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[Continued on next page]

(54) **Title**: DROPLET ACTUATOR DEVICES AND METHODS

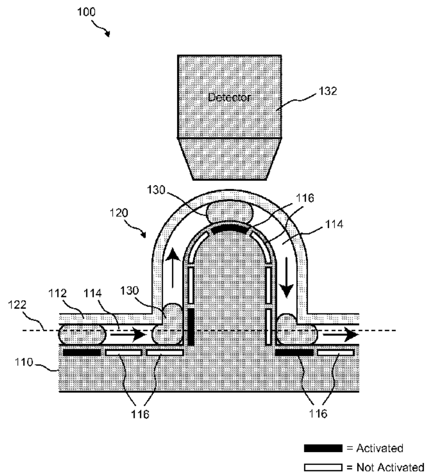

Figure 1

(57) **Abstract**: The invention provides droplet actuator devices for facilitating certain droplet actuated molecular techniques. In one embodiment, the invention provides droplet actuators that facilitate droplet operations in three dimensions. In another embodiment, the invention provides for cost-effective production of droplet actuators. In another embodiment, the invention provides for replacing one or more components of a droplet actuator. In another embodiment, the invention provides droplet actuators using printed conductive inks to form electrodes and/or ground planes. In another embodiment, the invention provides a magnetic clamping fixture for assembling droplet actuators. In another embodiment, the invention provides simple, low cost power sources for use in combination with microfluidic systems. In another embodiment, the invention provides an immunoassay multiplexing platform that uses digital microfluidics and real-time imaging of flash-based chemiluminescent signals. In still another embodiment, the invention provides droplet actuator devices that are fabricated using a plasma treatment process to raise substrate surface energy.

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Droplet Actuator Devices and Methods

1 Related Applications

In addition to the patent applications cited herein, each of which is incorporated herein by reference, this patent application is related to and claims priority to U.S. Provisional Patent Application Nos. 61/241,543, filed on 09/11/2009, entitled "Droplet Actuator Designs"; 61/254,865, filed on 10/26/2009, entitled "Digital Microfluidic Systems"; 61/222,185, filed on 07/01/2009, entitled "Real-Time Imaging of Luminescent Immunoassays on a Digital Microfluidic Platform"; 61/237,008, filed on 08/26/2009, entitled "Real-Time Imaging of Luminescent Immunoassays on a Digital Microfluidic Platform"; 61/225,582, filed on 07/15/2009, entitled "Droplet Actuators with Disposable and Non-Disposable Components"; 61/254,849, filed on 10/26/2009, entitled "Droplet Actuator Devices and Methods"; 61/234,114, filed on 08/14/2009, entitled "Droplet Actuator with Conductive Ink Ground"; 61/294,874, filed on 01/14/2010, entitled "Droplet Actuator with Conductive Ink Ground"; 61/238,512, filed on 08/31/2009, entitled "Magnetic Clamping Fixture for Assembling Droplet Actuators"; 61/288,665, filed on 12/21/2009, entitled "Magnetic Clamping Fixture for Assembling Droplet Actuators"; and 61/254,877, filed on 10/26/2009, entitled "Droplet Actuator Configurations and Methods of Conducting Droplet Operations"; the entire disclosures of which are incorporated herein by reference.

2 Field of the Invention

The invention generally relates to microfluidic systems. In particular, the invention is directed to droplet actuator devices for and methods of facilitating certain droplet actuated molecular techniques.

3 Background of the Invention

Droplet actuators are used to conduct a wide variety of droplet operations. 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 include electrodes for conducting droplet operations. The gap between the substrates is typically filled or coated with a filler fluid that is immiscible with the liquid that is to be subjected to droplet operations. Droplet operations are controlled by electrodes associated with the one or more substrates. Current designs of droplet actuators may have certain drawbacks, as follows.

Designs of droplet actuators have been limited to two-dimensional structures and/or features. To further expand the capabilities of droplet actuators, there is a need for droplet actuator designs that may include multi-planar structures and/or three-dimensional features. Further, current methods of assembling droplet actuators may result in an unreliable bond between the substrates and/or a non-uniform gap height. Consequently, there is a need for new approaches to assembling droplet actuators.

Additionally, because there is a need to produce droplets having more accurate and/or precise volumes for both samples and reagents, there is a need for alternative approaches to metering droplets in a droplet actuator. There is also a need for improved approaches to loading droplet operations fluids, such as samples and/or reagents, into and removing such fluids from a droplet actuator.

A droplet actuator typically includes (1) the bottom substrate with the control electrodes (electrowetting electrodes) insulated by a dielectric, (2) the conductive top substrate, and (3) the hydrophobic coating on the bottom and top substrates. Using current manufacturing processes, all three of these components and their associated processes contribute significantly to the overall cost of the cartridge. Consequently, there is a need for improved manufacturing processes that reduce the cost associated with each component and consequently reduces the cost of the droplet actuator.

Droplet actuators are typically designed to be disposable. Because they are disposable, inexpensive processing techniques, such as printed circuit board (PCB) processes, are often used which may compromise the performance of the droplet actuator. However, alternative processing techniques, such as glass or silicone processes, that may provide finer line widths and more controlled topography, are often more costly and may be prohibitive in manufacturing a disposable device. Consequently, there is a need for improved droplet actuators that include both disposable and non-disposable components that provide for easy replacement of one or more parts of a droplet actuator.

The substrates of a droplet actuator typically include electrodes and/or an electrical ground plane patterned thereon that are exposed to the droplet operations gap. The materials and/or processes for forming the electrodes and/or electrical ground planes may be costly. Consequently, there is a need for less costly materials and/or processes for forming the electrodes and/or electrical ground planes of droplet actuators.

Because high voltage circuits for supplying electrowetting voltages to droplet actuator systems may be complex and costly, there is a need for simple and low cost approaches to supplying electrowetting voltages. Additionally, in low-resource settings, such as in developing countries, reliable electrical power may not be available. Therefore, there is a need for alternative approaches to supplying power to droplet actuator systems that are not dependent on a power distribution grid.

4 Brief Description of the Invention

The invention is directed to droplet actuator devices for and methods of facilitating certain droplet actuated molecular techniques.

In one example, the invention provides droplet actuator designs that may facilitate droplet operations in three dimensions. For example, embodiments of the disclosure include droplet actuator designs that may include multi-planar structures and/or other three-dimensional (3D) features. Further, various mechanisms are disclosed for moving droplets from one level to another, thereby facilitating droplet operations in three dimensions. For example, droplets may be transported from one plane to another to facilitate droplet operations on multiple planes. In some cases, each plane will accommodate different functions, e.g., sample processing on one plane; thermal cycling and/or incubation on another plane; detection on another plane. Alternatively, each plane may support parallel operations, enabling parallelization of hundreds or even thousands of droplet operations in a compact three dimensional space.

In another example, the invention provides droplet actuators and methods for conducting droplet operations on a droplet actuator. For example, the invention provides droplet actuator configurations and techniques for improved droplet loading, splitting and/or dispensing in a droplet actuator. In some embodiments, electrode structures and/or configurations make use of (1) voltage and/or capacitance gradients across one or more electrodes, which may be useful for assisting droplet operations, (2) dielectric layers with different dielectric constants or thicknesses in different regions and/or a gradient in dielectric constant across a region, (3) variable gap heights and/or variable pressure regions, and (4) any combinations thereof.

In yet another example, the invention provides devices and methods for cost-effective production of droplet actuators. In one embodiment, the methods of the invention provide for cost-effective fabrication of the top substrate using polymer films and precision spacers. In another embodiment, the invention provides for cost-effective fabrication of the bottom substrate using an additive printing process on thermoplastic films. In yet another embodiment, the invention provides for cost-effective deposition of hydrophobic coating materials while maintaining or improving droplet operations performance.

In yet another example, the invention provides droplet actuator devices and methods for replacing one or more components of a droplet actuator. For example, the invention provides droplet actuator devices that may include the combination of both disposable components that may be readily replaced and non-disposable components that may be more expensive to manufacture. Ready replacement of one or more disposable components may also provide substantially unlimited re-use of a droplet actuator device or a portion of a droplet actuator device without concern for cross-contamination between applications. In one embodiment, moveable films may be used to readily replace substrate layers (e.g., dielectric and/or hydrophobic layers). In another embodiment, reversible attachment of a top substrate and a bottom substrate may be used to provide ready access to and replacement of one or more substrate layers. In yet another embodiment, a self-contained replaceable top cartridge may be used to provide a single-use, contaminant-free substrate. In yet another embodiment, selectively removable layered structures may be used to replace one or more dielectric and/or hydrophobic substrate layers. In yet another embodiment, a single-unit droplet actuator cartridge that is easily opened and closed may be used to provide a droplet actuator device wherein one or more substrate layers are readily removed and replaced.

In yet another example, the invention provides droplet actuators and methods of using printed conductive inks to form electrodes and/or ground planes, which uses inexpensive materials and/or processes.

In yet another example, the invention provides a magnetic clamping fixture for assembling droplet actuators. Installed in a base plate and a top plate of the magnetic clamping fixture are respective sets of magnets. When assembled, the positions of the respective sets of magnets are aligned. Further, the orientation of the magnets is such that the magnets of the base plate are attracted to the magnets of the top plate. Substrates of droplet actuators that are to be bonded together may be sandwiched between the base plate and top plate of the magnetic clamping fixture and held by compression due to the magnetic forces that are pulling the base plate and the top plate together.

In yet another example, the invention is related to simple, low cost power sources for use in combination with microfluidic systems. Certain embodiments of the invention incorporate low voltage power circuits, which are simple and low cost, thereby reducing, preferably eliminating the need for complex and/or expensive high voltage circuits and/or power supplies in microfluidic systems. Additionally, certain embodiments of the invention incorporate simple, fixed (i.e., non-programmable) mechanical mechanisms for connecting the electrowetting voltages to a droplet actuator. Further, alternative power sources that are not dependent on the presence of a power distribution grid are described in combination with microfluidic systems.

In yet another example, the invention provides an immunoassay multiplexing platform that uses digital microfluidics and real-time imaging of flash-based chemiluminescent signals. Various aspects of the invention may include, but are not limited to, flash-based chemiluminescence (CL), real-time imaging and interpretation, spatial multiplexing, and lab-on-a-chip. In one embodiment of the immunoassay multiplexing platform, the invention provides for 4-plexed flash-based chemiluminescent assays (e.g., 4 different cytokine assays, such as IL-6, TNF-alpha, IL-8, and IL-1beta) on each of 12 samples (i.e., 48 immunoassays). In another embodiment, the invention provides a dynamic smart imaging system configured to simultaneously read chemiluminescent signals from a row of 12 sample droplets and extract intensity information (e.g., algorithms).

In yet another embodiment of the immunoassay multiplexing platform, a high-throughput assay platform for an individual investigator that may circumvent the drawbacks associated with traditional multiplexing systems.

In yet another embodiment of the immunoassay multiplexing platform, it may be utilized by researchers conducting multi-center clinical trials to standardize diagnostic assays (i.e., reduce variability among testing laboratories), control costs, and/or decrease time-to-results.

In still another embodiment of the immunoassay multiplexing platform, it may be used for rapid and accurate profiling of cytokines for cancer immunotherapy. Multiparameter analysis of cytokine patterns may, for example, be used to design, optimize, and/or monitor cancer vaccines. Multiparameter analysis may be used to create “signatures” of the most desirable immune responses to anticancer targeted therapies.

In still another example, the invention provides droplet actuator devices that are fabricated using a plasma treatment process to raise the surface energy of the substrates. In particular, the higher surface energy makes the surfaces of the bottom of the top substrates hydrophilic so that aqueous fluids are attracted thereto. Additionally, being hydrophilic improves the adhesion of the conductive layer (e.g., PEDOT coating) to the surfaces of the substrates.

5 Definitions

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

"Activate" with reference to one or more electrodes means effecting a change in the electrical state of the one or more electrodes which, in the presence of a droplet, results in a droplet operation.

"Bead," with respect to beads on a droplet actuator, means any bead or particle that is capable of interacting with a droplet on or in proximity with a droplet actuator. Beads may be any of a wide variety of shapes, such as spherical, generally spherical, egg shaped, disc shaped, cubical and other three dimensional shapes. The bead may, for example, be capable of being transported in a droplet on a droplet actuator or otherwise configured with respect to a droplet actuator in a manner which permits a droplet on the droplet actuator to be brought into contact with the bead, on the droplet actuator and/or off the droplet actuator. Beads may be manufactured using a wide variety of materials, including for example, resins, and polymers. The beads may be any suitable size, including for example, microbeads, microparticles, nanobeads and nanoparticles. In some cases, beads are magnetically responsive; in other cases beads are not significantly magnetically responsive. For magnetically responsive beads, the magnetically responsive material may constitute substantially all of a bead or one component only of a bead. The remainder of the bead may include, among other things, polymeric material, coatings, and moieties which permit attachment of an assay reagent. Examples of suitable magnetically responsive beads include flow cytometry microbeads, polystyrene microparticles and nanoparticles, functionalized polystyrene microparticles and nanoparticles, coated polystyrene microparticles and nanoparticles, silica microbeads, fluorescent microspheres and nanospheres, functionalized fluorescent microspheres and nanospheres, coated fluorescent microspheres and nanospheres, color dyed microparticles and nanoparticles, magnetic microparticles and nanoparticles, superparamagnetic microparticles and nanoparticles (e.g., DYNABEADS® particles, available from Invitrogen Corp., Carlsbad, CA), fluorescent microparticles and nanoparticles, coated magnetic microparticles and nanoparticles, ferromagnetic microparticles and nanoparticles, coated ferromagnetic microparticles and nanoparticles, and those described in U.S. Patent Publication No. 20050260686, entitled, "Multiplex flow assays preferably with magnetic particles as solid phase," published on November 24, 2005, the entire disclosure of which is incorporated herein by reference for its teaching concerning magnetically responsive materials and beads. Beads may be pre-coupled with a biomolecule (ligand). The ligand may, for example, be an antibody, protein or antigen, DNA/RNA probe or any other molecule with an affinity for the desired target. Examples of droplet actuator techniques for immobilizing magnetically responsive beads and/or non-magnetically responsive beads and/or conducting droplet operations protocols using beads are described in U.S. Patent Application No. 11/639,566, entitled "Droplet-Based Particle Sorting," filed on December 15, 2006; U.S. Patent Application No. 61/039,183, entitled "Multiplexing Bead Detection in a Single Droplet," filed on March 25, 2008; U.S. Patent Application No. 61/047,789, entitled "Droplet Actuator Devices and Droplet Operations Using Beads," filed on April 25, 2008; U.S. Patent Application No. 61/086,183, entitled "Droplet Actuator Devices and Methods for Manipulating Beads," filed on August 5, 2008; International Patent Application No. PCT/US2008/053545, entitled "Droplet Actuator Devices and Methods Employing Magnetic Beads," filed on February 11, 2008; International Patent Application No. PCT/US2008/058018, entitled "Bead-based Multiplexed Analytical Methods and Instrumentation," filed on March 24, 2008; International Patent Application No. PCT/US2008/058047, "Bead Sorting on a Droplet Actuator," filed on March 23, 2008; and International Patent Application No. PCT/US2006/047486, entitled "Droplet-based Biochemistry," filed on December 11, 2006; the entire disclosures of which are incorporated herein by reference.

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

"Droplet Actuator" means a device for manipulating droplets. For examples of droplet actuators, see U.S. Patent 6,911,132, entitled "Apparatus for Manipulating Droplets by Electrowetting-Based Techniques," issued on June 28, 2005 to Pamula et al.; U.S. Patent Application No. 11/343,284, entitled "Apparatuses and Methods for Manipulating Droplets on a Printed Circuit Board," filed on filed on January 30, 2006; 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, both to Shenderov et al.; Pollack et al., International Patent Application No. PCT/US2006/047486, entitled "Droplet-Based Biochemistry," filed on December 11, 2006; and 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; the disclosures of which are incorporated herein by reference. Certain droplet actuators will include a substrate, droplet operations electrodes associated with the substrate, one or more dielectric and/or hydrophobic layers atop the substrate and/or electrodes forming a droplet operations surface, and optionally, a top substrate separated from the droplet operations surface by a gap. One or more reference electrodes may be provided on the top and/or bottom substrates and/or in the gap. 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 methods of controlling fluid flow that may be used in the droplet actuators of the invention include devices that induce hydrodynamic fluidic pressure, such as those that operate on the basis of mechanical principles (e.g. external syringe pumps, pneumatic membrane pumps, vibrating membrane pumps, vacuum devices, centrifugal forces, piezoelectric/ultrasonic pumps and acoustic forces); electrical or magnetic principles (e.g. electroosmotic flow, electrokinetic pumps, ferrofluidic plugs, electrohydrodynamic pumps, attraction or repulsion using magnetic forces and magnetohydrodynamic pumps); thermodynamic principles (e.g. gas bubble generation/phase-change-induced volume expansion); other kinds of surface-wetting principles (e.g. electrowetting, and optoelectrowetting, as well as chemically, thermally, structurally and radioactively induced surface-tension gradients); gravity; surface tension (e.g., capillary action); electrostatic forces (e.g., electroosmotic flow); centrifugal flow (substrate disposed on a compact disc and rotated); magnetic forces (e.g., oscillating ions causes flow); magnetohydrodynamic forces; and vacuum or pressure differential. In certain embodiments, combinations of two or more of the foregoing techniques may be employed in droplet actuators of the invention.

"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.

“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. The filler fluid may, for example, be a low-viscosity oil, such as silicone oil. Other examples of filler fluids are provided in International Patent Application No. PCT/US2006/047486, entitled, “Droplet-Based Biochemistry,” filed on December 11, 2006; International Patent Application No. PCT/US2008/072604, entitled “Use of additives for enhancing droplet actuation,” filed on August 8, 2008; and U.S. Patent Publication No. 20080283414, entitled “Electrowetting Devices,” filed on May 17, 2007; the entire disclosures of which are incorporated herein by reference. The filler fluid may fill the entire gap of the droplet actuator or may coat one or more surfaces of the droplet actuator. Filler fluid may be conductive or non-conductive.

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

“Magnetically responsive” means responsive to a magnetic field. “Magnetically responsive beads” include or are composed of magnetically responsive materials. Examples of magnetically responsive materials include paramagnetic materials, ferromagnetic materials, ferrimagnetic materials, and metamagnetic materials. Examples of suitable paramagnetic materials include iron, nickel, and cobalt, as well as metal oxides, such as Fe_3O_4, $BaFe_{12}O_{19}$, CoO, NiO, Mn_2O_3, Cr_2O_3, and CoMnP.

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

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

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

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

6 Brief Description of the Drawings

Figure 1 illustrates a side view of a portion of a droplet actuator that includes a 3D dome-shaped feature by which a droplet may be isolated;

Figure 2 illustrates a side view of a portion of a droplet actuator that includes the 3D dome-shaped feature of Figure 1 for isolating filler fluid to one portion of the droplet actuator;

Figure 3 illustrates a side view of a portion of a droplet actuator that includes intersecting paths of droplet operations electrodes that allow droplet operations to occur in the x, y, and z planes and allow droplets to pass from one plane to another;

Figures 4A and 4B illustrate a side view of a portion of a multi-level droplet actuator, which is an example of a droplet actuator that includes multiple droplet operations planes;

Figure 5 illustrates a side view of a portion of a multi-level droplet actuator, which is another example of a droplet actuator that includes multiple droplet operations planes;

Figure 6A illustrates a top view of an example of a pattern that is cut into a sheet of flexible circuit material on which lines of droplet operations electrodes are formed;

Figure 6B illustrates a side view of the sheet of flexible circuit material when in a non-flattened 3D state for supplying droplets to multiple levels and/or planes of a multi-level droplet actuator;

Figure 7A illustrates a top view of an example of a pattern that is cut into a sheet of flexible circuit material on which lines of droplet operations electrodes are formed;

Figure 7B illustrates a side view of the sheet of flexible circuit material when in a non-flattened 3D state for supplying droplets to multiple levels and/or planes of a multi-level droplet actuator,

Figure 8A illustrates a top view of an example of a strip of flexible circuit material on which lines of droplet operations electrodes are formed;

Figure 8B illustrates a side view of an example of using the strip of flexible circuit material;

Figures 9A and 9B show another example of using the strip of flexible circuit material of Figure 8A;

Figure 10 illustrates a side view of a portion of an example of a multi-level droplet actuator that includes a “wicking” mechanism for moving liquid from one level to another;

Figure 11 illustrates a side view of a portion of an example of a multi-level droplet actuator that uses various types of via holes for moving droplets from one level to another;

Figures 12A, 12B and 12C illustrate perspective views of an example of a 3D droplet actuator structure that is formed of multiple slotted droplet operations boards;

Figures 13A and 13B illustrate side views of examples of droplet actuators that include one or more double-sided substrates;

Figure 14 illustrates a side view of an example of a multi-level droplet actuator that is formed by folding and/or bending a sheet of flexible circuit material that is patterned with an array and/or lines of droplet operations electrodes;

Figure 15 illustrates a side view of an example of a multi-level droplet actuator that is formed of a stack of substrates that have openings therein for passing droplets from one level to another;

Figure 16 illustrates a side view of an example of a droplet actuator that is a 3D structure that includes wash and/or waste reservoirs at different levels with respect to the droplet operations plane;

Figure 17 illustrates a side view of an example of a droplet actuator that is a 3D structure for transporting droplets between two zones, such as between hot and cold zones;

Figure 18 illustrates a perspective view of an example of a droplet actuator that is a 3D structure that includes droplet operations tubes for transporting droplets between two levels and/or zones;

Figure 19 illustrates a perspective view of an example of a structure for stimulating vertical motion in droplets;

Figure 20 illustrates a perspective view of a portion of a multi-level droplet actuator, which is an example of a droplet actuator that includes any number of planes that are dedicated for certain functions;

Figures 21A, 21B, and 21C illustrate a side view of an example of a droplet actuator that includes a 3D feature in the top substrate and a process for allowing one droplet to pass another droplet in the gap of the droplet actuator;

Figure 22A illustrates a side view of an example of a droplet actuator that includes another 3D feature in the top substrate;

Figure 22B illustrates a side view of an example of a droplet actuator that includes another 3D feature in the top substrate;

Figure 23 illustrates a top view of an example of a portion of an electrode structure that may be formed by weaving, which is another example of a 3D structure for use in droplet actuators;

Figures 24, 25, 26, and 27 illustrate top and side views of non-limiting examples of resistive droplet operations electrodes having various geometries for developing linear and/or non-linear voltage and/or capacitance gradients, which may be used to assist droplet operations;

Figures 28A and 28B illustrate top views of the rectangle-shaped electrode of Figure 24 when in use during droplet operations;

Figures 29A through 29F illustrate top views of an electrode configuration and a process of using a voltage gradient to assist droplet operations;

Figures 30A through 30D illustrate side views of a portion of a droplet actuator and another process of using a voltage gradient to assist droplet operations;

Figure 31A illustrates a side view of a portion of a droplet actuator for using both a voltage gradient and capacitance gradient to assist droplet operations;

Figure 31B illustrates another side view of the droplet actuator of Figure 31A wherein the dielectric layer atop the wedge-shaped electrodes is modified;

Figures 32A and 32B illustrate side views of a portion of a droplet actuator for using capacitance gradient to assist droplet operations;

Figures 33A and 33B illustrate top views of a rectangle-shaped electrode when in use during droplet operations;

Figure 34 illustrates a top view of an electrode configuration that includes a resistance ladder structure for forming a voltage gradient, which may be used to assist droplet operations;

Figure 35 illustrates a perspective view of a 3D electrode configuration that includes a resistance ladder structure for forming a voltage gradient, which may be used to assist droplet operations;

Figure 36 illustrates a perspective view of a custom resistance droplet operations electrode for providing a customized voltage gradient, which may be used to assist droplet operations;

Figure 37 illustrates a top view of a droplet operations electrode for providing a certain voltage gradient, which may be used to assist droplet operations;

Figure 38 illustrates a top view of an electrode array of conductive and/or resistive electrodes for providing a voltage gradient of a certain predetermined profile, which may be used to assist droplet operations;

Figure 39 illustrates a top view of a droplet operations electrode for providing a certain capacitance gradient, which may be used to assist droplet operations;

Figures 40A and 40B illustrate top views of embodiments of a droplet operations electrode for providing a certain capacitance gradient, which may be used to assist droplet operations;

Figure 41 illustrates a perspective view of an example of a droplet operations electrode that when activated has a voltage gradient due to doping, which may be used to assist droplet operations;

Figures 42A through 42D illustrate top views of an example of an electrode configuration and a process of dispensing by use of different dielectric constants;

Figures 43A through 43D illustrate side views of a portion of a droplet actuator and a process of dispensing a droplet by use of a dielectric layer that has regions of different thicknesses;

Figures 44A and 44B illustrate a side view and top view, respectively, of a portion of a droplet actuator that may be used to dispense multiple droplets by use of a dielectric layer that has regions of different thicknesses;

Figures 45A through 45D illustrate side views of a portion of a droplet actuator and a process of dispensing a droplet by use of a two-piece dielectric layer that provides regions of different dielectric constants;

Figure 46 illustrates a side view of a portion of a droplet actuator that includes a two-piece dielectric layer that provides a dielectric constant gradient for assisting droplet operations;

Figures 47A, 47B, and 47C illustrate top views of an example of an electrode configuration and a process of dispensing by use of different dielectric constants in combination with different pressure regions;

Figures 48A, 48B, and 48C illustrate side views of a portion of a droplet actuator and a process of dispensing and/or splitting droplets by use of different pressure regions created by varying the gap height;

Figures 49A, 49B, and 49C illustrate side views of a portion of a droplet actuator and a process of transporting droplets by use of pressure gradients created by gradients in gap height;

Figure 50 illustrates a side view of an example of a droplet actuator that includes noncomplex features that are easy to manufacture;

Figure 51 illustrates a side view of another example of a droplet actuator that includes noncomplex features that are easy to manufacture;

Figure 52 illustrates a side view of yet another example of a droplet actuator that includes noncomplex features that are easy to manufacture;

Figure 53 illustrates a top view of an electrode configuration for providing an efficient single layer multi-phase bus configuration;

Figure 54 illustrates a side view of a portion of a droplet actuator that includes a top substrate with integrated spacer structures and a transparent conducting layer;

Figure 55 illustrates a side view of a portion of the droplet actuator of Figure 54 that includes a top substrate with a translucent or opaque conducting layer;

Figure 56 illustrates a side view of a portion of a droplet actuator that includes a top substrate with a precision spacer formed on a printed conducting layer;

Figure 57 illustrates a side view of a portion of the droplet actuator of Figure 3 that includes a top substrate with a precision spacer formed on a conductive polymer film;

Figure 58 illustrates a side view of a portion of a top substrate;

Figure 59 illustrates a side view of a portion of a bottom substrate that includes two conducting layers printed on the same side of a polymer film;

Figure 60 illustrates a side view of a portion of a bottom substrate that includes two conducting layers printed on opposite sides of a polymer film;

Figure 61 shows a bar graph of hydrophobic coating thickness with respect to different fluid flow rates;

Figure 62 shows a plot of the increase in substrate weight with respect to the number of spray coating passes;

Figures 63A and 63B illustrate side and top views, respectively, of a portion of a droplet actuator that includes replaceable flexible film layers arranged upon reel-to-reel mechanisms;

Figure 64 illustrates a side view of a portion of a droplet actuator that includes a rigid top substrate and a moveable film for replacement of bottom substrate layers;

Figures 65A and 65B illustrate side views of portions of droplet actuators and examples of methods for enhancing the contact between a flexible film and a substrate surface;

Figure 66 illustrates a cross-sectional view of a portion of a droplet actuator and a method for using vacuum for coupling and decoupling the top and bottom substrates;

Figure 67 illustrates a cross-sectional view of a portion of a droplet actuator and a method for using a temperature-sensitive material for coupling and decoupling the top and bottom substrates;

Figures 68A and 68B illustrate side views of a portion of a droplet actuator that includes a bottom substrate and a top substrate, wherein the top substrate is a self-contained replaceable cartridge;

Figures 69A, 69B, and 69C illustrate side views of a portion of a droplet actuator that includes a layered electrowettable structure and a method for readily regenerating the surface of a droplet actuator;

Figures 70A and 70B illustrate side views of portions of an example of a droplet actuator wherein a rigid-flex process may be used to form a single unit droplet actuator cartridge;

Figure 71 illustrates a cross-sectional view of an example of a portion of a droplet actuator that uses printed conductive inks to form electrodes and/or ground planes;

Figure 72 illustrates an example of a conductive ink pattern that is printed on the surface of a substrate;

Figure 73 illustrates a perspective view of an example of a magnetic clamping fixture for assembling droplet actuators;

Figure 74 illustrates a view of the inner surface of an example of a base plate of the magnetic clamping fixture of the invention;

Figures 75A, 75B, 76A, and 76B illustrate various views of an example of a top plate of the magnetic clamping fixture of the invention;

Figure 77 illustrates a perspective view of an example of the orientation and alignment of opposing magnets of the magnetic clamping fixture of the invention;

Figure 78 illustrates a cross-sectional view of an the magnetic clamping fixture of the invention taken along line A-A of Figure 73, again showing the orientation and alignment of opposing magnets;

Figure 79 illustrates a flow diagram of an example of a method of using the magnetic clamping fixture of the invention;

Figure 90 illustrates a side view of the magnetic clamping fixture when in use and a bond line between substrates of a droplet actuator;

Figures 81A, 81B, and 81C illustrate functional block diagrams of examples of simple and inexpensive ways to supply power to microfluidic systems;

Figure 82A illustrates a schematic diagram of one example of a simple, low cost power conditioning circuit for use in microfluidic systems;

Figure 82B illustrates a plot of the input voltage vs. output voltage of the power conditioning circuit of Figure 82A;

Figure 83A illustrates a schematic diagram of another example of a simple, low cost power conditioning circuit for use in microfluidic systems;

Figure 83B illustrates a plot of the input voltage vs. output voltage of the power conditioning circuit of Figure 83A;

Figure 84A illustrates a schematic diagram of yet another example of a simple, low cost power conditioning circuit for use in microfluidic systems;

Figure 84B illustrates a plot of the input voltage vs. output voltage of the power conditioning circuit of Figure 84A;

Figure 85 illustrates a schematic diagram of yet another example of a simple, low cost power conditioning circuit for use in microfluidic systems;

Figure 86 illustrates a schematic diagram of still another example of a simple, low cost power conditioning circuit for use in microfluidic systems;

Figures 87A and 87B illustrate schematic diagrams of examples of voltage multiplier circuits for use in certain power conditioning circuits of microfluidic systems;

Figures 88A and 88B illustrate a top view and cross-sectional view, respectively, of a portion of a droplet actuator and shows one example of a simple, fixed (i.e., non-programmable) mechanical mechanism for controlling the electrowetting voltage to the channels thereof;

Figure 89 illustrates a top view of a portion of a droplet actuator and shows another example of a simple, fixed (i.e., non-programmable) mechanical mechanism for controlling the electrowetting voltage to the channels thereof;

Figure 90 illustrates a perspective view of an example of a structure that uses static electricity for supplying power to microfluidic systems;

Figure 91 illustrates a perspective view of an example of a microfluidics assay multiplexing platform and a process of performing high-throughput, flash-based chemiluminescent profiling;

Figure 92 illustrates a schematic diagram of a process of performing dynamic smart imaging on a digital microfluidic platform;

Figure 93 illustrates a top view of a portion of an electrode configuration that is used for merging and detection of activated droplets in a flash-based chemiluminescent assay;

Figure 94 shows a plot of averaged (n=4) standard curve data of AE-NHS concentration versus chemiluminescent signal generated using the electrode configuration of Figure 93;

Figure 95 shows a plot of standard curve data of IL-6 and TNF-alpha concentrations generated by use of glow-based chemiluminescent immunoassays on a digital microfluidic droplet actuator platform;

Figure 96 illustrates a perspective view of an imaging luminometer system for simultaneous detection of chemiluminescent signals from multiple droplets;

Figure 97 shows a plot of kinetics (time-dependent) intensity profiles of on-chip ATP mediated luciferase reactions.

7 Detailed Description of the Invention

7.1 Droplet Actuator Designs

The invention is droplet actuator designs that may facilitate droplet operations in three dimensions. For example, embodiments of the disclosure include droplet actuator designs that may include multi-planar structures and/or other 3D features. In certain embodiments, droplet actuator designs include a stack of multiple substrates for performing droplet operations independently on any level. In certain other embodiments, droplet actuator designs include combinations of parallel-arranged and/or orthogonal-arranged substrates for facilitating droplet operations in three dimensions. Embodiments may include, for example, printed circuit board (PCB)-based substrates and/or flexible circuit-based substrates. Further, various mechanisms are disclosed for moving droplets from one level to another, thereby facilitating droplet operations in three dimensions. These mechanisms may include, but are not limited to, fluid wicking mechanisms, flexible circuit material having droplet operations electrodes, vertically oriented substrates having droplet operations electrodes, vertically oriented tubes and/or channels having droplet operations electrodes, and the like.

7.1.1 Three-Dimensional (3D) Droplet Actuator Structures and/or Features

The benefits of using droplet actuators that may facilitate droplet operations in three dimensions and, in particular, that may include multiple levels and/or planes for performing droplet operations may include, but are not limited to, the ability to provide different kinds of filler oil on different levels, the ability to provide different kinds of surfactants on different levels, the ability to provide different electrode sizes and/or shapes on different levels, the ability to isolate droplets in proximity to certain sensors on different levels, the ability to maximize droplets operations in a certain space, the ability to provide different droplet operation functions on different levels (e.f., incubation, thermal cycling, sample processing, bead washing, detection, etc., on different levels), and the like.

<u>**Figure 1**</u> illustrates a side view of a portion of a droplet actuator 100 that includes a 3D dome-shaped feature by which a droplet may be isolated at, for example, a detector to reduce interference from other droplets. Droplet actuator 100 may include a bottom substrate 110 and a top substrate 112 that are separated by a gap 114. Bottom substrate 110 may include an array and/or lines of droplet operations electrodes 116 (e.g., electrowetting electrodes) that are configured for droplet operations. A 3D dome-shaped feature 120 is formed substantially orthogonally to a plane 122, where plane 122 is substantially parallel to bottom substrate 110. Gap 114 and droplet operations electrodes 116 follow the profile of 3D dome-shaped feature 120 in a manner to maintain a continuous droplet operations path with gap 114 and droplet operations electrodes 116 that are along plane 122 of droplet actuator 100. More specifically, at a certain point along droplet operations electrodes 116 of bottom substrate 110, there is a change in

direction of gap 114 and droplet operations electrodes 116. That is, one or more droplets 130 may be transported along droplet operations electrodes 116 of bottom substrate 110 in the x- and/or y-axis of droplet actuator 100 (e.g., along plane 122). However, once encountering 3D dome-shaped feature 120, droplets 130 may be transported along the z-axis of droplet actuator 100 within gap 114 of 3D dome-shaped feature 120. The apex or topmost portion of 3D dome-shaped feature 120 may be, for example, a designated detection spot. For example, a detector 132 may be arranged in close proximity to the apex or topmost portion of 3D dome-shaped feature 120. This arrangement is an example of a 3D structure and/or feature that may be useful for isolating a droplet at a detector in order to reduce interference from other droplets when.

Figure 2 illustrates a side view of a portion of a droplet actuator 200 that includes the 3D dome-shaped feature 120 that is described in Figure 1 and also includes the ability to isolate filler fluid to one portion of the droplet actuator and to remove droplets from filler fluid. Droplet actuator 200 is substantially the same of droplet actuator 200 of Figure 1, except that a waste reservoir 210 (instead of droplet operations electrodes 116) is arranged on one side of 3D dome-shaped feature 120, which is hereafter referred to as the exit side of 3D dome-shaped feature 120. The arrangement of droplet operations electrodes 116 leads into the entry side of 3D dome-shaped feature 120. Additionally, a certain amount of filler fluid 212 may be in gap 114 at the entry side of 3D dome-shaped feature 120, while no filler fluid 212 is in waste reservoir 210 the exit side of 3D dome-shaped feature 120. In this way, 3D dome-shaped feature 120 provides a mechanism for isolating filler fluid 212 to one portion of droplet actuator 200.

In operation, using droplet operations electrodes 116, droplets 130 are transported in filler fluid 212 that is in gap 114 leading to the entry side of 3D dome-shaped feature 120. However, once encountering 3D dome-shaped feature 120, droplets 130 may be transported along the z-axis of droplet actuator 100 within gap 114 of 3D dome-shaped feature 120 and out of fluid 212. A detection operation may take place near detector 132. Once the detection operation is complete, droplets 130 may be transported out of 3D dome-shaped feature 120 and into waste reservoir 210, thereby forming a waste droplet 214 in waste reservoir 210.

Figure 3 illustrates a side view of a portion of a droplet actuator 300 that includes intersecting paths of droplet operations electrodes that allow droplet operations to occur in the x, y, and z planes and allow droplets to pass from one plane to another. Droplet actuator 300 may include a first structure 310 that is formed of a bottom substrate 312 and a top substrate 314 that are separated by a gap 316. Bottom substrate 312 may include an array and/or lines of droplet operations electrodes 318 (e.g., electrowetting electrodes) that are configured for droplet operations. Additionally, droplet actuator 300 may include a second structure 320 that is formed of a bottom substrate 322 and a top substrate 324 that are separated by a gap 326. Likewise, bottom substrate 322 may include an array and/or lines of droplet operations electrodes 328 (e.g., electrowetting electrodes) that are configured for droplet operations. In one example, first structure 310 and second structure 320 may be PCB structures.

First structure 310 may be oriented along a certain plane 330 and second structure 320 may be oriented along a different plane 332 in a manner such that gap 316 of first structure 310 intersects with gap 326 of second structure 320. Plane 330 and plane 332 and, thus, gap 316 and gap 326 may intersect at any angle. In one example, plane 330 may represent the xy-plane of droplet actuator 300 and plane 332 may represent the z-plane of droplet actuator 300. In this example, planes 330 and 332 may be about orthogonal to one another.

Because gap 316 of first structure 310 intersects with gap 326 of second structure 320, droplets 340 may be transported from one plane to another and back, as shown in Figure 3. For example, Figure 3 shows a branch or intersection point 342 at which droplet operations may be used to select the desired transport path of droplets 340. Droplet operations may occur independently along gap 316 of first structure 310 and gap 326 of second structure 320.

Figures 4A and 4B illustrate a side view of a portion of a multi-level droplet actuator 400, which is an example of a droplet actuator that includes multiple droplet operations planes. Multi-level droplet actuator 400 may include multiple substrates 410, such as a substrate 410a, 410b, 410c, 410d, and 410e. Each substrate 410 may include an array and/or lines of droplet operations electrodes 412 (e.g., electrowetting electrodes) that are configured for droplet operations. In one example, substrates 410a, 410b, 410c, 410d, and 410e may be arranged in parallel to one another in a stacked configuration in which there is a gap 414 between each substrate 410. For example, a gap 414a is between the electrode side of substrate 410a and the non-electrode side of substrate 410b; a gap 414b is between the electrode side of substrate 410b and the non-electrode side of substrate 410c; a gap 414c is between the electrode side of substrate 410c and the non-electrode side of substrate 410d; and a gap 414d is between the electrode side of substrate 410d and the non-electrode side of substrate 410e. Using droplet operations electrodes 412, each gap 414 facilitates droplet operations along each respective substrate 410.

In order to supply droplets, such as droplets 420, to the multiple droplet operations planes of multi-level droplet actuator 400, multi-level droplet actuator 400 may also include a substrate 416. Substrate 416 may include an array and/or lines of droplet operations electrodes 418 (e.g., electrowetting electrodes) that are configured for droplet operations. In one example, substrate 416 may be oriented about 90 degrees with respect to the orientation of substrates 410a, 410b, 410c, 410d, and 410e. Additionally, the electrode side of substrate 416 may be oriented to form a gap 422 with respect to the ends of substrates 410a, 410b, 410c, 410d, and 410e, as shown. Further, gap 422 intersects with gaps 414a through 414d of the stack of substrates 410a through 410e.

In one example, the multiple substrates 410 may be arranged horizontally in a vertical stack, while substrate 416 is arranged vertically to correspond to the vertical stack of substrates 410. In this example and referring to Figure 4A, droplets 420 may be transported vertically along gap 422 using droplet operations electrodes 418 of substrate 416. Referring to Figure 4B, as droplets 420 are being transported vertically along gap 422, certain droplet operations electrodes 412 at certain levels of substrates 410 may be activated in order to pull (via droplet operations) certain droplets 420 into their respective gaps 414. In this 3D droplet actuator configuration, substrate 416 provides a “ladder” function, by which droplets may “climb” up and down from one level to another. In this way, droplet operations may be facilitated at multiple planes of multi-level droplet actuator 400. Droplet operations may occur independently along gaps 414 of substrates 410 and gap 422 of substrate 416. Another example of a multi-level droplet actuator is shown with reference to Figure 5.

Figure 5 illustrates a side view of a portion of a multi-level droplet actuator 500, which is another example of a droplet actuator that includes multiple droplet operations planes. Multi-level droplet actuator 500 may include multiple substrates 410, which are described with reference to Figures 4A and 4B. However, in this embodiment, each substrate 410, such as substrate 410a, 410b, and 410c, may be expanded to include an array and/or multiple lines of droplet operations electrodes 412. For example, each substrate 410 may include a line of droplet operations electrodes 412a through a line of droplet operations electrodes 412f. Further, substrate 416 may be expanded to include an array and/or multiple lines of droplet operations electrodes 418. For example, substrate 416 may include a line of droplet operations electrodes 418a through a line of droplet operations electrodes 418f.

In this embodiment, substrate 410a, 410b, and 410c provide three levels or planes to which multiple droplets 420 may be supplied simultaneously due to the presence of multiple lines of droplet operations electrodes 418 at substrate 416. Similar to that described in Figures 4A and 4B, in this 3D droplet actuator configuration, substrate 416 provides a "ladder" function, by which droplets may "climb" up and down from one level to another. For example and referring to Figure 5, a droplet 420A is being supplied from the line of droplet operations electrodes 418a of substrate 416 to the line of droplet operations electrodes 412a of substrate 410a. At the same time, a droplet 420B is being supplied from the line of droplet operations electrodes 418a of substrate 416 to the line of droplet operations electrodes 412a of substrate 410b. At the same time, a droplet 420C is being supplied from the line of droplet operations electrodes 418c of substrate 416 to the line of droplet operations electrodes 412c of substrate 410b. At the same time, a droplet 420D is being supplied from the line of droplet operations electrodes 418e of substrate 416 to the line of droplet operations electrodes 412e of substrate 410c. In summary, droplet operations may occur independently along any line of droplet operations electrodes 412 of any substrate 410 and any line of droplet operations electrodes 418 of substrate 416. In this way, droplet operations may be facilitated at multiple planes of multi-level droplet actuator 500.

Figure 6A illustrates a top view of an example of a pattern that is cut into a sheet 600 of flexible circuit material on which lines of droplet operations electrodes are formed. Figure 6A shows sheet 600 in a flattened two-dimensional (2D) state. Sheet 600 may be formed of, for example, polyimide and/or polyester flexible printed circuit board material, which may be commercially available material that is well known in the flex circuit industry. In this example, three parallel lines of droplet operations electrodes 612 (e.g., electrowetting electrodes) are formed in sheet 600. The pattern that is cut into sheet 600 consists of a first cut 616 between the first and second lines of droplet operations electrodes 612 and a second cut 618 between the second and third lines of droplet operations electrodes 612. Cuts 616 and 618 may begin at one end of sheet 600 and extend toward the opposite end of sheet 600, but only a partial distance across the length of sheet 600. By way of example, Figure 6A shows cuts 616 and 618 stopping at about between the 12th and 13th droplet operations electrodes 612 of three lines. In this example, as a result of cuts 616 and 618, three "fingers," (e.g., fingers 614a, 614b, and 614c) are formed in the flattened sheet 600.

Figure 6B illustrates a side view of sheet 600 when in a non-flattened 3D state, which is suitable for incorporating into a droplet actuator for supplying droplets to multiple levels and/or planes of a multi-level droplet actuator, such multi-level droplet actuator 400 of Figures 4A and 4B and multi-level droplet actuator 500 of Figure 5. Sheet 600 may be flexed into the 3D structure shown in Figure 6B because of the pattern that is cut into sheet 600 when flattened (Figure 6A). For example, if the flattened sheet 600 represents the x-plane and y-plane, the pattern that is cut into sheet 600 also allows fingers 614a, 614b, and 614c of sheet 600 to be unflattened and, thereby, shaped in the z-plane. By way of example, Figure 6B shows that finger 614a may remain in a flattened state in order to supply droplets (not shown) to, for example, a LEVEL 1 of a multi-level droplet actuator (not shown). Figure 6B also shows that finger 614b may be shaped in the z-direction a certain distance to form a ramp-like shape in order to supply droplets to, for example, a LEVEL 2 of the multi-level droplet actuator. Figure 6B further shows that finger 614c may be shaped in the z-direction a certain distance that is greater than that of finger 614b to form another ramp-like shape in order to supply droplets to, for example, a LEVEL 3 of the multi-level droplet actuator.

Referring again to Figures 6A and 6B, sheet 600 that has fingers 614a, 614b, and 614c is an example of a flat 2D structure that may be cut and then flexed to form a 3D structure that is suitable for use in, for example, multi-level droplet actuator applications.

Figure 7A illustrates a top view of an example of a pattern that is cut into a sheet 700 of flexible circuit material on which lines of droplet operations electrodes are formed. Figure 7A shows sheet 700 in a flattened 2D state. Sheet 700 may be formed of, for example, polyimide and/or polyester flexible printed circuit board material, which may be commercially available material that is well known in the flex circuit industry. Sheet 700 has a first end 705 and a second end 710. In this example, four parallel lines of droplet operations electrodes 712 (e.g., electrowetting electrodes) are formed in sheet 700, such as a line 714a, a line 714b, a line 714c, and a line 714c of droplet operations electrodes 712. Additionally, other droplet operations electrodes, which are hereafter referred to as transition electrodes 716 are formed in sheet 700, which provide a droplet operations path between the lines 714 of droplet operations electrodes 712. For example, a transition electrode 716a is provided between lines 714a and 714b at second end 710 of sheet 700. A transition electrode 716b is provided between lines 714b and 714c at first end 705 of sheet 700. A transition electrode 716c is provided between lines 714c and 714d at second end 710 of sheet 700. As a result, a top down view of sheet 700 shows a continuous path of droplet operations electrodes in a serpentine type of pattern.

The pattern that is cut into sheet 700 consists of three cuts – A first cut 718 between lines 714a and 714b of droplet operations electrodes 712, beginning at first end 705 of sheet 700 and ending just short of transition electrode 716a at second end 710. A second cut 720 between lines 714b and 714c of droplet operations electrodes 712, beginning at second end 710 of sheet 700 and ending just short of transition electrode 716b at first end 705. A third cut 722 between lines 714c and 714d of droplet operations electrodes 712, beginning at first end 705 of sheet 700 and ending just short of transition electrode 716c at second end 710.

Figure 7B illustrates a side view of sheet 700 when in a non-flattened 3D state, which is suitable for incorporating into a droplet actuator for supplying droplets to multiple levels and/or planes of a multi-level droplet actuator, such multi-level droplet actuator 400 of Figures 4A and 4B and multi-level droplet actuator 500 of Figure 5. Sheet 700 may be flexed (accordion style) into the 3D structure shown in Figure 7B because of the pattern that is cut into sheet 700 when flattened (Figure 7A). For example, if the flattened sheet 700 represents the x-plane and y-plane, the pattern that is cut into sheet 700 also allows lines 714a, 714b, 714c, and 714c of droplet operations electrodes 712 to be unflattened and, thereby, shaped in the z-plane. For example, by anchoring line 714a of droplet operations electrodes 712 at first end 705 of sheet 700 and then lifting line 714d of droplet operations electrodes 712 at first end 705 of sheet 700, sheet 700 may be flexed accordion style into the 3D structure shown in Figure 7B.

More specifically, Figure 7B shows that at first end 705 of sheet 700, line 714a of droplet operations electrodes 712 may be flexed into a position for supplying droplets (not shown) to, for example, a LEVEL 1 of a multi-level droplet actuator (not shown). Figure 7B also shows that at second end 710 of sheet 700, line 714a and line 714b of droplet operations electrodes 712 may be flexed into a position for supplying droplets to, for example, a LEVEL 2 of a multi-level droplet actuator. Figure 7B also shows that at first end 705 of sheet 700, line 714b and line 714c of droplet operations electrodes 712 may be flexed into a position for supplying droplets to, for example, a LEVEL 3 of a multi-level droplet actuator. Figure 7B also shows that at second end 710 of sheet 700, line 714c and line 714d of droplet operations electrodes 712 may be flexed into a position for supplying droplets to, for example, a LEVEL 4 of a multi-level droplet actuator. Figure 7B also shows that at first end 705 of sheet 700, line 714d of droplet operations electrodes 712 may be flexed into a position for supplying droplets to, for example, a LEVEL 5 of a multi-level droplet actuator.

Referring again to Figures 7A and 7B, sheet 700 is an example of a flat 2D structure that may be cut and then flexed to form a 3D structure that is suitable for use in, for example, multi-level droplet actuator applications.

Figure 8A illustrates a top view of an example of a strip 800 of flexible circuit material on which lines of droplet operations electrodes are formed. Figure 8A shows strip 800 in a flattened 2D state. Strip 800 may be formed of, for example, polyimide and/or polyester flexible printed circuit board material, which may be commercially available material that is well known in the flex circuit industry. Strip 800 has a first end 805 and a second end 810. In this example, one line of droplet operations electrodes 812 (e.g., electrowetting electrodes) is formed in strip 800. However, this is exemplary only. Any number of lines of droplet operations electrodes may be formed in strip 800.

Figure 8B illustrates a side view of strip 800 and an example of using strip 800 in a non-flattened 3D state. In this example, strip 800 may be flexed into a horseshoe shape in order to provide a means for transporting droplets between two planes of, for example, a double-sided droplet actuator. Further to the example, Figure 8B shows a double-sided droplet actuator 820 that is formed of a substrate 822 that has droplet operations electrodes 824 arranged on both surfaces thereof. In this example, strip 800 may be flexed such that its first end 805 is coupled to one side of droplet actuator 820 and its second end 810 is coupled to the opposite side of droplet actuator 820. In this way, strip 800 is flexed in a horseshoe shape such that droplet operations electrodes 812 on strip 800 provide a droplet operations link between both sides of double-sided droplet actuator 820.

Figures 9A and 9B show another example of using strip 800 of Figure 8A in a non-flattened 3D state. For example, Figures 9A and 9B illustrate a top view and a side view, respectively, of strip 800 that is flexed axially with half twist. Referring to Figure 9A, because of the half twist that is present in strip 800, the endmost droplet operations electrode 812 at first end 805 of strip 800 and the endmost droplet operations electrode 812 at second end 810 are facing in opposite directions. This flexed state may be useful for providing a droplet operations path between two opposite-facing droplet actuators, an example of which is shown in Figure 9B.

More specifically, Figure 9B shows strip 800 that is flexed with a half twist spanning a substrate 910 of a first droplet actuator that is facing, for example, upward and a substrate 920 of a second droplet actuator that is facing, for example, downward.

In another example of using strip 800 of Figure 8A in a non-flattened 3D state, strip 800 may be flexed into a spiral shape (not shown) for providing a droplet operations path (in a spiral staircase fashion) between two levels of a multi-level droplet actuator.

Figure 10 illustrates a side view of a portion of an example of a multi-level droplet actuator 1000 that includes a "wicking" mechanism for moving liquid from one level to another. For example, a first level of multi-level droplet actuator 1000 may include a bottom substrate 1010 and a top substrate 1012 that are separated by a gap 1014. Bottom substrate 1010 may include an array and/or lines of droplet operations electrodes 1016 (e.g., electrowetting electrodes) that are configured for droplet operations. Further, a second level of multi-level droplet actuator 1000 may include a bottom substrate 1020 and a top substrate 1022 that are separated by a gap 1024. Bottom substrate 1020 may include an array and/or lines of droplet operations electrodes 1026 (e.g., electrowetting electrodes) that are configured for droplet operations.

In this example, one end of the first level of multi-level droplet actuator 1000 may be oriented toward one end of the second level of multi-level droplet actuator 1000 such that, for example, gap 1014 of the first level is offset vertically from gap 1024 of the second level. A channel 128 for linking gap 1014 of the first level and gap 1024 of the second level is provided in multi-level droplet actuator 1000. Channel 128 may be filled with a material 130 that may provide wicking action when a liquid, such as the liquid of a droplet 140, is introduced therein.

For example, material 130 may be a porous gel substance, a synthetic gauze, and the like that is capable of soaking up droplet 140, like a sponge. Due to capillary action, any fluid, such as the fluid of droplet 140, that comes into contact with material 130 seeps through the material from gap 1014 of the first level to gap 1024 of the second level. Once at the second level, the liquid emerges from material 130 and into gap 1024 in order to reform droplet 140. Droplet operations electrodes 1016 and/or droplet operations electrodes 1026 may be sequenced off and on in such as way at to make this design act like a pump. Fluid may seep through material 130 in either direction.

Figure 11 illustrates a side view of a portion of an example of a multi-level droplet actuator 1100 that uses various types of via holes for moving droplets from one level to another. For example, multi-level droplet actuator 1100 may include an arrangement of multiple levels of multiple substrates 1110 that are separated by a gap. Each substrate 1110 may include an array and/or lines of droplet operations electrodes 1112 (e.g., electrowetting electrodes) that are configured for droplet operations. In one implementation, substrates 1110 may be individual PCB-based substrates that may be arranged vertically and/or horizontally. In another implementation, multi-level droplet actuator 1100 may be a multi-layer PCB, where each level of substrates 1110 may be a certain layer of the PCB.

In the circuit board industry, whether it is PCB technology or ceramic technology, via holes that are plated (or entirely filled) with electrically conductive material facilitate electrical connections between layers of a multilayer structure. With respect to the various types of via holes, the following definitions apply. A "through" via hole (hereafter referred to as a "through-via") means any via hole that passes all the way through a multilayer structure (i.e., passes all the way through from one outer layer to the opposite outer layer). A "blind" via hole (hereafter referred to as a "blind-via") means any via hole that starts on an outer layer but terminates on an inner layer of a multilayer structure. A "buried" via hole (hereafter referred to as a "buried-via") means any via hole that exists only between inner layers of a multilayer structure and do not begin or terminate on an outer layer.

Applying the via hole concepts to multi-level droplet actuator 1100, through-vias, blind-vias, and/or buried-vias may be used for passing droplets from one level to another. An example of a blind-via is blind-via 1114. An example of a buried-via is buried-via 1116. Through-vias (not shown); blind-vias, such as blind-via 1114; and buried-vias, such as buried-via 1116 may also have droplet operations electrodes 1112 arranged along the length thereof for performing droplet operations. For example, droplet operations electrodes 1112 in the through-vias, blind-vias, and/or buried-vias may be used for applying electrowetting forces to droplets, such as droplets 1120, in order to transport the droplets from one level to another. Droplet operations electrodes 1112 may be formed as a full or partial ring around the via holes. In multi-level droplet actuator 1100, droplets may be passed from one level to another and droplet operations may occur independently along any substrate 1110 at any level.

In one implementation, the through-vias, blind-vias, and/or buried-vias may be formed, for example, of flexible printed circuit board material that is flexed to form a tube and is then placed between the various levels of substrates 1110. In another implementation in which multi-level droplet actuator 1100 is a multi-layer PCB, each level of substrates 1110 may be a certain layer of the PCB. In this implementation, the through-vias, blind-vias, and/or buried-vias may be formed using standard PCB technology.

Figures 12A, 12B and 12C illustrate perspective views of an example of a 3D droplet actuator structure 1200 that is formed of multiple slotted droplet operations boards. Figure 12A shows 3D droplet actuator structure 1200 when the slotted droplet operations boards are assembled. Figure 12B shows 3D droplet actuator structure 1200 when the slotted droplet operations boards are disassembled.

For example, 3D droplet actuator structure 1200 may include a droplet operations backplane 1210 that includes slots that are designed to interlock with slots of one or more droplet operations daughter boards 1212. More specifically, droplet operations backplane 1210 may include an array and/or lines of droplet operations electrodes 1214 (e.g., electrowetting electrodes) that are configured for droplet operations. Droplet operations backplane 1210 may also include one or more slots 1216 for interlocking with one or more operations daughter boards 1212. In one example, droplet operations backplane 1210 includes slots 1216a and 1216b for interlocking with droplet operations daughter boards 1212a and 1212b, respectively. A transition electrode 1218 is located in proximity to a certain droplet operations electrode 1214 and also to each slot 1216, such as a transition electrode 1218a near slot 1216a and a transition electrode 1218b near slot 1216b.

Similarly, each droplet operations daughter board 1212 may also include an array and/or lines of droplet operations electrodes 1214 (e.g., electrowetting electrodes) that are configured for droplet operations. Each droplet operations daughter board 1212 may also include one or more slots 1220 for interlocking with droplet operations backplane 1210. In one example and referring to Figure 12B, slot 1220a of droplet operations daughter board 1212a may interlock with slot 1216a of droplet operations backplane 1210. Also, slot 1220b of droplet operations daughter board 1212b may interlock with slot 1216b of droplet operations backplane 1210. Each droplet operations daughter board 1212 may also include a clearance hole 1222 that is located at the innermost end of its slot 1220. When droplet operations backplane 1210 and droplet operations daughter boards 1212 are assembled, clearance holes 1222 provide an opening through which droplets may pass.

Figure 12C shows an expanded view of a Detail A of Figure 12A, which shows more details of the operation of 3D droplet actuator structure 1200. Detail A shows that droplets 1230 may be transported along droplet operations electrodes 1214 of droplet operations backplane 1210 and/or droplet operations daughter board 1212b. In one example, droplets 1230 may be transported along the plane of droplet operations backplane 1210 by passing through clearance hole 1222b. In another example, droplets 1230 may be transported from along the plane of droplet operations backplane 1210 and then change direction in order to be transported along the plane of droplet operations daughter board 1212b. The change in direct occurs by transporting a certain droplet 1230 to, for example, transition electrode 1218b instead of through clearance hole 1222b. Once the certain droplet 1230 is at transition electrode 1218b, it may then pass (via droplet operations) to the nearest droplet operations electrode 1214 of droplet operations daughter board 1212b, affecting the change in direction to another plane of 3D droplet actuator structure 1200. In summary, droplet operations may occur independently along any plane of 3D droplet actuator structure 1200.

3D droplet actuator structure 1200 is not limited to the arrangement shown in Figures 12A, 12B, and 12C. This arrangement is exemplary only. 3D droplet actuator structure 1200 may be a heavily interconnected structure that includes multiple droplet operations backplanes 1210 and multiple droplet operations daughter boards 1212. Additionally, there may be droplet operations electrodes 1214 on both sides of the droplet operations backplanes 1210 and droplet operations daughter boards 1212.

Figures 13A and 13B illustrate side views of examples of droplet actuators that include one or more double-sided substrates. In one example, Figure 13A shows a droplet actuator 1300 that includes a double-sided substrate 1310 that has droplet operations electrodes 1312 arranged on both surfaces thereof. A top substrate 1314 is oriented in relation to one surface of double-sided substrate 1310 such that there is a gap 1316 therebetween. Another top substrate 1318 is oriented in relation to the opposite surface of double-sided substrate 1310 such that there is a gap 1320 therebetween. In this embodiment, droplet operations may occur independently on both sides of double-sided substrate 1310. For example, Figure 13A shows a droplet 1330a being transported via droplet operations along gap 1316. Figure 13A also shows a droplet 1330b being transported via droplet operations along gap 1320.

In another example, Figure 13B shows a droplet actuator 1350 that includes a stack of any number of double-sided substrates 1352, where each substrate 1352 has droplet operations electrodes 1354 arranged on both surfaces thereof. Further, each substrate 1352 has a droplet operations electrode 1356 at each end thereof. The droplet operations electrode 1356 may be used for transporting droplets, such as droplets 1370, around the edges of each substrate 1352. In this way, droplets 1370 may move between both sides of substrates 1352.

By way of example, Figure 13B shows double-sided substrates 1352a, 1352b, and 1352c that are arranged in parallel. Double-sided substrates 1352a and 1352b are separated by a gap 1358. Double-sided substrates 1352b and 1352c are separated by a gap 1360. Also shown are multiple droplets 1370 moving independently on any level of droplet actuator 1350. The transport of droplets 1370 from one level to the next is facilitated by use of droplet operations electrode 1356 at the ends of substrates 1352.

Droplets 1370 are shown as unit size droplets. However, smaller droplets, such as quarter-sized droplets 1372 may be moving independently along the same gap. For example, a droplet 1372a may be transported along the upper surface of substrate 1352a in gap 1358. At the same time, a droplet 1372b may be transported along the lower surface of substrate 1352b, also in gap 1358. Droplets 1372a and 1372b are suitably sized to be able to pass by one another in the same gap without interference.

Figure 14 illustrates a side view of an example of a multi-level droplet actuator 1400 that is formed by folding and/or bending a sheet 1410 of flexible circuit material. Sheet 1410 is patterned with an array and/or lines of droplet operations electrodes 1412 (e.g., electrowetting electrodes) that are configured for droplet operations. Sheet 1410 may be formed of, for example, polyimide and/or polyester flexible printed circuit board material, which may be commercially available material that is well known in the flex circuit industry.

By way of example, multi-level droplet actuator 1400 is formed by folding sheet 1410 into multiple segments 1414 to form, for example, a serpentine shaped 3D structure. In the example shown in Figure 14, a segment 1414a is arrange horizontally, then sheet 1410 continues with a segment 1414b that is folded vertically, then sheet 1410 continues with a segment 1414c that is folded horizontally, then sheet 1410 continues with a segment 1414d that is folded vertically, then sheet 1410 continues with a segment 1414e that is folded horizontally, then sheet 1410 continues with a segment 1414f that is folded vertically, and then sheet 1410 continues with a segment 1414f that is folded horizontally. In this example, segment 1414a provides a LEVEL 1 of multi-level droplet actuator 1400. Segment 1414c provides a LEVEL 2 of multi-level droplet actuator 1400. Segment 1414e provides a LEVEL 3 of multi-level droplet actuator 1400. Segment 1414g provides a LEVEL 4 of multi-level droplet actuator 1400. In operation, one or more droplets 1420 may be transported vertically and/or horizontally along droplet operations electrodes 1412 of any segments 1414 of multi-level droplet actuator 1400. Droplet operations may occur independently along any level and/or plane of multi-level droplet actuator 1400.

Multi-level droplet actuator 1400 is an example in which six folds in sheet 1410 are about 90 degrees. However, sheet 1410 may be folded and/or bent any number of times and at any angles to form any shape and, thus, multi-level droplet actuator 1400 is not limited to the serpentine shape. In another embodiment, segments 1414 of multi-level droplet actuator 1400 are formed of individual substrates that are abutted one to another at any desired angle.

Figure 15 illustrates a side view of an example of a multi-level droplet actuator 1500 that is formed of a stack of substrates that have openings therein for passing droplets from one level to another. In one example, multi-level droplet actuator 1500 may include a substrate 1510, a substrate 1512, a substrate 1514, and a substrate 1516 that are arranged in parallel in a stack. One side of substrates 1510, 1512, and 1514 may include an array and/or lines of droplet operations electrodes 1518 (e.g., electrowetting electrodes) that are configured for droplet operations. In one example, the electrode side of substrate 1510 is facing the non-electrode side of substrate 1512 and there is a gap 1520 between substrate 1510 and substrate 1512. Additionally, the electrode side of substrate 1512 is facing the non-electrode side of substrate 1514 and there is a gap 1522 between substrate 1512 and substrate 1514. Additionally, the electrode side of substrate 1514 is facing a top substrate 1516 and there is a gap 1524 between substrate 1514 and top substrate 1516.

Multi-level droplet actuator 1500 includes openings for passing droplets from one level to another. For example, substrate 1512 may include an opening 1526 by which droplets, such as droplets 1540, may pass back and forth between substrate 1510 and substrate 1512. Additionally, substrate 1514 may include an opening 1528 by which droplets, such as droplets 1540, may pass back and forth between substrate 1512 and substrate 1514. Optionally, top substrate 1516 may include an electrode 1530 located in proximity to opening 1528 in order to assist in pulling a certain droplet 1540 through opening 1528.

The size of, for example, openings 1526 and 1528 may be optimized with respect to the size of gaps 1520, 1522, and/or 1524 in order to optimize the amount of force that is required for pulling droplets back and forth through the openings. Further, the size of gaps 1520, 1522, and/or 1524 need not be uniform. The size of the gaps may vary, for example, from bottom to top of the stack of substrates. Droplet operations may occur independently along any level and/or plane of multi-level droplet actuator 1500.

Figure 16 illustrates a side view of an example of a droplet actuator 1600 that is a 3D structure that includes wash and/or waste reservoirs at different levels with respect to the droplet operations plane. Droplet actuator 1600 may include a bottom substrate 1610 and a top substrate 1612 that are separated by a gap 1614. Bottom substrate 1610 may include an array and/or lines of droplet operations electrodes 1616 (e.g., electrowetting electrodes) that are configured for droplet operations. Droplet actuator 1600 may also include a fluid reservoir 1618 that feeds an opening of top substrate 1612. A quantity of wash fluid 1620 may be supplied from fluid reservoir 1618 into gap 1614. A reservoir electrode 1622 on bottom substrate 1610 may be associated with fluid reservoir 1618.

Bottom substrate 1610 may also include an opening 1624 that feeds a fluid channel 1626 the may lead, for example, to a waste reservoir 1628, which is on a different plane with respect to gap 1614 of droplet actuator 1600. Further, droplet operations electrodes 1616 may be arranged along the walls of fluid channel 1626.

Additionally, bottom substrate 1610 may include an opening 1630 that feeds a fluid channel 1632 the may lead, for example, to a wash reservoir 1634, which is on a different plane with respect to gap 1614 of droplet actuator 1600. Further, droplet operations electrodes 1616 may be arranged along the walls of fluid channel 1632.

One way to supply wash fluid, such as wash fluid 1620 to droplet actuator 1600 is to dispense droplets 1636 from wash fluid 1620 that is supplied from fluid reservoir 1618. Another way to supply wash fluid to droplet actuator 1600 is to use droplet operations electrodes 1616 along fluid channel 1632 to pull a finger of fluid from the wash solution in wash reservoir 1634 and into gap 1614 of droplet actuator 1600 to form droplets 1636 that may then be transported in any direction. In this scenario, because of the weight of a column of liquid flowing up from wash reservoir 1634 the liquid may automatically snap off leaving a droplet in gap 1614. The result is a pull and snap sequence of events. Further, because the force of gravity is substantially constant, the volume of the droplets that are snapped off are substantially uniform.

One way to expel droplets to waste is to use droplet operations electrodes 1616 along fluid channel 1626 to pull droplets 1636 from gap 1614 of droplet actuator 1600 into waste reservoir 1628. In this scenario, gravity may assist the transport of droplets through fluid channel 1626 to waste reservoir 1628.

Figure 17 illustrates a side view of an example of a droplet actuator 1700 that is a 3D structure for transporting droplets between two zones, such as between hot and cold zones. Droplet actuator 1700 may include at least two block shaped substrates 1710, such as a substrate 1710a and 1710b. Each block shaped substrate 1710 may include on at least three sides thereof an array and/or lines of droplet operations electrodes 1712 (e.g., electrowetting electrodes) that are configured for droplet operations. In this example, substrate 1710a and 1710b are arranged side by side with a gap 1714 therebetween to form, for example, a vertical a channel.

Droplet actuator 1700 may also include a substrate 1716 that is arranged in parallel with a lower surface of substrates 1710a and 1710b with a gap 1718 therebetween. Additionally, droplet actuator 1700 may include a substrate 1720 that is arranged in parallel with an upper surface of substrates 1710a and 1710b with a gap 1722 therebetween. The result of this example arrangement is that gaps 1718 and 1722 are arranged in parallel and connected by gap 1714, which is orthogonal to gaps 1718 and 1722.

In one example, substrate 1716 may be a cold temperature source and substrate 1720 may be a hot temperature source. Therefore, droplets, such as a droplet 1730, may be transported (via droplet operations) back and forth between the cold substrate 1716 and the hot substrate 1720 via gap 1714. Droplet operations may occur independently along gap 1714, gap 1718, and gap 1722 of droplet actuator 1700.

Figure 18 illustrates a perspective view of an example of a droplet actuator 1800 that is a 3D structure that includes droplet operations tubes for transporting droplets between two levels and/or zones. Droplet actuator 1800 may include a bottom substrate 1810 and a block 1812 that are separated by a gap in which droplet operations may occur. Bottom substrate 1810 may include an array and/or lines of droplet operations electrodes 1814 (e.g., electrowetting electrodes) that are configured for droplet operations. Block 1812 may be a block of thermally conductive material for supplying a temperature gradient from the surface that is nearest bottom substrate 1810 to the surface that is farthest from bottom substrate 1810. In one example, the surface of block 1812 that is nearest bottom substrate 1810 is hot and the surface of block 1812 that is farthest from bottom substrate 1810 is cold, with a temperature gradient therebetween.

Further, block 1812 may include one or more droplet operations tubes 1816 that are arranged orthogonally with respect to the surface of bottom substrate 1810. Each droplet operations tube 1816 may include an arrangement of droplet operations electrodes 1818 along their length. Droplet operations electrodes 1818 may be formed as a partial ring (see Detail A) or a full ring (see Detail A) around droplet operations tubes 1816. In operation, droplet operations tubes 1816 may be used for transporting droplets, such as droplets 1820, up and down along the temperature gradient that may be present in block 1812. Droplet operations may occur independently along any droplet operations tube 1816 of droplet actuator 1800.

Figure 19 illustrates a perspective view of an example of a structure 1900 for stimulating vertical motion of droplets. Structure 1900 may include a first plate 1910 and a second plate 1912. First plate 1910 and second plate 1912 may be formed of any material that is both electrically conductive and thermally conductive, such as copper, aluminum, and gold. An alternating current (AC) voltage source 1914 may be connected between first plate 1910 and second plate 1912. Additionally, a hot temperature source may be connected to first plate 1910 and a cold temperature source may be connected to second plate 1912. Using this arrangement, one or more droplets 1920 that are present between first plate 1910 and second plate 1912 may be stimulated to "bounce" between the two plates due to the presence of AC voltage source 1914, which is providing a voltage that is switching at a certain frequency. In this way, the one or more droplets 1920 may bounce between the hot source and cold source. A structure, such as structure 1900, may be useful in polymerase chain reaction (PCR) applications and other applications.

Figure 20 illustrates a perspective view of a portion of a multi-level droplet actuator 2000, which is an example of a droplet actuator that includes any number of planes that are dedicated for certain functions. In one example, multi-level droplet actuator 2000 may includes a stack of substrates, such as a stack that includes a substrate 2010, a substrate 2012, a substrate 2014, and a substrate 2016. Each substrate may include an array and/or lines of droplet operations electrodes 2018 (e.g., electrowetting electrodes) that are configured for droplet operations.

In multi-level droplet actuator 2000, substrate 2012 may, for example, be a plane that is dedicated to performing droplet incubation activities; while substrate 2014 may, for example, be a plane that is dedicated to performing droplet washing activities; and while substrate 2016 may, for example, be a plane that is dedicated to performing droplet detection activities; and the like. The advantage of having planes that are dedicated to certain functions is that the amount of contamination may be reduced, preferably entirely eliminated. Droplet operations may occur independently along any level and/or plane of multi-level droplet actuator 2000.

Additionally, multi-level droplet actuator 2000 may provide different kinds of filler oil on different planes, different kinds of surfactants on different planes, different electrode sizes and/or shapes on different planes, and the ability to isolate droplets in proximity to certain sensors on different planes, and so on.

Figures 21A, 21B, and 21C illustrate a side view of an example of a droplet actuator 2100 that includes a 3D feature in the top substrate and a process for allowing one droplet to pass another droplet in the gap of the droplet actuator. Droplet actuator 2100 may include a bottom substrate 2110 and a top substrate 2112 that are separated by a gap 2114. Bottom substrate 2110 may include an array and/or lines of droplet operations electrodes 2116 (e.g., electrowetting electrodes) that are configured for droplet operations. Additionally, a notch 2118 is formed on the surface of top substrate 2112 that is facing gap 2114. Further, the placement of notch 2118 substantially aligns with a certain droplet operations electrode 2116.

Notch 2118 provides, for example, a rectangular-shaped void in top substrate 2112 that is suitably sized to fit the volume of a droplet therein. The void that is caused by notch 2118 may be useful for capturing one droplet (e.g., droplet 2120a) and allowing another droplet (e.g., droplet 2120b) to pass while both are in the same gap 2114. By way of example, Figures 21A, 21B, and 21C illustrate the process for allowing one droplet to pass another droplet in the gap of a droplet actuator. This process may include, but is not limited to, the following steps.

In a first step and referring to Figure 21A, droplet 2120a is being transported along droplet operations electrodes 2116 toward notch 2118 in top substrate 2112. Additionally, droplet 2120b is following droplet 2120a in the same direction.

In a second step and referring to Figure 21B, when droplet 2120a arrives at the droplet operations electrode 2116 that is aligned with notch 2118, this droplet operations electrode 2116 may be deactivated. By deactivating this droplet operations electrode 2116, droplet 2120a levitates into notch 2118 because of its buoyancy. Because droplet 2120a is now positioned in notch 2118 in top substrate 2112, a clear path is provided in gap 2114 by which droplet 2120b may pass by droplet 2120b.

In a third step and referring to Figure 21C, droplet 2120b is now being transported along droplet operations electrodes 2116 in advance of droplet 2120a, instead of behind droplet 2120a.

Additionally, notch 2118 in top substrate 2112 may be useful for other purposes. For example, when top substrate 2112 is transparent, notch 2118 may be used to secure droplets for the purpose of performing detection. That is, notch 2118 may be used as a designated detection spot.

Figure 22A illustrates a side view of an example of a droplet actuator 2200 that includes another 3D feature in the top substrate. Droplet actuator 2200 may include a bottom substrate 2210 and a top substrate 2212 that are separated by a gap 2214. Bottom substrate 2210 may include an array and/or lines of droplet operations electrodes 2216 (e.g., electrowetting electrodes) that are configured for droplet operations. Additionally, a wedge-shaped notch 2218 is formed on the surface of top substrate 2212 that is facing gap 2214. The two surfaces of wedge-shaped notch 2218 may form, for example, about a 90 degree angle. Further, the placement of wedge-shaped notch 2218 substantially aligns with a certain droplet operations electrode 2216.

Wedge-shaped notch 2218 provides a corresponding a wedge-shaped void in top substrate 2212 that is suitably sized to fit about the volume of a droplet therein. The void that is caused by wedge-shaped notch 2218 may be useful for capturing the volume of a droplet (e.g., droplet 2220) and then performing a detection operation thereon (assuming a transparent top substrate 2212). For example, an excitation light may be directed at one side of wedge-shaped notch 2218 and into the volume of liquid that is trapped in wedge-shaped notch 2218. The resulting emission light from this volume of liquid is then emitted from the opposite side of the wedge-shaped notch 2218 and may be measured via a detector (not shown).

Figure 22B illustrates a side view of an example of a droplet actuator 2245 that includes another 3D feature in the top substrate. Droplet actuator 2200 may include a bottom substrate 2250 and a top substrate 2252 that are separated by a gap 2254. Bottom substrate 2250 may include an array and/or lines of droplet operations electrodes 2256 (e.g., electrowetting electrodes) that are configured for droplet operations. Additionally, a tower-shaped structure 2258 is formed in top substrate 2252. The placement of tower-shaped structure 2258 substantially aligns with a certain droplet operations electrode 2256.

Tower-shaped structure 2258 provides, for example, a rectangular-shaped void that is suitably sized to fit a droplet therein. The void that is caused by tower-shaped structure 2258 may be useful for capturing a droplet (e.g., a droplet 2260) and then performing a detection operation thereon (assuming a transparent top substrate 2252). For example, an excitation light may be directed at one side of tower-shaped structure 2258 and into droplet 2260 that is trapped in tower-shaped structure 2258. The resulting emission light droplet 2260 is then emitted from the opposite side of the tower-shaped structure 2258 and may be measured via a detector (not shown).

In another implementation, tower-shaped structure 2258 may be useful for measuring the absorbance of an opaque substance, i.e., the absorbance of droplet 2260. For example, a certain intensity light may be directed at one side of tower-shaped structure 2258, which then passes directly through droplet 2260 that is trapped in tower-shaped structure 2258. The intensity of the light that emerges from the opposite side of the tower-shaped structure 2258 and may then be measured in order to determine the absorbance of droplet 2260.

Figure 23 illustrates a top view of an example of a portion of an electrode structure 2300 that may be formed by weaving, which is another example of a 3D structure for use in droplet actuators. Electrode structure 2300 may include multiple tapes, strands, and/or wires that are suitably flexible to be weaved together. In particular, certain tapes, strands, and/or wires may be electrically conductive and certain other tapes, strands, and/or wires are not electrically conductive (i.e., are insulators).

By weaving together a combination of multiple conductive and nonconductive tapes, strands, and/or wires, a mesh 2310 may be formed. Certain patches of the electrically conductive tapes, strands, and/or wires are exposed in an orderly and repeatable pattern to form a line and/or path of droplet operations electrodes 2312. For example, droplet operations electrode 2312a is a patch of a certain tape, strand, and/or wire that is exposed, droplet operations electrode 2312b is a patch of another tape, strand, and/or wire that is exposed, droplet operations electrode 2312c a patch of yet another tape, strand, and/or wire that is exposed, and droplet operations electrode 2312d a patch of still another tape, strand, and/or wire that is exposed.

The individual tapes, strands, and/or wires may be electrically connected to respective control signals (not shown). In operation, to move droplets, the different tapes, strands, and/or wires may be activated at different times. Because electrode structure 2300 is a woven structure, it is essentially a textile product that can be woven into many 3D shapes. In one example, the mesh 2310 that is formed may take the shape of a tube through which droplets may be moved.

7.2 Droplet Actuator Configurations and Methods of Conducting Droplet Operations

The invention provides droplet actuators and methods for conducting droplet operations on a droplet actuator. For example, the invention provides droplet actuator configurations and techniques for improved droplet loading, splitting and/or dispensing in a droplet actuator. In some embodiments, electrode structures and/or configurations make use of voltage and/or capacitance gradients across one or more electrodes, which may be useful for assisting droplet operations. In some embodiments, electrode structures and/or configurations make use of dielectric layers with different dielectric constants in different regions and/or a gradient in dielectric constant across a region, which may be useful for assisting droplet operations. In other embodiments, electrode structures and/or configurations make use of variable gap heights and/or variable pressure regions, again for assisting droplet operations. Other embodiments of the invention will be apparent from the ensuing discussion in light of the definitions provided above.

