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61/360,074 30 June 2010 (30.06.2010) US(71) Applicant (for all designated States except US): **THE GOVERNORS OF THE UNIVERSITY OF ALBERTA** [CA/CA]; #4000 TEC Centre, 10230 Jasper Avenue, Edmonton, Alberta T5J 4P6 (CA).

(72) Inventors; and

(75) Inventors/ Applicants (for US only): **TSUI, Ying, Yin** [CA/CA]; #4000 TEC Centre, 10230 Jasper Avenue, Edmonton, Alberta T5J 4P6 (CA). **SU, Xuantao** [CA/CA]; #4000 TEC Centre, 10230 Jasper Avenue, Edmonton, Alberta T5J 4P6 (CA).(74) Agent: **PARLEE MCLAWS LLP**; 1500 Manulife Place, 10180-101 Street, Edmonton, Alberta T5J 4K1 (CA).

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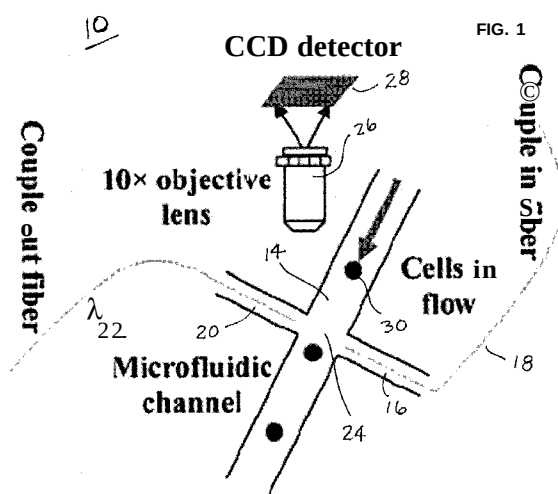
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(54) Title: APPARATUS AND METHOD FOR MICROSCOPE-BASED LABEL-FREE MICROFLUIDIC CYTOMETRY



(57) Abstract: An apparatus and method is provided for a microscope-based label-free microfluidic cytometry device. The device was developed to obtain two dimensional scattered light patterns from single cells or particles passing through a channel in a microfluidic chip without having a label attached to the cells or particles, where analysis of the scattered light pattern can determine information such as cell or particle size, orientation and inner nanostructure. The device can obtain light scattering patterns from the smallest mature human blood cells (platelets) and large cells (CFU-GM and cord blood CD34+ cells).



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TITLE:**APPARATUS AND METHOD FOR MICROSCOPE-BASED LABEL-FREE
MICROFLUTDIC CYTOMETRY****INVENTORS:**

Ying Yin Tsui and Xuantao Su

CROSS-REFERENCE TO RELATED APPLICATIONS:

This application claims priority of U.S. provisional patent application serial no. 61/360,074 filed June 30, 2010, and hereby incorporates the same provisional application by reference herein in its entirety.

TECHNICAL FIELD:

[0001] The present disclosure is related to the field of cytometry, in particular, label-free cytometry.

BACKGROUND:

[0002] Flow cytometry has wide applications in biology and medicine such as cell sorting and counting, HIV monitoring and tumor cell determination [1-3]. Recent research relating to the integration of optics with microfluidics [4, 5] has potential applications, particularly in flow cytometry [6-8]. Microfluidic flow cytometers, the result of an amalgamation of microfluidics with flow cytometry, can have several advantages over conventional cytometers. The microfluidic channel can have a cross-section dimension of micrometers that dramatically reduces the requirements for total sample volume in the experiments. Secondly, the use of laser tweezers [9], dielectrophoresis [10] or near-field methods [11] in a microfluidic flow has allowed single cells or even molecules to be manipulated. The development of lab-on-a-chip (LOC) [12] techniques can introduce the portable cytometers as a next generation of commercially available diagnostics tools. Large-scale production of portable cytometers could greatly reduce the cost of these devices.

[0003] Conventional flow cytometry measures fluorescence signals to obtain the inner organelle information in single biological cells [1, 3]; however, the fluorescence labelling or staining of the biological cells requires complex and expensive procedures. Labelling cells by using fluorophores can also alter the biological cell functions. In addition, the ability to properly label or stain biological cells is dependent on the expertise and experience of the lab technician carrying out the labelling of the cells. The use of dyes or nano-particles to label or stain biological cells can also be toxic to biological cells. In addition, not all dyes or nano-particles are universal in their ability to label or stain a biological cell or particle.

[0004] Developments in microscopy techniques have enabled the observation of organelles within cells at the nanometer scale, as well as viruses that are interacting with cells [31-34]. These methods for obtaining cellular information fall into two categories. First, fluorescence labeling has been used to enhance the contrast of the cell components, thus allowing otherwise transparent phase samples to be observed and even super resolution viewing of cells at tens of nanometers by using the stochastic optical reconstruction microscopy (STORM) technique [33]. A second method for obtaining high resolution images involves using shorter wavelength illumination, for example, the use of transmission electron microscopy (TEM) on thinly sliced biological cell specimens [34]. All the above methods are invasive to the cells.

[0005] Hematopoietic stem cells have the remarkable capability to differentiate into mature blood cells of all lineages (erythroid, myeloid and lymphoid) while maintaining their ability to self-renew [35]. Hence they have been used to re-establish the hematopoietic function in patients with damaged or defective bone marrow or immune system [36]. Human hematopoietic stem cells and progenitors express the surface

glycoprotein CD34, a marker that allows their isolation by immunomagnetic labelling [37].

[0006] It is, therefore, desirable to provide an apparatus and method for flow cytometry of particles or biological cells that does not require the labelling or staining of the particles or cells.

SUMMARY:

[0007] In some embodiments, a light scattering method may be used as a non-invasive, label-free technique for obtaining cellular information [38-47]. Light scattering spectroscopy ("LSS") has been used for obtaining information of submicrometer organelles noninvasively [41-44]. Recently, LSS has been combined with confocal microscopy, the confocal light absorption and scattering spectroscopic microscopy (CLASS), for cellular observation without exogenous labels [43, 44]. Alternatively, it has been demonstrated that two-dimensional ("2D") light scattering intensity patterns can be analyzed to obtain submicron-scale information of the cells [45, 46, 48]. Analysis of the light scattering patterns can provide very useful cellular information. In some embodiments, 2D light scattering patterns may be used for cell discrimination or the recognition of various physiological states in cells of the same kind.

[0008] In some embodiments, an apparatus and method is provided for microscope-based label-free microfluidic cytometry. Compared with fluorescence method, light scattering methods that are label-free can be used for the study of biological cells [7, 8, 13-18]. Light scattering measurements can be performed in conventional cytometers for particle or cell size approximation, by integrating the small angle forward scattering light intensity in a 5 degree cone angle, and by measuring the light scattered around the

90 degree polar angle, which is perpendicular to the incident light propagation direction [15, 17, 19]. The 2D pattern of the scattered light, especially the side scattered light (scattering around the 90 degree polar angle) from coherent light sources, can have significant information about internal organelles in biological cells [7]. A combination of light scattering with microfluidic cytometry can be used to find potential label-free applications in the fields of education, research and clinical diagnostics.

[0009] Motivated by the need for low-cost, point-of-care portable cytometers, a 2D light scattering microfluidic cytometric technique is presented herein. A cytometer apparatus can be employed to collect scattered light of a large cone angle from a cell. The 2D scattered light pattern can be used to accurately determine the size of a cell using a fast Fourier transform (FFT)-based method [8]. The 2D scattered light pattern also contains information about the distribution of mitochondria in a single cell [7]. The main source for the background noise in this cytometer can be the light scattering from the contaminants in the fluid and the sidewalls of a channel disposed on a microfluidic chip that could dominate the signals from the single scatter that is of interest. To improve the signal to noise ratio ("SNR") of the 2D cytometer, a substrate can be first coated with a thin film and then etched to fabricate a small observation window few hundred in size to limit the background scattered light reaching the light detector. Even with an improved SNR, it can be difficult to detect some important cells which are weak scatterers such as platelets and very small embryo-like ("VSEL") stem cells [20] due to their small sizes cause the scattered light to be indistinguishable from the background noise. To mitigate this low SNR problem, the inventors discovered that background

light can be greatly reduced when a microscope objective was placed in front of the light detector allowing the detection of small cells such as platelets [22].

[0010] In some embodiments, a fiber-coupled microfluidic cytometer can be used for measuring 2D light scattering patterns from single cells. In some embodiments, 2D light scattering patterns can be obtained from different cells including leukemic cell lines (Jurkat and THP-1) and normal hematopoietic cells (CD34+ cells and lymphocytes). Detailed numerical simulations can be performed to examine the effect of varying the number, the size, and the distribution of mitochondria in order to aid in the understanding of these light scattering patterns. By analyzing the 2D light scattering patterns, criteria can be established that can be used to discriminate between different types of cells. In some embodiments, these criteria may be used in the future to determine the physiological status of cells or for cell sorting.

[0011] Incorporated by reference into this application is a paper co-written by the within inventors/applicants entitled, "Microscope-based label-free microfluidic cytometry", as published on-line on December 24, 2010 at www.opticsinfobase.org/oe/home.cfm, and in the 3 January 2011, Vol. 19, No. 1 edition of the journal, Optics Express, pp. 387-397 as published by the Optical Society of America.

[0012] Also incorporated by reference into this application is a paper co-written by the within inventors/applicants entitled, "Label-free and noninvasive optical detection of the distribution and nanometer-size mitochondria in single cells", as published in the Journal of Biomedical Optics 16(6), 067003 (June 2011).

[0013] Broadly stated, in some embodiments, a method is provided for carrying out microscope-based label-free cytometry, the method comprising the steps of: providing

an apparatus, comprising: a microfluidic chip further comprising a channel disposed thereon, the channel substantially parallel to a top surface of the microfluidic chip, an optical port disposed on the microfluidic chip, the optical port substantially parallel to the top surface, the optical port intersecting the channel thereby defining an observation point at the intersection thereof, a light source configured to emit collimated visible or near-visible light, means for operatively coupling the light source to the optical port wherein at least some light emitted from the light source passes through the coupling means to illuminate the observation point, a light detection device disposed adjacent to the observation port, the light detection device comprising an incident surface, the light detection device configured to produce an electrical signal in response to light striking the incident surface, and means for directing light scattered from a biological cell or a particle disposed at the observation point onto the light detection device; passing the biological cell or particle through the channel to the observation point; illuminating the observation point with light emitted from the light source; and detecting light scattered from the biological cell or particle with the light detection device.

[0014] Broadly stated, in some embodiments, a method is provided for producing a scatter pattern from a biological cell or particle, the method comprising the steps of: providing an apparatus, comprising: a microfluidic chip further comprising a channel disposed thereon, the channel substantially parallel to a top surface of the microfluidic chip, an optical port disposed on the microfluidic chip, the optical port substantially parallel to the top surface, the optical port intersecting the channel thereby defining an observation point at the intersection thereof, a light source configured to emit collimated visible or near-visible light, means for operatively coupling the light source to the optical

port wherein at least some light emitted from the light source passes through the coupling means to illuminate the observation point, a light detection device disposed adjacent to the observation port, the light detection device comprising an incident surface, the light detection device configured to produce an electrical signal in response to light striking the incident surface, and means for directing light scattered from a biological cell or a particle disposed at the observation point onto the light detection device; passing the biological cell or particle through the channel to the observation point; illuminating the observation point with light emitted from the light source; detecting light scattered from the biological cell or particle with the CCD; and producing the scatter pattern from the electrical signal produced in response to the scattered light detected by the light detection device.

[0015] Broadly stated, in some embodiments, an apparatus is provided for microscope-based label-free cytometry, comprising: a microfluidic chip further comprising a channel disposed thereon, the channel substantially parallel to a top surface of the microfluidic chip; an optical port disposed on the microfluidic chip, the optical port substantially parallel to the top surface, the optical port intersecting the channel thereby defining an observation point at the intersection thereof; a light source configured to emit collimated visible or near-visible light; means for operatively coupling the light source to the optical port wherein at least some light emitted from the light source passes through the coupling means to illuminate the observation point; a light detection device disposed adjacent to the observation port, the light detection device comprising an incident surface, the light detection device configured to produce an electrical signal in response to light striking the incident surface; and means for directing light scattered from a

biological cell or a particle disposed at the observation point onto the light detection device.

[0016] Broadly stated, in some embodiments, an apparatus is provided for producing a scatter pattern from a biological cell or particle, comprising: a microfluidic chip further comprising a channel disposed thereon, the channel substantially parallel to a top surface of the microfluidic chip; an optical port disposed on the microfluidic chip, the optical port substantially parallel to the top surface, the optical port intersecting the channel thereby defining an observation point at the intersection thereof; a light source configured to emit collimated visible or near-visible light; means for operatively coupling the light source to the optical port wherein at least some light emitted from the light source passes through the coupling means to illuminate the observation point; a light detection device disposed adjacent to the observation port, the light detection device comprising an incident surface, the light detection device configured to produce an electrical signal in response to light striking the incident surface; means for directing light scattered from a biological cell or a particle disposed at the observation point onto the light detection device; and means for producing the scatter pattern from the electrical signal produced in response to the scattered light detected by the light detection device.

[0017] Broadly stated, in some embodiments, a scatter pattern of a biological cell or particle is provided when made by a process comprising the steps of: providing an apparatus, comprising: a microfluidic chip further comprising a channel disposed thereon, the channel substantially parallel to a top surface of the microfluidic chip, an optical port disposed on the microfluidic chip, the optical substantially parallel to the top

surface, the optical port intersecting the channel thereby defining an observation point at the intersection thereof, a light source configured to emit collimated visible or near-visible light, means for operatively coupling the light source to the optical port whereby at least some light emitted from the light source passes through the coupling means to illuminate the observation point, a light detection device disposed adjacent to the observation port, the light detection device comprising an incident surface, the light detection device configured to produce an electrical signal in response to light striking the incident surface, and means for directing light scattered from a biological cell or a particle disposed at the observation point onto the light detection device; passing the biological cell or particle through the channel to the observation point; illuminating the observation point with light emitted from the light source; detecting light scattered from the biological cell or particle with the light detection device; and producing the scatter pattern from the electrical signal produced in response to the scattered light detected by the light detection device.

BRIEF DESCRIPTION OF THE DRAWINGS:

[0018] Figure 1 is a schematic diagram depicting a microscope-based label-free microfluidic light scattering cytometer.

[0019] Figure 2 is an illustration depicting the obtaining of the 2D scattered light pattern of a 4 μ m bead flowing through the cytometer of Figure 1.

[0020] Figure 3 is a cross-sectional elevation view depicting the cytometer of Figure 1.

[0021] Figure 4 is an illustration depicting 2D scattered light patterns of 4 μ m and 9 μ m beads flowing through the cytometer of Figure 1.

[0022] Figure 5 is an illustration depicting 2D scattered light patterns of platelet cell orientation.

[0023] Figure 6 is an illustration depicting 2D scattered light patterns of CFU-GM and CB CD34+ cells.

[0024] Figure 7 is an X-Y graph depicting real space analysis of the intensity maxima in the 2D scattered light patterns for potential cell discrimination.

[0025] Figure 8(a) is a schematic diagram depicting a second embodiment of a microscope-based label-free microfluidic light scattering cytometer.

[0026] Figure 8(b) is a top plan view depicting the observation window of the microfluidic chip of the cytometer of Figure 8(a).

[0027] Figure 9 is a schematic diagram depicting a third embodiment of a microscope-based label-free microfluidic light scattering cytometer.

[0028] Figure 10 is a series of 16 images depicting the effects of the number and size of mitochondria on the 2D light scattering patterns. Figures 10(a)-(h) depict the cell models used for the AETHER simulations. Figures 10(a')-(h') depict the corresponding 2D light scattering patterns.

[0029] Figure 11 is a series of 6 images depicting 2D light scattering patterns from Jurkat and CD34+ cells. Figures 11(a)-(b) depict the representative experimental 2D light scattering patterns from a Jurkat cell and a CD34+ cell, respectively. Figures 11(c)-(d) depict the simplified optical properties of the two cells. Figures 11(e)-(f) depict the 2D finite-difference time-domain light scattering patterns for the cell models in Figures 11(c) and (d), respectively.

