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(54) **CAPTURING SPECIFIC NUCLEIC ACID MATERIALS FROM INDIVIDUAL BIOLOGICAL CELLS IN A MICRO-FLUIDIC DEVICE**

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(57) **ABSTRACT**

Individual biological cells can be selected in a micro-fluidic device and moved into isolation pens in the device. The cells can then be lysed in the pens, releasing nucleic acid material, which can be captured by one or more capture objects in the pens. The capture objects with the captured nucleic acid material can then be removed from the pens. The capture objects can include unique identifiers, allowing each capture object to be correlated to the individual cell from which the nucleic acid material captured by the object originated.

Related U.S. Application Data

(63) Continuation-in-part of application No. 14/133,361, filed on Dec. 18, 2013.

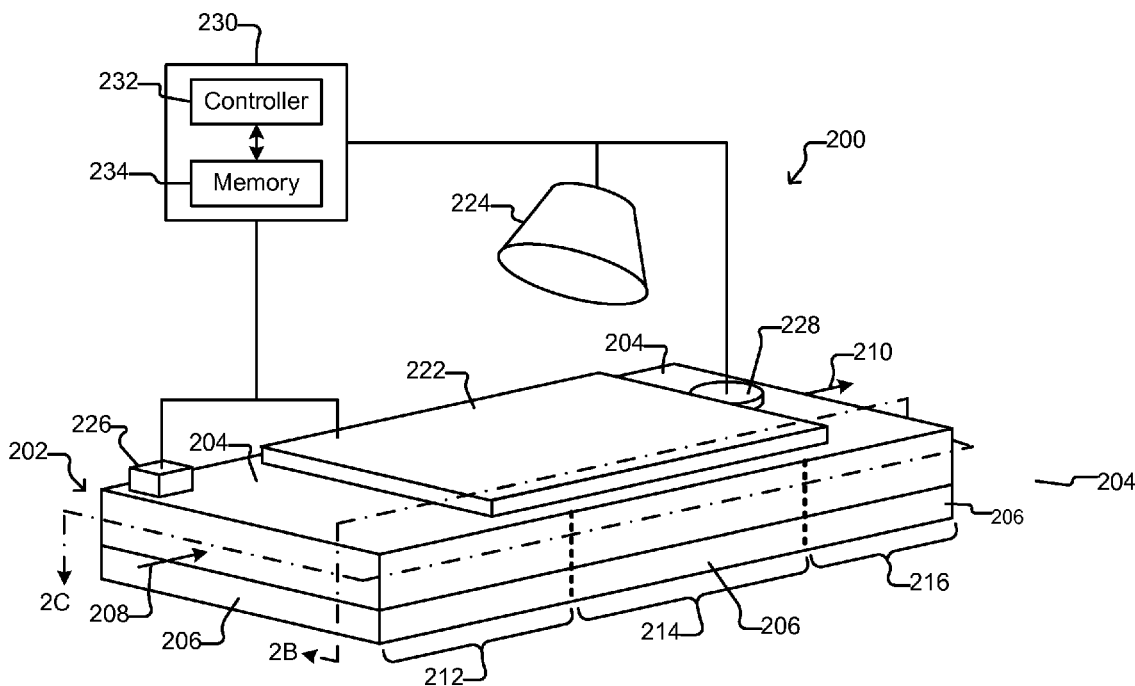


Figure 1

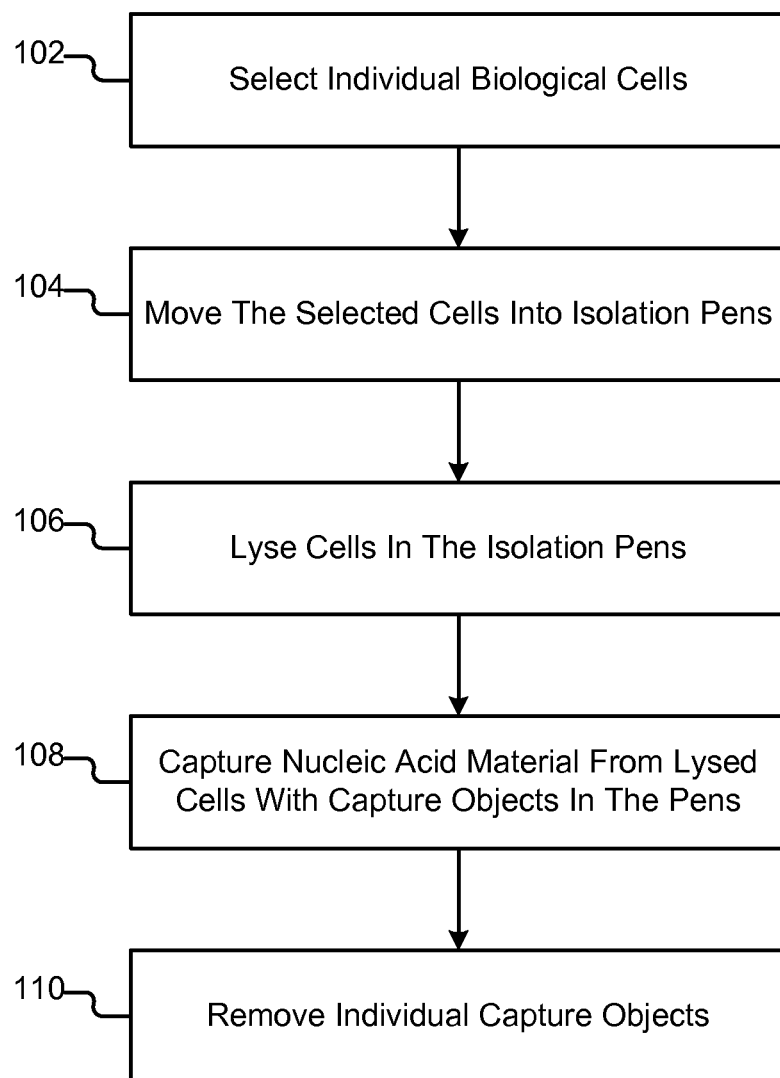


Figure 2A

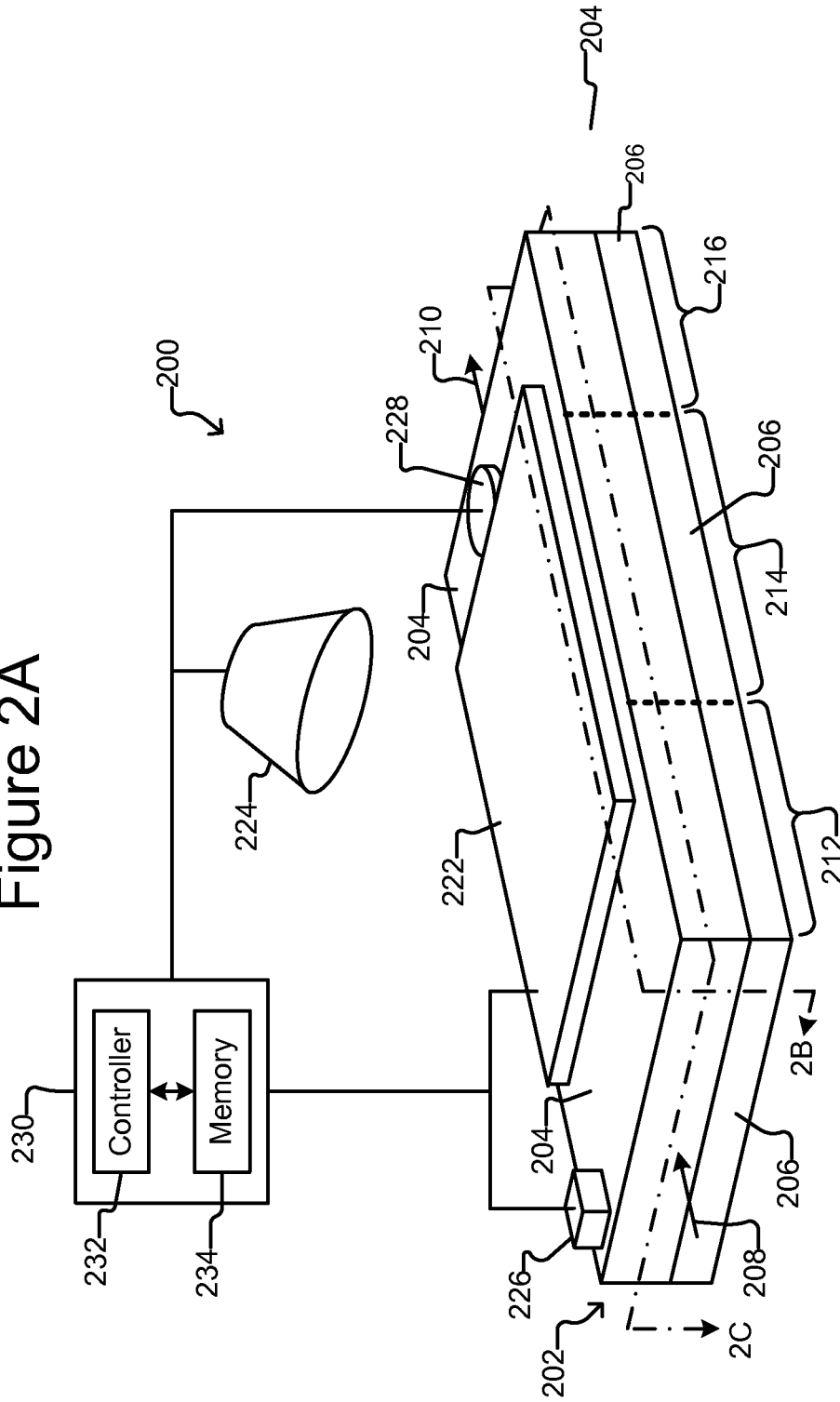


Figure 2B

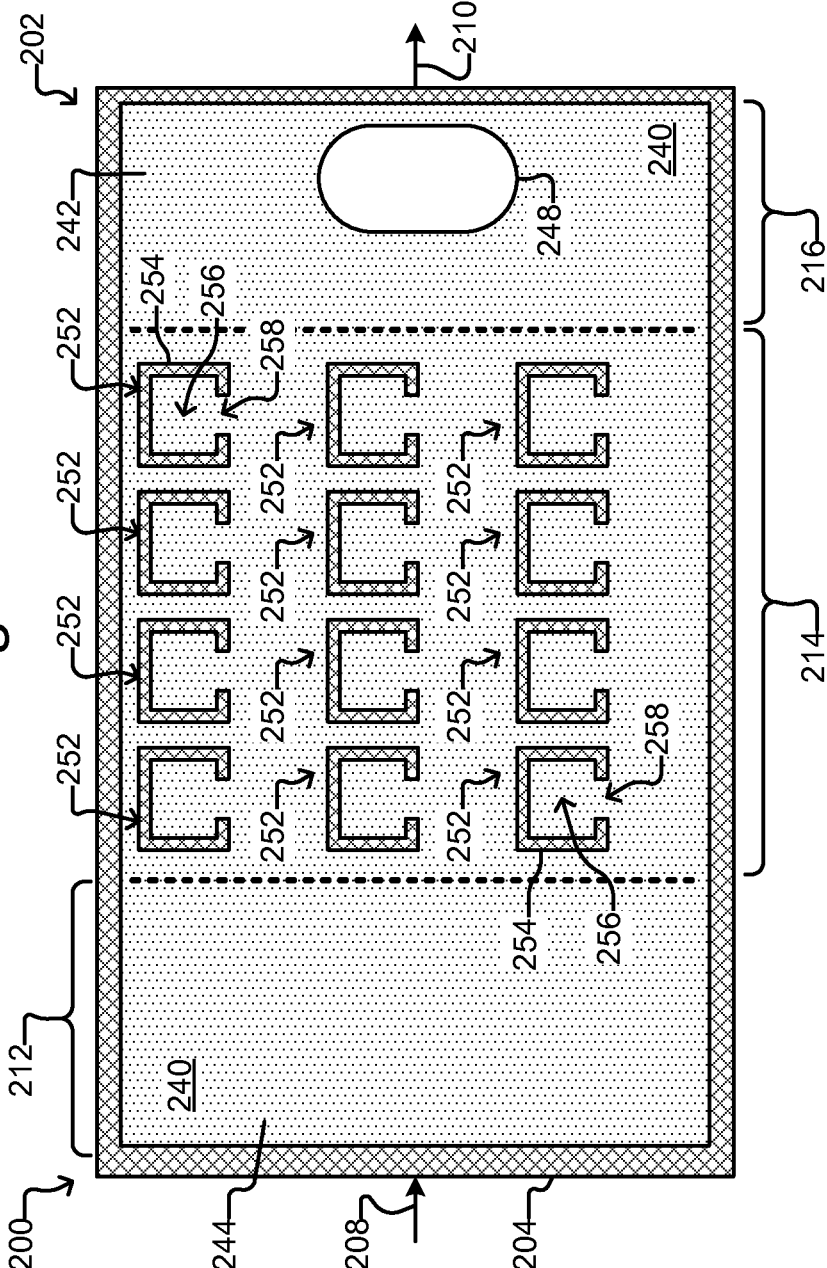


Figure 2C

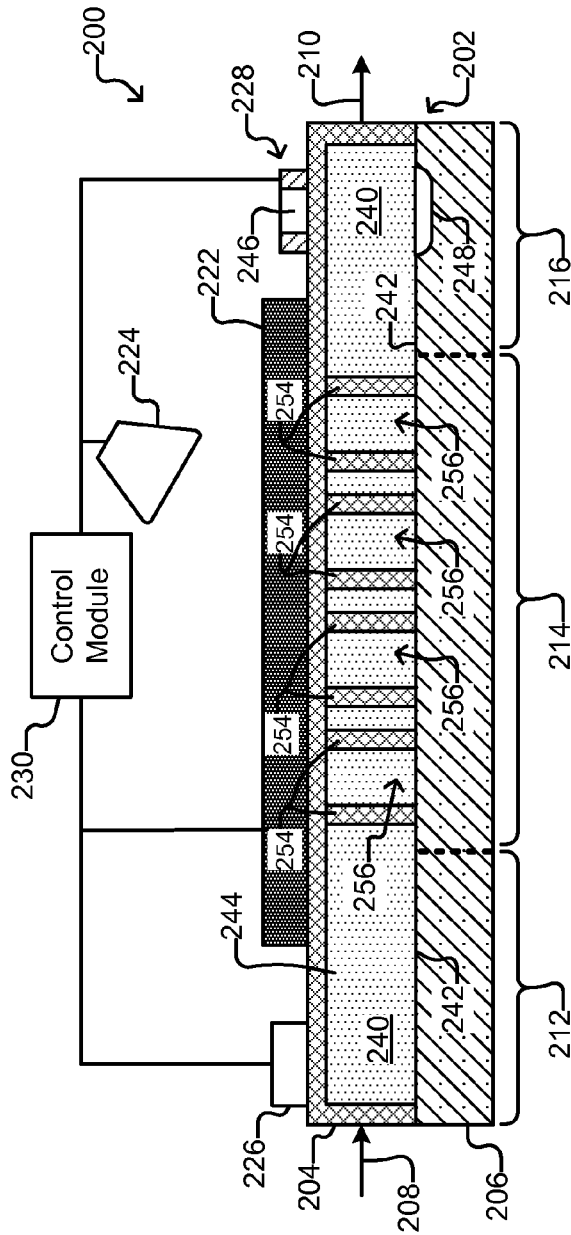


Figure 3

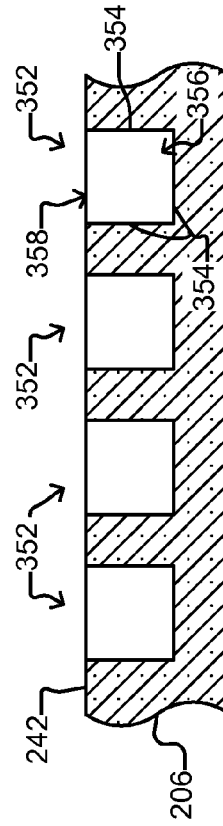


Figure 4A

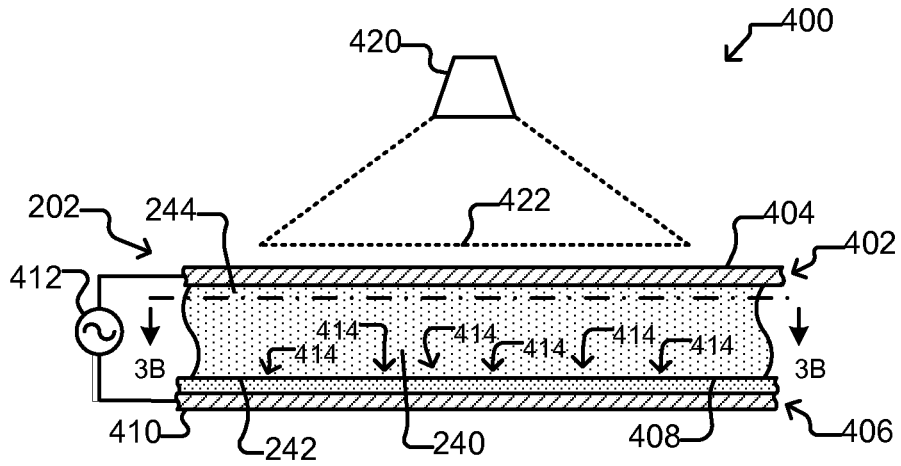
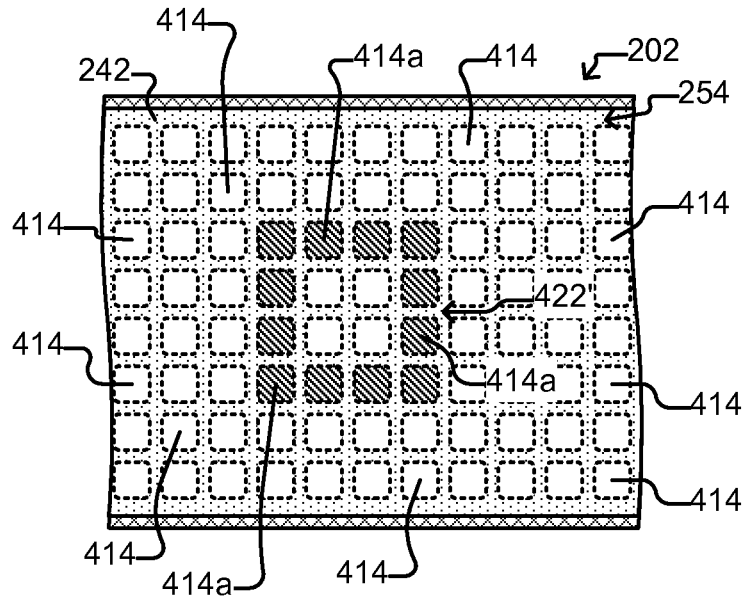
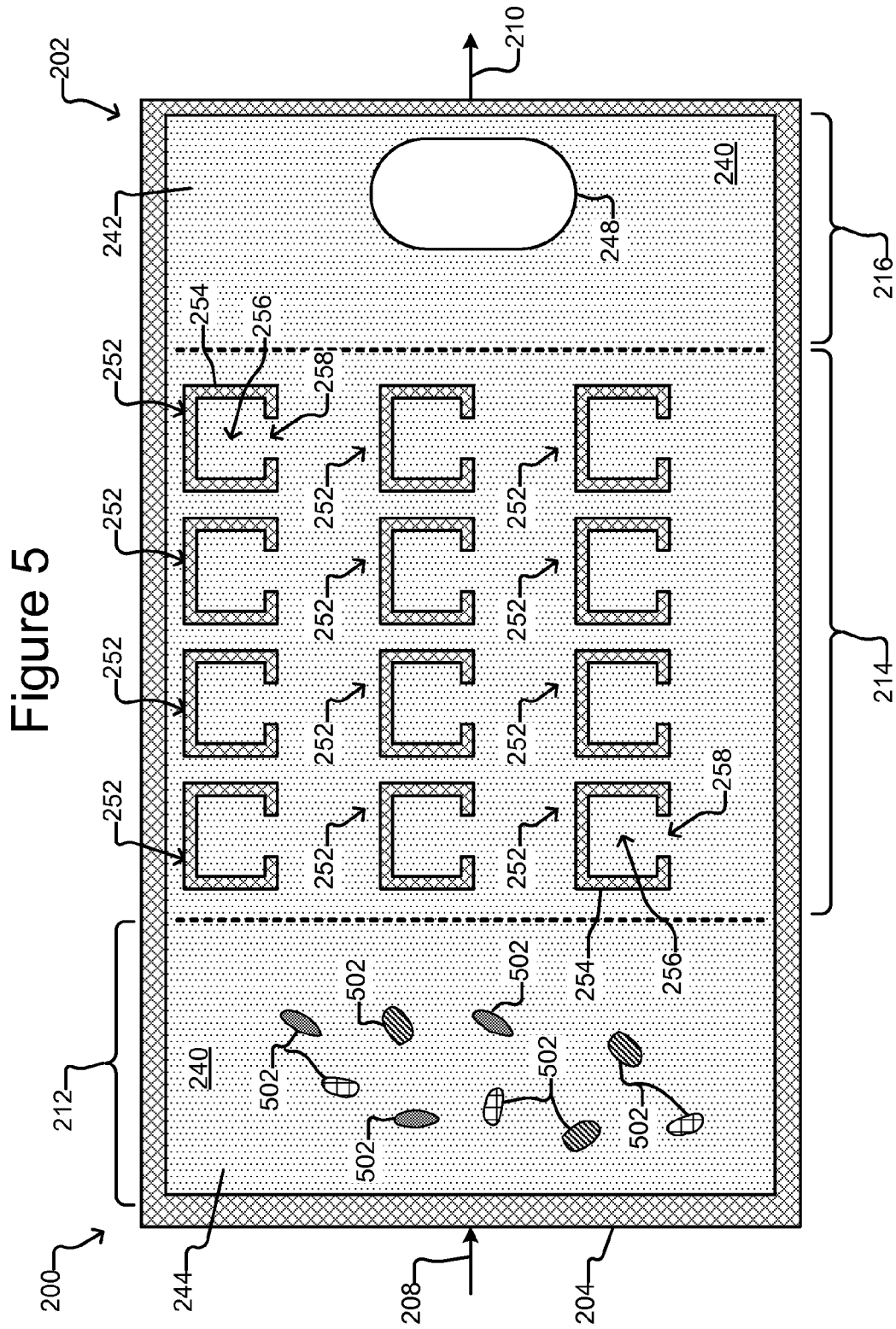