7.2.1 Voltage and Capacitance Gradients for Assisting Droplet Operations

In a droplet actuator, a droplet essentially forms one plate of a capacitor with the electrode forming the other plate of the capacitor. The amount of capacitive energy present may effect the movement of droplets along the electrodes of the droplet actuator. Capacitive energy = $(CV^2)/2$, where C is capacitance and V is voltage. Therefore, according to this equation, the capacitance and/or the voltage may be varied in order to vary the capacitive energy. The following embodiments of the invention describe electrode structures and/or configurations for creating voltage and/or capacitance gradients across one or more electrodes, which may be used to assist droplet operations. For example, the following embodiments describe electrode structures and/or configurations that may include resistive electrodes, conductive electrodes, electrodes having various footprints, electrodes having various cross-sectional geometries, electrodes installed in combination with various dielectric layers, and any combinations thereof to provide various voltage and/or capacitance gradients for assisting droplet operations.

Figures 24, 25, 26, and 27 illustrate top and side views of non-limiting examples of resistive droplet operations electrodes having various footprints and cross-sectional geometries for developing linear and/or non-linear voltage and/or capacitance gradients, which may be used to assist droplet operations. Further, the resistive droplet operations electrodes may be installed in combination with various dielectric layers, which again may be used to assist droplet operations.

Figure 24 illustrates a top and side view of a rectangle-shaped electrode 2400. Rectangle-shaped electrode 2400 is an example of an electrically resistive droplet operations electrode that may be formed of an electrically resistive material. In this example, rectangle-shaped electrode 2400 is rectangle-shaped with respect to its footprint. Rectangle-shaped electrode 2400 may have any specified length L, any specified width w that is substantially uniform along its length, and any specified thickness t that is substantially uniform along its length. Because the dimensions of rectangle-shaped electrode 2400 are substantially uniform, its resistance R is substantially linear along its length. Consequently, when rectangle-shaped electrode 2400 is electrically connected between two voltages, such as between ground and a certain higher voltage, a substantially linear voltage gradient develops along its length.

When activated during droplet operations, a droplet (not shown) tends to move from the lower voltage end of rectangle-shaped electrode 2400 to the higher voltage end. Additionally, because the voltage gradient is substantially linear, the rate of droplet movement is substantially linear along its length. In this way, the voltage gradient of rectangle-shaped electrode 2400 may be used to assist droplet operations.

Figure 25 illustrates a top and side view of a generally triangle-shaped electrode 2500. Triangle-shaped electrode 2500 is another example of an electrically resistive droplet operations electrode that may be formed of an electrically resistive material. In this example, triangle-shaped electrode 2500 is triangle-shaped with respect to its footprint. Triangle-shaped electrode 2500 may have any specified length *L*, any specified width that varies from a first width $w1$ at one end to a second width $w2$ at the opposite end, and any specified thickness t that is substantially uniform along its length. Because at least one dimension of triangle-shaped electrode 2500 varies, its resistance *R* is non-linear along its length. More specifically, the resistance is greatest at the narrow end of each triangle-shaped electrode 2500 and least at the wide end of each triangle-shaped electrode 2500. This is because the amount of material present in each triangle-shaped electrode 2500 is least at the narrow end and greatest at the wide end (i.e., the less material, the greater the resistance; the more material, the lesser the resistance). Further, because capacitance is a function of area, varying the width of triangle-shaped electrode 2500 from $w1$ to $w2$ along its length *L* also provides a capacitance gradient.

Figure 26 illustrates a top and side view of a wedge-shaped electrode 2600. Wedge-shaped electrode 2600 is yet another example of an electrically resistive droplet operations electrode that may be formed of an electrically resistive material. In this example, wedge-shaped electrode 2600 is wedge-shaped with respect to its lateral cross section. Wedge-shaped electrode 2600 may have any specified length *L*, any specified width w that is substantially uniform along its length, and any specified thickness that varies from a first thickness $t1$ at one end to a second thickness $t2$ at the opposite end. Because at least one dimension of wedge-shaped electrode 2600 varies, its resistance *R* is non-linear along its length. More specifically, the resistance is greatest at the thin end of each wedge-shaped electrode 2600 and least at the thick end of each wedge-shaped electrode 2600.

Figure 27 illustrates a top and side view of a pyramid-shaped electrode 2700. Pyramid-shaped electrode 2700 is yet another example of an electrically resistive droplet operations electrode that may be formed of an electrically resistive material. In this example, pyramid-shaped electrode 2700 is pyramid-shaped with respect to its overall geometry from one end to the other. Pyramid-shaped electrode 2700 may have any specified length L, any specified width that varies from a first width $w1$ at one end to a second width $w2$ at the opposite end, and any specified thickness that varies from a first thickness $t1$ at one end to a second thickness $t2$ at the opposite end. Because at least one dimension of pyramid-shaped electrode 2700 varies, its resistance R is non-linear along its length. More specifically, the resistance is greatest at the narrow and thin end of each pyramid-shaped electrode 2700 and least at the wide and thick end of each pyramid-shaped electrode 2700.

Referring again to Figures 24, 25, 26, and 27, when triangle-shaped electrode 2500, wedge-shaped electrode 2600, and/or pyramid-shaped electrode 2700 are electrically connected between two voltages, such as between ground and a certain higher voltage, a non-linear voltage gradient develops along the length thereof because of the non-linear resistance. When activated during droplet operations, a droplet (not shown) tends to move from the lower voltage end thereof to the higher voltage end thereof. Additionally, because the voltage gradient is non-linear, the rate of droplet movement may be non-linear along the length thereof. In this way, the voltage gradient of triangle-shaped electrode 2500, wedge-shaped electrode 2600, and/or pyramid-shaped electrode 2700 may be used to assist droplet operations.

The voltage and/or capacitance gradients provided by, for example, rectangle-shaped electrode 2400, triangle-shaped electrode 2500, wedge-shaped electrode 2600, and/or pyramid-shaped electrode 2700 provide another level of control that is not otherwise present in conductive droplet operations electrodes. Further, triangle-shaped electrode 2500, wedge-shaped electrode 2600, and/or pyramid-shaped electrode 2700 are examples of modifying the electrode geometry in order to provide yet another level of control that is not otherwise present in electrodes that have uniform dimensions, such as rectangle-shaped electrode 2400 of Figure 24.

Further, still another level of control may be achieved via the electrical connections at each end of the non-linear resistance electrodes. For example, when the ground and voltage are connected one way with respect to the lateral geometry, the movement of the droplet may begin at a certain rate at the lower voltage end and decrease toward the higher voltage end. However, when the ground and voltage are connected the opposite way with respect to the lateral geometry, the movement of the droplet may begin at a certain rate at the lower voltage end and increase toward the higher voltage end.

Additionally, because the capacitance may vary as a function of area, triangle-shaped electrode 2500 (i.e., triangle-shaped footprint) may provide the additional attribute of a capacitance gradient along its length. Further, the geometries of rectangle-shaped electrode 2400, triangle-shaped electrode 2500, wedge-shaped electrode 2600, and/or pyramid-shaped electrode 2700 are not limited to resistive electrodes only. These geometries may be used to form conductive electrodes for substantially any purpose with respect to droplet operations. Further, these geometries may be combined with various dielectric layers when installed. Figures 28A through 32B below show non-limiting examples of incorporating electrodes, such as described in Figures 24 through 27, in droplet actuators for the purpose of developing certain voltage and/or capacitance gradients, which may be used to assist droplet operations.

Figures 28A and 28B illustrate top views of rectangle-shaped electrode 2400 of Figure 24 when in use during droplet operations. For example, Figure 28A shows a droplet 2810 initially at the lower voltage end of rectangle-shaped electrode 2400. Figure 28B shows that when rectangle-shaped electrode 2400 is activated, a substantially linear voltage gradient develops along the length thereof. As a result, droplet 2810 tends to automatically move at a substantially linear rate from the lower voltage end to the higher voltage end of rectangle-shaped electrode 2400.

Figures 29A through 29F illustrate top views of an electrode configuration 2900 and a process of using a voltage gradient to assist droplet operations. In this example, electrode configuration 2900 may include a path or line of one or more triangle-shaped electrodes 2500 that are described in Figure 25. For example, a triangle-shaped electrode 2500a, 2500b, and 2500c are arranged on a substrate (not shown) of a droplet actuator. Triangle-shaped electrodes 2500 (e.g., electrowetting electrodes) are configured to perform droplet operations. Triangle-shaped electrodes 2500 are arranged such that the narrow end of each triangle-shaped electrode 2500 is oriented toward the wide end of its neighboring triangle-shaped electrode 2500. In this example, the narrow end of each triangle-shaped electrode 2500 may be the lower voltage end. Therefore, the wide end of each triangle-shaped electrode 2500 may be the higher voltage end. Figures 29A through 29F also show a droplet 2910 to be transported along triangle-shaped electrodes 2500 via droplet operations.

Figure 29A shows a first step in a process of using a voltage gradient to assist droplet operations. In this step, droplet 2910 is at the lower voltage end of triangle-shaped electrode 2500a, triangle-shaped electrode 2500a is turned ON, triangle-shaped electrode 2500b is turned OFF, and triangle-shaped electrode 2500c is turned OFF.

Figure 29B shows the next step in the process of using a voltage gradient to assist droplet operations. In this step, because a voltage gradient develops along the length of triangle-shaped electrode 2500a when it is turned ON, droplet 2910 tends to automatically move from the lower voltage end of triangle-shaped electrode 2500a to the higher voltage end thereof.

Figure 29C shows the next step in the process of using a voltage gradient to assist droplet operations. In this step, triangle-shaped electrode 2500a is turned OFF, triangle-shaped electrode 2500b is turned ON, and triangle-shaped electrode 2500c is turned OFF. Because triangle-shaped electrode 2500a is turned OFF and triangle-shaped electrode 2500b is turned ON, droplet 2910 tends to automatically move from the higher voltage end of triangle-shaped electrode 2500a to the lower voltage end of triangle-shaped electrode 2500b.

Figure 29D shows the next step in the process of using a voltage gradient to assist droplet operations. In this step, because a voltage gradient develops along the length of triangle-shaped electrode 2500b when it is turned ON, droplet 2910 tends to automatically move from the lower voltage end of triangle-shaped electrode 2500b to the higher voltage end thereof.

Figure 29E shows the next step in the process of using a voltage gradient to assist droplet operations. In this step, triangle-shaped electrode 2500a is turned OFF, triangle-shaped electrode 2500b is turned OFF, and triangle-shaped electrode 2500c is turned ON. Because triangle-shaped electrode 2500b is turned OFF and triangle-shaped electrode 2500c is turned ON, droplet 2910 tends to automatically move from the higher voltage end of triangle-shaped electrode 2500b to the lower voltage end of triangle-shaped electrode 2500c.

Figure 29F shows the next step in the process of using a voltage gradient to assist droplet operations. In this step, because a voltage gradient develops along the length of triangle-shaped electrode 2500c when it is turned ON, droplet 2910 tends to automatically move from the lower voltage end of triangle-shaped electrode 2500c to the higher voltage end thereof. The steps of the process of transporting a droplet along the triangle-shaped electrodes may continue in like manner for any number of electrodes, while being assisted by the presence of a voltage gradient at each electrode.

Figures 30A through 30D illustrate side views of a portion of a droplet actuator 3000 and another process of using a voltage gradient to assist droplet operations. In this example, droplet actuator 3000 may use wedge-shaped electrodes 2600 that are described in Figure 26. Droplet actuator 3000 may include a bottom substrate 3010 that is separated from a top substrate 3012 by a gap 3014. Bottom substrate 3010 may include a path or line of wedge-shaped electrodes 2600 (e.g., electrowetting electrodes) that are configured to perform droplet operations. By way of example, droplet actuator 3000 may include wedge-shaped electrodes 2600a, 2600b, and 2600c. Wedge-shaped electrodes 2600 are arranged such that the thin end of each wedge-shaped electrode 2600 is oriented toward the thick end of its neighboring wedge-shaped electrode 2600. In this example, the narrow end of each wedge-shaped electrode 2600 may be the lower voltage end. Therefore, the wide end of each wedge-shaped electrode 2600 may be the higher voltage end. Additionally, the surface of each wedge-shaped electrode 2600 that is nearest gap 3014 is substantially parallel to the droplet operations surface along gap 3014. Therefore, the dielectric properties of the dielectric layer atop wedge-shaped electrode 2600 are substantially uniform along its length. Figures 30A through 30D also show a droplet 3016 to be transported along wedge-shaped electrodes 2600 via droplet operations.

Figure 30A shows a first step in a process of using a voltage gradient to assist droplet operations. In this step, droplet 3016 is at the lower voltage end of wedge-shaped electrode 2600a, wedge-shaped electrode 2600a is turned ON, wedge-shaped electrode 2600b is turned OFF, and wedge-shaped electrode 2600c is turned OFF.

Figure 30B shows the next step in the process of using a voltage gradient to assist droplet operations. In this step, because a voltage gradient develops along the length of wedge-shaped electrode 2600a when it is turned ON, droplet 3016 tends to automatically move from the lower voltage end of wedge-shaped electrode 2600a to the higher voltage end thereof.

Figure 30C shows the next step in the process of using a voltage gradient to assist droplet operations. In this step, wedge-shaped electrode 2600a is turned OFF, wedge-shaped electrode 2600b is turned ON, and wedge-shaped electrode 2600c is turned OFF. Because wedge-shaped electrode 2600a is turned OFF and wedge-shaped electrode 2600b is turned ON, droplet 3016 tends to automatically move from the higher voltage end of wedge-shaped electrode 2600a to the lower voltage end of wedge-shaped electrode 2600b. Subsequently, because a voltage gradient develops along the length of wedge-shaped electrode 2600b when it is turned ON, droplet 3016 tends to automatically move from the lower voltage end of wedge-shaped electrode 2600b to the higher voltage end thereof.

Figure 30D shows the next step in the process of using a voltage gradient to assist droplet operations. In this step, wedge-shaped electrode 2600a is turned OFF, wedge-shaped electrode 2600b is turned OFF, and wedge-shaped electrode 2600c is turned ON. Because wedge-shaped electrode 2600b is turned OFF and wedge-shaped electrode 2600c is turned ON, droplet 3016 tends to automatically move from the higher voltage end of wedge-shaped electrode 2600b to the lower voltage end of wedge-shaped electrode 2600c. Subsequently, because a voltage gradient develops along the length of wedge-shaped electrode 2600c when it is turned ON, droplet 3016 tends to automatically move from the lower voltage end of wedge-shaped electrode 2600c to the higher voltage end thereof.

Figure 31A illustrates a side view of a portion of a droplet actuator 3100 for using both a voltage gradient and capacitance gradient to assist droplet operations. In this example, droplet actuator 3100 may use wedge-shaped electrodes 2600 that are described in Figure 26. Droplet actuator 3100 may include a bottom substrate 3110 that is separated from a top substrate 3112 by a gap 3114. Bottom substrate 3110 may include a path or line of wedge-shaped electrodes 2600 (e.g., electrowetting electrodes) that are configured to perform droplet operations. By way of example, droplet actuator 3100 may include the wedge-shaped electrodes 2600a, 2600b, and 2600c that are described with reference to droplet actuator 700 of Figure 7. In this example, the wide end of each wedge-shaped electrode 2600 may be the lower voltage end. Therefore, the narrow end of each wedge-shaped electrode 2600 may be the higher voltage end. Figure 31A also shows a droplet 3118 to be transported along wedge-shaped electrodes 2600 via droplet operations.

In this embodiment, the surface of each wedge-shaped electrode 2600 that is nearest gap 3114 is *not* substantially parallel to the droplet operations surface along gap 3114. However, a dielectric layer 3120 that is atop wedge-shaped electrodes 2600 is held substantially planar. As a result, a dielectric thickness T of dielectric layer 3120 varies and, therefore, the dielectric properties of dielectric layer 3120 vary along the length of each wedge-shaped electrode 2600. This has the result of developing a capacitance gradient along the length of each wedge-shaped electrode 2600. In particular, the capacitance is greatest at the end of each wedge-shaped electrode 2600 having the smallest dielectric thickness. The capacitance is least at the end of each wedge-shaped electrode 2600 having the largest dielectric thickness. Additionally, when activated, a voltage gradient develops along the length of each wedge-shaped electrode 2600, as described in Figure 26. The effects of the capacitance gradient and voltage gradient are complimentary to one another with respect to assisting droplet operations.

The combination of the capacitance gradient and voltage gradient may be used to assist droplet operations along a line of wedge-shaped electrodes 2600 because it has the effect of automatically drawing the droplet from one end to the other. That is, because both the capacitance gradient and voltage gradient develop in a complimentary fashion along the length of each wedge-shaped electrode 2600 when turned ON, droplet 3118 tends to automatically move from the higher capacitance/lower voltage end of wedge-shaped electrode 2600 toward the lower capacitance/higher voltage end thereof.

Figure 31B illustrates another side view of droplet actuator 3100 of Figure 31A wherein the dielectric layer atop the wedge-shaped electrodes is modified. In this embodiment, again the surface of each wedge-shaped electrode 2600 that is nearest gap 3114 is *not* substantially parallel to the droplet operations surface along gap 3114. However, in this embodiment, the dielectric layer 3120 that is atop wedge-shaped electrodes 2600 is *not* held substantially planar. Instead, dielectric layer 3120 conforms to the topology of wedge-shaped electrodes 2600 while the dielectric thickness T is held substantially uniform. As a result, the dielectric properties of dielectric layer 3120 are held substantially uniform along the length of each wedge-shaped electrode 2600 and, thus, no capacitance gradient is developed. Therefore, in this embodiment, when activated, a voltage gradient only develops along the length of each wedge-shaped electrode 2600, as described in Figure 26. This voltage gradient may be used to assist droplet operations along the line of wedge-shaped electrodes 2600 because it has the effect of automatically drawing the droplet (not shown) from one end to the other.

Figures 32A and 32B illustrate side views of a portion of a droplet actuator 3200 for using capacitance gradient to assist droplet operations. Droplet actuator 3200 may include a bottom substrate 3210 that is separated from a top substrate 3212 by a gap 3214. Bottom substrate 3210 may include a path or line of droplet operations electrodes 3216 (e.g., electrowetting electrodes) that are configured to perform droplet operations.

In this embodiment, the width of each droplet operations electrode 3216 is held substantially uniform along its length. Likewise, the thickness of each droplet operations electrode 3216 is held substantially uniform along its length. Additionally, each droplet operations electrode 3216 may be formed of conductive material and connected to a control voltage in the standard manner.

Further, each droplet operations electrode 3216 is installed at an angle with respect to the droplet operations surface along gap 3214. As a result, the dielectric thickness *T* varies, which has the result of developing a capacitance gradient along the length of each droplet operations electrode 3216, as described in Figure 31A. In particular, the capacitance is greatest at the end of each droplet operations electrode 3216 having the smallest dielectric thickness. The capacitance is least at the end of each droplet operations electrode 3216 having the largest dielectric thickness. Therefore, the capacitance gradient may be used to assist droplet operations along a line of droplet operations electrode 3216 because it has the effect of automatically drawing the droplet from one end to the other.

Figure 32A shows an arrangement wherein the droplet operations electrodes 3216 have little to no overlap one to another. Figure 32B shows an alternative arrangement wherein the droplet operations electrodes 3216 overlap to some degree in order to assist the movement of droplets from one electrode to the next.

In another embodiment, droplet operations electrode 3216 of droplet actuator 3200 may be replaced with resistive electrodes, such as rectangle-shaped electrodes 2500 of Figure 25. In this embodiment, both a capacitance gradient and a voltage gradient are provided in order to assist droplet operations.

Figures 33A and 33B illustrate top views of a rectangle-shaped electrode 3300 when in use during droplet operations. Rectangle-shaped electrode 3300 may be substantially the same as rectangle-shaped electrode 2400 of Figure 24 except that multiple connection points 3310 are provided along resistance R. For example, connection points 3310a, 3310b, 3310c, 3310d, 3310e, and 3310f are provided along the length of resistance R. In this way, one voltage connection, such as the ground connection, may be moveable along resistance R instead of fixed. The result is that the voltage gradient is defined only between a certain connection point 3310 and the opposite end of rectangle-shaped electrode 3300. The position of the droplet, such as a droplet 3320, is confined within this defined voltage gradient. Therefore, this moveable connection provides the ability to selectably size rectangle-shaped electrode 3300. For example, Figure 33A shows the ground connected at connection point 3310b and droplet 3320 positioned accordingly within the defined voltage gradient, effectively establishing a certain electrode size.

By moving the ground to, for example, connection point 3310d, as shown in Figure 33B, droplet 3320 moves accordingly within the defined voltage gradient, effectively establishing another electrode size.

The implementation of the multiple connection points along the resistance R is not limited to a rectangle-shaped electrode. The implementation of the multiple connection points is applicable to any geometry of resistive electrodes, such as but not limited to, rectangle-shaped electrode 2400 of Figure 24, triangle-shaped electrode 2500 of Figure 25, wedge-shaped electrode 2600 of Figure 26, and/or pyramid-shaped electrode 2700 of Figure 27.

Figure 34 illustrates a top view of an electrode configuration 3400 that includes a resistance ladder structure for forming a voltage gradient, which may be used to assist droplet operations. In one example, electrode configuration 3400 may include a grid or array of droplet operations electrodes 3410. Droplet operations electrodes 3410 may be conductive electrodes that have impedance elements, such as resistors 3412, arranged therebetween. Alternatively, resistors 3412 may be replaced with capacitors or replaced with a combination of resistors and capacitors. In another embodiment, droplet operations electrodes 3410 may be resistive electrodes, such as those described with reference to Figures 24, 25, 26, and 27. In this case, electrode configuration 3400 may or may not include the impedance elements, such as resistors 3412, arranged between droplet operations electrodes 3410.

At least two voltage nodes, such as voltage nodes V1, V2, V3, and V4, may be provided in any locations within electrode configuration 3400. In this way, certain voltage potentials may be selectably provided in order to create a desired voltage gradient along electrode configuration 3400. In operation during droplet operations, droplets (not shown) tend to automatically move from the low voltage region of electrode configuration 3400 to the high voltage region of electrode configuration 3400. In one example, when V1 is ground, V2 is floating, V3 is floating, and V4 is a certain higher voltage, droplets move from the droplet operations electrodes 3410 near V1 toward the droplet operations electrodes 3410 near V4. In another example, when V1 and V2 are grounded and V3 and V4 are a certain higher voltage, droplets move from the droplet operations electrodes 3410 near V1 and V2 toward the droplet operations electrodes 3410 near V3 and V4.

Figure 35 illustrates a perspective view of a 3D electrode configuration 3500 that includes a resistance ladder structure for forming a voltage gradient, which may be used to assist droplet operations. 3D electrode configuration 3500 may be formed within a droplet actuator device by, for example, two or more layers of the electrode configuration 3400 of Figure 34. When droplet operations electrodes 3410 are conductive electrodes, the impedance elements, such as resistors 3412, are arranged therebetween in all directions. Alternatively, when droplet operations electrodes 3410 are resistive electrodes, such as those described with reference to Figures 24, 25, 26, and 27, 3D electrode configuration 3500 may or may not include the impedance elements, such as resistors 3412, arranged between droplet operations electrodes 3410.

Again, at least two voltage nodes, such as voltage nodes V1, V2, V3, and V4, may be provided in any locations within 3D electrode configuration 3500. In this way, certain voltage potentials may be selectably provided in order to create a desired 2D and/or 3D voltage gradient along 3D electrode configuration 3500.

Figure 36 illustrates a perspective view of a custom resistance droplet operations electrode 3600 for providing a customized voltage gradient, which may be used to assist droplet operations. In one embodiment, droplet operations electrode 3600 includes two or more resistance regions of varying resistivity. For example, droplet operations electrode 3600 may include a first resistance region 3612, a second resistance region 3614, and a third resistance region 3616. The resistivity of first resistance region 3612, second resistance region 3614, and third resistance region 3616 may differ one from another. The different resistance regions may be accomplished, for example, via known silicon doping techniques. The presence of the different resistance regions, such as first resistance region 3612, second resistance region 3614, and third resistance region 3616, allow a customized voltage gradient to be formed within droplet operations electrode 3600 when connected between at least two voltages. Two or more voltage nodes may be provided at any locations of droplet operations electrode 3600. During droplet operations, the customized voltage gradient may be used to assist droplet operations.

Figure 37 illustrates a top view of a droplet operations electrode 3700 for providing a certain voltage gradient, which may be used to assist droplet operations. Droplet operations electrode 3700 may be formed of resistive material, such as described with reference to Figures 24, 25, 26, and 27. In this embodiment, droplet operations electrode 3700 is formed of a wave pattern (e.g., square wave, sign wave, sawtooth pattern) of resistive material, wherein the spacing of the waves varies from one end to the other. For example, there is a certain spacing of the wave pattern at one end of droplet operations electrode 3700 and the spacing gradually increases toward the opposite end. In the region of the closest spacing the effective resistance is smallest. In the region of the farthest spacing the effective resistance is greatest. Therefore, when a voltage is applied at each end, such as ground and any suitably higher voltage, a non-linear voltage gradient is developed across droplet operations electrode 3700, which may be used to assist droplet operations. For example, Figure 37 shows a droplet 3710 moving from the lower voltage end to the higher voltage end of droplet operations electrode 3700. The spacing of the wave pattern of droplet operations electrode 3700 may be customized in any fashion in order to create any linear and/or non-linear voltage gradient when in use.

Figure 38 illustrates a top view of an electrode array 3800 of conductive and/or resistive electrodes for providing a voltage gradient of a certain predetermined profile, which may be used to assist droplet operations. For example, electrode array 3800 may include the combination of conductive droplet operations electrodes 3810 and resistive droplet operations electrodes 3812. The numbers and locations of conductive droplet operations electrodes 3810 and resistive droplet operations electrodes 3812 may be customized. Each droplet operations electrode 3810 and resistive droplet operations electrode 3812 is connected to its neighboring electrodes. Additionally, the degree of resistivity of the resistive droplet operations electrodes 3812 may be customized and may vary within electrode array 3800.

Electrode array 3800 provides a way to create a customized resistance profile over an area that may be optimized for a certain use. For example, the placement of conductive droplet operations electrodes 3810 and the placement and resistivity of resistive droplet operations electrodes 3812 in electrode array 3800 may be selected and optimized for a certain resistance profile that results in a certain voltage gradient profile across the overall array area.

Figure 39 illustrates a top view of a droplet operations electrode 3900 for providing a certain capacitance gradient, which may be used to assist droplet operations. Droplet operations electrode 3900 may be formed of conductive material. In this embodiment, droplet operations electrode 3900 is formed of a wave pattern (e.g., square wave, sign wave, sawtooth pattern) of conductive material, wherein the spacing of the waves varies from one end to the other. For example, there is a certain spacing of the wave pattern at one end of droplet operations electrode 3900 and the spacing gradually decreases toward the opposite end. In the region of the closest spacing the effective capacitance is greatest. In the region of the farthest spacing the effective capacitance is smallest. Therefore, a non-linear capacitance gradient is developed across droplet operations electrode 3900, which may be used to assist droplet operations. For example, when droplet operations electrode 3900 is activated during droplet operations there is uniform voltage potential throughout droplet operations electrode 3900. A droplet, such as a droplet 3910, tends to automatically move from the higher capacitance end to the lower capacitance end of droplet operations electrode 3900. The spacing of the wave pattern of droplet operations electrode 3900 may be customized in any fashion in order to create any linear and/or non-linear capacitance gradient when in use.

Figures 40A and 40B illustrate top views of embodiments of a droplet operations electrode 4000 for providing a certain capacitance gradient, which may be used to assist droplet operations. Droplet operations electrode 4000 may be formed of conductive material. In this embodiment, droplet operations electrode 4000 is formed of a line of conductive segments 4010. In one embodiment, Figure 40A shows that the width of the various segments 4010 varies from one end to the other of droplet operations electrode 4000. For example, there is a certain width of segments 4010 at one end of droplet operations electrode 4000 and the width gradually increases toward the opposite end. In the region of the smallest width the effective capacitance is greatest. In the region of the greatest width the effective capacitance is smallest. Therefore, a non-linear capacitance gradient is developed across droplet operations electrode 4000, which may be used to assist droplet operations. For example, when droplet operations electrode 4000 is activated during droplet operations there is uniform voltage potential throughout droplet operations electrode 4000. A droplet, such as a droplet 4012, tends to automatically move from the higher capacitance end to the lower capacitance end of droplet operations electrode 4000. The widths of segments 4010 of droplet operations electrode 4000 may be customized in any fashion in order to create any linear and/or non-linear capacitance gradient when in use.

In another embodiment, Figure 40B shows that the width of the various segments 4010 is held substantially uniform from one end to the other of droplet operations electrode 4000, while the spacing varies. For example, there is a certain spacing of segments 4010 at one end of droplet operations electrode 4000 and the spacing gradually increases toward the opposite end. In the region of the smallest spacing the effective capacitance is greatest. In the region of the greatest spacing the effective capacitance is smallest. Therefore, a non-linear capacitance gradient is developed across droplet operations electrode 4000, which may be used to assist droplet operations. For example, when droplet operations electrode 4000 is activated during droplet operations there is uniform voltage potential throughout droplet operations electrode 4000. A droplet, such as a droplet 4012, tends to automatically move from the higher capacitance end to the lower capacitance end of droplet operations electrode 4000. The spacing of segments 4010 of droplet operations electrode 4000 may be customized in any fashion in order to create any linear and/or non-linear capacitance gradient when in use.

Referring again to Figures 24 through 40, the invention is not limited to the electrode structures and/or configurations shown therein. Any combination of electrode structures and/or configurations is possible for assisting droplet operations. For example, any combination of resistive electrodes, conductive electrodes, electrodes having various footprints, electrodes having various cross-sectional geometries, and/or electrodes installed in combination with various dielectric layers is possible.

Figure 41 illustrates a perspective view of an example of a droplet operations electrode 4100 that when activated has a voltage gradient due to doping, which may be used to assist droplet operations. For example, silicon doping techniques may be used to form droplet operations electrode 4100. In this example, when activated, the voltage gradient across a homogeneous electrode is due to the doping process rather than due to a change in geometry, such as described, for example, with reference to Figure 24 through 31B. The doping process allows the resistivity of droplet operations electrode 4100 to vary over a certain area thereof. As a result, when activated, the voltage distribution varies in a corresponding way across the area thereof.

In operation, a voltage may be applied at different points, such as at different points 4110, along droplet operations electrode 4100. Any droplet (not shown) that is present atop droplet operations electrode 4100 will tend to move along the gradient in the direction from lowest voltage to highest voltage. Applying at least two voltages at certain points 4110 allows a customized voltage gradient to be formed within droplet operations electrode 4100. During droplet operations, the customized voltage gradient may be used to assist droplet operations.

7.2.2 Variable Dielectric Constants for Assisting Droplet Operations

Figures 42A through 42D illustrate top views of an example of an electrode configuration 4200 and a process of dispensing by use of different dielectric constants. Electrode configuration 4200 may include, for example, a reservoir electrode 4210 and a dispensing electrode 4212. There is a layer of dielectric material (not shown) atop electrode configuration 4200 in which different regions thereof have different dielectric constants (k). The dielectric constant (k) of a material is the ratio of the flux density produced by an electric field in a given dielectric to the flux density produced by that field in a vacuum.

In one example, reservoir electrode 4210 is associated with a fluid reservoir and has two regions having two different dielectric constants. For example, reservoir electrode 4210 has a high-k region 4214 that has a certain dielectric constant. Reservoir electrode 4210 also has a low-k region 4216 that has a dielectric constant that is lower than that of high-k region 4214. High-k region 4214 corresponds to an area of reservoir electrode 4210 for holding a large volume of fluid 4218. By contrast, low-k region 4216 is a smaller localized area of reservoir electrode 4210 that is near dispensing electrode 4212. Additionally, dispensing electrode 4212 is a high-k region. In another embodiment, reservoir electrode 4210 and dispensing electrode 4212 of electrode configuration 4200 may be a single electrode that has a high-k region and a low-k region in the fluid reservoir and another high-k region for dispensing.

With respect to droplet operations, the higher the dielectric constant at a certain electrode, the lower the required electrowetting voltage for performing droplet operations. Conversely, the lower the dielectric constant at a certain electrode, the higher the required electrowetting voltage for performing droplet operations. Therefore, with respect to electrode configuration 4200 a higher voltage is required to "wet" low-k region 4216 of reservoir electrode 4210 than is required to wet both high-k region 4214 and dispensing electrode 4212 (a high-k region). In operation, a certain voltage may be applied that causes high-k region 4214 of reservoir electrode 4210 and dispensing electrode 4212 to be wetted, yet low-k region 4216 of reservoir electrode 4210 is not wetted. Additionally, a certain higher voltage may be applied that causes both high-k region 4214 and low-k region 4216 of reservoir electrode 4210, as well as dispensing electrode 4212 to be wetted (i.e., substantially all of electrode configuration 4200 is wetted). Taking advantage of these dielectric properties in a droplet actuator, a process of dispensing by use of different dielectric constants may include, but is not limited to, the following steps.

Referring to Figure 42B, a certain amount of fluid 4218 is initially held at high-k region 4214 of reservoir electrode 4210 by applying a certain voltage, V-low. V-low is applied in common to high-k region 4214 and low-k region 4216 of reservoir electrode 4210 and dispensing electrode 4212. V-low is suitably high to hold fluid 4218 at high-k region 4214, but suitably low that low-k region 4216 is not wetted with fluid 4218.

Referring to Figure 42C, a certain higher voltage, V-high, is applied in common to high-k region 4214 and low-k region 4216 of reservoir electrode 4210 and dispensing electrode 4212. V-high is suitably high to cause low-k region 4216 of reservoir electrode 4210 to be wetted. Therefore, fluid 4218 flows from high-k region 4214 of reservoir electrode 4210 and onto low-k region 4216 of reservoir electrode 4210 and then onto dispensing electrode 4212. In this way a finger of fluid 4218 is pulled out of the fluid reservoir and onto dispensing electrode 4212.

Referring to Figure 42D, V-low is again applied in common to high-k region 4214 and low-k region 4216 of reservoir electrode 4210 and dispensing electrode 4212. V-low is suitably low to cause low-k region 4216 of reservoir electrode 4210 to "dewet," while high-k region 4214 of reservoir electrode 4210 and dispensing electrode 4212 remain wetted. As a result of fluid 4218 pulling away from low-k region 4216 of reservoir electrode 4210 and retracting toward high-k region 4214 of reservoir electrode 4210 and dispensing electrode 4212, a droplet 4220 is pinched off and left behind at dispensing electrode 4212.

Figures 43A through 43D illustrate side views of a portion of a droplet actuator 4300 and a process of dispensing a droplet by use of a dielectric layer that has regions of different thicknesses. Droplet actuator 4300 may include a bottom substrate 4310 that is separated from a top substrate 4312 by a gap 4314. Bottom substrate 4310 may include a droplet operations electrode 4316. Additionally, droplet operations electrode 4316 may include a reservoir region 4318 and a dispensing region 4320. In another embodiment, droplet operations electrode 4316 may be formed of two separate electrodes, i.e., a reservoir electrode and a dispensing electrode.

Atop droplet operations electrode 4316 is a dielectric layer 4322. Generally, dielectric layer 4322 has a certain thickness *t1*. However, a cavity is formed in a portion of dispensing region 4320 of droplet operations electrode 4316. This cavity is void of electrode material and is filled with dielectric material of dielectric layer 4322 to a thickness *t2*, which is greater than the thickness *t1*. In one example, thickness *t2* is about two times thickness *t1*. In effect, the dielectric constant along dielectric layer 4322 is being adjusted by varying the thickness thereof. This is because the dielectric constant is inversely proportional to the thickness of the material. More specifically, with respect to droplet actuator 4300, the portion of dispensing region 4320 of droplet operations electrode 4316 at which dielectric layer 4322 has a thickness *t1* may be considered a high-k region 4326. Further, the portion of dispensing region 4320 of droplet operations electrode 4316 at which dielectric layer 4322 has a thickness *t2* may be considered a low-k region 4324. Additionally, the entirety of reservoir region 4318 may be considered a high-k region.

The usefulness of high-k regions and low-k regions for dispensing droplets is described with reference to Figures 19A through 19D. Similarly, which respect to droplet actuator 4300, a process of dispensing by use of different dielectric constants may include, but is not limited to, the following steps.

Referring to Figure 43A, a certain amount of fluid 4330 is initially held at reservoir region 4318 of droplet operations electrode 4316 by applying a certain voltage, V-low. V-low is applied in common to reservoir region 4318 and dispensing region 4320 of droplet operations electrode 4316. V-low is suitably high to hold fluid 4330 at reservoir region 4318, which is a high-k region, but suitably low that low-k region 4324 of dispensing region 4320 is not wetted with fluid 4330.

Referring to Figure 43B, a certain higher voltage, V-high, is applied in common to reservoir region 4318 and dispensing region 4320 of droplet operations electrode 4316. V-high is suitably high to cause low-k region 4324 of dispensing region 4320 to be wetted. Therefore, fluid 4330 flows from reservoir region 4318 of droplet operations electrode 4316 and onto low-k region 4324 of dispensing region 4320 and then onto high-k region 4326 of dispensing region 4320. In this way a finger of fluid 4330 is pulled out of reservoir region 4318 of droplet operations electrode 4316 and along the length of dispensing region 4320.

Referring to Figures 43C and 43D, V-low is again applied in common to reservoir region 4318 and dispensing region 4320 of droplet operations electrode 4316. V-low is suitably low to cause low-k region 4324 of dispensing region 4320 to "dewet," while high-k region 4326 of dispensing region 4320 and reservoir region 4318, which is also a high-k region, remain wetted. As a result of fluid 4330 pulling away from low-k region 4324 of dispensing region 4320 and retracting toward reservoir region 4318 and high-k region 4326 of dispensing region 4320, a droplet 4332 is pinched off and left behind at high-k region 4326 of dispensing region 4320.

Figures 44A and 44B illustrate a side view and top view, respectively, of a portion of a droplet actuator 4400 that may be used to dispense multiple droplets by use of a dielectric layer that has regions of different thicknesses. Droplet actuator 4400 may include a bottom substrate 4410 that is separated from a top substrate 4412 by a gap 4414. Bottom substrate 4410 may include a reservoir electrode 4416 and a droplet operations electrode 4418. In another embodiment, the combination of reservoir electrode 4416 and droplet operations electrode 4418 may be formed by a single electrode.

Atop reservoir electrode 4416 and droplet operations electrode 4418 is a dielectric layer 4420. Generally, dielectric layer 4420 has a certain thickness *t1*. However, similar to droplet operations electrode 4316 of Figures 43A through 43D, one or more cavities are formed in droplet operations electrode 4418. Again, these cavities are void of electrode material and are filled with dielectric material of dielectric layer 4420 to a thickness *t2*, which is greater than the thickness *t1*. In one example, thickness *t2* is about two times thickness *t1*. Again, the dielectric constant along dielectric layer 4420 is being adjusted by varying the thickness thereof. More specifically, with respect to droplet actuator 4400, the portions of droplet operations electrode 4418 at which dielectric layer 4420 has a thickness *t1* may be considered high-k regions 4422. Further, the portion of droplet operations electrode 4418 at which dielectric layer 4420 has a thickness *t2* may be considered low-k regions 4424. Additionally, the entirety of reservoir electrode 4416 may be considered a high-k region.

Droplet actuator 4400 operates substantially the same as droplet actuator 4300 of Figures 43A through 43D with respect to sequencing, for example, V-low and V-high to dispense droplets. However, droplet actuator 4400 provides the additional capability to dispense multiple droplets, such as a droplet at each high-k region 4422 of droplet operations electrode 4418.

While Figure 44B shows that droplet operations electrode 4418 of droplet actuator 4400 includes an array of high-k regions 4422 onto which droplets may be dispensed, alternatively, droplet operations electrode 4418 may include only a single line (or path) of high-k regions 4422.

Figures 45A through 45D illustrate side views of a portion of a droplet actuator 4500 and a process of dispensing a droplet by use of a two-piece dielectric layer that provides regions of different dielectric constants. Droplet actuator 4500 may include a bottom substrate 4510 that is separated from a top substrate 4512 by a gap 4514. Bottom substrate 4510 may include a droplet operations electrode 4516. Additionally, droplet operations electrode 4516 may include a reservoir region 4518 and a dispensing region 4520. In another embodiment, droplet operations electrode 4516 may be formed of two separate electrodes, i.e., a reservoir electrode and a dispensing electrode. Atop droplet operations electrode 4516 is a high-k dielectric layer 4522. However, a low-k dielectric segment 4524 is integrated into high-k dielectric layer 4522 at a position that aligns with a portion of dispensing region 4520 of droplet operations electrode 4516.

The usefulness of high-k regions and low-k regions for dispensing droplets is described with reference to Figures 42A through 42D. Similarly, which respect to droplet actuator 4500, a process of dispensing by use of different dielectric constants may include, but is not limited to, the following steps.

Referring to Figure 45A, a certain amount of fluid 4530 is initially held at reservoir region 4518 of droplet operations electrode 4516 by applying a certain voltage, V-low. V-low is applied in common to reservoir region 4518 and dispensing region 4520 of droplet operations electrode 4516. V-low is suitably high to hold fluid 4530 at reservoir region 4518, which is a high-k region, but suitably low that the portion of dispensing region 4520 at low-k dielectric segment 4524 is not wetted with fluid 4530.

Referring to Figure 45B, a certain higher voltage, V-high, is applied in common to reservoir region 4518 and dispensing region 4520 of droplet operations electrode 4516. V-high is suitably high to cause the portion of dispensing region 4520 at low-k dielectric segment 4524 to be wetted. Therefore, fluid 4530 flows from reservoir region 4518 of droplet operations electrode 4516 and onto the portion of dispensing region 4520 at low-k dielectric segment 4524 and then onto the remaining high-k portion of dispensing region 4520. In this way a finger of fluid 4530 is pulled out of reservoir region 4518 of droplet operations electrode 4516 and along the length of dispensing region 4520.

Referring to Figures 45C and 45D, V-low is again applied in common to reservoir region 4518 and dispensing region 4520 of droplet operations electrode 4516. V-low is suitably low to cause the portion of dispensing region 4520 at low-k dielectric segment 4524 to “dewet,” while the remaining high-k portion of dispensing region 4520 and reservoir region 4518, which is also a high-k region, remain wetted. As a result of fluid 4530 pulling away from low-k dielectric segment 4524 and retracting onto reservoir region 4518 and the high-k portion of dispensing region 4520, a droplet 4532 is pinched off and left behind at the high-k portion of dispensing region 4520.

Referring again to Figure 45, droplet actuator 4500 operates substantially the same as droplet actuator 4300 of Figures 43A through 43D, except that in droplet actuator 4500, the electrode is uniform and the dielectric constant is varied. While in droplet actuator 2000 of Figure 20, the dielectric constant is uniform and the electrode geometry is varied. Additionally, the combination of droplet actuator 4300 of Figures 43A through 43D and droplet actuator 4500 of Figures 45A through 45D may be used. Further, the multi-droplet dispensing that is described with reference to droplet actuator 4400 of Figures 44A and 44B may be achieved using the approach of Figure 45.

Figure 46 illustrates a side view of a portion of a droplet actuator 4600 that includes a two-piece dielectric layer that provides a dielectric constant gradient for assisting droplet operations. Droplet actuator 4600 may include a bottom substrate 4610 that is separated from a top substrate 4612 by a gap 4614. Bottom substrate 4610 may include a droplet operations electrode 4616. Atop droplet operations electrode 4616 is a dielectric layer 4618. Additionally, multiple dielectric segments 4620 are integrated into dielectric layer 4618 at various positions along the length of droplet operations electrode 4616. In one example, droplet actuator 4600 includes dielectric segments 4620A through 4620I that are distributed along the length of droplet operations electrode 4616. Further, the surface area of the different dielectric segments 4620 may vary. For example, dielectric segment 4620A may have a certain surface area and then the surface areas of subsequent dielectric segments 4620B through 4620I increase gradually. In other words, dielectric segment 4620A has the smallest surface area, while dielectric segment 4620I has the largest surface area.

Additionally, dielectric layer 4618 may have a certain dielectric constant $k1$, while the multiple dielectric segments 4620 have a certain dielectric constant $k2$. Dielectric constant $k1$ may be equal to, greater than, or less than $k2$, depending on the intended use of droplet actuator 4600.

The combination of the different dielectric constants $k1$ and $k2$ and the different sized dielectric segments 4620 provide a droplet actuator that has a variable effective dielectric constant along the length of the two-piece dielectric, i.e., along the length of droplet operations electrode 4616. The characteristics of this variable effective dielectric constant may be used for assisting droplet operations along the length of droplet operations electrode 4616.

In one example, when dielectric constant $k2$ of dielectric segments 4620 is less than dielectric constant $k1$ of dielectric layer 4618, the effective dielectric constant decreases along the length of droplet operations electrode 4616 from dielectric segment 4620A toward dielectric segment 4620I. That is, the effective dielectric constant is greatest at dielectric segment 4620A and least at dielectric segment 4620I.

In another example, when dielectric constant $k2$ of dielectric segments 4620 is greater than dielectric constant $k1$ of dielectric layer 4618, the effective dielectric constant increases along the length of droplet operations electrode 4616 from dielectric segment 4620A toward dielectric segment 4620I. That is, the effective dielectric constant is least at dielectric segment 4620A and greatest at dielectric segment 4620I.

Figures 47A, 47B, and 47C illustrate top views of an example of an electrode configuration 4700 and a process of dispensing by use of different dielectric constants in combination with different pressure regions. Electrode configuration 4700 may include, for example, a reservoir electrode 4710, a dispensing electrode 4712, and a droplet operations electrode 4714. There is a layer of dielectric material (not shown) atop electrode configuration 4700 in which different regions thereof have different dielectric constants (k). In one example, reservoir electrode 4710 may be a high-k region, dispensing electrode 4712 may be a low-k region, and droplet operations electrode 4714 may be a high-k region. In this respect, electrode configuration 4700 operates substantially the same as electrode configuration 1900 of Figures 19A through 19D that takes advantage of the presence of high-k regions and low-k regions for dispensing droplets.

Additionally, electrode configuration 4700 is bounded by gasket material 4716. The geometry of gasket material 4716 defines certain low pressure and high pressure regions along the path of droplet operations. For example, the geometry of gasket material 4716 defines a channel that is widest at reservoir electrode 4710 and droplet operations electrode 4714 and is narrowest at dispensing electrode 4712, as shown in Figures 47A, 47B, and 47C. As a result, the vicinity of reservoir electrode 4710 and droplet operations electrode 4714 are low pressure regions, while the vicinity of dispensing electrode 4712 is a high pressure region. When there is a gradient in the width of the fluid channel, the resulting pressure differences automatically draw the fluid toward the wider portions of the channel.

The usefulness of high-k regions and low-k regions for dispensing droplets is described with reference to Figures 42A through 42D. Similarly, which respect to electrode configuration 4700, a process of dispensing by use of different dielectric constants, but in combination with different pressure regions may include, but is not limited to, the following steps.

Referring to Figure 47A, a certain amount of fluid 4718 is initially held at reservoir electrode 4710 by applying a certain voltage, V-low. V-low is applied in common to reservoir electrode 4710, dispensing electrode 4712, and droplet operations electrode 4714. V-low is suitably high to hold fluid 4718 at reservoir electrode 4710 (a high-k region), but suitably low that dispensing electrode 4712 (a low-k region), is not wetted with fluid 4718. Additionally, fluid 4718 tends to stay at reservoir electrode 4710 due to capillary action because reservoir electrode 4710 is a low pressure region.

Referring to Figure 47B, a certain higher voltage, V-high, is applied in common to reservoir electrode 4710, dispensing electrode 4712, and droplet operations electrode 4714. V-high is suitably high to cause dispensing electrode 4712 (a low-k region) to be wetted. Therefore, fluid 4718 flows from reservoir electrode 4710 (a high-k region) and onto dispensing electrode 4712 (a low-k region) and then onto droplet operations electrode 4714 (a low-k region). Additionally, dispensing electrode 4712 is used to pull fluid 4718 through this high pressure region. Once fluid 4718 passes by dispensing electrode 4712, fluid 4718 tends to flow toward droplet operations electrode 4714 due to capillary action because droplet operations electrode 4714 is a low pressure region. In this way a finger of fluid 4718 is pulled from reservoir electrode 4710 and onto droplet operations electrode 4714.

Referring to Figure 47C, V-low is again applied in common to reservoir electrode 4710, dispensing electrode 4712, and droplet operations electrode 4714. V-low is suitably low to cause dispensing electrode 4712 (a low-k region) to "dewet," while reservoir electrode 4710 (a high-k region) and droplet operations electrode 4714 (a high-k region) remain wetted. Assisting the dispensing action, fluid 4718 tends to flow toward reservoir electrode 4710 and droplet operations electrode 4714 due to capillary action because these are low pressure regions. As a result of fluid 4718 flowing away from dispensing electrode 4712 and to the low pressure regions at reservoir electrode 4710 and droplet operations electrode 4714, a droplet 4720 is pinched off and left behind at droplet operations electrode 4714.

7.2.3 Variable Gap Heights for Assisting Droplet Operations

Figures 48A, 48B, and 48C illustrate side views of a portion of a droplet actuator 4800 and a process of dispensing and/or splitting droplets by use of different pressure regions created by varying the gap height. Droplet actuator 4800 may include a bottom substrate 4810 that is separated from a top substrate 4812 by a gap 4814. Bottom substrate 4810 may include a reservoir electrode 4816, a dispensing electrode 4818, and a droplet operations electrode 4820. Additionally, opposing hump features 4822 are formed on the respective surfaces of bottom substrate 4810 and top substrate 4812 that are facing gap 4814. While gap 4814 has a certain height, a narrow channel 4824 is formed between hump features 4822 that is less than the height of gap 4814. Further, the hump features 4822 and, thus, narrow channel 4824 is located at dispensing electrode 4818.

Because there is a gradient in the height of gap 4814 due the presence of narrow channel 4824, different pressure regions are formed. For example, any portion of gap 4814 on either side of narrow channel 4824 is low pressure because the gap is largest. By contrast, the gap at narrow channel 4824 is high pressure because the gap is smallest. Capillary action due to pressure differences tends to automatically draw fluid toward the larger portions of gap 4814 and away from narrow channel 4824. This characteristic may be used to assist dispensing and/or splitting droplets. For example, a process of dispensing and/or splitting droplets by use of different pressure regions may include, but is not limited to, the following steps.

Referring to Figure 48A, reservoir electrode 4816 is activated and a certain amount of fluid 4830 is held at reservoir electrode 4816. Dispensing electrode 4818 and droplet operations electrode 4820 are deactivated. Additionally, fluid 4830 tends to stay at reservoir electrode 4816 due to capillary action because reservoir electrode 4816 is a low pressure region.

Referring to Figure 48B, reservoir electrode 4816, dispensing electrode 4818, and droplet operations electrode 4820 are activated. In this step, dispensing electrode 4818 is used to pull fluid 4830 through narrow channel 4824, which is a high pressure region. Once fluid 4830 passes through narrow channel 4824, fluid 4830 tends to flow toward droplet operations electrode 4820 due to capillary action because droplet operations electrode 4820 is a low pressure region. In this way a finger of fluid 4830 is pulled from reservoir electrode 4816 and onto droplet operations electrode 4820.

Referring to Figure 48C, dispensing electrode 4818 is deactivated, while reservoir electrode 4816 and droplet operations electrode 4820 remain activated. Because dispensing electrode 4818 is turned off, fluid 4830 tends to flow toward reservoir electrode 4816 and droplet operations electrode 4820 due to capillary action because these are low pressure regions. As a result of fluid 4830 flowing away from narrow channel 4824 and to the low pressure regions at reservoir electrode 4816 and droplet operations electrode 4820, a droplet 4832 is pinched off and left behind at droplet operations electrode 4820.

In other embodiments, droplet actuator 4800 does not include an electrode on either side of narrow channel 4824. Only dispensing electrode 4818 at narrow channel 4824 is present. In this embodiment, a droplet tends to stay in the larger portions of gap 4814 due to the lower pressure. However, the droplet may be pulled into and held at narrow channel 4824 (a higher pressure region) by activating dispensing electrode 4818. Then by deactivating dispensing electrode 4818, the droplet automatically returns to the larger portions of gap 4814. In summary, there are two states: (1) active electrode moves droplet to smaller gap and (2) inactive electrode, whereby capillary action moves the droplet to larger gap. Another example of using a variable gap height is described with reference to Figures 49A through 49D.

In yet other embodiments, droplet actuator 4800 may use different pressure regions created, for example, by hump features 4822 to form narrow channel 4824 in combination with different dielectric constant regions, as described with reference to Figures 42A through 47C.

Figures 49A, 49B, and 49C illustrate side views of a portion of a droplet actuator 4900 and a process of transporting droplets by use of pressure gradients created by gradients in gap height. Droplet actuator 4900 may include a bottom substrate 4910 that is separated from a top substrate 4912 by a gap 4914. The topology of, for example, the surface of bottom substrate 4910 that is facing gap 4914 is such that the height of gap 4914 varies between heights $h1$ and $h2$ in a repeating fashion, where height $h1$ is a certain height and height $h2$ is greater than height $h1$. In other words, the height of gap 4914 is smallest at $h1$ and greatest at $h2$. While Figures 49A, 49B, and 49C show that the topography is established by the bottom substrate, alternatively, the topography can be established by the top substrate or by both substrates.

Because of the gradient in gap height, which is repeating, a repeating pressure gradient is created. That is, the pressure in gap 4914 is highest at $h1$ and lowest at $h2$. This is because the pressure is inversely proportional to the height. As a result, there is a pressure transition from low pressure to high pressure at the transition from h2 and h1 in the repeating pattern in the topology of bottom substrate 4910. A physical step is present in the topology of bottom substrate 4910 at each low-to-high pressure interface.

In operation, due to capillary action, a droplet automatically tends to move toward the low pressure region of gap 4914. Therefore, in order to assist a droplet to move across the low-to-high pressure interface, droplet actuator 4900 includes a droplet operations electrode 4916 that is positioned where the height of gap 4914 is smallest at $h1$.

In the process of transporting a droplet, such as a droplet 4920, Figures 49A and 49B show droplet 4920 initially at the high pressure portion of gap 4914 (at *h1*) and automatically moving by capillary action toward the low pressure portion of gap 4914 (at *h2*). In Figures 49A and 49B, droplet operations electrode 4916 is not activated. Figure 49B shows droplet 4920 stopped at the *h2* portion of gap 4914 and at rest against the step in the topology of bottom substrate 4910. Referring to Figure 49C, droplet operations electrode 4916 is then activated in order to assist droplet 4920 to move across the low-to-high pressure interface (i.e., up and over the step in the topology of bottom substrate 4910 from the *h2* portion to the *h1* portion of gap 4914). Droplet operations electrode 4916 is used to overcome the high pressure at the *h1* portion of gap 4914 in order to pull droplet 4920 into this high pressure region via electrowetting forces. Referring to Figure 49D, droplet operations electrode 4916 is then deactivated, which allows capillary forces to take over and droplet 4920 automatically moves toward the next low pressure portion of gap 4914 (at *h2*). In summary, there are two states: (1) active electrode moves droplet to smaller gap and (2) inactive electrode, whereby capillary action moves the droplet to larger gap.

7.2.4 Droplet Actuators that are Easy and Inexpensive to Manufacture

Figure 50 illustrates a side view of a portion of a droplet actuator 5000, which is an example of a droplet actuator that includes noncomplex features that are easy to manufacture. Droplet actuator 5000 may include a bottom substrate 5010 that is separated from a top substrate 5012 by a gap 5014. Bottom substrate 5010 may include a droplet operations electrode 5016 that is installed along a PLANE A of droplet actuator 5000. Bottom substrate 5010 may also include droplet operations electrodes 5018 and 5020 that are installed along a PLANE B of droplet actuator 5000. PLANE B is slightly closer to gap 5014 than is PLANE A and, thus, droplet operations electrodes 5018 and 5020 are slightly closer to gap 5014 than is droplet operations electrode 5016. Additionally, there is a certain space between droplet operations electrodes 5018 and 5020 at PLANE B. Droplet operations electrode 5016 at PLANE A is positioned about midway of droplet operations electrodes 5018 and 5020.

Atop droplet operations electrodes 5018 and 5020 at PLANE B and droplet operations electrode 5016 at PLANE A is a dielectric layer 5022. The thickness of dielectric layer 5022 is held substantially uniform even though droplet operations electrodes 5018 and 5020 and droplet operations electrode 5016 are at different planes. Therefore, dielectric layer 5022 substantially follows the topology of the arrangement of droplet operations electrodes 5018 and 5020 at PLANE B and droplet operations electrode 5016 at PLANE A. As a result, a small dimple or cavity 5024 is formed in dielectric layer 5022 between droplet operations electrodes 5018 and 5020 and at about the position of droplet operations electrode 5016. At cavity 5024 the height of gap 5014 is slightly increased. As a result, there is a slight reduction in pressure at cavity 5024 as compared with the pressure outside of cavity 5024.

Additionally, because the thickness of dielectric layer 5022 is held substantially uniform, its dielectric constant (k) is substantially the same at each electrode. However, in other embodiments, the surface of dielectric layer 5022 may be held planar across the arrangement of droplet operations electrodes 5018 and 5020 at PLANE B and droplet operations electrode 5016 at PLANE A. In this case, dielectric layer 5022 is thicker at droplet operations electrode 5016 than at droplet operations electrodes 5018 and 5020. Consequently, the dielectric constant (k) of dielectric layer 5022 may be different at droplet operations electrode 5016 than at droplet operations electrodes 5018 and 5020.

In droplet actuator 5000, the spacing between droplet operations electrodes 5018 and 5020 at PLANE B is suitably large that a droplet (not shown) may not move across this distance using droplet operations of droplet operations electrodes 5018 and 5020 only. Rather, droplet operations electrode 5016 at PLANE A may be activated in order to assist the movement of a droplet across the distance between droplet operations electrodes 5018 and 5020. Further, the slightly reduced pressure at cavity 5024 provides additional assistance to moving a droplet between droplet operations electrodes 5018 and 5020, as a droplet tends to automatically move to the lowest pressure by capillary action.

Figure 51 illustrates a side view of a portion of a droplet actuator 5100, which is another example of a droplet actuator that includes noncomplex features that are easy to manufacture. Droplet actuator 5100 is substantially the same as droplet actuator 5000 of Figure 50 except that the planes at which droplet operations electrode 5016 and droplet operations electrodes 5018 and 5020 are installed is reversed. For example, in droplet actuator 5100, droplet operations electrode 5016 is installed at PLANE B instead of PLANE A and droplet operations electrodes 5018 and 5020 are installed at PLANE A instead of PLANE B.

Again, the thickness of dielectric layer 5022 is held substantially uniform atop the arrangement of droplet operations electrode 5016 and droplet operations electrodes 5018 and 5020 and, thus, the dielectric constant (k) is substantially the same at each electrode. Because of the reverse electrode arrangement, cavity 5024 is not present in droplet actuator 5100. Instead, this arrangement results in two different gap heights, a gap height *h1* at droplet operations electrodes 5018 and 5020 and a gap height *h2* at droplet operations electrode 5016. Gap height *h2* is smaller than gap height *h1* and, thus, the pressure in gap 5014 at droplet operations electrode 5016 is higher than the pressure at droplet operations electrodes 5018 and 5020.

Like droplet actuator 5000 of Figure 50, the spacing between droplet operations electrodes 5018 and 5020 at PLANE A is suitably large that a droplet (not shown) may not move across this distance using droplet operations of droplet operations electrodes 5018 and 5020 only. Rather, droplet operations electrode 5016 at PLANE B may be activated in order to assist the movement of a droplet across the distance between droplet operations electrodes 5018 and 5020.

Figure 52 illustrates a side view of a portion of a droplet actuator 5200, which is yet another example of a droplet actuator that includes noncomplex features that are easy to manufacture. Droplet actuator 5200 is substantially the same as droplet actuator 5100 of Figure 51 except that it includes two dielectric layers instead of one. For example, droplet actuator 5200 includes a first dielectric layer 5210 atop droplet operations electrodes 5018 and 5020 that are installed at PLANE A. Additionally, droplet actuator 5200 includes a second dielectric layer 5212 atop droplet operations electrode 5016 that is installed at PLANE B.

In this electrode arrangement, there is a double thickness of dielectric material atop droplet operations electrodes 5018 and 5020 and only a single thickness of dielectric material atop droplet operations electrode 5016. This results in low-k regions at droplet operations electrodes 5018 and 5020 and a high-k region at droplet operations electrode 5016. Therefore, including two layers of dielectric provides another way of achieving a variable dielectric constant in a droplet actuator.

Referring again to Figures 50, 51, and 52, an advantage of the electrode arrangements of droplet actuators 5000, 5100, and 5200, respectively, is that the substrates thereof have large simple features that are spaced far apart. This allows the manufacturing process to be easy and inexpensive compared with devices that have, for example, small, complex, and/or closely spaced features.

7.2.5 Droplet Operations Control Bus Configuration

Figure 53 illustrates a top view of an electrode configuration 5300 for providing an efficient single layer multi-phase bus configuration. Electrode configuration 5300 includes a spiral like bus configuration for minimizing the number of control lined for controlling multiple droplet operations electrodes. In one example, electrode configuration 5300 may include a first terminal 5310A that drives a first control line 5312A, a second terminal 5310B that drives a second control line 5312B, and a third terminal 5310C that drives a third control line 5312C. Control lines 5312A, 5310B, and 5312C are arranged in a spiral like pattern, where each control line may connect to multiple droplet operations electrodes. For example, terminal 5310A and control line 5312A may be electrically connected to droplet operations electrodes 5320A, 5320B, 5320C, 5320D, and 5320E. Terminal 5310B and control line 5312B may be electrically connected to droplet operations electrodes 5322A, 5322B, 5322C, and 5322D. Terminal 5310C and control line 5312C may be electrically connected to droplet operations electrodes 5324A, 5324B, 5324C, and 5324D. These electrode operations electrodes may be arranged an alternating fashion along a path or line within the spiral like control lines. This allows droplet operations to occur along multiple droplet operations electrodes using a minimal number of control lines. In this example, three control lines only are needed to control thirteen droplet operations electrodes. Further, this bus configuration may be implemented in a single layer of a droplet actuator substrate, such as a single layer of a PCB.

In one example, by use of only three control lines, two droplets are transported simultaneously from the outer portion to the center portion of electrode configuration 5300 and merged at the center portion. For example, a droplet 5330 is initially at droplet operations electrodes 5320A and a droplet 5332 is initially at droplet operations electrodes 5320B. Then, by sequencing first control line 5312A, second control line 5312B, and third control line 5312C, both droplets may be transported toward droplet operations electrodes 5320E, which is at the center portion of electrode configuration 5300, and merged at droplet operations electrodes 5320E.

7.3 Droplet Actuator Devices and Methods

The invention provides devices and methods for cost-effective production of droplet actuators. In one embodiment, the methods of the invention provide for cost-effective fabrication of the top substrate using polymer films and precision spacers. In another embodiment, the invention provides for cost-effective fabrication of the bottom substrate using an additive printing process on thermoplastic films. In yet another embodiment, the invention provides for cost-effective deposition of hydrophobic coating materials while maintaining or improving droplet operations performance.

7.3.1 Top Substrate

Plastics are preferred materials for fabrication of the top substrate of a droplet actuator due to their improved manufacturability and potentially lower costs. In one example, the top substrate may be formed of injection molded polycarbonate material that has liquid wells (e.g., sample and reagent wells) on one side and is flat on the other side. The top substrate may also include a conductive layer. In one embodiment, the conductive layer may be formed by vacuum deposition of a conductive material. In another embodiment, the conductive layer may be formed using conductive polymer films.

The top substrate may also include a spacer that separates the top substrate from the bottom substrate. The spacer sets the gap between a bottom substrate and a top substrate and determines the height of the droplet. Precision in the spacer thickness is required in order to ensure precision in droplet volume, which is necessary for accuracy in an assay. Islands of spacer material are typically required for control of gap height across large cartridges. In one embodiment, the spacer may be integrated within the injection molded polycarbonate material. In another embodiment, the spacer may be formed on the injection molded polycarbonate material by screen printing. Screen printing may be used to form a precision spacer that has small feature sizes and to form isolated spacer islands. A preferred spacer thickness is from about 0.010 inches to about 0.012 inches. In yet another embodiment, the spacer may be screen printed onto a conductive polymer film and laminated onto injection molded polycarbonate material.

Figure 54 illustrates a side view of a portion of a droplet actuator 5400 that includes a top substrate with integrated spacer structures and a transparent conducting layer. In this embodiment, the conductive layer is formed by vacuum deposition of indium tin oxide (ITO). Vacuum deposition of ITO is a relatively expensive process.

Droplet actuator 5400 may include a bottom substrate 5410 and a top substrate 5412 that are separated by a gap 5414. Bottom substrate 5410 may include an arrangement of droplet operations electrodes 5416 (e.g., electrowetting electrodes). Droplet operations may be mediated by electrodes 5416 on a droplet operations surface. Bottom substrate 5410 may, for example, be formed of a PCB. Top substrate 5412 may, for example, be formed of an injection molded plastic material, such as injection molded polycarbonate. Top substrate 5412 may include one or more integrated spacer structures 5418 that may be formed in the molding process. Spacer structures 5418 may be used to set the size of gap 5414. Top substrate 5412 may also include one or more sample and reagent wells (not shown) that are formed on the surface of top substrate 5412 that is facing away from gap 5414.

A conductive layer 5420 may be disposed on the surface of top substrate 5412 that is facing gap 5414. Conductive layer 5420 may, for example, be formed of a transparent conductive material, such as ITO. Conductive layer 5420 (e.g., ITO) may be disposed on the surface of top substrate 5412 by vacuum deposition. Because of the geometry of top substrate 5412, vacuum deposition of ITO on top substrate 5412 is typically preformed in a batch process. The cost incurred using vacuum deposition is dictated by the chamber size and cycle time. Economies of scale generally do not apply and the reduction in cost due to increased manufacturing volume may be marginal.

Figure 55 illustrates a side view of a portion of a droplet actuator 5500 that includes a top substrate that has a translucent or opaque conductive layer instead of the transparent ITO conductive layer as shown in Figure 54.

In one embodiment, a conductive layer 5505 may, for example, be formed of a translucent material, such as DuPont 7162 or 7164. In another example, conductive layer 5505 may be formed of an opaque material such as DuPont 7152 or 7162. Conductive layer 5505 may, for example, be disposed on the surface of top substrate 5412 using a printing process, such as screen printing, pad printing, stencil printing, and/or other rotary printing methods.

In another embodiment, conductive layer 5505 may be formed by vacuum deposition of low cost translucent or opaque conductive materials. For example, aluminum may be used as the conductive material.

Because conductive layer 5505 is formed of a translucent or opaque material, a detection window 5510 may be patterned in conductive layer 5505. Detection window 5510 may be aligned with a certain droplet operations electrode 5416 (e.g., 5416D) on droplet actuator 5500. Detection window 5510 provides an optical path for a light signal, such as a fluorescent signal from a sample positioned on droplet operations electrode 5416D to an external imaging device (not shown).

Figure 56 illustrates a side view of a portion of a droplet actuator 5600 that includes a top substrate that has a precision spacer formed on a printed conductive layer. In this embodiment, a conductive layer may be formed on the top substrate prior to fabrication of the precision spacer.

Droplet actuator 5600 may include a bottom substrate 5610 and a top substrate 5612 that are separated by a gap 5614. Bottom substrate 5610 may include an arrangement of droplet operations electrodes 5616 (e.g., electrowetting electrodes). Droplet operations may be conducted atop electrodes 5616 on a droplet operations surface. Bottom substrate 5610 may, for example, be formed of a PCB. Top substrate 5612 may, for example, be formed of injection molded plastic material, such as polycarbonate. In one example, top substrate 5612 is substantially flat on one side and includes one or more wells, such as sample and reagent wells (not shown), on the opposite side. A conductive layer 5618 may be printed on the flat surface of top substrate 5612 that is facing gap 5614. Conductive layer 5618 may, for example, be formed of a translucent or opaque conductive material. Because conductive layer 5618 is formed of a translucent or opaque material, a detection window 5620 may be patterned in conductive layer 5618 as described above in reference to Figure 55.

One or more spacers 5622 may be disposed on the surface of conductive layer 5618 and protruding into gap 5614. In one example, spacers 5622 may be formed by screen printing. Screen printing typically deposits thin layers of ink (e.g., about 0.002 to about 0.003 inches thick) and multiple passes may be required to achieve a spacer of sufficient thickness (from about 0.010 to about 0.012 inches). Alternatively, the spacer material (e.g., printing ink) and printing process may be optimized to maximize the thickness achievable in a single pass. For example, the viscosity of the ink and the screen mesh size may be varied and the average thickness and standard deviation of the spacer layer may be determined (e.g., measured using micrometers and profilometry).

Spacers 5622 may, for example, be formed of a UV-curable material that does not require mixing and is rapidly cured. Suitable materials include the DSL 100 series lacquers from Peters GmbH (Germany), which are available in a variety of viscosities and can generate 80-500μm thick layers in a single pass. Because spacers 5622 may be formed by screen printing, individual features, such as spacer islands (e.g., 5624), may readily be formed. In another embodiment, spacers 5622 may be die cut laminated on the surface of conductive layer 5618. In certain embodiments, spacers 5622 may be formed on bottom substrate 5610 only, top substrate 5612 only, or on both bottom substrate 5610 and top substrate 5612.

Figure 57 illustrates a side view of a portion of the droplet actuator 5700 that includes a top substrate that has a precision spacer formed on a conductive polymer film. Droplet actuator 5700 may include a bottom substrate 5710 and a top substrate 5712 that are separated by a gap 5714. Bottom substrate 5710 may include an arrangement of droplet operations electrodes 5716 (e.g., electrowetting electrodes). Droplet operations may be conducted atop electrodes 5716 on a droplet operations surface. Bottom substrate 5710 may, for example, be formed of a PCB. Top substrate 5712 may, for example, be formed of injection molded plastic material, such as polycarbonate.

In this embodiment, one or more spacers 5722 may be screen printed onto a conductive polymer film and laminated onto the top substrate. Spacers 5722 may be disposed on conductive layer 5718 as described in reference to Figure 57. In a preferred example, conductive layer 5718 may be an ITO coated polyester film (ITO-PET). Because conductive layer 5718 is a transparent layer, detection window 5720 may not be required. In another example conductive layer 5718 may be a gold or copper coated polyester film. In yet another example, conductive layer 5718 may be a foil.

An adhesive layer 5726 may be used to laminate conductive layer 5718 onto the surface of top substrate 5712 that is facing gap 5714. Adhesive 5726 may, for example, be a heat seal adhesive, a pressure sensitive adhesive (PSA), or applied as a liquid.

Using plastic as a material for the top substrate improves the manufacturability and therefore lowers the cost of the final cartridge, as compared with glass. In one example, a plastic top substrate may be constructed of an ITO-PET-polycarbonate laminate.

Figure 58 illustrates a side view of a portion of a top substrate 5800 that is constructed of an ITO-PET-polycarbonate laminate. For example, a 0.007-inch thick ITO-PET layer 5810 is laminated to 0.060-inch thick polycarbonate layer 5812 using a 0.002-inch 3M 467MP pressure sensitive adhesive (PSA) layer 5814. A laser cut 0.007-inch polyester layer 5816 that is laminated to a heat sealable 0.004-inch thick 3M 583 adhesive layer 5818 provides a spacer. Measurements have been taken to evaluate the consistency of the gap created by the spacer. The average spacer thickness measured across 9 structures was 247 um with a standard deviation of 20 um (8%). Top substrate 5800 has been evaluated using simple droplet dispensing and transport protocols on a well plate sized cartridge using biologically relevant sample matrices (data not shown). No fundamental failures in droplet operations were observed, suggesting that a plastic top substrate is a viable alternative to glass.

The adhesive layer and lamination processes may be optimized to prevent the formation of air bubbles at the lamination bond line (polycarbonate/ITO-PET) during subsequent high temperature curing cycles. There are two potential sources of air at the lamination bond line. Air may be trapped during lamination and expand or the adhesive may out-gas during the thermal curing cycle. To avoid the trapping of air during lamination, a heated platen press may be used to provide a higher lamination pressure. To minimize out-gassing from the adhesive, an adhesive with an ultra low volatile organic content may be used. An example of a suitable adhesive is Adhesives Research ARClean EL90420 permanent adhesive film. EL90240 has ultra low volatile organic content and is also rated for high temperatures. Additionally, optically clear liquid adhesives may be used.

7.3.2 Bottom Substrate

Currently, the bottom substrates of droplet actuators are formed of a PCB that includes electrodes arranged for conducting droplet operations. The PCB process used to fabricate the bottom substrate is relatively inexpensive (compared with semiconductor photolithography) and may be used to achieve a cost of about $0.10/square inch for a two-layer device produced in extremely high volumes. The cost of the bottom substrate may be further reduced by using a roll-based process as opposed to the traditional panel processing typically used by PCB vendors. In addition, some of the materials that are currently used in PCB-based bottom substrates, such as FR4, may be replaced with lower cost thermoplastic alternatives. FR4 is a glass filled high temperature (about 170 °C) material that is required to withstand harsh plating processes and high temperature soldering. Because solderability is not a requirement for the microfluidic devices of the invention and an additive printing process may be used to avoid plating, FR4 may be readily replaced. Thermoplastic materials are also recyclable. Additive processes are also inherently more environmentally friendly because there is minimal wastage of material.

An additive printing process may be used to manufacture a two-layer microfluidic chip substrate. The microfluidic device may be fabricated on a plastic substrate, such as a thermoplastic polymer film. In one embodiment, two conductive layers may be printed on one side of the polymer. In an alternative embodiment, two conductive layers may be printed on opposite sides of the polymer.

Figure 59 illustrates a side view of a portion of a bottom substrate 5900 that includes two conductive layers printed on the same side of a polymer film. Bottom substrate 5900 may include a plastic substrate 5910. Substrate 5910 may, for example, be heat stabilized polyester, polycarbonate, PEN or LCP. PEN and LCP can withstand temperatures > 150 °C. Polyester and polycarbonate are low cost materials. Polyester is a commonly used material. A conductive interconnect layer 5912 may be printed on one side of substrate 5910. An interlevel dielectric layer 5914 may be printed on top of interconnect layer 5912 with regions exposed to form one or more via holes 5916. Via holes 5916 may be filled with a conductive material 5918. A layer of droplet operations electrodes 5920 (e.g., electrowetting electrodes) may be printed on top of dielectric layer 5914 that has via holes integrated therein. Via holes 5916 that are filled with conductive material 5918 are used to electrically connect interconnect layer 5912 to certain droplet operations electrodes 5920. A primary dielectric layer 5922 may be printed on top of droplet operations electrodes 5920 to complete the device.

Interconnect layer 5912, conductive material 5918, and droplet operations electrodes 5920 may, for example, be formed of a silver conductive material, such as DuPont 5025. Interlevel dielectric layer 5914 and primary dielectric layer 5922 may, for example, be formed of a UV-curable dielectric material, such as DuPont 5018.

Figure 60 illustrates a side view of a portion of a bottom substrate 6000 that includes two conductive layers printed on opposite sides of a polymer film. The polymer film and conductive and dielectric materials may be the same as those described in reference to Figure 59.

Bottom substrate 6000 may include a plastic substrate 6010. One or more via holes 6012 may be formed through substrate 6010. In one example, via holes 6012 may be formed using laser ablation. A conductive layer 6014 may be printed on one side of substrate 6010. A dielectric layer 6016 may be printed on top of conductive layer 6014. Via holes 6012 may be filled with a conductive material 6018. A layer of droplet operations electrodes 6020 (e.g., electrowetting electrodes) may be printed on the side of substrate 6010 that is opposite conductive layer 6014 and dielectric layer 6016. Via holes 6012 that are filled with conductive material 6018 are used to electrically connect conductive layer 6014 to certain droplet operations electrodes 6020. A primary dielectric layer 6022 may be printed on top of electrode layer 6020 to complete the device.

7.3.3 Deposition of Hydrophobic Material

Spray coating may be used as an alternative to dip coating to apply hydrophobic materials to the components of a droplet actuator (e.g., bottom substrate, top substrate, and well plate). Dip coating is an excellent method to obtain uniform coating when the part to be coated is flat and planar, such as a glass substrate. However, when using a low cost PCB technology for fabrication of the bottom substrate it is not possible to achieve the flatness and planarity of glass. Because of the irregularity of the substrate surface, application of the coating material may be sensitive to part orientation, surface topology, and flatness. Further, dip coating, an immersion process, also coats areas of the droplet actuator components that do not need a hydrophobic coating, such as the back sides of the bottom and top substrates. Dip coating has other disadvantages such as a large dead volume of coating material and is more prone to contamination because all parts are dipped in the same solution. The coating thickness may be controlled to some extent by varying the withdrawal speed, but to achieve large changes the coating concentration has to be changed.

Because spray coating is a metered coating process, any thickness of coating material may be readily applied. Further, spray coating allows for coating of only essential surfaces and thereby reduces the cost of the assembled droplet actuator. Spray coating processes typically have transfer efficiencies between 20-80%. The remaining material is carried away from the part due to air currents at the surface or deposited outside the part in regions which may, in some cases, be masked. Factors which may affect the transfer efficiency include coating liquid flow rate, nozzle air pressure, and distance between part and spray nozzle. Other parameters which may affect the coating include number of passes and solvent boiling point.

Amorphous fluoropolymers such as CYTOP® and Teflon® are almost exclusively used by the entire electrowetting community to provide a low hysteresis hydrophobic surface. These fluoropolymers are extremely expensive (about $50 per gram) and is one of the most expensive components of the droplet actuator. Therefore, lowering the wastage of material and increasing the transfer efficiency of the coating process is useful for lowering the overall cost of this process.

In order to reduce the coating material costs and the process costs while maintaining or improving droplet operations performance, a spray coating method has been developed using an air atomizing fan spray nozzle (781S-SS-WF from EFD Inc.). The height of the nozzle from the droplet actuator component was chosen to allow an entire well plate sized bottom or top substrate to be coated in a single pass. The parameters which were varied were fluid flow rate and atomizing air pressure. For a fixed coating, fluid flow rate dictates how much material is available to coat a substrate. For a fixed flow rate, increasing the nozzle pressure decreased the mean droplet size. For a fixed nozzle pressure, increasing the flow rate increased the mean droplet size resulting in larger droplets covering the spray area in a more discrete fashion.

