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(54) Title: SYSTEMS AND METHODS FOR CONTAINING BIOLOGICAL SAMPLES

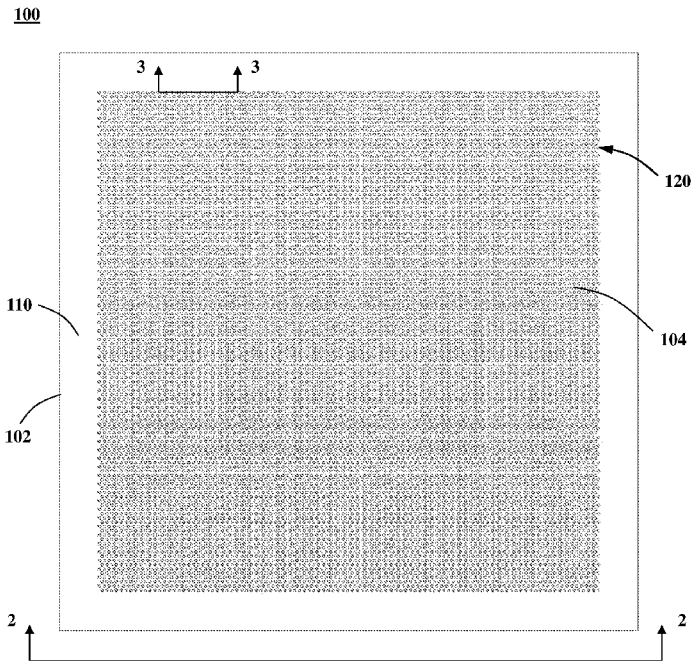

FIG. 1

(57) Abstract: An article for holding a plurality of biological samples includes a substrate a substrate comprising a first surface and an opposing second surface and a plurality of reaction sites in the substrate. Each of the reaction sites extends from an opening in the first surface to an opening in the second surface. The reaction sites comprise a hexagonal shape and are configured to provide sufficient surface tension by capillary action to hold respective biological samples. The reaction sites have a density over at least a portion of the surfaces that is at least 170 holes per square millimeter. At least one of the surfaces may have a surface roughness characterized by an arithmetic average roughness (Ra) that is less than or equal to 5 nanometers.

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Systems and Methods for Containing Biological Samples

Background of the Invention

Field of the Invention

The present invention relates generally to devices, systems, and methods for containing biological samples, and more specifically to devices, systems, and methods for containing biological samples in a plurality of reaction sites for assessment.

Description of the Related Art

The use of microtiter plates have been used for monitoring, measuring, and/or analyzing multiple biological and biochemical reactions during a single experiment or assay. Such plates are commonly used in sequencing, genotyping, polymerase chain reactions (PCR), and other biochemical reactions to monitor progress and provide quantitative data. For example, an optical excitation beam may be used during real-time PCR (qPCR) processes to illuminate fluorescent DNA-binding dyes or fluorescent probes to produce fluorescent signals indicative of the amount of a target gene or other nucleotide sequence. Increasing demands to provide greater numbers of reactions per experiment or assay have resulted in instruments that are able to conduct much large numbers of reactions simultaneously.

Newer approaches such as digital PCR (dPCR) have increased the demand for devices, systems, and methods involving ever greater numbers of reaction sites that are much smaller than those used in more traditional quantitative PCR (qPCR). There is a need for systems and sample formats that will provide reliable, high quality, data in high-density sample formats with sample sites having volumes on the order of nanoliters or picoliters or even smaller.

Brief Description of the Drawings

Embodiments of the present invention may be better understood from the following detailed description when read in conjunction with the accompanying drawings. Such embodiments, which are for illustrative purposes only, depict novel and non-obvious aspects of the invention. The drawings include the following figures:

Figure 1 is a top view of an article of manufacture according to an embodiment of the present invention.

Figure 2 is a side view of the article shown in Figure 1.

Figure 3 is a cross-sectional view of a portion of the article shown in Figure 1.

Figure 4 is a schematic representation of model of an embodiment of the present invention

Figure 5 is a schematic representation result using the model shown in Figure 4

Figure 6 is a representation of a pattern for distribution of reaction sites according to an embodiment of the present invention

Figure 7 is a representation of the geometric layout showing a comparison between circular reaction sites and hexagonal reaction sites.

Figure 8 is a cross-sectional view of a substrate according to an embodiment of the present invention.

Figure 9 is a perspective view of a portion of a substrate according to an embodiment of the present invention.

Figure 10 is a top view of an article of manufacture according to an embodiment of the present invention.

Figure 11 is a flow chart of a method according to an embodiment of the present invention.

Figure 12 is a cross-sectional view of a carrier and associated substrate according to an embodiment of the present invention.

Figure 13 is a top view of the carrier and substrate shown in Figure 12.

Figure 14 is a perspective view of a carrier according to an embodiment of the present invention.

Figure 15 is a perspective view of a carrier according to an embodiment of the present invention.

Figure 16 is a flow chart of a method according to an embodiment of the present invention.

Figure 17 is a schematic representation of a system according to an embodiment of the present invention.

Figures 18 is a front view of an article of manufacture according to an embodiment of the present invention.

Figures 19-20 are magnified front views of the article of manufacture shown in Figure 18.

Figure 21 is a front view of the article of manufacture shown in Figure 18 showing various dimensions of the article.

Figure 22 is a front view of an article of manufacture according to an embodiment of the present invention.

Figure 23 is a front views of the article of manufacture shown in Figure 22 showing various dimensions of the article.

Figure 24 is a flow chart illustrating a method of making an article of manufacture according to an embodiment of the present invention.

Figures 25A-C are a cross-sectional views of an embodiment of the article shown in FIGS. 18-21 illustrating an embodiment of the method shown in FIG. 24.

Figure 26 is an article of manufacture and associated case or holder according to an embodiment of the present invention.

Detailed Description of the Drawings

Embodiments of the present invention are generally directed devices, instruments, systems, and methods for monitoring or measuring a biological reactions for a large number of samples or solutions located at a plurality of reaction regions or reaction sites. Embodiments include the use of polymerase chain reaction (PCR) processes, assays, and protocols. While generally applicable to dPCR (digital PCR) or qPCR (real-time or quantitative PCR) where a large number of samples are being processed, it should be recognized that any suitable PCR method may be used in accordance with various embodiments described herein. Suitable PCR methods include, but are not limited to allele-specific PCR, asymmetric PCR, ligation-mediated PCR, multiplex PCR, nested PCR, quantitative or real-time PCR (qPCR), cast PCR, genome walking, bridge PCR, digital PCR (dPCR), or the like.

While embodiments of the present invention are generally directed to dPCR and qPCR, the present invention may be applicable to any PCR processes, experiment, assays, or protocols where a large number of samples or test volumes are processed, observed, and/or measured. In a dPCR assay or experiment according to embodiments of the present invention, a dilute

solution containing a relatively small number of at least one target polynucleotide or nucleotide sequence is subdivided into a large number of small test samples or volumes, such that at least some of these samples or volumes contains none of the target nucleotide sequence. When the samples are subsequently thermally cycled in a PCR assay, process, or experiment, individual samples containing one or more molecules of the target are amplified and produce a positive, detectable signal, while those containing none of the target(s) do not produce a signal, or a produce a signal that is below a predetermined threshold or noise level. Using Poisson statistics, the number of target nucleotide sequences in the original solution may be correlated to the number of samples producing a positive detection signal. In some embodiments, the detected signal may be used to determine a number, or number range, of target molecules contained in an individual sample or volume. For example, a detection system may be configured to distinguish between samples containing one target molecule and samples containing two or at least two target molecules. Additionally or alternatively, the detection system may be configured to distinguish between samples containing a number of target molecules that is at or below a predetermined amount and samples containing more than the predetermined amount. In certain embodiments, both qPCR and dPCR processes, assays, or protocols are conducted using a single device, instrument, or system.

In various embodiments, the devices, instruments, systems, and methods described herein may be used to detect one or more types of biological components or targets of interest that are contained in an initial sample or solution. These biological components or targets may be any suitable biological target including, but are not limited to, DNA sequences (including cell-free DNA), RNA sequences, genes, oligonucleotides, molecules, proteins, biomarkers, cells (e.g., circulating tumor cells), or any other suitable target biomolecule. In various embodiments, such biological components may be used in conjunction with one or more PCR methods and systems in applications such as fetal diagnostics, multiplex dPCR, viral detection, quantification standards, genotyping, sequencing assays, experiments, or protocols, sequencing validation, mutation detection, detection of genetically modified organisms, rare allele detection, and/or copy number variation.

According to embodiments of the present invention, one or more samples or solutions containing at least one biological target of interest may be distributed or divided between a

plurality of small sample volumes or reaction sites. The sample volumes or reaction sites disclosed herein are generally illustrated as through-holes located in a substrate material; however, where applicable, sample volumes or reaction sites according to embodiments of the present invention may include wells or indentations formed in a substrate, spots of solution distributed on the surface a substrate, or samples or solutions located within test sites or volumes of a microfluidic system, or within or on small beads or spheres.

In certain embodiments, a dPCR protocol, assay, process, or experiment included distributing or dividing an initial sample or solution into at least ten thousand reaction sites, at least a hundred thousand reaction sites, at least one million reaction sites, or at least ten million of reaction sites. Each reaction site may have a volume of a few nanoliters, about one nanoliter, or that is less than or equal to one nanoliter (e.g., less than or equal to 100 picoliters, less than or equal to 10 picoliters, and/or less than or equal to one picoliter). When the number of target nucleotide sequences contained in the initial sample or solution is very small (e.g., less than 1000 target molecules, less than 100 target, less than 10 target molecules, or only one or two target molecules), it may also be important in certain cases that the entire content, or nearly the entire content, of the initial solution be contained in or received by the sample volumes or reaction sites being processed. For example, where there are only a few target nucleotides present in the initial solution, some or all of these target nucleotide could potentially be contained in a small residual fluid volume that are not located in any of the reaction sites and, therefore, would not be detected, measured, or counted. Thus, efficient transfer of the initial solution may aid in reducing the chances or possibility of a miscalculation in the number count of a rare allele or target nucleotide or of failing to detect the presences at all a rare allele or target nucleotide if none of the target molecules are successfully located into one of the designated reaction sites. Accordingly, embodiments of the present invention may be used to provide a high loading efficiency, where loading efficiency is defined as the volume or mass of an initial sample or solution received within the reaction sites divided by the total volume or mass of the initial sample or solution.

Referring to FIGS. 1-3, in certain embodiments of the present invention, an article, device, substrate, slide, or plate 100 comprises a substrate 102 containing a plurality of partitions, through-holes, reaction regions, or reaction sites104 located in substrate 102. In

certain embodiments, article 100 may comprise a chip. Additionally or alternatively, article 100 may comprise a microfluidic device which, for example, may further include a plurality of channels or paths for transferring reagents and/or test solutions to reaction sites 104. In other embodiments, reaction sites 104 comprise a plurality of droplets or beads and article 100 may comprise one or more chambers and/or channels containing some or all of the droplets or beads 104. In such embodiments, droplets or beads 104 may form an emulsion, where some or all of droplets or beads 104 contain one or more target of at least one polynucleotide or nucleotide sequence. Where reaction sites 104 are beads, the beams may optionally include an attached optical signature or label. Droplets or beams 104 may be inspected, monitored, or measured either one at time or in groups containing one or more droplets or beads 104, for example using an imaging system according to embodiments of the present invention.

In the illustrated embodiment, article 100 comprises a first surface 110 and an opposing second surface 112. In the illustrated embodiment, each reaction site 104 extends from an opening 114 in first surface 110 to an opening 116 in second surface 112. While the illustrated embodiment shown in FIG. 3 shows a substrate containing through-holes 104, substrate 102 may additionally or alternatively comprise other types of reaction sites. For example, reaction sites 104 may include reaction volumes located within wells or indentations formed in substrate 102, spots of solution distributed on the surfaces 110 or 112, or other types of reaction chambers or formats, such as samples or solutions located within test sites or volumes of a microfluidic system, or within or on small beads or spheres.

Reaction sites 104 may be configured to provide sufficient surface tension by capillary action to draw in respective amounts of liquid or sample containing a biological components of interest. Article 100 may have a general form or construction as disclosed in any of USPN's 6,306,578; 7,332,271; 7,604,983; 7,6825,65; 6,387,331; or 6,893,877, which are herein incorporated by reference in their entirety as if fully set forth herein. Substrate 102 may be a flat plate or comprise any form suitable for a particular application, assay, or experiment. Substrate 102 may comprise any of the various materials known in the fabrication arts including, but not limited to, a metal, glass, ceramic, silicon, or the like. Additionally or alternatively, substrate 102 may comprise a polymer material such as an acrylic, styrene, polyethylene, polycarbonate, and polypropylene material. Substrate 102 and reaction sites 104 may be formed by one or

more of machining, injection molding, hot embossing, laser drilling, photolithography, or the like.

In certain embodiments, surfaces 110, 112 may comprise a hydrophobic material, for example, as described in US Patent Application Publication Numbers 2006/0057209 or 2006/0105453, which are herein incorporated by reference in their entirety as if fully set forth herein. In such embodiments, reaction sites 104 may comprise a hydrophilic material that attracts water or other liquid solutions. An array of such hydrophilic regions may comprise hydrophilic islands on a hydrophobic surface and may be formed on or within substrate 102 using any of various micro-fabrication techniques including, but are not limited to, depositions, plasmas, masking methods, transfer printing, screen printing, spotting, or the like.

It has been discovered that a high reaction site density may be configured to reduce the amount of a solution that is left on surface 110, 112 during a loading process, thus leading to higher loading efficiency or transfer of the initial solution. For example, by reducing ratio of the value of the spacing between adjacent well to the value of the well diameter, the amount of solution left on the surface of a plate may be significantly reduced so that, all, or nearly all, of an initial solution or sample containing biological components of interest is located inside reaction sites 104. In this way the possibility is reduced of missing a rare allele or other target molecule, since it would be less likely that one or more target molecule would remain on the substrate surface instead of being received in one of the designated reaction sites 104.

