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- (54) **Title:** METHOD AND APPARATUS FOR FRACTIONATING GENETICALLY DISTINCT CELLS AND CELLULAR COMPONENTS

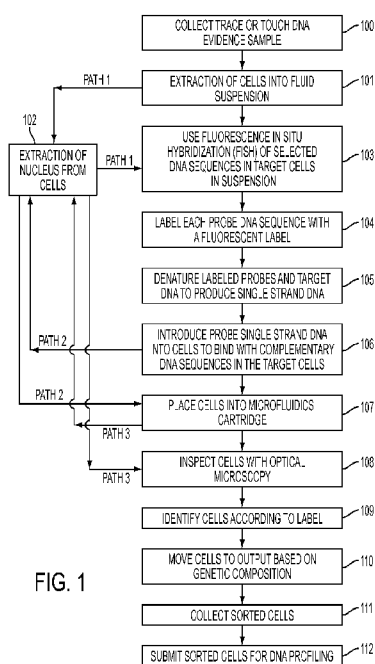


FIG. 1

- (57) **Abstract:** The present invention relates to a method of processing forensic trace or touch DNA evidence in order to differentially label cells, and then isolate them into distinct fractions which can be processed using standard downstream DNA profiling methods. A mixture of cells and/or cellular components in fluid suspension are first labeled with one or more markers which bind specifically to genetic sequences of interest, and which can be detected optically. The labeled cells/nuclei are placed into a fluidic cartridge, and are transported via fluid flow to an inspection and separation region, observed and identified according to their labels using optical microscopy, and are moved to output channels corresponding to the different labels, degree of labeling, or combination of labels, and to a different subset of possible genetic profiles. The separated cell fractions are extracted from the cartridge via their respective outputs, and can be genetically profiled via short- tandem-repeat (STR) analysis.



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METHOD AND APPARATUS FOR FRACTIONATING GENETICALLY DISTINCT CELLS AND CELLULAR COMPONENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present invention claims priority from U.S. Provisional Patent Application No. 61/457,870, filed June 24, 2011, the contents of which are herein incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a technique for fractionating cells and cellular components, by sorting mixed forensic samples containing cellular contributions from multiple individuals, such as from trace or touch DNA forensic samples, into subgroups which have a high likelihood of deriving from single contributors. The standard DNA analysis techniques are then applied to each subgroup, to identify the individual contributors.

2. Description of the Related Art

Trace DNA refers to the collection of minute biological samples and the extraction of tiny amounts of genetic material – often less than 100 pg - from the sample. Touch DNA refers to the collection of trace DNA deposited by touch. In one presently used method, as outlined in “Forensic trace DNA: a review”, by Roland AH van Oorschot et al., *Investigative Genetics* 2010, 1:14, trace samples are collected using swabs or tape, and laser microdissection techniques are used to isolate relevant target cells from other overwhelming cell types. Microscopically, different cell types are

distinguished based upon morphological characteristics, various chemical staining or fluorescence labeling techniques. A clear DNA profile can be derived from the minor cell type alone. Further, cells derived from a male contributor and a female contributor can be distinguished from each other using fluorescent probes and separated accordingly, using the laser microdissection methodology. However, drawbacks include damage to the cells from preparation of the sample using coated glass slides, in transferring the sample to the slide, and the fact that identification of relevant cell types is currently done manually and is very time consuming. Thus, laser capture microdissection is currently impractical for almost all real-world forensic application.

Another approach to separating contributors in sample mixtures is the use of flow cytometry/cell sorting methods which are used in non-forensic applications. Methods include simultaneous separation of up to four distinct populations of cells, based either on morphology of the cell or use of specific antibodies against each cell type. Single or multiple cells can be sorted directly into PCR tubes or onto glass slides for low volume PCR applications. However, flow cytometry requires larger volumes and has higher waste, so it is not an attractive choice for most forensic samples. Although some success has been obtained with sperm cells, technical difficulties remain to be overcome with flow cytometry.

In other methods outside criminal forensics, flow cytometry has been used with fluorescence in situ hybridization (FISH) - which is a process in which a labeled fragment of single-stranded DNA or RNA is used as a probe to label complementary DNA sequences on an intact chromosome - to study bacterial community structure, composition, and activity. Thus, FISH has only recently been applied for use with flow

cytometry in the separation of methanotroph (bacterial) populations (see Kalyuzhnaya et al., “*Fluorescence In Situ Hybridization-Flow Cytometry-Cell Sorting-Based Method for Separation and Enrichment of Type I and Type II Methanotroph Populations*”, Applied and Environmental Microbiology, June 2006). However, as noted above, there are difficulties with flow cytometry, and these methods are not of general use or of wide applicability to forensic human identification.

Forensic DNA mixtures are generally a challenge with distinct limitations and costs associated with them. In a mixture representing two individuals, if one individual is much more highly represented than the other, the less represented individual can mostly or completely “drop out” from the amplification process and not be represented. In this case, one of the two individuals can generally be identified and the other cannot.

Alternately, if the two individuals are equally represented, there is no discernible signal on which to distinguish which alleles are from which individuals and neither individual can be determined. There is a narrow range where one individual is more prominent than the other but not much more prominent than the other. In this case, with careful and time-consuming analysis, each individual can be characterized. However, the confidence level may fall, sometimes to very low confidence levels. With mixtures involving more than two individuals, the difficulties are much greater. For this reason, there would be great value in being able to process mixtures into fractions which represent a single individual or a smaller number of individuals than the original mixture.

Thus, a practical method of fractionating mixtures of cells from forensic samples into genetically differentiated sub-populations, especially a method which may be suitable for handling very small quantities of cells and can be automated, is needed. Such

a method would be of very high value to the criminal forensics community and would enable high-confidence human identification in samples comprising mixtures, which is fairly common in sexual assault evidence and routine in trace or “touch DNA” samples.

SUMMARY OF THE INVENTION

The present invention relates to a method and apparatus to reliably isolate cells and cellular components from mixtures of cells with different genetic content, such as having or lacking particular sequences of bases or having a different number of occurrences of a particular sequence of bases. The mixture of cells/cellular components is separated into fractions which are more likely to contain cells or cellular components, with a single genetic profile than the original mixture.

In one example, the present invention focuses on a method of processing forensic trace or touch DNA evidence – that is, mixtures of epithelial cells (or cellular components) collected from evidence that may have been touched by multiple persons, and thus, contain cells contributed by each of said persons – in order to differentially label cells and then isolate them into distinct fractions which can be processed using standard downstream DNA profiling methods.

In brief, given a mixture of cells and/or cellular components in fluid suspension which are to be fractionated, the cells (nuclei) are first labeled with one or more markers which bind specifically to genetic sequences of interest, and which can be detected optically (e.g., by machine vision or human eyes). Alternatively, among other embodiments, cell nuclei may be extracted from the cells prior to labeling, and the nuclei are labeled.

The labeled cells (or cell nuclei) are then placed into a fluidic cartridge, and are transported via fluid flow to an inspection and separation region. The cells (or cell nuclei) are observed and identified according to their labels, and are moved to output channels corresponding to the labels they present. Each output corresponds to a different label, degree of labeling, or combination of labels, and thus, to a different subset of possible genetic profiles. The isolated cell fractions are then extracted from the fluidic cartridge via their respective outputs, and can be genetically profiled via short-tandem-repeat (STR) analysis or other methods. Each separated fraction will have a higher probability of deriving from a single contributor, or at least from fewer contributors than the original mixture, and therefore a higher probability of yielding an interpretable genetic profile or a profile which can yield higher-confidence identification.

The fluidic cartridge of the present invention includes a plurality of outputs – i.e., two or four outputs - but may be designed with additional outputs. The fluid suspension of cells and/or cell components is introduced into a sample input/reservoir which is part of a recirculation loop which is connected to an inspection and separation region containing a chamber. The recirculating flow prevents the cells/cellular components from sedimenting in the sample input and fluidic channels. The fluid suspension is pumped into the inspection and separation region using a valve system with pumps and actuators. Pneumatic channels control the valves to the fluid layer of the fluidic cartridge to allowing fluid to flow, blocking flow, or driving flow. The valves are connected to a pressure supply.

Buffer is inputted through buffer inputs that are formed in a U-shaped design, with the bottom of the U-shape being a dropoff area connected to the inspection and

separation region. Buffer is pumped through the U-shaped buffer channels to prime the system.

The labeled cells/cellular components are inspected using optical microscopy in the chamber and an optical trapping apparatus traps and moves the labeled cells/cellular components to the dropoff areas, to enable the isolation of one or two unique fractions from of the cell/cellular component mixture. A reservoir includes unsorted samples pumped from the inspection region.

All optical trapping and imaging is conducted through the optically-clear (e.g., COC polymer molded or embossed using a highly polished metal mold) window that forms the bottom of the chamber in the observation and inspection region of the fluidic cartridge.

Thus has been outlined, some features consistent with the present invention in order that the detailed description thereof that follows may be better understood, and in order that the present contribution to the art may be better appreciated. There are, of course, additional features consistent with the present invention that will be described below and which will form the subject matter of the claims appended hereto.

In this respect, before explaining at least one embodiment consistent with the present invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and to the arrangements of the components set forth in the following description or illustrated in the drawings. Methods and apparatuses consistent with the present invention are capable of other embodiments and of being practiced and carried out in various ways. Also, it is to be understood that the

phraseology and terminology employed herein, as well as the abstract included below, are for the purpose of description and should not be regarded as limiting.

As such, those skilled in the art will appreciate that the conception upon which this disclosure is based may readily be utilized as a basis for the designing of other structures, methods and systems for carrying out the several purposes of the present invention. It is important, therefore, that the claims be regarded as including such equivalent constructions insofar as they do not depart from the spirit and scope of the methods and apparatuses consistent with the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a flow chart which provides the major steps in the method consistent with the present invention. Note that step 103 will generally incorporate steps 104-106, but these steps are listed separately here for clarity.

FIG. 2 is a schematic diagram which shows the difference between direct labeling and indirect labeling of FISH probes, in the method consistent with the present invention.

FIG. 3 is a schematic diagram showing the steps in fluorescence in situ hybridization (FISH) technique, consistent with the method of the present invention.

FIG. 4 is a Table from Semrock Inc., showing the relative fluorophore contributions to the transmitted emission through different filters in Semrock Inc.'s BrightLine® filter series.

FIG. 5A is a schematic diagram of the overall cellular isolation apparatus consistent with the present invention.

FIGS. 5B-5C are schematic diagrams showing two embodiments of nuclei labeling, isolation, and sorting using genetic labeling, in a fluidic cartridge, according to one embodiment consistent with the present invention.

FIG. 6A is a top view of a schematic diagram of the fluidic layer of a two output fluidic cartridge, according to one embodiment consistent with the present invention.

FIG. 6B is a top view of a schematic diagram of the fluidic layer of a four output fluidic cartridge, according to one embodiment consistent with the present invention.

FIG. 7A is a perspective view of a schematic diagram of a fluidic cartridge inserted in a substage, and stage, according to one embodiment consistent with the present invention.

FIG. 7B is a perspective view of a schematic diagram of a fluidic cartridge showing the pneumatic system, according to one embodiment consistent with the present invention.

FIG. 8 is an exploded perspective view of the fluidic cartridge, showing its layers, according to one embodiment consistent with the present invention.

FIG. 9A is a top view and cross-sectional view of a schematic diagram of the valve (in the closed state) of the fluidic cartridge, according to one embodiment consistent with the present invention.

FIG. 9B is a cross-sectional view of a schematic diagram of the valve (in the open state) of the fluidic cartridge, according to one embodiment consistent with the present invention.

FIG. 10A is a schematic diagram of the operation of three valves of the fluidic cartridge to achieve peristaltic pumping, according to one embodiment consistent with the present invention.

FIG. 10B is a schematic diagram of the valve arrangement of the sample recirculating region of the fluidic cartridge, according to one embodiment consistent with the present invention.

DESCRIPTION OF THE INVENTION

The present invention relates to a method and apparatus to isolate cells and cellular components with specific genetic commonalities from a mixture of cells with differing genetic content. In one embodiment of the present invention, the mixture of cells includes epithelial cells extracted as forensic evidence from items that have been handled by multiple people (i.e., “trace or touch DNA”). Trace or touch DNA includes samples from objects someone has touched, and is used to solve a variety of forensic cases including burglary, rape, homicide, etc. Since humans constantly shed skin cells, the bulk of trace or touch DNA evidence samples include skin cells and DNA extracted from those cells. Other forensic samples of interest may contain any of various bodily fluids, such as blood, saliva, mucous, semen, vaginal fluid, tears, or other bodily fluids.

In this application, a cell is defined as a single cell or a portion of a cell which includes the cell’s nuclear or genetic material or which otherwise contains DNA or RNA. Further, in the present invention, genetic profiling is defined as the procedure of analyzing DNA or RNA for the purpose of identification, whereas a contributor is

defined as an individual whose genetic material is present in a cell mixture to be sorted and profiled.

In one embodiment, the present invention focuses on methods of processing forensic trace or touch DNA samples in order to differentially label cells or cell nuclei and then isolate them into distinct fractions which can be processed using standard DNA profiling methods.

Sample Collection/Preparation

A. Collection of Trace or Touch DNA Evidence

Several standard methods are used by forensic investigators to extract trace or touch DNA samples from physical evidence. In FIG. 1, step 100, trace or touch DNA samples may be collected via the “cut” method from soft surfaces such as fabric and clothing where one expects to potentially find skin cells (such as the collars of a shirt or dress) or bodily fluids, or by using tape on such soft surfaces. Alternatively, swabs may be used for collecting cells from hard-surfaced objects such as cell phones and door knobs or from individuals, such as a victim of a crime.

In the swab method, human biological samples, such as epithelial cells or nucleated blood cells, are collected via the swabbing of touched objects via standard forensic evidence-collection methods for such objects. For example, moistened (with water or isopropanol, etc.) and dry DNA-free sterile swabs, may be used to traverse the target area to collect the samples from objects of interest. These evidence swabs may be preserved by freezing or drying.

B. Preparation of Cells in Fluid Suspension

The present invention requires the cells that are to be analyzed and fractionated to be suspended in fluid. Since trace or touch DNA evidence is typically stored on evidence swabs, cut fabric, etc., it is necessary to elute the cells from the collection/storage medium. That is, in FIG. 1, step 101, the cells must be extracted from the swab or other medium into fluid suspension. One known method for doing this involves agitating the swab in a phosphate buffered saline (PBS) solution to release the cellular material. To increase the yield, the swab can be submersed in fresh PBS several times. Other extraction methods may be employed, some of which may be well known in the art. Centrifugation or other techniques may be used to help isolate cells from unwanted debris and free DNA.

C. Nucleus Isolation

In the present invention, certain downstream steps can be facilitated by extracting the nuclei from the cells (see step 102, FIG. 1) to be analyzed, prior to insertion into the fluidic cartridge 600/620 (i.e., a device which is capable of having fluidic channels of dimension 5mm or smaller – see FIGS. 6A-6B).

