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(54) Title: INTEGRATED DROPLET ACTUATOR FOR GEL ELECTROPHORESIS AND MOLECULAR ANALYSIS

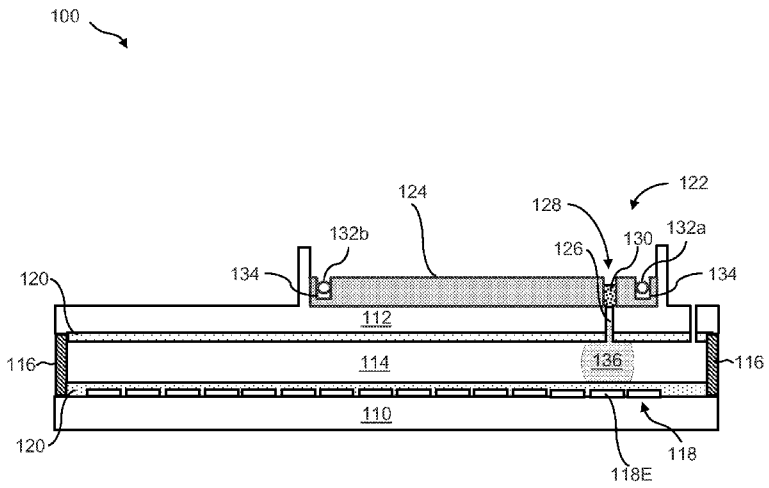


Figure 1

(57) Abstract: The invention is directed to a droplet actuator device and methods for integrating gel electrophoresis analysis with pre or post-analytical sample handling as well as other molecular analysis processes. Using digital microfluidics technology, the droplet actuator device and methods of the invention provide the ability to perform gel electrophoresis and liquid handling operations on a single integrated device. The integrated liquid handling operations may be used to prepare and deliver samples to the electrophoresis gel, capture and subsequently process products of the electrophoresis gel or perform additional assays on the same sample materials which are analyzed by gel electrophoresis. In one embodiment, one or more molecular assays, such as nucleic acid (e.g., DNA) quantification by real-time PCR, and one or more sample processing operations such as sample dilution is performed on a droplet actuator integrated with an electrophoresis gel. In one embodiment, an electrophoresis gel may be integrated on the top substrate of the droplet actuator.

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# Integrated Droplet Actuator for Gel Electrophoresis and Molecular Analysis

## 1 Related Applications

In addition to the patent applications cited herein, each of which is incorporated herein by reference, this patent application is related to and claims priority to U.S. Provisional Patent Application No 61/258,827, filed on November 6, 2009, entitled "Integrated Droplet Actuator for Gel Electrophoresis and Molecular Analysis", the entire disclosure of which are incorporated herein by reference.

## 2 Field of the Invention

The invention generally relates to an integrated droplet actuator device and techniques. In particular, the invention is directed to an integrated droplet actuator device and techniques for gel electrophoresis and molecular analysis.

## 3 Background

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.

Droplet actuators are used in a variety of analytical settings, such as biomedical research and clinical diagnostics. Depending on the type of analysis performed, one or more different molecular techniques are often implemented together, providing a wide range of field-specific applications. Many of the molecular techniques typically used to analyze a biological sample, such as a DNA sample, are often implemented in conjunction with gel electrophoresis. Gel electrophoresis is a technique routinely used to separate nucleic acids (and other biological molecules) based on size. In one example, gel electrophoresis may be used to assess the quality (e.g., measured as a function of average fragment size) of a DNA sample prior to more complex analysis such as genotyping. In another example, gel electrophoresis may be used to analyze

specific DNA restriction enzyme digestion patterns or polymerase chain reaction (PCR) amplification fragments. Specific DNA bands may also be isolated from a gel matrix for subsequent manipulations, such as cloning or sequencing. However, this approach of combined analyses is labor-intensive and expensive because it requires each technique to be performed separately and often sequentially. There is a need for improved methods for implementing one or more molecular techniques that provides for flexibility in assay design and for comprehensive sample handling and analysis.

## 4 Summary of the Invention

The invention is directed to an integrated droplet actuator device and techniques for gel electrophoresis and molecular analysis.

In one embodiment, the invention provides an integrated droplet actuator device including a top substrate and a bottom substrate; the two substrates configure to form a droplet operations gap. The integrated droplet actuator device may further include electrodes associated with one or both of the bottom substrate and the top substrate, and configured for conducting droplet operations in the gap; a gel arranged on the top substrate; a pair of electrodes in electrical contact with the gel, the electrodes arranged at first and second locations in relation to the gel; and one or more fluid paths extending from inside the gap to the gel.

In another embodiment, the invention provides a method of preparing and delivering a sample droplet for gel electrophoresis in an integrated droplet actuator device. The method preferably includes providing an integrated droplet actuator device, including a top substrate and a bottom substrate, where the two substrates are configured to form a droplet operations gap; electrodes associated with one or both of the bottom substrate and the top substrate, and configured for conducting droplet operations in the gap; a gel arranged on the top substrate; a pair of electrodes in electrical contact with the gel, the electrodes arranged at first and second locations in relation to the gel; and one or more fluid paths extending from inside the gap to the gel. The method may further include loading a droplet containing a sample material on at least one of the one or more electrodes, where at least one electrode is in proximity to the one or more fluid paths; transferring the sample material into a loading buffer; and transferring the sample

material from the loading buffer into the gel. The method may further include concentrating the sample material include delivering, using droplet operations, additional droplets containing the sample material to at least one of the one or more electrodes in proximity to the one or more fluid paths; and transferring the sample material, from the additional droplets, into the loading buffer until a desired concentration of sample material is achieved.

In yet another embodiment, the invention provides a method of preparing and delivering a sample droplet for gel eletrophoresis in an integrated droplet actuated device. The method preferably includes providing an integrated droplet actuated device, including a top substrate and a bottom substrate, the two substrates configured to form a droplet operations gap; electrodes associated with one or both of the bottom substrate and the top substrate, and configured for conducting droplet operations in the gap; a gel arranged on the top substrate, wherein at least a portion of the gel extends into the gap of the integrated droplet actuator; a pair of electrodes in electrical contact with the gel, the electrodes arranged at first and second locations in relation to the gel; and one or more fluid paths extending from inside the gap to the gel. The method may further include loading a droplet containing a sample material on at least one of the one or more electrodes, where the at least one electrode is in proximity to the one or more fluid paths; transferring the sample material into a loading buffer retained in the one or more fluid paths; and transferring the sample material from the loading buffer into the gel. The method may further include concentrating the sample material include delivering, using droplet operations, additional droplets containing the sample material to at least one of the one or more electrodes in proximity to the one or more fluid paths; and transferring the sample material, from the additional droplets, into the loading buffer until a desired concentration of sample material is achieved.

In still yet another embodiment, the intervention provides an integrated droplet actuator device for conducting molecular assays, including a top substrate and a bottom substrate, the two substrates configured to form a droplet operations gap; electrodes associated with one or both of the bottom substrate, and configure for conducting droplet operations in the gap; a gel arranged on the top sub straight; a pair of electrodes in electrical contact with the gel, the electrodes arranged at first and second locations in relation to the gel;

one or more fluid paths extending from inside the gap to the gel; and at least a first reaction zone and a second reaction zone.

In still yet another embodiment, the invention provides a method of conducting a molecular analysis and integrated droplet actuator device. The method may include providing an integrated droplet actuator device for conducting molecular analysis, including a top substrate and a bottom substrate, the two substrates configured to form a droplet operations gap; electrodes associated with one or both of the bottom substrate and the top substrate, and configure for conducting droplet operations in the gap; a gel arranged on the top substrate; a pair of electrodes in electrical contact with the gel, the electrodes arranged at first and second locations in relation to the gel; one or more fluid paths extending from inside the gap to the gel; and at least a first reaction zone and second reaction zone. The method may further include positioning a reaction droplet on an electrode in a first reaction zone and incubating the reaction droplet in the first reaction zone for a period of time; transporting the reaction droplet to the second reaction zone and incubating the reaction droplet in the second reaction zone for a period of time; and cycling the reaction droplet between at least the first reaction zone and at least the second reaction zone until a desired result is achieved.

In still yet another embodiment, the invention provides an electrode configuration for real-time PCR on a multi-channel integrated droplet actuator device. The device may include controlled electrode loops configured for conducting droplet operations arranged on a substrate of the integrated droplet actuator device, wherein each of the controlled electrode loops have at least a first reaction zone and a second reaction zone; and reservoir wells in fluidic connection with the controlled electrode loops, where the reservoir wells are adapted to dispense sample droplets on one or more electrodes of the controlled electrode loops.

In still yet another embodiment, the invention provides a method of conducting real-time PCR in a multi-channel integrated droplet actuated device. The method may include, dispensing a sample droplet containing a quantity of sample material from a first reservoir onto one or more electrodes of a controlled electrode loop arranged on a substrate of the integrated droplet actuator device; dispensing a PCR reagent sample

droplet from a second reservoir onto one or more electrodes of a controlled electrode loop arranged on a substrate of the integrated droplet actuator device; merging and mixing the sample droplet and the PCR reagent sample droplet to form a combined droplet, thermocycling the combined droplet between at least two temperature zones positioned on the controlled electrode loop; and detecting a quantity of amplified sample material within the combined droplet.

In still yet another embodiment, the invention provides a method of diluting a sample in an integrated droplet actuator. The method may include dispensing a droplet containing sample material on one or more substrates of an integrated droplet actuator; merging the droplet with a buffer droplet and mixing to form a combined droplet; splitting the combined droplet into two essentially equal diluted droplets; and repeating until a value within a range sufficient for sample analysis is achieved.