Referring to FIG. 4, this increase in loading efficiency was demonstrated with a computer model of a hydrophobic surface containing a plurality of hydrophilic reaction sites. The model was used to analyze the distribution of a sample into the plurality of reaction sites as a function of the reaction site pitch (or density) for through-holes having a diameter of 75 micrometers. FIG. 5 demonstrates that as the spacing between reaction sites is decreased (increased density), a greater percentage of an initial liquid sample is captured by the reaction sites, and a lesser amount of residual liquid is left behind on the hydrophobic surface after the loading process. Thus, a higher density of reaction sites 104 of a given cross-sectional dimension provides both an increase in the number of test samples for a given size substrate 102 and decreases or eliminates residual fluid left on surfaces 110, 112 (which may contain a rare allele or other target molecule of interest).

In certain embodiments, a lower bound in the spacing between adjacent reaction sites may exist, for example, due to optical limitations when reaction sites 104 are being imaged by an optical system. For example, the lower bound in spacing between adjacent reaction sites may exist because of limitations in the ability of the optical system to distinctly image adjacent reaction sites. To increase the density of reaction sites 104 in a substrate 102, a close-packed hexagonal matrix pattern may be used, for example, as illustrated in FIGS. 6 and 7.

It has been discovered that reaction sites having a non-circular cross-section may advantageously reduce an average distance or spacing between adjacent reaction sites 104, leading to a reduction in the amount of residual liquid or solution left behind on surfaces 110, 112 after loading of a test solution or sample. Referring to FIGS. 6 and 7, an array of hexagonal reaction sites 104 having a vertex-to-vertex diameter D are arranged in a hexagonal pattern in which the spacing or pitch between adjacent reaction sites is P. In certain embodiments, cross-talk between adjacent reaction sites in an optical system used to measure a fluorescence signal from the reaction sites 104 is a function of a minimum edge distance S between adjacent reaction sites. Thus, the geometry shown in FIG. 7 represents a minimum pitch P between reaction sites that can be used and still maintain the cross-talk between adjacent reaction sites at or below a predetermined value. A dash-lined circle is also shown in FIG. 7 inside each hexagon. This represents a circular reaction site of diameter D' having the same values of pitch P and the same edge spacing S as that of the hexagonal reaction site. The grayed portion in FIG. 7 shows the area between adjacent reaction sites over some width W for both the circular and hexagonal reaction sites. As is clearly seen in FIG. 7, the area between adjacent reaction sites over width W is greater for the circular reaction sites than between the hexagonal reaction sites, when the pitch P and the edge spacing S are the same. The modeling results discussed in regards to FIGS. 4 and 5 show that a smaller area between adjacent reaction sites lead to higher loading efficiency. Thus, based on the results illustrated in FIG. 7, a higher loading efficiency is provided, under the same spacing conditions (P and S), for a hexagonal shaped reaction site than for a circular reaction site.

This result also provides an unexpected advantage for an optical system configured to inspect the reaction sites. Since the minimum edge spacing S in FIG. 7 is the same for both the circular and hexagonal reaction sites, the cross-talk between adjacent reaction sites would be the

same or similar for either type of reaction site. However, the cross-sectional area of the hexagonal reaction sites is greater than that of the circular reaction sites, for the same pitch P and edge spacing S. Thus, the image produced by an optical system would have a greater area for hexagonal reaction sites than for circular reaction sites. Accordingly, the larger image produced by the hexagonal reaction site may potentially span a greater number of pixels. A greater number of pixels per reaction site aids in making a more accurate calculation of the signal produced a reaction site. Thus, in addition to providing a higher loading efficiency, the use of hexagonal reaction sites, as shown in FIGS. 6 and 7, may also produce more accurate measurement or calculation of an optical signal or output produce by each reaction site 104 (e.g., measurement or calculation of a fluorescence signal produced in proportion to an amount of a target or dye molecule).

In the illustrated embodiment shown in FIG. 1, article 100 has a square shape and an overall dimension of 15 millimeter by 15 millimeter . Article 100 also has an active area, region, or zone 120 with a dimension of 13 millimeter by 13 millimeter . As used herein, the term "active area", "active region", or "active zone" means a surface area, region, or zone of an article, such as the article 100, over which reaction sites or solution volumes are contained or distributed. In certain embodiments, the active area of article 100 may be increased to 14 millimeter by 14 millimeter or larger, for example on a 15 millimeter by 15 millimeter substrate dimension, in order to increase the total number of reaction sites contained on substrate 102. Article 100 may have other shapes and dimensions. For example, surfaces 110, 112 may be rectangular, triangular, circular, or some other geometric shape. The overall dimensions of article 100 and active area 120 may be smaller or larger than that for the illustrated embodiment in FIG. 1, depending on the particular design parameters for a given system, assay, or experiment.

In the illustrated embodiment of FIG. 1, reaction sites 104 may have a characteristic diameter of 75 micrometer and be distributed over active area 120 with a pitch of 125 micrometers between adjacent reaction sites. In other embodiments, reaction sites 104 have a characteristic diameter of that is less than or equal 75 micrometers, for example, a characteristic diameter that is less than or equal to 60 micrometers or less than or equal to 50 micrometers. In

other embodiments, reaction sites 104 have a characteristic diameter that is less than or equal to 20 micrometers, less than or equal to 10 micrometers, less than or equal to 1 micrometer, or less than or equal to 100 nanometers. The pitch between reaction sites may be less than 125 micrometers, for example, less than or equal to 100 micrometers, less than or equal to 30 micrometers, less than or equal to 10 micrometers, or less than or equal to 1 micrometer.

In certain embodiments, substrate 102 has a thickness between surface 110 and surface 112 that is equal to or about 300 micrometer, so that each reaction site 104 has a volume of about 1.3 nanoliters. Alternatively, the volume of each reaction site 104 may be less than 1.3 nanoliters, for example, by decreasing the diameter of reaction sites 104 and/or the thickness of substrate 102. For example, each reaction site 104 may have a volume that is less than or equal to 1 nanoliter, less than or equal to 100 picoliters, less than or equal to 30 picoliters, or less than or equal to 10 picoliters. In other embodiments, the volume some or all of the reaction site 104 is in a range from 1 nanoliter to 20 nanoliters.

In certain embodiments, the density of reaction sites 104 over surfaces 110, 112 is at least 100 reaction sites per square millimeter. Higher densities are also anticipated. For example, a density of reaction sites 104 over surfaces 110, 112 may be greater than or equal to 150 reaction sites per square millimeter, greater than or equal to 200 reaction sites per square millimeter, greater than or equal to 500 reaction sites per square millimeter, greater than or equal to 1,000 reaction sites per square millimeter, greater than or equal to 10,000 reaction sites per square millimeter, or greater than or equal to 1,000,000 reaction sites per square millimeter.

Advantageously, all the reaction sites 104 in active area 120 may be simultaneously imaged and analyzed by an optical system. In certain embodiments, active area 120 imaged and analyzed by the optical system comprises at least 12,000 reaction sites 104. In other embodiments, active area 120 imaged and analyzed by the optical system comprises at least 15,000, at least 20,000, at least 30,000, at least 100,000, at least 1,000,000 reaction sites, or at least 10,000,000 reaction sites.

In certain embodiments, reaction sites 104 comprise a first plurality of the reaction sites characterized by a first characteristic diameter, thickness, and/or volume, and a second plurality of the reaction sites characterized by a second characteristic diameter, thickness, and/or volume that is different than that of the corresponding the first characteristic diameter, thickness, or

volume. Such variation in reaction site size or dimension may be used, for example, to simultaneously analyze two or more different nucleotide sequences that may have different concentrations. Additionally or alternatively, a variation in reaction site 104 size on a single substrate 102 may be used to increase the dynamic range of a dPCR process, assay, or experiment. For example, article 100 may comprise two or more subarrays of reaction sites 104, where each group is characterized by a diameter or thickness that is different a diameter or thickness of the reaction sites 104 of the other or remaining group(s). Each group may be sized to provide a different dynamic range of number count of a target polynucleotide. The subarrays may be located on different parts of substrate 102 or may be interspersed so that two or more subarrays extend over the entire active area of article 100 or over a common portion of active area of article 100.

In certain embodiments, at least some of the reaction sites 104 are tapered over all or a portion of their walls. For example, referring to FIG. 8, at least some of reaction sites 104 may comprise a chamfer 130 at surface 110. Additionally or alternatively, at least some of reaction sites 104 may comprise a chamfer 130 at surface 112 (not shown). The use of chamfered and/or tapered reaction sites have been found to reduce the average distance or total area between adjacent reaction sites 104, yet without exceeding optical limitations for minimum spacing between solution sites or test samples. As discussed above in relation to FIG. 5, a decrease in the area between adjacent reaction sites 104 may result in a reduction in the amount liquid solution that is left behind on surfaces 110, 112 during a loading process. Thus, a higher sample loading efficiency may be obtained, while still maintaining a larger effective spacing between adjacent solution sites or test samples for the optical system.

In the embodiment shown in FIG. 9, an article, device, array, slide, or plate 100a includes an inactive area, region, or zone 132a that does not contain any reaction sites 104a. The inactive area may be a peripheral zone that surrounds the active zone containing reaction sites 104a. Alternatively, the inactive area may comprise an area that boarders the active zone on one, two, or more sides or zones. In the illustrated embodiment shown in FIG. 9, article 100a has a thickness equal to, or about equal to, 0.3 millimeter and the distance from the edge of the inactive area to the active area is equal to, or about equal to, 1 millimeter; however, other dimensions may be used. In the illustrated embodiment shown in FIG. 9, reaction sites 104a

have a diameter that is equal to, or about equal to, 0.075 millimeter and a pitch spacing that is equal to, or about equal to, 0.100 millimeter; however, other dimensions may be used. Where appropriate, features and/or dimensions discussed above in relation to article 100 may be incorporated into article 100a, or vice versa.

Referring to FIG. 10, in certain embodiments, an article, device, array, slide, or plate 100b includes an active area, region, or zone 120b comprising a plurality of reaction sites and an inactive area 132b, wherein inactive area 132b comprises a partition, divider, or separator, 134b that is located between adjacent active areas 120b. As illustrated in FIG. 10, inactive zone 132b may also include a peripheral zone that surrounds active zone 120b. The dimensions shown in FIG. 10 for the various features of article 100b are an example of a particular embodiment and may be different, depending on the requirements of a particular design. For example, partition 134b may have a thickness between active areas 120b that less than or equal to 500 micrometers, less than or equal to 1 millimeter, or less than or equal to 2 millimeters or 3 millimeters.

Partition 134b may be configured to aid in isolating the reaction sites in one active area, region, or zone from those in a separate active area, region, or zone. Such configurations may be used, for example, to facilitate the loading of a first sample in a first active area and a different second sample in a second active area, where the two areas are separated by partition 134b. In certain embodiments, the surface of active areas 120b and partition 134b are flush with one another on one or both faces of article 100b. Additionally or alternatively, at least a portion of partition 134b may be raised or offset from active areas 120b on one or both faces of article 100b. In other embodiments, at least a portion of partition 134b forms a trough relative to active areas 120b for one or both faces of article 100b. Where appropriate, features and/or dimensions discussed above in relation to articles 100, 100a may be incorporated into article 100b, or vice versa.

In certain embodiments, substrate 102 comprises a photostructurable material, such as certain glass or ceramic materials. In such embodiments, a method 140 shown in FIG. 11 may be used to fabricate substrate 102. Advantageously, last optional element of method 140 shown in FIG. 11 may be used to provide a substrate 102 that is opaque or nearly opaque, so that light emitted from one reaction site 104 does not enter an adjacent reaction site 104.

Method 140 may be used to provide a substrate 102 having an opacity sufficient prevent any, or nearly any, light emitted in one reaction site 104 from being transmitted into an adjacent reaction site 104. Method 140 may further comprise removing material from substrate 102 by an amount sufficient to reduce thickness between surfaces 110, 112, for example, removing material from substrate 104 by an amount sufficient to reduce the thickness between surfaces 110, 112 by at least 20 percent over an initial thickness or by at least 30 percent or 40 percent over an initial thickness. Method 140 may also include heating substrate 102 to a temperature of at least 500 degrees Celsius during fabrication. In certain embodiments, the patterned mask used in method 140 comprises a quartz plate with chrome pattern. The mask may be removed prior to exposing the at least portion of the substrate to the corrosive agent. The corrosive material used in method 140 may be hydrofluoric acid.

Referring to FIGS. 12 and 13, in certain embodiments, article 100 is housed within a carrier 150 comprising a first cover 152 having a bottom surface 154 and a second cover 156 having a top surface 158. Carrier 150 may further include one or more side walls 159 configured to maintain a predetermined spacing between the covers 152, 154. The covers 152, 154 and the walls 159 together form a cavity 160 sized to contain article 100. During use, article 100 is disposed within the cavity 160 formed between surfaces 154, 158. The thickness of cavity 160 may be greater than the thickness of article 100 such that there is a gap between article 100 and bottom surface 154 and/or between article 100 and top surface 158. As shown in the illustrated embodiment of FIG. 12, there may also be a gap between one or more of side walls 159. Additionally or alternatively, at a portion of article 100 may be attached to one or more of covers 152, 156 and one or more of side walls 159.

Carrier 150 may be made or formed from a metallic material, such as stainless steel, aluminum, copper, silver, or gold, or a semimetal such as graphite. Additionally or alternatively, all or portions of carrier 150 may be made of a non-metallic material including, but are not limited to, glass, acrylics, styrenes, polyethylenes, polycarbonates, and polypropylenes. In certain embodiments, at least one of the covers 152, 156 comprises a suitably transparent material for providing a window configured to allow optical access to and/or from reaction sites 104. Additionally or alternatively, the entire carrier 150 may be made of one or more transparent or nearly transparent materials.

Referring to FIG. 14, in certain embodiments, a carrier 150a comprises an aperture, port, or opening 162 that may be disposed generally perpendicular to a cover or optical access window 152a and sized to allow passage of article 100 into carrier 150a. Carrier 150a may further comprise a wiper or blade 164 disposed along at least one long edge of opening 162. Blade 164 may be configured to contact or engage at least one of surfaces 110, 112 of article 100 when article 100 is loaded into carrier 150a. Carrier 150a may further comprise a film or membrane (not shown) disposed over all or a portion of opening 162 that helps to seal cavity 160a and is pierced when article 100 is loaded into carrier 150a. In certain embodiments, the membrane and blade 164 form a single piece.