Figure 4B





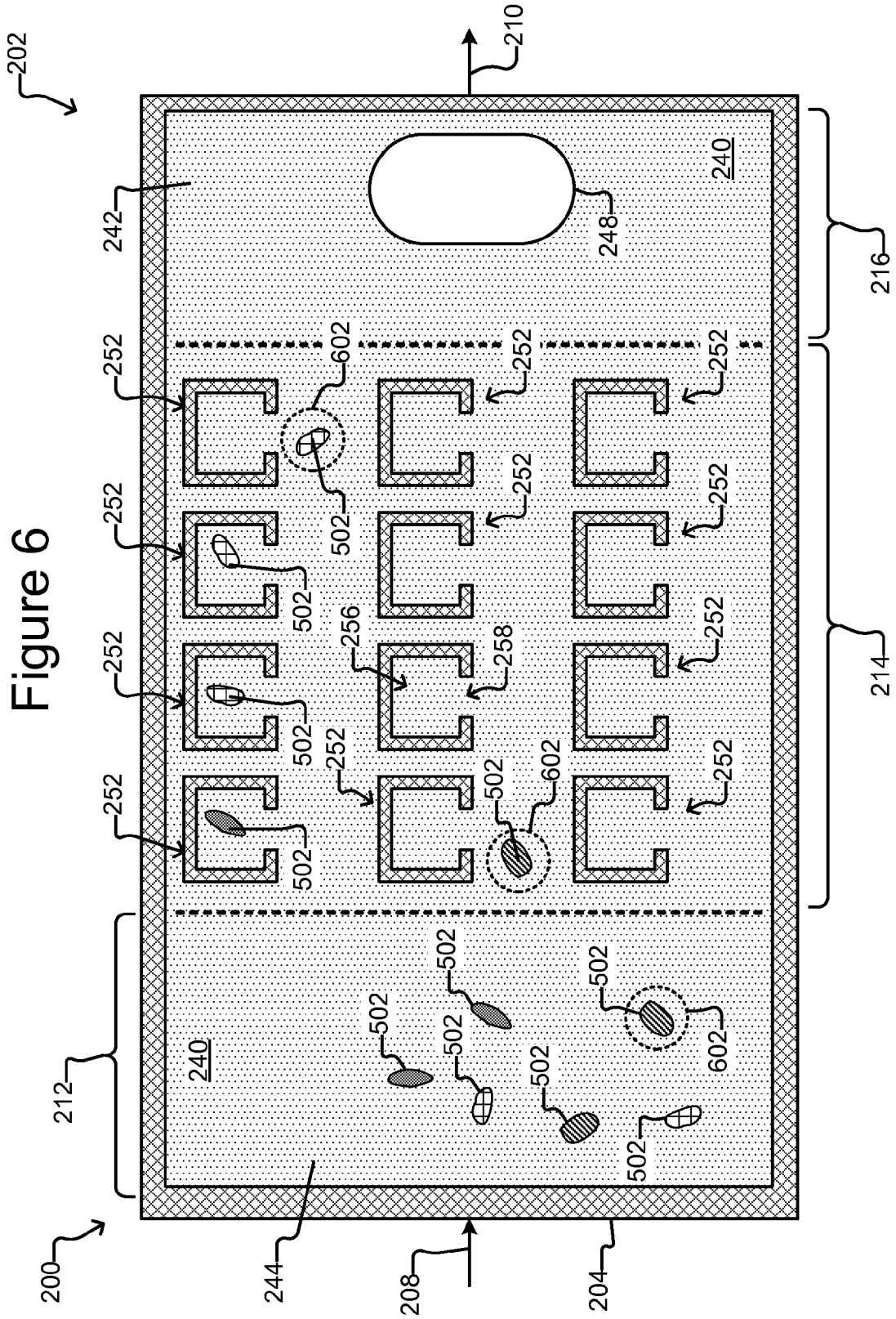


Figure 6

Figure 7

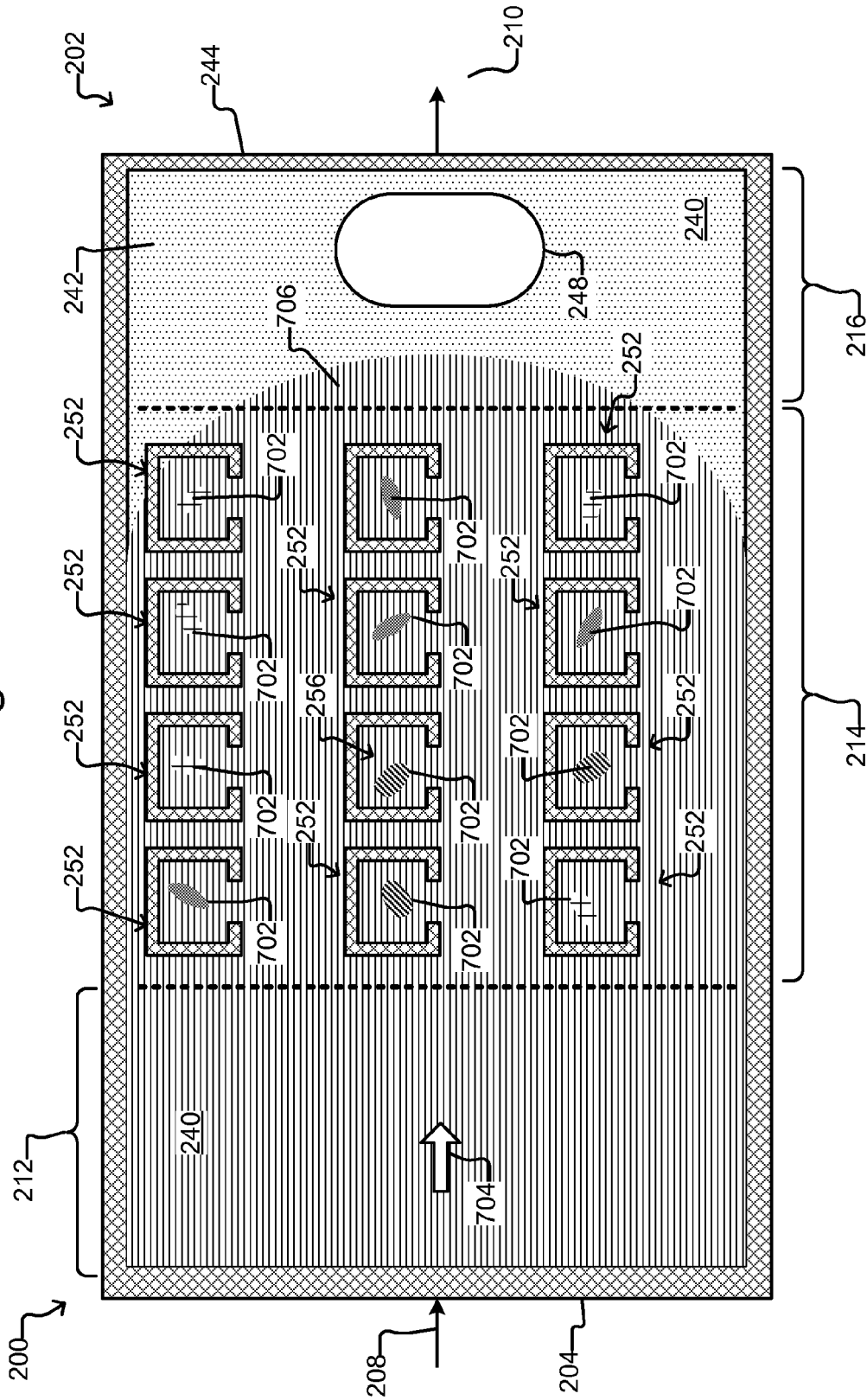


Figure 8

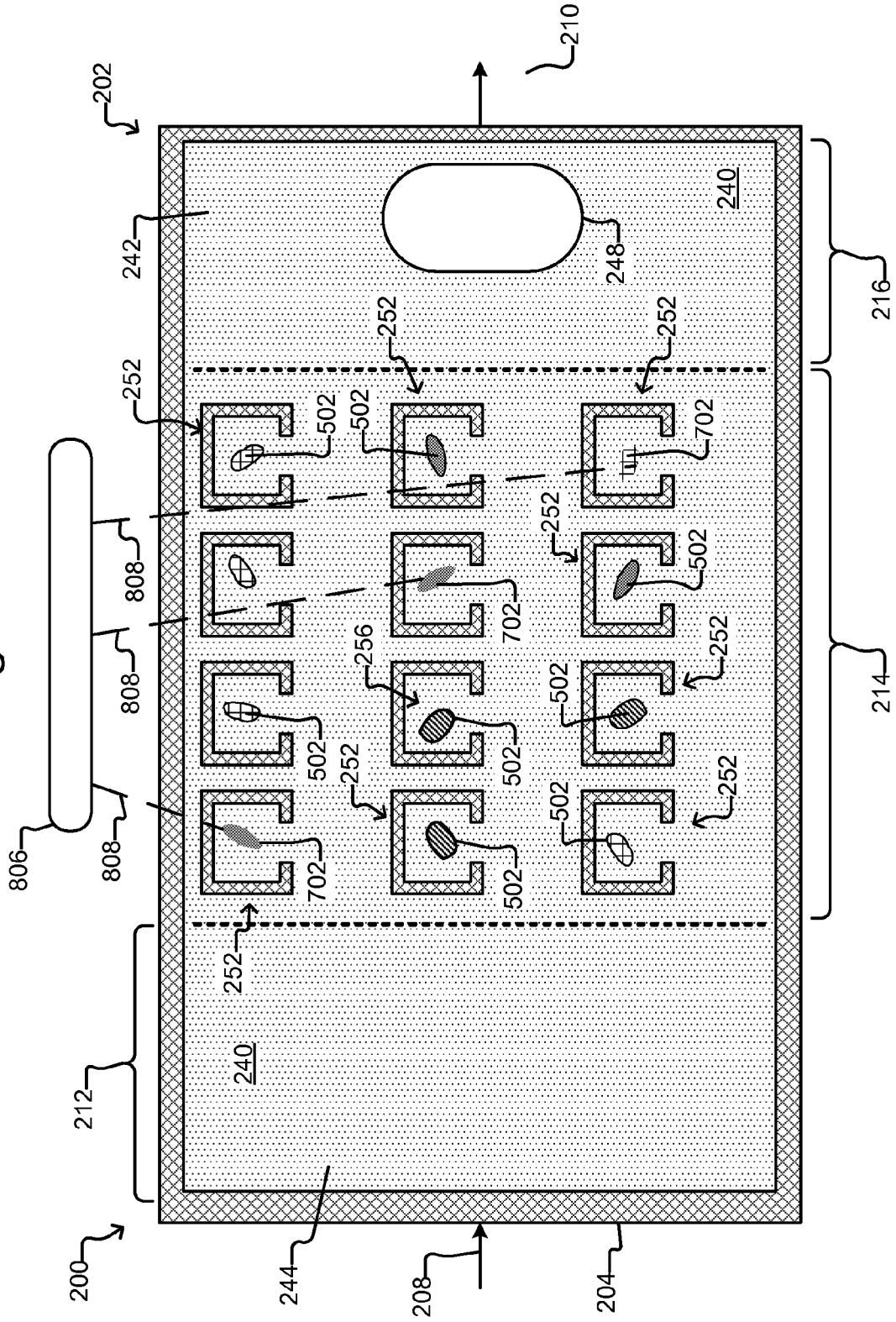


Figure 9

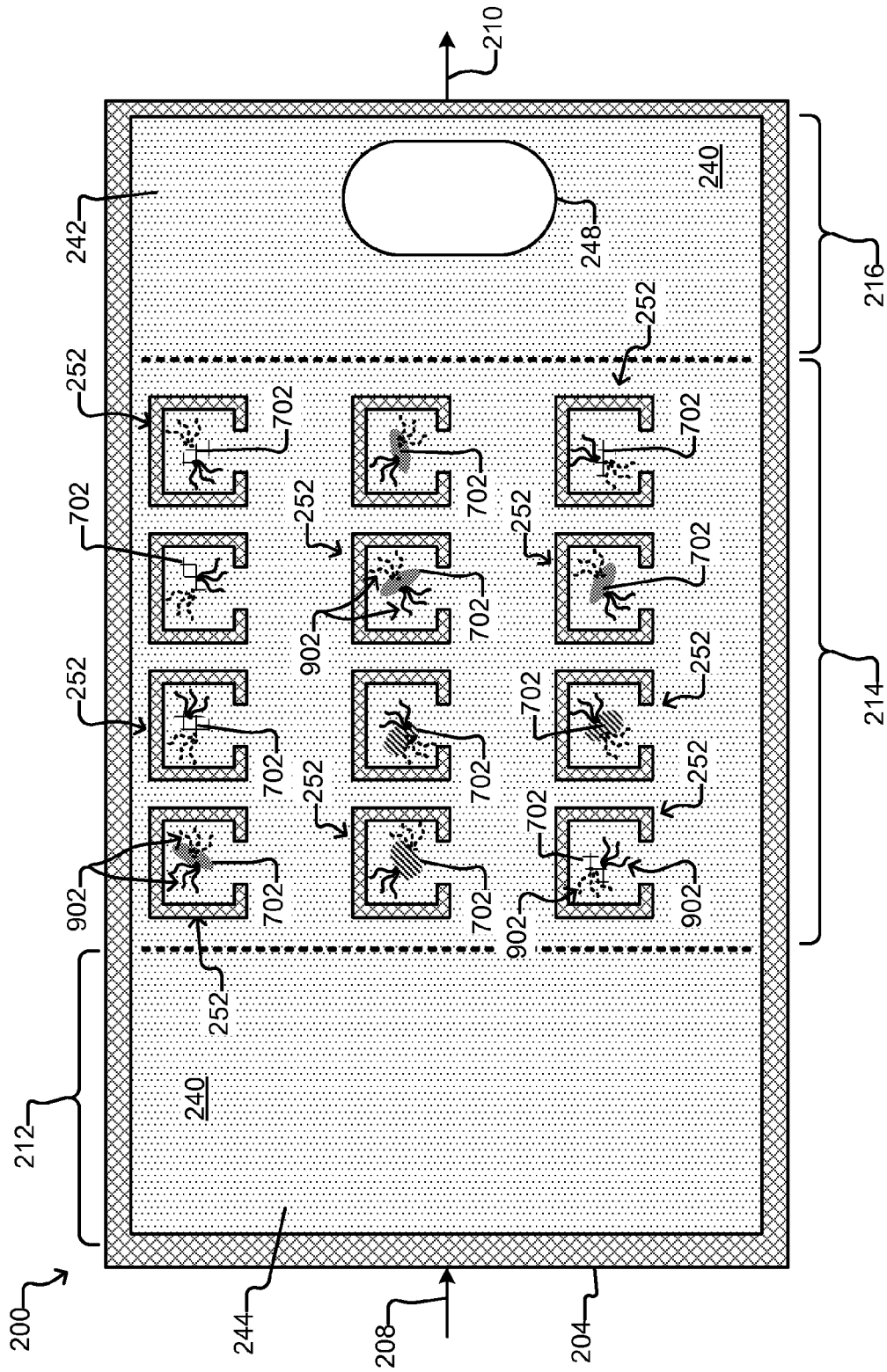


Figure 10

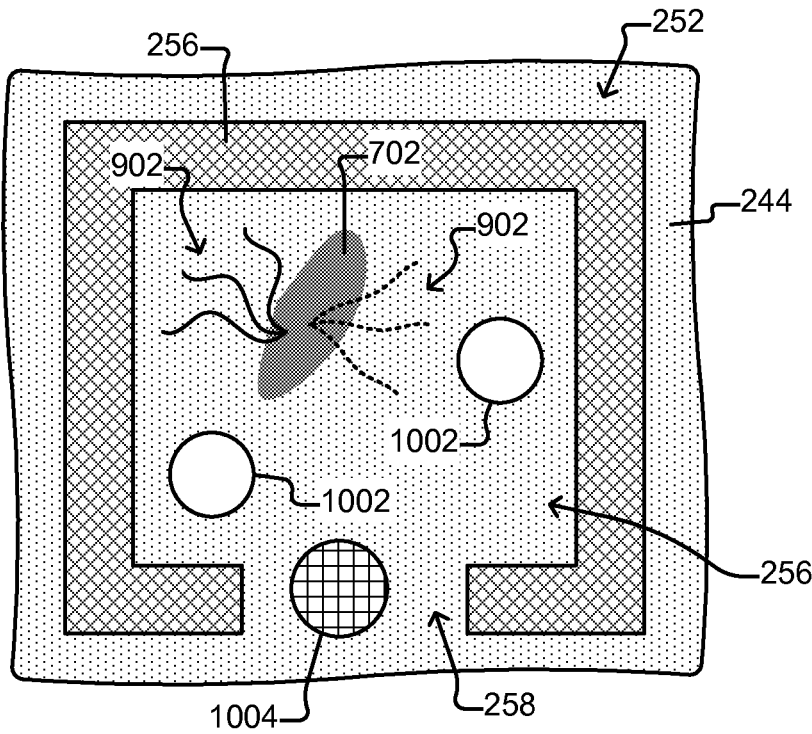


Figure 11

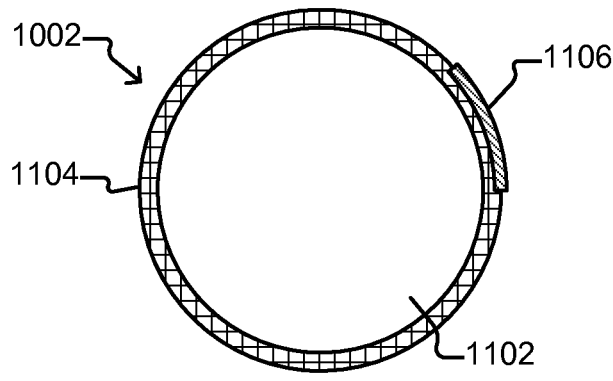


Figure 12A

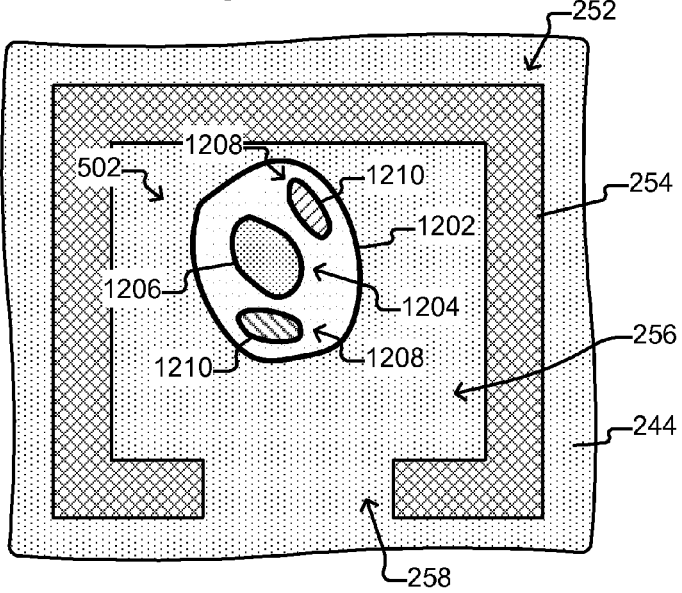


Figure 12B

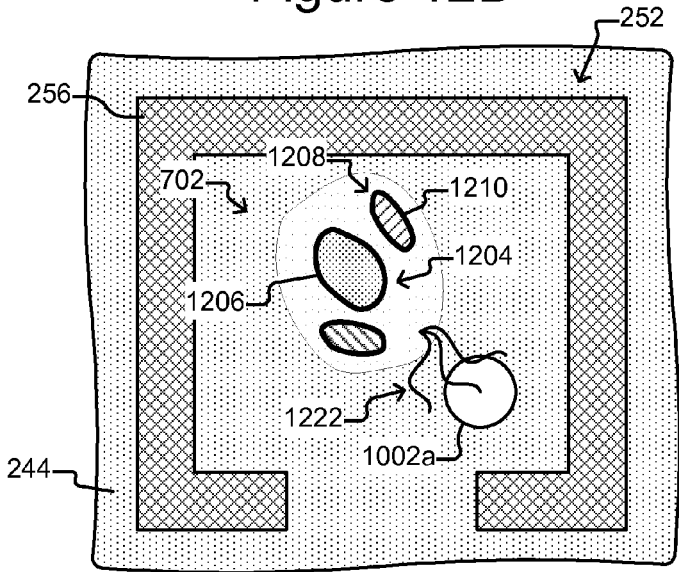


Figure 12C

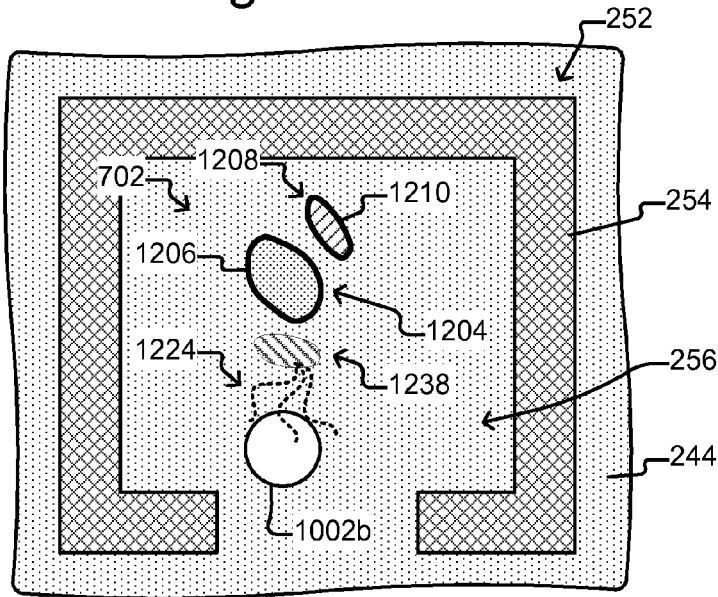


Figure 12D

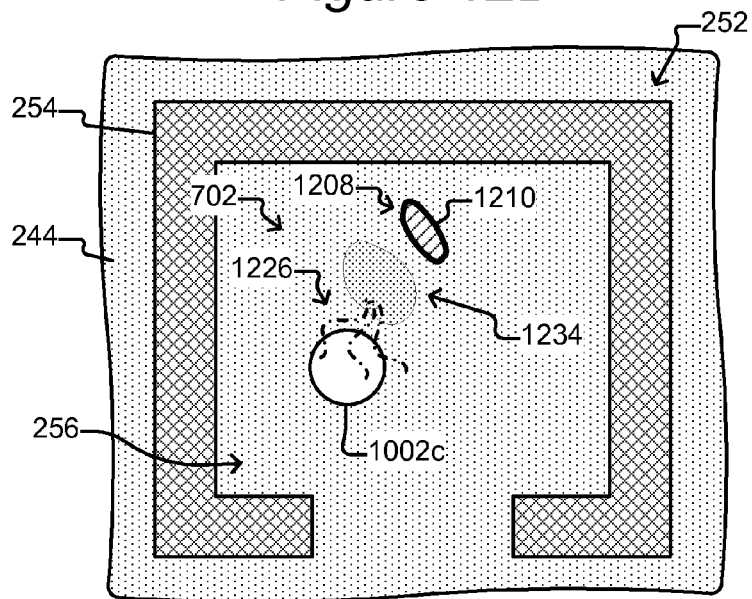


Figure 13

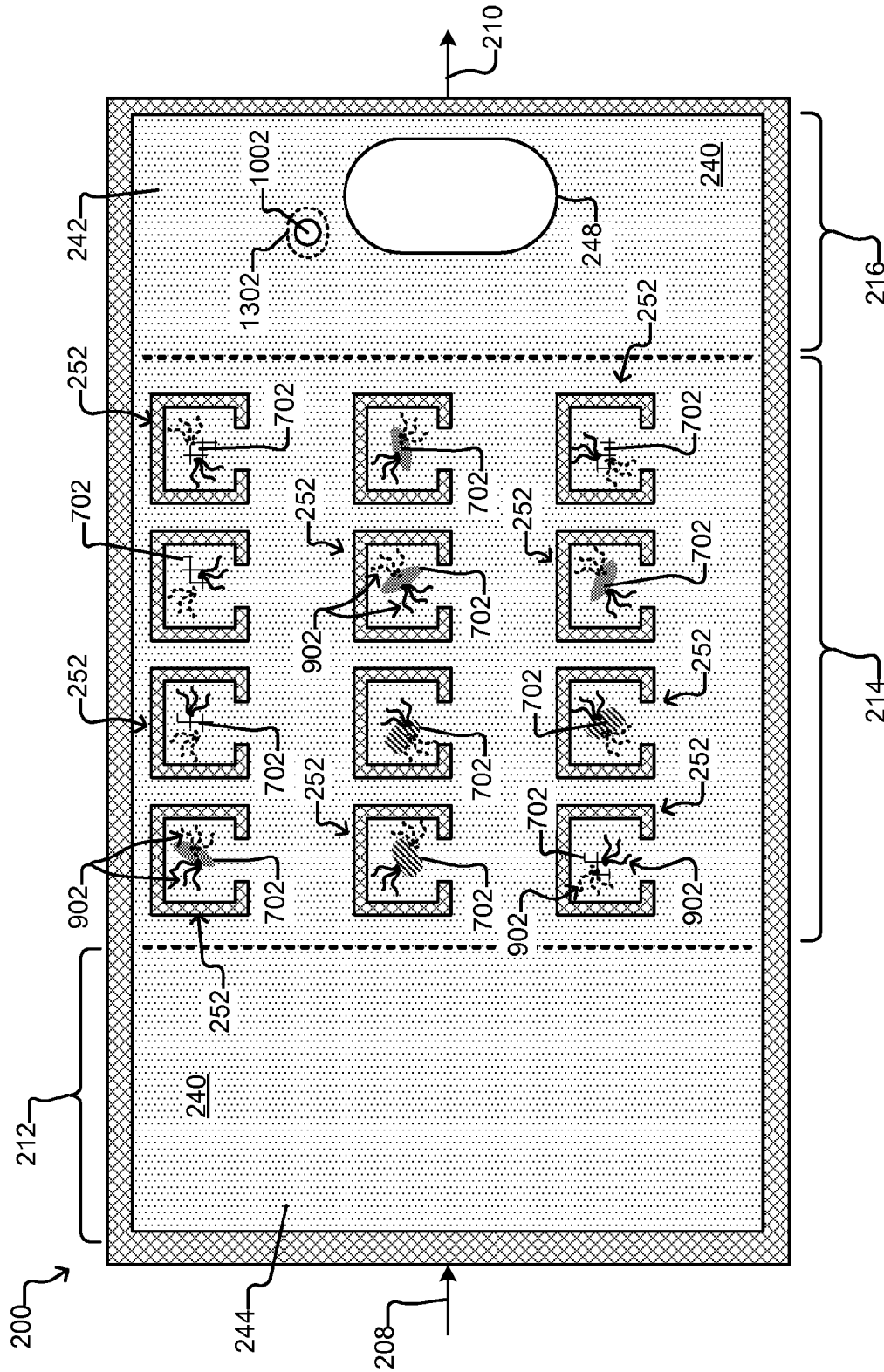


Figure 14

1400

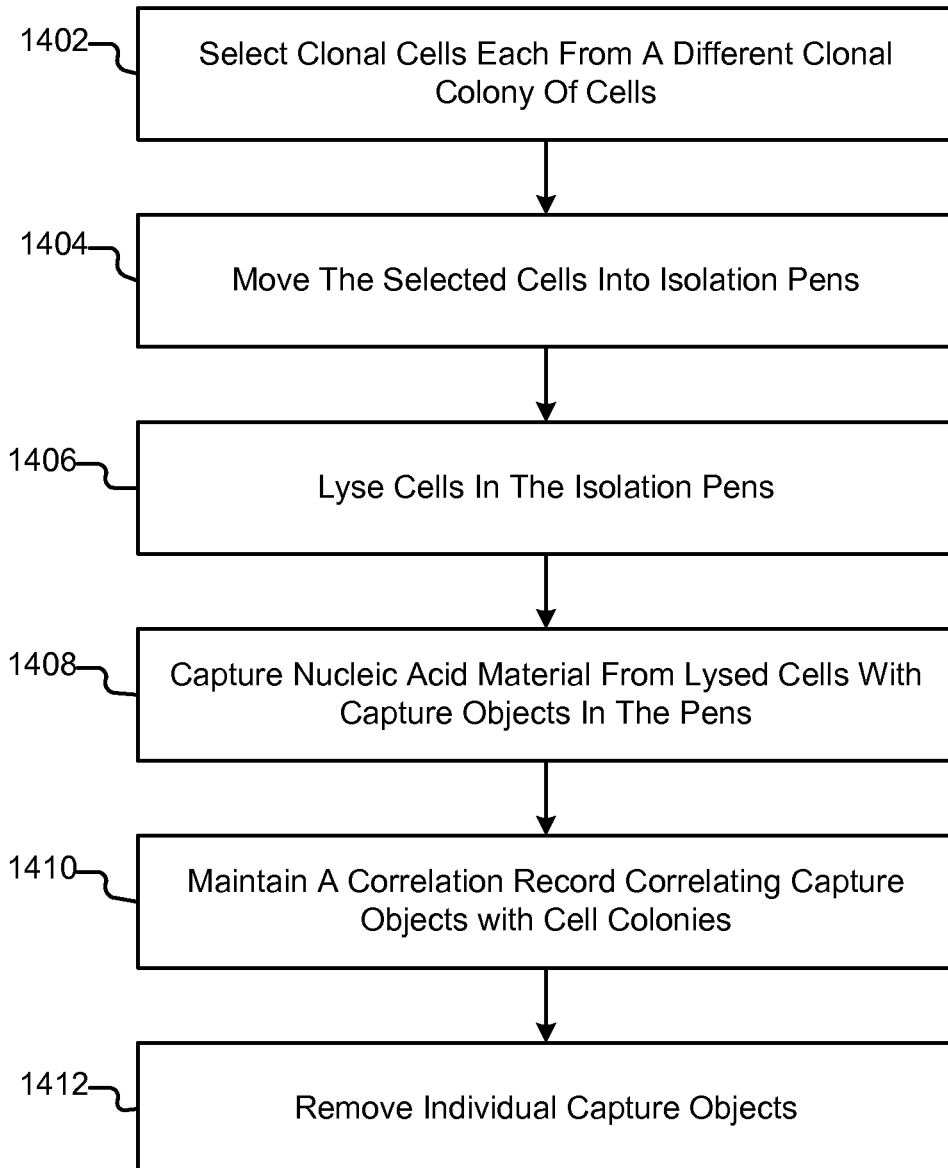


Figure 15

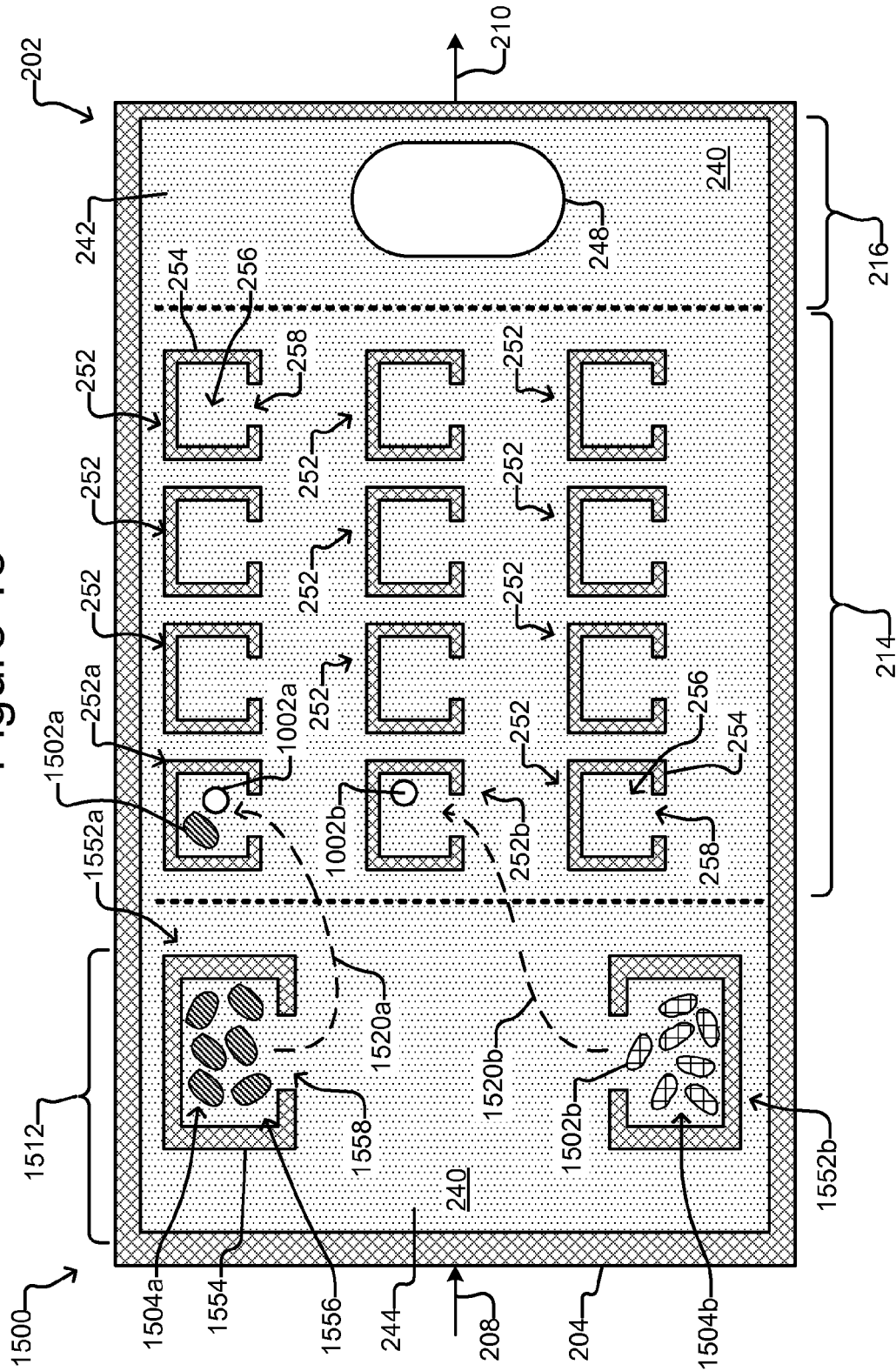


Figure 16

Fig. 16A

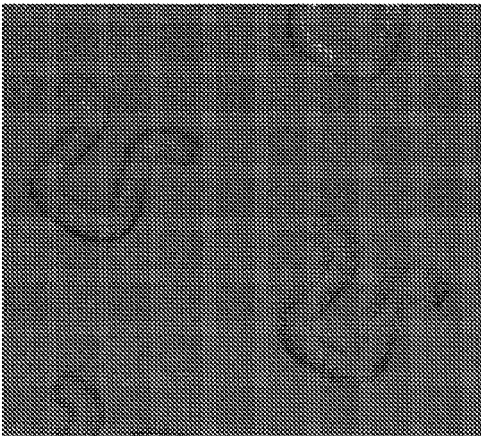


Fig. 16B

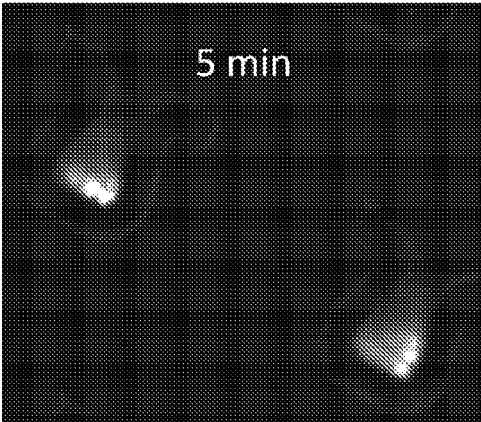
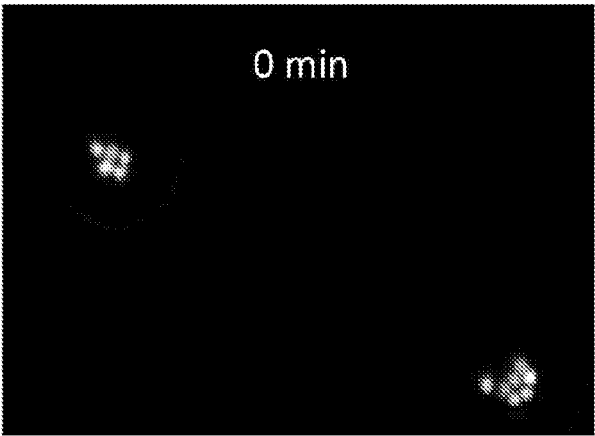


Fig. 16C

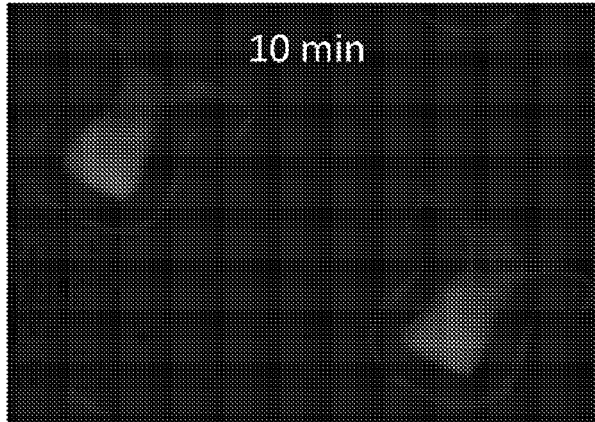


Fig. 16D

**CAPTURING SPECIFIC NUCLEIC ACID
MATERIALS FROM INDIVIDUAL
BIOLOGICAL CELLS IN A MICRO-FLUIDIC
DEVICE**

BACKGROUND

[0001] In biological fields, it can be useful to extract and capture nucleic acid materials from biological cells. Examples of such nucleic acid materials include deoxyribonucleic acid (DNA), ribonucleic acid (RNA), polymers of DNA or RNA, organelles containing DNA or RNA, organelles containing polymers or oligomers of DNA or RNA, and the like. Embodiments of the present invention include devices and processes for extracting and selectively capturing specific types of nucleic acid materials from individual biological cells.

SUMMARY

[0002] In some embodiments of the invention, a process of capturing nucleic acid material from individual biological cells can include disposing individual biological cells into different isolation pens in a micro-fluidic device. The process can also include lysing one of the cells in the isolation pens and capturing with a capture object in the isolation pen nucleic acid material from the lysed cell. The process can further include removing the capture object from the isolation pen.

[0003] In some embodiments of the invention, a micro-fluidic device can include a common space, isolation pens, capture objects, and selecting means. The capture objects can be sized to be placed in one of the isolation pens. Each of the capture objects can comprise a capture material that binds to a particular type of nucleic acid material with at least two times greater specificity than it binds to other types of nucleic acid material. The selecting means can be for moving the selected individual cells into different isolation pens.

[0004] In some embodiments of the invention, a micro-fluidic device can include isolation pens, moving means, and correlation means. The isolation pens can be sized to contain a biological cell and a capture object, which can be configured to capture nucleic acid from the biological cell. The moving means can be for moving individual biological cells into the isolation pens. The correlation means can be for generating a correlation record correlating capture objects in the isolation pens with clonal cell colonies from which the biological cells in the isolation pens originated.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] FIG. 1 is an example of a process for selectively capturing nucleic acid material from biological cells according to some embodiments of the invention.

[0006] FIG. 2A is a perspective view of a micro-fluidic device with which the process of FIG. 1 can be performed according to some embodiments of the invention.

[0007] FIG. 2B is a top, cross-sectional view of the micro-fluidic device of FIG. 2A.

[0008] FIG. 2C is a side, cross-sectional view of the micro-fluidic device of FIG. 2A.

[0009] FIG. 3 is a partial, side cross-sectional view of the base of the micro-fluidic device of FIG. 2A illustrating examples of isolation pens configured as cavities into the base according to some embodiments of the invention.

[0010] FIG. 4A is a partial side, cross-sectional view of the micro-fluidic device of FIGS. 2A-2C in which the manipulator is configured as an opto-electronic tweezer (OET) device according to some embodiments of the invention.

[0011] FIG. 4B is a partial top, cross-sectional view of FIG. 4A.

[0012] FIG. 5 illustrates an example of a plurality of cells in a selection portion of the micro-fluidic device of FIGS. 2A-2C according to some embodiments of the invention.

[0013] FIG. 6 is an example of selecting individual biological cells in the selection portion of the micro-fluidic device of FIGS. 2A-2C and moving the selected cells into isolation pens in the device according to some embodiments of the invention.

[0014] FIG. 7 shows an example of lysing cells in the isolation pens of the micro-fluidic device of FIGS. 2A-2C with a lysing reagent according to some embodiments of the invention.

[0015] FIG. 8 is an example of lysing cells in the isolation pens of the micro-fluidic device of FIGS. 2A-2C with a lysing mechanism according to some embodiments of the invention.

