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(54) Title: TECHNIQUES AND DROPLET ACTUATOR DESIGNS FOR REDUCING BUBBLE FORMATION

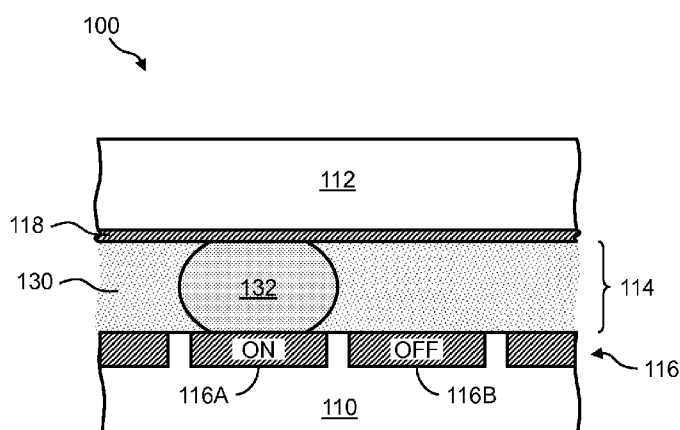


Figure 1A

(57) Abstract: During droplet operations in a droplet actuator, bubbles often form in the filler fluid in the droplet operations gap and interrupt droplet operations. The present invention provides methods and systems for performing droplet operations on a droplet in a droplet actuator comprising maintaining substantially consistent contact between the droplet and an electrical ground while conducting multiple droplet operations on the droplet in the droplet operations gap and/or reducing the accumulation of electrical charges in the droplet operations gap during multiple droplet operations. The methods and systems reduce or eliminate bubble formation in the filler fluid of the droplet operations gap, thereby permitting completion of multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap.



Techniques and Droplet Actuator Designs for Reducing Bubble Formation

1 Related Applications

5 In addition to the patent applications cited herein, each of which is incorporated herein by reference, this patent application is related to and claims priority to U.S. Provisional Patent Application No. 61/664,980, filed on June 27, 2012, entitled “Methods of Providing a Reliable Ground Connection to Droplets in a Droplet Actuator and Thereby Reduce or Eliminate Air Bubble Formation”; U.S. Provisional Patent Application No. 61/666,417, filed on June 29, 2012, entitled “Reduction of Bubble Formation in a Droplet Actuator”; and U.S. Provisional Patent Application No. 61/678,263, filed on August 1, 2012 entitled “Techniques and Droplet Actuator Designs for Reducing Bubble Formation”; the entire disclosures of which are incorporated herein by reference.

2 Field of the Invention

15 The invention relates to methods and systems for reducing or eliminating bubble formation in droplet actuators, thereby permitting completion of multiple droplet operations without interruption by bubble formation.

3 Background

20 A droplet actuator typically includes one or more substrates configured to form a surface or gap for conducting droplet operations. The one or more substrates establish a droplet operations surface or gap for conducting droplet operations and may also include electrodes arranged to conduct the droplet operations. The droplet operations substrate or the gap between the substrates may be coated or filled with a filler fluid that is immiscible with the liquid that forms the droplets. Bubble formation in the filler fluid in a droplet actuator can interfere with functionality of the droplet actuator. There is a need for techniques for preventing unwanted bubbles from forming in the filler fluid in a droplet actuator.

4 Brief Description of the Invention

30 A method of performing droplet operations on a droplet in a droplet actuator is provided, the method including: (a) providing a droplet actuator including a top substrate and a bottom substrate separated to form a droplet operations gap, where the droplet actuator further includes an arrangement of droplet operations electrodes

arranged for conducting droplet operations thereon; (b) filling the droplet operations gap of the droplet actuator with a filler fluid; (c) providing a droplet in the droplet operations gap; (d) conducting multiple droplet operations on the droplet in the droplet operations gap, where the droplet is transported through the filler fluid in the droplet operations gap; and (e) maintaining substantially consistent contact between the droplet and an electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap; where the substantially consistent contact between the droplet and the electrical ground permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap. In certain embodiments, the method further includes heating the droplet in the droplet operations gap, particularly heating the droplet to at least sixty percent of boiling point. In other embodiments, the droplet is heated to a minimum temperature of seventy five degrees Celsius. In other embodiments, the droplet is heated to within twenty degrees Celsius of boiling point. In certain embodiments, conducting the multiple droplet operations without the interruption by the bubble formation in the filler fluid in the droplet operations gap includes conducting at least 10, at least 100, at least 1,000, or at least 100,000 droplet operations. In other embodiments, conducting the multiple droplet operations without the interruption by the bubble formation in the filler fluid in the droplet operations gap includes completing an assay or completing multiple cycles of a polymerase chain reaction. In other embodiments, the droplet includes multiple droplets in the droplet operations gap, and substantially consistent contact is maintained between multiple droplets and the electrical ground while conducting multiple droplet operations on the multiple droplets in the droplet operations gap. In another embodiment, the filler fluid is an electrically conductive filler fluid.

In other embodiments, maintaining substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap includes grounding the top substrate of the droplet actuator to the electrical ground and maintaining substantially consistent contact between the droplet and the top substrate. In other embodiments, maintaining substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap includes texturing the surface of the top substrate. In other embodiments, maintaining substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap includes adjusting a height of the droplet operations gap, particularly reducing the

height of the droplet operations gap. In some embodiments, the height of the droplet operations gap may be adjusted with a spring. In certain embodiments, maintaining substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap includes moving the electrical ground toward the droplet. In certain embodiments, maintaining substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap includes merging the droplet with another droplet.

In certain embodiments, the method of performing droplet operations on a droplet in a droplet actuator further includes: (i) heating the droplet in a zone of the droplet operations gap; and (ii) arranging the electrical ground coplanar to the droplet operations electrodes in the zone to maintain the substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap.

In other embodiments, the droplet operations electrodes are arranged on one or both of the bottom and/or top substrates. In other embodiments, maintaining substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap includes providing the droplet operations electrodes in various arrangements, including an overlapping arrangement, an interdigitated arrangement, or a triangular arrangement.

In certain embodiments, the method of performing droplet operations on a droplet in a droplet actuator further includes: (i) bounding the droplet operations gap with a sidewall and an opposite sidewall to create a droplet operations channel; (ii) arranging the droplet operations electrodes on the sidewall; (iii) arranging one or more ground electrodes along the opposite sidewall; and (iv) connecting the one or more ground electrodes to the electrical ground; where the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap is unaffected by gravity. In some embodiments, the sidewall includes a first rail and the opposite sidewall includes a second rail, where the first rail and second rail are elongated three-dimensional (3D) structures that are arranged in parallel with each other. The method may further include offsetting positions of the droplet operations electrodes and the position of the one or more ground electrodes. The method may also include where the one or more ground electrodes are a continuous strip. The method may further include oppositely arranging each droplet operations electrode to each one or more ground electrode.

5 In other embodiments, the method of performing droplet operations on a droplet in a droplet actuator further includes: (i) bounding the droplet operations gap with a sidewall and an opposite sidewall to create a droplet operations channel; (ii) arranging the droplet operations electrodes on the sidewall; (iii) arranging one or more ground
10 electrodes along the bottom substrate; and (iv) connecting the one or more ground electrodes to the electrical ground; where the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap is unaffected by gravity. In some embodiments, the sidewall includes a first rail and the opposite sidewall includes a second rail, where
15 the first rail and second rail are elongated three-dimensional (3D) structures that are arranged in parallel with each other.

In certain embodiments, the method of performing droplet operations on a droplet in a droplet actuator further includes: (i) applying a voltage to transport the droplet from an unactivated electrode to an activated electrode; and (ii) reducing electrical charges
20 in the droplet operations gap as the droplet is transported to the activated electrode; where bubble formation in the filler fluid in the droplet operations gap is reduced or eliminated. In other embodiments, the method further includes heating the droplet in the droplet operations gap. In certain embodiments, the electrical charges may be reduced by adjusting a height of the droplet operations gap, particularly reducing the
25 height of the droplet operations gap, or texturing the surface of the top substrate.

In other embodiments, the method of performing droplet operations on a droplet in a droplet actuator further includes: (i) applying a voltage to transport the droplet from an unactivated electrode to an activated electrode; and (ii) reducing discharge of electrical charges as the droplet is transported to the activated electrode; where bubble
30 formation in the filler fluid in the droplet operations gap is reduced or eliminated. In other embodiments, the method further includes heating the droplet in the droplet operations gap. In certain embodiments, the discharge of electrical charges may be reduced by adjusting a height of the droplet operations gap, particularly reducing the height of the droplet operations gap, or texturing the surface of the top substrate.

30 In certain embodiments, a method of performing droplet operations on a droplet in a droplet actuator is provided, including: (a) providing a droplet actuator including a top substrate and a bottom substrate separated to form a droplet operations gap, where the droplet actuator further includes an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon; (b) filling the droplet operations
35 gap of the droplet actuator with a filler fluid; (c) providing a droplet in the droplet

operations gap; (d) heating the droplet to within twenty degrees Celsius of boiling to produce a heated droplet; (e) conducting multiple droplet operations on the heated droplet in the droplet operations gap, where the heated droplet is transported through the filler fluid in the droplet operations gap; and (f) reducing accumulation of electrical charges in the droplet operations gap as the heated droplet is transported through the filler fluid in the droplet operations gap; where the reduced accumulation of electrical charges in the droplet operations gap permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap.

Systems for performing droplet operations on a droplet in a droplet actuator are also provided. In some embodiments, the system includes a processor for executing code and a memory in communication with the processor, and code stored in the memory that causes the processor at least to: (a) provide a droplet in the droplet operations gap of a droplet actuator, where the droplet actuator includes a top substrate and a bottom substrate separated to form the droplet operations gap, and where the droplet actuator further includes an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon; (b) fill the droplet operations gap of the droplet actuator with a filler fluid; (c) heat the droplet in a zone of the droplet operations gap to within twenty degrees Celsius of boiling to produce a heated droplet; (d) conduct multiple droplet operations on the heated droplet in the droplet operations gap, where the heated droplet is transported through the filler fluid in the zone of the droplet operations gap; and (e) maintain substantially consistent contact between the heated droplet and an electrical ground while conducting the multiple droplet operations on the heated droplet in the zone of the droplet operations gap; where the substantially consistent contact between the heated droplet and the electrical ground permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the zone of the droplet operations gap. In some embodiments, the code causing the processor to conduct the multiple droplet operations without the interruption by the bubble formation in the filler fluid in the zone of the droplet operations gap includes conducting at least 10, at least 100, at least 1,000, or at least 100,000 droplet operations. In further embodiments, the code further causes the processor to complete an assay or to complete multiple cycles of a polymerase chain reaction without the interruption by the bubble formation in the filler fluid in the zone of the droplet operations gap.

In certain embodiments of the system for performing droplet operations on a droplet in a droplet actuator, the code further causes the processor to ground the top substrate of the droplet actuator to the electrical ground, where maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for maintaining substantially consistent contact between the heated droplet and the top substrate while conducting the multiple droplet operations on the heated droplet in the zone of the droplet operations gap. In some embodiments, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for adjusting a height of the droplet operations gap, particularly reducing the height of the droplet operations gap. In some embodiments, the means for adjusting the height of the droplet operations gap includes a spring. In other embodiments, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for texturing the surface of the top substrate of the droplet operations gap. In some embodiments, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for moving the electrical ground toward the droplet. In other embodiments, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for arranging the electrical ground coplanar to the droplet operations electrodes in the zone. In certain embodiments, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for merging the droplet with another droplet.

In other embodiments of the system for performing droplet operations on a droplet in a droplet actuator, the droplet operations electrodes are arranged on one or both of the bottom and/or top substrates. In other embodiments of the system, maintaining substantially consistent contact between the heated droplet and the electrical ground while conducting the multiple droplet operations on the heated droplet in the zone of the droplet operations gap includes providing the droplet operations electrodes in various arrangements, including an overlapping arrangement, an interdigitated arrangement, or a triangular arrangement. In certain embodiments, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for decreasing a distance between adjacent droplet operations electrodes.

In other embodiments of the system, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for: (i) bounding the droplet operations gap with a sidewall and an opposite sidewall to

create a droplet operations channel; (ii) arranging the droplet operations electrodes on the sidewall; (iii) arranging one or more ground electrodes along the bottom substrate; and (iv) connecting the one or more ground electrodes to the electrical ground; where the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap is unaffected by gravity. In some embodiments, the sidewall includes a first rail and the opposite sidewall includes a second rail, where the first rail and second rail are elongated three-dimensional (3D) structures that are arranged in parallel with each other. In other embodiments of the system, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for offsetting positions of the droplet operations electrodes to the positions of the one or more ground electrodes. In other embodiments of the system, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for arranging the one or more ground electrodes as a continuous strip. In other embodiments of the system, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for oppositely arranging each droplet operations electrode to each one or more ground electrodes.

In other embodiments of the system, maintaining substantially consistent contact between the heated droplet and the electrical ground includes means for: (i) bounding the droplet operations gap with a sidewall and an opposite sidewall to create a droplet operations channel; (ii) arranging the droplet operations electrodes on the sidewall; (iii) arranging one or more ground electrodes along the bottom substrate; and (iv) connecting the one or more ground electrodes to the electrical ground; where the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap is unaffected by gravity. In some embodiments, the sidewall includes a first rail and the opposite sidewall includes a second rail, where the first rail and second rail are elongated three-dimensional (3D) structures that are arranged in parallel with each other.

In another embodiment, a system for performing droplet operations on a droplet in a droplet actuator is provided, including a processor for executing code and a memory in communication with the processor, the system including code stored in the memory that causes the processor at least to: (a) provide a droplet in the droplet operations gap of a droplet actuator, where the droplet actuator includes a top substrate and a bottom substrate separated to form the droplet operations gap, and

where the droplet actuator further includes an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon; (b) fill the droplet operations gap of the droplet actuator with a filler fluid; (c) provide a droplet in the droplet operations gap; (d) heat the droplet to within twenty degrees Celsius of boiling to produce a heated droplet; (e) conduct multiple droplet operations on the heated droplet in the droplet operations gap, where the heated droplet is transported through the filler fluid in the droplet operations gap; and (f) reduce accumulation of electrical charges in the droplet operations gap as the heated droplet is transported through the filler fluid in the droplet operations gap; where the reduced accumulation of electrical charges in the droplet operations gap permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap.

A computer readable medium storing processor executable instructions for performing a method of performing droplet operations on a droplet in a droplet actuator is also provided, the method including: (a) providing a droplet actuator including a top substrate and a bottom substrate separated to form a droplet operations gap, and where the droplet actuator further includes an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon; (b) filling the droplet operations gap of the droplet actuator with a filler fluid; (c) providing a droplet in the droplet operations gap; (d) conducting multiple droplet operations on the droplet in the droplet operations gap, where the droplet is transported through the filler fluid in the droplet operations gap; and (e) maintaining substantially consistent contact between the droplet and an electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap; where the substantially consistent contact between the droplet and the electrical ground permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap.

In another embodiment, a computer readable medium storing processor executable instructions for performing a method of performing droplet operations on a droplet in a droplet actuator is also provided, the method including: (a) providing a droplet actuator including a top substrate and a bottom substrate separated to form a droplet operations gap, and where the droplet actuator further includes an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon; (b) filling the droplet operations gap of the droplet actuator with a filler fluid; (c) providing a droplet in the droplet operations gap; (d) heating the droplet to within

twenty degrees Celsius of boiling to produce a heated droplet; (e) conducting multiple droplet operations on the heated droplet in the droplet operations gap, where the heated droplet is transported through the filler fluid in the droplet operations gap; and (f) reducing accumulation of electrical charges in the droplet operations gap as the heated droplet is transported through the filler fluid in the droplet operations gap; where the reduced accumulation of electrical charges in the droplet operations gap permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap.

A droplet actuator is also provided, including: (a) a top substrate and a bottom substrate separated to form a droplet operations gap, where the droplet operations gap is filled with a filler fluid; (b) a sidewall and an opposite sidewall bounding the droplet operations gap, thereby creating a droplet operations channel; (c) an arrangement of droplet operations electrodes on the sidewall; and (d) an arrangement of one or more ground electrodes along the opposite sidewall, where the one or more ground electrodes are connected to an electrical ground; where multiple droplet operations may be conducted on one or more droplets in the droplet operations gap while maintaining substantially consistent contact between the one or more droplets and the one or more ground electrodes, thereby permitting completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap, and where the multiple droplet operations are unaffected by gravity. In some embodiments, the sidewall includes a first rail and the opposite sidewall includes a second rail, where the first rail and second rail are elongated three-dimensional (3D) structures that are arranged in parallel with each other.

These and other embodiments are described more fully below.

5 Brief Description of the Drawings

Figures 1A, 1B, 1C, and 1D illustrate side views of a portion of a droplet actuator and a droplet operations process in which the droplet loses contact with the ground or reference electrode of the top substrate;

Figure 2 illustrates a side view of the droplet actuator at the moment in time of the droplet operations process in which the droplet loses contact with the top substrate and bubbles;

Figures 3A and 3B illustrate side views of examples of a droplet actuator that include a region in which the droplet operations gap height is reduced to assist the droplet to be in reliable contact with the ground or reference of the droplet actuator;

5 Figures 4A and 4B illustrate side views of examples of a droplet actuator that include a region in which the surface of the top substrate is textured to assist the droplet to be in reliable contact with the ground or reference of the droplet actuator;

Figures 5A and 5B illustrate side views of a droplet actuator that includes a set of adjustable ground probes to assist the droplet to be in reliable contact with the ground or reference of the droplet actuator;

10 Figures 6A and 6B illustrate a side view and top view, respectively, of a droplet actuator that includes a ground or reference that is coplanar to the droplet operations electrodes to assist the droplet to be in reliable contact with the ground or reference of the droplet actuator;

15 Figures 7A and 7B illustrate side views of a droplet actuator whose droplet operations gap height is adjustable, wherein the droplet operations gap height can be reduced as needed to assist the droplet to be in reliable contact with the ground or reference of the droplet actuator;

Figures 8A and 8B illustrate side views of droplet actuators that utilize electrical conductivity in the filler fluid to assist the droplet to discharge to the droplet;

20 Figure 9 illustrates a side view of a droplet actuator that includes a ground wire in the droplet operations gap to assist the droplet to be in reliable contact with the ground or reference of the droplet actuator;

25 Figure 10 illustrates a side view of a droplet actuator that utilizes 2X or larger droplets to assist the droplets to be in reliable contact with the ground or reference of the droplet actuator;

Figures 11, 12A, 12B, 12C, and 12D illustrate top views of examples of electrode arrangements that utilize interdigitated droplet operations electrodes to smooth out the transport of droplets from one interdigitated electrode to the next;

30 Figures 13A and 13B illustrate top views of examples of electrode arrangements that utilize triangular droplet operations electrodes to smooth out the transport of droplets from one triangular electrode to the next;

Figures 14A and 14B illustrate a side view and a top down view, respectively, of a droplet actuator in which the droplet operations electrodes are tailored for increasing the speed of droplet operations;

5 Figures 15 through 22B illustrate various views of a droplet actuator that includes a droplet operations channel, wherein the sidewalls of the droplet operations channel includes electrode arrangements to assist the droplet to be in reliable contact with the ground or reference of the droplet actuator;

10 Figure 23 illustrates a side view of a droplet actuator at the moment in time of the droplet operations process in which the droplet loses contact with the top substrate and Taylor cones are formed; and

Figure 24 illustrates a functional block diagram of an example of a microfluidics system that includes a droplet actuator.

6 Definitions

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

15 “Activate,” with reference to one or more electrodes, means affecting a change in the electrical state of the one or more electrodes which, in the presence of a droplet, results in a droplet operation. Activation of an electrode can be accomplished using alternating or direct current. Any suitable voltage may be used. For example, an electrode may be activated using a voltage which is greater than about 150 V, or
20 greater than about 200 V, or greater than about 250 V, or from about 275 V to about 1000 V, or about 300 V. Where alternating current is used, any suitable frequency may be employed. For example, an electrode may be activated using alternating current having a frequency from about 1 Hz to about 10 MHz, or from about 10 Hz to about 60 Hz, or from about 20 Hz to about 40 Hz, or about 30 Hz.

25 “Bubble” means a gaseous bubble in the filler fluid of a droplet actuator. In some cases, bubbles may be intentionally included in a droplet actuator, such as those described in U.S. Patent Pub. No. 20100190263, entitled “Bubble Techniques for a Droplet Actuator,” published on July 29, 2010, the entire disclosure of which is incorporated herein by references. The present invention relates to undesirable
30 bubbles which are formed as a side effect of various processes within a droplet actuator, such as evaporation or hydrolysis of a droplet in a droplet actuator. A bubble may be at least partially bounded by filler fluid. For example, a bubble may

be completely surrounded by filler fluid or may be bounded by filler fluid and one or more surfaces of the droplet actuator. As another example, a bubble may be bounded by filler fluid, one or more surfaces of the droplet actuator, and/or one or more droplets in the droplet actuator.

5 “Droplet” means a volume of liquid on a droplet actuator that is at least partially bounded by a filler fluid. Droplets may, for example, be aqueous or non-aqueous or may be mixtures or emulsions including aqueous and non-aqueous components. Droplets may take a wide variety of shapes; nonlimiting examples include generally
10 disc shaped, slug shaped, truncated sphere, ellipsoid, spherical, partially compressed sphere, hemispherical, ovoid, cylindrical, combinations of such shapes, and various shapes formed during droplet operations, such as merging or splitting or formed as a result of contact of such shapes with one or more surfaces of a droplet actuator. For examples of droplet fluids that may be subjected to droplet operations using the approach of the invention, see International Patent Application No. PCT/US
15 06/47486, entitled, “Droplet-Based Biochemistry,” filed on December 11, 2006. In various embodiments, a droplet may include a biological sample, such as whole blood, lymphatic fluid, serum, plasma, sweat, tear, saliva, sputum, cerebrospinal fluid, amniotic fluid, seminal fluid, vaginal excretion, serous fluid, synovial fluid, pericardial fluid, peritoneal fluid, pleural fluid, transudates, exudates, cystic fluid,
20 bile, urine, gastric fluid, intestinal fluid, fecal samples, liquids containing single or multiple cells, liquids containing organelles, fluidized tissues, fluidized organisms, liquids containing multi-celled organisms, biological swabs and biological washes. Moreover, a droplet may include a reagent, such as water, deionized water, saline solutions, acidic solutions, basic solutions, detergent solutions and/or buffers. Other
25 examples of droplet contents include reagents, such as a reagent for a biochemical protocol, such as a nucleic acid amplification protocol, an affinity-based assay protocol, an enzymatic assay protocol, a sequencing protocol, and/or a protocol for analyses of biological fluids. A droplet may include one or more beads.

