバイスは、シリコン基板３４をエッチングすることにより製造され、試料ストリーム入口溝３５、抽出ストリーム入口溝３６、生産物ストリーム出口溝３７および副産物ストリーム出口溝３８、ならびに抽出チャネル７を設ける。ガラスカバー３３は、抽出チャネル７を閉じる役割をする。図５において、試料ストリーム入口１に向かう下向きの矢印は、試料ストリーム１のフローを示す。同様に、抽出ストリーム入口５に向かう下向きの矢印は、抽出ストリーム４のフローを示す。生産物出口１４から上に向かう矢印は生産物ストリーム１６のフローを示し、副産物出口１５から上に向かう矢印は副産物ストリーム１２のフローを示す。抽出チャネル７の長さはＬとして示し、チャネルの幅

は黒矢印でwとして示す。抽出チャネル７の深さをｄとして示す。図６に示す開口部を備えた結合マニホルド(coupling manifold)３２は、試料ストリーム入口溝３５の深さにわたって延びることによって試料ストリームチャネル３および試料ストリーム入口１を形成し、抽出ストリーム入口溝３６の深さにわたって延びることによって抽出ストリームチャネル６および抽出ストリーム入口５を形成し、生産物ストリーム流出溝３７の深さにわたって延びることによって生産物出口チャネル１１および生産物出口１４を形成し、副産物ストリーム流出溝３８の深さにわたって延びることによって副産物出口チャネル１０および副産物流出口１５

を形成する。

図６に示す拡散（濃度勾配）によって作動する平坦抽出システム設計において、左上の矢印で示される試料ストリーム２は、試料ストリーム入口１に入り、試料ストリームチャネル３中を流れる。抽出ストリーム４は、抽出ストリーム入口５に入る矢印で示され、抽出ストリーム入口チャネル６に流れ込む。試料ストリーム２は、層流抽出ストリーム９下において、抽出チャネル７中の層流試料ストリーム８となって流れる。層流試料ストリーム８は、長さＬにわたって抽出チャネル７中の層流抽出ストリーム９と接触する。層流抽出ストリーム９中の点で示される層流試料ストリーム８からの小さい（「所望の」）粒子は、生産物ストリーム１３となって生産物出口チャネル１１に流れ込む。生産物ストリーム１３は、生産物出口１４において上向きの矢印で示されるように流出する。副産物ストリーム１２は、生産物ストリーム１６を越えた層流試料ストリーム８の続きであり、生産物ストリーム１６中に拡散しなかった、大きな（「所望でない」）粒子と、小さい（「所望の」）粒子の一部との両方を含有する。副産物ストリーム１２は、副産物出口チャネル１０中を通って流れ、副産物出口１５を通って流れ出す。

本明細書において説明する原理に従って適切なチャネル長さが得られるようにチャネルの配置を調整することにより、流速および試料ストリームと抽出ストリームとの接触時間、ならびに試料ストリーム中に残って生産物ストリーム中に拡

散する粒子のサイズを制御することができる。必要な接触時間は、粒子の拡散係数Ｄ（一般に粒子の線形サイズ(linear size)で変化する）の関数として計算され得る。また、粒子が拡散するべき距離ｄは、ｔ＝ｄ^2／Ｄによって計算され得る。Ｄより大きい拡散係数を有する粒子または分子は、流出生産物ストリーム中に存在し、Ｄより実質的に小さい拡散係数を有する粒子または分子は、流出生産物ストリーム中に存在しないことになる。分離される大きい粒子の拡散係数がＤより約１０倍小さければ、生産物は大きい粒子をほぼ全ったく有さないはずである。

単純な計算により、Ｄ＝w_{fb}^2ｖ／Ｌより小さい拡散係数を有する粒子または分子はほとんど流出生産物ストリーム中に見いだされないことがわかる。ここで、w_{fb}は流体障壁の幅であり、ｖは層流試料ストリームの平均流速であり、Ｌは抽出チャネルの長さである。Ｄ＝ｗ^{2}ｖ／Ｌより大きい拡散係数を有する粒子または分子は（ｗは抽出チャネルの幅である）、副産物ストリーム中と同じ濃度で流出生産物ストリーム中に存在することになる。

本発明のデバイスを分析システムの一部として用いる場合などにおける、供給液体をデバイスに注入するための手段が提供される。このような手段は、標準的なシリンジおよびチューブを包含する。毛管現象、圧力、重力その他上述の分野で公知の手段によって流れ出す流体の受け容器(receptacle)を備える、生産物流出口から流体を除去するための手段もまた、提供され得る。そのような受け部材は、生産物ストリームをさらに処理するための分析その他のデバイスの一部であってもよい。

図７は、抽出チャネル７を示すとともに、速度$\dot{V}_{cs}$で移動する層流抽出ストリーム９と、速度$\dot{V}_{ss}$で移動しストリーム高さ（拡散方向座標）Z_sを有する層流試料ストリーム８とを示す（抽出チャネル７の入口近傍において層流試料ストリーム８と層流抽出ストリーム９との間の界面ストリームライン位置（点線）を規定する）。両ストリームを合わせた高さ、すなわち抽出チャネル７の深さを、ｄとして示す。曲線は、速度プロフィール(velocity profile)の形状を表す。こ

れらストリームが抽出チャネル７の長さ方向に沿って移動するにつれ、層流試料ストリーム８は、速度$\dot{V}_{bps}$で移動し、副産物ストリーム１２と生産物ストリーム１３との間の界面ストリームライン位置（点線）を規定するストリーム高さ（拡散方向座標）Z_pを有する、副産物ストリーム１２になる。層流抽出ストリーム９は、速度$\dot{V}_{ps}$で移動する生産物ストリーム１６になる。

流体混合物の化学的アッセイで普通に行われるいくつかの工程は、（１）正確な混合物の希釈；（２）特定の構成要素の抽出；（３）インジケータ試薬または試験プローブ（例えば蛍光標識ポリマービーズ）の混合および；（４）インジケータまたはプローブの非侵襲的検出（例えば吸光度または蛍光分光分析）である。

本発明の抽出デバイスは、例えば図８に示す、微細製造された「チップ上実験室(lab-on-a-chip)」などの、トータルな分析システム内に集積(integrate)されてもよい。

図８は、１個のシリコンウェハ上に製造された、本発明の拡散ベースの抽出デバイス１００を示す。試料ストリーム流速$\dot{V}_{ss}$および試料ストリーム要素ｉ濃度$C_{i,ss}$を有する試料ストリーム２は、抽出ストリーム流速$\dot{V}_{es}$を有する抽出ストリーム４とともに、拡散ベースの抽出デバイス中に流れ込む。副産物ストリーム流速$\dot{V}_{bps}$および副産物ストリーム要素ｉ濃度$C_{i,bps}$を有する副産物ストリーム１２は、システムから除去される。生産物ストリーム流速$\dot{V}_{ps}$および生産物ストリーム要素ｉ濃度$C_{i,ps}$を有する生産物ストリーム１３は、同じチップ上に微細製造された拡散ベースの混合デバイス４３中に流れ込む。また、インジケータ色素ストリーム流速$\dot{V}_{ind}$およびインジケータ色素濃度$C_{dyc,ind}$を有するストリームインジケータ色素ストリーム３９も、拡散ベースの混合デバイス

４３中に流れ込む。検出ストリーム(detector stream)４０は、拡散ベースの混合デバイス４３から流出して検出チャンバ中に流れ混み、光学的検出手段４１が作動して、検出ストリーム４０が検出チャンバ中４４にある間に、好ましくは蛍光シグナルであるシグナル４２を検出する。次に、検出ストリーム４０は、検出ストリーム流速V_{ds}、検出ストリーム要素ｉ濃度$C_{i,ds}$およびインジケータ色素濃度$C_{dyc,ind}$にて、検出チャンバ４４から流出する。

図８に示す検出ストラテジーは、粒子を含んだ(particulate laden)試料からの要素抽出、希釈された分析物と蛍光インジケータとの混合、および蛍光光学的検出を必要とする。推定技術の正確な運用に重要なのは、システム中の全てのストリーム流速を正確に調節することである。蛍光強度と要素濃度との間の較正、ならびに要素抽出物およびインジケータ混合希釈比を正確に規定している情報を用いて、元の試料ストリーム中の要素の濃度を見積もる。完全なシステムはまた、データ縮小(data reduction)、圧力調節、および廃棄物回収を含む。集積化トータル分析システム中の正確なフロー制御は、その一部分、オンチップマイクロポンプを用いて達成し得る（Gravesen, P.ら(1993)、「Microfluidics - a review」、J. Micromechanics and Microengineering 3(4)：168－182；Elwenspoek, M.ら(1994)、「Towards integrated microliquid handling systems」、J. Micromechanics and Microengineering 4(4):227-245；およびForster, F.K.ら(1995)、「Design, Fabrication and Testing of Fixed-Valve Micro-Pumps」、ASME International Mechanical Engineering Congress & Exposition、サンフランシス

コ、ASME）。

抽出システムの、実施例で説明した図２などのような「Ｈ」型設計と、図５および６の平坦抽出システムとの両方において、拡散要素は、抽出ストリーム４中に泳動し、抽出チャネル７全体にわたってほぼ均一な濃度になる傾向がある。試料流速、抽出流速および副産物流速は、外部的に調節されることにより、生産物ストリーム流速を固定する。本明細書の実施例に説明するように、製造される図２の設計において、拡散方向のチャネル寸法（ｄ）は、実施例においては100μｍ

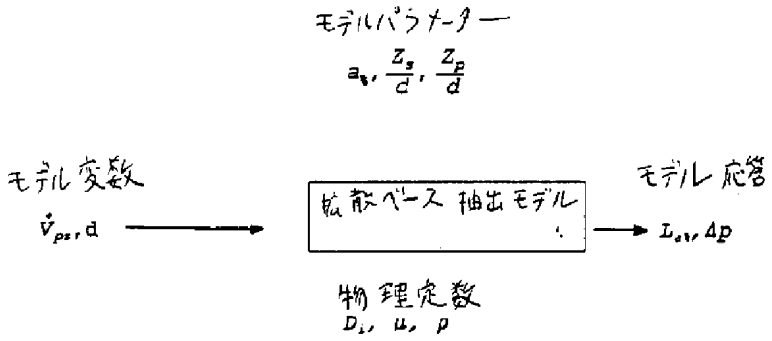
未満であり、拡散方向およびフロー方向に対して垂直方向のチャネル寸法（ｗ）をチャネル深さ（ｄ）で割ったものと規定されるアスペクト比は、１未満である。図５および６の平坦拡散抽出システムにおいて、アスペクト比ｗ／ｄ（ｄはやはり約100μｍ未満である）は、１より大きいが、５０よりずっと小さい。

抽出中の要素が、微細チャネルの断面全体にわたって固定パーセントの平衡濃度内の濃度を達成するために必要な距離を、平衡化長(equilibration length)と定義する。微細チャネル内の要素濃度は、１－Ｄ分析拡散モデルを用いて計算される。平衡化長は、抽出される要素に対して特異的な、一連のプロセス空間設計曲線(process space design curves)を構築するために用いられる。最適化目的関数(optimization objective function)は、生産物ストリームの体積流速を、シリコンチップ上に微細製造されたシステムの課する制約内において最大化する設計を同定するために、規定される。

ほぼ水の粘度を有するキャリア試料ストリームからアルブミン（ヒト血液中に存在するタンパク質要素）を抽出するための最適なデバイスの設計に、この方法論を適用する。全血は典型的には、体積にして４０～５０％の赤血球(RBC)成分（RBCは楕円体形状および８μｍの主軸寸法を有する）および、約１５～２５μｍの呼び直径を有する白血球を有する。本説明において、単一の粘度、単一の拡散性プロセスモデルを考慮することにより、分析を単純化している。複数粘度の場合に関する考慮は後に述べる。ここで述べるデバイスは、１％の平衡化長についての特定のものである（無限長のデバイスに対してアルブミン平衡化濃度１％内）。このプロセス感度情報は、上流および下流の流動成分(fluidic component)に対する設計要求を提供し、デバイスを「チップ上実験室」化学分析システム

に集積するためには必須である。

プロセスモデルは、そのパラメータ、物理定数、独立変数、従属変数、およびプロセスをモデル化するために用いる式によって規定される。本文において検分する抽出プロセスを、以下に図示する。



物理定数は、デバイスの設計またはその制御のいずれによっても変化し得ない。上に示した３つの物理定数がある。すなわち、２成分要素拡散性(binary constituent diffusivity)D_i、粘度μ、および密度ρである。定数パラメータは、完全な要素平衡に対する所望のパーセンテージa_g、正規化(normalized)試料－抽出ストリームライン界面位置z_s/d、および正規化副産物－生産物ストリームライン界面位置z_p/dである。変数モデルパラメータは、生産物ストリーム流

速$\dot{V}_{ps}$および拡散方向チャネル深さｄである。この定義に基づき、モデル出力は、a_gを達成するために必要なチャネル長L_{ag}、およびフロー方向における、抽出チャネルにわたる圧力差Δｐである。

抽出プロセスの、２－Ｄフローおよび要素輸送モデルを述べる。一般的な３－Ｄ輸送問題を述べることから説明を始める。次に、２－Ｄ近似に対して単純化仮定事項を定義し、これらを適用する。次に、結果として得られる記述的モデル化式の解と関連する境界条件との解を、非粘性フローの場合について述べ、粘性フローの数値解の場合について述べる。

一般的３－Ｄ質量輸送モデル式。拡散および対流輸送の両方による要素の輸送を記述する一般式は、以下の通りである（Cussler, E.L. (1984)、Diffusion, Mass Transfer in Fluid Systems、Cambridge, Cambridge University Press）：

$$\frac{\partial c_i}{\partial t}+v_x\frac{\partial c_i}{\partial x}+v_y\frac{\partial c_i}{\partial y}+v_z\frac{\partial c_i}{\partial z}=D_i\left[\frac{\partial^2 c_i}{\partial x^2}+\frac{\partial^2 c_i}{\partial y^2}+\frac{\partial^2 c_i}{\partial z^2}\right]+r_i \qquad (3)$$

上式において、C_iはｉ番目の要素の濃度であり、D_iはｉ番目の要素の２成分拡散係数であり、$v_x v_y v_z$は速度ベクトル成分であり、r_iはｉ番目の要素の、混合物中における化学反応による生成速度である。

２－Ｄ定常フロー近似。本説明において用いるモデル化仮定事項を表す数学的関係を、式４に示す。

$$\frac{\partial C_i}{\partial t}=0, \qquad 4(a)$$

$$v_y=v_z=0, \qquad 4(b)$$

$$\frac{\partial^2 C_i}{\partial x^2}=\frac{\partial^2 C_i}{\partial y^2}=0, \qquad 4(c)$$

$$r_i=0 \qquad 4(d)$$

式４(a)は、定常状態のデバイス動作を仮定したものを表す。抽出デバイスはダイナミックな動作を意図しているが、最終構成設計構成を目標として定常状態の動作を用いる。フローは、式４(b)に反映されるように、単一の座標方向で起こる。２つの議論を用いることにより式４(c)は正当化される。すなわち、（１）拡散の空間的スケールは、チャネルフロー方向（ｘ座標）よりも、拡散抽出方向（ｚ座標）において、１桁小さい（距離１にわたって拡散するために必要な時間は、l^2/Dに比例する）；（２）チャネル幅方向（ｙ座標）における拡散により、粘性フローの場合の濃度プロフィールは平坦になる傾向があり、解が、

同一の平均流速を有する非粘性フローの場合における拡散により近く近似される。本説明において問題にするアッセイについて、フローストリーム中の種の変化を反映する化学的平衡動力学は存在しないため、式４(d)は正当化される。これは常に成り立つわけではない。式４を式３に適用することにより、以下のように単純化された関係が得られる。

$$\frac{\partial C_i}{\partial x}=\frac{D_i}{v_x}\frac{\partial^2 C_i}{\partial z^2}, \qquad (5)$$

無次元形態(non-dimensional form)。以下の変数の無次元的変化(non-dimensional change)を定義することにより、式５を試料ストリーム要素濃度および拡散

チャネル深さに関して正規化することができる。

$$\tilde{c}_i = \frac{c_i}{c_{i,0}}, \quad \tilde{x} = \frac{x}{d}, \quad \tilde{z} = \frac{z}{d}, \qquad (6)$$

上式において、$c_{0,i}$は、試料ストリーム中の要素ｉの濃度であり、ｄはチャネル深さである。式６を式５に代入することにより、以下が得られる。

$$\frac{\partial \tilde{c}_i}{\partial \tilde{x}} = \left[\frac{D_i}{v_x d}\right] \frac{\partial^2 \tilde{c}_i}{\partial \tilde{z}^2}. \qquad (7)$$

式７の括弧内の項はペクレ数の逆数である。ペクレ数は、対流質量輸送の、拡散質量輸送に対する相対的な意義の、有用なゲージを提供するものであり、以下のように定義される。

$$Pe = \frac{v_x d}{D_i} = \frac{\text{対流輸送}}{\text{拡散輸送}} \qquad (8)$$

濃度は従って、正規化された位置とペクレ数との関数である。

$$\tilde{c}_i(\tilde{x}, \tilde{z}, Pe).$$

定常フロー流入(entrance)境界条件。抽出デバイスの入口において試料および抽出ストリームを分離するストリームラインの位置は、Z_sである。抽出チャネル入口における境界条件$\tilde{x}=0$は、一定である。抽出ストリーム正規化濃度はゼロであり、

$$\tilde{c}_i(0, \tilde{z}) = \begin{cases} 1, & 0 < \tilde{z} < \frac{Z_s}{h} \\ 0, & \frac{Z_s}{h} < \tilde{z} < 1 \end{cases} \qquad (9)$$

となる。

無限長チャネル遠距離領域(Far Field)境界条件。遠距離領域境界条件は、無限に長い抽出チャネルを想定することにより定義される。そのようなチャネルについては、チャネル断面にわたって全ての拡散要素が平衡化しなければならない。従って、

$$\tilde{c}_i(\infty, \tilde{z}) = \xi \qquad (10)$$

である。上式において、ξは平衡正規化濃度である。正規化平衡濃度は、以下のように与えられる。

$$\xi = \frac{\dot{V}_{ss}}{\dot{V}_{ss}+\dot{V}_{es}}. \qquad (11)$$

非浸透(impermeable)チャネル壁境界条件。デバイスが定状動作する間、デバイス表面上の要素の吸着は、平衡化したものと仮定されるため、デバイス境界によって規定されるコントロール表面における質量フラックスは、ゼロである。このことからフィックの法則を用いて、境界における濃度勾配はゼロでなければならず、

$$\frac{\partial \tilde{c}_i(\tilde{x},0)}{\partial \tilde{z}} = \frac{\partial \tilde{c}_i(\tilde{x},1)}{\partial \tilde{z}} = 0. \qquad (12)$$

となる。

非粘性フロー（プラグフロー）。非粘性フローを仮定すると、z方向におけるチャネルにわたる速度は、一定になる。このモデル化近似により、試料ストリームと抽出ストリームとの間のストリームライン界面の位置は、

$$\frac{z_s}{d} = \xi. \qquad (13)$$

として与えられる。式９、式１０、式１２およびストリームライン界面位置（式９）によって与えられる境界条件に従った式７の解は、以下のように導出され与えられる。

$$\tilde{c}_i(\tilde{x},\tilde{z}) = \xi + \sum_{n=1}^{\infty} \frac{2}{\pi(2n-1)} \sin[(2n-1)\pi\xi]\tilde{x} \exp\left[-(2n-1)^2\pi^2\left(\frac{D_i}{V_x d}\right)\tilde{x}\right]\cos[(2n-1)\pi\tilde{z}]. \qquad (14)$$

式１４は、変数分離の方法を用いて導出した。この方法の詳細な記載およびその物理システムにおける応用については、Folland, G.B. (1992)Fourier Analysis and its Applications、Pacific Grove Wadsworth&Brooks/Cole Advanced Books and Softwareを参照のこと。

粘性フロー単一粘性流体。ある１つの粘性フロー速度プロフィールについての

、試料ストリームと抽出ストリームとを分離するストリームラインの位置は、質量保存を用いて達成される。単一粘性流体ストリームの速度プロフィールは、以下のように与えられる。

$$v_x(z) = -\frac{d^2}{2\mu}\frac{dP}{dz}\left[\left(\frac{z}{d}\right)-\left(\frac{z}{d}\right)^2\right]. \qquad (15)$$

深さｄおよび幅ｗのチャネルにおける全体積フローは、試料ストリーム流速と抽出ストリーム流速との和に等しい。速度プロフィール的によって、この正味チャネル流速は以下のように与えられる。

$$\dot{V}_{ss}+\dot{V}_{es} = w\int_{z=0}^{z=d} v_x(z)\,dz = -\frac{d^3b}{12\mu}\frac{dP}{dz} \qquad (16)$$

抽出チャネルの試料ストリーム部分における体積流速は、以下のように与えられる。

$$\dot{V}_{ss} = w\int_{z=0}^{z=z_s} v_x(z)\,dz \qquad (17)$$

上式において、z_sは、試料ストリームと抽出ストリームとを分離する平衡ストリームラインの位置である。ある１つの粘性フロープロフィールに対して、全試料ストリーム体積フローは、領域$0<z<z_s$中になければならない。式１７を式１６および１５を用いて解くことにより、以下の３乗関係式が得られる。

$$2\left(\frac{z_s}{d}\right)^3 - 2\left(\frac{z_s}{d}\right)^2 + \xi = 0. \qquad (18)$$

任意の便利な平方根解法を用いることにより、試料ストリームと抽出ストリームとを分離する分離ストリームラインの位置z_sを決定することができる。

非粘性フローを仮定することに関連する誤差を調べるために、２－Ｄ数量モデルを書き用いることにより、非粘性フローモデルによって示唆される「最適」設計のフロープロフィールを分析した。数量シミュレーションモデルにおいて、式の解は以下のように与えられる。

$$\frac{\partial^2 \bar{c}_i}{\partial \bar{z}^2} = \left[\frac{v_x(\bar{z})\,d}{D_i}\right]\frac{\partial \bar{c}_i}{\partial \bar{x}}, \qquad (19)$$

上式におけるペクレ数は、今度は、粘性流速プロフィールによるフローチャネル

内の位置の関数である。$\tilde{z}$の中心差分(centered finite difference)および$\tilde{x}$の上流差を用いて、上式を数量的に解いた。$\tilde{z}_s = z_s$ d＝0.5について、同一の正味チャネル流速に関して、必要抽出チャネル長の２０％の縮小が観察された。従って、非粘性仮定を用いて設計曲線を発生させることにより、抽出に必要なデバイスのサイズの控えめな(conservative)計算値が得られるはずである。

最適化目的関数(optimization objective function)。この設計最適化問題の目標は、生産物ストリームの、単位フィルターチャネル幅ｗ毎の体積流速を最大化することであった。この設計目的を記述する関数は、以下のように与えられる。

$$maxF(d, L_{a\%}) = \hat{V}_{ps}(d, L_{a\%}). \qquad (20)$$

上式において、ｄはチャネル深さであり、$L_{a\%}$はａ％平衡化長である。式２０は、設計目的を記述し、そして最大のデバイススループットを確実にする。他のアプリケーションにおいて、多目的(multiobjective)設計目的関数を用いた、競合する設計目的を考慮してもよい。この場合、主観的重み付け(weight)を用いて競合する設計目的を順序付けることにより、複合多目的関数を形成する。ミクロスケールにおいては、特定のアプリケーションにおいて、体積流速の単位デバイス体積に対する比を最大にする一方で、同時に、微細流動デバイス(micro－fluidic device)の単位デバイス体積に対する表面積を最小化すること（あるいは単位表面積に対する体積流速を同等に最大化する）が、有効であろう。これらの比は主として、いかなるデバイス設計にも直接結合する(couple)、拡散方向深さの関数である。加えて、デバイスを実現するために必要なシリコン実面積(silicon real estate)を同時に最小化することもまた必要になり得る。同時に最適化されなければならない各設計目的について、追加的な主観的重み付けが必要になる。適切な重みの選択は、設計配置によって変化する。

設計制約条件。微細流動デバイスを作成するために用いられるシリコンウェハは有限のサイズを有するため、最大可能フィルタ長には実用的制限が存在する。

$a_{\%}$平衡長L_aは、最大実用フィルタ長L_{max}未満でなければならない。すなわち、

$$L_{a=1\%} < L_{max}. \qquad (21)$$

である。同様に、試料ストリームおよび抽出ストリーム中に存在するいかなる粒子も、チャネルにおいて幾何学的制約のためだけに抽出ストリームを外れる(violate)ことがないように、チャネルは十分に深くなくてはいけない、$d > d_{min}$。さらに、チャネルは、シリコンウェハの強度が過度に妥協されるほど深くてはならない、$d < d_{max}$。これらの２つの制約条件を組み合わせることにより、以下の単一の制約条件式が得られる。

$$d_{min} < d < d_{max}. \qquad (22)$$

最後に、抽出およびそれに続く分析動作の１セットを完了するために許される最大時間により、そのデバイスにおける容認可能な最小の生産物ストリーム流速が決定される。すなわち、

$$\dot{V}_{ps} > \dot{V}_{ps,min}. \qquad (23)$$

である。図９および１０は、a_g＝１％用に設計された一連の拡散抽出デバイスの、プロセス空間を表す。

図９は、１％の平衡化長を達成する、全血からアルブミンを抽出するための、４mm幅の平行フロー拡散抽出デバイスのための設計空間を示す。設計空間は、試料ストリームと抽出ストリームとの間に１：１のフロー比を仮定し、流体粘性10^{-3}[Pas]および流体密度10^{3}[kg/m^3]を仮定して計算されている。この研究に用いた食塩水溶液中のアルブミンの拡散係数は、$D_{alubumin}$＝７・10^{-11}[m^2/s]である。

物理定数は、D_i＝７・10^{-11}m^2/s（アルブミン）、μ＝１０$^{-3}$Ｐａ／ｓ（水）、およびｐ＝10^3ｋｇ／ｍ^3（水）である。これらの性質は、ある１つの

アルブミンの希釈水溶液に関して不変である。これらの定数は、他の化学アッセイを考慮する場合にのみ変化する。この設計最適化について固定に選択されたパラメータは、ａ＝１％、z_s／ｄ＝0.5、およびｗ＝４mmである。これらの値は、本アプリケーションを表すものとして選択されたのであり、特定の目的の達成のために変更し得る。例えば、総フロースループットを増加するために、チャネル

幅を大きくすることが可能である。

図９において、領域Ａは、プロセスにおける制約を受けるパラメータを図示しており、この領域の右上の大きい黒点はチャネル長40mm、チャネル深さ５０μｍおよび生産物ストリーム流速(v_{ps})約0.23μl/sであり、最も最適な設計を示している。40mmより大きいチャネル長さを必要とする領域Ｂは、これらのチャネル長さがシリコンチップの40mm幅を越えるため、最適領域外である（$L > L_{max}$）。必要チャネル深さが100μｍを越える領域Ｃは、チャネル深さが効率的な拡散に許容される深さを越えるため、最適設計範囲外である（$d > d_{max}$）。チャネル深さが50μｍ未満である領域Ｄは、通常の細胞要素を通過させるにはチャネルが浅すぎるため、最適設計範囲外である（$d < d_{min}$）。生産物ストリーム流速が０から約0.10μl/sである領域Ｅは、生産物流速が小さすぎるため、最適設計範囲外である（$Q_{product} < Q_{product.min}$）。

図１０は、図９において示した条件における最適設計パラメータを、フロー方向の抽出チャネルを横切る圧力差について示している。図９に説明したように流速およびチャネル深さに関して定義された領域Ａが、最適設計領域である。この領域の右上の大きい黒点はやはり、圧力差0.5kPaにおける最適設計を示している。

図示の平衡化長（$L_{a=1\%}$）は、所与のチャネル深さ（ｄ）における$\dot{V}_{ps}$の線形関数である。式１４は、$\tilde{x}$に対する濃度の指数的減衰を示している。拡散性は問題になる所与の要素に対して定数であるので、v_xおよびｄが指数的減衰の割合を制御する。ファクター$1/Pe = D_t/\dot{v}_x d$は、時定数のように作用する。ｄが減少するにつれてv_xが増加して同じ1／Peが得られるようになるとすれば、$L_{a\%=1\%}$は一定になる。所与のｄについて$\dot{V}_{ps}$が線形的に増加するにつれ、v_xは比例して増加し、1／Peが線形的に減少するために$L_{a\%=1\%}$は線形的に増加する。対流は拡散に対してより重要になっており、平衡に達するためにはより長いチャネル長が必要である。

所与の平衡化長における流速を最大化するためには、制約されたプロセス空間の右上のすみに行き、小さなチャネル深さ（図９）および高圧力差（図１０）で動作させることになる。領域要求を最小にするためには、図１０の左下においてずっと低い圧力差で動作させるように設計するとよい。表面効果を避け得る限り、ｄをできるだけ減らすべきである。

以下の議論において考慮している２つの流体は、異なる粘性を有しておりかつ均質で非相溶性の、ニュートン流体として振る舞う流体である仮定する。２粘性の場合をモデル化して設計パラメータおよび結果を得るために、３つの別々のステップが必要である。以下において、試料ストリームは領域２として示され、抽出ストリームは領域１として示す。領域１の絶対粘性の領域２のそれに対する比はｍであり、領域１方向における、半チャネル幅の部分としての中間チャネル(mid-channel)からの流体界面位置は、αである。抽出チャネルの高さは２ωとしている。第１のステップは、両ストリームにわたる速度プロフィールをｍおよびαについて計算することである。第２のステップは、速度プロフィールを用いて、

αの数値および、所与の体積流速$\dot{V}_{es}/\dot{V}_{ss}\equiv F$における各ストリームの平均

速度の比を決定することである。第３のステップは、界面(inerface)の位置、各ストリーム中の平均速度、および各ストリーム中の問題の粒子の拡散係数に基づいて拡散式を解くことである。

第１のステップを完成するために、長方形のダクト中のニュートン流体の１次元２相のよく発達した(fully-developed)安定なフローについて、ナビエ・ストークス(Navier-Stokes)式を解くことにより、軸速度プロフィールｕ(ｚ)を決定する。この場合の式は、以下のように縮小される（White, F.M.（1994）Fluid Mechanics）：

$$\nabla p+\mu\nabla^2 u = 0. \qquad (24)$$

結果として得られる、$\omega^2\Delta p/\mu_1 L$によって無次元化(non-dimensionalized)され中央チャネルから領域１への測定されたｚ＝ｚ／ωによる速度プロフィール

は、

$$\bar{U}_1(\bar{z}) = \frac{1}{2}\left(-\bar{z}^2 + \frac{\bar{z}(\alpha^2 m - \alpha^2 + 1 - m) - \alpha^2 m + 2m + \alpha^2 - \alpha + m\alpha}{m + m\alpha - \alpha + 1}\right) \quad \alpha < \bar{z} < 1 \qquad (25)$$

および

$$\bar{U}_2(\bar{z}) = \frac{1}{2}\left(-m\bar{z}^2 + \frac{m\bar{z}(\alpha^2 m - \alpha^2 + 1 - m) + m(\alpha^2 m - \alpha^2 - \alpha + m\alpha + 2)}{m + m\alpha - \alpha + 1}\right) \quad -1 < \bar{z} < \alpha, \qquad (26)$$

によって与えられる。

第２のステップは、以下の式におけるαを解くことにより、αの数値をＦの特定の値について計算し、

$$P = \frac{\int_{\alpha}^{1} \overline{U_1} d\bar{z}}{\int_{-1}^{\alpha} \overline{U_2} d\bar{z}}, \qquad (27)$$

そして、このαの値を用いて、

$$\frac{\overline{U_1}(1-\alpha)}{\overline{U_2}(1+\alpha)} = F \quad . \qquad (28)$$

から各領域の平均フローの比を計算することである。

最後のステップは、式（９）、（１０）および（１２）によって与えられる境界条件ならびに、濃度の連続性および界面における拡散種の質量保存を要求する更なる２つの界面条件に従って、各領域中における拡散式（７）を解くことである。界面から領域１への測定されたｚを用いて、これらの条件は、

$$\bar{c}_{i1}(\bar{x}, 0+) = \bar{c}_{i2}(\bar{x}, 0-), \qquad (29)$$

および

$$D_1 \frac{\partial \bar{c}_{i1}(\bar{x}, 0+)}{\partial \bar{z}} = D_2 \frac{\partial \bar{c}_{i2}(\bar{x}, 0+)}{\partial \bar{z}} \quad . \qquad (30)$$

である。

これによって得られる、チャネル全体にわたる質量濃度に関する式は、

$$\bar{c}_1(\bar{x}, \bar{z}) = \xi + \sum_{n=1}^{\infty} K_n f_n(\bar{z}) \exp(-\lambda^2 \bar{x}/Pe_1) \qquad (31)$$

によって与えられ、上式において$\tilde{x}=x/\omega$であり、固有関数$f_n(\tilde{z})$は、

$$f_n(\tilde{z}) = \begin{cases} \cos k\lambda_n\beta_2 \cos\lambda_n(\tilde{z}-\beta_1) & 0<\tilde{z}<\beta_1 \\ \cos\lambda_n\beta_1 \cos k\lambda_n(\tilde{z}+\beta_2) & -\beta_2<\tilde{z}<0 \end{cases}, \qquad (32)$$

によって与えられ、固有値λ_nは、以下の特徴式の解であり、

$$\tan\lambda_n(\beta_1) + \sigma\tan k\lambda_n(\beta_2) = 0, \qquad (33)$$

定数K_nは以下によって与えられ、

$$K_n = 4\frac{-\xi\cos k\lambda_n\beta_2\sin\lambda_n\beta_1 + (1-\xi)\sigma\cos\lambda_n\beta_1\sin k\lambda_n\beta_2}{\cos^2 k\lambda_n\beta_2(\sin 2\lambda_n\beta_1 + 2\lambda_n\beta_1) + \sigma\cos^2\lambda_n\beta_1(\sin 2k\lambda_n\beta_2 + 2k\lambda_n\beta_2)} \qquad (34)$$

ここで、$\beta_1=1-\alpha$、$\beta_2=1-\alpha$、$k=\sqrt{Pe_2/Pe_1}$、かつ$\sigma=k(D_2/D_1)$である。

上述の技術を異なる粘性のストリームについて使用することの例として、抽出ストリーム（１）が水であり、抽出ストリーム（２）が水の粘性の３倍を有する流体である場合を考える。さらに、体積流速の比が等しく、Ｆ＝１である場合を考える。さらに、$m\simeq 1/3$、かつ$D_2/D_1\simeq 1/2$であると仮定する。上式からα＝0.0960、$\overline{U}_1/\overline{U}_2=1.21$であり、チャネルを横切る速度プロフィールは図１１に示される。図１２において、これらの流体の２粘性モデルおよび、同じ界面位置を仮定しながら各ストリーム中に粘性または拡散性の差がないモデルの比較を示している。チャネル高さにわたる濃度の比較は、抽出チャネルの上流端近く（$x/w/Pe_1=0.01$）および比較的遠くの下流（$x/w/Pe_1=1.0$）で行った。２粘性計算値は実線の通りであり、単純な１粘性計算値は点線の通りである。特に、下流位置において曲線間に大きな差があることに注意されたい。これらの結果は、各ストリーム中において異なる粘性を有する流体を用いた場合における、差動抽出デバイスの設計および定量的使用に関する上述の技術の重要性を示している。

実施例

本発明のデバイスを作成するための好適なプロセスにおいて、１μm厚さの含水熱性酸化物(wet thermal oxide)を３”のシリコンウェハ中において成長する。この酸化物をフローチャネルを有するようにフォトリソグラフ的にパターニングし、深さ60nmにエッチングする。ウェハに再びフォトレジストを塗布し、スルーホール接続部を有するようにパターニングする。このパターンから酸化物を完全に除去する。EDPエッチングにより、ウェハを完全に通してエッチングする（約400μm）。酸化物エッチングを行うことにより、酸化物400nmを水から均一に除去する。フローチャネルをシリコン内に約10μmの深さでエッチングする。最後に、ウェハを、Pyrex glassの３”ディスクにアノード結合させる。

以下の例は、シリコン中に微細製造されたミクロンサイズのデバイスを用いて、粒子を含有する試料ストリームから拡散要素を分離するための拡散ベースの抽出を示している。図２を参照せよ。0.5μmの蛍光ポリスチレン球およびフロオレセイン色素を含有する試料ストリームから、フロオレセイン色素を抽出した。この動作は、蛍光球による抽出ストリームの汚染がゼロであることを示した。このデバイスは、総抽出チャネル流体体積が約１フェムトリットル(femtoliter)であった。本例は、正確なフローストリーム調節に適切に注意を払えば、フェムトリットルスケールでの分離が可能であることを示している。また、アスペクト比が50よりずっと小さい抽出チャネルおよび、拡散方向寸法が100μmよりずっと小さいチャネルにおいて、効率的な分離が可能であることを示している。w/d＜＜50、ｄ＜100μmの抽出デバイスは、シリコン微細製造技術および超低レイノルズ数フ

ロー(ultra-low Reynolds number flow)の本質的属性を用いて製造された微細流動システムの有効性を示した。

デバイスを製造するために、２マスクレベルのプロセスが必要であった。第１のレベルは接続ポートを規定し、ウェハをシリコンの背面まで完全に貫通してエッチングされた。第２のレベルは、流体輸送チャネルを規定した。

我々の規格に基づき、４インチクロームマスクおよび500nmのSiO_2を上に成長

した3"ウェハ（｛100｝、n型）が、Photo Sciences, Inc.（カリフォルニア州Torrance）により作成された。

処理前に、ウェハをピラニヤ浴（H_2SO_4およびH_2O_2）（2：1）で清掃した。プライマー（300rpmで回転されたHMDS）を用いてフォトレジスト接着を高めた。約1μmのAZ-1370-SF（Hoechst）フォトレジストをスピンコーティング（3000rpm）によって堆積し、これに続いてソフトベークを行った（90℃で30分間）。

コンタクト位置揃え器(contact aligner)を用いて、ウェハを位置揃えおよび露光した。最良の結果を得るように露光時間を変化させた。露光後ベークは行わなかった。AZ-351（4：1に希釈）（Hoechst）中でウェハを1分間現像し、DI水中ですすいだ。酸化物を酸化物エッチング液から保護するために、ブルータックテープ（Semiconductor Equipment Corporation, カリフォルニア州Moorpark）をウェハの裏面に塗布した。

ウェハを、緩衝化酸化物エッチング液（BOE、10:1 HF（49%）およびNH_4F(10%)）中に11分間浸すことによって保護されていない酸化物を完全にエッチング除去した。ブルータックテープを手で除去し、フォトレジストをアセトンリンス中で除去した。

再還流沸騰フラスコ中にセットアップしたエチレン－ジアミン、ピロ－カテコール、および水の混合物（EPW F-エッチング液）中にて、シリコンエッチングを行った。このエッチング液は、シリコンの｛100｝面を毎時約100μmのレートで攻撃する。流体取り付けポートを第1のステップにおいてエッチングした。流体ポートとフィルタ領域との間のフローチャネルを、第2のステップにおいてエッチングした。障壁を最後のステップにおいてエッチングした。

最後の処理後、ウェハを再びピラニヤ浴中で清掃し、DI水中ですすいだ。これらを次にダイシングして個々のデバイスとした。

本発明者らは、アノード結合（Wallis, G. およびPomerantz, D.I.(1969)、J. Appl. Physics 40: 3946-3949）を用いてPyrexガラスをシリコンデバイスに取り付けた。本発明者らは、1"平方のPyrexガラス片（100μm厚）をEsco Products Inc. (Oak Ridge、NJ)から入手した。まず、シリコンおよびPyrexガラスを、5

0℃に加熱したH_2O、NH_4OH、およびH_2O（1:4:6）の溶液中に浸した。このプロセスにより、表面上の有機物が除去され、かつ、表面を親水性にする。この溶液中で20分間の後、シリコンおよびPyrexをDI水中ですすいで乾かした。アノード結合は、400℃でガラスとシリコンとの間に400Vを印加しながら行った。

ウェハの裏面上において、ポートに流体接続部を作成した。流体ポート周囲に、ガラス管（1/8"内径、長さ約３cm）をエポキシ化した。流入ポートと流出ポートとの間の圧力差によってフローを起こした。H_2Oの３cm未満であるこの圧力差は、毎秒100μmを越える流速を得るために十分である。

Zeiss ICM-405倒立顕微鏡上で観察を行い、Dage シリコン強化ターゲットカメラ(silicon intensified target camera)を用いて記録した。まず、デバイスをイソプロピルアルコールで湿らせ、約70kPaの圧力を加えることによって閉じこめられた気泡をすべて除去した。次に、水、カルボキシフルオロセイン（Molecular Probes）および0.5μm直径の蛍光球(Duke Scientific)の混合物を、流体流入ポートの１つ中に導入した。純水を他方の流入ポート中に導入した。全ての0.5μm球体が、試料ストリーム用の流出チャネルに流れた。色素は、抽出チャネル全体にわたって拡散し、その一部は生産物ストリームとともに流出した。

本発明を特定の実施態様によって説明した。しかし、当業者には理解されるように、本明細書中で開示した特定の要素およびプロセスステップについて、様々な代替が可能である。本発明は、付属の請求項の範囲によってのみ限定を受ける。

【図１】

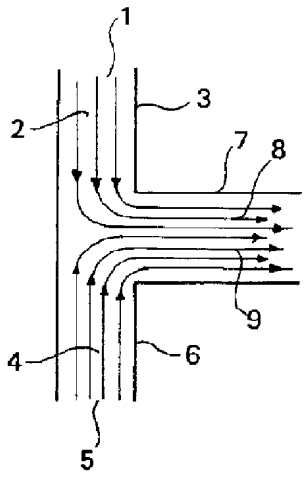


FIGURE 1

【図２】

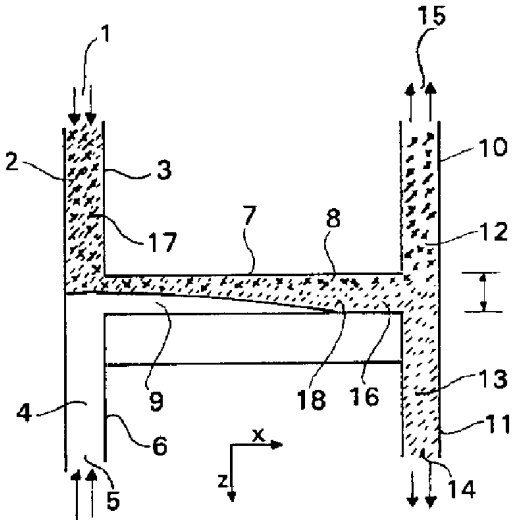


FIGURE 2

【図３】

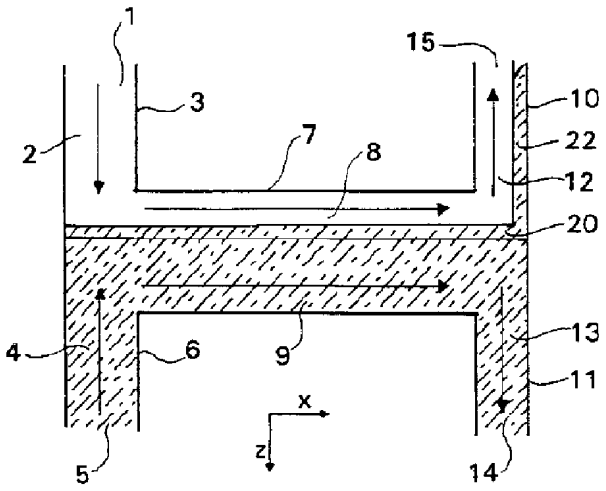


FIGURE 3

【図４】

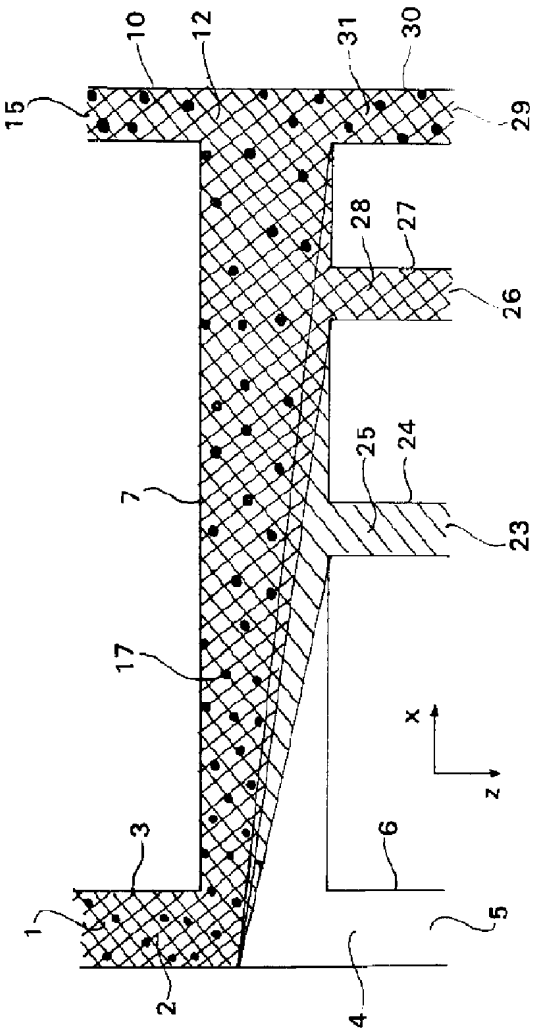


FIGURE 4

【図５】

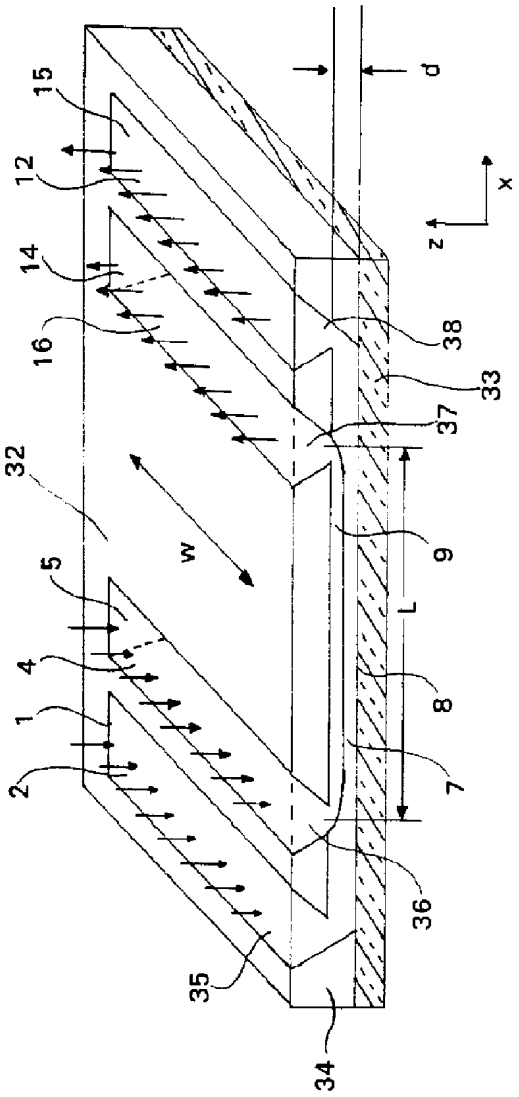


FIGURE 5

【図６】

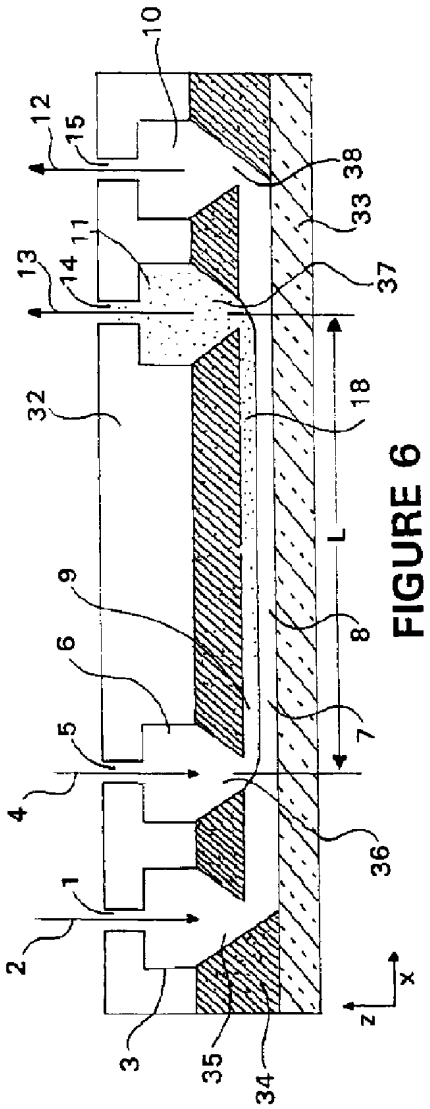


FIGURE 6

【図７】

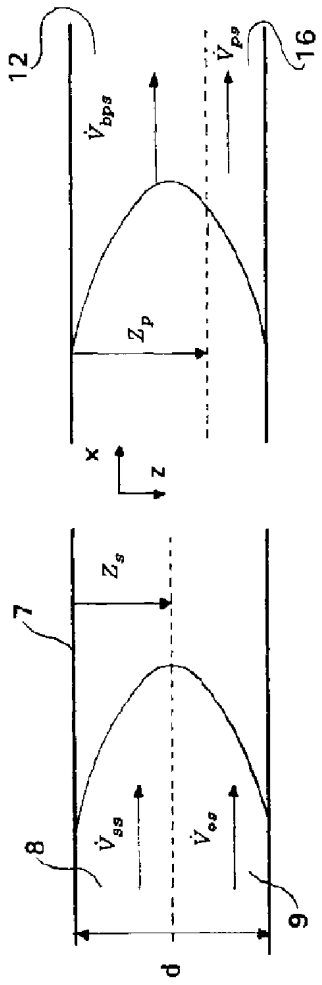


FIGURE 7

【図８】

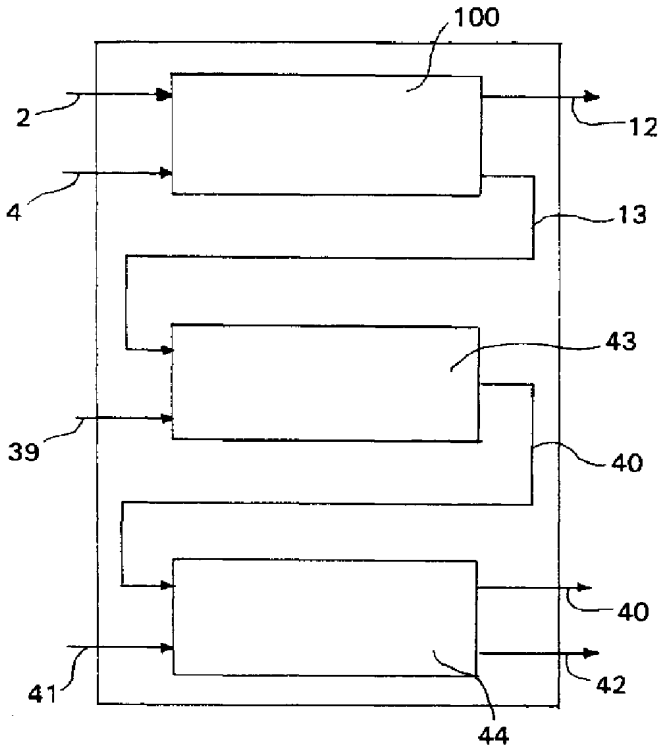


FIGURE 8

【図９】

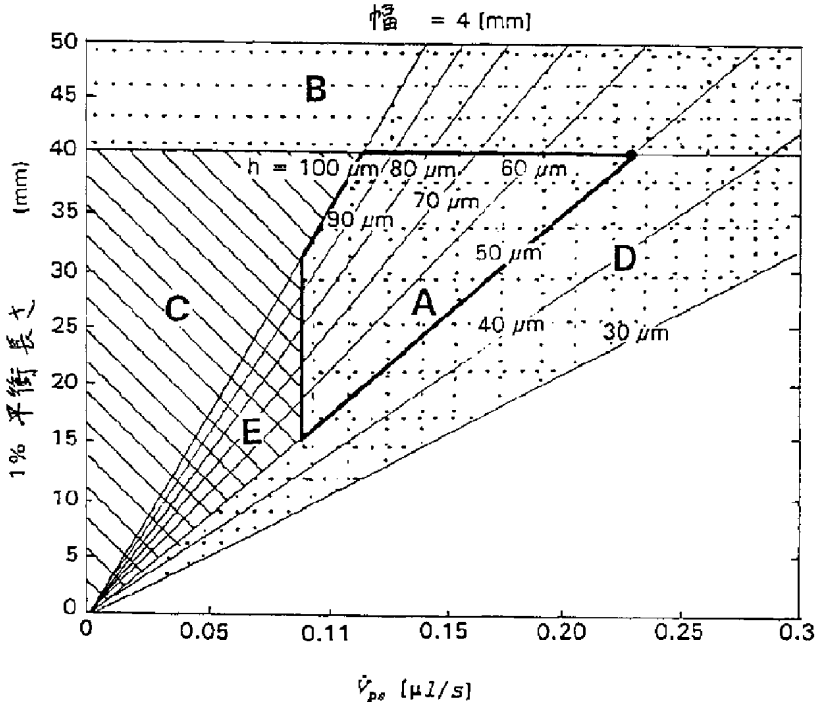

幅　= 4 [mm]
B
C
A
D
E
h = 100 µm
80 µm
60 µm
90 µm
70 µm
50 µm
40 µm
30 µm
1% 平衡長さ (mm)
0
5
10
15
20
25
30
35
40
45
50
0
0.05
0.11
0.15
0.20
0.25
0.3
$\dot{V}_{ps}$ [µl/s]

FIGURE 9

【図１０】

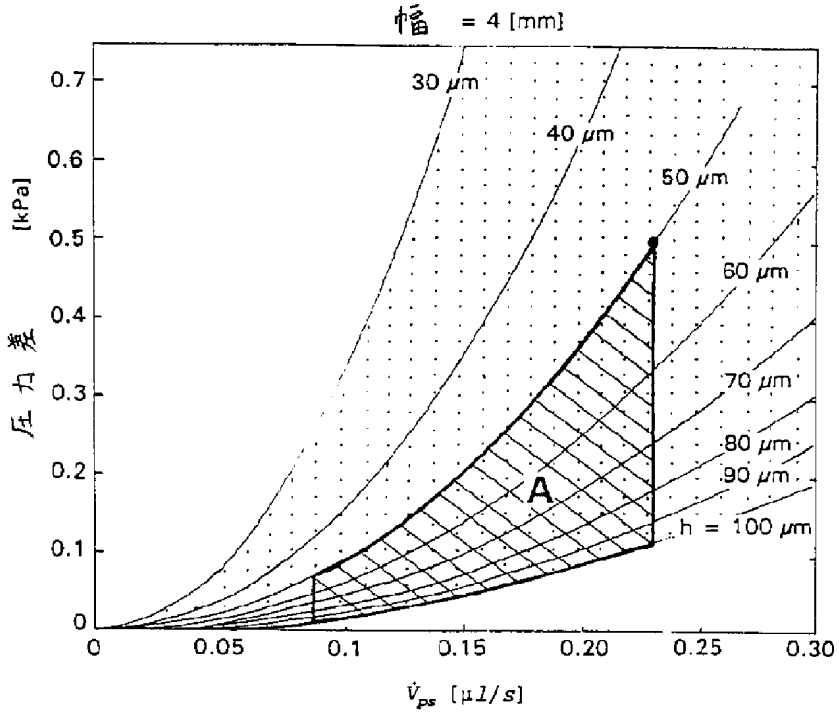


FIGURE 10

【図１１】

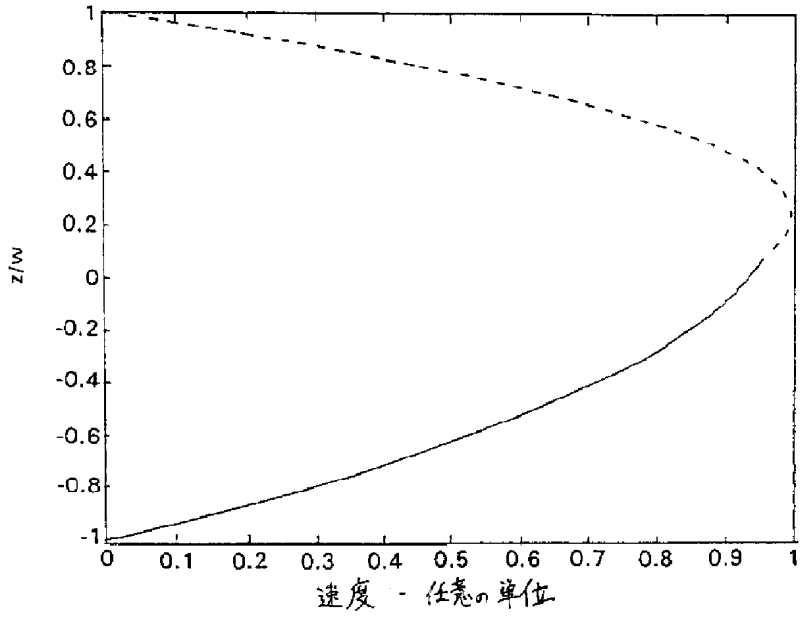


FIGURE 11

【図１２】

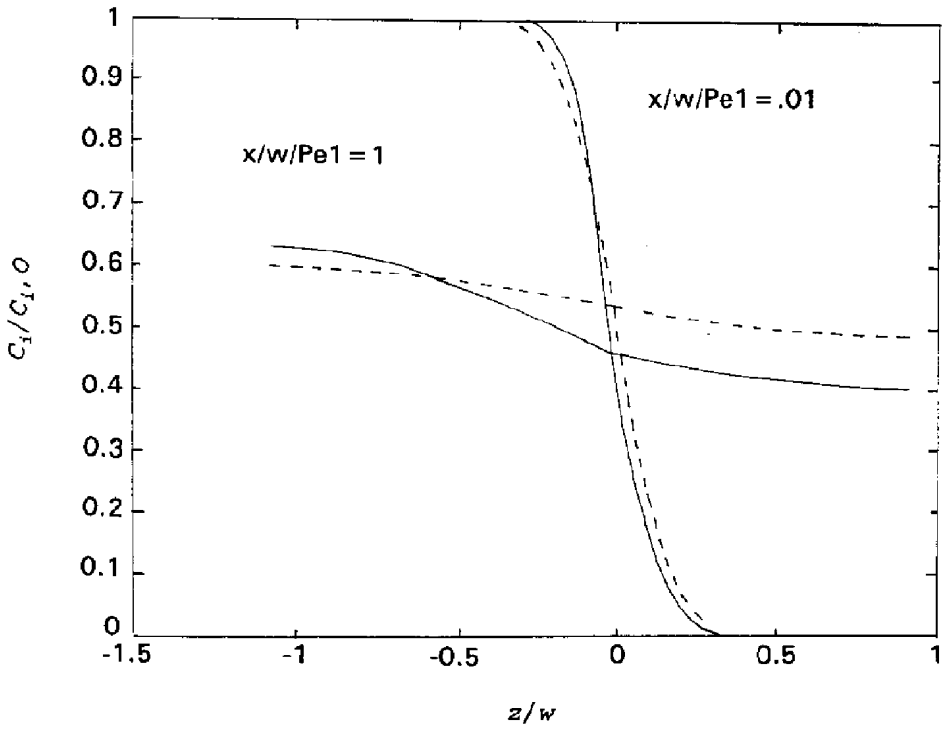


FIGURE 12

【国際調査報告】

INTERNATIONAL SEARCH REPORT

Interm al Application No
PCT/US 96/10308

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6　G01N30/00　G01N15/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6　G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANALYTICAL CHEMISTRY, vol. 59, no. 2, 15 January 1987, COLUMBUS US, pages 344-350, XP002018196 S.R. SPRINGSTON ET AL: "continuous particle fractionation based on gravitational sedimentation in split-flow thin cells"	1-3,18, 19
Y	see abstract see figures 1,2	15-17
X	US 4 737 268 A (GIDDINGS) 12 April 1988 see column 6, line 54-58 see column 9, line 57 - column 10, line 19	4
A	US 5 039 426 A (GIDDINGS) 13 August 1991 see column 11, line 19-41; figure 3	1-4

-/--

[X] Further documents are listed in the continuation of box C.　[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 November 1996	04. 12. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Zinngrebe, U

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INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/US 96/10308

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF MIRCHOMECHANICS AND MICROENGINEERING, vol. 4, 1994, pages 227-245, XP000601275 M. ELWENSPOEK: "towards integrated microliquid handling systems" cited in the application	1-4
Y	see page 232; figure 8 ---	15-17
A	JOURNAL OF CLINICAL CHEMISTRY, vol. 40, no. 1, 1994, pages 43-47, XP000601246 P. WILDING ET AL.: "manipulation and flow of biological fluids in straight channels micromachined in silicon" cited in the application see page 46, column 2, paragraph 2 ---	4
A	JOURNAL OF MIRCHOMECHANICS AND MICROENGINEERING, vol. 3, 1993, pages 168-182, XP000601274 P. GRAVENSEN ET AL.: "microfluidics - a review" cited in the application ---	
A	JOURNAL OF MIRCHOMECHANICS AND MICROENGINEERING, vol. 4, 1994, pages 257-265, XP000601273 A. MANZ ET AL.: "electroosmotic pumping and alectrophoretic separations for miniaturised chemical analysis systems" cited in the application -----	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern al Application No
PCT/US 96/10308

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4737268	12-04-88	NONE	
US-A-5039426	13-08-91	NONE	

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(51)Int.Cl.[6]	識別記号	ＦＩ	
Ｇ０１Ｎ　15/06		Ｇ０１Ｎ　15/06	Ｃ

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15/06		15/06	Ｚ

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(81)指定国　ＥＰ(ＡＴ，ＢＥ，ＣＨ，ＤＥ，ＤＫ，ＥＳ，ＦＩ，ＦＲ，ＧＢ，ＧＲ，ＩＥ，ＩＴ，ＬＵ，ＭＣ，ＮＬ，ＰＴ，ＳＥ)，ＡＵ，ＣＡ，ＪＰ，ＵＳ

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最終頁に続く

(54)【発明の名称】　マイクロ製造される拡散ベースの化学センサ

(57)【要約】
サンプルストリーム中の分析物粒子の存在を検出および／または測定するチャネルセルシステムであって、層流チャネル（１００）と、それぞれがインジケータストリーム（７０）を層流チャネル（１００）に案内する、層流チャネル（１００）と流体連通している２つの入口手段（３０、２０）と、層流チャネル（１００）からストリームを出すように案内して単一の混合ストリームを形成する出口手段（６０）とを含む、チャネルセルシステムが開示されている。インジケータストリーム（７０）は、分析物粒子に接触したときに検出可能な特性変化により分析物粒子の存在を示すインジケータ物質と、サンプルストリーム（８０）とを含み得る。層流チャネル（１００）は、ストリームの層流を可能にするに十分小さい深さと、分析物の粒子がインジケータストリーム中（７０）に拡散してサンプルストリーム（８０）中の相対的に大きい粒子を実質的に排除することを可能にするに十分な長さとを有し、それにより検出領域を形成する。

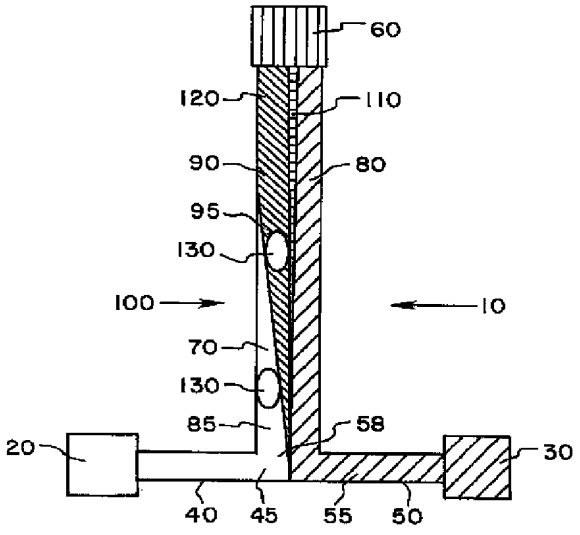

Fig. 1

【特許請求の範囲】

１．サンプルストリーム中の分析物粒子の存在を検出するチャネルセルシステムであって、

ａ）層流チャネルと、

ｂ）該層流チャネルに、それぞれ（１）インジケータストリームと、（２）該サンプルストリームとを案内する、該層流チャネルと流体連通している少なくとも２つの入口手段であって、

ｃ）該層流チャネルが該ストリームの層流を可能にするに十分小さい深さと、該分析物の粒子が該インジケータストリーム中に拡散して該サンプルストリーム中の相対的に大きな粒子を実質的に排除することを可能にするに十分な長さとを有し、それにより検出領域を形成する、入口手段と、

ｄ）該ストリームを該層流チャネルから出るように案内して単一の混合ストリームを形成する出口手段と、

を含む、チャネルセルシステム。

２．前記分析物粒子との接触の結果、前記インジケータストリーム中に担持されたインジケータ物質内の変化を検出する蛍光検出器をさらに含む、請求項１に記載のシステム。

３．試料ストリームを前記層流チャネルの長さに沿って、連続的間隔で前記インジケータストリームから出るように案内する手段と、該試料ストリームの各々中の該インジケータストリームからシグナルを測定する手段とを含み、それにより前記サンプルストリーム中の前記分析物の濃度が決定され得る、請求項１に記載のシステム。

４．前記層流チャネルと流体連通しているＶ溝チャネルをさらに含む、請求項１に記載のチャネルセルシステム。

５．前記層流チャネルと流体連通しているシースフローモジュールをさらに含む、請求項１に記載のチャネルセルシステム。

６．前記インジケータストリームがレポータービーズを含む、請求項１に記載のチャネルセルシステム。

７．前記層流チャネルが回旋する、請求項１に記載のチャネルセルシステム。

８．分析物検出領域を含む、請求項１に記載のチャネルセルシステム。

９．前記層流チャネルと流体連通しており、それにより溶解した粒子と溶解していない粒子との両方を検出する、少なくとも１つの分岐フローチャネルをさらに含む、請求項１に記載のチャネルセルシステム。

１０．前記サンプルストリーム中の前記分析物粒子の存在を伝達により検出する、基板内に形成された、請求項１に記載のチャネルセルシステムであって、

（ａ）前記入口手段と前記出口手段との間の分析物検出領域と、

（ｂ）該基板の両側にシールされた光学的に透明な板と、

を含み、

（ｃ）該分析物検出領域が、該基板の幅方向を横切る空間において、該透明板間に存在する、システム。

１１．請求項１に記載のシステムを用いて、サンプルストリーム中の分析物粒子の存在を検出する方法であって、

ａ）該サンプルストリームを層流チャネルに案内する工程と、

ｂ）インジケータストリームを該層流チャネルに案内し、該サンプルストリームと該インジケータストリームとが該チャネル内において隣接する層流ストリーム中で流れるようにする工程と、

ｃ）該分析物粒子が該インジケータストリーム中に拡散することを可能にする工程と、

ｄ）該インジケータストリーム中の該分析物粒子の存在を検出する工程と、

を含む、方法。

１２．前記インジケータストリームが、前記分析物粒子に接触したときに、検出可能な特性の変化により該分析物粒子の存在を示すインジケータ物質を含み、前記検出工程が該インジケータ物質の特性の変化を検出することを含む、請求項１１に記載の方法。

１３．請求項８に記載のチャネルセルを用いて反応のための動力学的速度定数を決定する方法であって、

（ａ）前記サンプルストリームと前記インジケータストリームとを、入口を有

する前記層流チャネルに案内する工程と、

（ｂ）前記分析物粒子が該インジケータストリーム中に拡散することを可能にして、分析物検出領域を形成する工程と、

（ｃ）該入口から公知の距離において、該インジケータストリーム中の該分析物の存在を検出する工程と、

を含む、方法。

【発明の詳細な説明】

マイクロ製造される拡散ベースの化学センサ

仮出願の相互参照

本発明は、米国陸軍によって与えられた陸軍研究契約DAMD17-94-J-4460に基づく政府援助によるものである。米国政府は本発明に関する特定の権利を享受する。

発明の分野

本発明は、広義には、拡散原理によって、小さな粒子および比較的大きな粒子の両方を含むストリーム中において小さな粒子の存在および濃度を分析するためのマイクロセンサおよび方法に関する。例えば、本発明は、血液を分析して、細胞を含むストリーム中において水素、ナトリウムまたはカルシウムイオン等の小さな粒子の存在を検出するのに有用である。

発明の背景

マクスウェルの有名なgedanken（思考）実験において、魔物(demon)は同じ温度の２つの気体が入った箱の間のドアを操作する。魔物は分子を選別し、比較的動きの速い分子を一方の箱に、そして比較的動きの遅い分子を他方の箱に維持する。これは熱力学の基本法則に反する。以降、このパラドックスは多くの様々な方法で解決されてきた。Leff, H.S.およびRex, A.F.（1990）「Resource letter md-1:Maxwell's demon」Am. J. Physics58:201-209。

類似の構成を用いて粒子を分離することができる。水の中に２つの異なる大きさの粒子を懸濁させた混合物を一方の箱の中に入れ、他方の箱の中に純水を入れた場合を考える。魔物が、大きい方の粒子がこの出入口を通って拡散しないように素早く、しかし小さい方の粒子が他方の箱に拡散できるような時間で箱の間のドアを開閉した場合、ある程度の分離が起こる。

最近、複数のブラウン粒子の存在下で、空間的に非対称の電位を定期的に印加する実験が２つ行われた。Faucheux, L.S.ら（1995）「Optical thermal ratchet」Physical Rev. Letters 74:1504-1507;Rousselet, J.ら（1994） Directional motion of brownian particles induced by a periodic asymmetric potentia

1」Nature 370:446-448。

これが、拡散係数に依存する速度での粒子の指向された移動を引き起こすことが分かっている。ある実験（Rousselet, J.ら(1994),「Directional motion of brownian particles induced by a periodic asymmetric potentia」Nature 370:446-448）では、顕微鏡のスライド上にマイクロ製造された電極を用いて、その電位の電界を印加した。このアイデアは、Adjari, A.らの1995年３月29日付け欧州特許公報第645169号「Separation of particles in a fluid using a saw-tooth electrode and an intermittent excitation field」の主題でもある。別の実験（Faucheux, L.S.ら(1995)「Optical thermal ratchet」Physical Rev. Letters 74:1504-1507）では、変調された光学ピンセット構成を用いた。

拡散は、大きなスケールでは容易に無視し得るプロセスであるが、マイクロスケールになると急に重要になる。ある分子がある距離ｄだけ拡散するのにかかる平均時間ｔは、ｔ＝ｄ^{2}／Ｄである。但し、Ｄはその分子の拡散係数である。タンパク質または他の大きな分子の場合、マクロスケールにおいて拡散は比較的ゆっくりである（例えば、水中、室温においてＤ＝７×１０$^{-7}$ｃｍ^{2}／ｓのヘモグロビンが１センチメートルのパイプを拡散するのにかかる時間は約１０^{6}秒（１０日間）であるが、１０ミクロンのチャネルを拡散するのにかかる時間は約１秒である）。

電子部品を小型化するために半導体産業において開発された道具を用いれば、チャネルの大きさがわずかに１ミクロンの入り組んだ流体システムを製造することが可能である。これらの装置は、安価に大量生産することができ、近い将来には単純な分析テストにおいて広く使用される見込みである。

マイクロスケールで作られてはいないが層流を生成するには十分に小さいチャネルを持つシステムにおいて、「フィールドフローフラクショネーション（field-flow fractionation）」（ＦＦＦ）と呼ばれるプロセスを用いて、単一インプットストリームの成分の分離および分析が行われている。濃度勾配を含む様々なフィールドを用いてフロー方向に垂直な力を生成し、これにより、インプットストリームの粒子の分離を引き起こす。例えば、Giddings, J.C.の1969年６月１７

日付け米国特許第3,449,938号「Method for Separating and Detecting Fluid Materials」；Giddings, J.C.の1979年４月３日付け米国特許第4,147,621号「Method and Apparatus for Flow Field-Flow Fractionation」；Giddings, J.C.の1980年７月29日付け米国特許第4,214,981号「Steric Fleld-Flow Fractionation」；Giddings, J.C.らの1981年２月10日付け米国特許第4,250,026号「Continuous Steric FFF Device for The Size Separation of Particles」；Giddings, J.C.らの(1983)「Outlet Stream Splitting for Sample Concentration in Field-Flow Fractionation」JSeparation Science and Technology 18：293-306；Giddings, J.C.の(1985)「Optimized Field-Flow Fractionation System Based on Dual Stream Splitters」Anal. Chem. 57:945-947；Giddings, J.C.の1989年３月16日付け米国特許第4,830,756号「High Speed Separation of Ultra-High Molecular Weight Polymers by Hyperlayer Field-Flow Fractionation」；Giddings, J.C.の1992年８月25日付け米国特許第4,141,651号「Pinched Channel Inlet System for Reduced Relaxation Effects and Stopless Flow Injection in Field-Flow Fractionation」；Giddings, J.C.の1992年10月20日付け米国特許第5,156,039号「Procedure for Determining the Size and Size Distribution of Particles Using Sedimentation Field-Flow Fractionation」；Giddings, J.C.の1993年３月16日付け米国特許第5,193,688号「Method and Apparatus for Hydrodynamic Relaxation and Sample Concentrationl in Field-Flow Fraction Using Permeable Wall Elements」；Caldwell, K.D.らの1993年８月31日付け米国特許第5,240,618号「Electrical Field-Flow Fractionation Using Redox Couple Added to Carrier Fluid」；Giddings, J.C.の(1993)「Field-Flow Fractionation:Analysis of Macromolecular, Colloidal and Particulate Materials」Science 260:1456-1465；Wada, Y.らの1995年11月14日付け米国特許第5,465,849号「Column and Method for Separating Particles in Accordance with their Magnetic Susceptibility」を参照。これらの参考文献はいずれも、粒子を含むインプットストリームから拡散した粒子を受けるための別のインプットストリームの使用を開示しない。

これに関連する粒子フラクショネーション方法は、「分流薄セル(Split Flow Thin Cell)」（ＳＰＬＩＴＴ）プロセスである。例えば、Williams, P.S.らの(1992)「Continuous SPLITT Fractionation Based on a Diffusion Mechanism」Ind. Eng. Chem. Res. 31:2172-2181、およびJ.C. Giddingsの米国特許第5,039,426を参照。これらの刊行物は、層流を生成するには十分に小さいチャネルを有するが、やはり入口ストリームを１つしか提供しないチャネルセルを開示する。J.C. Giddingsの別の米国特許第4,737,268号は、２つの入口ストリーム（図３）を有するＳＰＬＩＴＴフローセルを開示する。しかし、第２の入口ストリームはインジケータストリームではなく、無粒子ストリーム(particle-free stream)である。Giddingsの米国特許第4,984,146号も、２つのインプットストリームを有するＳＰＬＩＴＴフローセルを開示するが、インジケータストリームはない。これらの全てのＳＰＬＩＴＴフロー方法では、様々な粒子画分を分離するために１つより多くのアウトプットストリームが存在する必要がある。

上記の刊行物のいずれも、比較的大きな粒子、特に分析に使用されるインジケータに影響を与えることができる比較的大きな粒子を含む、非常に少量のサンプル中の小さな粒子を分析することができるチャネルシステムを開示しない。セルシステム内においてインジケータストリームを用いる装置または方法は全く記載されていない。

発明の要旨

マイクロ流体装置(microfluidic device)は、拡散を高速分離メカニズムとして利用することを可能にする。マイクロ構造におけるフロー挙動は、肉眼の世界におけるフロー挙動とは大きく異なる。このような構造における極端に小さな慣性力に起因して、マイクロ構造におけるフローは実際的に全て層流である。これにより、拡散以外を混合することなく、流体および粒子の互いに隣接する異なる層が１つのチャネル内を移動することが可能になる。一方、このようなチャネルにおける横方向の距離は小さいので、拡散は、通常その大きさの関数である分子および小さな粒子の拡散係数に応じて分子および小さな粒子を分離するための強力な道具となる。

本発明は、比較的大きな粒子をも含むサンプルストリーム中における分析物粒子の存在を検出するチャネルセルシステムを提供する。このチャネルセルシステムは、

ａ）層流チャネルと、

ｂ）上記層流チャネルに、それぞれ（１）インジケータ物質、例えば、上記分析物粒子に接触したときの検出可能な特性変化によって上記分析物粒子の存在を示すｐＨ感応性染料(pH-sensitive dye)を好ましくは含むインジケータストリーム、および（２）上記サンプルストリームとを案内する、上記層流チャネルと流体連通している少なくとも２つの入口手段であって、

ｃ）上記層流チャネルが、互いに近接した上記各ストリームの層流を可能にするに十分に小さい深さと、分析物粒子が上記インジケータストリーム中に拡散して上記サンプルストリーム中の上記比較的大きな粒子を実質的に排除することを可能にするに十分な長さとを有し、それにより検出領域を形成する、入口手段と、

ｄ）上記各ストリームを上記層流チャネルから出るように案内して単一の混合ストリームを形成する出口手段と、

を備えている。

本発明の最も単純な実施形態において、単一のインジケータストリームおよび単一のサンプルストリームが用いられる。しかし、本発明の方法および装置において、互いに対して層流となっている、複数のサンプルストリームおよび／またはインジケータストリーム、ならびに参照または較正ストリームを用いることも可能である。

本発明の好適な実施形態では液体ストリームを用いるが、本方法および本装置は、気体状ストリームの使用にも適している。用語「流体連通」とは、互いに流体連通された２つ以上の要素間の流体フローを意味する。

本願において使用される用語「検出」とは、特定の物質が存在すると判定することである。典型的には、特定の物質の濃度を求める。本発明の方法および装置を用いて、サンプルストリーム中の物質の濃度を求めることができる。

本発明のチャネルセルシステムは、分析物粒子との接触の結果生じた、インジ

ケータストリーム中を運ばれているインジケータ物質の変化を検出するための外

部検出手段を含み得る。検出および分析は、光学分光分析等の光学的手段、および吸収分光分析または蛍光等のその他の手段を含む当該分野において公知のあらゆる手段によって、分析物に曝されたときに色または他の特性が変化する化学的インジケータによって、免疫学的手段、装置に挿入された電極等の電気的手段、電気化学的手段、放射性手段、または、磁気共鳴技術を含む当該分野において公知である事実上全ての微量分析手段、または、イオン、分子、ポリマー、ウィルス、ＤＮＡ配列、抗原、微生物またはその他の因子等の分析物の存在を検出する当該分野において公知であるその他の手段によって行われる。好ましくは、光学的または蛍光手段を使用し、抗体、ＤＮＡ配列等を蛍光マーカーに付ける。

「粒子」という用語は、分子、細胞、懸濁され溶解された粒子、イオン、および原子を含む任意の粒子材料を指す。

インプットストリームは、同じまたは異なる大きさの粒子、例えば血液またはその他の体液、汚染された飲料水、汚染された有機溶媒、尿、例えば発酵ブロスなどのバイオテクノロジープロセスサンプル、などを含む任意のストリームであり得る。分析物は、デバイス内のインジケータストリームに拡散することができる、インプットストリーム中の任意のより小さい粒子であり得、例えば、水素、カルシウムもしくはナトリウムイオン、例えばアルブミンなどのタンパク質、有機分子、薬物、殺虫剤、およびその他の粒子であり得る。好適な実施形態では、サンプルストリームが全血であるとき、水素およびナトリウムなどの小さいイオンは、チャネルにわたって急速に拡散し、大きいタンパク質、血球などのより大きな粒子は、ゆっくりと拡散する。好ましくは、分析物粒子は、わずか約3マイクロメートルであり、より好ましくはわずか約0.5マイクロメートルであるか、または、わずか約1,000,000MWであり、より好ましくはわずか約50,000MWである。

システムはまた、インジケータ物質がその上に固定された、ポリマーまたはビーズなどの基質粒子を含む液体キャリアを含む入口手段のうちの１つに導入されるインジケータストリームを含み得る。システムはまた、インジケータ物質がそ

の上に固定された、ポリマービーズ、抗体などの基質粒子を含む分析物ストリームを含み得る。液体キャリアは、供給ストリームから拡散し、インジケータ物質を含む粒子を受け入れることができる任意の流体であり得る。好適なインジケータストリームは、水と、約10mMのNaCl、KClもしくはMgClの塩濃度を有する塩水などの等張液、または、アセトン、イソプロピルアルコール、エタノールのような有機溶媒、または、分析物がインジケータ物質もしくは検出手段に与える影響を妨害しないその他の任意の都合のよい液体とを含む。

チャネルセルは、当該技術分野において既知のマイクロ製造（microfabrication）法、例えば、本明細書において例示されるような、シリコンマイクロチップの表面に溝をエッチングしてこの表面上にガラスカバーを置くことなどによってシリコンマイクロチップにチャネルを形成する工程を包含する方法によって製造され得る。精密射出成形されたプラスチックもまた、製造のために用いられ得る。

本発明の方法は、すべてのフローが層流になるように行われるように設計される。一般に、これは、チャネル内のフローについてのレイノルズ数が約１よりも小さくなり、好ましくは約0.1よりも小さくなるような大きさのマイクロチャネルを含むデバイスにおいて達成される。レイノルズ数は、慣性の粘性に対する比である。低いレイノルズ数は、慣性が本質的に無視できるほどであり、乱れが本質的に無視できるほどであり、2つの隣接するストリームのフローが層流である、即ち、これらのストリームが上述のような粒子の拡散以外は混ざらないことを意味する。フローは、レイノルズ数が１よりも大きい層流であり得る。しかし、そのようなシステムは、例えばストリームの流速が変わる、またはストリームの粘度が変わるなど、フローのパターンが妨害されると、乱れを引き起こしやすい。

層流チャネルは、小さい分析物粒子がサンプルストリームから拡散して、インジケータ物質または検出手段に対して検出可能な影響を与えることを可能にするほど十分に長く、好ましくは、少なくとも約2mmの長さである。フローチャネルの長さは、そのジオメトリに依存する。多くの方法のうちのいずれの方法におい

ても、フローチャネルは、まっすぐであっても湾曲されていてもよい。１つの実施形態では、フローチャネルは、１つあるいはそれ以上の「ヘアピンターン」を含み得、密接した階段状のジオメトリを作り得る。別の実施形態では、フローチャネルは、きちんと巻き取られた庭のホースのようなコイルの形状であり得る。まっすぐでないチャネルジオメトリは、例えばシリコンマイクロチップなどの、チャネルが形成される基板プレートの大きさ／直径を増加させずにフローチャネルの長さを増加させることを可能にする。通常分析物の大きさに反比例する分析物の拡散係数は、所望のフローチャネル長に影響を及ぼす。所定の流速の場合、より小さい拡散係数を有する粒子は、インジケータストリームに拡散する時間を得るために、より長いフローチャネルを必要とする。

あるいは、拡散が起こるためのより多くの時間を与えるために、流速を減らすことができる。しかし、幾つかの要因により最小流速は制限されるため、より長いフローチャネルが望ましい場合もある。第１に、流速は、ポンピング手段または圧力源によって達成され、小さい拡散係数を有する粒子の拡散のための十分な時間を可能にするが、ポンピング手段または圧力源のなかには、望まれ得るほど低い圧力および流速を生成できないものもある。第２に、流速が十分に遅く、粒子の中に、周りの流体ストリームとは大幅に異なる密度を有する粒子がある場合、周りの流体ストリームよりもより密度の高い粒子が、フローチャネルの底に沈み得、周りの流体ストリームよりも密度の低い粒子が、フローチャネルの上に浮かび得る。流体力学的な力が、粒子がフローチャネルの底、上、または壁に付着することを実質的に防ぐほど、流速が十分に速いことが好ましい。第３に、圧力のわずかな変化が、より低い流速の場合の測定精度の大きな誤差につながる。第４に、低い流速では、流体の粘度の変化などのその他の要因が、測定精度の大きな誤差につながり得る。

フローチャネルは、まっすぐであっても、まっすぐでない、即ち、回旋状であってもよい。本明細書で用いられている回旋状フローチャネルという用語は、まっすぐでないフローチャネルを指す。回旋状チャネルは、例えば、らせん状に巻かれ得るか、あるいは、1つまたは複数の「ヘアピン」カーブを含み、方形波の

形状を作り得る。回旋状チャネルは、拡散が起こるためのより長い距離を与え、それにより、より大きい拡散係数を有する分析物、例えば典型的にはより大きい分析物の測定を可能にする。シリコンマイクロチップが、フローチャネルが形成される基板プレートである本発明の好適な実施形態では、まっすぐなフローチャネルのチャネル長は約5mmと約50mmとの間である。フローチャネルが回旋状である、即ち、まっすぐでない本発明の好適な実施形態では、フローチャネルの長さは、チャネルがエッチングもしくはその他の方法で形成されるマイクロチップまたはその他の基板プレートの大きさによってのみ規定または制限される。チャネル幅（拡散方向）は、好ましくは、約20マイクロメートルと約1mmとの間である。チャネルの幅は、より好ましくは比較的広くされ、例えば、少なくとも約200マイクロメートルにされる。そうすれば、単純な光学を用いたインジケータ蛍光の測定がより簡単に行えるようになり、粒子がチャネルを詰まらせる可能性がより低くなる。しかし、用いられている粒子でチャネルが詰まることを防ぎながら、チャネルの幅をできるだけ狭くすることができる。チャネルの幅を狭くすると、拡散がより素早く起こり、従って、検出をより迅速に行うことできる。チャネルの深さは、チャネル内での２つのストリームの層流を可能にするほど十分に浅く、好ましくは、わずか約1000マイクロメートルであり、より好ましくは約50マイクロメートルと約400マイクロメートルとの間である。

幾つかの実施形態では、層流チャネルは、インジケータおよびサンプルストリームが、チャネル内の分析物粒子に関して平衡状態に達することができるほど十分に長くてもよい。平衡状態は、より小さい粒子の最大量がインジケータストリーム中に拡散したときに起こる。

システムはまた、試料チャネル、インジケータストリームからの試料ストリームを、層流チャネルの長さに沿って連続的な間隔で伝えるための、より小さいチャネルなどの出口手段、およびビューポート（view port）と、各試料ストリーム中のインジケータ物質における変化を測定するための蛍光検出器であって、この測定によりサンプルストリーム中の分析物の濃度が決定され得る蛍光検出器とを含む手段を含んでいてもよい。

溶解されていない分析物および溶解された分析物の両方の検出を可能にする、本発明のデバイスの２重検出の実施形態もまた、提供される。溶解されていない分析物および溶解された分析物の両方の検出は、１つの２重検出デバイスにおいて達成することができる。即ち、溶解された粒子は、Ｔセンサのフローチャネルにおいて検出することができ、溶解されていない粒子は、Ｖ溝チャネルまたはシース（sheath）フローモジュールにおいて検出することができ、これらの粒子のいずれかまたは両方は、Ｔセンサのフローチャネルと流体連通され得る。分岐フローチャネルは、Ｔセンサのフローチャネルと、Ｖ溝チャネルおよび／またはシースフローモジュールとの間の流体連通を与えることができる。

本発明のチャネルセルシステムは、Ｖ溝フローチャネルと流体連通されることができる。好ましくは、このＶ溝フローチャネルは、その頂部では、粒子を１列にさせるほど十分に小さく、なおかつ最も大きい粒子を詰まらせずに通過させるほど十分広い幅を有する。Ｖ溝チャネルは、単結晶シリコンマイクロチップの異方性EPW（エチレンジアミン－ピロカテコール－水）エッチングによって形成され、マイクロチップの表面に関して高精度にエッチングされた角度を有する反射表面へのアクセスを与える（Petersen、Proc. IEEE 70(5)：420-457、1982）。（「Silicon Microchannel Optical Flow Cytometer」と題された1995年9月27日出願の米国特許出願シリアル番号第08/534,515号は、シリコンマイクロチップをマイクロ機械加工することによって形成されるＶ溝フローチャネルを含むフローサイトメーターを開示している。本明細書において、上記米国出願全体を参考として援用する。）そのようなチャネルの断面はＶ字型であり、従って、Ｖ溝チャネルと呼ばれる。Ｖ溝フローチャネルとともに使用するために適合されたレーザと、小角(small angle)および大角(large angle)光検出器とを含む光学ヘッドも使用することができる。米国特許出願シリアル番号第08/534,515号に記載されているように、プローブビームのうちでＶ溝の壁から反射された部分に関して小角および大角で配置される検出器は、細胞などの粒子を数え、これらの粒子を、（小角検出器によって）大きさおよび（大角検出器によって）構造／形態で区別するために用いることができる。適切なフィルタを大角検出器の前に配置すること

により、当業者が常套的に選択して決めることができる適切なレーザまたはLED源、例えば、青色レーザを用いて蛍光検出を行うことができる。

Tセンサのフローチャネルは、1つのデバイスで、溶解された1列の粒子および溶解されていない1列の粒子の2重検出を可能にするV溝チャネルと流体連通され得る。流体ストリームは、まずTセンサのフローチャネルを流れ、その後、分岐フローチャネルを介して、V溝チャネルを流れることができる。あるいは、流体ストリームは、まずV溝チャネルを流れて、その後に、Tセンサフローチャネルを流れてもよい。

フローチャネルを1列の粒子が流れるフローを達成する別の手段は、「Device and Method for 3-Dimensional Alignment of Particles in Microfabricated Flow Channels」と題された1997年3月26日出願の米国特許出願(Attorney Docket No. 32-96)に開示されているシースフローモジュールである。本明細書において、上記米国特許出願全体を特に参考として援用する。シースフローモジュールは、層流流体チャネルが形成された第1の材料プレートと、少なくとも2つの入口であって、各々の入口が接合部で層流チャネルを結合し、第1の入口の接合部の幅が第2の入口の接合部よりも広い入口と、フローチャネルからの出口とを含む。例えば透明なカバープレートなどの第2のプレートは、モジュールを密閉し、光学測定を可能にする。透明なカバープレートは、第1のプレートが反射性材料、例えばシリコンである場合、反射による光学測定を可能にする。第1の入口は、第1の流体のフローチャネルへの導入を可能にする。第1の流体は、シース流体である。第2の入口は、シース流体がフローチャネルを流れている間に第2の流体をシース流体に導入することを可能にする。第2の流体は、中心流体である。第2の入口の接合部の幅が第1の入口の接合部の幅よりも狭いため、中心流体は、その両側でシース流体に囲まれる。すべての流体が導入され、シースフローが達成されると、フローチャネルの深さを浅くして、垂直方向の流体力学的集束(vertical hydrodynamic focusing)を得ることがができる。オプションとして、フローチャネルの幅を小さくして、水平方向の流体力学的集束を得ることも可能である。深さおよび幅の低減は、徐々に行っても、急激に行ってもよい。

シースフローモジュールの流体力学的集束により、１列の粒子のフローが得られる。

シースフローモジュールは、本発明のチャネルセルシステムと流体連通され得る。この流体ストリームは、まずＴセンサのフローチャネルを流れ、その後、シースフローモジュールを流れることができる。あるいは、流体ストリームは、まずシースフローモジュールを流れて、その後、Ｔセンサのフローチャネルを流れてもよい。

本発明の好適な実施形態のチャネルセルシステムは、シリコンマイクロチップの表面に刻まれた中央トランクと２つのブランチとを有する「Ｔ」または「Ｙ」字形状のチャネル溝を備え、シリコンマイクロチップの表面は、その後、ガラス

シートで覆われる。中央溝は、「Ｔ」または「Ｙ」字形状のトランクから形成され、ブランチは、サンプルおよびインジケータストリームを層流チャネルへとそれぞれ導くための、層流チャネルと流体連通した入口手段である。

本発明のチャネルセルはまた、複数の入口ストリームを層流チャネルへと導くための、層流チャネルと流体連通した複数の入口ブランチを備え得る。これらは、「キャンデラブラ」状アレイで構成されてもよく、あるいは、「Ｔ」字構造の「クロスバー」または「Ｙ」字構造のブランチに沿って連続的に構成されていてもよく、唯一の制約は、全てのストリームの層流が維持されなければならないことである。

入口手段は、入口チャネルまたは「ブランチ」を含み、供給流体を装置に注入するための手段を提供する管およびシリンジ等の他の手段も含み得る。出口手段は、収集ポートおよび／または出口から流体を除去する手段（例えば、流体のレセプタクル、毛管作用、圧力、または重力によってフローを誘起する手段、および当該分野で公知の他の手段）を含む。このようなレセプタクルは、分析または検出装置の一部であり得る。

透過の際の光学測定を可能とする本発明の装置の実施形態を提供する。このような実施形態では、チャネルセルシステムまたは少なくとも分析物検出領域が、チャネルセルシステムが形成される基板プレートの幅を横切する。本明細書中で

使用されるような基板プレートは、本発明のチャネルセルシステムが形成される材料（例えば、シリコンウェハおよびプラスチックシート）の片を意味する。分析物検出領域および必要に応じてチャネルセルシステムの他の部分が、基板プレートの幅全体にわたる空間における光透過性板の間に配置される。本明細書中に使用されるような分析物検出領域は、分析物粒子がインジケータストリームにおいて検出可能な変化を生じさせるインジケータストリームの部分を意味する。

反射光を利用した光学測定は、本明細書中では反射による検出と呼ばれ、透過光を利用した光学測定は、本明細書中では透過による検出と呼ばれる。

より大きな粒子も含むサンプルストリーム（好ましくは、液体ストリーム）において分析物粒子の存在を検出するための方法も提供され、この方法は：

ａ）サンプルストリームを層流チャネルに案内する工程と；

ｂ）インジケータストリームを導く工程であって、インジケータストリームは、好適には、層流チャネル（ここでは、サンプルストリームおよびインジケータストリームがチャネル中で隣接した層流ストリームで流れる）に入る分析物粒子と接触した場合に、検出可能な性質上の変化によって分析物粒子の存在を示すインジケータ物質を含む、工程と；

ｃ）分析物粒子がインジケータストリーム中に拡散することを可能にする工程と；

ｄ）インジケータストリーム中の分析物粒子の存在を検出する工程と；

を包含する。

インプットストリームの流速は、好適には、約５マイクロメータ／秒と約５００マイクロメータ／秒との間であり、より好適には、約２５マイクロメータ／秒である。好適には、両方のストリームの流速が同じである。

本発明の方法およびシステムは、インジケータストリームまたはインジケータストリーム中のインジケータ物質において検出可能な変化を引き起こすインジケータストリームへと拡散するサンプルストリームから分析物粒子の層流チャネル内での位置を検出することによって、サンプルストリーム中の分析物粒子の濃度を決定する工程を含む。サンプルストリームおよびインジケータストリームは、

層流チャネル内で平衡に達することが可能となり得る。検出領域（すなわち、検出可能な濃度で拡散した粒子を含有するインジケータストリーム部分）と、影響を受けないインジケータストリームとの境界線位置が、流速および／またはサンプル濃度に関する情報を提供するために使用され得る。所与の分析物に関してチャネルにおけるこの境界線の物理的位置は、流速が一定で、且つサンプルが変化しない限り、時間が経過しても一定のままである。検出領域の位置および大きさは、検出のための信号を最適化するために、流速、サンプル濃度、および／またはインジケータ物質の濃度を変化させることによって変更され得る。

サンプルストリーム中の分析物粒子の濃度を決定するために有用な情報は、本明細書中に記載されるようなビューポートを備えたより小型のチャネルなどの層流チャネルの長さに沿って連続的間隔でインジケータストリームから試料ストリームを導くための手段を設けることによって得られ得る。上記にリストされたような検出手段は、インジケータストリームからの信号を測定するために使用される。試料チャネルから試料チャネルへの信号強度の変化が、オリジナルのサンプルにおける分析物粒子の濃度を計算するために使用され得る。

本発明の１実施形態による方法は、インジケータストリーム内で運ばれる粒子状基質上に固定化されるインジケータ物質の使用を包含する。インジケータ物質は、好適には、染料、酵素、および分析物濃度の関数として特性が変化する他の有機分子等の分析物粒子が存在する場合に、蛍光または色が変化する物質である。「インジケータ物質」という用語は、その上に固定化された染料または他のインジケータを有するポリマービーズまたは抗体等を指すためにも使用される。分析物粒子によって引き起こされるインジケータストリーム中での電気的、化学的、または他の変化を直接検出するような検出手段が使用される場合には、インジケータストリームが、インジケータ物質を含む必要はない。

このシステムの利点は、血液などの濁り、強く着色された溶液中の分析物が、沪過または遠心分離を前もって行う必要なしに光学的に測定され得る；より大きなサンプル成分に対するインジケータ染料のクロス感度（cross-sensitivity）（一般的な問題）が回避され得る；および最適な特性（例えば、ｐＨまたはイオ

ン強度に対するクロス感度が、強い緩衝液を用いることによって抑制され得る）を示す溶液中でインジケータが維持され得るという事実を含む。チャネルに沿った数カ所の位置でインジケータストリームを測定することによって、いくらかの残りのクロス感度が補償され得る。さらに、フローチャネルは幅が広くてもよく、それによって、単純な光学を用いてインジケータ蛍光を測定することが容易となる。メンブレンは必要なく、このシステムは、メンブレンシステムに比べて、生物付着および目詰まりを受けにくい。このシステムはまた、サンプルまたはインジケータストリーム濃度および／または流速が、検出されている信号を最適化するために変更され得る点で調整可能である。例えば、反応が約５秒かかる場合には、このシステムは、反応が装置の中央部分で見られるように調節され得る。

この方法は、サンプルおよびインジケータストリームの連続的貫流によって実施され得る。この方法の定常状態的性質により、より長い信号積分時間が可能となる。

サンプルストリームは、インジケータ物質にも敏感な、分析物粒子より大きな粒子を含有し得る。これらは、インジケータストリームには拡散せず、従って、分析物の検出を妨害することはない。

さらに、２つのストリームが合流するＴ接合部からのサンプルストリームおよびインジケータストリームの移動距離の関数として動力学的速度定数を決定するための方法が提供される。概して、動力学的測定は、濃度対時間、すなわち反応時間に関連する物理的特性をプロットすることによって行われる。時間の関数としてではなく、サンプルストリームおよびインジケータストリームの移動距離の関数として動力学的測定を行うための本明細書中に提供される方法は、以下の理由で有利である。ストリームの成分（すなわち粒子）と、フローチャネルにおけるある位置でのストリーム成分の濃度は、流速が一定であると仮定すると一定のままである。この方法により、経時的に検出からデータを積分すること、例えば光学測定が可能となり、その結果、収集されたデータの正確さ、従って、計算された／決定された速度定数の正確さが向上する。さらに、実験エラーが検出中（例えばデータの収集中）のある時点で生じた場合には、単に、エラーが生じたフ

ローチャネル中の距離／位置で再び検出測定を行うことができる。動力学的測定を行うための従来の方法では、ある時点のデータが実験エラーによって失われた場合には、これらのデータが、同じ実験中に再び収集されることは不可能である。

図面の簡単な説明

図１は、本発明によるＴセンサーチャネルセルの実施形態におけるフローおよび拡散の模式図である。

図２は、pH９の緩衝液（右入口）が装置に流入し、弱緩衝インジケータ染料溶液（pH５）が左から入る、本発明のＴセンサの蛍光顕微鏡写真図である。拡散が進むにつれて、ある形態から他の形態への染料の明瞭な転換が、明白に見える。

図３は、本発明のビューポート－Ｔセンサの実施形態のレイアウトを示す。この実施形態では、インジケータストリームは、右のＴ脚部から来て、pH９の低イオン強度緩衝液中のインジケータ染料溶液である。左から導入されるサンプルストリームは、ここでは、pH５の0.15Mの緩衝液である。インジケータ染料を含有するインジケータストリームの数カ所が、様々な位置での試料ストリームとしてチャネルから連続的に取り出される。

図４は、フローサイトメータ光学ヘッドに接続されたＶ溝フローチャネルを示す。

図５は、方形波形状の回旋フローチャネルを示す。

図６は、コイル形状の回旋フローチャネルを示す。

図７Ａは、曲線的Ｔ接合部を有するＴセンサを示す。

図７Ｂは、曲線的Ｔ接合部を有するビューポート－Ｔセンサを示す。

図８は、距離の関数として動力学的測定を行うための複数の検出領域を有する回旋フローチャネルを示す。

図９Ａ～図９Ｃで成る図９は、溶解および非溶解分析物両方の２重検出のためのブランチフローチャネルを有する実施形態を示す。

図１０Ａ～図１０Ｃで成る図１０は、シースフローモジュールを示す。

図１１は、分析物検出領域が、基板プレートの幅全体にわたってエッチングさ

れるＴセンサを示す。

発明の詳細な説明

本発明の微小規模チャネルセルは、サンプルストリームの中の大きな粒子から小さな粒子を分離するのに有用である。この微小規模チャネルセルは、粒子の拡散係数は粒子のサイズに実質的に反比例するため、大きな粒子は小さな粒子よりもゆっくりと拡散するという事実、拡散は、当業者に公知の大規模の分離よりも本発明の微小規模において、より急速に発生するという事実、および、層流の非乱流は、微小規模で隣接するストリームにおいて誘発されることが可能であるという事実に基づいて、粒子を分離する。

図１に示すように、「Ｔ」形のチャネルセルを設け、本明細書ではＴセンサ１０と呼ぶ。この装置は、シリコンマイクロチップをエッチングすることによって、マイクロ製造（microfabricate）されることが可能である。形態は、「Ｙ」のよ

うに、必ずしも「Ｔ」である必要はない。製造することが可能な任意の角度であれば十分である。上述のように、複数のインプットチャネルがあってもよい。全てのインプットチャネルが単一のフローチャネルに合流することのみが必要であり、全てのチャネルは十分に小さいので、全ての動作条件に関して層流が維持される。概して、システムのレイノルズ数は、１またはそれより小さい。関心の対象である小さな分子を含むサンプル、すなわちサンプルストリーム８０は、サンプルストリーム入口ポート３０を介して装置内に運ばれ、サンプルストリーム８０は、このサンプルストリーム入口ポート３０からサンプルストリーム入口チャネル５０に流れ込む。このサンプルストリーム入口５０では、サンプルストリーム８０はサンプル入口ストリーム５５と呼ばれる。インジケータストリーム７０は、インジケータストリーム入口ポート２０に運ばれ、このインジケータストリーム入口ポート２０からインジケータストリーム入口チャネル４０に流れ込む。このインジケータストリーム入口チャネル４０では、インジケータストリーム７０はインジケータ入口ストリーム４５と呼ばれる。

サンプル入口ストリーム５５は、フローチャネル１００の始点のＴ接合部５８

でインジケータ入口ストリーム４５と出合い、２つのストリームは、インジケータストリーム７０およびサンプルストリーム８０として平行な層流の形で出口ポート６０へ流れる。インジケータストリーム７０は、物理的特性における検出可能な変化によってサンプルストリーム８０中の分析物粒子と反応する、染料などのインジケータ物質を含む。図１に、インジケータストリーム７０を白で示す。小さなフローチャネル１００ではレイノルズ数が低いため、乱流誘発した混合は発生せず、２つのストリームは混合することなく互いに平行に流れる。しかし、距離が短いために、拡散がフロー方向に対して直角に作用する。そのため、サンプル成分（分析物粒子）は、左に拡散してインジケータストリーム７０中に拡散し、最終的には均一分析物粒子拡散領域１２０において、フローチャネル１００の全幅に亘って均一に分散する。

インジケータストリーム７０は、フローチャネル１００に流れ込んで、分析物粒子が拡散していない初期参照（reference）領域８５を形成する。サンプルストリーム８０からインジケータストリーム７０に拡散する分析物粒子は、分析物

検出領域９０を形成する。この分析物検出領域９０において、好適にはインジケータストリーム７０内部のインジケータ物質の性質に検出可能な変化を起こすことによって、分析物粒子はインジケータストリーム７０中で検出可能な変化を起こす。インジケータ物質の粒子、例えば染料粒子もまた、サンプルストリーム８０中に拡散して拡散インジケータ領域１１０を形成し得る。インジケータ物質の局部的な濃度におけるこの変化が幾つかの用途において問題になるならば、ポリマーまたはビーズ、例えばインジケータビーズ１３０への固定によって、その拡散速度を任意に小さくすることが可能である。

図１のＴセンサ１０では、サンプルストリーム８０、例えば血液と、インジケータ染料を含むインジケータストリーム７０とは、サンプルストリーム入口チャネル５０とインジケータストリーム入口チャネル４０との交差点で、フローチャネル１００（すなわちＴ接合部５８）と結合し、互いに層流で隣接して、出口ポート６０でこの構造から出るまで、フローチャネル１００内を流れる。H^+およびNa^+などの小さなイオンがフローチャネル１００の直径に亘って急速に拡散

するのに対して、染料アニオンなどの大きなイオンは、ゆっくりとしか拡散しない。糖、タンパク質、等および血球などの大きい粒子は、インジケータストリーム７０とサンプルストリーム８０とが互いに接触している時間中は、著しい拡散を示さない。小さなサンプル成分がより速く拡散し、Ｔ接合部５８付近で平衡に達するのに対して、大きな成分は、フローチャネル１００のさらに上方で平衡に達する。さらに、インジケータは特定の半飽和濃度（ｐＨ染料の場合はpK_a）を有するので、拡散がチャネルの上方で進み、検出領域９０を形成するにつれて、インジケータ染料の色または蛍光の変化の前面（front）または検出領域境界９５が現れる。実際には、検出領域境界９５および参照領域８５は、図２に最も良く見られる曲線を形成し得る。この前面の曲線の位置は、流速およびチャネル幅を変えてシグナルのサイズと強度とを最適化することによって調節される、「静止位置」を有することが可能である。

これはフローシステムであるが、所定の分析物用のフローチャネル１００における検出領域境界９５の物理的位置は、フローが一定し且つサンプルが変わらない限り、時間が経過しても同じ位置にある。分析物濃度は、実質的に平衡に達した後の均一分析物粒子拡散領域１２０におけるインジケータシグナルをモニタすることによって、または最も急勾配のインジケータの色変化の前面の位置を、例えば多元素検出器（図３参照）を用いて着目することのいずれかによって、決定される。分析物検出領域９０は、検出可能なインジケータシグナルを提供するのに必要なだけ大きくすることが可能である。同様に、参照領域８５は、検出可能な参照シグナルを提供するのに必要なだけ大きく形成することが可能である。これらの領域の調節は、分析物ならびにインジケータ物質の拡散係数、流速、およびチャネルのサイズに基づいて、後述のように行うことが可能である。

