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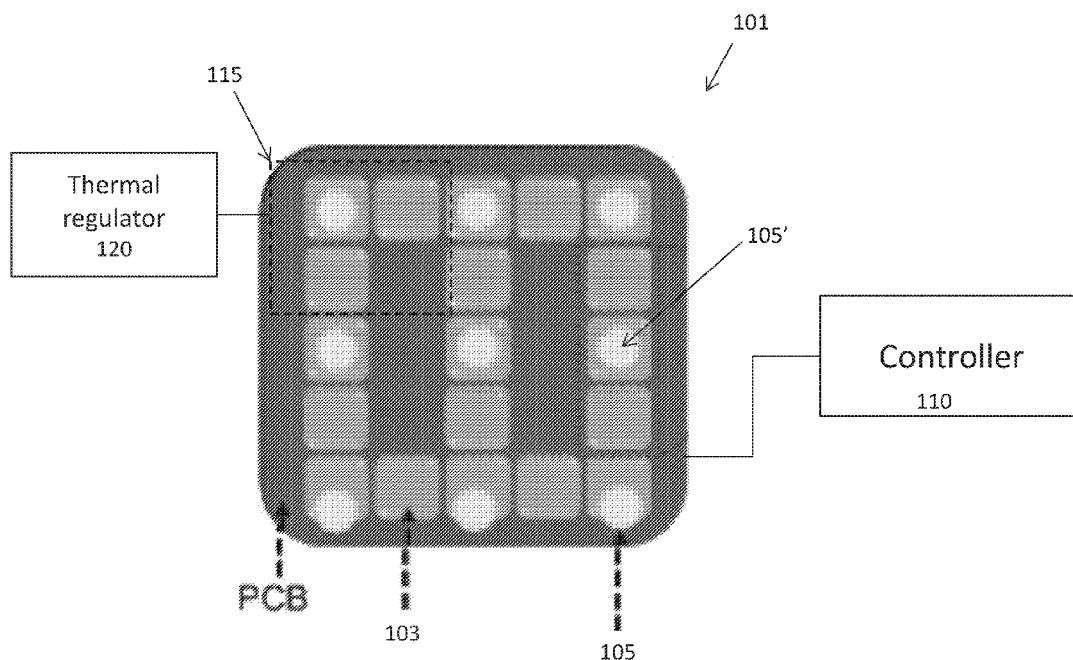


FIG. 1

(57) Abstract: Air-matrix digital microfluidics (DMF) apparatuses and methods of using them to prevent or limit evaporation and surface fouling of the DMF apparatus. In particular, described herein are air-matrix DMF apparatuses and methods of using them including thermally controllable regions with a wax material that may be used to selectively encapsulate a reaction droplet in the air gap of the apparatus; additional aqueous droplets may be combined with the encapsulated droplet even after separating from the wax, despite residual wax coating, by merging with an aqueous droplet having a coating of a secondary material (e.g., an oil or other hydrophobic material) that may remove the wax from the droplet and/or allow combining of the droplets.



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**DIGITAL MICROFLUIDIC APPARATUSES AND METHODS FOR
MANIPULATING AND PROCESSING ENCAPSULATED DROPLETS**

CROSS REFERENCE TO RELATED APPLICATIONS

5 [0001] This patent application claims priority to U.S. provisional patent applications no. 62481488, titled “DIGITAL MICROFLUIDICS APPARATUSES AND METHODS FOR MANIPULATING AND PROCESSING ENCAPSULATED DROPLETS,” and filed on April 4, 2017; U.S. provisional patent application no. 62553743, titled “DIGITAL MICROFLUIDICS DEVICES AND METHODS OF USING THEM,” filed on September 1, 2017; and U.S. 10 provisional patent application no. 62557714, titled “DIGITAL MICROFLUIDICS DEVICES AND METHODS OF USING THEM,” filed on September 12, 2017, each of which is herein incorporated by reference in its entirety.

[0002] This patent application may be related to U.S. patent application no. 15/579,455, titled “AIR-MATRIX DIGITAL MICROFLUIDICS APPARATUSES AND METHODS FOR 15 LIMITING EVAPORATION AND SURFACE FOULING,” filed on June 6, 2016, which claimed priority to U.S. Provisional Application 62/171,756 entitled, “DEVICE AND METHODS FOR LIMITING EVAPORATION AND SURFACE FOULING,” filed on June 5, 2015, which is herein incorporated by reference in its entirety.

20 **INCORPORATION BY REFERENCE**

[0003] All publications and patent applications mentioned in this specification are herein incorporated by reference in their entirety to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

25 **FIELD**

[0004] Air-matrix digital microfluidic (DMF) apparatuses and methods for manipulating and processing encapsulated droplets are described herein.

BACKGROUND

30 [0005] Microfluidics has transformed the way traditional procedures in molecular biology, medical diagnostics, and drug discovery are performed. Lab-on-a-chip and biochip type devices have drawn much interest in both scientific research applications as well as potentially for point-of-care applications because they carryout highly repetitive reaction steps with a small reaction volume, saving both materials and time. While traditional biochip-type devices utilize micro- or 35 nano- sized channels and typically require corresponding micropumps, microvalves, and

microchannels coupled to the biochip to manipulate the reaction steps, these additional components greatly increase cost and complexity of the microfluidic device.

[0006] Digital microfluidics (DMF) has emerged as a powerful preparative technique for a broad range of biological and chemical applications. DMF enables real-time, precise, and highly flexible control over multiple samples and reagents, including solids, liquids, and even harsh chemicals, without need for pumps, valves, or complex arrays of tubing. In DMF, discrete droplets of nanoliter to microliter volumes are dispensed from onto a planar surface coated with a hydrophobic insulator, where they are manipulated (transported, split, merged, mixed) by applying a series of electrical potentials to an embedded array of electrodes. Complex reaction steps can be carried out using DMF alone, or using hybrid systems in which DMF is integrated with channel-based microfluidics.

[0007] Despite significant advances, both evaporation, particularly in air-matrix DMF, and surface fouling remains issues. Surface fouling occur when components from the reaction mixture irreversibly adheres to surfaces of the microfluidic or DMF device after contacting these surfaces. Surface fouling is a particularly acute problem when operating a higher (e.g., greater than 37°C) temperatures. Various strategies have been proposed to prevent surface fouling, such as using polymers, glass, and metals to fabricate the microfluidic channels or chemical modification of material surfaces. However, these strategies have had limited success, particularly in the context of DMF, despite efforts to test and fabricate surfaces and surface coatings that are resistant to surface fouling. In some instances, a coating intended to prevent surface fouling may cause undesirable interactions and secondary reactions with the reaction mixture and/or reagents used. In general, it would be desirable to have a simple solution to minimizing surface fouling in microfluidic and DMF devices.

[0008] Evaporation is also a concern when performing reactions in an air-matrix DMF device. In general, an air-matrix DMF apparatus may refer to any non-liquid interface of the DMF apparatus in which the liquid droplet being manipulated by the DMF apparatus is surrounded by an air (or any other gas) matrix. As used herein, an air-matrix may also and interchangeably be referred to as a “gas-matrix” DMF apparatus; the gas does not have to be air, though it may be. Evaporation may be especially problematic in air-matrix DMF methods and that heat for a prolonged period of time (e.g., greater than 30 seconds). Evaporation limits the utility of air-matrix DMF, because enzymatic reactions are often highly sensitive to changes in reactant concentration. Largely for this reason, others have attempted to use oil-matrix DMF for biochemical applications, despite numerous drawbacks including: the added complexity of incorporating gaskets or fabricated structures to contain the oil; unwanted liquid-liquid extraction of reactants into the surrounding oil; incompatibility with oil-miscible liquids (e.g.,

organic solvents such as alcohols); and efficient dissipation of heat, which undermines localized heating and often confounds temperature-sensitive reactions. Another strategy for addressing evaporation has been to place the air-matrix DMF device in a closed humidified chamber, but this often results in unwanted condensation on the DMF surface, difficult and/or limited access to the device, and a need for additional laboratory space and infrastructure.

[0009] It has also been proposed to address evaporation by transferring reaction droplets from the air-matrix DMF device to microcapillaries, where they can be heated in dedicated off-chip modules without evaporation problems. However, this complicates design and manufacture of the air-matrix DMF device, and introduces the added complications of microcapillary interfaces and coordination with peripheral modules.

[0010] Thus, there exists a need for air-matrix DMF apparatuses and methods that may prevent or limit evaporation and/or prevent or limit surface fouling. Described herein are apparatuses and methods that may address this need.

SUMMARY OF THE DISCLOSURE

[0011] A typical DMF apparatus may include parallel plates separated by an air gap; one of the plates (typically the bottom plate) may contain a patterned array of individually controllable actuation electrodes, and the opposite plate (e.g., the top plate) may include one or more ground electrode. Alternatively, the one or more ground electrode(s) can be provided on the same plate as the actuating (e.g., high-voltage) electrodes. The surfaces of the plates in the air gap may include a hydrophobic material which may be dielectric or in some variations an additional dielectric layer may be included. The hydrophobic and/or dielectric layer(s) may decrease the wettability of the surface and add capacitance between the droplet and the control electrode.

Droplets may be moved or otherwise manipulated while in the air gap space between the plates.

The air gap may be divided up into regions, and some regions of the plates may include heating/cooling by a thermal regulator (e.g., a Peltier device, a resistive heating device, a convective heating/cooling device, etc.) that is in thermal contact with the region, and may be localized to that region. Reactions performed on with the air-matrix DMF apparatus may be detected, including imaging or other sensor-based detection, and may be performed at one or more localized regions or over all or over a majority of the air gap space of the air-matrix DMF apparatus.

[0012] Any of the apparatus variations described herein may include wax within the reaction chamber. As described herein, wax may be included in the air gap even if a separate reaction chamber apparatus is included. The wax may be present in a thermal zone (e.g., a thermally controlled sub-region of the air gap) as a solid (e.g., a wall, channel, cave, or other structure of

wax) that can be melted to form a liquid and combined with a reaction droplet. The liquid wax, upon mixing together with the reaction droplet, will typically form a coating over and around the liquid droplet, protecting it from evaporation.

5 [0013] Unfortunately, following the use of wax to encapsulate a droplet in an air gap to prevent evaporation, in some cases it may be difficult to fully separate the wax material from the aqueous droplet. Thus, adding additional aqueous droplets may be difficult or impossible, as the small amount of residual wax material may form an outer coating of wax that will prevent merging of the coated droplet with other aqueous droplets. Thus, described herein are methods and apparatuses for performing one or more droplet operations (including merger with additional
10 aqueous droplets) on an at least partially outer wax-coated aqueous droplet.

[0014] Any of the methods described herein may be referred to as a method of performing droplet operations on a droplet that is at least partially coated in wax and within an air-matrix digital microfluidic (DMF) apparatus (e.g., within the air gap of the air-matrix DMF apparatus). In general, these methods may include starting with the aqueous droplet (e.g., adding it into the
15 air gap of the DMF apparatus), and using electrowetting to move it within the air gap. The methods may also generally include encapsulating with a wax (wax material) as described in more detail. This may be used to perform a thermally-regulated procedure. Further, any of these methods may also typically include removing much or most of the wax from the aqueous droplet, e.g., by reducing the temperature to solidify the wax while moving the droplet away
20 (e.g., by electrowetting).

[0015] In addition, any of these methods may generally include: moving, by electrowetting, an aqueous reaction droplet having an outer coating of wax (which may be just a very thin layer at least partially coating the aqueous droplet) within an air gap of the air-matrix DMF apparatus (as expected, the air gap may be formed between a first plate and a second plate of the air-matrix
25 DMF apparatus). Any of these methods may also include merging the aqueous reaction droplet with a carrier droplet comprising an aqueous droplet coated with an oil or an organic solvent in the air gap to form a combined droplet. The oil or organic solvent may be coated in a thin (e.g., monolayer or thicker) layer on the second aqueous droplet but interact with the wax coating on the first aqueous droplet and permit the two to merge; in the absence of this oil or organic
30 solvent, the two droplets will not merge. Thereafter, the methods may include moving, by electrowetting, the combined droplet within the air gap. The second aqueous droplet may include any material (buffer, marker, beads, wash, etc.). This process may be repeated multiple times, e.g., by combining the combined droplet with additional aqueous droplets including an oil or organic solvent.

[0016] In some variations, the oil or organic solvent material may be separated from the combined droplet or target molecules in the combined droplet. For example, beads (e.g., magnetic beads) holding the target molecule(s) may be separated magnetically from the combined droplet, including the outer coating (e.g., wax and oil/organic solvent). The beads
5 may then be washed to remove any residual coating. Alternatively, in some variations, the coating may be mechanically removed.

[0017] Described herein are air-matrix DMF apparatuses that include a wax material in a solid state at room temperature and below, but may selectively and controllably combined with a reaction droplet within the air gap when the wax structure is heated. For example, described
10 herein are air-matrix digital microfluidic (DMF) apparatuses configured to prevent evaporation. The apparatus may include a first plate having a first hydrophobic layer; a second plate having a second hydrophobic layer; an air gap formed between the first and second hydrophobic layers; a plurality of actuation electrodes adjacent to the first hydrophobic layer, wherein each actuation electrode defines a unit cell within the air gap; one or more ground electrodes adjacent to
15 actuation electrode of the plurality of actuation electrodes; a thermal regulator arranged to heat a thermal zone portion of the air gap wherein a plurality of unit cells are adjacent to the thermal zone; a wax body within the thermal zone of the air gap; and a controller configured to regulate the temperature of the thermal zone to melt the wax body and to apply energy to actuation electrodes of the plurality of actuation electrode to move a droplet through the air gap.

