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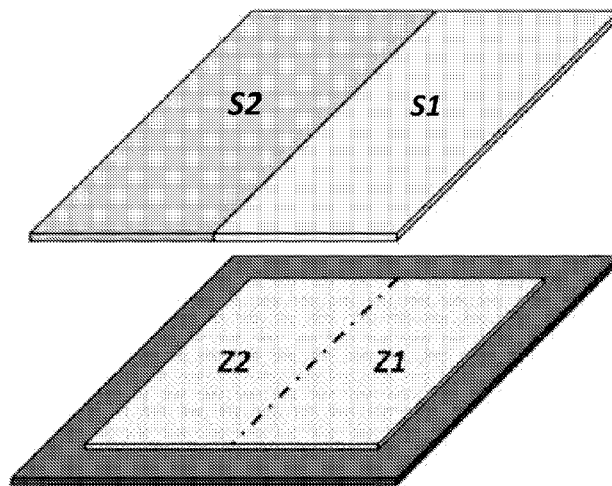


FIG. 7

(57) Abstract: A digital microfluidic device, comprising a bottom plate and a top plate. The bottom plate comprises a bottom electrode array comprising a plurality of digital microfluidic propulsion electrodes. The top plate comprises a segmented top electrode array comprising a plurality of separately voltage addressable top electrode segments. Each top electrode segment and at least two of the propulsion electrodes of the bottom electrode array form a zone within the device. A controller is operatively coupled to the top electrode array and to the bottom electrode array and is configured to provide propulsion voltages between the top plate segment and the bottom plate propulsion electrodes of at least one of the zones. The top plate and the bottom plate are provided in a spaced relationship defining a microfluidic region therebetween.



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SEGMENTED TOP PLATE FOR VARIABLE DRIVING AND SHORT PROTECTION FOR DIGITAL MICROFLUIDICS

RELATED APPLICATIONS

[Para 1] This application claims priority to U.S. Provisional Patent Application No. 63/015,738, filed April 27, 2020, which is incorporated herein by reference. All patents and publications disclosed herein are incorporated by reference in their entireties.

BACKGROUND

[Para 2] Digital microfluidic devices use independent electrodes to propel, split, and join droplets in a confined environment, thereby providing a “lab-on-a-chip.” Digital microfluidic devices are alternatively referred to as electrowetting on dielectric, or “EWoD,” to further differentiate the method from competing microfluidic systems that rely on electrophoretic flow and/or micropumps. A 2012 review of the electrowetting technology was provided by Wheeler in “Digital Microfluidics,” *Annu. Rev. Anal. Chem.* 2012, 5:413-40, which is incorporated herein by reference in its entirety. The technique allows sample preparation, assays, and synthetic chemistry to be performed with tiny quantities of both samples and reagents. In recent years, controlled droplet manipulation in microfluidic cells using electrowetting has become commercially-viable, and there are now products available from large life science companies, such as Oxford Nanopore.

[Para 3] Most of the literature reports on EWoD involve direct driving devices (a.k.a. “segmented” devices), whereby ten to twenty electrodes are directly driven with a controller. While segmented devices are easy to fabricate, the number of electrodes is limited by space and driving constraints. Accordingly, it is very difficult to perform massive parallel assays, reactions, etc., in direct drive devices. In comparison, “active matrix” devices (a.k.a. active matrix EWoD, a.k.a. AM-EWoD) devices can have many thousands, hundreds of thousands or even millions of addressable electrodes. The electrodes are typically switched by thin-film transistors (TFTs) and droplet motion is programmable with controllers so that AM-EWoD arrays can be used as general purpose devices that allow great freedom for controlling multiple droplets and executing simultaneous analytical processes.

[Para 4] Because of the need for higher performance, i.e., higher voltage driving and faster switching, most advanced AM-EWoD devices are constructed from polycrystalline silicon (a.k.a. polysilicon, a.k.a. poly-Si). However, polysilicon fabrication is substantially more

expensive than amorphous silicon fabrication, i.e., the type used in mass-produced active matrix TFTs for the LCD display industry. For example, Sharp Life Sciences depends upon the improved functionality of polysilicon to achieve AM-EWoD devices that include propulsion, sensing, and heating capabilities on a single active matrix. See, e.g., U.S. Patent Nos. 8,419,273, 8,547,111, 8,654,571, 8,828,336, 9,458,543, all of which are incorporated herein by reference in their entireties.

[Para 5] While poly-Si fabrication techniques allow implementation of complex AM-EWoD devices, the costs of poly-Si device production, combined with a global shortage of suitable fabrication facilities, has prevented the AM-EWoD technology from becoming widely available. There is a need for different designs that can take advantage of existing amorphous silicon fabrication capacity. Such devices could be produced at lower cost, and in great quantities, making them ideal for commonplace diagnostic testing, such as immunoassays.

SUMMARY OF INVENTION

[Para 6] The present application addresses the shortcomings of the prior art by providing an alternate architecture for a segmented top plate electrode. In one aspect, there is provided a digital microfluidic device, comprising a bottom plate and a top plate. The bottom plate includes a bottom electrode array comprising a plurality of digital microfluidic propulsion electrodes. A first dielectric layer covering the bottom electrode array. The top plate comprises a segmented top electrode array comprising a plurality of separately voltage addressable top electrode segments, wherein each top electrode segment and at least two of the bottom plate propulsion electrodes form a zone within the device. A second dielectric layer covers the top electrode array. The device also includes a controller operatively coupled to the top electrode array and to the bottom electrode array, wherein the controller is configured to provide propulsion voltages between the top plate segment and the bottom plate propulsion electrodes of at least one of the zones. The top plate and the bottom plate are provided in a spaced relationship defining a microfluidic region therebetween to permit droplet motion within the microfluidic region under application of propulsion voltages between the bottom electrode array and the segmented top electrode array.

[Para 7] In a second aspect, there is provided a method of driving a digital microfluidic system. The system includes: (a) a bottom plate array comprising: a transistor matrix, wherein each transistor of the matrix is operably connected to a gate line, a data line, and a propulsion electrode; a plurality of gate lines, wherein each gate line is operably connected to a gate driver;

a plurality of data lines, wherein each data line is operably connected to a data driver; (b) a segmented top electrode array comprising a plurality of separately voltage addressable top electrode segments, wherein each top electrode segment and at least two of the bottom plate propulsion electrodes form a zone within the device; (c) a plurality of top segment drivers, wherein each driver is configured to separately address a top electrode segment; (d) a controller operably connected to the processing unit, the gate drivers, the data drivers, and the top segment drivers; (e) a processing unit operably programmed to perform a microfluidic driving method. The method includes: receiving input instructions in the processing unit, the input instructions relating to a droplet operation to be performed by the digital microfluidic system; outputting top electrode segment instructions from the processing unit to the controller; outputting a top electrode segment signal from the controller to the driver of a first top electrode segment, to drive the first segment to a first voltage differing from the second voltage of a second top electrode segment; outputting gate line and data line selecting instructions from the processing unit to the controller; outputting a gate line signal from the controller to the driver of a gate line, to drive the gate line, and outputting a data line signal from the controller to the driver of a data line, to drive the data line.

BRIEF DESCRIPTION OF DRAWINGS

[Para 8] FIG. 1 shows a traditional microfluidic device including a common top electrode.

[Para 9] FIG. 2 is a schematic diagram of a TFT architecture for a plurality of propulsion electrodes of an EWoD device.

[Para 10] FIG. 3 is a schematic diagram of a portion of a AM-EWoD array, including a propulsion electrode, a thin film transistor, a storage capacitor, a dielectric layer, and a hydrophobic layer.

[Para 11] FIG. 4 is a schematic illustration of a segmented top electrode array.

[Para 12] FIG. 5 is a schematic illustration of a multi-layered, segmented top electrode array.

[Para 13] FIG. 6 is a schematic illustration of a top plate electrode array fitted with connectors from the fan out on the edge.

[Para 14] FIG. 7 illustrates a top electrode array including two segments, each segment defining a zone within the microfluidic device.

[Para 15] FIG. 8 illustrates exemplary pulse sequences driven between the electrodes of FIG. 7.

[Para 16] FIG. 9 illustrates a configuration including a reservoir zone and a standard actuation zone.

[Para 17] FIG. 10 illustrates an approach for turning off damaged zones in the device of FIG. 7.

[Para 18] FIG. 11 is a flow chart of a method for implementing a droplet operation on a microfluidic device of the present application.

DEFINITIONS

[Para 19] Unless otherwise noted, the following terms have the meanings indicated.

[Para 20] “Actuate” 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 manipulation of the droplet.

