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## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Michael G. Pollack, et al.

Application Number: 60/745,058

Confirmation No. 9367

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For: FILLER FLUIDS IN DROPLET-BASED MICROFLUIDIC SYSTEMS

Asst. Commissioner for Patents

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On April 18, 2006 our office electronically filed a provisional patent application referenced herein and paid the filing fee through electronic funds transfer (EFT). Upon review of our account and official filing receipt dated April 25, 2006, it appears that a large entity filing fee of \$200.00 was charged instead of the small entity filing fee of \$100.00, as noted on our cover sheet. Therefore, Applicants herein request a refund for one-half of the large entity filing fee to Moore & Van Allen's deposit account no. 13-4365 in the amount of \$100.00.

Respectfully submitted,

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**PROVISIONAL APPLICATION FOR PATENT COVER SHEET - Page 1 of 2**

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. \_\_\_\_\_

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**TITLE OF THE INVENTION (500 characters max):**

**FILLER FLUIDS IN DROPLET-BASED MICROFLUIDIC SYSTEMS**

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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**ENCLOSED APPLICATION PARTS (check all that apply)**

☐ Application Data Sheet. See 37 CFR 1.76 ☐ CD(s), Number of CDs \_\_\_\_\_

☒ Specification Number of Pages **13** ☐ Other (specify) \_\_\_\_\_

☒ Drawing(s) Number of Sheets **3**

**Fee Due:** Filing Fee of \$200 (\$100 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is \$250 (\$125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

**METHOD OF PAYMENT OF FILING FEE AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT**

☒ Applicant claims small entity status. See 37 CFR 1.27. TOTAL FEE AMOUNT (\$)

☐ A check or money order is enclosed to cover the filing fee and application size fee (if applicable). 100.00

☐ Payment by credit card. Form PTO-2038 is attached

☒ The Director is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account Number: **13-4365**. A duplicative copy of this form is enclosed for fee processing.

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PTO/SB/16 (10-05)

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SIGNATURE \_\_\_\_\_



Date April 18, 2006

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Docket Number: 020303-000023.1PRV2

## **FILLER FLUIDS IN DROPLET-BASED MICROFLUIDIC SYSTEMS**

### **1 Field of the Invention**

The invention relates generally to multi-phase microfluidic systems and specifically to electrowetting-based droplet microfluidic systems.

### **2 Related Applications**

This patent application was co-filed with, and incorporates by reference, U.S. Patent Application Nos. 60/\_\_\_\_, entitled “Apparatus and Methods for Droplet-Based Blood Chemistry” [Attorney Ref. 20303-006.3PRV]; 60/\_\_\_\_, entitled “Apparatus and Methods for Droplet-Based PCR” [Attorney Ref. 20303-008.5PRV]; 60/\_\_\_\_, entitled “Apparatus and Methods for Droplet-Based Immunoassay” [Attorney Ref. 20303-009.6PRV]; 60/\_\_\_\_, entitled “Apparatus and Methods for Droplet-Based Protein Crystallization” [Attorney Ref. 20303-0022.7PRV2]; 60/\_\_\_\_, entitled “Droplet-Based Multi-Well Plate” [Attorney Ref. 20303-018.15PRV].

This application also incorporates by reference related provisional US Patent Application Nos. 60/736,399, entitled “Filler Fluids for Droplet-Based Microfluidics” filed on November 14, 2005; and 60/744,780, entitled “Apparatus and Methods for Droplet-Based Protein Crystallization,” filed on April 13, 2006.

### **3 Background of the Invention**

In droplet-based microfluidic systems a second fluid phase is required to fill the spaces between the droplets and we refer to this liquid or gas as a filler fluid. The selection of the filler fluid has many important consequences with respect to the dynamics and stability of the droplet phase and plays a very important role in the overall performance or capability of the system.

### **4 Brief Description of the Invention**

The invention provides various improvements in the selection and use of the filler fluid phase which are applicable to any multi-phase or droplet-based microfluidic implementation (see Section 0). The invention also provides methods of introducing or using the filler fluid which are generally applicable to any type of filler fluid (see Section 6.2). In certain embodiments using electrowetting, the invention involves the use of electrowetting force exerted on a body of liquid

can be used to manipulate the surrounding filler fluid or other adjacent fluid or material (see Section 6.3). In other embodiments specific to the use of electrowetting, the invention makes use of electrowetting force to control the thickness of a fluid film lying between the droplet and chip surface (see Section 6.4).

## 5 Terminology

Generally, the “**droplet**” or “**primary**” phase refers to the liquid which is either 1) directly microactuated or is 2) the medium in which the chemistry is performed.

