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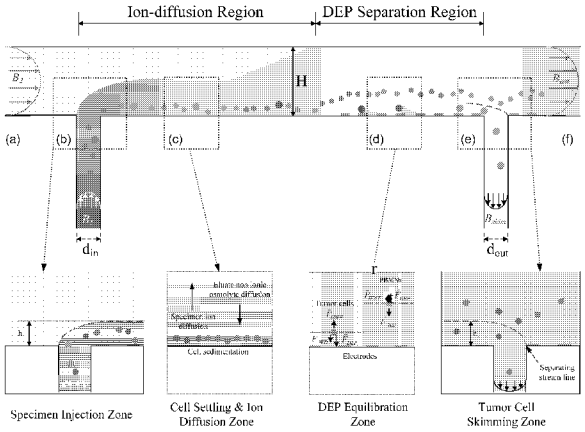

FIG. 4

(57) Abstract: The present disclosure includes apparatuses and methods for (e.g., improved and/or optimized) continuous flow dielectrophoretic separation of target matter (e.g., cells) from fluid (e.g., a blood sample). In some embodiments, the apparatus can include a body defining a chamber (e.g., a chamber having: one or more inlets and one or more outlets; a first portion; and a second portion between one end of the first portion and the outlets) and one or more electrodes configured to generate a non-uniform elec - trie field in the second portion of the chamber. The apparatus can be configured such that if a first fluid including target matter and a second fluid are introduced into the chamber through the inlets, the first fluid and the second fluid can flow substantially laminarly through the first portion to permit diffusion of solutes between the first fluid and the second fluid, and the one or more electrodes can generate a dielectrophoretic force on the target matter in the second portion to extract the target matter from the first fluid.

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DESCRIPTION

APPARATUSES AND METHODS FOR CONTINUOUS FLOW DIELECTROPHORETIC SEPARATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 61/577,088, filed December 18, 201 1, which is incorporated by reference herein in its entirety.

BACKGROUND

1. Field of the Invention

[0002] The present disclosure relates generally to discrimination and/or separation of target matter (e.g., from a biological sample). More particularly, but not by way of limitation, the present disclosure relates to continuous-flow dielectrophoretic separation of target matter.

2. Brief Description of Related Art

[0003] The ability to identify, characterize and purify cell subpopulations may be useful in various biological and medical applications, and may act as the starting point for research protocols and/or current and emerging clinical protocols. Cell separation may have numerous applications in areas such as medicine, biotechnology, biomedical research, environmental monitoring and bio/chemical warfare defense. For example, cell separation can make possible certain life-saving procedures such as autologous bone marrow transplantation for the remediation of advanced cancers where the removal of cancer-causing metastatic cells from a patient's marrow is necessitated (Fischer, 1993). In other applications, such as the study of signaling between blood cells (Stout, 1993; Cantrell et al., 1992), highly purified cell subpopulations permit studies that would otherwise be impossible. Current approaches to cell sorting often exploit differences in cell density (Boyum, 1974), specific immunologic targets (Smeland et al., 1992), or receptor-ligand interactions (Chess et al., 1976) to isolate particular cells. These techniques may often be inadequate.

[0004] The application of the principles of AC electrokinetics has been used for the dielectric characterization of mammalian cells through the method of electrorotation (ROT) (Arnold and Zimmermann, 1982; Fuhr, 1985; Holzel and Lamprecht, 1992; Wang et al., 1994) and for cell discrimination and sorting (Hagedorn et al., 1992; Huang et al., 1993; Gascoyne et al., 1992; Gascoyne et al., 1994; Huang et al., 1992). In these techniques, cells generally become

electrically polarized when they are subjected to an AC electric field. In ROT, a rotational electrical field is applied and the interaction between the cells' polarization and the applied field can result in cell rotation. If that field is inhomogeneous, then the cells can experience a lateral dielectrophoretic (DEP) force, the frequency response of which is a function of their intrinsic electrical properties (Gascoyne et al., 1992). In turn, these properties may depend strongly on cell composition and organization, features that generally reflect cell morphology and phenotype. Cells differing in their electrical polarizabilities can thus experience differential forces in the inhomogeneous electric field (Becker et al., 1994; Becker et al., 1995). Analysis of the dielectrophoretic motion of mammalian cells as a function of applied frequency permits cell membrane biophysical parameters, such as capacitance and surface conductance, to be probed. Because DEP can effectively map biophysical properties into a translational force whose direction and magnitude reflects cellular properties, DEP force may induce separation between particles of different characteristics. For example, DEP has been used on a microscopic scale to separate bacteria from erythrocytes (Markx et al., 1994), viable from nonviable yeast cells (Wang et al., 1993), and erythroleukemia cells from erythrocytes (Huang et al., 1992). However, the differences in the electrical polarizabilities of the cell types in those various mixtures were greater than those to be expected in many typical cell sorting applications.

[0005] Field flow fractionation (FFF) has also been employed for separation of matter, utilizing particle density, size, volume, diffusivity, and surface charge as parameters (Giddings, 1993). The technique can be used to separate different types of matter, for example, having a size from about 1 nm to more than about 100 micrometers, which may include, for example, biological and non-biological matter. Separation according to field flow fractionation generally occurs by differential retention in a stream of liquid flowing through a channel. FFF techniques may combine elements of chromatography, electrophoresis, and ultracentrifugation, and may utilize a flow velocity profile established when the fluid is caused to flow through the channel. Such a velocity profile may be, for example, linear or parabolic. In some FFF techniques, a field may be applied at right angles to the flow in the channel, and the field may serve to drive the matter into different positions within the flow velocity profile. The matter being displaced at different positions within the velocity profile can then be carried with the fluid flow through the chamber at differing velocities. Fields may be based on sedimentation, crossflow, temperature gradient, centrifugal forces, and the like. Some such techniques may suffer, however, from

producing insufficiently pure cell populations, being too slow, or being too limited in the spectrum of target cells or other matter.

[0006] One example of dielectrophoretic field flow fractionation (DEP-FFF) is described in U.S. Patent No. 6,641,708. DEP-FFF may be used, for example, to perform fractionation of circulating tumor cells (CTCs) from peripheral blood mononuclear cells (PBMNs) in batches of approximately one million cells on a 300 mm x 25 mm electrode array. In DEP-FFF methods, target cells do not need to be trapped by the electric field. However, when operated in batch mode, in which the target cells are eluted from the chamber in a later fraction than the PBMNs, the maximum practical specimen size for high isolation efficiency isolation of target cells is generally less than 5 million cells in each run or batch. Such batches are so small that the mixture of PBMNs and CTCs in a CTC isolation application using a 10 mL blood specimen would need to be split into as many as 20 aliquots in order to isolate all the CTCs. This can makes such a method impractical for at least some clinical applications. Principles of DEP field flow fractionation are described in U.S. Patent Nos. 5,888,370; 5,993,630; 5,993,632; 6,641,708; 6,790,330; and 7,033,473.

SUMMARY

[0007] The present disclosure includes apparatuses and methods for continuous flow dielectrophoretic separations. Embodiments can, for example, be optimized based on DEP and FFF.

[0008] Some embodiments of the present apparatuses comprise: a body defining a chamber having one or more inlets and one or more outlets, where the one or more inlets are configured to receive a first fluid including target matter and a second fluid. The chamber can include: a first portion having a first end, and a second end between the one or more inlets and the one or more outlets; and a second portion between the second end of the first portion and at least one of the one or more outlets. The apparatus can also include one or more electrodes configured to generate a non-uniform electric field in the second portion of the chamber. In some embodiments, the apparatus is configured such that if a first fluid including target matter and a second fluid are introduced into the chamber through the one or more inlets: the first fluid and the second fluid can flow substantially laminarly through the first portion to permit diffusion of solutes between the first fluid and the second fluid; and the one or more electrodes can generate a dielectrophoretic force on the target matter in the second portion to extract the target matter from the first fluid.

[0009] In some embodiments, the apparatus is further configured such that during the substantially laminar flow through the first portion, the cross-sectional area of the first fluid is smaller than the cross-sectional area of the second fluid. In some embodiments, the length of the first portion is large enough to permit the first fluid and the second fluid to substantially reach diffusion equilibrium in the first portion, where the diffusion in the first portion reduces the conductivity of the first fluid. In some embodiments, the chamber includes a bottom surface and side surfaces, and the one or more electrodes are disposed on or beneath the bottom surface. In some embodiments, the width of the chamber is larger than the height of the chamber. In some embodiments, the chamber is configured to direct the first and second fluids from the one or more inlets to the one or more outlets according to a predetermined velocity profile. In some embodiments, the second fluid may include one or more osmolytes configured to facilitate diffusion between the first fluid and the second fluid to reduce the conductivity of the first fluid.

[0010] In some embodiments, the first portion of the chamber does not include electrodes that are configured to generate a non-uniform electric field. In some embodiments, the one or more inlets comprise a first inlet configured to receive the first fluid and a second inlet configured to receive the second fluid; and, optionally, where the first inlet is angled relative to (e.g., perpendicular to) the direction of flow in the first portion of the chamber. In some embodiments, the one or more outlets comprise a first outlet configured to permit the target matter to exit the chamber and a second outlet configured to permit the remainder of the first and second fluids to exit the chamber; and, optionally, where the first outlet is angled relative to (e.g., perpendicular to) the direction of flow in the second portion of the chamber. In some embodiments, the apparatus further comprises a signal generator coupled to the one or more electrodes, the signal generator configured to generate an electric signal with a frequency and voltage for the non-uniform electric field.

[0011] In some embodiments, the apparatus includes a conductivity sensor configured to measure a conductivity of a fluid in the chamber and a controller configured to calculate one or more target properties of the non-uniform electric field based on at least one of measured conductivity of the first fluid and a property of the target matter. In some embodiments, the controller is further configured to adjust the electric signal generated by the signal generator to cause the non-uniform electric field to substantially include the one or more target properties. In some embodiments, the apparatus includes a current sensor configured to measure a current of the electric signal and a controller coupled to the current sensor and configured to compare the

measured current to a target current, and the controller is further configured to adjust the electric signal based on the comparison. In some embodiments, adjusting the electric signal includes changing at least one of the frequency and the voltage of the electric signal.

[0012] Some embodiments of the present methods includes steps necessary to carry out the functions described above with respect to the operation of the described apparatus.

[0013] Some embodiments of the present methods comprise: introducing a first fluid including target matter and a second fluid into a chamber of an apparatus, the chamber having one or more inlets and one or more outlets. In some embodiments, the chamber includes: a first portion having a first end, and a second end between the one or more inlets and the one or more outlets; and a second portion between the second end of the first portion and at least one of the one or more outlets. In some embodiments, the apparatus also includes one or more electrodes configured to generate a non-uniform electric field in the second portion of the chamber. In some embodiments, the method includes causing the first and second fluids to flow substantially laminarly through the first portion of the chamber such that diffusion between the first fluid and the second fluid lowers the conductivity of the first fluid, and applying an electric signal to the one or more electrodes to generate a dielectrophoretic force on the target matter to extract the target matter from the first fluid. In some embodiments, the method also includes outputting the target matter through a first outlet of the chamber and outputting the remainder of the first and second fluids through a second outlet of the chamber; and, optionally, where: the first fluid is introduced through a first inlet that is angled relative to the first portion of the chamber; and/or the first outlet is angled relative to the direction of flow in the second portion of the chamber.

[0014] In some embodiments, the method includes controlling the flow rate of the first fluid and the second fluid such that the first fluid and the second fluid flow through the first and second portions of the chamber according to a target velocity profile, where the target velocity profile is such that the first fluid and the second fluid substantially reach diffusion equilibrium in the first portion of the chamber.

[0015] In some embodiments, the method includes measuring conductivity of the first fluid; calculating one or more target properties of the non-uniform electric field based on at least one of: measured conductivity of the first fluid and a property of the target matter; and adjusting the electric signal to include the one or more target properties. In some embodiments, the method

includes measuring a current of the electric signal; comparing the measured current to a desired current; and adjusting the electric signal based on the comparison. In some embodiments, adjusting the electric signal includes changing at least one of the frequency and the voltage of the electric signal.

[0016] In some embodiments, the method includes adding one or more osmolarity-altering components, density-altering components, and/or viscosity-altering components to the first and/or second fluids to achieve desired osmolarity, density and/or viscosity relationships between the first and second fluids.

[0017] In some embodiments, there are methods and/or compositions related to antibody-independent isolation of circulating tumor cells by continuous-flow dielectrophoresis.

[0018] In some embodiments, dielectrophoresis is employed to discriminate between blood and tumor cells, for example, on the basis of cell size and/or membrane morphology that contribute to differences in cell membrane area and consequently to cell dielectric properties. In at least some embodiments, the separation criterion is independent of cell surface protein markers. In some embodiments, DEP allows isolation of CTCs that are applicable to a broad range of cancers.

[0019] In some embodiments, dielectric properties of a cancer cell type, including whether or not they are solid tumor cell types and regardless of their tissue of origin, are significantly different from those of PBMNs and allow their isolation from blood. Exemplary cancer types include lung, breast, prostate, pancreas, brain, skin, kidney, colon, liver, endometrium, thyroid, heart, nerve, stomach, spleen, testicular, ovarian, gall bladder, head and neck, blood, bone, liver, and so forth.

[0020] Some embodiments of the present methods are configured for and/or are capable of efficiently isolating cultured cells from PBMNs of healthy donors or efficiently isolated CTCs from clinical specimens derived from cancer patients.

[0021] Some embodiments of the present methods allow continuous flow microfluidic processing at rates of at least 10^6 nucleated cells/minute; in some cases, the rate is up to 10^7 cell/min. In some embodiments, a continuous flow microfluidic processing chamber is utilized into which a peripheral blood mononuclear cell fraction of a clinical specimen is slowly injected,

deionized by diffusion, and then subjected to a balance of DEP, sedimentation and hydrodynamic lift forces, for example. In some embodiments, these forces cause tumor cells to be transported close to the floor of a chamber of an apparatus while blood cells are carried about three cell diameters above them. The tumor cells may be isolated by skimming them from the bottom of the chamber while the blood cells flow to waste, in certain embodiments.

[0022] In some embodiments, there is isolation of circulating tumor cells from a clinical specimen and verification of the tumor origin of these cells by molecular analysis. Upon verification of the tumor origin of the cells, a medical provider may then draw prognostic inferences about the disease state of the individual from whom the specimen was derived and/or provide suitable therapy to the individual from whom the specimen was derived, including chemotherapy, surgery, immunotherapy, radiation, and so forth, that is tailored for the specific cancer.

[0023] In some embodiments, there is isolation of circulating tumor cells from a clinical specimen withdrawn for cancer screening purposes. Upon verification of the tumor origin of the cells, a medical provider may then infer that the individual from whom the specimen was derived has cancer.

[0024] In some embodiments, there is isolation of circulating tumor cells from a clinical specimen withdrawn for screening purposes from a patient who is in remission from cancer. Upon verification of the tumor origin of the cells, a medical provider may then infer that the individual from whom the specimen was derived has relapsed and once again has cancer.

[0025] Specimens utilized in embodiments of the present methods may originate from a variety of sources; such specimens may come from an individual, a hospital or medical facility, or a repository, for example. The specimens may or may not have been stored prior to processing of the specimens with one or more methods of the invention. The processing methods may be performed by the individual that collected the specimen(s) or they may be performed by a separate entity. The specimens may originate from a healthy individual or an individual that has cancer, is at risk for having cancer, or is in remission from cancer, or is suspected of having cancer, for example because of analysis from the same or other specimen(s) using an alternative method (biopsy, histology, immunoanalysis, and so forth).

[0026] Any embodiment of any of the present methods or apparatuses can consist of or consist essentially of - rather than comprise/include/contain/have - any of the described steps, elements, and/or features. Thus, in any of the claims, the term "consisting of or "consisting essentially of can be substituted for any of the open-ended linking verbs recited above, in order to change the scope of a given claim from what it would otherwise be using the open-ended linking verb.

[0027] The feature or features of one embodiment may be applied to other embodiments, even though not described or illustrated, unless expressly prohibited by this disclosure or the nature of the embodiments.

[0028] Details associated with the embodiments described above and others are presented below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] The following drawings illustrate by way of example and not limitation. For the sake of brevity and clarity, every feature of a given structure is not always labeled in every figure in which that structure appears. Identical reference numbers do not necessarily indicate an identical structure. Rather, the same reference number may be used to indicate a similar feature or a feature with similar functionality, as may non-identical reference numbers.

[0030] **FIG. 1A** is a schematic diagram illustrating one embodiment of an apparatus for optimized continuous flow dielectrophoretic separations.

[0031] **FIG. IB** is a schematic diagram illustrating one embodiment of a body of an apparatus for optimized continuous flow dielectrophoretic separations.

[0032] **FIG. 2** is a schematic flow chart illustrating one embodiment of a method optimized continuous flow dielectrophoretic separations.

[0033] **FIGS. 3A** and **3B** are schematic flow charts illustrating additional steps for one embodiment of a method optimized continuous flow dielectrophoretic separations.

[0034] **FIG. 4** depicts a side view of DEP-FFF isolation of tumor cells from PBMNs in an embodiment of the present continuous-flow chambers. The relative vertical scale of the extremely thin chamber is exaggerated by ~ 120-fold for clarity.

[0035] **FIG. 5** depicts COMSOL Multiphysics simulations of the fluid flow behavior at the DEP-FFF chamber withdrawal slot for different geometries and flow rates: (a) when the withdrawal slot width d_{out}, is small compared with the chamber height H, optimal skimming behavior is observed with negligible vortices or regions of low flow rate; (b) as the relative withdrawal slot width is increased, a vortex forms within the slot, streamlines from the main channel are depressed into the slot region, and zones of low flow rate appear; (c) the skimming height h_s accurately follows that predicted by simple Poiseuille flow theory. Vorticity (d) and depression of the streamlines from the main channel (e) increase with increasing slot width.

[0036] **FIG. 6** depicts COMSOL-multiphysics simulations of the conductivity distribution in the flow stream (a) at the specimen injection zone (with a specimen-containing first fluid entering a first, smaller inlet and having a conductivity of 1400 $mS.m^{-1}$, and a second fluid entering a larger, second inlet having a conductivity of 30 $mS.m^{-1}$), (b) at the midpoint of the cell settling and ion diffusion zone (in which diffusion has occurred and conductivity varies from about 64 $mS.m^{-1}$ at the bottom of the chamber to about 30 $mS.m^{-1}$ at the top of the chamber), and (c) at the cell skimming zone (in which conductivity is a substantially uniform 61.5 $mS.m^{-1}$ throughout the height of the chamber).

[0037] **FIG. 7** depicts height distributions of MDA-MB-23 1 human breast cancer cells and PBMNs as they flowed through the chamber in force-balance equilibrium during batch mode DEP-FFF separation. The height distribution was mapped from the cell elution times assuming that the transit velocities of the cells reflected their heights in the Poiseuille hydrodynamic flow profile inside the DEP-FFF chamber.