[Para 21] “Droplet” means a volume of liquid that electrowets a hydrophobic surface and is at least partially bounded by carrier fluid. For example, a droplet may be completely surrounded by carrier fluid or may be bounded by carrier fluid and one or more surfaces of an EWOD device. Droplets may take a wide variety of shapes; non-limiting 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 working surface of an EWOD device. Droplets may include typical polar fluids such as water, as is the case for aqueous or non-aqueous compositions, or may be mixtures or emulsions including aqueous and non-aqueous components. The specific composition of a droplet is of no particular relevance, provided that it electrowets a hydrophobic working surface. 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 one or more 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, a nucleic acid amplification protocol, an affinity-based assay protocol, an enzymatic assay protocol, a gene sequencing protocol, a protein sequencing protocol, and/or a protocol for analyses of biological fluids. Further example of reagents include those used in biochemical

synthetic methods, such as a reagent for synthesizing oligonucleotides finding applications in molecular biology and medicine, and/or one more nucleic acid molecules. The oligonucleotides may contain natural or chemically modified bases and are most commonly used as antisense oligonucleotides, small interfering therapeutic RNAs (siRNA) and their bioactive conjugates, primers for DNA sequencing and amplification, probes for detecting complementary DNA or RNA via molecular hybridization, tools for the targeted introduction of mutations and restriction sites in the context of technologies for gene editing such as CRISPR-Cas9, and for the synthesis of artificial genes by “synthesizing and stitching together” DNA fragments.

[Para 22] “Droplet operation” means any manipulation of one or more droplets on a microfluidic device. A droplet operation may, for example, include: loading a droplet into the microfluidic device; 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 microfluidic device; 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.

[Para 23] “Gate driver” is a power amplifier that accepts a low-power input from a controller, for instance a microcontroller integrated circuit (IC), and produces a high-current drive input for the gate of a high-power transistor such as a TFT. “Source driver” is a power amplifier producing a high-current drive input for the source of a high-power transistor. “Top segment driver” is a power amplifier producing a drive input for a top plane electrode segment of an EWoD device.

[Para 24] “Nucleic acid molecule” is the overall name for DNA or RNA, either single- or double-stranded, sense or antisense. Such molecules are composed of nucleotides, which are the monomers made of three moieties: a 5-carbon sugar, a phosphate group and a nitrogenous base. If the sugar is a ribosyl, the polymer is RNA (ribonucleic acid); if the sugar is derived from ribose as deoxyribose, the polymer is DNA (deoxyribonucleic acid). Nucleic acid molecules vary in length, ranging from oligonucleotides of about 10 to 25 nucleotides which are commonly used in genetic testing, research, and forensics, to relatively long or very long prokaryotic and eukaryotic genes having sequences in the order of 1,000, 10,000 nucleotides or more. Their nucleotide residues may either be all naturally occurring or at least in part chemically modified, for example to slow down *in vivo* degradation. Modifications may be made to the molecule backbone, e.g. by introducing nucleoside organothiophosphate (PS) nucleotide residues. Another modification that is useful for medical applications of nucleic acid molecules is 2' sugar modifications. Modifying the 2' position sugar is believed to increase the effectiveness of therapeutic oligonucleotides by enhancing their target binding capabilities, specifically in antisense oligonucleotides therapies. Two of the most commonly used modifications are 2'-O-methyl and the 2'-Fluoro.

[Para 25] 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.

[Para 26] When a droplet is described as being “on” or “loaded on” a microfluidic device, it should be understood that the droplet is arranged on the device in a manner which facilitates using the device to conduct one or more droplet operations on the droplet, the droplet is

arranged on the device 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.

[Para 27] "Each," when used in reference to a plurality of items, is intended to identify an individual item in the collection but does not necessarily refer to every item in the collection. Exceptions can occur if explicit disclosure or context clearly dictates otherwise.

DETAILED DESCRIPTION

[Para 28] As indicated above, the present application provides a digital microfluidic device including a segmented top plate and a bottom plate defining a microfluidic region. Traditional devices feature a top plate with a single continuous electrode, allowing for only one potential to be set for the top plate. As a result, propulsion electrodes that require a different voltage than their neighbors can only rely on their respective bottom plate electrode potentials for this purpose. Moreover, a defect encountered in the course of a droplet operation may induce current flows which force the top plate potential to change, thereby affecting all the pixels in the array. In such instances, a microfluidic device and/or a microfluidic operation may be ruined by only a few defects in a bottom array of propulsion electrodes.

[Para 29] As depicted herein, a bottom plate is fitted with an array of elements, where each element includes a digital microfluidic actuation electrode, to propel various droplets through the microfluidic region. The segmented top plate of this aspect of the invention includes a top electrode array partitioned into two or more separately voltage addressable segments. Circuitry coupled to the top electrode segments allows for each segment of the top electrode array to be driven independently from the others. Each segment and a subset of at least two elements of the bottom plate array form a zone including the segment, the propulsion electrodes of the elements in the subset, and the space there between. Typically, each segment forms a respective zone together with the propulsion electrodes of propulsion electrodes (a.k.a., pixels) directly across from it on the bottom plate, thereby including an area of the bottom electrode surface that is symmetrical to the shape of the segment and directly opposite thereto. This novel architecture allows for multiple top plane potentials to be set independently in different zones of the device. Multiple simultaneous voltage ranges may be achieved on neighboring pixels, a functionality beneficial for driving different types of droplets or different types of functional zones, for instance reservoirs vs. main array pixels. Moreover, defects formed during operation of the device may be isolated by switching a particular zone off, thereby containing the damage and allowing the remainder of the device to continue operating. In instances where top plane

switching is used, non-active parts of device may be switched completely off to minimize wear and preserve device performance.

[Para 30] The number of top electrode segments in a device can vary to provide the number of zones required by the application at hand, and the characteristics of each zone may be tailored to suit particular droplet formulations and operating voltages. A device may feature any from just two to ten or more segmented top electrode zones, for example to accommodate instances where several reactants are introduced as separate droplets from a number of reservoirs on the plate periphery. Similarly, the number and density of bottom plate propulsion electrodes in a given zone can fall within several different ranges, depending on factors such as the area of the zone and its electrode density, which may be constant or differing at various locations on the bottom plate. For example, a zone having an area of 1 in² and a bottom plate electrode density resolution of 100 PPI will encompass 10,000 propulsion electrodes. The same area at a higher resolution, for example 200 PPI or more, will result in a zone having 40,000 or more propulsion electrodes.

[Para 31] The use of “top” and “bottom” is merely a convention as the locations of the two plates can be switched, and the devices can be oriented in a variety of ways, for example, the top and bottom plates can be roughly parallel while the overall device is oriented so that the plates are normal to a work surface (as opposed to parallel to the work surface as shown in the figures). The top or the bottom plates may include additional functionalities, such as heating by commercial micro-heaters and thermocouples that are integrated with the microfluidic platform and/or temperature sensing.

[Para 32] In a representative embodiment, the bottom plate of the device includes an active matrix electrowetting on dielectric (AM-EWoD) device featuring a plurality of array elements, each array element including a propulsion electrode, although other configurations for driving the bottom plate electrodes are also contemplated. The AM-EWoD matrix may be in the form of a transistor active matrix backplane, for example a thin film transistor (TFT) backplane where each propulsion electrode is operably attached to a transistor and capacitor actively maintaining the electrode state while the electrodes of other array elements are being addressed. Top electrode circuitry independently drives each of the segments of the top plate.

[Para 33] A propulsion voltage is defined by a voltage difference between an array electrode and the top electrode segment directly opposed to it across the microfluidic region. By adjusting the frequency and amplitude of the signals driving the array electrodes and top electrode segments, the propulsion voltage of each pixel of the array may be controlled to

operate the AM-EWoD device at different modes of operation in accordance with different droplet manipulation operations to be performed. In one embodiment, the TFT array may be implemented with amorphous silicon (a-Si), thereby reducing the cost of production to the point that the devices can be disposable.

[Para 34] The fundamental operation of a traditional EWoD device is illustrated in the sectional image of FIG. 1. The EWoD **100** includes microfluidic region filled with an oil **102** and at least one aqueous droplet **104**. The microfluidic region gap depends on the size of droplets to be handled and is typically in the range 50 to 200 μm , but the gap can be larger. In a basic configuration, as shown in FIG. 1, a plurality of propulsion electrodes **105** are disposed on one substrate and a singular top electrode **106** is disposed on the opposing surface. The device additionally includes hydrophobic coatings **107** on the top and bottom surfaces, i.e., where they contact the oil layer, in addition to a dielectric layer **108** between the propulsion electrodes **105** and the hydrophobic coating **107**. (The upper substrate may also include a dielectric layer, but it is not shown in FIG. 1). The hydrophobic layer prevents the aqueous droplet from wetting the surface. When no voltage differential is applied between adjacent electrodes, the droplet will maintain a spheroidal shape to minimize contact with the hydrophobic surfaces (oil and hydrophobic layer). Because the droplets do not wet the surface, they are less likely to contaminate the surface or interact with other droplets except when that behavior is desired.