図２に、図１のＴセンサの蛍光顕微鏡写真を示す。このＴセンサは、ｐＨ５の弱く緩衝したインジケータ染料溶液であるインジケータ入口ストリーム４５、およびｐＨ９の緩衝溶液であるサンプル入口ストリーム５５を特徴とする。右側の明るい区域は、シリコン上で反射した光であり、サンプルおよびインジケータのストリームとは無関係である。サンプルストリーム８０は、右側に暗色の透明な

液体として現れる。左側の明るい区域は、分析物粒子がインジケータストリーム７０中にまだ拡散していない参照領域８５である。中央の灰色の領域は、サンプルストリーム８０からのＯＨ⁻イオンがインジケータストリーム７０中に拡散して形成される分析物検出領域９０である。灰色検出領域９０の右側のぼやけた縁は、サンプルストリーム８０中に拡散する染料粒子によって発生する。均一分析物粒子拡散領域は、ＯＨ⁻イオンが均一に拡散している１２０に示される。最も強いシグナルは、検出領域９０の中央に存在する。

図３に、本発明のＴセンサチャネルセル装置の別の実施形態を示す。この実施形態は、複数の試料チャネルと、フローチャネルの長さに沿って間隔を置いたビューポートとを有する。図３では、インジケータ入口ストリーム４５は、インジケータストリーム入口ポート２０において右側から入る（図１および図２のように左側ではない）。ｐＨ９の低イオン強度緩衝液中のインジケータ染料の溶液が使用される。ｐＨ５の０．１５Ｍ緩衝溶液であるサンプル入口ストリーム５５が、サンプルストリーム入口ポート３０において左側から入る。染料の濃度は、図２で使用した染料濃度のわずか約１０％である。インジケータおよびサンプルのストリーム４５および５５は各々、インジケータストリーム入口チャネル４０およ

びサンプルストリーム入口チャネル５０に沿って流れ、Ｔ接合部５８で出合い、フローチャネル１００に沿って共に層流で流れる。インジケータ染料を含むインジケータストリーム７０からの試料ストリーム１４５は、様々な位置でフローチャネル１００から連続的に取り出される。これらの試料ストリーム１４５は、ビューポート１４０として機能する拡大部を通って流れる。ビューポート１４０のサイズ（数平方ミリメートル）のため、蛍光強度は、蛍光顕微鏡を介してまたは光検知器を用いて直接容易にモニタすることが可能である。

Ｔ接合部５８に最も近いビューポートが主に乱されていない（undisturbed）染料溶液を含むのに対して、出口ポート６０に最も近いビューポートは、インジケータストリーム７０と完全に平衡に達したサンプルストリーム８０を含む。間に位置するビューポートは、様々な程度でサンプル成分と平衡に達しているイン

ジケータストリーム７０を含む。Ｔ接合部５８に近ければ近いほど、ビューポートがサンプルからの小さなイオンのみを含む可能性が高くなる。ビューポートの蛍光顕微鏡写真は、Ｔ接合部５８に最も近いビューポートにおける色が乱されていないインジケータ染料の塩基形態の赤色であるのに対して、出口ポート６０に最も近いビューポートの黄緑色は、拡散ベースの平衡が達成されたときにインジケータストリーム７０のｐＨが塩基性から酸性に変わった後の、染料の酸性形態を表すことを示す。

図３のビューポートＴセンサは、単純な参照技術に役立つ。１つ以上の波長のそれぞれのビューポートの積算（integral）蛍光強度は、蛍光顕微鏡を介して、または光ダイオードを用いて直接的に、容易に測定することが可能である。最も容易な場合では、他のサンプル成分に対してクロス感度（cross-sensitivity）を示さないインジケータ染料を用いて、選択したビューポート間の強度比率が、染料の濃度および励起光の強度から十分に独立した測定値を与える。１つより多くのビューポートで測定することによって、冗長性が増大し、従って測定の正確度が増大する。

大きなサンプル成分（例えば、アルブミンなどの大きな生体分子）に対してインジケータがクロス感度を示す場合には、この干渉は、異なるビューポートの比率を比較することによって参照されることが可能である。Ｔ接合部５８に近いビューポートが主に小さなサンプル成分を含むのに対して、フローチャネル１００のさらに上方のビューポートはまた、大きな粒子を含む。

本発明のＴセンサ装置をレポータービーズと併用して、生物学的流体中のｐＨ、酸素飽和度、およびイオン含有量を測定することが可能である。（本明細書において全体を参考のため援用する、米国特許出願第08/621,170号、「Fluorescent Reporter Beads for Fluid Analysis」は、蛍光性および吸収性レポーター分子並びにレポータービーズを開示している。）レポータービーズはまた、アルコール、農薬、ラクテートなどの有機塩、グルコースなどの糖、重金属、並びにサリチル酸、ハロタン、および麻酔薬などの薬物を検出および測定するために使用することが可能である。それぞれのレポータービーズは、基質ビーズを有し、こ

の基質ビーズ上に複数の少なくとも１つの型の蛍光性レポーター分子が固定されている。本明細書で使用する複数とは、１より多いことを意味する。強度、寿命、または波長などのレポータービーズの蛍光特性は、対応する分析物に対して敏感である。レポータービーズが流体サンプルに加えられ、そして分析物濃度が、例えばフローサイトメータにおいて、個々のビーズの蛍光を測定することによって決定される。あるいは、分析物濃度の関数としての吸光度を変化させる、吸収性レポーター分子を使用することが可能である。レポータービーズの使用は、複数の分析物を同時に測定することを可能にし、そして生物学的細胞の含有量を同時に測定することも可能にする。ビーズは、異なるレポーター分子を用いてタグ化されることが可能なので、複数の分析物を同時に測定することが可能である。

本発明の蛍光性レポーター分子は、特定の分析物または分析物のクラスの濃度の関数である蛍光特性を有する任意の蛍光分子であり得る。当該分野において公知の多くの染料および蛍光色素を本発明においてレポーター分子として使用することが可能である（例えば、R.P. Haugland, Handbook of Fluorescent Probes and Research Chemicals, 第５版、Molecular Probes Inc., Eugene, 1992年参照）。レポーター分子の選択に関する判定基準は、分子が基質ビーズ上に固定されることが可能であること、および分子の蛍光が分析物の濃度の関数であることである。１集合中のビーズの数が変化する、以前に使用されていた蛍光性ビーズと比較すると、米国特許出願第08/621,170号のレポータービーズは、レポーター

分子と結合する表面上に、リガンド、アンチリガンド、抗原、または抗体などの免疫試薬を有する必要がない。

蛍光レポーター分子は、レポーター分子の蛍光特性が変化するように分析物と相互作用を行う。いくつかの例では、ＡＢ５８０（Molecular Probes）によるアルブミン検出の場合のように、レポーター分子は分析物と反応する。場合によっては、相互作用は化学反応ではない。例えば、レポーター分子の蛍光は、ルテニウムジフェニルフェナントロリンによるO_2の検出の場合のように、分析物への非放射性エネルギー転移によってクエンチされ得る。いくつかのレポーター分子において、蛍光は流体中の極性変化に感応し、これは、水性流体中の有機溶媒お

よび炭化水素の検出に用いられ得る。相互作用はまた、他の溶媒効果を介して行われ得、この場合、溶媒のイオン強度が蛍光に影響を与える。溶媒効果は、すべての溶解したイオンの全濃度を決定するために用いられ得る。相互作用は、リガンド／アンチリガンドまたは抗原／抗体反応であり得る。相互作用は、好ましくは、他の粒子との凝集体を導かない。特に、複数のレポータービーズを含有する凝集体を生成しない。分析物のレポーター分子との相互作用により流体中の分析物濃度が顕著に変動しないことが好ましい。

蛍光レポータービーズの場合には、レポーター分子の少なくとも１つの蛍光特性は分析物濃度の関数である。レポータービーズに対して測定される特性は、蛍光強度、崩壊時間、またはスペクトルなどの、分析物のビーズとの相互作用によって影響を受ける特性であり得る。

もしくは、レポーター分子は、吸収インジケータ、例えば、基板ビーズ上に固定される生理学的ｐＨインジケータＮ９（Merck, Germany）であり得る。このようなインジケータはそれらの吸収を分析物濃度の関数として変化させる。典型的には、分子の色が変化する（すなわち、分子の吸収極大の波長が変化する）。

吸収レポーター分子を、基板ビーズ上の蛍光レポーター分子と組み合わせて用いることができ、また吸収ビーズを蛍光ビーズと組み合わせて用いることができる。

基板ビーズの機能は、単一ビーズの光学測定値により、分析物および場合によってはその濃度の検出を可能にすることである。ビーズのタイプが識別され得る

場合は、所定のサンプルを分析するために、複数のタイプのレポータービーズ、すなわち、様々な異なるレポーター分子が固定されているビーズが用いられ得る。ビーズは、様々な手段によって、すなわち、ビーズのサイズを用いる手段、例えば光の散乱によって、ビーズに接着される蛍光レポーター分子とは異なる励起波長および／または発光波長を有するビーズに接着される蛍光タグによって、またはビーズに接着される蛍光分子を直接識別することによって、識別され得る。これにより、一度に１つより多い分析物を検出することが可能である。基板ビーズはまた、レポーター分子を固定してこれらがサンプルストリーム内に拡散する

のを防ぐように機能する。レポーター分子は、基板ビーズの表面または基板ビーズ内に存在し得る。ビーズは様々な材料により製造され得、球体に限らずいかなる形状であってもよい。適切な材料としては、ガラス、ラテックス、ヒドロゲル、ポリスチレン、およびリポソームを含む。ビーズは、レポーター分子の接着を容易にするために追加の表面基を有し得る。例えば、ラテックスおよびアミノ修飾ポリスチレン上にカルボキシル基を加え得る

基板ビーズ上にレポーター分子を固定させるためには様々な技法が用いられ得る。吸着に基づくコーティングは、基板ビーズをレポーター分子溶液中に浸し、次に過剰なレポーター分子を洗い流すことによって行われ得る。同様に、レポーター分子は、孔が制御されたガラスビーズのキャビティ内に拡散され得る。レポーター分子はまた、レポーター分子を適切な基板ビーズの官能基に化学的に接着させることによって、共有結合により固定され得る。レポーター分子を含有する溶液中で重合化ビーズを形成し、これにより分子を固定ポリマーキャビティ内にトラップし得る。レポーター分子をリポソーム中に固定させるために、脂質をレポーター分子溶液と混合し、溶液を撹拌してリポソームを分離し得る。

本発明の方法においてレポータービーズを用いるためには、ビーズを流体サンプルと混合し、個々のビーズの蛍光または吸収を測定する。ビーズは、サンプルとの混合前は乾燥状態であり得るか、または流体中に散乱させ得る。マイクロ単位の測定では、ビーズおよびこれに伴う流体の追加量をサンプル量に比べて少なくして（例えば、＜１％）、サンプルの希釈が目立たないようにすることが好適である。

本発明のチャネルセルは当該分野において既知の技法によって、好ましくは、エッチングによりシリコンマイクロチップの水平面上にフローチャネルを形成して、エッチングされた基板上に、好ましくはガラスまたはシリコーンゴムシートなどの光透過性材料により製造される蓋を配置することによって形成され得る。本発明のチャネルセルを製造する他の手段としては、プラスチック技術、マイクロ機械加工技術、および他の当該分野において既知の技術で装置を成形する場合のテンプレートとしてシリコン構造体または他の材料を用いることを含む。精密

射出成形プラスチックを用いて装置を形成することもまた考慮される。マイクロ製造技術は当該分野において既知であり、以下にさらに特定して述べる。

本発明の１つの好適な実施形態では、本発明のチャネルセルは親水性表面を有し、これにより内部の液体の流れが容易になり、加圧を行う必要なく装置の動作が可能になる。基板は、チャネルの製造後、当該分野において既知の手段によって処理されて親水性が与えられる。蓋もまた、好ましくは、親水性が与えられるように処理される。

本発明のＴセンサチャネルシステムは、１つ以上のｖ溝チャネルと流体連通し得る。シリコンマイクロチップをエッチングして、チャネルの反射表面／壁と共にｖ溝を形成し得る。従って、光学測定値は、透過ではなく反射された入射光を利用し得る。検出は反射によって、すなわち反射光を検出することによって行われ得る。小角散乱光（チャネル内の粒子の表面から散乱）もまたｖ溝壁によって反射され、小角光検出器によって集光され得る。大角散乱光および蛍光光は反射せずにチャネル内に存在し、大角光検出器によって集光され得る。さらに、照射粒子の背後のｖ溝の反射壁により、蛍光集光効率が向上する。入射光、例えばレーザービームのうちのｖ溝チャネル内に入らない部分は、シリコン表面から小角または大角検出器のいずれかから離れる方向に反射される。光が蓋、例えば透明のカバープレートに直接結合されずに空中から入る場合には、蓋／カバープレートから反射される光部分もまた、小角および大角検出器から離れる方向に反射し、これにより測定値から望ましくないバックグラウンド光強度が差し引かれる。

ｖ溝フローチャネルは入射光を透過するのではなく反射するため、本発明のマイクロチャネルシステムの製造は極めて簡単である。マイクロチャネルは、一方の側のみにパターン化される単一のシリコンマイクロチップにより製造される。透明なカバープレートがマイクロチップの先端部に取り付けられ、チャネルを密封する。

図４は、ｖ溝フローチャネルおよびオプションとしての光学ヘッドを示す。シリコンマイクロチップ２１０は内部にｖ溝２１１を有する。ｖ溝という用語は、本明細書においては、シリコンマイクロチップの表面の実質的に「Ｖ」形状の溝

に対して用いられる。製造プロセスに依存して、「Ｖ」の先端部は平坦（台形の溝）であってもよいが、これは、この平坦部が、照射ビームのサンプルフローとの交差によって規定される分析物検出領域内にない場合に限る。好適な実施形態では、マイクロチップ２１０は＜１００＞表面方位を有し、溝２１１の壁は＜１１１＞面に沿っており、溝の壁とマイクロチップの表面の面との間に５４．７°の角度を提供する。透明カバープレート２２０がマイクロチップ２１０の表面に密封状態で取り付けられる。１つの好適な実施形態では、カバープレートはパイレックスにより製造され、陽極酸化によりシリコンマイクロチップに結合される。この好適な実施形態では、光源はダイオードレーザ３１０と光ファイバ３１２と集光ヘッド３１４とを含む。非散乱光、すなわち粒子によって散乱されていない光はチャネル２１１の壁によって鏡面反射され、経路３２２に沿って進行する。小角（前方）散乱光は経路３２２から僅かに外れ、小角検出器３２０に突き当たる。大角度で散乱した光の一部は経路３３２に沿って大角光検出器３３０に向かって進行する。これらの光検出器はフォトダイオードまたは光電子増倍管であり得る。大角検出器３３０は、大角散乱および／または蛍光を測定するために用いられ得る。

装置を通る供給流体の流れに圧力を加える手段もまた提供され得る。このような手段は供給入口および／または出口で（例えば、化学的または機械的手段によって加えられる真空として）提供され得る。このような圧力を加える手段は、例えば、Shoji, S.およびEsashi, M. (1994),「Microflow devices and systems」J. Micromechanics and Microengineering, 4:157-171に記載されているように当該分野において既知であり、水カラムまたは他の水圧、電気浸透力、光力、重力、および表面張力を加える手段を使用することを含む。システムの要件に依

存して、約10^{-6}ｐｓｉ～約１０ｐｓｉの圧力が用いられ得る。好ましくは、約10^{-3}ｐｓｉが用いられる。もっとも好適な圧力は、水圧約２ｍｍ～約１００ｍｍの間である。

多数のストリームを用いる実施形態の１つの例としては、層流として流れる３つの入口ストリームを有し、このうち中央のストリームが試薬ストリームである

チャネルセルが挙げられる。例えば、サンプルストリームは血液であり、中央ストリームはグルコースオキシダーゼであり、第３のストリームは、ｐＨ感応性染料を含有するインジケータストリームであり得る。グルコース粒子は試薬ストリームを通って拡散するときグルコン酸に変えられ、グルコン酸は、グルコン酸分子がインジケータストリームに拡散するとき、ｐＨ感応性染料によって検出される。多ストリームシステムの他の例としては、検出手段の較正のために分析物を異なる濃度で有するいくつかのサンプルストリームを含むシステムが挙げられる。サンプルストリームに隣接していないインジケータストリームはまたコントロールストリームとして使用され得る。

インジケータストリームは、粒子のストリームへの拡散が行われる前および行われた後、検出手段によって測定され、このような測定値は、インジケータストリームのその長さに沿った変化率と共に、分析物濃度をアッセイするために用いられ得る。さらに、インジケータストリームを測定するために、異なるタイプの多数の検出手段が用いられ得る。イオン感応性または化学感応性の電界効果が装置の様々な位置で測定され得る。

本発明のチャネルセルおよびセル内のチャネルは、検出が所望される粒子のサイズによって決定される大きさであり得る。当該分野において既知のように、分析物粒子の拡散係数は、一般に、粒子のサイズに逆に関連する。検出が所望される粒子の拡散係数が分かれば、２つのストリームの接触時間、中央チャネルのサイズ、各ストリームの相対量、各ストリームの圧力および速度は調整され得、これにより所望の拡散パターンが実現される。

流体の動的挙動はフローのレイノルズ数に直接関連する。レイノルズ数は、慣性力対粘性力の比率である。レイノルズ数が減少すると、フローパターンの粘性効果に対する依存度は大きくなり、慣性効果に対する依存度は小さくなる。所定

のレイノルズ数、例えば０．１より小さい場合は、慣性効果は実質的に無視され得る。本発明のマイクロ流体装置は慣性効果を必要とせずに仕事を行い、このため、装置の小型化がレイノルズ数効果によって内在的に制約されることはない。本発明の装置は、層流の非乱流フローを必要とし、上述の原理に従って設計され

、これによりレイノルズ数が低い、すなわちレイノルズ数が約１より低いフローを生成する。

レイノルズ数は、慣性力の粘性力に対する比である。レイノルズ数が減少するにつれて、フローのパターンはより粘性効果に依存しかつ慣性効果にはより依存しなくなる。あるレイノルズ数、例えば約１未満では（屈曲および管腔サイズ変化を有するチャネルを含むシステムについては管腔サイズに基づく）、慣性効果は実質的に無視し得る。本発明のマイクロ流体装置は慣性効果を必要とすることなしに達成することが可能であり、従って、レイノルズ数効果に起因する小型化に対する内在的な制限を何ら有さない。本発明者らのチャネルセル設計は、従来報告されている設計とは大きく異なるが、この範囲で動作する。本発明のこれらのマイクロ流体装置は、層状の非乱流を必要とし、前記原理に従って低レイノルズ数を有するフローを生成するように設計される。

本発明の好適な実施態様による装置は、数秒、例えば約３秒間以内に、約0.01マイクロリットル～約20マイクロリットル間のサイズのサンプルの分析が可能である。本装置はまた再使用が可能である。目詰まりは最小にされ、可逆的(reversible)である。例えば幅100μｍおよび100μｍ／ｓのサイズおよび速度は、レイノルズ数（$R_e = \rho l v / \eta$）が約10^{-2}であることを意味し、従って流体は、粘性が慣性に勝る状態にある。

円形状のチャネル（長さｌ、直径ｄ）を通した際に、絶対粘度ηおよび密度ｐを有する流体の平均速度ｖを得るために必要な圧力滴の大きさは、ポアズイユの法則から計算され得る（Batchelo、,G.K.、An Introduction to Fluid Dynamics、Cambridge Univ. Press 1967）：

$$\frac{P}{l} = \frac{32\eta v}{d^2}$$

ｖ＝100μｍ／secおよびｄ＝100μｍを用いると、チャネル長の１ｃｍ毎につき約0.3mmのH_2Oに等しい圧力滴が得られる。ポアズイユの式は円形状のフローチャネルにのみ厳密に有効である一方、本発明のチャネルの断面は実質的に矩形であるため、これは表記の変数間のおおよその関係としか考えられ得ないものである。

液体が装置中に導入される際、まず、加えられた圧力P_oと表面張力に起因する圧力

$$P_{st} = \frac{\gamma \cos\theta}{r}$$

との和に等しい実効圧$P_{eff} = P_o + P_{st}$が存在する。P_{st}は、流体の表面張力γ、表面に対する流体の接触角度θ、および流体表面の曲率半径rの関数である。

親水性表面においては、$\cos\theta$は1に近く、小さいチャネルにおいては装置を濡らすために圧力を加える必要がない。これは、「毛管現象による濡れ」と呼ばれる。しかし、装置がいったん完全に濡れると、出口領域における表面張力の心配をしなくてはならなくなる。本実施例に記載した装置において、出口領域における流体の曲率半径は数ミリメートルであり、表面張力に起因する圧力は無視し得るものであった。

チャネル幅が100μmならば、P_{st}は、約1cmのH_2Oであり、出口チャネル上の表面張力は問題になる。しかし、シリコンの<100>面を浸食する後述のEPW F-Etchのようなエッチャントを用いれば、エッチングされた角部は図に示したほど鋭くはなくなる。このためチャネルが約1mmまで漸進的に幅広となり、表面張力の効果が減少する。

上述の原理に基いて、チャネルの構成を適切なチャネル長が得られるように調整することにより、サンプルストリームとインジケータストリームの間の流速および接触時間、およびサンプルストリーム中に残存してインジケータストリーム中に拡散する粒子のサイズを制御することができる。必要な接触時間は、粒子の拡散係数Dと粒子が拡散しなければならない距離dとの関数として、$t = d^2/D$により計算することができる。Dより大きい拡散係数を有する粒子または分子

はインジケータストリーム中に拡散し、Dより実質的に小さい拡散係数を有する粒子または分子はインジケータストリーム中に拡散しない。大きい方の粒子の拡散係数がDよりおよそ10倍小さければ、インジケータストリームは大きい粒子をまったく含まないはずである。

所与の流速、比較的小さい拡散係数を有するある種の分析物において、直線状

チャネルセルシステム（Tセンサ）チャネル（好ましくは長さ５～50ｍｍ）では、拡散が十分に起こるほどの長いフローチャネルが得られない。典型的には、シリコンマイクロチップの直径は３インチ、４インチ、６インチ、または８インチである。このようなサイズのマイクロチップにエッチングされた直線状チャネルは、マイクロチップ直径よりも長くはなれない。比較的小さい拡散係数を有する分析物、例えば比較的大きい分析物または非球状分析物の検出には、好ましくは回旋状フローチャネルを用いる。本明細書において、回旋状フローチャネルとは、直線状でないフローチャネルを指す。図５および６に、典型的な３～４インチのシリコンマイクロチップ上においてより長いフローチャネルを可能にする２つの異なるチャネルジオメトリを示す。

図５のチャネルセルシステム（Tセンサ）において、左右のストリーム、例えばサンプルストリームおよびインジケータストリームは、同じ総パス長を有する。この実施態様において複数回の計測を行う場合は、両ストリームが同じ流速で流れかつ同じフロー距離を流れるように、センサの垂直な中心線に沿って行うべきである。回旋状フローチャネルが図５に示すような方形波形状を有しているこの実施態様において、ストリームは湾曲を通じて異なる速度で流れる。従って、異なる速度で流れるストリーム間の緊密／狭湾曲部および垂直力(sheer force)のために層流再循環が起こる地帯が発生し得るため、直線状フローチャネルにおいて用いられる速度よりも遅い流速を用いる方が好ましい場合がある。層流再循環は乱流ではなく、フローは以前として層流であり予測可能である。しかしながら層流再循環は好ましくないので、レイノルズ数を約１未満に維持することにより回避することができる。

図６のチャネルセルシステム（Tセンサ）は、コイル／スパイラル状のフローチャネルを示している。このジオメトリにおいて、各々長さ220ｍｍのフローチ

ャネルを有する４つの別々のTセンサを、１個の３インチマイクロチップ上に製造することができる。方形波状のジオメトリよりもこのジオメトリの方が屈曲半径が大きいため、層流再循環が起こる可能性はより少ない。左右のストリーム（サンプルストリームおよびインジケータストリーム）の相対流速の差が最小なた

め、２つのストリームが異なる粘度を有していても２つのストリーム間の垂直ストレスがより小さくなる。しかしこのチャネルジオメトリの場合、左右のストリームについて異なる総フロー距離となる。

図７Ａおよび７Ｂは、Ｔ接合部５８を丸めた、本発明のチャネルセルシステム（Ｔセンサ装置）を示している。図７Ａは、図１に示したものと同様なＴセンサを示しているが、図７Ａにおいてはｔ接合部５８が丸められている。図７Ｂは、図３に示したものと同様なビューポートＴセンサを示しているが、図７Ｂにおいてはｔ接合部５８が丸められている。丸められたＴ接合部は、レイノルズ数が約１より大きい場合に発生し得るＴ接合部における層流再循環を防止するため、好ましい。また丸められたＴ接合部は、サンプルストリームのインジケータストリームへ、あるいはその逆の汚染の確率を減少させる意味でも、好ましい。

本発明のチャネルセルシステムは、時間ではなく距離（Ｔ接合部からの）の関数として分析物の濃度を測定するために用い得る。距離の増分は時間の増分に比例する。層流であり流速がわかっている場合、距離の増分は時間の増分に変換することができる。

動力学的測定を行うための他の方法は、濃度、または濃度に起因する何らかの物理的特性、例えば吸光または蛍光を、時間に対してプロッティングすることを用いる。出発物質の濃度の経時的な減少または生成物の濃度の経時的な増加は、ある反応における動力学的速度定数を決定する。

本発明のＴセンサ装置を用いて、反応の速度または速度定数を決定し得る。１つ以上の分析物検出領域において、吸収または蛍光測定などの検出を行うことができる。図８を参照して、複数の分析物検出器410を、Ｔ接合部５８から様々な距離に位置することができる。または１つの検出器を用いて、Ｔ接合部５８から様々な距離にあるフローチャネルをモニタすることができる。図８は、方形波／蛇行形状のフローチャネルを示している。しかし層流を維持する任意のジオメトリのＴセンサを用いて、動力学的測定を、特に本明細書に開示した方法を用いて行うことができる。サンプルストリームはサンプルストリーム入口ポート３０を介して導入され、インジケータストリームはインジケータストリーム入口ポート

２０を介して導入される。２つのストリームはＴ接合部５８で合流する。サンプルストリームからの分析物は、インジケータストリーム中に拡散し始め、例えば蛍光の増加などの測定可能な変化が起こる。測定可能な変化は、分析物検出領域９０に示すように、分析物がインジケータストリーム中に拡散する結果として起こる。

分析物検出領域における蛍光または吸光の強度および分析物検出領域の幅は、Ｔ接合部５８から様々な距離において測定される。分析物検出領域の強度および幅は、測定される分析物の濃度の関数である。分析物がインジケータストリーム中に拡散する際、分析物検出領域において色の変化（すなわち吸光度の変化）または蛍光が起こる。この光学的変化は、Ｔ接合部からの距離が増加するにつれ、分析物およびインジケータが互いと相互作用した時間が増えるために、より強くなる。Ｔ接合部からの距離が増加するにつれ、分析物検出領域の幅もまた増加する。２つの独立の原因によりこの幅の増加が起こる。第１に、分析物は時間の増加につれ、従って距離の増加につれより拡散が進む。第２に、分析物とインジケータとの間の相互作用が進行するにつれ、分析物検出領域における吸光または蛍光が大きくなる。従って、吸光または蛍光が検出できる分析物検出領域の幅が増大する。

図８を参照して、Ｔ接合部５８からの距離が増大するにつれ、分析物検出領域９０はより幅広くかつより強くなる。

本発明の装置および方法を用いて、１回の測定、例えばＴ接合部から所定の距離における蛍光の測定だけで、反応の速度定数を決定することができる。当該分野において公知であるように、測定の回数を増やすことにより、それらの測定値から計算される動力学的速度定数の精度が増大する。

他の実施態様において、本発明のＴセンサチャネルセルシステムは、図９Ａに示すような分岐フローチャネル４０１および４０２を有し得る。目的の小さな分子を含有するサンプルは、サンプルストリーム入口ポート３０を通して装置に導

かれ、サンプルストリーム入口ポート３０からサンプルストリーム入口チャネル５０に流れる。インジケータストリームは、インジケータストリーム入口ポート

２０に導かれ、インジケータストリーム入口ポート２０からインジケータストリーム入口チャネル４０に流れる。２つのストリームは、互いに平行に層流で流れ、サンプルストリームからの小さな分子（分析物）は、インジケータストリームに拡散する。本明細書で用いる分岐フローチャネルとは、フローチャネル１００と流体連通するフローチャネルを指す。図９Ａおよび図９Ｂに示すＷ接合部４００は、分岐フローチャネル４０１および４０２をフローチャネル１００で補正するために用いられ得る。分岐フローチャネルによって、未溶解粒子および溶解粒子の両方が検出可能になる。好ましくは装置の上方または下方に配置される検出器は、フローチャネル１００およびｖ溝４０３または４０４をモニタする。この二重検出実施態様によって、ｖ溝内で一列に流れる未溶解粒子だけでなく、フローチャネル１００内の溶解粒子および未溶解粒子を検出することが可能である。粒子検出は、粒子が、分岐フローチャネル４０１および４０２とそれぞれ流体連通するｖ溝４０３または４０４の１つまたは両方を通して流れるときに、標準的な光学技術（例えば、撮像、光散乱、または分光技術）によって成し遂げられ得る。分岐フローチャネル４０１および４０２は、それぞれ、出口ポート４０５および４０６と流体連通する。

例えば、本実施態様において、サンプル（例えば、全血）は、サンプルストリーム入口ポート３０を介して導入され、サンプルストリーム入口ポート３０からサンプルストリーム入口チャネル５０に流れ得る。レポータービーズを含有する緩衝溶液は、インジケータストリーム入口ポート２０を介して導入され、インジケータストリーム入口ポート２０からインジケータストリーム入口チャネル４０に流れる。サンプルおよびインジケータストリームは、フローチャネル１００内を互いに平行に層流で流れる。サンプル内の小さな分析物（例えば、プロトン）は、インジケータストリームに拡散する。図９Ａを参照すると、サンプルは、分岐フローチャネル４０２に流れ、次にｖ溝４０４に流れ、粒子（例えば、赤血球および白血球）は、ｖ溝４０４を通して一列に流れる。同時に、レポータービーズは、分岐フローチャネル４０２、次にｖ溝４０３に流れ、ｖ溝４０３を通して

一列に流れる。好ましくは装置の上方または下方に配置される光学検出器は、フ

ローチャネル１００内の２つのストリームおよびｖ溝４０４内の未溶解サンプル粒子およびｖ溝４０３内のビーズを同時にモニタする。このビーズは、溶解サンプル分析物のインジケータである。

あるいは、インジケータストリームは、本装置のこの実施態様が実施されるときに、未溶解サンプル粒子をモニタする際にモニタされる溶解インジケータ染料を含み得る。溶解インジケータ染料は、ｖ溝においてモニタされる必要はない。従って、両分岐フローチャンネルは、図９Ｃに示すように、ｖ溝に接続される必要はない。

本発明の二重検出実施態様の他の実施例は、以下の通りである。全血のサンプルは、白血球の数を検出するために、ｖ溝チャネル内でモニタされ得る。次に、同じサンプルは、ｖ溝チャネルと流体連通するＴセンサに流れる。Ｔセンサでは、白血球は、抗体でタグ化された蛍光レポータービーズと反応する。次に、サンプルは、Ｔセンサと流体連通する他のｖ溝チャネルに流れる。このｖ溝チャネルにおいて、白血球は、蛍光によって識別される。

本発明のＴセンサチャネルシステムは、図９Ｂに示すように、排気ポート４０７をさらに有し得る。サンプルストリームのみが分岐フローチャネル４０２に入り、インジケータストリームのみが分岐フローチャネル４０１に入ることを確実にするために、各ストリームの一部は、排気ポート４０７に迂回させられ得る。各ストリームの一部を排気出口に迂回させるために、排気ポートは、Ｗ接合部でフローチャネルと流体が連通している。

図９Ｃは、分岐フローチャネルおよび排気ポートを有する本発明のチャネルシステムを通して流れるサンプルストリーム（ｘで示す）およびインジケータストリーム（白抜きの四角で示す）を示す。図９Ｃは、さらに、分岐フローチャネルが、環状にならずに、フローチャネル１００と平行に延びることを示す。分岐フローチャネルは、所望の任意の角度でフローチャネル１００に接続し得る。様々なチャネルを通したフローを同時に１つの検出器を用いてモニタするためには、分岐フローチャネルが、このようなモニタを可能にする角度でフローチャネル１００と接続することが好ましい。

この実施態様を用いる１つの装置内の溶解粒子および未溶解粒子の検出は、経済的に有利である。なぜなら、測定が、１セットのポンプおよび１つの検出器のみで行われ得るからである。

一列の未溶解粒子を検出する他の手段は、シースフロー（sheath flow）モジュールを用いる。サンプルは、まず、Ｔセンサのフローチャネルを通して流れ、そこで、サンプルは、レポータービーズと反応する。例えば、サンプル中の分析物は、レポータービーズを含有するインジケータストリームに拡散する。次に、レポータービーズを含有する流体は、Ｔセンサフローチャネルと流体連通するシースフローモジュールに流れ得る。シースフローモジュール内でビーズは集められ、一列で流れて検出される。

ｖ溝チャネルについては、シースフローモジュールとＴセンサの順番を逆にすることも可能である。即ち、流体は、シースフローモジュール、次にＴセンサを通して流れ得る。図１０Ａは、「マイクロ製造されたフローチャネルにおける粒子の３次元配列装置および方法」という名称の米国特許出願（１９９７年３月２６日付けで提出）に記載されるように、フローモジュールの中央に沿った長さ方向の断面図である。プレート５０１は、機械加工、成形またはエッチングされ、フローチャネルを形成する。プレートは、限定はされないが、シリコンウェハ、プラスチック（例えば、ポリプロピレン）、および鋳造材料を含むものから選択され得る。シリコンウェハをエッチングし、プラスチックを成形および機械加工する技術は、当該技術分野で周知である。層流チャネル５０８は、プレートの平坦面で形成される。第１入口５１０は、チャネルの上流端でプレートを通過し、第１入口接合部５１１でフローチャネルを接合する。出口５３０は、チャネルの下流端でプレートを通過し、出口接合部５３１でフローチャネルを接合する。第２入口５２０は、第１入口と出口との間のプレートを通過し、フローチャネルを第２入口接合部５２１で接合する。第２入口接合部５２１は、第１入口接合部よりも狭い。第２プレート５０５は、第１プレートの平坦面にシールされ、それによって、層流チャネルの一側部を形成する。図１０Ｂは、チャネル表面を示す。入口接合部、およびフローチャネル５０８のエッジ５１２の相対的な幅が示される。第２入口接合部５２１は、第１入口接合部５１１よりも狭い。図１０Ａおよ

び図１０Ｂを再び参照すると、シース流体は、第１入口５１０を介してフローチャネル５０８に導入され、フローチャネルを通して出口５３０に流れる。中央流体は、第２入口５２０を介して、好ましくはシース流体よりも低圧力および低速度で導入される。図１０Ｃは、図１０Ａおよび図１０Ｂのフローチャネルの断面図であり、本発明の一実施態様において成し遂げられるシースフローを示す。本実施態様において、フローチャネル５０８は、台形である。入口５２０から排出された中央流体５５４は、シース流体５５３によって両側（左および右）および上部を囲まれている。

上記のように、反射材料におけるチャネルシステムを形成することによって、反射による光学測定が可能となる。あるいは、透過による光学測定は、次に記載する実施態様において成し遂げられ得る。Ｔセンサチャネルシステムは、基板プレート（例えば、シリコンマイクロチップまたは材料の他のスラブ）を通して完全にエッチングされ得る。全チャネルシステムは、完全にエッチングされ得るので、基板プレートの幅を横断、即ち貫通して延びる。あるいは、分析物検出領域９０を含むチャネルシステムの部分のみが、完全にエッチングされ得るので、図１１に示すように、基板プレートの幅を貫通して延びる。インジケータストリーム入口ポート２０、サンプルストリーム入口ポート３０、および出口ポーロ６０も図示する。光学的に透明なプレート（例えば、カバープレート）は、マイクロチップの両側にシールされる。チャネルシステムの部分のみがマイクロチップを通して完全にエッチングされる場合、透明なプレートは、マイクロチップのその部分のみを覆うだけでよい。

本発明の他の実施態様において、装置の寸法は、層流が維持されるように選択される。本実施態様において、シリコンマイクロチップは、異方性ＥＰＷエッチングによってエッチングされる場合、チャネル直径が、層流を維持するのに十分小さく維持され得るように薄いマイクロチップを用いることが好ましい。異方性ＥＰＷエッチングは、チャネルの下部よりも上部が広いチャネルを形成する。マイクロチップを完全にエッチングすることによって、上部が望ましくなく広く、望ましくなく大きなチャネル直径のチャネルを形成し得る。チャネルの直径が望ましくなく大きいと、層流は維持され得ない。薄いマイクロチップの好ましい幅

は、１００ミクロンと３００ミクロンとの間であり、より好ましくは１００ミクロンと２００ミクロンとの間である。あるいは、シリコンをエッチングする他の方法、例えば、反応性イオンエッチングは、チャネル直径を層流を維持するのに十分小さく保つために用いられ得る。チャネルシステムを形成するのに機械加工または成形される他の材料（例えば、プラスチック）は、チャネル直径を小さく保つために必ずしも薄くする必要はない。

マイクロチップは、チャネルシステムを内部に形成する前にエッチングによって薄くされ得る。フォトレジストが形成されていないマイクロチップであるコーティングされていないマイクロチップは、マイクロチップをエッチング溶液に浸漬することによって薄くされ得る。次に、チャネルシステムまたは少なくとも分析物検出領域は、マイクロチップを通して完全にエッチングされ得る。

あるいは、低レイノルズ数を維持するＴセンサチャネルシステム、即ち、層流が形成され得、チャネルの深さは、幅よりも大きい。しかし、流速は、チャネル幅に対して放物線状、即ち、チャネル中央で速くなり、壁でゼロに近づくので、チャネル直径は、上部から下部および下部から上部への拡散が、この放物状の流速プロファイルを打ち消すような直径であることが好ましい。フローチャネルの深さを高めると、上部から下部および下部から上部への拡散の効果が減少する。

本明細書で記載した実施態様以外の多数の実施態様は、当業者に容易に明白であり、本発明の限度および範囲内である。本明細書で引用したすべての参考文献は、本明細書において参考のためにその全体を援用する。以下の実施例は、本発明を例示するが、本発明を限定するものではない。

実施例

実施例１．チャネルセルの形成

２マスクレベルプロセスを用いて、シリコンウエハ上に本発明のチャネルセルを形成した。チャネルセルは、幅４００マイクロメータで長さ２０ｍｍのフローチャネルを有していた。入口チャネルを構成する「Ｔ」の「ブランチ」またはクロスバーは、長さ３０ｍｍで幅２００マイクロメータの溝を有していた。チャネル深さは５０マイクロメータであった。

第１のマスクレベルは入口および出口ポートを規定した。入口および出口ポートを、ウエハを介してシリコンの裏面まで完全にエッチングした。第２のレベルは、流体搬送チャネルを規定した。

Photo Sciences, Inc. (Torrance, CA)による仕様書に従って４インチのクロムマスクを作成した。この上に成長させた５００ｎｍのＳｉO_2を含む３インチウエハ（｛１００｝、ｎ型）を用いた。

処理前に、Piranha浴（H_2SO_4およびH_2O_2）（２：１）中でウエハを洗浄した。プライマー（３０００ｒｐｍでＨＭＤＳスピンした）を用いてフォトレジストの付着を向上させた。ＡＺ－１３７０－ＳＦ(Hoechst)フォトレジストをスピンコーティング（３０００ｒｐｍ）によって約１μｍ堆積し、続いて軟焼成（９０℃で３０分間）を行った。

コンタクト整合器を用いてウエハを整合させ露出させた。露出時間を変えることにより最高の結果を得た。露出後の焼成は行わなかった。ウエハをＡＺ－３５１（４：１に希釈）(Hoechst)中で１分間現像し、ＤＩ水中でリンスした。酸化物エッチング液に対して酸化物を保護するために、ブルータックテープ(Semiconductor Equipment Corporation、Moorpark, CA)をウエハの裏面に塗布した。

緩衝化酸化物エッチング液(BOE、１０：１　ＨＦ（４９％）およびNH_4F（１０％））中にウエハを１１分間浸漬することにより、保護されていない酸化物を完全にエッチング除去した。ブルータックテープを手で除去し、フォトレジストをアセトンリンス液中で除去した。

シリコンのエッチングを、リフラックスボイリングフラスコ内にセットされたエチレン－ジアミン、ピロ－カテコール、および水の混合液（Reisman A.ら(1979)、J. Electrochem. Soc. 126:1406-1415に記載のＥＰＷ　Ｆ－ｅｔｃｈ）中で行った。このエッチングは、１時間当たり約１００μｍの速度でシリコンの｛１００｝面を攻撃する。第１の工程で、流体取付ポートを約３時間エッチングした。フォトレジストを再び塗布して、流体ポートとバリア領域との間のフローチャネルを含むマスクを露出させた。第２の工程で、ウエハを現像して約１時間エッチングした。

最終処理の後、ウエハを再びPiranha浴中で洗浄してＤＩ水でリンスした。そ

の後、ウエハを、約１ｃｍ×１ｃｍの個々の素子にダイシングした。

Wallis, G.およびPomerantz, D.I(1969)のJ. Appl. Physics 40:3946-3949による陽極酸化ボンディングを用いて、シリコン素子にPyrexガラスを取り付けた。Esco Products Inc. (Oak Ridge, NJ)からのPyrexガラス（厚み１００μｍ）の１インチ平方の片を用いた。まず、シリコンおよびPyrexガラスを、５０℃に加熱したH_2O_2、NH_4OH、およびH_2O（１：４：６）の溶液に浸漬した。このプロセスは、表面上のいずれの有機物質をも除去し、さらに表面を親水性にする。この溶液中に２０分間浸漬した後、シリコンおよびPyrexガラスをＤＩ水でリンスして乾燥した。陽極酸化ボンディングを、ガラスとシリコンとの間に４００Ｖを印加して、４００℃で行った。

実施例２．ｐＨによる蛍光色変化

分析グレード化学薬品(Aldrich)からｐＨ７．２、７．４、７．６、７．８および８．０の５種類の０．０１Ｍ　ＨＥＰＥＳ緩衝液を調製した。得られた液を、サンプルストリームとして連続的に用いた。この実験において注目すべき分析物は、H^+またはOH^-である。１ｍｇの蛍光ｐＨインジケータ染料カルボキシーＳＮＡＦＬ２(Molecular Probes、Eugune, OR)を２ｍｌのＤＭＳＯ(((.9%、Aldrich)中に溶解した。この溶液０．１ｍｌをｐＨ７．０の０．０００１Ｍ　ＨＥＰＥＳ緩衝液１ｍｌと混合した。得られた溶液をインジケータストリームとして用いた。

Ｔセンサチャネルセルを、Ｔセンサの接合部が対物レンズの視野内にあるように、顕微鏡のステージに取り付けた。入口ポートと出口ポートとの間に３０ｍｍ水カラムの圧力差があるように、入口ポートと出口ポートとをインジェクタループと、水で充満した直立チューブとに接続した。２つのストリームがＴ接合部の中央で合流して出口ポートに平行に流れるように、両方の入口ポートを同一の圧力に曝した。一方のインジェクタループをインジケータ染料溶液で充満させて、他方のループをサンプル溶液のうちの１つで充満させた。ループは、約１時間装置を動作させるのに十分な容量を含んでいた。

両方のインジェクションループがＴセンサに流入することが可能になった後且つ１分間の平衡およびフラッシング時間の後、顕微鏡のカメラアタッチメントに

より写真を撮った。励起フィルタ中央波長は４８０ｎｍであり、発光フィルタは、ロングパス５１０ｎｍフィルタであった。

実験により、インジケータストリームとサンプルストリームとの間の分析物検出領域の色がサンプルストリームのｐＨの関数である写真が得られた。ｐＨが８．０から７．２に低下するにつれて、色は赤からオレンジを経て黄色に変化した。コンピュータエンハンスト画像は、インジケータストリーム自体の色が黄色であり、ストリーム間の分析物検出領域が赤からオレンジの範囲であることを示したが、無色の大量のストリームは黒く見えた。カラーマッピングにより、システムを較正するために用いられる異なる色に数値を割り付ける。あるいは、光強度の変化を２つの波長で測定し、それによりｐＨの低下に伴う、スペクトルの赤い部分の減少と黄色い部分の増加とを測定する。

実施例３．距離の関数としての動力学的測定

血清中のアルカリホスファターゼと、０．１Ｍ　ＨＥＰＥＳ緩衝液、ｐＨ７．４０中の０．１Ｍρ－ニトロフェノールホスフェート（ＰＮＰＰ）（薄い黄色）とをＴセンサ装置に注入した。アルカリホスファターゼは、ＰＮＰＰの反応を触媒して、ρ－ニトロフェノール（濃い黄色）とホスフェートとにした。ρ－ニトロフェノールの生成（およびその速度）は、黄色の濃さの増加により検出された。Ｔ接合部からの距離の関数としての黄色の濃さの変化の速度は、酵素濃度の関数であり、速度定数の計算を可能にする。

【図１】

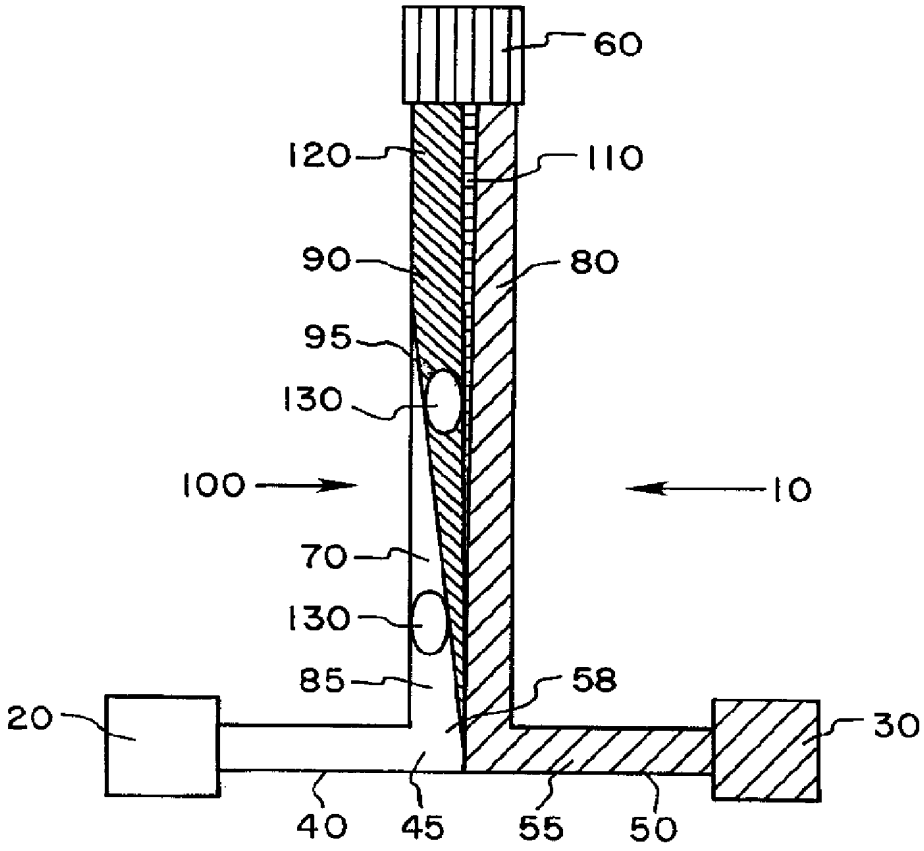

Fig. 1

【図２】

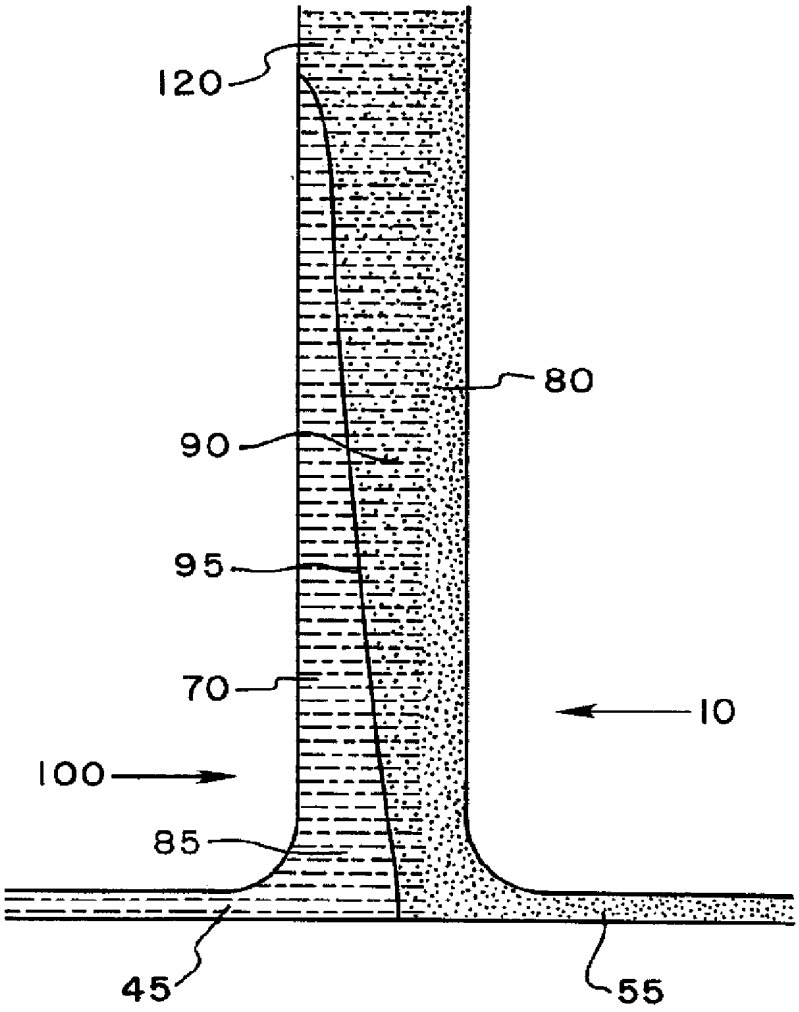

Fig. 2

【図３】

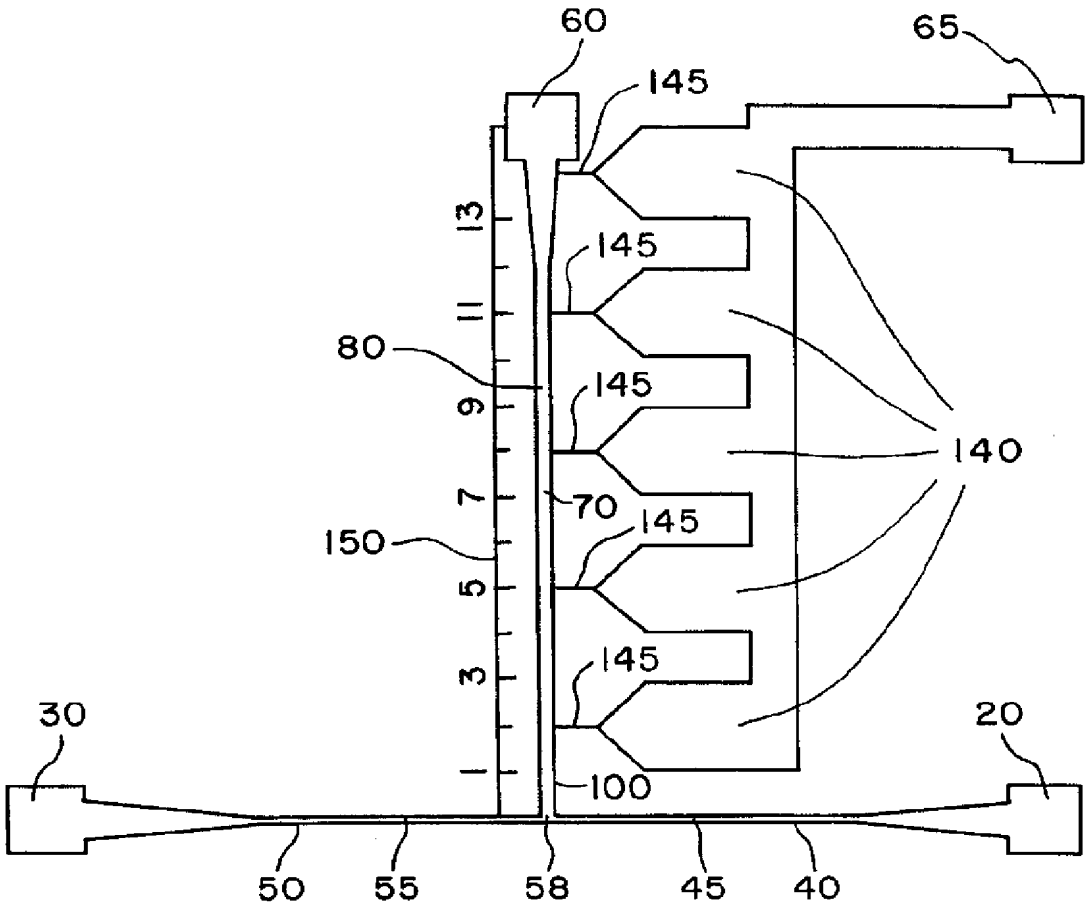
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Fig. 3

【図４】

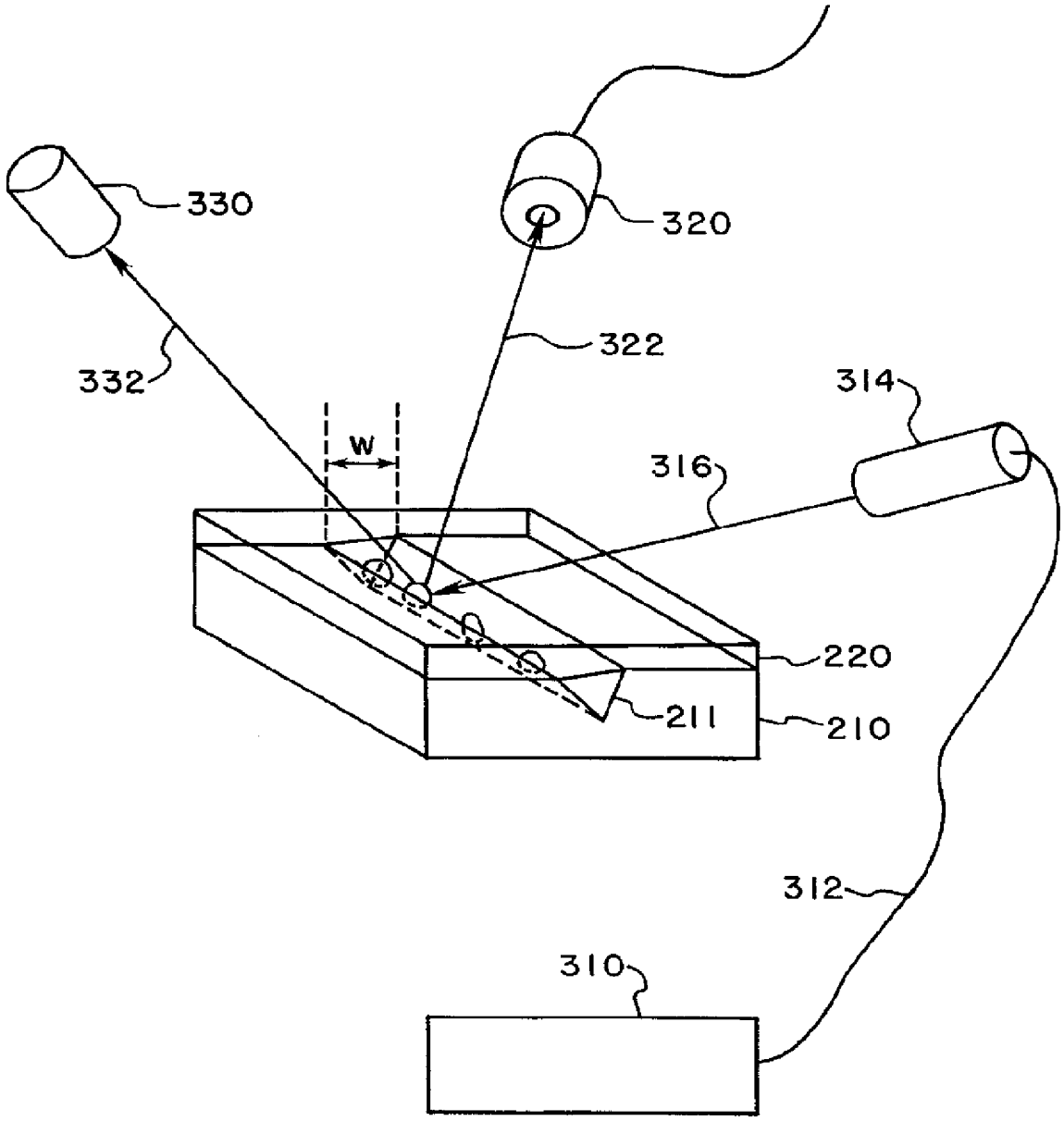

Fig. 4

【図５】

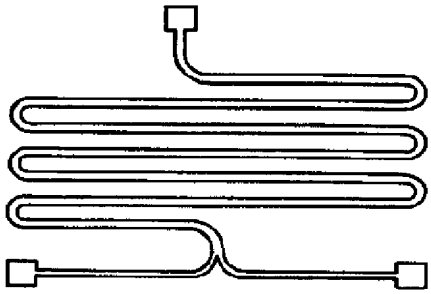

Fig. 5

【図６】

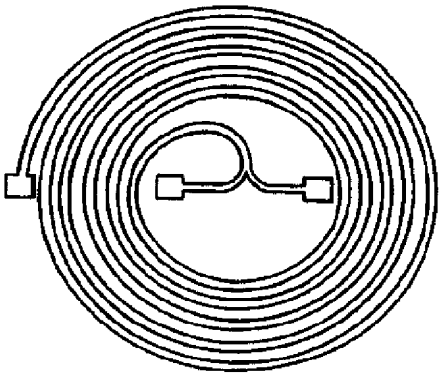

Fig. 6

【図７】

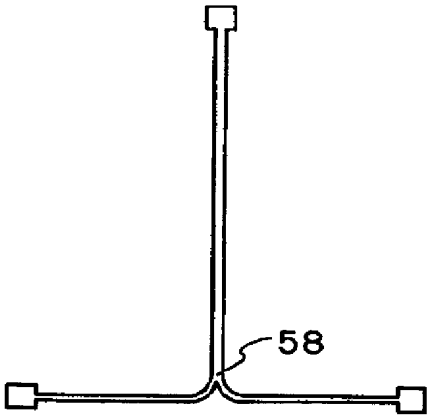

Fig. 7a

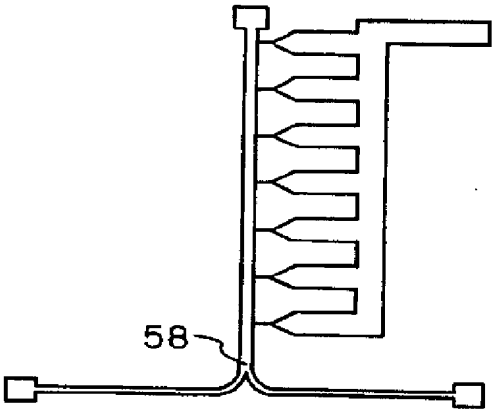

Fig. 7b

【図８】

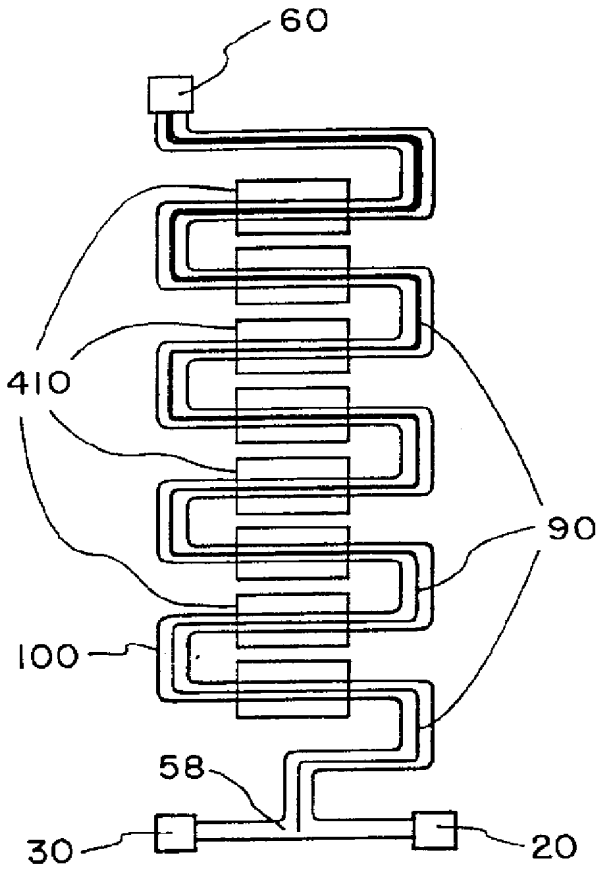

Fig. 8

【図９】

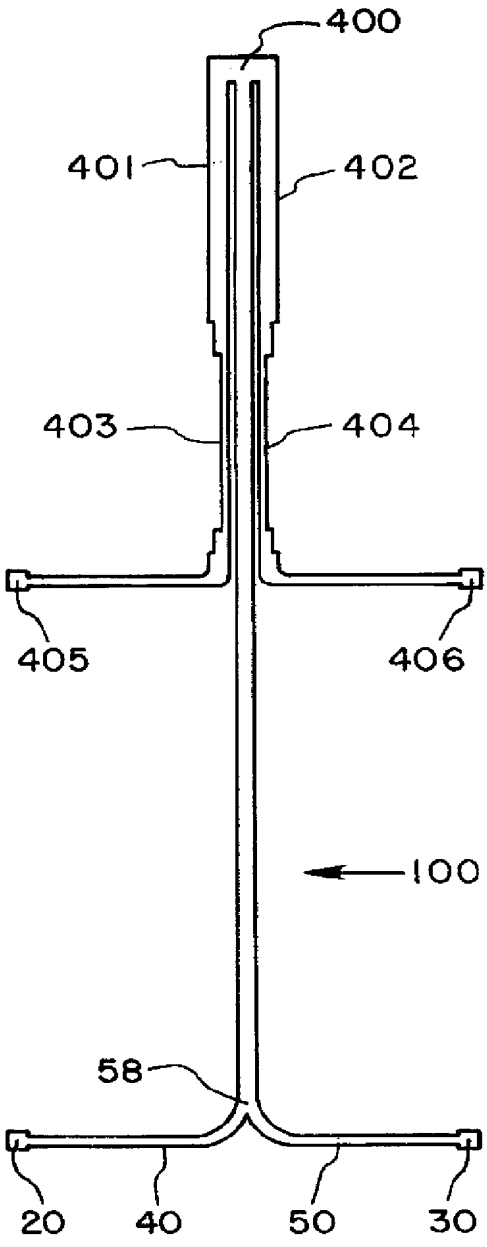

Fig. 9a

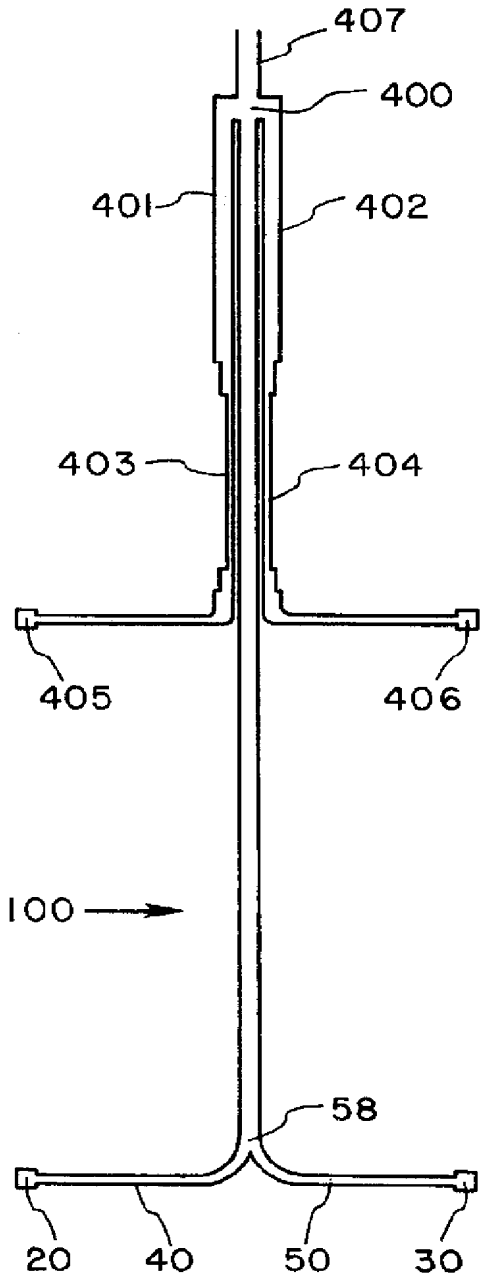

Fig. 9b

【図９】

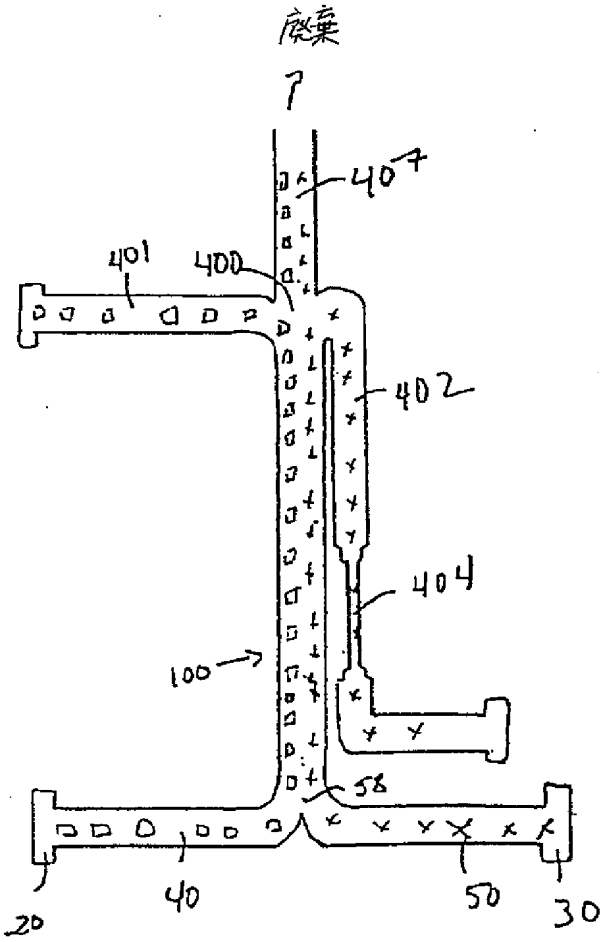

FIG. 9C

【図１０】

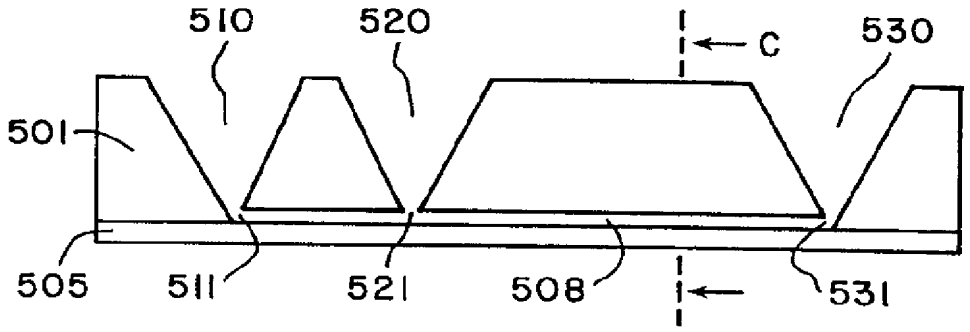

Fig. 10a

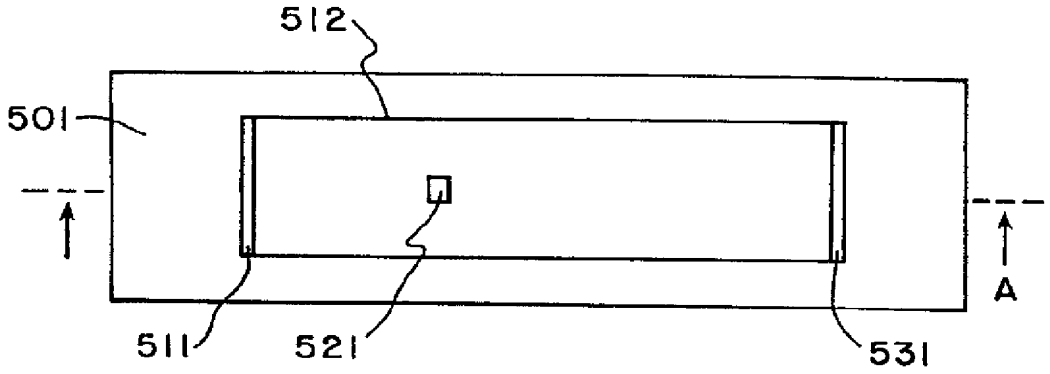

Fig. 10b

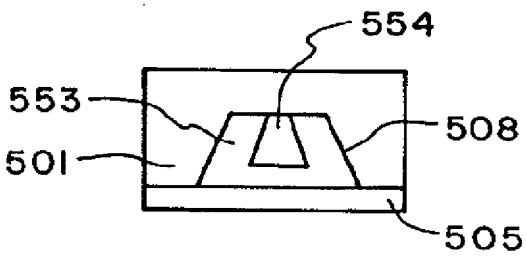

Fig. 10c

【図１１】

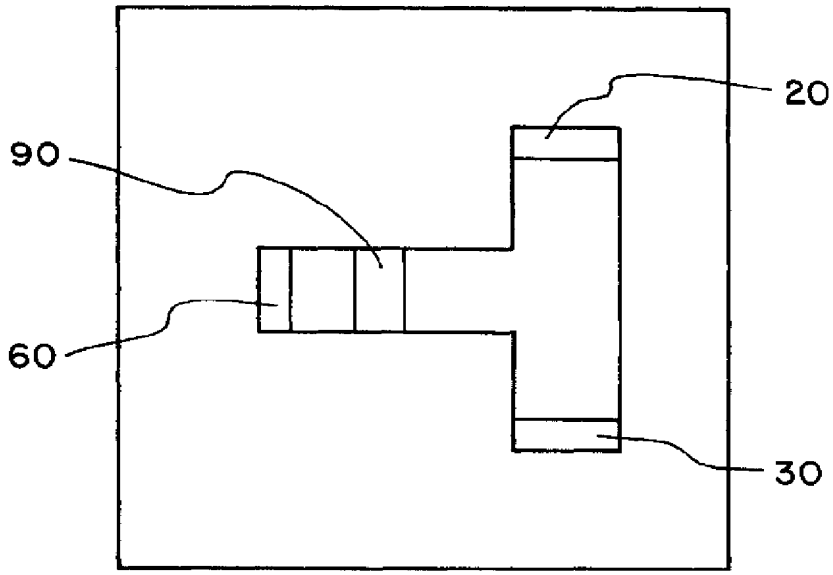

Fig. 11

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05245

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 21/64
US CL :436/172, 177, 180; 422/81
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 436/172, 177, 180; 422/81

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,683,212 A (UFFENHEIMER) 28 July 1987, entire document.	1-13
A	US 5,389,524 A (LARSEN et al) 14 February 1995, entire document.	1-13
A	US 4,737,268 A (GIDDINGS) 12 April 1988, entire document.	1-13
A	US 4,894,146 A (GIDDINGS) 16 January 1990, entire document.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 SEPTEMBER 1997	Date of mailing of the international search report 29 SEP 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JEFFREY R. SNAY Telephone No. (703) 308-0651

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1/00	１０１	1/00	１０１Ｑ	
15/14		15/14	Ｅ	
33/48		33/48	Ｍ	
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審査請求　未請求　予備審査請求　有　（全 68 頁）

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(33)優先権主張国　デンマーク（ＤＫ）
(31)優先権主張番号　０１５０／９７
(32)優先日　平成９年２月10日(1997.2.10)
(33)優先権主張国　デンマーク（ＤＫ）

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(74)代理人　弁理士　野河　信太郎

最終頁に続く

(54)【発明の名称】　粒子の分離と分析用のマイクロフローシステム

(57)【要約】
粒子を含有する流体流をフローチャンネルに案内する部材に画定されたフローチャンネル(5)、流体をフローチャンネルに導入するためフローチャンネルの一端に位置した第１の入口手段(2)、流体をフローチャンネルから排出するためフローチャンネルの他端に位置した第１出口手段(7)とを有する微細な構造の部材からなり、粒子を含有する流体流が、一時に１つの粒子がフローチャンネルの断面を通過するように制御され、かつ部材は、フローチャンネルに存在しかつフローチャンネルを横切る場に影響される粒子が場の方向に偏向されるように、フローチャンネルの縦軸に実質的に垂直である場に位置される粒子分離用のマイクロフローシステムが提供される。さらに、流体流をフローチャンネルに案内する部材に画定されたフローチャンネル、粒子をフローチャンネルに導入するための第１入口手段、流体をフローチャンネルから排出するための第１出口手段及び流体がフローチャンネルにある間に複数の成分を分析できる、フローチャンネルに位置し、固定化試薬からなる複数のアッセイ部位を有する微細な構造の部材からなる流体成分の分

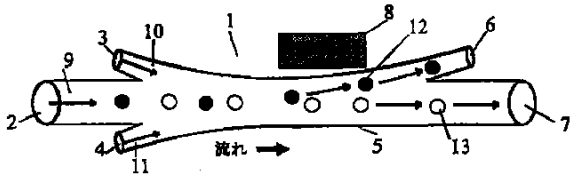


図 1

$$D = 3\pi\mu\, Ud \left(1 + \frac{3}{16} Re\right)^{\frac{1}{2}}$$

$$G = (\rho_{\text{粒子}} - \rho_{\text{液体}})g\frac{\pi}{6}d^3$$

$$U_\infty = \frac{(\rho_{\text{粒子}} - \rho_{\text{液体}})g\,d^2}{18\mu}$$

$$U(t) = U_\infty(1 - e^{-1\frac{g}{U_\infty}})$$

製造方法　材料：酸化ケイ素　SiO_2

写真平版

湿式化学エッチング

フローチャンネル

断面領域	巾 0.1～0.55mm x 深さ0.04～0.2mm
長さ	1.0～200mm
全流量 [μl/分]	1～200
流速度 [mm/分]	15～180
レイノルズ数	0.1～20
分離時間	0.1秒～6.0秒[2μl/分]

磁力

永久磁石	希土類サマリウムゲルマニウム 0.5x 0.5x 0.2mm
電磁石	ホールディング磁石25mm12VD.C. RS

$$E = \frac{\dfrac{\text{分離後の陽極粒子\%}}{\text{分離後の陰極粒子\%}}}{\dfrac{\text{分離前の陽極粒子\%}}{\text{分離前の陰極粒子\%}}}$$

常磁性ビーズ	大きさ μm	分離効率（%）[1] A)	B)	C)
Polyscience				
２５%鉄－酸化物	1－10	>99	>99	95
５０%鉄－酸化物	1－10	>99	>99	96.5
Paesel+Lorei				
磁性親和力	0.5－1.5	>99	>99	97.5
Boehringer				
Streptavidin Magnetic	1	90.5	88.7	89.5
Dynal				
Magnetic Mass Dyal M-450	1－10	98.0	>99	96.5

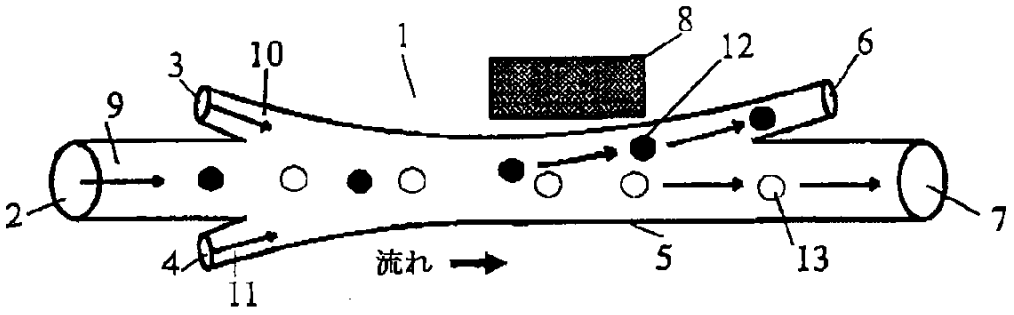


図 1

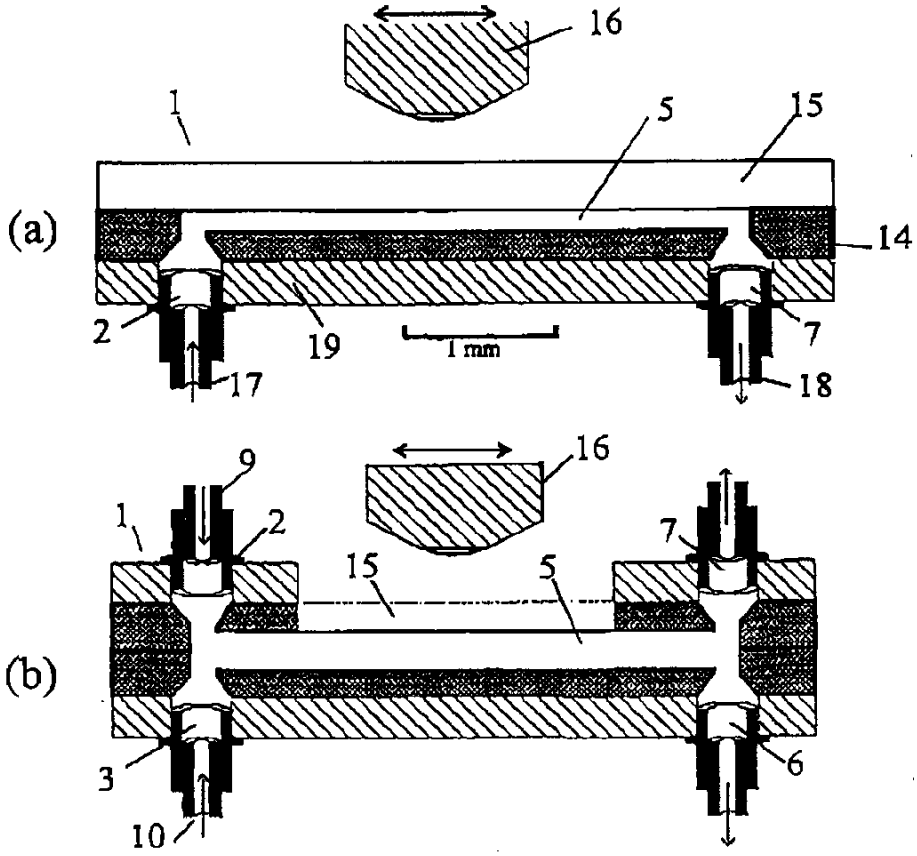
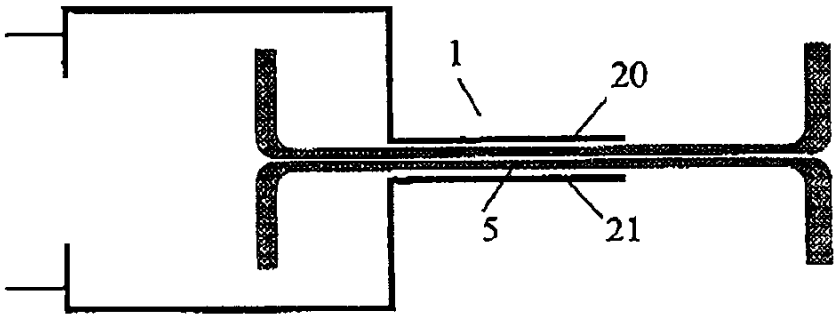


図 2



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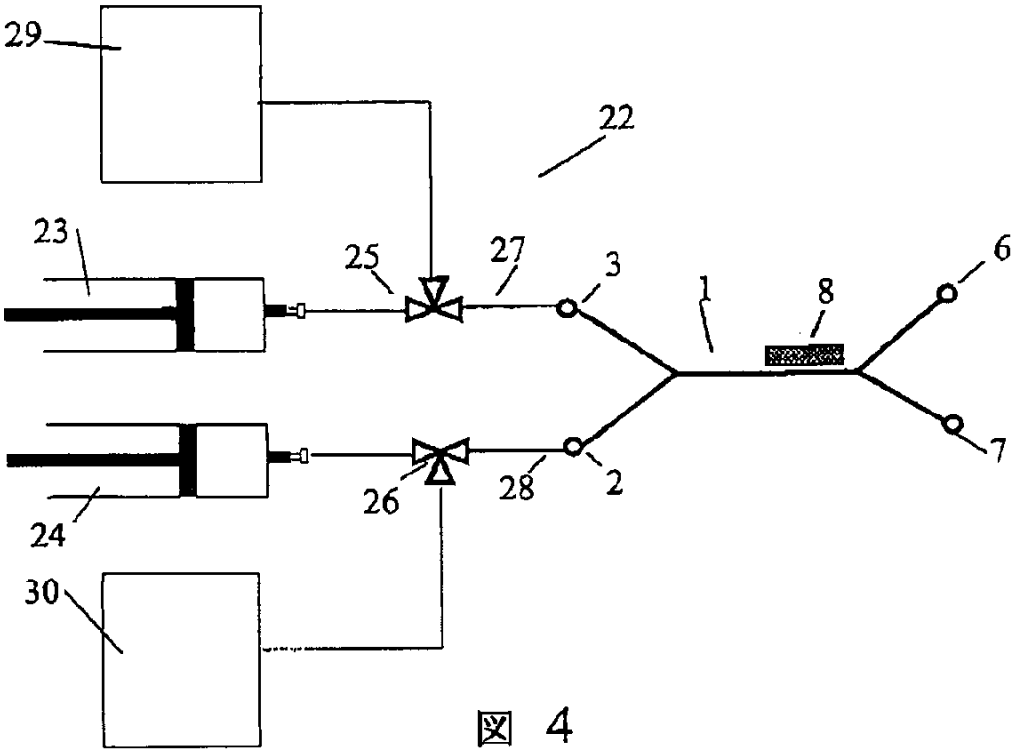
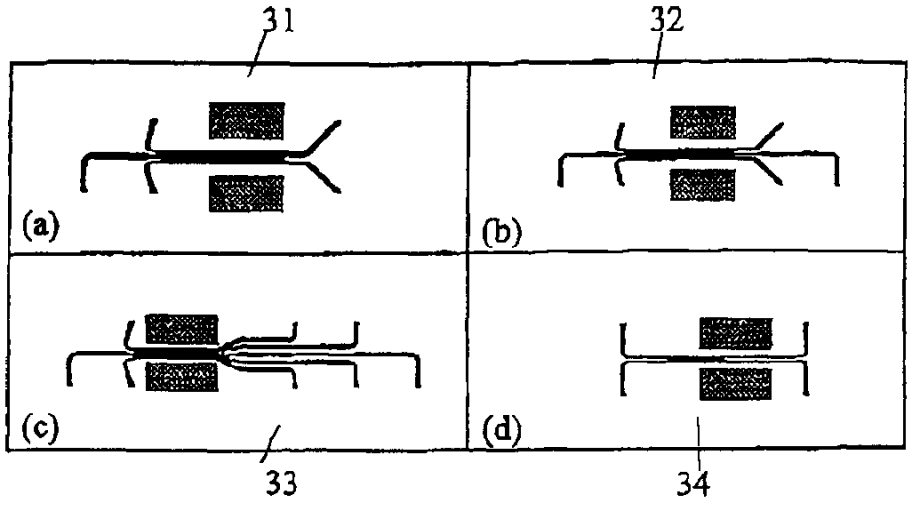


図 4



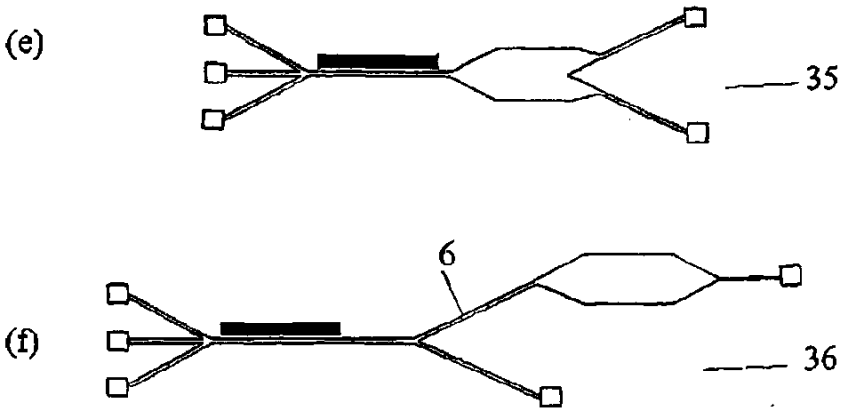


図 5

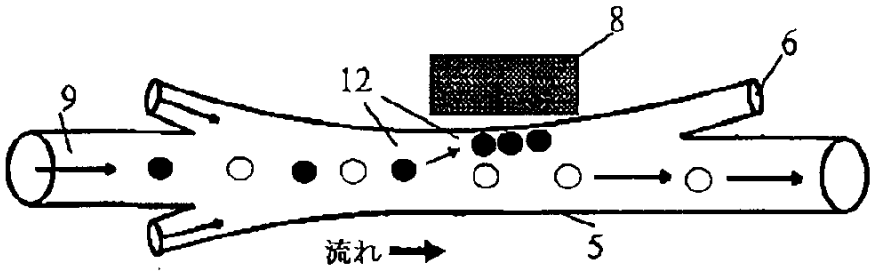


図 6

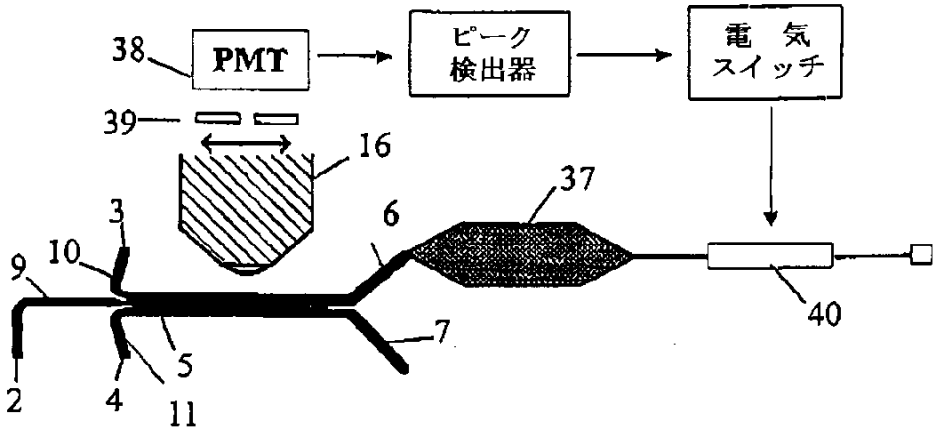
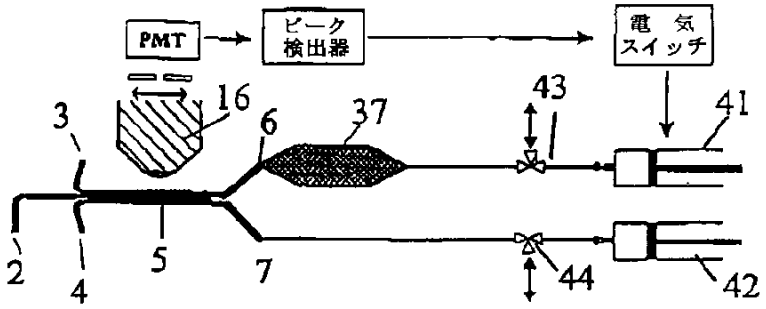


図 7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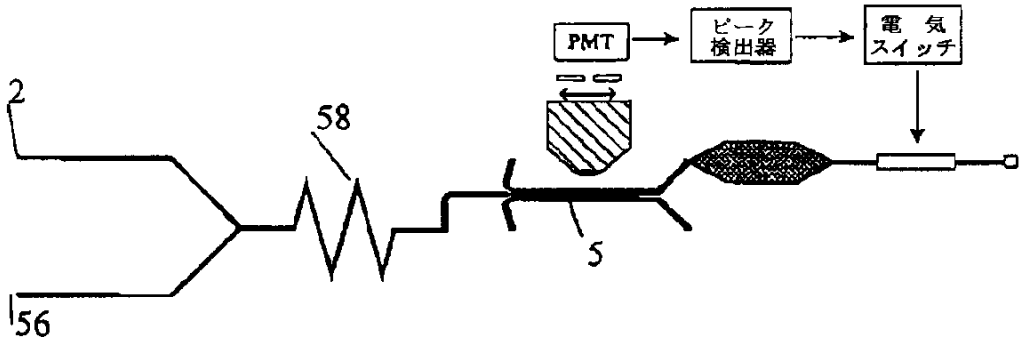
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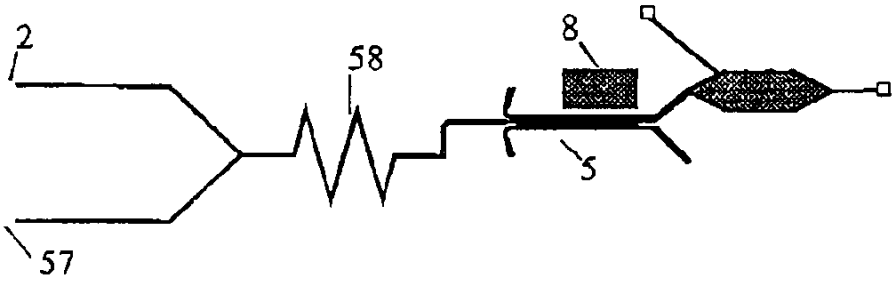
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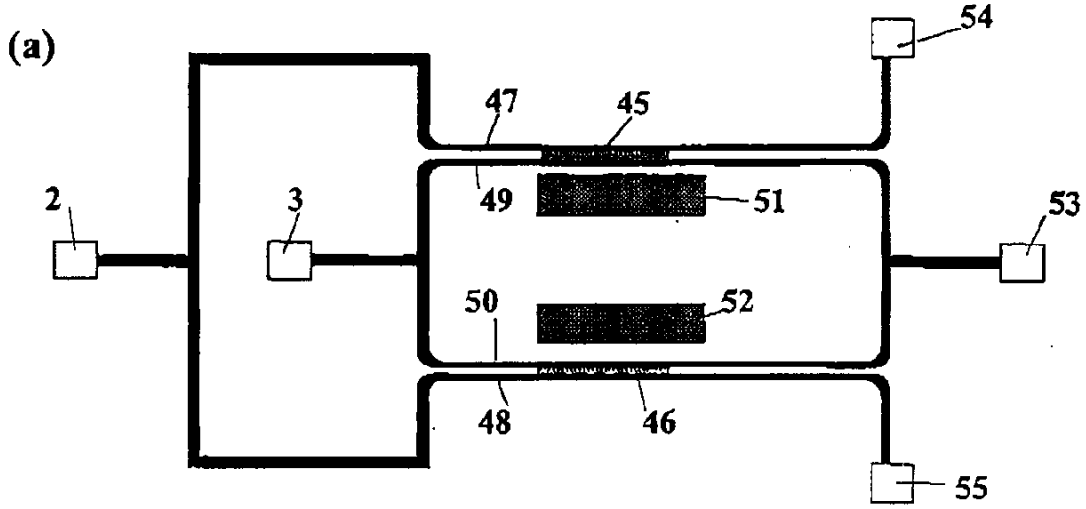
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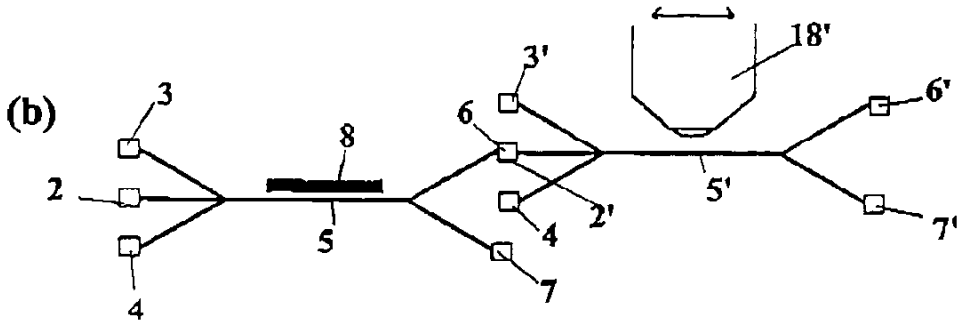
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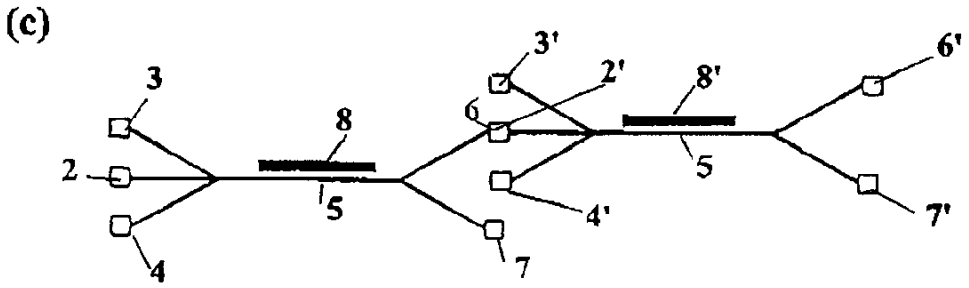
(c)
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4'
7'
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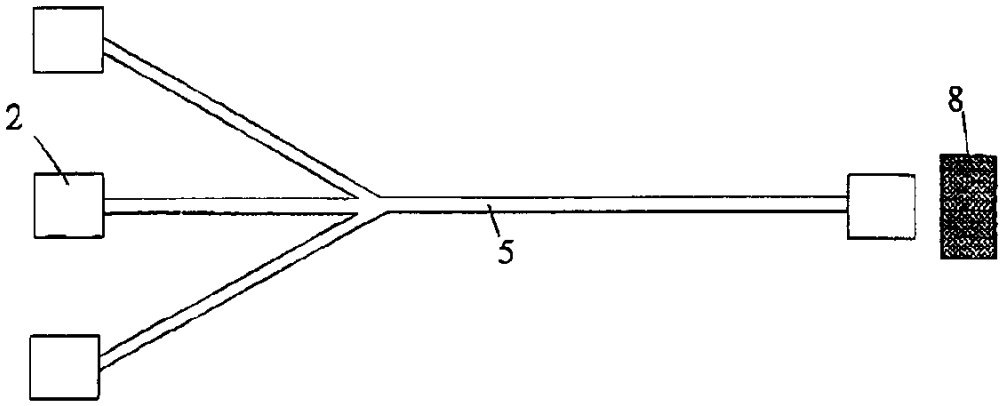
図 9



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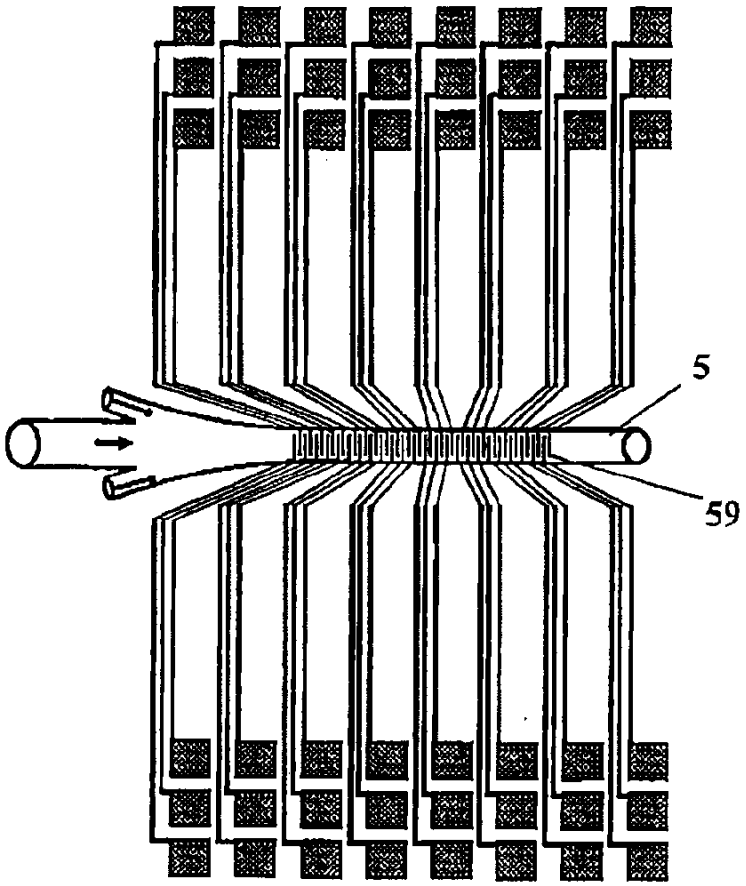

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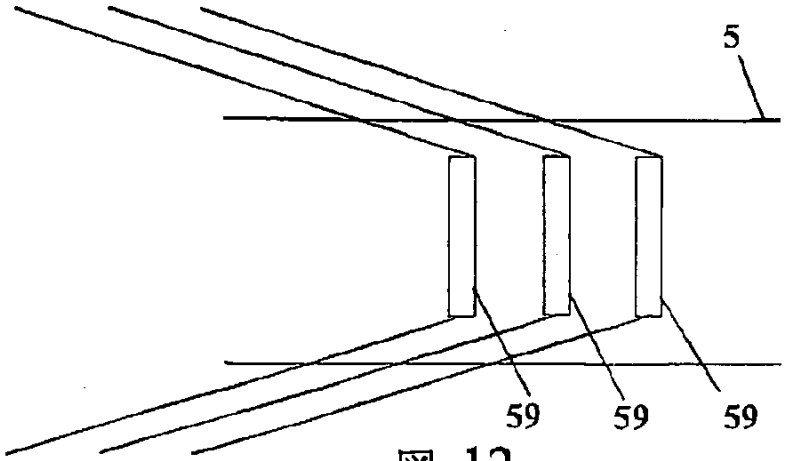

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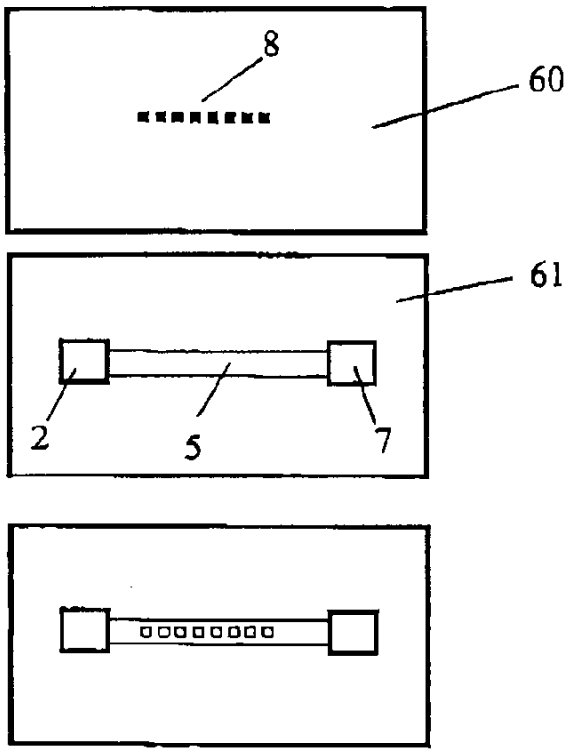

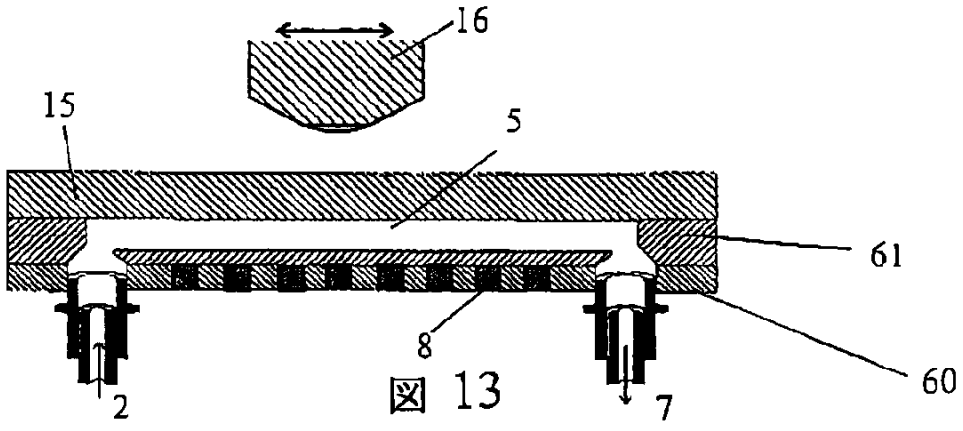

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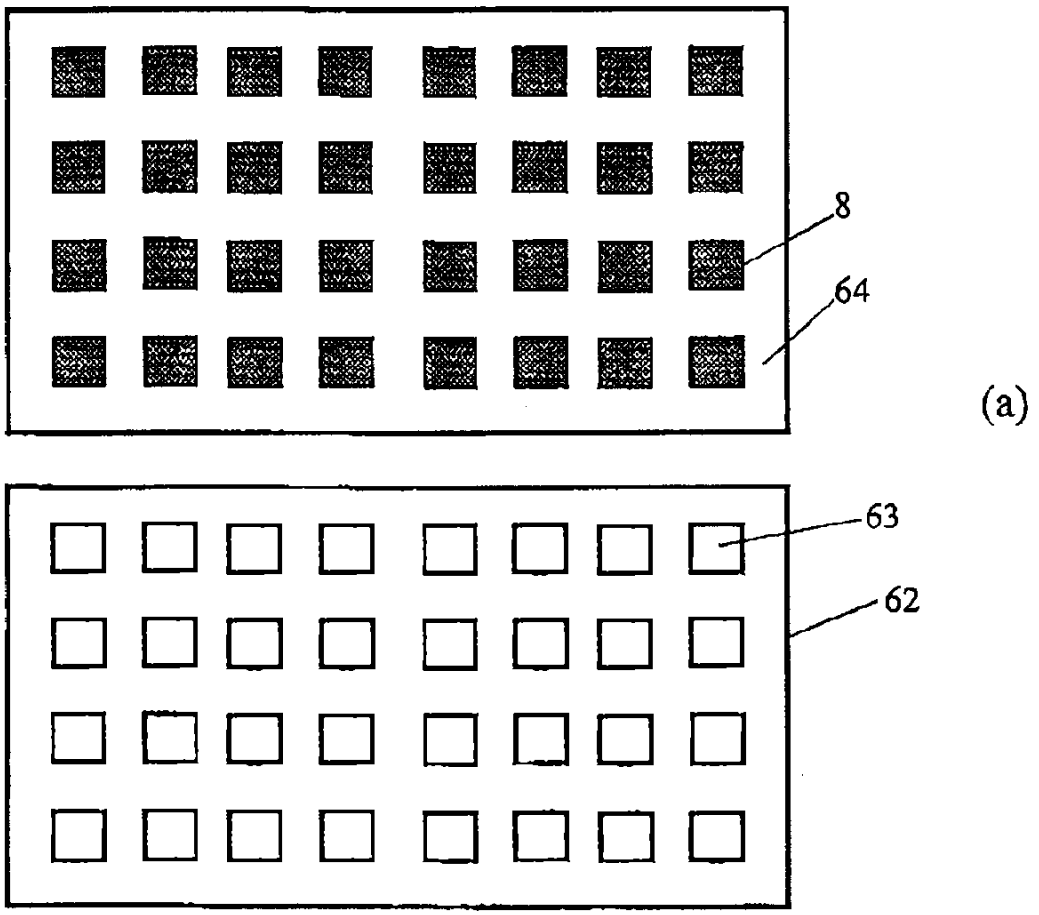

図 14

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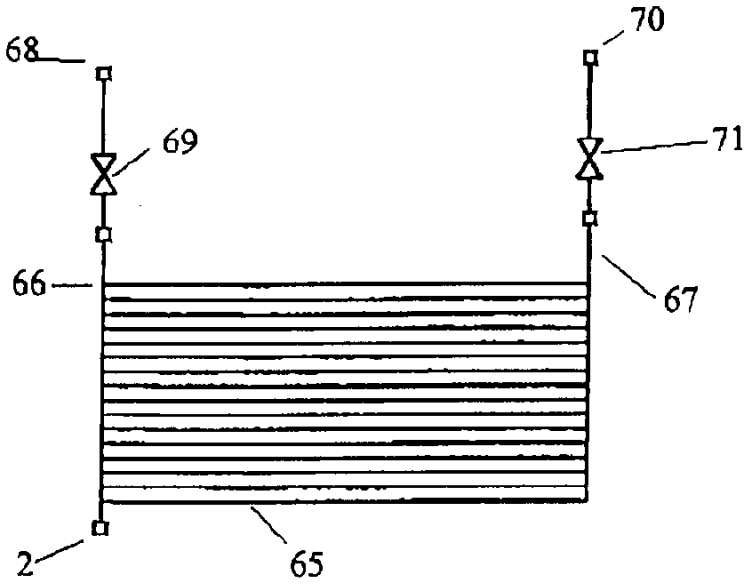

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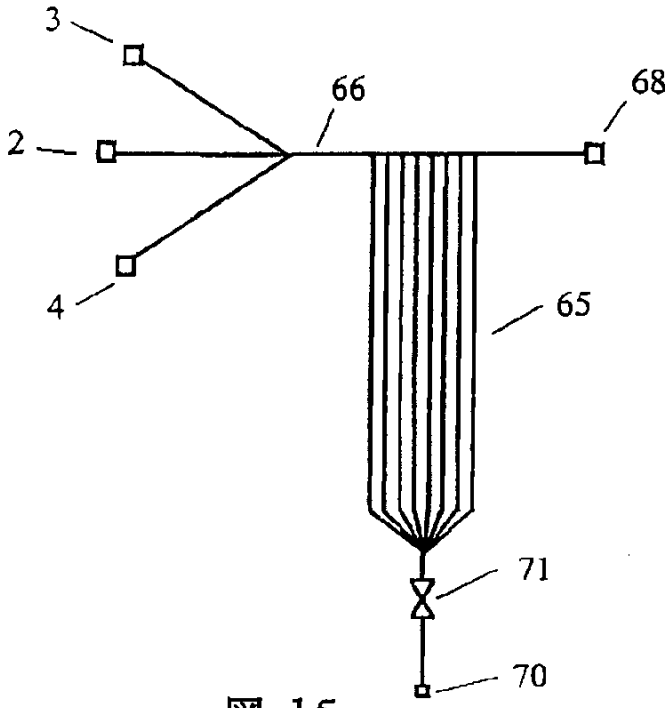

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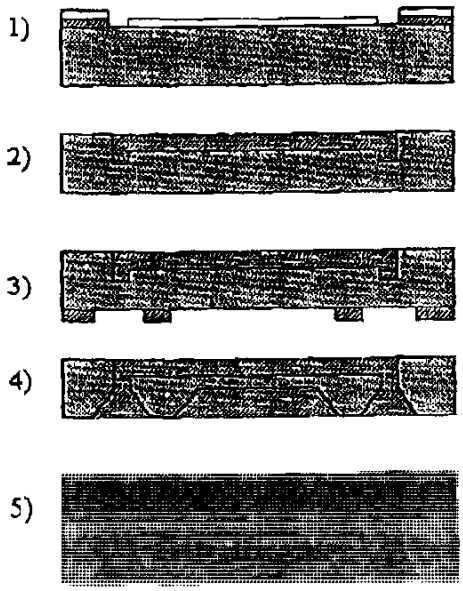

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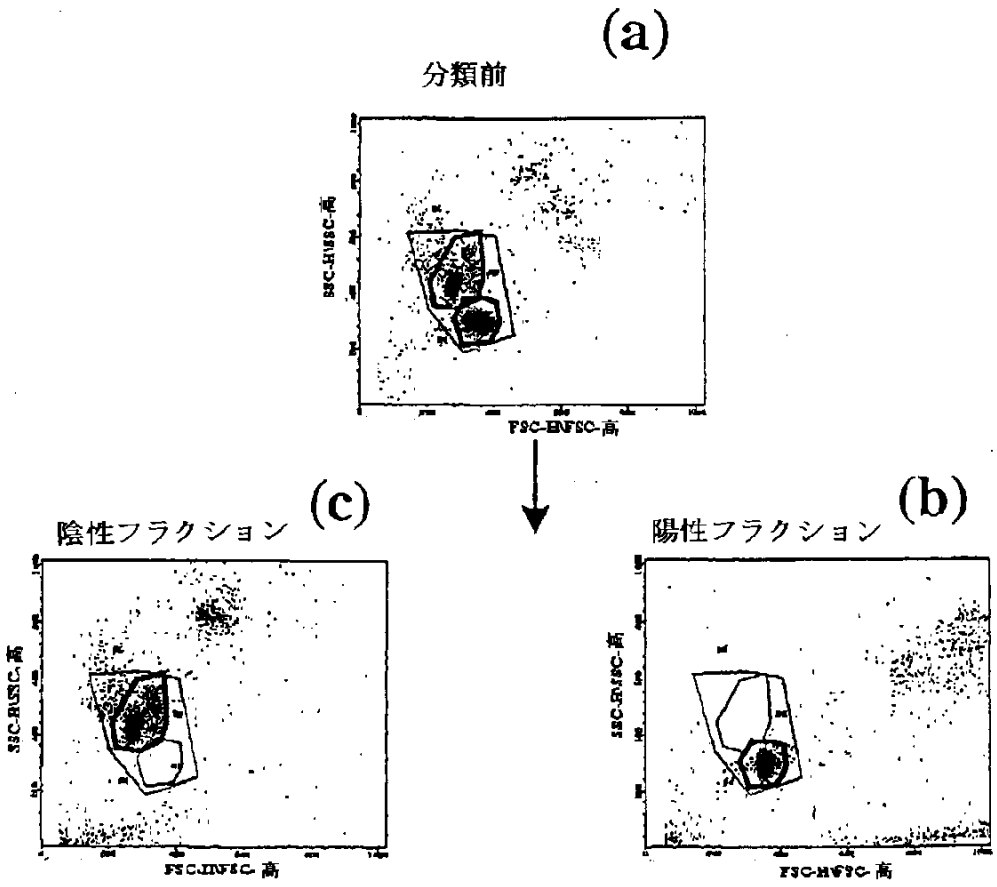

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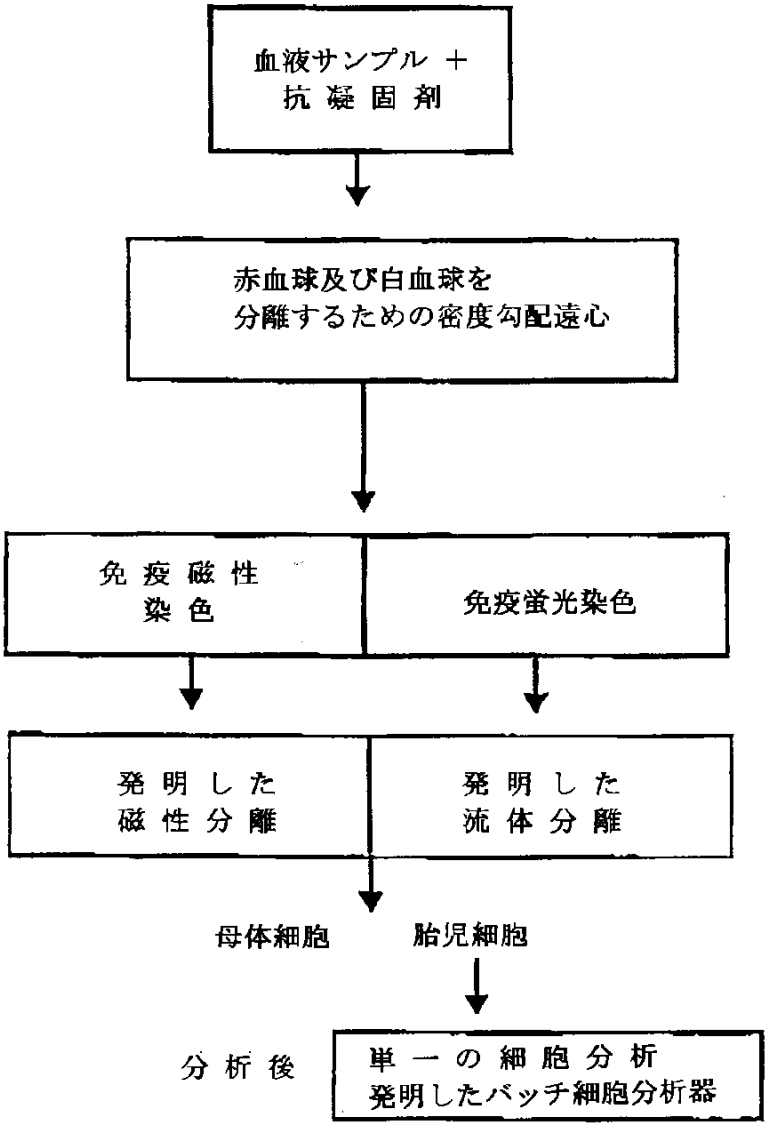
血液サンプル ＋
抗 凝 固 剤
赤血球及び白血球を
分離するための密度勾配遠心
免 疫 磁 性
染 色
免疫蛍光染色
発 明 し た
磁 性 分 離
発 明 し た
流 体 分 離
母体細胞
胎児細胞
分 析 後
単 一 の 細 胞 分 析
発明したバッチ細胞分析器

図 18

INTERNATIONAL SEARCH REPORT

Interm al Application No
PCT/DK 97/00368

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N15/14 G01N33/543 B03C1/035

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N B03C B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AHN C H ET AL: "A FULLY INTEGRATED MICROMACHINED MAGNETIC PARTICLE MANIPULATOR AND SEPARATOR" PROCEEDING OF THE WORKSHOP ON MICRO ELECTRO MECHANICAL SYSTEMS (MEM, OISO, JAN. 25 - 28, 1994, no. WORKSHOP 7, 25 January 1994, INSTITUTE OF ELECTRICAL AND ELECTRONICS ENGINEERS, pages 91-96, XP000528399 see the whole document --- -/--	1-4,9, 10,12, 17, 20-27, 29,30, 34,35

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 1997	-7. 01. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Brison, O

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/DK 97/00368

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3 827 555 A (KAMENTSKY L ET AL) 6 August 1974 see the whole document	1-4,9, 10,12, 17, 20-27, 29,30,34
X	---	37
Y	US 4 279 345 A (ALLRED JOHN C) 21 July 1981 see the whole document	12
Y	---	35
	WO 96 09489 A (MILTENYI BIOTECH INC) 28 March 1996 see page 5, line 10 - page 8, line 20	
Y	---	36
	US 4 910 148 A (SORENSEN OTTO ET AL) 20 March 1990 see the whole document	
Y	---	36
	US 3 560 754 A (KAMENTSKY LOUIS A) 2 February 1971 see the whole document	
X	---	38
	WO 94 15193 A (SIENNA BIOTECH INC) 7 July 1994 see abstract	
X	---	39
	WO 93 22058 A (UNIV PENNSYLVANIA) 11 November 1993 see page 11, line 1-14	
A	---	1
	US 5 053 344 A (ZBOROWSKI MACIEJ ET AL) 1 October 1991 see abstract	
A	---	1
	US 5 483 469 A (VAN DEN ENGH GER ET AL) 9 January 1996 see the whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna al Application No
PCT/DK 97/00368

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 3827555 A	06-08-74	US 3984307 A	05-10-76
US 4279345 A	21-07-81	NONE	
WO 9609409 A	28-03-96	AU 3593595 A	09-04-96
		CA 2200294 A	28-03-96
		EP 0778899 A	18-06-97
US 4910148 A	20-03-90	NONE	
US 3560754 A	02-02-71	BE 689813 A	02-05-67
		CH 446769 A	
		DE 1598634 A	15-04-71
		FR 1501326 A	29-01-68
		GB 1101063 A	
		NL 6615120 A	18-05-67
		SE 333264 B	08-03-71
WO 9415193 A	07-07-94	AU 670489 B	18-07-96
		AU 5873494 A	19-07-94
		CA 2151060 A	07-07-94
		CN 1092866 A	28-09-94
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		US 5589401 A	31-12-96
WO 9322058 A	11-11-93	US 5304487 A	19-04-94
		US 5296375 A	22-03-94
		AT 155711 T	15-08-97
		AT 140025 T	15-07-96
		AT 140880 T	15-08-96
		AU 677780 B	08-05-97
		AU 4222393 A	29-11-93
		AU 680195 B	24-07-97
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		AU 677781 B	08-05-97
		AU 4222693 A	29-11-93
		AU 4222793 A	29-11-93
		AU 677197 B	17-04-97
		AU 4223593 A	29-11-93

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna al Application No
PCT/DK 97/00368

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9322058 A		CA 2134474 A	11-11-93
		CA 2134475 A	11-11-93
		CA 2134476 A	11-11-93
		CA 2134478 A	11-11-93
		DE 69303483 D	08-08-96
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		DE 69303898 D	05-09-96
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		HK 16897 A	13-02-97
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		WO 9322054 A	11-11-93
		WO 9322421 A	11-11-93
		WO 9322055 A	11-11-93
		US 5635358 A	03-06-97
		US 5427946 A	27-06-95
		US 5498392 A	12-03-96
		US 5587128 A	24-12-96
		US 5486335 A	23-01-96
		US 5637469 A	10-06-97
US 5053344 A	01-10-91	NONE	
US 5483469 A	09-01-96	NONE	

Espacenet

Bibliographic data: JP2000512541 (A) — 2000-09-26

ABSORPTION-ENHANCED DIFFERENTIAL EXTRACTION DEVICE

Inventor(s):

Applicant(s):

Classification: **- international:** ***B01D57/00; B01J19/00; B03B5/00; C07K1/02; G01N1/02; G01N15/00; G01N30/00;*** *A61M1/36; G01N30/02; G01N30/58; G01N30/60;* (IPC1-7): B01D15/00; B01D57/00; C07K1/02; G01N1/02; G01N15/00

- cooperative: **B01J19/0093; B03B5/00; G01N30/0005;** A61M1/3679; B01J2219/00828; B01J2219/00952; B01J2219/00995; G01N2030/582; G01N30/02; G01N30/6095; Y10S366/01 more

Application number: JP19980501864 19970613

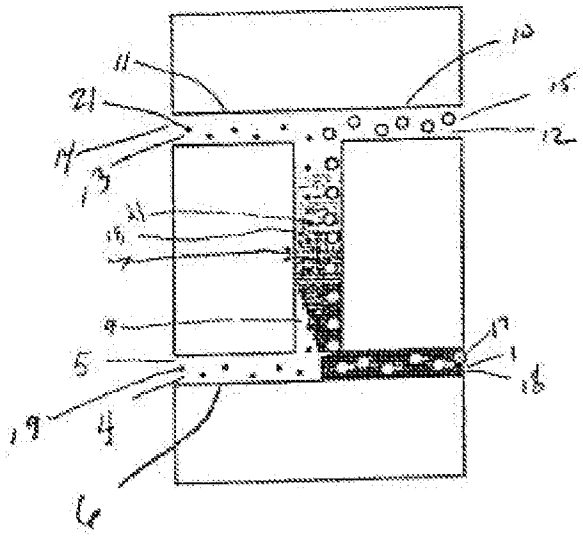
Priority number(s): WO1997US10307 19970613 ; US19960019904P 19960614

Also published as: WO9747390 (A1) US5971158 (A) US6695147 (B1) EP0910474 (A1) EP0910474 (A4) more

Abstract not available for JP2000512541 (A)
Abstract of corresponding document: WO9747390 (A1)

This invention provides an extraction device and method for extracting desired particles from a sample stream (2) containing the desired particles. The device comprises a sample stream inlet (1); an extraction stream inlet (5); an extraction channel (7) in fluid communication with the sample stream inlet (1) and the extraction stream inlet (5) for receiving a sample stream (2) from the sample stream inlet (1) in adjacent laminar flow with an extraction stream (4) from the extraction stream inlet (5); a sequestering material within the extraction channel (7) for capturing desired particles (18) in the extraction stream (9); a by-product stream outlet (15) in fluid communication with the extraction channel (7) for receiving a by-product stream (12) comprising at least a portion of the sample stream (2) from which desired particles (18) have been extracted; and a product outlet (14) in fluid communication with the extraction channel (7) for

receiving a product comprising the sequestering material and at least a portion of the desired particles (18).



* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
difference extracting apparatus whose absorptive power improved the [to which the present invention was given from the U.S. Army / U.S. Army research contract] -- it is made by the government largess based on DAMD17-94-J-No. 4460. The government has a fixed right in the present invention.
Cross-reference of related application An application concerned is patent application which claims the preference based on the provisional application 60th for which it applied on June 14, 1996 which uses the whole sentence for an application concerned as reference / No. 019 or 904.

Field of invention The present invention about the system and method of extraction of separating the analyte from the stream which contains other constituents in a broad sense by a difference transportation principle (differentialtransport principles) like diffusion and applying space, The method of having acted as Kougami which uses an absorbent or an absorbent into an extraction stream is provided. The equipment and the method of the present invention can be used for the purpose of diagnosis, and a therapy/treatment.
The background of invention Field-flow-fractionation equipment separates grain size using a single input stream. For example, Giddings, US,3,449,938,B "Method for Separating and Detecting Fluid Materials" as of June 17, 1969 of J.C., Giddings, US,4,147,621,B "Method and Apparatus for Field-Flow Fractionation" as of April 3, 1979 of J.C., Giddings, US,4,214,981,B "Steric Field-Flow Fractionation" as of July 29, 1980 of J.C., Giddings, US,4,250,026,B "Continuous Steric FFF Device for The Size Separation of Particles" as of February 10, 1981 of J.C. and others, Giddings, J.C. et al. (1983) "Outlet Stream Splitting for Sample Concentration inField-Flow Fractionation",Separation Science and Technology 18: 293-306, Giddings, J.C. "Optimized Field-Flow of (1985). Fractionation System. Based on Dual Stream. Splitters, "Anal.Chem.57:. 945-947, Giddings, J. United States patent 4th as of May 16, 1989 of C., 830, No. 756 "High Speed Separation of Ultra-High Molecular Weight Polymers by Hyperlayer Fie1d-Flow Fractionation", Giddings, J. United States patent 4th as of August 25, 1992 of C., 141, No. 651 "Pinched channel Inlet System for Reduced Relaxation Effects and Stopless Flow Injection in Field-Flow Fractionation", Giddings, J. United States patent 5th as of October 20, 1992 of C., 156, No. 039 "Procedure for Determining the Size and Size Distribution of Particles Using Sedimentation Field-Flow Fractionation", Giddings, J. United States patent 5th as of March 16, 1993 of C., 193, No. 688 "Method and Apparatus for Hydrodynamic Relaxation and Sample Concentration in Field-Flow Fraction Using Permeable Wall Elements", Caldwell, US,5,240,618,B as of August 31, 1993 of K D . and others, and "Electrical Field-Flow Fractionation Using Redox Couple Added. to Carrier Fluid", "Field-Flow Fractionation:Analysis of Macromole cular, Colloidal and Particulate Materials" Science of Giddings and J.C. (1993). 260:1456-1465, Wada, and November [1995 year] 14 attachment US,5,465,849,B"Column and Method for Separating Particles in Accordance of Y and others. with Their Magnetic Susceptibility", "Miniature Field-Flow Fractionation Systems for Analysis of Blood Cells" Clin.Chem.40:1810-1814, [of Yve, V. and others (1994)] Afromowitz and M.A.and. Samaras, J.E. (1989) "Pinch Field Flow Fractionation Using Flow Injection Techniques,"Separation Science andTechnology 24(5and6):325-339. Reference.

In the separation cell which has a thin channel, thin channel split flow fractionation (SPLITT) technology also provides particle separation. The power of a place is exerted in the direction vertical to the direction of a flow. Particles move to a non-particle stream through a transportation stream from a particle content stream. The equipment which performs this process is manufactured from two or more glass plates using the Teflon sheet generally used as a spacer for forming a channel. Therefore, the channel depth may be a size more than the spacer which is generally a thickness of about 100-120 micrometers. For example, Giddings, US,4,737,268,B as of April 12, 1988 of J.C., "Thin Channel Split Flow Continuous Equilibrium Process and Apparatus for Particle Fractionation", Giddings, US,4,894,146,B "Thin Channel Split Flow Process and Apparatus for Particle Fractionation" as of January 16, 1990 of J.C., Giddings, J. United States patent 5th as of August 13, 1991 of C., 093, No. 426 "Process for Continuous Particle and Polymer Separationin Split-Flow Thin Cells Using Flow-Dependent Lift Forces", "Continuous SPLITT Fractionation Based on a Diffusion mechanism" Ind.Eng.Chem.Res.31:2172-2181, [of Williams, P.S. and others (1992)] And Levin and S.and. Tawil, G. "Analytical SPLITT Fractionation in the Diffusion Mode Operating as a Dialysis-like System Devoid of Membrane. of (1993). Refer to Applicationto Drug-Carrying Liposomes and "Anal.Chem.65:2254-2261.

One of the the objects of this invention is providing the improved extraction system which can perform extraction of the analyte, detection, and quantification using a difference transportation principle. Another purpose of the present invention is to provide the extraction system by which it has been improved for refining of the fluid containing a body fluid like blood, and treatment.

All the publications mentioned in an application concerned, a patent, and patent application use the whole sentence as reference.

Summary of invention The above difference extracting apparatus enable the particles of a request into the extraction stream which flows as laminar flow parallel to an extraction stream from a sample stream to move. One of the simple embodiments of such a system uses the concentration gradient (gradient) over between two or more streams so that desired particles may diffuse into an extraction stream from a sample stream. It is also possible to use other inclination and power, for example, magnetism, electrical and electric equipment, gravity, dielectric, precipitate, shear, centrifugal forces, temperature, pressures, and cross flow inclination (cross-flow gradients).

One of the improvement points in the above-mentioned process provided in an application concerned is addition of blockade material (sequestering material) to an extraction stream. Provide the present invention and the extracting apparatus which extracts desired particles from the sample stream containing desired particles this equipment, a. Sample stream input and b. extraction stream input, c. Carry out fluid communicating to this sample stream input and this extraction stream input, The extraction channel for receiving the sample stream from this sample stream input of the laminar flow which adjoins the extraction stream from this extraction stream input, d. The blockade material for being in this extraction channel and catching desired particles in this extraction stream, e. The by-product stream tap hole for receiving the by-product stream containing at least 1 portion of this sample stream from which fluid communicating was carried out to this extraction channel, and desired particles were extracted, f. It is an extracting apparatus which carries out fluid communicating to this extraction channel, and includes the product stream exit for receiving the output containing this blockade material and at least 1 portion of the particles of this request.

Blockade material is a material to catch the desired absorption, combination or adhesion (sticking) to particles, or by absorbing them. Desired enzyme, antibody, antigen, and other ligands to particles are publicly known in the field concerned, and are a useful blockade material in the present invention. As a blockade material, all publicly known ligands may be used in the field concerned. Such ligand may be added to an extraction stream "as it is", or may be fixed on a substrate like other publicly known materials in a polymeric bead, the amount polymer of polymers, or the field concerned. "The amount polymer of polymers" refers to polymer of sufficient molecular weight for the degree which does not diffuse in a sample stream substantially, while moving in the inside of equipment. Although the amount dextran of polymers, the amount polypeptide of polymers, and the amount nucleic acid of polymers are contained in

the example of the amount polymer of polymers, limitation is not carried out to this. Blockade material may be activated carbon or an absorbent material like porous polymer. An absorbent or an absorbent may be specific in a specific particle type like an antibody, or may be nonspecific like activated carbon.
So that it may not become detectable in a by-product stream preferably. Or the blockade material should diffuse sufficient slowly so that an extraction stream may not be substantially exceeded into a sample stream to non-diffusibility, i.e., a significant degree, so that it may not interfere in analysis of the analyte in a by-product stream.
Blockade material catches desired particles by preventing desired particles' flowing out (exiting) and flowing out of equipment with a by-product stream. Blockade material may ****** desired particles into blockade material, as long as sufficient long time particles are held, although it prevents the particle flowing out with a by-product stream. Desired particles may be reversibly caught by combination or blockade material into blockade material, in order to make the reuse of blockade material possible so that it can remove for the further analysis or.
The difference extracting apparatus manufactured by microscale provides the many advantage which is not in above large-sized equipment. Such equipment by which micro manufacture was carried out is described in the application 08th for which it applied on June 14, 1996 / No. 663,916. This application is used specifically [that whole sentence] as reference to an application concerned with all the Reference documents applied there as reference. The definition of term currently used in the above-mentioned application applied to the microscale structure currently disclosed in the above-mentioned application is applicable not only to microscale structure but macroscale structure in an application concerned. In an application concerned, "macroscale structure" is a bigger structure than microscale structure, and is specified as a sufficient small structure to make laminar flow possible.
The particles of the request in the present invention are good in analyte ****, and may also be the analyte and a substance in which it interferes. The above-mentioned particles may also be collecting and using it for another purpose, or particles which are desired, or may also be the poison (poisons) in a patient's blood, or poison (toxins) like metabolite. For example, the poison is counteracted for blood using the present invention, for example, it is possible to remove a toxic metal from blood or to counteract the poison for other body fluids, for example, to perform hemodialysis. It is possible to use the present invention for sewage disposal, for example, removal of the impurity from water. Or it is also possible to use the present invention, and to remove other output generated by a drug or the microorganism in a fermentation reactor (fermentation reactor), for example, a bacterial cell, without doing injury to a microorganism. It is possible to perform such treatment continuously.
Provide the present invention and the method of extracting at least one copy of desired particles from the sample stream containing desired particles this method, a. The process of introducing this sample stream into the sample stream input of the above-mentioned extracting apparatus, b. The process of introducing an extraction stream into the extraction channel of this extracting apparatus, With this blockade material, introduce the blockade material for catching the particles of this request into this extraction channel, they are caught by the particles of this request, and c. This blockade material, It is the way this extraction stream containing at least 1 portion of the particles of this request leaves this equipment as an output stream, and this sample stream from which desired particles were extracted leaves this equipment as a by-product stream.
The equipment and the method of the present invention provide a means to perform affinity chromatography. Affinity chromatography refers to the method of refining or isolating a desired substance, and it includes carrying out the covalent bond of the specific ligand to an insoluble inactive base material in a broad sense so that I may be understood by the person skilled in the art. In the case of affinity chromatography, the ligand must have high compatibility to the desired substance so that a desired substance may be preferentially maintained by ligand, when passing through the inside of a solution through a column.
Although the present invention provides the equipment and the method of performing affinity chromatography, it has at least one advantage. Extraction of a desired substance (particles) can

be performed continuously. It can be continuously flowed through the sample stream and extraction stream of the present invention through equipment. In the field concerned, publicly known affinity chromatography, Two or more distance, the distance which loads with ligand on an inactive material, for example, and the distance which carries out flashing (flushing) of the column, In each distance, the loss of output usually arises including the distance which loads with a sample, the distance to rinse, and the distance which rinses again and releases a desired substance after that. the virus fixed on the bead in the equipment of the present invention in the extraction stream containing one antibody -- and, After it introduces the sample stream of whole blood in equipment and 2 virus particles move to an extraction stream, a virus can be extracted from whole blood by releasing these by changing the pH of a solution. A bead may be a magnetic body so that it may also be possible to choose for example, so that it may fall to the bottom of a channel or it can draw near to one side of a channel with a magnet.
Blockade material may exist in an extraction stream, before an extraction stream is introduced into an extraction channel. Or blockade material may be added to an extraction stream by suspending or disassembling the blockade material in the liquid introduced into the extraction stream which already exists in an extraction channel via extraction stream input.
The extraction system of the present invention in the easiest concept is a diffusion extraction device containing the micro channel of the "H" form. The mixture of the particles suspended in the sample stream goes into an extraction channel (crossbar of "H") from one of the arms (for example, left-hand side upper part), and an extraction stream (dilution stream) enters from the left back lower part. Although both two streams flow in an extraction channel, since the size of a channel is small, the flow is stratified and a stream is not mixed. A sample stream comes out from the right-hand side upper part as a by-product stream, and an extraction stream comes out from the right-hand side lower part as an output stream. While a stream is the laminar flow which adjoined within the extraction channel, the particles (particles smaller than those, such as albumin, sugar, and a small ion) which have a bigger diffusion coefficient have time to diffuse to an extraction stream, but particles (for example, blood cell) big on the other hand remain in a sample stream. The particles in the extraction stream (at this time, called an output stream) flowing out may be analyzed without receiving the interference from bigger particles.
Let the flow direction of a channel be the length (L) in this patent application. About the dimension of the channel in the transportation direction of right-angled particles, it is the depth (d) to length (L).
It carries out. Let the 3rd right-angled channel dimension be the width (w) to both length and the depth. Therefore, the surface of the interface of a sample stream and an extraction stream and the depth (d) cross at right angles. Table 1 shows the other abbreviations used on these Descriptions.

表1

V	容量
V_{ss}	サンプルストリーム流量（m^3／s）
V_{es}	抽出ストリーム流量（m^3／s）

V_{ps}	生成物ストリーム流量（m^3／ｓ）
V_{bps}	副生成物ストリーム流量（m^3／ｓ）
V_{ind}	インジケータ色素ストリーム流量（m^3／ｓ）
V_{ds}	検出ストリーム流量（m^3／ｓ）
$C_{i,ss}$	サンプルストリーム構成要素ｉ濃度（ｋｇ／ｋｇ）
$C_{i,es}$	抽出ストリーム構成要素ｉ濃度（ｋｇ／ｋｇ）
$C_{i,bps}$	副生成物ストリーム構成要素ｉ濃度（ｋｇ／ｋｇ）
$C_{i,ps}$	生成物ストリーム構成要素ｉ濃度（ｋｇ／ｋｇ）
$C_{dye,ind}$	インジケータストリーム色素濃度（ｋｇ／ｋｇ）
$C_{i,ds}$	検出ストリーム構成要素ｉ濃度（ｋｇ／ｋｇ）
d	拡散方向抽出チャネル深さ（ｍ）
w	抽出チャネル幅（ｍ）
L	抽出チャネル長さ（ｍ）
$a_{\%}$	平衡濃度からのパーセント偏差値
$L_{a\%}$	$a_{\%}$を成し遂げるのに必要な装置長さ（ｍ）
z_s	抽出チャネル流入口におけるサンプルストリームと抽出ストリームとの間のインターフェース流線位置（ｍ）
z_p	副生成物ストリームと生成物ストリームとの間のインターフェース流線位置（ｍ）
P	流体ストリーム内の絶対圧力（Ｐａ）
ΔP	抽出チャネルの流入口と流出口との間の圧力差（Ｐａ）
D_i	構成要素ｉの二成分拡散係数（m^2／ｓ）
μ	流体粘度（Ｐａ・ｓ）
ρ	流体密度（Ｋｇ／m^3）
ξ	無限長抽出チャネルの平衡正規化構成要素濃度（無次元）
$\hat{c}$	正規化構成要素濃度（無次元）
x	チャネル長方向座標（流れ方向）
y	チャネル幅方向座標
Z	拡散方向座標

$\tilde{x}, \tilde{z}$	非次元正規化変数（無次元）
w／d	アスペクト比
D	拡散係数
Re	レイノルズ数
T	温度
u	軸速度
$\overline{V}$	平均速度

The length and the extraction channel flow rates of an extraction channel are main parameters which determine the time when particles must diffuse to an extraction stream. Blockade material makes diffusion of desired particles increase by decreasing the effective concentration of the particles of the request in an extraction stream. That is, blockade material shifts the balance of diffusion of the particles of the request to an extraction stream (to positive direction).
From a sample stream, using diffusion as a transport mechanism, the particles in above-mentioned differ to an extraction stream, and are transported to it. Other means to differ and to transport desired particles may be used. The term "difference transportation" means that a part of desired particles are transported to an extraction stream from a sample stream, and it eliminates substantially the particles which are not desired. For example, magnetism, the electrical and electric equipment, or other power may be applied to an extraction stream, or a temperature gradient may be used, or also in absorbents, such as an antibody, an absorbent material is added to an extraction stream and the comb can catch desired particles.
A sample stream, extraction stream input, a by-product stream, and an output stream tap hole may contain a channel, a stores dept., a port, or other containers. Sample stream input is designed so that extracting in order to detect "a desired particle, for example, the existence," may accommodate the sample stream containing the particles for which it asks. A sample stream contains further other particles (in this Description, called "the particles which are not desired") which are not extracted. These particles that are not desired contain the particles which may interfere in detection of desired particles. In a preferable embodiment, a sample stream contains whole blood. Desired particles are albumin or other plasma constituents, and are obtained, and the particles which are not desired may be blood cells. Especially equipment is useful although the plasma constituent which does not contain the cell from whole blood is obtained. The solution or suspended solid of the DNA fragment of different length, protein of various sizes, or a foreign chemical reaction mixture is contained in other fluids with the useful present invention. In enforcement of the present invention, biological fluid, such as a fermentation broth, unsettled sewage, a liquefaction food sample, a soil sample and saliva, urine, and cerebrospinal fluid, is contained in a useful sample stream.
The term "particles" points out giant molecules, such as a molecule, a cell, protein, nucleic acid, and compound carbohydrate, the small molecule which contains some atoms from 1, and ion. Particles may suspend or dissolve into a stream. The term "stream" points out carrier fluid, such as water or other liquids containing desired particles and/or particles which are not desired, air, or other gas. The term "particles" used on these Descriptions does not contain the molecule of a carrier stream.
The term "extraction" points out transporting the portion in which it is possible, at least one copy, i.e., detection, of desired particles, to an extraction stream from a sample stream, and eliminating substantially the particles which are not desired. Although the particles which are not desired are particles which may be transported to an extraction stream and which can diffuse especially more quickly than desired particles, it is recognized as such existence of the particles which are not desired being recognized the minimum so that the particles which are not desired may not interfere in detection of the stream containing desired particles, or next processing.
Transmission from the sample stream of the particles which are not desired to an extraction

stream may be made into the minimum by filling up an extraction stream with such particles that are not desired previously. the operative condition whose by-product stream is the purpose being previously filled up with the particles which an extraction stream is not expected (for example, used or analyzed further) -- it sets like, and it is preferable and obtains. For example, when blood is returned to a patient's inside of the body, an extraction stream contains the electrolysis solution of suitable concentration in a person skilled in the art unsurprisingly preferably. Blockade material raises the efficiency which separates desired particles from a sample by decreasing the effective concentration of the particles of the request in an extraction stream.
The term "extraction efficiency" is transmitted to an extraction stream, and refers to the percent of the particles of the request in the sample discharged by the output stream. Extraction efficiency may increase by using blockade material.
The term "laminar flow" refers to flowing without recycling lining up side-by-side stably without mixing two streams. There is no zone of the recirculation and a turbulent flow is a degree which can be disregarded. Practice [as opposed to / so that it may be publicly known at the technical field concerned / ** and stickiness in the Reynolds number of a flow]
性力の比である。導管を流れるためには、レイノルズ数は、Ｒｅ＝ρｄ（$\bar{V}$／

It is calculated using mu. here -- Re -- Reynolds number and rho -- the volume of a fluid -- dense 度、ｄは、導管の形状による導管の典型的な断面寸法、$\bar{V}$は、導管断面を通る平

****** and mu are viscosity.
It comes to be dependent on a viscous effect, and a flow pattern stops being dependent on an inertia effect as Reynolds number decreases. The inertia effect of less than specific Reynolds number (it has a bend and based on the lumen size to the system of a channel from which lumen size changes) is insufficient for causing the phenomenon which shows significant existences, such as a stratified recycle zone and a turbulent flow. Therefore, the ordered stratified flow which is not recycled is generated in the extracting apparatus described in this Description. In such equipment, the minimum dispersion mixing is generated as a result of the viscous flow speed profile who exists in any stratified viscosity flow. This enables two stratified fluid streams which are not recycled to flow through an extraction channel in order to extract desired particles from one stream to other streams.
A stream may be separated by the end of a lead pipe in any position by adjusting the tap hole flow rate of a tap hole correctly. This is not more possible in high Reynolds number than not meeting the standard of un-recycling and nonturbulent flow.
Extraction stream input is designed to accommodate the extraction stream which can accommodate desired particles when contacting a sample stream by laminar flow. An extraction stream may be any fluid which can accept the particles transported from a sample stream. An extraction stream includes the blockade material which combines the particles of the request transported to the extraction stream from the sample stream. Preferable extraction streams are isosmotic solutions, such as water and a physiological saline. Other useful extraction streams contain organic solvents, such as acetone, isopropyl alcohol, carbon dioxide of a supercritical state (supercritical), or ethanol. Air and other gas may be used as a sample and an extraction stream carrier.
Including at least one copy of the sample stream from which desired particles were extracted, desired particles may contain a part of extraction stream carried from the sample stream, or there may be no by-product stream profitably [hidden] so that it may describe below. Blockade material makes desired particles extract from a sample more effectively, and a purer by-product stream is formed of it. When an excessive blockade material is used and it has a high association constant to desired particles, the particles of all the requests may be extracted from a sample stream only by processing a sample only once according to flow rate and extraction channel length substantially in a sample stream. When it assumes that there is no blockade material and the flow rate of a sample and an extraction fluid is equal, the equilibrium concentration of desired

particles is 50% in an extraction stream. That is, at most only 50% of desired particles diffuse to an extraction stream. Therefore, when there is no blockade material, in order to remove 97% of desired particles from a sample, a sample must be processed at least 5 times.
a by-product stream tap hole draws the by-product stream (a sample stream -- and probably it comprises a part of ****** stream) which is removed and discarded from an extraction channel and which is reused or is moved to other system components, and is designed to process further.
An output stream includes at least one copy and blockade material of desired particles. In the output stream which an output stream tap hole may contain an output stream channel as mentioned above, and contains the particles of a request of a detectable quantity, detection is also designed so that a comb may lead to the further treatment area or system component. Sufficient extraction stream of quantity must exist in an output stream including the particles of a request of sufficient quantity so that desired particles may become detectable within an output stream by a publicly known means by the technical field concerned.
An output stream may be led to a storage chamber or other equipment. Then, US,5,304,487,B of Wilding by which the output stream was published as of April 19, 1994, for example, P. and others (in an application concerned) Blockade material is separated from desired particles and it may be further processed by mixing, separation, analysis, heating, or another processing as it discloses for using for reference of this. A by-product stream may be processed by being carried out by being led to a storage chamber, other containers, or equipment again.
"Micro manufacture" of the equipment of the present invention may be carried out. This can be easily manufactured on an available silicon wafer to the person skilled in the art of silicon micro manufacture, LIGA, thermoplastic micro pattern transmission, micro casting that used resin as the base, The micro molding (MIMIC) in a capillary tube, wet isotropy, and anisotropic etching, the equipment which has the peculiar size and geometrical structures which can be manufactured by a method, such as other publicly known technology, in the field of laser assistance chemical etching (LACE), reactive ion etching (RIE), or micro manufacture is pointed out. Two or more equipment of the present invention is accommodated with two or more forms, so that a wafer becomes largely in silicon micro manufacture. Although it is a small number, standard wafer sizes are 3", 4", 6", and 8." Application of the principle shown to this Description using a new micro manufacturing method is in the range of a claim, and the meaning.
In a preferable embodiment called "H filter device" in this Description, width of an inflow channel and an outflow channel is for about 100 microns from about two to 3 times of a stream particle diameter at the time of the maximum size.
From about two to 3 times of the particle diameter at the time of the maximum size, the depth is in between below about 100 microns, and an extraction channel, Width is for [of about two to 3 times to wafer thickness of a particle diameter at the time of the maximum size / three] about 2/, the depth is in between below about 100 microns from about two to 3 times of a particle diameter at the time of the maximum size, and length is for 5 mm or less from about 4 times - about 10 times of a particle diameter at the time of the maximum size.

In the second embodiment which calls a particle transportation direction a "flat extracting apparatus" or a "flat filter device" in this Description rotated 90 degrees from "H filter device" design, setting an inflow channel at an entrance to an extraction channel -- width equal to extraction channel width -- having the width between about 500 microns from two to 3 particle diameter preferably, width is preferable and an extraction channel is for 5 mm or less from about two to 3 times of a diameter of particles at the time of the maximum size.
The depth is in between below about 100 microns from about two to 3 times of a diameter of particles at the time of the maximum size, and length is at least about 4 times the diameter of particles at the time of the maximum size.

An extraction channel receives the inflow (inflow) of a sample stream and an extraction stream from sample stream input and extraction stream input, and these streams as laminar flow which adjoined, Extraction into the extraction stream of desired particles is drawn covering sufficient

distance to make it possible. Like the flow channel disclosed in U.S. patent application 08th of Weigl and others of the application as of March 31, 1997 used in an application concerned for reference / No. 829,679, and PCT application the PCT/US97 of the application as of March 31, 1997 / No. 05245, The length of an extraction channel can be increased by forming in folding structures, such as the shape of a snake (set of a "hairpin" chip box), or a coil, for example. The width and the depth of an extraction stream channel and a product tap hole channel are sufficiently largely indispensable, in order to enable passage of any composites of desired particles, blockade material, and desired particles and blockade material.

In [when a width dimension is the direction of wafer thickness as in the embodiment of H filter device] the silicon micro manufacture embodiment of the microscale extracting apparatus of the present invention, The width of a sample channel, an extraction channel, a product channel, a by-product channel, input, and a tap hole is silicon wafer less than thickness, i.e., about 300 microns. Or a "flat extracting apparatus" when equipment is formed from other materials and a preferable material (moldable) of a plastic etc. which can be molded

In the case of an embodiment, there is no maximum community theoretical to width. 0.5 meter, 1 meter, and bigger width can be considered. If it assumes that the flow rate of each fluid is controllable covering the width of a channel, for example if it assumes that delivery to the equipment of a fluid (a sample stream and extraction stream) is controllable, there will be no theoretical maximum community in width.

The dimension of an extraction channel is chosen so that laminar flow and uniform flow rate may be maintained, for example, so that there may be no turbulent flow and there may be no deposition of particles on a channel-wall part again.

In the embodiment by which silicon micro manufacture of the microscale extracting apparatus of the present invention was carried out when a depth dimension was the direction of wafer thickness as in a "flat filter" embodiment, The depth of a sample channel, an extraction channel, a product channel, a by-product channel, input, and an exit is silicon wafer less than thickness, i.e., about 300 microns. In the equipment by which micro manufacture was carried out, the depth (especially extraction channel) is less than about 200 microns, and is less than about 100 microns more preferably.

Some of publicly known places in the field concerned which may be used for difference transportation of the particles in the equipment of the present invention. It is generated by the following. : - sedimentation (sedimentation), electric potential , and temperature gradient - cross-flow , dielectric inclination , shearing force , magnetism , and concentration gradient A means to generate the place of these is publicly known in the field concerned.

Since the size (depth) of the diffusion direction of the channel described in this Description is small, if diffusion or difference transportation of the particles of the request by other means is less than about 300 seconds and a request, for example, it will be performed in less than about 1 second very quickly. When blockade material exists in an extraction stream, the effective concentration of the particles of the request in an extraction stream is made to be deteriorated, transportation of desired particles increases, and the effective density difference between a sample stream and an extraction stream is maximized. Pure movement which is along the depth (diffusion dimension) of an extraction channel is maximized by this, and the rapid separation from the sample of desired particles is attained.

A sample stream and an extraction stream may have the characteristic that viscosity, density, surface energy, and homogeneity differ from chemical composition etc., for example, and this can affect difference transporting speed. In order to take these different characteristics into consideration, it can give that a system parameter can be adjusted and optimized without an excessive experiment clearly to a person skilled in the art.

A sample stream and an extraction stream are maintained by the contact state into sufficient time to make it possible the quantity of desired particles which can be analyzed at least, and to transport most (major portion) into an extraction stream preferably, and an extraction channel. In the equipment (for example, about 50 micrometers is exceeded) which has big width, the flow rate of the product stream from equipment is for not less than about 10ml/second from about 0.001 pico liter/second. For example, the optimal flow rate of a product stream may be about

200 nano a liter/second. In the field concerned, a spectroscopic analysis and other means can also detect the analyte of a very small quantity which exists in such few product streams publicly known.
平均流速$\overline{V}$は、以下の関係式を満たすように選択される：

$$\overline{V} < f\frac{DL}{d^2}$$

f is a time factor (proportionality constant) about between the length of which two streams must touch that it is mutual, in order for the particles of a request of a certain percentage to move to an extraction stream from a sample stream.
therefore, unit width -- (-- the capacity flow rate (Q) of per w) is limited to less than f(DL) / d される。ここで、$Q = \overline{V} w d$、$Q/w = \overline{V} d$と表されて、

$$\frac{Q}{w} < f\frac{DL}{d}$$

f= 1 is chosen, and it is convenient to calculate to calculate the maximum stream flow per unit width based on this, and it obtains. For example, in biotin (in a channel (length (L) =1cm and depth (diffusion dimension) (d) =10micrometer), they are diffusion coefficient [of D= 500 micrometers] ^{2} / second), the maximum stream flow per unit width is attached to per micrometer in width, and is about 500 pico liters/second.
The following expressions of relations are drawn from the above. : $d^2/D=2t$

This is 2t of average numbers of hours, and means that a molecule diffuses covering the distance d (depth of a channel).
Desired "most particles" is tops [particles / that exist in a sample stream / 50% of / these].
Blockade material raises the efficiency of an extraction process and enables extraction exceeding 50% (using the sample and extraction stream of equal volume and other blockade material and differences transport capacity (for example, a magnetic field and an electric field) business the greatest extraction obtained by the chair). Preferably, blockade material enables extraction exceeding about 50 to about 80% of desired particles. Blockade material enables about 75 to about 95% of extraction of desired particles more preferably. Blockade material enables about 85 to about 100% of extraction of desired particles most preferably.
In order to make successful the operation of invention described in this Description, in three of four channels (namely, a sample stream, an extraction stream, a product stream, and a by-product stream) of equipment, it is required to control a capacity flow rate correctly. It is not necessary to restrict the 4th channel and, and it should not be restricted.
It is because equipment is enabled to correspond to change [**********] of the volume of a sample for deltaV of mixing of a sample stream and an extraction stream by leaving this channel restricting [no]. The means for attaining the flow rate restricted correctly is publicly known in the field concerned.
Control of the size of the particles transported to the product stream in the spread type extraction system of the present invention is helped, and in order to decrease the appearance of big particles rather than being able to set in a product stream, a fluid barrier may be created to an extraction channel. That such a fluid barrier exists is a case where only sufficient volume for a part of extraction stream to make it flowing through a by-product exit with the by-product stream into which an extraction stream flows exists, as shown in Fig.3. Unless it goes over the width of this fluid barrier, small particles cannot flow out with a product stream, rather than having diffused in the extraction stream. Williams P.S. et al. (1992) which such a fluid barrier formed on a large scale uses in this Description for reference, "Continuous SPLITT Fractionation Based on a Diffusion Mechanism" and Ind.Eng.Chem.Res.2172-2181 describe.

By controlling the flow rate of a sample stream and an extraction stream, the ratio of the volume from each included in an extraction channel is controllable. The volume ratio of a sample stream and an extraction stream may be set up also by the structure of an outflow channel and an inflow channel again about the predetermined delivery pressure (delivery pressure) concerning a sample stream and an extraction stream. The capacity flow rate of a product and a by-product stream may be controlled again also operating product stream pressure or by-product stream pressure or by changing flow resistance of an inflow using any port (inflow) pressure. An inflow channel and the outflow channel must fulfill the conditions of the minimum channel dimension based on the size of the particles which are described in this Description and which should be processed regardless of control mode. A fluid barrier will be formed, if the volume of the extraction stream included in an extraction channel is larger than the volume of a sample stream and two outflow streams are the same. A fluid barrier is formed even if the capacity flow rate of a product stream is so small that it cannot respond to the full capacity flow of an extraction stream.

The extracting apparatus of the present invention may have a means for controlling the volume of the extraction stream in an extraction channel to the volume of a sample stream. Such a means has a product stream tap hole smaller than a size required to make it possible to make all the extraction streams flow out in cooperation with such a sufficient large by-product stream that an excessive extraction stream coped with. The extracting apparatus of the present invention may enable it to collect the product streams which contain the particles of a request of a different type by having two or more product stream tap holes.

The equipment of the present invention can be used as a sample pre processing system of an analyzing system including the sensing device for detecting the particles of the request in a product stream. Such a means contains the means for mixing with the indicator stream which enables an interaction with desired particles to detect a product stream by a publicly known sensing device in particles or the field concerned. In the field concerned, a publicly known sensing device in optical means rows, such as optical spectroscopic-analysis equipment, The chemical indicator to which the characteristic of a color or others is changed when it exposes to the particles of a request of other means, for example, spectrophotometric analysis equipment, or a fluorescence detection means, and the analyte, Electric means, such as an electrode inserted in an immunological means and equipment, an electrochemical means, In the field concerned to include, a radioactive means or magnetic resonance equipment Almost all publicly known micro analytical skills, Or the means for detecting existence of analyte particles, such as publicly known ion and molecule, polymer, a virus, a DNA sequence, an antigen, a microorganism, and other factors, in the other fields concerned is included. An antibody, a DNA sequence, etc. are made to adhere to a fluorescent marker preferably using an optical means or a fluorescence means. The indicator, the mixing means and detection by which micro manufacture was carried out, and a sensing device are described in U.S. application serial No.08/625,808 used in this Description for reference.

The present invention sets like 1 operative condition, and an above-mentioned difference extracting apparatus, The means for processing a product and/or a by-product stream further, For example, spread type mixing equipment for mixing a product stream with an indicator substance (as [describe / in U.S. application serial No.08/625,808 used in this Description for reference / for example,]), And it is internalized in the analyzing system which has a detection chamber for detecting existence of desired analyte particles. These additional processing means are preferably provided all over "the chip top laboratory (lab-on-a-chip)" created on the standard silicon wafer with the inside of a difference extracting apparatus. This system may have a fixed-quantity means for determination of the concentration of the analyte particles (a request or the particles which are not desired) in a product stream and/or a by-product stream, and/or determination of the concentration of the analyte particles in a sample stream. Such a means includes analysis spectrum equipment, a voltmeter, an ammeter, and dielectric relaxation equipment (dielectric relaxation equipment). The means **** calculation or the proofreading disclosed in the publicly known means and this Description in the field concerned can perform concentration determination.

When using for refining of a fluid of waste water treatment, hemodialysis, blood detoxication, etc. in another embodiment of the present invention, a lot of [, such as about 10ml/second,] sample streams can be processed. In this embodiment, preferably, although the width of an extraction channel can make it about 1 meter largely, the theoretical maximum of fixing of channel width does not exist as mentioned above. In processing of a lot of fluid capacitance, the device group of the present invention containing the equipment by which micro manufacture was carried out may be connected in parallel, and it may connect in series selectively.
In order to process equipment previously, namely, to improve performance so that it may describe in the following working examples, it is preferable to coat the wall of equipment previously and it obtains. In order that equipment may perform a wall separation of desired particles, before being used, it may be coated with the blockade material which should be used. Without desiring to be bound to any specific theory, it is considered to prevent blockade material from adhering to a wall to coat a wall with blockade material previously, when a sample and blockade material are behind introduced into equipment.
Or in order to perform the surface protection by the charge of a hydrophilic coating material, the wall of equipment may be coated previously. the charge of a hydrophilic coating material is marketed -- albumin (for example, bovine serum albumin, lactalbumin, and a human serum albumin) and a silanizing reagent publicly known in the field concerned -- although a polyethylene-glycol silane is included preferably, it is not limited to these.
Many substitution does to the component and process which are disclosed on these Descriptions, and the present invention is not limited to the specific embodiment described on these Descriptions so that I may be understood by the person skilled in the art.
The easy description Fig.1 of Drawings shows the micro channel structure which shows the laminar flow of two input streams which have low Reynolds number.
Fig.2 shows the micro channel structure from a sample stream to an extraction stream which shows diffusion of small particles relatively.
Fig.3 shows the micro channel structure which shows formation of the fluid barrier between a sample stream and an extraction stream.
Fig.4 is a figure showing the flow rate of a sample, extraction, output, and a by-product stream showing the input within an extraction channel, and a tap hole interface stream line.
Fig.5 shows the extracting apparatus which does not include blockade material.
Fig.6 shows the embodiment (embodiment of H filter) of the extracting apparatus of the present invention containing blockade material.
Fig.7 shows the temporal diffusion in the extracting apparatus of Fig.5 including Fig.7 A - Fig.7 D.

Fig.8 shows the temporal diffusion in the extracting apparatus of Fig.6 including Fig.8 A - Fig.8 D.

Fig.9 shows the flow direction (L), and the diffusion/transportation direction (depth) of equipment of the present invention.
Fig.10 shows one embodiment of the present invention with which two or more extracting apparatus are connected in parallel.
Fig.11 shows one embodiment of the present invention with which two or more extracting apparatus are connected in in-series.
Fig.12 is a perspective view of the flat diffusion extracting apparatus by which micro manufacture was carried out which has the diffusion direction rotated 90 degree from the "H" design shown in Fig.1 - Fig.3, Fig.5, and Fig.6.
Fig.13 is the plan view of a flat diffusion extraction system design in which micro manufacture of the Fig.12 was carried out.
Fig.14 is a graph which shows the relation of the fraction of urea and time with various numbers of flat filters connected in parallel which remain after processing.
Fig.15 is a graph which shows the relation of the fraction of urea and time by the total blood volume in a liter which remain after processing.
Fig.16 is a graph which shows the relation of the fraction of urea and time by extraction efficiency which remain after processing.

Detailed description of a preferable embodiment Diffusion of a small molecule takes place quickly [the typical dimension by which micro manufacture was carried out]. The relation between size r of particles, the diffusion coefficient D, and the temperature T is discovered by Einstein, and, in the case of the simplest spherical particle, this may be expressed as follows.

$$D = \frac{k_b T}{6\pi\mu r}.$$

Peculiar distance l in which the particles which have the diffusion coefficient D diffuse within the time t $l=\sqrt{Dt}$.

It comes out.
Table 2 shows some typical diffusion coefficients and proper time.
Table 2: Some typical values to different particles and molecule of size.
The proper time which diffuses 10 micrometers is shown.

粒子	D（２０℃）	t
０．５μｍ球	5×10^{-9} ｃｍ^2／秒	２００秒
タンパク質（ヘモグロビン）	7×10^{-7} ｃｍ^2／秒	１秒
小さい分子（フルオロセイン）	5×10^{-6} ｃｍ^2／秒	０．２秒

As shown in Fig.1, the sample stream 2 which can disregard an inertia force and goes into the sample stream input 1 as a result in the micro channel which has sufficient small dimension, It flows into the extraction channel 7 from the sample stream channel 3, without mixing with the extraction stream 4 which goes into the extraction stream input 5 and flows into the extraction channel 7 from the extraction stream input channel 6. Two streams in the extraction channel 7 form the stratified sample stream 8 and the stratified extraction stream 9.
In Fig.2, an upper left arrow shows the flow direction within the sample channel 3 of the sample stream 2 included in the sample stream input 1. A lower left arrow shows the flow direction within the sample channel 6 of the sample stream 4 included in the sample stream input 5. The sample stream 2 is relatively large ("not wished").
The small ("request") particles 18 (crosshatching shows) are included relatively to the particles 17. The sample stream 2 and the extraction stream 4 join within the extraction channel 7, serve as laminar flow, and form the stratified sample stream 8 and the stratified extraction stream 9. Relatively, the particles 18 of a small request begin to diffuse from the stratified sample stream 8 to the stratified extraction stream 9, and form the stratified output stream 16 which diffused and which contains the particles 18 of a small request relatively. The stratified sample stream 8 flows into the by-product tap hole channel 10, forms the by-product stream 12, and comes out of a channel via the by-product tap hole 15. The stratified extraction stream 9 receives the particles 18 of a relatively small request which diffused from the stratified sample stream 8, and turns into the stratified output stream 16. In the product stream outlet channel 11, the stratified output stream 16 turns into the output stream 13, and comes out of a channel via the product stream exit 14.
In Fig.3, an upper left arrow shows the flow direction within the sample stream channel 3 of the sample stream 2 which enters via the sample stream input 1. A lower left arrow shows the flow direction within the extraction stream input channel 6 of the extraction stream 4 which enters via the extraction stream input 5. Crosshatching shows the extraction stream 4. The arrow of the top within the extraction channel 7 shows the flow direction of the stratified sample stream

8, and the arrow of Shimo within the extraction channel 7 shows the flow direction of the stratified extraction stream 9. When there is more capacity of the extraction stream 4 than the quantity which may flow out via the product stream appearance channel 11 and the product stream exit 14, a part of stratified extraction stream 9 comes out via the by-product tap hole channel 10 and the by-product tap hole 15 as the excessive extraction stream 22. The excessive extraction stream 22 is laminar flow within the extraction channel 7.
The fluid barrier 20 is formed.
Relatively in the sample stream 2, the particles 18 (refer to the Fig.2 which is not shown in Fig.3) of a small request diffuse from the stratified sample stream 8 to the stratified extraction stream 9 via the fluid barrier 20, and form the output stream 16 (refer to the Fig.2 which is not shown in Fig.3).

簡単な計算が、$D = W_{fb}^2 \overline{V} / L$よりも小さい拡散係数を有する数個の粒子ま

It is shown that ** is found out in the output stream into which a molecule flows. the above-mentioned formula

いて、W_{fb}は流体バリアの幅であり、$\overline{V}$は層状サンプルストリームの平均流速

であり、Lは抽出チャネルの長さである。$D = w^2 \overline{V} / L$よりも大きい拡散係数

The particles or molecule which **** is the same concentration as the inside of a by-product stream, and exists in the output stream flowing out. In the above-mentioned formula, w is the width of an extraction channel.
A means to inject a feeding liquid in equipment is provided like the time of the equipment of the present invention being used as some analyzing systems. Such a means contains a standard syringe (fixed capacity per unit time), and a pipe (fixing pressure). A means to remove a fluid from an output exit is also provided, and, thereby, the above-mentioned means derives a flow by other publicly known means including the receptacle for fluids in capillary tube invitation, a pressure, attraction, and the field concerned mentioned above. Such receptacles may be some the analysis or other equipment which process an output stream further.
Fig.4 shows the extraction channel 7 which has the stratified extraction stream 9 which moves by speed V_{es}, and the stratified sample stream 8 which moves by speed V_{ss}. The extraction channel 7 has stream height (diffusion direction coordinates) Z_s which specifies the interface stream line position (dotted line) of Hazama of the stratified sample stream 8 and the stratified extraction stream 9. [/ near the entrance of the extraction channel 7] the height with which both streams were combined -- and -- therefore, d shows the depth of the extraction channel 7. A curve shows the form of a speed profile. If a stream moves in accordance with the length direction of the extraction channel 7, the stratified sample stream 8 will turn into the by-product stream 12. The by-product stream 12 has stream height (diffusion direction coordinates) Z_p which moves by speed V_{bps} and specifies the interface stream line position (dotted line) of Hazama of the by-product stream 12 and the output stream 13. The stratified extraction stream 9 turns into the output stream 13 which moves by speed V_{ps}.
Some processes performed by chemical assay of a fluid mixture, (1) It is non-invasive detection (for example, extinction or fluorescence spectroscopy) of precise mixture dilution, extraction of a specific component and (2) (3) indicator or precise mixing of a test probe (for example, polymer beads labeled fluorescently) and (4) indicators, or a probe.
Fig.5 shows the extracting apparatus which does not include blockade material. The particles 18 and the sample stream 2 which contains relatively the large particles 17 which are not desired of a small request are relatively introduced / injected via the sample stream tap hole 1. The extraction stream 4, for example, an aqueous buffer, is introduced / extracted via the extraction stream tap hole 5. Two streams flow through the inside of the extraction channel 7 into the layer, and the small particles 18 diffuse to the extraction stream 4 via an extraction channel from a sample stream in the meantime relatively as a result of a concentration gradient. Other

inclination, for example, magnetism, the electrical and electric equipment, and a centrifugal force may be used. The output stream 13 which contains the small particles 18 relatively [some] at least comes out from the product stream exit 14. Both the large particles 17 and by-product streams 12 which contain the small particles 18 relatively come out of a sample stream from the by-product exit 15 relatively. When [to enable the balance with a perfect extraction channel to use the sample stream and extraction stream which have the same flow rate and capacity, and to happen / sufficient] long, a maximum of 50% of the small particles in a sample stream diffuse to an extraction stream, and it comes out from an output exit. In the case of such equipment that does not include blockade material, diffusion of the small particles to an extraction stream may increase by injecting a little (or low flow rate) sample streams rather than an extraction stream. However, increase of diffusion is proportional to the ratio of the capacity/flow rate of the extraction stream to the capacity/flow rate of a sample. Therefore, the diffuser efficiency which rises as a result of having increased the ratio of the capacity/flow rate of the extraction stream to the capacity/flow rate of a sample is offset by the quantity (or flow rate) in which the sample which may be injected for every time decreased.
The concentration profile of the particles 18 of a relatively small request which diffuse within the equipment of Fig.5 is shown in Fig.7.In Fig.7, particle transportation takes place by diffusion. The curve 23 shows the relation between concentration and the position of the particles which diffuse. To 7B, time has passed in the pan since 7A to 7C, and the balance has taken place in Fig.7 D. In Fig.7 D, the particles with the same small concentration are in two streams (left and right). The concentration of each stream (both sides of the left and the right) constitutes 50% of the concentration of an initial sample stream.
in order to attain that an output stream removes the particles of the request from a sample stream exceeding 50% in the case of the equipment which does not include blockade material, if there is no multiple-times flow (run), it will not become about such equipment. for example, when it is assumed that the total balance is attained by each flow (run), in order to extract about 97% of desired small particles, a sample flows through such equipment 5 times (run), and if there is nothing, it will not become.
In the present invention, blockade material is adopted in an extraction channel.
Therefore, extraction efficiency which improved is provided.
Blockade material makes the effective concentration of the particles of the request in an extraction stream be deteriorated, and, thereby, enables more rapid and perfect diffusion to the extraction stream of desired particles.
Fig.6 shows the embodiment of the present invention. The sample stream which is shown in a dark slash region, which are relatively indicated to be the small particles 18 with a circle [white] and which contains the large particles 17 relatively is introduced / injected via the sample stream input 1. The extraction stream 4 containing the blockade material 19 is introduced / injected via the extraction stream input 5. Through the extraction channel 7, two streams flow into the layer and in the meantime, The complex 21 of the blockade material which the particles 18 (a slash region shows) of a small request diffuse to the extraction stream 4 via an extraction channel from a sample stream, combine with blockade material, and combine with desired particles relatively is formed as a result of a concentration gradient. Other inclination, for example, magnetism, the electrical and electric equipment, and a centrifugal force are used. The output stream 13 which a part combines with blockade material and forms the complex 21 and which contains the particles 18 of a small request relatively [some] at least comes out from the product stream exit 14. Both the large particles 17 and by-product streams 12 which probably contain the small particles 18 relatively [some] come out of a sample stream from the by-product tap hole 15 relatively.
The association constant and quantity of blockade material in an extraction stream determine the concentration of the particles of the separated request in an extraction stream. Diffusion of the particles of the request from a sample stream is proportional to a concentration gradient. As for the blockade material with a high association constant to desired particles, the binding site of blockade material provides effective concentration (or activity) equal to zero substantially, when excessive compared with desired particles. Therefore, desired particles continue the diffusion to

an extraction stream until blockade material is saturated. It begins to equilibrate for the first time after the saturation of blockade material in the stream whose free concentration of desired particles is two. (Comparing with the quantity of the particles of the request in a sample) When using the binding site of an excessive blockade material, the particles of all the requests are substantially extracted from a sample by the extraction stream.
Both the association constants to the quantity of blockade material and desired particles affect extraction efficiency. Extraction is so more efficient that an association constant is high, and it obtains. When to analyze particles under the nonexistence of blockade material, for example after extraction of desired particles depending on the case is wished, it is preferable that combination is reversible. Preferably, the association constant of blockade material is 10^{-1}M or 10^{-2}M at least, and this is within the limits of the association constant for the blockade material combined with unspecified, for example, activated carbon. In the case of a blockade material specific to the particles of a particular type, the association constant of abbreviation 10^{-6}M to abbreviation 10^{-8}M is preferable. Many antibodies are combined with an antigen with the association constant within the limits of this. An irreversible combination is substantially produced in the association constant of the range of 10^{-14}M to 10^{-15}M. The latter value is an association constant to the avidin of biotin. It will be recognized that an "irreversible" combination may also be reversed by changing the temperature, pH, and the solvent type of the system of reaction, for example if it is a person skilled in the art. As for the inversion (dissociation) of such combination, it is preferable to carry out after extraction of desired particles to analyze desired particles under the nonexistence of blockade material.
The concentration profile of the particles 18 of a request of the small diameter which is diffusing within the equipment of Fig.6 is shown in Fig.8.The curve 23 shows the relation between the concentration of diffusion particles, and a position. A lapse of time is shown in order of Fig.8 B from Fig.8 A, Fig.8 C, and Fig.8 D. In Fig.8 A, the desired particles 18 exist in the sample stream on the right-hand side of equipment, and the blockade material 19 exists in the extraction stream on the left-hand side of equipment. The particles 18 of a request of some quantity cross a channel, and diffuse, and Fig.8 B shows the state of combining with the blockade material 19 and forming the complex 21.
The particles 18 of a request of much more quantity cross a channel, and diffuse, and Fig.8 C shows the state of combining with the blockade material 19 and forming the complex 21 of much more quantity. The particles 18 of a request of further more much quantity cross a channel, and diffuse, and Fig.8 D shows the state of combining with the blockade material 19 and forming the complex 21 of further more much quantity. the free concentration of the particles 18 of the request in an extraction stream (left-hand side) is maintained by the very low state, and blockade material and the desired particles 18 are combined strongly -- it becomes efficiency top zero in this case. Blockade material exists in an excess and that a coupling coefficient is high can extract the particles 18 of all the requests from a sample to an extraction stream substantially.
The dimension of equipment is chosen so that laminar flow may be maintained within an extraction channel. As mentioned above, as shown in Fig.9, the flow direction of a channel is called length (L). Length may be about 1 (cm) to about 5-centimeter Hazama. The right-angled channel dimension of the direction of movement (diffusion which crosses an extraction channel) of particles is called the depth (d) to length (L). The depth is less than about 100 micrometers preferably.
He is about 20 to about 50-micrometer Hazama more preferably.
The 3rd right-angled channel dimension is called width (w) to length and the depth. By Fig.9, since a paper side and a width dimension cross at right angles, it is not shown. Width may be more than it to about 1 meter, and contains 500 micrometers, 1 mm, 5 cm, and 1/2 meter. Width is sufficient size for all the particles in the stream containing blockade material to pass. If width is long, the capacity processed within equipment can be enlarged. It is sufficient straitness for a diffusion direction (depth) to maintain laminar flow, and if it is sufficient length for diffusion with

effective length to take place, the width can lengthen 1 meter above extremely, for example.
If one side of following at least two composition is used, the sample of big capacity can be processed with the equipment of the present invention. First composition is that lengthen width of equipment and equipment holds a mass fluid as mentioned above. Second composition is that connect plurality, i.e., two or more pieces of equipment, in parallel, and each equipment processes a part of sample simultaneously, respectively. Fig.10 shows the composition which has arranged some extracting apparatus in parallel. Fluid connection of the sample stream input 1 of each equipment is carried out to the sample many line 24 via the sample connector 27, and it is carrying out fluid connection of the extraction stream input 5 of each equipment to the extraction many line 25 via the extraction connector 26. A by-product stream is left via the by-product tap hole line 28, and an output stream is left via the product stream outlet line 29. Altogether, it is connected to one reservoir and the by-product tap hole line 28 may flow into this.
Altogether, it is connected to another single reservoir and the product stream outlet line 29 may flow into this. By Fig.10, these pieces of equipment is shown that width is longer than the depth and length. Thus, in addition to comparatively long width being alternative and connecting some equipment in parallel, it can use instead of this and the sample capacity processed by unit time can be increased.
In order to improve separation of particles, it is in-series and two or more equipment can be connected. That is, as shown in Fig.11, fluid connection of the present output stream is carried out to the sample stream input of another equipment. Each extracting apparatus is shown by the reference number 100 in Fig.11. The by-product stream 12 leaves each equipment, and the output stream 13 turns into a sample stream of the equipment of the following by which the series connection was carried out.
Fig.12 is a perspective view of another embodiment of the present invention, and a "flat extracting apparatus."
Fig.13 is the plan view.
In this equipment, the embodiment which showed the diffusion direction in the extraction channel 7 to Fig.1 - Fig.3, Fig.5, and Fig.6 is the direction rotated 90 degrees.This embodiment provides the advantage that the capacity of the material which may be processed is not restrained by the width of the extraction channel 7.
Fig.12 and the flat extracting apparatus of Fig.13 are produced by etching the silicon substrate 34,The sample stream input slot 35, the extraction stream input slot 36, the output stream tap hole slot 37, the by-product stream tap hole slot 38, and the extraction channel 7 are arranged. The extraction channel 7 is closed by the glass cover 33. By Fig.12, is it Mukai and a downward arrow shows the flow of the sample stream 2 to the sample stream input 1. Similarly, is it Mukai and a downward arrow shows the flow of the extraction stream 4 to the extraction stream input 5. An upward arrow shows the flow of the output stream 16 from the product stream exit 14, and an upward arrow shows the flow of the by-product stream 12 from the by-product tap hole 15. The length of the extraction channel 7 is shown by L and the width of a channel is shown by the black arrow as w. The depth of the extraction channel 7 is shown by d. The coupling manifold 32 which is shown in Fig.13 and which has an opening, Extend the depth of the sample stream input slot 35, and the sample stream channel 3 and the sample stream input 1 are formed, Extend the depth of the extraction stream input slot 36, and the extraction stream channel 6 and the extraction stream input 5 are formed, The depth of the output stream tap hole slot 37 is extended, the product stream outlet channel 11 and the product stream exit 14 are formed, and the depth of the by-product stream tap hole slot 38 is extended, and the by-product tap hole channel 10 and the by-product tap hole 15 are formed.
In the flat extracting apparatus which is shown in Fig.13 and which operates by diffusion (concentration gradient), the sample stream 2 shown by an upper left arrow goes into the sample stream input 1, and flows through the inside of the sample stream channel 3. The extraction stream 4 is shown by the arrow included in the extraction stream input 5, and flows through the inside of the extraction stream input channel 6. The sample stream 2 flows under the stratified extraction stream 9 as the stratified sample stream 8 within the extraction channel 7. The

stratified sample stream 8 contacts the stratified extraction stream 9 over length L within the extraction channel 7. The particles ("request") 18 of the small diameter from the stratified sample stream 8 shown by the spot in the stratified extraction stream 9 flow into the product stream outlet channel 11 as the output stream 13, and they are left at the product stream exit 14 so that it may be shown by the upward arrow. The by-product stream 12 is a flow continuous from the stratified sample stream 8 after passing over the output stream 13. The by-product stream 12 contains a part of particles of the small diameter which did not diffuse to a large diameter ("not wished") particle and the output stream 13 ("request"). The by-product stream 12 leaves the by-product tap hole 15 through the by-product tap hole channel 10.

As mentioned above, the equipment of the present invention may be used for hemodialysis. Various details of the example and such a procedure of pointing out about the point taken into consideration when designing the equipment of the present invention below, and removing urea from blood are provided.

When using the extracting apparatus (the embodiment or the embodiment of a flat filter of H filter may be sufficient) of the present invention as hemodialyzer, blood is supplied to equipment via a shunt. Flow rate F_F in equipment is determined by the diffusion rate (speed which acted as Kougami by using blockade material preferably) of the particles of the request in question, and the form of equipment itself. Partially, the extraction efficiency of equipment determines the maximum flow rate of equipment with this overall depending on time required for diffusion of particles.

The diffusion coefficient of urea is $11.8 \times 10^{-6} cm^2/s$. First, it is not the absorption whose diffusion improved, i.e., the situation where blockade material is not used is taken into consideration. The variable of the following used in this example and the related assumption are as follows.

M_U: Total mass of urea in the living body. These influences are considered although generation and elimination are not mathematically taken into consideration in this application. It is assumed that this value is known (it is easily measurable with a dialysis patient).

V_B: Total blood volume (the quantity in the shunt in any moment is included). Typically, this quantity is 2-3L in 5-6L, and a child for an adult.

E: Extraction efficiency of equipment. Since the last output stream is in an equilibrium state when blockade material does not exist, this is 0.5. Equipment is designed so that it can be balanced (that is, the flow rate is made sufficient low so that it may be length for predetermined form with sufficient contact time).

It is interested what M_U carries out aging of the inventors. Although it turns out that the half of the urea which flowed is removed by passing equipment, in order the whole quantity in the living body is fixed and to decrease, it is difficult to determine the value of M_U. It is expected that a solution is exponential attenuation according to the fact that the second principle of the Fickian diffusion must be dispelled fundamentally. I hear that it is well mixed with the remaining blood of blood, and the blood which returns to the inside of the body has one final and decisive assumption. By forcible activity of the heart, this is an effective assumption.

The general formula of M_U is as follows.

$$M_U = M_{U(o)} e^{-kt}$$

The following formulas will be obtained if the differential coefficient to time is taken.

$$\frac{dM_U}{dt} = -kM_{u(o)} e^{-kt}$$

Time = this differential coefficient of zero is as follows.

$$\frac{dM_U}{dt}\Big|_{t=0} = -kM_{U(o)}$$

On this type of problem, k is a ratio between initial mass and an initial active mass typically.

Since initial mass is known, k may be determined by finding out the formula to initial quantity. Flow rate F_F in equipment expresses the quantity processed within equipment [capacity / from the inside of the body]. By carrying out multiplication with the initial concentration of urea, the amount of initial processings (initial sampling volume of desired particles) is given per right (mass/time). Consideration of that they must be a minus sign since extraction efficiency and this quantity express removal may show initial quantity as follows.

$$\frac{dM_{U(o)}}{dt}\Big|_{t=0} = -\left(\frac{M_{U(o)}}{V_B}\right)F_F E$$

Here, the item in a parenthesis is initial concentration. If it substitutes for the formula of an initial state, the formula about k will come out.

$$k = \left(\frac{F_F}{V_B}\right)E$$

And the following formulas will be obtained if it substitutes for the formula of mass.

$$M_U = M_{U(o)} e^{-\left[\left(\frac{F_F}{V_B}\right)E\right]t}$$

The increase in the coefficient t means quicker attenuation, and this means that removal from a system is quick. Since blood may be more quickly processed when flow rate F_F in equipment is made high, removal of urea becomes quick. Removal of urea becomes quick even if it makes extraction efficiency high. Because, it is because it will be more nearly sufficiently washed if the capacity which passes equipment is the same (i.e., since more urea is extracted). These the prediction of both is adjusted with a formula. Removal will become slow if total blood volume is increased. It is because more capacity is processed, and there is nothing if it is ****** in order for the concentration of urea to become thinner than ** and to realize the same removal. This prediction is also adjusted with a formula.

Consideration of the above-mentioned example which does not use blockade material will impose some restrictions on a design according to an equilibrium condition specific to urea. Preferably, the diffusion dimension d of equipment is shortened as much as possible. Thereby, a diffusion time becomes short and the maximum of flow rate becomes high. However, since it may be restrained by possibility that a channel is blockaded by red corpuscles (about 8 micrometers in diameter), generally this dimension is at least about 100 micrometers, when a sample is whole blood. Therefore, the mean distance which a diffusion molecule must move for a balance is the half of this value, i.e., 50 micrometers. Please take the formula of the following Brownian motion into consideration.

$$\frac{(\Delta x)^2}{\Delta t} = 2D$$

Here, D is a diffusion coefficient and this is $11.8 \times 10^{-6} cm^2/s$ in urea. A deltat=1.06s value is obtained by solving to an average diffusion time.

This is a minimum of the contact time of two streams. For the following calculations, the length L of equipment shall be 10 mm. In this case, the fluid must move 10 mm in 1.06 seconds or more, and, thereby, the following maximum mean velocity is obtained.

$$\bar{V} = \frac{L}{\Delta t} = \frac{10mm}{1.06s} = 9.434\frac{mm}{s}$$

Flow rate is a product of mean velocity and a cross-sectional area. Since it is what determines time quantity required for blood to contact an extraction stream, only the half of the channel which has introduced blood is taken into consideration.

The fundamental point of difference between the embodiment of H filter and the embodiment of a flat filter is the width dimension w. In H filter, if a substrate is silicon, width is about 50

micrometers preferably. In a flat filter, as mentioned above, width is theoretically infinite and a width of about 1 meter is planned.
In order to reduce hereafter contact time required for the hemodialysis which does not use the blockade material in both the embodiment of H filter, and the embodiment of a flat filter, and required contact time, the number of parallel and needed equipment is compared. And the effect of various extraction efficiency for comparison is given.
According to the embodiment of H filter which is w= 50 micrometers, flow rate is as follows.

$$F_F = \dot{V}_W \frac{d}{2} = (9.434\frac{mm}{s})\left(\frac{1x10^3\mu m}{mm}\right)(50\mu m)\left(\frac{100\mu m}{2}\right) = 2.358x10^7\frac{\mu m^3}{s}$$

If it converts to a standard unit, flow rate will serve as $2.358x10^{-5}$ml/s. This value can be assigned to the above-mentioned mass removal equation. What rearranged the equation slightly is useful.

$$\frac{M_U}{M_{U(0)}} = e^{-\left[\left(\frac{F_F}{V_B}\right)E\right]t}$$

The fraction in which the left-hand side item remains now (FR; what broke the present mass by initial mass)
******. A target fraction can be chosen and required contact time can be calculated. Generally, a target in the case of such an exponential process is 99% of perfection.
This corresponds to 0.01FR.
if F_F of the above-mentioned value, E= 0.5, and V_B=5L are used about a typical adult -- time -- $1.953x10^9$ -- a second -- namely, -- 61.9 -- a year -- it is . Or the target time (point estimate based on a typical hemodialysis period) of 4 hours can be chosen, and required F_F can be calculated. Many H filter may be connected in parallel. If it breaks by flow rate of one piece of the equipment of an application concerned, the number of required H filters will be determined. In the case of the contact time of 4 hours, F_F of 3.198 ml/s is needed. If it breaks by $2.358x10^{-5}$ml/s which is the flow rate of one piece of the equipment of an application concerned, it will be predicted that parallel H filter which exceeds 130,000 pieces is needed. This uses blockade material in H filter extracting apparatus, and shows the advantage of making extraction efficiency increase.
A case with a flat filter width of 1 meter which carries out the scale of the H filter effectively by the factor of 20,000 for comparison is considered (blockade material is not used). Thereby, flow rate F_F of one piece of equipment increases to a part for 0.4716 ml/s, i.e., 28.30 ml,/, i.e., 1.7L / time. Since it is not thought that 5 liters is "continuous" capacity, treatment time cannot only be determined for 1.7 liters only by division to five. Therefore, it must return to a mass equation and target FR of 0.01. When the flow rate of a flat filter, E= 0.5, and the blood volume of 5 liters are used, required contact time (treatment time) is 97,650 seconds, i.e., 27.1 hours. If some parallel flat filters are used, required contact time can be reduced. In the target time of 4 hours, required flow rate is 3.198ml/s (it is the same as the case of H filter). Thereby, it is predicted that seven parallel flat filters are required. Or one 7-meter-wide flat filter can be considered. However, this is considered not to be so preferable in view of a design. (** which does not use blockade material) The effect at the time of using many parallel flat filters is shown in Fig.14. The fraction of the urea which remains is decreasing as the number of parallel flat filters increases.
Total blood volume V_B is an important factor of a mass removal equation. From a small child, a patient's blood volume is a child and the range of an adult, and the ranges of it may be 1 liter - 6 liters actually. (** which does not use blockade material) The influence of the total blood volume at the time of it being parallel and using seven flat filters is shown in Fig.15. The fraction of the urea which remains is decreasing as the total blood volume decreases.
Fig.16 shows the effect of increasing extraction efficiency, when it is parallel and seven flat

filters are used. The fraction of the urea which remains is decreasing as the extraction effect of blockade material increases.
In the embodiment of a flat filter, it may be preferable to choose larger extraction channel length than 10 mm. A preferable embodiment is an embodiment 50 cm in length, and 50 cm in width. In this case, a filter becomes square [a comparable size] at the present hemodialysis machine. Only the factor of 25 increases the maximum stream flow in which this filter is possible, and, thereby, the fraction time of 0.01 is reduced from 27.1 hours to 1.1 hours. Therefore, a possibility that a cell will be sheared by high flow rate must be taken into consideration.
Considering the case where extraction efficiency is less than 1.0, the idea generally suggested is arranging a many flat filter in parallel and in series. The case of E= 0.5 is considered. If two flat filters are arranged in parallel, it will be increased to 0.75 by E. This is because only 1/4 of urea of a basis remains. Considering the case where a damping time constant decreases, an improvement factor is 1.5 in this case. However, since flow rate has doubled if two same pieces of the equipment (respectively E= 0.5) are connected in parallel, an improvement factor is 2.0. Therefore, in order to increase extraction efficiency, it is preferable to connect many equipment in series and in parallel.
A many embodiment becomes easy for a person skilled in the art clear [other than the embodiment mentioned here], and these embodiments are within the limits of the present invention. As for all the document quoted on these Descriptions, the whole is used as reference in this Description. Although the following working examples show the present invention, they do not limit the present invention by any methods.
Working-example working-example 1 In the field concerned, it is publicly known technology (Brody reaches, and it Yager(s) and). The extracting apparatus was made by etching a silicon wafer using Solid State Sensorand Actuator Workshop, South Carolina, leech ton head (Hilton Head), and 2-June 6, 1996. Channel length is about 100 micrometers and is the depth (size of diffusion) of a channel.
It was about 15 micrometers of **, and channel width was about 10 micrometers. What put the biotin (Sigma Chemical #B8889) (0.5 microg/(ml)) in which the sign was carried out by FITC (fluorescein) in distilled water was introduced into sample stream input. What put the avidin (Sigma Chemical #A3026) (160 microg/(ml)) in which the sign was carried out by the rhodamine in distilled water was introduced into extraction stream input. The flow rate which passes along an extraction channel was about 100 pico liters/second. It is known by the person skilled in the art that about 1 mg of avidin will combine with biotin which is 10-15microg. It was observed that the avidin by which the sign was carried out by the rhodamine is moving at the low rate substantially rather than flow rate, and it judged that this avidin had adhered to the wall of a channel, input, and a tap hole.
In order to bar adhesion in the wall of this avidin, the quantity of the avidin needed in order to coat the wall of equipment with the monolayer of avidin uniformly was calculated. The capacity/surface area ratio of equipment were about 10 micrometers. When it was assumed that all the avidins are absorbed into a wall, 1mg/ml of avidin solution was calculated as minimal dose by which the wall of the equipment which is about 10 micrometers is needed for capacity/surface area ratio at a wrap sake. (If the inner volume of equipment is calculated, it will be 15 pico liters, therefore this) It is the capacity of the solution needed in order to be filled up with equipment and to coat a wall. The solution (0.17mg/(ml)) (it is 1/6 of the concentration of a 1mg/ml solution) of distilled water and avidin was introduced into equipment with six aliquots. Front (front) coated with avidin is moving at a rate of the abbreviation 1/6 of an average stream flow.
It is shown that avidin has adhered to a wall.

Working-example 2 In the working example 2, the wall used the equipment coated with avidin (it prepares in working example 1) substantial completely and uniformly. The solution (10microL) of streptoavidin and distilled water which were fixed by the 1-micrometer iron oxide particle (Sigma Chemical #S2415) was introduced into extraction stream input. The solution (10 ng/ml) of biotin (Sigma Chemical #B8889) and distilled water was introduced into sample stream input. It was

clear that biotin was condensed in the extraction stream at the naked eye. However, since there was little molecularity of the streptoavidin fixed by iron oxide, fluorescence was low. Therefore, in order to maintain stoichiometrical excessive streptoavidin, low biotin concentration is needed. Streptoavidin makes fluorometry difficulty more in order to quench a fluorescent marker (FITC) partially.
In order to improve fluorometry, equipment is previously loaded with a polyethylene-glycol silane so that surface passivation may be attained. The streptoavidin which has a longer arm in front of the binding site for biotin helps to prevent quenching of fluorescence.

[Translation done.]

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(54)【発明の名称】　吸収力が向上した差違抽出装置

(57)【要約】
本発明は、所望の粒子を含むサンプルストリーム（２）から所望の粒子を抽出するための抽出装置およびその方法を提供する。装置は、サンプルストリーム流入口（１）と、抽出ストリーム流入口（５）と、サンプルストリーム流入口（１）および抽出ストリーム流入口（５）と流体連通し、抽出ストリーム流入口（５）からの抽出ストリーム（４）と隣接する層流の、サンプルストリーム流入口（１）からのサンプルストリーム（２）を受けるための抽出チャネル（７）と、抽出チャネル（７）内にあり、抽出ストリーム（９）において所望の粒子（１８）を捉えるための封鎖材料と、抽出チャネル（７）と流体連通し、所望の粒子（１８）が抽出されたサンプルストリーム（２）の少なくとも一部分を含む副産物ストリーム（１２）を受けるための副産物ストリーム流出口（１５）と、抽出チャネル（７）と流体連通し、封鎖材料と所望の粒子（１８）の少なくとも一部分とを含む生成物を受けるための生成物流出口（１４）とを含む。

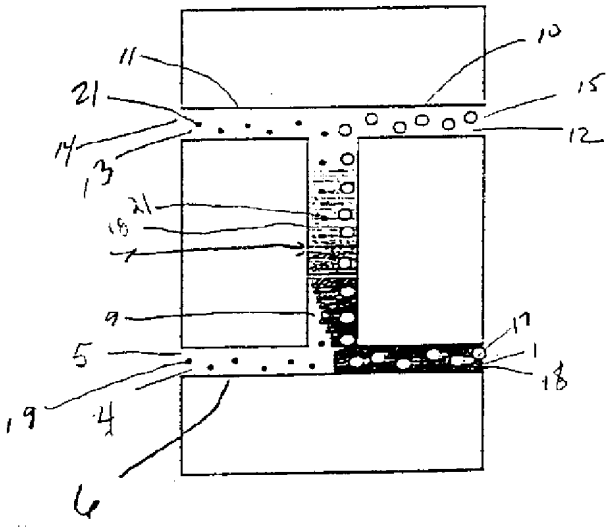


Fig. 6

【特許請求の範囲】

1．所望の粒子を含むサンプルストリームから、該所望の粒子を抽出するための抽出装置であって、

ａ．サンプルストリーム流入口と、

ｂ．抽出ストリーム流入口と、

ｃ．該サンプルストリーム流入口および該抽出ストリーム流入口と流体連通し、該抽出ストリーム流入口からの抽出ストリームと隣接する層流の、該サンプルストリーム流入口からのサンプルストリームを受けるための抽出チャネルと、

ｄ．該抽出チャネル内にあり、該抽出ストリームにおいて所望の粒子を捉えるための封鎖材料と、

ｅ．該抽出チャネルと流体連通し、所望の粒子が抽出された該サンプルストリームの少なくとも一部分を含む副産物ストリームを受けるための副産物ストリーム流出口と、

ｆ．該抽出チャネルと流体連通し、該封鎖材料と該所望の粒子の少なくとも一部分とを含む生成物を受けるための生成物流出口とを含む、抽出装置。

2．前記抽出装置は、マイクロ製造された装置である、請求項1に記載の装置。

3．前記封鎖材料が、前記装置において効果的に非拡散性である、請求項1に記載の装置。

4．前記封鎖材料が、前記所望の粒子と相互作用することができ、該所望の粒子の検出を可能にする、請求項1に記載の装置。

5．前記封鎖材料が、前記所望の粒子を解放することができ、該所望の粒子を、前記サンプルストリームからの抽出後に分析することを可能にする、請求項1に記載の装置。

6．約10マイクロメートルと約100マイクロメートルとの間の幅を有する、請求項1に記載の装置。

7．請求項1の装置を、前記封鎖材料によって捉えられた前記所望の粒子の存在を検出するための手段と組み合わせて含む、分析システム。

8．並列に接続された複数の請求項1に記載の装置を含む、装置。

９．所望の粒子を含むサンプルストリームから該所望の粒子の少なくとも一部分を抽出する方法であって、

ａ．該サンプルストリームを、請求項１に記載の抽出装置のサンプルストリーム流入口に導入する工程と、

ｂ．抽出ストリームを、該抽出装置の抽出チャネルに導入する工程と、

ｃ．該所望の粒子を捉えるための封鎖材料を該抽出チャネルに導入し、該所望の粒子が該封鎖材料によって捉えられ、該封鎖材料と、該所望の粒子の少なくとも一部分とを含む該抽出ストリームが、生成物ストリームとして該装置を出ていき、所望の粒子か抽出された該サンプルストリームが、副産物ストリームとして該装置を出ていく、方法。

１０．前記サンプルストリームが血液であり、前記所望の粒子が毒性の粒子である、請求項９に記載の方法。

【発明の詳細な説明】

吸収力が向上した差違抽出装置

本発明は、米国陸軍から与えられた米国陸軍研究契約第DAMD17-94-J-4460号に基づく政府援助によってなされている。政府は、本発明において一定の権利を有する。

関連出願の相互参照

本願は、その全文を本願に参考として援用する1996年６月14日に出願された仮出願第60/019,904号に基づく優先権を主張する特許出願である。

発明の分野

本発明は、広義には、拡散および印加場のような差違輸送原理(differential transport principles)によって他の構成成分を含むストリームから分析物を分離する抽出のシステムおよび方法に関し、抽出ストリーム中において吸収剤または吸収剤を使用する向上した方法を提供する。本発明の装置および方法は、診断および治療／処置の目的に使用し得る。

発明の背景

フィールドフローフラクショネーション装置は、単一の流入口ストリームを用いて粒子サイズの分離を行う。例えば、Giddings, J.C.の1969年６月17日付け米国特許第3,449,938号"Method for Separating and Detecting Fluid Materials"、Giddings, J.C.の1979年４月３日付け米国特許第4,147,621号"Method and Apparatus for Field-Flow Fractionation"、Giddings, J.C.の1980年７月29日付け米国特許第4,214,981号"Steric Field-Flow Fractionation"、Giddings, J.C.らの1981年２月10日付け米国特許第4,250,026号"Continuous Steric FFF Device for The Size Separation of Particles"、Giddings,J.C.ら（1983）の"Outlet Stream Splitting for Sample Concentration in Field-Flow Fractionation

",Separation Science and Technology 18:293-306、Giddings, J.C.（1985）の"Optimized Field-Flow Fractionation System Based on Dual Stream Splitters,"Anal. Chem. 57:945-947、Giddings, J.C.の1989年5月16日付け米国特許第4,830,756号"High Speed Separation of Ultra-High Molecular Weight Polymers

by Hyperlayer Field-Flow Fractionation"、Giddings, J.C.の1992年8月25日付け米国特許第4,141,651号"Pinched channel Inlet System for Reduced Relaxation Effects and Stopless Flow Injection in Field-Flow Fractionation"、Giddings, J.C.の1992年10月20日付け米国特許第5,156,039号"Procedure for Determining the Size and Size Distribution of Particles Using Sedimentation Field-Flow Fractionation"、Giddings, J.C.の1993年3月16日付け米国特許第5,193,688号"Method and Apparatus for Hydrodynamic Relaxation and Sample Concentration in Field-Flow Fraction Using Permeable Wall Elements"、Caldwell, K.D.らの1993年8月31日付け米国特許第5,240,618号, "Electrical Field-Flow Fractionation Using Redox Couple Added to Carrier Fluid"、Giddings,J.C.(1993)の"Field-Flow Fractionation:Analysis of Macromole cular, Colloidal and Particulate Materials,"Science 260:1456-1465、Wada,Yらの1995年11月14日付け米国特許第5,465,849号"Column and Method for Separating Particles in Accordance with Their Magnetic Susceptibility"、Yve,V.ら(1994)の"Miniature Field-Flow Fractionation Systems for Analysis of Blood Cells,"Clin. Chem. 40:1810-1814、Afromowitz, M.A. and Samaras,J.E.(1989)の"Pinch Field Flow Fractionation Using Flow Injection Techniques,"Separation Science and Technology 24(5and6):325-339を参照。

薄チャネルスプリットフローフラクショネーション（ＳＰＬＩＴＴ）技術も、薄チャネルを有する分離セル内において粒子分離を提供する。場の力は、フロー方向と垂直な方向に発揮される。粒子は、粒子含有ストリームから輸送ストリームを通って無粒子ストリームへと移動する。このプロセスを行う装置は、一般に、チャネルを形成するためのスペーサとして使用されるテフロンシートを用いて複数のガラス板から製造される。従って、チャネル深さは、一般に約１００～１２０μｍの厚さであるスペーサ以上の大きさであり得る。例えば、Giddings, J.C.