In certain embodiments, blade 164 is configured to aid in distributing sample fluid into some or all of reaction sites 104 as article 100 is inserted into carrier 150 through opening 162. For example, blade 164 may be configured to contact one or both surfaces 110, 112 during loading of article 100, so that liquid does not pass blade 164, but is instead pushed, and/or pulled by capillary forces, into reaction sites 104 as surface 110, 112 moves past blade 164. Additionally or alternatively, blade 164 may be configured to cover one or both surfaces 110, 112 of article 100 with a liquid, gel, or the like, for example to reduce or eliminate contamination and/or evaporation of sample fluid contained inside reaction sites 104.

Where appropriate, carrier 150a may incorporate any of the structures or features discussed above in relation to carrier 150, or vice versa.

Referring to FIG. 15, in certain embodiments, a carrier 150b comprises a body 170, which may include some or all of the structures and features of carrier 150 and/or carrier 150a. Carrier 150b further comprises a loader or insertion tool 172 for holding article 100, for aiding in loading article 100 into a body 170, and/or for loading a test solution into reaction sites 104. Tool 172 may have a U-shaped body, wherein article 100 is held inside the "U" prior to loading into body 170. Tool 172 may include tabs 174 on opposite arms 175 that are configured to engage or press into corresponding tabs or similar structure 176 of body 170.

Portions of cavity 160 between article 100 and surfaces 154, 158 may be filled with an immiscible fluid 170 (e.g., a liquid or a gel material) that does not mix with test solution contained in reaction sites 104 and configured to prevent or reduce evaporation of the test solution contained from reaction sites 104. One suitable fluid 170 for some applications is

Fluorinert, sold commercially by 3M Company. However, in certain embodiments, Fluorinert may be problematic for certain PCR applications due to its propensity to readily take up air that may be later released during PCR cycling, resulting in the formation of unwanted air bubbles.

Alternatively, in certain embodiments, it has been discovered that polydimethylsiloxane (PDMS) may be used in cavity 160 if the PDMS is not fully cross-linked. In such embodiment, PDMS has been found to have several characteristics that make it suitable for use with PCR, including low auto-fluorescing, thermal stability at PCR temperatures, and being non-inhibiting to polymerization processes. In addition, PDMS may contain an aqueous sample but be gas permeable to water vapor. A typical siloxane to cross linking agent used for general applications outside embodiments of the present invention is at a ratio of 10:1 (10 percent cross-linker) by weight.

It has been discovered that by under cross-linking a PDMS material, the resulting material can function as a suitable encapsulant for reducing evaporation, while also retaining the favorable attributes discussed above and associated with the fully cross linked material. More specifically, an under cross-linked PDMS material may be formed by using less than 10 percent of the cross-linker by weight. For example, a cross link level of less than or equal to 1% by weight has been shown to meet design requirements for certain PCR applications, such as for certain dPCR applications. Multiple dPCR responses have been demonstrated using a flat plate 100 that is encapsulated with an amount of cross-linker that is less than or equal to 0.8 percent by weight. Further, due to the higher viscosity of the under cross-linked PDMS material, as compared to Fluorinert, a PDMS encapsulant may also lend itself packaging requirements and customer workflow solutions.

Referring to FIG. 16, a method 200 of preparing a plurality of biological samples comprises providing a substrate of an article such as article 100, 100a, or 100b. Method 200 further comprises providing a carrier such as carrier 150, 150a, or 150b, and providing an insertion tool such as insertion tool 172, the insertion tool comprising a U-shaped body that includes a pair of arms configured to slideably engage the carrier. Method 200 also includes mounting or attaching the substrate to the insertion tool and the insertion tool to the carrier. In certain embodiments, the substrate is mounted or attached to the insertion tool, then the insertion tool and substrate together are mounted to the carrier. In other embodiments, the

insertion tool is mounted to the carrier without the substrate, then the substrate is later mounted to the insertion tool and/or the carrier.

Once the substrate is mounted or attached, method 200 includes sliding the insertion tool along the carrier by an amount sufficient to locate the substrate inside the carrier, for example, by inserting the substrate through an opening and/or membrane of the carrier. Using the method 200, a solution or sample may be applied to a face of the substrate in such a way that the solution is deposited or drawn into reaction sites or through-holes in the substrate as the substrate is inserted into the carrier. In addition, one of both surfaces of the substrate may be covered with a liquid or gel, for example, in order to protect the solution from contaminants and/or evaporation.

In certain embodiments, at least 99 percent of the liquid sample is received by at least some of reaction sites. In other embodiments, at least 99.5 percent or 99.9 percent of the liquid sample is received by at least some of reaction sites. In certain embodiments, the total volume of reaction sites 104 is selected to be greater than the volume of the liquid sample to be loaded into reaction sites 104. This has been found to increase the loading efficiency, which can be critical in certain circumstances, as discussed above. In certain embodiments, the ratio of the liquid volume sample to the total volume of all reaction sites 104 is less than or equal to 95 percent. In other embodiments, the ratio of the liquid volume sample to the total volume of all reaction sites 104 is less than or equal to 90 percent, less than or equal to 80 percent, or less than or equal to 70 percent. In certain embodiments, the value of this ratio depends on the percent of the total volume of each reaction site that is filled with liquid after loading. For example, if only 90 percent of each reaction site 104 contains liquid sample after loading, then the ratio of the liquid volume sample to the total volume of all reaction sites 104 may be less than or equal to 90 percent, less than or equal to 80 percent, less than or equal to 70 percent, or less than or equal to 60 percent.

Various methods and devices may be used to provide detection of one or more biological components of interest that are contained in reaction sites 104. For example, various fluorescent dyes may be incorporated into solutions containing one or more biological components of interest, which may then be detected using an optical system to determine the presence or amount of the one or more biological components. In other embodiments, the

presence of ions (positive or negative) may be detected and/or changes in pH, voltage, or current may be used to determine the presence or amount of one or more biological components of interest.

Referring to FIG. 17, a system 400 may be used to optically view, inspect, or measure one or more samples or solutions containing biological components of interest contained in reaction sites 104 of article 100. Article 100 may be contained in a carrier such as carrier 150, 150a, or 150b. System 400 comprises an optical head or system 402. System 400 further comprises a controller, computer, or processor 404 configured, for example, to operate various components of optical system 402 or to obtain and/or process data provided by system 400. For example, computer 404 may be used to obtain and/or process optical data provided by one or more photodetectors of optical system 402. In certain embodiments, processor 404 may transmit data to one or more computing systems for further processing. Data may be transmitted from processor 404 to the computing systems via an internet connection or some other network system.

In certain embodiments, system 400 further comprises a thermal control system 406 comprising, for example, a thermal cycler configured to perform a PCR procedure or protocol on at least some of the samples contained in article 100. Systems 402, 406 may combined or coupled together into a single unit, for example, in order to perform a qPCR and/or a dPCR procedure, assay, experiment, or protocol on at least some of the samples contained in article 100. In such embodiments, computer 404 may be used to control systems 402, 406 and/or to collect or process data provided or obtained by either or both systems 402, 406. Alternatively, thermal control system 406 may be completely separate from optical system 402 and/or from computer 404. In such embodiments, optical system 402 may be used to perform a dPCR or end-point PCR procedure on the samples contained in reaction sites 104 after thermal cycle has been performed on the samples using thermal control system 406 or some other thermal controller or thermal cycler. In certain embodiments, thermal control system 406 comprises a thermal cycler in which PCR is done using a traditional thermal cycler, isothermal amplification, thermal convention, infrared mediated thermal cycling, or helicase dependent amplification. In certain embodiments, at least a portion of thermal control system 406 may be integrated with or into article 100. For example, article 100 may include one or more heating

elements distributed along one or both surfaces 110, 112. Additionally or alternatively, at least portions of substrate 102 may be a heating element, for example, by being made of a material with an electrical resistance configured to provide resistive heating upon application of a voltage potential to substrate 102.

In certain embodiments, article 100 comprises an electronic chip comprising integrated circuits and semiconductor. In such embodiment, a detection system may also be integrated into the chip to determine the presence and/or quantity of a biological components of interest.

In certain embodiments, optical system 402 comprises a light source 410 and an associated excitation optic system 412 configured to illuminate at least some of samples contained in the reaction sites of article 100. Excitation optical system 412 may include one or more lenses 414 and/or one or more filters 416 for conditioning light directed to the samples. Optical system 402 may further comprise a photodetector 420 and an associated emission optic system 422 configured to receive optical data emitted by at least some of samples contained in the reaction sites of article 100. For example, when system 400 is configured to perform a qPCR and/or a dPCR assay or experiment, the sample may contain fluorescent dyes that provide a fluorescent signal that varies according to an amount of target nucleotide sequence contained in various of the reaction sites of article 100. Emission optical system 422 may include one or more lenses 424 and/or one or more filters 426 for conditioning light directed to the samples.

In the illustrated embodiment of FIG. 17, excitation/emission optical systems 412, 422 both comprise one or more common optical elements. For example, excitation/emission optical systems 412, 422 both comprise a beamsplitter 430 that reflects excitation light and transmits emission light from the samples to photodetector 420. In certain embodiments, excitation/emission optical systems 412, 422 both comprise a field lens (not shown) disposed between beamsplitter 430 and article 100, which may be used improve optical performance, for example, to provide more even illumination and reading of light to and from the samples contained in article 100. In certain embodiments, for example where even illumination is less critical (e.g., some dPCR applications), the common field lens may be omitted, as shown in the illustrated embodiment of FIG. 17. Omission of the field lens may help to reduce the size and complexity of optical system 402.

Photodetector 420 may comprise one or more photodiodes, photomultiplier tubes (PMTs), or the like. Such photodetectors may be used, for example, where optical system 402 is configured to scan individual reaction sites 104 or subsets of reaction sites 104. In other embodiments, photodetector 420 may comprise one or segmented detector arrays, for example, one or more CCD (charge coupled device) or CMOS (complementary metal-oxide semiconductor) arrays. Segmented detector arrays may be advantageously used where all or large groups of reaction sites 104 are simultaneously imaged or inspected. In order to provide a plurality of pixels per each reaction site, photodetector 420 may comprise at least 4,000,000 pixel or more than 10,000,000 pixels.

In certain embodiments, article 100 comprises an electronic chip comprising integrated circuits and semiconductor. In such embodiment, a detection system may also be integrated into the chip to determine the presence and/or quantity of a biological component of interest.

Referring to FIGS 18-21, in certain embodiments an article, device, array, slide, or plate 500 comprises a substrate 502 containing a plurality of through-holes or reaction sites 504 located in substrate 102. Substrate 502 comprises a first surface and an opposing second surface. In the illustrated embodiment, each reaction site 504 extends from an opening in the first surface to an opening in the second surface. As illustrated in FIGS. 19 and 20, reaction sites 504 may have a hexagonal shape and/or be arranged in a close-packed hexagonal matrix pattern. Alternatively, some or all of reaction sites 504 may have a shape, diameter, density, thickness, pitch spacing, or the like discussed above in relation to reaction sites 104. Article 500 further comprises one or more tabbed, cutout, or blank regions 506 in which no reaction sites 504 are present. As discussed below, blank regions 506 may be located in support regions for article 500. In the illustrated embodiment, blank regions define four semi-circular shape; however, other shapes and sizes are anticipated. In addition, article 500 may include a blank perimeter 508 in which no reaction sites are located.

In certain embodiments, substrate 502 comprise silicon, which may be configured to provide an even temperature distribution across article 500 during use. Alternatively, substrate 502 comprises a glass material, such as a photo-structured glass ceramic, or a metal, such as aluminum, copper, or stainless steel.

Referring to FIG. 19, reaction sites 504 may be arranged so as to define one or more dropout regions 509 located within the array of reaction sites 504. In some embodiments, drop regions 509 have a dimension suitable of viewing with an unaided eye (e.g., visible to the unaided eye without the use of a magnifying device). In the illustrated embodiment, article 500 comprises one dropout region 504 located in a first quadrant of article 500; however, multiple dropout regions on a single article 500 may be incorporated. One or more dropout regions 509 may define an overall shape that is longer along one axis than along an orthogonal axis, as illustrated in FIG. 19. Thus, the use of the single elongated dropout region 509 shown if FIG. 19 that is located away from a center of article 500 allows a uses to determine the orientation of article 500 (e.g., to determine which side is the front and back, and determine the proper orientation about an axis perpendicular to the page of FIGS. 18-20). Dropout regions 509 may also be configured to provide a reference signal, for example, a reference optical signal used during optical inspection of reaction sites 504.

In certain embodiments, a plurality of dropout regions 509 may be configured provide information about the article 500 based on, for example, the dropout shape(s), number of dropout regions 509, and/or the relative position of one dropout region 509 to another dropout region 509. For example, the number of dropout regions on a particular article 500 may be used to determined the diameter of reaction sites 504 and/or the distance between dropout regions 509, or the geometry of the dropout regions 509 to one another, may be used to determine the number of reaction sites 504 or the pitch between reaction sites 504. Many other combinations of dropout regions size, shape, and distribution are anticipated.

Referring to FIG. 21, article 500 may have an overall dimension of or about 10 mm by 10 mm. FIG. 21 also shows the value of other dimensions relevant to the particular embodiment shown in FIG. 21.

Referring to FIGS. 22 and 23, an article 500b may be configured similar to article 500 in FIG. 18, expect that article 500b also includes one or more landing regions 530 that may be size to provide more favorable loading properties. Thus, one or more edges of article 500b have wider zones without reaction sites 504 than other edges of article 500b.

Article 500 may incorporate, where appropriate, various of the elements and/or features discussed in relation to article 100, or vice versa. In addition, article 500 may be used in carrier

150 or other carriers according to embodiments of the current invention. Article 500 may be used in conjunction with system 400 or method 140 in ways similar to those in which article 100 has been disclose herein, as well as in or with other systems and methods disclosed herein in relation to article 100.

