



US 20100194408A1

(19) **United States**

(12) **Patent Application Publication**
Sturmer et al.

(10) **Pub. No.: US 2010/0194408 A1**

(43) **Pub. Date: Aug. 5, 2010**

(54) **CAPACITANCE DETECTION IN A DROPLET ACTUATOR**

Related U.S. Application Data

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(60) Provisional application No. 60/889,966, filed on Feb. 15, 2007, provisional application No. 60/980,520, filed on Oct. 17, 2007, provisional application No. 60/980,746, filed on Oct. 17, 2007.

Publication Classification

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(51) **Int. Cl.**
G01R 27/26 (2006.01)
(52) **U.S. Cl.** **324/664**

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(57) **ABSTRACT**

(21) Appl. No.: **12/527,208**

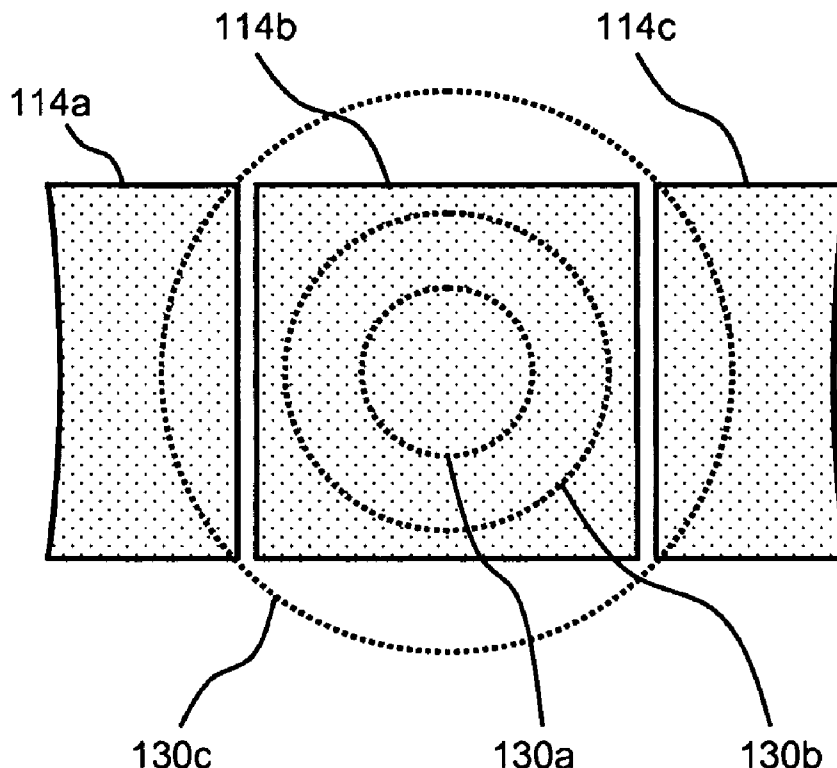
(22) PCT Filed: **Feb. 15, 2008**

(86) PCT No.: **PCT/US08/54134**

§ 371 (c)(1),
(2), (4) Date: **Apr. 19, 2010**

A method, circuit and apparatus for detecting capacitance on a droplet actuator, inter alia, for determining the presence, partial presence or absence of a droplet at an electrode on a droplet actuator by: (a) providing a droplet actuator comprising: (i) a substrate comprising electrodes arranged on the substrate for conducting droplet operations on a surface of the substrate; (ii) a capacitance detection circuit for detecting capacitance at the droplet operations surface at one or more of the electrodes; (b) detecting capacitance at the droplet operations surface at one or more of the electrodes; and (c) determining from the capacitance the presence, partial presence or absence of a droplet at the droplet operations surface at the electrode.

