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(54) **Title:** SAMPLE PREPARATION ON A DROPLET ACTUATOR

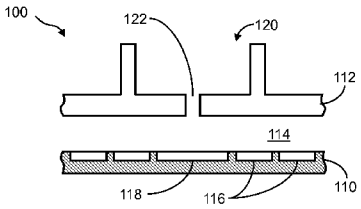


Figure 1A

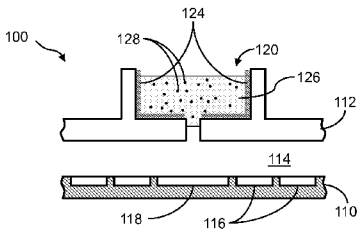


Figure 1C

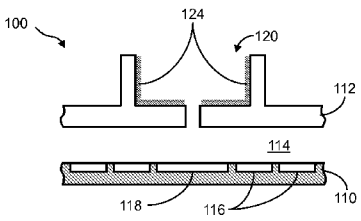


Figure 1B

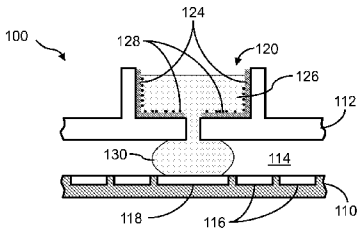


Figure 1D

**(57) Abstract:** The present invention relates to methods of sample preparation on a droplet actuator. The invention further relates to methods of plasma sample preparation on a droplet actuator by immunocapture. The invention also relates to methods of preparing plasma that contains HIV from whole blood without the need for filtration or centrifugation. Additionally, the invention relates to methods of sample preparation that use hydrophobic chromatography for purifying or removing unwanted materials from the sample. The invention further relates to a droplet actuator and methods of loading solutions of large volume into a droplet actuator under controlled conditions. The invention also relates to methods of processing cells on a droplet actuator.

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# Sample Preparation on a Droplet Actuator

## 1 Related Applications

In addition to the patent applications cited herein, each of which is incorporated herein by reference, this patent application is related to and claims priority to U.S. Provisional Patent Application No. 61/576,384, filed on December 16, 201 1, entitled "Sample Preparation on a Droplet Actuator," the entire disclosure of which is incorporated herein by reference.

## 2 Field of the Invention

The present invention generally relates to sample preparation on droplet actuators. In particular, the present invention is directed to simplified apparatuses and methods of sample preparation on droplet actuators.

## 3 Background of the Invention

A droplet actuator typically includes one or more substrates configured to form a surface or gap for conducting droplet operations. The one or more substrates establish a droplet operations surface or gap for conducting droplet operations and may also include electrodes arranged to conduct the droplet operations. The droplet operations substrate or the gap between the substrates may be coated or filled with a filler fluid that is immiscible with the liquid that forms the droplets. Certain sample preparation processes that are associated with droplet actuators may be somewhat complex. There is a need for simplified approaches to sample preparation with respect to droplet actuators.

## 4 Brief Description of the Invention

The present invention is directed to simplified apparatuses and methods of sample preparation on droplet actuators.

In one embodiment, a droplet actuator is provided. The droplet actuator includess: a bottom substrate and a top substrate that are separated by a gap; a fluid reservoir integrated into the top substrate; and an opening in the top substrate which is substantially aligned with a reservoir electrode on the bottom substrate and provides a fluid path from the fluid reservoir into the gap; where the fluid reservoir comprises a

chemically modified surface. In a particular embodiment, the chemically modified surface of the droplet actuator is modified by chemical conjugation of antibodies, particularly where the antibodies are anti-erythrocytes, leukocytes, or platelets antibodies. In another particular embodiment, the bottom substrate of the droplet actuator is a printed circuit board. In another particular embodiment, the top substrate of the droplet actuator is formed of at least one of glass, injection-molded plastic, silicon, and indium tin oxide. In a further particular embodiment, the bottom substrate of the droplet actuator comprises a configuration of droplet operations electrodes.

In another embodiment, a method of plasma sample preparation on a droplet actuator by immunocapture is provided. The method, includes: loading a quantity of a blood sample into a fluid reservoir, where the fluid reservoir comprises a chemically modified surface; incubating the blood sample in the fluid reservoir, where blood cells in the blood sample bind to the chemically modified surface to produce a plasma sample; and dispensing the plasma sample from the fluid reservoir into a droplet operations gap of the droplet actuator. In a particular embodiment, the chemically modified surface is modified by chemical conjugation of antibodies, particularly where the antibodies are anti-erythrocytes, leukocytes, or platelets antibodies. In another particular embodiment, the plasma sample is dispensed atop a reservoir electrode into the droplet operations gap of the droplet actuator. In a further particular embodiment, the plasma sample is substantially cell free. In another particular embodiment, the method comprises processing the plasma sample in the droplet actuator via electrowetting droplet operations.

In another embodiment, a method of preparing a plasma sample from a whole blood sample on a droplet actuator is provided. The method includes: loading a quantity of the whole blood sample into a fluid reservoir; adding a lectin to the whole blood sample, causing cells in the whole blood sample to agglutinate and fall out of solution to produce a plasma sample; and dispensing the plasma sample from the fluid reservoir onto the droplet actuator. In a particular embodiment, the whole blood sample and plasma sample contain human immunodeficiency virus (HIV). In another particular embodiment, the plasma sample is dispensed from the fluid reservoir into a droplet operations gap of the droplet actuator. In a further particular embodiment, the

plasma sample is dispensed atop a reservoir electrode in the droplet operations gap of the droplet actuator. In another particular embodiment, the plasma sample is combined with a lysis reagent to produce an HIV RNA prior to the step of dispensing the plasma sample from the fluid reservoir into a droplet operations gap of the droplet actuator. In a further particular embodiment, the lectin is lyophilized and stored on the droplet actuator.

In another embodiment, a method of preparing a substantially purified sample on a droplet actuator using hydrophobic chromatography is provided. The method includes: loading a quantity of the sample into a fluid reservoir, where the fluid reservoir contains magnetically responsive beads; washing and eluting the magnetically responsive beads to produce a substantially purified sample; and dispensing the substantially purified sample from the fluid reservoir. In a particular embodiment, the magnetically responsive beads serve as a matrix for hydrophobic chromatography. In another particular embodiment, the magnetically responsive beads are washed and eluted with a gradient of increasing hydrophobicity to produce the substantially purified sample. In a further particular embodiment, the magnetically responsive beads are modified with C-4, C-8, or C-18 alkyl chains. In another particular embodiment, the gradient of increasing hydrophobicity is in the range of about 0% to about 70% acetonitrile. In another particular embodiment, the substantially purified sample comprises a purified protein or small molecule analyte. In a further particular embodiment, the substantially purified sample is dispensed from the fluid reservoir into a droplet operations gap of a droplet actuator, particularly where the substantially purified sample is dispensed atop a reservoir electrode in the droplet operations gap of the droplet actuator. In another particular embodiment, the hydrophobic material is removed from the sample.

In another embodiment, a method of preparing a substantially purified sample on a droplet actuator using ion exchange chromatography is provided. The method includes: loading a quantity of the sample into a fluid reservoir, where the fluid reservoir contains magnetically responsive ion exchange beads; washing and eluting the magnetically responsive beads to produce a substantially purified sample; and dispensing the substantially purified sample from the fluid reservoir. In a particular embodiment, the magnetically responsive ion exchange beads comprise cations and

anions. In another particular embodiment, the magnetically responsive ion exchange beads serve as a matrix for ion exchange chromatography. In a further particular embodiment, the magnetically responsive ion exchange beads are washed and eluted with a gradient to produce the substantially purified sample, particularly where the the gradient comprises increasing ionic strength, or comprises increasing or decreasing pH. In another particular embodiment, the gradient is a step gradient or a linear gradient. In a further particular embodiment, the substantially purified sample comprises a substantially purified protein or small molecule analyte. In another particular embodiment, the substantially purified sample is dispensed from the fluid reservoir into a droplet operations gap of a droplet actuator, particularly where the substantially purified sample is dispensed atop a reservoir electrode in the droplet operations gap of the droplet actuator.

In another embodiment, a droplet actuator is provided. The droplet actuator includes: a bottom substrate and a top substrate that are separated by a gap; an opening in the top substrate which is substantially aligned with a reservoir electrode on the bottom substrate and provides a fluid path from the fluid reservoir into the gap; and a fluid reservoir integrated into the top substrate; where the fluid reservoir comprises a hollow tube extending through a portion of the fluid reservoir, where one end of the hollow tube is located outside of the fluid reservoir and the opposite end of the hollow tube is located inside of the fluid reservoir, and where the end of the hollow tube that is located inside of the fluid reservoir is held at a height with respect to a plane of the opening in the top substrate. In a particular embodiment, the fluid reservoir is enclosed. In another particular embdoment, the end of the hollow tube that is located inside of the fluid reservoir is substantially aligned with the opening. In a further particular embodiment, the fluid reservoir maintains a substantially constant and low head pressure at the opening in the top substrate. In another particular embodiment, the bottom substrate comprises a configuration of droplet operations electrodes. In a further particular embodiment, the fluid reservoir is filled by threading a smaller sample input tube through the hollow tube.

In another embodiment, a method of processing cells on a droplet actuator is provided. The method includes: loading a quantity of a sample into a fluid reservoir, wherein the sample includes cells; adding magnetically responsive beads to the

sample, wherein the magnetically responsive beads are coated with a biopolymer; and growing the cells on the coated magnetically responsive beads. In a particular embdoment, the biopolymer is a cell attachment promoter, particulary where the cell attachment promoter is collagen or polylysine. In another particular embodiment, processing the cells comprises at least one of growing and testing of cells. In a further particular embodiment, the cells comprise mammalian cells.

## 5 Definitions

As used herein, the following terms have the meanings indicated.

"Activate," with reference to one or more electrodes, means affecting a change in the electrical state of the one or more electrodes which, in the presence of a droplet, results in a droplet operation. Activation of an electrode can be accomplished using alternating or direct current. Any suitable voltage may be used. For example, an electrode may be activated using a voltage which is greater than about 150 V, or greater than about 200 V, or greater than about 250 V, or from about 275 V to about 375 V, or about 300 V. Where alternating current is used, any suitable frequency may be employed. For example, an electrode may be activated using alternating current having a frequency from about 1 Hz to about 100 Hz, or from about 10 Hz to about 60 Hz, or from about 20 Hz to about 40 Hz, or about 30 Hz.