[0038] **FIG. 8** depicts flow cytometric (FACS) scattergrams showing the recovery of tumor cells from PBMNs spiked with (a) 6000, (b) 2000 and (c) 500 MDA-MB-435 cultured cells prelabeled with CellTracker Green fluorescent dye.

[0039] **FIG. 9** depicts an image of circulating tumor cells collected by continuous flow DEP-FFF from the peripheral blood of a patient with colon cancer. The green (lighter) fluorescence reveals staining of cytokeratin in the tumor cells by F1TC-conjugated CK3-6H5 antibodies. PBMNs show only blue (darker) fluorescence due to DAPI staining of their nuclei.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0040] The term "coupled" is defined as connected, although not necessarily directly, and not necessarily mechanically; two items that are "coupled" may be unitary with each other. The terms "a" and "an" are defined as one or more unless this disclosure explicitly requires otherwise. The term "substantially" is defined as largely but not necessarily wholly what is specified (and includes what is specified; e.g., substantially 90 degrees includes 90 degrees and substantially parallel includes parallel), as understood by a person of ordinary skill in the art. In any disclosed embodiment, the terms "substantially," "approximately," and "about" may be substituted with "within [a percentage] of what is specified, where the percentage includes .1, 1, 5, and 10 percent.

[0041] The terms "comprise" (and any form of comprise, such as "comprises" and "comprising"), "have" (and any form of have, such as "has" and "having"), "include" (and any form of include, such as "includes" and "including") and "contain" (and any form of contain, such as "contains" and "containing") are open-ended linking verbs. As a result, an apparatus that "comprises," "has," "includes" or "contains" one or more elements possesses those one or more elements, but is not limited to possessing only those elements. Likewise, a method that "comprises," "has," "includes" or "contains" one or more steps possesses those one or more steps, but is not limited to possessing only those one or more steps.

[0042] Further, an apparatus, or a component of an apparatus, that is configured in a certain way is configured in at least that way, but it can also be configured in ways than those specifically described.

[0043] The present methods and apparatuses may be suitable for many applications in which it may be desirable to separate and/or isolate certain particles or packets or matter having certain properties (which may be referred to in this disclosure as "target matter" or "target particles"), from a mixture containing additional particles having one or more different properties (which may be referred in this disclosure as "background particles"). For example, in the life sciences and medicine, the present apparatuses and methods can be applied to the isolation of circulating tumor cells from the peripheral blood of cancer patients, and/or isolation of infected cells and/or infectious agents from the blood, urine, saliva or lavage specimens of individuals carrying disease or exposed to disease or to disease agents. The present apparatuses and methods can also be applied to the isolation of stem and/or progenitor cells or of other types of target cells from

mixtures containing other cell and/or particle types. In microparticle and nanoparticle production, the present apparatuses and methods can be applied to purifying particles having desired conductive and/or dielectric properties from particles lacking desired conductive and/or dielectric properties. In environmental sciences, the present apparatuses and methods can be applied to isolating pathogens, biowarfare or bioterrorism agents from liquid suspensions containing mixtures of particles that may contain dirt, pollen, and/or other non-target particles. In other environmental applications, the present apparatuses and methods can be applied to isolate one type of living organism from non living particles and/or from other types of living organisms (e.g., isolating target bacteria from dirt and/or from non-target bacteria). In mineral processing, the present apparatuses and methods can be applied to isolating metals and/or minerals having desired conductive and/or dielectric properties from particles lacking desired conductive and/or dielectric properties. In food processing, the present apparatuses and methods can be applied to the isolation of desired foodstuffs from non-desired contaminants, and/or to the isolation of pathogens and/or potential pathogens from foodstuffs in order to facilitate the detection and/or identification of said pathogens.

[0044] Embodiments of the present apparatuses and methods allow for adjustment of properties of a suspending medium (e.g., a first fluid) including target matter (e.g., cells) via diffusion between the first fluid and a second fluid in a chamber immediately prior to the first fluid entering a DEP portion or section of the chamber. In biological samples (e.g., whole blood), this temporal proximity of the adjustment of properties of the first fluid to the DEP sorting can result in reduced damage (e.g., cell rupture, reduced activity, and/or the like) to target matter (e.g., cells) than might otherwise be caused by traditional cell preparation.

1. Theoretical Overview

[0045] In embodiments of the present continuous flow DEP-FFF apparatuses and methods, particles are subjected to DEP, sedimentation and hydrodynamic lift forces as they move through a chamber towards one or more exits or outlets. At least one of these forces generally varies with distance from the chamber floor, and at least one of these forces is configured to oppose the other forces. The flow conditions are generally selected to enable particles to spend enough time traveling under the influence of the applied forces to attain equilibrium heights over the DEP electrode array. Attainment of equilibrium heights can assure that particles exit the chamber at a height that reflects their physical properties (e.g., independent of the position at which they entered the chamber). Fractionation in such continuous-flow DEP-FFF apparatuses and methods

can be achieved by withdrawing or permitting fluid to exit through two or more ports or outlets at the exit end or portion of the chamber. By regulating the flow rate through the withdrawal ports, the ports can be arranged to skim off fluid that contains particles within defined height ranges above the chamber floor. For example, the flow rate through a first exit port may be configured to cause particles up to a defined height above the chamber floor to be captured through a first exit port, and the flow rate through a second exit port may be configured to cause particles travelling above said the height of the first exit port to exit through a second exit port.

[0046] To operate efficiently and with high discrimination, DEP-FFF can be configured to allow the particles to reach an equilibrium height above the chamber floor at which the DEP, sedimentation (gravitational), and hydrodynamic lift forces substantially balance. If the particles travel through the chamber without contacting the chamber floor (so-called hyperlayer mode DEP-FFF), the height ***h*** above the accumulation wall at which a particle moves is determined by the balance of vertical DEP, sedimentation and hydrodynamic lift (HDL) forces:

$$F_{sed} + F_{DEP} + F_{HDL} = 0. \quad [1]$$

[0047] If the particles touch the chamber floor (so-called steric mode DEP-FFF), the particles come into contact with the accumulation wall and a vertical contact force $F_{contact}$ comes into play so that $F_{sed} + F_{DEP} + F_{contact} = 0$. In this case, steric (friction-like) interactions occur between the particle and the wall, slowing the lateral translation of the particles, and making their elution from the chamber a function of particle-wall contact interactions.

10048] F_{sed} is the sedimentation force $\frac{4}{3}\pi \cdot R^3(p_p - p_s)g$, where p_p and p_x are the densities of the particle and the DEP-FFF elutant medium, respectively, and g is the acceleration due to gravity. The cell density ρ_p for a given particle type is usually considered to be a fixed parameter, but alterations in suspension conditions such as osmolarity may impact it dynamically for cells, for example. It is evident from the expression for the sedimentation force that the density of the suspension medium p_s is a parameter that will generally impact the force balance in equation [1] and thereby influence the behavior of microparlicles and/or cells in DEP-FFF.

[0049] The hydrodynamic lift force F_{HDI} on deformable particles has been shown to depend on particle geometry and may be approximated according to the relationship:

$$F_{HDL} = -\eta_s \dot{v}_0 \frac{R^3}{h} \cdot \Phi(\nu) \quad [2]$$

where η_s is the dynamic viscosity of the eluate and $\phi(\nu)$ is a dimensionless geometry function ($0 < \Phi(\nu) < 1$). B is the flow rate through a chamber of height H and width W; $v_0 = BI(HW)$ is the mean fluid flow velocity and $\dot{v}_0 = \frac{6v_0}{H}$ is the flow shear rate at the floor of the chamber.

Equation [2] is an approximation, and it will be understood by those skilled in the art that more complex expressions may govern the hydrodynamic lift properties of microparticles and/or cells in specific cases. Nevertheless, the more complex expressions are also expected to depend upon the viscosity ¾ and shear rate $\dot{v}_0$ of the suspending medium. It follows that the viscosity ¾ is a parameter that will impact the force balance in equation |**1]** and thereby influence the behavior of microparticles and/or cells in DEP-FFF.

[0050] F_{DEP} arises from the dielectric response of a particle to an imposed inhomogeneous electric field. The field induces electric polarization whereby equal and opposite charges build up on opposite sides of the particle to form an electric dipole. Because the electric field varies spatially, the field intensities on the opposing charges on either side of the particle are different, imposing coulombic forces that do not balance. The residual net force, called the dielectrophoretic (DEP) force, acts on the particle even though it retains zero net charge. Because reversal of the field also causes reversal of the particle polarization, the DEP force direction is independent of the field sense. The buildup of charges in DEP is not spontaneous, but depends on both particle dielectric and geometric characteristic and the medium. Particle DEP responses to fields of different frequencies may be used to infer the particle properties and to impose separation forces on different particle types.

(0051] Suitable inhomogeneous electric fields for DEP can be created by an array of phased electrodes and, depending on the configuration and excitation phases, the electric field pattern may move through space (a so-called traveling wave) or form a fixed field pattern. Although DEP forces for many field types have been explored, in FFF F_{DEP}, is usually produced by a fixed electric field distribution created by energizing, in anti-phase, two interdigitated arrays of microelectrodes patterned all over the accumulation wall of the FFF chamber. When the electrode array consists of parallel plain microclectrode strips of equal width and spacing s, the

vertical component of the DEP force due to fringing fields above the electrode plane may be written as

$$F_{DEP} = 2\pi\varepsilon_s\varepsilon_0 R^3 \cdot \mathrm{Re}[f_{CM}(f)] \cdot q(h) \cdot [P_{eff}(f) \cdot V]^2, \quad [3]$$

where V is the AC voltage of frequency f that energizes the microelectrode array to provide the electric field. V and f may be adjusted to program the DEP response. The geometric scaling function

$$q(h) = -\left(\frac{176}{d^3}\right) \cdot \exp\left(-\frac{4\pi h}{d}\right) \quad [4]$$

defines the height dependency of the intensity of the DEP field above the electrode plane and depends on the electrode periodicity, $d = 4s$. $P_{eff}(f)$, which ideally approaches unity, defines the proportion of the applied excitation voltage that is effective in imposing a DEP force on particles within the eluate. As defined here, $P_{eff}(f)$ accounts for frequency-dependent voltage drops caused not only by electrode polarization at the electrode-eluate interface but also by electrode imperfections and stray impedance in the leads, buses and electrodes downstream of the voltage measurement point. Therefore, $P_{eff}(f)$ lumps together all the unknowns of the DEP configuration into a single parameter that can be readily determined experimentally from the DEP-FFF elution characteristics of standard particles such as polystyrene beads. It is the only calibration term needed to define the performance of a practical DEP-FFF instrument and, although it may alter over time, it can remain stable for many months of operation with good electrode care. Equation [3] shows that the voltage V is a parameter that will impact the force balance in equation [1] and thereby influence the behavior of microparticles and/or cells in DEP-FFF. Furthermore, $P_{eff}(f)$ is a parameter that will impact the force balance in equation [1]. Equation [3] shows further that it is possible to compensate for changes in $P_{eff}(f)$ by adjusting V so as to keep the product $P_{eff}(f) \cdot V$ constant.

[0052] $\mathrm{Re}[f_{CM}(f)]$, the real part of the Clausius-Mossotti factor, describes the frequency-dependent dielectric polarization properties of the particle of radius R within its eluate medium, which is assumed to have a dielectric permittivity $\varepsilon_\chi\varepsilon_0$. This factor for mammalian cells has been the subject of numerous papers. If mammalian cells are suspended in a medium having a

much lower conductivity than their cytoplasm and they have an intact membrane barrier ftinction, then their dielectric responses are dominated by the membrane. Over the approximate frequency range 1 kHz </ < 1 MHz, the real part of the Claussius-Mossotti factor can then be defined in terms of a single "crossover" frequency -^at which cells exhibit a null DEP response:

$$\mathrm{Re}[f_{CM}(f)] = \frac{f^2 - f_0^2}{f^2 + 2f_0^2}. \qquad [5]$$

[0053] To maintain a high differential conductance between the interior and exterior of the cell, the cell membrane barrier must be intact so that Equation [5] applies specifically to viable cells. f_0 is a characteristic crossover frequency at which a given cell type exhibits a null DEP response. f_0 depends both on the cell properties and on the conductivity of the medium in which the cell is suspended. More precisely, for a cell having a low plasma membrane conductivity,

$$f_0 = \frac{\sigma_s}{2^{\frac{1}{2}}\pi \cdot R \cdot C_{mem}}, \qquad [6]$$

where R is the cell radius, C_{mem} is the cell plasma membrane capacitance per unit area of membrane, and the cell is suspended in an eluate of conductivity σ_s. A related cell parameter that is independent of the eluate conductivity is $\Theta_0 = \frac{f_0}{\sigma_s}$, the cell crossover frequency per unit conductivity of the suspending medium. Θ_0 may be written in terms of C_{nKm} and of the cell total plasma membrane capacitance C_{tot}, respectively, as

$$\Theta_0 = \frac{1}{2^{\frac{1}{2}}\pi \cdot R \cdot C_{mem}} \quad \text{and} \quad \Theta_0 = \frac{2^{\frac{3}{2}}R}{C_{tot}}. \qquad [7]$$

[0054] The parameter Θ_0 is different for different cell types and the value of Θ_0 for a given cell type is a characteristic of that cell type that may be used to identify and predict its behavior in DEP analysis. To achieve high discrimination separation and isolation of cells by DEP methods, the frequency /of the applied electric field that creates the DEP forces must be controlled so that it bears a precise relationship to the characteristic Θ_0 values of the cell types in the mixture that is

being processed. For a given cell suspension of conductivity σ_χ, the crossover frequency of a given cell type having a crossover frequency per unit suspending medium conductivity of Θ_0 is

$$f_0 = \Theta_0 \cdot \sigma_s . \quad [8]$$

[0055] If the applied DEP frequency in the DEP process is to bear a well-defined relationship to the crossover frequency of the cells to achieve optimal separation of different cell types then the applied frequency f must be adjusted to take account of the suspending medium conductivity σ_s. It follows that the frequency f is a parameter that will impact the force balance in equation **[1]** and thereby influence the behavior of microparticles and/or cells in DEP-FFF. It follows, further, that the suspending medium conductivity σ_s is a parameter that will impact the force balance in equation [**1**] and thereby, in turn, influence the behavior of microparticles and/or cells in DEP-FFF.

[0056] Under physiological conditions, the conductivity of the cytoplasm of a mammalian cell approaches 1 $S.m^{-1}$ and has an osmolarity of ~320 mOs. For DEP processing, cells are usually suspended in a medium of conductivity σ_s that is much lower than 1 $S.m^{-1}$. More specifically, the eluate is typically chosen to have a conductivity in the range 10 to 100 mS. m^{-1}. The osmolarity of an aqueous solution containing only ions and having a conductivity of between 10 mS/m and 100 mS/m is very small compared with the physiological osmolarity of 290 $mOs.kg^{-1}$ needed to support mammalian cells without inflicting damage on them. In order to process mammalian cells without causing undesired osmotic stress, low conductivity eluate may be supplemented with a non-ionic osmolyte to adjust its osmolarity to a target osmolarity O_x that lies in the range of osmolarities within which cells have desired properties. The osmolyte supplement used to adjust the osmolarity to desired conditions in low conductivity DEP eluate suspension buffers is typically a cell membrane-impermeant sugar such as 9.5% sucrose, 5.1% mannitol, another sugar, or a zwitterionic osmolyte such as glycine. The conductivity of the final suspending medium may be adjusted to a target operating conductivity with salts such as sodium chloride or potassium chloride. Alternative osmolyte and ion combinations may be used to achieve the same purpose using principles and recipes well known in the art.

[0057] Reducing the conductivity of the cell suspending medium within the range 30 to 100 mS. m^{-1} not only leads to desirable DEP behavior of the cells as detailed in equations **[4]-[7]** but

also is advantageous because it lowers the electric current between the DEP electrodes. If the suspension has a physiological ion concentration and a corresponding conductivity in excess of 1 $S.m^{-1}$, the resulting current may lead to Joule heating and to the formation of convection currents in the fluid above the DEP electrodes as well as to electroosmotic and other flow effects. These flow effects may perturb microparlicle and cell discrimination in a DEP-dependent separations. By contrast, an ionic solution having a conductivity within the range 30 to 100 $mS.\ m^{-1}$ requires a relatively small electric current that may allow confounding fluid flow effects to be avoided.

[0058] To achieve cell suspension conditions for DEP processing of clinical specimens that meet desirable conductivity and osmolarity conditions as described above, steps must be taken to reduce the ionic conductivity and to compensate the osmolarity of the suspending medium before DEP-FFF fractionation can occur. These steps may involve centrifuging the cell specimen to remove cells from their physiological medium and resuspending the cells in a low conductivity medium containing an appropriate osmolyte. The resultant cell suspension maybe injected into a chamber for DEP analysis.

[0059] However, this approach presents difficulties when a large quantity of cells is to be processed. In particular, cells cannot be kept in a low-conductivity medium for an extended period of time without their biological and dielectric properties changing. Specifically, if cells are kept in low conductivity eluate for more than a few hundred seconds then ion leakage occurs from the cell cytoplasms into the suspending medium and non-ideal osmotic responses of the cells towards the osmolyte used to compensate the suspension to a target osmolarity O_s may alter cell size, shape and physiological function. As the result of such cell responses to low conductivity medium, the cell dielectric properties may alter and this, in turn, may modify the cell responses to DEP and confound the discrimination and separation of cells by DEP methods. Typically, DEP-FFF runs should be completed on cells that have been suspended in low conductivity medium for « 1000 seconds and ideally every cell analyzed should be suspended in low conductivity medium for the same period of time at the time it undergoes DEP processing to insure that the dielectric properties of all cells processed from a specimen are consistent. For applications such as the isolation of CTCs from blood specimens, the large number of background cells leads to specimen processing times considerably in excess of 1000 seconds. Therefore, it is disadvantageous to replace the suspending medium for a whole specimen with low conductivity medium prior to starting the DEP-FFF process because the properties of cells

undergoing DEP processing late in the DEP-FFF run may have dielectric properties that differ from cells processed early in the run as a result of the different cell exposure times to the low conductivity medium. These differences may cause cell isolation efficiency and consistency to alter during the DEP-FFF processing of the specimen.

[0060] From these considerations, it is evident that the behavior of microparticles and/or cells in DEP-based separators may depend upon the density p_s, conductivity σ_s, viscosity ¾ and osmolarity O_s of the suspending medium in which DEP forces are imposed and upon the voltage V and frequency f of the electric signal used to create the DEP force. Furthermore, the cells need to remain in the DEP-based separator for a time $T_{ransi(}$ that is sufficiently long to allow them to reach equilibrium heights in the applied force fields before they reach the exit ports. To achieve optimal separation, fractionation and/or isolation of target microparticles and/or cells from background microparticles and/or cells by DEP-FFF methods, it is necessary to control these parameters.

