



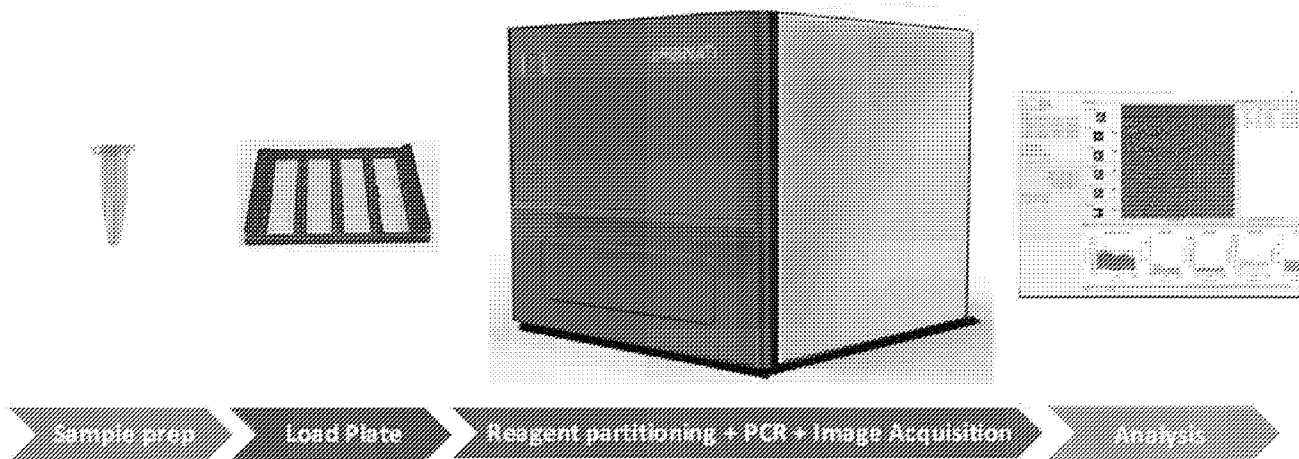
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(54) Titre : RESEAU MICROFLUIDIQUE POUR NUMERISATION D'ECHANTILLON  
 (54) Title: MICROFLUIDIC ARRAY FOR SAMPLE DIGITIZATION



**FIG. 10**

(57) **Abrégé/Abstract:**

The present disclosure provides systems, methods, and devices for processing a biological sample. The device may be a microfluidic device comprising a fluid flow path and a chamber. The fluid flow path may comprise a channel and an inlet port and no outlet port. The inlet port may be configured to direct a biological sample to the channel. The channel may be in fluid communication with the chamber. The chamber may be configured to receive a portion of the biological sample from the channel and retain the biological sample during processing.

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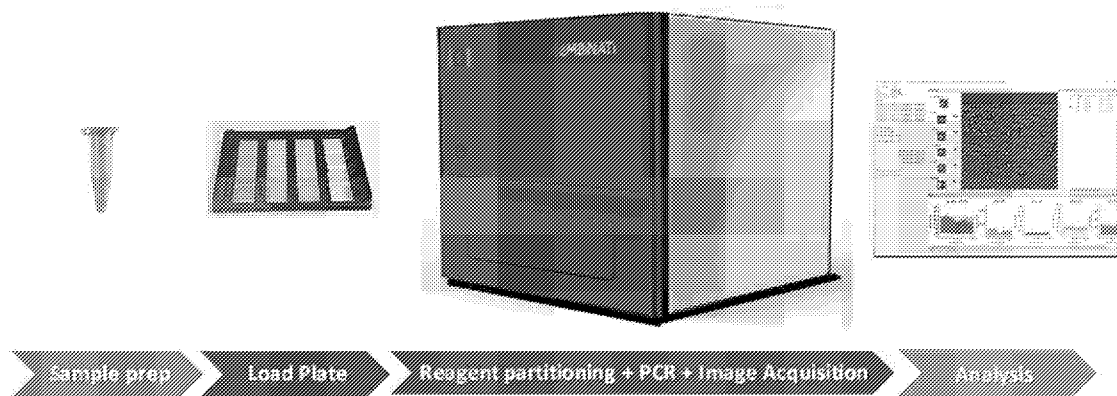


FIG. 10

(57) Abstract: The present disclosure provides systems, methods, and devices for processing a biological sample. The device may be a microfluidic device comprising a fluid flow path and a chamber. The fluid flow path may comprise a channel and an inlet port and no outlet port. The inlet port may be configured to direct a biological sample to the channel. The channel may be in fluid communication with the chamber. The chamber may be configured to receive a portion of the biological sample from the channel and retain the biological sample during processing.



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## MICROFLUIDIC ARRAY FOR SAMPLE DIGITIZATION

### CROSS-REFERENCE

**[0001]** This application claims the benefit of U.S. Provisional Patent Application No. 62/777,616, filed December 10, 2018, which is entirely incorporated herein by reference.

### GOVERNMENT INTEREST STATEMENT

**[0002]** This invention was made with government support under Small Business Innovation Research grant number 1R43CA221597-01A1 awarded by the National Cancer Institute. The U.S. government has certain rights in the invention.

### BACKGROUND

**[0003]** Microfluidic devices are devices that contain structures that handle fluids on a small scale. Typically, a microfluidic device operates on a sub-millimeter scale and handles micro-liters, nano-liters, or smaller quantities of fluids. One application of microfluidic structures is in digital polymerase chain reaction (dPCR). For example, a microfluidic structure with multiple partitions may be used to partition a nucleic acid sample for dPCR. In dPCR, a nucleic acid sample may be diluted such that one or less nucleic acid template is present in a partition and a PCR reaction may be performed in each partition. By counting the partitions in which the template was successfully PCR amplified and applying Poisson statistics to the result, the target nucleic acid may be quantified.

**[0004]** For genomic researchers and clinicians, dPCR is particularly powerful in rare mutation detection, quantifying copy number variants, and Next Gen Sequencing library quantification. The potential use in clinical settings for liquid biopsy with cell free DNA and viral load quantification further increases the value of dPCR technology.

### SUMMARY

**[0005]** Provided herein are methods and devices that may be useful for analysis of a biological sample, for example, amplifying and quantifying nucleic acids. The present disclosure provides methods, systems, and devices that may enable sample preparation, sample amplification, and sample analysis through the use of dPCR. Samples may be digitized and analyzed with little to no sample waste (e.g., zero or near zero sample dead volume). This may enable sample analysis, for example nucleic acid amplification and quantification, at a reduced cost and complexity as compared to other systems and methods.

**[0006]** In an aspect, the present disclosure provides a microfluidic device for processing a biological sample, comprising: a fluid flow path comprising a channel and an inlet port, wherein the fluid flow path does not include an outlet port and wherein the inlet port is configured to direct a solution comprising the biological sample to the channel; and a chamber in fluid communication with the channel, wherein the chamber is configured to receive at least a portion of the solution from the channel and retain the at least the portion of the solution during the processing.

**[0007]** In some embodiments, the microfluidic device further comprises a plurality of chambers in fluid communication with the channel, wherein the plurality of chambers comprises the chamber. In some embodiments, the channel comprises a first end and a second end, and wherein the first end and the second end are connected to a single inlet port. In some embodiments, the fluid flow path is circular. In some embodiments, the channel comprises a first end and a second end, and wherein the first end is connected to the inlet port and the second end is connected to a different inlet port.

**[0008]** In some embodiments, the chamber is configured to permit pressurized off-gassing. In some embodiments, the chamber comprises a film or membrane that permits the pressurized off-gassing. In some embodiments, the film or membrane is polymer film or membrane. In some embodiments, the polymer film or membrane does not comprise an elastomer. In some embodiments, the film or membrane has a thickness of less than about 100 micrometers ( $\mu\text{m}$ ). In some embodiments, the thickness is less than about 50  $\mu\text{m}$ . In some embodiments, the film or membrane is substantially impermeable to liquids.

**[0009]** In some embodiments, the fluid flow path or the chamber does not include a valve. In some embodiments, a volume of the chamber is less than or equal to about 250 picoliters. In some embodiments, a volume of the chamber is less than or equal to about 500 picoliters. In some embodiments, the chamber has a cross-sectional dimension of less than or equal to about 250  $\mu\text{m}$ . In some embodiments, the chamber has a depth of less than or equal to about 250  $\mu\text{m}$ . In some embodiments, the microfluidic device further comprises a siphon aperture disposed between the channel and the chamber, wherein the siphon aperture is configured to provide fluid communication between the channel and the chamber.

**[0010]** In another aspect, the present disclosure provides methods for processing a biological sample, comprising: providing a device comprising (i) a fluid flow path comprising a channel and an inlet port, wherein the fluid flow path does not include an outlet port, and (ii) a chamber in fluid communication with the channel; directing a solution comprising the biological sample

from the inlet port to the channel; and directing at least a portion of the solution from the channel to the chamber, which chamber retains the at least the portion of the solution during the processing.

**[0011]** In some embodiments, the device comprises a plurality of chambers in fluid communication with the channel, and wherein the plurality of chambers comprises the chamber. In some embodiments, the method further comprises applying a single pressure differential to the inlet port to direct solution from the inlet port to the channel and from the channel to the chamber. In some embodiments, the single pressure differential permits pressurized off-gassing of gas in the chamber.

**[0012]** In some embodiments, the method further comprises applying a first pressure differential to the inlet port to direct the solution from the inlet port to the channel. In some embodiments, the method further comprises applying a second pressure differential to the inlet port to direct the solution from the channel to the chamber. In some embodiments, the second pressure differential is greater than the first pressure differential. In some embodiments, the second pressure differential permits pressurized off-gassing of gas in the chamber. In some embodiments, the chamber comprises a film or membrane, and wherein the film or membrane permits pressurized off-gassing of the gas in the chamber.

**[0013]** In some embodiments, a volume of the solution is less than or equal to a volume of the chamber. In some embodiments, the device partitions the solution comprising the biological sample into the chamber such that no residual solution remains in the channel. In some embodiments, the method further comprises providing an immiscible fluid to the inlet port and directing the immiscible fluid to the channel. In some embodiments, a volume of the immiscible fluid is greater than a volume of the channel. In some embodiments, the biological sample is a nucleic acid molecule. In some embodiments, the method further comprises amplifying the nucleic acid molecule by thermal cycling the chamber. In some embodiments, the method further comprises controlling a temperature of the channel or the chamber. In some embodiments, the method further comprises detecting one or more components of the biological sample or a reaction with the one or more components of the biological sample in the chamber. In some embodiments, detecting the one or more components of the biological sample or the reaction comprises imaging the chamber.

**[0014]** In another aspect, the present disclosure provides systems for processing a biological sample, comprising: a device comprising (i) a fluid flow path comprising a channel and an inlet port, wherein the fluid flow path does not include an outlet port, and wherein the inlet port is

configured to direct a solution comprising the biological sample to the channel, and (ii) a chamber in fluid communication with the channel, wherein the chamber is configured to receive at least a portion of the solution from the channel and retain the at least the portion of the solution during the processing; a holder configured to receive and retain the device during the processing; and a fluid flow module configured to fluidically couple to the inlet port and supply a pressure differential to subject (i) the solution to flow from the inlet port to the channel and (ii) at least a portion of the solution to flow from the channel to the chamber.

**[0015]** In some embodiments, the device comprises a plurality of chambers in fluid communication with the channel, and wherein the plurality of chambers comprises the chamber. In some embodiments, the chamber of the device is configured to permit pressurized off-gassing of a gas in the chamber when the fluid flow module applies the pressure differential to the inlet port. In some embodiments, the chamber comprises a film or membrane that is configured to permit the pressurized off-gassing.

**[0016]** In some embodiments, the system further comprises one or more computer processors operatively coupled to the fluid flow module, wherein the one or more computer processors are individually or collectively programmed to direct the fluid flow module to supply the pressure differential when the fluid flow module is fluidically coupled to the inlet port, to thereby subject the solution to flow from the inlet port to the channel and direct the at least the portion of the solution from the channel to the chamber. In some embodiments, the system further comprises a thermal module in thermal communication with the chamber, wherein the thermal module is configured to control a temperature of the chamber during the processing. In some embodiments, the system further comprises a detection module in communication with the chamber, wherein the detection module is configured to detect a content of the chamber during the processing. In some embodiments, the detection module is an optical module in optical communication with the chamber. In some embodiments, the optical module is configured to image the chamber.

**[0017]** In another aspect, the present disclosure provides systems for processing a biological sample, comprising a holder configured to retain a device comprising (i) a fluid flow path comprising a channel and an inlet port, wherein said fluid flow path does not include an outlet port, and (ii) a chamber in fluid communication with said channel; and one or more computer processors configured to be operatively coupled to said device when said device is retained by said holder, wherein said one or more computer processors are individually or collectively programmed to (i) direct a solution comprising said biological sample from said inlet port to said

channel; and (ii) direct at least a portion of said solution from said channel to said chamber, which chamber retains said at least said portion of said solution during said processing.

**[0018]** In some embodiments, the system further comprises a fluid flow module operatively coupled to said one or more computer processors, wherein said fluid flow module is configured to to be operatively coupled to said device when said device is retained by said holder, and wherein said one or more computer processors are programmed to direct said fluid flow module to direct said solution from said inlet port to said channel.

**[0019]** In some embodiments, the system further comprises a thermal module configured to be in thermal communication with said chamber when said device is retained by said holder, wherein said thermal module is configured to control a temperature of said chamber during said processing.

**[0020]** In some embodiments, the system further comprises a detection module configured to be in communication with said chamber when said device is retained by said holder, wherein said detection module is configured to detect a content of said chamber during said processing. In some embodiments, the detection module is an optical module in optical communication. In some embodiments, the optical module is configured to image said chamber.

**[0021]** Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

### **INCORPORATION BY REFERENCE**

**[0022]** All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “figure” and “FIG.” herein), of which:

[0024] **FIGS. 1A – 1F** schematically illustrate an example microfluidic device and method for filling the microfluidic device; **FIG. 1A** schematically illustrates loading a sample and immiscible fluid into the microfluidic device; **FIG. 1B** schematically illustrates pressurizing the microfluidic device to load the sample into the channel; **FIG. 1C** schematically illustrates continued pressurization to degas the fluid flow path and continue to load the sample into the channel; **FIG. 1D** schematically illustrates partial digitization of the sample into the chambers, loading of oil into the channel, and displacement of air; **FIG. 1E** schematically illustrates further digitization and displacement of air; **FIG. 1F** schematically illustrates complete digitization of the sample;

[0025] **FIGS. 2A-2E** show an example image of a sample digitization in a microfluidic device; **FIG. 2A** shows an example microfluidic device; **FIG. 2B** shows an example pressurized loading of the sample into the microfluidic device; **FIG. 2C** shows an example of the sample and an immiscible fluid filling the channel; **FIG. 2D** shows an example of partial loading of the sample into the chambers; **FIG. 2E** shows an example of complete digitization of the sample;

[0026] **FIG. 3** schematically illustrates an example method for digitization of a sample;

[0027] **FIG. 4** schematically illustrates an example method for digital polymerase chain reaction (dPCR);

[0028] **FIG. 5** schematically illustrates an example system for digitizing and analyzing a sample;

[0029] **FIG. 6** shows a computer system that is programmed or otherwise configured to implement methods provided herein;

[0030] **FIGS. 7A and 7B** show a microfluid device comprising a plurality of slides, with each slide comprising a plurality of processing units;

[0031] **FIG. 8** shows microscope images of a single processing unit;

[0032] **FIGS. 9A-9D** show four different timepoints during the digitization process;

[0033] FIG. 10 shows a laboratory workflow integrating a reagent digitization process, as described herein; and

[0034] FIG. 11 shows a screenshot example of an image analysis software.

### DETAILED DESCRIPTION

[0035] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[0036] As used herein, the terms “amplification” and “amplify” are used interchangeably and generally refer to generating one or more copies or “amplified product” of a nucleic acid. Such amplification may be using polymerase chain reaction (PCR) or isothermal amplification, for example.

[0037] As used herein, the term “nucleic acid,” generally refers to biological polymer comprising nucleic acid subunits (e.g., nucleotides) of any length (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 100, 500, or 1000 nucleotides), either deoxyribonucleotides or ribonucleotides, or analogs thereof. A nucleic acid may include one or more subunits selected from adenosine (A), cytosine (C), guanine (G), thymine (T), and uracil (U), or variants thereof. A nucleotide can include A, C, G, T, or U, or variants thereof. A nucleotide can include any subunit that can be incorporated into a growing nucleic acid strand. Such subunit can be A, C, G, T, or U, or any other subunit that is specific to one of more complementary A, C, G, T, or U, or complementary to a purine (i.e., A or G, or variant thereof) or pyrimidine (i.e., C, T, or U, or variant thereof). In some examples, a nucleic acid may be single-stranded or double stranded, in some cases, a nucleic acid molecule is circular. Non-limiting examples of nucleic acids include deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acids can include coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA, short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), ribozymes, cDNA, recombinant nucleic acids, branched nucleic acids, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A nucleic acid may comprise one or more modified nucleotides, such as methylated nucleotides and nucleotide analogs.

**[0038]** As used herein, the terms “polymerase chain reaction reagent” or “PCR reagent” are used interchangeably and generally refer to a composition comprising reagents for the completion of a nucleic acid amplification reaction (e.g., DNA amplification), with non-limiting examples of such reagents including primer sets or priming sites (e.g., nick) having specificity for a target nucleic acid, polymerases, suitable buffers, co-factors (e.g., divalent and monovalent cations), dNTPs, and other enzymes. A PCR reagent may also include probes, indicators, and molecules that comprise probes and indicators.

**[0039]** As used herein, the term “probe,” generally refers to a molecule that comprises a detectable moiety, the presence or absence of which may be used to detect the presence or absence of an amplified product. Non-limiting examples of detectable moieties may include radiolabels, stable isotope labels, fluorescent labels, chemiluminescent labels, enzymatic labels, colorimetric labels, or any combination thereof.

**[0040]** As used herein, the term “extension,” generally refers to incorporation of nucleotides into a nucleic acid in a template directed fashion. Extension may occur via the aid of an enzyme. For example, extension may occur via the aid of a polymerase. Conditions at which extension may occur include an “extension temperature” that generally refers to a temperature at which extension is achieved and an “extension duration” that generally refers to an amount of time allotted for extension to occur.