30 “Droplet Actuator” means a device for manipulating droplets. For examples of droplet actuators, see Pamula et al., U.S. Patent 6,911,132, entitled “Apparatus for Manipulating Droplets by Electrowetting-Based Techniques,” issued on June 28, 2005; Pamula et al., U.S. Patent Application No. 11/343,284, entitled “Apparatuses and Methods for Manipulating Droplets on a Printed Circuit Board,” filed on January 30, 2006; Pollack et al., International Patent Application No.
35 PCT/US2006/047486, entitled “Droplet-Based Biochemistry,” filed on December 11,

2006; Shenderov, U.S. Patents 6,773,566, entitled "Electrostatic Actuators for Microfluidics and Methods for Using Same," issued on August 10, 2004 and 6,565,727, entitled "Actuators for Microfluidics Without Moving Parts," issued on January 24, 2000; Kim and/or Shah et al., U.S. Patent Application Nos. 10/343,261, 5 entitled "Electrowetting-driven Micropumping," filed on January 27, 2003, 11/275,668, entitled "Method and Apparatus for Promoting the Complete Transfer of Liquid Drops from a Nozzle," filed on January 23, 2006, 11/460,188, entitled "Small Object Moving on Printed Circuit Board," filed on January 23, 2006, 12/465,935, entitled "Method for Using Magnetic Particles in Droplet Microfluidics," filed on 10 May 14, 2009, and 12/513,157, entitled "Method and Apparatus for Real-time Feedback Control of Electrical Manipulation of Droplets on Chip," filed on April 30, 2009; Velev, U.S. Patent 7,547,380, entitled "Droplet Transportation Devices and Methods Having a Fluid Surface," issued on June 16, 2009; Sterling et al., U.S. Patent 7,163,612, entitled "Method, Apparatus and Article for Microfluidic Control 15 via Electrowetting, for Chemical, Biochemical and Biological Assays and the Like," issued on January 16, 2007; Becker and Gascoyne et al., U.S. Patent Nos. 7,641,779, entitled "Method and Apparatus for Programmable fluidic Processing," issued on January 5, 2010, and 6,977,033, entitled "Method and Apparatus for Programmable fluidic Processing," issued on December 20, 2005; Decre et al., U.S. Patent 20 7,328,979, entitled "System for Manipulation of a Body of Fluid," issued on February 12, 2008; Yamakawa et al., U.S. Patent Pub. No. 20060039823, entitled "Chemical Analysis Apparatus," published on February 23, 2006; Wu, International Patent Pub. No. WO/2009/003184, entitled "Digital Microfluidics Based Apparatus for Heat-exchanging Chemical Processes," published on December 31, 2008; Fouillet et al., 25 U.S. Patent Pub. No. 20090192044, entitled "Electrode Addressing Method," published on July 30, 2009; Fouillet et al., U.S. Patent 7,052,244, entitled "Device for Displacement of Small Liquid Volumes Along a Micro-catenary Line by Electrostatic Forces," issued on May 30, 2006; Marchand et al., U.S. Patent Pub. No. 20080124252, entitled "Droplet Microreactor," published on May 29, 2008; Adachi 30 et al., U.S. Patent Pub. No. 20090321262, entitled "Liquid Transfer Device," published on December 31, 2009; Roux et al., U.S. Patent Pub. No. 20050179746, entitled "Device for Controlling the Displacement of a Drop Between two or Several Solid Substrates," published on August 18, 2005; Dhindsa et al., "Virtual Electrowetting Channels: Electronic Liquid Transport with Continuous Channel 35 Functionality," Lab Chip, 10:832–836 (2010); the entire disclosures of which are incorporated herein by reference, along with their priority documents. Certain droplet actuators will include one or more substrates arranged with a droplet operations gap

between them and electrodes associated with (e.g., layered on, attached to, and/or embedded in) the one or more substrates and arranged to conduct one or more droplet operations. For example, certain droplet actuators will include a base (or bottom) substrate, droplet operations electrodes associated with the substrate, one or more dielectric layers atop the substrate and/or electrodes, and optionally one or more hydrophobic layers atop the substrate, the dielectric layers and/or the electrodes forming a droplet operations surface. A top substrate may also be provided, which is separated from the droplet operations surface by a gap, commonly referred to as a droplet operations gap. Various electrode arrangements on the top and/or bottom substrates are discussed in the above-referenced patents and applications and certain novel electrode arrangements are discussed in the description of the invention. During droplet operations it is preferred that droplets remain in continuous contact or frequent contact with a ground or reference electrode. A ground or reference electrode may be associated with the top substrate facing the gap, the bottom substrate facing the gap, and/or in the gap. Where electrodes are provided on both substrates, electrical contacts for coupling the electrodes to a droplet actuator instrument for controlling or monitoring the electrodes may be associated with one or both plates. In some cases, electrodes on one substrate are electrically coupled to the other substrate so that only one substrate is in contact with the droplet actuator. In one embodiment, a conductive material (e.g., an epoxy, such as MASTER BOND™ Polymer System EP79, available from Master Bond, Inc., Hackensack, NJ) provides the electrical connection between electrodes on one substrate and electrical paths on the other substrates, e.g., a ground electrode on a top substrate may be coupled to an electrical path on a bottom substrate by such a conductive material. Where multiple substrates are used, a spacer may be provided between the substrates to determine the height of the gap therebetween and define dispensing reservoirs. The spacer height may, for example, be from about 5 μm to about 600 μm , or about 100 μm to about 400 μm , or about 200 μm to about 350 μm , or about 250 μm to about 300 μm , or about 275 μm . The spacer may, for example, be formed of a layer of projections form the top or bottom substrates, and/or a material inserted between the top and bottom substrates. One or more openings may be provided in the one or more substrates for forming a fluid path through which liquid may be delivered into the droplet operations gap. The one or more openings may in some cases be aligned for interaction with one or more electrodes, e.g., aligned such that liquid flowed through the opening will come into sufficient proximity with one or more droplet operations electrodes to permit a droplet operation to be effected by the droplet operations electrodes using the liquid. The base (or bottom) and top substrates may in some

cases be formed as one integral component. One or more reference electrodes may be provided on the base (or bottom) and/or top substrates and/or in the gap. Examples of reference electrode arrangements are provided in the above referenced patents and patent applications. In various embodiments, the manipulation of droplets by a droplet actuator may be electrode mediated, e.g., electrowetting mediated or dielectrophoresis mediated or Coulombic force mediated. Examples of other techniques for controlling droplet operations that may be used in the droplet actuators of the invention include using devices that induce hydrodynamic fluidic pressure, such as those that operate on the basis of mechanical principles (e.g. external syringe pumps, pneumatic membrane pumps, vibrating membrane pumps, vacuum devices, centrifugal forces, piezoelectric/ultrasonic pumps and acoustic forces); electrical or magnetic principles (e.g. electroosmotic flow, electrokinetic pumps, ferrofluidic plugs, electrohydrodynamic pumps, attraction or repulsion using magnetic forces and magnetohydrodynamic pumps); thermodynamic principles (e.g. bubble generation/phase-change-induced volume expansion); other kinds of surface-wetting principles (e.g. electrowetting, and optoelectrowetting, as well as chemically, thermally, structurally and radioactively induced surface-tension gradients); gravity; surface tension (e.g., capillary action); electrostatic forces (e.g., electroosmotic flow); centrifugal flow (substrate disposed on a compact disc and rotated); magnetic forces (e.g., oscillating ions causes flow); magnetohydrodynamic forces; and vacuum or pressure differential. In certain embodiments, combinations of two or more of the foregoing techniques may be employed to conduct a droplet operation in a droplet actuator of the invention. Similarly, one or more of the foregoing may be used to deliver liquid into a droplet operations gap, e.g., from a reservoir in another device or from an external reservoir of the droplet actuator (e.g., a reservoir associated with a droplet actuator substrate and a flow path from the reservoir into the droplet operations gap). Droplet operations surfaces of certain droplet actuators of the invention may be made from hydrophobic materials or may be coated or treated to make them hydrophobic. For example, in some cases some portion or all of the droplet operations surfaces may be derivatized with low surface-energy materials or chemistries, e.g., by deposition or using in situ synthesis using compounds such as poly- or per-fluorinated compounds in solution or polymerizable monomers. Examples include TEFLON® AF (available from DuPont, Wilmington, DE), members of the cytop family of materials, coatings in the FLUOROPEL® family of hydrophobic and superhydrophobic coatings (available from Cytonix Corporation, Beltsville, MD), silane coatings, fluorosilane coatings, hydrophobic phosphonate derivatives (e.g., those sold by Aculon, Inc), and NOVEC™ electronic coatings

(available from 3M Company, St. Paul, MN), other fluorinated monomers for plasma-enhanced chemical vapor deposition (PECVD), and organosiloxane (e.g., SiOC) for PECVD. In some cases, the droplet operations surface may include a hydrophobic coating having a thickness ranging from about 10 nm to about 1,000 nm. Moreover, in some embodiments, the top substrate of the droplet actuator includes an electrically conducting organic polymer, which is then coated with a hydrophobic coating or otherwise treated to make the droplet operations surface hydrophobic. For example, the electrically conducting organic polymer that is deposited onto a plastic substrate may be poly(3,4-ethylenedioxythiophene) poly(styrenesulfonate) (PEDOT:PSS). Other examples of electrically conducting organic polymers and alternative conductive layers are described in Pollack et al., International Patent Application No. PCT/US2010/040705, entitled "Droplet Actuator Devices and Methods," the entire disclosure of which is incorporated herein by reference. One or both substrates may be fabricated using a printed circuit board (PCB), glass, indium tin oxide (ITO)-coated glass, and/or semiconductor materials as the substrate. When the substrate is ITO-coated glass, the ITO coating is preferably a thickness in the range of about 20 to about 200 nm, preferably about 50 to about 150 nm, or about 75 to about 125 nm, or about 100 nm. In some cases, the top and/or bottom substrate includes a PCB substrate that is coated with a dielectric, such as a polyimide dielectric, which may in some cases also be coated or otherwise treated to make the droplet operations surface hydrophobic. When the substrate includes a PCB, the following materials are examples of suitable materials: MITSUI™ BN-300 (available from MITSUI Chemicals America, Inc., San Jose CA); ARLON™ 11N (available from Arlon, Inc, Santa Ana, CA); NELCO® N4000-6 and N5000-30/32 (available from Park Electrochemical Corp., Melville, NY); ISOLA™ FR406 (available from Isola Group, Chandler, AZ), especially IS620; fluoropolymer family (suitable for fluorescence detection since it has low background fluorescence); polyimide family; polyester; polyethylene naphthalate; polycarbonate; polyetheretherketone; liquid crystal polymer; cyclo-olefin copolymer (COC); cyclo-olefin polymer (COP); aramid; THERMOUNT® nonwoven aramid reinforcement (available from DuPont, Wilmington, DE); NOMEX® brand fiber (available from DuPont, Wilmington, DE); and paper. Various materials are also suitable for use as the dielectric component of the substrate. Examples include: vapor deposited dielectric, such as PARYLENE™ C (especially on glass), PARYLENE™ N, and PARYLENE™ HT (for high temperature, ~300°C) (available from Parylene Coating Services, Inc., Katy, TX); TEFLON® AF coatings; cytop; soldermasks, such as liquid photoimageable soldermasks (e.g., on PCB) like TAIYO™ PSR4000 series, TAIYO™ PSR and AUS

series (available from Taiyo America, Inc. Carson City, NV) (good thermal characteristics for applications involving thermal control), and PROBIMER™ 8165 (good thermal characteristics for applications involving thermal control (available from Huntsman Advanced Materials Americas Inc., Los Angeles, CA); dry film
5 soldermask, such as those in the VACREL® dry film soldermask line (available from DuPont, Wilmington, DE); film dielectrics, such as polyimide film (e.g., KAPTON® polyimide film, available from DuPont, Wilmington, DE), polyethylene, and fluoropolymers (e.g., FEP), polytetrafluoroethylene; polyester; polyethylene naphthalate; cyclo-olefin copolymer (COC); cyclo-olefin polymer (COP); any other
10 PCB substrate material listed above; black matrix resin; and polypropylene. Droplet transport voltage and frequency may be selected for performance with reagents used in specific assay protocols. Design parameters may be varied, e.g., number and placement of on-actuator reservoirs, number of independent electrode connections, size (volume) of different reservoirs, placement of magnets/bead washing zones,
15 electrode size, inter-electrode pitch, and gap height (between top and bottom substrates) may be varied for use with specific reagents, protocols, droplet volumes, etc. In some cases, a substrate of the invention may derivatized with low surface-energy materials or chemistries, e.g., using deposition or in situ synthesis using poly- or per-fluorinated compounds in solution or polymerizable monomers. Examples
20 include TEFLON® AF coatings and FLUROPEL® coatings for dip or spray coating, other fluorinated monomers for plasma-enhanced chemical vapor deposition (PECVD), and organosiloxane (e.g., SiOC) for PECVD. Additionally, in some cases, some portion or all of the droplet operations surface may be coated with a substance for reducing background noise, such as background fluorescence from a PCB
25 substrate. For example, the noise-reducing coating may include a black matrix resin, such as the black matrix resins available from Toray industries, Inc., Japan. Electrodes of a droplet actuator are typically controlled by a controller or a processor, which is itself provided as part of a system, which may include processing functions as well as data and software storage and input and output capabilities. Reagents may
30 be provided on the droplet actuator in the droplet operations gap or in a reservoir fluidly coupled to the droplet operations gap. The reagents may be in liquid form, e.g., droplets, or they may be provided in a reconstitutable form in the droplet operations gap or in a reservoir fluidly coupled to the droplet operations gap. Reconstitutable reagents may typically be combined with liquids for reconstitution.
35 An example of reconstitutable reagents suitable for use with the invention includes those described in Meathrel, et al., U.S. Patent 7,727,466, entitled “Disintegratable films for diagnostic devices,” granted on June 1, 2010.

“Droplet operation” means any manipulation of a droplet on a droplet actuator. A droplet operation may, for example, include: loading a droplet into the droplet actuator; dispensing one or more droplets from a source droplet; splitting, separating or dividing a droplet into two or more droplets; transporting a droplet from one location to another in any direction; merging or combining two or more droplets into a single droplet; diluting a droplet; mixing a droplet; agitating a droplet; deforming a droplet; retaining a droplet in position; incubating a droplet; heating a droplet; vaporizing a droplet; cooling a droplet; disposing of a droplet; transporting a droplet out of a droplet actuator; other droplet operations described herein; and/or any combination of the foregoing. The terms “merge,” “merging,” “combine,” “combining” and the like are used to describe the creation of one droplet from two or more droplets. It should be understood that when such a term is used in reference to two or more droplets, any combination of droplet operations that are sufficient to result in the combination of the two or more droplets into one droplet may be used. For example, “merging droplet A with droplet B,” can be achieved by transporting droplet A into contact with a stationary droplet B, transporting droplet B into contact with a stationary droplet A, or transporting droplets A and B into contact with each other. The terms “splitting,” “separating” and “dividing” are not intended to imply any particular outcome with respect to volume of the resulting droplets (i.e., the volume of the resulting droplets can be the same or different) or number of resulting droplets (the number of resulting droplets may be 2, 3, 4, 5 or more). The term “mixing” refers to droplet operations which result in more homogenous distribution of one or more components within a droplet. Examples of “loading” droplet operations include microdialysis loading, pressure assisted loading, robotic loading, passive loading, and pipette loading. Droplet operations may be electrode-mediated. In some cases, droplet operations are further facilitated by the use of hydrophilic and/or hydrophobic regions on surfaces and/or by physical obstacles. For examples of droplet operations, see the patents and patent applications cited above under the definition of “droplet actuator.” Impedance or capacitance sensing or imaging techniques may sometimes be used to determine or confirm the outcome of a droplet operation. Examples of such techniques are described in Sturmer et al., International Patent Pub. No. WO/2008/101194, entitled “Capacitance Detection in a Droplet Actuator,” published on August 21, 2008, the entire disclosure of which is incorporated herein by reference. Generally speaking, the sensing or imaging techniques may be used to confirm the presence or absence of a droplet at a specific electrode. For example, the presence of a dispensed droplet at the destination electrode following a droplet dispensing operation confirms that the droplet

dispensing operation was effective. Similarly, the presence of a droplet at a detection spot at an appropriate step in an assay protocol may confirm that a previous set of droplet operations has successfully produced a droplet for detection. Droplet transport time can be quite fast. For example, in various embodiments, transport of a droplet from one electrode to the next may exceed about 1 sec, or about 0.1 sec, or about 0.01 sec, or about 0.001 sec. In one embodiment, the electrode is operated in AC mode but is switched to DC mode for imaging. It is helpful for conducting droplet operations for the footprint area of droplet to be similar to electrowetting area; in other words, 1x-, 2x- 3x-droplets are usefully controlled operated using 1, 2, and 3 electrodes, respectively. If the droplet footprint is greater than the number of electrodes available for conducting a droplet operation at a given time, the difference between the droplet size and the number of electrodes should typically not be greater than 1; in other words, a 2x droplet is usefully controlled using 1 electrode and a 3x droplet is usefully controlled using 2 electrodes. When droplets include beads, it is useful for droplet size to be equal to the number of electrodes controlling the droplet, e.g., transporting the droplet.

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

“Droplet Actuator Devices and Methods Employing Magnetic Beads,” published on August 14, 2008; and Monroe et al., U.S. Patent Publication No. 20080283414, entitled “Electrowetting Devices,” filed on May 17, 2007; the entire disclosures of which are incorporated herein by reference, as well as the other patents and patent applications cited herein.

“Reservoir” means an enclosure or partial enclosure configured for holding, storing, or supplying liquid. A droplet actuator system of the invention may include on-cartridge reservoirs and/or off-cartridge reservoirs. On-cartridge reservoirs may be (1) on-actuator reservoirs, which are reservoirs in the droplet operations gap or on the droplet operations surface; (2) off-actuator reservoirs, which are reservoirs on the droplet actuator cartridge, but outside the droplet operations gap, and not in contact with the droplet operations surface; or (3) hybrid reservoirs which have on-actuator regions and off-actuator regions. An example of an off-actuator reservoir is a reservoir in the top substrate. An off-actuator reservoir is typically in fluid communication with an opening or flow path arranged for flowing liquid from the off-actuator reservoir into the droplet operations gap, such as into an on-actuator reservoir. An off-cartridge reservoir may be a reservoir that is not part of the droplet actuator cartridge at all, but which flows liquid to some portion of the droplet actuator cartridge. For example, an off-cartridge reservoir may be part of a system or docking station to which the droplet actuator cartridge is coupled during operation. Similarly, an off-cartridge reservoir may be a reagent storage container or syringe which is used to force fluid into an on-cartridge reservoir or into a droplet operations gap. A system using an off-cartridge reservoir will typically include a fluid passage means whereby liquid may be transferred from the off-cartridge reservoir into an on-cartridge reservoir or into a droplet operations gap.

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

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

example, filler fluid can be considered as a film between such liquid and the electrode/array/matrix/surface.

5 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 Description

10 During droplet operations in a droplet actuator bubbles often form in the filler fluid in the droplet operations gap and interrupt droplet operations. Without wishing to be bound by a particular theory, the inventors have observed that during droplet operations, bubble formation can occur when the droplet loses contact with a reference or ground electrode of the droplet actuator. Further, bubble formation
15 appears to occur as the droplet begins to regain contact with the reference or ground electrode after losing contact. Electrical charges that cause bubble formation may accumulate in the droplet across the layer of filler fluid that is created when the droplet loses contact with the reference or ground electrode. As the droplet regains contact with the top substrate after losing contact this filler fluid layer thins and the charge is discharged. This discharge may be the cause of the bubbles. Figures 1A,
20 1B, 1C, 1D, and 2 illustrate the problem of bubble formation during a droplet transport operation on an electrowetting droplet actuator.

Figures 1A, 1B, 1C, and 1D illustrate side views of a portion of a droplet actuator 100 and a droplet operations process in which the droplet loses contact with the
25 ground or reference electrode of the top substrate. In this example, droplet actuator 100 includes a bottom substrate 110 and a top substrate 112 that are separated by a droplet operations gap 114. Bottom substrate 110 includes an arrangement of droplet operations electrodes 116 (e.g., electrowetting electrodes). Droplet operations electrodes 116 are on the side of bottom substrate 110 that is facing droplet operations gap 114. Top substrate 112 includes a conductive layer 118. Conductive layer 118 is
30 on the side of top substrate 112 that is facing droplet operations gap 114. In one example, conductive layer 118 is formed of indium tin oxide (ITO), which is a material that is electrically conductive and substantially transparent to light. Conductive layer 118 provides a ground or reference plane with respect to droplet

operations electrodes 116, wherein voltages (e.g., electrowetting voltages) are applied to droplet operations electrodes 116. Other layers (not shown), such as hydrophobic layers and dielectric layers, may be present on bottom substrate 110 and top substrate 112.

5 The droplet operations gap 114 of droplet actuator 100 is typically filled with a filler fluid 130. The filler fluid may, for example, include one or more oils, such as silicone oil, or hexadecane filler fluid. One or more droplets 132 in droplet operations gap 114 may be transported via droplet operations along droplet operations electrodes 116 and through the filler fluid 130.

10 Figure 1A, 1B, 1C, and 1D show an electrode sequence for transporting a droplet 132 from, for example, a droplet operations electrode 116A to a droplet operations electrode 116B. Initially and referring now to Figure 1A, droplet operations electrode 116A is turned ON and droplet operations electrode 116B is turned OFF. Therefore, droplet 132 is held atop droplet operations electrode 116A.