Figure 61 shows a bar graph 6100 of hydrophobic coating thickness with respect to different fluid flow rates. A stroke of 1 corresponds to a flow rate of 0.040mL/sec. Full speed was 25mm/sec. The thickness obtained using dip coating was 150nm and this was chosen as a target thickness. A nozzle pressure of 30psi, a flow rate of 0.040mL/sec and a coating speed of 6mm/sec was used to achieve this thickness.

Figure 62 shows a plot 6200 of the increase in substrate weight with respect to the number of spray coating passes. The transfer efficiency of the spray coating process was measured by coating multiple passes onto the same substrate and measuring the weight of the final coating as a fraction of the total weight of coating material consumed. The substrate size was chosen to be much larger than the coated area. The coating weight was measured after 10, 20, 30, 40 and 50 passes to verify that the thickness build up was linear. The transfer efficiency calculated after 50 passes was around 50% which is the maximum achievable for the tested setting. In practice the spray pattern is larger by 1.3-1.5x than the coated part to avoid edge effects and the transfer efficiency will be lower than 50% and is about 30-40%.

The transfer efficiency of the spray coating process may be further optimized in a series of experiments as outlined in Table 1. Factors which may affect the transfer efficiency include coating liquid flow rate, nozzle air pressure, and distance between part and spray nozzle. Other parameters which may affect the coating include number of passes and solvent boiling point. Using these factors as inputs and measuring various response parameters, such as coating thickness, roughness, thickness uniformity across the part, contact angle hysteresis, coating coverage, visual smoothness, visual reflectance/sheen and transfer efficiency, and the coating process may be optimized. CYTOP® prepared in a fluorinated solvent may be used as the hydrophobic material. Chrome coated glass may be used as the substrate for ease of measurement. Because spray coating is a metered deposition process, the selection of substrate is not expected to have a significant effect on the coating thickness. A minimum coating thickness of 150 nm is required for reliable electrowetting.

Table 1- Design of experiments to optimize transfer efficiency of coating

Std DOE order	Run order	Factor A [CYTOP] %	Factor B Nozzle P psi	Factor C Speed mm/s	Factor D Z setting (50% overlap) mm	Factor E Stroke psi
28	1	0.55	20	10	55	5.5
27	2	0.55	20	10	55	5.5
15	3	1	30	2	40	10
8	4	1	10	2	70	1
9	5	1	30	2	70	1
17	6	0.1	30	18	40	10
20	7	0.1	30	2	70	10
2	8	0.1	30	2	40	1
5	9	1	10	18	40	1
12	10	1	30	18	70	1
21	11	1	30	2	70	10
14	12	1	10	2	40	10
24	13	0.1	30	18	70	10
25	14	0.55	20	10	55	5.5
18	15	1	30	18	40	10
26	16	0.55	20	10	55	5.5
16	17	1	10	18	40	10
11	18	0.1	30	18	70	1
13	19	0.1	10	2	40	10
23	20	1	10	18	70	10
19	21	0.1	10	2	70	10
6	22	0.1	30	18	40	1
3	23	1	30	2	40	1
10	24	1	10	18	70	1
7	25	0.1	10	2	70	1
29	26	0.55	20	10	55	5.5
1	27	0.1	10	2	40	1
22	28	0.1	10	18	70	10
4	29	0.1	10	18	40	1

The coating thickness and roughness may be measured by profilometry. The average thickness may be used to estimate the transfer efficiency. Contact angle hysteresis may be measured using the KSV contact angle meter. Other parameters which result in high transfer efficiency and in the ability to independently control coating thickness and roughness may be identified. The predictive power of the model may be validated using a second set of experiments designed to produce coatings of selected thicknesses and roughness.

Higher transfer efficiency is generally expected at lower nozzle pressures since the droplet sizes are larger. However, if the droplet size is too large, solvent evaporation is slow and may cause wrinkles in the coating due to Marangoni effects. Also, for a given solvent boiling point there is usually an optimal nozzle pressure (and droplet size) at which the transfer efficiency is maximized along with coated film quality. In a second set of experiments the solvent boiling point may be varied to control the drying rate. Fluorinated solvents may be obtained in boiling points ranging from 50 °C to 250 °C.

As an alternative to CYTOP®, Teflon AF may be used as the hydrophobic coating material. In comparison to CYTOP®, Teflon AF adheres poorly to substrate surfaces. To improve adhesion of Teflon AF to substrate surfaces, oxygen plasma ashing followed by fluorosilane functionalization may be used. Further, the use of fluorosilane may have an additional benefit of reducing the coating thickness requirements since it will render most of the surface hydrophobic.

7.3.4 UV Adhesives for Cartridge Assembly

Light curable adhesives provide a cost-effective alternative to one or two part epoxies for bonding the bottom substrate to the top substrate during cartridge assembly. Light curable adhesives are available in a wide range of formulations. The key advantages of light curable adhesives are ease of use (e.g., one part, no mixing required, stable properties over time) and extremely short curing time (e.g., less than 10 seconds possible).

Nine different UV-curable adhesive candidates for bonding top and bottom substrates were evaluated based on the following parameters: dispensing characteristic, tack time (i.e., surface cure), fixture time (i.e., bulk cure), chemical resistance (i.e., immersion in oil), and thermal resistance (i.e., thermal cycling). Chemical resistance is an important parameter because the cartridges are typically filled with low surface tension oil (i.e., filler fluid) which may degrade the adhesive bond line. Thermal resistance is an important parameter for applications such as polymerase chain reaction (PCR) assays.

The results of the adhesive evaluations are summarized in Table 2. Of all the adhesives evaluated, only 3M LC-1212 passed all the criteria. However, adhesives which failed only the tack time parameter (i.e., Loctite 3106, Loctite 3556) may also be used. Because the presence of an oxygenated environment may slow down surface curing, tack time evaluations are not always conclusive.

Table 2 – Summary of UV adhesive testing studies

	Dispensing	Fixture Time	Chemical Resistance	Thermal Resistance	Tack Time
3M LC-1212	Pass	Pass	Pass	Pass	Pass
3M LC-1214	Pass	Fail	N/A	N/A	Fail
Dymax 3019-Gel	Pass	Pass	Pass	Fail	Fail
Dymax 3094-Gel-F	Pass	Pass	Pass	Fail	Fail
Loctite 3103	Pass	Fail	N/A	N/A	Fail
Loctite 3106	Pass	Pass	Pass	Pass	Fail
Loctite 3355	Fail	N/A	N/A	N/A	Fail
Loctite 3526	Pass	Fail	N/A	N/A	Fail
Loctite 3556	Pass	Pass	Pass	Pass	Fail

7.4 Droplet actuators with disposable and non-disposable components

The invention provides droplet actuator devices and methods for replacing one or more components of a droplet actuator. For example, the invention provides droplet actuator devices that may include the combination of both disposable components that may be readily replaced and non-disposable components that may be more expensive to manufacture. Ready replacement of one or more disposable components may also provide substantially unlimited re-use of a droplet actuator device or a portion of a droplet actuator device without concern for cross-contamination between applications. In one embodiment, moveable films may be used to readily replace substrate layers (e.g., dielectric and/or hydrophobic layers). In another embodiment, reversible attachment of a top substrate and a bottom substrate may be used to provide ready access to and replacement of one or more substrate layers. In yet another embodiment, a self-contained replaceable top cartridge may be used to provide a single-use, contaminant-free substrate. In yet another embodiment, selectively removable layered structures may be used to replace one or more dielectric and/or hydrophobic substrate layers. In yet another embodiment, a single-unit droplet actuator cartridge that is easily opened and closed may be used to provide a droplet actuator device wherein one or more substrate layers are readily removed and replaced.

7.4.1 Replaceable Films

Figures 63A and 63B illustrate side and top views, respectively, of a portion of a droplet actuator 6300 that includes replaceable flexible film layers arranged upon reel-to-reel mechanisms. Droplet actuator 6300 is an example of a droplet actuator wherein moveable films are used to readily replace one or more top substrate layers (e.g., ITO and/or hydrophobic) and/or bottom substrate layers (e.g., dielectric and/or hydrophobic) to provide a clean surface for conducting droplet operations.

Droplet actuator 6300 may include a bottom substrate 6310 that is separated from a film top substrate 6312 by a gap 6314. Gap 6314 may be filled with a filler fluid (not shown). Bottom substrate 6310 may, for example, be formed of a rigid material, such as a silicon-based material, glass, plastic, and/or any other suitable material. Bottom substrate 6310 may include a path or array of droplet operations electrodes 6318 (e.g., electrowetting electrodes). A replaceable film 6320 in proximity to droplet operations electrodes 6318 may be used to provide one or more functional layers on bottom substrate 6310. One or more spacers 6316 may be used to establish the size of gap 6314 (i.e., the distance between film top substrate 6312 and replaceable film 6320.

In one example, replaceable film 6320 may include a dielectric layer and a hydrophobic layer (not shown). In this example, a dielectric layer may be disposed on the surface of replaceable film 6320 that is facing bottom substrate 6310. A hydrophobic layer may be disposed on the surface of replaceable film 6320 that is facing gap 6314. Replaceable film 6320 may be moveable through droplet actuator 6300 by way of a reel-to-reel system formed by a pair of reels 6322. By use of reels 6322, replaceable film 6320 may be advanced automatically or manually (in any direction) to provide a clean surface for conducting droplet operations.

Flexible top substrate 6312 may, for example, be formed of a flexible substrate film that includes one or more functional layers. For example, film top substrate 6312 may include an ITO layer and a hydrophobic layer. Film top substrate 6312 may be moveable atop droplet actuator 6300 by way of a reel-to-reel system formed by a pair of reels 6324. By use of reels 6324, film top substrate 6312 may be advanced automatically or manually (in any direction) to provide a clean surface for conducting droplet operations. One or more openings 6326 may be provided in film top substrate 6312. Openings 6326 provide a fluid path from film top substrate 6312 into gap 6314 in sufficient proximity of certain droplet operations electrodes 6318. Figure 63B shows an arrangement of openings 6326 in top substrate 6312. In this example, a line of openings 6326 (e.g., four openings 6326) are arranged within a certain area defined by spacers 6316.

Figure 64 illustrates a side view of a portion of a droplet actuator 6400 that includes a rigid top substrate and a moveable film for replacement of bottom substrate layers. Droplet actuator 6400 is substantially the same as droplet actuator 6300 of Figures 63A and1B, except that film top substrate 6312 is replaced with a rigid top substrate 6410. Rigid top substrate 6410 may be formed of a rigid material, such as a silicon-based material, glass, plastic, and/or other suitably rigid materials. In one embodiment, rigid top substrate 6410 may be alternately raised and lowered to make contact with replaceable film 6320 on bottom substrate 6310. In another embodiment, rigid top substrate 6410 may be replaced either manually or automatically to provide a clean top substrate surface for conducting droplet operations.

Figures 65A and 65B illustrate side views of portions of droplet actuators and examples of methods for enhancing the contact between a flexible film and a substrate surface. For example, Figure 65A illustrates a side view of a portion of a droplet actuator 6500 and an example of a method for enhancing the contact between a flexible film and a substrate surface. Droplet actuator 6500 is substantially the same as droplet actuator 6400 of Figure 64, except that a contact layer 6510 is provided between replaceable film 6320 and droplet operations electrodes 6318 at bottom substrate 6310. Contact layer 6510 is provided to enhance the contact the physical contact between replaceable film 6320 and droplet operations electrodes 6318. Contact layer 6510 may be formed of, for example, a liquid that may be injected into gap (114 of droplet actuator 6500. In this embodiment, the quantity of liquid is of sufficient volume to fill empty spaces between bottom substrate 6310 and replaceable film 6320. In another embodiment, contact layer 6510 may be provided as an additional layer on replaceable film 6320. In this example, contact layer 6510 may be a compressible material, such as a compressible adhesive material. In yet another embodiment, contact layer 6510 may be a thin (e.g., about 50 microns) dielectric material.

Further, Figure 65B illustrates a side view of a portion of a droplet actuator 6550 and an example of another method for enhancing the contact between a flexible film and a substrate surface. Droplet actuator 6550 is substantially the same as droplet actuator 6400 of Figure 64, except that a set of openings 6552 are provided in bottom substrate 6310. The positions of openings 6552 substantially correspond to the respective positions of droplet operations electrodes 6318. In one embodiment, openings 6552 may be formed by the electrical via holes that may already be present in the PCB material that forms bottom substrate 6410. In this example, the electrical via holes are typically aligned with droplet operations electrodes 6416. Openings 6552 provide a channel by which a vacuum force may be applied to replaceable film 6320 of droplet actuator 6550, thereby pulling replaceable film 6320 into good contact with droplet operations electrodes 6318. An external vacuum source (not shown) may be connected to openings 6552.

7.4.2 Reversible Attachment

Typically, top and bottom substrates of a droplet actuator are bonded together. Because the top and bottom substrates are bonded together, disassembly of the droplet actuator for regeneration of substrate surfaces may be difficult.

Figure 66 illustrates a cross-sectional view of a portion of a droplet actuator 6600 and a method for using vacuum for coupling and decoupling the top and bottom substrates. In this embodiment, a vacuum seal instead of a permanent seal between top and bottom substrates provides for ready disassembly of droplet actuator 6600 for use in subsequent applications.

Droplet actuator 6600 may include a bottom substrate 6610 that is separated from a top substrate 6612 by a gap 6614. The size of gap 6614 may be of sufficient height for conducting droplet operations. Gap 6614 may be filled with a filler fluid (not shown). Bottom substrate 6610 may, for example, be formed of a PCB or a rigid material, such as a silicon-based material, glass, plastic, and/or any other suitable material. Bottom substrate 6610 may include a path or array of droplet operations electrodes 6616 (e.g., electrowetting electrodes). Bottom substrate 6610 may include one or more openings 6618. Openings 6618 provide a channel for connection to an external vacuum source (not shown).

Top substrate 6612 may, for example, be formed of silicon based materials, glass, plastic, and/or any other suitable material. A recessed area 6620 is provided within top substrate 6612 for providing a vacuum channel along the perimeter thereof. Recessed area 6620 is substantially aligned with openings 6618 of bottom substrate 6610. A vacuum seal 6622 around recessed area 6620 and openings 6618 may be provided between bottom substrate 6610 and top substrate 6612. Vacuum seal 6622 may be formed of, for example a soft gasket material suitable for forming a vacuum seal. Bottom substrate 6610 is coupled to top substrate 6612 by providing a vacuum force through openings 6618 into recessed areas 6620, thereby pulling a vacuum between bottom substrate 6610 and top substrate 6612 that is maintained because of vacuum seal 6622.

The use of vacuum force for coupling and decoupling the top and bottom substrates of a droplet actuator, such as droplet actuator 6600, provides the benefit of an easy way to assemble a droplet actuator without bonding as well as an easy method of disassembly after use.

Figure 67 illustrates a cross-sectional view of a portion of a droplet actuator 6700 and a method for using a temperature-sensitive material for coupling and decoupling the top and bottom substrates. Droplet actuator 6700 may include a bottom substrate 6710 that is separated from a top substrate 6712 by a gap 6714. The size of gap 6714 may be of sufficient height for conducting droplet operations. Gap 6714 may be filled with a filler fluid (not shown). Bottom substrate 6710 may, for example, be formed of a PCB. Bottom substrate 6710 may include a path or array of droplet operations electrodes 6716. Bottom substrate 6710 may include one or more heating electrodes 6718, which may be used as heating elements. In one example, heating electrodes 6718 may be droplet operations electrodes that are designated for heating. Heating electrodes 6718 may be formed, for example, of a resistive material that is designed to generate a certain amount of heat when a certain voltage is applied. Heating electrodes 6718 may be used to provide sufficient heat to certain areas of droplet actuator 6700.

Top substrate 6712 may, for example, be formed of silicon based materials, glass, plastic, and/or any other suitable material. A gasket 6720 may be used to provide a seal around the outer edge of droplet actuator 6700 and between bottom substrate 6710 and top substrate 6712. Gasket 6720 is substantially aligned with heating electrodes 6718. Gasket 6720 may, for example, be a heat-sensitive material, such as hot melt glue, that is suitable for forming a seal when melted. Bottom substrate 6710 is attached to top substrate 6712 by activation of heating electrodes 6718 to a sufficient temperature and for a sufficient period of time to melt gasket 6720. Heating electrodes 6718 may then be turned off. Top substrate 6712 may be readily detached from bottom substrate 6710 by reactivating heating electrodes 6718, which again melts gasket 6720 such that bottom substrate 6710 and top substrate 6712 may be separated.

In an alternative embodiment, instead of using heating electrodes 6718, an external heat source (not shown) may be used to apply heat to droplet actuator 6700 for melting gasket 6720.

7.4.3 Replaceable Top Cartridges

Figures 68A and 68B illustrate side views of a portion of a droplet actuator 6800 that includes a fixed bottom substrate and a removable top substrate, wherein the top substrate is a replaceable cartridge. The replaceable top cartridge of the invention is a self-contained cartridge, i.e., may include reagents, buffers, substrates and filler fluid required for a droplet actuator-based assay.

Droplet actuator 6800 may include a bottom substrate 6810, which may be fixed, and a replaceable top cartridge 6812. Bottom substrate 6810 may, for example, be formed of a PCB or a rigid material, such as a silicon-based material, glass, and/or any other suitable material. Bottom substrate 6810 may include a fixed array of droplet operations electrodes 6814 (e.g., electrowetting electrodes).

Top cartridge 6812 may be, for example, a plastic housing that is formed around an enclosed area 6816. Enclosed area 6816 may be of sufficient height for conducting droplet operations. In one embodiment, top cartridge 6812 may include a ground electrode 6818. In an alternative embodiment, ground electrode 6818 may be replaced with a hydrophobic layer (not shown) suitable for co-planar electrowetting operations. Top cartridge 6812 may include an opening 6820. Opening 6820 provides a fluid path from top cartridge 6812 into enclosed area 6816 in sufficient proximity of certain droplet operations electrodes 6814 on bottom substrate 6810. Opening 6820 may be used for loading one or more samples into top cartridge 6812. Positioning of top cartridge 6812 in sufficient proximity of certain droplet operations electrodes 6814 may, for example, be provided by alignment guides (not shown).

Referring to Figure 68A, top cartridge 6812 may include one or more pouches 6822. Pouches 6822 may be used as fluid reservoirs for holding a volume of a certain fluid 6823. Pouches 6822 may be formed of a material that may be punctured for releasing fluid 6823 into enclosed area 6816. Fluid 6823 may be, for example, one or more different reagents required for droplet actuator-based assays. In one example one or more pouches 6822 may contain a filler fluid such as silicone oil. In this example, a piercing mechanism may be used for puncturing pouches 6822 and dispensing a filler fluid there from into enclosed area 6816 during alignment and loading of top cartridge 6812 onto bottom substrate 6810. In another example, one or more pouches 6822 may include reagents, buffers, and substrates required for performing a molecular assay. An interface material 6824 is disposed between top cartridge 6812 and bottom substrate 6810. Interface material 6824 may be, for example, a thin layer of certain liquid, certain grease, a certain soft material, or certain reversible glue. Interface material 6824 may also serve as the dielectric layer atop droplet operations electrodes 6814 of bottom substrate 6810.

Referring to Figure 68B, top cartridge 6812 may include a dielectric layer 6828 that interfaces with droplet operations electrodes 6814. Because top cartridge 6812 is a replaceable cartridge, dielectric layer 6828 is also replaceable. Dielectric layer 6828 may be patterned according to a desired topology that may, for example, correspond to a certain arrangement of droplet operations electrodes 6814 on bottom substrate 6810. For example, certain features 6830 may be patterned into dielectric layer 6828 for fitting between droplet operations electrodes 6814 on bottom substrate 6810 when assembled.

In one example, a stamping process may be used to form features 6830 of dielectric layer 6828. More specifically, a stamp (not shown) may be provided that mimics the topology of bottom substrate 6810 that has droplet operations electrodes 6814 patterned thereon. Initially, dielectric layer 6828 is formed on top cartridge 6812 having a certain uniform thickness, and then the stamp may be brought into contact with dielectric layer 6828 of top cartridge 6812 under a certain amount of heat and/or pressure for a certain amount of time. In this way, a reverse impression of bottom substrate 6810 that has droplet operations electrodes 6814 patterned thereon is formed in dielectric layer 6828 of top cartridge 6812, thereby forming, for example, features 6830. The reverse impression of droplet operations electrodes 6814 of bottom substrate 6810 that is patterned into dielectric layer 6828 of top cartridges 6812 provides a tight coupling between bottom substrate 6810 and top cartridge 6812 when assembled.

7.4.4 Replaceable Hydrophobic and/or Dielectric Layers

A hydrophobic layer (i.e., an electrowettable coating) is typically the first surface layer on a droplet actuator contacted, for example, by a sample droplet and/or reagent droplets. Replacing the hydrophobic layer is typically sufficient to regenerate the device (i.e., provide clean, uncontaminated surfaces). In one embodiment, hydrophobic surfaces of a droplet actuator may be replaced by removing the spent hydrophobic layer with a suitable solvent and subsequently flowing fresh coating material into the droplet actuator.

In another embodiment, hydrophobic surfaces of a droplet actuator may be continuously replenished by providing a suitable coating material in the filler fluid of a droplet actuator.

In yet another embodiment, a coating material may be applied directly over the existing hydrophobic layer. Because hydrophobic coatings are typically sufficiently thin, relative to the thickness of the dielectric layer, a build-up of one or more hydrophobic layers has minimal direct effects on the electrowetting performance of the droplet actuator.

In yet another embodiment, a layered electrowettable coating structure may be used to readily regenerate the surface of a droplet actuator.

Figures 69A, 69B, and 69C illustrate side views of a portion of a droplet actuator 6900 that includes a layered electrowettable structure and a method for readily regenerating the surface of a droplet actuator. In one example, the layered structure includes one or more electrowettable coatings that are selectively soluble in different solvents. In another example, the layered structure includes one or more electrowettable coatings that are soluble in the same solvent. In yet another example, the layered structure includes one or more electrowettable films that are readily removed by peeling and/or etching.

Droplet actuator 6900 may include a bottom substrate 6910. Bottom substrate 6910 may, for example, be formed of a PCB. Bottom substrate 6910 may include a fixed array of droplet operations electrodes 6912 (e.g., electrowetting electrodes).

Referring to Figure 69A, an electrowettable coating 6914 may be disposed on the surface of bottom substrate 6910 atop droplet operations electrodes 6912. Electrowettable coating 6914 may, for example, be formed of a suitable material that is soluble in a certain solvent. An electrowettable coating 6916 may be disposed atop electrowettable coating 6914. Electrowettable coating 6916 may, for example, be formed of a suitable material that is soluble in a different solvent. An electrowettable coating 6918 may be disposed atop electrowettable coating 6916. Electrowettable coating 6918 may, for example, be formed of a suitable material that is soluble in yet another different solvent.

The layered structure of electrowettable coatings 6914, 6916, and 6918 provide a means to readily regenerate the surface of bottom substrate 6910 after one or more uses of droplet actuator 6900. For example, after a first use of droplet actuator 6900, electrowettable coating 6918 may be selectively removed with a certain solvent to expose electrowettable coating 6916. Electrowettable coating 6916 provides a fresh, uncontaminated hydrophobic layer on the surface of bottom substrate 6910. Similarly, electrowettable coating 6916 may be selectively removed after a second use of droplet actuator 6900, with a different solvent to expose electrowettable coating 6914. Electrowettable coating 6914 provides yet another fresh, uncontaminated hydrophobic layer on the surface of bottom substrate 6910. Electrowettable coating 6914 may be selectively removed after a third use of droplet actuator 6900 with yet another different solvent. The layered electrowettable coating structure may then be regenerated on bottom substrate 6910 by introducing suitable coating materials.

In another embodiment, the electrowettable coatings are selectively removed using different conditions, such as different temperatures.

Referring to Figure 69B, an electrowettable coating 6920a may be disposed on the surface of bottom substrate 6910 atop droplet operations electrodes 6912. Electrowettable coating 6920a may, for example, be formed of a suitable material that is soluble in a certain solvent. A sacrificial layer 6922a may be disposed atop electrowettable coating 6920a. Sacrificial layer 6922a may, for example, be formed of a suitable material (e.g., a material that may or may not be electrowettable) that is soluble in a different solvent. A second electrowettable coating 6920b may be disposed atop sacrificial layer 6922a. Electrowettable coating 6920b may be formed of the same material as coating 6920a or a different material that is soluble in the same solvent as coating 6920a. A second sacrificial layer 6922b may be disposed atop electrowettable coating 6920b. Sacrificial layer 6922b may be formed of the same material as sacrificial layer 6922a, or a different material that is soluble in the same solvent. A third electrowettable coating 6920c may be disposed atop sacrificial layer 6922b. Electrowettable coating 6920c may be formed of the same material as coating 6920a and 6920b or a different material that is soluble in the same solvent.

The layered structure of electrowettable coatings 6920 and sacrificial layers 6922 provide another means to readily regenerate the surface of bottom substrate 6910 of droplet actuator 6900. For example, a first solvent may be used to remove electrowettable coating 6920a. A second solvent may be used to remove sacrificial layer 6922a and expose a fresh, uncontaminated hydrophobic layer (i.e., electrowetting coating 6920b) on the surface of bottom substrate 6910. The process may be repeated to provide yet another fresh, uncontaminated hydrophobic layer (i.e., electrowetting coating 6920c) on the surface of bottom substrate 6910. The layered electrowettable coating structure may then be regenerated on bottom substrate 6910 by introducing suitable coating materials.

Referring to Figure 69C, multiple hydrophobic films may be laminated onto bottom substrate 6910. For example, three hydrophobic film layers 6924a, 6924b, and 6924c may be laminated onto bottom substrate 6910. Hydrophobic film layer 6924 may be formed of a suitable hydrophobic material that is readily removed, for example, by peeling. Multiple hydrophobic film layers 6924 (two or more film layers) may also provide dielectric properties. In another example, film layer 6924 may be formed of both a dielectric material and a hydrophobic material.

In yet another embodiment, the dielectric layer of a droplet actuator may also be replaced to regenerate the surface (e.g., eliminate contamination) of a droplet actuator. For example, the dielectric layer may be formed of a dissolvable and/or meltable material such as a wax, photoresist, or other organic polymers that may be readily removed and replaced after each use.

In yet another embodiment, dielectric and/or hydrophobic layers may be selectively removed and replaced in active areas of droplet actuator 6900, i.e., areas that include electrodes.

Droplet actuator 6900 of the invention is not limited the number and types of layers that are shown in Figures 69A, 69B, and 69C. The configurations of droplet actuator 6900 that are shown in Figures 69A, 69B, and 69C are exemplary only.

7.4.5 Single-unit Droplet Actuator Cartridge

Figures 70A and 70B illustrate side views of portions of a droplet actuator cartridge 7000. Droplet actuator cartridge 7000 is an example of a droplet actuator wherein a rigid-flex process may be used to form a single unit droplet actuator cartridge.

Cartridge 7000 may include a flexible substrate 7010. Flexible substrate 7010 may be selectively processed (e.g., rigid-flex processing) to provide certain regions for conducting droplet operations. For example, flexible substrate 7010 may include a bottom substrate region 7012 and a top substrate region 7014. Bottom substrate region 7012 and top substrate region 7014 may be separated by a hinge region 7016. Hinge region 7016 provides a mechanism to fold top substrate region 7014 into proximity of bottom substrate region 7012 (i.e., to close cartridge 7000). In the closed position, cartridge 7000 is ready for operation. Hinge region 7016 also provides a mechanism to readily open cartridge 7000. Cartridge 7000 may, for example, be readily opened at hinge region 7016 for removing and replacing one or more substrate layers.

Bottom substrate region 7012 may include a path or array of droplet operations electrodes 7018 (e.g., electrowetting electrodes). A dielectric layer 7020 may be selectively disposed atop droplet operations electrodes 7018 in bottom substrate region 7012. In one embodiment and referring to Figure 70B, dielectric layer 7020 may be an adhesive backed polyimide, such as a Pyralux LF coverlay composite (DuPont). In one example, Pyralux LF7013 may be used. Pyralux LF7013 includes an approximately 25 micrometer thick Dupont KAPTON® polyimide film and an approximately 25 micrometer thick acrylic adhesive. In another example, a Pyralux coverlay composite that includes a polyimide film and adhesive layer of a different thickness may be used.

Top substrate region 7014 may include a ground electrode 7022. Ground electrode 7022 may, for example, be formed of copper or another suitable material. A hydrophobic layer 7024 may be disposed as a final layer atop bottom substrate region 7012, top substrate region 7014, and hinge region 7016. In one embodiment and again referring to Figure 70B, hydrophobic layer 7024 may be a Cytop™ coating. Hydrophobic layer 7024 may, for example, be approximately 700nm to several microns in thickness.

An optional rigid layer 7026 may be disposed on the surface of flexible substrate 7010 that is opposite droplet operations electrodes 7016 and ground electrode 7022 and excluding hinge region 7014.

7.5 Droplet Actuator with Conductive Ink Electrodes and/or Ground Planes

The substrates of a droplet actuator typically include electrodes and/or an electrical ground plane patterned thereon that is exposed to the droplet operations gap. Current materials (e.g., ITO) and/or processes for forming the electrodes and/or electrical ground planes may be costly. The invention provides droplet actuators and methods of using printed conductive inks to form electrodes and/or ground planes, which uses less costly materials and/or processes.

Figure 71 illustrates a cross-sectional view of an example of a portion of a droplet actuator 7100 that uses printed conductive inks to form electrodes and/or ground planes. Droplet actuator 7100 may include a bottom substrate 7110 that is separated from a top substrate 7112 by a gap 7114. Bottom substrate 7110 may be formed, for example, of silicone, glass, plastic or PCB. Top substrate 7112 may be formed, for example, of any suitable material, e.g., glass; polycarbonate; COC (cyclo-olefin copolymer); COP (cyclo-olefin polymer); PMMA (polymethylmethacrylate); polystyrene or other plastics that are fabricated through injection-molding, lamination, printing, or by any other means; and any combinations thereof. Bottom substrate 7110 may include an arrangement of droplet operations electrodes 7116 (e.g., electrowetting electrodes). Droplet operations are conducted atop droplet operations electrodes 7116 on a droplet operations surface.

A dielectric layer 7118 is atop droplet operations electrodes 7116 of bottom substrate 7110. A hydrophobic layer 7120 is atop dielectric layer 7118 of bottom substrate 7110. The side of top substrate 7112 that is facing gap 7114 is coated with a conductive ink layer 7122, which is coated then coated with a hydrophobic layer 7124.

Hydrophobic layer 7120 of bottom substrate 7110 and hydrophobic layer 7124 of top substrate 7112 may, for example, be Teflon AF; a CYTOP coating; coatings in the F1uoropel family; silane coatings; f1uorosilane coatings; and 3M Novec electronic coatings. For example, a CYTOP™ spray-coating may be used to form hydrophobic layer 7120 and hydrophobic layer 7124.

The printing of conductive ink layer 7122 may be performed on the bare surface of top substrate 7112 that is facing gap 7114, prior to Cytop coating. The printing of conductive ink layer 7122 may be done using electrode path patterns. Line widths may vary (e.g., 0.001 – 100 mm range). Examples of suitable conductive inks include HC Starck's (Newton, MA) Inherently Conductive Polymers (PEDOT:PSS) (e.g., the Clevios™ line, ex-Baytron™ lines of products). Specific examples include inks such as Clevios™ "P Jet N", "P Jet HC", "P Jet N V2" and "P Jet HC V2", but other inks (including non-transparent ones) may be used.

The printing of conductive ink layer 7122 to form the ground plane of top substrate 7112 is an inexpensive material and process as compared with depositing an ITO layer to form the ground plane. A Dimatix Materials Printer DMP-2831 (an R&D grade benchtop inkjet printer from FUJIFILM Dimatix, Inc.) or any other printing equipment may be used to apply the conductive inks.

Figure 72 illustrates an example of a conductive ink pattern 7200 that is printed on the surface of a substrate 7210. Figure 72 shows an example of conductive ink that is printed onto substrate 7210 in areas where the droplets (not shown) will be moving. For example, conductive ink pattern 7200 may be patterned along droplet paths or in regions where droplet operations will be conducted. This is more economical than coating the substrate with, for example, an ITO conductive layer.

7.6 Magnetic Clamping Fixture for Assembling Droplet Actuators

The invention is a magnetic clamping fixture for assembling droplet actuators. The magnetic clamping fixture of the invention includes a base plate and a top plate. Installed in the base plate and top plate of the magnetic clamping fixture are respective sets of magnets. When assembled, the positions of the respective sets of magnets are aligned. Further, the orientation of the magnets is such that the magnets of the base plate are attracted to the magnets of the top plate. Substrates of droplet actuators that are to be bonded together may be sandwiched between the base plate and top plate of the magnetic clamping fixture and held by compression due to the magnetic forces that are pulling the base plate and the top plate together.

In one example, ultraviolet (UV) cure adhesive may be used for bonding together the substrates of droplet actuators. In this example, the magnetic clamping fixture may be used to hold the substrates during the application and curing of the UV cure adhesive. An aspect of the magnetic clamping fixture of the invention is that it provides substantially uniform pressure between the substrates while the adhesive is curing. Another aspect of the magnetic clamping fixture of the invention is that it ensures a substantially uniform gap height between the substrates while the adhesive is curing. Yet another aspect of the magnetic clamping fixture of the invention is that it ensures full exposure of, for example, the UV cure adhesive to UV light during the curing cycle. That is, no mechanical features of the magnetic clamping fixture are obstructing the path of the UV light.

Figure 73 illustrates a perspective view of an example of a magnetic clamping fixture 7300 for assembling droplet actuators. Magnetic clamping fixture 7300 may include a base plate 7310 and a top plate 7312. Top plate 7312 may further include one or more magnet placement holes 7314 into which magnets 7315 may be installed. Top plate 7312 may further include a hole 7316, which may be, for example, a threaded hole into which a handle (not shown) may be installed. Similarly, base plate 7310 includes one or more holes, which are not visible in Figure 73, into which opposing magnets, which are also not visible in Figure 73, may be installed. More details of base plate 7310 are described with reference to Figure 74.

During a substrate bonding process, the substrates of a droplet actuator may be sandwiched between base plate 7310 and a top plate 7312 of magnetic clamping fixture 7300 and held by the attraction forces of the magnets. For example, a bottom substrate 7320 may be installed against the inner surface of base plate 7310. One or more alignment pins 7322 may be provided for precisely positioning bottom substrate 7320 to base plate 7310. Additionally, a top substrate 7324 may be installed against the inner surface of top plate 7312, such that top substrate 7324 aligns with bottom substrate 7320 when compressed together. In one example, bottom substrate 7320 may be a PCB and top substrate 7324 may be formed of glass, plastic, or ITO. Further, one or more standoffs 7318 may be provided along the perimeter of top plate 7312 of magnetic clamping fixture 7300. All of or a portion of standoffs 7318 may be formed of rubber. More details of top plate 7312 are described with reference to Figures 75A, 75B, 76A, and 76B.

Additionally, Figure 73 shows that base plate 7310 of magnetic clamping fixture 7300 may include certain alignment holes 7326 that may be used for installing magnetic clamping fixture 7300 into any other equipment in the process of bonding the substrates of droplet actuators together. More details of an example of a method of using magnetic clamping fixture 7300 are described with reference to Figure 79.

Figure 74 illustrates a view of the inner surface of an example of base plate 7310 of magnetic clamping fixture 7300 of the invention. In this example, base plate 7310 may include one or more magnet placement holes 7328 into which magnets 7330 may be installed. The pattern of magnet placement holes 7328 in base plate 7310 is a mirror image of the pattern of magnet placement holes 7314 in top plate 7312. In one example and referring to Figures 73 and 74, magnet placement holes 7314 in top plate 7312 and magnet placement holes 7328 in base plate 7310 may have a diameter of about 77/16 inches for receiving magnets 7315 and magnets 7330, respectively. In this example, magnets 7315 and 7330 are also about 77/16 inches in diameter and may be press-fitted into magnet placement holes 7314 and 7328, respectively. Magnets 7315 and 7330 may be permanent magnets or electromagnets.

Figures 75A, 75B, 76A, and 76B illustrate various views of an example of top plate 7312 of magnetic clamping fixture 7300 of the invention. For example, Figure 75A illustrates a view of the inner surface of an example of top plate 7312 of magnetic clamping fixture 7300 shown in Figure 73. Figure 75A again shows hole 7316 and certain standoffs 7318 that are arranged around the perimeter of top plate 7312. However, in this view additional standoffs 7332 are shown that are substantially aligned with magnet placement holes 7314 of top plate 7312. All of or a portion of standoffs 7332 may be formed of rubber. Figure 75A also shows a rubber strip 7334 installed at one end of top plate 7312. Also, Figure 75B illustrates a side view of an example of top plate 7312 of magnetic clamping fixture 7300. Again, certain standoffs 7318 are shown.

Figure 76A illustrates a perspective view of the outer surface of an example of top plate 7312 of magnetic clamping fixture 7300. Again, Figure 76A shows magnet placement holes 7314, hole 7316, certain standoffs 7318, and rubber strip 7334. Also, Figure 76B illustrates an end view of an example of top plate 7312 of magnetic clamping fixture 7300. Again, certain standoffs 7318 and rubber strip 7334 are shown.

Figure 77 illustrates a perspective view of an example of the orientation and alignment of opposing magnets of magnetic clamping fixture 7300 of the invention. For example, a pattern of magnets 7315 along the plane of top plate 7312 is shown in alignment with a corresponding pattern of magnets 7330 along the plane of base plate 7310. In this example, the magnets are oriented in an alternating fashion with respect to polarity. The north poles of certain magnets 7315 face the south poles of corresponding magnets 7330. Likewise, the south poles of certain magnets 7315 face the north poles of corresponding magnets 7330. In this way, a magnetic attraction is formed between magnets 7315 of top plate 7312 and magnets 7330 of base plate 7310. This is further illustrated with reference to Figure 78.

Figure 78 illustrates a cross-sectional view of magnetic clamping fixture 7300 of the invention taken along line A-A of Figure 73, again showing the orientation and alignment of opposing magnets. In this view, bottom substrate 7320 and top substrate 7324 are shown sandwiched between base plate 7310 and top plate 7312. The magnetic strength of magnets 7315 and magnets 7330 is suitably large such that their magnetic force (e.g., magnetic force 7810) may pass through the combined thickness of bottom substrate 7320 and top substrate 7324. In this way, the attraction between magnets 7315 and magnets 7330 is facilitated, which has the result of pulling base plate 7310 and top plate 7312 together. The resulting compression force serves as a clamping mechanism for holding bottom substrate 7320 and top substrate 7324 in the process bonding them together.

Figure 79 illustrates a flow diagram of an example of a method 7900 of using a magnetic clamping fixture, such as magnetic clamping fixture 7300, of the invention. Method 7900 may include, but is not limited to, the following steps, which may be implemented in any order.

At step 7910, base plate 7310 of magnetic clamping fixture 7300 is installed into any equipment that may be used in the substrate bonding process. Alignment holes 7326 in base plate 7310 may be used for installing base plate 7310 into this equipment. In one example, bottom substrate 7320 and top substrate 7324 may be bonded together by use of UV cure adhesive. In this example, base plate 7310 of magnetic clamping fixture 7300 is installed into the UV bonding equipment. The UV bonding equipment may include, for example, a mechanism for dispensing UV cure adhesive and a UV light source for subsequently exposing and, thereby, curing the UV cure adhesive.

At step 7912, using alignment pins 7322, bottom substrate 7320 of the droplet actuator is aligned to and installed against the surface of base plate 7310 of magnetic clamping fixture 7300.

At step 7914, using the UV bonding equipment, UV cure adhesive is dispensed onto bottom substrate 7320 to form a bond line. In one example, **Figure 80** illustrates a side view of magnetic clamping fixture 7300 when in use and a bond line 8010 between, for example, bottom substrate 7320 and top substrate 7324 of the droplet actuator that is being assembled. In this example, bond line 8010 is formed by dispensing a line of UV cure adhesive onto bottom substrate 7320 in a continuous path that will substantially follow the perimeter of top substrate 7324 when installed.

The top plate of the fixture may be formed of any suitable material. Examples include metals, such as aluminum, resins, and plastics. The material may be selected to be transparent to UV light, e.g., transparent acrylic.

At step 7916, using magnetic fields, top substrate 7324 of the droplet actuator that is being assembled is aligned to bottom substrate 7320.

At step 7918, using magnets 7315 and 7330, top plate 7312 of magnetic clamping fixture 7300 is aligned to bottom substrate 7320 and top substrate 7324 of the droplet actuator that is being assembled and also aligned to base plate 7310 of magnetic clamping fixture 7300. The magnetic attraction between magnets 7315 and 7330 results in a uniform compression force between bottom substrate 7320 and top substrate 7324.

At step 7920, the bond line, such as bond line 8010 of Figure 80, is exposed to UV light in order to cure the UV cure adhesive. Referring again to Figure 80, preferably the entirety of bond line 8010 may be exposed to the UV light because there are no mechanical features of magnetic clamping fixture 7300 obstructing the path of the UV light from reaching bond line 8010. In one example, the UV light exposure time may be about one minute, which is a suitable amount of time for curing the UV cure adhesive. One example of UV cure adhesive is the BISCO® EC-2000 Series Conductive Silicone from Rogers Corporation (Rogers, CT).

At step 7922, magnetic clamping fixture 7300 may be removed from the UV bonding equipment. Subsequently, magnetic clamping fixture 7300 may be disassembled from the droplet actuator. More specifically, once the bonding of bottom substrate 7320 and top substrate 7324 of the droplet actuator that has been assembled is complete, base plate 7310 and top plate 7312 of magnetic clamping fixture 7300 are separated. For example, an outward force that is suitably strong to overcome the magnetic attraction forces of magnets 7315 and 7330 is applied to base plate 7310 and top plate 7312, thereby separating the two plates and allowing the droplet actuator to be removed.

In summary and referring again to Figures 73 through 80, magnetic clamping fixture 7300 of the invention uses magnets and standoffs (e.g., magnets 7315 and 7330 and standoffs 7318 and 7332) to provide substantially uniform pressure between the substrates while, for example, the UV cure adhesive is curing. Additionally, the use of magnetic clamping fixture 7300 of the invention ensures a substantially uniform gap height between the substrates while the adhesive is curing. Further, the use of magnetic clamping fixture 7300 of the invention ensures full exposure of, for example, bond line 8010 of Figure 80 to UV light during the curing cycle. This is because there are no mechanical features of magnetic clamping fixture 7300 obstructing the path of the UV light from reaching the entirety of bond line 8010. Yet further, because magnetic clamping fixture 7300 is particularly useful for bonding processes that use UV cure adhesive, certain thermal cycles of the droplet actuator that may otherwise be required by other bonding processed may be avoided. This is beneficial because thermal cycling of droplet actuators may be damaging.

7.7 Digital Microfluidic Systems

The invention is related to simple, low cost power sources for use in combination with microfluidic systems. Certain embodiments of the invention incorporate low voltage power circuits, which are simple and low cost, thereby reducing, preferably eliminating the need for complex and/or expensive high voltage circuits and/or power supplies in microfluidic systems. Additionally, certain embodiments of the invention incorporate simple, fixed (i.e., non-programmable) mechanical mechanisms for connecting the electrowetting voltages to a droplet actuator. Further, alternative power sources that are not dependent on the presence of a power distribution grid are described in combination with microfluidic systems.

Figures 81A, 81B, and 81C illustrate functional block diagrams of examples of simple and inexpensive ways to supply power to microfluidic systems. In one example, Figure 81A shows a microfluidics system 8100 that includes a direct current (DC) power source 8112. DC power source 8112 may be, for example, one or more rechargeable or disposable (non-rechargeable) batteries. In one example, DC power source 8112 may be one or more standard AA, AAA, C, and/or D size rechargeable or disposable batteries and/or one or more disc type rechargeable or disposable batteries (e.g., watch or hearing aid type batteries). The batteries may be packaged separately but connected to microfluidics system 8100. Alternatively, the batteries may be integrated directly into the physical instantiation of microfluidics system 8100. In one example, the batteries may be integrated directly into the packaging of a disposable droplet actuator cartridge. DC power source 8112 is one example of a power source for a microfluidics system that is not dependent on the presence of a power distribution grid.

The output of DC power source 8112 supplies a power conditioning circuit 8114. Power conditioning circuit 8114 may be specific to DC power source 8112. Power conditioning circuit 8114 may be any power conditioning circuit for, for example, stepping up or down the voltage output of DC power source 8112, for regulating the voltage output of DC power source 8112, for converting the voltage output of DC power source 8112 from DC to alternating current (AC), and the like. The output of power conditioning circuit 8114 supplies control electronics 8116 that then feeds, for example, a droplet actuator 8118. The design of control electronics 8116 may be dependent on the design of power conditioning circuit 8114. Control electronics 8116 may be any control circuitry for, for example, distributing electrowetting voltages to one or more channels of droplet actuator 8118, for providing a switching function of the electrowetting voltages, and the like.

In another example, Figure 81B shows a microfluidics system 8120 that includes a DC generator 8122. DC generator 8122 may be, for example, a low RPM (rotations per minute) permanent magnet DC generator, such as a hand crank DC generator or any other form of generator. Examples of permanent magnet DC generators may include, but are not limited to, DC generator model 443540 from Windstream Power LLC (N. Ferrisburg, VT) and DC generator model EM-8090 from Pascon® (Roseville, CA). By way of example, the output of the EM-8090 generator is about 12 volts DC at about 25 watts. DC generator 8122 may be provided separately but connected to microfluidics system 8120. Alternatively, DC generator 8122 may be integrated directly into the physical instantiation of microfluidics system 8120. DC generator 8122 is another example of a power source for a microfluidics system that is not dependent on the presence of a power distribution grid.

The output of DC generator 8122 supplies a power conditioning circuit 8124. Power conditioning circuit 8124 may be specific to DC generator 8122. Power conditioning circuit 8124 may be any power conditioning circuit for, for example, stepping up or down the voltage output of DC generator 8122, for regulating the voltage output of DC generator 8122, for converting the voltage output of DC generator 8122 from DC to AC, and the like. The output of power conditioning circuit 8124 supplies control electronics 8126 that then feeds, for example, the droplet actuator 8118. The design of control electronics 8126 may be dependent on the design of power conditioning circuit 8124. Control electronics 8126 may be any control circuitry for, for example, distributing electrowetting voltages to one or more channels of droplet actuator 8118, for providing a switching function of the electrowetting voltages, and the like.

In one embodiment, the output of DC generator 8122 may be stored in capacitors (not shown). By way of example, to supply 3.5 watts of power for about 20 minutes requires about 4,200 joules of energy. Assuming about 50% efficiency of drawing power off the capacitors, about 10,000 joules of energy needs to be stored. At about 2.5 volts DC, this requires about 3,000 farads of capacitance. 3,000 farads of capacitance may be achieved, for example, by the use of fifteen 200 farad capacitors.

In another example, Figure 81C shows a microfluidics system 8130 that includes an AC power source 8132. AC power source 8132 may be, for example, the standard 8110 volt AC power in a building that is accessible via a standard AC wall outlet. Unlike DC power source 8112 of Figure 81A and DC generator 8122 of Figure 81B, AC power source 8132 of Figure 81C is a power source for a microfluidics system that is dependent on the presence of a power distribution grid. However, the use of standard 110 volt AC power may reduce, preferably eliminate, the need for expensive power supplies in microfluidic systems.

The output of AC power source 8132 supplies a power conditioning circuit 8134. Power conditioning circuit 8134 may be specific to AC power source 8132. Power conditioning circuit 8134 may be any power conditioning circuit for, for example, stepping up or down the voltage output of AC power source 8132, for converting the voltage output of AC power source 8132 from AC to DC and then regulating the resulting DC voltage, and the like. The output of power conditioning circuit 8134 supplies control electronics 8136 that then feeds, for example, the droplet actuator 8118. The design of control electronics 8136 may be dependent on the design of power conditioning circuit 8134. Control electronics 8136 may be any control circuitry for, for example, distributing electrowetting voltages to one or more channels of droplet actuator 8118, for providing a switching function of the electrowetting voltages, and the like.

More details of examples of simple, low cost power circuits that are suitable for use in microfluidic systems, such as microfluidic systems 8100, 8120, and 8130, are described with reference to Figures 82A through 87B.

Referring again to Figures 81A, 81B, and 81C, microfluidic systems may include a DC power source, such as DC power source 8112 of Figure 81A, a DC generator, such as DC generator 8122 of Figure 81B, an AC power source, such as AC power source 8132 of Figure 81C, and any combinations thereof.

Figure 82A illustrates a schematic diagram of one example of a simple, low cost power conditioning circuit 8200 for use in microfluidic systems. Power conditioning circuit 8200 includes a DC power source 8210 for supplying a low voltage source V-IN. DC power source 8210 may be, for example, a DC power source, such as DC power source 8112 of Figure 81A. Power conditioning circuit 8200 also includes a transformer 8212, an oscillator 8214, and a solid state switch 8216.

DC power source 8210, oscillator 8214, which may provide, for example, a square wave, and solid state switch 8216 may be arranged as shown to develop a low voltage AC signal at the primary of transformer 8212. A standard rectifier circuit at the secondary of transformer 8212 provides a DC voltage output V-OUT, which is a higher voltage than V-IN. A feedback loop is provided from V-OUT to oscillator 8214. Further power distribution and switching (not shown) of V-OUT may be provided in order to use V-OUT to supply the electrowetting voltage to one or more channels of a droplet actuator (not shown) for controlling droplet operations.

Figure 82B illustrates a plot 8250 of V-IN vs. V-OUT of power conditioning circuit 8200 of Figure 82A. Plot 8250 shows that there may be a certain amount of noise on V-OUT. However, a certain amount of noise may be tolerated in electrowetting applications.

Figure 83A illustrates a schematic diagram of another example of a simple, low cost power conditioning circuit 8300 for use in microfluidic systems. Power conditioning circuit 8300 includes a DC generator 8310 for supplying a low voltage source V-IN. DC generator 8310 may be, for example, a DC generator, such as DC generator 8122of Figure 81B. Power conditioning circuit 8300 also includes a transformer 8312 and a fast mechanical switch 8316. Mechanical switch 8316 may be mechanically coupled to the rotation of DC generator 8310 via, for example, a gear arrangement. In this way, mechanical switch 8316 may be opened and closed based on the rotation of DC generator 8310. In one example, mechanical switch 8316 may be geared down with respect to the rotation of DC generator 8310. In other words, mechanical switch 8316 opens and closes at a slower rate than the rotation of DC generator 8310 by some ratio.

DC generator 8310 and mechanical switch 8316 may be arranged as shown to develop a low voltage AC signal at the primary of transformer 8312. A standard rectifier circuit at the secondary of transformer 8312 provides a DC voltage output V-OUT, which is a higher voltage than V-IN. A feedback loop is provided from V-OUT to mechanical switch 8316. Further power distribution and switching (not shown) of V-OUT may be provided in order to use V-OUT to supply the electrowetting voltage to one or more channels of a droplet actuator (not shown) for controlling droplet operations.

Figure 83B illustrates a plot 8350 of V-IN vs. V-OUT of power conditioning circuit 8300 of Figure 83A. Plot 8350 shows that there may be a certain amount of noise on V-OUT. Again, a certain amount of noise may be tolerated in electrowetting applications.

Figure 84A illustrates a schematic diagram of yet another example of a simple, low cost power conditioning circuit 8400 for use in microfluidic systems. Power conditioning circuit 8400 is substantially the same as power conditioning circuit 8300 of Figure 83A, except that it further includes a switch bank 8410 for distributing V-OUT. Switch bank 8410 may include multiple mechanical switches. Each mechanical switch of switch bank 8410 is electrically connected to V-OUT at one side and to a certain channel 8412 of a droplet actuator at the other side. In this way, the respective mechanical switches of switch bank 8410 are used to distribute V-OUT of power conditioning circuit 8400 to multiple channels 8412, respectively, of a droplet actuator for controlling droplet operations. Examples of mechanical switching mechanisms for controlling droplet operations are described with reference to Figures 88A, 88B, and 89.

As described in Figure 83A, DC generator 8310 is mechanically coupled to mechanical switch 8316 and, thus, mechanical switch 8316 may be opened and closed based on the rotation of DC generator 8310. Similarly, DC generator 8310 is mechanically coupled to the mechanical switches of switch bank 8410. In this way, each mechanical switch of switch bank 8410 may also be opened and closed based on the rotation of DC generator 8310. In one example, the gear ratios are such that mechanical switches of switch bank 8410 are opening and closing at a slower rate than mechanical switch 8316.

Figure 84B illustrates a plot 8450 of V-IN vs. V-OUT of power conditioning circuit 8400 of Figure 84A. Plot 8450 shows that there may be a certain amount of noise on V-OUT. Again, a certain amount of noise may be tolerated in electrowetting applications.

Figure 85 illustrates a schematic diagram of yet another example of a simple, low cost power conditioning circuit 8500 for use in microfluidic systems. Power conditioning circuit 8500 includes an AC power source 8510 that is electrically connected to a switch array 8512. Switch array 8512 may be, for example, an array of silicon-controlled rectifiers (SCRs), an array of triodes for alternating current (TRIACs), an array of bipolar junction transistors (BJTs), an array of conventional switches, an array of mechanical switches, and the like.

Switch array 8512 may drive multiple channels of, for example, a droplet actuator for controlling droplet operations. For example, Figure 85 shows switch array 8512 electrically connected to a substrate 8514 of a droplet actuator. AC power source 8510 may be, for example, the standard 110 volt AC power in a building that is accessible via a standard AC wall outlet. The use of standard 110 volt AC power may reduce, preferably eliminate, the need for expensive power supplies in microfluidic systems.

Figure 86 illustrates a schematic diagram of still another example of a simple, low cost power conditioning circuit 8600 for use in microfluidic systems. Power conditioning circuit 8600 includes a DC power source 8610 for supplying a low voltage source V-IN. DC power source 8610 may be, for example, a DC power source, such as DC power source 8112 of Figure 81A. Power conditioning circuit 8600 also includes a set of low voltage switches 8612. Low voltage switches 8612 may be, for example, solid state switches. Low voltage switches 8612 feed a set of voltage multipliers 8614 that are used to convert the low voltage signals to a higher voltage level that is suitable for electrowetting. Voltage multipliers 8614 may be any method of translating a low voltage to a high voltage in an effective and low cost way. Examples of voltage multipliers are described with reference to Figures 87A and 87B. Each voltage multiplier 8614 may drive one or more channels of a droplet actuator (not shown) for controlling droplet operations.

High voltage switches may be complex and costly. By contrast, power conditioning circuit 8600, which uses low voltage switches, provides a simple, low cost power circuit for use in microfluidic systems.

Figures 87A and 87B illustrate schematic diagrams of examples of voltage multiplier circuits for use in certain power conditioning circuits of microfluidic systems. In one example, Figure 87A shows a charge pump method of translating a low voltage to a high voltage. More specifically, Figure 87A shows a Cockcroft–Walton (CW) generator 8700, which is an example of a voltage multiplier that is suitable for voltage multipliers 8614 of Figure 86. As is well known, a CW generator is a voltage multiplier that converts AC or pulsing DC electrical power from a low voltage level to a higher DC voltage level. A CW generator is made up of a voltage multiplier ladder network of capacitors and diodes to generate high voltages.

In another example, Figure 87B shows an inductive method of translating a low voltage to a high voltage. More specifically, Figure 87B shows a transformer 8750, which may be a step up transformer. The primary of transformer 8750 may be fed by, for example, a low voltage square wave (V-low). A high voltage square wave (V-high) is produced at the secondary of transformer 8750. Transformer 8750 is an example of a voltage multiplier method that is suitable for voltage multipliers 8614 of Figure 86.

Figure 88A illustrates a top view of a portion of a droplet actuator 8800 and shows one example of a simple, fixed (i.e., non-programmable) mechanical mechanism for controlling the electrowetting voltage to the channels thereof. **Figure 88B** illustrates a cross-sectional view of droplet actuator 8800 that is taken along line AA of Figure 88A.

Droplet actuator 8800 includes a bottom substrate 8810 and a top substrate 8812 that are separated by a gap 8814. An arrangement of channel input/output (I/O) pads may be associated with one or both substrates. In one example, an arrangement of channel I/O pads 8816 is associated with bottom substrate 8810. Channel I/O pads 8816 are the I/O pads for connecting an electrowetting voltage to the channels of droplet actuator 8800 for controlling droplet operations. Additionally, a voltage electrode 8818 is associated with top substrate 8812. An electrowetting voltage 8820 may be electrically connected to voltage electrode 8818.

One or more ball bearings 8822 (e.g., ball bearings 8822A and 8822B) may be provided in gap 8814 of droplet actuator 8800. Each ball bearing 8822 may be formed of any metallic material that is electrically conductive; materials such as stainless steel, gold, and aluminum. Each ball bearing 8822 is sized such that it may make physical contact with both a channel I/O pad 8816 and a certain voltage electrode 8818 (see Figure 88B) and, thereby, provide an electrical connection therebetween. In this way, the electrowetting voltage 8820 may be electrically connected to any channel I/O pad 8816 according to the position of the one or more ball bearings 8822. By way of example, Figure 88A shows ball bearing 8822A positioned at channel I/O pad 8816A and ball bearing 8822B positioned at channel I/O pad 8816L. As a result, the electrowetting voltage 8820 is connected to channel I/O pads 8816A and 8816L, while the electrowetting voltage 8820 is not connected to the remaining channel I/O pads 8816.

In one example, the position of one or more ball bearings 8822 may be controlled by gravity. That is, droplet actuator 8800 may be tilted in a controlled manner to cause the one or more ball bearings 8822 to roll to certain channel I/O pad 8816 locations. The degree of tilt may also control the rate of movement of the one or more ball bearings 8822. Additionally, droplet actuator 8800 may be designed to have different regions with different gaps sizes and/or channels (not shown) that are along the arrangement of channel I/O pads 8816 and having different widths. In this scenario, droplet actuator 8800 may include different sized ball bearings 8822 for controlling the regions of droplet actuator 8800 in which they may operate. For example, a certain sized ball bearing 8822 is blocked when encountering a gap or channel that is too small to receive it.

The use of electrically conductive ball bearings 8822 is one example of a simple, fixed (i.e., non-programmable) mechanical mechanism for controlling the electrowetting voltage to the channels of a droplet actuator.

Figure 89 illustrates a top view of a portion of a droplet actuator 8900 and shows another example of a simple, fixed (i.e., non-programmable) mechanical mechanism for controlling the electrowetting voltage to the channels thereof.

Droplet actuator 8900 includes a bottom substrate 8910 and a top substrate 8912 that are separated by a gap. An arrangement of channel I/O pads and droplet operations electrodes may be associated with one or both substrates. For example, an arrangement of channel I/O pads 8914 and an arrangement of droplet operations electrodes 8916 (e.g., electrowetting electrodes) are associated with bottom substrate 8910. Channel I/O pads 8914 are the I/O pads for connecting an electrowetting voltage 8920 to droplet operations electrodes 8916 of droplet actuator 8900 for controlling droplet operations.

In one example, droplet actuator 8900 includes channel I/O pads 8914A, 8914B, 8914C, 8914D, and 8914E that are electrically connected to droplet operations electrodes 8916A, 8916B, 8916C, 8916D, and 8916E by certain wiring routes 8918. The wiring routes 8918 of droplet actuator 8900 may be customized to achieve certain droplet operations for performing certain protocols. In the example of Figure 89, wiring routes 8918 of droplet actuator 8900 are customized for performing a droplet merge operation using droplet operations on droplet actuator 8900. More specifically, channel I/O pad 8914A is wired to droplet operations electrode 8916A, channel I/O pad 8914B is wired to droplet operations electrode 8916E, channel I/O pad 8914C is wired to droplet operations electrode 8916B, channel I/O pad 8914D is wired to droplet operations electrode 8916D, and channel I/O pad 8914E is wired to droplet operations electrode 8916C.

In this example, electrowetting voltage 8920 may be applied sequentially from channel I/O pad 8914A to 8914E via, for example, a spring-loaded voltage probe 8922. In doing so, a process of merging, for example, a droplet 8924A with a droplet 8924B may be achieved. For example, the process of merging droplet 8924A with droplet 8924B may include, but is not limited to, the following steps.

In one step, voltage probe 8922 is contacted to channel I/O pad 8914A in order to energize droplet operations electrode 8916A. As a result, droplet 8924A is transported using droplet operations to droplet operations electrode 8916A from, for example, an adjacent droplet operations electrode 8916 that is not shown.

In another step, voltage probe 8922 is removed from channel I/O pad 8914A and is contacted to channel I/O pad 8914B in order to energize droplet operations electrode 8916E. As a result, droplet 8924B is transported using droplet operations to droplet operations electrode 8916E from, for example, an adjacent droplet operations electrode 8916 that is not shown.

In another step, voltage probe 8922 is removed from channel I/O pad 8914B and is contacted to channel I/O pad 8914C in order to energize droplet operations electrode 8916B. As a result, droplet 8924A is transported using droplet operations from droplet operations electrode 8916A to droplet operations electrode 8916B, which is in the direction of droplet 8924B.

In another step, voltage probe 8922 is removed from channel I/O pad 8914C and is contacted to channel I/O pad 8914D in order to energize droplet operations electrode 8916D. As a result, droplet 8924B is transported using droplet operations from droplet operations electrode 8916E to droplet operations electrode 8916D, which is in the direction of droplet 8924A. At the end of this step, droplet 8924A is at droplet operations electrode 8916B and droplet 8924B is at droplet operations electrode 8916D.

In another step, voltage probe 8922 is removed from channel I/O pad 8914D and is contacted to channel I/O pad 8914E in order to energize droplet operations electrode 8916C. As a result, droplet 8924A is transported using droplet operations from droplet operations electrode 8916B to droplet operations electrode 8916C. Likewise, droplet 8924B is transported using droplet operations from droplet operations electrode 8916D to droplet operations electrode 8916C. Consequently, droplet 8924A and droplet 8924B are merged using droplet operations at droplet operations electrode 8916C.

The aforementioned process is an example of using a simple, fixed (i.e., non-programmable) mechanical mechanism (e.g., a fixed sequence of moving voltage probe 8922) for controlling the electrowetting voltage to the channels of a droplet actuator. Certain droplet operations and protocols may be achieved by customizing the droplet actuator, while using the same fixed mechanical mechanism for supplying the electrowetting voltage. Further, the movement of voltage probe 8922 may be based on the rotation of a DC generator, such as described with reference to Figures 83A and 84A. For example, spring-loaded voltage probe 8922 may be one instantiation of switch bank 8410 of power conditioning circuit 8400 of Figure 84A, in which switch bank 8410 is mechanically coupled to the rotation of DC generator 8310.

In another embodiment, voltage probe 8922 may be replaced by the ball bearing mechanism that is described with reference to Figures 88A and 88B.

In yet other embodiments, the ball bearing mechanism of Figures 88A and 88B, as well as voltage probe 8922 of Figure 89 may be replaced by any mechanical switching mechanism that may be coupled, for example, to the rotation of a DC generator, such as described with reference to Figures 83A and 84A. In one example, the mechanical switching mechanism may be a player piano or music box type of cylinder that rotates. These cylinders are sometimes referred to as pinned barrels. The cylinder mechanism may be have a fixed arrangement of contactors (or pins) protruding from the surface thereof. In operation, the player piano or music box type of cylinder may be electrically connected to an electrowetting voltage and installed such that its contactors are aligned with certain I/O pads of a droplet actuator. When rotated, the contactors come into physical contact with the I/O pads in a certain fixed sequence for supplying the electrowetting voltage thereto in a fixed sequence. Again, the design of the droplet actuator may be customized to perform a certain protocol, while the mechanical switching mechanism remains a simple, fixed (i.e., non-programmable) mechanical mechanism for controlling the droplet operations.

Figure 90 illustrates a perspective view of an example of a structure 9000 that uses static electricity for supplying power to microfluidic systems. For example, structure 9000 may include a substrate 9010 that has one or more dielectric regions 9012. Dielectric regions 9012 may be formed of any material that is capable of storing a high voltage charge for long periods of time. For example, dielectric regions 9012 maybe formed of Teflon® dielectric material. These one or more dielectric regions 9012 may be charged with static electricity via, for example, a Van de Graaf generator 9014. In one example, Van de Graaf generator 9014 may be a hand crank Van de Graaf generator.

In operation, substrate 9010, which has the dielectric regions 9012 that are charged, may be positioned in close proximity to another substrate, such as a substrate 9016 that has an arrangement of droplet operations electrodes 9018 (e.g., electrowetting electrodes). Droplet operations electrodes 9018 of substrate 9016 may be patterned to align with the pattern of dielectric regions 9012. In doing so, certain droplet operations electrodes 9018 may be energized by the static charge of dielectric regions 9012 and droplet operations may occur. The position of substrate 9010 relative to substrate 9016 may be adjusted in order to transfer the charge from dielectric regions 9012 to the desired droplet operations electrodes 9018.

The use of charged dielectric regions on a substrate is another example of a simple, fixed (i.e., non-programmable) mechanical mechanism for controlling the electrowetting voltage to the channels and/or droplet operations electrodes of a droplet actuator. Further, structure 9000 is an example of a simple inexpensive power source that may replace expensive high voltage power supplies in microfluidic systems.

7.8 Real-Time Imaging of Luminescent Immunoassays on a Digital Microfluidic Platform

The invention is an immunoassay multiplexing platform that uses digital microfluidics and real-time imaging of flash-based chemiluminescent signals. Various aspects of the invention may include, but are not limited to, flash-based chemiluminescence (CL), real-time imaging and interpretation, spatial multiplexing, and lab-on-a-chip. In one embodiment, the invention provides for 4-plexed flash-based chemiluminescent assays (e.g., 4 different cytokine assays, such as IL-6, TNF-alpha, IL-8, and IL-1beta) on each of 12 samples (i.e., 48 immunoassays). In another embodiment, the invention provides a dynamic smart imaging system configured to simultaneously read chemiluminescent signals from a row of 12 sample droplets and extract intensity information (e.g., algorithms). The dynamic imaging array format provides for ready interrogation of the chemiluminescent signal in individual sample droplets in real time and makes decisions on droplet movement.

Digital microfluidics (e.g., spatial multiplexing) combined with dynamic smart imaging may be used to improve (i.e., decrease) time-to-result and overall throughput of a multiplexed immunoassay. In addition, the digital microfluidic platform of the invention provides numerous advantages including, but not limited to: low cost of instrument (reducing capital costs), higher levels of automation and integration (reducing labor costs), disposable droplet actuators (reducing maintenance costs), and any combinations thereof.

In yet another embodiment, the invention provides a high-throughput assay platform for an individual investigator that may circumvent the drawbacks associated with traditional multiplexing systems.

In yet another embodiment, the invention may be utilized by researchers conducting multi-center clinical trials to standardize diagnostic assays (i.e., reduce variability among testing laboratories), control costs, and/or decrease time-to-results.

The immunoassay platform of the invention may, in yet another embodiment, be used for rapid and accurate profiling of cytokines for cancer immunotherapy. Multiparameter analysis of cytokine patterns may, for example, be used to design, optimize, and/or monitor cancer vaccines. Multiparameter analysis may be used to create “signatures” of the most desirable immune responses to anticancer targeted therapies.

7.8.1 Flash-based Chemiluminescent Cytokine Profiling

The invention uses a microfluidic platform to manipulate (i.e., using droplet operations) and analyze individual droplets in a flash-based chemiluminescent immunoassay. Droplet operations include, for example, sample-processing, mixing, incubation, detection, and/or waste-handling. Droplet operations may be performed on large numbers of droplets simultaneously and independently, which allows complex protocols to be flexibly implemented directly through software control. The speed and agility with which droplets may be positioned enables extremely rapid throughput in a multiplexed immunoassay.

Figure 91 illustrates a perspective view of an example of a microfluidics assay multiplexing platform 9100 and a process of performing high-throughput, flash-based chemiluminescent cytokine profiling. The method of the invention of Figure 91 uses droplet-based microfluidics and dynamic smart imaging to interrogate a chemiluminescent signal in real time and make decisions on droplet movement. Because droplet-based microfluidics are used, chemiluminescent reactions may be readily initiated (e.g., by merging and mixing droplets) directly in view of an imaging system.

Multiplexing platform 9100 may include a multi-well droplet actuator 9110 in combination with an imaging system 9112. In one example, multi-well droplet actuator 9110 may be a 12-well droplet actuator configured to perform 4 different immunoassays. In one example, imaging system 9112 may be an imaging luminometer system that is configured to read from a row of 12 droplets that are undergoing simultaneous chemiluminescent reactions. An example of imaging system 9112 is described in more detail with reference to Figure 96.

A short exposure image of a row of droplets (e.g., 5 droplets) on droplet actuator 9110 is captured using imaging system 9112 (step 1). Image processing software is used to interpret the luminescence images of the droplets and feed the metrics to a decision tree algorithm to determine whether sufficient images have been acquired to make a quantitation call for any particular droplet (step 2). A signal(s) is sent to the droplet control electronics such that each droplet is independently manipulated based on metric data for a given droplet (step 3). In one example, droplets with low chemiluminescent signal may stay in an imaging zone for further analysis. Other droplets may be transported out of the imaging zone and into a waste reservoir. New droplets may be transported into the imaging zone (step 4). Because imaging system 9112 is a closed-loop that includes image capture, image processing, decision tree algorithms, and droplet control electronics, assay throughput may be optimized.

Figure 92 illustrates a schematic diagram of a process 9200 of performing dynamic, smart imaging on a digital microfluidic platform. In time frame, t_0, sample droplets (i.e., analyte droplets) that include bound chemical probes (S, red) and trigger droplets (T, yellow) are positioned at certain droplet operations electrodes. In Figure 92, arrows indicate the direction of droplet transport for the subsequent step. At time $t_0+\Delta t$, sample droplets and trigger droplets are merged and mixed within the imaging zone to form activated droplets (A, orange). Successive imaging exposures are continually acquired at time $t_i \ll \Delta t$. Between image acquisitions, the image processing software is interpreting the luminescence images of the activated droplets and feeding metrics to a decision tree algorithm to determine whether enough images have been acquired to make a quantitation call for any particular activated droplet. At time $t_0+2\Delta t$, the decision tree has determined that sufficient exposures have been taken of the third activated droplet (from the left). A signal is sent to the droplet control electronics to move (in direction of arrow) the activated, but now depleted, droplet out of the imaging zone and into a waste reservoir. Other droplets remain in the detection zone for further quantitation. At time $t_0+3\Delta t$, a new cycle of merging the next sample droplet in the queue with a trigger droplet is initiated. All sample droplets are independently manipulated and analyzed and new sample reactions subsequently initiated.

Figure 93 illustrates a top view of a portion of an electrode configuration 9300 of a droplet actuator that is used for merging and detection of activated droplets in a flash-based chemiluminescent assay. Electrode configuration 9300 includes a merging location 9310 and a detection spot 9312 (e.g., photomultiplier tube, PMT). Merging location 9310 is separated from detection spot 9312 by, for example, about 40 unit electrodes. In this example, detection spot 9310 is designed for the detection of a single activated droplet.

Acridinium-NHS (AE) is a flashed-based chemiluminescent label that is triggered by a combination of sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) (i.e., trigger solution). A stock solution of 5 mM AE-NHS was prepared by dissolving a lyophilized AE compound in 316μL of dimethyl formamide. Titration dilutions were made in a purification buffer. Trigger solution was prepared by mixing equal volumes of 0.2M NaOH and 0.06% H_2O_2. The merging and detection protocol performed using electrode configuration 9300 was to dispense one droplet of AE-NHS and one droplet of trigger solution mixture. The droplets were merged at location

9310 to form an activated droplet. The activated droplet was transported from merging location 9310 to detection spot 9312 at a switching speed of 10 electrodes per second. The chemiluminescence signal was detected by a photon counting PMT for 5 seconds at a rate of 4 Hz to obtain 20 data points for each concentration (dilution) of AE-NHS. Because a PMT was utilized, only one reaction (i.e., activated droplet) was read at a time. The area under the curve was calculated and a standard curve of AE-NHS concentration versus area under the curve was generated (e.g., the plot shown in Figure 94)

Figure 94 shows a plot 9400 of averaged (n=4) standard curve data of AE-NHS concentration versus chemiluminescent signal generated using the electrode configuration of Figure 93. Data was obtained from 4 separate standard curves generated on-chip where the error bars represent the standard error between measurements. The Limit of Detection (LOD) was calculated as three standard deviations above the mean of the background and was obtained as 0.95 attomoles in a 300nL droplet of AE-NHS.

In a different protocol performed on a different droplet actuator configuration, the AE-NHS droplet and the trigger droplet were merged directly underneath a detector. The chemiluminescent signal was measured while the droplet was shuttled between 3 electrodes underneath the detector. The inset in Figure 94 shows the generation and decay of the chemiluminescent signal for a 300nL droplet containing 1.5 femtomoles of AE-NHS.

As proof of concept, complete immunoassays for cardiac IL-6 and TNF-alpha have been performed. **Figure 95** shows a plot 9500 of standard curve data of IL-6 and TNF-alpha concentrations generated by use of glow-based chemiluminescent immunoassays on a digital microfluidic droplet actuator platform. The immunoassays used APS-5, a dioxetane based chemiluminescent substrate that results in a steady glow reaction (e.g., many minutes).

7.8.2 Detection System

Figure 96 illustrates a perspective view of imaging luminometer system 9600 for simultaneous detection of chemiluminescent signals from multiple microfluidic droplets. In one embodiment, imaging luminometer system 9600 may be a 12-droplet imaging system to simultaneously capture chemiluminescent signals from 12 different sample droplets aligned in a row. In the multi-well digital microfluidics format, sample droplets dispensed from individual wells (e.g., 12 wells) preferentially move in a manner such that the samples are maintained in their own independent paths. Different assay reagent droplets for each sample are preferentially moved in the vertical directions intersecting with the sample droplets. The sample droplets queue up at an imaging zone. Once the sample droplets have entered the imaging zone, they are mixed with flash trigger droplets. Mixing of flash trigger droplets with the sample droplets is maximized in this linear fashion (refer to Figure 92). In one example, when the center-to-center spacing in the detection zone is about 4.5 mm (384 well spacing), a field size of about 54 mm diameter is required for imaging 12 droplets in a row.

Imaging luminometer system 9600 may be configured for imaging a row of 12 sample droplets. Imaging luminometer system 9600 may include a rectangular CCD droplet actuator 9610. The rectangular shape of CCD droplet actuator 9610 substantially aligns with a linear row of sample droplets in the imaging zone. In one example, CCD droplet actuator 9610 may be Hamamatsu S7031-0960 with 32,768 pixels in a 512 x 64 pixel format (pixel size 24 μm). In this example, the active area of CCD droplet actuator 9610 is about 12.288 mm x 1.392 mm. Because of the size of CCD droplet actuator 9610 relative to the size of the imaging field (e.g., 54 mm long), a camera lens system 9612 may be used to demagnify the image onto CCD droplet actuator 9610. Camera lens system 9612 may, for example, demagnify an image by a factor of 4-5X. For example, a 1/5X magnification of a 54 mm long imaging field corresponds to 10.8 mm, an image size suitable for CCD droplet actuator 9610, such as Hamamatsu S7031-0960. In one example, camera lens system 9612 may be a Xenon 25 mm f/0.95 fixed-focus, 1" format lens system from Schneider Optics (Hauppauge, NY). The working f/# in the image plane with a magnification of 1/5X on this lens increases to f/1.3 and provides sufficient light collection to maximize throughput. Because of the length of the front working distance for this lens, a folding mirror 9614 may be used to provide a compact configuration of imaging luminometer system 9600.

A row of sample droplets 9618 (e.g., 5 droplets) may be positioned in proximity to imaging luminometer system 9600. The chemiluminescent signal from each sample droplet 9618 may be projected along the long-axis of CCD droplet actuator 9610 through camera lens system 9612. In one example, droplet images may be evaluated about every 100msec to determine which droplet has satisfied pre-established criteria on its kinetic intensity profile sufficient to establish the concentration of the sample analyte. Some droplets may have high-intensity at any given time interval and other droplets may have low intensity, as illustrated in the inset in Figure 96.

Luminescent measurements are typically collected as a series of data points over time beginning at the introduction of a trigger solution to the chemiluminescent-labeled analyte sample.

Typically, in glow-based reactions, data collection may extend over a sufficient period of time until the luminescence signal has significantly faded and/or the kinetic profile has reached either a steady state or a slowly decaying state. In a flash-based chemiluminescent assay, data collection may extend over shorter time intervals until a peak flash point is reached. Various metrics, such as peak height, slope, and/or area-under-the-curve, may be applied to correlate the data to a known standard set of analyte titrations. In flash-based immunoassays, the metrics for determining the amount of data (and therefore optical collection time) may be cytokine specific.

Figure 97 shows a plot 9700 of kinetics (time-dependent) intensity profiles of on-chip ATP mediated luciferase reactions. Data was generated from glow-based pyrosequencing reactions that use ATP driven luciferase-mediated conversion of luciferin to oxyluciferin that generates a visible glow in amounts that are proportional to the amount of ATP. The reactions run on a digital microfluidics platform demonstrate a highly reproducible intensity profile. The repeatability of the slow-glow assay kinetics may be due to the small droplet size and rapid mixing that occurs on a digital microfluidic droplet actuator.

7.9 Plasma Treated Substrates for Improved Surface Energy

The invention provides droplet actuator devices that are fabricated using a plasma treatment process to raise the surface energy of the bottom and top substrates. In particular, the higher surface energy makes the surfaces of the substrates hydrophilic so that aqueous fluids are attracted thereto. Additionally, the hydrophilic nature of the surfaces improves the adhesion of the conductive layer (e.g., PEDOT coating) to the surfaces of the substrates.

For example, the plasma treatment itself may be a Corona treatment, which is a high-voltage arc that is run over the substrate surface. The manner in which the arc is created and applied to the surface may vary with the different plasma treatment systems.

As stated above, the purpose of the Corona treatment process is to raise the surface energy of the substrates, which causes the surface to be hydrophilic and improves the adhesion of the conductive layer (e.g., PEDOT coating). The substrate material may be, for example, polymethyl methacrylate (PMMA), polycarbonate (PC), and cyclo-olefin polymer (COP). The substrates may be fed into the Corona treatment process with no other coatings present thereon. After the Corona treatment process is complete, the conductive layer (e.g., PEDOT coating) is applied directly to the treated surface of the substrates. After the conductive layer is applied, the hydrophobic coating may be applied.