**[0041]** As used herein, the term “indicator molecule,” generally refers to a molecule that comprises a detectable moiety, the presence or absence of which may be used to indicate sample partitioning. Non-limiting examples of detectable moieties may include radiolabels, stable isotope labels, fluorescent labels, chemiluminescent labels, enzymatic labels, colorimetric labels, or any combination thereof.

**[0042]** The term “sample,” as used herein, generally refers to any sample containing or suspected of containing a nucleic acid molecule. For example, a sample can be a biological sample containing one or more nucleic acid molecules. The biological sample can be obtained (e.g., extracted or isolated) from or include blood (e.g., whole blood), plasma, serum, urine, saliva, mucosal excretions, sputum, stool and tears. The biological sample can be a fluid or tissue sample (e.g., skin sample). In some examples, the sample is obtained from a cell-free bodily fluid, such as whole blood. In such instance, the sample may include cell-free DNA and/or cell-free RNA. In some examples, the sample can include circulating tumor cells. In some examples, the sample is an environmental sample (e.g., soil, waste, ambient air and etc.), industrial sample (e.g., samples from any industrial processes), and food samples (e.g., dairy

products, vegetable products, and meat products). The sample may be processed prior to loading into the microfluidic device. For example, the sample may be processed to lyse cells, purify the nucleic acid molecules, and/or to include reagents.

**[0043]** As used herein, the term “fluid,” generally refers to a liquid or a gas. A fluid cannot maintain a defined shape and will flow during an observable time frame to fill the container into which it is put. Thus, the fluid may have any suitable viscosity that permits flow. If two or more fluids are present, each fluid may be independently selected among essentially any fluids (liquids, gases, and the like) by those of ordinary skill in the art.

**[0044]** As used herein, the term “partition,” generally refers to a division into or distribution into portions or shares. For example, a partitioned sample is a sample that is isolated from other samples. Examples of structures that enable sample partitioning include wells and chambers.

**[0045]** As used herein, the term “digitized” or “digitization” may be used interchangeable and generally refers to a sample that has been distributed into one or more partitions. A digitized sample may or may not be in fluid communication with another digitized sample. A digitized sample may not interact or exchange materials (e.g., reagents, analytes, etc.) with another digitized sample.

**[0046]** As used herein, the term “microfluidic,” generally refers to a chip, area, device, article, or system including at least one channel, a plurality of siphon apertures, and an array of chambers. The channel may have a cross-sectional dimension less than or equal to about 10 millimeters (mm), less than or equal to about 5 mm, less than or equal to about 4 mm, less than or equal to about 3 mm, less than or equal to about 2 mm, less than or equal to about 1.5 mm, less than or equal to about 1 mm, less than or equal to about 750 micrometers ( $\mu\text{m}$ ), less than or equal to about 500  $\mu\text{m}$ , less than or equal to about 250  $\mu\text{m}$ , less than or equal to about 100  $\mu\text{m}$ , or less.

**[0047]** As used herein, the term “depth,” generally refers to the distance measured from the bottom of the channel, siphon aperture, or chamber to the thin film that caps the channel, plurality of siphon apertures, and array of chambers.

**[0048]** As used herein, the terms “cross-section” or “cross-sectional” may be used interchangeably and generally refer to a dimension or area of a channel or siphon aperture that is substantially perpendicularly to the long dimension of the feature.

**[0049]** As used herein, the terms “pressurized off-gassing” or “pressurized degassing” may be used interchangeably and generally refer to removal or evacuation of a gas (e.g., air, nitrogen,

oxygen, etc.) from a channel or chamber of the device (e.g., microfluidic device) to an environment external to the channel or chamber through the application of a pressure differential. The pressure differential may be applied between the channel or chamber and the environment external to the channel or chamber. The pressure differential may be provided by the application of a pressure source to one or more inlets to the device or application of a vacuum source to one or more surfaces of the device. Pressurized off-gassing or pressurized degassing may be permitted through a film or membrane covering one or more sides of the channel or chamber.

**[0050]** Whenever the term “at least,” “greater than,” or “greater than or equal to” precedes the first numerical value in a series of two or more numerical values, the term “at least,” “greater than” or “greater than or equal to” applies to each of the numerical values in that series of numerical values. For example, greater than or equal to 1, 2, or 3 is equivalent to greater than or equal to 1, greater than or equal to 2, or greater than or equal to 3.

**[0051]** Whenever the term “no more than,” “less than,” or “less than or equal to” precedes the first numerical value in a series of two or more numerical values, the term “no more than,” “less than,” or “less than or equal to” applies to each of the numerical values in that series of numerical values. For example, less than or equal to 3, 2, or 1 is equivalent to less than or equal to 3, less than or equal to 2, or less than or equal to 1.

**[0052]** The present disclosure provides microfluidic devices for sample processing and/or analysis. A microfluidic device of the present disclosure may be formed from a polymeric material (e.g., thermoplastic), and may include a thin film to allow for pressurized outgassing or degassing while serving as a gas barrier when pressure is released. The microfluidic device may be a chip or cartridge. A microfluidic device of the present disclosure may be a single-use and/or disposable device. As an alternative, the microfluidic device may be multi-use device. The use of polymers (e.g., thermoplastics) to form the microfluidic structure may allow for the use of an inexpensive and highly scalable injection molding processes, while the thin film may provide the ability to outgas via pressurization, avoiding the fouling problems that may be present some microfluidic structures that do not incorporate such thin films.

**[0053]** For example, as a microfluidic device operates on a sub-millimeter scale and handles micro-liters, nano-liters, or smaller quantities of fluids, a major fouling mechanism may be trapped air, or bubbles, inside the micro-structure. This may be particularly problematic when using a polymer material, such as a thermoplastic, to create the microfluidic structure, as the gas permeability of thermoplastics is very low. In order to avoid fouling by trapped air, other microfluidic structures use either simple straight channel or branched channel designs with

thermoplastic materials, or else manufacture the device using high gas permeability materials such as elastomers. However, simple designs limit possible functionality of the microfluidic device, and elastomeric materials are both difficult and expensive to manufacture, particularly at scale.

**[0054]** One use for this structure is a microfluidic design incorporating an array of dead-ended chambers connected by channels, formed out of thermoplastics. This design may be used in the detection and analysis of biological analytes. For example, the microfluidic design may be used for a digital polymerase chain reaction (dPCR) application to partition reagents into the array of chambers (e.g., chambers) and thereby used to quantify nucleic acids in dPCR.

### **Microfluidic device for analyzing biological samples**

**[0055]** In an aspect, the present disclosure provides a device (e.g., microfluidic device) for processing a biological sample. The device (e.g., microfluidic device) may include a unit, which comprises a fluid flow path and a chamber. The device may include at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more units. The fluid flow path may include a channel and an inlet port. The fluid flow path may not include an outlet port. The inlet port may be in fluid communication with the channel. The inlet port may be configured to direct a solution comprising the biological sample to the channel. The chamber may be in fluid communication with the channel. The chamber may be configured to receive at least a portion of the solution from the channel and retain the portion of the solution during processing.

**[0056]** The fluid flow path may include one channel or multiple channels. The fluid flow path may include at least 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, 25, 30, 40, 50 or more channels. Each channel may be fluidically isolated from one another. Alternatively, or in addition to, the multiple channels may be in fluidic communication with one another. The channel may include a first end and a second end. The first end and second end may be connected to a single inlet port. A channel with a first end and second end connected to a single inlet port may be in a circular and/or looped configuration such that the fluid entering the channel through the inlet port may be directed through the first end and second end of the channel simultaneously. Alternatively, the first end and second end may be connected to different inlet ports. The fluid flow path and/or the chamber may not include valves to stop or hinder fluid flow or to isolate the chamber(s).

**[0057]** The device may comprise a long dimension and a short dimension. The long dimension may be less than or equal to about 20 centimeters (cm), 15 cm, 10 cm, 8 cm, 6 cm, 5

cm, 4 cm, 3 cm, 2 cm, 1 cm, or less. The short dimension of the device may be less than or equal to about 10 cm, 8 cm, 6 cm, 5 cm, 4 cm, 3 cm, 2 cm, 1 cm, 0.5 cm, or less. In an example, the dimensions of the device (e.g., microfluidic device) are about 7.5 cm by 2.5 cm. The channel may be substantially parallel to the long dimension of the microfluidic device. Alternatively, or in addition to, the channel may be substantially perpendicular to the long dimension of the microfluidic device (e.g., parallel to the short dimension of the device). Alternatively, or in addition to, the channel may be neither substantially parallel nor substantially perpendicular to the long dimension of the microfluidic device. The angle between the channel and the long dimension of the microfluidic device may be at least about 5 °, 10 °, 15 °, 20 °, 30 °, 40 °, 50 °, 60 °, 70 °, or 90. In an example, the channel is a single long channel. Alternatively, or in addition to, the channel may have bends, curves, or angles. The channel may have a long dimension that is less than or equal to about 100 millimeters (mm), 75 mm, 50 mm, 40 mm, 30 mm, 20 mm, 10 mm, 8 mm, 6 mm, 4 mm, 2 mm, or less. The length of the channel may be bounded by the external length or width of the microfluidic device. The channel may have a depth of less than or equal to about 500 micrometers ( $\mu\text{m}$ ), 250  $\mu\text{m}$ , 100  $\mu\text{m}$ , 80  $\mu\text{m}$ , 60  $\mu\text{m}$ , 30  $\mu\text{m}$ , 20  $\mu\text{m}$ , 10  $\mu\text{m}$ , or less. The channel may have a cross-sectional dimension (e.g., width or diameter) of less than or equal to about 500  $\mu\text{m}$ , 250  $\mu\text{m}$ , 100  $\mu\text{m}$ , 75  $\mu\text{m}$ , 50  $\mu\text{m}$ , 40  $\mu\text{m}$ , 30  $\mu\text{m}$ , 20  $\mu\text{m}$ , 10  $\mu\text{m}$ , or less.

**[0058]** In some examples, the cross-sectional dimensions of the channel may be about 100  $\mu\text{m}$  wide by about 100  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 100  $\mu\text{m}$  wide by about 80  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 100  $\mu\text{m}$  wide by about 60  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 100  $\mu\text{m}$  wide by about 40  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 100  $\mu\text{m}$  wide by about 20  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 100  $\mu\text{m}$  wide by about 10  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 80  $\mu\text{m}$  wide by about 100  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 60  $\mu\text{m}$  wide by about 100  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 40  $\mu\text{m}$  wide by about 100  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 20  $\mu\text{m}$  wide by about 100  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 10  $\mu\text{m}$  wide by about 100  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 80  $\mu\text{m}$  wide by about 80  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 60  $\mu\text{m}$  wide by about 60  $\mu\text{m}$  deep. In

some examples, the cross-sectional dimensions of the channel may be about 40  $\mu\text{m}$  wide by about 40  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 20  $\mu\text{m}$  wide by about 20  $\mu\text{m}$  deep. In some examples, the cross-sectional dimensions of the channel may be about 10  $\mu\text{m}$  wide by about 10  $\mu\text{m}$  deep.

**[0059]** The cross-sectional shape of the channel may be any suitable cross-sectional shape including, but not limited to, circular, oval, triangular, square, or rectangular. The cross-sectional area of the channel may be constant along the length of the channel. Alternatively, or in addition to, the cross-sectional area of the channel may vary along the length of the channel. The cross-sectional area of the channel may vary from about 50% to 150%, 60% to 125%, 70% to 120%, 80% to 115%, 90% to 110%, 95% to 100%, or 98% to 102%. The cross-sectional area of the channel may be less than or equal to about 10,000 micrometers squared ( $\mu\text{m}^2$ ), 7,500  $\mu\text{m}^2$ , 5,000  $\mu\text{m}^2$ , 2,500  $\mu\text{m}^2$ , 1,000  $\mu\text{m}^2$ , 750  $\mu\text{m}^2$ , 500  $\mu\text{m}^2$ , 400  $\mu\text{m}^2$ , 300  $\mu\text{m}^2$ , 200  $\mu\text{m}^2$ , 100  $\mu\text{m}^2$ , or less.

**[0060]** The channel may have a single inlet or multiple inlets. The inlet(s) may have the same diameter or they may have different diameters. The inlet(s) may have diameters less than or equal to about 2.5 millimeters (mm), 2 mm, 1.5 mm, 1 mm, 0.5 mm, or less.

**[0061]** The device may include a plurality of chambers. The plurality of chambers may be an array of chambers. The device may include a single array of chambers or multiple arrays of chambers, with each array of chambers fluidically isolated from the other arrays. The array of chambers may be arranged in a row, in a grid configuration, in an alternating pattern, or in any other configuration. The microfluidic device may have at least 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, or more arrays of chambers. The arrays of chambers may be identical or the arrays of chambers may be different (e.g., have a different number or configuration of chambers). The arrays of chambers may all have the same external dimension (i.e., the length and width of the array of chambers that encompasses all features of the array of chambers) or the arrays of chambers may have different external dimensions. An array of chambers may have a width of less than or equal to about 100 mm, 75 mm, 50 mm, 40 mm, 30 mm, 20 mm, 10 mm, 8 mm, 6 mm, 4 mm, 2 mm, 1 mm, or less. The array of chambers may have a length of greater than or equal to about 50 mm, 40 mm, 30 mm, 20 mm, 10 mm, 8 mm, 6 mm, 4 mm, 2 mm, 1 mm, or less. In an example, the width of an array may be from about 1 mm to 100 mm or from about 10 mm to 50 mm. In an example, the length of an array may be from about 1 mm to 50 mm or from about 5 mm to 20 mm.

**[0062]** The array of chambers may have greater than or equal to about 1,000 chambers, 5,000 chambers, 10,000 chambers, 20,000 chambers, 30,000 chambers, 40,000 chambers, 50,000

chambers, 100,000 chambers, or more. In an example, the microfluidic device may have from about 10,000 to 30,000 chambers. In another example, the microfluidic device may have from about 15,000 to 25,000 chambers. The chambers may be cylindrical in shape, hemispherical in shape, or a combination of cylindrical and hemispherical in shape. Alternatively, or in addition to, the chambers may be cubic in shape. The chambers may have a cross-sectional dimension of less than or equal to about 500  $\mu\text{m}$ , 250  $\mu\text{m}$ , 100  $\mu\text{m}$ , 80  $\mu\text{m}$ , 60  $\mu\text{m}$ , 30  $\mu\text{m}$ , 15  $\mu\text{m}$ , or less. In an example, the chamber has a cross-sectional dimension (e.g., diameter or side length) that is less than or equal to about 250  $\mu\text{m}$ . In another example, the chamber has a cross-sectional dimension (e.g., diameter or side length) that is less than or equal to about 100  $\mu\text{m}$ . In another example, the chamber has a cross-sectional dimension (e.g., diameter or side length) that is less than or equal to about 50  $\mu\text{m}$ .

**[0063]** The depth of the chambers may be less than or equal to about 500  $\mu\text{m}$ , 250  $\mu\text{m}$ , 100  $\mu\text{m}$ , 80  $\mu\text{m}$ , 60  $\mu\text{m}$ , 30  $\mu\text{m}$ , 15  $\mu\text{m}$ , or less. In an example, the chambers may have a cross-sectional dimension of about 30  $\mu\text{m}$  and a depth of about 100  $\mu\text{m}$ . In another example, the chambers may have a cross-sectional dimension of about 35  $\mu\text{m}$  and a depth of about 80  $\mu\text{m}$ . In another example, the chambers may have a cross-sectional dimension of about 40  $\mu\text{m}$  and a depth of about 70  $\mu\text{m}$ . In another example, the chambers may have a cross-sectional dimension of about 50  $\mu\text{m}$  and a depth of about 60  $\mu\text{m}$ . In another example, the chambers may have a cross-sectional dimension of about 60  $\mu\text{m}$  and a depth of about 40  $\mu\text{m}$ . In another example, the chambers may have a cross-sectional dimension of about 80  $\mu\text{m}$  and a depth of about 35  $\mu\text{m}$ . In another example, the chambers may have a cross-sectional dimension of about 100  $\mu\text{m}$  and a depth of about 30  $\mu\text{m}$ . In another example, the chambers and the channel have the same depth. In an alternative embodiment, the chambers and the channel have different depths.

**[0064]** The chambers may have any volume. The chambers may have the same volume or the volume may vary across the microfluidic device. The chambers may have a volume of less than or equal to about 1000 picoliters (pL), 900 pL, 800 pL, 700 pL, 600 pL, 500 pL, 400 pL, 300 pL, 200 pL, 100 pL, 75 pL, 50 pL, 25 pL, or less picoliters. The chambers may have a volume from about 25 pL to 50 pL, 25 pL to 75 pL, 25 pL to 100 pL, 25 pL to 200 pL, 25 pL to 300 pL, 25 pL to 400 pL, 25 pL to 500 pL, 25 pL to 600 pL, 25 pL to 700 pL, 25 pL to 800 pL, 25 pL to 900 pL, or 25 pL to 1000 pL. In an example, the chamber(s) have a volume of less than or equal to 250 pL. In another example, the chambers have a volume of less than or equal to about 150 pL.

**[0065]** The volume of channel may be less than, equal to, or greater than the total volume of the chambers. In an example, the volume of the channel is less than the total volume of the chambers. The volume of the channel may be less than or equal to 95%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, or less than the total volume of the chambers.

**[0066]** The device may further include a siphon aperture disposed between the channel and the chamber. The siphon aperture may be one of a plurality of siphon apertures connecting the channel to a plurality of chambers. The siphon aperture may be configured to provide fluid communication between the channel and the chamber. The lengths of the siphon apertures may be constant or may vary across the device (e.g., microfluidic device). The siphon apertures may have a long dimension that is less than or equal to about 150  $\mu\text{m}$ , 100  $\mu\text{m}$ , 50  $\mu\text{m}$ , 25  $\mu\text{m}$ , 10  $\mu\text{m}$ , 5  $\mu\text{m}$ , or less. The depth of the siphon aperture may be less than or equal to about 50  $\mu\text{m}$ , 25  $\mu\text{m}$ , 10  $\mu\text{m}$ , 5  $\mu\text{m}$ , or less. The siphon apertures may have a cross-sectional dimension of less than or equal to about 50  $\mu\text{m}$ , 40  $\mu\text{m}$ , 30  $\mu\text{m}$ , 20  $\mu\text{m}$ , 10  $\mu\text{m}$ , 5  $\mu\text{m}$ , or less.