In still yet another embodiment, the invention provides a method of conducting multiple assays on an integrated droplet actuator. The method may include providing an integrated droplet actuator having one or more reagent reservoirs, at least one sample reservoir, and an electrophoresis gel; dispensing an initial sample droplet comprising a quantity of sample material from the at least one sample reservoir onto a substrate of the integrated droplet actuator; quantitating the concentration of the sample material in the dispensed initial sample droplet; determining, using the total quantified sample material concentration, a sample droplet volume having an appropriate amount of sample material required for conducting gel electrophoresis; dispensing the required sample droplet volume from the at least one sample reservoir onto the substrate of the integrated droplet actuator; transporting the required sample droplet volume to the electrophoresis gel and conducting electrophoresis-based assay; dispensing another sample droplet from the at least one sample reservoir onto the substrate of the integrated droplet actuator and mixing with a reagents droplet dispensed from the one or more reagents reservoir to form a combined droplet; and analyzing the combined droplet and a sample material standard droplet having a known sample material quantity, to determine the quantity of sample material present in the sample. Quantitating may include merging and mixing a sample droplet with a droplet comprising a quantitation reagent to form a combined droplet; measuring the florescence of the combined droplet; merging and mixing a standard

droplet having a known sample material concentration with a droplet comprising a quantitation reagent to form a combined standard droplet; measuring florescence of the standard droplet and of the combined standard droplet; and comparing the florescence of the combined droplet with the florescence of the combined standard droplet to determine the sample material concentration and the sample droplet.

## 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 an integrated droplet actuator for gel electrophoresis;

Figure 2 illustrates a side view of a portion of the integrated droplet actuator of Figure 1 that is configured for real-time PCR analysis;

Figure 3 illustrates a schematic diagram of an example of an electrode configuration for multi-channel real-time PCR on an integrated droplet actuator;

Figure 4 shows a plot of fluorescence data of a DNA standard measured in a PicoGreen® assay;

Figure 5 shows a plot of fluorescence data from a binary dilution series performed on-chip; and

Figure 6 illustrates a flow diagram of an example of a microfluidic protocol for multiplexed analysis of a DNA sample on an integrated droplet actuator.

# 7 Description

The present invention provides a droplet actuator device and methods for integrating gel electrophoresis analysis with pre or post-analytical sample handling as well as other molecular analysis processes. Using digital microfluidics technology, the droplet actuator device and methods of the invention provide the ability to perform gel electrophoresis and liquid handling operations on a single integrated device. The integrated liquid handling operations may be used to prepare and deliver samples to the electrophoresis gel, capture and subsequently process products of the electrophoresis gel or perform additional assays on the same sample materials which are analyzed by gel electrophoresis. In one embodiment, one or more molecular assays, such as nucleic acid (e.g., DNA) quantification by real-time PCR, and one or more sample processing operations such as sample dilution is performed on a droplet actuator integrated with an electrophoresis gel. In one embodiment, an electrophoresis gel slab may be integrated on the top substrate of the droplet actuator. In another embodiment, an electrophoresis gel slab may be integrated inside a microfluidic droplet actuator within an oil environment.

Because of the software programmability of digital microfluidics, essentially all of the parameters varied between and within different assay protocols, such as incubation times, sequences of reagent additions, washing protocols and thermal programs, may be configured on a single droplet actuator.

In one embodiment, the integrated microfluidic device may be used for DNA quality control testing.

## 7.1 Integration of Gel Electrophoresis on a Droplet actuator

**Figure 1** illustrates a side view of a portion of an integrated droplet actuator 100 for gel electrophoresis. Integrated droplet actuator 100 of Figure 1 is an example of a droplet actuator structure in which an electrophoresis gel slab is provided on the top substrate of the droplet actuator. The digital microfluidic layer and a gel electrophoresis layer are connected by through-holes fabricated in the top substrate of the droplet actuator.

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 be attached to top substrate 112 by an epoxy glue ring 116. Gap 114 may be filled with a filler fluid, such as silicone oil (not shown).

Bottom substrate 110 may include a path or array of droplet operations electrodes 118 (e.g., electrowetting electrodes). Bottom substrate 110 may, for example, be formed of a printed circuit board (PCB). Top substrate 112 may, for example, be formed of a plastic material with high transparency and low fluorescence in the wavelength range compatible with fluorescence detection (i.e., suitable for fluorimeter operation). For example, top substrate 112 may be formed of cyclo-olefin polymer (COP) and/or copolymer (COC). A hydrophobic layer 120 may be disposed on the surface of bottom substrate 110 that is facing gap 114 (i.e., atop droplet operations electrodes 118). Similarly, another hydrophobic layer 120 may be disposed on the surface of top substrate 112 that is facing gap 114.

Top substrate 112 may include a protruded structure 122 of sufficient size to accommodate a gel slab 124 for electrophoresis (e.g., agarose gel electrophoresis). One or more openings 126 are provided within top substrate 112. Openings 126 in top substrate 112 provide a fluid path from certain droplet operations electrodes 118 (e.g., droplet operations electrode 118E) to one or more corresponding nucleic acid loading slots 128 that are molded into gel slab 124. Loading slots 128 may be partially filled with a loading buffer 130. Loading buffer 130 is retained in loading slot 128 by surface tension forces. The alignment of opening 126 and loading slot 128 is such that a microfluidics-gel interface is formed. A pair of thin wire electrodes 132 (e.g., electrode 132a and electrode 132b) may be positioned at two ends of gel slab 124 and parallel to loading slots 128. Electrodes 132 may be directly attached to and integrated with top substrate 112 or may be positioned in a trench 134 that is molded into gel slab 124. Trench 134 may be filled with an electrophoresis buffer (not shown), such as TBE. Trench 134 is an electrical interface that provides a uniform electrical field in gel slab 124. In another example, electrodes 132 may be embedded in gel slab 124. Electrodes 132 may, for example, be platinum wires. Electrodes 132 may be connected to an electrophoresis power supply (not shown). Electrode 132a may, for example, be a negative electrode (anode). Electrode 132b may, for example, be a positive electrode (cathode). In one embodiment, electrophoresis may be conducted without submerging gel slab 124 in an electrophoresis buffer (e.g., TBE). In another embodiment, gel slab 124 may be submerged in an electrophoresis buffer.

A droplet 136 may be positioned, for example, at droplet operations electrode 118E. Droplet 136 may, for example, be an aqueous sample droplet that contains a quantity of nucleic acid to be sized by gel electrophoresis. Droplet 136 on droplet operations electrode 118E is in proximity of the microfluidics-gel interface formed at opening 126 and loading slot 128. At this interface, a surface energy gradient may be formed from the hydrophobic droplet actuator inner space through the less hydrophobic opening 126, to the hydrophilic loading slot 128 in gel slab 124.

Because of the surface energy gradient formed at the interface, droplet 136 automatically flows upward due to capillary action and is merged into loading buffer 130 retained in loading slot 128. In another embodiment, the gel slab 124 extends into the interior of the droplet actuator avoiding the need to transport the droplet 136 through the opening 126. Alternatively, droplet 136 may remain inside the inner space but in contact with the loading buffer 130 through opening 126 so that material (e.g. DNA) can be transferred between the two spaces without bulk liquid transfer. In one embodiment, material is transferred between droplet 136 to loading buffer 130 through chemical diffusion. In another embodiment, material is transferred between droplet 136 to loading buffer 130 by electrophoresis. In this embodiment an electrical field gradient is established throughout the continuous liquid body formed by droplet 136 and loading buffer 130 connected through opening 126 which causes charged molecules to be concentrated in one end of the continuous liquid body. In any of these embodiments the transfer of material may occur in either direction, i.e. from the sample droplet 136 into loading buffer 130 or from loading buffer 130 into sample droplet 136. From the loading buffer 130 material may be transferred into the gel 124 by electrophoresis. Alternatively, gel electrophoresis products from gel 124 may be transferred into loading buffer 130. Thus, a means is provided both to deliver sample materials from the droplet actuator to the gel for analysis and to receive products of the gel analysis for subsequent processing on the droplet actuator.

## 7.2 Real-Time PCR on an Integrated Droplet actuator

Integrated droplet actuator 100 of Figure 1 may be configured for one or more molecular assays. In one embodiment integrated droplet actuator 100 may be configured for real-time PCR.

**Figure 2** illustrates a side view of a portion of integrated droplet actuator 100 of Figure 1 that is configured for real-time PCR analysis. In this embodiment, integrated droplet actuator 100 further includes, for example, two temperature control or reaction zones 210, such as temperature control zone 210a and 210b. A pair of heater bars 212, such as heater bars 212a and 212b, may be used to control the temperature of filler fluid in vicinity of thermal reaction zones 210a and 210b, respectively. Each heater bar 212 may be, for example, an aluminum heater bar equipped with heating resistors and thermistor. In one example, heater bar 212a may be used to heat temperature control zone 210a to about 95 °C (melting temperature), which is a temperature sufficient for denaturation of DNA template and primers. Heater bar 212b may be used to heat temperature control zone 210b to about 55 °C to 65 °C, which is a temperature sufficient for annealing of primer to single-stranded DNA template and primer extension by DNA polymerase.

Temperature control zones 210a and 210b may be positioned at a sufficient distance from gel slab 124 that they do not affect the temperature inside the gel.

A PCR reaction droplet 214 may be positioned at a certain droplet operations electrode in temperature control zone 210a. Reaction droplet 214 includes the components required for PCR amplification of a target DNA template and fluorescence detection of amplified product (e.g., Eva Green). Reaction droplet 214 may be incubated within temperature control zone 210a for a period of time that is sufficient to dissociate the target DNA to free single stranded template and denature any primer-dimer pairs. Reaction droplet 214 may be transported using droplet operations along droplet operations electrodes 118 to temperature control zone 210b. Reaction droplet 214 may be incubated within temperature control zone 210b for a period of time that is sufficient for annealing of primers to the single stranded target DNA template and extension of the annealed primers by DNA polymerase. Reaction droplet 214 may be repeatedly transported between temperature control zones 210a and 210b any number of times sufficient for a desired level of DNA amplification. Because of the low thermal conductivity of bottom substrate 110 and top substrate 112, PCR thermocycling and gel electrophoresis may be executed simultaneously without overheating gel slab 124.

While a two temperature control zone is described herein, it is envisioned that multiple control zones (i.e., three or more) would be possible.