[0016] FIG. 9 shows nucleic acid material flowing from the lysed cells into the interior spaces of the isolation pens of the micro-fluidic device of FIGS. 2A-2C according to some embodiments of the invention.

[0017] FIG. 10 illustrates an example of capture objects in one of the pens of the micro-fluidic device of FIGS. 2A-2C according to some embodiments of the invention.

[0018] FIG. 11 shows an example configuration of a capture object according to some embodiments of the invention.

[0019] FIG. 12A illustrates an example of a cell in a pen of the micro-fluidic device of FIGS. 2A-2C showing the outer membrane of the cell and examples of elements internal to the cell.

[0020] FIG. 12B shows an example of lysing the cell of FIG. 12A according to some embodiments of the invention.

[0021] FIG. 12C is an example of lysing one of the internal elements of the cell of FIG. 12A according to some embodiments of the invention.

[0022] FIG. 12D shows an example of lysing the nucleus of the cell of FIG. 12A according to some embodiments of the invention.

[0023] FIG. 13 is an example of selecting and moving capture objects from the isolation pens to the export portion of the micro-fluidic device of FIGS. 2A-2C according to some embodiments of the invention.

[0024] FIG. 14 is an example of a process for selectively capturing nucleic acid material from clonal biological cells according to some embodiments of the invention.

[0025] FIG. 15 illustrates an example of selecting individual clonal biological cells from different clonal colonies in a micro-fluidic device and moving the selected cells into isolation pens in the device according to some embodiments of the invention.

[0026] FIGS. 16A-D are images of cells being lysed within isolation pens in a microfluidic device. FIG. 16A is an image of pens that contain cells prior to introduction of lysis buffer into the microfluidic device. FIGS. 16B, 16C, and 16D are images of the same pens at time t=0 minutes, 5 minutes, and 10 minutes, respectively, after introduction of the lysis buffer. Calcein AM staining of cells is used as a marker to monitor cell lysis.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

[0027] This specification describes exemplary embodiments and applications of the invention. The invention, however, is not limited to these exemplary embodiments and applications or to the manner in which the exemplary embodiments and applications operate or are described herein. Moreover, the figures may show simplified or partial views, and the dimensions of elements in the figures may be exaggerated or otherwise not in proportion. In addition, as the terms “on,” “attached to,” or “coupled to” are used herein, one element (e.g., a material, a layer, a substrate, etc.) can be “on,” “attached to,” or “coupled to” another element regardless of whether the one element is directly on, attached to, or coupled to the other element or there are one or more intervening elements between the one element and the other element. Also, directions (e.g., above, below, top, bottom, side, up, down, under, over, upper, lower, horizontal, vertical, “x,” “y,” “z,” etc.), if provided, are relative and provided solely by way of example and for ease of illustration and discussion and not by way of limitation. In addition, where reference is made to a list of elements (e.g., elements a, b, c), such reference is intended to include any one of the listed elements by itself, any combination of less than all of the listed elements, and/or a combination of all of the listed elements.

[0028] As used herein, “substantially” means sufficient to work for the intended purpose. The term “substantially” thus allows for minor, insignificant variations from an absolute or perfect state, dimension, measurement, result, or the like such as would be expected by a person of ordinary skill in the field but that do not appreciably affect overall performance. When used with respect to numerical values or parameters or characteristics that can be expressed as numerical values, “substantially” means within ten percent. The term “ones” means more than one.

[0029] As used herein, the term “disposed” encompasses within its meaning “located.”

[0030] As used herein, the term “capture object” can encompass one or more of the following: inanimate micro-objects such as microparticles, microbeads (e.g., polystyrene beads, Luminescent™ beads, or the like), magnetic beads, microrods, microwires, quantum dots, and the like; biological micro-objects such as cells, liposomes (e.g. synthetic or derived from membrane preparations), lipid nanorrafts, and the like; or a combination of inanimate micro-objects and biological micro-objects (e.g., microbeads attached to cells, liposome-coated micro-beads, liposome-coated magnetic beads, or the like). Lipid nanorrafts have been described, e.g., in Ritchie et al. (2009) “Reconstitution of Membrane Proteins in Phospholipid Bilayer Nanodiscs,” *Methods Enzymol.*, 464:211-231.

[0031] The term “cell” means a biological cell, which can be a plant cell, an animal cell, a bacterial cell, a fungal cell, embryos, oocytes, sperms, cells dissociated from a tissue, blood cells, hydridomas, cultured cells, cells from a cell line, cancer cells, infected cells, transfected and/or transformed cells, reporter cells, and the like. An animal cell can be, for example, from a mammal, such as a human, a mouse, a rat, a horse, a goat, a sheep, a cow, a primate, or the like.