For example, manipulation of the nucleus using optical traps (including holographic optical traps (HOT), as described in, for example, U.S. Patent Nos. 6,055,106, 6,863,406, 7,411,180 etc., which are herein incorporated by reference) is typically much easier for smaller, more compact, objects like a nucleus, than for much larger objects with lower protein concentrations like an intact epithelial cell.

Thus, the nuclei extraction procedure may be performed when intact epithelial cells are too large or vary too greatly in shape to be reliably and quickly manipulated with optical traps or some other conventional micro-scale techniques.

In particular, nuclei 533 may be isolated from the cells 532 of interest (see step 102, FIG. 1, path 1, and FIG. 5B) using established chemical and mechanical techniques (i.e., nucleus isolation), commercially-available kits, or variations thereon which are developed for particular cell types such as epithelial cells.

Alternatively, nuclei may be extracted after the cells have been introduced to the fluidic cartridge 600/620, through use of laser scissors to help liberate nuclei from cells (see step 102, path 3, FIG. 1). In this case, a high peak energy pulsed laser with a highly focused beam can be used to cut out the nucleus, according to known methods – see, for example, U.S. Patent No. 4,249,533, to Olympus Optical, which is herein incorporated by reference in its entirety.

However, although nucleus extraction can facilitate some of the later steps in the present invention, it is not required. In place of isolated cell nuclei, whole cells or any part of a cell containing the nucleus or genetic material of interest, may be used in all of the subsequent steps described herein.

In one embodiment, when nuclei extraction is performed, the outer cell membrane is disrupted and the internal skeleton is disrupted so as to liberate the nucleus 533 from each cell 532 (i.e., epithelial cell) (see FIG. 5B). This provides the benefits of having a smaller, more intact, and easier to manipulate object (i.e., the nucleus 533 of interest, rather than the entire cell 532). The nuclei are then labeled, as nuclei 534-537 (see FIG. 5A), and then sorted in the fluidic cartridge 600/620.

In one embodiment, the nucleus 533 (or nuclei) may be extracted from a cell(s) 532 using a commercial lysis buffer containing detergents such as Triton X-100, SDS, or NP-40, etc., and buffer at a suitable pH.

In another embodiment, selective lysis is further facilitated by the use of a Proteinase K, a serine protease. The nuclei 533, which may be labeled (see labeled nuclei 534-537 in FIG. 5C) according to known methods, and Proteinase K is added in increased concentration over time. With increased concentration of Proteinase K, the labeled cells 534-537 erupt and release the nuclei 534-537. The labeled nuclei 534-537 remain while the other parts of the cell 532 are disintegrated. This method can be carried out in typical liquid handling systems or in a fluidic cartridge 600/620 (see FIGS. 6A-6B and below). This method can be carried out in conjunction with mechanical methods for disrupting the cell membrane, cytoskeleton, or other structures.

In one embodiment, the nuclei extraction procedure further includes known methods of centrifugation at a low spin and controlled speed, or other steps to help physically liberate the nuclei 533 from other cellular material.

In another embodiment, the nuclei extraction procedure further includes processing the cells 532 through shear fluid flow to pull material away from the nuclei 533 through viscous shear forces. In one embodiment, this can be achieved through capillary flow. In another embodiment, this can be achieved through processing with a shear cell. In another embodiment, this can be achieved by flowing the solution through a small opening.

In another embodiment, the nuclei extraction procedure may also include laser scissors (not shown), known in the art, to help liberate nuclei 533 from cells 532, after the

cells 532 are placed in the fluidic cartridge 600/620, for example, FIG. 1, step 102, path 3). In this case, a high peak energy pulsed laser with a highly focused beam can be used to cut out the nucleus 533, according to known methods – see, for example, U.S. Patent No. 4,249,533, to Olympus Optical, which is herein incorporated by reference in its entirety. This method would be performed in the observation/separation area 606, 627 of the fluidic cartridge 600/627 (see FIGS. 6A-6B), prior to the separation step.

In other embodiments, the whole cell 532, including the nucleus 533, is used, and no nuclei extraction is performed – skipping this step entirely.

In other embodiments, the cell nuclei 533 are not completely isolated from the rest of the cell 532, and partial cells 532 that include intact nuclei 533 are used.

In another embodiment, mitochondrial DNA is used and thus, mitochondria are isolated.

Labeling of Target DNA Sequences with Fluorescent in-situ Hybridization (FISH)

A. FISH Labeling of Cells in Fluid Suspension

In another step of the present invention, target DNA sequences in the nucleus of each cell to be sorted are tagged with one or a number of fluorescent labels. In order to label a DNA sequence of interest, a molecule or array of molecules that selectively bind to that DNA sequence, and which can also be attached to a suitable label, are required. Furthermore, in the present invention with respect to trace or touch DNA, the processes used to achieve this binding must not require extracting the DNA from the nucleus to the degree that the DNA becomes freely disassociated prior to labeling, labeling as it is desirable to keep the DNA from different chromosomes associated.

To this end, the present invention focuses on the use of fluorescence in situ hybridization (FISH) of selected DNA sequences in target cells to distinguish cellular nuclei with different genetic features (see FIG. 1, step 103). Fluorescent in situ hybridization (FISH) is a process in which fluorescently labeled fragments of single-stranded DNA or RNA is used as probes to label complementary target DNA sequences. The FISH process, as applied to the present invention must employ labels that fluoresce brightly and without severe photobleaching when bound to a target DNA sequence and with good spectral resolution between labels. The probes must bind specifically and efficiently to the target sequences.

In the present invention, the FISH process is performed on interphase cells using DNA or RNA probes. Although DNA probes are discussed below, the methods are similar with RNA probes. Interphase is the default, everyday state of a cell, when it is not undergoing cell division. In interphase, a cell's chromosomes are not condensed, and the nuclear membrane is intact.

In traditional interphase FISH, the cells (or cellular components) of interest are fixed to glass slides prior to the hybridization step. The probes are prepared separately, in solution, and are labeled either directly with the desired fluorophores ("direct labeling") (see FIG. 2), or with antibodies to which the fluorophores will attach at a later point in the process ("indirect labeling") (see FIG. 2). Labeling of the probes can be accomplished with techniques such as nick translation (a tagging technique in molecular biology in which DNA polymerase is used to replace some of the nucleotides of a DNA sequence with their labeled analogs, creating a tagged DNA sequence which can be used as a probe in FISH or blotting techniques), polymerase chain reaction (PCR), or another suitable

method as is well known in the art. The probe is then introduced to the slide with the fixed cells, and allowed to hybridize to the target DNA sequences, a process of forming a double stranded nucleic acid from joining two complementary strands of DNA.

Hybridization requires first denaturing the cellular DNA at elevated temperature, and then incubating the system at a lower temperature for several hours. Methods and procedures for performing FISH are well-known in the art.

By contrast, in the present invention, the probes are prepared and labeled as in the conventional approach using direct or indirect labeling (see step 104, FIG. 1, and FIG. 2), but the cell preparation is performed in fluid suspension, and the probes are introduced to the cells in fluid suspension, without first fixing the cells to a substrate—a technique known as “suspension FISH” or S-FISH. While S-FISH is not widely used, it is taught in the art by for example, Steinhaeuser et al., “*Suspension (S)-FISH, A New Technique for Interphas Nuclei*,” *Journal of Histochemistry & Cytochemistry* 50 (December 1, 2002): 1697-1698.

This approach to FISH is used in studies of the three-dimensional chromosomal structure of cell nuclei, because fixing and drying the cells, as is done in the traditional FISH technique, tends to flatten the nuclei and distort their internal structure. It is necessary to use suspension FISH in the present invention, because the cells must be in fluid suspension in order to sort them using fluidic cartridges and optical trapping (described below). The process is described below.

In the present invention, each probe DNA sequence is labeled with a fluorescent label (see FIG. 1, step 104, FIGS. 2-3, and FIG. 5C). In one embodiment, the labels may be organic dye molecules (e.g., DAPI, FITC, Cy3, Cy5, Cy7, Texas Red) that are directly

incorporated into the nucleotide FISH probes (“direct labeling”) by known methods, or organic dye molecules or complexes of molecules that bind to haptens that, in turn, are directly incorporated into the FISH probes (e.g., via biotin-streptavidin linkage, where the biotin is incorporated into the FISH probe, and the labels are conjugated to streptavidin – “indirect labeling”) (see FIG. 2). Non-organic fluorophores such as quantum dots may also be used as labels. The emission characteristics of the fluorescent labels are chosen to minimize overlap among them across the detectable spectrum, as discussed below.

Quantum dots provide some compelling advantages including very narrow emission spectrum which would enable one to distinguish a large number of different probes, as well as very good efficiency and robustness against photobleaching.

The labeled probes and the target DNA—still contained in cell nuclei-- are then denatured by heating to produce single-strand DNA (see FIG. 1, step 105, and FIG. 3), and the probe single strand DNA is introduced to the cells (see FIG. 1, step 106). This enables the single strand DNA probes to bind with complementary DNA sequences in the target cells (see FIG. 1, step 106). The DNA denaturization and hybridization steps occur in suspension, and the resulting labeled cells are transferred to a fluidic cartridge for observation and measurement (step 107, FIG. 1).

In one embodiment, the nuclei are first extracted from the cells of interest (see FIG. 1, path 1, and FIG. 5B) by the methods provided above, and then suspension FISH methods (FIG. 1, step 103) as described herein, are practiced.

In another embodiment, the cells are FISH-labeled first according to the present method (see FIG. 1, steps 103-106, and FIG. 5C), then the nuclei are extracted (see FIG. 1, step 102, path 2) according to the previous methods.

In another embodiment, the cells are FISH-labeled first (see FIG. 1, steps 103-106) according to the present method, and are processed whole, according to the methods described below, without extracting the nuclei first.

B. Selection of Labels and Probes

In the present invention, which is directed to trace or touch DNA applications, the number of distinguishable fractions depends on the number of distinctly labeled probes that are employed. Since a given target DNA sequence will either be present or absent in a particular cell, the simultaneous use of N (number) probes will enable sorting of cells into 2^N fractions. For example, using 5 probes with distinct fluorescent labels enables cells to be identified as belonging to one of 32 possible groups. See also, for example, Fig 5B in which two probes are used to fractionate cell nuclei into 4 fractions. It follows that the greatest ability to sort cells into fractions that have a higher likelihood of corresponding to different contributors arises when probes are chosen which target DNA sequences that are present in approximately half the people in the population of interest. Thus, when selecting the set of probes to employ, it is advantageous to define the population of interest or to even define multiple populations of interest and create multiple sets of probes. In selecting probes, off-the-shelf probes may be employed (e.g., Abnova's loci-specific gene probes). However, custom probes may be developed to target specific base sequences of interest to attain a much greater performance at distinguishing cells from different individuals. A set of sequences should be chosen that are neither highly prevalent nor highly rare within the population of interest, and wherein the presence/absence of one sequence is not highly correlated with the presence/absence

of another sequence. In some cases, selecting repeated sequences may provide improved performance. In one embodiment, probes targeting genes determining red blood cell surface antigens (minor and major blood type antigens) may be employed.

In the present invention, as described above, suspension FISH is used to label the cell nuclei with multiple FISH probes (i.e., DNA or RNA segment for binding to genetic sequences of interest, plus the conjugated fluorophore) (see FIG. 1, step 103) in order to distinguish between genetically defined fractions. The nuclei are labeled with a number of fluorescent probes that, when multiplexed, can provide differing fluorescent signatures for different genetically defined fractions. Most protocols for single probe FISH can be modified to accommodate multiple probes, for either fixed or suspended cells. Using an appropriate set of fluorophores combined with a high-quality filter set, one can distinguish around five or six distinct fluorophores. The labeled cells or nuclei are then placed into a multi-output fluidic cartridge, for imaging, identification, and sorting into fractions (see FIG. 1, step 107-110).

The selection of fluorescent labels to use with the selected probes should be done in conjunction with the choice of imaging (emission) filters to be used in detection, as described below. The emission spectra of different fluorophores vary considerably across the detectable spectrum. The excitation spectra also vary, and in some cases it is possible to distinguish two fluorophores based on differing excitation spectra even if their emission profiles match. Generally, however, fluorophores are distinguished by capturing the band of light near their peak emissions and by ensuring that no other fluorophores have substantial emission in this range. Because the emission spectral widths vary, filter manufacturers (e.g. Semrock) provide a useful chart (see FIG. 4, for Semrock chart)

comparing the emission transmission spectra for dyes across the visible spectrum when viewed through different filter sets offered. From this Table in FIG. 4, a combination of DAPI, SpGreen, SpGold, SpRed, and Cy7 can be observed with no greater than 3% overlap between fluorophores. Cy5.5 could be added with only a 12% overlap through the Cy7 filter set. Assuming that 3% overlap is negligible for these purposes, this combination of five dyes would enable the detection of five different target DNA sequences, thus allowing each cell to be identified as belonging to one of 32 distinct groups.

Multiplexed FISH can be used to tag the presence or absence of a greater number of target sequences through the use of combinatorial labeling, albeit at the expense of extended analysis time and increased complexity of the labeling process. Combinatorial, or ratio labeling, means that rather than having a one-to-one match between probes and spectrally-distinct fluorophores, a probe can be labeled with a known ratio of multiple fluorophores, and probes can be distinguished by the relative intensities of the colors. For example, with two fluorophores, yellow (Y) and blue (B), and each probe having three fluorophore-binding sites, four distinguishable probes (YYY, YYB, YBB, BBB) can be created. Combinatorial labeling is used in experiments to observe the interphase chromosome structure of cells. Combinatorial labeling of the probes can lead to a much larger set of distinguishable probes and potentially a larger set of distinct cell fractions, provided the probes are spatially resolved in the cells.

In another embodiment, the number of probes which may be distinguished, is increased by using fluorophores which may have similar emission spectra but differ in

their fluorescence decay lifetime, and employing a detection system that can distinguish or measure the fluorescence decay lifetime as is known in the art.

In another embodiment, entire chromosomes are FISH-labeled (“chromosome painting”), rather than individual DNA sequences. This approach would be useful for gender sorting, or screening for chromosomal abnormalities (i.e., gene screening).

In another embodiment, the cells are labeled by another means other than FISH. For example, one may employ zinc fingers as a probe with fluorescent labels in a method which is generally similar to employing FISH. See, for example, U.S. Patent 6,348, 317, which is herein incorporated by reference. In another embodiment, a peptide utilizing a helix-turn-helix motif for binding DNA sequences is used as the probe. See, for example, U.S. Patent 6,348, 317.

C. Inspection and Fractionation of Cells According to Genetic Sequences

Once the cells or cellular components have been labeled according to the method of the previous section, the cell suspension (or a portion thereof) is placed in a fluidic cartridge 600/620 (see FIG. 5A, and FIG. 1, step 107, and FIGS. 6A-6B) which in turn is placed on an inverted microscope system 500 (see FIG. 5A). The fluidic cartridge 600/620 is disposed in a substage 701 which is part of an XY stage 702 (see also FIGS. 5A and 7B). A clamping plate 501 and clamps 502 hold the fluidic cartridge 600/620 firmly against the substage 701 (see FIG. 5A) so as to form airtight seals for the pneumatic control connections between the substage ports 703 (see FIG. 7A) and the pneumatic ports 802 (see FIG. 8), to ensure the fluidic cartridge 600/620 is level, and to maintain its position.

