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(54) Title: TARGETED SEPARATION OF CULTURED CELLS

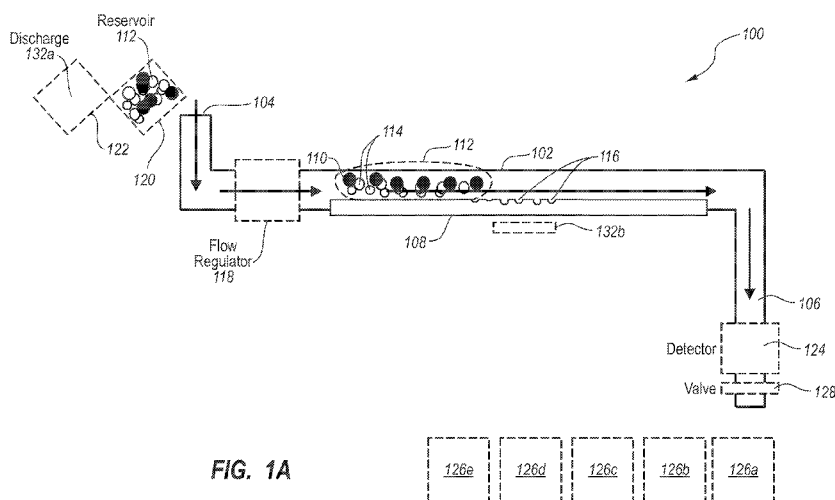


FIG. 1A

(57) Abstract: Embodiments described herein relate to separating and/or concentrating target cells from a carrier fluid that may include other non-target cells. Embodiments include a cell separator with a flow surface having indentations formed thereon. The indentations are configured to capture target cells by physical and/or chemical interactions. The indentations may also include a layer of support molecules, for example, photo-responsive molecules such as azobenzene that assist in releasing captured cells for collection.

TARGETED SEPARATION OF CULTURED CELLS

TECHNICAL FIELD

[0001] Embodiments described herein can broadly be used in technical fields implicating cell culturing systems to facilitate culturing and harvesting of cells.

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BACKGROUND

[0002] Generally, cells are cultured in vitro in either suspended cultures or adherent cultures. In suspended culture, cultured cells are grown while suspended in a growth media. Cell cultures grown in suspension are typically derived from cell types that naturally live in suspension without being attached to a surface or to one another. In
10 adherent cell culture environments, cultured cells adhere to surfaces (e.g., the surfaces of a culture vessel) and/or to one another as a support for cell growth.

[0003] Examples of suspended cells that can be cultured in vitro include blood cells, lymphocyte cells, and the like. Examples of adherent cells that can be cultured in vitro include epithelial cells, hematopoietic cells, mast cells, neurocytes, hepatocytes, hepatic
15 parenchymal cells, bone marrow cells, osteoblasts, fibroblasts, epidermal cells, many stem cell types, and the like.

[0004] When adherent cells are ready for harvesting, chemical means such as enzymes (e.g., collagenase) and/or physical means such as glass capillary colony cutters have generally been used to separate the cells from the growth surface and/or to separate
20 the cells from one another. Harvesting of cells grown in suspension or suspended adherent cells is typically performed by filtration, centrifugation, antibody processes, beads, pipetting, and the like. However use of chemicals, enzymes, and/or physical means to separate cultured cells can damage the cells.

[0005] In some cases, different types of cells are mixed in culture. For example, stem
25 cells may be cultured with feeder cells, or stimulating cells (e.g., antigen presenting cells

and a cancer cell line) may be co-cultured. In cases where mixed cultures are concerned, separation of the cells in the mixed culture can be additionally complicated and require additional physical and/or chemical processes, such as filtration, centrifugation, antibody, or beads. Again, these processes can damage the cells.

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SUMMARY

[0006] In one embodiment, the present disclosure describes an apparatus for cell separation. In one aspect, the apparatus includes a channel having an inlet and an outlet, the inlet configured to receive an initial carrier fluid containing at least a first cell, and a flow surface extending between the inlet and the outlet of the channel, wherein the flow surface is configured to selectively capture at least the first cell and to permit the carrier fluid flow therethrough.