[0032] As used with respect to a biological cell, “lyse” means to break, rupture, or otherwise compromise at least a membrane of the cell sufficiently to release nucleic acid material from the cell. When used with respect to a biological

cell, “internal element” means any element or component of a biological cell that is inside the outer membrane of the cell and bounded by its own membrane, and lysing an internal element means breaking, rupturing, or otherwise compromising the membrane of the element sufficiently to release nucleic acid from the element. Examples of internal elements of a cell include a nucleus of the cell and organelles.

[0033] In some embodiments of the invention, individual biological cells can be selected in a micro-fluidic device based on any of a number of different possible characteristics. Nucleic acid material can then be extracted from an individual cell while the cell is in an isolation pen in the micro-fluidic device. Capture objects in the pen can each capture a specific type of the nucleic acid material from the cell, after which the capture objects can be removed from the pen and, for example, exported from the micro-fluidic device. The capture objects can include unique identifiers, allowing each capture object to be correlated to the individual cell from which the nucleic acid material captured by the object originated. The unique identifiers can also provide additional information such as the type of nucleic acid material captured from the cell.

[0034] FIG. 1 illustrates an example of a process 100 in which individual biological cells can be selected in a micro-fluidic device at step 102 and moved into isolation pens in the device at step 104. Alternatively, individual cells already in the pens can be selected for one or more particular characteristics at step 102, and the cells in the pens that lack that characteristic or characteristics can be moved out of the pens at step 104, leaving selected cells in the pens. Regardless, the selected cells can be lysed in the isolation pens at step 106, releasing nucleic acid material from the lysed cells into the pens. At step 108, capture objects in the pens can capture specific types of the nucleic acid material. The capture objects can then be removed from the pens at step 110 and exported from, stored in, or further processed in the micro-fluidic device.

[0035] FIGS. 2A-2C show an example of a micro-fluidic device 200 on which the process 100 of FIG. 1 can be performed, and FIGS. 4A and 4B illustrate an example of the manipulator 222 of the device 200 configured as an opto-electronic tweezers (OET) device. FIGS. 5-12 illustrate an example of the process 100 of FIG. 1 performed on the micro-fluidic device 200 the manipulator 222 configured as an OET device, for example, as illustrated in FIGS. 4A and 4B. Before turning to the example of the process 100 performed with the device 200 illustrated in FIGS. 5-12, the micro-fluidic device 200 is discussed.

[0036] FIGS. 2A-2C illustrate an example of a micro-fluidic device 200 on which the process 100 can be performed. As shown, the micro-fluidic device 200 can comprise a housing 202, a manipulator 222, a detector 224, a flow controller 226, an export mechanism 228, and a control module 230.

[0037] As shown, the housing 202 can comprise one or more channels 240 for containing a liquid medium 244. FIG. 2B illustrates an inner surface 242 of the channel 240 on which the medium 244 can be disposed as even (e.g., flat) and featureless. The inner surface 242, however, can alternatively be uneven (e.g., not flat) and comprise features such as electric terminals (not shown).

[0038] The housing 202 can comprise one or more inlets 208 through which the medium 244 can be input into the

channel **240**. An inlet **208** can be, for example, an input port, an opening, a valve, another channel, fluidic connectors, or the like. The housing **202** can also comprise one or more outlets **210**. For example, medium **244** can be removed through the outlet **210**. An outlet **210** can be, for example, an output port, an opening, a valve, another channel, fluidic connectors, or the like. As another example, an outlet **210** can comprise a droplet outputting mechanism such as any of the outputting mechanisms disclosed in U.S. patent application Ser. No. 13/856,781 filed Apr. 4, 2013 (attorney docket no. BL1-US). All or part of the housing **202** can be gas permeable to allow gas (e.g., ambient air) to enter and exit the channel **240**.

[0039] Although one inlet **208** and one outlet **210** are illustrated, there can be more than one inlet **208** and/or more than one outlet **210**. Moreover, the inlets **208** and/or outlets **210** can be in different locations than shown in FIGS. 2A-2C. For example, there can be an outlet (not shown) from what will be described below as the selection portion **212** of the device **200** for waste such as unselected cells.