100 →



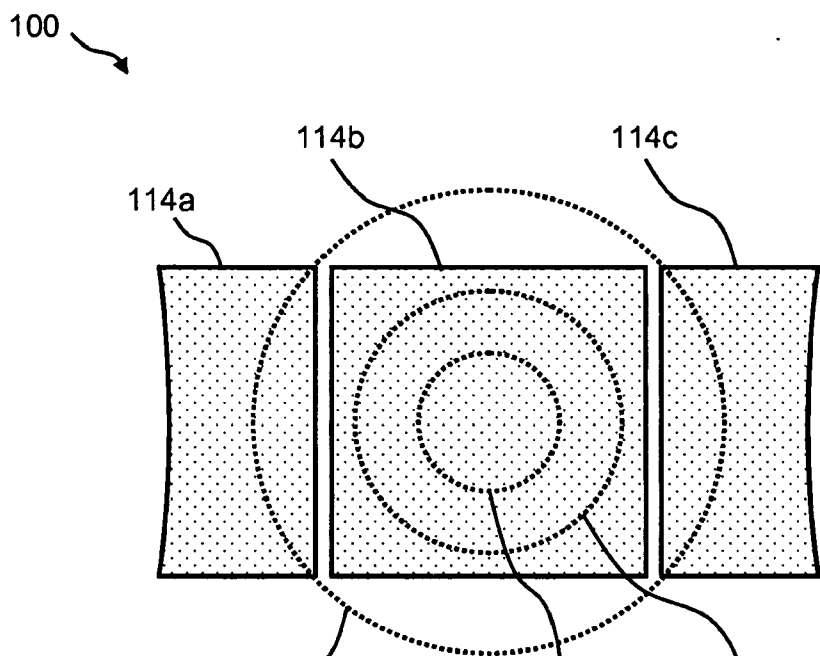


Figure 1A

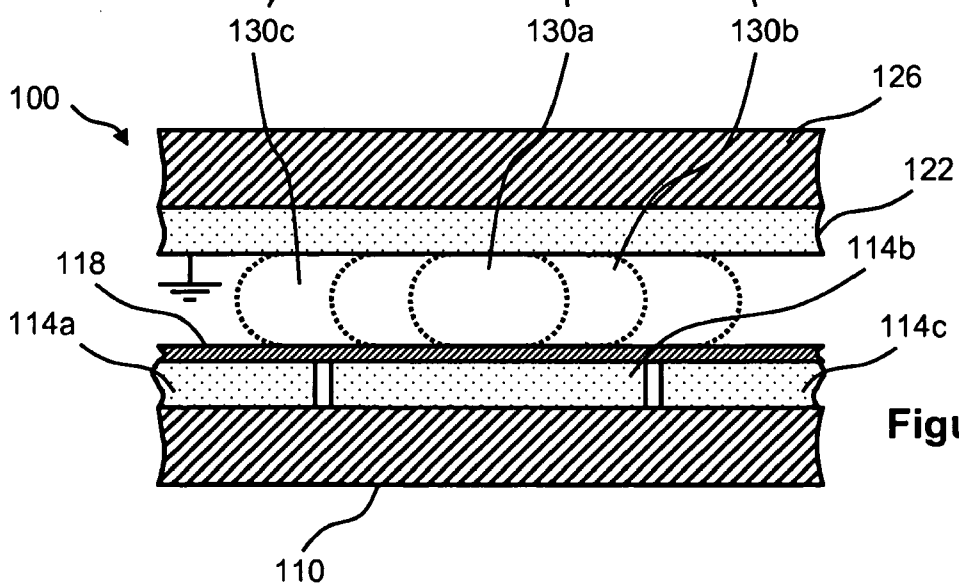


Figure 1B

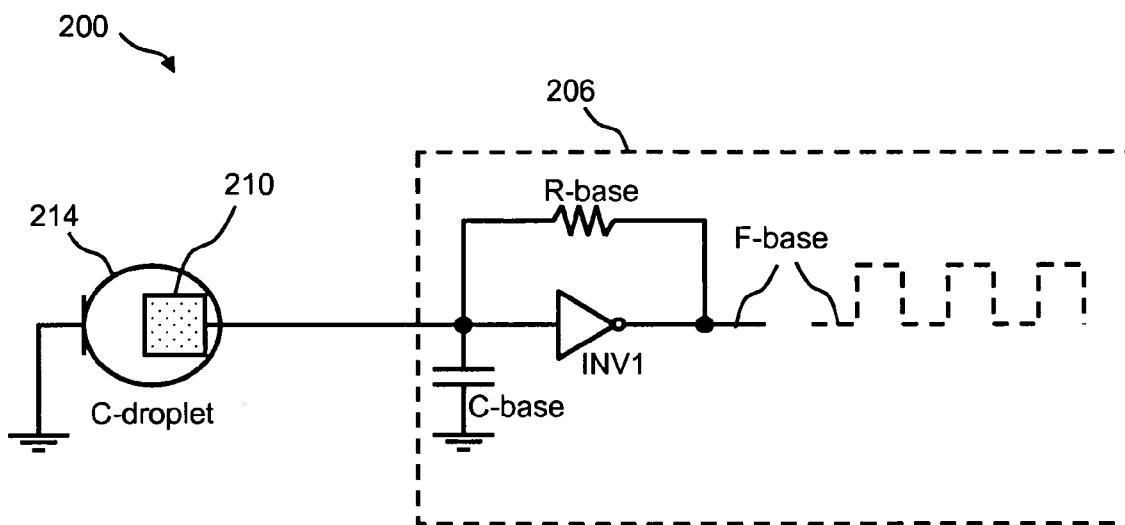


Figure 2

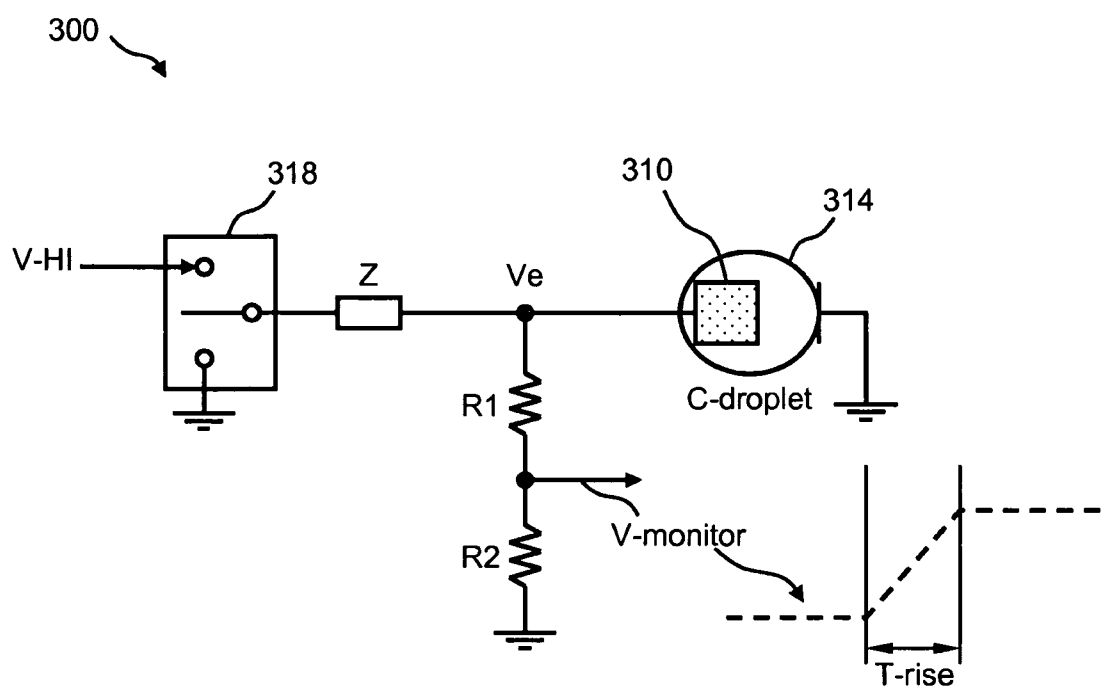


Figure 3

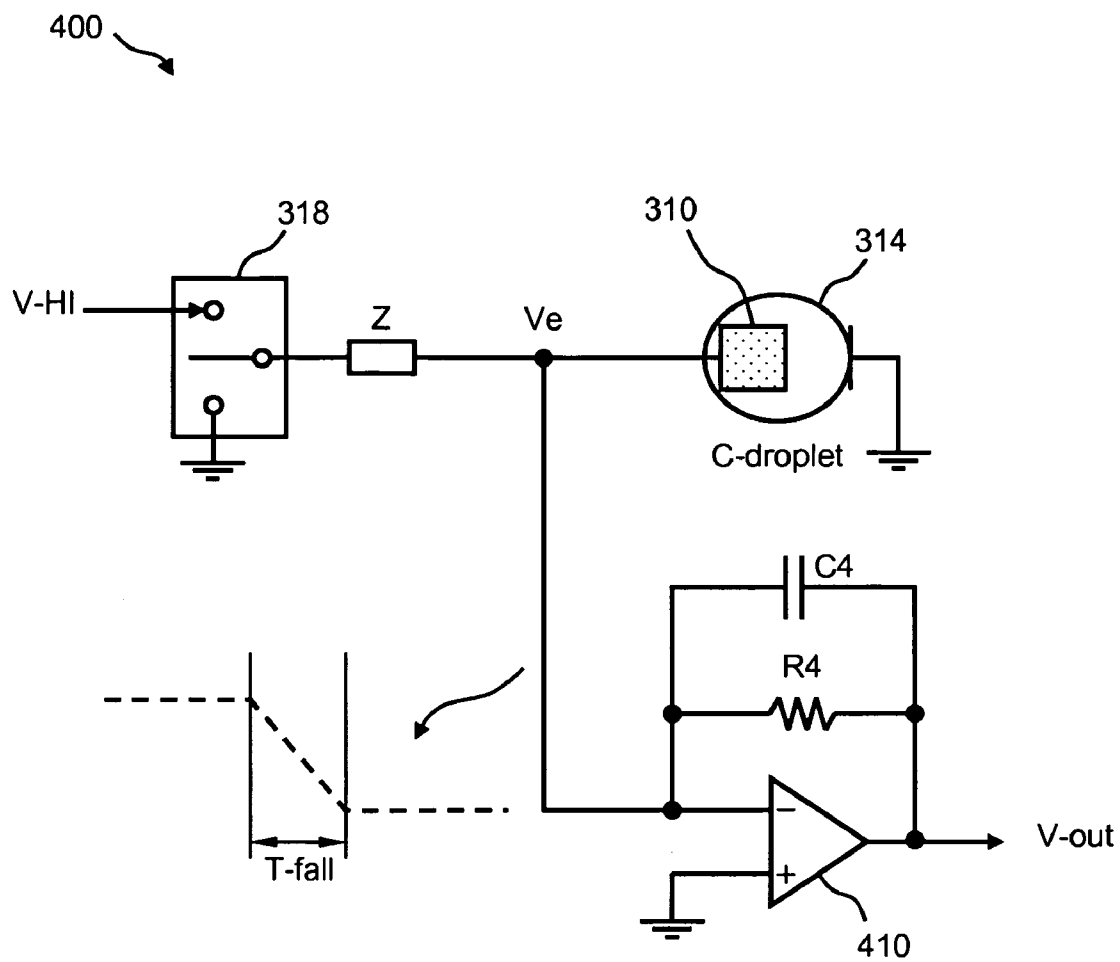


Figure 4

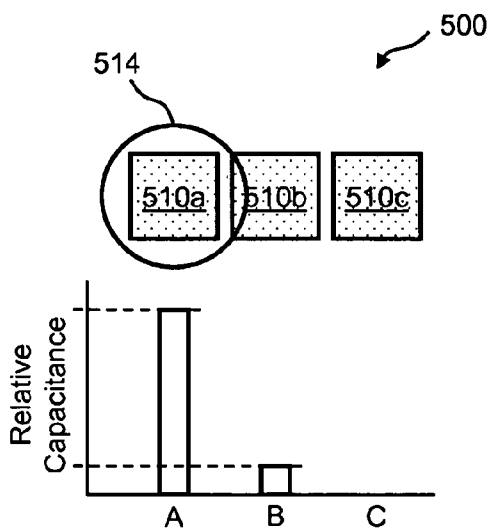


Figure 5A

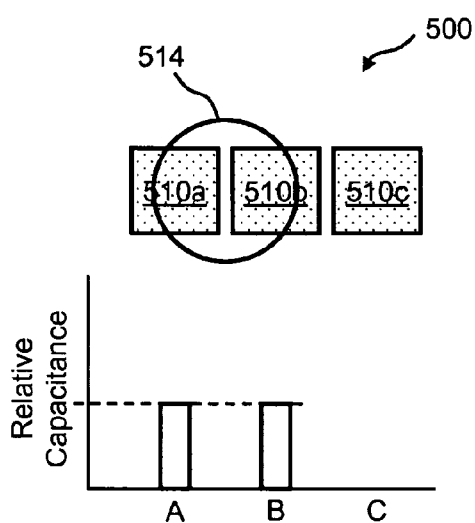


Figure 5B

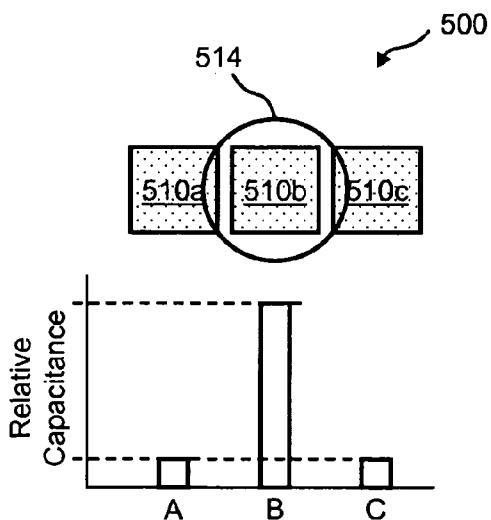


Figure 5C

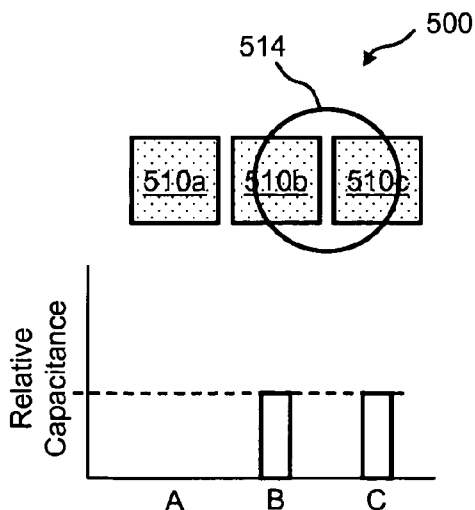


Figure 5D

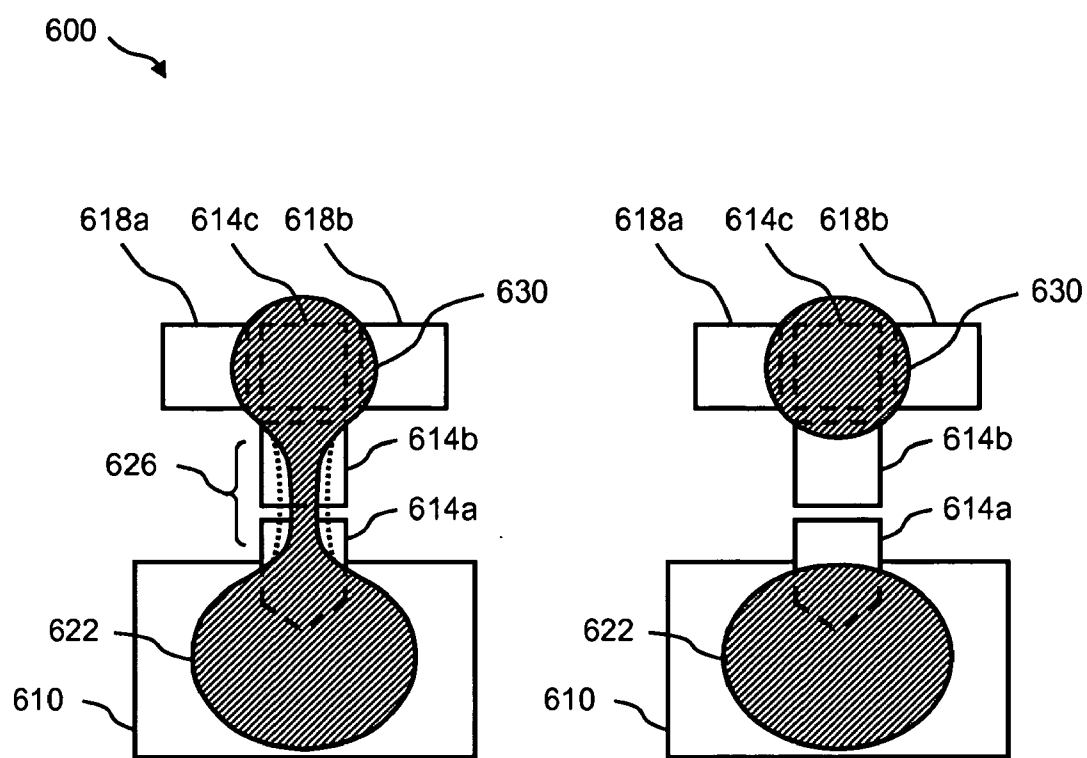


Figure 6A

Figure 6B

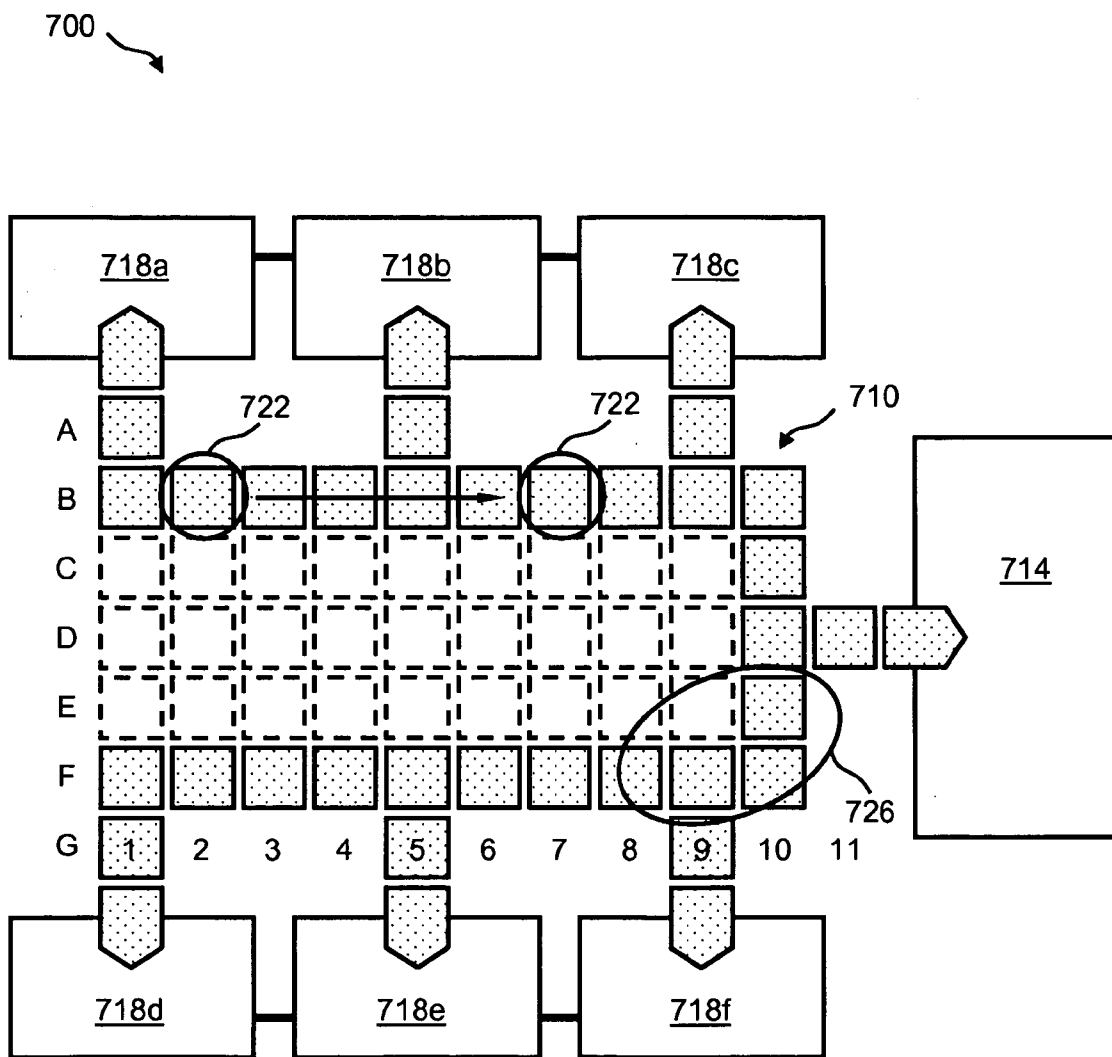


Figure 7

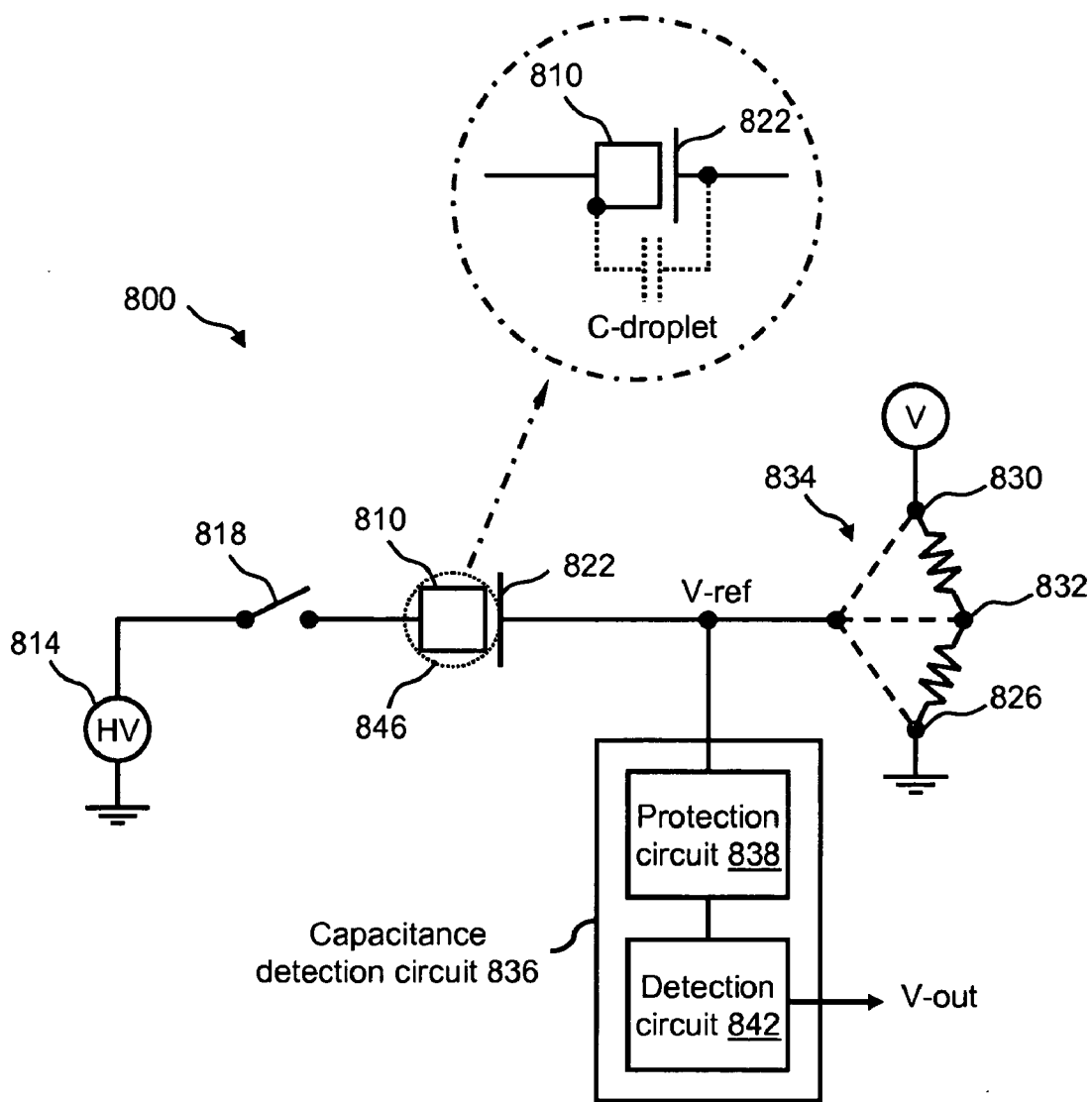


Figure 8

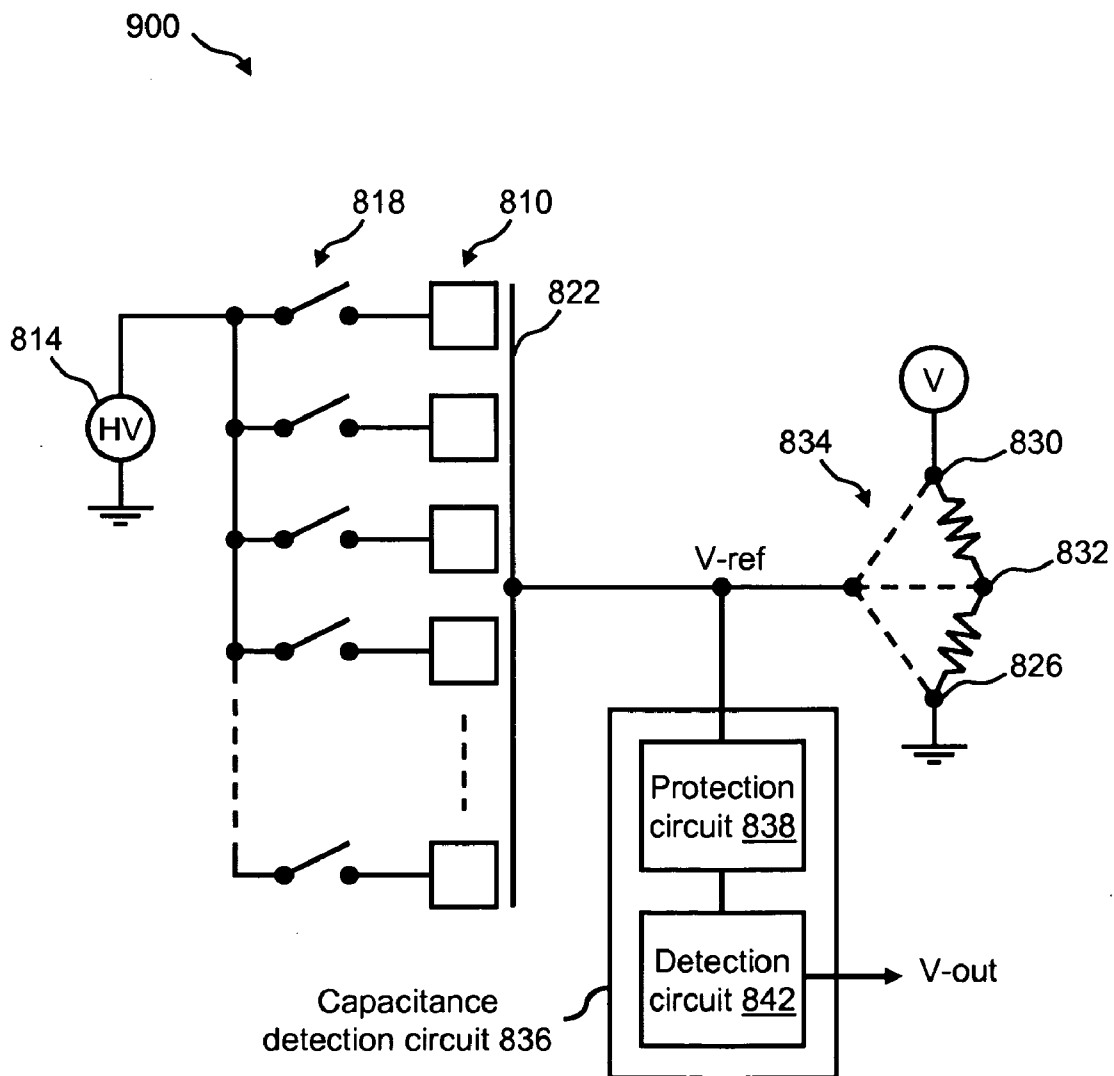


Figure 9

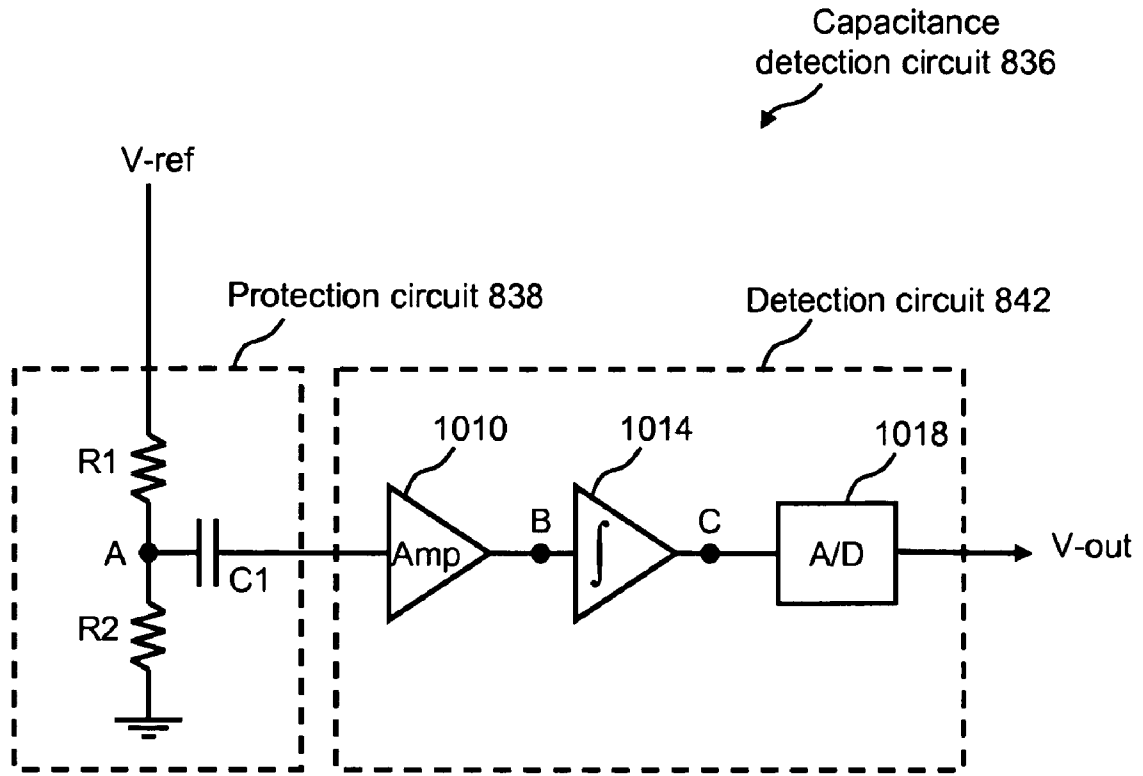


Figure 10A

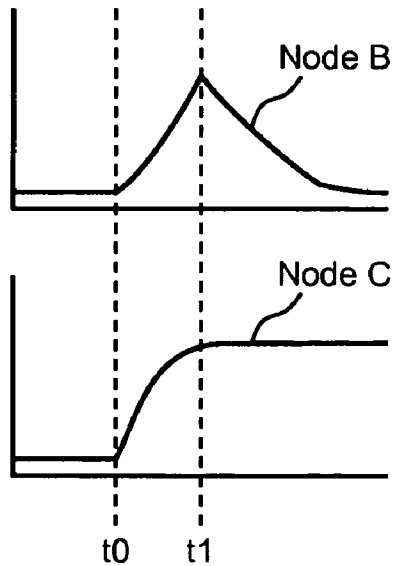


Figure 10B

CAPACITANCE DETECTION IN A DROPLET ACTUATOR

1 RELATED APPLICATIONS

[0001] This application claims priority to and incorporates by reference provisional U.S. patent application Nos. 60/889,966, filed on Feb. 15, 2007, entitled “Capacitance detection in a droplet actuator”; 60/980,520, filed on Oct. 17, 2007, entitled “Capacitance detection in a droplet actuator”; and 60/980,746, filed on Oct. 17, 2007, entitled “Capacitance detection in a droplet actuator.”

2 GRANT INFORMATION

[0002] This invention was made with government support DK066956-02 and GM072155-02 awarded by the National Institutes of Health of the United States. The government has certain rights in the invention.

3 FIELD OF THE INVENTION

[0003] The present invention generally relates to the field of conducting droplet operations in a droplet actuator. In particular, the present invention is directed to apparatus and methods for capacitance detection in a droplet actuator.

4 BACKGROUND OF THE INVENTION

[0004] Droplet actuators are used to conduct a wide variety of droplet operations. A droplet actuator typically includes two plates separated by a space. The plates include electrodes for conducting droplet operations. The space is typically filled with a filler fluid that is immiscible with the fluid that is to be manipulated on the droplet actuator. A droplet on the droplet actuator is separated from one or more of the electrodes by a dielectric layer. The droplet may be grounded. For a variety of reasons described more fully herein, it may be useful to measure the capacitance of the dielectric layer between the electrode(s) and the droplet.

