8-13-07

IFW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentees:	Rabbani *et al.*
U.S. Patent No.:	7,064,197 B1
Issued:	June 20, 2006
Serial No.:	08/486,070
Filed:	June 7, 1995
For:	**SYSTEM, ARRAY AND NON-POROUS SOLID SUPPORT COMPRISING FIXED OR IMMOBILIZED NUCLEIC ACIDS**

Group Art Unit: 1631

Primary Examiner: John S. Brusca

527 Madison Avenue, 9th Floor
New York, NY 10022-4304
August 10, 2007

FILED VIA EXPRESS MAIL
-- RETURN RECEIPT REQUESTED

Mail Stop -- Box Reconstruction
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Attention: **Deborah Dotson (Telephone 571-272-0520)**
Technology Center 1600

COMMUNICATION ACCOMPANYING PATENTEES' SUBMISSION OF CERTAIN MISSING DOCUMENTS FROM U.S. PATENT APPLICATION SERIAL NO. 08/486,070, FILED JUNE 7, 1995

Dear Sirs:

This Communication accompanies Patentees' submission of certain missing documents from the file history of U.S. Patent Application Serial No. 08/486,070, filed on June 7, 1995. Patentees' undersigned attorney was informed by a third party that these documents were missing. No Notice Under 37 CFR 1.251 or other formal or informal inquiry is believed to have been issued or made with respect to these missing documents.

EXPRESS MAIL CERTIFICATE
"Express Mail" Label No. EM008701605US Deposit Date AUGUST 10, 2007 I hereby certify that this paper (Communication Accompanying Patentees' Submission Of Certain Missing Documents From U.S. Patent Application Serial No. 08/486, Filed June 7, 1995) and the attachments herein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Box Reconstruction, P. O. Box 1450, Alexandria, VA 22313-1450. Ronald C. Fedus Reg. No. 32,567 AUG 10 2007 Date

REMARKS

Earlier this year it came to the undersigned's attention from a third party that several documents appeared to be missing from the USPTO's file wrapper for U.S. Patent Application Serial No. 08/486,070 ("the '070 Application), filed on June 7, 1995, the immediate application from which U.S. Patent No. 7,064,197 B1 ("the '197 Patent) issued on June 20, 2006. In three instances, the exhibits to filed declarations or a communication appeared to be missing. In three other instances, the entire declaration or information disclosure statements appeared to be missing.

According to the third party, the following documents were missing from the USPTO's file wrapper for the '070 Application:

1. Exhibits 5-10 to May 8, 2001 Declaration Of Dr. Cheryl H. Agris, Attorney At Law (In Support Of The Written Description Of The Invention Claimed In U.S. Patent Application Serial No. 08/486,070);

2. Exhibits 1-4 to May 30, 2001 Communication For Transmitting Charts In Support Of Applicant's Invention Claimed In U.S. Patent Application Serial No. 08/486,070;

3. June 17, 2002 Declaration Of Dr. Jannis G. Stavrianopoulos with Exhibits 1-26;

4. June 28, 2002 Supplemental Information Disclosure Statement Under 37 C.F.R. §§1.56 & 1.97-1.98 with Exhibits 1-16;

5. July 30, 2002 Second Supplemental Information Disclosure Statement Under 37 C.F.R. §§1.56 & 1.97-1.98 with Exhibits 1-28; and

6. Eight (8) Charts attached to December 31, 2002 Communication For Submitting Eight Charts In Support Of Applicant's Invention Claimed In U.S. Patent Application Serial No. 08/486,070.

Upon receiving this information from the third party, the undersigned immediately reviewed the contents of the instant Assignee's (Enzo Life Sciences, Inc.) files to determine whether the above-listed documents were present. These documents were found to be present in the Assignee's '070 Application file.

Before preparing this Communication and submitting the above-listed documents, however, the undersigned contacted Landon-IP, Inc. of Alexandria, Virginia, to request that they undertake to examine the contents of the '070 Application file maintained by the USPTO. Landon-IP later informed the undersigned that an inspection of the publicly available papers for the file history revealed that the requested papers could not be located. Landon-IP suggested, however, that the next option would be to order an electronic copy of the entire file with cited references on CD-Rom, and to conduct a further examination. According

to Landon-IP, this option would cover the situation where the missing papers may have been incorrectly scanned in the non-patent literature. The undersigned proceeded to order the electronic copy of the entire file for the '070 Application with cited references on CD-Rom. A short time later, Landon-IP confirmed with the undersigned that the requested papers (above-listed documents) are missing from the official USPTO copy of the file history for the '197 Patent.

Having thus confirmed that the above-listed documents are missing from the '070 Application and '197 Patent file, the undersigned proceeded to check the Code of Federal Regulations (notably 37 CFR 1.251 Unlocatable File), the Manual of Patent Examining Procedure (MPEP) §508.04 (MPEP Pages 500-32 through 500-34), and the USPTO website for information on handling the matter of incomplete file wrapper papers, but not a complete file wrapper. In the MPEP (page 500-34, left column, last paragraph, through right column, first paragraph), it states:

> 37 CFR 1.251 generally applies only to situations in which the file of an application or patent (not just certain documents) is unlocatable. When a document is missing from an application, Office practice is to call the applicant's representative and request submission (generally by facsimile) of a copy of the missing document. While the Office will generally treat missing documents in this relatively informal manner (rather than issuing a notice under 37 CFR 1.251), the Office may issue a notice under 37 CFR 1.251 to obtain a copy of a missing document if the Office's informal attempts to obtain a copy of the document are unsuccessful. The notice under 37 CFR 1.251 will include a printout of the contents entries from the Office's PALM system.

The undersigned also checked the Public PAIR for the '070 Application and the '197 Patent to determine whether a Notice Under 37 CFR 1.251 had been recently issued. No such notice was listed.

Lastly, before filing this Communication and submitting the above-listed documents, the undersigned also attempted to obtain information by contacting the USPTO at various numbers, including the Reconstruction Section (Tel. Nos. 571-

272-3150 and 571-272-1000) and Document Services (an option from the menu for Tel. No. 571-272-3150). A call was made on August 9, 2007 to Deborah Dotson, Technology Center 1600 (Telephone 571-272-0520) who suggested that the missing documents could be sent to her attention for proper scanning into the USPTO's system.[1] The undersigned acknowledges with appreciation the time and courtesy extended by Ms. Dotson in handling his inquiry about these missing documents.

Accordingly, the above-listed documents are attached as exhibits to this Communication as follows:

1. Exhibits 5-10 to May 8, 2001 Declaration Of Dr. Cheryl H. Agris, Attorney At Law (In Support Of The Written Description Of The Invention Claimed In U.S. Patent Application Serial No. 08/486,070) **[Exhibit 1]**;

2. Exhibits 1-4 to May 30, 2001 Communication For Transmitting Charts In Support Of Applicant's Invention Claimed In U.S. Patent Application Serial No. 08/486,070 **[Exhibit 2]**;

3. June 17, 2002 Declaration Of Dr. Jannis G. Stavrianopoulos with Exhibits 1-26 **[Exhibit 3]**;

4. June 28, 2002 Supplemental Information Disclosure Statement Under 37 C.F.R. §§1.56 & 1.97-1.98 with Exhibits 1-16 **[Exhibit 4]**;

5. July 30, 2002 Second Supplemental Information Disclosure Statement Under 37 C.F.R. §§1.56 & 1.97-1.98 with Exhibits 1-28 **[Exhibit 5]**; and

6. Eight (8) Charts attached to December 31, 2002 Communication For Submitting Eight Charts In Support Of Applicant's Invention Claimed In U.S. Patent Application Serial No. 08/486,070 **[Exhibit 6]**.

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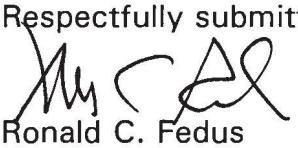
[1] In responding to a November 18, 2005 Office Communication initiating reconstruction pursuant to 37 C.F.R. §1.251 of U.S. Patent Application Serial No. 06/461,469, filed on January 27, 1983 (the first-filed application in the family leading to the '070 Application), the undersigned filed a Communication on December 8, 2005 directed to the attention of Deborah Dotson, Technology Center 1600.

Including the cover sheets (but not the tabs) for each of the six above-listed exhibits, a total of 880 pages are being submitted herewith to complete the USPTO's file history for the '070 Application.

No fee or fees are believed to be due in connection with this Communication. In the event that any fee or fees are due, however, The Patent and Trademark Office is hereby authorized to charge the amount of any such fee(s) to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney requests that he be contacted at the number provided below.

Respectfully submitted,



Ronald C. Fedus
Registration No. 32,567
Attorney for Patentees

ENZO LIFE SCIENCES, INC.
c/o ENZO BIOCHEM, INC.
527 Madison Avenue, 9th Floor
New York, NY 10022-4304
Telephone: (212) 583-0100
Facsimile: (212) 583-0150

Enz-7(P2)

U.S. PAT. NO. 7,064,197
(USSN 08/486,070)

EXHIBIT 6 TO AUGUST 10, 2007 COMMUNICATION

MISSING F/W ITEM NO. 6

EIGHT (8) CHARTS ATTACHED TO DECEMBER 31, 2002 COMMUNICATION FOR SUBMITTING EIGHT CHARTS IN SUPPORT OF APPLICANT'S INVENTION CLAIMED IN U.S. PATENT APPLICATION SERIAL NO. 08/486,070

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Stavrianopoulos et al.
Serial No.:	08/486,070
Filed:	June 7, 1995
For:	ARRAYS AND SYSTEMS COMPRISING ARRAYS FOR GENETIC ANALYSES AND OTHER APPLICATIONS (AS PREVIOUSLY AMENDED)

Group Art Unit: 1631

Ex'r: Ardin H. Marschel, Ph.D.

527 Madison Avenue (9th Floor)
New York, New York 10022-4304
December 31, 2002

FILED BY EXPRESS MAIL

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

COMMUNICATION FOR SUBMITTING EIGHT CHARTS IN SUPPORT OF APPLICANT'S INVENTION CLAIMED IN U.S. PATENT APPLICATION SERIAL NO. 08/486,070

Dear Sirs:

This Communication follows the PTO interview held on December 17, 2002 in connection with the above-identified application. This Communication also follows Applicants' December 6, 2002 Communication and their December 3, 2002 Amendment Under 37 C.F.R. §1.115. No communication or office action is outstanding in this application. Thus, no extension request or fee is believed due in connection with this Communication which is being timely filed.

Enz-7(P)(C3)

EXPRESS MAIL CERTIFICATE

"Express Mail" Label No. EV205342237US
Deposit Date December 31, 2002
I hereby certify that this paper and the attachments herein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington DC 20231.

Ronald C. Fedus
Reg. No. 32,567

DEC 31 2002
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Stavrianopoulos et al.

Serial No. 08/486,070 Group Art Unit: 1631

Filed: June 7, 1995 Examiner: Ardin H. Marschel, Ph.D.

Title: **ARRAYS AND SYSTEMS COMPRISING ARRAYS FOR GENETIC ANALYSES AND OTHER APPLICATIONS**
(As Previously Amended)

FILED BY EXPRESS MAIL

Honorable Commissioner of
Patents and Trademarks
Washington, D. C. 20231

Sir:

Transmitted herewith is a Communication For Submitting Eight Charts In Support Of Applicants' Invention Claimed In U.S. Patent Application Serial No. 08/486,070.

The fee has been calculated as shown below:

	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE	ADDITIONAL FEE
Total	585	Minus	1660	= 0	X $ 18	$ 0
Indep	6	Minus	11	= 0	X $ 80	$ 0
( )	First Presentation of Multiple Dependent Claims				+$270	$ 0
	TOTAL ADDITIONAL FEE					$ 0

( ) Charge Deposit Account No. 05-1135 in the amount of $_____.

( ) A check in the amount of $_____ is attached.

(X) The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 05-1135: any filing fees under 37 C.F.R. §1.16 for the presentation of extra claims and any patent application processing fees under 37 C.F.R. §1.17.

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"Express Mail" Label No. EV205342237US
Deposit Date December 31, 2002
I hereby certify that this paper and the attachments herein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington DC 20231.

Ronald C. Fedus
Reg. No. 32,567

DEC 31 2002
Date

Copies are being provided in triplicate.