**Figure 3** illustrates a schematic diagram of an example of an electrode configuration 300 for multi-channel real-time PCR on an integrated droplet actuator. The PCR-integrated droplet actuator may include four independently controlled electrode loops (e.g., loops 1 through 4) as well as reservoirs for dispensing DNA samples and PCR reagents. Each thermocycler loop may circulate a single droplet or a droplet train between two temperature zones (e.g., between a 55-65 °C zone and a 95 °C zone). A detection spot may be provided within the 55-65 °C temperature zones in each thermocycler loop. For a typical PCR assay, a 300-nL droplet of DNA sample and a 300-nL droplet of PCR master mix and fluorescence dye (e.g., Eva Green) are dispensed from separate reservoirs and mixed using droplet operations. The combined droplet is then thermocycled between the two temperature zones. The amount of amplified DNA may be determined during each amplification cycle using a fluorimeter.

In one embodiment, a multi-channel real-time PCR assay may be used to assess the purity of a DNA sample. For example, a multiplexed PCR protocol may be used to quantitate the relative amounts of human DNA and non-human DNA (i.e. bacterial contamination) in a human

biological sample (e.g., saliva). Contaminant DNA can be amplified and quantitated using primers pairs specific to particular common suspected bacterial contaminants (e.g., streptococcus, *Escherichia coli*), or by pooling multiple primers pairs each specific to a particular organism (i.e. multiplexed PCR) or by using primers which non-specifically amplify a broad class of organisms (i.e pan-bacterial or pan-fungal) by targeting highly-conserved sequences. Human DNA can be amplified and quantitated using primer pairs specific to particular human DNA sequences including many common reference genes. Relative quantitation of the human and non-human DNA present in a sample provides a means to assess the proportion of human DNA present in the total DNA sample. Alternatively, the amount of human or non-human DNA could be individually determined and compared to the amount of total DNA present in the sample (e.g. determined with Picogreen or Eva green fluorescent dye). Based on this quantitative assessment a sample may be rejected for further analysis if the contamination is determined to be unacceptable or the amount of total DNA used downstream may be adjusted to compensate for the presence of some fraction of non-human DNA. More generally, this approach can be used to quantitate the relative fraction of DNA contributed by any one organism or class or organisms within a mixed DNA sample (i.e. the sample need not be human and the contaminant need not be bacterial).

Sufficient sensitivity and specificity may be achieved by optimization of thermocycling conditions and PCR assay formulation. In particular, the type and concentration of the primer sets and polymerase, as well as the annealing temperature, may be chosen to selectively amplify human DNA in a bacterial DNA background or vice versa. The real-time detection may, for example, be by incorporation of a generic indicator, such as Eva Green. Alternatively, a specific indicator such as TaqMan probe or molecular beacon may be used to target specific sequences.

## 7.3 DNA Quantification on an Integrated Droplet actuator

In another embodiment, a serial dilution process and a DNA quantification assay may be combined on a droplet actuator, such as integrated droplet actuator 100 of Figure 1 and/or Figure 2. For example, a dilution protocol maybe applied prior to quantitation by a fluorescence assay, such as a PicoGreen™ assay if the undiluted sample is expected to be outside of the range of quantitation assay. Alternatively, if the undiluted sample is initially tested and found to be outside the range of the quantitation assay it may be diluted and retested. Alternatively, several different dilutions of the sample may be prepared initially and each one separately quantitated to provide several different values for each sample.

Dilution protocols may likewise be applied to a sample following the quantitation step. For example, a downstream process such as gel electrophoresis may require a particular concentration of DNA for optical performance. Based on the results on the quantitation step the sample may be diluted to achieve a particular amount or concentration in the sample droplet before being introduced into the gel. This could be performed on the same sample droplet analyzed in the quantitation step or, more likely on a separate aliquot from the same original sample.

In addition, a downstream process may require concentration rather than dilution to achieve a target amount or concentration of DNA. Concentration of DNA can generally be performed by several different methods on a droplet actuator including solid-phase capture and solvent evaporation. For gel electrophoresis, concentration can also be achieved by delivering multiple droplets to the loading well of the gel, i.e. the DNA contained in one or more droplets can be can combined in a well and "injected" as a single sample. Because of the substantially greater electrophoretic mobility of DNA in buffer compared to gel, the DNA within the loading well is effectively concentrated by electrophoresis.

**Figure 4** shows a plot 400 of fluorescence data of a DNA standard measured in a PicoGreen™ assay. A 500 ng/μL DNA sample was diluted off-chip to a series of concentrations ranging from 0.1 ng/μL to 0.7 ng/μL using TE buffer. A 300-nL droplet of each of the diluted DNA samples was dispensed and mixed with a 300-nL PicoGreen droplet. The fluorescence of the combined droplet was measured on a digital microfluidic chip using a fluorimeter. The data show a linear fluorescence response to the DNA concentrations between 0.1 and 0.7 ng/μL.

An on-chip binary dilution protocol may include, but is not limited to, the following steps:

In a first step, a 300-nL DNA sample droplet is merged and mixed using droplet operations with a 300-nL dilution buffer droplet (e.g., TE buffer) to yield a 600-nL combined droplet. In a second step, the 600-nL combined droplet is split into two identical 300-nL diluted droplets. In a third step, steps 1 and 2 are repeated on one of the diluted droplets formed in step 2. Steps 1 through 3 may be repeated any number of times (e.g., 1 to 10 times) sufficient for sample analysis.

Using fluoroscein tracer dye to demonstrate on-chip serial dilution because of its greater dynamic range, **Figure 5** shows a plot 500 of fluorescence data from a binary dilution series performed on-chip. The fluorescence of the dilution series was quantitated using a fluorimeter. The samples, from high concentration to low concentration, were transported sequentially to the same detection spot and measured using the same fluorimeter channel.

## 7.4 Fluorescence Detection

In one embodiment, a multi-channel, e.g., a four channel, fluorimeter module may be used for fluorescence detection on a droplet actuator, such as integrated droplet actuator 100 of Figure 1 and/or Figure 2. For example, one channel may be used for fluorescence detection of a DNA quantitation assay, such as a PicoGreen assay. The other three channels may be used for up to three real-time PCR reactions performed in parallel.

## 7.5 Example Application for an Integrated Droplet actuator

Because of the flexibility and programmability of the digital microfluidics platform, two or more different types of assays may be readily performed sequentially and/or simultaneously on a droplet actuator. In one embodiment, DNA quantification, multi-channel real-time PCR and gel electrophoresis may be performed on a single droplet actuator. For example, an integrated droplet actuator may be used to determine the quantity, quality (i.e., by sizing) and purity of one or more DNA samples, such as DNA samples provided through biobanks.

**Figure 6** illustrates a flow diagram of an example of a microfluidic protocol 600 for multiplexed analysis of a DNA sample on an integrated droplet actuator. The integrated droplet actuator may be provided with reagent reservoirs that are loaded with dilution buffer (e.g., TE buffer), PicoGreen solution (for DNA quantitation), real-time PCR reagents, and/or a DNA standard (e.g., human DNA or bacterial DNA standard). A 5 μL aliquot of a DNA sample (e.g., a Biobank DNA sample) is loaded onto a sample reservoir of a droplet actuator.

In a first step, a 300-nL sample droplet is dispensed from the sample reservoir and serially diluted using, for example, a binary dilution protocol (e.g., $2^n$ where n= 1-10).

In a second step, the $10^{th}$ (i.e., 1024-fold) dilution of the series, as well as the $10^{th}$ dilution of the DNA standard with known concentration is quantitated using a PicoGreen assay. A 300-nL droplet of the $10^{th}$ dilution of the DNA samples is mixed with a 300-nL 1X PicoGreen droplet, and the fluorescence measured using a fluorimeter. By comparing the fluorescence of the dilutions from the unknown DNA sample and the standard, the total DNA concentration in the original sample may be determined. Any variation in the dilution factor will not interfere with the quantification results. Optionally, the actual dilution factor may be verified by measuring additional dilutions, such as the $8^{th}$ and $9^{th}$ dilutions.

In a third step, the quantified total DNA concentration is used to calculate the sample volume which contains the appropriate amount of total DNA required for gel electrophoresis (e.g., about 500ng). The corresponding volume is dispensed from the original DNA sample and delivered to the gel for electrophoresis-based DNA quality assessment. In one example, the quality of genomic DNA may be evaluated. In this example, intact genomic DNA appears as a high molecular weight smear on the gel. Degraded genomic DNA appears as fragmented and/or a lower molecular weight smear on the gel.

In a fourth step, another 300-nL DNA sample droplet is dispensed from the sample reservoir and mixed with real-time PCR reagents. The combined droplet and a human and/or a bacterial DNA standard are analyzed by real-time PCR to determine the amount of human DNA in the sample. The gel electrophoresis and real-time PCR reaction may be conducted in parallel provided that the thermal isolation between the agarose gel and the interior of the droplet actuator is sufficient.

## 7.6 Systems

Referring to Figures 1 through 6, as will be appreciated by one of skill in the art, the invention may be embodied as a method, system, or computer program product. Accordingly, various 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 present invention. The term "the invention" or the like is used with reference to certain specific examples of the many alternative aspects or embodiments of the applicants' invention set forth in this specification, and neither its use nor its absence is intended to limit the scope of the applicants' invention or the scope of the claims. This specification is divided into sections for the convenience of the reader only. Headings should not be construed as limiting of the scope of the invention. The definitions are intended as a part of the description of the invention. It will be understood that various details of the present invention may be changed without departing from the scope of the present invention. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

# THE CLAIMS

We claim:

1. An integrated droplet actuator device comprising:

   (a) a top substrate and a bottom substrate, the two substrates configured to form a droplet operations gap;

   (b) electrodes associated with one or both of the bottom substrate and the top substrate, and configured for conducting droplet operations in the gap;

   (c) a gel arranged on the top substrate;

   (d) a pair of electrodes in electrical contact with the gel, the electrodes arranged at first and second locations in relation to the gel; and

   (e) one or more fluid paths extending from inside the gap to the gel.

2. The integrated droplet actuator device of any of claims 1 and following, further comprising one or more loading slots formed in the gel and arranged in fluid connection with one or more of the fluid paths.

3. The integrated droplet actuator device of any of claims 1 and following, wherein the gel comprises a gel slab.

4. The integrated droplet actuator device of any of claims 1 and following, wherein the gel comprises an electrophoresis gel.