15 Referring now to Figure 1B, droplet operations electrode 116A is turned OFF and droplet operations electrode 116B is turned ON and droplet 132 begins to move from droplet operations electrode 116A to droplet operations electrode 116B. Figure 1B shows droplet 132 beginning to deform, whereas a finger of fluid begins to pull from droplet operations electrode 116A onto droplet operations electrode 116B.

20 With droplet operations electrode 116A remaining OFF and droplet operations electrode 116B remaining ON, Figure 1C shows the moment in time at which more of the volume of droplet 132 is transferred from droplet operations electrode 116A onto droplet operations electrode 116B, whereas the volume of fluid is spread across both droplet operations electrode 116A and droplet operations electrode 116B in a manner
25 that causes the droplet 132 to lose contact with top substrate 112 and more particularly to lose contact with conductive layer 118.

With droplet operations electrode 116A remaining OFF and droplet operations electrode 116B remaining ON, Figure 1D shows the moment in time at which the full volume of droplet 132 is atop droplet operations electrode 116B and thus droplet 132
30 has regained contact with conductive layer 118 of top substrate 112.

Figure 2 illustrates a side view of droplet actuator 100 at the moment in time of the droplet operations process in which droplet 132 approaches re-contact with top substrate 112 and bubbles 215 form.

5 The inventors have observed that bubbles can appear at low temperature, even room temperature; however, bubble formation is most prevalent and problematic at elevated temperatures, such as greater than about 80 °C, or greater than 90 °C, or greater than about 95 °C. The inventors have observed that bubbles can appear at low temperature, even room temperature; however, bubble formation is most prevalent and problematic at elevated temperatures, such as greater than about 60% of the droplet's boiling point, or greater than about 70% of the droplet's boiling point, or greater than about 80% of the droplet's boiling point, or greater than about 90% of the droplet's boiling point, or greater than about 95% of the droplet's boiling point.

10 Figure 2 shows an optional heating zone 210 that is associated with droplet actuator 100. As a droplet, such as droplet 132, is transported through heating zone 210 the droplet is heated and bubbles form during droplet operations.

15 In one embodiment, techniques and designs of the invention improve reliability of electrical ground connection to droplets in a droplet actuator to reduce or eliminate bubble formation in the droplet actuator, thereby permitting completion of multiple droplet operations without interruption by bubble formation. In one embodiment, conducting the multiple droplet operations comprises conducting at least ten droplet operations without the interruption by the bubble formation in the filler fluid in the droplet operations gap. In other embodiments, conducting the multiple droplet operations comprises conducting at least 100, at least 1,000, or at least 100,000 droplet operations without the interruption by the bubble formation in the filler fluid in the droplet operations gap.

7.1 Droplet Grounding Techniques

25 Figures 3A and 3B illustrate side views of examples of a droplet actuator 300 that include a region in which the droplet operations gap height is reduced to assist the droplet to be in reliable contact with the ground or reference of the droplet actuator. Referring to Figure 3A, droplet actuator 300 includes a bottom substrate 310 and a top substrate 312 that are separated by a droplet operations gap 314. Bottom substrate 310 includes an arrangement of droplet operations electrodes 316 (e.g., electrowetting electrodes). Top substrate 312 includes a conductive layer 318, such as an ITO layer. Conductive layer 318 provides a ground or reference plane with respect to droplet operations electrodes 316, wherein voltages (e.g., electrowetting voltages) are applied to droplet operations electrodes 316. Additionally, Figure 3A shows a dielectric layer 320 atop conductive layer 318 of top substrate 312. The

droplet operations gap 314 of droplet actuator 300 is filled with a filler fluid 330. A heating zone 340 is associated with droplet actuator 300. As a droplet, such as a droplet 332, is transported through heating zone 340 the droplet is heated.

5 In this example, droplet actuator 300 includes a gap height transition region 345 in which the height of droplet operations gap 314 is reduced in heating zone 340 to assist droplet 332 to be in reliable contact with conductive layer 318, which is the ground or reference of droplet actuator 300. Because the gap height is reduced in heating zone 340, droplet 332 is more likely to maintain contact with conductive layer 318 throughout the entirety of droplet operations process, thus reducing or
10 eliminating bubbles, thereby permitting completion of multiple droplet operations without interruption by bubble formation.

In Figure 3A, which is one example implementation, the surface of top substrate 312 that is facing droplet operations gap 314 has a step feature to accomplish the reduced gap height in heating zone 340. Conductive layer 318 and dielectric layer 320
15 substantially follow the topography of top substrate 312. In Figure 3B, which is another example implementation, the thickness of dielectric layer 320 is varied to accomplish the reduced gap height in heating zone 340. The thickness of dielectric layer 320 is increased in heating zone 340.

Figures 4A and 4B illustrate side views of examples of droplet actuator 300 that include a region in which the surface of top substrate 312 is textured to assist the droplet to be in reliable contact with conductive layer 318, which is the ground or reference. For example, in this embodiment of droplet actuator 300, dielectric layer 320 is textured to assist the droplet to be in reliable contact with conductive layer 318.
20 In the example shown in Figure 4A, dielectric layer 320 has a texture 410 that is a sawtooth texture. In the example shown in Figure 4B, texture 410 of dielectric layer 320 is formed by an arrangement of ridges, projections, or protrusions. In one example, substantially the entire surface area of dielectric layer 320 includes the texture 410. In another example, only the area of dielectric layer 320 in the heating zone 340 includes the texture 410.

30 In another example, needles or wires (not shown) may extend from top substrate 312 into droplet operations gap 314. In yet another example, the conductive layer 318 itself may include ridges, projections, or protrusions (not shown) that extend through dielectric layer 320 and into droplet operations gap 314, wherein the ridges, projections, or protrusions maintain contact with the droplet during droplet

operations, thus reducing or eliminating bubbles, thereby permitting completion of multiple droplet operations without interruption by bubble formation.

5 The texturing may take any form or configuration. The texture 410, for example, may be one or more dimples that outwardly extend into the gap 314. The texturing 410 may be randomly or uniformly created to reduce formation of bubbles. The texturing may have a random height or extension into the gap 314, such that adjacent texturing features (*e.g.*, dimples, ridges, or teeth) may have different apex heights and/or shapes. Alternatively, the texturing may have uniform features, such that all the features are substantially similar. The texturing may also include depressions, craters, or valleys extending into the top surface.

Figures 5A and 5B illustrate side views of droplet actuator 300 that includes a set of adjustable ground probes to assist the droplet to be in reliable contact with conductive layer 318, which is the ground or reference. Here electrical ground may be moved or slid to maintain substantial contact with the droplet. As Figure 5A illustrates, droplet actuator 300 may include a plate 510 that further includes a set of probes 512. Plate 510 and probes 512 are formed of electrically conductive material and are electrically connected to the electrical ground of droplet actuator 300. Probes 512 are, for example, a set of cylindrical point probes or a set of parallel-arranged plates or fins that protrude from plate 510. Openings are provided in top substrate 312 for fitting probes 512 therethrough in a slideable fashion. Because probes 512 are fitted into top substrate 312 in a slideable fashion, the position of the tips of the probes 512 may be adjusted with respect to the droplet operations gap 314. For example, plate 510 may be spring-loaded.

25 In operation, when plate 510 is pushed toward or against top substrate 312, the tips of the probes 512 extend slightly into droplet operations gap 314 and maintain contact with the droplet during droplet operations. In so doing, a ground connection is reliably maintained with the droplet during droplet operations, thus reducing or eliminating bubbles, thereby permitting completion of multiple droplet operations without interruption by bubble formation. However, when desired, plate 510 can be lifted away from top substrate 312 such that the tips of the probes 512 retract out of droplet operations gap 314.

30 In one embodiment, plate 510 and probes 512 are provided in the heated regions only of the droplet actuator. In another embodiment, plate 510 and probes 512 are provided in both the heated regions and unheated regions of the droplet actuator.

5 The electrical ground may be moved or slid using pneumatic, hydraulic, and/or electrical actuators. Any of these actuators may extend the electrical ground into contact with the droplet. When extension is no longer needed, the electrical ground may be retracted away from the droplet. A controller of the droplet actuator may control an actuator, thus controlling a position of the electrical ground.

10 **Figures 6A and 6B** illustrate a side view and top view, respectively, of an example of droplet actuator 300 that includes a ground or reference that is coplanar to droplet operations electrodes 316 to assist the droplet to be in reliable contact with the ground or reference of droplet actuator 300. In this example, in the portion of droplet actuator 300 that is in heating zone 340, the spacing between the droplet operations electrodes 316 is increased to allow a ground or reference plane 610 to be implemented in the same plane as droplet operations electrodes 316 on bottom substrate 310. For example, ground or reference plane 610 is an arrangement of wiring traces that substantially surround each droplet operations electrodes 316.

15 Ground or reference plane 610 is electrically connected to the electrical ground of droplet actuator 300. In this way, while a droplet, such as droplet 332, transitions from one droplet operations electrode 316 to the next, a ground connection of the droplet to ground is maintained, thus reducing or eliminating bubbles, thereby permitting completion of multiple droplet operations without interruption by bubble formation.

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In one example, ground or reference plane 610 is implemented according to Figure 1A of U.S. Patent Publication No. 20060194331, entitled "Apparatuses and methods for manipulating droplets on a printed circuit board," published on August 31, 2006, the entire disclosure of which is incorporated herein by reference.

25 While the presence of ground or reference plane 610 consumes more surface area than the biplanar approach (i.e., conductive layer 318 only), ground or reference plane 610 can be limited to the heated regions of the droplet actuator. In the example shown in Figures 6A and 6B, droplet actuator 300 includes both conductive layer 318 and ground or reference plane 610 in the heated regions. However, in another example, droplet actuator 300 includes only the ground or reference plane 610 in the heated regions and conductive layer 318 in the unheated regions. In yet another example, droplet actuator 300 includes the ground or reference plane 610 throughout the entirety of bottom substrate 310 and there is no conductive layer 318 on any portion of top substrate 312.

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Figures 7A and 7B illustrate side views of an example of droplet actuator 300 whose droplet operations gap height is adjustable. Namely, the height of droplet operations gap 314 can be reduced as needed to assist the droplet to be in reliable contact with conductive layer 318, which is the ground or reference. In one example, a spring force exists between bottom substrate 310 and top substrate 312. For example, multiple springs 710 are provided in droplet operations gap 314. The gap height can be reduced by compressing bottom substrate 310 and top substrate 312 slightly together. Namely, by holding bottom substrate 310 stationary and applying force to top substrate 312, by holding top substrate 312 stationary and applying force to bottom substrate 310, or by applying force to both simultaneously. The force may be applied during the heating of a droplet, or while droplets are in a heated region, in order to reduce the gap height and ensure that the droplet maintains contact with conductive layer 318 of top substrate 312, thus reducing or eliminating bubbles, thereby permitting completion of multiple droplet operations without interruption by bubble formation.

Figures 8A and 8B illustrate side views of examples of droplet actuator 300 that utilize electrical conductivity in the filler fluid to discharge to the droplet. In one example, Figure 8A shows that the droplet operations gap 314 of droplet actuator 300 is filled with a filler fluid 810 that is electrically conductive. Providing an electrically conductive filler fluid permits the droplet to discharge even when it is not in contact with top substrate 312. An example of electrically conductive fluid is a ferrofluid, such as a silicone oil based ferrofluid. Other examples of ferrofluids are known in the art, such as those described in U.S. Patent 4,485,024, entitled "Process for producing a ferrofluid, and a composition thereof," issued on November 27, 1984; and U.S. Patent 4,356,098, entitled "Stable ferrofluid compositions and method of making same," issued on October 26, 1982; the entire disclosures of which are incorporated herein by reference.

In another example, Figure 8B shows that the droplet operations gap 314 of droplet actuator 300 is filled with a filler fluid 820 that contains electrically conductive particles. The electrically conductive particles in the filler fluid permit the droplet to discharge even when it is not in contact with top substrate 312. Examples of electrically conductive particles are known in the art, such as those described in U.S. Patent Publication No. 20070145585, entitled "Conductive particles for anisotropic conductive interconnection," published on June 8, 2007, the entire disclosure of which is incorporated herein by reference.

Figure 9 illustrates a side view of an example of droplet actuator 300 that includes a ground wire 910 in the droplet operations gap 314 to discharge to the droplet. Ground wire 910 is electrically connected to the electrical ground of droplet actuator 300. Ground wire 910 is, for example, formed of copper, aluminum, silver, or gold.

5 The ground wire 910 in the filler fluid extends through the droplet and thus permits the droplet to discharge even when it is not in contact with top substrate 312. In one example, ground wire 910 exists without the presence of conductive layer 318 and therefore alone serves as the ground or reference electrode of droplet actuator 300. In another example, ground wire 910 exists in combination with conductive layer 318

10 and together they serve as the ground or reference electrode of droplet actuator 300. In yet another example, ground wire 910 exists in the heated regions only of the droplet actuator. In still another example, ground wire 910 exists in both the heated regions and unheated regions of the droplet actuator.

Examples of liquid moving along a wire are known in the art, such as those described

15 in U.S. Patent 7,052,244, entitled "Device for displacement of small liquid volumes along a micro-catenary line by electrostatic forces," issued on May 10, 2006; the entire disclosure of which is incorporated herein by reference.

Figure 10 illustrates a side view of droplet actuator 300 that utilizes 2X or larger droplets to assist the droplets to be in reliable contact with conductive layer 318,

20 which is the ground or reference. For example, in advance of heating zone 340, two or more 1X droplets 332 can be merged using droplet operations to form, for example, 2X or 3X droplets 332. The 2X or 3X droplets 332 are then transported into heating zone 340. Droplet operations in heating zone 340 are then conducted using the 2X or 3X droplets 332. In this way, reliable contact between the 2X or 3X

25 droplets 332 and conductive layer 318 is maintained, thus reducing or eliminating bubbles, thereby permitting completion of multiple droplet operations without interruption by bubble formation.

In other embodiments, the viscosity of the droplet can be increased to help maintain contact with conductive layer 318 of top substrate 312. If the droplet viscosity is

30 greater, it is more likely to displace oil in contact with top substrate 312. Further, droplet movement will be slower, and the droplet will be distorted less during droplet operations, thereby helping to maintain contact with conductive layer 318. In yet other embodiments, the viscosity of the filler fluid can be decreased, which helps the droplet stay in contact with top substrate 312.

7.2 Droplet Operations Electrodes for Improved Droplet Transport

Figure 11 illustrates a top view of an example of an electrode arrangement 1100 that utilizes interdigitated droplet operations electrodes to smooth out the transport of droplets from one interdigitated electrode to the next. “Smooth out” means to perform droplet operations with less droplet deformation than when interdigitated electrodes are not provided. For example, electrode arrangement 1100 includes an arrangement of droplet operations electrodes 1110. The edges of each of the droplet operations electrodes 1110 include interdigitations 1112. Droplet operations electrodes 1110 are designed such that the interdigitations 1112 of one droplet operations electrode 1110 are fitted together with the interdigitations 1112 of an adjacent droplet operations electrode 1110, as shown in Figure 11. Examples of interdigitated droplet operations electrodes are known in the art, such as those described in Figure 2 of U.S. Patent No. 6,565,727, entitled “Actuators for microfluidics without moving parts,” issued on May 20, 2003, the entire disclosure of which is incorporated herein by reference.

Droplet operations electrodes 1110 that include interdigitations 1112 have the effect of smoothing out the transport of the droplet from one electrode to the next electrode. This is due to the overlap between electrode surfaces. As a result, during droplet operations the droplet is more likely to remain in contact with the ground or reference electrode of the top substrate (e.g. conductive layer 318 of top substrate 312), thus reducing or eliminating bubbles, thereby permitting completion of multiple droplet operations without interruption by bubble formation. In the example shown in Figure 11, the interdigitations are fairly shallow, meaning they do not extend deep into the base portion of the adjacent electrode.

Figures 12A, 12B, 12C, and 12D illustrate top views of other examples of electrode arrangements that utilize interdigitated droplet operations electrodes to smooth out the transport of droplets from one interdigitated electrode to the next. In these examples, the interdigitations extend to at least the halfway point of the base portion of the adjacent electrode. In one example, an electrode arrangement 1200 of Figure 12A includes an arrangement of droplet operations electrodes 1205. Extending from one side of each droplet operations electrode 1205 is an interdigitation 1210. The side of each droplet operations electrode 1205 that is opposite the interdigitation 1210 includes a cutout 1215. In this example, interdigitation 1210 is an elongated rectangular-shaped finger and, therefore, cutout 1215 is an elongated rectangular-shaped cutout region. When arranged in a line, interdigitation 1210 of one droplet

operations electrode 1205 is fitted into cutout 1215 of the adjacent droplet operations electrode 1205, as shown in Figure 12A.

5 In another example, an electrode arrangement 1220 of Figure 12B includes an arrangement of the droplet operations electrodes 1205. However, in this example, each droplet operations electrode 1205 includes two interdigitations 1210 and two corresponding cutouts 1215. Again, when arranged in a line, the two interdigitations 1210 of one droplet operations electrode 1205 are fitted into the two cutouts 1215 of the adjacent droplet operations electrode 1205, as shown in Figure 12B.

10 In yet another example, an electrode arrangement 1240 of Figure 12C includes an arrangement of droplet operations electrodes 1245. Extending from one side of each droplet operations electrode 1245 is an interdigitation 1250. The side of each droplet operations electrode 1245 that is opposite the interdigitation 1250 includes a cutout 1255. In this example, interdigitation 1250 is an elongated triangular-shaped finger and, therefore, cutout 1255 is an elongated triangular-shaped cutout region. When
15 arranged in a line, interdigitation 1250 of one droplet operations electrode 1245 is fitted into cutout 1255 of the adjacent droplet operations electrode 1245, as shown in Figure 12C.

In still another example, an electrode arrangement 1260 of Figure 12D includes an arrangement of the droplet operations electrodes 1245. However, in this example,
20 each droplet operations electrode 1245 includes two interdigitations 1250 and two corresponding cutouts 1255. Again, when arranged in a line, the two interdigitations 1250 of one droplet operations electrode 1245 are fitted into the two cutouts 1255 of the adjacent droplet operations electrode 1245, as shown in Figure 12D.

25 Droplet operations electrodes 1205 and droplet operations electrodes 1245 are not limited to only one or two interdigitations and cutouts and are not limited to the shapes shown in Figures 12A, 12B, 12C, and 12D. Droplet operations electrodes 1205 and droplet operations electrodes 1245 can include any number and any shapes of interdigitations and cutouts. A main aspect of the electrode arrangements shown in Figures 12A, 12B, 12C, and 12D is that they include interdigitations that extend to at
30 least the halfway point of the base portion of the adjacent droplet operations electrode. For example, the interdigitations extend at least 50%, 60%, 70%, 80%, 90% or more across the base portion of the adjacent droplet operations electrode. The base portion means the portion of the electrode that is not the interdigitation itself.

Figures 13A and 13B illustrate top views of examples of electrode arrangements that utilize triangular droplet operations electrodes to smooth out the transport of droplets from one triangular electrode to the next. Figure 13A shows an electrode arrangement 1300 that includes a line of triangular droplet operations electrodes 1310. During droplet operations, greatest benefit is achieved when the droplet 332 travels in the direction that is away from the apex of the originating triangular droplet operations electrode 1310 and toward the apex of the destination triangular droplet operations electrode 1310. Therefore, in a heated region of a droplet actuator, droplet transport along triangular droplet operations electrodes 1310 may be in one direction. However, outside the heated region triangular droplet operations electrodes 1310 could be used to transport in either direction. Alternatively, triangular droplet operations electrodes 1310 may be provided only in the heated region. Further, triangular droplet operations electrodes 1310 may be provided in a loop, as shown in Figure 13B, in order to transport in both directions.

Figures 14A and 14B illustrate a side view and a top down view, respectively, of droplet actuator 300 in which droplet operations electrodes 316 are tailored for increasing the speed of droplet operations. Each droplet operations electrode 316 has a length L and a width W, wherein the length L is the dimension of the droplet operations electrode 316 that coincides with the direction of droplet travel. Typically, the width W and length L of droplet operations electrodes are about equal. However, in this example, the length L is less than the width W. In one example, the length L is about one half the width W. In this electrode arrangement the travel distance across each droplet operations electrode 316 is reduced and thus the speed of droplet operations is increased. By increasing the speed of droplet operations, the droplet is more likely to maintain contact with conductive layer 318 throughout the entirety of droplet operations process, thus reducing or eliminating bubbles, thereby permitting completion of multiple droplet operations without interruption by bubble formation.

7.3 Droplet Operations Channels

In one embodiment, the droplet operations gap of a droplet actuator is bounded with sidewalls (e.g., a sidewall and an opposite sidewall) to create a droplet operations channel.

Figure 15 illustrates an isometric view of a droplet actuator 1500 that includes a droplet operations channel, wherein the sidewalls of the droplet operations channel include electrode arrangements to assist the droplet to be in reliable contact with the

ground or reference of the droplet actuator. Droplet actuator 1500 includes a bottom substrate 1510 and a top substrate 1512 that are separated by a gap 1514.

Referring now to **Figure 16**, which is an isometric view of bottom substrate 1510 alone, bottom substrate 1510 further includes a first rail 1520 and a second rail 1522. First rail 1520 and second rail 1522 are elongated three-dimensional (3D) structures that are arranged in parallel with each other. There is a space s between first rail 1520 and second rail 1522. First rail 1520 and second rail 1522 have a height h . The space s between first rail 1520 and second rail 1522 forms a droplet operations channel 1524. More particularly, the side of first rail 1520 that is facing droplet operations channel 1524 and the side of second rail 1522 that is facing droplet operations channel 1524 provide droplet operations surfaces. Accordingly, an arrangement of droplet operations electrodes 1530 are provided on the surface of first rail 1520 that is facing droplet operations channel 1524. Similarly, an arrangement of ground or reference electrodes 1532 are provided on the surface of second rail 1522 that is facing droplet operations channel 1524. As a result, droplet operations can be conducted along droplet operations channel 1524 using droplet operations electrodes 1530 and ground or reference electrodes 1532. The space s and the height h of droplet operations channel 1524 are set such that a droplet (e.g., droplet 332) of a certain volume may be manipulated along droplet operations channel 1524.

Referring now to **Figure 17**, which is a cross-sectional view of a portion of droplet actuator 1500 taken along line A-A of Figure 15, there is a gap between top substrate 1512 and the topmost surfaces of first rail 1520 and second rail 1522 that allows the full volume between bottom substrate 1510 and top substrate 1512 to be filled with filler fluid 330.