The objective lens 503 of the microscope system 500 is disposed below the cartridge 600/620, on a Z stage 504, and an illumination source 505 (i.e., brightfield illumination) is disposed above the fluidic cartridge 600/620 to view and illuminate, respectively, the observation and inspection region 606, 627 (see FIGS. 6A-6B). A more detailed description of the setup of the apparatus of the present invention is described further below.

The sample solution of labeled cells or cellular components, is pipetted into the sample inputs of the fluidic cartridge 600/620 (see below and also FIGS. 5B-5C and 6A-6B). Buffer solution (generally an isotonic solution with a pH which is compatible with the probes and labels chosen) is also inserted by pipette into buffer inputs of the fluidic cartridge 600/620 (see below and also FIGS. 5B-5C, and 6A-6B). The sample fluid containing the cells/cellular components is flowed into an observation and separation chamber 606/627 (FIG. 5B-5C, and FIGS. 6A-6B).

The chamber of the observation and inspection region 606, 627 should have optical characteristics suitable for fluorescence and bright-field microscopy and optical trapping, as explained below. Further, for embodiments employing optical trapping, this region should be compatible with optical trapping, as explained below.

The cells or cellular components are allowed to settle or sediment to the bottom of the chamber of the observation and inspection region 606/627 of the cartridge 600/620, over an amount of time determined by the chamber height.

The cells/cellular components are inspected with fluorescence microscopy (see FIG. 1, step 108), classified according to their genetic labels (step 109, FIG. 1), and each is moved to an output channel according to its labels and thus its genetic composition

(step 110, FIG. 1). Sorted cells are then collected from each output channel (step 111, FIG. 1), and the fractions can then be submitted for standard DNA profiling or other diagnostic analysis (step 112, FIG. 1).

In one embodiment, as shown in FIGS. 5B-5C, labeled nuclei 534-537 are introduced into sample input 625, and then flowed into the observation and inspection region 627. The nuclei are sorted according to their genetic labels, and then moved into outputs 630 for collection.

At the very least, the techniques used in the present invention are compatible with optical microscope imaging, optical trapping, and fluidic devices.

Fluidic Cartridge

For an application such as this involving micro-scale manipulation, exemplary embodiments of a fluidic cartridge 600/620 (see FIGS. 6A-6B which show the fluidic layer) provide the link between the separation abilities of the optical trapping apparatus (see FIG. 5A) and the necessary inputs and outputs accessible to manual or automated pipettes and other typical sample transport mechanisms.

While means of moving fluids around a cartridge are known in the art, it should be noted that embodiments employing optical trapping pose particular constraints which make the fluidic cartridge more challenging. In particular, the lower surface of areas where optical trapping will be done should have optical quality, flat smooth surfaces, uniform thickness, and low absorption of the laser wavelength used. For this reason, it is difficult to place a valve between the places where optical traps pick up cells or nuclei and the place where they are dropped off. Thus, valves should be placed carefully, be of appropriate design, and should have sufficient performance to prevent flows between

areas which do not have valves separating them. Further, because a sample may have large quantities of free-floating DNA or other undesirable debris or contamination, optimal designs will minimize free-floating DNA in the sample solution from reaching the isolated fraction outputs. Due to these constraints and challenges, considerable detail is given below on the design, manufacture, and use of these cartridges. Note that the following are exemplary embodiments, and other embodiments may be designed to achieve the same outcome as shown in FIGS. 6A-6B.

A. First Embodiment

This exemplary embodiment shown in FIG. 6A, has two dropoff areas 601-602, and would thus enable the isolation of one or two unique fractions from a cell or cellular component mixture. In the illustrated fluidic device 600, a fluid suspension containing labeled cells (and/or cellular components) of interest is introduced into the device 600 via sample input 607 or reservoir. Buffer fluid is inputted through buffer inputs 603. To prime the channels, the buffer fluid is first pumped through channels 609A to inspection and separation region 606 and towards reservoir 605 in an exemplary curved path, and then the buffer fluid is pumped through channels 609A to channels 609B to prime channels 609B. Optionally, the buffer may also be pumped through additional channels to prime them as well.

Labeled cells/cellular components will naturally start to sediment to the bottom of the sample input 607. However, a suspension mechanism, such as a recirculating flow of fluid from sample input 607 through the channels 614, or other flowing or mixing feature which acts to mix or drive components with shear, keeps the labeled cells/cellular

components from sedimenting to the bottom of the channels 614 and input reservoir 607 prematurely.

The fluid suspension containing the labeled cells is pumped from the reservoir 607 via valves 613, to the inspection region 606, using pumps and actuators as described below with respect to FIGS. 8, 9A-9B, and 10A-10B. Pneumatic channels 806 (see FIG. 8) control valves 612, 613 to the fluid layer of the cartridge 600 allowing fluid to flow, blocking flow, or driving flow (see FIGS. 9A and 9B). The valves 612, 613 are connected to a pressure supply (pumps 711 in FIG. 7B).

Labeled cells/cellular components are pumped into the inspection and separation region 606 and allowed to sediment to the surface of the chamber so that they reside in or near the plane which is imaged and in which optical trapping and imaging (see further below) can be performed. Reservoir 605 includes unsorted samples which are pumped from the inspection region 606, noting that the process of loading region 606 with solution and sorting certain cells or components to dropoff regions 601-602 may be performed repeatedly with the unsorted solution being pushed towards reservoir 605 for each cycle.

All optical trapping and imaging is conducted through the optically-clear (e.g., COC polymer, molded or embossed using a highly polished metal mold) window that forms the bottom of the chamber in the observation and inspection region 606 of the fluidic cartridge 600.

The labeled cells/cellular components are inspected and identified as described below, using optical microscopy, and are then moved via e.g., optical trapping and possibly stage motion, to one of two dropoff areas 601, 602, based on their labels.

To extract the separated labeled cells or cellular components, the fluid is pumped using pumps and actuators (see below for detailed description) from the dropoff areas 601, 602 through channels 609B to the appropriate two outputs 611, where the isolated fractions of labeled cells/cellular components, are extracted.

B. Second Embodiment

FIG. 6B shows another exemplary embodiment of a fluidic channel structure 620 that could be used in the present invention.

This exemplary embodiment shown in FIG. 6B, has four dropoff areas 621-624 and four outputs 630, and would thus enable the isolation of four fractions of labeled cells or cellular components from a mixture. In the illustrated fluidic device 620, a fluid suspension containing labeled cells/cellular components of interest is introduced into the device 620 via sample input 628.

As noted above, a suspension mechanism, such as a recirculating flow of fluid from sample input 628 through the channels 634, or other flowing or mixing feature which acts to mix or drive components with shear, keeps the labeled cells/cellular components from sedimenting to the bottom of the channels 634 and input reservoir 628 prematurely.

Buffer fluid is inputted through buffer inputs 626, and the buffer fluid flows through channels 629A to inspection and separation region 627 in an exemplary curved path. The buffer fluid primes the buffer channels 609A, 609B prior to the sample being inputted.

The fluid suspension containing the labeled cells is pumped from the sample input 628 via valves 631 in channels 634, to the inspection region 627, using pumps and

actuators as described below with respect to FIGS. 8, 9A-9B, and 10A-10B. Pneumatic channels 806 (see FIG. 8) control valves 631, 632 to the fluid layer of the cartridge 620 to allowing fluid to flow, blocking flow, or driving flow (see FIGS. 9A and 9B).

Labeled cells/cellular components are pumped into the inspection and separation region 627 and allowed to sediment to the surface of the chamber so that they reside in or near the plane which is imaged and in which optical trapping (see further below) can be performed. Reservoir 625 receives unselected components of the sample from inspection region 627.

All optical trapping and imaging is conducted through the optically-clear (e.g., COC polymer, molded or embossed using a highly polished metal mold) window that forms the bottom of the chamber in the observation and inspection region 627 of the fluidic cartridge 620.

The labeled cells/cellular components are inspected and identified as described below, using optical microscopy, and are then moved via e.g., optical trapping and possibly stage motion, to one of four dropoff areas 621-624, based on their genetic labels.

To extract the separated labeled cells or cellular components, the fluid is pumped using pumps and actuators (see below for detailed description) from the dropoff areas 621-624 through channels 629B to the appropriate four outputs 630, where the isolated fractions of labeled cells/cellular components, are extracted.

The cartridge 600/620 designs are exemplary, and may have additional outputs required for isolating a larger number of fractions, but one of ordinary skill in the art would know how to provide such additional outputs.

Fluidic Cartridge Assembly

In one embodiment of the present invention, the fluidic cartridge 600/620 is disposable and is mounted on a sub-stage 701 (see FIG. 7A) that places the fluidic cartridge 600/620 in a microscope optical train (see FIG. 5A, 500, 505, 511, 521, and 518, for example) and connects the pneumatic channels of the fluidic cartridge 600/620 to the pneumatic control system 506, including control valves 509, pumps 507, tubing 510, and electronics (see below for further description, and FIG. 5A).

FIG. 7A shows a substage assembly 700, where the fluidic cartridge 600/620 is inserted into a substage 701, which is part of a larger stage 702. Specifically, the fluidic cartridge 600/620 is positioned above the mounting substage 701 and oriented for mounting. The substage 701 has a plurality of pneumatic connection ports 703 connecting to corresponding ports 802 of the pneumatic layer 801 of the fluidic cartridge 600/620 (see FIG. 8). When connected, the pneumatic control system of the assembly 704 may deliver pressure or vacuum via ports 802 to valves 612/613 and 631/632 of the fluidic cartridge 600/620 as needed.

In order to make a hermetic connection between the fluidic cartridge pneumatic ports 802 and substage ports 703, the raised substage ports 703 enter the pneumatic ports 802, and the clamping pressure from the clamping plate 501 compresses the harder substage against the softer silicone layer of the fluidic cartridge 600/620 which acts like a gasket, creating a seal. The substage assembly 701 is mounted on a larger stage 702, such as a Prior H117 stage 702 (see also FIG. 5A).

FIG. 7B shows the substage and pneumatic control system assembly 704. The substage 701 is attached to a pneumatic manifold 705 which has a plurality of solenoid valves 706 directly mounted onto it. Two air tubes 707, 708 (one vacuum 707 and one

pressure line 708) connect the manifold 705 to a control board 709 which has tubes 710 connecting to a group of pneumatic pumps 711. Solenoid valves 706 are all programmed and controlled by the host computer system 712 through the control board 709 (data cables are not shown). When the computer system 712 is on, the manifold 705 is supplied with a positive pressure (5-10 psi) and a negative pressure (vacuum of -10 psi). Since each pneumatic port 703 (FIG. 7A) of the substage 701 is connected to the manifold 705 through a dedicated 3-way solenoid valve 706, either positive pressure or negative pressure can be delivered through each pneumatic port 703 at predetermined timings via the computer-controlled solenoid valve 706.

Valve Operation

FIGS. 9A and 9B illustrate one embodiment of a normally-closed pumping valve arrangement that is used in the fluidic cartridge 600/620 of the present invention. The valves correspond to the valves 612/613 and 631/632 in the fluidic channels described in FIGS. 6A-6B. The valves 612/613 and 631/632 are disposed at the interface between pneumatic layer 903 and fluidic layer 907 (see 801, 803 in FIG. 8).

In the closed position of FIG. 9A, the membrane 900, which is adhered to pneumatic layer 903 by adhesive 906, seals the fluidic channel 901 so that liquid cannot flow. A positive pneumatic pressure (e.g., 5-10 psi) may be applied to the membrane 900 via a pneumatic channel 902 of the pneumatic layer 903 (see also FIG. 8) to ensure proper sealing. When a negative pressure (e.g., a vacuum of -10 psi) is applied, the membrane 900 is pulled into the valve seat 904A in the pneumatic layer 903 (see FIG. 9B), thus opening the valve 905 and permitting fluidic flow.

Because the opening of the valve 905 can draw liquid into the newly exposed valve chamber 904, subsequent closing of the valve 905 can displace this volume of liquid back into the fluidic channel 901 to create a pumping action. In order to create a directional flow of liquid through the fluidic channel system of the fluidic cartridge 600/620, a cycle of valve operations can be executed to induce peristaltic flow (e.g., in the direction of the arrow in FIG. 10A) within a fluidic circuit as follows.

The valve operating sequence starts with all valves 905 closed by sending a high voltage signal (V) on all solenoid valves 706. The sequence is as follows: (i) Valve 1 (FIG. 10A) is opened by sending a high (5v) voltage signal to the corresponding solenoid valve 706. (ii) Valve 2 is opened. (iii) Valve 1 is closed. (iv) Valve 3 is opened. (v) Valve 2 is closed. (vi) Valve 3 is closed.

With respect to the recirculating flow, when the labeled cells/cellular components are in the on-cartridge sample input 607, 628, they may tend to settle or sediment over time. As a result, when the cells/cellular components are dispensed to the chamber in the inspection region 606, 627, they may exit the sample input 607, 628 with a non-uniform concentration, very low concentration, or they may not exit at all.

FIG. 10B shows a scheme for mixing/resuspending the labeled cells/cellular components prior to dispensing to the chamber of the inspection region 606, 627. In the embodiment of FIG. 10B, valve 7, which leads to the observation and inspection chamber, remains closed throughout the mixing sequence. Valves 4, 5 and 6 are operated in a sequence similar to the one described above and shown in FIG. 10A, to create a circular flow in the direction of the arrow. This continual flow keeps the cells/cellular

components suspended and purges any air bubbles into the sample input 607, 628 (particularly where the sample input is open-top) where the bubbles can leave the system.

In operation, as described above, valve control software run by computer system 712 (for controlling opening and closing timings for all valves 706, 612, 613, 631, 632), runs to prime various channels and regions 609A, 609B, 629A, 629B, 606, 627 with buffer solution from buffer inputs 603, 626 (see FIGS. 6A-6B) in the fluidic cartridge 600/620.

The software then runs the mixing pump sequence (see FIGS. 10A, 10B) for a few cycles to flow sample solution from the sample input 607, 628 to mix/resuspend sample solutions that have sedimented to the bottom of the sample input 607, 628 and recirculation channels 614, 634. Alternately, the priming of channels with the buffer can be performed simultaneously with the continual operation of the mixing sequence so as to minimize the opportunity for sedimentation in the sample input 607, 628 and recirculation channel 630, 634.

The sample solution is then pumped from sample input 607, 628 to the interrogation region 606, 627. Samples in the chamber of the interrogation region 606, 627 are inspected using microscopic imaging equipment and software (see below for description).

The labeled cells/cellular components are isolated according to their genetic labels, and moved by software-controlled optical trapping, and stage motion where necessary, to the dropoff regions 601/602 and 621-624.

The buffer solution is pumped from the buffer inputs 603, 626 to move the sorted labeled cells or cellular components, to outputs 611, 630. Sorted labeled cells/cellular

components are collected and removed from the outputs 611, 630 using pipettes or other liquid handling devices. Unsorted samples are pumped from the inspection region 606, 627 to reservoir 605, 625 where it may be stored or removed.