5. The integrated droplet actuator device of any of claims 2 and following, wherein the one or more loading slots comprise nucleic acid loading slots.

6. The integrated droplet actuator device of any of claims 2 and following, wherein the one or more loading slots are loaded with a loading buffer.

7. The integrated droplet actuator device of any of claims 6 and following, wherein the loading buffer is retained in the one or more loading slots by surface tension forces.

8. The integrated droplet actuator device of any of claims 2 and following, wherein the one or more fluid paths further provide a fluidic path from one or more designated electrodes to one or more corresponding loading spots.

9. The integrated droplet actuator device of any of claims 2 and following, wherein a microfluidics-gel interface is formed where the one or more fluid paths and the one or more loading slots interface.

10. The integrated droplet actuator device of any of claims 1 and following, wherein the gel is in electrical contact with the pair of electrodes, wherein the pair of electrodes are arranged at first and second locations in relation to the gel and parallel to the one or more loading slots.

11. The integrated droplet actuator device of any of claims 1 and following, wherein the pair of electrodes are directly attached to and integrated with the top substrate.

12. The integrated droplet actuator device of any of claims 1 and following, wherein the pair of electrodes are positioned in a corresponding pair of trenches molded into the gel, wherein the trenches are arranged at first and second locations in relation to the gel and facilitate an electrical interface that provides an essentially uniform electrical field in the gel.

13. The integrated droplet actuator device of any of claims 12 and following, wherein the pair of trenches comprises an electrophoresis buffer.

14. The integrated droplet actuator device of any of claims 13 and following, wherein electrophoresis buffer comprises Tris-Borate-Edta (TBE).

15. The integrated droplet actuator device of any of claims 1 and following, wherein the pair of electrodes are arranged at first and second locations in contact with the gel.

16. The integrated droplet actuator device of any of claims 1 and following, wherein the pair of electrodes comprises platinum wire.

17. The integrated droplet actuator device of any of claims 1 and following, wherein one of the pair of electrodes is a negative electrode and one of the pair of electrodes is a positive electrode.

18. The integrated droplet actuator device of any of claims 1 and following, wherein electrophoresis is conducted without submerging the gel in an electrophoresis buffer.

19. The integrated droplet actuator device of any of claims 1 and following, wherein the gel is arranged in a protruded structure formed on the top substrate.

20. The integrated droplet actuator device of any of claims 1 and following, wherein the gel is submerging in an electrophoresis buffer during electrophoresis.

21. The integrated droplet actuator device of any of claims 1 and following, wherein the top substrate and the bottom substrate are attached to one another by an epoxy glue ring.

22. The integrated droplet actuator device of any of claims 1 and following, wherein the electrodes comprise an array of electrodes.

23. The integrated droplet actuator device of any of claims 1 and following, wherein the electrodes comprise a path of electrodes.

24. The integrated droplet actuator device of any of claims 1 and following, wherein the electrodes comprise electrowetting electrodes.

25. The integrated droplet actuator device of any of claims 1 and following, wherein the bottom substrate comprises a printed circuit board.

26. The integrated droplet actuator device of any of claims 1 and following, wherein the top substrate comprises a high transparency and low fluorescence plastic material.

27. The integrated droplet actuator device of any of claims 26 and following, wherein the plastic material is suitable for fluorimeter operation.

28. The integrated droplet actuator device of any of claims 26 and following, wherein the plastic material comprises cyclo-olefin polymer and copolymer.

29. The integrated droplet actuator device of claim 1, wherein the droplet operations gap comprises a filler fluid.

30. The integrated droplet actuator device of claim 29, wherein the filler fluid comprises oil.

31. The integrated droplet actuator device of claim 29, wherein the oil comprises silicone oil.

32. The integrated droplet actuator device of any of claims 1 and following, wherein the bottom substrate comprises a hydrophobic layer disposed on a surface of the bottom substrate facing the gap.

33. The integrated droplet actuator device of any of claims 32 and following, wherein the hydrophobic layer is formed on top of the electrodes.

34. The integrated droplet actuator device of any of claims 1 and following, wherein the top substrate comprises a hydrophobic layer disposed on a surface of the top substrate facing the gap.

35. The integrated droplet actuator device of any of claims 1 and following, wherein the top substrate and bottom substrate comprise a single integral substrate.

36. The integrated droplet actuator device of any of claims 35 and following, wherein the single integral substrate is folded over on itself to form a top portion and a bottom portion comprising the top substrate and bottom substrate respectively.

37. A method of preparing and delivering a sample droplet for gel electrophoresis in an integrated droplet actuator device comprising:

(a) providing an integrated droplet actuator device comprising:

(i) a top substrate and a bottom substrate, the two substrates configured to form a droplet operations gap;

(ii) electrodes associated with one or both of the bottom substrate and the top substrate, and configured for conducting droplet operations in the gap;

(iii) a gel arranged on the top substrate;

(iv) a pair of electrodes in electrical contact with the gel, the electrodes arranged at first and second locations in relation to the gel; and

(v) one or more fluid paths extending from inside the gap to the gel.

(b) loading a droplet containing a sample material on at least one of the one or more electrodes, wherein the at least one electrode is in proximity to the one or more fluid paths;

(c) transferring the sample material into a loading buffer; and

(d) transferring the sample material from the loading buffer into the gel.

38. The method of any of claims 37 and following, wherein the sample droplet comprises a quantity of nucleic acid.

39. The method of any of claims 38 and following, wherein the quantity of nucleic acid comprises DNA.

40. The method of claims 37 and following, wherein the integrated droplet actuator device, further comprising one or more loading slots formed in the gel and arranged in fluid connection with one or more of the fluid paths.

41. The method of claims 40 and following, wherein the loading buffer is retained in the one or more loading slots.

42. The method of claims 37 and following, wherein the sample droplet containing the sample material is merged into the loading buffer.

43. The method of any of claims 42 and following, wherein the sample droplet is merged into the loading buffer through capillary action.

44. The method of any of claims 40 and following, wherein the capillary action is effected by a surface energy gradient formed from a hydrophobic gap of the integrated droplet

actuator through the one or more fluid paths being less hydrophobic than the gap of the integrated droplet actuator to the one or more loading slots being hydrophilic.

45. The method of claims 37 and following, wherein the sample material is transferred into the loading buffer through chemical diffusion.

46. The method of claims 37 and following, wherein the sample material is transferred into the loading buffer through electrophoresis.

47. The method of claims 37 and following, wherein the sample material is transferred into the sample droplet from the loading buffer.

48. The method of claims 47 and following, wherein the sample material is transferred into the sample droplet from the loading buffer through chemical diffusion.

49. The method of claims 47 and following, wherein sample material is transferred into the sample droplet from the loading buffer through electrophoresis.

50. The method of claims 37 and following, wherein sample material is transferred into the gel from the loading buffer by electrophoresis.

51. The method of any of claims 37 and following, wherein the sample material is concentrated, wherein concentrating the sample material comprises:

(a) delivering, using droplet operations, additional droplets containing the sample material to at least one of the one or more electrodes in proximity to the one or more fluid paths; and

(b) transferring the sample material, from the additional droplets, into the loading buffer until a desired concentration of sample material is achieved.

52. A method of preparing and delivering a sample droplet for gel electrophoresis in an integrated droplet actuator device comprising:

(a) providing an integrated droplet actuator device comprising:

(i) a top substrate and a bottom substrate, the two substrates configured to form a droplet operations gap;

(ii) electrodes associated with one or both of the bottom substrate and the top substrate, and configured for conducting droplet operations in the gap;

(iii) a gel arranged on the top substrate, wherein at least a portion of the gel extends into the gap of the integrated droplet actuator;

(iv) a pair of electrodes in electrical contact with the gel, the electrodes arranged at first and second locations in relation to the gel; and

(v) one or more fluid paths extending from inside the gap to the gel.

(b) loading a droplet containing a sample material on at least one of the one or more electrodes, wherein the at least one electrode is in proximity to the one or more fluid paths;

(c) transferring the sample material into a loading buffer retained in the one or more fluid paths; and

(d) transferring the sample material from the loading buffer into the gel.

53. The method of any of claims 52 and following, wherein the sample droplet comprises a quantity of nucleic acid.

54. The method of any of claims 53 and following, wherein the quantity of nucleic acid comprises DNA.

55. The method of claims 52 and following, wherein the sample droplet containing the sample material is merged into the loading buffer.

56. The method of claims 52 and following, wherein the sample material is transferred into the loading buffer through chemical diffusion.

57. The method of claims 52 and following, wherein the sample material is transferred into the loading buffer through electrophoresis.

58. The method of claims 52 and following, wherein sample material is transferred into the sample droplet from the loading buffer.

59. The method of claims 58 and following, wherein sample material is transferred into the sample droplet from the loading buffer through chemical diffusion.

60. The method of claims 58 and following, wherein the sample material is transferred into the sample droplet from the loading buffer through electrophoresis.

61. The method of claims 52 and following, wherein the sample material is transferred into the gel slab from the loading buffer by electrophoresis.

62. The method of any of claims 52 and following, wherein the sample material is concentrated, wherein concentrating the sample material comprises:

(a) delivering, using droplet operations, additional droplets containing the sample material to the at least one of the one or more electrodes in proximity to the one or more fluid paths; and

(b) transferring the sample material, from the additional droplets, into the loading buffer until a desired concentration of sample material is achieved.

63. An integrated droplet actuator device for conducting molecular assays comprising:

(a) a top substrate and a bottom substrate, the two substrates configured to form a droplet operations gap;

(b) electrodes associated with one or both of the bottom substrate and the top substrate, and configured for conducting droplet operations in the gap;

(c) a gel arranged on the top substrate;

(d) a pair of electrodes in electrical contact with the gel, the electrodes arranged at first and second locations in relation to the gel;

(e) one or more fluid paths extending from inside the gap to the gel; and

(f) at least a first reaction zone and a second reaction zone.

64. The integrated droplet actuator device of any of claims 63 and following, further comprising one or more loading slots formed in the gel and arranged in fluid connection with one or more of the fluid paths.