[0018] The wax body may span one or more (e.g., a plurality of adjacent) unit cells. The wax body may comprise a wall of wax within the air gap. In some variations the wax body forms a channel or vessel within the air gap. For example, the wax body may form a concave shape in the air gap, which may help it combine with a reaction droplet when heated. In general, the wax body may be melted immediately before combining with the reaction droplet. In some
25 variations the wax body may itself be a droplet (wax droplet) that is moved into position by the air-matrix DMF apparatus so that it can combine with the reaction droplet.

[0019] The wax body may be formed of any appropriate wax that is typically solid at room temperature, such as, e.g., paraffin wax. Other waxes may generally include hydrophobic, malleable solids near ambient temperatures such as higher alkanes and lipids, typically with
30 melting points above about 40°C (104°F), that may melt to give low viscosity liquids. Examples of waxes include natural waxes (beeswax, plant waxes, petroleum waxes, etc.).

[0020] Any of these apparatuses may include features such as those described above, e.g., at least one temperature sensor in thermal communication with the thermal regulator. The plurality of actuation electrodes may form a portion of the first plate. The one or more ground electrodes
35 may be adjacent to the second hydrophobic layer, across the air gap from the first plate. The

apparatus may also include a dielectric between the first hydrophobic layer and the plurality of actuation electrodes (or in some variations the dielectric layer is the hydrophobic layer, as some hydrophobic layers are also dielectric materials). As mentioned above, a thermal regulator may be a thermoelectric heater.

5 [0021] Also described herein are methods of preventing droplet evaporation within an air-matrix digital microfluidic (DMF) apparatus, the method may include: introducing a reaction droplet into an air gap of the air-matrix DMF apparatus which is formed between a first plate and a second plate of the air-matrix DMF apparatus; melting a wax body within the air gap of the air-matrix DMF; combining the reaction droplet with the melted wax body to protect the reaction
10 droplet from evaporation; and allowing a reaction to proceed within the reaction droplet.

[0022] Melting the wax body typically comprises increasing the temperature of a portion of the air gap comprising a thermal zone to a temperature above the melting point of the wax forming the wax body. In some variations, melting the wax body comprises melting a solid wax body formed into a wall or open chamber within the air gap.

15 [0023] Introducing the reaction droplet into an air gap may comprise combining multiple droplets to form a reaction droplet within the air gap. The first plate may comprise a plurality of adjacent actuation electrodes, and wherein combining the reaction droplet with the melted wax body comprises applying energy to a subset of the actuation electrodes of the plurality of adjacent actuation electrodes to move the reaction droplet in contact with the wax body prior to
20 melting the wax body.

[0024] The first plate may comprise a plurality of adjacent actuation electrodes, wherein combining the reaction droplet with the melted wax body may comprise applying energy to a subset of the actuation electrodes of the plurality of adjacent actuation electrodes to move the reaction droplet in contact with the melted wax body.

25 [0025] Allowing a reaction to proceed may comprise heating portion of the air gap containing the reaction droplet. As mentioned, any of these methods may include detecting a product within the reaction droplet.

[0026] Although the majority of the devices described herein are air-matrix DMF apparatuses that include two parallel plates forming the air gap, any of the techniques (methods and apparatuses) may be adapted for operation as part of a one-plate air-matrix DMF apparatus.
30 In this case, the apparatus includes a single plate and may be open to the air above the single (e.g., first) plate; the "air gap" may correspond to the region above the plate in which one or more droplet may travel while on the single plate. The ground electrode(s) may be positioned adjacent to (e.g., next to) each actuation electrode, e.g., in, on, or below the single plate. The
35 plate may be coated with the hydrophobic layer (and an additional dielectric layer maybe

positioned between the hydrophobic layer and the dielectric layer, or the same layer may be both dielectric and hydrophobic). The methods and apparatuses for correcting for evaporation may be particularly well suited for such single-plate air-matrix DMF apparatuses.

[0027] In some embodiments, an air-matrix digital microfluidic (DMF) apparatus configured to prevent evaporation is provided. The apparatus includes a first plate having a first hydrophobic layer; a second plate having a second hydrophobic layer; and an air gap formed between the first and second hydrophobic layers. The apparatus further includes a plurality of actuation electrodes adjacent to the first hydrophobic layer; a thermal regulator arranged to heat a portion of the air gap configured as a thermal zone; a wax body within the thermal zone of the air gap; and a controller. The controller is programmed to actuate the plurality of actuation electrodes to transport an aqueous reaction droplet through the air gap to the thermal zone; regulate the temperature of the thermal zone to melt the wax body into a liquid wax that encapsulates the aqueous reaction droplet; regulate the temperature of the thermal zone to perform a reaction protocol within the liquid wax encapsulated aqueous reaction droplet; actuate the plurality of actuation electrodes to transport the aqueous reaction droplet away from the thermal zone; actuate the plurality of actuation electrodes to bring a carrier droplet comprising an oil or an organic solvent coated aqueous droplet to the aqueous reaction droplet; and merge the carrier droplet with the aqueous reaction droplet.

[0028] In some embodiments, the wax body spans a plurality of adjacent actuation electrodes of the plurality of actuation electrodes. In some embodiments, the wax body comprises a wall of wax within the air gap. In some embodiments, the wax body forms a channel or vessel within the air gap. In some embodiments, the wax body comprises paraffin wax.

[0029] In some embodiments, the apparatus further includes at least one temperature sensor in thermal communication with the thermal regulator.

[0030] In some embodiments, the plurality of actuation electrodes form a portion of the first plate.

[0031] In some embodiments, the thermal regulator comprises a thermoelectric heater.

[0032] In some embodiments, the carrier droplet comprises beads. In some embodiments, the beads are magnetic. In some embodiments, the beads are configured to bind to a molecule selected from the group consisting of DNA, RNA, and proteins.

[0033] In some embodiments, the carrier droplet comprises a reagent, a primer, a dilution buffer, an enzyme, a protein, a nanopore, a wash buffer, an alcohol, formamide, or a detergent.

[0034] In some embodiments, the wax body is disposed on a removable sheet that can be removably attached to either the first plate or the second plate.

[0035] In some embodiments, the first plate or the second plate are part of a removable cartridge.

[0036] In some embodiments, a method of preventing droplet evaporation within an air-matrix digital microfluidic (DMF) apparatus is provided. The method includes introducing an aqueous reaction droplet into an air gap of the air-matrix DMF apparatus which is formed between a first plate and a second plate of the air-matrix DMF apparatus; transporting the aqueous reaction droplet to a thermal zone of the air gap, the thermal zone comprising a wax; melting the wax within the thermal zone to encapsulate the aqueous reaction droplet with the wax; regulating the temperature of the encapsulated reaction droplet to allow a reaction to proceed within the aqueous reaction droplet; transporting the aqueous reaction droplet away from the thermal zone after the reaction is completed; introducing a carrier droplet comprising an oil or an organic solvent coated aqueous droplet into the air gap; and merging the aqueous reaction droplet with the carrier droplet to form a merged droplet .

[0037] In some embodiments, melting the wax comprises melting a solid wax formed into a wall or open chamber within the air gap.

[0038] In some embodiments, introducing the aqueous reaction droplet into an air gap comprises combining multiple droplets to form the aqueous reaction droplet within the air gap.

[0039] In some embodiments, the first plate comprises a plurality of adjacent actuation electrodes, and wherein combining the aqueous reaction droplet with the melted wax comprises applying energy to a subset of the actuation electrodes of the plurality of adjacent actuation electrodes to move the aqueous reaction droplet in contact with the wax prior to melting the wax.

[0040] In some embodiments, the first plate comprises a plurality of adjacent actuation electrodes, and wherein combining the aqueous reaction droplet with the melted wax comprises applying energy to a subset of the actuation electrodes of the plurality of adjacent actuation electrodes to move the aqueous reaction droplet in contact with the melted wax after melting the wax.

[0041] In some embodiments, the method further includes detecting a product within the aqueous reaction droplet.

[0042] In some embodiments, the method further includes mixing the reaction droplet with a plurality of beads after the carrier droplet has been merged with the aqueous reaction droplet.

[0043] In some embodiments, the method further includes immobilizing the beads after the carrier droplet has been merged with the aqueous reaction droplet.

[0044] In some embodiments, the method further includes moving the merged carrier droplet and aqueous reaction droplet away from the immobilized beads.

[0045] In some embodiments, the method further includes re-suspending the immobilized beads with an aqueous droplet.

[0046] In some embodiments, the method further includes separating the beads from the merged carrier droplet and aqueous reaction droplet by moving a magnet away from the merged carrier droplet and aqueous reaction droplet.

BRIEF DESCRIPTION OF THE DRAWINGS

[0047] The novel features of the invention are set forth with particularity in the claims that follow. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0048] FIG. 1 is a top view of an example of a portion of an air-matrix DMF apparatus, showing a plurality of unit cells (defined by the underlying actuating electrodes) and reaction chamber openings (access holes).

[0049] FIG. 2A shows the top view of FIG. 1 and FIGS. 2B-2D show side views of variations of reaction chamber wells that may be used in an air-matrix DMF apparatus. In FIG. 2B the reaction chamber well comprises a centrifuge tube; in FIG. 2C the reaction chamber well comprises a well plate (which may be part of a multi-well plate); and in FIG. 2D the reaction chamber well is formed as part of the plate of the air-matrix DMF apparatus.

[0050] FIGS. 3A-3E illustrate movement (e.g., controlled by a controller of an air-matrix DMF apparatus) into and then out of a reaction chamber, as described herein. In this example, the reaction chamber well is shown in a side view of the air-matrix DMF apparatus and the reaction chamber is integrally formed into a plate (e.g., a first or lower plate) of the air-matrix DMF apparatus which includes actuation electrodes (reaction well actuation electrodes) therein.

[0051] FIG. 4A shows a time series of photos of an air matrix DMF apparatus including a wax (in this example, paraffin) body which is melted and covers a reaction droplet.

[0052] FIG. 4B is an example of a time series similar to that shown in FIGS. 4A(3) and 4A(4), without using a wax body to cover the reaction droplet, showing significant evaporation.

[0053] FIG. 5 is a graph comparing an amplification reaction by LAMP with and without a wax covering as described herein, protecting the reaction droplet from evaporation.

[0054] FIG. 6A show graphical results of LAMP using paraffin-mediated methods; this may be qualitatively compared to the graph of FIG. 6B shows graphical results of LAMP using conventional methods.

[0055] FIGS. 7A and 7B show the encapsulation of a droplet within wax in a thermal zone and the subsequent separation of the droplet from the liquid wax.

[0056] FIGS. 8A-8C show the merging of a carrier droplet with beads with the droplet from FIGS. 7A and 7B and the subsequent separation and re-suspension of the beads.

5

DETAILED DESCRIPTION

[0057] Any of the methods (including user interfaces) described herein may be implemented as software, hardware or firmware, and may be described as a non-transitory computer-readable storage medium storing a set of instructions capable of being executed by a processor (e.g.,
10 computer, tablet, smartphone, etc.), that when executed by the processor causes the processor to control perform any of the steps, including but not limited to: displaying, communicating with the user, analyzing, modifying parameters (including timing, frequency, intensity, etc.), determining, alerting, or the like.

[0058] Described herein are air-matrix digital microfluidics (DMF) methods and apparatuses
15 that may minimize the effect of surface fouling and/or evaporation. An air-matrix DMF apparatus as described herein may be particularly useful when heating the reaction droplets being processed.

[0059] In general, an air-matrix DMF apparatus as disclosed herein may have any appropriate shape or size. As used herein, the term “surface fouling” may refer to accumulation
20 of unwanted materials on solid surfaces, including with the air gap of the air matrix DMF apparatus (e.g., upper and/or lower plate surfaces). Surface fouling materials can consist of either living organisms (biofouling) or a non-living substance (inorganic or organic). Surface fouling is usually distinguished from other surface-growth phenomena in that it occurs on a surface of a component, or system and that the fouling process impedes or interferes with
25 function.

[0060] The air-matrix DMF apparatuses described herein generally includes at least one hydrophobic surface and a plurality of activation electrodes adjacent to the surface; either the hydrophobic surface may also be a dielectric material or an additional dielectric material/layer may be positioned between the actuation electrodes and the hydrophobic surface. For example,
30 in some variations, the air-matrix DMF includes a series of layers on a printed circuit board (PCB) forming a first or bottom plate. The outer (top) surface of this plate is the hydrophobic layer. Above this layer is the air gap (air gap region) along which a reaction droplet may be manipulated. In some variations a second plate may be positioned opposite from the first plate, forming the air gap region between the two. The second plate may also include a hydrophobic
35 coating and in some variations may also include a ground electrode or multiple ground

electrodes opposite the actuation electrodes. The actuation electrodes may be configured for moving droplets from one region to another within the DMF device, and may be electrically coupled to a controller (e.g., control circuitry) for applying energy to drive movement of the droplets in the air gap. As mentioned, this plate may also include a dielectric layer for increasing the capacitance between the reaction droplet and the actuation electrodes. The reaction starting materials and reagents, as well as additional additive reagents may be in reservoirs that may be dispensed into the air gap, where the reaction mixture is typically held during the reaction. In some instances the starting materials, reagents, and components needed in subsequent steps may be stored in separate areas of the air gap layer such that their proximity from each other prevents them from prematurely mixing with each other. In other instances, the air gap layer may include features that are able to compartmentalize different reaction mixtures such that they may be close in proximity to each other but separated by a physical barrier. In general, the floor of the air gap is in the first plate, and is in electrical contact with a series of actuation electrodes.