The “**filler fluid**” or “**secondary**” phase is an ambient fluid which bathes the droplets in the system and is generally used to fill the space within the chip which is not occupied by droplets. “**Droplet**” in this context refers to any liquid body of any size and shape which has at least one free interface.

“**Chip**” refers generically to any solid structure which contains the droplets to operate as a microfluidic system.

## 6 Detailed Description of the Invention

In some embodiments the invention employs droplet manipulation structures and techniques described in 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 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., the disclosures of which are all incorporated herein by reference.

The digital microfluidic devices of our invention can be fabricated using PCB processes, such as the methods described in U.S. Patent Application No. 11/343,284, entitled “Apparatuses and Methods for Manipulating Droplets on a Printed Circuit Board,” filed on January 30, 2006, the entire disclosure of which is incorporated herein by reference. In some embodiments, devices of the invention provide a platform technology that avoids continuous-flow microfluidic technologies. PCB technology already has an extensive manufacturing base and the whole electronics industry is constantly pushing to advance the technology.

## 6.1 Use of Filler Fluid as an Inert Phase in Droplet Microfluidic Systems

Droplet-based microfluidic systems by definition have one or more free (i.e. fluid-fluid) interfaces consisting of a liquid-liquid or liquid-gas interface and these systems are described as multi-phase systems. Typically chemistry is performed in the primary (droplet) phase and the secondary phase merely serves as a filler fluid separating the droplets from each other. The secondary phase can be either a gas (e.g. air) or a liquid which is sufficiently immiscible with the primary liquid phase and can have a large influence on the overall performance of the system Figure 1. Most typically the primary phase is an aqueous solution and the secondary phase is air or an oil which is immiscible with water.

### 6.1.1 Factors influencing the Choice of a Gas Versus a Liquid Filler Fluid

*Trapping of air bubbles.* One advantage of using a gas filler fluid is that it is not susceptible to problems associated with the trapping or generation of gas bubbles. Unwanted gas bubbles can be introduced into a liquid filler fluid either at the interface between the chip and external atmosphere (for example, air can be introduced when loading fluid through an open access port) or can be generated internally by electrolysis, vapor heating or chemical reaction. Once introduced into a liquid-filled system, unwanted gas bubbles can be difficult to remove or dislodge and may interfere with microfluidic operations on the chip by preventing the flow or transport of the droplets. In liquid filled systems gas bubbles can be a major cause of system failure or irreproducible results.

*Control of droplet volume.* One of the primary advantages of using a liquid filler is that evaporation of the droplets can be prevented when the liquid filler completely bathes all surfaces of the droplet. When a gas is used as the filler fluid evaporation of the droplets may be a problem unless the gas pressure of the primary phase solvent in the gas phase is controlled -- for example, by humidifying the air in a water/air system. It should be emphasized that at submicroliter and nanoliter volumes the potential exists for the entire droplet volume to evaporate in minutes or even seconds unless precautions are taken to minimize the effects of evaporation. However, the degree to which evaporation presents a problem also depends on the expected time scale of the assay or microfluidic procedure. Evaporation reduces droplet volume which can interfere with microfluidic operations on the chip – for example a droplet may become too small to be transported to an adjacent electrode in an electrowetting-based system – in other types of systems changes in droplet volumes may likewise alter the fluid dynamics or operability of the system. Evaporation can also lead to concentration of certain species with the droplets which may alter

biochemical reactions on the chip or lead to less reproducible results. While the use of a liquid filler may prevent evaporation of the droplet, any miscibility between the two liquids can also result in shrinking (or swelling) of the droplets over time. Shrinking or swelling of droplets can, however, be prevented by saturating one or both phases with the equilibrium concentration of the other phase. In either case, whether a liquid or gas is used as the filler fluid, transport of material across the phase boundary should be taken into account and managed if necessary to maintain a constant droplet volume to insure reliable microfluidic operation and assay results.

