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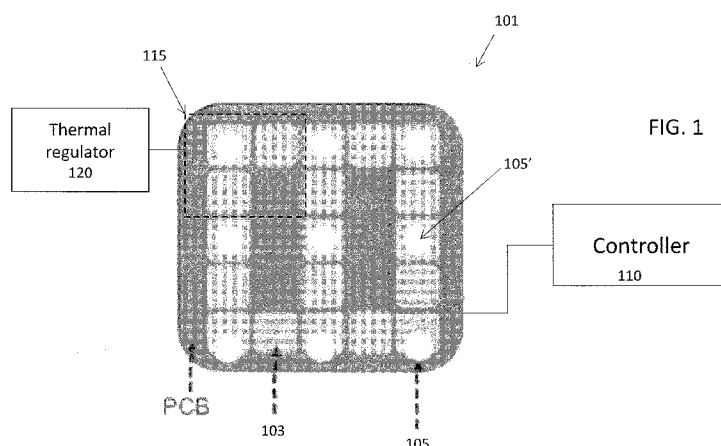
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(54) Title: AIR-MATRIX DIGITAL MICROFLUIDICS APPARATUSES AND METHODS FOR LIMITING EVAPORATION AND SURFACE FOULING



(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 in which a separate well that is accessible from the air gap of the DMF apparatus isolates a reaction droplet by including a cover to prevent evaporation. The cover may be a lid or cap, or it may be an oil or wax material within the well. The opening into the well and/or the well itself may include actuation electrodes to allow the droplet to be placed into, and in some cases removed from, the well. Also 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.

AIR-MATRIX DIGITAL MICROFLUIDICS APPARATUSES AND METHODS FOR LIMITING EVAPORATION AND SURFACE FOULING

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application 62/171,756 entitled,
5 “DEVICE AND METHODS FOR LIMITING EVAPORATION AND SURFACE FOULING,” filed on
June 5, 2015, which is herein incorporated by reference in its entirety.

INCORPORATION BY REFERENCE

[0002] All publications and patent applications mentioned in this specification are herein
10 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.

FIELD

[0003] Air-matrix digital microfluidic (DMF) apparatuses and methods that limit or prevent
15 evaporation and/or surface fouling are described herein.

BACKGROUND

[0004] Microfluidics has come a long way to transform the way traditional procedures in molecular
biology, medical diagnostics, and drug discovery are performed. Lab-on-a-chip and biochip type devices
20 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 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
25 microfluidic device.

[0005] 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
30 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.

[0006] Despite significant advances, both evaporation, particularly in air-matrix DMF, and surface
35 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.

[0007] 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.

[0008] 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.

[0009] 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

[00010] Described herein are air-matrix DMF apparatuses that minimize surface fouling and/or evaporation.

[00011] 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.

[00012] In general, any of the air-matrix apparatuses described herein may include a reaction chamber opening that extends transversely through a plate of the apparatus. The reaction chamber opening is typically configured to put a reaction chamber well in fluid communication with the air gap. The opening through the plate may be referred to as the reaction chamber opening and the chamber into which this opening exits may be referred to as the reaction chamber well (or simply 'reaction chamber'). A reaction chamber opening may be at least partially surrounded by an actuation electrode from the plurality of actuation electrodes; these electrodes may extend down into the opening, out of the plane of the plate. In any of these variations, the reaction chamber opening may pass through an actuation electrode.

[00013] A reaction chamber opening may be any appropriate size and shape. The opening may be round, oval, square, triangular, hexagonal, rectangular, etc. The reaction chamber opening may be between 0.1 mm and 20 mm wide (e.g., between 1 and 15 mm, between 2 and 12 mm, wide, etc.).

[00014] The air-matrix DMF apparatuses and methods described herein may enable facile and reliable execution of biochemical reactions over a range of temperatures (including but not limited to 4-95°C) and incubation times (including but not limited to times up to 6 hr. or more, e.g., up to 1 hr., up to 2 hr., up to 3 hr., etc.). The air-matrix DMF apparatuses described herein may include at least one reaction chamber well, and/or may be configured to couple to one or more reaction chamber wells. The reaction chamber wells may be in close proximity to at least one actuating electrode. In some variations the reaction chamber well may include one or more actuation electrodes that are out of the plane of the plurality of actuation electrodes operation in the air gap. The reaction chamber well may be in fluid communication with other areas of the air gap region of the DMF apparatus.

[00015] The air gap region of the air matrix DMF apparatus may include one or more regions for holding starting reaction solutions as well as regions for other solutions that may include subsequent components for the reaction (e.g., enzymes, nucleotides, etc.) or additional reaction solution (solvent). One or more reservoirs may be in communication with the air gap, and in some instances there may be access holes for introducing solutions having starting material, reagent solution, and so forth. Access holes may be connected to external sources of starting components, other reaction component, reaction

reagents, and so on. In other instances, the DMF apparatus may be provided having all the reaction materials needed.

[00016] A reaction chamber well may be located in close proximity to one or more actuating electrodes. For example, an actuating electrode may be in contact with the reaction chamber opening to enable transportation of a droplet into the reaction compartment. In some instances, the reaction chamber well(s) may be removable from air gap DMF apparatus; for example, the reaction chamber well may be a centrifuge tube (e.g., microcentrifuge tubes, Eppendorf tubes, etc., of any appropriate volume, such as 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml, 1 ml, 1.2 ml, 1.4 ml, 1.5 ml, 1.8 ml, 2 ml, etc.) or a multi-well plate (e.g., microtiter plates, etc. of any appropriate size and volume, such as 96 well plates, 384 well plates, 1536 well plates, etc.) that may be securely coupled to the reaction chamber opening. In some variations the reaction chamber well may be formed within either the first or second plate, such as formed of a printed circuit board (PCB) that may also act as a substrate for the actuating electrodes.

[00017] For example, described herein are air-matrix digital microfluidic (DMF) apparatuses configured to prevent evaporation and surface fouling. The air-matrix DMF 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 arranged in a first plane adjacent to the first hydrophobic layer; one or more ground electrodes; and a reaction chamber opening that extends transversely through the first plate, the reaction chamber opening configured to put a reaction chamber well in fluid communication with the air gap, wherein the reaction chamber opening is at least partially surrounded by an actuation electrode from the plurality of actuation electrodes.

[00018] An air-matrix digital microfluidic (DMF) apparatus configured to prevent evaporation and surface fouling 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 arranged in a first plane adjacent to the first hydrophobic layer; one or more ground electrodes; a reaction chamber opening that extends through first plate, the reaction chamber opening configured to put a reaction chamber well in fluid communication with the air gap, wherein the reaction chamber opening is at least partially surrounded by an actuation electrode from the plurality of actuation electrodes; a reaction chamber cover adjacent to the reaction chamber opening, the reaction chamber cover configured to be actuated to close over the reaction chamber opening and separate the reaction chamber from the air gap; and a controller electrically coupled to the plurality of actuation electrodes and configured to apply energy to the plurality of actuation electrodes to move a droplet into and out of the reaction chamber. An air-matrix digital microfluidic (DMF) apparatus configured to prevent evaporation and surface fouling 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 arranged in a first plane adjacent to the first hydrophobic layer; one or more ground electrodes; a reaction chamber opening that extends through the first plate transverse to the first plane, the reaction chamber opening configured to put a reaction chamber well in fluid communication

with the air gap, wherein the reaction chamber opening is at least partially surrounded by an actuation electrode from the plurality of actuation electrodes; one or more reaction chamber actuation electrodes within the reaction chamber opening, wherein the one or more reaction chamber actuation electrodes are positioned or extend out of the first plane; a reaction chamber cover adjacent to the reaction chamber opening, the reaction chamber cover configured to be actuated to close over the reaction chamber opening and separate the reaction chamber from the air gap; and a controller electrically coupled to the plurality of actuation electrodes and the one or more reaction chamber actuation electrodes and configured to apply energy to move a droplet into and out of the reaction chamber.

[00019] Any of these apparatuses may include a reaction chamber cover. In general, the term 'cover' is broadly understood to be any cover, including a cover configured to close off the reaction chamber from the air gap, and/or sealing the reaction chamber, and one or more substances in the reaction chamber (e.g., oil, wax, etc.) that cover the reaction droplet when it is within the reaction chamber well.

[00020] In some variations the reaction chamber cover is positioned adjacent to the reaction chamber opening, and is configured to be actuated to close over the reaction chamber opening and separate the reaction chamber from the air gap.