5 BRIEF DESCRIPTION OF THE INVENTION

[0005] The invention provides example methods of performing capacitance detection on a droplet actuator. A capacitor may be formed by the combination of a conductive droplet, an insulator layer, and one or more transport electrodes within a droplet actuator. At any given electrode, the capacitance measured is proportional to the footprint area of a droplet thereon. In some embodiments, the capacitance detection methods described herein may be used as a real-time verification tool in order to detect the absence, presence, and/or partial presence of a droplet at an electrode; analysis of droplet properties; measurement of droplet size or volume; optimization of the speed of droplet operations; and detection of air bubbles.

[0006] Additionally, the invention provides a capacitance detection circuit, droplet actuator chips and systems comprising the circuit, and related methods. The circuit is useful for performing capacitance detection in a droplet actuator. Capacitance detection permits analysis of a variety of operations in a droplet actuator. For example, capacitance detection may be used to determine at a designated location whether a droplet is present, partially present or absent. Capacitance at the location will vary depending on the presence, partial presence or absence of the droplet. This capability provides, among other things, a means of verifying whether a certain

droplet operation or protocol is progressing as expected. Additionally, by use of existing droplet actuator infrastructures, such as the existing voltage reference electrode of the top plate, which is common to all electrodes of the bottom plate, and the existing droplet actuation control switches, the invention facilitates the use of a single detection circuit for performing capacitance measurements at multiple electrodes.