*Contamination.* Cross-contamination between droplets is a major consideration when choosing a filler fluid. Generally, contamination within a chip can occur by two routes – 1) transfer of material between droplets through the filler fluid phase 2) transfer of material between droplets by contamination of chip surfaces. The use of gas as a filler fluid is advantageous for preventing contamination by diffusion through the filler fluid, although contamination of certain volatile species may still occur by vaporization into the gas phase. A liquid filler fluid is susceptible to contamination when contaminating or interfering molecules exhibit some solubility in both liquid phases and are able to move between droplets by diffusion. For example, when an oil is used as a filler fluid for an aqueous phase, oil soluble molecules may transfer between droplets by diffusion in the oil phase. Additionally, some molecules may exhibit a strong preference for one phase, so that even if contamination is not a concern, depletion of a particular molecular species may interfere which chemical reactions on the chip – for example oil-soluble reaction components may be depleted within the droplets in an oil/water system. Similarly, the choice of filler fluid may depend on the ability of a particular molecular species to be dissolved into and carried by the filler fluid, for example, an oxygen carrying fluid can be desirable to provide aerobic conditions for the droplets. To prevent cross-contamination by transfer of material from the liquid to solid phase (i.e. by sticking to the chip), a liquid filler fluid may be advantageous over a gas filler fluid since under certain conditions a liquid filler fluid minimizes contact between the droplet and chip surfaces. That is, a film of liquid may exist between the droplet and surface which prevents material within the droplet from coming into contact with and sticking to the surface and therefore being transferred to other droplets by this route – whereas in a gas filled system the solid and liquid will be direct contact. In addition to cross-contamination of material between droplets, a liquid film may also help prevent spoiling of the chip surface due to absorption or reaction of the droplet with the chip surface which may render the surface unusable. For example, it has been observed that high concentrations of certain proteins in water droplets readily stick to certain hydrophobic surfaces spoiling the hydrophobic nature of these surfaces – whereas the

same droplets can be moved across the same surfaces without appreciable sticking of proteins if bathed in an oil which minimizes contact between the two surfaces.

*Surface lubrication.* A liquid filler fluid may be advantageous for providing a lubricating layer between the droplet and chip surface. When the filler fluid forms a film between the droplet and chip surface this can have the effect of lubricating the droplet by preventing friction-like physical interactions between the droplet and surface.

### **6.1.2 Hybrid System Strategies**

One strategy for achieving a combination of the advantages of both a liquid and a gas filler fluid is to use a thin coating of a liquid filler fluid layer in an otherwise gas filled system. For example, the invention provides a microfluidic system including an enclosed system including a thin layer of oil layered on a chip, wherein the system is otherwise filled with a gas. The oil is of sufficient thickness to provide lubrication and reduce contamination through chip surfaces. Preferably the oil is selected to minimize transport of material between the droplet and oil phases.

Treatment of a Teflon AF surface with silicone oil can provide some of the lubrication benefit of silicone oil filler fluid even when operating in air. This approach can be used to prime the chip with a lubricating layer of oil, followed by replacement with air to allow samples to be loaded without introduction of bubbles, followed by re-introduction of oil to prevent evaporation of the samples. Thus the benefits of each kind of system are available depending on the type of microfluidic processing to be carried out.

Suitable liquid filler fluids include, without limitation, silicone oils, aliphatic and aromatic alkanes such as dodecane, hexadecane, and cyclohexane, hydrocarbon oils, mineral oils, paraffin oils, halogenated oils including fluorinated oils (e.g. 3M Fluorinert liquids).

Suitable gas filler fluids include, without limitation, air, argon, nitrogen, carbon dioxide, oxygen, humidified air, any inert gases.

In another embodiment, the filler fluid can be completely exchanged at different steps within a protocol. For example, a gas filler fluid can be introduced during sample loading to prevent trapping of air bubbles and then a liquid filler fluid can be pumped in to prevent evaporation of the liquid. Different types of filler fluid can be pumped into or out of the system depending on the particular assay steps to be performed.



In yet another embodiment, multiple filler fluids can be used within a single system. For example, a chip can be selected to have separate gas filled and liquid filled regions. Operations or certain types of droplets can be segregated between the different filler fluid regions.

### **6.1.3 Other Factors Affecting the Selection of Filler Fluids**

A combination of electrical, thermal, chemical, mechanical or optical properties may influence the choice or selection of the filler fluid. Some examples include:

*Selection based on refractive index.* The filler fluid may be selected based on its refractive index to either match the droplet to prevent refraction of light passing through or near the droplet. Alternatively the filler fluid may be selected with a refractive index that differs from the droplet to provide contrast for certain types of optical measurements or optical manipulations. For example, a filler fluid may be chosen to have a lower index of refraction than the primary liquid so that light can be transmitted through the primary liquid by total internal reflection. The primary phase can include highly elongated droplets which can serve as “light pipes” to convey light between two locations Figure 2.

*Selection based on color.* The filler fluid may be selected based on its color to provide contrast between the droplet and the filler fluid. This approach can enhance visualization of the different phases, for example to distinguish droplets from filler fluid or from air bubbles. In optical applications, the differential absorbance of the two phases can be used to modulate the color of light passing through the system. As another example, in applications where fluorescence measurements are made within droplets it may be desirable for the oil to be dyed to absorb the emitted wavelength of light to minimize cross-talk between reactions occurring in adjacent droplets.