[0030] Figure 12 is a series of 2 images depicting an optical properties model and simulated 2D light scattering pattern. Figure 12(a) depicts an optical property model of 72 mitochondria of diameter of $1\ \mu\text{m}$ distributed randomly throughout a cell. Figure 12(b) depicts a corresponding simulated 2D light scattering pattern of the cell.

[0031] Figure 13 is a series of 6 images depicting representative confocal fluorescence images of Jurkat and CD34+ cells. Figures 13(a)-(b) depict the mitochondria and nucleus in a Jurkat cell, respectively. Figure 13(c) depicts the overlay of Figures 13(a) and (b). Figures 13(d)-(e) depict the mitochondria and nucleus in a CD34+ cell, respectively. Figure 13(f) depicts the overlay of Figures 13(d) and (e).

[0032] Figure 14 is a series of 4 images depicting an analysis of experimental and simulated 2D light scattering patterns. Figures 14(a)-(d) depict the pattern analysis results of Figures 11(a), (b), (e) and (f), respectively.

[0033] Figure 15 is an X-Y graph depicting an analysis of experimental and simulated light scattering patterns for Jurkat and CD34+ cells.

[0034] Figure 16 is a schematic depicting a third embodiment of a microscope-based label-free microfluidic light scattering cytometer.

DETAILED DESCRIPTION OF EMBODIMENTS

[0035] In some embodiments, a light scattering microfluidic cytometer can comprise three major components: an illumination light source, a microfluidic chip and a detector. The coupling of light into the microfluidic channel is very important and has been studied intensively. For example, a tapered microchannel has been fabricated that can couple laser light into a microfluidic channel [49]. Another way is to use a prism to couple a laser beam into a microfluidic channel [46]. In some embodiments, a fiber

coupling technique can be adopted to illuminate a single scatterer within the microfluidic flow. In so doing, three major advancements can be achieved: (1), the rigorous micro-channel fabrication is no longer necessary as compared with Ref. [49]; (2), the coupling of the laser light to the microfluidic channel can be easier, while the tapered waveguide structure (Ref. [49]) and the prism coupling technique (Ref. [46]) require tedious alignment of the laser system with the microfluidic chip; and (3), with the optical fiber, the chip can be moved around in the experimental area with the laser-fiber coupling system kept fixed, which can be very convenient for bio-experiments.

[0036] In some embodiments, the method can comprise the combination of two techniques: 2D light scattering from a microfluidic cytometer and optical microscope imaging, into a method that the inventors define as microscope-based label-free microfluidic cytometry ("LFMC"). By incorporating a microscope objective lens (or "microscope objective") into a microfluidic cytometer, an apparatus can be provided that has the capability to first image a single cell in the microfluidic flow, and then to obtain its 2D scatter patterns by defocusing through this same microscope objective. The numerical aperture ("NA") of the microscope objective helps to reduce the background stray light and can improve the detection capability of the LFMC for the smallest mature human blood cells (such as platelets). Because the background stray light can be reduced with the microscope objective lens, fabrication of a micrometer-sized observation window is no longer necessary. Thus, the microscope-based LFMC can allow for low-cost fabrication techniques to be used to prepare the microfluidic chips, without the necessity of standard microfabrication tools and clean room facilities such as sputtering machines. In some embodiments, information obtained by the

microscope-based LFMC can be verified by comparing the experimental scatter patterns of standard micrometer-sized polystyrene beads with finite-difference time-domain ("FDTD") [7, 23-27] simulations. Applying this LFMC for the analysis of biological cells, the inventors find that in some embodiments the cell size, orientation and inner nanostructure of single cells can be determined without any labelling.

[0037] Experiments and Methods

[0038] In some embodiments, the components of the LFMC can comprise the fiber coupling of a light source into the side of a microfluidic channel to illuminate a single bead or cell, the microfluidic channel to propagate the cell (or bead) to the illumination point, and the microscope objective for imaging the cell (or bead) and obtaining its scatter pattern onto a CCD sensor array or detector. A schematic of the microfluidic and optical components of one embodiment of microfluidic cytometer 10 is shown in Figure 1. Laser light can be coupled into microfluidic channel 16 via optical fiber 18. A 2D scatter pattern from a single cell or particle 30 can be obtained by CCD detector 28 via objective lens 26.

[0039] Microfluidic chip 12 can be fabricated by cutting a 600 μm wide channel 14 out from a 100 μm thick polymer sheet. Two additional channels 16 and 20 were cut out on either side of microfluidic channel 14, forming intersection 24, to accept the placement of standard optical fibers 18 and 22, respectively, (such as the 105/125 μm multimode fiber, Thorlab, NJ, USA): one for guiding light from the laser to illuminate a cell (or a particle) 30 and one for guiding laser light out of the channel. A UV-curable epoxy edge-bonding method can be used to sandwich the polymer sheet between two standard microscope slides. A sample solution is diluted to approximately 2000 cells (or

beads) per millilitre to control the cell concentration to one cell within the observation volume. The flow in channel 14 can be pressure-driven by using a syringe. As a cell 30 arrives at intersection 24, it can be immobilized by manipulating the syringe. Coherent light from a 532 nm laser diode (DPSS laser, Laserglow Technologies, ON, Canada) operating at 2 mW can be coupled into one of the fibers to illuminate the cells or beads flowing through the channel. The scattered light can be collected by a microscope objective with an NA of 0.25 onto a CCD detector (ICX204AK, Sony, Japan).

[0040] The detection capability of the LFMC for scattered light angular range can be determined by geometric analysis. Figure 3 is a diagram depicting the propagation of a scattered light ray from cell or particle 30 in the LFMC through a layer of water in channel 14 (100 μm , refractive index 1.334), glass substrate 34 (1.0 mm, refractive index 1.5) and the air (refractive index 1.0), into objective lens 26, with the light ray subsequently detected by CCD detector 28. Note that an objective lens with a NA of 0.25 will limit the angular range that can be detected by CCD sensor. Based on geometric analysis, this 2D cytometer was capable of obtaining light scattered over a 22 degree cone angle (79 degree to 101 degree in polar angle, as compared with the detection of light scattering in a 60 degree cone angle centered around the 90 degree scattering by using the lens-free cytometer [8]).

[0041] In some embodiments, an illustration of an experimental setup of light scattering microfluidic cytometer 10 is shown in Figure 8. In Figure 8(a), the laser light 38 (532 nm, DPSS laser, Laserglow Technologies, ON, Canada) can be coupled into one end of fiber 18 (105/125 μm , Thorlabs, NJ, USA) via 4 $\times$ microscope objective lens 36 with a numerical aperture (NA) of 0.1 into fiber coupler 40. In some embodiments, an

approximately 2 mW input laser power can be used. CCD detector 28 (ICX204AK, Sony, Japan) can be in close contact with microfluidic chip 12 to maximize the observation angle. The imaging system can comprise of microscope objective lens 26 and CCD detector 28 placed on the opposite side of microfluidic chip 12. The imaging system can be used to locate a scatterer of interest. An immobilized single scatterer 30 can be examined by the image system on top of microfluidic chip 12, and CCD detector 29 underneath microfluidic chip 12 can obtain the 2D light scattering patterns. Upper XYZ translation stage 42 can help for coupling laser light 39 into optical fiber 18, while lower XYZ translation stage 43 can be used to locate the single scatterer 30 and to align the system. Figure 8(b) shows a detailed illustration of one embodiment of the sensing area of microfluidic channel 14. Microfluidic channel 14 can be fabricated by sandwiching three layers: a glass slide on the top, a gasket in the middle, and a glass slide with a thin coated chrome film of approximately 80 nm in the bottom. The three layers can be bonded together using UV curable epoxy [46]. The flow in channel 14 can be pressure-driven by using a syringe. As a cell arrives at observation area 44, it can be immobilized by manipulating the syringe to apply positive and negative pressures to the flow. In some embodiments, there can be two fibers: fiber 18 to couple the laser light into microfluidic channel 14 via channel 16 to excite the single scatterer that has been immobilized, and fiber 22 to couple the light that is transmitted through the single scatterer out from channel 14 via channel 20. The couple-out fiber 22 can help to reduce the background noise that is due to scattering from the surroundings, such as that originating from the rough channel edges. In some embodiments, fiber channels 16 and 20 can be perpendicular to the flow direction in channel 14. Microsize

observation window 44 can be located in the center of the 600 μm wide microfluidic channel 14, and can be approximately 400 μm in diameter. Microsize window 44 can help reduce the scattering of the background light in microfluidic channel 14 into CCD sensor 28. The optical fiber can have a NA of 0.22, and the fiber end can be approximately 2 mm from the center of the observation window. The laser beam from the optical fiber can expand to approximately 700 μm while arriving at the observation window area. For a 10 μm cell excited by a 700 μm width beam, a plane wave excitation for the light scattering analysis can be assumed.

[0042] In Figure 9, a further embodiment of light scattering microfluidic cytometer 10 is shown. In this embodiment, cytometer 10 can further comprise neutral density filter 48 disposed between laser 46 and objective lens 36. Laser light 38 emitted from laser 46 passes through filter 48 to become filtered laser light 37 that can enter objective lens 37. Laser light 39 exiting objective lens 37 can couple into fiber 18 via fiber coupler 40. Side scattered light can be detected by CCD detector 28 via objective lens 26 receiving the side scattered light from observation window 44. Forward scattered light can be detected by photodiode detector 50 receiving the scattered light from optical fiber 22 exiting microfluidic chip 12.

[0043] Referring to Figure 16, another embodiment of microfluidic chip 52 that can be used in label-free cytometry is shown. From top to bottom, the first circled region illustrates a hydrodynamic focusing system, which can use higher velocity side flow of fluid to confine the cells to the middle of the microfluidic channel. Specifically, microfluidic chip 52 can comprise coupler 56 further comprising channels 60, 61 and 62 that converge into channel 64. Cells or particles can be injected into channel 60 from

syringe or cell delivery system 54. Higher velocity fluid delivered by syringe or fluid delivery system 58 into channels 61 and 62 can act to confine or center cells arriving into coupler 56

[0044] The second circled region illustrates channel coupler 66 where on-chip waveguides 68, 69, 70, 72 and 74 can be located. One of waveguides 70, 72 and 74 can be used for delivering the laser light to interrogate a cell in microfluidic channel 68 whereas the other waveguides of this group can be used to collect scattering light from the cell. Light can be coupled into or out of waveguides 70, 72 and 74 via couplers 76. A CCD detector with and without an objective lens can be placed on top and bottom, respectively, of second circled region 66 to collect the 2D scattered light pattern. With an objective lens, the collection angle is reduced but the unwanted background noise can also be greatly reduced. The third circled region illustrates channel coupler 78 having a hydrodynamic cell diverter system. Channel 82, shown in the far left, can be a side-flow channel and when a supply of side-flow fluid from syringe or delivery system 86 is activated through tube 84 into channel 82, it can push a cell into channel 92, shown in the far right, through tube 94 to receptacle 98. If the side-flow is not activated, the cell can go to middle channel 88 and received in receptacle 90. As an example, the cell diverter system can be used to sort cancerous and noncancerous cells for further examination.

[0045] In some embodiments, the method of using the microscope-based LFMC to obtain 2D scatter patterns from a single cell can comprise the steps of illuminating the cell, and viewing the illuminated cell perpendicularly with a viewing system and then defocusing the system to obtain a 2D scatter pattern. In Figures 2 (a) and (b),

procedures are shown for obtaining the 2D scatter patterns from a 4 μm polystyrene bead (Invitrogen, CA, USA). In Figure 2(a), an image of a 4 μm bead in the microfluidic channel is shown when the optical system was in focus. In Figure 2(b), a scatter pattern of the 4 μm bead is shown when the optical system was defocused.

[0046] When a single bead is immobilized in the microfluidic channel, the microscope objective can be translated approximately 300 μm from its best focal position to obtain a 2D scatter pattern. At its best focal position, the resolution of the microscope objective is approximately 1.3 μm at 532 nm illumination, according to the Rayleigh criterion. The 2D scatter patterns obtained by defocusing may contain information about the internal nanostructures in a single cell with sizes significantly smaller than 1.3 μm as will be discussed below.

[0047] The numerical 2D scatter patterns can be obtained by using AETHER [7, 26], FDTD numerical code created by the inventors based on Yee's algorithm [23]. The AETHER solved Maxwell's equations and gave numerical solutions for light scattering from a single scatterer, including non-homogeneous, irregular-shaped biological cells [7]. A scatterer was located in a 3D grid and a Liao boundary condition terminated the 3D grid [23]. Because of the small size of cells, the incident laser light can be modelled as a plane wave, propagating along the +z direction and polarized along x direction. The AETHER was implemented in a FORTRAN 90 code and was run on the WestGrid clusters. For a typical 3D simulation of a 10 μm cell in diameter, the simulation required approximately 24 h using 64 GB of memory with a spatial step size of 40 nm.

[0048] In some experiments, the Mie theory to analyze the 2D light scattering patterns obtained from various sizes of polystyrene beads [46]. These 2D light scattering

patterns can have a symmetric fringe structure, which can be due to the homogeneous spherical property of the beads, as also observed by other groups [51]. In some experiments, the light scattering from hematopoietic cells was studied. Compared with polystyrene beads, cells can have various organelles with different optical properties, resulting in a heterogeneous structure. It can be a challenge to apply Mie theory for the exact simulation of light scattering from cells, due to the boundary conditions required to solve the Maxwell's equations. For the study of cells with various organelles, a numerical solution such as the FDTD method can be adopted [52-58].

[0049] A FDTD code (AETHER) was developed for the simulation of light scattering from irregularly-shaped, heterogeneous cells [45,55]. The AETHER FDTD code can solve the Maxwell's equations in a three-dimensional ("3D") grid in the near-field of the scatterer. A Liao absorption boundary condition can be applied to terminate the 3D calculations [59]. In AETHER, a near-field to far-field transformation can be performed so that the experimental light scattering patterns, which are in the far field, can be compared with those from FDTD simulations.

[0050] The inventors performed experiments with various human cells. Platelet concentrates were obtained from donors at the Canadian Blood Services (Edmonton, AB, Canada). In some experiments, the human hematopoietic cell lines Jurkat (acute T-cell leukemia) and THP-1 (acute monocytic leukemia) were obtained from the American Type Culture Collection (Rockville, MD, USA) and grown in RPMI media (Invitrogen, Burlington, ON, Canada) supplemented with 10% bovine growth serum (BGS, Hyclone, ThermoFisher Scientific, Nepean, ON, Canada).

[0051] In other experiments, cord blood ("CB") was obtained with mothers' informed consent after delivery, all in accordance with the guidelines approved by the University of Alberta Health Research Ethics Board. Light-density mononuclear cells ("MNC") were separated by centrifugation using a 60% Percoll density gradient (1.077 g/mL, Amersham, Uppsala, Sweden). CD34⁺ cells were isolated from the light-density MNC interphase using the Miltenyi MACS system (Miltenyi Biotec, Auburn, CA, USA) as described previously [50]. Lymphocytes were obtained from whole blood after density gradient centrifugation using Lymphocyte-poly (1.113 g/mL, Cedarlane Laboratories, Hornby, ON, Canada) according to the manufacturer's instructions. Cord blood hematopoietic stem/progenitor cells ("HSPC"), which express the CD34 antigen, were separated by immunomagnetic selection using MACS technology according to the manufacturer's instructions (Miltenyi Biotec, Auburn, CA, USA). Cord blood CD34⁺ cells were also *ex vivo* expanded and differentiated towards the myeloid lineage in a serum-free liquid culture (StemSpan, StemCell Technologies Inc., Vancouver, British Columbia, Canada) in the presence of recombinant human (rh) interleukin-3 (10 ng/mL) and rh granulocyte macrophage-colony stimulating factor (5 ng/mL) (both from Peprotech, Rocky Hill, NJ, USA). Cell cultures were incubated at 37°C in a humidified atmosphere supplemented with 5% CO₂ for up to 11 days (CFU-GM cells). Under these conditions, on day 11, almost 100% of cells expressed CD33, a marker of the myeloid lineage. In some experiments, Jurkat and CD34⁺ cells were suspended (0.5 x 10⁶/mL) in pre-warmed (37°C) RPMI medium containing 500 nM Mito Tracker Red (M-7512, Molecular Probes, Invitrogen) and incubated for 30 min at in a humidified atmosphere at 37°C and 5% CO₂. After incubation, the cells were plated on poly-L-lysine (Sigma, St.