の1988年４月12日付け米国特許第4,737,268号、"Thin Channel Split Flow Continuous Equilibrium Process and Apparatus for Particle Fractionation"、Gi

ddings, J.C.の1990年1月16日付け米国特許第4,894,146号"Thin Channel Split Flow Process and Apparatus for Particle Fractionation"、Giddings, J.C.の1991年8月13日付け米国特許第5,093,426号"Process for Continuous Particle and Polymer Separationin Split-Flow Thin Cells Using Flow-Dependent Lift Forces"、Williams, P.S.ら(1992)の"Continuous SPLITT Fractionation Based on a Diffusion mechanism,"Ind. Eng. Chem. Res. 31:2172-2181、およびLevin, S. and Tawil, G. (1993)の"Analytical SPLITT Fractionation in the Diffusion Mode Operating as a Dialysis-like System Devoid of Membrane. Application to Drug-Carrying Liposomes,"Anal.Chem. 65:2254-2261を参照。

本発明の目的の１つは、差違輸送原理を用いて、分析物の抽出、検出および定量化を行うことができる改善された抽出システムを提供することである。本発明の別の目的は、血液のような身体流体を含む流体の精製および処置のための改善された抽出システムを提供することである。

本願において言及される全ての刊行物、特許および特許出願は、その全文を参考として援用する。

発明の要旨

上記のような差違抽出装置は、サンプルストリームから、抽出ストリームと平行な層流として流れる抽出ストリーム内へと所望の粒子が移動することを可能にする。このようなシステムの単純な実施形態の１つは、所望の粒子がサンプルストリームから抽出ストリーム内へと拡散するように、複数のストリーム間にわたる濃度勾配(gradient)を使用する。他の勾配および力、例えば、磁気、電気、重力、誘電性、沈殿、剪断、遠心力、温度、圧力および直交流勾配(cross-flow gradients)を用いることも可能である。

本願において提供される上記プロセスにおける改良点の１つは、抽出ストリームに対する封鎖材料(sequestering material)の追加である。

本発明は、所望の粒子を含むサンプルストリームから所望の粒子を抽出する抽出装置を提供し、この装置は、

ａ．サンプルストリーム流入口と、

ｂ．抽出ストリーム流入口と、

ｃ．該サンプルストリーム流入口および該抽出ストリーム流入口と流体連通し、該抽出ストリーム流入口からの抽出ストリームと隣接する層流の、該サンプルストリーム流入口からのサンプルストリームを受けるための抽出チャネルと、

ｄ．該抽出チャネル内にあり、該抽出ストリームにおいて所望の粒子を捉えるための封鎖材料と、

ｅ．該抽出チャネルと流体連通し、所望の粒子が抽出された該サンプルストリームの少なくとも一部分を含む副産物ストリームを受けるための副産物ストリーム流出口と、

ｆ．該抽出チャネルと流体連通し、該封鎖材料と該所望の粒子の少なくとも一部分とを含む生成物を受けるための生成物流出口とを含む、抽出装置である。

封鎖材料は、所望の粒子に対する吸収、結合または粘着(sticking)によって、あるいはそれらを吸収することによって、捕捉する材料である。所望の粒子に対する酵素、抗体、抗原および他のリガンドは、当該分野において公知であり、本発明において有用な封鎖材料である。封鎖材料として、当該分野において公知のあらゆるリガンドが使用され得る。このようなリガンドは、「そのまま」抽出ストリームに加えてもよいし、または、ポリマー性ビード、高分子量ポリマーまたは当該分野において公知の他の材料のような基板上に固定化してもよい。「高分子量ポリマー」とは、装置中を移動中に実質的にサンプルストリーム内に拡散しない程度に十分な分子量のポリマーを指す。高分子量ポリマーの例には、高分子量デキストラン、高分子量ポリペプチド、および高分子量核酸が含まれるが、これに限定はされない。封鎖材料は、活性炭または多孔性ポリマーのような吸収性材料であってもよい。吸収剤または吸収剤は、抗体のように特定の粒子タイプに特異的であってもよいし、あるいは活性炭のように非特異的であってもよい。

好ましくは、副産物ストリーム中で検出可能にならないように、あるいは、副産物ストリーム中における分析物の分析に干渉しないように、封鎖材料は、実質的に非拡散性、即ち、有意な程度にサンプルストリーム内へと抽出ストリームを越えないように十分にゆっくりと拡散するべきである。

封鎖材料は、所望の粒子が流出(exiting)副産物ストリームとともに装置から流出することを防ぐことによって所望の粒子を捕捉する。所望の粒子は、封鎖材料が、その粒子が副産物ストリームとともに流出することを防ぐのに十分に長い時間粒子を保持する限りにおいて、封鎖材料に緩結合し得る。所望の粒子は、さらなる分析のために除去できるように、または封鎖材料の再使用を可能にするために、封鎖材料に可逆的に結合、あるいは封鎖材料に捕捉され得る。

マイクロスケールに製造された差違抽出装置は、上記の大型装置にはない多数の利点を提供する。このようなマイクロ製造された装置は、1996年6月14日に出願された出願第08/663,916号に記載されている。この出願は、そこに参考として援用されている全ての参考文献とともに、参考としてその全文が本願に具体的に援用される。上記出願に開示されているマイクロスケール構造に適用されている上記出願において使用されている用語の定義は、本願において、マイクロスケール構造だけでなく、マクロスケール構造にも適用可能である。本願において「マクロスケール構造」は、マイクロスケール構造よりも大きな構造であって、かつ層流を可能にするのには十分に小さい構造として規定される。

本発明における所望の粒子は、分析物であってもよいし、分析物と干渉する物質でもあり得る。上記粒子は、回収して別の目的に使用することか望まれる粒子でもあり得るし、または患者の血液中の毒(poisons)または代謝物質のような毒物(toxins)でもあり得る。例えば、本発明を用いて、血液を解毒する、例えば、血液から毒性金属を除去すること、または他の身体流体を解毒する、例えば、血液透析を行うことが可能である。本発明を汚水処理、例えば、水からの不純物の除去に用いることが可能である。あるいは、本発明を用いて、発酵リアクター(fermentation reactor)中において、薬物、または微生物によって生成される他の生成物、例えば細菌細胞を、微生物に傷害を与えることなく除去することも可能である。このような処置を、連続的に行うことが可能である。

本発明は、所望の粒子を含むサンプルストリームから所望の粒子の少なくとも一部を抽出する方法をも提供し、この方法は、

ａ．該サンプルストリームを、上記の抽出装置のサンプルストリーム流入口に

導入する工程と、

ｂ．抽出ストリームを、該抽出装置の抽出チャネルに導入する工程と、

ｃ．該所望の粒子を捉えるための封鎖材料を該抽出チャネルに導入し、該所望の粒子が該封鎖材料によって捉えられ、該封鎖材料と、該所望の粒子の少なくとも一部分とを含む該抽出ストリームが、生成物ストリームとして該装置を出ていき、所望の粒子が抽出された該サンプルストリームが、副産物ストリームとして該装置を出ていく、方法である。

本発明の装置および方法は、アフィニティクロマトグラフィーを行う手段を提供する。当業者には理解されるように、アフィニティクロマトグラフィーとは、所望の物質を精製または単離する方法を指し、広義には、不溶性の不活性支持体に特定のリガンドを共有結合させることを含む。アフィニティクロマトグラフィーの場合、カラムを通って溶液中を通過する際に所望の物質がリガンドによって優先的に維持されるように、リガンドは、所望の物質に対して高い親和性を有していなければならない。

本発明は、アフィニティクロマトグラフィーを行う装置および方法を提供するものであるが、少なくとも１つの利点を有する。所望の物質（粒子）の抽出は、連続的に行うことができる。本発明のサンプルストリームおよび抽出ストリームは、装置を通って連続的に流れることができる。当該分野において公知のアフィニティクロマトグラフィーは、複数の行程、例えば、リガンドを不活性材料上に装填する行程と、カラムをフラッシング(flushing)する行程と、サンプルを装填する行程と、水洗する行程と、その後、再び水洗して所望の物質を解放する行程とを含み、通常は各行程において生成物の損失が生じる。本発明の装置において、例えば、１）抗体を含む抽出ストリームをビード上に固定化されたウィルスに、そして、全血のサンプルストリームを装置内に導入し、２）ウィルス粒子が抽出ストリームに移動した後、溶液のｐＨを変化させることによってこれらを解放することによって、全血からウィルスを抽出することができる。ビードは、例えば、チャネルの底に落ちるように選択することも可能であるし、あるいは、マグネットによってチャネルの一方に引き寄せられるように磁性体であり得る。

封鎖材料は、抽出ストリームが抽出チャネルに導入される前に抽出ストリームに存在し得る。あるいは、封鎖材料は、抽出チャネル内にすでに存在する抽出ストリームに抽出ストリーム流入口を介して導入される液体中の封鎖材料を懸濁または分解することによって、抽出ストリームに加えられ得る。

最も簡単な概念における本発明の抽出システムは、「H」形のマイクロチャネルを含む拡散抽出デバイスである。サンプルストリーム中に懸濁された粒子の混合物は、アームの1つ(例えば、左側上部)から抽出チャネル(「H」のクロスバー)に入り、抽出ストリーム(希釈ストリーム)は左側下部から入る。2つのストリームは、抽出チャネルにおいて共に流れるが、チャネルのサイズが小さいため、流れは層状であり、かつストリームは混合しない。サンプルストリームは、右側上部から副生成物ストリームとして出て、抽出ストリームは、右側下部から生成物ストリームとして出る。ストリームが抽出チャネル内で隣接した層流である間、より大きな拡散係数を有する粒子(アルブミン、糖、および小イオンなどのより小さな粒子)は、抽出ストリームに拡散する時間があるが、一方大きな粒子(例えば、血液細胞)は、サンプルストリームにとどまる。流出する抽出ストリーム(この時点では、生成物ストリームと呼ばれる)における粒子は、より大きな粒子からの干渉を受けずに分析され得る。

本特許出願において、チャネルの流れ方向は、その長さ(L)とする。長さ(L)に対して直角な粒子の輸送方向におけるチャネルの寸法を、その深さ(d)とする。長さおよび深さの両方に対して直角な第3のチャネル寸法は、その幅(w)とする。従って、深さ(d)は、サンプルストリームおよび抽出ストリームのインターフェースの面と直交する。表1は、本明細書で用いるその他の略語を示す。

表1

V	容量
V_{ss}	サンプルストリーム流量(m^3/s)
V_{es}	抽出ストリーム流量(m^3/s)

V_{ps}	生成物ストリーム流量（m^3／ｓ）
V_{bps}	副生成物ストリーム流量（m^3／ｓ）
V_{ind}	インジケータ色素ストリーム流量（m^3／ｓ）
V_{ds}	検出ストリーム流量（m^3／ｓ）
$C_{i,ss}$	サンプルストリーム構成要素ｉ濃度（ｋｇ／ｋｇ）
$C_{i,es}$	抽出ストリーム構成要素ｉ濃度（ｋｇ／ｋｇ）
$C_{i,bps}$	副生成物ストリーム構成要素ｉ濃度（ｋｇ／ｋｇ）
$C_{i,ps}$	生成物ストリーム構成要素ｉ濃度（ｋｇ／ｋｇ）
$C_{dye,ind}$	インジケータストリーム色素濃度（ｋｇ／ｋｇ）
$C_{i,ds}$	検出ストリーム構成要素ｉ濃度（ｋｇ／ｋｇ）
d	拡散方向抽出チャネル深さ（ｍ）
w	抽出チャネル幅（ｍ）
L	抽出チャネル長さ（ｍ）
$a_{\%}$	平衡濃度からのパーセント偏差値
$L_{a\%}$	$a_{\%}$を成し遂げるのに必要な装置長さ（ｍ）
z_s	抽出チャネル流入口におけるサンプルストリームと抽出ストリームとの間のインターフェース流線位置（ｍ）
z_p	副生成物ストリームと生成物ストリームとの間のインターフェース流線位置（ｍ）
P	流体ストリーム内の絶対圧力（Ｐａ）
ΔP	抽出チャネルの流入口と流出口との間の圧力差（Ｐａ）
D_i	構成要素ｉの二成分拡散係数（m^2／ｓ）
μ	流体粘度（Ｐａ・ｓ）
ρ	流体密度（Ｋｇ／m^3）
ξ	無限長抽出チャネルの平衡正規化構成要素濃度（無次元）
$\tilde{c}$	正規化構成要素濃度（無次元）
x	チャネル長方向座標（流れ方向）
y	チャネル幅方向座標
Z	拡散方向座標

$\tilde{x}, \tilde{z}$	非次元正規化変数（無次元）
w／d	アスペクト比
D	拡散係数
Re	レイノルズ数
T	温度
u	軸速度
$\overline{V}$	平均速度

抽出チャネルの長さおよび抽出チャネル流速は、粒子が抽出ストリームに拡散しなければならない時間を決定する主要なパラメータである。封鎖材料は、抽出ストリームにおける所望の粒子の有効濃度を減少させることによって、所望の粒子の拡散を増加させる。即ち、封鎖材料は、抽出ストリームへの所望の粒子の拡散の平衡を（正の方向に）シフトさせる。

上記の場合の粒子は、サンプルストリームから抽出ストリームへ、輸送機構として拡散を用いて異なって輸送される。所望の粒子を異なって輸送する他の手段も用いられ得る。用語「差違輸送」とは、所望の粒子の一部が、サンプルストリームから抽出ストリームに輸送され、望まれない粒子を実質的に排除することを意味する。例えば、磁気、電気または他の力が抽出ストリームにかけられ得るか、温度勾配が用いられ得るか、または抗体などの吸収剤もくしは吸収材料が、抽出ストリームに加えられ、所望の粒子を捕捉し得る。

サンプルストリームおよび抽出ストリーム流入口、ならびに副生成物ストリームおよび生成物ストリーム流出口は、チャネル、貯蔵部、ポート、または他のコンテナを含み得る。サンプルストリーム流入口は、「所望の粒子」、例えば、その存在を検出するために抽出することが所望される粒子を含むサンプルストリームを収容するように設計される。サンプルストリームはさらに、抽出されない他の粒子（本明細書では、「望まれない粒子」と呼ばれる）を含む。これらの望まれない粒子は、所望の粒子の検出に干渉し得る粒子を含む。好ましい実施態様において、サンプルストリームは、全血を含む。所望の粒子は、アルブミンまたは他の血漿構成成分であり得、望まれない粒子は血液細胞であり得る。装置は、特

に、全血からの細胞を含まない血漿構成成分を得るのに有用である。本発明が有用な他の流体には、異なる長さのＤＮＡ断片、様々なサイズのタンパク質、または外来の化学反応混合物の溶液または懸濁物が含まれる。本発明の実施において有用なサンプルストリームには、発酵ブロス、未処理の下水、液化食物サンプル、土壌サンプル、ならびに唾液、尿および脳脊髄液などの生物学的流体が含まれる。

用語「粒子」は、分子、細胞、タンパク質、核酸および複合炭水化物などの巨大分子、１から数個の原子を含む小分子、およびイオンを指す。粒子は、ストリーム中に懸濁または溶解され得る。用語「ストリーム」は、所望の粒子および／または望まれない粒子を含む、水もしくは他の液体などのキャリア流体、空気または他のガスを指す。本明細書で用いる用語「粒子」は、キャリアストリームの分子を含まない。

用語「抽出」は、所望の粒子の少なくとも一部、即ち、検出可能な部分を、サンプルストリームから抽出ストリームに輸送し、望まれない粒子を実質的に排除することを指す。望まれない粒子は、抽出ストリームに輸送され得る、特に所望の粒子よりも速く拡散し得る粒子であるが、このような望まれない粒子の存在は、望まれない粒子が、所望の粒子を含むストリームの検出または後の処理に干渉しないように最小限にされると認識される。望まれない粒子のサンプルストリームから抽出ストリームまでの転送は、抽出ストリームにこのような望まれない粒子を予め充填することによって最小限にされ得る。抽出ストリームに望まれない粒子を予め充填することは、副生成物ストリームが目的である（例えば、さらに使用または分析される）実施態様において好適であり得る。例えば、血液が患者の体内に戻される場合、抽出ストリームは、好ましくは、当業者には当然のことながら、適切な濃度の電解液を含む。封鎖材料は、抽出ストリームにおける所望の粒子の有効濃度を減少させることによって、所望の粒子をサンプルから分離する効率を高める。

用語「抽出効率」とは、抽出ストリームに転送され、生成物ストリームに排出されるサンプル中の所望の粒子のパーセントを指す。抽出効率は、封鎖材料を用いることによって増加し得る。

用語「層流」とは、２つのストリームが混合しないで、安定して横並びに再循環せずに流れることを指す。再循環のゾーンはなく、乱流は無視できる程度である。当該技術分野で公知のように、流れのレイノルズ数は、は、粘力に対する慣性力の比である。導管を流れるためには、レイノルズ数は、$Re=\rho d(\bar{V}/\mu)$を用いて計算される。ここで、Ｒｅは、レイノルズ数、ρは、流体の体積密度、ｄは、導管の形状による導管の典型的な断面寸法、$\bar{V}$は、導管断面を通る平均速度、μは粘度である。

レイノルズ数が減少するにつれて、流れパターンは、粘性効果に依存するようになり、慣性効果には依存しなくなる。特定のレイノルズ数（湾曲部を有し、管腔サイズが変化するチャネルのシステムに対する管腔サイズに基づく）未満では、慣性効果は、層状再循環ゾーンおよび乱流などの有意な存在を示す現象を引き起こすのに不充分である。従って、乱れていない層状の再循環しない流れは、本明細書に記載する抽出装置において発生する。このような装置では、最小の分散混合は、任意の層状粘性フロー内に存在する粘性フロー速度プロファイルの結果発生する。これによって、所望の粒子を１つのストリームから他のストリームに抽出する目的で、２つの層状の再循環しない流体ストリームは抽出チャネルを流れることが可能になる。

ストリームは、流出口の流出口流量を正確に調節することによって任意の位置で導管の端部で分離され得る。これは、非再循環および非乱流の基準を満たさないより高いレイノルズ数においては可能ではない。

抽出ストリーム流入口は、サンプルストリームと層流で接触するとき、所望の粒子を収容することができる抽出ストリームを収容するように設計される。抽出ストリームは、サンプルストリームから輸送される粒子を受け入れることが可能な任意の流体であり得る。抽出ストリームは、サンプルストリームから抽出ストリームへ輸送された所望の粒子を結合する封鎖材料を含む。好ましい抽出ストリームは、水および生理食塩水などの等浸透圧溶液である。他の有用な抽出ストリ

ームは、アセトン、イソプロピルアルコール、超臨界状態（supercritical）の二酸化炭素またはエタノールなどの有機溶媒を含む。空気および他のガスもまた、サンプルおよび抽出ストリームキャリアとして使用され得る。

副生成物ストリームは、所望の粒子が抽出されたサンプルストリームの少なくとも一部を含み、以下に記載するように、所望の粒子がサンプルストリームから運搬された抽出ストリームの一部を含み得るかまたは含み得ない場合もある。封鎖材料は、サンプルから所望の粒子をより効果的に抽出させ、それによって、より純粋な副生成物ストリームが形成される。過剰な封鎖材料が使用され、所望の粒子に対して高い結合定数を有する場合、サンプルストリーム中の実質的にすべての所望の粒子は、流量および抽出チャネル長に応じて、サンプルを一回だけ処理するだけでサンプルストリームから抽出され得る。封鎖材料がなく、サンプルおよび抽出流体の流量が等しいと仮定すると、所望の粒子の平衡濃度は、抽出ストリームにおいて５０％である。即ち、所望の粒子の多くともわずか５０％が、抽出ストリームに拡散する。従って、封鎖材料がない場合、所望の粒子の９７％をサンプルから除去するためには、サンプルは、少なくとも５回処理されなければならない。

副生成物ストリーム流出口は、抽出チャネルから除去されて廃棄される、再利用されるまたは他のシステム構成要素に移される副生成物ストリーム（サンプルストリーム、および恐らくは抽出ストリームの一部で構成される）を導き、さらに処理するように設計される。

生成物ストリームは、所望の粒子の少なくとも一部および封鎖材料を含む。生成物ストリーム流出口は、上記のように、生成物ストリームチャネルを含み得、検出可能な量の所望の粒子を含む生成物ストリームを検出もくしはさらなる処理領域またはシステム構成要素に導くように設計される。充分な量の抽出ストリームは、所望の粒子が当該技術分野で公知の手段によって生成物ストリーム内で検出可能となるように、充分な量の所望の粒子を含んで、生成物ストリーム内に存在しなければならない。

生成物ストリームは、貯蔵チャンバ、または他の装置に導かれ得る。そこで、

生成物ストリームは、例えば、１９９４年４月１９日付けで発行されたＷｉｌｄｉｎｇ，Ｐ．らの米国特許第５，３０４，４８７号（本願では、これを参考のために援用する）に開示されているように、例えば、所望の粒子から封鎖材料を分離し、混合、分離、分析、加熱または別の処理によってさらに処理され得る。副生成物ストリームはまた、貯蔵チャンバまたは他のコンテナまたは装置に導かれ

されに処理され得る。

本発明の装置は、「マイクロ製造」され得る。これは、シリコンマイクロ製造の当業者に容易に利用可能なシリコンウェハ上で製造可能で、ＬＩＧＡ、熱可塑性マイクロパターン転送、樹脂をベースとしたマイクロ鋳造、毛細管におけるマイクロ成形（ＭＩＭＩＣ）、ウェット等方性および異方性エッチング、レーザ援助化学エッチング（ＬＡＣＥ）、反応性イオンエッチング（ＲＩＥ）、またはマイクロ製造の分野で公知の他の技術などの方法によって製造可能な固有サイズおよび幾何学構造を有する装置を指す。シリコンマイクロ製造の場合、ウェハが大きくなるほど、本発明の複数の装置を複数の形態で収容する。少数ではあるが標準ウェハサイズは３”、４”、６”および８”である。新規のマイクロ製造方法を用いた本明細書に提示する原理の応用は、請求項の範囲および趣旨内である。

本明細書において「Ｈフィルタ装置」と呼ぶ好適な実施態様において、流入チャネルおよび流出チャネルは、幅が最大サイズ時のストリーム粒子直径の約２～３倍から約１００ミクロンの間であり、深さが最大サイズ時の粒子直径の約２～３倍から約１００ミクロン未満の間であり、抽出チャネルは、幅が最大サイズ時の粒子直径の約２～３倍からウェハ厚の約２／３の間であり、深さが最大サイズ時の粒子直径の約２～３倍から約１００ミクロン未満の間であり、長さが最大サイズ時の粒子直径の約４倍～約１０倍から５ｍｍ以下の間である。

粒子輸送方向を「Ｈフィルタ装置」設計から９０°回転させた、本明細書において「フラット抽出装置」または「フラットフィルタ装置」と呼ぶ第２の実施態様において、流入チャネルは抽出チャネルへの入口において抽出チャネル幅と等しい幅、好ましくは２～３粒子直径から約５００ミクロンの間の幅を有し、抽出チャネルは、幅が好ましくは最大サイズ時の粒子の直径の約２～３倍から５ｍｍ

以下の間であり、深さが最大サイズ時の粒子の直径の約２～３倍から約１００ミクロン未満の間であり、長さが最大サイズ時の粒子の直径の少なくとも約４倍である。

抽出チャネルは、サンプルストリーム流入口および抽出ストリーム流入口からサンプルストリームおよび抽出ストリームの流入(inflow)を受け取り、これらのストリームを隣接した層流として、所望の粒子の抽出ストリーム中への抽出を可

能にするのに十分な距離にわたって、導く。本願において参考のために援用する１９９７年３月３１日付け出願のWeiglらの米国特許出願第08/829,679号および１９９７年３月３１日付け出願のＰＣＴ出願第PCT/US97/05245号に開示されたフローチャネルと同様に、抽出チャネルの長さは、例えば蛇状（「ヘアピン」折の集合）またはコイルなどの折り畳み構造に形成することによって増やすことができる。

抽出ストリームチャネルおよび生産物流出口チャネルの幅および深さは、所望の粒子、封鎖材料、および所望の粒子と封鎖材料とのいかなる複合物の通過も可能にするために十分大きくなければならない。

Ｈフィルタ装置の実施態様におけるように幅寸法がウェハ厚方向である場合、本発明のマイクロスケール抽出装置のシリコンマイクロ製造実施態様において、サンプルチャネル、抽出チャネル、生産物チャネル、および副産物チャネル、ならびに流入口および流出口の幅は、シリコンウェハ厚、すなわち約３００ミクロン未満である。あるいは、装置が他の材料、好ましくはプラスチックなどの成型可能な(moldable)材料から形成されている場合、すなわち「フラット抽出装置」実施態様の場合には、幅に理論的な最大限界は無い。０．５メートル、１メートル、およびより大きな幅が考えられる。流体（サンプルストリームおよび抽出ストリーム）の装置への送達を制御できると仮定すれば、例えばチャネルの幅にわたって各流体の流量を制御できると仮定すれば、幅に理論的な最大限界は無い。抽出チャネルの寸法は、層流および均一な流量を維持するように、例えば乱流が無くまたチャネル壁部上に粒子の堆積が無いように、選択される。

「フラットフィルタ」実施態様におけるように、深さ寸法がウェハ厚方向であ

る場合、本発明のマイクロスケール抽出装置のシリコンマイクロ製造された実施態様において、サンプルチャネル、抽出チャネル、生産物チャネル、および副産物チャネル、ならびに流入口および出口の深さは、シリコンウェハ厚、すなわち約３００ミクロン未満である。好ましくは、マイクロ製造された装置において、深さ（特に抽出チャネルの）は、約２００ミクロン未満であり、より好ましくは約１００ミクロン未満である。

本発明の装置中の粒子の差違輸送に用いられ得る、当該分野で公知の場のいくつかは、以下によって生成されるものである：

・沈降(sedimentation)

・電位

・温度勾配

・クロスフロー

・誘電勾配

・剪断力

・磁力

・濃度勾配

これらの場を生成する手段は、当該分野において公知である。

本明細書に記載したチャネルの拡散方向のサイズ（深さ）が小さいため、拡散またはその他の手段による所望の粒子の差違輸送は、非常に急速に、例えば約３００秒未満および所望であれば約１秒未満で行われる。抽出ストリーム中に封鎖材料が存在することにより、抽出ストリーム中の所望の粒子の実効濃度を低下させて所望の粒子の輸送が増大し、サンプルストリームと抽出ストリームとの間の実効濃度差を最大化する。これにより、抽出チャネルの深さ（拡散寸法）に沿っての純移動が最大化され、所望の粒子のサンプルからの急速な分離が可能になる。

サンプルストリームおよび抽出ストリームは、例えば粘度、密度、表面エネルギー、均一性、化学組成などが異なる特性を有し得、これは差違輸送速度に影響を与え得る。これらの異なる特性を考慮するためにシステムパラメータを調節お

よび最適化する必要があり得ることは当業者には明らかであり、また過大な実験なしに行い得る。

サンプルストリームおよび抽出ストリームは、所望の粒子の少なくとも分析可能な量、そして好ましくは大部分(major portion)が抽出ストリーム中に輸送されることを可能にするために十分な時間、抽出チャネル中において接触状態に保たれる。装置からの生産物ストリームの流量は、大きな幅を有する装置（例えば約５０μｍを越える）においては、約０．００１ピコリットル／秒から約１０ｍｌ／秒以上の間である。例えば、生産物ストリームの最適な流量は約２００ナノリットル／秒であり得る。当該分野において公知のように、そのようなわずかな生産物ストリーム中に存在する非常に少ない量の分析物でも、分光分析その他の手段により検出し得る。

平均流速$\overline{V}$は、以下の関係式を満たすように選択される：

$$\overline{V} < f\frac{DL}{d^2}$$

ｆは、あるパーセンテージの所望の粒子がサンプルストリームから抽出ストリームへ移動するためには、２つのストリームがどれだけの長さの間互いと接触していなければならないかに関する、時間係数（比例定数）である。

従って、単位幅（ｗ）当たりの容量流量（Ｑ）は、ｆ（ＤＬ）／ｄ未満に限定される。ここで、$Q=\overline{V}wd$、$Q/w=\overline{V}d$と表されて、

$$\frac{Q}{w} < f\frac{DL}{d}$$

ｆ＝１を選び、これに基づいて単位幅当たりの最大流量を計算することが計算に便利であり得る。例えば、ビオチン（長さ（Ｌ）＝１ｃｍおよび深さ（拡散寸法）（ｄ）＝１０μｍのチャネルにおいて拡散係数Ｄ＝５００μm^2／秒）において、単位幅当たりの最大流量は、幅１μｍ当たりにつき約５００ピコリットル／秒である。

上記から、以下の関係式が導かれる：

$$d^2/D=2t$$

これは、平均時間２ｔで、分子が距離ｄ（チャネルの深さ）にわたって拡散することを意味する。

所望の粒子の「大部分」とは、サンプルストリーム中に存在する該粒子の５０％より上である。

封鎖材料は抽出プロセスの効率を高め、５０％（等しい体積のサンプルおよび

抽出ストリームを用い、かつ封鎖材料その他の差違輸送力（例えば磁場および電場）を用いすに得られる最大の抽出）を越える抽出を可能にする。好ましくは、封鎖材料は、所望の粒子の約５０％から約８０％を越える抽出を可能にする。より好ましくは、封鎖材料は、所望の粒子の約７５％から約９５％の抽出を可能にする。最も好ましくは、封鎖材料は、所望の粒子の約８５％から約１００％の抽出を可能にする。

本明細書に記載した発明の動作を成功させるためには、装置の４つのチャネル（すなわちサンプルストリーム、抽出ストリーム、生産物ストリーム、および副産物ストリーム）のうち３つにおいて、容量流量を正確に制御することが必要である。４つ目のチャネルは、規制する必要はなく、また規制されるべきでない。なぜなら、このチャネルを無規制のままにしておくことにより、サンプルストリームおよび抽出ストリームの混合のΔＶのために、装置がサンプルの体積の予測不可能な変化に対応することが可能になるからである。正確に規制された流量を達成するための手段は、当該分野において公知である。

本発明の拡散型抽出システムにおける生産物ストリームに輸送されている粒子のサイズの制御を助け、生産物ストリーム中におけるより大きな粒子の出現を減少させるため、抽出チャネルに流体障壁を作成してもよい。このような流体障壁は存在するのは、図３に示すように、抽出ストリームが、流出する副産物ストリームとともに抽出ストリームの一部が副産物出口を通って流れさせるのに十分な体積だけ存在している場合である。抽出ストリーム中に拡散したより小さな粒子は、この流体障壁の幅をわたらないと、生産物ストリームとともに流れ出ること

ができない。大規模に形成されたこのような流体障壁が、本明細書において参考のために援用するWilliams P.S.ら（１９９２）、"Continuous SPLITT Fractionation Based on a Diffusion Mechanism"、Ind. Eng. Chem. Res. 2172-2181に説明されている。

サンプルストリームおよび抽出ストリームの流量を制御することにより、抽出チャネルに入る各々からの体積の比を制御することができる。サンプルストリームおよび抽出ストリームの体積比はまた、サンプルストリームおよび抽出ストリームにかかる所定の送達圧(delivery pressure)について、流出チャネルおよび

流入チャネルの構造によっても、設定され得る。生産物および副産物ストリームの容量流量はまた、生産物ストリーム圧または副産物ストリーム圧を操作すること、または任意のポート（流入）圧を用いて流入のフロー抵抗を変化させることによっても制御され得る。制御モードに関わらず、流入チャネルおよび流出チャネルは、本明細書において記載する処理されるべき粒子のサイズに基づいた、最小チャネル寸法の条件を満たさなければならない。抽出チャネルに入る抽出ストリームの体積がサンプルストリームの体積よりも大きく、かつ２つの流出ストリームが同一であれば、流体障壁が形成される。生産物ストリームの容量流量が、抽出ストリームの全容量フローに対応しきれないほど小さくても、流体障壁は形成される。

本発明の抽出装置は、抽出チャネル中の抽出ストリームの体積をサンプルストリームの体積に対して制御するための手段を有してもよい。そのような手段は、余剰の抽出ストリームに対処できるほど十分に大きい副産物ストリームと協同して全抽出ストリームを流出させることを可能にするのに必要な大きさよりも小さい、生産物ストリーム流出口を有する。本発明の抽出装置は、複数の生産物ストリーム流出口を有することにより、異なるタイプの所望の粒子を含有する生産物ストリームが回収できるようにしてもよい。

本発明の装置は、生産物ストリーム中の所望の粒子を検出するための感知手段を含む分析システムの、サンプル前処理システムとして利用し得る。そのような手段は、生産物ストリームを、所望の粒子との相互作用により粒子か当該分野に

おいて公知の感知手段によって検出されることを可能にするインジケータストリームと混合するための手段を含む。当該分野において公知の感知手段は、光学分光分析設備などの光学的手段ならびに、他の手段、例えば、吸光分光分析設備あるいは蛍光検出手段、分析物の所望の粒子に曝されたときに色またはその他の特性を変化させる化学的インジケータ、免疫学的手段、装置に挿入された電極などの電気的手段、電気化学的手段、放射性手段、あるいは磁気共鳴設備を含む当該分野において公知のほとんどすべてのマイクロ分析技術、またはその他の当該分野において公知の、イオン、分子、ポリマー、ウィルス、ＤＮＡ配列、抗原、微生物その他のファクタなどの分析物粒子の存在を検出するための手段を含む。好

ましくは、光学手段または蛍光手段を用いて、抗体およびＤＮＡ配列などを蛍光マーカーに付着させる。インジケータおよびマイクロ製造された混合手段ならびに検出および感知手段は、本明細書において参考のために援用する米国出願シリアルＮｏ．08/625,808に記載されている。

本発明の一実施態様において、上述の差違抽出装置は、生産物および／または副産物ストリームをさらに処理するための手段、例えば生産物ストリームをインジケータ物質と混合するための拡散型混合装置（例えば本明細書において参考のために援用する米国出願シリアルＮｏ．08/625,808に記載されるような）、ならびに所望の分析物粒子の存在を検出し得るための検出チャンバを有する、分析システム中に内在化される。これらの追加的な処理手段は好ましくは、差違抽出装置中とともに、標準的なシリコンウェハ上に作成された「チップ上実験室(lab-on-a-chip)」中に設けられる。このシステムは、生産物ストリームおよび／または副産物ストリーム中の分析物粒子（所望のあるいは望まれない粒子）の濃度の決定および／またはサンプルストリーム中の分析物粒子の濃度の決定のための、定量手段を有し得る。そのような手段は、分析分光設備、電圧計、電流計、および誘電緩和設備(dielectric relaxation equipment)を含む。濃度決定は、当該分野において公知の手段および本明細書に開示した手段よる計算または較正によって行い得る。

本発明の別の実施態様において排水処理、血液透析、血液解毒などの、流体の

精製に用いる場合、約１０ｍｌ／秒などの大量のサンプルストリームを処理し得る。この実施態様において、好ましくは、抽出チャネルの幅が大きく、例えば約１メートル程度にし得るが、上述のように、チャネル幅の固定の理論的な最大値は存在しない。大量の流体容量の処理においては、マイクロ製造された装置を含む本発明の装置群を、並列に接続してもよく、および選択的に直列に接続してもよい。

以下の実施例に記載するように、装置を予め処理する、すなわち性能を向上させるために装置の内壁を予めコーティングすることが好適であり得る。壁は、装置が所望の粒子の分離を実行するために用いられる前に、用いるべき封鎖材料でコーティングされ得る。いずれの特定の理論にも束縛されることを望むことなく、

壁を封鎖材料で予めコーティングすることは、サンプルおよび封鎖材料が後に装置に導入されるときに、壁に封鎖材料が付着することを防止すると考えられる。あるいは、親水性コーティング材料による表面保護を実行するために、装置の内壁が予めコーティングされ得る。親水性コーティング材料は、市販されており、アルブミン（例えば、ウシ血清アルブミン、ラクトアルブミン、およびヒト血清アルブミン）および当該分野で公知のシラン処理試薬、好適にはポリエチレングリコールシランを含むがこれらには限定されない。

当業者には理解されるように、本明細書で開示するコンポーネントおよび工程に対して多くの置換がなされ得、本発明は本明細書で述べる特定の実施形態には限定されない。

図面の簡単な説明

図１は、低レイノルズ数を有する２つの入力ストリームの層流を示すマイクロチャネル構造を示す。

図２は、サンプルストリームから抽出ストリームへの相対的に小さい粒子の拡散を示すマイクロチャネル構造を示す。

図３は、サンプルストリームと抽出ストリームとの間の流体バリアの形成を示すマイクロチャネル構造を示す。

図４は、サンプル、抽出、生成物および副生成物ストリームの流量を示す、抽出チャネル内の流入口および流出口界面ストリームラインを示す図である。

図５は、封鎖材料を含まない抽出装置を示す。

図６は、封鎖材料を含む、本発明の抽出装置の実施形態（Ｈフィルタの実施形態）を示す。

図７は、図７Ａ～図７Ｄを含み、図５の抽出装置内の経時的な拡散を示す。

図８は、図８Ａ～図８Ｄを含み、図６の抽出装置内の経時的な拡散を示す。

図９は、本発明の装置の流れ方向（Ｌ）と拡散／輸送方向（深さ）とを示す。

図１０は、複数の抽出装置が並列的に連結されている、本発明の１実施形態を示す。

図１１は、複数の抽出装置が直列的に連結されている、本発明の１実施形態を示す。

図１２は、図１～図３、図５および図６に示す「Ｈ」設計から９０度回転した拡散方向を有するマイクロ製造された平坦な拡散抽出装置の斜視図である。

図１３は、図１２のマイクロ製造された平坦な拡散抽出システム設計の平面図である。

図１４は、並列的に連結された様々な数の平坦フィルタによる、処理後に残存する尿素の画分と時間との関係を示すグラフである。

図１５は、リットルでの総血液容量による、処理後に残存する尿素の画分と時間との関係を示すグラフである。

図１６は、抽出効率による、処理後に残存する尿素の画分と時間との関係を示すグラフである。

好適な実施形態の詳細な説明

小さい分子の拡散は、典型的なマイクロ製造されたディメンションに亘って急速に起こる。粒子のサイズｒ、拡散係数Ｄ、および温度Ｔの関係は、アインシュタインによって発見され、最も単純な球状粒子の場合、これは以下のように表され得る。

$$D = \frac{k_b T}{6\pi\mu r}.$$

拡散係数Dを有する粒子が時間t内で拡散する固有距離lは、

$$l = \sqrt{Dt}.$$

である。

表２は、いくつかの典型的な拡散係数と固有時間とを示す。

表２：

異なるサイズの粒子および分子に対するいくつかの典型的な値。

１０μｍを拡散する固有時間が示される。

粒子	D（２０℃）	t
０．５μｍ球	5×10^{-9}ｃｍ^2／秒	２００秒
タンパク質（ヘモグロビン）	7×10^{-7}ｃｍ^2／秒	１秒
小さい分子（フルオロセイン）	5×10^{-6}ｃｍ^2／秒	０．２秒

図１に示すように、十分小さいディメンションを有するマイクロチャネルにおいて、慣性力は無視でき、その結果、サンプルストリーム流入口１に入るサンプルストリーム２は、抽出ストリーム流入口５に入って抽出ストリーム流入口チャネル６から抽出チャネル７に流入する抽出ストリーム４と混合することなく、サンプルストリームチャネル３から抽出チャネル７に流入する。抽出チャネル７内の２つのストリームは、層状サンプルストリーム８と層状抽出ストリーム９とを形成する。

図２において、左上の矢印は、サンプルストリーム流入口１に入るサンプルストリーム２の、サンプルチャネル３内における流方向を示す。左下の矢印は、サンプルストリーム流入口５に入るサンプルストリーム４の、サンプルチャネル６内の流方向を示す。サンプルストリーム２は、相対的に大きい（「望まれない」

）粒子１７と相対的に小さい（「所望の」）粒子１８（クロスハッチングで示す）とを含む。サンプルストリーム２および抽出ストリーム４は、抽出チャネル７内で合流して層流となり、層状サンプルストリーム８および層状抽出ストリーム９を形成する。相対的に小さい所望の粒子１８は、層状サンプルストリーム８から層状抽出ストリーム９に拡散し始めて、拡散された相対的に小さい所望の粒子１８を含む層状生成物ストリーム１６を形成する。層状サンプルストリーム８は、副生成物流出口チャネル１０に流入して、副生成物ストリーム１２を形成し、副生成物流出口１５を介してチャネルから出る。層状抽出ストリーム９は、層状サンプルストリーム８から拡散した、相対的に小さい所望の粒子１８を受け取り、層状生成物ストリーム１６になる。層状生成物ストリーム１６は、生成物流出口チャネル１１において、生成物ストリーム１３になり生成物流出口１４を介してチャネルから出る。

図３において、左上の矢印は、サンプルストリーム流入口１を介して入るサンプルストリーム２の、サンプルストリームチャネル３内での流方向を示す。左下の矢印は、抽出ストリーム流入口５を介して入る抽出ストリーム４の、抽出ストリーム流入口チャネル６内における流方向を示す。抽出ストリーム４を、クロスハッチングで示す。抽出チャネル７内の上の矢印は層状サンプルストリーム８の流方向を示し、抽出チャネル７内の下の矢印は層状抽出ストリーム９の流方向を示す。抽出ストリーム４の容量が、生成物流出チャネル１１および生成物流出口１４を介して流出し得る量よりも多いとき、層状抽出ストリーム９の一部が、過剰抽出ストリーム２２として副生成物流出口チャネル１０および副生成物流出口１５を介して出る。過剰抽出ストリーム２２は、抽出チャネル７内では層流であり、流体バリア２０を形成する。サンプルストリーム２内の相対的に小さい所望の粒子１８（図３には示さない、図２を参照のこと）は、層状サンプルストリーム８から流体バリア２０を介して層状抽出ストリーム９に拡散し、生成物ストリーム１６（図３には示さない、図２を参照のこと）を形成する。

簡単な計算が、$D = W_{fb}^{2}\overline{V} / L$よりも小さい拡散係数を有する数個の粒子ま

たは分子が流出する生成物ストリーム内に見い出されることを示す。上記式において、W_{fb}は流体バリアの幅であり、$\overline{V}$は層状サンプルストリームの平均流速であり、Ｌは抽出チャネルの長さである。$D = w^2\overline{V}/L$よりも大きい拡散係数を有する粒子または分子は、副生成物ストリーム内と同一の濃度で、流出する生成物ストリーム内に存在する。上記式において、ｗは抽出チャネルの幅である。

本発明の装置が分析システムの一部として用いられるとき同様、フィード液体を装置内に注入する手段が提供される。このような手段は、標準的シリンジ（単位時間当たりの固定容量）および管（固定圧力）を含む。流体を生成物出口から除去する手段もまた提供され、上記手段は流体用レセプタクルを含み、それにより毛細管誘引、圧力、引力、および上述した当該分野で公知の他の手段により流れを誘導する。このようなレセプタクルは、生成物ストリームを更に処理する分析または他の装置の一部であり得る。

図４は、速度V_{es}で移動する層状抽出ストリーム９と速度V_{ss}で移動する層状サンプルストリーム８とを有する抽出チャネル７を示す。抽出チャネル７は、

抽出チャネル７の入口近傍における層状サンプルストリーム８と層状抽出ストリーム９との間の界面ストリームライン位置（点線）を規定するストリーム高さ（拡散方向座標）Z_sを有する。両方のストリームの組み合わされた高さ、および従って抽出チャネル７の深さをｄで示す。曲線は、速度プロフィールの形状を示す。ストリームが抽出チャネル７の長さ方向に沿って移動すると、層状サンプルストリーム８が副生成物ストリーム１２となる。副生成物ストリーム１２は、速度V_{bps}で移動し、且つ副生成物ストリーム１２と生成物ストリーム１３との間の界面ストリームライン位置（点線）を規定するストリーム高さ（拡散方向座標）Z_pを有する。層状抽出ストリーム９は、速度V_{ps}で移動する生成物ストリーム１３になる。

流体混合物の化学的アッセイで行われるいくつかの工程は、（１）精密な混合物希釈、（２）特定の成分の抽出、（３）指示薬またはテストプローブ（例えば、蛍光標識されたポリマービーズ）の精密な混合、および（４）指示薬またはプ

ローブの非侵襲的検出（例えば、吸光または蛍光分光法）である。

図５は、封鎖材料を含まない抽出装置を示す。相対的に小さい所望の粒子１８及び相対的に大きい望まれない粒子１７を含むサンプルストリーム２が、サンプルストリーム流出口１を介して導入／注入される。抽出ストリーム４、例えば、水性緩衝液が抽出ストリーム流出口５を介して導入／抽出される。２つのストリームは、抽出チャネル７内を層様に流れ、その間に、濃度勾配の結果、サンプルストリームからの相対的に小さい粒子１８が抽出チャネルを介して抽出ストリーム４に拡散する。他の勾配、例えば、磁気、電気、および遠心力が用いられ得る。少なくともいくつかの相対的に小さい粒子１８を含む生成物ストリーム１３は、生成物流出口１４から出る。共にサンプルストリームからの相対的に大きい粒子１７及び相対的に小さい粒子１８を含む副生成物ストリーム１２は、副生成物出口１５から出る。同一の流量および容量を有するサンプルストリームと抽出ストリームとが用いられ且つ抽出チャネルが完全な平衡が起こることを可能にするに十分長い場合、サンプルストリーム内の小さい粒子の最高５０％が抽出ストリームに拡散して生成物出口から出る。封鎖材料を含まないこのような装置の場合、抽出ストリームへの小さい粒子の拡散は、抽出ストリームよりも少量（または低

流量）のサンプルストリームを注入することにより増大され得る。しかし、拡散の増大は、サンプルの容量／流量に対する抽出ストリームの容量／流量の割合に比例する。従って、サンプルの容量／流量に対する抽出ストリームの容量／流量の割合を増加した結果として上昇する拡散効率は、１回ごとに注入され得るサンプルの減少した量（または流量）により相殺される。

図５の装置内で拡散する、相対的に小さい所望の粒子１８の濃度プロフィールを図７に示す。図７において、粒子輸送は拡散によって起こる。曲線２３は、濃度と拡散する粒子の位置との関係を示す。時間は７Ａから７Ｂへ、さらに７Ｃへと経過し、平衡は図７Ｄにおいて起こっている。図７Ｄにおいて、同一濃度の小さい粒子は、２つのストリーム（左および右）内にある。各ストリーム（左と右との両側）の濃度は、初期サンプルストリームの濃度の５０％である。

封鎖材料を含まない装置の場合、生成物ストリームは、サンプルストリームからの所望の粒子を５０％を越えて除去することを達成するためには、このような装置を複数回流れ（ｒｕｎ）なければならない。例えば、総平衡が各流（ｒｕｎ）で達成されると仮定した場合、所望の小さい粒子の約９７％を抽出するためには、サンプルは、このような装置を５回流れ（ｒｕｎ）なければならない。

本発明は、抽出チャネル内に封鎖材料を採用することにより、向上した抽出効率を提供する。封鎖材料は、抽出ストリーム内の所望の粒子の実効濃度を低下させ、それにより所望の粒子の、抽出ストリームへの、より急速で且つ完全な拡散を可能にする。

図６は、本発明の実施形態を示す。暗い斜線領域で示す、相対的に小さい粒子１８と、白丸で示す相対的に大きい粒子１７とを含むサンプルストリームが、サンプルストリーム流入口１を介して導入／注入される。封鎖材料１９を含む抽出ストリーム４は、抽出ストリーム流入口５を介して導入／注入される。２つのストリームは、抽出チャネル７を通って層様に流れ、その間に、濃度勾配の結果、サンプルストリームからの相対的に小さい所望の粒子１８（斜線領域で示す）が抽出チャネルを介して抽出ストリーム４に拡散し、封鎖材料に結合して、所望の粒子に結合する封鎖材料の複合体２１を形成する。さらに、他の勾配、例えば磁気、電気、および遠心力が用いられる。一部分が封鎖材料に結合して複合体２１

を形成する少なくともいくつかの相対的に小さい所望の粒子１８を含む生成物ストリーム１３は、生成物流出口１４から出る。共にサンプルストリームからの、相対的に大きい粒子１７及びおそらくいくつかの相対的に小さい粒子１８を含む副生成物ストリーム１２は副生成物流出口１５から出る。

抽出ストリーム中の封鎖材料の結合定数および量が、抽出ストリーム中の遊離した所望の粒子の濃度を決定する。サンプルストリームからの所望の粒子の拡散は濃度勾配に比例する。所望の粒子に対する結合定数が高い封鎖材料は、封鎖材料の結合サイトが所望の粒子に比べて過剰である場合は、実質的にゼロに等しい有効濃度（または活性）を提供する。従って、所望の粒子は、封鎖材料が飽和状態になるまで抽出ストリームへの拡散を続ける。封鎖材料の飽和後初めて、所望

の粒子の自由濃度が２つのストリーム中で平衡化し始める。（サンプル中の所望の粒子の量に比べて）過剰な封鎖材料の結合サイトを用いる場合は、実質的にすべての所望の粒子が、サンプルから抽出ストリームに抽出される。

封鎖材料の量および所望の粒子に対する結合定数は共に、抽出効率に影響を及ぼす。結合定数が高いほど、抽出はより効率的であり得る。場合によっては、例えば、所望の粒子の抽出後、封鎖材料の非存在下で粒子を分析することが所望される場合には、結合が可逆であることが好ましい。好ましくは、封鎖材料の結合定数は、少なくとも10^{-1}Mまたは10^{-2}Mであり、これは不特定に結合する封鎖材料、例えば活性炭にとっての結合定数の範囲内である。特定タイプの粒子に特異的である封鎖材料の場合には、約10^{-6}Mから約10^{-8}Mの結合定数が好ましい。多くの抗体はこの範囲内の結合定数で抗原に結合する。実質的に不可逆の結合は、10^{-14}Mから10^{-15}Mの範囲の結合定数で生じる。後者の値はビオチンのアビジンに対する結合定数である。「不可逆」の結合でも、例えば反応系の温度、ｐＨおよび溶媒タイプを変えることによって逆転させ得ることは、当業者であれば認識される。このような結合の逆転（解離）は、所望の粒子を封鎖材料の非存在下で分析したい場合に所望の粒子の抽出後に行うのが好ましい。

図６の装置内で拡散している小径の所望の粒子１８の濃度プロフィールを図８に示す。曲線２３は拡散粒子の濃度と位置との関係を示す。図８Ａから図８Ｂ、図８Ｃ、図８Ｄの順に時間の経過を示す。図８Ａでは、所望の粒子１８は、装置の右側のサンプルストリーム中に存在し、封鎖材料１９は装置の左側の抽出ストリーム中に存在する。図８Ｂは、いくらかの量の所望の粒子１８がチャネルを横断して拡散し、封鎖材料１９と結合して複合体２１を形成している状態を示す。図８Ｃは、もっと多くの量の所望の粒子１８がチャネルを横断して拡散し、封鎖材料１９と結合してもっと多くの量の複合体２１を形成している状態を示す。図８Ｄは、さらにもっと多くの量の所望の粒子１８がチャネルを横断して拡散し、封鎖材料１９と結合してさらにもっと多くの量の複合体２１を形成している状態を示す。抽出ストリーム（左側）中の所望の粒子１８の自由濃度は非常に低い状態に維持され、封鎖材料と所望の粒子１８とが強く結合されるこの場合には、実

効上ゼロになる。封鎖材料が過剰に存在し、結合係数が高いことにより、実質的にすべての所望の粒子１８を、サンプルから抽出ストリームへと抽出することができる。

装置の寸法は、抽出チャネル内で層流が維持されるように選択される。上記のように、また図９に示すように、チャネルの流れの方向を長さ（Ｌ）と呼ぶ。長さは約１センチメートル（ｃｍ）から約５センチメートルの間であり得る。長さ（Ｌ）に対して直角の、粒子の移動（抽出チャネルを横断する拡散）の方向のチャネル寸法を、深さ（ｄ）と呼ぶ。深さは、好ましくは約１００マイクロメートル未満であり、より好ましくは約２０マイクロメートルから約５０マイクロメートルの間である。長さおよび深さに対して直角の第３のチャネル寸法を、幅（ｗ）と呼ぶ。図９では、幅寸法は紙面に直交するため示されていない。幅は約１メートルまでまたはそれ以上であり得、５００マイクロメートル、１ｍｍ、５ｃｍ、および１／２メートルを含む。幅は、封鎖材料を含むストリーム中のすべての粒子が通過し得るのに十分な大きさである。幅が長いと、装置内で処理される容量を大きくすることができる。拡散方向（深さ）が層流を維持するのに十分な狭さであり、長さが効果的な拡散が起こり得るのに十分な長さであるならば、幅は、例えば１メートル以上のように極めて長くすることができる。

以下の少なくとも２つの構成のうちの一方を用いれば、本発明の装置によって大きな容量のサンプルを処理することができる。第１の構成は、上述のように、装置の幅を長くして、装置が大容量の流体を保持するようにすることである。第

２の構成は、複数の、すなわち２つ以上の装置を並列に接続して、各装置がそれぞれサンプルの一部を同時に処理するようにすることである。図１０は、いくつかの抽出装置を並列に配置した構成を示す。各装置のサンプルストリーム流入口１は、サンプルコネクタ２７を介してサンプル多岐ライン２４に流体接続し、各装置の抽出ストリーム流入口５は、抽出コネクタ２６を介して抽出多岐ライン２５に流体接続している。副生成物ストリームは副生成物流出口ライン２８を介して退出し、生成物ストリームは生成物流出口ライン２９を介して退出する。副生成物流出口ライン２８はすべて、１つのレザバーに接続されこれに流入し得る。

生成物流出口ライン２９はすべて、別の単一のレザバーに接続され、これに流入し得る。図１０では、これらの装置は幅が深さおよび長さより長いように示されている。このように比較的長い幅は選択的なものであり、いくつかの装置を並列に接続することに加えて、またはこれの代わりに用いて、単位時間で処理されるサンプル容量を増大させることができる。

粒子の分離を向上させるために、複数の装置を直列で接続し得る。すなわち、図１１に示すように、現在の生成物ストリームを別の装置のサンプルストリーム流入口と流体接続させる。図１１では、各抽出装置は参照番号１００で示される。副生成物ストリーム１２は各装置を退出し、生成物ストリーム１３は、直列接続された次の装置のサンプルストリームとなる。

図１２は、本発明の別の実施形態、「フラット抽出装置」の斜視図であり、図１３はその平面図である。この装置では、抽出チャネル７での拡散方向は、図１～図３、図５および図６に示した実施形態とは９０°回転した方向である。この実施形態は、処理され得る材料の容量が抽出チャネル７の幅によって制約されることはないという利点を提供する。

図１２および図１３のフラット抽出装置は、シリコン基板３４をエッチングすることによって作製され、サンプルストリーム流入口溝３５、抽出ストリーム流入口溝３６、生成物ストリーム流出口溝３７，および副生成物ストリーム流出口溝３８、ならびに抽出チャネル７が配備される。ガラスカバー３３により抽出チャネル７が閉鎖される。図１２では、サンプルストリーム流入口１に向かって下向きの矢印は、サンプルストリーム２の流れを示す。同様に、抽出ストリーム流

入口５に向かって下向きの矢印は、抽出ストリーム４の流れを示す。生成物流出口１４から上向きの矢印は、生成物ストリーム１６の流れを示し、副生成物流出口１５から上向きの矢印は、副生成物ストリーム１２の流れを示す。抽出チャネル７の長さはＬで示され、チャネルの幅は、ｗとして黒い矢印で示される。抽出チャネル７の深さはｄで示される。図１３に示す、開口部を有するカップリング多岐管３２は、サンプルストリーム流入口溝３５の深さを延びてサンプルストリームチャネル３およびサンプルストリーム流入口１を形成し、抽出ストリーム流

入口溝３６の深さを延びて抽出ストリームチャネル６および抽出ストリーム流入口５を形成し、生成物ストリーム流出口溝３７の深さを延びて生成物流出口チャネル１１および生成物流出口１４を形成し、そして副生成物ストリーム流出口溝３８の深さを延びて副生成物流出口チャネル１０および副生成物流出口１５を形成する。

図１３に示す、拡散（濃度勾配）によって動作するフラット抽出装置では、左上の矢印によって示されるサンプルストリーム２がサンプルストリーム流入口１に入り、サンプルストリームチャネル３内を流れる。抽出ストリーム４は、抽出ストリーム流入口５に入る矢印によって示され、抽出ストリーム流入口チャネル６内を流れる。サンプルストリーム２は、抽出チャネル７内で層状サンプルストリーム８として層状抽出ストリーム９の下を流れる。層状サンプルストリーム８は、抽出チャネル７内で長さＬにわたって層状抽出ストリーム９と接触する。層状抽出ストリーム９内の斑点によって示される層状サンプルストリーム８からの小径の（「所望の」）粒子１８は、生成物ストリーム１３として生成物流出口チャネル１１へと流れ、上向きの矢印によって示されるように生成物流出口１４で退出する。副生成物ストリーム１２は、生成物ストリーム１３を過ぎた後の層状サンプルストリーム８から連続する流れである。副生成物ストリーム１２は大径の（「望まれない」）粒子と、生成物ストリーム１３へと拡散しなかった小径の（「所望の」）粒子の一部とを含む。副生成物ストリーム１２は、副生成物流出口チャネル１０を通って副生成物流出口１５から退出する。

上述のように、本発明の装置は、血液透析のために用いられ得る。以下に、本発明の装置の設計に際して考慮される点について指摘し、また血液から尿素を除去する例およびこのような手順の様々な詳細を提供する。

本発明の抽出装置（Ｈフィルタの実施形態でもフラットフィルタの実施形態でもよい）を血液透析器として用いる場合、血液はシャントを介して装置に供給される。装置内の流速F_Fは、問題の所望の粒子の拡散速度（好ましくは、封鎖材料を用いることによって向上した速度）および装置自体の形状によって決定される。装置の抽出効率は、部分的には、粒子の拡散に必要な時間に依存し、これが

全体的な装置の最大流速を決定する。

尿素の拡散係数は１１．８×１０$^{-6}$ｃｍ^{2}／ｓである。先ず、拡散が向上した吸収ではない、すなわち封鎖材料が用いられていない状況を考慮する。この例で用いられる以下の変数、および関連する仮定は以下の通りである。

M_U：体内の尿素の全質量。この適用では生成および排泄は数学的には考慮されないが、これらの影響については検討される。この値は既知である（透析患者では容易に測定可能である）と仮定する。

V_B：全血液量（任意の瞬間におけるシャント内の量を含む）。この量は、典型的には、大人では５～６Ｌ、子供では２～３Ｌである。

Ｅ：装置の抽出効率。封鎖材料が存在しない場合、最終の出力ストリームは均衡状態にあるため、これは０．５である。装置は、均衡が可能であるように設計される（すなわち、接触時間が所定の形状にとって十分な長さであるように流速は十分に低くされる）。

本発明者らはM_Uがどのように経時変化するかに興味がある。流入した尿素の半分は装置を通過することにより除去されることは分かっているが、体内の全量は一定して減少するため、M_Uの値を決定することは困難である。このことにより、または基本的にフィックの拡散の第二法則を解かなければならないという事実により、解は指数関数的な減衰であると予想する。１つの最終的で決定的な仮定は、体内に戻る血液は、血液の残りの血液とうまく混合されるということである。心臓の力強い活動により、これは有効な仮定である。

M_Uの一般式は以下の通りである。

$$M_U = M_{U(o)}e^{-kt}$$

時間に対する導関数をとると、以下の式が得られる。

$$\frac{dM_U}{dt} = -kM_{u(o)}e^{-kt}$$

時間＝０でのこの導関数は以下のようになる。

$$\frac{dM_U}{dt}\Big|_{t=0} = -kM_{U(o)}$$

このタイプの問題では典型的であるように、ｋは初期質量と初期活動量との間の比率である。初期質量は既知であるため、ｋは初期量に対する式を見いだすことによって決定され得る。装置内の流量$Ｆ_{F}$は、体内からの容量が装置内で処理される量を表す。尿素の初期濃度で乗算することによって、初期処理量（所望の粒子の初期抽出量）が正しい単位（質量／時間）で与えられる。抽出効率、およびこの量は除去を表すため負の記号でなければならないことを考慮すると、初期量は以下のように示され得る。

$$\frac{dM_{U(o)}}{dt}\Big|_{t=0} = -\left(\frac{M_{U(o)}}{V_B}\right)F_F E$$

ここで、括弧内の項は初期濃度である。初期状態の式に代入すると、ｋについての式がでる。

$$k = \left(\frac{F_F}{V_B}\right)E$$

そして、質量の式に代入すると、以下の式が得られる。

$$M_U = M_{U(o)}e^{-\left[\left(\frac{F_F}{V_B}\right)E\right]t}$$

係数ｔの増加はより速い減衰を意味し、これは、系からの除去が速いことを意味する。装置内の流量$Ｆ_{F}$を高くすると、血液はより速く処理され得るため、尿素の除去が速くなる。抽出効率を高くしても尿素の除去が速くなる。何故なら、装置を通過する容量が同じならばより十分に洗浄されるため、すなわちより多くの尿素が抽出されるためである。これらの予測は共に式と整合する。全血液量を増やすと、除去が遅くなる。何故なら、尿素の濃度ががより薄くなり、同じ除去を実現するためにはより多くの容量を処理しなければならないからである。この予測もまた式と整合する。

封鎖材料を用いない上記の例を考慮すると、尿素に特異的な平衡条件により、設計にいくつかの制約が課せられる。好ましくは、装置の拡散寸法ｄは可能な限

り短くされる。これにより、拡散時間が短くなり、流量の上限が高くなる。しかし、この寸法は、赤血球（直径約８μｍ）によりチャネルが閉塞される可能性によって制約され得るため、サンプルが全血である場合には、一般に少なくとも約１００μｍである。従って、平衡のために拡散分子が移動しなければならない平均距離は、この値の半分、すなわち５０μｍである。以下のブラウン運動の式を考慮されたい。

$$\frac{(\Delta x)^2}{\Delta t} = 2D$$

ここで、Ｄは拡散係数であり、これは尿素では１１．８×１０$^{-6}$ｃｍ^2／ｓである。平均拡散時間に対して解くことにより、Δｔ＝１．０６ｓの値が得られる。これは、２つのストリームの接触時間の下限である。以下の計算のために、装置の長さＬを１０ｍｍとする。この場合には、流体は１０ｍｍを１．０６秒以上で移動しなければならず、これにより以下の最大平均速度が得られる。

$$\bar{V} = \frac{L}{\Delta t} = \frac{10mm}{1.06s} = 9.434\frac{mm}{s}$$

流量は、平均速度と断面積との積である。血液が抽出ストリームと接触するのに必要な時間量を決定するものであるため、血液を導入しているチャネルの半分のみが考慮される。

Ｈフィルタの実施形態と、フラットフィルタの実施形態との基本的な相違点は、幅寸法ｗである。Ｈフィルタでは、基板がシリコンであれば、幅は、好ましくは約５０μｍである。フラットフィルタでは、上記のように、幅は、理論的には無限であり、約１メートルの幅が企図される。

以下、Ｈフィルタの実施形態およびフラットフィルタの実施形態の両方における、封鎖材料を使用しない血液透析のために必要な接触時間と、必要な接触時間を低減するために並列で必要とされる装置の数とを比較する。そして、比較のために、様々な抽出効率の効果を与える。

ｗ＝５０μｍであるＨフィルタの実施形態では、流量は以下のようになる。

$$F_F = \dot{\overline{V}}_W \frac{d}{2} = \left(9.434\frac{mm}{s}\right)\left(\frac{1x10^3\mu m}{mm}\right)(50\mu m)\left(\frac{100\mu m}{2}\right) = 2.358x10^7\frac{\mu m^3}{s}$$

標準単位に変換すると、流量は２．３５８×１０$^{-5}$ｍｌ／ｓとなる。この値は、上記の質量除去方程式に代入することができる。その方程式をわずかに並べ替えたものが有用である。

$$\frac{M_U}{M_{U(o)}} = e^{-\left[\left(\frac{F_F}{V_B}\right)E\right]t}$$

今、左側の項は、残っている画分（ＦＲ；現在の質量を初期質量で割ったもの）を表す。目標画分を選択することができ、必要な接触時間を計算することができる。一般に、そのような指数関数的なプロセスの場合の目標は、９９％の完全さであり、これは、０．０１ＦＲに対応する。典型的な成人について上記値のＦ$_F$、Ｅ＝０．５およびＶ$_B$＝５Ｌを使用すると、時間は、１．９５３×１０^{9}秒、即ち、６１．９年である。あるいは、４時間の目標時間（典型的な血液透析期間に基づく推定値）を選択することができ、必要なＦ$_F$を計算することができる。多数のＨフィルタは、並列に接続され得る。本願の１つの装置の流量で割ると、必要なＨフィルタの数が決定される。４時間の接触時間の場合、３．１９８ｍｌ／ｓのＦ$_F$が必要とされる。本願の１つの装置の流量である２．３５８×１０$^{-5}$ｍｌ／ｓで割ると、１３０，０００個を上回る並列のＨフィルタが必要とされることが予測される。これは、Ｈフィルタ抽出装置において封鎖材料を使用し、抽出効率を増加させるという利点を示す。

比較のために、２０，０００のファクタでＨフィルタを効果的にスケールする１メートルのフラットフィルタ幅の場合を考える（封鎖材料は使用しない）。これにより、１つの装置の流量Ｆ$_F$が、０．４７１６ｍｌ／ｓ、即ち２８．３０ｍｌ／分、即ち１．７Ｌ／時間に増加する。５リットルは、「連続的な」容量であるとは考えられないため、単に１．７リットルを５つに分けだけでは処置時間を決定することができない。従って、質量方程式、および０．０１の目標ＦＲに戻らなければならない。フラットフィルタの流量、Ｅ＝０．５、および５リットル

の血液容量を用いた場合、必要な接触時間（処置時間）は９７，６５０秒、即ち、２７．１時間である。並列の幾つかのフラットフィルタを使用すると、必要な接触時間を低減することができる。４時間の目標時間では、必要な流量は３．１９８ｍｌ／ｓである（Ｈフィルタの場合と同じ）。これにより、７個の並列のフラットフィルタが必要であると予測される。あるいは、幅７メートルの１つのフラットフィルタが考えられる。しかし、これは、設計の観点からあまり好ましくないと思われる。（封鎖材料を使用せずに）多数の並列のフラットフィルタを使用した場合の効果は、図１４に示される。並列のフラットフィルタの数が増加するに従って、残っている尿素の画分は減少している。

総血液容量V_Bは、質量除去方程式の重要な因子である。患者の血液容量は、幼児から小児、そして成人の範囲で、現実的には１リットル～６リットルの範囲であり得る。（封鎖材料を使用せずに）７個のフラットフィルタを並列で使用した場合の総血液容量の影響は、図１５に示される。総血液容量が減少するに従って、残っている尿素の画分は減少している。

図１６は、７個のフラットフィルタを並列で使用した場合に抽出効率を増加する効果を示す。封鎖材料の抽出効果が増加するに従って、残っている尿素の画分は減少している。

フラットフィルタの実施形態では、１０ｍｍよりも大きい抽出チャネル長を選択することが好ましい場合がある。好適な実施形態は、長さ５０ｃｍ、幅５０ｃｍの実施形態である。この場合、フィルタは、現在の血液透析機に匹敵する大きさの正方形となる。さらに、このフィルタは、可能な最大流量を２５のファクタだけ増大し、これにより、０．０１の画分時間が、２７．１時間から１．１時間に低減される。従って、高い流量で細胞が剪断される可能性を考慮しなければならない。

抽出効率が１．０未満である場合を考えると、一般に示唆される考えは、多数のフラットフィルタを並列ではなく直列に配置することである。Ｅ＝０．５の場合を考える。２つのフラットフィルタを並列に配置すると、Ｅは０．７５に増加される。これは、もとの尿素の４分の１しか残らないからである。時定数が減少した場合を考えると、この場合、改善度は１．５である。しかし、２つの同じ装

置（それぞれ、Ｅ＝０．５）を並列に接続すると、流量が２倍になっているため、改善度は２．０である。従って、抽出効率を増加するためには、多数の装置を、直列ではなく並列に接続することが好ましい。

ここで言及される実施形態の他に、多数の実施形態が当業者に容易に明らかとなり、これらの実施形態は、本発明の範囲内である。本明細書で引用されたすべての文献は、その全体が、本明細書において、参考として援用される。以下の実施例は本発明を示しているが、本発明をいかなる方法でも限定するものではない。

実施例

実施例１

当該分野において公知の技術（BrodyおよびYager、Solid State Sensor and Actuator Workshop、サウスカロライナ州、ヒルトンヘッド（Hilton Head）、1996年6月2〜6日）を用いてシリコンウェハをエッチングすることにより、抽出装置を作った。チャネル長は約１００μｍであり、チャネルの深さ（拡散の大きさ）は約１５μｍであり、チャネル幅は約１０μｍであった。ＦＩＴＣ（フルオレセイン）により標識されたビオチン（Sigma Chemical #B8889）（０．５μｇ／ｍｌ）を蒸留水中に入れたものを、サンプルストリーム流入口に導入した。ローダミンにより標識されたアビジン（Sigma Chemical #A3026）（１６０μｇ／ｍｌ）を蒸留水中に入れたものを、抽出ストリーム流入口に導入した。抽出チャネルを通る流量は、約１００ピコリットル／秒であった。約１ｍｇのアビジンが１０〜１５μｇのビオチンと結合することが、当業者に知られている。ローダミンで標識されたアビジンが、流量よりも実質的に低いレートで移動しているのが観察され、このアビジンが、チャネル、流入口、および流出口の壁に付着していると判断した。

このアビジンの壁への付着を妨げるために、装置の壁をアビジンの単層で均一にコーティングするために必要とされるアビジンの量を計算した。装置の容量／表面積比は、約１０μｍであった。すべてのアビジンが壁に吸収されると仮定した場合、１ｍｇ／ｍｌのアビジン溶液が、容量／表面積比が約１０μｍの装置の壁を覆うために必要とされる最小量として計算された。（装置の内部容量を計算

すると１５ピコリットルであり、従って、これが、装置を充填し、且つ、壁をコーティングするために必要とされる溶液の容量である。）蒸留水とアビジンとの溶液（０．１７ｍｇ／ｍｌ）（１ｍｇ／ｍｌの溶液の濃度の１／６である）を、６アリコートで装置に導入した。アビジンでコーティングされたフロント（front）は、平均流量の約１／６のレートで移動しており、アビジンが壁に付着していることを示している。

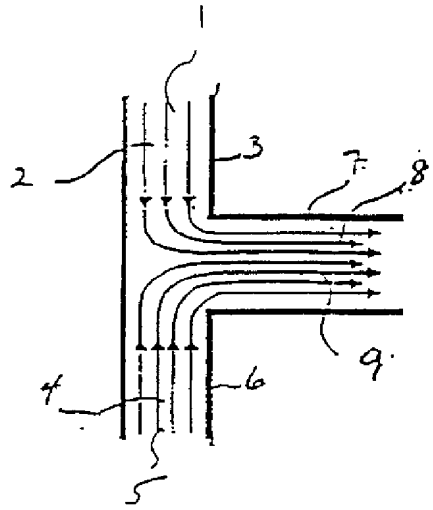
実施例２

実施例２では、壁がアビジン（実施例１で調製）で実質的に完全に且つ均一にコーティングされた装置を使用した。１μｍの酸化鉄粒子（Sigma Chemical #S2415）に固定化されたストレプトアビジンと蒸留水との溶液（１０μＬ）を、抽出ストリーム流入口に導入した。ビオチン（Sigma Chemical #B8889）と蒸留水との溶液（１０ｎｇ／ｍｌ）を、サンプルストリーム流入口に導入した。抽出ストリームにおいてビオチンが濃縮されたことは裸眼で明らかであった。しかし、酸化鉄に固定化されたストレプトアビジンの分子数が少なかったため、蛍光性は低かった。従って、化学量論的な過剰のストレプトアビジンを維持するためには、低いビオチン濃度が必要とされる。さらに、ストレプトアビジンは、蛍光マーカー（ＦＩＴＣ）を部分的にクエンチするため、蛍光測定をより困難にしている。

蛍光測定を向上するために、装置には、表面パッシベーションを達成するように、ポリエチレングリコールシランが予め装填される。ビオチンのための結合部位の前に、より長いアームを有するストレプトアビジンは、蛍光のクエンチを防止する助けとなる。

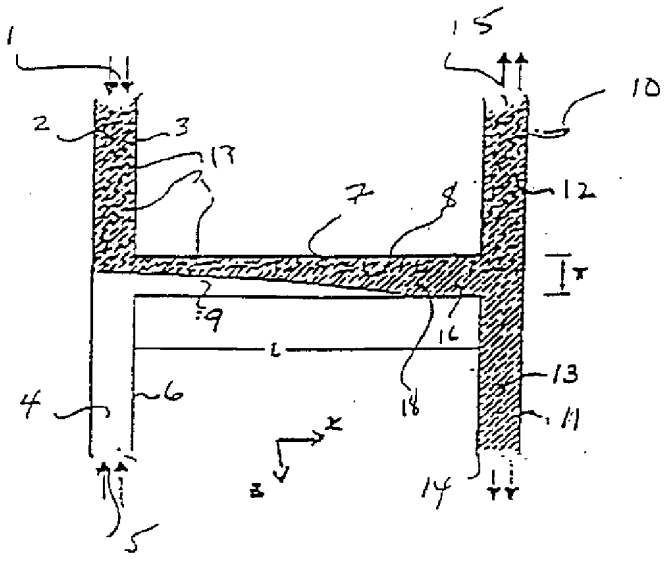
【図１】

Fig. 1



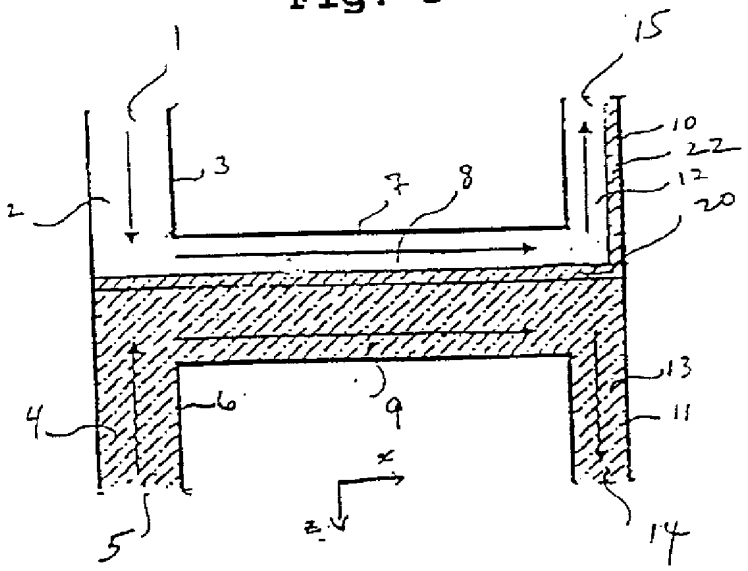
【図２】

Fig. 2



【図３】

Fig. 3



【図４】

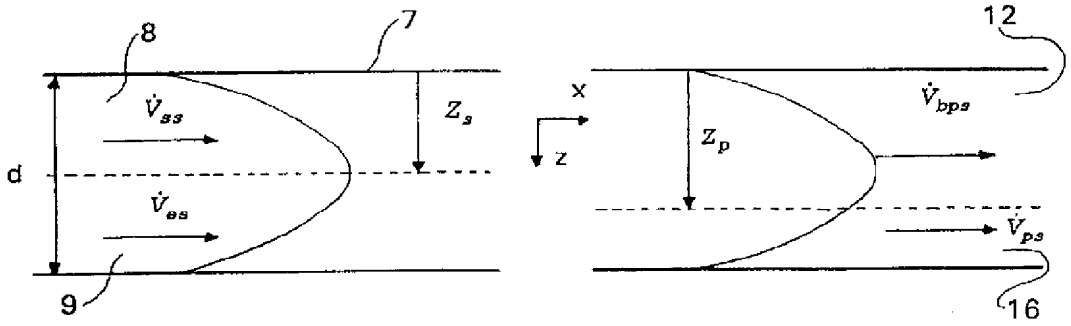


Fig. 4

【図５】

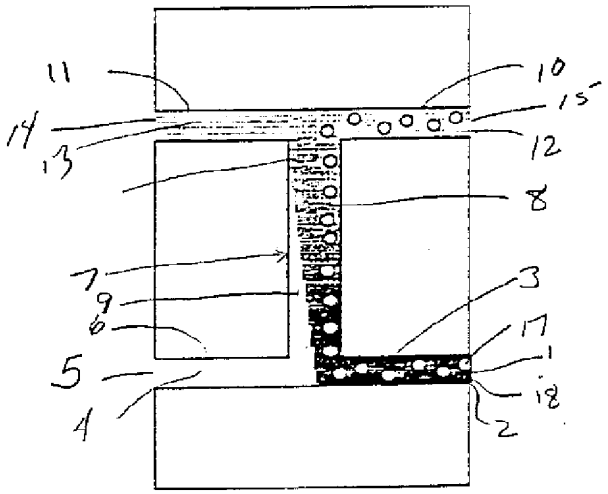


Fig. 5

【図６】

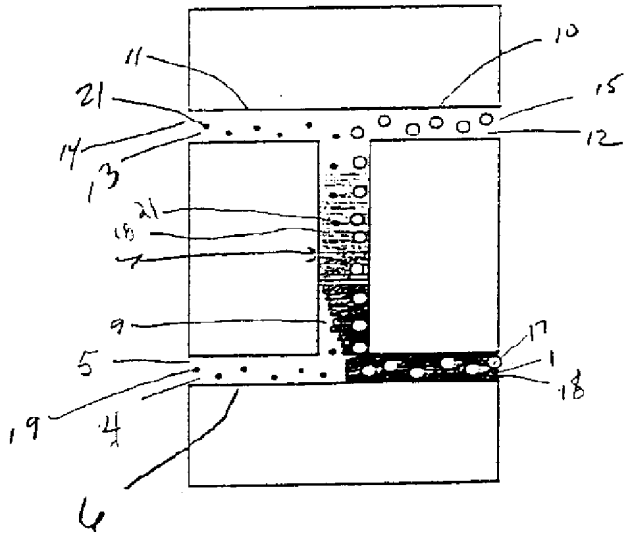


Fig. 6

【図７】

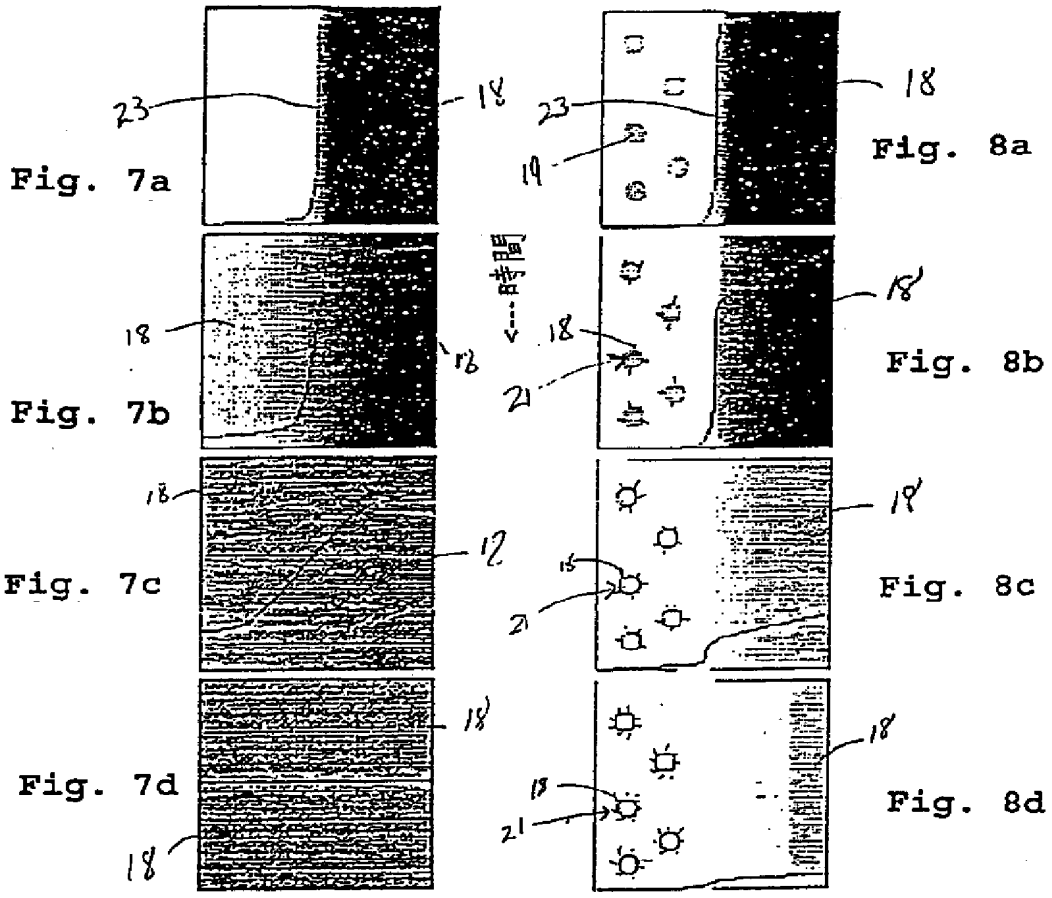


Fig. 7a

Fig. 7b

Fig. 7c

Fig. 7d

Fig. 8a

Fig. 8b

Fig. 8c

Fig. 8d

【図８】

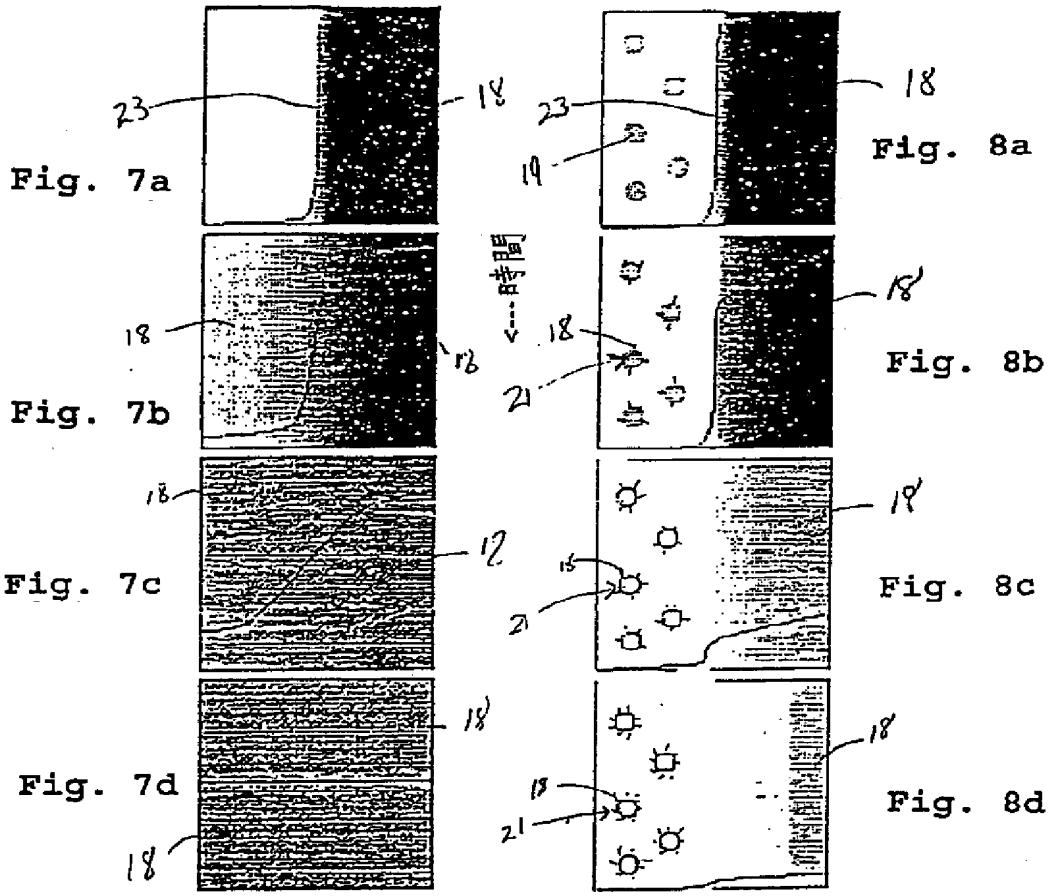


Fig. 7a

Fig. 8a

Fig. 7b

Fig. 8b

Fig. 7c

Fig. 8c

Fig. 7d

Fig. 8d

【図９】

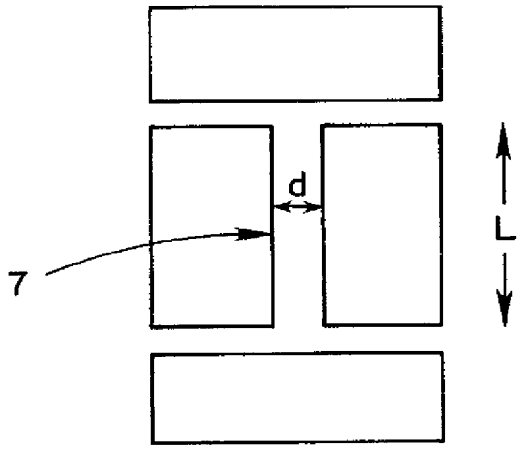


Fig. 9

【図１０】

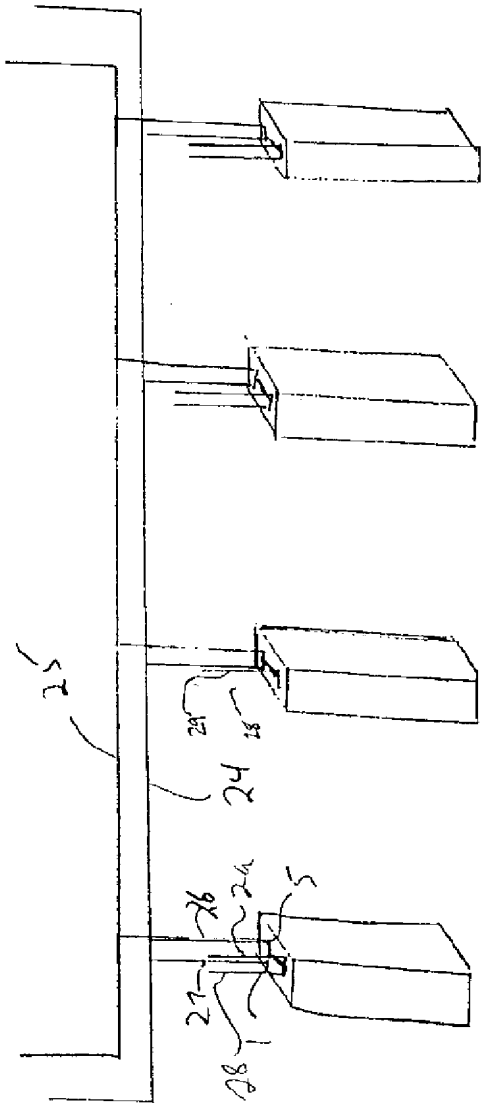


Fig. 10

【図１１】

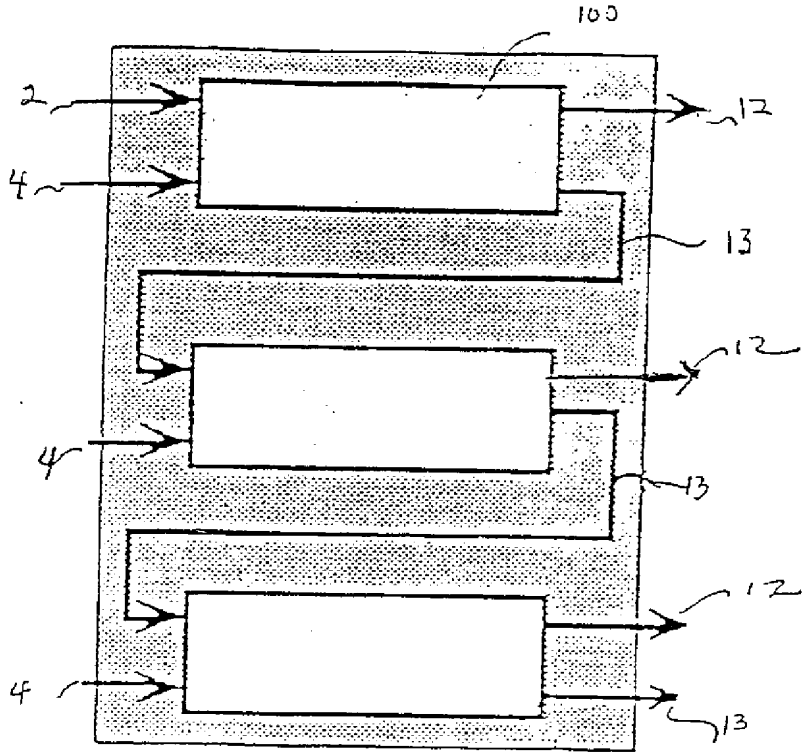


Fig. 11

【図１２】

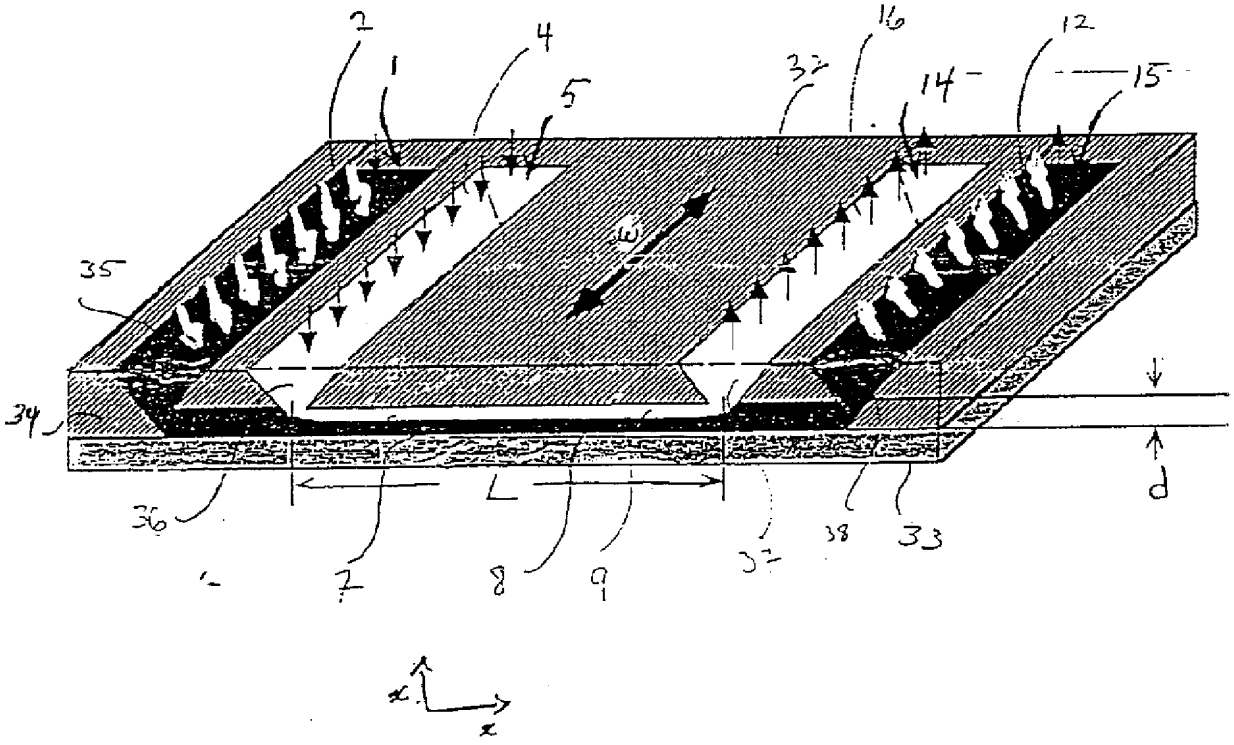


Fig. 12

【図１３】

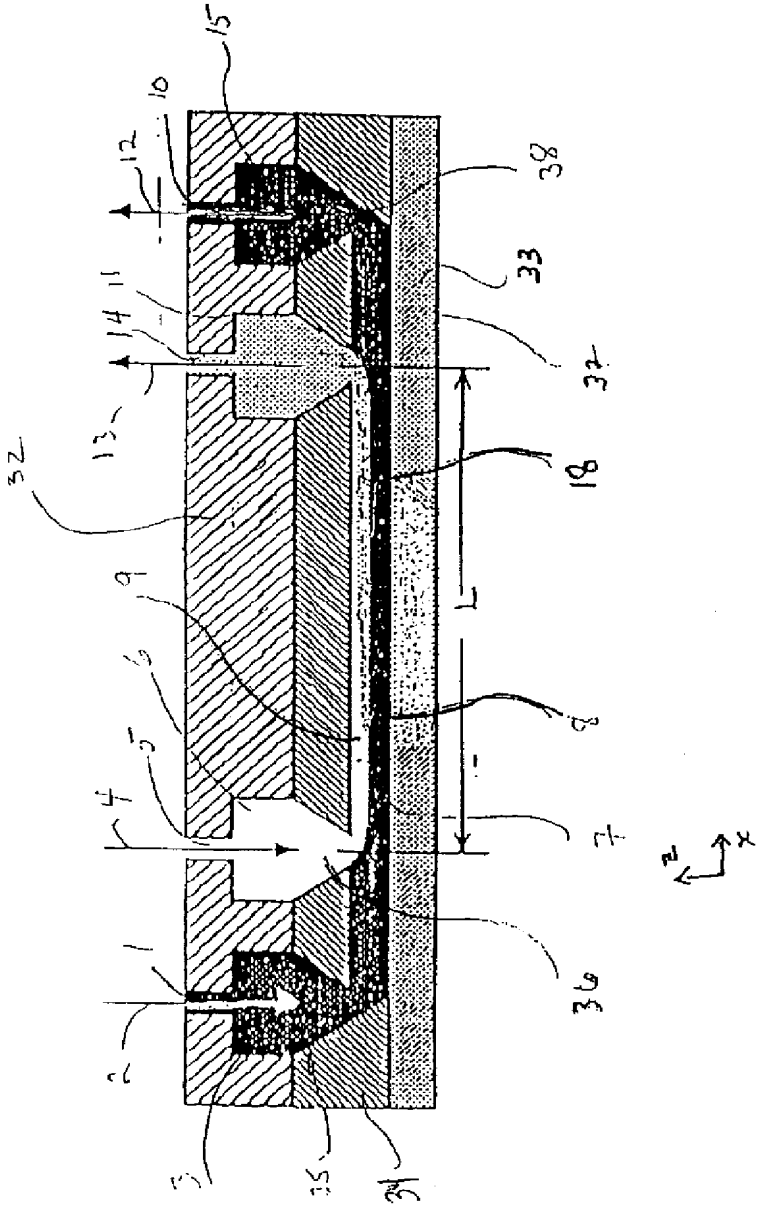


Fig. 13

【図１４】

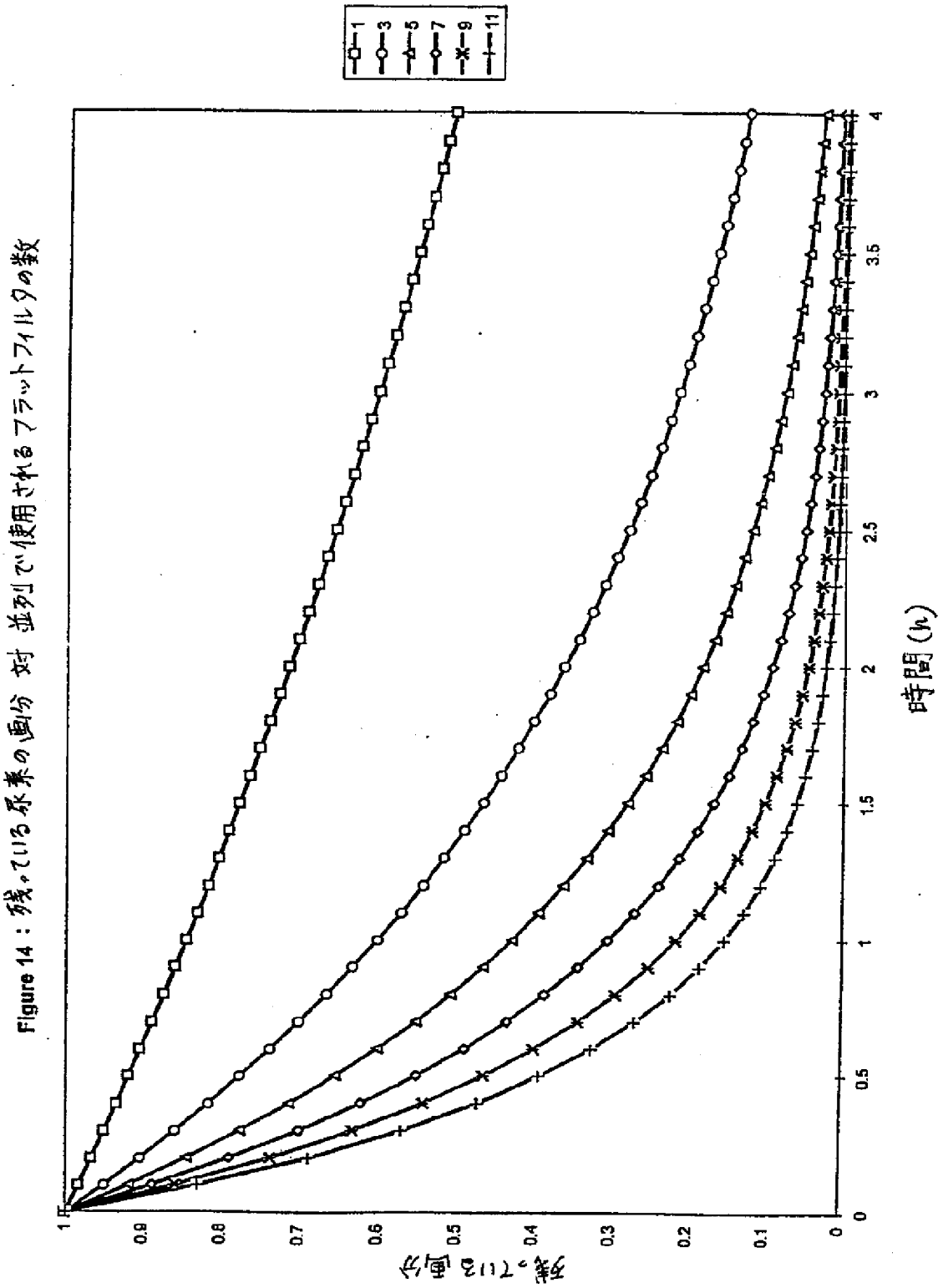


Figure 14：残っている系の画分 対 並列で使用されるフラットフィルタの数

【図１５】

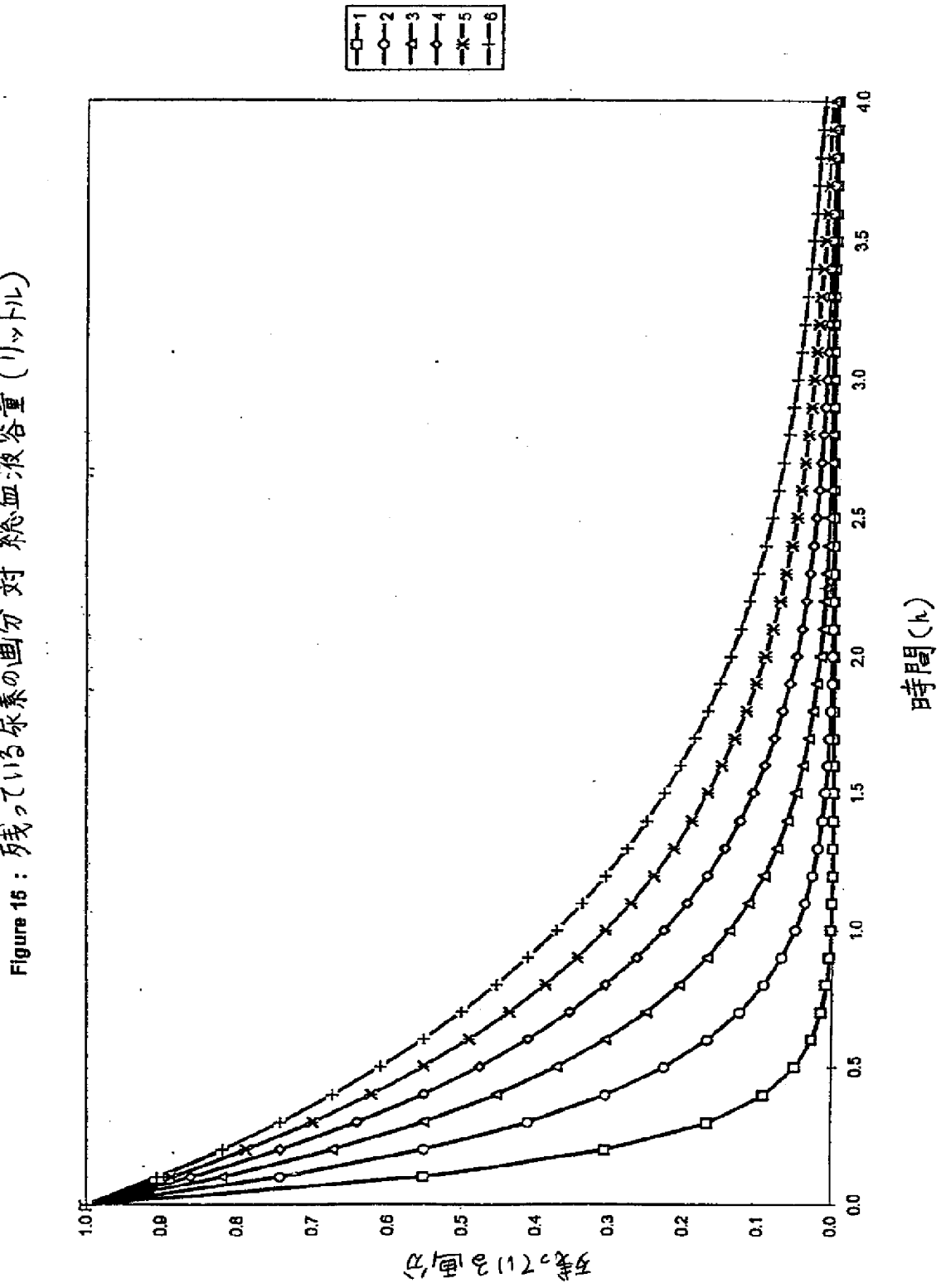


Figure 15：残っている尿素の画分 対 総血液容量（リットル）

【図１６】

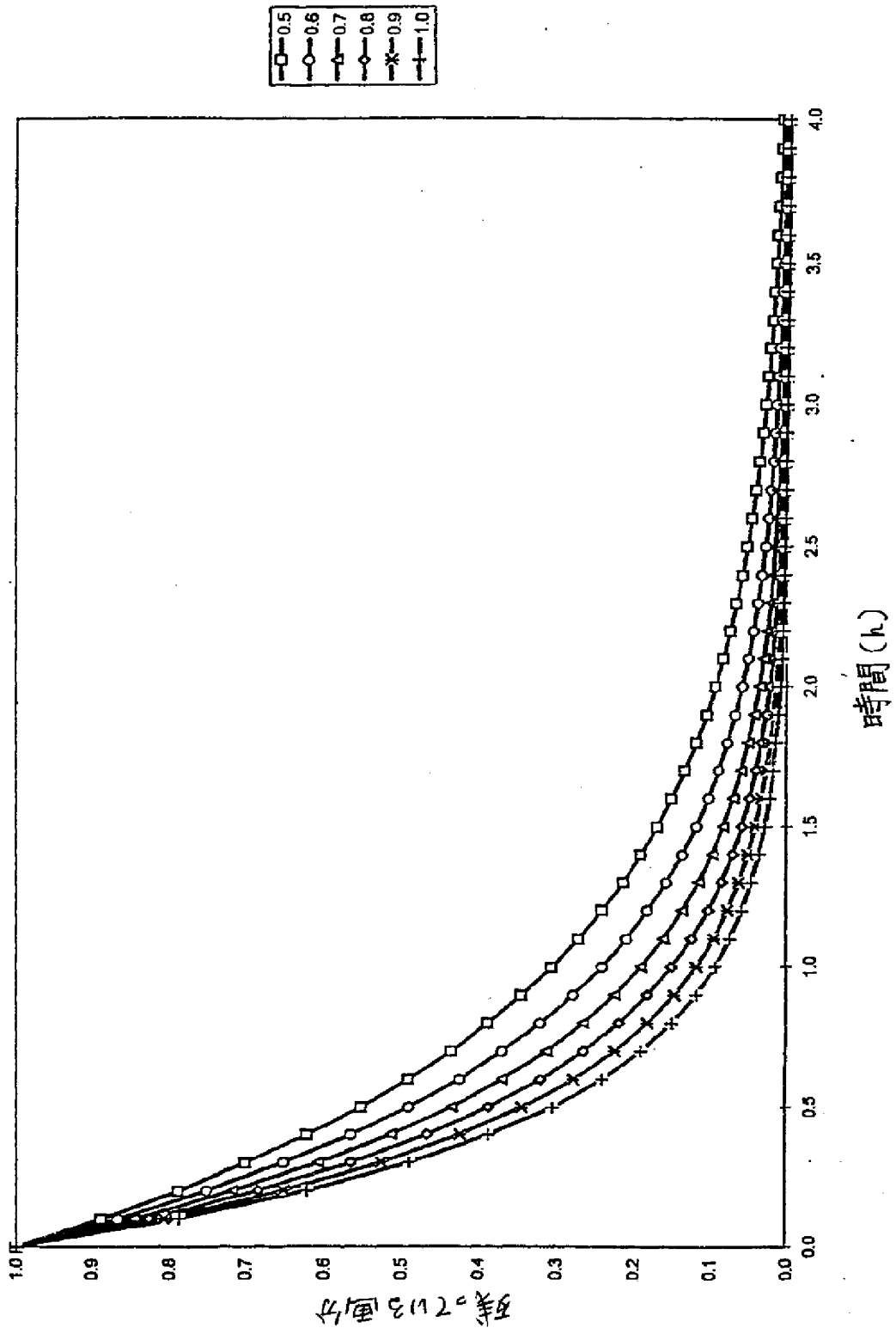


Figure 16 : 残っている尿素の画分 対 抽出効率

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/10307

A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)

U.S. : 209/2, 12.1, 18, 155, 208, 209, 210, 458; 210/656, 659, 723, 726, 198.2, 198.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 5,039,426 A (GIDDINGS) 13 August 1991	
A	US 4,894,146 A (GIDDINGS) 16 January 1990	
A	US 4,737,268 A (GIDDINGS) 12 April 1988	
A	US 4,830,756 A (GIDDINGS) 16 May 1989	
A	US 3,449,938 A (GIDDINGS) 17 June 1969	

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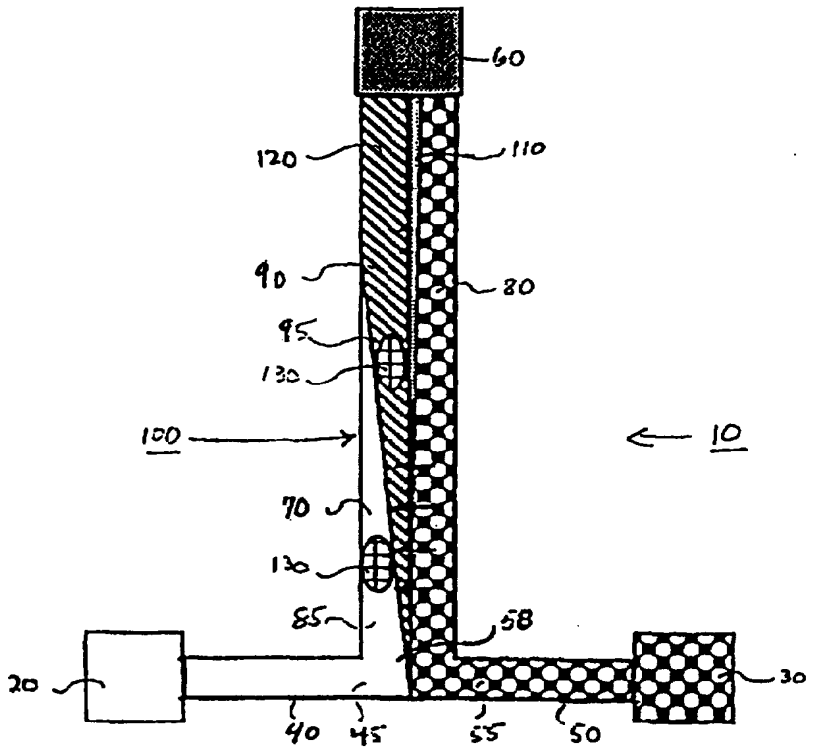
(54) Title: MICROFABRICATED DIFFUSION-BASED CHEMICAL SENSOR

(57) Abstract

A channel-cell system is provided for detecting the presence and/or measuring the presence of analyte particles in a sample stream comprising a laminar flow channel (100), two inlet means (30, 20) in fluid connection with said laminar flow channel (100) for respectively conducting into the laminar flow channel (100) an indicator stream (70) which may comprise an indicator substance which indicates the presence of said analyte particles by a detectable change in property when contacted with said analyte particles, and the sample stream (80), wherein the laminar flow channel (100) has a depth sufficiently small to allow laminar flow of the streams and a length sufficient to allow particles of the analyte to diffuse into said indicator stream (70) to the substantial exclusion of said larger particles in the sample stream (80) to form a detection area; and outlet means (60) for conducting the streams out of the laminar flow channel (100) to form a single mixed stream.



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MICROFABRICATED DIFFUSION-BASED CHEMICAL SENSOR

This invention was made with government support under Army research contract DAMD17-94-J-4460 awarded by the U.S. Army. The government has certain rights in the invention.

FIELD OF THE INVENTION

This invention relates generally to microsensors and methods for analyzing the presence and concentration of small particles in streams containing both these small particles and larger particles by diffusion principles. The invention is useful, for example, for analyzing blood to detect the presence of small particles such as hydrogen, sodium or calcium ions in a stream containing cells.

BACKGROUND OF THE INVENTION

In Maxwell's famous gedanken (thought) experiment, a demon operates a door between two boxes of gas at the same temperature. The demon sorts the molecules keeping the faster molecules in one box and the slower in the other, violating the basic laws of thermodynamics. This paradox has since been resolved in many different ways. Leff, H.S. and Rex, A.F. (1990), "Resource letter md-1: Maxwell's demon," Am. J. Physics 58:201-209.

A similar arrangement can be used to separate particles. Consider a mixture of particles of two different sizes suspended in water in one box and pure water in the other. If the demon opens and closes the door between the boxes quickly enough so that none of the larger particles have time to diffuse through the doorway, but long enough so that some of the smaller particles have enough time to diffuse into the other box, some separation will be achieved.

Recently two experiments have been done where a spatially asymmetric potential is periodically applied in the presence of a number of brownian particles. Faucheux, L.S., et al. (1995), "Optical thermal ratchet," Physical Rev. Letters 74:1504-1507; Rousselet, J., et al. (1994), "Directional motion of brownian particles induced by a periodic asymmetric potential," Nature 370:446-448.

This has been shown to lead to a directed motion of the particles at a rate depending on the diffusion coefficient. One experiment (Rousselet, J., et al. (1994), "Directional motion of brownian particles induced by a periodic asymmetric potential," Nature 370:446-448) used microfabricated electrodes on a microscope slide to apply an electric field for the potential. This idea is also the subject of European Patent Publication 645169 of March 29, 1995, for "Separation of particles in a fluid using a saw-tooth electrode and an intermittent excitation field," Adjari, A., et al. The other experiment (Faucheux, L.S., et al. (1995), "Optical thermal ratchet," Physical Rev. Letters 74:1504-1507) used a modulated optical tweezer arrangement.

Diffusion is a process which can easily be neglected at large scales, but rapidly becomes important at the microscale. The average time t for a molecule to diffuse across a distance d is $t = d^2/D$ where D is the diffusion coefficient of the molecule. For a protein or other large molecule, diffusion is relatively slow at the macro-scale (e.g. hemoglobin with D equal to 7×10^{-7} cm^2/s in water at room temperature takes about 10^6 seconds (ten days) to diffuse across a one centimeter pipe, but about one second to diffuse across a ten micron channel).

Using tools developed by the semiconductor industry to miniaturize electronics, it is possible to fabricate intricate fluid systems with channel sizes as small as a micron. These devices can be mass-produced inexpensively and are expected to soon be in widespread use for simple analytical tests.

A process called "field-flow fractionation" (FFF) has been used to separate and analyze components of a single input stream in a system not made on the microscale, but having channels small enough to produce laminar flow. Various fields, including concentration gradients, are used to produce a force perpendicular to the direction of flow to cause separation of particles in the input stream. See, e.g. Giddings, J.C., U.S. Patent 3,449,938, June 17, 1969, "Method for Separating and Detecting Fluid Materials;" Giddings, J.C., U.S. Patent 4,147,621, April 3, 1979, "Method and Apparatus for Flow Field-Flow Fractionation;" Giddings, J.C., U.S. Patent 4,214,981, July 29, 1980), "Steric Field-Flow Fractionation; "Giddings, J.C., et al., U.S. Patent 4,250,026, February 10, 1981,

"Continuous Steric FFF Device for The Size Separation of Particles;" Giddings, J.C., et al., (1983), "Outlet Stream Splitting for Sample Concentration in Field-Flow Fractionation," Separation Science and Technology 18:293-306; Giddings, J.C. (1985), "Optimized Field-Flow Fractionation System Based on Dual Stream Splitters," Anal. Chem. 57:945-947; Giddings, J.C., U.S. Patent 4,830,756, May 16, 1989, "High Speed Separation of Ultra-High Molecular Weight Polymers by Hyperlayer Field-Flow Fractionation;" Giddings, J.C., U.S. Patent 4,141,651, August 25, 1992, "Pinched Channel Inlet System for Reduced Relaxation Effects and Stopless Flow Injection in Field-Flow Fractionation; "Giddings, J.C., U.S. Patent 5,156,039 October 20, 1992, "Procedure for Determining the Size and Size Distribution of Particles Using Sedimentation Field-Flow Fractionation;" Giddings, J.C., U.S. Patent 5,193,688, March 16, 1993, "Method and Apparatus for Hydrodynamic Relaxation and Sample Concentration in Field-Flow Fraction Using Permeable Wall Elements;" Caldwell, K.D. et al., U.S. Patent 5,240,618, August 31, 1993, "Electrical Field-Flow Fractionation Using Redox Couple Added to Carrier Fluid;" Giddings, J.C. (1993), "Field-Flow Fractionation: Analysis of Macromolecular, Colloidal and Particulate Materials," Science 260:1456-1465; Wada, Y., et al., U.S. Patent 5,465,849, November 14, 1995, "Column and Method for Separating Particles in Accordance with their Magnetic Susceptibility." None of these references disclose the use of a separate input stream to receive particles diffused from a particle-containing input stream.

A related method for particle fractionation is the "Split Flow Thin Cell" (SPLITT) process. See, e.g., Williams, P.S., et al. (1992), "Continuous SPLITT Fractionation Based on a Diffusion Mechanism," Ind. Eng. Chem. Res. 31:2172-2181; and J.C. Giddings U.S. Patent 5,039,426. These publications disclose channel cells with channels small enough to produce laminar flow, but again only provide for one inlet stream. A further U.S. patent to J.C. Giddings, U.S. Patent No. 4,737,268, discloses a SPLITT flow cell having two inlet streams (Figure 3); however the second inlet stream is not an indicator stream, but rather a particle-free stream. Giddings U.S. Patent 4,894,146 also discloses a SPLITT flow cell having two input streams, but no indicator stream. All these SPLITT flow methods require the presence of more than one output stream for separating various particle fractions.

None of the foregoing publications describe a channel system capable of analyzing small particles in very small quantities of sample containing larger particles, particularly larger particles capable of affecting the indicator used for the analysis. No devices or methods using indicator streams within the cell system are described.

SUMMARY OF THE INVENTION

Microfluidic devices allow one to take advantage of diffusion as a rapid separation mechanism. Flow behavior in microstructures differs significantly from that in the macroscopic world. Due to extremely small inertial forces in such structures, practically all flow in microstructures is laminar. This allows the movement of different layers of fluid and particles next to each other in a channel without any mixing other than diffusion. On the other hand, due to the small lateral distances in such channels, diffusion is a powerful tool to separate molecules and small particles according to their diffusion coefficients, which is usually a function of their size.

This invention provides a channel cell system for detecting the presence of analyte particles in a sample stream also comprising larger particles comprising:

a) a laminar flow channel;

b) at least two inlet means in fluid connection with said laminar flow channel for respectively conducting into said laminar flow channel (1) indicator stream, said indicator stream preferably comprising an indicator substance, for example, a pH-sensitive dye, which indicates the presence of said analyte particles by a detectable change in property when contacted with said analyte particles, and (2) said sample stream;

c) wherein said laminar flow channel has a depth sufficiently small to allow laminar flow of said streams adjacent to each other and a length sufficient to allow analyte particles to diffuse into said indicator stream to the substantial exclusion of said larger particles in said sample stream to form a detection area;

d) outlet means for conducting said streams out of said laminar flow channel to form a single mixed stream.

In the simplest embodiment of this invention, a single indicator stream and a single sample stream are used; however, the methods and devices of this invention may also use multiple sample and/or indicator streams, and reference or calibration streams, all in laminar flow with each other.

The preferred embodiments of this invention utilize liquid streams, although the methods and devices are also suitable for use with gaseous streams. The term "fluid connection" means that fluid flows between the two or more elements which are in fluid connection with each other.

The term "detection" as used herein means determination that a particular substance is present. Typically, the concentration of a particular substance is determined. The methods and apparatuses of this invention can be used to determine the concentration of a substance in a sample stream.

The channel cell system of this invention may comprise external detecting means for detecting changes in an indicator substance carried within the indicator stream as a result of contact with analyte particles. Detection and analysis is done by any means known to the art, including optical means, such as optical spectroscopy, and other means such as absorption spectroscopy or fluorescence, by chemical indicators which change color or other properties when exposed to the analyte, by immunological means, electrical means, e.g. electrodes inserted into the device, electrochemical means, radioactive means, or virtually any microanalytical technique known to the art including magnetic resonance techniques, or other means known to the art to detect the presence of an analyte such as an ion, molecule, polymer, virus, DNA sequence, antigen, microorganism or other factor. Preferably optical or fluorescent means are used, and antibodies, DNA sequences and the like are attached to fluorescent markers.

The term "particles" refers to any particulate material including molecules, cells, suspended and dissolved particles, ions and atoms.

The input stream may be any stream containing particles of the same or different size, for example blood or other body fluid, contaminated drinking water, contaminated organic solvents, urine, biotechnological process samples, e.g. fermentation broths, and the like. The analyte may be any smaller particle in the input stream which is capable of diffusing into the indicator stream in the device, e.g. hydrogen, calcium or sodium ions, proteins, e.g. albumin, organic molecules, drugs, pesticides, and other particles. In the preferred embodiment when the sample stream is whole blood, small ions such as hydrogen and sodium diffuse rapidly across the channel, whereas larger particles such as those of large proteins, blood cells, etc. diffuse slowly. Preferably the analyte particles are no larger than about 3 micrometers, more preferably no larger than about 0.5 micrometers, or are no larger than about 1,000,000 MW, and more preferably no larger than about 50,000 MW.

The system may also include an indicator stream introduced into one of the inlet means comprising a liquid carrier containing substrate particles such as polymers or beads having an indicator substance immobilized thereon. The system may also include an analyte stream comprising substrate particles such as polymer beads, antibodies and the like on which an indicator substance is immobilized. The liquid carrier can be any fluid capable of accepting particles diffusing from the feed stream and containing an indicator substance. Preferred indicator streams comprise water and isotonic solutions such as salt water with a salt concentration of about 10 mM NaCl, KCl or MgCl, or organic solvents like acetone, isopropyl alcohol, ethanol, or any other liquid convenient which does not interfere with the effect of the analyte on the indicator substance or detection means.

The channel cell may be fabricated by microfabrication methods known to the art, e.g. as exemplified herein, a method comprising forming channels in a silicon microchip, such as by etching grooves into the surface of the silicon microchip and placing a glass cover over the surface. Precision injection molded plastics may also be used for fabrication.

The method of this invention is designed to be carried out such that all flow is laminar. In general, this is achieved in a device comprising microchannels of a size such that the Reynolds number for flow within the channel is below about 1, preferably below about 0.1. Reynolds number is the ratio of inertia to viscosity. Low Reynolds number means that inertia is essentially negligible, turbulence is essentially negligible, and, the flow of the two adjacent streams is laminar, i.e. the streams do not mix except for the diffusion of particles as described above. Flow can be laminar with Reynolds number greater than 1. However, such systems are prone to developing turbulence when the flow pattern is disturbed, e.g., when the flow speed of a stream is changed, or when the viscosity of a stream is changed.