*Selection based on thermal properties.* The filler fluid may be selected to have particular thermal properties that can either thermally insulate the droplets or conduct heat away from the droplets. For example, in applications where the temperature of the droplet is varied, such as PCR, a thermally conductive or low heat capacity filler fluid is desirable to permit rapid changes in temperature. For applications where a steady temperature is required a thermally insulating or high heat capacity filler fluid can be used to provide temperature stability.

*Selection based on phase change properties.* The filler fluid may be selected to undergo a phase change upon presentation of an appropriate stimulus. For example, a wax-like filler fluid (e.g. paraffin wax or octadecane) can be used where the filler fluid is changed from solid to liquid

form by application of heat. Lowering the temperature would return the filler fluid to a solid so that droplets would be contained within a solid matrix. This would be useful for certain applications which require long reaction or incubation steps, such as protein crystallization where a solid matrix would provide more stable conditions for the reaction. Encapsulation of the liquid phase within a solid would also facilitate storage and handling of the droplets in certain applications or allow for safe and convenient disposal of the materials following an assay. Alternatively, the phase change between solid and liquid can be used for valving purposes on the chip. For example, the filler fluid can be stored as a solid which is then heated to allow it to flow into and fill the chip. Or the immiscible filler fluid can be selected to be crosslinkable (by UV, heat, moisture or chemically) to create capsules of liquids within a solid shell.

*Selection based on gas permeability properties.* The filler fluid may be selected to have particular gas permeability or saturation properties. In certain applications a reaction occurring inside the droplet may consume oxygen or other gas which may need to be replenished by gas contained within or transported through the filler fluid. For example, some fluorinated oils have useful gas permeability properties for such applications. Alternatively, the filler fluid may be selected to exclude certain gases from the droplet, for example to maintain anaerobic conditions within the droplet. The filler fluid may be selected to have a certain degree of miscibility or partitioning into the droplet phase. Usually, complete lack of miscibility between the droplet and filler fluid is desired, but some applications may benefit from some limited degree of miscibility between the phases or partitioning of particular molecules between the phases. For example, in liquid-liquid extraction applications.. In certain applications where dissolved gases in the filler fluid may be problematic, a means for degassing the filler fluid prior to or during use may need to be provided. For example, filler fluid may be degassed by incubation under vacuum, heating, sparging or by centrifugation..

*Selection based on surface tension properties.* The filler fluid may be selected to have a particular surface or interfacial tension with the droplet phase or with the chip surfaces. The liquid-liquid surface tension may be selected to control the ease with which droplets can be split apart during dispensing or splitting operations. For example, filler fluids containing surfactants would be expected to reduce the liquid-liquid interfacial tension which in turn would reduce the amount of energy required to deform or stretch that interface would facilitate dispensing, splitting and other operations where the droplet is made to assume a non-spherical shape. The liquid-liquid interfacial tension has a major effect on the behavior of liquid films that may be present between the droplet and solid phases. Surfactants can be added to the filler fluid to stabilize these thin

films. Examples of suitable surfactants include Triton X-15 or Span 85. For example, in one embodiment the filler fluid is 2 cSt silicone oil and the surfactant is Triton X-15 at 0.1% w/w. Alternatively, the solid-liquid interfacial tension may be selected to control the wetting of the filler fluid on the chip surfaces, for example, to control the formation, thickness or behavior of thin films of the filler fluid between the droplet and chip surfaces or to control the wetting behavior of the fluid when filling or emptying it from the chip.

*Selection based on viscosity or volatility.* The filler fluid may be selected to have a particular viscosity or volatility. For example, a low viscosity liquid (e.g. 0.65 cSt. Silicone oil) facilitates transport of droplets through the oil while a low volatility filler fluid (e.g., 2, 5 or 10 cSt. Silicone oil) may be desirable to prevent loss of filler fluid by evaporation. In some applications, evaporation of the filler fluid can be desired, in which case the filler fluid can be selected to have a low volatility. Or the filler fluid may be selected to have a particular viscosity dependence on temperature, since the viscosity of the filler fluid affects the fluid dynamics and the temperature on the chip may vary. The filler fluid may be selected to have a particular molecular weight which would be important in certain applications like mass spectroscopy where the filler fluid can contaminate the measurement.