65. The integrated droplet actuator device of any of claims 63 and following, wherein the gel comprises a gel slab.

66. The integrated droplet actuator device of any of claims 63 and following, wherein the gel slab comprises an electrophoresis gel.

67. The integrated droplet actuator device of any of claims 63 and following, further comprising at least a first heat source and a second heat source corresponding to the at least first reaction zone and a second reaction zone respectively.

68. The integrated droplet actuator of any of claims 67 and following, wherein the at least first heat source and second heat source each comprise a heater bar.

69. The integrated droplet actuator of any of claims 68 and following, wherein each heater bar comprises an aluminum heater bar, the aluminum heater bar further comprising one or more heating resistors and thermistors.

70. The integrated droplet actuator of any of claims 63 and following, wherein one of the at least first or second reaction zones is heated to a melting temperature.

71. The integrated droplet actuator of any of claims 70 and following, wherein the melting temperature comprises a temperature sufficient for denaturing of DNA template and primers.

72. The integrated droplet actuator of any of claims 70 and following, wherein the melting temperature comprises about 95 °C.

73. The integrated droplet actuator of any of claims 63 and following, wherein one of the at least first or second reaction zones is heated to a temperature sufficient for annealing of DNA template and primer extension.

74. The integrated droplet actuator of any of claims 73 and following, wherein the temperature sufficient for annealing of DNA template and primer extension comprises a temperature in a range of about 55 °C to about 65 °C.

75. The integrated droplet actuator of any of claims 63 and following, wherein the at least first reaction zone and second reaction zone are positioned at a sufficient distance from the gel so as to not affect the gel internal temperature.

76. The integrated droplet actuator device of any of claims 63 and following, wherein the gel comprises an electrophoresis gel.

77. The integrated droplet actuator device of any of claims 63 and following, wherein the gel comprises a gel slab.

78. The integrated droplet actuator device of any of claims 63 and following, wherein the gel comprises one or more loading slots formed in the gel.

79. The integrated droplet actuator device of any of claims 78 and following, wherein the one or more loading slots are aligned with and in fluid connection with the one or more fluid paths.

80. The integrated droplet actuator device of any of claims 78 and following, wherein the one or more loading slots comprise nucleic acid loading slots.

81. The integrated droplet actuator device of any of claims 78 and following, wherein the one or more loading slots are loaded with a loading buffer.

82. The integrated droplet actuator device of any of claims 78 and following, wherein the loading buffer is retained in the one or more loading slots by surface tension forces.

83. The integrated droplet actuator device of any of claims 78 and following, wherein the one or more fluid paths further provide a fluidic path from one or more designated electrodes to one or more corresponding loading spots.

84. The integrated droplet actuator device of any of claims 78 and following, wherein a microfluidics-gel interface is formed where the one or more fluid paths and the one or more loading slots interface.

85. The integrated droplet actuator device of any of claims 78 and following, wherein the gel is in electrical contact with a pair of electrodes, wherein the electrodes are arranged at first and second locations in relation to the gel and parallel to the one or more loading slots.

86. The integrated droplet actuator device of any of claims 63 and following, wherein the pair of electrodes are directly attached to and integrated with the top substrate.

87. The integrated droplet actuator device of any of claims 63 and following, wherein the pair of electrodes are positioned in a corresponding pair of trenches molded into the gel slab, wherein the trenches are arranged at first and second locations in relation to the gel and facilitate an electrical interface that provides a uniform electrical field in the gel.

88. The integrated droplet actuator device of any of claims 87 and following, wherein the pair of trenches comprises an electrophoresis buffer.

89. The integrated droplet actuator device of any of claims 88 and following, wherein electrophoresis buffer comprises Tris-Borate-Edta (TBE).

90. The integrated droplet actuator device of any of claims 63 and following, wherein the pair of electrodes are arranged at first and second locations in contact with the gel.

91. The integrated droplet actuator device of any of claims 63 and following, wherein the pair of electrodes comprises platinum wire.

92. The integrated droplet actuator device of any of claims 63 and following, wherein one of the pair of electrodes is a negative electrode and one of the pair of electrodes is a positive electrode.

93. The integrated droplet actuator device of any of claims 63 and following, wherein electrophoresis is conduct without submerging the gel in an electrophoresis buffer.

94. The integrated droplet actuator device of any of claims 63 and following, wherein the gel is arranged in a protruded structure formed on the top substrate.

95. The integrated droplet actuator device of any of claims 63 and following, wherein the gel is submerging in an electrophoresis buffer during electrophoresis.

96. The integrated droplet actuator device of any of claims 63 and following, wherein the top substrate and the bottom substrate are attached to one another by an epoxy glue ring.

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

98. The integrated droplet actuator device of any of claims 63 and following, wherein the electrodes comprise a path of electrodes.

99. The integrated droplet actuator device of any of claims 63 and following, wherein the electrodes comprise electrowetting electrodes.

100. The integrated droplet actuator device of any of claims 63 and following, wherein the bottom substrate comprises a printed circuit board.

101. The integrated droplet actuator device of any of claims 63 and following, wherein the top substrate comprises a high transparency and low fluorescence plastic material.

102. The integrated droplet actuator device of any of claims 101 and following, wherein the plastic material is suitable for fluorimeter operation.

103. The integrated droplet actuator device of any of claims 101 and following, wherein the plastic material comprises cyclo-olefin polymer and/or copolymer.

104. The integrated droplet actuator device of claim 63, wherein the droplet operations gap comprises a filler fluid.

105. The integrated droplet actuator device of claim 104, wherein the filler fluid comprises oil.

106. The integrated droplet actuator device of claim 105, wherein the oil comprises silicone oil.

107. The integrated droplet actuator device of any of claims 63 and following, wherein the bottom substrate comprises a hydrophobic layer disposed on a surface of the bottom substrate facing the gap.

108. The integrated droplet actuator device of any of claims 107 and following, wherein the hydrophobic layer is formed on top of the electrodes.

109. The integrated droplet actuator device of any of claims 63 and following, wherein the top substrate comprises a hydrophobic layer disposed on a surface of the top substrate facing the gap.

110. A method of conducting molecular analysis in an integrated droplet actuator device comprising:

(a) providing an integrated droplet actuator device for conducting molecular analysis comprising:

(i) a top substrate and a bottom substrate, the two substrates configured to form a droplet operations gap;

(ii) electrodes associated with one or both of the bottom substrate and the top substrate, and configured for conducting droplet operations in the gap;

(iii) a gel arranged on the top substrate;

(iv) a pair of electrodes in electrical contact with the gel, the electrodes arranged at first and second locations in relation to the gel;

(v) one or more fluid paths extending from inside the gap to the gel; and

(vi) at least a first reaction zone and a second reaction zone.

(b) positioning a reaction droplet on an electrode in a first reaction zone and incubating the reaction droplet in the first reaction zone for a period of time;

(c) transporting the reaction droplet to the second reaction zone and incubating the reaction droplet in the second reaction zone for a period of time; and

(d) cycling the reaction droplet between at least the first reaction zone and at least the second reaction zone until a desired result is achieved.

111. The method of any of claims 110 and following, wherein the integrated droplet actuator device further comprises one or more loading slots formed in the gel and arranged in fluid connection with one or more of the fluid paths.

112. The method of any of claims 110 and following, wherein the gel comprises a gel slab.

113. The method of any of claims 110 and following, wherein the gel comprises an electrophoresis gel.

114. The method of any of claims 110 and following, wherein the integrated droplet actuator device further comprises at least a first heat source and a second heat source corresponding to the at least first reaction zone and a second reaction zone respectively.

115. The method of any of claims 110 and following, wherein the molecular analysis comprises real-time PCR.

116. The method of any of claims 115 and following, wherein the cycling comprises PCR thermocycling.

117. The method of any of claims 110 and following, wherein the reaction droplet comprises a PCR reaction droplet.

118. The method of any of claims 117 and following, wherein the PCR reaction droplet comprises components required for PCR amplification of a target DNA template.

119. The method of any of claims 118 and following, wherein the PCR reaction droplet further comprises components required for fluorescence detection of the amplified target DNA template.

120. The method of any of claims 119 and following, wherein the component for fluorescence detection comprises EvaGreen®.

121. The method of any of claims 110 and following, wherein the reaction droplet is incubated in the first reaction zone for a period of time sufficient for denaturing of DNA template and primers.

122. The method of any of claims 110 and following, wherein the reaction droplet is incubated in the second reaction zone for a period of time sufficient for annealing of DNA template and primer extension.

123. The method of any of claims 110 and following, wherein the first reaction zone is heated to a melting temperature.

124. The method of any of claims 123 and following, wherein the melting temperature comprises a temperature sufficient for denaturing of DNA template and primers.

125. The method of any of claims 123 and following, wherein the melting temperature comprises about 95 °C.

126. The method of any of claims 110 and following, wherein the second reaction zones is heated to a temperature sufficient for annealing of DNA template and primer extension.

127. The method of any of claims 126 and following, wherein the temperature sufficient for annealing of DNA template and primer extension comprises a temperature in a range of about 55 °C to about 65 °C.

128. The method of any of claims 110 and following, wherein transporting and cycling is mediated by electrodes by an electrowetting effect.

129. The method of any of claims 116 and following, wherein PCR thermocycling and gel electrophoresis is executed simultaneously on the integrated droplet actuator device.

130. An electrode configuration for real-time PCR on a multi-channel integrated droplet actuator device comprising:

(a) controlled electrode loops configured for conducting droplet operations arranged on a substrate of the integrated droplet actuator device, wherein each of the controlled electrode loops have at least a first reaction zone and a second reaction zone; and

(b) reservoir wells in fluidic connection with the controlled electrode loops, wherein the reservoir wells are adapted to dispense sample droplets on one or more electrodes of the controlled electrode loops.

131. The electrode configuration of any of claims 130 and following, further comprising at least one detection zone positioned within each of the controlled electrode loops.

132. The electrode configuration of any of claims 130 and following, wherein at least one of the sample droplets comprises a quantity of nucleic acid.

133. The electrode configuration of any of claims 132 and following, wherein the quantity of nucleic acid comprises DNA.

134. The electrode configuration of any of claims 130 and following, wherein at least one of the sample droplets comprises PCR reagents.