[00021] Any of the apparatus variations described herein may include oil or 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.

[00022] The reaction chamber opening may be surrounded and pass through an actuation electrode from the plurality of actuation electrodes. For example, a reaction chamber opening may be a hole through an actuation electrode and any other layers of a plate. Any of the reaction wells described herein may also or alternatively include one or more reaction chamber actuation electrodes within the reaction chamber opening. The reaction chamber activation electrodes may be configured to connect to the same controller controlling and/or coordinating activation of the plurality of actuation electrodes, e.g., to move one or more droplets within the air gap. For example, the DMF apparatus may include one or more reaction chamber actuation electrodes within the reaction chamber opening, wherein the one or more reaction chamber actuation electrodes are positioned out of the first plane. The DMF apparatus may also or alternatively include one or more reaction chamber actuation electrodes within the reaction chamber opening, wherein the one or more reaction chamber actuation electrodes are positioned within the reaction chamber. All or a part of the reaction chamber well may include an outer hydrophobic surface. For example, the outer surface may be coated with a hydrophobic coating layer (which may also be a dielectric material/coating). In some variations, just the region over (immediately adjacent to) the actuation electrodes is hydrophobic; for example the reaction chamber activation electrodes may be coated with a dielectric and hydrophobic coating.

[00023] The reaction chamber cover may be coupled to the second plate. For example, when the reaction chamber cover includes a plastic, metal or other foreign material that is adapted (e.g., sized, shaped, etc.) to fit into the reaction chamber opening, and in some cases seal it off. The second plate may be flexible and configured to be pushed to seal the reaction chamber cover over the reaction chamber opening. In some variation the second plate is itself acts as a reaction chamber lid, deflecting to close off the reaction chamber opening. In general, the second plate may be flexible and configured to be pushed to seal over the reaction chamber opening.

[00024] Any of the air matrix DMF apparatuses described herein may include a thermal regulator adjacent to the first plate and configured to control the temperature of a portion of the air gap and the reaction chamber.

[00025] As mentioned above, the air matrix DMF may include any appropriate reaction chamber wells that form part of or are connected to a plate of the apparatus. For example, a reaction chamber well may be formed within a thickness of the plate (e.g., within a substrate such as the PCB forming a plate). Thus, the first plate may comprise a reaction chamber within a thickness of the first plate. The first plate may include a printed circuit board (PCB) and the reaction chamber is formed as a well within the PCB. Alternatively, the air-matrix DMF apparatus may include a first plate that is configured to mate with a multi-well plate so that the reaction chamber opening mates with a well of the multi-well plate. Alternatively or additionally, the DMF apparatus's first plate may be configured to mate with a centrifuge tube so that the reaction chamber opening mates with the centrifuge tube.

[00026] The air gap layer may generally include at least one sample region and at least one reagent region.

[00027] In any of the variations described herein, the reaction chamber opening may be flush with the hydrophobic layer and/or dielectric layer. The reaction chamber well's internal surface may be hydrophilic or hydrophobic. For example, in variations in which there are no actuating electrodes in the reaction chamber well (that may actively move a droplet into a well), it may be beneficial that the surface be hydrophilic to attract the droplet and pull it inside the well. In variations in which there are one or more actuating electrodes in the reaction chamber well, the surface may be hydrophobic. As mentioned, any of the reaction chamber wells (which may be referred to herein as just 'reaction chambers' for simplicity) may include one or more electrode, e.g., actuation electrodes.

[00028] Also described herein are methods of preventing or reducing evaporation using an air gap DMF apparatus. For example, a method of operating a matrix digital microfluidic (DMF) apparatus to prevent evaporation and surface fouling 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; transporting the reaction droplet along the air gap and through a reaction chamber opening that extends through the first plate, and into a reaction chamber well; covering the reaction droplet within the reaction chamber well to protect the reaction droplet from evaporation; and allowing a reaction to proceed within the reaction droplet.

[00029] A method of operating a matrix digital microfluidic (DMF) apparatus to prevent evaporation and surface fouling 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, wherein the first plate comprises a plurality of adjacent actuation electrodes; transporting the reaction droplet along the air gap and through a reaction chamber opening that extends through the first plate, and into a reaction chamber well by applying energy to a subset of the actuation electrodes of the plurality of actuation electrodes; covering the reaction droplet within the reaction chamber well to protect the reaction droplet from evaporation; and heating the reaction chamber well so that a reaction may proceed within the reaction droplet.

[00030] A method of operating a matrix digital microfluidic (DMF) apparatus to prevent evaporation and surface fouling 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, wherein the first plate comprises a plurality of adjacent actuation electrodes; transporting the reaction droplet along the air gap and through a reaction chamber opening that extends through the first plate, and into a reaction chamber well by applying energy to a subset of the actuation electrodes of the plurality of actuation electrodes; sealing the reaction chamber well opening with a cover to close off the reaction chamber opening from the air gap to protect the reaction droplet from evaporation; and heating the reaction chamber well so that a reaction may proceed within the reaction droplet.

[00031] Introducing the reaction droplet into an air gap may generally include combining multiple droplets to form a reaction droplet within the air gap.

[00032] In any of these examples, the first plate may include a plurality of adjacent actuation electrodes, and wherein transporting the reaction droplet comprises applying energy to a subset of the actuation electrodes of the plurality of adjacent actuation electrodes. In any of these examples, covering the reaction droplet may include sealing the reaction chamber well opening with a cover to close off the reaction chamber opening from the air gap to protect the reaction droplet from evaporation.

[00033] Covering the reaction chamber may generally comprise combining the reaction droplet with a droplet of oil or wax within the reaction chamber. As mentioned, the oil or wax may mix with the reaction droplet and enclose it, protecting from from evaporation. The droplet may still be visualized through the cover, including any wax or oil cover formed by combining with a reaction droplet.

[00034] In any of the variations described herein, allowing a reaction to proceed may comprise heating the reaction chamber well, e.g., using the same or a different thermal controller than described previously for heating and/or cooling the thermal zone.

[00035] In some variations, sealing the reaction chamber well opening comprises pushing on the second plate to cover the reaction chamber well opening.

[00036] Any of these methods may also include moving the reaction chamber droplet from the reaction well and into the air gap by applying energy to at least one actuation electrode within the reaction well. Any of these methods may also include detecting a product within the reaction droplet. The product may be detected visually and/or (e.g., using a colorimetric test, etc.) or like.

[00037] Also 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 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 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.

[00038] 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 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.

[00039] 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 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.).

[00040] 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 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.

[00041] 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 droplet from evaporation; and allowing a reaction to proceed within the reaction droplet.

[00042] 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.

5 [00043] 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 melting the wax body.

10 [00044] 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.

[00045] 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.

15 [00046] 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. In this case, the apparatus
20 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 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
25 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[00047] 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
30 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:

[00048] 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).

35 [00049] 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.

[00050] FIGS. 3A-3E illustrate movement (e.g., controlled by a controller of an air-matrix DMF apparatus) into an 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.

[00051] FIGS. 4A and 4B show side views of air-matrix DMF apparatuses in which a reaction chamber well includes an inert coating substance (e.g., oil, wax, etc.) that may combine with and cover the reaction droplet.

[00052] FIGS. 5A-5B show a side view of one example of a portion of an air-matrix DMF apparatus in which the top plate is flexible and includes a cover (e.g., plug, cap, lid, etc.) for the reaction chamber opening that is coupled to the plate. FIG. 5A shows the reaction chamber well (configured as a tube) before the reaction chamber opening is sealed, and FIG. 5B shows the reaction chamber well after pushing on the flexible top plate to seal the reaction chamber opening with the plug.

[00053] FIGS. 5C-5D illustrate sealing of another variation of a reaction chamber well using a flexible top plate having an integrated cover opposite from the reaction chamber well opening. FIG. 5C shows the reaction chamber well before it is sealed and FIG. 5D shows the reaction chamber well after pushing down on the top plate to seal it.

[00054] FIG. 6A 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.

[00055] FIG. 6B is an example of a time series similar to that shown in FIGS. 6A(3) and 6A(4), without using a wax body to cover the reaction droplet, showing significant evaporation.

[00056] FIG. 7 is a graph comparing an amplification reaction by LAMP with and without a wax covering as described herein, protecting the reaction droplet from evaporation.

[00057] FIG. 8A show graphical results of LAMP using paraffin-mediated methods; this may be qualitatively compared to the graph of FIG. 8B shows graphical results of LAMP using conventional methods.