"Bead," with respect to beads on a droplet actuator, means any bead or particle that is capable of interacting with a droplet on or in proximity with a droplet actuator. Beads may be any of a wide variety of shapes, such as spherical, generally spherical, egg shaped, disc shaped, cubical, amorphous and other three dimensional shapes. The bead may, for example, be capable of being subjected to a droplet operation in a droplet on a droplet actuator or otherwise configured with respect to a droplet actuator in a manner which permits a droplet on the droplet actuator to be brought into contact with the bead on the droplet actuator and/or off the droplet actuator. Beads may be provided in a droplet, in a droplet operations gap, or on a droplet operations surface. Beads may be provided in a reservoir that is external to a droplet operations gap or situated apart from a droplet operations surface, and the reservoir may be associated with a fluid path that permits a droplet including the beads to be brought into a droplet operations gap or into contact with a droplet operations surface. Beads may be manufactured using a wide variety of materials, including for example, resins, and polymers. The beads may be any suitable size, including for example, microbeads, microparticles, nanobeads and nanoparticles. In some cases, beads are magnetically responsive; in other

cases beads are not significantly magnetically responsive. For magnetically responsive beads, the magnetically responsive material may constitute substantially all of a bead, a portion of a bead, or only one component of a bead. The remainder of the bead may include, among other things, polymeric material, coatings, and moieties which permit attachment of an assay reagent. Examples of suitable beads include flow cytometry microbeads, polystyrene microparticles and nanoparticles, functionalized polystyrene microparticles and nanoparticles, coated polystyrene microparticles and nanoparticles, silica microbeads, fluorescent microspheres and nanospheres, functionalized fluorescent microspheres and nanospheres, coated fluorescent microspheres and nanospheres, color dyed microparticles and nanoparticles, magnetic microparticles and nanoparticles, superparamagnetic microparticles and nanoparticles (e.g., DYNABEADS® particles, available from Invitrogen Group, Carlsbad, CA), fluorescent microparticles and nanoparticles, coated magnetic microparticles and nanoparticles, ferromagnetic microparticles and nanoparticles, coated ferromagnetic microparticles and nanoparticles, and those described in U.S. Patent Publication Nos. 20050260686, entitled "Multiplex flow assays preferably with magnetic particles as solid phase," published on November 24, 2005; 20030132538, entitled "Encapsulation of discrete quanta of fluorescent particles," published on July 17, 2003; 20050118574, entitled "Multiplexed Analysis of Clinical Specimens Apparatus and Method," published on June 2, 2005; 20050277197. Entitled "Microparticles with Multiple Fluorescent Signals and Methods of Using Same," published on December 15, 2005; 20060159962, entitled "Magnetic Microspheres for use in Fluorescence-based Applications," published on July 20, 2006; the entire disclosures of which are incorporated herein by reference for their teaching concerning beads and magnetically responsive materials and beads. Beads may be pre-coupled with a biomolecule or other substance that is able to bind to and form a complex with a biomolecule. Beads may be pre-coupled with an antibody, protein or antigen, DNA/RNA probe or any other molecule with an affinity for a desired target. Examples of droplet actuator techniques for immobilizing magnetically responsive beads and/or non-magnetically responsive beads and/or conducting droplet operations protocols using beads are described in U.S. Patent Application No. 11/639,566, entitled "Droplet-Based Particle Sorting," filed on December 15, 2006; U.S. Patent Application No. 61/039,183, entitled "Multiplexing Bead Detection in a Single Droplet," filed on March 25, 2008; U.S. Patent Application No. 61/047,789, entitled "Droplet Actuator Devices and Droplet Operations Using Beads," filed on April 25, 2008; U.S. Patent Application No. 61/086,183, entitled "Droplet Actuator Devices and Methods for Manipulating Beads," filed on August 5, 2008; International Patent Application No. PCT/US2008/053545, entitled "Droplet Actuator Devices and Methods Employing Magnetic Beads," filed on February 11, 2008; International Patent Application No. PCT/US2008/058018, entitled "Bead-based Multiplexed Analytical Methods and

Instrumentation," filed on March 24, 2008; International Patent Application No. PCT/US2008/058047, "Bead Sorting on a Droplet Actuator," filed on March 23, 2008; and International Patent Application No. PCT/US2006/047486, entitled "Droplet-based Biochemistry," filed on December 11, 2006; the entire disclosures of which are incorporated herein by reference. Bead characteristics may be employed in the multiplexing aspects of the invention. Examples of beads having characteristics suitable for multiplexing, as well as methods of detecting and analyzing signals emitted from such beads, may be found in U.S. Patent Publication No. 20080305481, entitled "Systems and Methods for Multiplex Analysis of PCR in Real Time," published on December 11, 2008; U.S. Patent Publication No. 20080151240, "Methods and Systems for Dynamic Range Expansion," published on June 26, 2008; U.S. Patent Publication No. 20070207513, entitled "Methods, Products, and Kits for Identifying an Analyte in a Sample," published on September 6, 2007; U.S. Patent Publication No. 20070064990, entitled "Methods and Systems for Image Data Processing," published on March 22, 2007; U.S. Patent Publication No. 20060159962, entitled "Magnetic Microspheres for use in Fluorescence-based Applications," published on July 20, 2006; U.S. Patent Publication No. 20050277197, entitled "Microparticles with Multiple Fluorescent Signals and Methods of Using Same," published on December 15, 2005; and U.S. Patent Publication No. 200501 18574, entitled "Multiplexed Analysis of Clinical Specimens Apparatus and Method," published on June 2, 2005.

"Droplet" means a volume of liquid on a droplet actuator. Typically, a droplet is at least partially bounded by a filler fluid. For example, a droplet may be completely surrounded by a filler fluid or may be bounded by filler fluid and one or more surfaces of the droplet actuator. As another example, a droplet may be bounded by filler fluid, one or more surfaces of the droplet actuator, and/or the atmosphere. As yet another example, a droplet may be bounded by filler fluid and the atmosphere. Droplets may, for example, be aqueous or non-aqueous or may be mixtures or emulsions including aqueous and non-aqueous components. Droplets may take a wide variety of shapes; nonlimiting examples include generally disc shaped, slug shaped, truncated sphere, ellipsoid, spherical, partially compressed sphere, hemispherical, ovoid, cylindrical, combinations of such shapes, and various shapes formed during droplet operations, such as merging or splitting or formed as a result of contact of such shapes with one or more surfaces of a droplet actuator. For examples of droplet fluids that may be subjected to droplet operations using the approach of the invention, see International Patent Application No. PCT/US 06/47486, entitled, "Droplet-Based Biochemistry," filed on December 11, 2006. In various embodiments, a droplet may include a biological sample, such as whole blood, lymphatic fluid, serum, plasma, sweat, tear, saliva, sputum, cerebrospinal fluid, amniotic fluid, seminal fluid, vaginal excretion, serous fluid, synovial

fluid, pericardial fluid, peritoneal fluid, pleural fluid, transudates, exudates, cystic fluid, bile, urine, gastric fluid, intestinal fluid, fecal samples, liquids containing single or multiple cells, liquids containing organelles, fluidized tissues, fluidized organisms, liquids containing multi-celled organisms, biological swabs and biological washes. Moreover, a droplet may include a reagent, such as water, deionized water, saline solutions, acidic solutions, basic solutions, detergent solutions and/or buffers. Other examples of droplet contents include reagents, such as a reagent for a biochemical protocol, such as a nucleic acid amplification protocol, an affinity-based assay protocol, an enzymatic assay protocol, a sequencing protocol, and/or a protocol for analyses of biological fluids.

"Droplet Actuator" means a device for manipulating droplets. For examples of droplet actuators, see Pamula et al., U.S. Patent 6,911,132, entitled "Apparatus for Manipulating Droplets by Electrowetting-Based Techniques," issued on June 28, 2005; Pamula et al., U.S. Patent Application No. 11/343,284, entitled "Apparatuses and Methods for Manipulating Droplets on a Printed Circuit Board," filed on filed on January 30, 2006; Pollack et al., International Patent Application No. PCT/US2006/047486, entitled "Droplet-Based Biochemistry," filed on December 11, 2006; Shenderov, U.S. Patents 6,773,566, entitled "Electrostatic Actuators for Microfluidics and Methods for Using Same," issued on August 10, 2004 and 6,565,727, entitled "Actuators for Microfluidics Without Moving Parts," issued on January 24, 2000; Kim and/or Shah et al., U.S. Patent Application Nos. 10/343,261, entitled "Electrowetting-driven Micropumping," filed on January 27, 2003, 11/275,668, entitled "Method and Apparatus for Promoting the Complete Transfer of Liquid Drops from a Nozzle," filed on January 23, 2006, 11/460,188, entitled "Small Object Moving on Printed Circuit Board," filed on January 23, 2006, 12/465,935, entitled "Method for Using Magnetic Particles in Droplet Microfluidics," filed on May 14, 2009, and 12/513,157, entitled "Method and Apparatus for Real-time Feedback Control of Electrical Manipulation of Droplets on Chip," filed on April 30, 2009; Velev, U.S. Patent 7,547,380, entitled "Droplet Transportation Devices and Methods Having a Fluid Surface," issued on June 16, 2009; Sterling et al., U.S. Patent 7,163,612, entitled "Method, Apparatus and Article for Microfluidic Control via Electrowetting, for Chemical, Biochemical and Biological Assays and the Like," issued on January 16, 2007; Becker and Gascoyne et al., U.S. Patent Nos. 7,641,779, entitled "Method and Apparatus for Programmable fluidic Processing," issued on January 5, 2010, and 6,977,033, entitled "Method and Apparatus for Programmable fluidic Processing," issued on December 20, 2005; Deere et al., U.S. Patent 7,328,979, entitled "System for Manipulation of a Body of Fluid," issued on February 12, 2008; Yamakawa et al., U.S. Patent Pub. No. 20060039823, entitled "Chemical Analysis Apparatus," published on February 23, 2006; Wu, International Patent Pub. No. WO/2009/003184, entitled "Digital Microfluidics Based

Apparatus for Heat-exchanging Chemical Processes," published on December 31, 2008; Fouillet et al., U.S. Patent Pub. No. 20090192044, entitled "Electrode Addressing Method," published on July 30, 2009; Fouillet et al., U.S. Patent 7,052,244, entitled "Device for Displacement of Small Liquid Volumes Along a Micro-catenary Line by Electrostatic Forces," issued on May 30, 2006; Marchand et al., U.S. Patent Pub. No. 20080124252, entitled "Droplet Microreactor," published on May 29, 2008; Adachi et al., U.S. Patent Pub. No. 20090321262, entitled "Liquid Transfer Device," published on December 31, 2009; Roux et al., U.S. Patent Pub. No. 20050179746, entitled "Device for Controlling the Displacement of a Drop Between two or Several Solid Substrates," published on August 18, 2005; Dhindsa et al., "Virtual Electrowetting Channels: Electronic Liquid Transport with Continuous Channel Functionality," *Lab Chip,* 10:832-836 (2010); the entire disclosures of which are incorporated herein by reference, along with their priority documents. Certain droplet actuators will include one or more substrates arranged with a gap therebetween and electrodes associated with (e.g., layered on, attached to, and/or embedded in) the one or more substrates and arranged to conduct one or more droplet operations. For example, certain droplet actuators will include a base (or bottom) substrate, droplet operations electrodes associated with the substrate, one or more dielectric layers atop the substrate and/or electrodes, and optionally one or more hydrophobic layers atop the substrate, dielectric layers and/or the electrodes forming a droplet operations surface. A top substrate may also be provided, which is separated from the droplet operations surface by a gap, commonly referred to as a droplet operations gap. Various electrode arrangements on the top and/or bottom substrates are discussed in the above-referenced patents and applications and certain novel electrode arrangements are discussed in the description of the invention. During droplet operations it is preferred that droplets remain in continuous contact or frequent contact with a ground or reference electrode. A ground or reference electrode may be associated with the top substrate facing the gap, the bottom substrate facing the gap, in the gap. Where electrodes are provided on both substrates, electrical contacts for coupling the electrodes to a droplet actuator instrument for controlling or monitoring the electrodes may be associated with one or both plates. In some cases, electrodes on one substrate are electrically coupled to the other substrate so that only one substrate is in contact with the droplet actuator. In one embodiment, a conductive material (e.g., an epoxy, such as MASTER BOND™ Polymer System EP79, available from Master Bond, Inc., Hackensack, NJ) provides the electrical connection between electrodes on one substrate and electrical paths on the other substrates, e.g., a ground electrode on a top substrate may be coupled to an electrical path on a bottom substrate by such a conductive material. Where multiple substrates are used, a spacer may be provided between the substrates to determine the height of the gap therebetween and define dispensing reservoirs. The spacer height may, for example, be from about 5 μιη to about 600