[0040] The housing **202** can also comprise a micro-fluidic structure **204** disposed on a base (e.g., a substrate) **206**. The micro-fluidic structure **204** can comprise a flexible material (e.g. rubber, plastic, an elastomer, silicone, polydimethylsiloxane (“PDMS”), or the like), which can be gas permeable. Alternatively, the micro-fluidic structure **204** can comprise other materials including rigid materials, or combinations of flexible and rigid materials. Examples of micro-fluidic structures that define microfluidic elements, such as channels and chambers (or pens), which are bounded at least in part by flexible (e.g., deformable) surfaces are described in U.S. Provisional Patent Application 62/089,065 (filed Dec. 8, 2014), the entire contents of which are incorporated herein by reference. The base **206** can comprise one or more substrates. Although illustrated as a single structure, the base **206** can comprise multiple interconnected structures such as multiple substrates. The micro-fluidic structure **204** can similarly comprise multiple interconnected structures.

[0041] The micro-fluidic structure **204** and the base **206** can define a channel **240**, and/or one or more chambers (e.g., isolation pens **252**). Although one channel **240** is shown in FIGS. 2A-2C, the micro-fluidic structure **204** and the base **206** can define multiple such channels, chambers, and/or the like for the medium **244**, and such channels and chambers can be interconnect to form micro-fluidic circuits.

[0042] As shown in FIGS. 2B and 2C, isolation pens **252** can be disposed in the channel **240**. For example, each isolation pen **252** can comprise an enclosure **254** that defines an interior space **256** and an opening **258** from the channel **240** to the interior space **256**. There can be many such isolation pens **252** in the channel **240** disposed in any pattern, the isolation pens **252** can be any of many different sizes and shapes, and the pens **252** can have more than one opening **258**. The opening **258** of each isolation pen **252** can be sized and positioned to allow for the natural exchange of liquid medium **244** in a pen **252** and liquid medium **244** flowing past the opening **258** of the pen **252** by, for example, diffusion. Alternatively, the opening **258** of each isolation pen **252** can be sized and positioned to allow droplets of aqueous medium (e.g., containing one or more cells, one or more capture objects, and/or reagents, such as lysis buffer) to be moved into or out of the isolation pens **252**. Otherwise, however, the enclosures **254** can sufficiently enclose the interior spaces **256** of the pens **252** to prevent biological

material or objects (not shown) (e.g., biological cells, secreted material, nucleic acid material, or the like) in the interior space **256** of one pen **252** from mixing with such biological material or objects in the interior space **256** of any another pen **252**, and as will be described, prevent mixing of capture objects in one pen **256** from mixing with capture objects of another pen **256**.

[0043] Although twelve pens **252** disposed in three rows are shown, there can be more or fewer pens **252**, and the pens **252** can be disposed in other patterns. Moreover, the pens **252** can have different shapes, sizes, orientations, or the like than shown. For example, the pens **252** can have any of the shapes, sizes, or orientations or be disposed in any of the patterns disclosed in US2014/0116881 (filed Oct. 22, 2013) or U.S. patent application Ser. No. 14/520,568 (filed Oct. 22, 2014), the entire contents of which are incorporated herein by reference.

[0044] Isolation pens **252** comprising enclosures **254** that, as illustrated in FIG. 2C, extend the entire height of the channel **240** (e.g., from the surface **242** of the base **206** to the top of the micro-fluidic structure **204**) are but an example and variations are contemplated. For example, the enclosures **254** need not extend the entire height of the channel **240**.

[0045] FIG. 3 illustrates another example in which isolation pens **352** comprise cavities in the base **206** rather than enclosures **254**. For example, as shown, each pen **352** can comprise an interior space **356** defined by sidewalls **354** of a cavity into the base **206**. The opening **358** of each such pen **352** can be at the surface **242** of the base **206**. Herein, any mention, discussion, illustration, or the like of a pen **252** can be replaced with a pen **352** in which the sidewalls **354**, the interior space **356**, and the opening **358** can correspond, respectively, to the enclosure **254**, interior space **256**, and opening **258** of a pen **252**.

[0046] Medium **244** can be flowed (e.g., from the inlet **208** to the outlet **210**) past the openings **258** in the isolation pens **252**. Such a flow of medium **244** can, for example, provide nutrients to biological objects (not shown) in the isolation pens **252**. As another example, the flow of medium **244** can also provide for the removal of waste from the isolation pens **252**. As will also be seen, the flow of medium **244** can cause material in the medium (e.g., a lysing reagent **706** as illustrated in FIG. 7, which is discussed below), to mix with medium **244** in the pens **252**. Alternatively, the medium **244** can be an oil-based medium that contains droplets of aqueous medium. The droplets can contain cells, capture objects, and/or reagents (e.g., lysis buffer) that can be moved into the isolation pens **252**, and optionally combined therein.

[0047] The manipulator **222** can be configured to create selectively electrokinetic forces on objects (not shown) in the medium **244**. For example, the manipulator **222** can be configured to selectively activate (e.g., turn on) and deactivate (e.g., turn off) dielectrophoresis (DEP) electrodes at the inner surface **242** of the channel **240**. The DEP electrodes can be each connected to an electrical connection through which current and/or voltage levels can be changed to individually activate and deactivate each electrode. As another example, the DEP electrodes can be light activated and deactivated such as in the example illustrated in FIGS. 4A and 4B and discussed below. Regardless, the DEP electrodes can create forces in the medium **244** that attract

or repel objects (not shown) in the medium **244**, and the manipulator **222** can thus select and move one or more objects in the medium **244**.

[0048] For example, the manipulator **222** can comprise one or more optical (e.g., laser) tweezers devices, one or more optoelectronic tweezers (OET) devices (e.g., as disclosed in U.S. Pat. No. 7,612,355 (which is incorporated in its entirety by reference herein) or US2014/0124370, filed Oct. 10, 2013 (which is also incorporated in its entirety by reference herein)), and/or one or more devices having phototransistors (e.g., lateral bipolar transistors). As yet another example, the manipulator **222** can include one or more devices (not shown) for moving a droplet of the medium **244** in which one or more of objects are suspended. Such devices (not shown) can include electrowetting devices such as optoelectronic wetting (OEW) devices (e.g., as disclosed in U.S. Pat. No. 6,958,132, the entire contents of which are incorporated herein by reference), single-sided OEW devices (e.g., as disclosed in US2012/0024708, filed Jul. 31, 2011, or U.S. Provisional Application No. 62/088,532, filed Dec. 5, 2014, both of which are incorporated herein by reference in their entirety), or other electrowetting devices. The manipulator **222** can thus be characterized as a DEP device in some embodiments.

[0049] FIGS. **4A** and **4B** illustrate an example in which the manipulator **222** comprises an OET device **400**, which is a type of DEP device. As shown, the OET device **400** can comprise a first electrode **404**, a second electrode **410**, an electrode activation substrate **408**, a power source **412** (e.g., an alternating current (AC) power source), and a light source **420**. Medium **244** in the channel **240** and the electrode activation substrate **408** can separate the electrodes **404**, **410**. Changing patterns of light **422** from the light source **420** can selectively activate and deactivate changing patterns of DEP electrodes at regions **414** of the inner surface **242** of the channel **240**. (Hereinafter the regions **414** are referred to as “electrode regions.”)

[0050] In the example illustrated in FIG. **4B**, a light pattern **422'** directed onto the inner surface **242** of the base **206** illuminates the cross-hatched electrode regions **414a** in the square pattern shown. The other electrode regions **414** are not illuminated and are hereinafter referred to as “dark” electrode regions **414**. The electrical impedance across the electrode activation substrate **408** from each dark electrode region **414** to the second electrode **410** is greater than the impedance from the first electrode **404** across the medium **244** in the channel **240** to the dark electrode region **414**. Illuminating an electrode region **414a**, however, reduces the impedance across the electrode activation substrate **408** from the illuminated electrode region **414a** to the second electrode **410** to less than the impedance from the first electrode **404** across the medium **244** in the channel **240** to the illuminated electrode region **414a**.

[0051] With the power source **412** activated, the foregoing creates an electric field gradient in the medium **244** between illuminated electrode regions **414a** and adjacent dark electrode regions **414**, which in turn creates local DEP forces that attract or repel nearby objects (not shown) in the medium **244**. DEP electrodes that attract or repel objects in the medium **244** can thus be selectively activated and deactivated at many different such electrode regions **414** at the inner surface **242** of the channel **240** by changing light patterns **422** projected from a light source **420** (e.g., a laser source, a high intensity discharge lamp, or other type of light

source) into the micro-fluidic device **200**. Whether the DEP forces attract or repel nearby objects can depend on such parameters as the frequency of the power source **412** and the dielectric properties of the medium **244** and/or the objects (not shown).

[0052] The square pattern **422'** of illuminated electrode regions **414a** illustrated in FIG. **4B** is an example only. Any pattern of the electrode regions **414** can be illuminated by the pattern of light **422** projected into the device **200**, and the pattern of illuminated electrode regions **422'** can be repeatedly changed by changing the light pattern **422**.

[0053] In some embodiments, the electrode activation substrate **408** can be a photoconductive material, and the inner surface **242** can be featureless. In such embodiments, the DEP electrodes **414** can be created anywhere and in any pattern on the inner surface **242** of the channel **240** in accordance with the light pattern **422** (see FIG. **4A**). The number and pattern of the electrode regions **414** are thus not fixed but correspond to the light pattern **422**. Examples are illustrated in the aforementioned U.S. Pat. No. 7,612,355 in which the un-doped amorphous silicon material **24** shown in the drawings of the foregoing patent can be an example of photoconductive material that can compose the electrode activation substrate **408**.

[0054] In other embodiments, the electrode activation substrate **408** can comprise a circuit substrate such as a semiconductor material comprising a plurality of doped layers, electrically insulating layers, and electrically conductive layers that form semiconductor integrated circuits such as is known in semiconductor fields. In such embodiments, electric circuit elements can form electrical connections between the electrode regions **414** at the inner surface **242** of the channel **240** and the second electrode **410** that can be selectively activated and deactivated by the light pattern **422**. Non-limiting examples of such configurations of the electrode activation substrate **408** include the phototransistor-based OET device **400** illustrated in FIGS. **21** and **22** of U.S. Pat. No. 7,956,339 and the OET devices illustrated throughout the drawings in the aforementioned U.S. patent application Ser. No. 14/051,004 (attorney docket no. BL9-US). The phototransistors can be, for example, lateral bipolar phototransistors.

[0055] In some embodiments, the first electrode **404** can be part of a first wall **402** of the housing **202**, and the electrode activation substrate **408** and second electrode **410** can be part of a second wall **406** of the housing **202** generally as illustrated in FIG. **4A**. As shown, the channel **240** can be between the first wall **402** and the second wall **406**. The foregoing, however, is but an example. In other embodiments, the first electrode **404** can be part of the second wall **406** and one or both of the electrode activation substrate **408** and/or the second electrode **410** can be part of the first wall **402**. As another example, the first electrode **404** can be part of the same wall **402** or **406** as the electrode activation substrate **408** and the second electrode **410**. For example, the electrode activation substrate **408** can comprise the first electrode **404** and/or the second electrode **410**. Moreover, the light source **420** can alternatively be located below the housing **202**.

[0056] Configured as the OET device **400** of FIGS. **4A** and **4B**, the manipulator **222** can thus select an object (not shown) in the medium **244** in the channel **240** by projecting a light pattern **422** into the device **200** to activate one or more DEP electrodes at electrode regions **414** of the inner

surface 242 of the channel 240 in a pattern that captures the object. The manipulator 222 can then move the captured object by moving the light pattern 422 relative to the device 200. Alternatively, the device 200 can be moved relative to the light pattern 422. Examples are illustrated in FIGS. 6 and 12 and discussed below. Although the enclosures 254 that define the isolation pens 252 are illustrated in FIGS. 2B and 2C and discussed above as physical enclosures, the enclosures 254 can alternatively be virtual enclosures comprising DEP forces activated by the light pattern 422.

[0057] As mentioned, the OET device 400 of FIGS. 4A and 4B is but an example of the manipulator 222. For example, although the electrode regions 414 are illustrated and discussed above as being activated and deactivated by a changing light pattern 422, device 400 can instead provide electrical connections (not shown) to each electrode region 414 (which can comprise an electrically conductive terminal at the surface 242) and individually activate and deactivate each electrode region 414 by controlling the voltage and/or current provided to each electrode region 414 through the electrical connections. So configured, the device 400 need not include the light source 420 or direct the light pattern 422 into the device 400. Another alternative is an OEW device, such as a single-sided OEW device, or a combined OET/OEW device, such as described in U.S. application Ser. No. 14/262,140, filed Apr. 25, 2014, or U.S. application Ser. No. 14/262,200, filed Apr. 25, 2014, both of which are incorporated herein by reference in their entirety. In addition, forces that can be applied uniformly across a micro-fluidic device, such as gravity, can be used in conjunction with any of the foregoing, as described in U.S. Provisional Application No. 62/090,303, filed Dec. 10, 2014, the entire contents of which are incorporated herein by reference.

[0058] With reference again to FIGS. 2A-2C, it is noted that the detector 224 can be a mechanism for detecting events in the channel 240. For example, the detector 224 can comprise a photodetector capable of detecting one or more radiation characteristics (e.g., due to fluorescence or luminescence) of an object (not shown) in the medium. Such a detector 224 can be configured to detect, for example, that one or more objects (not shown) in the medium 244 are radiating electromagnetic radiation and/or the approximate wavelength, brightness, intensity, or the like of the radiation. Examples of suitable photodetectors include without limitation photomultiplier tube detectors and avalanche photodetectors.

[0059] The detector 224 can alternatively or in addition comprise an imaging device for capturing digital images of the channel 240 including objects (not shown) in the medium 244. Examples of suitable imaging devices that the detector 224 can comprise include digital cameras or photosensors such as charge coupled devices and complementary metal-oxide-semiconductor imagers. Images can be captured with such devices and analyzed (e.g., by the control module 230). Such images can also be displayed on a display device such as a computer monitor (not shown).

[0060] The flow controller 226 can be configured to control a flow of the medium 244 in the channel 240. For example, the flow controller 226 can control the direction and/or velocity of the flow. Non-limiting examples of the flow controller 226 include one or more pumps or fluid actuators. In some embodiments, the flow controller 226 can include additional elements such as one or more sensors (not

shown) for sensing, for example, the velocity of the flow of the medium 244 in the channel 240.

[0061] The export mechanism 228 can facilitate export of objects (not shown) from the micro-fluidic device 200. For example, as illustrated in FIGS. 2B and 2C, the export mechanism 228 can comprise a staging area 248 and a passage 246 through the housing 202. The passage 246 can alternatively be through the base 206 or a sidewall of the micro-fluidic structure 204. Objects (not shown) can be moved to the staging area 248 and exported from the device 200 through the passage 246. The export mechanism 228 can be, for example, like any of the examples of export mechanisms disclosed in U.S. patent application Ser. No. 14/520,510 (filed Oct. 22, 2014). Alternatively, the export mechanism 228 can simply comprise an outlet 210.

[0062] The control module 230 can be configured to receive signals from and control the manipulator 222, the detector 224, the flow controller 226, and/or the export mechanism 228. As shown, the control module 230 can comprise a controller 232 and a memory 234. In some embodiments, the controller 232 can be a digital electronic controller (e.g., a microprocessor, microcontroller, computer, or the like) configured to operate in accordance with machine readable instructions (e.g., software, firmware, microcode, or the like) stored as non-transitory signals in the memory 234, which can be a digital electronic, optical, or magnetic memory device. Alternatively, the controller 232 can comprise hardwired digital circuitry and/or analog circuitry or a combination of a digital electronic controller operating in accordance with machine readable instructions and hardwired digital circuitry and/or analog circuitry.

[0063] As illustrated, the micro-fluidic device 200 can comprise a selection portion 212 (which can be an example of a common space in the device 200), an isolation portion 214, and/or an export portion 216. These portions 212, 214, 216 can be represent physical partitions of the device 200 or merely conceptual partitions. Regardless, as will be seen, biological cells (not shown) can be loaded into the selection portion 212, where individual ones of the biological cells (not shown) can be identified and selected. The isolation portion 214 can comprise the isolation pens 252, where the individual biological cells (not shown) selected in the selection portion 212 can be placed and isolated one from another.

[0064] As noted, FIGS. 5-12 illustrate an example of operation of the process 100 on the micro-fluidic device 200 of FIGS. 2A-2C. The process 100 is now discussed with reference to examples illustrated in FIGS. 5-12.

[0065] As shown in FIG. 1, at step 102, the process 100 can select individual biological cells. FIGS. 5 and 6 illustrate an example. As shown in FIG. 5, there can be biological cells 502 in the selection portion 212 of the micro-fluidic device 200. The cells 502 can all be the same type of cell. Alternatively, the cells 502 can comprise a variety of different types of cells. Regardless, the cells 502 can be loaded into the micro-fluidic device 200 through, for example, an inlet 208.

[0066] The process 100 can select one or more of the cells 502 individually based on any of a variety of different criteria or desired characteristics. For example, the process 100 can, as part of step 102, test the cells 502 in the selection portion 212 of the device 200 for one or more particular characteristics and select ones of the cells 502 determined to have the characteristic or characteristics. As another

example, the process 100 can select ones of the cells 502 determined not to have the characteristic or characteristics.

[0067] Examples of characteristics that can be tested for as part of step 102 include the size and/or morphology (e.g., form and structure) of the cells 502. Thus, for example, the detector 224 can capture images of the cells 502 in the selection portion 212 of the device 200. The captured images of the cells 502 can then be analyzed to identify ones of the cells 502 that meet one or more predetermined size or morphology characteristics. For example, the captured images of the cells 502 can be analyzed to identify ones of the cells 502 that meet one or more of the following characteristics related to size: larger than, smaller than, or substantially equal to a predetermined threshold size or within a range of sizes between a high threshold size and a low threshold size. As another example, the captured images of the cells 502 can be analyzed to identify ones of the cells 502 that meet one or more predetermined morphology characteristics relating to the form and/or structure of the cells 502. Regardless, the captured images of the cells 502 can be displayed (e.g., on an electronic display device (not shown)) and analyzed by a human operator. Alternatively or in addition, the captured images of the cells 502 can be analyzed by the control module 230. For example, the control module 230 can comprise machine readable instructions (e.g., software, firmware, microcode, or the like) stored in the memory 234 and/or hardwired electrical circuits (not shown) for analyzing such images and identifying ones of the cells 502 that meet particular criteria regarding size or morphology.