DETAILED DESCRIPTION

[00058] Described herein are air-matrix digital microfluidics (DMF) methods and apparatuses 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.

[00059] 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 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 function.

[00060] 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, 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 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 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.

[00061] 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.

[00062] 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.

- 5 **[00063]** 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.
- 10 **[00064]** 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)
- 15 by the apparatus to perform a reaction when covered. The cover may be a mechanical cover (e.g., a cover the 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.
- 20 **[00065]** 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.
- 25 **[00066]** 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
- 30 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
- 35 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.

[00067] In the air-matrix apparatuses described herein, the first (lower) plate also includes 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 through 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.

[00068] 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 105, 105' 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.

[00069] 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 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).

[00070] 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. 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 product droplet may be analyzed directly within the reaction chamber.

[00071] 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 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 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). 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 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.

[00072] 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 mentioned, an electrode (actuation electrode) may apply a potential to move the droplets into and/or out of the well.

[00073] In general, the actuation electrodes may bring the droplet into the well in a controlled manner and 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 shows the droplet being moved up the 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.

[00074] 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.

[00075] 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 in resistance correlated with position of a droplet).

[00076] 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 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 biochemical type reactions in microfluidic and DMF devices to address unwanted evaporation. One major drawback of using an oil in the DMF reaction is the added complexity of incorporating additional structures to contain the oil.

[00077] The well or reaction chamber structure described herein provides a simple solution for maintaining oil in a DMF device. FIGS. 4A and 4B shows the DMF devices having a well or tube integrated into its structure (reaction chamber well) where the well or tube may including a protective/coating material such as oil 405, 407. This may allow the reaction steps that require heating to be performed in an oil environment where the oil is able to limit evaporation of the reaction mixture. If additional steps are still required, the reaction droplet may be brought out of the tube or well through use of the actuation electrodes to move the reaction droplet to a different location. Other covers comprising coating or covering agents may include wax materials (e.g., paraffin, etc.) or the like, which may be melted to combine with and cover/coat the reaction droplet. The temperature may be adjusted to add (or in some variations, remove) the wax material from the droplet. As described in greater detail below, a

wax material may be used in the air gap directly, without a dedicated reaction chamber (or forming the reaction 'chamber' in the air gap from the wax material).

[00078] Covering the droplet may allow performance of reaction steps (particularly those that require heating) and may limit evaporation. For example, the reaction chamber well may be mechanically covered by covering the reaction chamber opening. FIGS. 5A-5C suggest one way of covering a reaction chamber opening using a cap, plug, or lid 505 that is coupled to the upper plate 503. In this example, the top plate of the DMF apparatus may be flexible. When force 510 is applied to an outer surface of the top plate, the plate becomes deformed and the cap 505 is pushed down into the reaction well opening, sealing the reaction chamber well 501, as shown in FIG. 5B. In the example of FIGS. 5A and 5B the reaction chamber well is a tube 505 (e.g., microfuge tube, etc.). The air-matrix DMF apparatus includes a lower plate with actuation electrodes 507 covered by a hydrophobic (and in this example, dielectric) layer 509.

[00079] Similarly, FIGS. 5C and 5D illustrate a variation in which the reaction chamber/well 551 is formed into the lower plate and the upper plate 503 also includes a cover 505 (e.g., cap, lid, plug, etc.). Alternatively, the upper plate may be lowered over the cover to seal the reaction chamber/well opening even if it does not include a separate cover, etc. For example the upper plate itself may cover the opening. In general, it is not necessary that the upper plate be flexible, as the entire upper plate may be lowered to seal the reaction chamber well (not shown); the upper plate may later be restored to the same air gap width if desired, and the droplet may be returned to the air gap or not, as illustrated in FIGS. 3A-3E for additional processing/detection. Being able to perform a heated step of a reaction in a closed chamber may aid with evaporation prevention and, unlike instances where the entire DMF device is placed in a humidifying chamber, does not cause unwanted condensation in other regions of the DMF device.

[00080] Another method that has been developed to combat evaporation is the use of wax (e.g. paraffin) in minimizing evaporation during a reaction. A wax substance may include substances that are composed of long alkyl chains. Waxes typically solids at ambient temperatures having a melting point of approximately 46°C to approximately 68°C depending upon the amount of substitution within the hydrocarbon chain. In some instances higher melting point waxes may be purifying crude wax mixtures.

[00081] As mentioned above, the 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. 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.

[00082] 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 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 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 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.

[00083] FIG. 6A shows a time-sequence images (numbered 1-5) 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. 6B, images 1-2). In FIG. 6A, 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. 6A, or the wax material may be melted first (not shown). In FIG. 6A 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. 6A images 3 and 4. Using this approach, in the example shown in FIG. 6A 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. 6B. The left picture (image 1) in FIG. 6B shows the reaction droplet 603' at time zero at 63°C and the right picture of FIG. 6B 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.

[00084] 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.

[00085] As mentioned, the wax-based evaporation methods described may be used in conjunction with the DMF devices having a reaction chamber feature. In those instances, 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.

- 5 **[00086]** The methods and apparatuses described herein may be used for preventing 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
10 Dependent Amplification-HDA, Nicking 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-LMPCR,
15 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).

EXAMPLE 1: Device Fabrication and Assembly

- 20 **[00087]** 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
25 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)
30 and then baked at 100 °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.

[00088] Prototype devices fabricated as described above performed better or as well as air-gap DMF apparatuses without reaction chambers.

- 35 Example 2: Quantifying evaporation prevention using waxes

[00089] 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. 7, the LAMP assay amplified miR-451, and the C_t 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. 6A). 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. 8A shows a LAMP assay using paraffin-mediated methods, while FIG. 8B shows a LAMP assay using conventional methods. In FIG. 8A, 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. 8B, 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 C_t values to those generated by conventional LAMP in tubes as shown in FIGS. 8A and 8B.

[00090] 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. 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.

[00091] Terminology used herein is for the purpose of describing particular embodiments 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 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 "/".

[00092] 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 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 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",
5 "vertical", "horizontal" and the like are used herein for the purpose of explanation only unless specifically indicated otherwise.

[00093] Although the terms "first" and "second" may be used herein to describe various 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
10 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 invention.

[00094] 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
15 components can be co-jointly employed in the methods and articles (e.g., compositions and apparatuses including device and methods). For example, the term "comprising" will be understood to imply the inclusion of any stated elements or steps but not the exclusion of any other elements or steps.

[00095] 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
20 "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 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), +/-
25 10% of the stated value (or range of values), etc. Any numerical range recited herein is intended to include all sub-ranges subsumed therein.

[00096] Although various illustrative embodiments are described above, any of a number of 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
30 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. 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.

[00097] The examples and illustrations included herein show, by way of illustration and not of
35 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 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 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.

CLAIMS

What is claimed is:

1. An air-matrix digital microfluidic (DMF) apparatus configured to prevent evaporation and surface fouling, the DMF apparatus comprising:
 - 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 arranged in a first plane adjacent to the first hydrophobic layer;
 - one or more ground electrodes; and
 - a reaction chamber opening that extends transversely through the first plate, the reaction chamber opening configured to put a reaction chamber well in fluid communication with the air gap, wherein the reaction chamber opening is at least partially surrounded by an actuation electrode from the plurality of actuation electrodes.
2. An air-matrix digital microfluidic (DMF) apparatus configured to prevent evaporation and surface fouling, the DMF apparatus comprising:
 - 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 arranged in a first plane adjacent to the first hydrophobic layer;
 - one or more ground electrodes;
 - a reaction chamber opening that extends through first plate, the reaction chamber opening configured to put a reaction chamber well in fluid communication with the air gap, wherein the reaction chamber opening is at least partially surrounded by an actuation electrode from the plurality of actuation electrodes;
 - a reaction chamber cover adjacent to the reaction chamber opening, the reaction chamber cover configured to be actuated to close over the reaction chamber opening and separate the reaction chamber from the air gap; and
 - a controller electrically coupled to the plurality of actuation electrodes and configured to apply energy to the plurality of actuation electrodes to move a droplet into and out of the reaction chamber.
3. An air-matrix digital microfluidic (DMF) apparatus configured to prevent evaporation and surface fouling, the DMF apparatus comprising:
 - 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 arranged in a first plane adjacent to the first
hydrophobic layer;
5 one or more ground electrodes;
a reaction chamber opening that extends through the first plate transverse to the first
plane, the reaction chamber opening configured to put a reaction chamber well in
fluid communication with the air gap, wherein the reaction chamber opening is at
least partially surrounded by an actuation electrode from the plurality of actuation
10 electrodes;
one or more reaction chamber actuation electrodes within the reaction chamber opening,
wherein the one or more reaction chamber actuation electrodes are positioned or
extend out of the first plane;
a reaction chamber cover adjacent to the reaction chamber opening, the reaction chamber
15 cover configured to be actuated to close over the reaction chamber opening and
separate the reaction chamber from the air gap; and
a controller electrically coupled to the plurality of actuation electrodes and the one or
more reaction chamber actuation electrodes and configured to apply energy to move
a droplet into and out of the reaction chamber.