μιη, or about 100 μιη to about 400 μιη, or about 200 μιη to about 350 μιη, or about 250 μιη to about 300 μιη, or about 275 μιη. The spacer may, for example, be formed of a layer of projections form the top or bottom substrates, and/or a material inserted between the top and bottom substrates. One or more openings may be provided in the one or more substrates for forming a fluid path through which liquid may be delivered into the droplet operations gap. The one or more openings may in some cases be aligned for interaction with one or more electrodes, e.g., aligned such that liquid flowed through the opening will come into sufficient proximity with one or more droplet operations electrodes to permit a droplet operation to be effected by the droplet operations electrodes using the liquid. The base (or bottom) and top substrates may in some cases be formed as one integral component. One or more reference electrodes may be provided on the base (or bottom) and/or top substrates and/or in the gap. Examples of reference electrode arrangements are provided in the above referenced patents and patent applications. In various embodiments, the manipulation of droplets by a droplet actuator may be electrode mediated, e.g., electrowetting mediated or dielectrophoresis mediated or Coulombic force mediated. Examples of other techniques for controlling droplet operations that may be used in the droplet actuators of the invention include using devices that induce hydrodynamic fluidic pressure, such as those that operate on the basis of mechanical principles (e.g. external syringe pumps, pneumatic membrane pumps, vibrating membrane pumps, vacuum devices, centrifugal forces, piezoelectric/ultrasonic pumps and acoustic forces); electrical or magnetic principles (e.g. electroosmotic flow, electrokinetic pumps, ferrofluidic plugs, electrohydrodynamic pumps, attraction or repulsion using magnetic forces and magnetohydrodynamic pumps); thermodynamic principles (e.g. gas bubble generation/phase-change-induced volume expansion); other kinds of surface-wetting principles (e.g. electrowetting, and optoelectrowetting, as well as chemically, thermally, structurally and radioactively induced surface-tension gradients); gravity; surface tension (e.g., capillary action); electrostatic forces (e.g., electroosmotic flow); centrifugal flow (substrate disposed on a compact disc and rotated); magnetic forces (e.g., oscillating ions causes flow); magnetohydrodynamic forces; and vacuum or pressure differential. In certain embodiments, combinations of two or more of the foregoing techniques may be employed to conduct a droplet operation in a droplet actuator of the invention. Similarly, one or more of the foregoing may be used to deliver liquid into a droplet operations gap, e.g., from a reservoir in another device or from an external reservoir of the droplet actuator (e.g., a reservoir associated with a droplet actuator substrate and a fluid path from the reservoir into the droplet operations gap). Droplet operations surfaces of certain droplet actuators of the invention may be made from hydrophobic materials or may be coated or treated to make them hydrophobic. For example, in some cases some portion or all of the droplet operations surfaces may be derivatized with low surface-energy materials or chemistries, e.g., by

deposition or using in situ synthesis using compounds such as poly- or per-fluorinated compounds in solution or polymerizable monomers. Examples include TEFLON® AF (available from DuPont, Wilmington, DE), members of the cytop family of materials, coatings in the FLUOROPEL® family of hydrophobic and superhydrophobic coatings (available from Cytonix Corporation, Beltsville, MD), silane coatings, fluorosilane coatings, hydrophobic phosphonate derivatives (e.g.., those sold by Aculon, Inc), and NOVEC™ electronic coatings (available from 3M Company, St. Paul, MN), and other fluorinated monomers for plasma-enhanced chemical vapor deposition (PECVD). In some cases, the droplet operations surface may include a hydrophobic coating having a thickness ranging from about 10 nm to about 1,000 nm. Moreover, in some embodiments, the top substrate of the droplet actuator includes an electrically conducting organic polymer, which is then coated with a hydrophobic coating or otherwise treated to make the droplet operations surface hydrophobic. For example, the electrically conducting organic polymer that is deposited onto a plastic substrate may be poly(3,4-ethylenedioxythiophene) poly(styrenesulfonate) (PEDOT:PSS). Other examples of electrically conducting organic polymers and alternative conductive layers are described in Pollack et al., International Patent Application No. PCT/US20 10/040705, entitled "Droplet Actuator Devices and Methods," the entire disclosure of which is incorporated herein by reference. One or both substrates may be fabricated using a printed circuit board (PCB), glass, indium tin oxide (ITO)-coated glass, and/or semiconductor materials as the substrate. When the substrate is ITO-coated glass, the ITO coating is preferably a thickness in the range of about 20 to about 200 nm, preferably about 50 to about 150 nm, or about 75 to about 125 nm, or about 100 nm. In some cases, the top and/or bottom substrate includes a PCB substrate that is coated with a dielectric, such as a polyimide dielectric, which may in some cases also be coated or otherwise treated to make the droplet operations surface hydrophobic. When the substrate includes a PCB, the following materials are examples of suitable materials: MITSUI™ BN-300 (available from MITSUI Chemicals America, Inc., San Jose CA); ARLON™ 1IN (available from Arlon, Inc, Santa Ana, CA).; NELCO® N4000-6 and N5000-30/32 (available from Park Electrochemical Corp., Melville, NY); ISOLA™ FR406 (available from Isola Group, Chandler, AZ), especially IS620; fluoropolymer family (suitable for fluorescence detection since it has low background fluorescence); polyimide family; polyester; polyethylene naphthalate; polycarbonate; polyetheretherketone; liquid crystal polymer; cyclo-olefm copolymer (COC); cyclo-olefm polymer (COP); aramid; THERMOUNT® nonwoven aramid reinforcement (available from DuPont, Wilmington, DE); NOMEX® brand fiber (available from DuPont, Wilmington, DE); and paper. Various materials are also suitable for use as the dielectric component of the substrate. Examples include: vapor deposited dielectric, such as PARYLENE™ C (especially on glass) and PARYLENE™ N (available from Parylene

Coating Services, Inc., Katy, TX); TEFLON® AF coatings; cytop; soldermasks, such as liquid photoimageable soldermasks (e.g., on PCB) like TAIYO™ PSR4000 series, TAIYO™ PSR and AUS series (available from Taiyo America, Inc. Carson City, NV) (good thermal characteristics for applications involving thermal control), and PROBIMER™ 8165 (good thermal characteristics for applications involving thermal control (available from Huntsman Advanced Materials Americas Inc., Los Angeles, CA); dry film soldermask, such as those in the VACREL® dry film soldermask line (available from DuPont, Wilmington, DE); film dielectrics, such as polyimide film (e.g., KAPTON® polyimide film, available from DuPont, Wilmington, DE), polyethylene, and fluoropolymers (e.g., FEP), polytetrafluoroethylene; polyester; polyethylene naphthalate; cyclo-olefm copolymer (COC); cyclo-olefm polymer (COP); any other PCB substrate material listed above; black matrix resin; and polypropylene. Droplet transport voltage and frequency may be selected for performance with reagents used in specific assay protocols. Design parameters may be varied, e.g., number and placement of on-chip reservoirs, number of independent electrode connections, size (volume) of different reservoirs, placement of magnets^ead washing zones, electrode size, inter-electrode pitch, and gap height (between top and bottom substrates) may be varied for use with specific reagents, protocols, droplet volumes, etc. In some cases, a substrate of the invention may derivatized with low surface-energy materials or chemistries, e.g., using deposition or in situ synthesis using poly- or per-fluorinated compounds in solution or polymerizable monomers. Examples include TEFLON® AF coatings and FLUOROPEL® coatings for dip or spray coating, and other fluorinated monomers for plasma-enhanced chemical vapor deposition (PECVD). Additionally, in some cases, some portion or all of the droplet operations surface may be coated with a substance for reducing background noise, such as background fluorescence from a PCB substrate. For example, the noise-reducing coating may include a black matrix resin, such as the black matrix resins available from Toray industries, Inc., Japan. Electrodes of a droplet actuator are typically controlled by a controller or a processor, which is itself provided as part of a system, which may include processing functions as well as data and software storage and input and output capabilities.

"Droplet operation" means any manipulation of a droplet on a droplet actuator. A droplet operation may, for example, include: loading a droplet into the droplet actuator; dispensing one or more droplets from a source droplet; splitting, separating or dividing a droplet into two or more droplets; transporting a droplet from one location to another in any direction; merging or combining two or more droplets into a single droplet; diluting a droplet; mixing a droplet; agitating a droplet; deforming a droplet; retaining a droplet in position; incubating a droplet; heating a droplet; vaporizing a droplet; cooling a droplet; disposing of a droplet; transporting a droplet out of a droplet actuator; other droplet operations described herein;

and/or any combination of the foregoing. The terms "merge," "merging," "combine," "combining" and the like are used to describe the creation of one droplet from two or more droplets. It should be understood that when such a term is used in reference to two or more droplets, any combination of droplet operations that are sufficient to result in the combination of the two or more droplets into one droplet may be used. For example, "merging droplet A with droplet B," can be achieved by transporting droplet A into contact with a stationary droplet B, transporting droplet B into contact with a stationary droplet A, or transporting droplets A and B into contact with each other. The terms "splitting," "separating" and "dividing" are not intended to imply any particular outcome with respect to volume of the resulting droplets (i.e., the volume of the resulting droplets can be the same or different) or number of resulting droplets (the number of resulting droplets may be 2, 3, 4, 5 or more). The term "mixing" refers to droplet operations which result in more homogenous distribution of one or more components within a droplet. Examples of "loading" droplet operations include microdialysis loading, pressure assisted loading, robotic loading, passive loading, and pipette loading. Droplet operations may be electrode-mediated. In some cases, droplet operations are further facilitated by the use of hydrophilic and/or hydrophobic regions on surfaces and/or by physical obstacles. For examples of droplet operations, see the patents and patent applications cited above under the definition of "droplet actuator." Impedance or capacitance sensing or imaging techniques may sometimes be used to determine or confirm the outcome of a droplet operation. Examples of such techniques are described in Stunner et al., International Patent Pub. No. WO/2008/101194, entitled "Capacitance Detection in a Droplet Actuator," published on August 21, 2008, the entire disclosure of which is incorporated herein by reference. Generally speaking, the sensing or imaging techniques may be used to confirm the presence or absence of a droplet at a specific electrode. For example, the presence of a dispensed droplet at the destination electrode following a droplet dispensing operation confirms that the droplet dispensing operation was effective. Similarly, the presence of a droplet at a detection spot at an appropriate step in an assay protocol may confirm that a previous set of droplet operations has successfully produced a droplet for detection. Droplet transport time can be quite fast. For example, in various embodiments, transport of a droplet from one electrode to the next may exceed about 1 sec, or about 0.1 sec, or about 0.01 sec, or about 0.001 sec. In one embodiment, the electrode is operated in AC mode but is switched to DC mode for imaging. It is helpful for conducting droplet operations for the footprint area of droplet to be similar to electrowetting area; in other words, lx-, 2x-3x-droplets are usefully controlled operated using 1, 2, and 3 electrodes, respectively. If the droplet footprint is greater than the number of electrodes available for conducting a droplet operation at a given time, the difference between the droplet size and the number of electrodes should typically not be greater than 1; in other words, a 2x droplet is usefully

controlled using 1 electrode and a 3x droplet is usefully controlled using 2 electrodes. When droplets include beads, it is useful for droplet size to be equal to the number of electrodes controlling the droplet, e.g., transporting the droplet.

"Filler fluid" means a fluid associated with a droplet operations substrate of a droplet actuator, which fluid is sufficiently immiscible with a droplet phase to render the droplet phase subject to electrode-mediated droplet operations. For example, the gap of a droplet actuator is typically filled with a filler fluid. The filler fluid may, for example, be a low-viscosity oil, such as silicone oil or hexadecane filler fluid. The filler fluid may fill the entire gap of the droplet actuator or may coat one or more surfaces of the droplet actuator. Filler fluids may be conductive or non-conductive. Filler fluids may, for example, be doped with surfactants or other additives. For example, additives may be selected to improve droplet operations and/or reduce loss of reagent or target substances from droplets, formation of microdroplets, cross contamination between droplets, contamination of droplet actuator surfaces, degradation of droplet actuator materials, etc. Composition of the filler fluid, including surfactant doping, may be selected for performance with reagents used in the specific assay protocols and effective interaction or non-interaction with droplet actuator materials. Examples of filler fluids and filler fluid formulations suitable for use with the invention are provided in Srinivasan et al, International Patent Pub. Nos. WO/2010/027894, entitled "Droplet Actuators, Modified Fluids and Methods," published on March 11, 2010, and WO/2009/021173, entitled "Use of Additives for Enhancing Droplet Operations," published on February 12, 2009; Sista et al., International Patent Pub. No. WO/2008/098236, entitled "Droplet Actuator Devices and Methods Employing Magnetic Beads," published on August 14, 2008; and Monroe et al., U.S. Patent Publication No. 20080283414, entitled "Electrowetting Devices," filed on May 17, 2007; the entire disclosures of which are incorporated herein by reference, as well as the other patents and patent applications cited herein.