Louis, MO, USA)-coated cover glass slips. The cells were fixed with freshly prepared 3.7% paraformaldehyde (Sigma) and permeabilized by incubating in 0.2% Triton X-100 (EMD Chemicals, Gibbstown, NJ, USA) for 5 min. The nucleic acid stain SYTOX Green (Molecular Probes) was then added (100 nM). The slide was kept covered in foil until confocal microscopic analysis.

[0052] Results and Discussion

[0053] The detection capability of the LFMC for scattered light angular range can be determined by geometric analysis. Figure 3 is the schematic of the scattered light propagation in the LFMC. The light scattered from a single scatterer can propagate through a multi-layer of medium onto the CCD detector. The scattered light angular range that can be detected is determined by the medium that the light propagates and by the numerical aperture of the objective lens.

[0054] As shown in Fig. 3, the scattered light propagates through a layer of water (100 μm , refractive index 1.334), a glass substrate (1.0 mm, refractive index 1.50) and the air (refractive index 1.0) into the microscope lens, and is subsequently detected by a CCD sensor. Note that the microscope lens with an NA of 0.25 can limit the angular range that can be detected by the CCD sensor. Based on geometric analysis, it was found that this 2D cytometer is capable of obtaining light scattering from a 22 degree cone angle (79 degree to 101 degree in polar angle, as compared with the detection of light scattering in a 60 degree cone angle centered around the 90 degree scattering by using a lens-free cytometer [8]).

[0055] In order to demonstrate that the LFMC can obtain the light scattering patterns, experiments were first performed using the standard 4 μm and 9.6 μm diameter

polystyrene beads (Invitrogen, CA, USA). Figures 4 (a) and (b) show the 2D scatter patterns from the 4 μm and 9.6 μm beads, respectively. Figures 4 (c) and (d) show simulated scatter patterns for the 4 μm and 9.6 μm beads, respectively. The collection angles for the scatter pattern are from 79 to 101 degrees.

[0056] Finite-difference time-domain light scattering simulations can be performed on these beads. They have included far-field transformation of the scattered light and the angular range is determined by ray tracing simulations to describe light propagation in the optical elements of the cytometer, cf. Figure 3. The refractive index used for the beads was 1.591 at laser wavelength of 532 nm. The surrounding medium was assumed to have a refractive index of 1.334. Figures 4(c) and (d) show the AETHER generated 2D scatter patterns for the 4 μm and 9.6 μm beads collected in a 22 degree cone angle, respectively. From both experimental and simulation results, four fringes for the 4 μm bead, and nine fringes for the 9.6 μm bead were observed. Good agreement between experimental and simulation results confirms that the LFMC obtained light scattering patterns in a cone angle of approximately 22 degrees, which is in agreement with the geometric analysis as described above.

[0057] This 2D LFMC has potential for label-free single cell characterizations. The inventors expect the 2D scatter pattern to contain significant information on the internal organelles in a single biological cell, such as the cell nucleus, mitochondria, and cytoplasm. These organelles are the main contributors to the total light scattering from biological cells. Other organelles such as ribosome or lysosome contribute less to the total scattering due to their smaller volume fraction in the biological cells [7, 17]. Among the blood cells, some have many organelles (e.g., white blood cells), some have no

nucleus (e.g., red blood cells and platelets), and some have few mitochondria (e.g., platelets). Hence, it is of interest to study representative cell types using this 2D LFMC. The blood cells the inventors chose to study are human platelets, CB CD34+ and CFU-GM cells.

[0058] The inventors' second objective was to verify whether the LFMC could be used to obtain 2D light scattering patterns from the smallest human blood cells. Platelets are the smallest mature cells in human blood, a disc-shaped cell with a diameter of about 3 μm and a thickness of about 1 μm . The experimentally obtained platelet scatter patterns, as shown in Figs. 5(a), (b) and (c), which were compared with the AETHER light scattering simulations. Figs. 5(a), (b) and (c) show the representative experimental 2D scatter patterns from platelets. Figs. 5(d), (e) and (f) show the cell models for platelets at different orientations. Figs. 5(g), (h) and (i) show the AETHER 2D scatter patterns. The platelet scatter patterns have fringe structures (Figs. 5(a), (c), (g) and (i)) from the cell microstructures or no fringes (Figs. 5(b) and (h)) when the effective cell microstructure is small.

[0059] For the simulation of platelets, the cell was assumed to be ellipsoidal (Fig. 5(d)), the projection on the xz plane being a circular region with a 3 μm diameter, and the projection on the yz plane being an ellipse with a minor axis along y of 1 μm and a major axis along z of 3 μm . The direction of fluid flow and laser propagation were assumed to be along the x axis and z axis, respectively. It was assumed that there are three spherical mitochondria in the platelet model, each with a diameter of 500 nm. The refractive index of the mitochondrion was 1.42 inside a cell with a refractive index of 1.38 [7]. The surrounding medium had a refractive index of 1.334. Figure 5 (e) shows a

platelet with a different orientation in the microfluidic channel, rotated 90 degrees counter clockwise from the previous case (Figure 5(d)). Figure 5(f) shows a platelet with an orientation in between these two cases. In Figures 5(d), (e) and (f), the different cell components are shown in different colors: the cell cytoplasm is magenta and the mitochondria are blue. The corresponding AETHER 2D scatter patterns for the platelet models (d), (e) and (f) are shown in Figures 5(g), (h) and (i), respectively. The experimental results agree reasonably well with the AETHER simulations. Since the cell microstructures (nucleus and cytoplasm) give fringe structures in 2D scatter patterns [7] and the platelets have no nucleus, the platelet cell cytoplasm is the main contributor to the 2D scatter patterns of the platelets. The fringe numbers increase when the effective size (size of the platelet projection along the z axis) of the platelet increases. Three fringes (Figure 5(g)), two fringes (Figure 5(i)), and no fringe (Figure 5(h)) were predicted for different orientations. For the experimental platelet scatter patterns with the 3 fringes (see Figure 5(a)) and with no obvious fringe (see Figure 5(b)), according to simulations operated by the inventors, the 3 fringes correspond to an effective size of $3\ \mu\text{m}$, while the scatter pattern with no obvious fringe is for an effective size of $1\ \mu\text{m}$. This agrees with the size parameters for platelets, which have a disc-shape structure with the disc diameter of $3\ \mu\text{m}$ and a thickness of $1\ \mu\text{m}$. The inventors have also obtained platelet scatter pattern with two fringes (Figure 5(c)), which may be explained with the cell model of Figure 5(f) that gives a scatter pattern as in Figure 5(i). The study of cell orientation are important for the understanding of light scattering from biological cells [28] and the active response of biological cells to external stimuli [29].

The light scattering method shown here can be used for the label-free determination of cell orientation in a microfluidic flow.

[0060] The inventors have shown in their previous publication [8] that the highest dominant frequency component in the Fourier spectra of the light scattering signal can be used for size determination of cells. Alternatively, as shown in this report, one can simply count number of diffraction fringes in order to determine the size of beads (Figure 4) or in case of the platelets (Figure 5) to gain information about the orientation of cells. The FFT based method [8] can also be successfully applied for the size determination of plastic beads at different flow velocities in a recent publication from another group [22]. The results in Figure 5 show that the orientation effects should be considered when using the fringes of the scatter patterns for size determination of a single scatterer.

[0061] Since the scatter light intensity increases with the size of a cell, the successful detection of platelets indicating the microscope-based label-free cytometer device is capable of measuring the scatter patterns from many human blood cells. The next objective was to obtain scattered light patterns from larger blood cells with many mitochondria, as the mitochondria have been reported as controlling apoptosis and are significant for increasing our understanding of diseases such as cancer [30].

[0062] Figure 6(a) shows the representative experimental scatter pattern from a CFU-GM cell obtained by using the LFMC. Figure 6(a) shows a representative experimental scatter pattern from a CB CD34+ cell. Figure 6(b) shows a CFU-GM cell scatter pattern. Figures 6(c) and (d) show the cell models for the CFU-GM cell and CB CD34+

cells, respectively. Figures 6(e) and (f) show the AETHER 2D scatter patterns of the cell models (c) and (d), respectively.

[0063] The CFU-GM cells were harvested on day 11, which are representative as well-developed cells. In order to determine the scatter patterns from the CB CD34+ cells, experiments were run on controlled CB CD34+ samples with a purity of approximately 90%. For the scatter patterns obtained from the 90% purity sample, scatter patterns were obtained similar to Figure 6(b) (85%) and those similar to Figure 6(a) (~15%). There were no scatter patterns similar to platelets obtained from the CB CD34+ sample. Notice that the CB stem cells can differentiate into many cell lines, such as the CFU-GM, which can give a scatter pattern similar to Figure 6(a). The inventors believe that the majority of the pure CB CD34+ cells will give scatter pattern as shown in Figure 6(b), which is representative for the CB CD34+ cells.

[0064] The scatter patterns from CFU-GM and CB CD34+ cells (Figure 6) are quite different as compared with the platelet scatter patterns. The CFU-GM and CB CD34+ cell scatter patterns are dominated by small-scale 2D structures in contrast to the fringe patterns observed from the platelets. The difference between the platelet scatter patterns and those of the CFU-GM and the CB CD34+ cells can be explained with the recently reported results, where the homogeneous microstructures in biological cells give 2D fringe scatter patterns and the randomly distributed nanometer scale mitochondria generate the small scale 2D structures. [7].

[0065] A simple cell model was used for the AETHER simulation of the light scattering from the CFU-GM cells (Figure 6(c)). In the model, a CFU-GM cell was assumed to be a 10 μm diameter sphere with a 6 μm diameter nucleus (in cyan) located at the center

of the cell. There were also 120 mitochondria arbitrarily distributed inside the cell, each with a diameter of $1\text{ }\mu\text{m}$. The refractive index for the CFU-GM cell plasma, nucleus, and mitochondria were assumed to be 1.35, 1.39, and 1.42, respectively [7]. Figure 6(e) shows the simulated scatter pattern for the CFU-GM cell model (Figure 6(c)), which qualitatively agrees with the experimental results (quantitative analysis was performed as shown in Figure 7). Both the experimental and simulation scatter patterns are dominated by the small scale 2D structures, showing that the mitochondria can be the main contributors to the 2D scattered light patterns of the CFU-GM cells.

[0066] However the inventors have noticed the difference between the scatter patterns of the CFU-GM cell (Figure 6(a)) and the CB CD34+ cell (Figure 6(b)). As the homogenous nucleus in a biological cell will give fringe patterns as for the beads and the platelets, the inventors postulate that the difference between Figures 6(a) and (d) can be due to the mitochondria distributions. Because the randomly distributed mitochondria are the main contributors for the 2D structures in Figures 6(a) and (b), a simple cell model was used for the CB CD34+ cells where we consider only the contributions from the aggregated mitochondria. In Figure 6(d), there are 70 mitochondria with a diameter of $1\text{ }\mu\text{m}$ and a refractive index of 1.42, arbitrarily distributed in an ellipsoid centered at the origin with two long axes of 8 μm , and a short axis of 4 μm . The short axis is in the yz plane and rotated 45° from the $+z$ axis. Figure 6(f) shows the AETHER 2D scatter pattern for the CB CD34+ cell model (Figure 6(d)), which agrees well with the experimental CB CD34+ cell scatter pattern as will be analyzed in Figure 7.

[0067] So far, the inventors have shown scattered light diffraction fringes from beads or platelets and have further discussed the methods of determining the particle size or cell orientation. Note that the beads or platelets can be treated as homogenous spheres or ellipsoids, thus giving the regular 2D fringe patterns. When the scattered light angular spectrum is dominated by irregularly distributed small-scale 2D structures as in Figure 6, corresponding to the scatter pattern produced by mitochondria, the real space analysis of the intensity maxima may become very effective. Set out below, a method is shown for cell differentiations by analyzing the 2D scatter patterns in spatial domain.

[0068] The inventors analyzed the 2D scatter patterns in Figure 6. In Figure 7, two parameters can be used for the cell discrimination by using the label-free light scattering method. In this figure, the normal CB CD34+ cells may be discriminated from the well developed CFU-GM cells. One parameter is the number of the local 2D intensity maxima in a 2D scatter pattern, and the other parameter is the averaged area for the total small-scale 2D structures. In Figure 7, the inventors performed a same analysis for scatter patterns of 10 different CB CD34+ cells and 10 CFU-GM cells. The open triangles and open squares are for the CB CD34+ cells and the CFU-GM cells, respectively. The solid signs indicate the averaged values for both groups of cells. It is noticed that the CB CD34+ cells are distinctive from the CFU-GM cells. The CB CD34+ cells are with an averaged value at (6, 1.7545) with a standard deviation (SD) of (1.3499, 0.4601) for the number and the integrated area, respectively. For the CFU-GM cells, the average value is (23, 0.8045) with an SD of (7.7488, 0.2561). Compared with the CFU-GM cells, there are less small-scale 2D structures and the averaged area of these small-scale 2D structures is larger in the CB CD34+ cells. The AETHER results

are shown in Fig. 7 as plus and cross signs for the CFU-GM cell and the CB CD34+ cell, respectively. The parameters for the CFU-GM cell pattern (Figure 6(e)) are (23, 0.8525), and those for the CB CD34+ cell pattern are (8, 1.8425). The AETHER simulation results agree well with the statistical results from the experimental patterns for both the CFU-GM cells and the CB CD34+ cells. The results show that, by analyzing the 2D scatter patterns in real space, it may be possible to discriminate the cell lineages of the stem cell differentiation. To do so, the study of the biological cells, especially for physiological determination, would require a large sample of cells.

Comparison of the 2D experimental light scattering patterns with AETHER simulations

[0069] Recent studies have experimentally demonstrated that the mitochondria can be the main contributors for light scattering from cells [39, 45, 60]. Mitochondria have been implicated in many diseases such as cancer, Alzheimer's and Parkinson's [61, 62], hence understanding light scattering from mitochondria in single cells is of value in the future treatment of these diseases [63, 64]. Mitochondria are organelles of nanometer to micrometer size and may be distributed throughout a cell. Because of the diffraction limit, optical microscopy cannot produce a well-resolved image of mitochondria. However, information about mitochondria can be obtained from studying the 2D light scattering patterns from single cells [45]. In some embodiments, a detailed analysis of AETHER simulations can be used to determine the various mitochondrial contributions to the 2D light scattering patterns. The experimental 2D light scattering patterns from Jurkat cells and CD34+ cells can then be compared with simulations. In the simulations, simplified artificial models for optical properties of cells can be used. In order to confirm

that the choice of these models is reasonable, laser scanning confocal fluorescence imaging of the Jurkat and the CD34+ cells can be performed (see below).

[0070] In order to study the mitochondrial contributions to the 2D light scattering patterns, the Jurkat cell can be assumed to be spherical with randomly distributed mitochondria, and a nucleus located at the center. The cytoplasm (as shown in Figure 10, in the cell models) can have a refractive index of 1.35 with a cell diameter of 12 μm . The nucleus diameter can be 8 μm , with a refractive index of 1.39. The refractive index for the mitochondria can be 1.42. The surrounding medium can have a refractive index of 1.334. These refractive index values of the cell components are based on recent publications [39,40,45,53,55,60].

[0071] Referring to Figure 10, images (a), (b), (c), (d), (e), (f), (g), and (h) are cell models used for the AETHER simulations and images (a'), (b'), (c'), (d'), (e'), (f), (g') and (h') are the corresponding 2D light scattering patterns. There are 15, 30, 60, 90 and 120 mitochondria of diameter 1 μm in images (a), (b), (c), (d), and (e), respectively. In image (f), there are 35 mitochondria of diameter 1.5 μm . In image (g), there are 20 mitochondria of diameter 1.8 μm . In image (h), there are 15 mitochondria of diameter 1.8 μm . Figures 10 (e), (f), and (g) have approximately the same total volume of mitochondria. Figures 10 (d) and (h) have approximately the same total volume of mitochondria. The scale bar for the cell model is 1 μm , and is 500 μm for the 2D light scattering patterns.