[0007] In another embodiment, the present disclosure describes an apparatus for separating or concentrating target cells. In one aspect, the apparatus may include a channel having an inlet and an outlet, the inlet configured to receive an initial carrier fluid containing a plurality of first target cells, and a flow surface extending between the inlet and the outlet of the channel, the flow surface configured to include one or more indentations configured to capture one or more of the plurality of first target cells when the initial carrier fluid passes over the flow surface, leaving extraneous carrier fluid to flow to the outlet of the channel.

[0008] In yet another embodiment, the present disclosure describes a method for separating target cells from a carrier fluid. In one aspect, the method may include passing an initial carrier fluid containing a plurality of first target cells through an inlet of a channel to pass over a flow surface of the channel, the flow surface having at least one indentation sized with a diameter that substantially matches a diameter of at least a portion of the first target cell to capture one or more of the plurality of first target cells as

the initial carrier fluid passes over the flow surface, leaving extraneous carrier fluid to flow to an outlet of the channel, and applying a first energy source to initiate release of the captured one or more target cells from the at least one indentation such that the released one or more target cells flow to the outlet of the channel.

- 5 [0009] The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, embodiments, and features described above, further aspects, embodiments, and features will become apparent by reference to the following detailed description.

BRIEF DESCRIPTION OF THE FIGURES

- 10 [00010] **Figure 1A** is a schematic diagram of an illustrative embodiment of a cell separator, in a first stage of operation.

[00011] **Figure 1B through 1D** are schematic diagram of the illustrative embodiment of a cell separator of Figure 1A, in various stages of operation.

- [00012] **Figure 2A through 2E** are schematic diagrams of illustrative embodiments of
15 flow surfaces having indentations and/or materials to form flow surfaces having indentations.

DETAILED DESCRIPTION

- [00013] In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify
20 similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented herein. It will be readily understood that the aspects of the present disclosure, as generally described herein, and
25 illustrated in the Figures, can be arranged, substituted, combined, separated, and designed

in a wide variety of different configurations, all of which are explicitly contemplated herein.

Introduction

[00014] Embodiments herein are directed to separation (e.g., harvesting) of cultured
5 cells without damaging the cells. Embodiments described herein provide compositions, apparatuses, and methods for culturing and harvesting cells without use of conventional chemical and physical harvesting methods. Cells are suspended in a carrier fluid from which it is desired to separate one or more target cells and, optionally, from other non-target cells contained in the carrier fluid. In one aspect, this may be accomplished by
10 providing an apparatus that can selectively capture then subsequently release cells while allowing the carrier fluid to flow through the apparatus.

[00015] The carrier fluid can be, in one embodiment, a culture medium in which cultured cells were suspended to mature, such as, but not limited to saline, bovine serum, fetal calf serum, Dulbecco's modified Eagle's medium (DMEM), nutrient broths, and the
15 like. The carrier fluid can include any combination of adherent cells, floating cells, feeder cells, stimulating cells, and the like. Alternatively, the carrier fluid may have only target cells carried therein, in which it is desired to concentrate or separate the target cells from the carrier fluid. In any case, the term "target cell" will be used to refer to a particular type of cell or aggregation of cells that is desired to be separated from the rest of the
20 carrier fluid and/or non-target cells.

[00016] Various types of adherent cells can be cultured including, but not limited to, stem cells, osteocyte, epidermal cells, organ cells (e.g., blood vein cells, vascular cells, liver cells (i.e., hepatocytes), cardiac cells, etc), or other tissue engineering cells, and the like. Additional examples of adherent cells include, but are not limited to, epithelial cells,
25 ciliated epithelial cell, chondrocyte (e.g., fibrocartilage, elastic cartilage), osteoblasts,

osteocytes, muscle cells (e.g., smooth muscle cells, skeletal muscle cells, myocardial cells), neurocytes (e.g., glia cells, schwan cells, myelin sheath cells), hematopoietic cells, mast cells, hepatocytes, hepatic parenchymal cells, and bone marrow cells. Examples of stem cells include, but are not limited to, embryonic stem cells (ESCs), pluripotent stem
5 cells, induced pluripotent stem cells, embryonic stem cells, adult stem cells, and multipotent and induced multipotent stem cells such as neural cells, mesencymal stem cells, epithelial stem cells, bone marrow stem cells, neural stem cells, hematopoietic stem cells, germ stem cells, somatic stem cells, modified stem cells, and the like.