[0068] Other examples of characteristics that can be tested for as part of step 102 include determining whether the cells 502 comprise or produce (e.g., express or secrete) one or more particular substances (e.g., a particular protein, a particular antibody, or the like). For example, the cells 502 can be treated (before or after being loaded into the selection portion 212 of the device 200) with a reagent that reacts in a distinct, detectable manner to the presence of one or more of such particular substances. Examples of such reagents include markers that stain cells 502 that comprise or produce a particular substance. The detector 224 can capture images of the treated cells 502 in the selection portion 212 of the device 200, and the images of the cells 502 can be analyzed to identify ones of the cells 502 that indicate the presence (or absence) of the particular substance. As noted, the images of the cells 502 can be displayed for and analyzed by a human user and/or analyzed by the control module 230 generally as discussed above. Methods of detecting cellular characteristics, such as size, morphology, and/or protein expression (e.g., antibody expression) have been described, for example, in U.S. application Ser. Nos. 14/520,568 and 14/521,447, both filed Oct. 22, 2014, and both of which are incorporated herein by reference in their entirety.

[0069] The detector 224 and/or the controller 230 programmed to analyze images of the cells 502 in the selection portion 212 of the device 200 can be an example of a means for identifying individual biological cells for a particular characteristic.

[0070] Thus, at step 102, the process 100 can test the cells 502 in the selection portion 212 of the device 200 for one or more specific characteristics (which can be different characteristics) and select one or more of the cells 502 that test positive for one or more of those specific characteristics.

Alternatively, the process 100 can, at step 102, select one or more of the cells 502 that test negative for such characteristics.

[0071] Regardless, at step 104, the process 100 can move cells 502 selected at step 102 from the selection portion 212 of the device 200 into isolation pens 252 in the isolation portion 214 of the device 200. For example, each selected cell 502 can be moved into a different pen 252 such that each pen 252 contains one and only one of the cells 502 selected at step 102.

[0072] FIG. 6 illustrates an example of selecting individual cells 502 in the selection portion 212 of the device 200 (which can be part of step 102) and moving the selected individual cells 502 into isolation pens 252 (step 104). As shown in FIG. 6, the process 100 can select at step 102 a specific, individual cell 502 by trapping a desired cell 502 with a light trap 602 in the selection portion 212 of the device 200. For example, the manipulator 222 (see FIGS. 2A-2C) configured as the OET device 400 of FIGS. 4A and 4B can generate light traps 602 that trap individual cells 502. The OET device 400 can then move the light traps 602 into the pens 252, which moves the trapped cells 502 into the pens 252. As illustrated, each cell 502 can be individually trapped and moved into a holding pen 252.

[0073] The light traps 602 can be part of a changing pattern 422 of light projected onto an inner surface 242 of the channel 240 of the micro-fluidic device 200 as discussed above with respect to FIGS. 4A and 4B. Once a selected cell 502 is in a pen 252, the light trap 602 corresponding to that cell 502 can be turned off. The detector 224 can capture images of all or part of the channel 240 including images of the cells 502 and the pens 252, and those images can facilitate trapping and moving specific, individual cells 502 into specific pens 252. The detector 224 and/or the manipulator 222 (e.g., configured as the OET device of FIGS. 4A and 4B) can thus be one or more examples of a means for selecting and moving individual cells 502 from the selection portion 212 into pens 252 in the isolation portion 214 of the device 200.

[0074] The manipulator 222 is an example of a means for selecting individual biological cells 502 (e.g., in the selection portion 212 and/or the pens 252 of the device 200) and moving the selected individual cells 502 (e.g., into or out of isolation pens 252). Any configuration (including but not limited to the OET device illustrated in FIGS. 4A and 4B) of the manipulator 222 illustrated, discussed, or disclosed herein is thus an example of means for selecting individual biological cells 502 in the device 200 and/or moving the selected individual cells 502 in the device 200. A globally acting force such as gravity (e.g., applied by means of a tilted or tiltable support for the microfluidic device 200) can be used to assist with moving the cells 502. Alternatively, individual cells 502 that are contained within droplets of aqueous medium can be selected and moved into a holding pen 252 using an OEW device.

[0075] As noted above, alternatively, cells 502 can be in the pens 252 prior to step 102, and the process 100 can select at step 102 cells 502 that are in the pens 252 for one of more characteristics generally as discussed above. The process 100 can then, at step 104, move unselected cells 502 out of the pens 252, leaving selected cells 502 in the pens 252.

[0076] Returning again to FIG. 1, at step 106, the process 100 can lyse cells 502 in the isolation pens 252. FIGS. 7 and 8 illustrate examples of lysing cells 502 in pens 252, which

can thus be examples of lysing pens. Cells **502** that are lysed at step **106** are labeled **702** in FIGS. 7-12.

[0077] As shown in FIG. 7, cells **502** in isolation pens **252** can be lysed to produce lysed cells **702** by flowing **704** a lysing reagent **706** through the isolation portion **214** of the device **200**. For example, the lysing reagent **706** can be flowed from the inlet **208** to the outlet **210** for a sufficient time period for the lysing reagent **706** to enter into the interior spaces **256** of the pens **252** (e.g., by diffusion through the openings **258** of the pens **252**) and lyse cells **502** in the pens **252**. Although not shown, thereafter medium **244** can be flowed through the isolation portion **214** of the device sufficient to flush the lysing reagent **706** from the device **200**. Alternatively, one or more droplets of lysing reagent **706** can be moved into each pen **252** (e.g., using an OEW device) and merged with a droplet containing a cell **502** to be lysed.

[0078] Lysing reagent **706** can be any suitable lysis buffer (or combined lysis/nucleic acid binding buffer) known in the art. For example, the lysis buffer can include a buffering agent, a chelating agent, salt, a detergent or chaotropic agent, an RNase inhibitor, a protease, a denaturant, or any combination thereof. The buffering agent can be, for example, a Tris buffer such as TrisHCl (e.g., at a concentration of about 10 mM to about 100 mM). The buffering agent can provide a physiologically-compatible pH (e.g., about pH 7.0 to about pH 8.5). The chelating agent can be, for example, a divalent cation chelating agent, such as EDTA or EGTA (e.g., at a concentration of about 1 mM to about 10 mM). The salt can be, for example, a chloride salt, such as LiCl, NaCl, or KCl (e.g., at a concentration of about 100 mM to about 1 M). The detergent can be, for example, an ionic detergent, such as sodium dodecyl sulfate (SDS), lithium dodecyl sulfate (LiDS), or the like (e.g., at a concentration of about 0.1% to about 1.0%), a non-ionic detergent, such as Triton X-100, NP-40, a Tween detergent (e.g., Tween 20), or the like (e.g., at a concentration of about 0.1% to about 2.0%). The chaotropic agent can, for example, comprise guanidine (e.g., guanidine HCl or guanidine isothiocyanate) or urea (e.g., at a concentration of about 0.1 M to about 6.0 M). The RNase inhibitor can be at a concentration of about 0.1 to 2.0 units per microliter. The protease can be, e.g., Proteinase K or the like (e.g., at a concentration of about 100 ng/ml to about 1 mg/ml). The denaturant can include, for example, formamide or DTT (e.g., at a concentration of about 0.01 M to about 1 M). Thus, in certain embodiments, the lysing reagent **706** can comprise a buffering agent (e.g., Tris HCl), a chloride salt (e.g., NaCl), an ionic and/or non-ionic detergent (e.g., SDS), a protease, and an RNase inhibitor. In other embodiments, the lysing reagent **706** can comprise a buffering agent (e.g., Tris HCl), a chloride salt (e.g., LiCl), a divalent cation chelating agent (e.g., EDTA), a denaturant (e.g., DTT), and an ionic and/or non-ionic detergent (e.g., LiDS).

[0079] FIG. 8 illustrates another example of lysing cells **502** in the pens **252** to produce lysed cells **702**. As shown, FIG. 8 includes a lysing mechanism **806**, which can be part of or separate from the device **200**. The lysing mechanism **806** can be controlled to direct lysing beams **808** at one or more of the cells **502** in the pens **252** to produce lysed cells **702**. Each lysing beam **808** can comprise sufficient energy to lyse one of the cells **502**. The lysing mechanism **806** can be, for example, a laser mechanism, and the lysing beams **808** can comprise laser beams. The lysing mechanism **806** can be

controlled (e.g., by the control module **230** of FIG. 2A) to direct a lysing beam **808** at a specific one of the cells **502**.

[0080] The lysing mechanism **806** can be controlled to lyse selectively individual cells **502** one at a time. For example, the lysing mechanism **806** can be controlled to lyse cells **502** in the pens **252** sequentially one at a time. As another example, the lysing mechanism **806** can be controlled to lyse a subset of more than one but less than all of the cells **502** in the pens **252** substantially in parallel. As yet another example, the lysing mechanism **806** can be controlled to lyse all of the cells **502** in the pens **252** substantially simultaneously.

[0081] FIGS. 7 and 8 illustrate examples of lysing cells **502** in the pens **252**. Other examples of lysing include applying electroporation, temperature (e.g., heat that exceeds an upper lysing threshold or cold that is less than a lower lysing threshold), electric field energy, or acoustic energy to one or more of the cells **502** in the pens **252**. For example, the lysing mechanism **806** can be replaced with a similar mechanism for applying electroporation, electric field energy, or acoustic energy to or controlling the temperature of one or more of the cells **502** sufficiently to lyse the cells **502**. Another example of an alternative way to lyse cells **502** is capturing and moving (e.g., with the manipulator **222** of FIGS. 2A-2C) cells **502** into contact with a mechanical piercing device (not shown) such as a knife structure, a spear structure, or the like. Any of the foregoing or other devices and processes can be used to lyse one or more of the cells **502** in the pens **252** at step **106** to produce lysed cells **702**.

[0082] Regardless of how lysed, the membrane of a lysed cell **702** is sufficiently disrupted that nucleic acid material from the lysed cell **702** is free to flow out of the lysed cell **702** and into the interior space **256** of the corresponding pen **252** (or within an aqueous droplet contained within the corresponding pen **252**). An example is shown in FIG. 9, which shows nucleic acid material **902** from lysed cells **702** in pens **252**. As noted, the isolation pens **252** can prevent nucleic acid material **902** from a lysed cell **702** in one pen **252** from flowing into and mixing with nucleic acid material **902** from a different lysed cell **702** in another pen **252**. The isolation pens **252** can also prevent droplets, materials, elements, or objects (e.g., capture objects **1002** to be discussed below) in one pen **252** for mixing with droplets, materials, elements, or objects in the other pens **252**.

[0083] The nucleic acid material **902** can comprise, for example, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or the like. Such DNA can be any type of DNA including mitochondrial DNA (mitDNA), nuclear DNA (nDNA), or exome DNA. Such RNA can be any type of RNA including micro RNA (miRNA), messenger RNA (mRNA), ribosomal RNA (rRNA), small nuclear RNA (snRNA), or transfer RNA (tRNA).

[0084] The lysing mechanism **806** (e.g., a laser) configured to generate and direct lysing energy **808** (e.g., laser beams) at individual cells **502** in the isolation pens **252**, an electroporation device configured to electroporate cells **502** in the isolation pens **252**, a temperature control device configured to heat or cool cells **502** in the isolation pens **252** sufficiently to lyse the cells **502**, or an acoustic device configured to apply sufficient acoustic energy to cells **502** in the isolation pens **252** to lyse the cells **502** are all examples of lysing means for lysing cells **502** in the isolation pens **252**.

[0085] In some embodiments, the process 100 can, as part of step 106, control the time of lysing of one or more of the cells 502 in the pens 252.

[0086] For example, as part of step 106, the process 100 can time the lysing of one or more cells 502 in the pens 252 to correspond to one or more of the characteristics of the cells 502 utilized at step 102 to select the cells 502. Thus, the process 100 can control the timing of the lysing of one or more cells 502 in the pens 252 to correspond to a particular morphology or size of the cells 502 or material composing or secreted from the cells 502 as detected as part of step 102. Thus, for example, one or more cells 502 in the pens 252 having a size in a first size range can be lysed at a first time, then one or more cells 502 in the pens having a size in a second size range (which can be different than the first size range) can be lysed at a second time (which can be different than (e.g., later or earlier in time) than the first time), etc. As another example, cells 502 in the pens 252 having a particular morphology characteristic can be lysed at a first time, then one or more cells 502 in the pens 252 having a different morphology characteristic can be lysed at a second time (which can be different than (e.g., later or earlier in time) than the first time), etc. In certain embodiments, the amount of time that it takes to lyse one or more cells can be, for example about 1 to about 10 minutes (e.g., about 5 to about 10 minutes).

[0087] As another example of controlling the timing of lysing at step 106, the process 100 can time the lysing of one or more cells 502 in the pens 252 to correspond to a particular event. For example, step 106 can include monitoring the pens 252 and/or the selection region 212 for a particular event, and the process 100 can then time lysing of one or more cells 502 in the pens 252 from the detected event. Examples of the event can include a change in morphology or secretion or dividing of one or more cells 502 in the pens 252 or the selection region 212. The selection region 212 and/or the pens 252 can be monitored for such events by capturing images of the pens 252 and/or the selection region 212 with the detector 224, and the images can be analyzed by a human operator and/or the control module 230 configured (e.g., programmed with software, microcode, firmware, or the like) to analyze such images generally as discussed above.

[0088] The timing of lysing can be controlled by controlling any of the lysing mechanisms discussed above. For example, a human user and/or the control module 230 can control the lysing mechanism 806 to lyse particular cells 502 in the pens 252 at specific times. As another example, although not shown, the device 200 can comprise multiple channels like channel 240, and each of those channels 240 can include a set of isolation pens 252. The lysing time of cells 502 in the pens 252 in each such channel 240 can be controlled by selectively controlling application of lysing to each channel 240. For example, a lysing reagent (e.g., like 706) can be flowed at different times through each individual channel 240. As another example, a lysing temperature, lysing electric field energy, lysing acoustic energy, or the like can be selectively applied at different times to each channel 240.

[0089] Referring again to FIG. 1, at step 108, one or more types of the nucleic acid material from cells lysed at step 106 can be captured with one or more capture objects in the pens. FIG. 10, which depicts one of the pens 252, illustrates an example.

[0090] As shown in FIG. 10, one or more capture objects 1002 (two are shown but there can be more or fewer) can be disposed in the interior space 256 of a pen 252 with a lysed cell 702. As will be seen, each such capture object 1002 can be configured to bind a particular type of nucleic acid material 902 from the lysed cell 702 in the pen 252. There can be one or more similar capture objects in each of the pens 252 in the device 200.

[0091] FIG. 11 illustrates an example configuration of an object 1002. That is, each capture object 1002 in any of the pens 252 of the device 200 can be configured like the capture object 1002 illustrated in FIG. 11.

[0092] As shown in FIG. 11, a capture object 1002 can comprise a base 1102 and a capture material 1104. The base 1102 can be a micro-object such as a micro-bead, a micro-rod, or the like. The base can be, for example, a streptavidin coated bead, a magnetic bead, or the like. The capture material 1104 can comprise a material that binds a specific type of nucleic acid material with a significantly greater (e.g., two, three, five, ten, or more times greater) specificity than any other type of nucleic acid material. For example, the capture material 1104 can bind a specific type of DNA or RNA (e.g., any of the types of DNA or RNA identified above) with a greater (e.g., two, three, five, ten, or more times greater) specificity than any other type of DNA or RNA. Each capture object 1002 in a pen 252 with a lysed cell 702 can have a different capture material 1104 and thus capture a different type of the nucleic acid material (e.g., DNA or RNA) from the lysed cell 702 in the pen 252. Alternatively, each capture object 1002 in a pen 252 with a lysed cell 702 can have the same capture material 1104. In one example, poly-dT oligos can be used to bind mRNA. Alternatively, the oligos can specifically bind to the conserved regions of mRNAs that encode antibody heavy chains and/or light chains.

[0093] As also shown in FIG. 11, each capture object 1002 can comprise an identifier 1106, which can comprise a code that uniquely identifies the capture object 1002. Each capture object 1002 in the pens 252 can thus have a unique identifier 1106 so that all of the capture objects 1002 in the device 200 can be uniquely identified one from another.

[0094] The identifier 1106 can be any element or material that can uniquely identify a capture object 1002 and facilitate distinguishing one capture object 1002 from another capture object 1002. For example, the identifier 1106 can comprise a biological substance that uniquely identifies the capture object 1002. Synthetic nucleic acid material, such as oligonucleotides (e.g., relatively short, single-stranded DNA or RNA molecules), manufactured to have a unique, user-specified sequence is an example of such an identifier 1106. The identifier 1106 of each of a plurality of capture objects 1002 can have a different such user-specified sequence, allowing the capture objects 1002 to be readily distinguished one from another. As another example, the identifier 1106 can comprise an electronically, optically, or magnetically readable element with a code that uniquely identifies the capture object 1002.

[0095] Capture objects 1002 can be placed into the pens 252 as part of step 108 of FIG. 1. Alternatively, capture objects 1002 can be placed into the pens 252 before, during, or after any of steps 102-106. The capture objects 1002 can be placed into pens 252 along with a binding buffer that is conducive to binding between the capture objects 1002 and target nucleic acids.