6 DEFINITIONS

[0007] As used herein, the following terms have the meanings indicated.

[0008] “Activate” with reference to one or more electrodes means effecting a change in the electrical state of the one or more electrodes which results in a droplet operation.

[0009] “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 and other three dimensional shapes. The bead may, for example, be capable of being transported 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 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 or one component only 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 magnetically responsive beads are described in U.S. Patent Publication No. 2005-0260686, entitled, “Multiplex flow assays preferably with magnetic particles as solid phase,” published on Nov. 24, 2005, the entire disclosure of which is incorporated herein by reference for its teaching concerning magnetically responsive materials and beads. The beads may include one or more populations of biological cells adhered thereto. In some cases, the biological cells are a substantially pure population. In other cases, the biological cells include different cell populations, e.g., cell populations which interact with one another.

[0010] “Droplet” means a volume of liquid on a droplet actuator that is at least partially bounded by filler fluid. For example, a droplet may be completely surrounded by filler fluid or may be bounded by filler fluid and one or more surfaces of the droplet actuator. 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, 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.

[0011] “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 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 size of the resulting droplets (i.e., the size 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.

[0012] “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 to permit execution of a splitting operation on a droplet, yielding one droplet with substantially all of the beads and one droplet substantially lacking in the beads.

[0013] “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 , $\text{BaFe}_{12}\text{O}_{19}$, CoO , NiO , Mn_2O_3 , Cr_2O_3 , and CoMnP .

[0014] “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 partially complete, 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 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. Other embodiments are described elsewhere herein, and still others will be immediately apparent in view of the present disclosure.

Examples of suitable approaches to washing include, without limitation, those described in U.S. patent application Nos. 60/900,653, filed on Feb. 9, 2007, entitled “Immobilization of magnetically-responsive beads during droplet operations”; 60/980,772, filed on Oct. 17, 2007, entitled “Immobilization of magnetically-responsive beads in droplets”; 60/969,736, filed on Sep. 4, 2007, entitled “Droplet actuator assay improvements”; and 60/980,762, filed on Oct. 17, 2007, entitled “Droplet actuator assay improvements”; and International Patent Application No. International Patent Application No. PCT/US2006/47486, filed on Dec. 11, 2006, entitled “Droplet-Based Biochemistry.”

[0015] The terms “top” and “bottom” are used throughout the description with reference to the top and bottom substrates of the droplet actuator for convenience only, since the droplet actuator is functional regardless of its position in space.

[0016] When a given component, such as a layer, region or substrate, is referred to herein as being disposed or formed “on” another component, that given component can be directly on the other component or, alternatively, intervening components (for example, one or more coatings, layers, interlayers, electrodes or contacts) can also be present. It will be further understood that the terms “disposed on” and “formed on” are used interchangeably to describe how a given component is positioned or situated in relation to another component. Hence, the terms “disposed on” and “formed on” are not intended to introduce any limitations relating to particular methods of material transport, deposition, or fabrication.

[0017] 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.

[0018] 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.

7 BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIGS. 1A and 1B illustrate a top view and side view, respectively, of a droplet actuator;

[0020] FIG. 2 illustrates a nonlimiting example of a capacitance detection circuit for determining C-droplet;

[0021] FIG. 3 illustrates another nonlimiting example of a capacitance detection circuit for determining the capacitance of a droplet within a droplet actuator;

[0022] FIG. 4 illustrates yet another nonlimiting example of a capacitance detection circuit for determining the capacitance of a droplet within a droplet actuator;

[0023] FIGS. 5A, 5B, 5C, and 5D illustrate a nonlimiting example of using capacitance detection in a droplet actuator;

[0024] FIGS. 6A and 6B illustrate another nonlimiting example of using capacitance detection in a droplet actuator;

[0025] FIG. 7 illustrates yet another nonlimiting example of using capacitance detection in a droplet actuator;

[0026] FIG. 8 illustrates a schematic diagram of an embodiment of a droplet actuation circuit of the invention;

[0027] FIG. 9 illustrates a schematic diagram of an embodiment of a droplet actuation circuit that includes a capacitance detection circuit;

[0028] FIG. 10A illustrates a schematic diagram of an embodiment of a capacitance detection circuit of the invention that may be used in a droplet actuator for the purpose of performing droplet detection; and

[0029] FIG. 10B illustrates an input voltage curve and an output voltage curve of a charge integrating amplifier that is suitable for use in the capacitance detection circuit of the invention;

8 DETAILED DESCRIPTION OF THE INVENTION

[0030] The present invention relates to a droplet actuator configured to detect capacitance of fluids loaded thereon and to methods of making and using such a droplet actuator.

8.1 Capacitance Detection Circuits

[0031] FIGS. 1A and 1B illustrate a top view and side view, respectively, of a droplet actuator 100. Droplet actuator 100 includes a first substrate 110, which may be, for example, a glass substrate or a printed circuit board; a plurality of electrodes 114, such as electrodes 114a, 114b, and 114c; an insulator layer 118, which may be, for example, a hydrophobic dielectric layer, and a reference electrode 122 disposed upon a second substrate 126, which may be, for example, a glass substrate. In one example, the plurality of electrodes 114 may include a grid or array of electrodes 114, and the reference electrode 122 may be substantially equidistant from each of the actuator electrodes, wherein the measurement at each of the actuator electrodes in the grid or array may be determined based on the single reference electrode.

[0032] A gap between insulator layer 118 and reference electrode 122 forms a fluid path through which one or more droplets of various size and/or footprint may flow. A droplet positioned in the gap between insulator layer 118 and reference electrode 122 at the position of electrode 114b displaces a portion of the filler fluid (e.g. air, silicone oil) that would otherwise occupy that space and therefore results in a change in capacitance measured between electrode 114b and reference electrode 122. A non-conductive droplet results in a change in measured capacitance if the dielectric properties of the droplet differ from the medium being displaced. For example, an oil droplet displacing air filler within the gap at the position of electrode 114b would result in an increased measured capacitance because the dielectric constant of oil is typically higher than air. Similarly, the introduction of an air bubble at the position of electrode 114b when the actuator is filled with oil would reduce the capacitance measured between electrode 114b and reference electrode 122. Because the capacitance contributed by the combination of droplet/bubble/filler within the gap is arranged in series with the capacitance contributed by solid dielectric 118, the relative magnitude of the change in capacitance would depend on the properties of dielectric 118 as well as any other capacitances in the system. It is also noted that presence of filler liquid trapped between the droplet and either of the actuator surfaces could also affect the measured capacitance.

[0033] When the droplet positioned between electrode 114b and reference 122 is substantially conductive and is in

electrical communication with reference 122, then another capacitive effect is observed. In this case, the droplet effectively “shorts-out” the capacitor formed by the filler liquid between the surface of dielectric 118 and reference 122. That is, the capacitive contribution of the liquid layer at the position of the droplet is effectively reduced such that the dielectric 118 contributes substantially all of the capacitance measured between electrode 114b and reference 122 at the position of the droplet. The capacitance associated with the overlap of the droplet and electrode is arranged in parallel with the capacitance associated with the portions of electrode 114b not overlapping the droplet and being covered with filler fluid. There is a certain amount of capacitance associated with the droplet fully covering the electrode and a certain amount of capacitance associated with the droplet being fully absent from the electrode. Between these two extremes the amount of capacitance measured is proportional to the amount of overlap between the droplet and electrode. Although fringing electrical fields exist at the electrode edges, in most cases the contribution of these fields can be neglected so the measured capacitance is directly proportional to the amount of overlap. The total amount of area included in the overlap between the base of the droplet and the surface of the dielectric at the position of an electrode is referred to as the footprint of the droplet.

[0034] In one example, FIGS. 1A and 1B show a droplet 130a that is fully contained within the lateral extent of electrode 114b and that forms a certain footprint on electrode 114b; droplet 130b that is of a certain larger footprint than droplet 130a and which has a size that is roughly proportional to the size of electrode 114b; and droplet 130c that is of a certain larger footprint than both droplets 130a and 130b and is atop electrode 114b and overlaps onto adjacent electrodes 114a and 114c.

[0035] The combination of an insulator layer that is arranged between a conductive droplet, which may be connected to a reference potential, and another conductive layer effectively forms a parallel plate capacitor. More specifically and referring again to FIGS. 1A and 1B, insulator layer 118, which is the dielectric layer, is arranged between droplet 130a, 130b, or 130c, which has a certain amount of electrical conductivity, and one or more electrodes 114, thereby forming a plate capacitor. Droplet 130a, 130b, or 130c may be electrically connected to a reference electrode 122 and electrodes 114 may be electrically connected to a bias voltage. It is further understood that in other embodiments, the reference electrode can be in a co-planar relationship with the electrodes.

[0036] The amount of capacitance C-droplet measured due to the presence or absence of a droplet is a function of the droplet footprint area on that electrode. Because capacitance $C = \epsilon(A/d)$; where C is the capacitance in farads, F; ϵ is the permittivity of the insulator used; A is the area of each plate (in square meters); and d is the separation between the plates (in meters). Therefore and referring again to FIGS. 1A and 1B, the area of the footprint of droplet 130c on electrode 11b > the area of the footprint of droplet 130b on electrode 114b > the area of the footprint of droplet 130a on electrode 114b and, thus, the capacitance measured between droplet 130c and electrode 114b > the capacitance measured between droplet 130b and electrode 114b > the capacitance measured between droplet 130a and electrode 114b.

[0037] In the situation where a fluid droplet is located over the actuator electrode, a processor, for example, can initiate a

measurement of an amount by which a portion of the droplet overlaps the actuator electrode.

[0038] FIG. 2 illustrates a nonlimiting example of a capacitance detection circuit 200 for determining C-droplet. In particular, capacitance detection circuit 200 performs an active capacitance measurement by providing a reference signal that is applied to an electrode.

[0039] For example, capacitance detection circuit 200 includes a ring oscillator circuit 206 that is formed of an inverter INV1 in combination with a base resistance R-base and a base capacitance C-base, which are arranged as shown in FIG. 2. Resistance R-base and capacitance C-base form an RC circuit that determines a base oscillation frequency F-base. The input of ring oscillator circuit 206 is electrically connected to an electrode 210 upon which may be disposed on droplet 214, which may be connected to a reference potential. The droplet, such as droplet 214, controls a certain capacitance C-droplet between sensing electrode 210 and the reference potential that is in parallel with capacitance C-base. Consequently the capacitance C-droplet adds to capacitance C-base, which controls the frequency F-base. A change in frequency F-base, which is the result of a change in capacitance C-droplet due to motion of the droplet 210, may be measurable by, for example, a pulse counter (not shown) that is connected to the output of ring oscillator circuit 206. The change in frequency F-base is inversely proportional to the change in capacitance C-droplet, i.e., the frequency F-base decreases as capacitance C-droplet increases. By calculating the difference between frequency F-base with and without the droplet present, a capacitance value may be determined, which may be correlated to the absence, presence, and/or partial presence of, for example, droplet 214 at electrode 210. Note that in this example, electrode 210 may be either biased or unbiased during the capacitance measurement.

[0040] FIG. 3 illustrates another nonlimiting example of a capacitance detection circuit 300 for determining the capacitance of a droplet within a droplet actuator. In particular, capacitance detection circuit 300 performs a passive capacitance measurement by monitoring the charge time of capacitance C-droplet. For example, capacitance detection circuit 300 includes a transport electrode 310 upon which may be disposed a droplet 314, which may be grounded. When droplet 314 is fully or partially present it has a capacitance C-droplet. The control line of transport electrode 310 has a certain impedance Z and may be connected to either a bias voltage V-HI or to ground via a switch 318. Switch 318 may be any electronic switch mechanism.

[0041] When droplet 314 is fully or partially present, capacitance C-droplet is charged when transport electrode 310 is connected to bias voltage V-HI. By contrast, capacitance C-droplet is discharged when transport electrode 310 is connected to ground. An electrode voltage V_e , which may be a high voltage, at transport electrode 310 may be monitored by use of a voltage divider circuit, in order to provide a low voltage monitor. In one example, a resistor R1 and R2 are arranged in series between electrode voltage V_e and ground, and a voltage V-monitor is provided at a node between resistors R1 and R2. A rise time T-rise of voltage V-monitor when transport electrode 310 is switched from ground to bias voltage V-HI may be monitored. Consequently, when droplet 314 is fully or partially present at transport electrode 310, the capacitance C-droplet that is introduced causes the rise time T-rise of voltage V-monitor to increase. The change in T-rise, which is the result of introducing capacitance C-droplet, may

be measurable by, for example, an analog-to-digital (A/D) converter (not shown) that is connected to voltage V-monitor. The change in T-rise at voltage V-monitor is proportional to the amount of capacitance C-droplet, i.e., T-rise increases as capacitance C-droplet increases. By calculating the difference between T-rise at voltage V-monitor with and without capacitance C-droplet present, a capacitance C-droplet value may be determined, which may be correlated to the absence, presence, and/or partial presence of, for example, droplet 314 at transport electrode 310.