[00017] Examples of mammalian cells that can be cultured in suspension (i.e., floating
10 cells) include, but are not limited to cells that are present in the blood stream. Cells that are present in the blood stream include, but are not limited to, granulocytes (e.g., neutrophils, eosinophils, basophils), lymphocytes (e.g., B lymphocytes, T lymphocytes, NK (natural killer) cells), monocyte, and the like. Many non-mammalian cells have also been adapted for growth in suspension. For example, cells isolated from ovarian tissue of
15 the fall army worm, *Spodoptera frugiperda*, and from embryonic tissue from the *Trichoplusia ni* have been adapted for growth in suspension and they are used in many laboratories. Many microorganisms are also adapted for growth in suspension. For example, many species of bacteria (e.g., E. Coli, S. aureus, S. typhimurium, etc), archea (e.g., Sulfolobus solfataricus, halobacteria (e.g., Halobacterium salinarum)), protists (e.g.,
20 algae, dinoflagellates, and euglena), and fungi (e.g., baker's yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*)) are adapted for growth in suspended culture.

[00018] One example of cells that are commonly co-cultured include stem cells and feeder cells. A feeder cell is generally a non-dividing cell that is co-cultured with a
25 multiplying cell type (e.g., a stem cell) for the purpose of providing necessary growth

factors, nutrients, and other co-factors to the dividing cells. Suitable examples of feeder cells include, but are not limited to, mouse embryonic fibroblast cells, mouse liver cells, xenogenetic feeder cells, allogenic feeder cells, allogenic fibroblast cells, artificial feeder cells, and combinations thereof. Another example of cells that are commonly co-

5 cultured are stimulating cells. For example, for stimulation a Mixed Lymphoid Reaction(MLR) is commonly done (e.g., T-lymphocytes and dendritic cells plus antigen, or T-lymphocytes and a cancer cell line).

[00019] Embodiments thus provide compositions, apparatuses, and methods for separation of cultured cells. One aspect includes a cell separator having a channel with an

10 inlet and an outlet. The channel has a flow surface extending between the inlet and the outlet. The channel can have any of various shapes including being a closed channel in which a wall substantially surrounds the channel from the inlet to the outlet. Or, the channel could have an open top. The channel can have any of various cross sections including, but not limited to, circular, elliptical, square, triangular, or other polygonal

15 shape, or any partial polygonal shape, and the like. The channel can be substantially straight, or have a curved portion. The channel could be substantially horizontal, or could rest at an incline. Basically, the channel can have various configurations that will be apparent to one of skill in the art.

[00020] The flow surface separates target cells from an initial carrier fluid and/or other

20 non-target cells in the initial carrier fluid when the initial carrier fluid passes over the flow surface. The flow surface may be configured to selectively capture at least a first cell and to permit the carrier fluid to flow therethrough. For example, the flow surface may be configured to include indentations sized and configured for selectively capturing target cells from the initial carrier fluid when the carrier fluid passes over the flow surface.

25 In one aspect, the indentations can be sized with a diameter substantially similar to at

least a portion of a target cells such that the target cells can fit into the indentations and be captured thereby. In another aspect, the indentations can be sized with a diameter sufficient to encompass 1/8, 1/5, 1/4, 1/3, or 1/2 of the mass of the target cells at least a portion of the target cells can fit into the indentations and be captured thereby. The inlet
5 of the channel is configured to receive an initial carrier fluid containing target cells. In one embodiment, the indentations on the flow surface capture target cells when the initial carrier fluid passes over the flow surface, leaving extraneous carrier fluid to flow to the outlet of the channel.

[00021] In one embodiment, the flow surface can be configured to release the cells
10 subsequent to their have been selectively captured. For example, the indentations may be configured to release the captured target cells responsive to a first energy source to allow the released target cells to flow to the outlet of the channel. For example, the indentations can be formed from a material having a photo-responsive region configured to change conformation and/or chemical state in response to exposure to the first energy source. In
15 one aspect, the indentations can be configured to change conformation between at least a more hydrophobic state and more hydrophilic state in response to exposure to the first energy source. When the indentations are in a more hydrophobic state, the indentations have a first conformation that is more accessible and/or suitable for association with and optionally adherence by cultured cells. In contrast, when the indentations have a more
20 hydrophilic state, the indentations have a second conformation that is less accessible and/or less conducive to association with and optionally adherence by cultured cells. In one embodiment, the first energy source is applied at a particular time that causes the released target cells to flow to the outlet of the channel at a delayed period after the extraneous carrier fluid flows to the outlet of the channel.