**[0067]** The cross-sectional shape of the siphon aperture may be any suitable cross-sectional shape including, but not limited to, circular, oval, triangular, square, or rectangular. The cross-sectional area of the siphon aperture may be constant along the length of the siphon aperture. Alternatively, or in addition to, the cross-sectional area of the siphon aperture may vary along the length of the siphon aperture. The cross-sectional area of the siphon aperture may be greater at the connection to the channel than the cross-sectional area of the siphon aperture at the connection to the chamber. Alternatively, the cross-sectional area of the siphon aperture at the connection to the chamber may be greater than the cross-sectional area of the siphon aperture at the connection to the channel. The cross-sectional area of the siphon aperture may vary from about 50% to 150%, 60% to 125%, 70% to 120%, 80% to 115%, 90% to 110%, 95% to 100%, or 98% to 102%. The cross-sectional area of the siphon aperture may be less than or equal to about 2,500  $\mu\text{m}^2$ , 1,000  $\mu\text{m}^2$ , 750  $\mu\text{m}^2$ , 500  $\mu\text{m}^2$ , 250  $\mu\text{m}^2$ , 100  $\mu\text{m}^2$ , 75  $\mu\text{m}^2$ , 50  $\mu\text{m}^2$ , 25  $\mu\text{m}^2$ , or less. The cross-sectional area of the siphon aperture at the connection to the channel may be less than or equal to the cross-sectional area of the channel. The cross-sectional area of the siphon aperture at the connection to the channel may be less than or equal to about 98 %, 95 %, 90%, 85 %, 80%, 75 %, 70%, 60%, 50%, 40%, 30 %, 20 %, 10%, 5%, 1%, 0.5%, or less of the cross-sectional area of the channel. The siphon apertures may be substantially perpendicular to the channel. Alternatively, or in addition to, the siphon apertures are not substantially perpendicular to the channel. An angle between the siphon apertures and the channel may be at least about 5°, 10°, 15°, 20°, 30°, 40°, 50°, 60°, 70°, or 90°.

**[0068]** The microfluidic device may be configured to permit pressurized off-gassing or degassing of the channel, chamber, siphon aperture, or any combination thereof. Pressurized off-gassing or degassing may be provided by a film or membrane configured to permit pressurized off-gassing or degassing. The film or membrane may be permeable to gas above a pressure threshold. The film or membrane may not be permeable to (e.g., is impermeable or substantially impermeable to) liquids such as, but not limited to, aqueous fluids, oils, or other solvents. The channel, the chamber, the siphon aperture, or any combination thereof may comprise the film or membrane. In an example, the chamber comprises the gas permeable film or membrane and the channel and/or siphon aperture does not comprise the gas permeable film or membrane. In another example, the chamber and siphon aperture comprise the gas permeable film or membrane and the channel does not comprise the gas permeable film or membrane. In another example, the chamber, channel, and siphon aperture comprise the gas permeable film or membrane.

**[0069]** The film or membrane may be a thin film. The film or membrane may be a polymer. The film may be a thermoplastic film or membrane. The film or membrane may not comprise an elastomeric material. The gas permeable film or membrane may cover the fluid flow path, the channel, the chamber, or any combination thereof. In an example, the gas permeable film or membrane covers the chamber. In another example, the gas permeable film or membrane covers the chamber and the channel. The gas permeability of the film may be induced by elevated pressures. The thickness of the film or membrane may be less than or equal to about 500 micrometers ( $\mu\text{m}$ ), 250  $\mu\text{m}$ , 200  $\mu\text{m}$ , 150  $\mu\text{m}$ , 100  $\mu\text{m}$ , 75  $\mu\text{m}$ , 50  $\mu\text{m}$ , 25  $\mu\text{m}$ , or less. In an example, the film or membrane has a thickness of less than or equal to about 100  $\mu\text{m}$ . In another example, the film or membrane has a thickness of less than or equal to about 50  $\mu\text{m}$ . In another example, the film or membrane has a thickness of less than or equal to about 25  $\mu\text{m}$ . The thickness of the film or membrane may be from about 0.1  $\mu\text{m}$  to about 200  $\mu\text{m}$ , 0.5  $\mu\text{m}$  to 150  $\mu\text{m}$ , or 25  $\mu\text{m}$  to 100  $\mu\text{m}$ . In an example, the thickness of the film or membrane is from about 25  $\mu\text{m}$  to 100  $\mu\text{m}$ . The thickness of the film may be selected by manufacturability of the film, the air permeability of the film, the volume of each chamber or partition to be out-gassed, the available pressure, and/or the time to complete the partitioning or digitizing process.

**[0070]** The film or membrane may be configured to employ different permeability characteristics under different applied pressure differentials. For example, the thin film may be gas impermeable at a first pressure differential (e.g., low pressure) and at least partially gas permeable at a second pressure differential (e.g., high pressure). The first pressure

differential (e.g., low pressure differential) may be less than or equal to about 8 pounds per square inch (psi), 6 psi, 4 psi, 2 psi, 1 psi, or less. In an example, the film or membrane is substantially impermeable to gas at a pressure differential of less than 4 psi. The second pressure differential (e.g., high pressure differential) may be greater than or equal to about 1 psi, 2 psi, 4 psi, 6 psi, 8 psi, 10 psi, 12 psi, 14 psi, 16 psi, 20 psi, or more. In an example, the film or membrane is substantially gas permeable at a pressure of greater than or equal to 4 psi.

### **Method for analyzing biological samples**

**[0071]** In another aspect, the present disclosure provides methods for processing a biological sample. The method may include providing a device (e.g., microfluidic device). The device may include a fluid flow path and a chamber. The fluid flow path may comprise a channel and an inlet port. The fluid flow path may not include an outlet port. The chamber may be in fluid communication with the channel. A solution comprising the biological sample may be directed from the inlet port to the channel. At least a portion of the solution may be directed from the channel to the chamber. The chamber may retain the portion of the sample during processing of the solution and biological sample.

**[0072]** The device may include a chamber or a plurality of chambers. The device may include a single inlet port or multiple inlet ports. In an example, the device includes a single inlet port. In another example, the device includes two or more inlet ports. The device may be as described elsewhere herein.

**[0073]** The method may further include applying a single or multiple pressure differentials to the inlet port to direct the solution from the inlet port to the channel. Alternatively, or in addition to, the device may include multiple inlet ports and the pressure differential may be applied to the multiple inlet ports. The inlet of the device (e.g., microfluidic device) may be in fluid communication with a fluid flow module, such as a pneumatic pump, vacuum source or compressor. The fluid flow module may provide positive or negative pressure to the inlet. The fluid flow module may apply a pressure differential to fill the device with a sample and partition (e.g., digitize) the sample into the chamber. Alternatively, or in addition to, the sample may be partitioned into a plurality of chambers as described elsewhere herein. Filling and partitioning of the sample may be performed without the use of valves between the chambers and the channel to isolate the sample. For example, filling of the channel may be performed by applying a pressure differential between the sample in the inlet port and the channel. This pressure differential may be achieved by pressurizing the sample or by

applying vacuum to the channel and or chambers. Filling the chambers and partitioning the solution comprising the sample may be performed by applying a pressure differential between the channel and the chambers. This may be achieved by pressurizing the channel via the inlet port(s) or by applying a vacuum to the chambers. The solution comprising the sample may enter the chambers such that each chamber contains at least a portion of the solution.

**[0074]** In some cases, one single pressure differential may be used to deliver the solution with the biological sample (including molecule targets of interest) to the channel, and the same pressure differential may be used to continue to digitize (i.e., delivering the solution from the channel to the chamber) the chamber with the solution. Moreover, the single pressure differential may be sufficiently high to permit pressurized off-gassing or degassing of the channel and/or chamber. Alternatively, or in addition to, the pressure differential to deliver the solution with sample to the channel may be a first pressure differential. The pressure differential to deliver the solution from the channel to the chamber(s) may be a second pressure differential. The first and second pressure differentials may be the same or may be different. In an example, the second pressure differential is greater than the first pressure differential. Alternatively, the second pressure differential may be less than the first pressure differential. The first pressure differential, the second pressure differential, or both may be sufficiently high to permit pressurized off-gassing or degassing of the channel and/or chamber. In some cases, a third pressure differential may be used to permit pressurized off-gassing or degassing of the channel and/or chamber. Pressurized off-gassing or degassing of the channel or chamber(s) may be permitted by a film or membrane. For example, when a pressure threshold is reached the film or membrane may permit gas to travel from the chamber and/or channel through the film or membrane to an environment outside of the chamber and/or channel.

**[0075]** The film or membrane may employ different permeability characteristics under different applied pressure differentials. For example, the film or membrane may be gas impermeable at the first pressure differential (e.g., low pressure) and gas permeable at the second pressure differential (e.g., high pressure). The first and second pressure differentials may be the same or they may be different. During filling of the microfluidic device, the pressure of the inlet port may be higher than the pressure of the channel, permitting the solution in the inlet port to enter the channel. The first pressure differential (e.g., low pressure) may be less than or equal to about 8 psi, 6 psi, 4 psi, 2 psi, 1 psi, or less. In an example, the first pressure differential may be from about 1 psi to 8 psi. In another example,

the first pressure differential may be from about 1 psi to 6 psi. In another example, the first pressure differential may be from about 1 psi to 4 psi. The chambers of the device may be filled by applying a second pressure differential between inlet and the chambers. The second pressure differential may direct fluid from the channel into the chambers and gas from the channel and/or chambers to an environment external to the channel and/or chambers. The second pressure differential may be greater than or equal to about 1 psi, 2 psi, 4 psi, 6 psi, 8 psi, 10 psi, 12 psi, 14 psi, 16 psi, 20 psi, or more. In an example, the second pressure differential is greater than about 4 psi. In another example, the second pressure differential is greater than about 8 psi. The and the microfluidic device may be filled and the sample partitioned by applying the first pressure differential, second pressure differential, or a combination thereof for less than or equal to about 20 minutes, 15 minutes, 10 minutes, 5 minutes, 3 minutes, 2 minutes, 1 minute, or less.

**[0076]** The sample may be partitioned by removing the excess sample from the channel by backfilling the channel with a gas or a fluid immiscible with an aqueous solution comprising the biological sample. The immiscible fluid may be provided after providing the solution comprising the sample such that the solution enters the channel first followed by the immiscible fluid. The immiscible fluid may be any fluid that does not mix with an aqueous fluid. The gas may be oxygen, nitrogen, carbon dioxide, air, a noble gas, or any combination thereof. The immiscible fluid may be an oil or an organic solvent. For example, the immiscible fluid may be silicone oil or other types of oil/organic solvent that have similar characteristics compared to the silicone oil. Alternatively, removing sample from the channel may prevent reagents in one chamber from diffusing through the siphon aperture into the channel and into other chambers. Sample within the channel may be removed by partitioning the sample into the chambers such that no sample remains in the channel or by removing excess sample from the channel.

**[0077]** Directing the solution from the channel to the chamber or chambers may partition the sample. The device may permit partitioning of the sample into the chambers, or digitizing the samples, such that no residual solution remains in the channel and/or siphon apertures (e.g., such that there is no or substantially no sample dead volume). The solution comprising the sample may be partitioned such that there is zero sample dead volume (e.g., all sample and reagent input into the device are fluidically isolated within the chambers), which may prevent or reduce waste of sample and reagents. Alternatively, or in addition to, the sample may be partitioned by providing a sample volume that is less than a volume of the chamber(s). The volume of the channel may be less than the total volume of the chambers such that all sample loaded into the

channel is distributed to the chambers. The total volume of the solution comprising the sample may be less than the total volume of the chambers. The volume of the solution may be 100%, 99%, 98%, 95%, 90%, 85%, 80%, or less than the total volume of the chambers. The solution may be added to the inlet port simultaneously with or prior to a gas or immiscible fluid being added to the inlet port. The volume of the gas or immiscible fluid may be greater than or equal to the volume of the channel to fluidically isolate the chambers. A small amount of the gas or immiscible fluid may enter the siphon apertures or chambers.

**[0078]** FIGS. 1A – 1F schematically illustrate an example method for filling the microfluidic device. FIG. 1A schematically illustrates loading a sample and immiscible fluid into the microfluidic device. The microfluidic device includes an input port 101, channel 102, and chambers 103. The channel and the chambers of the microfluidic device are filled with air 104. The sample 105 is directed or injected to the input port 101. FIG. 1B schematically illustrates pressurizing the microfluidic device to load the sample 105 into the channel 102. In this example, the microfluidic device includes a single inlet port connected to both ends of the channel in a loop configuration. As pressure is applied, the sample 105 is directed through both ends of the channel simultaneously. FIG. 1C schematically illustrates continued pressurization to degas the fluid flow path and continue to load the sample into the channel. As the sample 105 enters the chambers 103, a portion of the channel 103 is filled with an immiscible fluid 106, such as oil or gas, that may be added simultaneously with the sample or sequentially (e.g., sample followed by immiscible fluid). As the sample 105 and immiscible fluid 106 fills the channel and chambers, the air 104 is directed through the film or membrane and out of the device. FIG. 1D schematically illustrates partial digitization of the sample 105 into the chambers 103 and continued loading of the immiscible fluid 106 into the channel 102. As the sample 105 enters the chambers 103 the air 104 within the chambers 103 is displaced through the film or membrane. FIG. 1E schematically illustrates further digitization and displacement of air 103. As the immiscible fluid 106 fills the channel from both ends, sample is directed into the chambers 103 and the volume of the sample 105 within the channel is reduced; FIG. 1F schematically illustrates complete digitization of the sample 105 in which the immiscible fluid 106 fills the entire channel 102 and the sample 105 is isolated in the chambers 103. In another example, the device has multiple inlet ports and the sample and immiscible fluid are applied to each port simultaneously to fill the channel and chambers.

**[0079]** FIGS. 2A-2E show an example image of a sample digitization in a microfluidic device. FIG. 2A shows an example microfluidic device with two inlet ports. The sample and

immiscible fluid, in this example oil, are applied to both inlet ports simultaneously. **FIG. 2B** shows pressurized loading of the sample and oil into the microfluidic device. Both inlet ports are pressurized simultaneously to evenly direct the sample and oil into the channel of the device. **FIGs. 2C** and **2D** shows the sample and an oil progressively filling the channel and chambers of the device. **FIG. 2E** shows the example device after complete digitization or partitioning of the sample within the device.

**[0080]** **FIG. 3** schematically illustrates an example method for digitization of a sample. A sample and immiscible fluid may be provided **301** at the inlet port(s) of the microfluidic device. The inlet port(s) may be pressurized **302** to load the sample and immiscible fluid into the channel. The inlet port may be further pressurized to load the sample into the chambers and fill the channel with the immiscible fluid to provide complete digitization of the sample **304**.

**[0081]** Partitioning of the sample may be verified by the presence of an indicator within the reagent. An indicator may include a molecule comprising a detectable moiety. The detectable moiety may include radioactive species, fluorescent labels, chemiluminescent labels, enzymatic labels, colorimetric labels, or any combination thereof. Non-limiting examples of radioactive species include  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{22}\text{Na}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  $^{42}\text{K}$ ,  $^{45}\text{Ca}$ ,  $^{59}\text{Fe}$ ,  $^{123}\text{I}$ ,  $^{124}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ , or  $^{203}\text{Hg}$ . Non-limiting examples of fluorescent labels include fluorescent proteins, optically active dyes (e.g., a fluorescent dye), organometallic fluorophores, or any combination thereof. Non-limiting examples of chemiluminescent labels include enzymes of the luciferase class such as Cypridina, Gaussia, Renilla, and Firefly luciferases. Non-limiting examples of enzymatic labels include horseradish peroxidase (HRP), alkaline phosphatase (AP), beta galactosidase, glucose oxidase, or other types of labels.

**[0082]** The indicator molecule may be a fluorescent molecule. Fluorescent molecules may include fluorescent proteins, fluorescent dyes, and organometallic fluorophores. In some embodiments, the indicator molecule is a protein fluorophore. Protein fluorophores may include green fluorescent proteins (GFPs, fluorescent proteins that fluoresce in the green region of the spectrum, generally emitting light having a wavelength from 500-550 nanometers), cyan-fluorescent proteins (CFPs, fluorescent proteins that fluoresce in the cyan region of the spectrum, generally emitting light having a wavelength from 450-500 nanometers), red fluorescent proteins (RFPs, fluorescent proteins that fluoresce in the red region of the spectrum, generally emitting light having a wavelength from 600-650 nanometers). Non-limiting examples of protein fluorophores include mutants and spectral variants of AcGFP, AcGFP1, AmCyan, AmCyan1, AQ143, AsRed2, Azami Green, Azurite, BFP, Cerulean, CFP, CGFP, Citrine, copGFP, CyPet,

dKeima-Tandem, DsRed, dsRed-Express, DsRed-Monomer, DsRed2, dTomato, dTomato-Tandem, EBFP, EBFP2, ECFP, EGFP, Emerald, EosFP, EYFP, GFP, HcRed-Tandem, HcRed1, JRed, Katuska, Kusabira Orange, Kusabira Orange2, mApple, mBanana, mCerulean, mCFP, mCherry, mCitrine, mECFP, mEmerald, mGrape1, mGrape2, mHoneydew, Midori-Ishi Cyan, mKeima, mKO, mOrange, mOrange2, mPlum, mRaspberry, mRFP1, mRuby, mStrawberry, mTagBFP, mTangerine, mTeal, mTomato, mTurquoise, mWasabi, PhiYFP, ReAsH, Sapphire, Superfolder GFP, T-Sapphire, TagCFP, TagGFP, TagRFP, TagRFP-T, TagYFP, tdTomato, Topaz, TurboGFP, Venus, YFP, YPet, ZsGreen, and ZsYellow1.