Also enclosed: _____

Respectfully submitted,

December 31, 2002
Date

Ronald C. Fedus
Registration No. 32,567
Attorney for Applicants

ENZO LIFE SCIENCES, INC.
(formerly named Enzo Diagnostics, Inc.)
c/o Enzo Biochem, Inc.
527 Madison Avenue (9th Fl.)
New York, New York 10022
Tel. (212) 583-0100
Attorney's Docket No.: Enz-7(P)(C3)

REMARKS

Claims 1576-2160 as amended previously by Applicants' December 3, 2002 Amendment Under 37 C.F.R. §1.115 are still pending and remain under examination in this application. No claim changes have been effected by this Communication.

As indicated by the title, the purpose of this Communication is to submit eight charts in support of Applicants' array invention that is claimed in the present application. These eight charts are attached as Exhibits 1-8, respectively.

With minor changes, Charts 1-4 (Exhibits 1-4) correspond to the charts presented in Applicants' May 30, 2000 Communication.

In Charts 5 and 6 (Exhibits 5 and 6), Applicants have illustrated the various embodiments of non-porous supports or substrates disclosed in the specification. Such non-porous supports or substrates fall into flat surfaces and curved surfaces. Chart 6 (Exhibit 6) contains citations to the specification for each listed embodiment.

Charts 7 and 8 (Exhibits 7 and 8) are also new. Chart 7 (Exhibit 7) illustrates the reactive groups or binding sites in Applicants' claimed array invention. Such reactive groups or binding sites can take at least three forms: amines, epoxides and hydroxyls. Also listed in Chart 7 (Exhibit 7) are examples of such amines, epoxides and hydroxyls.

Chart 8 (Exhibit 8) is a Venn diagram illustrating the relationship between treated plastic and treated glass as disclosed in the specification. As shown in the Venn diagram, certain treatments are specific to plastic and others are specific to glass. In the case of two treatments, epoxy glue or solution, and ammonium

acetate (NH_4OAc), these are applicable to both plastic and glass, as shown in the intersecting portion of the two circles in the Venn diagram of Chart 8 (Exhibit 8).

Applicants respectfully request that full consideration be given to each of the eight charts (Exhibits 1-8) that are being submitted in support of Applicants' claimed array invention.

Favorable action is respectfully requested.

* * * * * * *

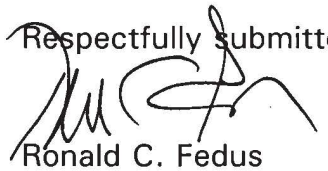
SUMMARY AND CONCLUSIONS

Claims 1576-2160 continue to be presented for further examination in this application. No changes to the claims have been effected by this Communication, the purpose of which is to present eight charts bearing on the written description of Applicants' claimed array subject matter.

No fee or fees are believed to be due in connection with this Communication. In the event that any other fee or fees are due, however, authorization is hereby given to charge the amount of any such fee(s) to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,



Ronald C. Fedus
Registration No. 32,567
Attorney for Applicants

ENZO LIFE SCIENCES, INC.
(formerly named Enzo Diagnostics, Inc.)
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New York, New York 10022
Telephone: (212) 583-0100
Facsimile: (212) 583-0150

Enz-7(P)(C3)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Stavrianopoulos et al.

Serial No. 08/486,070 Group Art Unit: 1631

Filed: June 7, 1995 Examiner: Ardin H. Marschel, Ph.D.

Title: **ARRAYS AND SYSTEMS COMPRISING ARRAYS FOR GENETIC ANALYSES AND OTHER APPLICATIONS (As Previously Amended)**

FILED BY EXPRESS MAIL

Mail Stop -- Non-Fee Amendments
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith is a Supplemental Amendment To Applicants' December 3, 2002 Amendment Under 37 C.F.R. §1.115 in the above-identified patent application.

The fee has been calculated as shown below:

	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE	ADDITIONAL FEE
Total	587	Minus	1660	= 0	X $ 18	$ 0
Indep	6	Minus	11	= 0	X $ 80	$ 0
( )	First Presentation of Multiple Dependent Claims				+$270	$ 0
	TOTAL ADDITIONAL FEE					$ 0

( ) Charge Deposit Account No. 05-1135 in the amount of $_____.
( ) A check in the amount of $_____ is attached.
(X) The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 05-1135: any filing fees under 37 C.F.R. §1.16 for the presentation of extra claims and any patent application processing fees under 37 C.F.R. §1.17.

Enz-7(P)(C3)

Copies are being provided in triplicate.
Also enclosed: _____

Respectfully submitted,

May 8, 2003
Date

Ronald C. Fedus
Registration No. 32,567
Attorney for Applicant(s)

ENZO LIFE SCIENCES, INC.
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Tel. (212) 583-0100
Attorney's Docket No.: Enz-7(P)(C3)

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Deposit Date May 8, 2003
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Ronald C. Fedus
Reg. No. 32,567

MAY 8 2003
Date

Enz-7(P)(C3)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Stavrianopoulos et al.	
Serial No.:	08/486,070	Group Art Unit: 1631
Filed:	June 7, 1995	Ex'r: Ardin H. Marschel, Ph.D.
For:	**ARRAYS AND SYSTEMS COMPRISING ARRAYS FOR GENETIC ANALYSES AND OTHER APPLICATIONS** (As Previously Amended)	

527 Madison Avenue (9th Floor)
New York, NY 10022-4304
May 8, 2003

FILED BY EXPRESS MAIL

MailStop -- Non-Fee Amendments
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL AMENDMENT TO APPLICANTS' DECEMBER 3, 2002 AMENDMENT UNDER 37 C.F.R. §1.115
(FOLLOWING THEIR DECEMBER 31, 2002 COMMUNICATION FOR SUBMITTING EIGHT CHARTS IN SUPPORT OF APPLICANTS' INVENTION CLAIMED IN U.S. PATENT APPLICATION SERIAL NO. 08/486,070)

Dear Sirs:

This is a supplemental response to Applicants' December 3, 2002 Amendment Under 37 C.F.R. §1.115, and it follows their December 21, 2002 Communication For Submitting Eight Charts In Support Of Applicants' Invention Claimed In U.S. Patent Application Serial No. 08/486,070. No deadline is believed to be pending for taking action in this case, a Request Under 37 C.F.R. §1.129(a) For Withdrawal Of The Finality Of The October 10, 2001 Office Action having been filed on November 8, 2001. Accordingly, no extension request or fee is believed due in connection with this response.

Enz-7(P)(C3)

EXPRESS MAIL CERTIFICATE

"Express Mail" Label No. EL634886271US
Deposit Date May 8, 2003
I hereby certify that this paper and the attachments herein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington DC 20231.

MAY 8 2003

Ronald C. Fedus
Reg. No. 32,567

Date

Enz-7(P)(C3)

AMEND THE ABOVE-IDENTIFIED APPLICATION AS FOLLOWS:

In The Claims:

Please enter replacement claims 1576 and 1670 as follows:

1576. (Twice Amended) An array comprising a non-porous substrate having surfaces, each surface comprising at least one double-stranded nucleic acid fixed or immobilized to one or more reactive groups or binding sites on said surface, wherein at least one nucleic acid strand or a sequence therefrom comprises one or more non-radioactive chemical labels which comprise a non-radioactive signaling moiety or moieties which are quantifiable or detectable, and wherein said non-porous substrate comprises siliceous matter or polymeric material.

1670. (Twice Amended) An array comprising a non-porous substrate having surfaces, each surface comprising at least one nucleic acid strand fixed or immobilized to one or more reactive groups or binding sites on said surface, and wherein said non-porous substrate comprises siliceous matter or polymeric material.

Add new claims 2161-2162 as follows:

-- 2161. (NEW) The array of claim 1576, wherein at least one nucleic acid strand or a sequence therefrom in one of said surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another surface. --

Enz-7(P)(C3)

-- 2162. (NEW) The array of claim 1670, wherein at least one nucleic acid strand or a sequence therefrom in one of said surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another surface. --

* * * * * * *

REMARKS

Reconsideration of this application is respectfully requested.

Claims 1576-2160 were previously pending in this application. Claims 1576 and 1670 have been amended and new claims 2161 and 2162 have been added. No claims have been canceled by this paper. Accordingly, as amended and added above, claims 1576-2162 are being presented for further examination in this application.

Claim Amendments

In a further sincere effort to define their invention more clearly, Applicants have entered new replacement claims 1576 and 1670, both of which are independent and are directed to their claimed array invention. As set forth above, replacement claim 1576 now recites "[a]n array comprising a non-porous substrate having surfaces, each surface comprising at least one double-stranded nucleic acid fixed or immobilized to one or more reactive groups or binding sites on said surface, wherein at least one nucleic acid strand or a sequence therefrom comprises one or more non-radioactive chemical labels which comprise a non-radioactive signaling moiety or moieties which are quantifiable or detectable, and wherein said non-porous substrate comprises siliceous matter or polymeric material." In the case of claim 1670, Applicants are now claiming "[a]n array comprising a non-porous substrate having surfaces, each surface comprising at least one nucleic acid strand fixed or immobilized to one or more reactive groups or binding sites on said surface, and wherein said non-porous substrate comprises siliceous matter or polymeric material." The changes to claims 1576 and 1670 are believed to better reflect the

patentable nature of Applicants' claimed array which, among other things, comprises a non-porous substrate comprising siliceous matter or polymeric material.[1]

A marked-up version of amended claims 1576 and 1670 is attached as Exhibit 1. This marked-up version is entitled "Marked-Up Version Of Amended Claims."

Reference to Applicants' claimed array subject matter taken in its historical context might be helpful to understanding the basis for the above amendments to claims 1576 and 1670. Array claims (143-182) were first added to this application by Applicants' July 21, 1998 Amendment Under 37 C.F.R. §1.115. Claim 143 was independent and recited "[a]n array of substrate surfaces, said array comprising a plurality of nucleic acid strands fixed or immobilized to said substrate surfaces." Later, in their May 18, 1999 Third Supplemental Amendment, Applicants canceled their array claims 143-182 in favor of new claims 325-376.[2] In this later set, claim 325 was independent and it recited "[a]n array of substrate surfaces, each substrate surface comprising at least one double-stranded nucleic

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[1] In effect, the following language has been deleted from claims 1576 and 1670:

. . . wherein at least one nucleic acid strand or a sequence therefrom in one of said surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another surface . . .

[2] Other array embodiments were included among claims 325-373. For example, claim 374 was directed to "[a] collection or set comprising the array of any of claims 325 to 373, wherein said substrate surface is porous or non-porous." Further, claim 375 was directed to "[a] transparent non-porous or translucent non-porous system capable of retaining or containing a fluid or solution, which system comprises the array of any of claims 325 to 373." Lastly, claim 376 depended from claim 375, and it recited "[t]he system of claim 375, wherein said substrate surfaces are contained within the transparent non-porous or translucent non-porous system."

acid fixed or immobilized thereto, wherein at least one strand comprises one or more chemical labels which comprises a signaling entity or entities which are quantifiable or detectable, and wherein at least one nucleic acid strand or a sequence therefrom in one of said substrate surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another substrate surface." The subject of these later array claims was listed as item no. 4 among the issues discussed at the March 23, 1999 interview (see page 53 in Applicants' May 18, 1999 Third Supplemental Amendment). The array claims were also discussed on the next page (54) in the same paper.

More recently, in Applicants' April 10, 2002 Amendment Under 37 C.F.R. §1.116, array claims 1266-1424 were added in which the claimed subject matter recited that "said [array substrate] surfaces comprise siliceous matter or polymeric material." In Applicants' June 17, 2002 Supplemental Amendment to their April 10, 2002 Amendment, array claims 1576-1761 were added in place of claims 1266-1424. Like their predecessor claims, array claims 1576-1761 were also limited to "siliceous matter or polymeric material." Unlike their predecessor claims, however, array claims 1576-1761 were further limited to "an array comprising a ***non-porous*** substrate having surfaces . . ."

In summary, since the first presentation of their array claims in July 1998, Applicants have seen fit to add two features to their claimed array subject matter. First, the claimed subject matter is now defined both in the preamble and in the body of the claim as "[a]n array comprising a <u>non-porous</u> substrate having surfaces." Second, the non-porous substrate in their claimed array subject matter is further defined as comprising <u>siliceous matter or polymeric material</u>.