In operation and referring to Figures 15, 16, and 17, because droplet operations are conducted between droplet operations electrodes 1530 and ground or reference electrodes 1532, which are arranged on the sidewalls of first rail 1520 and second rail 1522, respectively, gravity does not come into play (as shown in Figure 2) to cause droplet 332 to lose contact with ground during any phase of the droplet operations. In this way, reliable contact between droplet 332 and, for example, ground or reference electrodes 1532 is maintained, thus reducing or eliminating bubbles, thereby permitting completion of multiple droplet operations without interruption by bubble formation.

Droplet actuator 1500 and more particularly droplet operations channel 1524 is not limited to the electrode arrangements shown in Figures 15, 16, and 17. Other electrode arrangements may be used in droplet operations channel 1524, examples of which are described below with reference to Figures 18 through 22B.

5 In one example, whereas Figures 15, 16, and 17 show droplet operations electrodes 1530 of first rail 1520 and ground or reference electrodes 1532 of second rail 1522 aligned substantially opposite one another, **Figure 18** illustrates a top down view of a portion of bottom substrate 1510 in which droplet operations electrodes 1530 and ground or reference electrodes 1532 are staggered or offset from one another.

10 In another example, **Figure 19** illustrates a top down view of a portion of bottom substrate 1510 in which the line of multiple ground or reference electrodes 1532 is replaced with a continuous ground or reference electrode 1532.

In yet another example, **Figure 20** illustrates a top down view of a portion of bottom substrate 1510 in which droplet operations electrodes 1530 and ground or reference electrodes 1532 are alternating along both first rail 1520 and second rail 1522. Additionally, in this arrangement, each droplet operations electrode 1530 on one sidewall is opposite a ground or reference electrode 1532 on the opposite sidewall.

15 In yet another example, **Figure 21** illustrates a top down view of a portion of bottom substrate 1510 in which ground or reference electrodes 1532 (or a continuous ground or reference electrode 1532) are provided along both first rail 1520 and second rail 1522 and the droplet operations electrodes 1530 are provided on the floor of droplet operations channel 1524. More details of this configuration are shown with respect to Figures 22A and 22B. Namely, **Figure 22A** illustrates an isometric view of the bottom substrate 1510 shown in Figure 21 and **Figure 22B** illustrates a cross-sectional view of a portion of bottom substrate 1510 taken along line A-A of Figure 22A. Again, Figures 22A and 22B show droplet operations electrodes 1530 arranged on the floor of droplet operations channel 1524 instead of on the sidewalls of droplet operations channel 1524.

20 Referring now to Figures 15 through 22B, in one embodiment, one or more droplet operations channels 1524 are provided in heated regions only of a droplet actuator and used to maintain reliable contact of droplets to ground, thus reducing or eliminating bubbles, thereby permitting completion of multiple droplet operations without interruption by bubble formation. In another embodiment, one or more

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droplet operations channels 1524 are provided in both heated regions and unheated regions of a droplet actuator.

7.4 Taylor Cones and Bubble Formation

5 In a liquid, it is widely assumed that when the critical potential ϕ_0^* has been reached and any further increase will destroy the equilibrium, the liquid body acquires a conical shape referred to as the Taylor cone. For example, when a small volume of liquid is exposed to an electric field the shape of the liquid starts to deform from the shape caused by surface tension alone. As the voltage is increased the effect of the electric field becomes more prominent and as it approaches exerting a similar amount
10 of force on the droplet as does the surface tension a cone shape begins to form with convex sides and a rounded tip. An example of Taylor cones forming in a droplet actuator are described below in Figure 23.

Figure 23 illustrates a side view of droplet actuator 300 at the moment in time of the droplet operations process in which droplet 332 loses contact with top substrate 312 and Taylor cones are formed. For example, a Detail A of Figure 23 shows one or
15 more Taylor cones 2310 formed between droplet 332 and top substrate 312 of droplet actuator 300.

As previously described, it has been observed that bubble formation can occur when the droplet loses contact with the top substrate. More particularly, bubble formation
20 appears to occur as the droplet begins to regain contact with the top substrate after losing contact. This contact is made through a Taylor cone or “cone jet” which is a tiny finger of liquid extracted from the droplet interface because of the high electric field that is present between the droplet and the top substrate. Since a Taylor cone is very small and localized, the charges that go through the Taylor cone are also very
25 localized and the film of filler fluid between the droplet and the substrate can become very thin, resulting in break down of the filler fluid or joule heating and therefore bubbles form, particularly at elevated temperatures.

In order to reduce or eliminate bubbles from forming due to Taylor cones certain solutions may be implemented. In one example, if the contact of the droplet to the
30 ground electrode is again made on a large area, i.e., greater than the area covered by a Taylor cone (e.g., about 10 μ m), no bubbles will form. In another example, the shape, frequency, and/or magnitude of the electrical signal can be controlled in a manner that results in no Taylor cones being formed and thus no bubbles being formed. For

example, frequency must be at least the cone frequency, such as at least about 10 kHz.

7.5 Systems

Figure 24 illustrates a functional block diagram of an example of a microfluidics system 2400 that includes a droplet actuator 2405. Digital microfluidic technology conducts droplet operations on discrete droplets in a droplet actuator, such as droplet actuator 2405, by electrical control of their surface tension (electrowetting). The droplets may be sandwiched between two substrates of droplet actuator 2405, a bottom substrate and a top substrate separated by a droplet operations gap. The bottom substrate may include an arrangement of electrically addressable electrodes. The top substrate may include a reference electrode plane made, for example, from conductive ink or indium tin oxide (ITO). The bottom substrate and the top substrate may be coated with a hydrophobic material. Droplet operations are conducted in the droplet operations gap. The space around the droplets (i.e., the gap between bottom and top substrates) may be filled with an immiscible inert fluid, such as silicone oil, to prevent evaporation of the droplets and to facilitate their transport within the device. Other droplet operations may be effected by varying the patterns of voltage activation; examples include merging, splitting, mixing, and dispensing of droplets.

Droplet actuator 2405 may be designed to fit onto an instrument deck (not shown) of microfluidics system 2400. The instrument deck may hold droplet actuator 2405 and house other droplet actuator features, such as, but not limited to, one or more magnets and one or more heating devices. For example, the instrument deck may house one or more magnets 2410, which may be permanent magnets. Optionally, the instrument deck may house one or more electromagnets 2415. Magnets 2410 and/or electromagnets 2415 are positioned in relation to droplet actuator 2405 for immobilization of magnetically responsive beads. Optionally, the positions of magnets 2410 and/or electromagnets 2415 may be controlled by a motor 2420. Additionally, the instrument deck may house one or more heating devices 2425 for controlling the temperature within, for example, certain reaction and/or washing zones of droplet actuator 2405. In one example, heating devices 2425 may be heater bars that are positioned in relation to droplet actuator 2405 for providing thermal control thereof.

A controller 2430 of microfluidics system 2400 is electrically coupled to various hardware components of the invention, such as droplet actuator 2405, electromagnets

2415, motor 2420, and heating devices 2425, as well as to a detector 2435, an impedance sensing system 2440, and any other input and/or output devices (not shown). Controller 2430 controls the overall operation of microfluidics system 2400. Controller 2430 may, for example, be a general purpose computer, special purpose
5 computer, personal computer, or other programmable data processing apparatus. Controller 2430 serves to provide processing capabilities, such as storing, interpreting, and/or executing software instructions, as well as controlling the overall operation of the system. Controller 2430 may be configured and programmed to control data and/or power aspects of these devices. For example, in one aspect, with
10 respect to droplet actuator 2405, controller 2430 controls droplet manipulation by activating/deactivating electrodes.

Detector 2435 may be an imaging system that is positioned in relation to droplet actuator 2405. In one example, the imaging system may include one or more light-emitting diodes (LEDs) (i.e., an illumination source) and a digital image capture
15 device, such as a charge-coupled device (CCD) camera.

Impedance sensing system 2440 may be any circuitry for detecting impedance at a specific electrode of droplet actuator 2405. In one example, impedance sensing system 2440 may be an impedance spectrometer. Impedance sensing system 2440 may be used to monitor the capacitive loading of any electrode, such as any droplet
20 operations electrode, with or without a droplet thereon. For examples of suitable capacitance detection techniques, see Sturmer et al., International Patent Publication No. WO/2008/101194, entitled "Capacitance Detection in a Droplet Actuator," published on Aug. 21, 2008; and Kale et al., International Patent Publication No. WO/2002/080822, entitled "System and Method for Dispensing Liquids," published
25 on Oct. 17, 2002; the entire disclosures of which are incorporated herein by reference.

Droplet actuator 2405 may include disruption device 2445. Disruption device 2445 may include any device that promotes disruption (lysis) of materials, such as tissues, cells and spores in a droplet actuator. Disruption device 2445 may, for example, be a sonication mechanism, a heating mechanism, a mechanical shearing mechanism, a
30 bead beating mechanism, physical features incorporated into the droplet actuator 2405, an electric field generating mechanism, a thermal cycling mechanism, and any combinations thereof. Disruption device 2445 may be controlled by controller 2430.

It will be appreciated that various aspects of the invention may be embodied as a method, system, computer readable medium, and/or computer program product.

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

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

Program code for carrying out operations of the invention may be written in an object oriented programming language such as Java, Smalltalk, C++ or the like. However, the program code for carrying out operations of the invention may also be written in conventional procedural programming languages, such as the “C” programming language or similar programming languages. The program code may be executed by a processor, application specific integrated circuit (ASIC), or other component that executes the program code. The program code may be simply referred to as a software application that is stored in memory (such as the computer readable medium discussed above). The program code may cause the processor (or any processor-

controlled device) to produce a graphical user interface (“GUI”). The graphical user interface may be visually produced on a display device, yet the graphical user interface may also have audible features. The program code, however, may operate in any processor-controlled device, such as a computer, server, personal digital assistant, phone, television, or any processor-controlled device utilizing the processor and/or a digital signal processor.

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

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

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

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

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

10 8 Concluding Remarks

15 The foregoing detailed description of embodiments refers to the accompanying drawings, which illustrate specific embodiments of the invention. Other embodiments having different structures and operations do not depart from the scope of the present invention. The term “the invention” or the like is used with reference to certain specific examples of the many alternative aspects or embodiments of the applicants’ invention set forth in this specification, and neither its use nor its absence is intended to limit the scope of the applicants’ invention or the scope of the claims. This specification is divided into sections for the convenience of the reader only. Headings should not be construed as limiting of the scope of the invention. The definitions are intended as a part of the description of the invention. It will be 20 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.

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The Claims

We claim:

1. A method of performing droplet operations on a droplet in a droplet actuator,
5 comprising:
 - (a) providing a droplet actuator comprising a top substrate and a bottom substrate separated to form a droplet operations gap, wherein the droplet actuator further comprises an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon;
 - 10 (b) filling the droplet operations gap of the droplet actuator with a filler fluid;
 - (c) providing a droplet in the droplet operations gap;
 - (d) conducting multiple droplet operations on the droplet in the droplet operations gap, wherein the droplet is transported through the filler fluid in the droplet operations gap; and
 - 15 (e) maintaining substantially consistent contact between the droplet and an electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap;wherein the substantially consistent contact between the droplet and the electrical ground permits completion of the multiple droplet operations without interruption by
20 bubble formation in the filler fluid in the droplet operations gap.
2. The method according to claim 1, further comprising heating the droplet in the droplet operations gap.
- 25 3. The method according to claim 1, further comprising heating the droplet in the droplet operations gap to at least sixty percent of boiling point.
4. The method according to claim 1, wherein conducting the multiple droplet operations comprises conducting at least 10 droplet operations without the interruption by the
30 bubble formation in the filler fluid in the droplet operations gap.
5. The method according to claim 1, further comprising heating the droplet in the droplet operations gap to at least sixty percent of boiling point, wherein conducting the multiple droplet operations comprises conducting at least 10 droplet operations
35 without the interruption by the bubble formation in the filler fluid in the droplet operations gap.

6. The method according to claim 5, wherein conducting the multiple droplet operations comprises conducting at least 100 droplet operations without the interruption by the bubble formation in the filler fluid in the droplet operations gap.
- 5 7. The method according to claim 5, wherein conducting the multiple droplet operations comprises conducting at least 1,000 droplet operations without the interruption by the bubble formation in the filler fluid in the droplet operations gap.
8. The method according to claim 5, wherein conducting the multiple droplet operations
10 comprises conducting at least 100,000 droplet operations without the interruption by the bubble formation in the filler fluid in the droplet operations gap.
9. The method according to claim 1, further comprising heating the droplet in the
15 the multiple droplet operations comprises completing an assay without the interruption by the bubble formation in the filler fluid in the droplet operations gap.
10. The method according to claim 1, further comprising heating the droplet in the
20 the multiple droplet operations comprises completing multiple cycles of a polymerase chain reaction without the interruption by the bubble formation in the filler fluid in the droplet operations gap.
11. The method according to claim 1, further comprising heating the droplet in the
25 the droplet operations gap to a minimum temperature of seventy five degrees Celsius, wherein conducting the multiple droplet operations comprises completing an assay without the interruption by the bubble formation in the filler fluid in the droplet operations gap.
12. The method according to claim 1, further comprising heating the droplet in the
30 the droplet operations gap to within twenty degrees Celsius of boiling point, wherein conducting the multiple droplet operations comprises completing an assay without the interruption by the bubble formation in the filler fluid in the droplet operations gap.

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13. The method according to claim 1, wherein the droplet comprises multiple droplets in the droplet operations gap, and wherein substantially consistent contact is maintained between multiple droplets and the electrical ground while conducting multiple droplet operations on the multiple droplets in the droplet operations gap.
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14. The method according to claim 1, further comprising grounding the top substrate of the droplet actuator to the electrical ground and maintaining substantially consistent contact between the droplet and the top substrate while conducting the multiple droplet operations on the droplet in the droplet operations gap.
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15. The method according to claim 1, further comprising texturing the surface of the top substrate to maintain the substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap.
- 15
16. The method according to claim 1, further comprising adjusting a height of the droplet operations gap to maintain the substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap.
- 20
17. The method according to claim 16, further comprising reducing the height of the droplet operations gap to maintain the substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap.
- 25
18. The method according to claim 16, further comprising adjusting the height of the droplet operations gap with a spring to maintain the substantially consistent contact between the droplet and electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap.
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19. The method according to claim 1, further comprising moving the electrical ground toward the droplet to maintain the substantially consistent contact while conducting the multiple droplet operations on the droplet in the droplet operations gap.
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20. The method according to claim 1, wherein the droplet operations electrodes are arranged on one or both of the bottom and/or top substrates.

21. The method according to claim 1, further comprising:
- (i) heating the droplet in a zone of the droplet operations gap; and
 - (ii) arranging the electrical ground coplanar to the droplet operations electrodes in the zone to maintain the substantially consistent contact between the droplet and the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap.
22. The method according to claim 1, wherein the filler fluid is an electrically conductive filler fluid.
23. The method according to claim 1, further comprising merging the droplet with another droplet to maintain the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap.
24. The method according to claim 1, further comprising providing the droplet operations electrodes in an overlapping arrangement to maintain the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap using the droplet operations electrodes.
25. The method according to claim 1, further comprising providing the droplet operations electrodes in an interdigitated arrangement to maintain the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap using the droplet operations electrodes.
26. The method according to claim 1, further comprising providing the droplet operations electrodes in a triangular arrangement to maintain the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap using the droplet operations electrodes.
27. The method according to claim 1, further comprising:
- (i) bounding the droplet operations gap with a sidewall and an opposite sidewall to create a droplet operations channel;
 - (ii) arranging the droplet operations electrodes on the sidewall;
 - (iii) arranging one or more ground electrodes along the opposite sidewall; and
 - (iv) connecting the one or more ground electrodes to the electrical ground,

wherein the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap is unaffected by gravity.

28. The method according to claim 27, wherein the sidewall comprises a first rail and the opposite sidewall comprises a second rail, wherein the first rail and second rail are elongated three-dimensional (3D) structures that are arranged in parallel with each other.
29. The method according to claim 27, further comprising offsetting positions of the droplet operations electrodes and the position of the one or more ground electrodes.
30. The method according to claim 27, wherein the one or more ground electrodes are a continuous strip.
31. The method according to claim 27, further comprising oppositely arranging each droplet operations electrode to each one or more ground electrode.
32. The method according to claim 1, further comprising:
 - (i) bounding the droplet operations gap with a sidewall and an opposite sidewall to create a droplet operations channel;
 - (ii) arranging the droplet operations electrodes on the sidewall;
 - (iii) arranging one or more ground electrodes along the bottom substrate; and
 - (iv) connecting the one or more ground electrodes to the electrical ground,wherein the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap is unaffected by gravity.
33. The method according to claim 32, wherein the sidewall comprises a first rail and the opposite sidewall comprises a second rail, wherein the first rail and second rail are elongated three-dimensional (3D) structures that are arranged in parallel with each other.

34. The method according to claim 1, further comprising:
- (i) applying a voltage to transport the droplet from an unactivated electrode to an activated electrode; and
 - (ii) reducing electrical charges in the droplet operations gap as the droplet is transported to the activated electrode;
- wherein bubble formation in the filler fluid in the droplet operations gap is reduced or eliminated.
35. The method according to claim 34, further comprising heating the droplet in the droplet operations gap.
36. The method according to claim 35, further comprising adjusting a height of the droplet operations gap to reduce the electrical charges.
37. The method according to claim 36, further comprising reducing the height of the droplet operations gap to reduce the electrical charges.
38. The method according to claim 35, further comprising texturing the surface of the top substrate to reduce the electrical charges.
39. The method according to claim 1, further comprising:
- (i) applying a voltage to transport the droplet from an unactivated electrode to an activated electrode; and
 - (ii) reducing discharge of electrical charges as the droplet is transported to the activated electrode;
- wherein bubble formation in the filler fluid in the droplet operations gap is reduced or eliminated.
40. The method according to claim 39, further comprising heating the droplet in the droplet operations gap.
41. The method according to claim 40, further comprising adjusting a height of the droplet operations gap to reduce the discharge of electrical charges.
42. The method according to claim 41, further comprising reducing the height of the droplet operations gap to reduce the discharge of electrical charges.

43. The method according to claim 40, further comprising texturing the surface of the top substrate of the droplet operations gap to reduce the discharge of electrical charges.

44. A method of performing droplet operations on a droplet in a droplet actuator, comprising:

- (a) providing a droplet actuator comprising a top substrate and a bottom substrate separated to form a droplet operations gap, wherein the droplet actuator further comprises an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon;
- (b) filling the droplet operations gap of the droplet actuator with a filler fluid;
- (c) providing a droplet in the droplet operations gap;
- (d) heating the droplet to within twenty degrees Celsius of boiling to produce a heated droplet;
- (e) conducting multiple droplet operations on the heated droplet in the droplet operations gap, wherein the heated droplet is transported through the filler fluid in the droplet operations gap; and
- (f) reducing accumulation of electrical charges in the droplet operations gap as the heated droplet is transported through the filler fluid in the droplet operations gap;

wherein the reduced accumulation of electrical charges in the droplet operations gap permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap.

45. A system for performing droplet operations on a droplet in a droplet actuator, comprising a processor for executing code and a memory in communication with the processor, the system comprising code stored in the memory that causes the processor at least to:

- (a) provide a droplet in the droplet operations gap of a droplet actuator, wherein the droplet actuator comprises a top substrate and a bottom substrate separated to form the droplet operations gap, and wherein the droplet actuator further comprises an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon;
- (b) fill the droplet operations gap of the droplet actuator with a filler fluid;
- (c) heat the droplet in a zone of the droplet operations gap to within twenty degrees Celsius of boiling to produce a heated droplet;

- (d) conduct multiple droplet operations on the heated droplet in the droplet operations gap, wherein the heated droplet is transported through the filler fluid in the zone of the droplet operations gap; and
- (e) maintain substantially consistent contact between the heated droplet and an electrical ground while conducting the multiple droplet operations on the heated droplet in the zone of the droplet operations gap;

wherein the substantially consistent contact between the heated droplet and the electrical ground permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the zone of the droplet operations gap.

- 46. The system according to claim 45, wherein the code further causes the processor to conduct at least 10 droplet operations without the interruption by the bubble formation in the filler fluid in the zone of the droplet operations gap.
- 47. The system according to claim 45, wherein the code further causes the processor to conduct at least 100 droplet operations without the interruption by the bubble formation in the filler fluid in the zone of the droplet operations gap.
- 48. The system according to claim 45, wherein the code further causes the processor to conduct at least 1,000 droplet operations without the interruption by the bubble formation in the filler fluid in the zone of the droplet operations gap.
- 49. The system according to claim 45, wherein the code further causes the processor to conduct at least 100,000 droplet operations without the interruption by the bubble formation in the filler fluid in the zone of the droplet operations gap.
- 50. The system according to claim 45, wherein the code further causes the processor to complete an assay without the interruption by the bubble formation in the filler fluid in the zone of the droplet operations gap.
- 51. The system according to claim 45, wherein the code further causes the processor to complete multiple cycles of a polymerase chain reaction without the interruption by the bubble formation in the filler fluid in the zone of the droplet operations gap.

52. The system according to claim 45, wherein the code further causes the processor to ground the top substrate of the droplet actuator to the electrical ground, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for maintaining substantially consistent contact between the heated droplet and the top substrate while conducting the multiple droplet operations on the heated droplet in the zone of the droplet operations gap.
53. The system according to claim 45, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for adjusting a height of the gap.
54. The system according to claim 53, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for reducing a height of the gap.
55. The system according to claim 45, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for texturing the surface of the top substrate of the droplet operations gap.
56. The system according to claim 45, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for moving the electrical ground toward the droplet.
57. The system according to claim 45, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for arranging the electrical ground coplanar to the droplet operations electrodes in the zone.
58. The system according to claim 45, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for adjusting a height of the gap with a spring.
59. The system according to claim 45, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for merging the droplet with another droplet.