[Para 35] While it is possible to have a single layer for both the dielectric and hydrophobic functions, such layers typically require thick inorganic layers (to prevent pinholes) with resulting low dielectric constants, thereby requiring more than 100V for droplet movement. To achieve low voltage propulsion, it is often better to have a thin inorganic layer for high capacitance and to be pinhole free, topped by a thin organic hydrophobic layer. With this combination it is possible to have electrowetting operation with voltages in the range +/-10 to +/-50V, which is in the range that can be supplied by conventional TFT arrays built upon amorphous silicon.

[Para 36] Hydrophobic layers may be manufactured from hydrophobic materials formed into coatings by deposition onto a surface via suitable techniques. Depending on the hydrophobic material to be applied, example deposition techniques include spin coating, molecular vapor deposition, and chemical vapor deposition. Hydrophobic layers may be more or less wettable as usually defined by their respective contact angles. Unless otherwise specified, angles are herein measured in degrees ($^{\circ}$) or radians (rad), according to context. For the purpose of measuring the hydrophobicity of a surface, the term “contact angle” is understood to refer to

the contact angle of the surface in relation to deionized (DI) water. If water has a contact angle between $0^\circ < \theta < 90^\circ$, then the surface is classed as hydrophilic, whereas a surface producing a contact angle between $90^\circ < \theta < 180^\circ$ is considered hydrophobic. Usually, moderate contact angles are considered to fall in the range from about 90° to about 120° , while high contact angles are typically considered to fall in the range from about 120° to about 150° . In instances where the contact angle is $150^\circ < \theta$ then the surface is commonly known as superhydrophobic or ultrahydrophobic. Surface wettabilities may be measured by analytical methods well known in the art, for instance by dispensing a droplet on the surface and performing contact angle measurements using a contact angle goniometer. Anisotropic hydrophobicity may be examined by tilting substrates with gradient surface wettability along the transverse axis of the pattern and examining the minimal tilting angle that can move a droplet.

[Para 37] Hydrophobic layers of moderate contact angle typically include one or a blend of fluoropolymers, such as PTFE (polytetrafluoroethylene), FEP (fluorinated ethylene propylene), PVF (polyvinylfluoride), PVDF (polyvinylidene fluoride), PCTFE (polychlorotrifluoroethylene), PFA (perfluoroalkoxy polymer), FEP (fluorinated ethylenepropylene), ETFE (polyethylenetetrafluoroethylene), and ECTFE (polyethylenechlorotrifluoroethylene). Commercially available fluoropolymers include Cytop® (AGC Chemicals, Exton, PA) and Teflon® AF (Chemours, Wilmington, DE). An advantage of fluoropolymer films is that they can be highly inert and can remain hydrophobic even after exposure to oxidizing treatments such as corona treatment and plasma oxidation.

[Para 38] When a voltage differential is applied between adjacent electrodes, the voltage on one electrode attracts opposite charges in the droplet at the dielectric-to-droplet interface, and the droplet moves toward this electrode, also as illustrated in FIG. 1. The voltages needed for acceptable droplet propulsion depend on the properties of the dielectric and hydrophobic layers. AC driving is used to reduce degradation of the droplets, dielectrics, and electrodes by various electrochemistries. Operational frequencies for EWOD can be in the range 100 Hz to 1 MHz, but lower frequencies of 1 kHz or lower are preferred for use with TFTs that have limited speed of operation.

[Para 39] Returning to FIG. 1, the top electrode **106** is a single conducting layer normally set to zero volts or a common voltage value (VCOM) to take into account offset voltages on the propulsion electrodes **105** due to capacitive kickback from the TFTs that are used to switch the voltage on the electrodes (see FIG. 3). The top electrode can also have a square wave applied to increase the voltage across the liquid. Such an arrangement allows lower propulsion voltages to be used for the TFT connected propulsion electrodes **105** because the top plate voltage **106**

is additional to the voltage supplied by the TFT. In many instances, the top electrode is constructed from a light-transmissive material, such as indium tin oxide (ITO) sputtered on a film of polyethylene terephthalate (PET), however, the top electrode does not have to be light-transmissive for most electrowetting on dielectric applications. Other simple conductive films, such as metal films, may be used, provided that they are sufficiently protected from the materials of the droplet layer, e.g., the oil and the aqueous droplet.

[Para 40] As illustrated in FIG. 2, an active matrix of propulsion electrodes can be arranged to be driven with data and gate (select) lines much like an active matrix in a liquid crystal display. The gate (select) lines are scanned for line-at-a time addressing, while the data lines carry the voltage to be transferred to propulsion electrodes for electrowetting operation. If no movement is needed, or if a droplet is meant to move away from a propulsion electrode, then 0V will be applied to that (non-target) propulsion electrode. If a droplet is meant to move toward a propulsion electrode, an AC voltage will be applied to that (target) propulsion electrode.

[Para 41] The architecture of an amorphous silicon, TFT-switched, propulsion electrode is shown in FIG. 3. The dielectric **308** must be thin enough and have a dielectric constant compatible with low voltage AC driving, such as available from conventional image controllers for LCD displays. For example, the dielectric layer may comprise a layer of approximately 20-40 nm SiO₂ over-coated with 200-400 nm plasma-deposited silicon nitride. Alternatively, the dielectric may comprise atomic-layer-deposited Al₂O₃ between 2 and 100 nm thick, preferably between 20 and 60 nm thick. The TFT may be constructed by creating alternating layers of differently-doped a-Si structures along with various electrode lines, with methods known to those of skill in the art. The hydrophobic layer **307** can be constructed from materials such as Teflon® AF (Sigma-Aldrich, Milwaukee, WI) and FlurorPel™ coatings from Cytonix (Beltsville, MD), which can be spin coated over the dielectric layer **308**.

[Para 42] As anticipated above, the novel device of this aspect of the invention features a top electrode array partitioned into a plurality of elements, where each element includes a voltage addressable segment. FIG. 4 is a schematic illustration of a segmented top electrode array **400** which may be manufactured from a transparent conducting material partitioned into a plurality of strips **402**. (Again, the conducting material need not be transparent for electrowetting applications. It does make it easier to assess the progress of the various processes taking place on the device, however.) Each strip is separate voltage addressable and is connected to the bottom plane by circuitry contacting the strip at contact point **404**. A small gap spacing is interposed between adjacent electrode segments to insulate the segments from each other. To

avoid discontinuities where propulsion electrodes might lack a corresponding top plane electrode segment located directly overhead, the gap spacing is preferably smaller than the electrode size on the bottom plate array. Optionally, the gap spacing may be lined with an electrically insulator substance, the sidewalls of the strips may be covered with an insulator coating, or both.

[Para 43] The top electrode array design may rely on standard transparent conducting materials that are well-known in the art, such as indium-doped tin oxide (ITO), fluorine-doped zinc oxide or titanium dioxide to achieve a segmented top electrode layer. It is possible to extend the design to two or more layers, for example by combining the segmented electrode layer with a trace layer. In the representative embodiment schematically illustrated in FIG. 5, **L1** depicts traces laid on a first insulating substrate, followed by intermediate insulating layer **L2**, and via connections made to segmented top electrode layer **L3**. The materials of **L3** may be chosen from among the standard transparent conducting materials listed above, and typically come into contact with droplets in the microfluidic region between the top and bottom plates. Typically, the bottom surface of **L3** is coated with a hydrophobic layer to prevent direct contact between the electrodes and liquids or gases in the microfluidic region.

[Para 44] Layer **L2** may be an insulator material, produced by a vacuum deposition technique, of which there are several types including physical vapor deposition (PVD) and chemical vapor deposition (CVD) in one of its variants: low-pressure chemical vapor deposition (LPCVD), plasma-enhanced chemical vapor deposition (PECVD), and plasma-assisted CVD (PACVD). In several instances, a combination of PVD and CVD are used in the same or connected processing chambers. In one exemplary embodiment, the insulator material is parylene, the name for a variety of chemical vapor deposited poly(p-xylylene) polymers used as moisture and dielectric barriers. Among them, Parylene C, bearing one chlorine atom substituent to the phenyl moiety per repeat polymeric unit, is the most popular due to its combination of barrier properties, cost, and other processing advantages. Parylene N and C have a low degree crystallinity and are therefore usually suitable as optical materials. Other optically transparent or at least partially transparent polymeric and ceramic insulators may also be used.