- 20 4. The DMF apparatus of claim 1, further comprising a reaction chamber cover adjacent to the
reaction chamber opening, the reaction chamber cover configured to be actuated to close over
the reaction chamber opening and separate the reaction chamber from the air gap.
5. The DMF apparatus of claim 1, further comprising oil or wax within the reaction chamber.
- 25 6. The DMF apparatus of claim 1, 2 or 3, wherein the reaction chamber opening is surrounded and
passes through an actuation electrode from the plurality of actuation electrodes.
7. The DMF apparatus of claim 1 or 2, further comprising one or more reaction chamber actuation
electrodes within the reaction chamber opening.
- 30 8. The DMF apparatus of claim 1 or 2, further comprising one or more reaction chamber actuation
electrodes within the reaction chamber opening, wherein the one or more reaction chamber
actuation electrodes are positioned out of the first plane.
9. The DMF apparatus of claim 1 or 2, further comprising one or more reaction chamber actuation
electrodes within the reaction chamber opening, wherein the one or more reaction chamber
actuation electrodes are positioned within the reaction chamber.

10. The DMF apparatus of claim 1 or 2, wherein the reaction chamber cover is coupled to the second plate, further wherein the second plate is flexible and configured to be pushed to seal the reaction chamber cover over the reaction chamber opening.
- 5 11. The DMF apparatus of claim 1 or 2, wherein the second plate is flexible and configured to be pushed to seal over the reaction chamber opening.
12. The DMF apparatus of claim 1, 2 or 3, further comprising a thermal regulator adjacent to the first plate and configured to control the temperature of a portion of the air gap and the reaction chamber.
- 10 13. The DMF apparatus of claim 1, 2 or 3, wherein the first plate comprises the reaction chamber within a thickness of the first plate.
14. The DMF apparatus of claim 1, 2 or 3, wherein the first plate comprises a printed circuit board (PCB) and wherein the reaction chamber is formed as a well within the PCB.
- 15 15. The DMF apparatus of claim 1, 2 or 3, wherein the DMF wherein the first plate is configured to mate with a multi-well plate so that the reaction chamber opening mates with a well of the multi-well plate.
16. The DMF apparatus of claim 1, 2 or 3, wherein the DMF wherein the first plate is configured to mate with a centrifuge tube so that the reaction chamber opening mates with the centrifuge tube.
- 20 17. The DMF apparatus of claim 1, 2 or 3, where the air gap layer comprising at least one sample region and at least one reagent region.
18. The DMF apparatus of claim 1, 2 or 3, wherein the reaction chamber opening is flush with the second hydrophobic layer.
19. The DMF apparatus of claim 1, 2 or 3, wherein the reaction chamber internal surface is hydrophobic.
- 25 20. The DMF apparatus of claim 1, 2 or 3, wherein the reaction chamber internal surface is hydrophillic.
21. A method of operating a matrix digital microfluidic (DMF) apparatus to prevent evaporation and surface fouling, the method comprising:
introducing a reaction droplet into an air gap of the air-matrix DMF apparatus which is
30 formed between a first plate and a second plate of the air-matrix DMF apparatus;

transporting the reaction droplet along the air gap and through a reaction chamber opening that extends through the first plate, and into a reaction chamber well; covering the reaction droplet within the reaction chamber well to protect the reaction droplet from evaporation; and
5 allowing a reaction to proceed within the reaction droplet.

22. A method of operating a matrix digital microfluidic (DMF) apparatus to prevent evaporation and surface fouling, the method comprising:

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,
10 wherein the first plate comprises a plurality of adjacent actuation electrodes;

transporting the reaction droplet along the air gap and through a reaction chamber opening that extends through the first plate, and into a reaction chamber well by applying energy to a subset of the actuation electrodes of the plurality of actuation electrodes;

15 covering the reaction droplet within the reaction chamber well to protect the reaction droplet from evaporation; and

heating the reaction chamber well so that a reaction may proceed within the reaction droplet.

23. A method of operating a matrix digital microfluidic (DMF) apparatus to prevent evaporation and surface fouling, the method comprising:

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,
20 wherein the first plate comprises a plurality of adjacent actuation electrodes;

transporting the reaction droplet along the air gap and through a reaction chamber opening that extends through the first plate, and into a reaction chamber well by applying energy to a subset of the actuation electrodes of the plurality of actuation electrodes;

25 sealing the reaction chamber well opening with a cover to close off the reaction chamber opening from the air gap to protect the reaction droplet from evaporation; and

30 heating the reaction chamber well so that a reaction may proceed within the reaction droplet.

24. The method of claim 21, 22 or 23, wherein introducing the reaction droplet into an air gap comprises combining multiple droplets to form a reaction droplet within the air gap.

25. The method of claim 21, wherein the first plate comprises a plurality of adjacent actuation electrodes, and wherein transporting the reaction droplet comprises applying energy to a subset of the actuation electrodes of the plurality of adjacent actuation electrodes.
35

26. The method of claim 21 or 22, wherein covering the reaction droplet comprises sealing the reaction chamber well opening with a cover to close off the reaction chamber opening from the air gap to protect the reaction droplet from evaporation.

27. The method of claim 21 or 22, wherein covering the reaction chamber comprises combining the reaction droplet with a droplet of oil or wax within the reaction chamber.

28. The method of claim 21, wherein allowing a reaction to proceed comprises heating the reaction chamber well.

29. The method of claim 23, wherein sealing the reaction chamber well opening comprises pushing on the second plate to cover the reaction chamber well opening.

30. The method of claim 21, 22 or 23, further comprising moving the reaction chamber droplet from the reaction well and into the air gap by applying energy to at least one actuation electrode within the reaction well.

31. The method of claim 21, 22 or 23, further comprising detecting a product within the reaction droplet.

32. 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;

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 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.

33. The apparatus of claim 32, wherein the wax body spans a plurality of adjacent unit cells.

34. The apparatus of claim 32, wherein the wax body comprises a wall of wax within the air gap.

35. The apparatus of claim 32, wherein the wax body forms a channel or vessel within the air gap.

36. The apparatus of claim 32, wherein the wax body comprises paraffin wax.
37. The apparatus of claim 32, further comprising at least one temperature sensor in thermal communication with the thermal regulator.
38. The apparatus of claim 32, wherein the plurality of actuation electrodes form a portion of the first plate.
39. The apparatus of claim 32, wherein the one or more ground electrodes are adjacent to the second hydrophobic layer, across the air gap from the first plate.
40. The apparatus of claim 32, further comprising a dielectric between the first hydrophobic layer and the plurality of actuation electrodes.
41. The apparatus of claim 32, wherein the thermal regulator comprises a thermoelectric heater.
42. A method of preventing droplet evaporation within an air-matrix digital microfluidic (DMF) apparatus, the method comprising:
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 droplet from evaporation; and
allowing a reaction to proceed within the reaction droplet.
43. The method of claim 42, wherein melting the wax body 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.
44. The method of claim 42, wherein melting the wax body comprises melting a solid wax body formed into a wall or open chamber within the air gap.
45. The method of claim 42, wherein introducing the reaction droplet into an air gap comprises combining multiple droplets to form a reaction droplet within the air gap.
46. The method of claim 42, wherein the first plate comprises 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 melting the wax body.

47. The method of claim 42, wherein the first plate comprises 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 melted wax body.
- 5 48. The method of claim 42, wherein allowing a reaction to proceed comprises heating portion of the air gap containing the reaction droplet.
49. The method of claim 42, further comprising detecting a product within the reaction droplet.