It is believed that prior to the introduction of the presently claimed array subject matter, the recitation that "wherein at least one nucleic acid strand or a

sequence therefrom in one of said surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another surface" was helpful if not important to distinguish Applicants' array invention from the prior art. Having recently added the characteristics that the substrate is non-porous and that the non-porous substrate comprises siliceous matter or polymeric material, the previous recitation that one nucleic acid strand or sequence is different from another nucleic acid strand or sequence is no longer believed to be necessary to distinguish the present array invention over the prior art. Merely, by way of illustration, Grunstein and Hogness (1975) ["Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene," Proc. Natl. Acad. Sci. USA 72:3961-3965] and Benton and Davis (1977) ["Screening λgt Recombinant Clones by Hybridization to Single Plaques in situ," Science 196:180-182][3] are disclosures relating to screening methods for hybrid plasmids and single plaques of recombinant phage, respectively. In short, the Grunstein and Benton publications concern the use of replica plating onto porous membranes. Clearly, both publications are "pre-array" disclosures confined to the use of porous membranes. As noted above, Applicants' claimed arrays are directed to non-porous substrates comprising siliceous matter or polymeric material. Thus, their present array invention is distinguishable from such prior art.

New Claims

New claims 2161 and 2162 have been added above. Both new claims recite the language that was deleted from claims 1576 and 1670. Thus, both claims

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[3] Copies of Grunstein and Hogness (1975) and Benton and Davis (1977) are attached to this paper as Exhibits 2 and 3, respectively.

recite "wherein at least one nucleic acid strand or a sequence therefrom in one of said surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another surface."

Because language has been removed in two independent claims and is now recited in two dependent claims that have been added, it is believed that no issue of new matter is presented by these claim changes. Entry of these amendments and the two new claims is respectfully requested.

Early and favorable action is respectfully requested.

* * * * * * *

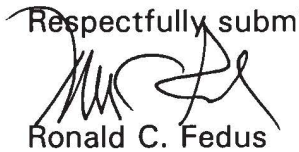
SUMMARY AND CONCLUSIONS

Claims 1576-2162 are presented for further examination. Of these, claims 1576 and 1670 have been amended and new claims 2161-2162 have been added. No claims have been canceled by this paper.

The claim fee for adding new claims 2161 and 2162 is $36. The Patent and Trademark Office is hereby authorized to charge the requisite $36 claim fee to Deposit Account No. 05-1135. No other fee or fees are believed due in connection with this Supplemental Amendment. In the event that any other fee or fees are due, however, The Patent and Trademark Office is hereby authorized to charge the amount of any such other fee(s) to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,



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Enz-7(P)(C3)

MARKED-UP VERSION OF AMENDED CLAIMS

1576. (Twice Amended) An array comprising a non-porous substrate having surfaces, each surface comprising at least one double-stranded nucleic acid fixed or immobilized to one or more reactive groups or binding sites on said surface, wherein at least one nucleic acid strand or a sequence therefrom comprises one or more non-radioactive chemical labels which comprise a non-radioactive signaling moiety or moieties which are quantifiable or detectable, [~~wherein at least one nucleic acid strand or a sequence therefrom in one of said surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another surface,~~] and wherein said non-porous substrate comprises siliceous matter or polymeric material.

1670. (Twice Amended) An array comprising a non-porous substrate having surfaces, each surface comprising at least one nucleic acid strand fixed or immobilized to one or more reactive groups or binding sites on said surface, [~~wherein at least one nucleic acid strand or a sequence therefrom in one of said surfaces is different from at least one other nucleic acid strand or a sequence therefrom in another surface,~~] and wherein said non-porous substrate comprises siliceous matter or polymeric material.

* * * * * * *

Enz-7(P)(C3)

ARTIFACT SHEET

Enter artifact number below. Artifact number is application number + artifact type code (see list below) + sequential letter (A, B, C ...). The first artifact folder for an artifact type receives the letter A, the second B, etc.. Examples: 59123456PA, 59123456PB, 59123456ZA, 59123456ZB

08486070 C.B

Indicate quantity of a single type of artifact received but not scanned. Create individual artifact folder/box and artifact number for each Artifact Type.

☐ CD(s) containing computer program listing
Doc Code: Computer Artifact Type Code: P

☒ Stapled Set(s) of Extra Color Drawings/Photographs
Doc Code: Artifact Artifact Type Code: C

☐ CD(s) containing pages of specification ☐
and/or sequence listing ☐
Doc Code: Artifact Artifact Type Code: S

☐ CD(s) with content unspecified
Doc Code: Artifact Artifact Type Code: U

☐ Microfilm(s)
Doc Code: Artifact Artifact Type Code: F

☐ Video tape(s)
Doc Code: Artifact Artifact Type Code: V

☐ Model(s)
Doc Code: Artifact Artifact Type Code: M

☐ Bound Document(s)
Doc Code: Artifact Artifact Type Code: B

☐ Other, description: _____
Doc Code: Artifact Artifact Type Code: Z

ARTIFACT SHEET

Enter artifact number below. Artifact number is application number + artifact type code (see list below) + sequential letter (A, B, C ...). The first artifact folder for an artifact type receives the letter A, the second B, etc..
Examples: 59123456PA, 59123456PB, 59123456ZA, 59123456ZB

08486070CA

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☒ Stapled Set(s) of Extra Color Drawings/Photographs
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☐ CD(s) containing pages of specification ☐ and/or sequence listing ☐
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☐ Model(s)
Doc Code: Artifact Artifact Type Code: M

☐ Bound Document(s)
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☐ Other, description: _____
Doc Code: Artifact Artifact Type Code: Z

Support within Specification for Claimed Genus "Array of Substrate Surfaces"

U.S. Pat. Appl. SN 08/486,070–Stavrianopoulos et al., filed Jun. 7, 1995 claiming priority of SN 06/461,469 (filed Jan. 21, 1983) & SN 06/732,374 (filed May 9, 1985)

SEPTEMBER 7, 2000 – OFFICE ACTION

Glass or plastic arrays having depressions or wells.

SUPPORT FOR "MEANS FOR CONTAINING A FLUID"

- Apparatus comprising **a plurality of devices for containing a fluid**, in which at least one such device contains the above-described immobilized polynucleotide sequence, polynucleotide or oligonucleotide probe, . . .
  Specification, page 14, lines 21-26
- Conventional apparatus employed in diagnostic laboratories, i.e., **plastic or glass wells, tubes, cuvettes** or **arrangements of wells, tubes or cuvettes**.
  Specification, page 13, last 2 lines, through page 14, 1st line
- The portion of the device for containing the fluid is desirably **a well, a tube, or a cuvette**.
  Specification, page 14, lines 19-20
- An apparatus comprising **a plurality of means for containing a fluid**, wherein at least one of said means comprises:
  (i) an immobilized polynucleotide sequence hybridized to a polynucleotide or oligonucleotide probe, . . .
  Specification, originally filed claim 23
- Said means for containing a fluid is selected from the group consisting of **a well, a tube, and a cuvette**.
  Specification, originally filed claim 21

SUPPORT FOR "SURFACES" IN EXAMPLES

Specification, pages 16-19 & 20-23

- Example 2 "...glass **surface**..."
- Example 3 "...glass **surface**..."
- Example 5 "...plastic **surface**..." or
- "...non-porous plastic **surface**..."
- Example 6 "...glass or polystyrene **surfaces**..."
- "...**surfaces or wells**."

COMPOSITE

Glass or plastic arrays having depressions or wells or **other surfaces** or being **a plurality of devices or means for containing a fluid** which are desirably **wells, tubes or cuvettes or an arrangement of wells, tubes or cuvettes.**

Claim reciting all of the species cited

Support within Specification for Claimed "Array Comprising A Non-Porous Substrate Having Surfaces"

U.S. Pat. Appl. SN 08/486,070–Stavrianopoulos et al., filed Jun. 7, 1995 claiming priority of SN 06/461,469 (filed Jan. 27, 1983) & SN 06/732,374 (filed May 9, 1985)

SEPTEMBER 7, 2000 & OCTOBER 10, 2001 OFFICE ACTIONS

Glass or plastic arrays having depressions or wells.

SUPPORT FOR "SURFACES" IN EXAMPLES

- Example 2 "...glass **surface**..."
- Example 3 "...glass **surface**..."
- Example 5 "...plastic **surface**..." or
- "...non-porous plastic **surface**..."
- Example 6 "...glass or polystyrene **surfaces**..."
- "...**surfaces or wells**."

Specification, pages 16-19 & 20-23

ARRAY CLAIMS 1576 & 1670

Array comprising a non-porous substrate having surfaces, . . . wherein said non-porous substrate comprises siliceous matter or polymeric material.

Array Claim Reciting Non-Porous Substrate

SUPPORT FOR NON-POROUS FLAT SURFACES

Because polystyrene various various batches or sources exhibits different binding capacities, the adherence or fixing of DNA to a polystyrene surface is improved . . . Previous experiments demonstrated that addition of duodecadiamine (DDA) to polystyrene resulted in an uniform binding coefficient of ***polystyrene plates*** of different batches. . .

Specification, Example 5, Page 20, Lines 23-31

In a further example of the method, denatured adenovirus 2 DNA, the analyte, was bound to ***polystyrene plates*** as described above. . .

Specification, Example 5, Page 21, Lines 16-18

In further tests, radioactively-labeled DNA was prepared by nick translation with [3H]dATP. The labelled, non-biotinylated denatured DNA [2000 ng to 5 ng] was applied to DDA-coated ***polystyrene plates***. . .

Specification, Example 6, Page 21, Last 4 Lines, Through Page 22, First 2 Lines

An improved capability for fixing or immobilization of DNA to ***non-porous siliceous solid supports***, such as ***glass and plastic***, is also provided by treatment with a coating of an epoxy resin. For example, treatment of ***glass or polystyrene surfaces*** with commercially available epoxy glues, such as a solution of epoxy glue in ethanol [1 percent w/v] serves this purpose. These epoxy solutions are applied to the ***surfaces*** or wells, and the solvent, ethanol, evaporated thereupon at a temperature of 37°C, thereby providing a polyamine polymeric coating on the treated surface. These surfaces were found to absorb ^{3}H-labeled DNA from aqueous solution at pH less than 9.5.

Specification, Example 6, Page 22, Last 4 Lines, Through Page 23, Line 10

SUPPORT FOR NON-POROUS CURVED SURFACES

Examples of devices useful in the spectrophotometric analysis of the signal include conventional apparatus employed in diagnostic laboratories, i.e., *plastic or glass wells, tubes, cuvettes or arrangements of wells, tubes or cuvettes.*

Specification, Page 13, Last 4 Lines, Through First Line On Page 14

porous glass [Weetal and Filbert (1974)]

Specification, Example 1, Page 15, Last Paragraph, Through Page 16, First Paragraph

One set of *tubes* is checked . . .
To another set of *tubes* is delivered . . .
To the third set of *tubes* is delivered . . .
. . . the *tubes* are incubated . . .
. . . one component of the signalling moiety gives a positive visible color reaction, upon reaction with its chromogen, only in *tubes* containing "probe" T4 DNA and bridging moiety, ConA, but was washed off from the *tubes* which contained only ConA or ConA and calf thymus DNA.

Specification, Example 2, Page 17, First Full Paragraph, Continuing Through First Two Paragraphs on Page 18

In test involving the fixing of DNA to a plastic surface, biotinylated DNA (b-DNA) was denatured and aliquoted into Dynatech, Immulon II™ removeable wells...

Specification, Example 5, Page 20, Last 2 Lines, Through Page 21, Line 2

In other tests, *polystyrene microfilter wells* were nitrated using the procedure of Filipsson and Hornby, Biochem. J. 120, 215 (1970). The polystyrene wells were immersed . . .

Specification, Example 6, Page 22, First Full Paragraph

. . . Amino-derivitized *polystyrene microfilter wells* lined with this solution were allowed to react at room temperature for 4 hours and then washed with distilled water. The resulting treated wells absorbed 3H-labeled DNA from aqueous solution at pH less than 9.5.

Specification, Example 6, Page 22, Penultimate Paragraph

Yet another example of the method of the present invention, including fixing the polynucleotide analyte sequence directly to a non-porous solid support, such as a *conventional microtiter well*, may be performed according to the procedures outlined below.
Conventional microtiter well plates can be pre-rinsed with 1M ammonium acetate (NH_4OAc), in an amount . . .