[Para 45] In digital microfluidic devices with sensors built into the bottom plate array, for example as disclosed in U.S. Patent No. 8,828,336 to Hadwen *et al.*, such that requirements on optical detection are relaxed, more conventional materials may be used for **L1**, for example glass-reinforced epoxy laminate materials such as FR-4, other epoxy-based materials, and semiconductors. For **L2**, non-transparent polymers and ceramics may be used, and **L3** may be

an electrode grid composed of oxidation-resistant metals and alloys, for instance gold, platinum, or nickel-chrome (NiCr), or highly doped semiconductor materials.

[Para 46] Circuitry for connecting and/or controlling the voltages of the top plate segments may be housed in the top plate itself, in the bottom plate, for example on the edges of the electrode array, or elsewhere in the device depending on the needs and constraints of the application at hand. In one embodiment, the connections to the top electrode array are housed in the top plate with a separate connector. In another embodiment, the device is fitted with a connection interface with the bottom electrode array, for example connectors from the fan out on the edge, as illustrated in FIG. 6. In this instance, filled vias are made in the top electrode segments and are fanned out to a location where a connection can be made. For more complex designs, the top plate electrode may comprise two or more layers, thereby allowing for various geometries such as square, hexagonal, or triangular formed with top electrode segment arrays that can be independently addressed.

[Para 47] In the basic design schematically illustrated in FIG. 7, the top electrode array is composed of two segments labeled **S1** and **S2**, respectively. Segment **S1** overlays the bottom layer pixels of zone **Z1** and is driven at a voltage $VS1$, while segment **S2** is above those of zone **Z2** and is driven at a voltage $VS2$. One or more of the bottom plate electrodes in zone **Z1** are driven at a voltage $V1$ while one or more of the bottom plate electrodes in zone **Z2** are driven at a voltage $V2$. The voltages may be measured with respect to the common ground of the bottom plate array. As illustrated in the pulse sequences of FIG. 8, the electrodes of **Z1** may be driven at an AC potential $V1 = VS1 - VZ1$ between lower threshold voltage $-V3$ to upper limit $+V3$ at a frequency of 20 Hz, whereas zone **Z2** electrodes may be driven at AC potential $V2 = VS2 - VZ2$ between lower and upper threshold voltages $-V4$ to $+V4$. In operation, zones **Z1** and **Z2** may have differing driving requirements, either in polarity, amplitude, or frequency, for example to achieve differing electrowetting forces in separate parts of the bottom plate. Accordingly, each of $VS1$, $VS2$, $VZ1$, and $VZ2$ may be adjusted independently to suit such requirements, and each may be a DC potential or a time-varying AC potential. It is also possible to ground **S1** and **Z1** to 0V, thus requiring no driving of these electrodes, whereas in a traditional device having a single top electrode, if the pixels of **Z2** were to be active, a voltage would need to be applied to the pixels of **Z1** to match the voltage of the top plate in order to keep the overall voltage difference equal to 0V for the electrodes of **Z1**. In some embodiments, the controller is configured to “turn off” a zone by taking the top and bottom electrodes to 0V to prevent further damage to this area or to assure that droplets undergoing processes on other areas of the device are not routed into this area. For example, if there are a number of failed

propulsion electrodes in the zone beyond a predetermined amount, e.g., greater than 1%, e.g., greater than 2%, e.g., greater than 5%, e.g., greater than 10%, the controller will turn off that zone.

[Para 48] It has been known in the art that relatively thin dielectrics subjected to repeated low voltage actuation have a tendency to be more susceptible to failure than thicker dielectrics at higher voltages. (Sensors & Actuators B: 2012 October, 173: 338-345). The present application addresses this problem with the configuration schematically illustrated in FIG. 9, where **Z1** is a “reservoir zone” including reservoirs R1 and R2 while **Z2** includes standard actuation electrodes. This configuration may include a TFT backplane for the **Z2** electrodes, and either the same TFT backplane for the **Z1** electrodes or customized direct drive electrodes. The top plate is segmented such that a first voltage V1 may be applied to opposite to zone **Z1** and a second voltage V2 to zone **Z2**, independently.

[Para 49] In one example embodiment of this aspect, **Z1** is made operate at higher voltages than **Z2**. To this end, dielectrics having different thicknesses may be deposited on different zones of the device surfaces, thereby covering **Z1** with a thicker dielectric layer capable of withstanding higher voltages. By employing a combination of thicker dielectric with higher voltages, this design is able to greatly reduce the strain on dispensing zones that undergo many dispensing cycles, thus reducing their likelihood of failure. This approach may be further extended to allow for any number of zones, each zone having its specific voltage and dielectric thickness depending on whether a given zone requires more or fewer actuation cycles. In addition, TFT arrays are usually best suited to operate at low voltage ranges, so the design of this embodiment allows to restrict high voltage operation to specific zones, thereby minimizing wear on the rest of an array.

[Para 50] When in operation, the dielectric stack of the bottom plate may experience a catastrophic failure that may impact the voltage of the top electrode in instances where the defect creates an electrical connection between the top and bottom plates. In devices featuring a segmented top plate, if the impacted zone is no longer in operating condition, it may be turned off, and will not affect the functioning of other zones free of defects. This approach is schematically illustrated in FIG. 10, where the top plate segment **S1** is turned off, leaving **Z2** unaffected by the damage. In one embodiment, if the area of the bottom electrode array is labeled as “A”, and the defect density is denoted as “x”, the number of zones needed to guarantee at least one operable zone is equal to $Ax+1$. Therefore, the bottom plate electrode array may be subdivided into zones of area $A/(Ax+1)$, assuming the defects force an entire zone to be inoperable. In a representative embodiment, the defect density threshold for turning off

a zone may be set, for example, at about 25 defects per cm^2 , which translates to approximately 1 defect per 10×10 pixel droplet.

[Para 51] It is also possible to establish a metric “F” which represents the operable area fraction of the array area A. In this instance, the required number of segments will be: $x/(1-F)*A$, where x is the defect density as set out above. For example, if the defect density is equal to 2 defects per cm^2 , the number of segments required for 50% guaranteed operation on a 20 cm^2 electrode array is equal to 80 in the worst case scenario. However, depending on the statistical clustering of defects, this number might be lower.

[Para 52] Electrode driving sequences for specific top plate and bottom plate electrodes can be calculated and implemented based on the requirements of a droplet operation to be performed on the EWoD device. The flow chart of FIG. 11 illustrates an example embodiment of this process. In step **110**, a user inputs a desired droplet operation in the form of instructions which are stored in a computer-readable medium that is accessed by a processing unit of the device. The processing unit is programmed to perform driving sequences featuring multiple top electrode potentials. The instructions cause the processing unit to calculate the polarity, frequency, and amplitude of each pulse applied to the top plate segments and bottom plate propulsion electrodes which are to take part in the droplet operation (**112**). Then, the processing unit outputs instructions to the controller (**114**), and the controller outputs signals to the drivers of the top plate electrode segments and bottom plate propulsion electrodes (**116**). In instances where the bottom plate includes an array of TFT electrodes, the controller outputs gate line signals to the drivers of gate lines and data line signals to data line drivers, thereby driving the intended propulsion electrodes. The selected top electrode segments and bottom plate propulsion electrodes are then driven to perform the droplet operation (**118**).

[Para 53] In an exemplary driving sequence, a first top electrode segment is driven to a first voltage, and a second top electrode segment is driven to a second voltage differing from the first voltage in at least one of polarity, frequency, and amplitude. However, the top plate may include more than two segments, and each segment may be set at a different potential to suit the application at hand. The controller functionality may be provided by a first sub-controller for addressing the top electrode segments and a second sub-controller deputed to driving the bottom plate propulsion electrodes.

[Para 54] From the foregoing, it will be seen that the present invention allows for different voltages to be applied independently across the device, which in turn enables fully turning off pixel zones during top plane operation, thereby reducing strain on driving circuitry. The invention also protects against full failure on potentially very expensive microfluidic cartridges

by turning off defective zones. The presence of independent voltage zones increases the functional versatility of digital microfluidic devices, and reduced driving requirements for zones kept in the “off” state, which can potentially comprise large portions of the device, may reduce failure rates. As an added benefit, the present invention makes it possible to mediate dielectric stack density by having the ability to turn off defective zones while not affecting the remainder of the array.

[Para 55] It will be apparent to those skilled in the art that numerous changes and modifications can be made in the specific embodiments of the invention described above without departing from the scope of the invention. Accordingly, the whole of the foregoing description is to be interpreted in an illustrative and not in a limitative sense.