60. The system according to claim 45, wherein the droplet operations electrodes are arranged on one or both of the bottom and/or top substrates.
61. The system according to claim 45, wherein the droplet operations electrodes are provided in an overlapping arrangement to maintain the substantially consistent contact between the heated droplet and the electrical ground while conducting the multiple droplet operations on the heated droplet in the zone of the droplet operations gap using the droplet operations electrodes .
62. The system according to claim 45, wherein the droplet operations electrodes are provided in an interdigitated arrangement to maintain the substantially consistent contact between the heated droplet and the electrical ground while conducting the multiple droplet operations on the heated droplet in the zone of the droplet operations gap using the droplet operations electrodes.
63. The system according to claim 45, the droplet operations electrodes are provided in a triangular arrangement to maintain the substantially consistent contact between the heated droplet and the electrical ground while conducting the multiple droplet operations on the heated droplet in the zone of the droplet operations gap using the droplet operations electrodes.
64. The system according to claim 45, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for decreasing a distance between adjacent droplet operations electrodes.
65. The system according to claim 45, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for:
- (i) bounding the droplet operations gap with a sidewall and an opposite sidewall to create a droplet operations channel;
 - (ii) arranging the droplet operations electrodes on the sidewall;
 - (iii) arranging one or more ground electrodes along the bottom substrate; and
 - (iv) connecting the one or more ground electrodes to the electrical ground;
- wherein the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap is unaffected by gravity.

66. The system according to claim 65, wherein the sidewall comprises a first rail and the opposite sidewall comprises a second rail, wherein the first rail and second rail are elongated three-dimensional (3D) structures that are arranged in parallel with each other.
67. The system according to claim 65, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for offsetting positions of the droplet operations electrodes to the positions of the one or more ground electrodes.
68. The system according to claim 65, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for arranging the one or more ground electrodes as a continuous strip.
69. The system according to claim 65, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for oppositely arranging each droplet operations electrode to each one or more ground electrodes.
70. The system according to claim 65, wherein maintaining substantially consistent contact between the heated droplet and the electrical ground comprises means for:
- (i) bounding the droplet operations gap with a sidewall and an opposite sidewall to create a droplet operations channel;
 - (ii) arranging the droplet operations electrodes on the sidewall;
 - (iii) arranging one or more ground electrodes along the bottom substrate; and
 - (iv) connecting the one or more ground electrodes to the electrical ground;
- wherein the substantially consistent contact with the electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap is unaffected by gravity.
71. The system according to claim 70, wherein the sidewall comprises a first rail and the opposite sidewall comprises a second rail, wherein the first rail and second rail are elongated three-dimensional (3D) structures that are arranged in parallel with each other.

72. A system for performing droplet operations on a droplet in a droplet actuator, comprising a processor for executing code and a memory in communication with the processor, the system comprising code stored in the memory that causes the processor at least to:
- (a) provide a droplet in the droplet operations gap of a droplet actuator, wherein the droplet actuator comprises a top substrate and a bottom substrate separated to form the droplet operations gap, and wherein the droplet actuator further comprises an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon;
 - (b) fill the droplet operations gap of the droplet actuator with a filler fluid;
 - (c) provide a droplet in the droplet operations gap;
 - (d) heat the droplet to within twenty degrees Celsius of boiling to produce a heated droplet;
 - (e) conduct multiple droplet operations on the heated droplet in the droplet operations gap, wherein the heated droplet is transported through the filler fluid in the droplet operations gap; and
 - (f) reduce accumulation of electrical charges in the droplet operations gap as the heated droplet is transported through the filler fluid in the droplet operations gap;
- wherein the reduced accumulation of electrical charges in the droplet operations gap permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap.
73. A computer readable medium storing processor executable instructions for performing a method of performing droplet operations on a droplet in a droplet actuator, the method comprising:
- (a) providing a droplet actuator comprising a top substrate and a bottom substrate separated to form a droplet operations gap, and wherein the droplet actuator further comprises an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon;
 - (b) filling the droplet operations gap of the droplet actuator with a filler fluid;
 - (c) providing a droplet in the droplet operations gap;
 - (d) conducting multiple droplet operations on the droplet in the droplet operations gap, wherein the droplet is transported through the filler fluid in the droplet operations gap; and

- (e) maintaining substantially consistent contact between the droplet and an electrical ground while conducting the multiple droplet operations on the droplet in the droplet operations gap;

wherein the substantially consistent contact between the droplet and the electrical ground permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap.

74. A computer readable medium storing processor executable instructions for performing a method of performing droplet operations on a droplet in a droplet actuator, the method comprising:

- (a) providing a droplet actuator comprising a top substrate and a bottom substrate separated to form a droplet operations gap, and wherein the droplet actuator further comprises an arrangement of droplet operations electrodes arranged for conducting droplet operations thereon;
- (b) filling the droplet operations gap of the droplet actuator with a filler fluid;
- (c) providing a droplet in the droplet operations gap;
- (d) heating the droplet to within twenty degrees Celsius of boiling to produce a heated droplet;
- (e) conducting multiple droplet operations on the heated droplet in the droplet operations gap, wherein the heated droplet is transported through the filler fluid in the droplet operations gap; and
- (f) reducing accumulation of electrical charges in the droplet operations gap as the heated droplet is transported through the filler fluid in the droplet operations gap;

wherein the reduced accumulation of electrical charges in the droplet operations gap permits completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap.

75. A droplet actuator comprising:

- (a) a top substrate and a bottom substrate separated to form a droplet operations gap, wherein the droplet operations gap is filled with a filler fluid;
- (b) a sidewall and an opposite sidewall bounding the droplet operations gap, thereby creating a droplet operations channel;
- (c) an arrangement of droplet operations electrodes on the sidewall; and

(d) an arrangement of one or more ground electrodes along the opposite sidewall, wherein the one or more ground electrodes are connected to an electrical ground;

wherein multiple droplet operations may be conducted on one or more droplets in the droplet operations gap while maintaining substantially consistent contact between the one or more droplets and the one or more ground electrodes, thereby permitting completion of the multiple droplet operations without interruption by bubble formation in the filler fluid in the droplet operations gap, and wherein the multiple droplet operations are unaffected by gravity.

76. The droplet actuator according to claim 75, wherein the sidewall comprises a first rail and the opposite sidewall comprises a second rail, wherein the first rail and second rail are elongated three-dimensional (3D) structures that are arranged in parallel with each other.

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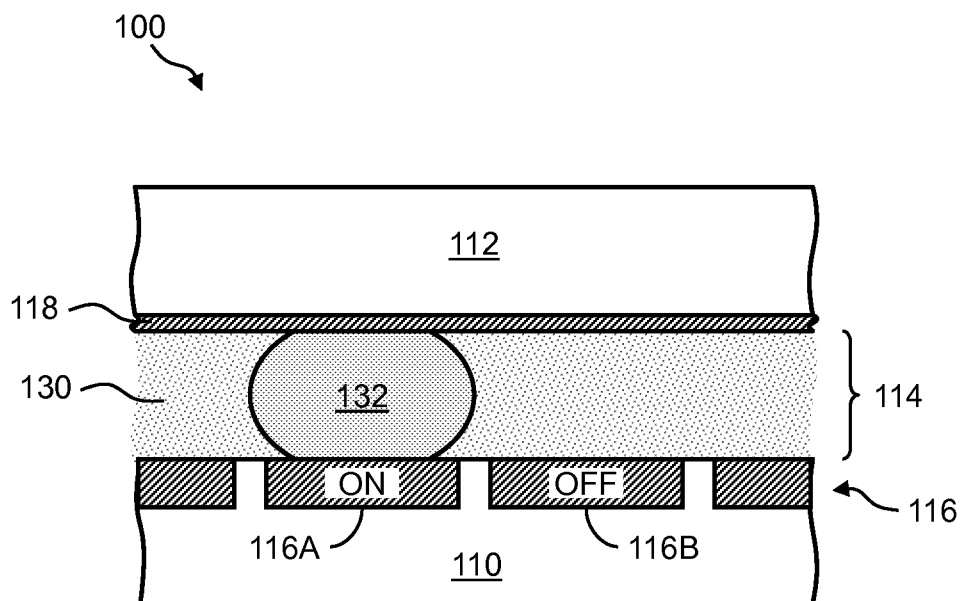


Figure 1A

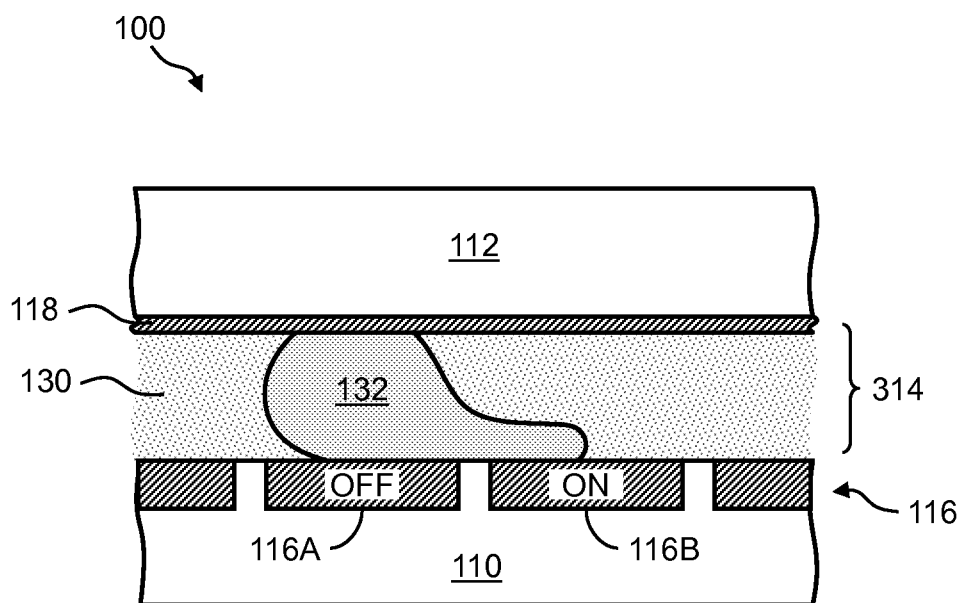


Figure 1B

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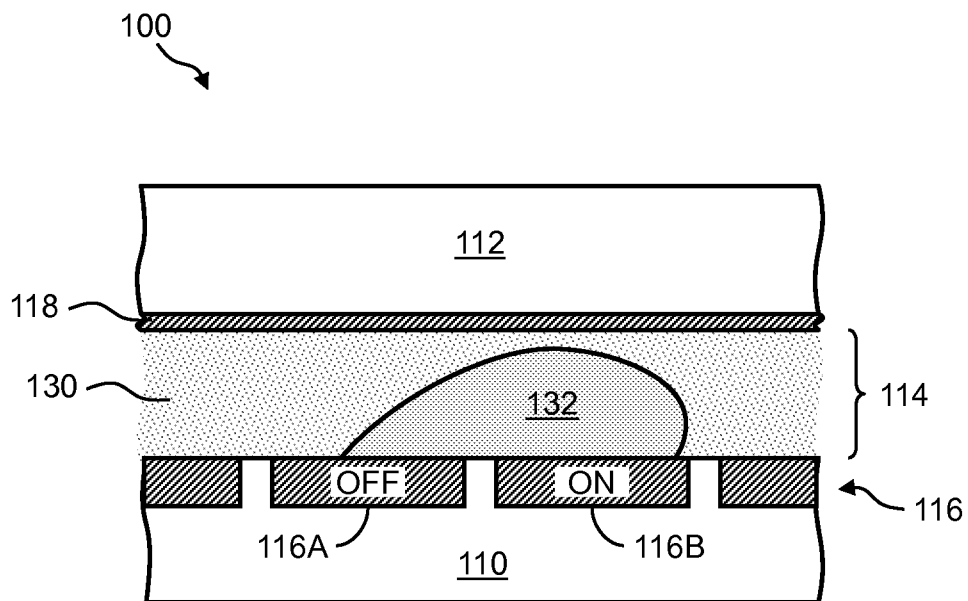


Figure 1C

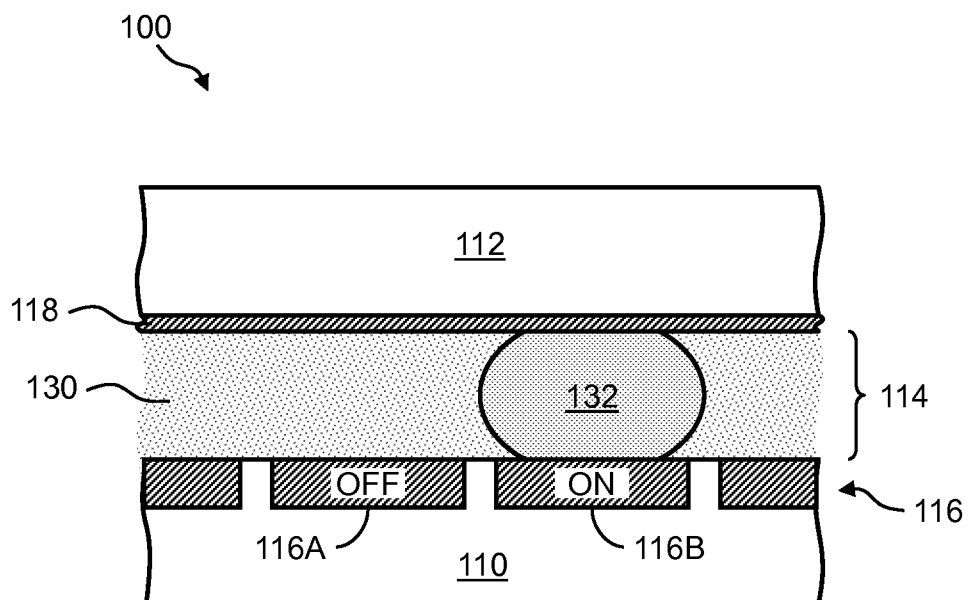


Figure 1D

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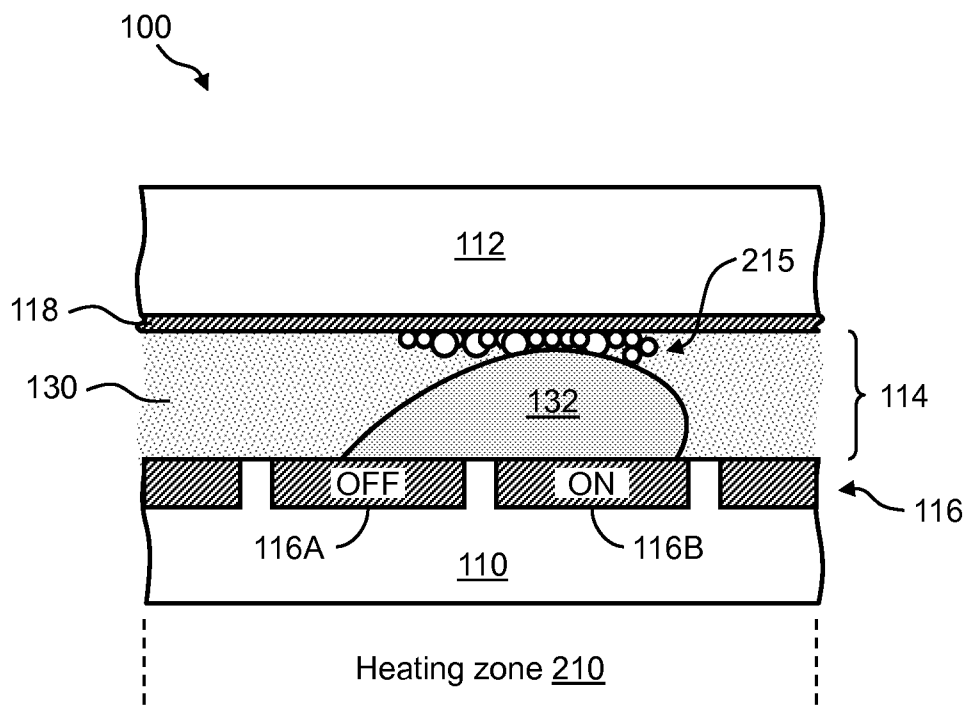


Figure 2

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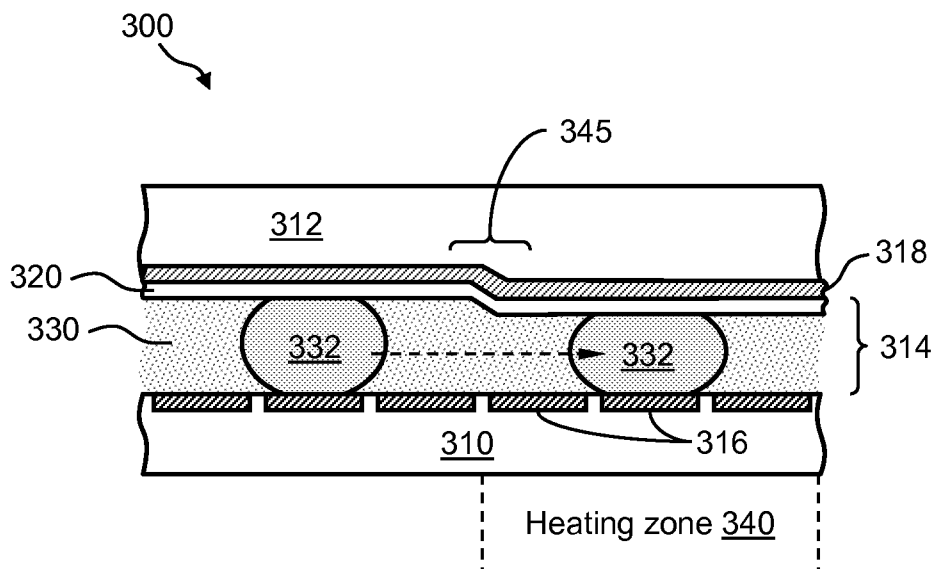


Figure 3A-

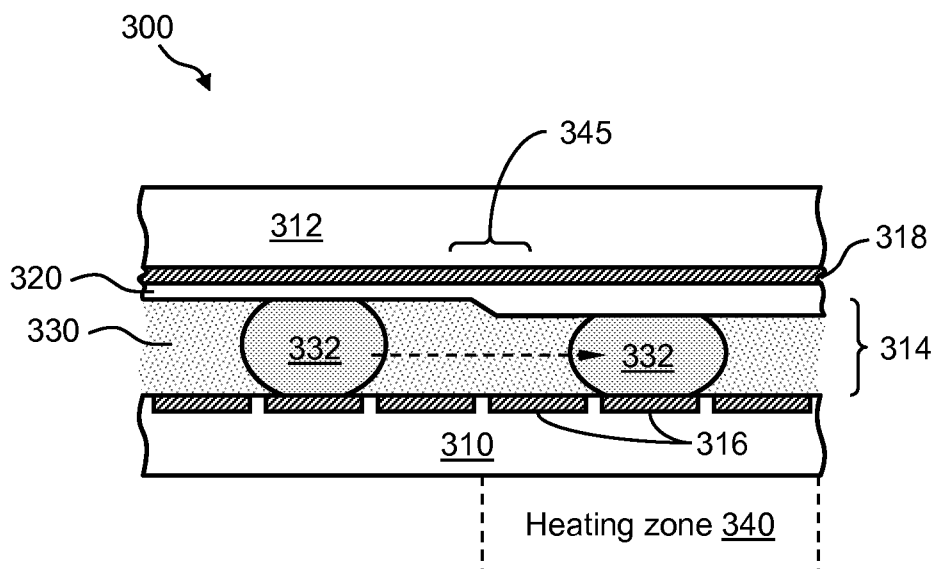


Figure 3B

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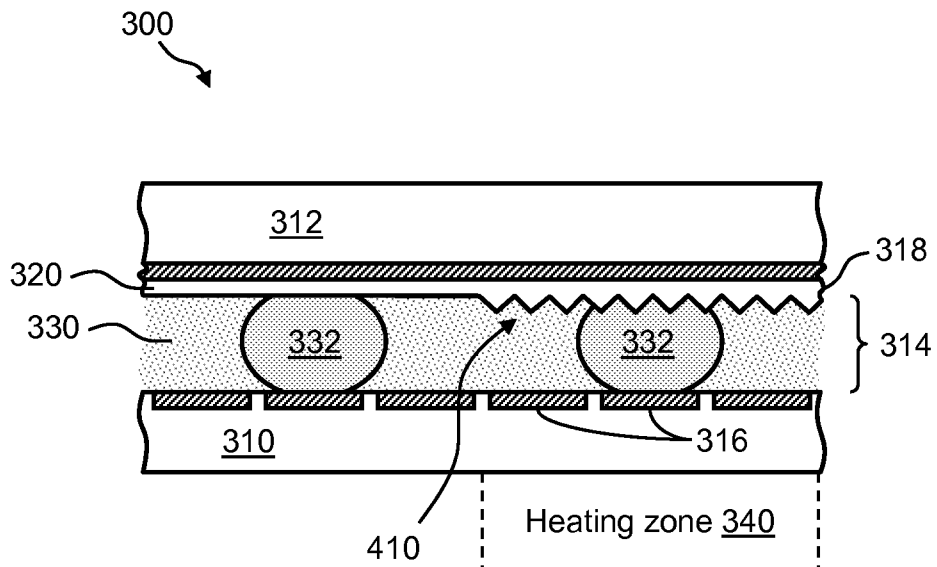