135. The electrode configuration of any of claims 134 and following, wherein the PCR reagents comprise PCR master mix and fluorescence dye.

136. The electrode configuration of any of claims 135 and following, wherein the fluorescence dye comprises EvaGreen®.

137. The electrode configuration of any of claims 130 and following, wherein the controlled electrode loops are independently controlled.

138. The electrode configuration of any of claims 130 and following, wherein one of the at least first or second reaction zones comprises a temperature in a range of about 55 °C to about 65 °C.

139. The electrode configuration of any of claims 130 and following, wherein one of the at least first or second reaction zones comprises a temperature of about 95 °C.

140. The electrode configuration of any of claims 131 and following, wherein the detection zone is positioned in at least one of the at least first reaction zone and second reaction zone.

141. The electrode configuration of any of claims 140 and following, wherein the detection zone is positioned within the at least first or second reaction zones that comprise a temperature in the range of about 55 °C to about 65 °C.

142. A method of conducting real-time PCR in a multi-channel integrated droplet actuator device comprising:

(a) dispensing a sample droplet containing a quantity of sample material from a first reservoir onto one or more electrodes of a controlled electrode loop arranged on a substrate of the integrated droplet actuator device;

(b) dispensing a PCR reagent sample droplet from a second reservoir onto one or more electrodes of a controlled electrode loop arranged on a substrate of the integrated droplet actuator device;

(c) merging and mixing the sample droplet and the PCR reagent sample droplet to form a combined droplet;

(d) thermocycling the combined droplet between at least two temperature zones positioned on the controlled electrode loop; and

(e) detecting a quantity of amplified sample material within the combined droplet.

143. The method of any of claims 142 and following, wherein the sample droplet comprises a quantity of DNA.

144. The method of any of claims 142 and following, wherein the PCR reagent sample droplet comprises PCR master mix and fluorescence dye.

145. The method of any of claims 144 and following, wherein the fluorescence dye comprises EvaGreen®.

146. The method of any of claims 142 and following, wherein at least one of the at least two temperature zones comprise a temperature in a range of about 55 °C to about 65 °C.

147. The method of any of claims 142 and following, wherein at least one of the at least two temperature zones comprise a temperature of about 95 °C.

148. The method of any of claims 142 and following, wherein the amount of amplified sample material is detected using a fluorimeter.

149. The method of any of claims 127 and following, wherein real-time PCR is conducted in at least one channel of the multi-channel integrated droplet actuator and one or more sample processing operations are conducted in at least one or more of the other channels of the multi-channel integrated droplet actuator.

150. The method of any of claims 149 and following, wherein the one or more sample processing operations comprise sample dilution.

151. The method of any of claims 149 and following, wherein the one or more sample processing operations comprise fluorescence detection of a sample material quantitation assay.

152. The method of any of claims 151 and following, wherein the sample material quantitation assay comprises a PicoGreen® assay.

153. A method of diluting a sample in an integrated droplet actuator comprising:

(a) dispensing a droplet containing sample material on one or more substrates of an integrated droplet actuator;

(b) merging the droplet with a buffer droplet and mixing to form a combined droplet;

(c) splitting the combined droplet into two essentially equal diluted droplets; and

(d) repeating (a) - (c) until a value within a range sufficient for sample analysis is achieved.

154. The method of any of claims 153 and following, wherein one or more of combining, mixing and splitting is mediated by electrodes.

155. The method of any of claims 154 and following, wherein one or more of combining, mixing and splitting is mediated by electrodes by an electrowetting effect.

156. A method of conducting multiple assays on an integrated droplet actuator comprising:

(a) providing an integrated droplet actuator having one or more reagent reservoirs, at least one sample reservoir; and an electrophoresis gel;

(b) dispensing an initial sample droplet comprising a quantity of sample material from the at least one sample reservoir onto a substrate of the integrated droplet actuator;

(c) quantitating the concentration of the sample material in the dispensed initial sample droplet;

(d) determining, using the total quantified sample material concentration of (c), a sample droplet volume having an appropriate amount of sample material required for conducting gel electrophoresis;

(e) dispensing the required sample droplet volume determined in (d) from the at least one sample reservoir onto the substrate of the integrated droplet actuator;

(f) transporting the required sample droplet volume to the electrophoresis gel and conducting electrophoresis-based assay;

(g) dispensing another sample droplet from the at least one sample reservoir onto the substrate of the integrated droplet actuator and mixing with a reagents droplet dispensed from the one or more reagents reservoir to form a combined droplet; and

(h) analyzing the combined droplet and a sample material standard droplet having a known sample material quantity, to determine the quantity of sample material present in the sample.

157. The method of any of claims 156 and following, wherein quantitating comprises:

(a) merging and mixing a sample droplet with a droplet comprising a quantitation reagent to form a combined droplet;

(b) measuring fluorescence of the combined droplet;

(c) merging and mixing a standard droplet having a known sample material concentration with a droplet comprising a quantitation reagent to form a combined standard droplet;

(d) measuring fluorescence of the standard droplet and of the combined standard droplet; and

(e) comparing the fluorescence of the combined droplet with the fluorescence of the combined standard droplet to determine the sample material concentration in the sample droplet.

158. The method of any of claims 157 and following, wherein the quantitation reagent comprises PicoGreen®.

159. The method of any of claims 156 and following, wherein analyzing comprises real-time PCR.

160. The method of any of claims 156 and following, wherein the reagents droplet comprises a PCR reagents droplet.

161. The method of any of claims 156 and following, wherein the initial sample droplet is diluted using a dilution protocol.

162. The method of any of claims 161 and following, wherein the dilution protocol comprises a binary dilution protocol.

163. The method of any of claims 156 and following, wherein the multiple assays are conducted sequentially on the integrated droplet actuator.

164. The method of any of claims 156 and following, wherein the multiple assays are conducted simultaneously on the integrated droplet actuator.

165. The method of any of claims 156 and following, wherein the multiple assays comprise one or more of, or a combination of DNA quantification, real-time PCR, and gel electrophoresis.

166. The method of any of claims 156 and following, wherein the one or more reagent reservoirs comprise one or more of the following dilution buffer, PicoGreen® solution, PCR reagents, and a DNA standard.

167. The method of any of claims 166 and following, wherein the dilution buffer comprises TE buffer.

168. The method of any of claims 166 and following, wherein the DNA standard comprises human DNA.

169. The method of any of claims 166 and following, wherein the DNA standard comprises bacterial DNA.

170. The method of any of claims 156 and following, wherein the quantity of sample material comprises a quantity nucleic acid.

171. The method of any of claims 170 and following, wherein the quantity of nucleic acid comprises a DNA.

172. The method of any of claims 156 and following, wherein mixing is mediated by electrodes by an electrowetting effect.

173. The method of any of claims 156 and following, wherein transporting is mediated by electrodes by an electrowetting effect.

174. The method of any of claims 156 and following, wherein merging is mediated by electrodes by an electrowetting effect.

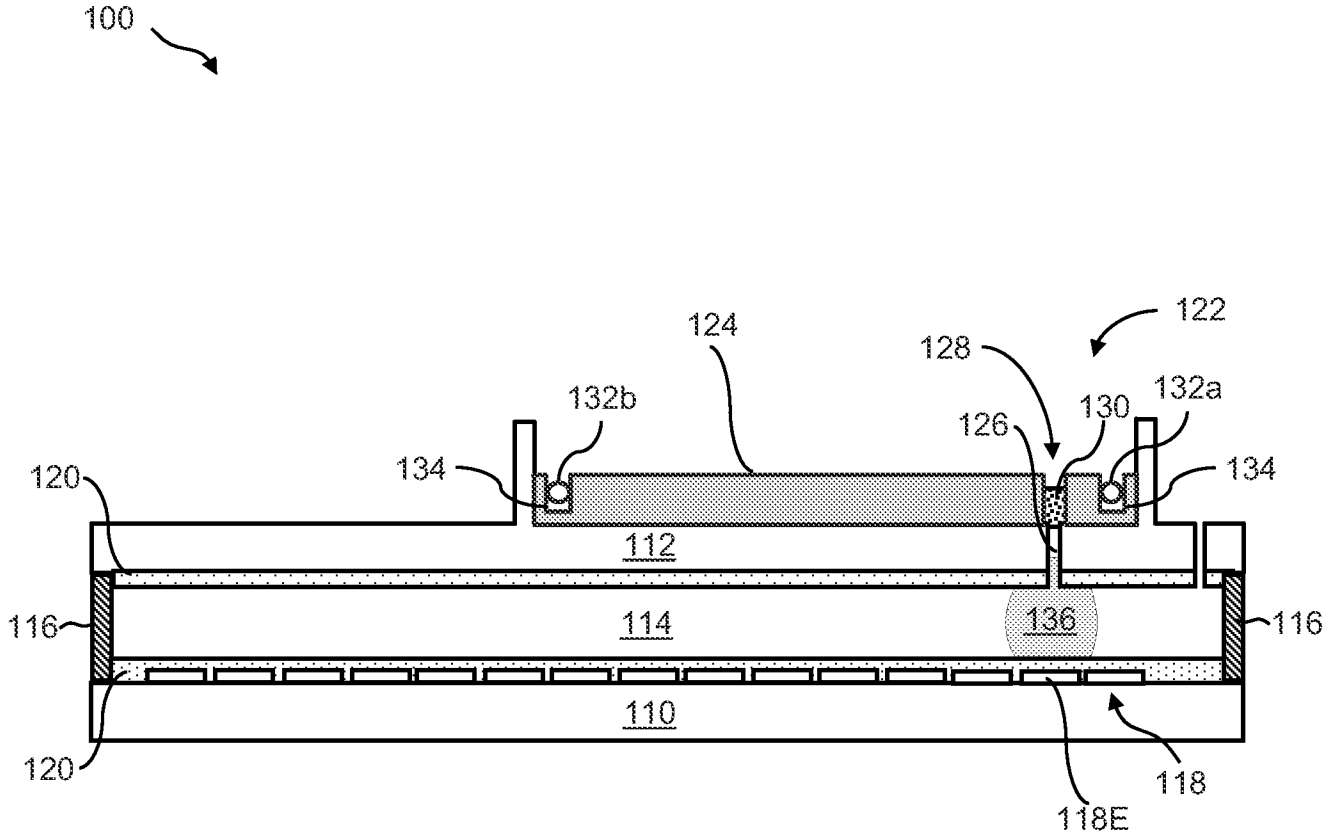
100
122
124
128
132b
132a
130
126
134
134
120
112
116
114
136
116
110
120
118
118E