CLAIMS

1. A digital microfluidic device, comprising:
 - (a) a bottom plate comprising:
 - a bottom electrode array comprising a plurality of digital microfluidic propulsion electrodes; and
 - a first dielectric layer covering the bottom electrode array;
 - (b) a top plate comprising:
 - a segmented top electrode array comprising a plurality of separately voltage addressable top electrode segments,
 - wherein each top electrode segment and at least two of the bottom plate propulsion electrodes form a zone within the device;
 - a second dielectric layer covering the top electrode array, and
 - (c) a controller operatively coupled to the top electrode array and to the bottom electrode array, wherein the controller is configured to provide propulsion voltages between the separately voltage addressable top electrode segments and the plurality of digital microfluidic propulsion electrodes in at least one of the zones;
 - wherein the top plate and the bottom plate are provided in a spaced relationship defining a microfluidic region there between to permit droplet motion within the microfluidic region under application of propulsion voltages between the separately voltage addressable top electrode segments and the plurality of digital microfluidic propulsion electrodes.
2. The digital microfluidic device of claim 1, wherein the segmented top electrode array includes from at least three to at most ten top separately voltage addressable electrode segments.
3. The digital microfluidic device of claim 1, wherein the device is configured to turn off a zone in the event that the zone becomes inoperable.
4. The digital microfluidic device of claim 3, wherein the device is further configured to turn off the at least one of the zones when the zone reaches a defect density in the bottom electrode array exceeding a predetermined threshold.

5. The digital microfluidic device of claim 1, wherein circuitry for connecting the top electrode array to the bottom electrode array comprises a connection interface.

6. The digital microfluidic device of claim 1, wherein the top plate further comprises a trace layer adjacent the segmented top electrode array, and an insulating layer between the trace layer and the segmented top electrode array, wherein traces on the trace layer are connected to segments of the segmented top electrode array through vias in the insulating layer.

7. The digital microfluidic device of claim 1, wherein the bottom plate further comprises a transistor active matrix backplane, with each transistor of the backplane being operably connected to a gate driver, a data line driver, and one of the digital microfluidic propulsion electrodes.

8. The digital microfluidic device of claim 7, wherein the transistors of the active matrix backplane are thin film transistors (TFT).

9. The digital microfluidic device of claim 1, wherein the bottom plate further comprises a reservoir area and the segmented top electrode array comprises a reservoir segment, wherein the reservoir segment and reservoir area are provided in a spaced relationship defining a reservoir zone.

10. The digital microfluidic device of claim 9, wherein the first dielectric layer comprises a first portion and a second portion, the first portion having a first thickness and covering a portion of the plurality of digital microfluidic propulsion electrodes, the second portion having a second thickness and covering the reservoir area, wherein the second thickness is greater than the first thickness.

11. The digital microfluidic device of claim 1, wherein the controller includes a first sub-controller operably connected to the segmented top electrode array, and a second sub-controller operably connected to the bottom electrode array.

12. The digital microfluidic device of claim 1, wherein each zone comprises at least 10 digital microfluidic propulsion electrodes.

13. A method of driving a digital microfluidic system, the system comprising:
- (a) a bottom plate array comprising:
 - a transistor matrix, wherein each transistor of the matrix is operably connected to a gate line, a data line, and a separate propulsion electrode;
 - a plurality of gate lines, wherein each gate line is operably connected to a gate driver; and
 - a plurality of data lines, wherein each data line is operably connected to a data driver;
 - (b) a segmented top electrode array comprising a plurality of separately voltage addressable top electrode segments,
 - wherein each separately voltage addressable top electrode and at least two of the propulsion electrodes form a zone within the device;
 - (c) a plurality of top electrode segment drivers, wherein each driver is configured to separately address a top electrode segment;
 - (d) a controller operably connected to the gate driver, the data driver, and the top segment drivers;
 - (e) a processing unit operably connected to the controller and programmed to perform a microfluidic driving method, the method comprising:
 - receiving input instructions in the processing unit, the input instructions relating to a droplet operation to be performed by the digital microfluidic system;
 - outputting top electrode segment instructions from the processing unit to the controller;
 - outputting a top electrode segment signal from the controller to the driver of a first top electrode segment, to drive the first segment to a first voltage differing from the second voltage of a second top electrode segment;
 - outputting gate line and data line selecting instructions from the processing unit to the controller;
 - outputting a gate line signal from the controller to the gate driver, to drive the gate line, and
 - outputting a data line signal from the controller to the data driver, to drive the data line.

14. The method of driving a microfluidic system according to claim 13, wherein the top electrode array includes from at least three to at most ten separately voltage addressable top electrode segments.

15. The method of driving a microfluidic system according to claim 13, wherein a first voltage of a first top electrode segment differs from a second voltage of a second top electrode segment in polarity, frequency, or amplitude.

16. The method of driving a microfluidic system according to claim 13, wherein the second top electrode segment is grounded to a voltage of 0 V.

17. The method of driving a microfluidic system according to claim 13, further comprising turning off a second top electrode segment in the event that a second zone becomes inoperable.

18. The method of driving a microfluidic system according to claim 17, wherein the processing unit is further programmed to turn off the second top electrode segment in the event that a portion of the bottom plate array in the second zone reaches a defect density exceeding a predetermined threshold.

19. The method of driving a microfluidic system according to claim 13, wherein the transistors of the matrix are thin film transistors.

20. The method of driving a microfluidic system according to claim 13, wherein the droplet operation comprises moving a droplet including nucleic acid molecules.