[0072] For the simulations in this disclosure, an incident wavelength of 532 nm was used. The 2D light scattering patterns can be obtained by assuming that the cell models are in a microfluidic channel as shown in Figures 1, 8 or 9. The scattered light

from a single cell can travel through a layer of water (100 μm , refractive index 1.334), a glass substrate (1.1 μm , 1.47), a layer of air (300 μm , 1.0), the CCD cover glass (0.75 μm , 1.5), and another layer of air (1.25 μm , 1.0) onto a CCD sensor (Silica, refractive index 4.15). The light scattering pattern can have a dimension of about 2.0 mm horizontally, which can correspond to the light scattering in a 30-degree cone angle (from 75 to 105 degrees in polar angle).

[0073] The various optical properties models for Jurkat cells and their corresponding simulated 2D light scattering patterns are shown in Figure 10. In Figures 10(a) to (e), the mitochondria number is increased from 15, 30, 60, 90, to 120, while keeping the size (1.0 Mm) unchanged. From Figure 10(a'), it is noticed that the 2D light scattering patterns can have both the small-scale 2D structures (blob-like structures) and the fringes. When the number of mitochondria changes to 30, similar light scattering patterns (Figure 10(b')) can be obtained as in Figure 10(a'). However, when the number of mitochondria increases to 60 or more in Figures 10(c'), 10(d') and 10(e'), only small-scale 2D structures can be obtained. In Figures 10(e), 10(f) and 10(g), the volume of the mitochondria is fixed but with varying size and number. There are 35 mitochondria with a diameter of 1.5 μm in Figure 10(f), and 20 mitochondria of a diameter of 1.8 μm (Figure 10(g)). We observe that they give similar small-scale 2D structure patterns. When we reduce the mitochondria number from 20 (Figure 10(g)) to 15 (Figure 10(h)), the 2D light scattering patterns are still dominated by the small-scale 2D structures.

[0074] It was observed that when the mitochondria volume fraction in a cell is above a certain value, here 3.5% (Figure 10(d)), the cell light scattering pattern will be dominated by small-scale 2D structures. In typical cells, the mitochondria volume

fraction is above 5%, thus it is reasonable to expect small-scale 2D structures to be present in the experimental light scattering patterns providing the mitochondria are main contributors to the 2D light scattering patterns. It is understood that real cells have much more complex organelle distributions than what we assumed here in Figure 10. However, given that the indices of refraction for mitochondria typically have the largest values among the cellular organelles, simplified cell models can be used to perform simulations. It was also observed that when the volume fraction of the mitochondria is fixed, the simulated 2D light scattering patterns are insensitive to the number of mitochondria (see Figures 10(e'), 10(f) and 10(g')). No significant change of the light scattering patterns was observed even after the volume fraction is changed by 25% (see Figure 10(g') and Figure 10(h')). These observations from the FDTD simulation results can help understand the difference between the 2D light scattering patterns from the Jurkat and the CD34+ cells.

[0075] The comparisons between the experimental and simulated 2D light scattering patterns from the Jurkat and CD34+ cells are shown in Figure 11. Figures 11(a) and (b) show the representative experimental 2D light scattering pattern from a Jurkat cell and a CD34+ cell, respectively. In Figure 11(a), the light scattering pattern is dominated by the many small-scale 2D structures. In Figure 11(b), the size of the 2D structures increases and their number decreases as compared with Figure 11(a). From the detailed numerical study discussed above, it was found that the light scattering patterns can be insensitive to the change of mitochondria number and volume. The experimental light scattering pattern of a Jurkat cell (Figure 11(a)) is similar to the simulated light scattering patterns shown in Figures 10(e)-(h). However, the

experimental light scattering pattern of a CD 34+ cell (Figure 11(b)) is significantly different from those shown in Figures 10(e)-(h) in terms of both the number and the sizes of their small-scale 2D structures.

[0076] Figures 11(c) and (d) are the simplified optical properties models for the two cells. In this figure, the cytoplasm is $12\text{ }\mu\text{m}$ in diameter and the nucleus is $8\text{ }\mu\text{m}$ in diameter. In Figure 11(c), there are 120 mitochondria, each with a diameter of $1\text{ }\mu\text{m}$. In Figure 11(d), there are 72 mitochondria, each with a diameter of $1\text{ }\mu\text{m}$. Figures 11(e) and (f) represent the 2D FDTD light scattering patterns for the cell models in Figures 11(c) and (d), respectively.

[0077] In some embodiments, the differences in light scattering patterns between Jurkat and CD34+ cells can be attributed to their mitochondrial distributions. Figure 11(d) shows a cell model for CD34+ cell. In this model, we assume there are 72 mitochondria with a diameter of $1\text{ }\mu\text{m}$, aggregated in an ellipsoid with two long axes of $8\text{ }\mu\text{m}$ along x and y , and a short axis of $4\text{ }\mu\text{m}$ along z . The corresponding simulated 2D light scattering pattern is shown in Figure 11(f). The simulated 2D light scattering pattern reproduced the key features of the experimental 2D light scattering pattern of a CD34+ cell. To further explore the effects of mitochondrial aggregation on the 2D scatter patterns, the aggregated mitochondria in Figure 11(d) can be re-distributed randomly as shown in Figure 12(a). Figure 12(b) shows the corresponding simulated 2D light scattering pattern for randomly distributed mitochondrial model of Figure 12(a). Figure 12 shows the same number of mitochondria as in Figure 11(d) but in this case, the mitochondria are randomly distributed throughout the entire cell, while in Figure 11(d), the mitochondria are aggregated.

[0078] The randomly distributed mitochondria can give a pattern similar to those of Jurkat cell light scattering patterns. The above analysis indicates that the difference between the experimental scatter patterns of Figures 11(a) and (b) may be due to how the mitochondria are distributed within the cells as suggested by our numerical study. In particular, the numerical simulations suggest that the mitochondria in a Jurkat cell would have a random distribution and an aggregated distribution for a CD34+ cell.

Laser Scanning Confocal Fluorescence Imaging of Cells

[0079] In the above disclosure, the light scattering patterns from Jurkat and CD34+ cells were compared with the experimental results from FDTD simulations. The results suggested that the mitochondrial distribution for a CD34+ cell may be aggregated while that for a Jurkat cell may be random.

[0080] Figure 13 shows the laser scanning confocal fluorescence imaging of Jurkat and CD34+ cells. The experiments were performed on a Fluoview300 confocal microscope (Olympus, Germany). The cells were labeled for nucleus and mitochondria, and a sequential scanning of the cells was performed. Shown in Figure 13(a) is the confocal image of the labeled mitochondria in a Jurkat cell, and Figure 13(b) is the labeled nucleus in the Jurkat cell. Figure 13(c) is the overlay of Figures 13(a) and (b). Similarly, Figures 13(d), (e) and (f) show the results for the CD34+ cell. From the confocal imaging results, it was observed that the mitochondria in the Jurkat cell are randomly distributed in the whole cell. However, in the CD34+ cell, there are aggregated mitochondria in the lower part of the cell. The phenomenon of aggregated mitochondria in cells has also been observed by others in the study of cell apoptosis [65-67].

Analysis of the 2D Light Scattering Patterns

[0081] In conventional flow cytometry, the fluorescence signals from the labeled organelles inside cells are used for cell sorting and cell determination. The results set out in this disclosure have shown that the light scattering patterns from different cells can be distinctive and that useful parameters may be obtained by analyzing the 2D light scattering patterns. It is expected that these parameters or observables may have similar functions as those obtained in commercial flow cytometers, but with the advantage of being label-free. Consequently, 2D light scattering patterns can be analyzed to extract characteristics that can further be used for cell discrimination.

[0082] In some embodiments, a speckle analysis of the obtained 2D light scattering patterns can be adopted. Whenever a coherent light of a laser probe is scattered by randomly distributed centers, the light collected on the 2D screen of a CCD sensor can form interference pattern that varies randomly in space and is known as speckle (more precisely, the maxima, here, the small-scale 2D structures are 2D cross-sections of the speckles, which are 3D objects). A comparison between the spectra in Figures 11(e) and 11(f) shows how transition from the randomly distributed mitochondria in Figure 11(c) to the aggregated distribution of mitochondria in 11(d) modifies speckle distributions. The random pattern of Figure 11(e) has evolved towards the interference pattern Figure 11(f) reminiscent of the scattering on the large structures such as cell cytoplasm or nucleus. Comparisons between Figure 11(f) and Figure 12(b) demonstrate how the speckle patterns can be formed with the increasing randomness of the mitochondria distributions.

[0083] The statistical properties of these 2D speckle cross-sections can be used in the quantitative analysis of the numerical and experimental pattern of scattered light. In some embodiments, a method can comprise the steps of first counting how many local intensity maxima are in each 2D light scattering pattern, and then calculating their average area. These two observables, i.e., the number of speckles and the average area of their cross-sections can be used as parameters for cell determination.

[0084] Figure 14 shows the analysis of the 2D light scattering patterns in Figures 11(a), (b), (e) and (f). For the Jurkat cell experimental light scattering pattern (Figure 14(a)), there are 46 speckles in total with an average area of 0.0037 mm^2 . In Figure 14(b), the CD34+ cell experimental light scattering pattern has 9 local maxima with an average area of 0.0146 mm^2 . The simulated light scattering patterns using the Jurkat cell model (Figure 14(c)) produces 43 speckles with an area parameter of 0.0084 mm^2 , while in the CD34+ cell model (Figure 14(d)), there are 9 maxima with an average area of 0.0131 mm^2 .

[0085] The parameters obtained from different cells were plotted in Figure 15, which reveals that CD34+ cells and Jurkat cells can form well-separated clusters in the plots, similar to a conventional flow cytometric plot. This can be due to the fact that CD34+ cells and Jurkat cells are very different cells. The AETHER simulation results for the CD34+ and the Jurkat cell models are in reasonable agreement as compared with the experimental results. In this case, the speckle number and the average area for their cross sections in the 2D light scattering patterns may be used for cell determination. Since the CD34+ cells are normal cells, while the Jurkat cells are malignant cells, the obtained results agree with the recent studies showing that in normal cells the

mitochondria aggregate, causing apoptosis, while in malignant cells the mitochondria are randomly distributed in the whole cells [65-67]. The method presented herein can detect those two mitochondrial distributions in single cells in a label-free manner.

[0086] Results obtained from acute monocytic leukemia THP-1 cells and normal lymphocytes are also shown in Figure 15. It was found that the THP-1 cell results can form a cluster next to the cluster of the Jurkat cell results, while the cluster of the normal lymphocytes can be located in between those of CD34+ cells and the THP-1 cells. The FDTD result for the 72 randomly distributed mitochondria (Figure 12(a)) correlates well with the THP-1 cell results. Inclusion of these malignant and normal cells illustrates the value of the method developed here for its use in future discrimination between these various cell types.

[0087] Figure 15 also shows the mean and the standard deviation (SD) for Jurkat cells, THP-1 cells, normal lymphocytes and CD34+ cells as open triangles with different orientations. The Jurkat cells have 43 ± 7 maxima, with an average area of $0.0038 \pm 0.0008 \text{ mm}^2$. The THP-1 cells have 30 ± 9 speckles and the average cross-sectional area is $0.0047 \pm 0.0008 \text{ mm}^2$. The speckle number and the average cross-sectional area for the CD34+ cells are 7 ± 3 , and $0.0153 \pm 0.0042 \text{ mm}^2$, respectively. Parameters for normal lymphocytes are 19 ± 3 , and $0.0061 \pm 0.0016 \text{ mm}^2$. Thus, generally speaking, the normal cells (CD34+ cell and normal lymphocytes) can be discriminated from leukemic cells (Jurkat and THP-1 cells).

[0088] In summary, a microscope-based label-free microfluidic cytometric (LFMC) technique is provided that can be used to obtain 2D scattered light patterns from a wide range of blood cells. The comparisons between the AETHER generated and the

experimental polystyrene bead scatter patterns showed that the microscope-based LFMC can be used for accurate cell size determination. To achieve such a good agreement between simulations and experimental data, the inventors have also introduced into AETHER the optical ray tracing subroutine that models light propagation in the optical system of the cytometer [7]. Using this LFMC technique, the inventors can obtain 2D scatter patterns from platelets, CFU-GM cells and CB CD34+ cells. The 2D scatter patterns from the platelets (the smallest mature human blood cells) have been used for the determination of the platelet cell orientation in a microfluidic channel. The nanoscale organelle information (for example, mitochondria) in CB CD34+ cells and the CFU-GM cells can be identified by using the LFMC, which may not be achieved by using the 10x objective lens according to the Rayleigh criterion. Analyzing the experimental CB CD34+ and CFU-GM cell scatter patterns showed that the determination of stem cell differentiation can be achieved by using our LFMC technique.

[0089] In some embodiments, the experimental setup can be simple and compact: it can comprise a CCD detector, a microfluidic chip, a diode laser source coupled into the channel by an optical fiber and an objective lens. These equipment components are standard in most laboratories, so the set-up is widely accessible. The microscope-based LFMC technique also eliminates the necessity of the microfabrications for the detection of the elastically scattered light. This inexpensive LFMC may be of interest to researchers studying the inner structures of live cells. Future development of the LFMC may lead to a new generation of cytometers that could have applications in medicine, including the study of stem cell differentiation or early detection of malignant cells.

[0090] In addition, a label-free technique based on 2D light scattering patterns for the determination of mitochondrial distributions in single cells in a microfluidic platform is provided. The acquisition of 2D light scattering patterns can be achieved by employing a microfluidic cytometer in which laser light was fiber-coupled into the microfluidic channel. Experimental 2D light scattering patterns from different cells were compared with those obtained from the simulations using our AETHER FDTD code. The results suggest that the different experimental light scattering patterns obtained for Jurkat and CD34+ cells may be due to the fact that the mitochondria are randomly distributed in Jurkat cells, while they are aggregated in CD34+ cells. The cell models used in the simulations can be good mimics as confirmed by the confocal fluorescence cell images. The label-free technique presented herein cell determination can be based on two observables, namely, the number of the speckles, and their average cross-sectional area in a 2D light scattering pattern. It has been presented that this method can be used for the discrimination between normal hematopoietic cells (CD34+ cells and lymphocytes) and leukemic cells (Jurkat and THP-1 cells). In some embodiments, this technique can be used in the physiological monitoring of human blood cells in clinics for detection of hematologic malignancy.

[0091] In some embodiments, the apparatuses and methods described herein can be used to count the number of cells or particles in a given volume of fluid to determine the concentration level of the cells or particles. In other embodiments, the apparatus and method can be used to determine the size of cells or particles. In further embodiments, digital imaging hardware and software as well known to those skilled in the art can be used to interpret electronic signals produced by the CCD in response to scattered light

striking the incident surface of the CCD and produce images of patterns produced by the scattered light striking the incident surface of the CCD. This can produce data in the form of scattered light patterns, which can be compiled in a database as a form of "scattered light pattern signature" of healthy biological cells. In some embodiments, the signatures can be taken throughout the life cycle of a healthy biological cell so as to catalogue the cell lineage process. For example, it is known that stem cells can develop into other cells. The apparatuses and methods described herein can be used in some embodiments to determine the type of cell, and can be further used to determine what type of cell a stem cell is transforming into.

[0092] In other embodiments, the signatures can be taken of unhealthy or diseased biological cells throughout the life cycles of the cells. In so doing, a database can be compiled of diseased cells that can be used as part of a clinical diagnostic procedure in determining whether biological cells taken from a patient are healthy or diseased. In some embodiments, the apparatus and method can be used to detect cancerous cells. In other embodiments, the apparatus and method can be used to detect other disease cells.

[0093] In further embodiments, the apparatus and method can be used as a teaching tool for educational purposes. The simplicity of some embodiments of the apparatuses and methods disclosed herein enable the apparatus to be a relatively low-cost device that can be used in schools in teaching science to young students. In other embodiments, the apparatus and method can be used as a tool in research of biological cells. In further embodiments, the apparatus and method can be used as a clinical diagnostic tool by health care professionals on patients.

[0094] While the apparatuses and methods described herein refer to using a CCD to produce 2D scattered light patterns, it is obvious to those skilled in the art that two or more CCDs can be used to produce 3D scattered light patterns, which can provide further information and data in respect of the applications and embodiments of the apparatus and method.

[0095] In some embodiments, the apparatuses and methods described herein in respect of label-free cytometry can be used to measure the orientation of a non-spherical cell flowing down a fluidic channel of such an apparatus, as has been demonstrated in respect of platelets.