Specification, Example 7, Page 23, First Two Paragraphs

U.S. Patent Application SN 08/486,070 – Stavrianopoulos et. al., Filed June 7, 1995

Claiming Priority of: SN 06/461,469 (filed January 27, 1983) & SN 06/732,374 (filed May 9, 1985)

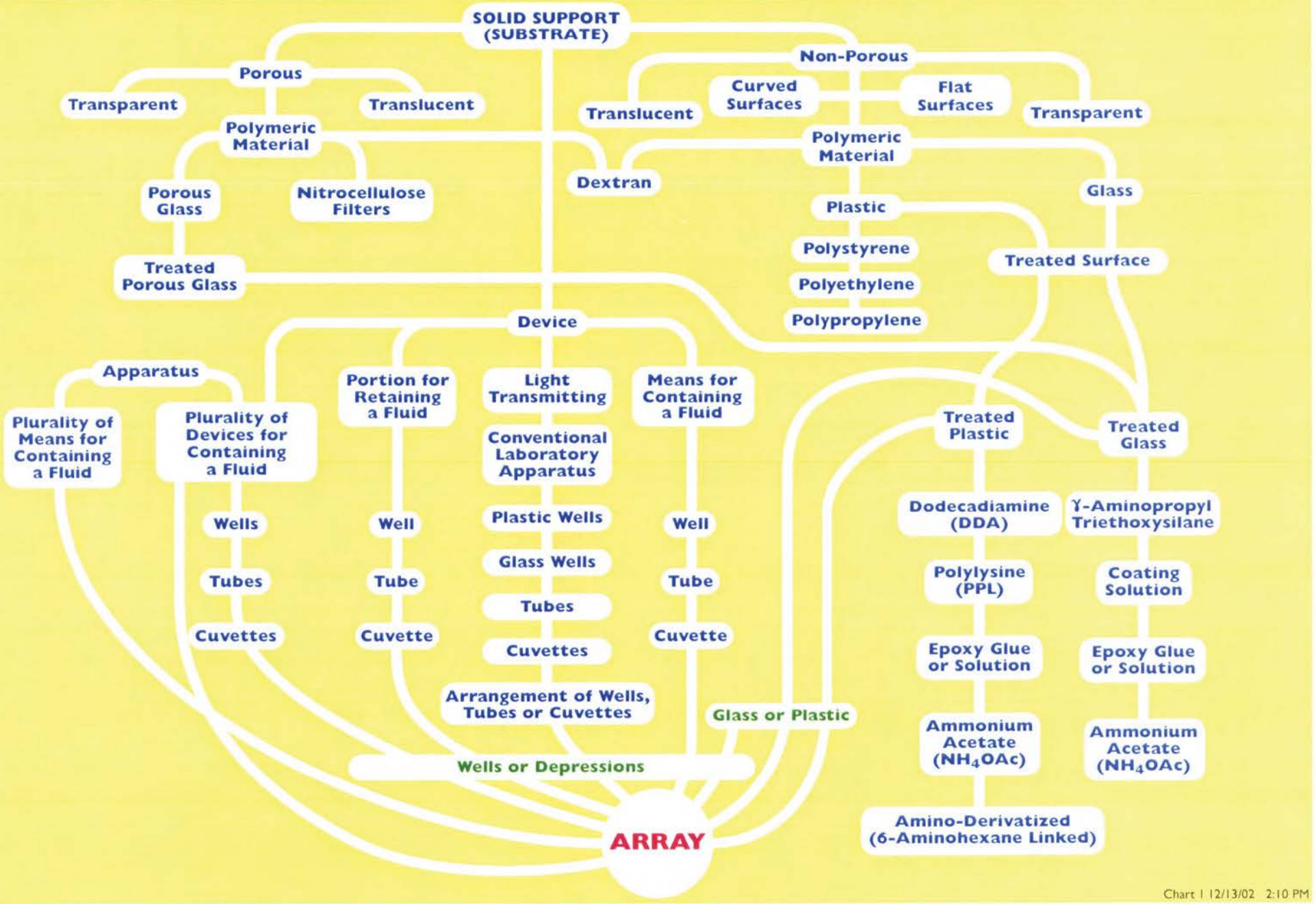
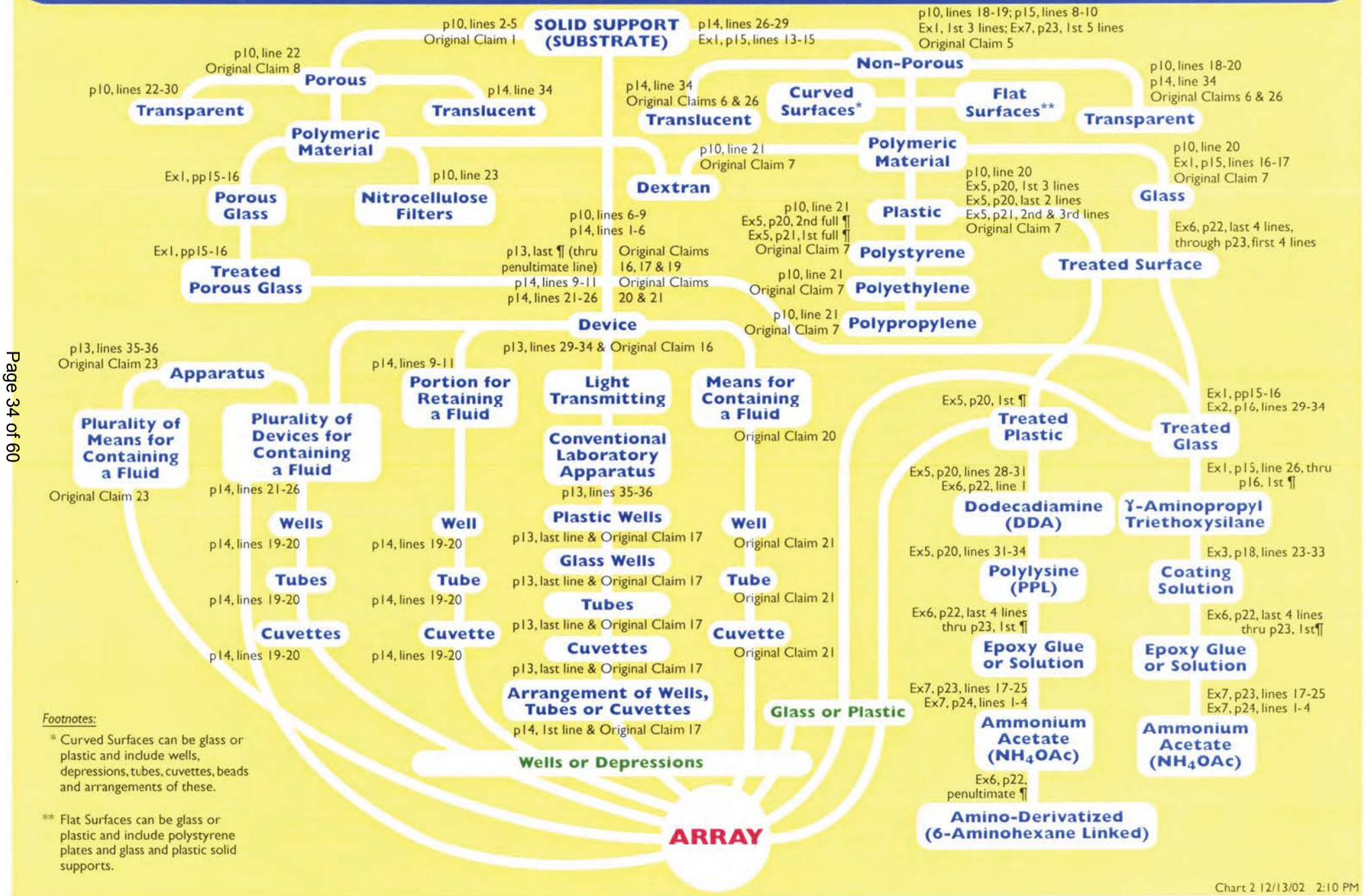


Chart 1 12/13/02 2:10 PM

U.S. Patent Application SN 08/486,070 – Stavrianopoulos et. al., Filed June 7, 1995

Claiming Priority of: SN 06/461,469 (filed January 27, 1983) & SN 06/732,374 (filed May 9, 1985)



Footnotes:

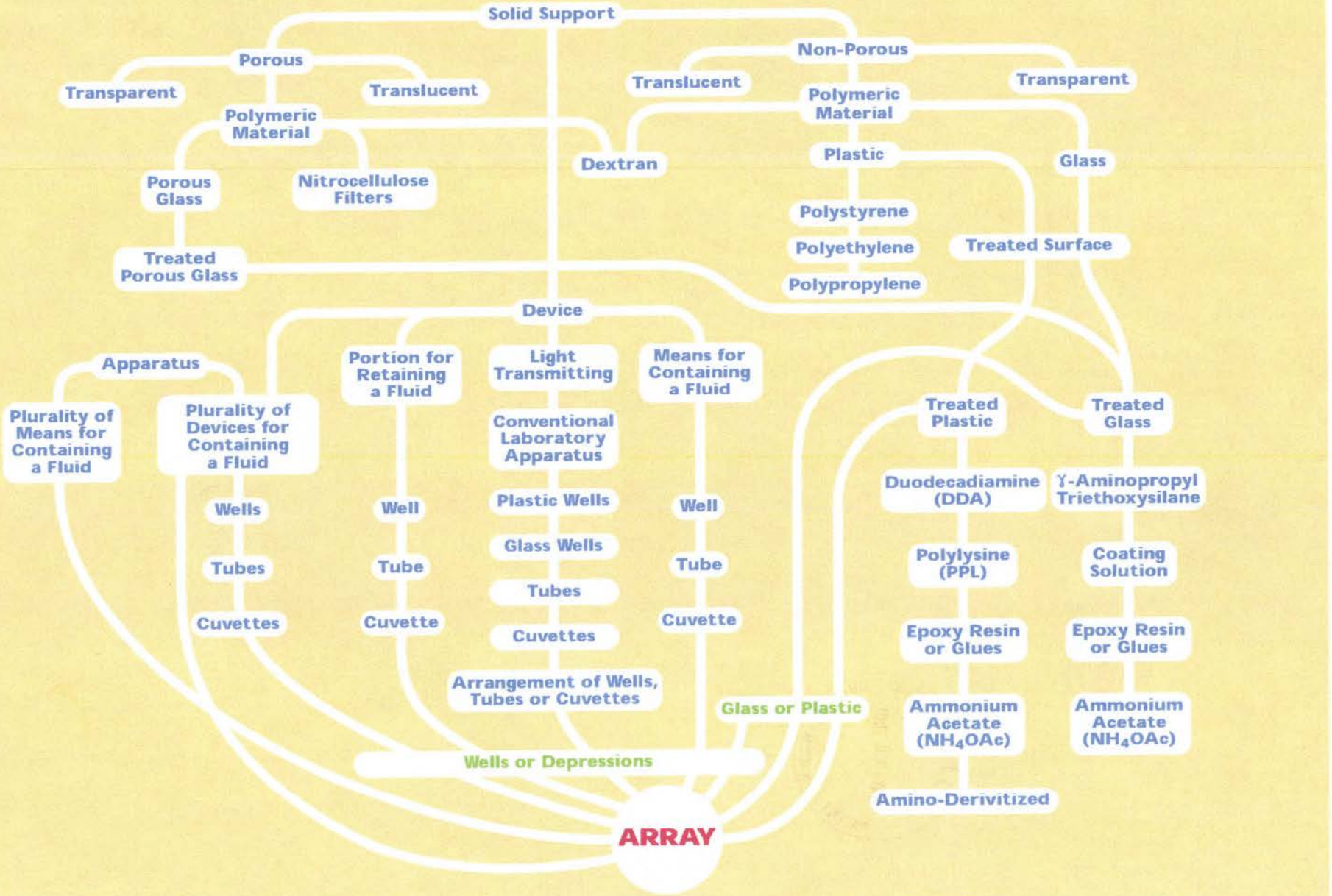
* Curved Surfaces can be glass or plastic and include wells, depressions, tubes, cuvettes, beads and arrangements of these.