[0096] The binding buffer can be the same as the lysis buffer, as described above. Thus, for example, a combined lysis/binding buffer can be used for both steps 106 and 108 of the method of FIG. 1. In certain embodiments, a suitable lysis/binding buffer can comprise a buffering agent, a chelating agent, salt, a detergent, a denaturant, or any combination thereof. The buffering agent can be, for example, a Tris buffer such as TrisHCl (e.g., at a concentration of about 10 mM to about 100 mM). The buffering agent can provide a physiologically-compatible pH (e.g., about pH 7.0 to about pH 8.5). The chelating agent can be, for example, a divalent cation chelating agent, such as EDTA or EGTA (e.g., at a concentration of about 1 mM to about 10 mM). The salt can be, for example, a chloride salt, such as LiCl, NaCl, or KCl (e.g., at a concentration of about 100 mM to about 1 M). The detergent can be, for example, an ionic detergent, such as sodium dodecyl sulfate (SDS), lithium dodecyl sulfate (LiDS), or the like (e.g., at a concentration of about 0.1% to about 1.0%), a non-ionic detergent, such as Triton X-100, NP-40, a Tween detergent (e.g., Tween 20), or the like (e.g., at a concentration of about 0.1% to about 2.0%). The denaturant can include, for example, formamide or DTT (e.g., at a concentration of about 0.01 M to about 1 M). Thus, for example, the combined lysis/binding buffer can comprise a buffering agent (e.g., Tris HCl), a chloride salt (e.g., LiCl), a divalent cation chelating agent (e.g., EDTA), a denaturant (e.g., DTT), and an ionic and/or non-ionic detergent (e.g., LiDS).

[0097] In certain embodiments, a suitable binding buffer can comprise a buffering agent, a chelating agent, salt, or any combination thereof. The buffering agent can be, for example, a Tris buffer such as TrisHCl (e.g., at a concentration of about 10 mM to about 100 mM). The buffering agent can provide a physiologically-compatible pH (e.g., about pH 7.0 to about pH 8.5). The chelating agent can be, for example, a divalent cation chelating agent, such as EDTA or EGTA (e.g., at a concentration of about 1 mM to about 10 mM). The salt can be, for example, a chloride salt, such as LiCl, NaCl, or KCl (e.g., at a concentration of about 100 mM to about 1 M). Thus, for example, the binding buffer can comprise a buffering agent (e.g., Tris HCl), a chloride salt (e.g., LiCl), and a divalent cation chelator (e.g., EDTA).

[0098] Specific individual capture objects 1002 can be placed in each of the pens 252, for example, in the same way selected cells 502 are placed into the pens 252: capture objects 1002 can be loaded through the inlet 208 into the selection portion 212 of the device 200, and specific individual capture objects 1002 can be individually trapped with a light trap (not shown) and moved into a specific pen 252 generally like a selected cell 502 can be trapped by a light trap 602 and moved into a pen 252 as discussed above. Alternatively, capture objects 1002 can be contained within aqueous droplets and the droplets can be moved into the pens 252, for example, using OEW. The individual capture objects 1002 can be moved into a pen 252, and such movement can be in parallel, serially one at a time, or in part in parallel and in part serially.

[0099] As noted, each of the one or more objects 1002 in a pen 252 with a lysed cell 702 can have a different capture material 1104 and thus capture a different, specific type of nucleic acid material from the lysed cell 702. The process 100 can thus capture any one or more specific types of nucleic acid material from the lysed cell 702 in a pen 252.

[0100] As also noted, the enclosure 254 of each pen 252 can be configured to keep the nucleic acid material 902 within the interior space 256 of the pen 252. Alternatively or in addition, a blocking object 1004 can be placed generally in the opening 258 of a pen 252, for example, as illustrated in FIG. 10. The blocking object 1004 can be generally similar to a capture object 1002 except that the blocking object 1004 can be configured to bind with a relatively high specificity most or all of the different types of nucleic acid material 902 from the lysed cell 702 in the pen 252. In still other alternatives, oil used for an OEW-type configuration can be located in the space between isolation pens 252 (e.g., a channel) and optionally in the opening 258 of the pens 252. The blocking object 1004 or oil (not shown) can thus further prevent nucleic acid material 902 from a lysed cell 702 in a pen 252 from escaping the pen 252 and mixing with nucleic acid material 902 in another pen 252.

[0101] The blocking object 1004 can be similar to a capture object 1002. For example, the blocking object 1004 can comprise a base (not shown but can be like base 1102 of FIG. 11) and a capture material (not shown but can be like capture material 1104). As noted, however, the capture material (not shown) of the blocking object 1004 can be configured to bind most or all of the nucleic acid material 902 from a lysed cell 702 in the pen 252.

[0102] In the examples illustrated in FIGS. 7-10, the outer membrane of a cell 502 in a pen 252 and any number from zero to all of the membranes of elements internal to the cell 502 can be lysed at step 1006 of FIG. 1. Each lysed cells 702 can thus have its outer membrane and none, some, or all of any internal membranes inside the cell 702 lysed at step 106, and the nucleic acid material 902 can comprise some or all of the nucleic acid material 902 from anywhere inside a lysed cell 702. As discussed above, at step 108, specific types of the nucleic acid material 902 in the pen 252 can be captured with one or more capture objects 1002 in the pen 252.

[0103] FIGS. 12A and 12B illustrate an example in which step 106 of FIG. 1 can be performed such that only a selected one or more of the membranes of a cell 502, but not all of the membranes, are lysed.

[0104] FIG. 12A (which, like FIG. 10, shows one of the pens 252 in the device 200) illustrates example components of a cell 502 in the pen 252. Components of the cell 502 can include a nucleus 1204 and organelles 1208 (two are shown but there can be more or fewer). As is known, an outer membrane 1202 bounds the cell 502, a nuclear membrane 1206 bounds the nucleus 1204, and a mitochondrial membrane 1210 bounds each organelle 1208.

[0105] As shown in FIG. 12B, rather than lyse all of the membranes 1202, 1206, 1210 of the cell 502 in the pen 252 at step 106, one or more but less than all of the membranes 1202, 1206, 1210 can be lysed at step 106. In the example, illustrated in FIG. 12B, the outer membrane 1202, but not the nuclear membrane 1206 or any of the mitochondrial membranes 1210, of the cell 502 is lysed at step 106. The released nucleic acid material 1222 will thus not include nucleic acid material from inside the nucleus 1204 or the organelles 1208. Thus, in the example illustrated in FIG. 12B, the released nucleic acid material 1222 can be RNA (e.g., any of the types of RNA identified above).

[0106] Step 108 can then be performed generally as discussed above to capture one or more of the types of nucleic acid material 1222 released from the now lysed cell 702. For

example, as shown in FIG. 12B, one or more capture objects **1002a** (one is shown but there can be more) configured to capture one or more types of the nucleic acid material **1222** released from the lysed cell **702** can be in the pen **252**.

[0107] As illustrated in FIGS. 12C and 12D, steps **106** and **108** can be repeated one or more times to lyse one or more additional membranes of the now lysed cell **702** in the pen **252** and thus release and capture additional types of nucleic acid material released as each additional membrane is lysed.

[0108] In the example illustrated in FIG. 12C, the mitochondrial membrane **1210** of one of the organelles **1208** is lysed at a repetition of step **106** of FIG. 1, which can release nucleic acid material **1224** from the now lysed organelle **1238**. (A lysed organelle **1208** is labeled **1238** in FIG. 12C.) The released nucleic acid material **1224** can comprise nucleic acid material, such as mtDNA, such as is typically found in organelles. Step **108** of FIG. 1 can then be repeated generally as discussed above to capture one or more types of the nucleic acid material **1224** released from the lysed organelle **1238**. For example, as shown in FIG. 12C, one or more capture objects **1002b** (one is shown but there can be more) configured to capture one or more types of the nucleic acid material **1224** released from the lysed organelle **1238** can be in the pen **252**. In this example in which an organelle **1208** is lysed before lysing the nucleus **1204**, highly enriched mtDNA from the lysed organelle **1208** can be captured because there is no free nuclear DNA from the nucleus **1204** in the interior space **256** of the pen **252**.

[0109] In the example illustrated in FIG. 12D, the nuclear membrane **1206** of the nucleus **1204** can be lysed at another repetition of step **106** of FIG. 1, which can release nucleic acid material **1226** from the now lysed nucleus **1234**. (The lysed nucleus **1204** is labeled **1234** in FIG. 12D.) The released nucleic acid material **1226** can comprise nucleic acid material, such as various types of DNA, typically found in the nucleus of a cell. Step **108** can then be repeated again generally as discussed above to capture one or more types of the nucleic acid material **1226** released from the lysed nucleus **1234**. For example, as shown in FIG. 12D, one or more capture objects **1002c** (one is shown but there can be more) configured to capture one or more types of the nucleic acid material **1226** released from the lysed nucleus **1234** can be in the pen **252**.

[0110] In the examples illustrated in FIGS. 12A-12D, the membranes **1202**, **1206**, **1208** can be lysed and the capture objects **1002a**, **1002b**, **1002c** can be moved into the pen **252** in any manner illustrated or discussed above. Moreover, each capture object **1002a**, **1002b**, **1002c** can be removed from the pen **252** (e.g., generally as shown in FIG. 13 and discussed below) at the end of each repetition of step **108**, or all of the capture objects **1002a**, **1002b**, **1002c** can be removed (e.g., generally as shown in FIG. 13 and discussed below) from the pen **252** after the last repetition of step **108**.

[0111] Although FIGS. 12C and 12D illustrate lysing an organelle **1208** and then lysing the nucleus **1204**, other orders are possible. For example, the nucleus **1204** can be lysed (as illustrated in FIG. 12D) before lysing an organelle **1208** (as illustrated in FIG. 12C). As another example, multiple organelles **1208** can be lysed (each as shown in FIG. 12C), and the nuclear membrane **1206** can be lysed (as shown in FIG. 12D) between the lysing of two of the organelles **1208**. Although FIGS. 12A-12D illustrate only one pen **252** of the device **100**, the lysing and capturing with capture objects **1002** illustrated in those figures can also be

performed in others of the pens **252** in the device **100**. Also, although the example cell **502** in FIGS. 12A-12D is illustrated as having a nuclear membrane **1206** and thus being an eukaryote cell, the cells **502** illustrated in the drawings and discussed herein can be other types of cells such as prokaryote cells.

[0112] Returning again to FIG. 1, at step **110**, the process **100** can remove one or more of the capture objects **1002** from one or more of the pens **252**. FIG. 13 illustrates an example in which light cages **1302** can trap capture objects **1002** in the pens **252** and move the capture objects **1002** into the export portion **216** of the device **200**. (Any of the DEP devices discussed or mentioned above, including an OET device configured as illustrated in FIGS. 4A and 4B or, alternatively, and OEW device, is thus an example of a means for selecting individual capture objects **1002** (or droplets containing such capture objects) in the isolation pens **252** of the device **200** and moving the selected capture objects **1002** out of the isolation pens **252**.) For example, the capture objects **1002** can be moved to the staging area **248** of the export mechanism **228** and exported from the device **200** through the passage **246**. The foregoing can be performed in any manner, for example, disclosed in the aforementioned US patent application serial no. U.S. patent application Ser. No. 14/520,510 (filed Oct. 22, 2014) (attorney docket no. BL14-US). Alternatively, capture objects **1002** can be exported from the device **200** through an outlet **210**. As yet another alternative, capture objects **1002** removed from the pens **252** at step **110** can be stored and/or further processed at other locations in the device **200**.

[0113] The process **100** of FIG. 1 can thus identify and select from a group of cells in a micro-fluidic device **200** specific individual cells **502** determined to have one or more particular characteristic, and the process **100** can place the selected cells **502** individually into isolation pens **252** in the device **200** such that each of the pens **252** contains only one of the selected cells **502**. The process **100** can then extract nucleic acid material from a single cell **502** in one of the pens **252** and capture with one or more capture objects **1002** in the pen **252** one or more specific types of nucleic acid material (e.g., any one or more of the types of DNA or RNA identified above) from the single cell **502**. Alternatively, the process **100** can place more than one cell **502** in a pen **252** and/or a single cell **502** in a pen can grow and multiple into multiple such cells in a pen **252**. Regardless, the process **100** can then individually remove capture objects **1002**, and thus the nucleic acid material captured by the capture objects **1002**, from the pens **252** and export the capture objects **1002** from the device **200**, store the capture objects **1002** in other locations in the device **200**, or further process the capture objects **1002** in the device **200**.

[0114] As noted, each capture object **1002** can comprise a unique identifier **1006**, which can facilitate correlating the nucleic acid material on each capture object **1002** with the cell **502** from which the nucleic acid material originated. For example, the control module **230** can be programmed to maintain a digital record (e.g., stored in the memory **234**) of each of the unique identifiers **1106** of the capture objects **1002** and, for each capture object **1002**, information regarding nucleic acid material captured by the capture object **1002**. For example, the controller **230** can store in the memory **234** any of the following information associated with the unique identifier **1106** of a particular capture object **1002**: an identification of the particular pen **252** in which the

nucleic acid material was captured, characteristics of the cell 502 from which the nucleic acid material was captured, the type of nucleic acid material captured, processing conditions in which the nucleic acid material was captured, and/or the like. The controller 230, programmed as described above, can thus be an example of a means for storing a correlation between the capture objects and data regarding the nucleic acid material captured by each capture object.

[0115] Indeed, the control module 230 of FIG. 2A can be configured (e.g., programmed with software, firmware, microcode, or the like; hardwired; or the like) to control or can provide for control by a human operator of some, most, or all of the process 100. For example, the control module 230 can be configured to control operation of the manipulator 222, the detector 224, the flow controller 226, and/or the output mechanism 228 to carry out any or all of the steps 102-110 of the process 100 in any way described above.

[0116] The process 100 shown in FIG. 1 and the operation of the process 100 illustrated in FIGS. 5-13 are examples only, and variations are contemplated. For example, one or more of the steps 102-110 can be performed in a different order than shown in FIG. 1. As another example, not all of the steps 102-110 need be performed, and the process 100 can thus comprise less than all of the steps 102-110. As yet another example, steps in addition to steps 102-110 can be performed. For example, one or more washing steps can be performed before, during, or after any of the steps 102-110 to, for example, wash one or more of the capture objects 1002. As still another example, although process 100 is illustrated and discussed above as placing only one cell 502 in a pen 252 and then extracting and capturing nucleic acid material from only a single cell 502 in each pen 252, the process 100 can alternatively place multiple cells 502 in a pen 252 and extract and capture nucleic acid material from the multiple cells in the pen 252. As yet another example, an individual cell 502 can be placed in a pen 252 and allowed to grow and multiple into multiple cells prior to releasing and capturing nucleic acid material from one or more of the cells 502 thus grown and then lysed. Additional cells 502 that are not lysed can be exported from the pen 252 as living progeny of the lysed cell 702.

[0117] FIG. 14 illustrates another example of a process 1400 for extracting and capturing nucleic acid from biological cells. As will be seen, the process 1400 can move selected clonal cells from clonal cell colonies into isolation pens, where the process 1400 can lyse the clonal cells and capture with capture objects in the pens nucleic acid released from the cells. The process can also store a correlation record correlating each such capture object to the clonal cell colonies from which the clonal cell whose nucleic acid is captured by the capture object was taken.

[0118] FIG. 15 shows a top cross-sectional view of an example of a micro-fluidic device 1500 on which the process 1400 can be performed. The device 1500 can be generally the same as the device 200 (e.g., as illustrated in FIGS. 2A-2C including any variation illustrated in any of FIGS. 3, 4A, 4B, 7, and 8) except device 1500 can include a culturing portion 1512 rather than (or in addition to) the selection portion 212. As shown, there can be culturing pens 1552 (two are shown but there can be more or fewer) in the culturing portion 1512. Other than the culturing pens 1552, the culturing portion 1512 can be generally the same as or similar to the selection portion 212 of FIGS. 2A-2C including any variation illustrated or described herein.

[0119] Examples of the culturing pens 1552 are illustrated in FIG. 15. As shown, each culturing pen 1552 can be generally similar to an isolation pen 252. For example, a culturing pen 1552 can comprise an enclosure 1554 that defines an interior space 1556 and an opening 1558 from the channel 240 to the interior space 1556. The enclosure 1554, interior space 1556, and opening 1558 can be generally similar, respectively, to the enclosure 254, interior space 256, and interior space 256 (including any variation illustrated or described herein) of the device 200 of FIGS. 2A-2C. For example, the enclosure 1554 can comprise any of the materials mentioned above with respect to the enclosure 254. As another example, the opening 1558 of each isolation pen 1552 can be sized and positioned to allow for the natural exchange of liquid medium 244 in a pen 1552 and liquid medium 244 flowing past the opening 1558 of the pen 1552. Otherwise, however, the enclosures 1554 can enclose the interior spaces 1556 of the culturing pens 1552 sufficiently to prevent biological material, cells, or objects in the interior space 1556 of one culturing pen 1552 from mixing with such biological material, cells, or objects in the interior space 1556 of any another culturing pen 1552.

[0120] The number, pattern, and configuration of the culturing pens 1552 illustrated in FIG. 15 is an example, and variations are possible. For example, each culturing pen 1552 can instead be like the pens 352 illustrated in FIG. 3.

[0121] Generally as illustrated in FIG. 15, a colony of clonal cells 1504 can be cultured in one or more of the culturing pens 1552. In the example of FIG. 15, a first colony 1504a of clonal cells 1502a is cultured in a first culturing pen 1552a, and a second colony 1504b of clonal cells 1502b is cultured in a second culturing pen 1552b. As noted, there can be more than two culturing pens 1552, and a different colony 1504 of clonal cells 1502 can be cultured in each of any number of the culturing pens 1552.

[0122] Each such colony 1504 can be created in one of the culturing pens 1552 by placing a parent cell into the pen 1552 and allowing the parent cell to produce daughter cells in the pen 1552. For example, the parent cell and resulting daughter cells can be cultured in a pen 1552 by providing a flow of nutrients in a flow of medium 244 in the channel 240 past the opening 1558 of the culturing pen 1552. Such nutrients can flow into and cell waste can flow out of the pen 1552 by, for example, diffusion of medium 244 through the opening 1558.

[0123] All of the cells 1502 in a particular culturing pen 1552 can thus consist solely of the parent cell placed into the pen 1552 and daughter cells produced by or from the parent cell. Thus, for example, all of the cells 1502a in the first colony 1552 in the first culturing pen 1552a can be either a parent cell or progeny of the parent cell. The first colony 1504a can thus be a clonal colony, and all of the cells 1502a of the first colony 1504a can be clonal cells. Similarly, all of the cells 1502b in the second colony 1504b in the second culturing pen 1552b can be either a parent cell or progeny of the parent cell. The second colony 1504b can thus be a clonal colony, and all of the cells 1502b of the second colony 1504b can be clonal cells.