Manufacture of Fluidic Cartridge

The fluidic cartridge 600/620 of the present invention should be fabricated of materials with low light absorption of the wavelengths used for optical trapping, so that the optical traps formed (see below) will not become blocked or heat the fluidic cartridge 600/620, and thus, the sample to an unacceptable level. Further, the materials should not be processed in such a way as to make them absorbing at the wavelengths used for optical trapping. Additionally, materials in the cartridge 600/620 should not fluoresce to the point of obscuring fluorescence measurements. In addition, the bottom surface of the fluidic cartridge's 600/620 inspection and separation region 606, 627 should be optically clear and must be sufficiently thin to allow a high numerical aperture, short working distance objective lens of an optical trapping apparatus 510 (see FIG. 5A) to focus inside the chamber, and should be transparent to light wavelengths used to excite and observe the fluorescent labels discussed previously. The channel structure of the cartridge 600/620 should be designed to minimize sample loss inside the cartridge 600/620, for example due to cells or cellular components such as nuclei, becoming trapped in corners or against surfaces. Bubble formation and leaks must be prevented since they can create undesired flows. A detailed description of the manufacture of the fluidic cartridge 600/620 is provided below.

One example of a fluidic cartridge 600/620 design showing the manufacture and internal layers suitable for use with the present invention is shown in FIG. 8. The fluidic cartridge 600/620 fabrication and assembly steps are as follows.

- 1) Laminate one side of a 200 μm PMMA sheet (e.g., Asta Products, 0.2mm thickness, clear uncoated) with a 25 μm silicone PSA sheet (Pressure sensitive adhesive, e.g., Dielectric Polymers, Inc., Trans-Sil Silicone Transfer Adhesive, Product # 1001-1) at room temperature (RT) using a laminator (e.g., Tah Hsin Industrial Corp.; TCC-2700 ITE Laminator), to achieve a laminated material 804.
- 2) Laser cut the laminated material 804 using, for example, M-300 Universal Laser System, to make the top layer.
- 3) Laser cut the pneumatic channels 806 (0.2 ~ 0.7 mm) and ports 802 (0.7 ~ 0.9 mm) in a 1.5 mm PMMA sheet 805 (e.g., McMaster Carr; clear cast acrylic 0.06" Product # 8560K171) to make the middle layer.
- 4) Apply parylene coating on one side of a 250 μm silicone elastomer (e.g., Marian Chicago; 010" HT-6240, 40 Durometer Solid Silicone, Transparent; Parylene coating: Specialty coating systems, parylene coating 0.60 μm thickness).
- 5) Laminate the parylene coated side of the 250 μm silicone elastomer with a 25 μm silicone PSA at room temperature (RT) using the laminator, to achieve a laminated material 807.
- 6) Laser cut the laminated silicone elastomer material 807 to make the membrane layer 807.

- 7) Align and press the three layers (i.e., top layer, middle layer and membrane layer) together and apply 7000 lbs force for 1 min at RT to make the pneumatic layer 801.
- 8) Add 1-1.5 gram of COC resin pellets (e.g., ZEON Chemical, Zenor 1020r) into a mold for the desired fluid channel pattern and place the mold inside a pneumatic press (e.g., Tetrahedron, model 100). The press then operates at 420°F and 10 psi for 30-40 minutes. The molded COC layer 808 is cooled and released from the mold. The finished COC layer 808 is about 0.3mm thick.
- 9) Treat molded COC layer 808 with 60W oxygen plasma (e.g., SPI Plasma Prep II) for 30 sec and place it in an aqueous solution of 1% v/v APTES for 20 min. (see V. Sunkara et al., Lab Chip, 2011, 11, 962-965).
- 10) Wash the resulting fluidic layer 803 with DI water and completely dry it using an air stream.
- 11) The bonded pneumatic layer 801 is placed in the plasma instrument with the silicone layer facing up. Treat the pneumatic layer 801 with 60W oxygen plasma for 30 sec.
- 12) Carefully align the fluidic layer 803 on top of the pneumatic layer 801. Then press and bond the fluidic layer 803 onto the pneumatic layer 801. Mount the assembled fluidic cartridge 600/620 onto a custom vacuum stage which has a vacuum chamber connecting to all pneumatic ports 802 of the cartridge 600/620. The vacuum chamber is connected to vacuum pump.
- 13) Bake the assembled cartridge 600/620 for 40 min at 90°C while applying vacuum to the cartridge 600/620 through the vacuum stage. Vacuum is applied to the

cartridge 600/620 to avoid direct contact of the silicone elastomer layer 807 to the COC layer 808 in the valve areas (to prevent unwanted bonding of the membrane to the fluidic layer below the valve seat 904A).

Thus, layers of Poly(methyl methacrylate) (PMMA), pressure-sensitive adhesive, silicone, and COC are bonded together to form a chip 600/620 effectively, with a pneumatic layer 801 and a fluidic layer 803. The pneumatic layer 801 relies on diaphragm pumps, valve manifolds, and precise computer control over valve timings to create controlled flows in the fluidic layer 803 over the course of a separation process.

Inspection and Identification of Cells

Once the fluid suspension containing cells to be analyzed has been introduced to the imaging/separation region 606, 627 of the fluidic cartridge 600/620 (see FIGS. 6A-6B), and the cells or cellular components are allowed to settle to the bottom of the region 606, 627, they are inspected using optical microscopy, in order to identify how each cell or cellular component is labeled, and thus determine which output fraction it belongs to, as well as how many fractions are present among the observed cells or cellular components (i.e., how many output fractions there should be in total).

In the present invention, which uses fluorescently labeled probes specific to sequences of DNA/RNA bases, this inspection is accomplished via fluorescence microscopy, or a combination of fluorescence microscopy and bright-field microscopy - techniques which are well known in the art.

Images of the cells or cellular components are acquired via an optical sensor array (e.g., CCD or CMOS camera 522 of the imaging system 521 – see FIG. 5A) incorporated into the optical system 505, 500, 511, 518, 521, 524, 528. Direct inspection of the

cells/cellular components is performed by the optical sensor array 522, or possibly a human operator, in order to identify the cells/nuclei which have different labels, or to identify a presence, absence, quantity or relative quantity of the DNA or RNA probes. Identification and classification of cells/cellular components may be performed by a computer program (see computer system with display 530/531), or by a human operator.

In the present invention, a number of different targets in the cell nuclei should be simultaneously tagged with distinguishable fluorescent labels. In the preferred embodiment, these will be fluorescent dye molecules with different excitation and emission spectra (colors). Conventional fluorescence microscopy, using organic fluorophores - as described previously - requires the use of specialized optical filter sets to isolate emission light (the signal to be detected) from excitation and background light. Several options for the filters that may be used in the present invention are as follows:

- a. If only a small number (i.e., currently 1-4, or more) of labels is to be detected, a single multiband filter set (comprising an excitation filter, a dichroic reflector, and an emission filter) may be used to simultaneously observe all the colored labels.
- b. If a larger number of labels is to be detected, a sequence of observations may be made, using sequential imaging of multiple labels using a series of single-band and/or several multi-band filter sets, that are moved into place for each observation. This may be accomplished via a rotating filter cube turret, filter wheel 527/529 (see FIG. 5A), and other such mechanisms known in the art.
- c. Alternatively, one could perform spectral imaging of multiple labels using either a single-band filter set, or liquid crystal or acousto-optical tunable filters, which permit continuous filter adjustment without mechanical motion, along a wide range of

wavelengths. Tunable filters offer some advantages over mechanically scanning dispersive devices (i.e., filter wheels, monochromators) because they are fast, compact, and demonstrate increased spectral selectivity, spectral purity, and flexibility. Examples of such filters are Meadowlark Optics' Liquid Crystal Tunable Filter and CRI's VariSpec line. However, their light transmission is much lower than for simple dielectric filters.

One embodiment of the present invention uses conventional (non-tunable) optical filters, as described in points (a) and (b) above, as this is a well-established and readily available technology. Furthermore, it is likely that it will enable the brightest possible fluorescent signals to reach the detector (e.g., camera 522), and avoid the significantly lower transmission of the continually-adjustable filters. Since multiple filter sets are necessary to evaluate a larger number of fluorescent labels, automated filter switching is desirable. This is readily available, and Nikon, for example, offers a motorized filter cube turret that can switch between filter sets in 0.3 s.

In order to image the cells to be categorized, an autofocus system 518 (see FIG. 5A) autofocuses on the imaging system on the cells. In one embodiment, the autofocusing system 518 employs a laser 519, collimator 520, and beamsplitter 532 to create a focused point of laser light in the sample plane, allowing the system to adjust the focus to bring this focused point of light into sharp focus on an imager 522. The advantage to this method is that it can be done without cells or debris present, and it can detect and distinguish the lower air-COC interface and the upper COC-water interface. In other embodiments, other known methods for autofocusing can be employed to focus the system at or near the COC-water interface to where the sample sediments.

The cells will then be imaged using a series of filter combinations, illumination intensities, and camera 522 settings, in order to examine the labels present in each nucleus. In the preferred embodiment, this is done in an automated fashion, using a motorized microscopy system 504, 530 and a computer-controlled imaging device 521 (i.e., with camera 522, tube lens 523, and mirror 532) (see FIG. 5A). In another embodiment, some or all of the steps are performed by a human operator.

In one embodiment, simultaneous imaging of multiple color labels using a single multiband filter set (e.g., 3-4 colors) is used. In another embodiment, sequential imaging of multiple labels using a series of single-band and/or multi-band filter sets, is used. In another embodiment, spectral imaging of multiple labels using a tunable excitation light system 524 (including broadband light source 525, collimator 526, beamsplitter 532, and filter wheel 527) and a tunable emission filter system 528 (see FIG. 5A), is used. In another embodiment, a combination of tunable and non-tunable filters is used. In some embodiments, the beamsplitter 532 is also tunable or switchable between multiple discrete beamsplitters.

Fractionation of Cells using Optical Trapping

Once the cells have been imaged and categorized according to their fluorescent labels, they are moved from the observation/separation area 606, 627 of the fluidic cartridge 600/620 to dropoff areas 601/602, 621-624 (see FIGS. 6A-6B). Each output 611/630 corresponds to a different genetic composition, that has been identified by the system as indicated by the labels presented and possibly their intensities, intensity ratios, or other attributes (e.g., fluorescence decay lifetime).

In the present invention, in one embodiment, as stated above, holographic optical trapping (HOT) 510 (see U.S. Patent Nos. 6,055,106, 6,863,406, 7,411,180 etc.), and possibly stage 702 movement, is used to move cells from the inspection area of the microfluidic cartridge 600/620 to their designated output areas 601/602, 621-624. HOT allows visualization-based single cell sorting (see U.S. Patent Nos. 8,067,170 and 7,998,676), and is compatible for use with cells labeled with fluorescent biomarkers. HOT has been shown to work effectively to trap a number of different cell types such as epithelial cells, macrophages, red blood cells, white blood cells and platelets, cells of neuronal type such as PC 12 cells, and sperm. Furthermore, HOT has been demonstrated to trap extracted nuclei from a variety of different cell types. Optical trapping has been shown to not damage DNA, if care is taken to employ reasonably low light power and certain (generally infrared) wavelengths for trapping, and therefore, is suitable for handling cells that are to be subjected to genetic analysis. Further, optical trapping of fluorescently labeled sperm has been shown to be compatible with downstream forensic DNA profiling.

As shown in FIG. 5A, the HOT system 511, which includes a laser 512, collimator 513 (i.e., collimating lens), mirror 514, spatial light modulator 516, beamsplitter 532, and other lenses 517, is connected to the objective lens 503 of the microscope system 500.

In this embodiment, optical traps (using HOT) are created at the locations of cells within the inspection region 606, 627 of the chip 600/620 (see FIGS. 6A-6B) which have been identified as targets. Using these traps, the cells/cellular components are lifted above the bottom surface of the fluidic cartridge 600/620, so that they can be moved without

interference from other cells/cellular components or debris that have settled on the bottom of the chamber of the observation/separation area 606, 627. Lifting the cells also enables faster movement by reducing hydrodynamic coupling to the surface, provided spherical aberrations do not become too severe. HOT may also be used to rearrange the configuration of trapped cells/cellular components to improve the efficiency of long-distance movement. Finally, stage motion, where necessary, is used to transport the trapped cells/cellular components over large (greater than approximately 100 μm) distances, to designated dropoff areas 601/602, 621-624 of the fluidic cartridge. Differently-labeled cells are moved to different dropoff areas 601/602, 621-624, and to different outputs 611, 630.

In another embodiment, a single optical trap is used. In another embodiment, multiple optical traps are produced using HOT and are used to move the labeled cells/cellular components. In another embodiment, multiple optical traps are produced with other multi-trap techniques, and used on the labeled cells/cellular components. In another embodiment, non-Gaussian optical trapping methods, such as Bessel beams, may be used for trapping and sorting the cells/cellular components, as described in U.S. Patent No. 7,574,076 which is herein incorporated by reference in its entirety.

If necessary, based upon chip design, cell concentration, and the number of desired output cells/cellular components, this process is repeated on multiple regions of the inspection/separation region 606, 627 until either all available labeled cells or cellular components in the inspection region 606, 627 have been processed, or the desired number of labeled cells/cellular components in each output group has been collected from outputs 611, 630. If the inspection region 606, 627 has been depleted of cells/cellular

components, but more cells/cellular components are needed, a new quantity of the sample cell suspension can be flowed into the inspection region 606, 627, and the process repeated. Finally, once a sufficient number of cells/cellular components has been collected at a given dropoff area 601/602, 621-624, the fluidic system 600/620 is used to transport those separated cells/nuclei to a region where they can be manually extracted from the outputs 611, 630 of the cartridge 600/620.

In one embodiment, the trapping and separation procedures are automated and computer-controlled by a computer system 530 with display 531 (see FIG. 5A). In another embodiment, some or all steps could be performed by a human operator.

In the present invention, the optical trapping and cell visualization procedures use many of the same optical components, and therefore any shared optical components should be chosen to be compatible with both optical trapping and fluorescence microscopy.

Fractionation of Cells by Non-Optical Forces

With suitable modifications to the design of the fluidic cartridge, other methods besides optical trapping and its variations may be used to separate the cells/cellular components into fractions based on their labels. For example, the following methods may be used to fractionate the cells/cellular components upon preparing in solution, genetic labeling, reading the labeling of the cells or cell components, and sorting in accordance with the groups defined by the genetic labeling:

a. Microfluidic Cytometry: The cells/nuclei may be sorted in an active microfluidic system, such as a fluidic cartridge which has actuators, valves, or other mechanisms to steer the fluids between multiple fluid paths. This may be done, for

example, using piezoelectric actuators to direct flow. See, for example, Mueth et al., “*Method and apparatus for sorting cells*”, U.S. Patent 7,545,491, which is herein incorporated by reference in its entirety. In one such embodiment, the cells may be arranged to follow each other down the faster internal flow of a channel by using an external sheath fluid (as described in aforementioned patent), interrogated optically, and sorted by directing the solution between one of multiple output paths.

b. **Dielectrophoresis and Electrowetting:** The cells or nuclei of interest may be isolated using dielectrophoretic forces upon the cells/cellular components or upon the fluid the cells/cellular components are in. For example, the cells/cellular components are disposed in droplets which are guided or moved along a surface using dielectrophoretic forces or by locally changing the wetting properties of the surface. These methods exploit the interaction of electric fields (frequently AC electric fields) with materials or fluids, causing them to move to areas of higher or lower field strength or field gradient. See, for example, Pamula et al, “Apparatus for manipulating droplets by electrowetting-based techniques”, U.S. Patent 6,911,132 (commercialized by Advanced Liquid Logic).

c. **Combined Opto-electronic Forcing:**

The cells/nuclei may be moved, or the cells/nuclei separated, by a combination of optical and electrically induced forces. See, for example, Chuang et al, “Open Optoelectrowetting droplet actuation device and method”, U.S. Patent Application Publication No. 2012/0091003.