Examples of Corona treatment equipment may include, but are not limited to, the Dyne-A-Mite 3D treater, Dyne-A-Mite HP air plasma surface treater, and Dyne-A-Mite IT air plasma surface treater, all from Enercon Industries Corporation (Menomonee Falls, WI); and the Plasma-Jet 3D surface-treating system from Corotec Corporation (Farmington, CT).

7.10 Systems

Referring to Figures 1 through 97, the invention may be embodied as a method, system, 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. 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.

Computer 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 computer 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 execute entirely on the user's computer, partly on the user's computer, as a stand-alone software package, partly on the user's computer and partly on a remote computer or entirely on the remote computer or server. In the latter scenario, the remote computer may be connected to the user's computer through a local area network (LAN) or a wide area network (WAN), or the connection may be made to an external computer (for example, through the Internet using an Internet Service Provider).

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 computer program instructions. These computer program instructions may be provided to a processor of a general purpose computer, special purpose computer, or other programmable data processing apparatus to produce a machine, such that the instructions, which execute via the processor of the computer or other programmable data processing apparatus, create means for implementing the functions/acts specified in the methods.

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

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

8 Concluding Remarks

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

THE CLAIMS

We claim:

1. A droplet actuator device for conducting droplet operations, comprising:

   (a) two substrates comprising a top substrate and a bottom substrate, separated to form a gap of sufficient height to conduct droplet operations; and

   (b) electrodes arranged on the bottom substrate,

   wherein the top substrate comprises a three dimensional (3D) structure formed substantially orthogonally to the bottom substrate plane, wherein the gap and bottom substrate, with electrodes arranged thereon, follow the profile of the 3D structure such that a continuous gap size is maintained to form a continuous droplet operations path.

2. A droplet actuator device of claim 1, wherein one or more droplets are transported along the electrodes arranged on the bottom substrate in an x and/or y axis direction of the droplet actuator device, wherein once the one or more droplet encounter the 3D structure the one or more droplets are transported along the z-axis of the droplet actuator device within the gap of the 3D structure.

3. A droplet actuator device of any of claims 1 and following, wherein the 3D structure comprises a dome-shaped structure.

4. A droplet actuator device of any of claims 1 and following, further comprising a detector wherein the detector is positioned at the topmost portion of the 3D structure.

5. A droplet actuator device of any of claims 1 and following, wherein the electrodes comprise an array of electrodes.

6. A droplet actuator device of any of claims 1 and following, wherein the electrodes comprise one or more lines of electrodes.

7. A droplet actuator device for conducting droplet operations, comprising:

(a) two substrates comprising a top substrate and a bottom substrate, separated to form a gap of sufficient height to conduct droplet operations, wherein the bottom substrate comprises a first region and a second region;

(b) electrodes arranged on the first region of the bottom substrate; and

(c) a reservoir positioned at the second region of the bottom substrate,

wherein the top substrate comprises a three dimensional (3D) structure formed substantially orthogonally to the bottom substrate plane, wherein the gap and bottom substrate follow the profile of the 3D structure such that a continuous gap size is maintained to form a continuous droplet operations path.

8. A droplet actuator device of any of claims 7 and following, wherein the gap comprises filler fluid in the first region of the bottom substrate, and no filler fluid in the reservoir.

9. A droplet actuator device of any of claims 7 and following, wherein one or more droplets are transported along the electrodes arranged on the bottom substrate in an x and/or y axis direction of the droplet actuator device, wherein once the one or more droplet encounter the 3D structure the one or more droplets are transported along the z-axis of the droplet actuator device within the gap of the 3D structure.

10. A droplet actuator device of any of claims 7 and following, wherein the reservoir comprises a waste reservoir.

11. A droplet actuator device of any of claims 7 and following, wherein the 3D structure comprises a dome-shaped structure.

12. A droplet actuator device of any of claims 7 and following, further comprising a detector wherein the detector is positioned at the topmost portion of the 3D structure.

13. A droplet actuator device of any of claims 7 and following, wherein the electrodes comprise an array of electrodes.

14. A droplet actuator device of any of claims 7 and following, wherein the electrodes comprise one or more lines of electrodes.

15. A method of isolating one or more droplets during droplet operations, the method comprising:

(a) providing a droplet actuator device for conducting droplet operations, comprising:

(i) two substrates comprising a top substrate and a bottom substrate, separated to form a gap of sufficient height to conduct droplet operations, wherein the bottom substrate comprises a first region and a second region;

(ii) electrodes arranged on the first region of the bottom substrate; and

(iii) a reservoir positioned at the second region of the bottom substrate,

(iv) wherein the top substrate comprises a three dimensional (3D) structure formed substantially orthogonally to the bottom substrate plane, wherein the gap and bottom substrate follow the profile of the 3D structure such that a continuous gap size is maintained to form a continuous droplet operations path.

(b) transporting droplets in an x or y direction using one or more electrodes to the 3D structure, wherein at the 3D structure one or more droplets are further transported, using the one or more electrodes, along the z-axis of the droplet actuator device within the gap of the 3D structure;

(c) performing a detection operation on the one or more droplets at a top most portion of the 3D structure; and

(d) transporting the one more or more droplets into a reservoir.

16. The method of isolating one or more droplets of any of claims 15 and following, wherein the reservoir comprises a waste reservoir.

17. The method of isolating one or more droplets of any of claims 15 and following, wherein the gap formed along the first region of the top substrate if filled with a filler fluid.

18. The method of isolating one or more droplets of any of claims 15 and following, wherein the gap formed along the second region of the top substrate is not filled with a filler fluid.

19. A droplet actuator device for conducting droplet operations, comprising:

(a) a first structure formed of a first bottom substrate and a first top substrate, separated to form a gap of sufficient height to conduct droplet operations;

(b) a second structure formed of a second bottom substrate and a second top substrate separated to form a second gap

(c) electrodes arranged on the first and second bottom substrate;

wherein, the first structure is oriented along a certain plane and the second structure is oriented along a different plane in an orientation such that the first gap of the first structure intersects the second gap of the second structure.

20. The droplet actuator device of any of claims 19 and following, wherein the first structure is oriented along the xy-plane of the droplet actuator and the second structure is orientated along the z-plane of the droplet actuator.

21. The droplet actuator device of any of claims 19 and following, wherein the electrodes comprise an array of electrodes.

22. The droplet actuator device of any of claims 19 and following, wherein the electrodes comprise one or more lines of electrodes.

23. The droplet actuator device of any of claims 19 and following, wherein the first structure and second structure comprise PCB.

24. A droplet actuator device for conducting droplet operations, comprising:

(a) a plurality of first substrates arranged in parallel to one another in a stacked configuration and separated to form first gaps of sufficient height to conduct droplet operations between each of the plurality of first substrates, wherein each of the plurality of first substrates comprise an electrode side and a non-electrode side;

(b) electrodes arranged on the electrode side of the plurality of first substrates, wherein the first gap between each of the plurality of first substrates is formed between the electrode side of each of the plurality of first substrates and the non-electrode side of an adjacent stacked first substrate; and

(c) a second substrate having electrodes arranged on one side thereon, wherein the second substrate is orientated such that the electrode side of the second substrate forms a second gap with respect to ends of the plurality of first substrates such that the second gap formed by the second substrate with respect to ends of the plurality of first substrates intersects the first gaps formed between each of the plurality of first substrates.

25. The droplet actuator device of any of claims 24 and following, wherein the second substrate is orientated at about 90 degrees with respect to the orientation of the plurality of stacked substrates.

26. The droplet actuator device of any of claims 24 and following, wherein the electrodes comprise an array of electrodes.

27. The droplet actuator device of any of claims 24 and following, wherein the electrodes comprise one or more lines of electrodes.

28. The droplet actuator device of any of claims 24 and following, wherein one or more droplets are transported along the second gap via the electrodes of the second substrate, wherein certain droplets of the one or more droplets may be transported from the second gap along a certain first gap of a certain level of the plurality of first substrates via the electrodes arranged thereon.

29. The droplet actuator device of any of claims 24 and following, wherein droplet operations may occur independently along the first gaps of the plurality of first substrates and along the second gap of the second substrate.

30. The droplet actuator device of any of claims 24 and following, wherein the droplets are transported up and/or down the plurality of first substrates via electrodes arranged on the second substrate and electrodes arranged on the plurality of first substrates.

31. The droplet actuator device of any of claims 24 and following, wherein multiple droplet operations are conducted independently at the same time.

32. The droplet actuator device of any of claims 24 and following, wherein multiple droplets may be supplied simultaneously to the plurality of first substrates and second substrate.

33. The droplet actuator device of any of claims 24 and following, wherein the electrodes are formed from flexible circuit material.

34. The droplet actuator device of any of claims 33 and following, wherein the flexible circuit material comprises at least one of polyimide and polyester flexible printed circuit board material.

35. The droplet actuator device of any of claims 33 and following, wherein the flexible circuit material is patterned such that it is in a 2D state when in a flattened state and a 3D state when in a non-flattened state, wherein the flexible circuit material in its 3D state is suitable for incorporating into a droplet actuator device for supplying droplets to any one or more of the plurality of first substrates.

36. A flexible circuit sheet patterned for supplying droplets to multiple levels of a multi-level droplet actuator device comprising:

(a) a sheet of flexible circuit material having a first end and a second end;

(b) a plurality of parallel electrode lines arranged on the sheet of flexible circuit material; and

(c) one or more transition electrodes formed on the sheet of flexible circuit material, wherein the one or more transition electrodes provide a droplet operations path between the plurality of parallel electrode lines,

wherein parallel cuts are patterned between each of the plurality of parallel electrode lines, wherein the cuts alternate between starting at the first end of the flexible circuit sheet and the second end of the flexible circuit sheet, and wherein the parallel cuts do not extend through the one or more transition electrodes, such that a continuous electrode path in a serpentine pattern is formed, wherein the sheet of flexible circuit material is flexed to a 3D structure suitable for supplying droplets to multiple levels of a multi-level droplet actuator device.

37. The flexible circuit sheet of any of claims 36 and following, wherein the flexible circuit material comprises at least one of polyimide and polyester flexible printed circuit board material.

38. A flexible circuit sheet patterned for supplying droplets to a droplet actuator device comprising:

(a) a sheet of flexible circuit material having a first end and a second end; and

(b) one or more electrode lines arranged on the sheet of flexible circuit material, wherein the sheet of flexible circuit material is flexed into a 3D horseshoe shape, wherein the first end of the flexible circuit material is coupled to a first side of a double-sided droplet actuator and the second end is coupled to a second side of the double-sided droplet actuator providing a droplet operations link between each side of the double-sided droplet actuator, wherein the double-sided droplet actuator comprises a substrate comprising electrodes arranged on the first and second side thereof.

39. The flexible circuit sheet of any of claims 38 and following, wherein the flexible circuit material comprises at least one of polyimide and polyester flexible printed circuit board material.

40. A flexible circuit sheet patterned for supplying droplets to a droplet actuator device comprising:

(a) a sheet of flexible circuit material having a first end and a second end; and

(b) one or more electrode lines arranged on the sheet of flexible circuit material, wherein the sheet of flexible circuit material is flexed such that the end most electrode on the first end of the sheet of flexible circuit material and the end most electrode on the second end of the sheet of flexible circuit material are facing opposite directions relative to each other, wherein the sheet of flexible circuit material is coupled to a first droplet actuator at the first end and a second droplet actuator facing in the opposite direction of the first droplet actuator at the second end thereby providing a droplet operations link between the first droplet actuator and second opposite facing droplet actuator.

41. The flexible circuit sheet of any of claims 40 and following, wherein the flexible circuit material is flexed axially by a half twist.

42. The flexible circuit sheet of any of claims 40 and following, wherein the flexible circuit material is flexed in a spiral direction.

43. The flexible circuit sheet of any of claims 40 and following, wherein the flexible circuit material comprises at least one of polyimide and polyester flexible printed circuit board material.

44. A flexible circuit sheet patterned to form a 3D structure when in a flexed state is suitable for supplying droplets to multiple levels of a multi-level droplet actuator device.

45. A multi-level droplet actuator device for conducting droplet operations, comprising:

(a) a first level comprising a bottom substrate and a top substrate separated to form a gap of sufficient height to conduct droplet operations;

(b) electrodes arranged on the bottom substrate of the first level;

(c) a second level comprising a bottom substrate and a top substrate separated to form a gap;

(d) electrodes arranged on the bottom substrate of the second level, wherein an end of the first level is orientated with an end of the second level, such that the gap of the first level is set off vertically from the gap of the second level; and

(e) a channel coupling the gap of the first level to the gap of the second level, wherein the channel comprises a wicking material.

46. The droplet actuator device of any of claims 45 and following, wherein the wicking material comprises

47. The droplet actuator device of any of claims 45 and following, wherein the electrodes comprise an array of electrodes.

48. The droplet actuator device of any of claims 45 and following, wherein the electrodes comprise one or more lines of electrodes.

49. A method of transporting a droplet through a multi-level droplet actuator device, comprising:

(a) introducing a droplet into a first level of a multi-level droplet actuator device;

(b) transporting the droplet to a channel containing wicking material connecting the first level of a multi-level droplet actuator device to a second level of the multi-level droplet actuator device by sequencing off and on the appropriate electrodes arranged on a substrate of the first level of the multi-level droplet actuator device;

(c) wicking the droplet through the wicking material from the first level to the second level of multi-level droplet actuator device; and

(d) reforming the droplet on the second level by sequencing on and off the electrodes arranged on a substrate of the second level of the multi-level droplet actuator device to create a pump function.

50. A multi-level droplet actuator device for conducting droplet operations, comprising:

(a) a plurality of levels comprising a plurality substrates each one of a plurality of substrates separated to form a gap between each of the plurality of substrates

(b) electrodes arranged on the multiple substrates; and

(c) one or more vias, wherein the one or more vias transport droplets from one level of the plurality of levels to another level.

51. The multi-level droplet actuator device of any of claims 50 and following, wherein the plurality of substrates comprise individual PCB-based substrates.

52. The multi-level droplet actuator device of any of claims 50 and following, wherein the plurality of substrates are arranged vertically.

53. The multi-level droplet actuator device of any of claims 50 and following, wherein the plurality of substrates are arranged horizontally.

54. The multi-level droplet actuator device of any of claims 50 and following, wherein the plurality of substrates are arranged vertically and horizontally.

55. The multi-level droplet actuator device of any of claims 50 and following, wherein the multi-level droplet actuator comprises a multi-layer PCB, where each level of the plurality of substrates comprises a certain layer of the multi-layer PCB.

56. The multi-level droplet actuator device of any of claims 50 and following, wherein the one or more vias have electrodes arranged along the vias length thereof.

57. The multi-level droplet actuator device of any of claims 50 and following, wherein the electrodes arranged along the vias are formed as a full ring around the one or more vias length.

58. The multi-level droplet actuator device of any of claims 50 and following, wherein the electrodes arranged along the vias are formed as a partial ring the one or more vias length.

59. The multi-level droplet actuator device of any of claims 50 and following, wherein the vias comprise a flexible printed circuit board material that is flexed to form a tube placed between the a plurality of levels of substrates

60. A 3D droplet actuator structure for conducting droplet operations, comprising:

(a) one or more droplet operations backplanes comprising one or more slots;

(b) one or more droplet operations daughter boards comprising one or more slots and one or more clearance holes positioned at the innermost end of the slots, wherein the slots of the one or more droplet operations daughter boards interlock with the slots of the one or more droplet operations backplanes and the clearance holes provide an opening through which droplets may pass when the one or more droplet operations daughter boards are interlocked with one or more droplet operations backplanes;

(c) electrodes arranged on the one or more droplet operations backplanes and the one or more droplet operations daughter boards; and

(d) transition electrodes positioned in proximity to a certain electrode on the droplet operations backplane and each slot of droplet operations backplane.

61. The droplet actuator device of any of claims 60 and following, wherein the electrodes comprise an array of electrodes.

62. The droplet actuator device of any of claims 60 and following, wherein the electrodes comprise one or more lines of electrodes.

63. A droplet actuator device for conducting droplet operations, comprising:

(a) a double-side substrate having a first side and a second side;

(b) a first top substrate, wherein the first top substrate and the first side of the double-sided substrate are separated to form a gap of sufficient height to conduct droplet operations;

(c) a second top substrate, wherein the second top substrate and the second side of the double-sided substrate are separated to form a gap of sufficient height to conduct droplet operations; and

(d) electrodes arranged on both the first side and second side of the double-sided substrate configured for conducting one or more droplet operations.

64. The droplet actuator device of any of claims 63 and following, wherein the electrodes comprise an array of electrodes.

65. The droplet actuator device of any of claims 63 and following, wherein the electrodes comprise one or more lines of electrodes.

66. The droplet actuator device of any of claims 63 and following, wherein droplet operations can be conducted independently on both the first and second sides of the double-sided substrate.

67. A droplet actuator device for conducting droplet operations, comprising:

(a) a plurality of double-sided substrates, wherein the plurality of double-sided substrates are in a stacked configuration to form levels

(b) electrodes arranged on both surfaces of each of the plurality of double-sided substrates and on the ends of each of the plurality of double-sided substrates, the electrodes configured for conducting one or more droplet operations, wherein the electrodes on the ends of each of the plurality of double-sided substrates transport droplets around the ends of each of the plurality of double-sided substrates to allow droplets to move up and down the levels of double-sided substrates.

68. The droplet actuator device of any of claims 67 and following, wherein the electrodes comprise an array of electrodes.

69. The droplet actuator device of any of claims 67 and following, wherein the electrodes comprise one or more lines of electrodes.

70. The droplet actuator device of any of claims 67 and following, wherein droplet operations can be conducted independently on different levels of the plurality of double-sided substrates.

71. A multi-level droplet actuator device for conducting droplet operations, comprising:

(a) a sheet of flexible circuit material; and

(b) electrodes patterned on the flexible circuit material configured for droplet operations, wherein the sheet of flexible circuit material is formed in a 3D structure with multiple levels.

72. The droplet actuator device of any of claims 71 and following, wherein the electrodes comprise an array of electrodes.

73. The droplet actuator device of any of claims 71 and following, wherein the electrodes comprise one or more lines of electrodes.

74. The droplet actuator device of any of claims 71 and following, wherein the sheet of flexible circuit material comprises at least one of polyimide and polyester flexible printed circuit board material.

75. The droplet actuator device of any of claims 71 and following, wherein the multi-level droplet actuator is formed by folding the sheet of flexible circuit material into multiple segments to form a serpentine shaped 3D structure with multiple levels.

76. The droplet actuator device of any of claims 75 and following, wherein droplet operations can be conducted independently along any level of the multi-level droplet actuator.

77. A droplet actuator device for conducting droplet operations, comprising:

(a) a bottom substrate and a top substrate separated to form a gap of sufficient height to conduct droplet operations;

(b) electrodes arranged on the bottom substrate configured for droplet operations;

(c) a fluid reservoir in fluid connection with the top substrate, wherein a quantity of fluid is supplied from the fluid reservoir into the gap;

(d) a reservoir electrode arranged on the bottom substrate associated with the fluid reservoir;

(e) a first fluid channel in fluid connection with a waste reservoir and the gap formed by the bottom and top substrates, wherein the first fluid channel is on a different plane with respect to the gap formed by the bottom and top substrates;

(f) A second fluid channel in fluid connection with a wash reservoir and the gap formed by the bottom and top substrates, wherein the second fluid channel is on a different plane with respect to the gap formed by the bottom and top substrates; and

(g) electrodes arranged along the walls of the first and second fluid channels configured for droplet operations.

78. The droplet actuator device of any of claims 77 and following, wherein the electrodes comprise an array of electrodes.

79. The droplet actuator device of any of claims 77 and following, wherein the electrodes comprise one or more lines of electrodes.

80. A 3D droplet actuator device for conducting droplet operations, comprising:

(a) a first block shaped substrate and a second block shaped substrate separated to form a center gap of sufficient height to conduct droplet operations;

(b) electrodes arranged on at least three sides the first block shaped substrate and the second block shaped substrate configured for droplet operations;

(c) a bottom substrate arranged in parallel with a lower surface portion of the first block shaped substrate and the second block shaped substrate separated to form a bottom gap therebetween of sufficient height to conduct droplet operations; and

(d) a top substrate arranged in parallel with an upper surface portion of the first block shaped substrate and the second block shaped substrate, separated to form an upper gap therebetween of sufficient height to conduct droplet operations, wherein the bottom gap and upper gap are arranged parallel with respect tone another and are connected by the center gap, wherein the center gap is orthogonal to the bottom gap and the upper gap.

81. The 3D droplet actuator device of any of claims 80 and following, wherein one of the bottom substrate and the top substrate comprise a high temperature source and one of the bottom substrate and the top substrate comprise a cold temperature source.

82. The 3D droplet actuator device of any of claims 80 and following, wherein the electrodes comprise an array of electrodes.

83. The 3D droplet actuator device of any of claims 80 and following, wherein the electrodes comprise one or more lines of electrodes.

84. A 3D droplet actuator device for conducting droplet operations, comprising:

(a) a bottom substrate and a block shaped substrate separated to form a gap of sufficient height to conduct droplet operations;

(b) electrodes arranged on the bottom substrate configured for droplet operations; and

(c) one or more tubes arranged orthogonally with respect to the surface of the bottom substrate, wherein the one or more tubes have electrodes, configured for droplet operations, arranged along the length of the one or more tubes, and wherein the one or more tubes are arranged to transport droplets along a portion of the block shaped substrate.

85. The 3D droplet actuator device of any of claims 84 and following, wherein the block shaped substrate comprises a thermally conductive material, wherein the thermally conductive material provides a temperature gradient from a surface of the block shaped substrate in closest proximity to the bottom substrate to a surface of the block shaped substrate in farthest proximity from the bottom substrate.

86. The 3D droplet actuator device of any of claims 84 and following, wherein the surface of the block shaped substrate in closest proximity to the bottom substrate is hot and the surface of the block shaped substrate in farthest proximity from the bottom substrate is cold, wherein there comprises a temperature gradient therebetween.

87. The 3D droplet actuator device of any of claims 84 and following, wherein the surface of the block shaped substrate in closest proximity to the bottom substrate is cold and the surface of the block shaped substrate in farthest proximity from the bottom substrate is hot, wherein there comprises a temperature gradient therebetween.

88. The 3D droplet actuator device of any of claims 84 and following, wherein the electrodes comprise an array of electrodes.

89. The 3D droplet actuator device of any of claims 84 and following, wherein the electrodes comprise one or more lines of electrodes.

90. The 3D droplet actuator device of any of claims 84 and following, wherein the electrodes arranged along the length of the one or more tubes are arranged as a partial ring around the one or more tubes.

91. The 3D droplet actuator device of any of claims 84 and following, wherein the electrodes arranged along the length of the one or more tubes are arranged as a full ring around the one or more tubes.

92. A droplet actuator device for conducting droplet operations, comprising:

(a) a first plate and a second plate separated to form a gap therebetween of sufficient height to conduct droplet operations;

(b) an alternating current (AC) voltage source electrically connected between the first plate and the second plate;

(c) a hot temperature source coupled to the first plate; and

(d) a cold temperature source coupled to the second plate,

wherein, one or more droplets positioned between the first plate and the second plate may be are stimulated to move between the first plate and the second plate due to the presence of the AC voltage source providing a voltage switching at a certain frequency, and wherein the one or more droplets move between the hot temperature source and the cold temperature source in a controlled manner for conducting droplet operations.

93. The droplet actuator device of any of claims 92 and following, wherein the first plate and second plate comprise material that is electrically and thermally conductive.

94. The droplet actuator device of any of claims 92 and following, wherein the material comprise at least one of copper, aluminum, and gold.

95. A multi-level droplet actuator device for conducting droplet operations, comprising:

(a) substrates arranged in a stack configuration forming multiple planes; and

(b) electrodes arranged on the substrates configured for droplet operations,

wherein each substrate plane is dedicated to conducting a certain droplet operation.

96. The multi-level droplet actuator device of any of claims 95 and following, wherein the electrodes comprise an array of electrodes.

97. The multi-level droplet actuator device of any of claims 95 and following, wherein the electrodes comprise one or more lines of electrodes.

98. A droplet actuator device for conducting droplet operations, comprising:

(a) a top substrate and a bottom substrate separated to form a gap therebetween of sufficient height to conduct droplet operations;

(b) electrodes arranged on the bottom substrate configured for droplet operations; and

(c) a notch formed on a surface of the top substrate facing the gap, wherein the notch substantially aligns with a certain electrode arranged on the bottom substrate, and wherein the notch is substantially sized to fit a droplet volume therein.

99. The droplet actuator device of any of claims 98 and following, wherein the electrodes comprise an array of electrodes.

100. The droplet actuator device of any of claims 98 and following, wherein the electrodes comprise one or more lines of electrodes.

101. The droplet actuator device of any of claims 98 and following, wherein the top substrate comprises transparent material

102. The droplet actuator device of any of claims 98 and following, wherein the notch comprises a detection site.

103. The droplet actuator device of any of claims 98 and following, further comprising a detector aligned with the notch for conducting detection operations.

104. The droplet actuator device of any of claims 98 and following, wherein the notch comprises a wedge-shape.

105. The droplet actuator device of any of claims 98 and following, wherein the notch comprises a tower-shape.

106. A method for transporting a droplet past a second droplet in a droplet actuator device, comprising:

(a) transporting a first droplet along a gap using electrodes toward a notch formed in a top substrate of the droplet actuator device, wherein a second droplet is following the first droplet in the same direction;

(b) deactivating a certain electrode when the first droplet is aligned with the notch causing the first droplet to be transported into the notch providing a clear path in the gap allowing the second droplet to pass by the first droplet; and

(c) transporting the second along a gap using electrodes in advance of the first droplet.

107. The method for transporting a droplet past a second droplet of any of claims 106 and following, further comprising performing detection operations within the notch, wherein the top substrate comprises a transparent material and the notch comprises a detection site.

108. A droplet actuator device for conducting droplet operations, comprising:

(a) a bottom substrate and a top substrate separated to form a gap of sufficient height to conduct droplet operations;

(b) electrodes arranged on the bottom substrate configured for conducting droplet operations;

(c) a notch formed on a surface of the top substrate that is facing the gap, wherein the notch substantially aligns with a certain electrode arranged on the bottom substrate, and wherein the notch is substantially sized to fit a droplet volume therein

109. The droplet actuator device of any of claims 108 and following, wherein the electrodes comprise an array of electrodes.

110. The droplet actuator device of any of claims 108 and following, wherein the electrodes comprise one or more lines of electrodes.

111. An electrode structure for conducting droplet operations in a droplet actuator device, comprising electrically conductive and electrically non-conductive material weaved together to form a mesh pattern of electrodes suitable for conducting defined droplet operations, wherein the electrode structure is electrically coupled to control signals.

112. A droplet actuator comprising a substrate and an associated electrode, wherein the substrate and the electrode are configured such that an alteration in one or more properties of the voltage applied to the electrode causes a droplet operation to be effected by the electrode upon a droplet operations surface of the substrate.

113. The droplet actuator of claim 112, wherein the electrode underlies the substrate.

114. The droplet actuator of any of claims 112 and following wherein the electrode and substrate are configured to effect a voltage or capacitance gradient which effects the droplet operation upon the alteration in voltage.

115. The droplet actuator of any of claims 112 and following wherein the electrode is configured to effect a voltage gradient across its surface that causes a droplet to be transported along the voltage gradient atop the electrode.

116. The droplet actuator of any of claims 112 and following wherein the width of the electrode increases and/or decreases along the length of the electrode.

117. The droplet actuator of any of claims 112 and following wherein the width of the electrode is patterned to effect the droplet operation atop the electrode upon alteration in the one or more properties of the voltage applied to the electrode.

118. The droplet actuator of any of claims 112 and following wherein the thickness of the electrode increases and/or decreases along the length of the electrode.

119. The droplet actuator of any of claims 112 and following wherein the thickness of the electrode is patterned to effect the droplet operation atop the electrode upon alteration in the one or more properties of the voltage applied to the electrode.

120. The droplet actuator of any of claims 112 and following wherein the length and thickness of the electrode each increases or decreases along the length of the electrode.

121. The droplet actuator of any of claims 112 and following wherein the length and thickness of the electrode is patterned to effect the droplet operation atop the electrode upon alteration in the one or more properties of the voltage applied to the electrode.

122. The droplet actuator of any of claims 112 and following wherein the electrode is oriented relative to the droplet operations surface in an orientation which effects the droplet operation atop the electrode upon alteration in the one or more properties of the voltage applied to the electrode.

123. The droplet actuator of any of claims 112 and following wherein the electrodes are coated with a dielectric material.

124. The droplet actuator of any of claims 119 and following wherein the thickness of the dielectric material increases and/or decreases along the length of the electrode.

125. The droplet actuator of any of claims 119 and following wherein the thickness of the dielectric material is patterned atop the electrode to effect the droplet operation upon alteration in the voltage applied to the electrode.

126. The droplet actuator of any of claims 119 and following wherein the properties of the dielectric material are patterned atop the electrode to effect the droplet operation upon alteration in the voltage applied to the electrode.

127. The droplet actuator of any of claims 112 and following wherein the substrate comprises a substrate of a droplet actuator device and comprises an array of two or more the electrodes, the array optionally including other electrodes.

128. The droplet actuator of any of claims 112 and following wherein the substrate is provided as a first substrate of a droplet actuator also comprising a second substrate separated from the first substrate to provide a droplet operations gap.

129. The droplet actuator of any of claims 112 and following wherein the droplet operation comprises transport of the droplet from one region to another region of the electrode.

130. The droplet actuator of any of claims 112 and following wherein the droplet operation comprises splitting of the droplet atop the electrode.

131. The droplet actuator of any of claims 112 and following wherein the droplet operation comprises dispensing of the droplet atop the electrode.

132. A method of effecting a droplet operation comprising alternating one or more properties of the voltage applied to the electrode of any of claims 112 and following to effect the droplet operation.

133. The method of any of claims 126 and following wherein the altering effects transport of the droplet from one region to another region of the electrode.

134. The method of any of claims 126 and following wherein the altering effects splitting of the droplet atop the electrode.

135. The method of any of claims 126 and following wherein the altering effects dispensing of the droplet atop the electrode.

136. A droplet actuator device for conducting droplet operations, comprising:

(a) a top substrate and a bottom substrate, wherein the top substrate and the bottom substrate are separated to form a gap of sufficient height to conduct droplet operations;

(b) a conductive layer disposed on the gap facing side of the top substrate; and

(c) one or more electrodes arranged on the bottom substrate configured for conducting one or more droplet operations.

137. The droplet actuator device of any of claims 136 and following, wherein the conductive layer comprises a transparent material.

138. The droplet actuator device of any of claims 137 and following, wherein the conductive layer comprises an ITO coated polyester film.

139. The droplet actuator device of any of claims 136 and following, wherein the conductive layer comprises gold coated polyester film.

140. The droplet actuator device of any of claims 136 and following, wherein the conductive layer comprises a copper coated polyester film.

141. The droplet actuator device of any of claims 136 and following, wherein the conductive layer comprises foil.

142. The droplet actuator device of any of claims 136 and following, wherein the conductive layer is formed on the top substrate using vacuum deposition.

143. The droplet actuator device of any of claims 136 and following, wherein the conductive layer is formed on the top substrate using a printing process.

144. The droplet actuator device of any of claims 136 and following, wherein the one or more spacer substrates are integrated on the gap facing side of the top substrate.

145. The droplet actuator device of any of claims 136 and following, wherein the one or more spacer substrates have a thickness in the range of about 0.010 inches to about 0.012 inches.

146. The droplet actuator device of any of claims 136 and following, wherein the top substrate comprises an injection molded plastic material.

147. The droplet actuator device of any of claims 136 and following, wherein the top substrate comprises polycarbonate.

148. The droplet actuator device of any of claims 136 and following, wherein the bottom substrate comprises a PCB.

149. The droplet actuator device of any of claims 136 and following, wherein the top substrate further comprises one or more reagent and/or sample wells formed on the non gap facing surface of the top substrate.

150. The droplet actuator device of any of claims 136 and following, wherein the electrodes comprise electrowetting electrodes.

151. The droplet actuator device of any of claims 150 and following, wherein the droplet operations are mediated by the electrowetting electrodes.

152. The droplet actuator device of any of claims 136 and following, wherein the conductive layer comprises a translucent conductive material.

153. The droplet actuator device of any of claims 152 and following, wherein the translucent conductive material comprises material selected from the group consisting of DuPont 7162 and DuPont 7164.

154. The droplet actuator device of any of claims 136 and following, wherein the conductive layer comprises an opaque conductive material.

155. The droplet actuator device of any of claims 154 and following, wherein the opaque conductive material comprises material selected from the group consisting of DuPont 7152 and DuPont 7162.

156. The droplet actuator device of any of claims 136 and following, wherein the conductive layer further comprises a detection window.

157. The droplet actuator device of any of claims 156 and following, wherein the detection window is aligned with a defined electrode, wherein the detection window provides an

optical path for a light signal from a sample droplet positioned on the defined electrode to an external imaging device.

158. The droplet actuator device of any of claims 136 and following, wherein the one or more spacer substrates are disposed on the surface of the conductive layer, wherein the spacer substrates protrude from the surface of the conductive layer into the gap.

159. The droplet actuator device of any of claims 158 and following, wherein the one or more spacer substrates are formed on the conductive layer by screen printing.

160. The droplet actuator device of any of claims 158 and following, wherein the one or more spacer substrates have a thickness in the range of about 0.010 inches to about 0.012 inches.

161. The droplet actuator device of any of claims 158 and following, wherein the one or more spacer substrates comprises a UV-curable material.

162. The droplet actuator device of any of claims 136 and following, wherein the one or more spacer substrates are formed on the gap facing side of the bottom substrate.

163. The droplet actuator device of any of claims 136 and following, wherein the one or more spacer substrates are formed on the gap facing sides of the bottom substrate and top substrate.

164. The droplet actuator device of any of claims 136 and following, wherein the conductive layer is attached to the gap facing side of the top substrate by an adhesive layer.

165. The droplet actuator device of any claim 164 and following, wherein the adhesive layer comprises a heat seal adhesive.

166. The droplet actuator device of any of claims 164 and following, wherein the adhesive layer comprises a pressure sensitive adhesive.

167. The droplet actuator device of any of claims 164 and following, wherein the adhesive layer comprises a liquid adhesive.

168. The droplet actuator device of any of claims 136 and following, wherein the one or more spacers are attached to the gap facing side of the conductive layer by an adhesive layer.

169. The droplet actuator device of any of claims 168 and following, wherein the adhesive layer comprises a heat seal adhesive.

170. The droplet actuator device of any of claims 168 and following, wherein the adhesive layer comprises a pressure sensitive adhesive.

171. The droplet actuator device of any of claims 168 and following, wherein the adhesive layer comprises a liquid adhesive.

172. The droplet actuator device of any of claims 136 and following, wherein the bottom substrate comprises:

(a) a substrate;

(b) a conductive layer disposed on one side of the substrate;

(c) a first dielectric layer disposed on top of the conductive layer;

(d) one or more electrodes disposed on the first dielectric layer;

(e) one or more vias formed through the first dielectric layer electrically connecting the conductive layer and the one or more electrodes, wherein the one or more vias comprise conductive material; and

(f) a second dielectric layer disposed on top of the one or more electrodes.

173. The droplet actuator device of any of claims 172 and following, wherein the substrate comprises at least one of heat stabilized polyester, polycarbonate, PEN and LCP.

174. The droplet actuator device of any of claims 172 and following, wherein the conductive layer comprises silver conductive material.

175. The droplet actuator device of any of claims 172 and following, wherein the conductive material comprises silver conductive material.

176. The droplet actuator device of any of claims 172 and following, wherein the one or more electrodes comprises silver conductive material.

177. The droplet actuator device of any of claims 172 and following, wherein the first dielectric layer comprises UV-curable dielectric material.

178. The droplet actuator device of any of claims 172 and following, wherein the second dielectric layer comprises UV-curable dielectric material.

179. The droplet actuator device of any of claims 136 and following, wherein the bottom substrate comprises:

(a) a substrate;

(b) a conductive layer disposed on one side of the substrate;

(c) a first dielectric layer disposed on top of the conductive layer;

(d) one or more electrodes disposed on a side of the substrate that is opposite the conductive layer and first dielectric layer;

(e) one or more vias formed through the substrate electrically connecting the conductive layer and the one or more electrodes, wherein the one or more vias comprise conductive material; and

(f) a second dielectric layer disposed on top of the one or more electrodes.

180. The droplet actuator device of any of claims 179 and following, wherein the substrate comprises at least one of heat stabilized polyester, polycarbonate, PEN and LCP.

181. The droplet actuator device of any of claims 179 and following, wherein the conductive layer comprises silver conductive material.

182. The droplet actuator device of any of claims 179 and following, wherein the one or more electrodes comprises silver conductive material.

183. The droplet actuator device of any of claims 179 and following, wherein the first dielectric layer comprises UV-curable dielectric material.

184. The droplet actuator device of any of claims 179 and following, wherein the second dielectric layer comprises UV-curable dielectric material.

185. The droplet actuator device of any of claims 136 and following, wherein the top substrate and bottom substrate are bonded together using a light curable adhesive.

186. The droplet actuator device of any of claims 185 and following, wherein the light curable adhesive is selected from the group consisting of 3M LC-1212, LOCTITE 3106 and LOCTITE 3556.

187. A droplet actuator device for conducting droplet operations, comprising:

(a) two substrates separated to form a gap of sufficient height to conduct droplet operations, at least one substrate comprising electrodes configured for conducting one or more droplet operations;

(b) at lease one substrate layer comprising a moveable replaceable film; and

(c) openings providing a fluid path into the gap.

188. The droplet actuator device of any of claims 187 and following, wherein both substrate layers comprise respectively, a moveable replaceable film.

189. The droplet actuator device of any of claims 188 and following, wherein the moveable replaceable films are arranged on reel-to-reel mechanisms.

190. The droplet actuator device of any of claims 187 and following, wherein the at least one moveable replaceable film comprises a dielectric layer and a hydrophobic layer.

191. The droplet actuator device of any of claims 188 and following, wherein the substrate layers comprise a top substrate and a bottom substrate, and wherein the top substrate includes one or more functional layers.

192. The droplet actuator device of any of claims 191 and following, wherein the functional layers comprise an indium tin oxide layer and a hydrophobic layer.

193. The droplet actuator device of any of claims 187 and following, further comprising openings in the top substrate providing a fluid path from the top substrate into the gap in sufficient proximity to selected electrodes.

194. The droplet actuator device of any of claims 193 and following, further comprising spacers in proximity to the openings to define a predetermined area for fluid to enter the gap.

195. The droplet actuator device of any of claims 187 and following, wherein the two substrates comprise respectively, a top substrate and a bottom substrate, and wherein the bottom substrate comprises the moveable replaceable film, and the top substrate comprising a rigid substrate.

196. The droplet actuator device of any of claims 195 and following, wherein the rigid substrate is formed of at least one of silicon-based material, glass and plastic.

197. The droplet actuator device of any of claims 195 and following, wherein the rigid substrate is moveable in an up and down direction, and can be lowered to make contact with the lower substrate.

198. The droplet actuator device of any of claims 195 and following, wherein the electrodes are in proximity to the bottom substrate, and further comprising a contact layer positioned for enhancing physical contact between the bottom substrate and the electrodes.

199. The droplet actuator device of any of claims 195 and following, wherein the bottom substrate further comprises openings for providing a channel through which a vacuum may be applied, the electrodes being in proximity to the bottom substrate, and the vacuum serving to place the bottom substrate in contact with the electrodes.

200. A droplet activator device for conducting droplet operations, comprising:

(a) a top substrate and a bottom substrate, two substrates separated to form a gap of sufficient height to conduct droplet operations;

(b) electrodes arranged on one of the bottom substrate and the top substrate, and at least one opening on at least one substrate for connection to a vacuum source; and

(c) a vacuum seal around the at least one opening for sealing the top substrate to the bottom substrate open a vacuum being drawn through the opening.

201. A droplet actuator device of any of claims 200 and following, wherein the bottom substrate comprises at least one of silicon-based material, glass and plastic.

202. A droplet actuator device of any of claims 200 and following, further comprising a recessed area within one of the substrates and adjacent to at least one opening for providing a vacuum channel along a perimeter of the substrate having the recessed area.

203. A droplet actuator device of any of claims 200 and following, wherein the top substrate comprises at least one of silicon-based materials, glass and plastic.

204. A droplet actuator device of any of claims 200 and following, wherein the vacuum seal is made of soft gasket material.

205. A droplet actuator device of any of claims 200 and following, wherein the electrodes comprise an array of electrodes.

206. A droplet actuator device for conducting droplet operations, comprising:

(a) two substrates comprising a top substrate and a bottom substrate, separated to form a gap of sufficient height to conduct droplet operations;

(b) electrodes arranged on the bottom substrate;

(c) one or more heating electrodes on one of the substrates; and

(d) a gasket providing a seal around an outer edge of the actuator device between the bottom substrate and the top substrate, and aligned with the one or more heating electrodes.

207. The droplet actuator device of any of claims 206 and following, wherein the gasket comprises a heat sensitive material.

208. The droplet actuator device of any of claims 206 and following, wherein the one or more heating electrodes is arranged on the bottom substrate.

209. The droplet actuator device of any of claims 206 and following, wherein the electrodes comprise an array of electrodes.

210. The droplet actuator device of any of claims 206 and following, wherein the bottom substrate comprises PCB.

211. The droplet actuator device of any of claims 206 and following, wherein the bottom substrate comprises at least one of silicon-based materials, glass and plastic.

212. A droplet actuator device for conducting droplet operations, comprising:

(a) a fixed bottom substrate and a removable top substrate;

(b) electrodes arranged on the bottom substrate; and

(c) the top substrate comprising a self-contained cartridge for a droplet actuator-based array.

213. The droplet actuator device of any of claims 212 and following, wherein the bottom substrate comprises at least one of silicon-based material, glass and PCB.

214. The droplet actuator device of any of claims 212 and following, wherein the cartridge comprises a housing formed around an enclosed area.

215. The droplet actuator device of any of claims 212 and following, wherein the cartridge comprises a ground electrode.

216. The droplet actuator device of any of claims 212 and following, wherein the top cartridge comprises a hydrophobic layer.

217. The droplet actuator device of any of claims 212 and following, further comprising at least one opening in the cartridge for providing a fluid path into the enclosed area.

218. The droplet actuator device of any of claims 212 and following, further comprising alignment guides for positioning the cartridge in proximity to the electrodes.

219. The droplet actuator device of any of claims 212 and following, wherein the cartridge further comprises at least one pouch for holding a volume of predetermined fluid.

220. The droplet actuator device of any of claims 216 and following, wherein the at least one pouch is constructed for being punctured, and further comprising a puncturing device.

221. The droplet actuator device of any of claims 212 and following, further comprising an interface material between the cartridge and the bottom substrate.

222. The droplet actuator device of any of claims 212 and following, further comprising a dielectric layer interfacing with the electrodes.

223. The droplet actuator device of any of claims 221 and following, wherein the interface material comprises a dielectric layer interfacing with the electrodes.

224. The droplet actuator device of any of claims 222 and following, wherein the dielectric layer is replaceable.

225. The droplet actuator device of any of claims 222 and following, wherein the dielectric layer is patterned.

226. A droplet actuator device for conducting droplet operations, comprising:

(a) a bottom substrate;

(b) an array of droplet operations electrodes; and

(c) at least a first electrowettable coating disposed on a surface of the bottom substrate.

227. The droplet actuator device of any of claims 226 and folowing, further comprising plural electrowettable coating layers arranged in sequence on the bottom substrate.

228. The droplet actuator device of any of claims 226 and folowing, wherein the at least first electrowettable coating is soluble in a predetermined solvent.

229. The droplet actuator device of any of claims 226 and folowing, wherein the first electrowettable coating is disposed on the surface of the bottom substrate atop the array of droplet operation electrodes.

230. The droplet actuator device of any of claims 226 and folowing, further comprising at least one sacrificial layer disposed atop the at least a first electrowettable coating.

231. The droplet actuator device of any of claims 226 and folowing, further comprising multiple hydrophobic films laminated onto the bottom substrate.

232. The droplet actuator device of any of claims 231 and following, wherein the hydrophobic films are removable.

233. The droplet actuator device of any of claims 231 and following, wherein the hydrophobic films have dielectric properties.

234. A droplet actuator device for conducting droplet operations, comprising:

(a) a flexible substrate comprised of a bottom substrate region and a top substrate region;

(b) a hinge region in the flexible substrate connecting the bottom substrate region and the top substrate region;

(c) an array of electrodes on the bottom substrate region; and

(d) a dielectric layer disposed on top of the array of electrodes on the bottom substrate region.

235. The droplet actuator device of any of claims 234 and following, further comprising a ground electrode on the top substrate region.

236. The droplet actuator device of any of claims 234 and following, further comprising a hydrophobic layer disposed on top of the dielectric layer.

237. The droplet actuator device of any of claims 234 and following, wherein the dielectric layers comprises an adhesive backed polyimide.

238. A droplet actuator device for conducting droplet operations, comprising:

(a) two substrates comprising a top substrate and a bottom substrate, separated to form a gap of sufficient height to conduct droplet operations;

(b) electrodes arranged on the bottom substrate;

(c) a dielectric layer disposed on top of the array of electrodes on the bottom substrate; and

(d) a conductive ink layer disposed on the gap facing side of the top substrate.

239. The droplet actuator device of any of claims 238 and following, wherein the electrodes comprise an array of electrodes.

240. The droplet actuator device of any of claims 238 and following, wherein the electrodes comprise electrowetting electrodes.

241. The droplet actuator device of any of claims 238 and following, wherein the bottom substrate comprises at least one of silicon-based material, glass, plastic and PCB.

242. The droplet actuator device of any of claims 238 and following, wherein the top substrate comprises at least one of glass, polycarbonate, COC, COP, PMMA, polystyrene and plastic.

243. The droplet actuator device of any of claims 238 and following, further comprising a hydrophobic layer disposed on top of the dielectric layer and/or on top of the conductive ink layer.

244. The droplet actuator device of any of claims 243 and following, wherein the hydrophobic layer is formed on top of the dielectric layer and/or on top of the conductive ink layer by spray coating.

245. The droplet actuator device of any of claims 243 and following, wherein the hydrophobic layer material is selected from the group consisting of CYTOP and Teflon AF.

246. The droplet actuator device of any of claims 238 and following, wherein the conductive ink layer is printed on the gap facing side of the top substrate.

247. The droplet actuator device of any of claims 246 and following, wherein the conductive ink layer is printed on the gap facing side of the top substrate using electrode path patterns.

248. The droplet actuator device of any of claims 247 and following, wherein line widths of the conductive ink layer electrode path patterns are in the range of 0.001 mm to 100 mm.

249. The droplet actuator device of any of claims 238 and following, wherein the conductive ink layer comprises PEDOT:PSS.

250. The droplet actuator device of any of claims 238 and following, wherein the conductive ink layer comprises at least one of CLEVOS P Jet N, CLEVOS P Jet HC, CLEVOS P Jet N V2 and CLEVOS P Jet HC V2.

251. A clamping fixture for assembling droplet actuators, the clamping fixture comprising:

(a) a base plate having magnets installed therein;

(b) a top plate having magnets installed therein at selected locations aligned with the magnets of the base plate; and

(c) the number of the magnets in the base plate and the top plate being positioned for providing sufficient pressure between substrates of a droplet actuator being adhered together when clamped between the base plate and the top plate.

252. The clamping fixture of any of claims 251 and following, further comprising at least one alignment pin for positioning a bottom substrate of a pair of substrates on the base plate.

253. The clamping fixture of any of claims 251 and following, wherein the base plate further comprises alignment holes for installing the clamping fixture on another piece of equipment.

254. The clamping fixture of any of claims 251 and following, wherein a pattern of magnets in placement holes in the base plate comprises a mirror image of a pattern of magnets in placement holes in the top plate.

255. The clamping fixture of any of claims 251 and following, wherein the magnets are permanent magnets or electromagnets.

256. The clamping fixture of any of claims 251 and following, further comprising standoffs around the perimeter of the top plate on a face thereof facing the base plate when assembled.

257. The clamping fixture of any of claims 256 and following, further comprising additional standoffs substantially aligned with magnet placement holes in the top plate.

258. The clamping fixture of any of claims 256 and following, wherein the standoffs are made of rubber.

259. The clamping fixture of any of claims 251 and following, wherein the magnets of the base plate and the top plate are arranged to have opposing poles face each other.

260. The clamping fixture of any of claims 251 and following, wherein the magnets are sufficiently large such that the magnetic force thereof is sufficient to pass through the substrates being clamped between the base plate and top plate.

261. A method of bonding substrates of droplet actuators together, the method comprising:

(a) providing a clamping fixture comprising a base plate having magnets installed therein, a top plate having magnets installed therein at selected locations aligned with the magnets of the base plate, the number of magnets in the base plate and the top plate being positioned for providing sufficient pressure between substrates of a droplet actuator being adhered together when clamped between the base plate and the top plate;

(b) aligning a bottom substrate of a droplet actuator on the base plate of the clamping fixture;

(c) dispensing adhesive onto the bottom substrate;

(d) aligning a top substrate of a droplet actuator with the bottom substrate;

(e) aligning the top plate of the magnetic clamping fixture with the droplet actuator substrates and the base plate, and placing the top plate in contact therewith to provide a uniform compression force between the bottom substrate and the top substrate; and

(f) removing the clamping fixture after the substrates have bonded.

262. The method of any of claims 261 and following, wherein the dispensed adhesive comprises a UV cure adhesive, and further comprising exposing the adhesive to UV light to cure the adhesive.

263. The method of any of claims 261 and following, further comprising aligning the bottom substrate on the base plate through the use of alignment pins.

264. The method of any of claims 261 and following, further comprising aligning the top substrate on the bottom substrate through the use of alignment pins.

265. The method of any of claims 261 and following, wherein the top plate is aligned with the top and bottom substrates and the base plate by using the magnets.

266. The method of any of claims 261 and following, wherein the base plate further comprises alignment holes, and further comprising installing the clamping fixture on another piece of equipment using the alignment holes.

267. The method of any of claims 261 and following, further comprising providing standoffs around the perimeter of the top plate on a face thereof facing the base plate when assembled.

268. A clamping fixture for assembling droplet actuators, the clamping fixture comprising:

(a) a base plate having magnets installed therein in a pattern, and having alignment holes for installing the clamping fixture on another piece of equipment;

(b) a top plate having magnets installed therein at selected locations aligned with the magnets of the base plate, a pattern of magnets of the top plate being a mirror image of the pattern of magnets on the base plate; and

(c) the number of magnets in the base plate and the top plate being positioned for providing sufficient pressure between substrates of a droplet actuator being adhered together when clamped between the base plate and the top plate.

269. The clamping fixture of any of claims 268 and following, further comprising means for attaching a handle.

270. The clamping fixture of any of claims 268 and following, further comprising standoffs around the perimeter of the top plate on a face thereof facing the base plate when

assembled, and additional standoffs substantially aligned with magnet placement holes in the top plate.

271. A microfluidic system for conducting droplet operations, comprising:

(a) one or more power sources;

(b) a power conditioning circuit electrically connected to the power source, wherein the power source provides a voltage source V-IN to the power conditioning circuit;

(c) control electronics electronically connected to the power conditioning circuit; and

(d) a droplet actuator device electrically connected to the control electronics, wherein the droplet actuator conducts droplet operations.

272. The microfluidic system of any of claims 271 and following, wherein the one or more power sources comprises a direct current (DC) power source.

273. The microfluidic system of any of claims 272 and following, wherein the DC power source comprises one or more batteries.

274. The microfluidic system of any of claims 272 and following, wherein the DC power source is packaged separately from the microfluidic system while remaining electrically connected to the microfluidics system.

275. The microfluidic system of any of claims 272 and following, wherein the DC power source is integrated directly into the physical instantiation of the microfluidics system.

276. The microfluidic system of any of claims 271 and following, wherein the power conditioning circuit regulates voltage output of the one or more power sources.

277. The microfluidic system of any of claims 271 and following, wherein the power conditioning circuit converts voltage output of the one or more power sources from DC to alternating current (AC).

278. The microfluidic system of any of claims 271 and following, wherein the power conditioning circuit converts voltage output of the one or more power sources from AC to DC.

279. The microfluidic system of any of claims 271 and following, wherein the control electronics distribute electrowetting voltages to one or more channels of the droplet actuator.

280. The microfluidic system of any of claims 272 and following, wherein the DC power source comprises a DC generator.

281. The microfluidic system of any of claims 280 and following, wherein the DC generator comprises a low RPM permanent magnet DC generator.

282. The microfluidic system of any of claims 280 and following, wherein the DC generator is packaged separately from the microfluidic system while remaining electrically connected to the microfluidics system.

283. The microfluidic system of any of claims 280 and following, wherein the DC generator is integrated directly into the physical instantiation of the microfluidics system.

284. The microfluidic system of any of claims 280 and following, wherein the DC generator output is stored in one or more capacitors.

285. The microfluidic system of any of claims 271 and following, wherein the one or more power sources comprises an AC power source.

286. The microfluidic system of any of claims 272 and following, wherein the power conditioning circuit comprises a transformer having a primary region and a secondary region; an oscillator; and a solid state switch, wherein the DC power source, oscillator, transformer and the solid state switch are electrically coupled and arranged to develop a

low voltage signal at the primary region of the transformer, wherein a standard rectifier circuit electrically coupled to the secondary region of the transformer provides a DC voltage output V-OUT that is a higher voltage than V-IN.

287. The microfluidic system of any of claims 286 and following, wherein the power conditioning circuit further comprises a feedback loop from V-OUT to the oscillator.

288. The microfluidic system of any of claims 286 and following, wherein the V-OUT supplies electrowetting voltage to one or more channels of the droplet actuator device.

289. The microfluidic system of any of claims 280 and following, wherein the power conditioning circuit comprises a transformer having a primary region and a secondary region; and a fast mechanical switch, wherein the transformer and fast mechanical switch are electrically coupled to the DC generator and arranged to develop a low voltage signal at the primary region of the transformer, wherein a standard rectifier circuit positioned at the secondary region of the transformer provides a DC voltage output V-OUT that is a higher voltage than V-IN.

290. The microfluidic system of any of claims 289 and following, wherein the power conditioning circuit further comprises a feedback loop from V-OUT to the fast mechanical switch.

291. The microfluidic system of any of claims 289 and following, wherein the V-OUT supplies electrowetting voltage to one or more channels of the droplet actuator device.

292. The microfluidic system of any of claims 289 and following, wherein the fast mechanical switch is mechanically coupled to the DC generator, wherein the fast mechanical switch is opened or closed based on rotation of the DC generator.

293. The microfluidic system of any of claims 292 and following, wherein the fast mechanical switch is geared down with respect to the rotation of the DC generator, wherein the fast mechanical switch opens and closes at a slower rate than the rotation of the DC generator.

294. The microfluidic system of any of claims 289 and following, wherein the power conditioning circuit further comprises a switch bank, wherein the switch bank comprises

a plurality of mechanical switches, wherein each of the plurality of mechanical switches is electrically coupled to V-OUT and to a defined channel of the droplet actuator devise, wherein in the closed position the respective mechanical switches of the plurality of mechanical switches distributes V-OUT to the respective one or more channels of the droplet actuator device.

295. The microfluidic system of any of claims 294 and following, wherein the plurality of mechanical switches of the switch bank and the fast mechanical switch are mechanically coupled to the DC generator, wherein each of the mechanical switches of the plurality of mechanical switches and the fast mechanical switch are opened or closed based on rotation of the DC generator.

296. The microfluidic system of any of claims 295 and following, wherein the fast mechanical switch is geared down with respect to the rotation of the DC generator, wherein the fast mechanical switch opens and closes at a slower rate than the rotation of the DC generator, and wherein the plurality of mechanical switches of the switch bank are geared at a ratio wherein the mechanical switches of the switch bank opens and closes at a slower rate than that of the fast mechanical switch.

297. The microfluidic system of any of claims 285 and following, wherein the power conditioning circuit comprises a switch array electrically coupled to the AC power source.

298. The microfluidic system of any of claims 297 and following, wherein the switch array drives one or more channels of the droplet actuator device.

299. The microfluidic system of any of claims 298 and following, wherein the switch array is electrically couple to a substrate of the droplet actuator device.

300. The microfluidic system of any of claims 297 and following, wherein the switch array comprises an array of silicon-controlled rectifiers (SCRs).

301. The microfluidic system of any of claims 297 and following, wherein the switch array comprises an array of triodes for alternating current (TRIACs).

302. The microfluidic system of any of claims 297 and following, wherein the switch array comprises an array of bipolar junction transistors (BJTs).

303. The microfluidic system of any of claims 297 and following, wherein the switch array comprises an array of switches.

304. The microfluidic system of any of claims 303 and following, wherein the array of switches comprises mechanical switches.

305. The microfluidic system of any of claims 272 and following, wherein the power conditioning circuit comprises one or more low voltage switches electrically coupled to the DC power source; and one or more voltage multipliers electrically couple to the one or more low voltage switches, wherein the one or more voltage multipliers convert low voltage signals to higher voltage signals.

306. The microfluidic system of any of claims 305 and following, wherein the one or more switches comprise solid state switches.

307. The microfluidic system of any of claims 305 and following, wherein the one or more voltage multipliers are electrically coupled to one or more channels of a droplet actuator devise for controlling droplet operations.

308. The microfluidic system of any of claims 305 and following, wherein the one or more voltage multipliers comprises a charge pump.

309. The microfluidic system of any of claims 305 and following, wherein the one or more voltage multipliers comprises a Cockcroft-Walton (CW) generator.

310. The microfluidic system of any of claims 305 and following, wherein the one or more voltage multipliers comprises a step up transformer wherein the step up transformer has a primary region and a secondary region, wherein a low voltage square wave (V-low) is introduced to the primary region of the transformer and a high voltage square wave (V-high) is produced at the secondary region of the transformer.

311. A droplet actuator device for conducting droplet operations, comprising:

(a) a top substrate and a bottom substrate, separated to form a gap of sufficient height to conduct droplet operations;

(b) channel input/output (I/O) pads arranged on the gap facing surface of the bottom substrate, wherein the channel IO pads are electrically coupled to one or more channels of the droplet actuator device and provide electrowetting voltage to the one or more channels; and

(c) one or more voltage electrodes arranged on the gap facing surface of the top substrate.

312. The droplet actuator device of any of claims 311 and following, wherein the channel input/output pads are arranged on the top substrate.

313. The droplet actuator device of any of claims 311 and following, wherein the channel input/output pads are arranged on both the top substrate and bottom substrate.

314. The droplet actuator device of any of claims 311 and following, wherein electrowetting voltage is provided to the one or more voltage electrodes.

315. The droplet actuator device of any of claims 311 and following, further comprising one or more ball bearings arranged in the gap, wherein the one or more ball bearings are in physical contact with both one or more certain channel I/O pads and one or more certain voltage electrodes wherein the physical contact provides an electrical connection between the one or more certain channel I/O pads and the one or more certain voltage electrodes.

316. The droplet actuator device of any of claims 315 and following, wherein the one or more ball bearings comprise an electrically conductive material.

317. The droplet actuator device of any of claims 316 and following, wherein the electrically conductive material comprises at least one of stainless steel, gold and aluminum.

318. The droplet actuator device of any of claims 315 and following, wherein the position of the one or more ball bearings within the droplet actuator device is controlled by tilting the

droplet actuator device, wherein the tilting of the droplet actuator device in a controlled manner causes the one or ball bearings to roll to certain channel I/O pad.

319. The droplet actuator device of any of claims 315 and following, further comprising regions, wherein the regions comprise different size gaps and/or channels that are along the arrangement of the channel I/O pads, wherein the regions further comprise ball bearings of corresponding size to the gaps and/or channels, such that the one or more ball bearings corresponding to a certain region do not enter a that of a different region.

320. A droplet actuator device for conducting droplet operations, comprising:

(a) a top substrate and a bottom substrate, separated to form a gap of sufficient height to conduct droplet operations; and

(b) channel I/O pads and electrodes arranged on the bottom substrate, wherein the channel I/O pads are electrically coupled to the electrodes to provide electrowetting voltage to the droplet actuator electrodes.

321. The droplet actuator device of any of claims 320 and following, wherein the channel I/O pads and electrodes are arranged on the top substrate.

322. The droplet actuator device of any of claims 320 and following, wherein the channel I/O pads and electrodes are electrically coupled by certain wiring routes.

323. The droplet actuator device of any of claims 322 and following, wherein the wiring routes are customized to perform defined protocols.

324. The droplet actuator device of any of claims 320 and following, wherein the electrowetting voltage is supplied to the channel I/O pad via a voltage probe.

325. The droplet actuator device of any of claims 320 and following, wherein the electrowetting voltage is supplied to the channel I/O pad via a mechanical switch.

326. The droplet actuator device of any of claims 325 and following, wherein the mechanical switch comprises a pinned barrel.

327. A droplet actuator device for conducting droplet operations, comprising:

(a) a first substrate comprising one or more charged dielectric regions arranged thereon; and

(b) a second substrate comprising one or more electrodes arranged thereon, wherein the first and second substrate are positioned in close physical proximately to one another and wherein the one or more electrodes are aligned with the one or more dielectric regions to facilitate energizing the electrodes by static charge of the one or more dielectric regions for conducting droplet operations.

328. The droplet actuator device of any of claims 327 and following, wherein the position of the first substrate is adjusted to transfer the static charge from the one or more dielectric regions to the desired one or more electrodes.

329. A microfluidics array multiplexing platform for using droplet operations for performing flash-based chemiluminescent profiling, the platform comprising:

(a) a multi-well droplet actuator configured to perform more than one different immunoassays ; and

(b) an imaging system configured to read images from a row of droplets in the multi-well droplet actuator undergoing simultaneous chemiluminescent reactions.

330. The microfluidics platform of any of claims 329 and following, wherein the multi-well droplet actuator comprises a 12-well droplet actuator configured to perform 4 different immunoassays.

331. The microfluidics platform of any of claims 329 and following, wherein the imaging system is configured for capturing a short exposure image of a row of droplets for being processed and interpreted.

332. The microfluidics platform of any of claims 331 and following, further comprising a processing unit having image processing software for interpreting luminescent images captured from the droplets in the row.

333. The microfluidics platform of any of claims 332 and following, further comprising droplet control electronics for manipulating each droplet based on metric data acquired by the imaging system for a given droplet.

334. The microfluidics platform of any of claims 332 and following, wherein the image processing software further comprises a decision tree algorithm configured for determining if sufficient images have been acquired to make a quantitative determination for a predetermined droplet.

335. The microfluidics platform of any of claims 333 and following, further configured for sending a signal to the droplet control electronics for independently manipulating each droplet based on metrics data obtained for each droplet.

336. The microfluidics platform of any of claims 335 and following, further comprising means for maintaining droplets with low chemiluminescent signals in an imaging zone for further analysis.

337. A method of performing flash-based chemiluminescent profiling operations in a microfluidics array multiplexing platform using droplet operations, the method comprising:

(a) providing a multi-well droplet actuator configured to perform more than one different immunoassays; and

(b) acquiring at least one image with an imaging system, from a row of droplets in the multi-well droplet actuator undergoing simultaneous chemiluminescent immunoassays.

338. The method of any of claims 337 and following, wherein the droplet actuator comprises a 12-well droplet actuator configured to perform four (4) different immunoassays.

339. The method of any of claims 337 and following, further comprising capturing a short exposure image of a row of droplets with the imaging system for being processed and interpreted.

340. The method of any of claims 339 and following, further comprising transmitting the captured image to a processing unit having image processing software for interpreting the captured luminescent images, and interpreting the captured images therewith.

341. The method of any of claims 340 and following, further comprising manipulating each droplet with droplet control electronics based on metrics data acquired by the imaging system for a given droplet.

342. The method of any of claims 340 and following, further comprising determining with a decision tree algorithm if sufficient images have been acquired to make a quantitative determination for a predetermined droplet.

343. The method of any of claims 341 and following, further comprising sending a signal to the droplet control electronics and independently manipulating each droplet based on metric data obtained for each droplet.

344. The method of any of claims 343 and following, further comprising maintaining droplets with low chemiluminescent signals in an imaging zone for further analysis.

345. A method of performing dynamic, smart imaging on a digital microfluidic platform using droplet operations, the method comprising:

(a) placing sample droplets at predetermined droplet electrodes of a microfluidic array multiplexing platform for performing flash-based chemiluminescent profiling;

(b) transporting sample droplets and merging and mixing the sample droplets with trigger droplets at a time $t_0 + \Delta t$ in an imaging zone to form activated droplets;

(c) acquiring successive image exposures of the activated droplets at a time $t_1 << \Delta t$; and

(d) interpreting luminescent images of the activated droplets with image exposure software and feeding metrics thereof to a decision tree algorithm to determine whether enough images have been acquired to make a quantitative call for any activated droplet.

346. The method of any of claims 345 and following, further comprising at time $t_0 + 2\Delta t$, determining with the decision tree if sufficient images have been acquired of a third activated droplet.

347. The method of any of claims 346 and following, further comprising moving each activated droplet out of the imaging zone when depleted.

348. The method of any of claims 347 and following, further comprising merging a next sample droplet with a trigger droplet at a time $t_1 + 3\Delta t$ and then repeating the method.

349. An electrode configuration for a droplet actuator for use in merging and detection of activated droplets in a flash-based chemiluminescent array, the configuration comprising:

(a) an electrode configuration with a plurality of electrodes, a merging location for merging of sample droplets to result in activated droplets, and a detection spot; and

(b) the detection spot being separated from the merging location by a predetermined number of electrodes.

350. The electrode configuration of any of claims 349 and following, wherein the detection spot is separated from the merging location by about 40 unit electrodes.

351. The electrode configuration of any of claims 349 and following, wherein the detection spot is configured for detection of a single activated droplet.

352. A method of merging and detecting activated droplets in a flash-based chemiluminescent array having an electrode configuration for a droplet actuator, the method comprising:

(a) providing an electrode configuration with a plurality of electrodes, a merging location, and a detection spot;

(b) merging a sample droplet and a trigger droplet at the merging location to form an activated droplet;

(c) transporting the activated droplet to the detection spot; and

(d) detecting a chemiluminescent signal from the activated droplet at the detection spot.

353. The method of any of claims 352 and following, wherein the chemiluminescent signal is detected by a photon counting PMT for a predetermined amount of time to obtain a desired number of data points.

354. The method of any of claims 352 and following, wherein the activated droplet is transported to the detection spot at a switching speed of about 10 electrodes per second.

355. The method of any of claims 352 and following, wherein a standard curve of concentration versus area is generated from the detected chemiluminescent signal.

356. The method of any of claims 352 and following, wherein the sample droplet and trigger droplet are merged at the detection spot to form the activated droplet, and then shuttled between a predetermined number of electrodes under a detector, and further comprising detecting the chemiluminescent signal with the detector.

357. A luminometer system for simultaneous detection of chemiluminescent signals from multiple microfluidic droplets aligned in a row in wells in a droplet actuator, the system comprising:

(a) a droplet actuator for being in alignment with a row of sample droplets in an imaging zone;

(b) a camera lens system for projecting an image onto the droplet actuator; and

(c) a mirror positioned for directing images from a row of sample droplets to the camera lens system, and to the droplet actuator.

358. The system of any of claims 357 and following, wherein the droplet actuator comprises a CCD droplet actuator.

359. The system of any of claims 357 and following, wherein the camera lens system is configured to demagnify images onto the droplet actuator.