The laminar flow channel is long enough to permit small analyte particles to diffuse from the sample stream and have a detectable effect on an indicator substance or detection means, preferably at least about 2 mm long. The length of the flow channel depends on its geometry. The flow channel can be straight or curved in any of a number of ways. In one embodiment, the flow channel can include one or more "hairpin turns," making a tight stairstep geometry. In another embodiment, the flow channel can be in the shape of a coil, like a neatly wound up garden hose. Non-straight channel geometries allow for increasing the length of the flow channel without increasing the size/diameter of the substrate plate in which the channel is formed, e.g., a silicon microchip. The diffusion coefficient of the analyte, which is usually inversely proportional to the size of the analyte, affects the desired flow channel length. For a given flow speed, particles with smaller diffusion coefficients require a longer flow channel to have time to diffuse into the indicator stream.

Alternatively, to allow more time for diffusion to occur, the flow rate can be decreased. However, several factors limit the minimum flow rate and therefore make a longer flow channel desirable in some cases. First, the flow rate is achieved by a pumping means or pressure source, some of which cannot produce as low a pressure and flow rate as may be desired, to allow enough time for diffusion of particles with small diffusion coefficients. Second, if the flow rate is slow enough and some particles are of significantly different density from the surrounding fluid streams, particles denser than the surrounding fluid streams may sink to the bottom of the flow channel and particles less dense than the surrounding fluid streams may float to the top of the flow channel. It is preferable that the

flow rate be fast enough that hydrodynamic forces substantially prevent particles from sticking to the bottom, top, or walls of the flow channel. Third, a small change in pressure leads to larger errors in measurement accuracy at lower flow rates. Fourth, at low flow rates, other factors, such as changes in viscosity of fluids, can lead to larger errors in measurement accuracy.

The flow channel can be straight or non-straight, i.e., convoluted. A convoluted flow channel as used herein refers to a flow channel which is not straight. A convoluted channel can be, for example, coiled in a spiral shape or comprise one or a plurality of "hairpin" curves, yielding a square wave shape. Convoluted channels provide longer distances for diffusion to occur, thereby allowing for measurement of analytes with larger diffusion coefficients, e.g., typically larger analytes. In preferred embodiments of this invention wherein a silicon microchip is the substrate plate in which the flow channel is formed, the channel length of a straight flow channel is between about 5 mm and about 50 mm. In preferred embodiments of this invention wherein the flow channel is convoluted, i.e., non-straight, the length of the flow channel is defined or limited only by the size of the microchip or other substrate plate into which the channel is etched or otherwise formed. The channel width (diffusion direction) is preferably between about 20 micrometers and about 1 mm. The channel is more preferably made relatively wide, e.g. at least about 200 micrometers, which makes it easier to measure indicator fluorescence with simple optics, and less likely for particles to clog the channel. However, the channel can be made as narrow as possible while avoiding clogging the channel with the particles being used. Narrowing the width of the channel makes diffusion occur more rapidly, and thus detection can be done more rapidly. The channel depth is small enough to allow laminar flow of two streams therein, preferably no greater than about 1000 micrometers and more preferably between about 50 micrometers and about 400 micrometers.

In some embodiments, the laminar flow channel may be long enough to allow the indicator and sample streams to reach equilibrium with respect to the analyte particles within the channel. Equilibrium occurs when the maximum amount of smaller particles have diffused into the indicator stream.

The system may also comprise specimen channel and outlet means such as smaller channels for conducting specimen streams from the indicator stream at successive intervals along the length of the laminar flow channel, and means including viewports and fluorescence detectors for measuring changes in an indicator substance in each specimen stream, whereby concentration of the analyte in the sample stream may be determined.

Dual detection embodiments of the device of the present invention which allow for detection of both undissolved and dissolved analytes are also provided. Detection of both undissolved and dissolved analytes can be achieved in one dual detection device: dissolved particles can be detected in the flow channel of the T-sensor and undissolved particles can be detected in a v-groove channel or sheath flow module, either or both of which can be in fluid connection with a T-sensor flow channel. Branching flow channels can provide for fluid connection between a T-sensor flow channel and a v-groove channel and/or sheath flow module.

The channel cell systems of this invention can be in fluid connection with a v-groove flow channel, which preferably has a width at the top small enough to force the particles into single file but large enough to pass the largest particles without clogging. V-groove channels are formed by anisotropic EPW (ethylenediamine-pyrocatechol-water) etching of single crystalline silicon microchips, providing access to reflective surfaces with precisely etched angles relative to the surface of the microchip (Petersen, Proc. IEEE 70 (5): 420-457, 1982). (U.S. Patent Application Ser. No. 08/534,515, "Silicon Microchannel Optical Flow Cytometer," filed September 27, 1995, which is incorporated by reference herein in its entirety, discloses a flow cytometer comprising a v-groove flow channel formed by micromachining a silicon microchip.) The cross-section of such a channel is like a letter V, and thus is referred to as a v-groove channel. An optical head comprising a laser and small and large angle photodetectors adapted for use with a v-groove flow channel can be employed as well. As described in U.S. Patent Application Ser. No. 08/534,515, detectors placed at small and large angles with respect to the portion of the probe beam reflected from the v-groove wall can be used to count particles, such as cells, and distinguish them by size (via small angle detector) and structure/morphology (via large angle detector). Using an appropriate laser or LED source, e.g., a blue laser, which can be determined by routine

choice by those of ordinary skill in the art, fluorescence detection can be performed by placing an appropriate filter in front of the large angle detector.

The flow channel of the T-sensor can be in fluid connection with a v-groove channel allowing for dual detection of dissolved and undissolved, single-file particles with one device. The fluid streams can flow first through a T-sensor flow channel and then through a v-groove channel, via branching flow channels. Alternatively, the fluid stream can flow first through a v-groove channel and then through a T-sensor flow channel.

An alternative means of achieving single file particle flow through a flow channel is the sheath flow module disclosed in U.S. Patent Application (Attorney Docket No. 32-96) "Device and Method for 3-Dimensional Alignment of Particles in Microfabricated Flow Channels," filed March 26, 1997 and specifically incorporated in its entirety by reference herein. The sheath flow module includes a first plate of material having formed therein a laminar fluid flow channel; at least two inlets, each inlet joining the laminar flow channel at a junction, the first inlet junction being wider than the second inlet junction, and an outlet from the flow channel. A second plate, e.g., a transparent cover plate, seals the module and allows for optical measurements. A transparent cover plate allows for optical measurements by reflection, in cases where the first plate is a reflective material, e.g., silicon. A first inlet allows for introduction of a first fluid into the flow channel. The first fluid is the sheath fluid. A second inlet allows for introduction of a second fluid into the sheath fluid while it is flowing through the flow channel. The second fluid is the center fluid. Because the second inlet junction is narrower than the first inlet junction, the center fluid becomes surrounded on both sides by the sheath fluid. After all fluids have been introduced and sheath flow has been achieved, the depth of the flow channel can be decreased, leading to vertical hydrodynamic focusing. Optionally, the width of the flow channel can be decreased, leading to horizontal hydrodynamic focusing. The decrease in depth and width can be gradual or abrupt. Hydrodynamic focusing in the sheath flow module leads to single file particle flow.

The sheath flow module can be in fluid connection with the channel cell system of the present invention. The fluid streams can flow first through a T-sensor flow channel and then

through a sheath flow module. Alternatively, the fluid stream can flow first through a sheath flow module and then through a T-sensor flow channel.

The channel cell system of a preferred embodiment of this invention comprises channel grooves in the form of a "T" or a "Y" having a central trunk and two branches etched into the surface of a silicon microchip, which surface is thereafter covered with a glass sheet. The central groove is formed of the trunk of the "T" or "Y", and the branches are the inlet means in fluid connection with the laminar flow channel for respectively conducting the sample and indicator streams into the laminar flow channel.

Channel cells of this invention may also include multiple inlet branches in fluid connection with the laminar flow channel for conducting a plurality of inlet streams into said channel. These may be arranged in a "candelabra"-like array or may be arranged successively along a "crossbar" for the "T" or the branches of the "Y" configuration, the only constraint being that laminar flow of all the streams must be preserved.

Inlet means include the inlet channels or "branches" and may also include other means such as tubes, syringes, and the like which provide means for injecting feed fluid into the device. Outlet means include collection ports, and/or means for removing fluid from the outlet, including receptacles for the fluid, means inducing flow by capillary action, pressure, gravity, and other means known to the art. Such receptacles may be part of an analytical or detection device.

Embodiments of the device of the present invention which allow for optical measurements in transmission are provided. In such embodiments, the channel cell system, or at least the analyte detection area, transects the width of the substrate plate in which the channel cell system is formed. Substrate plate as used herein refers to the piece of material in which the channel cell system of this invention is formed, e.g., a silicon wafer and a plastic sheet. The analyte detection area, and optionally other parts of the channel cell system, lie between optically transparent plates in a space which cuts through the entire width of the substrate plate. Analyte detection area as used herein refers to that portion of the indicator stream where analyte particles create a detectable change in the indicator stream.

Optical measurements exploiting reflected light are referred to herein as detection by reflection, whereas optical measurements exploiting transmitted light are referred to herein as detection by transmission.

A method is also provided for detecting the presence of analyte particles in a sample stream, preferably a liquid stream, also comprising larger particles comprising:

a) conducting said sample stream into a laminar flow channel;

b) conducting an indicator stream, said indicator stream preferably comprising an indicator substance which indicates the presence of said analyte particles, by a detectable change in property when contacted with particles of said analyte into said laminar flow channel, whereby said sample stream and said indicator stream flow in adjacent laminar streams in said channel;

c) allowing analyte particles to diffuse into said indicator stream;

d) detecting the presence of particles of the analyte in said indicator stream.

The flow rate of the input streams is preferably between about 5 micrometers/second and about 5000 micrometers/second, more preferably about 25 micrometers/second. Preferably the flow rate for both streams is the same.

The method and system of this invention include determining the concentration of the analyte particles in the sample stream by detecting the position within the laminar flow channel of analyte particles from the sample stream diffusing into the indicator stream causing a detectable change in the indicator stream or in an indicator substance in the indicator stream. The sample stream and the indicator stream may be allowed to reach equilibrium within the laminar flow channel. The location of the boundary of the detection area (i.e. that portion of the indicator stream containing diffused particles at a detectable concentration) with the unaffected indicator stream may be used to provide information about flow speed and/or sample concentration. The physical location of this boundary in the

channel for a given analyte stays the same over time as long as the flow speed is constant and the sample unchanged. The location and size of the detection area can be varied by varying flow rate, sample concentration, and/or concentration of an indicator substance so as to optimize the signal for detection.

Information useful for determining the concentration of the analyte particles in the sample stream may be obtained by providing means for conducting specimen streams from the indicator stream at successive intervals along the length of the laminar flow channel, such as smaller channels equipped with viewports as described herein. Detection means such as those listed above are used to measure signals from the indicator stream. Changes in the intensity of the signals from specimen channel to specimen channel may be used to calculate the concentration of analyte particles in the original sample.

The method of one embodiment of this invention includes the use of an indicator substance which is immobilized on a particulate substrate carried within the indicator stream. The indicator substance is preferably a substance which changes in fluorescence or color in the presence of analyte particles, such as a dye, enzymes, and other organic molecules that change properties as a function of analyte concentration. The term "indicator substance" is also used to refer to polymeric beads, antibodies or the like having dyes or other indicators immobilized thereon. It is not necessary that the indicator stream comprise an indicator substance when detection means such as those directly detecting electrical, chemical or other changes in the indicator stream caused by the analyte particles are used.

Advantages of this system include the fact that analytes can be determined optically in turbid and strongly colored solutions such as blood, without the need for prior filtering or centrifugation; cross-sensitivities of indicator dyes to larger sample components (a common problem) can be avoided; and the indicator can be kept in a solution in which it displays its optimal characteristics (e. g., cross-sensitivities to pH or ionic strength can be suppressed by using strongly buffered solutions). Measurements of the indicator stream at several locations along the channel can compensate for some remaining cross-sensitivities. In addition, the flow channel can be wide, which makes it easy to measure the indicator fluorescence with simple optics. No membrane is needed; the system is less subject to

biofouling and clogging than membrane systems. The system is also tunable in that sample or indicator stream concentrations and/or flow rates can be varied to optimize the signal being detected. For example, if a reaction takes about five seconds, the system can be adjusted so that the reaction will be seen in the central portion of the device.

The method can be conducted by a continuous flow-through of sample and indicator streams. The steady-state nature of this method makes longer signal integration times possible.

The sample stream may contain particles larger than the analyte particles which are also sensitive to the indicator substance. These do not diffuse into the indicator stream and thus do not interfere with detection of the analyte.

Additionally, a method for determining kinetic rate constants as a function of distance traveled by the sample stream and indicator stream from the T-joint where the two streams meet is provided. Generally, kinetic measurements are made by plotting a physical property related to concentration versus time, i.e., time of reaction. The method provided herein for making kinetic measurements as a function of distance traveled by the sample and indicator stream, rather than as a function of time, is advantageous for the following reasons. The constituents of the streams, i.e., the particles, and the concentrations thereof, at a given position in the flow channel remain constant, given that the flow rate is constant. This method allows for integrating the data from detection, e.g., optical measurements, over time, thereby increasing the accuracy of the data collected and hence of the calculated/determined rate constants. Furthermore, if an experimental error occurs during detection, e.g. in the collection of data, at a given time, one can merely perform the detection measurement again, at the distance/position in the flow channel where the error occurred. In prior art methods of making kinetic measurements, if data at a given time point are lost due to experimental error, those data cannot be collected again during the same experiment.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of flow and diffusion within the T-sensor channel cell embodiment of this invention.

Figure 2 is a fluorescence micrograph of a T-sensor of this invention in which a buffer solution of pH 9 (right inlet) is flowing into the device, and a weakly buffered indicator dye solution (pH 5) enters from the left. The distinct conversion of the dye from one form to the other as diffusion proceeds is clearly visible.

Figure 3 shows the layout of the viewport-T-sensor embodiment of this invention. In this embodiment the indicator stream comes from the right T-leg, and is a solution of indicator dye in a low ionic strength buffer of pH 9. The sample stream, which is introduced from the left, here is a 0.15 M buffer solution of pH 5. Several portions of the indicator stream which contains the indicator dye are continuously taken out of the channel as specimen streams at various locations.

Figure 4 shows a v-groove flow channel coupled with a flow cytometer optical head.

Figure 5 shows a convoluted flow channel in a square wave shape.

Figure 6 shows a convoluted flow channel in a coiled shape.

Figure 7A shows a T-sensor with a rounded T-joint.

Figure 7B shows a viewport-T-sensor with a rounded T-joint.

Figure 8 shows a convoluted flow channel with a plurality of detection areas for making kinetic measurements as a function of distance.

Figure 9, comprising Figures 9A-9C, shows embodiments with branching flow channels for dual detection of both dissolved and undissolved analytes.

Figure 10, comprising Figures 10A-10C, shows a sheath flow module.

Figure 11 shows a T-sensor in which the analyte detection area is etched all the way through the width of the substrate plate.

DETAILED DESCRIPTION OF THE INVENTION

The microscale channel cells of this invention are useful to separate smaller particles from larger particles in a sample stream based on the fact that the diffusion coefficient of a particle is substantially inversely proportional to the size of the particle so that larger particles diffuse more slowly than smaller particles, on the fact that diffusion occurs more quickly at the microscale of this invention than in larger scale separation devices known to the art and on the fact that laminar, non-turbulent flow can be induced in adjacent streams at the microscale.

As shown in Figure 1, a channel cell in the form of a "T" is provided, referred to herein as T-sensor **10**. The device can be microfabricated by etching on a silicon microchip. The geometry need not necessarily be a "T," as a "Y." Any angle that can be fabricated will also suffice. As discussed above, there may be a plurality of input channels. It is necessary only that all input channels merge into a single flow channel, and all channels be sufficiently small that laminar flow is preserved for all operating conditions. In general, the Reynolds number of the system is less than 1. The sample containing small molecules of interest, sample stream **80**, is brought into the device through sample stream inlet port **30**, from whence it flows into sample stream inlet channel **50**, where it is referred to as sample inlet stream **55**. An indicator stream **70** is brought into indicator stream inlet port **20**, from whence it flows into indicator stream inlet channel **40**, where it is referred to as indicator inlet stream **45**.

Sample inlet stream **55** meets indicator inlet stream **45** at T-joint **58** at the beginning of flow channel **100**, and the two streams flow in parallel laminar flow as indicator stream **70** and sample stream **80** to exit port **60**. The indicator stream **70** contains an indicator substance such as a dye which reacts with analyte particles in the sample stream **80** by a detectable change in physical properties. Indicator stream **70** is shown in white in Figure 1.

Due to the low Reynolds number in the small flow channel **100**, no turbulence-induced mixing occurs and the two streams flow parallel to each other without mixing. However, because of the short distances involved, diffusion does act perpendicular to the flow direction, so sample components (analyte particles) diffuse to the left into indicator stream **70** and eventually become uniformly distributed across the width of flow channel **100** at uniform analyte particle diffusion area **120**.

The indicator stream **70** flows into flow channel **100** to form an initial reference area **85** into which analyte particles have not yet diffused. Analyte particles from sample stream **80** diffusing into indicator stream **70** form an analyte detection area **90** where analyte particles create a detectable change in the indicator stream **70**, preferably by causing a detectable change in property in an indicator substance within the indicator stream **70**. Particles of an indicator substance, e.g. dye particles, may also diffuse into sample stream **80** to form a diffused indicator area **110**. If this change in local concentration of the indicator substance is a problem in some applications, its diffusion rate can be made arbitrarily small by immobilization on polymers or beads, e.g. indicator beads **130**.

In the T-sensor **10** of Figure 1, a sample stream **80**, e.g. blood, and an indicator stream **70** containing an indicator dye are joined at the intersection of sample stream inlet channel **50** and indicator stream inlet channel **40**, with flow channel **100** (i.e., T-joint **58**) and flow laminarly next to each other in flow channel **100** until they exit the structure at exit port **60**. Small ions such as H^+ and Na^+ diffuse rapidly across the diameter of flow channel **100**, whereas larger ions such as the dye anion diffuse only slowly. Larger particles such as sugars, proteins, and the like and blood cells show no significant diffusion within the time the indicator stream **70** and sample stream **80** are in contact with each other. The smaller sample components diffuse more rapidly and equilibrate close to the T-joint **58**, whereas larger components equilibrate further up in flow channel **100**. Furthermore, as the indicator has a particular half-saturation concentration (pK_a, in the case of a pH dye), a front or detection area boundary **95** of indicator dye color or fluorescence change exists as diffusion proceeds up the channel to form detection area **90**. In practice the detection area boundary **95** and reference area **85** may form a curved line best seen in Figure 2. The location and

curvature of the front can have its "resting location" adjusted by changing flow speed and channel width to optimize signal size and intensity.

Although this is a flow system, the physical location of the detection area boundary **95** in flow channel **100** for a given analyte stays the same over time as long as the flows are constant and the sample unchanged. Analyte concentration is determined either by monitoring indicator signal at uniform analyte particle diffusion area **120** after substantial equilibration, or by noting the position of the front of steepest indicator color change, for example with a multi-element detector (see Figure 3). The analyte detection area **90** can be as large as necessary to provide a detectable indicator signal. Similarly reference area **85** can be made to be as large as necessary to provide a detectable reference signal. Adjustments of these areas can be made as described below based on the diffusion coefficients of the analyte and indicator substance, flow rates and channel sizes.

Figure 2 shows a fluorescence microscope photograph of the T-sensor of Figure 1 featuring an indicator inlet stream **45** which is a weakly buffered indicator dye solution of pH 5, and a sample inlet stream **55** which is a buffer solution of pH 9. The bright zone at the right is light reflecting on the silicon and does not relate to the sample and indicator streams. The sample stream **80** appears as a dark clear fluid on the right. The bright zone on the left is reference area **85** where analyte particles have not yet diffused into indicator stream **70**. The grey area in the middle is analyte detection area **90** where OH^- ions from the sample stream **80** have diffused into indicator stream **70** to form detection area **90**. The fuzzy right edge of the grey detection area **90** is caused by dye particles diffusing into the sample stream **80**. Uniform analyte particle diffusion area is shown at **120** where the OH^- ions are uniformly diffused. The strongest signal is in the middle of detection area **90**.

Figure 3 shows another embodiment of the T-sensor channel cell device of this invention having multiple specimen channels and viewports spaced along the length of the flow channel. In Figure 3 an indicator inlet stream **45** enters from the right (rather than the left as in Figures 1 and 2) at indicator stream inlet port **20**. A solution of indicator dye in a low ionic strength buffer of pH 9 is used. A sample inlet stream **55** which is a 0.15 M buffer solution of pH 5, enters from the left at sample stream inlet port **30**. The

concentration of the dye is only about 10% of the dye concentration used in Figure 2. The indicator and sample streams **45** and **55** respectively, flow along indicator stream inlet channel and sample stream inlet channel **40** and **50** respectively, to meet at T-joint **58** and flow laminarly together along flow channel **100**. Specimen streams **145** from indicator stream **70** which contain the indicator dye are continuously taken out of flow channel **100** at various locations. These specimen streams **145** flow through widenings which serve as viewports **140**. Due to the size of the viewports **140** (several square millimeters), the fluorescence intensity can be easily monitored through a fluorescence microscope, or directly with a photodetector.

The viewport closest to T-joint **58** contains mainly undisturbed dye solution, whereas the viewport closest to exit port **60** contains the sample stream **80** completely equilibrated with the indicator stream **70**. The viewports in between contain the indicator stream **70** in various degrees of equilibration with the sample components. The closer to T-joint **58**, the more likely the viewport is to contain only small ions from the sample. A fluorescence micrograph of the viewports shows that the color in the viewport closest to T-joint **58** is the red color of the base form of the undisturbed indicator dye, whereas the yellow-green color of the viewports closest to exit port **60** represent the acid form of the dye, after the pH of the indicator stream **70** was altered from basic to acidic when diffusion-based equilibration has been reached.

The viewport T-sensor of Figure 3 lends itself to simple referencing techniques. The integral fluorescence intensity of each viewport at one or more wavelengths can easily be measured through a fluorescence microscope, or directly, with photodiodes. In the easiest case, with an indicator dye showing no cross-sensitivity to other sample components, the intensity ratio between selected viewports gives a measurement value largely independent of dye concentration and excitation light intensity. Measuring at more than one viewport increases the redundancy and therefore the measurement accuracy.

In cases of cross-sensitivity of the indicator to larger sample components (e.g. larger biomolecules such as albumin), this interference can be referenced out by comparing the ratios of the different viewports. The viewports closer to T-joint **58** will contain mainly

smaller sample components, whereas the viewports further up flow channel **100** will also contain larger particles.

The T-sensor device of the present invention can be used with reporter beads to measure pH, oxygen saturation and ion content, in biological fluids. (U.S. Patent Application Ser. No. 08/621,170 "Fluorescent Reporter Beads for Fluid Analysis," which is incorporated by reference herein in its entirety, discloses fluorescent and absorptive reporter molecules and reporter beads.) Reporter beads can also be used to detect and measure alcohols, pesticides, organic salts such as lactate, sugars such as glucose, heavy metals, and drugs such as salicylic acid, halothane and narcotics. Each reporter bead comprises a substrate bead having a plurality of at least one type of fluorescent reporter molecules immobilized thereon. Plurality as used herein refers to more than one. A fluorescent property of the reporter bead, such as intensity, lifetime or wavelength, is sensitive to a corresponding analyte. Reporter beads are added to a fluid sample and the analyte concentration is determined by measuring fluorescence of individual beads, for example, in a flow cytometer. Alternatively, absorptive reporter molecules, which change absorbance as a function of analyte concentration, can be employed. The use of reporter beads allows for a plurality of analytes to be measured simultaneously, and for biological cells, the cell content can also be measured simultaneously. A plurality of analytes can be measured simultaneously because the beads can be tagged with different reporter molecules.

The fluorescent reporter molecules of this invention can be any fluorescent molecules having fluorescence properties which are a function of the concentration of a particular analyte or class of analytes. Many dyes and fluorochromes known in the art can be used as reporter molecules in this invention (see, for example, R.P. Haugland, Handbook of Fluorescent Probes and Research Chemicals, 5th Edition, Molecular Probes Inc., Eugene, 1992). The criteria for reporter molecule selection are that the molecules can be immobilized on a substrate bead and that their fluorescence is a function of the concentration of an analyte. In contrast with previously used fluorescent beads, wherein the number of beads in an aggregate changes, the reporter beads of U.S. Patent Application Ser. No. 08/621,170 are not required to have an immunoreagent, such as a ligan, antiligand, antigen or antibody, on the surface in combination with the reporter molecules.

Fluorescent reporter molecules interact with the analyte in a way that changes the fluorescent properties of the reporter molecule. In some instances the reporter molecule reacts with the analyte, as in the case of albumin detection by AB 580 (Molecular Probes). In some cases the interaction is not a chemical reaction. For example the reporter molecule fluorescence can be quenched by nonradiative energy transfer to the analyte, as in the case of O_2 detection by ruthenium diphenyl phenanthroline. For some reporter molecules the fluorescence is sensitive to polarity changes in the fluid, which can be used to detect organic solvents and hydrocarbons within an aqueous fluid. The interaction can also be through other solvent effects, wherein the ionic strength of the solvent affects the fluorescence. Solvent effects can be used to determine the total concentration of all dissolved ions. The interaction can be a ligand/antiligand or antigen/antibody reaction. The interaction preferably does not lead to an aggregate with other particles and, in particular, does not create an aggregate containing a plurality of reporter beads. It is preferred that the interaction of the analyte with the reporter molecules does not significantly perturb the analyte concentration in the fluid.

In the case of fluorescent reporter beads, at least one fluorescence property of the reporter molecules is a function of analyte concentration. The property measured for the reporter beads can be any property which is affected by the analyte interaction with the beads, such as the fluorescence intensity, decay time or spectrum.

Alternatively, the reporter molecules can be absorption indicators, for example the physiological pH indicator N9 (Merck, Germany) immobilized on a substrate bead. Such indicators change their absorption as a function of analyte concentration. Typically the color of the molecules changes (i.e., the wavelength of their absorption maximum changes).

Absorptive reporter molecules can be used in combination with fluorescent reporter molecules on a substrate bead, and absorptive beads can be used in combination with fluorescent beads.

The substrate bead function is to allow the detection of an analyte, and optionally its concentration, with optical measurements of single beads. More than one type of reporter bead, i.e., beads with different reporter molecules immobilized thereon, can be used to

analyze a given sample, provided that the bead type can be identified. Beads can be identified by various means, including means employing bead size, e.g., light scattering; fluorescent tag(s) attached to the bead which has a different excitation and/or emission wavelength from that of the fluorescent reporter molecule attached to that bead; or by directly identifying the fluorescent molecule attached to the bead. This allows for detection of more than one analyte at a time. The substrate bead also functions to immobilize the reporter molecules to prevent their diffusion into the sample stream. The reporter molecules can be on the surface of or within the substrate bead. The beads can be fabricated from a variety of materials and can have any shape, not limited to spherical. Suitable materials include glass, latex, hydrogels, polystyrene and liposomes. The beads can have added surface groups to facilitate attaching reporter molecules, such as carboxyl groups on latex and amino-modified polystyrene.

Various techniques can be employed to immobilize the reporter molecules on the substrate bead. Adsorption based coatings can be prepared by immersing the substrate beads in a reporter molecule solution and then washing off excess reporter molecules. Reporter molecules can similarly be diffused into the cavity of controlled pore glass beads. Reporter molecules can also be covalently immobilized by chemically attaching them to functional groups of suitable substrate beads. Polymerized beads can be formed in a solution containing reporter molecules, thereby trapping the molecules in a fixed polymer cavity. To immobilize reporter molecules in a liposome, lipids can be mixed with a reporter molecule solution, the solution shaken, and the liposomes separated.

To employ reporter beads in the methods of this invention, the beads are mixed with a fluid sample and the fluorescence or absorption of individual beads is measured. The beads can be dry before mixing with the sample or can be dispersed in a fluid. For microscale measurements it is preferred that the added volume of beads and any accompanying fluid be small compared to the sample volume (for example $<1\%$) so that sample dilution is insignificant.

The channel cells of this invention may be formed by any techniques known to the art, preferably by etching the flow channels onto the horizontal surface of a silicon microchip

and placing a lid, preferably of an optically clear material such as glass or a silicone rubber sheet, on the etched substrate. Other means for manufacturing the channel cells of this invention include using silicon structures or other materials as a template for molding the device in plastic, micromachining, and other techniques known to the art. The use of precision injection molded plastics to form the devices is also contemplated. Microfabrication techniques are known to the art, and more particularly described below.

In a preferred embodiment of this invention, channel cells of this invention have hydrophilic surfaces to facilitate flow of liquid therein and allow operation of the device without the necessity for pressurization. The substrate may be treated by means known to the art following fabrication of the channels, to render it hydrophilic. The lid is also preferably treated to render it hydrophilic.

The T-sensor channel system of this invention can be in fluid connection with one or more v-groove channels. A silicon microchip can be etched to form a v-groove with reflective surfaces/walls of the channels. Thus, optical measurements can exploit reflected, rather than transmitted, incident light. Detection can be achieved by reflection, that is by detecting reflected light. Small angle scattered light (scattered off the surfaces of any particles in the channel) is also reflected by the v-groove wall and can be collected by a small angle photodetector. Large angle scattered light and fluorescent light can exit the channel without reflection and can be collected by the a large angle photodetector. In addition, the reflective wall of the v-groove behind the illuminated particle enhances the fluorescence collection efficiency. Any part of the incident light, e.g., laser beam, that is not within the v-groove channel is reflected from the silicon surface in a direction away from either the small or large angle detectors. The fraction of light reflected from the lid, e.g., transparent cover plate, in a case wherein light enters from air without being directly coupled into the lid/cover plate, is also directed away from the small and large angle detectors thereby reducing undesirable background light intensity from the measurements.

Because the v-groove flow channel reflects the incident light, rather than transmitting it, fabrication of the microchannel system of this invention is extremely simple. The

microchannel is fabricated from a single microchip of silicon which is patterned on a single side. A transparent cover plate is attached to the top of the microchip to seal the channel.

Figure 4 shows a v-groove flow channel and optional optical head. Silicon microchip **210** has v-groove **211** therein. The term v-groove is used herein for a substantially "V" shaped groove in the surface of a silicon microchip. Depending on the fabrication process the point of the "V" can be flat (a trapezoidal groove), but only if the flat portion does not fall within the analyte detection area defined by the interception of the illumination beam with the sample flow. In a preferred embodiment, microchip **210** has a $<100>$ surface orientation and the walls of groove **211** are along $<111>$ planes, providing an angle of 54.7° between the walls of the groove and the plane of the surface of the microchip. Transparent cover plate **220** is sealed to the surface of microchip **210**. In a preferred embodiment the cover plate is made of pyrex and is anodically bonded to the silicon microchip. In the illustrated embodiment the light source includes diode laser **310**, optical fiber **312** and focusing head **314**. Non-scattered light, i.e., light which has not been scattered by a particle, is specularly reflected by a wall of channel **211** and travels along path **322**. Small angle (forward) scattered light deviates slightly from path **322** and impinges on small angle detector **320**. Some of the light scattered at large angles travels along path **332** to large angle photodetector **330**. The photodetectors can be photodiodes or photomultipliers. Large angle detector **330** can be used to measure large angle scattering and/or fluorescence.

Means for applying pressure to the flow of the feed fluids through the device can also be provided. Such means can be provided at the feed inlets and/or the outlet (e.g. as vacuum exerted by chemical or mechanical means). Means for applying such pressure are known to the art, for example as described in Shoji, S. and Esashi, M. (1994), "Microflow devices and systems," J. Micromechanics and Microengineering, **4**:157-171, and include the use of a column of water or other means of applying water pressure, electroendoosmotic forces, optical forces, gravitational forces, and surface tension forces. Pressures from about 10^{-6} psi to about 10 psi may be used, depending on the requirements of the system. Preferably about 10^{-3} psi is used. Most preferred pressures are between about 2 mm and about 100 mm of water pressure.

An example of an embodiment using multiple streams is a channel cell having three inlet streams flowing in laminar flow wherein the middle stream is a reagent stream. For example, the sample stream may be blood, the middle stream glucose oxidase, and the third stream an indicator stream containing pH sensitive dye. As glucose particles diffuse through the reagent stream they are changed to gluconic acid which is detected by a pH-sensitive dye when the gluconic acid molecules diffuse into the indicator stream. Other examples of multiple-stream systems include systems having several sample streams with analyte at different concentrations for calibration of the detection means. Indicator streams not adjacent to the sample streams may also be used as control streams.

The indicator stream can be measured by the detection means before and after diffusion of particles into the stream has taken place, and such measurements as well as the rate of change of the indicator stream along its length can be used to assay analyte concentration. In addition, multiple detection means of different types can be used to measure the indicator stream. Field effects which are ion or chemical sensitive can be measured at different locations in the device.

The channel cells of this invention and the channels therein can be sized as determined by the size of the particles desired to be detected. As is known in the art, the diffusion coefficient for the analyte particles is generally inversely related to the size of the particle. Once the diffusion coefficient for the particles desired to be detected is known, the contact time of the two streams, size of the central channel, relative volumes of the streams, pressure and velocities of the streams can be adjusted to achieve the desired diffusion pattern.

Fluid dynamic behavior is directly related to the Reynolds number of the flow. The Reynolds number is the ratio of inertial forces to viscous forces. As the Reynolds number is reduced, flow patterns depend more on viscous effects and less on inertial effects. Below a certain Reynolds number, e.g., 0.1, inertial effects can essentially be ignored. The microfluidic devices of this invention do not require inertial effects to perform their tasks, and therefore have no inherent limit on their miniaturization due to Reynolds number effects. The devices of this invention require laminar, non-turbulent flow and are designed according

to the foregoing principles to produce flow having low Reynolds numbers, i.e. Reynolds numbers below about 1.

The Reynolds number is the ratio of inertial forces to viscous forces. As the Reynolds number is reduced, flow patterns depend more on viscous effects and less on inertial effects. Below a certain Reynolds number, e.g. below about 1, (based on lumen size for a system of channels with bends and lumen size changes), inertial effects can essentially be ignored. The microfluidic devices of this invention do not require inertial effects to perform their tasks, and therefore have no inherent limit on their miniaturization due to Reynolds number effects. Applicants' channel cell designs, while significantly different from previous reported designs, operate in this range. These microfluidic devices of this invention require laminar, non-turbulent flow and are designed according to the foregoing principles to produce flows having low Reynolds numbers.

The devices of the preferred embodiment of this invention are capable of analyzing a sample of a size between about 0.01 microliters and about 20 microliters within a few seconds, e.g. within about three seconds. They also may be reused. Clogging is minimized and reversible. The sizes and velocities of 100 μm wide and 100 μm/s, for example, indicate a Reynolds number ($R_e = plv/\eta$) of about 10^{-2} so that the fluid is in a regime where viscosity dominates over inertia.

The magnitude of the pressure drop needed to obtain an average velocity, v, of a fluid with absolute viscosity, η, and density, p, through a circular channel (length, 1, diameter, d) can be calculated from Poiseuille's Law (Batchelor, G.K., An Introduction to Fluid Dynamics, Cambridge Univ. Press 1967),

$$\frac{P}{l} = \frac{32\eta v}{d^2}$$

Using v = 100 μm/sec and d = 100 μm, we get a pressure drop equivalent to about 0.3 mm of H_2O per cm of channel length. Since Poiseuille's equation is strictly valid only for circular flow channels and the channels of this invention are substantially rectangular in

cross-section it can be considered only as an approximate relation between the variables represented.

When a liquid is introduced into a device there is at first an effective pressure, $P_{eff} = P_o + P_{st}$, equal to the sum of the applied pressure, P_o, and a pressure due to the surface tension,

$$P_{st} = \frac{\gamma \cos\Theta}{r} .$$

P_{st} is a function of the surface tension of the fluid, γ, the contact angle of the fluid with the surface, Θ, and the radius of curvature of the fluid surface, r.

For hydrophilic surfaces, cos Θ is close to 1, and for small channels no applied pressure is needed to wet the device. This is referred to as "wetting by capillary action." However, once the device is completely wet, one has to worry about the surface tension at the exit area. In the device described in the example hereof, the radius of curvature of the fluid in the exit area was several millimeters, so that the pressure due to the surface tension was negligible

With a channel width of 100 μm, P_{st} is about 1 cm of H_2O, so surface tension on the exit channel is significant. However, using an etchant such as EPW F-Etch as described below, which attacks the <100> planes of silicon, means that the corners as etched are not as sharp as shown in the figures. This results in a gradual widening of the channel to about 1 mm which reduces the effect of the surface tension.

By adjusting the configuration of the channels in accordance with the principles discussed above to provide an appropriate channel length, flow velocity and contact time between the sample stream and the indicator stream, the size of the particles remaining in the sample stream and diffusing into the indicator stream can be controlled. The contact time required can be calculated as a function of the diffusion coefficient of the particle D and the distance d over which the particle must diffuse by $t = d^2/D$. Particles or molecules that have diffusion coefficients larger than D will diffuse into the indicator stream, and particles or molecules having a diffusion coefficient substantially smaller than D will not. If the diffusion

coefficient of the larger particles is about ten times smaller than D, the indicator stream should be entirely free of the large particles.

For a given flow speed, some analytes with relatively small diffusion coefficients, a straight channel cell system (T-sensor) channel, preferably 5-50 mm in length, does not provide a long enough flow channel for diffusion to occur adequately. Typically, silicon microchips are 3 inches, 4 inches, 6 inches, or 8 inches in diameter. A straight channel etched into a microchip of such size can be no longer than the microchip diameter. Detection of analytes with relatively small diffusion coefficients, e.g. relatively large analytes or non-spherical analytes, preferably employs a convoluted flow channel. A convoluted flow channel as used herein refers to a flow channel which is not straight. Figures 5 and 6 show two different channel geometries which allow for longer flow channels on a typical 3-4 inch silicon microchip.

In the channel cell system (T-sensor) of Figure 5, the left and right streams, e.g., sample and indicator streams, have the same overall pathlength. If multiple measurements are taken in this embodiment, they should be taken along the vertical center line of the sensor so that both streams are flowing at the same flow speed and have had the same flow distance. In this embodiment, wherein the convoluted flow channel has a square wave shape like that in Figure 5, the streams flow at different speeds through the curves. Therefore, it may be preferable to use slower flow speeds than the speeds used in straight flow channels because the tight/narrow curves and sheer forces between the streams flowing at different speeds can cause zones in which laminar recirculation occurs. Laminar recirculation is not turbulence; the flow is still laminar and predictable. Nonetheless, laminar recirculation is not preferable and can be avoided by maintaining a Reynolds number below about 1.

The channel cell system (T-sensor) of Figure 6 shows a coiled/spiral flow channel. In this geometry, four separate T-sensors each having a 220 mm long flow channel, can be fabricated on a single 3 inch microchip. Because the bending radius is larger in this geometry than in the square wave geometry, laminar recirculation is less likely to occur. The difference in relative flow speeds of the left and right streams (sample and indicator streams) is minimal, leading to less sheer stress between the two streams if the two streams

have different viscosities. This channel geometry does, however, create different overall flow distances for the left and right streams.

Figures 7A and 7B illustrate channel cell systems (T-sensor devices) of this invention wherein the T-joint **58** is rounded. Figure 7A shows a T-sensor similar to the one shown in Figure 1, except that the T-joint **58** is rounded in Figure 7A. Figure 7B shows a viewport T-sensor similar to the one shown in Figure 3, except that the T-joint **58** is rounded in Figure 7B. A rounded T-joint is preferable because it helps prevent laminar recirculation in the T-joint which can occur at Reynolds number above about 1. A rounded T-joint is preferable also because it decreases the chance of contamination of the sample stream with the indicator stream, and vice versa.

The channel cell system of this invention can be used to measure concentration of an analyte as a function of distance (from the T-joint) rather than time. An increment of distance is proportional to an increment of time. With laminar flow and a known flow speed, an increment of distance can be converted to an increment of time.

Other methods for making kinetic measurements employ plotting concentration, or some physical property resulting from concentration, e.g., absorbance or fluorescence, versus time. The decrease in concentration of a starting material, or increase in concentration of a product, with time determines the kinetic rate constant for a reaction.

The rate of, or rate constant for, a reaction can be determined using the T-sensor device of this invention. Detection, e.g., absorption or fluorescence measurements, can be performed at one or more analyte detection area. Referring to Figure 8, a plurality of analyte detectors **410** can be positioned at various distances from the T-joint **58.** Alternatively, one detector can be used to monitor the flow channel at various distances from the T-joint **58**. Figure 8 shows a square-wave/serpentine shaped flow channel. However, a T-sensor of any geometry which maintains laminar flow can be employed to make kinetic measurements, particularly according to the methods disclosed herein. A sample stream is introduced via sample stream inlet port **30** and an indicator stream is introduced via indicator stream inlet port **20.** The two streams meet at T-joint **58.** Analytes from the sample stream

begin to diffuse into the indicator stream, and a measurable change, e.g., increase in fluorescence, occurs. A measurable change occurs as a result of analytes diffusing into the indicator stream, shown at analyte detection areas **90**.

The intensity of fluorescence or absorbance in the analyte detection area and the width of the analyte detection area are measured at various distances from the T-joint **58.** The intensity and width of the analyte detection area are a function of the concentration of the analyte being measured. As the analyte diffuses into the indicator stream, a change in color (i.e. change in optical absorbance) or fluorescence occurs in the analyte detection area. This optical change becomes more intense with increasing distance from the T-joint, because the analyte and the indicator have had a longer time to interact with each other. The width of the analyte detection area also increases with increasing distance from the T-joint. Two independent causes lead to this increase in width. First, the analytes diffuse farther with increasing time, and therefore with increasing distance. Second, the more the interaction between the analyte and indicator has progressed, the greater the absorbance or fluorescence at the analyte detection area. Hence, absorbance or fluorescence can be detected at a greater width in the analyte detection area.

Referring to Figure 8, the analyte detection area **90** becomes wider and more intense with increasing distance from the T-joint **58.**

Using the device and methods of this invention, a rate constant for a reaction can be determined with as few as one measurement, e.g., fluorescence at a certain distance from the T-joint. As is known in the art, increasing the number of measurements leads to increased accuracy of the kinetic rate constant calculated from such measurements.

In another embodiment, the T-sensor channel cell system of this invention can comprise branching flow channels **401** and **402** as illustrated in Figure 9A. The sample containing small molecules of interest is brought into the device through sample stream inlet port **30,** from whence it flows into sample stream inlet channel **50**. An indicator stream is brought into indicator stream inlet port **20**, from whence it flows into indicator stream inlet channel **40**. The two streams flow parallel to one another in laminar flow, and small

molecules (analytes) from the sample stream diffuse into the indicator stream. Branching flow channels as used herein refer to flow channels in fluid connection with the flow channel **100**. A W-joint **400** as shown in Figures 9A and 9B may be used to correct the branching flow channels **401** and **402** with flow channel **100**. Branching flow channels allow for detection of both undissolved and dissolved particles. A detector, preferably positioned above or below the device, monitors the flow channel **100** and v-grooves **403** or **404**. This dual detection embodiment can detect dissolved and undissolved particles in the flow channel **100** as well as undissolved particles flowing in single file fashion in the v-groove(s). Particle detection can be performed by standard optical techniques, e.g., imaging, light scattering, or spectroscopy, as the particles flow through one or both of the v-grooves **403** or **404**, which are in fluid connection with branching flow channels **401** and **402**, respectively. Branching flow channels **401** and **402** are in fluid connection with exit ports **405** and **406**, respectively.

For example, in this embodiment a sample, e.g., whole blood, can be introduced via sample stream inlet port **30** from whence it flows into sample stream inlet channel **50** and a buffered solution containing reporter beads can be introduced via indicator stream inlet port **20** from when it flows into indicator stream inlet channel **40**. The sample and indicator stream flow parallel to each other in laminar flow in flow channel **100**. Small analytes in the sample, e.g., protons, diffuse into the indicator stream. Referring to Figure 9A, the sample flows into branching flow channel **402** and then into v-groove **404**, through which particles, e.g., red and white blood cells, flow in single file fashion. At the same time, the reporter beads flow into branching flow channel **402** and then into v-groove **403**, through which the beads flow in single file fashion. An optical detector, preferably positioned above or below the device simultaneously monitors the two streams in flow channel **100** and the undissolved sample particles in v-groove **404** and beads in v-groove **403**, the beads being indicators of dissolved sample analytes.

Alternatively, the indicator stream can include a dissolved indicator dye which is monitored with the monitoring of the undissolved sample particles when this embodiment of the present device is employed. A dissolved indicator dye does not need to be monitored in

a v-groove. Hence, both branching flow channels need not be connected to v-grooves, as illustrated in Figure 9C.

Another example of the dual detection embodiment of this invention is the following. A sample of whole blood can be monitored in a v-groove channel to detect the number of white blood cells. Then the same sample flows into a T-sensor in fluid connection with the v-groove channel. In the T-sensor the white blood cells react with fluorescent reporter beads tagged with an antibody. Then the sample flows into another v-groove channel in fluid connection with the T-sensor. In this v-groove channel the white blood cells are identified by fluorescence.

The T-sensor channel system of the present invention can further comprise a waste port **407**, as illustrated in Figure 9B. To insure that only sample stream enters branching flow channel **402**, and that only indicator stream enters branching flow channel **401**, a portion of each stream can be diverted to a waste port **407**. The waste port is in fluid connection with the flow channels at the W-joint to divert a portion of each stream to a waste outlet.

Figure 9C illustrates sample stream (represented by x) and indicator stream (represented by squares) flowing through the channel system of this invention comprising branching flow channels and a waste port. Figure 9C further illustrates that the branching flow channels do not have to loop back and run parallel to the flow channel **100**. Branching flow channels can connect to the flow channel **100** in any angle desired. In order to monitor the flow through the various channels simultaneously and with one detector it is preferable that the branching flow channels connect with the flow channel **100** at an angle which allows for such monitoring.

Detection of dissolved and undissolved particles in one device employing this embodiment is economically advantageous, as measurements can be performed with only one set of pumps and one detector.

Another means for detecting undissolved particles in single file flow employs a sheath flow module. A sample can first flow through a flow channel of a T-sensor where the sample reacts with reporter beads, e.g., an analyte in the sample diffuses into an indicator stream containing reporter beads. The fluid containing reporter beads can then flow into a sheath flow module in fluid connection with the T-sensor flow channel. In the sheath flow module the beads are focused so that they flow in single file fashion for detection.

As with the v-groove channel, the order of the sheath flow module and the T-sensor can be reversed, i.e., the fluids can flow first through the sheath flow module and then through the T-sensor. Figure 10A is a lengthwise section through the center of a flow module, as described in U.S. Patent Application "Device and Method for 3-Dimensional Alignment of Particles in Microfabricated Flow Channels," (filed March 26, 1997). Plate **501** is machined, molded or etched to form the flow channel. The plate can be selected from the following which include, but are not limited to, silicon wafers, plastics, e.g., polypropylene, and casting materials. Techniques for etching silicon wafers and molding and machining plastics are well-known in the art. A laminar flow channel **508** is formed in a flat plane of the plate. A first inlet **510** passes through the plate at the upstream end of the channel and joins the flow channel at first inlet junction **511**. An outlet **530** passes through the plate at the downstream end of the channel and joins the flow channel at outlet junction **531.** A second inlet **520** passes through the plate between the first inlet and the outlet and joins the flow channel at second inlet junction **521**, which is narrower than the first inlet junction. A second plate **505** is sealed to the flat plane of the first plate, thereby forming one side of the laminar flow channel. A view of the channel surface is illustrated in Figure 10B. The relative widths of the inlet junctions are shown, as well as the edge **512** of the flow channel **508**. The second inlet junction **521** is narrower than the first inlet junction **511**. Referring again to Figures 10A and 10B, a sheath fluid is introduced into the flow channel **508** via the first inlet **510** and flows through the flow channel toward the outlet **530**. A center fluid is introduced via the second inlet **520**, preferably at lower pressure and speed than the sheath fluid. Figure 10C is a cross section of the flow channel of Figures 10A and 10B, illustrating the sheath flow attained in one embodiment of the present invention. In this embodiment flow channel **508** is trapezoidal. A center fluid **554**, injected from inlet **520**, is surrounded on both sides (left and right) and on top by a sheath fluid **553**.

As discussed above, forming the channel system in a reflective material allows for optical measurements by reflection. Alternatively, optical measurements by transmission can be performed in the embodiment described next. A T-sensor channel system can be etched all the way through a substrate plate, e.g., a silicon microchip or other slab of material. The entire channel system can be etched all the way through, and therefore transect, that is, extend through the width of, the substrate plate. Alternatively, only that part of the channel system comprising the analyte detection area **90** can be etched all the way through, and therefore extend through the width of, the substrate plate, as shown in Figure 11. Indicator stream inlet port **20**, sample stream inlet port **30**, and exit port **60** are shown also. An optically transparent plate, e.g., a cover plate, is sealed to both sides of the microchip. If only part of the channel system is etched all the way through the microchip, then the transparent plate need cover only that part of the microchip.

As in the other embodiments of this invention, the dimensions of the device are chosen so that laminar flow is maintained. In this embodiment, if a silicon microchip is etched by anisotropic EPW etching, it is preferable to use a thin microchip so that the channel diameters can be kept small enough to maintain laminar flow. The anisotropic EPW etching creates channels which are wider at the top than at the bottom of the channel. Etching all the way through a microchip can create a channel which is undesirably wide at the top and therefore with an undesirably large channel diameter. Undesirably large channel diameters may not maintain laminar flow. Preferable widths of a thin microchip are between 100 and 300 microns, and more preferably between 100 and 200 microns. Alternatively, other methods of etching silicon, e.g., reactive ion etching, can be used to keep channel diameters small enough to maintain laminar flow. Other materials, e.g., plastics, which are machined or molded to form the channel system need not necessarily be thin to keep channel diameters small.

A microchip can be made thinner by etching prior to formation of the channel system therein. An uncoated microchip, that is a microchip with no photoresist on it, can be made thinner by submerging it in etching solution. A channel system, or at least the analyte detection area, can then be etched all the way through the microchip.

Alternatively, a T-sensor channel system which maintains a low Reynolds number, i.e. laminar flow, can be formed wherein the depth of the channel is greater than the width. However, because the flow speed is parabolic with respect to the channel width, i.e., it is fastest in the center of the channel and approaches zero at the walls, it is preferable that the channel dimensions are such that diffusion from top to bottom and bottom to top counteracts this parabolic flow speed profile. Increasing the depth of the flow channel decreases the effect of diffusion from top to bottom and bottom to top.

Numerous embodiments besides those mentioned herein will be readily apparent to those skilled in the art and fall within the range and scope of this invention. All references cited in this specification are incorporated in their entirety by reference herein. The following examples illustrate the invention, but are in no way intended to limit the invention.

EXAMPLES

Example 1. Fabrication of Channel Cell.

A two-mask level process was used to fabricate a channel cell of this invention on a silicon wafer. The channel cell had a flow channel 400 micrometers wide and 20 mm long. The "branches" or crossbar of the "T" comprising the inlet channels was a groove 30 mm long and 200 micrometers wide. Channel depth was 50 micrometers.

The first mask level defined the inlets and outlet ports, which were etched completely through the wafer to the rear side of the silicon. The second level defined the fluid transport channels.

Four inch chrome masks were made to these specifications by Photo Sciences, Inc. (Torrance, CA) and 3" wafers ({100}, n-type) with 500 nm of SiO_2 grown on them were used.

Wafers were cleaned in a Piranha bath (H_2SO_4 and H_2O_2) (2:1) before processing. A primer (HMDS spun on at 3000 rpm) was used to enhance photoresist adhesion. About one μm of AZ-1370-SF (Hoechst) photoresist was deposited by spin coating (3000 rpm), and this was followed by a soft bake (30 min at 90°C).

A contact aligner was used to align and expose wafers. Exposure time was varied to yield best results. No post-exposure bake was done. Wafers were developed in AZ-351 (diluted 4:1) (Hoechst) for one minute and rinsed in DI water. Blue tack tape (Semiconductor Equipment Corporation, Moorpark, CA) was applied to the backsides of the wafers to protect the oxide from the oxide etch.

The wafers were immersed in a buffered oxide etch (BOE, 10:1 HF (49%) and NH_4F (10%)) for eleven minutes to completely etch away the unprotected oxide. The blue tack tape was removed by hand, and the photoresist was removed in an acetone rinse.

Silicon etching was done in a mixture of ethylene-diamine, pyro-catechol, and water (EPW F-etch as described in Reisman, A., et al. (1979) J. Electrochem. Soc. **126**:1406-1415) set up in a reflux boiling flask. This etch attacks the {100} planes of silicon at a rate of about 100 μm an hour. Fluid attachment ports were etched in the first step for about three hours. Photoresist was again applied, and the mask containing flow channels between fluid ports and the barrier region was exposed. The wafers were developed and etched in this second step for about one hour.

After final processing, the wafers were once again cleaned in a Piranha bath and rinsed in DI water. They were then diced into individual devices about 1 cm by 1 cm.

Anodic bonding according to Wallis, G. and Pomerantz, D.I (1969) J. Appl. Physics **40**:3946-3949, was used to attach Pyrex glass to the silicon devices. One inch square pieces of Pyrex glass (100 μm thickness) from Esco Products Inc. (Oak Ridge, NJ) were used. First, the silicon and Pyrex glass were immersed in a solution of H_2O_2, NH_4OH, and H_2O (1:4:6) heated to 50°C. This process removes any organic matter on the surfaces and also makes the surfaces hydrophilic. After 20 minutes in this solution, the silicon and Pyrex were rinsed with DI water and dried. Anodic bonding was done at 400°C with 400 V applied between the glass and the silicon.

Example 2. Fluorescence Color changes with pH.

Five 0.01 M HEPES Buffer solutions, with pH 7.2, 7.4, 7.6, 7.8 and 8.0 were prepared from analytical grade chemicals (Aldrich). The resulting solutions were used consecutively as sample streams. The analyte in question in this experiment is H^+ or OH^-. 1 mg of the fluorescent pH indicator dye carboxy-SNAFL 2 (Molecular Probes, Eugene, OR), was dissolved in 2 ml of DMSO (((.9%, Aldrich). 0.1 ml of this solution was mixed with 1 ml of a 0.0001 M HEPES Buffer of pH 7.0. The resulting solution was used as the indicator stream.

The T-sensor channel cell was attached to the stage of a microscope so that the joint of the T-sensor was in the view field of the objective. The inlet ports and the outlet port were connected to injector loops and to upright tubes which were filled with water so that there was a pressure difference of 30 mm water column between the inlet ports and the outlet port. Both inlet ports were exposed to identical pressure so that the two streams joined in the middle of the T-joint, and were flowing parallel to the outlet port. One injector loop was filed with indicator dye solution, the other loop was filled with one of the sample solutions. The loops contained enough volume to operate the device for roughly one hour.

After both injection loops were allowed to flow into the T-sensor, and after 1 min of equilibration and flushing time, photographs were taken through a camera attachment on the microscope. The excitation filter center wavelength was 480 nm, the emission filter was a longpass 510 nm filter.

The experiment yielded photographs in which the color of the analyte detection area between the indicator stream and the sample stream was a function of the pH of the sample stream. The color changed from red over orange to yellow as the pH decreased from 8.0 to 7.2. Computer-enhanced images showed the color of the indicator stream per se to be yellow, and the analyte detection area between the streams to range from red to orange, whereas the colorless ample stream appeared black. By color mapping, numeric values are assigned to the different colors which are used to calibrate the system. Alternatively, light intensity change is measured at two wavelengths, thereby measuring the decrease of the red portion and the increase of the yellow portion of the spectrum with decreasing pH.

Example 3. Kinetic Measurements as a Function of Distance

Alkaline phosphatase in serum and 0.1 M *p*-nitrophenol phosphate (PNPP)(weakly yellow) in 0.1 M HEPES buffer, pH 7.40, were injected into a T-sensor device. The alkaline phosphatase catalyzed the reaction of PNPP to *p*-nitrophenol (strongly yellow) and phosphate. The formation, (and rate thereof), of *p*-nitrophenol was detected by an increase in yellow color. The rate of change of yellow color intensity as a function of distance from the T-joint was a function of enzyme concentration, enabling calculation of a rate constant.

CLAIMS

1. A channel cell system for detecting the presence of analyte particles in a sample stream also comprising larger particles comprising:

a) a laminar flow channel;

b) at least two inlet means in fluid connection with said laminar flow channel for respectively conducting into said laminar flow channel (1) an indicator stream and (2) said sample stream;

c) wherein said laminar flow channel has a depth sufficiently small to allow laminar flow of said streams and a length sufficient to allow particles of said analyte to diffuse into said indicator stream to the substantial exclusion of said larger particles in said sample stream to form a detection area;

d) outlet means for conducting said streams out of said laminar flow channel to form a single mixed stream.

2. The system of claim 1 also comprising a fluorescence detector for detecting changes in an indicator substance carried within said indicator stream as a result of contact with analyte particles.

3. The system of claim 1 comprising means for conducting specimen streams from the indicator stream at successive intervals along the length of said laminar flow channel and means for measuring signals from the indicator stream in each specimen stream whereby concentration of the analyte in the sample stream may be determined.

4. The channel cell system of claim 1 further comprising a v-groove channel in fluid connection with said laminar flow channel.

5. The channel cell system of claim 1 further comprising a sheath flow module in fluid connection with said laminar flow channel.

6. The channel cell system of claim 1 wherein said indicator stream comprises reporter beads.

7. The channel cell system of claim 1 wherein said laminar flow channel is convoluted.

8. The channel cell system of claim 1 comprising an analyte detection area.

9. The channel system of claim 1 further comprising at least one branching flow channel in fluid connection with said laminar flow channel whereby both dissolved and undissolved particles are detected.

10. The channel cell system of claim 1 formed in a substrate plate, for detecting by transmission the presence of analyte particles in a sample stream also comprising larger particles comprising:

(a) an analyte detection area between said inlet means and said outlet means;

(b) optically transparent plates sealed to both sides of said substrate plate; and

(c) wherein said analyte detection area lies between said transparent plates in a space cut through the width of said substrate plate.

11. A method for detecting the presence of analyte particles in a sample stream also comprising larger particles using the system of claim 1, comprising the steps of:

a) conducting said sample stream into a laminar flow channel;

b) conducting an indicator stream into said laminar flow channel, whereby said sample stream and said indicator stream flow in adjacent laminar streams in said channel;

c) allowing analyte particles to diffuse into said indicator stream;

d) detecting the presence of analyte particles in said indicator stream.

12. The method of claim 11 wherein said indicator stream comprises an indicator substance which indicates the presence of said analyte particles by a detectable change in property when contacted with particles of said analyte, and said detecting step comprises detecting a change in property of said indicator substance.

13. A method of determining the kinetic rate constant for a reaction using the channel cell system of claim 8, comprising the steps of:

(a) conducting said sample stream and said indicator stream into said laminar flow channel having an inlet;

(b) allowing analyte particles to diffuse into said indicator stream to form an analyte detection area; and

(c) detecting the presence of said analyte in said indicator stream at a known distance from said inlet.

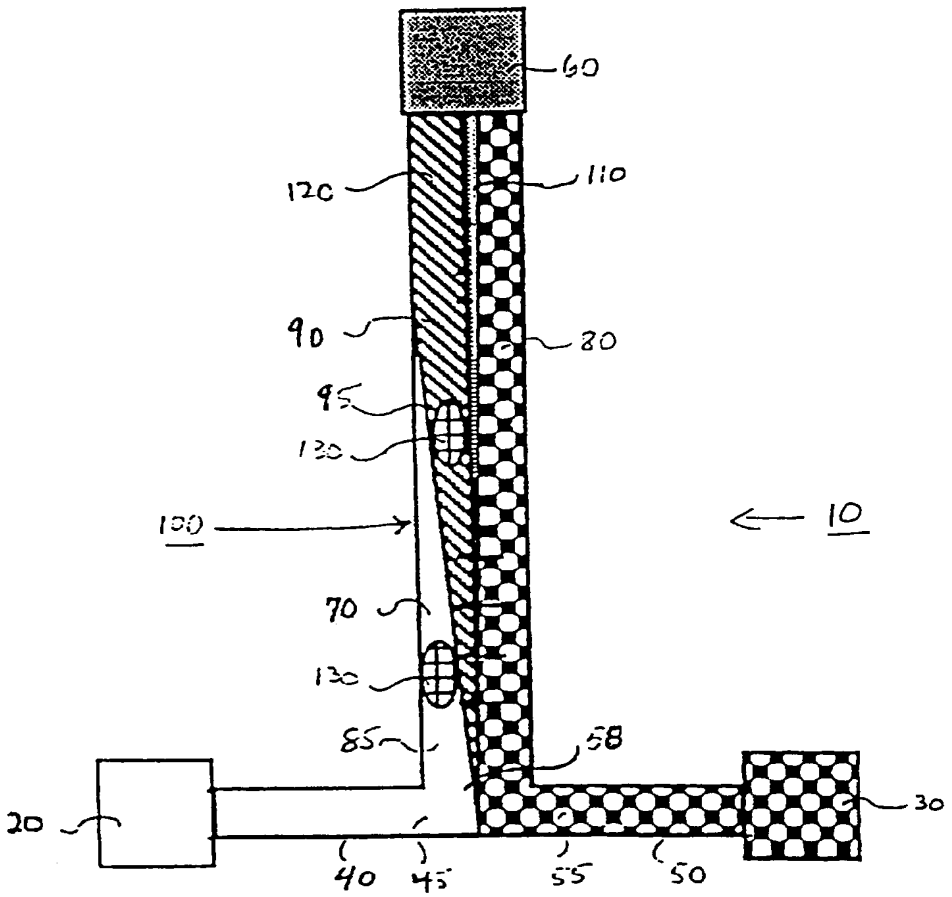

FIG. 1

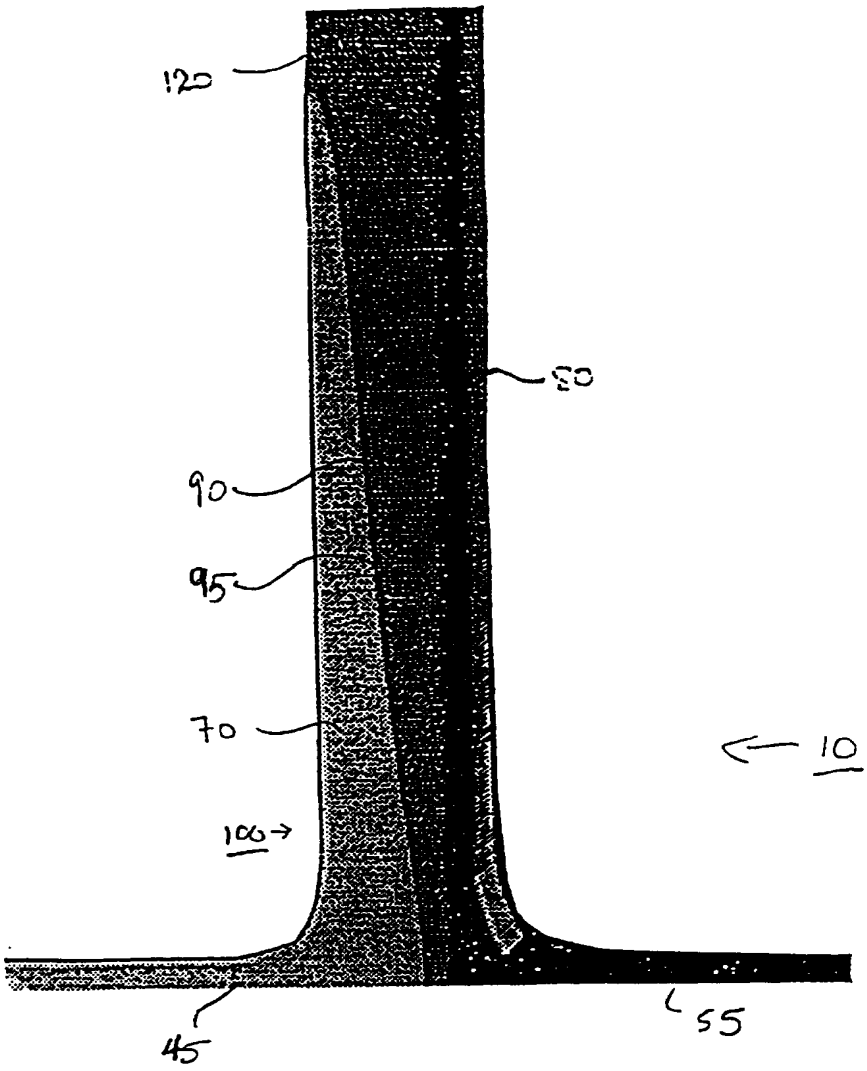

FIG. 2

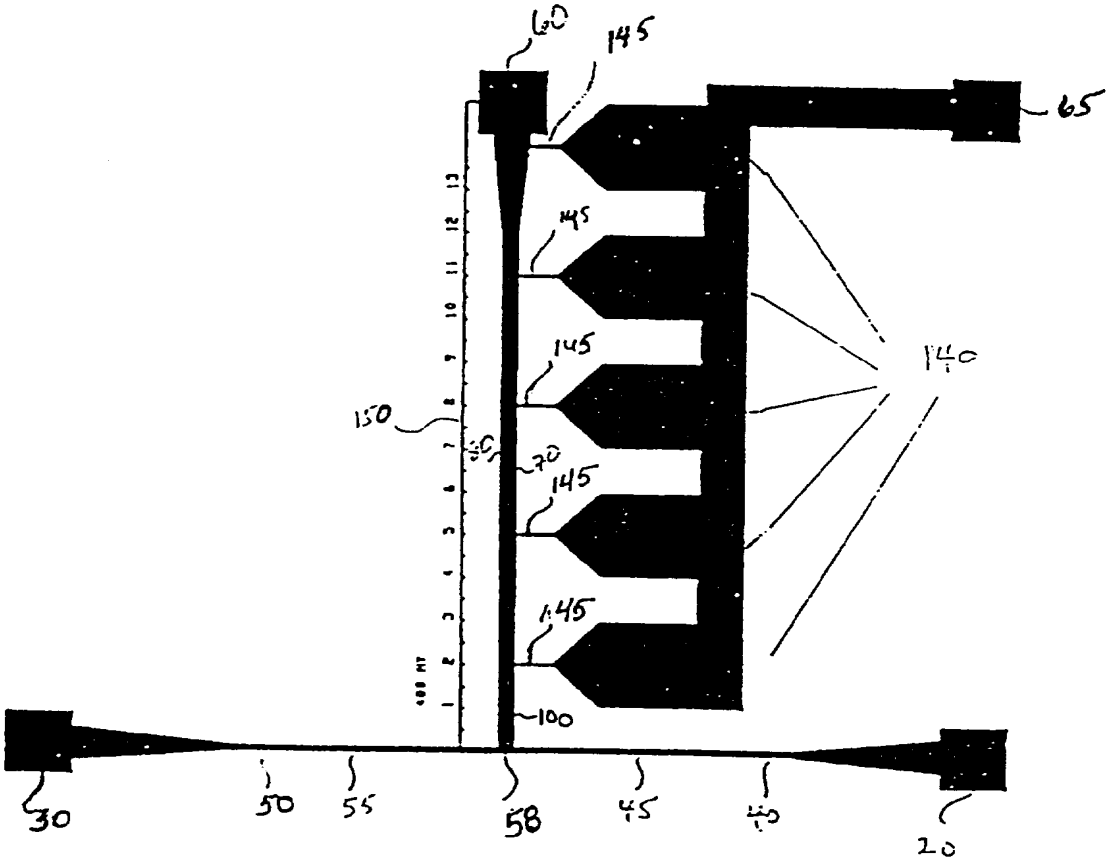

FIG. 3

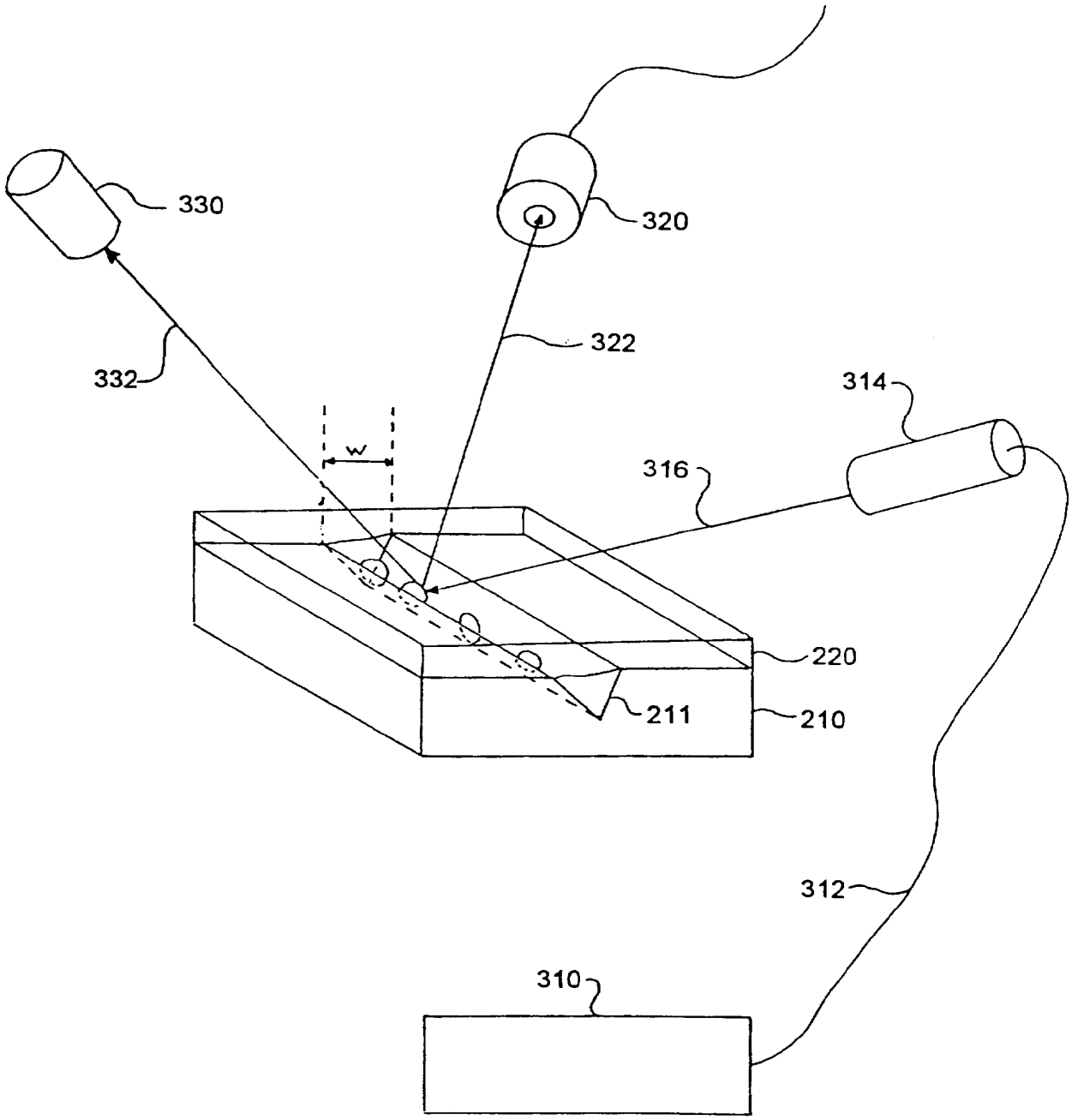

FIG. 4

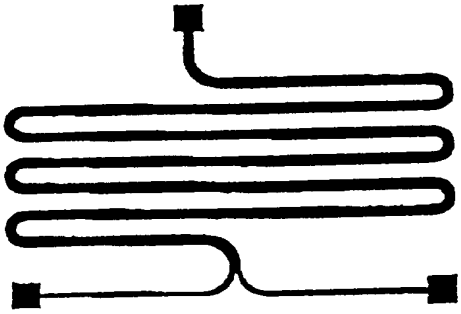

FIG. 5

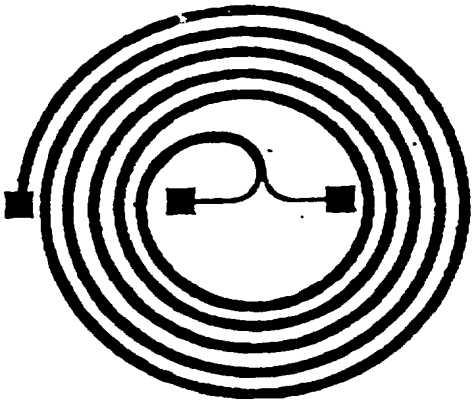

FIG. 6

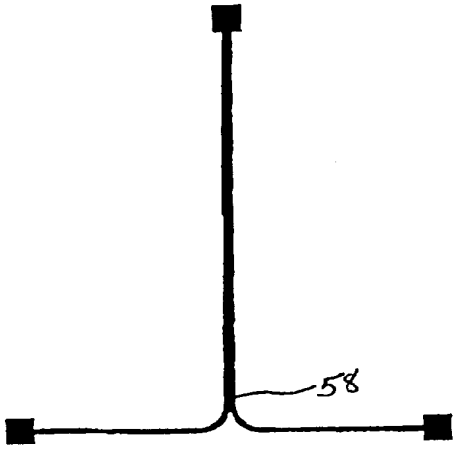

FIG. 7A

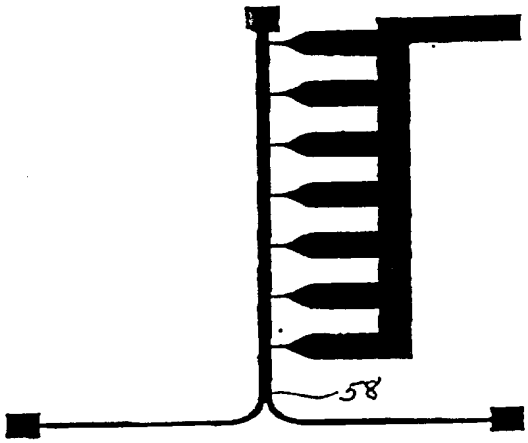

FIG. 7B

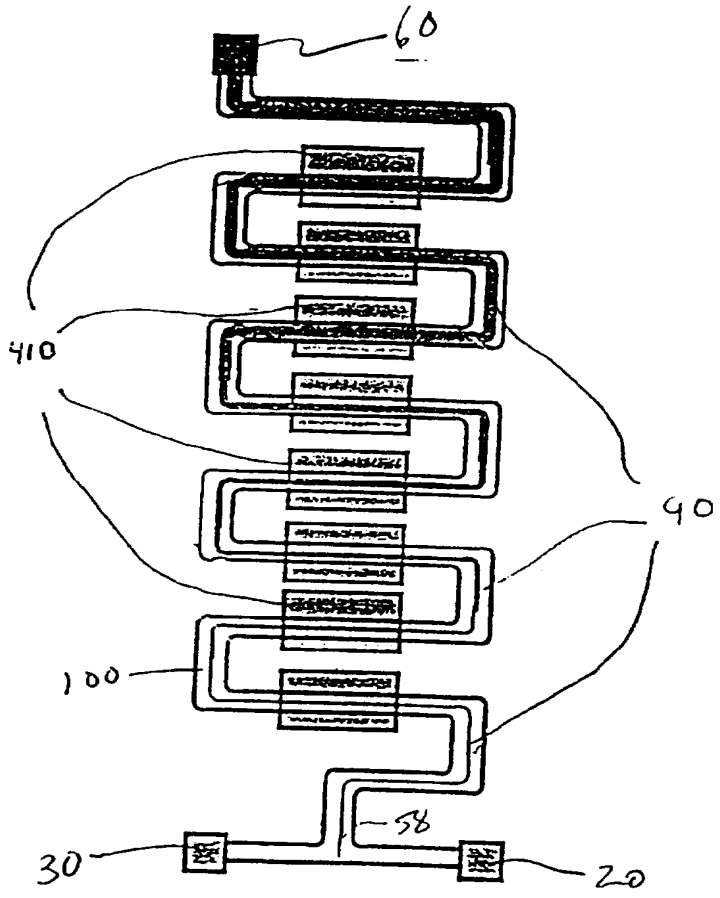

FIG. 8

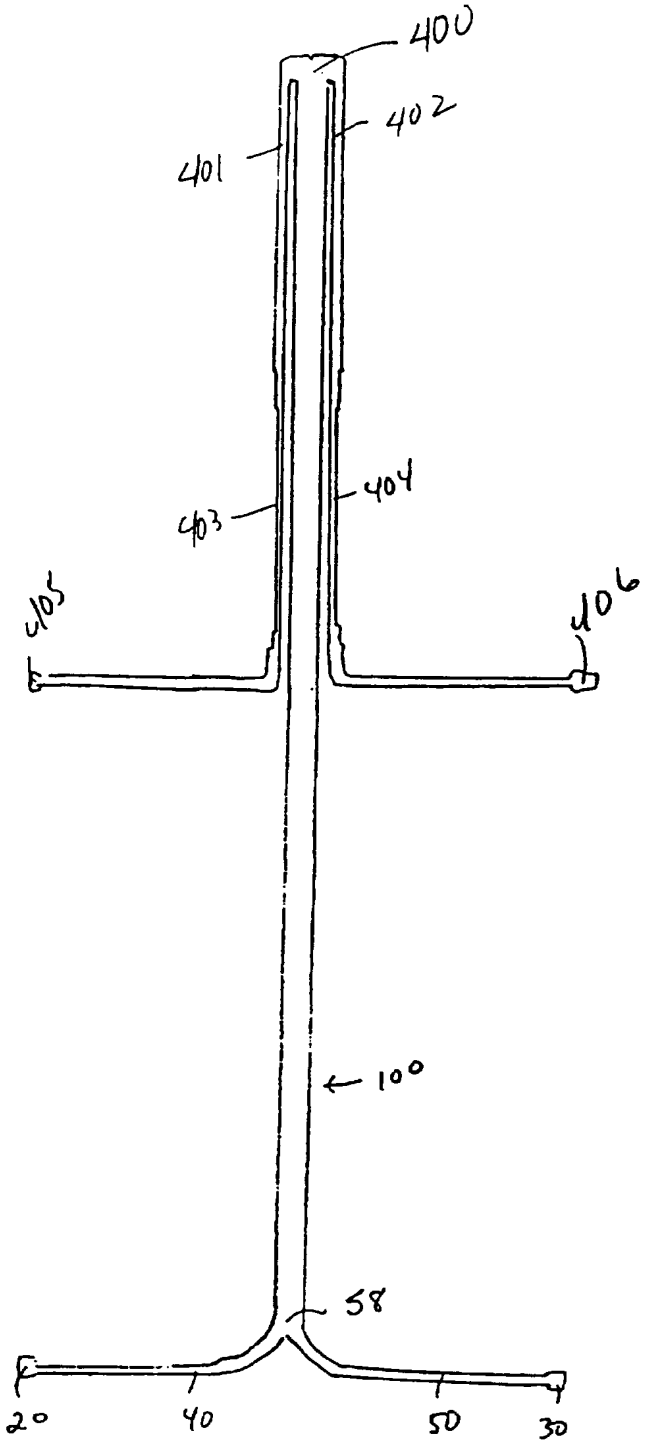
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FIG. 9A

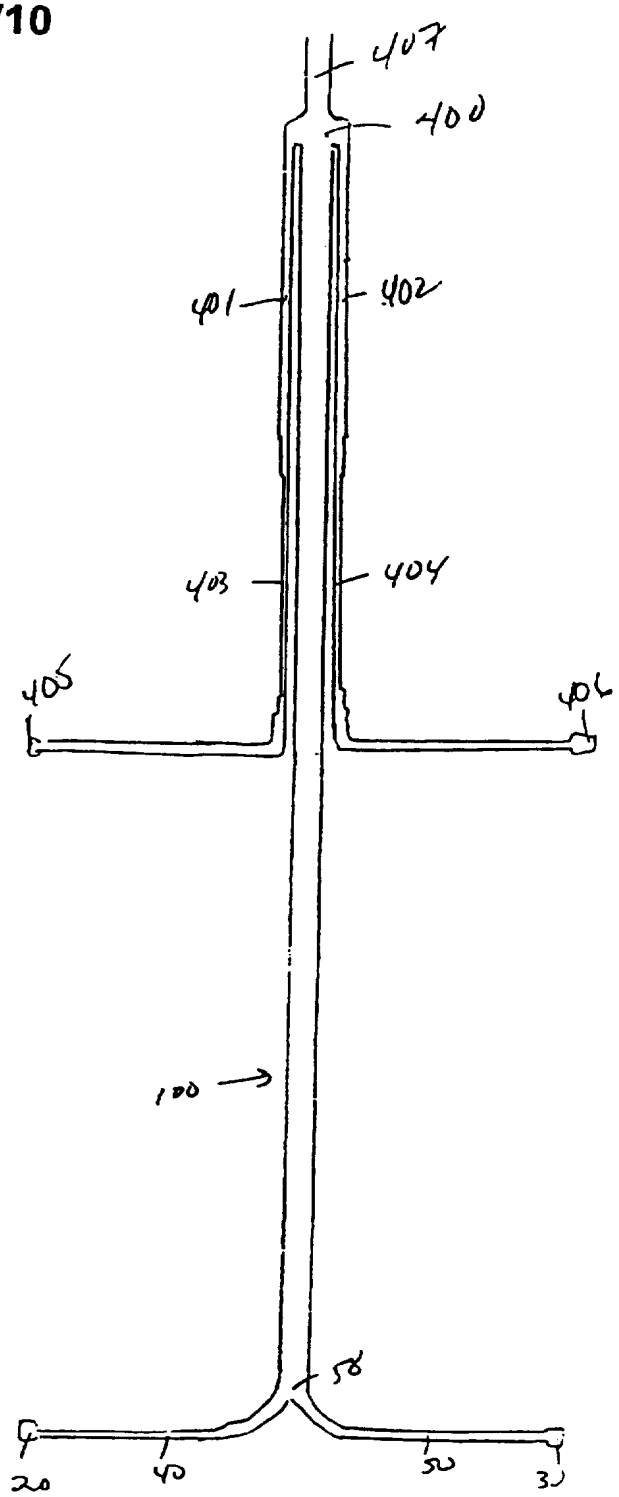
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FIG. 9B

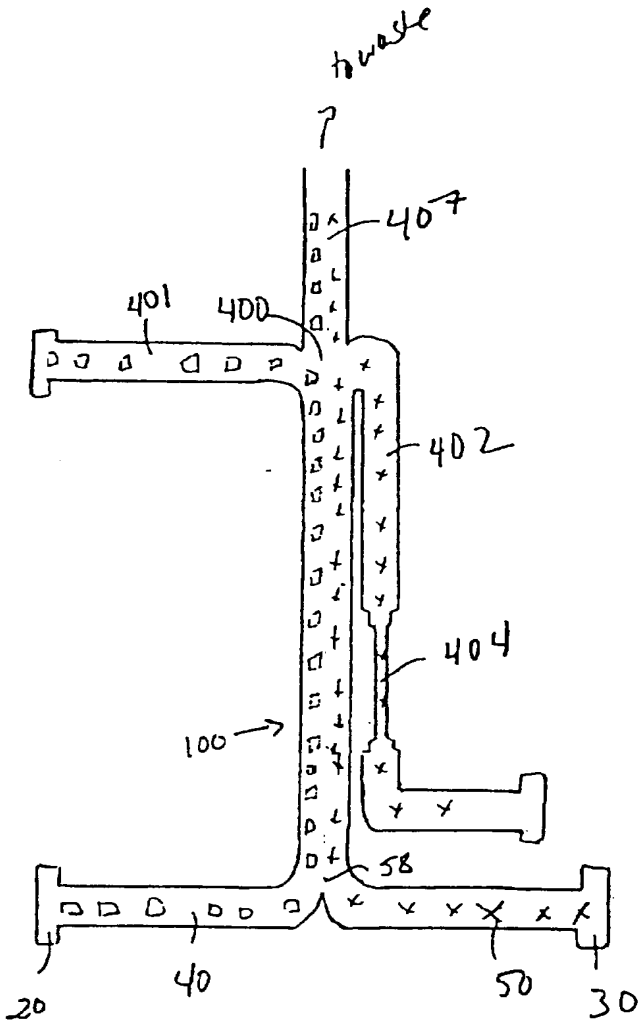


FIG. 9C

FIG. 10A

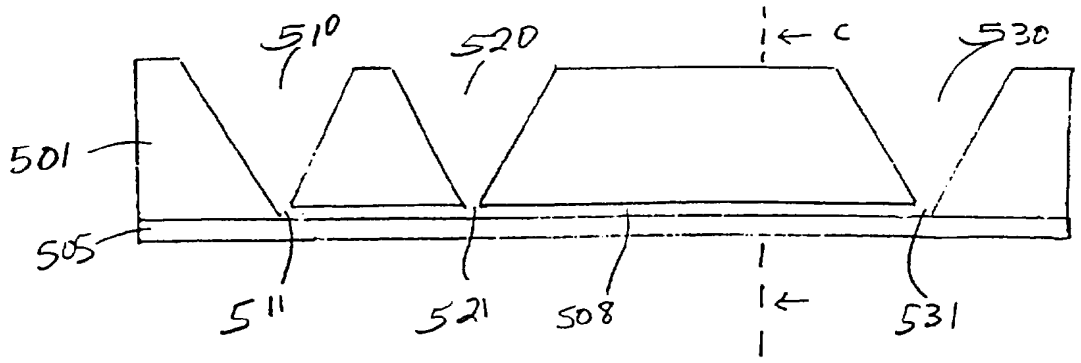


FIG. 10B

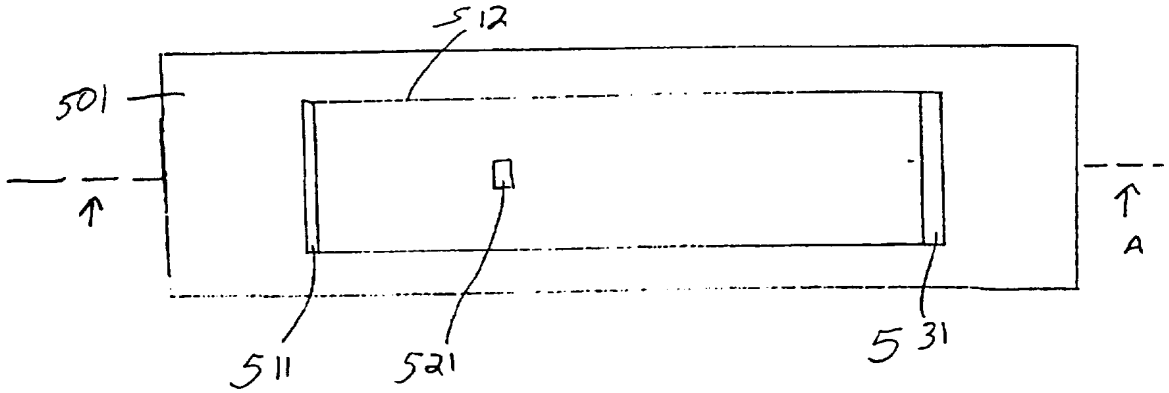
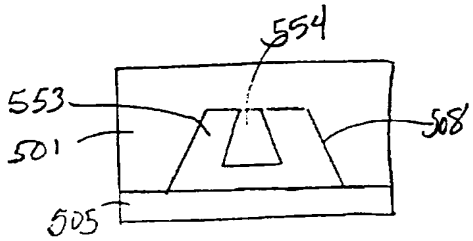


FIG. 10C



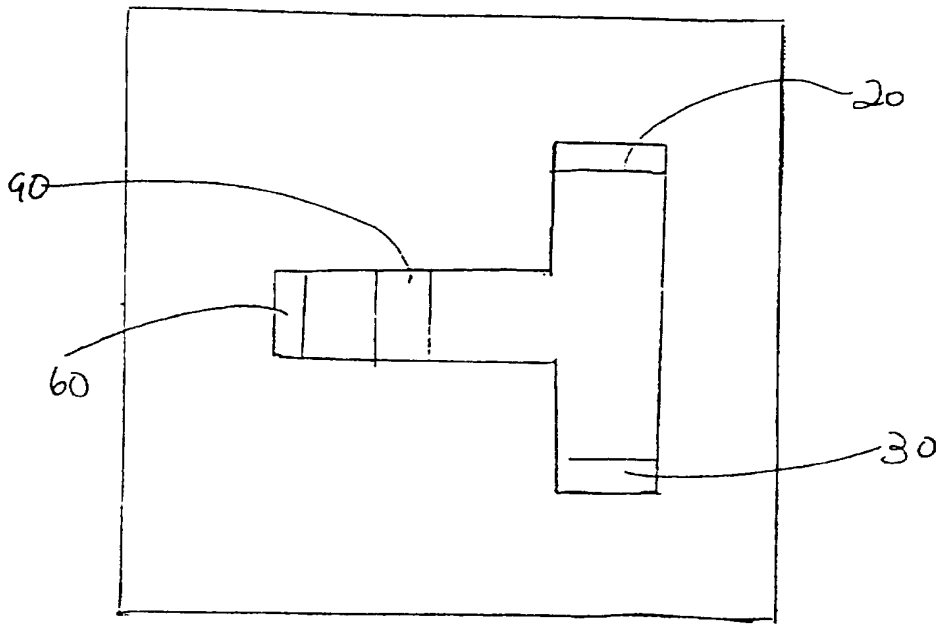


FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05245

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 21/64
US CL :436/172, 177, 180; 422/81

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/172, 177, 180; 422/81

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,683,212 A (UFFENHEIMER) 28 July 1987, entire document.	1-13
A	US 5,389,524 A (LARSEN et al) 14 February 1995, entire document.	1-13
A	US 4,737,268 A (GIDDINGS) 12 April 1988, entire document.	1-13
A	US 4,894,146 A (GIDDINGS) 16 January 1990, entire document.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 SEPTEMBER 1997	Date of mailing of the international search report 29 SEP 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JEFFREY R. SNAY Telephone No. (703) 308-0651

Form PCT/ISA/210 (second sheet)(July 1992)*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Daniel MUETH et al.

Attorney Docket No.: 81527.0018

Application No.: 14/169,927

Group Art Unit: 2881

Filed: January 31, 2014

Examiner: Kiet Tuan NGUYEN

Customer No.: 13155

Confirmation No.: 2069

For: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION (as amended)

INFORMATION DISCLOSURE STATEMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the provisions of 37 C.F.R. 1.56, 1.97 and 1.98, the attention of the U.S. Patent and Trademark Office is hereby directed to the references listed on the attached Form PTO/SB/08A. It is requested that the information on Form PTO/SB/08A be considered during the prosecution of this application, and that the references be made of record and appear among the "References Cited" on any issued patent.

Listed on Form PTO/SB/08A are twelve (12) references, which were cited in the attached Japanese Office Action, Reference No. 13, which was issued by the Japanese Patent Office on October 28, 2014, in connection with Japanese Patent Application No. 2011-256171, which is related to the present application. The attached English language abstracts and/or translations of the cited foreign references and Japanese Office Action should meet the requirements of a concise statement of relevance under 37 CFR 1.56(c).

___ This Information Disclosure Statement is being submitted:

___(1) within three months of the filing date of the application; or

____(2) after the filing date of the application and before the mailing date of a first Office Action on the merits; or

___(3) before the mailing of a first Office Action after the filing of a Request for Continued Examination; and thus, no Statement under 37 C.F.R. §1.97(e) or fee under 37 C.F.R. §1.17(p) is required.

___This Information Disclosure Statement is being submitted after the above periods (1)-(3), but before a Final Office Action or Notice of Allowance, a Statement under 37 CFR 1.97(e) is provided (see below), and no fee under 37 CFR 1.17(p) is due.

____ This Information Disclosure Statement is being submitted after the above periods (1)-(3), but before a Final Office Action or Notice of Allowance, and no Statement under 37 CFR 1.97(e) is provided, the fee under 37 CFR 1.17(p) is enclosed.

_X_ This Information Disclosure Statement is being submitted after the mailing date of a Final Office Action or Notice of Allowance, along with a Request for Consideration of an Information Disclosure Statement Filed after Payment of the Issue Fee Under the QPIDS Pilot Program and a Statement under 37 CFR 1.97(e) is provided (see below), and the fee under 37 CFR 1.17(p) is enclosed.

_X_ Statement under 37 CFR 1.97(e)(1): Each item of information contained in this Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the Information Disclosure Statement.

____ Statement under 37 CFR 1.97(e)(2): No item of information contained in this Information Disclosure Statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to our knowledge, after making reasonable inquiry, no item of information contained in the Information Disclosure Statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the Information Disclosure Statement.

_X_ Fee under 37 CFR 1.17(p) is enclosed (see Fee Transmittal).

The submission of the listed documents is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicants do not waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

Applicants hereby petition for any extension of time that may be required to maintain the pendency of this case, and any required fee for such an extension is to be charged to Deposit Account No. 50-5497.

Respectfully submitted,

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Date: November 17, 2014

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/169,927	12/02/2014	8901481	81527.0018	2069

13155 7590 11/12/2014

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ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

PREMIUM GENETICS (UK) LTD., Nantwich, UNITED KINGDOM, Assignee (with 37 CFR 1.172 Interest);
Daniel MUETH, Chicago, IL;
Joseph PLEWA, Park Ridge, IL;
Jessica SHIREMAN, Kansas City, MO;
Amy ANDERSON, Palatine, IL;
Lewis GRUBER, Chicago, IL;
Neil ROSENBAUM, Chicago, IL;

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/169,927	01/31/2014	Daniel MUETH	81527.0018	2069

13155 7590 10/30/2014

Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

EXAMINER	
NGUYEN, KIET TUAN	
ART UNIT	PAPER NUMBER
2881	

NOTIFICATION DATE	DELIVERY MODE
10/30/2014	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipgeneral@edwardsneils.com

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address : COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
14/169,927	31 January, 2014	MUETH ET AL.	81527.0018

Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

EXAMINER	
KIET T. NGUYEN	
ART UNIT	PAPER
2881	20141022

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

The Information Disclosure Statement (IDS) filed on 10-08-2014 has been considered. See the IDS form attached herewith.

/KIET T NGUYEN/
Primary Examiner, Art Unit 2881

PTO-90C (Rev.04-03)

IFW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Daniel MUETH et al.

Attorney Docket No.: 81527.0018

Application No.: 14/169,927

Group Art Unit: 2881

Filed: January 31, 2014

Examiner: Kiet Tuan NGUYEN

Customer No.: 13155

Confirmation No.: 2069

Continuation Patent Application of
U.S. Patent Application No.: 13/412,969 filed March 6, 2012

For: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION (as amended)

INFORMATION DISCLOSURE STATEMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sirs:

In accordance with the provisions of 37 C.F.R. 1.56, 1.97 and 1.98, the attention of the U.S. Patent and Trademark Office (USPTO) is hereby directed to the references listed on the attached Form PTO/SB/08A. It is requested that the information on Form PTO/SB/08A be considered during the prosecution of this application, and that the references be made of record and appear among the "References Cited" on any issued patent.

Listed on Form PTO/SB/08A are references 1-2, United States Patent Nos. 4,919,817 and 8,158,122, which are first cited in the Office Action issued by the USPTO on July 17, 2014.

Listed on Form PTO/SB/08B is reference 3, the cited Office Action issued by the USPTO on July 17, 2014 in connection with U.S. Patent Application No. 14/317,738, on which the present application is based.

10/09/2014 SMOHAMME 00000016 14169927
01 FC:1806 180.00 OP

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

The U.S. References cited in this submission are not included herewith as it is assumed that the USPTO has these references readily available.

____ This Information Disclosure Statement is being submitted:

____ (1) within three months of the filing date of the application; or

____ (2) after the filing date of the application and before the mailing date of a first Office Action on the merits; or

____ (3) before the mailing of a first Office Action after the filing of a Request for Continued Examination; and thus, no Statement under 37 C.F.R. §1.97(e) or fee under 37 C.F.R. §1.17(p) is required.

____ This Information Disclosure Statement is being submitted after the above periods (1)-(3), but before a Final Office Action or Notice of Allowance, a Statement under 37 CFR 1.97(e) is provided (see below), and no fee under 37 CFR 1.17(p) is due.

____ This Information Disclosure Statement is being submitted after the above periods (1)-(3), but before a Final Office Action or Notice of Allowance, and no Statement under 37 CFR 1.97(e) is provided, the fee under 37 CFR 1.17(p) is enclosed.

__X__ This Information Disclosure Statement is being submitted after the mailing date of a Final Office Action or Notice of Allowance, but before payment of the Issue Fee, and a Statement under 37 CFR 1.97(e) is provided (see below), and the fee under 37 CFR 1.17(p) is enclosed.

____ Statement under 37 CFR 1.97(e)(1): Each item of information contained in this Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the Information Disclosure Statement.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

__X__ Statement under 37 CFR 1.97(e)(2): No item of information contained in this Information Disclosure Statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to our knowledge, after making reasonable inquiry, no item of information contained in the Information Disclosure Statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the Information Disclosure Statement.

__X__ Fee under 37 CFR 1.17(p) is enclosed (see Fee Transmittal).

The submission of the listed documents is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicant does not waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

Applicants hereby petition for any extension of time that may be required to maintain the pendency of this case, and any required fee for such an extension is to be charged to Deposit Account No. 50-5497.

Respectfully submitted,

Jean C. Edwards

Jean C. Edwards
Registration No. 41,728

(13155)
EDWARDS NEILS PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191
Telephone: 703-466-0150
Facsimile: 703-537-8149
Date: October 8, 2014

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	14/169,927
Filing Date	January 31, 2014
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	Kiet Tuan NGUYEN
Attorney Docket Number	81527.0018

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	US- 4,919,817	04-24-1990	Schoendorfer et al.	
	2	US- 8,158,122	04-17-2012	Hampson et al.	
		US-			
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

Examiner Signature	/Kiet Tuan Nguyen/	Date Considered	10/22/2014

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

PTO/SB/08b (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	14/169,927
Filing Date	January 31, 2014
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	Kiet Tuan NGUYEN
Attorney Docket Number	81527.0018

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	3	Office Action issued by USPTO on July 17, 2014 in connection with related U.S. Patent Application No. 14/317,738	✓

Examiner Signature	/Kiet Tuan Nguyen/	Date Considered	10/22/2014

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached.

The PTO did not receive the following listed item(s)

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
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INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

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Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/169,927	01/31/2014	Daniel MUETH	81527.0018	2069

TITLE OF INVENTION: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	10/20/2014

EXAMINER	ART UNIT	CLASS-SUBCLASS
NGUYEN, KIET TUAN	2881	250-251000

10/17/2014 LNGUYEN2 00000035 14169927
01 FC:1501 960.00 OP
02 FC:1801 24.00 OP

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47: Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Jean C. Edwards, Esq.
2 Edwards Neils PLLC
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: PREMIUM GENETICS (UK) LTD.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Cheshire, UNITED KINGDOM

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
- ☒ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☒ Advance Order - # of Copies 8 (eight)

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ☐ A check is enclosed.
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- ☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 50-5497 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
  NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
  NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ☐ Applicant changing to regular undiscounted fee status.
  NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature Jean C. Edwards Date October 16, 2014

Typed or printed name Jean C. Edwards, Esq. Registration No. 41,728

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
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P.O. Box 1450
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Edwards Neils PLLC
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(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/169,927	01/31/2014	Daniel MUETH	81527.0018	2069

TITLE OF INVENTION: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	10/20/2014

EXAMINER	ART UNIT	CLASS-SUBCLASS
NGUYEN, KIET TUAN	2881	250-251000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
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2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Jean C. Edwards, Esq.
2 Edwards Neils PLLC
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

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(B) RESIDENCE: (CITY and STATE OR COUNTRY): Cheshire, UNITED KINGDOM

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
- ☒ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☒ Advance Order - # of Copies 8 (eight)

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
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NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature Jean C. Edwards Date October 16, 2014

Typed or printed name Jean C. Edwards, Esq. Registration No. 41,728

Doc Code: TRAN.LET

Document Description: Transmittal Letter

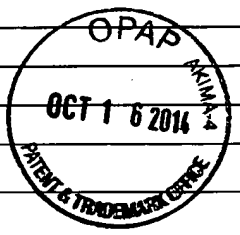
PTO/SB/21 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	14/169,927
Filing Date	January 31, 2014
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	Kiet Tuan NGUYEN
Attorney Docket Number	81527.0018

Total Number of Pages in This Submission



ENCLOSURES *(Check all that apply)*

- [x] Fee Transmittal Form
  - [x] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):
  -PTOL-85 Part B Fee Transmittal (in duplicate);
  -Credit Card Payment Form

Remarks

- Please charge any deficiences or credit any overpayments to deposit account no. 50-5497.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	EDWARDS NEILS PLLC		
Signature	Jean C. Edwards		
Printed name	Jean C. Edwards, Esq.		
Date	October 16, 2014	Reg. No.	41,728

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name		Date	

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Daniel MUETH et al.

Attorney Docket No.: 81527.0018

Application No.: 14/169,927

Group Art Unit: 2881

Filed: January 31, 2014

Examiner: Kiet Tuan NGUYEN

Customer No.: 13155

Confirmation No.: 2069

Continuation Patent Application of
U.S. Patent Application No.: 13/412,969 filed March 6, 2012

For: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION (as amended)

INFORMATION DISCLOSURE STATEMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sirs:

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Listed on Form PTO/SB/08B is reference 3, the cited Office Action issued by the USPTO on July 17, 2014 in connection with U.S. Patent Application No. 14/317,738, on which the present application is based.

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____ (1) within three months of the filing date of the application; or

_____ (2) after the filing date of the application and before the mailing date of a first Office Action on the merits; or

_____ (3) before the mailing of a first Office Action after the filing of a Request for Continued Examination; and thus, no Statement under 37 C.F.R. §1.97(e) or fee under 37 C.F.R. §1.17(p) is required.

____ This Information Disclosure Statement is being submitted after the above periods (1)-(3), but before a Final Office Action or Notice of Allowance, a Statement under 37 CFR 1.97(e) is provided (see below), and no fee under 37 CFR 1.17(p) is due.

_____ This Information Disclosure Statement is being submitted after the above periods (1)-(3), but before a Final Office Action or Notice of Allowance, and no Statement under 37 CFR 1.97(e) is provided, the fee under 37 CFR 1.17(p) is enclosed.

__X__ This Information Disclosure Statement is being submitted after the mailing date of a Final Office Action or Notice of Allowance, but before payment of the Issue Fee, and a Statement under 37 CFR 1.97(e) is provided (see below), and the fee under 37 CFR 1.17(p) is enclosed.

_____ Statement under 37 CFR 1.97(e)(1): Each item of information contained in this Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the Information Disclosure Statement.

__X__ Statement under 37 CFR 1.97(e)(2): No item of information contained in this Information Disclosure Statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to our knowledge, after making reasonable inquiry, no item of information contained in the Information Disclosure Statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the Information Disclosure Statement.

__X__ Fee under 37 CFR 1.17(p) is enclosed (see Fee Transmittal).

The submission of the listed documents is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicant does not waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

Applicants hereby petition for any extension of time that may be required to maintain the pendency of this case, and any required fee for such an extension is to be charged to Deposit Account No. 50-5497.

Respectfully submitted,

Jean C. Edwards

Jean C. Edwards
Registration No. 41,728

(13155)
EDWARDS NEILS PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191
Telephone: 703-466-0150
Facsimile: 703-537-8149
Date: October 8, 2014

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	14/169,927
Filing Date	January 31, 2014
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	Kiet Tuan NGUYEN
Attorney Docket Number	81527.0018

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	US- 4,919,817	04-24-1990	Schoendorfer et al.	
	2	US- 8,158,122	04-17-2012	Hampson et al.	
		US-			
		US-			
		US-			
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		US-			

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08b (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	14/169,927
Filing Date	January 31, 2014
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	Kiet Tuan NGUYEN
Attorney Docket Number	81527.0018

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	3	Office Action issued by USPTO on July 17, 2014 in connection with related U.S. Patent Application No. 14/317,738	✓

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached.

The PTO did not receive the following listed Item(s) MPH

Doc Code: TRAN.LET

Document Description: Transmittal Letter

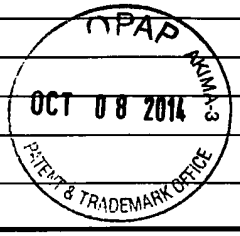
PTO/SB/21 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	

Application Number	14/169,927
Filing Date	January 31, 2014
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	Kiet Tuan NGUYEN
Attorney Docket Number	81527.0018



ENCLOSURES *(Check all that apply)*

- ☑ Fee Transmittal Form
  - ☑ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☑ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☑ Other Enclosure(s) (please identify below):
  - -IDS w/ Form PTO/SB/08A and PTO/SB/08B;
  - -Fee Transmittal Form;
  - -Credit Card Payment Form;
  - -2 (two) cited references

Remarks

- Please charge any deficiences or credit any overpayments to deposit account no. 50-5497.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	EDWARDS NEILS PLLC		
Signature	Jean C. Edwards		
Printed name	Jean C. Edwards, Esq.		
Date	October 8, 2014	Reg. No.	41,728

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name		Date	

PTO/SB/17 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995 no persons are required to respond to a collection of information unless it displays a valid OMB control number

FEE TRANSMITTAL

Complete if known	
Application Number	14/169,927
Filing Date	January 31, 2014
First Named Inventor	Daniel MUETH
Examiner Name	Kiet Tuan NGUYEN
Art Unit	2881
Practitioner Docket No.	81527.0018

☐ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

TOTAL AMOUNT OF PAYMENT ($) 180.00

METHOD OF PAYMENT (check all that apply)

☐ Check ☑ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☑ Deposit Account Deposit Account Number: 50-5497 Deposit Account Name: Edwards Neils PLLC

For the above-identified deposit account, the Director is hereby authorized to (check all that apply):

☐ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except** for the filing fee

☑ Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17 ☑ Credit any overpayment of fee(s)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)

	FILING FEES			SEARCH FEES			EXAMINATION FEES			
Application Type	U ($)	S ($)	M ($)	U ($)	S ($)	M ($)	U ($)	S ($)	M ($)	Fees Paid ($)
Utility	280	140*	70	600	300	150	720	360	180	
Design	180	90	45	120	60	30	460	230	115	
Plant	180	90	45	380	190	95	580	290	145	
Reissue	280	140	70	600	300	150	2,160	1,080	540	
Provisional	260	130	65	0	0	0	0	0	0	

* The $140 small entity status filing fee for a utility application is further reduced to $70 for a small entity status applicant who files the application via EFS-Web.