[0061] The air gap DMF apparatuses described herein may also include other elements for providing the needed reaction conditions. For instance, the air gap DMF apparatuses may include one or more thermal regulators (e.g., heating or cooling element such as thermoelectric modules) for heating and cooling all or a region (thermal zone) of the air gap. In other instances, heating or cooling may be provided by controlling endothermic or exothermic reactions to regulate temperature. The air gap DMF apparatuses may also include temperature detectors (e.g., resistive temperature detector) for monitoring the temperature during a reaction run. In addition, the DMF apparatuses may also include one or more magnets that can be used to manipulate magnetic beads in an on demand fashion. For example, the magnet(s) can be an electromagnet that is controlled by a controller to generate a magnetic field that can agitate or immobilize magnetic beads.

[0062] Thus, the air gap DMF apparatuses described herein may include one or more thermal zones. Thermal zones are regions on the air gap DMF apparatuses (e.g., the air gap) that may be heated or cooled, where the thermal zones may transfer the heating or cooling to a droplet within the thermal zone through one or more surfaces in contact with the air gap region in the zone (e.g., the first plate). Heating and cooling may be through a thermal regulator such as a thermoelectric module or other type of temperature-modulating component. The temperature of one or many thermal zones may be monitored through a temperature detector or sensor, where the temperature information may be communicated to a computer or other telecommunication device. The temperature is typically regulated between 4°C and 100°C, as when these apparatuses are configured to perform one or more reactions such as, but not limited to: nucleic acid amplifications, like LAMP, PCR, molecular assays, cDNA synthesis, organic synthesis, etc.

[0063] An air gap DMF apparatus may also include one or more thermal voids. Thermal voids may be disposed adjacent to the different thermal zones. The thermal voids are typically regions in which heat conduction is limited, e.g., by removing part of the plate (e.g., first plate) (forming the “void”). These voids may be strategically placed to isolate one thermal zone from another which allows the correct temperatures to be maintained within each thermal zone.

[0064] In general, any of the air-matrix DMF apparatuses described herein may include a separate reaction chamber that is separate or separable from the air gap of the apparatus, but may be accessed through the air gap region. The reaction chamber typically includes a reaction chamber opening that is continuous with the lower surface of the air gap (e.g., the first plate), and a reaction chamber well that forms a cup-like region in which a droplet may be controllably placed (and in some variations, removed) by the apparatus to perform a reaction when covered. The cover may be a mechanical cover (e.g., a cover that seals or partially seals the reaction chamber opening, or a cover that encapsulates, encloses or otherwise surrounds the reaction droplet, such as an oil or wax material that mixes with (then separates from and surrounds) the reaction droplet when the two are combined in the reaction chamber.

[0065] In general, the reaction chamber opening may be any shape or size (e.g., round, square, rectangular, hexagonal, octagonal, etc.) and may pass through the first (e.g., lower) plate, and into the reaction chamber well. In some variations, the reaction chamber opening passes through one or more actuation electrodes; in particular, the reaction chamber opening may be completely or partially surrounded by an actuation electrode.

[0066] FIG. 1 shows a top view of an exemplary air-matrix DMF apparatus 101. As shown, the DMF device may include a series of paths defined by actuation electrodes. The actuation electrodes 103 are shown in FIG. 1 as a series of squares, each defining a unit cell. These actuation electrodes may have any appropriate shape and size, and are not limited to squares. For example, the unit cells formed by the actuation electrodes in the first layer may be round, hexagonal, triangular, rectangular, octagonal, parallelogram-shaped, etc. In the example of FIG. 1, the squares representing the unit cells may indicate the physical location of the actuation electrodes in the DMF device or may indicate the area where the actuation electrode has an effect (e.g., an effective area such that when a droplet is situated over the denoted area, the corresponding actuation electrode may affect the droplet’s movement or other physical property). The actuation electrodes 103 may be placed in any pattern. In some examples, actuation electrodes may span the entire corresponding bottom or top surface the air gap of the DMF apparatus. The actuation electrodes may be in electrical contact with starting sample chambers (not shown) as well as reagent chambers (not shown) for moving different droplets to different regions within the air gap to be mixed with reagent droplets or heated.

[0067] In the air-matrix apparatuses described herein, the first (lower) plate may also include one or more reaction chamber openings (access holes) 105, 105'. Access to the reaction chamber wells may allow reaction droplets to be initially introduced or for allowing reagent droplets to be added later. In particular, one or more reaction droplets may be manipulate in the air gap (moved, mixed, heated, etc.) and temporarily or permanently moved out of the air gap and into a reaction chamber well though a reaction chamber opening. As shown, some of the reaction chamber openings 105' pass through an actuation electrode. As will be shown in greater detail herein, the reaction chamber may itself include additional actuation electrodes that may be used to move a reaction chamber droplet into/out of the reaction chamber well. In some variations one or more actuation electrodes may be continued (out of the plane of the air gap) into the reaction chamber well.

[0068] In general, one or more additional reagents may be subsequently introduced either manually or by automated means in the air gap. In some instances, the access holes may be actual access ports that may couple to outside reservoirs of reagents or reaction components through tubing for introducing additional reaction components or reagents at a later time. As mentioned, the access holes (including reaction chamber openings) may be located in close proximity to a DMF actuation electrode(s). Access holes may also be disposed on the side or the bottom of the DMF apparatus. In general, the apparatus may include a controller 110 for controlling operation of the actuation electrodes, including moving droplets into and/or out of reaction chambers. The controller may be in electrical communication with the electrodes and it may apply power in a controlled manner to coordinate movement of droplets within the air gap and into/out of the reaction chambers. The controller may also be electrically connected to the one or more temperature regulators (thermal regulators 120) to regulate temperature in the thermal zones 115. One or more sensors (e.g., video sensors, electrical sensors, temperature sensors, etc.) may also be included (not shown) and may provide input to the controller which may use the input from these one or more sensors to control motion and temperature.

[0069] As indicated above, surface fouling is an issue that has plagued microfluidics, including DMF devices. Surface fouling occurs when certain constituents of a reaction mixture irreversibly adsorbs onto a surface that the reaction mixture is in contact with. Surface fouling also appears more prevalent in samples containing proteins and other biological molecules. Increases in temperature may also contribute to surface fouling. The DMF apparatuses and methods described herein aim to minimize the effects of surface fouling. One such way is to perform the bulk of the reaction steps in a reaction chamber that is in fluid communication with the air gap layer. The reaction chamber may be an insert that fits into an aperture of the DMF device as shown in FIGS. 2B and 2C. FIG. 2B shows the floor (e.g., first plate) of an air gap

region coupled to a centrifuge (e.g., Eppendorf) tube 205 while FIG. 2C incorporates a well-plate 207 (e.g., of a single or multi-well plate) into the floor of the air gap region. A built-in well 209 may also be specifically fabricated to be included in the air-matrix DMF apparatus as shown in FIG. 2D. When a separate or separable tube or plate is used, the tubes may be coupled to the

5 DMF device using any suitable coupling or bonding means (e.g., snap-fit, friction fit, threading, adhesive such as glue, resin, etc., or the like).

[0070] In general, having a dedicated reaction chamber within the DMF device minimizes surface fouling especially when the reaction is heated. Thus, while surface fouling may still occur within the reaction chamber, it may be mainly constrained to within the reaction chamber.

10 This allows the majority of the air gap region floor to remain minimally contaminated by surface fouling and clear for use in subsequent transfer of reagents or additional reaction materials if needed, thus allowing for multi-step or more complex reactions to be performed. When the reaction step or in some instances, the entire reaction is completed, the droplet containing the product may be moved out of the reaction chamber to be analyzed. In some examples, the

15 product droplet may be analyzed directly within the reaction chamber.

[0071] In order to bring the droplet(s) containing the starting materials and the reagent droplets into the reaction chamber, additional actuation electrodes, which may also be covered/coated with a dielectric and a hydrophobic layer (or a combined hydrophobic/dielectric layer), may be used. FIGS. 3A-3E shows a series of drawings depicting droplet 301 movement

20 into and out of an integrated well 305. As this series of drawings show, in addition to lining the floor of the air gap layer, additional actuation electrodes 307 line the sides and the bottom of the well. In some variations, the same actuation electrode in the air gap may be extended into the reaction chamber opening. The actuation electrodes 307 (e.g., the reaction chamber actuation electrodes) may be embedded into or present on the sides and bottom of the well for driving the

25 movement of the droplets into/out of the reaction chamber well. Actuation electrodes may also cover the opening of the reaction chamber. In FIG. 3A, a droplet 301 (e.g., reaction droplet) in the air gap layer may be moved (using DMF) to the reaction chamber opening. The actuation electrodes 307 along the edge of the well and the sides of the well maintain contact with the droplet as it moved down the well walls to the bottom of the well (shown in FIGS. 3B and 3C).

30 Once in the reaction chamber well, the droplet may be covered (as described in more detail below, either by placing a cover (e.g., lid, cap, etc.) over the reaction chamber opening and/or by mixing the droplet with a covering (e.g., encapsulating) material such as an oil or wax (e.g., when the droplet is aqueous). In general, the droplet may be allowed to react further within the well, and may be temperature-regulated (e.g., heated, cooled, etc.), additional material may be

35 added (not shown) and/or it may be observed (to detect reaction product). Alternatively or

additionally, the droplet may be moved out of the well using the actuation electrodes; if a mechanical cover (e.g., lid) has been used, it may be removed first. If an encapsulating material has been used it may be left on.

5 [0072] In some variations contacts may penetrate the surfaces of the reaction chamber. For example, there may be at least ten electrical insertion points in order to provide sufficient electrical contact between the actuation electrodes and the interior of the reaction chamber. In other examples there may need to be at least 20, 30, or even 40 electrical insertion points to provide sufficient contact for all the interior surfaces of the reaction chamber. The interior of the reaction chamber may be hydrophobic or hydrophilic (e.g., to assist in accepting the droplet). As
10 mentioned, an electrode (actuation electrode) may apply a potential to move the droplets into and/or out of the well.

[0073] In general, the actuation electrodes may bring the droplet into the well in a controlled manner that minimizes dispersion of the droplet as it is moved into the well and thus maintaining as cohesive a sample droplet as possible. FIGS. 3D and 3E show the droplet being moved up the
15 wall of the well and then out of the reaction chamber. This may be useful for performing additional subsequent steps or for detecting or analyzing the product of interest within the droplet, although these steps may also or alternatively be performed within the well. Actuation electrodes may be on the bottom surface, the sides and the lip of the well in contact with the air gap layer; some actuation electrodes may also or alternatively be present on the upper (top) layer.

20 [0074] In instances where the reaction compartment is an independent structure integrated with the DMF devices as those shown in FIGS. 2A and 2B, the thickness of the substrate (e.g., PCB) may be similar to what is commonly used in DMF fabrication. When the reaction compartment is an integrated well structure fabricated in the bottom plate of the DMF device as shown in FIG. 2D, the thickness of the substrate may be equivalent to the depth of the well.

25 [0075] In another embodiment, the electrodes embedded in the reaction compartments can include electrodes for the electrical detection of the reaction outputs. Electrical detection methods include but are not limited to electrochemistry. In some instances, using the changes in electrical properties of the electrodes when the electrodes contact the reaction droplet, reagent droplet, or additional reaction component to obtain information about the reaction (e.g., changes
30 in resistance correlated with position of a droplet).

[0076] The apparatuses described herein may also prevent evaporation. Evaporation may result in concentrating the reaction mixture, which may be detrimental as a loss of reagents in the reaction mixture may alter the concentration of the reaction mixture and result in mismatched concentration between the intermediate reaction droplet with subsequent addition of other
35 reaction materials of a given concentration. In some variations, such as with enzymatic

reactions, enzymes are highly sensitive to changes in reaction environment and loss of reagent may alter the effectiveness of certain enzymes. Evaporation is especially problematic when the reaction mixture has to be heated to above ambient temperature for an extended period of time. In many instances, microfluidics and DMF devices utilizes an oil-matrix for performing
5 biochemical type reactions in microfluidic and DMF devices to address unwanted evaporation. One major drawback of using an oil matrix in the DMF reaction is the added complexity of incorporating additional structures to contain the oil.

[0077] In general, described herein are methods and apparatuses for combating evaporation by the use of wax (e.g., paraffin) in minimizing evaporation during a reaction. A wax substance
10 may include substances that are composed of long alkyl chains. Waxes are typically solids at ambient temperatures and have a melting point of approximately 46°C to approximately 68°C depending upon the amount of substitution within the hydrocarbon chain. However, low melting point paraffins can have a melting point as low as about 37°C, and some high melting point waxes can have melting points about 70-80°C. In some instances higher melting point waxes
15 may be purifying crude wax mixtures.

[0078] As mentioned, wax is one type of sealing material that may be used as a cover (e.g., within a reaction chamber that is separate from the plane of the air gap). In some variations, wax may be used within the air gap. In particular, the wax may be beneficially kept solid until it is desired to mix it with the reaction droplet so that it may coat and protect the reaction droplet.
20 Typically the wax material (or other coating material) may be mixed with the reaction droplet and enclose (e.g., encapsulate, surround, etc.) the aqueous reaction droplet.