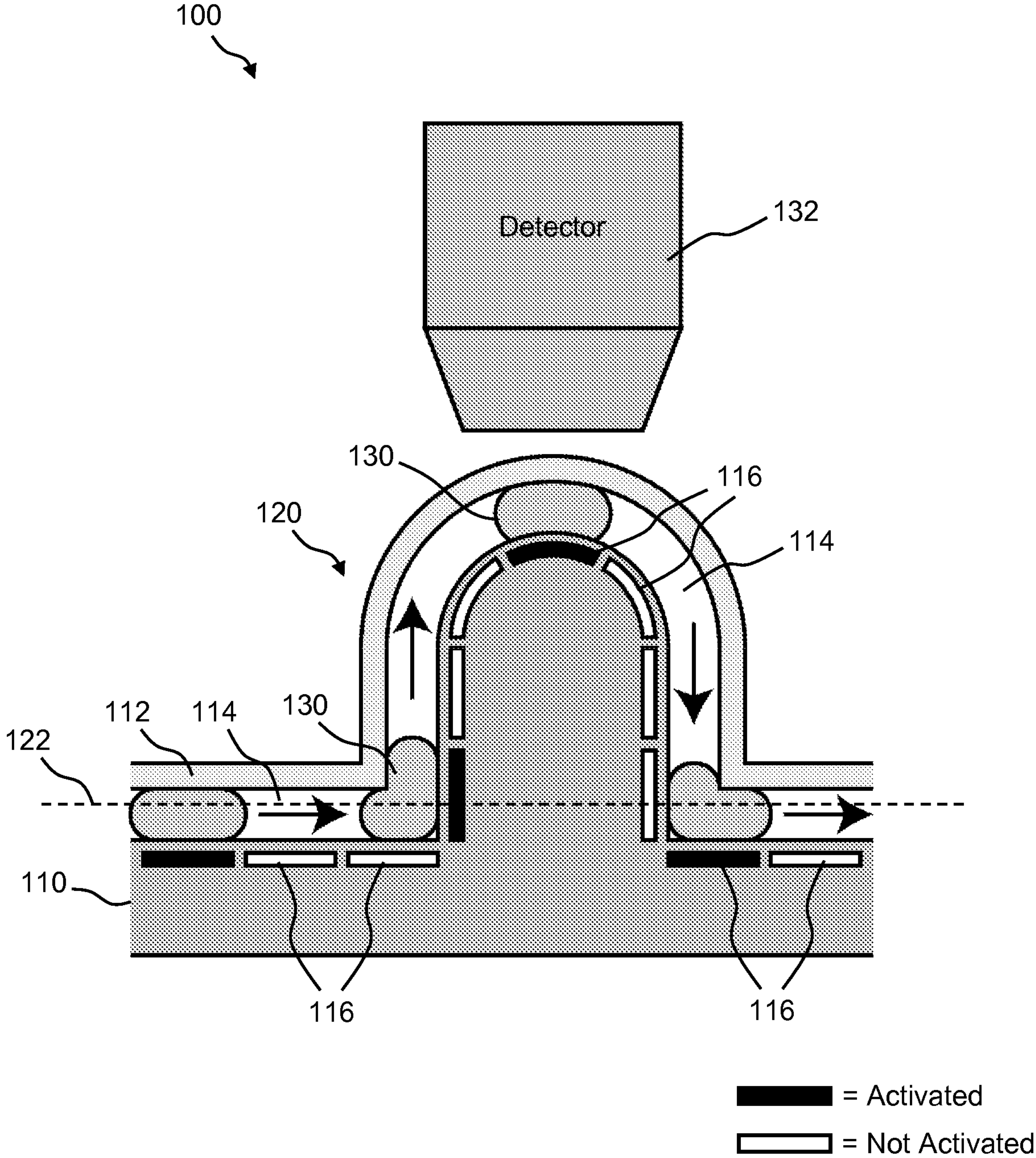

100
Detector
132
130
116
120
114
112
114
130
122
110
116
116
= Activated
= Not Activated

Figure 1

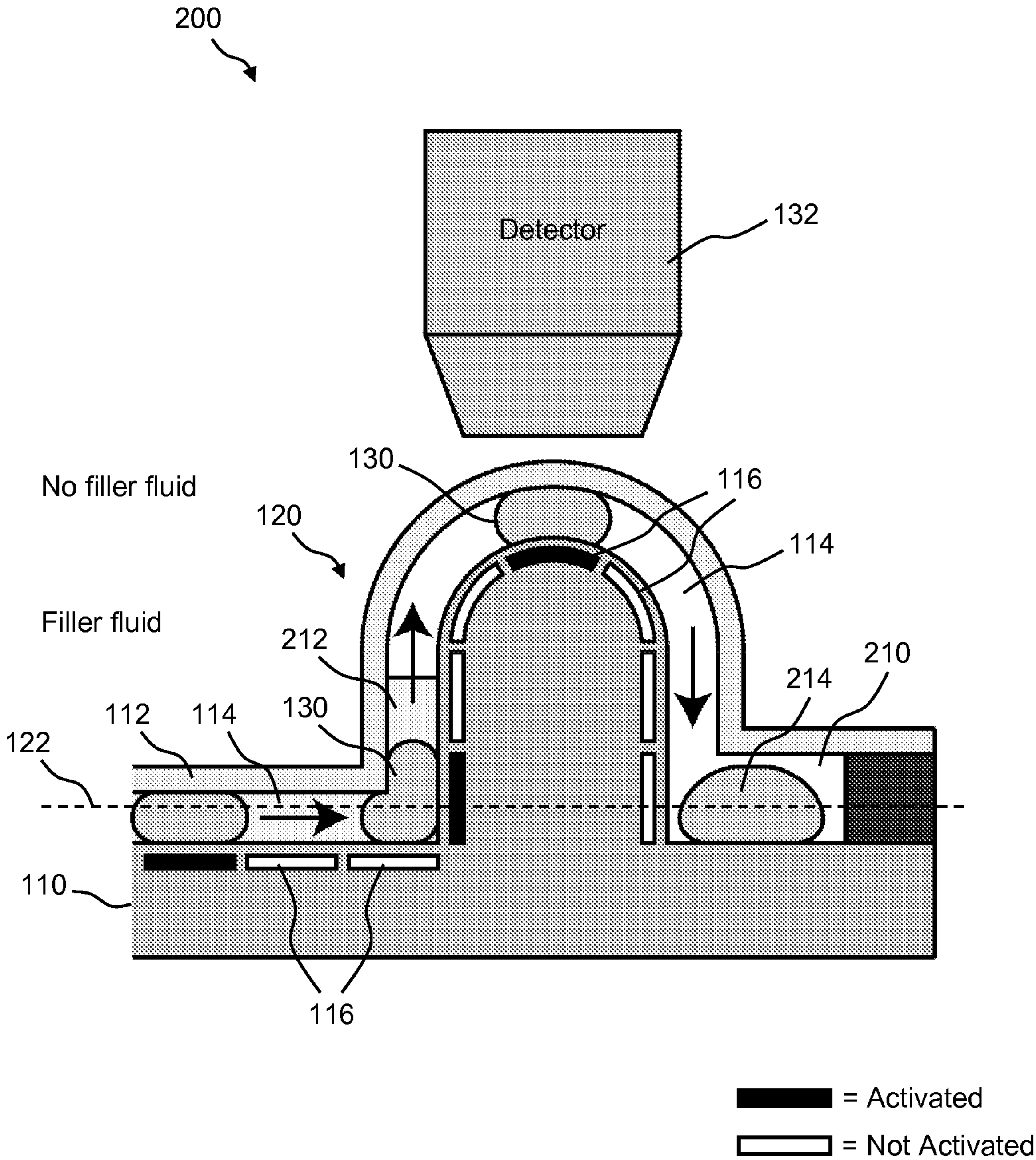

200
Detector
132
130
No filler fluid
116
120
114
Filler fluid
212
210
214
112
114
130
122
110
116
= Activated
= Not Activated

Figure 2

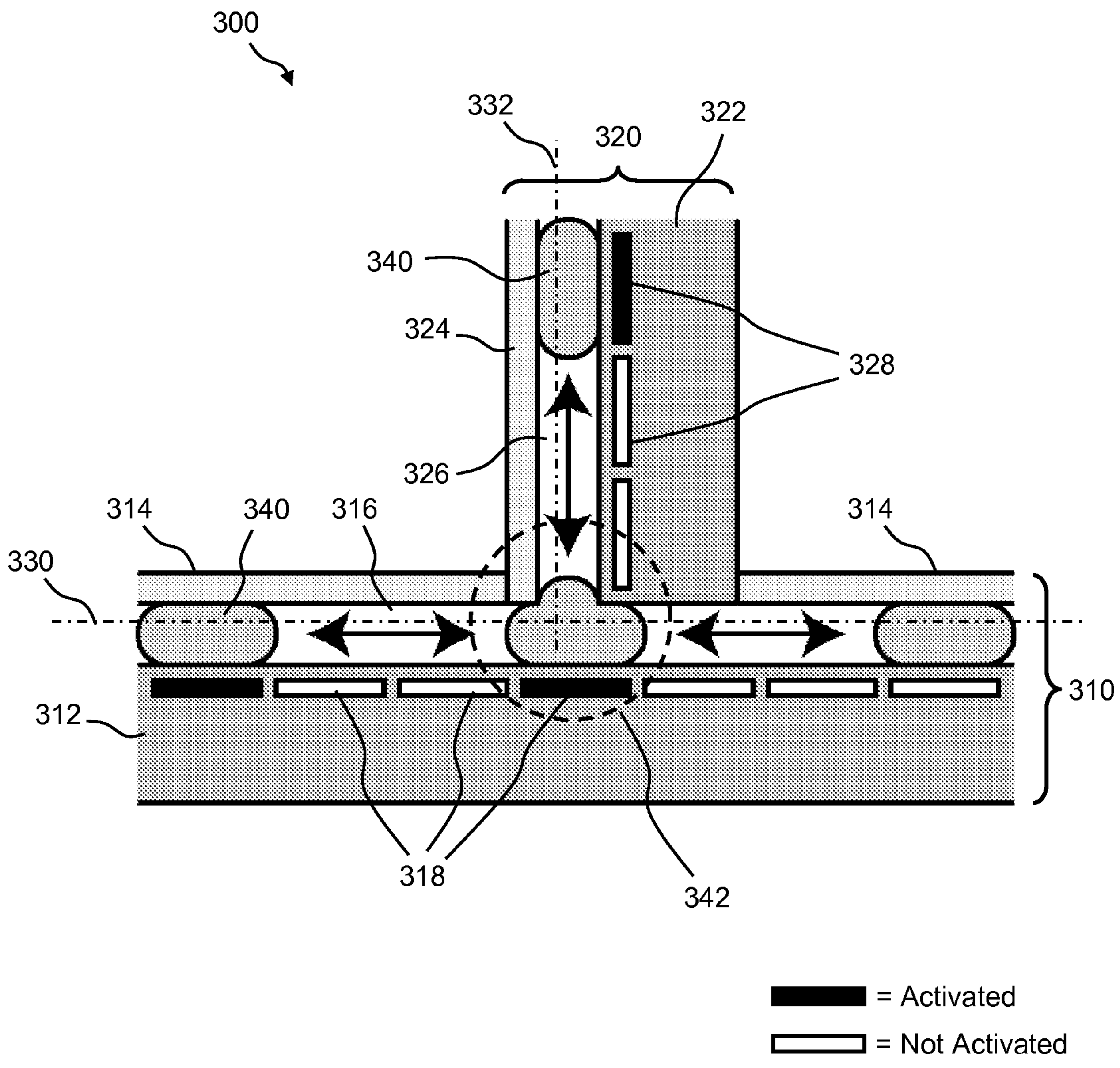

300
332
320
322
340
324
328
326
314
340
316
314
330
310
312
318
342
= Activated
= Not Activated

Figure 3

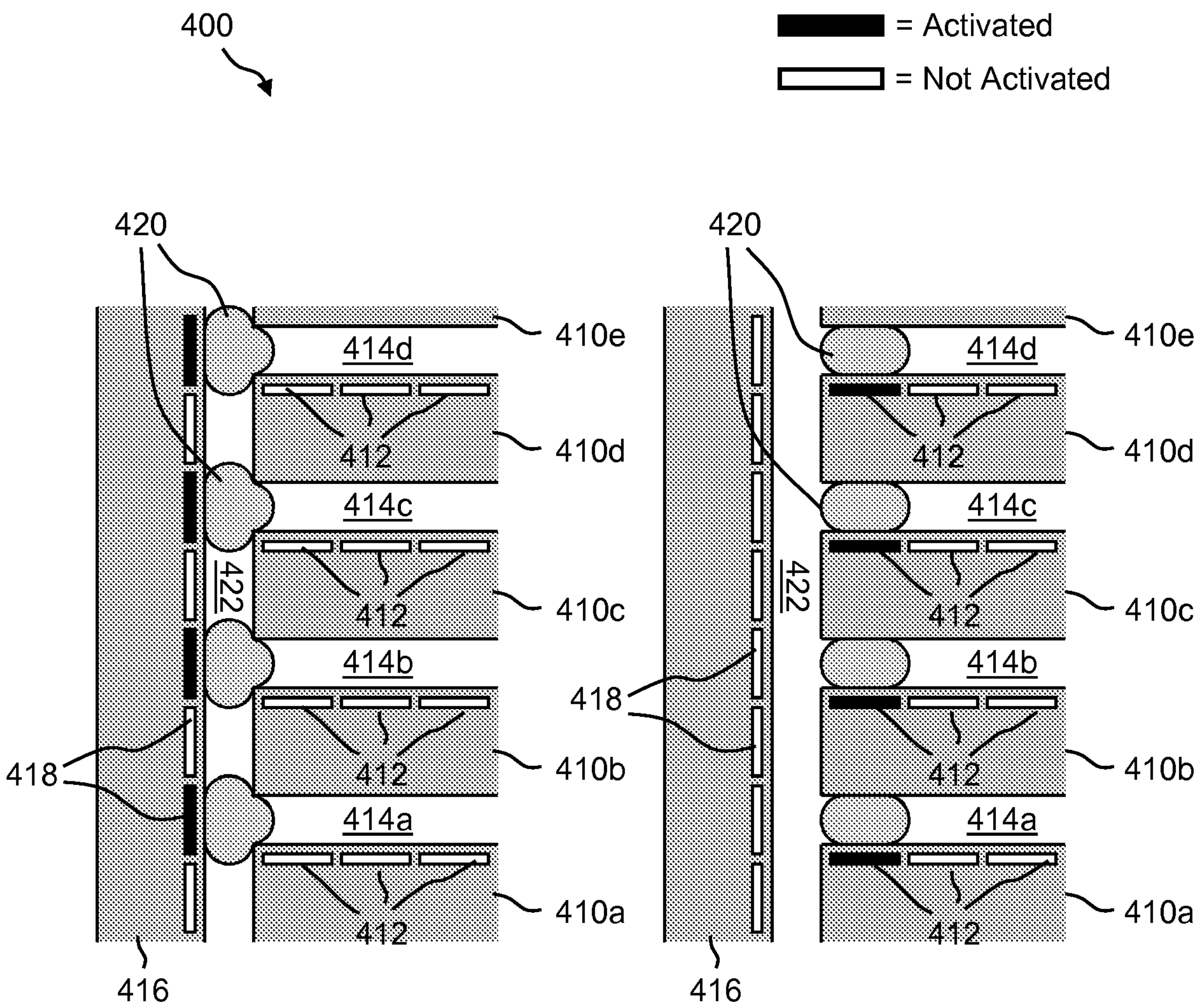


Figure 4A

Figure 4B

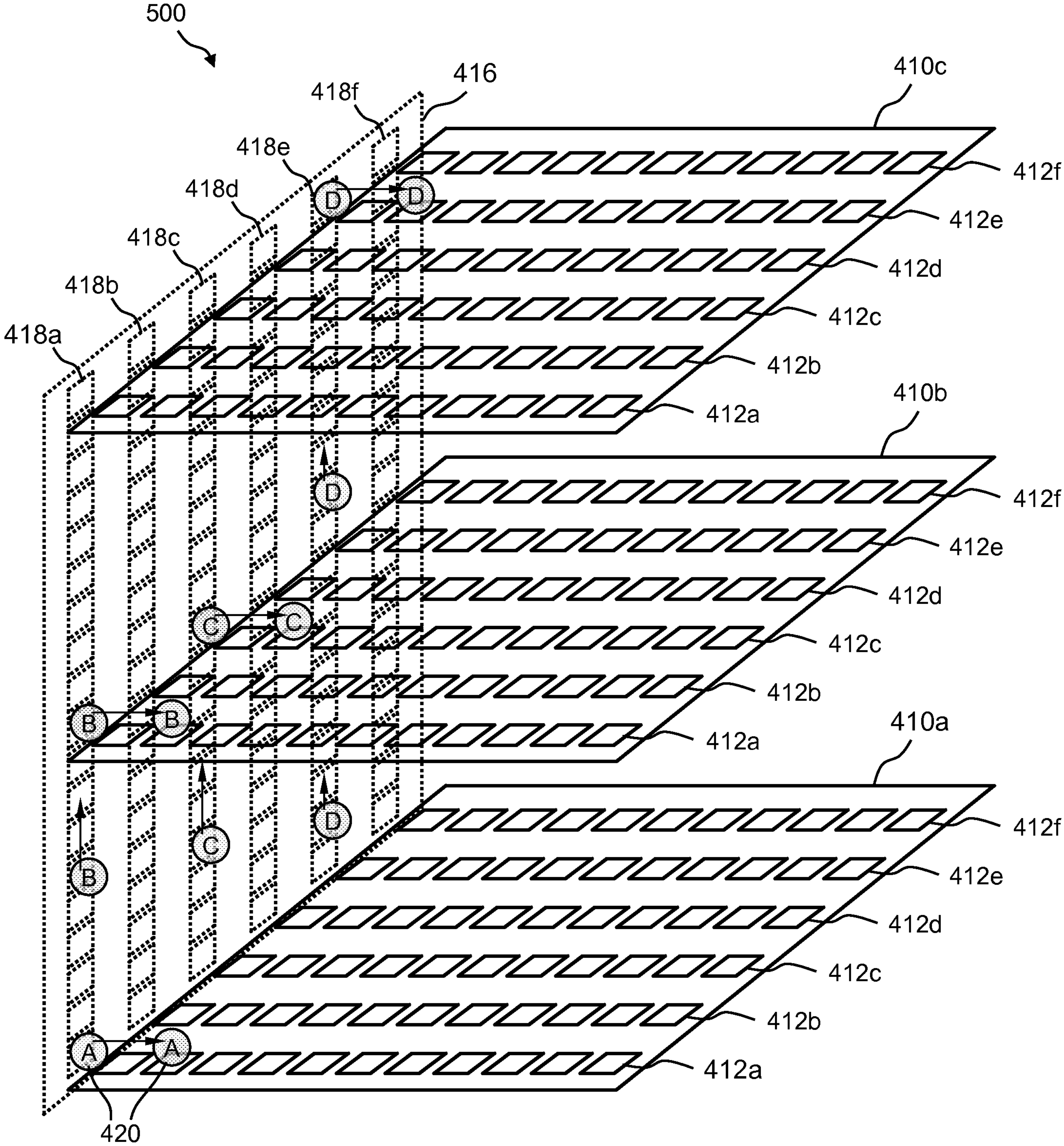

500
416
418f
418e
418d
418c
418b
418a
410c
410b
410a
412f
412e
412d
412c
412b
412a
A
B
C
D
420

Figure 5

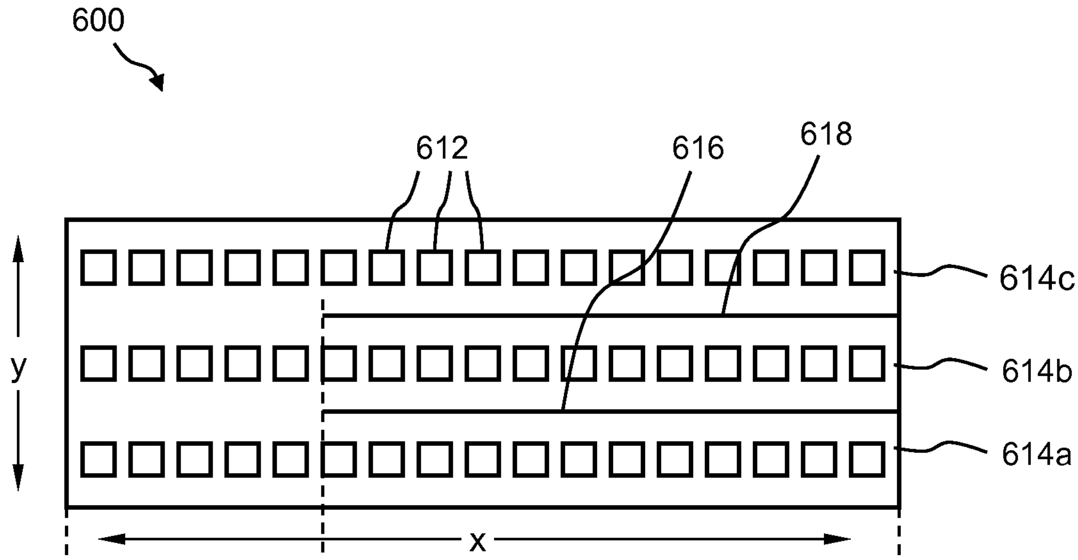


Figure 6A

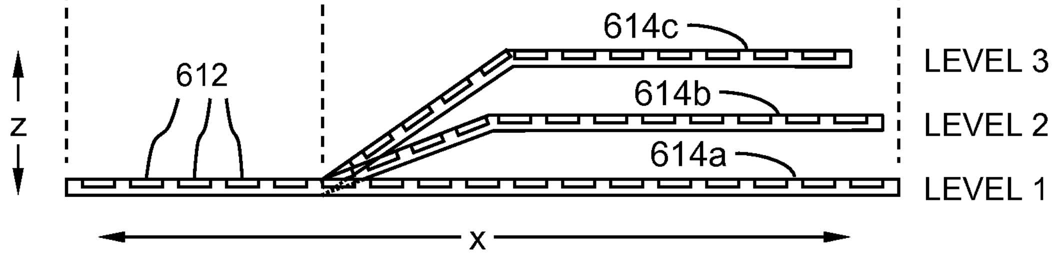


Figure 6B

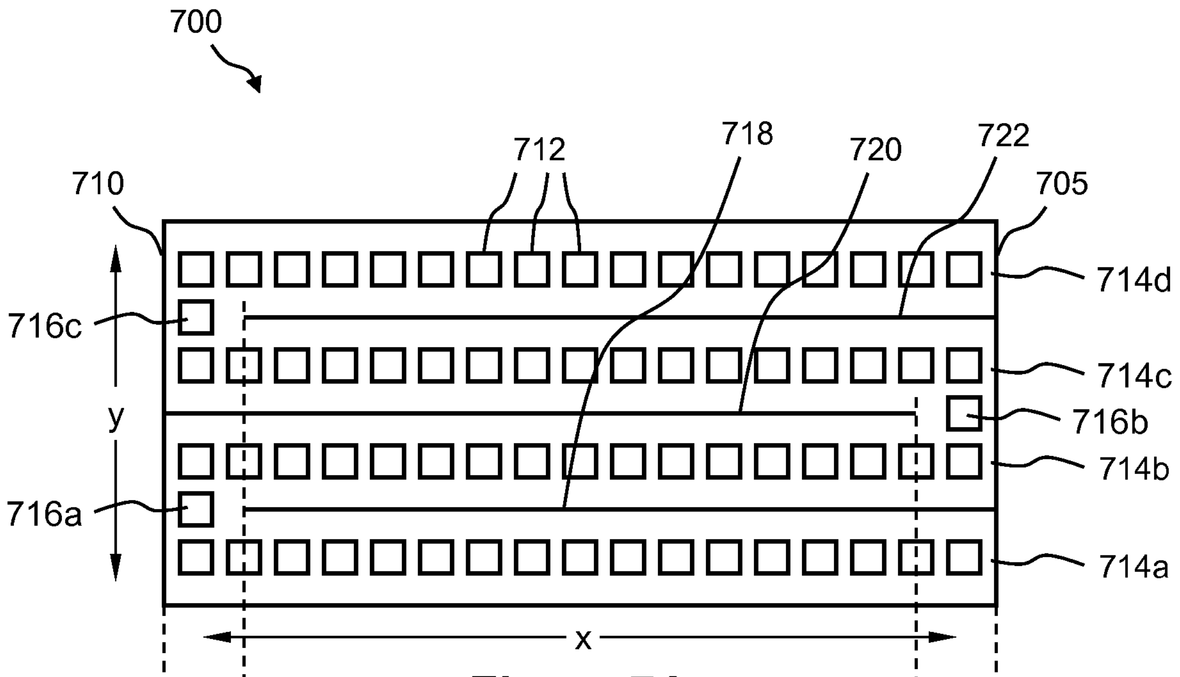


Figure 7A

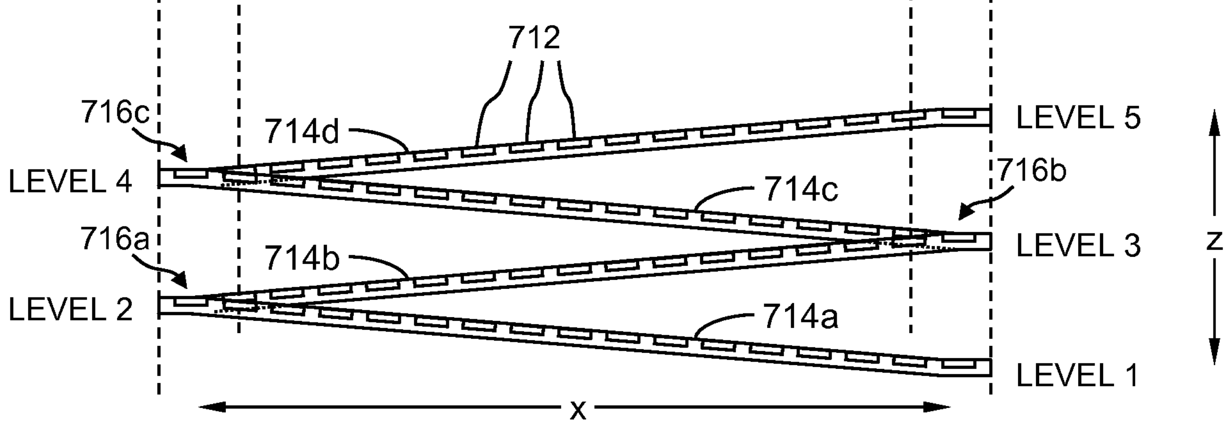


Figure 7B

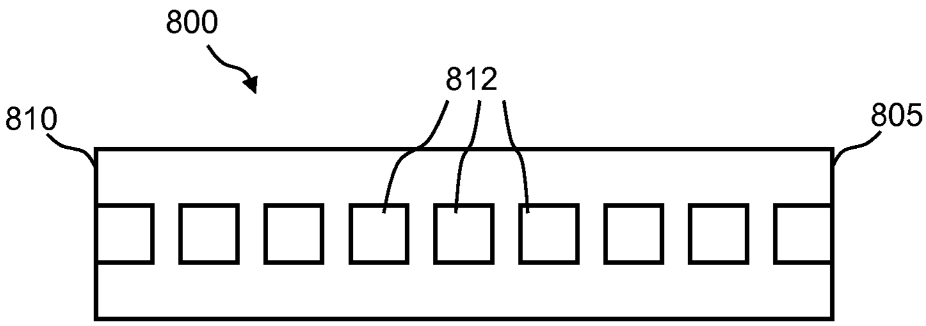


Figure 8A

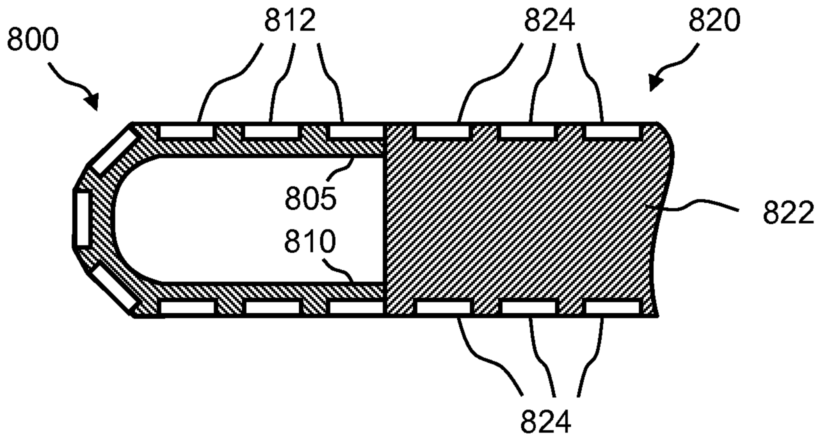


Figure 8B

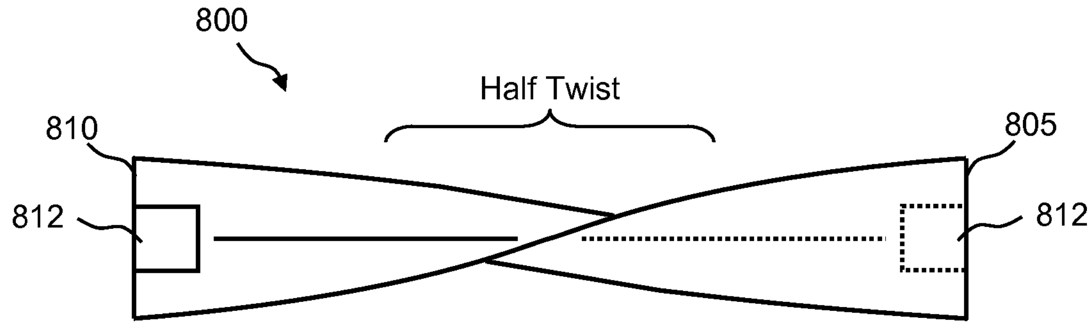


Figure 9A

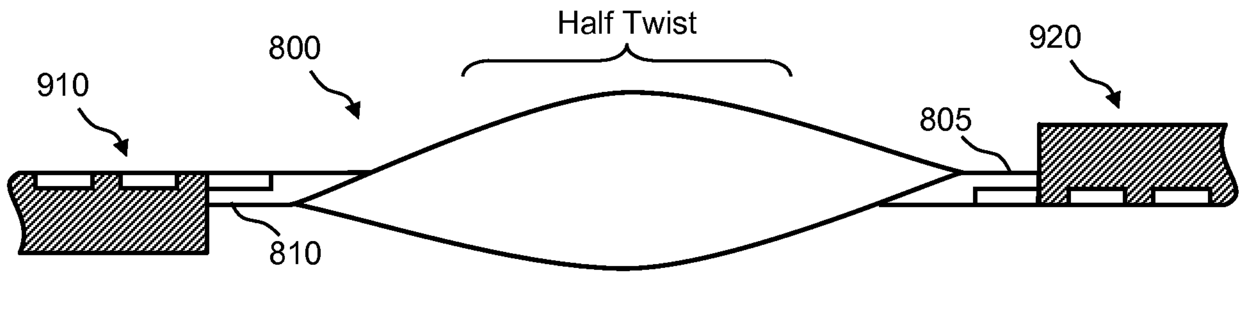


Figure 9B

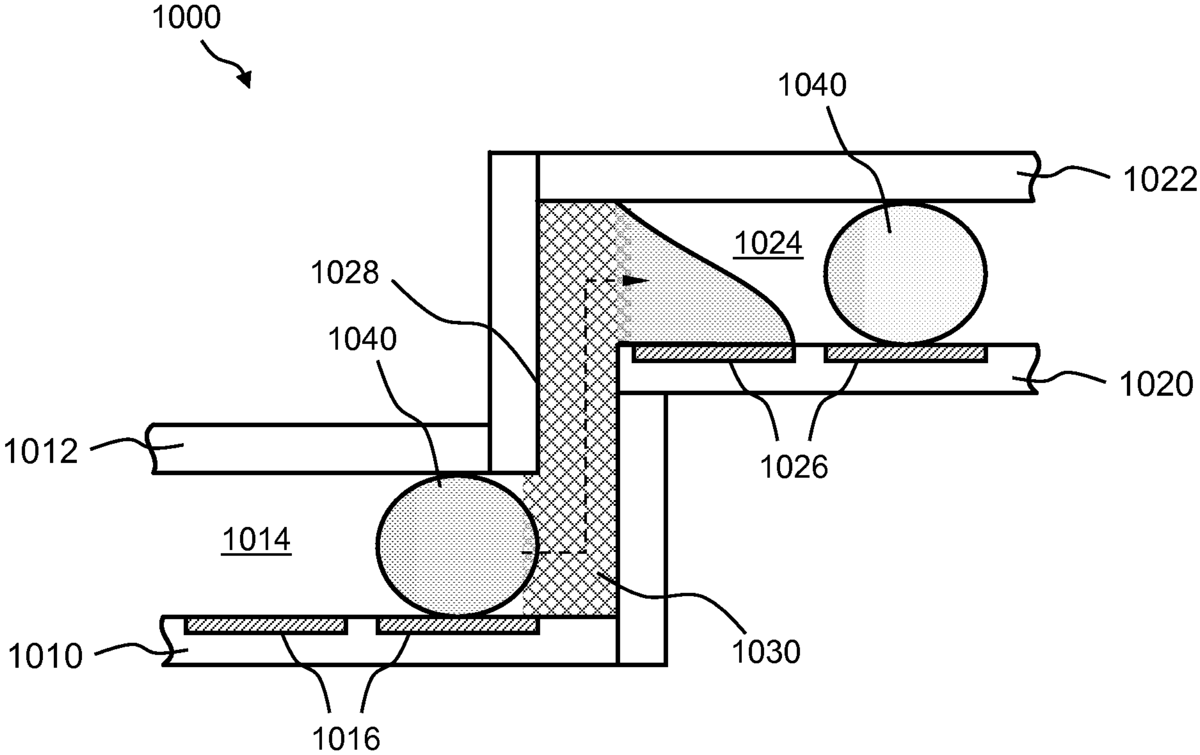

1000
1040
1022
1024
1028
1040
1020
1012
1026
1014
1030
1010
1016

Figure 10

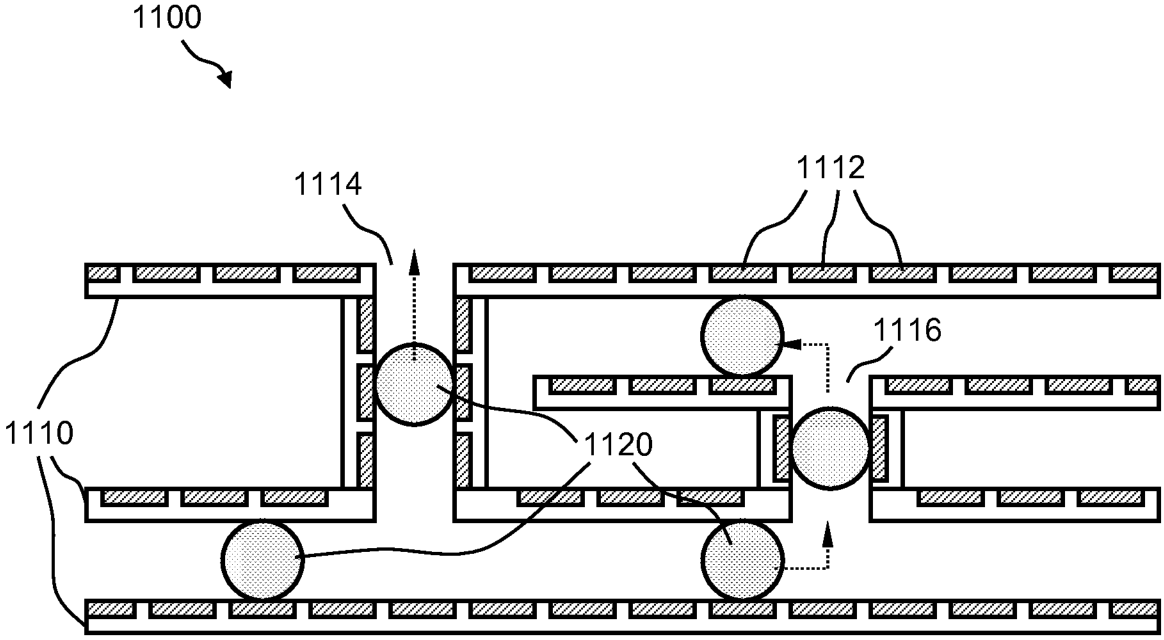


Figure 11

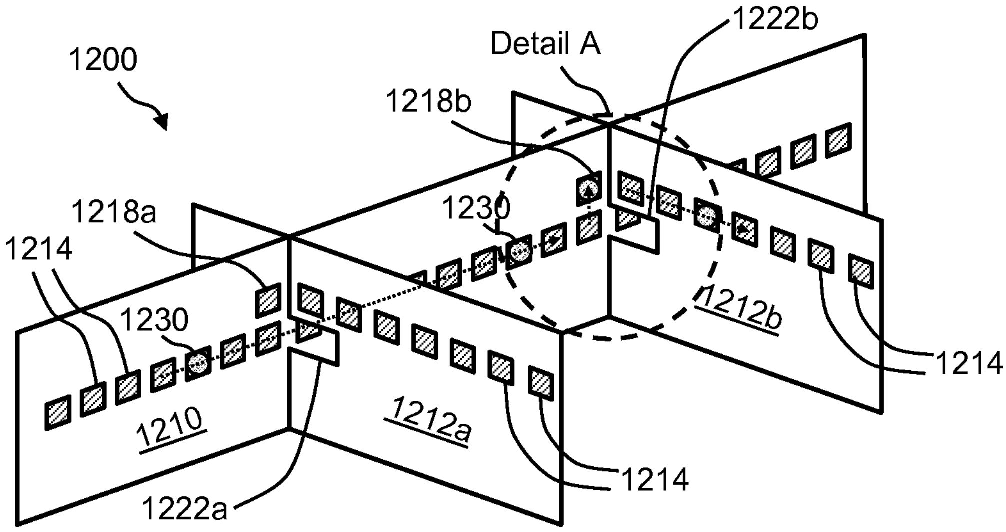


Figure 12A

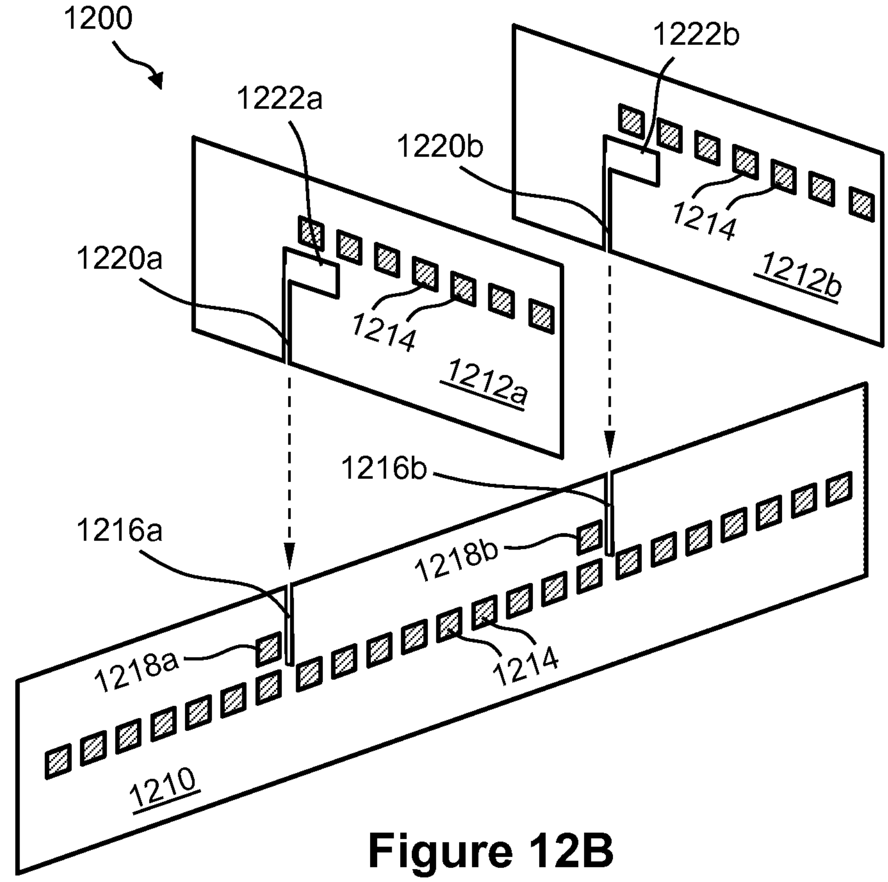


Figure 12B

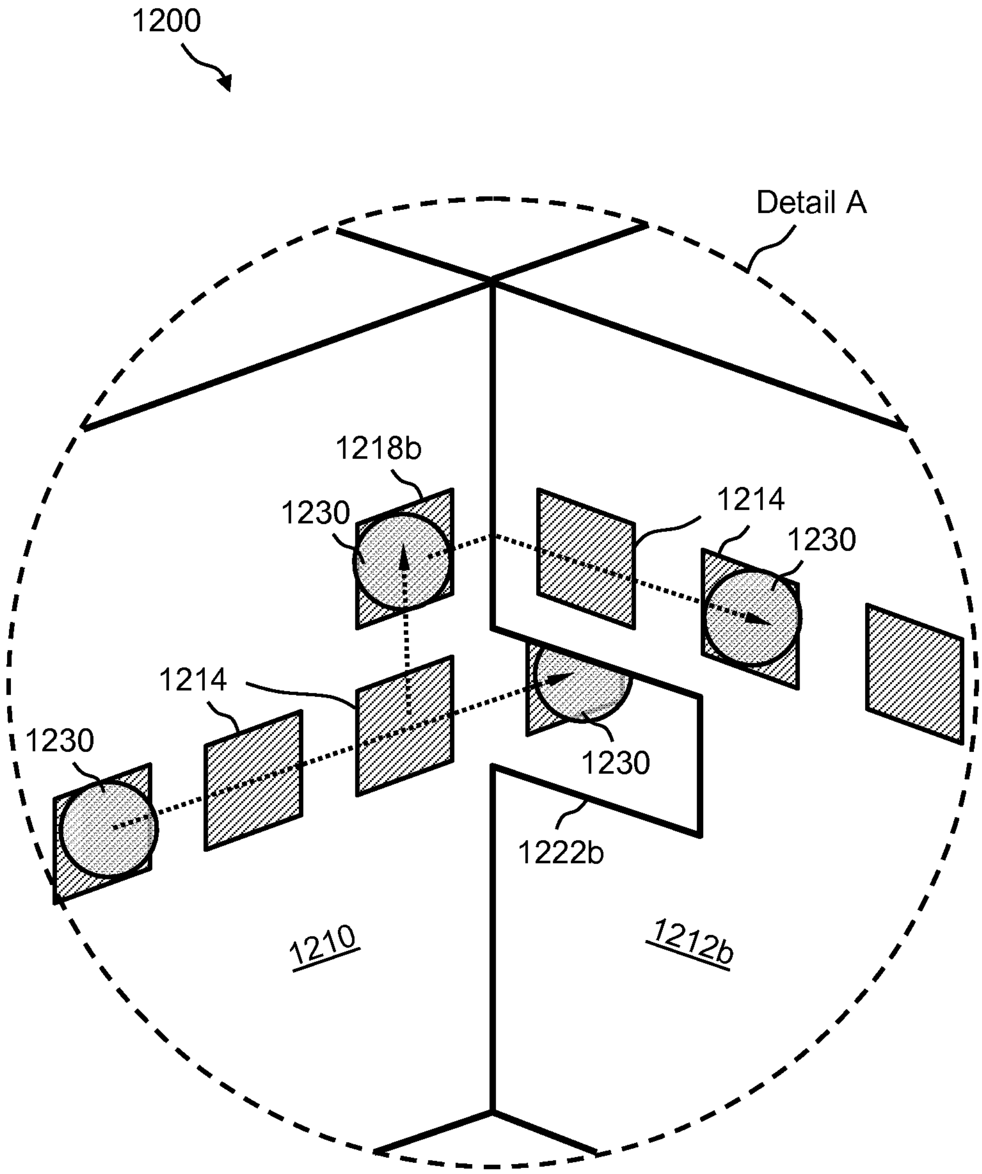

1200
Detail A
1218b
1230
1214
1230
1214
1230
1230
1222b
1210
1212b

Figure 12C

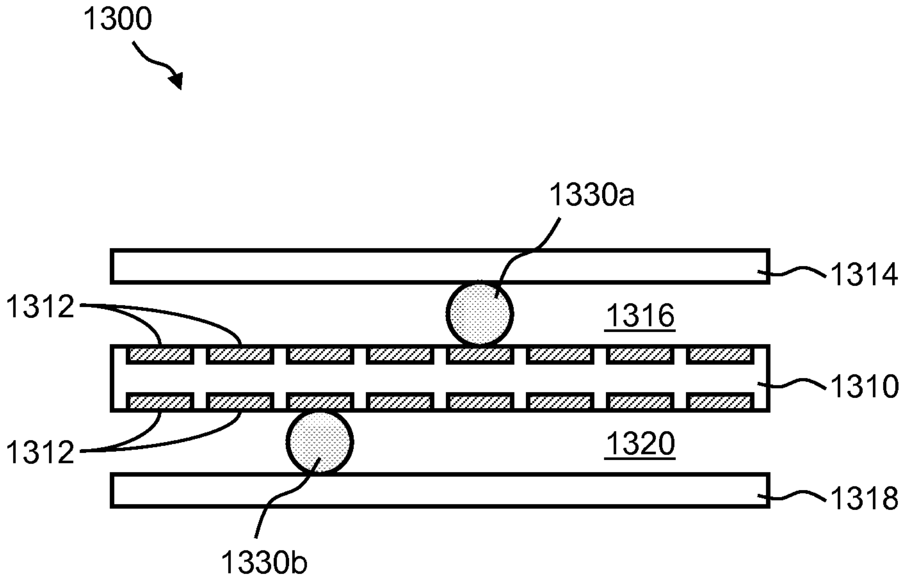


Figure 13A

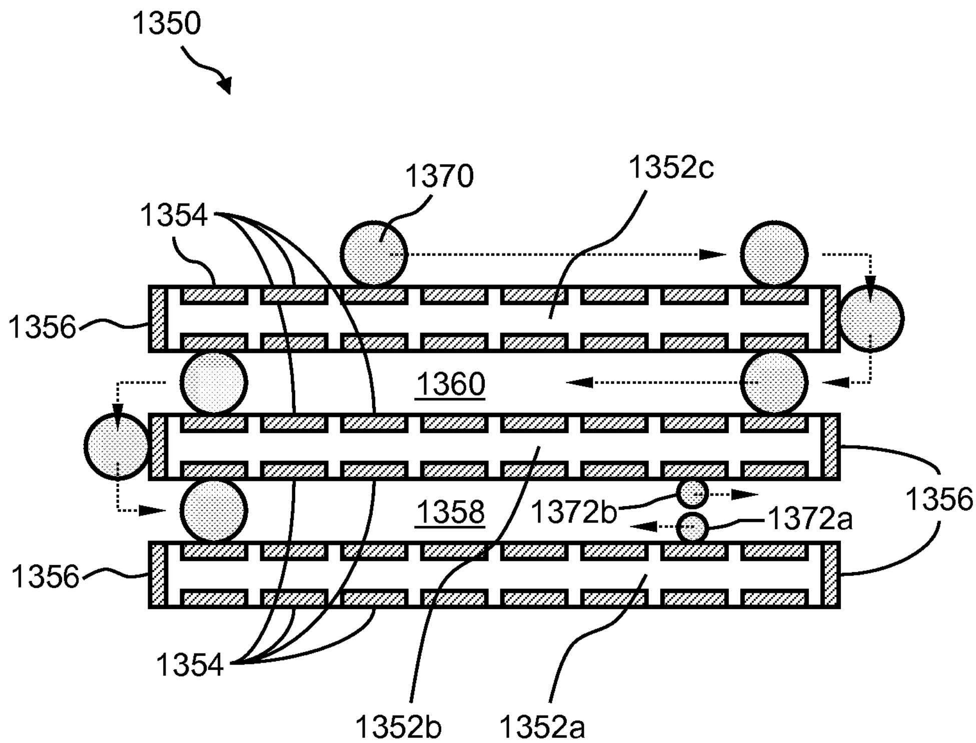


Figure 13B

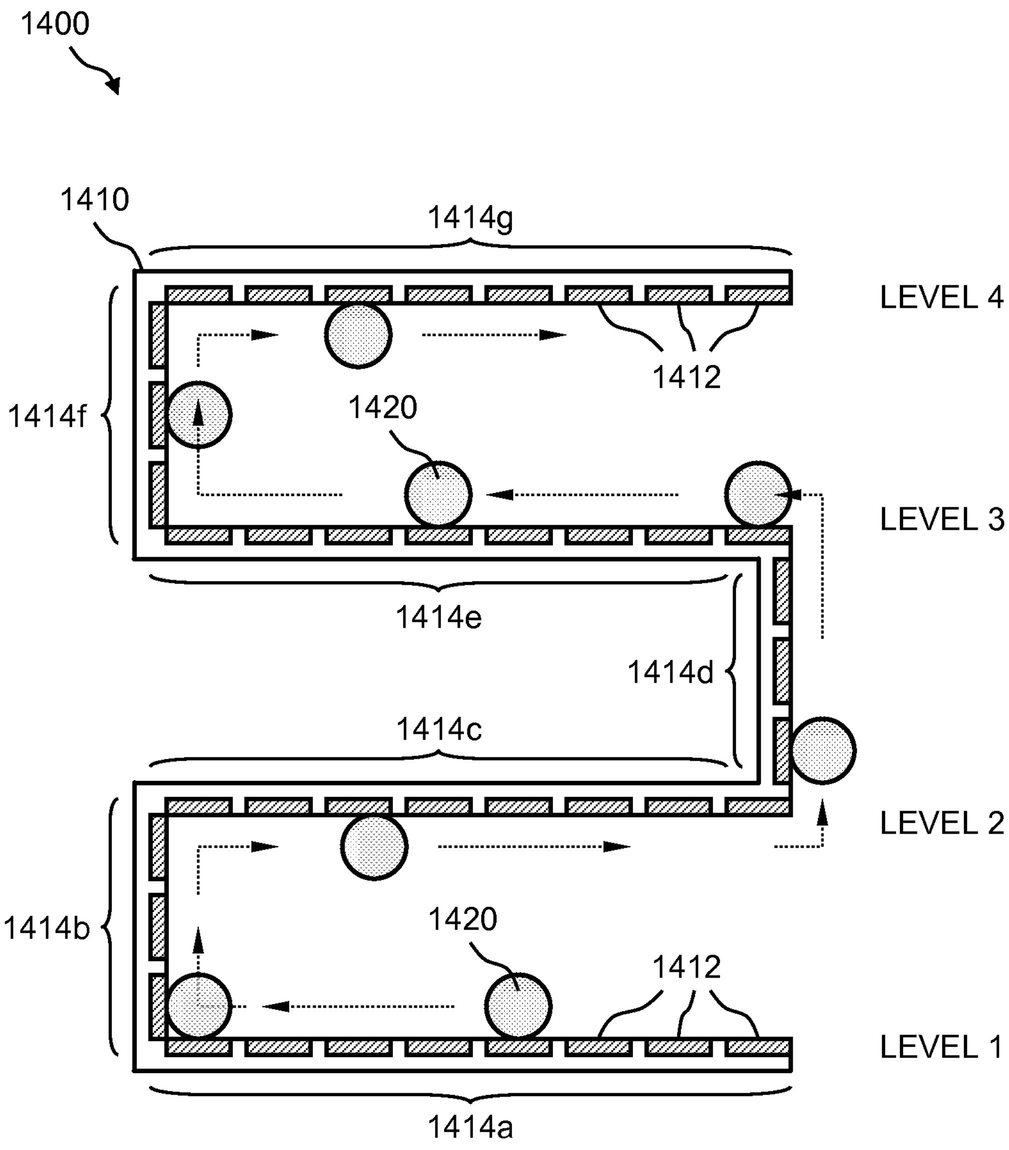

1400
1410
1414g
LEVEL 4
1412
1414f
1420
LEVEL 3
1414e
1414d
1414c
LEVEL 2
1414b
1420
1412
LEVEL 1
1414a

Figure 14

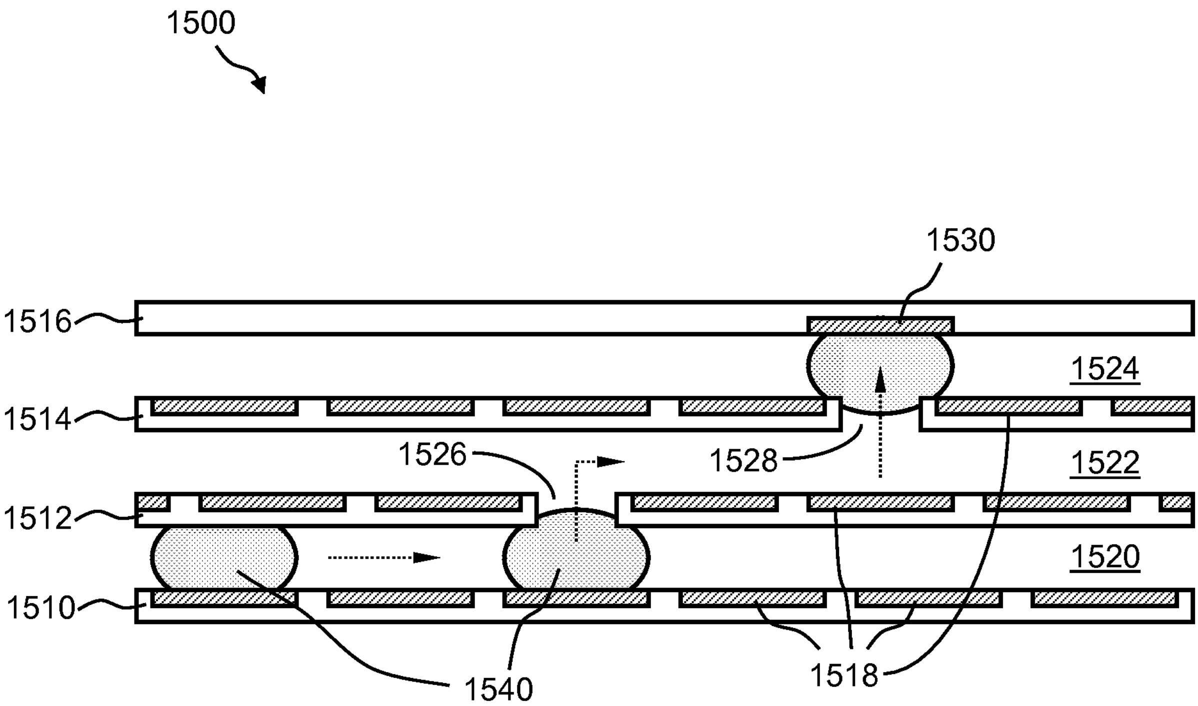

1500
1530
1516
1524
1514
1526
1528
1522
1512
1520
1510
1540
1518

Figure 15

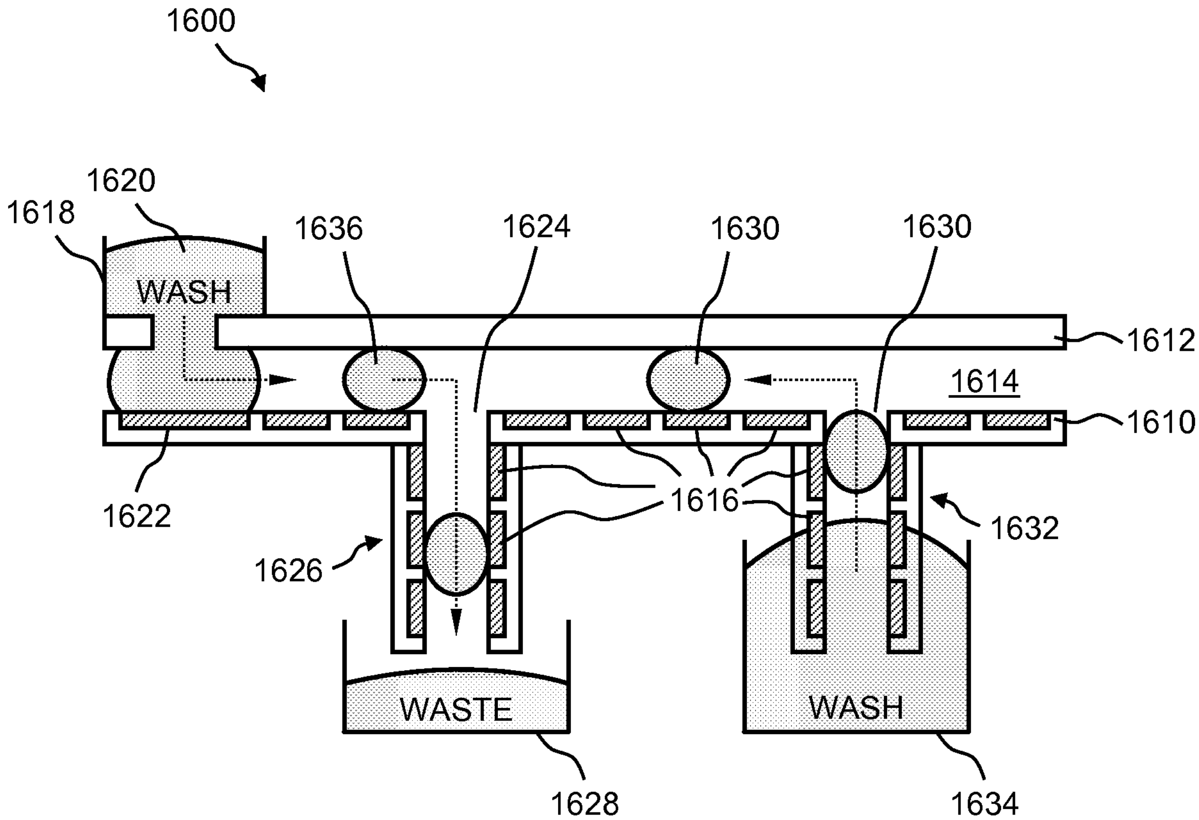

1600
1620
1618
WASH
1636
1624
1630
1630
1612
1614
1610
1622
1616
1632
1626
WASTE
WASH
1628
1634

Figure 16

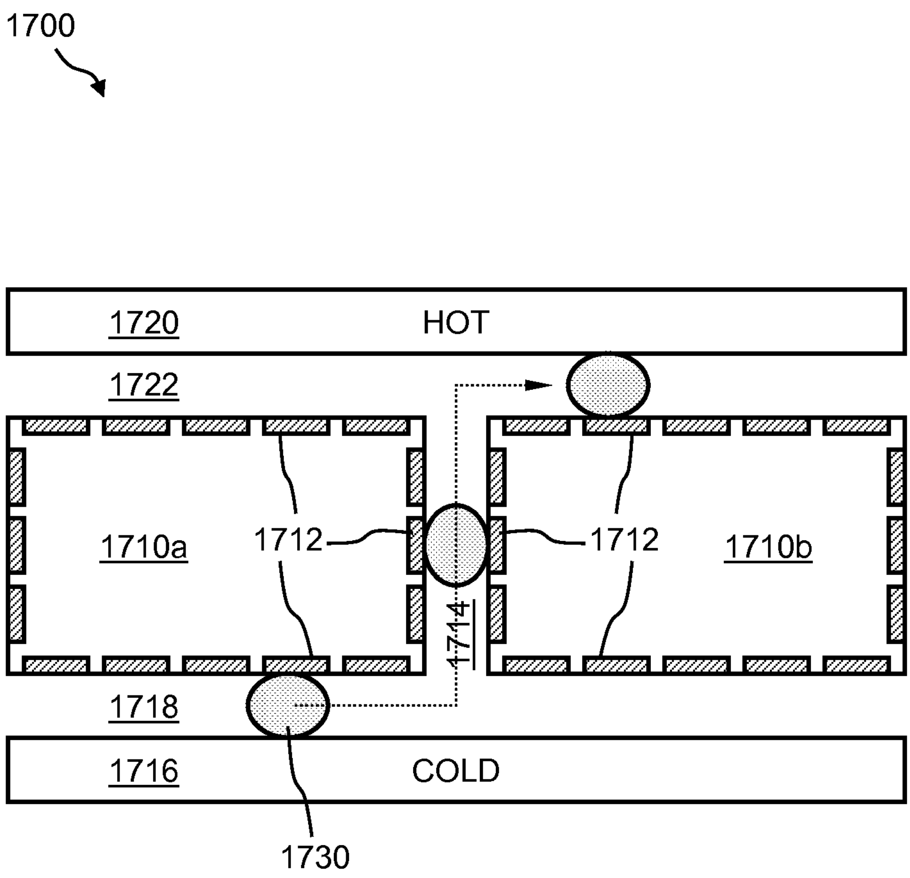

1700
1720
HOT
1722
1710a
1712
1712
1710b
1714
1718
1716
COLD
1730

Figure 17

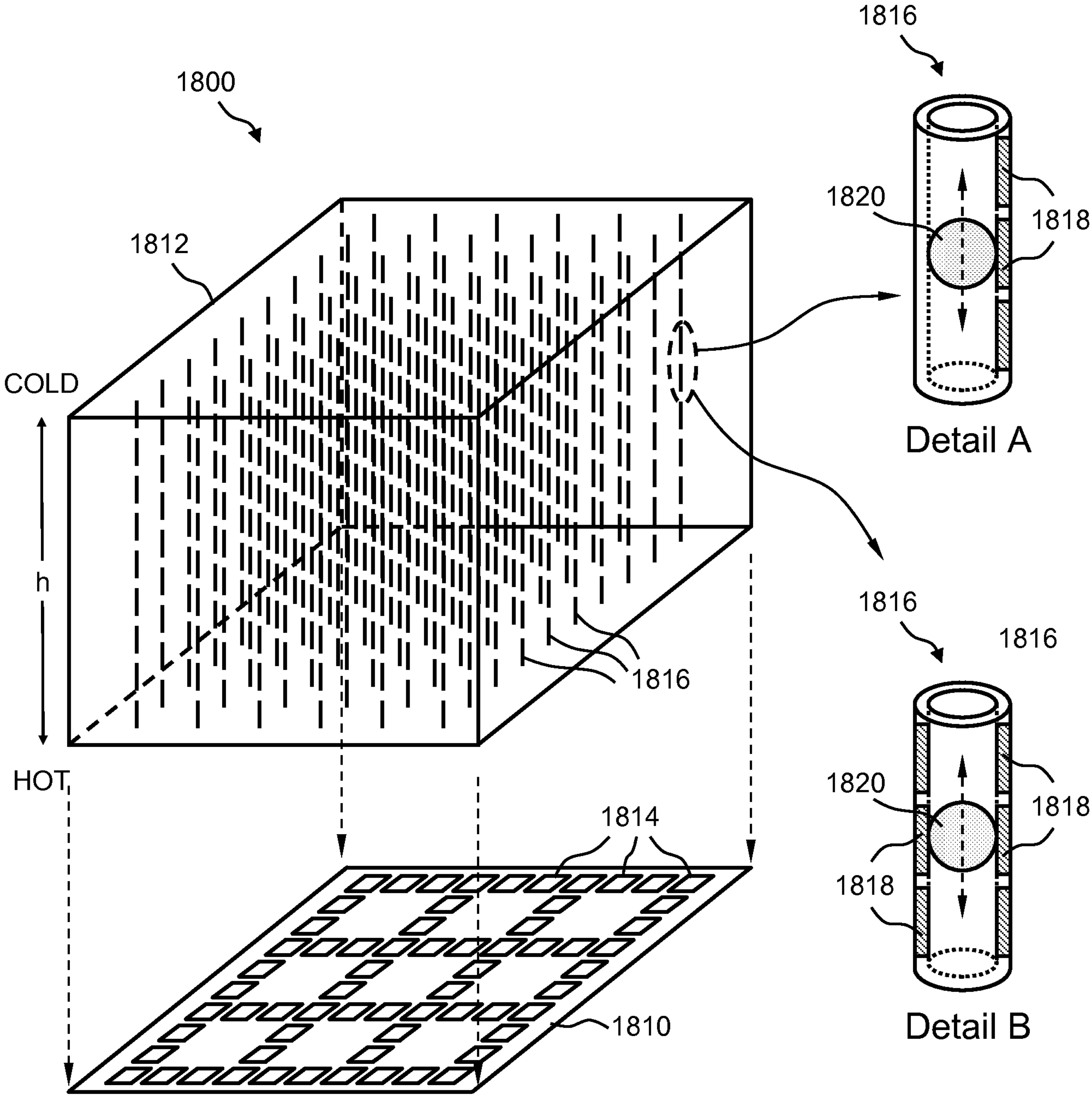

1800
1812
COLD
h
HOT
1816
1814
1810
1816
1820
1818
Detail A
1816
1816
1820
1818
1818
Detail B

Figure 18

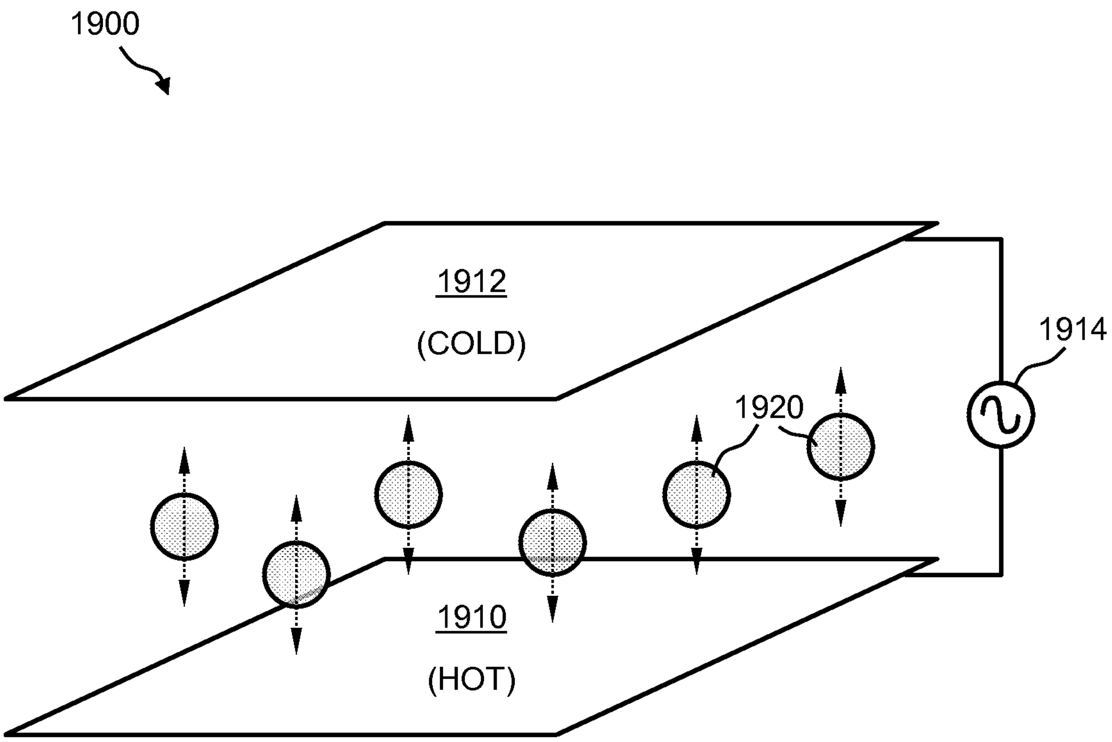


Figure 19

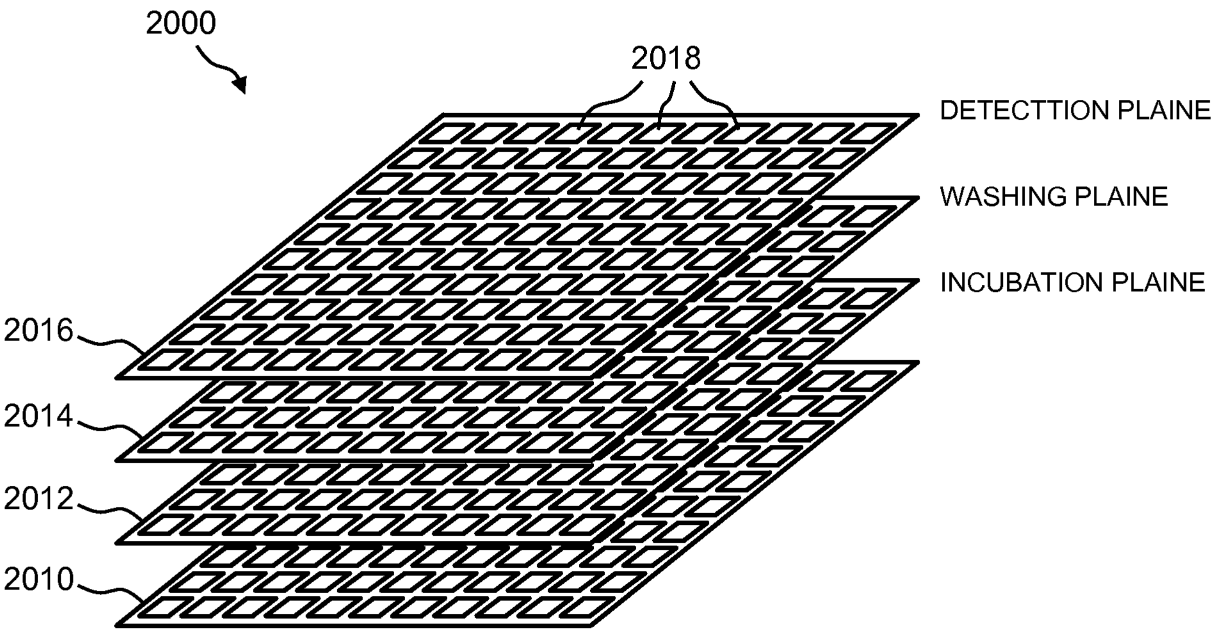

2000
2018
DETECTTION PLAINE
WASHING PLAINE
INCUBATION PLAINE
2016
2014
2012
2010

Figure 20

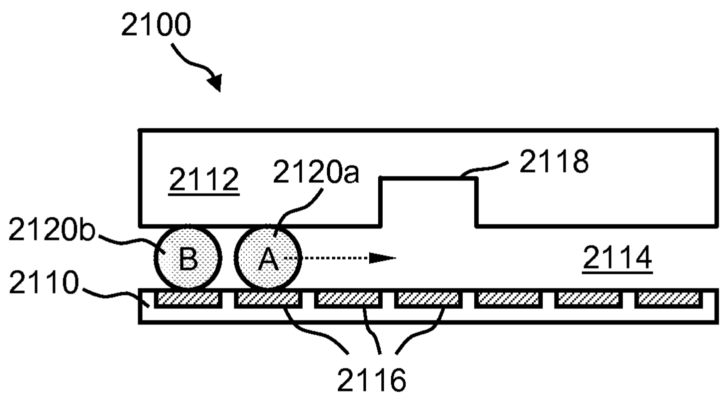


Figure 21A

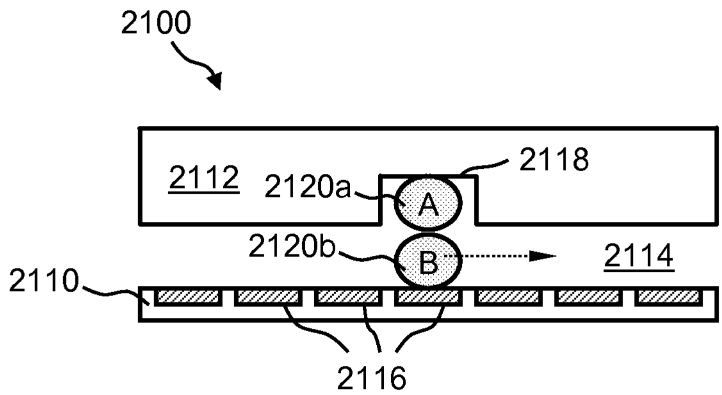


Figure 21B

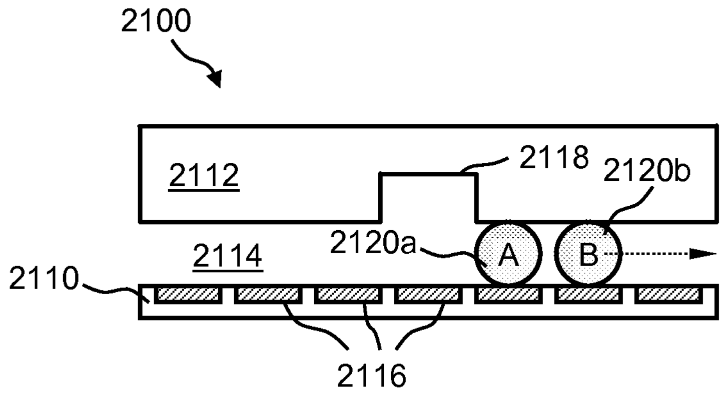


Figure 21C

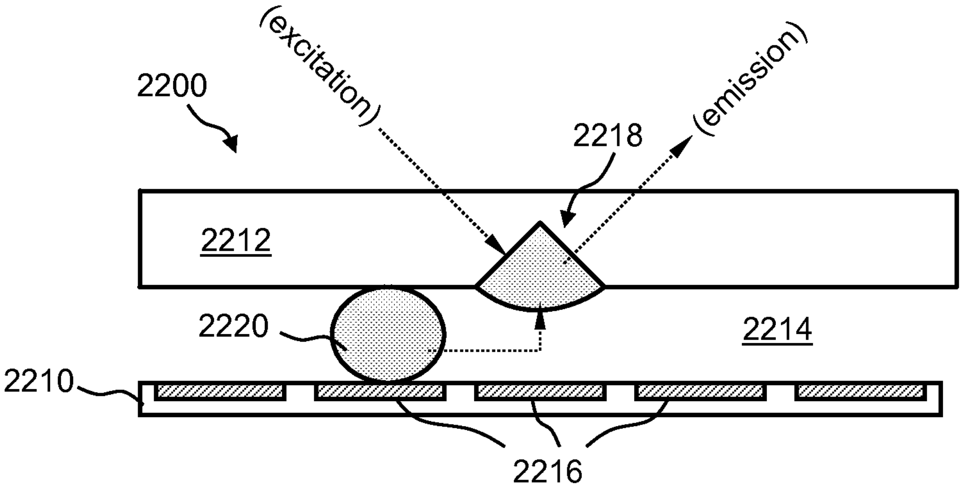


Figure 22A

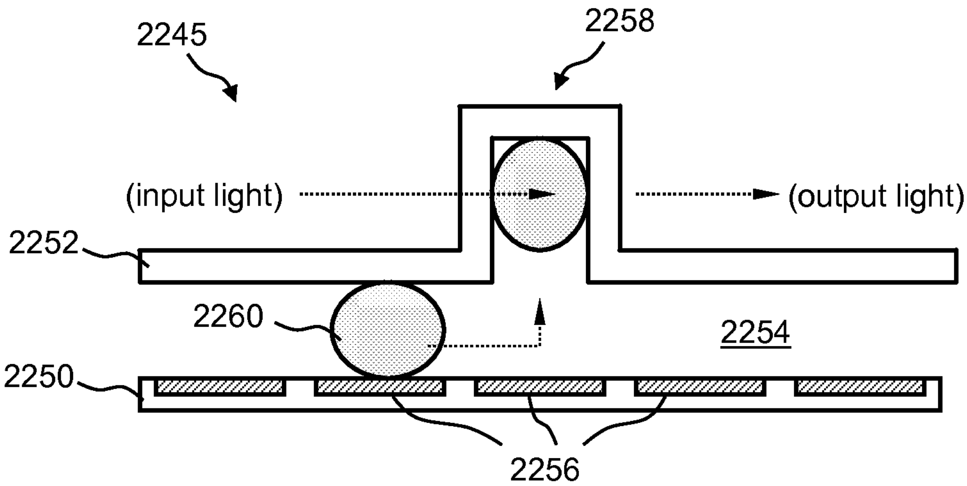


Figure 22B

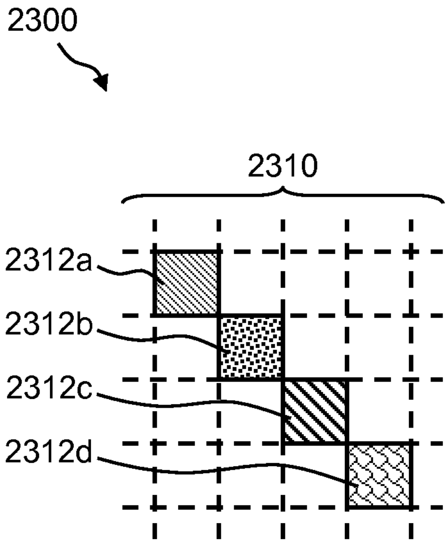


Figure 23

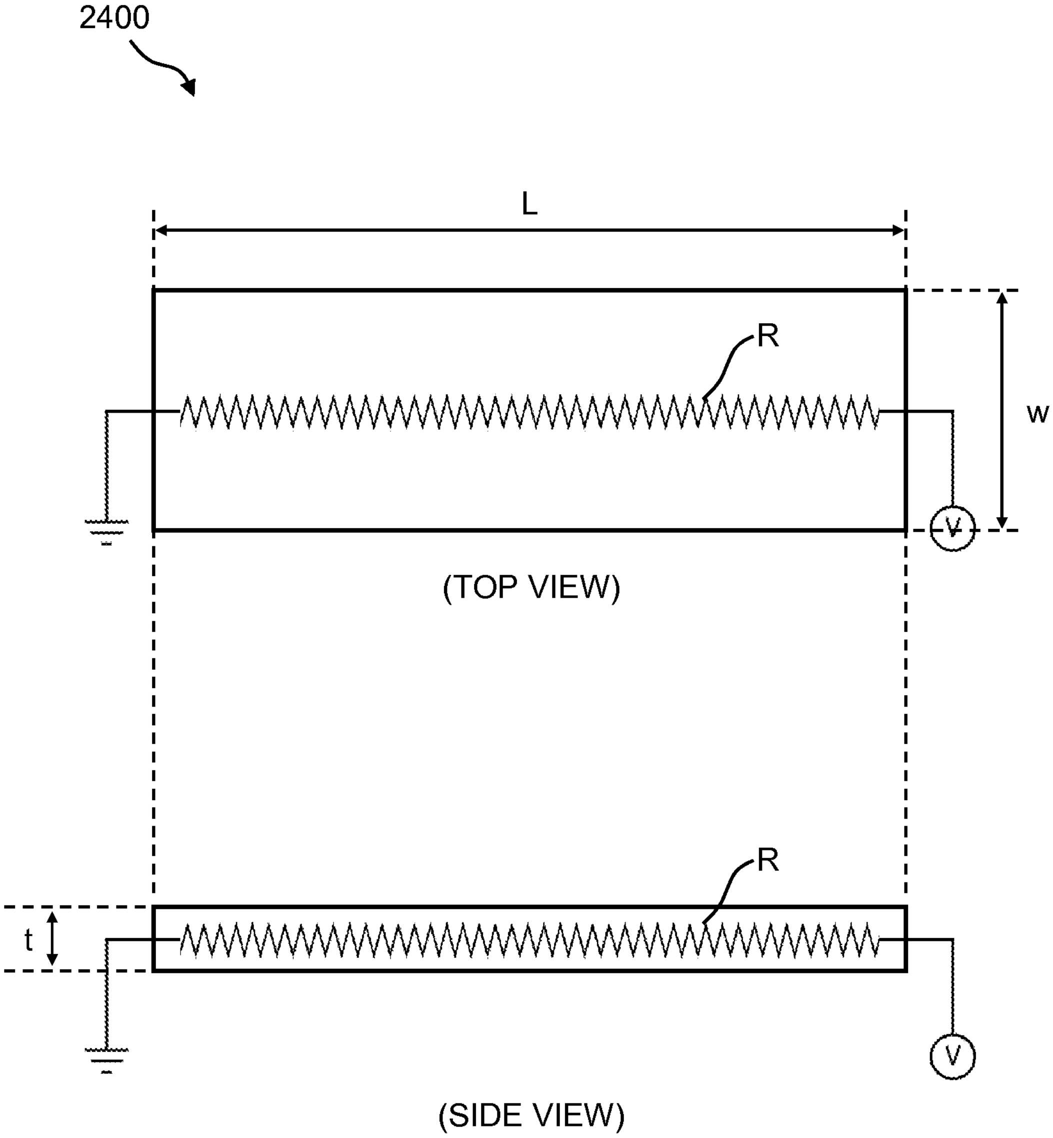


Figure 24

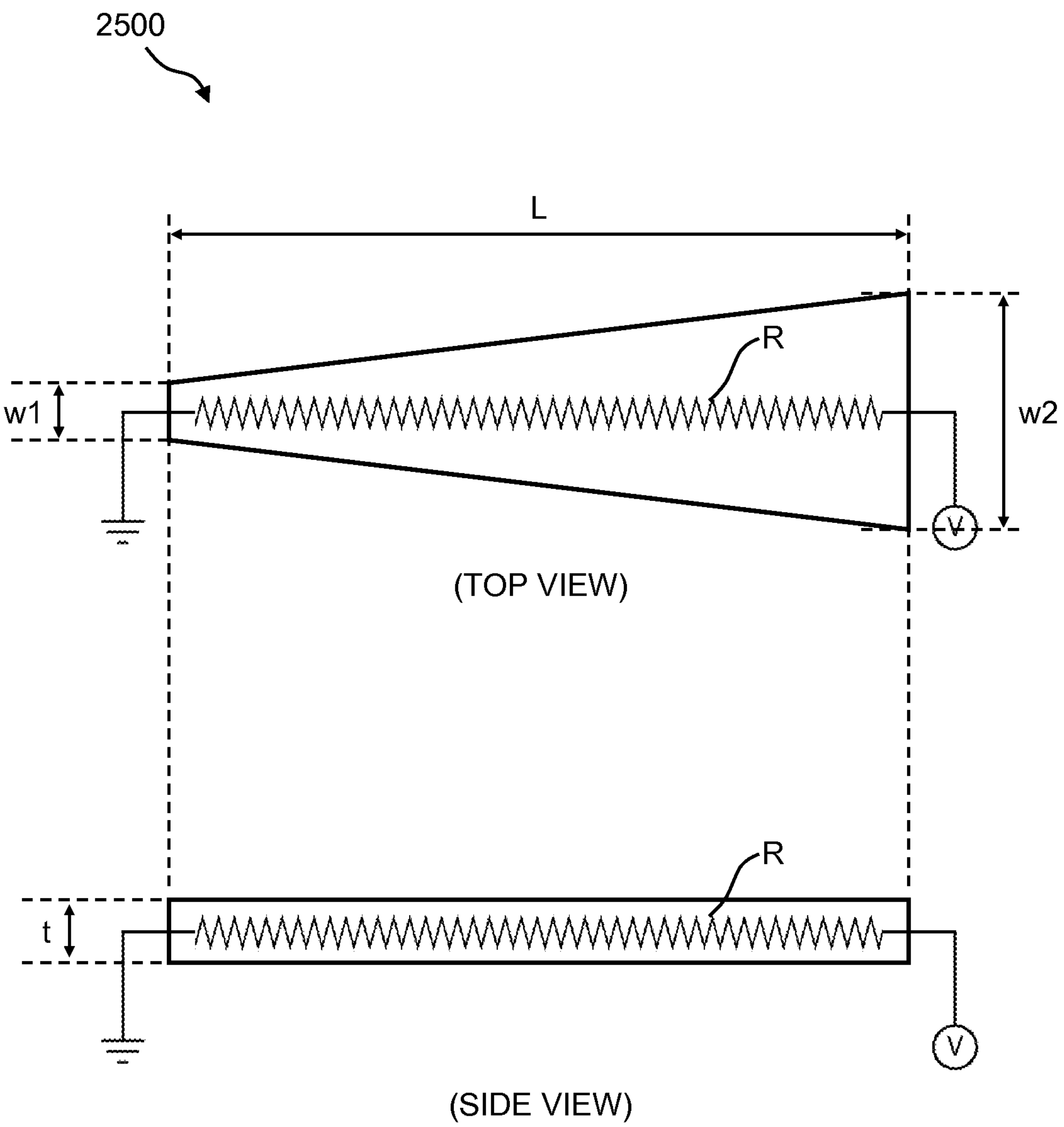


Figure 25

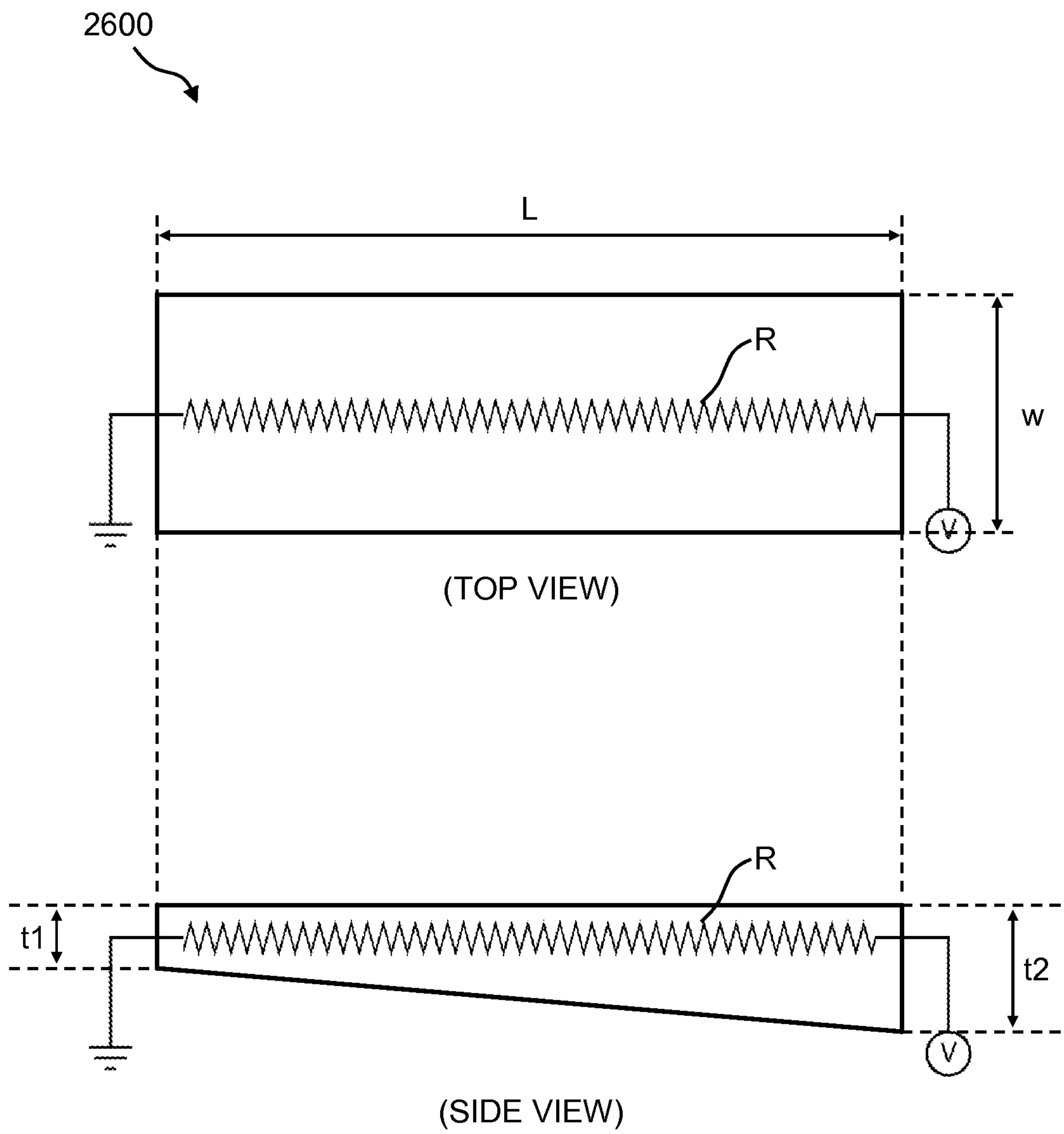


Figure 26

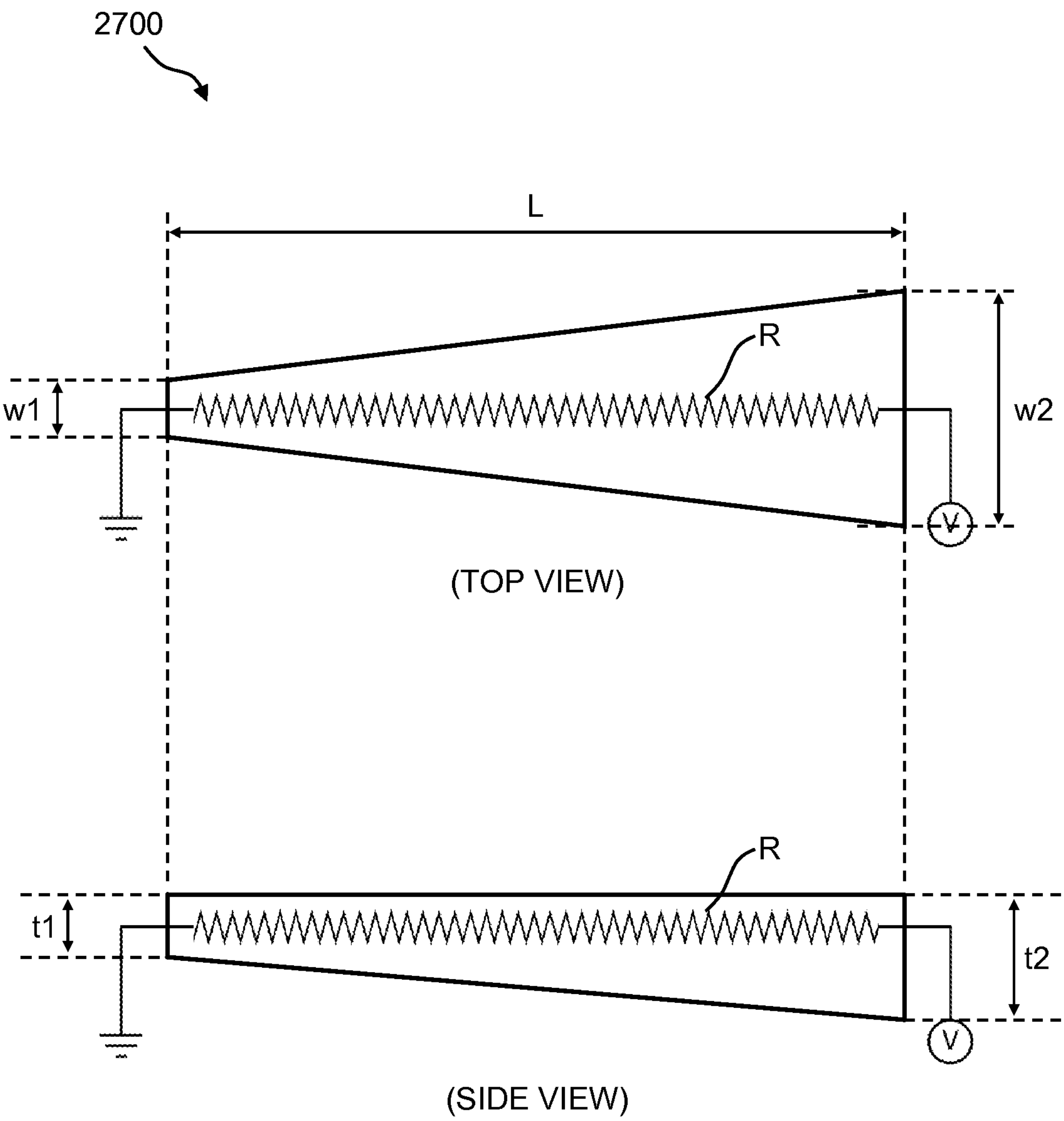


Figure 27

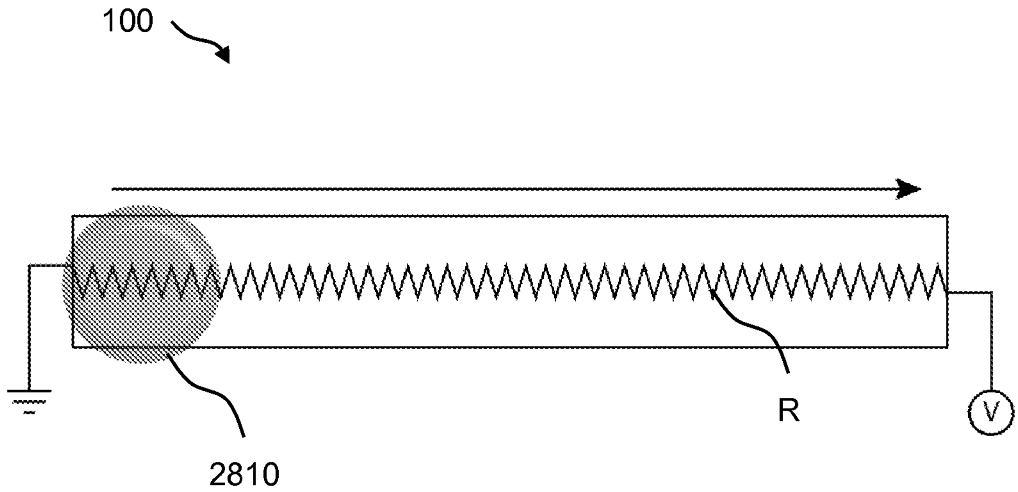


Figure 28A

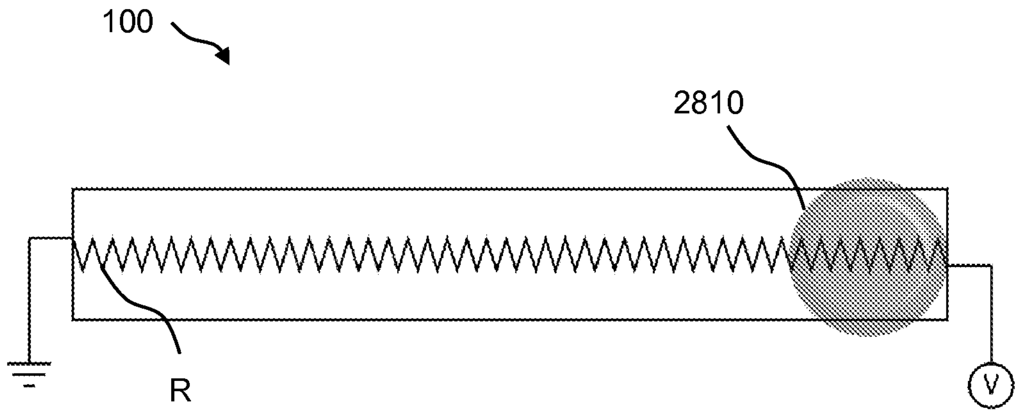
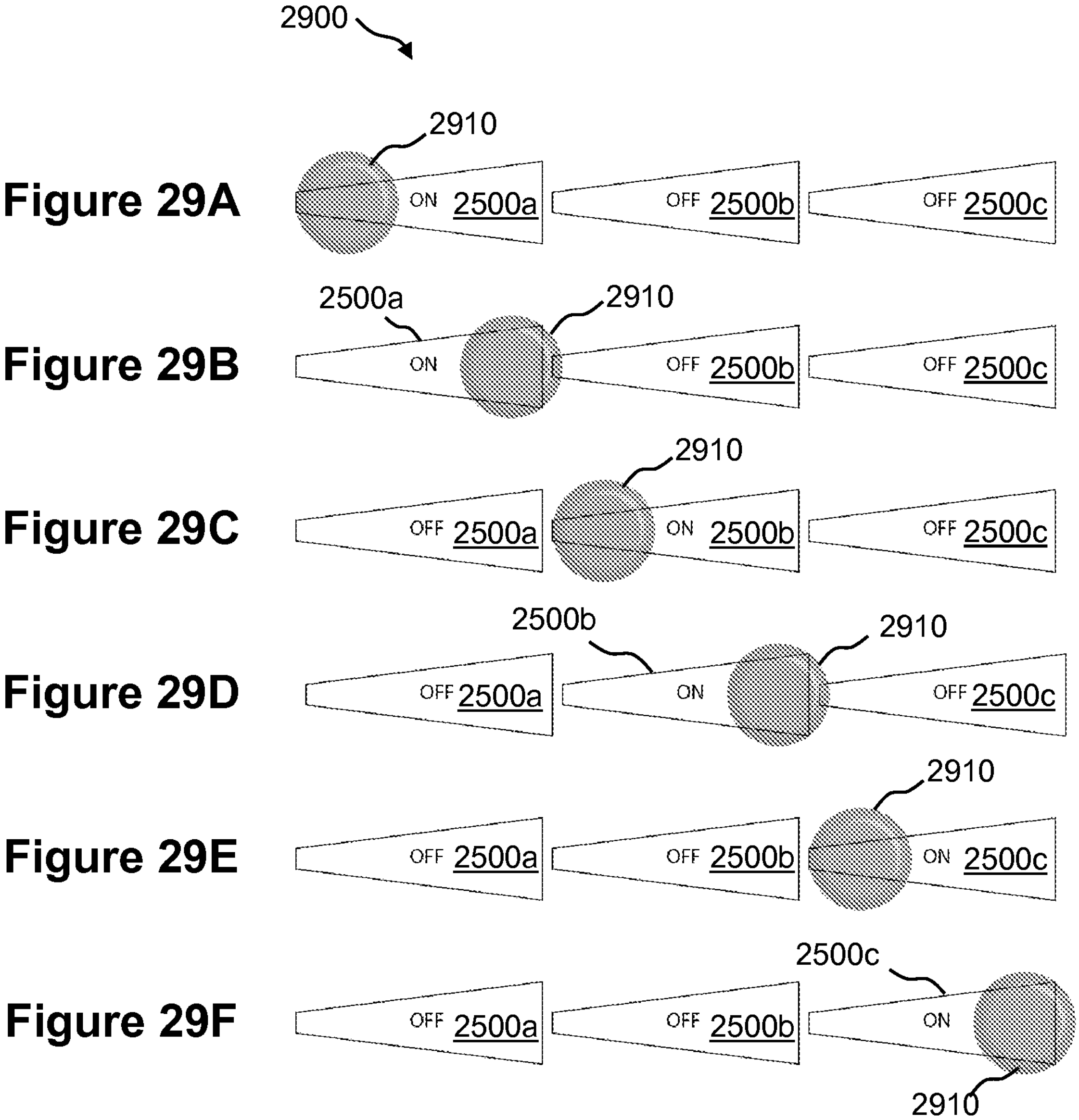