[0096] In some embodiments, the apparatuses and methods described herein in respect of label-free cytometry can be used to distinguish the various mitochondria distributions in single cells without labelling.

[0097] Although a few embodiments have been shown and described, it will be appreciated by those skilled in the art that various changes and modifications might be made without departing from the scope of the invention. The terms and expressions used in the preceding specification have been used herein as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding equivalents of the features shown and described or portions thereof, it being recognized that the scope of the invention is defined and limited only by the claims that follow.

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WE CLAIM:

1. A method for carrying out microscope-based label-free cytometry, the method comprising the steps of:
 - a) providing an apparatus, comprising:
 - i) a microfluidic chip further comprising a channel disposed thereon, the channel substantially parallel to a top surface of the microfluidic chip,
 - ii) an optical port disposed on the microfluidic chip, the optical port substantially parallel to the top surface, the optical port intersecting the channel thereby defining an observation point at the intersection thereof,
 - iii) a light source configured to emit collimated visible or near-visible light,
 - iv) means for operatively coupling the light source to the optical port wherein at least some light emitted from the light source passes through the coupling means to illuminate the observation point,
 - v) a light detection device disposed adjacent to the observation port, the light detection device comprising an incident surface, the light detection device configured to produce an electrical signal in response to light striking the incident surface, and
 - vi) means for directing light scattered from a biological cell or a particle disposed at the observation point onto the light detection device;

- b) passing the biological cell or particle through the channel to the observation point;
 - c) illuminating the observation point with light emitted from the light source; and
 - d) detecting light scattered from the biological cell or particle with the light detection device.
2. The method as set forth in claim 1, further comprising the step of creating a scatter pattern from the electrical signal produced in response to the scattered light detected by the light detection device.
 3. The method as set forth in claim 2, wherein the scatter pattern comprises a two-dimensional scatter pattern.
 4. The method as set forth in any one of claims 1 to 3, further comprising the step of passing the biological cell or particle through the channel suspended in a fluid.
 5. The method as set forth in claim 4, wherein the fluid comprises an aqueous solution.
 6. The method as set forth in any one of claims 1 to 5, further comprising the step of focusing the biological cell or particle as it passes by the observation point.
 7. The method as set forth in claim 6, wherein the focusing step further comprises hydro-dynamically focusing the biological cell or particle.
 8. The method as set forth in any one of claims 1 to 7, wherein the directing means comprises an objective lens disposed between the observation point and the light detection device, the objective lens configured to direct light scattered from the biological cell or particle onto the light detection device.

9. The method as set forth in any one of claims 1 to 8, wherein the light source comprises a laser light source.
10. The method as set forth in any one of claims 1 to 9, wherein the coupling means comprises an optical fiber.
11. The method as set forth in any one of claims 1 to 10, wherein the incident surface of the light detection device is substantially parallel to the top surface.
12. The method as set forth in any one of claims 1 to 11, wherein light detection device comprises a charged coupled device ("CCD").
13. A method for producing a scatter pattern from a biological cell or particle, the method comprising the steps of:
 - a) providing an apparatus, comprising:
 - i) a microfluidic chip further comprising a channel disposed thereon, the channel substantially parallel to a top surface of the microfluidic chip,
 - ii) an optical port disposed on the microfluidic chip, the optical port substantially parallel to the top surface, the optical port intersecting the channel thereby defining an observation point at the intersection thereof,
 - iii) a light source configured to emit collimated visible or near-visible light,
 - iv) means for operatively coupling the light source to the optical port wherein at least some light emitted from the light source passes through the coupling means to illuminate the observation point,

- v) a light detection device disposed adjacent to the observation port, the light detection device comprising an incident surface, the light detection device configured to produce an electrical signal in response to light striking the incident surface, and
 - vi) means for directing light scattered from a biological cell or a particle disposed at the observation point onto the light detection device;
 - b) passing the biological cell or particle through the channel to the observation point;
 - c) illuminating the observation point with light emitted from the light source;
 - d) detecting light scattered from the biological cell or particle with the CCD; and
 - e) producing the scatter pattern from the electrical signal produced in response to the scattered light detected by the light detection device.
14. The method as set forth in claim 13, wherein the scatter pattern comprises a two-dimensional scatter pattern.
15. The method as set forth in claim 13 or claim 14, further comprising the step of passing the biological cell or particle through the channel suspended in a fluid.
16. The method as set forth in claim 15, wherein the fluid comprises an aqueous solution.
17. The method as set forth in any one of claims 13 to 16, further comprising the step of focusing the biological cell or particle as it passes by the observation point.
18. The method as set forth in claim 17, wherein the focusing step further comprises hydro-dynamically focusing the biological cell or particle.

19. The method as set forth in any one of claims 13 to 18, wherein the directing means comprises an objective lens disposed between the observation point and the light detection device, the objective lens configured to direct light scattered from the biological cell or particle onto the light detection device.
20. The method as set forth in any one of claims 13 to 19 wherein the light source comprises a laser light source.
21. The method as set forth in any one of claims 13 to 20, wherein the coupling means comprises an optical fiber.
22. The method as set forth in any one of claims 13 to 21, wherein the incident surface of the light detection device is substantially parallel to the top surface.
23. The method as set forth in any one of claims 13 to 22, wherein light detection device comprises a charged coupled device ("CCD").
24. An apparatus for microscope-based label-free cytometry, comprising:
 - a) a microfluidic chip further comprising a channel disposed thereon, the channel substantially parallel to a top surface of the microfluidic chip;
 - b) an optical port disposed on the microfluidic chip, the optical port substantially parallel to the top surface, the optical port intersecting the channel thereby defining an observation point at the intersection thereof;
 - c) a light source configured to emit collimated visible or near-visible light;
 - d) means for operatively coupling the light source to the optical port wherein at least some light emitted from the light source passes through the coupling means to illuminate the observation point;

- e) a light detection device disposed adjacent to the observation port, the light detection device comprising an incident surface, the light detection device configured to produce an electrical signal in response to light striking the incident surface; and
 - f) means for directing light scattered from a biological cell or a particle disposed at the observation point onto the light detection device.
25. The apparatus as set forth in claim 14, further comprising means for creating a scatter pattern from the electrical signal produced in response to scattered light detected by the light detection device.
26. The apparatus as set forth in claim 15, wherein the scatter pattern comprises a two-dimensional scatter pattern.
27. The apparatus as set forth in any one of claims 14 to 16, further comprising means for suspending the biological cell or particle in a fluid.
28. The apparatus as set forth in claim 17, wherein the fluid comprises an aqueous solution.
29. The apparatus as set forth in any one of claims 14 to 18, further comprising means for focusing the biological cell or particle as it passes by the observation point.
30. The apparatus as set forth in claim 19, wherein the focusing means further comprises means for hydro-dynamically focusing the biological cell or particle.
31. The apparatus as set forth in any one of claims 24 to 30, wherein the directing means comprises an objective lens disposed between the observation point and

the light detection device, the objective lens configured to direct light scattered from the biological cell or particle onto the light detection device.

32. The apparatus as set forth in any one of claims 24 to 31, wherein the light source comprises a laser light source.
33. The apparatus as set forth in any one of claims 24 to 32, wherein the coupling means comprises an optical fiber.
34. The apparatus as set forth in any one of claims 24 to 33, wherein the incident surface of the light detection device is substantially parallel to the top surface.
35. The apparatus as set forth in any one of claims 24 to 34, wherein light detection device comprises a charged coupled device ("CCD").
36. An apparatus for producing a scatter pattern from a biological cell or particle, comprising:
 - a) a microfluidic chip further comprising a channel disposed thereon, the channel substantially parallel to a top surface of the microfluidic chip;
 - b) an optical port disposed on the microfluidic chip, the optical port substantially parallel to the top surface, the optical port intersecting the channel thereby defining an observation point at the intersection thereof;
 - c) a light source configured to emit collimated visible or near-visible light;
 - d) means for operatively coupling the light source to the optical port wherein at least some light emitted from the light source passes through the coupling means to illuminate the observation point;
 - e) a light detection device disposed adjacent to the observation port, the light detection device comprising an incident surface, the light detection device

configured to produce an electrical signal in response to light striking the incident surface;

- f) means for directing light scattered from a biological cell or a particle disposed at the observation point onto the light detection device; and
 - g) means for producing the scatter pattern from the electrical signal produced in response to the scattered light detected by the light detection device.
37. The apparatus as set forth in claim 15, wherein the scatter pattern comprises a two-dimensional scatter pattern.
38. The apparatus as set forth in claim 36 or claim 37, further comprising means for suspending the biological cell or particle in a fluid.
39. The apparatus as set forth in claim 38, wherein the fluid comprises an aqueous solution.
40. The apparatus as set forth in any one of claims 36 to 39, further comprising means for focusing the biological cell or particle as it passes by the observation point.
41. The apparatus as set forth in claim 40, wherein the focusing means further comprises means for hydro-dynamically focusing the biological cell or particle.
42. The apparatus as set forth in any one of claims 36 to 41, wherein the directing means comprises an objective lens disposed between the observation point and the light detection device, the objective lens configured to direct light scattered from the biological cell or particle onto the light detection device.
43. The apparatus as set forth in any one of claims 36 to 42 wherein the light source comprises a laser light source.

44. The apparatus as set forth in any one of claims 36 to 43, wherein the coupling means comprises an optical fiber.
45. The apparatus as set forth in any one of claims 36 to 44, wherein the incident surface of the light detection device is substantially parallel to the top surface.
46. The apparatus as set forth in any one of claims 36 to 45, wherein light detection device comprises a charged coupled device ("CCD").
47. A scatter pattern of a biological cell or particle when made by a process comprising the steps of:
 - a) providing an apparatus, comprising:
 - i) a microfluidic chip further comprising a channel disposed thereon, the channel substantially parallel to a top surface of the microfluidic chip,
 - ii) an optical port disposed on the microfluidic chip, the optical substantially parallel to the top surface, the optical port intersecting the channel thereby defining an observation point at the intersection thereof,
 - iii) a light source configured to emit collimated visible or near-visible light,
 - iv) means for operatively coupling the light source to the optical port whereby at least some light emitted from the light source passes through the coupling means to illuminate the observation point,
 - v) a light detection device disposed adjacent to the observation port, the light detection device comprising an incident surface, the light

detection device configured to produce an electrical signal in response to light striking the incident surface, and

- vi) means for directing light scattered from a biological cell or a particle disposed at the observation point onto the light detection device;
 - b) passing the biological cell or particle through the channel to the observation point;
 - c) illuminating the observation point with light emitted from the light source;
 - d) detecting light scattered from the biological cell or particle with the light detection device; and
 - e) producing the scatter pattern from the electrical signal produced in response to the scattered light detected by the light detection device.
48. The scatter pattern as set forth in claim 47, wherein the scatter pattern comprises a two-dimensional scatter pattern.
49. The scatter pattern as set forth in claim 47 or claim 48, wherein the process further comprises the step of passing the biological cell or particle through the channel suspended in a fluid.
50. The scatter pattern as set forth in claim 49, wherein the fluid comprises an aqueous solution.
51. The scatter pattern as set forth in any one of claims 47 to 50, wherein the process further comprises the step of focusing the biological cell or particle as it passes by the observation point.
52. The scatter pattern as set forth in claim 52, wherein the focusing step further comprises hydro-dynamically focusing the biological cell or particle.

53. The scatter pattern as set forth in any one of claims 47 to 52, wherein the directing means comprises an objective lens disposed between the observation point and the light detection device, the objective lens configured to direct light scattered from the biological cell or particle onto the light detection device.
54. The scatter pattern as set forth in any one of claims 47 to 53 wherein the light source comprises a laser light source.
55. The scatter pattern as set forth in any one of claims 47 to 54, wherein the coupling means comprises an optical fiber.
56. The scatter pattern as set forth in any one of claims 47 to 55, wherein the incident surface of the light detection device is substantially parallel to the top surface.
57. The scatter pattern as set forth in any one of claims 47 to 56, wherein light detection device comprises a charged coupled device ("CCD").

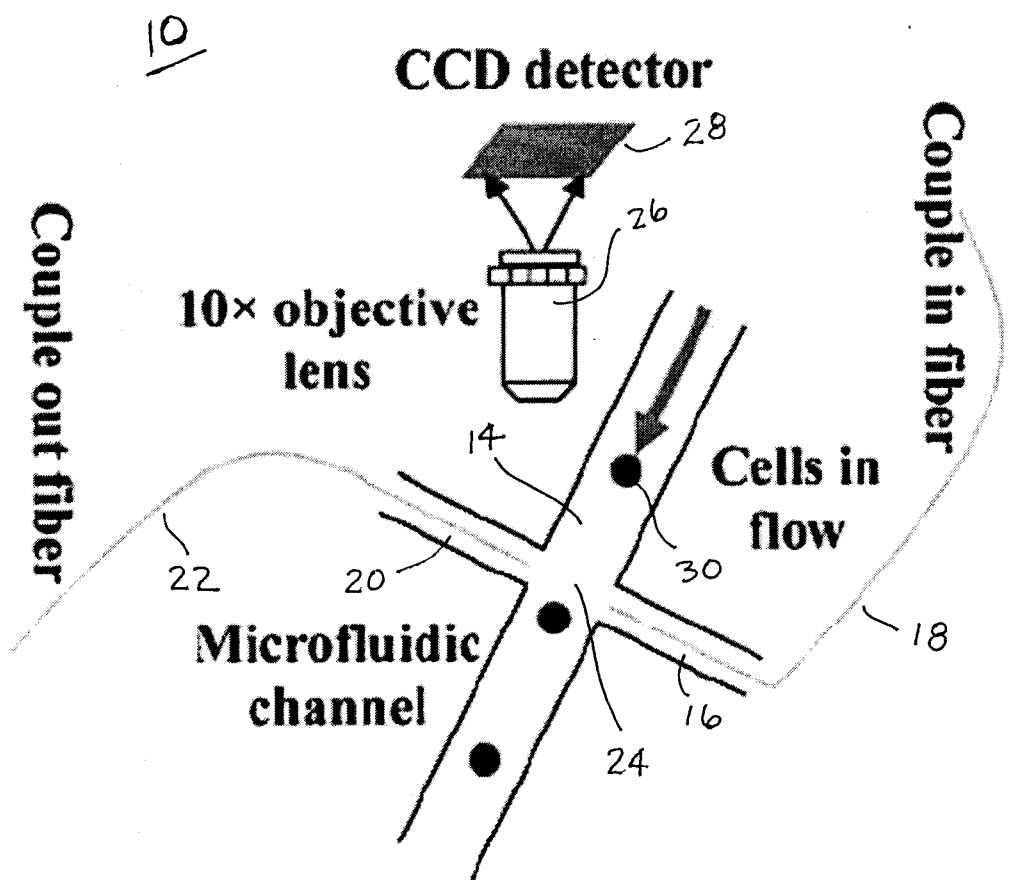
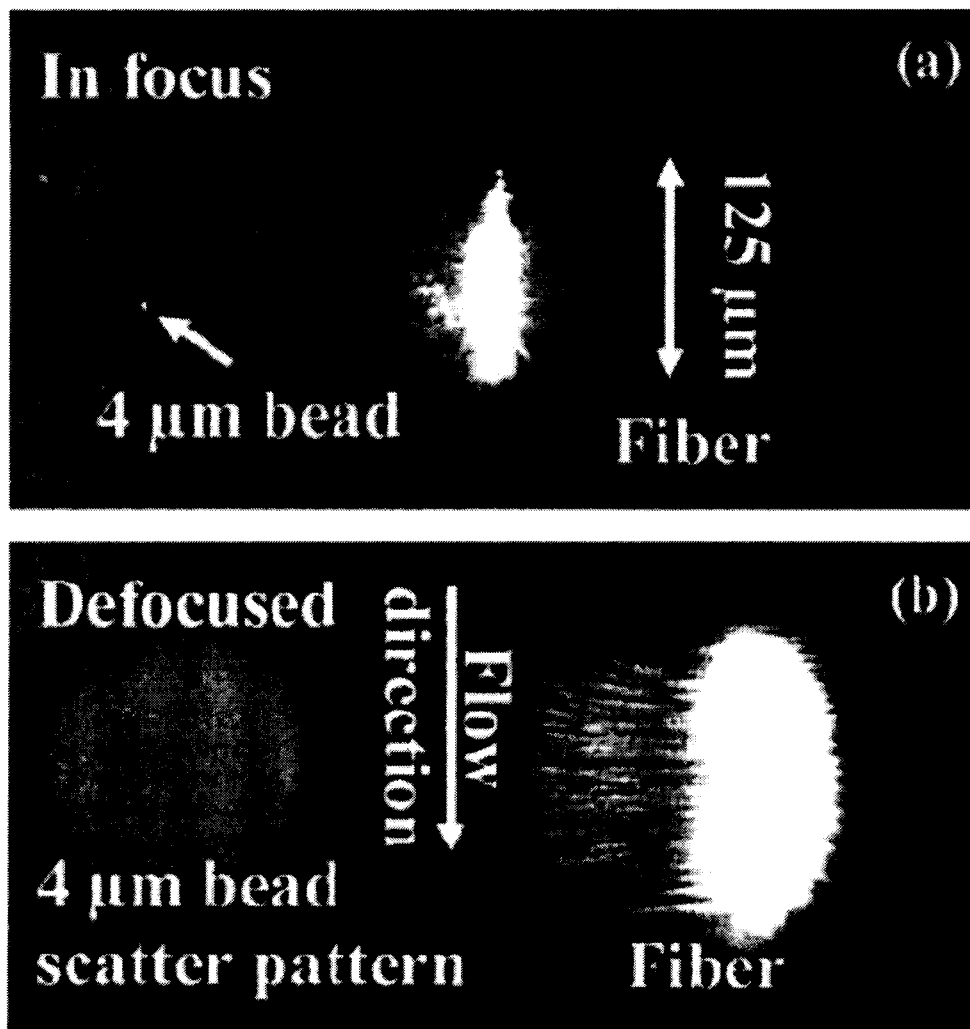