[00022] Any combination of target cells and/or non-target cells may be contained in the initial carrier fluid. When the initial carrier fluid includes non-target cells along with target cells, the non-target cells pass over the flow surface with the extraneous carrier fluid. The non-target cells can be collected separately from the target cells. In one
5 embodiment, the first energy source is applied at a particular time that causes the released target cells to flow to the outlet of the channel at a delayed period after the non-target cells flow to the outlet of the channel.

[00023] The apparatus can further include a detector in communication with the outlet of the channel that detects flow of extraneous carrier fluid flowing from the outlet of the
10 channel and/or flow of the released target cells flowing from the outlet of the channel. The apparatus can include a flow regulator in fluid communication with the inlet of the channel that regulates a flow of the initial carrier fluid and/or a flow of discharge fluid into the inlet of the channel. In one embodiment, the discharge fluid is configured to provide a first energy source to release the captured target cells from the indentations. In
15 one embodiment, the discharge fluid is applied at a particular time that causes the released target cells to flow to the outlet of the channel at a delayed period after the extraneous carrier fluid. In another embodiment, a stimulus, such as an optical device, provides the first energy source to release the captured target cells from the indentations. The apparatus can include a reservoir connected to the flow regulator and configured to
20 hold initial carrier fluid and/or discharge fluid.

[00024] One aspect includes a method that includes passing an initial carrier fluid containing target cells through an inlet of a channel to pass over a flow surface of the channel. In one aspect, the flow surface has indentations sized with a diameter that substantially matches a diameter of the target cells such that the target cells can fit into
25 the indentations and be captured thereby. In another aspect, the indentations can be sized

with a diameter sufficient to encompass 1/8, 1/5, 1/4, 1/3, or 1/2 of the mass of the target cells at least a portion of the target cells can fit into the indentations and be captured thereby. The indentations on the flow surface capture target cells as the initial carrier fluid passes over the flow surface, leaving extraneous carrier fluid to flow to the outlet of the channel. A first energy source is applied to initiate release of the captured target cells from the indentations such that the released target cells flow to the outlet of the channel. In one embodiment, the first energy source is applied at a particular time that causes the released target cells to flow to the outlet of the channel at a delayed period after the extraneous carrier fluid flows to the outlet of the channel. In one embodiment, the first energy source is a discharge fluid being applied to the inlet of the channel. In another embodiment, the first energy source is a stimuli, such as an optical device, being applied to the flow surface.

[00025] The flow surface of the channel can include a substrate that is made from various non-corrosive and bio-safe materials including, but not limited to, glass, quartz, polycarbonate, plastic, silicon, aluminum, stainless steel, ceramic, and the like. Other materials for making the substrate will be readily apparent to those skilled in the art, thus further description will not be provided to avoid obscuring the concepts herein.

[00026] The indentations in the flow surface can be made using various polymer techniques, soft lithography techniques, BioMEMS techniques, μ TAS techniques, and the like. In one embodiment, the process of making indentations results in the indentations including a functional group configured to interact with target cells having complementary functional groups. In another embodiment, the indentations can be formed being at least partially coated with support molecules having a first region configured to bind to a surface of the indentation and a photo-responsive region configured to change conformation between a first conformation and a second

conformation responsive to irradiation from an optical energy source. The use of the term “support molecule” simply refers to molecules that modify the indentations to enhance the ability of the indentations to interact with and/or release the target cells. The term “support” when used in reference to molecules is not intended to refer to any particular structure or characteristic of the molecules themselves.

[00027] In view of the foregoing, apparatuses for targeted separation of cultured cells, methods for targeted separation, and methods for manufacturing flow surfaces having indentations for use in targeting separation of cells will now be described in further detail with reference to the drawings.