DNA Analysis/Profiling

For final analysis, each cell fraction is subjected to DNA profiling using standard techniques known in the art (step 112, FIG. 1). This may be done on the same fluidic cartridge 600/620, in other embodiments. In the case of the present invention for processing forensic trace or touch DNA evidence, profiling is directed towards identifying individual contributors. Additionally, the profiling may reveal genetic or potentially phenotypic characteristics about the individual contributors.

Techniques that may be used to extract DNA profiles from small amounts of genetic material, such as would be the outputs of the present invention, include:

- a. Polymerase chain reaction (PCR) amplification and detection of short tandem repeat (STR) alleles. This is the current standard method for forensic DNA profiling, and many commercial products exist for performing the reactions and analyzing the results.
- b. SNPs (Single Nucleotide Polymorphisms) are another type of DNA sequence that can be used to identify or classify individuals, like STRs. Also, they can be used to identify ethnic origins of DNA sources, by comparing against population databases.
- c. Y-chromosome DNA analysis (using SNPs or STRs), commercial products exist for this class of analysis
- d. Mitochondrial DNA analysis, commercial products exist for this class of analysis
- e. Whole genome amplification (WGA) techniques, in conjunction with PCR and STR or SNP analysis

f. Other techniques

Because the present invention sorts cells/cellular components into a finite number of fractions based on selected genetic markers, it is not guaranteed that each output fraction corresponds to a single contributor. The present invention, rather, decreases the likelihood that an output fraction will contain DNA from multiple contributors, and each fraction is likely to contain DNA from fewer contributors than did the original mixture. The DNA profiles derived from the above analysis methods must still be interpreted using statistical methods known in the art.

Applications

Particular applications of the present invention in forensics include:

- a. Classifying epithelial cells or cell nuclei in “touch” evidence into multiple groups and isolating the cells or nuclei into distinct subgroups, for downstream profiling of the individual or subset of individuals in that group.
- b. Classifying sperm in sexual assault evidence into multiple groups and isolating the cells in a given group from the rest of the sample, for downstream profiling of the individual or subset of individuals in that group.
- c. Classifying epithelial cells in sexual assault evidence into multiple groups and isolating the cells in a given group from the rest of the sample, for downstream profiling of the individual or subset of individuals in that group.
- d. Classifying other cells types in forensic evidence into multiple groups and isolating one or more cells in one or more groups from the rest of the sample, for downstream profiling of one or more individuals in that group.

Beyond the forensics field, particular applications in medical diagnostics and medicine include:

- a. Isolating cells with certain qualities for genetic testing, such as possibly-cancerous cells.
- b. Isolating cells with certain qualities for non-genetic testing, such as testing possibly-infected or diseased cells for certain antigens or for their response to certain chemicals or pharmaceuticals.
- c. Isolating cells with particular qualities for non-testing use, such as culturing, for therapeutic use, or for other non-testing purposes. For applications involving live cells, FISH may not be suitable and the use of zinc fingers or another molecule which does not require denaturing of the target DNA will be preferred.

It should be emphasized that the above-described embodiments of the invention are merely possible examples of implementations set forth for a clear understanding of the principles of the invention. Variations and modifications may be made to the above-described embodiments of the invention without departing from the spirit and principles of the invention. All such modifications and variations are intended to be included herein within the scope of the invention and protected by the following claims.

What is claimed is:

1. A method of sorting cells and/or cellular components into multiple populations, comprising:
 - preparing a plurality of cells and/or cellular components in fluid suspension;
 - preparing a plurality of probes which bind to predetermined DNA or RNA sequences in said cells and/or cellular components;
 - introducing said plurality of probes to target cells and/or cellular components in a fluid suspension so as to specifically bind said probes to their corresponding sequences of DNA or RNA in said cells and/or cellular components;
 - moving said cells and/or cellular components to an input of a fluidic cartridge;
 - wherein said probes are probes labeled during said preparing step or after said introducing step or said moving step, by using predetermined fluorophores or immunoglobins to which said fluorophores will attach; and
 - sorting said labeled cells and/or cellular components in said fluidic cartridge into output fractions according to their genetic composition.
2. The method of claim 1, further comprising:
 - performing a fluorescence in situ hybridization (FISH) process with said probes and said target cells and/or cellular components in suspension, wherein said FISH process includes:
 - producing single-strand DNA or RNA in said probes and in said nuclei of said target cells and/or cellular components;

introducing said single-strand DNA or RNA probes to said target cells and/or cellular components, allowing the single-strand DNA or RNA probes to bind with complementary DNA or RNA in said target cells and/or cellular components.

3. The method of claim 2, further comprising:

labeling at least a portion of said predetermined DNA or RNA sequences of each specificity of said probes with a different fluorescent label or a different combination or ratio of fluorescent labels.

4. The method of claim 3, wherein said labels of said probes are organic dye molecules which are directly incorporated into said probes.

5. The method of claim 3, wherein labels of said probes are organic dye molecules or complexes of molecules that bind to haptens that are directly incorporated into said probes, including by biotin-streptavidin linkage; and
wherein said labels are conjugated to streptavidin.

6. The method of claim 3, wherein said nuclei of said target cells are labeled with a number of fluorescent probes that, when multiplexed, can provide differing fluorescent signatures for different genetic profiles.

7. The method of claim 1, further comprising:

extracting nuclei from said target cells prior to performing said labeling step.

8. The method of claim 1, further comprising:
labeling said probes, prior to introducing said probes to said target cells and/or cellular components.
9. The method of claim 7, further comprising:
introducing said probes to said target cells and/or cellular components, prior to extracting said nuclei from said target cells.
10. The method of claim 6, wherein emission characteristics of said fluorescent labels are predetermined to minimize overlap among them across the detectable spectrum.
11. The method of claim 6, further comprising:
using an appropriate set of fluorophores combined with a high-quality imaging filter set, such that five or six distinct fluorophores can be distinguished.
12. The method of claim 6, wherein multiplexed fluorescence in situ hybridization (FISH) in suspension is used to tag a presence or absence of a greater number of said predetermined DNA or RNA sequences in said target cells and/or cellular components through a use of combinatorial labeling.

13. The method of claim 1, wherein entire chromosomes are labeled using FISH rather than individual DNA or RNA sequences in said target cells and/or cellular components, for gender sorting or screening for chromosomal abnormalities.

14. The method of claim 1, wherein said cells and/or cellular components are labeled by non-FISH means, including zinc fingers.

15. The method of claim 1, further comprising:
placing said fluidic cartridge in an inverted microscope system used as an optical microscopy system to perform said optical microscopy.

16. The method of claim 15, further comprising:
flowing said cells and/or cellular components into an observation and separation chamber from a sample input of said fluidic cartridge for inspection; and
inspecting said cells and/or cellular components in said chamber using optical microscopy to identify how each of said labeled cells is labeled, to determine to which of said output fractions said labeled cells belong, and which genetic labels are present amongst said labeled cells and/or cellular components.

17. The method of claim 16, further comprising:
sorting into said output fractions by moving said labeled cells and/or cellular components into separate output channels or regions according to their genetic labels; and

moving said labeled cells and/or cellular components from said output channels or regions to separate outputs.

18. The method of claim 17, further comprising:
collecting said output fractions from each of said outputs; and
submitting said output fractions for DNA profiling and diagnostic analysis.

19. The method of claim 16, wherein said inspection of said labeled cells and/or cellular components in said chamber is accomplished via fluorescence microscopy, laser fluorescence, or a combination of fluorescence microscopy and bright-field microscopy.

20. The method of claim 16, further comprising:
acquiring images of said labeled cells and/or cellular components via an optical sensor array incorporated into said optical microscopy system.

21. The method of claim 20, further comprising:
identifying and classifying said labeled cells and/or cellular components using a computer system.

22. The method of claim 10, wherein imaging filters are used to isolate emission light from excitation and background illumination.

23. The method of claim 22, wherein a single multiband filter set is used to simultaneously observe all colored labeled cells and/or cellular components, when no more than five labels are to be detected.

24. The method of claim 22, wherein a set of said imaging filters includes an excitation filter, a dichroic reflector, and an emission filter.

25. The method of claim 22, wherein a series of single-band and/or a plurality of multi-band filter sets, are used via a rotating filter cube turret or filter wheel.

26. The method of claim 22, wherein spectral imaging of multiple labels is performed using one of a single-band filter set, or a liquid crystal or acousto-optical tunable filters.

27. The method of claim 22, wherein a non-tunable optical filter is used.

28. The method of claim 20, wherein said cells are imaged and categorized by a fluorescent imaging system which performs said fluorescence microscopy, said fluorescent imaging system which autofocuses on said labeled cells and/or cellular components.

29. The method of claim 28, wherein said autofocusing is performed under bright-field or fluorescence illumination or using a focused laser which reflects off of material interfaces to provide a target on which to focus.

30. The method of claim 20, wherein said labeled cells and/or cellular components are imaged using a series of filter combinations, illumination intensities, and camera settings, in order to examine fluorescent characteristics thereof.

31. The method of claim 20, wherein said imaging is automated using a motorized optical microscopy system and a computer-controlled imaging device.

32. The method of claim 19, further comprising:
inspecting multiple fluorescent labels using tunable excitation light and a tunable emission filter.

33. The method of claim 19, wherein a combination of tunable and non-tunable filters is used for inspecting multiple fluorescent labels.

34. The method of claim 17, wherein said moving steps include:
optical trapping said cells and/or cellular components to move said cells and/or cellular components from said chamber of said fluidic cartridge to a designated one of said output channels and to its designated output.

35. The method of claim 34, wherein said optical trapping is holographic optical trapping.

36. The method of claim 35, further comprising:
rearranging a configuration of said trapped cells and/or cellular components to improve efficiency of long-distance movement.

37. The method of claim 34, wherein stage motion is used to transport said trapped cells and/or cellular components to said designated output of said fluidic cartridge.

38. The method of claim 34, wherein a single optical trap is used to trap one of said labeled cells and/or cellular components.

39. The method of claim 35, wherein multiple optical traps are produced using holographic optical trapping to move said labeled cells and/or cellular components.

40. The method of claim 34, wherein non-Gaussian optical trapping methods are used for moving and sorting.

41. The method of claim 34, wherein said optical trapping and sorting procedures are automated and computer-controlled by a computer system.

42. The method of claim 17, wherein said cells and/or cellular components are separated into said output fractions using microfluidic cytometry using actuators or valves which steer fluids in said fluidic cartridge between multiple fluid paths or channels.

43. The method of claim 17, wherein said cells and/or cellular components are separated into fractions using dielectrophoresis and electrowetting, or combined opto-electronic forcing.

44. The method of claim 18, wherein methods for said DNA profiling include Polymerase chain reaction amplification (PCR) and detection of short tandem repeat (STR) alleles, Single Nucleotide Polymorphisms (SNP) analysis, Y-chromosome DNA analysis, Mitochondrial DNA analysis, whole genome amplification techniques, or a combination thereof.

45. The method of claim 1, wherein said cells include at least one one of epithelial cells, sperm cells, cancerous cells, diseased cells, or cells for non-testing use.

46. The method of claim 1, wherein a plurality of said probes are used so as to define specific groups of individuals.

47. The method of claim 21, wherein said cells and/or cellular components are sensed using said optical sensor array to identify a presence, absence, quantity, or relative quantity of said probes.

48. The method of claim 3, wherein said fluorophores are quantum dots.

49. The method of claim 34, wherein multiple optical traps are produced using methods other than holographic optical trapping, to move said labeled cells and/or cellular components.

50. The method of claim 16, further comprising:
providing a recirculating flow from said sample input to remix said cells and/or cellular components in said sample input and fluidic channels from said sample input, more uniformly.

51. The method of claim 17, further comprising:
pumping unsorted labeled cells and/or cellular components from said chamber into a reservoir.

52. The method of claim 16, wherein sample fluid is inserted directly into said sample input from an open-top.

53. The method of claim 9, further comprising:

processing the cells and/or cellular components through shear fluid flow to pull material away from said nuclei of said cells and/or cellular components, through viscous shear forces.

54. The method of claim 53, wherein said processing step is accomplished through one of capillary flow or a shear cell.

55. The method of claim 9, wherein laser scissors are used to liberate said nuclei from said cells and/or cellular components, after said cells and/or cellular components are disposed in said fluidic cartridge.

56. The method of claim 21, further comprising:
using fluorophores which have similar emission spectra and which differ in their fluorescence decay lifetime; and
employing a detection system which distinguishes or measures said fluorescence decay lifetime, to increase a number of said probes which may be distinguished.

57. The method of claim 1, wherein said probes are peptides utilizing a helix-turn-helix motif for binding DNA sequences.

58. The method of claim 1, wherein probes bind to the target DNA through a means other than hybridization to single-stranded target DNA.

59. The method of claim 1, wherein non-imaging detection is used to separate said labeled cells and/or cell components into said dropoff regions.

60. The method of claim 18, wherein said DNA profiling is performed on said fluidic cartridge.

61. A system which sorts cells and/or cellular components into multiple populations, comprising:

a fluidic cartridge including:

a sample input through which a fluid suspension containing cells and/or cellular components is inputted;

at least one buffer input through which buffer fluid is inputted into a respective one of a plurality of buffer channels;

an inspection and separation region containing a chamber, into which said fluid suspension of genetically labeled cells and/or cellular components is flowed from said sample input; and

a suspension mechanism which prevents sedimentation of the cells and/or cellular components, said suspension mechanism including a circulation channel or other flowing or mixing feature which acts to mix or drive components with shear.

62. The system of claim 61, further comprising:

a plurality of dropoff areas connected to said inspection and separation region, into which said labeled cells and/or cellular components are moved.

63. The system of claim 61, further comprising:
a reservoir for unsorted labeled cells and/or cellular components, connected to said inspection and separation region.
64. The system of claim 61, further comprising:
a plurality of valves and actuators which pump said sample fluid throughout channels of the fluidic cartridge.
65. The system of claim 62, wherein said chamber includes an optically clear window at a bottom thereof.
66. The system of claim 65, further comprising:
an optical microscopy system which images said cells and/or cellular components through said window.
67. The system of claim 66, further comprising:
an optical trapping system which traps and moves said labeled cells and/or cellular components from said inspection region to said dropoff areas.
68. The system of claim 67, wherein said system is a holographic optical trapping system.

69. The system of claim 67, further comprising a stage, which is used in conjunction with said optical trapping system, to move said cells and/or cellular components through said channels of said fluidic cartridge.