FIG. 1



FIGS. 2(a) and 2(b)

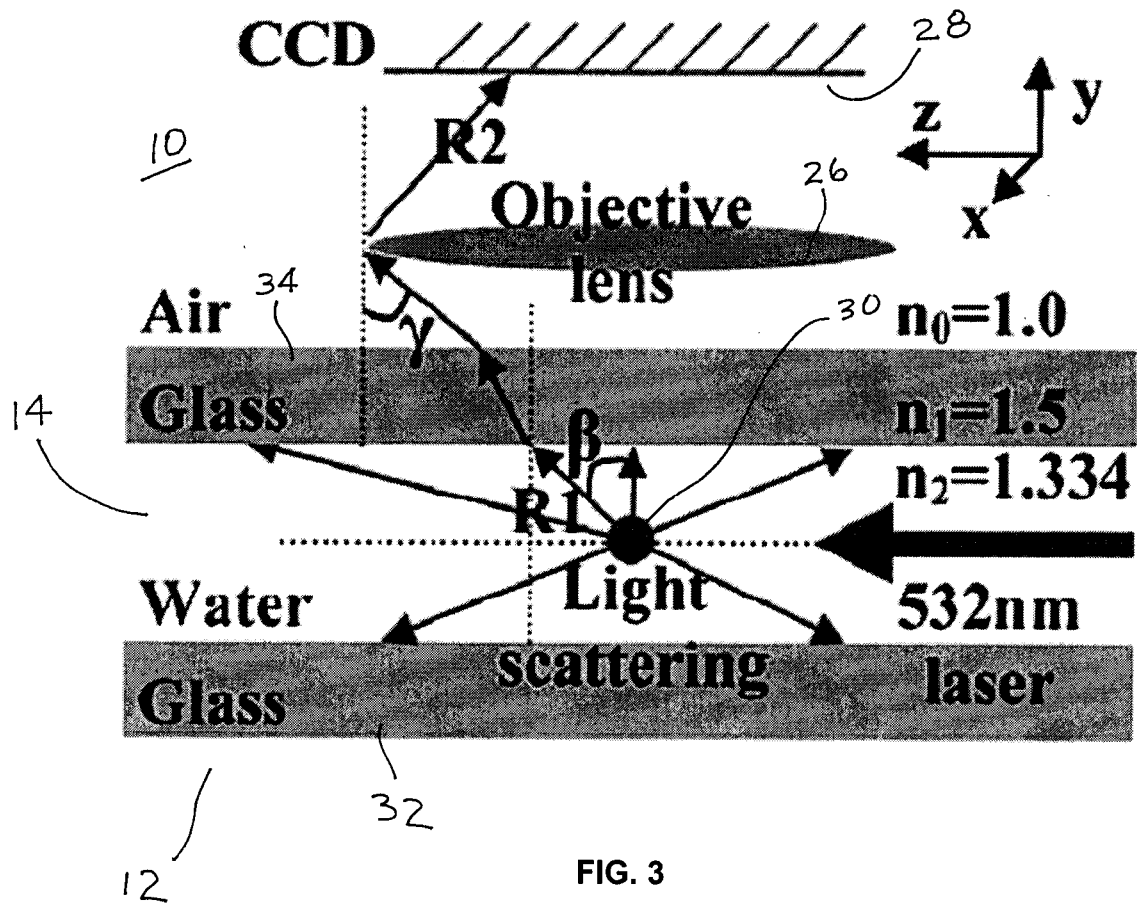
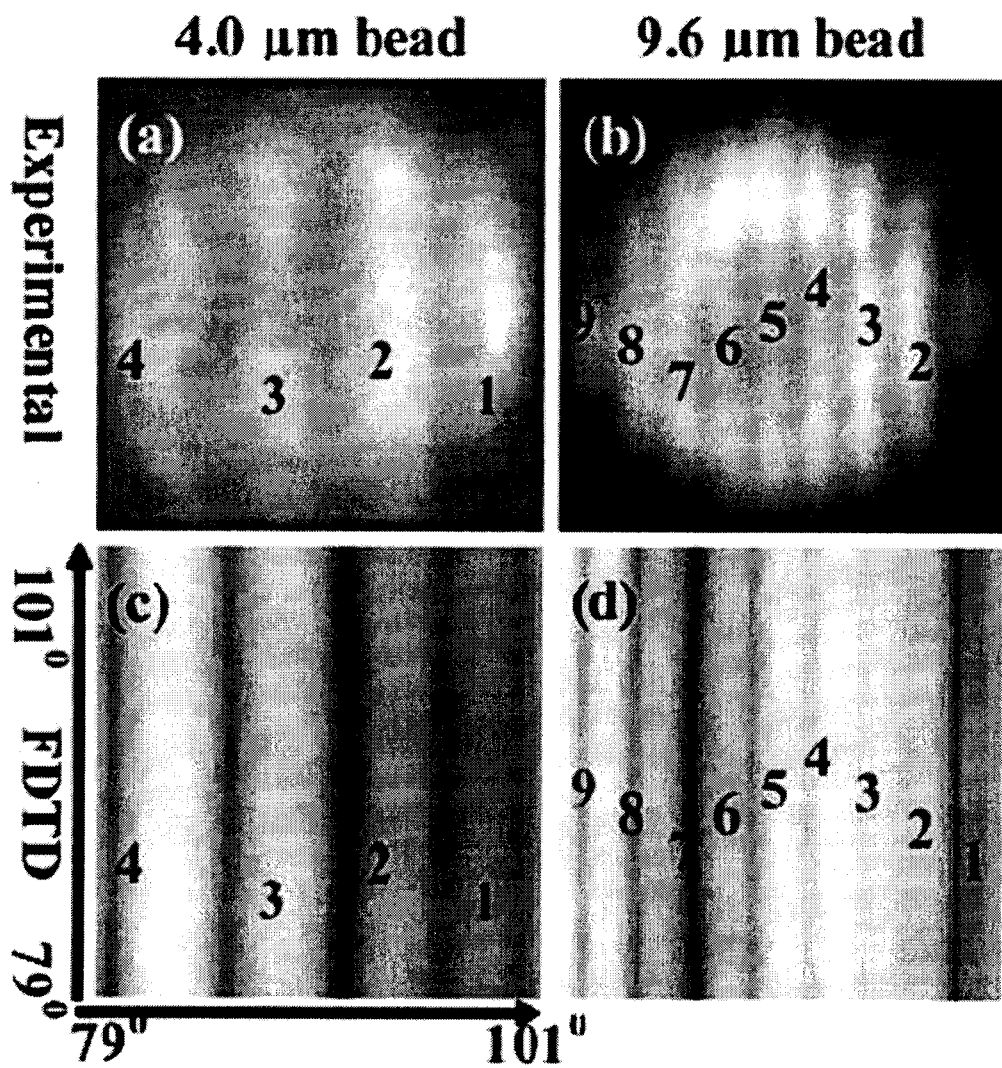


FIG. 3



FIGS. 4(a), (b), (c) and (d)

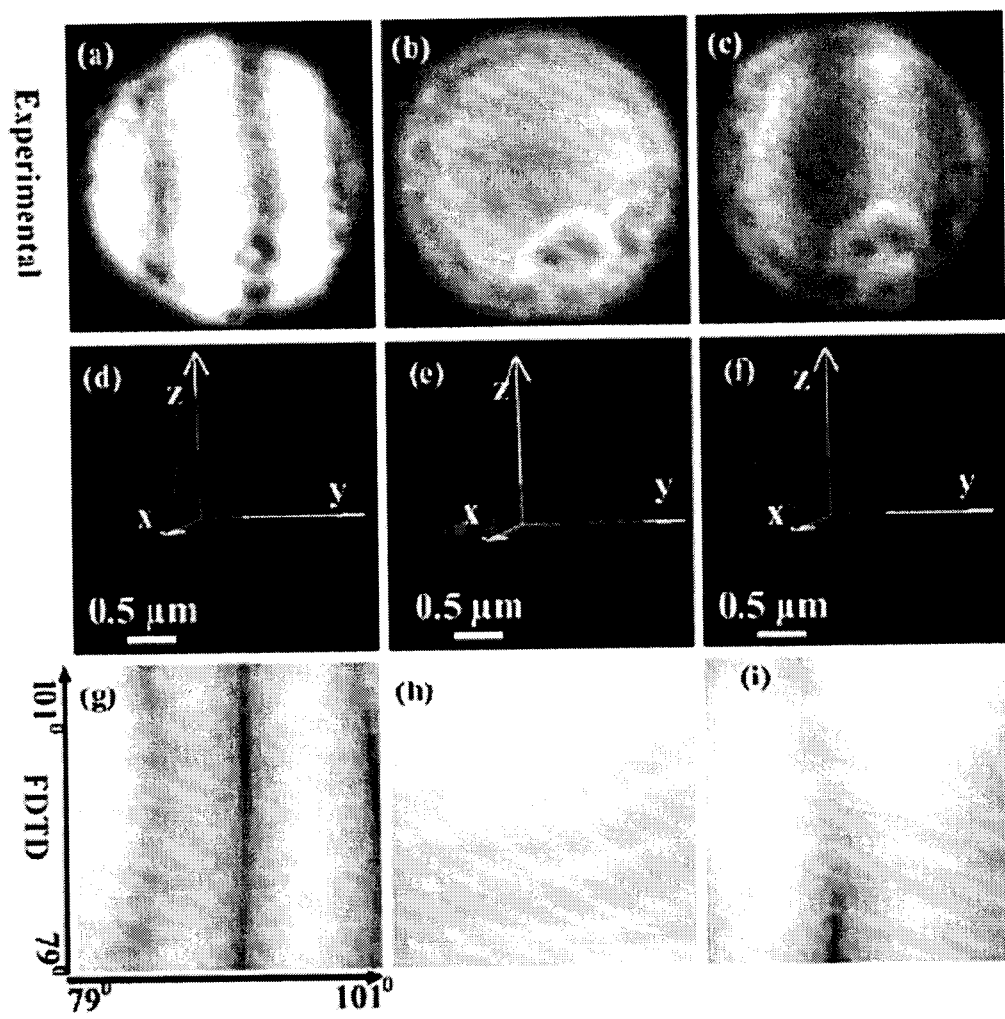
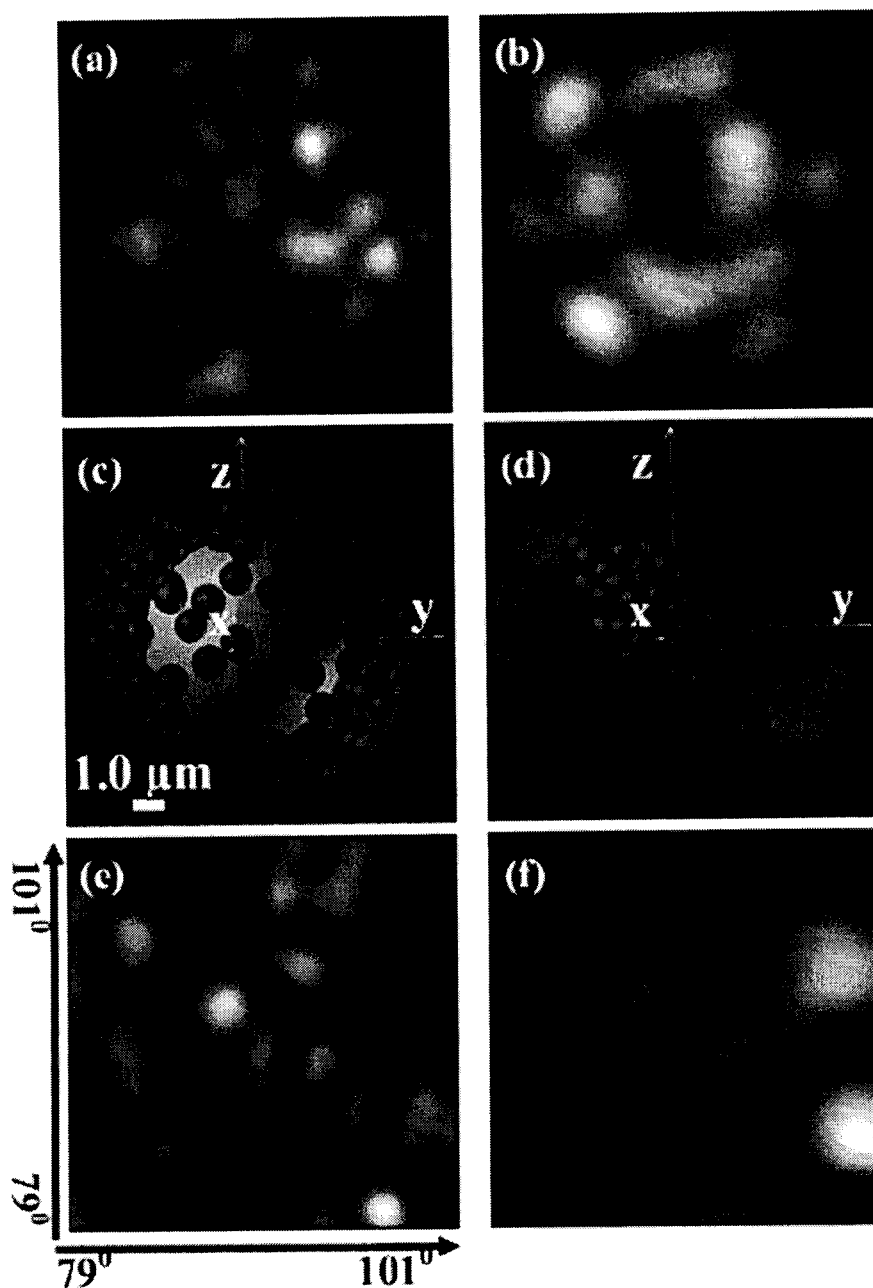


FIG. 5



FIGS. 6(a), (b), (c), (d), (e) and (f)