[0042] FIG. 4 illustrates yet another nonlimiting example of a capacitance detection circuit 400 for determining the capacitance of a droplet within a droplet actuator. In particular, capacitance detection circuit 400 performs a passive capacitance measurement by monitoring the discharge time of capacitance C-droplet. For example, capacitance detection circuit 400 is substantially the same as capacitance detection circuit 300 of FIG. 3 except that it does not include a voltage divider circuit. Instead, electrode voltage V_e of capacitance detection circuit 400 is monitored directly via a charge integrating amplifier 410, which outputs a voltage V-out that is the integral of its input voltage. However, alternatively, the elements of capacitance detection circuit 300 and capacitance detection circuit 400 may be combined.

[0043] Transport electrode 310 is first connected to bias voltage V-HI via switch 318 for a period of time that allows capacitance C-droplet to be fully charged to a certain voltage. After capacitance C-droplet is fully charged, transport electrode 310 is then connected to ground via switch 318, which discharges capacitance C-droplet and, thus, electrode voltage V_e falls from the certain voltage to ground with a fall time of T-fall. Consequently, when droplet 314 is fully or partially present at transport electrode 310, the capacitance C-droplet that is introduced causes the fall time T-fall of electrode voltage V_e to increase. The integral of T-fall may be analyzed at V-out of charge integrating amplifier 410 by, for example, an A/D converter (not shown). The change in T-fall of electrode voltage V_e is proportional to the amount of capacitance C-droplet, i.e., T-fall increases as capacitance C-droplet increases. By calculating the difference between T-fall of electrode voltage V_e with and without capacitance C-droplet present, a capacitance C-droplet value may be determined, which may be correlated to the absence, presence, and/or partial presence of, for example, droplet 314 at transport electrode 310.

8.2 Uses of Capacitance Detection

[0044] Capacitance detection in a droplet actuator can be employed to affect a variety of useful results. Examples follow.

8.2.1 Analysis of Basic Microfluidic Functions

[0045] FIGS. 5A, 5B, 5C, and 5D illustrate a nonlimiting example of using capacitance detection in a droplet actuator. More specifically, FIGS. 5A, 5B, 5C, and 5D illustrate a set of nonlimiting exemplary steps of a droplet operation process 500, which demonstrates a simple inexpensive analysis of basic microfluidic functions by use of capacitance detection. In particular, FIGS. 5A, 5B, 5C, and 5D show the real-time progression of an exemplary droplet 514 moving along a line of transport electrodes 510, such as transport electrodes 510a, 510b, and 510c. In this example, each of transport electrodes 510a, 510b, and 510c are connected to a capacitance detec-

tion mechanism, such as, but not limited to, capacitance detection circuit 200 of FIG. 2, capacitance detection circuit 300 of FIG. 3, and capacitance detection circuit 400 of FIG. 4, for measuring the capacitance C-droplet. In doing so, the absence, presence, partial presence, and/or location of droplet 514 along the line of transport electrodes 510 may be determined in real time. For each step shown by FIGS. 5A, 5B, 5C, and 5D, respectively, a bar graph of the relative capacitance C-droplet at each of transport electrodes 510a, 510b, and 510c is provided.

[0046] FIG. 5A shows droplet 514 at a first location along the line of transport electrodes 510a, 510b, and 510c. More specifically, droplet 514 is centered upon transport electrode 510a and shows that the footprint area of droplet 514 is larger than the area of transport electrode 510a. Therefore, while droplet 514 is centered upon transport electrode 510a, it also overlaps slightly the adjacent transport electrode 510b. The bar graph for FIG. 5A of the relative amount of capacitance C-droplet shows that maximum capacitance C-droplet is detected at transport electrode 510a, a small capacitance C-droplet is detected at transport electrode 510b, and no capacitance C-droplet is detected at transport electrode 510c. As a result, without the need for visualization, it may be concluded that the location of droplet 514 is substantially at transport electrode 510a.

[0047] FIG. 5B shows droplet 514 at a second location along the line of transport electrodes 510a, 510b, and 510c. More specifically, droplet 514 is bridging transport electrodes 510a and 510b. Therefore, a substantially equal portion of droplet 514 is upon each of transport electrodes 510a and 510b. The bar graph for FIG. 5B of the relative amount of capacitance C-droplet shows that approximately half the maximum capacitance C-droplet is detected at each of transport electrodes 510a and 510b and no capacitance C-droplet is detected at transport electrode 510c. As a result, without the need for visualization, it may be concluded that the movement of droplet 514 from transport electrode 510a to 510b is progressing as expected.

[0048] FIG. 5C shows droplet 514 at a third location along the line of transport electrodes 510a, 510b, and 510c. More specifically, droplet 514 is centered upon transport electrode 510b and shows that the footprint area of droplet 514 is larger than the area of transport electrode 510b. Therefore, while droplet 514 is centered upon transport electrode 510b, it also overlaps slightly the adjacent transport electrodes 510a and 510c. The bar graph for FIG. 5C of the relative amount of capacitance C-droplet shows that a small amount of capacitance C-droplet is detected at transport electrode 510a, maximum capacitance C-droplet is detected at transport electrode 510b, and a small amount of capacitance C-droplet is detected at transport electrode 510c. As a result, without the need for visualization, it may be concluded that the movement of droplet 514 to substantially the position of transport electrode 510b has occurred as expected.

[0049] FIG. 5D shows droplet 514 at a fourth location along the line of transport electrodes 510a, 510b, and 510c. More specifically, droplet 514 is bridging transport electrodes 510b and 510c. Therefore, a substantially equal portion of droplet 514 is upon each of transport electrodes 510b and 510c. The bar graph for FIG. 5D of the relative amount of capacitance C-droplet shows that no capacitance C-droplet is detected at transport electrode 510a and approximately half the maximum capacitance C-droplet is detected at each of transport electrodes 510b and 510c. As a result, without the need for

visualization, it may be concluded that the movement of droplet 514 from transport electrode 510b to 510c is progressing as expected.

8.2.2 Droplet Splitting Operations

[0050] FIGS. 6A and 6B illustrate another nonlimiting example of using capacitance detection in a droplet actuator. More specifically, FIGS. 6A and 6B illustrate a nonlimiting example of a droplet actuator 600 that uses capacitance detection in a droplet splitting operation for determining droplet uniformity. In particular, FIG. 6A shows the droplet splitting operation in progress and FIG. 6B shows the droplet splitting operation when complete. Droplet actuator 600 includes a reservoir electrode 610 that outlets to a line of transport electrodes 614a, 614b, and 614c. Adjacent to and on either side of transport electrode 614c is a transport electrode 618a and 618b. In this example, each of transport electrodes 614a, 614b, 614c, 618a and 618b are connected to a capacitance detection mechanism, such as, but not limited to, capacitance detection circuit 200 of FIG. 2, capacitance detection circuit 300 of FIG. 3, and capacitance detection circuit 400 of FIG. 4, for detecting the capacitance C-droplet.

[0051] Referring again to FIGS. 6A and 6B, a volume of fluid 622 is provided at reservoir electrode 610. During the droplet splitting operation, transport electrode 614c is activated and fluid 622 from reservoir electrode 610 is pinched off across a split zone 626 along transport electrodes 614a and 614b to form a droplet 630 at transport electrode 614c. The size of droplet 630 may vary, for example, because as the volume of fluid 622 at reservoir electrode 610 varies, the amount of fluid pinched off may vary. However, capacitance detection may be used in order to monitor the droplet splitting operation and provide uniform droplet dispensing. For example, by applying capacitance detection at transport electrode 614c and transport electrode 618a and transport electrode 618b the relative position and distribution of the liquid across each electrode may be determined. The progression of fluid 622 as it flows across portions of transport electrode 614a, transport electrode 614b, transport electrode 614c, transport electrode 618a and transport electrode 618b may be monitored in real-time. Similarly, as transport electrode 614a and transport electrode 614b are deactivated, the progression of the fluid as it drains back to reservoir electrode 610 can similarly be determined. Based on this, the size of droplet 630 may be determined and adjustments to the process may be performed in order to ensure a reproducible droplet geometry at transport electrode 614c. Additionally, by applying capacitance detection at reservoir electrode 610 and transport electrode 614a and 614b the volume of fluid at reservoir electrode 610 and at split zone 626 may be determined and adjustments to the process may be performed in order to ensure a reproducible droplet geometry at transport electrode 614c. For example, if droplet 630 is too small, certain actions or adjustments to the droplet operation process may be performed, such as, but not limited to, returning the droplet to the reservoir, adding more volume to reservoir, adjusting the electrode bias voltage, adjusting the electrode bias time, and any combinations thereof. Adjustments may also be made in real-time as the droplet splitting process is being performed based on capacitance-based feedback from each of the electrodes participating in the process. For example, the amount of voltage on a particular electrode could be adjusted to maintain a particular rate of liquid drainage or certain electrodes could

activated or deactivated at particular times depending on the location of the liquid and progression of the droplet splitting process.

8.2.3 Droplet Transport Fault Detection

[0052] FIG. 7 illustrates yet another nonlimiting example of using capacitance detection in a droplet actuator. More specifically, FIG. 7 illustrates a nonlimiting example of a droplet actuator 700 that uses capacitance detection in a droplet transport fault detection application and/or a quality control application. Droplet actuator 700 includes a set of transport electrodes that are arranged, for example, in a grid. In one example, droplet actuator 700 includes an array of transport electrodes 710 that are arranged along rows A through G and columns 1 through 11 and that are in fluid connection with a reservoir 714 and multiple receptacles 718, such as receptacles 718a through 718f. In this example, all or certain selected transport electrodes 710 are connected to a capacitance detection mechanism, such as, but not limited to, capacitance detection circuit 200 of FIG. 2, capacitance detection circuit 300 of FIG. 3, and capacitance detection circuit 400 of FIG. 4, for detecting the droplet capacitance C-droplet.

[0053] Referring again to FIG. 7, in a droplet transport fault detection application, capacitance detection may be used for determining whether an electrode has failed (e.g., due to open electrical connection). More specifically, capacitance detection may be used to monitor the flow within droplet actuator 700. In one example, FIG. 7 shows a droplet 722 moving from, for example, grid location B2 to B7. If the expected change in capacitance is not measured at a certain selected transport electrode 710 along the path, a fault is detected, which may prompt certain action, such as, but not limited to, routing droplet 722 from grid location B2 to B7 via an alternate path. In one example, when a droplet transport fault is detected along the path from grid location B2 to B7, droplet 722 may be alternatively routed from grid location B2 to C2, then from C2 to C7, then from C7 to B7.

[0054] Referring again to FIG. 7, in a quality control application at the time of manufacture or operation of the device, when filling with oil the fluid path within a droplet actuator, such as within droplet actuator 700, the presence of air bubbles may be determined using capacitance detection. In one example, FIG. 7 shows an air bubble 726 that is trapped within droplet actuator 700 near one or more transport electrodes 710, which is problematic. Analyzing the capacitance profile of each transport electrode 710 in oil may provide an indication of whether an air bubble is present and its position and extent within the droplet actuator. When a bubble is detected, the device may be reloaded with oil to remedy the problem.

[0055] In another quality control application at the time of manufacture, a droplet actuator, such as droplet actuator 700, may be filled with a conductive fluid, such as water. Then the capacitance profile of each transport electrode 710 in a conductive fluid may be analyzed in order to determine whether the capacitance profile for each transport electrode 710 matches an expected capacitance profile. In this way, an open transport electrode 710 or a shorted transport electrode 710 may be detected.

8.2.4 Droplet Transport Speed Detection

[0056] Capacitance detection of the presence, absence or partial presence of a droplet at the position of a particular

electrode may be used as a basis for measuring the speed of droplet transport in a droplet actuator. Position measurements made at different points in time can be used to calculate the average velocity of droplet motion in a particular interval. For example, a signal may be sent to activate an electrode adjacent to a droplet and the time required for the droplet to move onto that activated electrode may be determined by monitoring the capacitance at that electrode over time due to the footprint of the droplet. Certain threshold levels of capacitance may be defined to facilitate measurements of this type. For example, one could define a transport time based on the time required for the capacitance to change from 10% to 90% where 0% represents the minimum footprint value and 100% represents the maximum footprint value. Many other types of measurements of instantaneous or average droplet position, velocity or acceleration may likewise be made. Furthermore, the measurement need not be made on the activated receiving electrode, but could be made on the deactivated source electrode (i.e. the rate at which the droplet moves away from the source is determined) or could be made using a third electrode. For example, the time required for the droplet to traverse an activated electrode and to overlap the next adjacent electrode to a could be measured.