In certain embodiments, substrate 502 comprises silicon material and reaction sites 504 may comprise through-holes that are formed using a hexamethyldisilazane (HMDS) vapor coating process. Referring to FIG. 24, a method 600 may be used to form through-hole reaction sites 504 in the silicon substrate 502. Method 600 includes a process 605 comprising applying or forming a pattern on a first surface of a substrate material. Method 600 further includes a process 610 comprising using the pattern to etch a plurality of wells in the first surface of the substrate. Method 600 also includes a process 615 comprising removing material from a second surface that is opposite the first surface. Method 600 additionally includes a process 620 comprising etching and/or polishing the second surface. Method 600 further includes a process 625 comprising coating at least one of the surfaces, for example, to form a hydrophobic surface.

Referring to FIGS. 25A-C, method 600 may comprise a deep reactive-ion etching (DRIE) process to form reaction sites 504 in article 500. As seen in FIG. 25A, article 500 comprises a first surface 510 and an opposing second surface 512. FIG. 25A shows a mask 550 applied to second surface 512 in accordance with method 600. Using at etching process such as DRIE, mask 550 may be configured to form a plurality of wells 504' in second surface 512. For illustrative purposes, article 500 is shown in a horizontal orientation, with second surface 512 being located below first surface 510; however, it will be appreciated that during fabrication and/or use, second surface 512 may be located above first surface 510 and/or article 500 may have a different orientation, such as a vertical orientation. As seen in FIG. 25B, wells 504' do not penetrate through to first surface 510, but have a depth that is less than the thickness of article 500. Alternatively, etching process 610 may produce through-holes that completely penetrate the thickness of article 500. In such embodiments, further processing of first surface 510 may or may not be conducted in accordance with method 500.

Referring to FIG. 25C, first surface 510 may be further processed in accordance with process 615 of method 600 so as to reduce the thickness of article 500 by an amount sufficient to form through-holes 504 from wells 504'. First surface 512 may be further processed in

accordance with processes 620 and/or 625 of method 600 so as be prepare first surface 510 so that a sample or solution applied to first surface 510 is efficiently received by through-holes 504. Additionally or alternatively, processes 620 and/or 625 may be performed on second surface 512. In such embodiments, etching process 615 may be excluded altogether or may performed so that the final form of article 500 is a substrate with the plurality of wells 504' instead of the through-holes 504 shown in FIG. 25C.

In certain embodiments, processes 620 and/or 625 are performed so that at least one of the surfaces 510, 512 has a roughness that is below a predetermined value. For example, it has been found that after a solution or sample has been introduced into reaction sites 504, a residual thin film of the solution or sample may be left behind or later formed (e.g., during a PCR thermal cycling process) on first surface 510. This residual film may provide a "bridge" between adjacent or neighboring reaction sites 504. The bridging layer can result in contamination of one reaction site 504 by one or more adjacent or neighboring reaction sites 504. To solve this problem, it has been discovered when first surface 510 is polished and/or coated to have roughness is less than or equal to a predetermined value, this bridging problem can be solved or eliminated. For example, when article 500 comprises a silicon material and a reaction site geometry as shown in FIG. 20, it has been determined that the bridging problem is eliminated if the roughness of first surface 510 meets any of the following roughness criteria:

- Ra (arithmetic average): less than or equal to 5 nanometers.
- Rv (maximum valley depth): less than or equal to 15 nanometers.
- Rp (maximum peak height): less than or equal to 9 nanometers.
- Rt(maximum peak to trench): less than or equal to 24 nanometers.

In certain embodiments, articles 100 or 500 may be configured for use in any of the enclosures, housings, or cases disclosed in copending U.S. provisional application number 61/723,710, which is herein incorporated by reference in its entirety. For example, as shown in FIG. 26, article 500 may be arranged in an enclosure, housing, or case 700, according to an embodiment of the present invention and of provisional application number 61/723,710. Case 700 may comprise a base 702 and a cover or lid 704 configured to sealably engage base 702. Base 702 and cover 704 may be joined together to form a cavity or chamber 708, which may receive or contain a article 500. Article 500 may be part of base 702, or may be separate and/or

distinct from base 702 and be configured to be mounted or held by base 702. In the illustrated embodiment, tabs

Base 702 may comprise a plurality of bosses, tabs, staking sites, or support pads 720 (e.g., tabs 720a and 720b in the illustrated embodiment) that are configured to hold and/or locate article 500 within base 702 and cavity 708. One or more tabs 182 may be staked so that material from the tab is deformed or moved to hold article 500 firmly within base 702. Additionally or alternatively, article 500 may be glued to one or more tabs 182 using an adhesive, epoxy, or glue. In certain embodiments, gluing is used in conjunction with a glass or silicon article 500 in order to avoid possible cracking or damage to such holder materials, which might be induced by use of a crimping or holding force produced by tabs 720. In the illustrated embodiment, tabs 720a correspond with blank regions 506 of article 500. In certain embodiments, tabs 620a and blank regions 506 are large enough to provide proper support of article 500, but small enough so that the active area of corresponding article 500 provide a desired predetermined active area containing a predetermined number of reaction sites 504.

The above presents a description of the best mode contemplated of carrying out the present invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains to make and use this invention. This invention is, however, susceptible to modifications and alternate constructions from that discussed above which are fully equivalent. Consequently, it is not the intention to limit this invention to the particular embodiments disclosed. On the contrary, the intention is to cover modifications and alternate constructions coming within the spirit and scope of the invention as generally expressed by the following claims, which particularly point out and distinctly claim the subject matter of the invention.

Exemplary systems for methods related to the various embodiments described in this document include those described in following U.S. provisional patent applications:

U.S. provisional application number 61/612,087, filed on March 16, 2012; and

U.S. provisional application number 61/723,759, filed on November 7, 2012; and

U.S. provisional application number 61/612,005, filed on March 16, 2012; and

U.S. provisional application number 61/612,008, filed on March 16, 2012; and

U.S. provisional application number 61/723,658, filed on November 7, 2012; and

U.S. provisional application number 61/723,738, filed on November 7, 2012; and
U.S. provisional application number 61/659,029, filed on June 13, 2012; and
U.S. provisional application number 61/723,710, filed on November 7, 2012; and
U.S. provisional application number 61/774,499, filed on March 7, 2013; and
Life Technologies Docket Number LT00656 PCT, filed March 15, 2013; and
Life Technologies Docket Number LT00657 PCT, filed March 15, 2013; and
Life Technologies Docket Number LT00658 PCT, filed March 15, 2013; and
Life Technologies Docket Number LT00668 PCT, filed March 15, 2013; and
Life Technologies Docket Number LT00699 PCT, filed March 15, 2013.

All of these applications are also incorporated herein in their entirety by reference.

WHAT IS CLAIMED IS:

1. An article for holding a plurality of biological samples, the article comprising:

a substrate comprising a first surface and an opposing second surface; and

a substrate comprising a first surface and an opposing second surface; and

a plurality of reaction sites in the substrate, each of the reaction sites extending from an opening in the first surface to an opening in the second surface, the reaction sites being configured to provide sufficient surface tension by capillary action to hold respective biological samples;

wherein a density of reaction sites over at least a portion of one of surfaces is at least 170 holes per square millimeter.

2. The article of claim 1, wherein at least one of the surfaces has a surface roughness characterized by one or more of an arithmetic average roughness (Ra) that is less than or equal to 5 nanometers, a maximum valley depth roughness (Rv) that is less than or equal to 15 nanometers, a maximum peak height roughness (Rp) that is less than or equal to 9 nanometers, or a maximum peak to trench roughness (Rt) that is less than or equal to 24 nanometers.

3. The article of claim 1, wherein two or more of the reaction sites comprise a non-circular shape at one of the surfaces or at each of the surfaces.

4. The article of claim 1, wherein two or more of the reaction sites comprise a hexagonal shape at one of the surfaces or at each of the surfaces.

5. The article of claim 1, wherein the reaction site is a through-hole, a well, an indentation in one of the surfaces, a chemically modified spot on one of the surface, a hydrophilic spot on an otherwise hydrophobic surface.

6. The article of claim 1, wherein the substrate comprises a flat plate.

7. The article of claim 1, wherein each of the opposing surfaces comprises a hydrophobic material and two or more of the reaction sites comprise a wall comprising a hydrophilic material.

8. The article of claim 1, wherein the openings of each surface are arranged in a close-packed hexagonal matrix.

9. The article of claim 1, wherein the substrate has a thickness that is less than or equal to 300 micrometers.

10. The article of claim 1, wherein each of the plurality of reaction sites is characterized by a diameter that is less than or equal to 60 micrometers.

11. The apparatus of claim 1, wherein a reaction site diameter of at a first plurality of the reaction sites is different than a reaction site diameter of at a second plurality of the reaction sites.

12. The article of claim 1, wherein each of the walls of the two or more of the reaction sites is tapered between the surfaces.

13. The article of claim 12, wherein the taper is greater than 3 degrees.

14. The article of claim 1, wherein each of the walls of the two or more of the reaction sites includes a chamfer located at one of the surfaces or at each of the surfaces.

15. The article of claim 1, wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 10 nanoliter.

16. The article of claim 1, wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 1 nanoliter.

17. The article of claim 1, wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 100 picoliters.

18. The article of claim 1, wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 30 picoliters.

19. The apparatus of claim 1, wherein a reaction site volume of at a first plurality of the reaction sites is different than a reaction site volume of at a second plurality of the reaction sites.

20. The article of claim 1, wherein the density of reaction sites is at least 170 holes per square millimeter.

21. The article of claim 1, wherein the density of reaction sites is at least 200 holes per square millimeter.

22. The article of claim 1, wherein a minimum spacing between adjacent reaction sites of the plurality of reaction sites is less than or equal to 85 micrometers.

23. The article of claim 1, wherein surfaces of the substrate have a dimension of at least 14 millimeters by 14 millimeters, the surfaces defining an active area containing the plurality of reaction sites, the active area having an extent of at least 13 millimeters by 13 millimeters.

24. The article of claim 1, wherein the substrate contains at least 20,000 reaction sites.

25. The article of claim 1, wherein the substrate contains at least 30,000 reaction sites.

26. The article of claim 1, wherein the substrate contains at least 100,000 reaction sites.

27. The article of claim 1, wherein the substrate contains at least 1,000,000 reaction sites.

28. The article of claim 1, wherein the substrate comprises a photo-structured glass ceramic material.

29. The article of claim 1, wherein the substrate comprises a silicon material.

30. The apparatus of claim 1, further comprising a fiducial disposed on at least one of the surfaces.

31. An article for holding biological samples for analysis, the article comprising:

a substrate having a pair of opposing surfaces; and

a plurality of reaction sites in the substrate, each of the reaction sites extending from an opening in one of the opposing surfaces of the substrate to an opening in the other one of the opposing surfaces, the reaction sites being configured to provide sufficient surface tension by capillary action to hold respective biological samples;

wherein at least some of the reaction sites have a volume between the opposing surfaces that is less than or equal to 1 nanoliter.

32. An apparatus, comprising:

the article of claim 1; and

a carrier comprising:

a first cover comprising a bottom surface; and

a second cover comprising a top surface;

wherein the article is disposed between the top surface and the bottom surface;

wherein there is a space between the first surface of the substrate and the top surface;

wherein there is a space between the second surface of the substrate and the bottom surface.

33. The apparatus of claim 32, further comprising a gel material disposed within the spaces.

34. The apparatus of claim 32, further comprising a PDMS material disposed within the spaces.

35. The article of claim 32, wherein at least one of the covers comprise a window configure to provide optical access to the reaction sites.

36. The article of claim 32, wherein the carrier comprises an aperture disposed perpendicular to the surfaces and sized to allow passage of the substrate.

37. The article of claim 36, wherein the aperture comprises a piercable membrane that is broken when the substrate is moved into the carrier.

38. The apparatus of claim 32, further comprising:

an insertion tool comprising:

a U-shaped body configured to hold the article for loading into the carrier;

a pair of arms configured to slideably engage the carrier;

wherein the insertion tool is configured for transferring the article into the carrier.

39. The apparatus of claim 38, wherein the carrier comprises a wiper blade configured contact at least one of the surfaces of the article during loading of the article into the carrier.

40. A system comprising:

the apparatus of claim 32;

a light source configured to provide an excitation beam;

a photodetector configured to receive emission light from two or more of the reaction sites when the excitation beam interacts with a biological solution contained in the two or more of the reaction sites;

an optical system comprising an excitation optical system configured to deliver at least a portion of the excitation beam to the two or more of the reaction sites and emission optical system to deliver at least some of the emission light from the two or more of the reaction sites to the detector.

41. The system of claim 40, further comprising a thermal control unit to control a temperature of the substrate or within the substrate.

42. The article of claim 40, further comprising a microprocessor configure to provide one or more of:

control of the light source;

control of the photodetector;

control of an optical element of the optical system; or

control a temperature of one or more of the apparatus or a solution contained inside at least one of the reaction sites; or

processing of data obtained by the photodetector of .

43. The article of claim 40, wherein the photodetector comprises a CCD array or a CMOS array.

44. The article of claim 43, photodetector comprises at least 4,000,000 pixels.

45. The article of claim 43, photodetector comprises at least 10,000,000 pixels.

46. A method of making an article for holding biological samples for analysis, the method comprising:

providing a substrate comprising glass and having a first surface and an opposing second surface;

covering the glass with a mask comprising a pattern;

passing an ultraviolet beam through the mask and onto at least portions of the substrate;

exposing the at least portion of the substrate to a corrosive agent;

removing material to provide a plurality of reaction sites extending from one of the surfaces of the substrate to the other one of the surfaces;

subsequently exposing the substrate to an ultraviolet beam so as to change a characteristic of the glass;

47. The method of claim 46, wherein the glass is a photostructurable material.

48. The method of claim 46, wherein the characteristic is an opacity of the glass and the change is an increase in the opacity of the glass by an amount sufficient prevent light emitted in one reaction site of the plurality of reaction sites from being transmitted through the glass and into an adjacent reaction site of the plurality of reaction sites.

49. The method of claim 46, further comprising removing material from the substrate by an amount sufficient to reduce thickness between the surfaces of the substrate.

50. The method of claim 46, further heating the substrate with reaction sites to a temperature of at least 500 degrees Celsius.

51. The method of claim 46, further comprising removing material from the substrate by an amount sufficient to reduce thickness between the surfaces of the substrate by at least 30% over an initial thickness between the surfaces.