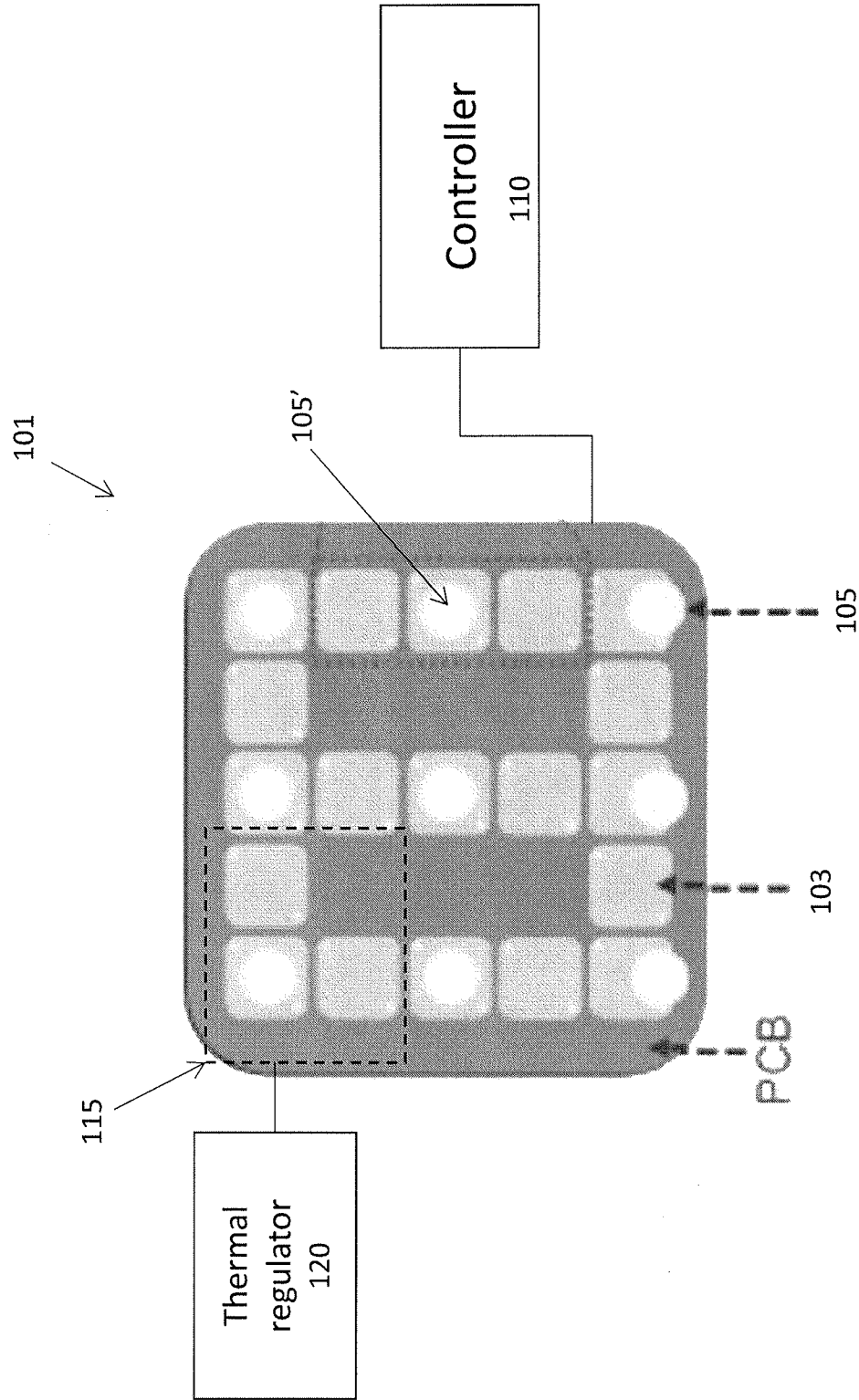
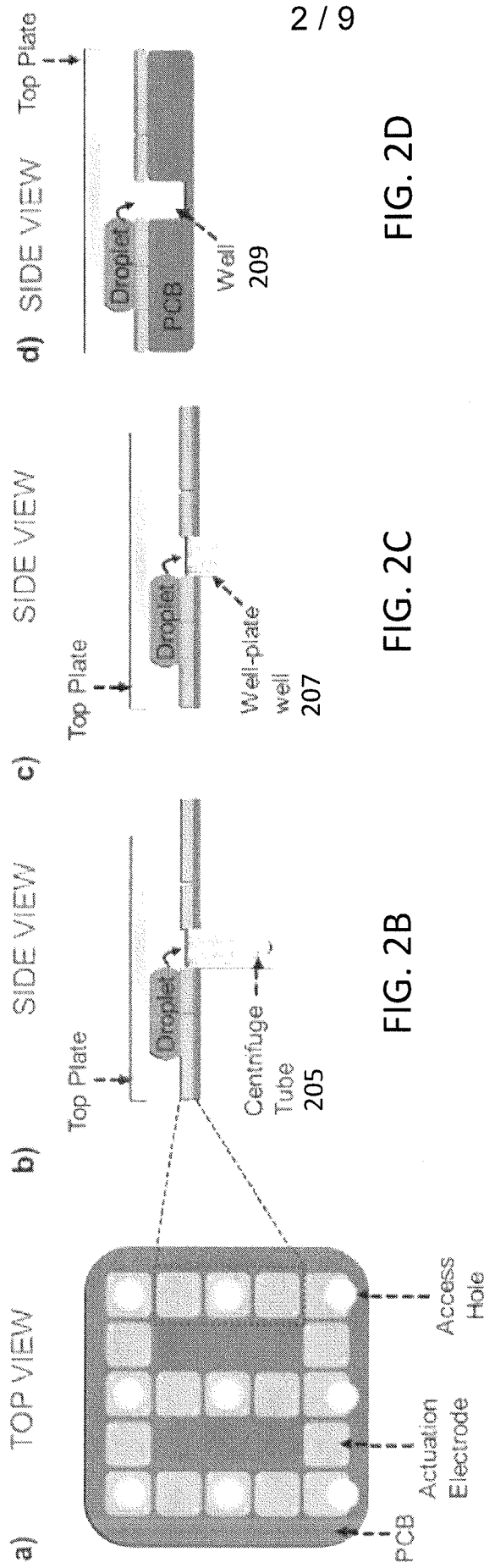