Figure 1

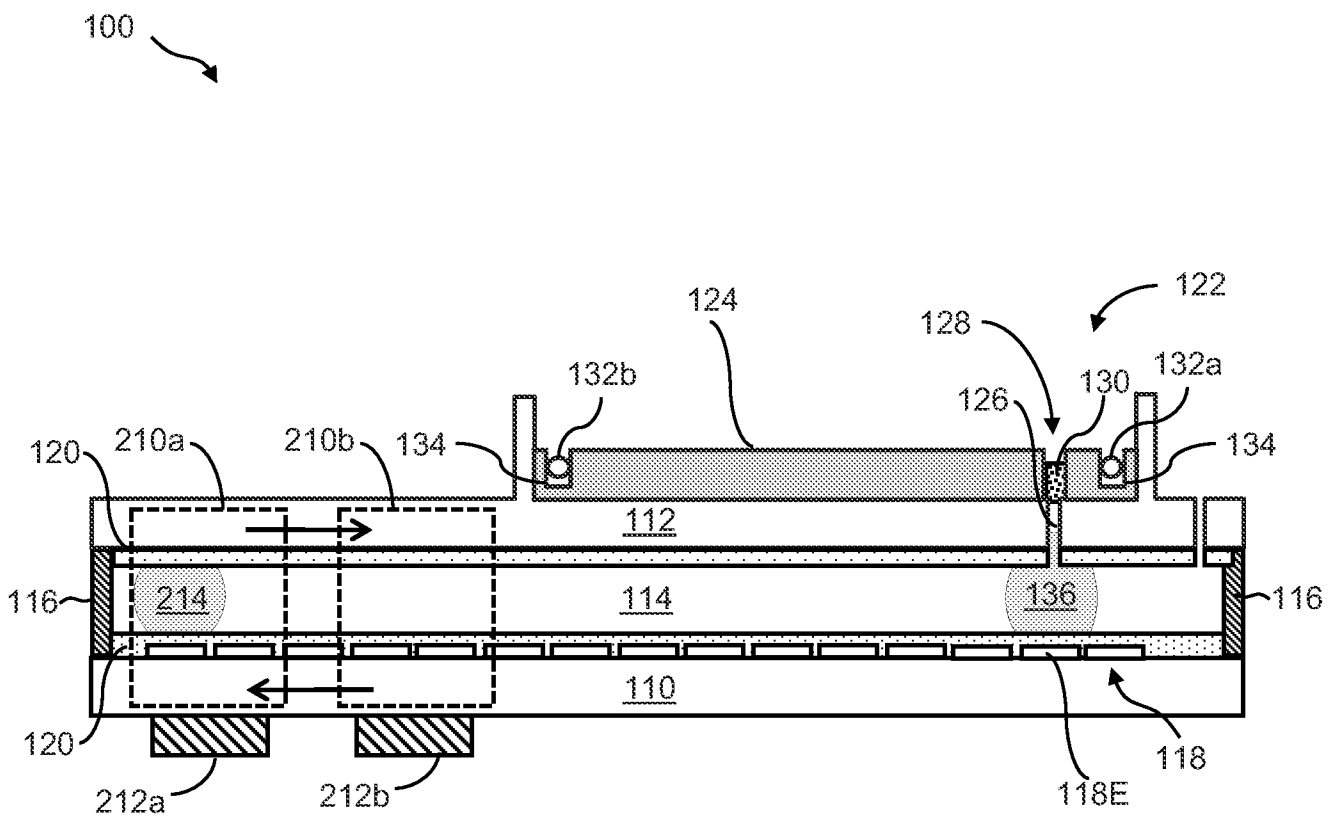
100
122
124
128
132b
126
130
132a
120
210a
210b
134
134
112
116
214
114
136
116
110
120
118
212a
212b
118E

Figure 2

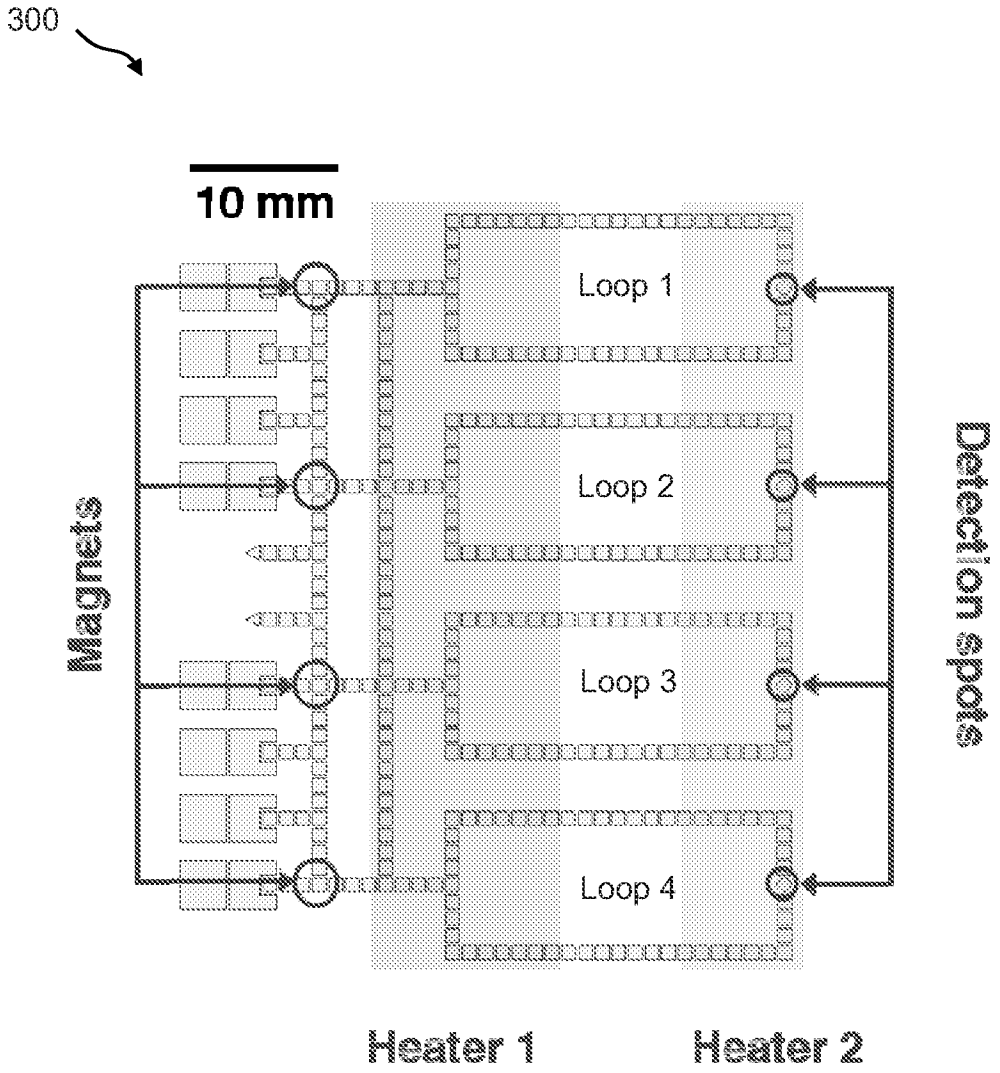
300
10 mm
Loop 1
Loop 2
Loop 3
Loop 4
Magnets
Detection spots
Heater 1
Heater 2

Figure 3

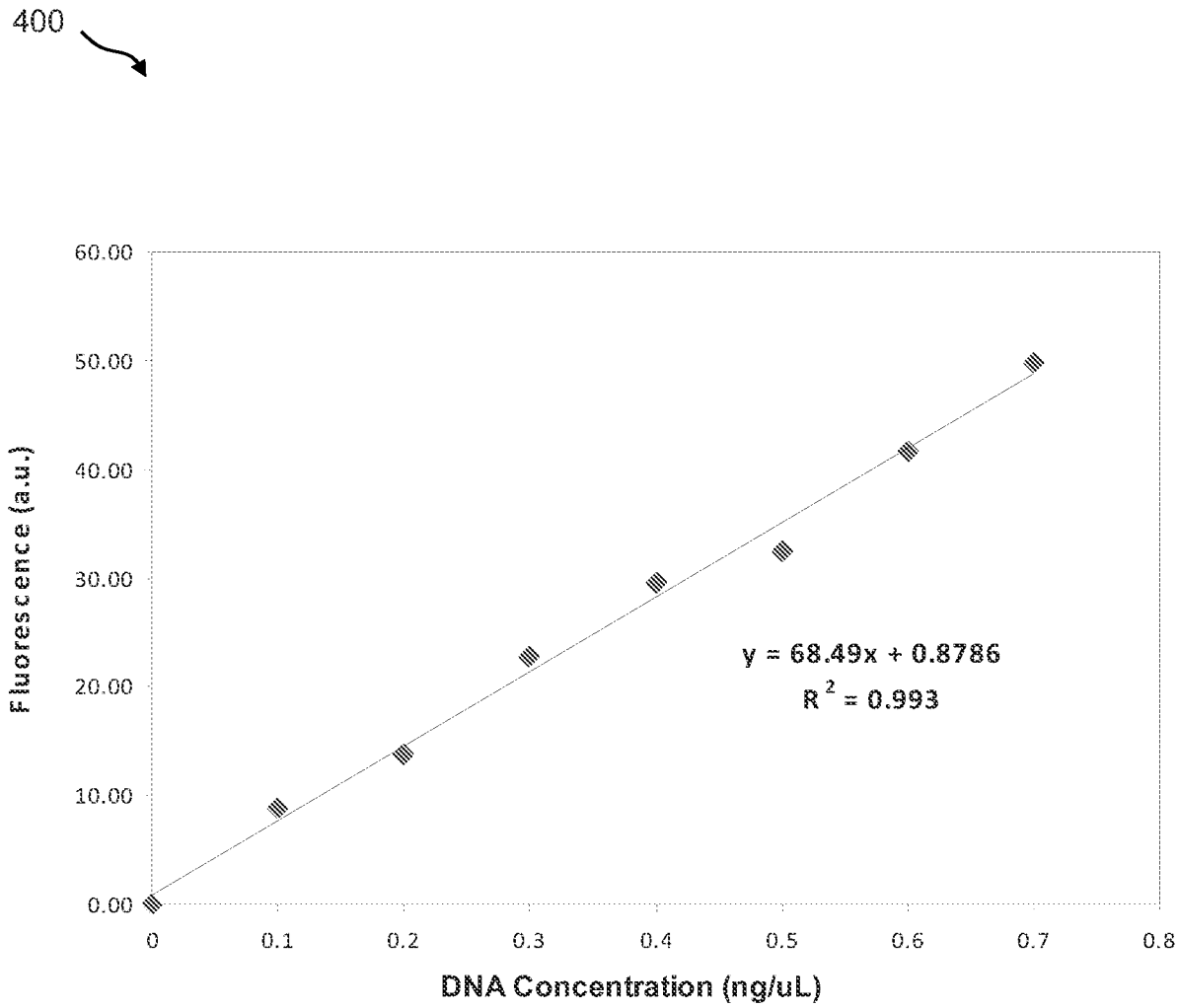
400
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Fluorescence (a.u.)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
DNA Concentration (ng/uL)
y = 68.49x + 0.8786
R 2 = 0.993