Figure 4A

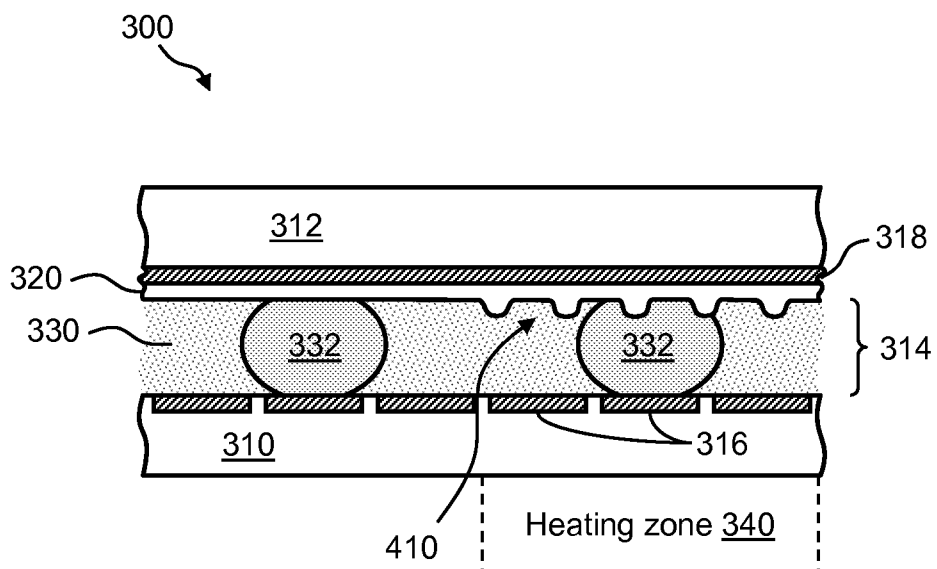


Figure 4B

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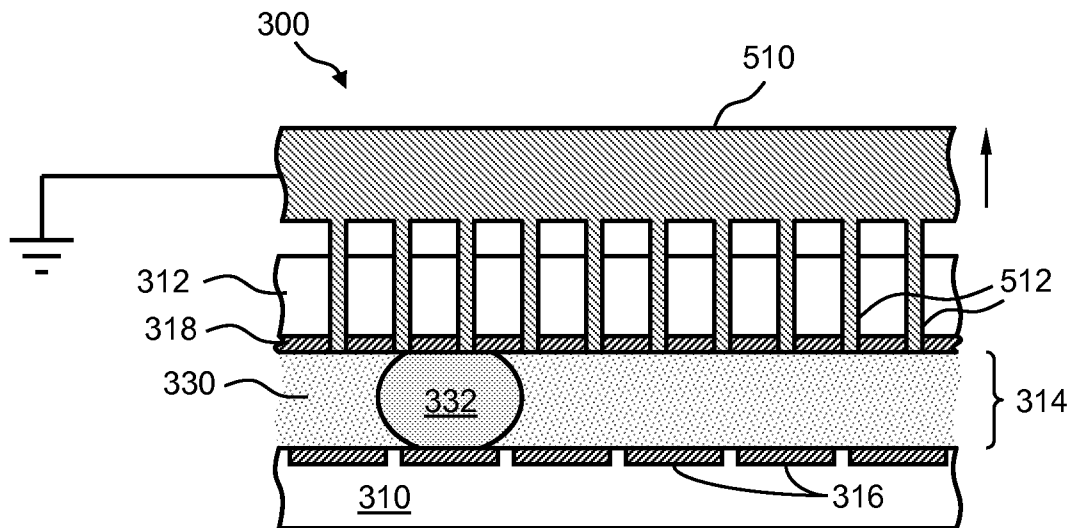


Figure 5A

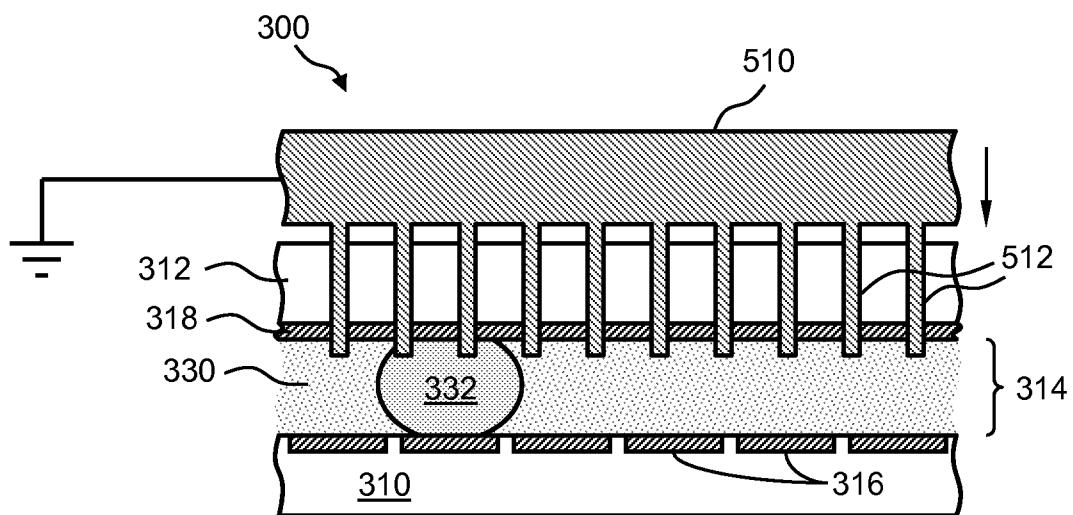


Figure 5B

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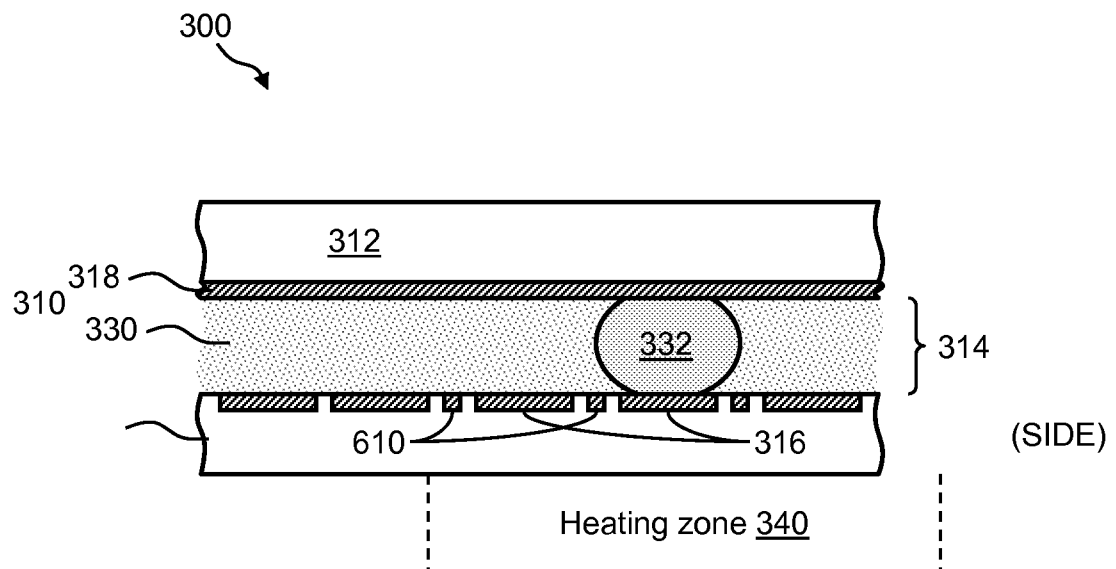


Figure 6A

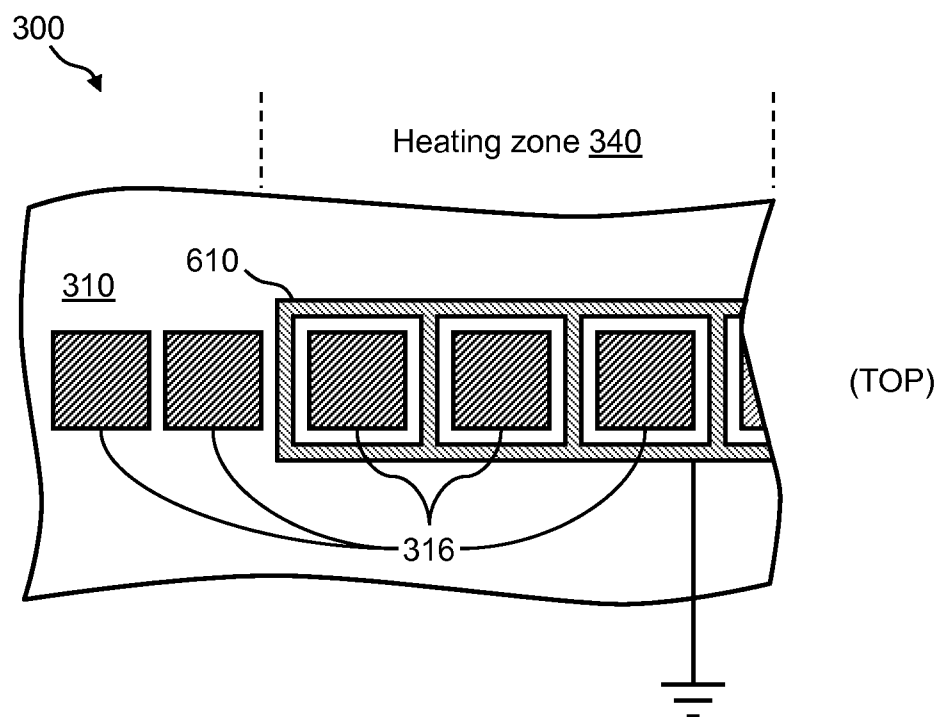
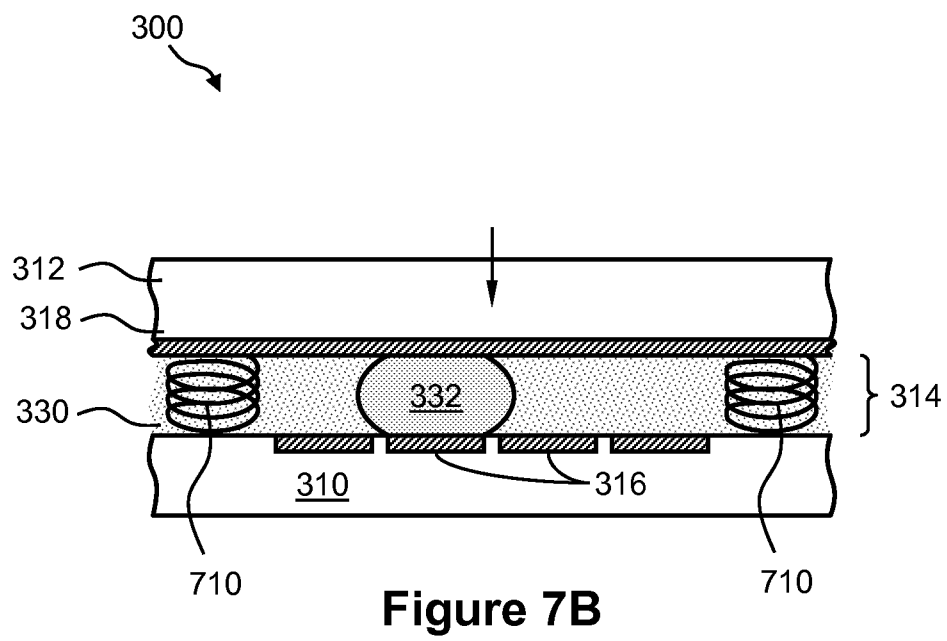
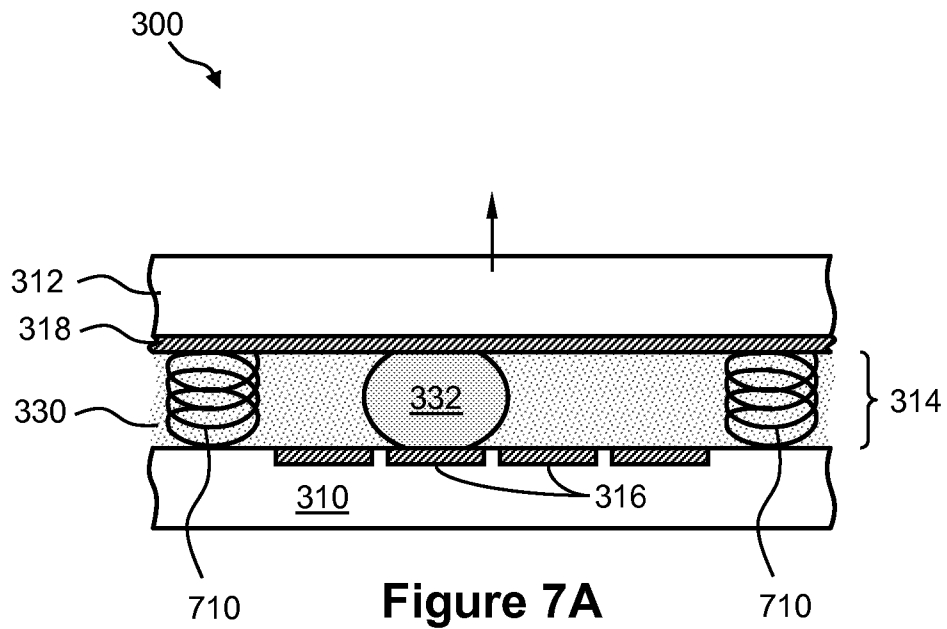


Figure 6B

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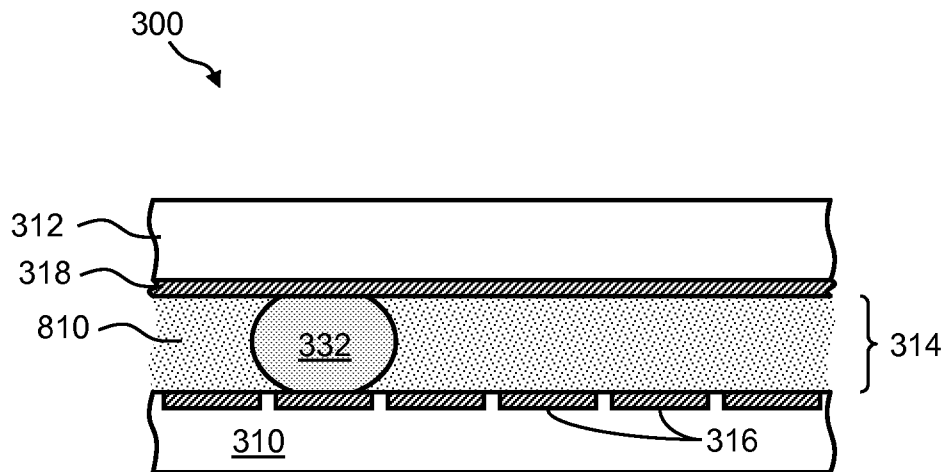


Figure 8A

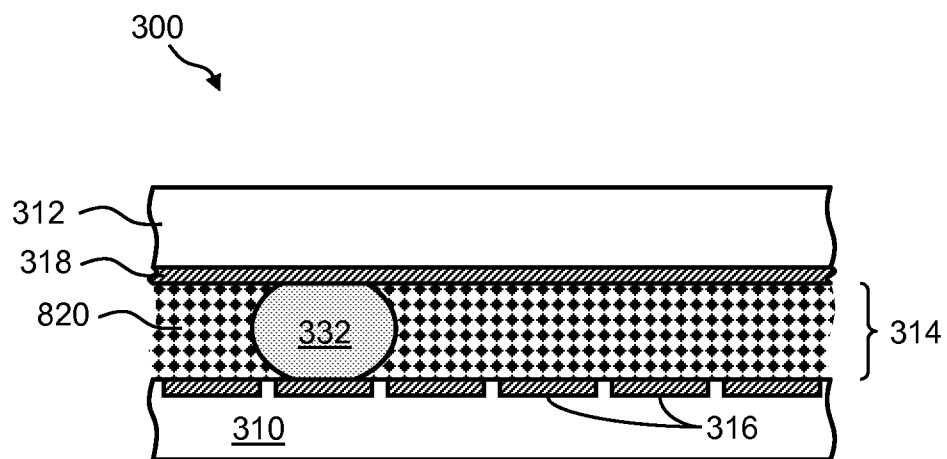


Figure 8B

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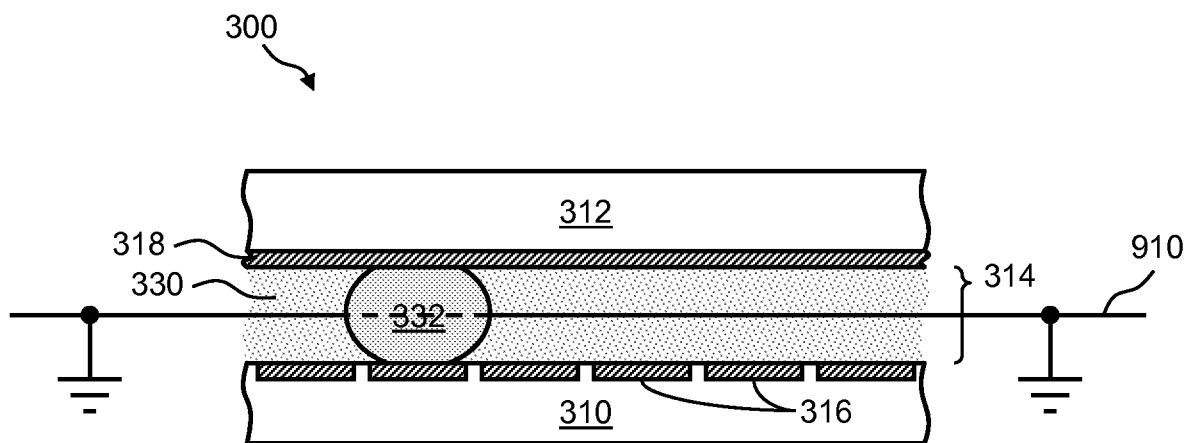


Figure 9

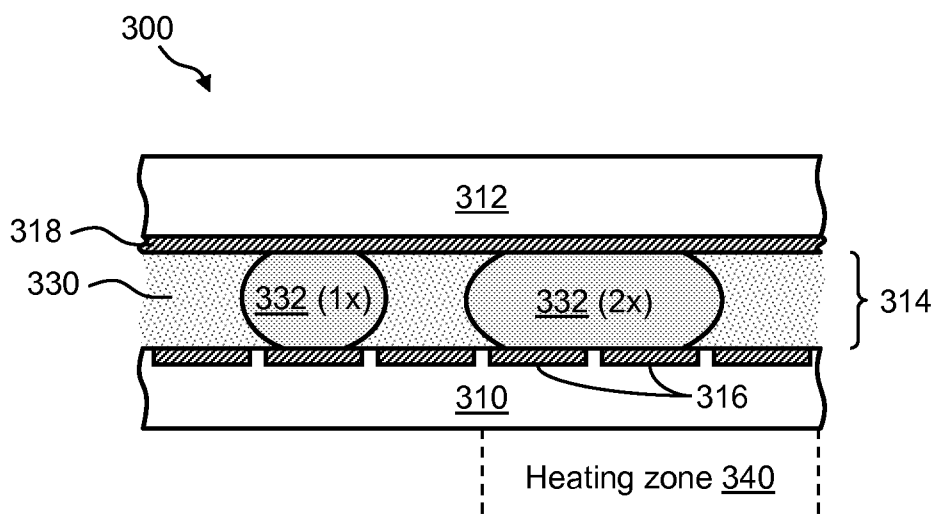


Figure 10

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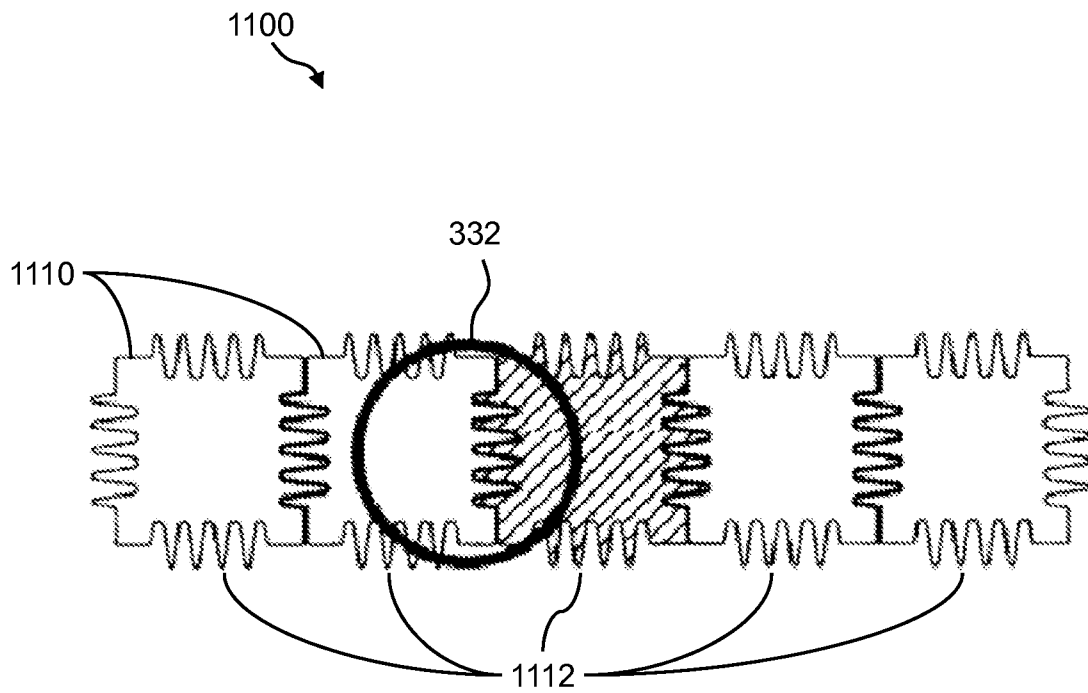