**[0083]** The indicator molecule may be a fluorescent dye. Non-limiting examples of fluorescent dyes include SYBR green, SYBR blue, DAPI, propidium iodine, Hoeste, SYBR gold, ethidium bromide, acridines, proflavine, acridine orange, acriflavine, fluorcoumanin, ellipticine, daunomycin, chloroquine, distamycin D, chromomycin, homidium, mithramycin, ruthenium polypyridyls, anthramycin, phenanthridines and acridines, ethidium bromide, propidium iodide, hexidium iodide, dihydroethidium, ethidium homodimer-1 and -2, ethidium monoazide, and ACMA, Hoechst 33258, Hoechst 33342, Hoechst 34580, DAPI, acridine orange, 7-AAD, actinomycin D, LDS751, hydroxystilbamidine, SYTOX Blue, SYTOX Green, SYTOX Orange, POPO-1, POPO-3, YOYO-1, YOYO-3, TOTO-1, TOTO-3, JOJO-1, LOLO-1, BOBO-1, BOBO-3, PO-PRO-1, PO-PRO-3, BO-PRO-1, BO-PRO-3, TO-PRO-1, TO-PRO-3, TO-PRO-5, JO-PRO-1, LO-PRO-1, YO-PRO-1, YO-PRO-3, PicoGreen, OliGreen, RiboGreen, SYBR Gold, SYBR Green I, SYBR Green II, SYBR DX, SYTO-40, -41, -42, -43, -44, -45 (blue), SYTO-13, -16, -24, -21, -23, -12, -11, -20, -22, -15, -14, -25 (green), SYTO-81, -80, -82, -83, -84, -85 (orange), SYTO-64, -17, -59, -61, -62, -60, -63 (red), fluorescein, fluorescein isothiocyanate (FITC), tetramethyl rhodamine isothiocyanate (TRITC), rhodamine, tetramethyl rhodamine, R-phycoerythrin, Cy-2, Cy-3, Cy-3.5, Cy-5, Cy5.5, , Cy-7, Texas Red, Phar-Red, allophycocyanin (APC), Sybr Green I, Sybr Green II, Sybr Gold, CellTracker Green, 7-AAD, ethidium homodimer I, ethidium homodimer II, ethidium homodimer III, ethidium bromide, umbelliferone, eosin, green fluorescent protein, erythrosin, coumarin, methyl coumarin, pyrene, malachite green, stilbene, lucifer yellow, cascade blue, dichlorotriazinylamine fluorescein, dansyl chloride, fluorescent lanthanide complexes such as those including europium and terbium, carboxy tetrachloro fluorescein, 5 and/or 6-carboxy fluorescein (FAM), 5- (or 6-) iodoacetamidofluorescein, 5-{[2(and 3)-5-(Acetylmercapto)-succinyl]amino} fluorescein (SAMSA-fluorescein), lissamine rhodamine B sulfonyl chloride, 5 and/or 6 carboxy rhodamine (ROX), 7-amino-methyl-coumarin, 7-Amino-4-methylcoumarin-3-acetic acid (AMCA), BODIPY fluorophores, 8-methoxypyrene-1,3,6-trisulfonic acid trisodium salt, 3,6-Disulfonate-4-

amino-naphthalimide, phycobiliproteins, AlexaFluor 350, 405, 430, 488, 532, 546, 555, 568, 594, 610, 633, 635, 647, 660, 680, 700, 750, and 790 dyes, DyLight 350, 405, 488, 550, 594, 633, 650, 680, 755, and 800 dyes, or other fluorophores.

**[0084]** The indicator molecule may be an organometallic fluorophore. Non limiting examples of organometallic fluorophores include lanthanide ion chelates, nonlimiting examples of which include tris (dibenzoylmethane) mono(1,10-phenanthroline) europium(III), tris (dibenzoylmethane) mono(5-amino-1,10-phenanthroline) europium (III), and Lumi4-Tb cryptate.

**[0085]** **FIG. 4** schematically illustrates an example method for using the microfluidic device for a digital polymerase chain reaction (dPCR). The sample and reagents may be partitioned **401** as shown in **FIGS. 2A-2E**. The sample and reagent may be subjected to thermal cycling **402** to run the PCR reaction on the reagent in the chambers. Thermal cycling may be performed, for example, using a flat block thermal cycler. Image acquisition **403** may be performed to determine which chambers have successfully run the PCR reaction. Image acquisition may, for example, be performed using a three-color probe detection unit. Poisson statistics may be applied **404** to the count of chambers determined in **403** to convert the raw number of positive chambers into a nucleic acid concentration.

**[0086]** The method may further include detecting one or more components of the solution, one or more components of the biological sample, or a reaction with one or more components of the biological sample. Detecting the one or more components of the solution, one or more components of the biological sample or the reaction may include imaging the chamber. The images may be taken of the microfluidic device. Images may be taken of single chambers, an array of chambers, or of multiple arrays of chambers concurrently. The images may be taken through the body of the microfluidic device. The images may be taken through the film or membrane of the microfluidic device. In an example, the images are taken through both the body of the microfluidic device and through the thin film. The body of the microfluidic device may be substantially optically transparent. Alternatively, the body of the microfluidic device may be substantially optically opaque. In an example, the film or membrane may be substantially optically transparent. The images may be taken prior to filling the microfluidic device with sample. The Images may be taken after filling of the microfluidic device with sample. The images may be taken during filling the microfluidic device with sample. The images may be taken to verify partitioning of the sample. The images may be taken during a reaction to monitor products of the reaction. In an example, the products of the reaction comprise amplification products. The images may be taken at specified intervals. Alternatively, or in addition to, a video

may be taken of the microfluidic device. The specified intervals may include taking an image at least about every 300 seconds, 240 seconds, 180 seconds, 120 seconds, 90 seconds, 60 seconds, 30 seconds, 15 seconds, 10 seconds, 5 seconds, 4 seconds, 3 seconds, 2 seconds, 1 second, or more frequently during a reaction.

**[0087]** The biological sample may be any biological analyte such as, but not limited to, a nucleic acid molecule, protein, enzyme, antibody, or other biological molecule. In an example, the biological sample includes one or more nucleic acid molecules. Processing the nucleic acid molecules may further include thermal cycling the chamber or chambers to amplify the nucleic acid molecules. The method may further include controlling a temperature of the channel or the chamber(s). The method for using a microfluidic device may further comprise amplification of a nucleic acid sample. The microfluidic device may be filled with an amplification reagent comprising nucleic acid molecules, components used for an amplification reaction, an indicator molecule, and an amplification probe. The amplification may be performed by thermal cycling the plurality of chambers. Detection of nucleic acid amplification may be performed by imaging the chambers of the microfluidic device. The nucleic acid molecules may be quantified by counting the chambers in which the nucleic acid molecules are successfully amplified and applying Poisson statistics. In some embodiments, nucleic acid amplification and quantification may be performed in a single integrated unit.

**[0088]** A variety of nucleic acid amplification reactions may be used to amplify the nucleic acid molecule in a sample to generate an amplified product. Amplification of a nucleic acid target may be linear, exponential, or a combination thereof. Non-limiting examples of nucleic acid amplification methods include primer extension, polymerase chain reaction, reverse transcription, isothermal amplification, ligase chain reaction, helicase-dependent amplification, asymmetric amplification, rolling circle amplification, and multiple displacement amplification. In some embodiments, the amplification product is DNA or RNA. For embodiments directed towards DNA amplification, any DNA amplification method may be employed. DNA amplification methods include, but are not limited to, PCR, real-time PCR, assembly PCR, asymmetric PCR, digital PCR, dial-out PCR, helicase-dependent PCR, nested PCR, hot start PCR, inverse PCR, methylation-specific PCR, miniprimer PCR, multiplex PCR, overlap-extension PCR, thermal asymmetric interlaced PCR, touchdown PCR, and ligase chain reaction. In some embodiments, DNA amplification is linear, exponential, or any combination thereof. In some embodiments, DNA amplification is achieved with digital PCR (dPCR).

**[0089]** Reagents used for nucleic acid amplification may include polymerizing enzymes, reverse primers, forward primers, and amplification probes. Examples of polymerizing enzymes include, without limitation, nucleic acid polymerase, transcriptase, or ligase (i.e., enzymes which catalyze the formation of a bond). The polymerizing enzyme can be naturally occurring or synthesized. Examples of polymerases include a DNA polymerase, and RNA polymerase, a thermostable polymerase, a wild-type polymerase, a modified polymerase, E. coli DNA polymerase I, T7 DNA polymerase, bacteriophage T4 DNA polymerase  $\Phi$ 29 (phi29) DNA polymerase, Taq polymerase, Tth polymerase, Tli polymerase, Pfu polymerase Pwo polymerase, VENT polymerase, DEEPVENT polymerase, Ex-Taq polymerase, LA-Taw polymerase, Sso polymerase Poc polymerase, Pab polymerase, Mth polymerase ES4 polymerase, Tru polymerase, Tac polymerase, The polymerase, Tma polymerase, Tca polymerase, Tih polymerase, Tfi polymerase, Platinum Taq polymerases, Tbr polymerase, Tfl polymerase, Pfutubo polymerase, Pyrobest polymerase, KOD polymerase, Bst polymerase, Sac polymerase, Klenow fragment polymerase with 3' to 5' exonuclease activity, and variants, modified products and derivatives thereof. For a Hot Start polymerase, a denaturation cycle at a temperature from about 92 °C to 95 °C for a time period from about 2 minutes to 10 minutes may be used.

**[0090]** The amplification probe may be a sequence-specific oligonucleotide probe. The amplification probe may be optically active when hybridized with an amplification product. In some embodiments, the amplification probe is only detectable as nucleic acid amplification progresses. The intensity of the optical signal may be proportional to the amount of amplified product. A probe may be linked to any of the optically-active detectable moieties (e.g., dyes) described herein and may also include a quencher capable of blocking the optical activity of an associated dye. Non-limiting examples of probes that may be useful as detectable moieties include TaqMan probes, TaqMan Tamara probes, TaqMan MGB probes, Lion probes, locked nucleic acid probes, or molecular beacons. Non-limiting examples of quenchers that may be useful in blocking the optical activity of the probe include Black Hole Quenchers (BHQ), Iowa Black FQ and RQ quenchers, or Internal ZEN Quenchers. Alternatively or in addition to, the probe or quencher may be any probe that is useful in the context of the methods of the present disclosure.

**[0091]** The amplification probe is a dual labeled fluorescent probe. The dual labeled probe may include a fluorescent reporter and a fluorescent quencher linked with a nucleic acid. The fluorescent reporter and fluorescent quencher may be positioned in close proximity to each other. The close proximity of the fluorescent reporter and fluorescent quencher may block the optical

activity of the fluorescent reporter. The dual labeled probe may bind to the nucleic acid molecule to be amplified. During amplification, the fluorescent reporter and fluorescent quencher may be cleaved by the exonuclease activity of the polymerase. Cleaving the fluorescent reporter and quencher from the amplification probe may cause the fluorescent reporter to regain its optical activity and enable detection. The dual labeled fluorescent probe may include a 5' fluorescent reporter with an excitation wavelength maximum of at least about 450 nanometers (nm), 500 nm, 525 nm, 550 nm, 575 nm, 600 nm, 625 nm, 650 nm, 675 nm, 700 nm, or higher and an emission wavelength maximum of about 500 nm, 525 nm, 550 nm, 575 nm, 600 nm, 625 nm, 650 nm, 675 nm, 700 nm, or higher. The dual labeled fluorescent probe may also include a 3' fluorescent quencher. The fluorescent quencher may quench fluorescent emission wavelengths between about 380 nm and 550 nm, 390 nm and 625 nm, 470 nm and 560 nm, 480 nm and 580 nm, 550 nm and 650 nm, 550 nm and 750 nm, or 620 nm and 730 nm.

**[0092]** The nucleic acid amplification may be performed by thermal cycling the chambers of the microfluidic device. Thermal cycling may include controlling the temperature of the microfluidic device by applying heating or cooling to the microfluidic device. Heating or cooling methods may include resistive heating or cooling, radiative heating or cooling, conductive heating or cooling, convective heating or cooling, or any combination thereof. Thermal cycling may include cycles of incubating the chambers at a temperature sufficiently high to denature nucleic acid molecules for a duration followed by incubation of the chambers at an extension temperature for an extension duration. Denaturation temperatures may vary depending upon, for example, the particular nucleic acid sample, the reagents used, and the reaction conditions. A denaturation temperature may be from about 80 °C to 110 °C, 85 °C to about 105 °C, 90 °C to about 100 °C, 90 °C to about 98 °C, 92 °C to about 95 °C. The denaturation temperature may be at least about 80 °C, 81 °C, 82 °C, 83 °C, 84 °C, 85 °C, 86 °C, 87 °C, 88 °C, 89 °C, 90 °C, 91 °C, 92 °C, 93 °C, 94 °C, 95 °C, 96 °C, 97 °C, 98 °C, 99 °C, 100 °C, or higher.

**[0093]** The duration for denaturation may vary depending upon, for example, the particular nucleic acid sample, the reagents used, and the reaction conditions. The duration for denaturation may be less than or equal to about 300 seconds, 240 seconds, 180 seconds, 120 seconds, 90 seconds, 60 seconds, 55 seconds, 50 seconds, 45 seconds, 40 seconds, 35 seconds, 30 seconds, 25 seconds, 20 seconds, 15 seconds, 10 seconds, 5 seconds, 2 seconds, or 1 second.

**[0094]** Extension temperatures may vary depending upon, for example, the particular nucleic acid sample, the reagents used, and the reaction conditions. An extension temperature may be from about 30 °C to 80 °C, 35 °C to 75 °C, 45 °C to 65 °C, 55 °C to 65 °C, or 40 °C to 60 °C.

An extension temperature may be at least about 35 °C, 36 °C, 37 °C, 38 °C, 39 °C, 40 °C, 41 °C, 42 °C, 43 °C, 44 °C, 45 °C, 46 °C, 47 °C, 48 °C, 49 °C, 50 °C, 51 °C, 52 °C, 53 °C, 54 °C, 55 °C, 56 °C, 57 °C, 58 °C, 59 °C, 60 °C, 61 °C, 62 °C, 63 °C, 64 °C, 65 °C, 66 °C, 67 °C, 68 °C, 69 °C, 70 °C, 71 °C, 72 °C, 73 °C, 74 °C, 75 °C, 76 °C, 77 °C, 78 °C, 79 °C, or 80 °C.

**[0095]** Extension time may vary depending upon, for example, the particular nucleic acid sample, the reagents used, and the reaction conditions. In some embodiments, the duration for extension may be less than or equal to about 300 seconds, 240 seconds, 180 seconds, 120 seconds, 90 seconds, 60 seconds, 55 seconds, 50 seconds, 45 seconds, 40 seconds, 35 seconds, 30 seconds, 25 seconds, 20 seconds, 15 seconds, 10 seconds, 5 seconds, 2 seconds, or 1 second. In an alternative embodiment, the duration for extension may be no more than about 120 seconds, 90 seconds, 60 seconds, 55 seconds, 50 seconds, 45 seconds, 40 seconds, 35 seconds, 30 seconds, 25 seconds, 20 seconds, 15 seconds, 10 seconds, 5 seconds, 2 seconds, or 1 second. In an example, the duration for the extension reaction is less than or equal to about 10 seconds.

**[0096]** Nucleic acid amplification may include multiple cycles of thermal cycling. Any suitable number of cycles may be performed. The number of cycles performed may be more than about 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100 cycles, or more. The number of cycles performed may depend upon the number of cycles necessary to obtain detectable amplification products. For example, the number of cycles necessary to detect nucleic acid amplification during dPCR may be less than or equal to about 100, 90, 80, 70, 60, 50, 40, 30, 20, 15, 10, 5 cycles, or less. In an example, less than or equal to about 40 cycles are used and the cycle time is less than or equal to about 20 minutes.

**[0097]** The time to reach a detectable amount of amplification product may vary depending upon the particular nucleic acid sample, the reagents used, the amplification reaction used, the number of amplification cycles used, and the reaction conditions. In some embodiments, the time to reach a detectable amount of amplification product may be about 120 minutes or less, 90 minutes or less, 60 minutes or less, 50 minutes or less, 40 minutes or less, 30 minutes or less, 20 minutes or less, 10 minutes or less, or 5 minutes or less. In an example, a detectable amount of amplification product may be reached in less than 20 minutes.

**[0098]** In some embodiments, the ramping rate (i.e., the rate at which the chamber transitions from one temperature to another) is important for amplification. For example, the temperature and time for which an amplification reaction yields a detectable amount of amplified product may vary depending upon the ramping rate. The ramping rate may impact the time(s), temperature(s), or both the time(s) and temperature(s) used during amplification. In some

embodiments, the ramping rate is constant between cycles. In some embodiments, the ramping rate varies between cycles. The ramping rate may be adjusted based on the sample being processed. For example, optimum ramping rate(s) may be selected to provide a robust and efficient amplification method.

### **System for analyzing biological samples**

**[0099]** In another aspect, the present disclosure may provide systems for processing a biological sample. The system may include a device (e.g., microfluidic device), a holder, and a fluid flow channel. The device may include a fluid flow path and a chamber. The fluid flow path may include a channel and an inlet port. The fluid flow path may not include an outlet port. The inlet port may be configured to direct a solution comprising the biological sample into the channel. The chamber may be in fluid communication with the channel. The chamber may be configured to receive at least a portion of the solution comprising the biological sample from the channel and retain the solution during processing. The holder may be configured to receive and retain the device during processing. The fluid flow module may be configured to fluidically couple to the inlet port and supply a pressure differential to subject the solution to flow from the inlet port to the channel. Additionally, the fluid flow module may be configured to supply a pressure differential to subject at least a portion of the solution to flow from the channel to the chamber.

**[00100]** The holder may be a shelf, receptacle, or stage for holding the device. In an example, the holder is a transfer stage. The transfer stage may be configured input the microfluidic device, hold the microfluidic device, and output the microfluidic device. The microfluidic device may be any device described elsewhere herein. The transfer stage may be stationary in one or more coordinates. Alternatively, or in addition to, the transfer stage may be capable of moving in the X-direction, Y-direction, Z-direction, or any combination thereof. The transfer stage may be capable of holding a single microfluidic device. Alternatively, or in addition to, the transfer stage may be capable of holding at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more microfluidic devices.

**[00101]** The fluid flow module may be a pneumatic module and/or a vacuum module. The fluid flow module may be configured to be in fluid communication with the inlet port(s) of the microfluidic device. The fluid flow module may have multiple connection points capable of connecting to multiple inlet port(s). The fluid flow module may be able to fill, backfill, and partition a single array of chambers at a time or multiple arrays of chambers in tandem. The fluid flow module may be a pneumatic module combined with a vacuum module. The

fluid flow module may provide increased pressure to the microfluidic device or provide vacuum to the microfluidic device.