** Flat Surfaces can be glass or plastic and include polystyrene plates and glass and plastic solid supports.

Chart 2 12/13/02 2:10 PM

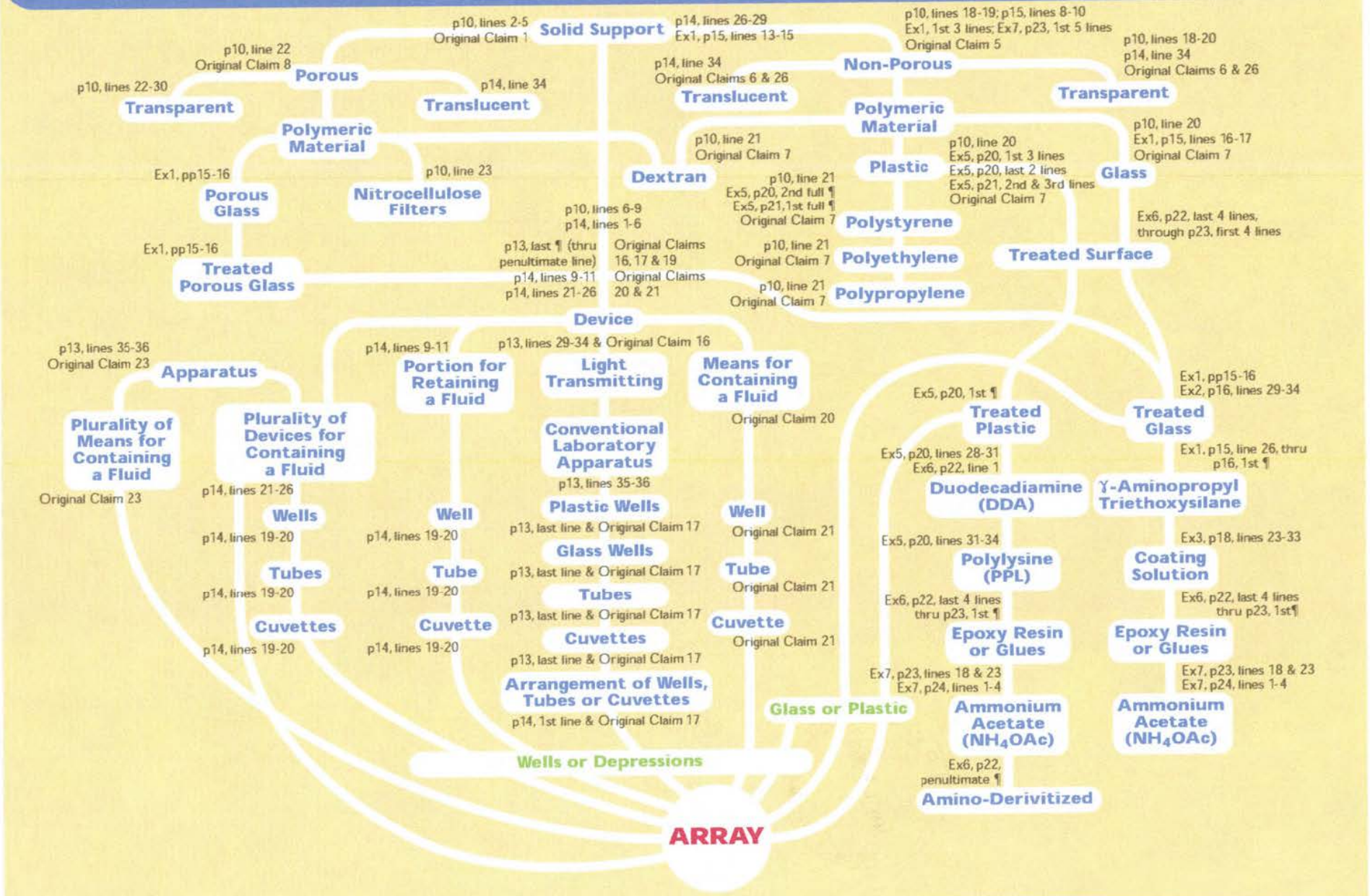
U.S. Patent Application SN 08/486,070 – Stavrianopoulos et. al., Filed June 7, 1995

Claiming Priority of: SN 06/461,469 (filed January 21, 1983) & SN 06/732,374 (filed May 9, 1985)



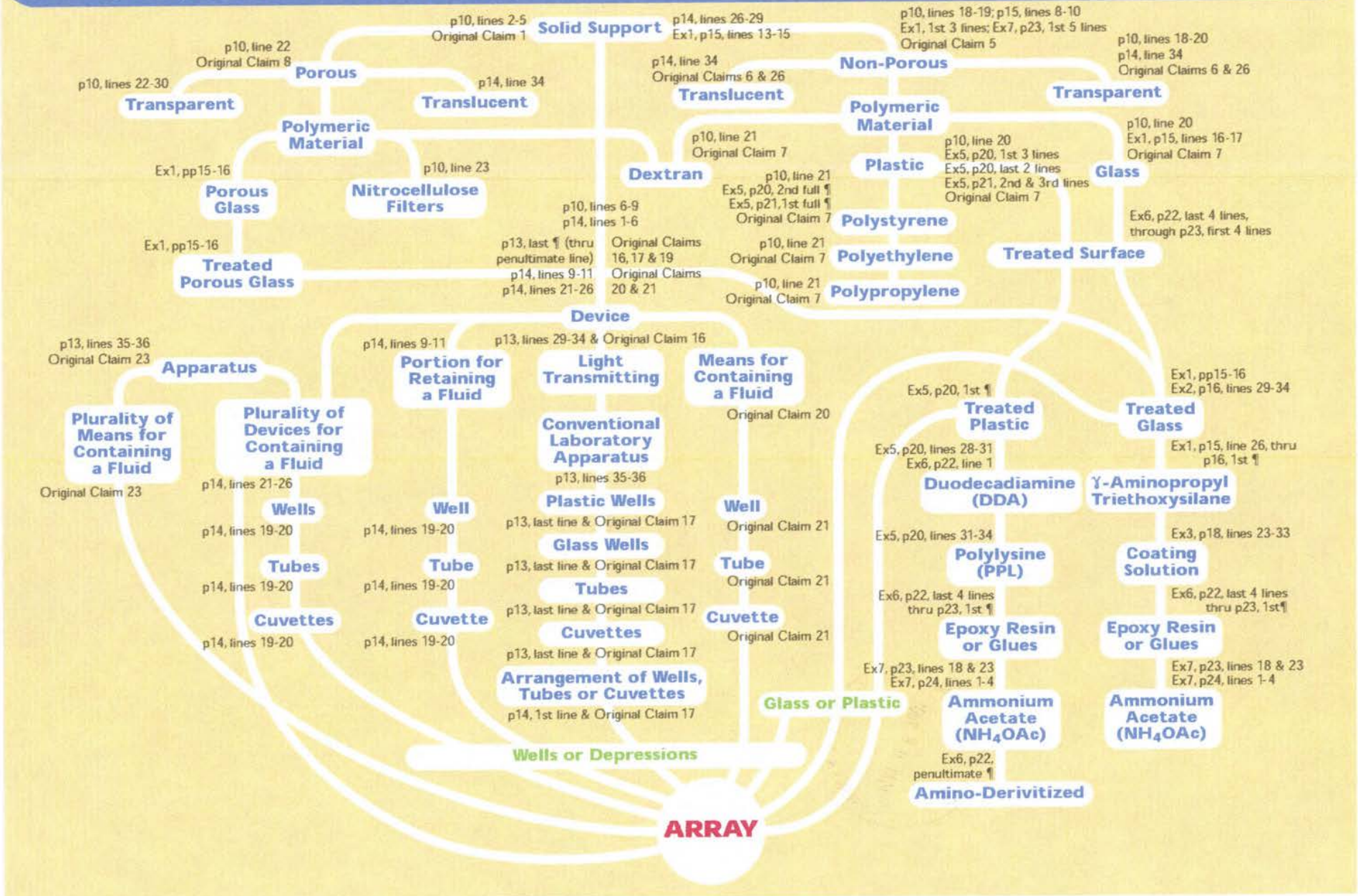
U.S. Patent Application SN 08/486,070 – Stavrianopoulos et. al., Filed June 7, 1995

Claiming Priority of: SN 06/461,469 (filed January 21, 1983) & SN 06/732,374 (filed May 9, 1985)



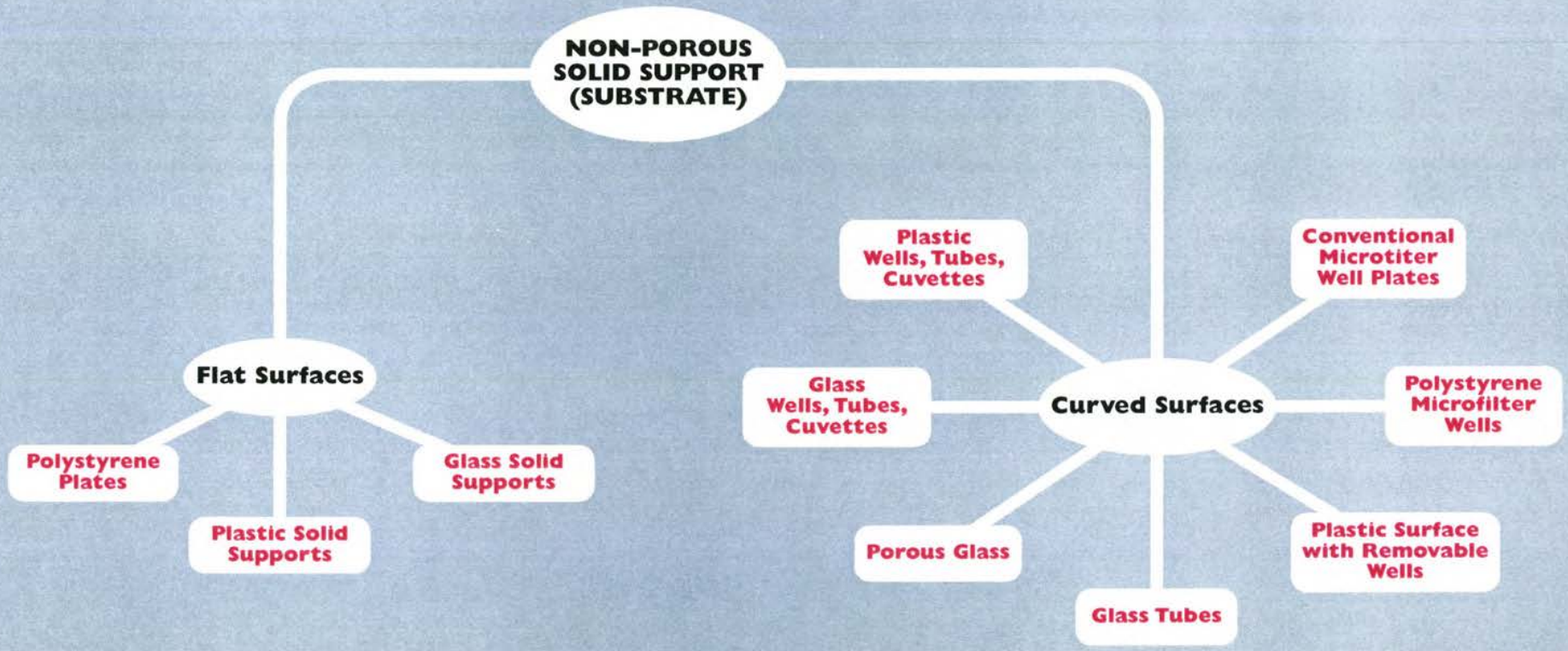
U.S. Patent Application SN 08/486,070 – Stavrianopoulos et. al., Filed June 7, 1995

Claiming Priority of: SN 06/461,469 (filed January 21, 1983) & SN 06/732,374 (filed May 9, 1985)