Figure 11

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Figure 12A

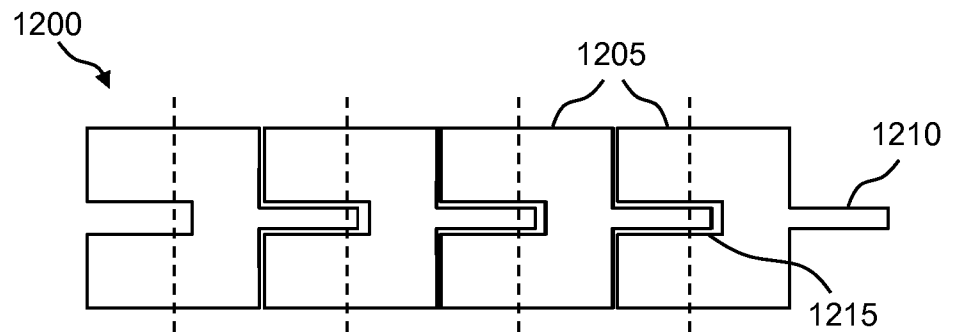


Figure 12B

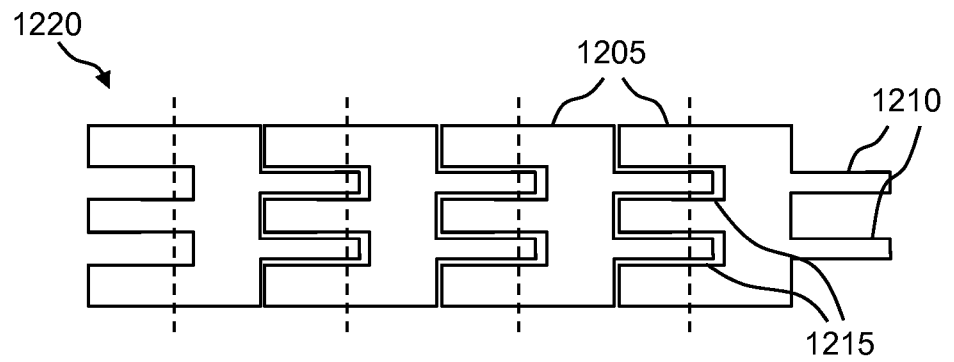


Figure 12C

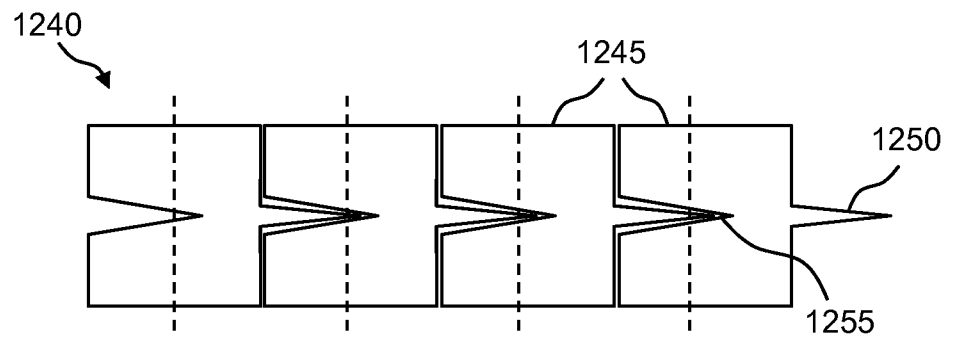
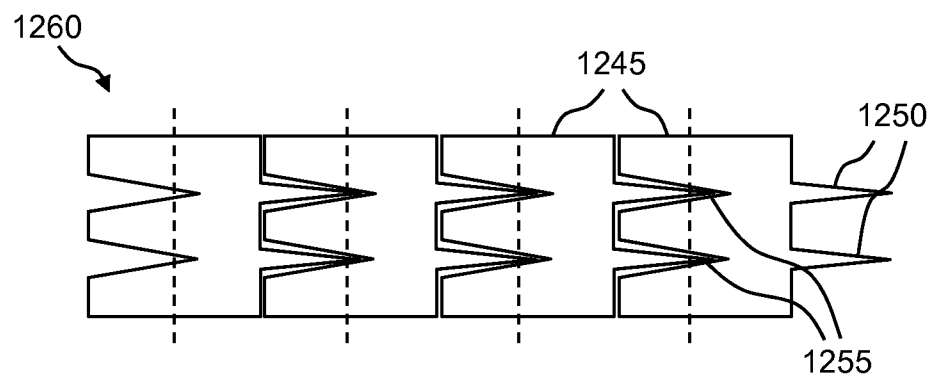


Figure 12D



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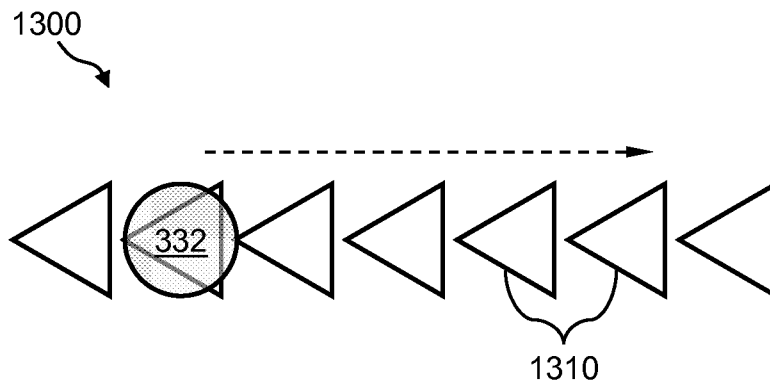


Figure 13A

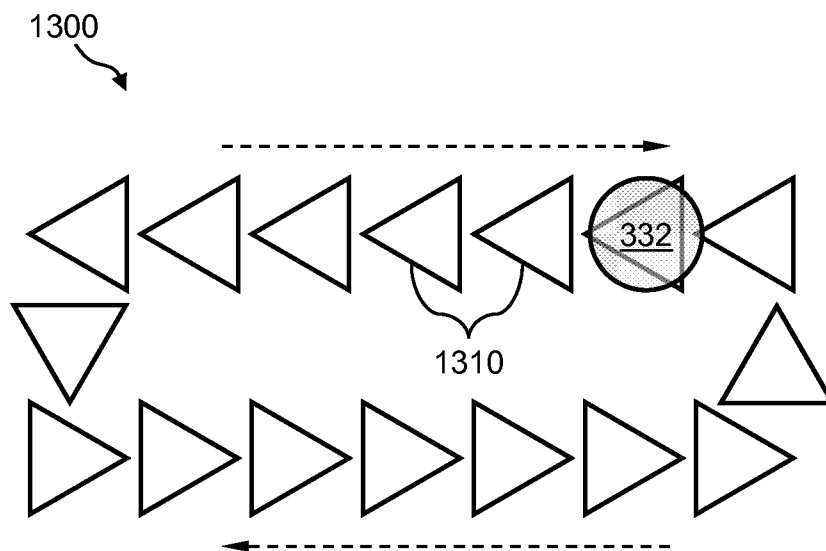


Figure 13B

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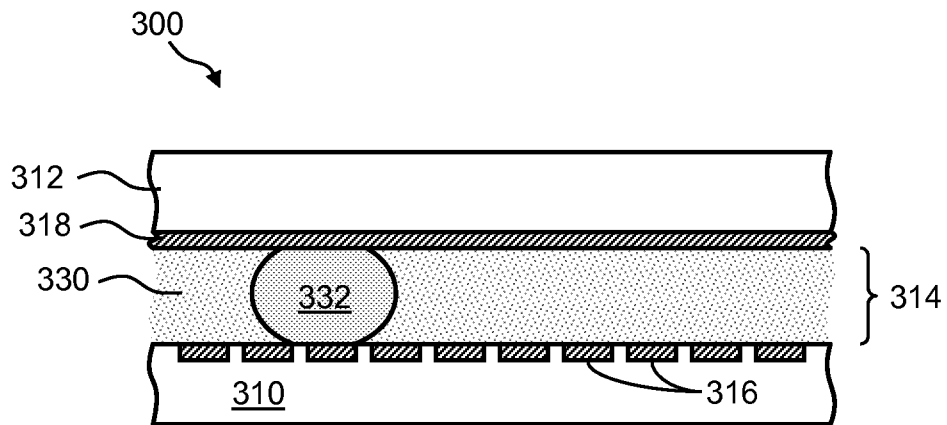


Figure 14A

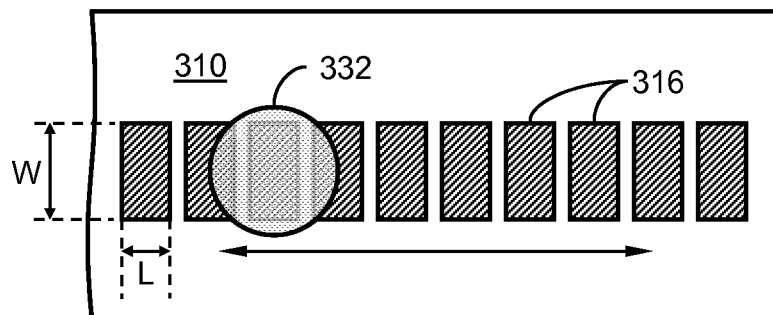


Figure 14B

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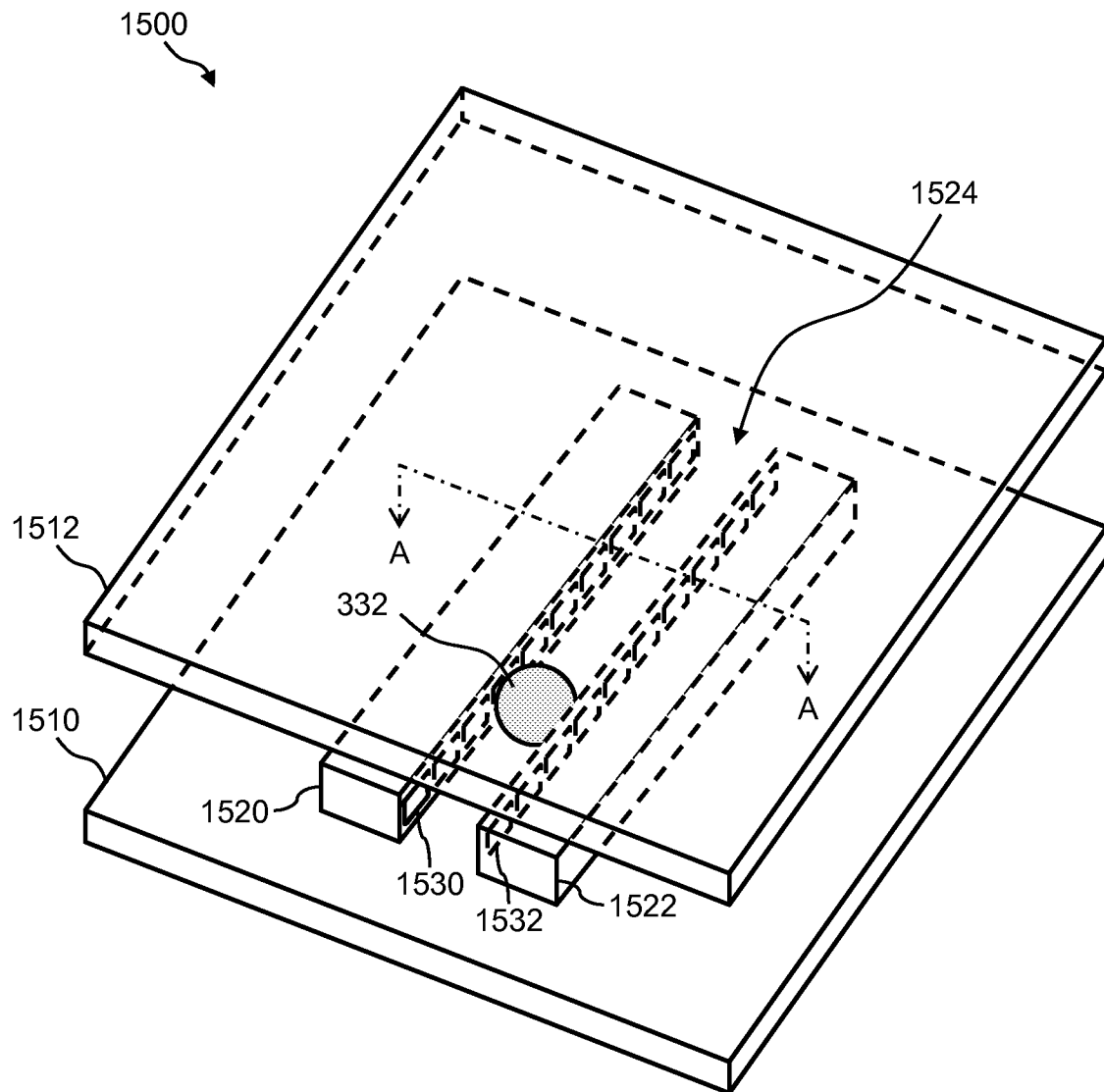


Figure 15

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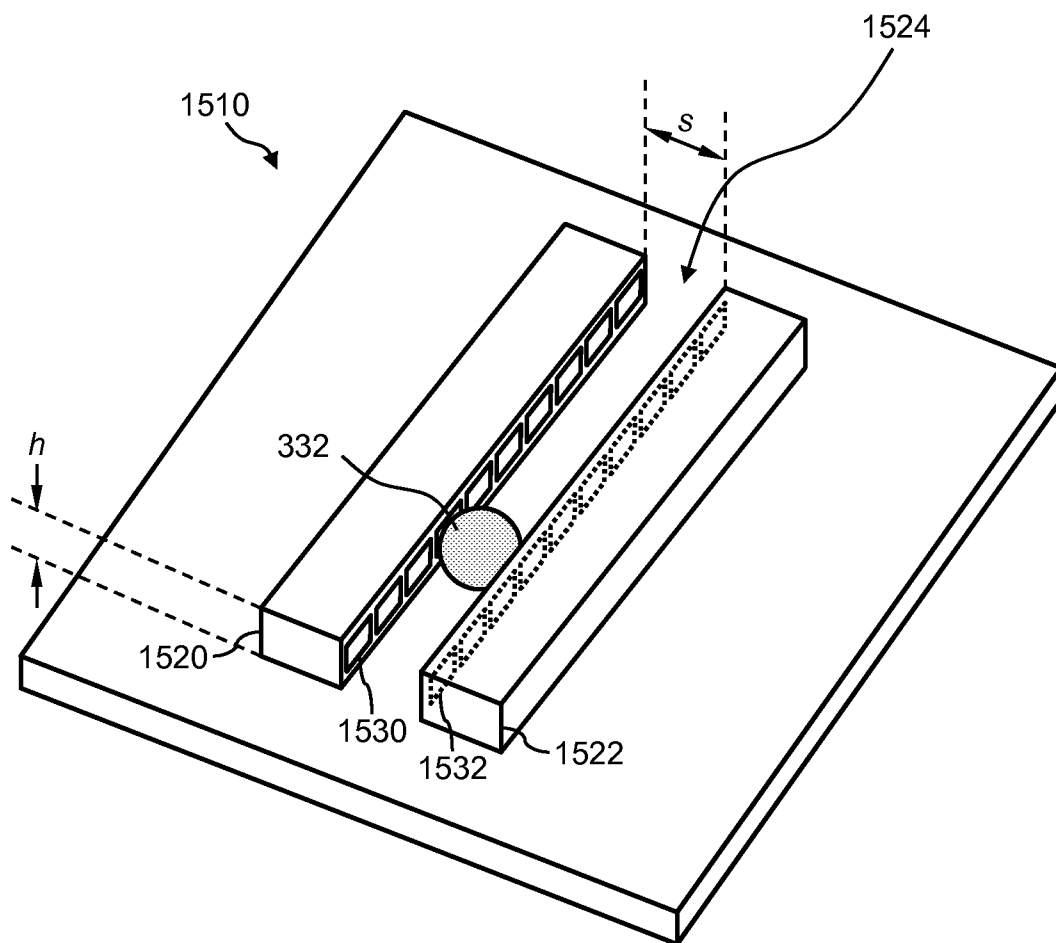


Figure 16

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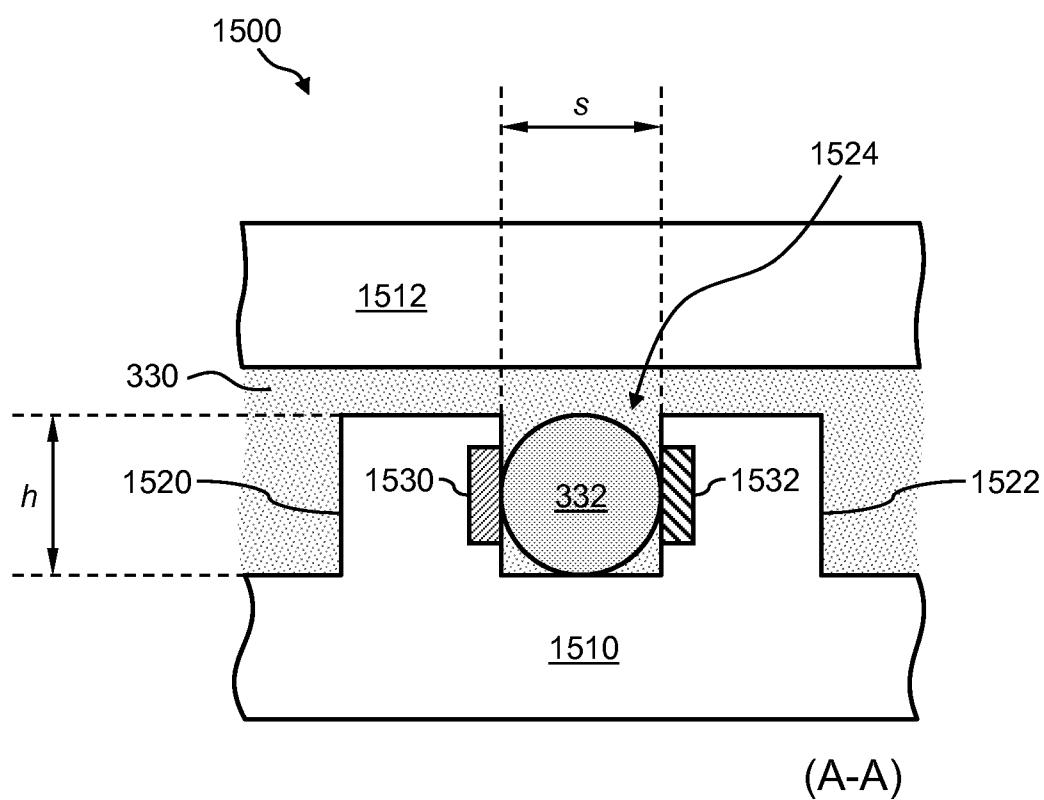


Figure 17

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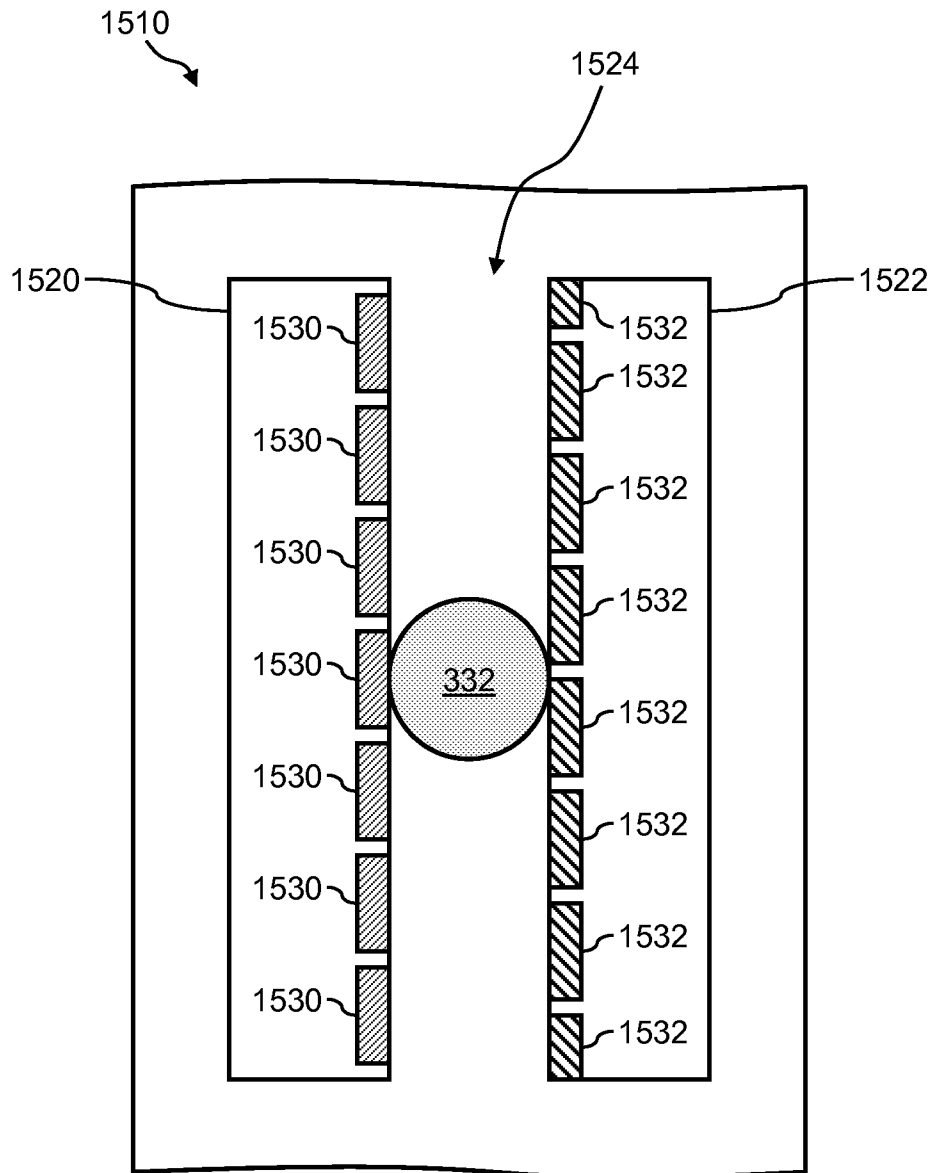