*Selection based on electrical properties.* The filler fluid may be selected to have particular electrical properties. For example, certain applications including electrowetting-on-dielectric favor the use of a filler fluid that is non-conductive (e.g., silicone oil). Or the dielectric permittivity can be selected to control the coupling of electrical energy into the system from external electrodes. In certain applications a non-conductive filler fluid can be employed as an electrical insulator or dielectric in which the droplet floats just above the electrodes without physically contacting them. For example, in an electrowetting system a layer of filler fluid (e.g., silicone oil) between the droplet and electrode can be used to provide electrostatic control of the droplet.

*Selection based on density.* The filler fluid may be selected to have a particular density relative to the droplet phase. A difference in density between the two phases can be used to control or exploit buoyancy forces acting upon the droplets. Examples of two-phase systems useful in this aspect of the invention include water/silicone oil, water/flourinert, and water/fluorosilicone oil. When one phase is buoyant, then that effect can be exploited in a vertical configuration as a means to transport one phase through the other. For example, a waste or collection well can exist at the top or bottom of the chip where droplets are delivered to that reservoir by simply releasing

them at an appropriate point and allowing them to float or sink to the target destination. Density differences can also be used as a means to control or engineer contact between the droplets and chip surfaces. For example, a droplet not normally contacting a top-plate can be released to sink or float to that surface to contact it. Density differences and buoyancy effects can also be exploited for sensing applications in which the movement of droplets is detected and related to a change in position, orientation or acceleration.

*Selection based on colloidal properties.* The filler fluid can be selected to be a colloid. Solid particles can be mixed with the filler fluid for a number of purposes including to provide a high extinction coefficient for effective absorption of light (e.g., titanium dioxide particles mixed in silicone oil). For example, solid particles can provide more effective absorption of light than a dye dispersed in the liquid. Alternatively, the particles can be selected to absorb or capture contaminants in the filler fluid or to release particular molecules into the filler fluid.

*Selection based on compatibility with chip surfaces.* The filler fluid should generally be selected for material compatibility with the chip surfaces. For example, certain filler fluids can etch, dissolve, contaminate, absorb into or otherwise be incompatible with certain chip materials. For example, fluorinated hydrocarbons such as Fluorinert may be incompatible with Teflon AF or Cytop surfaces because of their tendency to dissolve these materials, while silicone oils may be incompatible with PDMS surfaces due to the tendency of these materials to dissolve each other.

*Selection based on compatibility with chip surfaces.* The filler fluid should generally be selected for biochemical compatibility with materials located within the droplet. For example, the fluid can be selected for low cytotoxicity when live cells are contained within the droplet.

## **6.2 Introduction and Management of Filler Fluids in Droplet Microfluidic Systems**

The invention also provides means for controlling the introduction or circulation of the filler fluid within the system. In one mode of operation the filler fluid is injected once during the initialization of chip operation. The filler fluid may be provided from an external source using a syringe, dropper, pipettor, capillary, tube or other means. Alternatively, the filler fluid may be provided from a reservoir internal to the chip assembly or cartridge. As an example, the fluid can be contained within a sealed pouch which is punctured or pressed to transfer the liquid into the chip.

In another mode of operation a means can be provided for multiple introductions or recirculation of one or more filler fluids within the chip. A secondary fluid-handling system can be provided to inject and to remove fluid from within the chip. Pressure, gravity or other means such as the use of thermal gradients can be used to transport the filler fluid into or out of the chip. Such a system can be used for the following purposes:

1. To replenish filler fluid lost to evaporation or leakage over time. A slow steady flow or periodic injection of filler fluid can be employed to make up for any loss of filler fluid volume.
2. To provide “clean” filler fluid either continually or periodically to reduce contamination between droplets. The filler fluid can be cleaned either by completely replacing it or by circulating it through a filter or bed of absorbent material selected to remove contaminants.
3. To provide a means for transporting droplets to waste. For example, at the end of an assay, droplets can be released and allowed to flow with the filler fluid to the outlet providing a means to “flush” the chip. Flushing the chip can be performed to reset the status of the chip in preparation to perform additional assays.
4. To exchange the filler fluid when different fluids may be desired for certain steps, for example to replace oil with air to allow drying of droplets, or to replace one oil with a different oil.
5. To provide a means of controlling the temperature of the droplets by heating or cooling the fluid as it is circulated through the chip. For example, PCR can be performed in droplets containing the appropriate PCR reagents (e.g., primers, nucleotides, and polymerase) by circulating temperature controlled filler fluid through the chip to perform thermocycling. The temperature of the filler fluid entering and leaving the chip can be directly measured and the temperature and flow rate of the filler fluid can be adjusted to provide optimal temperature control inside the chip.