[0079] When a reaction droplet is maintained within a paraffin coating, not only is evaporation minimized, but the paraffin may also insulate the reaction droplet from other potentially reaction interfering factors. In some instances, a solid piece of paraffin or other wax
25 substance may be placed within a thermal zone of the air gap layer of the DMF device. For example, during a reaction, actuation electrodes may move a reaction droplet to a wax (e.g., paraffin) body. Upon heating to a melting temperature, the wax body may melt and cover the reaction droplet. The reaction then may continue for an extended period of time (including at elevated temperatures) without need to replenish the reaction solvents, while preventing loss by
30 evaporation. For example wax-encapsulated droplet may be held and/or moved to a thermal zone to control the temperature. The temperature may be decreased or increased (allowing control of the phase of the wax as well, as the wax is typically inert in the reactions being performed in the reaction droplet). The temperature at that particular thermal zone may be further increased to melt the paraffin and release the reaction droplet. The reaction droplet may
35 be analyzed for the desired product when encapsulated by the liquid or solid wax, or it may be

moved to another region of the DMF device for further reaction steps after removing it from the wax covering. Paraffins or other wax materials having the desired qualities (e.g. melting point above the reaction temperature) may be used. For example, paraffins typically have melting points between 50 and 70 degrees Celsius, but their melting points may be increased with increasing longer and heavier alkanes.

[0080] FIG. 4A shows a time-sequence images (numbered 1-4) taken from an example using a wax body within the air matrix as discussed above, showing profound reduction in evaporation as compared to a control without wax (shown in FIG. 4B, images 1-2). In FIG. 4A, the first image, in the top right, shows an 8 μ L reaction droplet 603 that has been moved by DMF in the air matrix apparatus to a thermal zone (“heating zone”) containing a solid wax body (e.g., paraffin wall 601). Once in position, the reaction droplet may be merged with a solid paraffin wall (e.g., thermally printed onto DMF), as shown in image 2 of FIG. 4A, or the wax material may be melted first (not shown). In FIG. 4A image 3, the thermal zone is heated (63 °C) to or above the melting point of the wax material thereby melting the paraffin around the reaction droplet, and the reaction droplet is surrounded/encapsulated by the wax material, thus preventing the droplet from evaporation as shown in FIG. 4A images 3 and 4. Using this approach, in the example shown in FIG. 4A image 4, the volume of reaction droplets was maintained roughly constant at 63 °C for an incubation time approximately two hours long (120 min). An equivalent experiment without the paraffin wall was performed, and shown in FIG. 4B. The left picture (image 1) in FIG. 4B shows the reaction droplet 603’ at time zero at 63°C and the right picture of FIG. 4B shows the reaction droplet after 60 minutes at 63 °C. As shown, the reaction droplet almost completely evaporated within approximately an hour’s time at 63 °C.

[0081] Through this approach of enclosing a droplet in a shell of liquid wax, the reaction volume and temperature are maintained constant without the use of oil, a humidified chamber, off-chip heating, or droplet replenishment methods. Waxes other than paraffin can be used to prevent droplet evaporation as long as their melting temperature is higher than the ambient temperature, but lower or equal to the reaction temperature. Examples of such waxes include paraffin, bees and palm waxes. The wax-like solids can be thermally printed on the DMF device surface by screen-, 2D- or 3D-printing. This wax-mediated evaporation prevention solution is an important advancement in developing air-matrix DMF devices for a wide variety of new high-impact applications.

[0082] As mentioned, the wax-based evaporation methods described may be used in conjunction with the DMF devices having a reaction chamber feature, or they may be used without separate reaction chambers. When used within a reaction chamber, the wax may be present in the reaction chamber and the reaction droplet may be moved to the reaction chamber

containing wax for performing the reaction steps requiring heating. Once the heating step has completed, the reaction droplet may be removed from the reaction chamber for detection or to perform subsequent reaction steps within the air gap layer of the DMF device.

[0083] The methods and apparatuses described herein may be used for preventing
5 evaporation in air-matrix DMF devices and may enable facile and reliable execution of any chemistry protocols on DMF with the requirement for a temperature higher than the ambient temperature. Such protocols include, but are not limited to, DNA/RNA digestion/fragmentation, cDNA synthesis, PCR, RT-PCR, isothermal reactions (LAMP, rolling circle amplification-RCA, Strand Displacement Amplification-SDA, Helicase Dependent Amplification-HDA, Nicking
10 Enzyme Amplification reaction-NEAR, Nucleic acid sequence-based amplification-NASBA, Single primer isothermal amplification-SPIA, cross-priming amplification-CPA, Polymerase Spiral Reaction-PSR, Rolling circle replication-RCR), as well as ligation-based detection and amplification techniques (ligase chain reaction-LCR, ligation combined with reverse transcription polymerase chain reaction-RT PCR, ligation-mediated polymerase chain reaction-
15 LMPCR, polymerase chain reaction/ligation detection reaction-PCR/LDR, ligation-dependent polymerase chain reaction-LD-PCR, oligonucleotide ligation assay-OLA, ligation-during-amplification-LDA, ligation of padlock probes, open circle probes, and other circularizable probes, and iterative gap ligation-IGL, ligase chain reaction-LCR, over a range of temperatures (37-100 °C) and incubation times (≥ 2 hr). Additional protocols that can be executed using the
20 systems and methods described herein include hybridization procedures such as for hybrid capture and target enrichment applications in library preparation for new generation sequencing. For these types of applications, hybridization can last up to about 3 days (72h). Other protocols include end-repair, which can be done, for example, with some or a combination of the following enzymes: DNA Polymerase I, Large (Klenow) Fragment (active at 25°C for 15 minutes), T4
25 DNA Polymerase (active at 15°C for 12 minutes), and T4 Polynucleotide Kinase (active at 37°C for 30 minutes). Another protocol includes A-Tailing, which can be done with some or a combination of the following enzymes: Taq Polymerase (active at 72°C for 20 minutes), and Klenow Fragment (3'→5' exo-) (active at 37°C for 30 minutes). Yet another protocol is ligation by DNA or RNA ligases.

30

Manipulation and Processing of Encapsulated Droplets

[0084] Although the encapsulation of droplets in wax may prevent or reduce evaporation while executing chemistry protocols at elevated temperatures, after protocol completion, it has been discovered that when the droplet is removed and separated from the wax, e.g., by driving
35 the droplet using the electrodes of the DMF apparatus, a small amount of liquid wax remains

with the droplet as a coating even when the aqueous droplet is moved away from the wax, and that this wax coating may prevent or interfere with subsequent processing and analysis of the reaction droplet, particularly as the droplet cools and the wax solidifies around the droplet after the droplet is moved out of the heating zone. Therefore, in some embodiments, the wax
5 encapsulated reaction droplet can be accessed through the wax coating using the systems and methods described herein, which enables facile and reliable execution of downstream biochemical processes.

[0085] To access the reaction droplet through the wax coating after the reaction droplet has been separated from the bulk liquid wax in the heating zone, an additional hydrophobic (e.g., oil)
10 material may be added to the reaction droplet to help dissolve the solidified wax encapsulated the reaction droplet. For example, a carrier droplet (i.e., an aqueous droplet enclosed in a thin layer of oil) can be merged with the encapsulated reaction droplet. The carrier droplet gains access to the reaction droplet by having the oil from the carrier droplet dissolve and/or merge with the thin wax layer encapsulating the reaction droplet. Other materials other than oil may be used by the
15 carrier droplet to break through the wax layer encapsulating the reaction droplet. For example, materials that are immiscible with aqueous reaction droplet and are capable of dissolving wax may be used, such as carbon tetrachloride, chloroform, cyclohexane, 1,2-dichloroethane, dichloromethane, diethyl ether, dimethyl formamide, ethyl acetate, heptane, hexane, methyl-tert-butyl ether, pentane, toluene, 2,2,4-trimethylpentane, and other organic solvents. Other materials
20 that may be used to break through the wax layer include ionic detergents such as cetyltrimethylammonium bromide, Sodium deoxycholate, n-lauroylsarcosine sodium salt, sodium n-dodecyl Sulfate, sodium taurochenodeoxycholic; and non-ionic detergents such as dimethyldecylphosphine oxide (APO-10), dimethyldodecylphosphine oxide (APO-12), n-Dodecyl- β -D-maltoside (ULTROL®), n-dodecanoylsucrose, ELUGENT™ Detergent,
25 GENAPOL® C-100, HECAMEG®, n-Heptyl β -D-glucopyranoside, n-Hexyl-b-D-glucopyranoside, n-Nonyl-b-D-glucopyranoside, NP-40 Alternative, n-Octanoylsucrose, n-Octyl-b-D-glucopyranoside, n-Octyl-b-D-thioglucopyranoside, PLURONIC® F-127, Saponin, TRITON® X-100, TRITON® X-114, TWEEN® 20, TWEEN® 80, Tetronic 90R4. At
30 temperatures where a wax remains liquid, a carrier droplet encapsulated with wax may also be used to break through the wax encapsulating the reaction droplet. However, for lower temperatures where the wax solidifies, a carrier droplet coated with wax generally cannot be used since solid wax will prevent droplet movement.

[0086] For example, FIG. 7A illustrates a setup similar or the same as that shown in FIG. 4A. The setup includes a DMF device interfaced to a heating element placed below or within the
35 bottom DMF substrate, hence generating discrete heating zones 900 on the bottom DMF

substrate. Alternatively, the heating element can be placed above or within the top substrate to form a heating zone on the top substrate. However, forming the heating zone on the bottom substrate allows visual access. On the bottom substrate, a hydrophilic region 902 is printed or otherwise formed or disposed around the actuating electrodes in the electrode array 904 that are in the heating zone 900. One or more wax walls 906 or wax structures, which can be solid at room temperature, can be assembled on the top substrate by, for example, thermal printing to overlay a portion of the hydrophilic region 902 adjacent to the electrodes in the heating zone 900 on the bottom plate when the DMF device is assembled. Alternatively, the wax walls 906 or wax structures can be formed directly on the bottom plate around the electrodes in the heating zone 900. In yet another embodiment, the wax walls 906 can be placed on a removable sheet that can be removably attached to either the top plate or the bottom plate. The removable sheet can have a hydrophobic surface on one side for interacting with the droplet and an adhesive on the other side for adhering to the top or bottom plate. Reagents and other materials can also be placed on the removable sheet to interact with the droplets. In some embodiments, the top plate or the bottom plate can be part of a removable cartridge that is combined with the other plate and electronics to form the working DMF device. As described herein, a reaction droplet 908 can be transported to the heating zone 900 along a path of actuating electrodes, which may be a relatively narrow path formed by a single line of actuating electrodes to the heating zone 900. Then the heating zone 900 is heated, and the wax wall 906 surrounding the heating zone 900 and reaction droplet 908 melts to encapsulate the reaction droplet 908 in liquid wax 910 as shown in FIG. 7B (frame i), thereby preventing or reducing evaporation from the reaction droplet 908 during the reaction protocol. The hydrophilic region 902 surrounding the heating zone 900 functions to pin or localize the liquid wax 910 in place in the heating zone 900 and allows the reaction droplet 908 to break away as described below.

[0087] As shown in FIG. 7B (frames ii-iv), the process of breaking away or separating the encapsulated reaction droplet 908 from liquid wax 910 can be accomplished by driving the aqueous reaction droplet 908 away from the heating zone 900 and the liquid wax 910 by actuating the actuating electrodes in the heating zone and path. As the aqueous reaction droplet 908 is actuated away from the heating zone 900, the hydrophilic region 902 surrounding the liquid wax 910 helps hold the liquid wax 910 in place as the reaction droplet 908 moves away from the heating zone 900, which causes the liquid wax 910 encasing the droplet 908 to begin to neck and eventually break off from the droplet 908, thereby leaving trace or small quantities of liquid wax 910 surrounding the separated reaction droplet 908. In general, the heating zone 900 is single use only to avoid cross-contamination. However, in situations where cross-

contamination is not an issue, the heating zone 900 may be reused by heating and melting the wax within the heating zone and then moving the next droplet into the reheated liquid wax 910.

[0088] Because the reaction droplet may be surrounded by a thin layer of liquid wax 910 after separation from the heating zone 900, it may be difficult to merge the reaction droplet 908 with another aqueous droplet since the liquid wax 910 coating may act as a barrier. In addition, the liquid wax 910 may solidify as the droplet cools to form a physical barrier that impedes merger with another droplet. Therefore, to facilitate merging of a liquid wax 910 coated reaction droplet 908 or a cooled reaction droplet 908 with a solid wax coating with another droplet, a carrier droplet 912 can be used to merge with the reaction droplet 908 as shown in FIG. 7B

(frame v). The carrier droplet 912 can be an aqueous droplet that is coated with a thin layer of oil or another organic solvent as described above. The aqueous portion of the carrier droplet 912 can include additional reagents, beads coated (or not) with DNA/RNA probes or antibodies or antigens for performing separations, uncoated beads, magnetic beads, beads coated with a binding moiety, solid phase reversible immobilization (SPRI) beads, water for dilution of the reaction droplet, enzymes or other proteins, nanopores, wash buffers, ethanol or other alcohols, formamide, detergents, and/or other moieties for facilitating further processing of the reaction droplet 908. As shown in FIG. 8A (frames i-iv), when the carrier droplet 912 and the reaction droplet 908 are moved by the actuating electrodes to the same location, the thin layer of oil surrounding the carrier droplet 912 can merge with the thin layer of liquid wax surrounding the reaction droplet 908, thereby facilitating the merger of the aqueous portions of the two droplets 908, 912 to form a combined droplet 914.