[0061] The exchange or removal of solutes is a common requirement in biological processing. The most common approaches to solute exchange are the replacement of the suspending medium following centrifugation or filtration (for particles of sufficiently high density of large enough to be retained by filtration), dialysis across a semipermeable membrane (in the case of removing small solutes from blood and proteins, for example), or diafiltration in which a new solute is infused with the particle mixture while the particle suspension travels along a tube perforated by small pores that allow the suspending mixture to leak out yet retain particles and/or cells. These approaches are difficult to realize in microfluidic applications. An alternative approach is the use of solute diffusion across fluid junctions.

[0062] A thin layer of fluid will be referred to as a fluid lamina. At the small Reynolds numbers typical of fluid flow rates used for DEP separation applications, fluid flow in the DEP separation chamber is laminar and fluid streams from two or more inlet ports may be merged into a composite flow in which the two individual fluid flow stream move as separate fluid laminas travelling in parallel and in contact with one another throughout their passage through the chamber (see Figure 1). In this case, a transfer of solutes across the interface between the two parallel fluid laminas may occur by diffusion. Fick's first law of diffusion relates the diffusive flux of a solute to its concentration and, in one dimension, the flux may be written as

$$J = -D\frac{\partial \phi}{dx} \qquad [9]$$

where J is the diffusion flux [(moles of solute) $m^{-2}.s^{-1}$], D is the diffusion coefficient [$m^2.s^{-1}$], ϕ is the solute concentration in dimensions of [moles.m^{-3}], and x is the position [m], D is proportional to the squared velocity of the diffusing molecules of the solute, which in turn depends on the temperature, viscosity of the fluid and the size of the particles. The Einstein-Smolokowskii diffusion equation describes the relationship between diffusion distance, time and effective size of diffusing entities as

$$\bar{x}^2 = \frac{kTAt}{3\pi\eta r} \qquad \textbf{[10]}$$

where $\bar{x}$ is the mean diffusion distance, Δt is time, η is the fluid viscosity, T is the absolute temperature, k is the Boltzmann constant, and r is the effective hydrodynamic radius. r is approximately equal to the mean radius for microparticles and cells. For macromolecules and solutes, r may be approximated in terms of the molecular weight M of the diffusing entity in Daltons as $r \approx 8.94 \times 10^{-10}.M^{\frac{1}{3}}$.

[0063] In dilute aqueous solutions the diffusion coefficients of most ions are of similar order of magnitude with values in the range of 0.6×10^{-9} to 2×10^{-9} $m^2.s^{-1}$ at room temperature. Sucrose, a small molecular solute, has a diffusion coefficient around 0.3×10^{-9} $m^2.s^{-1}$. In contrast, the diffusion coefficients for biological macromolecules (for which M is high) lie between 10^{-11} to 10^{-10} $m^2.s^{-1}$. Finally, for cells and microparticles, for which hydrodynamic radii are very large compared to molecules, the diffusion coefficients are less than 10^{-15} $m^2.s^{-1}$. It follows that ions and small molecules diffuse many orders of magnitude more rapidly than cells and microparticles.

[0064] Exploiting these diffusion characteristics, it is feasible to arrange for two fluid laminas to flow in contact with one another through a channel and for solute exchange to occur across the interface between the two laminas. Furthermore, if one lamina contains cells and/or microparticles as well as a solute, it is possible for the diffusion of solute from one lamina to the other to occur while cells and/or microparticles undergo negligible transfer. Thus, poorly diffusing microparticles and/or cells may be retained within a flow lamina while diffusible undesired solute is depleted by diffusion into the second flow lamina.

[0065] The design parameters for this type of separation, including the relative thickness and flow rates of the first and second flow laminas and the length of the interface region between them, may be chosen based on the diffusion coefficient of the undesired solute to insure that the undesired solute may be sufficiently depleted to meet desired conditions for DEP. Note that the maximum depletion of a diffusible, undesired solute in this scheme will occur when the concentration of the undesired solute is the same as it would have been had the first flow lamina been mixed completely with the second flow lamina. Therefore, if the thicknesses of said adjacent first and second flow laminas are h_1 and /¾, respectively, and the concentrations of the undesired solute in said adjacent first and second flow laminas are C, and C_2, respectively, then, by conservation of mass principles, the minimum achievable concentration of the undesired solute in said first flow lamina will be

$$C_{\min} = \left(\frac{C_1 h_1 + C_2 h_2}{h_1 + h_2} \right). \qquad \textbf{[11]}$$

[0066] The importance in maintaining separate, adjacent laminas and allowing diffusion to drive the solute dilution is that the micoparticles and/or cells are not diluted but are retained at their initial concentration in the first fluid lamina. Therefore, the dilution effect of the undesired solute is analogous to the process of dialysis against a fixed volume of eluate, where the minimum achievable concentration of the dialysable entity occurs when it equilibriates across the dialysis membrane throughout the specimen and eluate volumes. In the case of adjacent fluid laminas, the laminas are flowing parallel to one another and diffusion occurs across the interface region without a dialysis membrane. Therefore, a great advantage of the microfluidic approach shown here is that no dialysis membrane is required. This not only greatly simplifies the arrangement needed to achieve solute depletion but also greatly accelerates the rate of solute depletion relative to dialysis because there is no need for the solute to cross a semipermeable membrane. Diffusion across a membrane is typically orders of magnitude slower than unimpeded diffusion across a fluid interface.

[0067] To achieve rapid depletion of ions in the specimen, the height of the first fluid lamina containing the microparticles and/or cells should be small. As is well known in the art and embodied in equation **[9],** the rate of diffusion through a region of space depends inversely on the square of the distance across it. Sodium ions in water at room temperature, for example, will diffuse about 50 micrometers in one second yet they will take about 100 seconds to cross 500

micrometers and 10,000 seconds to diffuse 5mm. It follows that laminar flow-based solute depletion will benefit greatly from reduction of the thickness of the fluid lamina that contains the microparticles and/or cells and undesired solute.

[0068] The heights of the laminas may be found from the equations of lamina flow. Assume that the first fluid lamina arises from fluid injected at a flow rate $/J,$ into the bottom inlet slot and joins the faster eluate flow rate B_2 from the top inlet that forms the second fluid lamina. The proportion of the chamber height $\frac{h}{H}$ that is filled by the first fluid lamina containing the microparticles and/or cells during this injection process can be found from

$$\frac{B_1}{B_2} = \frac{3h^2}{H^2} - \frac{2h^3}{H^3}, \qquad \textbf{[12]}$$

showing that the height to which the first fluid lamina containing the microparticles and/or cells fills the chamber is

$$h \approx II \cdot \sqrt{\frac{B_1}{V3F}} \quad \text{when } \frac{h}{H} \text{ is small.} \qquad [13]$$

[0069] An analogous expression describes the height h_x from which fluid will be skimmed through an outlet port that withdraws fluid at a rate B_{skim}.

[0070] The height at which cells and/or micoparticles traverse the microelectrode array in DEP-FFF depends on the DEP frequency and voltage and the suspension medium conductivity and density. Providing the cell and/or microparticle suspension injection rate B_1 is sufficiently small for the cells and/or microparticles to undergo solute depletion at an eluate flow rate B_2 such that the injected cells and/or microparticles reach desired equilibrium heights over the microelectrode array before reaching the outlet port, target microparticle and/or cell populations may be collected by adjusting B_{skim} appropriately.

[0071] The principles and basic equations provided here apply to any particle mixture for which diffusion is negligible and for which electrolyte and osmolyte diffusion is sufficiently rapid on the time scale of separation. Typically, diffusion can be assumed to be negligible for particles of the order of 1 micron in diameter or larger. Such particles include many types of

cells, bacteria, large organelles, cell nuclei, sedimentary particles and silts, minerals, ores, conductive versus nonconductive versus semiconducting particles, plastics, glasses, etc. For microparticles that are surrounded by a thin membrane, the single shell model described here is applicable together with the analytical methods presented for deriving particle parameters physical. In particular, living organisms normally depend on membrane barriers to control the flux of nutrients and metabolites and constrain the locality of biomolecules. Therefore DEP, which can be used to characterize and exploit the properties of membrane barriers, would appear to offer possibilities for additional applications in the life sciences also. According to specific particle types in a separation application, a different form of dielectric model from that shown in equations (5) and (6) may be applicable as is known in the art.

2. Apparatuses

[0072] Referring now to the drawings, and more particularly to FIG. 1A, shown there and designated by the reference numeral 10 is one embodiment of the present apparatuses for (e.g., optimized) continuous flow dielectrophoretic separations. In the embodiment shown, apparatus 10 comprises a body 14 that defines a chamber 18 having a first portion 22 and a second portion 26. In the embodiment shown, chamber 18 has a bottom 30, and two sides 34 (only one shown) that define a substantially U-shaped chamber or channel. In the embodiment shown, a width (between and perpendicular to sides 34) of the chamber may be larger than a height 38 of the chamber. In some embodiments, the chamber 18 may also include one or more inlets and one or more outlets. For example, in the depicted embodiment, body 14 includes a first inlet 42, a second inlet 46, a first outlet 50 and a second outlet 54. In the embodiment shown, first portion 22 includes a first end 58 and a second end 62 between the one or more inlets (e.g., inlets 42 and 46, in the embodiment shown) and the one or more outlets (e.g., outlets 50 and 54, in the embodiment shown). In the embodiment shown, second portion 26 is disposed between second end 62 of first portion 22 and at least one of the outlets (e.g., both of outlets 50 and 54, in the embodiment shown).

[0073] In some embodiments, apparatus 10 further includes one or more electrodes 66 configured to generate a non-uniform electric field in second portion 26 of chamber 18. For example, in the embodiment shown, apparatus 10 comprises a plurality of electrodes 66. Electrodes 66 may, for example, be disposed on or beneath bottom 30 of chamber 18. In the embodiment shown, first portion 22 does not include electrodes 66 that are configured to generate a non-uniform electric field. In some embodiments, first portion 22 may include

detection electrodes that are configured to detect or measure one or more properties of fluids (e.g., liquids) in chamber 18 (e.g., in conjunction with a controller or sensor coupled to such detection electrodes).

[0074] In the embodiment shown, first inlet 42 is configured to receive a first fluid 70 including a target matter (e.g., cells, malignant cells, and/or other particles or packets of matter), and second inlet 46 is configured to receive a second fluid 74. Second fluid 74 may include one or more components configured to diffuse, or facilitate diffusion, between first fluid 70 and second fluid 74 (e.g., to reduce the conductivity of the first fluid 70). In some embodiments, apparatus 10 may be configured such that if a first fluid 70 (including target matter) and a second fluid 74, are introduced into chamber 18 through inlets 42 and 46, first fluid 70 and second fluid 74 can flow substantially laminarly through first portion 22 to permit diffusion of solutes between first fluid 70 and second fluid 74.

[0075] The chamber 18 may be configured to direct first and second fluids 120 and 122 from inlets 42 and 46, respectively, to outlets 50 and 54 according to a predetermined velocity profile. The predetermined velocity profile may be such that first fluid 70 and second fluid 74 substantially reach diffusion equilibrium in first portion 22 of chamber 18. For example, in the embodiment shown, a length 78 of first portion 22 is large enough to permit first fluid 70 and second fluid 74 to substantially reach diffusion equilibrium in first portion 22. In the embodiment shown, length 78 of first portion 22 is greater than length 82 of second portion 26. In other embodiments, lengths 78 and 82 may be substantially equal, or length 82 may be greater than length 78. Diffusion between first and second fluids 70 and 74 in first portion 22 may, for example, reduce the conductivity of the first fluid 70 (e.g., to improve the sensitivity of separation of the target matter in second portion 26). In the embodiment shown, apparatus 10 is configured such that first fluid 70 flows below second fluid 74 through chamber 18. To increase the rate of diffusion between first and second fluids 70 and 74 (and therefore reduce the time and length of first portion 22 required to reach diffusion equilibrium), apparatus 10 can be configured such that during the substantially laminar flow through first portion 22, the cross-sectional area of first fluid 70 is smaller than the cross-sectional area of second fluid 74. For example, in the embodiment shown, the lamina of first fluid 70 has a height 86 that is shorter than (e.g., less than or between any of: 5%, 10%, 15%, 20% of) height 90 of the lamina of second fluid 74.

[0076] In some embodiments, apparatus 10 is configured such that electrodes 66 can generate a non-uniform or spatially inhomogeneous electric field in second portion 26 to generate a dielectrophorelic force on target matter (in first fluid 70) in second portion 26, such that the target matter is extracted from first fluid 70. For example, in the embodiment shown, apparatus 10 includes a signal generator 94 coupled to electrodes 66, and the signal generator is configured to generate an electric signal with a designated frequency and voltage for delivery to electrodes 66 to cause the electrodes to generate the non-uniform electric field in second portion 26 for dielectrophoretic separation of target matter from first fluid 70. In this embodiment, first outlet 50 is configured to permit the target matter to exit the chamber, and second outlet 54 is configured to permit the remainder of first and second fluids 70 and 74 to exit the chamber.

[0077] In some embodiments, such as the one shown, apparatus 10 can include a conductivity sensor 98 that is configured to measure the conductivity of the fluid in the chamber 18(e.g., first fluid 70 after at least some—up to and including all—of the diffusion in first portion 22 takes place). For example, in the embodiment shown, conductivity sensor 98 is configured to measure the conductivity of first fluid 70 in second portion 26; but in other embodiments, may be configured to measure the conductivity of the fluid in first portion 22 and/or in second portion 26. The fluid of which the conductivity is measured may be in contact with electrodes 66, and/or one or more other detection electrodes may be included that are configured to contact the fluid.

[0078] In some embodiments, such as the one shown, apparatus 10 can also include a controller 102 (e.g., a microcontroller, processor, and/or the like) configured to perform one or more functions that support operation (e.g., semi- and/or fully-automated operation) of the apparatus. For example, in the embodiment shown, controller 102 is configured to calculate one or more target properties of the non-uniform electric field (e.g., one or more properties of the electric field that are likely to result in separation of the target matter from the first fluid in second portion 26 of the chamber) based on at least one of the measured conductivity of first fluid 70 and a property of the target matter (e.g., conductivity, density, and/or the like). In some embodiments, controller 102 can also be configured to adjust the electric signal generated by signal generator 94 to cause the non-uniform electric field to substantially include the one or more target properties. For example, the controller may calculate one or more properties of the electric field that are likely to cause separation of the target matter in a fluid having the measured conductivity, and send a control signal to signal generator 94 so that signal generator 94 adjusts

the generated electric signal accordingly. Such an adjustment of the electric signal may include, for example, the frequency and/or the voltage of the electric signal.

[0079] In some embodiments, such as the one shown, apparatus 10 can include a current sensor 106 that is configured to measure the current of the electric signal generated by signal generator 94. In such an embodiment, controller 102 (e.g., coupled to current sensor 106) can be configured to compare the measured current to a target current, and to to adjust the electric signal based on the comparison (e.g., can increase frequency and/or voltage of the electric signal if the measured current is below a target current).

[0080] Some embodiments of the present apparatuses do not include either of conductivity sensor 98 or current sensor 106, or include only one of conductivity sensor 98 or current sensor 106.

[0081] FIG. 1B illustrates an alternative embodiment of a body 12' for some embodiments of the present apparatuses. In the depicted embodiment, body 12' differs from body 12 in that body 12' includes first and second outlets 50' and 54' that differ in configuration relative to first and second outlets 50 and 54 of body 12. More particularly, in the embodiment shown, first outlet 50' diverts target matter in a direction that is angularly disposed relative to direction 110 of flow through the chamber. In the embodiment shown, the direction of first outlet 50' is substantially perpendicular to direction 110. In other embodiments, the direction of first outlet 50' may be disposed at any suitable angle (e.g., greater than or between any two of: 15, 30, 45, 60, 75, and/or 90 degrees) relative to direction 110. Additionally, in the depicted embodiment, length 78' of first portion 22' is larger than length 78, and length 82' of second portion 26' is shorter than length 82. As such, the ratio of length 78' to length 82' is larger than the ratio of length 78 to length 82.

[0082] FIG. 1B also illustrates the separation of target matter from the remainder of first fluid 70 and second fluid 74 that is representative of target matter that may also be isolated or separated in the embodiment of FIG. 1A. For example, in the embodiment shown, first fluid 70 includes a blood sample that includes blood cells 114 and malignant cells 118, with malignant cells 118 being the target matter that is removed from the blood sample through first outlet 50'.

3. Methods

[0083] The schematic flow chart diagrams that follow are generally set forth as logical flow chart diagrams. As such, the depicted order and labeled steps are indicative of one embodiment

of the presented method. Other steps and methods may be conceived that are equivalent in function, logic, or effect to one or more steps, or portions thereof, of the illustrated method. Additionally, the format and symbols employed are provided to explain the logical steps of the method and are understood not to limit the scope of the method. Although various arrow types and line types may be employed in the flow chart diagrams, they are understood not to limit the scope of the corresponding method. Indeed, some arrows or other connectors may be used to indicate only the logical flow of the method. For instance, an arrow may indicate a waiting or monitoring period of unspecified duration between enumerated steps of the depicted method. Additionally, the order in which a particular method occurs may or may not strictly adhere to the order of the corresponding steps shown.

[0084] FIG. 2 illustrates one embodiment of a method 200 for optimized continuous flow dielectrophoretic separations. In one embodiment, the method 200 may include a step 202 of introducing a first fluid 70 including target matter (e.g., a blood sample including malignant cells) and a second fluid 74 into a chamber (e.g., 18) of an apparatus. The second fluid may include one or more osmolytes configured to facilitate the diffusion between the first fluid and the second fluid to reduce the conductivity of the first fluid. The apparatus may, for example, be one that is depicted in and described with reference to FIG. 1A or 1B. That is, the chamber 18 of apparatus 10 may include one or more inlets (e.g., first and second inlets 42 and 46) and one or more outlets (e.g., first and second outlets 50 and 54). The chamber 18 may also include a first portion 22 having a first end 58 , and a second end 62 between the first and second inlets (42 and 46) and the first and second outlets (50 and 54), and a second portion 26 between second end 62 of first portion 22 and at least one the first and second outlets (e.g., first outlet 50). Apparatus 10 may also include one or more electrodes (e.g., electrodes 66) configured to generate a non¬uniform electric field in the second portion (26) of the chamber. In some embodiments, the heights and/or sizes of the inlets (42 and 46) may be configured such that the input streams of first and second fluids 70 and 74 are thin enough to achieve rapid diffusion between the first and second fluids 70 and 74 (e.g., such that first and second fluids 70 and 74 substantially reach diffusion equilibrium in first portion 22).