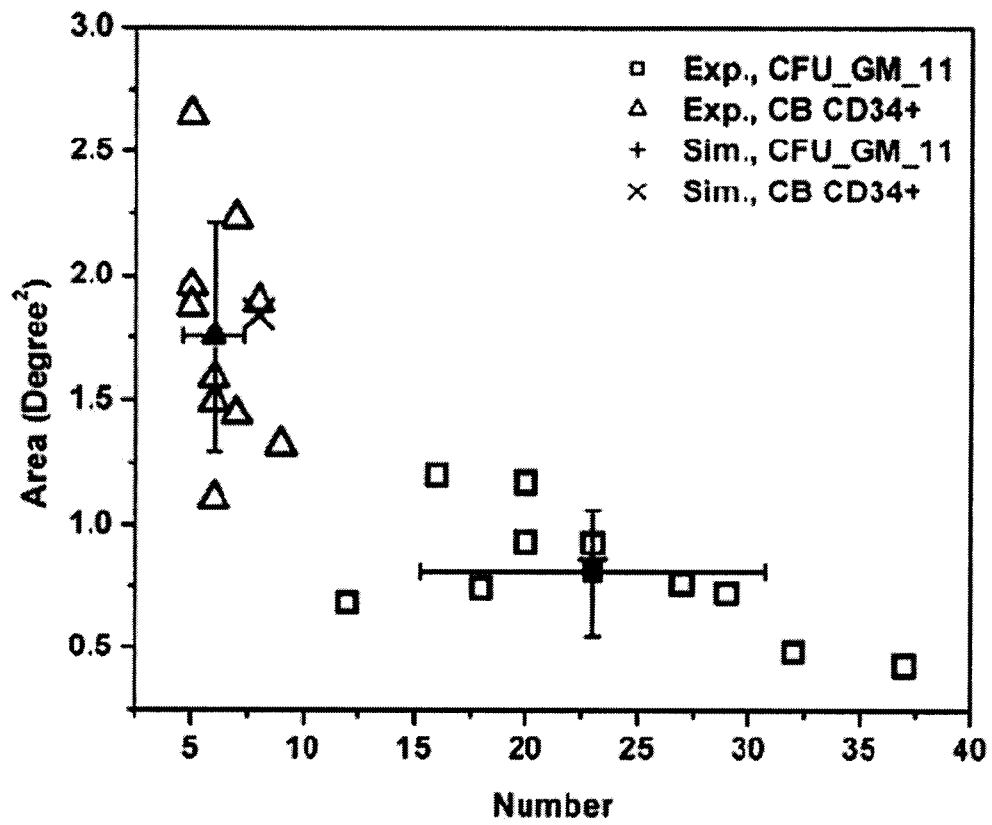
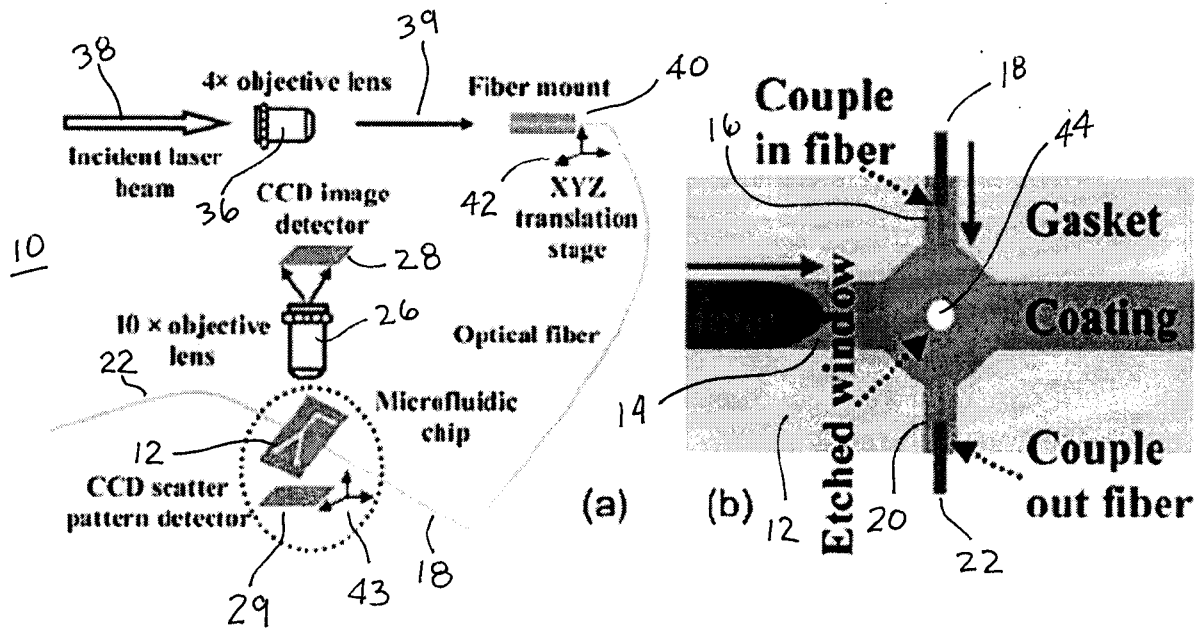


FIG. 7



FIGS. 8 (a) - (b)

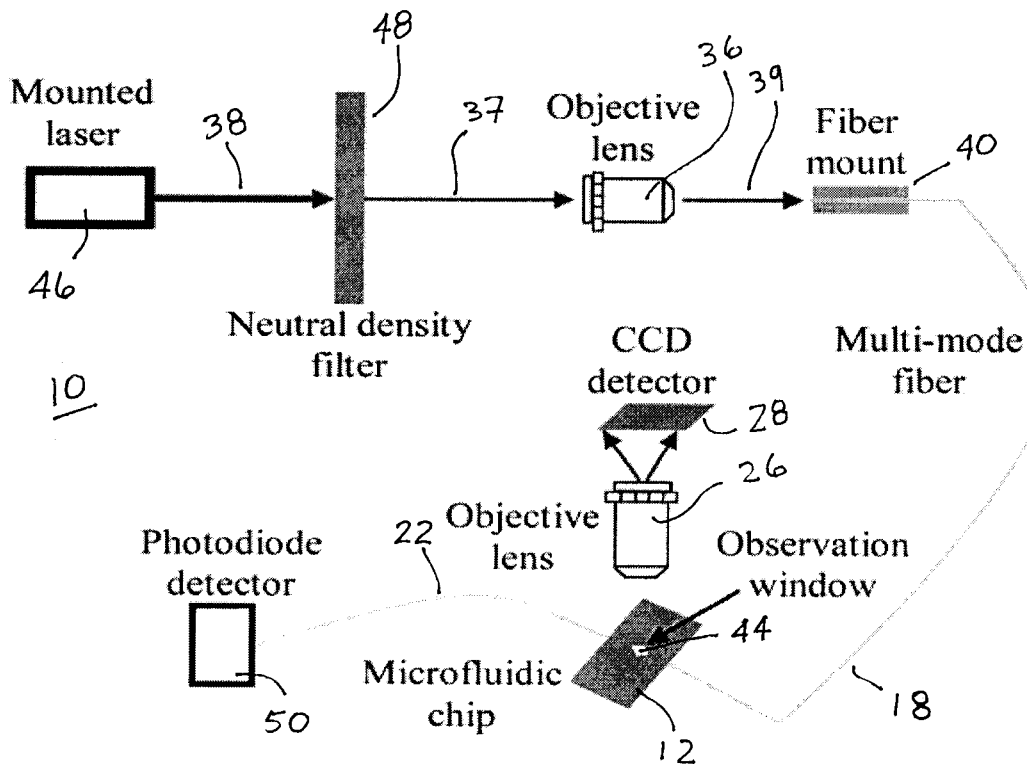
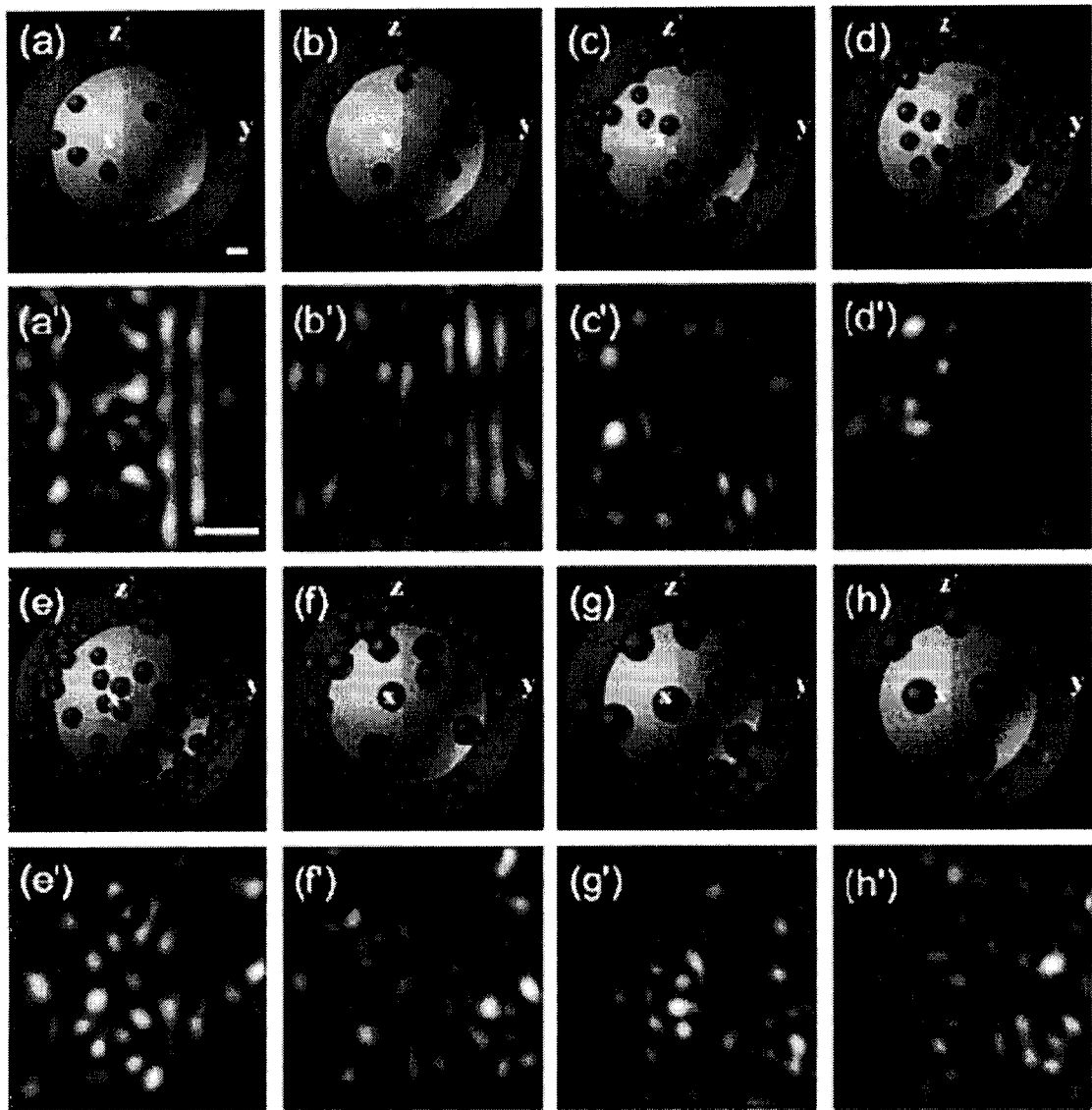
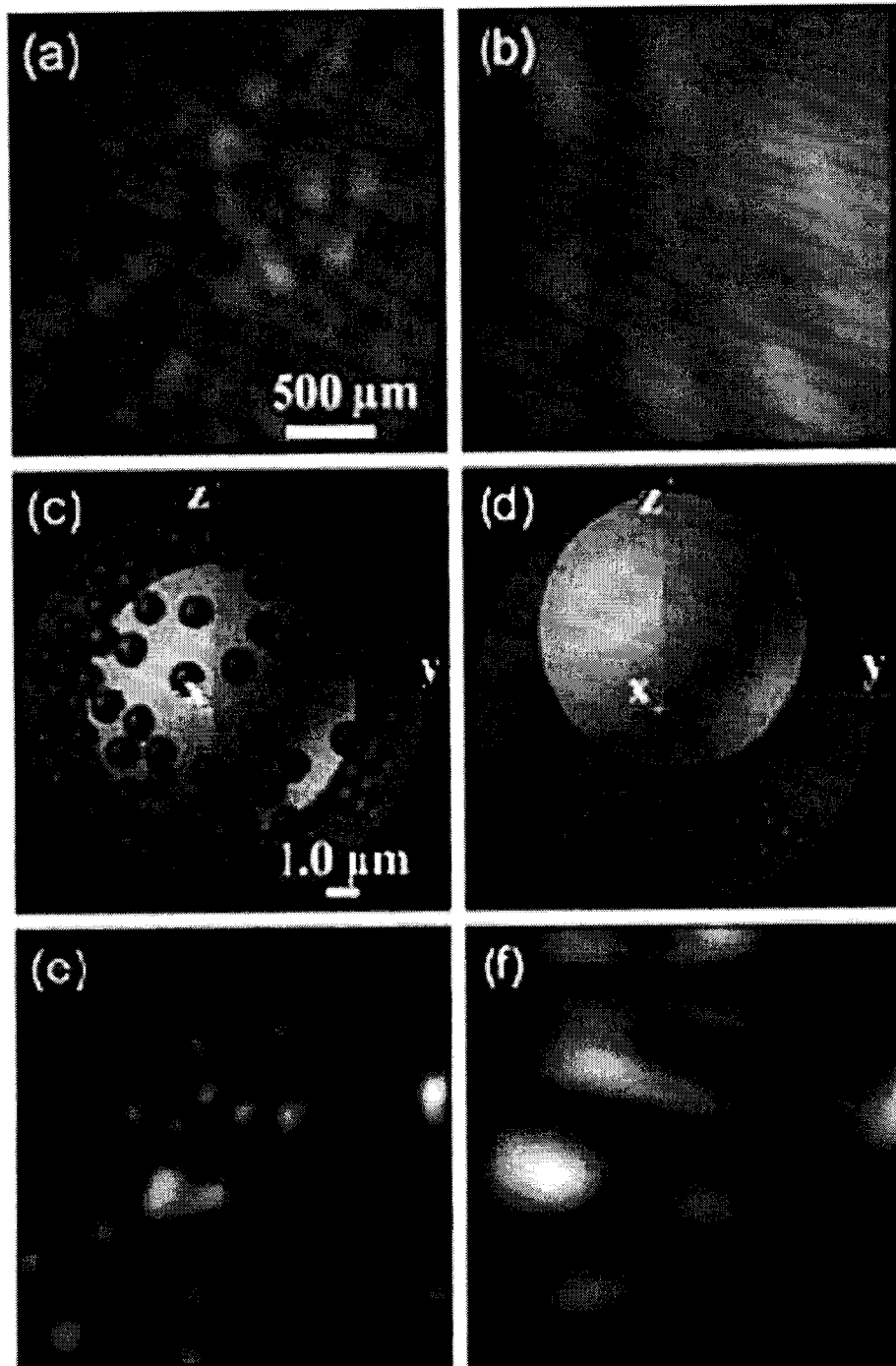