[0089] After the carrier droplet 912 has been merged with the reaction droplet 908, further processing of the combined droplet 914 can proceed, such as extracting an analyte from the combined droplet 914 and/or perform other steps such as hybridizing capture probes, digesting the reaction product using an enzyme, amplifying the reaction product with a set of primers, and the like. For example, the carrier droplet 912 can be carrying beads for extracting the analyte, e.g., DNA or RNA or proteins. When the droplets are merged, the beads, which can be magnetic, can be used to mix the combined droplet 914 by application of a magnetic field. The target analyte binds to the beads, which can be immobilized against the substrate by the magnetic field to form a bead pellet 916, as shown in FIG. 8B (frame i). Next, the combined droplet 914 can be moved away from the immobilized bead pellet 916, leaving the bead pellet 916 with bound analyte on the substrate, as shown in FIG. 8B (frames ii-iii). The combined droplet 914 can be moved away from the immobilized bead pellet 916 by actuating the electrodes.

Alternatively, the combined droplet 914 can be held in place while the bead pellet 916 is moved away from the combined droplet 914. The bead pellet 916 can be moved away and separated

from the combined droplet 914 by, for example, moving the magnetic field (e.g., by moving the magnet generating the magnetic field) that is engaging the bead pellet 916 away from the combined droplet 914. In some embodiments, the combined droplet 914 can be actively immobilized through actuation of the electrodes in contact with the droplet and/or surrounding the droplet. Alternatively or in addition, the droplet 914 can be passively immobilized through natural adhesive forces between the droplet and substrate on which the droplet is contacting, as well as physical structures, such as retaining walls that partially surround the combined droplet 914 while having an opening for passing the bead pellet 916. As shown in FIG. 8C (frames i and ii), an aqueous droplet 918 can be moved over the bead pellet 916 to resuspend the beads with the bound analyte. See Example 3 described below for an embodiment of this procedure used for miRNA purification.

EXAMPLE 1: Device Fabrication and Assembly

[0090] DMF apparatuses that include embedded centrifuge tubes and/or well-plate wells (e.g., FIGS. 2B, 2C) were constructed by drilling 5.5 mm diameter holes into 3 mm thick PCB substrates, bearing copper (43 μm thick) plated with nickel (185 μm) and gold (3.6 μm) for electrodes and conductive traces. Tubes and wells were then inserted into holes. DMF devices with embedded wells (e.g., FIG. 2D) were fabricated with holes (5 mm diameter, 10 mm depth) drilled in 15 mm thick PCB substrates. Actuation electrodes (each 10 mm \times 10 mm) were formed by conventional photolithography and etching, and were coated with soldermask (~15 μm) as the dielectric. As shown in FIGS. 3A-3E, some of the electrodes were formed around and adjacent to the hole which served as the access point to reaction compartments. The electrical contact pads were masked with polyimide tape (DuPont; Hayward, CA), and the substrate was spin-coated with a 50 nm layer of Teflon-AF (1% wt/wt in Fluorinert FC-40, 1500 rpm for 30 sec) and then baked at 100 $^{\circ}\text{C}$ for 3 h. The top plate of the DMF device, consisting of a glass substrate coated uniformly with unpatterned indium tin oxide (ITO) (Delta Technologies Ltd; Stillwater, MN) with 5.5 mm diameter PDMS plugs was spin-coated with 50 nm of Teflon-AF, as described above.

[0091] Prototype devices fabricated as described above performed better or as well as air-gap DMF apparatuses without reaction chambers.

EXAMPLE 2: Quantifying evaporation prevention using waxes

[0092] To qualitatively evaluate the effect of wax bodies to prevent evaporation in our assays, loop mediated amplification (LAMP) reactions were executed while covered in liquid paraffin wax in tubes on the benchtop using a real-time PCR Machine. As shown in FIG. 5, the LAMP assay amplified miR-451, and the Ct values with and without paraffin were comparable

(~13 cycles), indicating no significant effect on the assay. For LAMP on DMF, the reaction droplet (8 μ L) was driven to heating zone (as shown in FIG. 4A). There, the droplet wets the solid paraffin wax wall which under conditional heating at 63 °C will melt into liquid wax to encircle the reaction volume and maintain it intact throughout the incubation time at 63 °C. FIG. 6A shows a LAMP assay using paraffin-mediated methods, while FIG. 6B shows a LAMP assay using conventional methods. In FIG. 6A, the two upper traces are for a hemolyzed sample while the two lower traces are for a non-hemolyzed sample. The two traces of each are to show repeatability of the runs using wax-mediated air matrix DMF. In FIG. 6B, the conventional LAMP assay for a hemolyzed sample are shown in upper two traces while the non-hemolyzed LAMP runs are shown in lower two traces. Again, the two upper and two lower traces each are to show result repeatability. The wax-mediated approach on DMF generated results comparable in Ct values to those generated by conventional LAMP in tubes as shown in FIGS. 6A and 6B.

EXAMPLE 3: miRNA purification

[0093] Human Panel A beads from the TaqMan® miRNA ABC Purification Kit (Thermo Fisher Scientific). Aliquots of miRNA (4ul), or “reaction droplets”, were loaded onto the DMF platform and brought to an array of electrodes overlaying the heating zone such that the droplet came into contact with the paraffin wall. The heating zone was then heated (65°C, 2 min) to melt the paraffin around the droplet. Once the paraffin melted, the reaction droplets were driven away from the heating zone and merged with miRNA Binding Beads (4 x 10⁶ beads; Fig 3A) in 2ul of mineral oil (i.e., carrier droplet). After mixing, the droplets were incubated (30°C, 30 min) to allow miRNA to bind to the miRNA Binding Beads. Beads were captured by engaging an external magnet positioned below the bottom plate. Once a pellet was formed, the beads were recovered from solution by moving the magnet laterally along the bottom plate while simultaneously actuating the electrodes positioned below the reaction droplet (Fig 3B). The miRNA Binding Beads were then resuspended in water (4ul) using the DMF platform and transferred to a centrifuge tube for elution of miRNA (70°C, 3 min; Fig 3C). The efficiency of miRNA recovery from paraffin-encased miRNA droplets was evaluated against recovery from miRNA droplets without paraffin, but only in oil. RT-qPCR analysis of miRNA prepared by the system from samples with and without paraffin encasement generated comparable Ct values.

[0094] When a feature or element is herein referred to as being "on" another feature or element, it can be directly on the other feature or element or intervening features and/or elements may also be present. In contrast, when a feature or element is referred to as being "directly on" another feature or element, there are no intervening features or elements present. It will also be understood that, when a feature or element is referred to as being "connected", "attached" or

"coupled" to another feature or element, it can be directly connected, attached or coupled to the other feature or element or intervening features or elements may be present. In contrast, when a feature or element is referred to as being "directly connected", "directly attached" or "directly coupled" to another feature or element, there are no intervening features or elements present.

5 Although described or shown with respect to one embodiment, the features and elements so described or shown can apply to other embodiments. It will also be appreciated by those of skill in the art that references to a structure or feature that is disposed "adjacent" another feature may have portions that overlap or underlie the adjacent feature.

[0095] Terminology used herein is for the purpose of describing particular embodiments
10 only and is not intended to be limiting of the invention. For example, as used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" and/or "comprising," when used in this specification, specify the presence of stated features, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or
15 more other features, steps, operations, elements, components, and/or groups thereof. As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items and may be abbreviated as "/".

[0096] Spatially relative terms, such as "under", "below", "lower", "over", "upper" and the like, may be used herein for ease of description to describe one element or feature's relationship
20 to another element(s) or feature(s) as illustrated in the figures. It will be understood that the spatially relative terms are intended to encompass different orientations of the device in use or operation in addition to the orientation depicted in the figures. For example, if a device in the figures is inverted, elements described as "under" or "beneath" other elements or features would then be oriented "over" the other elements or features. Thus, the exemplary term "under" can
25 encompass both an orientation of over and under. The device may be otherwise oriented (rotated 90 degrees or at other orientations) and the spatially relative descriptors used herein interpreted accordingly. Similarly, the terms "upwardly", "downwardly", "vertical", "horizontal" and the like are used herein for the purpose of explanation only unless specifically indicated otherwise.

[0097] Although the terms "first" and "second" may be used herein to describe various
30 features/elements (including steps), these features/elements should not be limited by these terms, unless the context indicates otherwise. These terms may be used to distinguish one feature/element from another feature/element. Thus, a first feature/element discussed below could be termed a second feature/element, and similarly, a second feature/element discussed below could be termed a first feature/element without departing from the teachings of the present
35 invention.

[0098] Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising” means various components can be co-jointly employed in the methods and articles (e.g., compositions and apparatuses including device and methods). For example, the term
5 “comprising” will be understood to imply the inclusion of any stated elements or steps but not the exclusion of any other elements or steps.

[0099] In general, any of the apparatuses and methods described herein should be understood to be inclusive, but all or a sub-set of the components and/or steps may alternatively be exclusive, and may be expressed as “consisting of” or alternatively “consisting essentially of”
10 the various components, steps, sub-components or sub-steps.

[0100] As used herein in the specification and claims, including as used in the examples and unless otherwise expressly specified, all numbers may be read as if prefaced by the word “about” or “approximately,” even if the term does not expressly appear. The phrase “about” or “approximately” may be used when describing magnitude and/or position to indicate that the
15 value and/or position described is within a reasonable expected range of values and/or positions. For example, a numeric value may have a value that is +/- 0.1% of the stated value (or range of values), +/- 1% of the stated value (or range of values), +/- 2% of the stated value (or range of values), +/- 5% of the stated value (or range of values), +/- 10% of the stated value (or range of values), etc. Any numerical values given herein should also be understood to include about or
20 approximately that value, unless the context indicates otherwise. For example, if the value “10” is disclosed, then “about 10” is also disclosed. Any numerical range recited herein is intended to include all sub-ranges subsumed therein. It is also understood that when a value is disclosed that “less than or equal to” the value, “greater than or equal to the value” and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the
25 value “X” is disclosed the “less than or equal to X” as well as “greater than or equal to X” (e.g., where X is a numerical value) is also disclosed. It is also understood that the throughout the application, data is provided in a number of different formats, and that this data, represents endpoints and starting points, and ranges for any combination of the data points. For example, if
30 a particular data point “10” and a particular data point “15” are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed as well as between 10 and 15. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[0101] Although various illustrative embodiments are described above, any of a number of
35 changes may be made to various embodiments without departing from the scope of the invention

as described by the claims. For example, the order in which various described method steps are performed may often be changed in alternative embodiments, and in other alternative embodiments one or more method steps may be skipped altogether. Optional features of various device and system embodiments may be included in some embodiments and not in others.

5 Therefore, the foregoing description is provided primarily for exemplary purposes and should not be interpreted to limit the scope of the invention as it is set forth in the claims.

[0102] The examples and illustrations included herein show, by way of illustration and not of limitation, specific embodiments in which the subject matter may be practiced. As mentioned, other embodiments may be utilized and derived there from, such that structural and logical
10 substitutions and changes may be made without departing from the scope of this disclosure. Such embodiments of the inventive subject matter may be referred to herein individually or collectively by the term “invention” merely for convenience and without intending to voluntarily limit the scope of this application to any single invention or inventive concept, if more than one is, in fact, disclosed. Thus, although specific embodiments have been illustrated and described
15 herein, any arrangement calculated to achieve the same purpose may be substituted for the specific embodiments shown. This disclosure is intended to cover any and all adaptations or variations of various embodiments. Combinations of the above embodiments, and other embodiments not specifically described herein, will be apparent to those of skill in the art upon reviewing the above description.

20

CLAIMS

What is claimed is:

1. A method of performing droplet operations on a droplet at least partially coated in wax within an air-matrix digital microfluidic (DMF) apparatus, the method comprising:
5 moving, by electrowetting, an aqueous reaction droplet having an outer coating of wax within an air gap of the air-matrix DMF apparatus which is formed between a first plate and a second plate of the air-matrix DMF apparatus; merging the aqueous reaction droplet with a carrier droplet comprising an aqueous droplet coated with an oil or an organic solvent in the air gap to form
10 a combined droplet; and moving, by electrowetting, the combined droplet within the air gap.
2. The method of claim 1, wherein moving, by electrowetting, the aqueous reaction droplet comprises initially transporting the aqueous reaction droplet to a thermal zone of the air gap, the thermal zone comprising a wax and melting the wax within the
15 thermal zone to at least partially encapsulate the aqueous reaction droplet with the wax.
3. The method of claim 2, further comprising regulating the temperature of the at least partially encapsulated reaction droplet to allow a reaction to proceed within the aqueous reaction droplet before transporting the aqueous reaction droplet having the
20 outer coating of wax within the air gap.
4. The method of claim 1, wherein moving, by electrowetting, the aqueous reaction droplet comprises transferring the aqueous reaction droplet away from away from a thermal zone comprising a wax material so that at least some of the wax is left behind.
- 25 5. The method of claim 2, wherein melting the wax comprises melting a solid wax formed into a wall or open chamber within the air gap.
6. The method of claim 1, further comprising detecting a product within the aqueous reaction droplet or the combined droplet.
7. The method of claim 1, wherein merging the aqueous reaction droplet with the carrier
30 droplet comprises moving one or both of the aqueous reaction droplet and the carrier droplet into contact with each other by electrowetting.