**[00102]** The system may further comprise a thermal module. The thermal module may be configured to be in thermal communication with the chambers of the microfluidic devices. The thermal module may be configured to control the temperature of a single array of chambers or to control the temperature of multiple arrays of chambers. Each array of chambers may be individually addressable by the thermal module. For example, thermal module may perform the same thermal program across all arrays of chambers or may perform different thermal programs with different arrays of chambers. The thermal module may be in thermal communication with the microfluidic device and/or the chambers of the microfluidic device. The thermal module may heat or cool the microfluidic device. One or more surfaces of the microfluidic device may be in direct contact with the thermal module. Alternately, or in addition to, a thermally conductive material may be disposed between the thermal module and the microfluidic device. The thermal module may maintain the temperature across a surface of the microfluidic device such that the variation is less than or equal to about 2 °C, 1.5 °C, 1 °C, 0.9 °C, 0.8 °C, 0.7 °C, 0.6 °C, 0.5 °C, 0.4 °C, 0.3 °C, 0.2 °C, 0.1 °C, or less. The thermal module may maintain a temperature of a surface of the microfluidic device that is within about plus or minus 0.5 °C, 0.4 °C, 0.3 °C, 0.2 °C, 0.1 °C, 0.05 °C, or closer to a temperature set point.

**[00103]** The system may further include a detection module. The detection module may provide electronic or optical detection. In an example, the detection module is an optical module providing optical detection. The optical module may be configured to emit and detect multiple wavelengths of light. Emission wavelengths may correspond to the excitation wavelengths of the indicator and amplification probes used. The emitted light may include wavelengths with a maximum intensity around about 450 nm, 500 nm, 525 nm, 550 nm, 575 nm, 600 nm, 625 nm, 650 nm, 675 nm, 700 nm, or any combination thereof. Detected light may include wavelengths with a maximum intensity around about 500 nm, 525 nm, 550 nm, 575 nm, 600 nm, 625 nm, 650 nm, 675 nm, 700 nm, or any combination thereof. The optical module may be configured to emit more than one, two, three, four, or more wavelengths of light. The optical module may be configured to detect more than one, two, three, four, or more wavelengths of light. One emitted wavelength of light may correspond to the excitation wavelength of an indicator molecule. Another emitted wavelength of light may correspond to the excitation wavelength of an amplification probe. One detected wavelength of light may correspond to the emission

wavelength of an indicator molecule. Another detected wavelength of light may correspond to an amplification probe used to detect a reaction within the chambers. The optical module may be configured to image sections of an array of chambers. Alternatively, or in addition to, the optical module may image an entire array of chambers in a single image. In an example, the optical module is configured to take video of the device.

**[00104]** FIG. 5 illustrates a system 500 for performing the process of FIG. 4 in a single system. The system 500 includes a fluid flow module 501, which may contain pumps, vacuums, and manifolds and may be moved in a Z-direction, operable to perform the application of pressure as described in FIGS. 1A-1F. System 500 may also include a thermal module 502, such as a flat block thermal cycler, to thermally cycle the microfluidic device and thereby cause the polymerase chain reaction to run. System 500 further includes an optical module 503, such as an epi-fluorescent optical module, which can optically determine which chambers in the microfluidic device have successfully run the PCR reaction. The optical module 503 may provide this information to a processor 504, which may use Poisson statistics to convert the raw count of successful chambers into a nucleic acid concentration. A holder 505 may be used to move a given microfluidic device between the various modules and to handle multiple microfluidic devices simultaneously. The microfluidic device described above, combined with the incorporation of this functionality into a single machine, may reduce the cost, workflow complexity, and space requirements for dPCR over other implementations of dPCR.

**[00105]** The system may further include a robotic arm. The robotic arm may move, alter, or arrange a position of the microfluidic device. Alternatively, or in addition to, the robotic arm may arrange or move other components of the system (e.g., fluid flow module or detection module). The detection module may include a camera (e.g., a complementary metal oxide semiconductor (CMOS) camera) and filter cubes. The filter cubes may alter or modify the wavelength of excitation light and/or the wavelength of light detected by the camera. The fluid flow module may comprise a manifold (e.g., pneumatic manifold) and/or one or more pumps. The manifold may be in an upright position such that the manifold does not contact the microfluidic device. The upright position may be used when loading and/or imaging the microfluidic device. The manifold may be in a downward position such that the manifold contacts the microfluidic device. The manifold may be used to load fluids (e.g., samples and reagents) into the microfluidic device. The manifold may apply a pressure to the microfluidic device to hold the device in place and/or to prevent warping, bending, or other stresses during

use. In an example, the manifold applies a downward pressure and holds the microfluidic device against the thermal module.

**[00106]** The system may further include one or more computer processors. The one or more computer processors may be operatively coupled to the fluid flow module, holder, thermal module, detection module, robotic arm, or any combination thereof. In an example, the one or more computer processors is operatively coupled to the fluid flow module. The one or more computer processors may be individually or collectively programmed to direct the fluid flow module to supply a pressure differential to the inlet port when the fluid flow module is fluidically coupled to the inlet port to subject the solution to flow from the inlet port to the channel and/or from the channel to the chamber(s) and, thereby, partition through pressurized out-gassing of the chambers.

**[00107]** The present disclosure is not to be limited in scope by the specific embodiments described herein. Indeed, other various embodiments of and modifications to the present disclosure, in addition to those described herein, will be apparent to those of ordinary skill in the art from the foregoing description and accompanying drawings.

**[00108]** For example, while described in the context of a dPCR application, other microfluidic devices which may require a number of isolated chambers filled with a liquid, that are isolated via a gas or other fluid, may benefit from the use of a thin thermoplastic film to allow outgassing to avoid gas fouling while also providing an advantage with respect to manufacturability and cost. Other than PCR, other nucleic acid amplification methods such as loop mediated isothermal amplification can be adapted to perform digital detection of specific nucleic acid sequences according to embodiments of the present disclosure. The chambers can also be used to isolate single cells with the siphoning apertures designed to be close to the diameter of the cells to be isolated. In some embodiments, when the siphoning apertures are much smaller than the size of blood cells, embodiments of the present disclosure can be used to separate blood plasma from whole blood.

**[00109]** The system described may be used with any device (e.g., microfluidic device) described elsewhere herein. Additionally, the system described may be used to implement any of the methods described elsewhere herein.

### **Computer systems**

**[00110]** The present disclosure provides computer systems that are programmed to implement methods of the disclosure. **FIG. 6** shows a computer system **601** that is programmed or

otherwise configured for processing and analyzing a biological sample (e.g., nucleic acid molecule). The computer system **601** can regulate various aspects of the systems and methods of the present disclosure, such as, for example, loading, digitizing, and analyzing a biological sample. The computer system **601** can be an electronic device of a user or a computer system that is remotely located with respect to the electronic device. The electronic device can be a mobile electronic device capable of or otherwise configured to monitor and control the biological analysis system.

**[00111]** The computer system **601** includes a central processing unit (CPU, also “processor” and “computer processor” herein) **605**, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system **601** also includes memory or memory location **610** (e.g., random-access memory, read-only memory, flash memory), electronic storage unit **615** (e.g., hard disk), communication interface **620** (e.g., network adapter) for communicating with one or more other systems, and peripheral devices **625**, such as cache, other memory, data storage and/or electronic display adapters. The memory **610**, storage unit **615**, interface **620** and peripheral devices **625** are in communication with the CPU **605** through a communication bus (solid lines), such as a motherboard. The storage unit **615** can be a data storage unit (or data repository) for storing data. The computer system **601** can be operatively coupled to a computer network (“network”) **630** with the aid of the communication interface **620**. The network **630** can be the Internet, an internet and/or extranet, or an intranet and/or extranet that is in communication with the Internet. The network **630** in some cases is a telecommunication and/or data network. The network **630** can include one or more computer servers, which can enable distributed computing, such as cloud computing. The network **630**, in some cases with the aid of the computer system **601**, can implement a peer-to-peer network, which may enable devices coupled to the computer system **601** to behave as a client or a server.

**[00112]** The CPU **605** can execute a sequence of machine-readable instructions, which can be embodied in a program or software. The instructions may be stored in a memory location, such as the memory **610**. The instructions can be directed to the CPU **605**, which can subsequently program or otherwise configure the CPU **605** to implement methods of the present disclosure. Examples of operations performed by the CPU **605** can include fetch, decode, execute, and writeback.

**[00113]** The CPU **605** can be part of a circuit, such as an integrated circuit. One or more other components of the system **601** can be included in the circuit. In some cases, the circuit is an application specific integrated circuit (ASIC).

**[00114]** The storage unit **615** can store files, such as drivers, libraries and saved programs. The storage unit **615** can store user data, e.g., user preferences and user programs. The computer system **601** in some cases can include one or more additional data storage units that are external to the computer system **601**, such as located on a remote server that is in communication with the computer system **601** through an intranet or the Internet.

**[00115]** The computer system **601** can communicate with one or more remote computer systems through the network **630**. For instance, the computer system **601** can communicate with a remote computer system of a user (e.g., laboratory technician, scientist, researcher, or medical technician). Examples of remote computer systems include personal computers (e.g., portable PC), slate or tablet PC's (e.g., Apple® iPad, Samsung® Galaxy Tab), telephones, Smart phones (e.g., Apple® iPhone, Android-enabled device, Blackberry®), or personal digital assistants. The user can access the computer system **601** via the network **630**.

**[00116]** Methods as described herein can be implemented by way of machine (e.g., computer processor) executable code stored on an electronic storage location of the computer system **601**, such as, for example, on the memory **610** or electronic storage unit **615**. The machine executable or machine-readable code can be provided in the form of software. During use, the code can be executed by the processor **605**. In some cases, the code can be retrieved from the storage unit **615** and stored on the memory **610** for ready access by the processor **605**. In some situations, the electronic storage unit **615** can be precluded, and machine-executable instructions are stored on memory **610**.

**[00117]** The code can be pre-compiled and configured for use with a machine having a processor adapted to execute the code, or can be compiled during runtime. The code can be supplied in a programming language that can be selected to enable the code to execute in a pre-compiled or as-compiled fashion.

**[00118]** Aspects of the systems and methods provided herein, such as the computer system **601**, can be embodied in programming. Various aspects of the technology may be thought of as “products” or “articles of manufacture” typically in the form of machine (or processor) executable code and/or associated data that is carried on or embodied in a type of machine readable medium. Machine-executable code can be stored on an electronic storage unit, such as memory (e.g., read-only memory, random-access memory, flash memory) or a hard disk. “Storage” type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software

programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry such waves, such as wired or wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible “storage” media, terms such as computer or machine “readable medium” refer to any medium that participates in providing instructions to a processor for execution.

**[00119]** Hence, a machine readable medium, such as computer-executable code, may take many forms, including but not limited to, a tangible storage medium, a carrier wave medium or physical transmission medium. Non-volatile storage media include, for example, optical or magnetic disks, such as any of the storage devices in any computer(s) or the like, such as may be used to implement the databases, etc. shown in the drawings. Volatile storage media include dynamic memory, such as main memory of such a computer platform. Tangible transmission media include coaxial cables; copper wire and fiber optics, including the wires that comprise a bus within a computer system. Carrier-wave transmission media may take the form of electric or electromagnetic signals, or acoustic or light waves such as those generated during radio frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media therefore include for example: a floppy disk, a flexible disk, hard disk, magnetic tape, any other magnetic medium, a CD-ROM, DVD or DVD-ROM, any other optical medium, punch cards paper tape, any other physical storage medium with patterns of holes, a RAM, a ROM, a PROM and EPROM, a FLASH-EPROM, any other memory chip or cartridge, a carrier wave transporting data or instructions, cables or links transporting such a carrier wave, or any other medium from which a computer may read programming code and/or data. Many of these forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution.

**[00120]** The computer system **601** can include or be in communication with an electronic display **635** that comprises a user interface (UI) **640** for providing, for example, processing parameters, data analysis, and results of a biological assay or reaction (e.g., PCR). Examples of UI's include, without limitation, a graphical user interface (GUI) and web-based user interface.

[00121] Methods and systems of the present disclosure can be implemented by way of one or more algorithms. An algorithm can be implemented by way of software upon execution by the central processing unit 605. The algorithm can, for example, regulate or control the system or implement the methods provided herein (e.g., sample loading, thermal cycling, detection, etc.).

### Examples

#### Example 1: A Microfluidic Device Comprising Multiple Processing Units

[00122] FIGS. 7A and 7B show an example of a microfluid device for processing a biological sample. The microfluid device 701 comprises a plurality of slides, for example, 4 slides as shown in FIG. 7B. The microfluid device 701 may contain at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more slides. A plurality of slides may be bonded to an automation compatible plate frame by welding. The plate frame may be a standard format plate frame with single inlet wells as shown in FIG. 7B. Other suitable methods may also be employed to bond the plurality of slides together. A single slide 702 (a four-unit array with about 20,000 partitions per unit) comprises a plurality of processing units, for example, 4 processing units as shown in FIG. 7A. Further, the slide 702 may include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more processing units.

[00123] Further, a single processing unit comprises about 20,000 chambers/partitions, and a single partition/chamber 704 is shown in FIG. 7A. Also, each processing unit contains a channel as illustrated in FIGS. 1A-1F. Each processing unit, as described herein, includes a single inlet port. The single chamber/partition 704 has a length of about 85  $\mu\text{m}$ , a width of about 65  $\mu\text{m}$ , and a height of about 100  $\mu\text{m}$ . Further, the single chamber/partition 704 may comprise a micro-channel 705, which has a depth of about 10  $\mu\text{m}$  and a width of about 15  $\mu\text{m}$ . As a result of each processing unit's dimension, for example, the processing unit 703, may comprise a total of analyzing volume of about 11.5  $\mu\text{L}$ . Furthermore, each processing unit contains less than 10% dead volume.

#### Example 2: Loading Biological Samples and Reaction Reagents into a Microfluidic Device

[00124] FIG. 8 shows microscope images of a single processing unit 801. FIG. 8 further includes a 650  $\mu\text{m}$  scale bar indicated by a solid line located at a corner of the microscope images. As shown by various configurations 802, 803, and 804 of main channels, a main channel 805 is configured to lead to a plurality of microchannels configured to connect to and be in fluid communication with one or more partitions/chambers.

[00125] For example, for loading a processing unit, a combination liquid of biological samples and reaction reagents is first flowed into the processing unit, followed by an immiscible

fluid, such as inert silicone oil. The immiscible fluid is configured to clear main channels of the combination liquid and isolate individual partition/chamber for PCR reactions, such as thermal cycling, and subsequent detection analysis.

**[00126]** Further, to resolve issues of overloading fluid, each processing unit may comprise one or more sacrificial chambers 806, which are configured to connect to and be in fluid communication with the main channel 805. Each main channel may connect to and be in fluid communication with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more sacrificial chambers. A sacrificial chamber may be of different shapes and may contain different volumes. When a processing unit is overloaded with fluid and some residual liquid remains in main channels, especially towards the end of the flow of the fluid, one or more neighboring partitions/chambers become in fluid communication with the overloading processing unit. Further, one or more target molecules contained in the overloading fluid may bleed into adjacent partitions/chambers during PCR amplification process. One or more sacrificial chambers 806 positioned in each processing unit are configured to capture excess fluid and enable the immiscible fluid to fully purge main channels. As a result, sacrificial chambers help to account for slight variation in loaded combination fluid (e.g., biological samples and reagent reagents) volume (e.g., the slight variation may be caused by a pipetting error). Sacrificial chambers 806 may also help to isolate PCR reactions in each partition/chamber so that signals generated from the PCR reactions may be accurately quantified.

### **Example 3: Digitize a Combination Fluid of Biological Samples and Reaction Reagents**

**[00127]** FIGS. 9A-9D show the loading and digitization of a combination fluid of biological samples and reaction reagents using a single inlet with any outlet. A pneumatic controller (not shown in FIGS. 9A-9D) is utilized and PCR reagent mix containing the common qPCR calibration dye ROX is used to visualize the digitization process.

**[00128]** The digitization process may be completed using either a single pressure step or multiple pressure steps. For example, to load each processing unit of the microfluidic device 701 (FIG. 7A), about 10  $\mu$ L of reaction reagent (include target nucleic acid molecules from a biological sample) is loaded (e.g., by pipetting) into an inlet well 706 (FIG. 7A) on the microfluidic device 701. Further, about 7  $\mu$ L of isolation buffer/silicone oil is loaded (e.g., by pipetting) into the inlet well 706 on the microfluidic device 701. Subsequently, using the pneumatic controller to apply about 50 PSI positive pressure to the inlet well 706 for about 60 minutes or until completion of the reagent digitization. The pressure of about 50 PSI may be ramped up gradually. The isolation buffer/silicone oil is configured to serve as an overlay liquid

and rest above the PCR reagents during the loading process ensuring the PCR reagents enter the microfluidic array first because it has a density lower than water. After the digitization process is completed, the partitions/chambers are fluidically isolated allowing them to perform independent PCR when activated.

**[00129]** Further, FIGS. 9A-9D show 4 different timepoints during the digitization process. FIG. 9A shows the first time point when the reagent first enters the array. FIG. 9B shows the second time point when the reagent has almost crossed the entire array and the loading channels in the array are now filled with reagents while the microchambers remaining largely empty. FIG. 9C shows the third time point when the reagent has entered the microchambers, although the chambers are still fluidically connected at this moment, and FIG. 9D shows the fourth time point after the depletion of reagent, oil moves through the array, displacing all the reagent in the main fluidic channels. The first row of FIGS. 9A-9D demonstrates fluorescence images of an entire array at 4 timepoints in the progression as reagent begins to fill the array, fills most of the main and loading channels, enters the microchambers, and gets displaced by silicone oil in the main channels only. The middle row of FIGS. 9A-9D shows a schematic representation of the microchamber array at the 4 above mentioned timepoints. The bottom row of FIGS. 9A-9D shows magnified images of the timepoint progression.

#### **Example 4: Integration of the Digitization Process into a Laboratory Workflow**

**[00130]** The digitization process can be easily integrated into common laboratory workflow for nucleic acid assays as illustrated by FIG. 10. Biological sample preparation (sample prep step) may be performed as in other PCR-based workflows that includes nucleic acid isolation and combining of Master Mix and primers/probes with the biological sample. The microfluid device 701 is loaded (load plate step) as described herein (e.g., pipetting of sample mixture followed by pipetting of an oil overlay) followed by being placed into an instrument that integrates pneumatic loading/digitization of the reagents, thermocycling and data/image acquisition (reagent partitioning + PCR + image acquisition step and analysis step). The acquired data of the PCR reaction can then be analyzed by software downstream to provide results such as concentration of target genes in the original biological sample.