FIG. 1



b) SIDE VIEW

c) SIDE VIEW

d) SIDE VIEW

FIG. 2B

FIG. 2C

FIG. 2D

FIG. 2A

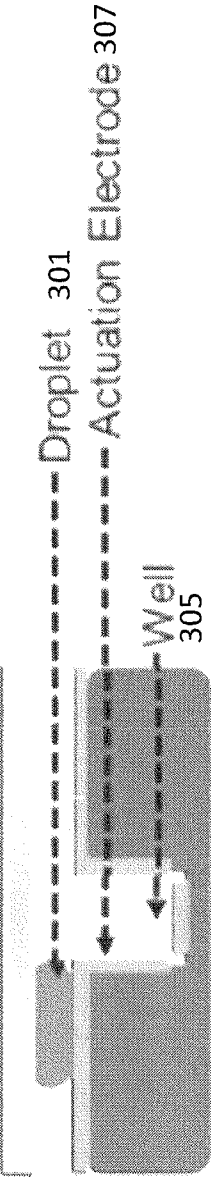


FIG. 3A

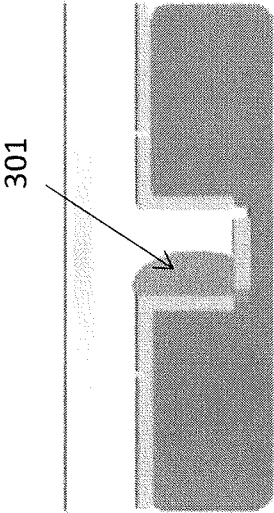


FIG. 3B

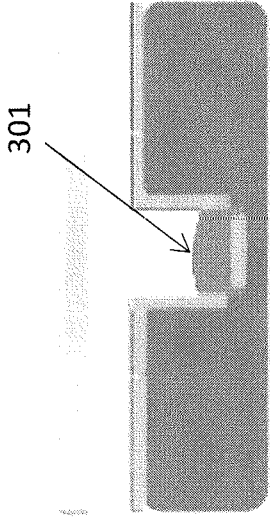


FIG. 3C

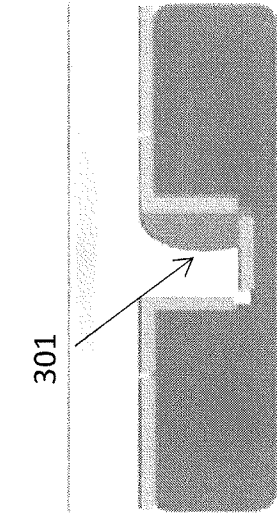


FIG. 3D

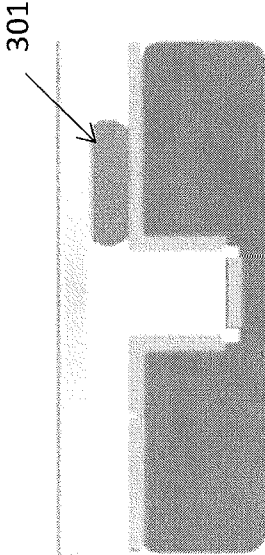


FIG. 3E

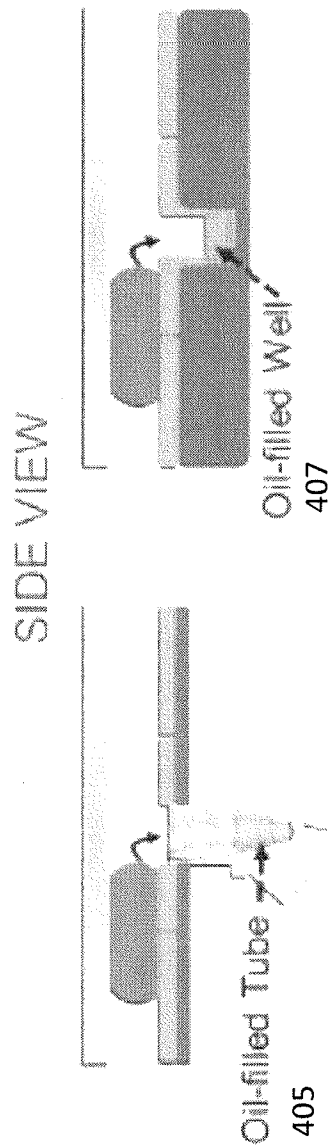


FIG. 4B

FIG. 4A

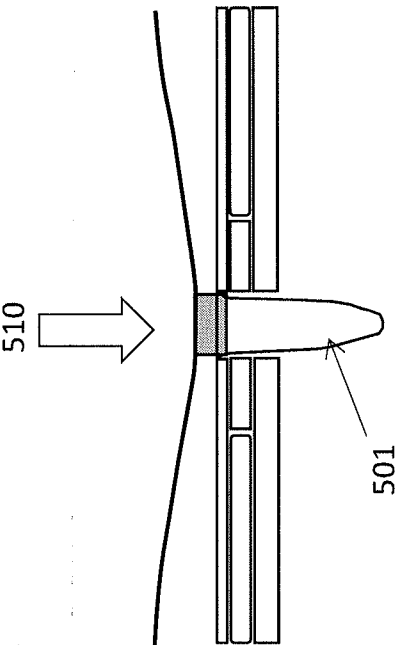


FIG. 5A

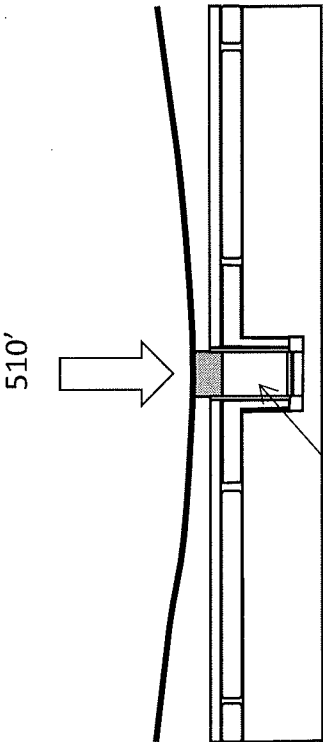


FIG. 5B

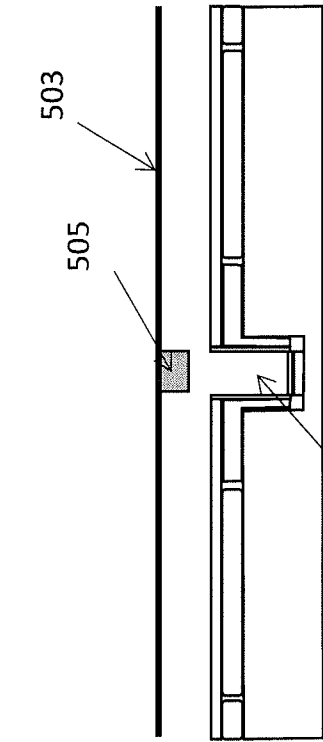


FIG. 5C

FIG. 5D

FIG. 5E

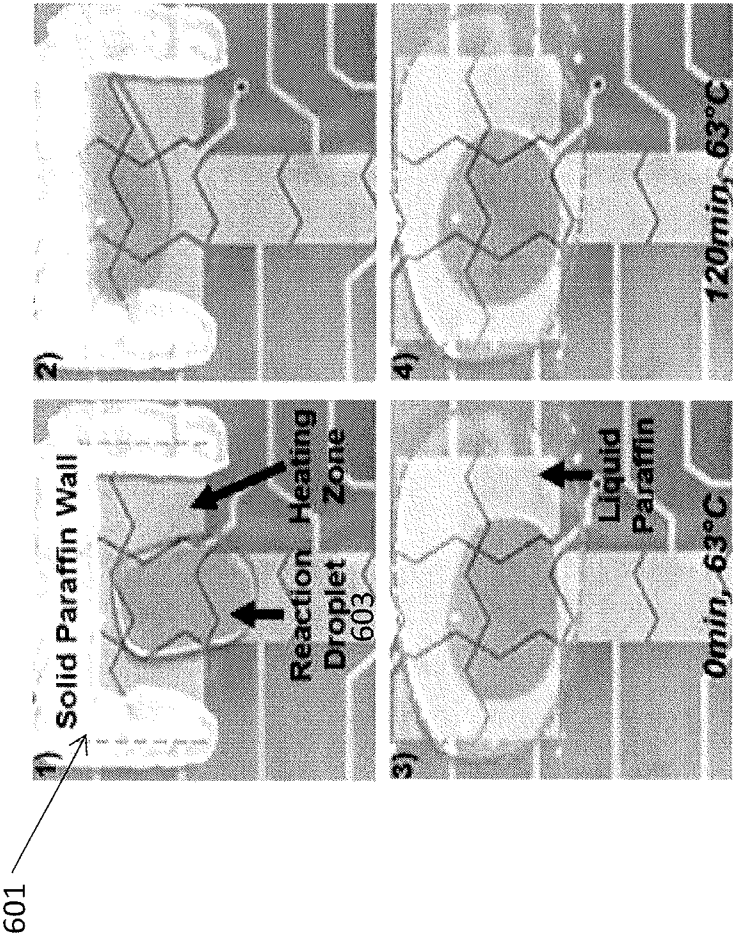


FIG. 6A

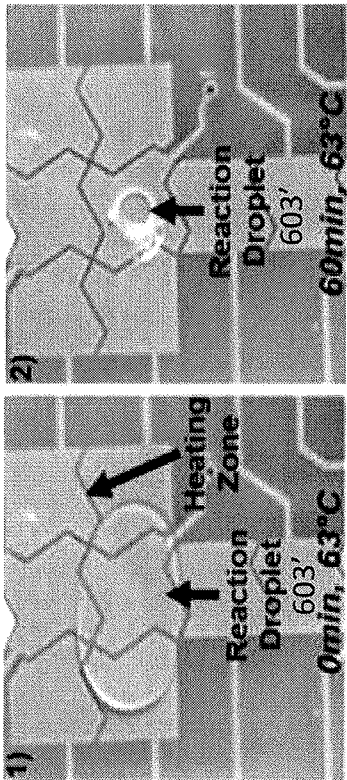


FIG. 6B

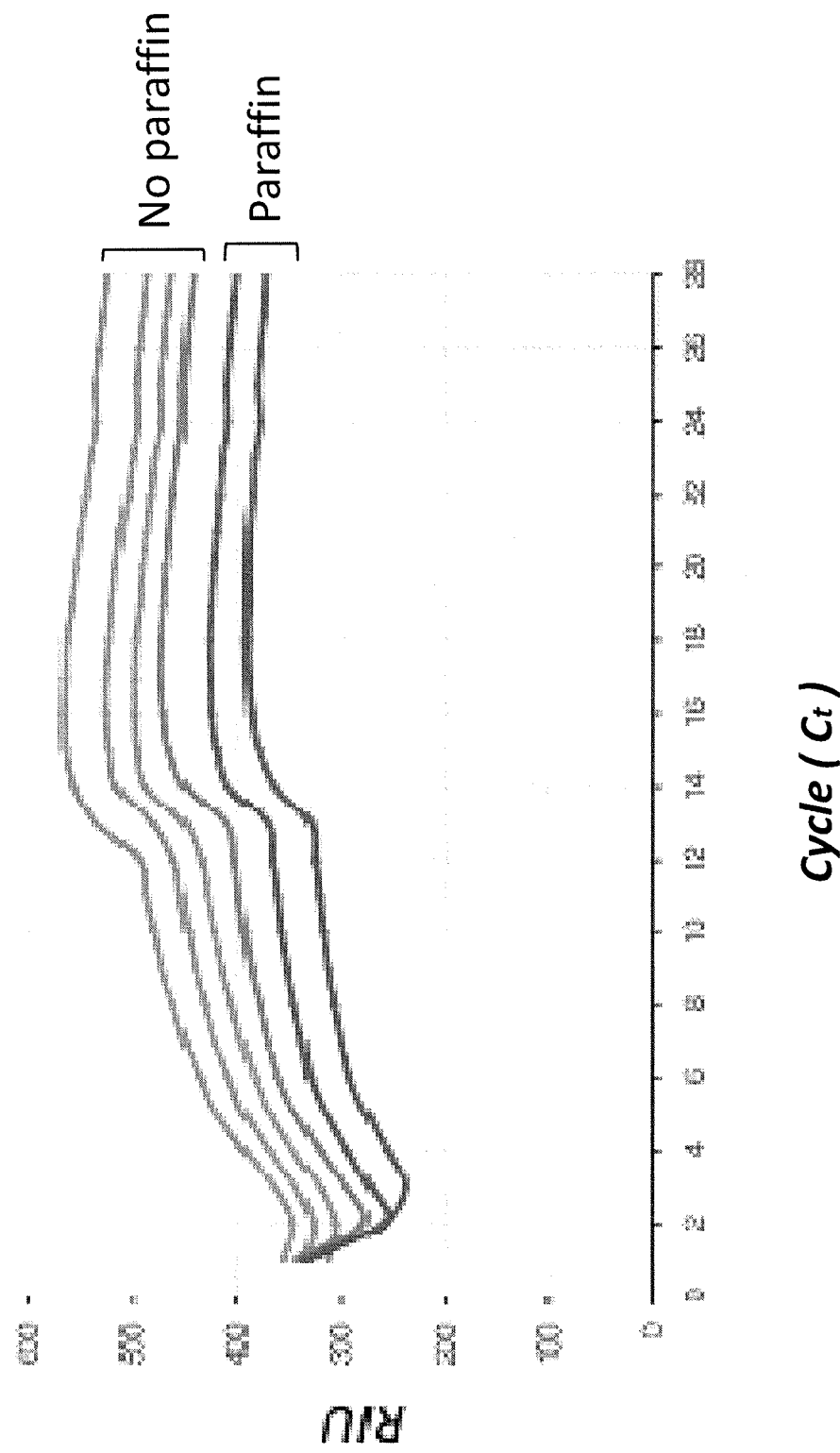


FIG. 7

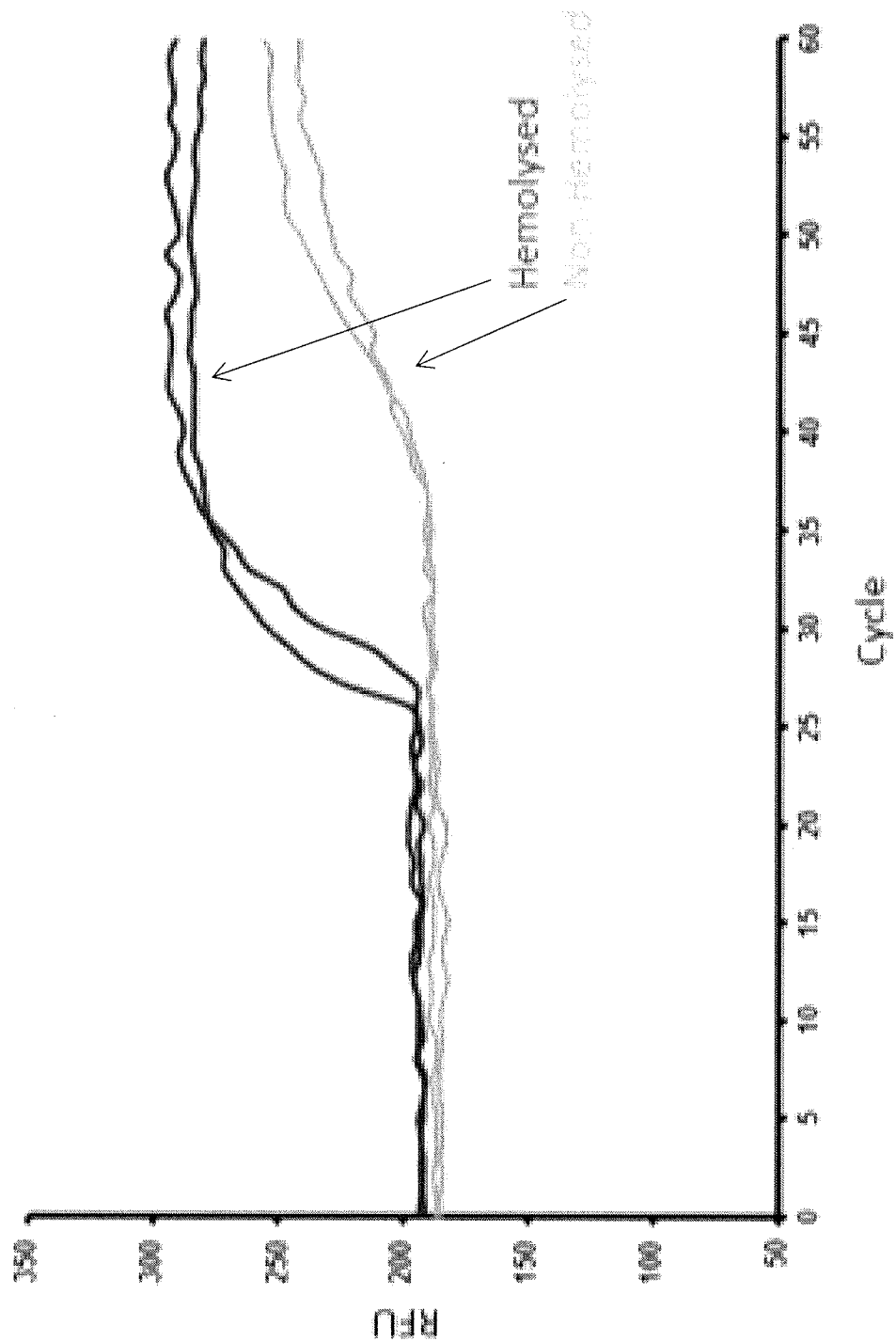


FIG. 8A

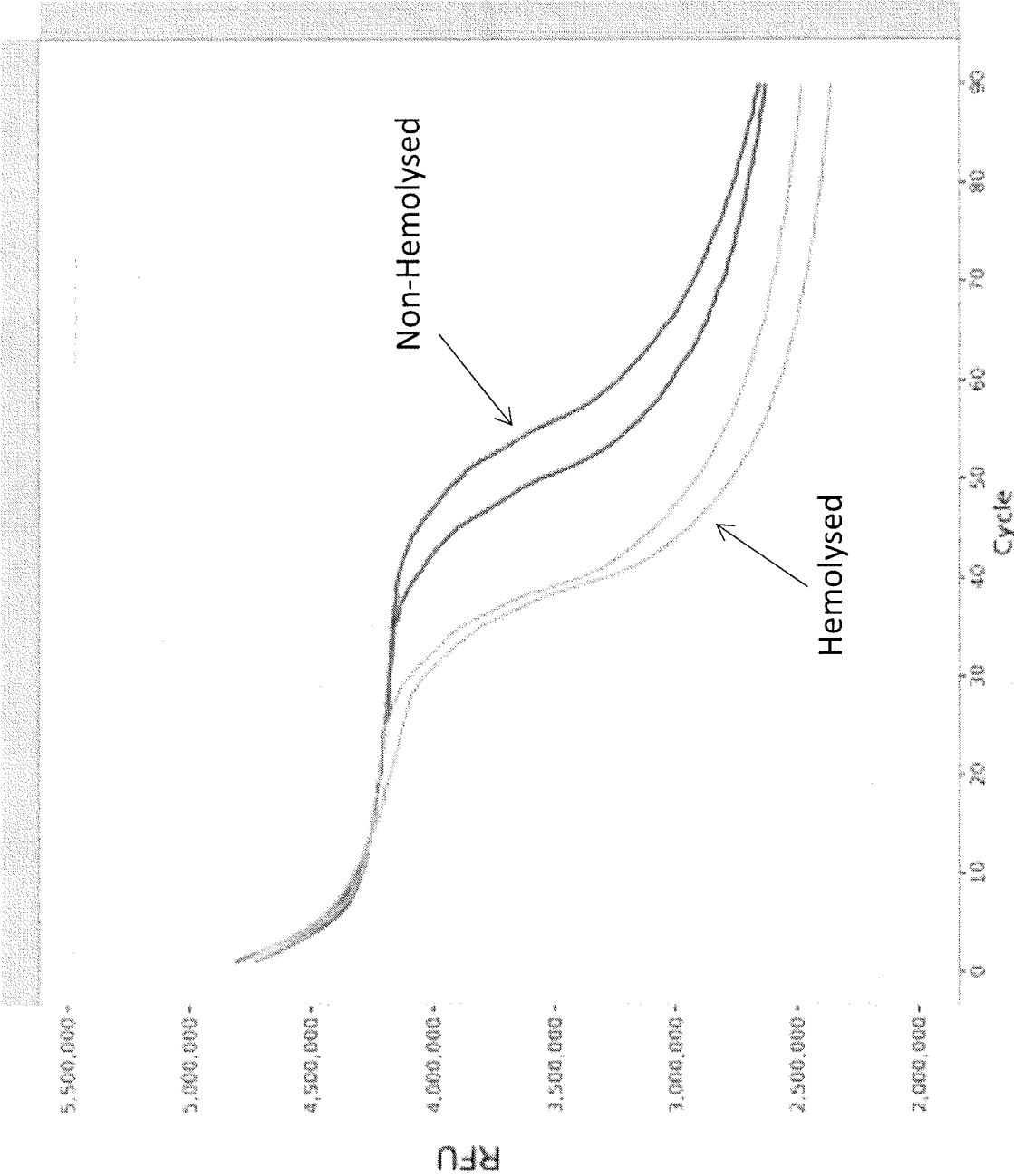


FIG. 8B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/36015

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12M 1/00, 1/38; B01L 3/00 (2016.01)

CPC - C12M 1/00, 23/16; B01L 3/5027, 3/502792

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)

IPC(8) Classifications: C12M 1/00, 1/38; B01L 3/00 (2016.01)

CPC Classifications: C12M 1/00, 23/16; B01L 3/5027, 3/502792

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

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

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google Scholar; Google; EBSCO; PubMed
digital microfluidic device, evaporation, actuation electrodes, sealing, reaction wells, reaction chambers

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	(JEBRAIL, MJ et al.) A solvent replenishment solution for managing evaporation of biochemical reactions in air-matrix digital microfluidics devices. Lab Chip. 2015. Vol. 15. Pages 151-158 (First published online 01 October 2014); figures 1a, 3a-3c; page 152, left column, last paragraph; page 153, left column, last paragraph, right column, 1st paragraph; page 156, left column, last paragraph, right column, 2nd paragraph	1-5, 6/1-3, 7/1-2, 8/1-2, 9/1-2, 10/1-2, 11/1-2, 12/1-3, 13/1-3, 14/1-3, 15/1-3, 16/1-3, 17/1-3, 18/1-3, 19/1-3, 20/1-3, 21-23, 24/21-23, 25, 26/21-22, 27/21-22, 28-29, 30/1-3, 31/1-3
Y	WO 2013/176767 A1 (THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL) 28 November 2013; paragraphs [0007], [0059]; figures 5A-5B	1-5, 6/1-3, 7/1-2, 8/1-2, 9/1-2, 10/1-2, 11/1-2, 12/1-3, 13/1-3, 14/1-3, 15/1-3, 16/1-3, 17/1-3, 18/1-3, 19/1-3, 20/1-3, 27/21-22