8.3 Additional Examples of Capacitance Detection Architectures

[0057] FIG. 8 illustrates a schematic diagram of an embodiment of a droplet actuation circuit 800 of the invention. Droplet actuation circuit 800 includes a capacitance detection circuit and may be used for performing a capacitance measurement at any electrode of a droplet actuator, e.g., for performing droplet detection. Droplet actuation circuit 800 may include an electrode 810, e.g., droplet actuation electrode, for performing droplet operations. Electrode 810 is electrically connected to a high-voltage supply 814, e.g., at an electrowetting voltage, via an electronic switch 818. Electronic switch 818 may be the droplet actuation switch for connecting/disconnecting the voltage of high-voltage supply 814 to/from electrode 810. Electrode 810, high-voltage supply 814, and electronic switch 818 may in some embodiments be associated with the bottom plate (not shown) of a droplet actuator (not shown). Additionally, the droplet actuator may have arrays and/or paths of electrodes 810 for performing droplet operations. FIG. 9, described hereinbelow, illustrates additional details of a droplet actuation circuit that includes multiple electrodes.

[0058] Droplet actuation circuit 800 further includes a reference electrode 822 that may be electrically connected to multiple nodes via an electronic switch. In one example, reference electrode 822 may be electrically connected to a ground node 826, a voltage node 830, or a high-impedance node 832 via an electronic switch 834, e.g., a 10 position electronic switch. Reference electrode 822, ground node 826, voltage node 830, high-impedance node 832, and electronic switch 834 may in some embodiments be associated with the top plate (not shown) of a droplet actuator. When reference electrode 822 is electrically connected to ground node 826, it serves as a ground reference plane for the droplet actuator. When reference electrode 822 is electrically connected to voltage node 830, it serves as a voltage reference plane for the droplet actuator. When reference electrode 822 is electrically connected to high-impedance node 832, it is substantially disconnected from ground node 826 and voltage node 830 and is, thus, considered in a "float" state.

[0059] The combination of electrode **810**, high-voltage supply **814**, electronic switch **818**, reference electrode **822**, ground node **826**, voltage node **830**, high-impedance node **832**, and electronic switch **834** is included in the typical infrastructure of a droplet actuator. However, in addition to these typical elements of a droplet actuator, the invention provides a capacitance detection circuit **836** that includes a protection circuit **838** and a detection circuit **842**. More specifically, a voltage, V-ref, at reference electrode **822** is electrically connected to an input of protection circuit **838** of capacitance detection circuit **836**. An output of protection circuit **838** is electrically connected to an input of detection circuit **842** of capacitance detection circuit **836**. An output voltage, V-out, of detection circuit **842** is provided for monitoring by external resources (not shown). Protection circuit **838** is provided to protect detection circuit **842** from damage due to high voltage when electronic switch **834** is connected to voltage node **830**.

[0060] FIG. **8** also shows that when a droplet **846** is present at electrode **810**, the droplet **846** has a certain capacitance, C-droplet, between electrode **810** and reference electrode **822**. By contrast, when droplet **846** is not present at electrode **810**, capacitance, C-droplet, does not exist between electrode **810** and reference electrode **822**.

[0061] In operation, during droplet operations, reference electrode **822** may be electrically connected, for example, to ground node **826** via electronic switch **834** and droplet operations may occur at electrode **810** under the control of electronic switch **818**. However, during droplet detection operations, reference electrode **822** is electrically connected to high-impedance node **832** via electronic switch **834**, to place reference electrode **822** in a “float” state. Additionally, electronic switch **818** that is associated with electrode **810** serves as a rising edge generator. More specifically, a rising edge at electrode **810** is generated by toggling electronic switch **818** from an open state to a closed state, thereby causing a voltage transition to occur at electrode **810** from about 0 volts to about the value of high-voltage supply **814**. In this way, the capacitive energy that is caused by the presence of capacitance, C-droplet, of droplet **846** at electrode **810** is coupled to reference electrode **822**, which then is coupled to protection circuit **838** and passed to detection circuit **842** of capacitance detection circuit **836**. This capacitive energy generated is a voltage pulse at V-ref that is proportional to the capacitance, C-droplet.

[0062] The voltage pulse that is present at the V-ref node, which may be a high voltage pulse, is processed via protection circuit **838** and detection circuit **842** of capacitance detection circuit **836** to provide a digital V-out value that reflects the magnitude of capacitance, C-droplet. In one example, when the digital V-out value of detection circuit **842** is about 0 volts, this indicates that there is no droplet **846** present at electrode **810**. In another example, when the digital V-out value of detection circuit **842** is a certain expected value that is greater than about 0 volts, this indicates that droplet **846** is present at electrode **810**. In this way, capacitance detection circuit **836** provides a way to detect the presence or absence of a droplet at a certain electrode by detecting the presence or absence of capacitance, C-droplet. FIGS. **10A** and **10B**, described hereinafter, illustrate more details of an example capacitance detection circuit that includes a detection circuit and a protection circuit.

[0063] FIG. **9** illustrates a schematic diagram of an embodiment of a droplet actuation circuit **900** that includes a capaci-

tance detection circuit. The capacitance detection circuit may, for example, be used for a capacitance measurement at any electrode of a droplet actuator, e.g., for performing droplet detection. Droplet actuator circuit **900** is substantially the same as droplet actuator circuit **800** of FIG. **8**, except for the illustration of multiple electrodes **810** and the associated bank of electronic switches **818**. FIG. **9** shows that all electronic switches **818** are connected to a common high voltage of high-voltage supply **814**. In this example, a rising edge may be generated by activating the electronic switch **818** that is associated with an electrode **810** of interest and capacitance detection circuit **836** may be used to detect the presence or absence of capacitance, C-droplet, at the electrode **810** of interest. A sequential operation may occur, i.e., sequencing from one electrode **810**/electronic switch **818** pair to the next, by which capacitance detection takes place from one electrode **810** to the next.

[0064] FIG. **10A** illustrates a schematic diagram of an embodiment of a capacitance detection circuit, such as capacitance detection circuit **836**, of the invention that may be used in a droplet actuator for the purpose of performing droplet detection. Capacitance detection circuit **836** includes protection circuit **838** and detection circuit **842**. More specifically, the input of protection circuit **838** is fed, for example, by voltage V-ref of droplet actuator circuit **800** or **900** of FIG. **8** or **9**, respectively. The output of protection circuit **838** feeds the input of detection circuit **842**, which provides a digital V-out value.

[0065] Additionally, protection circuit **838** of capacitance detection circuit **836** includes a voltage divider network, such as a resistor R1 and R2 that are electrically connected in series, as shown in FIG. **10A**. A voltage node A between resistor R1 and R2 is electrically connected to one side of a capacitor C1. The opposite side of capacitor C1 is electrically connected to the input of detection circuit **842**. Because of the action of the voltage divider network, which is formed by resistors R1 and R2, a fraction of the voltage value of V-ref is present at voltage node A. The values of resistors R1 and R2 are such that a suitably safe, low-voltage at node A feeds the input of detection circuit **842**, to ensure that a high voltage at V-ref does not damage the components of detection circuit **842**. Additionally, capacitor C1 provides an alternating current (AC) coupling mechanism for coupling the AC components only of V-ref to detection circuit **842**.

[0066] Additionally, detection circuit **842** of capacitance detection circuit **836** includes an amplifier **1010**, a charge integrating amplifier **1014**, and an analog-to-digital (A/D) converter **1018**, which are electrically connected as shown in FIG. **10A**. Amplifier **1010** may, for example, be a conventional operational amplifier device that scales its input voltage either up or down to any suitable voltage for feeding the next signal processing stage, the charge integrating amplifier **1014**. Alternatively, amplifier **1010** may serve as a buffer only, to convert the input signal impedance to a certain impedance value that is suited to pass to the next signal processing stage, charge integrating amplifier **1014**. Charge integrating amplifier **1014** may, for example, be a conventional charge integrating amplifier that generates an output voltage (e.g., voltage node C) that is the integral of its input voltage (e.g., voltage node B), which is illustrated in FIG. **10B**. A reason for integrating the output of amplifier **1010** is to render the signal less sensitive to stray capacitances that may be present at electrode **810**, while still capturing the capacitance across droplet **846**. A/D converter **1018** may, for example, be a

conventional n-bit A/D converter device for converting an analog input voltage to an n-bit digital word. For example, A/D converter **1018** may be an 8-bit, 10-bit, or 16-bit A/D converter, depending on a desired resolution.

[0067] Referring again to FIGS. **8**, **9**, **10A**, and **10B**, the operation of capacitance detection circuit **836** may be summarized as follows. Reference electrode **822** is electrically connected to high-impedance node **832** via electronic switch **834**, to place reference electrode **822** in a “float” state, which provides electrical isolation from ground node **826** and voltage node **830** via a high resistance (e.g., Megaohms). For an electrode **810** of interest, its associated electronic switch **818** is toggled from open to closed to generate a rising edge at the electrode **810** of interest. Assuming a droplet **846** is present at the electrode **810** of interest, capacitive energy is coupled to reference electrode **822** that is proportional to capacitance, C-droplet. Protection circuit **838** of capacitance detection circuit **836** reduces the amplitude of V-ref to a suitably low voltage via resistors **R1** and **R2**. Capacitor **C1** then couples the low-voltage pulse at node **A** to amplifier **1010**, which scales the low-voltage pulse to any usable value for feeding charge integrating amplifier **1014**. Charge integrating amplifier **1014** generates an output voltage (e.g., voltage node **C**) that is the integral of its input voltage (e.g., voltage node **B**), as shown in FIG. **10B**. A/D converter **1018** performs an analog-to-digital conversion of the output of charge integrating amplifier **1014**. A/D converter **1018** may be sampled, for example, at some time after time **t1** (see FIG. **10B**) and its digital V-out value is captured by an external processor (not shown) for analysis. In one example, A/D converter **1018** may be sampled once only at some time after time **t1** (see FIG. **10B**) to arrive at a measurement of capacitance, C-droplet. In another example, A/D converter **1018** may be sampled multiple times after time **t1** and then the multiple digital V-out values may be averaged to arrive at a measurement of capacitance, C-droplet.

[0068] In one example application, a capacitance detection circuit of the invention may be used for validating one or more droplet operations on a droplet actuator. For example, the circuit may be used to verify whether one or more droplet operations in a certain protocol have been achieved. In one embodiment, as a certain droplet is moved via droplet operations from one electrode to the next and a capacitance detection operation may occur after each movement to verify that the droplet has moved as expected.

[0069] In another example application, a capacitance detection circuit, such as capacitance detection circuit **836**, may be used for performing a droplet actuator characterization operation. For example, a droplet may be moved along a line of electrodes toward a designated detection location at a certain droplet actuation frequency. At the end of the sequence, a capacitance detection operation may occur at the designated detection location, to verify that the droplet arrived successfully. This sequence may be repeated at higher and higher droplet actuation frequencies until the droplet actuator fails. In performing this characterization operation using the capacitance detection circuit of the invention, the droplet actuation frequency specification of the droplet actuator may be established.

8.4 Droplet Actuator

[0070] For examples of droplet actuator architectures that are suitable for use with the present invention, see U.S. Pat. No. 6,911,132, entitled, “Apparatus for Manipulating Drop-

lets by Electrowetting-Based Techniques,” issued on Jun. 28, 2005 to Pamula et al.; U.S. patent application Ser. No. 11/343,284, entitled, “Apparatuses and Methods for Manipulating Droplets on a Printed Circuit Board,” filed on Jan. 30, 2006; U.S. Pat. Nos. 6,773,566, entitled, “Electrostatic Actuators for Microfluidics and Methods for Using Same,” issued on Aug. 10, 2004 and 6,565,727, entitled, “Actuators for Microfluidics Without Moving Parts,” issued on Jan. 24, 2000, both to Shenderov et al.; and International Patent Application No. PCT/US 06/47486, entitled, “Droplet-Based Biochemistry,” filed by Pollack et al. on Dec. 11, 2006, the disclosures of which are incorporated herein by reference.

8.5 Fluids

[0071] For examples of fluids that may be subjected to droplet operations and capacitance detection according to the invention, see the patents listed in section 8.4, especially International Patent Application No. PCT/US 06/47486, entitled, “Droplet-Based Biochemistry,” filed on Dec. 11, 2006. In some embodiments, the droplet is a sample fluid, such as 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, fluidized tissues, fluidized organisms, biological swabs and biological washes. In some embodiment, the fluid that includes a reagent, such as water, deionized water, saline solutions, acidic solutions, basic solutions, detergent solutions and/or buffers. In some embodiments, the fluid includes a reagent, such as a reagent for a biochemical protocol, such as a nucleic acid amplification protocol, an affinity-based assay protocol, a sequencing protocol, and/or a protocol for analyses of biological fluids.

8.6 Filler Fluids

[0072] The gap is typically filled with a filler fluid. The filler fluid may, for example, be a low-viscosity oil, such as silicone oil. Other examples of filler fluids are provided in International Patent Application No. PCT/US2006/47486, filed on Dec. 11, 2006, entitled “Droplet-Based Biochemistry”.