[0085] In some embodiments, such as the one shown, method 200 may also include a step 204 of causing first and second fluids 70 and 74 to flow substantially laminarly through first portion 22 of the chamber such that diffusion between first fluid 70 and second fluid 74 lowers the conductivity of first fluid 70. In one embodiment, first and second fluids 70 and 74 flows

continuously through first and second portions 22 and 26 of chamber 18. As described above, length 78 of first portion 22 of the chamber may be large enough to permit first fluid 70 and second fluid 74 to substantially reach diffusion equilibrium in first portion 22, and during the laminar flow through first portion 22, the cross-sectional area of first fluid 70 may be smaller than the cross-sectional area of second fluid 74.

[0086] In some embodiments, method 200 may further include a step 206 of applying an electric signal to the one or more electrodes 66 of the apparatus to generate a dielectrophoretic (DEP) force on the target matter to extract the target matter from the first fluid. Electrodes 66 of the apparatus (e.g., when an appropriately configured electric signal is applied) may generate a non-uniform electric field in second portion 26 of the chamber, which may in turn generate a DEP force on the target matter in the first fluid. The DEP force applied on the target matter in an appropriate direction, may balance out the sedimentation force, hydrodynamic lift force, and/or on the target matter, and the residual DEP force may act on the target matter to direct them to exit from a desired outlet (e.g., first outlet 50) of the chamber. Method 200, may also include a step 210 of outputting the target matter through a first outlet of the chamber and the remainder of the first and second fluids through a second outlet of the chamber.

[0087J The sedimentation force acting on microparticles and/or cells may be at orders of magnitude greater than the sedimentation force acting on electrolytes and osmolytes. Therefore, if the first fluid 70 containing the target matter (e.g. cells and/or microparticles) is configured so as to flow underneath the second fluid 74 containing the osmolyte, then the target matter may tend to sediment away from the interface between the fluids and cause the target matter to be confined within the lower fluid. This may facilitate bringing target matter into juxtaposition with the on or more electrodes 66 that imposes DEP forces, thereby facilitating a stronger influence of DEP on cell behavior. At the same time, the effects of sedimentation forces on the electrolytes and osmolyte may be negligible and may not significantly affect the diffusion of those materials across the interface region. Alternatively, if the fluids move in a vertical direction, then sedimentation effects may act along the direction of flow and not across the interface region. In this case, sedimentation may not play a role in influencing the flow behavior of the target matter across either flow of the first and second fluids.

[0088J In some embodiments, method 200 may further include an optional step 208 that can include one or more of various components illustrated in FIGS. 3A-3B. As illustrated in FIGS.

3A-3B, in some embodiments, method 200 can include a sub-step 302 of controlling the flow rate of the first fluid and the second fluid (e.g., at a target flow rate). For example, the target flow rate may be such that first fluid 70 and second fluid 74 flow through first and second portions 22 and 26 of the chamber according to a target velocity profile. The target velocity profile may, for example, be such that first fluid 70 and second fluid 74 substantially reach diffusion equilibrium in first portion 22 of the chamber. The diffusion between the first and second fluids may, for example, adjust the conductivity of the first fluid. When first fluid 70 flows adjacent to second fluid 74, it allows an undesired solute in first fluid 70 to become depleted by diffusion of the undesired solute into second fluid 74 (across the interface between the first and second fluids).

[0089] In some embodiments, method 200 can include a substep 304 of measuring the conductivity of the first fluid (e.g., with conductivity sensor 98); a substep 306 of calculating (e.g., with controller 102) one or more target properties of the non-uniform electric field based on at least one of measured conductivity of the first fluid and a property of the target matter; and/or a substep 308 of adjusting (e.g., with controller 102) the electric signal to include the one or more target properties. The adjustment of the electric signal may include changing at least one of the frequency and the voltage of the electric signal. For example, in some embodiments, an optimal frequency f_0 can be computed from a product of a target frequency-per-unit conductivity parameter Θ_0 · and the measured fluid conductivity σ_χ. An electric signal may then be generated at the optimal frequency f_0 and applied to the one or more electrodes 66 to provide the DEP forces to the target matter in the chamber 18.

[0090] In some embodiments, method 200 can include a substep 310 of measuring a current of the electric signal (e.g., with current sensor 106); a substep 312 of comparing the measured current to a desired current (e.g., with controller 102); and/or a substep 314 of adjusting the electric signal based on the comparison (e.g., with controller 102). The adjustment of the electric signal may include changing at least one of the frequency and the voltage of the electric signal. For example, the electric current I_{DEP} that is flowing through the DEP electrodes 66 as a result of an applied signal of frequency f_0 and voltage V from the signal generator 94 may be detected and compared to a demand current that is known to likely to provide optimal DEP performance at the fluid conductivity σ_s and frequency f_Q. If the measured current I_{DEP} differs from the demand current, then a voltage correction may be computed and the signal generator may be

adjusted through a feedback network so as to provide an electric signal at a compensated voltage V that brings I_{DEP} to the demand current value.

[0091] In some embodiments, the processes of conductivity sensing and current sensing may be carried out at the same time to achieve a desired (e.g., improved and/or optimal) frequency and voltage for the electric signal generated by the signal generator 94. For example, the conductivity σ_s of the fluid that flows over electrodes 66 may be determined by conductivity sensor 98. The optimal frequency f_0 may be computed from a product of a target frequency per unit conductivity parameter Θ_0 ·and the fluid conductivity σ_s. The signal generator frequency may be adjusted to f_0 through a first feedback scheme thereby insuring that the frequency of the electric signal applied to the electrodes 66 is appropriate for the fluid conductivity σ_χ. In parallel with this frequency feedback, the electric current I_{DEP} that is flowing through DEP electrodes 66 as a result of the applied signal of frequency f_0 and voltage V from the signal generator maybe sensed by current sensor 106 and compared by controller 102 to a demand current that is likely to provide desired (e.g., optimal) DEP voltage at the fluid conductivity σ_s and frequency f_0 . If the measured current I_{DEP} differs from the demand current, then signal generator 94 may be adjusted through a second feedback scheme that compensates the DEP signal voltage V in order to bring I_{DEP} to the demand current value. Working in parallel, the two feedback schemes insure that both the applied frequency and voltage from signal generator 94 are desired (e.g., optimal) and automatically compensated for changes in the conductivity of the fluid and the condition of electrodes 66.

[0092] In some embodiments, method 200 can include a substep 316 of adding one or more osmolarity-altering components to first fluid 70 (e.g., such that the osmolarity of first fluid 70 bears a desired relationship to the osmolarity of second fluid 74); and/or a substep 318 of adding one or more osmolarity-altering components to second fluid 74 (e.g., such that the osmolarity of second fluid 74 bears a desired relationship to the osmolarity of first fluid 70). The osmolarity-altering components may, for example, include salts such as sodium chloride or potassium chloride. Alternative osmolyte and ion combinations may be used to achieve similar effects. Osmolarity adjustment may, for example, achieved by flowing first fluid 70 adjacent to second fluid 74 to allow an osmolyte contained in one of the first and second fluids to diffuse to the other

of the first and second fluids across the interface between the first and second fluids, thereby altering the osmolarity in first fluid 70.

(0093] In some embodiments, method 200 can include a substep 320 of adding one or more density-altering components to first fluid 70 such that the density of first fluid 70 bears a desired relationship to the density of second fluid 74; and/or a substep 322 of adding one or more density-altering components to second fluid 74 such that the density of second fluid 74 bears a desired relationship to the density of first fluid 70. The desired density relationship may be such that convection is reduced and/or substantially prevented between first and second fluids 70 and 74. For example, undesired flow patterns of and between the first and second fluids may be reduced and/or obviated by adjusting the density of the first and/or second fluids such that a desired density relationship exists between the first and second fluids (e.g., that inhibits convection). Similarly, undesired sedimentation behavior of microparticles and/or cells in the flow channel may be reduced and/or obviated by adjusting the density of the first and/or second fluids such that a desired density relationship exists between the first and second fluids (e.g., in accordance with and/or compatible with the density characteristics of such microparticles and/or cells).

[0094J In some embodiments, method 200 can include a substep 324 of adding one or more viscosity-altering components to first fluid 70 such that the viscosity of first fluid 70 bears a desired relationship to the viscosity of second fluid 74; and/or a substep 326 of adding on or more viscosity -altering components to second fluid 74 such that the viscosity of second fluid 74 bears a desired relationship to the viscosity of first fluid 70. The desired density relationship may be such that convection is reduced and/or substantially between the first and second fluids.

Example 1: Isolation of CTCs

[0095] As one specific example, the blood of patients having the disease of cancer may contain cancer cells, and it may be desirable to isolate said cancer cells from the blood for purposes of prognosis, diagnosis, treatment and/or management of the cancer. Such cancer cells may be circulating in the blood at a very small concentration compared with the many normal blood cells within the peripheral blood stream of the patient. Such cancer cells mixed with the blood are generally referred to as "circulating tumor cells" or CTCs. ´The isolation of CTCs from the peripheral blood of cancer patients is generally considered to be of importance for the

prognosis and treatment of breast, prostate, ovarian, colon, and other cancers. The CTC concentrations in the peripheral blood of patients vary in relation to the stage of the disease but are often extremely low compared with the background count of PBMNs. It has been concluded, for example, that a concentration above five CTCs per 7.5 mL of peripheral blood generally correlates to a worsening outcome in breast cancer. A number of methods exist for isolating CTCs from blood, but most rely on the cell surface marker EpCAM that targets epithelial cells. Because not all cancers, even of the same type, express EpCAM, those methods are not universally applicable. Furthermore, to derive detailed information about the metastatic potential of CTCs, it is desirable to collect them in an intact and viable state to allow for complete morphological, phenotypic, cytokinetic and molecular characterization.

(0096] To isolate CTCs in the continuous flow implementation, 10 mL of blood was first subjected to centrifugation on a histopaque density gradient to remove erythrocytes. The supernanant layer of cells consisting of peripheral blood mononuclear cells (PBMNs) and rare CTCs was suspended in 1 mL of RPMI medium whose density has been adjusted to 1036 kg.m-3 with iodixanol (Optiprep, Axis-Shield, Norway). This suspension was injected into the bottom inlet port of a continuous DEP-FFF chamber at a rate of 25 uL/min. Eluate consisting of 9.5% sucrose at 30 mS/m was flowed from the inlet to the outlet end of the chamber at a rate of 1 mL/min and met the influx of cell suspension to form an upper fluid lamina (that is, a thin layer of fluid) . Under these conditions, the blood cell suspension had the same density as the eluate and filled the bottom 40 um of the chamber while the eluate flowed above it. The cell suspension flowed over a DEP electrode from the moment it entered the chamber, however the first 40 mm of the electrode was not energized. This was to allow the physiological concentration of ions having a conductivity of ~ 1.4 S/m to diffuse away from the electrodes as the suspension moved along the chamber floor so as to bring the conductivity in the first fluid lamina to ~60 mS/m. In the non-energized part of the chamber the cells sedimented and reached equilibrium heights based on the balance of sedimentation and hydrodynamic lift (HDL) forces alone. After flowing over an electrically-non-energized region of the chamber, the first fluid lamina crossed an additional 40 mm of DEP electrode that was energized by an electric signal of 95 kHz frequency and 3.5 Vp-p voltage before arriving at the first outlet or exit port. Once cells reached the energized region, the target CTCs experienced a positive DEP force because the applied signal was above their DEP crossover frequency. This DEP force pulled the CTCs towards the electrode but was not sufficient to overcome HDL forces. Therefore the CTCs were not trapped

on the DEP electrode by DEP forces but instead moved slowly at a height of about 5 micrometers above the chamber floor towards the first outlet or exit port. Simultaneously, the applied electric signal frequency was well below the crossover frequency for the PBMNs and these background cells were levitated about 22 micrometers above the chamber floor and moved rapidly towards the second outlet or exit port. Fluid was withdrawn through the first outlet or exit port at 15 uL/min by a syringe pump and was collected on a filter. The target CTCs were thereby captured. The vast majority of PBM background cells passed over the first outlet or exit port and was carried out of the second outlet or exit port to waste.

[0097] It is to be understood that the same strategy used to isolate CTCs from PBMNs may also be used to eliminate cancer cells from desired blood cells. In this case, the target cells are the blood cells and the undesired cells are the cancer cells. To recover the desired blood cells in this case, the blood cells passing over the first outlet or exit port are collected at the second, downstream or exit port. The cancer cells withdrawn through the first outlet or exit port may be then used for diagnostic, prognostic and/or or risk assessment purposes or discarded.

[0098] The strategies described above for isolating desired cancer cells from blood cells or for isolating desired blood cells from cancer cells may also be used to eliminate other types of undesired cells from desired target cells providing the desired and undesired cells have different DEP crossover frequencies. In general for both strategies, the cell type with the lower crossover frequency will always be collected at the first outlet or withdrawal port.

Example 2: Overview of Separation Strategy

[0099] To achieve discrimination between different cell types by DEP, cells are suspended in a low conductivity medium of physiological osmolarity. This can stress mammalian cells and elicit changes in their dielectric properties as a result of ionic leakage from the cytoplasm if they are suspended in the low conductivity medium for more than 10^3 seconds (Gascoyne et al., 2009). This is not a problem in batch-mode DEP-FFF where the cells may be suspended in the eluate before DEP processing begins and processing is completed in around 10^3 seconds. In continuous flow DEP-FFF, however, a large specimen is processed gradually using a continuous injection approach that takes up to an hour. Suspension of the whole specimen in low conductivity medium prior to starting this slow injection process is not feasible if the dielectric properties of

the cells are to remain unchanged and cell discrimination is to remain consistent throughout the run.

[00100] To provide for this consistency, the inventors developed the continuous flow DEP-FFF approach shown in the figures (e.g., FIG. 4). Continuous flow of a low-conductivity eluate of physiological osmolarity is provided from an inlet port (a) to an outlet port (f), establishing a Poisseuille flow profile in a separation channel of, for example, 200 mm length, 25 mm width and 314 μm height. In the Specimen Injection Zone (b), the specimen, comprising a cell suspension in physiological medium, is fed through a slot in the bottom of the chamber at a continuous flow rate B_1, which is much lower than the eluate inlet flow rate B_2. As a result of the relative flow rates, the specimen forms a thin lamina of height h_{in} flowing adjacent to the floor of the chamber beneath the main eluate flow stream. As the thin specimen lamina travels along the chamber floor in the Ion-diffusion Region, cells find themselves subjected to sedimentation and weak hydrodynamic lift forces and settle to equilibrium heights very close to the chamber floor (see FIG. 4(c)). Meanwhile, ions diffuse from the thin specimen lamina into the main eluate flow stream while non-ionic osmolytes counter-diffuse from the eluate into the specimen lamina. As a result, the high conductivity of the specimen lamina is reduced while the osmolarity near the cells is maintained at a physiological level. After flowing a sufficient distance L_{mix} along the chamber for diffusion to reach equilibrium, the ion concentration, and resultant electrical conductivity of the medium, becomes independent of height in the chamber and small enough for DEP separation to be undertaken. The flow then enters the DEP Separation Region, where microelectrodes on the chamber floor are energized by an appropriate AC voltage to impose DEP forces on the cells. As they flow over the microelectrodes, tumor cells are pulled towards the chamber floor by positive DEP forces while PBMNs are repelled and levitated by negative DEP forces. Cells eventually move to heights at which the DEP, sedimentation and hydrodynamic lift forces balance (see FIG. 4(d)). Finally, as the flow enters the Tumor Cell Skimming Zone (e), fluid is withdrawn through a slot in the chamber floor at a rate B_{skim}, skimming off a thin lamina of fluid from the bottom of the chamber up to a height h_s. Tumor cells, which have reached equilibrium heights close to the chamber floor, are thereby captured through the withdrawal slot while PBMNs, which reached equilibrium heights above the skim height h_s, are carried over the slot and exit to waste with the main eluate exit flow B_{out}, at (f). The detailed design and operation of these stages are described below.

Example 3: Continuous Injection and Skimming

[00101] As indicated, one embodiment for continuous flow DEP-FFF is to transport the specimen along the floor of the separation chamber in a thin lamina that flows beneath the main eluate stream. For the parabolic Poiseuille flow velocity profile, the thickness of this specimen lamina, /?,,,, above the chamber floor is related to the injection flow rate, $B_{/}$ and the eluate inlet rate B_2 according to the expression $Bi\,l(Bi + B_1) = 3(h_{in} / H)^2 - 2(h_{in} / H)^3$, where H is the height of the chamber. For a very thin specimen lamina, $B_1 << B_2$, and $h_{in} \approx H(B_1 I\, 3B_2)^{1/2}$. Similarly, at the withdrawal port, a thin lamina of fluid may be skimmed from the chamber floor up to a height h_s by withdrawing fluid at a rate ¾ ,,,, where $h_s \approx /1(B_s*_n»/3B_{out})^{1/2}$. Downstream from the withdrawal port, the residual fluid flows at a rate B_{out}.

[001 02] In practice, $B_{/}$ and B_{skim} are generally very small and cell sedimentation can result in imperfect injection and skimming behavior if there are either vortices or positions of low flow rate at the injection or withdrawal slots. In order to optimize the slot design, the inventors simulated flow profiles for various chamber heights, slot widths and flow rates using COMSOL Multiphysics software (Stockholm, Sweden) assuming that the fluid was incompressible and had a density of 1036 kg.m^{-3} and a dynamic viscosity of 1.3 1 x 10^{-3} Pa.s, reflecting the properties of the eluate medium used in the inventors' cell-isolation experiments. The results for the withdrawal slot simulations arc shown in FIG. 5. The behavior at the inlet slot region is a mirror image of that at the withdrawal slot.

[00103] Desirable skimming behavior was observed when the slot width d_{out}, was small compared with the chamber height H (FIG. 5 (a)). In this case, a well-defined separation region at the slot opening cleanly split the thin lamina of height h_s at the chamber bottom from the main chamber flow. Streamlines above the skim height h_s travelled essentially horizontally across the slot and no regions of low flow rale or significant vortices were generated in the separation region. In this case, tumor cells close to the chamber floor would be skimmed cleanly into the withdrawal slot, while PBMNs in the fluid above the skim height would be rapidly carried over the slot without being thrown into the slot by inertial forces or having the opportunity to sediment into the slot from regions of low flow rate.