"Immobilize" with respect to magnetically responsive beads, means that the beads are substantially restrained in position in a droplet or in filler fluid on a droplet actuator. For example, in one embodiment, immobilized beads are sufficiently restrained in position in a droplet to permit execution of a droplet splitting operation, yielding one droplet with substantially all of the beads and one droplet substantially lacking in the beads.

"Magnetically responsive" means responsive to a magnetic field. "Magnetically responsive beads" include or are composed of magnetically responsive materials. Examples of magnetically responsive materials include paramagnetic materials, ferromagnetic materials,

ferrimagnetic materials, and metamagnetic materials. Examples of suitable paramagnetic materials include iron, nickel, and cobalt, as well as metal oxides, such as $Fe_3O_4$, $BaFe_{12}O_{19}$, CoO, NiO, $Mn_2O_3$, $Cr_2O_3$, and CoMnP.

"Transporting into the magnetic field of a magnet," "transporting towards a magnet," and the like, as used herein to refer to droplets and/or magnetically responsive beads within droplets, is intended to refer to transporting into a region of a magnetic field capable of substantially attracting magnetically responsive beads in the droplet. Similarly, "transporting away from a magnet or magnetic field," "transporting out of the magnetic field of a magnet," and the like, as used herein to refer to droplets and/or magnetically responsive beads within droplets, is intended to refer to transporting away from a region of a magnetic field capable of substantially attracting magnetically responsive beads in the droplet, whether or not the droplet or magnetically responsive beads is completely removed from the magnetic field. It will be appreciated that in any of such cases described herein, the droplet may be transported towards or away from the desired region of the magnetic field, and/or the desired region of the magnetic field may be moved towards or away from the droplet. Reference to an electrode, a droplet, or magnetically responsive beads being "within" or "in" a magnetic field, or the like, is intended to describe a situation in which the electrode is situated in a manner which permits the electrode to transport a droplet into and/or away from a desired region of a magnetic field, or the droplet or magnetically responsive beads is/are situated in a desired region of the magnetic field, in each case where the magnetic field in the desired region is capable of substantially attracting any magnetically responsive beads in the droplet. Similarly, reference to an electrode, a droplet, or magnetically responsive beads being "outside of or "away from" a magnetic field, and the like, is intended to describe a situation in which the electrode is situated in a manner which permits the electrode to transport a droplet away from a certain region of a magnetic field, or the droplet or magnetically responsive beads is/are situated away from a certain region of the magnetic field, in each case where the magnetic field in such region is not capable of substantially attracting any magnetically responsive beads in the droplet or in which any remaining attraction does not eliminate the effectiveness of droplet operations conducted in the region. In various aspects of the invention, a system, a droplet actuator, or another component of a system may include a magnet, such as one or more permanent magnets (e.g., a single cylindrical or bar magnet or an array of such magnets, such as a Halbach array) or an electromagnet or array of electromagnets, to form a magnetic field for interacting with magnetically responsive beads or other components on chip. Such interactions may, for example, include substantially immobilizing or restraining movement or flow of magnetically responsive beads during

storage or in a droplet during a droplet operation or pulling magnetically responsive beads out of a droplet.

"Washing" with respect to washing a bead means reducing the amount and/or concentration of one or more substances in contact with the bead or exposed to the bead from a droplet in contact with the bead. The reduction in the amount and/or concentration of the substance may be partial, substantially complete, or even complete. The substance may be any of a wide variety of substances; examples include target substances for further analysis, and unwanted substances, such as components of a sample, contaminants, and/or excess reagent. In some embodiments, a washing operation begins with a starting droplet in contact with a magnetically responsive bead, where the droplet includes an initial amount and initial concentration of a substance. The washing operation may proceed using a variety of droplet operations. The washing operation may yield a droplet including the magnetically responsive bead, where the droplet has a total amount and/or concentration of the substance which is less than the initial amount and/or concentration of the substance. Examples of suitable washing techniques are described in Pamula et al., U.S. Patent 7,439,014, entitled "Droplet-Based Surface Modification and Washing," granted on October 21, 2008, the entire disclosure of which is incorporated herein by reference.

The terms "top," "bottom," "over," "under," and "on" are used throughout the description with reference to the relative positions of components of the droplet actuator, such as relative positions of top and bottom substrates of the droplet actuator. It will be appreciated that the droplet actuator is functional regardless of its orientation in space.

When a liquid in any form (e.g., a droplet or a continuous body, whether moving or stationary) is described as being "on", "at", or "over" an electrode, array, matrix or surface, such liquid could be either in direct contact with the electrode/array/matrix/surface, or could be in contact with one or more layers or films that are interposed between the liquid and the electrode/array/matrix/surface.

When a droplet is described as being "on" or "loaded on" a droplet actuator, it should be understood that the droplet is arranged on the droplet actuator in a manner which facilitates using the droplet actuator to conduct one or more droplet operations on the droplet, the droplet is arranged on the droplet actuator in a manner which facilitates sensing of a property of or a signal from the droplet, and/or the droplet has been subjected to a droplet operation on the droplet actuator.

## 6 Brief Description of the Drawings

Figures 1A through 1D illustrate side views of a portion of an example of a droplet actuator and a process of plasma sample preparation on a droplet actuator by immunocapture;

Figure 2 illustrates a flow diagram of an example of a method of preparing plasma that contains HIV from whole blood without the need for filtration or centrifugation;

Figure 3 illustrates a flow diagram of an example of a method of sample preparation that uses hydrophobic chromatography for purifying and/or removing unwanted materials from the sample;

Figure 4 illustrates a flow diagram of another example of a method of sample preparation that uses hydrophobic chromatography for purifying and/or removing unwanted materials from the sample;

Figures 5A through 5D illustrate side views of a portion of an example of a droplet actuator and a process of loading solutions of large volume into a droplet actuator under controlled conditions; and

Figure 6 illustrates a flow diagram of an example of a method of processing cells on a droplet actuator.

## 7 Detailed Description of the Invention

The invention provides methods of sample preparation on a droplet actuator. In one example, the invention provides a method of plasma sample preparation on a droplet actuator by immunocapture. In another example, the invention provides a method of preparing plasma that contains HIV from whole blood without the need for filtration or centrifugation. In yet another example, the invention provides methods of sample preparation that use hydrophobic chromatography for purifying and/or removing unwanted materials from the sample. The invention also provides a droplet actuator and method of loading solutions of large volume into a droplet actuator under controlled conditions. Further, the invention provides a method of processing cells on a droplet actuator.

### 7.1 Sample Preparation on a Droplet Actuator

**Figures 1A through ID** illustrate cross sections of a droplet actuator 100 and a process of plasma sample preparation on a droplet actuator by immuno capture. Droplet actuator 100 may include a bottom substrate 110 and a top substrate 112 that are separated by a gap 114. Bottom substrate 110 may, for example, be a printed circuit board (PCB). Top substrate 112 may, for example, be formed of glass, injection-molded plastic, silicon, and/or indium tin oxide (ITO). Bottom substrate 110 may include a configuration of droplet operations electrodes 116 (e.g., electrowetting electrodes). Droplet operations are conducted atop droplet operations electrodes 116 on a droplet operations surface. Bottom substrate 110 may also include a reservoir electrode 118, which may be arranged in the configuration of droplet operations electrodes 116.

A fluid reservoir 120 may be integrated into top substrate 112 for holding a certain amount of sample fluid to be loaded into droplet actuator 100. An opening 122 in top substrate 112, which is substantially aligned with reservoir electrode 118, provides a fluid path from fluid reservoir 120 to gap 114 of droplet actuator 100.

The invention provides a process of plasma sample preparation on a droplet actuator by immunocapture. For example, Figures 1A through ID show a method of removing blood cells from a blood sample in order to prepare plasma. The method of removing blood cells from a blood sample in order to prepare plasma may include, but is not limited to, the following steps.

Referring to Figure 1A, fluid reservoir 120 is shown in its original manufactured state and with no sample fluid therein.

Figure IB shows a chemically modified surface 124 of fluid reservoir 120. For example, the surface of fluid reservoir 120 may be modified by chemical conjugation of anti-erythrocytes, leukocytes, and platelets antibodies.

Referring to Figure 1C, a quantity of blood sample 126 is loaded into fluid reservoir 120 and allowed to incubate for a certain amount of time. Blood sample 126 includes blood cells 128.

Referring to Figure ID, as a result of incubation, blood cells 128 bind to the antibodies on chemically modified surface 124 of fluid reservoir 120. In this way, blood cells 128 are retained at fluid reservoir 120 and substantially removed from blood sample 126. Then, substantially cell free-plasma 130 may be dispensed atop reservoir electrode 118 and into gap

114 of droplet actuator 100. The substantially cell free-plasma 130 may then be processed in droplet actuator 100 via droplet operations, e.g., electrowetting droplet operations.

The invention provides a simple method to prepare plasma on a droplet actuator that requires no magnets, no filters, and no centrifugation. Additionally, the method of the invention is very gentle to avoid cell lysis. Further, the method provides maximum recovery of plasma.

**Figure 2** illustrates a flow diagram of an example of a method 200 of preparing plasma from whole blood without the need for filtration or centrifugation. The method of the invention is dependent on the differential expression of complex carbohydrates on the plasma membrane of cells that are found in blood and HIV. All cells in blood contain on their surface a mixture of N- and O-linked oligosaccharide chains that have unique carbohydrate structures. HIV contains glycoproteins, which are made of only N-linked carbohydrate chains. The method makes use of the exquisite carbohydrate specificity of lectins. Method 200 may include, but is not limited to, the following steps.

At step 210, a lectin, which is specific for the sugars found in O-linked oligosaccharide chains, is added to the whole blood sample. This causes the cells to rapidly agglutinate.

At step 212, in a matter of a few minutes, the agglutinated cells condense into a massive cell pellet and fall out of solution without the need for centrifugation.

At step 214, the plasma may be dispensed onto the droplet actuator for use in immunoassays.

In some embodiments, the plasma contains human immunodeficiency virus (HIV). Step 212 may leave HIV virus in the remaining liquid plasma fraction. Prior to step 214, the plasma may be combined with a lysis reagent (on or off the droplet actuator) for the preparation of HIV RNA.

The invention provides a method of preparing plasma that contains HIV from whole blood without the need for centrifugation or filtration through small pore membranes. Further, the method is fast, inexpensive, and simple. Lectins can be lyophilized for long term storage stability on a droplet actuator.

**Figure 3** illustrates a flow diagram of a method 300, which is one example of a sample preparation process that uses hydrophobic chromatography for purifying and/or removing unwanted materials from the sample. Method 300 may be used to enrich and/or purify a

protein and/or small molecule analyte. Method 300 may also be used to remove hydrophobic material from the sample that may interfere with some downstream process or assay. Method 300 may include, but is not limited to, the following steps.

At step 310, magnetically responsive beads are modified with, for example, C-4, C-8, and/or C-18 alkyl chains, which may serve as a matrix for hydrophobic chromatography on a droplet actuator.

At step 312, the magnetically responsive beads may be washed and eluted with a gradient of increasing hydrophobicity, such as going from about 0% to about 70% acetonitrile. Thereby enriching and/or purifying a protein and/or small molecule analyte; and thereby, removing hydrophobic material from the sample that may interfere with some downstream process or assay.