52. The method of claim 46, wherein the mask comprises a quartz plate with chrome pattern.

53. The method of claim 46, wherein the corrosive material is hydrofluoric acid.

54. The method of claim 46, further comprising removing the mask prior to exposing the at least portion of the substrate to the corrosive agent.

55. A method of making an article for holding biological samples for analysis, the method comprising:

providing a substrate comprising silicon and having a first surface and an opposing second surface;

applying a pattern to one of the surfaces;

etching a plurality of wells the first surface;

removing material from the second surface to form at least one through-hole from a corresponding well of the plurality of wells;

forming a surface roughness on at least one of the surfaces, the surface roughness characterized by one or more of an arithmetic average roughness (Ra) that is less than or equal to 5 nanometers, a maximum valley depth roughness (Rv) that is less than or equal to 15 nanometers, a maximum peak height roughness (Rp) that is less than or equal to 9 nanometers, or a maximum peak to trench roughness (Rt) that is less than or equal to 24 nanometers.

56. The method of claim 55, further comprising at least partially filling at least one of the at least one through-hole with a liquid solution containing a nucleotide sequence and a fluorescent dye.

57. A method of preparing a plurality of biological samples, the method comprising:

providing the article of claim 1;

providing a carrier comprising:

a first cover comprising a bottom surface; and

a second cover comprising a top surface;

an aperture sized to receive the article;

providing an insertion tool comprising:

a U-shaped body;

a pair of arms configured to slideably engage the carrier;

attaching the article within the U-shaped body;

sliding the insertion tool by an amount sufficient to dispose the article between the covers;

58. The method of claim 57, further comprising:

providing a wiper blade configured contact at least one of the surfaces of the article;

placing an amount of liquid onto one of the surfaces of the article, the liquid containing a biological sample to be deposited into the reaction sites of the article;

depositing the liquid into at least some of the plurality of reaction sites while sliding the insertion tool by wiping the wiper blade across the at least one of the surfaces.

59. The method of claim 57, further comprising removing the insertion tool from the article and the carrier.

60. The method of claim 57, further comprising attaching the insertion tool onto the carrier, then subsequently attaching the article within the U-shaped body.

61. The method of claim 57, further comprising attaching the insertion tool and the article together onto the carrier.

62. The method of claim 57, wherein at least 99 percent of the liquid sample is received by at least some of reaction sites.

63. The method of claim 57, wherein the at least 99 percent of the liquid sample is received by less than 95 percent of the reaction sites.

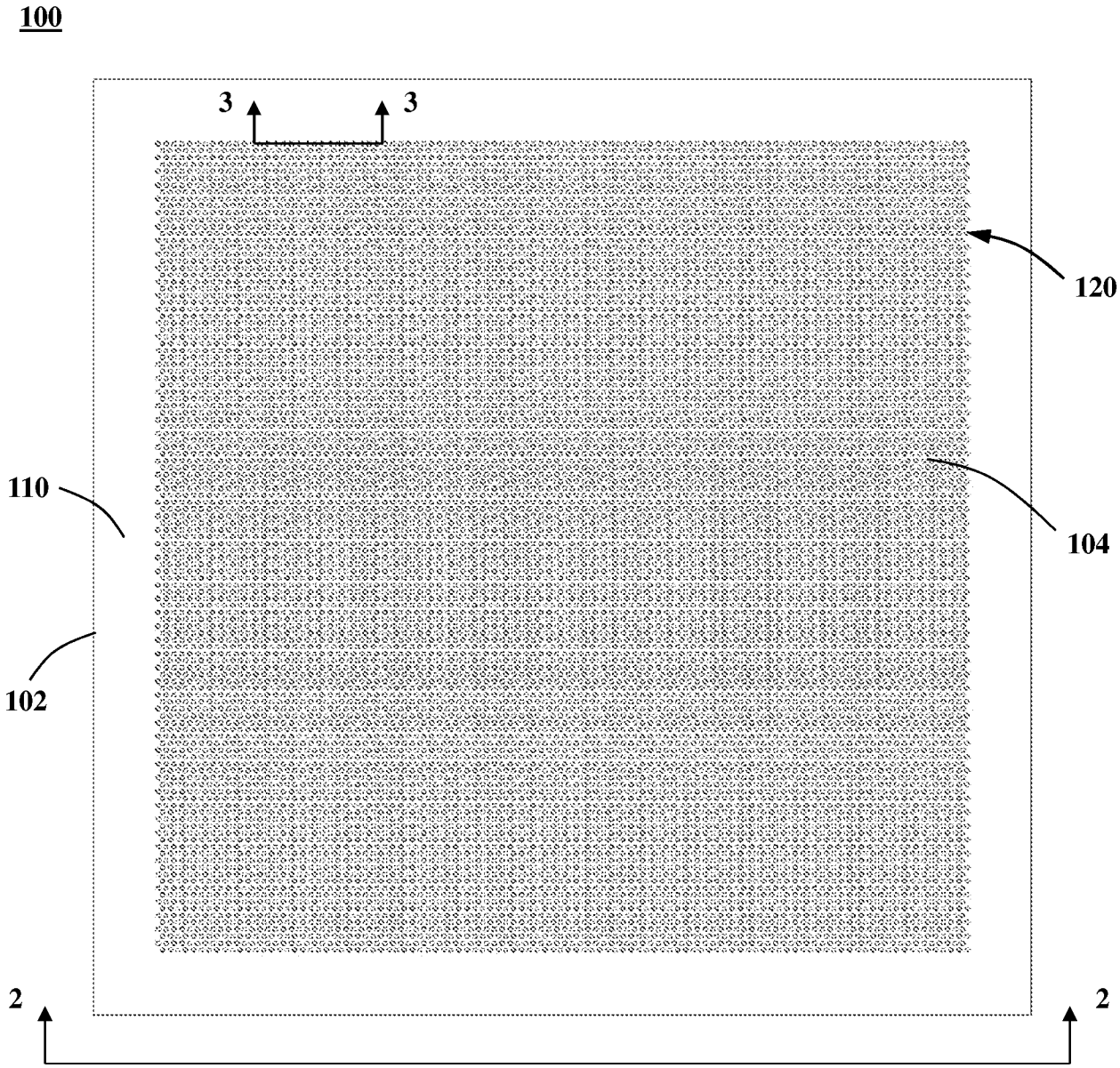


FIG. 1

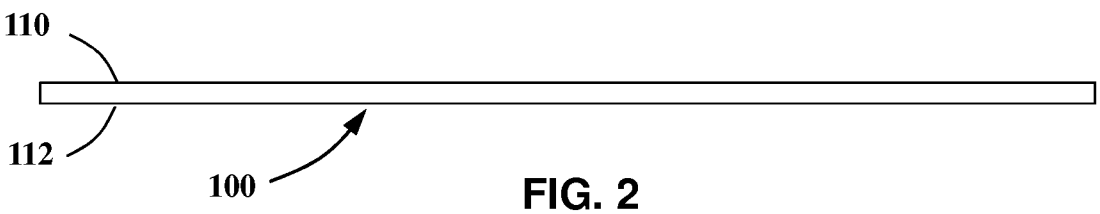


FIG. 2

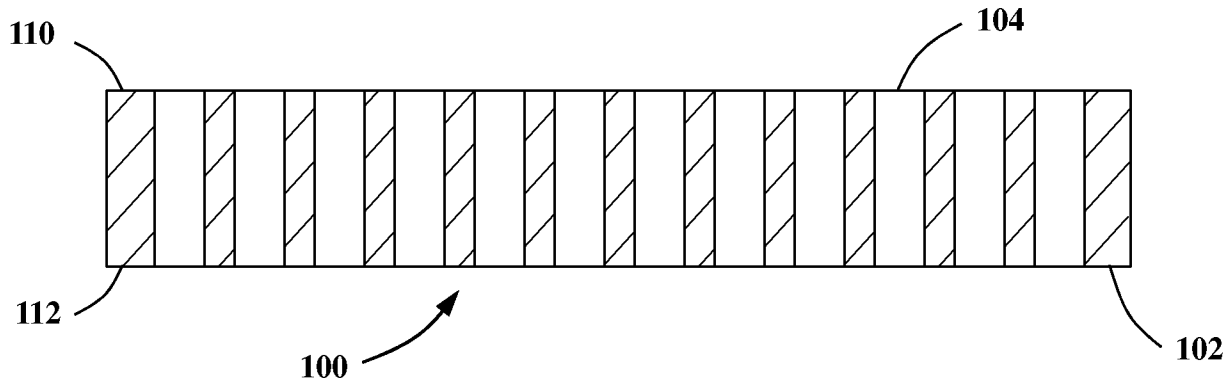


FIG. 3

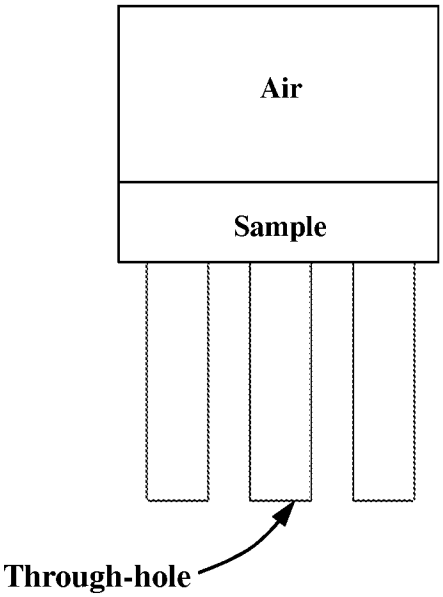
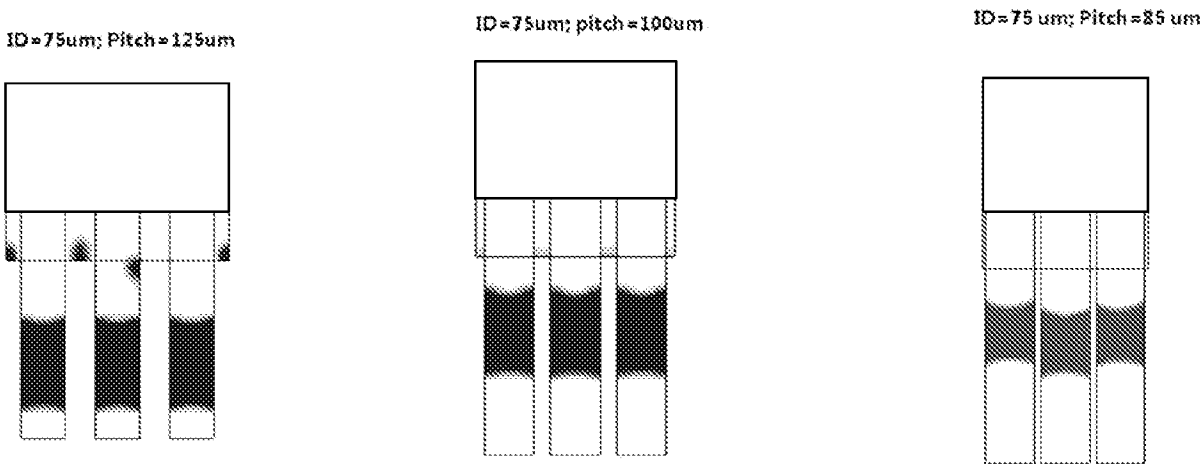