70. The system of claim 67, wherein said fluidic cartridge includes a plurality of layers, a single layer which is employed to both form said window for imaging and optical trapping, and to form lower and side walls of said fluidic channels.

71. The system of claim 61, wherein said fluidic cartridge is disposable.

72. The system of claim 69, further comprising:
a substage into which said fluidic cartridge is mounted, and which is disposed in said stage, said substage which places said fluidic cartridge in an optical train of said optical trapping system, and connects pneumatic channels of said fluidic cartridge to a pneumatic system.

73. The system of claim 72, further comprising:
a computer system which controls said pneumatic system.

74. The system of claim 70, wherein said single layer is polymeric and formed through molding or embossing to achieve a high quality optical surface.

75. The system of claim 70, wherein the single layer is bonded to a membrane layer with a chemical bond.

76. The system of claim 75, wherein said membrane layer is coated with parylene or another polymer with low air permeability.

77. The system of claim 75, wherein said single layer is treated by plasma etching and APTES treatment.

78. The system of claim 62, wherein non-imaging detection is used to separate said labeled cells and/or cell components into said dropoff regions.

79. The system of claim 61, wherein DNA profiling is performed on said fluidic cartridge.

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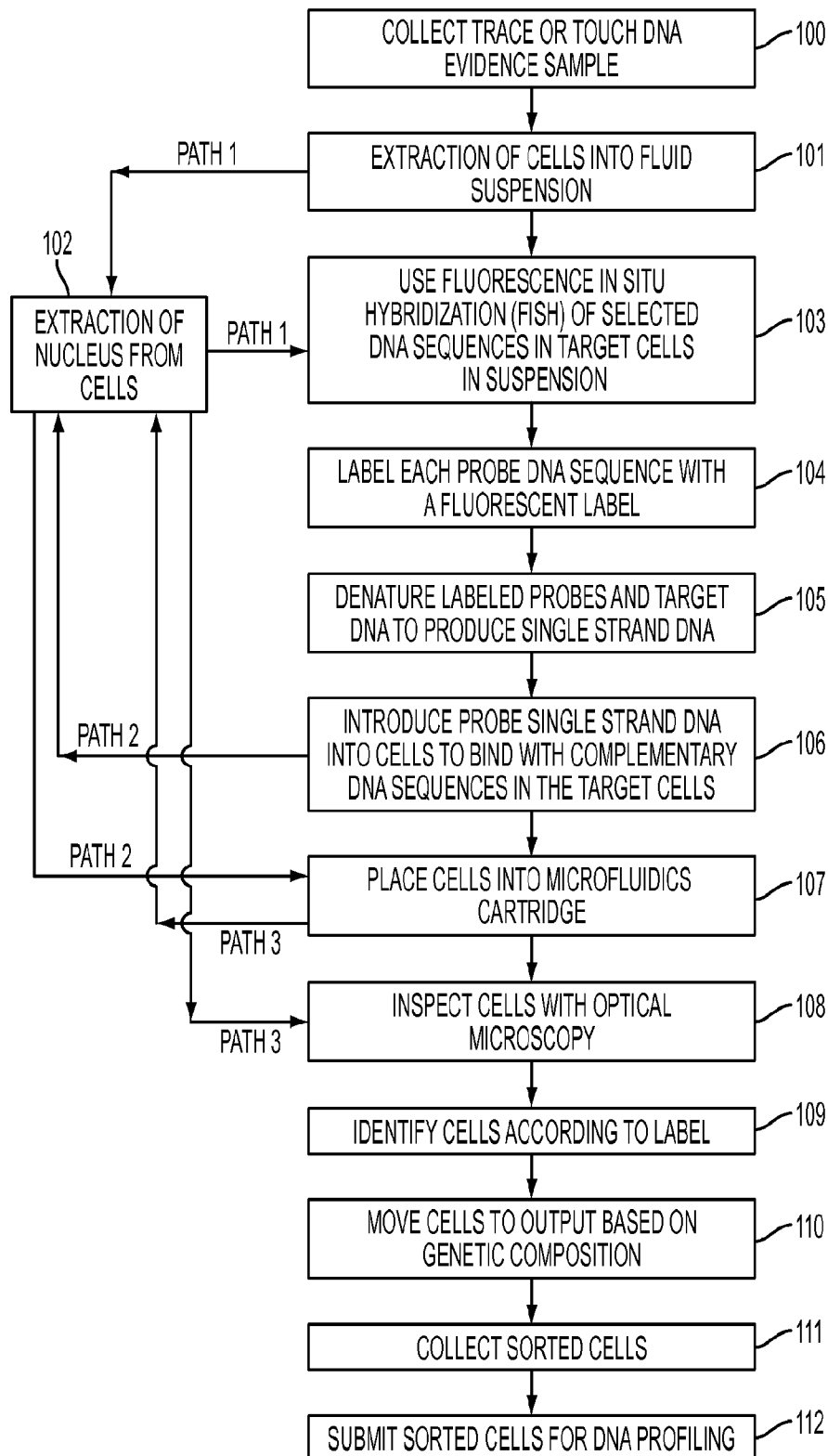


FIG. 1

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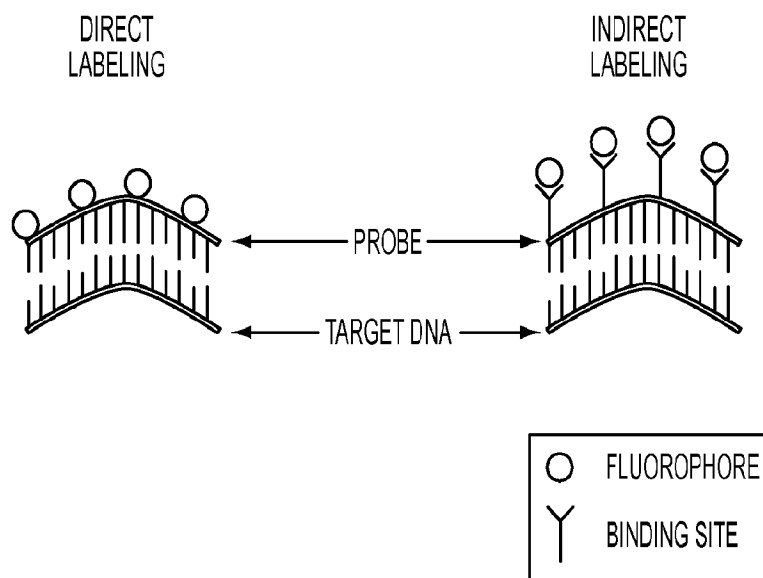


FIG. 2

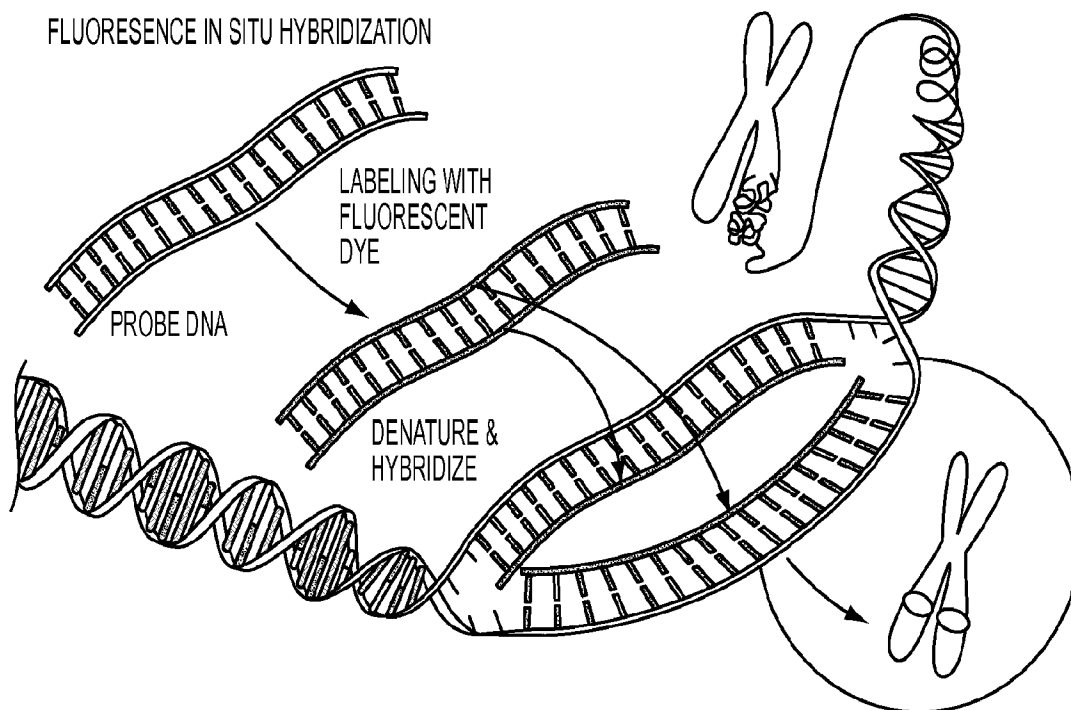


FIG. 3

FLUOROPHORE		RELATIVE FLUOROPHORE CONTRIBUTIONS FOR EACH FILTER SET									
FILTER SET	DAPI	SpAqua	SpGreen	SpGold	SpOrange	SpRed	Cy5 / FRed	Cy5.5	Cy7		
DAPI	100%	30%	0%								
SpAqua	0%	100%	1%	0%							
SpGreen	0%	0%	100%	3%	0%						
SpGold		0%	2%	100%	49%	1%					
SpOrange			0%	36%	100%	11%	0%				
SpRed				0%	15%	100%	1%	0%			
Cy5 / FRed					0%	100%	100%	53%	1%		
Cy5.5						0%	53%	100%	6%		
Cy7						0%	0%	12%	100%		
GREY COMBINATIONS ARE NOT RECOMMENDED											

FIG. 4

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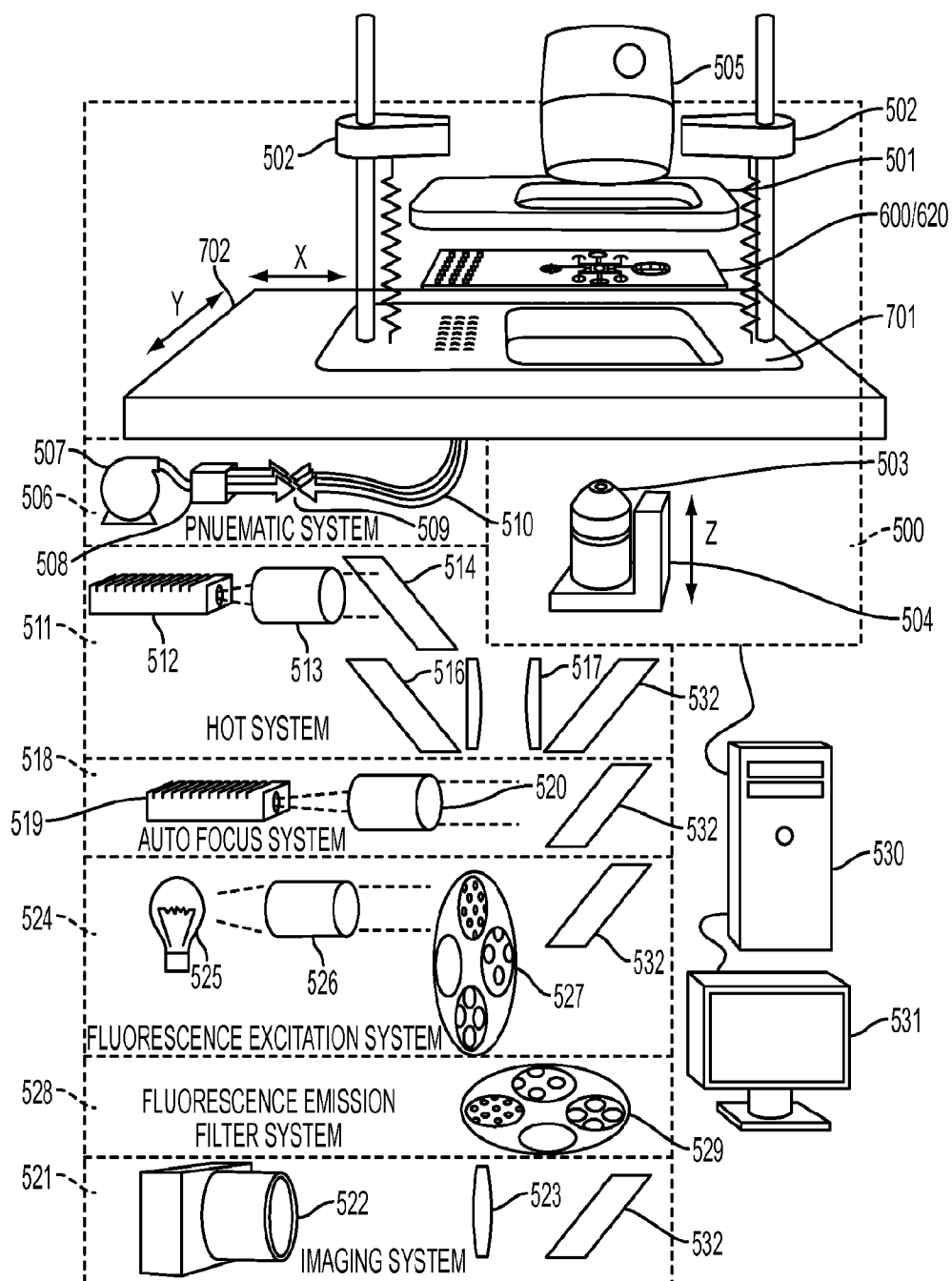