Figure 18

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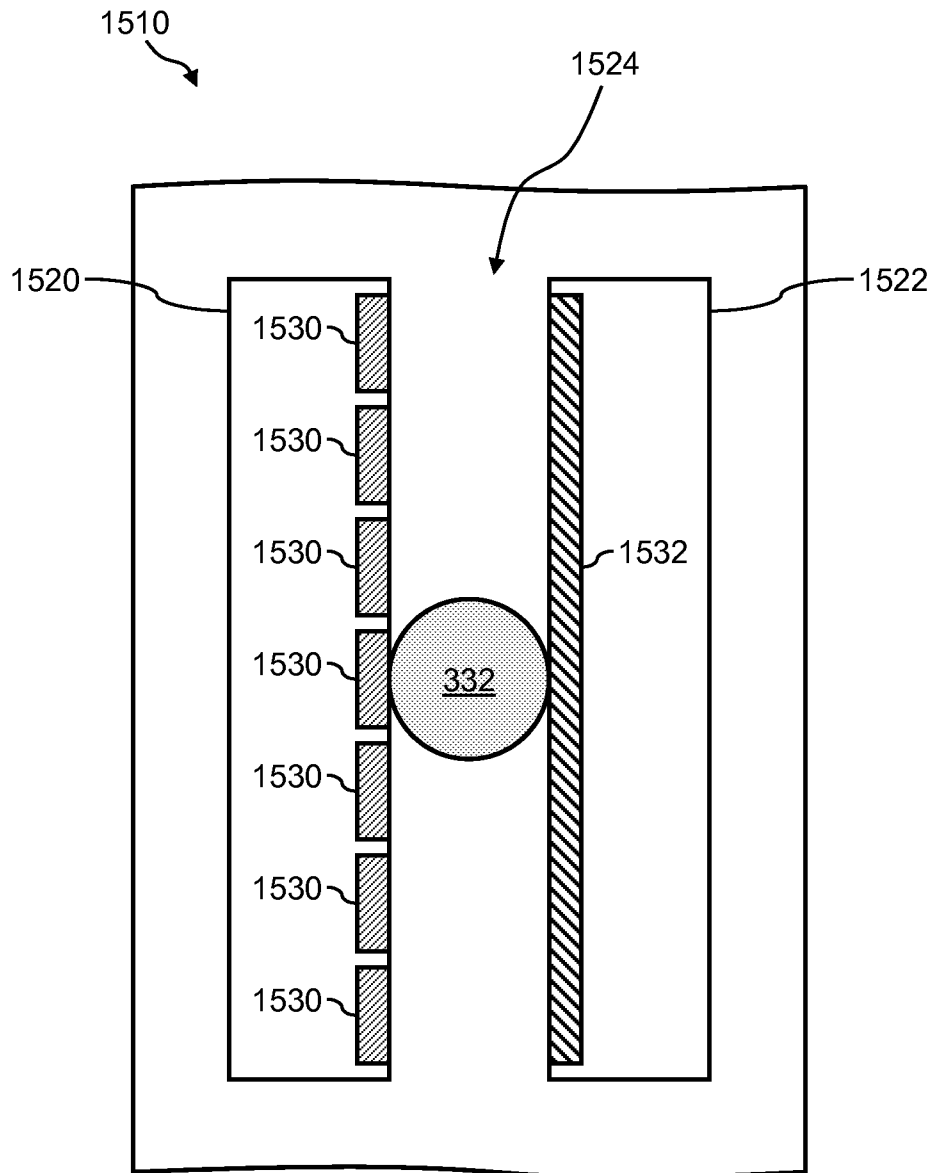


Figure 19

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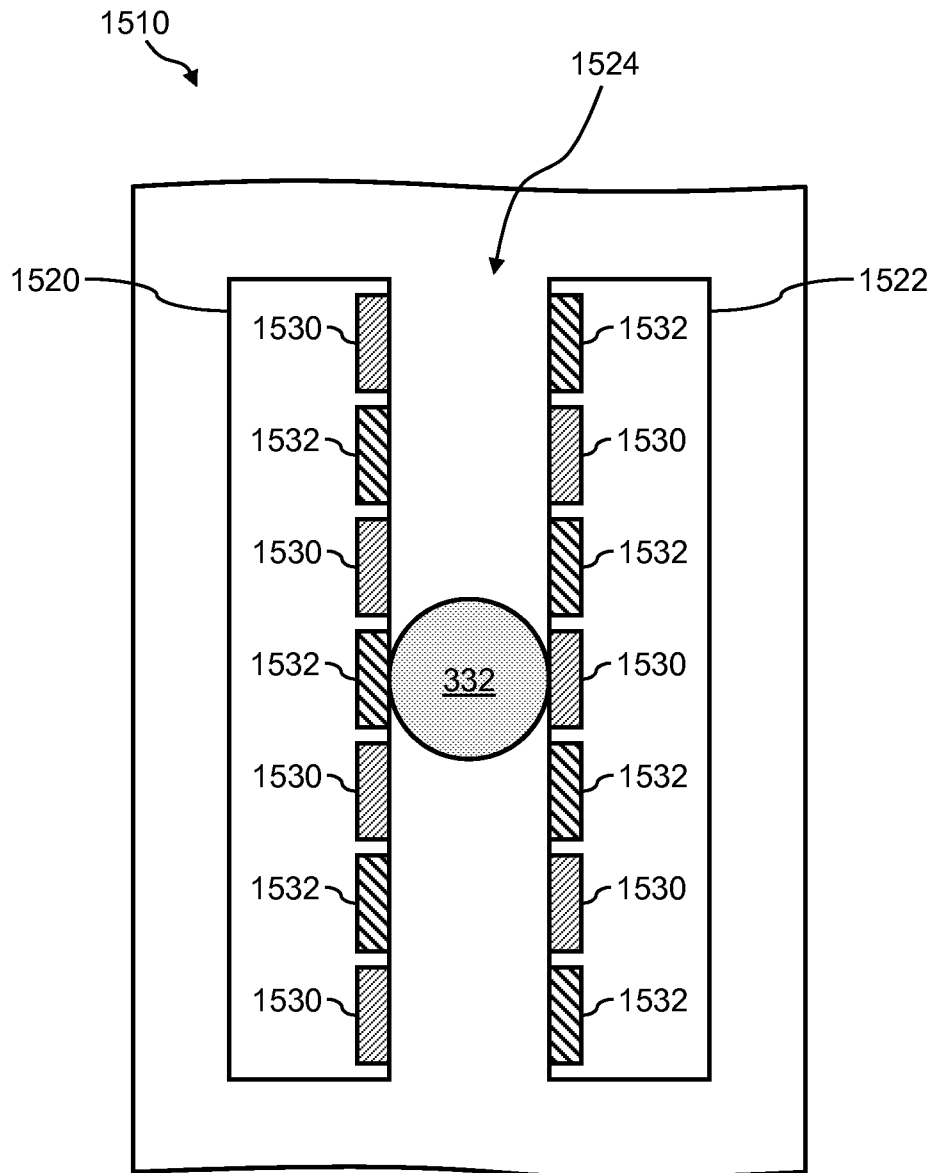


Figure 20

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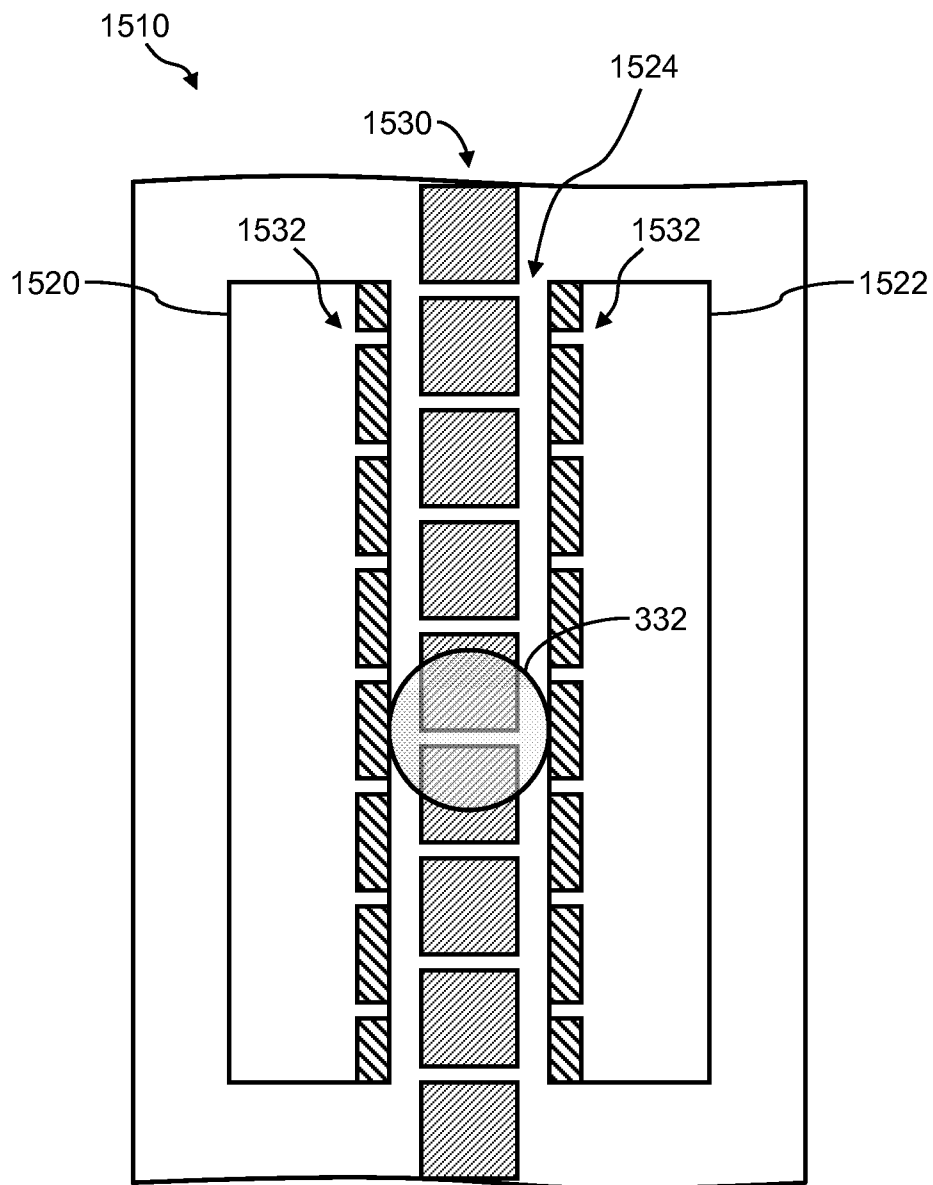


Figure 21

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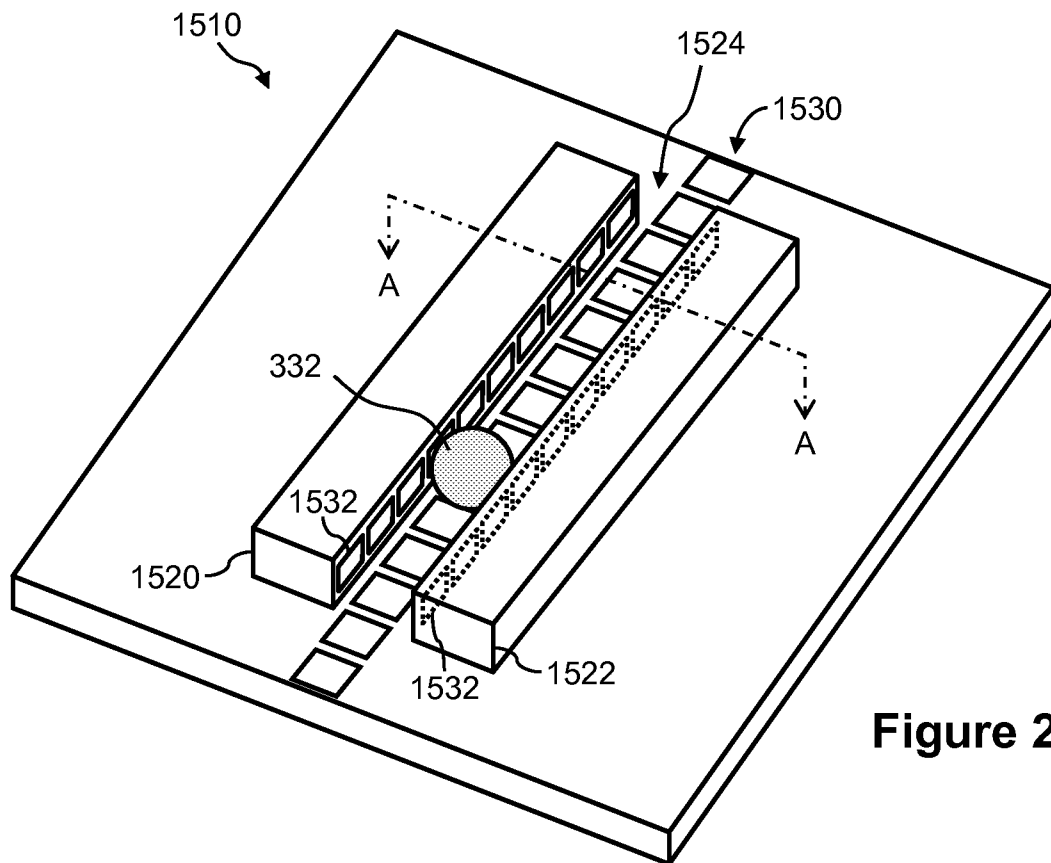
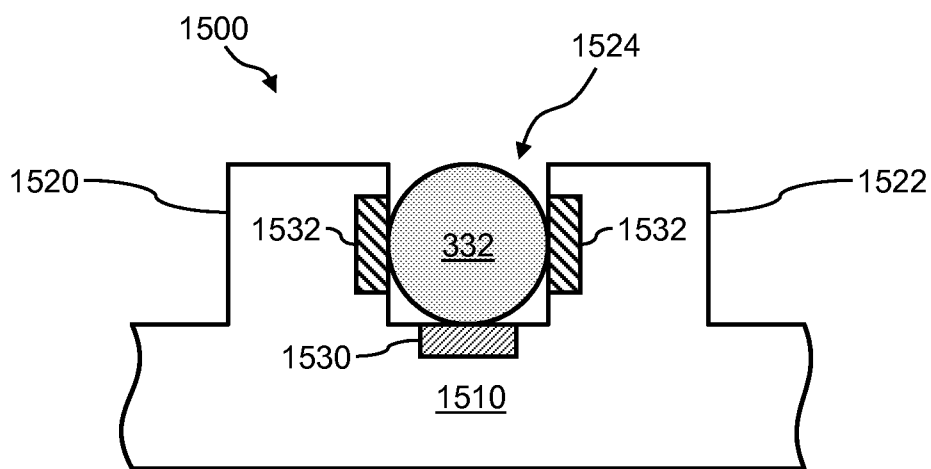


Figure 22A



(A-A)

Figure 22B

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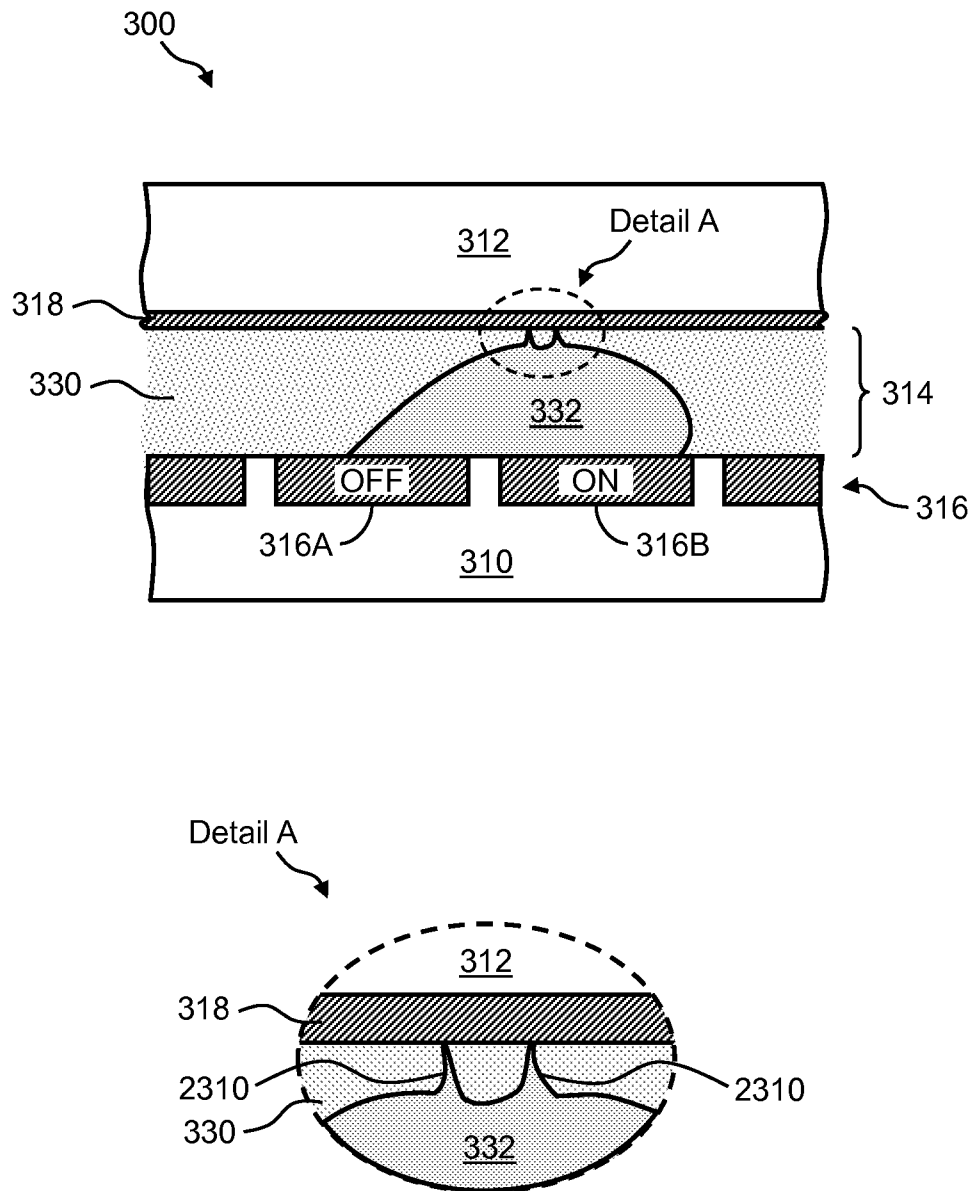


Figure 23

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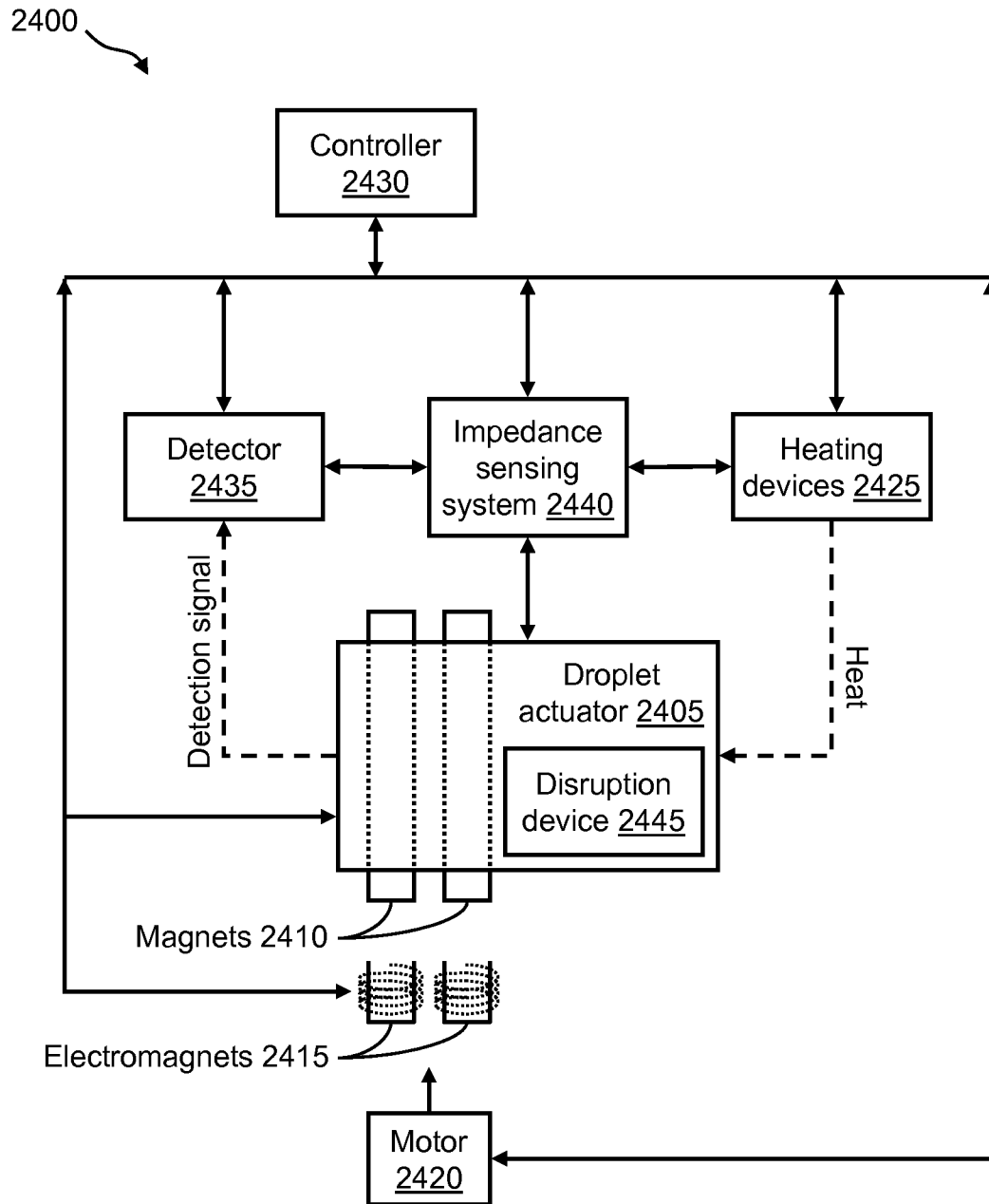


Figure 24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/048319**A. CLASSIFICATION OF SUBJECT MATTER****G01N 1/00(2006.01)i, B01D 57/02(2006.01)i, B01L 3/00(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N 1/00; C12M 1/00; B01F 13/00; G01N 15/06; G01N 27/26; B01D 57/02; C12Q 1/68; B32B 5/02; B01L 3/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: droplet, actuator, electrode, heat and bubble

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010-0307917 A1 (SRINIVASAN et al.) 09 December 2010 See paragraphs 20-28; claims 39-47 and figures 1-3.	1-76
A	US 2010-0291578 A1 (POLLACK et al.) 18 November 2010 See paragraphs 12-34; claims 17-20 and figure 1.	1-76
A	US 7163612 B2 (STERLING et al.) 16 January 2007 See column 1, line 50 - column 2, line 28 and figure 1.	1-76
A	US 2010-0120130 A1 (SRINIVASAN et al.) 13 May 2010 See paragraphs 4-29 and figure 1.	1-76
A	US 7189359 B2 (YUAN et al.) 13 March 2007 See column 3, line 49 - column 5, line 12 and figures 14(a)-14(b).	1-76



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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