#### **Example 5: Image Analysis**

**[00131]** FIG. 11 shows a screenshot of a user interface of software that performs analysis on acquired images. FIG. 11 demonstrates actual results from a dPCR assay with four different indicators (indicated by four different fluorescent colors) using human genomic DNA as the sample. The analysis settings displayed in panel 1101 are selectable by a user. The result from

one single processing unit out of sixteen processing units on the microfluid device 701 – in this case unit C3 – is shown in the form of scatter plots in panel 1104 and concentration values in panel 1103. The central image in panel 1102 is a composite image overlaying the positives from each of the four optical channels used to detect target genes. A fifth optical channel is used as a quality control channel.

**[00132]** While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

## CLAIMS

### WHAT IS CLAIMED IS:

1. A microfluidic device for processing a biological sample, comprising:  
a fluid flow path comprising a channel and an inlet port, wherein said fluid flow path does not include an outlet port, and wherein said inlet port is configured to direct a solution comprising said biological sample to said channel; and  
a chamber in fluid communication with said channel, wherein said chamber is configured to receive at least a portion of said solution from said channel and retain said at least said portion of said solution during said processing.
2. The microfluidic device of claim 1, further comprising a plurality of chambers in fluid communication with said channel, wherein said plurality of chambers comprises said chamber.
3. The microfluidic device of claim 1, wherein said channel comprises a first end and a second end, and wherein said first end and said second end are connected to a single inlet port.
4. The microfluidic device of claim 3, wherein said fluid flow path is circular.
5. The microfluidic device of claim 1, wherein said channel comprises a first end and a second end, and wherein said first end is connected to said inlet port and said second end is connected to a different inlet port.
6. The microfluidic device of claim 1, wherein said chamber is configured to permit pressurized off-gassing.
7. The microfluidic device of claim 6, wherein said chamber comprises a film or membrane that permits said pressurized off-gassing.
8. The microfluidic device of claim 7, wherein said film or membrane is a polymer film or membrane.
9. The microfluidic device of claim 8, wherein said polymer film or membrane does not comprise an elastomer.
10. The microfluidic device of claim 7, wherein said film or membrane has a thickness of less than about 100 micrometers ( $\mu\text{m}$ ).
11. The microfluidic device of claim 10, wherein said thickness is less than about 50  $\mu\text{m}$ .
12. The microfluidic device of claim 7, wherein said film or membrane is substantially impermeable to liquids.

13. The microfluidic device of claim 1, wherein said fluid flow path or said chamber does not include a valve.
14. The microfluidic device of claim 1, wherein a volume of said chamber is less than or equal to about 500 picoliters.
15. The microfluidic device of claim 1, wherein a volume of said chamber is less than or equal to about 250 picoliters.
16. The microfluidic device of claim 1, wherein said chamber has a cross-sectional dimension of less than or equal to about 250  $\mu\text{m}$ .
17. The microfluidic device of claim 1, wherein said chamber has a depth of less than or equal to about 250  $\mu\text{m}$ .
18. The microfluidic device of claim 1, further comprising a siphon aperture disposed between said channel and said chamber, wherein said siphon aperture is configured to provide fluid communication between said channel and said chamber.
19. The microfluid device of claim 1, further comprising a sacrificial chamber that is in fluid communication with said channel.
20. The microfluid device of claim 19, wherein said sacrificial chamber is configured to retain an excess portion of a solution comprising said biological sample.
21. A method for processing a biological sample, comprising:
  - (a) providing a device comprising (i) a fluid flow path comprising a channel and an inlet port, wherein said fluid flow path does not include an outlet port, and (ii) a chamber in fluid communication with said channel;
  - (b) directing a solution comprising said biological sample from said inlet port to said channel; and
  - (c) directing at least a portion of said solution from said channel to said chamber, which chamber retains said at least said portion of said solution during said processing.
22. The method of claim 21, wherein said device comprises a plurality of chambers in fluid communication with said channel, and wherein said plurality of chambers comprises said chamber.

23. The method of claim 21, further comprising applying a single pressure differential to said inlet port to direct said solution from said inlet port to said channel and from said channel to said chamber.
24. The method of claim 23, wherein said single pressure differential permits pressurized off-gassing of gas in said chamber.
25. The method of claim 21, further comprising applying a first pressure differential to said inlet port to direct said solution from said inlet port to said channel.
26. The method of claim 25, further comprising applying a second pressure differential to said inlet port to direct said solution from said channel to said chamber.
27. The method of claim 26, wherein said second pressure differential is greater than said first pressure differential.
28. The method of claim 26, wherein said second pressure differential permits pressurized off-gassing of gas in said chamber.
29. The method of claim 24 or claim 28, wherein said chamber comprises a film or membrane, and wherein said film or membrane permits pressurized off-gassing of said gas in said chamber.
30. The method of claim 21, wherein a volume of said solution is less than or equal to a volume of said chamber.
31. The method of claim 30, wherein said device partitions said solution comprising said biological sample into said chamber such that no residual solution remains in said channel.
32. The method of claim 21, further comprising providing an immiscible fluid to said inlet port and directing said immiscible fluid to said channel.
33. The method of claim 32, wherein a volume of said immiscible fluid is greater than a volume of said channel.
34. The method of claim 21, wherein said biological sample is a nucleic acid molecule.
35. The method of claim 34, further comprising amplifying said nucleic acid molecule by thermal cycling said chamber.
36. The method of claim 21, further comprising controlling a temperature of said channel or said chamber.

37. The method of claim 21, further comprising detecting one or more components of said biological sample or a reaction with said one or more components of said biological sample in said chamber.

38. The method of claim 37, wherein detecting said one or more components of said biological sample or said reaction comprises imaging said chamber.

39. A system for processing a biological sample, comprising:

a device comprising (i) a fluid flow path comprising a channel and an inlet port, wherein said fluid flow path does not include an outlet port, and wherein said inlet port is configured to direct a solution comprising said biological sample to said channel, and (ii) a chamber in fluid communication with said channel, wherein said chamber is configured to receive at least a portion of said solution from said channel and retain said at least said portion of said solution during said processing;

a holder configured to receive or retain said device during said processing; and

a fluid flow module configured to fluidically couple to said inlet port and supply a pressure differential to subject (i) said solution to flow from said inlet port to said channel and (ii) at least a portion of said solution to flow from said channel to said chamber.

40. The system of claim 39, wherein said device comprises a plurality of chambers in fluid communication with said channel, and wherein said plurality of chambers comprises said chamber.

41. The system of claim 39, wherein said chamber of said device is configured to permit pressurized off-gassing of a gas in said chamber when said fluid flow module applies said pressure differential to said inlet port.

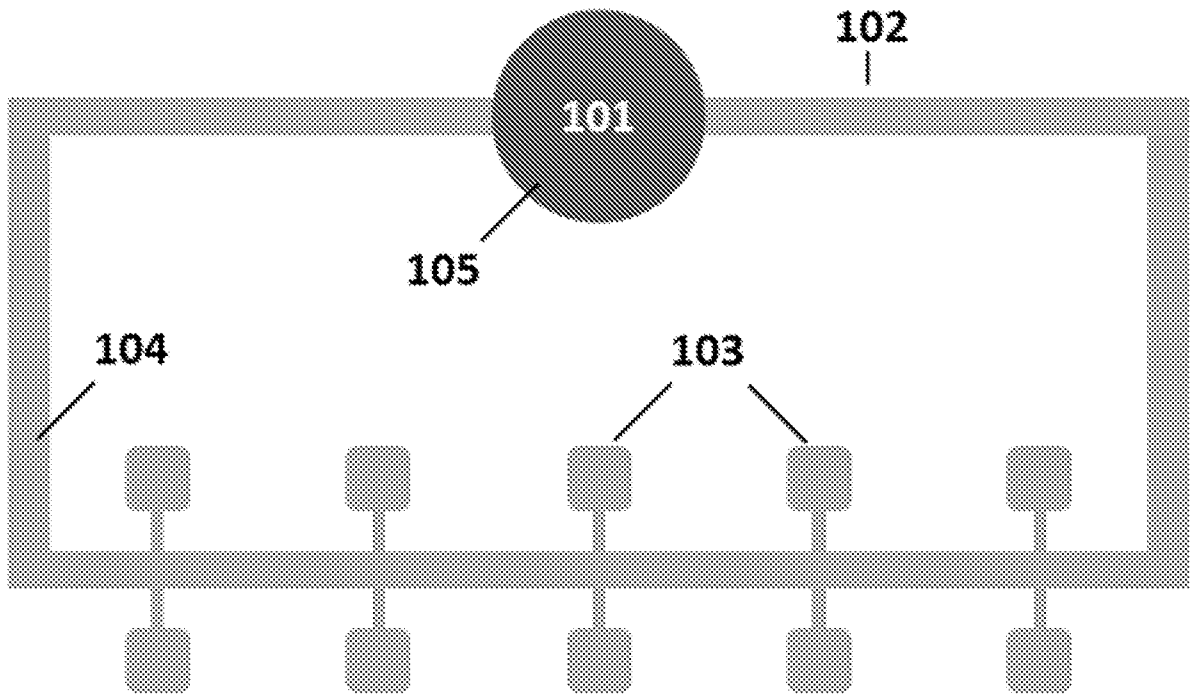
42. The system of claim 41, wherein said chamber comprises a film or membrane that is configured to permit said pressurized off-gassing.

43. The system of claim 39, further comprising one or more computer processors operatively coupled to said fluid flow module, wherein said one or more computer processors are individually or collectively programmed to direct said fluid flow module to supply said pressure differential when said fluid flow module is fluidically coupled to said inlet port, to thereby subject said solution to flow from said inlet port to said channel and direct said at least said portion of said solution from said channel to said chamber.

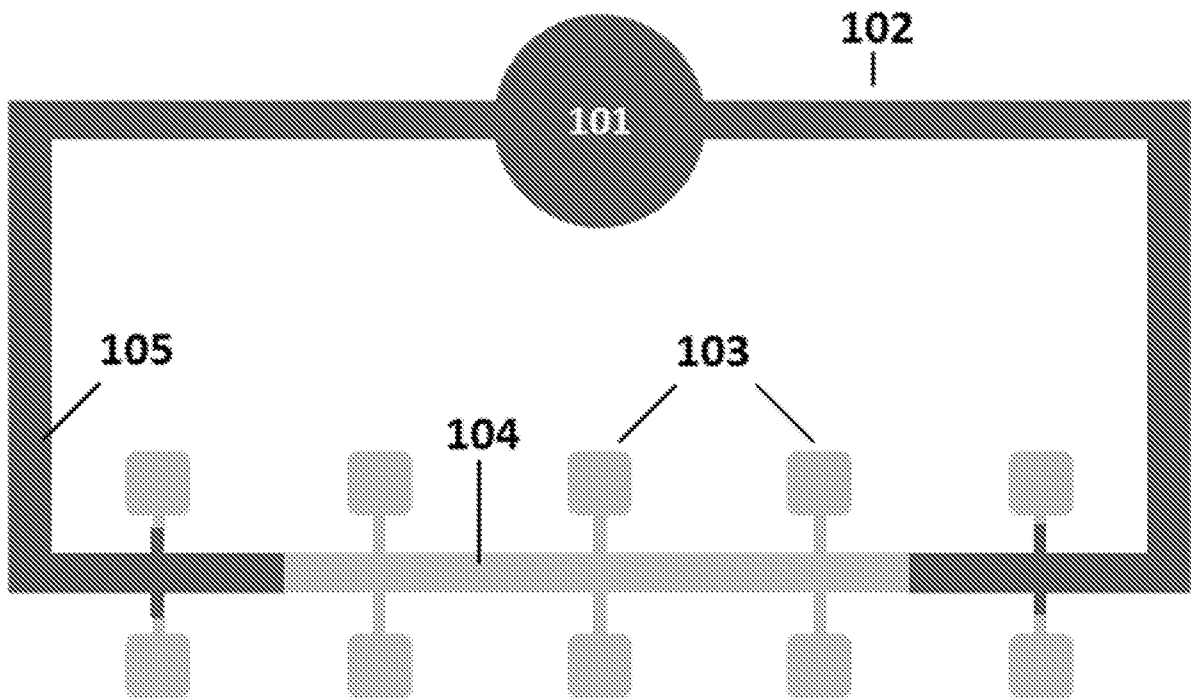
44. The system of claim 39, further comprising a thermal module in thermal communication with said chamber, wherein said thermal module is configured to control a temperature of said chamber during said processing.
45. The system of claim 39, further comprising a detection module in communication with said chamber, wherein said detection module is configured to detect a content of said chamber during said processing.
46. The system of claim 45, wherein said detection module is an optical module in optical communication with said chamber.
47. The system of claim 48, wherein said optical module is configured to image said chamber.
48. A system for processing a biological sample, comprising:  
a holder configured to retain a device comprising (i) a fluid flow path comprising a channel and an inlet port, wherein said fluid flow path does not include an outlet port, and (ii) a chamber in fluid communication with said channel; and  
one or more computer processors configured to be operatively coupled to said device when said device is retained by said holder, wherein said one or more computer processors are individually or collectively programmed to (i) direct a solution comprising said biological sample from said inlet port to said channel; and (ii) direct at least a portion of said solution from said channel to said chamber, which chamber retains said at least said portion of said solution during said processing.
49. The system of claim 48, further comprising a fluid flow module operatively coupled to said one or more computer processors, wherein said fluid flow module is configured to be operatively coupled to said device when said device is retained by said holder, and wherein said one or more computer processors are programmed to direct said fluid flow module to direct said solution from said inlet port to said channel.
50. The system of claim 48, further comprising a thermal module configured to be in thermal communication with said chamber when said device is retained by said holder, wherein said thermal module is configured to control a temperature of said chamber during said processing.
51. The system of claim 48, further comprising a detection module configured to be in communication with said chamber when said device is retained by said holder, wherein said detection module is configured to detect a content of said chamber during said processing.

52. The system of claim 51, wherein said detection module is an optical module in optical communication.
53. The system of claim 52, wherein said optical module is configured to image said chamber.