Illustrative Embodiments for Cell Separator Apparatus

[00028] Figure 1A is a schematic diagram of an illustrative embodiment of a cell separator 100. Cell separator 100 includes a channel 102 with an inlet 104 and an outlet 106. The channel 102 has a flow surface 108 extending between the inlet 104 and the outlet 106. The channel 102 can have any of various shapes including being a closed channel (shown in Figure 1A) in which a wall substantially surrounds the channel from the inlet 104 to the outlet 106. Or, the channel 102 could have an open top. The channel 102 can have any of various cross sections including, but not limited to, circular, elliptical, square, triangular, or other polygonal shapes. Furthermore, when the channel 102 has an open top, the cross section can be a partial circle, partially elliptical, partially square, partially triangular, or other partial polygonal shapes. The channel 102 can be substantially straight (shown in Figure 1A), or have a curved portion. The channel 102 could be substantially horizontal (shown in Figure 1A), or could rest at an incline. Illustrative dimensions for the channel 102 include a length of about 5 cm to about 50 cm, or about 5 cm to about 30 cm, or about 5 cm to about 15 cm, with a diameter of about 0.1 cm to about 1 cm, or about 0.3 cm to about 1 cm, or about 0.5 cm to about 1 cm.

Basically, the channel 102 can have any of various configurations, so the shape of the channel will not be discussed in further detail to avoid obscuring the concepts herein.

[00029] The flow surface 108 separates target cells 110 from an initial carrier fluid 112 (shown by the dashed line) and/or other non-target cells 114 contained in the initial carrier fluid 112 when the initial carrier fluid 112 passes over the flow surface 108. The flow surface 108 is configured to include indentations 116 sized with a diameter substantially similar to but not smaller than at least a portion of a target cell 110. The flow surface 108 can have an area of any shape and usually conforms to the cross-section of the channel 102. The flow surface 108 could cover the entire channel 102, or only a portion of the channel 102. Illustrative dimension of an area of the flow surface 108 can be about 0.1-1 cm by about 3-30 cm square. Illustrative sizes for the indentations will be described further below. Basically, the size and shape of the surface area of flow surface 108 can have any of various configurations, so will not be discussed in further detail to avoid obscuring the concepts herein.

[00030] Optionally, the apparatus 100 can include a flow regulator 118 that is in fluid communication with the inlet 104 of the channel 102. The flow regulator 118 regulates a flow of the initial carrier fluid 112 from a first reservoir 120 (optional) that is connected to the flow regulator 118 and holds initial carrier fluid 112. The flow regulator 118 may also be connected to a second reservoir 122 (optional) that holds discharge fluid 132a that can flow into the inlet 104 of the channel 102. In one embodiment, the flow regulator 118 can operate the flow of from the first reservoir 120 at a different flow rate than the flow from the second reservoir 122. In one embodiment, the flow regulator 118 is a pump such as, but not limited to a peristaltic pump, diaphragm pump, and the like. In one embodiment, the flow regulator 118 operates at a flow rate ranging from about 0.5 mm³/min to about 200 cm³/min, or about 1 mm³/min to about 10 cm³/min, or about 10

mm³/min to about 1 cm³/min, or about 100 mm³/min to about 1 cm³/min, as illustrative examples. Various types of flow regulators are commercially available. Example flow regulators include, but are not limited to, ultrasonic flowmeter (Transonic Systems Inc., Ithaca, NY), electromagnetic blood flowmeter (NIHON KOHDEN CORPORATION, Tokyo, Japan) liquid flow meter ASL1600 (Sensirion AG, Zurich). The flow meter can also include a separate flow pump and a flow meter for achieving flow control.

[00031] In one embodiment, the first reservoir 120 and second reservoir 122 may be the same device and may hold target cells and/or non-target cells separately from the carrier fluid. In one embodiment, the carrier fluid is used to carry the target cells and/or non-target cells through the channel 102 and also serves as the discharge fluid. In other embodiments, a separate liquid is used as the discharge fluid. Examples of carrier fluids include, but are not limited to saline, bovine serum, fetal calf serum, Dulbecco's modified Eagle's media, nutrient broths, and the like. Examples of discharge fluids include, but are not limited to, saline, bovine serum, fetal calf serum, Dulbecco's modified Eagle's media, nutrient broths, and the like.