FIG. 5A

5/13

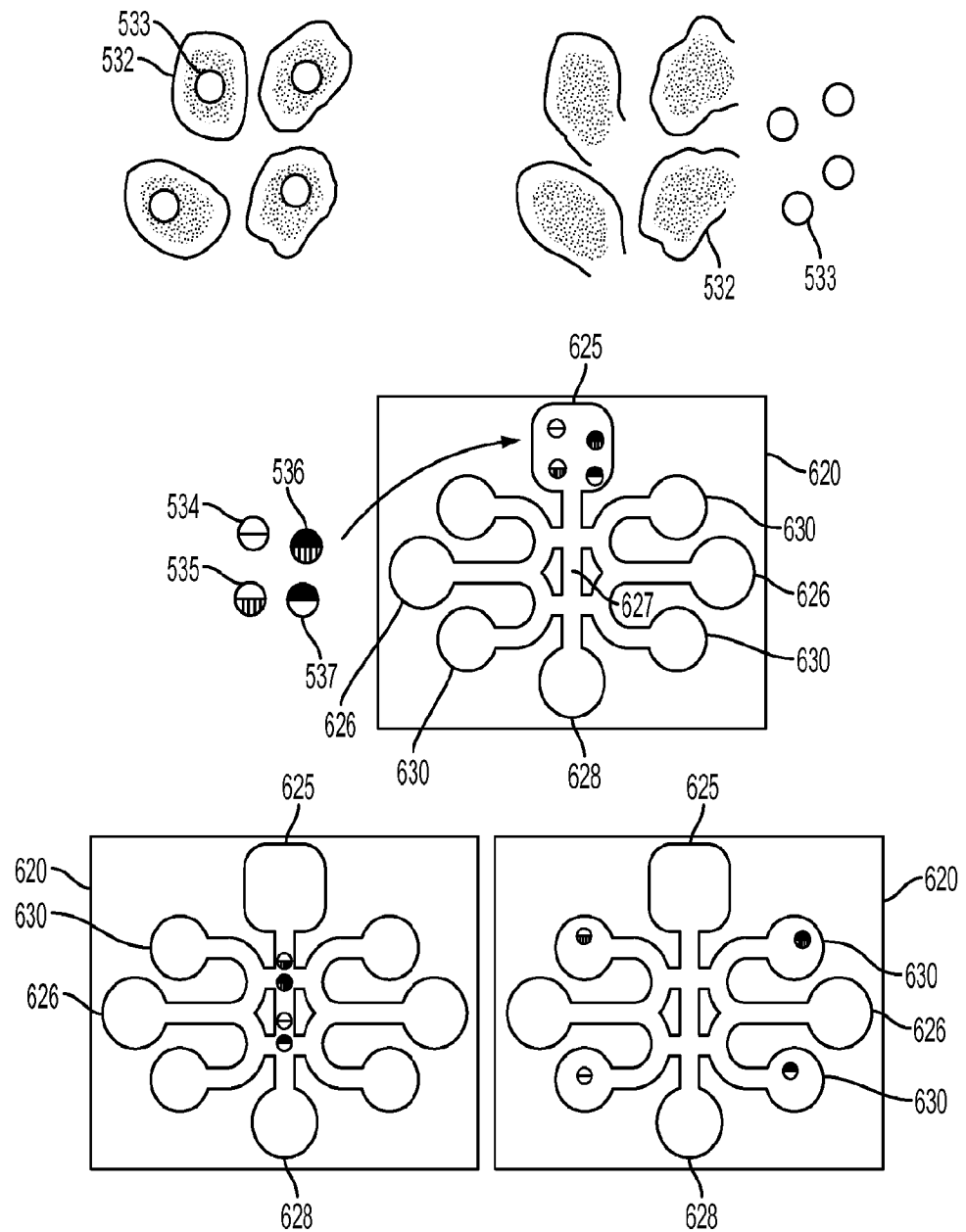


FIG. 5B

6/13

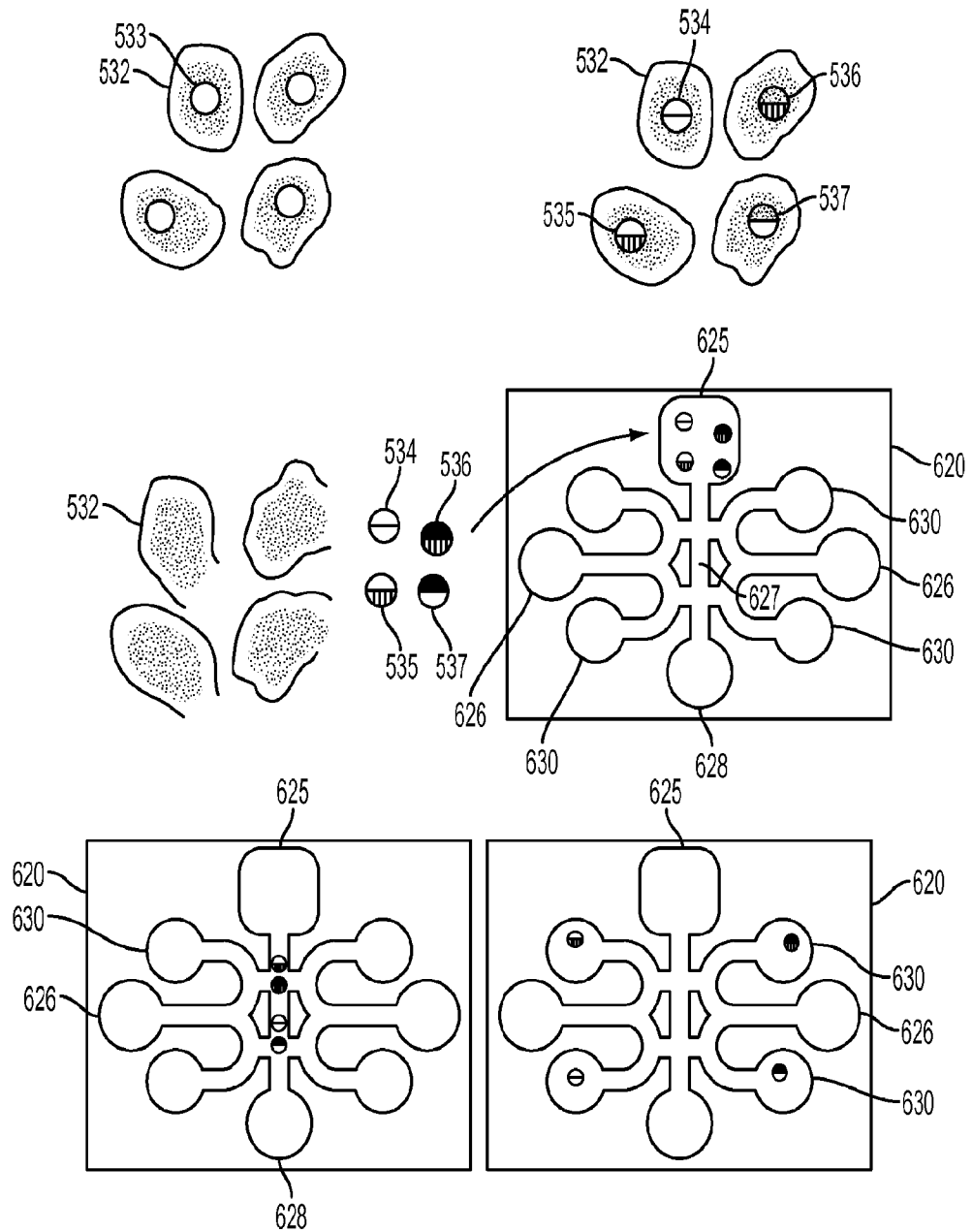


FIG. 5C

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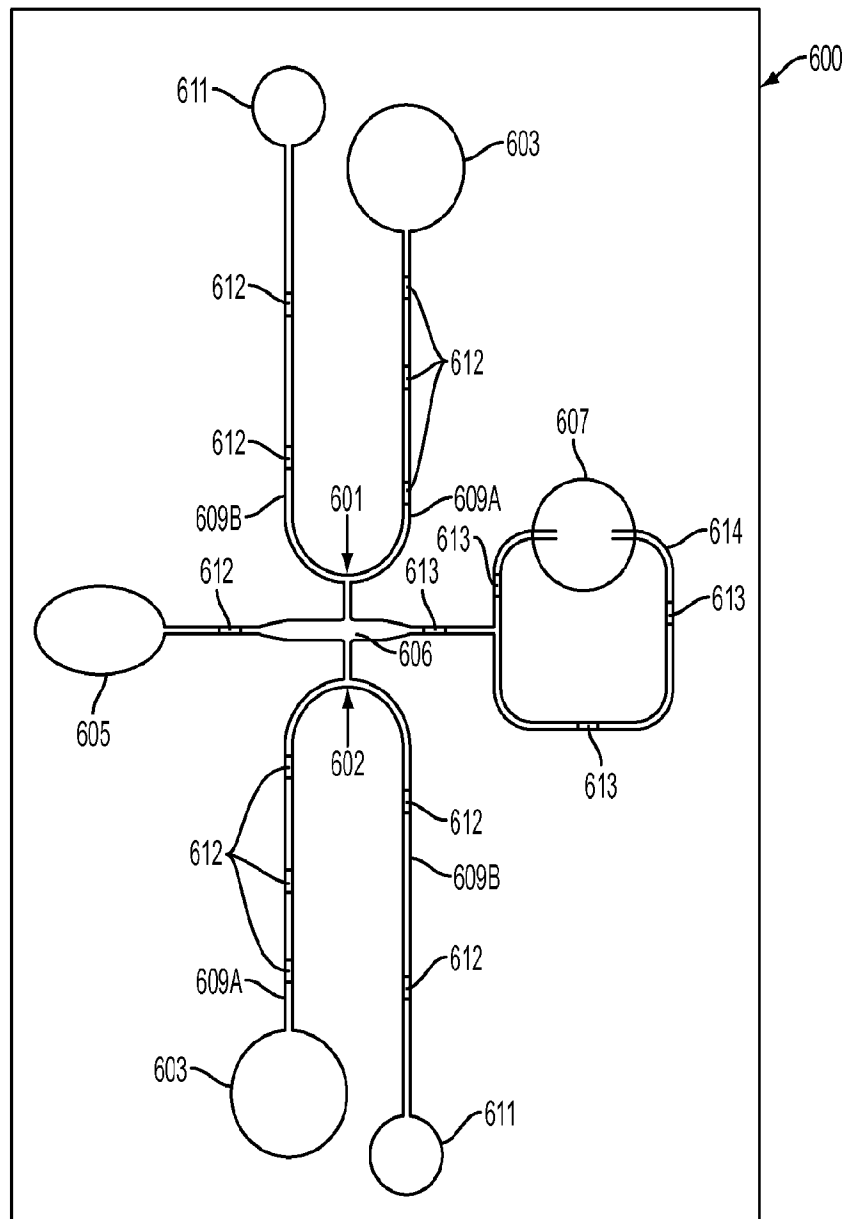