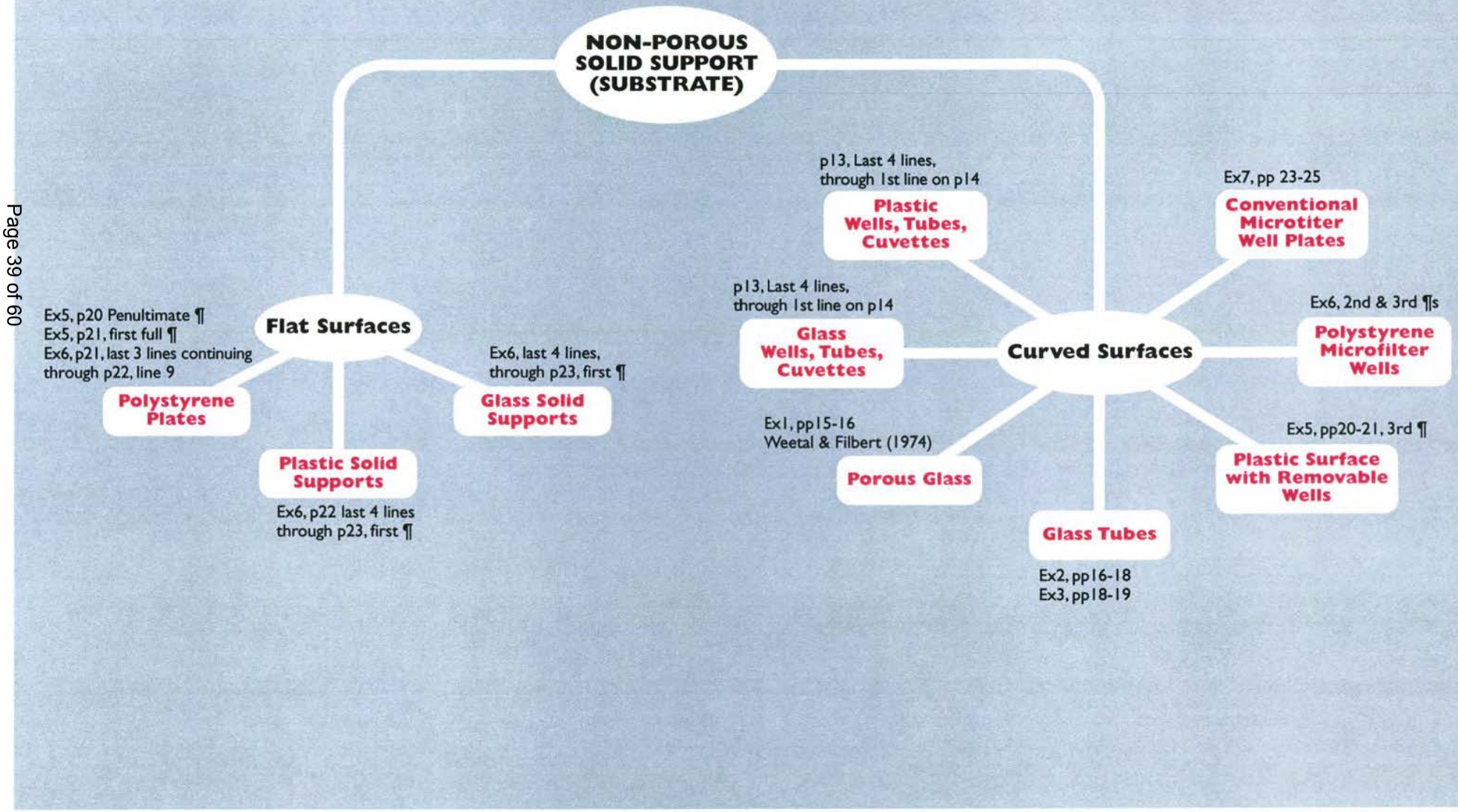
U.S. Patent Application SN 08/486,070 – Stavrianopoulos et. al., Filed June 7, 1995

Claiming Priority of: SN 06/461,469 (filed January 27, 1983) & SN 06/732,374 (filed May 9, 1985)



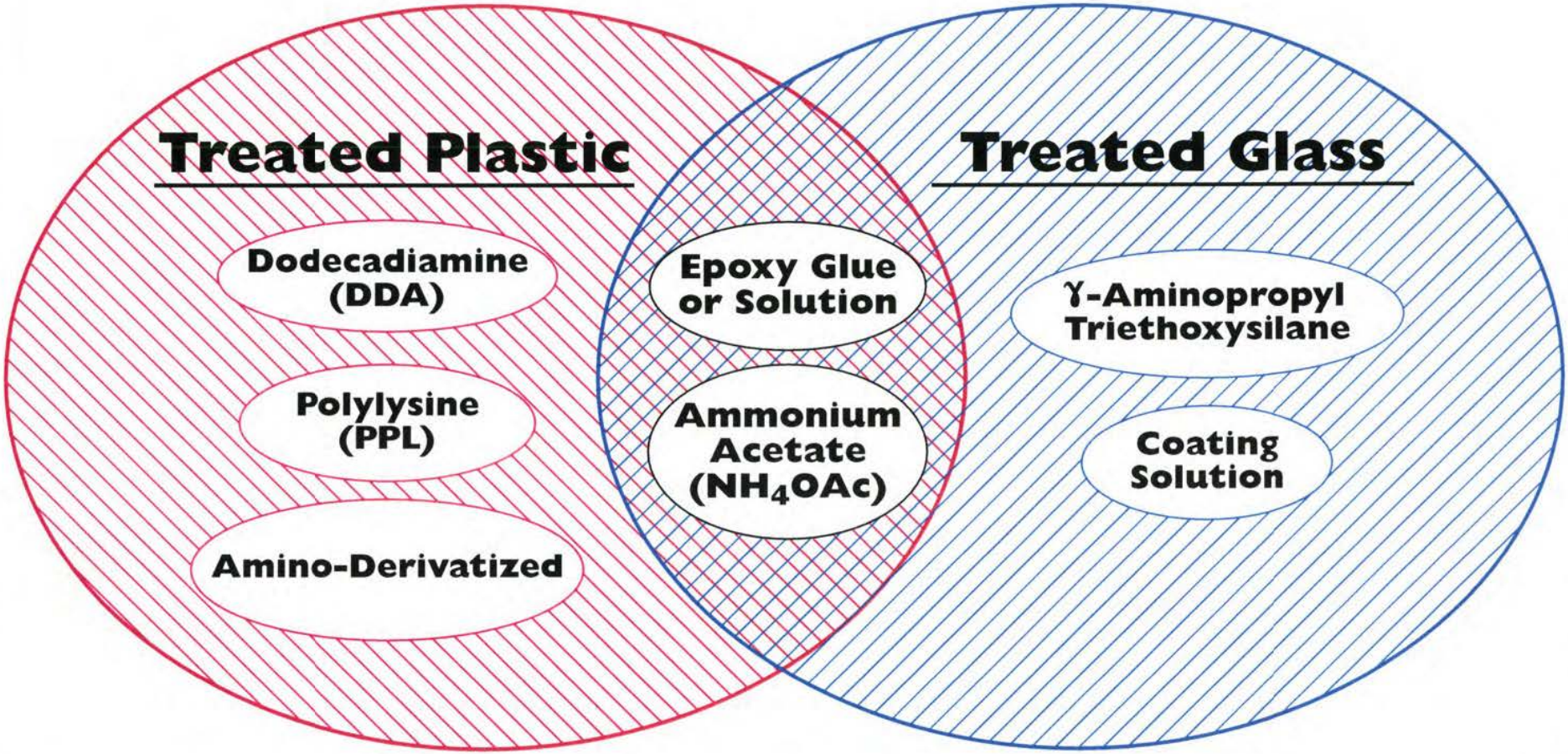
U.S. Patent Application SN 08/486,070 – Stavrianopoulos et. al., Filed June 7, 1995

Claiming Priority of: SN 06/461,469 (filed January 27, 1983) & SN 06/732,374 (filed May 9, 1985)



U.S. Patent Application SN 08/486,070 – Stavrianopoulos et. al., Filed June 7, 1995
Claiming Priority of: SN 06/461,469 (filed January 27, 1983) & SN 06/732,374 (filed May 9, 1985)

"One or More Reactive Groups or Binding Sites on Said Surface"*



Footnote:

*As amended on December 3, 2002, claims 1576 and 1670 recite "one or more reactive groups or binding sites on said surface,..."

Chart 8 12/13/02 5:02 PM

U.S. Patent Application SN 08/486,070 – Stavrianopoulos et. al., Filed June 7, 1995

Claiming Priority of: SN 06/461,469 (filed January 27, 1983) & SN 06/732,374 (filed May 9, 1985)

Reactive Groups or Binding Sites*

Amines

Ex5, p20, lines 28-31
Ex6, p22, line 1
Ex5, p20, lines 31-34
Ex6, p22, last 4 lines thru p23, 1st ¶
Ex7, p23, lines 17-25
Ex7, p24, lines 1-4
Ex6, p22, penultimate ¶

Epoxides

Ex6, p22, last 4 lines thru p23, 1st ¶

Hydroxyls

Ex1, lines 16-23

Footnote:

*As amended on December 3, 2002, claims 1576 and 1670 recite "one or more reactive groups or binding sites on said surface,..."

Chart 7 12/13/02 3:50 PM

The Scientist 15[24]:36, Dec. 10, 2001

PROFILE

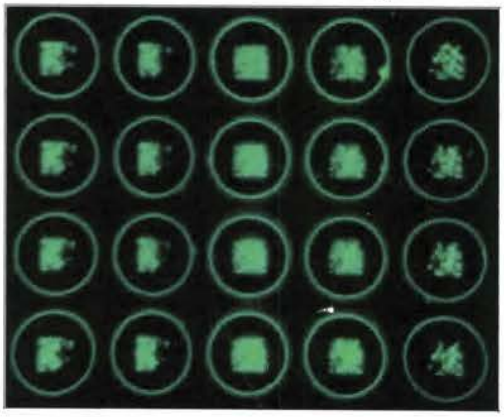
E-mail article

Microarray Readers: Pushing the Envelope

Companies adopt many strategies to overcome technical challenges

By Jorge D. Cortese

Courtesy of UVP Inc.



Fluorescein-labeled cDNA in a 10x10 spotted microarray. Shown are 20 wells of a 96-well microplate imaged with overhead 365-nm UV excitation, a 475-575-nm interference filter, and a 1.4 megapixel CCD camera.

To truly reap the benefits of the flood of information coming out of sequencing factories worldwide, investigators must move beyond the traditional notion of "one-gene, one-experiment," in favor of highly parallel, automation-friendly, and miniaturized assays. One such tool is the microarray--a matrix of biomaterials attached to a support such as glass or plastic.[1-3] Using microarrays, scientists can perform hundreds or thousands of experiments in parallel, all thanks to a chip usually no bigger than a microscope slide.

DNA microarrays, as the name suggests, are arrays of oligonucleotides, cDNAs, or other DNA targets, which promise to advance several biomedical goals. First, microarrays allow researchers to track global gene expression patterns characteristic of normal and disease states, and to identify genes that are up- or down-regulated when a drug or signaling factor is added.[1] Such information can advance drug discovery efforts. Second, since gene expression patterns vary between physiological states or in different regions of an organ, such as the brain, there is considerable interest in the generation of comparative three-dimensional maps of gene expression in normal and diseased tissues.[4] These maps can be produced by combining laser capture microdissection (LCM),[5] single-cell DNA amplification, and microarray analysis. Finally, arrays can aid single-nucleotide polymorphism (SNP)[3] and mutation pattern mapping, thus potentially individualizing medical treatments.

Scientists can label samples for microarray analysis with either radionuclides or fluorophores. Direct or indirect fluorescence labeling is generally preferred, because it enables direct sample-to-sample comparison on a single chip. Direct labeling involves the introduction of a covalently linked fluorophore in the nucleic acid sequence. For differential, two-color labeling, probe families with similar chemical structure but different spectroscopic properties are desirable. Examples include the cyanine (Cy) dyes (e.g., Cy3 and Cy5), as well as Eugene, Ore.-based **Molecular Probes Inc.**'s Alexa™ probes.[6] Indirect labeling techniques create labeling sites on the biopolymer, followed in some systems by amplification of these linking sites and labeling with Cy3/Cy5.

Once the array is labeled, however, researchers need an imager or scanner to collect all of the data. Continuing *The Scientist*'s ongoing series on microarray tools,[7-9] LabConsumer now reviews the instrumentation used to read nucleic acid-derived microarray assays.

Scanners vs. Imagers

Microarray readers can be either scanners or imagers. Typically, scanners use one or more lasers and scan dual-fluorophore samples either sequentially or simultaneously; however, simultaneous scanning produces fewer registration errors. Union City, Calif.-based **Axon Instruments Inc.** offers the GenePix™ 4000 B Array Scanner that simultaneously reads at two excitation wavelengths, 532 and 635 nm.