Figure 28B


2900
Figure 29A
2910
ON 2500a
OFF 2500b
OFF 2500c
Figure 29B
2500a
2910
ON
OFF 2500b
OFF 2500c
Figure 29C
2910
OFF 2500a
ON 2500b
OFF 2500c
Figure 29D
2500b
2910
OFF 2500a
ON
OFF 2500c
Figure 29E
2910
OFF 2500a
OFF 2500b
ON 2500c
Figure 29F
2500c
OFF 2500a
OFF 2500b
ON
2910

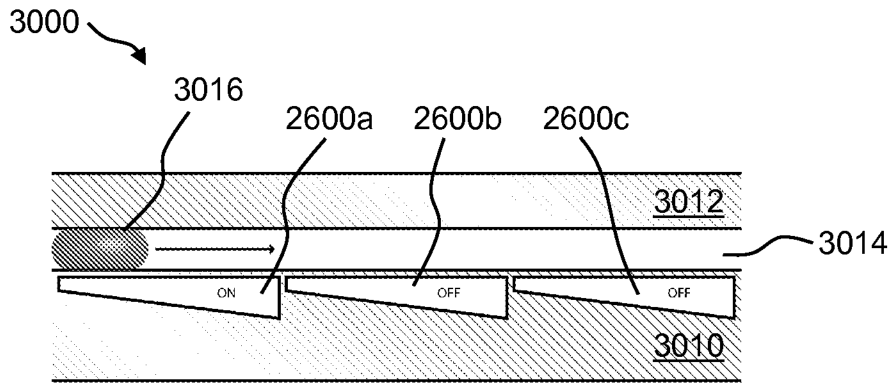


Figure 30A

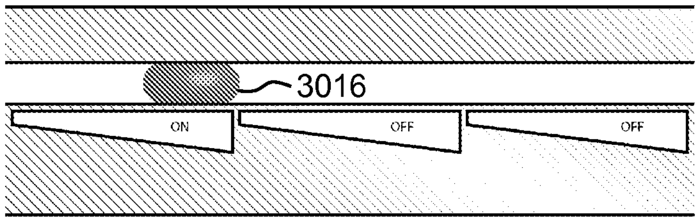


Figure 30B

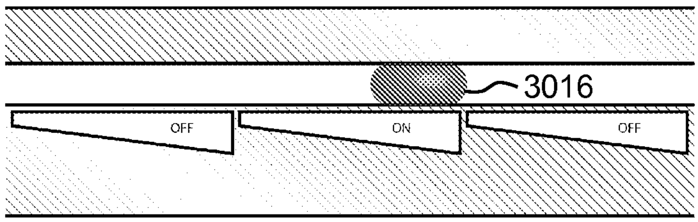


Figure 30C

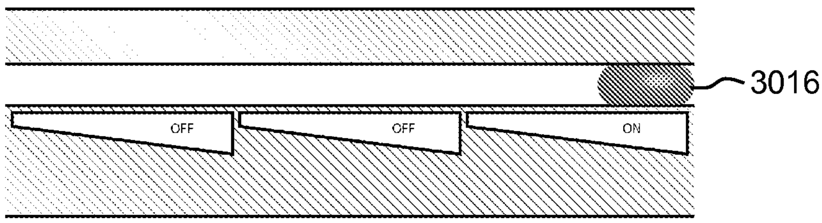


Figure 30D

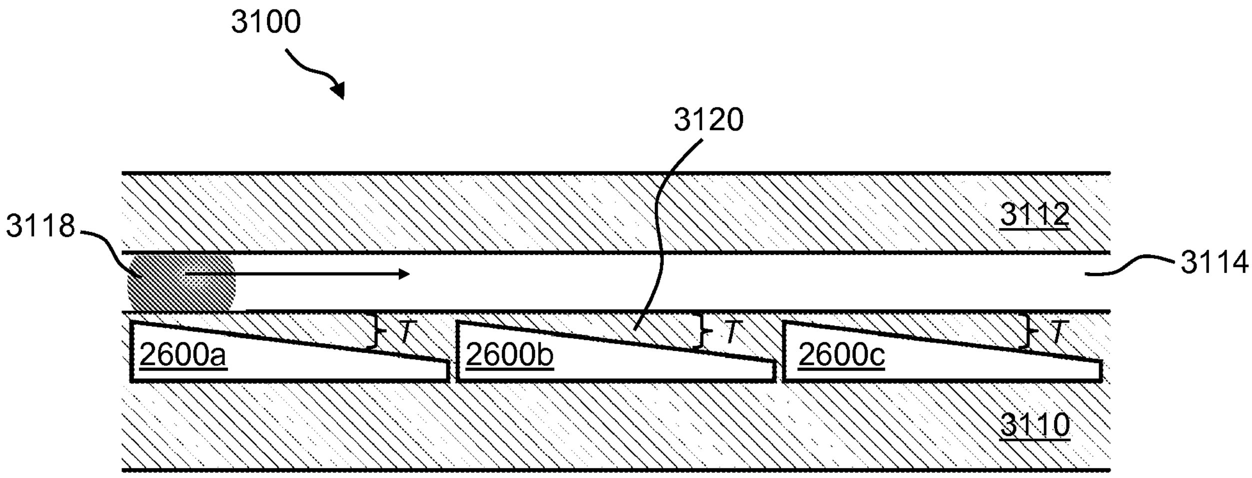


Figure 31A

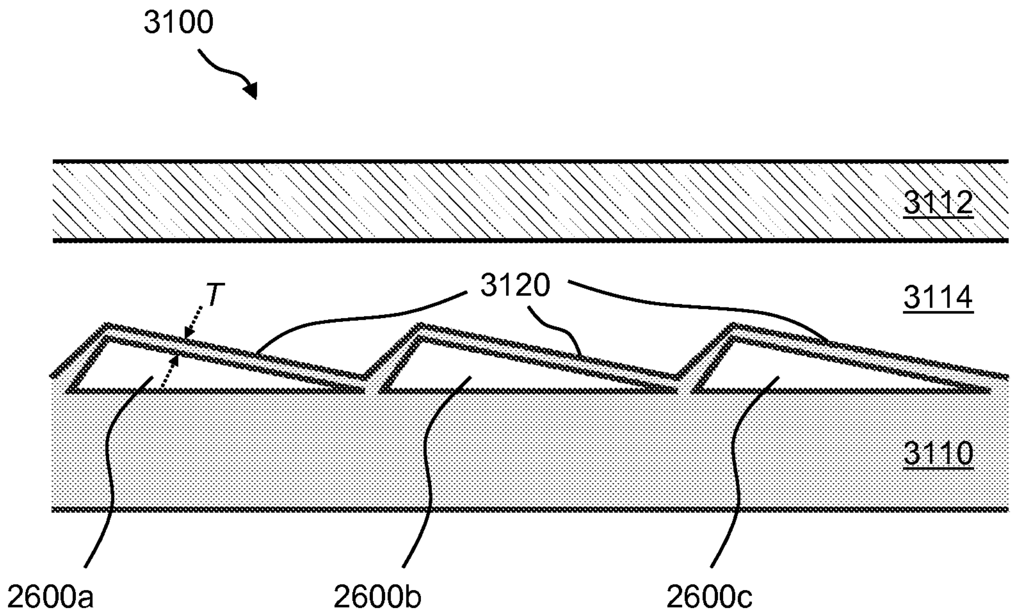


Figure 31B

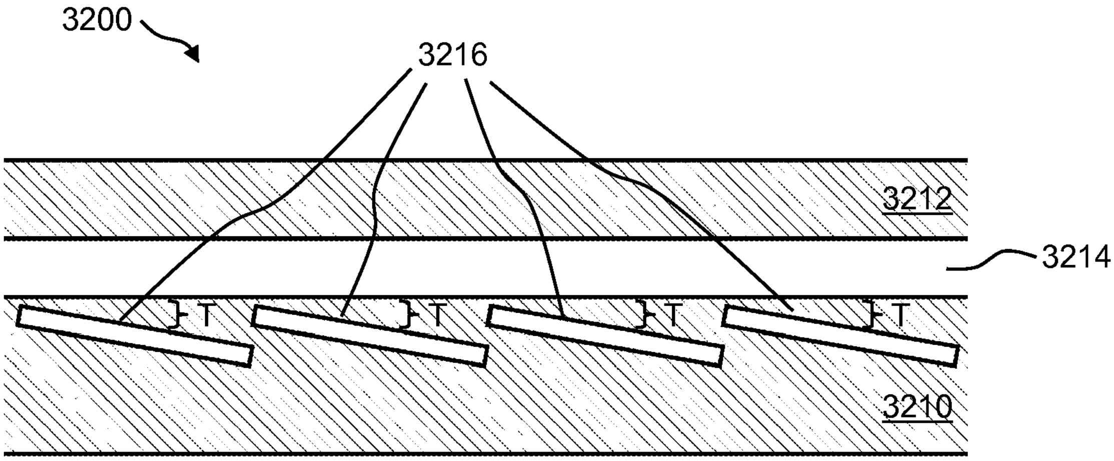


Figure 32A

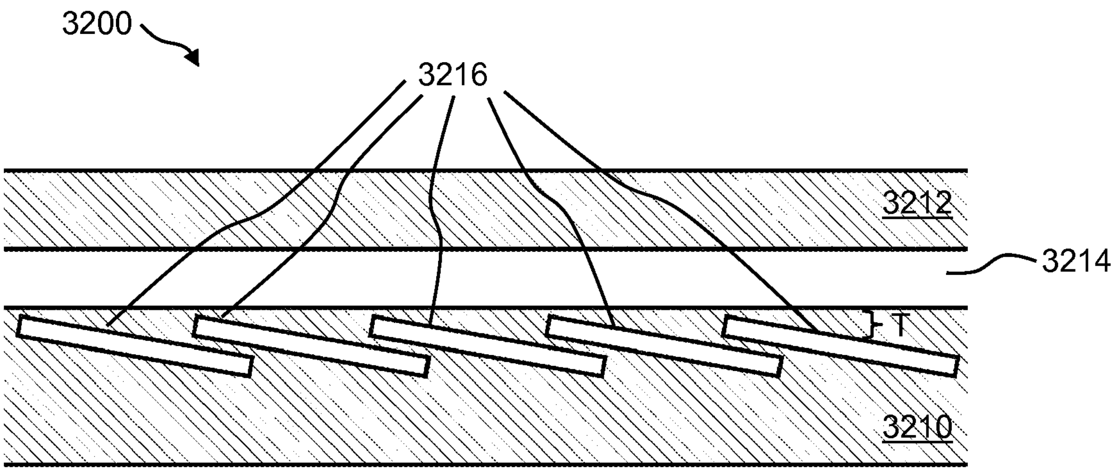


Figure 32B

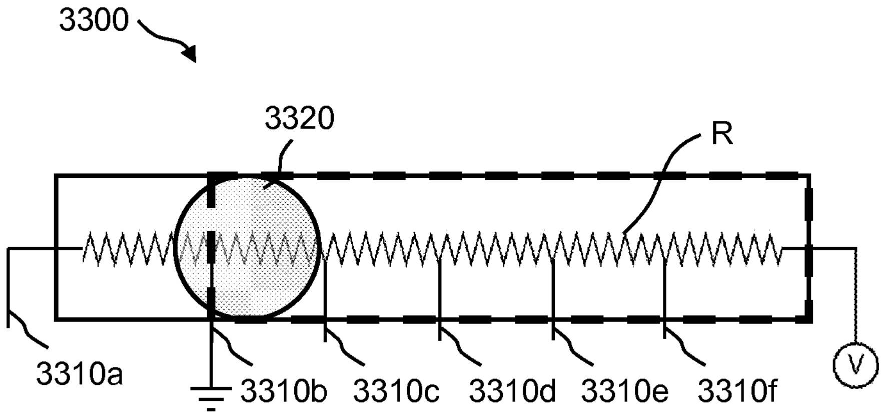


Figure 33A

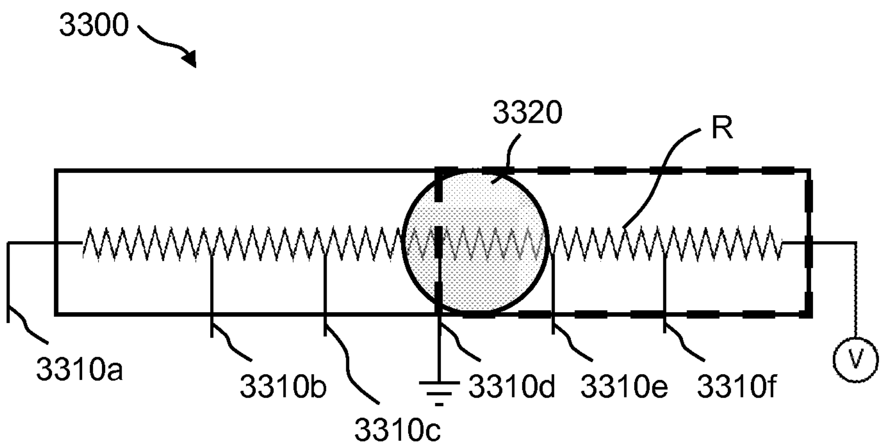


Figure 33B

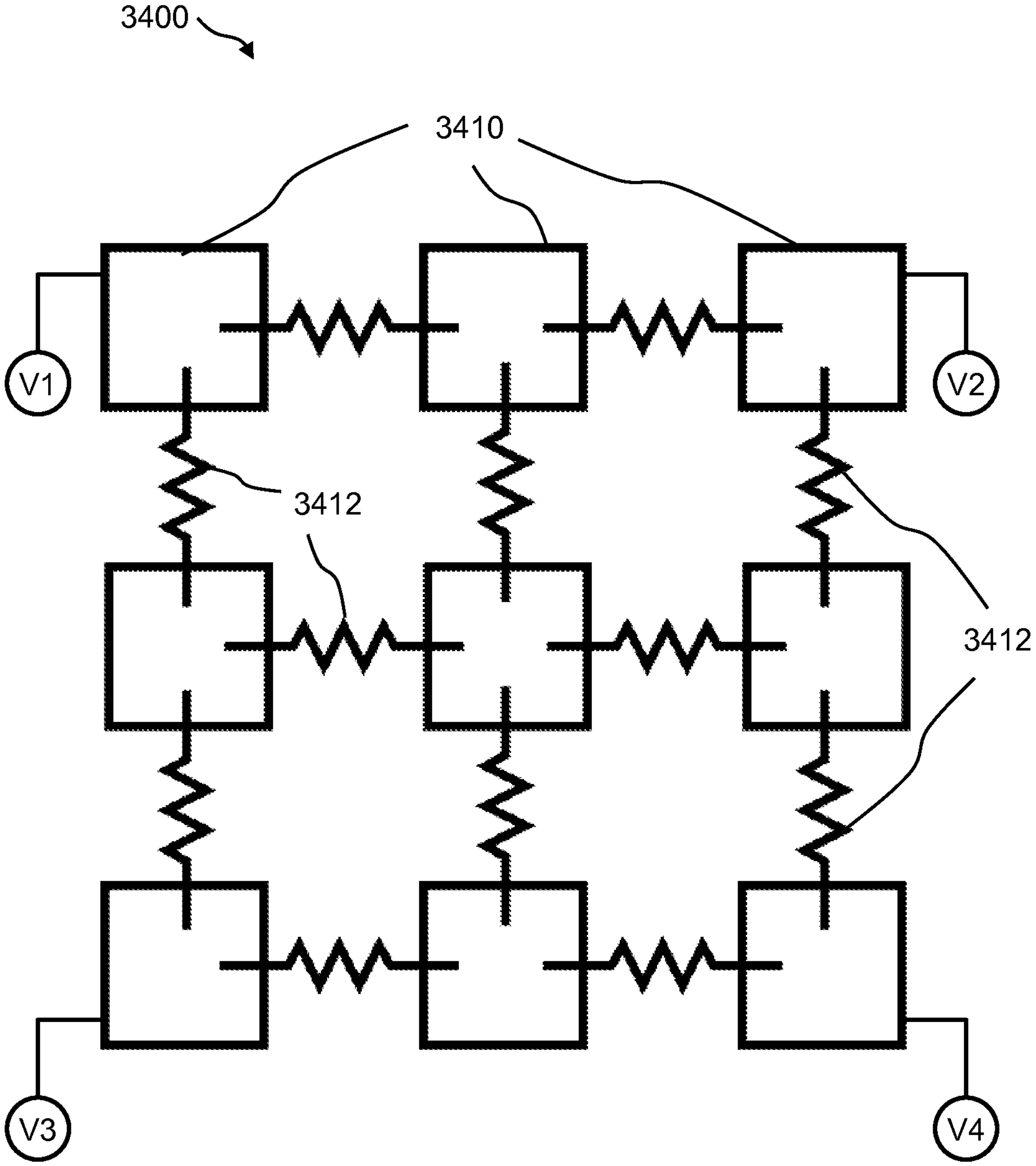
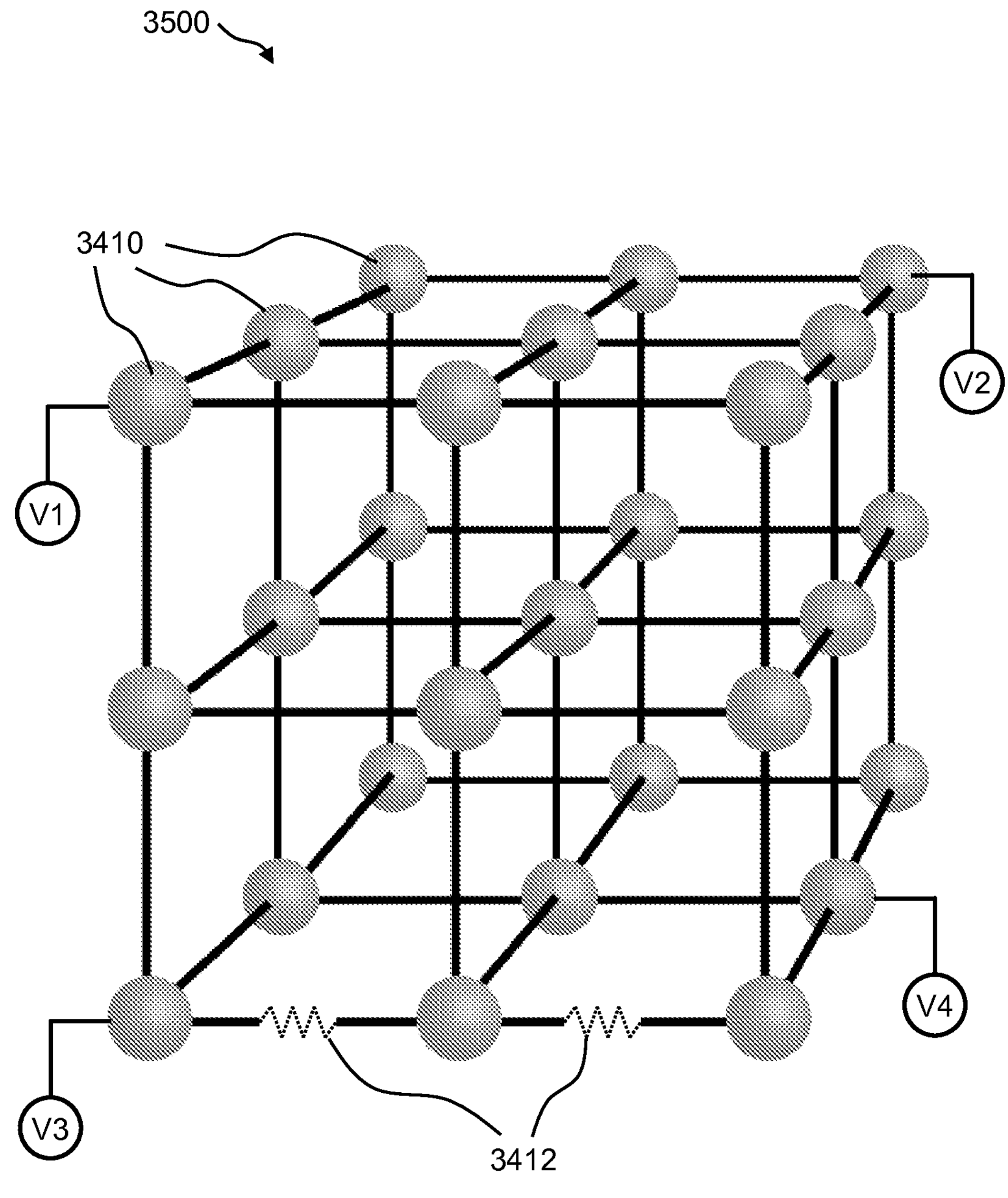


Figure 34



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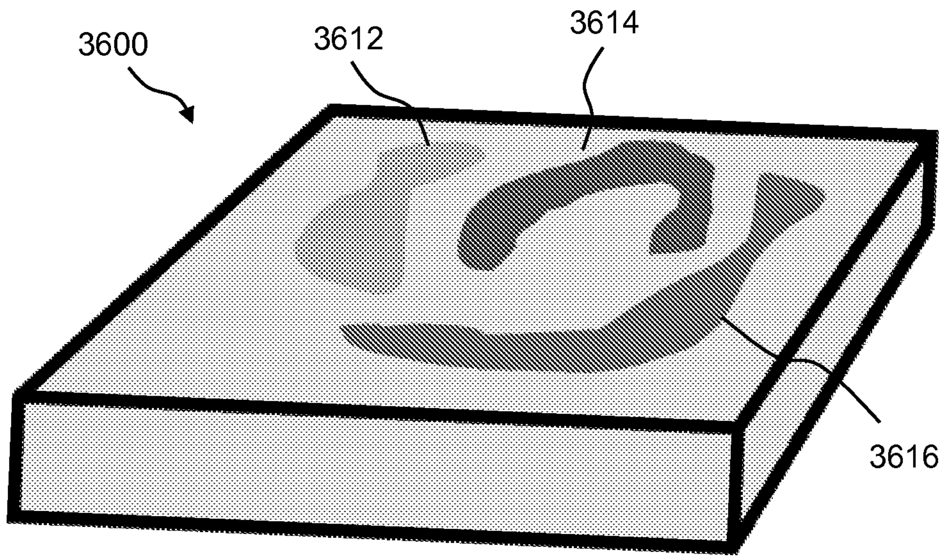

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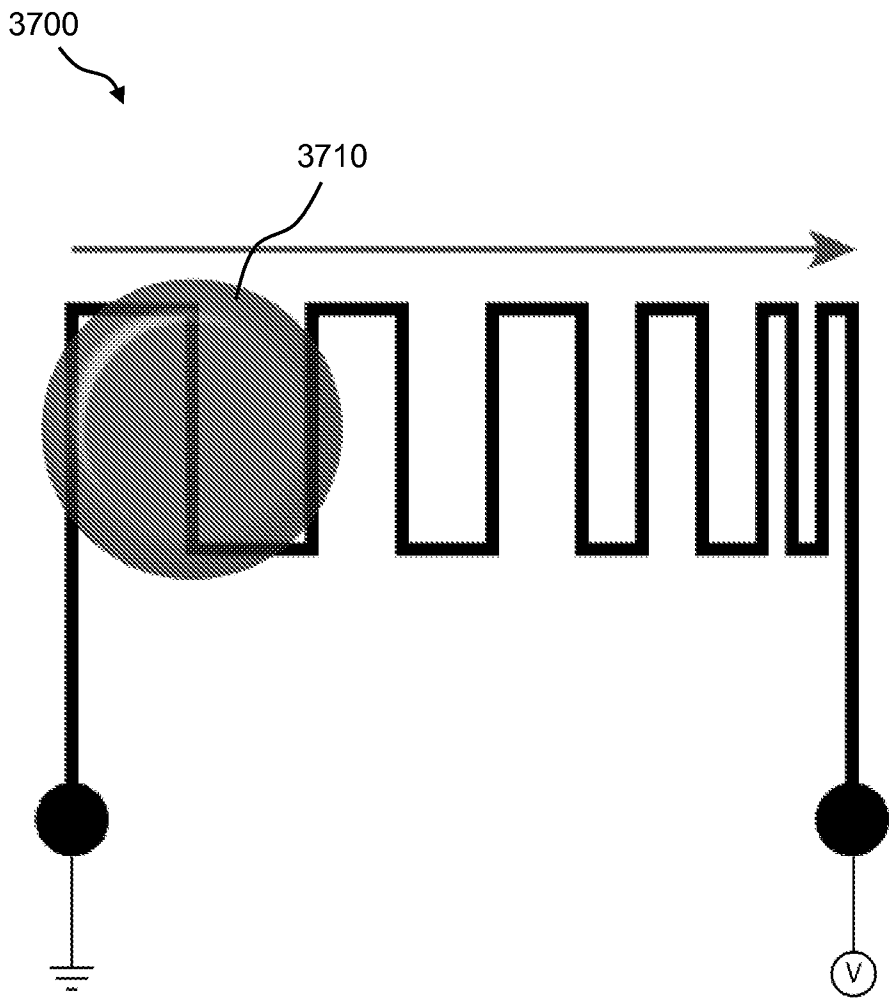

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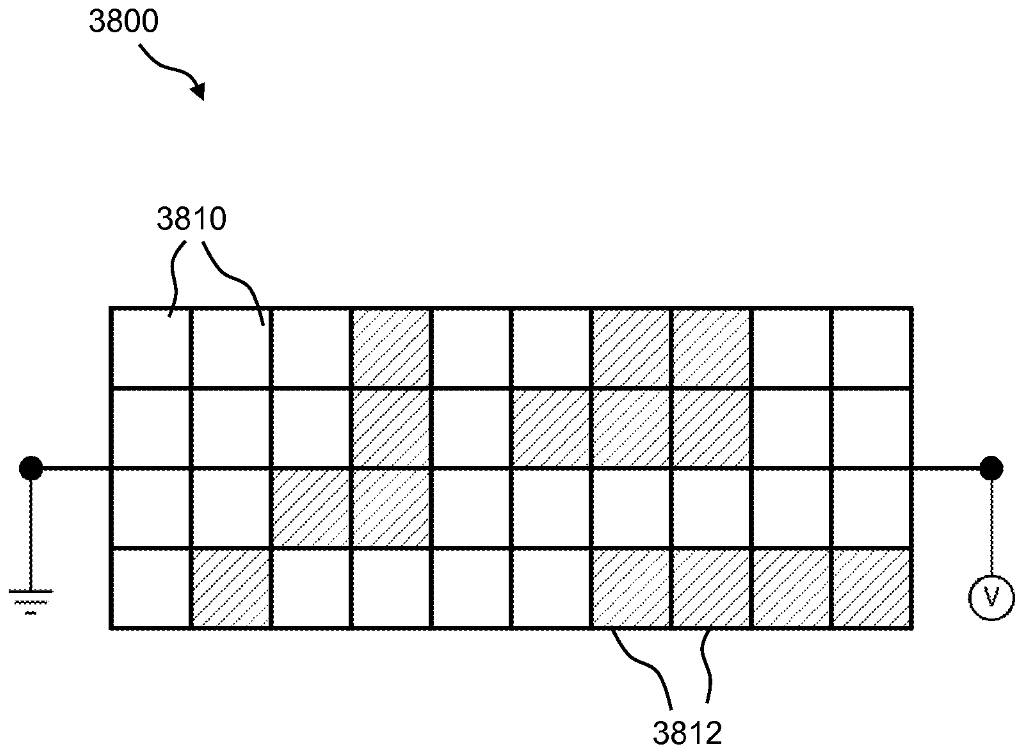

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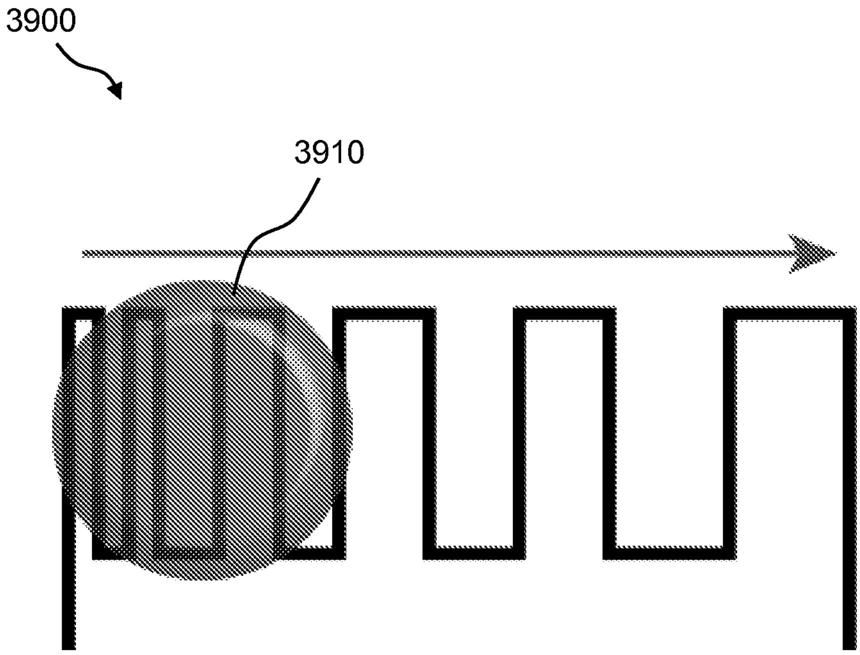

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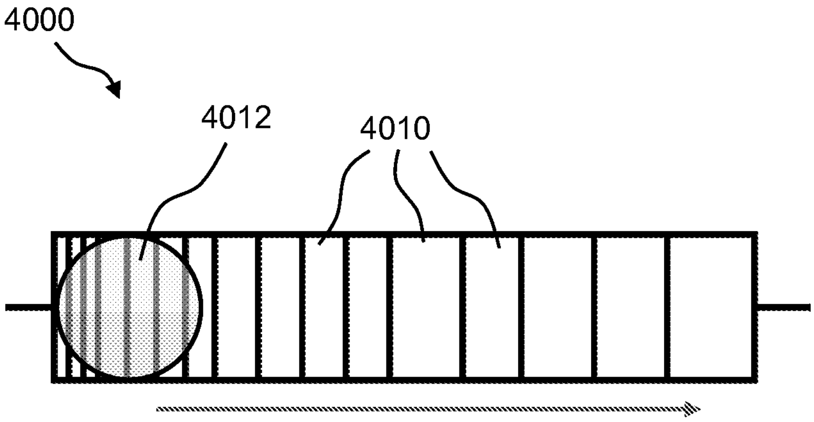

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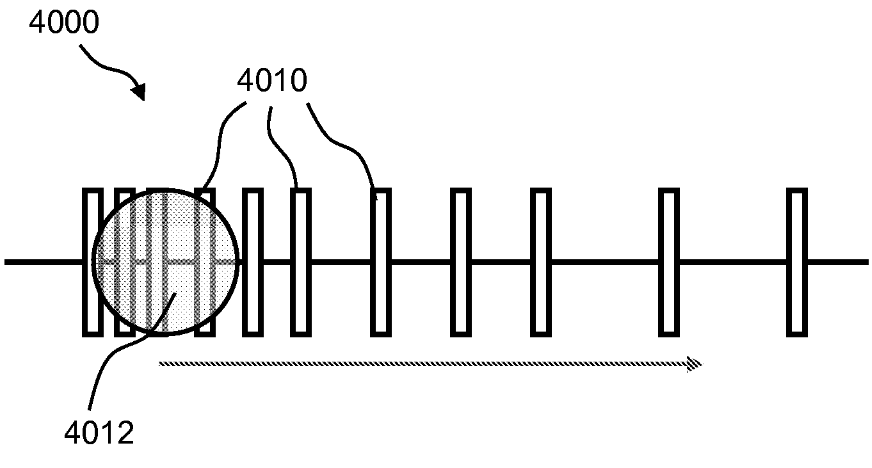
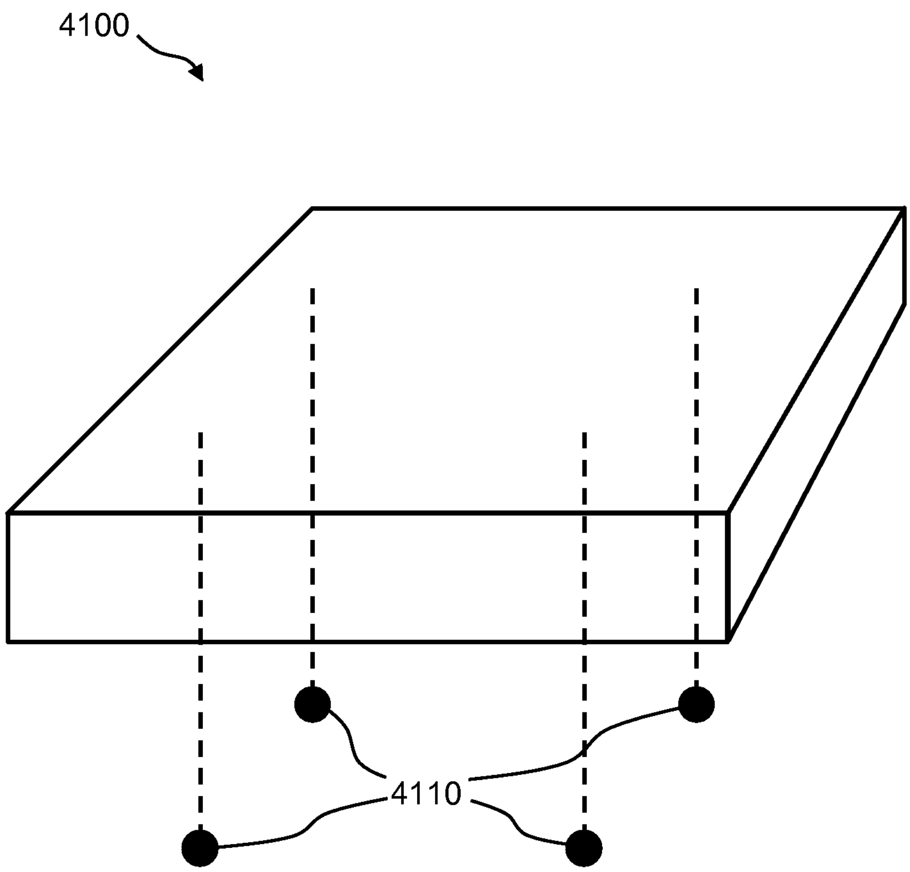


Figure 40B



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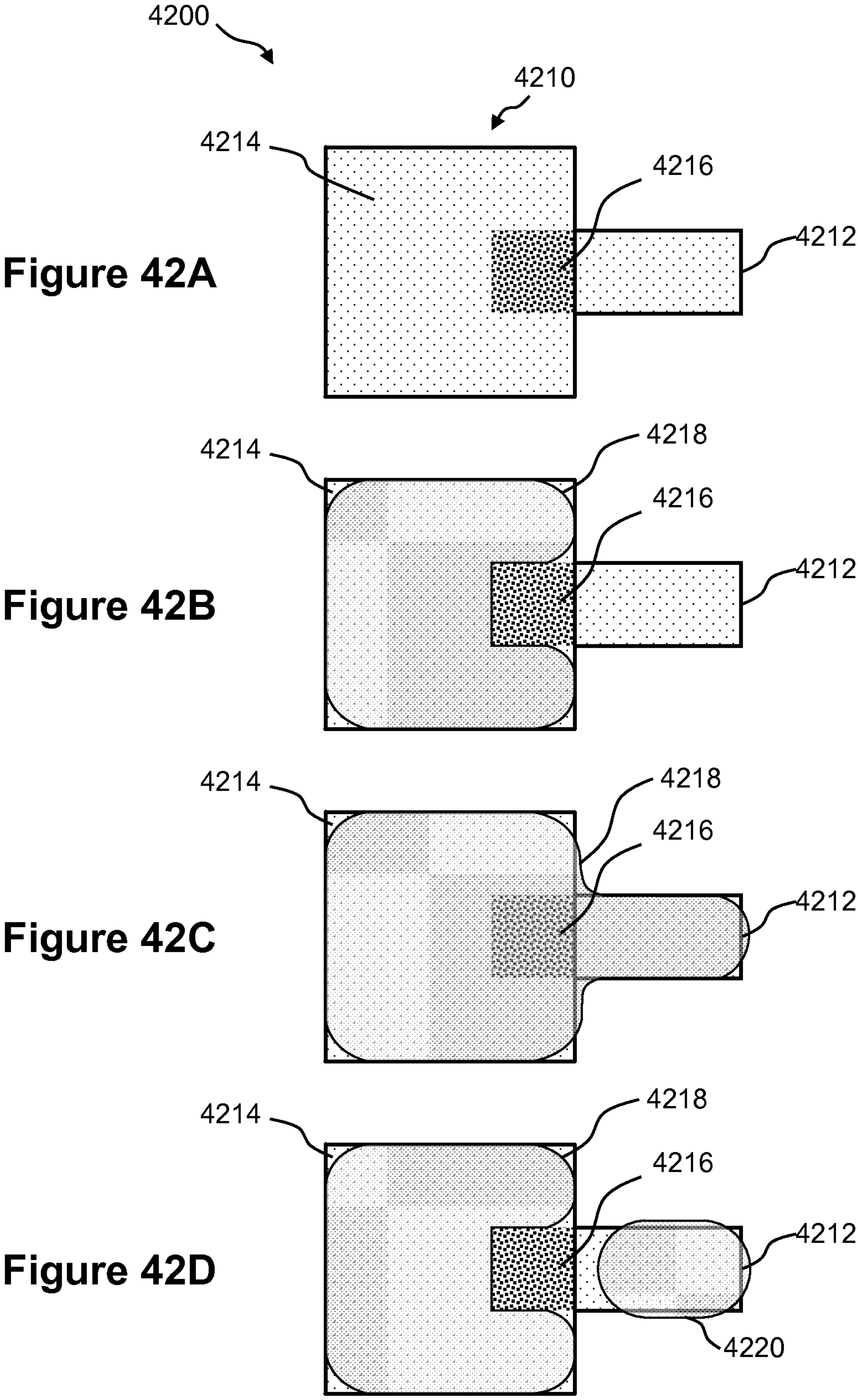
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Figure 42A
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Figure 42B
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Figure 42C
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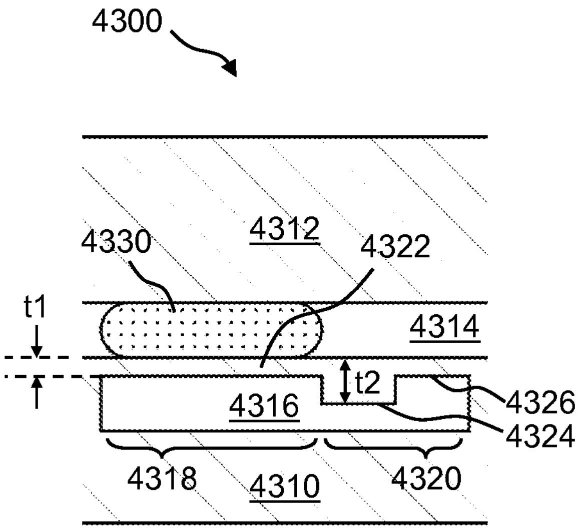
Figure 42D



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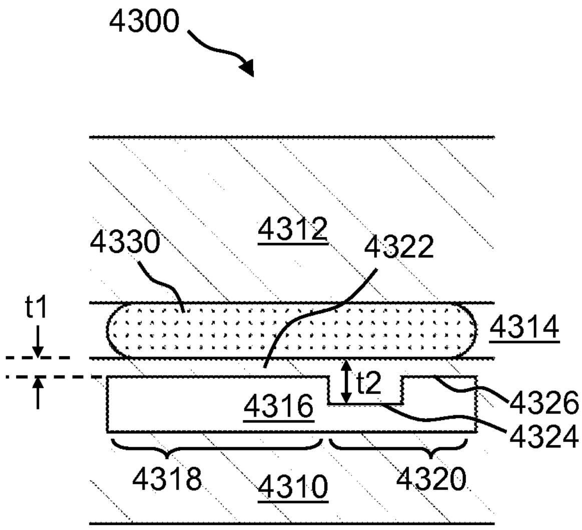
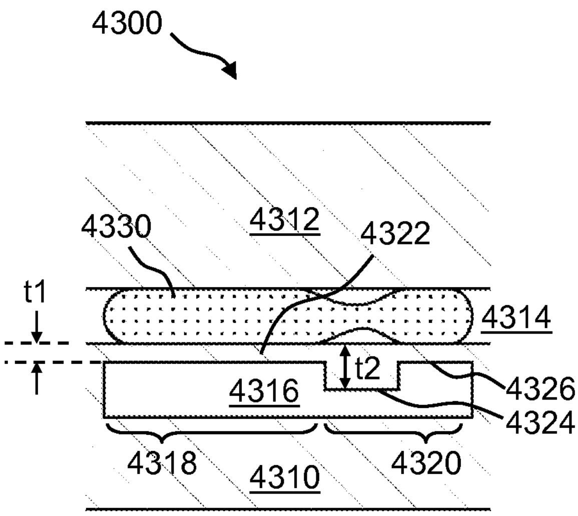


Figure 43B



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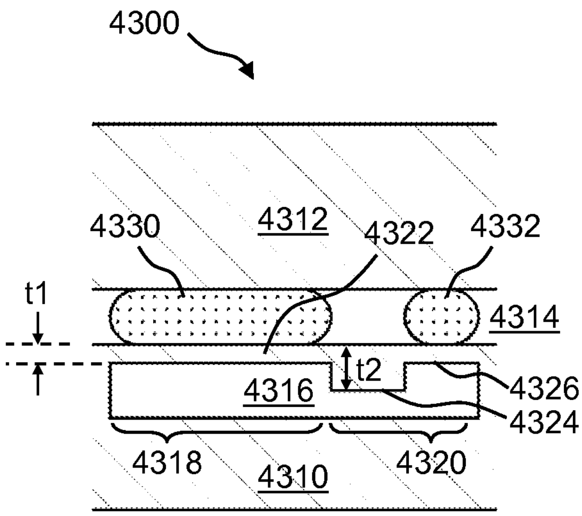

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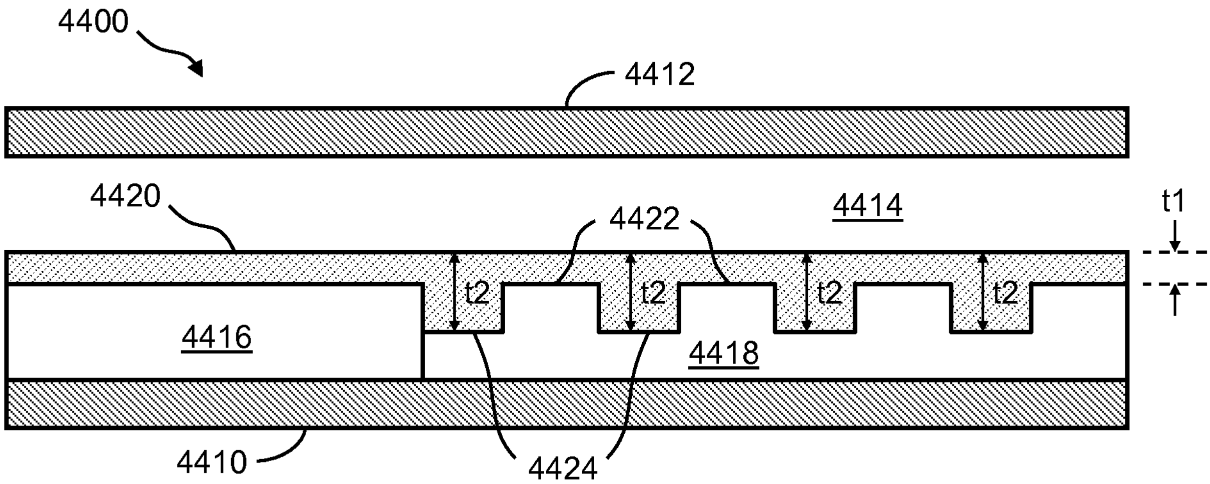


Figure 44A

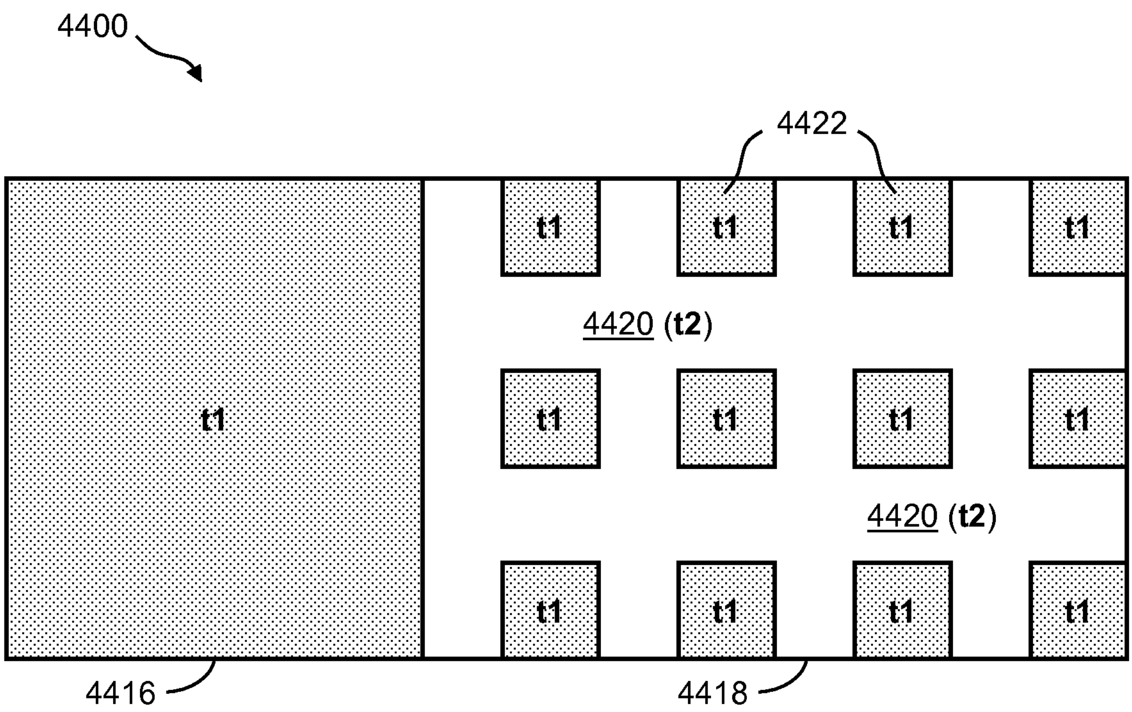


Figure 44B

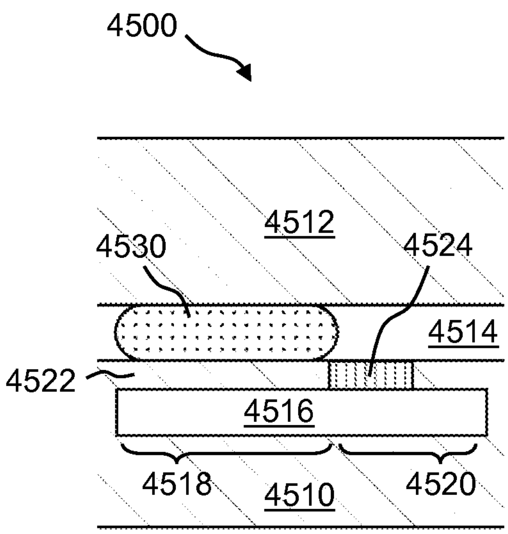
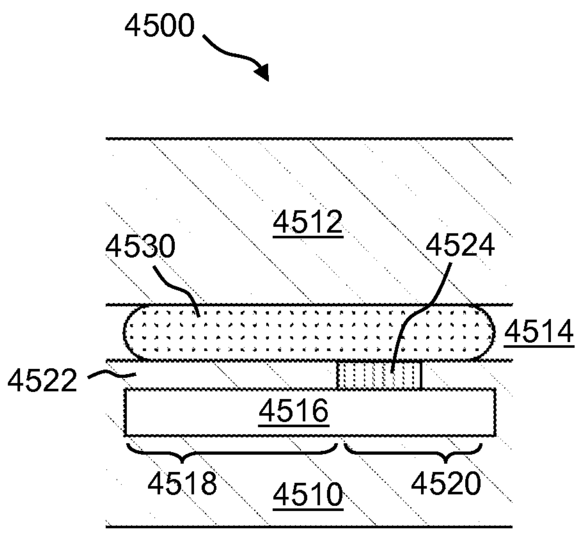


Figure 45A



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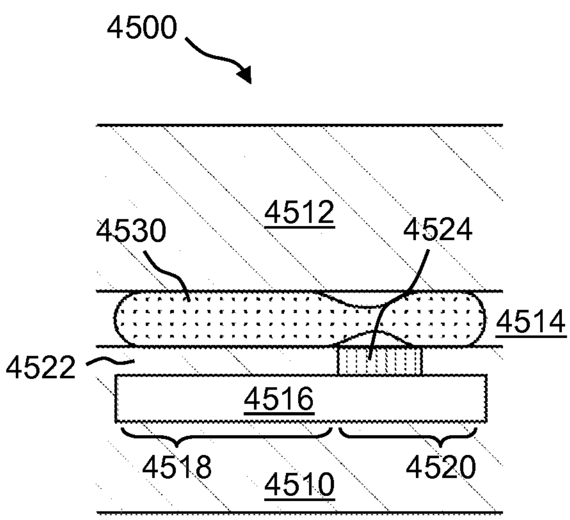

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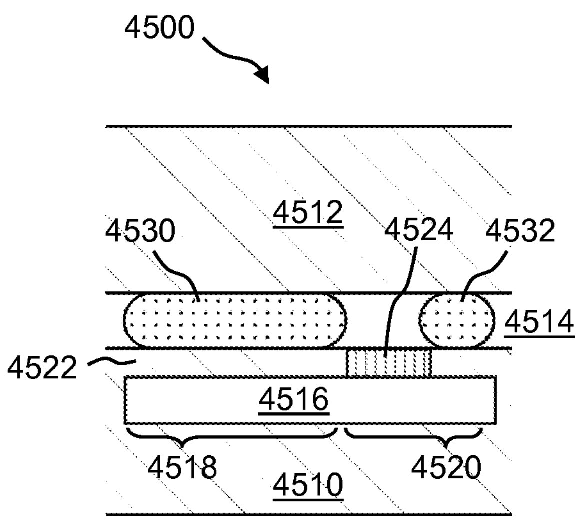

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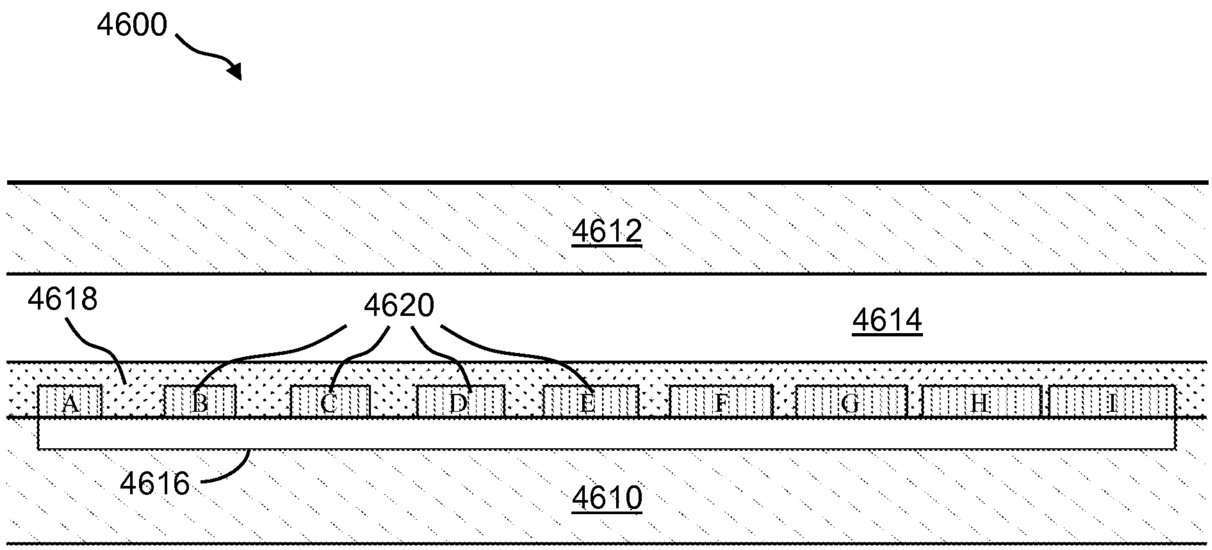

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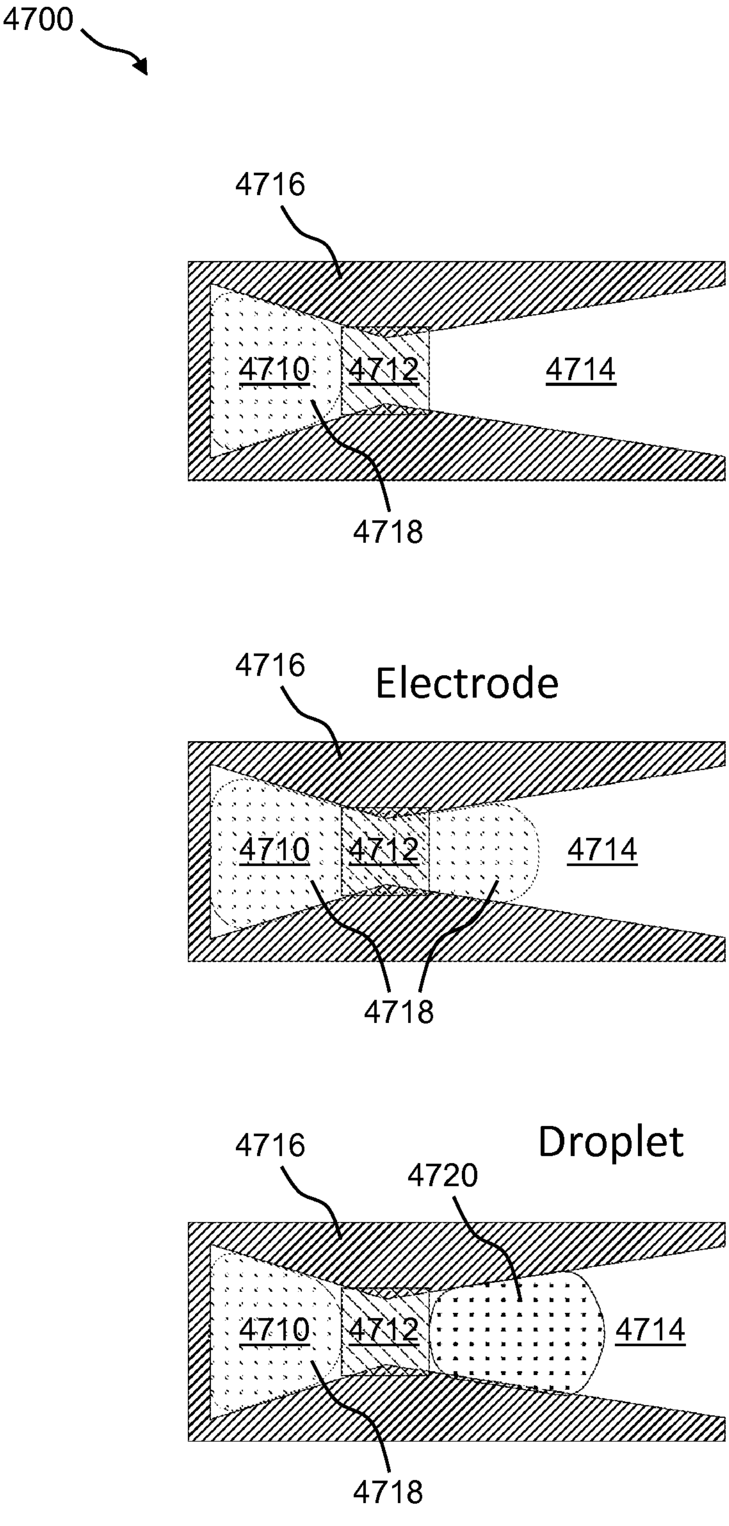
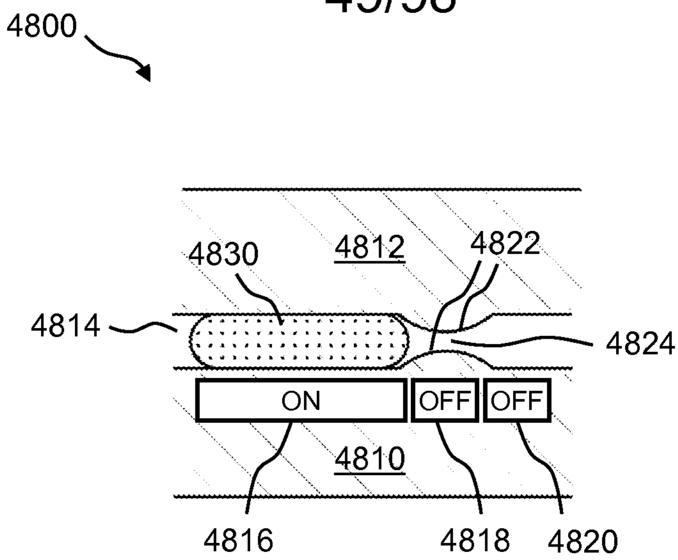


Figure 47A

Figure 47B

Figure 47C



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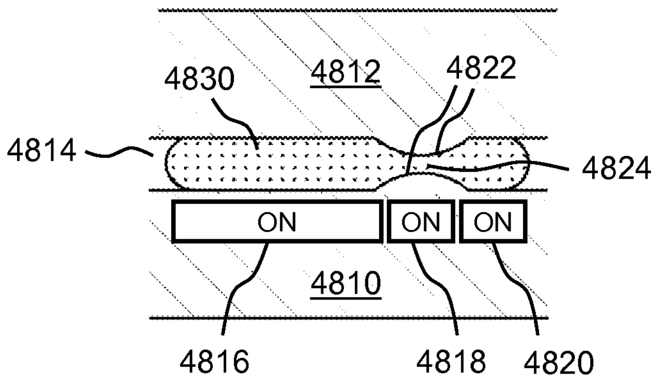
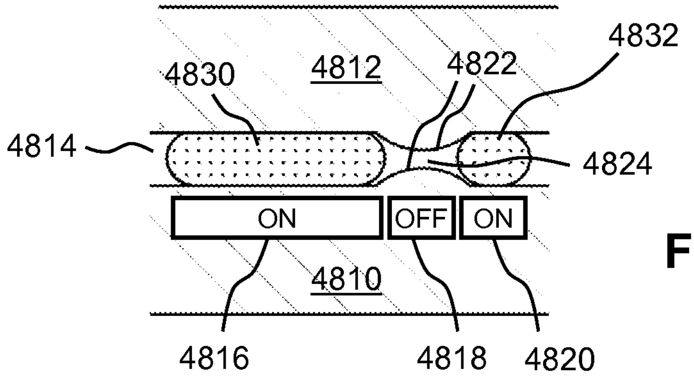


Figure 48B



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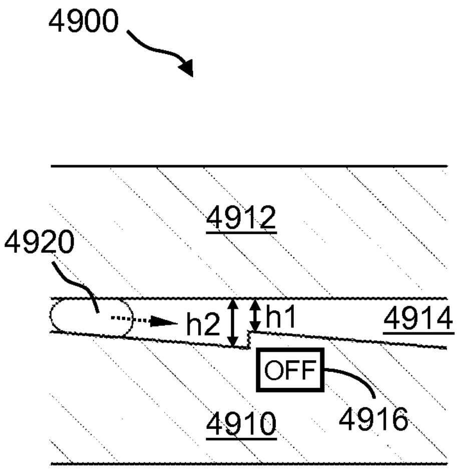

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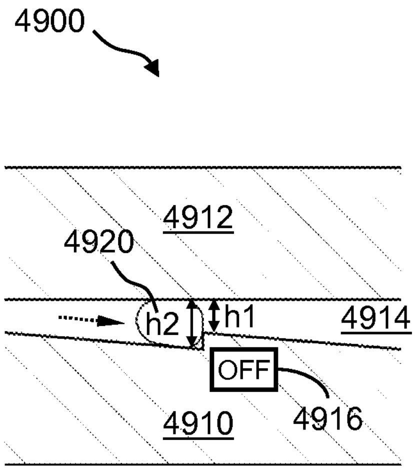


Figure 49B

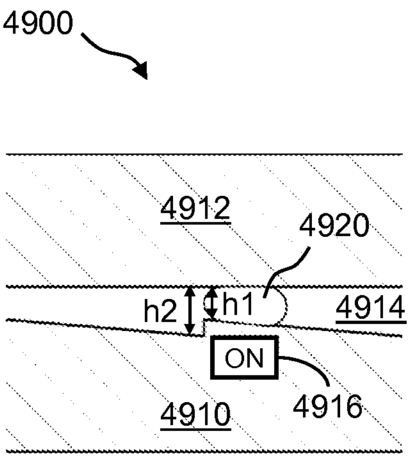


Figure 49C

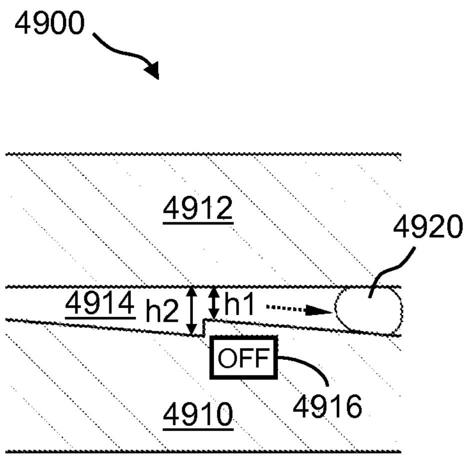
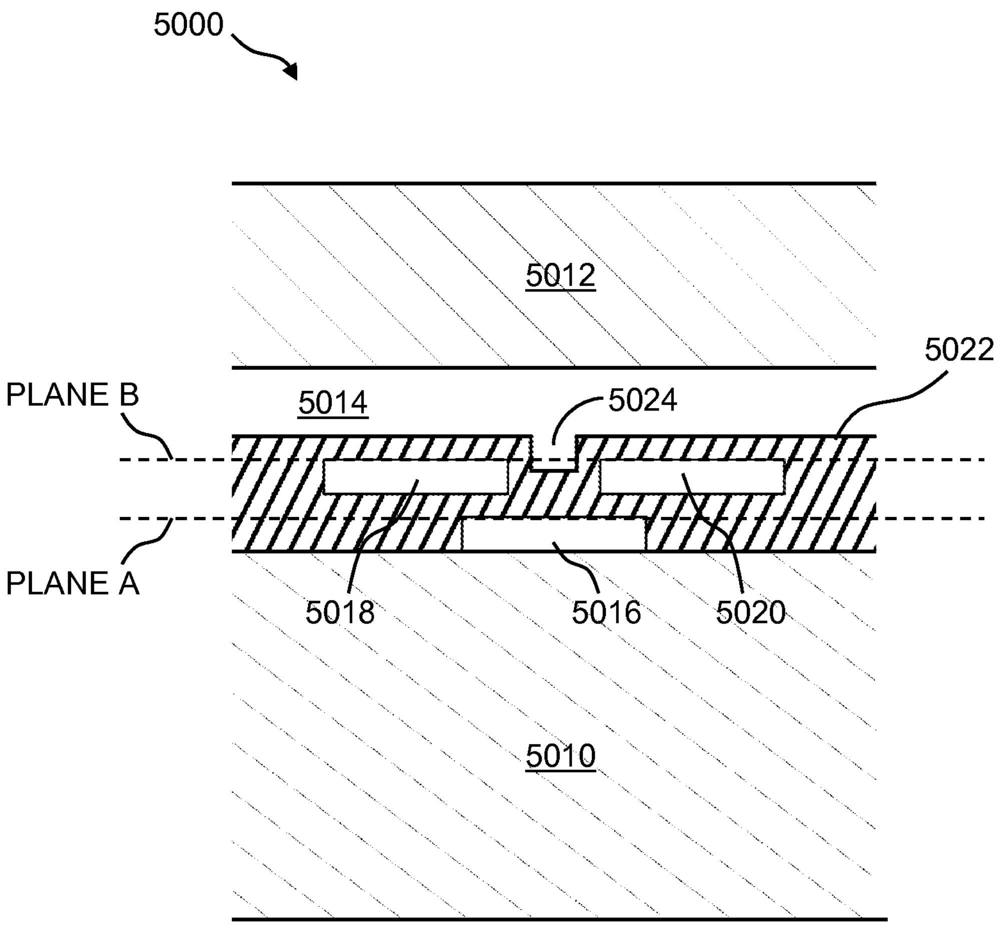


Figure 49D



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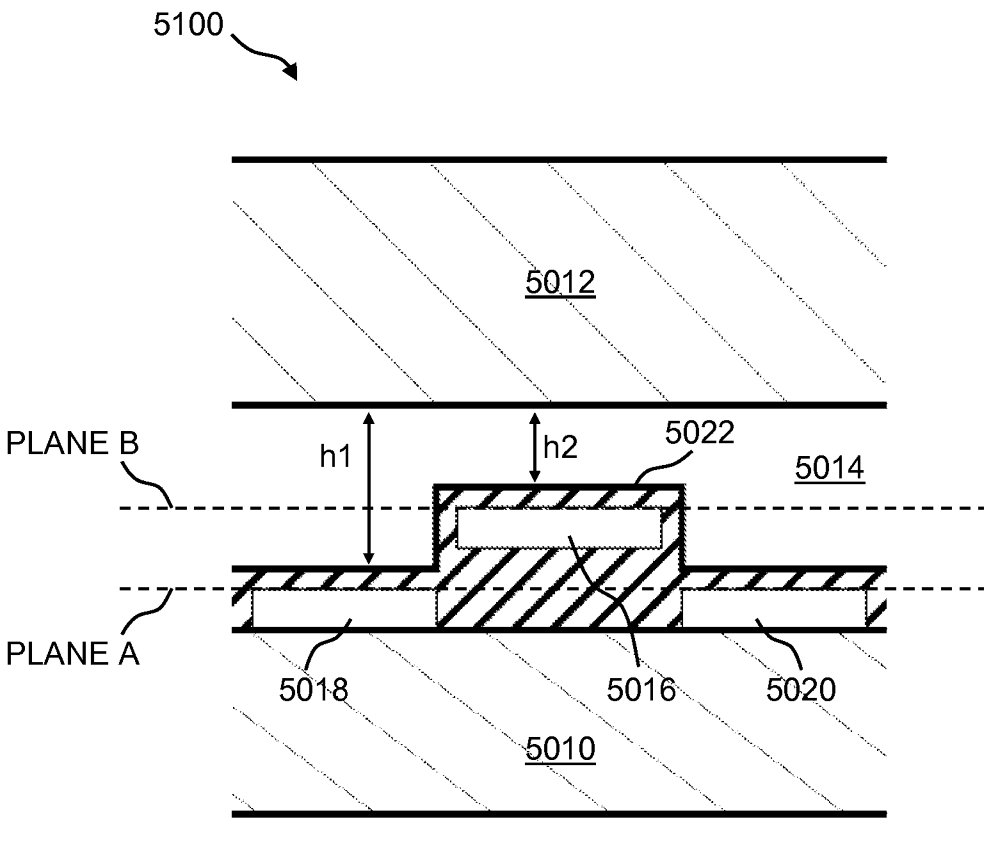

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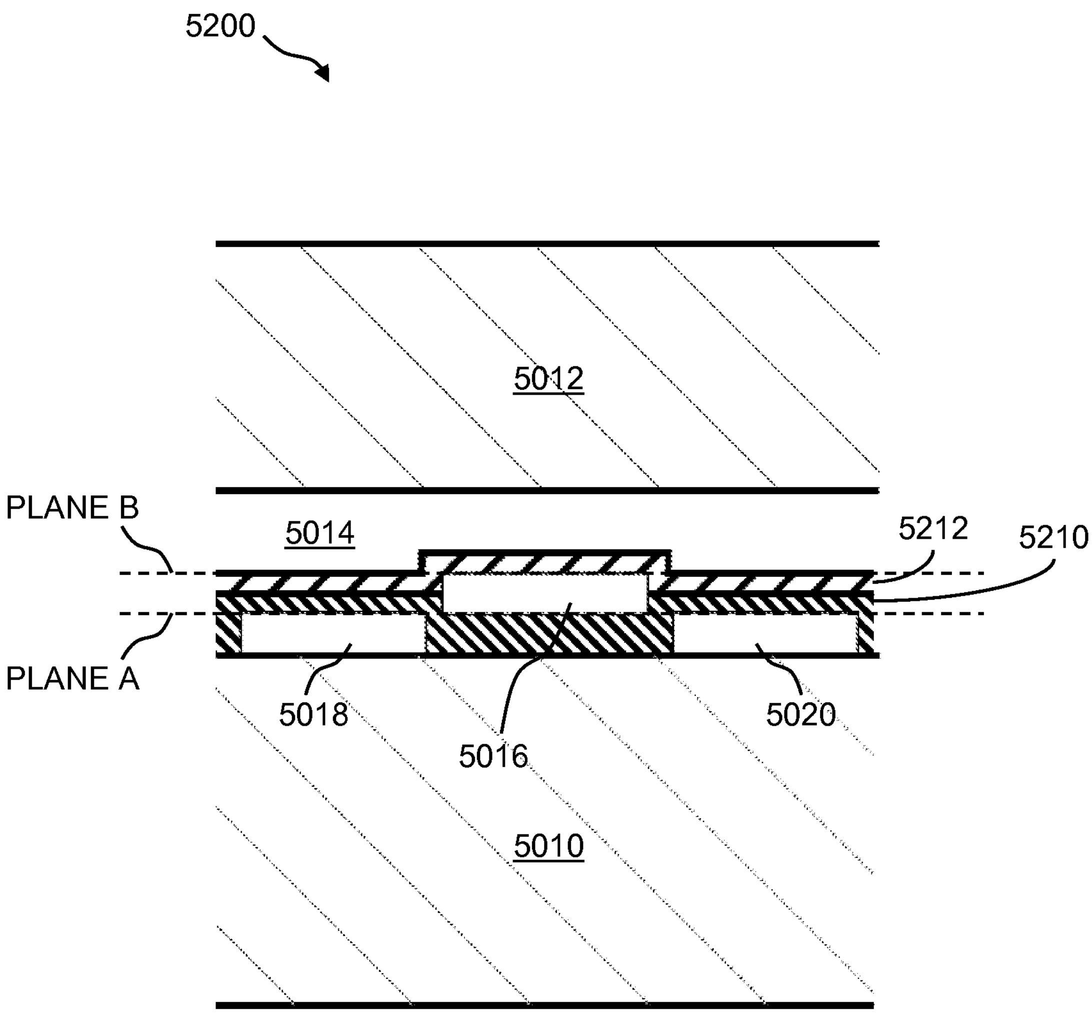