Alternatively, rather than using a global pool of filler fluid which bathes all droplets in the system. Local regions of filler fluid or even individual units of filler fluid for each droplet can be used. For example aqueous droplets can be encapsulated in an individual shell of oil which moves along with that droplet. Each such droplet would then have its own local bath of oil with the

space between encapsulated droplets filled with third immiscible liquid such as air or fluorsilicone oil. This approach can be used to minimize the transfer of material between droplets in the system by partitioning into the oil phase while retaining the advantageous properties of the oil with respect to evaporation and fouling of the surface. As shown in Figure 3 it has been demonstrated that a droplet can “drag” a shell or capsule of liquid filler fluid (e.g. oil) surrounding it. The shells of oil can be created by simply moving the droplet through an oil interface, pinching off a unit of oil as the droplet creates a bulge along the interface.

Hybrid systems can be implemented in which different regions of the chip are filled with different fluids. For example, samples can be processed under oil and then transported into an air portion to be evaporated for subsequent analysis by MS. Conversely, a sample can be collected in air and then processed under oil.

### **6.3 Indirect Manipulation of Materials by Electrowetting**

Certain liquid exhibit an electrowetting effect which is sufficient to directly enable transport of droplets of those liquids as “electrowettable”. Other fluids which are non-responsive to electrowetting we refer to as “non-electrowettable”. Non-electrowettable fluids include the oils and hydrocarbons typically used as liquid filler fluids as well as gases and solid materials. Non-electrowettable liquids may also include electrowettable solvents which have been rendered partially or completely non-electrowettable by the addition of certain materials such as proteins, surfactants, particles, or by the application of elevated temperature or other effects.

Although non-electrowettable fluids cannot be directly manipulated by electrowetting forces, an electrowettable liquid can be employed as an intermediate for manipulation of non-electrowettable fluids by the electrowetting effect, e.g., by one of the following methods:

1. Droplets of the electrowettable liquid phase can be used to “push” droplets or bubbles of the non-electrowettable liquid Figure 4. That is the translational force of the droplet due to electrowetting is directly communicated to the non-electrowettable bubble, droplet or liquid body. For example, air bubbles can be introduced into the system. Air bubbles can be transported, divided and otherwise manipulated indirectly using the actuated fluid. Air bubbles can be introduced from the external environment or generated on chip by electrolysis or heating.
2. Droplets of the electrowettable liquid phase can be used to encapsulate solid-particles (e.g., latex microspheres) or droplets of non-electrowettable fluids and serve as

microcontainers for transportation and micromanipulation of the non-electrowettable material Figure 4. For example, an oil droplet can be engulfed by a water droplet with the water droplet then serving as a vehicle for transporting the oil droplet with a third fluid used as a filler fluid.

3. A segment of liquid can be used to produce a hydraulic force on a filler fluid in a channel. This hydraulic force can be transmitted through the channel to manipulate a non-electrowettable fluid in the channel. Fluid manipulation can also be performed by reverse-electrowetting where non-compatible solids, or gasses are manipulated indirectly by displacement using a compatible surrounding medium.
4. An electrowetting fluid can be used to create dynamic walls or reservoirs for separating different non-electrowetting fluids in a microfluidic system. These dynamic walls can be moved or extended or contracted to control the mixing or interaction of the non-electrowetting fluids. Or the electrowetting fluid can be used to form virtual wells for containing liquids or separating different liquids.

Multi-phase systems consisting of co-immiscible liquid phases and multiple solid phases can also be employed. Solid phases can exist as the surfaces of beads or surfaces of the chip. Liquid phases can include immiscible liquids, e.g., as the electrowetting fluid or filler fluid.

#### **6.4 Manipulation of Fluid Film Thickness**

When a liquid filler fluid is used in a droplet-based microfluidic system, film of the liquid may be present between the droplet and solid surface of the chip Figure 5. The behavior of this film may significantly affect the behavior of droplets within the system by mediating surface interactions between the droplet and chip surfaces. For example, the film may prevent fouling of the chip surface by minimizing contact between the surface and surface-fouling molecules located within the droplet. The film may also provide a lubricating layer to facilitate movement of liquid in the system.

The thickness of the fluid film if it exists can be influenced by an electrical field applied between the droplet and an electrode located within the chip. The application of a voltage between two conducting surfaces capable of storing energy (e.g. a capacitance) creates an electrostatic attraction force between the two surfaces. If one of the conducting surfaces is free to move, then this electric field has the effect of drawing the two surfaces together (i.e. “squeezing” out the liquid film). Assuming that a retarding force exists which opposes the electrostatic force, then the

gap between the two surfaces will be dependent on the magnitude of the applied voltage. Thus the thickness of a film of filler fluid can be electrostatically tuned to vary the proximity or degree of contact between the two surfaces. This gives rise to several possible applications in droplet-based microfluidic systems.