The alternative to scanning arrays is imaging them with a charge-coupled device (CCD) camera.[10] Unlike scanners, CCD-based imagers can link images covering a very large surface area, so that many slides can be imaged sequentially. Scanners can focus more energy to excite fluorophores and thus collect more light in less time. The resulting shorter integration times should be a big advantage over CCD imaging, however, this higher power output is not necessarily advantageous, since fluorophores can reach a saturation point at which further excitation leads to photobleaching or a non-linear emission response.

Mark Rand, senior product manager and applications scientist for **Applied Precision Inc**. of Issaquah, Wash., has carried out extensive studies on the process

of imaging arrays, and explains that "a key difference with scanners is that CCD cameras can collect far more photons per pixel without reaching saturation." This makes the imagers' longer integration times and lower excitation power a benefit, rather than a drawback.

San Leandro, Calif.-based **Alpha Innotech Corp.**'s Alpha Array™ Reader is a high-throughput imager. Its optical system is enhanced by the NovaRay™ Light Management System, which reduces overall acquisition time: The instrument can image a 16 x 22-mm array in under one second. The instrument also overcomes some of the inherent problems with laser scanning, especially lateral displacement noise and photobleaching effects. With eight excitation wavelengths, the Alpha Array Reader can detect numerous fluorophores.

Some companies derive microarray imagers from general imaging devices. For example, **Amersham Biosciences** of Piscataway, N.J., recently entered this market with the Typhoon 9410, a high-end imager with extensive capabilities to tackle one- and two-dimensional gels, blots, tissue sections, macroarrays, and microarrays. The instrument can also be "flavored" to undertake specific needs in storage-phosphor, fluorescence, or chemiluminescence detections.

UVP Inc. of Upland, Calif., also bridges the gap between general lab imaging and microarray work. Three of the company's eight imaging workstations can gather microarray data. The BioMicro™ System is a microscope-compatible imager that can carry out high-resolution gel- or plate-imaging analysis, fluorescence microscopy, and microarray measurements. The BioChemi™ System has a cooled CCD camera and faster optics capable of visible light or colorimetric measurements, fluorescence detection, and chemiluminescence or chemifluorescence imaging. UVP's OptiChem™ System has a 1.4 mega-pixel, super-cooled CCD device (75°C below ambient) with five orders of magnitude linear dynamic range. The OptiChem is also capable of imaging and analyzing fluorescent microarrays, chemiluminescent membrane arrays, and microplate arrays.

Unlike laser scanners, imagers are not limited to single-wavelength excitation. For example, Applied Precision's arrayWor$_x$™ instruments use white light.[11] A white light beam--one that contains all visible wavelengths--is directed through an excitation filter in a filter wheel to give monochromatic illumination to the sample. The filter wheel allows researchers to acquire up to four wavelengths while the slide is stationary, eliminating spatial registration artifacts. The cooled scientific-grade CCD provides low noise and high quantum efficiency. The camera collects partial images (panels) from the designated area and reconstructs them using Stitch-by-Position™ Image Registration Technology.

Several arrayWoR$_x$ instruments are available. The arrayWoR$_x^e$ Biochip Reader-Basic handles up to four-color simultaneous analysis, and includes two standard channels, Cy3 and Cy5; the arrayWoR$_x^e$ Biochip Reader-Standard runs full area scanning even at 3.25-µm resolution and handles image files up to 500 MB in size. The Automated arrayWoR$_x$ Microarray Scanners include the same capabilities of the other arrayWoR$_x$ scanners as well as automatic slide loading and analysis for up to 25 slides. **Vysis Inc.** of Downers Grove, Ill., offers another CCD imaging microarray

reader. The instrument, which uses a Xenon-lamp source, is part of the company's GenoSensor™ System, which includes the GenoSensor Array 300 consisting of 287 targets for postnatal and cancer research applications.

Companies can construct general scanning/imaging systems to detect both fluorophores and radionuclides. Stamford, Conn.-based **Fujifilm Medical Systems USA Inc.**'s FLA-5000 Science Imaging System can scan both fluorescent and radioisotopic labels in an area of up to 40 x 46 cm. With three standard lasers (437/532/635 nm) and ports for another two, researchers can use the device to read most fluorophores. An optional second photomultiplier allows simultaneous detection of two dyes in a single scan. The new Fujifilm FLA-8000 Fluorescence Image Analyzer can efficiently image macroarrays and a variety of other applications, such as labeled cells and radiolabeled samples; it has two standard lasers and one optional laser. Resolution can be as low as 5 µm for fluorescence images and 10 µm for radioisotope images. Although most microarrays are read using fluorescence, this instrument incorporates a newly designed Phosphor Imaging Plate (IP™ BAS-SR0813) that is scanned by the FLA-8000's red laser and emits blue fluorescence by photo-stimulated luminescence.

Overcoming Technical Hurdles

Courtesy of Tecan

Tecan's LS 200 scanner

By their very nature, microarrays push imaging equipment to their detection limits. These arrays can contain tens of thousands of 50-150-µm diameter spots per square centimeter, but they tend to be biased toward known and abundant gene products, even though many interesting genes are often expressed at relatively low levels. In practice, microarray readers can usually detect transcripts with a relative abundance of between one copy in 100,000 and one copy in 500,000, or 3 to 10 copies per cell.[3,4] The sensitivity required for this low-level fluorescence detection is approximately two-to-five molecules per square micrometer, with a linear dynamic range of five orders of magnitude.

Image optimization increases demands on equipment. For instance, researchers often try to minimize fluorophore photobleaching (photochemical damage) and "bleed-through" (contamination of detected emission with that of additional fluorophores). Pixel size and resolution are also critical parameters for microarray scanners and imagers. In general, resolutions of 5-10 µm are needed to resolve common features. Santa Clara, Calif.-based **Affymetrix**'s GeneArray™ Scanner can focus its argon-ion laser (488 nm) down to 3 µm.

Scanners use standard microscope objectives, producing images akin to moderately magnified fluorescent micrographs. Unfortunately, the large lateral focal plane is not homogenous, so the objective must be moved across the microarray or the array must be moved across the objective's field. This is a technical problem that increases non-uniformity across an array, and each instrument solves it in a slightly different manner.

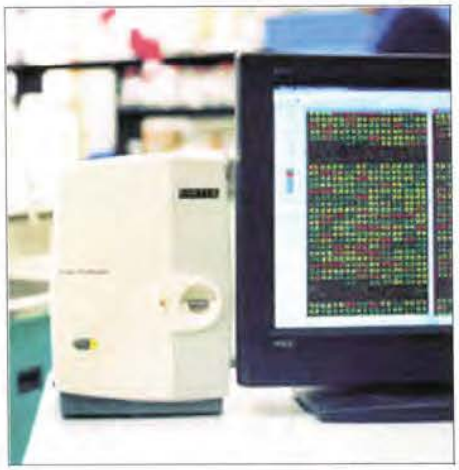
Alameda, Calif.-based **MiraiBio**'s FMBIO® IIe tackles this problem by moving the slide under a fixed objective. It uses a high-power, solid-state laser, and the optical unit moves under the sample as it is scanned using a high-speed rotating polygonal mirror; two optical fiber arrays and two photomultipliers accelerate the reading process. The device can read up to 48 medium-density, multicolor arrays simultaneously.

Another problem is the signal-to-noise ratio of array quantification: How does the instrument differentiate data from junk? One approach is to use confocal microscopy. In principle, confocal optics reject fluorescence from non-scanned focal planes, leading to lower backgrounds.[12] But this requires the use of precisely aligned pinholes, heavy-duty moving components, and alignment that is maintained in every scan. Furthermore, scientists may find working with 2-µm confocal slices impractical when a depth-of-field of 20-30 µm suffices for most current quantitative purposes.

GeneFocus®, a division of **Biomedical Photometrics Inc**., based in Waterloo, Ontario, produces several microarray confocal-laser scanners in its DNAscope™ line: DNAscope IV, V, and LM. GeneFocus also offers an open- systems version of its confocal-based scanners, the Open Frame Research DNAscope, suitable for the development of new solid or "wet" biochips for a wide selection of fluorescent probes; high-resolution versions can image Affymetrix's GeneChips®.

Background contributions can also be minimized by non-confocal techniques. **PerkinElmer Life Science** of Boston offers two instruments with dark-field illumination to enhance background rejection. The GeneTAC™ 2000 Microarray Analyzer is part of the GeneTAC Biochip Solution from **Genomic Solutions** of Ann Arbor, Mich.[7] The GeneTAC 2000 is compatible with various substrates including glass, plastic, ceramic, and metal. It can also detect luminescence. The alternative GeneTAC LSIV Microarray Analyzer has four excitation lasers and two additional photomultipliers. Both instruments use a 24-slide carousel for high-throughput applications.

Courtesy of Virtek Vision Corp.



Virtek's ChipReader Microarray Scanner

Another way to eliminate background noise and photobleaching is to optimize the microarray reader to operate at low laser power. Waterloo, Ontario-based **Virtek Vision Corp.**'s ChipReader™ Microarray Scanner is a very small, single-slide reader that uses this approach. In addition, researchers can control each channel's output continuously and independently during scanning. The ChipReader is a two-color scanner available at various resolutions, down to 3 µm. The ChipReader also has high sensitivity as it can detect less than 2.6 femtograms of labeled DNA on a 100-µm diameter spot with a reproducible signal-to-noise ratio greater than nine.

The final technical issue is the quality of the microarray itself. If the slide is not perfectly flat, the images will not be uniform or consistent, regardless of resolving power. **Agilent Technologies Inc**. of Wilmington, Del., offers a solution to this problem with its DNA Microarray Scanner, which uses dynamic auto focus. By adjusting the focus continually during scanning, the instrument minimizes the effect of glass slide aberrations, improving uniformity and enhancing sensitivity.

One typical test of array quality is the "concordance" correlation: Labeling an array with the same source RNA divided in two pools and labeled with two probes (e.g., Cy3 and Cy5) should lead to an array with a theoretical differential gene expression ratio of 1.0. From these experiments, users can estimate the minimal differential gene expression value that the array can detect.

Final Notes

A number of issues still exist regarding array-reading technologies. The first is data quality. Comparing data obtained in different labs can be problematic, and part of the blame rests on the array readers. However, analysis of a single array with instruments based on different reading principles indicates that inter-laboratory comparisons of experimental data are warranted.[13] Such comparisons should help researchers take advantage of both their collaborators' unpublished work as well as research appearing

in the literature.

Another issue is the convergence between imaging and scanning. Scanners will likely dominate in the short-term, but imaging is bound to become the principal form of analysis. Scanners may have an edge for very high-density arrays (if required for massive clinical testing). Alternatively, researchers may adopt techniques such as two-photon or near-infrared confocal microscopy,[14] endowed with more restricted focus depth and low background.

As microarray usage becomes commonplace, microarray images will join the standard record-keeping photographs of Western blots, agarose gels, or tissue sections. Thus, a general-purpose imager will be ideal, especially from a financial point of view. Some already exist, such as MiraiBio's FMBIO II, which can detect DNA in both agarose gels and sequencing gels, in addition to scanning microarrays. Array reading can be incorporated as a module of operation in many general imagers, but it seems unlikely that the most sophisticated microarray readers will take this all-in-one approach.

Courtesy of Packard BioScience

Packard BioScience's ScanArray Express

Since the needs of microarray laboratories vary greatly between individual labs and university-level facilities, several companies offer lines of microarray readers so that labs can purchase equipment to meet their needs. For example, Meriden, Conn.-based **Packard BioScience Co.**'s ScanArray® line of upgradeable instruments ranges from the ScanArray LITE Microarray Analysis System, a two-color scanner, to high-end instruments such as the five-laser ScanArray 5000 XL. These instruments support applications ranging from standard microarray reading to sophisticated four-color SNP genotyping. The instrument features sequential scanning, the ability to decrease scan laser power to diminish photobleaching, and a focus depth optimized to eliminate reading artifacts caused by dust particles or fingerprints.

For all the hype surrounding them, microarrays are like a large collection of test tubes, miniaturized on a microscope slide. They answer a single question: What genes are expressed in a given sample? Yet they are part of a new era of multitasking that

can and will change the way researchers do science.