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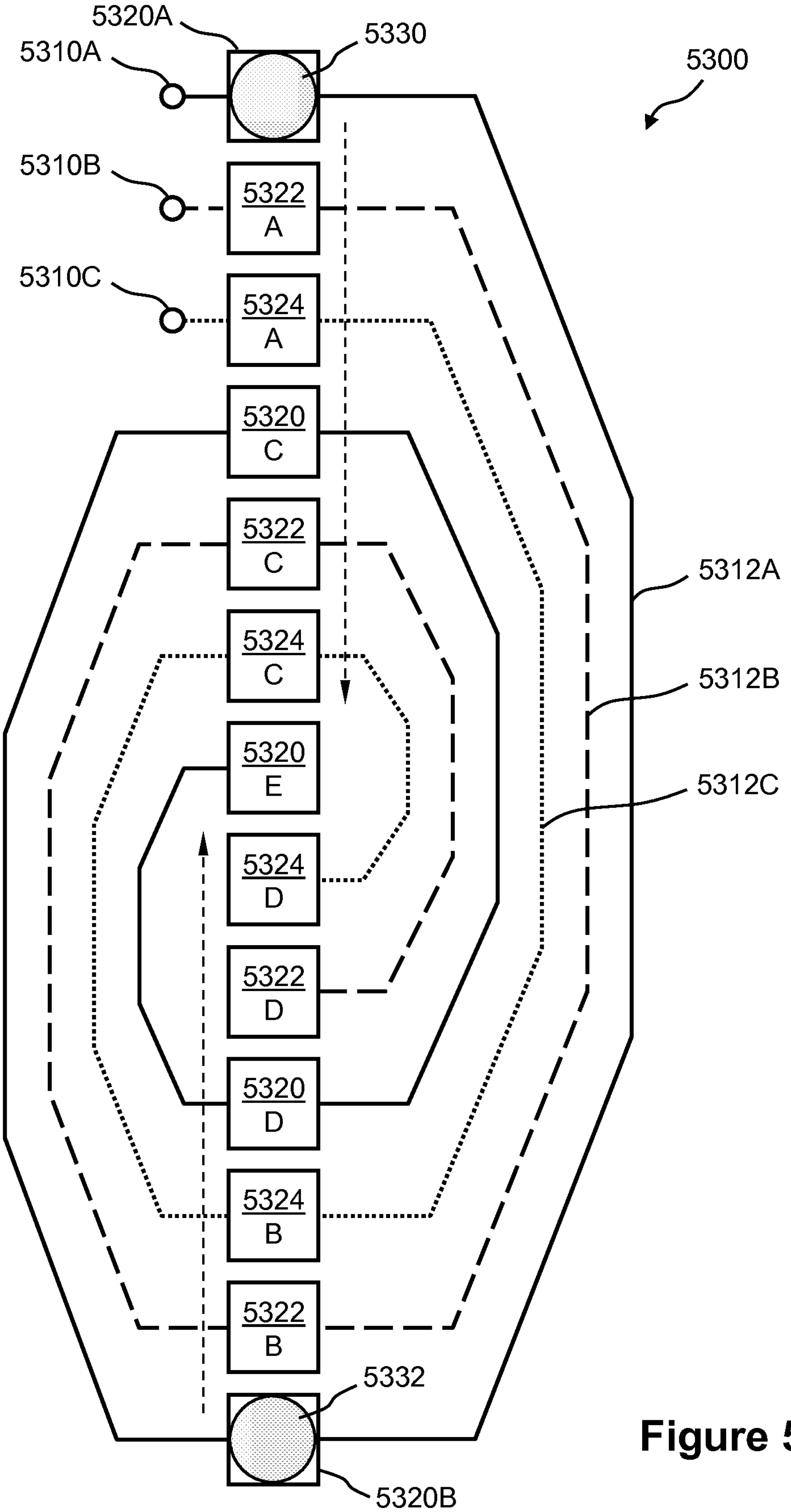
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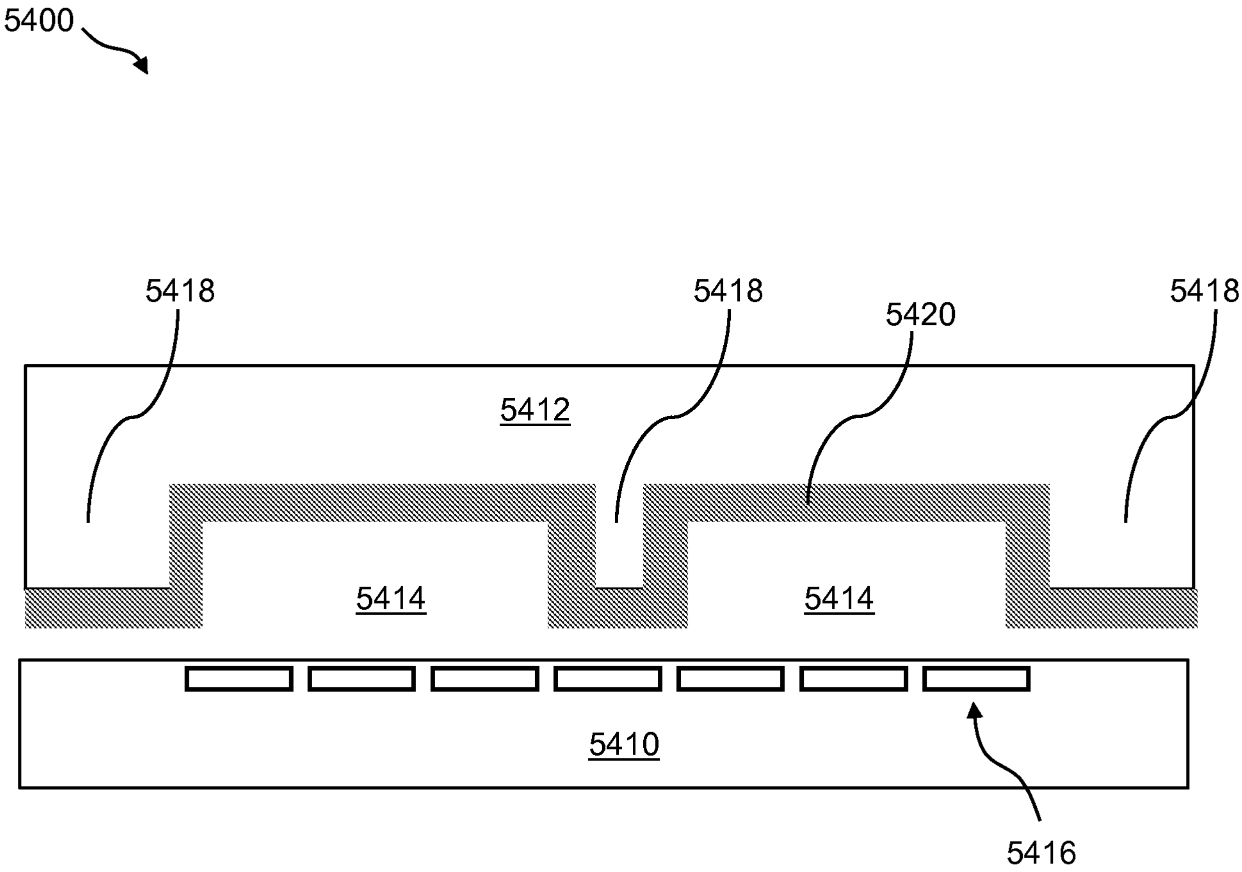
Figure 53



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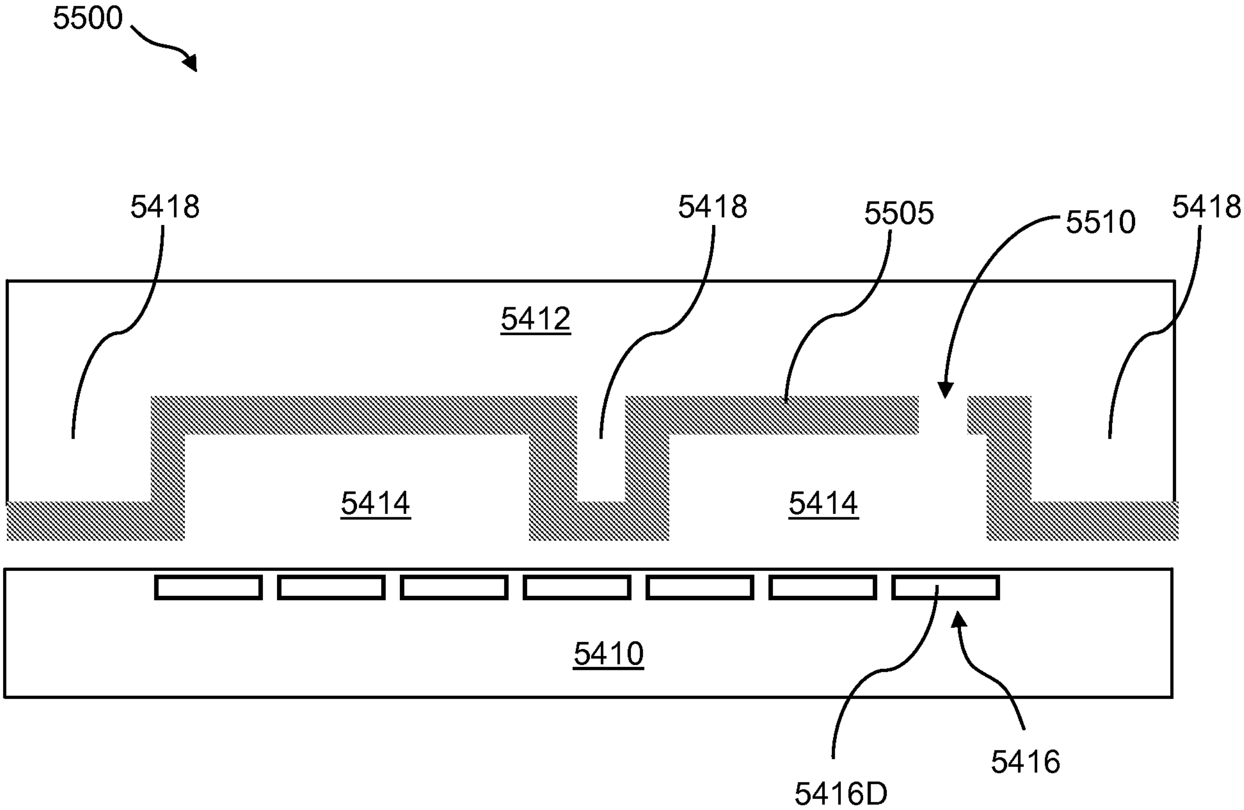

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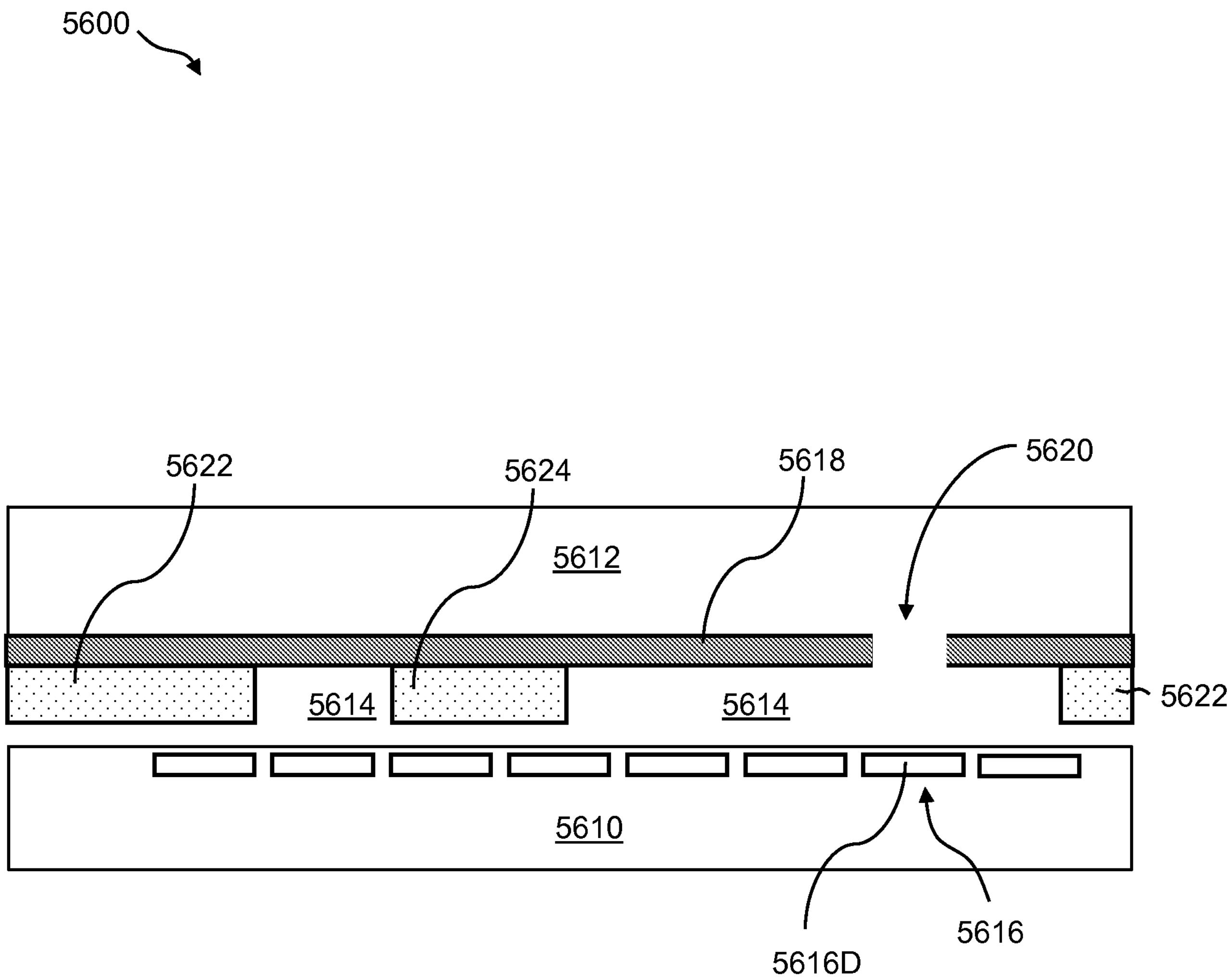

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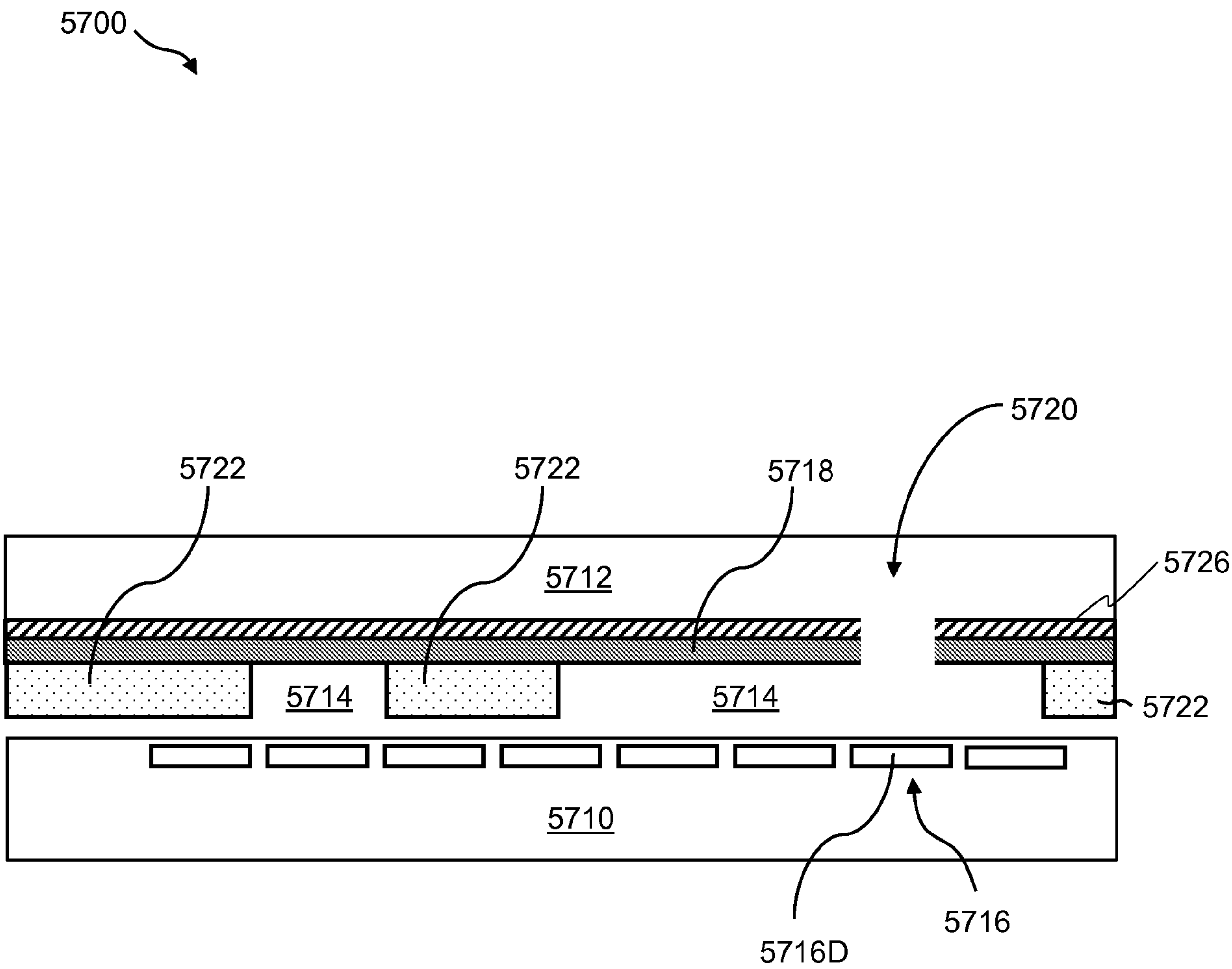
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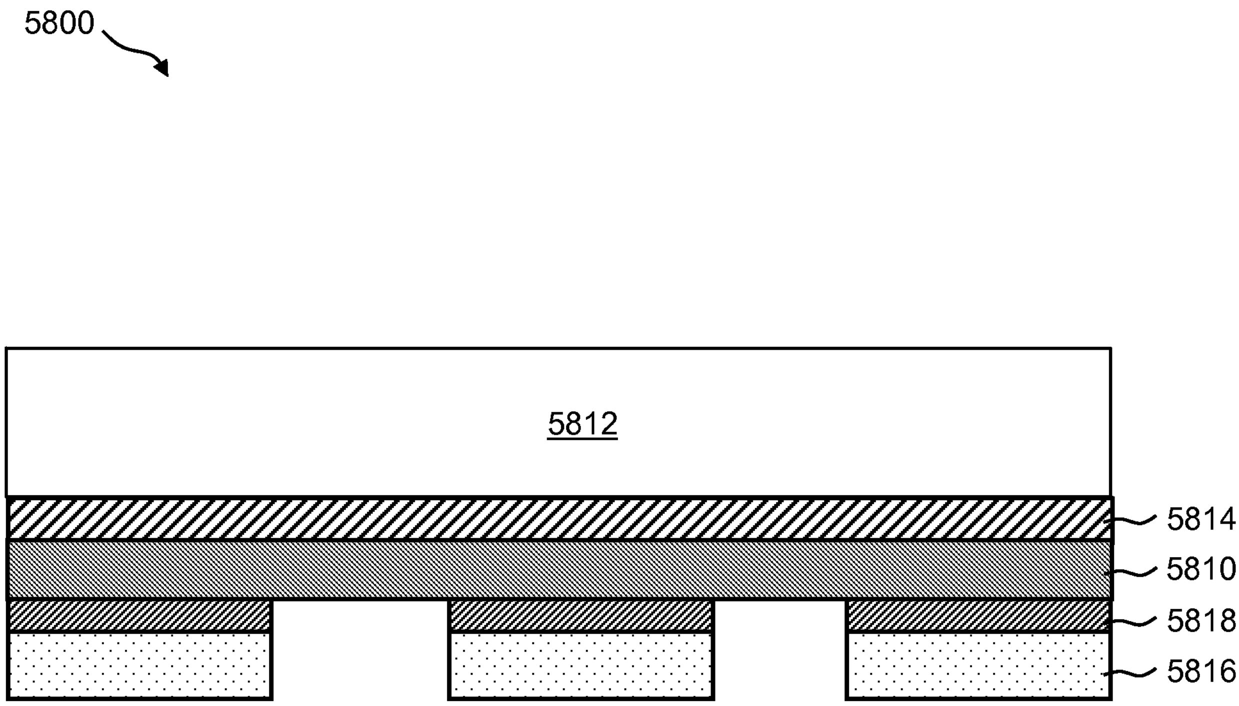
Figure 57



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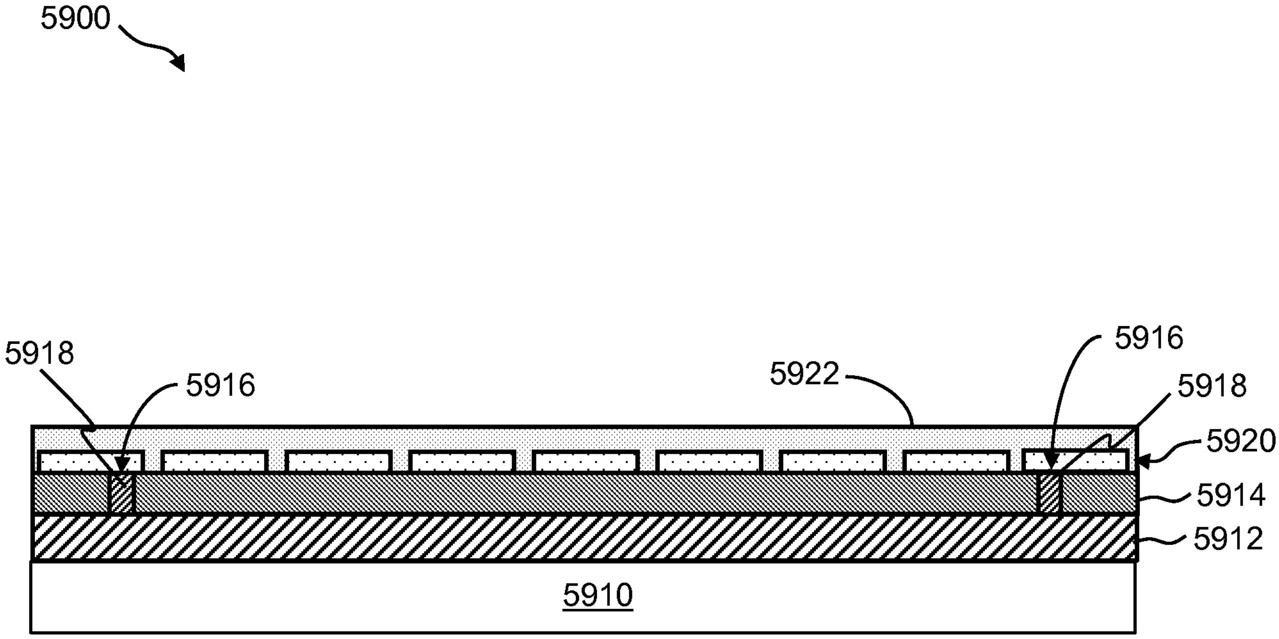

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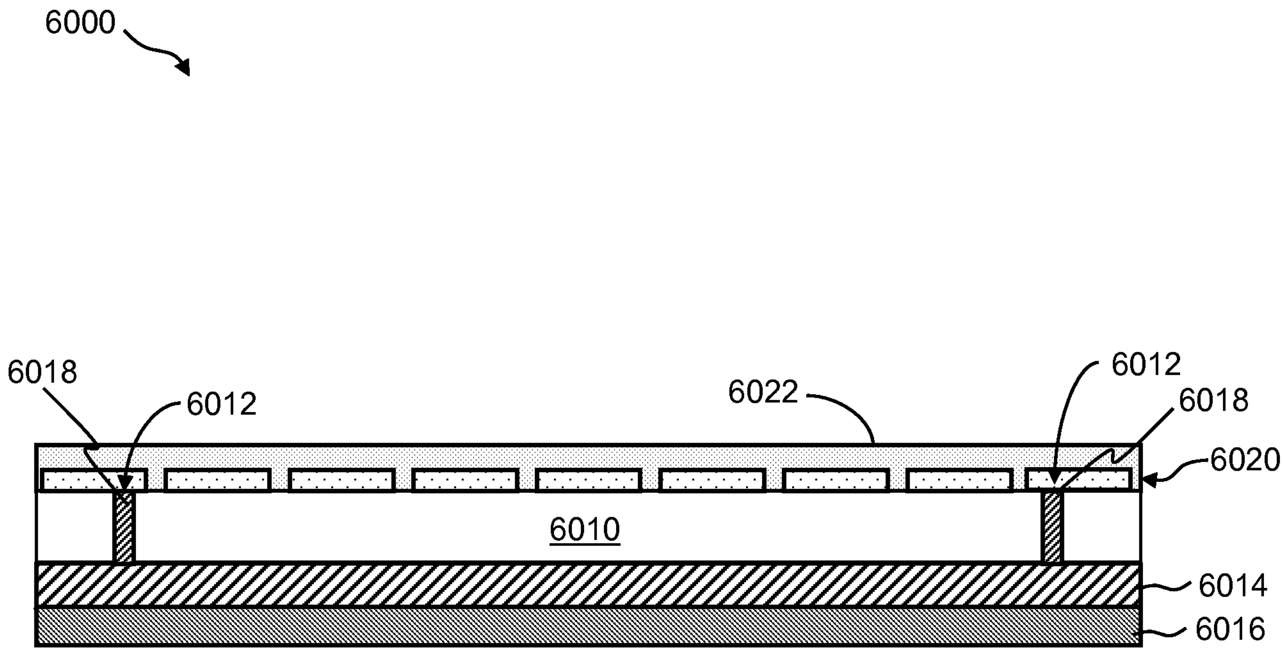

Figure 60

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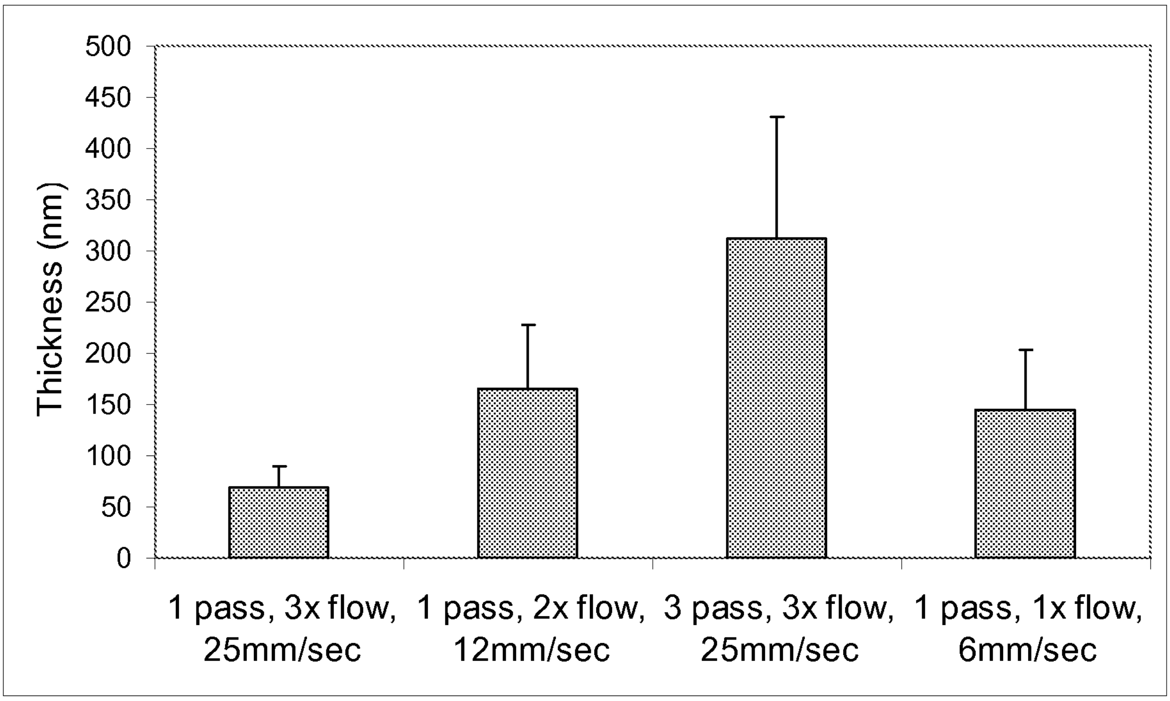

Figure 61

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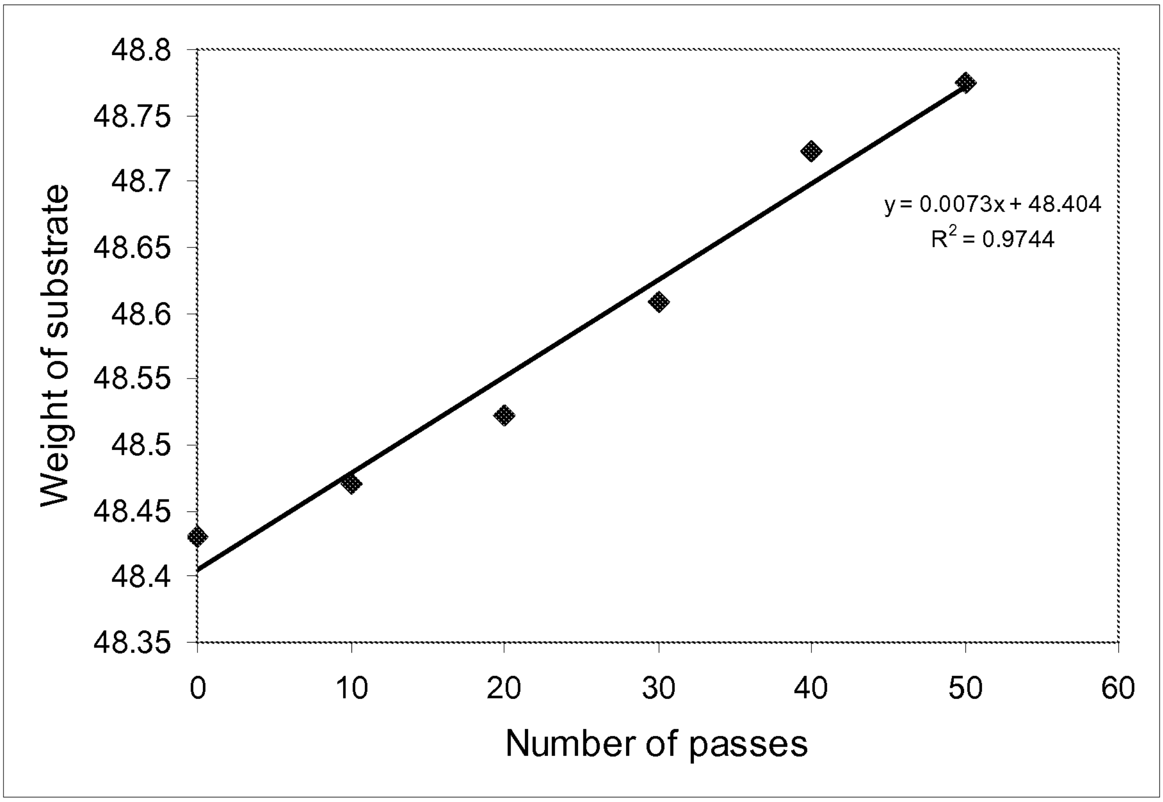


Figure 62

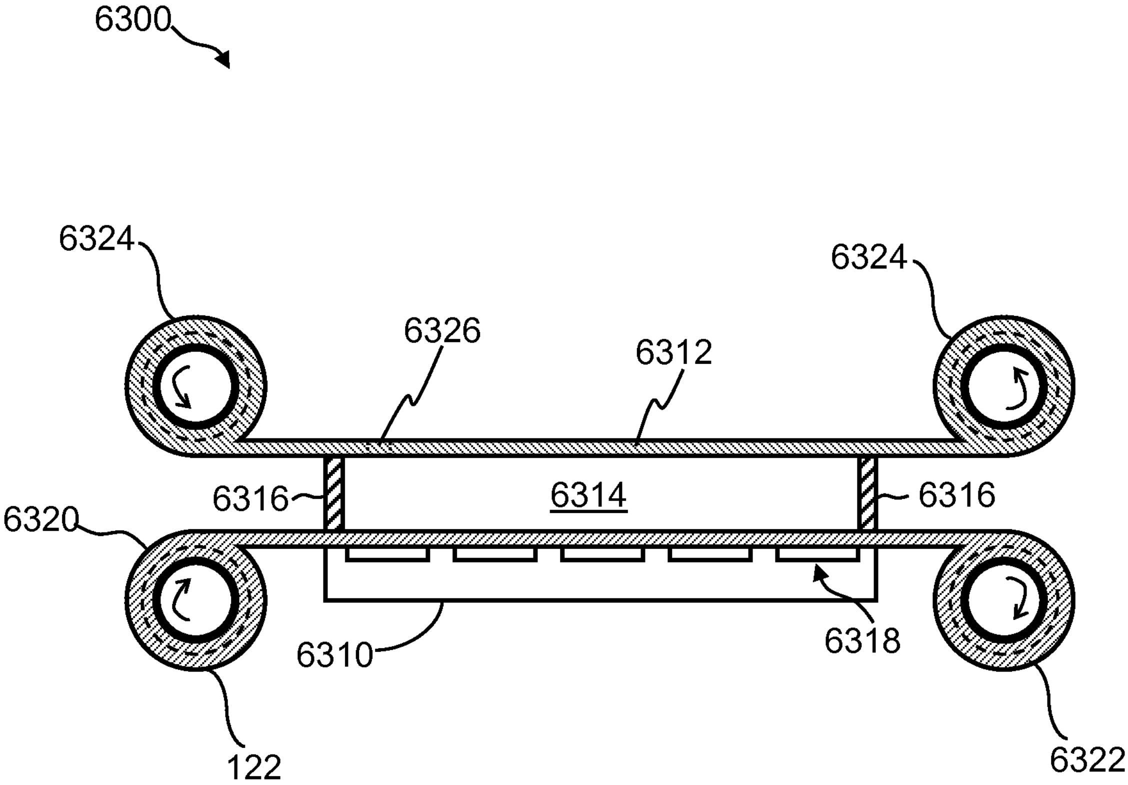


Figure 63A

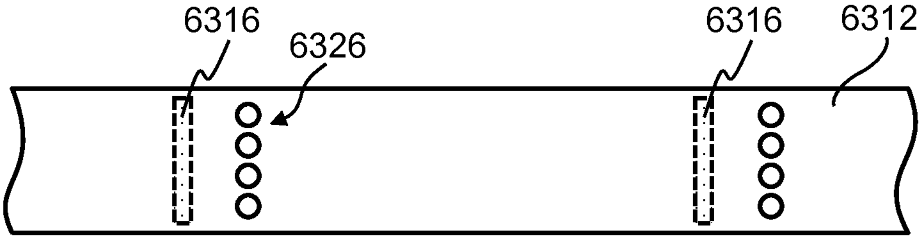
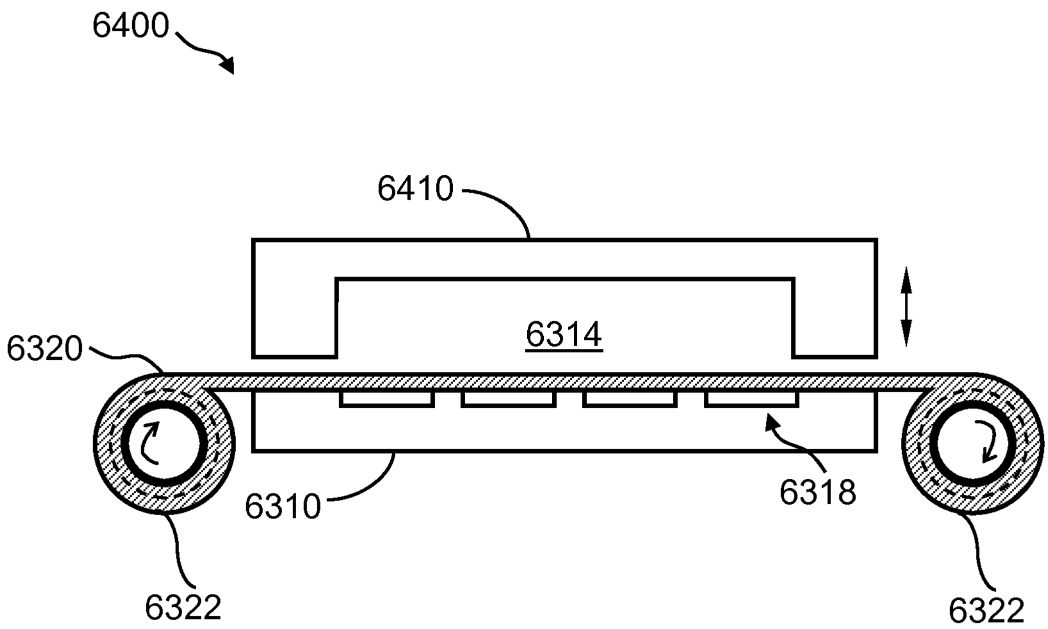


Figure 63B



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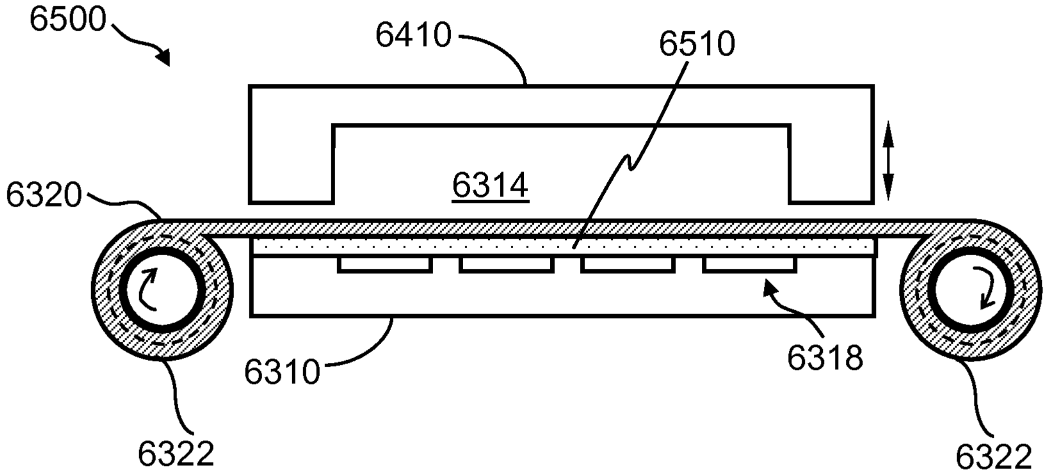


Figure 65A

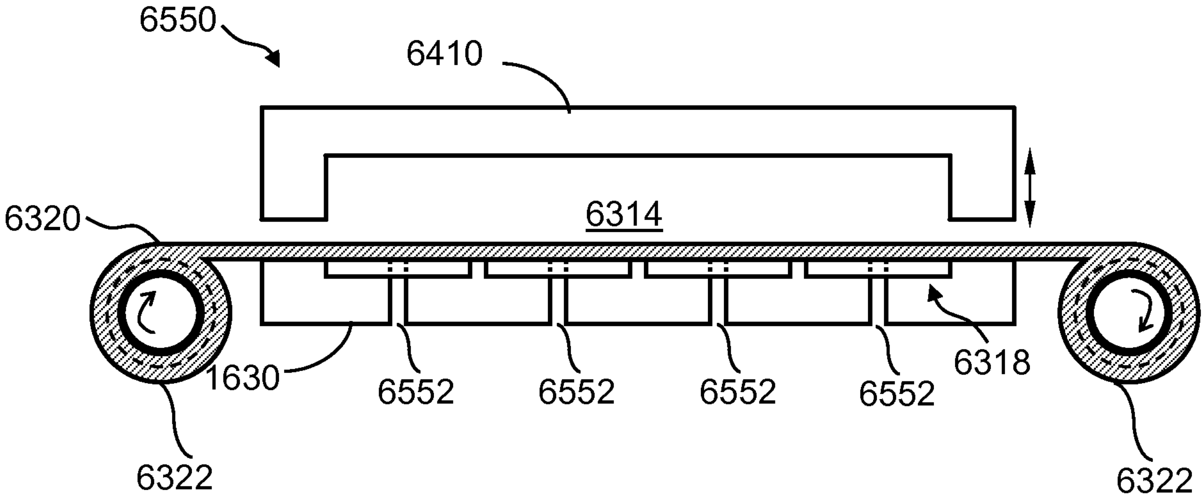


Figure 65B

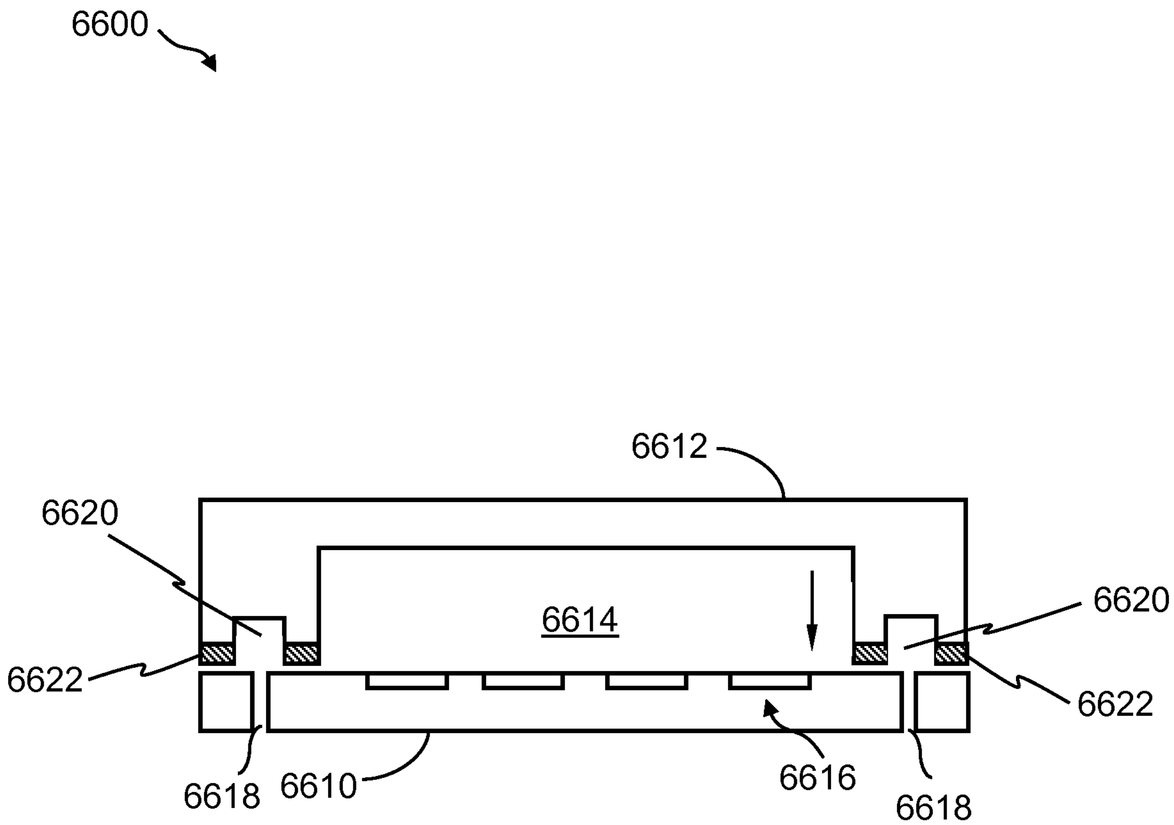
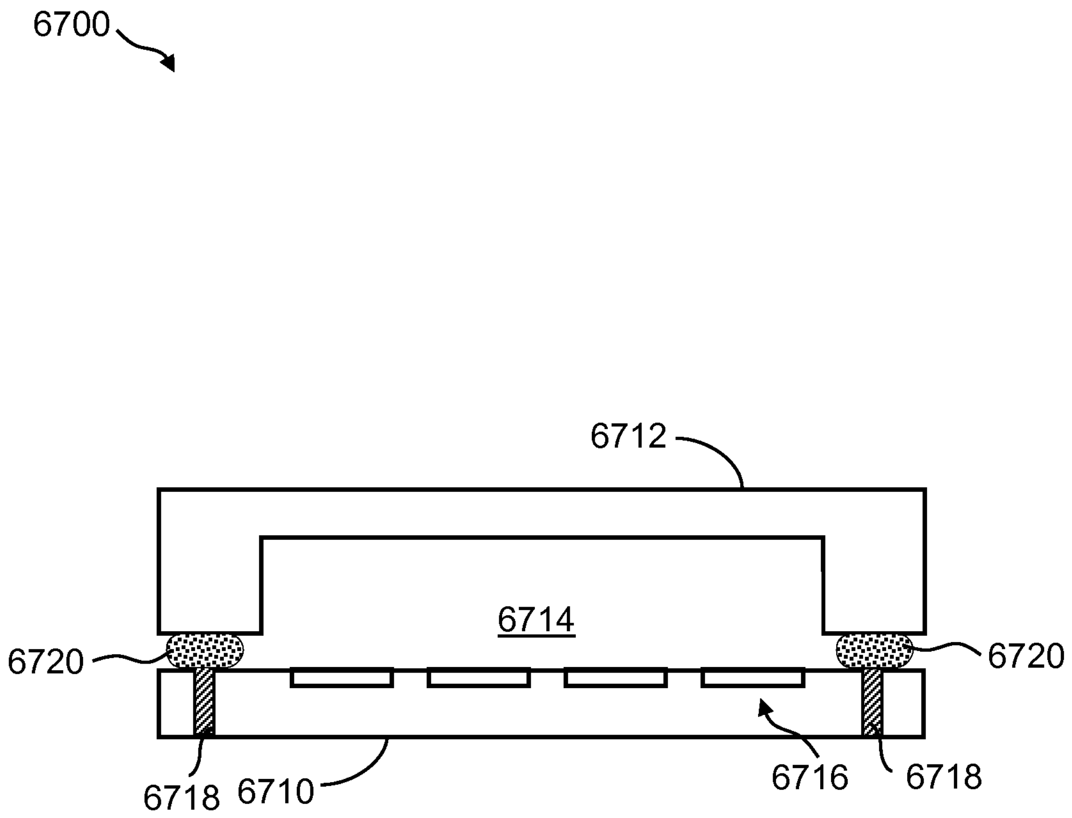


Figure 66



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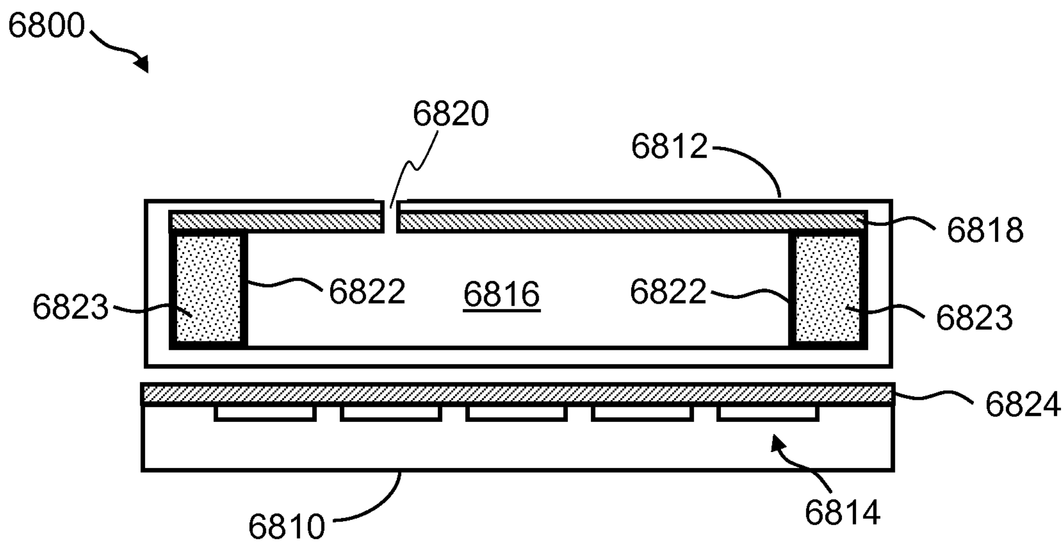


Figure 68A

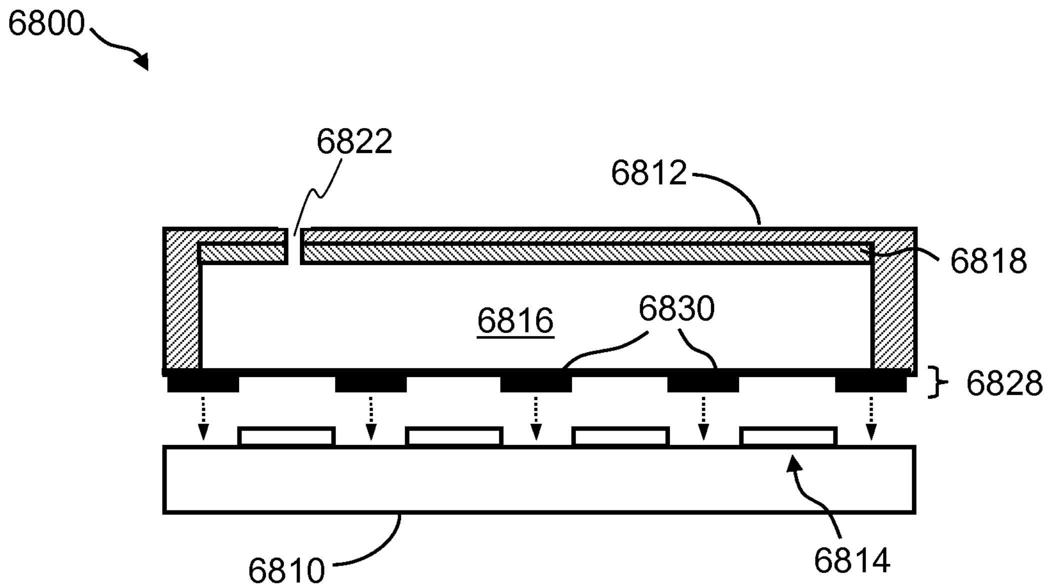


Figure 68B

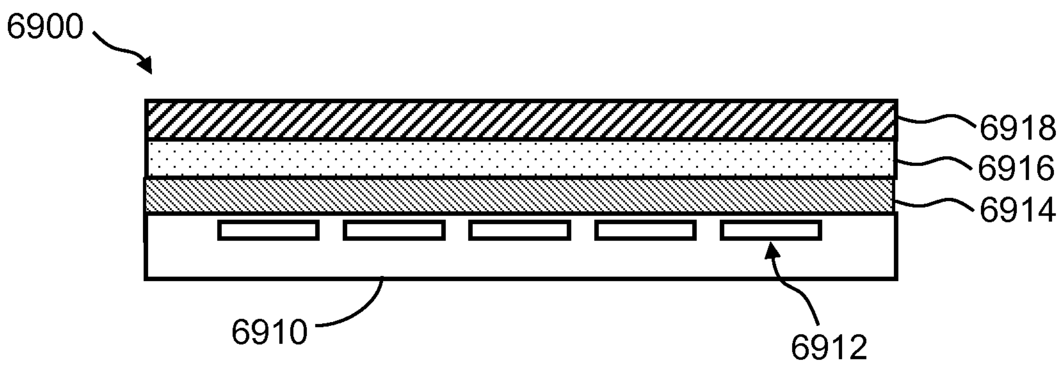


Figure 69A

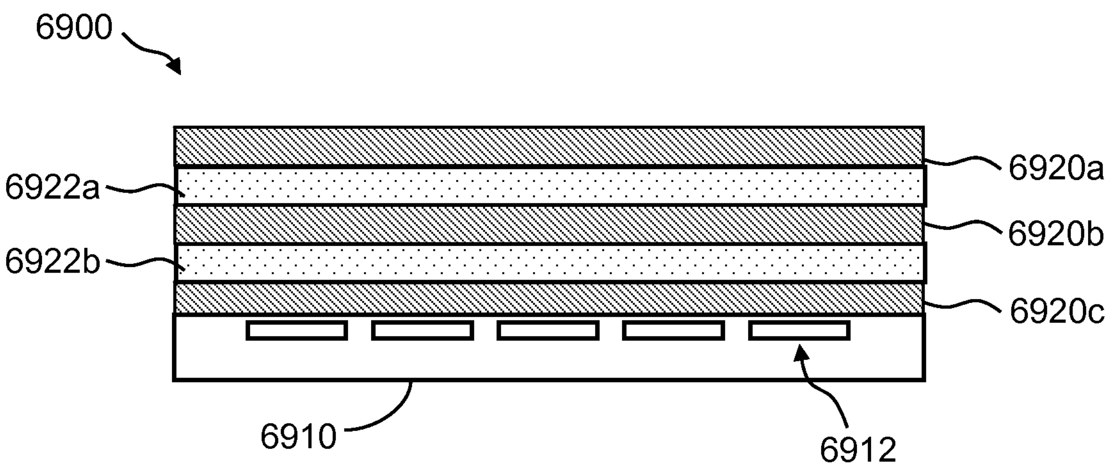


Figure 69B

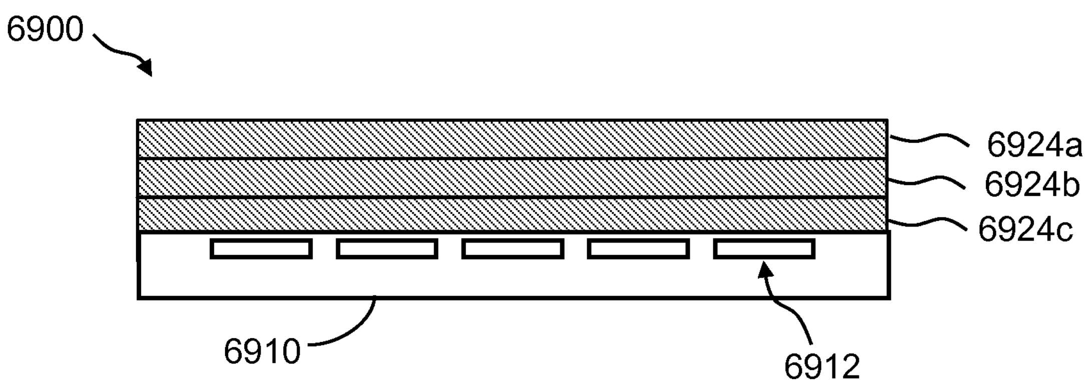
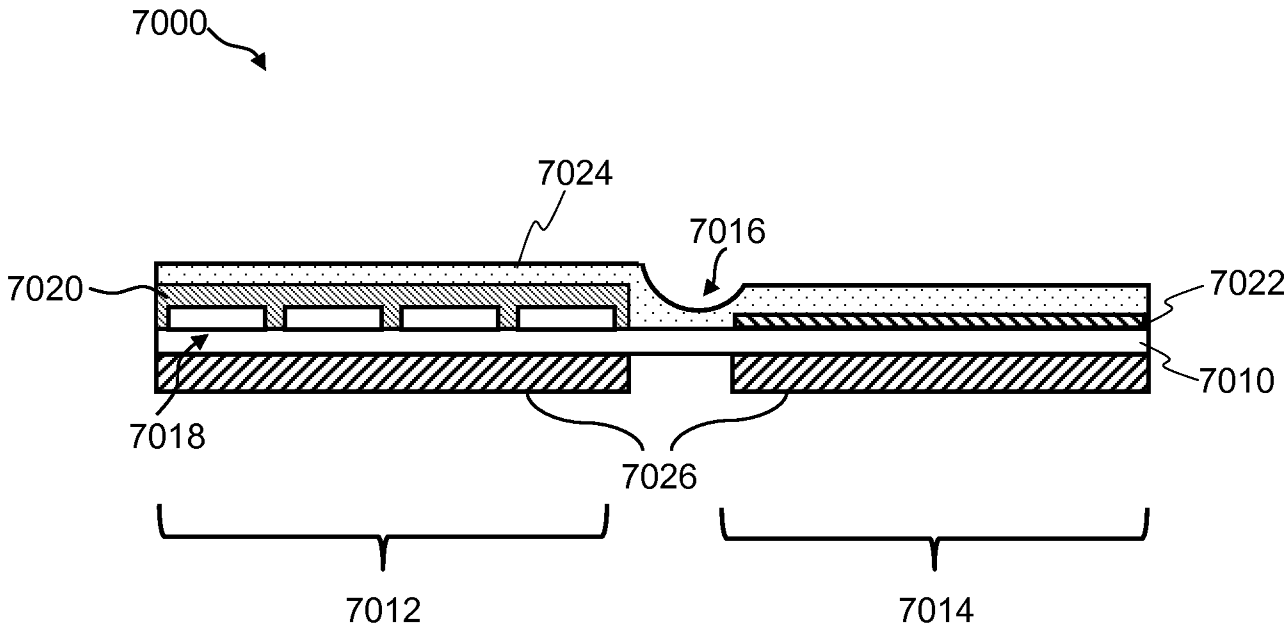


Figure 69C



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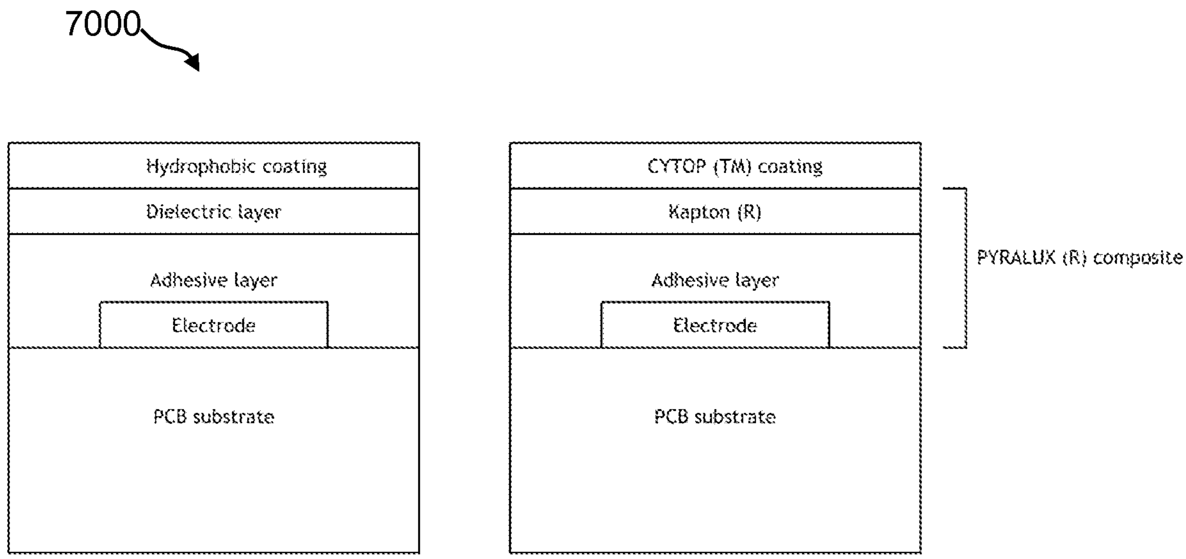
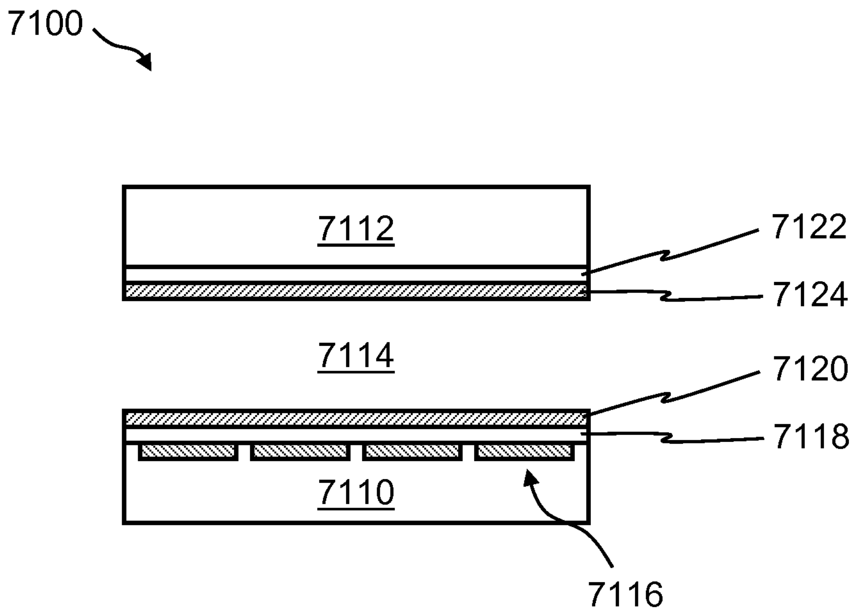


Figure 70B



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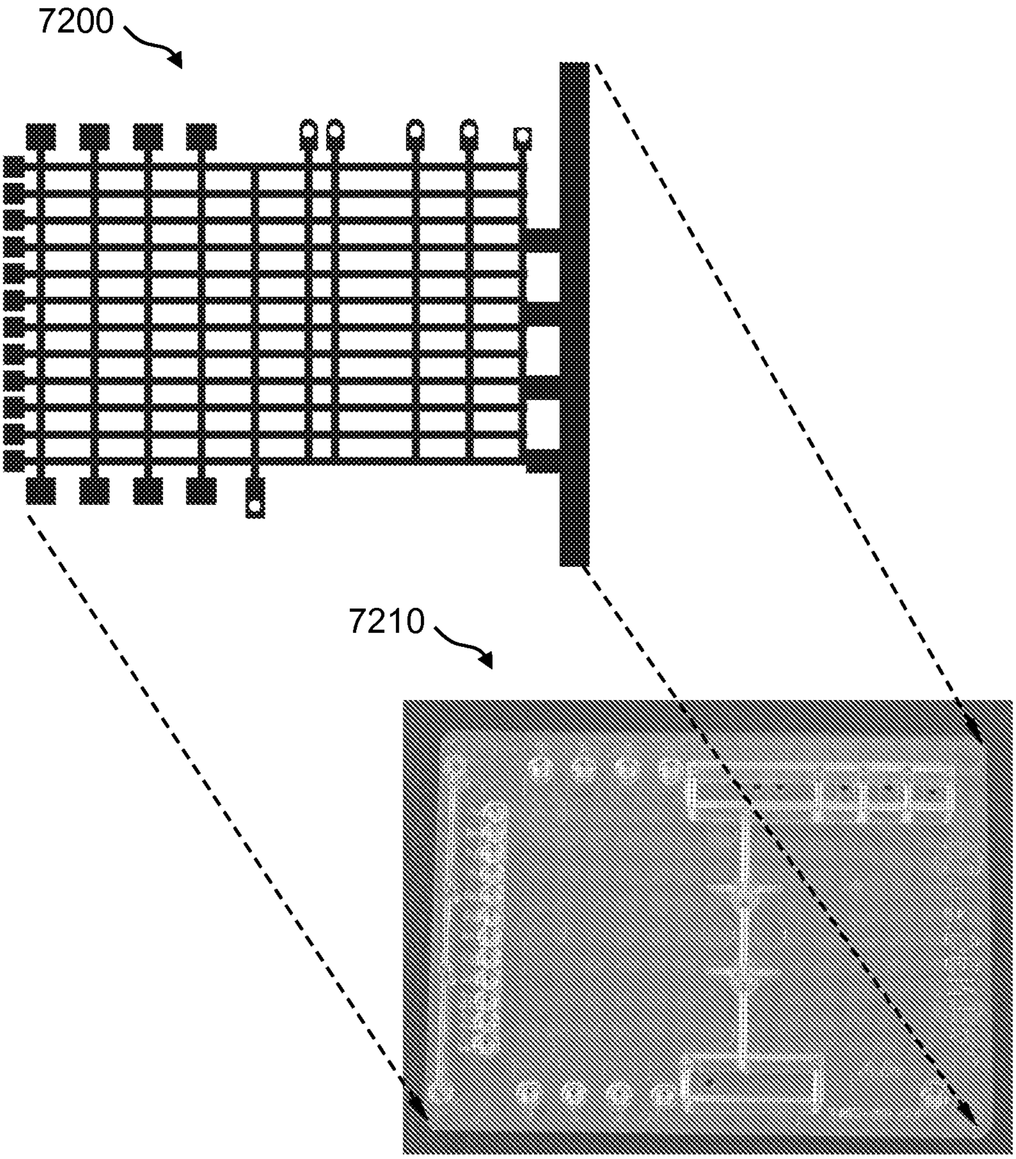

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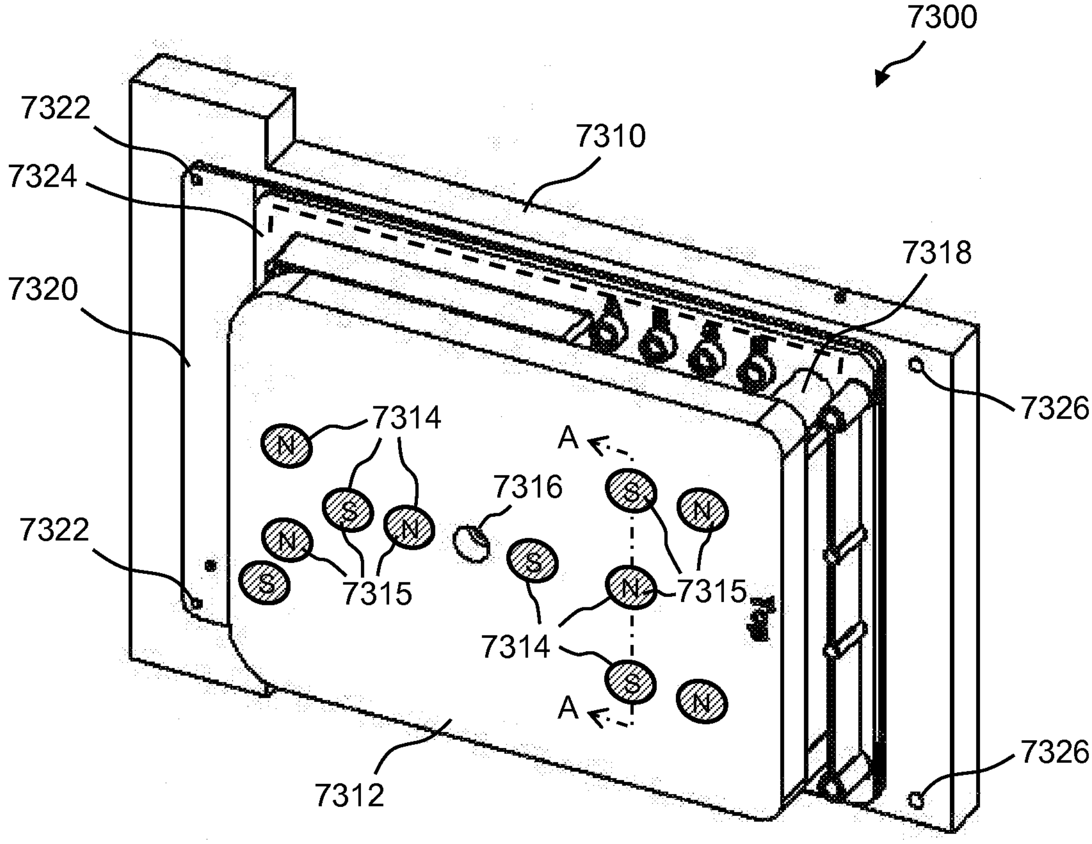
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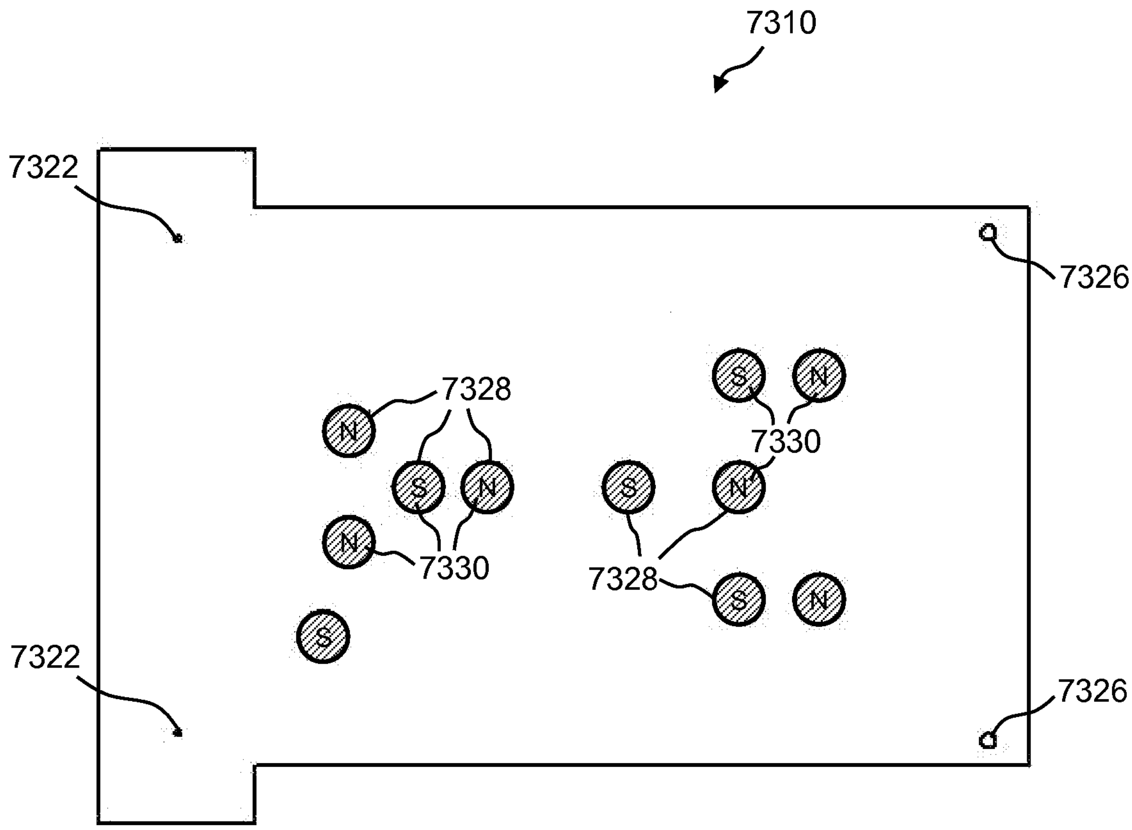
Figure 73



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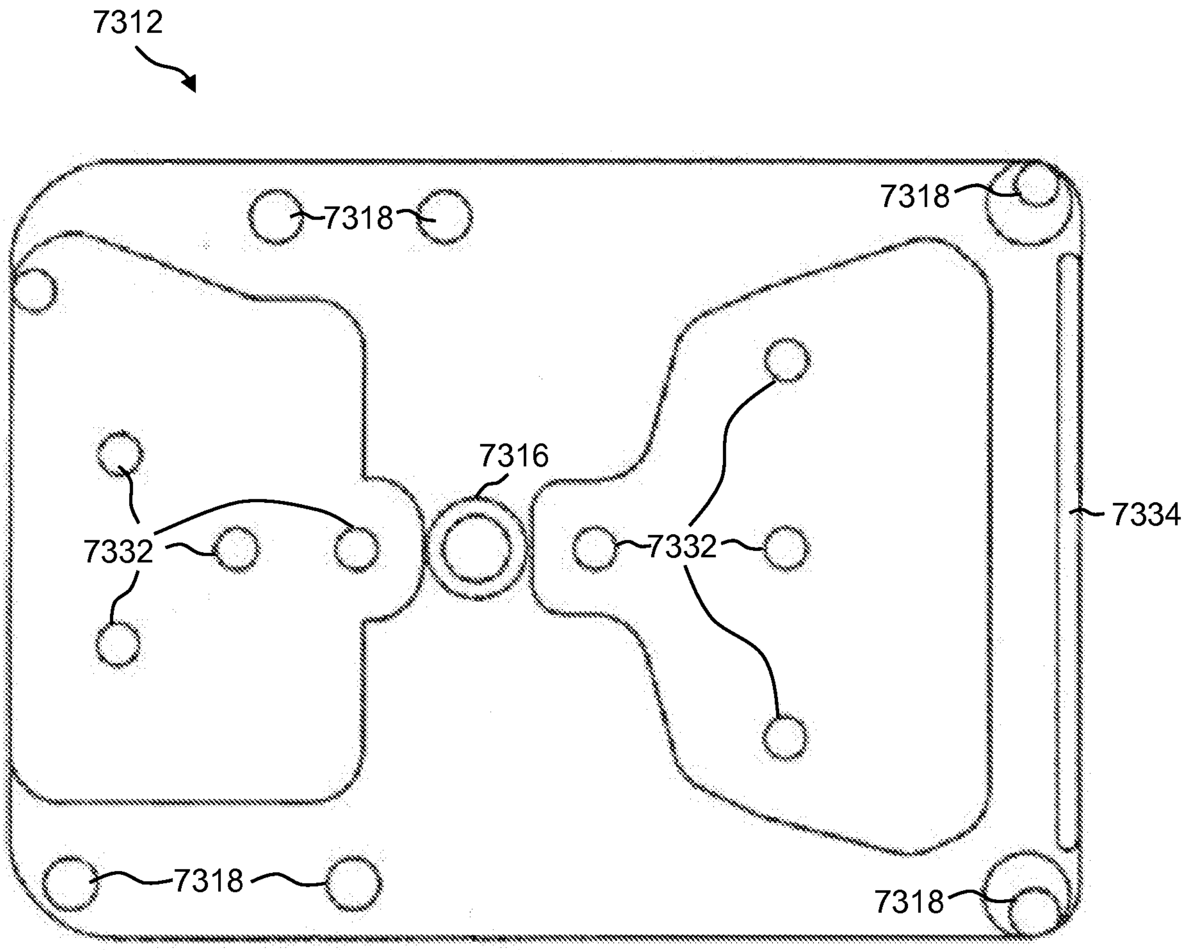


Figure 75A

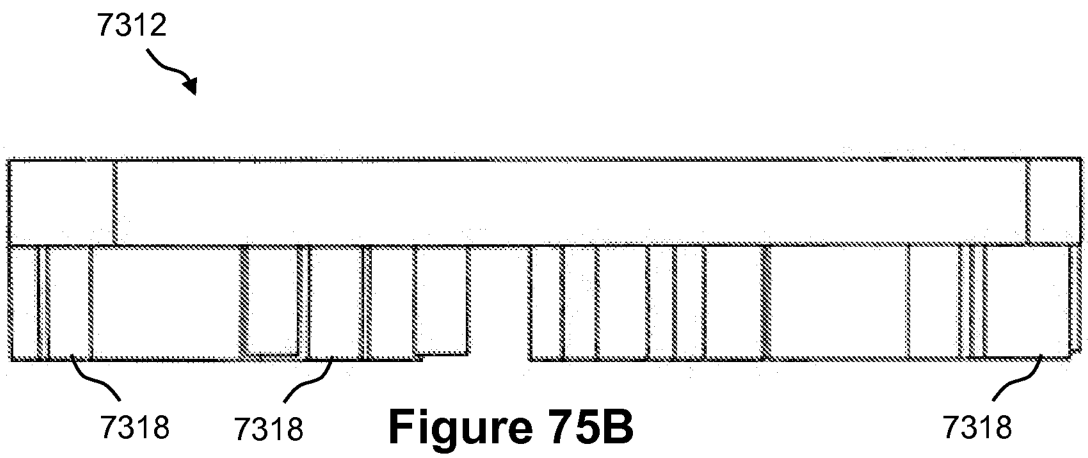
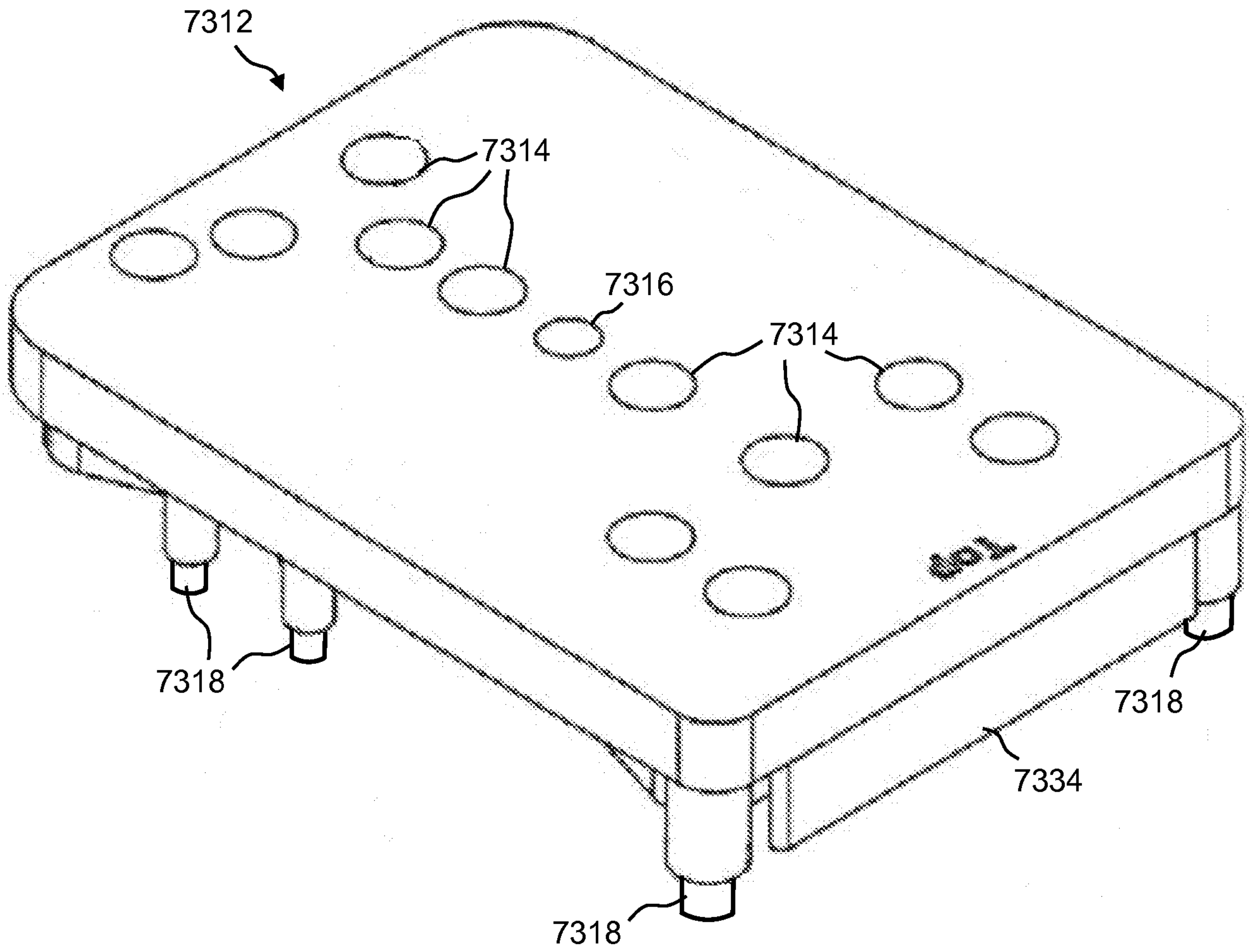