[0124] Referring now to FIG. 14, at step 1402, the process 1400 can select individual clonal cells 1502 from the colonies 1504 in the culturing pens 1552 in the device 1500, and at step 1404, the process 1400 can move the selected individual clonal cells 1502 into isolation pens 252 in the isolation portion 214 of the device 1500. FIG. 15 illustrates

an example. As shown in FIG. 15, a single, individual cell 1502a from the first colony 1504a can be selected in and moved 1520a from the first culturing pen 1552a to a first one of the isolation pens 252a. In certain embodiments, the isolation portion 214 of the device 1500 can be configured for OEW and the movement of individual cell 1502a from the first colony 1504a can involve creating a droplet of aqueous medium containing the individual cell 1502a in an oil medium, and moving the droplet into the isolation pen 252a. As previously noted, the isolation pens 252 can be examples of lysing pens. Similarly, a single, individual cell 1502b from the second colony 1504b can be selected in and moved 1520b from the second culturing pen 1552b to a second one of the isolation pens 252b. As noted, there can be more than two such culturing pens 1552, and a clonal cell 1502 from a clonal cell colony 1504 can be thus placed in a plurality (e.g., all) of the isolation pens 252. For example, one and only one clonal cell 1502 can be placed in each of a plurality of the isolation pens 252, and each such clonal cell 1502 can be from a different clonal cell colony 1504 in a different culturing pen 1552. Alternatively, more than one clonal cell 1502 can be placed in an isolation pen 252, but all of the clonal cells 1502 placed in any one isolation pen 252 can be from the same clonal cell colony 1504.

[0125] Each clonal cell 1502 can be selected from its cell colony 1504 randomly or using any selection criteria discussed above with respect to step 102 of FIG. 2. The clonal cells 1502 can be selected in and moved from the culturing pen 1552 in any way discussed above with respect to step 104. For example, each clonal cell 1502 can be trapped with a light trap (not shown in FIG. 15) like light trap 602, which can be generated and manipulated as discussed above with respect to FIG. 6.

[0126] Alternatively, or in addition, OEW can be used to create a droplet of aqueous medium that contains the clonal cell 1502. In still other alternatives, the cell colonies 1504 can be located outside of the device 1500, and individual clonal cells 1502 from the colonies 1504 can be imported into the device 1500 (e.g., through the inlet 208). Step 1402 can thus be skipped or left out of the process 1400. Once imported into the device 1500, the clonal cells 1502 can be selected and moved into the isolation pens 252 (e.g., generally as shown in FIGS. 5 and 6).

[0127] Regardless, after steps 1402 and/or 1404, one or more clonal cells 1502 are now in each of a plurality of the isolation pens 252 of the device 1500, and the one or more clonal cells 1502 in each pen 252 can be from the same clonal colony 1504. As will be seen, the cells 1502 can then be lysed at step 1406, and released nucleic acid material from the lysed cells 1502 can be captured at step 1408. As discussed below, steps 1406 and 1408 can be performed generally like steps 106 and 108 of FIG. 1.

[0128] For example, at step 1406, cells 1502 in the isolation pens 252 can be lysed to produce lysed cells (not shown in FIG. 15). Cells 1502 can be lysed in the isolation pens 252 in any of the ways discussed above with respect to step 106 for lysing cells 502 in the isolation pens 252. For example, one or more cells 1502 can be lysed in the isolation pens 252 as illustrated in FIG. 7 or FIG. 8 or in any alternative discussed above. Lysing at step 1406 can include lysing any one or more of the membranes of the cells 1502 (sequentially and/or substantially simultaneously) generally as illustrated in FIGS. 7, 8, and 12A-12D. Generally as illustrated in FIGS. 9 and 12A-12D, lysing at step 1502 can release

nucleic acid material from the cells 1502 into interior spaces 256 of the isolation pens 252.

[0129] At step 1408, one or more types of the nucleic acid material from cells 1502 lysed at step 1406 can be captured with one or more capture objects 1002 in the pens 252. Step 1408 can be performed in the same way as step 108 is performed including any variation as illustrated and discussed herein. For example, one or more specific types of nucleic acid material released from the lysed cells 1502 can be captured in the isolation pens 252 with one or more capture objects 1002 in the pens 252 as discussed above with respect to step 108.

[0130] At step 1410, the process 1400 can create and/or maintain a correlation record correlating each capture objects 1002 in the isolation pens 252 to the cell colony 1504 from which the cell 1502 whose nucleic acid material is captured by the capture object 1002 originated. For example, for each capture object 1002 in the isolation pens 252, the correlation record can correlate a unique identifier (e.g., the identifier 1106 shown in FIG. 11) of the capture object 1002 with any of the following information about the cell 1502 whose nucleic acid material was captured by the capture object 1002: the identity (e.g., location such as the culturing pen 1552) of the clonal cell colony 1504 from which the cell 1502 was taken, one or more characteristics of the cell 1502, and/or the like.

[0131] FIG. 15 shows a first capture object 1002a in the first isolation pen 252a with the first cell 1502a from the first cell colony 1504a. After the first cell 1502a is lysed at step 1406, the first capture object 1002a can thus capture nucleic acid material released from the first cell 1502a. Similarly, a second capture object 1002b in the second isolation pen 252b can capture nucleic acid material released after the second cell 1502b is lysed. The correlation record created at step 1410 of FIG. 10 can thus comprise a unique identifier of the first capture object 1002a correlated with an identification of the first cell colony 1504a and/or its culturing pen 1552a, and the correlation record can also include a unique identifier of the second capture object 1002b correlated with an identification of the second cell colony 1504b and/or its culturing pen 1552b. In some embodiments, the control module 230 can be programmed (e.g., with machine readable instructions (e.g., software, firmware, or microcode) and/or hardwired circuitry) to create, store (e.g., in the memory 234), and maintain (e.g., update) such a correlation record.

[0132] At step 1412, the process 1400 can remove one or more of the capture objects and thus the nucleic acid material captured by the capture objects, from one or more of the isolation pens 252. Step 1412 can be performed generally like step 110 of FIG. 1 including any variation thereof illustrated or discussed herein.

[0133] The process 1400 is an example only, and variations are contemplated. For example, one or more of the steps 1402-1412 can be performed in a different order than shown in FIG. 14. As another example, not all of the steps 1402-1412 need be performed, and the process 1400 can thus comprise less than all of the steps 1402-1412. As yet another example, steps in addition to steps 1402-1412 can be performed. For example, one or more washing steps can be performed before, during, or after any of the steps 1402-1412 to, for example, wash one or more of the capture objects. Although specific embodiments and applications of

the invention have been described in this specification, these embodiments and applications are exemplary only, and many variations are possible.

EXAMPLES

Example 1

Cell Lysis in Pens

[0134] To test cell lysis in isolation pens of a microfluidic device, cells were loaded into isolation pens, a lysis buffer was flowed through the device, and cell lysis was monitored using Calcein AM stain. The lysis buffer was as follows:

[0135] RNase inhibitor: 2 units/microliter;

[0136] NaCl: 0.135 M;

[0137] Tris-HCl (pH 8.0): 9 mM;

[0138] Dithiothreitol (DTT) 4.5 mM; and

[0139] SDS: 1%.

[0140] As shown in FIGS. 16A-D, cells loaded into the pens (FIG. 16A) are detectable using Calcein AM at t=0 minutes after introduction of the lysis buffer (FIG. 16B). At 5 minutes after introduction of the lysis buffer, the cells are still largely intact but starting to lyse (FIG. 16C). At 10 minutes after introduction of the lysis buffer, cell lysis appears complete (FIG. 16D).

[0141] Any of the foregoing components of the lysis buffer can be substituted with equivalent buffers, salts, and chelating agents, as understood by persons skilled in the art.

Example 2

Binding Conditions

[0142] Following lysis of cells in isolation pens of a microfluidic device (e.g., as described in Example 1), binding of target nucleic acids to capture objects can be performed by flowing a binding buffer through the microfluidic device while the capture objects are in the presence of cell lysate. The binding buffer can be as follows:

[0143] Tris-HCl (pH 7.5): 20 mM;

[0144] LiCl: 1.0 M; and

[0145] EDTA: 2 mM.

[0146] Any of the foregoing components of the binding buffer can be substituted with equivalent buffers, salts, and chelating agents, as understood by persons skilled in the art.

Example 3

Use of a Combined Lysis/Binding Buffer

[0147] As an alternative to using separate lysis and binding buffers, cell lysis and nucleic acid capture can be accomplished using a combined lysis/binding buffer. Thus, cell can be loaded into isolation pens in a microfluidic device, and a combined lysis/binding buffer can be flowed through the device for a sufficient time to achieve cell lysis. The lysis/binding buffer can be flowed through prior to, at substantially the same time, or after capture objects are disposed adjacent to the cells that are to be lysed. The combined lysis/binding buffer can be as follows:

[0148] Tris-HCl (pH 7.5): 100 mM;

[0149] LiCl: 500 mM;

[0150] EDTA: 10 mM;

[0151] LiDS: 1%; and

[0152] DTT: 5 mM.

[0153] Any of the foregoing components of the combined lysis/binding buffer can be substituted with equivalent buffers, salts, and chelating agents, as understood by persons skilled in the art.

We claim:

1. A process of capturing nucleic acid material from individual biological cells, said process comprising:

placing an individual biological cell into an isolation pen in a micro-fluidic device;

lysing said individual cell in said isolation pen;

capturing with a capture object in said isolation pen nucleic acid material from said lysed cell; and

after said capturing, removing said capture object from said isolation pen.

2. The process of claim 1, wherein said placing step comprises:

selecting said individual biological cell from a group of biological cells in a common space in said micro-fluidic device, and

moving said individual cell from said common space into said isolation pen in said micro-fluidic device.

3. The process of claim 2, wherein said selecting step comprises testing said group of biological cells in said micro-fluidic device for a particular characteristic.

4. The process of claim 3, wherein said individual biological cell is one of said biological cells in said group that tests positive for said particular characteristic.

5. The process of claim 3, wherein said individual biological cell is one of said biological cells in said group that tests negative for said particular characteristic.

6. The process of any one of claims 2 to 5, wherein said selecting step further comprises creating an individual light trap trapping said individual cell by projecting a light pattern into said common space inside said micro-fluidic device.

7. The process of claim 6, wherein said moving step comprises moving said individual light trap from said common space into said isolation pen.

8. The process of claim 3, wherein said particular characteristic comprises a size of said biological cells or a morphology of said biological cells.

9. The process of claim 3, wherein said particular characteristic comprises whether said biological cells comprise a particular material or whether said biological cells produce a particular material.

10. The process of any one of claims 1 to 9, wherein a plurality of individual cells are placed into a corresponding plurality of isolation pens such that each isolation pen includes a single individual cell.

11. The process of claim 10, wherein said lysing step comprises lysing said plurality of said individual cells in said isolation pens simultaneously.

12. The process of any one of claims 1 to 9, wherein said lysing step comprises:

flowing a lysing reagent through a channel in said micro-fluidic device in which said isolation pen is located, directing a beam of electromagnetic energy at said individual cell,

electroporating said individual cell,

changing a temperature of said individual cell sufficiently to lyse said individual cell, or

applying sufficient acoustic energy to said individual cell to lyse said individual cell.

13. The process of claim 10, wherein said lysing step comprises:

selecting a specific individual one of said cells in said isolation pens, and

lysing said specific individual one of said cells without also simultaneously lysing any others of said cells in said isolation pens.

14. The process of claim **13**, wherein said lysing step further comprises sequentially lysing a plurality of said cells in said pens by sequentially repeating a plurality of times said step of selecting a specific individual one of said cells in said isolation pens followed by said step of lysing said specific individual one of said cells.

15. The process of any one of claims **1** to **9**, wherein: said lysing step comprises compromising an outer membrane of said individual cell without compromising a membrane of a first internal element of said individual cell,

said compromising said outer membrane releases a first type of nucleic acid from said individual cell, and said capture object is a first capture object configured to capture said first type of nucleic acid.

16. The process of claim **15** further comprising repeating said lysing step and said capturing step as follows:

lysing in said isolation pen said first internal element of said individual cell by compromising said membrane of said first internal element, and

capturing with a second capture object in said isolation pen a second type of nucleic acid material released by said lysing said first internal element.

17. The process of claim **16**, wherein said first internal element is one of a nucleus or an organelle of said one of said cells.

18. The process of claim **17**, wherein said lysing said one of said cells further comprises compromising said outer membrane of said one of said cells without compromising a membrane of a second internal element of said one of said cells,

19. The process of claim **18** further comprising repeating again said lysing step and said capturing step as follows:

lysing in said isolation pen said second internal element of said individual cell by compromising said membrane of said second internal element, and

capturing with a third capture object in said isolation pen a third type of nucleic acid material released by said lysing said second internal element.

20. The process of claim **19**, wherein:

one of said first internal element or said second internal element is a nucleus of said one of said cells, and another of said first internal element or said second internal element is an organelle of said one of said cells.

21. The process of claim **20**, wherein:

said first type of nucleic acid material is a different type of nucleic acid material than said second type of nucleic acid material,

said second type of nucleic acid material is a different type of nucleic acid material than said third type of nucleic acid material, and

said first type of nucleic acid material is a different type of nucleic acid material than said third type of nucleic acid material.

22. The process of claim **10** or **11** wherein:

said lysing step comprises lysing said plurality of individual cells in said plurality of isolation pen,

said capturing step comprises capturing with a plurality of capture objects in said plurality of isolation pens nucleic acid material from said lysed cells, and said removing step comprises removing said plurality of capture objects from said plurality of isolation pens.

23. The process of claim **22**, wherein:

each of said capture objects comprises an identifier that uniquely identifies each said capture object from every other capture object of said plurality, and

said process further comprises storing in a memory device a correlation between each said capture object and data regarding nucleic acid material captured by said capture object.

24. The process of claim **23**, wherein said data comprises a type of said nucleic acid material captured by said capture object.

25. The process of claim **23**, wherein said correlation comprises a characteristic of one of said lysed cells from which said nucleic acid material captured by said capture object originated.

26. The process of any one of claims **1** to **9** further comprising placing a blocking object substantially in an opening of said isolation pen, wherein said blocking object is configured to capture nucleic acid material from said lysed cell.

27. The process of any one of claims **1** to **9**, wherein said individual biological cell is a cell from a clonal cell colony.

28. The process of claim **27**, wherein said placing comprises moving said individual cell from a clonal cell colony in a culturing pen in said micro-fluidic device into said isolation pen.

29. The process of claim **28** wherein:

said lysing step comprises lysing said individual cell in said isolation pen,

said capturing step comprises capturing with one or more capture objects in said isolation pen nucleic acid material from said lysed cell, and

said removing step comprises removing said one or more capture objects from said isolation pen.

30. The process of claim **29**, wherein:

each of said capture objects comprises an identifier that uniquely identifies each said capture object from every other one of said capture objects, and

said process further comprises storing in a memory device a correlation between said identifier of each said capture object and an identification of said clonal cell colony from which said one of said cells whose nucleic acid material is captured by said capture object originated.

31. The process of claim **10** or **11**, wherein:

said lysing comprises lysing said one of said cells in said one of said isolation pens at a first time,

said process further comprises lysing a second one of said cells in a second one of said isolation pens at a second time, and

said second time is after said first time.

32. The process of claim **31** further comprising monitoring said micro-fluidic device for an event inside said micro-fluidic device, wherein:

said first time is a first time period from detection of said event, and

said second time is a second time period from said detection of said event.

33. The process of claim **31** further comprises testing a group of biological cells in said micro-fluidic device for particular characteristics, wherein:

said one of said cells tested positive for a first one of said characteristics, and
said second one of said cells tested positive for a second one of said characteristics.

34. A micro-fluidic device comprising:

an electrode activation substrate comprising dielectrophoresis (DEP) electrodes at a surface of said substrate, wherein each said electrode is configured to be selectively activated and deactivated;

a micro-fluidic structure that, with said surface of said substrate, defines a micro-fluidic channel;

isolation pens disposed in said channel; and
capture objects sized to be placed in one of said isolation pens, each said capture object comprising a capture material that has at least a two times greater specificity for a particular type of nucleic acid material than other types of nucleic acid material.

35. The device of claim **34** further comprising lysing means for lysing biological cells in said isolation pens.

36. The device of claim **34** or **35**, wherein activated ones of said electrodes generate sufficient DEP forces to trap a biological cell in said channel adjacent to said activated ones of said electrodes.

37. The device of any one of claims **34** to **36**, wherein said electrodes are virtual electrodes on said surface of said substrate.

38. The device of any one of claims **34** to **36**, wherein each said electrode comprises a fixed electrically conductive terminal at said surface of said substrate.

39. The device of any one of claims **34** to **36**, wherein each said electrode is selectively activated and deactivated in response to a changing pattern of light directed onto said surface of said substrate.

40. The device of any one of claims **34** to **39**, wherein each of said capture object comprises an identifier that uniquely identifies said capture object from every other one of said capture objects.

41. A controller for controlling a micro-fluidic device comprising isolation pens each sized to contain a biological cell and a capture object configured to capture nucleic acid from said biological cell, said controller comprising:

selecting/moving means for selecting individual ones of biological cells in said micro-fluidic device and moving said selected ones of said cells into said isolation pens;

a control module configured to control lysing of said biological cells in said isolation pens; and

correlation means for generating a correlation record correlating each one of a plurality of said capture objects in said isolation pens with a corresponding one of biological cells in said isolation pens from which nucleic acid material captured by said one of said capture objects originated.

42. The controller of claim **41**, wherein said correlation record correlates each one of said capture objects with a clonal colony of cells from which said corresponding one of said cells originated.

43. The device of claim **41** or **42**, wherein said moving/selecting means is part of a dielectrophoresis (DEP) device for generating DEP forces in said device that selectively trap any desired one of said biological cells in said device.

44. The device of any one of claims **41** to **43**, wherein said DEP device comprises an optoelectronic tweezers device.

45. The device of claim **41**, wherein said DEP device comprises an OEWE device.

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