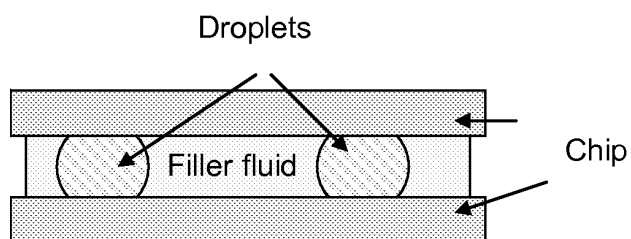
For example, a droplet may contain material, such as a protein, that one wishes to selectively deposit on a chip surface. The droplet can be transported to a desired location and then brought into intimate contact with the surface through electrostatic means to deposit a material onto the surface, or to allow material on the chip surface to be transferred to the droplet or to allow a component of the droplet to react with a component located on the chip surface Figure 6.

When light is carried by the droplet phase, the filler fluid film thickness can be varied to control the escape of light from the chip. For example, a light pipe in which light is contained by total internal reflection can be created where the filler fluid serves as the cladding. Contact with the droplet and surface can be controlled to permit light to escape wherever the droplet directly contacts the surface.

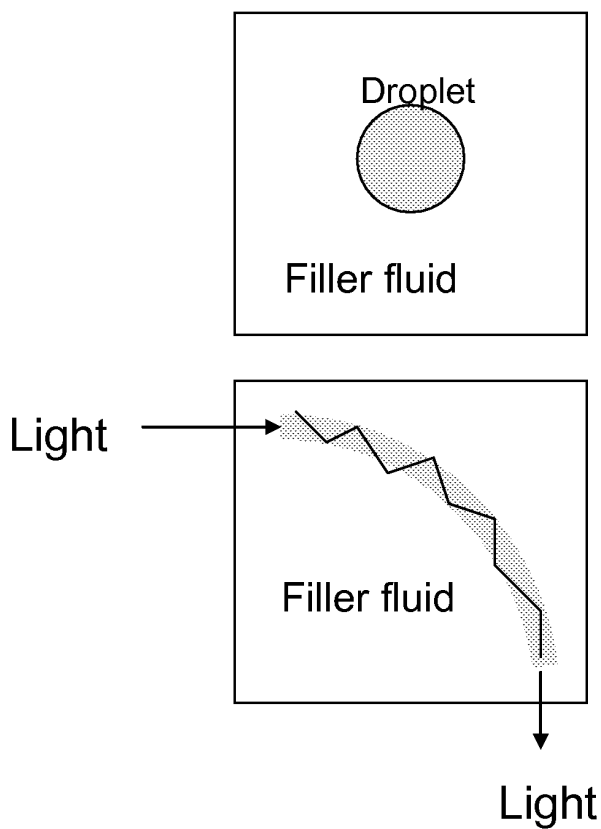
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*This specification is divided into sections for the convenience of the reader only. Headings should not be construed as limiting of the scope of the invention.*