The invention provides a chromatography/extraction method on a droplet actuator that is compatible with electrowetting up to the upper limit of tolerance for hydrophobic solvents.

**Figure 4** illustrates a flow diagram of a method 400, which is a sample preparation process that uses ion exchange chromatography for purifying and/or removing unwanted materials from the sample. Method 400 may be used to enrich and/or purify a protein and/or small molecule analyte. Method 400 may also be used to remove contaminating and/or unwanted material from the sample that may interfere with some downstream process or assay. Method 400 may include, but is not limited to, the following steps.

At step 410, magnetically responsive ion exchange beads (e.g., cation and anion) are provided in the sample fluid. The beads may serve as a matrix for ion exchange chromatography on a droplet actuator.

At step 412, the magnetically responsive ion exchange beads may be washed or eluted with a gradient of increasing ionic strength or increasing or decreasing pH. Thereby enriching and/or purifying a protein and/or small molecule analyte; and thereby removing contaminating and/or unwanted material from the sample that may interfere with some downstream process or assay.

An aspect of the method of the invention is that it is easy to use with magnetically responsive beads on a droplet actuator. Step or linear gradients of salt or pH may be readily set up on a droplet actuator for washing and elution of material from the beads.

### 7.2 Fluid Loading on a Droplet Actuator

**Figures 5A through 5D** illustrate side views of a portion of an example of a droplet actuator 500 and a process of loading solutions of large volume into a droplet actuator under controlled conditions. Droplet actuator 500 may include a bottom substrate 510 and a top substrate 512 that are separated by a gap 514. Bottom substrate 510 may include droplet operations electrodes 516 (e.g., electrowetting electrodes) arranged for conducting droplet operations. Droplet operations are conducted atop droplet operations electrodes 516 on a droplet operations surface. Bottom substrate 510 may also include a reservoir electrode 518, which may be arranged in the configuration of droplet operations electrodes 516.

A fluid reservoir 520 may be integrated into top substrate 512 for holding a certain amount of sample fluid to be loaded into droplet actuator 500. An opening 522 in top substrate 512 provides a fluid path from fluid reservoir 520 to gap 514 of droplet actuator 500. Fluid reservoir 520 is an enclosed fluid reservoir. A hollow tube 524 extends through, for example, the top portion of fluid reservoir 520. One end of tube 524 is outside of the enclosed fluid reservoir 520. The opposite end of tube 524 is located inside of fluid reservoir 520. The end of tube 524 that is inside of fluid reservoir 520 is held at a height *hi* with respect to, for example, the plane of opening 522.

Fluid reservoir 520 is designed to hold a large volume of fluid. Without the presence of tube 524 in fluid reservoir 520, the head pressure at opening 522 will vary depending on the amount of fluid in fluid reservoir 520. More specifically, without tube 524 the head pressure is directly proportional to the amount of fluid in fluid reservoir 520. That is, the greater the volume of fluid in fluid reservoir 520, the greater the head pressure. Consequently, without the presence of tube 524 in fluid reservoir 520, there may be a risk of "flooding" of the droplet actuator. However, fluid reservoir 520 is designed to deliver large volumes of fluids to a droplet actuator under controlled conditions. For example, fluid reservoir 520 is designed to deliver large volumes of fluids to a droplet actuator while maintaining a substantially constant and suitably low head pressure at opening 522 for reducing, preferably entirely eliminating, the risk of "flooding" the droplet actuator.

According to the invention, the presence of tube 524 in fluid reservoir 520 is used to reduce head pressures at opening 522 to suitably low levels that prevent "flooding" of the droplet actuator. This is because the head pressure on the fluid that is in fluid reservoir 520 is only the pressure that is present from the base of fluid reservoir 520 (at opening 522) to the end of tube 524 that is near opening 522 and set at height *hi*. The head pressure on the fluid in fluid

reservoir 520 remains constant even when the fluid level changes as long as the end of tube 524 is in the liquid (see Figures 5B, 5C, and 5D). Fluid reservoir 520 may be filled by threading a smaller sample input tube (not shown) through the hollow tube 524.

For example, Figure 5B shows fluid reservoir 520 that is substantially full of fluid 526. Yet there is a certain head pressure at opening 522 of top substrate 512 that is dependent on height *hi* of tube 524. Figure 5C shows fluid reservoir 520 with a lesser amount of fluid 526 therein as compared with the amount of fluid 526 shown in Figure 5B. Yet the head pressure at opening 522 of top substrate 512, which is dependent on height *hi* of tube 524, remains substantially unchanged. Figure 5D shows fluid reservoir 520 with a yet lesser amount of fluid 526 therein as compared with the amount of fluid 526 shown in Figures 5B and 5C. Yet the head pressure at opening 522 of top substrate 512, which is dependent on height *hi* of tube 524, remains substantially unchanged.

An aspect of the droplet actuator and fluid reservoir of the invention is that it provides a simple structure that delivers solutions of large volumes to a droplet actuator under controlled conditions.

### 7.3 Processing Cells on a Droplet Actuator

**Figure** 6 illustrates a flow diagram of a method 600 of processing cells on a droplet actuator. Growth and testing of mammalian cells in a droplet actuator has widespread utility. The cells must be fed fresh media at regular intervals and many cells are anchorage dependent and require a surface on which to grow. Method 600 provides a surface for cells to grow on in a droplet actuator, wherein the surface is not a surface of the bottom or top substrate. Method 600 may include, but is not limited to, the following steps.

At step 610, magnetically responsive beads, which are coated with biopolymers, such as collagen or other cell attachment promoters (e.g., polylysine), are provided in the sample fluid in a droplet actuator.

At step 612, because the surface of the magnetically responsive beads is favorable for the anchoring and growth of cells on a droplet actuator, cells may be seeded and allowed to grow on the magnetically responsive beads. This greatly simplifies cell washing and replacing media for nutrients and oxygen.

An aspect of the method of the invention is that it provides a cell attachment strategy to support growth on a droplet actuator, wherein the surface on which the cells grow is not a surface of the bottom or top substrate.

## 7.4 Systems

It will be appreciated that various aspects of the invention may be embodied as a method, system, computer readable medium, and/or computer program product. Aspects of the invention may take the form of hardware embodiments, software embodiments (including firmware, resident software, micro-code, etc.), or embodiments combining software and hardware aspects that may all generally be referred to herein as a "circuit," "module" or "system." Furthermore, the methods of the invention may take the form of a computer program product on a computer-usable storage medium having computer-usable program code embodied in the medium.

Any suitable computer useable medium may be utilized for software aspects of the invention. The computer-usable or computer-readable medium may be, for example but not limited to, an electronic, magnetic, optical, electromagnetic, infrared, or semiconductor system, apparatus, device, or propagation medium. The computer readable medium may include transitory and/or non-transitory embodiments. More specific examples (a non-exhaustive list) of the computer-readable medium would include some or all of the following: an electrical connection having one or more wires, a portable computer diskette, a hard disk, a random access memory (RAM), a read-only memory (ROM), an erasable programmable read-only memory (EPROM or Flash memory), an optical fiber, a portable compact disc read-only memory (CD-ROM), an optical storage device, a transmission medium such as those supporting the Internet or an intranet, or a magnetic storage device. Note that the computer-usable or computer-readable medium could even be paper or another suitable medium upon which the program is printed, as the program can be electronically captured, via, for instance, optical scanning of the paper or other medium, then compiled, interpreted, or otherwise processed in a suitable manner, if necessary, and then stored in a computer memory. In the context of this document, a computer-usable or computer-readable medium may be any medium that can contain, store, communicate, propagate, or transport the program for use by or in connection with the instruction execution system, apparatus, or device.

Program code for carrying out operations of the invention may be written in an object oriented programming language such as Java, Smalltalk, C++ or the like. However, the program code for carrying out operations of the invention may also be written in conventional

procedural programming languages, such as the "C" programming language or similar programming languages. The program code may be executed by a processor, application specific integrated circuit (ASIC), or other component that executes the program code. The program code may be simply referred to as a software application that is stored in memory (such as the computer readable medium discussed above). The program code may cause the processor (or any processor-controlled device) to produce a graphical user interface ("GUI"). The graphical user interface may be visually produced on a display device, yet the graphical user interface may also have audible features. The program code, however, may operate in any processor-controlled device, such as a computer, server, personal digital assistant, phone, television, or any processor-controlled device utilizing the processor and/or a digital signal processor.

The program code may locally and/or remotely execute. The program code, for example, may be entirely or partially stored in local memory of the processor-controlled device. The program code, however, may also be at least partially remotely stored, accessed, and downloaded to the processor-controlled device. A user's computer, for example, may entirely execute the program code or only partly execute the program code. The program code may be a stand-alone software package that is at least partly on the user's computer and/or partly executed on a remote computer or entirely on a remote computer or server. In the latter scenario, the remote computer may be connected to the user's computer through a communications network.

The invention may be applied regardless of networking environment. The communications network may be a cable network operating in the radio-frequency domain and/or the Internet Protocol (IP) domain. The communications network, however, may also include a distributed computing network, such as the Internet (sometimes alternatively known as the "World Wide Web"), an intranet, a local-area network (LAN), and/or a wide-area network (WAN). The communications network may include coaxial cables, copper wires, fiber optic lines, and/or hybrid-coaxial lines. The communications network may even include wireless portions utilizing any portion of the electromagnetic spectrum and any signaling standard (such as the IEEE 802 family of standards, GSM/CDMA/TDMA or any cellular standard, and/or the ISM band). The communications network may even include powerline portions, in which signals are communicated via electrical wiring. The invention may be applied to any wireless/wireline communications network, regardless of physical componentry, physical configuration, or communications standard(s).

Certain aspects of invention are described with reference to various methods and method steps. It will be understood that each method step can be implemented by the program code and/or by machine instructions. The program code and/or the machine instructions may create means for implementing the functions/acts specified in the methods.

The program code may also be stored in a computer-readable memory that can direct the processor, computer, or other programmable data processing apparatus to function in a particular manner, such that the program code stored in the computer-readable memory produce or transform an article of manufacture including instruction means which implement various aspects of the method steps.

The program code may also be loaded onto a computer or other programmable data processing apparatus to cause a series of operational steps to be performed to produce a processor/computer implemented process such that the program code provides steps for implementing various functions/acts specified in the methods of the invention.

## 8 Concluding Remarks

The foregoing detailed description of embodiments refers to the accompanying drawings, which illustrate specific embodiments of the invention. Other embodiments having different structures and operations do not depart from the scope of the present invention. The term "the invention" or the like is used with reference to certain specific examples of the many alternative aspects or embodiments of the applicants' invention set forth in this specification, and neither its use nor its absence is intended to limit the scope of the applicants' invention or the scope of the claims. This specification is divided into sections for the convenience of the reader only. Headings should not be construed as limiting of the scope of the invention. The definitions are intended as a part of the description of the invention. It will be understood that various details of the present invention may be changed without departing from the scope of the present invention. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

## The Claims

We claim:

1. A droplet actuator comprising:

(a) a bottom substrate and a top substrate that are separated by a gap;

(b) a fluid reservoir integrated into the top substrate, wherein the fluid reservoir comprises a chemically modified surface; and

(c) an opening in the top substrate which is substantially aligned with a reservoir electrode on the bottom substrate and provides a fluid path from the fluid reservoir into the gap.

2. The droplet actuator of claim 1, wherein the chemically modified surface is modified by chemical conjugation of antibodies.

3. The droplet actuator of claim 2, wherein the antibodies are anti-erythrocytes, leukocytes, or platelets antibodies.

4. The droplet actuator of any of claims 1-3, wherein the bottom substrate is a printed circuit board.

5. The droplet actuator of any of claims 1-4, wherein the top substrate is formed of at least one of glass, injection-molded plastic, silicon, and indium tin oxide.