FIG. 4

FIG. 5



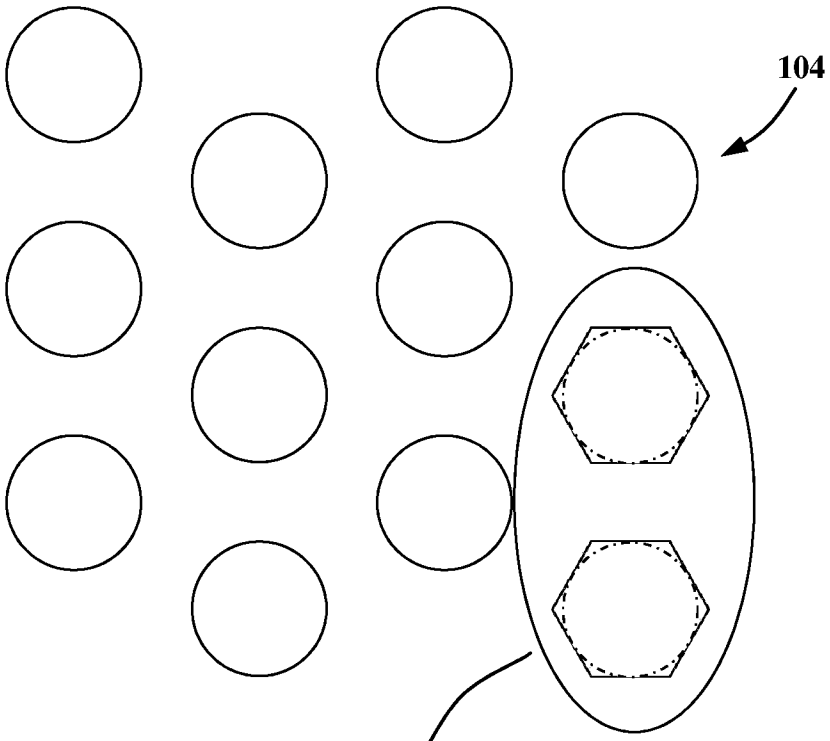


FIG. 6

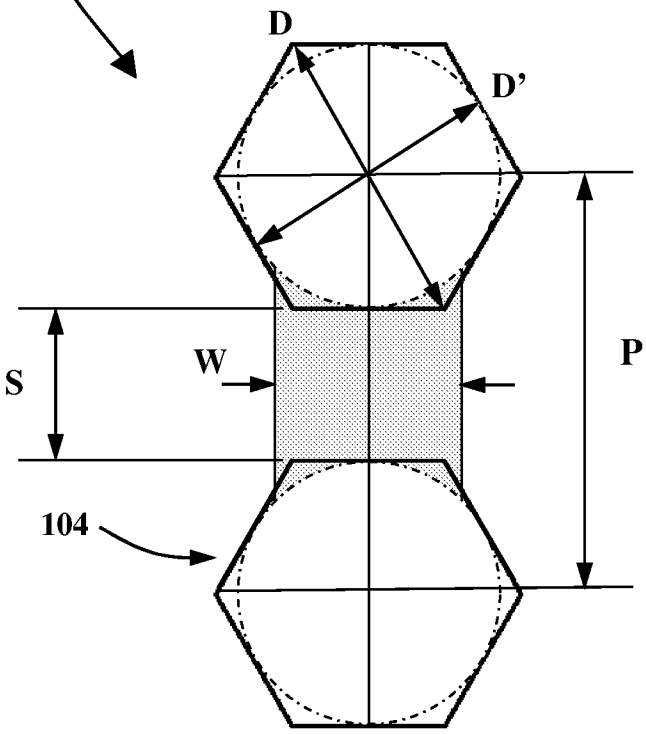


FIG. 7

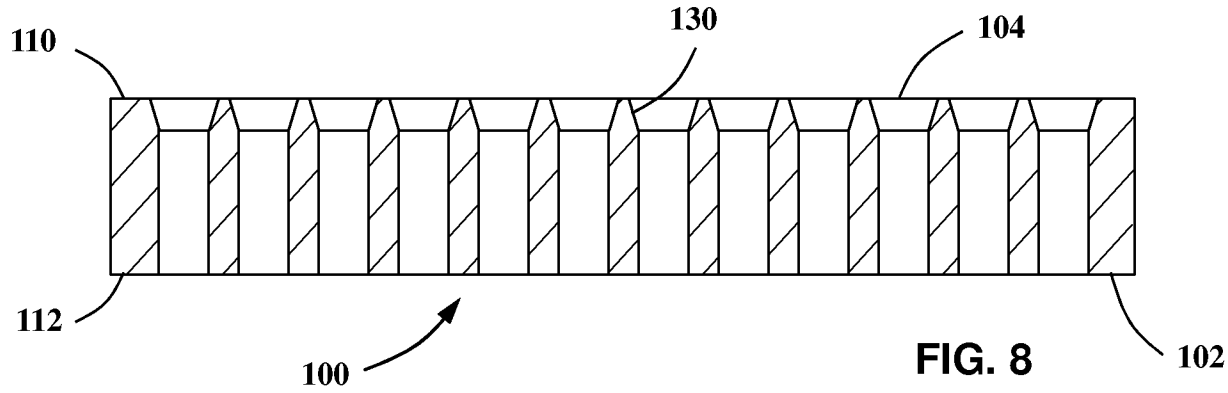
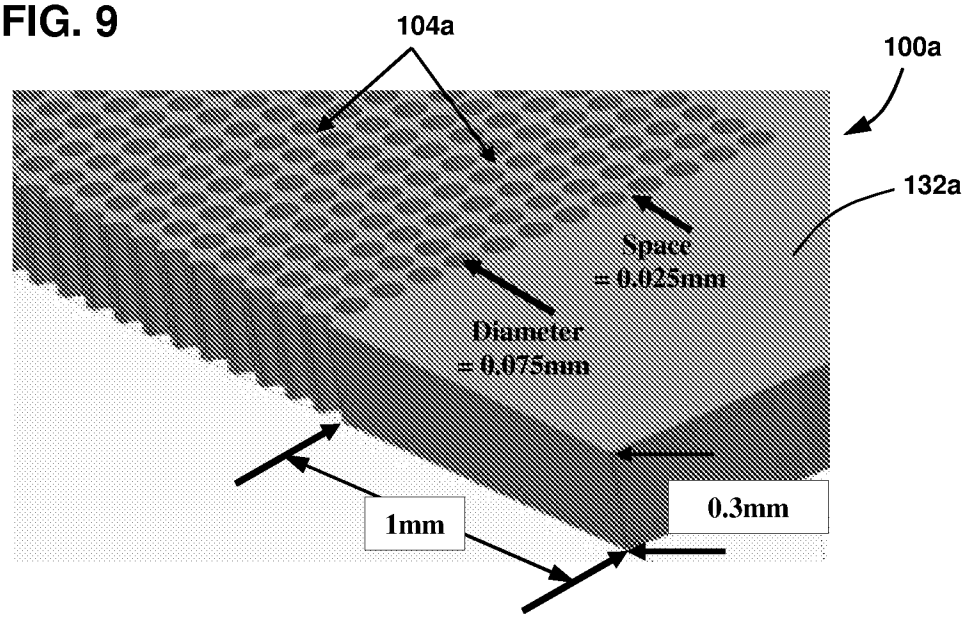


FIG. 8

FIG. 9



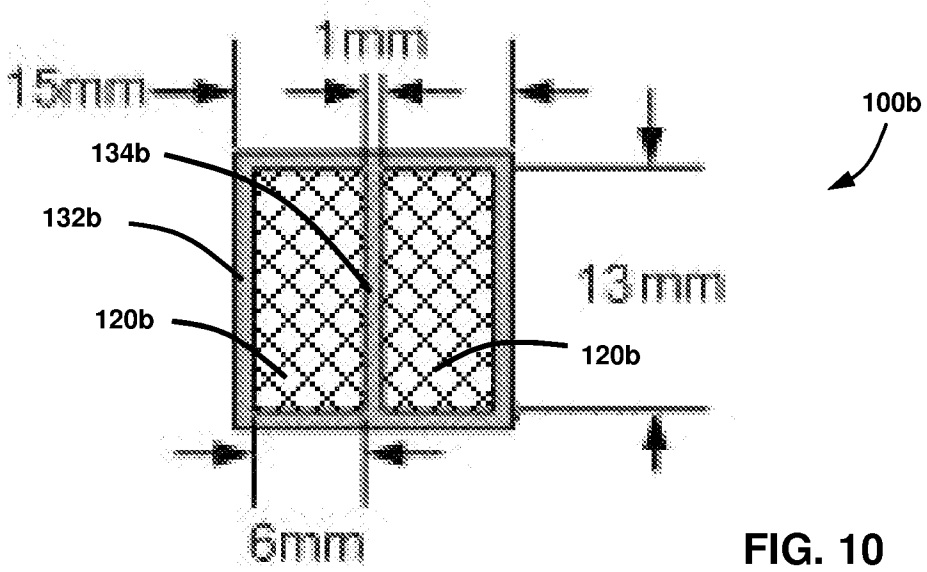


FIG. 10

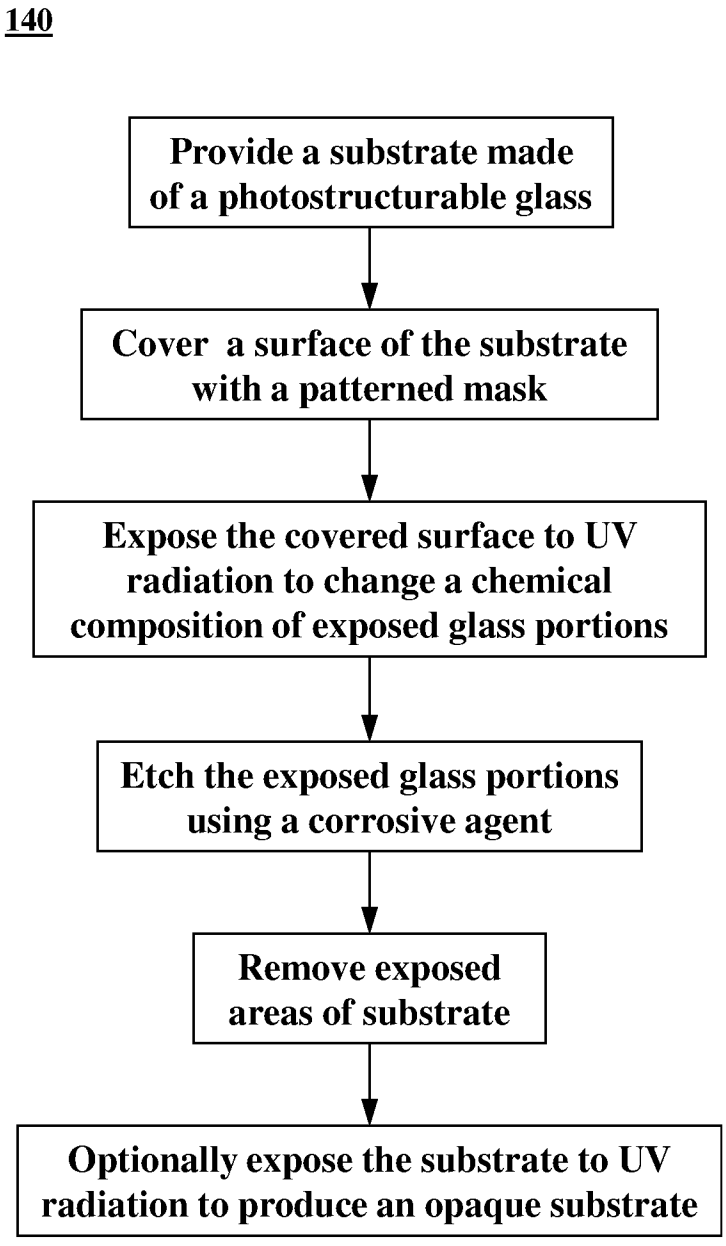

140
Provide a substrate made of a photostructurable glass
Cover a surface of the substrate with a patterned mask
Expose the covered surface to UV radiation to change a chemical composition of exposed glass portions
Etch the exposed glass portions using a corrosive agent
Remove exposed areas of substrate
Optionally expose the substrate to UV radiation to produce an opaque substrate

FIG. 11

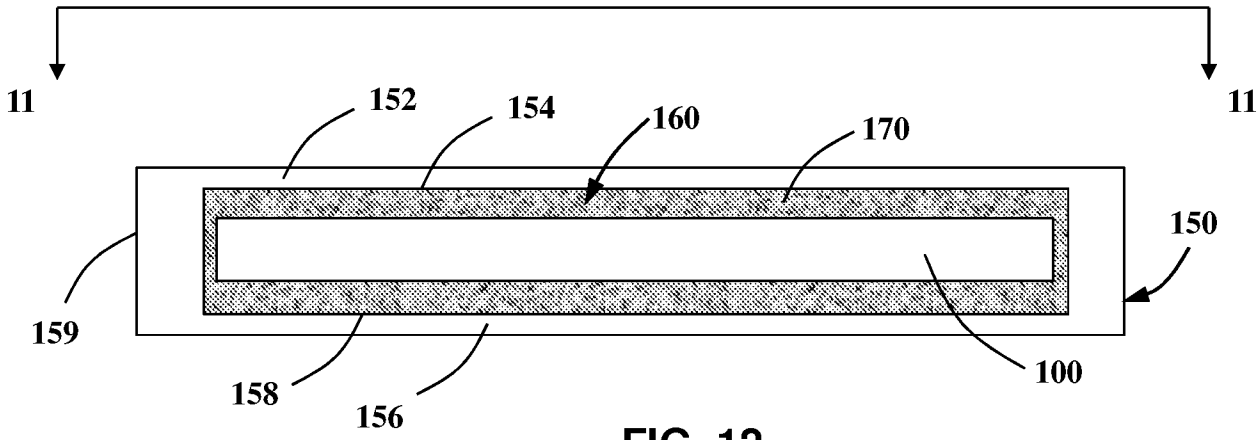


FIG. 12

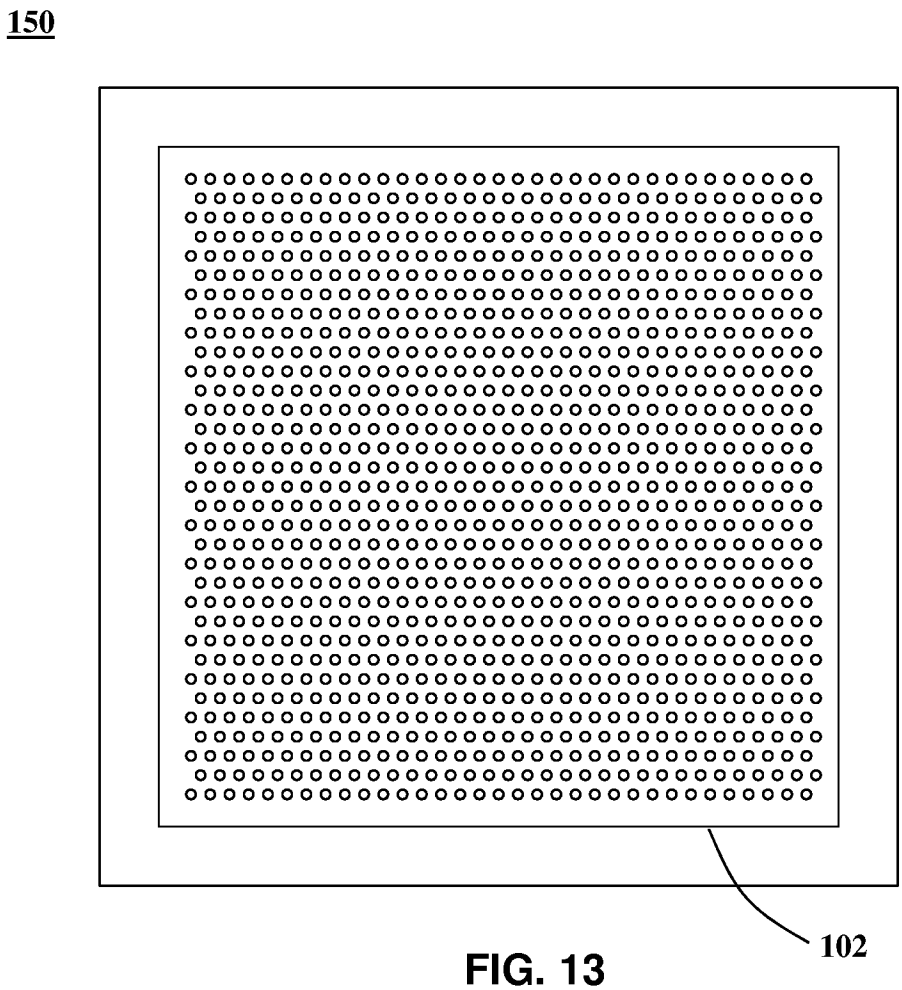
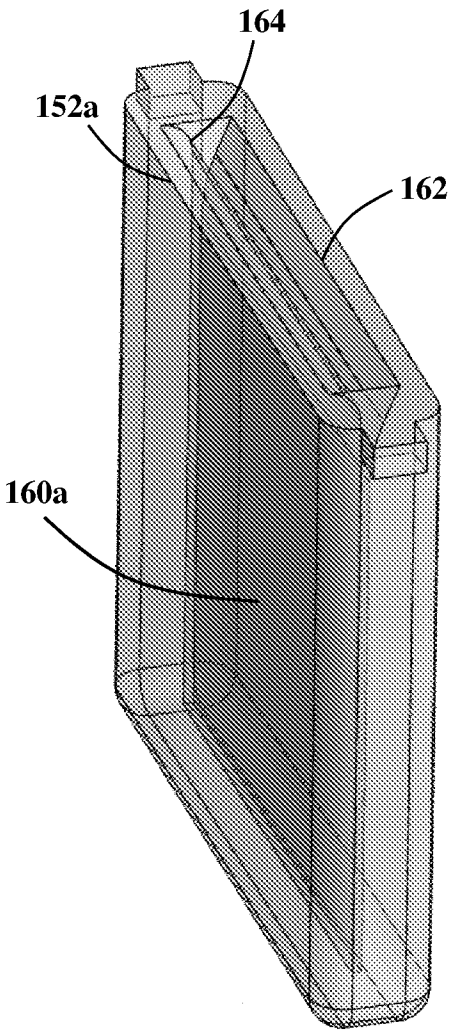