8.7 Example Method Detecting Capacitance

[0073] One approach for providing capacitance detection in a droplet actuator may include, but is not limited to, the steps of providing a mechanism for monitoring the electrode voltage V_e , switching on the electrode voltage V_e and measuring its rise time with no conductive droplet present at a transport electrode of interest, switching off the electrode voltage V_e , providing a conductive droplet at the transport electrode of interest in order to introduce capacitance C-droplet, switching on the electrode voltage V_e and measuring its rise time with conductive droplet present at the transport electrode of interest, calculating the difference between the two rise time measurements, correlating the difference between the two rise time measurements with a capacitance value, and correlating the capacitance value with a droplet footprint area.

[0074] Capacitance detection, in general, is particularly suited for most electrowetting applications given the typical physical spacing between electrodes. Moreover, capacitance detection provides a more direct correlation between bead and/or droplet properties than does the measurement of other

electrical quantities, such as inductance and impedance. Such other properties typically require additional processing for reactance, time constants and electron propagation factors, among other considerations. In that sense, embodiments determining capacitance require relatively less processing and hardware equipment, while delivering simpler and more accurate calculations than do measurements of other properties. In any case, one skilled in the art will appreciate that preferred embodiments described herein are merely exemplary, and other embodiments consistent with the underlying principles of the present invention may measure capacitance in a number of other manners known in the industry. Furthermore, while various exemplary embodiments are described herein with reference to capacitance, it is understood that other methods can be performed using other types of impedance circuits, such as resistance.

9 CONCLUDING REMARKS

[0075] 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.

[0076] 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.

[0077] 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, as the present invention is defined by the claims as set forth hereinafter.

We claim:

1. A method of determining the presence, partial presence or absence of a droplet at an electrode on a droplet actuator, the method comprising:

- (a) providing a droplet actuator comprising:
 - (i) a substrate comprising electrodes arranged on the substrate for conducting droplet operations on a surface of the substrate;
 - (ii) a capacitance detection circuit for detecting capacitance at the droplet operations surface at one or more of the electrodes;
- (b) detecting capacitance at the droplet operations surface at one or more of the electrodes; and
- (c) determining from the capacitance the presence, partial presence or absence of a droplet at the droplet operations surface at the electrode.

2. The method of claim 1 wherein the electrodes arranged on the substrate for conducting droplet operations are configured for mediating droplet operations by a means comprising electrowetting.

3. The method of claim 1 wherein the electrodes arranged on the substrate for conducting droplet operations are not configured for mediating droplet operations by a means comprising dielectrophoresis.

4. The method of claim 2 comprising determining in real time the absence, presence, partial presence, and/or location of a droplet along a path of transport electrodes.

5. The method of claim 4 wherein the absence, presence, partial presence, and/or location of the droplet along the path of transport electrodes is determined without visualization of the droplet's location.

6. The method of claim 2 comprising:

- (a) determining among several electrodes which electrode has maximum capacitance relative to the others of the several electrodes; and
- (b) concluding based on such determination that the droplet is at the electrode having the maximum capacitance.

7. The method of claim 6 further comprising conducting a subsequent droplet operation on the same or a different droplet based on the determined location of the droplet.

8. A method of transporting a droplet on a droplet actuator, the method comprising:

- (a) conducting one or more droplet operations to transport a droplet on the droplet actuator; and
- (b) executing the method of claim 1 to determine whether the droplet has been transported.

9. A method of identifying a faulty electrode on a droplet actuator, the method comprising:

- (a) attempting a droplet transport operation and using the method of claim 2 to determine whether the droplet transport operation is successful; and
- (b) where the droplet operation is not successful at a particular electrode, identifying the electrode as faulty.

10. A method of re-routing a droplet around a faulty electrode on a droplet actuator, the method comprising:

- (a) identifying a faulty electrode according to the method of claim 9; and
- (b) re-routing the droplet around the faulty electrode.

11. The method of claim 10 wherein the re-routing is controlled by a controller or processor.

12. The method of claim 10 further comprising re-routing a subsequent droplet around the target electrode.

13. A method of providing a sub-droplet from a droplet on a droplet actuator, the method comprising:

- (a) providing a droplet actuator comprising:
 - (i) a substrate comprising electrodes arranged on the substrate for forming the sub-droplet from the droplet on a surface of the substrate; and
 - (ii) a capacitance detection circuit for detecting capacitance at the droplet operations surface at one or more of the electrodes;
- (b) activating one or more electrodes to elongate some portion or all of the droplet while using the capacitance detection circuit to determine the location of one or more portions of the droplet; and
- (c) when the location of one or more portions of the droplet reaches a predetermined position as determined by the capacitance detection circuit, deactivating an intermediate one of the one or more electrodes along the elongated droplet to form the sub-droplet.

14. A method of determining whether a droplet comprises one or more beads, the method comprising:

- (a) providing a droplet on an electrode on a droplet actuator;
- (b) determining capacitance at the electrode;
- (c) comparing the capacitance with an expected capacitance in the presence of one or more beads; and
- (d) determining whether one or more beads is present.

15. The method of claim 14 wherein the step of providing a droplet on an electrode on a droplet actuator is mediated by a means comprising electrowetting.

16. The method of claim **14** wherein the step of providing a droplet on an electrode on a droplet actuator is not mediated by a means comprising dielectrophoresis.

17. The method of claim **14** wherein the determination:

- (a) follows a droplet splitting operation in a bead washing protocol; and
- (b) identifies whether bead loss has occurred during the splitting operation.

18. A method of determining whether a droplet comprises one or more biological cells, the method comprising:

- (a) providing a droplet on an electrode on a droplet actuator;
- (b) determining capacitance at the electrode;
- (c) comparing the capacitance with an expected capacitance in the presence of one or more cells; and
- (d) determining whether one or more cells is present.

19. The method of claim **18** wherein the step of providing a droplet on an electrode on a droplet actuator is mediated by a means comprising electrowetting.

20. The method of claim **18** wherein the step of providing a droplet on an electrode on a droplet actuator is not mediated by a means comprising dielectrophoresis.

21. A method of quantifying or estimating the quantity of beads in a droplet, the method comprising:

- (a) providing a droplet on an electrode on a droplet actuator;
- (b) determining capacitance at the electrode;
- (c) comparing the capacitance with a curve for expected capacitance relative to number of beads; and
- (d) quantifying or estimating the quantity of beads in the droplet.

22. The method of claim **21** wherein the step of providing a droplet on an electrode on a droplet actuator is mediated by a means comprising electrowetting.

23. The method of claim **21** wherein the step of providing a droplet on an electrode on a droplet actuator is not mediated by a means comprising dielectrophoresis.

24. A method of quantifying or estimating cells in a droplet, the method comprising:

- (a) providing a droplet on an electrode on a droplet actuator;
- (b) determining capacitance at the electrode;
- (c) comparing the capacitance with a curve for expected capacitance relative to number of cells; and
- (d) quantifying or estimating cells in the droplet.

25. The method of claim **24** wherein the step of providing a droplet on an electrode on a droplet actuator is mediated by a means comprising electrowetting.

26. The method of claim **24** wherein the step of providing a droplet on an electrode on a droplet actuator is not mediated by a means comprising dielectrophoresis.

27. A method of assessing viability of cells in a droplet, the method comprising:

- (a) providing a droplet on an electrode on a droplet actuator;
- (b) determining capacitance at the electrode;
- (c) comparing the capacitance expected capacitance in the presence of viable cells and/or non-viable cells; and
- (d) assessing viability cells in the droplet.

28. The method of claim **27** wherein the step of providing a droplet on an electrode on a droplet actuator is mediated by a means comprising electrowetting.

29. The method of claim **27** wherein the step of providing a droplet on an electrode on a droplet actuator is not mediated by a means comprising dielectrophoresis.

30. A method of assessing apoptosis of cells in a droplet, the method comprising:

- (a) providing a droplet on an electrode on a droplet actuator;
- (b) determining capacitance at the electrode;
- (c) comparing the capacitance expected in the presence of cells undergoing apoptosis and/or cells not undergoing apoptosis; and
- (d) assessing apoptosis of cells in the droplet.

31. The method of claim **30** wherein the step of providing a droplet on an electrode on a droplet actuator is mediated by a means comprising electrowetting.

32. The method of claim **30** wherein the step of providing a droplet on an electrode on a droplet actuator is not mediated by a means comprising dielectrophoresis.

33. A method of identifying a bead type in a droplet, the method comprising:

- (a) providing a droplet on a droplet actuator comprising multiple bead types, each bead type having a different capacitance;
- (b) dispensing from the droplet onto an electrode a sub-droplet comprising one or a few beads;
- (c) determining capacitance at the electrode;
- (d) comparing capacitance at the electrode to expected capacitance in the presence some or all of the bead types; and
- (e) identifying the bead type.

34. The method of claim **33** wherein the step of providing a droplet on an electrode on a droplet actuator is mediated by a means comprising electrowetting.

35. The method of claim **33** wherein the step of providing a droplet on an electrode on a droplet actuator is not mediated by a means comprising dielectrophoresis.

36. The method of claim **33** wherein the step of dispensing from the droplet onto an electrode a sub-droplet comprising one or a few beads is mediated by a means comprising electrowetting.

37. The method of claim **33** wherein the step of dispensing from the droplet onto an electrode a sub-droplet comprising one or a few beads is not mediated by a means comprising dielectrophoresis.

38. A method of identifying a bead type in a droplet, the method comprising:

- (a) providing on an electrode on a droplet actuator a droplet comprising a bead of a specific unknown type and having a unique capacitance;
- (b) determining capacitance at the electrode;
- (c) comparing capacitance at the electrode to expected capacitance in the presence one or more bead types; and
- (d) identifying the bead type.

39. The method of claim **38** wherein the step of providing a droplet on an electrode on a droplet actuator is mediated by a means comprising electrowetting.

40. The method of claim **38** wherein the step of providing a droplet on an electrode on a droplet actuator is not mediated by a means comprising dielectrophoresis.

41. A method of providing a sub-droplet from a droplet on a droplet actuator, the method comprising determining the location of one or more portions of the droplet during a splitting or dispensing droplet operation by detecting capacitance at one or more electrodes on the droplet actuator and

adjusting one or more parameters of the splitting or dispensing operation to influence the volume of the sub-droplet.

42. The method of claim **41** wherein the parameter is selected from the group consisting of reservoir volume, electrode bias voltage, electrode bias time, and any combinations thereof.

43. The method of claim **41** wherein the splitting or dispensing droplet operation is mediated by a means comprising electrowetting.

44. The method of claim **41** wherein the splitting or dispensing droplet operation is not mediated by a means comprising dielectrophoresis.

45. A method of determining the presence of air bubbles in a droplet actuator, the method comprising:

- (a) attempting to fill some portion of the droplet actuator with a filler fluid to provide a filled portion of the droplet actuator;
- (b) testing capacitance at electrodes in the filled portion of the droplet actuator; and
- (c) identifying the presence of one or more air bubbles based on a capacitance at one or more of the electrodes that differs from the expected capacitance at the electrode in the presence of the filler fluid.

46. The method of claim **45** further comprising reloading the portion of the droplet actuator with the filler fluid.

47. The method of claim **45** wherein the electrodes are configured for mediating droplet operations by a means comprising electrowetting.

48. The method of claim **45** wherein the electrodes are configured for mediating droplet operations by a means not comprising dielectrophoresis.

49. A method of testing electrodes on a droplet actuator, the method comprising:

- (a) filling at least a portion of the droplet actuator with a fluid having a known capacitance to provide a filled portion of the droplet actuator;
- (b) testing capacitance at electrodes in the filled portion of the droplet actuator; and
- (c) identifying faulty electrodes based on a capacitance that differs from the expected capacitance at the electrode in the presence of the fluid.

50. The method of claim **49** wherein the electrodes are configured for mediating droplet operations by a means comprising electrowetting.

51. The method of claim **49** wherein the electrodes are configured for mediating droplet operations by a means not comprising dielectrophoresis.

52. A droplet actuator for manipulating a fluid using an electrical field, comprising:

- (a) an actuator electrode configured to communicate the electrical field to the fluid;
- (b) a reference electrode in communication with the actuator electrode and in selective communication with at least one of a ground node, a high voltage node and a high impedance node having an intermediate potential;
- (c) a capacitance detection circuit in communication with the reference electrode and configured to detect a capacitance of the fluid; and
- (d) a processor in communication with the capacitor detection circuit and configured to determine a position of the fluid based on the detected capacitance of the fluid.

53. The droplet actuator of claim **52** wherein the actuator electrode is configured for mediating droplet operations by a means comprising electrowetting.

54. The droplet actuator of claim **52** wherein the actuator electrode is configured for mediating droplet operations by a means not comprising dielectrophoresis.

55. The droplet actuator of claim **52** further comprising a switch configured to selectively enable communication between the actuator and reference electrodes.

56. The droplet actuator of claim **52** further comprising a plurality of actuator nodes in communication with the reference electrode.

57. The droplet actuator of claim **56** wherein the plurality of actuator nodes sequentially communicate with the reference electrode.

58. The droplet actuator of claim **52** further comprising a voltage source in communication with the actuator electrode.