[00104] As the withdrawal-slot width d_{o_m} was increased, the separation behavior became less ideal (FIG. 5 (b)). Although the skim height h_s still followed the Poiseuille model (equations

above), the flow in the slot opening became more complex. Streamlines above the skim height h_s travelled downwards into the mouth of the slot causing a depression by a distance d_s of the region where the withdrawal and main chamber flow streams split. Furthermore, a vortex of diameter W_v formed in the withdrawal tube and significant regions of low flow rate were generated in the separation region. In this case, tumor cells close to the chamber floor would be skimmed into the withdrawal slot but then potentially recirculated in the vortex. Because of the curvature of the streamlines, some may be thrown by inertial forces into the depressed streamlines of the main chamber flow, carried back into the main chamber, and lost to waste. Meanwhile, PBMNs traveling above the skim height that followed the depressed streamlines from the main channel into the chamber mouth would not only have a tendency to be thrown into the withdrawal flow by the curvature of the streamlines but also would need to be carried uphill from their depressed locations well down inside the mouth of the withdrawal slot back into the main chamber against the sedimentation forces acting on them. As a result of these effects, some PBMNs would enter the withdrawal stream and contaminate the tumor cell fraction.

[00105] The simulations establish criteria for designing efficient withdrawal slots and show, in particular, that a withdrawal slot of width $d_{out} < H/3$, regardless of the flow rate, exhibits negligible depression of the main chamber streamlines into the mouth of the withdrawal port (FIG. 5(e)) as well as low vorticity (FIG. 5 (d)). Similar principles apply to the injection slot design to prevent the potential for cell accumulation in the mouth of the injection slot. For the inventors' experiments, a chamber height of 314 μm and injection and withdrawal slot widths of 127 μm were chosen, corresponding to the flow conditions shown in FIG. 5(a). At the inlet, the specimen was injected at a rate of Q_1=25 μL.min^{-1} with a main eluate flow rate Q_2=1000 μL.min^{-1}, leading to a specimen lamina thickness h_{in}=29.3 μm at the inlet. At the outlet, the withdrawal rate was Q_{skim}=20 μL.min^{-1} leading to a skimming height of h_s=26A μm. In the experiments, the injection and withdrawal flows were provided by **1** mL syringes driven by digital syringe pumps (KDS2**10**, KD Scientific, Holliston, Massachusetts) and the eluate flow by a gear pump (Ismatec, Glattbrugg, Switzerland).

[00106] The concept of combining and splitting lamina flow streams in a microfluidic channel is the basis of the so-called H filler design in which two inlet ports enter from opposite sides at one end of the chamber to combine lamina flows and two withdrawal ports exit from opposite sides at the other end of the chamber to split lamina flows. In cell-separation applications,

however, sedimentation of cells is a significant problem at low flow rales and the channels and tubing that carry the cells into and out of the separation chamber should be oriented vertically to avoid cell loss resulting from settling and adherence to tubing surfaces. In the H-filter design (Gascoyne, 2009), regions of stagnant flow can occur at both ends of the chamber at the interfaces of the flow streams. At the low flow velocities required for isolation of tumor cells, sedimentation of PBMNs would occur in the slow-moving region of fluid heading towards the top withdrawal port in the H-filter configuration and some PBMNs would inevitably fall into the lower withdrawal port and contaminate the tumor cell isolate. In the π configuration of the continuous flow DEP-FFF chamber described here and depicted in FIG. 4 (in which the specimen injection port (b) and skimming outlet port (e) are angled relative to (e.g., perpendicular to, as shown) the direction of flow in the chamber at ports (b) and (e), as well as, in at least some embodiments, to the inlet (a) and outlet (b)), the possibility of contamination is greatly reduced because stagnant flow is eliminated from the fluid interface regions. Tumor cells flow directly downward through the withdrawal slot in the chamber floor while PBMNs travel horizontally. Furthermore, microelectrodes are positioned on the chamber floor on both the upstream the downstream sides of the withdrawal slot in the design. These continue to levitate PBMNs by DEP forces in the slot region and on the far side of the slot. This helps reduce still further the likelihood PBMNs will fall into the withdrawal slot and contaminate the tumor cell isolate. In these ways, the π configuration improves significantly upon the H-filter design concept for applications involving sedimentary particles such as cells.

Example 4: Deionization

[00107] In order to pull tumor cells towards the microelectrodes on the floor of the separation chamber by positive DEP while simultaneously repelling PBMNs high into the eluate flow stream by negative DEP, as required in at least some embodiments of the present cell-isolation strategies, the cell suspending medium conductivity must be much lower than the cell cytoplasmic conductivity (Gascoyne and Vykoukal, 2002). The starting conductivity of the specimens was 1.4 $S.m^{-1}$, approximately the same as that of the cell cytoplasms, and this needed to be lowered to a target value of about 60 $mS.m^{-1}$ before the cells could be subjected to DEP separation. The reduction in conductivity was accomplished by using diffusion to deplete the ions in the specimen as it moved through the Cell Settling and Ion Diffusion Zone shown in FIG. 4 (c). The length of the Ion Diffusion Zone was chosen in accordance with the chamber height H and flow rate B_2 to insure that ions had sufficient time to diffuse throughout the chamber height before the sample entered the DEP zone. The required mixing length L_{mix} for a diffusible species

has been analyzed for the H-filter microfluidic configuration (Bruus, 2008) and, by analogy for the π configuration device, may be written as $L_{mix} \approx H(B_2 + B_1)/(WD)$, where Wis the width of the chamber in the direction perpendicular to the plane of FIG. 4 and D is the diffusion coefficient of the diffusing species.

[00108] The cell specimens contained between 20 x 10^6 and 40 x 10^6 PBMNs (containing trace levels of tumor cells) collected from whole blood by centrifugation over Histopaque 1077 (Cat 10771-lOOmL, Sigma-Aldrich, St Louis, USA) and were suspended in 1 mL RPMI medium that had been adjusted to a density of 1036 kg.m^{-3} by adding iodixanol (Optiprep™ Density Medium D1556, Sigma-Aldrich, St Louis) to a concentration of 11% (Gascoyne et al., 2009). The inventors used the eluate recipe developed in earlier DEP-FFF studies (Gascoyne et al., 2009; Shim et al., 2011) composed of an aqueous solution of 9.5% sucrose (S7903, Sigma-Aldich, St Louis, MO), 0.1 mg mL^{-1} dextrose (S73418-1, Fisher, Fair Lawn, NJ), 0.1% pluronic F68 (PI 300, Sigma-Aldich, St Louis, MO), 0.1% bovine serum albumin (A7906, Sigma-Aldich, St Louis, MO), 1 mM phosphate buffer pH 7.0, 0.1 mM CaAcetate, 0.5 mM MgAcetate and 100 units mL^{-1} catalase (C30, SigmaAldich, St Louis, MO). This mixture was adjusted to a conductivity of 30 mS m^{-1} with KCl. Pluronic F-68 provided mechanical stabilization of cell membranes under flow conditions (Wu et al., 1999), catalase protected cells from reactive oxygen intermediates (Wang et al., 1999), and bovine serum albumin inhibited cell adhesion to tubing and chamber surfaces. The specimen and eluate densities were both 1036 kg.m^{-3}, as a result of their iodixanol and sucrose contents, respectively, and this parity of densities was chosen to avoid the possibility of convection mixing when the flow streams were combined in the continuous flow chamber. After injection into the chamber, the Na^+, K^+ and Cl^- ions that dominated the high conductivity of the specimen diffused throughout the eluate. Sucrose counter diffused from the eluate to maintain the osmolarity of the cells at a physiological level so they were not osmolically stressed, which would have impacted cell size and membrane dielectric characteristics (Shim et al., 2011).

[00109] The inventors simulated the diffusion of ions and sucrose in the depicted embodiment of continuous-flow devices using the COMSOL Multiphysics software and the results for the conductivity distributions in key zones of the chamber are shown in FIG. 6. In the specimen injection zone (FIG. 6 (a)), a very large conductivity gradient exists where the specimen stream (1400 mS.m^{-1}) first joins the main eluate flow (30 mS.m^{-1}). At a distance 18 mm downstream from the specimen inlet, in the cell settling and ion diffusion zone (FIG. 6 (b)), the ion

conductivity is clearly spreading upwards through the chamber and by the time the flow reaches the DEP equilibration zone 40mm downstream from the specimen inlet and the tumor cell skimming zone 80mm downstream from the specimen inlet (FIG. 6 (c)), ion diffusion is essentially complete and the conductivity is homogeneous throughout the chamber height at about 61.5 mS. m^{-1}. Sucrose diffusion is also completed before the specimen reaches the DEP equilibration zone.

[00110] To verify these simulation results, the inventors measured the AC current drawn by the microelectrode array. This current depends much more sensitively on the conductivity immediately adjacent to the microelectrode that on the conductivity high in the chamber. The microelectrode current was the same under continuous specimen injection conditions as when the chamber was filled with a homogeneous medium having a conductivity of 61.5 $mS.m^{-1}$. This showed that the ion concentration at the microelectrode plane was the same in both cases, proving that diffusion was complete. Furthermore, the inventors conducted an experiment in which fluid leaving the ion diffusion region was skimmed from the chamber floor up to different heights by altering the withdrawal flow rate. The conductivity of the withdrawn fluid was 61.5 $mS.m^{-1}$ regardless of the skim height, also showing that diffusion was complete.

[001 111 This method of reducing the conductivity during flow through the ion diffusion zone insured that all cells were subjected to exposure to low conductivity conditions for the same, relatively short, period of time regardless of when they entered the chamber during the 40 minute specimen processing time. Passage of cells through this zone took about 180 seconds and this afforded sufficient time for cells to sediment to equilibrium positions close to the chamber floor so that they entered the DEP equilibration zone at identical heights and all were subjected to similar electric field conditions.

Example 5: DEP Stage

[001 12] The frequency and magnitude of the electrical signal applied to the microelectrodes in the DEP equilibration zone (FIG. 4 (d)) are chosen so that the tumor cells are pulled towards the chamber floor while the PBMNs are repelled high above it. This is possible because the direction of the DEP force is determined by whether the applied electric field frequency is greater or less than the characteristic "crossover frequency" f_{co} of a given cell type (Pethig, 2010; Jones and Kallio, 1979; Chan et al., 1997). In the accompanying article (Shim et al., 2013), it is shown that the crossover Irequencies of all cell types derived from solid tumors in the widely representative

NCI-60 panel of cancers lie far below those of all the subpopulations of peripheral blood cells. By choosing a signal frequency that is in between the crossover frequencies of tumor cells and PBMNs, it is possible to impose the desired differential DEP forces that drive cell separation and allow tumor cells to be isolated by skimming them from the chamber floor. At the equilibrium eluate target conductivity of 60 $mS.m^{-1}$ used for the continuous flow DEP-FFF tumor cell isolation experiments, the crossover frequencies f_{co} of all solid tumor cell types were well below 100 kHz while those of all the blood cell subpopulations were above 200 kHz. Therefore, a DEP signal frequency of 130 kHz was chosen for the inventors' tumor cell isolation experiments. (Note that the crossover frequency is proportional to the cell medium suspension conductivity, so that the f_{co} values measured by batch mode DEP-FFF at 30 $mS.m^{-1}$ in the accompanying article (Shim et al., 2013) are half the corresponding -^"values at the 60 $mS.m^{-1}$ target conductivity value used for continuous mode isolations herein).

[00113] The DEP force imposed on cells depends on the square of the applied voltage V, suggesting it might be advantageous to use a high DEP voltage to increase the height differential between tumor and PBMNs leaving the DEP equilibration zone (Shim et al., 2013). However, the electric field that gives rise to the DEP force also induces a transmembrane potential difference that can stress cells and cause them to become leaky towards ions and/or electroporated, which could alter their DEP properties and confound their separation characteristics. The magnitude of the induced transmembrane potential difference depends upon the electric field strength, the cell diameter and the applied electric field frequency (Wang et al., 1999). Because of their larger diameter and closer proximity to the microelectrodes during continuous DEP-FFF isolation, tumor cells are more prone than PBMNs to damage by a high voltage in the inventors' DEP-FFF cell isolation experiments. However, one could use an applied voltage of 4V p-p in the experiments to provide strong DEP forces without evidence of diminished tumor cell isolation efficiency. Catalase was included in the eluate buffer as in previous work (Gascoyne et al., 2009; Shim et al., 201 1) to protect the cells from a low concentration of reactive oxygen intermediates that may be produced by electrochemical processes at the microelectrodes (Wang et al., 1999).

[001 14] To confirm the differential height distributions of tumor cells and PBMNs under force balance conditions, the inventors ran a batch-mode DEP-FFF experiment using a chamber similar to those described in earlier work (Shim et al., 201 1; Vykoukal et al., 2008) and recorded the

time-dependent elution profile of a mixture of PBMNs from healthy donors and MDA-MB-231 human breast cancer cells for a DEP frequency that corresponded to the present continuous flow separation conditions (Shim et al., 2013). The cell elution profile was determined using a laser particle counter (PC2400D, ChemTrac Systems, Norcross, Georgia, USA) to count and size the cells as they left the batch-mode chamber. Because the cells were carried through the chamber at the velocities of the eluate at their equilibrium heights, the cell elution times could be mapped directly to the cell equilibrium height distribution in the chamber. The results are shown in FIG. 7 and it may be seen that the MDA-MB-23 1 cells traveled through the DEP-FFF chamber between 5 and 13 μηι above the chamber floor while the PBMNs were transported at between 14 and >40 μm height. Tumor cells may be isolated from PBMNs in continuous flow DEP-FFF by using a skim height that is between the tumor cell and PBMNs transport heights under the prevailing DEP field conditions.

[001 15] In FIG. 7, the batch mode DEP-FFF chamber had a microelectrode array area of 7500 mm^2 and the power output capacity of the signal generator limited the maximum DEP voltage to 2.8 V p-p. For continuous flow DEP-FFF, the chamber had a microelectrode array area only one third that size, which permitted the inventors to use the higher operating voltage of 4 V p-p. The DEP-FFF force balance equation (Shim et al., 201 3; Shim et al., 201 1; Gascoyne, 2012) showed that the levitation height for PBMNs under those conditions was > 27 μηι. Accordingly, the inventors chose a skimming height of h_s=26 μη by setting the withdrawal rate to $B_s k_{im}$=2Q μE.miη^{-1} for continuous flow DEP-FFF cell isolation experiments (as in FIG. 5(a)).

|001 16] In principle, fluid convection cells could form adjacent to energized DEP electrodes as the result of temperature gradients associated with Joule heating of the fluid. Convection cells on the periodic DEP microelectrode array would be expected to have the same spatial periodicity as the DEP force field and it might in certain aspects it is possible for convection and DEP fields to act in combination to alter the height at which cells are transported over the microelectrode array and thereby impact the cell isolation characteristics. This possibility was neglected in the analytical equations we used to describe DEP-FFF and the effect, if present, seems to be relatively small because one result of it would be to shift the apparent crossover frequency at which the direction of the DEP force on cells appears to reverse in DEP-FFF. In this case the crossover frequency deduced from DEP-FFF would differ from the true crossover frequency measured by conventional DEP methods. Even though the inventors did not observe such a

frequency shift, it is considered that this phenomenon is further characterized in which the modeling work is extended to include coupling of thermal, hydrodynamic and electrokinetic parameters in determining the fluid flow and cell transit behaviors. Characterization including such coupling potentiates further improvements to continuous mode DEP-FFF, in particular aspects.

Example 6: Exemplary Tests with Clinical Specimens

[001 17] Tests were run of continuous flow DEP-FFF isolation of MDA-MB-435, MDA-MB-231 and other cultured tumor cells spiked into PBMNs from healthy donors to compare with earlier batch-mode DEP-FFF experiments (Gascoyne et al., 2009). Measurements were conducted using a DEP-FFF chamber 160 mm long having a width of 25 mm, a height of 314 μm, and an inlet to outlet slot spacing of 90 mm. The chamber floor was lined by a microelectrode array based on the design detailed earlier (Vykoukal et al, 2008) in which parallel gold-on-copper microelectrodes of 50 μm width and spacing were patterned on a kaptan substrate. However, only the last 45 mm of the microelectrode array leading up to the outlet slot was energized. The DEP and flow conditions were as described in earlier sections, namely 2000 $\mu L.min^{-1}$ and 20 $\mu L.min^{-1}$ for eluate and injection/withdrawal rates, respectively, a DEP signal of 130 kHz at 4 V p-p, and a conductivity of the emerging eluate of about 64 $mS.m^{-1}$.

[001181 Cultured tumor cells were pre-labeled with fluorescent dye (CellTracker Green CMFDA, Life Technologies, Grand Island, New York) and spiked into PBMNs from 10 mL blood specimens at concentrations ranging from 50 to 600 per mL blood. Using the method the inventors developed earlier for characterizing batch-mode DEP-FFF separations (Huang et al., 1999), the output port of the DEP-FFF chamber was connected to a modified FACS instrument and the labeled tumor cells were gated and counted by their fluorescence (Ex 488 nm, Em 517 nm) as they emerged. These measurements confirmed that, independently of spiking density, tumor cell isolation efficiencies of 70- 80% were achieved. FIG. 8 shows scatter plots obtained for MDA-MB-435 cells at three different spiking concentrations. The average collection efficiency was found to be 75% and independent of spiking density.

[00119] Despite these encouraging results using spiked samples, it was considered that the dielectric properties of the blood of cancer patients might be modified by their disease and treatment regimen. For example, leukocytosis, thrombocytosis and increased acute phase protein levels in the blood are typically part of a chronic systemic inflammatory response to late stage

malignancy (Alexandrakis et al., 2002). These factors have the potential to lead to anomalous blood cell subpopulations that might contaminate CTCs during DEP-FFF isolation. Therefore, the inventors conducted a preliminary trial using a limited number of clinical specimens from late stage cancer patients to establish whether CTCs could be isolated from blood under realistic clinical conditions. Peripheral blood specimens were obtained as part of the Initiative for Molecular Profiling in Advanced Cancer Therapy (IMPACT) Trial at The University of Texas M.D. Anderson Cancer Center with informed patient consent and the approval of Institutional Biosafety Committee. Specimens of at least 7 mL volume were collected from patients in 10 mL BD purple cap (EDTA) vacutainers and processed within 3 hours. The PBMNs, putatively containing CTCs, were separated from the patient specimens over Histopaque 1077 and subjected to continuous DEP-FFF also using the experimental conditions described in the sections above. The isolate from the withdrawal slot was collected in a 1 mL syringe for each specimen over the approximately 40 minute processing time.

[00120] The FACS approach is generally not feasible for analyzing CTCs isolated from clinical specimens because a 10 mL blood specimen may contain as few as ten CTCs and very rarely more than hundreds and downstream immunostaining in cell suspension (as opposed to fluorescent dye preloading used to prepare cells before spiking experiments) followed by subsequent FACS analysis are too lossy to use to count such small cell populations accurately. Therefore, a Cytopro™ instrument (Wescor Model 7620, Logan, Utah) was used to mount the cells from this isolate onto two microscope slides. One slide was immunostained for cytokeratin using FITC-conjugated CK3-6H5 antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany) combined with nuclear staining by DAPI (D1306 Molecular Probes, Eugene, Oregon). An example is shown in FIG. 8, where putative CTCs show green fluorescence due to the presence of cytokeratin and both CTC and PBMN nuclei exhibit blue fluorescence.