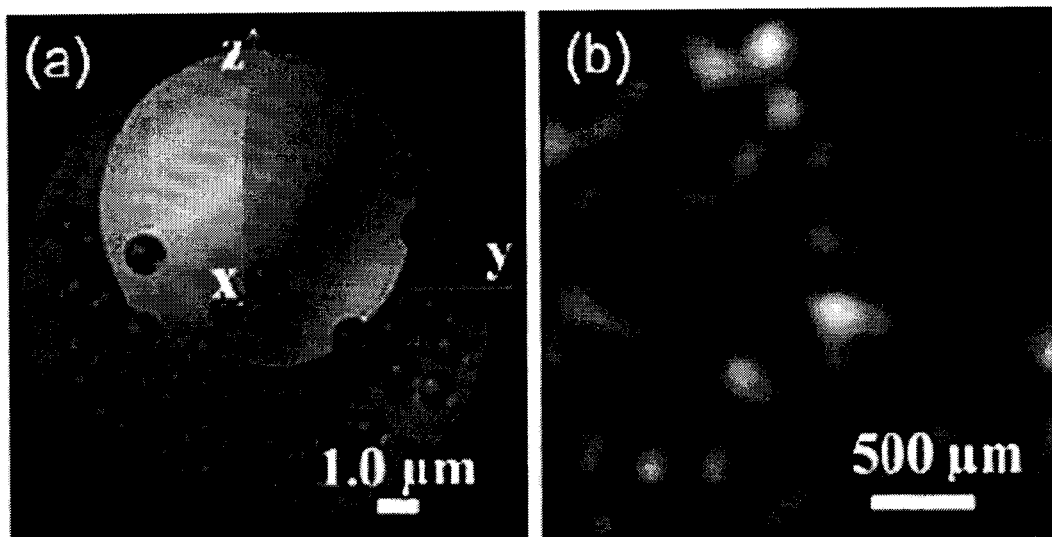
FIG. 9



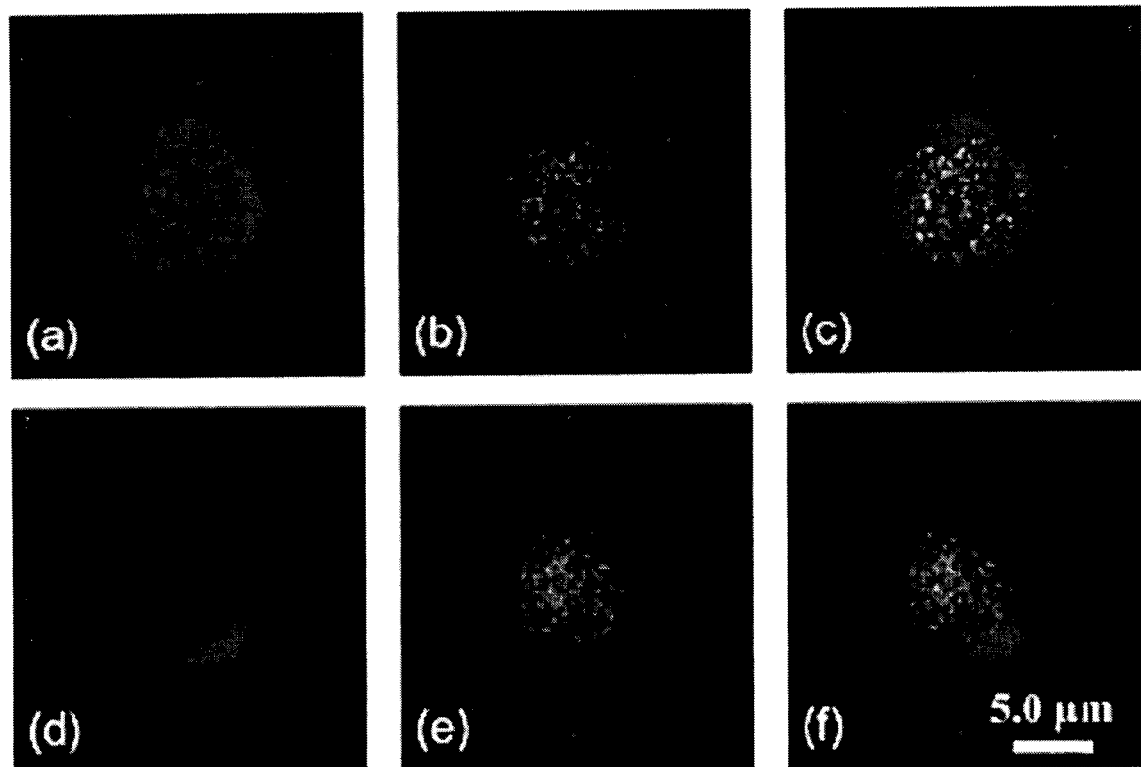
FIGS. 10 (a) – (h) and 10 (a') – (h')



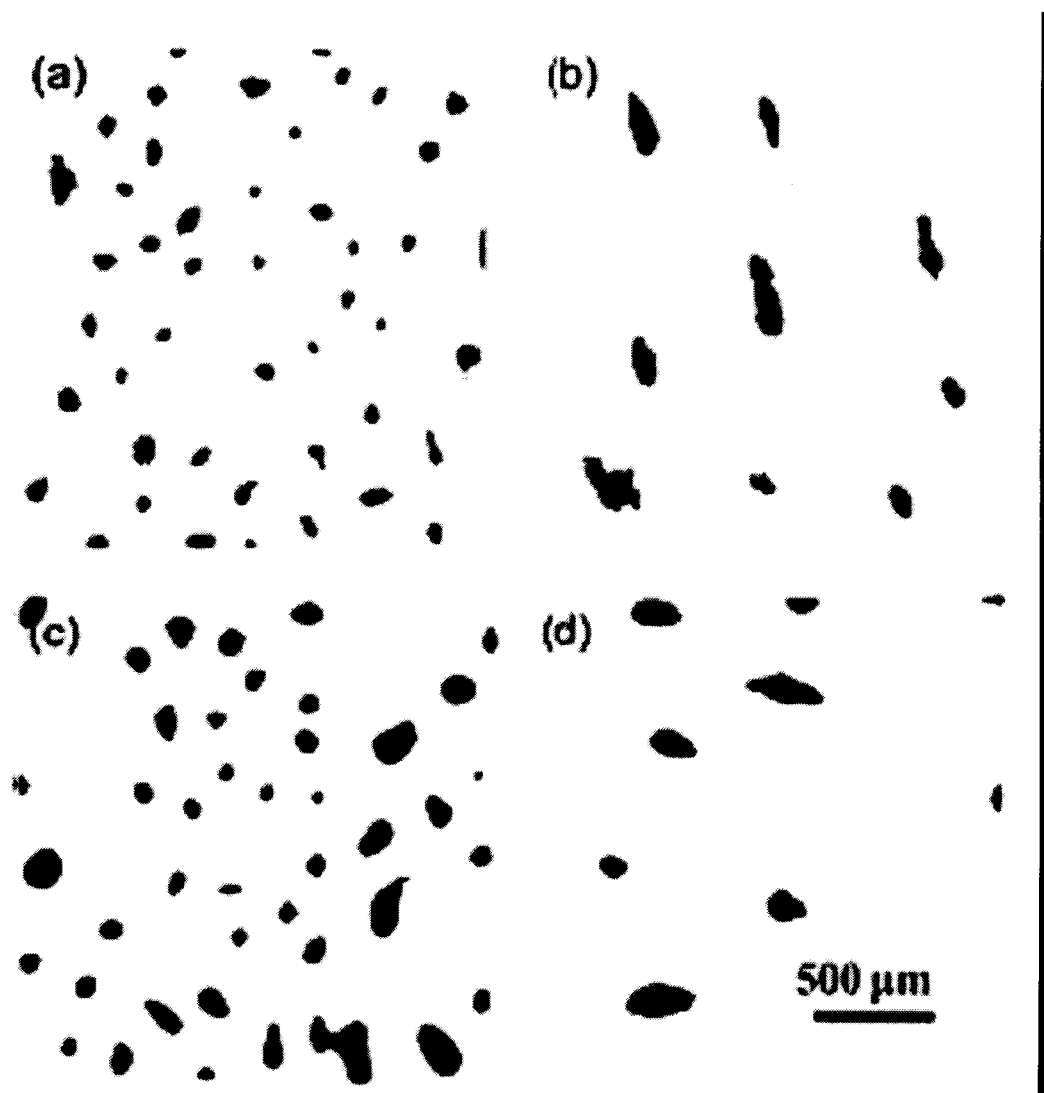
FIGS. 11 (a) – (f)



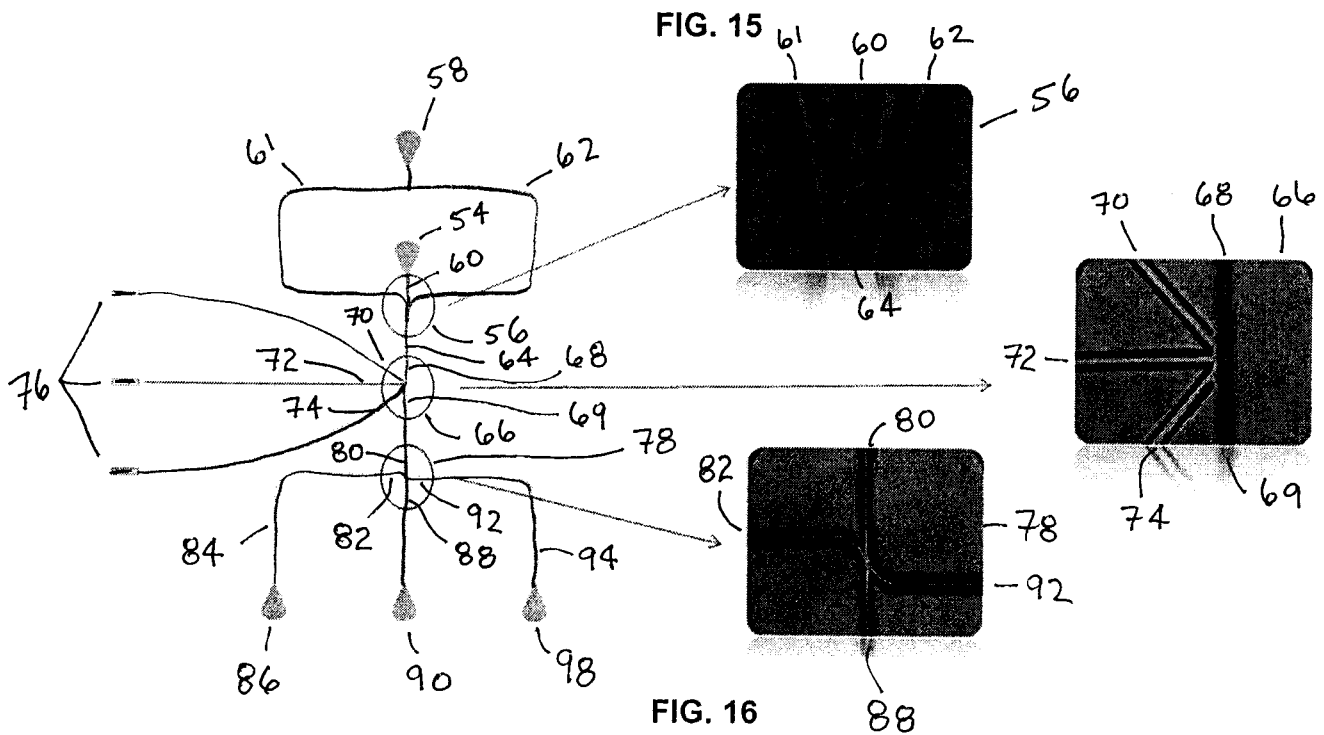
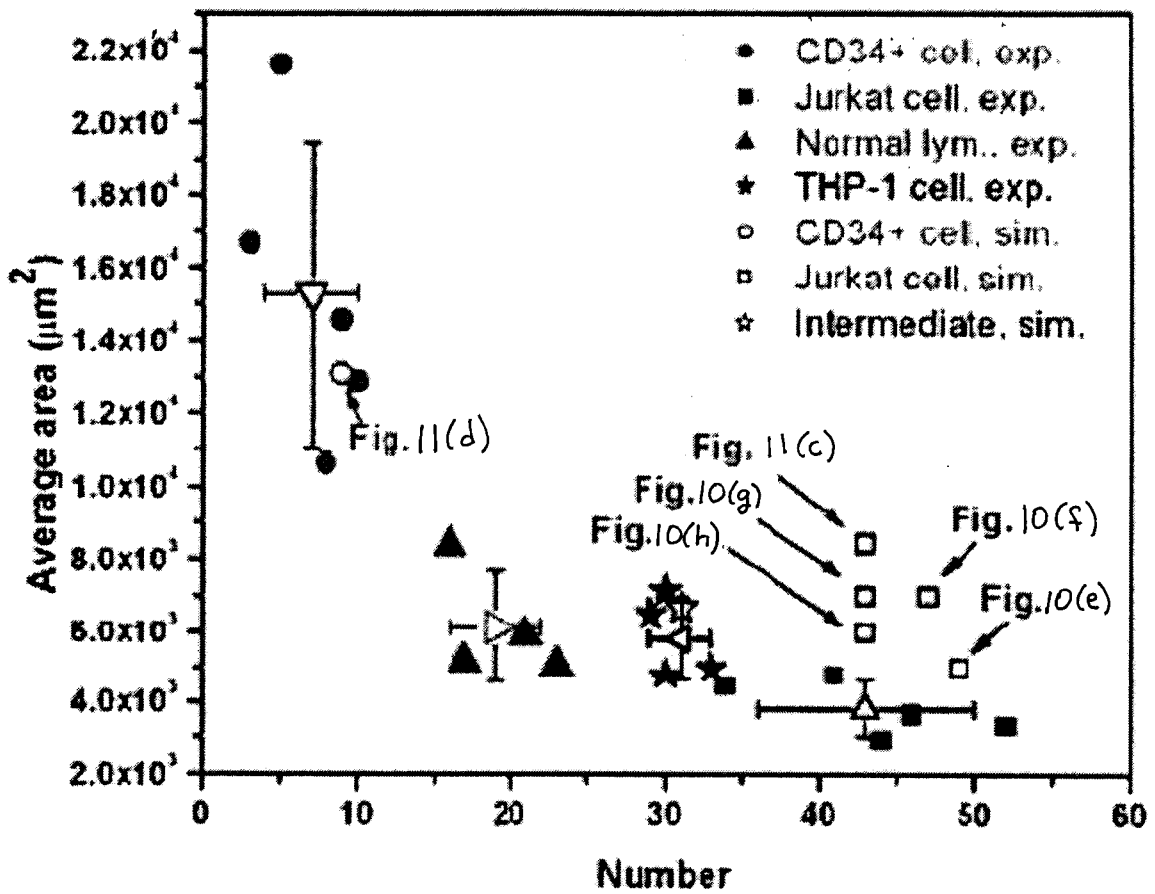
FIGS. 12 (a) - (b)



FIGS. 13 (a) – (f)



FIGS. 14 (a) – (d)



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA20 11/000775

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **GOIN 15/14** (2006.01)

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(2006.01): G01N 15/14

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

EPOQIJE (Epodoc), IEEE, Canadian Patent Database

(Keywords: microfluidic, scatter, pattern, microscope, label, stain)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SU, X. et al. Measurements of light scattering in an integrated microfluidic waveguide cytometer. Journal of Biomedical Optics, March/April 2008, 13(2), 024024.	1-7, 9, 11-18, 20, 22-30, 32, 34-41, 43, 45-52, 54, 56 and 57
Y	* Abstract, pages 024024-2 to 024024-3, Figures 1 and 2 *	8, 10, 19, 21, 31, 33, 42, 44, 53 and 55
X	SU, X. et al. Side scatter light for micro-size differentiation and cellular analysis. 2007, Proceedings of SPIE, v ol. 6446, 64460W.	1-5, 9, 12-16, 20, 23-28, 32, 35-39, 43, 46-50, 54 and 57
Y	* Abstract, page 64460W-2, second and third paragraphs, page 64460W-8, fourth paragraph and page 64460W-5, first paragraph *	8, 10, 19, 21, 31, 33, 42, 44, 53 and 55
X	SU, X. et al. 2D light scattering patterns of mitochondria in single cells. Optics Express, August 2007, Vol. 15, No. 17, 10562-10575.	1-4, 9, 12-15, 20, 23-27, 32, 35-38, 43, 46-49, 54 and 57
Y	* Abstract, page 10564, third paragraph *	8, 10, 19, 21, 31, 33, 42, 44, 53 and 55
Y	SU, X. et al. Light scattering characterization of single biological cells in a microfluidic cytometer. 3 August 2009, Proceedings of SPTfi, Vol. 7386, 738602.	8, 10, 19, 21, 31, 33, 42, 44, 53 and 55
	* Abstract and section 2.1 *	

[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 application or patent but 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
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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	

Date of the actual completion of the international search

1 August 2011 (01-08-2011)

Date of mailing of the international search report

15 September 2011 (15-09-2011)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
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Jacinthe Moreau (819) 953-4091

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2011/000775

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	SU, X. et al. Label-free and noninvasive optical detection of the distribution of nanometer-size mitochondria in single cells. June 2011, Journal of Biomedical Optics. Vol. 16(6). 067003.	1-57
X, P	SU, X. et al. Microscope-based label-free microfluidic cytometry. 24 December 2010 [Retrieved on 2011-08-01]. Retrieved from the Optics Express database of the OpticsInfoBase website using Internet: <URL: http://www.opticsinfobase.org/oe/home.cfm >. Also available as: Optics Express. Vol. 19, Issue 1, pp. 387-398 (2011).	1-57