8. The method of claim 1, further comprising mixing the combined droplet, wherein the combined droplet comprises a plurality of beads.
9. The method of claim 8, further comprising immobilizing the beads.
10. The method of claim 9, further comprising moving the combined droplet away from the immobilized beads.
- 5
11. The method of claim 10, further comprising re-suspending the immobilized beads within an aqueous droplet.
12. The method of claim 8, further comprising separating the beads from the combined droplet by moving a magnetic field away from the combined droplet to magnetically draw the beads away from the combined droplet.
- 10
13. An air-matrix digital microfluidic (DMF) apparatus configured to prevent evaporation, the apparatus comprising:
- a first plate having a first hydrophobic layer;
 - a second plate having a second hydrophobic layer;
 - 15 an air gap formed between the first and second hydrophobic layers;
 - a plurality of actuation electrodes adjacent to the first hydrophobic layer;
 - a thermal regulator arranged to heat a portion of the air gap configured as a thermal zone;
 - a wax material within the air gap; and
 - 20 a controller programmed to:
 - actuate the plurality of actuation electrodes to transport an aqueous reaction droplet through the air gap to the wax material and encapsulate the aqueous reaction droplet with the wax material;
 - regulate the temperature of the thermal zone to perform a reaction protocol
 - 25 within the wax-encapsulated aqueous reaction droplet;
 - actuate the plurality of actuation electrodes to transport the aqueous reaction droplet away from the thermal zone;
 - actuate the plurality of actuation electrodes to merge a carrier droplet comprising an oil or an organic solvent coated aqueous droplet with the
 - 30 aqueous reaction droplet.
14. The apparatus of claim 13, wherein the wax body is within the thermal zone of the air gap.

15. The apparatus of claim 13, wherein the first plate and the second plate comprise a cartridge that is removably coupleable to the plurality of actuation electrodes.
16. The apparatus of claim 13, wherein the wax body spans a plurality of adjacent actuation electrodes of the plurality of actuation electrodes.
- 5 17. The apparatus of claim 13, wherein the wax body comprises a wall of wax within the air gap.
18. The apparatus of claim 13, wherein the wax body forms a channel or vessel within the air gap.
19. The apparatus of claim 13, wherein the wax body comprises paraffin wax.
- 10 20. The apparatus of claim 13, wherein the plurality of actuation electrodes form a portion of the first plate.
21. The apparatus of claim 13, wherein the carrier droplet comprises beads.
22. The apparatus of claim 21, wherein the beads are magnetic.
23. The apparatus of claim 21, wherein the beads are configured to bind to a molecule
15 selected from the group consisting of DNA, RNA, and proteins.
24. The apparatus of claim 13, wherein the carrier droplet comprises a reagent, a primer, a dilution buffer, an enzyme, a protein, a nanopore, a wash buffer, an alcohol, formamide, or a detergent.
25. The apparatus of claim 13, wherein the wax body is disposed on a removable sheet that
20 can be removably attached to either the first plate or the second plate.
26. A method of preventing droplet evaporation within an air-matrix digital microfluidic (DMF) apparatus, the method comprising:
- 25 introducing an aqueous reaction droplet into an air gap of the air-matrix DMF apparatus which is formed between a first plate and a second plate of the air-matrix DMF apparatus;
- transporting the aqueous reaction droplet to a thermal zone of the air gap, the thermal zone comprising a wax;
- melting the wax within the thermal zone to encapsulate the aqueous reaction droplet with the wax;

- regulating the temperature of the encapsulated reaction droplet to allow a reaction to proceed within the aqueous reaction droplet;
- transporting the aqueous reaction droplet away from the thermal zone after the reaction is completed;
- 5 introducing a carrier droplet comprising an oil or an organic solvent coated aqueous droplet into the air gap; and
- merging the aqueous reaction droplet with the carrier droplet to form a merged droplet.
27. The method of claim 26, wherein melting the wax comprises melting a solid wax formed into a wall or open chamber within the air gap.
- 10 28. The method of claim 26, wherein introducing the aqueous reaction droplet into an air gap comprises combining multiple droplets to form the aqueous reaction droplet within the air gap.
29. The method of claim 26, wherein the first plate comprises a plurality of adjacent actuation electrodes, and wherein combining the aqueous reaction droplet with the melted wax comprises applying energy to a subset of the actuation electrodes of the plurality of adjacent actuation electrodes to move the aqueous reaction droplet in contact with the wax prior to melting the wax.
- 15 30. The method of claim 26, wherein the first plate comprises a plurality of adjacent actuation electrodes, and wherein combining the aqueous reaction droplet with the melted wax comprises applying energy to a subset of the actuation electrodes of the plurality of adjacent actuation electrodes to move the aqueous reaction droplet in contact with the melted wax after melting the wax.
- 20 31. The method of claim 26, further comprising detecting a product within the aqueous reaction droplet.
- 25 32. The method of claim 26, further comprising mixing the reaction droplet with a plurality of beads after the carrier droplet has been merged with the aqueous reaction droplet.
33. The method of claim 26, further comprising immobilizing the beads after the carrier droplet has been merged with the aqueous reaction droplet.
- 30 34. The method of claim 22, further comprising moving the merged carrier droplet and

aqueous reaction droplet away from the immobilized beads.

35. The method of claim 26, further comprising re-suspending the immobilized beads with an aqueous droplet.

5 36. The method of claim 26, further comprising separating the beads from the merged carrier droplet and aqueous reaction droplet by moving a magnet away from the merged carrier droplet and aqueous reaction droplet.