6. The droplet actuator of any of claims 1-5, wherein the bottom substrate comprises a configuration of droplet operations electrodes.

7. A method of plasma sample preparation on a droplet actuator by immunocapture, comprising:

(a) loading a quantity of a blood sample into a fluid reservoir, wherein the fluid reservoir comprises a chemically modified surface;

(b) incubating the blood sample in the fluid reservoir, wherein blood cells in the blood sample bind to the chemically modified surface to produce a plasma sample; and

(c) dispensing the plasma sample from the fluid reservoir into a droplet operations gap of the droplet actuator.

8. The method of claim 7, wherein the chemically modified surface is modified by chemical conjugation of antibodies.

9. The method of any of claims 7, wherein the plasma sample is dispensed atop a reservoir electrode into the droplet operations gap of the droplet actuator

10. The method of claim 8, wherein the antibodies are anti-erythrocytes, leukocytes, or platelets antibodies.

11. The method of claims 7-10, wherein the plasma sample is substantially cell free.

12. The method of any of claims 7-11, further comprising processing the plasma sample in the droplet actuator via electrowetting droplet operations.

13. A method of preparing a plasma sample from a whole blood sample on a droplet actuator, comprising:

(a) loading a quantity of the whole blood sample into a fluid reservoir;

(b) adding a lectin to the whole blood sample, causing cells in the whole blood sample to agglutinate and fall out of solution to produce a plasma sample; and

(c) dispensing the plasma sample from the fluid reservoir onto the droplet actuator.

14. The method of claim 13, wherein the whole blood sample and plasma sample contain human immunodeficiency virus (HIV).

15. The method of claim 13, wherein the plasma sample is dispensed from the fluid reservoir into a droplet operations gap of the droplet actuator.

16. The method of claim 15, wherein the plasma sample is dispensed atop a reservoir electrode in the droplet operations gap of the droplet actuator.

17. The method of claim 14, wherein prior to (c) the plasma sample is combined with a lysis reagent to produce an HIV RNA.

18. The method of any of claims 13-17, wherein the lectin is lyophilized and stored on the droplet actuator.

19. A method of preparing a substantially purified sample on a droplet actuator using hydrophobic chromatography, comprising:

(a) loading a quantity of the sample into a fluid reservoir, wherein the fluid reservoir contains magnetically responsive beads;

(b) washing and eluting the magnetically responsive beads to produce a substantially purified sample; and

(c) dispensing the substantially purified sample from the fluid reservoir.

20. The method of claim 19, wherein the magnetically responsive beads serve as a matrix for hydrophobic chromatography.

21. The method of claim 19, wherein the magnetically responsive beads are washed and eluted with a gradient of increasing hydrophobicity to produce the substantially purified sample.

22. The method of claim 19, wherein the magnetically responsive beads are modified with C-4, C-8, or C-18 alkyl chains.

23. The method of claim 21, wherein the gradient of increasing hydrophobicity is in the range of about 0% to about 70% acetonitrile.

24. The method of any of claims 19-23, wherein the substantially purified sample comprises a purified protein or small molecule analyte.

25. The method of any of claims 19-24, wherein the substantially purified sample is dispensed from the fluid reservoir into a droplet operations gap of a droplet actuator.

26. The method of claim 25, wherein the substantially purified sample is dispensed atop a reservoir electrode in the droplet operations gap of the droplet actuator.

27. The method of any of claims 19-26, wherein hydrophobic material is removed from the sample.

28. A method of preparing a substantially purified sample on a droplet actuator using ion exchange chromatography, comprising:

(a) loading a quantity of the sample into a fluid reservoir, wherein the fluid reservoir contains magnetically responsive ion exchange beads;

(b) washing and eluting the magnetically responsive beads to produce a substantially purified sample; and

(c) dispensing the substantially purified sample from the fluid reservoir.

29. The method of claim 28, wherein the magnetically responsive ion exchange beads comprise cations and anions.

30. The method of claim 28, wherein the magnetically responsive ion exchange beads serve as a matrix for ion exchange chromatography.

31. The method of any of claims 28-30, wherein the magnetically responsive ion exchange beads are washed and eluted with a gradient to produce the substantially purified sample.

32. The method of claim 31, wherein the gradient comprises increasing ionic strength.

33. The method of claim 31, wherein the gradient comprises increasing or decreasing pH.

34. The method of any of claims 31-33, wherein the gradient is a step gradient.

35. The method of any of claims 31-33, wherein the gradient is a linear gradient.

36. The method of any of claims 28-35, wherein the substantially purified sample comprises a substantially purified protein or small molecule analyte.

37. The method of any of claims 28-35, wherein the substantially purified sample is dispensed from the fluid reservoir into a droplet operations gap of a droplet actuator.

38. The method of claim 25, wherein the substantially purified sample is dispensed atop a reservoir electrode in the droplet operations gap of the droplet actuator.

39. A droplet actuator comprising:

(a) a bottom substrate and a top substrate that are separated by a gap;

(b) an opening in the top substrate which is substantially aligned with a reservoir electrode on the bottom substrate and provides a fluid path from the fluid reservoir into the gap; and

(c) a fluid reservoir integrated into the top substrate, wherein the fluid reservoir comprises a hollow tube extending through a portion of the fluid reservoir, wherein one end of the hollow tube is located outside of the fluid reservoir and the opposite end of the hollow tube is located inside of the fluid reservoir, and wherein the end of the hollow tube that is located inside of the fluid reservoir is held at a height with respect to a plane of the opening in the top substrate.

40. The droplet actuator of claim 39, wherein the fluid reservoir is enclosed.

41. The droplet actuator of claim 39, wherein the end of the hollow tube that is located inside of the fluid reservoir is substantially aligned with the opening.

42. The droplet actuator of claims 39-41, wherein the fluid reservoir maintains a substantially constant and low head pressure at the opening in the top substrate.

43. The droplet actuator of any of claims 39-42, wherein the bottom substrate comprises a configuration of droplet operations electrodes.

44. The droplet actuator of any of claims 39-43, wherein the fluid reservoir is filled by threading a smaller sample input tube through the hollow tube.

45. A method of processing cells on a droplet actuator, comprising:

(a) loading a quantity of a sample into a fluid reservoir, wherein the sample comprises cells;

(b) adding magnetically responsive beads to the sample, wherein the magnetically responsive beads are coated with a biopolymer; and

(c) growing the cells on the coated magnetically responsive beads.

46. The method of claim 45, wherein the biopolymer is a cell attachment promoter.

47. The method of claim 46, wherein the cell attachment promoter is collagen or polylysine.

48. The method of any of claims 45-47, wherein processing the cells comprises at least one of growing and testing of cells.