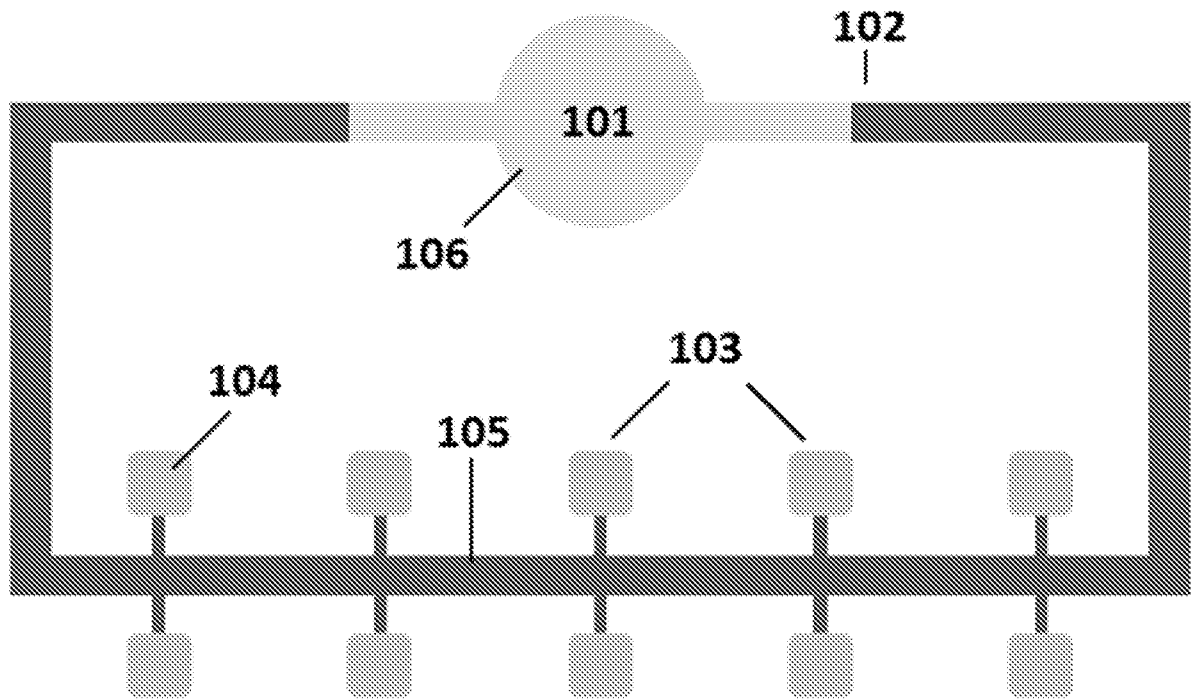
1/13



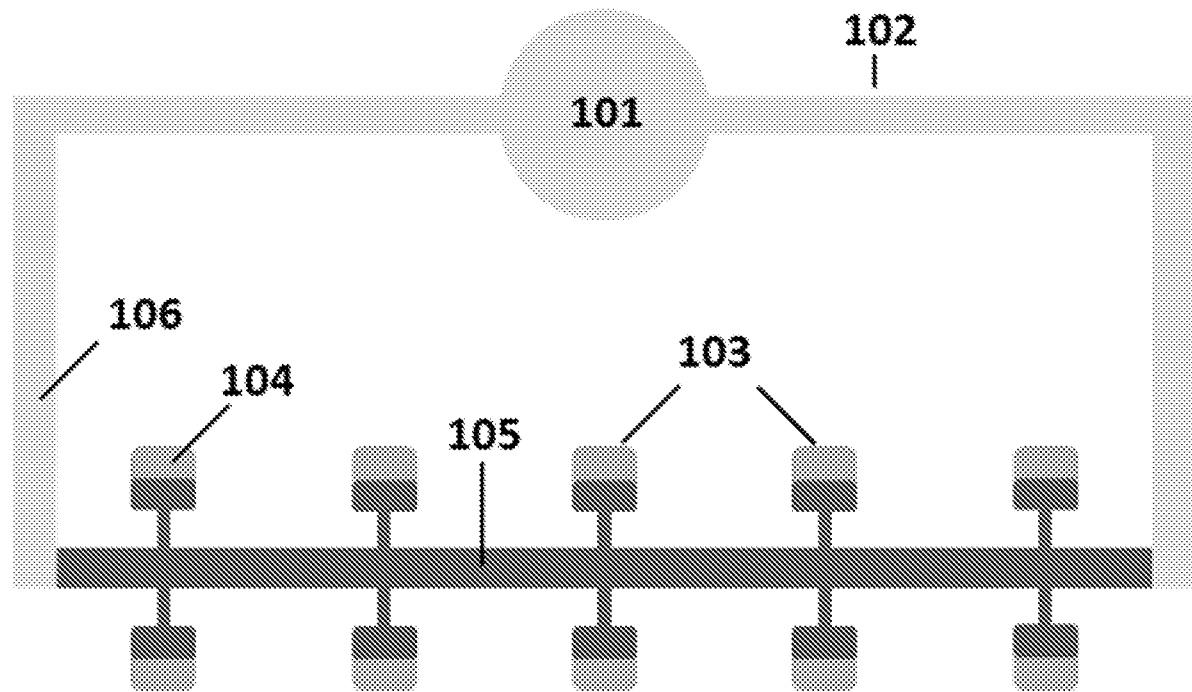
*FIG. 1A*



*FIG. 1B*



*FIG. 1C*



*FIG. 1D*

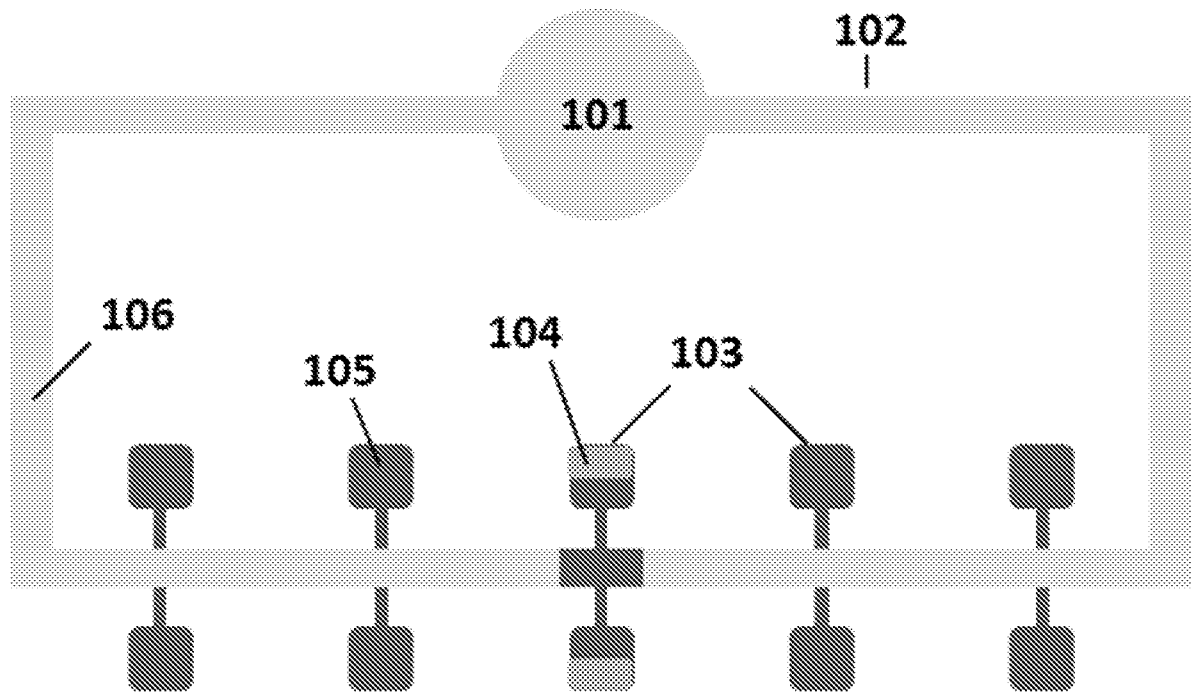


FIG. 1E

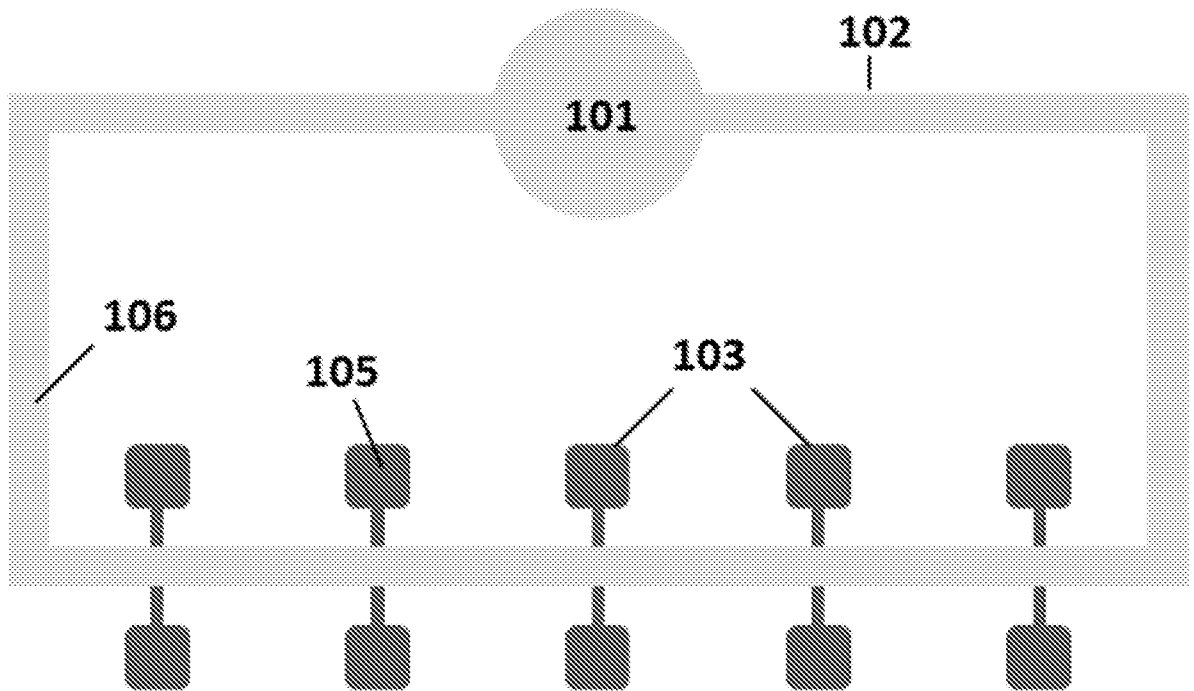
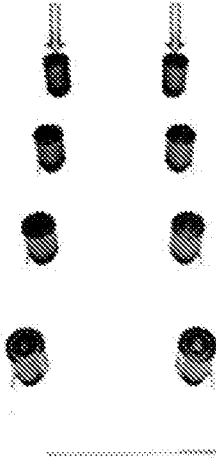
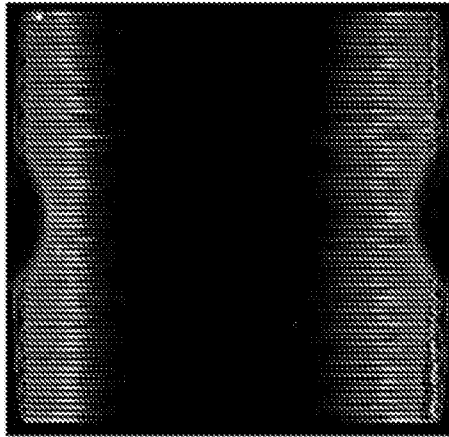


FIG. 1F

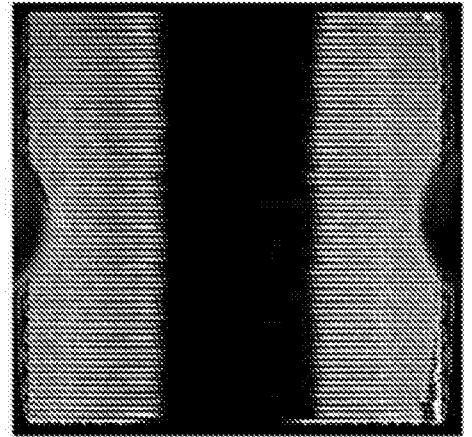
Input sample and  
oil into both  
inlet/outlet



Pressurize both inlet/outlet  
to load in reagents



Continue and fill from both  
side of the array

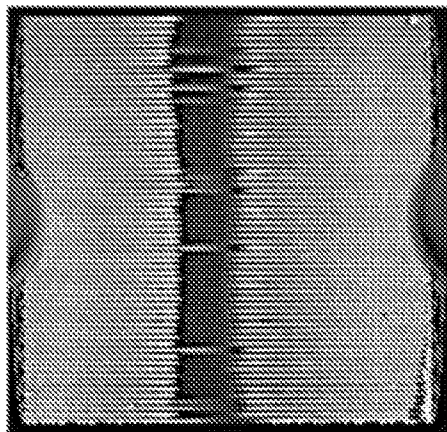


*FIG. 2A*

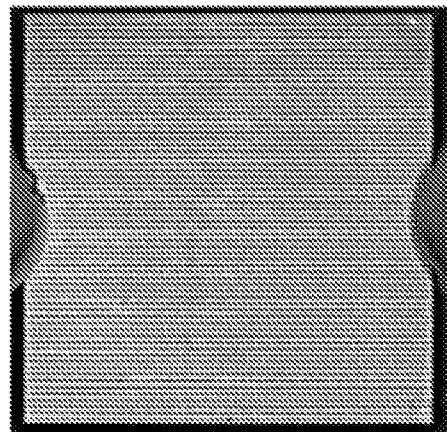
*FIG. 2B*

*FIG. 2C*

Continue and fill from both  
side of the array



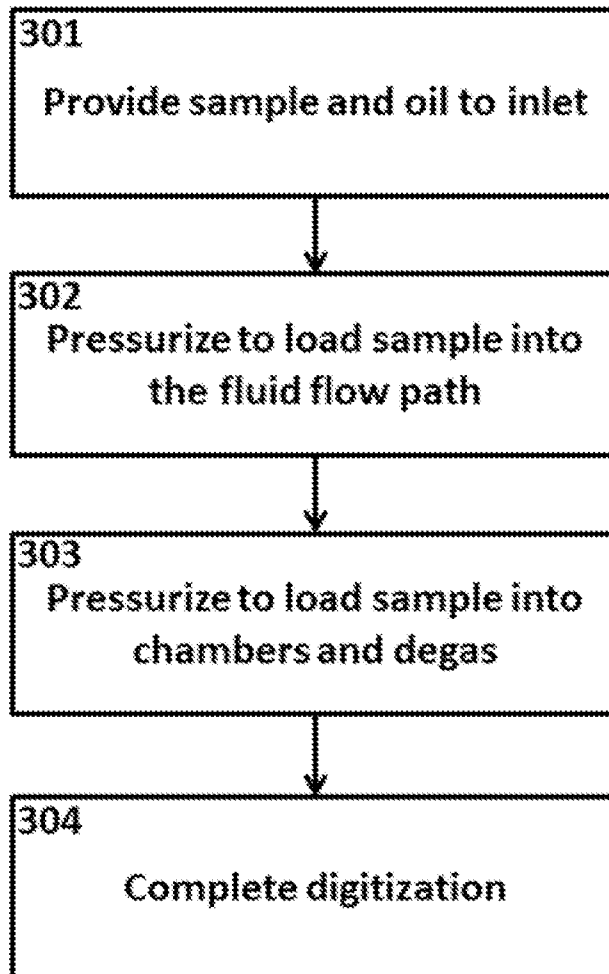
Complete loading and  
digitization once oil fills the  
channels



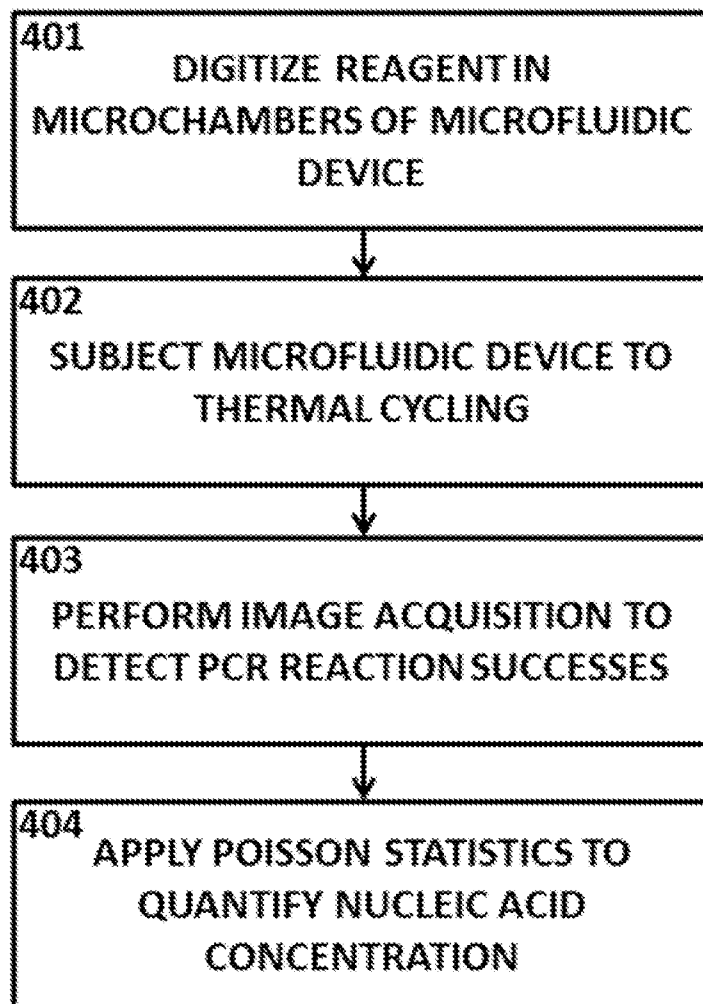
*FIG. 2D*

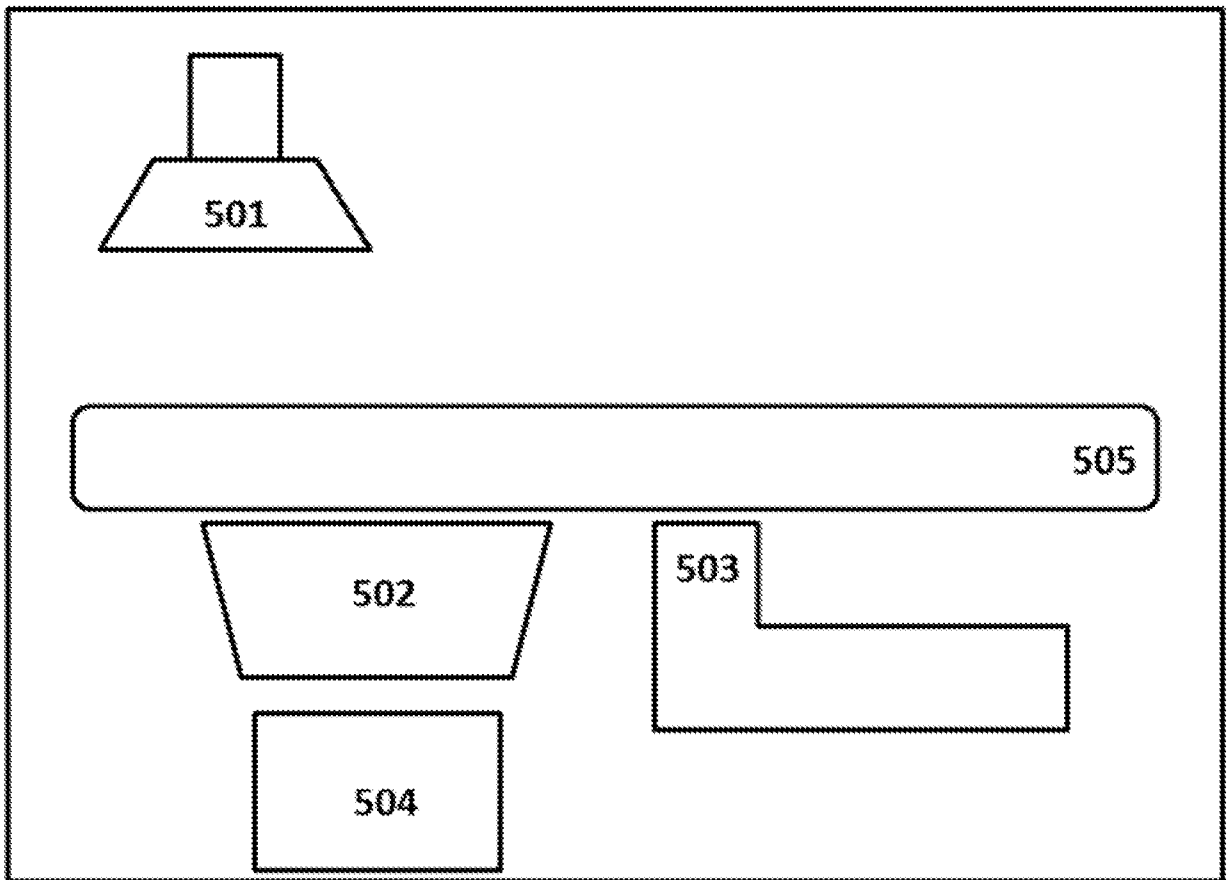
*FIG. 2E*

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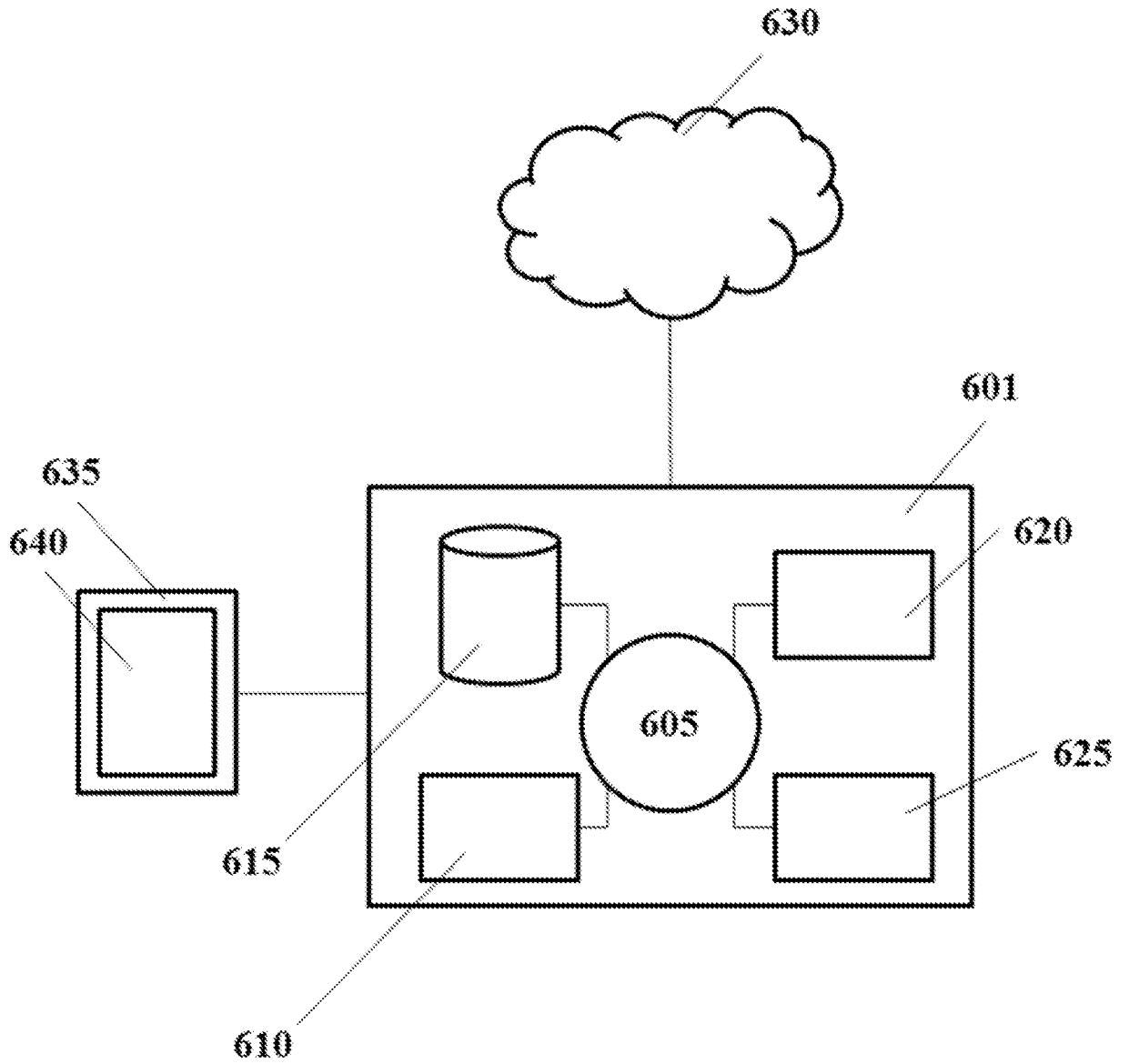
*FIG. 3*