Y	WO 01/25137 A1 (NANOSTREAM, INC.) 12 April 2001; page 5, lines 26-28; figure 2	13/1-3, 14/1-3
Y	US 7390463 B2 (HE, L et al.) 24 June 2008; figure 2; column 3, lines 48-53; column 5, lines 19-21; column 6, lines 42-46; column 7, lines 62-63; claim 1	15/1-3, 21-23, 24/21-23, 25, 26/21-22, 27/21-22, 28-29, 30/21-23, 31/21-23
Y	US 6401552 B1 (ELKINS, CD) 11 June 2002; claim 1	16/1-3
Y	US 2014/0273100 A1 (SEIKO EPSON CORPORATION) 18 September 2014; paragraphs [0029]-[0031]	16/1-3

☒ 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

24 October 2016 (24.10.2016)

Date of mailing of the international search report

03 NOV 2016

Name and mailing address of the ISA/

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P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/36015

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2014/108185 A1 (TECAN TRADING AG) 17 July 2014; claim 1	1-5, 6/1-3, 7/1-2, 8/1-2, 9/1-2, 10/1-2, 11/1-2, 12/1-3, 13/1-3, 14/1-3, 15/1-3, 16/1-3, 17/1-3, 18/1-3, 19/1-3, 20/1-3, 21-23, 24/21-23, 25, 26/21-22, 27/21-22, 28-29, 30/1-3, 31/1-3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/36015

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-31 are directed toward an air-matrix digital microfluidic (DMF) apparatus configured to prevent evaporation and surface fouling.

Group II: Claims 32-49 are directed toward an air-matrix digital microfluidic (DMF) apparatus configured to prevent evaporation.

Continued Within the Next Supplemental Box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-31

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

-Continued from Box III: Observations where unity of invention is lacking-

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

the special technical features of Group I include a reaction chamber opening that extends transversely through the first plate, the reaction chamber opening configured to put a reaction chamber well in fluid communication with the air gap, wherein the reaction chamber opening is at least partially surrounded by an actuation electrode from the plurality of actuation electrodes; a reaction chamber cover adjacent to the reaction chamber opening, the reaction chamber cover configured to be actuated to close over the reaction chamber opening and separate the reaction chamber