49. The method of any of claim 45-48, where in the cells comprise mammalian cells.

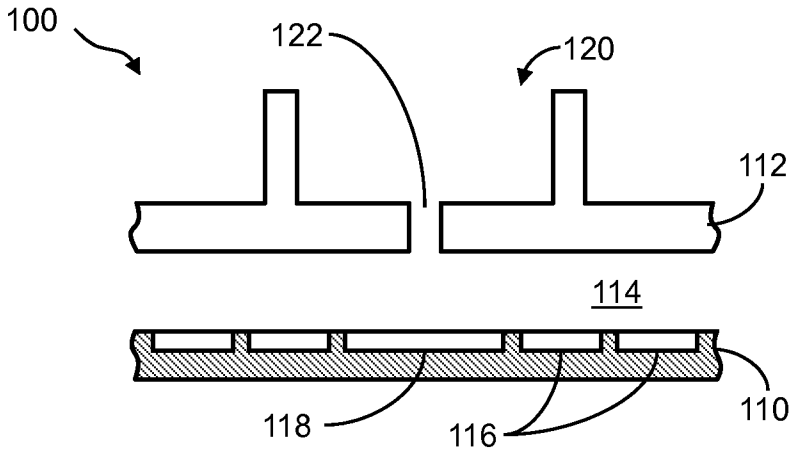


Figure 1A

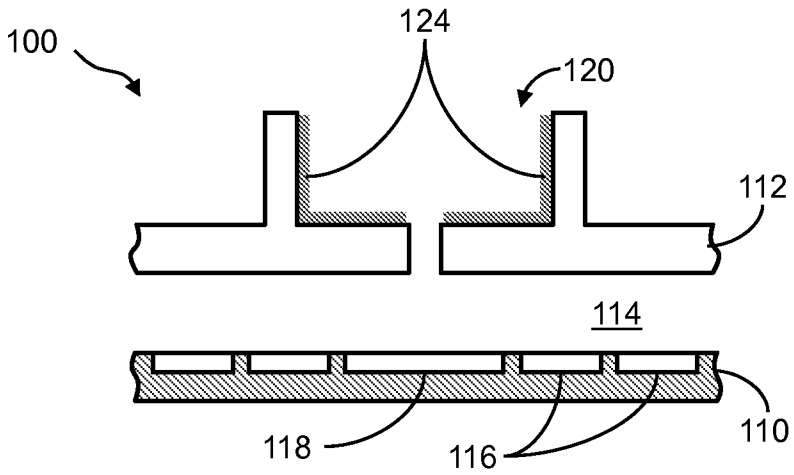


Figure 1B

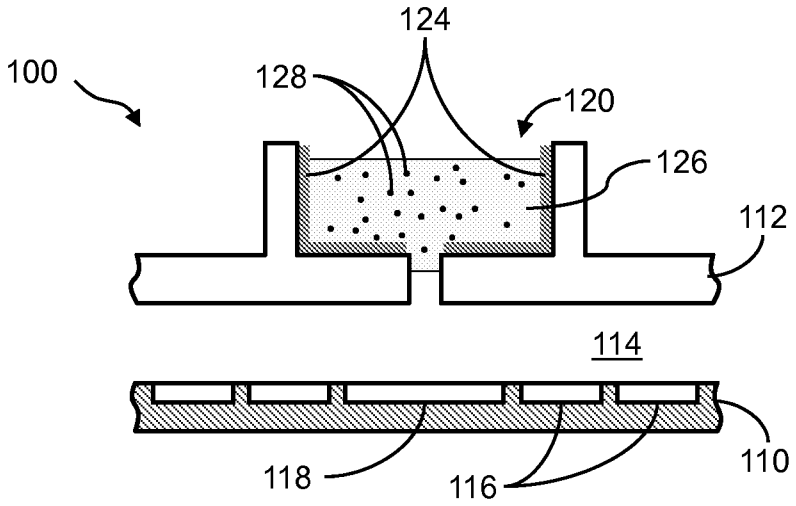


Figure 1C

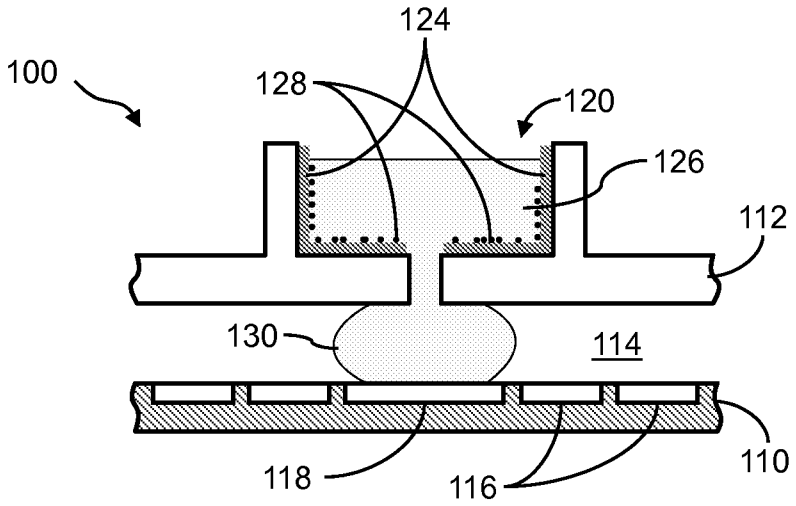


Figure 1D

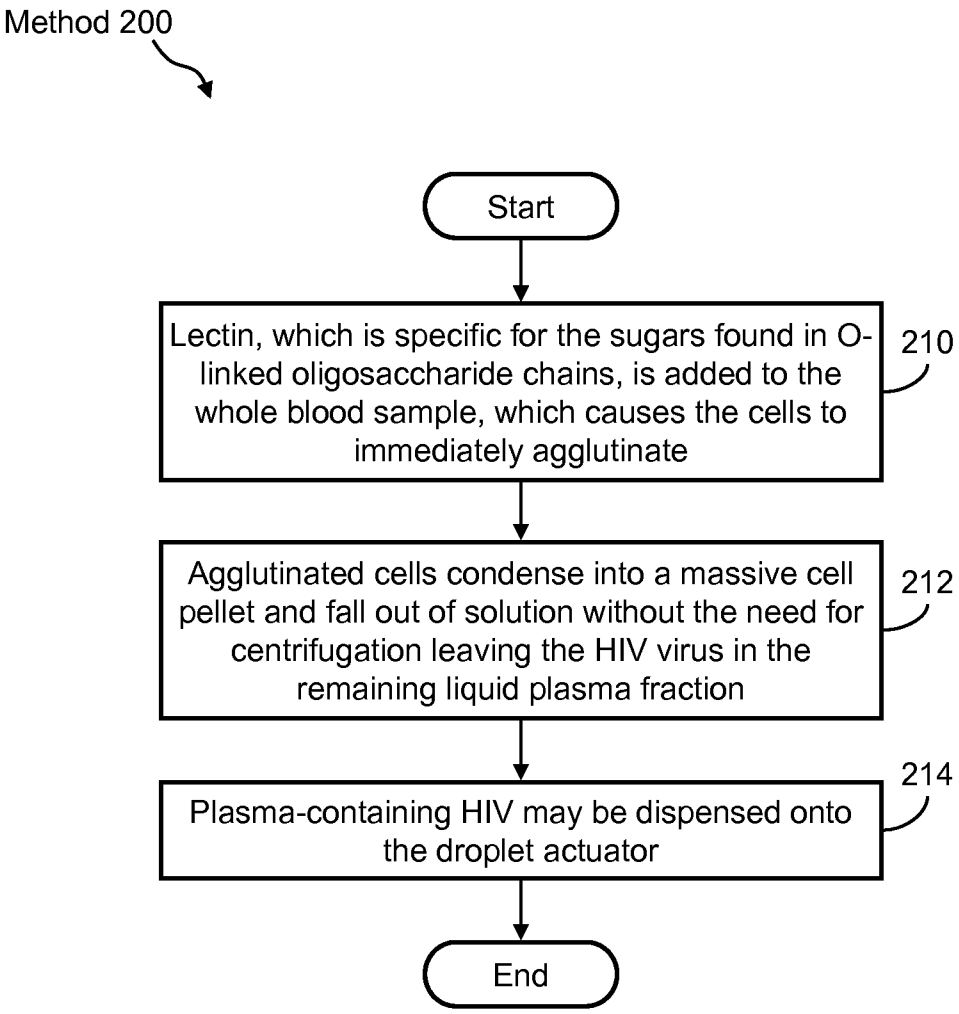
Method 200
Start
Lectin, which is specific for the sugars found in O-linked oligosaccharide chains, is added to the whole blood sample, which causes the cells to immediately agglutinate
210
Agglutinated cells condense into a massive cell pellet and fall out of solution without the need for centrifugation leaving the HIV virus in the remaining liquid plasma fraction
212
Plasma-containing HIV may be dispensed onto the droplet actuator
214
End

**Figure 2**

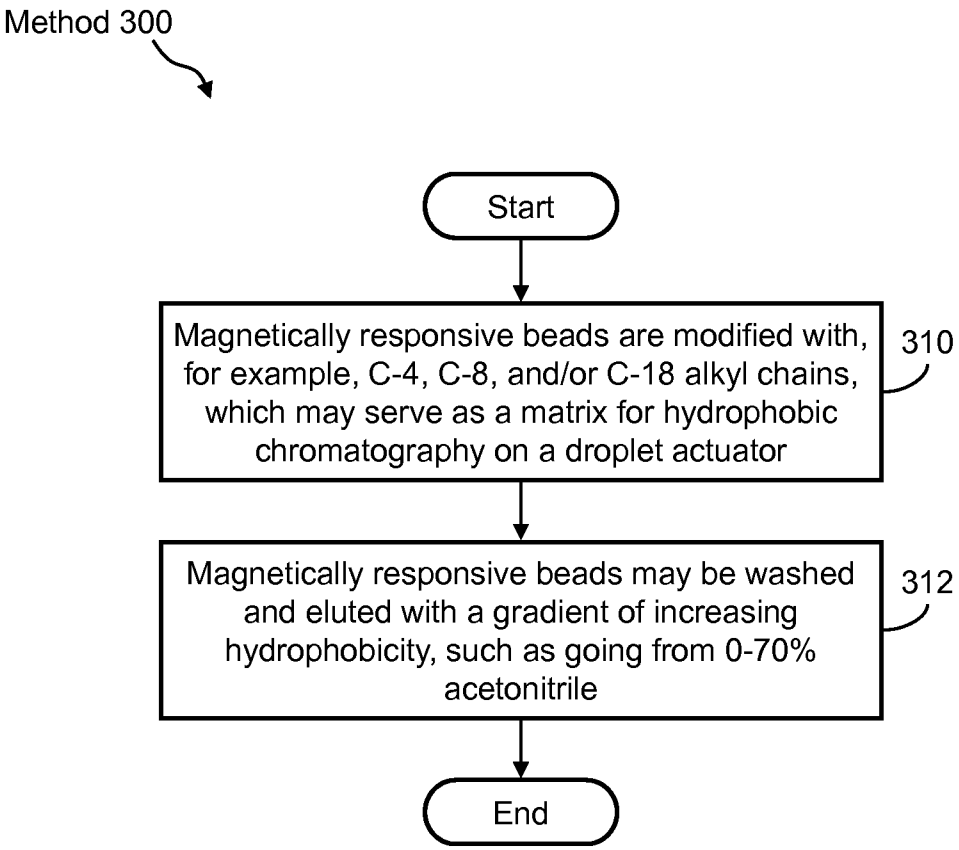
Method 300
Start
Magnetically responsive beads are modified with, for example, C-4, C-8, and/or C-18 alkyl chains, which may serve as a matrix for hydrophobic chromatography on a droplet actuator
310
Magnetically responsive beads may be washed and eluted with a gradient of increasing hydrophobicity, such as going from 0-70% acetonitrile
312
End

**Figure 3**

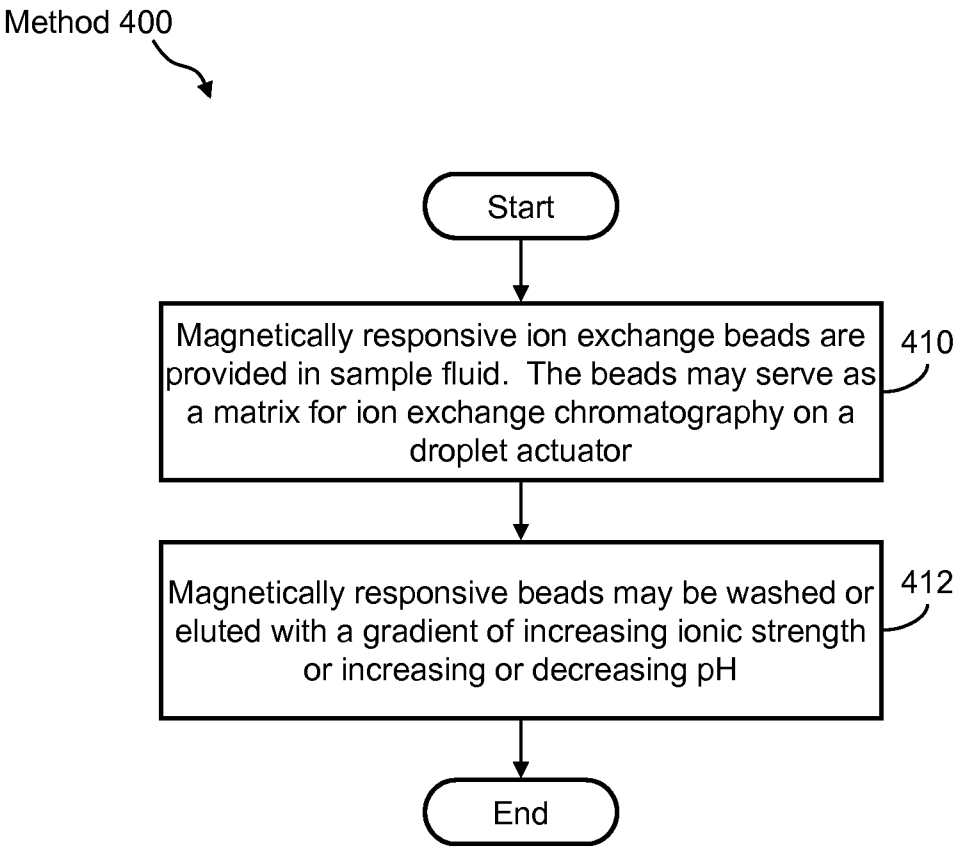
Method 400
Start
Magnetically responsive ion exchange beads are provided in sample fluid. The beads may serve as a matrix for ion exchange chromatography on a droplet actuator
410
Magnetically responsive beads may be washed or eluted with a gradient of increasing ionic strength or increasing or decreasing pH
412
End