FIG. 13

FIG. 14



150b

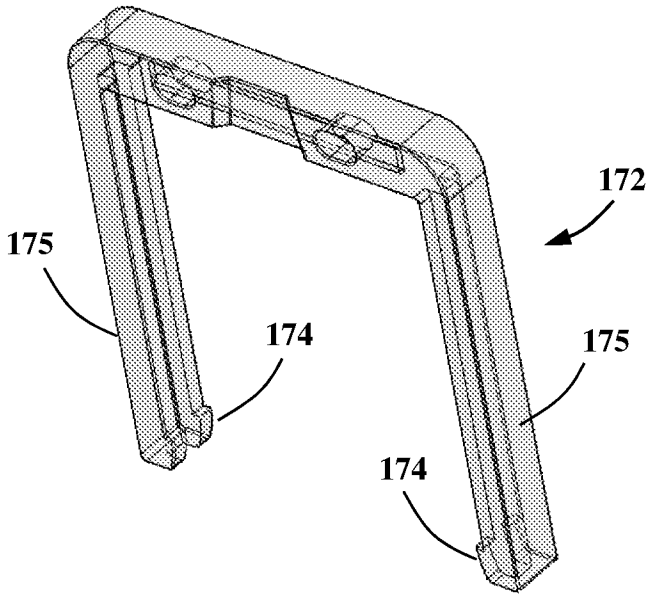
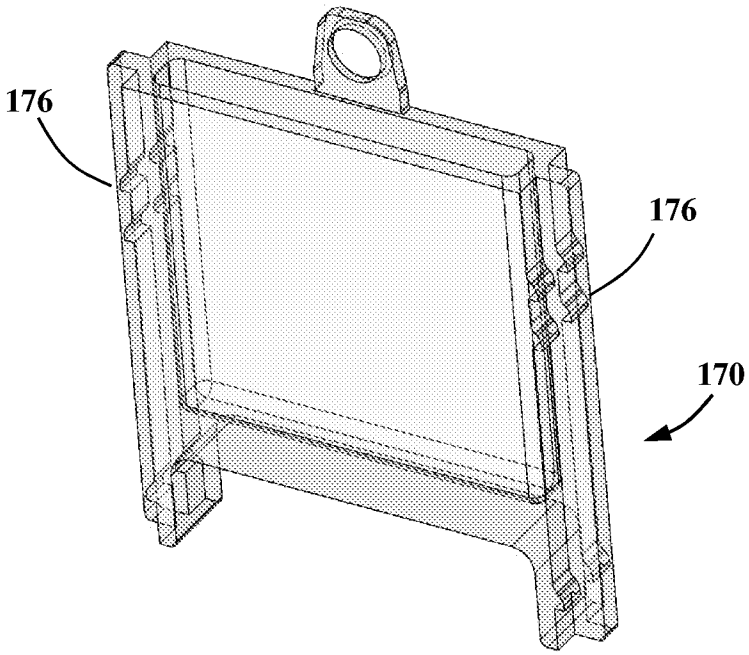


FIG. 15



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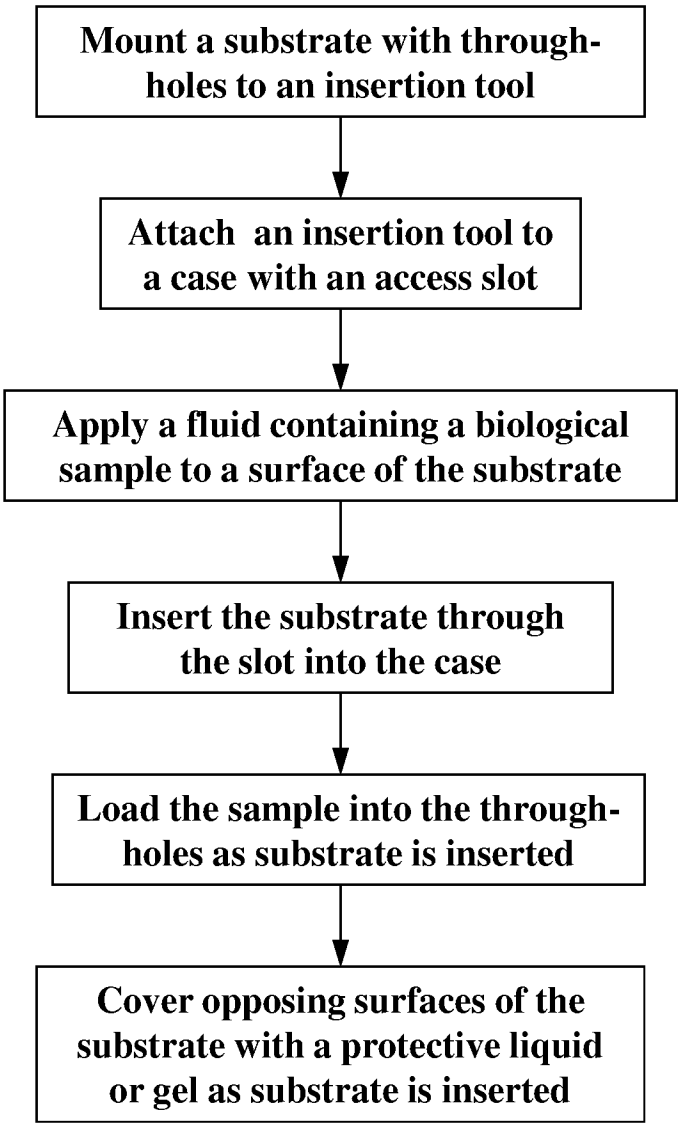

Mount a substrate with through-holes to an insertion tool
Attach an insertion tool to a case with an access slot
Apply a fluid containing a biological sample to a surface of the substrate
Insert the substrate through the slot into the case
Load the sample into the through-holes as substrate is inserted
Cover opposing surfaces of the substrate with a protective liquid or gel as substrate is inserted