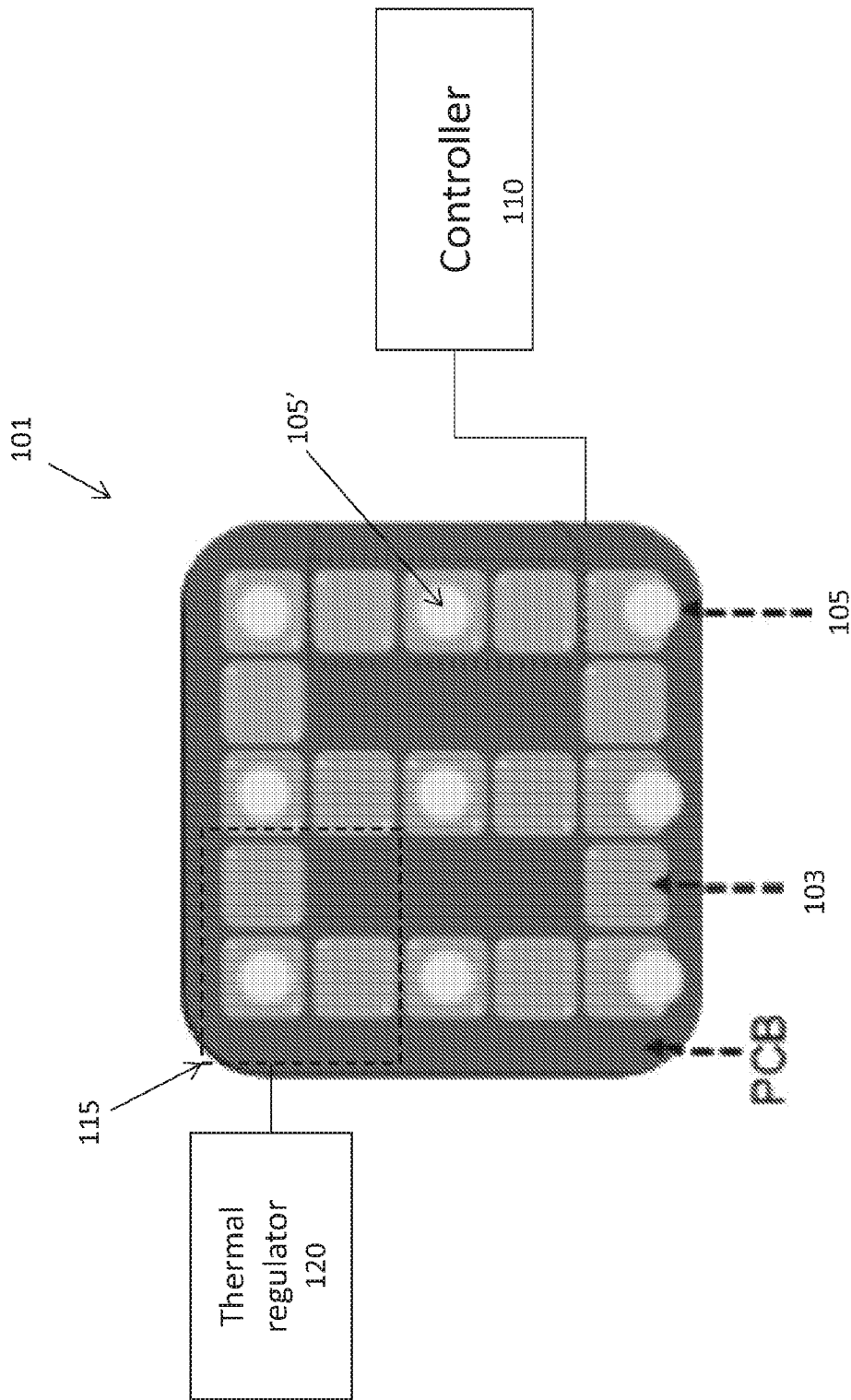


FIG. 1

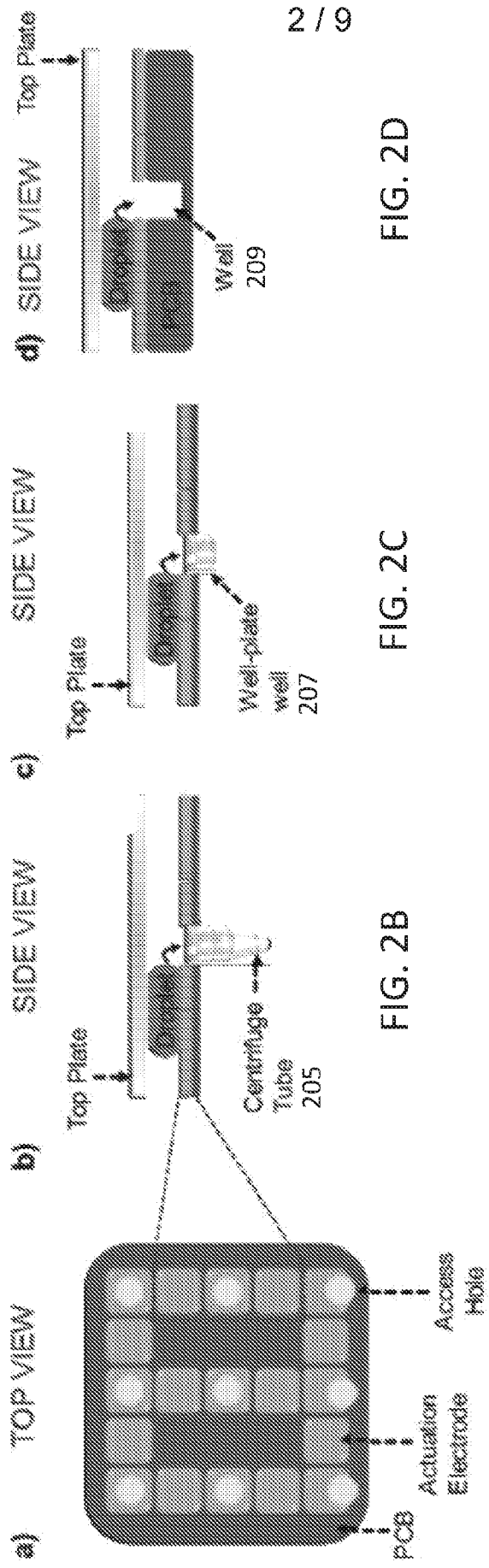


FIG. 2D

FIG. 2C

FIG. 2B

FIG. 2A

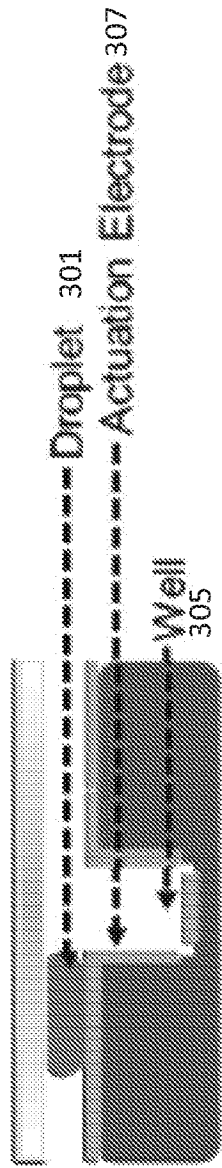


FIG. 3A

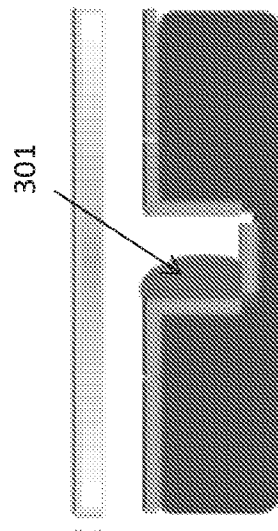


FIG. 3B

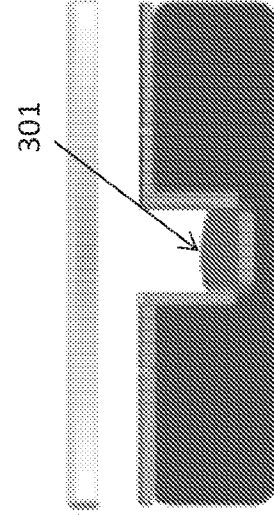


FIG. 3C

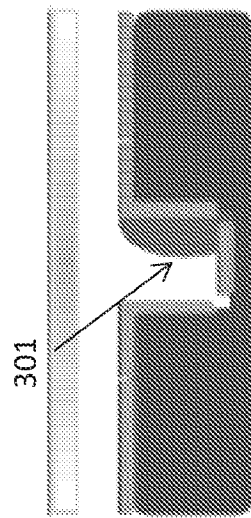


FIG. 3D

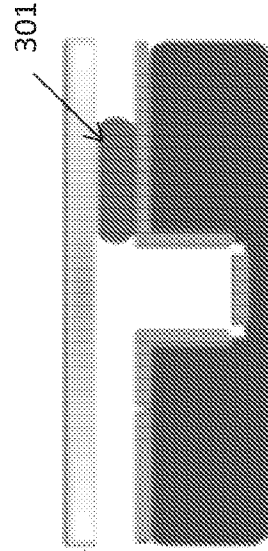


FIG. 3E

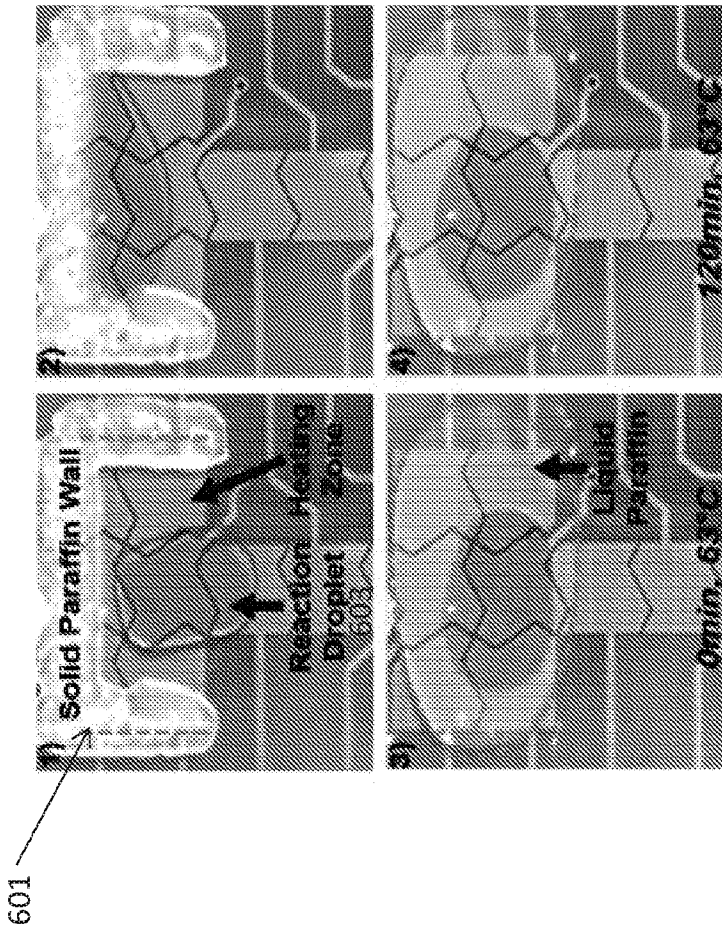


FIG. 4A

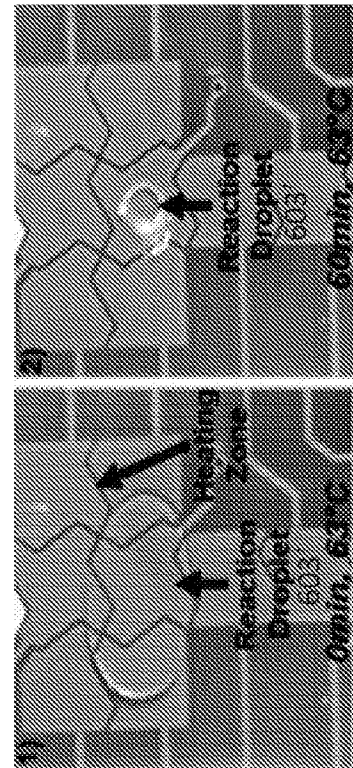


FIG. 4B

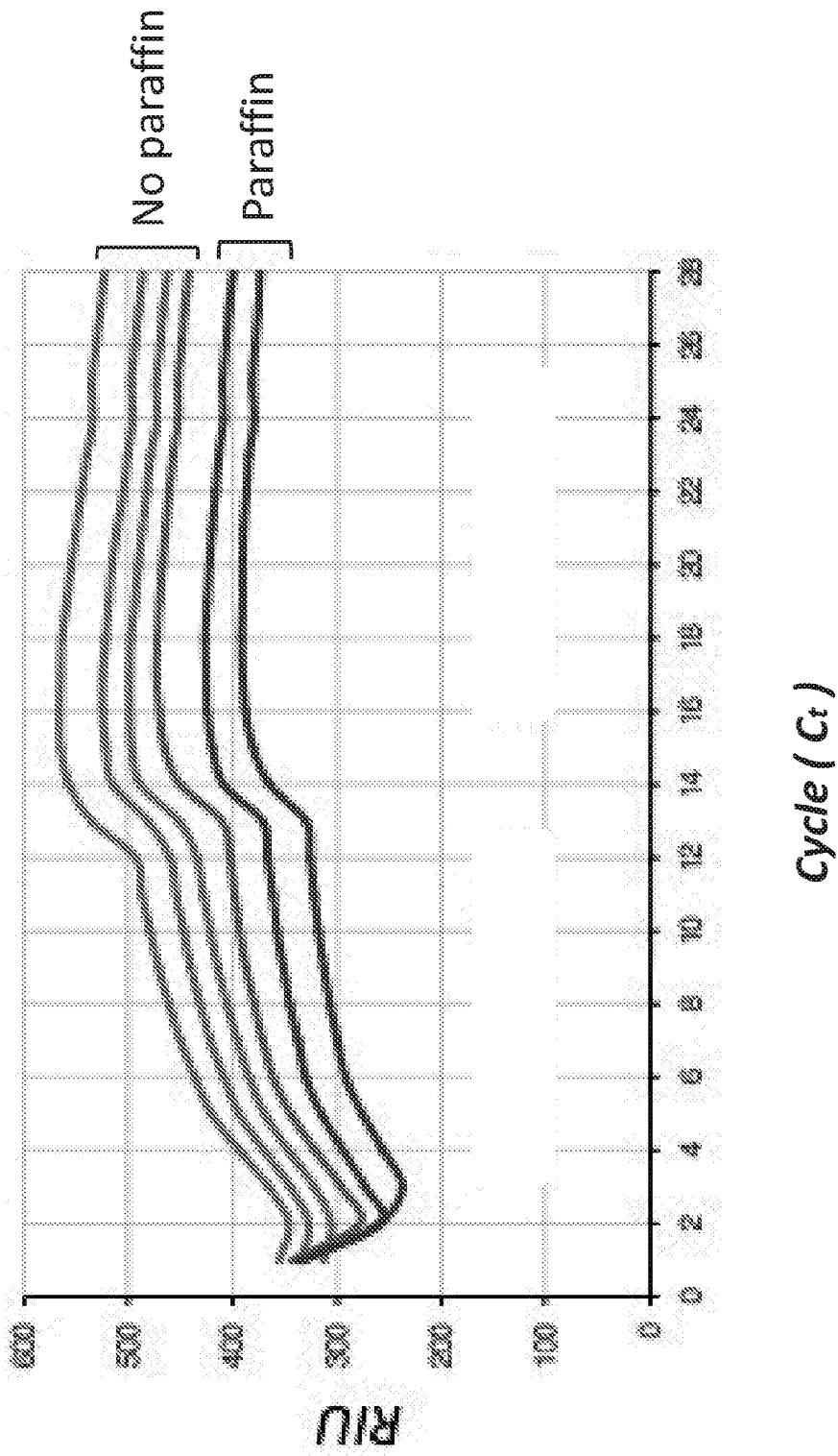


FIG. 5

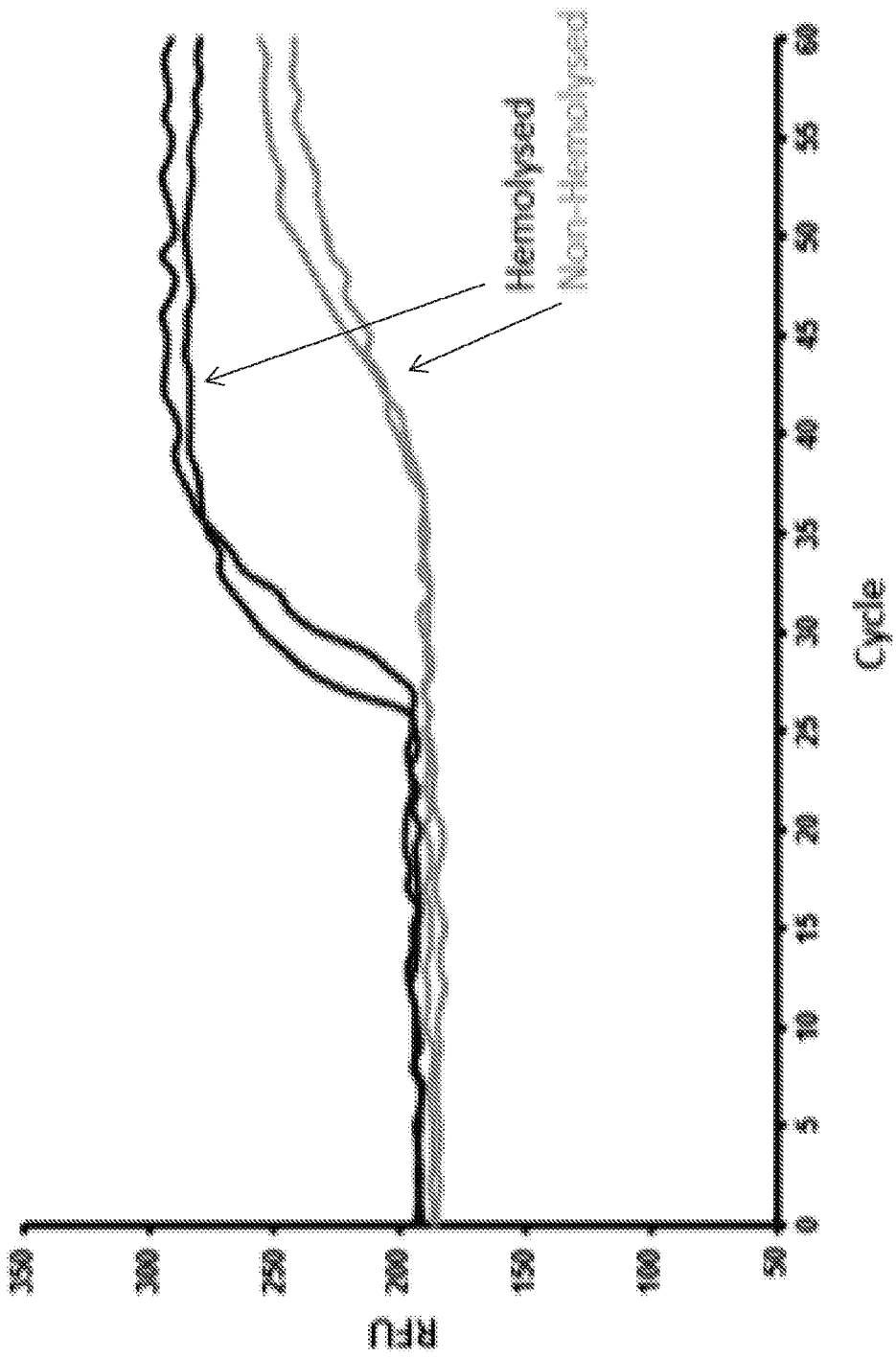


FIG. 6A

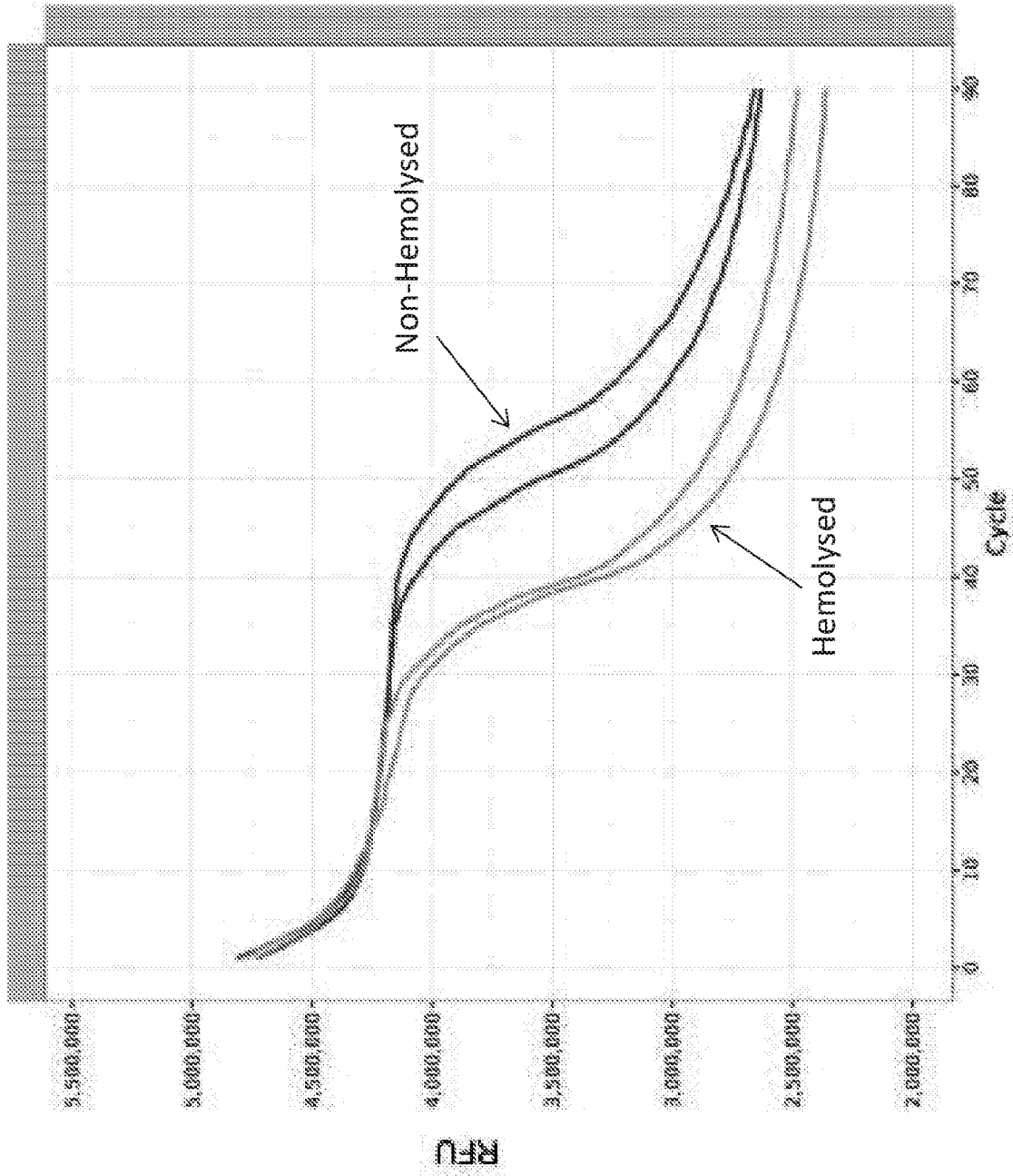


FIG. 6B

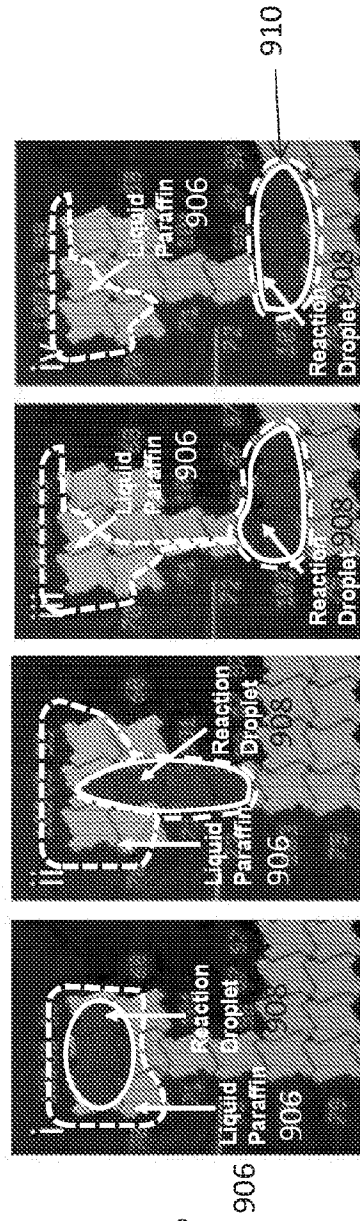
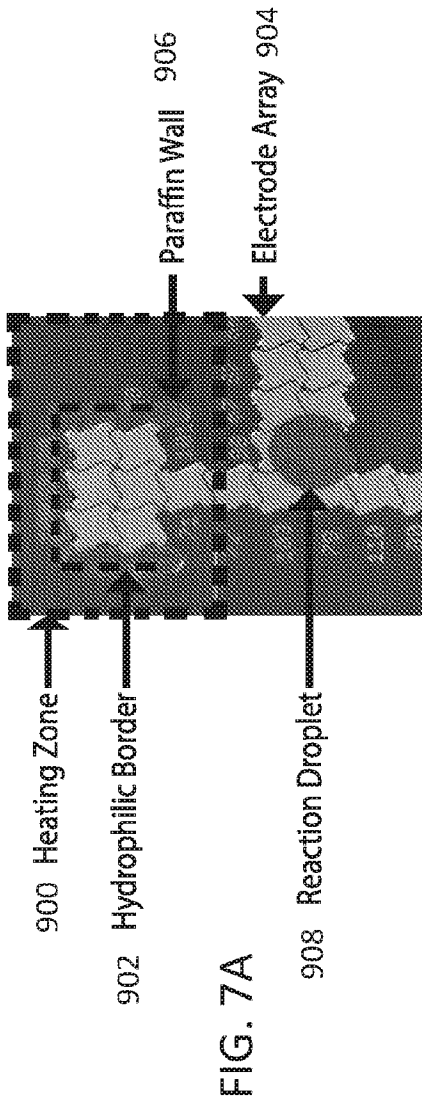
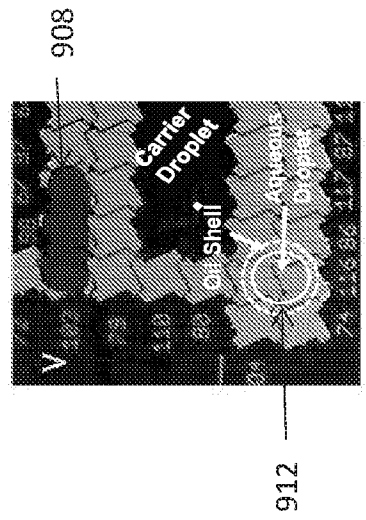
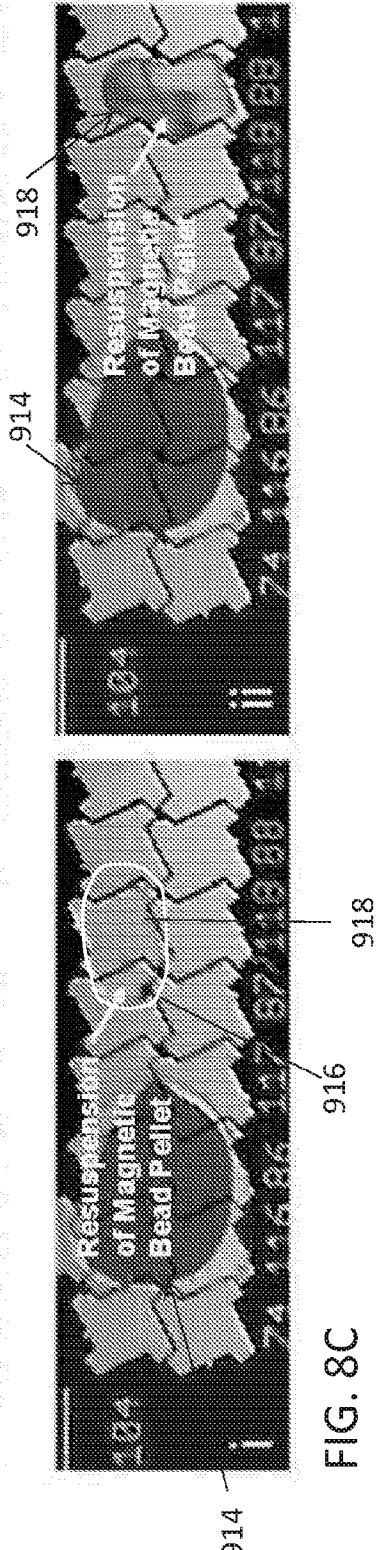
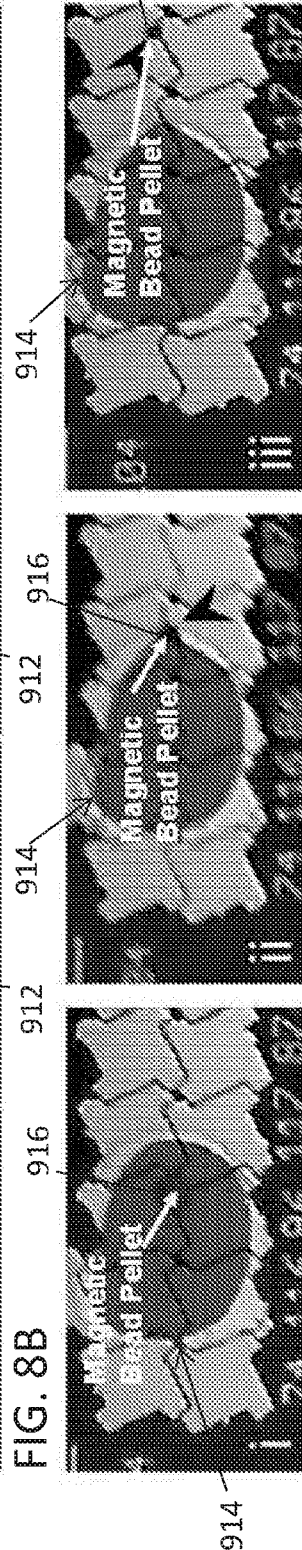
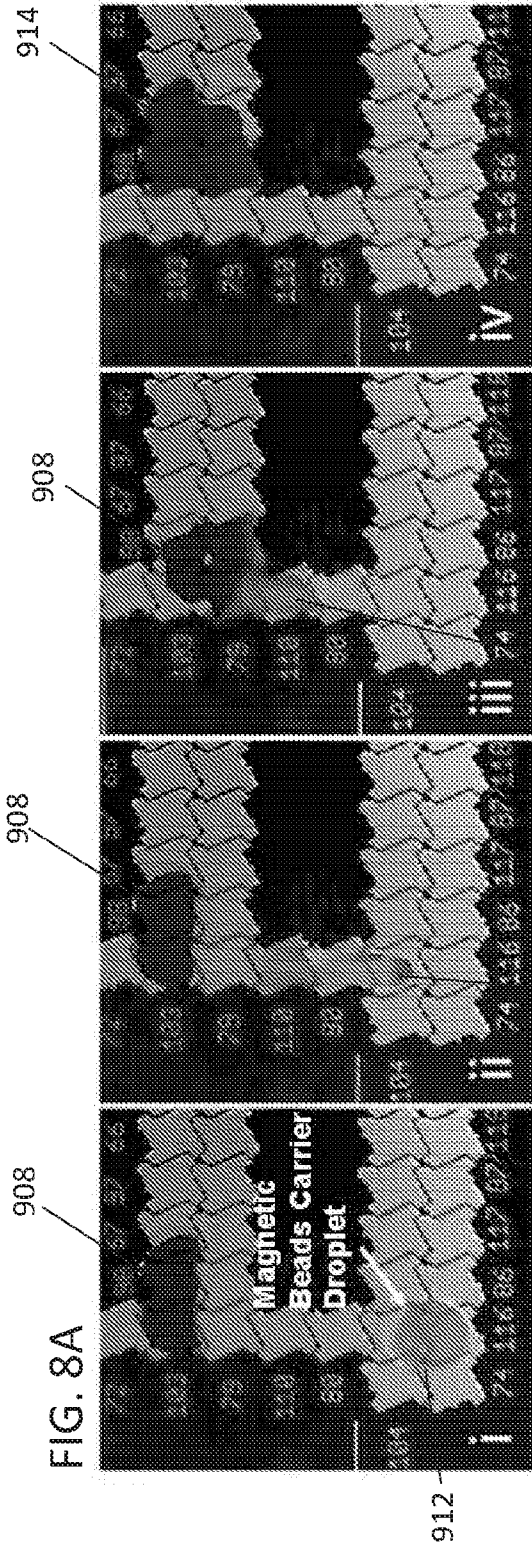


FIG. 7B





INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/026095

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - B01L 3/00; G01N 27/44 (2018.01)

CPC - B01L 3/502792; B01L 2200/142; G01N 27/453 (2018.05)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 204/450; 204/451 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016/197103 A1 (MIROCULUS INC.) 08 December 2016 (08.12.2016) entire document	1-36
Y	US 2015/0258520 A1 (THE BROAD INSTITUTE INC. et al) 17 September 2015 (17.09.2015) entire document	1-36
Y	US 2013/0203606 A1 (POLLACK et al) 08 August 2013 (08.08.2013) entire document	8-12, 21-23, 32-36
Y	US 2010/0081578 A1 (WHEELER et al) 01 April 2010 (01.04.2010) entire document	15, 25

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

06 June 2018

Date of mailing of the international search report

28 JUN 2018

Name and mailing address of the ISA/US

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