**Figure 4**

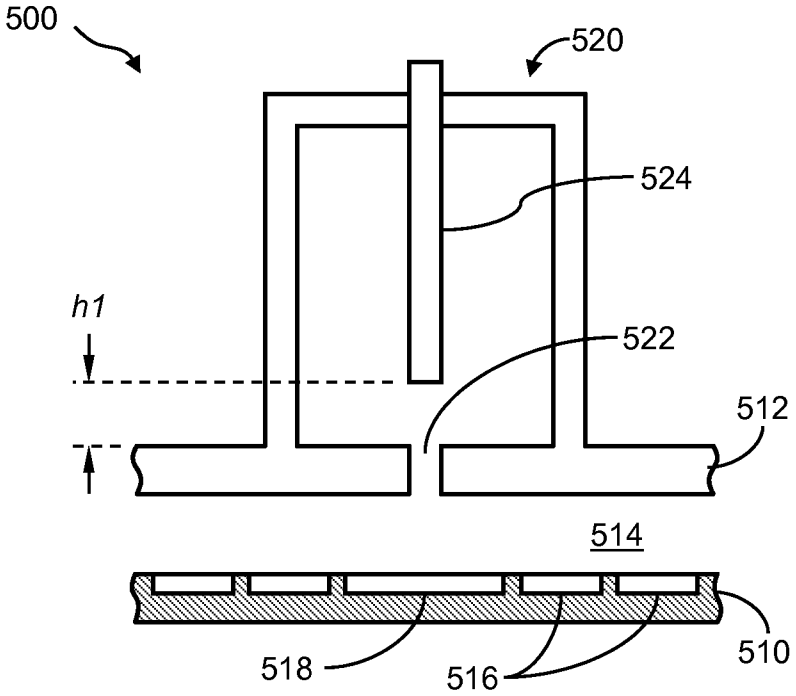


Figure 5A

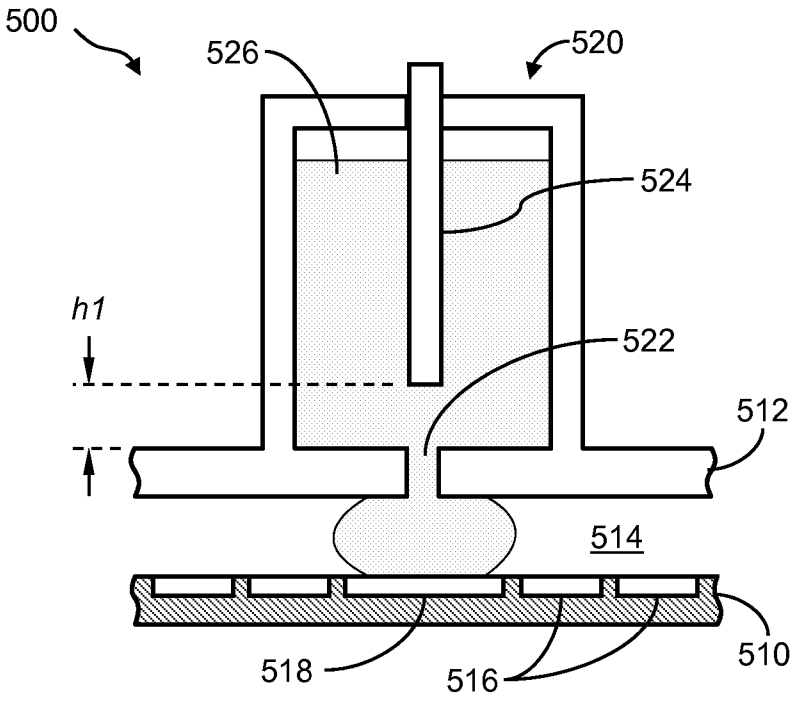


Figure 5B

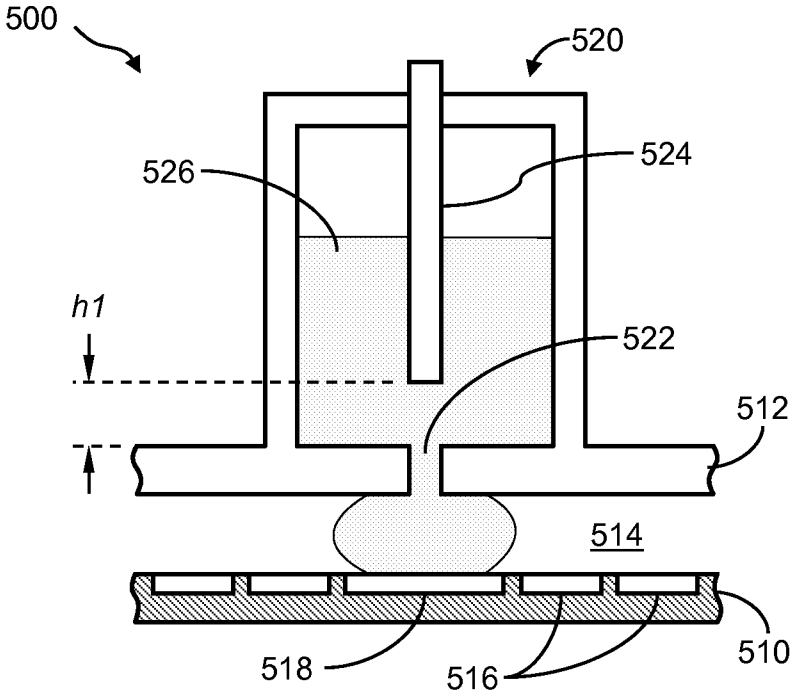


Figure 5C

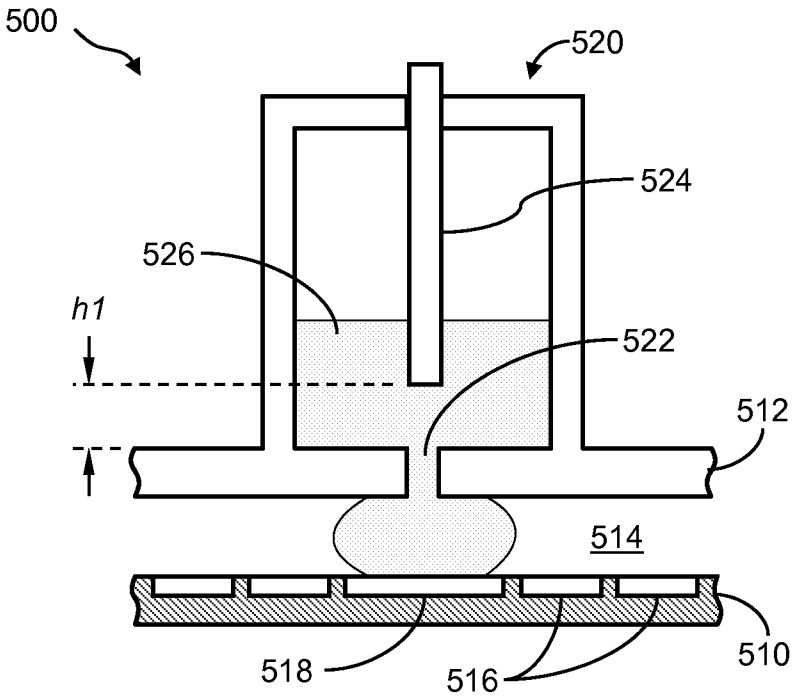


Figure 5D

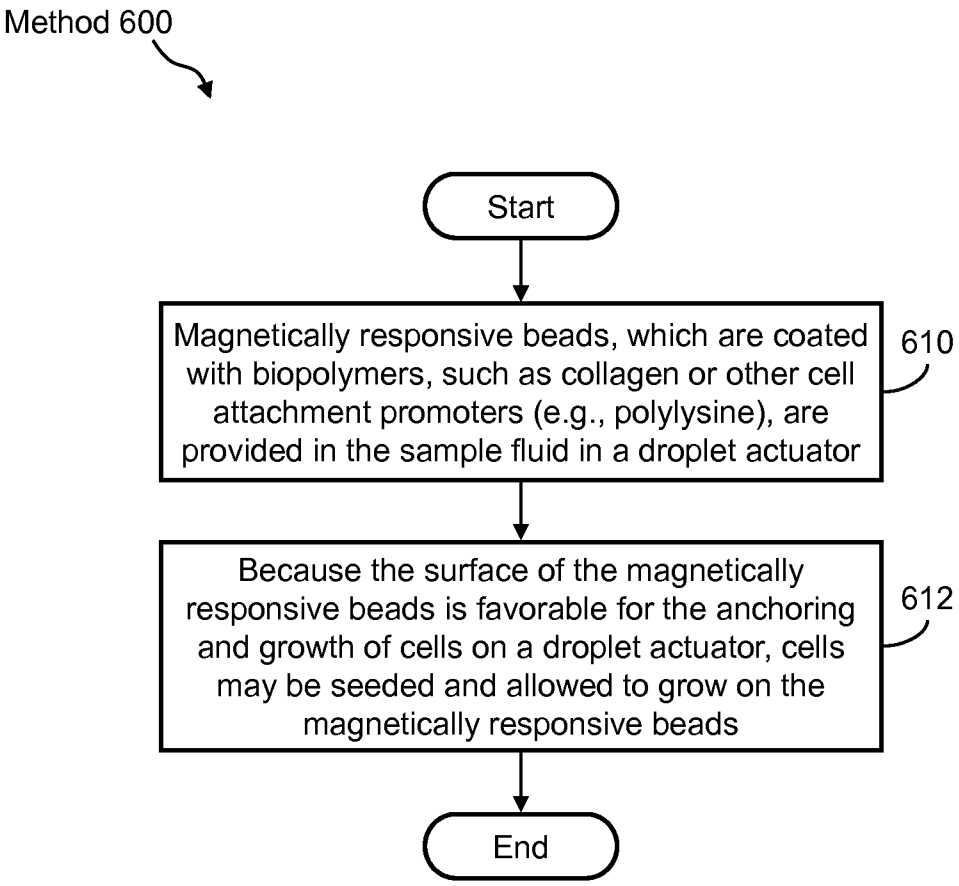
Method 600
Start
Magnetically responsive beads, which are coated with biopolymers, such as collagen or other cell attachment promoters (e.g., polylysine), are provided in the sample fluid in a droplet actuator
610
Because the surface of the magnetically responsive beads is favorable for the anchoring and growth of cells on a droplet actuator, cells may be seeded and allowed to grow on the magnetically responsive beads
612
End

**Figure 6**

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/US2012/070055**

**A. CLASSIFICATION OF SUBJECT MATTER**

***GOIN 33/50(2006. 01)i, GOIN 33/53(2006.01)1, GOIN 35/08(2006.01)1, GOIN 1/10(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 33/50; C07K 1/26; GOIN 27/26; B01J 19/00; C12M 3/00; C12Q 1/68

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:droplet, modified reservoir, chromatography, purified sample, beads, hollow tube, blood

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>A | US 2010-0282608 Al (SRINIVASAN et al.) 11 November 2010<br>See paragraphs [0021],[0032]-[0033],[0035],[0050],[0060]; and figure IB. | 1,4,7,9<br>2,3,8,10,13-24,28-33,39-41,45-48 |
| A | EP 2016091 Bl (ADVANCED LIQUID LOGIC, INC.) 08 December 2010<br>See claims 1,3; and paragraphs [0034],[0044],[0050],[0075],[0082],[0139]. | 1-4,7-10,13-24,28-33,39-41,45-48 |
| A | SHIKIDA et al., Using wettability and interfacial tension to handle droplets of magnetic beads in a micro-chemical-analysis system, Sensors and Actuators B : Chemical, 2006, Vol.113, pp. 563-569<br>See pages 564-565; and figure 2. | 1-4,7-10,13-24,28-33,39-41,45-48 |
| A | CIFTLIK et al., A direct injection method for blood cells into microchannels from pure blood droplets with switchable in-situ distillation of erythrocytes, IEEE Research in Microelectronics and Eletronics, 2008, pp. 29-32<br>See pages 29-30. | 1-4,7-10,13-24,28-33,39-41,45-48 |
| A | Sista et al., Heterogeneous immunoassays using magnetic beads on a digital microfluidic platform, Lab chip, 2009, pp. 2188-2196<br>See pages 1, 3; and figures 1-2. | 1-4,7-10,13-24,28-33,39-41,45-48 |

☒ 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
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
CHANG, Bong Ho
Telephone No. 82-42-481-3353

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

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/US2012/070055**

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 2007-0184463 Al (MOLHO et al.) 09 September 2007<br>See claims 1,2,4,5; and paragraph [0010] . | 1-4 ,7-10 , 13-24 ,28-33 ,39-41 ,45-48 |

# INTERNATIONAL SEARCH REPORT

International application No.
**PCT/US2012/070055**

**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.: **26, 38**
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:

Claims 26 and 38 refer to multiple dependent claims that refer to other multiple dependent claims. Therefore, the meaning of the technical feature to which they refer are vague and unclear.

3. ☒ Claims Nos.: **5,6, 11, 12, 25, 27, 34-37, 42-44, 49**
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:

Group I: Claims 1-12 are directed to a modified surface of reservoir of droplet actuator and a method of preparing plasma using the modified reservoir.

Group II: Claims 13-18 are directed to a method of preparing plasma by adding a lectin to a sample.

Group III: Claims 19-38 are directed to a method of preparing plasma in the reservoir of droplet acator using a chromatography.

Group IV: Claims 39-44 are directed to a reservoir of droplet actuator comprising a hollow tube.

Group V: Claims 45-49 are directed to a method of processing cell in the reservoir of droplet actuator.

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**

Information on patent family members

International application No.

**PCT/US2012/070055**

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| | | WO 2009-032863 A3 | 02.07.2009 |
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| | | AT 542921 T | 15.02.2012 |
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| | | CA 2680532 A1 | 25.10.2007 |
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| | | EP 2016091 A4 | 09.09.2009 |
| | | EP 2016189 A2 | 21.01.2009 |
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| | | EP 2016189 B1 | 25.01.2012 |
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