FIG. 16

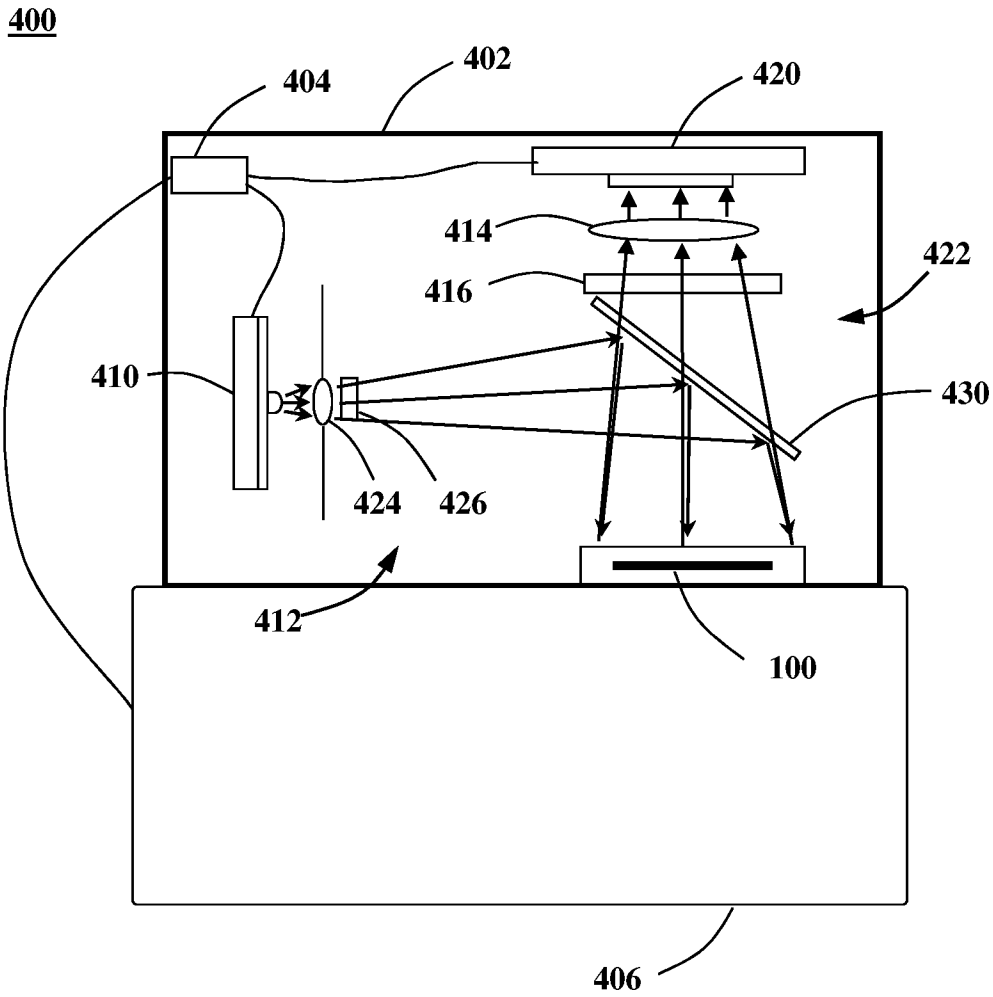


FIG. 17

FIG. 18

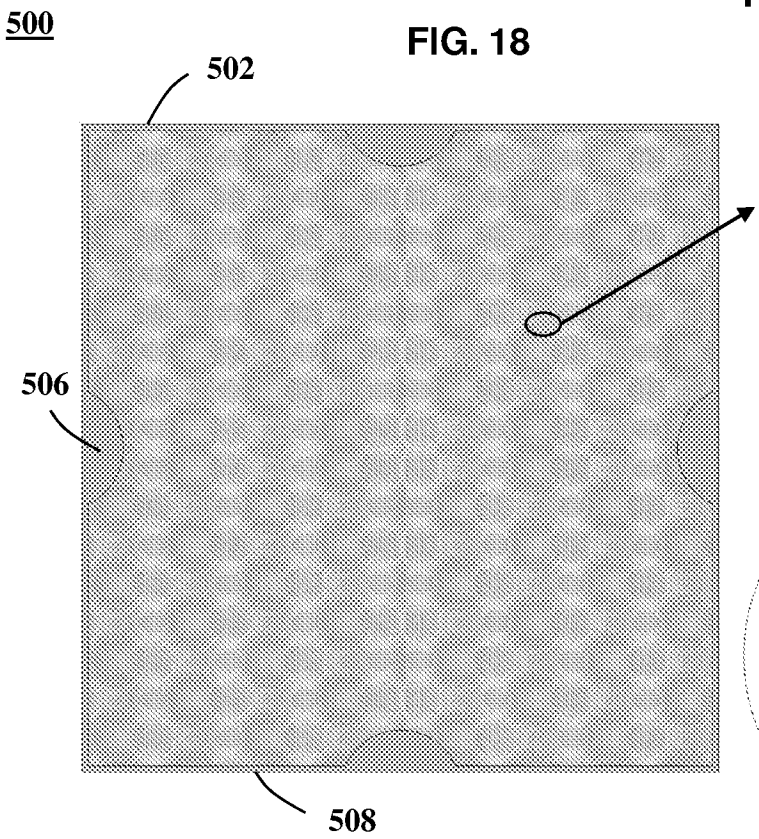


FIG. 19

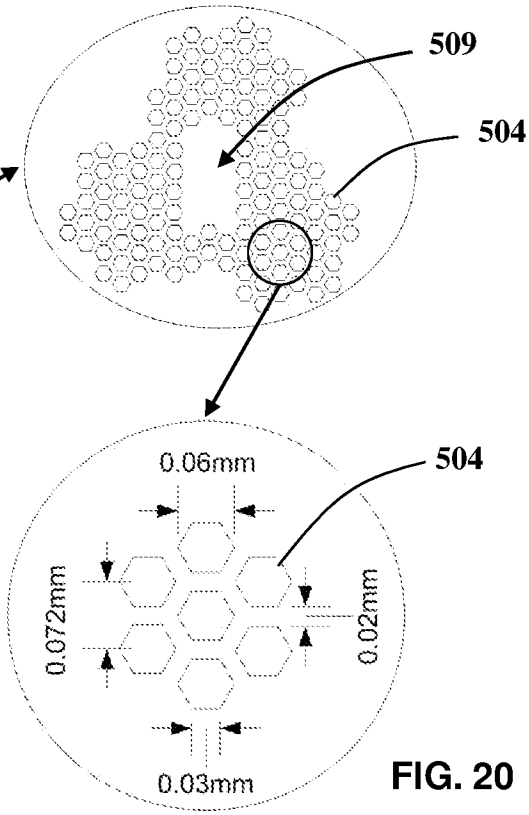


FIG. 20

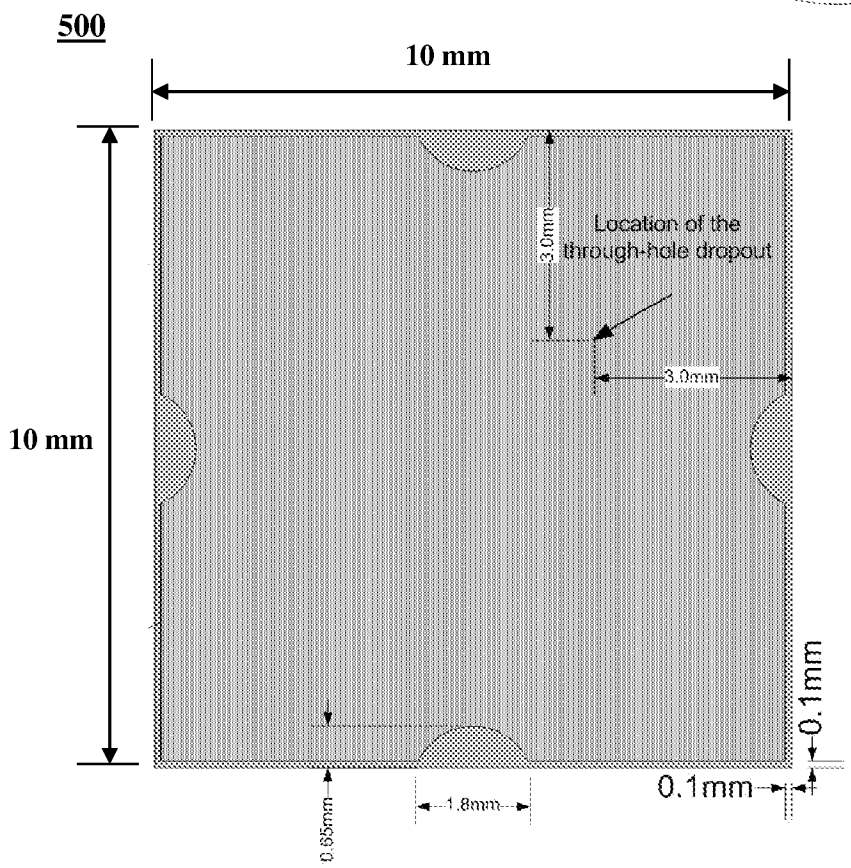


FIG. 21

FIG. 22

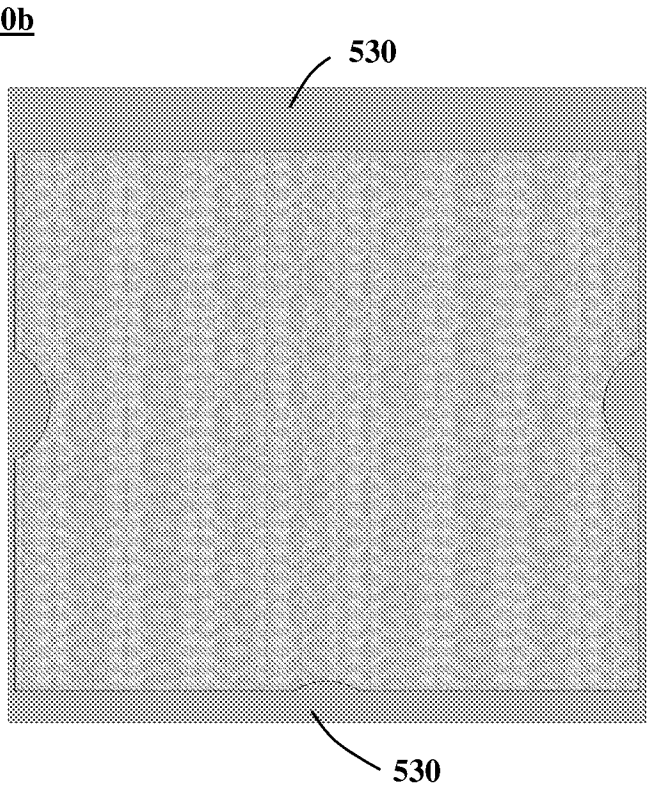
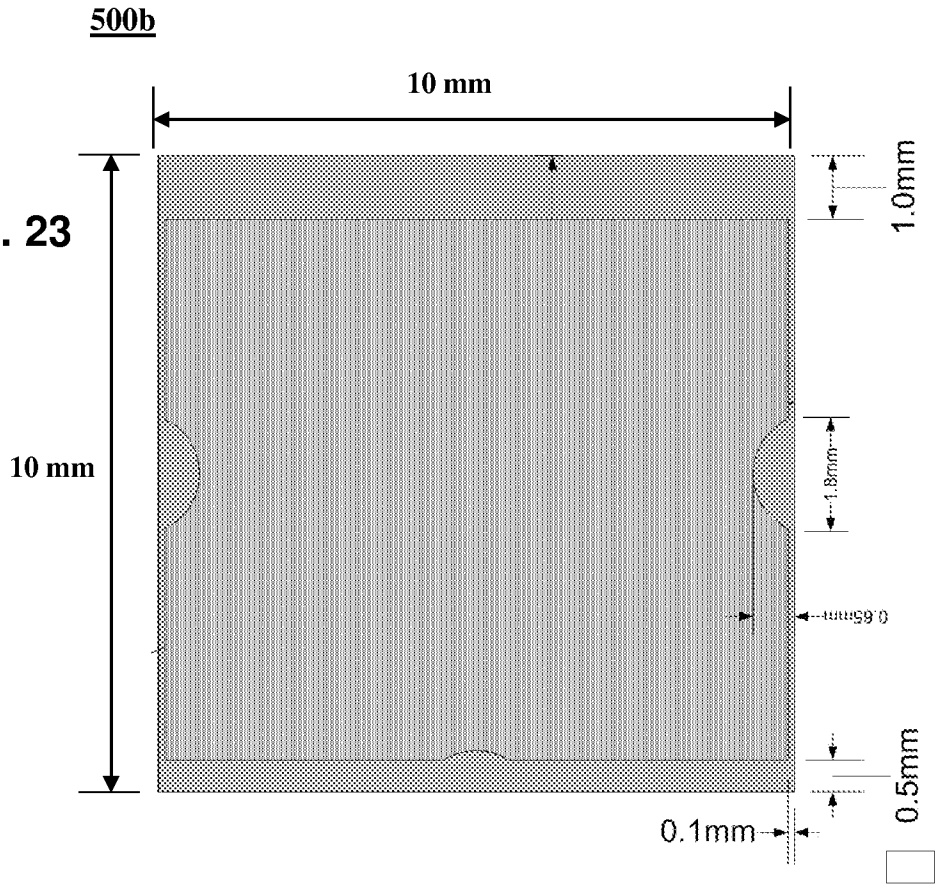


FIG. 23



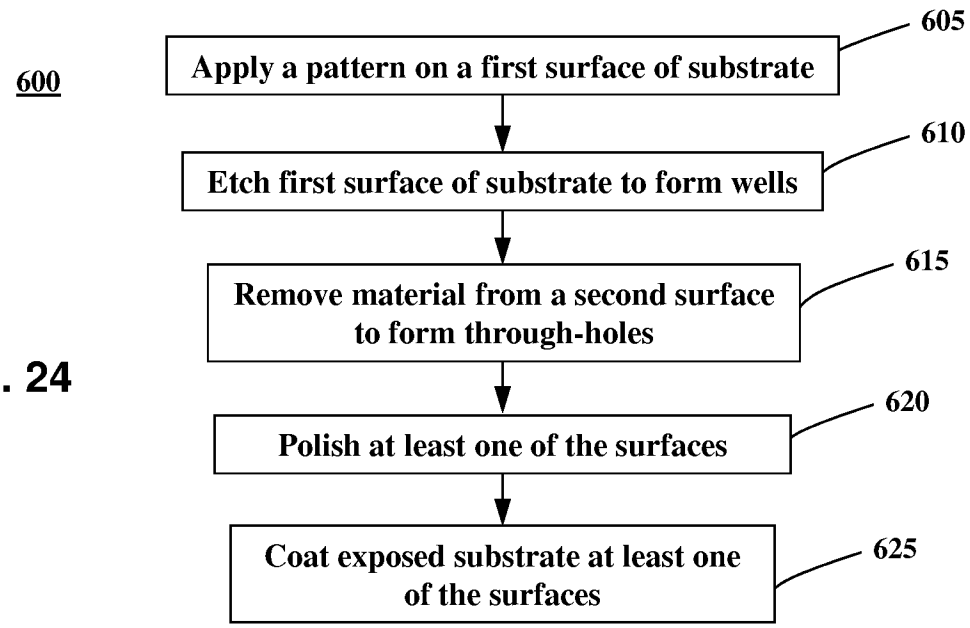


FIG. 24

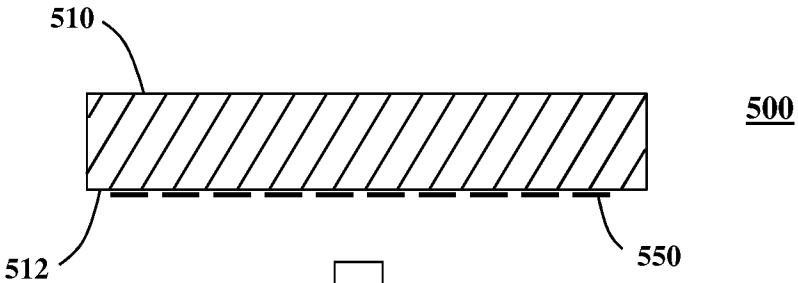


FIG. 25A

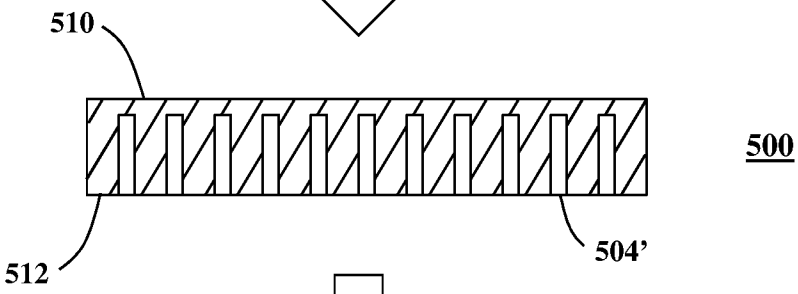


FIG. 25B

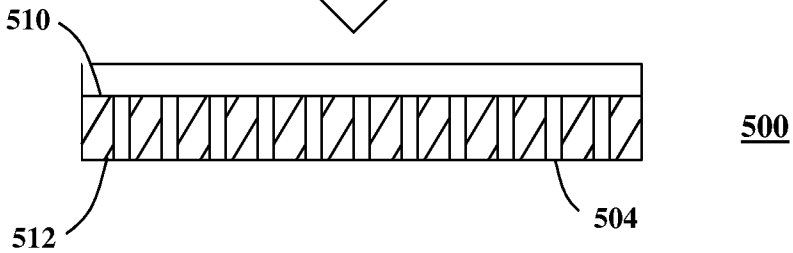


FIG. 25C

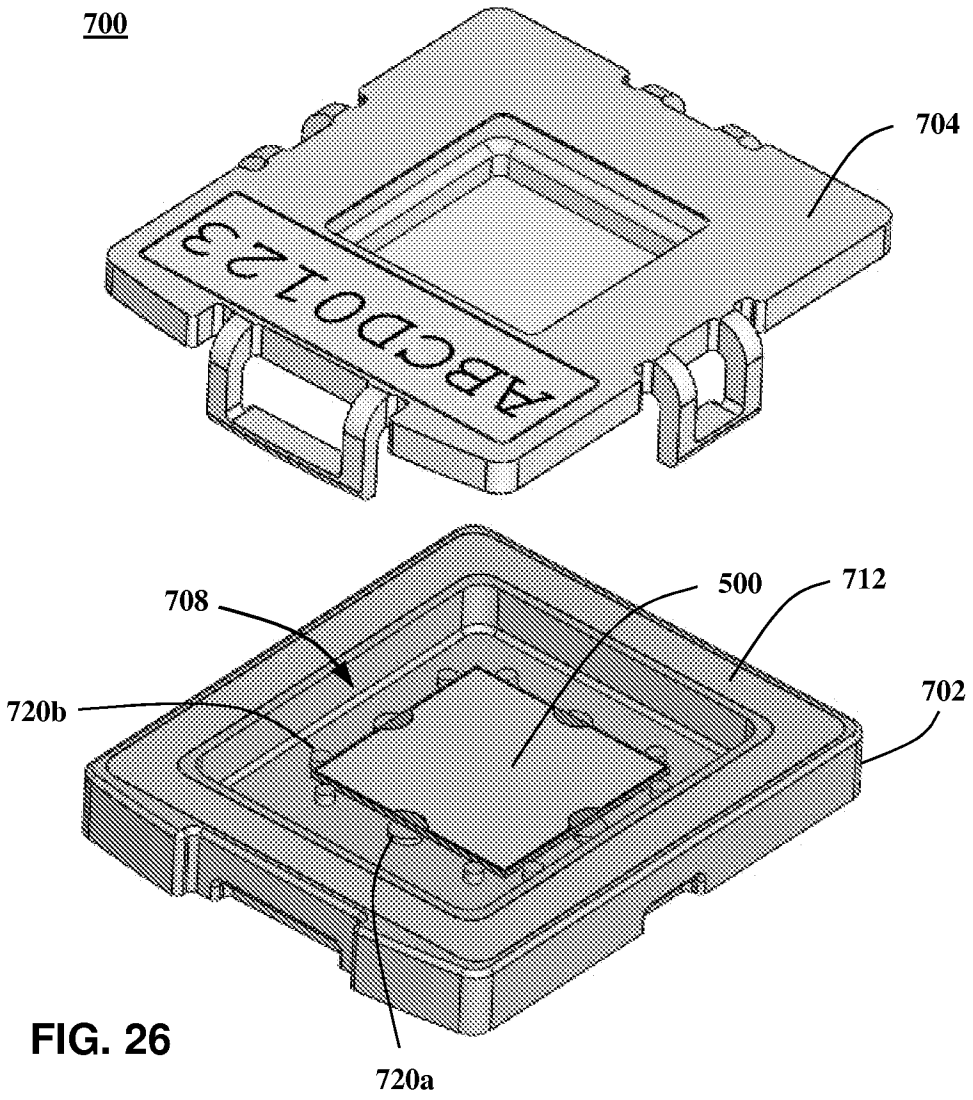


FIG. 26