[00121] The primary tumors of all patients enrolled in the IMPACT trial are subjected to screening for somatic mutations associated with their primary cancers. In order to verify the presence of CTCs in the isolates obtained with the present methods, the unstained slide was subjected to molecular analysis. DNA was extracted from the slide using PicoPure (cat 11815-00, Applied Biosystems) then further cleaned with a QIAamp DNA Micro Kit (cat 56304, Qiagen). The DNA was preamplified using the following primers: Forward: ATGACTGAATATAAACTTGTGGTAGTTGGA (SEQ ID NO:1), Reverse: GAATTAGCTGTATCGTCAAGGCACT (SEQ ID NO:2), Vic Reporter:

CTTGCCTACGCCACCAG (SEQ ID NO:3), FAM Reporter: CTTGCCTACGTCACCAG (SEQ ID NO:4). The Taqman Pre-Amp Master Mix (Cat 4391128) was employed according to the protocol specified by Fluidigm. The sample from the slide, together with a positive and a negative control were tested using a Fluidigm 48.48 Genotyping Array (cat BMK-M-48.48GT, Fluidigm) according to Fluidigm's protocol. For the colon cancer specimen shown in FIG. 9, the somatic mutation in the primary tumor was KRAS G13D. MDA-MB-231 was used as a positive control because it possesses this mutation. The specimen exhibited a positive result with a signal intensity that indicated approximately 10% of the cells on the slide had the KRAS G13D mutation, mirroring the proportion of cells that stained positively for cytokeratin in FIG. 9. This not only verified that sufficient CTCs were present on the slide to identify the cancer-causing gene but also shows that the continuous flow DEP-FFF method coupled with molecular analysis may be suitable for clinical screening purposes. A significant advantage of this DEP-FFF CTC isolation approach is that it is label-free and that the isolated CTCs are viable and unmodified. Therefore these cells are also suitable for a wide range of alternative molecular investigational techniques including gene expression analysis.

[00122] The above specification and examples provide a complete description of the structure and use of exemplary embodiments. Although certain embodiments have been described above with a certain degree of particularity, or with reference to one or more individual embodiments, those skilled in the art could make numerous alterations to the disclosed embodiments without departing from the scope of this invention. As such, the various illustrative embodiments of the present devices are not intended to be limited to the particular forms disclosed. Rather, they include all modifications and alternatives falling within the scope of the claims, and embodiments other than the one shown may include some or all of the features of the depicted embodiment. For example, components may be combined as a unitary structure, and/or components may be substituted with other components or types of components that permit the described function. Further, where appropriate, aspects of any of the examples described above may be combined with aspects of any of the other examples described to form further examples having comparable or different properties and addressing the same or different problems. Similarly, it will be understood that the benefits and advantages described above may relate to one embodiment or may relate to several embodiments.

[00123] The claims are not intended to include, and should not be interpreted to include, means-plus- or step-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase(s) "means for" or "step for," respectively.

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CLAIMS

1. An apparatus comprising:

a body defining a chamber having one or more inlets and one or more outlets, the one or more inlets configured to receive a first fluid including target matter and a second fluid, where the chamber comprises:

a first portion having a first end, and a second end between the one or more inlets and the one or more outlets; and

a second portion between the second end of the first portion and at least one of the one or more outlets; and

one or more electrodes configured to generate a non-uniform electric field in the second portion;

where the apparatus is configured such that if a first fluid including target matter and a second fluid are introduced into the chamber through the one or more inlets:

(i) the first fluid and the second fluid can flow substantially laminarly through the first portion to permit diffusion of solutes between the first fluid and the second fluid; and

(ii) the one or more electrodes can generate a dielectrophoretic force on the target matter in the second portion to extract the target matter from the first fluid.

2. The apparatus of claim 1, where the length of the first portion is large enough to permit the first fluid and the second fluid to substantially reach diffusion equilibrium in the first portion.

3. The apparatus of claim 1, where chamber includes a bottom surface and side surfaces, and the one or more electrodes are disposed on the bottom surface.

4. The apparatus of claim 3, where a width of the chamber is larger than a height of the chamber.

5. The apparatus of claim 1, where the apparatus is configured such that during the substantially laminar flow through the first portion, the cross-sectional area of the first fluid is smaller than the cross-sectional area of the second fluid.

6. The apparatus of claim 1, where the diffusion in the first portion adjusts the conductivity of the first fluid.

7. The apparatus of claim 1, where the diffusion in the first portion maintains or adjusts the osmolarity of the first fluid.

8. The apparatus of claim 1, where the first portion does not include electrodes that are configured to generate a non-uniform electric field.

9. The apparatus of claim 1, where the one or more inlets comprise a first inlet configured to receive the first fluid and a second inlet configured to receive the second fluid; and, optionally, where the first inlet is angled relative to the direction of flow in the first portion of the chamber.

10. The apparatus of claim 9, where the one or more outlets configured to permit the target matter to exit the chamber have a width less than one half the chamber height.

11 The apparatus of claim 1, where the one or more outlets comprise a first outlet configured to allow undesired matter to exit the chamber and a second outlet configured to allow the remainder of the first and second fluids containing the target matter to exit the chamber; and, optionally, where the first outlet is angled relative to the direction of flow in the second portion of the chamber.

12. The apparatus of claim 1, where the one or more outlets comprise a first outlet configured to permit the target matter to exit the chamber and a second outlet configured to permit the remainder of the first and second fluids to exit the chamber; and, optionally, where the first outlet is angled relative lo the direction of flow in the second portion of the chamber.

13. The apparatus of claim 1, further comprising:

a first fluid including a target matter;

a second fluid comprising one or more osmolytes configured to facilitate diffusion between the first fluid and the second fluid to reduce the conductivity of the first fluid.

14. The apparatus of claim 1, further comprising:

a first fluid including target matter;

a second fluid comprising one or more osmolyles configured to facilitate diffusion between the first fluid and the second fluid to adjust or maintain the osmolarity of the first fluid.

15. The apparatus of claim 1, where the chamber is configured to direct the first and second fluids from the one or more inlets to the one or more outlets according to a predetermined velocity profile.

16. The apparatus of claim 1, further comprising a signal generator coupled to the one or more electrodes, the signal generator configured to generate an electric signal with a frequency and voltage for the non-uniform electric field.

17. The apparatus of claim 16, further comprising:

a conductivity sensor configured to measure a conductivity of a fluid in the chamber; and

a controller configured to calculate one or more target properties of the non-uniform electric field based on at least one of measured conductivity of the first fluid and a property of the target matter, the controller further configured to adjust the electric signal generated by the signal generator to cause the non-uniform electric field to substantially include the one or more target properties.

18. The apparatus of claim 17, where the conductivity sensor is configured to measure the conductivity of fluid in contact with the one or more electrodes.

19. The apparatus of claim 17, where adjusting includes changing at least one of the frequency and the voltage of the electric signal.

20. The apparatus of claim 16, further comprising:

a current sensor configured to measure a current of the electric signal; and

a controller coupled to the current sensor and configured to compare the measured current to a target current, and the controller further configured to adjust the electric signal based on the comparison.

21. The apparatus of claim 20, where adjusting includes changing at least one of the frequency and the voltage of the electric signal.

22. A method for discriminating matter, the method comprising:

introducing a first fluid including target matter and a second fluid into a chamber of an apparatus, the chamber having one or more inlets and one or more outlets, the chamber comprising:

a first portion having a first end, and a second end between the one or more inlets and the one or more outlets; and

a second portion between the second end of the first portion and at least one of the one or more outlets; and

where the apparatus comprises one or more electrodes configured to generate a non-uniform electric field in the second portion of the chamber;

causing the first and second fluids to flow substantially laminarly through the first portion of the chamber such that diffusion between the first fluid and the second fluid adjusts the conductivity of the first fluid;

applying an electric signal to the one or more electrodes to generate a dielectrophoretic force on the target matter to extract the target matter from the first fluid.

23. The method of claim 22, further comprising controlling the flow rate of the first fluid and the second fluid such that the first fluid and the second fluid flow through the first and second portions of the chamber according to a target spatial profile.

24. The method of claim 23, where the target spatial profile and fluid flow rate is such that the first fluid and the second fluid substantially reach diffusion equilibrium in the first portion of the chamber.

25. The method of claim 22, where the second fluid comprises one or more osmolytes configured to facilitate the diffusion between the first fluid and the second fluid to reduce the conductivity of the first fluid.

26. The method of claim 22, where the second fluid comprises one or more osmolytes configured to facilitate the diffusion between the first fluid and the second fluid to maintain or adjust the osmolarity of the first fluid.

27. The method of claim 22, further comprising:

measuring conductivity of the first fluid;

calculating one or more target properties of the non-uniform electric field based on at least one of measured conductivity of the first fluid and a property of the target matter; and

adjusting the electric signal to include the one or more target properties.

28. The method of claim 27, where adjusting includes changing at least one of the frequency and the voltage of the electric signal.

29. The apparatus of claim 27, further comprising:

measuring a current of the electric signal;

comparing the measured current to a desired current; and

adjusting the electric signal based on the comparison.

30. The method of claim 29, where adjusting comprises changing at least one of the frequency and the voltage of the electric signal.

31. The method of claim 22, further comprising:

adding one or more osmolarity-altering components to the first fluid such that the osmolarity of the first fluid bears a desired relationship to the osmolarity of the second fluid.

32. The method of claim 22, further comprising:

adding one or more osmolarity-altering components to the second fluid such that the osmolarity oflhe second fluid bears a desired relationship to the osmolarity of the first fluid.

33. The method of claim 22, further comprising:

adding one or more density-altering components to the first fluid such that the density of the first fluid bears a desired relationship to the density of the second fluid.

34. The method of claim 33, where the desired relationship substantially prevents convection between the first and second fluids.

35 . The method of claim 22, further comprising:

adding one or more density-altering components to the second fluid such that the density of the second fluid bears a desired relationship to the density of the first fluid.

36. The method of claim 35, wherein the desired relationship substantially prevents convection between the first and second fluids.

37. The method of claim 22, further comprising:

adding one or more viscosity-altering components to the first fluid such that the viscosity of the first fluid bears a desired relationship to the viscosity of the second fluid.

38. The method of claim 22, further comprising:

adding one or more viscosity -altering components to the second fluid such that the viscosity of the second fluid bears a desired relationship to the viscosity of the first fluid.

39. The method of claim 22, further comprising:

outputting the target matter through a first outlet of the chamber and outputting the remainder of the first and second fluids through a second outlet of the chamber; and, optionally, where:

the first fluid is introduced through a first inlet that is angled relative to the first portion of the chamber; and/or

the first outlet is angled relative to the direction of flow in the second portion of the chamber.

40. The method of claim 22, wherein the target matter comprises circulating tumor cells (CTCs).

4 1. The method of claim 22, wherein the target matter is derived from blood.

42. The method of claim 40, wherein the target matter is obtained from an individual at risk for having cancer, known to have cancer, or suspected of having cancer.

43. ´The method of claim 42, further comprising the step of obtaining the target matter from the individual.

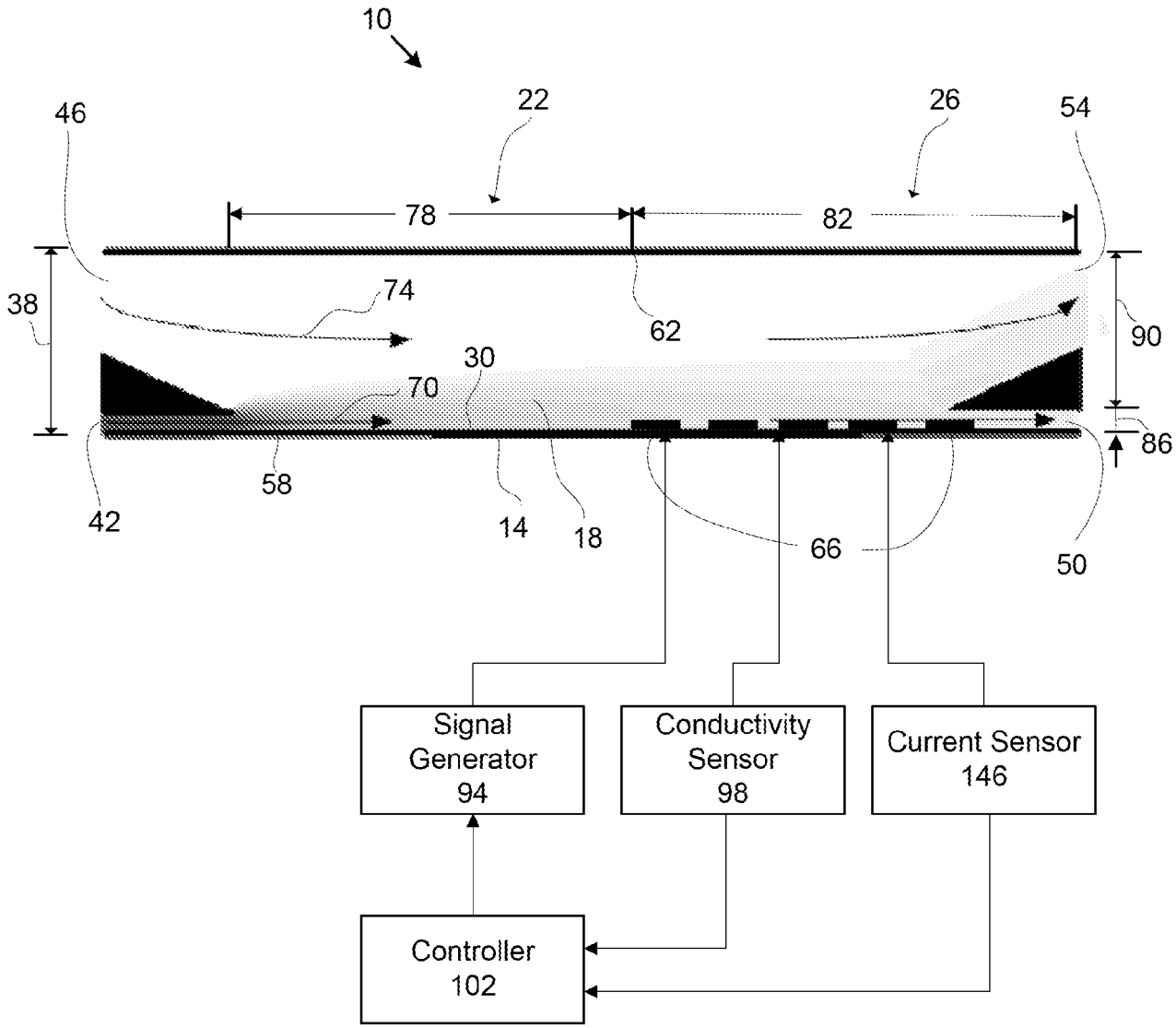
44. The method of claim 43, wherein the target matter comprises CTCs and the tissue of origin for the CTCs of the cancer is identified.

45. The method of claim 22, further comprising the step of obtaining the target matter from an individual.

46. The method of claim 22, lurther comprising the step of providing cancer treatment to the individual specific for the cancer.

47. The method of claim 42, further comprising the step of analyzing the CTCs.

48. The method of claim 47, wherein the analyzing comprises immunohistochemistry, gene expression analysis, histology, karyotyping, or a combination thereof.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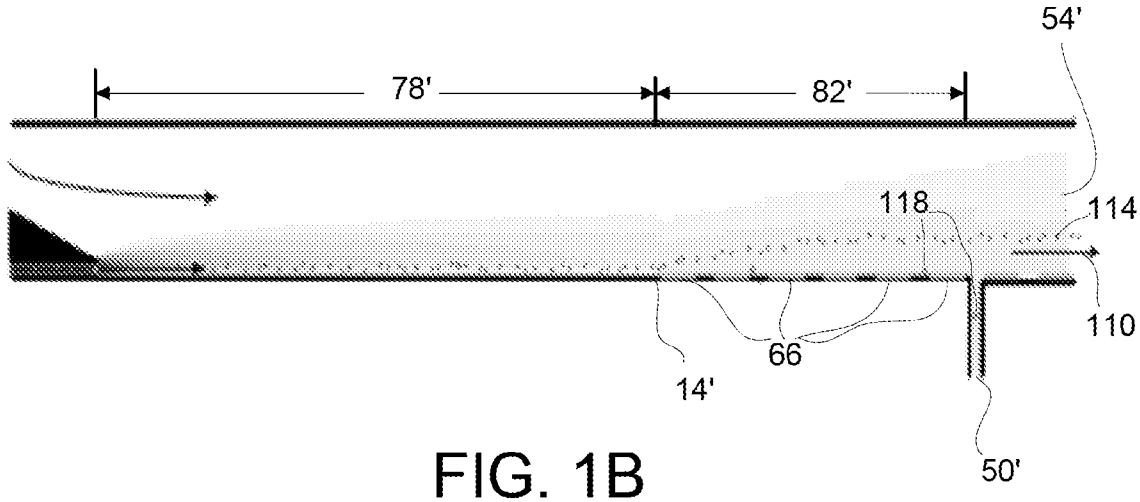
Signal Generator 94
Conductivity Sensor 98
Current Sensor 146
Controller 102

FIG. 1A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82'
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114
110
66
14'
50'