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Supplemental Materials

table **Suppliers of Microarray Readers**

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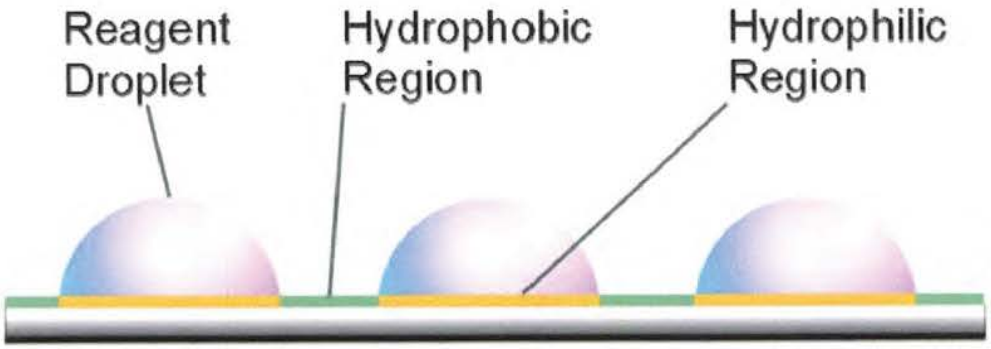
SURFACE TENSION

Protogene's array technology is based upon the segregation of fluids on a flat surface by differential surface tension. Surface tension characterizes the extent to which a given fluid will wet a surface.

In the macroscopic world, DNA chemistry is typically performed on glass beads held in a physical container or well. The container segregates the fluid reactants of one reaction from another. Protogene's fundamental invention is that one can segregate fluids on a flat surface by differences in surface tension imparted by chemical coatings. Once so segregated, DNA synthesis of thousands of different DNA features at higher quality than any other method can be achieved by inexpensive inkjet printing of reagents.



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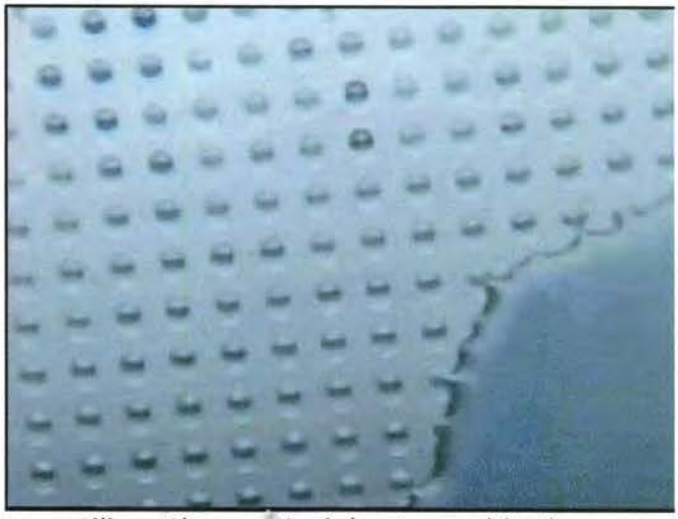
Protogene's technology

Protogene has developed a uniquely innovative approach to microarray technology employing differential surface tension. This technology permits *in situ* synthesis of high quality oligonucleotides on glass slides, using rapid inkjet printing methods. In the absence of surface tension properties, reagents delivered to the reaction site of a microarray would normally spread across the surface, resulting in a non-uniform, low quality array.

Protogene's key innovation was to realize that reagents could be localized to very clearly defined regions on the surface of a glass slide using differences in surface tension. A surface that is highly lipophobic (e.g. fluorosilane) tends to repel liquid reagents through forces generated by surface tension. If a non lipophobic area is nearby, reagents will be driven by surface tension to the non lipophobic area.



Protogene utilizes these principles to enable the manufacture of high quality microarrays using inkjet printing technology. A lipophobic layer with an array pattern of non lipophobic features is printed on the surface of a glass slide prior to synthesis of oligonucleotides. On this surface, reagents localize to very specific areas on the slide, defined by the non lipophobic pattern. This localization (self registration) prevents mixing of reagents from different reaction sites, results in better defined features, provides for higher volumes of reagent to be applied (enhancing

reaction efficiency) and speeds manufacturing as reagent delivery does not have to be as precise.

Using piezoelectric nozzles and proprietary engineering, which are controlled by advanced integrated software, oligonucleotide sequences are synthesized on the microarray. As a result, changes in sequences of oligonucleotides can be effected without any change in the set-up of the manufacturing line.

In situ synthesis combined with Protogene's proprietary surface tension technology:

1. enables a highly efficient, rapid turnaround, flexible manufacturing process
2. produces high quality microarrays
3. enables a new web based design and distribution model to be implemented providing a very high degree of accessibility for customers
4. supports market expansion by enabling rapid, iterative, massively parallel genomic analysis driven by availability of custom microarrays at an affordable price

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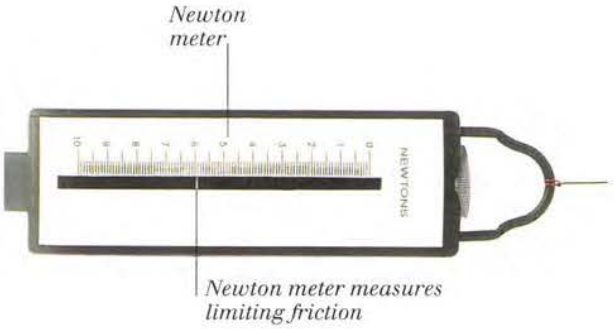
GYROSCOPE



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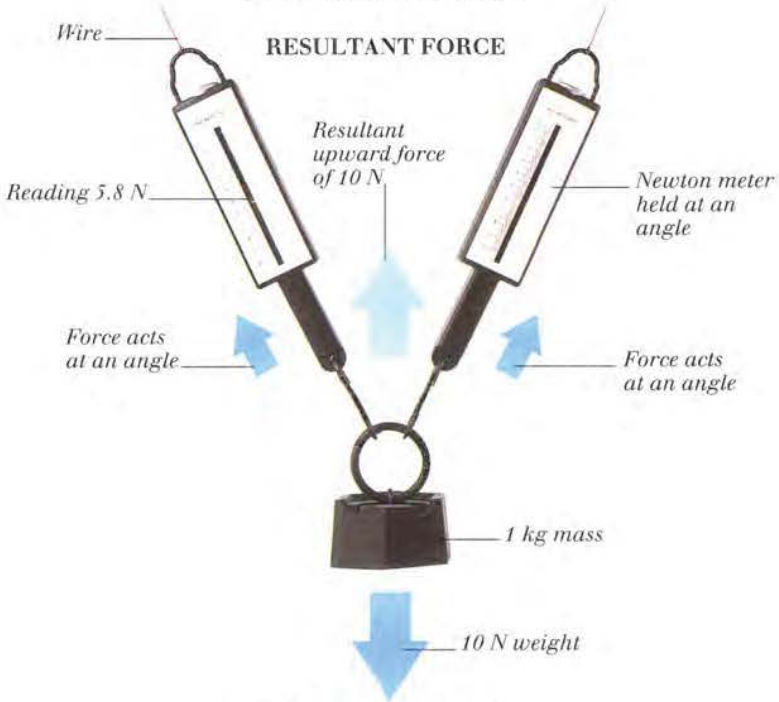

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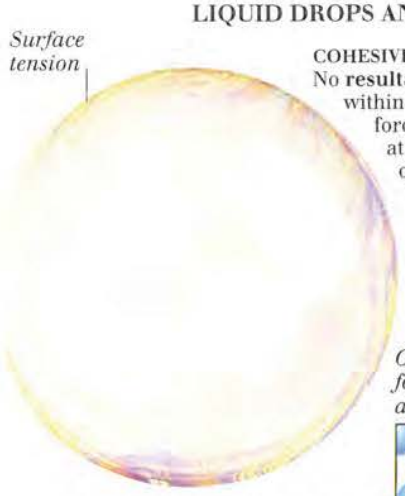
Liquids

UNLIKE SOLIDS, LIQUIDS CAN FLOW. Their particles move almost independently of each other but are not as free as the particles of a gas. Forces of attraction called **cohesive** forces act between the particles of a liquid. These forces create **surface tension**, which pulls liquid drops into a spherical shape. If the surface tension of water is reduced by dissolving soap in it, then pockets of air can stretch the surface into a thin film, forming a bubble. Forces of attraction between liquid particles and adjoining matter are called **adhesive** forces. The balance between cohesive and adhesive forces causes **capillary action**, and the formation of a **meniscus** curve at the boundary between a liquid and its container. Liquids exert pressure on any object immersed in them; the pressure acts in all directions and increases with depth, creating **upthrust** on an immersed object. If the upthrust is large enough, the object will float.

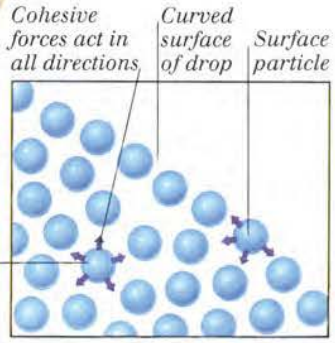
LIQUID DROPS AND BUBBLES



SPHERICAL SOAP BUBBLE

COHESIVE FORCES
No **resultant** force acts on any particle within the liquid, because cohesive forces pull it in every direction. But at the surface, the resultant force on each particle pulls it inward. This causes surface tension, which pulls drops and bubbles into spheres. A water drop on a surface will be flattened slightly by gravity.

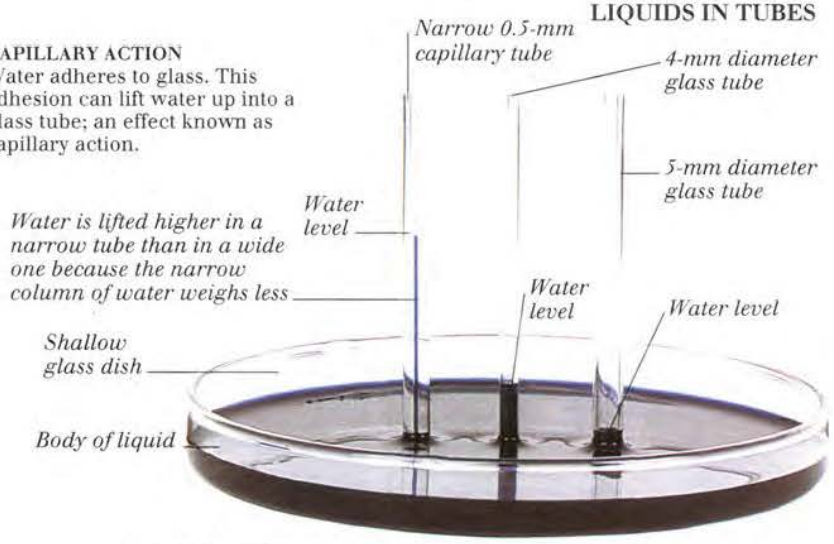
WATER DROP ON A SURFACE



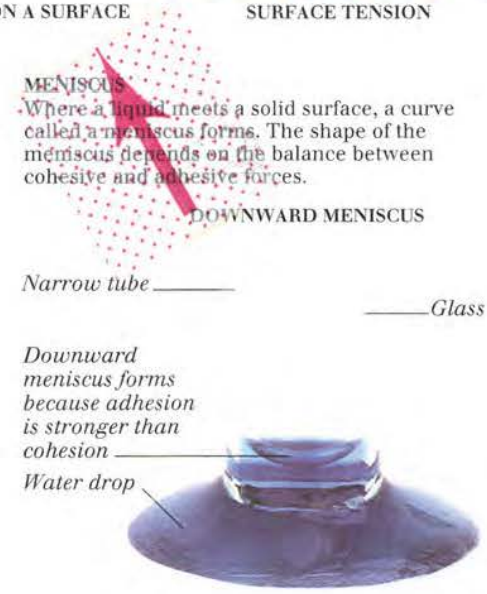
SURFACE TENSION

LIQUIDS IN TUBES

CAPILLARY ACTION
Water adheres to glass. This adhesion can lift water up into a glass tube; an effect known as capillary action.



MENISCUS
Where a liquid meets a solid surface, a curve called a meniscus forms. The shape of the meniscus depends on the balance between cohesive and adhesive forces.



MOLECULAR VIEW
Capillary action is caused by adhesive and cohesive forces between particles of glass and water. Here, water molecules adhere to glass and the adhesive force lifts the edge of the water up the glass. The cohesive forces between water molecules means that this lifted edge also raises water molecules lying farther out from the edge of the glass.