SYSTEM AND METHOD OF CELL SORTING USING HOLOGRAPHIC OPTICAL TRAPPING

The present invention relates to a system and method of cell sorting using holographic optical trapping.

BACKGROUND OF THE INVENTION

In United States industry, there is a large number of unmet sorting and separation needs involving material made up of particles or units smaller than 50 microns. These needs range across industries from particle sizing and sample preparation in the specialty chemicals and materials fields, to protein selection and purification in the pharmaceutical and biotechnology industries. Other examples include cell sorting and selection, in the medical, diagnostic and agriculture sectors.

The importance of these needs can be seen by exploring the annual expenditures in areas where specialized or partial solutions have been developed, as well as by estimating the market value of sorted/separated/purified output in areas where there is currently not even a partial solution. As an example of the former, the biotechnology and pharmaceutical industries annually spend in excess of a billion dollars on equipment and supplies for protein purification.

As an example of the latter, in the agricultural sector, there is currently no way to efficiently select the gender of offspring in farm animals; however, it is estimated that in the cattle area alone, over a billion dollars in value would be added by enabling such sperm selection as a part of the current artificial insemination process widely used in the industry.

Outside of the animal husbandry market, the purification process of islet cells from human pancreases is currently a large concern of medical scientists developing new treatment methods for Type I diabetes. Significant progress in islet transplantation methods has been made, but the purification problem is one of the remaining stumbling blocks. Traditional methods for purifying islet cells are inefficient and result in damage to the cells.

Islet cell transplantation is important because, in the Type I form of diabetes, the existing islet cells in the patient's pancreas have become damaged and no longer produce the insulin which is required for human survival. The current treatment for Type I diabetes involves injection of insulin 1 to 5 times per day. In spite of the treatment, the disease often leads to complications including blindness, blood flow problems requiring amputation, renal failure, and death. Greater purity and reduced contaminants for islet cells used in transplantation is expected to reduce the occurrence of these complications.

Of the approximately 1 million current sufferers of Type I diabetes in the United States, at least 50,000 sufferers per year would submit to islet cell

transplantation if it were available. Islet cell purification, at $1000 to $3000 per person, could result in $50 to $150 million in annual income per year. Upon large-scale acceptance of islet cell transplantation as an effective therapy, these numbers would be expected to jump substantially. The jump would be driven by the difficulty of using today's treatment method (frequent injections) and the severe consequences even when the current treatment is adequately administered.

Thus, islet purification is but one important problem requiring the highly selective sorting of human cells in a non-damaging, non-invasive way.

Another problem that needs to be addressed is the purification of normal cells from cancer cells in the bone marrow of persons undergoing whole-body radiation treatment for cancer.

Still another is the selection of stem cells for research into the causes of, and therapies for, diseases such as Parkinson's disease.

Yet another concern is developing new ways to automatically interrogate large numbers of human cells and select ones having characteristics not amenable to fluorescent tagging, which would enormously widen the scope and power of medical diagnoses.

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. "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.

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.

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.

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.

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.

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 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 can 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.

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."

Some implementations of the third approach have used a fixed transmission hologram to create between 16 and 400 individual trapping centers.

A fixed hologram was 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 intensity distribution of the liquid crystal grating may be easily controlled in real time by a computer, thus permitting a variety of dynamic manipulations.

Other types of traps that can 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.

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.)

The light cage (Neal in 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. However, unlike a vortex, no-zero electric field

area is created. An optical vortex, although similar in use to an optical trap, operates on an opposite principle.

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 can 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.

Accordingly, 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 (cell) sorting and purification is required. Still further, there is a need to separate sperm cells in the animal husbandry market.

SUMMARY OF THE INVENTION

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, preferably less than 0.8, more preferably less than 0.7, even more preferably less than 0.6, yet more preferably less than 0.5, even yet more preferably less than 0.4, further more preferably less than 0.3, even further more preferably less than 0.2 and most preferably less than 0.1.

The present invention provides a way of implementing a parallel approach to cell sorting using holographic optical trapping.

Optical trapping is used to address cell sorting and purification in three ways: 1) as reviewed earlier, the forces exerted by optical traps on a material are sensitive to the exact distribution of the dielectric value in that material - the optical force therefore depends on the composition and shape of the object; 2) 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; and, 3) localizing an object at a known position allows additional methods of automated interrogation such as high speed imaging and particle-specific scattering measurements.

In an embodiment, the present invention performs cell sorting of X and Y sperm for animal husbandry.

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