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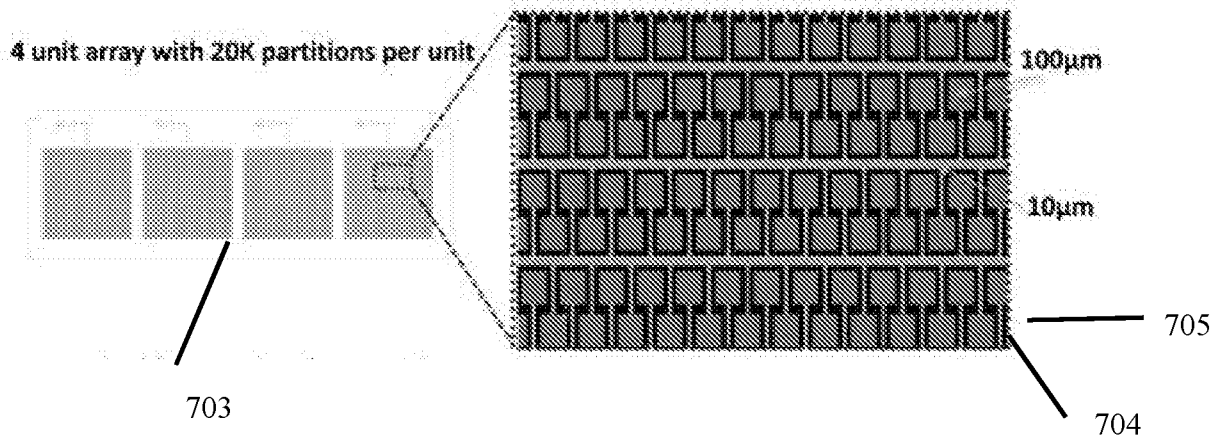
*FIG. 4*



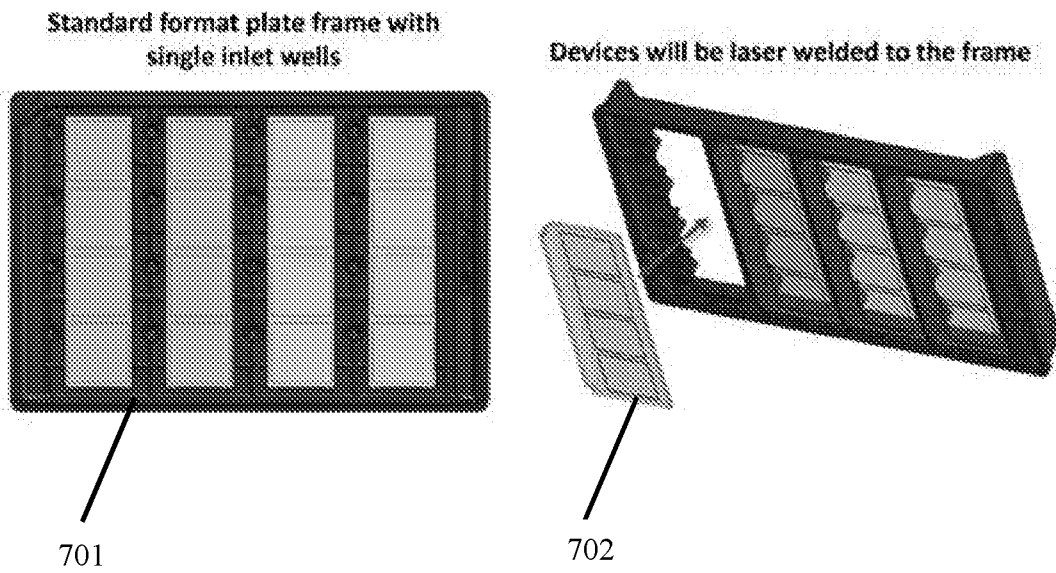
**FIG. 5**



**FIG. 6**



**FIG. 7A**



**FIG. 7B**

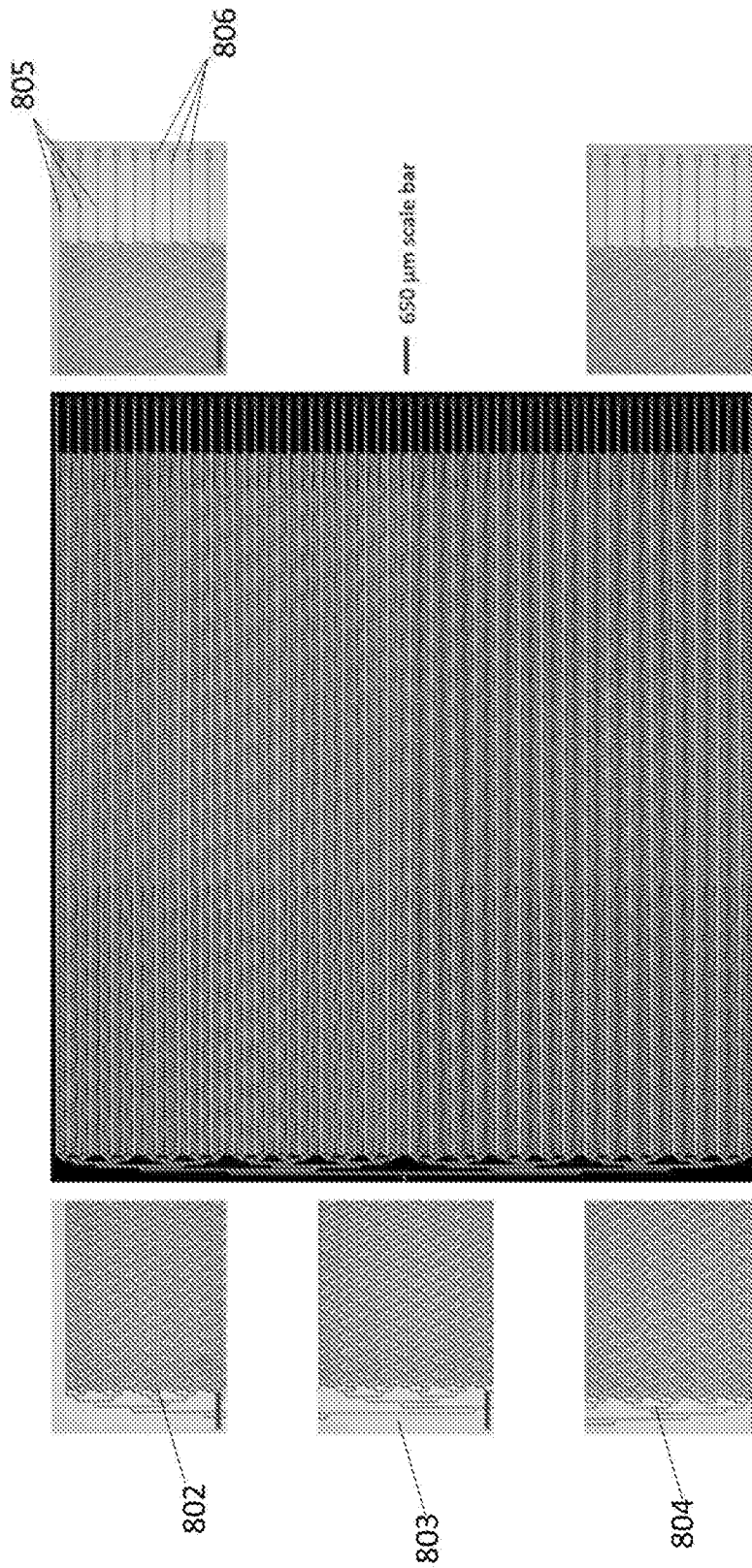


FIG. 8

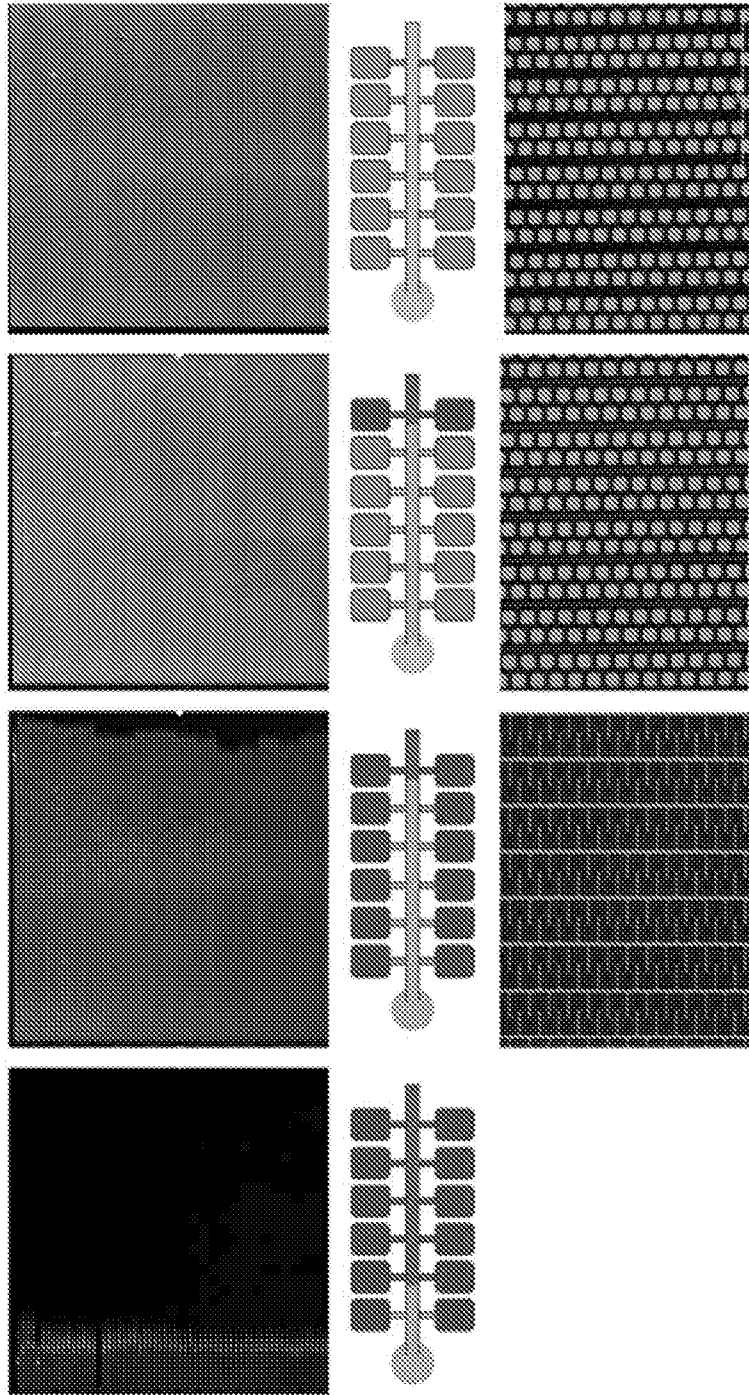
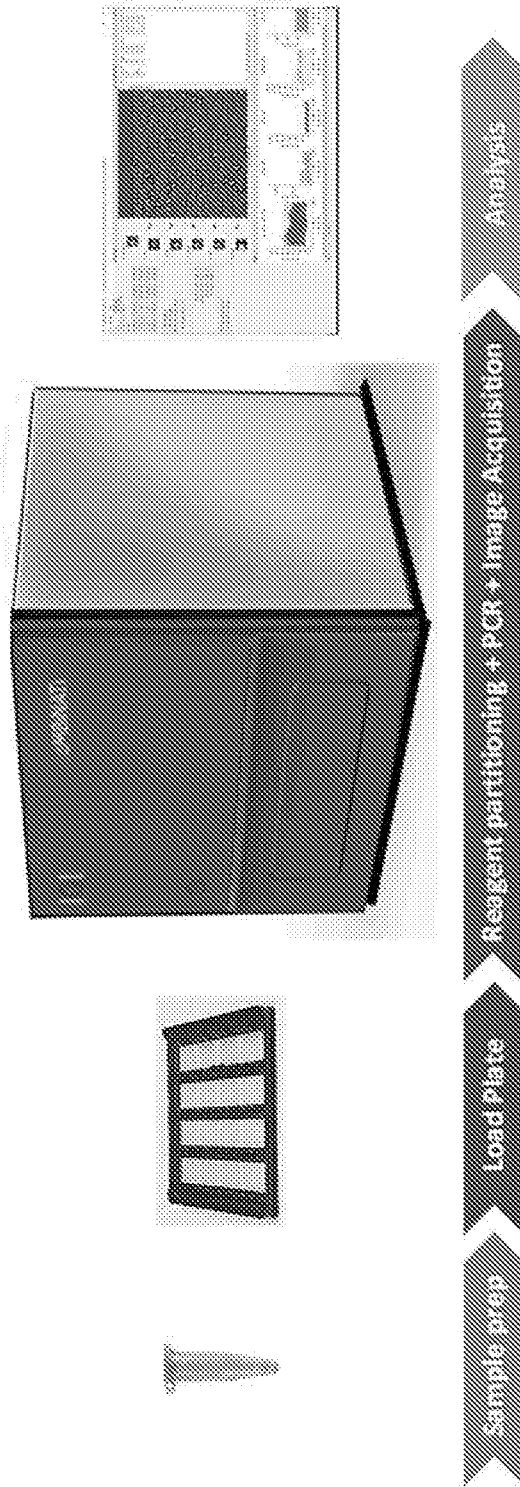


FIG. 9D

FIG. 9C

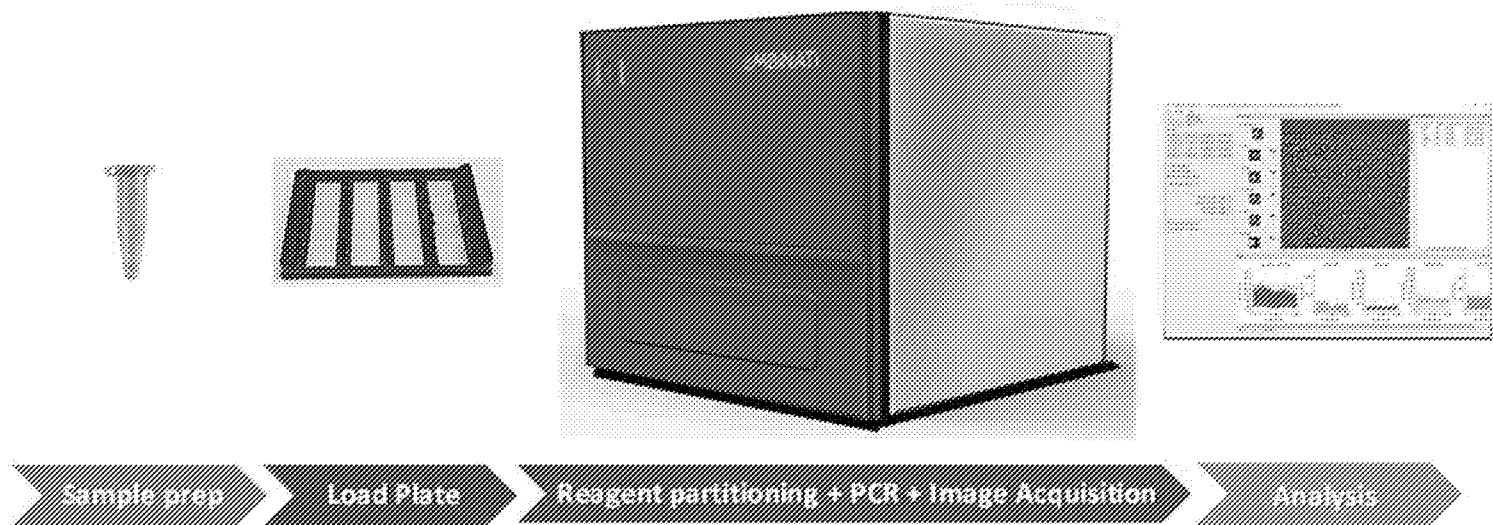
FIG. 9B

FIG. 9A



**FIG. 10**





*FIG. 10*