2. EXCESS CLAIM FEES

Fee Description	Undiscounted Fee ($)	Small Entity Fee ($)	Micro Entity Fee ($)
Each claim over 20 (including Reissues)	80	40	20
Each independent claim over 3 (including Reissues)	420	210	105
Multiple dependent claims	780	390	195

Total Claims		Extra Claims	Fee ($)		Fee Paid ($)
_____	-20 or HP =	_____	x _____	=	_____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims		Extra Claims	Fee ($)		Fee Paid ($)
_____	-3 or HP =	_____	x _____	=	_____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	Fee ($)	Fee Paid ($)
	_____	_____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $400 ($200 for small entity) ($100 for micro entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets		Extra Sheets		Number of each additional 50 or fraction thereof		Fee ($)		Fee Paid ($)
_____	- 100 =	_____	/ 50 =	_____ (round up to a whole number)	x	_____	=	_____

4. OTHER FEE(S) Fees Paid ($)

Non-English specification, $130 fee (no small or micro entity discount) _____

Non-electronic filing fee under 37 CFR 1.16(t) for a utility application, $400 fee ($200 small or micro entity) _____

Other (e.g., late filing surcharge): -Information Disclosure Fee 180.00

SUBMITTED BY

Signature	Jean C. Edwards	Registration No. (Attorney/Agent) 41,728	Telephone 703-466-0150
Name (Print/Type)	Jean C. Edwards, Esq.		Date October 8, 2014

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

13155 7590 07/18/2014

Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

EXAMINER
NGUYEN, KIET TUAN

ART UNIT	PAPER NUMBER
2881	

DATE MAILED: 07/18/2014

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/169,927	01/31/2014	Daniel MUETH	81527.0018	2069

TITLE OF INVENTION: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	10/20/2014

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

13155 7590 07/18/2014
Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/169,927	01/31/2014	Daniel MUETH	81527.0018	2069

TITLE OF INVENTION: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	10/20/2014

EXAMINER	ART UNIT	CLASS-SUBCLASS
NGUYEN, KIET TUAN	2881	250-251000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29
  NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27
  NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status.
  NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/169,927	01/31/2014	Daniel MUETH	81527.0018	2069

13155 7590 07/18/2014

Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

EXAMINER	
NGUYEN, KIET TUAN	
ART UNIT	PAPER NUMBER
2881	

DATE MAILED: 07/18/2014

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 14/169,927	Applicant(s) MUETH ET AL.	
	Examiner KIET T. NGUYEN	Art Unit 2881	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☐ This communication is responsive to _____.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *73-86*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 3/26/14
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/KIET T NGUYEN/
Primary Examiner, Art Unit 2881

The present application is being examined under the pre-AIA first to invent provisions.

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

In The Claims

Claim 72 has been cancelled.

Authorization for this examiner's amendment was given in a telephone interview with Jean C. Edwards on 06-26-2014.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kiet T. Nguyen whose telephone number is 571-272-2479. The examiner can normally be reached on Monday-Friday 8-6.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert H. Kim can be reached on 571-272-2293. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Kiet T. Nguyen/
Primary Examiner, Art Unit 2881

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2147	((sort$5 or separat$5 or select$5 or identif$5 or examin$5 or operat$3) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1) adj30 (fluid$1 or liquid$1 or blood$1)) and ((buffer$5 or solution$1) adj30 (input$5 or inlet$5)) and (channel$1 or port$1 or flow$5) and (flow$5 adj20 direction$1)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/06/30 04:35
L2	26283	(detect$5 adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1)) and laser$1 and ((damag$5 or kill$5 or destro$5) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/06/30 04:36
L3	262	l1 and l2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/06/30 04:36
L4	1422	((sort$5 or separat$5 or select$5 or identif$5 or examin$5 or operat$3) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1)) and (fluid$1 or liquid$1 or blood$1) and (chamber$1 or enclosur$5) and ((input$5 or inlet$5) adj20 (channel$1 or port$1 or flow$5)) and (sheath adj10 (buffer$5 or solution$1 or fluid$1 or liquid$1))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/06/30 04:37
L5	16	((rate$1 or pressure$1) adj20 sheath) and (orthogonal$3 adj10 direct$3) and detect$5 and ((damag$5 or kill$5 or destro$5) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1)) and laser$1	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/06/30 04:38
L6	6	l4 and l5	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/06/30 04:38

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp

L7	204846	"l1" and "l2"	US-PGPUB; USPAT; UPAD	OR	ON	2014/06/30 04:40
L8	31754	"l4" and "l5"	US-PGPUB; USPAT; UPAD	OR	ON	2014/06/30 04:41

6/ 30/ 2014 4:41:56 AM
C:\ Users\ knguyen2\ Documents\ EAST\ Workspaces\ default.wsp

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14169927	MUETH ET AL.
	Examiner	Art Unit
	KIET T NGUYEN	2881

CPC- SEARCHED		
Symbol	Date	Examiner
B01D	6/30/14	KN

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner
N/A	6/30/14	KN

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
250	251	6/30/14	KN
356	244,246	6/30/14	KN
494	36,45	6/30/14	KN
435	173.1	6/30/14	KN
210	732,800,802	6/30/14	KN

SEARCH NOTES		
Search Notes	Date	Examiner
EAST attached	6/30/14	KN

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
250;356;494;435;210/B01D	251;244,246;36,45;173.1;732,800,802/21/26	6/30/14	KN

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14169927	MUETH ET AL.
	Examiner	Art Unit
	KIET T NGUYEN	2881

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/30/2014								
	1	-								
	2	-								
	3	-								
	4	-								
	5	-								
	6	-								
	7	-								
	8	-								
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Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14169927	MUETH ET AL.
	Examiner	Art Unit
	KIET T NGUYEN	2881

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/30/2014								
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	38	-								
	39	-								
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	72	-								

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14169927	MUETH ET AL.
	Examiner	Art Unit
	KIET T NGUYEN	2881

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	06/30/2014								
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4	76	=								
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7	79	=								
10	80	=								
11	81	=								
8	82	=								
9	83	=								
12	84	=								
13	85	=								
14	86	=								

PTO/SB/08a (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	14/169,927
Filing Date	January 31, 2014
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	~~January 31, 2014~~ Kiet T. Nguyen
Attorney Docket Number	81527.0018

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	~~1~~	~~US 6,416,190 B1~~	~~07-09-2002~~	~~Grier et al.~~	
		US- (See the IDS filed on 01-31-2014)			
		US-			
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		US-			
		US-			

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	2	JPH-06-265452-A	09-22-1994	PRIMA MEAT PACKERS...	Abstract	✓

Examiner Signature	/Kiet Tuan Nguyen/	Date Considered	06/30/2014

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

Issue Classification

Application/Control No.	Applicant(s)/Patent Under Reexamination
14169927	MUETH ET AL.
Examiner	**Art Unit**
KIET T NGUYEN	2881

CPC							
Symbol						**Type**	**Version**
G03H		1	/	2294		A	2013-01-01
G03H		2001	/	0077		A	2013-01-01
G02B		21	/	32		I	2013-01-01
G01N		33	/	487		F	2013-01-01
B01D		21	/	01		I	2013-01-01
G03H		2225	/	32		A	2013-01-01
G03H		1	/	08		A	2013-01-01
H05H		3	/	04		I	2013-01-01
G03H		2001	/	085		A	2013-01-01
			/				
			/				
			/				
			/				
			/				
			/				

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	14	
/KIET T NGUYEN/ Primary Examiner.Art Unit 2881	6/30/14	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	5

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14169927	MUETH ET AL.
	Examiner	**Art Unit**
	KIET T NGUYEN	2881

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION	
CLASS	SUBCLASS	CLAIMED	NON-CLAIMED
250	251	B 0 1 D 21 / 26 (2006.01.01)	

CROSS REFERENCE(S)					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				
356	244	246			
494	36	45			
435	173.1				
210	732	800	802		

NONE	Total Claims Allowed:	
(Assistant Examiner) (Date)	14	
/KIET T NGUYEN/ Primary Examiner.Art Unit 2881 6/30/14	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner) (Date)	1	5

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14169927	MUETH ET AL.
	Examiner	**Art Unit**
	KIET T NGUYEN	2881

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
	1		17		33		49		65	11	81				
	2		18		34		50		66	8	82				
	3		19		35		51		67	9	83				
	4		20		36		52		68	12	84				
	5		21		37		53		69	13	85				
	6		22		38		54		70	14	86				
	7		23		39		55		71						
	8		24		40		56		72						
	9		25		41		57	1	73						
	10		26		42		58	2	74						
	11		27		43		59	3	75						
	12		28		44		60	4	76						
	13		29		45		61	5	77						
	14		30		46		62	6	78						
	15		31		47		63	7	79						
	16		32		48		64	10	80						

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 14	
/KIET T NGUYEN/ Primary Examiner.Art Unit 2881 (Primary Examiner)	6/30/14 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 5

Electronic Patent Application Fee Transmittal

Application Number:	14169927
Filing Date:	31-Jan-2014
Title of Invention:	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
First Named Inventor/Applicant Name:	Daniel MUETH
Filer:	Jean Ceceille Edwards./Kenneth Sowers
Attorney Docket Number:	81527.0018

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 1 month with $0 paid	1251	1	200	200

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**200**

Electronic Acknowledgement Receipt

EFS ID:	19430883
Application Number:	14169927
International Application Number:	
Confirmation Number:	2069
Title of Invention:	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
First Named Inventor/Applicant Name:	Daniel MUETH
Customer Number:	13155
Filer:	Jean Ceceille Edwards.
Filer Authorized By:	
Attorney Docket Number:	81527.0018
Receipt Date:	27-JUN-2014
Filing Date:	31-JAN-2014
Time Stamp:	10:21:00
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$200
RAM confirmation Number	8354
Deposit Account	505497
Authorized User	NEILS, PAUL F

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Fee Worksheet (SB06)	fee-info.pdf	30605 ffc1c32e0b53c2d50c1355d87042679168bed130	no	2

Warnings:

Information:

Total Files Size (in bytes):	30605

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/169,927	01/31/2014	Daniel MUETH	81527.0018

CONFIRMATION NO. 2069

13155
Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

PUBLICATION NOTICE

OC000000068703863

Title:MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

Publication No.US-2014-0147881-A1
Publication Date:05/29/2014

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/169,927	01/31/2014	Daniel MUETH	81527.0018

CONFIRMATION NO. 2069

13155
Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

NOTICE

OC000000066602055

2-18-14

Date Mailed: 02/18/2014

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

A new inventor's oath or declaration that identifies this application (e.g., by Application Number and filing date) is required. The inventor's oath or declaration does not comply with 37 CFR 1.63 in that it:

- does not state that the above-identified application was made or authorized to be made by the person executing the oath or declaration.
  - Daniel MUETH
  - Joseph PLEWA
  - Jessica SHIREMAN
  - Amy ANDERSON
  - Lewis GRUBER
  - Neil ROSENBAUM
- does not include an acknowledgement that any willful false statement made in such declaration or statement is punishable under section 1001 of title 18 by fine or imprisonment of not more than five (5) years, or both.
  - Daniel MUETH
  - Joseph PLEWA
  - Jessica SHIREMAN
  - Amy ANDERSON
  - Lewis GRUBER
  - Neil ROSENBAUM

Electronic Patent Application Fee Transmittal

Application Number:	14169927
Filing Date:	31-Jan-2014
Title of Invention:	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
First Named Inventor/Applicant Name:	Daniel MUETH
Filer:	Jean Ceceille Edwards./David Engstrom
Attorney Docket Number:	81527.0018

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Late Filing Fee for Oath or Declaration	1051	1	140	140
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**140**

Electronic Acknowledgement Receipt

EFS ID:	18753270
Application Number:	14169927
International Application Number:	
Confirmation Number:	2069
Title of Invention:	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
First Named Inventor/Applicant Name:	Daniel MUETH
Customer Number:	13155
Filer:	Jean Ceceille Edwards./David Engstrom
Filer Authorized By:	Jean Ceceille Edwards.
Attorney Docket Number:	81527.0018
Receipt Date:	14-APR-2014
Filing Date:	31-JAN-2014
Time Stamp:	14:25:28
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$140
RAM confirmation Number	795
Deposit Account	505497
Authorized User	NEILS, PAUL F

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	81527-0018_TTL.pdf	29325 7ae8bce02d35f70f385e3cb49f08434ac36285b1	no	2
Warnings:					
Information:					
2	Oath or Declaration filed	81527-0018_EXECUTED-DECLARATION-INVENTOR-1.pdf	108271 036d40e49ae537af78e4b87840cebfe86be89bb6	no	2
Warnings:					
Information:					
3	Oath or Declaration filed	81527-0018_EXECUTED-DECLARATION-INVENTOR-2.pdf	111702 8d4eebcf55002a5f5e67b20c96fb3cebbc1dbaf4	no	2
Warnings:					
Information:					
4	Oath or Declaration filed	81527-0018_EXECUTED-DECLARATION-INVENTOR-3.pdf	108119 b5ec62a9daaa4e1c8b72217584a19fc2ae216782	no	2
Warnings:					
Information:					
5	Oath or Declaration filed	81527-0018_EXECUTED-DECLARATION-INVENTOR-4.pdf	107033 154ef231a86ff782b9632c372eb65588eac5d946	no	2
Warnings:					
Information:					
6	Oath or Declaration filed	81527-0018_EXECUTED-DECLARATION-INVENTOR-5.pdf	109902 465ddebb9d4b0d6c02d471dea500d06d530489cf	no	2
Warnings:					
Information:					
7	Oath or Declaration filed	81527-0018_EXECUTED-DECLARATION-INVENTOR-6.pdf	108720 4329b6e4b1e381722a71df41a9bbda171e35dbdd	no	2
Warnings:					
Information:					
8	Miscellaneous Incoming Letter	81527-0018_INFORMATIONAL-NOTICE-TO-APPLICANT.pdf	71495 9c9b10652d006581452fdbe465545c229fc06895	no	1

Warnings:					
Information:					
9	Fee Worksheet (SB06)	fee-info.pdf	30374 1ad9ef8af34bdce4aed6f236519cf3d2e877b2ec	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	784941	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Daniel MUETH et al.	Group Art Unit: 2881
Application No.: 14/169,927	Examiner: Kiet Tuan NGUYEN
Filed: January 31, 2014	Atty Dkt No.: 81527.0018
Confirmation No.: 2069	Customer No.: 13155

For: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION (as amended)

TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Please find enclosed:

- Executed Declarations of 'Daniel MUETH', 'Amy ANDERSON', 'Jessica SHIREMAN', 'Joseph PLEWA', 'Neil ROSENBAUM', and 'Lewis GRUBER'
- Copy of Informational Notice to Applicant dated February 18, 2014

Please charge any deficiencies or credit any overpayments to Deposit Account No. 50-5497.

In the event the credit card payment submitted herewith fails to process, please charge the filing fees to deposit account No. 50-5497.

Applicants hereby petition for any extension of time that may be required to maintain the pendency of this case, and any required fee for such an extension is to be charged to Deposit Account No. 50-5497.

Respectfully submitted,

/Jean C. Edwards/
Jean C. Edwards
Registration No. 41,728

(13155)
EDWARDS NEILS PLLC
12020 Sunrise Valley Drive
Suite 200
Reston, VA 20191
Telephone: 703-466-0150
Facsimile: 703-537-8149
Date: April 14, 2014

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☒ United States application or PCT international application number 14/169,927 filed on January 31, 2014.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

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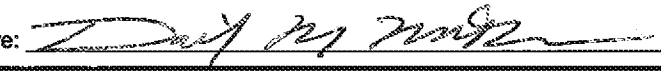
LEGAL NAME OF INVENTOR

Inventor: Daniel MUETH Date (Optional): March 15, 2014

Signature: 

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/AIA/01 form for each additional inventor.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☒ United States application or PCT international application number 14/169,927 filed on January 31, 2014.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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LEGAL NAME OF INVENTOR

Inventor: Amy ANDERSON Date (Optional): 31 MAR2014

Signature: [signature]

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/AIA/01 form for each additional inventor.

Privacy Act Statement

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

As the below named inventor, I hereby declare that:

This declaration is directed to:

- ☐ The attached application, or
- ☒ United States application or PCT international application number 14/169,927 filed on January 31, 2014

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

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LEGAL NAME OF INVENTOR

Inventor: Jessica SHIREMAN Date (Optional): 3/24/14

Signature: Jessica Shireman

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/AIA/01 form for each additional inventor.

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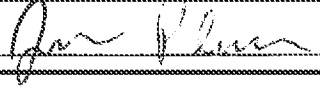
LEGAL NAME OF INVENTOR

Inventor: Joseph PLEWA Date (Optional): _____

Signature: 

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 14/169,927

filed on January 31, 2014.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Neil ROSENBAUM Date (Optional): 3/27/2014

Signature: Neil Rosenbaum

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/AIA/01 form for each additional inventor.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

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LEGAL NAME OF INVENTOR

Inventor: Lewis GRUBER Date (Optional): APRIL 4, 2014

Signature: LEWIS S GRUBER

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/AIA/01 form for each additional inventor.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Daniel MUETH et al.

Attorney Docket No.: 81527.0018

Application No.: 14/169,927

Group Art Unit: 2881

Filed: January 31, 2014

Examiner: Kiet Tuan NGUYEN

Customer No.: 13155

Confirmation No.: 2069

Continuation Patent Application of
U.S. Patent Application No.: 13/412,969 filed March 6, 2012

For: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION (as amended)

INFORMATION DISCLOSURE STATEMENT

MAIL STOP APPLICATION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Madam:

In accordance with the provisions of 37 C.F.R. 1.56, 1.97 and 1.98, the attention of the U.S. Patent and Trademark Office is hereby directed to the references listed on the attached Form PTO/SB/08A. It is requested that the information on Form PTO/SB/08A be considered during the prosecution of this application, and that the references be made of record and appear among the "References Cited" on any issued patent.

Listed on Form PTO/SB/08A are references 1-2, which were cited in a First Office Action issued by the Chinese State Intellectual Property Office (SIPO) on March 17, 2014 in connection with Chinese Patent Application No. 201180024683.9, which corresponds to the present application. The attached English language abstract of the cited foreign reference should

meet the requirements of a concise statement of relevance under 37 CFR 1.56(c). The U.S. Reference cited in this submission is not included herewith as it is assumed that the USPTO has this reference readily available.

__X__ This Information Disclosure Statement is being submitted:

__X__ (1) within three months of the filing date of the application; or

_____ (2) after the filing date of the application and before the mailing date of a first Office Action on the merits; or

_____ (3) before the mailing of a first Office Action after the filing of a Request for Continued Examination; and thus, no Statement under 37 C.F.R. §1.97(e) or fee under 37 C.F.R. §1.17(p) is required.

_____ This Information Disclosure Statement is being submitted after the above periods (1)-(3), but before a Final Office Action or Notice of Allowance, a Statement under 37 CFR 1.97(e) is provided (see below), and no fee under 37 CFR 1.17(p) is due.

_____ This Information Disclosure Statement is being submitted after the above periods (1)-(3), but before a Final Office Action or Notice of Allowance, and no Statement under 37 CFR 1.97(e) is provided, the fee under 37 CFR 1.17(p) is enclosed.

_____ This Information Disclosure Statement is being submitted after the mailing date of a Final Office Action or Notice of Allowance, but before payment of the Issue Fee, and a Statement under 37 CFR 1.97(e) is provided (see below), and the fee under 37 CFR 1.17(p) is enclosed.

_____ Statement under 37 CFR 1.97(e)(1): Each item of information contained in this Information Disclosure Statement was first cited in any communication from a foreign patent

office in a counterpart foreign application not more than three months prior to the filing of the Information Disclosure Statement.

____ Statement under 37 CFR 1.97(e)(2): No item of information contained in this Information Disclosure Statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to our knowledge, after making reasonable inquiry, no item of information contained in the Information Disclosure Statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the Information Disclosure Statement.

____ Fee under 37 CFR 1.17(p) is enclosed (see Fee Transmittal).

The submission of the listed documents is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicant does not waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

Applicants hereby petition for any extension of time that may be required to maintain the pendency of this case, and any required fee for such an extension is to be charged to Deposit Account No. 50-5497.

Respectfully submitted,

Jean C. Edwards

Jean C. Edwards
Registration No. 41,728

(13155)
EDWARDS NEILS PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191
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Date: March 26, 2014

PTO/SB/08a (07-09)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	14/169,927
Filing Date	January 31, 2014
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	January 31, 2014
Attorney Docket Number	81527.0018

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1	US- 6,416,190 B1	07-09-2002	Grier et al.	
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	2	JPH-06-265452-A	09-22-1994	PRIMA MEAT PACKERS...	Abstract	✓

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

Europäisches Patentamt
European Patent Office
Office européen des brevets

Espacenet

Bibliographic data: JPH06265452 (A) — 1994-09-22

AUTOMATIC INSPECTION DEVICE EQUIPPED WITH SAMPLE SURFACE TAKEOUT

No documents available for this priority number.

Inventor(s): MATSUYOSHI NAOTO; KUDO KENICHI; HIGUCHI TOSHIRO ± (MATSUYOSHI NAOTO, ; KUDO KENICHI, ; HIGUCHI TOSHIRO)

Applicant(s): PRIMA MEAT PACKERS LTD; HIGUCHI TOSHIRO ± (PRIMA MEAT PACKERS LTD, ; HIGUCHI TOSHIRO)

Classification: - international:***G01N1/06; G01N1/28; G01N21/84; G01N35/00;*** (IPC1-7): G01N1/06; G01N1/28; G01N35/00

- cooperative:

Application number: JP19910218243 19910829

Priority number(s): JP19910218243 19910829

Also published as: JPH07109384 (B2)

Abstract of JPH06265452 (A)

PURPOSE:To perform the continuous actions of a specimen formation of samples and both observation and analysis processes and the automation by setting up an observer and an analyzer adjacently in a sample former. CONSTITUTION:A sample S, three disk driving mechanisms 51 to 53, six stage driving mechanisms 54 to 59 are all installed at one side of a cutter attached disk 1, and a microscope 4 observing and analyzing a sample surface, a camera 10 and two sensors 12 and 14 are all set up at the opposite side. In addition, this disk 1 is rotated by rotation of a turning shaft 53 by the disk driving motor 51, and the stage 58 advances at slow speed with rotation of a supporting shaft 57 by the stage driving motor 54, extruding the sample S in a direction of the disk 1.; When this disk 1 rotates in a state that this sample S is extruded out, a cutter edge of the disk 1 cuts the sample S thinly, whereby a fresh sample surface comes out. Accordingly, those of microscope 4, camera 10, and both these sensors 12, 14 observe and analyze this fresh sample surface. With this constitution, a time lag between specimen formation and observation of the sample is reduced to some extent, and a continuous action of each process is thus made possible to be done.

(19)日本国特許庁（ＪＰ）　(12)**公開特許公報**（Ａ）　(11)特許出願公開番号

特開平6－265452

(43)公開日　平成６年(1994)９月22日

(51)Int.Cl.[5]	識別記号	庁内整理番号	ＦＩ	技術表示箇所
Ｇ０１Ｎ　1/06		Ｚ　7519－2Ｊ		
1/28		Ｆ　7519－2Ｊ		
35/00		Ａ　7370－2Ｊ		

審査請求　有　　請求項の数５　ＯＬ　（全 11 頁）

(21)出願番号　特願平3－218243

(22)出願日　平成３年(1991)８月29日

(71)出願人　000113067
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東京都千代田区霞が関３丁目２番５号

(71)出願人　000235576
樋口　俊郎
神奈川県横浜市港北区茅ケ崎南４－14－１－109

(72)発明者　松芳　直人
茨城県土浦市中向原635　ブリマハム株式会社中央研究所内

(74)代理人　弁理士　清水　守　（外３名）

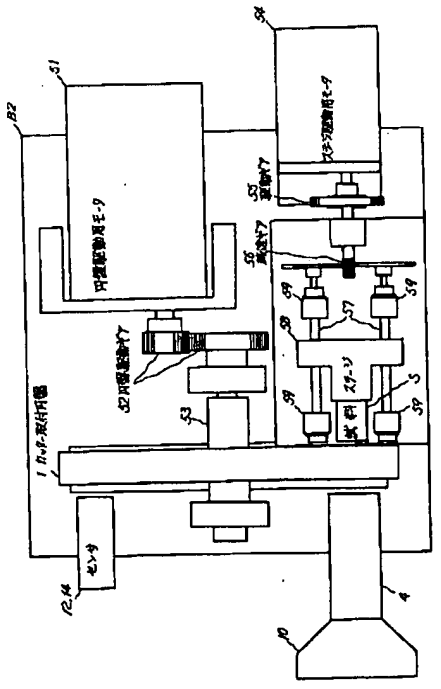
最終頁に続く

(54)【発明の名称】　試料面取り出し装置を具備する自動検査装置

(57)【要約】

【目的】　試料の任意の断面における長手方向を含む全体像において、撮像による画像データ、およびセンサによる分析データを表示して、人手による工程を除き、一連の検査、分析の自動化を行なう。

【構成】　ミクロトーム等の薄片の試料を形成するカッター取付円盤１に隣接させて、観察装置４，１０や分析装置を設け、画像データおよび分析データから薄片間にわたるデータを形成し、試料Ｓの内部状態の全体像を表示するデータを形成するデータ処理装置を、観察装置４，１０や分析装置に続けて接続し、その出力を表示装置や記憶装置に出力する。



【特許請求の範囲】
【請求項１】　試料の画像、物理的特性、化学的特性を検査する自動装置において、（ａ）試料内部の試料面を取り出す装置と、（ｂ）該試料面取り出す装置に隣接される撮像装置と、（ｃ）前記試料面取り出す装置に隣接されるセンサと、（ｄ）前記撮像装置と前記センサとから得られるデータから前記試料内部の状態を表すデータを演算するデータ処理装置と、（ｅ）該データ処理装置の出力データを表示する表示装置とを有する試料面取り出し装置を具備する自動検査装置。
【請求項２】　請求項１記載の自動検査装置において、前記試料内部の試料面を取り出す装置は、ミクロトームであることを特徴とする試料面取り出し装置を具備する自動検査装置。
【請求項３】　請求項１記載の自動検査装置において、前記試料内部の状態は、試料の長手方向の任意の断面であることを特徴とする試料面取り出し装置を具備する自動検査装置。
【請求項４】　請求項１記載の自動検査装置において、前記表示装置は、試料における表示断面位置を同時に表示することを特徴とする試料面取り出し装置を具備する自動検査装置。
【請求項５】　請求項１記載の自動検査装置において、前記試料内部の状態は、モード選択装置により選択可能であることを特徴とする試料面取り出し装置を具備する自動検査装置。
【発明の詳細な説明】
【０００１】
【産業上の利用分野】本発明は、理科学試料分析、食品分析、医療分析、工業材料分析等の検査、分析を行なう自動検査装置に関し、特に被分析試料材料から厚さの薄い試験片を作成する装置を有し、その試験片作成装置で作成された試験片の検査、分析を行なう自動検査装置に関する。
【０００２】
【従来の技術】従来、理科学試料分析、食品分析、医療分析、工業材料分析等を顕微鏡等により検査、分析を行なう際には、試料から厚さの薄い試験片を作成して、その試料を顕微鏡等により観察したり、試料を切断して被測定断面を露出させ、その被測定断面を各種のセンサにより測定していた。例えば、細胞組織の観察を行なうために、細胞組織をミクロトームにより薄切り、顕微鏡により観察することが行なわれている。
【０００３】また、医用工学の分野において、計算機による信号処理により、ＸＣＴスキャナや超音波を用いたリアルタイムの断層撮影装置のように、試料の内部を切断することなく非破壊的に検査することも行なわれている。
【０００４】
【発明が解決しようとする課題】しかしながら、試料から薄い試験片を作成して、その試料を顕微鏡等により観察するには、その試料の標本の作成が必要であって、その作成にはかなりの時間と技術を要していた。そのため、試料の標本の作成と観察の間、あるいは、試料の標本の作成と近赤光法やｐｈセンサ、その他のセンサによる分析との間には、かなりの時間的ずれがあり、これらの試料の標本の作成と観察、分析の工程を連続的な動作により行なうことはできなかった。この時間的ずれは、変質しやすい試料の分析においては問題であり、試料作成時の観察が行ないにくい、あるいは、多数の試料を観察、分析する場合には、試料間に差が生じるといった欠点があった。また、これらの工程は人手により行なわれており、一連の検査、分析の自動化が求められていた。
【０００５】本発明の第１の目的は、上記状況に鑑みて、試料の標本の作成と観察との間の時間的ずれを少なくし、試料の標本の作成と観察、分析の工程を連続的な動作により行ない得る試料面取り出し装置を具備する自動検査装置を提供することにある。また、本発明の第２の目的は、これらの検査工程から人手による部分を除き、一連の検査、分析の自動化を推進する試料面取り出し装置を具備する自動検査装置を提供することにある。
【０００６】更に、本発明の第３の目的は、試料の任意の断面における長手方向を含む全体像を表示し、観察することにより、表示面においては、データに基づいた表示とともに、表示断面の試料における位置を表示し得る試料面取り出し装置を具備する自動検査装置を提供するにある。
【０００７】
【課題を解決するための手段】本発明は、上記目的を達成するために、試料の画像、物理的特性、化学的特性を自動的に検査する装置において、試料内部の試料面を取り出す装置と、前記試料面取り出し装置に隣接される撮像装置と、前記試料面取り出し装置に隣接されるセンサと、前記撮像装置とセンサとから得られるデータから試料内部の状態を表すデータを演算するデータ処理装置と、前記データ処理装置の出力データを表示する表示装置とから成る。
【０００８】
【作用】本発明によれば、ミクロトーム等の薄片の試料を形成する装置に隣接させて、観察装置や分析装置を設けることにより構成される。観察装置や分析装置に続いて、画像データおよび分析データの処理装置が接続され、表示装置、記憶装置に出力される。さらに、データ処理装置は、記憶装置に記憶された各薄片ごとのデータから薄片間にわたるデータを形成し、試料の内部状態の全体像のデータを形成する装置も含んでいる。
【０００９】分析装置に用いるセンサとしては、例えば温度、圧力、色などを測定する物理センサや化学物質を計測する化学センサや、イオン選択性電極、ガスセンサ、バイオセンサ等を測定対象に応じて選択して用い

る。表示装置は、試料の画像データとともに、分析データを同一画面上に重ね合わせて表示することができる。

【００１０】

【実施例】以下、本発明の実施例について図１乃至図１１を参照しながら詳細に説明する。図１は本発明の実施例を示す自動検査装置の斜視図であり、回転式ミクロトームに適応した例を示している。図２はその自動検査装置の平面図、図３はその自動検査装置のカメラ側よりの一部破断側面図である。

【００１１】これらの図に示すように、基台上に配置されるベースＢ１上には、試料Ｓを薄く切るためのカッター刃２を回転可能にするカッター取り付け円盤１と、該円盤１を回転駆動させる円盤駆動機構５１、５２、５３と、試料Ｓを微動させるためのステージ５８を直進駆動させるステージ駆動機構５４～５９と、カッター刃２により新たに切り出された試料面を観察し分析するための機構等が搭載されている。試料Ｓとステージ５８およびステージ駆動機構５４～５９は、ベースＢ１上に設けられた第二のステージ送り装置ベース板Ｂ２上に搭載されている。なお、図１には観察、分析機構は示されていない。

【００１２】図２において、カッター取付円盤１に対して一方の側には試料面を観察し分析する顕微鏡４、カメラ（撮像装置）１０および各種のセンサ１２、１４が設置され、カッター取付円盤１の反対側には試料Ｓと、円盤駆動機構５１、５２、５３と、ステージ駆動機構５４～５９が設置されている。ここで、円盤駆動機構は、円盤駆動用モータ５１と、円盤駆動用モータの回転をカッター取付円盤１の回転軸５３に伝達する円盤駆動ギヤ５２により構成され、カッター取付円盤１を回転させ、カッター刃２により試料Ｓをスライスする。

【００１３】一方、ステージ駆動機構は、ステージ駆動用モータ５４と、該ステージ駆動用モータ５４の回転駆動力を伝達する駆動ギヤ５５と、該駆動ギヤ５５の駆動をステージ５８をスライド可能に支持する支持軸５７に伝達する減速ギヤ５６と、試料Ｓをカッター取付円盤１の方向に押し出すステージ５８と、支持軸５７を支持するよう支持軸５７の両端に設置された軸支持部５９とから成る。支持軸５７は、２本の軸から成り、試料Ｓの両側に該試料Ｓを挟むよう互いに並行に軸支持部５９により支持され、減速ギヤ５６により減速されて回転する。支持軸５７の回転により、ステージ５８は微速前進し、試料Ｓをカッター取付円盤１の方向に押し出す。カッター取付円盤１の方向に押し出された状態で、カッター取付円盤１が回転すると、試料Ｓはカッター刃２により薄く切り取られ、新しい試料面が現れる。顕微鏡４、カメラ（撮像装置）１０および各種のセンサ１（１２）・・・，センサＫ（１４）は、その新しい試料面を観察、分析する。

【００１４】図３は図２において顕微鏡４、カメラ（撮像装置）１０および各種のセンサ１（１２）・・・，センサＫ（１４）のある側から見た図であり、顕微鏡４、カメラ（撮像装置）１０および各種のセンサ１（１２）・・・，センサＫ（１４）を略して示してある。カッター取付円盤１は、回転軸５３により支持され、カッター刃２が取り付けられている。また、カッター取付円盤１には、弧状の切り欠き部３が開けられている。弧状の切り欠き部３は、該部分３を介して試料Ｓと顕微鏡４、カメラ（撮像装置）１０および各種のセンサ１（１２）・・・，センサＫ（１４）が対向する位置に形成されている。

【００１５】カッター取付円盤１が回転してカッター刃２が試料Ｓを通過すると、試料Ｓは薄切されて新たな試料面が現れる。さらにカッター取付円盤１が回転すると、弧状の切り欠き部３が試料面の位置に移動し、該弧状の切り欠き部３を介して、顕微鏡４、カメラ（撮像装置）１０および各種のセンサ１（１２）・・・，センサＫ（１４）が試料面が対向し、観察および分析が行なわれる。

【００１６】顕微鏡４、カメラ（撮像装置）１０、および各種のセンサ１（１２）・・・，センサＫ（１４）による観察および分析が終了すると、再びカッター取付円盤１が回転してカッター刃２が試料Ｓを薄切し、新たなスライス面における観察および分析が行なわれる。それぞれのスライス面における観察および分析のデータはメモリに記憶され、それらのデータを処理することにより、試料の断面の解析像を表示することができる。

【００１７】図４において、本発明の装置の信号処理の概略を示す。撮像装置１０が試料面と対向すると、走査制御装置１６により試料面は走査されて画像信号を出力する。撮像装置１０の画像信号は、Ａ／Ｄ変換回路１７によりデジタル信号に変換され、バッファメモリ２０に記憶される。バッファメモリ２０には、走査制御装置１６のアドレス信号が入力され、画像デジタル信号がアドレス設定されて記憶される。このバッファメモリ２０におけるアドレスをアドレス（Ｌ，Ｏ，Ｍ，Ｎ）とする。アドレス（Ｍ，Ｎ）は撮像装置１０で画像をＭ×Ｎのマトリックス画素により走査して得られる画像信号のアドレスであり、アドレス（Ｌ０）は記憶されているデータが画像データであることを示している。

【００１８】また、センサ１（１２）が試料面と対向すると、走査制御装置１６により試料面は走査されて画像信号を出力する。センサ１２の検出信号は、Ａ／Ｄ変換回路１７によりデジタル信号に変換され、バッファメモリ２２に記憶される。バッファメモリ２２には走査制御装置１６のアドレス信号が入力され、検出デジタル信号がアドレス設定されて記憶される。このバッファメモリ２２におけるアドレスをアドレス（Ｌ１，Ｍ，Ｎ）とする。アドレス（Ｍ，Ｎ）は、センサ１（１２）で画像をＭ×Ｎのマトリックス画素により走査して得られる画像

信号のアドレスであり、アドレス（Ｌ１）は記憶されているデータがセンサ１（１２）の検出データであることを示している。

【００１９】センサ１（１２）と同様にしてセンサＫ（１４）の検出データがアドレス（ＬＫ，Ｍ，Ｎ）によりバッファメモリ２４に記憶される。撮像装置１０、センサ１（１２）、・・・センサＫ（１４）の走査を制御するそれぞれの走査制御装置１６からは、スライス面の走査が終了したことを知らせる信号が出力され、論理回路１８に入力される。論理回路１８は、撮像装置１０、センサ１（１２）、・・・センサＫ（１４）のすべての走査が完了したことを検知し、走査完了信号をミクロトーム駆動制御装置５０に送る。

【００２０】ミクロトーム駆動制御装置５０は、走査完了信号を受け取ると、円盤駆動用モータ５１およびステージ駆動用モータ５４を駆動して試料Ｓの新しい試料面を薄切し、次のスライス面の画像データおよび検出データの抽出の準備を行なう。ミクロトーム駆動制御装置５０は、円盤駆動用モータ５１およびステージ駆動用モータ５４に駆動信号を送るとともに、入出力制御回路３２に制御信号を送る。

【００２１】制御信号を受けた入出力制御回路３２は、さらにバッファメモリ２０，２２，２４に制御信号を送り、バッファメモリ２０，２２，２４に記憶されている画像データ、検出データをデータメモリ２６に送る。ミクロトーム駆動制御装置５０は、入出力制御回路３２に制御信号を送る際、アドレス（Ｓｉ）で表されるアドレス信号を付与し、バッファメモリ２０の画像データはアドレス（Ｓｉ，Ｌ０，Ｍ，Ｎ）のアドレスにより設定される。

【００２２】また、バッファメモリ２２の検出データはアドレス（Ｓｉ，Ｌ１，Ｍ，Ｎ）のアドレスにより設定される。同様にバッファメモリ２４の検出データは（Ｓｉ，ＬＫ，Ｍ，Ｎ）のアドレスにより設定される。ここでアドレス（Ｓｉ）は、ミクロトームにより薄切される試料Ｓのｉ番目の試料面Ｓｉを表している。ｉ番目の試料面Ｓｉの画像データおよび検出データの抽出が終了すると、それらの画像データおよび検出データを前記のようにアドレスを指定してバッファメモリ２０，２２，２４からデータメモリ２６への伝送が行なわれ、次に試料Ｓのｉ＋１番目の試料面Ｓｉ＋１の画像データおよび検出データの抽出が行なわれる。

【００２３】同様にして、順次試料Ｓの試料面の観察および分析が行なわれ、それらのデータはデータメモリ２６に記憶される。最後の試料面の観察および分析が終了すると、データメモリ２６には試料Ｓの全体の画像データ、検出データが記憶されることになる。図５に上述の関係が示されている。試料Ｓは、輪切りにされた試料面が順に並べられたものとしてみることができ、ミクロトームによる薄切りによりにその試料面が順に観察および分析されることになる。

【００２４】次いで、データメモリ２６に記憶されたデータは、データ処理装置３０を介して信号処理された後、バッファメモリ２８を介してディスプレイ４０、記録装置６０に出力される。データ処理装置３０では、例えば画像データにおいては、境界線、辺、縁の抽出や画像の領域分割、解析や線の追跡や線図形のコード化、リスト表現や図形構造のシンタックス表現等の画像の特徴抽出、あるいは、テクスチャーパラメータ計測や面積、濃度、モーメント等幾何学的特徴量計測や線図形の位相的性質の計測や色彩パラメータ計測や各種統計量の計算等の画像特徴量計測等のデータ処理が行なわれる。

【００２５】また、検出データにおいては、温度、圧力、色などを測定する物理センサや化学物質を計測する化学センサ、イオン選択性電極、ガスセンサ、バイオセンサ等のセンサの出力の分析が行なわれる。センサの種類はこれに限られるわけではなく、必要に応じて任意のものを用いることができる。上記のデータ処理装置３０の処理の内容は、モード選択装置３４からの選択信号により選択される。モード選択装置３４からの選択信号は、入出力制御回路３２に入力され、処理の内容に応じて必要とするデータをデータメモリ２６から選択し、データ処理装置３０において処理が行なわれる。データメモリ２６からのデータの選択およびデータ処理の手順は、モード選択装置３４内のメモリに予め記憶されており、モード選択を行なうことによりメモリの読み出しが行なわれる。

【００２６】また、バッファメモリ２０を直接ディスプレイ４０に接続して撮像装置１０の画像データを表示してもよい。これにより、センサ１（１２）・・・センサＫ（１４）のデータ抽出に要する時間の間ディスプレイ４０に表示を行なうことができる。これは一般に撮像装置１０の画像データに要する時間は、センサ１（１２）・・・センサＫ（１４）のデータ抽出に要する時間よりも短いからである。

【００２７】本発明の特徴の一つである、試料の任意の断面の解析像の表示は、モード選択装置３４の選択信号を入出力制御回路３２、データ処理装置３０、および表示制御装置３６に入力することにより行なわれる。モード選択装置３４の選択信号により解析したい断面を指定すると、それに対応したデータが入出力制御回路３２を介してデータメモリ２６から取り出し、該データをデータ処理装置３０において処理し、表示制御装置３６によりディスプレイ４０に表示あるいは記録装置６０に出力される。また、ディスプレイ４０あるいは記録装置６０には解析断面の位置を同時に表示してデータの理解を容易にさせることもできる。

【００２８】表示の例を、試料がハムの場合をとって図６～図１１に示す。図６は試料がロースハムやボンレスハムの例である。図において、符号４２はスライス面Ｓ

ｉの断面であり、符号４１は解析断面である。解析断面４１には符号４２が表示しているスライス面Ｓｉを示すマーカ４５が併設されている。また、解析断面４１の試料に対する位置、角度を示す表示４３が成されている。これにより、試料においてどの断面の解析であるかを知ることができる。

【００２９】ロースハムは豚のロース（背肉）を用い、ボンレスハムはもも肉を用いて、血絞り、塩せき、くん煙、湯煮、冷却等の加工により形成されるが、ハムの構成要素の性質をセンサの出力により識別して、解析断面上において表示することができる。このセンサの出力の識別は、データ処理装置３０において行なわれる。図に示すロースハムやボンレスハムの例では、斜線部分は脂肪の部分を示し、その他の部分は赤身の部分を示している。

【００３０】図7は、図6と同様に試料がロースハムやボンレスハムの例であり、符号４６は塩濃度を検出するセンサの出力を表示したものである。図６と同様に符号４２はスライス面Ｓｉの断面であり、表示４３は符号４６で表される解析断面の試料に対する位置、角度を表す他の表示方法である。また、マーカ４５は、符号４２が表示しているスライス面Ｓｉを示す他の表示方法である。

【００３１】図8は、図6において脂肪の部分のみを表示した例である。図9は試料があらびきウィンナーの例であり、図６と同様な表示が成されている。図９においては、画像データと分析データが同一の場所に重ねて表示されている。図１０は試料がファインメッシュの例であり、図６と同様な表示が成されている。ファインメッシュは、脂身と赤身が細かく粉砕され均一に分布しているものであるが、図において斜線部分は粉砕が不十分な部分をしめている。本発明の装置においては、化学的に異なる性質のみならず、物理的に異なる性質についても識別し表示することができる。

【００３２】図１１は、試料がプレスハムの例であり、図６と同様な表示が成されている。プレスハムは、豚肉のほか、牛肉、馬肉、マトンなどの肉片を混ぜ合わせてケーシングに詰め、一つの肉塊からできたようにしたもので、原料肉の配合割合や、肉塊と肉塊の間に使われるつなぎの量などによって特級、上級、標準に分けられる。したがって、図の斜線部分で表されるつなぎの部分とその他の肉塊部分の識別を表示している。

【００３３】図１２は、試料が魚の例であり、固形化処理された魚が、図６のＳｉ断面と同様に表示されている。すなわち、図１２（ａ）におけるＡ断面が図１２（ｂ）に、Ｂ断面が図１２（ｃ）に、Ｃ断面が図１２（ｄ）にそれぞれ表示されている。これらの図において、５１は背鰭、５２は脊柱、５３は肋骨、５４は浮袋、５５は肝臓、５６は胃、５７は腸、５８は腎臓、５９は生殖線、６０は尻鰭である。

【００３４】図１３は、試料が蚕の例であり、固形化処理された蚕が、図６のＳｉ断面と同様に表示されている。すなわち、図１３（ａ）におけるＡ断面が図１３（ｂ）に、Ｂ断面が図１３（ｃ）に、Ｃ断面が図１３（ｄ）に、Ｄ断面が図１３（ｅ）にそれぞれ表示されている。これらの図において、６１は頭、６２は脳、６３は食道、６４は神経、６５は胸脚、６６は胸、６７は絹糸腺、６８は心臓、６９は生殖線原基、７０は胃、７１はマルピーギ管、７２は腹部、７３は後腸、７４は尾脚、７５は肛門である。

【００３５】図１４は、試料がミミズの例であり、固形化処理されたミミズが、図６のＳｉ断面と同様に表示されている。すなわち、図１４（ａ）におけるＡ断面が図１４（ｂ）に、Ａ－Ｂ線断面が図１４（ｃ）にそれぞれ表示されている。これらの図において、８０は表皮、８１はクチクラ層、８２は腎管、８３は体腔、８４は腹行血管、８５は背行血管、８６は複神経、８７は環状筋、８８は縦走筋、８９は体腔上皮、９０は卵、９１は消化管、９２は隔膜である。

【００３６】上記したように、ミクロトーム等の薄片の試料を形成する装置の駆動の制御と、観察装置や分析装置のデータ処理装置とは関連性を有して駆動される。試料はミクロトーム等の装置により薄片の試料片に形成され、薄片を形成するごとにその装置に隣接して設けられた観察装置や分析装置により観察、分析が行なわれて、画像データおよび分析データが得られる。各薄片ごとの観察装置からの画像データや分析装置からの分析データは、直接あるいは画像処理、演算処理等のデータ処理が施されたのち、記憶装置に記憶されたり、表示装置において表示される。分析センサは、試料の測定特性に応じて選択され、カッターにより試料の新しい断面が現れるとすぐに分析が行なわれる。

【００３７】画像データおよび分析データは、Ａ／Ｄ変換器によりデジタル信号に変換された後、試料の立体的なアドレスとともにデータメモリに記憶される。データメモリに記憶されたデータは、データの種類と、スライス面の位置と、画素の位置に関するアドレスを指定することにより特定される。アドレスを指定することにより特定されたデータは、画像処理や分析処理を行なうデータ処理装置において信号処理され、表示あるいは記憶される。

【００３８】また、モード選択装置により観察したい試料の断面部を指定することにより、アドレスを選択し、データメモリからのデータの読み出しを行ない、試料の任意の面でのデータ処理及び各種のデータの表示を行うことができる。なお、上記実施例は回転式ミクロトームを用いたが、滑走式ミクロトームや他の装置を用いて本発明の装置を実施することができることは、言うまでもない。

【００３９】また、本発明は上記実施例に限定されるも

のではなく、本発明の趣旨に基づき種々の変形が可能であり、それらを本発明の範囲から排除するものではない。

【0040】

【発明の効果】以上、詳細に説明したように、本発明によれば、試料の標本の作成と観察との間の時間的ずれを少なくし、試料の標本の作成と観察、分析の工程を連続的な動作により行なうことができる。これにより変質しやすい試料の分析においては問題を解消でき、多数の試料を観察、分析する場合の試料間の差をなくすことができる。

【0041】また、これらの工程から人手による部分を除き、一連の検査、分析の自動化を行なうことができる。更に、試料の任意の断面における長手方向を含む全体像を表示し、観察することができる。表示面においては、データに基づいた表示とともに、表示断面の試料における位置を表示することができる。

【図面の簡単な説明】

【図1】本発明の実施例を示す自動検査装置の斜視図である。

【図2】本発明の実施例を示す自動検査装置の平面図である。

【図3】本発明の実施例を示す自動検査装置のカメラ側よりの一部破断側面図である。

【図4】本発明の実施例を示す自動検査装置の信号処理の概略を示す図である。

【図5】本発明の装置において試料とスライス面との関係を示す概略図である。

【図6】本発明の装置において試料がロースハムやボンレスハムの第1の例である。

【図7】本発明の装置において試料がロースハムやボンレスハムの第2の例である。

【図8】本発明の装置において脂肪の部分のみを表示した例である。

【図9】本発明の装置において試料があらびきウィンナーの例である。

【図10】本発明の装置において試料がファインメッシュの例である。

【図11】本発明の装置において試料がプレスハムの例である。

【図12】本発明の装置において試料が魚の例である。

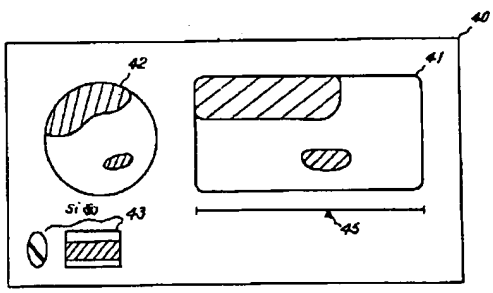
【図13】本発明の装置において試料が蚕の例である。

【図14】本発明の装置において試料がミミズの例である。

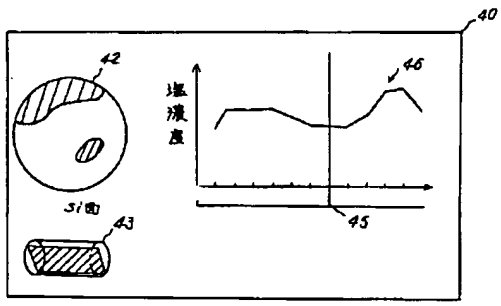
【符号の説明】

S　試料
1　カッター取付円盤
2　カッター刃
3　弧状の切り欠き部
4　顕微鏡
10　カメラ，撮像装置
12，14　センサ
16　走査制御装置
17　A／D変換回路
18　論理回路
20，22，24，28　バッファメモリ
26　データメモリ
30　データ処理装置
32　入出力制御回路
34　モード選択回路
40　ディスプレイ
50　ミクロトーム駆動制御装置
51　円盤駆動用モータ
52　円盤駆動ギヤ
53　回転軸
54　ステージ駆動用モータ
55　駆動ギヤ
56　減速ギヤ
57　支持軸
58　ステージ
59　軸支持部
60　記録装置

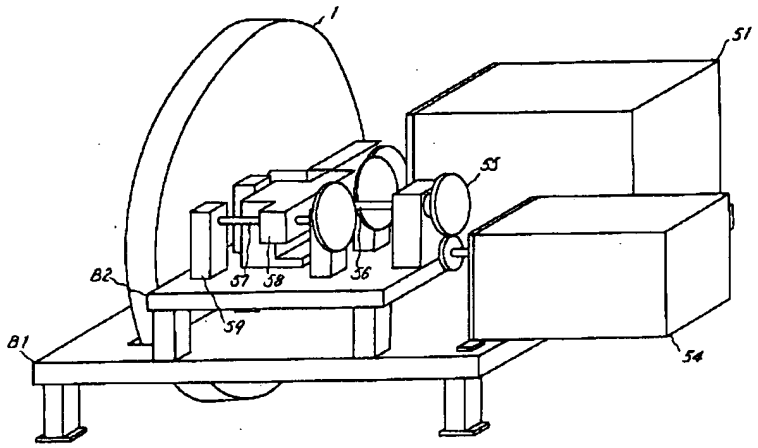
【図6】



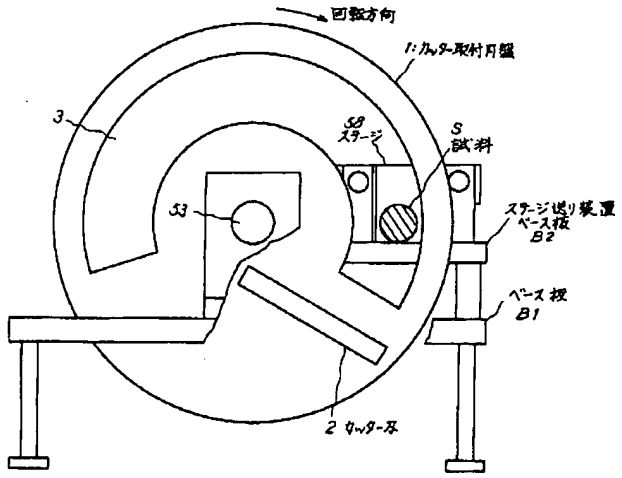
【図7】



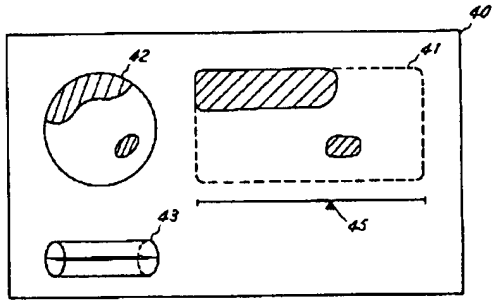
【図1】



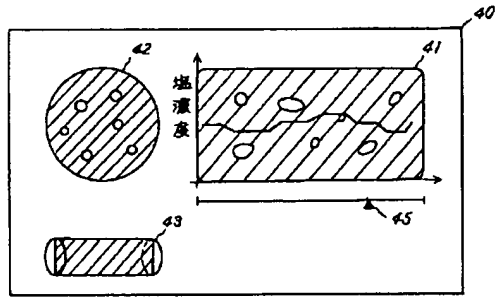
【図3】



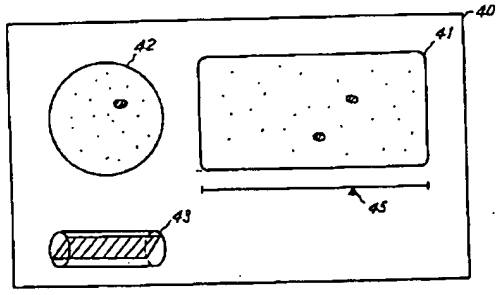
【図8】



【図9】



【図10】



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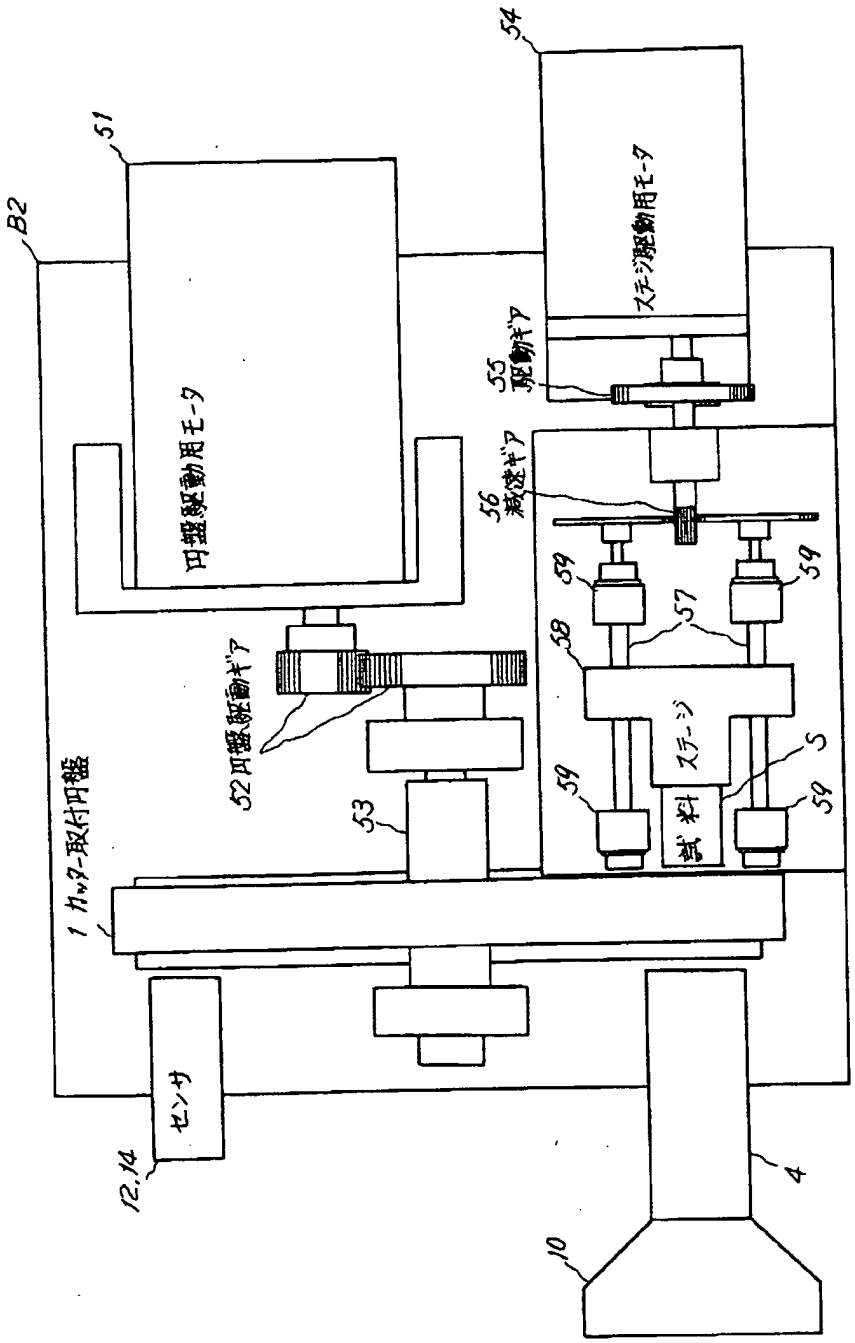
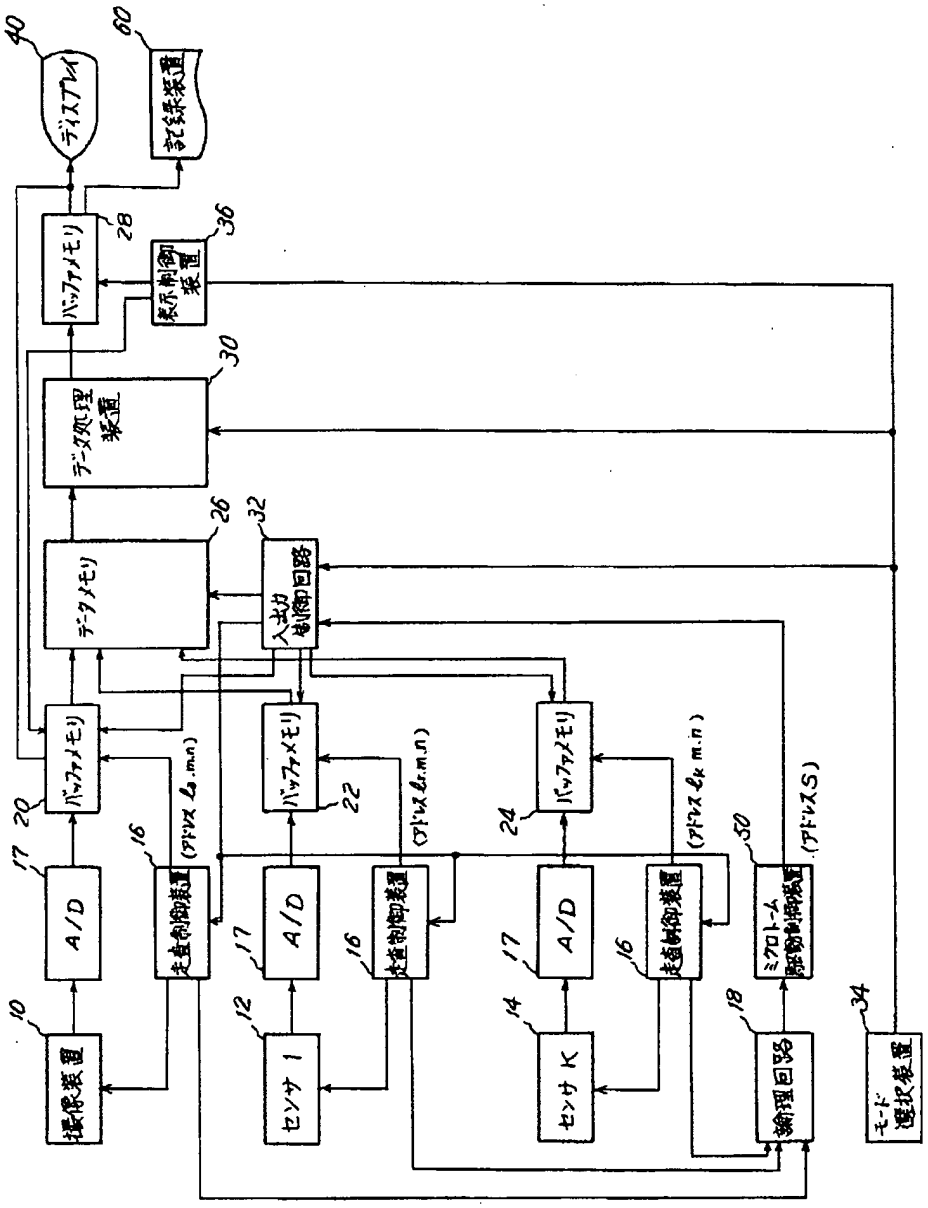

54
51
B2
ステージ駆動用モータ
円盤駆動用モータ
55 駆動ギア
56 減速ギア
59
57
58
ステージ
S
試料
52 円盤駆動ギア
53
1 カッター取付円盤
センサ
12,14
4
10

【図4】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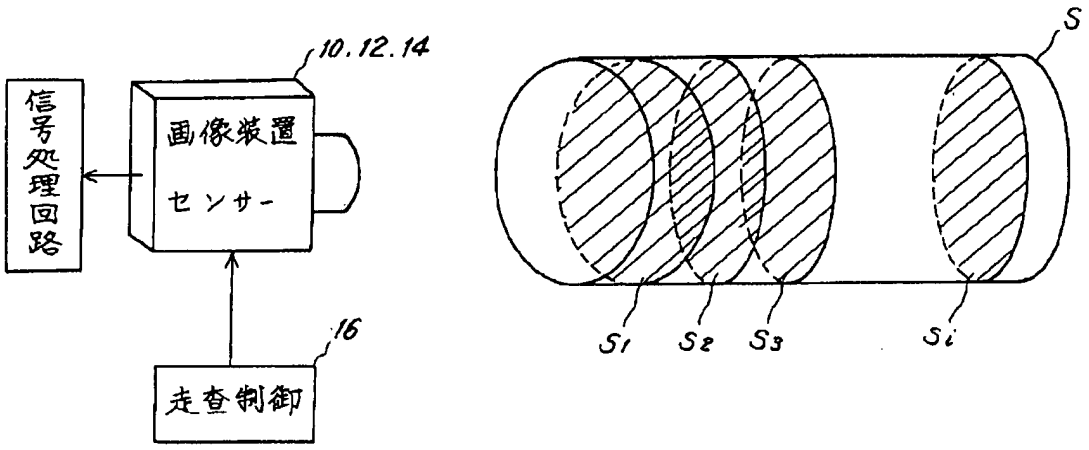
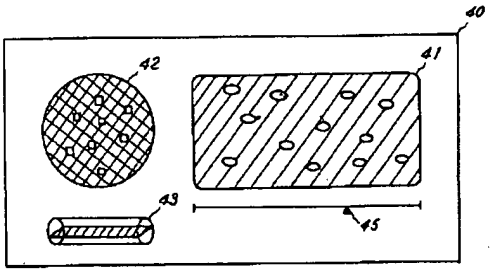
ディスプレイ
60
記録装置
28
バッファメモリ
36
表示制御装置
30
データ処理装置
26
データメモリ
32
入出力制御回路
20
バッファメモリ
22
バッファメモリ
24
バッファメモリ
17
A/D
16
走査制御装置
(アドレス La.m.n)
(アドレス Lb.m.n)
(アドレス Lk.m.n)
(アドレスS)
10
撮像装置
12
センサ 1
14
センサ K
18
論理回路
34
モード選択装置

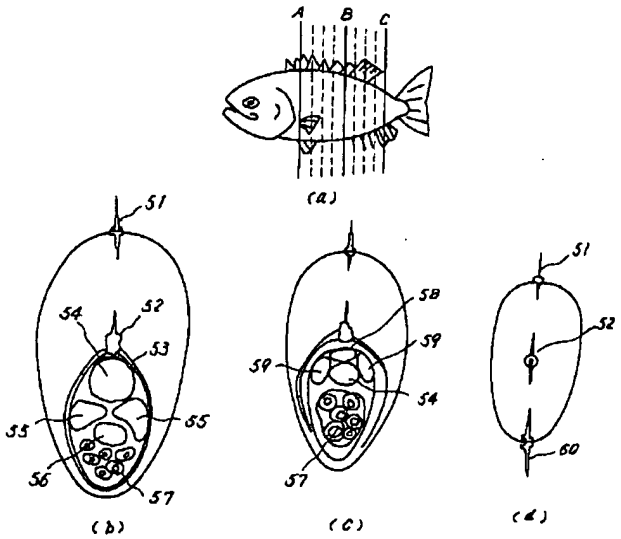
【図5】


10.12.14
信号処理回路
画像装置
センサー
16
走査制御
S
S1
S2
S3
Si

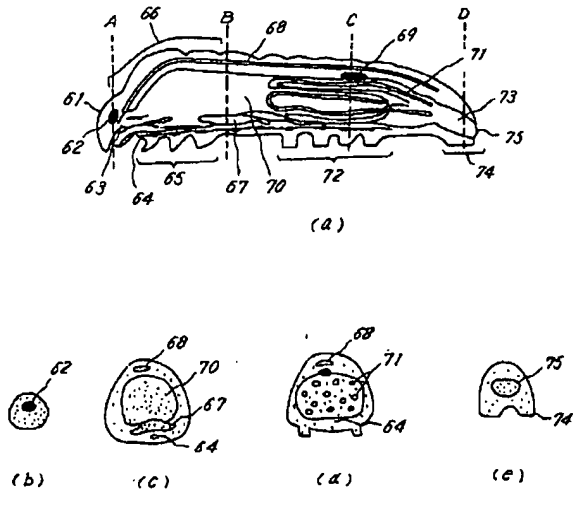
【図11】


40
42
41
43
45

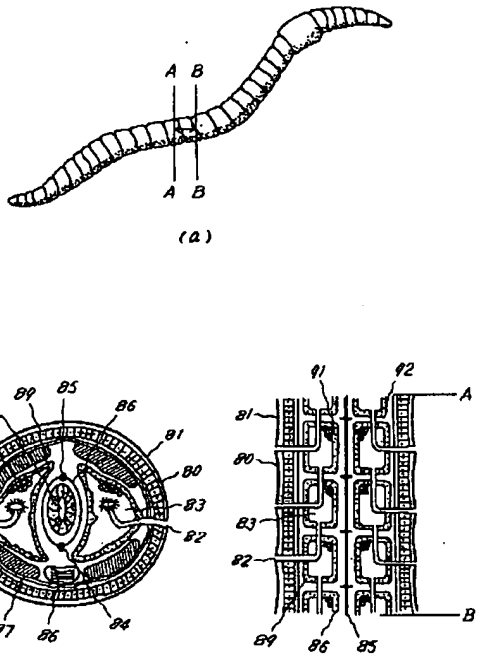
【図12】


A
B
C
(a)
51
54
52
53
55
55
56
57
(b)
58
59
59
54
57
(c)
51
52
60
(d)

【図13】



【図14】



フロントページの続き

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First Named Inventor	Daniel MUETH
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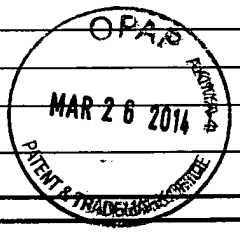

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  - ☐ Fee Attached
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  - ☐ After Final
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- ☑ Information Disclosure Statement
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  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
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- ☐ Status Letter
- ☑ Other Enclosure(s) (please Identify below):
  - IDS w/ Form PTO/SB/08A
  - One (1) cited foreign reference w/ English abstract

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SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	EDWARDS NEILS PLLC		
Signature	Jean C. Edwards		
Printed name	Jean C. Edwards, Esq.		
Date	March 26, 2014	Reg. No.	41,728

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ipgeneral@edwardsneils.com

Office Action Summary	Application No. 14/169,927	Applicant(s) MUETH ET AL.	
	Examiner KIET T. NGUYEN	Art Unit 2881	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01-31-2014*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *72-86* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☒ Claim(s) *73-86* is/are allowed.

7)☒ Claim(s) *72* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *1/31/14*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

The present application is being examined under the pre-AIA first to invent provisions.

Rejection Under 35 U.S.C. 102(b)

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 72 is rejected under 35 U.S.C. 102(b) as being anticipated by Blankenstein (6,432,630).

Blankenstein (6,432,630) discloses, in figs. 1-18, a micro flow apparatus for separating and identifying at least one component from a plurality of components in a fluid mixture. The apparatus includes a first input channel having a first flow for introducing a fluid mixture of a plurality of components (see figs. 5b and 5c); a plurality of buffer input channels having additional flows for introducing buffer solution (see figs. 5b and 5c); the first flow and the additional flows having a flow direction 5 along a length of the apparatus from one end of the apparatus to another end of the apparatus (see figs. 5b and 5c); the first flow and the additional flows which are interfaced with one another to form a channel 5 in a substantially laminar flow (see figs. 1, 2 and 6; and col. 4, lines 15-22 and 28-32); a plurality of selection channels disposed at the another end of the apparatus for selectively receiving components of the plurality of components (see figs. 5b and 5c), the selected components which are selectively removed from the first flow to the additional flows (see figs. 1, 5b, 5c and 6; and col. 4, lines 23-37, col. 6,

lines 22-35); a waste channel 7 for receiving unselected components removed from the first flow (see col. 15, lines 28-39); a plurality of pumps 23 and 24 connected respectively to a plurality of input reservoirs 29 and 30 to control flow rates of the first flow and the additional flows entering the first input channel and the plurality of buffer channels of the apparatus, respectively (see fig. 4; and col. 15, lines 3-27 and col. 18, lines 45-49); and a computer connected to the apparatus for providing user inputting a control of a selection of one of the plurality of components from the fluid mixture (see col. 18, lines 45-49).

Claims 73-86 are allowed.

Reasons for indicating allowable subject matter

The prior art fails to disclose an apparatus and/or method for identifying, examining and selectively operating at least one component from a plurality of components in a fluid mixture, which includes a laser beam for damaging, destroying or killing selected components or target cells of the plurality of components as recited in claims 73 and 74.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

1) North, Jr. (5,030,002) discloses an apparatus for sorting particles; and

2) Mueth et al. (7,355,696) discloses an apparatus for sorting cells.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kiet T. Nguyen whose telephone number is 571-272-2479. The examiner can normally be reached on Monday-Friday 8-6.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert H. Kim can be reached on 571-272-2293. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Kiet T. Nguyen/
Primary Examiner, Art Unit 2881

Notice of References Cited	Application/Control No. 14/169,927	Applicant(s)/Patent Under Reexamination MUETH ET AL.	
	Examiner KIET T. NGUYEN	Art Unit 2881	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,030,002	07-1991	North, Jr., Howard L.	356/73
*	B	US-7,355,696	04-2008	Mueth et al.	356/244
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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CONFIRMATION NO. 2069

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
14/169,927	01/31/2014 RULE	250	2881	81527.0018

APPLICANTS

PREMIUM GENETICS (UK) LTD., Nantwich, UNITED KINGDOM, Assignee (with 37 CFR 1.172 Interest);

INVENTORS

Daniel MUETH, Chicago, IL;
Joseph PLEWA, Park Ridge, IL;
Jessica SHIREMAN, Kansas City, MO;
Amy ANDERSON, Palatine, IL;
Lewis GRUBER, Chicago, IL;
Neil ROSENBAUM, Chicago, IL;

**** CONTINUING DATA ***************************

This application is a CON of 13/412,969 03/06/2012 PAT 8653442
which is a CON of 12/659,277 03/02/2010 PAT 8158927
which is a DIV of 12/213,109 06/13/2008 PAT 7699767
which is a DIV of 11/543,773 10/06/2006 PAT 7402131
which is a DIV of 10/934,597 09/03/2004 PAT 7118676
which is a CIP of 10/867,328 06/13/2004 PAT 7150834
which is a CIP of 10/630,904 07/31/2003 PAT 7241988
which claims benefit of 60/399,386 07/31/2002
and claims benefit of 60/435,541 12/20/2002
and said 10/934,597 09/03/2004
claims benefit of 60/571,141 05/14/2004
and claims benefit of 60/499,957 09/04/2003
and claims benefit of 60/511,458 10/15/2003

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

02/12/2014

Foreign Priority claimed	35 USC 119(a-d) conditions met	Verified and Acknowledged	Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ Yes ☑ No	☐ Yes ☑ No	/KIET NGUYEN/ Examiner's Signature	☐ Initials	IL	22	15	3

ADDRESS

Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191
UNITED STATES

TITLE

MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

		☐ All Fees
		☐ 1.16 Fees (Filing)

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14169927	MUETH ET AL.
	Examiner	Art Unit
	KIET T NGUYEN	2881

CPC- SEARCHED		
Symbol	Date	Examiner
B01D	2/23/14	KN

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner
N/A	2/23/14	KN

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
250	251	2/23/14	KN
356	244,246	2/23/14	KN
494	36,45	2/23/14	KN
435	173.1	2/23/14	KN
210	732,800,802	2/23/14	KN

SEARCH NOTES		
Search Notes	Date	Examiner
EAST attached	2/23/14	KN

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
250;356;494;435;210/B01D	251;244,246;36,45;173.1;732,800,802/21/26	2/23/14	KN

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	874	((sort$5 or separat$5 or select$5 or identif$5 or examin$5 or operat$3) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1) adj30 (fluid$1 or liquid$1 or blood$1)) and ((buffer$5 or solution$1) adj30 (input$5 or inlet$5) adj30 (channel$1 or port$1 or flow$5)) and (flow$5 adj20 direction$1)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/02/23 00:42
L2	1139	((output$5 or outlet$5 or select$5) adj30 (channel$1 or port$1 or flow$5) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1)) and (waste$1 adj30 (channel$1 or port$1 or flow$5) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/02/23 00:44
L3	2038	(pump$5 adj30 (reservoir$1 or contain$5)) and ((flow$5 adj10 rate$1) adj30 (buffer$5 or solution$1) adj30 (channel$1 or port$1 or flow$5))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/02/23 00:45
L4	3	l1 and l2 and l3	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/02/23 00:46
L5	874	((sort$5 or separat$5 or select$5 or identif$5 or examin$5 or operat$3) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1) adj30 (fluid$1 or liquid$1 or blood$1)) and ((buffer$5 or solution$1) adj30 (input$5 or inlet$5) adj30 (channel$1 or port$1 or flow$5)) and (flow$5 adj20 direction$1)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/02/23 00:46
L6	25311	(detect$5 adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1)) and laser$1 and ((damag$5 or kill$5 or destro$5) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/02/23 00:47
L7	183	l5 and l6	US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	2014/02/23 00:48

			JPO; DERWENT; IBM_TDB			
L8	1928	((sort$5 or separat$5 select$5 or identif$5 or examin$5 or operat$3) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1)) and (fluid$1 or liquid$1 or blood$1) and ((input$5 or inlet$5) adj20 (channel$1 or port$1 or flow$5)) and (sheath adj10 (buffer$5 or solution$1 or fluid$1 or liquid$1))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/02/23 00:48
L9	1660	(flow$5 adj10 (rate$1 or pressure$1)) and orthogonal and detect$5 and ((damag$5 or kill$5 or destro$5) adj30 (component$1 or cell$1 or platelet$1 or debris$5 or particle$1 or element$1)) and laser$1	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/02/23 00:50
L10	147	l8 and l9	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/02/23 00:50

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L11	90979	"l1" and "l2" and "l3"	US-PGPUB; USPAT; UPAD	OR	ON	2014/02/23 00:51
L12	20613	"l5" and "l6"	US-PGPUB; USPAT; UPAD	OR	ON	2014/02/23 00:51
L13	7488	"l8" and "l9"	US-PGPUB; USPAT; UPAD	OR	ON	2014/02/23 00:51

2/23/2014 12:52:47 AM
C:\Users\knguyen2\Documents\EAST\Workspaces\default.wsp

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	14/169,927
Filing Date	2014-01-31
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	Kiet T. Nguyen
Attorney Docket Number	81527.0018

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	3390449		1968-07-02	R.J. Fox	
	2	3649829		1972-03-14	Randolph	
	3	4325706		1982-04-20	Gershman et al.	
	4	4409106		1983-10-11	Furuta et al.	
	5	4424132		1984-01-03	Iriguchi	
	6	4660971		1987-04-28	Sage et al.	
	7	4667830		1987-05-26	Nozaki, Jr. et al.	
	8	5007732		1991-04-16	Ohki et al.	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

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	Examiner Name	Kiet T. Nguyen
	Attorney Docket Number	81527.0018

	9	5100627		1992-03-31	Buican et al.	
	10	5180065		1993-01-19	Touge et al.	
	11	5194909		1993-03-16	Tycko	
	12	5229297		1993-07-20	Schnipelsky et al.	
	13	5483469		1996-01-09	Van den Engh et al.	
	14	5620857		1997-04-15	Weetall et al.	
	15	5674743		1997-10-07	Ulmer	
	16	5800690		1998-09-01	Chow et al.	
	17	5837115		1998-11-17	Austin et al.	
	18	5849178		1998-12-15	Holm et al.	
	19	5879625		1999-03-09	Roslaniec et al.	

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	20	5966457		1999-10-12	Lemelson	
	21	6053856		2000-04-25	Hlavinka	
	22	6071442		2000-06-06	Dean et al.	
	23	6185664		2001-02-06	Jeddeloh	
	24	6368871		2002-04-09	Christel et al.	
	25	6416190		2002-07-09	Grier et al.	
	26	6432630		2002-08-13	Blankenstein	
	27	6451264		2002-09-17	Bhullar et al.	
	28	6506609		2003-01-14	Wada et al.	
	29	6524860		2003-02-25	Seidel et al.	
	30	6637463		2003-10-28	Lei et al.	

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	Art Unit	2881
	Examiner Name	Kiet T. Nguyen
	Attorney Docket Number	81527.0018

	31	6727451		2004-04-27	Fuhr et al.	
	32	6833542		2004-12-21	Wang et al.	
	33	6838056		2005-01-04	Foster	
	34	6944324		2005-09-13	Tran et al.	
	35	7029430		2006-04-18	Hlavinka et al.	
	36	7241988		2007-07-10	Gruber et al.	
	37	7482577		2009-01-27	Gruber et al.	
	38	H0001960		2001-06-05	Conrad et al.	
	39	7472794		2009-01-06	Oakey et al.	

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear

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	First Named Inventor	Daniel MUETH
	Art Unit	2881
	Examiner Name	Kiet T. Nguyen
	Attorney Docket Number	81527.0018

	1	20020058332		2002-05-16	Quake et al.	
	2	20020176069		2002-11-28	Hansen et al.	
	3	20030032204		2003-02-13	Walt et al.	
	4	20030047676		2003-03-13	Grier et al.	
	5	20030186426		2003-10-02	Brewer et al.	
	6	20050061962		2005-03-24	Mueth et al.	
	7	20050121604		2005-06-09	Mueth et al.	
	8	20060058167		2006-03-16	Regusa et al.	
	9	20060152707		2006-07-13	Kanda	

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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	2881
	Examiner Name	Kiet T. Nguyen
	Attorney Docket Number	81527.0018

	1	99/39223	WO	A1	1999-08-05	Arch Development Corp.		☒
	2	2798557	FR	A1	2001-03-23	Christine Nicolino	Abstract	☒
	3	19952322	DE	A1	2001-05-17	Evotec Biosystems AG	Abstract	☒
	4	2004/012133	WO	A2	2004-02-05	Arryx, Inc.		☒
	5	57-131451	JP	A	1982-08-14	Asahi Kasei Kogyo KK		☒
	6	0057907	EP	A1	1982-08-18	Asahi Kasei Kogyo KK		☒
	7	58-090513	JP	A	1983-05-30	Asahi Chem Ind Co Ltd	Abstract	☒
	8	2005-502482	JP	A	2005-01-27	University of Chicago		☒
	9	07-024309	JP		1995-01-27	Canon Inc	Abstract	☒
	10	2002-153260	JP		2002-05-28	Japan Science & Tech		☒
	11	01/18400	WO	A1	2001-03-15	Alup-Kompressoren		☒

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	2881
	Examiner Name	Kiet T. Nguyen
	Attorney Docket Number	81527.0018

	12	06-327494	JP		1994-11-29	Rikagaku Kenkyusho		☒
	13	0679325	EP	A1	1995-11-02	Shutze Raimund		☒

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NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
	1	HORI M. et al., "Cell fusion by optical trapping with laser - involves contacting different cells...", WPI/Thomson, December 27, 1991, Abstract	☒
	2	S. TAKAYAMA et al., "Patterning cells and their environments using multiple laminar fluid flows...", Proc. Natl. Acad. Sci. USA, May 1999, pgs. 5545-5548, Vol. 96	☒
	3	PAUL O.P. TS'O, "Basic Principles in Nucleic Acid Chemistry", National Library of Medicine, 1974, pgs. 311-387, Academic Press Inc., New York, NY	☐
	4	STEPHEN P. SMITH et al., "Inexpensive Optical Tweezers for Undergraduate Laboratories, Am. J. Phys., January 1999, Vol. 67	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature	/Kiet Tuan Nguyen/	Date Considered	02/20/2014

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	14/169,927
Filing Date	2014-01-31
First Named Inventor	Daniel MUETH
Art Unit	2881
Examiner Name	Kiet T. Nguyen
Attorney Docket Number	81527.0018

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Jean C. Edwards/	Date (YYYY-MM-DD)	2014-01-31
Name/Print	Jean C. Edwards, Esq.	Registration Number	41728

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /K.N./

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14169927	MUETH ET AL.
	Examiner	Art Unit
	KIET T NGUYEN	2881

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	02/24/2014								
	1	-								
	2	-								
	3	-								
	4	-								
	5	-								
	6	-								
	7	-								
	8	-								
	9	-								
	10	-								
	11	-								
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	29	-								
	30	-								
	31	-								
	32	-								
	33	-								
	34	-								
	35	-								
	36	-								

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14169927	MUETH ET AL.
	Examiner	Art Unit
	KIET T NGUYEN	2881

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	02/24/2014								
	37	-								
	38	-								
	39	-								
	40	-								
	41	-								
	42	-								
	43	-								
	44	-								
	45	-								
	46	-								
	47	-								
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	64	-								
	65	-								
	66	-								
	67	-								
	68	-								
	69	-								
	70	-								
	71	-								
	72	✓								

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14169927	MUETH ET AL.
	Examiner	Art Unit
	KIET T NGUYEN	2881

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	02/24/2014								
	73	=								
	74	=								
	75	=								
	76	=								
	77	=								
	78	=								
	79	=								
	80	=								
	81	=								
	82	=								
	83	=								
	84	=								
	85	=								
	86	=								

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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/169,927	01/31/2014		1600	81527.0018	15	3

CONFIRMATION NO. 2069

13155
Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

FILING RECEIPT

OC000000066602054

Date Mailed: 02/18/2014

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Daniel MUETH, Chicago, IL;
Joseph PLEWA, Park Ridge, IL;
Jessica SHIREMAN, Kansas City, MO;
Amy ANDERSON, Palatine, IL;
Lewis GRUBER, Chicago, IL;
Neil ROSENBAUM, Chicago, IL;

Applicant(s)

PREMIUM GENETICS (UK) LTD., Nantwich, UNITED KINGDOM

Assignment For Published Patent Application

PREMIUM GENETICS (UK) LTD., Nantwich, UNITED KINGDOM

Power of Attorney: The patent practitioners associated with Customer Number 13155

Domestic Priority data as claimed by applicant

This application is a CON of 13/412,969 03/06/2012 PAT 8653442
which is a CON of 12/659,277 03/02/2010 PAT 8158927
which is a DIV of 12/213,109 06/13/2008 PAT 7699767
which is a DIV of 11/543,773 10/06/2006 PAT 7402131
which is a DIV of 10/934,597 09/03/2004 PAT 7118676
which is a CIP of 10/867,328 06/13/2004 PAT 7150834
which is a CIP of 10/630,904 07/31/2003 PAT 7241988
which claims benefit of 60/399,386 07/31/2002
and claims benefit of 60/435,541 12/20/2002
and said 10/934,597 09/03/2004
claims benefit of 60/571,141 05/14/2004
and claims benefit of 60/499,957 09/04/2003

and claims benefit of 60/511,458 10/15/2003

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 02/12/2014

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/169,927**

Projected Publication Date: 05/29/2014

Non-Publication Request: No

Early Publication Request: No
Title

MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign

patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/169,927	01/31/2014	Daniel MUETH	81527.0018

CONFIRMATION NO. 2069

13155
Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

NOTICE

OC000000066602055

Date Mailed: 02/18/2014

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

A new inventor's oath or declaration that identifies this application (e.g., by Application Number and filing date) is required. The inventor's oath or declaration does not comply with 37 CFR 1.63 in that it:

- does not state that the above-identified application was made or authorized to be made by the person executing the oath or declaration.
  - Daniel MUETH
  - Joseph PLEWA
  - Jessica SHIREMAN
  - Amy ANDERSON
  - Lewis GRUBER
  - Neil ROSENBAUM
- does not include an acknowledgement that any willful false statement made in such declaration or statement is punishable under section 1001 of title 18 by fine or imprisonment of not more than five (5) years, or both.
  - Daniel MUETH
  - Joseph PLEWA
  - Jessica SHIREMAN
  - Amy ANDERSON
  - Lewis GRUBER
  - Neil ROSENBAUM

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 14/169,927

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	15 minus 20 =	*			OR	x 80 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*				x 420 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1600

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/169,927	01/31/2014	Daniel MUETH	81527.0018

CONFIRMATION NO. 2069

13155
Edwards Neils PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191

POA ACCEPTANCE LETTER

OC000000066601970

Date Mailed: 02/18/2014

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 01/31/2014.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/mpaulos/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PTO/AIA/82A (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B or equivalent) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5. If the Power of Attorney by Applicant form is not accompanied by this transmittal form or an equivalent, the Power of Attorney will not be recognized in the application.

Application Number	Not Yet Assigned
Filing Date	January 31, 2014
First Named Inventor	Daniel MUETH
Title	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	81527.0018

SIGNATURE of Applicant or Patent Practitioner

Signature	/Jean C. Edwards/	Date	January 31, 2014
Name	Jean C. Edwards, Esq.	Telephone	703-466-0150
Registration Number	41,728		

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications.

☐ *Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/82B(07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

POWER OF ATTORNEY BY APPLICANT

THIS POWER OF ATTORNEY HAS BEEN EXECUTED A DEED AND REMAINS IN FORCE UNTIL REVOKED.

I hereby revoke all previous powers of attorney given in the application identified in the attached transmittal letter.

☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A or equivalent):

13155

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A or equivalent):

Name	Registration Number	Name	Registration Number

Please recognize or change the correspondence address for the application identified in the attached transmittal letter to:

☒ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant:

☐ Inventor or Joint Inventor

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor

☒ Assignee or Person to Whom the Inventor is Under an Obligation to Assign

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (*e.g.*, a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document)

SIGNATURE of Applicant for Patent

Signature		Date	22 MAY 2013
Name	David CROSBY	Telephone	01256 345930
Title and Company	Director, PREMIUM GENETICS (UK) LTD		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms for more than one signature, see below *.

☐ *Total of _____ forms are submitted.

WITNESSED BY:

NAME: CARA CRICHTON

ADDRESS: BELVEDERE HOUSE, BASING VIEW, BASINGSTOKE, HANTS RG21 4HG

DATE: 22 MAY 2013

DECLARATION

As a below-named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am an original and first inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR SEPARATION WITH LASER STEERING

the specification filed on **September 3, 2004** as application **Serial No. 10/934,597**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

(Application Serial No.)	(Filing Date)
60/571,141	**05/14/2004**
60/499,957	**09/04/2003**
60/511,458	**10/15/2003**

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Nos.	Country	Foreign Filing Date	Priority Not Claimed	Certified Copy Attached? Yes	No
_____	_____		☐	☐	☐

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability

as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status-patented, pending, abandoned)
10/867,328	**06/13/2004**	**pending**
10/630,904	**07/31/2003**	**pending**

Inventor: Daniel Mueth
Inventor's Signature: [signature]
Date: Jan 31, 2005
Country of Citizenship: United States
Residence and Post Office Address: 1700 East 56th Street, Apt. 1908
Chicago, Illinois 60037

Inventor: Joseph Plewa
Inventor's Signature [signature]
Date: 1/31/05
Country of Citizenship: United States
Residence and Post Office Address: 1017 North Delphia Avenue
Park Ridge, Illinois 60068

Inventor: Jessica Shireman
Inventor's Signature:
Date:
Country of Citizenship: United States
Residence and Post Office Address: 13601 East 55th Terrace
Kansas City, Missouri 64133

Inventor: Amy Anderson
Inventor's Signature: [signature]
Date: 2.5.05
Country of Citizenship: United States
Residence and Post Office Address: 1425 South Wolfe Road, #124
Prospect Heights, Illinois 60070

Inventor: Lewis Gruber
Inventor's Signature: [signature]
Date: 2-5-05
Country of Citizenship: United States
Residence and Post Office Address: 400 East Randolph, Apartment 3911
Chicago, Illinois 60601

as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status-patented, pending, abandoned)
10/867,328	06/13/2004	pending
10/630,904	07/31/2003	pending

Inventor: Daniel Mueth
Inventor's Signature:
Date:
Country of Citizenship: United States
Residence and Post Office Address: 1700 East 56th Street, Apt. 1908
Chicago, Illinois 60037

Inventor: Joseph Plewa
Inventor's Signature
Date:
Country of Citizenship: United States
Residence and Post Office Address: 1017 North Delphia Avenue
Park Ridge, Illinois 60068

Inventor: Jessica Shireman
Inventor's Signature:
Date: 2-6-05
Country of Citizenship: United States
Residence and Post Office Address: 13601 East 55th Terrace
Kansas City, Missouri 64133

Inventor: Amy Anderson
Inventor's Signature:
Date:
Country of Citizenship: United States
Residence and Post Office Address: 1425 South Wolfe Road, #124
Prospect Heights, Illinois 60070

Inventor: Lewis Gruber
Inventor's Signature:
Date:
Country of Citizenship: United States
Residence and Post Office Address: 400 East Randolph, Apartment 3911
Chicago, Illinois 60601

Inventor:	Neil Harris Rosenbaum
Inventor's Signature:	Neil Rosenbaum
Date:	1/3/2005
Country of Citizenship:	United States
Residence and Post Office Address:	800 South Wells Street, Apartment 1141 Chicago, Illinois 60607-4539

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Daniel MUETH et al.

Application No.: Not yet assigned

Filed: January 31, 2014

Customer No.: 13155

Attorney Docket No.: 81527.0018

Group Art Unit: 2881 (parent)

Examiner: Kiet Tuan NGUYEN (parent)

Confirmation No.: Not yet assigned

Continuation Patent Application of

U.S. Patent Application No.: 13/412,969 filed March 6, 2012

For: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION (as amended)

TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Please find enclosed:

- Application Data Sheet (eight (8) pages)
- Preliminary Amendment
- 85 pages of Specification, Claims, and Abstract
- 22 pages of Formal Drawings (Figs. 1-27)
- Claim of Priority
- Information Disclosure Statement along with Form PTO/SB/08A
- Inventors' Declaration (four (4) pages)
- Power of Attorney (two (2) pages)
- Fee payment of $1,600.00 (Claims 15/3)

Please charge any deficiencies or credit any overpayments to Deposit Account No. 50-5497. In the event the credit card payment submitted herewith fails to process, please charge the filing fees to deposit account No. 50-5497.

Respectfully submitted,

/Jean C. Edwards/
Jean C. Edwards
Registration No. 41,728

(13155)
EDWARDS NEILS PLLC
12020 Sunrise Valley Drive, Suite 200
Reston, VA 20191
Telephone: 703-466-0150
Facsimile: 703-537-8149
Date: January 31, 2014

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Daniel MUETH et al.

Attorney Docket No.: 81527.0018

Application No.: Not yet assigned

Group Art Unit: 2881 (parent)

Filed: January 31, 2014

Examiner: Kiet Tuan NGUYEN (parent)

Customer No.: 13155

Confirmation No.: Not yet assigned

Continuation Patent Application of
U.S. Patent Application No.: 13/412,969 filed March 6, 2012

For: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION (as amended)

PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INTRODUCTORY REMARKS

Sir:

Prior to the examination of the present application, and prior to the calculation of fees, the Examiner is respectfully requested to enter the following amendments:

AMENDMENT TO THE TITLE:

Please amend the Title of the Specification as follows:

MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR ~~SEPARATION WITH LASER STEERING~~ INDENTIFICATION

AMENDMENT TO THE SPECIFICATION:

On page 1, please amend the first paragraph, as follows:

Cross-Reference to Related Applications and Priority Claims

[0001] The present application is a continuation application of parent U.S. Patent Application No. 13/412,969 filed March 6, 2012,now U.S. Patent No. 8,653,442, which is a continuation application of U.S. Patent Application No. 12/659,277, filed March 2, 2010, now U.S. Patent No. 8,158,927, which is a divisional application of U.S. Patent Application No. 12/213,109, filed June 13, 2008, now U.S. Patent No. 7,699,767, which is a divisional application of U.S. Patent Application No. 11/543,773, filed October 6, 2006, now U.S. Patent No. 7,402,131, which is a divisional application of U.S. Patent Application No. 10/934,597, filed September 3, 2004, now U.S. Patent No. 7,118,676, which is a continuation-in-part of ~~Daniel M. Mueth et al.,~~ U.S. Patent Application Patent Application Serial No. 10/867,328, filed June 13, 2004, now U.S. Patent No. 7,150,834, ~~entitled "Multiple Laminar Flow-Based Rate Zonal Or Isopycnic Separation With Holographic Optical Trapping of Blood Cells And Other Static Components",~~ which is a continuation-in-part of U.S. Patent Application No. 10/630,904, filed July 31, 2003, now U.S. Patent No. 7,241,988, and claims priority via U.S. Patent Application No. 10/630,904, to U.S. Provisional Patent Application No. 60/399,386, filed July 31, 2002, and 60/435,541, filed December 20, 2002, and claims priority via U.S. Patent Application No. 10/934,597, to U.S. Provisional Patent Application No. 60/571,141, filed May 14, 2004, U.S. Provisional Patent Application No. 60/499,957, filed September 4, 2003, and U.S. Provisional Patent Application No. 60/511,458, filed October 15, 2003, commonly assigned herewith, the contents of all of which are incorporated by reference herein~~, with priority claimed for all commonly disclosed subject matter (the "first related application")~~.

AMENDMENTS TO THE CLAIMS:

This listing of claims replaces all prior versions, and listings, of claims in the application:

1.-71. (Canceled)

72. (New) An apparatus to identify at least one component from a plurality of components in a fluid mixture, the apparatus comprising:

a first input channel into which a first flow is introduced, said first flow which contains the fluid mixture of the plurality of components;

a plurality of buffer input channels, into which additional flows of buffer solution are introduced, said plurality of buffer channels which are disposed on either side of said first input channel;

wherein said first flow and said additional flows have a flow direction along a length of the apparatus from one end of the apparatus to another end of the apparatus;

a plurality of selection channels disposed at said another end of the apparatus, said plurality of selection channels which are adapted to receive said additional flows enriched by selected components of the plurality of components, said selected components which are selectively removed from said first flow to said additional flows;

a waste channel through which unselected components are removed from said first flow which is depleted of said selected components;

a plurality of pumps connected respectively to a plurality of input reservoirs, to control flow rates of said first flow and said additional flows entering said first input channel and said plurality of buffer channels of the apparatus, respectively; and

a computer connected to the apparatus, said computer which is adapted to provide user input for control of a selection of one of the plurality of components from the fluid mixture.

73. (New) An apparatus to identify at least one component from a plurality of components in a fluid mixture, the apparatus comprising:

a first input channel into which a first flow is introduced, said first flow which contains the fluid mixture of the plurality of components;

a plurality of buffer input channels, into which additional flows of buffer solution are introduced, said plurality of buffer channels which are disposed on either side of said first input channel;

wherein said first flow and said additional flows have a flow direction along a length of the apparatus from one end of the apparatus to another end of the apparatus;

a detector apparatus which detects and identifies selected components of the plurality of components;

a laser which emits a laser beam which damages or kills selected components of the plurality of components; and

at least one channel disposed at said another end of the apparatus, said at least one channel which is adapted to receive said first flow and said additional flows after operation of said laser on said selected components.

74. (New) A method for examining and selectively operating on cells, comprising:

inputting a stream of sample fluid into an inlet adapted to received said sample fluid, into an input region of a flow chamber, said sample fluid containing cells to be processed;

inputting a plurality of streams of sheath fluids into a plurality of inlets adapted to receive said plurality of streams of sheath fluids, into said input region of said flow chamber;

wherein said sample fluid is in a contiguous relationship on all available sides with said sheath fluids, from said input region through to a selective operation region of said flow chamber;

wherein at least one of flow rates or pressures of said sheath fluids are chosen such that said sample fluid is constricted in two orthogonal directions, thereby allowing said sample fluid to form a relatively narrow stream in at least a detector region of said flow chamber, thereby causing the cells to be flattened and aligned such that flat sides of the cells are oriented parallel to confronting walls of the flow chamber;

distinguishing target cells from non-target cells in said detector region using a detector apparatus; and

damaging or destroying said target cells in said sample fluid using a laser beam, in said selective operation region of said flow chamber which is disposed downstream from said detector region.

75. (New) The method of claim 74, further comprising:

optically illuminating the cells in said detector region with a light beam from a laser.

76. (New) The method of claim 75, wherein said detector in said detector region detects said target cells from said non-target cells.

77. (New) The method of claim 76, wherein said cells are sperm cells.

78. (New) The method of claim 77, wherein said sperm cells are one of X-chromosome or Y-chromosome sperm cells.

79. (New) The method of claim 78, wherein said target cells are one of X-chromosome sperm cells or Y-chromosome sperm cells, and said non-target cells are the other of said X-chromosome sperm cells or Y-chromosome sperm cells.

80. (New) The method of claim 75, wherein the cells output light in response to said light beam, and said detector comprises an optical detector responsive to light emitted by the cells.

81. (New) The method of claim 80, wherein the output light comprises at least one of light scattered from the cells, and fluorescent light produced by the cells.

82. (New) The method of claim 77, further comprising:

performing gender discrimination of said sperm cells.

83. (New) The method of claim 82, wherein said sample fluid and said sheath fluids are disposed in a flat sorter.

84. (New) The method of claim 74, further comprising:

pumping said sample fluid and said sheath fluids into said input region.

85. (New) The method of claim 84, wherein a constant flow rate and pressure are maintaining during said pumping by a pumping system.

86. (New) The method of claim 74, further comprising:

adapting a computer to provide user input for control of a selection of one of the cells in said sample fluid.

AMENDMENT TO THE ABSTRACT:

Please replace the Abstract of the disclosure as follows:

Abstract of the Disclosure

The present invention provides a method and apparatus to identify at least one component from a plurality of components in a fluid mixture, the apparatus including a first input channel into which a first flow containing a plurality of components is introduced; a plurality of buffer input channels, into which additional flows of buffer solution are introduced, disposed on either side of the first input channel; wherein the first flow and the additional flows have a flow direction along a length of the apparatus; a detector apparatus which detects and identifies selected components of the plurality of components; a laser which emits a laser beam which damages or kills selected components of the plurality of components; and at least one channel disposed at the another end of the apparatus which is adapted to receive the first flow and the additional flows after operation of the laser on the selected components.

REMARKS

Entry of this Preliminary Amendment prior to examination of the above-identified application is respectfully requested.

Claims 72-86 are presently pending in this application. Claims 1-71 have been canceled without prejudice or disclaimer.

The Specification was amended to amend the cross-reference to related applications. The Abstract was amended to put in proper form for U.S. practice.

No new matter has been entered.

Applicants hereby petition for the Director to charge any additional fees or any underpayment of fees which may be required to maintain the pendency of this case at any time during prosecution, or to credit any overpayment to Deposit Account No. 50-5497.

Applicants hereby petition for any extension of time that may be required to maintain the pendency of this case, and any required fee for such an extension is to be charged to Deposit Account No. 50-5497.

Respectfully submitted,

/Jean C. Edwards/
Jean C. Edwards
Registration No. 41,728

(13155)
EDWARDS NEILS PLLC
12020 Sunrise Valley Drive
Suite 200
Reston, VA 20191
Telephone: 703-466-0150
Facsimile: 703-537-8149
Date: January 31, 2014

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Daniel MUETH et al.

Attorney Docket No.: 81527.0018

Application No.: Not yet assigned

Group Art Unit: 2881 (parent)

Filed: January 31, 2014

Examiner: Kiet Tuan NGUYEN (parent)

Customer No.: 13155

Confirmation No.: Not yet assigned

Continuation Patent Application of
U.S. Patent Application No.: 13/412,969 filed March 6, 2012

For: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION (as amended)

CLAIM OF PRIORITY

MAIL STOP APPLICATION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sirs:

The present application is a continuation application of parent U.S. Patent Application No. **13/412,969**, filed **March 6, 2012**, now U.S. Patent No. 8,653,442, which is a continuation application of parent U.S. Patent Application No. **12/659,277**, filed **March 2, 2010**, now U.S. Patent No. **8,158,927**, which is a divisional application of U.S. Patent Application No. **12/213,109**, filed **June 13, 2008**, now U.S. Patent No. **7,699,767**, which is a divisional application of U.S. Patent Application No. **11/543,773**, filed **October 6, 2006**, now U.S. Patent No. **7,402,131**, which is a divisional application of U.S. Patent Application No. **10/934,597**, filed **September 3, 2004**, now U.S. Patent No. **7,118,676**, which is a continuation-in-part of U.S. Patent Application No. **10/867,328**, filed **June 13, 2004**, now U.S. Patent No. **7,150,834**, which

is a continuation-in-part of U.S. Patent Application No. **10/630,904**, filed **July 31, 2003**, now U.S. Patent No. **7,241,988**, and claims priority via U.S. Patent Application No. **10/630,904**, from U.S. Provisional Patent Application No. **60/399,386**, filed **July 31, 2002**, and **60/435,541**, filed **December 20, 2002**, and claims priority via U.S. Patent Application No. **10/934,597**, from U.S. Provisional Patent Application No. **60/571,141**, filed **May 14, 2004**, U.S. Provisional Patent Application No. **60/499,957**, filed **September 4, 2003**, and U.S. Provisional Patent Application **No. 60/511,458**, filed **October 15, 2003**, commonly assigned herewith, the contents of all of which are incorporated by reference herein.

No fee is believed to be required. Should any fee be required, please charge any deficiency to Deposit Account No. 50-5497.

Respectfully submitted,

/Jean C. Edwards/
Jean C. Edwards
Registration No. 41,728

(13155)
EDWARDS NEILS PLLC
12020 Sunrise Valley Drive
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Inventors: Daniel MUETH et al.

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Application No.: Not yet assigned

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Continuation Patent Application of
U.S. Patent Application No.: 13/412,969 filed March 6, 2012

For: MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION (as amended)

INFORMATION DISCLOSURE STATEMENT

MAIL STOP APPLICATION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sirs:

In accordance with the provisions of 37 C.F.R. 1.56, 1.97 and 1.98, the attention of the U.S. Patent and Trademark Office is hereby directed to the references listed on the attached Form PTO/SB/08a. It is requested that the information on Form PTO/SB/08a be considered during the prosecution of this application, and that the references be made of record and appear among the "References Cited" on any issued patent.

Listed on Form PTO/SB/08a are sixty-five (65) references, which were cited in parent application no. 13/412,969 filed March 6, 2012. Copies of these references are not included in this submission as it is assumed that the USPTO has these references available from the parent application.

__X__ This Information Disclosure Statement is being submitted:

__X__ (1) within three months of the filing date of the application; or

_____ (2) after the filing date of the application and before the mailing date of a first Office Action on the merits; or

_____ (3) before the mailing of a first Office Action after the filing of a Request for Continued Examination; and thus, no Statement under 37 C.F.R. §1.97(e) or fee under 37 C.F.R. §1.17(p) is required.

_____ This Information Disclosure Statement is being submitted after the above periods (1)-(3), but before a Final Office Action or Notice of Allowance, a Statement under 37 CFR 1.97(e) is provided (see below), and no fee under 37 CFR 1.17(p) is due.

_____ This Information Disclosure Statement is being submitted after the above periods (1)-(3), but before a Final Office Action or Notice of Allowance, and no Statement under 37 CFR 1.97(e) is provided, the fee under 37 CFR 1.17(p) is enclosed.

_____ This Information Disclosure Statement is being submitted after the mailing date of a Final Office Action or Notice of Allowance, but before payment of the Issue Fee, and a Statement under 37 CFR 1.97(e) is provided (see below), and the fee under 37 CFR 1.17(p) is enclosed.

_____ Statement under 37 CFR 1.97(e)(1): Each item of information contained in this Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the Information Disclosure Statement.

_____ Statement under 37 CFR 1.97(e)(2): No item of information contained in this Information Disclosure Statement was cited in a communication from a foreign patent office in a

counterpart foreign application, and, to our knowledge, after making reasonable inquiry, no item of information contained in the Information Disclosure Statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the Information Disclosure Statement.

_____ Fee under 37 CFR 1.17(p) is enclosed (see Fee Transmittal).

The submission of the listed documents is not intended as an admission that any such document constitutes prior art against the claims of the present application. Applicant does not waive any right to take any action that would be appropriate to antedate or otherwise remove any listed document as a competent reference against the claims of the present application.

Applicants hereby petition for any extension of time that may be required to maintain the pendency of this case, and any required fee for such an extension is to be charged to Deposit Account No. 50-5497.