FIG. 1B

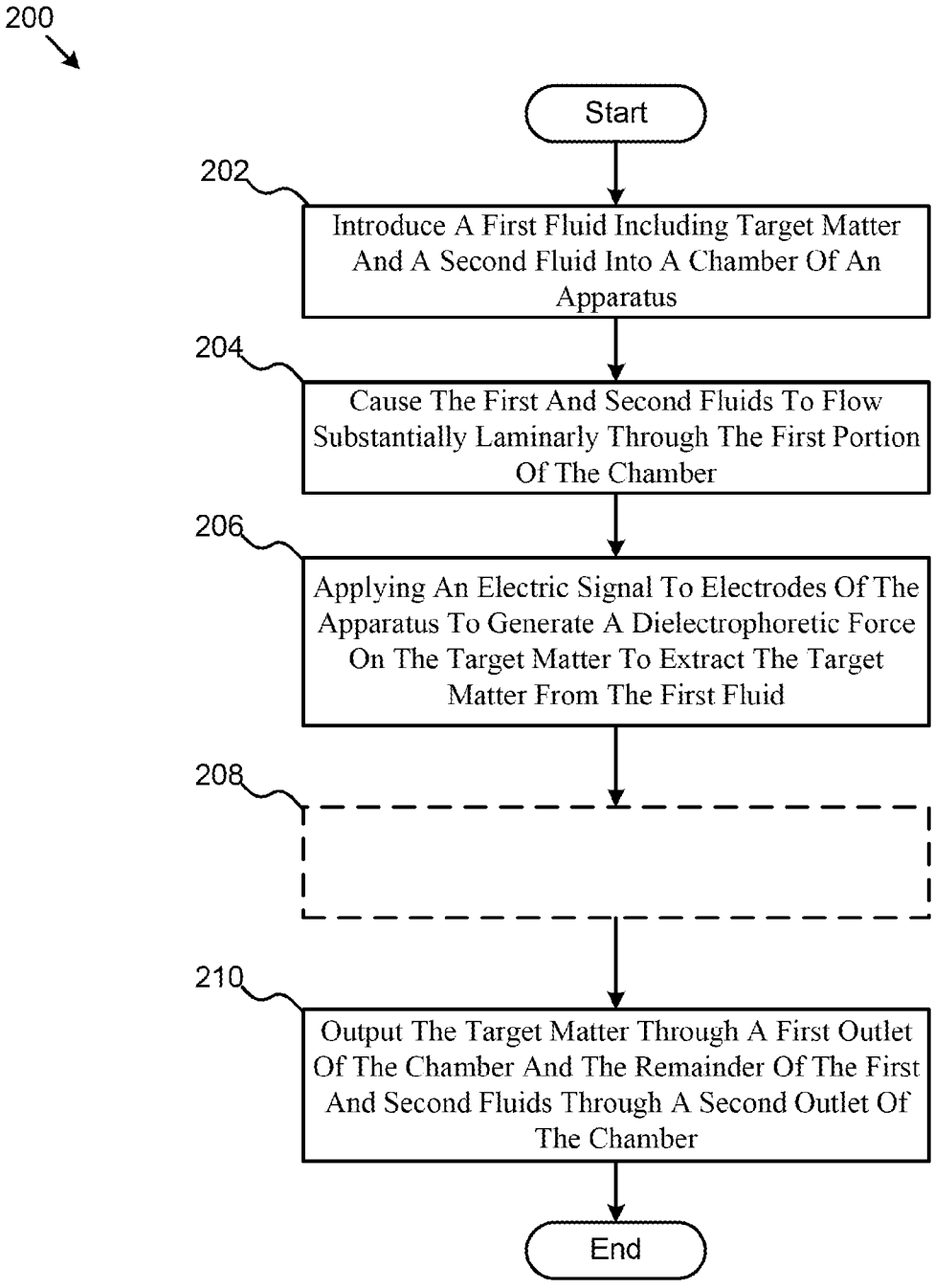
200
Start
202
Introduce A First Fluid Including Target Matter And A Second Fluid Into A Chamber Of An Apparatus
204
Cause The First And Second Fluids To Flow Substantially Laminarly Through The First Portion Of The Chamber
206
Applying An Electric Signal To Electrodes Of The Apparatus To Generate A Dielectrophoretic Force On The Target Matter To Extract The Target Matter From The First Fluid
208
210
Output The Target Matter Through A First Outlet Of The Chamber And The Remainder Of The First And Second Fluids Through A Second Outlet Of The Chamber
End

FIG. 2

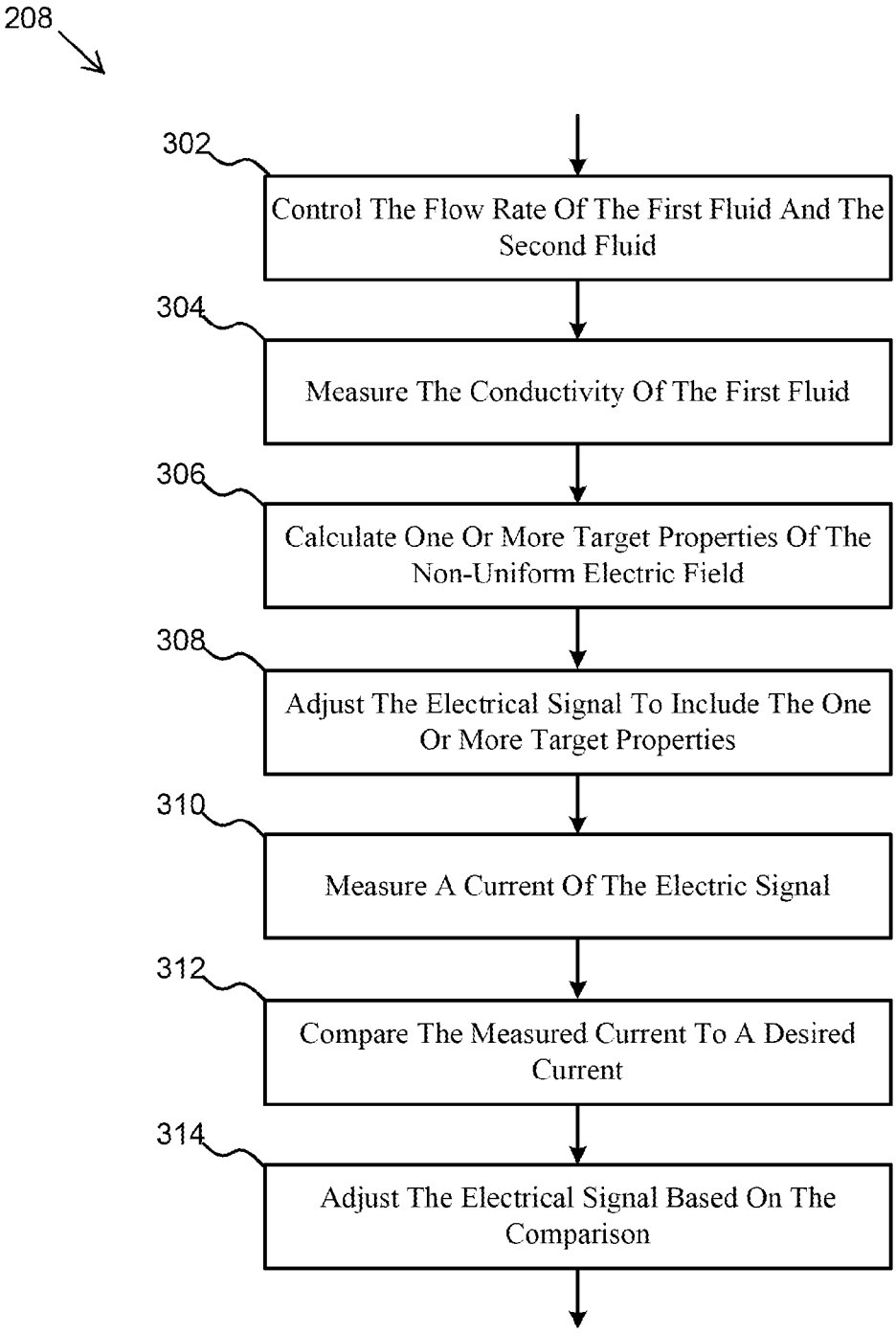
208
302
Control The Flow Rate Of The First Fluid And The Second Fluid
304
Measure The Conductivity Of The First Fluid
306
Calculate One Or More Target Properties Of The Non-Uniform Electric Field
308
Adjust The Electrical Signal To Include The One Or More Target Properties
310
Measure A Current Of The Electric Signal
312
Compare The Measured Current To A Desired Current
314
Adjust The Electrical Signal Based On The Comparison

FIG. 3A

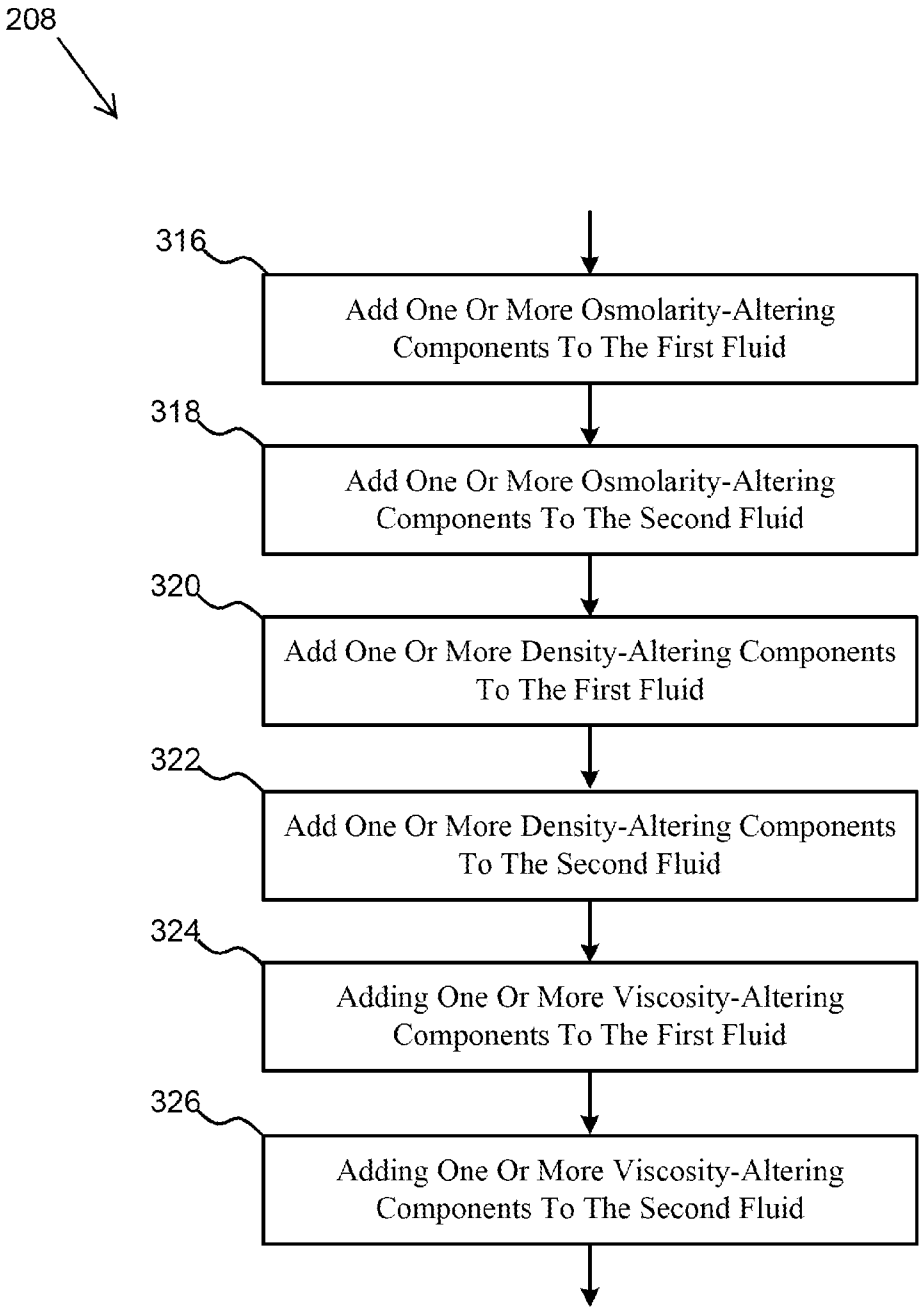
208
316
Add One Or More Osmolarity-Altering Components To The First Fluid
318
Add One Or More Osmolarity-Altering Components To The Second Fluid
320
Add One Or More Density-Altering Components To The First Fluid
322
Add One Or More Density-Altering Components To The Second Fluid
324
Adding One Or More Viscosity-Altering Components To The First Fluid
326
Adding One Or More Viscosity-Altering Components To The Second Fluid

FIG. 3B

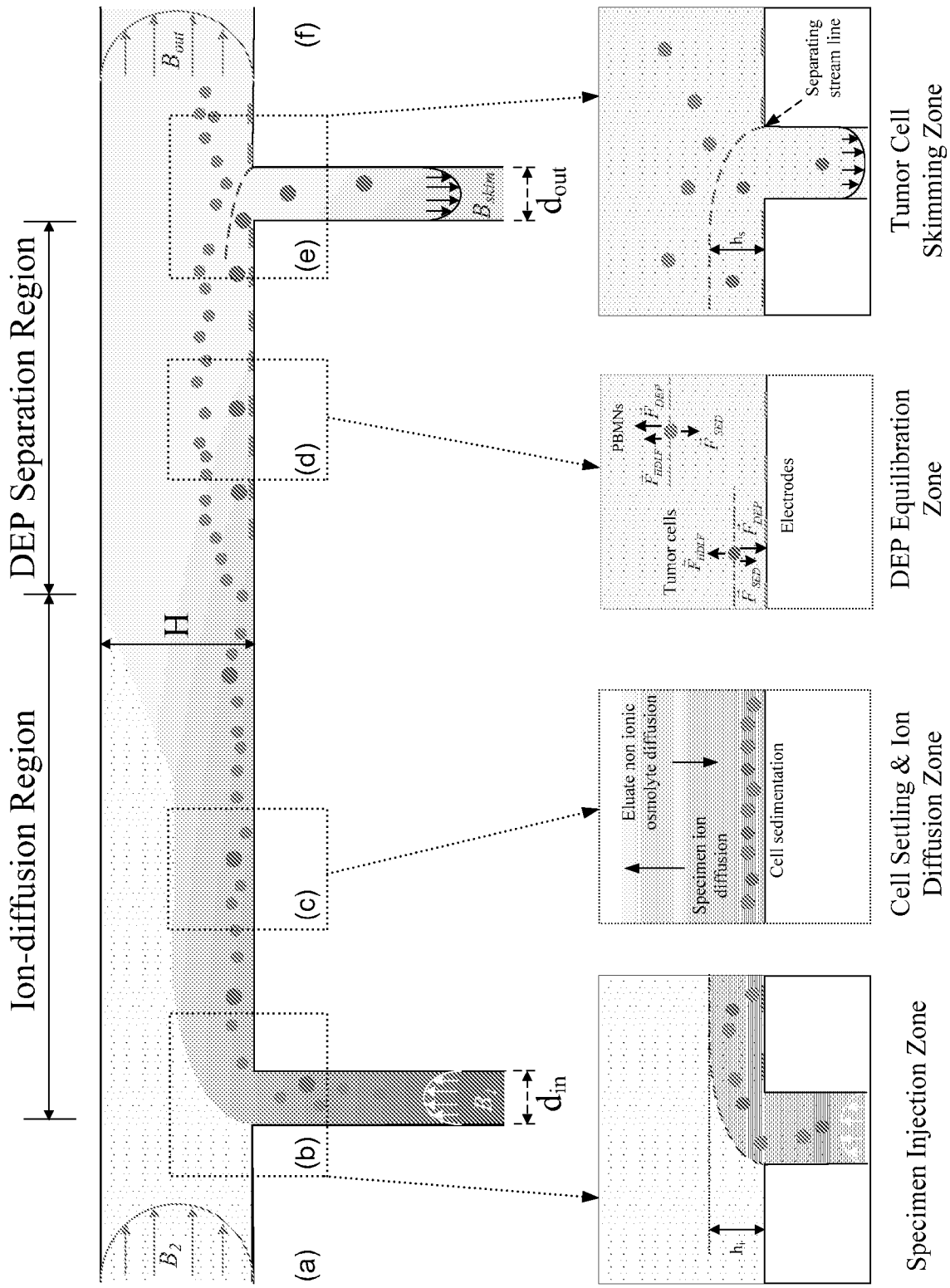
Ion-diffusion Region
DEP Separation Region
H
(a)
(b)
(c)
(d)
(e)
(f)
B_2
B_{out}
B_{skim}
d_{in}
d_{out}
h_i
h_s
Eluate non ionic osmolyte diffusion
Specimen ion diffusion
Cell sedimentation
PBMNs
Tumor cells
Electrodes
Separating stream line
Specimen Injection Zone
Cell Settling & Ion Diffusion Zone
DEP Equilibration Zone
Tumor Cell Skimming Zone

FIG. 4

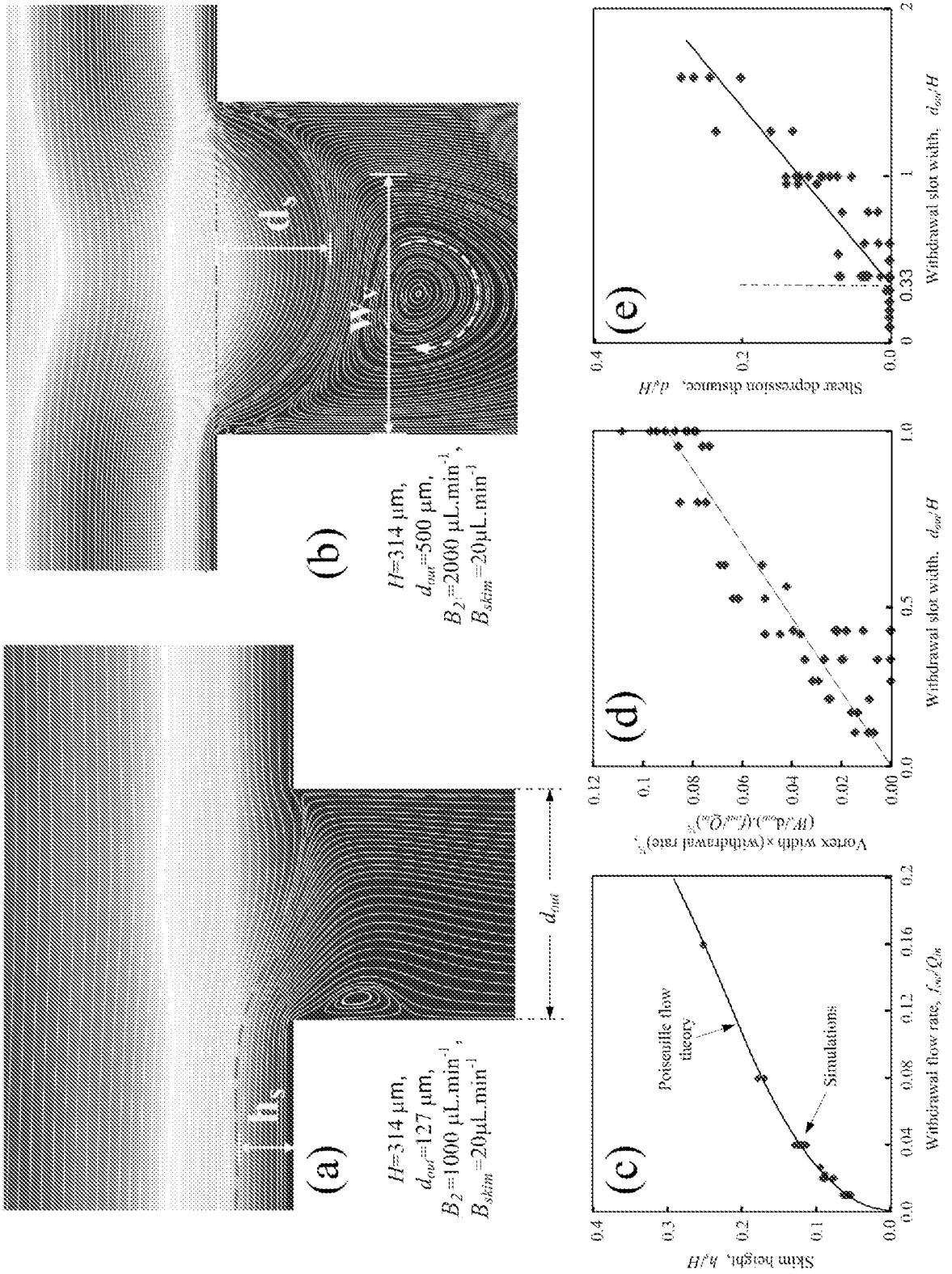
(a)
H=314 μm,
d_{out}=127 μm,
B_2=1000 μL.min^{-1},
B_{skim} =20μL.min^{-1}
d_{out}
(b)
H=314 μm,
d_{out}=500 μm,
B_2=2000 μL.min^{-1},
B_{skim} =20μL.min^{-1}
d_s
W_v
(c)
Skim height, h/H
Withdrawal flow rate, f_{out}/Q_{in}
Poiseuille flow theory
Simulations
(d)
Vortex width x(withdrawal rate)$^{1/2}$, $(W/d_{out})\sqrt{(f_{out}/Q_{in})}$
Withdrawal slot width, d_{out}/H
(e)
Shear depression distance, d/H
Withdrawal slot width, d_{out}/H