Figure 4

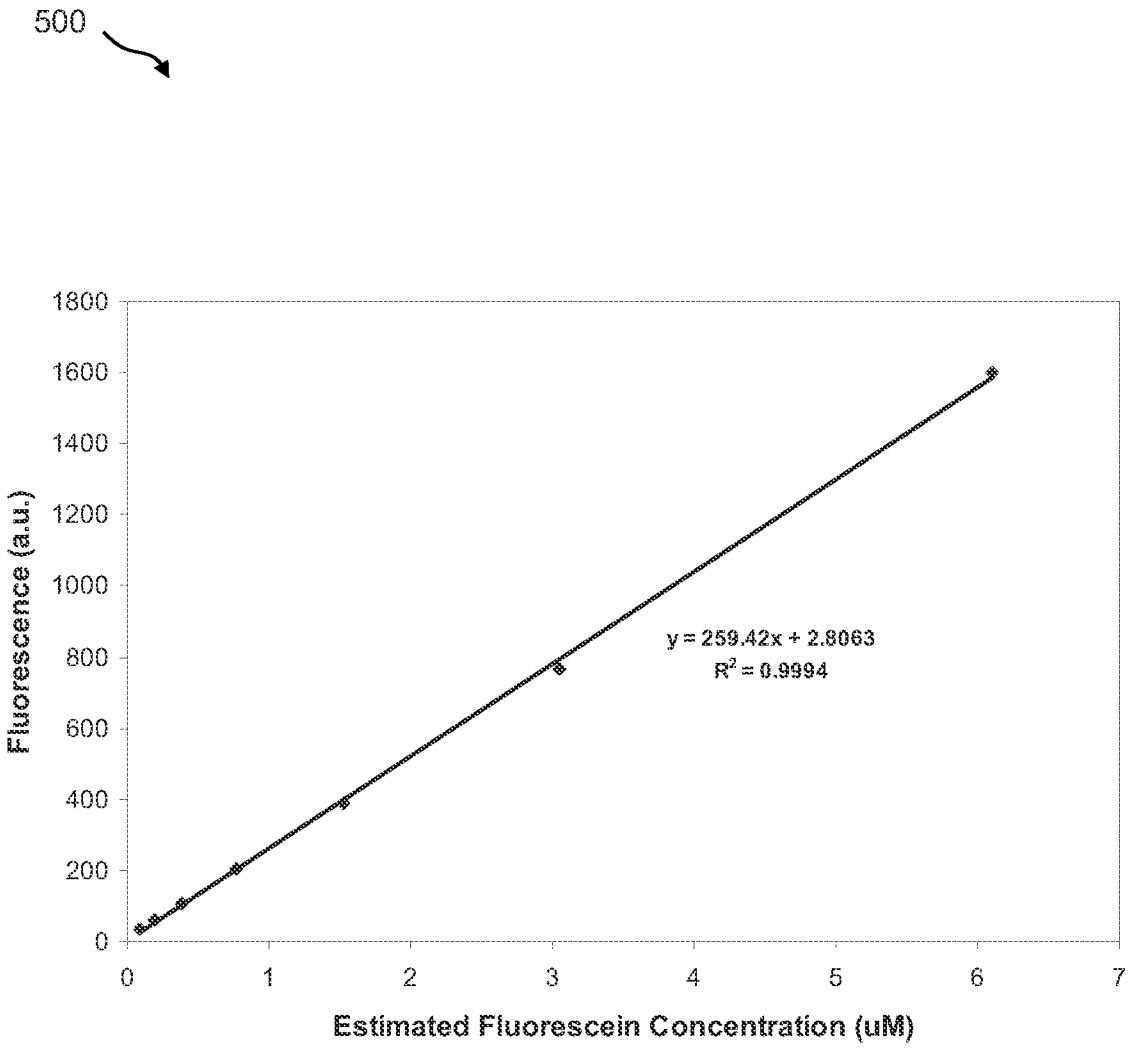
500
1800
1600
1400
1200
1000
800
600
400
200
0
Fluorescence (a.u.)
0
1
2
3
4
5
6
7
Estimated Fluorescein Concentration (uM)
y = 259.42x + 2.8063
R2 = 0.9994

Figure 5

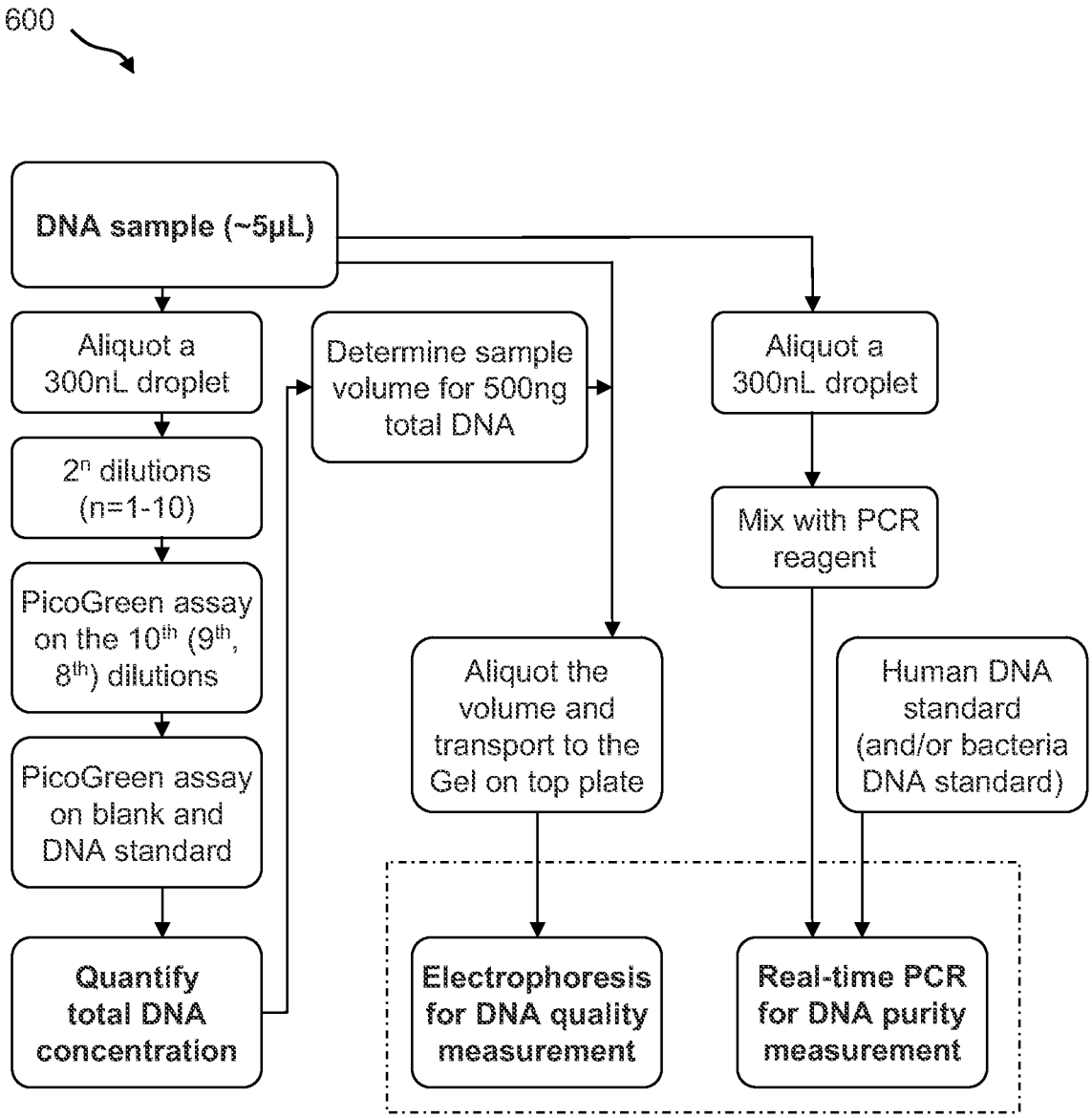
600
DNA sample (~5μL)
Aliquot a 300nL droplet
2^n dilutions (n=1-10)
PicoGreen assay on the 10th (9th, 8th) dilutions
PicoGreen assay on blank and DNA standard
Quantify total DNA concentration
Determine sample volume for 500ng total DNA
Aliquot the volume and transport to the Gel on top plate
Aliquot a 300nL droplet
Mix with PCR reagent
Human DNA standard (and/or bacteria DNA standard)
Electrophoresis for DNA quality measurement
Real-time PCR for DNA purity measurement

Figure 6