FIG. 6A

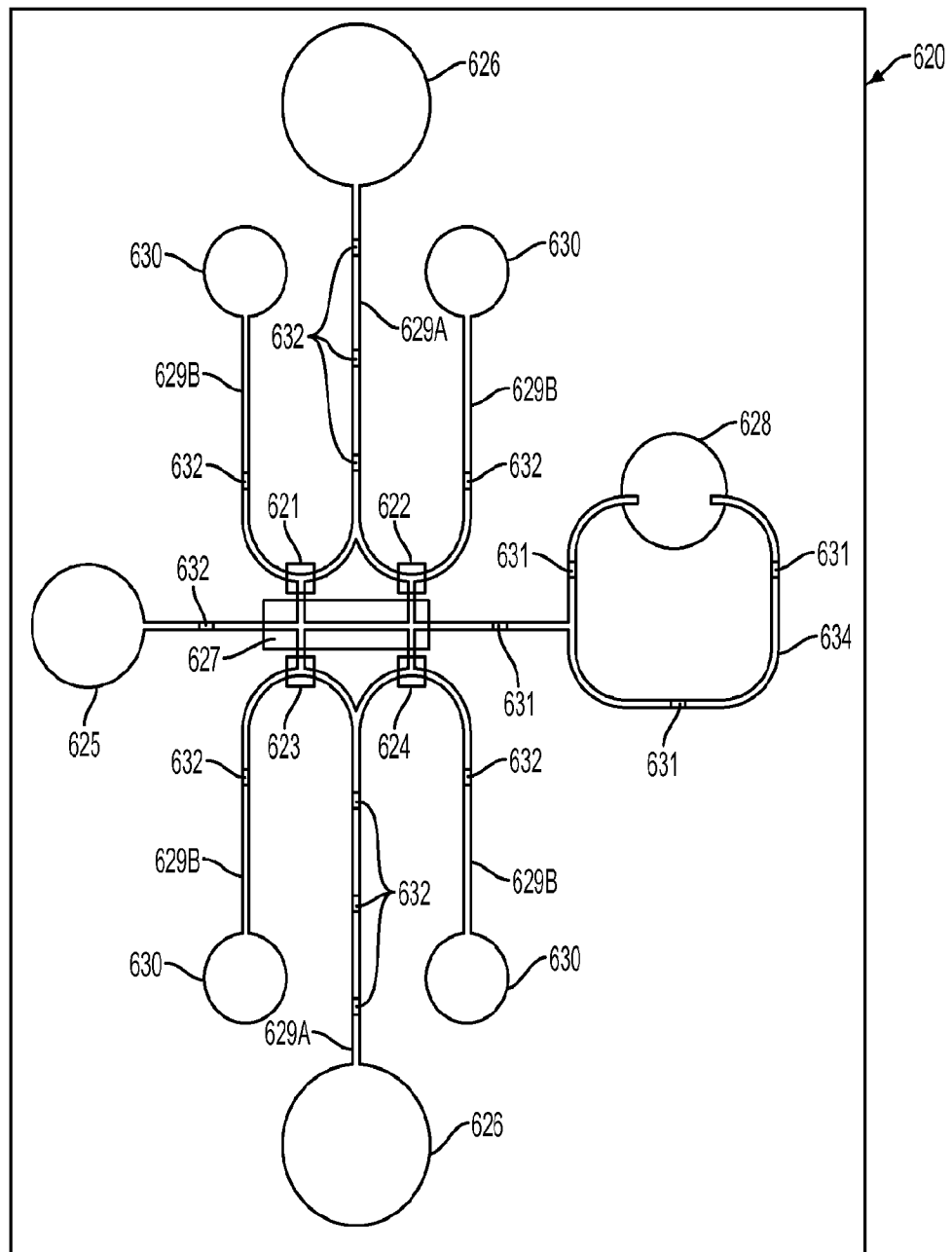


FIG. 6B

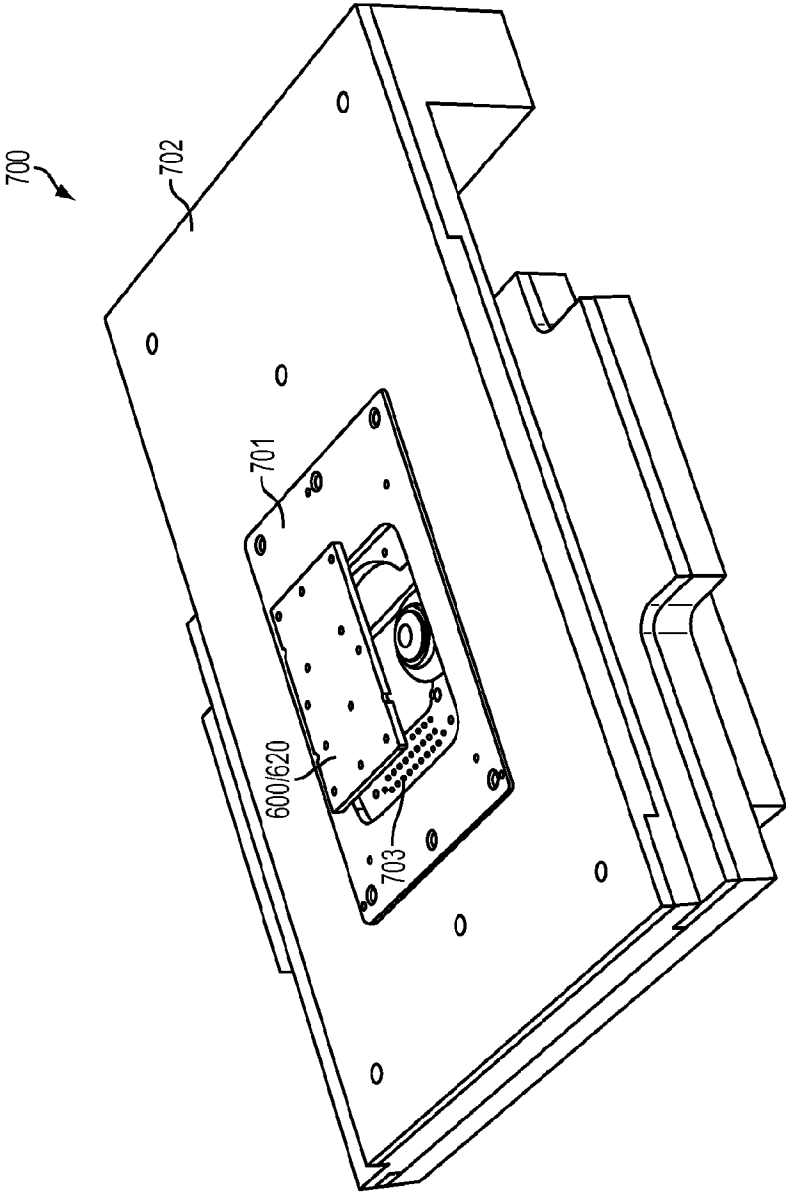


FIG. 7A

10/13

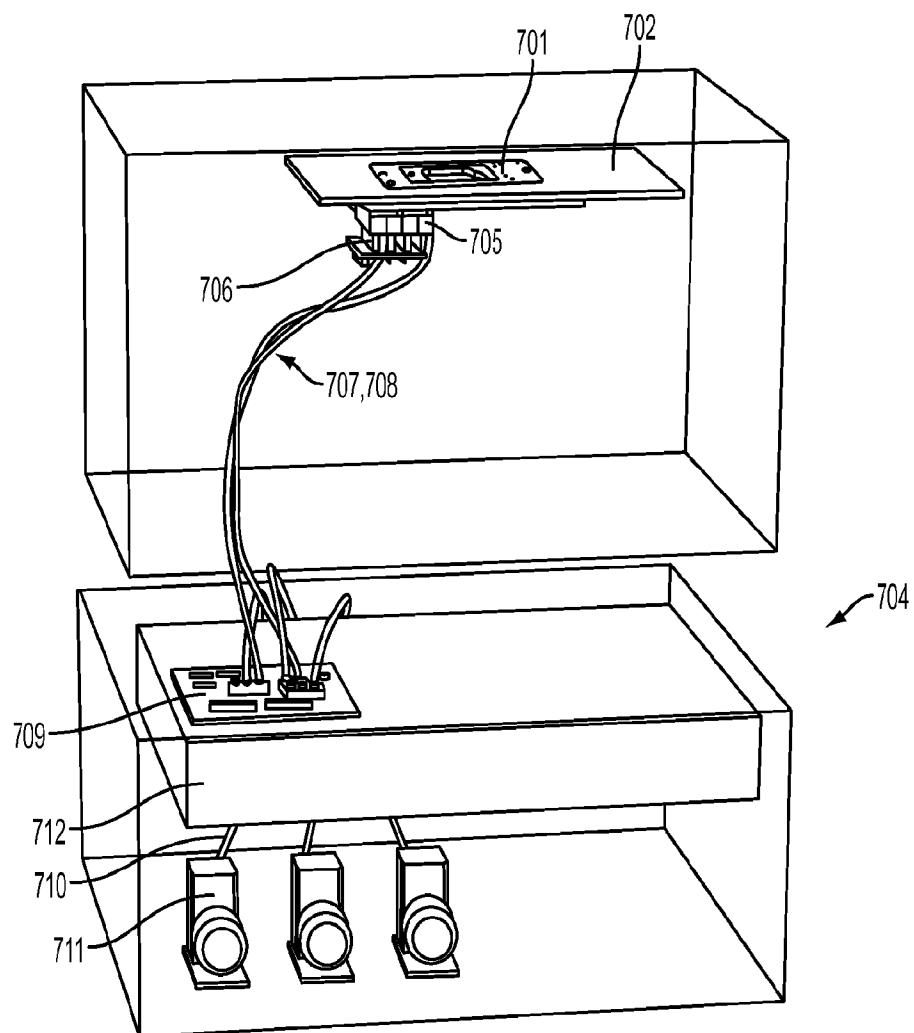


FIG. 7B

11/13

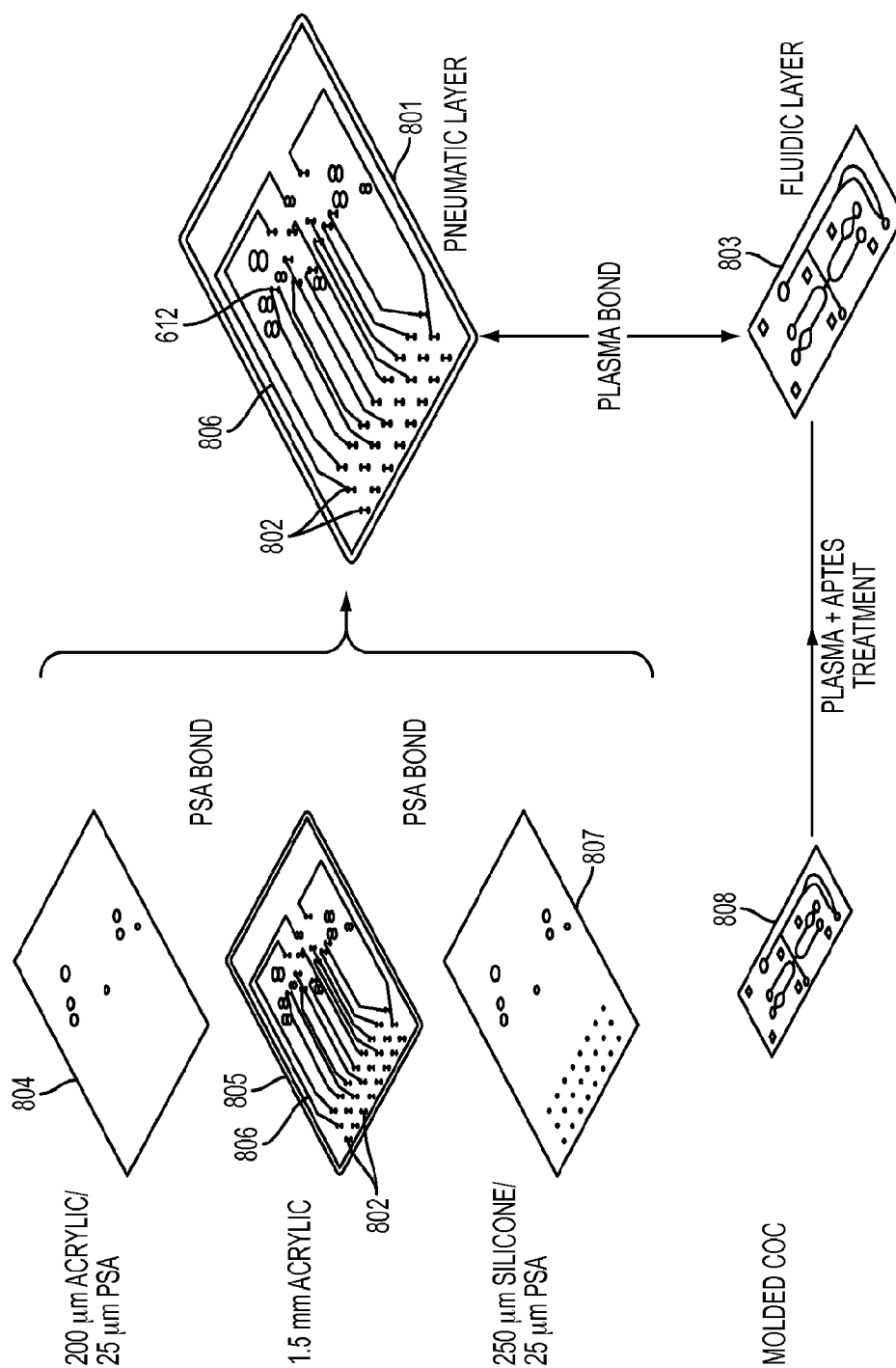


FIG. 8

12/13

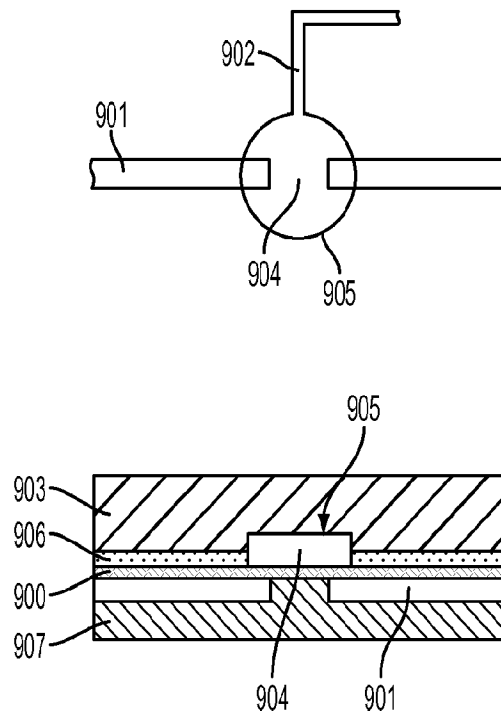


FIG. 9A

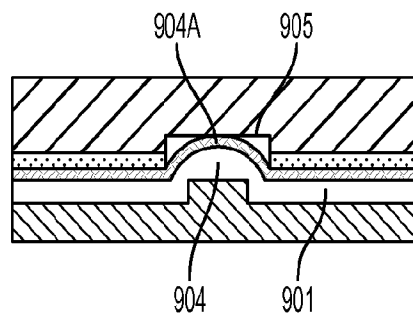


FIG. 9B

13/13

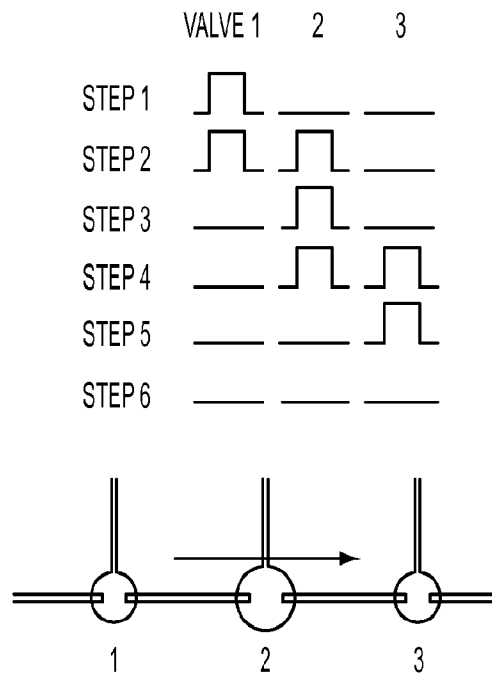


FIG. 10A

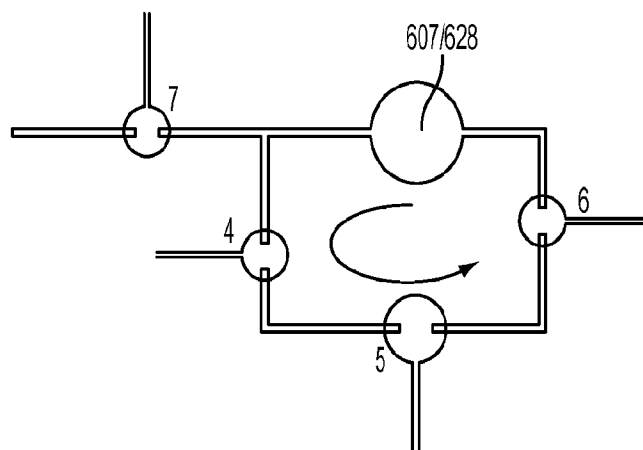


FIG. 10B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/44011

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/48 (2012.01)

USPC - 435/6.1, 6.11, 287.1

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(8) - G01N 33/48 (2012.01)

USPC - 435/6.1, 6.11, 287.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 436/94, 164

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

PubWest (PGPB,USPT,USOC,EPAB,JPAB) ; PubMed (MEDLINE)

sorting, cells, cartridge, label, FISH, holographic optical trapping, stage, pneumatic, shear cell, viscosity, laser scissors, fluorescence, decay, helix-turn-helix

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/0175980 A1 (HAYENGA et al.) 18 September 2003 (18.09.2003) para [0004], [0007], [0010]-[0011], [0021]-[0022], [0025]-[0026], [0028]-[0029], [0035], [0037]-[0039], [0047]	1, 61-67, 70-71
Y		2-60, 68-69, 72-79
Y	US 2004/0091880 A1 (WIEBUSCH et al.) 13 May 2004 (13.05.2004) para [0003], [0017], [0024]-[0028], [0037]-[0038], [0042]-[0043], [0056], [0070], [0073], [0077], [0080], [0122], [0139], [0178], [0182]-[0183], [0187], [0193], [0204]-[0205]	2-13, 15-56, 59, 60, 78-79
Y	US 2011/0026009 A1 (KNUTSON et al.) 03 February 2011 (03.02.2011) para [0013], [0083], [0106], [0112], [0116], [0120]-[0121], [0127], [0130]-[0131], [0133], [0135]	22-29, 31, 35-37, 39, 41, 68-69, 72-77
Y	US 2009/0269244 A1 (CUNNINGHAM et al.) 29 October 2009 (29.10.2009) para [0009], [0070], [0088]	26-27, 32-33
Y	US 2007/0095669 A1 (LAU et al.) 03 May 2007 (03.05.2007) para [0009], [0017], [0025]-[0026], [0029]	43, 49
Y	US 2003/0113709 A1 (ALIVISATOS et al.) 19 June 2003 (19.06.2003) para [0013], [0019], [0174]-[0175]	48
Y	US 2003/0108911 A1 (KLIMANT et al.) 12 June 2003 (12.06.2003) para [0019], [0030]-[0033]	56
Y	US 2006/0246490 A1 (ANDERSON et al.) 02 November 2006 (02.11.2006) para [0009], [0131], [0147], [0151]	76

☒ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

4 September 2012 (04.09.2012)

Date of mailing of the international search report

27 SEP 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

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PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2006/0286549 A1 (SOHN et al.) 21 December 2006 (21.12.2006) para [0017], [0064], [0069]	77
Y	US 6,594,076 B2 (SATO) 15 July 2003 (15.07.2003) col 3, ln 39-46; col 13, ln 59 - col 14, ln 9	25
Y	GEBSKA et al. "High-affinity binding sites for heparin generated on leukocytes during apoptosis arise from nuclear structures segregated during cell death" Blood; 15 March 2002; Vol. 99, No. 6; pg. 2221-2227. Entire document, especially pg. 2222, section entitled "Flow cytometry" and "Cell Sorting". pg. 2222 col 2, para 4-pg. 2223 col 1, para 1.	53, 54
Y	US 2004/0185453 A1 (MYERSON et al.) 23 September 2004 (23.09.2004) abstract; para [0043]; [0044]	14, 57, 58