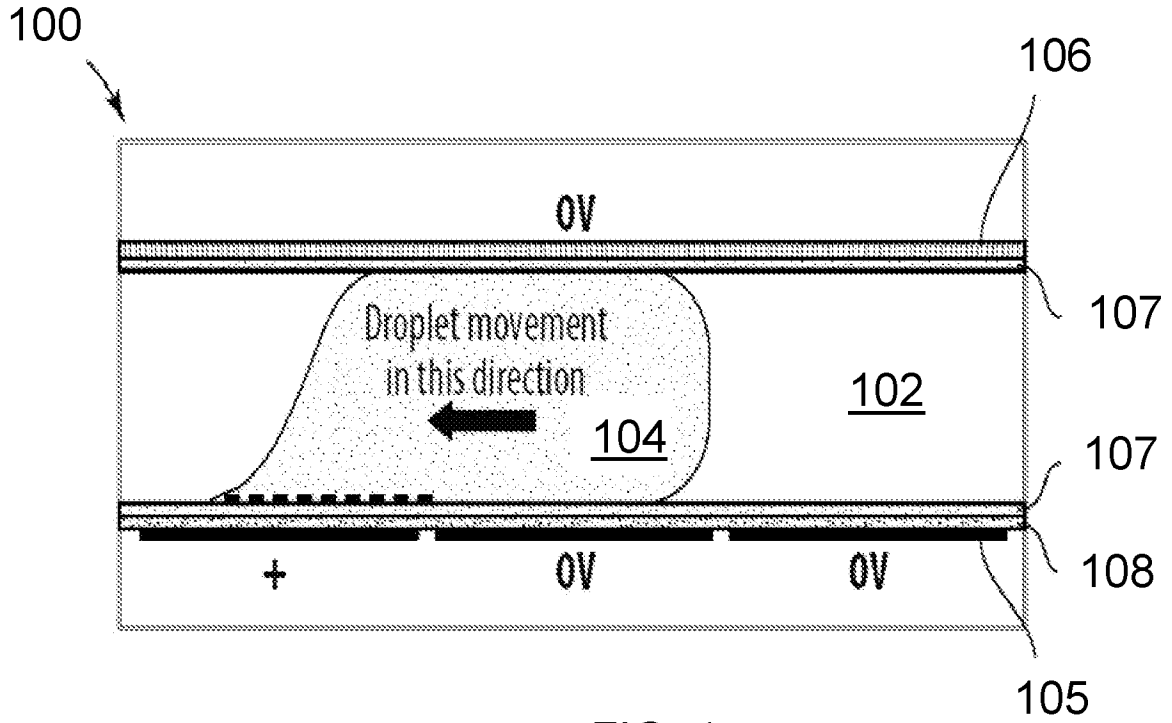


FIG. 1

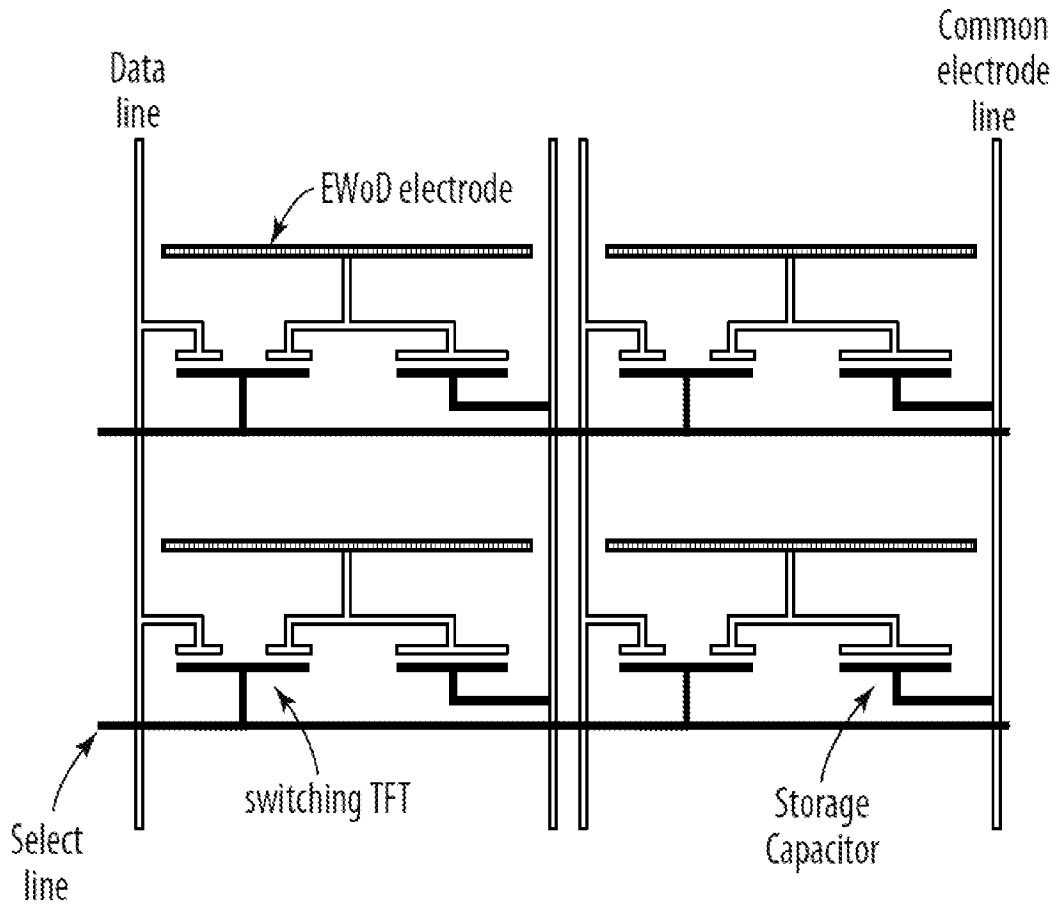


FIG. 2

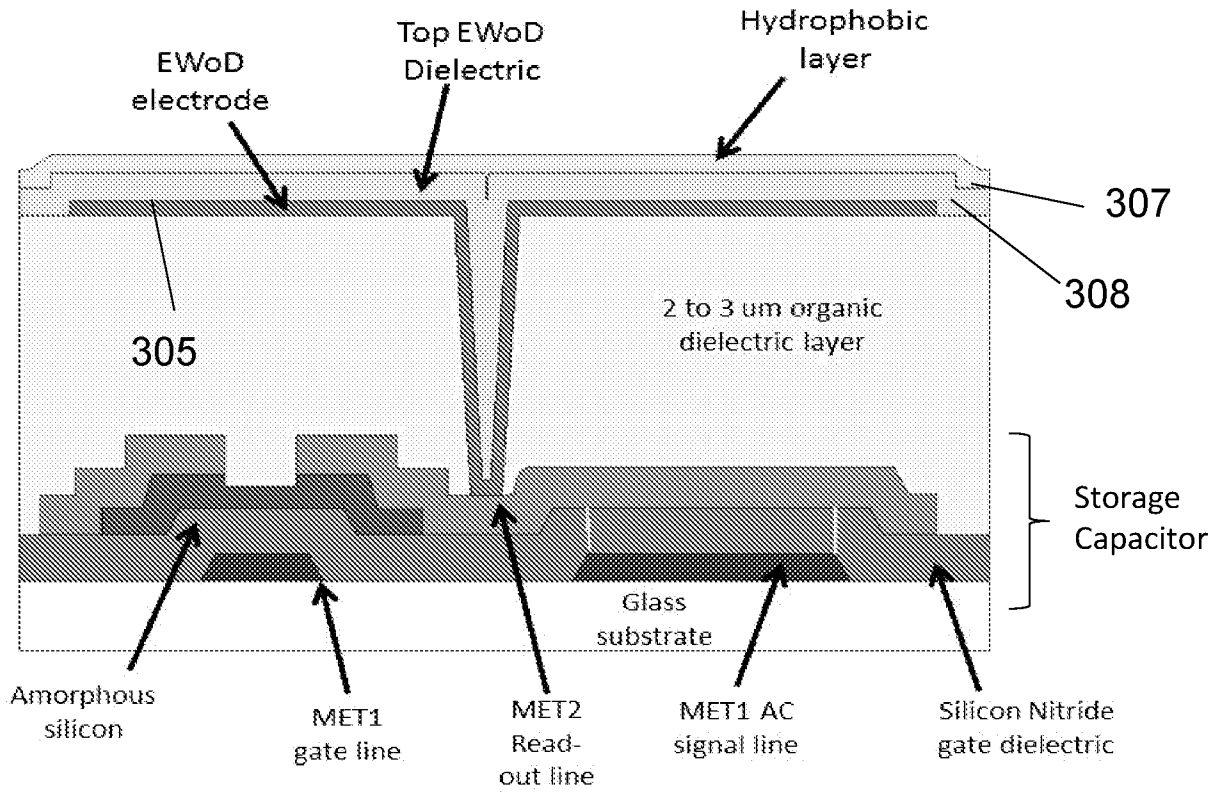


FIG. 3

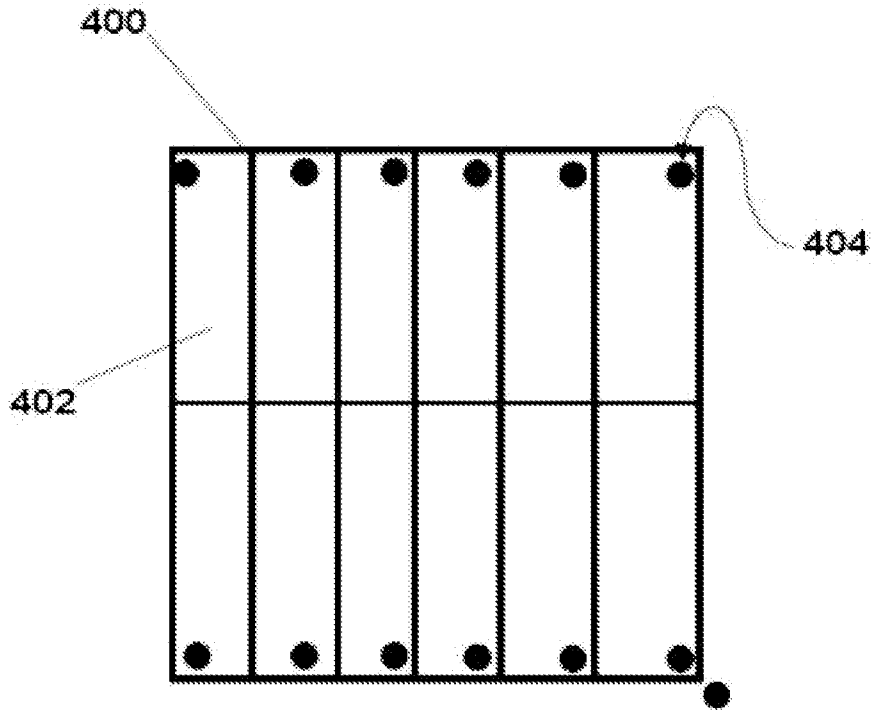


FIG. 4

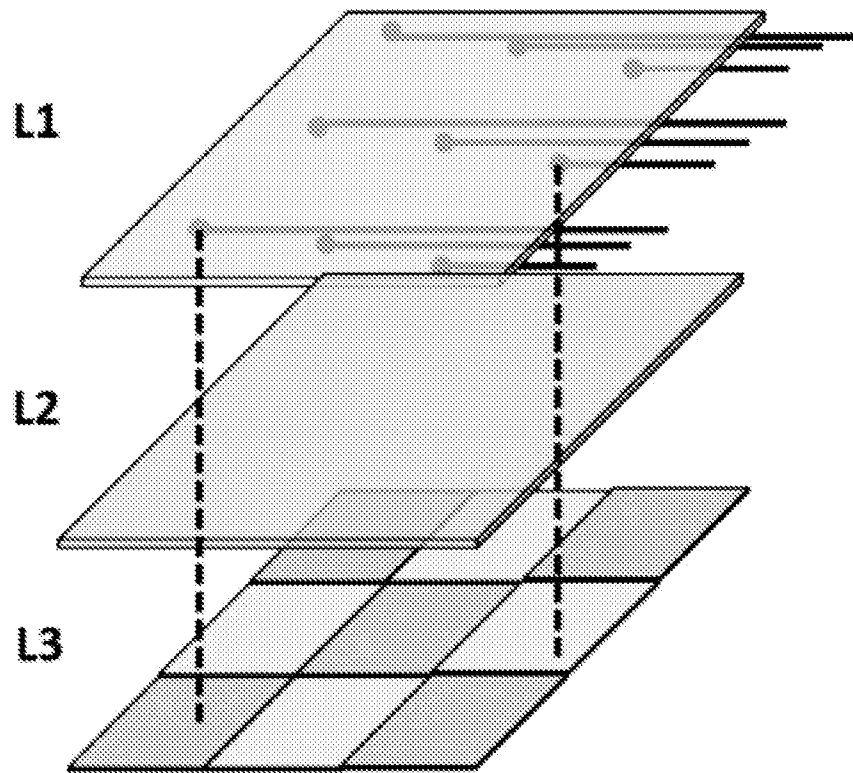


FIG. 5

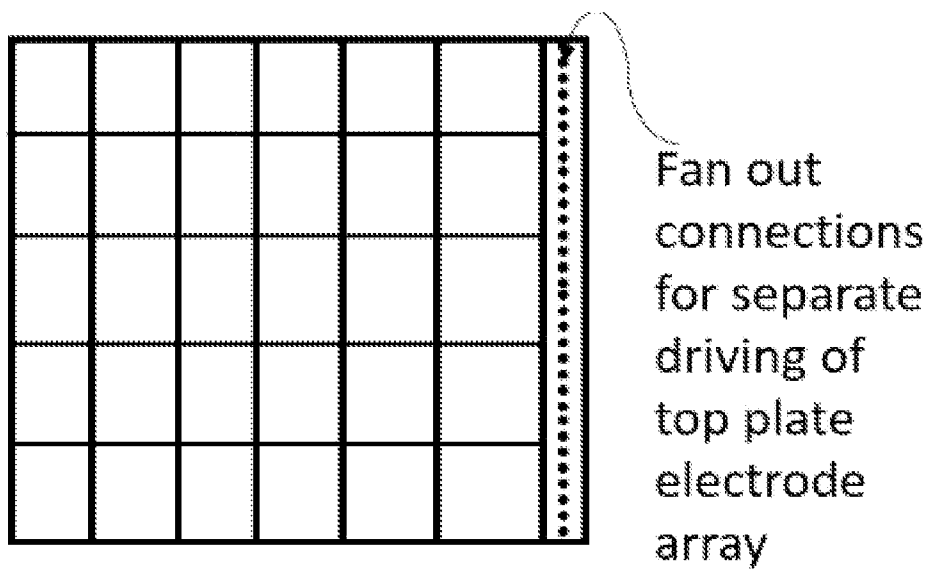


FIG. 6

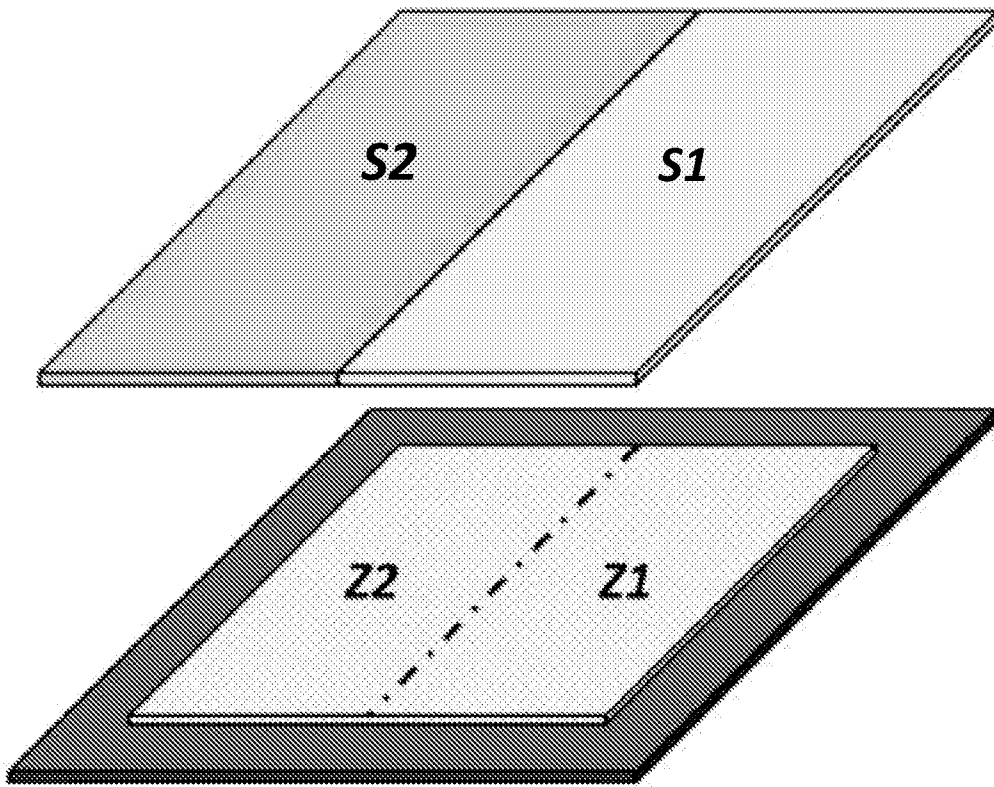


FIG. 7

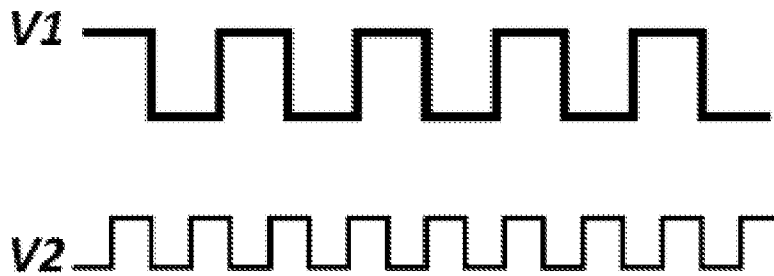


FIG. 8

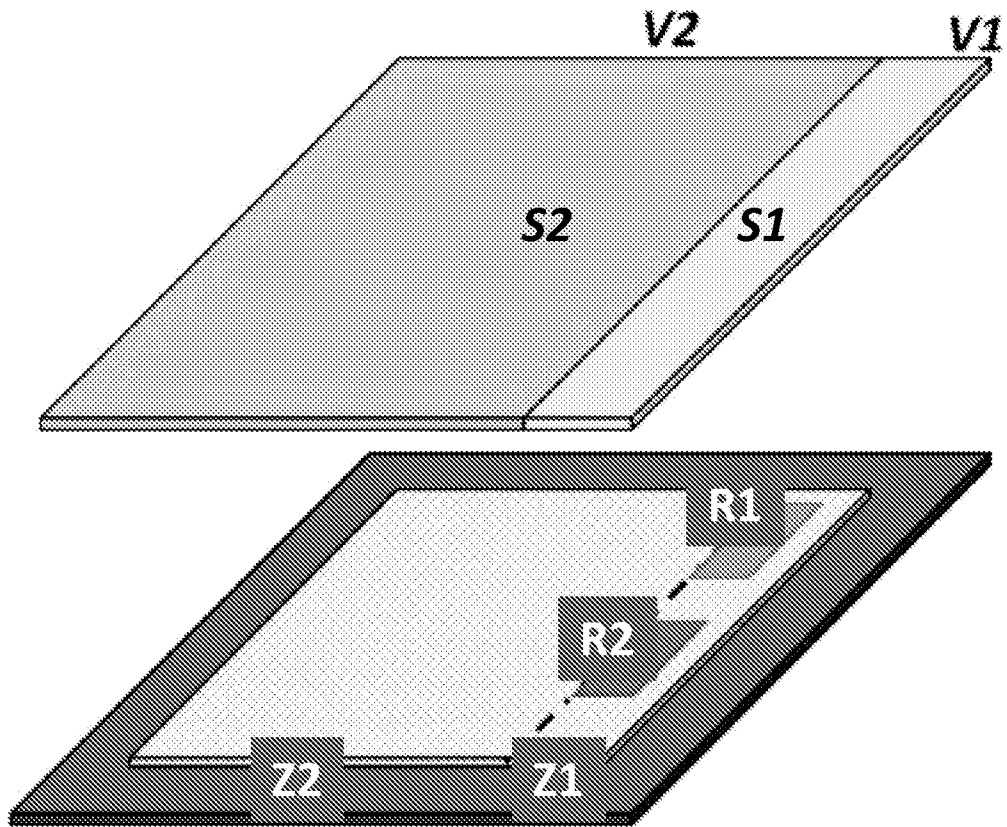


FIG. 9

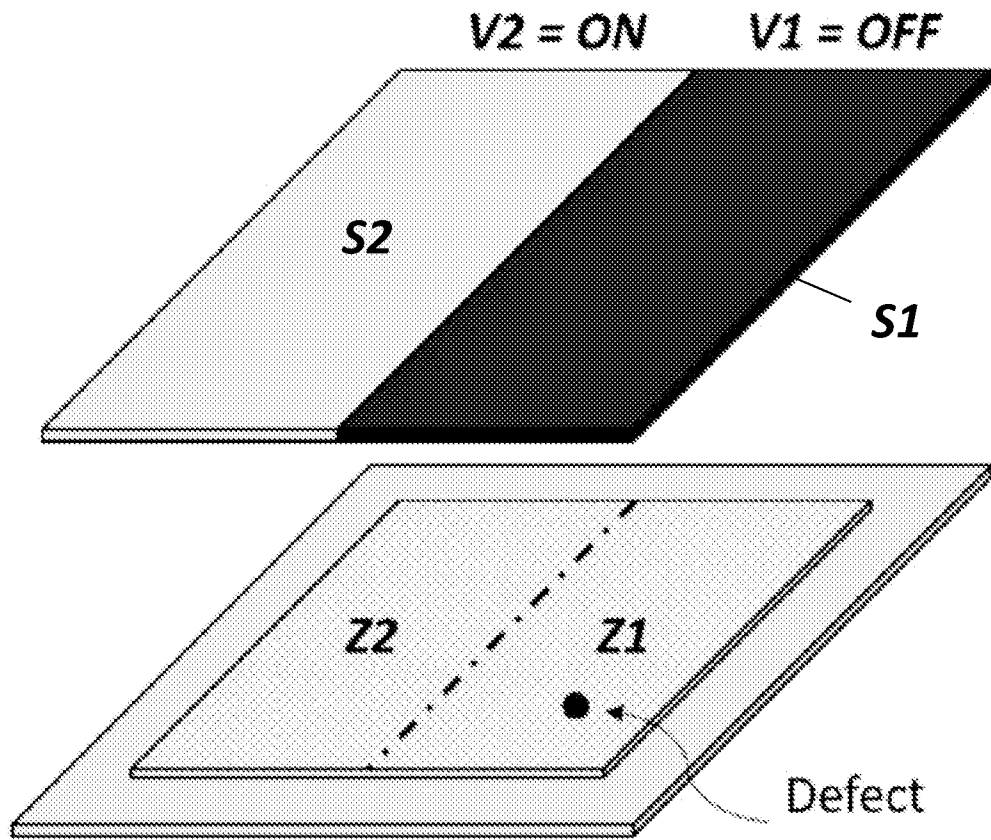


FIG. 10

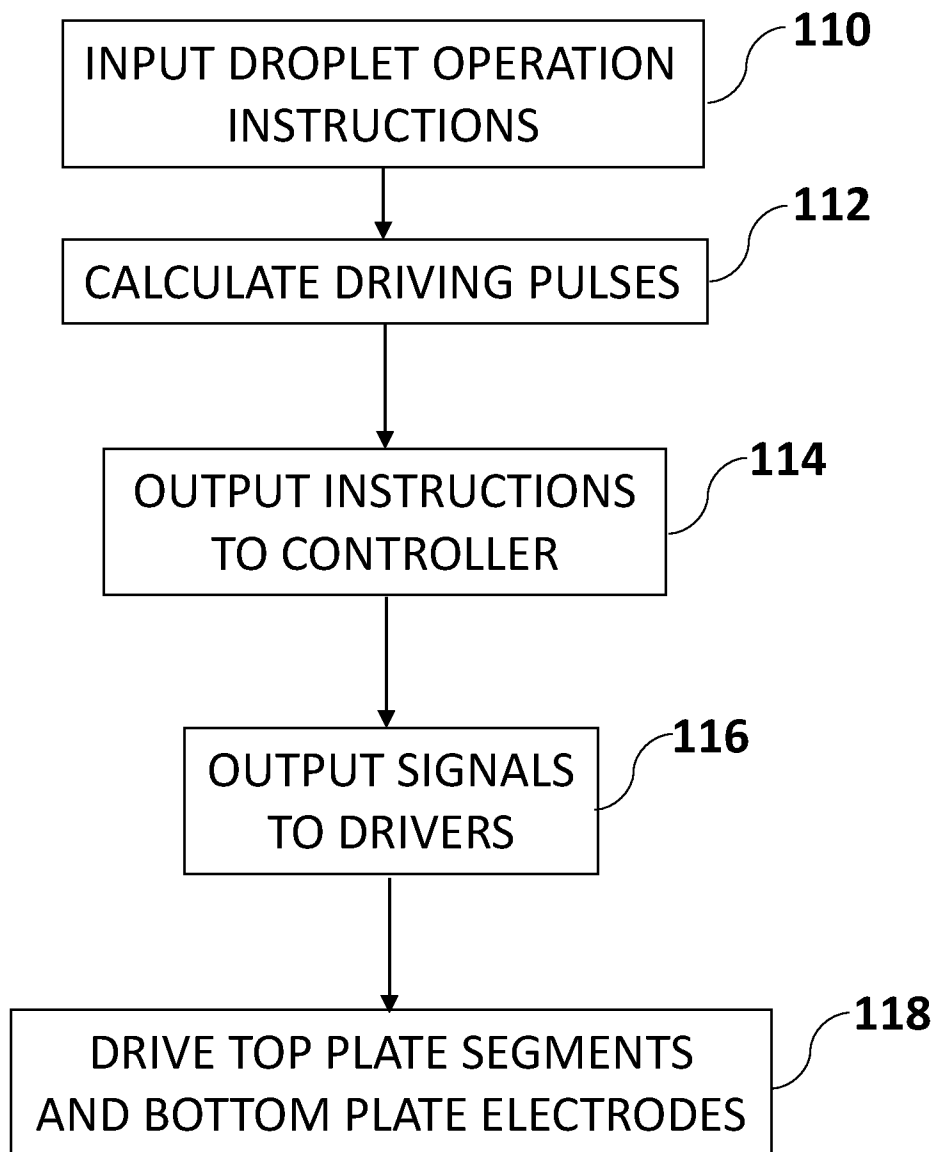


FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/029071

A. CLASSIFICATION OF SUBJECT MATTER		
B01L 3/00(2006.01)i		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) B01L 3/00(2006.01); C12Q 1/02(2006.01); C25B 9/00(2006.01); G01N 27/447(2006.01); G01N 