Respectfully submitted,

/Jean C. Edwards/
Jean C. Edwards
Registration No. 41,728

(13155)
EDWARDS NEILS PLLC
12020 Sunrise Valley Drive
Suite 200
Reston, VA 20191
Telephone: 703-466-0150
Facsimile: 703-537-8149
Date: January 31, 2014

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
First Named Inventor/Applicant Name:	Daniel MUETH
Filer:	Jean Ceceille Edwards./Kenneth Sowers
Attorney Docket Number:	81527.0018

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1600**

Electronic Acknowledgement Receipt

EFS ID:	18084568
Application Number:	14169927
International Application Number:	
Confirmation Number:	2069
Title of Invention:	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION
First Named Inventor/Applicant Name:	Daniel MUETH
Customer Number:	13155
Filer:	Jean Ceceille Edwards.
Filer Authorized By:	
Attorney Docket Number:	81527.0018
Receipt Date:	31-JAN-2014
Filing Date:	
Time Stamp:	16:34:31
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	$1600
RAM confirmation Number	3322
Deposit Account	505497
Authorized User	NEILS, PAUL F

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	81527-0018_ADS.pdf	1562368 bad53100bbc799791cb0a9d2fd49b4fe9606498d	no	9
Warnings:					
Information:					
2		81527-0018_Specification.pdf	2897066 bc5f89091a30f03167a6bfd95dfcf4fcfa5186eb	yes	85

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Specification	1	68
	Claims	69	84
	Abstract	85	85

Warnings:					
Information:					
3	Drawings-only black and white line drawings	81527-0018_Drawings.pdf	9175206 59863483e1e824837a9e12b215b1a3edeb5d566d	no	22
Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	81527-0018_SB08a.pdf	614589 3999f68428a49f300b5179b3472a3b69ac9a0e08	no	9
Warnings:					
Information:					
5	Power of Attorney	81527-0018_POA.pdf	572187 89b452266babda02e76d2759509f7a565f71f0d3	no	2
Warnings:					
Information:					
6	Oath or Declaration filed	81527-0018_DECLARATION.pdf	393956 ea59e98e3767ccb40674fa900dcf856e97a724d0	no	4
Warnings:					

Information:					
7	Transmittal of New Application	81527-0018_CONTINUATION_TTL.pdf	21202 4b3e445f3d2583dacbc1c5718229ece6d1b35451	no	1
Warnings:					
Information:					
8		81527-0018_Preliminary-Amendment.pdf	59836 847fbdab8f0a60011369d399ab90eec9e1ecbf2d	yes	10

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
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Information:					
9	Miscellaneous Incoming Letter	81527-0018_Claim-of-Priority.pdf	29736 555890b5477c9a02b554be6a384d8a2cb8133f0c	no	2
Warnings:					
Information:					
10	Transmittal Letter	81527-0018_IDS.pdf	32937 4f8dd6a2c6082e3c16d0b9f9866d767376d1eb22	no	3
Warnings:					
Information:					
11	Fee Worksheet (SB06)	fee-info.pdf	33118 4a164ac83fab95f19d8be4f4119164df16c0c4c7	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	15392201	

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/14 (12-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	81527.0018
	Application Number	
Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Daniel		MUETH	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

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Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Joseph		PLEWA	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

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Inventor 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Jessica		SHIREMAN	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	81527.0018
	Application Number	
Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION	

City	Kansas City	State/Province	MO	Country of Residence i	US

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Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Amy		ANDERSON	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

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Postal Code	60074	Country i	IL

Inventor 5 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Lewis		GRUBER	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	Chicago	State/Province	IL	Country of Residence i	US

Mailing Address of Inventor:

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Postal Code	60601	Country i	US

Inventor 6 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Neil		ROSENBAUM	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	Chicago	State/Province	IL	Country of Residence i	US

PTO/AIA/14 (12-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	81527.0018
	Application Number	
Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION	

Mailing Address of Inventor:			
Address 1	800 South Wells St., Apartment 1141		
Address 2			
City	Chicago	**State/Province**	IL
Postal Code	60607	**Country** i	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	13155		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION		
Attorney Docket Number	81527.0018	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	22	**Suggested Figure for Publication (if any)**	1

Filing By Reference :

Only compete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country i

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

PTO/AIA/14 (12-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	81527.0018
	Application Number	
Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION	

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	(•) Customer Number	( ) US Patent Practitioner	( ) Limited Recognition (37 CFR 11.9)
Customer Number	13155		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
	Continuation of	13412969	2012-03-06	8653442	2014-02-18
Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
13412969	Continuation of	12659277	2010-03-02	8158927	2012-04-17
Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
12659277	Division of	12213109	2008-06-13	7699767	2010-04-20
Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
12213109	Division of	11543773	2006-10-06	7402131	2008-07-22
Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
11543773	Division of	10934597	2004-09-03	7118676	2006-10-10
Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
10934597	Continuation in part of	10867328	2004-06-13	7150834	2006-12-19
Prior Application Status	Patented			Remove	

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	81527.0018
	Application Number	
Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION	

Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
10867328	Continuation in part of	10630904	2003-07-31	7241988	2007-07-10

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
10630904	Claims benefit of provisional	60399386	2002-07-31
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
10630904	Claims benefit of provisional	60435541	2002-12-20
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
10934597	Claims benefit of provisional	60571141	2004-05-14
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
10934597	Claims benefit of provisional	60499957	2003-09-04
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
10934597	Claims benefit of provisional	60511458	2003-10-15

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) [i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country [i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	81527.0018
	Application Number	
Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (12-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	81527.0018
	Application Number	
Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION	

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

(•) Assignee	( ) Legal Representative under 35 U.S.C. 117	( ) Joint Inventor
( ) Person to whom the inventor is obligated to assign.	( ) Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. [X]

Organization Name	PREMIUM GENETICS (UK) LTD.		
Mailing Address Information:			
Address 1	Alpha Building, London Road		
Address 2			
City	Nantwich	**State/Province**	
Country [i]	UK	Postal Code	CW57JW
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. [X]

PTO/AIA/14 (12-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	81527.0018
	Application Number	
Title of Invention	MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR IDENTIFICATION	

Organization Name	PREMIUM GENETICS (UK) LTD.

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1	Alpha Building, London Road		
Address 2			
City	Nantwich	**State/Province**	
Country i	UK	Postal Code	CW57JW
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications

Signature	/Jean C. Edwards/			Date (YYYY-MM-DD)	2014-01-31
First Name	Jean C.	Last Name	Edwards	Registration Number	41728

Additional Signature may be generated within this form by selecting the Add button. Add

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR SEPARATION WITH LASER STEERING

Cross-Reference to Related Applications and Priority Claims

[0001] The present invention is a continuation-in-part of Daniel M. Mueth et al., U.S. Patent Application Serial No. 10/867,328, filed June 13, 2004, entitled "Multiple Laminar Flow-Based Rate Zonal Or Isopycnic Separation With Holographic Optical Trapping Of Blood Cells And Other Static Components", commonly assigned herewith, the contents of which are incorporated by reference herein, with priority claimed for all commonly disclosed subject matter (the "first related application").

[0002] The present invention is related to Jessica Shireman et al., U.S. Provisional Patent Application Serial No. 60/571,141, filed May 14, 2004, entitled "System and Method of Sorting Blood Cells Using Holographic Laser Steering", commonly assigned herewith, the contents of which are incorporated by reference herein, with priority claimed for all commonly disclosed subject matter (the "second related application").

[0003] The present invention is related to and a conversion to a full utility application of Daniel M. Mueth, U.S. Patent Application Serial No. 60/499,957, filed September 4, 2003, entitled "Passive Fluidic Sorter", commonly assigned herewith, the contents of which are incorporated by reference herein, with priority claimed for all commonly disclosed subject matter (the "third related application").

[0004] The present invention is related to and a conversion to a full utility application of Daniel M. Mueth, U.S. Patent Application Serial No. 60/511,458, filed October 15, 2003, entitled "Passive Fluidic Sorter", commonly assigned herewith, the contents of which are incorporated by reference herein, with priority claimed for all commonly disclosed subject matter (the "fourth related application").

[0005] The present invention is related to Lewis Gruber et al., U.S. Patent Application Serial No. 10/630,904, filed July 31, 2003, entitled "System and Method of Sorting Materials Using Holographic Laser Steering", commonly assigned herewith, the

contents of which are incorporated by reference herein, with priority claimed for all commonly disclosed subject matter (the "fifth related application").

Field of the Invention

[0006] The present invention relates generally to techniques and systems for separation of particulate or cellular materials such as blood, semen and other particles or cells into their various components and fractions, using multiple laminar flows which further may be coupled with laser steering such as holographic optical trapping and manipulation.

Background of the Invention

[0007] There are several categories of blood cells. Erythrocyte or red blood cell (RBC) counts are for women 4.8 million cells/μl and men 5.4 million cells/μl. RBCs make up 93% of the solid element in blood and about 42% of blood volume. Platelets are 2μm-3μm in size. They represent 7% of the solid elements in blood and about 3% of the blood volume, corresponding to about 1.5 to $4x10^{11}$ cells per liter. There are 5 general types of white blood cells (WBCs) or leukocytes accounting for about1.5 to $4x10^{9}$ cells per liter. The WBCs comprise: 50-70% Neutrophils (12-15 μm in size); 2-4% Eosinophils (12-15 μm in size); 0.5-1% Basophils (9-10 μm in size); 20-40% Lymphocytes (25% B-cells and 75% T-cells) (8-10 μm in size); and 3-8% Monocytes (16-20 μm in size). They comprise 0.16% of the solid elements in the blood, and approximately 0.1% of the blood volume corresponding to around 4 to 12 $x10^{9}$ per liter. A subject with an infection might have a WBC count as high as $25x10^{9}$ per liter.

[0008] Platelets are the smallest cells in the blood and are important for releasing proteins into the blood that are involved in clotting. Patients with immune diseases that cause lower counts (such as cancer, leukemia and other chemotherapy patients) sometimes need platelet transfusions to prevent their counts from becoming too low. The platelet count in adults is normally between 140,000-440,000 cells/μl, and this number should not fall below 50,000 cells/μL because platelets play an integral role in blood clotting.

[0009] Blood separation techniques have traditionally employed discrete centrifugation processes. More particularly, a certain volume of blood is removed from a donor at a particular time. That volume of blood is then subjected to different levels of centrifugation to provide corresponding blood fractions for blood components such as plasma, platelets, red blood cells, and white blood cells. This process is discrete, rather than continuous, such that if more blood from the donor is to be processed, another volume is removed from the donor, and the process is repeated.

[0010] The steps in platelet collection are: collection of blood from donor: addition of anticoagulant; separation via centrifugation; return of red cells, leukocytes and plasma to the donor. A collection normally contains about 200-400 ml of plasma, which is reduced to avoid incompatibility. This collection normally contains about 8 to 8.5×10^{10} platelets. A donor normally gives approximately 10% of his/her platelets with no loss in clotting ability, although a larger number of platelets could be separated from the blood. These platelets must be used within five days of collection.

[0011] Plateletpheresis, called apheresis, is a state of the art process by which platelets are separated [Haemonetics Component Collection System (CCS) and Multi Component System (Multi)(Haemonetics, Braintree, MA)]. This automated machine separates platelets from blood over a period of 1.5 to 2 hours (assuming 10% donation). This process is faster than traditional approaches and is completely automated and can be used for single or double platelet doses. Nevertheless, the process is slow relative to the patience of donors and is capable of improvement for the purity of the separated platelet fraction.

[0012] Other procedures are also time consuming, often taking several hours, particularly when unused blood fractions are to be returned to the donor. For example, platelet donation make take several hours, as whole blood is removed from the donor, fractionated through centrifugation to obtain the platelets, and the remaining blood components are then injected back into the donor. This centrifugation process is also comparatively harsh, also can result in damage to a proportion of the harvested cells, effectively reducing the usable yield of the blood fractions.

[0013] Other types of separations are also either time consuming or cannot process large volumes of material in a timely fashion. For example, sperm sorting, in

which viable and motile sperm are isolated from non-viable or non-motile sperm, is often a time-consuming task, with severe volume restrictions.

[0014] As discussed below in greater detail in describing the present invention, manipulations of particles, such as that described in the second and fifth related applications, may also be part of a novel separation technique. One conventional technique in manipulating microscopic objects is optical trapping. An accepted description of the effect of optical trapping is that tightly focused light, such as light focused by a high numerical aperture microscope lens, has a steep intensity gradient. Optical traps use the gradient forces of a beam of light to trap a particle based on its dielectric constant.

[0015] To minimize its energy, a particle having a dielectric constant higher than the surrounding medium will move to the region of an optical trap where the electric field is the highest. Particles with at least a slight dielectric constant differential with their surroundings are sensitive to this gradient and are either attracted to or repelled from the point of highest light intensity, that is, to or from the light beam's focal point. In constructing an optical trap, optical gradient forces from a single beam of light are employed to manipulate the position of a dielectric particle immersed in a fluid medium with a refractive index smaller than that of the particle, but reflecting, absorbing and low dielectric constant particles may also be manipulated.

[0016] The optical gradient force in an optical trap competes with radiation pressure which tends to displace the trapped particle along the beam axis. An optical trap may be placed anywhere within the focal volume of an objective lens by appropriately selecting the input beam's propagation direction and degree of collimation. A collimated beam entering the back aperture of an objective lens comes to a focus in the center of the lens' focal plane while another beam entering at an angle comes to a focus off-center. A slightly diverging beam focuses downstream of the focal plane while a converging beam focuses upstream. Multiple beams entering the input pupil of the lens simultaneously each form an optical trap in the focal volume at a location determined by its angle of incidence. The holographic optical trapping technique uses a phase modifying diffractive optical element to impose the phase pattern for multiple beams onto the wavefront of a single input beam, thereby transforming the single beam into multiple traps.

[0017] Phase modulation of an input beam is preferred for creating optical traps because trapping relies on the intensities of beams and not on their relative phases. Amplitude modulations may divert light away from traps and diminish their effectiveness.

[0018] When a particle is optically trapped, optical gradient forces exerted by the trap exceed other radiation pressures arising from scattering and absorption. For a Gaussian TEM_{00} input laser beam, this generally means that the beam diameter should substantially coincide with the diameter of the entrance pupil. A preferred minimum numerical aperture to form a trap is about 0.9 to about 1.0.

[0019] One difficulty in implementing optical trapping technology is that each trap to be generated generally requires its own focused beam of light. Many systems of interest require multiple optical traps, and several methods have been developed to achieve multiple trap configurations. One existing method uses a single light beam that is redirected between multiple trap locations to "time-share" the beam between various traps. However, as the number of traps increases, the intervals during which each trap is in its "off" state may become long for particles to diffuse away from the trap location before the trap is re-energized. All these concerns have limited implementations of this method to less than about 10 traps per system.

[0020] Another traditional method of creating multi-trap systems relies on simultaneously passing multiple beams of light through a single high numerical aperture lens. This is done by either using multiple lasers or by using one or more beam splitters in the beam of a single laser. One problem with this technique is that, as the number of traps increases, the optical system becomes progressively more and more complex. Because of these problems, the known implementations of this method are limited to less than about 5 traps per system.

In a third approach for achieving a multi-trap system, a diffractive optical element (DOE) (e.g., a phase shifting hologram utilizing either a transmission or a reflection geometry) is used to alter a single laser beam's wavefront. This invention is disclosed in U.S. Patent No. 6,055,106 to Grier et al. The wavefront is altered so that the downstream laser beam essentially becomes a large number of individual laser beams with relative positions and directions of travel fixed by the exact nature of the diffractive optical element. In effect,

the Fourier transform of the DOE produces a set of intensity peaks each of which act as an individual trap or "tweezer."

[0021] Some implementations of the third approach have used a fixed transmission hologram to create between 16 and 400 individual trapping centers.

[0022] A fixed hologram has been used to demonstrate the principle of holographic optical trapping but using a liquid crystal grating as the hologram permitted 'manufacture' of a separate hologram for each new distribution of traps. The spatially varying phase modulation imposed on the trapping laser by the liquid crystal grating may be easily controlled in real time by a computer, thus permitting a variety of dynamic manipulations.

[0023] Other types of traps that may be used to optically trap particles include, but are not limited to, optical vortices, optical bottles, optical rotators and light cages. An optical vortex produces a gradient surrounding an area of zero electric field which is useful to manipulate particles with dielectric constants lower than the surrounding medium or which are reflective, or other types of particles which are repelled by an optical trap. To minimize its energy, such a particle will move to the region where the electric field is the lowest, namely the zero electric field area at the focal point of an appropriately shaped laser beam. The optical vortex provides an area of zero electric field much like the hole in a doughnut (toroid). The optical gradient is radial with the highest electric field at the circumference of the doughnut. The optical vortex detains a small particle within the hole of the doughnut. The detention is accomplished by slipping the vortex over the small particle along the line of zero electric field.

[0024] The optical bottle differs from an optical vortex in that it has a zero electric field only at the focus and a non-zero electric field in all other directions surrounding the focus, at an end of the vortex. An optical bottle may be useful in trapping atoms and nanoclusters which may be too small or too absorptive to trap with an optical vortex or optical tweezers. (See J. Arlt and M.J. Padgett. "Generation of a beam with a dark focus surrounded by regions of higher intensity: The optical bottle beam," Opt. Lett. 25, 191-193, 2000.)

[0025] The light cage (U.S. Patent No. 5,939,716) is loosely, a macroscopic cousin of the optical vortex. A light cage forms a time-averaged ring of optical traps to

surround a particle too large or reflective to be trapped with dielectric constants lower than the surrounding medium.

When the laser beam is directed through or reflected from the phase patterning optical element, the phase patterning optical element produces a plurality of beamlets having an altered phase profile. Depending on the number and type of optical traps desired, the alteration may include diffraction, wavefront shaping, phase shifting, steering, diverging and converging. Based upon the phase profile chosen, the phase patterning optical element may be used to generate optical traps in the form of optical traps, optical vortices, optical bottles, optical rotators, light cages, and combinations of two or more of these forms.

[0026] Researchers have sought indirect methods for manipulating cells, such as tagging the cells with diamond micro-particles and then tweezing the diamond particles. Cell manipulations have included cell orientation for microscopic analysis as well as stretching cells. Tissue cells have also been arranged with tweezers *in vitro* in the same spatial distribution as *in vivo*.

[0027] In addition to the cells themselves, optical tweezers have been used to manipulate cellular organelles, such as vesicles transported along microtubules, chromosomes, or globular DNA. Objects have also been inserted into cells using optical tweezers.

[0028] Accordingly, as an example of new types of sorting using laser steered optical traps, a method of cell sorting using a technique which isolates valuable cells from other cells, tissues, and contaminants is needed. Further, a way of achieving a unique contribution of optical trapping to the major industrial needs of blood cell sorting and purification is required. Still further, there is a need to separate sperm cells in the animal husbandry market.

[0029] As a consequence, a need remains for a separation technique and apparatus which is continuous, has high throughput, provides time saving, and which causes negligible or minimal damage to the various components for separation. In addition, such techniques should have further applicability to biological or medical areas, such as for separations of blood, sperm, other cellular materials, as well as viral, cell organelle, globular structures, colloidal suspensions, and other biological materials.

Summary of the Invention

[0030] The exemplary embodiments of the present invention provide for separating components in a mixture, such as separating the various blood components of whole blood into corresponding fractions, such as a platelet fraction, a red blood cell fraction, a white blood cell fraction, and a plasma fraction. The various embodiments of the present invention provide separation of components on a continuous basis, such as within a continuous, closed system, without the potential damage and contamination of prior art methods, particularly for fractionation of blood components. The continuous process of the present invention also provides significant time savings and higher throughput for blood fractionation. In addition, the various embodiments may also include additional means for separating and manipulating the components, particularly holographic optical manipulation and separation. The various embodiments may also be applied to separations of other types of cellular and biological materials, such as sperm, viruses, bacteria, cell debris, cell organelles, globular structures, colloidal suspensions, cellular debris, and other biological materials.

[0031] As used herein, "Particle" refers to a biological or other chemical material including, but not limited to, oligonucleotides, polynucleotides, chemical compounds, proteins, lipids, polysaccharides, ligands, cells, antibodies, antigens, cellular organelles, lipids, blastomeres, aggregations of cells, microorganisms, peptides, cDNA, RNA and the like.

[0032] An exemplary method of separating blood into components includes providing a first flow having a plurality of blood components; providing a second flow; contacting the first flow with the second flow to provide a first separation region; and differentially sedimenting a first blood cellular component of the plurality of blood components into the second flow while concurrently maintaining a second blood cellular component of the plurality of blood components in the first flow. The second flow having the first blood cellular component is then differentially removed from the first flow having the second blood cellular component.

[0033] The various sedimentation steps of the present invention may be rate zonal or isopycnic. In addition, the first flow and the second flow are substantially non-turbulent, and may also be substantially laminar.

[0034] In a selected embodiment, the first blood cellular component is a plurality of red blood cells and a plurality of white blood cells, and the second blood cellular component is a plurality of platelets. For the first blood cellular component, the plurality of white blood cells may be holographically separated (through laser steering) from the plurality of red blood cells. Other holographic manipulations of the present invention include holographically removing a plurality of contaminants from the first flow, holographically separating biological debris from the first flow, and holographically separating a plurality of second blood cellular components from the first flow.

[0035] Additional separation stages may also be included, with the exemplary method providing a third flow; contacting the first flow with the third flow to provide a second separation region; and differentially sedimenting the second blood cellular component of the plurality of blood components to sediment into the third flow while concurrently maintaining a third blood component of the plurality of blood components in the first flow. In selected embodiments, the second blood cellular component is a plurality of platelets and wherein the third blood component is plasma.

[0036] A plurality of separation stages may also be combined to form more complicated structures having multiple separation stages, connected in series, connected in parallel, or in combinations of both.

[0037] A second exemplary method of separating a fluid mixture into constituent, non-motile components, in accordance with the present invention, includes: providing a substantially laminar first flow having the fluid mixture, the fluid mixture having a plurality of components, the plurality of components having a corresponding plurality of sedimentation rates; providing a substantially laminar second flow; contacting the first flow with the second flow to provide a first separation region, the first flow and the second flow having a substantially non-turbulent interface within the separation region; differentially sedimenting from the first flow a first component of the plurality of components into the second flow to form an enriched second flow and a depleted first flow, while concurrently maintaining a second component of the plurality of components

in the first flow, the first component having a first sedimentation rate of the plurality of sedimentation rates and the second component having a second sedimentation rate of the plurality of sedimentation rates, wherein the first sedimentation rate is comparatively greater than the second sedimentation rate; differentially removing the enriched second flow from the depleted first flow; and holographically manipulating the second component in the depleted first flow.

[0038] The second exemplary method may also include additional separation stages, such as a holographic separation, including: providing a third flow; contacting the depleted first flow with the third flow to provide a second separation region; and holographically trapping the second component and moving the second component from the depleted first flow into the third flow while concurrently maintaining a third component of the plurality of components in the depleted first flow.

[0039] An exemplary apparatus embodiment of the invention for separating a fluid mixture into constituent, non-motile components includes: a first sorting channel having a first inlet for a first flow and a second inlet for a second flow; the first sorting channel further having a first outlet for the first flow and a second outlet for the second flow, the first sorting channel further having means to maintain the first flow and second flow substantially non-turbulent, the first sorting channel adapted to allow a first component in the first flow, of a plurality of components in the first flow, to sediment into the second flow to form an enriched second flow and a depleted first flow, while concurrently maintaining a second component of the plurality of components in the first flow; a second, optically transparent sorting channel having a first optical inlet coupled to the first outlet for the first flow and having a first optical outlet, the second, optically transparent sorting channel further having a second optical inlet for a third flow and a second optical outlet for the third flow; and a holographic optical trap coupled to the second, optically transparent sorting channel, the holographic optical trap adapted to generate a holographic optical trap to select and move the second component from the first flow into the third flow. The various components which are separated, for example, may be the various blood fractions or other biological materials, such as separations of motile from non-motile sperm.

[0040] Another apparatus or system for separating a plurality of components in a fluid comprises: an optically transparent sorting channel having a first inlet for a first flow and a second inlet for a second flow, the optically transparent sorting channel further having a first outlet for the first flow and a second outlet for the second flow; and a holographic optical trap system coupled to the optically transparent sorting channel, the holographic optical trap system adapted to generate a holographic optical trap to select and move a first component in the first flow, of a plurality of components in the first flow, into the second flow to form an enriched second flow and a depleted first flow, while a second component of the plurality of components is concurrently maintained in the first flow.

[0041] Another method embodiment provides for separating a plurality of cells, comprising: providing a first flow having the plurality of cells; providing a second flow; contacting the first flow with the second flow to provide a first separation region; and differentially sedimenting a first cell of the plurality of cells into the second flow while concurrently maintaining a second cell of the plurality of cells in the first flow. The method generally also includes differentially removing the second flow having the first cell from the first flow having the second cell. The method may also provide for providing a third flow; contacting the first flow with the third flow to provide a second separation region; and differentially sedimenting the second cell of the plurality of cells into the third flow while concurrently maintaining a third cell of the plurality of cells in the first flow. In addition, a plurality of second cells may be holographically separated from the first flow, and a plurality of contaminants or biological debris may be holographically removed from the first flow.

[0042] In another embodiment consistent with the present invention, optical trapping (or laser steering), which is a technology which has been used as a tool for manipulating microscopic objects, is used. An accepted description of the effect is that tightly focused light, such as light focused by a high numerical aperture microscope lens, has a steep intensity gradient. Optical traps use the gradient forces of a beam of light to trap a particles based on its dielectric constant To minimize its energy, a particle having a dielectric constant higher than the surrounding medium will move to the region of an optical trap where the electric field is the highest.

[0043] Optical trapping of the present invention is used to address cell sorting and purification (*e.g.*, from contaminants such as viruses and bacteria) in several ways. For example, the forces exerted by optical traps on a material are sensitive to the exact distribution of the dielectric constant in that material - the optical force therefore depends on the composition and shape of the object.

[0044] Further, other forces on the object are sensitive to the hydrodynamic interaction between the object and the surrounding fluid - control of the fluid flow probes material shape, size and such features as surface rugosity.

[0045] Still further, localizing an object at a known position allows additional methods of automated interrogation such as high speed imaging and particle-specific scattering measurements.

[0046] In one embodiment consistent with the present invention, in achieving a multi-trap system, a diffractive optical element ("DOE", *i.e.*, a phase shifting hologram utilizing either a transmission or a reflection geometry) is used to alter a single laser beam's wavefront. The wavefront is altered so that the downstream laser beam essentially becomes a large number of individual laser beams with relative positions and directions of travel fixed by the exact nature of the diffractive optical element.

[0047] The present invention provides optical trapping by focusing a laser beam with a lens to create an optical trap wherein the lens has a numerical aperture less than 0.9, and preferably decreases until it is most preferably less than 0.1.

[0048] Sorting using holographic laser steering involves establishing classes of identification for objects to be sorted, introducing an object to be sorted into a sorting area, and manipulating the object with a steered laser according to its identity class. The manipulation may be holding, moving, rotating, tagging or damaging the object in a way which differs based upon its identity class. Thus, the present invention provides a way of implementing a parallel approach to blood cell sorting and sperm cell sorting using holographic optical trapping.

[0049] In one embodiment of the present invention, spectroscopy of a sample of biological material may be accomplished with an imaging illumination source suitable for either inelastic spectroscopy or polarized light back scattering, the former being useful for assessing chemical identity, and the latter being suited for measuring dimensions of

internal structures such as the nucleus size. Using such spectroscopic methods, in some embodiments, cells are interrogated. The spectrum of those cells which had positive results (*i.e.*, those cells which reacted with or bonded with a label) may be obtained by using this imaging illumination.

[0050] A computer program may analyze the spectral data to identify the desired targets (i.e., cells bearing either an X or Y chromosome, or a suspected cancerous, pre-cancerous and/or non-cancerous cell types, etc.), then may apply the information to direct the phase patterning optical element (i.e., optical traps) to segregate or contain those desired or selected targets (i.e., cell types). The contained cells may be identified based on the reaction or binding of the contained cells with chemicals, or by using the natural fluorescence of the object, or the fluorescence of a substance associated with the object, as an identity tag or background tag. Upon completion of the assay, selection may be made, via computer and/or operator, of which cells to discard and which to collect.

[0051] Manipulation of cells in general, is made safer by having multiple beams available. Like a bed of nails, multiple tweezers ensure that less power is introduced at any particular spot in the cell. This eliminates hot spots and reduces the risk of damage. Any destructive two-photon processes benefit greatly since the absorption is proportional to the square of the laser power. Just adding a second tweezer decreases two-photon absorption in a particular spot by a factor of four. Trapping large cells involves a large amount of laser power for effective trapping. Putting the power into a single trap may cause immediate damage to the cell.

[0052] The manipulation of even just a single cell is greatly enhanced by utilizing holographic optical trapping, for example. A single cell may be manipulated by a line of tweezers, which lift the cell along the perimeter on one side. The resulting rotation allows a 360 degree view of the cell. In addition to the advantage for viewing of biological samples, there also exists the ability to orient samples stably, which has clear benefit for studies such as scattering experiments which have a strong dependence on orientation of the sample.

[0053] Sorting with a wide field of view has many advantages such as higher throughput. However, standard tweezing in a WFOV (wide field of view) may fail due to excessive radiation pressure.. Tweezing with a wide field of view using holographic

optical trapping may permit the ability to form exotic modes of light which greatly reduce the radiation pressure of the light beam. Vortex traps, for example, have a dark center because the varying phases of light cancel in the center of the trap. This dark center means most of the rays of light which travel down the center of the beam no longer exist. It is exactly these beams which harbor most of the radiation pressure of the light, so their removal greatly mitigates the difficulty in axial trapping. Other modes, e.g., donut modes, have the same advantage.

[0054] In one embodiment consistent with the present invention, the method and system lends itself to a semi-automated or automated process for tracking the movement and contents of each optical trap. In one embodiment consistent with the present invention, movement may be monitored via an optical data stream which can be viewed, or converted to a video signal, monitored, or analyzed by visual inspection of an operator, spectroscopically, and/or by video monitoring. The optical data stream may also be processed by a photodectector to monitor intensity, or any suitable device to convert the optical data stream to a digital data stream adapted for use by a computer and program. The computer program controls the selection of cells and the generation of optical traps.

[0055] In other embodiments consistent with the present invention, the movement of cells is tracked based on predetermined movement of each optical trap caused by encoding the phase patterning optical element. Additionally, in some embodiments, a computer program maintains a record of each cell contained in each optical trap.

[0056] There has thus been outlined, rather broadly, some features consistent with the present invention in order that the detailed description thereof that follows may be better understood, and in order that the present contribution to the art may be better appreciated. There are, of course, additional features consistent with the present invention that will be described below and which will form the subject matter of the claims appended hereto.

[0057] In this respect, before explaining at least one embodiment consistent with the present invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and to the arrangements of the components set forth in the following description or illustrated in the drawings. Methods and apparatuses consistent with the present invention are capable of other embodiments and of being

practiced and carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein, as well as the abstract included below, are for the purpose of description and should not be regarded as limiting.

[0058] As such, those skilled in the art will appreciate that the conception upon which this disclosure is based may readily be utilized as a basis for the designing of other structures, methods and systems for carrying out the several purposes of the present invention. It is important, therefore, that the claims be regarded as including such equivalent constructions insofar as they do not depart from the spirit and scope of the methods and apparatuses consistent with the present invention.

[0059] Numerous other advantages and features of the present invention will become readily apparent from the following detailed description of the invention and the embodiments thereof, from the claims and from the accompanying drawings.

Brief Description of the Drawings

[0060] The objects, features and advantages of the present invention will be more readily appreciated upon reference to the following disclosure when considered in conjunction with the accompanying drawings, in which:

[0061] Figure (or "FIG.") 1 is a lateral view of an apparatus 100 in accordance with one embodiment consistent with the present invention.

[0062] Figure 2 is an illustration of optical trapping for component separation in an apparatus 200.

[0063] Figure 3 is a diagram illustrating a closed, two-stage system 300 for blood component separation in accordance with one embodiment consistent with the present invention.

[0064] Figure 4 schematically illustrates a holographic optical trapping system in accordance with one embodiment consistent with the present invention.

[0065] Figure 5 is a schematic diagram of a holographic optical trapping system for sorting objects in accordance with one embodiment consistent with the present invention.

[0066] Figure 6 (divided into Figure 6A and Figure 6B) is a flow diagram illustrating a method embodiment of consistent with the present invention.

[0067] Figures 7A and 7B are a side (lateral) view schematic diagram and a top view schematic diagram, respectively, showing a sample being introduced into sample holder, in accordance with one embodiment consistent with the present invention.

[0068] Figure 8 depicts a scanning electron micrograph of a sample chamber in accordance with one embodiment consistent with the present invention.

[0069] Figure 9 shows an enlarged view of the working area of a sample chamber in accordance with one embodiment consistent with the present invention.

[0070] Figure 10 illustrates an example of lateral deflection for sorting in accordance with one embodiment consistent with the present invention.

[0071] Figures 11A and 11B illustrate schematic front and side views, respectively, of the funneling traps in accordance with one embodiment consistent with the present invention.

[0072] Figure 12 illustrates a spinning disc-based cell sorter in accordance with one embodiment consistent with the inventions of the second and fifth related applications.

[0073] Figure 13 illustrates optical peristalsis in accordance with one embodiment consistent with the present invention.

[0074] Figure 14 illustrates a sorting system in accordance with one embodiment consistent with the present invention.

[0075] Figure 15 illustrates a sorting system in accordance with one embodiment consistent with the present invention.

[0076] Figure 16 is a lateral view of a high-aspect ratio flat sorter in accordance with one embodiment consistent with the present invention.

[0077] Figure 17 is a plan view of a high-aspect ratio flat sorter in accordance with one embodiment consistent with the present invention.

[0078] Figure 18 is a perspective view of a three-dimensional sorting device having a plurality of flat sorters in accordance with one embodiment consistent with the present invention.

[0079] Figure 19 is a plan view of a multi-channel sorter in accordance with one embodiment consistent with the present invention.

[0080] Figure 20 is a plan view of a sorter having a narrow waste flow region in accordance with one embodiment consistent with the present invention.

[0081] Figure 21 is a plan view of a sorter using different flow rates for various channels in accordance with one embodiment consistent with the present invention.

[0082] Figure 22 is a plan view of a sorter having multiple selection channels in accordance with one embodiment consistent with the present invention.

[0083] Figure 23 is a plan view of a sorter having a constricted sorting region in accordance with one embodiment consistent with the present invention.

[0084] Figure 24A is a lateral view of a multi-layer laminar flow sorter in accordance with one embodiment consistent with the present invention.

[0085] Figure 24B is a plan view of a multi-layer laminar flow sorter in accordance with one embodiment consistent with the present invention.

[0086] Figure 25 illustrates the results of bovine sperm viability or motility sorting using the various embodiments of the present invention.

[0087] Figure 26 is a block diagram illustrating an exemplary sorting and separation system in accordance with one embodiment consistent with the present invention.

[0088] Figure 27 is a block diagram illustrating an exemplary bioreactor product purification and separation system in accordance with one embodiment consistent with the present invention.

Detailed Description of the Exemplary Embodiments

[0089] While the present invention is susceptible of embodiment in many different forms, there are shown in the drawings and will be described herein in detail specific embodiments thereof, with the understanding that the present disclosure is to be considered as an exemplification of the principles of the invention and is not intended to limit the invention to the specific embodiments illustrated.

[0090] As indicated above, the various embodiments of the present invention provide for separating components in a mixture, such as separating the various blood components of whole blood into corresponding fractions, such as a platelet fraction, a red blood cell fraction, a white blood cell fraction, and a plasma fraction. The various

embodiments, as described below, utilize one or more sorting channels, having a plurality of substantially laminar flows, allowing one or more components to differentially sediment from one flow into another, thereby separating the components into corresponding flows. In addition, the various components may be sorted further using optical mechanisms, such as holographic optical trapping. The various embodiments of the present invention thereby provide separation of components on a continuous basis, such as within a continuous, closed system, without the potential damage and contamination of prior art methods, particularly for fractionation of blood components. The continuous process of the present invention also provides significant time savings for blood fractionation.

[0091] In addition to whole blood sorting and fractionation applications, the present invention is also suitable for other cell sorting applications, such as separations of cancer cells from normal or healthy cells in, for example, bone marrow extractions. The various embodiments of the present invention have further applicability to other biological or medical areas, such as for separations of cells, sperm, viruses, bacteria, cellular organelles or subparts, globular structures, colloidal suspensions, lipids and lipid globules, gels, immiscible particles, blastomeres, aggregations of cells, microorganisms, and other biological materials. For example, the component separation in accordance with the present invention may include cell "washing", in which contaminants (such as bacteria) are removed from cellular suspensions, which may be particularly useful in medical and food industry applications. Significantly, prior art flow-based techniques have not recognized any applicability to sorting or separation of non-motile cellular components using variable sedimentation rates and optical manipulation.

[0092] While discussion below focuses on the sorting of blood components to create different blood fractions, the apparatus, methods and systems of the present invention may be extended to other types of particulate, biological or cellular matter, which are capable of sedimenting or creaming within a fluid flow, or which are capable of being manipulated optically between different fluid flows. For example, the methodology of the present invention could be utilized to separate non-motile or non-viable sperm cells from viable cells, by allowing the non-motile cells to sediment from a first flow into a second flow while retaining the motile cells in the first flow or allowing

the motile cells to move to a third flow. Other sorts of cell separation may also be performed, such as separating islet cells from other types of pancreatic cells, or otherwise separating islet cell clusters of different sizes, through either or both flow separation or optical tweezing (trapping). Viruses, proteins and other large molecules having different sedimentation rates may also be separated with the present invention. The holographic optical trapping utilized with the various separation stages may also be particularly useful in these other types of cell or particle separations.

[0093] The present invention has other medical applications as well. For example, the various laminar flows discussed below may be utilized as part of a kidney dialysis process, in which whole blood is cleansed of waste products and returned to the patient. As a consequence, in addition to particle separations based upon relative density, for example, the present invention may be utilized for separations based upon diffusion, motility, and other types of gradients.

[0094] For example, the present invention may be utilized to move a species from one solution to another solution where separation by filtering or centrifugation is not practical or desirable. In addition to the applications discussed above, additional applications include isolating colloids of a given size from colloids of other sizes (for research or commercial applications), and washing particles such as cells, egg cells, etc. (effectively replacing the medium in which they are contained and removing contaminants), or washing particles such as nanotubes from a solution of salts and surfactants with a different salt concentration or without surfactants, for example.

[0095] The action of separating species may rely on a number of physical properties of the objects including self-motility, self-diffusivity, free-fall velocity, or action under an external force, such as an electromagnetic field or a holographic optical trap. The properties which may be sorted upon include cell motility, cell viability, object size, object mass, object density, the tendency of particles to attract or repel one another or other objects in the flow, object charge, object surface chemistry, and the tendency of certain molecules to adhere to the object.

[0096] While the present invention is discussed in detail with respect to the apparatus 100, 200 and 300, it should be understood that this discussion applies equally to the various other embodiments illustrated in Figures 13-24 and 26-27.

[0097] Figure 1 is an illustration of a lateral view of an apparatus 100 in accordance with the present invention. As illustrated in Figure 1, the sorting apparatus 100 includes a sorting channel 110, a plurality of inlets 120 and a plurality of outlets 130. A corresponding fluid flow, such as illustrated flows W, X, Y and Z, enters one of the inlets 120 and flows, substantially non-turbulently or otherwise as a laminar flow, across the sorting channel (or sorting region) 110, and out through a corresponding outlet 130, as illustrated.

[0098] The apparatus 100 (and 200, below) may be constructed of a plurality of materials, integrally or as discrete components, using a wide variety of materials, such as metals, ceramics, glass, and plastics. Various materials and fabrication methods are discussed in the third and fourth related applications, and include, for example, use of various polymers which cure under UV exposure. Other details are also provided in the third and fourth related applications, such as the use and selection of different types of pumps, such as syringe pumping, peristaltic pumping, gravity-driven pumping, and various combinations of pumping actions.

[0099] In selected embodiments, when coupled with holographic trapping or other form of optical tweezing, the apparatus 100 (or 200) is transparent to the selected wavelength of the holographic generator, such as optically transparent when the holographic generator utilizes visible wavelengths. Depending upon the selected application, the apparatus 100 (200) should also be sterile and may also have a controlled temperature. The various fluid flows may be fed into the inlets 120 through a wide variety of means known to those of skill in the art and are within the scope of the present invention, including use of peristaltic pumps or a gravity feed, for example, and such means may also be utilized to control the flow rates of the various flows W, X, Y and Z. When peristaltic pumps are utilized, to maintain a constant flow rate and pressure, bubble-traps may be incorporated at the inlets 120 of the apparatus 100 (or 200).

[0100] The various fluids utilized in the separation flows may be diluted or concentrated, to increase or decrease the volume of one of the solutions, or to impact the concentration of some dissolved or suspended material, or to impact physical properties of the solution such as its viscocity, temperature, or density. Examples for the apparatus 100, when used for blood sorting, include: (a) dilution of the blood to reduce clogging or

hydrodynamic interaction between blood cells, (b) extension of the volume of the blood or a blood fraction, (c) modification of the density of the blood, a blood fraction, or another solution which impacts the flow properties or separation behavior, (d) extension of the volume of a solution to maintain in increase fluid volume, especially in circumstances when fluid volume is being removed from the system. As discussed in greater detail below, various chemical attractants and repellants may be added to the fluids, which may also be at different temperatures and viscosity levels, to improve sperm sorting.

[0101] The various fluids utilized in the separation flows also may be "activated", such that some process is activated within the solution by some external influence or mixing with an external solution. Examples of external influences include: (a) applying an electric field, (b) applying a magnetic field, (c) exposing to light, (d) modifying the temperature, (e) introducing a chemical, (f) introducing a biological material, (g) shearing the solution, and (h) vibrating the solution. Examples of the activation which is caused by the external solution include: (a) alignment of particles, molecules, or cells, (b) polarization of one or more components of the solution, (c) cross-linking, (d) initiation or termination of chemical reaction, (e) initiation or termination of a biological response, (f) changing the type or rate of a chemical, physical, or biological response, or (g) causing a response or separation which depends upon the character of the particular component which is responding. Examples for the apparatus 100, when used for blood sorting, include: (a) addition of an agent to reduce clotting; (b) addition of agents to preserve viability or health of the blood solution or its components; (c) addition of agents which may augment the sorting process, such as by binding or collecting near certain components, thereby influencing one or more of their physical properties, including the addition of beads or other particles or polymers which may adhere to one or more species, and also including the introduction of salts or other materials which may influence the electrostatic interaction of materials or the hydrodynamic size or character of the materials; (d) addition of agents which may influence the flow properties, such as by changing the density, viscosity, surface tension, or other parameters; (e) addition of agents to enhance or suppress the aggregation of certain materials; and (f) addition of

agents to enhance or suppress the adherence of certain materials to other materials or parts of the flow device.

[0102] In accordance with the invention, one of the fluid flows, such as the illustrated flow W, contains a plurality of components A, B, C and E. For example, when the fluid is whole blood, these components may be red blood cells ("RBC"), white blood cells ("WBC"), platelets, cellular debris and contaminants, all in plasma. Typically, many of the plurality of components have different sedimentation rates, typically measured using a Svedberg coefficient. For example, RBCs have a comparatively greater sedimentation rate than platelets, and will be expected to sediment faster on a passive basis, such as due to gravitational or buoyant forces, without the intervention of other, active mechanisms, such as centrifugation. As the various flows W, X, Y and Z flow through the sorting region 110, based upon different sedimentation rates, the plurality of components (such as cells or other particles) will sediment, moving from one flow to another. As illustrated, component A having the comparatively greatest sedimentation rate is illustrated as having moved from flow W to the lowest flow Z, component B having the comparatively next highest sedimentation rate is illustrated as having moved from flow W to the flow Y (above Z), component C having a comparatively smaller sedimentation rate is illustrated as having moved from flow W to the flow X (above Y), while component E having the comparatively smallest sedimentation rate, is illustrated as having remained in flow W (above Y). Using these different sedimentation properties, each of these components may be separated into a corresponding flow, and isolated from each other as each flow exits through its corresponding outlet 130. As each flow W, X, Y and Z exits through its corresponding outlet 130, that flow is differentially removed from the other flows, *i.e.*, the flow is removed while the other flow remains intact or is otherwise separately removed from the remaining flows. In addition, this differential removal may be concurrent, namely, all flows removed concurrently or continuously.

[0103] Continuing to refer to Figure 1, using whole blood with an anticoagulant (such as sodium citrate or heparin) as the fluid flow W, for example, the various blood fractions may be separated from each other, with red blood cells sedimenting fastest and represented by component A (*e.g.*, 4.59 μm/s), white blood cells sedimenting at a slightly

lower rate and represented by component B (*e.g.*, 2.28 μm/s), platelets sedimenting at a comparatively slower rate and represented by component C (*e.g.*, 0.055 μm/s), and plasma continuing to comprise flow W and represented by component E. Each blood fraction may then be removed through a corresponding outlet 130.

[0104] Not separately illustrated in Figure 1, due to buoyant forces and relative density considerations, there may be particles or components in one or more of the fluid flows which will flow up to a higher flow (*e.g.*, creaming). For example, less dense particles entering through flow X may rise into flow W, and exit with flow W through a corresponding outlet 130.

[0105] Illustrated in lateral view, the sorting channel (or sorting region) 110 of apparatus 11 has a varied length "L" parallel to the direction of flow, a depth "D" perpendicular to the direction of flow, and a width "WW", illustrated as extending into the page (and designated WW to avoid confusion with the W flow). These various dimensions may be selected based on a plurality of factors, particularly the flow rates and the sedimentation rates of the components of interest. For example, for a selected flow rate, the total length of the sorting channel should be long enough to differentially remove the component having the comparatively slowest sedimentation rate, illustrated as component C in Figure 2, with shorter lengths corresponding to other flows for separation of components having faster sedimentation rates, as illustrated for flow Z having component A and flow Y having component B.

[0106] The present invention is further distinguished from the prior art by having considerably more latitude or tolerance for aspect ratios, while nonetheless maintaining a substantially laminar flow. The aspect ratio of length to width, for example, may vary from about 5 (or more) to 1 (5:1), with the length being greater than the width, to about 1 (or less) to 2 (the length being smaller than the width). A preferred length to width aspect ratio is about 2:1, and may vary from 3:1 to 1:2.

[0107] Flow rates may also vary between the plurality of flows utilized in apparatus 100. For example, higher flow rates in the lower level flows (such as Y and Z) may tend to compress the flows W and X, resulting in a shorter distance that certain components must traverse to sediment into the flows Y and Z.

[0108] In addition, the sedimentation of components through the various flows of the apparatus 100 is typically rate zonal, that is, based upon both relative density and size of the components to be separated, as well as the material's shape and electrostatic properties. Under other conditions, however, such as slower flow rates, thinner flow depths, and/or longer sorting channels 100, the sedimentation may also be isopycnic, that is, based only upon relative density of the components.

[0109] When isopycnic separation is desired, the various fluids comprising the flows W, X, Y and Z may be selected and adjusted to create desired density gradients to match the component densities for the selected separations. For example, the various fluids comprising the flows W, X, Y and Z may be selected and adjusted to each have a different, increasing or decreasing density, creating a stepped density gradient, with various particles sedimenting to the appropriate step. In addition, through use of a sufficient number of fluid flow layers, the density gradient will effectively become continuous, with a corresponding ability for fine-grained separation.

[0110] The various fluids comprising the generally laminar flows, such as flows W, X, Y and Z, may also be selected based on suitable criteria for the particular desired component separation. For example, for blood separation, the various flows may be comprised of whole blood, such as for flow W, and plasma or buffering solutions for the remaining flows. The various fluids may also be preprocessed prior to entry through the inlets 120, such as through dilution, addition of other components such as additives (such as anticoagulants, flocculants, or binding agents), viscosity or other flow property manipulation, or preprocessed through other separation techniques. Also for example, whole blood may be preprocessed to initially remove some red blood cells or to add an anticoagulant such as sodium citrate.

[0111] While illustrated with four flows or channels, it should be understood that the apparatus 100 (or 200, below) may be implemented with any number of flows and corresponding fluid inlets 120 and outlets 130. One limitation to the number of fluid flows is based on the ability to maintain each flow in a substantially laminar or non-turbulent manner, such that each interface between flows is substantially non-turbulent, to minimize any unwanted mixing of flows. In addition, there also may be relative

density considerations for the fluids comprising the flows which could also result in limiting the number of flows utilized in a given stage of separation.

[0112] The various apparatus 100 (or 200, below) may be further coupled to additional apparatus 100 (200, below), in parallel for higher throughput, and in series for additional separation stages, such as for increased purity levels. In addition, the various apparatus 100 may also be combined with non-sedimentation separations, or be coupled in series with subsequent separations using non-sedimentation mechanisms, with additional separation of components between flows accomplished, for example, using optical forces such as holographic optical trapping of the fifth related application, incorporated herein by reference. These various apparatus 100, 200 or 300, moreover, may have different dimensions and different numbers of channels or flows.

[0113] Figure 2 (and also Figure 13) is a general illustration of using such optical forces created by holographic or optical trapping for additional component separation in an apparatus 200. Creation and manipulation of the plurality of holographic optical traps 210 is explained in greater detail below with reference to Figures 4 and 5, with apparatus 200 forming the sample 506 of Figure 5. Two flows W and X are illustrated in Figure 2, with flow W initially having two components A and B. Holographic optical traps 210 (illustrated as conic sections in Figure 2) are then utilized to capture "A" components, and move them into flow X. Such optical trapping is particularly useful for increased purification of a particular fraction, particularly for fractions having insufficient differentiation based on sedimentation rates. Such optical trapping is also particularly useful for removal of undesirable components, such as cellular debris and other impurities. In addition, where mixing or remixing of components may have occurred during the rate zonal laminar flow separations discussed above, the optical trapping may be particularly accurate in removing undesired components. For example, a comparatively small portion of white blood cells may not have sedimented fast enough, resulting in some white blood cell contamination of a platelet fraction. Optical trapping may be utilized to select and move the white blood cells into a separate flow, increasing the purity of the platelet fraction.

[0114] For blood sorting applications, it should be understood that platelets and RBC optically manipulate (or "tweeze") better than white blood cells. Using lower

numerical apertures in the system 500 (discussed below), however, significantly improves optical manipulation of white blood cells.

[0115] When implemented in conjunction with optical traps, the apparatus 100, 200 or 300 should be embodied utilizing an optically transparent material, for the selected optical wavelength. When the holographic traps are implemented at other wavelengths, other correspondingly transparent materials may be utilized which are suitable for the selected wavelength. The apparatus 100, 200 or 300 is then implemented and placed in the location of the sample 506 illustrated in Figure 5, with the system 500 utilized to perform the holographic optical trapping as one of or as part of a separation stage of the present invention.

[0116] Figure 3 is a diagram illustrating a closed, two-stage system 300 for blood or other component separation in accordance with the present invention. In a first stage 305, blood components from a selected donor flow through inlet 315 to form a first flow, and plasma is returned (or primed on initial start up) through inlet 320 to form a second flow. The first and second flows are non-turbulent and otherwise laminar flows, and make non-turbulent contact with each other in first separation region 325, forming a non-turbulent interface region between the two flows. In the first separation region 325, both red blood cells and white blood cells sediment from the first flow into the second flow, and are collected in reservoir 330 for other uses (such as medical uses for packed cells) or for return to the selected donor. As indicated above, both the length of the first separation region 325 and the flow rate of the first flow are predetermined such that both red blood cells and white blood cells have sufficient time to passively sediment into the second flow, under gravitational and buoyant forces.

[0117] Continuing to refer to Figure 3, the first flow, now substantially depleted of both red blood cells and white blood cells, flows non-turbulently on a continuous path into a second separation stage 310. In the second separation stage 310, the first flow enters a second separation region 340 with a third flow from inlet 335. The third flow is also comprised of plasma from the selected donor in the exemplary embodiment. In the second separation region 340, the platelets remaining in flow one passively sediment into flow three, and are collected with the plasma of flow three in reservoir 345 for medical use, for example. The further depleted flow one is then recirculated from outlet 350 back

to inlets 320 and 335, to form the first and third flows, respectively. As indicated above, the system 300 may be primed with donor plasma at system start-up by, for example, centrifuging a portion of the selected donor's blood, or by initially using another biocompatible, non-toxic liquid until a depleted flow one (substantially or predominantly plasma) is generated at outlet 350.

[0118] Not separately illustrated in Figure 3, an additional holographic trapping separation stage may also be utilized to aid in the separation of these blood fractions. For example, a holographic trapping separation stage may be utilized in lieu of, or in addition to, second stage 310. In addition, while the second stage separation has been illustrated using flow one, in other embodiments, flow two may be subjected to a second (or more) stage separation, in addition to or in lieu of the additional separation stage of flow one. Moreover, additional separation stages may be utilized in series or in parallel.

[0119] More generally, separation regions are regions where materials are partially or fully sorted or separated based upon some material property. The separation regions may employ one or more of the following techniques, individually, serially, or simultaneously, in any of the various flows in the embodiments of the invention:

(a) Sedimentation Rate Zonal Separation: separation by sedimentation rate. The sedimentation rate is generally a function of the material's size and density, as well as the material's shape and electrostatic properties. For this separation, laminar flow is set up and separation occurs under gravity, or in some cases through other inertial forces such as spinning in a centrifuge-like device.

(b) Isopycnic Separation: separation by density. For this separation, a linear, step, smoothed-step, or alternate density gradient is established and the materials are allowed to sediment and/or cream in the gradient until the material reaches or nearly reaches an area where the material is in an environment of matched density.

(c) Diffusivity Separation: separation by diffusivity. For this separation, materials are fractionated based upon the distance they diffuse in a given amount of time.

(d) Motility Separation: separation by motility. In this separation, materials are fractionated based on the distance the material travels under its own motility in a given amount of time.

(e) Optical Fractionation: separation using optical forces, typically without feedback mechanisms to inform and influence the optical system based upon investigation of the individual objects.

(f) Direct Optical Separation: separation using optical forces, typically using feedback mechanisms to inform and influence the optical system based upon investigation of the individual objects.

(g) Dielectrophoretic Separation: separation using dielectrophoresis. The forces exerted upon an object depends upon its position in the imposed electric field and the dielectric response of the material and its environment.

(h) Electrophoretic Separation: separation using electrophoresis. The forces exerted upon an object depends upon its position in the imposed electric field and the charge of the material and its environment.

(i) Magnetic Separation: separation using magnetic forces. The forces exerted upon an object depends upon its position in the imposed magnetic field and the magnetic properties of the object.

(j) Surface Tension Separation: separation using the surface tension or surface chemistry of a material. This may, for example, involve the creating of fluid interfaces which certain materials may be attracted to or stable at.

[0120] More particularly, exemplary separations for blood sorting include: (a) separation of one or more blood cell types from some or all of the other blood cell types and/or from the blood plasma or fluid medium by sedimentation rate zonal separation; (b) separation as in (a) but with isopycnic separation; (c) removal of just the red blood cells (RBCs) or the RBCs and white blood cells (WBCs) from the solution by sedimentation; (d) concentration of the platelets from the plasma using sedimentation; (e) extraction of the RBCs using dielectrophoresis, electrophoresis, or magnetic separation; (f) concentration or extraction of platelets from a solution using optical techniques including optical tweezers and optical fractionation; and (g) separation of blood components using agents which may bind to a particular cell type (such as functionalized beads) and be

acted upon by any of the above separation techniques, after which the agent may or may not be unbound from the cell type.

[0121] For blood sorting, a combined approach may be the most effective, such as: first extract most of the RBCs and WBCs using sedimentation rate zonal separation, then extraction and concentration of the platelets using optical fractionation, discussed below. The optical fractionation step will act not only to concentrate the platelets (which could also, for example, be done by a centrifugation step at the end), but will provide a second step which will exert strong suppression on the WBCs accidentally collected with the platelets, for the example of platelet aphoresis. Such concentration steps may also include filtering, such as to filter WBC from a platelet fraction or a plasma fraction.

[0122] While apparatus 300 has been described with respect to blood fractionation, it will be understood by those of skill in the art that the apparatus 300 may be utilized for a wide variety of separations, in addition to such blood fractionation. In addition, apparatus 300 may also be considered one particular embodiment of series-connected separation stages of the present invention.

[0123] Cell "washing" is also a significant application of the apparatus 100, 200 or 300. Such washing may include a change of media, for storage, preservation, or other medical purposes. Such washing may also consist of removing a media containing contaminants such as bacteria, by separating the cells of interest into another media flow free of such contamination. As indicated above, sperm separation is also a significant application of the apparatus 100, 200 or 300.

[0124] Portions of, or outputs from, the sorting device 100, 200 or 300 may be inspected optically. This may be direct visual imaging, such as with a camera, utilizing direct bright-light imaging or fluorescent imaging. Or, it may be more sophisticated techniques such as spectroscopy, transmission spectroscopy, spectral imaging, or scattering such as dynamic light scattering or diffusive wave spectroscopy. In many cases, these inspection regions may be incorporated directly into the flow device to characterize the inputs, outputs, or intermediate steps. They may be for diagnostics or record-keeping, or they may be used to inform the overall process, such as for feedback on how processing is done or on the speed of flow or amount of each solution to use. In some cases, the optical inspection regions may be used in conjunction with additives,

such as chemicals which bind to or affect parts of the solution or beads which are functionalized to bind and/or fluoresce in the presence of certain materials or diseases. For the example of blood sorting, these techniques may be used to measure cell concentrations, to detect disease, or to detect other parameters which characterize the blood.

[0125] Portions of, or outputs from, the sorting device 100, 200 or 300 also may be characterized electronically. For example, a portion of the sorting device may have electronic devices embedded. Example electronic devices may include: (a) capacitors, (b) electronic flow meters, (c) resistance meters for determining the bulk conductivity of the fluid, from which concentrations or compositions may be measured, or (d) pH measuring devices. For the application of blood sorting, measurements of cell concentration, iron content, flow rates, total cell counts, electrolyte concentration, pH, and other parameters may be a valuable part of a sorting device.

[0126] The flow components of the sorting device 100, 200 or 300 may be passive, being completely controlled externally by the flow rates of the inputs and outputs. Alternatively, there may be active flow components embedded in the device (not separately illustrated), such as valves which may be partially or fully opened or closed using electronic, optical, thermal, mechanical, or other influence. For the application of blood sorting, the sorting device 100, 200 or 300 may have an integrated method for storing and/or delivering one or more solutions. For example, a consumable sorting device may be manufactured to have a deformable membrane on a side of a reservoir. This reservoir may be filled with a solution, such as a buffering agent which is biologically compatible with the patient and which may be used to dilute the blood. Another example is that it could be filled with an anti-coagulant. The delivery and/or use of one such fluid may be actively controlled by mechanical influence, pressing on the membrane to deliver the fluid. Alternately, another mechanism may be used to deliver the fluid. Of particular interest, for the sake of simplicity and cost-saving, is the integration of various fluid solutions needed at differing steps in the sorting. Integrating these components may result in substantial simplification and reduction of the total cost of ownership and operation. It may also reduce the risk of contamination and error. Such

reservoirs holding input and buffering solutions or fluids are illustrated, for example, in Figures 14 and 15.

[0127] The device 100, 200 or 300 may include areas (not separately illustrated) where biological or chemical investigation of one or more of the fluids or fluid components. This may include measurements of pH, the presence of certain biological or chemical materials, or other measurements. For the application of blood sorting, this may include detection of disease, characterization of concentrations of various cell types or materials in the plasma, characterization of iron content, determination of blood type, or other evaluation of blood quality, type, or category.

[0128] The device 100, 200 or 300 may include a region (not separately illustrated) which sterilizes the solution using optical methods, such as exposure to UV light, or other methods. The sterilization may act upon solutions which are initially part of the device or added to it for buffering, washing, diluting, or other impacts on the sample solution. Or, the sterilization may act upon part or all of the sample solution being processed. For the application of blood sorting, optical sterilization of the solutions used in the device other than whole blood may be important. Also, sterilization of the blood or certain fractions of the blood may be important.

[0129] The device 100, 200 or 300 may be comprised of materials such that one or more surfaces have been constructed so as to interact physically or chemically with certain materials. For example, a surface may be functionalized so that certain materials adhere to it, for the purpose of extracting these materials from the solution or for the purpose of diagnostics. For the application of blood sorting, functionalized surfaces may be used to extract unwanted materials from certain fractions. Alternately, they may be used to collect materials which are at low concentration for the purpose of measuring the degree to which a material or a type of material is present, such as for disease detection.

[0130] The sorting device 100, 200 or 300 may contain regions where sorting acts in parallel but without physical walls to separate the flows. For example, a parallel sorting region may have multiple inlets 120 and outlets 130, some of which are functionally similar to each other. Instead of having physical dividers distinguishing the multiple parallel sorters, the division occurs as a consequence of the physical properties of the solutions and the flow. The contacting parallelized sorters regions may yield high

sorting rates with more simple and cheap devices. They may also act to avoid contact with surfaces. For blood sorting, these regions would be used to minimize contact with surfaces and to maximize sorting rate while reducing costs and complexity.

[0131] The device 100, 200 or 300 may have regions which are designed to regulate flow rate or the flow character (not separately illustrated). For example, in many cases laminar flow is required, and often a particular flow profile is desired. In other cases, several regions of the device should have identical flow rates and behavior. For these reasons, areas with shapes and other properties to influence flow behavior are often needed. In some cases, this is done by making very symmetric flow designs. In other cases, large changes in the diameter of flow regions and/or the existence of reservoirs help to maintain uniform flow rates. In other cases, very carefully designed channels provide the exact balancing of flow rates needed. To maintain laminar flow, areas where slow changes in channel size occur may be important. Obstacles or dividers may also act to maintain laminar flow. For blood sorting, flow regulation is important to achieve high sorting rates while maintaining the yield and purity of the fractions.

[0132] Not separately illustrated, the device 100, 200 or 300 may contain regions which are designed for mechanical mixing of the fluids, such as regions which encourage turbulence. For example, a region with a fast narrow stream entering a region with a large dimension may produce turbulent flow and mixing. For blood sorting, a mixing region may mix a diluent or anticoagulant with the blood, or may mix other solutions together as needed.

[0133] The device 100, 200 or 300 may contain regions which align cells or materials in a certain way (not separately illustrated). This is sometimes done through shear flows, but may also be done by imposing external fields such as electric fields.

[0134] The device 100, 200 or 300 may contain regions designed to lyse cells or break up materials (not separately illustrated). This may be done through shear flows, vibration, forcing through an orifice, electrical, or other means. For blood sorting, this may be valuable for the elimination of certain cell types or aggregates which may form. It may also be valuable for diagnostic purposes, such as disease detection or measurement of parameters which pertain to the contents of cells.

[0135] The device may contain regions (not separately illustrated) which swell or dehydrate cells or objects, such as by introducing agents which change the osmotic pressure or by changing the physical pressure. This may be done, for example, as a step prior to isopycnic sorting to adjust the density of the cells or objects. It may also be done to kill or shock certain components. For the example of blood sorting, this may be done as a later stage purification step to remove or neutralize undesired components.

[0136] The device 100, 200 or 300 may contain regions which heat or cool one or more solutions (not separately illustrated). This may be done for its impact on physical properties, such as viscosity or density. Or, it may be done for its impact on chemical properties, such as chemical reaction rates or chemical stability. Or, it may be done for its impact on biological properties, such as motility, metabolism rate, or viability. For example, one fluid flow may be at a higher temperature than another, causing motile sperm to move away from the hotter fluid to the cooler fluid. Also, it may be done for system-level compatibility, such as in preparation for the following processing step. For the example of blood sorting, solutions which are returned to the patient may be maintained at an appropriate temperature to avoid chilling the patient. Solutions which are to be stored may be cooled during processing to preserve those fractions or prepare them for the next processing or storage step.

[0137] The device 100, 200 or 300 may contain reservoirs which serve to store solutions which will be used during the process run, or which may be generated during the process run (not separately illustrated). Having these reservoirs integral to the sorting device simplifies the use of the device and reduces the need for additional parts. For the example of blood sorting, reservoirs may contain anticoagulants, diluents, dilutants, and any other solutions needed in the process. Reservoirs may also be incorporated which will hold the sorted fractions or waste fractions.

[0138] The device 100, 200 or 300 may contain regions which enhance mixing by diffusion (not separately illustrated). For example, when mixing by contacting two laminar flows, parallelizing into many narrow contacting flows enhances the overall mixing rate by diffusion. For the example of blood sorting, diffusive mixing regions may be used to mix diluent, anticoagulant, or other solutions with whole blood or blood fractions.

[0139] Also not separately illustrated, the device may contain regions with bubble traps to remove air bubbles from the system. This may be done by having a region where air bubbles are able to rise from a region with flow to a region above the primary flow region. For the example of blood sorting, this may be done in a simple way to guarantee that small air bubbles from the loading or running of the system do not pass on to the patient or the collection samples.

[0140] As indicated above, the device 100, 200 or 300 may contain regions which act to suppress any pulsation in the flow (not separately illustrated), such as that which occurs when peristaltic pumps are used. One way to suppress pulsation is to incorporate a "bubble-trap" into the device. The presence of an air pocket, which is in contact with the fluid, allows for compression of the air pocket as pressure in the fluid increases and decreases. Thus, the air pocket acts as a shock absorber, smoothing out the flow. Other devices may be used as well, such as a flexible membrane which may bend under higher pressures, thereby smoothing out the pressure and flow rate. For the application of blood sorting, pulsation reduction regions will yield more precise and smooth flows, and therefore higher sorting rates, purity, and yield.

[0141] The device 100, 200 or 300 may contain regions which reveal the state of the device (not separately illustrated). For a consumable, it may indicate whether the device has been sterilized or whether it has been used. For the application of blood sorting, one would want indicators to confirm both that the sorting device has been sterilized and that the device has not yet been used or contaminated.

[0142] The sorting device 100, 200 or 300, or overall sorting system, may contain mechanisms for precise leveling of the flow sorter. This is important because for some sorters, buoyant forces may cause unintentional flows and have negative impacts on sorting yields and purity in cases where the device is not precisely leveled. Additionally, the sorting device may contain components which reveal whether it is well-balanced, or the degree to which it is balanced. For example, it may have an electronic or gravity-based balance incorporated in the sorting device itself. One example of such a device is a shaped channel with fluid and an air bubble in it. The position of the air bubble may reveal the angle of the tilt of the device. Another such device may use a metal ball in a track to reveal the tilt angle. Another manifestation is to use an optical alignment, such

as bouncing a light source off a surface or passing a light source through a wedge to identify its orientation. For the application of blood sorting, leveling controls and indicators are significant to guarantee high-yield and high-purity products.

[0143] The sorting device 100, 200 or 300, or overall sorting system, may contain mechanisms for maintaining uniform and/or constant temperatures of the device and/or the solution (not separately illustrated). This is important to eliminate thermally-induced buoyant forces which may cause unintentional flows and have negative impacts on sorting yields and purity. Additionally, the sorting device may have indicators in it, or in the sorting system as a whole, which indicate the temperature and/or temperature uniformity of one or more components. For the application of blood sorting, temperature uniformity controls and indicators may be significant to guarantee high-yield and high-purity products.

[0144] The sorting device 100, 200 or 300, or overall sorting system, may contain mechanisms for measuring the level and concentration of one or more input or output solution (not separately illustrated). These measurements may be used to gauge the speed of operation, completion time, error state, for general monitoring, or for other applications. For the application of blood sorting, level and concentration indicators may be used to identify when sufficient sample has been collected or to detect when a failure or depletion of a solution has occurred.

[0145] Lastly, the sorting device 100, 200 or 300, or overall sorting system, may have a method for priming the system with one or more fluids using standard bottom-up filling or evacuation. Purging may similarly be done by draining the device or by flowing a solution through it. At the early stages of a sorting run, the priming solution may be discarded until a time when the priming solution has been mostly exhausted and the desired solution is obtained. Similarly, at the late stages of a sorting run, a fluid may be used to push the sorted material through the system to minimize waste and maximize yield.

[0146] The various sorting devices 100, 200 or 300 may also be utilized in a system providing a general purpose device which allows a user to extract one or more fractions of a solution, determined by a range of S values (size, density, or size*density). Figure 26 is a block diagram illustrating an exemplary sorting and separation system

2600 in accordance with one embodiment consistent with the present invention. This general purpose sorter would vary the flow rates on each input and output channel in response to user controls (user input 2610). The user controls would allow the user to determine the range of S values that leave through each output channel. This device would be a valuable lab tool for performing various purifications, washing, separations, and diagnostic evaluations of samples. It would also be an important platform for research, development, and prototyping applications.

[0147] The sorting hardware may be contained within a single enclosure which may or may not be temperature controlled. This hardware consists of: (1) Consumable flow plate 2620 (*e.g.*, sorting device 100, 200 or 300) with several inputs and outputs, (2) several computer-controllable peristaltic pumps 2625, (3) temperature control and monitoring apparatus 2630, (4) reservoir holders (or reservoirs 2635 and 2640). The sorting is controlled by a computer 2650 which monitors and controls the flow rates, temperature, and any other additional components which may be included for controlling or diagnostics purposes.

[0148] The flow plate 2620 may be a simple general-purpose device, suitable for many applications, as discussed above. It may have two to four inputs and two to ten outputs. It may be provided in a sterile, primed, and sealed state. The reservoirs may be part of the flow plate, or they may be separate sterile components which are attached to the flow plate before use. All or a portion of the central sorting region may be covered with a cover glass to allow manipulation with optical tweezers, optical dielectrophoresis, or laser killing/cutting of samples.

[0149] The computer 2650 control does measurements and control of the hardware for pumping and temperature control. It may also interface other hardware components that may be added. The user software provides a very convenient front end to the simultaneous control of all the pumps. It would do the necessary math to control each pump rate to give the user the desired flows for the particular sorting application.

[0150] This system 2600 would be general enough to allow for many sorting applications, either independently or in conjunction with an additional laser apparatus. These applications include but is not limited to: cell identification by fluorescence, cell killing by high intensity laser exposure, cell fractionation using optical dielectrophoresis,

motile cell fractionation based on motility, passive sorting of objects by diffusivity, and passive fluidic zonal density sorting. The passive fluidic zonal density sorting applications, which are numerous and extend over a broad range, can generally be done with minimal or no additional hardware.

[0151] Exemplary uses and applications for such a general purpose sorting system 2600, using passive fluidic sorting and/or use of optical gradient forces (discussed below) are outlined in the following table:

Category	General	Specific
Washing - Removing Components	Cell Washing for Disease Removal	Removing debris, bacteria, and viruses from human sperm Removing debris, bacteria, and viruses from animal sperm
	Aggregate Removal	Removing undesirable clumps of materials in industrial processes Removing aggregated colloids in solution Removing coarsened droplets in an emulsion
	Precursor Removal	Removing precursor materials from a halted growth process Separating differentiated cells Removing blastocytes from incubating sperms and eggs
Washing - Changing Media	Cell Processing	Changing osmotic conditions - Cell swelling or shrinking Cell staining Automated multi-step cell experiments or processing Sperm fluoridation to suppress activity
	Material Processing	Bead dying
	Assays	Object-solution response assays

Category	General	Specific
Diagnostics	Characterizing Monodisperse Solutions	Measuring S (Svedberg coefficient) of particles
		Measuring size of particles of known material
		Measuring density of particles of known size
		Measuring composition of particles
	Characterizing Polydisperse Solutions	Measuring distribution of S, size, density, composition, etc.
		Measuring average S, size, density, composition, etc.
	Disease Detection	Extracting a sample of bacteria from infected tissue
		Extracting a sample of bacteria from stool samples
		Extracting a sample of bacteria from blood
		Extracting cells that are infected with viruses from healthy cells
	Environmental	Extracting spores from sample to determine spore count in air
		Extracting particulate matter from water or air for environmental monitoring
		Monitoring water safety in drinking or swimming water
		Extracting spres from environmental samples to quantify mold levels in infected homes
	Food Safety	Extracting a sample of bacteria from food for diagnosis
Purifying - Reducing Variance	Viruses	Purifying a virus sample
Purifying - Extracting Components	Cell Components	Separating different cell components from solution of lysed cells

Category	General	Specific
	Cell Components	Separating organelles
	Bacteria	Extracting a sample of bacteria from infected tissue
	Spores	Extracting a sample of spores from a solution
Filtering	Filtering Large Components From Foods	Removing yeast from beer Removing yeast from wine Removing fat from milk
	Industrial Filtering	Removing particulates from water Removing particulates from machine oil
	Pharmaceutical Filtering	Removing undissolved clumps from solution to prevent overdosing
	Medical Filtering	Dialysis, using a membrane, for kidney dialysis
Sorting	Cell Type Sorting	Sorting blood cells from blood to extract the plasma Sorting platelet cells from blood for aphoresis Sorting cells based on presence of given antibody (may use functionalized beads) Sorting natural killer cells from blood as a treatment for AIDS Sorting different types of cells within a tissue or organ (eg. sorting osteoclasts, osteoplasts, osteoblasts) Sorting white blood cells as a treatment for white blood cell diseases (e.g., high WBC such as leukemia or lymphomia, or low WBC) Sorting sickle cells from normal red blood cells as a treatment for sickle-cell anemia

Category	General	Specific
	Cell Cluster Sorting	Sorting islet cell clusters by size (diabetes) Removing cell clusters from single cells
	Cell State Sorting	Removing infected cells from healthy cells Removing living cells from dead cells Separating proliferating and non-proliferating cells Isolating viable sperm from inviable sperm
	Isolating Cells From Biopsies	Purifying bone marrow cells from blood in marrow biopsy
	Colloids Fractionation	Sorting colloids by size, density, composition, S, etc.
	Sorting Variants of One Cell Type	Sorting multizygotic sperm from normal sperm Removing most severe RBCs in sickle cell anemia patients

[0152] Figure 27 is a block diagram illustrating an exemplary bioreactor product purification and separation system 2700 in accordance with one embodiment consistent with the present invention. A bioreactor is used in the production of monoclonal antibodies, recombinant protein products, viruses and viral antigens, and viable cell mass. The most common use for a bioreactor is in the production of various drug therapies. A bioreactor basically works by allowing cells to grow in high concentrations in ideal conditions. While the cells are growing a culture medium is flowed through the bioreactor. This medium collects the cell's waste products, as well as provides nutrition for the cells. The medium that has been flowed through the bioreactor is then processed for the product. In order to collect the product, the waste filled medium is filtered through many different processes, one of which is through bead column chromatography. This process takes a long time to complete, is very expensive, and does not yield a large percentage of the final product. The present invention solves all three of these problems.

[0153] The bioreactor product purifier 2700 would quickly, easily, and accurately remove the wanted product from the waste medium. The waste media would be flowed into the sorter through one channel 2701 At the same time a solution of beads coated with the proper affinity sites would be flowed into the sorter through the second channel 2702. Both channels would lead to a first mixer 2705 in which they would be mixed together. During the mixing the product would bind to the sites on the beads. The mixture would then flow through a first separation region 2710 along with an input buffer (input channel 2715). Through the use of passive diffusive sorting, the beads with selected product output through channel 2718 and the waste solution would be output through channel 2716 and discarded. Passive diffusive sorting succeeds because the large beads stay in one channel while the lighter molecules diffuse to the other side of the channel. The bead solution is then be flowed through channel 2718 as a denaturing solution is flowed through a second channel 2720. Both of these channels would flow to a second mixer 2725 where they would be mixed together. While they were being mixed, the denaturing solution would break the bond between the product and the beads. After mixing, the solution would be flowed through an output channel 2735 along with another buffer solution through channel 2730, into second separation region 2740. Through the use of passive diffusive sorting, the beads would be flowed through the bottom channel 2745 and be discarded as waste. The purified and recovered product would be flowed through the top channel 2750. This method of purification should reduce costs, reduce time, and increase the yield of final product.

[0154] Figure 4 schematically illustrates a holographic optical trapping system 400, generally used in conjunction with an apparatus 100, 200 or 300, according to one embodiment consistent with the present invention. Additional detail concerning holographic optical trapping is available in the fifth related application. In a holographic optical trapping apparatus or system 400 as illustrated in Figure 4, light is incident from a laser system, and enters as shown by the downward arrow, to power the system 400.

[0155] A phase patterning optical element 401 is preferably a dynamic optical element (DOE), with a dynamic surface, which is also a phase-only spatial light modulator (SLM) such as the "PAL-SLM series X7665," manufactured by Hamamatsu of Japan, the "SLM 512SA7" or the "SLM 512SA15" both manufactured by Boulder

Nonlinear Systems of Lafayette, Colorado. These dynamic phase patterned optical elements 401 are computer-controlled to generate beamlets by a hologram encoded in the medium which may be varied to generate the beamlets and select the form of the beamlets. A phase pattern 402 generated on the lower left of Figure 4 produces the traps 403 shown in the lower right filled with 1 μm diameter silica spheres 404 suspended in water-405. Thus, the system 400 is controlled by the dynamic hologram shown below on the left.

[0156] The laser beam travels through lenses 406, 407, to dichroic mirror 408. The beam splitter 408 is constructed of a dichroic mirror, a photonic band gap mirror, omni directional mirror, or other similar device. The beam splitter 408 selectively reflects the wavelength of light used to form the optical traps 403 and transmits other wavelengths. The portion of light reflected from the area of the beam splitter 408 is then passed through an area of an encoded phase patterning optical element disposed substantially in a plane conjugate to a planar back aperture of a focusing (objective) lens 409.

[0157] In single beam optical trapping (also called laser or optical tweezers) it had been thought, prior to the invention of the fifth related application, that a high numerical aperture lens was necessary for acceptable optical traps. A basis for this thinking was that, for optical trapping, one uses the gradient in the electric field of the impinging light to trap the particle. In order to have a large trapping force it has been thought necessary to have a large gradient in the electric field (or number density of rays). The way that one usually accomplishes this is to pass the light field through a high numerical aperture lens.

[0158] A concern with observation and trapping of samples within a large field of view is that such observation and trapping would involve an objective lens with a low numerical aperture. Contrary to prior teaching, the invention of the fifth related application provides a low numerical aperture lens as, for example, the objective lens 409 in Figure 4. The ability to observe and trap in this situation could be useful in any application where one would benefit from a large field of view given by a low magnification lens, such as placing microscopic manufactured parts or working with large numbers of objects, such as cells, for example.

[0159] As an example according to the present invention, 3 micron silica spheres 104 suspended in water 105 were trapped with lenses 109 with an unprecedented low numerical aperture. The lenses 109 used were manufactured by Nikon: (a) Plan 4x with an NA of 0.10 ; and (b) Plan 10x with an NA of 0.25.

[0160] Suitable phase patterning optical elements are characterized as transmissive or reflective depending on how they direct the focused beam of light or other source of energy. Transmissive diffractive optical elements transmit the beam of light or other source of energy, while reflective diffractive optical elements reflect the beam.

[0161] The phase patterning optical element 401 may also be categorized as having a static or a dynamic surface. Examples of suitable static phase patterning optical elements include those with one or more fixed surface regions, such as gratings, including diffraction gratings, reflective gratings, and transmissive gratings, holograms, including polychromatic holograms, stencils, light shaping holographic filters, polychromatic holograms, lenses, mirrors, prisms, waveplates and the like. The static, transmissive phase patterning optical element is characterized by a fixed surface.

[0162] In some embodiments, however, the phase patterning optical element 401 itself is movable, thereby allowing for the selection of one more of the fixed surface regions by moving the phase patterning optical element 401 relative to the laser beam to select the appropriate region.

[0163] The static phase patterning optical element may be attached to a spindle and rotated with a controlled electric motor (not shown). The static phase patterning optical element has a fixed surface and discrete regions. In other embodiments of static phase patterning optical elements, either transmissive or reflective, the fixed surface has a non-homogeneous surface containing substantially continuously varying regions, or a combination of discrete regions, and substantially continuously varying regions.

[0164] Examples of suitable dynamic phase patterning optical elements having a time dependent aspect to their function include computer-generated diffractive patterns, phase-shifting materials, liquid crystal phase-shifting arrays, micro-mirror arrays, including piston mode micro-mirror arrays, spatial light modulators, electro-optic deflectors, accousto-optic modulators, deformable mirrors, reflective MEMS arrays and

the like. With a dynamic phase patterning optical element 401, the medium 405 which comprises the phase patterning optical element 401 encodes a hologram which may be altered, to impart a patterned phase shift to the focused beam of light which results in a corresponding change in the phase profile of the focused beam of light, such as diffraction, or convergence. Additionally, the medium 405 may be altered to produce a change in the location of the optical traps 403. It is an advantage of dynamic phase patterning optical elements 401, that the medium 405 may be altered to independently move each optical trap 403.

[0165] In those embodiments in which the phase profile of the beamlets is less intense at the periphery and more intense at regions inward from the periphery, overfilling the back aperture by less than about 15 percent is useful to form optical traps with greater intensity at the periphery, than optical traps formed without overfilling the back aperture.

[0166] In some embodiments, the form of an optical trap may be changed from its original form to that of a point optical trap, an optical vortex, Bessel beam, an optical bottle, an optical rotator or a light cage The optical trap may be moved in two or three dimensions. The phase patterning optical element is also useful to impart a particular topological mode to the laser light, for example, by converting a Gaussian into a Gauss-Laguerre mode. Accordingly, one beamlet may be formed into a Gauss-Laguerre mode while another beamlet may be formed in a Gaussian mode. The utilization of Gauss-Laguerre modes greatly enhances trapping by reducing radiation pressure.

1. Imaging system

[0167] The current instrument design uses a high resolution CCD camera for the primary imaging system 110. The main advantage of the CCD camera (see reference numeral 511 in Figure 5) is the favorable cost/performance ratio since this technology is a mature one. Another advantage of CCD cameras is their wide dynamic range and the ease of generating digital output.

The images are viewed on a computer screen (see reference numeral 510 in Figure 5) to provide both a frame of reference for selecting the location of the traps as well as to minimize the possibility of inadvertent exposure of the operator to the laser.

2. User Interface

a. Object Display

[0168] The user interface consists of a computer screen which displays the field of view acquired by the CCD camera. The user designates the loci of the traps with a mouse. There is also an option to delete a location.

[0169] As described in greater detail below, the user is also able to specify the power per trap so as to be able to avoid specimen damage. In addition it is desirable to be able to vary trap power because trapping depends upon the difference between the index of refraction of the specimen and the suspending medium which can be expected to vary from specimen to specimen.

b. The Hologram

[0170] The purpose of designating the loci of the traps is to provide input for the hologram calculation. The hologram is essentially a function whose Fourier transform produces the desired trap array. However in the case of the liquid crystal display this function is a phase object (i.e., an object that changes the phase of the wavefront without absorbing any energy).

c. Methods for choosing the set of traps

[0171] Often one wishes to use the traps to move an object in a particular direction. This may be accomplished by using the mouse to create a line (by dragging). The computer program interprets a line as calling for a series of traps to be deployed sequentially and sufficiently close together so as to move the target in small steps without losing the lock on the target.

[0172] The present invention also includes the capability of changing the height of the traps. If a laser beam is parallel to the optical axis of the objective lens 409, then a trap forms at the same height as the focal plane of the lens 409. Changing the height of a trap is accomplished by adjusting the hologram so that the beam of light forming a trap is slightly converging (or diverging) as it enters the objective lens 409 of the microscope. Adjusting the height of a trap is possible using lenses but only a holographic optical trapping (HOT) allows the height of each individual trap to be adjusted independently of any other trap. This is accomplished by the computer program adjusting the phase modulation caused by the liquid crystal hologram.

3. Sample Holder

a. General

[0173] The sample chamber 700 (see FIGS. 7A and 7B) of the present invention is inexpensive and disposable. Although the sample chamber 700 of the present invention is described below, another object of the present invention is to create a flexible design that may be changed for differing applications. In addition to the sample chamber 700, the various other separation stages of the invention may be utilized, such as an apparatus 100, 200 or 300, or the other separation stages discussed below with reference to Figures 13- 24 and 26-27.

[0174] The sample chamber 700 lies on the surface of a microscope slide 701. The sample chamber 700 contains a series of channels 703 for introducing specimens or objects. The channels 703 are connected to supply and collection reservoirs by thin tubing 704 (commercially available). Samples or objects will be suspended in a liquid medium and will be introduced into the working area via the channels 703. The sample chamber 700 is covered by a cover slip 705.

b. Manufacture of the Sample Chamber

[0175] In one embodiment consistent with the present invention, a poly(dimethyl siloxane) (PDMS) resin is used to fabricate the chamber 700. The process involves creating the desired pattern of channels 703 on a computer using standard CAD/CAM methods and transferring the pattern to a photomask using conventional photoresist/etching techniques. The photomask is then used as a negative mask to create an inverse pattern of channels which are etched on a silicon wafer. The depth of the channels 703 is controlled by the etch time. The silicon wafer is a negative replica of the actual sample chamber 700. The final step consists of creating the positive sample chamber 700 by pouring PDMS onto the wafer and polymerizing. This results in a PDMS mold which is bonded to a glass slide 701 and overlaid with a cover slip 705. The glass to PDMA bonding is effected with an oxygen etch which activates the exposed surfaces.

[0176] A number of additional steps are necessary to ensure consistent quality. For instance the PDMS solution/hardner is maintained under a vacuum in order to prevent bubble formation. The silicon wafer is silanized to prevent the PDMS from

sticking to the wafer. There are a variety of steps involving cleaning the replicas and maintaining proper environmental controls. These represent standard technology.

[0177] The channels 703 are connected to microbore tubing 704 using small syringe needles 706 held using glue 714, which are inserted through the PDMS mold into small circular wells 707 which connect to each channel 703. Sample solutions are introduced into the channel 703 using micropumps 708.

[0178] Figure 7B shows a diagram of a typical arrangement for the introduction of a sample via the syringe pump 708 at 710. The medium is introduced at 711, and waste is collected at 71 and the desired collections at 713.

[0179] Figure 8 presents a representation of a scanning electron micrograph of the diagram in Figure 7B as actually created from the process described above. The channels are approximately 50 microns wide and 50 microns deep. Figure 9 presents a representation of a scanning electron micrograph of the 'working' volume where manipulations of the specimen under study would occur. The diagrams clearly show that the channels 703 are smooth and clean. Although the channels 703 are rectangular in cross-section, other shapes may be devised as well. The channels 703 are designed to allow samples to be flowed to a 'working area' whose shape may be custom designed for experimental requirements.

e. Holographic Optical Traps

[0180] Unlike scanned optical traps which address multiple trapping points in sequence, and thus are time-shared, holographic optical traps illuminate each of their traps continuously. For a scanned optical trap to achieve the same trapping force as a continuously illuminated trap, it must provide at least the same time-averaged intensity. This means that the scanned trap has to have a higher peak intensity by a factor proportional to at least the number of trapping regions. This higher peak intensity increases the opportunities for optically-induced damage in the trapped material. This damage may arise from at least three mechanisms: (1) single-photon absorption leading to local heating, (2) single-photon absorption leading to photochemical transformations, and (3) multiple-photon absorption leading to photochemical transformations. Events (1) and (2) may be mitigated by choosing a wavelength of light which is weakly absorbed by the trapping material and by the surrounding fluid medium. Event (3) is a more

general problem and is mitigated in part by working with longer-wavelength light. Thus holographic optical traps may manipulate delicate materials more gently with greater effect by distributing smaller amounts of force continuously among a number of points on an object rather than potentially damaging the object by exerting the total force on a single point or at a higher intensity for a period of time.

[0181] In one embodiment consistent with the present invention, the design is flexible in that any desired pattern of channels 703 may be designed with a standard CAD/CAM computer program. The complexity of the pattern is not a factor as long as the channels 703 are far enough apart so as not to impinge on one another. As may be seen in FIGS. 7B and 8, multiple sets of channels 703 may be easily accommodated so that a single chip may be used for more than one experiment. In addition, once a mold is made it may be used to fabricate thousands of sample chambers so the methodology is readily adaptable to mass production techniques. It is estimated that the marginal cost of a single chamber would be of the order of a few cents when in mass production.

4. Optical System

a. Synthesizing the Hologram

[0182] Early versions of the holographic optical traps used fixed holograms fabricated from a variety of materials. These were adequate to demonstrate the principle of using holograms to create up to several hundred traps. However the major shortcoming of these holograms was that they were static and it took hours to make a single hologram. With the advent of the hardware to create computer-driven liquid crystal displays capable of forming holograms many times per second, the use of optical traps as a dynamic device has become a practical reality. Software control permits automation of separation and trapping by simple implementation of programs to control laser beam steering. The principle for computing the hologram is described below.

b. The Microscope

[0183] The optical system 410 consists of a standard high quality light microscope. The objective is a high numerical aperture lens 409 coupled with a long working distance condenser lens. The high numerical aperture objective lens 409 is used for trapping. While the long working distance condenser lens may somewhat reduce the resolution in the images, it does not compromise trapping and provides extra space near

the sample slide to accommodate plumbing and receptacles. The objects may be moved by holding them with traps and moving the stage of the microscope vertically or laterally.

[0184] In one embodiment consistent with the present invention, approximately 2 mW of laser power is employed to produce 200 microwatts at the trap. The power level available from a 2W laser is adequate to create about 1000 traps. A green laser (532nm) is used, but other wavelengths may also be used, including, for example, a far red laser to work with materials absorbing near the 532 nm value.

[0185] Trapping depends upon the refractive index gradient so that materials with refractive indices close to that of the surrounding medium need traps with higher power levels. In addition, the tolerance of materials to damage will vary with trap power, so it is desirable for the user to be able to control this parameter. The user may increase the power level in any particular trap using a 'power slider' displayed on the graphical interface.

c. The Liquid Crystal Hologram (also referred to as a Spatial Light Modulator or SLM)

[0186] The spatial light modulator 408 is essentially a liquid crystal array controlled by an electrostatic field which, in turn may be controlled by a computer program. The liquid crystal array has the property that it retards the phase of light by differing amounts depending upon the strength of the applied electric field.

[0187] Nematic liquid crystal devices are used for displays or for applications where a large phase-only modulation depth is needed (2Π or greater). The nematic liquid crystal molecules usually lie parallel to the surface of the device giving the maximum retardance due to the birefringence of the liquid crystal. When an electric field is applied, the molecules tilt parallel to the electric field. As the voltage is increased the index of refraction along the extraordinary axis, and hence the birefringence, is effectively decreased causing a reduction in the retardance of the device.

d. The Laser

[0188] Useful lasers include solid state lasers, diode pumped lasers, gas lasers, dye lasers, alexandrite lasers, free electron lasers, VCSEL lasers, diode lasers, Ti-Sapphire lasers, doped YAG lasers, doped YLF lasers, diode pumped YAG lasers, and flash lamp-pumped YAG lasers. Diode-pumped Nd:YAG lasers operating between 10

mW and 5 W are preferred. The preferred wavelengths of the laser beam used to form arrays for investigating biological material include the infrared, near infrared, visible red, green, and visible blue wavelengths, with wavelengths from about 400 nm to about 1060 nm being most preferred.

[0189] Figure 5 is a schematic diagram of a holographic optical trapping system for sorting objects, and is used in conjunction with an apparatus 100, 200 or 300, according to one embodiment in accordance with the present invention. In one such embodiment, an optical trapping system 500 (see Figure 5) (such as the BioRyx system sold by Arryx, Inc., Chicago, Illinois) includes a Nixon TE 2000 series microscope 501 into which a mount for forming the optical traps using a holographic optical trapping unit 505 has been placed. The nosepiece 502 to which is attached a housing, fits directly into the microscope 501 via the mount. For imaging, an illumination source 503 is provided above the objective lens 504 to illuminate the sample 506. In accordance with the present invention, the sample 506 is one of the separation stages of the apparatus 100, 200 or 300.

[0190] In one embodiment, the optical trap system 400 (see Figures 4 and 5) includes one end of the first light channel which is in close proximity to the optical element, and the other end of the first light channel which intersects with and communicates with a second light channel formed perpendicular thereto. The second light channel is formed within a base of a microscope lens mounting turret or "nosepiece". The nosepiece is adapted to fit into a Nixon TE 200 series microscope. The second light channel communicates with a third light channel which is also perpendicular to the second light channel. The third light channel traverses from the top surface of the nosepiece through the base of the nosepiece and is parallel to an objective lens focusing lens 409. The focusing lens 409 has a top and a bottom forming a back aperture. Interposed in the third light channel between the second light channel and the back aperture of the focusing lens is a dichroic mirror beam splitter 408.

[0191] Other components within the optical trap system for forming the optical traps include a first mirror, which reflects the beamlets emanating from the phase patterning optical element 401 through the first light channel, a first set of transfer optics 406 disposed within the first light channel, aligned to receive the beamlets reflected by

the first mirror, a second set of transfer optics 407 disposed within the first light channel, aligned to receive the beamlets passing through the first set of transfer lenses, and a second mirror 408, positioned at the intersection of the first light channel and the second light channel, aligned to reflect beamlets passing through the second set of transfer optics and through the third light channel.

[0192] To generate the optical traps, a laser beam is directed from a laser 507 (see FIG. 5) through a collimator and through an optical fiber end 508 and reflected off the dynamic surface of the diffractive optical element 509. The beam of light exiting the collimator end of the optical fiber is diffracted by the dynamic surface of the diffractive optical element into a plurality of beamlets. The number, type and direction of each beamlet may be controlled and varied by altering the hologram encoded in the dynamic surface medium. The beamlets then reflect off the first mirror through the first set of transfer optics down the first light channel through the second set of transfer optics to the second mirror; and are directed at the dichroic mirror 509 up to the back aperture of the objective lens 504, are converged through the objective lens 504, thereby producing the optical gradient conditions necessary to form the optical traps. That portion of the light which is split through the dichroic mirror 509, for imaging, passes through the lower portion of the third light channel forming an optical data stream (see Figure 4).

[0193] Spectroscopy of a sample of biological material may be accomplished with an imaging illumination source 503 suitable for either spectroscopy or polarized light back scattering, the former being useful for assessing chemical identity, and the later being suited for measuring dimensions of internal structures such as the nucleus size. Using such spectroscopic methods, in some embodiments, cells are interrogated. A computer 510 may be used to analyze the spectral data and to identify cells bearing either an X or Y chromosome, or a suspected cancerous, pre-cancerous and/or non-cancerous cell types, or identify various types of blood cells, for example. The computer program then may apply the information to direct optical traps to contain selected cell types. The contained cells then may be identified based on the reaction or binding of the contained cells with chemicals.

[0194] The present method and system lends itself to a semi-automated or automated process for tracking the movement and contents of each optical trap. The

movement may be monitored, via video camera 511, spectrum, or an optical data stream and which provides a computer program controlling the selection of cells and generation of optical traps.

[0195] In other embodiments, the movement of cells is tracked based on predetermined movement of each optical trap caused by encoding the phase patterning optical element. Additionally, in some embodiments, a computer program is used to maintain a record of each cell contained in each optical trap.

[0196] The optical data stream may then be viewed, converted to a video signal, monitored, or analyzed by visual inspection of an operator, spectroscopically, and/or video monitoring. The optical data stream may also be processed by a photodetector to monitor intensity, or any suitable device to convert the optical data stream to a digital data stream adapted for use by a computer.

[0197] In an approach which does not employ an SLM (spatial light modulator), movement is accomplished by transferring the objects from a first set of optical traps to a second, third, and then fourth etc.. To move the objects from the first position to a second position, a static phase patterning optical element is rotated around a spindle to align the laser beam with a second region which generates the second set of optical traps at a corresponding second set of predetermined positions. By constructing the second set of optical traps in the appropriate proximity to the first position, the probes may be passed from the first set of optical traps to the second set of optical traps. The sequence may continue passing the probes from the second set of predetermined positions to a third set of predetermined positions, from the third set of positions to a fourth set of predetermined positions, and from the fourth set of predetermined positions and so forth by the rotation of the phase patterning optical element to align the appropriate region corresponding to the desired position. The time interval between the termination of one set of optical traps and the generation of the next is of a duration to ensure that the probes are transferred to the next set of optical traps before they drift away.

[0198] In a staggered movement of the objects from a wide to narrow proximity the staggered movement of the cells occurs in a similar fashion. However, as the objects are passed from a first set of optical traps to a second set and moved to second and subsequent positions, the staggered arrangement of the traps allows the objects to be

packed densely without placing a set of traps in too close a proximity to two objects at the same time which could cause the objects to be contained by the wrong optical trap

[0199] Once an object or cell has interacted with a trap, spectral methods may be used to investigate the cell. The spectrum of those cells which had positive results (*i.e.*, those cells which reacted with or bonded with a label) may be obtained by using imaging illumination such as that suitable for either inelastic spectroscopy or polarized light back scattering. A computer may analyze the spectral data to identify the desired targets and direct the phase patterning optical element to segregate those desired targets. Upon completion of the assay, selection may be made, via computer and/or operator, of which cells to discard and which to collect.

[0200] Optical peristalsis (see FIG. 13) is an existing process employing parallel lines of traps 1300 in a microfluidic channel 1301 arranged so that the spacing between the lines permits particles 1302 trapped in one line to be pulled into traps in the other line when the first line of traps is turned off. Optical peristalsis may be used as an alternative to and in conjunction with fluorescent labels (as described later regarding Applications). The process operates by timing the extinction of lines of traps timed so that particles are moved in desired directions specified by the arrangement of the lines of traps. By choosing whether a line of traps on one side or the other of a particle are on or off, the particle may be moved forward or back in a direction. By employing large numbers of traps, large numbers of particles may thus be moved in concert in a given direction. Thus, particles attracted to the traps may be moved to a given area and, if desired, collected there. This process may also be utilized in the various fluid flows utilized with the apparatus 100, 200 or 300.

[0201] Similarly, by gradually reducing the spacing between traps in lines toward a given direction and/or varying the curvature of the lines of traps, particles may be swept into a focusing pattern to concentrate them. Reversing such a pattern would disperse the particles.

[0202] Spacing between lines of traps may be relatively larger to speed up movement of the particles, or relatively narrower to slow them down. Similarly, varying the intensity of selected traps or lines, and hence their effect on particles, may also be employed. By converging or diverging flows, particles may be combined or separated.

In addition, optical peristalsis may be combined with differential effects of viscous drag or electrical fields to produce complex and specific sets of parameter values for finely separating materials, for example. By opposing the trapping and other forces, the balance point of the two forces determines whether a particle moves with the trap or the other force.

[0203] In one embodiment consistent with the present invention, optical peristalsis may be implemented with a holographic system which cycles through a sequence of phase patterns to implement a corresponding sequence of holographic optical trapping patterns.. Such patterns may be encoded in the surface relief of reflective diffractive optical elements mounted on the face of a prism, wherein each pattern is rotated into place by a motor. Likewise, transmissive diffractive optical elements may be placed on the perimeter of a disk and rotated to cycle through the patterns. Switchable phase gratings and phase holograms encoded on film may also be used.

[0204] For particles driven past a rectilinear array by an external bias force, such as fluid flow, where the trapping force is considerably greater than the external driving force, the particles are trapped. Where the bias force is greater, the particles flow past the array. Between these extremes, the bias force exceeds the trapping force to a differing degree for different fractions of the particles, causing the particles to hop from trap to trap along the direction of the principal axis of the array. A zero net deflection may be observed where the array is rotated to 45° because: (1) positive and negative displacements occur with equal probability; or (2) the particles become locked into the [11] direction, jumping diagonally through the array.

[0205] Particles affected to a greater degree by an array may be deflected to greater angles than the particles affected to a greater degree by the bias force. The optical gradient force exerted on particles varies roughly as a^3, where a = radius. Stokes drag on the particles varies as "a". Thus, larger particles are disproportionately affected by trap arrays, while the smaller particles experience smaller deflection. Orienting the array near the angle of optimal deflection and adjusting the intensity to place the largest particles in the hopping condition, and, hence at greater deflection than smaller particles. Differentially deflected particles may be collected or further fractionated by additional arrays downstream of the first.

[0206] Some conventional techniques for fractionation achieve separation in the direction of an applied force. However, such techniques operate on batches of samples rather than continuously.

[0207] Other conventional techniques for microfractionation employ microfabricated sieves consisting of a two dimensional lattice of obstacles or barriers. For example, an asymmetric placement of barriers rectifies the Brownian motion of particles that pass through the sieve, causing the particles to follow paths that depend on the diffusion coefficients of the particles. However, use of a microfabricated lattices clog and are not tunable for particle size and type.

[0208] In FIG. 10, an example of sorting of particles according to the present invention is exemplified. Although the illustrated example exemplifies lateral deflection, optical peristalsis may be obtained in the same system. A representation of a video image shows light-based separation of material, in this case, tuned to separate objects based on particle size. The flow in the upper left channel contains 1, 2.25, and 4.5 μm particles and another flow enters from the lower left. The superimposed lines respectively indicate each of the channels' flow when the system laser power is off. When the laser power is turned on, light in the interaction region (indicated by the superimposed green box), extracts the 4.5 μm particles from the upper flow and delivers them to the lower-right channel as indicated by the superimposed white path.

6. Application in Blood Cell Sorting

a. Background

[0209] In one application consistent with the present invention, a high-resolution, high-throughput cell sorter by using optical trapping technology is implemented. The need for implementing this technology as a new basis for cell sorting is evidenced by the failure of traditional flow cytometers to perform the high-resolution determinations of cell characteristics necessary in many sorting problems

b. Sorting using Holographic Optical Traps

[0210] The method of implementing high-resolution, high-throughput cell sorting of the present invention, has the following components: microfluidic development, optical-trap system development (trapping component for the funnel system and the trap

component for the separation system), high-resolution fluorescence measurement, system control (including hologram calculation), and mechanical design.

[0211] The first component is a flow cell that has a fluid input channel, carrying the input sample, and two output channels carrying cells separated out of the input channel. The second component is a set of traps that perform the "funneling" function (this "funneling function" is the equivalent of the nozzle forming the droplet flow in a traditional flow cytometer). The third component is the detection system and, finally, the fourth component is the sorting system. FIGS. 11A-11B illustrate the relationship among these four components. Similar functions can be implemented with the fluid flows utilized with the apparatus 100, 200 or 300.

[0212] The essential trait allowing this proposed embodiment of the present invention to achieve high throughputs is its inherent capacity to run material in parallel lines simultaneously and in close proximity to one another. For this initial implementation, a flow system with 10 input lines 1100 each separated by 10 microns is created. This sets an overall width to the flow from the input reservoir of 110 microns. The output channels 1102, 1103 are each the same 110 micron width as the input channel 301 and they run parallel to the input channel 301 as is shown in FIGS. 11A and 11B. Introduced into the "output channels" 1102, 1103 is a buffer solution that is fed into these channels at the same flow rate as is maintained in the input channel 1101. All three of these channels 1101, 1102, 1103 are designed to maintain laminar flow over the flow ranges of interest. The sorting stages discussed above with respect to the apparatus 100, 200 or 300 may also be utilized. In the sorting region, where specific cells are transferred from the input channel 1101 to one of the output channels 1102, 1103, all three flows are adjacent with no mechanical separation between them. The laminar flows keep any material in their respective flows unless a specific external force is introduced to transfer that material from one flow channel to another.

[0213] The funneling traps 1105 act on the input cells 1106 so they both travel in well defined lines of flow and so the input cells 1106 are separated from one another by a minimum distance 1106 to be set by the operator. The flow rates in the channels 1101, 1102, 1103 are set by this minimum distance 1106, by the "update" rate of the device that is performing the separation function, and by the overall cell processing rate desired

[0214] The funneling system is composed of a pattern of low intensity traps 1105 established by a set of static holograms that are mounted in a rotating wheel so that the pattern changes as a function of the rotation pattern. The most down stream funneling traps are of fixed intensity and position, serving only to maintain the separation between the cells' lines of flow. The upstream traps 1105 are allowed to change both intensity and position with time to act so as to disturb the flow on clumped cells and pass through individual, or un-clumped, cells.

[0215] The measurement upon which the sorting determination is made may occur in the downstream region of the funneling traps 1105 or it may occur in a region further beyond the funneling system. For this initial system, the measurement will consist of high resolution fluorescence detection. In the future, however, other active sorting criteria may be implemented, such as scattering measurements, or passive techniques may be employed such as those using optical deflection as outlined earlier.

[0216] The final component of the device is the separation system in which the sorting criteria is utilized to divert cells into one of the output channels 1102, 1103 or to allow them to remain in the flow of the input channel 1101. The crucial parameter for this component is the field-of-view of the high-numerical-aperture objective lens 1104 used to implement the array of dynamic traps 1105 driving the separation. The width of this field-of-view is the same 110 microns as the individual channels' widths. The length, however, depends upon the flow rates, the channel depths, and the update rates of the optical device used to control these traps.

[0217] Currently, one embodiment consistent with the present invention includes spatial light modulators that create phase masks which are highly effective in driving optical trapping systems. These devices have update rates of 30 Hz or more. With an estimated channel depth of 10 microns, and assuming that the sperm cells should be moved in 1 micron steps, 10 updates of the spatial light modulator are employed to move a cell from the center of the input channel 1101 to the center of either output channel 1102, 1103. With an update value of 30 Hz, the implementation of these 10 steps will occur in 1/3 second. At a flow rate of 3 mm / second, these 10 steps are implemented on a length of 1 mm in the direction of flow. The objective lens 1104 for the separation component would therefore have a working area of 110 microns × 1000 microns. An

important development area of this project is the design of this lens assembly. The trade-off in lens design generally is between field-of-view and numerical aperture. That is, for a lens assembly of a particular complexity, a significant performance increase in one of these areas will come with a decrease in performance in the other area. It is for this reason that the high-performance lenses used in areas such as the high-resolution lithographic production of integrated-circuit electronics are quite complex. The present invention; however, does not require the full performance levels of these lens assemblies.

7. Disclosure on Wide-Field Vortex Tweezing

[0218] Tweezing with a wide field of view involves microscope objective lenses that have a relatively low numerical aperture. The ability to optically trap objects in the axial direction relies on focusing a light beam down in a manner that will have the largest gradients in the axial direction. This implies that a cone of light be formed with the broadest possible radius. The radius of the cone is directly determined by the numerical aperture of the objective, *i.e.*, high numerical aperture means a broad cone radius. This is in direct conflict with the requirements for wide field of view. This has traditionally made tweezing with a wide field of view in the axial direction difficult. One of the major contributions to the difficulty in axial tweezing is the radiation pressure of the focused light beam. Especially for particles that are well matched in density to the surrounding medium, for example polystyrene microspheres, radiation pressure may blow particles out of the trap. With a low numerical aperture objective, it is difficult to overcome the radiation pressure with sufficient tweezing force in the axial direction. However, holographic optical traps have the ability to form exotic modes of light which greatly reduce the radiation pressure of the light beam. Vortex traps, for example, have a dark center because the varying phases of light cancel in the center of the trap. This dark center means most of the rays of light which travel down the center of the beam no longer exist. It is exactly these beams which harbor most of the radiation pressure of the light, so their removal greatly mitigates the difficulty in axial trapping. Other modes, e.g. donut modes, have the same advantage.