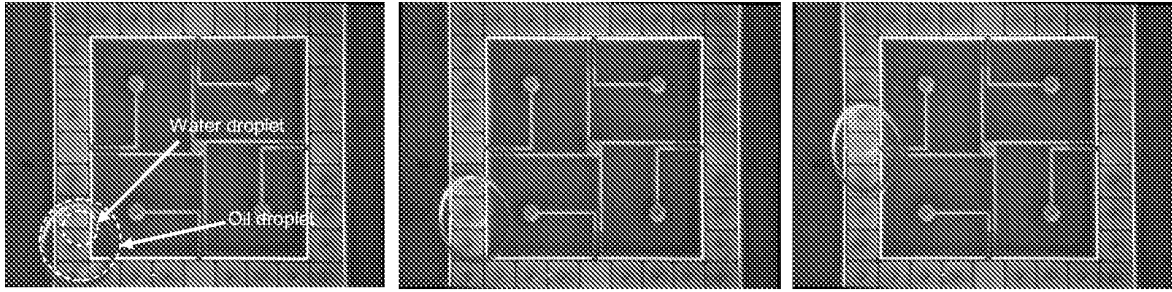




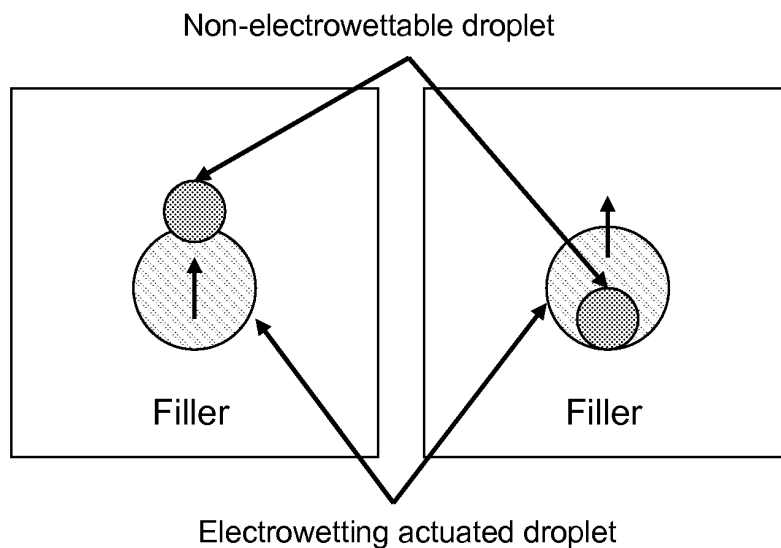
**Figure 1:** Example of filler fluid in a droplet-based microfluidic system (side view).



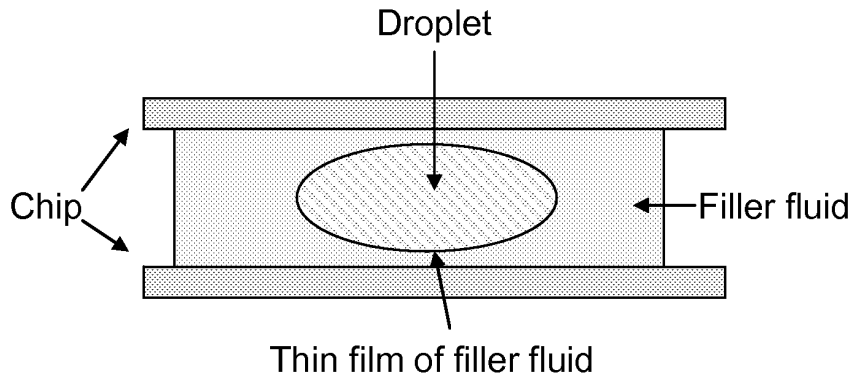
**Figure 2:** A liquid droplet is deformed to create a “light-pipe” (top-view). Because the filler fluid has a lower index of refraction than the droplet, light may experience total internal reflection and is conveyed through the path formed by the droplet liquid.



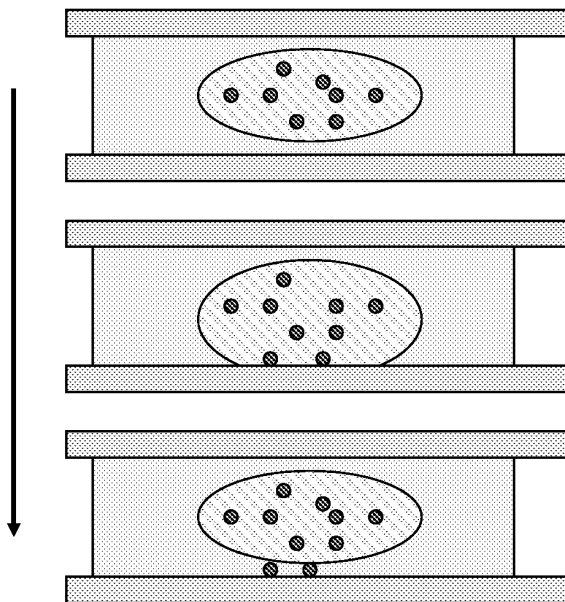
**FIGURE 3:** Successive frames from a video showing a water droplet surrounded by a capsule of silicone oil in turn surrounded by air being transported on an electrowetting array



**FIGURE 4:** An electrowetting-actuated droplet may be used to exert a force on an external liquid droplet or bubble (left) or may be used as a vessel to internally transport a droplet of another liquid or bubble of gas (right).



**FIGURE 5:** Even when the droplet is sandwiched between two chip surfaces, a thin layer or film of filler fluid may exist between the droplet and chip surface (side view).



**FIGURE 6:** Deposition of material from the droplet onto chip surface by momentarily bringing the surfaces into intimate contact (side view).

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Filer:		William A. Barrett/Katrina Holland		
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1	Transmittal letter	Provisional_Application_Cover_Sheet.pdf	87535	no	2
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