59. The droplet actuator of claim **58** further comprising a switch configured to enable selective communication between the voltage source and the actuator electrode.

60. The droplet actuator of claim **52** wherein the reference electrode further comprises another actuator electrode.

61. The droplet actuator of claim **52** further comprising a switch configured to selectively enable communication between the actuator electrode and at least one of the ground, high voltage and high impedance nodes.

62. The droplet actuator of claim **52** wherein the processor is further configured to conduct droplet processes while the actuator electrode is in communication with the ground node.

63. The droplet actuator of claim **52** wherein the processor is further configured to conduct droplet detection processes while the actuator electrode is in communication with the high impedance node.

64. The droplet actuator of claim **52** wherein the processor is further configured to enable communication between the actuator electrode and the voltage source while the actuator electrode is in communication with the high impedance node.

65. The droplet actuator of claim **52** wherein the capacitance detection circuit further comprises a protection circuit configured to regulate a voltage entering the capacitance detection circuit to minimize damage caused by a high voltage condition.

66. The droplet actuator of claim **65** wherein the capacitance detection circuit comprises at least one of a voltage divider and a capacitor.

67. The droplet actuator of claim **52** wherein the capacitance detection circuit comprises at least one of an operational amplifier, an integrating amplifier and an analog-to-digital converter.

68. The droplet actuator of claim **52** wherein the processor is further configured to determine that the fluid is not present at the actuator electrode in response to receiving an electrical value associated with the detected capacitance.

69. The droplet actuator of claim **52** wherein the processor is further configured to determine that the fluid is present at the actuator electrode in response to receiving an electrical value associated with the detected capacitance.

70. The droplet actuator of claim **52** wherein the fluid comprises a droplet.

71. The droplet actuator of claim **52** wherein the fluid comprises a droplet comprising beads.

72. The droplet actuator of claim **52** wherein the fluid comprises a droplet comprising cells.

73. The droplet actuator of claim **52** wherein the fluid comprises a filler fluid.

74. The droplet actuator of claim **52** wherein the fluid comprises a filler fluid comprising an oil.

75. The droplet actuator of claim **52** further comprising first and second substrates separated by the fluid, wherein the first substrate comprises the actuator electrode and the second substrate comprises the reference electrode.

76. The droplet actuator of claim **75** wherein the fluid comprises a droplet and the droplet is located over the actuator electrode.

77. The droplet actuator of claim **76** wherein the processor is further configured to initiate a measurement of an amount by which a portion of the droplet overlaps the actuator electrode.

78. The droplet actuator of claim **75** wherein the first substrate comprises a plurality of actuator electrodes and each actuator electrode is equidistant from the reference electrode.

79. The droplet actuator of claim **78** wherein the first substrate comprises a grid or array of actuator electrodes and the second substrate comprises a single reference electrode that is substantially equidistant from each of the actuator electrodes, wherein the measurement at each of the actuator electrodes in the grid or array may be determined based on the single reference electrode.

80. The droplet actuator of claim **78** wherein the first substrate comprises a grid or array of actuator electrodes and the detected capacitance measurement is based upon two or more of the actuator electrodes.

81. A method of determining fluid droplet overlap comprising:

- (a) providing a droplet actuator comprising:
 - (i) an actuator electrode configured to communicate the electrical field to the fluid;
 - (ii) a reference electrode in communication with the actuator electrode and in selective communication with at least one of a ground node, a high voltage node and a high impedance node having an intermediate potential;
 - (iii) a capacitance detection circuit in communication with the reference electrode and configured to detect a capacitance of the fluid; and
 - (iv) a processor in communication with the capacitor detection circuit and configured to determine a position of the fluid based on the detected capacitance of the fluid;
- (b) locating a fluid droplet over the actuator electrode; and
- (c) initiating a processor measurement of an amount by which a portion of the droplet overlaps the actuator electrode.

82. A method of detecting fluid within a droplet actuator having a plurality of actuator electrodes each configured to communicate an electric field to the fluid, the method comprising:

- (a) sensing a capacitance of the fluid at an actuator electrode of the plurality of actuator electrodes; and
- (b) determining that the fluid is present at the actuator electrode using the sensed capacitance.

83. The method of claim **82** wherein the actuator electrode is configured for mediating droplet operations by a means comprising electrowetting.

84. The method of claim **82** wherein the actuator electrode is configured for mediating droplet operations by a means not comprising dielectrophoresis.

85. The method of claim **82** wherein sensing the capacitance further comprises selectively enabling communication between the actuator electrode and a reference electrode.

86. The method of claim **85** wherein sensing the capacitance further comprises selectively enabling communication between the reference electrode and at least one of a ground node, a high voltage node and a high impedance node having an intermediate potential.

87. The method of claim **82** wherein sensing the capacitance further comprises selectively enabling communication between the actuator and a voltage source.

88. The method of claim **85** or **86** wherein sensing the capacitance further comprises concurrently enabling communication between the reference electrode and the high impedance node, and between the actuator electrode and the voltage source.

89. The method of claim **85** wherein determining that the fluid is present further comprises sequentially sensing a capacitance at each of the plurality of electrodes.

90. The method of claim **85** wherein sensing the capacitance further comprises sensing the capacitance while the reference electrode is in communication with the high impedance node.

91. The method of claim **82** further comprising regulating a voltage entering a capacitance detection circuit of the droplet actuator to minimize damage caused by a high voltage condition.

92. The droplet actuator of claim **82** wherein the fluid comprises a droplet.

93. The droplet actuator of claim **82** wherein the fluid comprises a droplet comprising beads.

94. The droplet actuator of claim **82** wherein the fluid comprises a droplet comprising cells.

95. The droplet actuator of claim **82** wherein the fluid comprises a filler fluid.

96. The droplet actuator of claim **82** wherein the fluid comprises a filler fluid comprising an oil.

97. A capacitance detection circuit for detecting a capacitance of fluid present in a droplet actuator, comprising:

- (a) an amplifier in communication with an electrode of the droplet actuator;
- (b) an analog-to-digital converter in communication with the amplifier and a processor configured to determine a position of the fluid based on the detected capacitance of the fluid; and
- (c) a protection circuit configured to regulate a voltage entering the capacitance detection circuit to minimize damage caused by a high voltage condition.

98. The capacitance detection circuit of claim **97** wherein the electrode of the droplet actuator is configured for mediating droplet operations by a means comprising electrowetting.

99. The capacitance detection circuit of claim **97** wherein the electrode of the droplet actuator is configured for mediating droplet operations by a means not comprising dielectrophoresis.

100. A method of determining the presence, partial presence or absence of a droplet at an electrode on a droplet actuator, the method comprising:

- (a) providing a droplet actuator comprising:
 - (i) a substrate comprising droplet operations electrodes arranged on the substrate for conducting droplet operations on a surface of the substrate; and
 - (ii) an impedance detection circuit coupled to:
 - (1) one or more of the droplet operations electrodes; and

- (2) a second electrode and arranged for detecting capacitance at the droplet operations surface at one or more of the electrodes;
 - (b) detecting impedance at the droplet operations surface at one or more of the electrodes; and
 - (c) determining from the impedance the presence, partial presence or absence of a droplet at the droplet operations surface at the electrode.
- 101.** The method of claim **100** wherein the impedance detection circuit comprises a capacitance detection circuit.
- 102.** The method of claim **100** wherein the impedance detection circuit comprises a resistance detection circuit.
- 103.** The method of claim **100** wherein:
- (a) at least a subset of the droplet operations electrodes comprises two or more of the droplet operations electrodes each coupled to an impedance detection circuit; and
 - (b) all of such impedance detection circuits are coupled to a common second electrode.
- 104.** The method of claim **100** wherein:
- (a) the droplet:
 - (i) has a location on the substrate; and
 - (ii) overlaps multiple droplet operations electrodes; and
 - (b) the method further comprises determining the location of the droplet by measuring the overlap of the droplet with each of the multiple droplet operations electrodes by measuring impedance at each of the multiple droplet operations electrodes.
- 105.** The method of claim **100** wherein:
- (a) the droplet:
 - (i) has a location on the substrate; and
 - (ii) overlaps multiple droplet operations electrodes; and
 - (b) the method further comprises:
 - (i) determining the location of the droplet by measuring the overlap of the droplet with each of the multiple droplet operations electrodes by measuring impedance at each of the multiple droplet operations electrodes; and
 - (ii) based on the overlap of the droplet with each of the multiple droplet operations electrodes, determining the volume of the droplet.
- 106.** The method of claim **100** wherein:
- (a) the droplet is smaller than an operations electrode; and
 - (b) the method comprises determining the size of the droplet based on the capacitance.
- 107.** The method of claim **100** wherein the impedance detection circuit is coupled to electrodes in a substantially common plane.
- 108.** A method of determining the velocity of deformation of a droplet, the method comprising detecting a change in impedance at a locus of a droplet actuator during a droplet operation that results in deformation of the droplet, where the locus is in impedance detecting proximity to the droplet.
- 109.** The method of claim **108** wherein the droplet operation comprises a droplet transport operation.
- 110.** A method of enhancing droplet transport rate on a droplet actuator, the method comprising:
- (a) determining the velocity of droplet transport according to the method of claim **109**; and
 - (b) adjusting droplet transport electrode switching rate and/or voltage of droplet transport electrodes to suit the droplet transport velocity.
- 111.** The method of claim **110** comprising enhancing the droplet transport rate of two or more droplets on a single

droplet actuator, the two or more droplets comprising at least two droplets each having different properties, and the method comprising independently adjusting droplet transport electrode switching rate and/or voltage of droplet transport electrodes to yield a suitable droplet transport velocity of each droplet.

112. A method of transporting a droplet onto a droplet transport electrode from an adjacent electrode on a droplet operations surface of a droplet actuator, the method comprising:

- (a) monitoring impedance at the droplet transport electrode as a droplet is being transported on the droplet operations surface onto the adjacent electrode; and
- (b) activating the droplet transport electrode when the impedance at the droplet transport electrode indicates that the droplet is in sufficient proximity with the droplet operations electrode to result in transport of the droplet from the adjacent electrode upon activation of the droplet transport electrode and deactivation of the adjacent electrode.

113. A method of transporting a droplet on a droplet operations surface of a droplet actuator, wherein:

- (a) the droplet actuator comprises:
 - (i) a first electrode;
 - (ii) a second electrode adjacent to the first electrode; and
 - (iii) a third electrode adjacent to the second electrode; and
- (b) the method comprises:
 - (i) monitoring impedance at the first electrode, the second electrode and/or the third electrode during a droplet operation comprising activating the second electrode to transport a droplet from the first electrode to the second electrode; and
 - (ii) sequentially or simultaneously activating the third electrode and deactivating the second electrode when at least one of the following conditions is satisfied:
 - (1) impedance at the first electrode indicates that the droplet is sufficiently on the second electrode to result in transport of the droplet from the second electrode to the third electrode upon activation of the third electrode and deactivation of the second electrode;
 - (2) impedance at the second electrode indicates that the droplet is sufficiently on the second electrode to result in transport of the droplet from the second electrode to the third electrode upon activation of the third electrode and deactivation of the second electrode; and
 - (3) impedance at the third electrode indicates that the droplet is sufficiently on the second electrode to result in transport of the droplet from the second electrode to the third electrode upon activation of the third electrode and deactivation of the second electrode.

114. A method of quality control for a droplet actuator, the method comprising:

- (a) transporting a droplet on a droplet operations surface of the droplet actuator and determining velocity of transport across and/or onto one or more of the droplet operations electrodes; and
- (b) making a quality determination based on deviations in transport velocity from a predetermined standard.

115. A method of determining a property of a droplet, the method comprising determining the velocity of deformation

of a droplet during a droplet operation on a droplet actuator correlating the velocity with a property of the droplet.

116. The method of claim **115** wherein the property comprises viscosity.

117. The method of claim **115** wherein the property comprises surface tension.

118. The method of claim **115** wherein the droplet operation comprises a droplet transport operation wherein the droplet is transported from one electrode to another electrode.

119. A method of controlling droplet location on a droplet actuator, the method comprising determining a droplet footprint of the droplet on a droplet operations surface of the droplet actuator and activating and/or deactivating one or more electrodes of the droplet actuator in order reposition the droplet.

120. The method of claim **119** wherein the repositioning of the droplet improves droplet location relative to sensing by a sensor.

121. A method of determining the location of a droplet on a droplet actuator, the method comprising:

(a) providing a droplet actuator comprising:

(i) a substrate comprising droplet operations electrodes arranged on the substrate for conducting droplet operations on a surface of the substrate and one or more droplets arranged on one or more of the droplet operations electrodes; and

(ii) an impedance detection circuit coupled to:

(1) one or more of the droplet operations electrodes; and/or

(2) one or more impedance detection electrodes; and

(b) detecting, using one or more of the impedance detection circuits, the location of a droplet on the droplet operations electrodes.

122. The method of claim **121** wherein the droplet operations electrodes are configured to mediate droplet operations by an electrowetting effect.

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