35/10(2006.01)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Korean utility models and applications for utility models Japanese utility models and applications for utility models		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) eKOMPASS(KIPO internal) & Keywords: digital microfluidic device, propulsion electrodes, segmented top electrode array, controller		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2013-0143312 A1 (THE GOVERNING COUNCIL OF THE UNIVERSITY OF TORONTO) 06 June 2013 (2013-06-06) See paragraph [0053]; claim 1; figure 2(a).	1-20
A	US 2013-0026040 A1 (CHENG, XING et al.) 31 January 2013 (2013-01-31) See the entire document.	1-20
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A	WO 2015-170268 A1 (DH TECHNOLOGIES DEVELOPMENT PTE. LTD.) 12 November 2015 (2015-11-12) See the entire document.	1-20
A	ZULKEPLI, SITI NOOR IDORA SYAFINAZ et al., 'Droplet Velocity Measurement Based on Dielectric Layer Thickness Variation Using Digital Microfluidic Devices', Biosensors, 2018, vol. 8, pages 1-12 See the entire document.	1-20
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "D" document cited by the applicant in the international application "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 06 August 2021		Date of mailing of the international search report 09 August 2021
Name and mailing address of the ISA/KR Korean Intellectual Property Office 189 Cheongsa-ro, Seo-gu, Daejeon 35208, Republic of Korea Facsimile No. +82-42-481-8578		Authorized officer Jung, Da Won Telephone No. +82-42-481-5373

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US2021/029071

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