FIG. 5

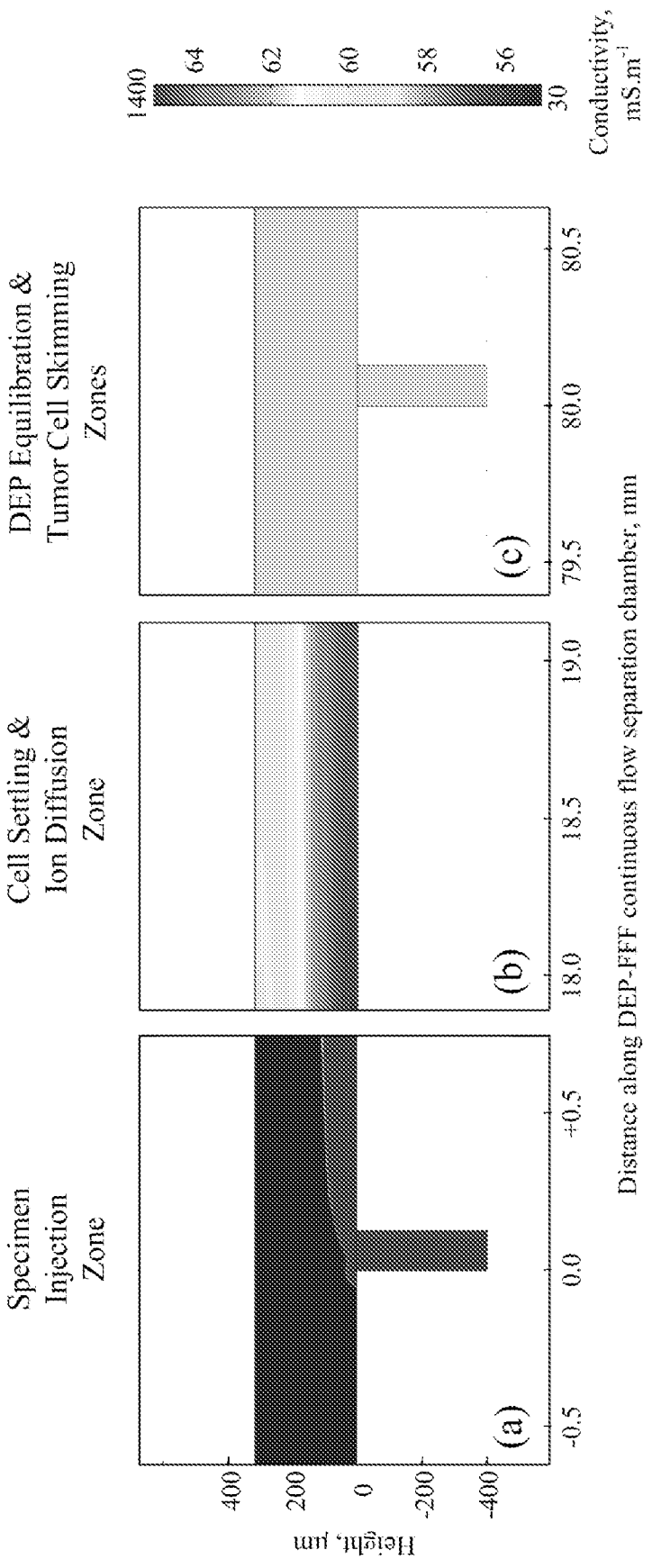
Specimen Injection Zone
Cell Settling & Ion Diffusion Zone
DEP Equilibration & Tumor Cell Skimming Zones
Height, μm
400
200
0
-200
-400
(a)
(b)
(c)
-0.5
0.0
+0.5
18.0
18.5
19.0
79.5
80.0
80.5
Distance along DEP-FFF continuous flow separation chamber, mm
1400
64
62
60
58
56
30
Conductivity, mS.m-1

FIG. 6

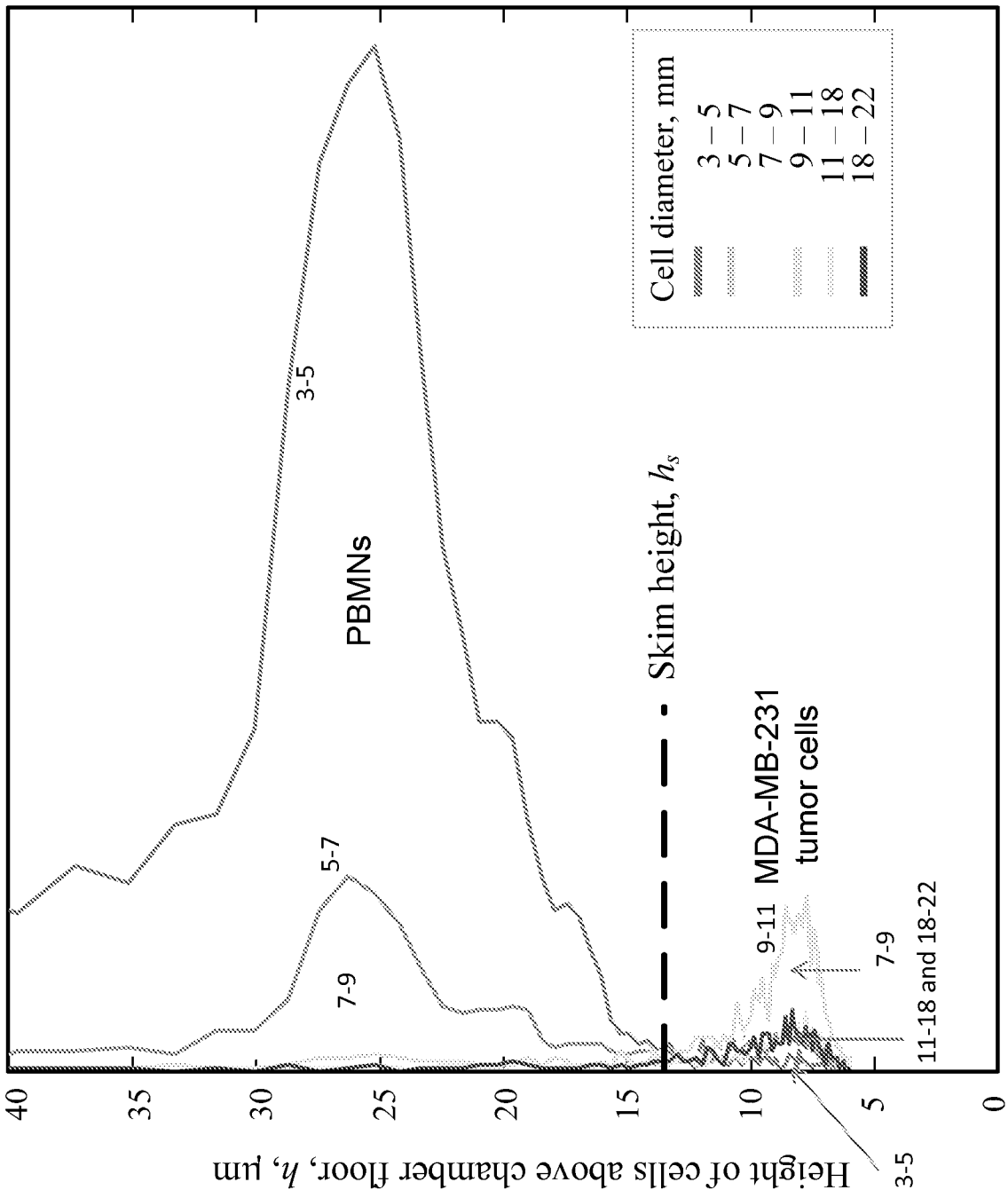
Height of cells above chamber floor, h, μm
Proportion of cells at given height
Skim height, h_s
PBMNs
MDA-MB-231 tumor cells
Cell diameter, mm
3 – 5
5 – 7
7 – 9
9 – 11
11 – 18
18 – 22
3-5
5-7
7-9
9-11
11-18 and 18-22
0
5
10
15
20
25
30
35
40

FIG. 7

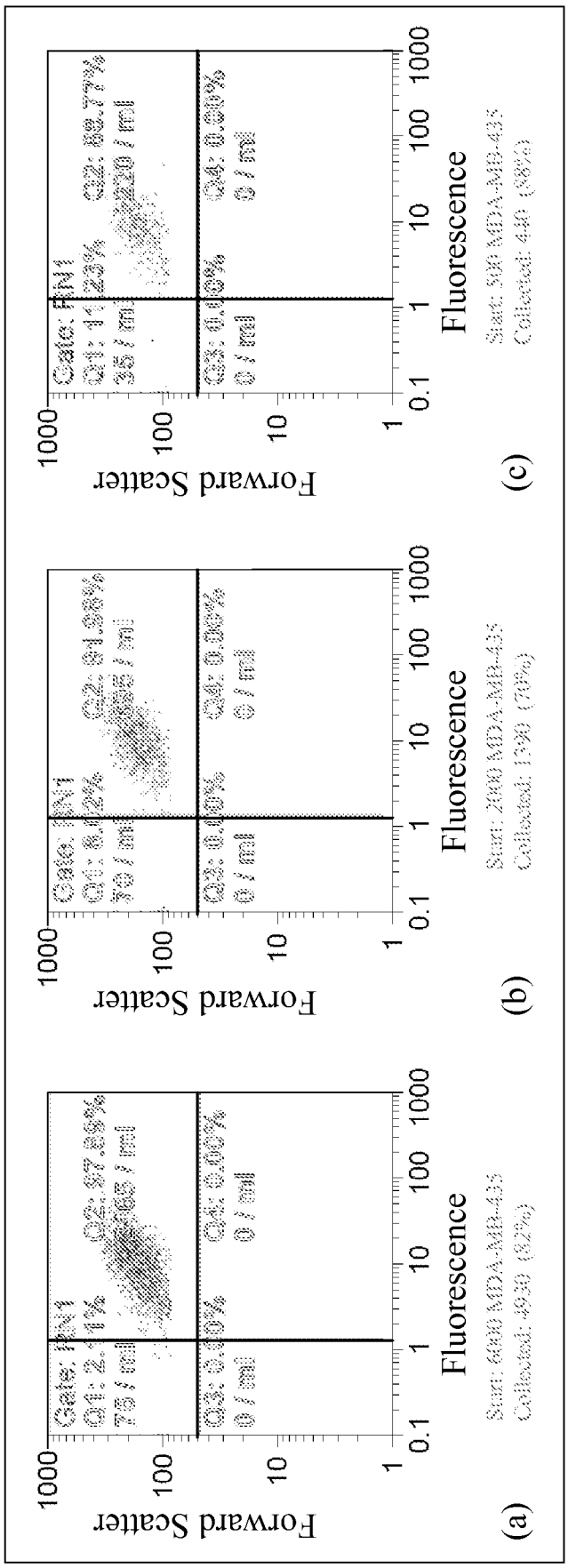
Forward Scatter
Fluorescence
1000
100
10
1
0.1
1
10
100
1000
Gate: RN1
Q1: 2.1%
Q2: 97.86%
Q3: 0.00%
Q4: 0.00%
0 / ml
Start: 6000 MDA-MB-435
Collected: 4930 (82%)
(a)
Gate: RN1
Q1: 8.2%
Q2: 91.38%
Q3: 0.00%
Q4: 0.00%
Start: 2000 MDA-MB-435
Collected: 1390 (70%)
(b)
Gate: RN1
Q1: 11.23%
Q2: 88.77%
Q3: 0.00%
Q4: 0.00%
0 / ml
Start: 500 MDA-MB-435
Collected: 440 (88%)
(c)

FIG. 8

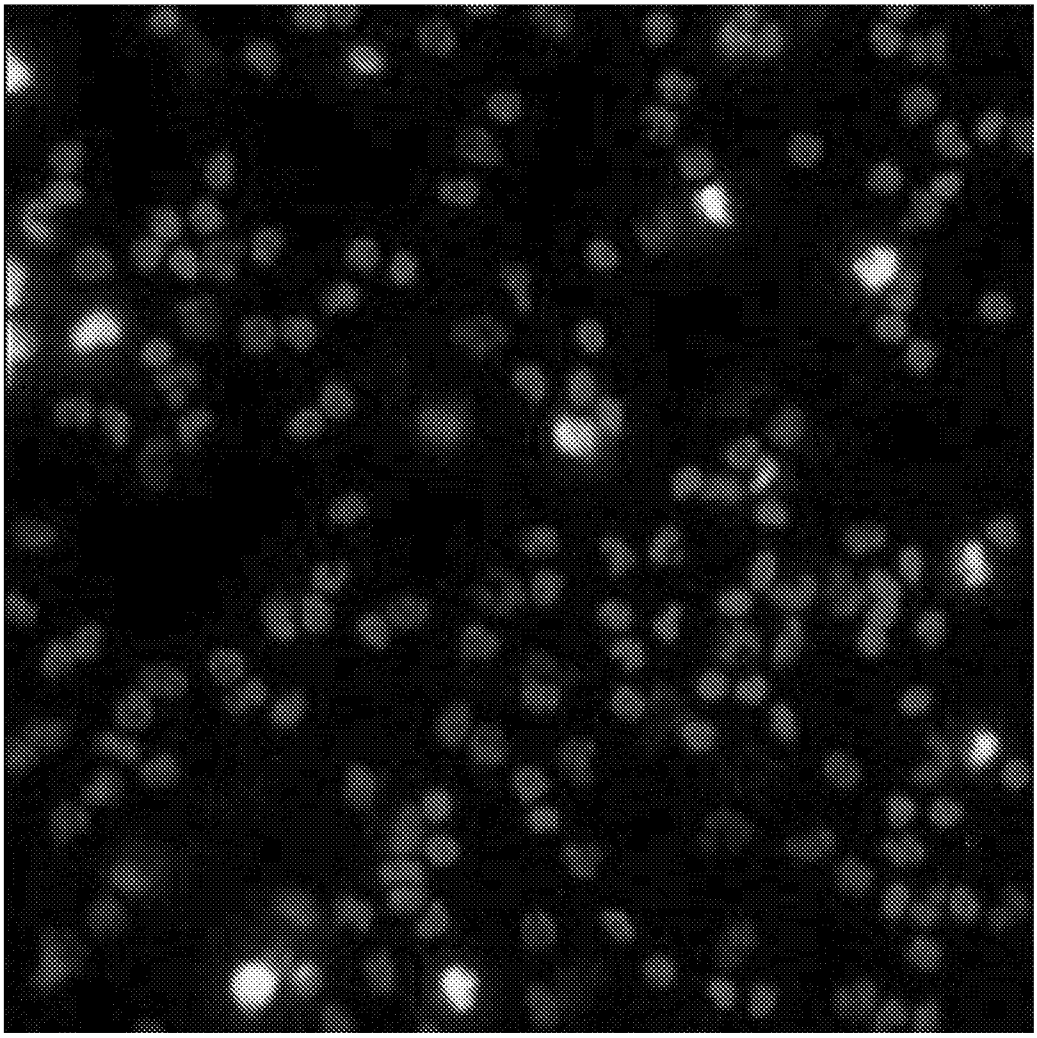

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/070333

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of iteml.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of :

a. **a sequence listing filed or furnished**
- ☐ on paper
- ☒ in electronic form

b. time of filing or furnishing
- ☐ contained in the international application as filed
- ☒ filed together with the international application in electronic form
- ☐ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/070333

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 46
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 46 pertains to methods for treatment of a human, and thus relates to a subject matter which this International Searching Authority is not required to search under Article 17(2)(a)(i) and Rule 39.1(iv) PCT.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. Ill Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/070333

A. CLASSIFICATION OF SUBJECT MATTER

GOIN 27/447(2006.01)i, GOIN 33/49(2006.01)1, C12Q 1/02(2006.01)1, GOIN 35/08(2006.01)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)

GOIN 27/447; BOID 13/02; C12M 1/00; BOID 37/00; B01D 57/00; G01N 27/72; G01N 27/26; G01N 27/27; C12M 1/42

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: dielectrophoretic, separation, osmolytes, diffusion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4349429 A (RHODES et al.) 14 September 1982 See column 1, lines 21-23, column 2, line 67, column 3, lines 1-10, 21-25, and column 6, lines 38-46.	1-45,47-48
A	US 2004-0011651 Al (BECKER et al.) 22 January 2004 See paragraphs [0013], [0020], [0050].	1-45,47-48
A	US 7033473 B2 (GASC0YNE et al.) 25 April 2006 See abstract, column 3, line 63 - column 4, line 6, and figure 2A.	1-45,47-48
A	KR 10-2009-0083655 A (INJE UNIVERSITY INDUSTRY-ACADEMIC COOPERATION FOUNDATION) 04 August 2009 See claim 1 and figure 10.	1-45,47-48
A	US 7318902 B2 (OAKEY et al.) 15 January 2008 See column 1, lines 39-67 and figures 7B, 7C.	1-45,47-48

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

* Special categories of cited documents:

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

"E" earlier 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 citation or other special reason (as specified)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 April 2013 (15.04.2013)	**16 April 2013 (16.04.2013)**

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office 189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-70 1, Republic of Korea Facsimile No. 82-42-472-7140	PARK, Tin Ho Telephone No. 0424818398

Form PCT/ISA/210 (second sheet) **(July** 2009)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2012/070333

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 2000-68002 B2	14. 10,2004
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