from the air gap; and a controller electrically coupled to the plurality of actuation electrodes and configured to apply energy to the plurality of actuation electrodes to move a droplet into and out of the reaction chamber, which are not present in Group II; and the special technical features of Group II include 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, which are not present in Group I.

The common technical features of Groups I-II are an air-matrix digital microfluidic (DMF) apparatus configured to prevent evaporation and surface fouling, the DMF apparatus comprising: 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 arranged in a first plane adjacent to the first hydrophobic layer; one or more ground electrodes.

These common technical features are disclosed by the document entitled "A solvent replenishment solution for managing evaporation of biochemical reactions in air-matrix digital microfluidics devices" by Jebrail, et al. (hereinafter 'Jebrail'). Jebrail discloses an air-matrix digital microfluidic (DMF) apparatus (air-matrix DMF device; figure 1a) configured to prevent evaporation and surface fouling (a solution for counteracting evaporation in an air-matrix DMF device, solution is just-in-time replenishment of the reaction volume using pre-heated droplets of solvent; page 152, left column, last paragraph), the DMF apparatus comprising: a first plate having a first hydrophobic layer (hydrophobic surface of DMF plates, where plates include a bottom plate (first plate); figure 1a; page 156, right column, 2nd paragraph); a second plate having a second hydrophobic layer (hydrophobic surface of DMF plates, where plates include a top plate (second plate); figure 1a; page 156, right column, 2nd paragraph); an air gap formed between the first and second hydrophobic layers (air gap is formed between the top plate and the bottom plate; figure 1a), a plurality of actuation electrodes arranged in a first plane adjacent to the first hydrophobic layer (actuation electrodes adjacent to the bottom plate, and the DMF device has an array of 100 actuation electrodes; page 152, right column, 2nd paragraph; figure 1a); one or more ground electrodes (DMF device containing ground electrodes; figure 1a);

Since the common technical features are previously disclosed by Jebrail, these common features are not special and so Groups I-II lack unity.