[0219] Manipulation (pushing, steering, sorting) of objects or cells in general , is made safer by having multiple beams available. Like a bed of nails, multiple tweezers

ensure that less power is introduced at any particular spot in the cell. This eliminates hot spots and reduces the risk of damage. Any destructive two-photon processes benefit greatly since the absorption is proportional to the square of the laser power. Just adding a second tweezer decreases two-photon absorption in a particular spot by a factor of four

[0220] Finally, manipulation of even just a single cell is greatly enhanced by utilizing holographic optical trapping. A single cell may be manipulated by a line of tweezers, which lift the cell along the perimeter on one side. The resulting rotation allows a 360 degree view of the cell. In addition to the advantage for viewing of biological samples, there also exists the ability to orient samples stably, which has clear benefit for studies such as scattering experiments which have a strong dependence on orientation of the sample.

8. Spinning Disk-Based Cell Sorter

[0221] The technology for using lasers to access a large number of sites quickly already exists in the form of a spinning laser disc, CD player, or DVD player. These devices combine rotational motion of the disc with radial motion of the laser to access sites with incredibly high speeds. For example, the typical DVD player may access approximately 4 billion separate "bits" on the disc in about two hours. Combining this spinning disc approach with optical trapping (see FIG. 12) allows access to cells at similar rates, and holographic optical trapping increases these rates by factors of 100 or even higher.

[0222] Figure 12 illustrates a spinning disc-based cell sorter in accordance with one embodiment consistent with the inventions of the second and fifth related applications. As shown in FIG. 12, objects or cells are introduced at the sample intake 1200, and using an appropriate sample delivery system 1201, the cells are provided to the sample distribution disc 1202 which is rotated by a motor control. The imaging and trapping system 1203, which is connected to a control and analysis system 1204, sorts the cells and they are collected in sample chambers 1205 and 1206.

[0223] There are many mechanisms for distributing the cells over the surface of the disc. Fluid chambers which house individual cells, gels which immobilize the cells, sticky or waxy surfaces which bind the cells, or even freezing the cells into a solid mass,

are all methods that may be employed. Once the cells are situated such that they maintain their relative positions, they may be appropriately measured. Optical trapping may then be used to free either the desired or unwanted cells from the surface or volume. In situations where sorting into more than two groups is desired, each group may be released in a single pass, and multiple passes may be executed.

9. Sorting of Cells and Non-Biological Material Using Meltable Substrates

[0224] Technologies such as Fluorescence-Activated Cell Sorting (FACS), although well-established, suffer from the fact that they are serial processing methods. Because of the ubiquity of labeling dyes in biology, sorting on the basis of these dyes is possible. These dyes often create a difference in absorption of some wavelength or range of wavelengths between dyed and undyed specimens, assuming that groups that are to be sorted do not already inherently exhibit such an absorption difference. Holographic optical traps may then be used to both heat and manipulate the specimen into a substrate which melts from the raised temperature of the specimen. The specimen which is embedded may then be released later with an increase in the bulk temperature. In addition, a faster, even more parallel processing method is possible in which the cells are illuminated by a broad, high power light source which processes the entire array of specimens simultaneously. The same set of methods may be applied to non-biological samples which differ in the absorption spectra, or may be selectively made to do so.

10. Gel-based Sorting

[0225] Holographic optical laser traps construe a great advantage on the manipulation of objects in that they are able access and move objects in three dimensions. As biological sorting applications become more advanced, larger numbers of specimens need to be sorted, often in small amounts of time. The three-dimensional access of holographic optical traps means that these sorting applications may be realized. Quantities of cells and other specimens of biological interest which would be cumbersome or impossible to sort serially or on a two-dimensional substrate, may be effectively sorted.

[0226] One implementation of such three dimensional sorting relies on a reversible gelation process. The cells are gelled in a network, and then either wanted or unwanted cells are extracted from the gel using holographic optical traps. The heat from the traps may be used to melt the gel and provide exit pathways.

[0227] Alternatively, cells are selectively killed based on some criterion with the holographic optical laser traps. The entire gel is then melted and the live cells are separated from the dead. Instead of just killing, a more destructive thermal explosion may be generated, which disintegrates the cell into much smaller components, and then sorting on the basis of size may be effected, grouping or connecting certain cells together again.

11. Killing of Biological Specimens

[0228] A large variety of applications benefit from the ability to selectively kill biological specimens. Removing pathogens from blood is one such application. Cell sorting is another application. Cells are identified, one or more groups of cells are killed, and then the dead cells are removed. The killing is performed by the light energy from the lasers themselves, and do not necessarily require optical traps to perform this function.

[0229] Essentially, the cells are heated or the medium around the cells are heated with the laser beam, damaging and killing the cell. Holographic optical traps, because of their versatility and three-dimensional control, allow selective, massively parallel killing of cells.

12. Example

[0230] Using a BioRyx 200 System (Arryx, Inc., Chicago, IL) platelets may be tweezed from whole blood. The platelets tweeze at a low laser power (0.2W) for 532 nm and they move easily in 3-D. It is preferable to use a slightly higher power for sending the platelets through automated traps, although 0.8W is sufficient. Even in the presence of anti-coagulant, the platelets still have short strings of fibrin attached to them. Over long periods of time, the platelets may irreversibly bind to the cover slip. Platelets are roughly 2-3 micrometers in size, and they tweeze almost as well as 2-3 micron silica at

532 nm. When the RBCs are in the same viewing frame as the platelets, they tend to be repelled by the out-of-focus light cone, even if the RBCs are well away from the traps. However, if the red blood cells come into contact with the laser, the laser will puncture them and often cause them to explode, depending on the osmolarity of the medium and the laser power. Different types of WBCs respond slightly differently to the laser tweezers. In general, WBCs are slightly repelled from them. Such differential responses to optical traps provide a basis for separating types of cells by their reaction to trapping beams. For example, platelets may be trapped and moved with steered laser beams while RBCs and certain WBCs are repelled and yet other WBCs are trapped and moved to an intermediate degree.

[0231] By combining techniques described above, it may be calculated that one may separate blood cell components at a rate of 10^{11} platelets per 20 minutes. Higher rates of sorting may be achieved by further combining these techniques with the laminar flow sorting of the apparatus 100, 200 or 300.

[0232] The various techniques described above may be used for sorting a wide variety of matter. For sorting sperm, for example, sperm may be sorted based upon motility or viability, such as by motile sperm moving or swimming into a selection stream, or by non-motile or nonviable sperm sedimenting into a waste stream. Sperm may also be sorted into multiple channels, each having different average motility. Sperm may also be isolated and separated from various pathogens or otherwise undesirable materials in the semen mixture. The separation described above may also be utilized for washing and/or cooling processes. In addition, yields or motile or viable sperm may be improved, for example, by manipulating the temperature of the various flows, and the chemical content of the flows, such as by adding attractants or repellants.

[0233] Figure 25 illustrates the results of bovine sperm viability or motility sorting using the various embodiments of the present invention, in which a high motility and viability sample was generated from a lower viability and motility sample. Frozen sperm were thawed and rinsed in saline to remove glycerine, test yolk, and other materials, in order to provide density matching to the buffer solution or saline, PEG and BSA utilized in a second flow. Alternatively, glycerine may be added to the buffer flow). Flow rates of 0.01 to 0.1 ml/min were used, with 0.025 ml/min used most commonly, in a

sorter such as the apparatus 200. Sperm concentrations of approximately 5 million cells/ml with viabilities of 5-60% or higher were utilized as the input flow solution. Following sorting, the selected flow was found to have up to 80% viability, with results anticipated to approach 90-100% viability and motility.

[0234] Various other sorter configurations also may be utilized, and improved results may also occur through the use of laser steering in conjunction with the laminar flow-based sorting. For example, increasing buffer flow speed relative to input flow increases the width of the buffer channel in the separation region, decreasing the distance that sperm must move to enter the buffer flow (and increasing the distance to exit the buffer flow), increasing yield in the buffer flow (as the selected flow). Increasing the waste channel flow rate may also improve yield, forcing any dead sperm in the buffer layer near the input channel to be reintroduced into the waste channel.

[0235] The sorting described above may also utilize gradients to enhance sorting efficiency, with different flows having different properties, creating gradients such as, for example, temperature gradients, velocity gradients, viscosity gradients, and diffusion gradients.

[0236] In addition to sorting, the various embodiments of the invention may also be utilized to change concentrations of particles or cells, for example, such as increasing a concentration of particles in the selection stream, or diluting a concentration through an input buffer solution. Diffusion coefficients may also be manipulated, altering the diffusivity (or motility) of the objects in the various separation streams, such as through altering temperature, chemical concentrations, fluid viscosity, fluid density, salt concentrations, use of surfactants, etc. to, for example, alter the hydrodynamic radius or surface attraction of an object.

[0237] As a consequence, in accordance with the present invention, the plurality of holographic optical traps, which are capable of being independently manipulated, can be utilized in conjunction with an apparatus 100, 200 or 300, to manipulate components or particles, such as blood cells and other blood components, from one flow to another flow, as part of a separation stage. For example, components of interest in flow one may be identified and moved by the holographic optical traps into flow two, and thereby separated from the other components of flow one.

[0238] Figure 6 is a flow diagram illustrating a method embodiment of the present invention, and provides a useful summary. Beginning with start step 600, the method provides a first flow having a plurality of components, step 605, such as a plurality of blood components. A second flow is provided, step 610, and the first flow is contacted with the second flow to provide a first separation region, step 615. A first component of the plurality of components is differentially sedimented into the second flow, step 620, while a second component of the plurality of components is concurrently maintained in the first flow, step 625. The second flow having the first component is differentially removed from the first flow having the second component, step 630. When no additional separations (or stages) are to occur, step 635, the method may end, return step 680.

[0239] When an additional separation is to occur, step 635, the method proceeds to step 640, and a third flow is provided. The first flow is contacted with the third flow to provide a second separation region, step 645. When holographic manipulation is to be utilized in the second, additional separation, step 650, the method proceeds to step 655, and a plurality of holographic traps are generated, typically using optical wavelengths. Using the holographic traps, the second component of the plurality of components is differentially moved into the third flow, step 660. When holographic manipulation is not to be utilized in the second, additional separation, step 650, the method proceeds to step 665, and the second component of the plurality of components is differentially sedimented into the third flow. Following either step 660 or 665, a third component of the plurality of components is concurrently maintained in the first flow, step 670. The third flow having the second component is then differentially removed from the first flow having the third component, step 675, and the method may end, return step 680. While not separately illustrated in Figure 6, it should be understood that the method may continue for additional separation stages, such as a third fourth, fifth, and so on.

[0240] Additional embodiments of the invention are illustrated in Figures 14-24. Figures 14 and 15 illustrate sorting systems 1400 and 1500 employing the various sorting stages of the apparatus 100, 200 or 300, including the use of reservoirs and peristaltic pumps for fluid flow. Figure 16 is a lateral view and Figure 17 is a plan view of a high-aspect ratio flat sorter 1600. Such high-aspect ratio sorters may be utilized to provide a

comparatively large laminar flow separation surface between the various flows, providing a greater area of contact for component separation between the flows.

[0241] Figure 18 is a perspective view of a three-dimensional sorting device having a plurality of flat sorters (with one illustrated, such as sorter 1600). Figure 19 is a plan view of a multi-channel sorter 1700. Figure 20 is a plan view of a sorter 1800 having a narrow waste flow region 1801. Figure 21 is a plan view of a sorter 1900 using different flow rates for the various channels. Figure 22 is a plan view of a sorter 2000 having multiple selection channels 2001. Figure 23 is a plan view of a sorter 2100 having a constricted sorting region 2105.

[0242] Figure 24A is a lateral view and Figure 24B is a plan view of a multi-layer laminar flow sorter 2200, with input channels in layer 1 (2210), layer 2 providing a plurality of sorting stages (2200), and layer 3 providing output channels (2230). These various sorting stages may also be connected in innumerable series and parallel connections. Numerous other variations of such a multi-stage sorter will be readily apparent to those of skill in the art.

[0243] Other shapes and configurations of channels may also be utilized. For example, a tortuous or "snake"-like channel configuration may be utilized to provide a longer interaction region in a small area or volume. Flow control posts may also be utilized in the separation region, to regulate the fluid flow in the channels. For example, vertical posts provide obstacles to fluid flow, effectively reducing channel size and the Reynolds number, and improving laminar flow.

[0244] Also in summary, and by way of example, the first component of the plurality of components may be a plurality of red blood cells and a plurality of white blood cells, while the second component is a plurality of platelets. In the second separation, the plurality of white blood cells may be holographically separated from the plurality of red blood cells, using techniques such as holographic (optical) trapping. Holographic trapping may also be utilized to holographically remove a plurality of contaminants from the first flow, or to holographically separate biological debris from the first flow. In the various embodiments, the first flow may substantially comprise whole blood from a donor and an anticoagulant, and the second flow may substantially comprise

plasma from the donor. The various sedimentation steps may be rate zonal or isopycnic. The various flows are substantially non-turbulent, and may also be substantially laminar.

[0245] The first and second separation regions each have a predetermined length substantially parallel to a direction of flow and a predetermined depth substantially perpendicular to the direction of flow, the predetermined length and predetermined depth having been determined from a first sedimentation rate of the first component, from a second sedimentation rate of the second component, from a first flow rate of the first flow, and from a second flow rate of the second flow. The first flow and the second flow may have substantially the same flow rates. Alternatively, the first flow may have a first flow rate and the second flow may have a second flow rate, in which the second flow rate is comparatively greater than the first flow rate.

[0246] Also in summary, the present invention further provides an apparatus for separating a fluid mixture into constituent, non-motile components, including: (1) a first sorting channel (110 or 325) having a first inlet (120 or 315) for a first flow and a second inlet (120 or 320) for a second flow; the first sorting channel further having a first outlet (130 or the continuous channel of Figure 3) for the first flow and a second outlet 130 (or 330) for the second flow, the first sorting channel adapted to allow a first component in the first flow, of a plurality of components in the first flow, to sediment into the second flow to form an enriched second flow and a depleted first flow, while concurrently maintaining a second component of the plurality of components in the first flow; (2) a second, optically transparent sorting channel (110 or 340) having a first optical inlet coupled to the first outlet (the continuous channel of Figure 3) for the first flow and having a first optical outlet (350), the second, optically transparent sorting channel further having a second optical inlet (335) for a third flow and a second optical outlet for the third flow (345); and (3) a holographic optical trap system (400, 500) coupled to the second, optically transparent sorting channel, the holographic optical trap system adapted to generate a holographic optical trap to select and move the second component from the first flow into the third flow.

[0247] Another apparatus or system for separating a plurality of components in a fluid comprises: an optically transparent sorting channel 100, 200 or 300 having a first inlet for a first flow and a second inlet for a second flow, the optically transparent sorting

channel further having a first outlet for the first flow and a second outlet for the second flow; and a holographic optical trap system coupled to the optically transparent sorting channel, the holographic optical trap system 500 adapted to generate a holographic optical trap to select and move a first component in the first flow, of a plurality of components in the first flow, into the second flow to form an enriched second flow and a depleted first flow, while a second component of the plurality of components is concurrently maintained in the first flow.

[0248] Lastly, another method embodiment provides for separating a plurality of cells, comprising: providing a first flow having the plurality of cells; providing a second flow; contacting the first flow with the second flow to provide a first separation region; and differentially sedimenting a first cell of the plurality of cells into the second flow while concurrently maintaining a second cell of the plurality of cells in the first flow. The method generally also includes differentially removing the second flow having the first cell from the first flow having the second cell. The method may also provide for providing a third flow; contacting the first flow with the third flow to provide a second separation region; and differentially sedimenting the second cell of the plurality of cells into the third flow while concurrently maintaining a third cell of the plurality of cells in the first flow. In addition, a plurality of second cells may be holographically separated from the first flow, and a plurality of contaminants or biological debris may be holographically removed from the first flow.

[0249] While discussion above has focused on the sorting of blood components to create different blood fractions, the apparatus, methods and systems of the present invention may be extended to other types of particulate, biological or cellular matter which are non-motile, which are capable of sedimenting or creaming within a fluid flow, or which are capable of being manipulated optically. For example, the methodology of the present invention could be utilized to separate non-motile or non-viable sperm cells from viable cells, by allowing the non-motile cells to sediment from a first flow into a second flow. Other sorts of cell separation may also be performed, such as separating islet cells from other types of pancreatic cells, or otherwise separating islet cell clusters of different sizes, through either or both flow separation or optical tweezing (trapping). Viruses, proteins and other large molecules having different sedimentation rates may also

be separated with the present invention. The holographic optical trapping utilized with the various separation stages may also be particularly useful in these other types of cell or particle separations.

[0250] From the foregoing, it will be observed that numerous variations and modifications may be effected without departing from the spirit and scope of the novel concept of the invention. It is to be understood that no limitation with respect to the specific methods and apparatus illustrated herein is intended or should be inferred. It is, of course, intended to cover by the appended claims all such modifications as fall within the scope of the claims.

It is claimed:

1. A method of separating blood into components, the method comprising:

providing a first flow having a plurality of blood components;

providing a second flow;

contacting the first flow with the second flow to provide a first separation region; and

differentially sedimenting a first blood cellular component of the plurality of blood components into the second flow while concurrently maintaining a second blood cellular component of the plurality of blood components in the first flow.

2. The method of claim 1, further comprising:

differentially removing the second flow having the first blood cellular component from the first flow having the second blood cellular component.

3. The method of claim 1, wherein the first blood cellular component is a plurality of red blood cells and a plurality of white blood cells.

4. The method of claim 3, further comprising:

holographically separating the plurality of white blood cells from the plurality of red blood cells.

5. The method of claim 1, wherein the second blood cellular component is a plurality of platelets.

6. The method of claim 1, further comprising:

providing a third flow;

contacting the first flow with the third flow to provide a second separation region; and

differentially sedimenting the second blood cellular component of the plurality of blood components into the third flow while concurrently maintaining a third blood component of the plurality of blood components in the first flow.

7. The method of claim 6, further comprising:

holographically trapping the second blood cellular component.

8. The method of claim 6, wherein the second blood cellular component is a plurality of platelets and wherein the third blood component is plasma.

9. The method of claim 6, further comprising:

recirculating the first flow to form the second flow.

10. The method of claim 1, further comprising:

holographically separating a plurality of second blood cellular components from the first flow.

11. The method of claim 1, further comprising:

holographically removing a plurality of contaminants or biological debris from the first flow.

12. The method of claim 1, wherein the first flow substantially comprises whole blood from a donor and an anticoagulant, and the second flow substantially comprises plasma from the donor.

13. The method of claim 1, wherein the sedimentation step is rate zonal.

14. The method of claim 1, wherein the sedimentation step is isopycnic.

15. The method of claim 1, wherein the first flow and the second flow are substantially non-turbulent.

16. The method of claim 1, wherein the first flow and the second flow are substantially laminar.

17. The method of claim 1, wherein the separation region has a predetermined length substantially parallel to a direction of flow of the first and second flows and a predetermined depth substantially perpendicular to the direction of flow of the first and second flows, the predetermined length and predetermined depth having been determined from a first sedimentation rate of the first blood cellular component, from a second sedimentation rate of the second blood cellular component, from a first flow rate of the first flow, and from a second flow rate of the second flow.

18. The method of claim 1, wherein the first flow and the second flow have substantially the same flow rates.

19. The method of claim 1, wherein the first flow has a first flow rate and the second flow has a second flow rate, the second flow rate comparatively greater than the first flow rate.

20. The method of claim 1, wherein a first volume of the first flow in the separation region is comparatively less than a second volume of the second flow in the separation region.

21. An apparatus which performs the method of claim 1.

22. A method of separating a fluid mixture into constituent components, the method comprising:

providing a substantially laminar first flow having the fluid mixture, the fluid mixture having a plurality of components, the plurality of components having a corresponding plurality of sedimentation rates;

providing a substantially laminar second flow;

contacting the first flow with the second flow to provide a first separation region, the first flow and the second flow having a substantially non-turbulent interface within the separation region;

differentially sedimenting from the first flow a first component of the plurality of components into the second flow to form an enriched second flow and a depleted first flow, while concurrently maintaining a second component of the plurality of components in the first flow, the first component having a first sedimentation rate of the plurality of sedimentation rates and the second component having a second sedimentation rate of the plurality of sedimentation rates, wherein the first sedimentation rate is comparatively greater than the second sedimentation rate;

differentially removing the enriched second flow from the depleted first flow; and

holographically manipulating the second component in the depleted first flow.

23. The method of claim 22, wherein the fluid mixture is whole blood, wherein the first component is a plurality of red blood cells and white blood cells, and wherein the second component is a plurality of platelets.

24. The method of claim 22, wherein the fluid mixture is semen, wherein the first component is a plurality of non-motile or nonviable spermatozoa cells, and wherein the second component is a plurality of motile or viable spermatozoa cells.

25. The method of claim 22, wherein the optical manipulation step further comprises:

holographically trapping the second component to remove the second component from the depleted first flow.

26. The method of claim 22, further comprising:

holographically removing a plurality of contaminants or biological debris from the first flow.

27. The method of claim 22, further comprising:

providing a third flow;

contacting the depleted first flow with the third flow to provide a second separation region; and

holographically trapping the second component and moving the second component from the depleted first flow into the third flow while concurrently maintaining a third component of the plurality of components in the depleted first flow.

28. The method of claim 27, further comprising:

recirculating the depleted first flow to form the second flow.

29. An apparatus which performs the method of claim 22.

30. A method of separating a fluid mixture into constituent components, the method comprising:

providing a substantially laminar first flow having the fluid mixture, the fluid mixture having a plurality of components, the plurality of components having a corresponding plurality of sedimentation rates;

providing a substantially laminar second flow;

providing a substantially laminar third flow;

contacting the first flow with the second flow and with the third flow to provide a first separation region, the first flow and the second flow having a substantially non-turbulent first interface within the separation region, and the first flow and the third flow having a substantially non-turbulent second interface within the separation region;

differentially removing from the first flow a first component of the plurality of components into the second flow to form an enriched second flow and a depleted first flow;

differentially removing from the first flow a second component of the plurality of components into the third flow to form an enriched third flow and a further depleted first flow;

differentially removing the enriched second flow from the depleted first flow; and

differentially removing the enriched third flow from the depleted first flow.

31. The method of claim 30, wherein the first flow, the second flow, and the third flow each have a different density to provide a stepped density gradient in the first separation region.

32. The method of claim 30, wherein the removal of the first component and the second component from the first flow is performed by diffusion.

33. The method of claim 30, wherein the removal of the first component and the second component from the first flow is performed by isopycnic or rate zonal sedimentation.

34. The method of claim 30, wherein the removal of the second component from the first flow is performed by holographic optical trapping manipulation.

35. The method of claim 30, wherein the removal of the first component and the second component from the first flow is performed electromagnetically.

36. The method of claim 30, wherein the fluid mixture is whole blood, wherein the first component is a plurality of red blood cells and white blood cells, and wherein the second component is a plurality of platelets.

37. The method of claim 30, wherein the fluid mixture is semen, wherein the first component is a plurality of non-motile or nonviable spermatozoa cells, and wherein the second component is a plurality of motile or viable spermatozoa cells.

38. The method of claim 30, wherein the step of differentially removing from the first flow the second component further comprises:

holographically trapping the second component to remove the second component from the depleted first flow.

39. An apparatus for separating a fluid mixture into constituent components, the apparatus comprising:

means for providing a substantially laminar first flow having the fluid mixture, the fluid mixture having a plurality of components, the plurality of components having a corresponding plurality of sedimentation rates;

means for providing a substantially laminar second flow;

means for providing a substantially laminar third flow;

means for contacting the first flow with the second flow and with the third flow to provide a first separation region, the first flow and the second flow having a substantially non-turbulent first interface within the separation region, and the first flow and the third flow having a substantially non-turbulent second interface within the separation region;

means for differentially removing from the first flow a first component of the plurality of components into the second flow to form an enriched second flow and a depleted first flow;

means for differentially removing from the first flow a second component of the plurality of components into the third flow to form an enriched third flow and a further depleted first flow;

means for differentially removing the enriched second flow from the depleted first flow; and

means for differentially removing the enriched third flow from the depleted first flow.

40. The apparatus of claim 39, wherein the first flow, the second flow, and the third flow each have a different density to provide a stepped density gradient in the first separation region.

41. The apparatus of claim 39, wherein the means for removal of the first component and the second component from the first flow is diffusion.

42. The apparatus of claim 39, wherein the means for removal of the first component and the second component from the first flow is performed by isopycnic or rate zonal sedimentation.

43. The apparatus of claim 39, wherein the means for removal of the second component from the first flow is a holographic optical trap.

44. The apparatus of claim 39, wherein the fluid mixture is whole blood, wherein the first component is a plurality of red blood cells and white blood cells, and wherein the second component is a plurality of platelets.

45. The apparatus of claim 39, wherein the fluid mixture is semen, wherein the first component is a plurality of non-motile or nonviable spermatozoa cells, and wherein the second component is a plurality of motile or viable spermatozoa cells.

46. A method of separating blood into components, the method comprising:

providing a substantially laminar first flow having a plurality of blood components;

providing a substantially laminar second flow;

contacting the first flow with the second flow to provide a first separation region, a size of the first separation region having been predetermined based on a plurality of sedimentation rates of the plurality of blood components;

differentially rate zonal sedimenting a plurality of red blood cells and a plurality of white blood cells, of the plurality of blood components, into the second flow while concurrently maintaining a plurality of platelets and plasma, of the plurality of blood components, in the first flow;

differentially removing the second flow having the first blood cellular component from the first flow having the second blood cellular component;

providing a third flow;

contacting the first flow with the third flow to provide a second separation region;

differentially rate zonal sedimenting the plurality of platelets into the third flow while concurrently maintaining plasma in the first flow; and

recirculating the first flow to form the second flow.

47. An apparatus for separating a plurality of components in a fluid, the apparatus comprising:

an optically transparent sorting channel having a first inlet for a first flow and a second inlet for a second flow, the optically transparent sorting channel further having a first outlet for the first flow and a second outlet for the second flow; and

a holographic optical trap system coupled to the optically transparent sorting channel, the holographic optical trap system adapted to generate a holographic optical trap to select and move a first component in the first flow, of a plurality of components in the first flow, into the second flow to form an enriched second flow and a depleted first flow, while a second component of the plurality of components is concurrently maintained in the first flow.

48. An apparatus for separating a fluid mixture into constituent, non-motile components, the apparatus comprising:

a first sorting channel having a first inlet for a first flow and a second inlet for a second flow; the first sorting channel further having a first outlet for the first flow and a second outlet for the second flow, the first sorting channel adapted to allow a first component in the first flow, of a plurality of components in the first flow, to sediment into the second flow to form an enriched second flow and a depleted first flow, while concurrently maintaining a second component of the plurality of components in the first flow;

a second, optically transparent sorting channel having a first optical inlet coupled to the first outlet for the first flow and having a first optical outlet, the second, optically transparent sorting channel further having a second optical inlet for a third flow and a second optical outlet for the third flow; and

a holographic optical trap system coupled to the second, optically transparent sorting channel, the holographic optical trap system adapted to generate a holographic optical trap to select and move the second component from the first flow into the third flow.

49. A method of separating a plurality of cells, the method comprising:

providing a first flow having the plurality of cells;

providing a second flow;

contacting the first flow with the second flow to provide a first separation region; and

differentially removing a first cell of the plurality of cells into the second flow while concurrently maintaining a second cell of the plurality of cells in the first flow.

50. The method of claim 49, further comprising:

differentially removing the second flow having the first cell from the first flow having the second cell.

51. The method of claim 49, further comprising:

providing a third flow;

contacting the first flow with the third flow to provide a second separation region; and

differentially removing the second cell of the plurality of cells into the third flow while concurrently maintaining a third cell of the plurality of cells in the first flow.

52. The method of claim 49, further comprising:

holographically separating a plurality of second cells from the first flow.

53. The method of claim 49, further comprising:

holographically removing a plurality of contaminants or biological debris from the first flow.

54. The method of claim 49, wherein the first flow and the second flow each have a different density to provide a stepped density gradient in the first separation region.

55. The method of claim 49, wherein the removal of the first cell from the first flow is performed by isopycnic or rate zonal sedimentation.

56. The method of claim 55, wherein the first cell is a nonviable or non-motile spermatozoa cell.

57. The method of claim 49, wherein the removal of the first cell from the first flow is performed by self-motility.

58. The method of claim 57, wherein the first cell is a viable or motile spermatozoa cell.

59. An apparatus for separating a plurality of cells, the method comprising:

means for providing a first flow having the plurality of cells;

means for providing a second flow;

means for contacting the first flow with the second flow to provide a first separation region; and

means for differentially removing a first cell of the plurality of cells into the second flow while concurrently maintaining a second cell of the plurality of cells in the first flow.

60. The apparatus of claim 59, further comprising:

means for differentially removing the second flow having the first cell from the first flow having the second cell.

61. The apparatus of claim 59, further comprising:

means for providing a third flow;

means for contacting the first flow with the third flow to provide a second separation region; and

means for differentially removing the second cell of the plurality of cells into the third flow while concurrently maintaining a third cell of the plurality of cells in the first flow.

62. The apparatus of claim 59, further comprising:

means for holographically separating a plurality of second cells from the first flow.

63. The apparatus of claim 59, wherein the first flow and the second flow each have a different density to provide a stepped density gradient in the first separation region.

64. The apparatus of claim 59, wherein the means for removal of the first cell from the first flow is isopycnic or rate zonal sedimentation.

65. The apparatus of claim 64, wherein the first cell is a nonviable or non-motile spermatozoa cell.

66. The apparatus of claim 59, wherein the means for removal of the first cell from the first flow is self-motility.

67. The apparatus of claim 66, wherein the first cell is a viable or motile spermatozoa cell.

68. An apparatus for separating a component from a plurality of components in a fluid, the apparatus having an aspect ratio of length to width, the apparatus comprising:

a first sorting channel having a first inlet for a first flow and a second inlet for a second flow; the first and second flows each having a flow direction along the length of the apparatus, the first sorting channel further having a first outlet for the first flow and a second outlet for the second flow, the first sorting channel adapted to allow a first component in the first flow, of a plurality of components in the first flow, to be selectively removed into the second flow to form an enriched second flow and a depleted first flow, while concurrently maintaining a second component of the plurality of components in the first flow; and

wherein the aspect ratio of length to width is less than about 2:1.

69. The apparatus of claim 68, wherein the aspect ratio of length to width is from about 2:1 to 1:2.

70. The apparatus of claim 68, further comprising:

a second, optically transparent sorting channel having a first optical inlet coupled to the first outlet for the first flow and having a first optical outlet, the second, optically transparent sorting channel further having a second optical inlet for a third flow and a second optical outlet for the third flow; and

a holographic optical trap system coupled to the second, optically transparent sorting channel, the holographic optical trap system adapted to generate a holographic optical trap to select and move the second component from the first flow into the third flow.

71. A system for separation of a plurality of components from a fluid mixture, the system comprising:

a flow plate having a plurality of inlets for a first flow and a second flow and having a plurality of outlets for the first flow and the second flow, the flow plate further having at least one separation region in which the first flow and the second flow are substantially laminar;

a plurality of pumps to control the flow rates of the first flow and the second flow;

a temperature control to control the temperature of the first flow and the second flow; and

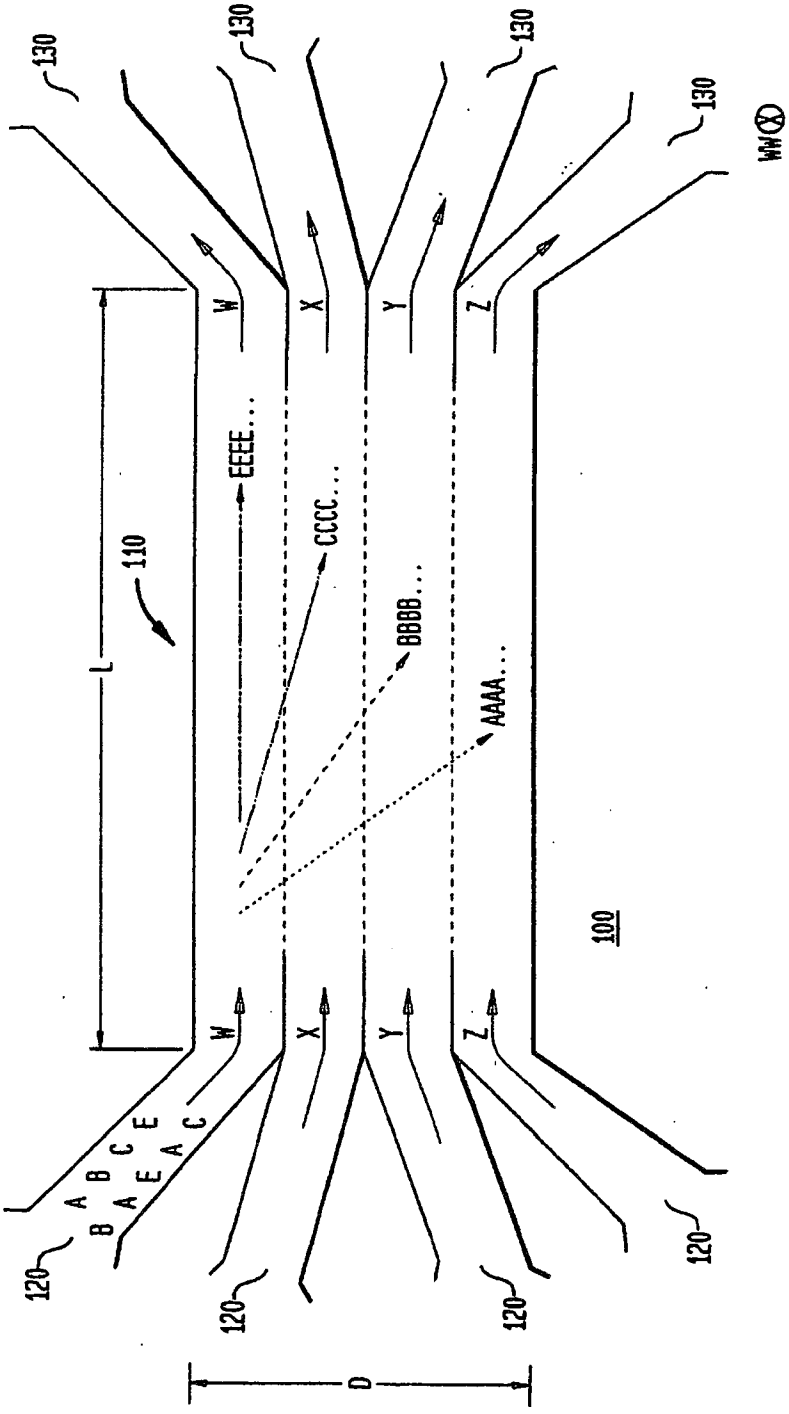
a computer adapted to provide user input for control of the separation of the plurality of components from the fluid mixture in the separation region.

MULTIPLE LAMINAR FLOW-BASED PARTICLE AND CELLULAR SEPARATION WITH LASER STEERING

Abstract of the Disclosure

The invention provides a method, apparatus and system for separating blood and other types of cellular components, and can be combined with holographic optical trapping manipulation or other forms of optical tweezing. One of the exemplary methods includes providing a first flow having a plurality of blood components; providing a second flow; contacting the first flow with the second flow to provide a first separation region; and differentially sedimenting a first blood cellular component of the plurality of blood components into the second flow while concurrently maintaining a second blood cellular component of the plurality of blood components in the first flow. The second flow having the first blood cellular component is then differentially removed from the first flow having the second blood cellular component. Holographic optical traps may also be utilized in conjunction with the various flows to move selected components from one flow to another, as part of or in addition to a separation stage.

FIG. 1


130
130
130
130
WW
W
X
Y
Z
EEEE...
CCCC...
BBBB...
AAAA...
110
L
100
A
B
C
E
E
A
C
120
120
120
120
D

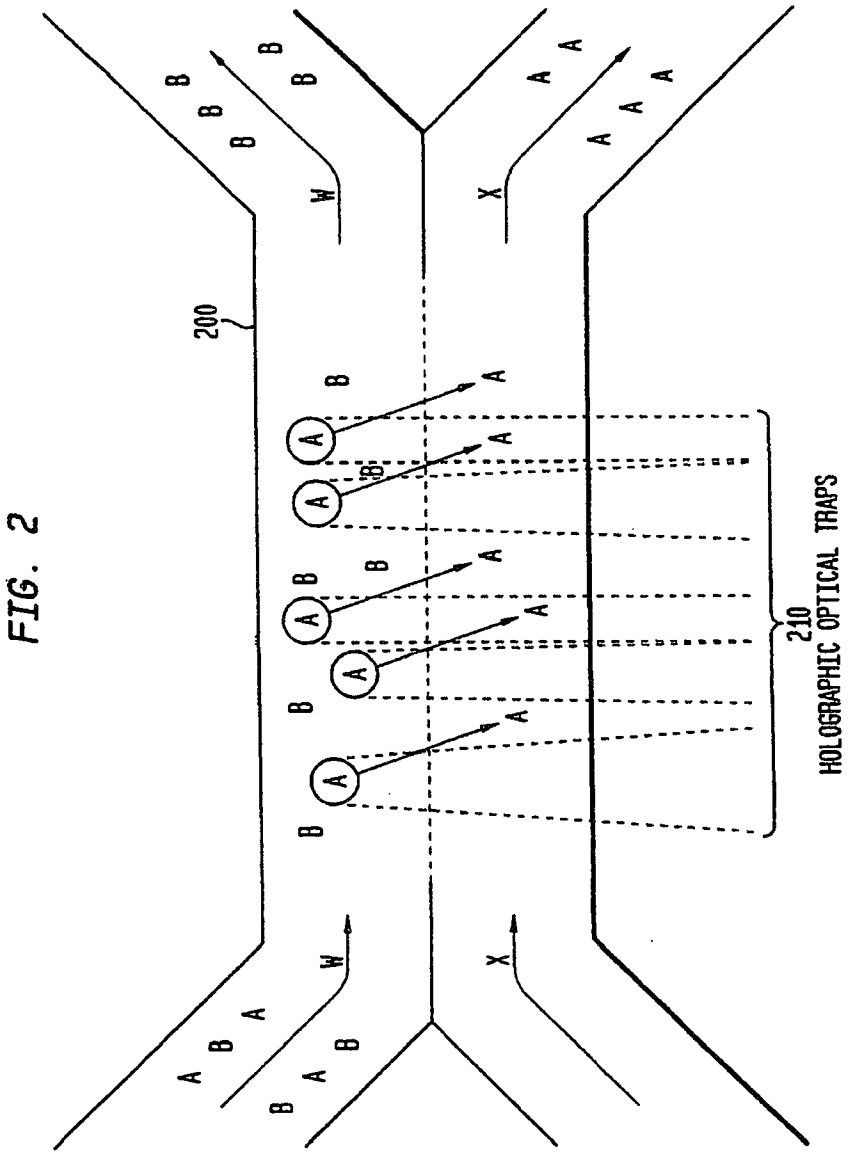


FIG. 2

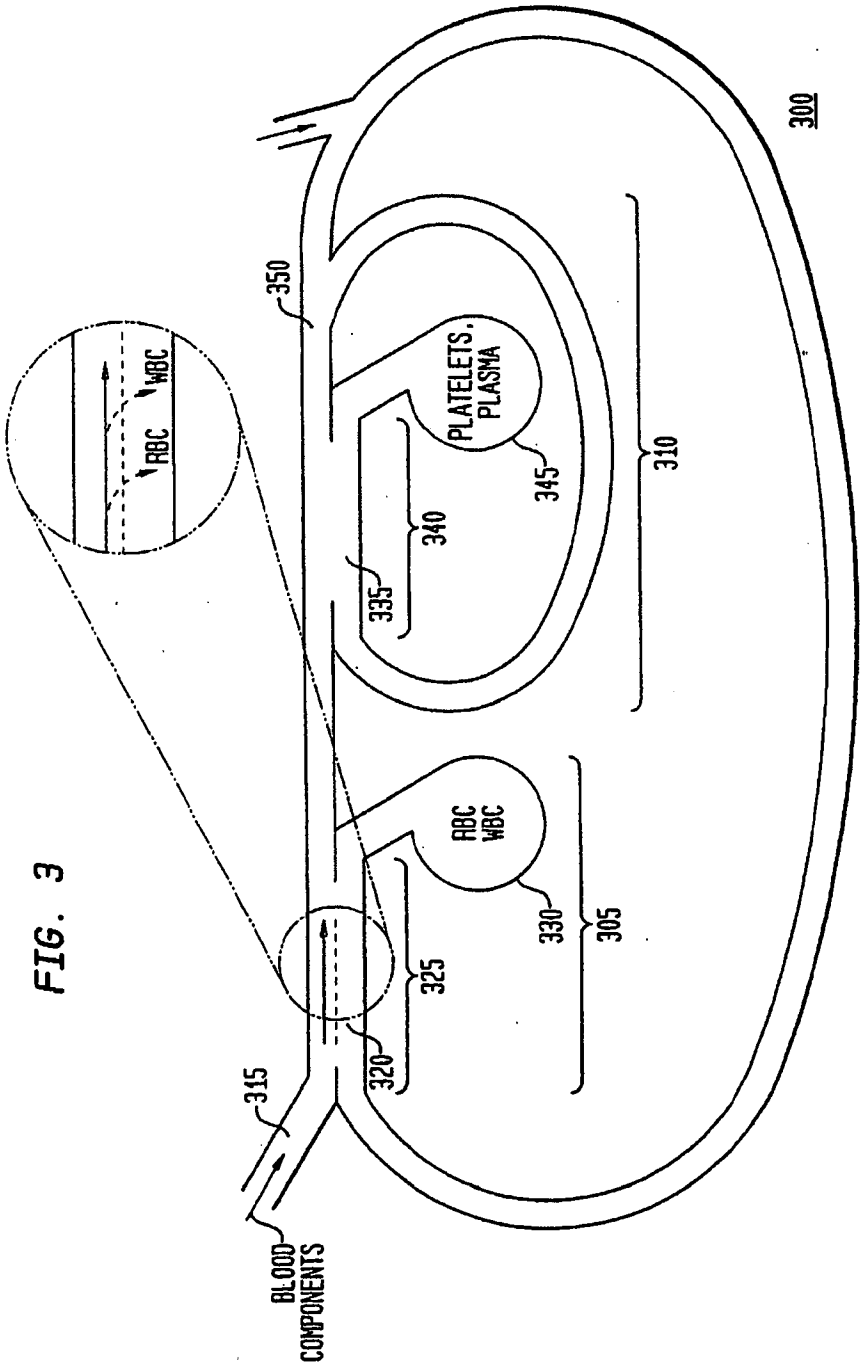
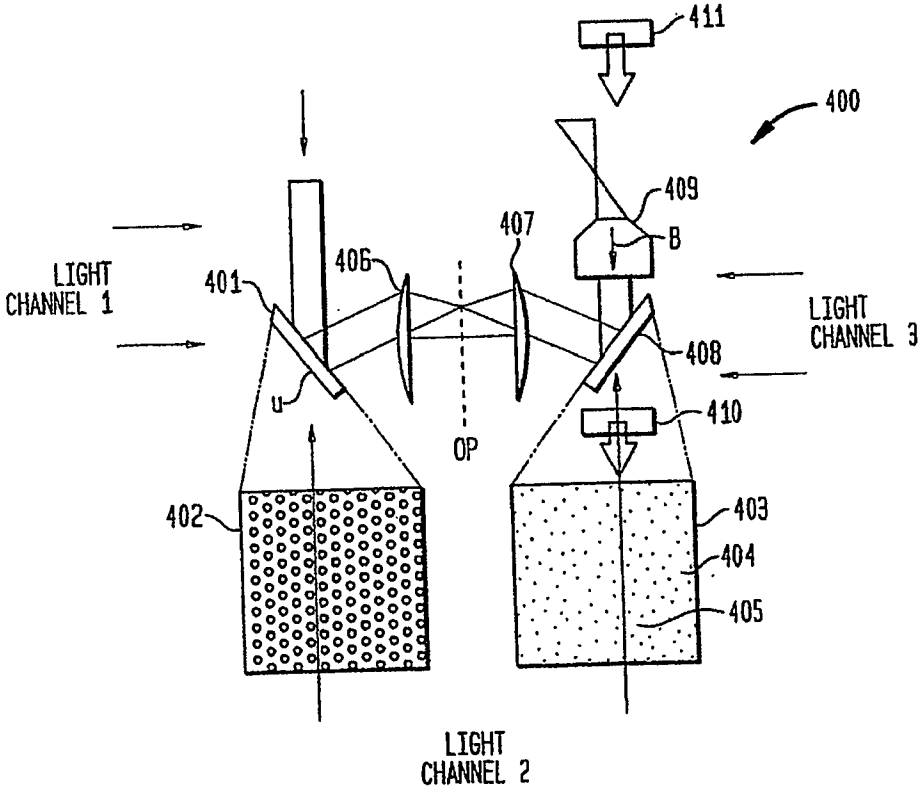

300
350
PLATELETS, PLASMA
345
310
340
335
RBC
WBC
RBC
WBC
330
305
325
320
315
BLOOD COMPONENTS

FIG. 3

FIG. 4


411
400
409
B
407
406
401
LIGHT
CHANNEL 1
LIGHT
CHANNEL 3
408
u
410
OP
402
403
404
405
LIGHT
CHANNEL 2

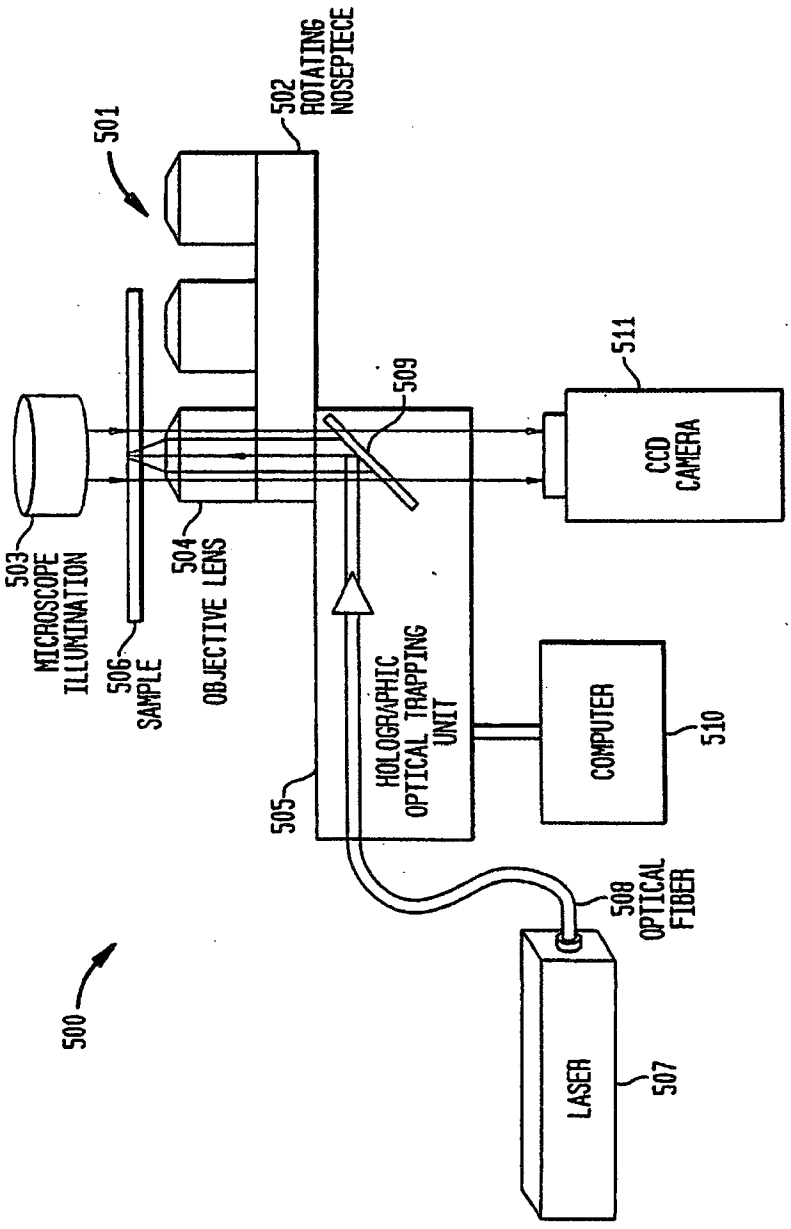

501
502
ROTATING NOSEPIECE
503
MICROSCOPE ILLUMINATION
504
OBJECTIVE LENS
505
506
SAMPLE
507
LASER
508
OPTICAL FIBER
509
510
COMPUTER
511
CCD CAMERA
HOLOGRAPHIC OPTICAL TRAPPING UNIT
500

FIG. 5

FIG. 6A

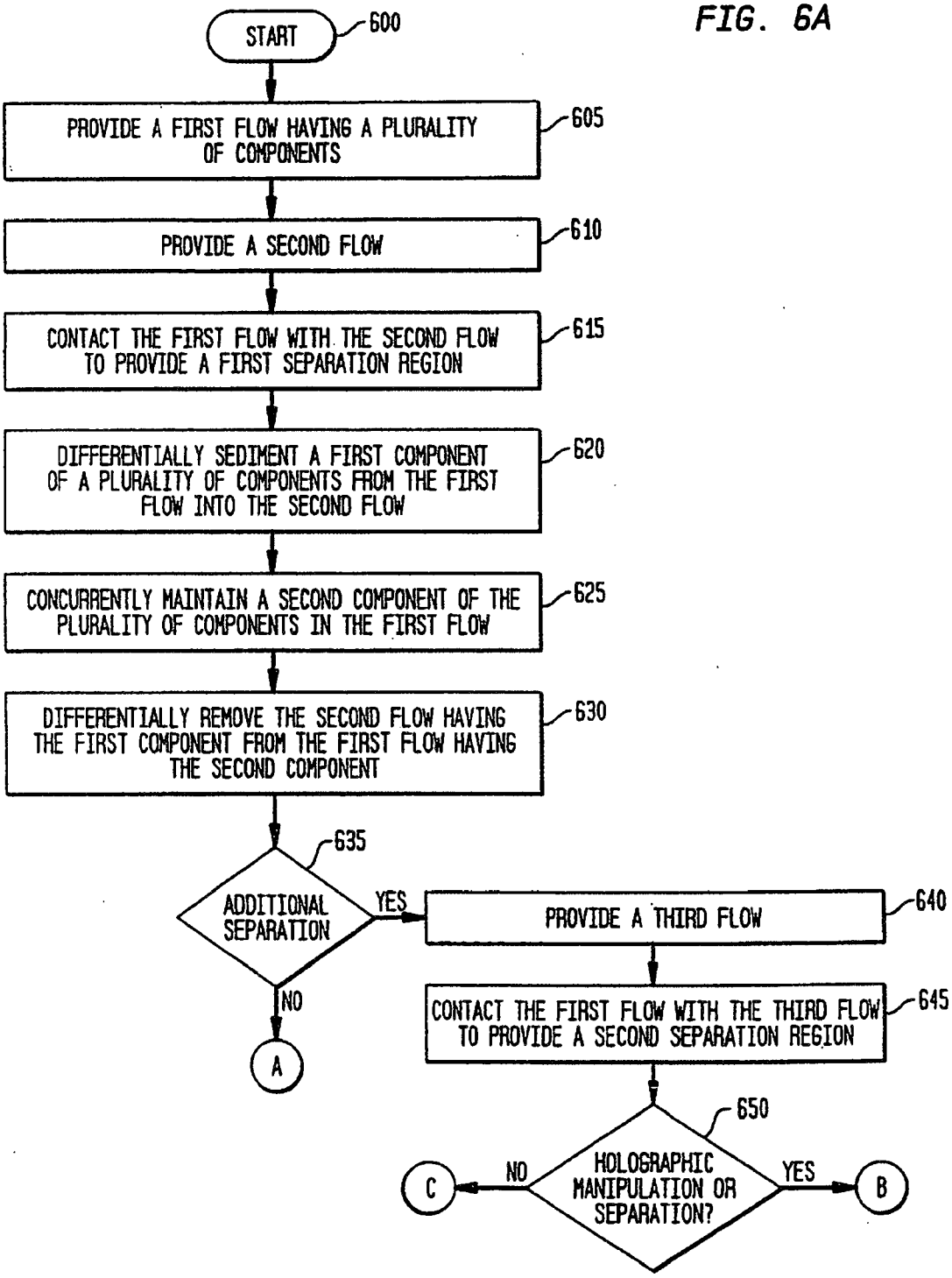

START
600
PROVIDE A FIRST FLOW HAVING A PLURALITY OF COMPONENTS
605
PROVIDE A SECOND FLOW
610
CONTACT THE FIRST FLOW WITH THE SECOND FLOW TO PROVIDE A FIRST SEPARATION REGION
615
DIFFERENTIALLY SEDIMENT A FIRST COMPONENT OF A PLURALITY OF COMPONENTS FROM THE FIRST FLOW INTO THE SECOND FLOW
620
CONCURRENTLY MAINTAIN A SECOND COMPONENT OF THE PLURALITY OF COMPONENTS IN THE FIRST FLOW
625
DIFFERENTIALLY REMOVE THE SECOND FLOW HAVING THE FIRST COMPONENT FROM THE FIRST FLOW HAVING THE SECOND COMPONENT
630
635
ADDITIONAL SEPARATION
YES
NO
A
PROVIDE A THIRD FLOW
640
CONTACT THE FIRST FLOW WITH THE THIRD FLOW TO PROVIDE A SECOND SEPARATION REGION
645
650
HOLOGRAPHIC MANIPULATION OR SEPARATION?
NO
YES
C
B

FIG. 6B

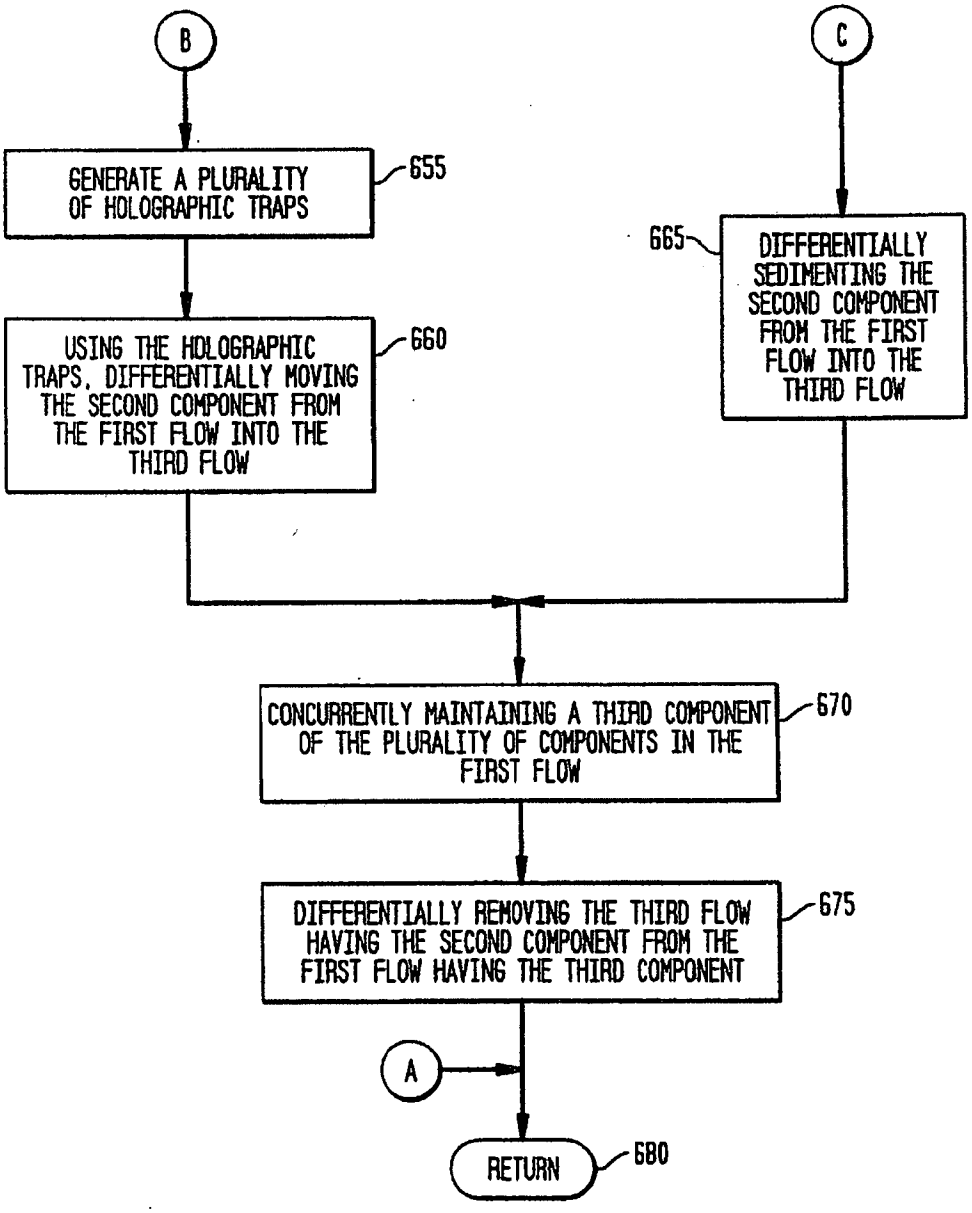

B
C
GENERATE A PLURALITY OF HOLOGRAPHIC TRAPS
655
USING THE HOLOGRAPHIC TRAPS, DIFFERENTIALLY MOVING THE SECOND COMPONENT FROM THE FIRST FLOW INTO THE THIRD FLOW
660
665
DIFFERENTIALLY SEDIMENTING THE SECOND COMPONENT FROM THE FIRST FLOW INTO THE THIRD FLOW
CONCURRENTLY MAINTAINING A THIRD COMPONENT OF THE PLURALITY OF COMPONENTS IN THE FIRST FLOW
670
DIFFERENTIALLY REMOVING THE THIRD FLOW HAVING THE SECOND COMPONENT FROM THE FIRST FLOW HAVING THE THIRD COMPONENT
675
A
RETURN
680

FIG. 7A

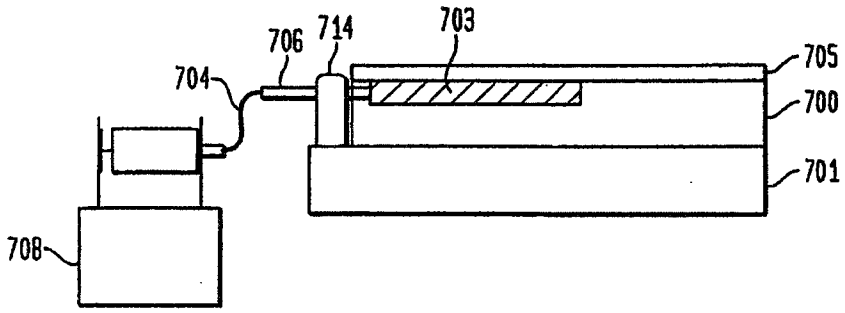

706
714
703
704
705
700
701
708

FIG. 7B

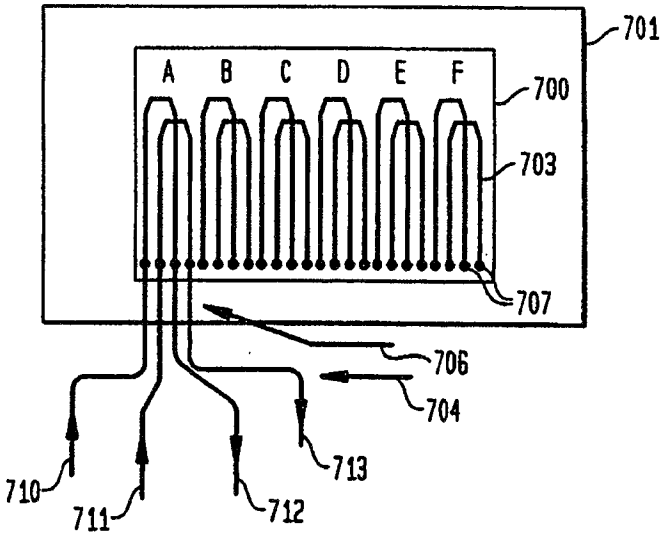

701
A
B
C
D
E
F
700
703
707
706
704
710
711
712
713

FIG. 8

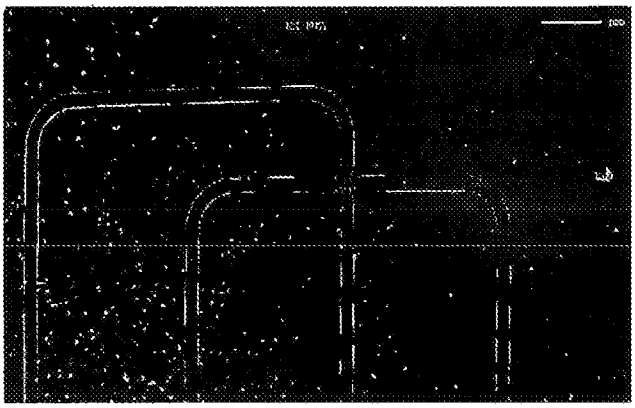


FIG. 9

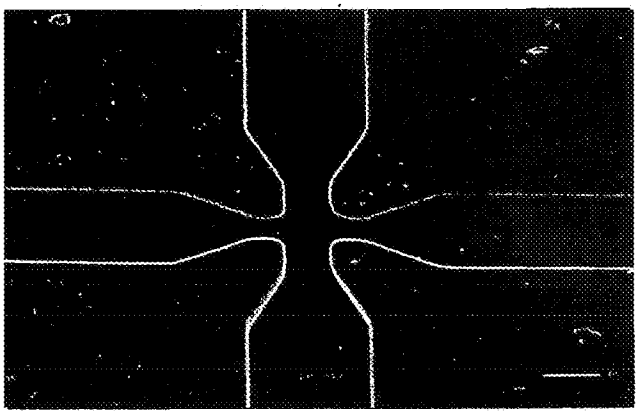
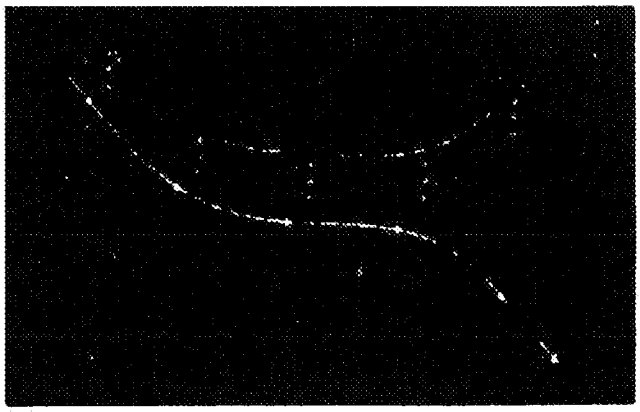


FIG. 10



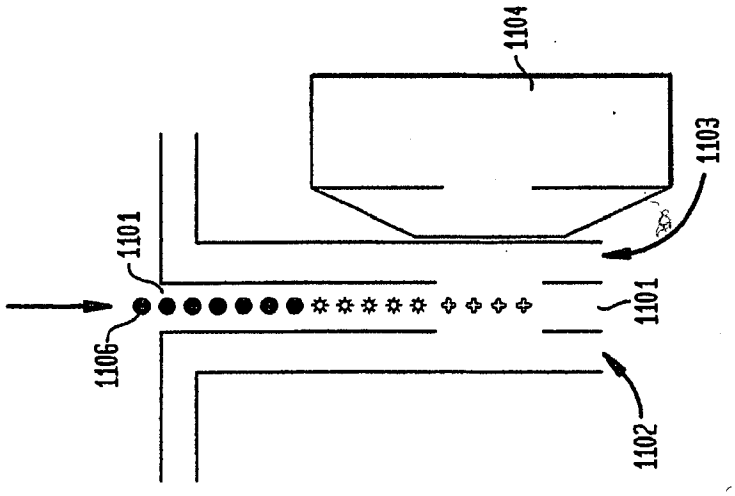


FIG. 11B

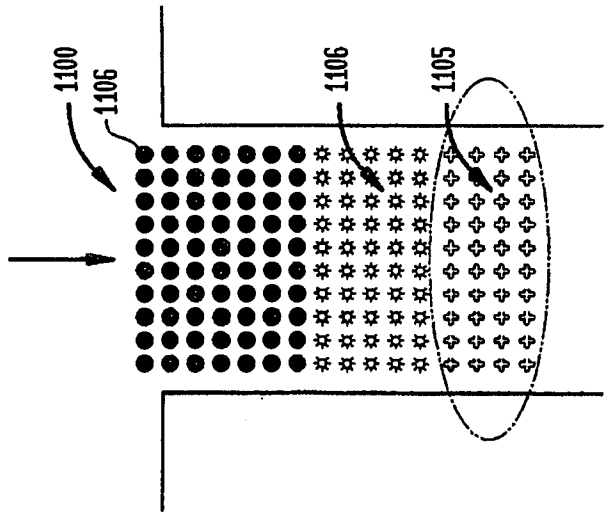


FIG. 11A

FIG. 12

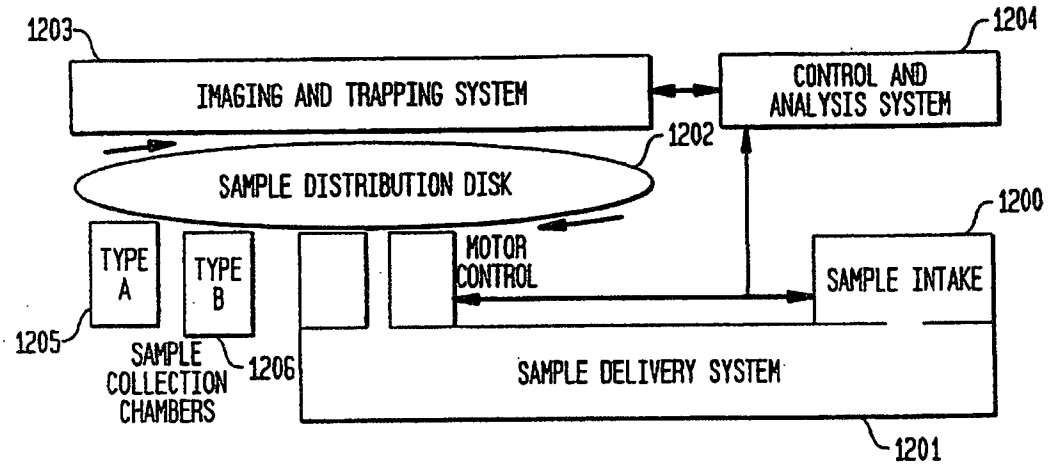
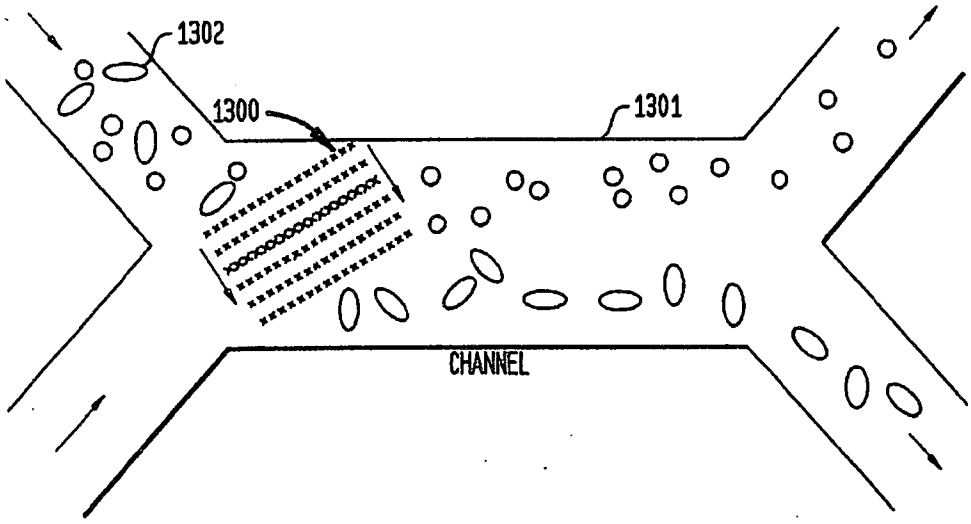

1203
IMAGING AND TRAPPING SYSTEM
1204
CONTROL AND ANALYSIS SYSTEM
1202
SAMPLE DISTRIBUTION DISK
1200
TYPE A
TYPE B
MOTOR CONTROL
SAMPLE INTAKE
1205
SAMPLE COLLECTION CHAMBERS
1206
SAMPLE DELIVERY SYSTEM
1201

FIG. 13


1302
1300
1301
CHANNEL

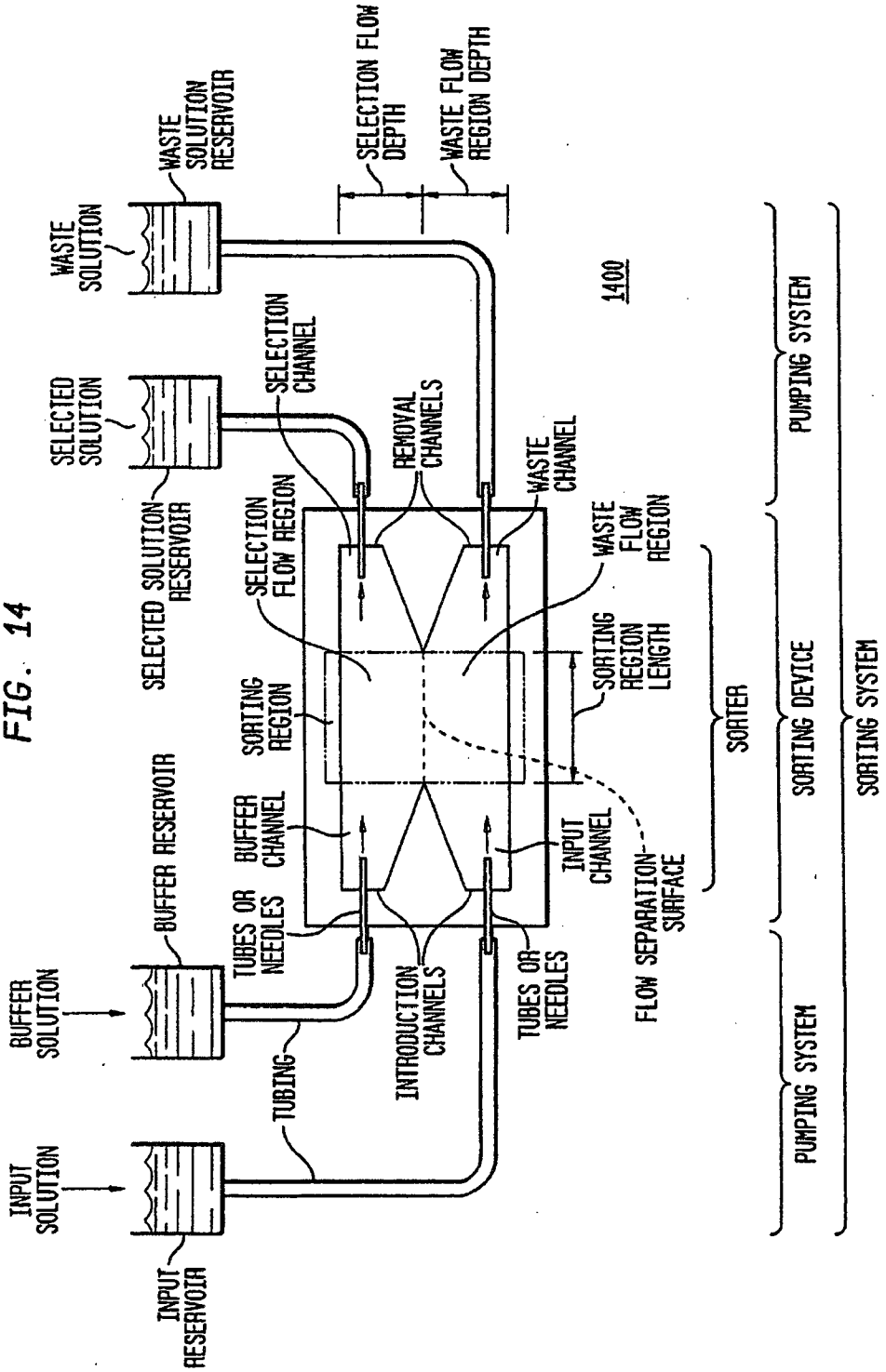

WASTE SOLUTION RESERVOIR
SELECTION FLOW DEPTH
WASTE FLOW REGION DEPTH
WASTE SOLUTION
SELECTED SOLUTION
SELECTION CHANNEL
1400
REMOVAL CHANNELS
WASTE CHANNEL
SELECTED SOLUTION RESERVOIR
SELECTION FLOW REGION
WASTE FLOW REGION
SORTING REGION
SORTING REGION LENGTH
BUFFER RESERVOIR
BUFFER CHANNEL
INPUT CHANNEL
SORTER
TUBES OR NEEDLES
FLOW SEPARATION SURFACE
BUFFER SOLUTION
INTRODUCTION CHANNELS
TUBES OR NEEDLES
PUMPING SYSTEM
SORTING DEVICE
TUBING
INPUT SOLUTION
PUMPING SYSTEM
SORTING SYSTEM
INPUT RESERVOIR

FIG. 14

FIG. 15

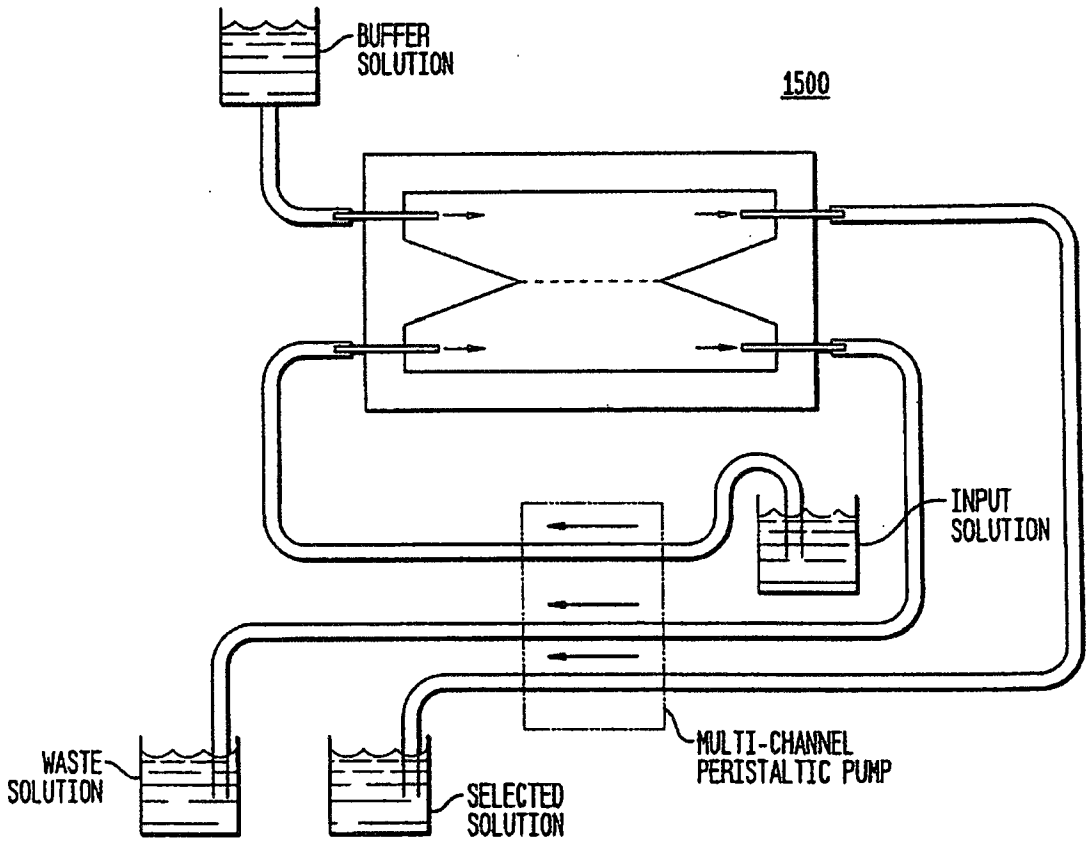

BUFFER SOLUTION
1500
INPUT SOLUTION
MULTI-CHANNEL PERISTALTIC PUMP
WASTE SOLUTION
SELECTED SOLUTION

FIG. 16

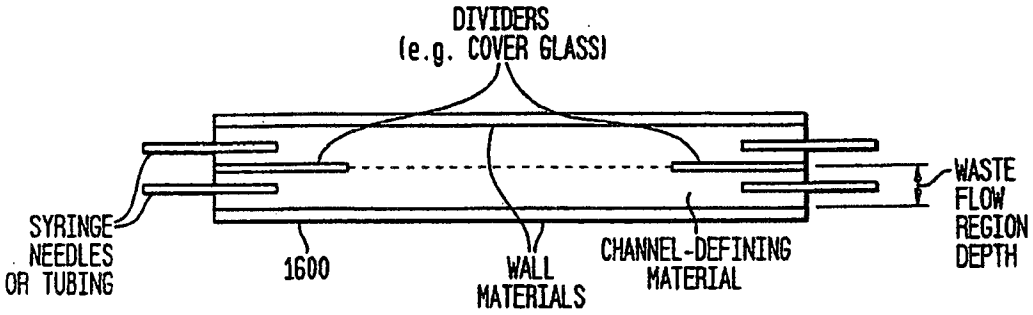

DIVIDERS
(e.g. COVER GLASS)
SYRINGE NEEDLES OR TUBING
1600
WALL MATERIALS
CHANNEL-DEFINING MATERIAL
WASTE FLOW REGION DEPTH

FIG. 17

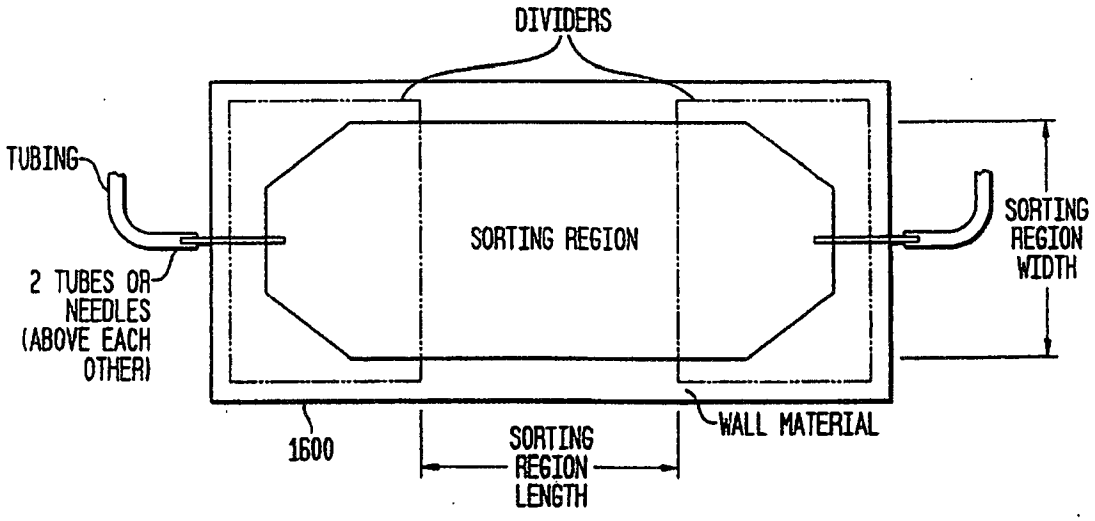

DIVIDERS
TUBING
2 TUBES OR NEEDLES (ABOVE EACH OTHER)
SORTING REGION
SORTING REGION WIDTH
1600
SORTING REGION LENGTH
WALL MATERIAL

FIG. 18

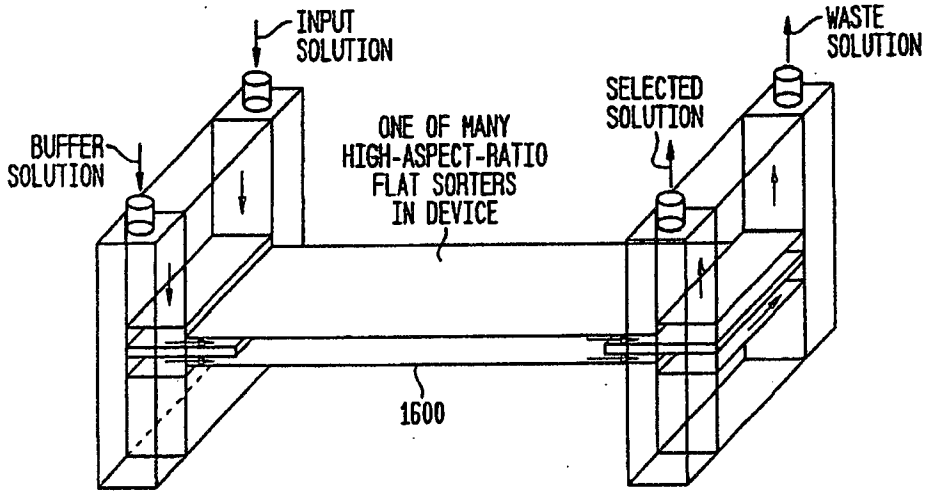

INPUT SOLUTION
WASTE SOLUTION
SELECTED SOLUTION
BUFFER SOLUTION
ONE OF MANY HIGH-ASPECT-RATIO FLAT SORTERS IN DEVICE
1600

FIG. 19

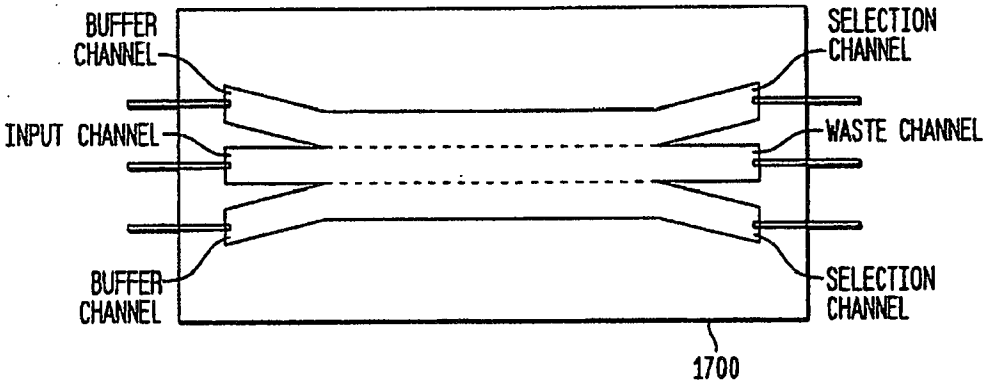

BUFFER CHANNEL
SELECTION CHANNEL
INPUT CHANNEL
WASTE CHANNEL
BUFFER CHANNEL
SELECTION CHANNEL
1700

FIG. 20

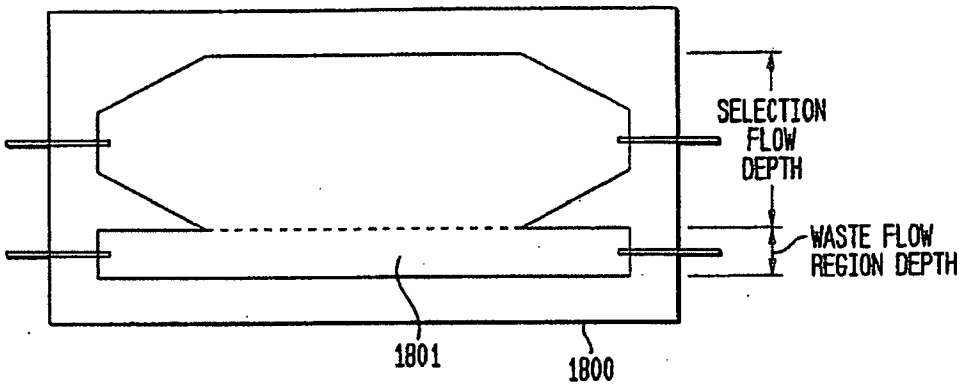

SELECTION
FLOW
DEPTH
WASTE FLOW
REGION DEPTH
1801
1800

FIG. 21

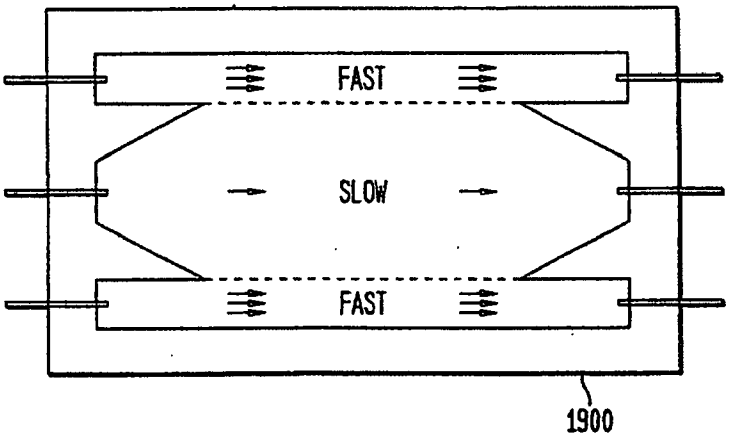

FAST
SLOW
FAST
1900

FIG. 22

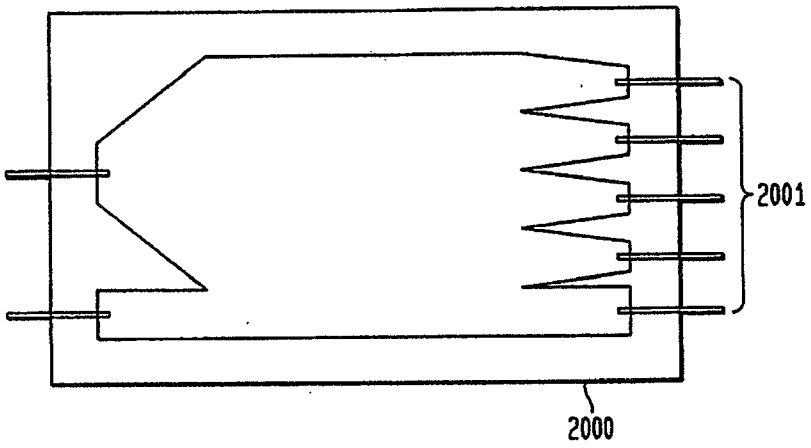


FIG. 23

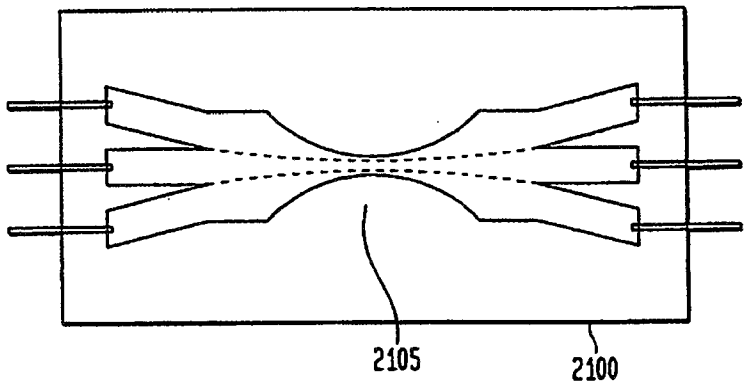
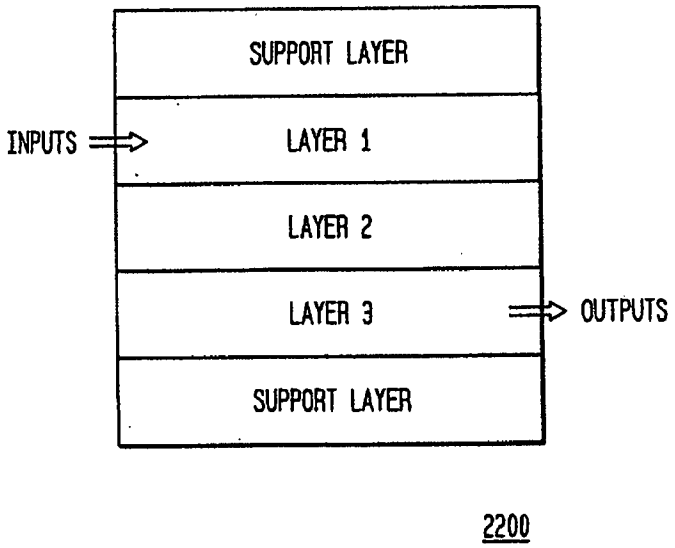


FIG. 24A


SUPPORT LAYER
INPUTS
LAYER 1
LAYER 2
LAYER 3
OUTPUTS
SUPPORT LAYER
2200

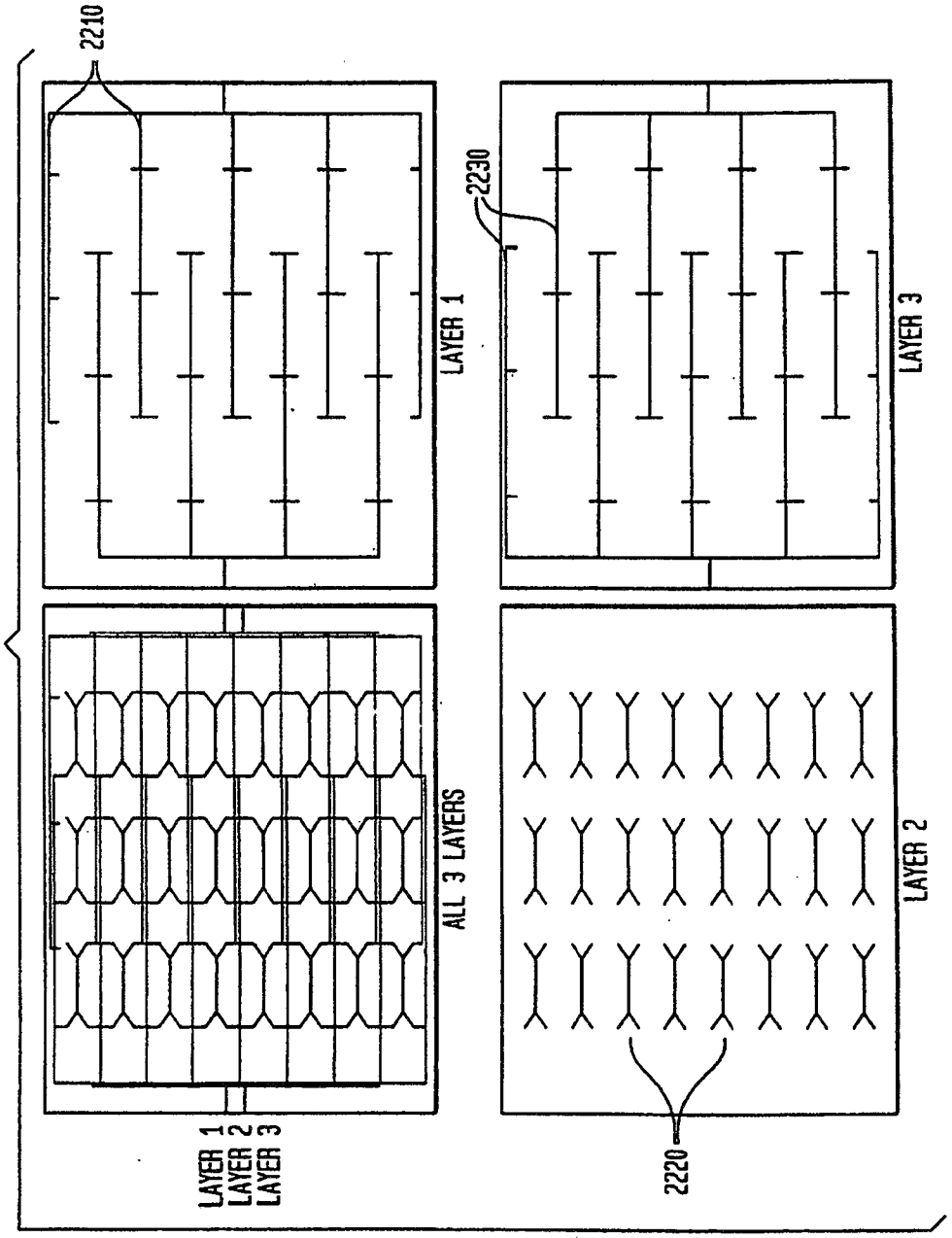

2210
LAYER 1
2230
LAYER 3
ALL 3 LAYERS
LAYER 2
LAYER 1
LAYER 2
LAYER 3
2220

FIG. 24B

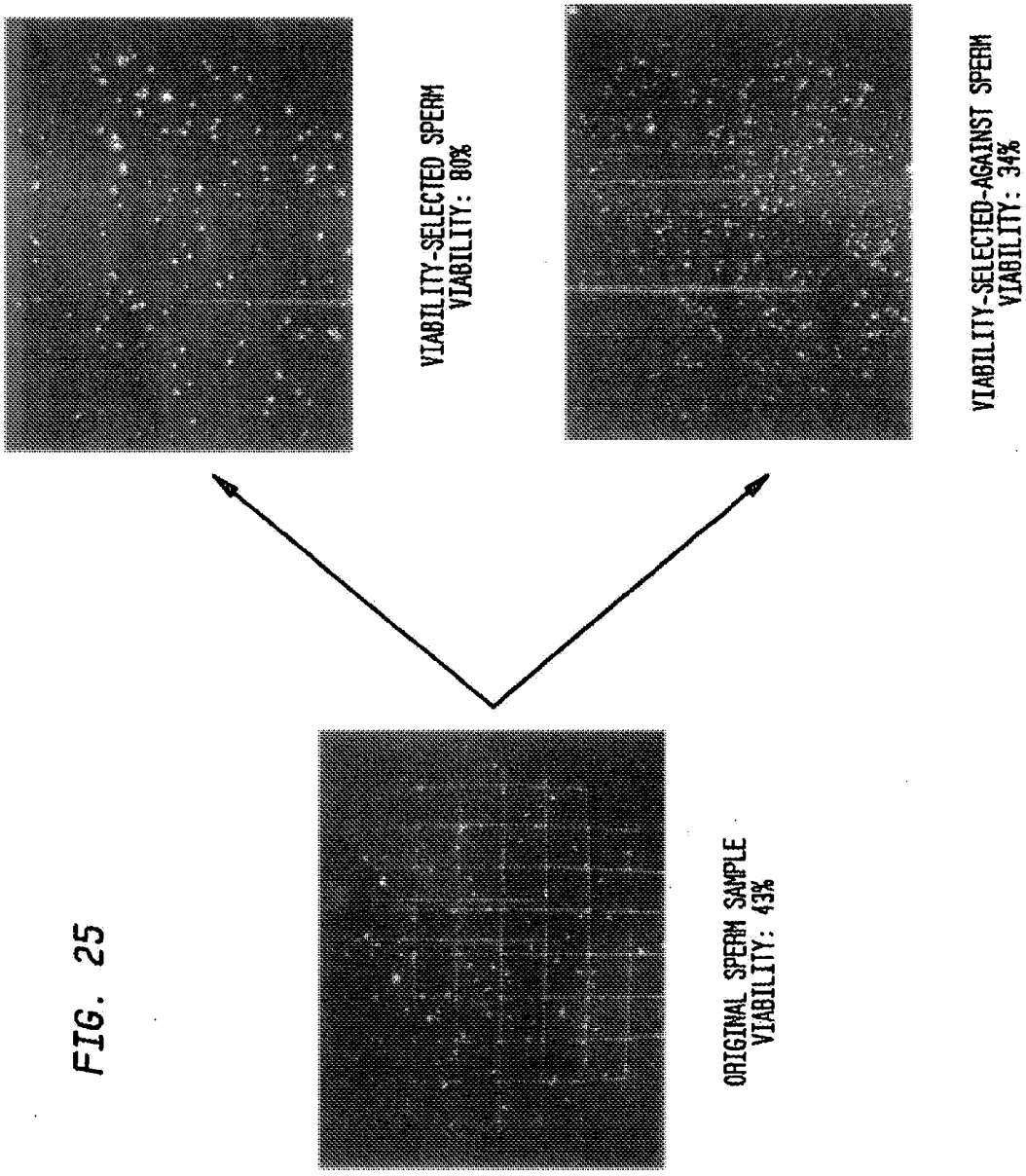
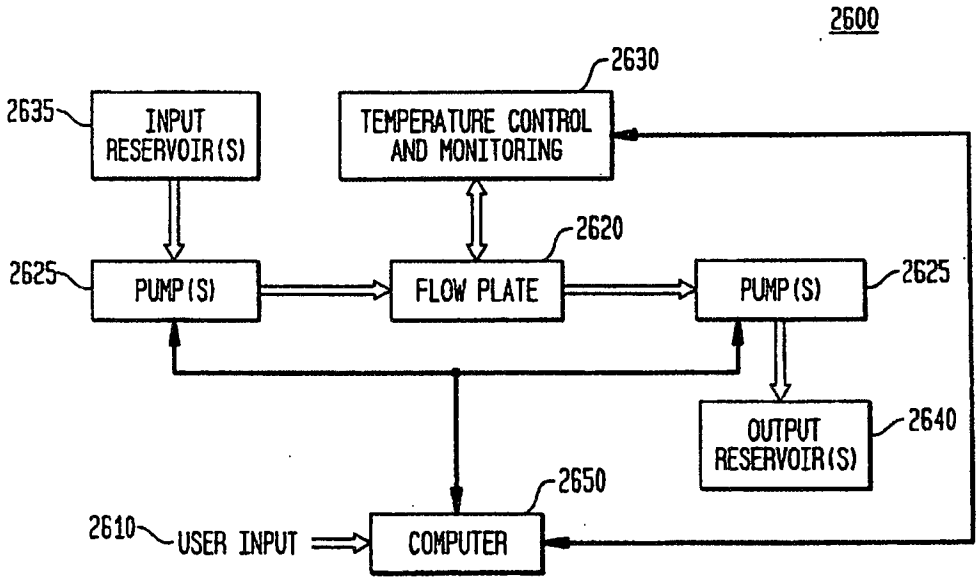

VIABILITY-SELECTED SPERM
VIABILITY: 80%
VIABILITY-SELECTED-AGAINST SPERM
VIABILITY: 34%
ORIGINAL SPERM SAMPLE
VIABILITY: 43%

FIG. 25

FIG. 26


2600
2630
2635
INPUT RESERVOIR(S)
TEMPERATURE CONTROL AND MONITORING
2620
2625
PUMP(S)
FLOW PLATE
PUMP(S)
2625
OUTPUT RESERVOIR(S)
2640
2650
2610
USER INPUT
COMPUTER

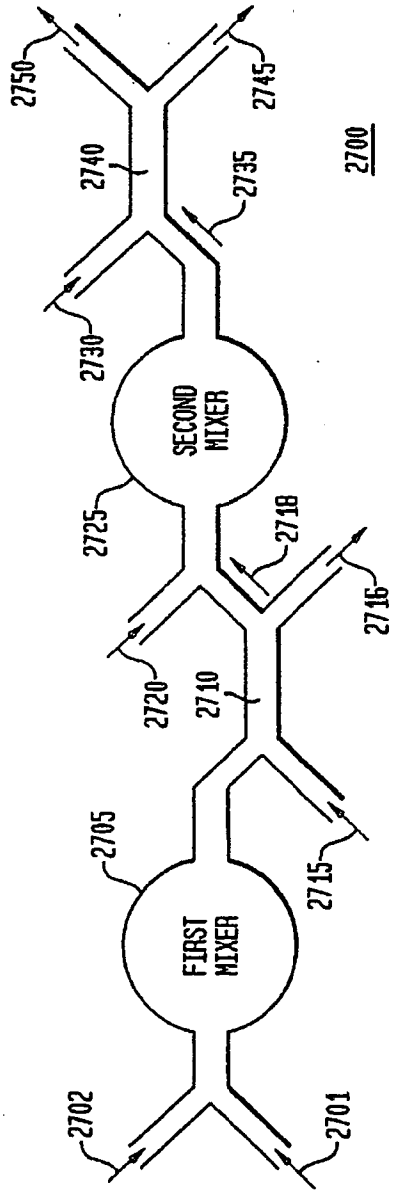

2750
2745
2740
2735
2700
2730
SECOND MIXER
2725
2718
2716
2720
2710
2705
2715
FIRST MIXER
2702
2701

FIG. 27

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	
Filing Date	2014-01-31
First Named Inventor	Daniel MUETH
Art Unit	
Examiner Name	
Attorney Docket Number	81527.0018

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	3390449		1968-07-02	R.J. Fox	
	2	3649829		1972-03-14	Randolph	
	3	4325706		1982-04-20	Gershman et al.	
	4	4409106		1983-10-11	Furuta et al.	
	5	4424132		1984-01-03	Iriguchi	
	6	4660971		1987-04-28	Sage et al.	
	7	4667830		1987-05-26	Nozaki, Jr. et al.	
	8	5007732		1991-04-16	Ohki et al.	

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	First Named Inventor	Daniel MUETH
	Art Unit	
	Examiner Name	
	Attorney Docket Number	81527.0018

	9	5100627		1992-03-31	Buican et al.	
	10	5180065		1993-01-19	Touge et al.	
	11	5194909		1993-03-16	Tycko	
	12	5229297		1993-07-20	Schnipelsky et al.	
	13	5483469		1996-01-09	Van den Engh et al.	
	14	5620857		1997-04-15	Weetall et al.	
	15	5674743		1997-10-07	Ulmer	
	16	5800690		1998-09-01	Chow et al.	
	17	5837115		1998-11-17	Austin et al.	
	18	5849178		1998-12-15	Holm et al.	
	19	5879625		1999-03-09	Roslaniec et al.	

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	First Named Inventor	Daniel MUETH
	Art Unit	
	Examiner Name	
	Attorney Docket Number	81527.0018

	20	5966457		1999-10-12	Lemelson	
	21	6053856		2000-04-25	Hlavinka	
	22	6071442		2000-06-06	Dean et al.	
	23	6185664		2001-02-06	Jeddeloh	
	24	6368871		2002-04-09	Christel et al.	
	25	6416190		2002-07-09	Grier et al.	
	26	6432630		2002-08-13	Blankenstein	
	27	6451264		2002-09-17	Bhullar et al.	
	28	6506609		2003-01-14	Wada et al.	
	29	6524860		2003-02-25	Seidel et al.	
	30	6637463		2003-10-28	Lei et al.	

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	Application Number	
	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	
	Examiner Name	
	Attorney Docket Number	81527.0018

	31	6727451		2004-04-27	Fuhr et al.	
	32	6833542		2004-12-21	Wang et al.	
	33	6838056		2005-01-04	Foster	
	34	6944324		2005-09-13	Tran et al.	
	35	7029430		2006-04-18	Hlavinka et al.	
	36	7241988		2007-07-10	Gruber et al.	
	37	7482577		2009-01-27	Gruber et al.	
	38	H0001960		2001-06-05	Conrad et al.	
	39	7472794		2009-01-06	Oakey et al.	

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	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	
	Examiner Name	
	Attorney Docket Number	81527.0018

	1	20020058332		2002-05-16	Quake et al.	
	2	20020176069		2002-11-28	Hansen et al.	
	3	20030032204		2003-02-13	Walt et al.	
	4	20030047676		2003-03-13	Grier et al.	
	5	20030186426		2003-10-02	Brewer et al.	
	6	20050061962		2005-03-24	Mueth et al.	
	7	20050121604		2005-06-09	Mueth et al.	
	8	20060058167		2006-03-16	Regusa et al.	
	9	20060152707		2006-07-13	Kanda	

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	Application Number	
	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	
	Examiner Name	
	Attorney Docket Number	81527.0018

	1	99/39223	WO	A1	1999-08-05	Arch Development Corp.		☒
	2	2798557	FR	A1	2001-03-23	Christine Nicolino	Abstract	☒
	3	19952322	DE	A1	2001-05-17	Evotec Biosystems AG	Abstract	☒
	4	2004/012133	WO	A2	2004-02-05	Arryx, Inc.		☒
	5	57-131451	JP	A	1982-08-14	Asahi Kasei Kogyo KK		☒
	6	0057907	EP	A1	1982-08-18	Asahi Kasei Kogyo KK		☒
	7	58-090513	JP	A	1983-05-30	Asahi Chem Ind Co Ltd	Abstract	☒
	8	2005-502482	JP	A	2005-01-27	University of Chicago		☒
	9	07-024309	JP		1995-01-27	Canon Inc	Abstract	☒
	10	2002-153260	JP		2002-05-28	Japan Science & Tech		☒
	11	01/18400	WO	A1	2001-03-15	Alup-Kompressoren		☒

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	Application Number	
	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	
	Examiner Name	
	Attorney Docket Number	81527.0018

	12	06-327494	JP		1994-11-29	Rikagaku Kenkyusho		☒
	13	0679325	EP	A1	1995-11-02	Shutze Raimund		☒

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	1	HORI M. et al., "Cell fusion by optical trapping with laser - involves contacting different cells...", WPI/Thomson, December 27, 1991, Abstract	☒
	2	S. TAKAYAMA et al., "Patterning cells and their environments using multiple laminar fluid flows...", Proc. Natl. Acad. Sci. USA, May 1999, pgs. 5545-5548, Vol. 96	☒
	3	PAUL O.P. TS'O, "Basic Principles in Nucleic Acid Chemistry", National Library of Medicine, 1974, pgs. 311-387, Academic Press Inc., New York, NY	☐
	4	STEPHEN P. SMITH et al., "Inexpensive Optical Tweezers for Undergraduate Laboratories, Am. J. Phys., January 1999, Vol. 67	☐

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	Application Number	
	Filing Date	2014-01-31
	First Named Inventor	Daniel MUETH
	Art Unit	
	Examiner Name	
	Attorney Docket Number	81527.0018

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☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Jean C. Edwards/	Date (YYYY-MM-DD)	2014-01-31
Name/Print	Jean C. Edwards, Esq.	Registration Number	41728

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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