Figure 75B



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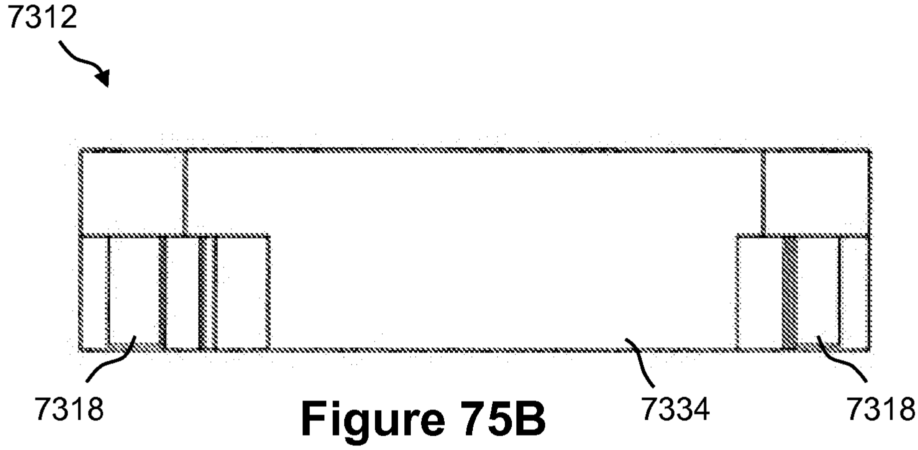
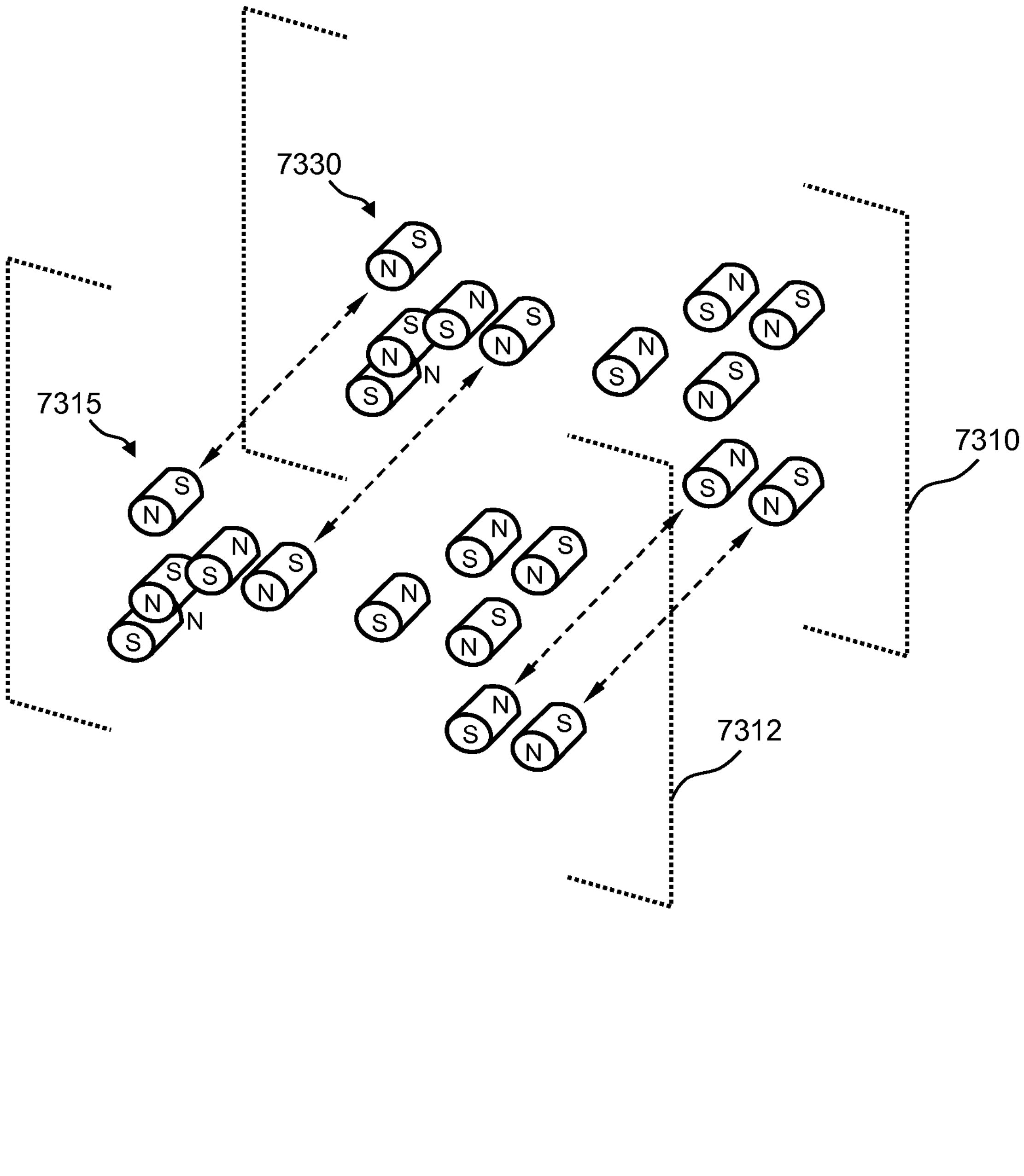


Figure 75B



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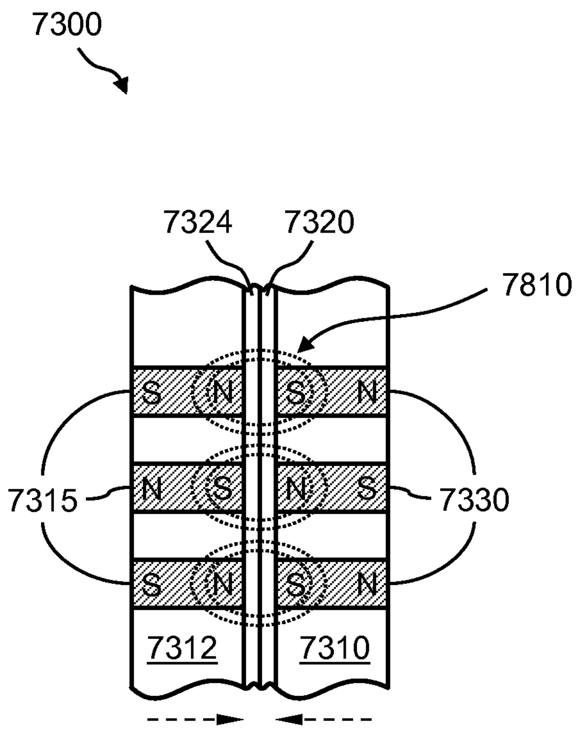

Figure 78

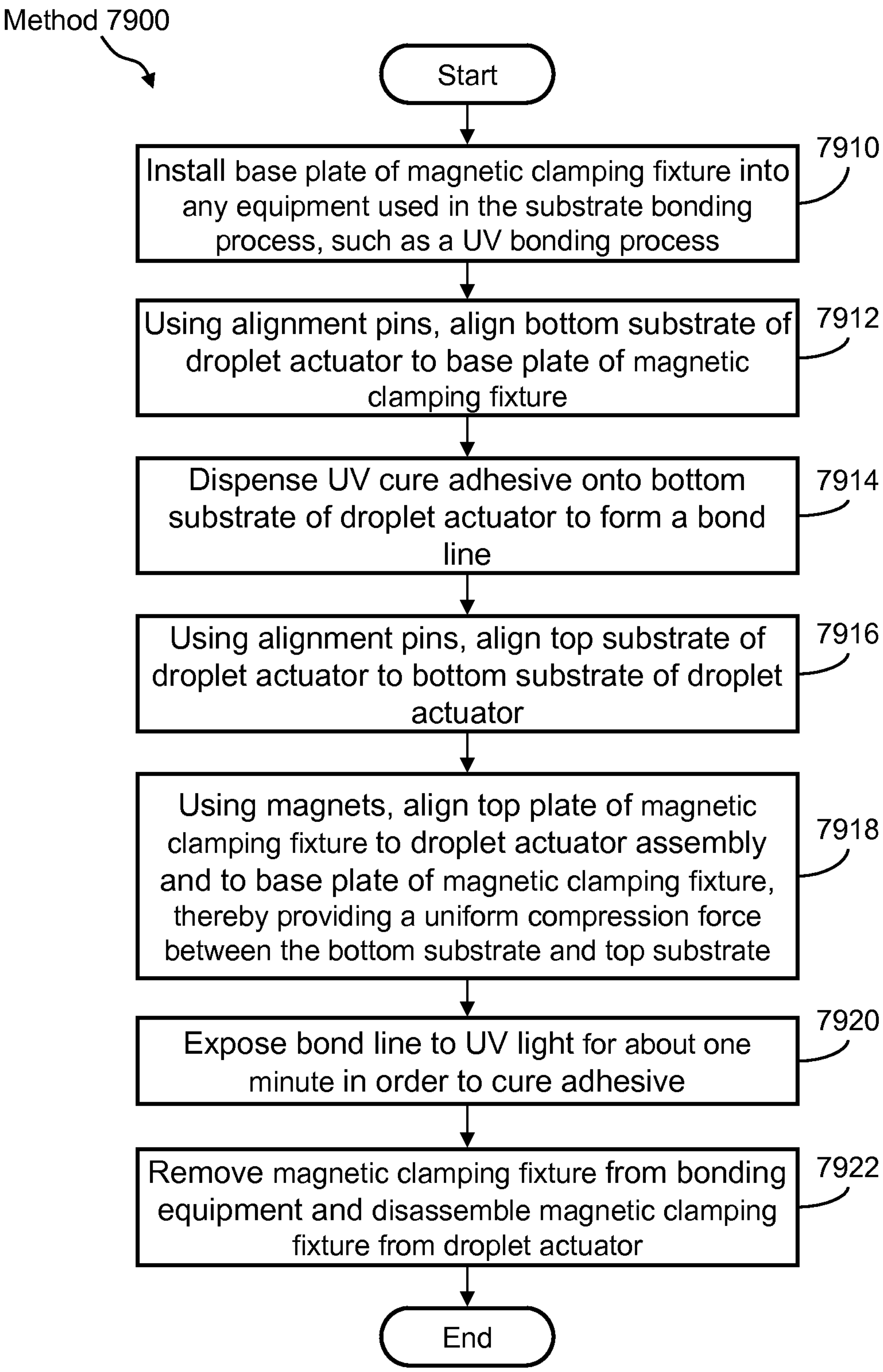
Method 7900
Start
Install base plate of magnetic clamping fixture into any equipment used in the substrate bonding process, such as a UV bonding process
7910
Using alignment pins, align bottom substrate of droplet actuator to base plate of magnetic clamping fixture
7912
Dispense UV cure adhesive onto bottom substrate of droplet actuator to form a bond line
7914
Using alignment pins, align top substrate of droplet actuator to bottom substrate of droplet actuator
7916
Using magnets, align top plate of magnetic clamping fixture to droplet actuator assembly and to base plate of magnetic clamping fixture, thereby providing a uniform compression force between the bottom substrate and top substrate
7918
Expose bond line to UV light for about one minute in order to cure adhesive
7920
Remove magnetic clamping fixture from bonding equipment and disassemble magnetic clamping fixture from droplet actuator
7922
End

Figure 79

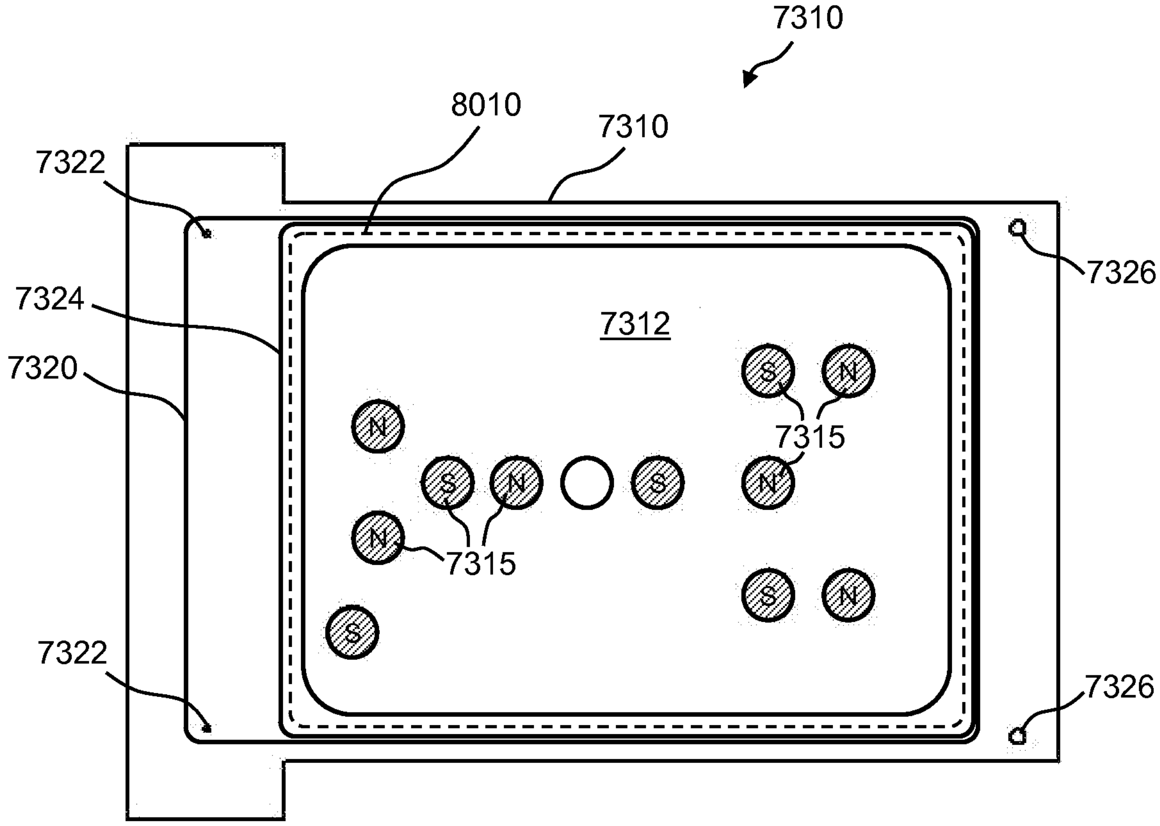
7310
8010
7310
7322
7326
7324
7312
7320
S
N
N
7315
S
N
S
N
N
7315
S
N
S
7322
7326

Figure 80

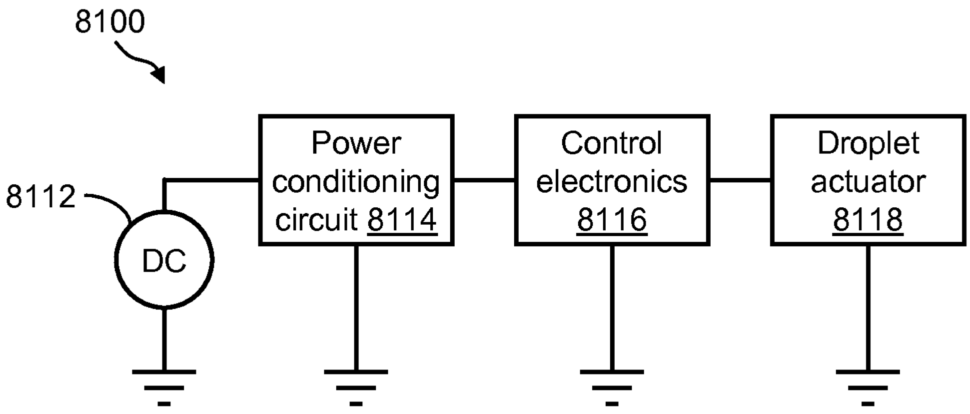

Figure 81A

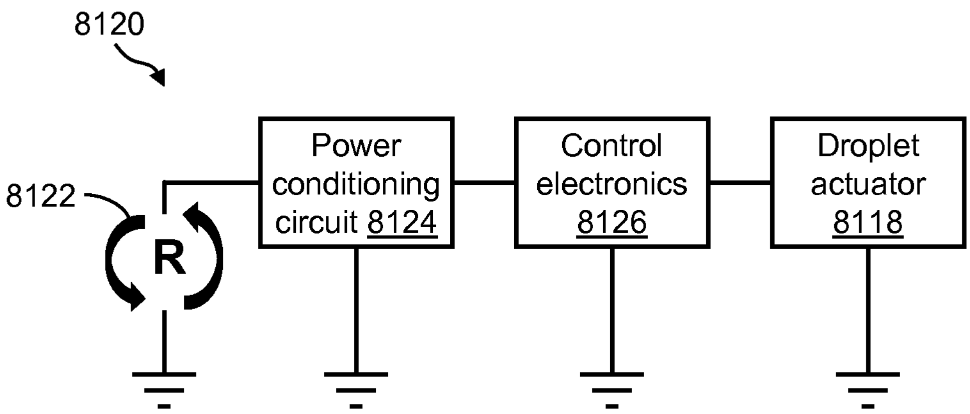

Figure 81B

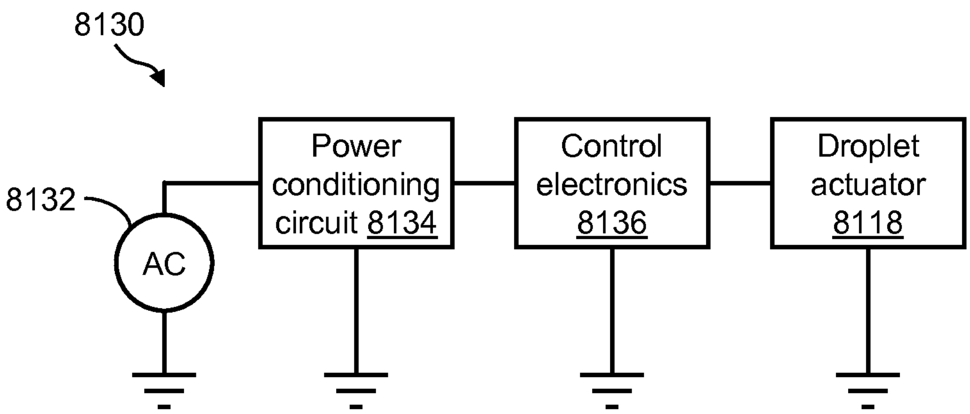

Figure 81C

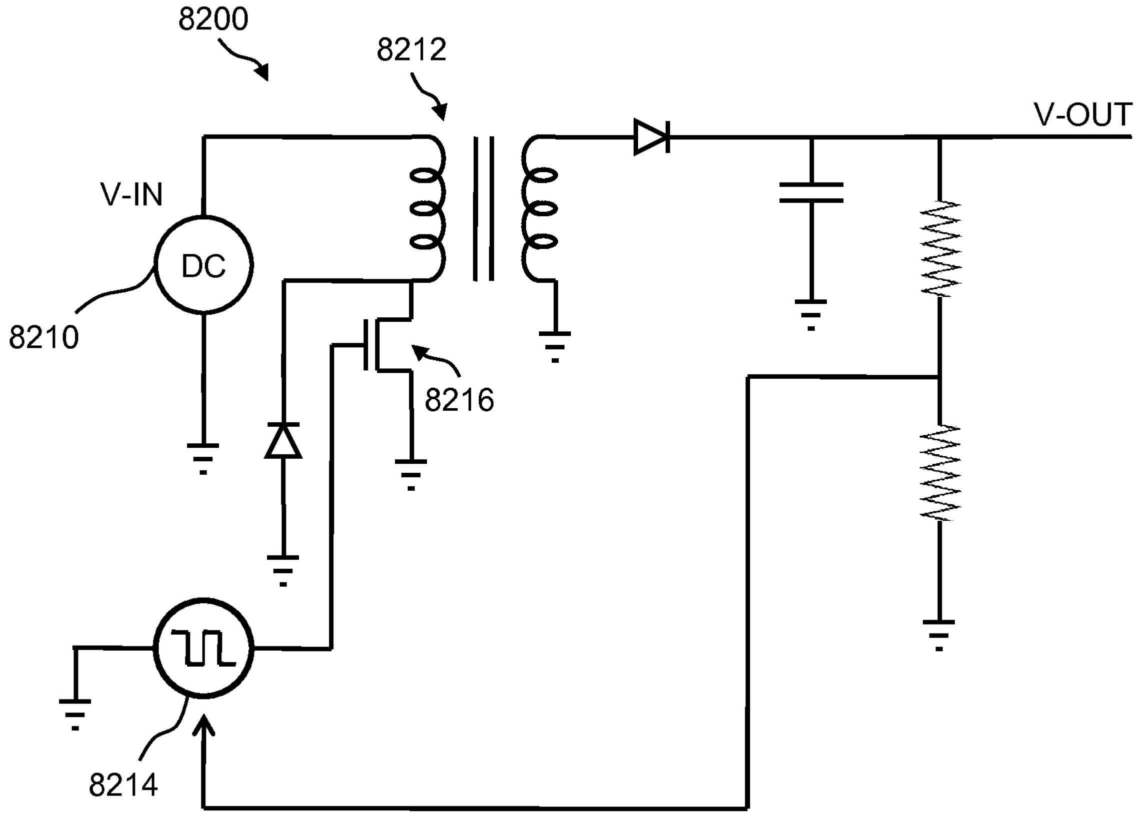

Figure 82A

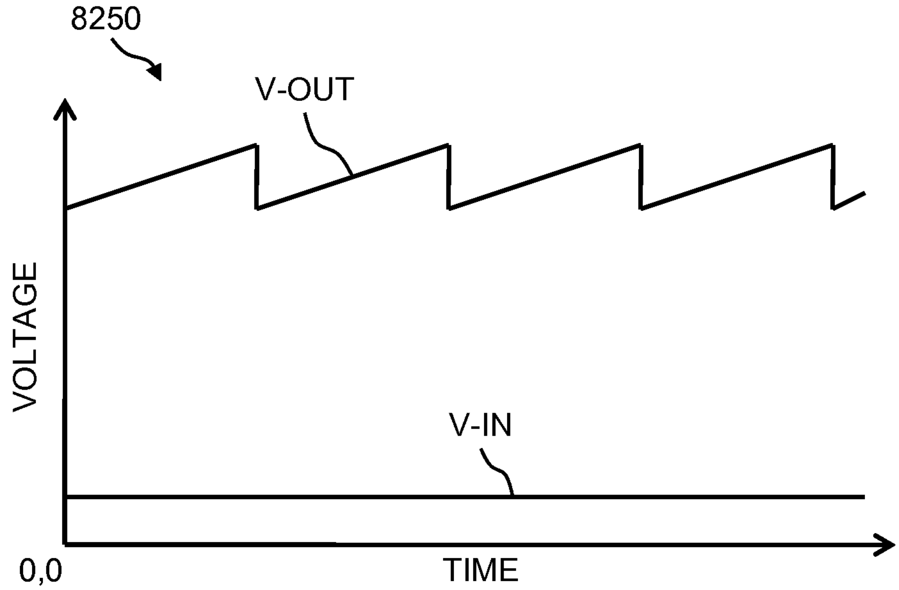

Figure 82B

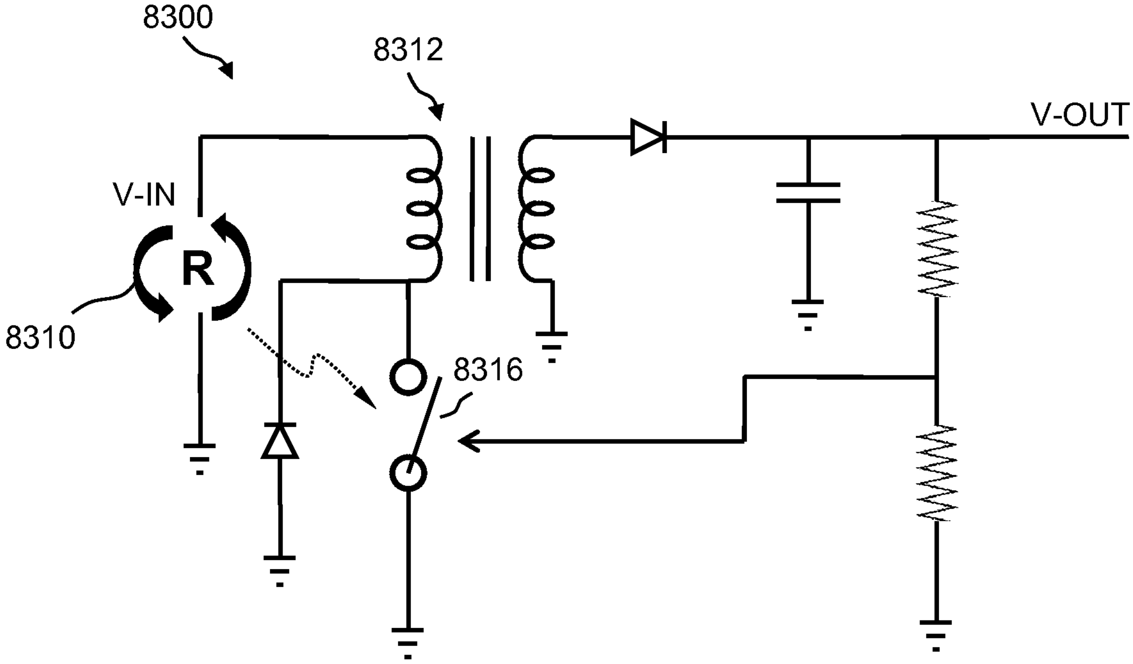

Figure 83A

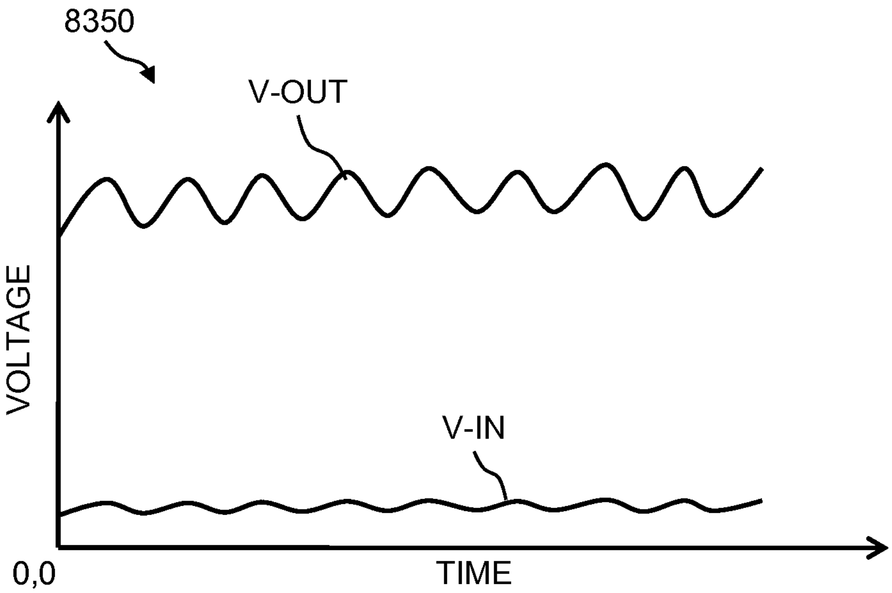

Figure 83B

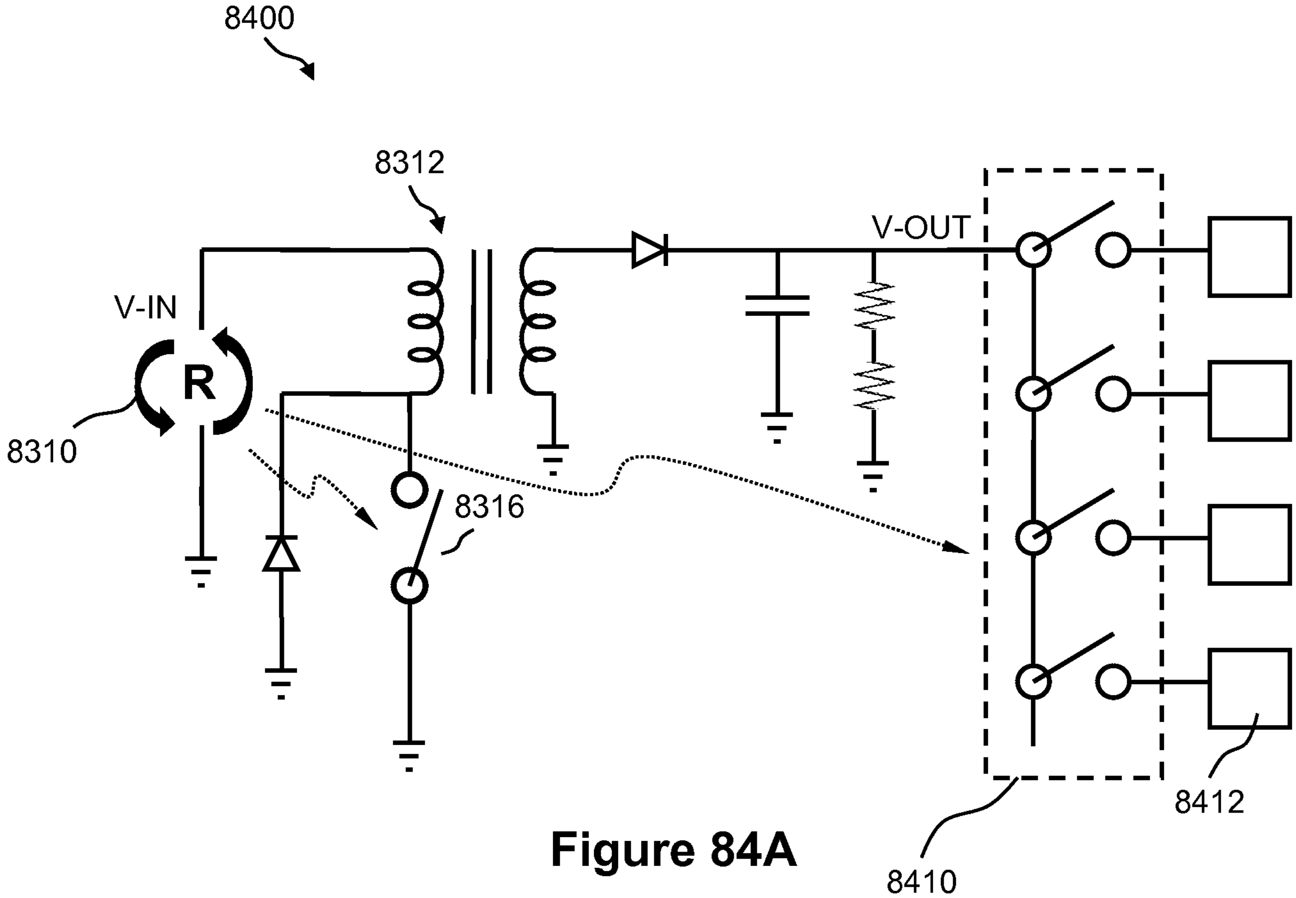

Figure 84A

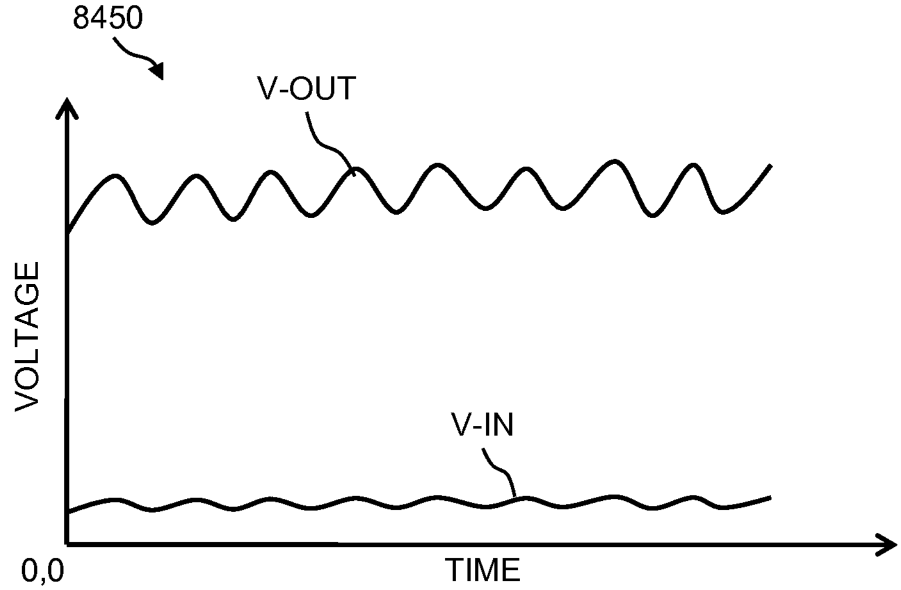

Figure 84B

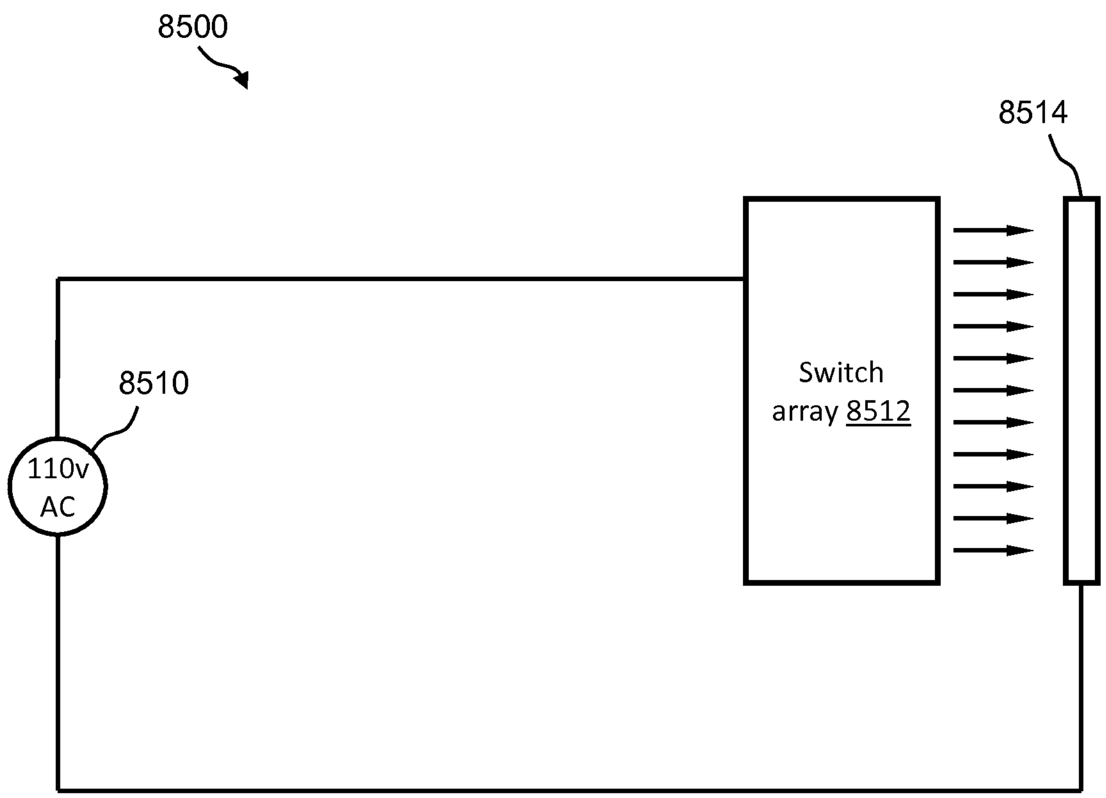

Figure 85

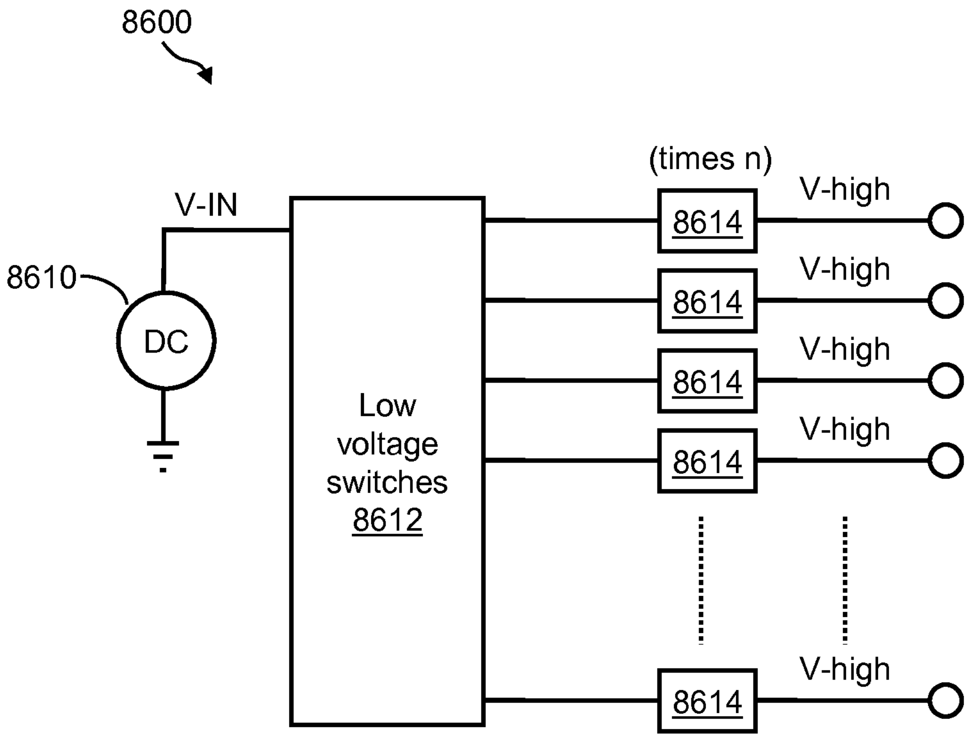
8600
(times n)
V-IN
8610
DC
Low voltage switches 8612
8614
8614
8614
8614
8614
V-high
V-high
V-high
V-high
V-high

Figure 86

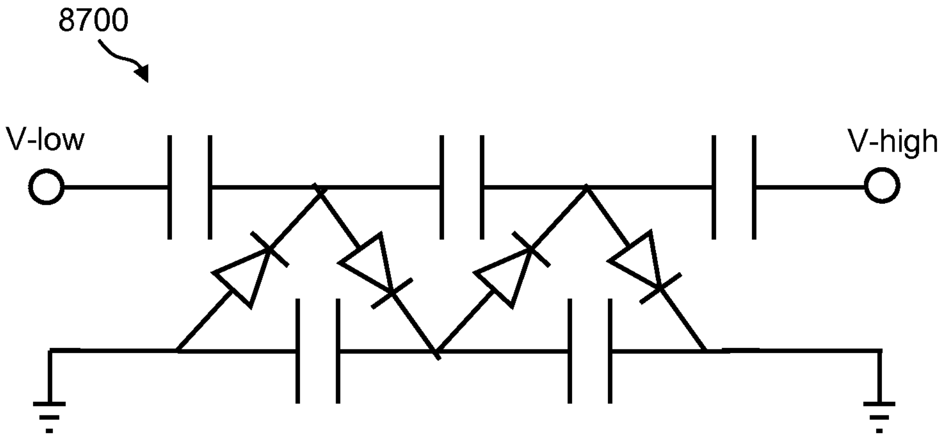

Figure 87A

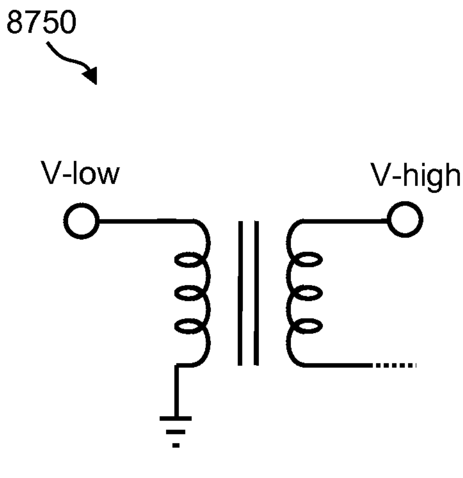

Figure 87B

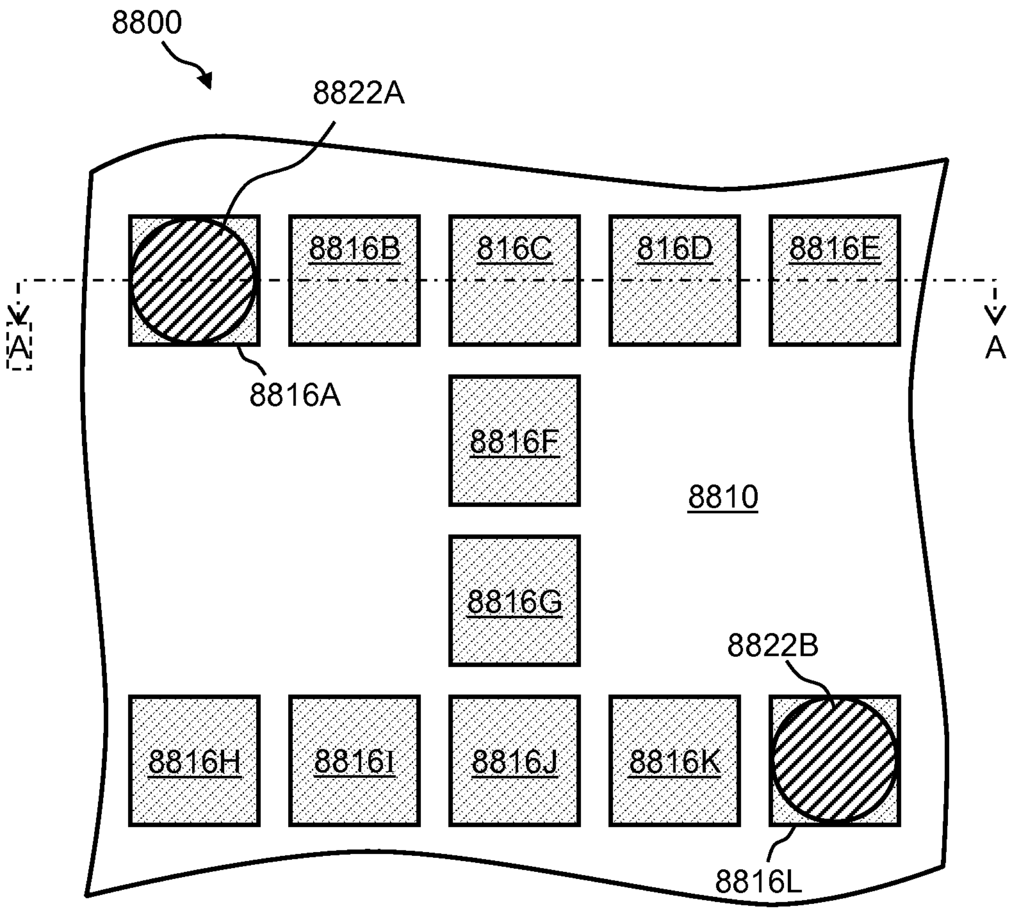

Figure 88A

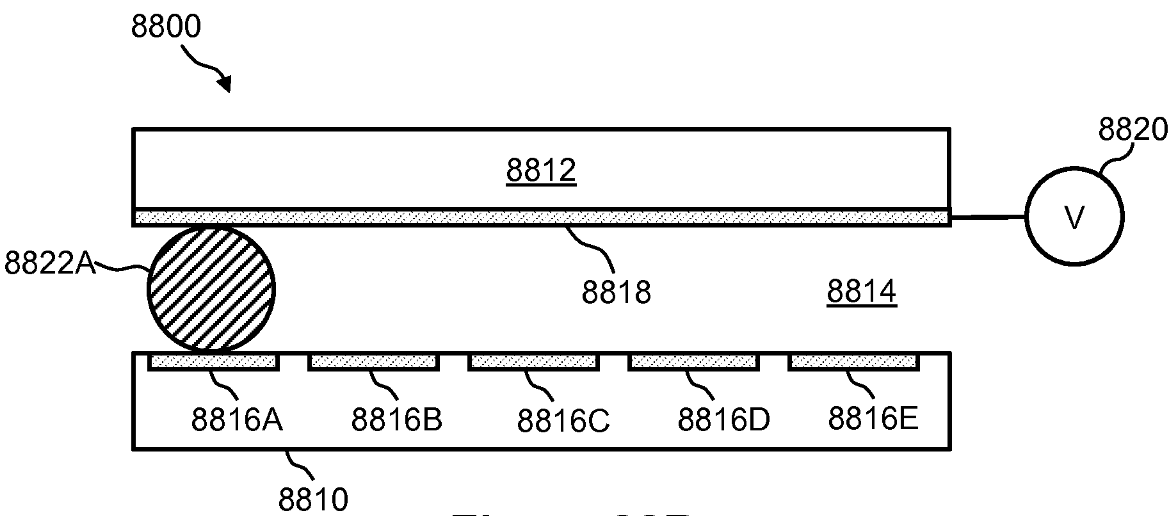

Figure 88B

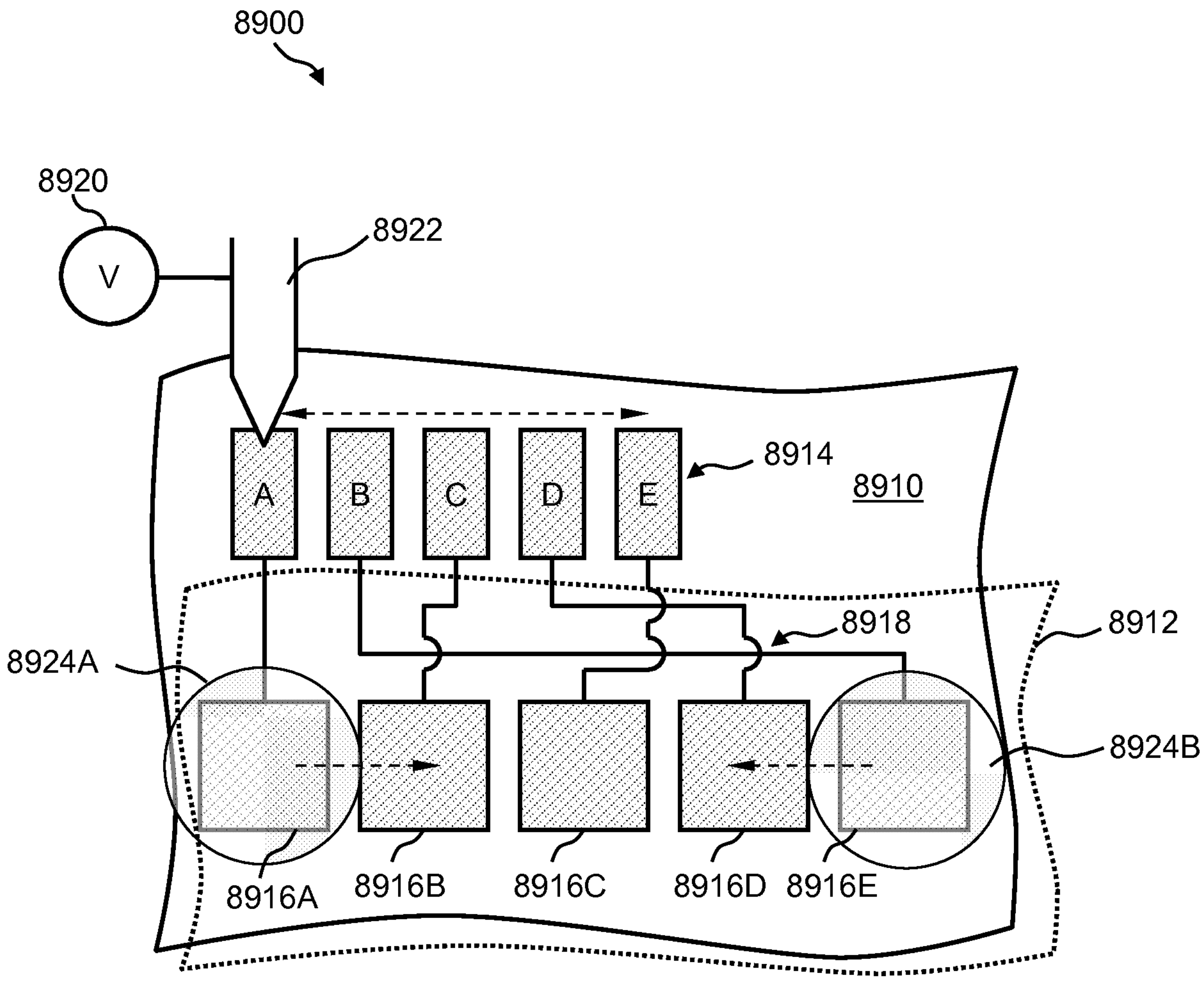

Figure 64 **Figure 89**

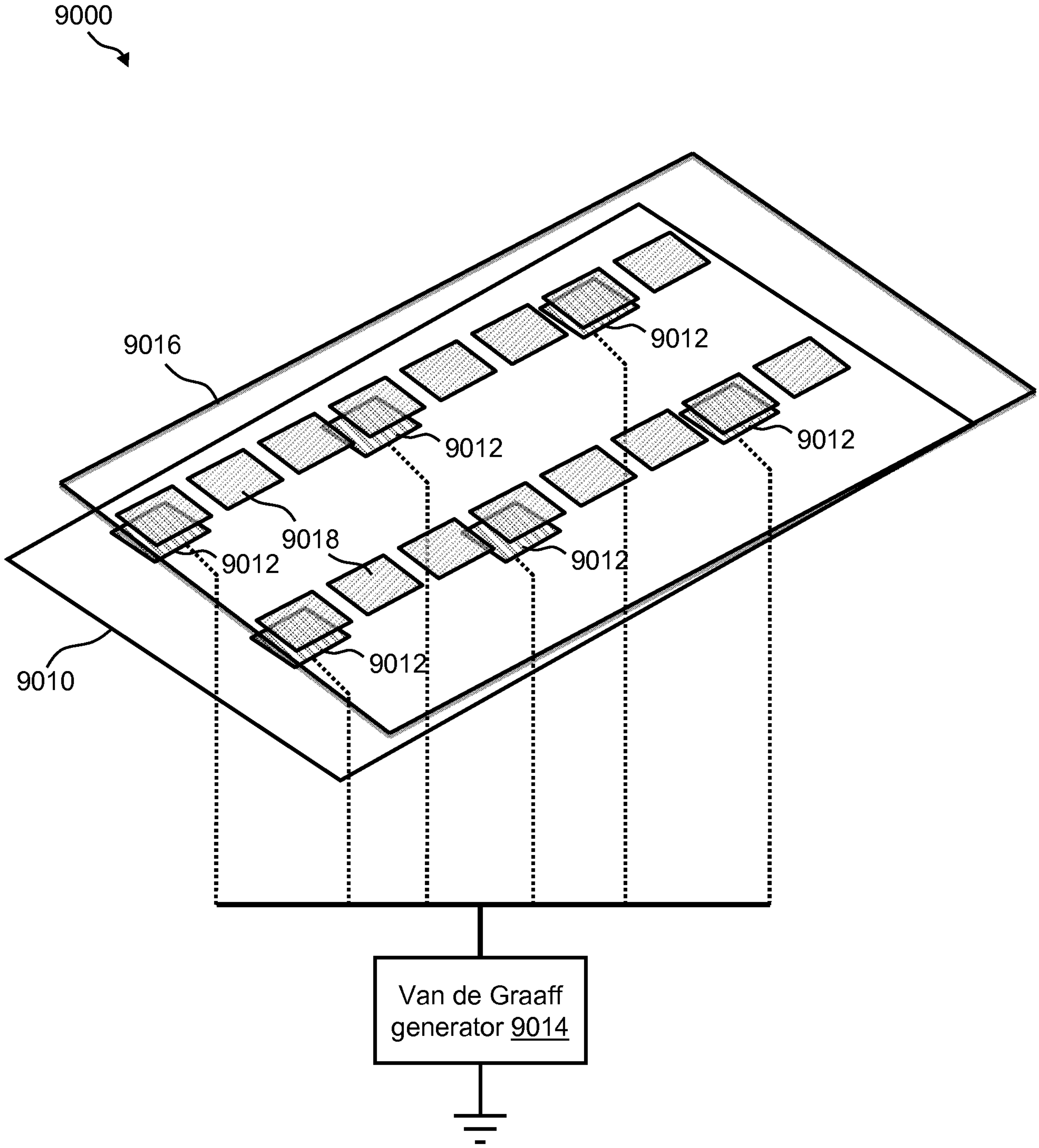
9000
9016
9012
9012
9012
9018
9012
9012
9012
9010
Van de Graaff generator 9014

Figure 90

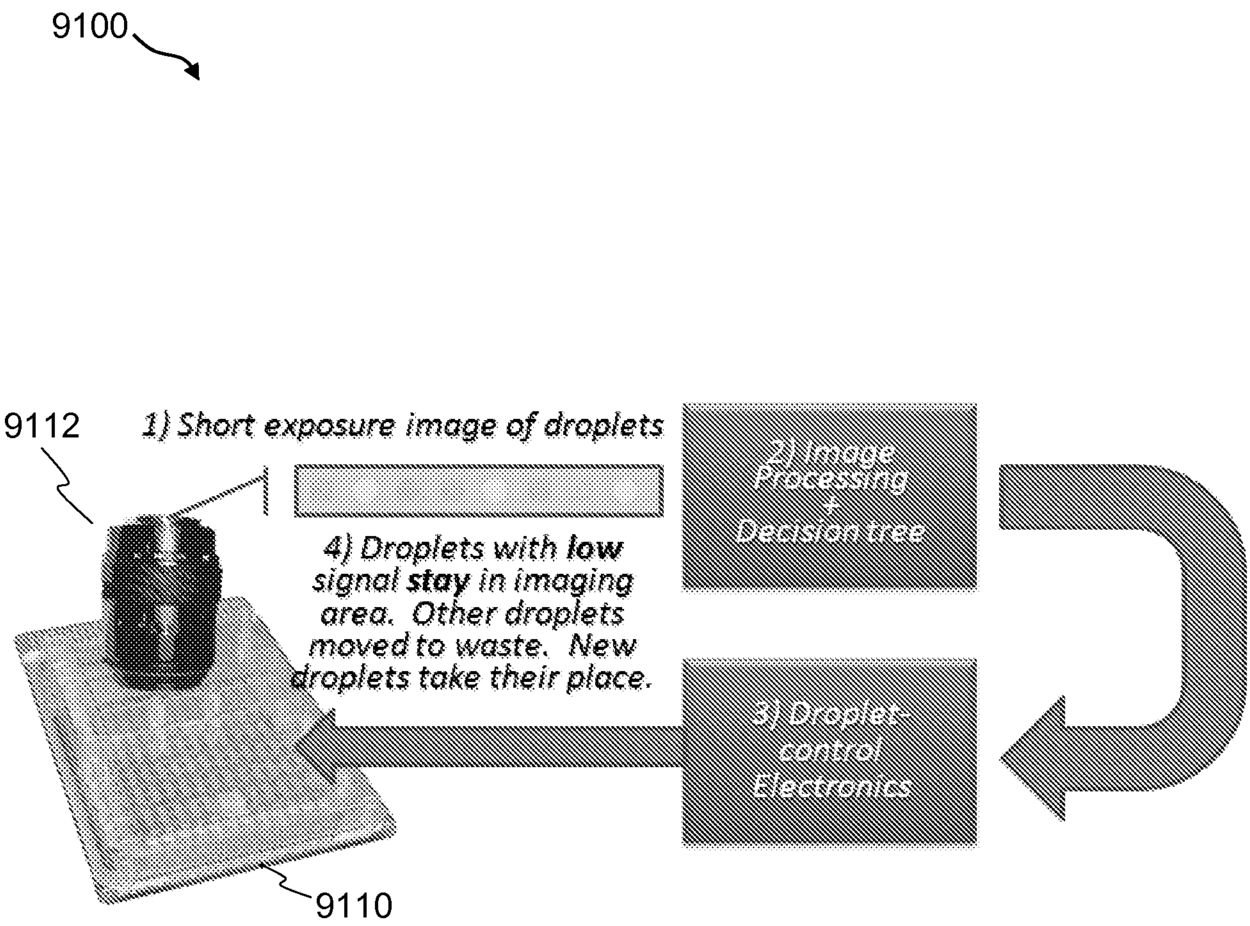
9100
9112
1) Short exposure image of droplets
2) Image Processing + Decision tree
4) Droplets with **low** signal **stay** in imaging area. Other droplets moved to waste. New droplets take their place.
3) Droplet-control Electronics
9110

Figure 91

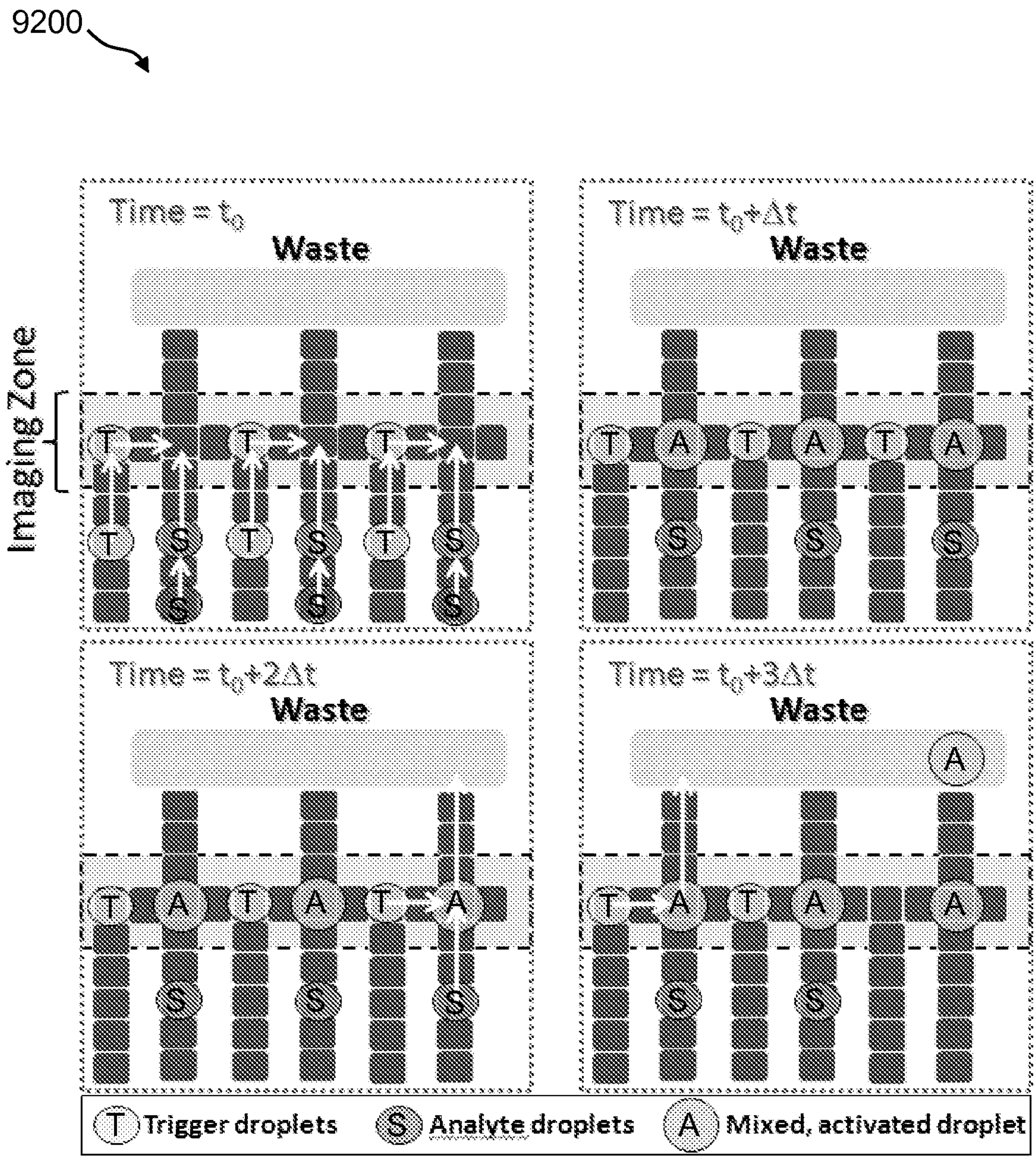
9200
Imaging Zone
Time = t_0
Waste
Time = $t_0+\Delta t$
Waste
Time = $t_0+2\Delta t$
Waste
Time = $t_0+3\Delta t$
Waste
T
S
A
Trigger droplets
Analyte droplets
Mixed, activated droplet

Figure 92

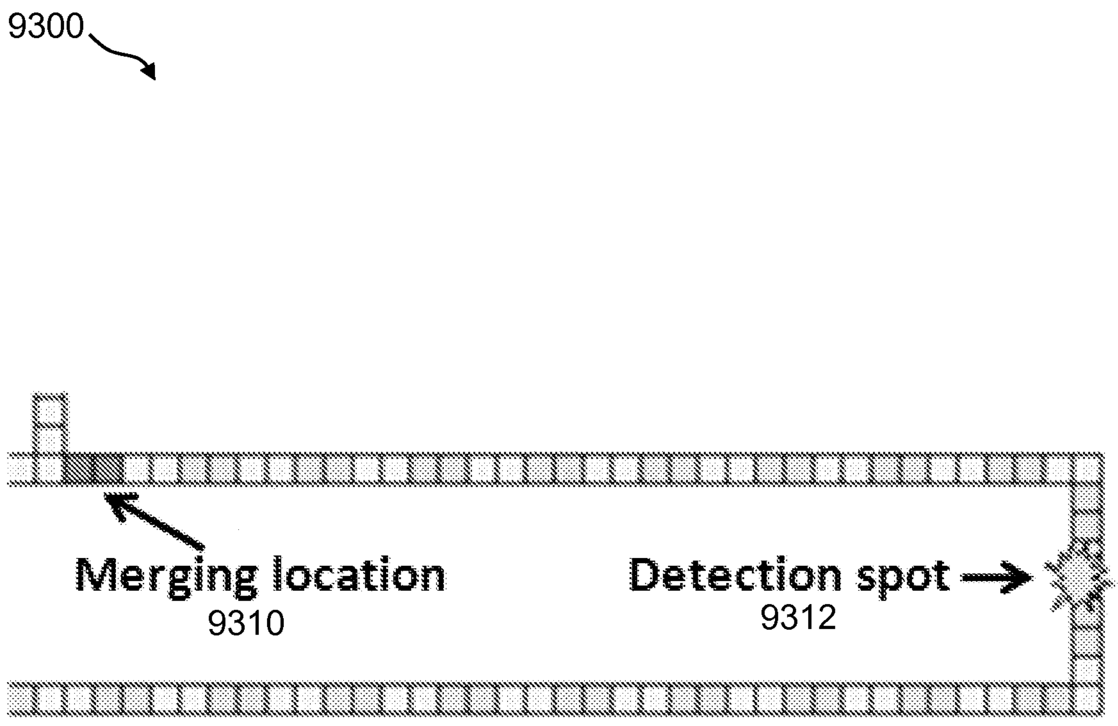

Figure 93

9400

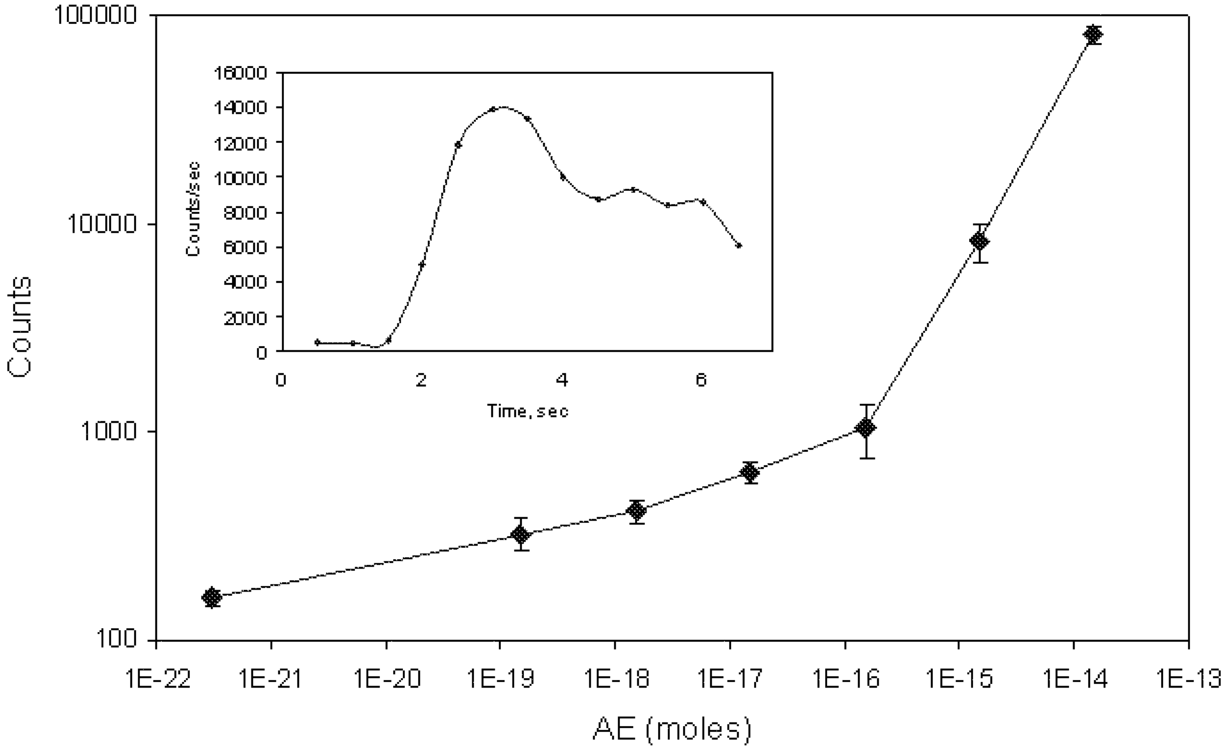

Figure 94

9500

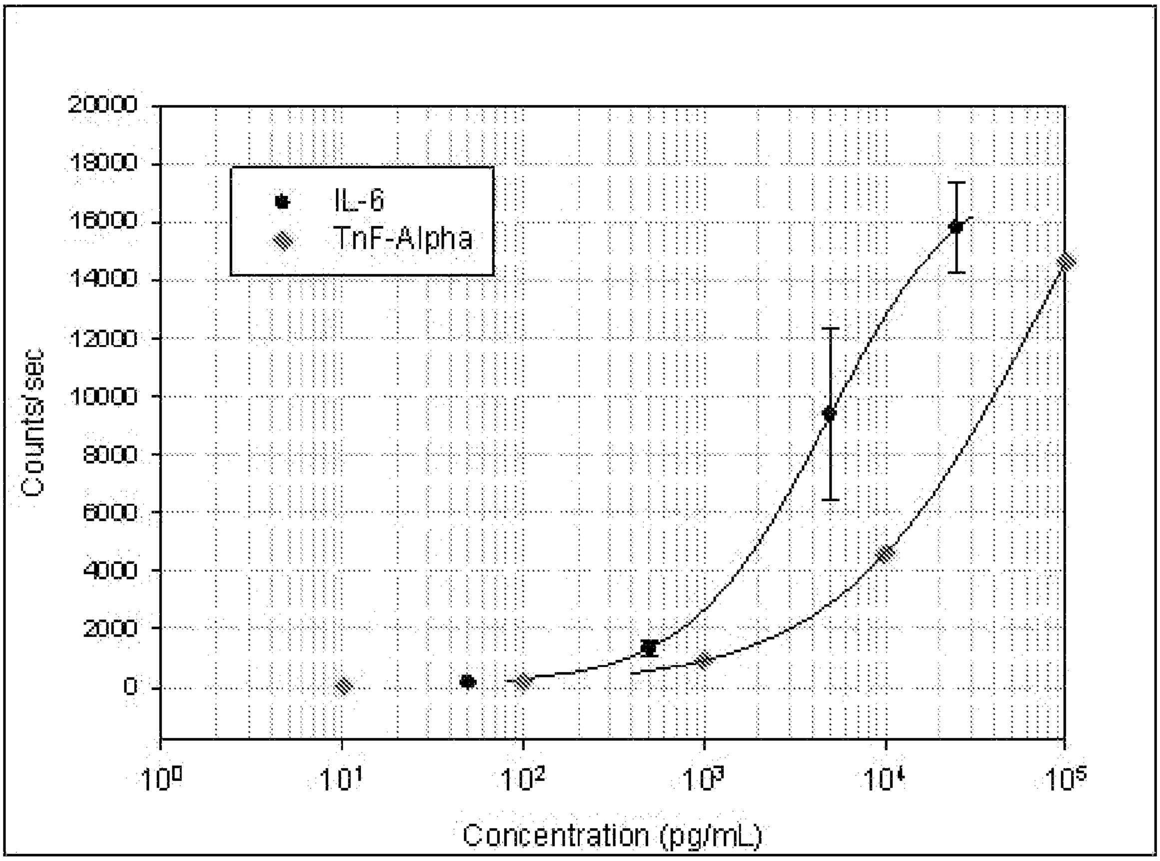

Figure 95

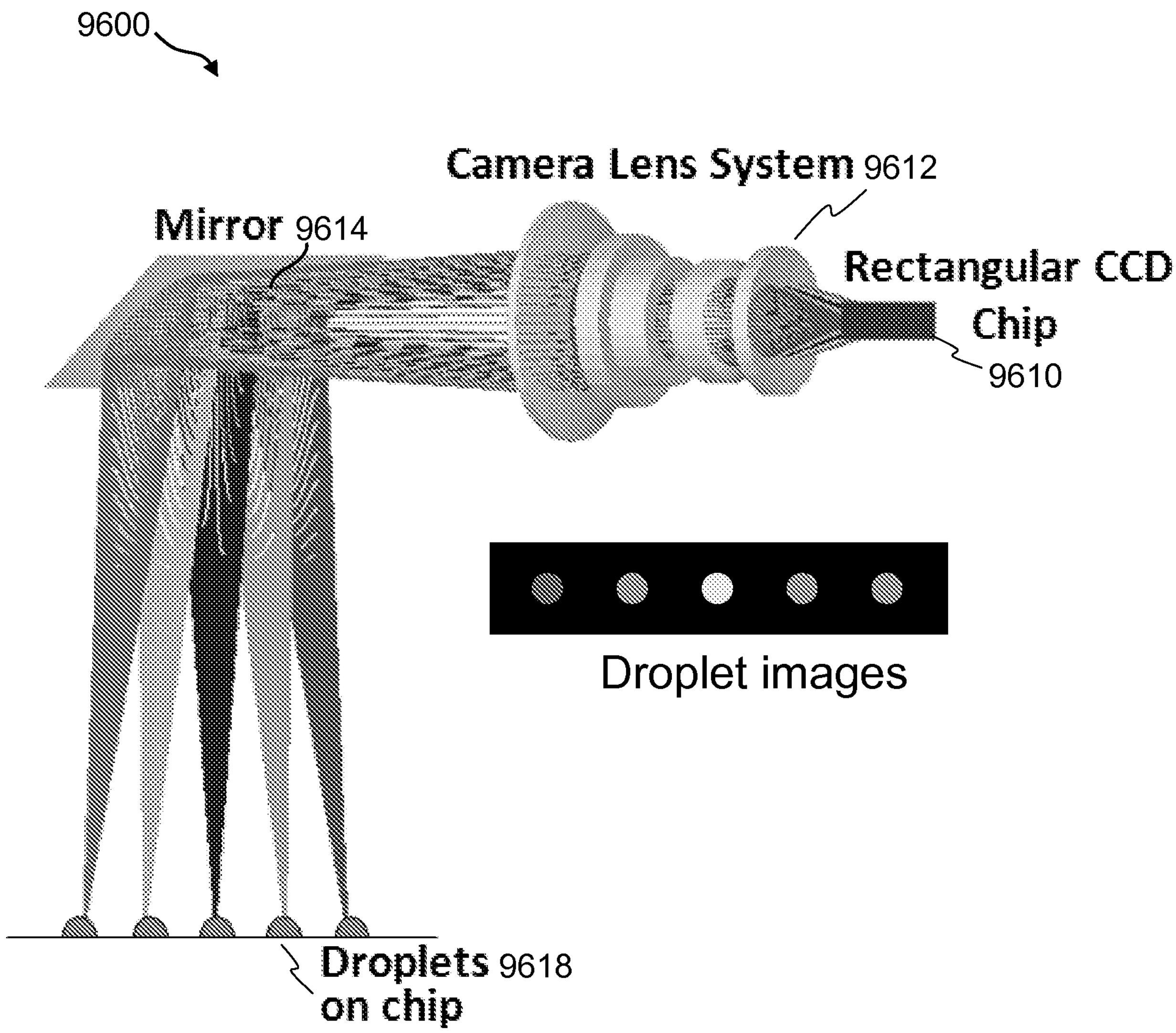
9600
Camera Lens System 9612
Mirror 9614
Rectangular CCD Chip
9610
Droplet images
Droplets 9618
on chip

Figure 96

9700

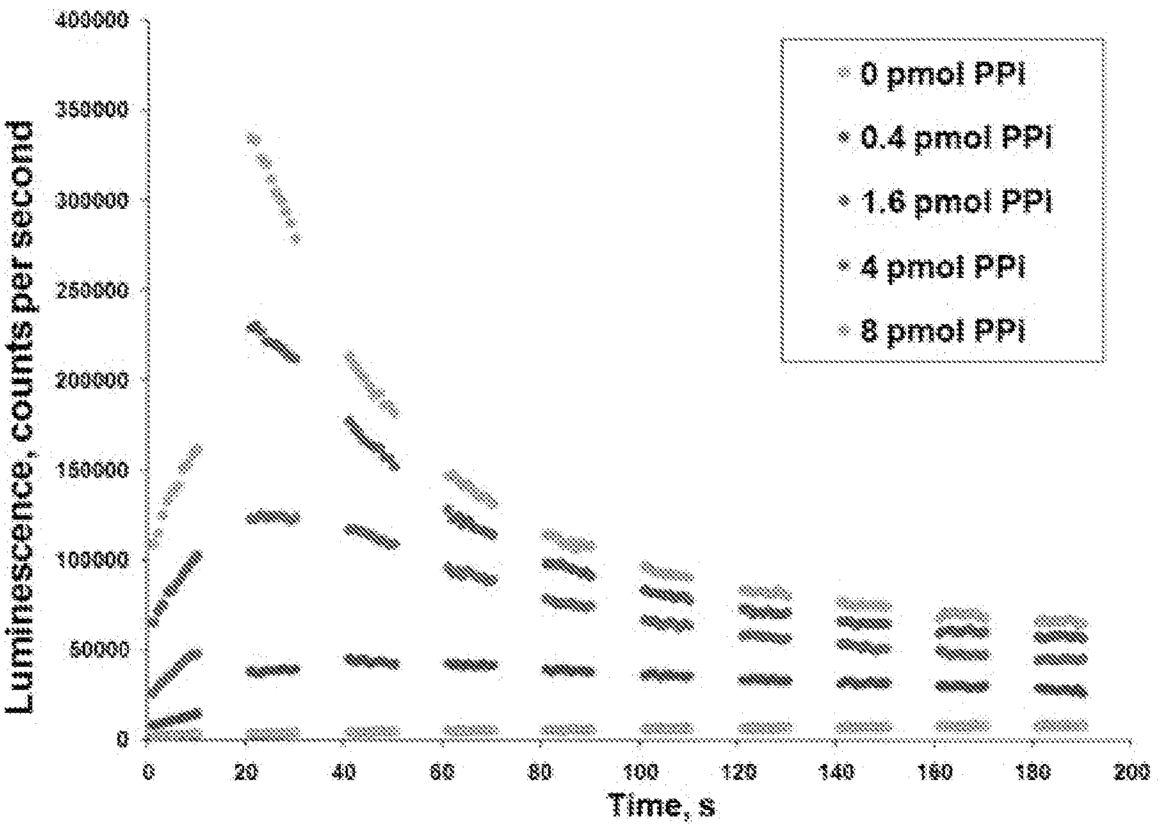

Figure 97