[00032] Optionally, the apparatus 100 can further include a detector 124 in communication with the outlet 106 of the channel 102 that detects flow of carrier fluid, discharge fluid, target cells, and/or non-target cells flowing from the outlet 106 of the channel 102. In one embodiment, the detector 124 is one of an optical density detector, an absorption spectrometer, or a turbidimeter. In another embodiment, the detector 124 is an autosampler that can both detect flow of carrier fluid, discharge fluid, target cells, and/or non-target cells flowing from the outlet as well as dispense cells into different sampling containers (described below). Examples of autosamplers are commercially manufactured and available by Varian Medical Systems and Beckman Coulter. Various

types of detectors 124 are commercially available so will not be described in further detail to avoid obscuring the concepts herein.

[00033] Optionally, the apparatus 100 can also include one or more sampling containers 126a through 126e positioned near the outlet 106 of the channel 102. Suitable examples of sampling containers include, but are not limited to, culture dishes, test tubes, microcentrifuge tubes (e.g., Eppendorf tubes), conical centrifuge tubes, culture flasks, and the like. In one embodiment, the sampling containers 126a through 126e are configured to rotate positioning underneath the outlet 106 so that each sampling container 126 collects different materials flowing through the channel 102, as described further below.

10 [00034] When the detector 124 does not have an autosampling function, a flow regulating valve 128 can be installed below the detector 102. When detector 102 detects passage of the desired target cells, then regulating valve 128 can be closed, stopping fluid flow from the outlet 106 of the channel 102. A clean sampling container can be positioned under the outlet 106 and then the flow regulating valve 128 opened to collect the target cells. In this embodiment, preferably the detector 124 is placed along channel 102 at a location sufficient to allow the regulating valve 128 to close before the target cells reach an end of the outlet 106.

[00035] Finally, the apparatus 100 can optionally include a stimuli device 132b disposed in communication with the flow surface 108 to apply a stimulus to the flow surface 108, as described below in further detail.

Illustrative Operations for Separating Target Cells

[00036] Figures 1A through 1D are schematic drawings of the illustrative embodiment of the apparatus 100 in various stages of operation. In Figure 1A, the reservoir 120 contains initial carrier fluid 112. To prepare the initial carrier fluid, target cells 110 and/or non-target cells 114 are selected from a culture in which it is desired to concentrate

and/or separate the target cells 110. Adherent cells can be detached a culture dish using, for example, trypsin EDTA. The trypsin can be deactivated using an equal amount of Dubelcco's Modified Eagle's Medium and 10% fetal bovine serum. Cells can then be centrifuged to a pellet and resuspended in phosphate buffered saline pH 7.4. The
5 suspension forms the initial carrier fluid. The suspension can additionally include adhesion-promoting proteins that assist target cells to adhere to hydrophobic surfaces. Other methods for preparing initial carrier fluid are commercially known and available.

[00037] The regulator 118 can be operated to initiate a flow of initial carrier fluid 112 containing at least target cells 110 from the reservoir 120. The inlet 104 of the channel
10 102 receives the initial carrier fluid 112 containing target cells 110. Any combination of target cells 110 and/or non-target cells 114 may be contained in the initial carrier fluid 112. The initial carrier fluid 112 passes over the flow surface 108 of the channel 102. In one embodiment, the initial carrier fluid 112 has a combination of target cells 110 and non-target cells 114.

15 [00038] Turning to Figure 1B, when the initial carrier fluid (Figure 1A) passes over the flow surface, the indentations 116 on the flow surface 108 capture target cells 110. This separates the target cells 110 from the initial carrier fluid, leaving extraneous carrier fluid 130 to flow to the outlet 106 of the channel 102. As shown in Figure 1B, capturing the target cells 110 creates a time difference between when the extraneous carrier fluid 130
20 and the captured target cells 110 arrive at the outlet 106. The extraneous carrier fluid 130 thus arrives at the outlet 106 before the target cells 110.

[00039] Figure 1B also shows that when the initial carrier fluid (Figure 1A) includes non-target cells 114 along with target cells 110, the non-target cells 114 pass over the flow surface 102, are not captured by the indentations 116, and flow to the outlet 106

along with the extraneous carrier fluid 130. This allows non-target cells 114 to be collected separately from the target cells 110.

[00040] After the target cells 110 are captured by the indentations 116, an energy source 132a and/or 132b are applied to the flow surface 108 at a particular time that triggers release of the target cells 110. The indentations 116 are responsive to the energy source 132a and/or 132b to allow the released target cells to flow to the outlet of the channel. The energy source 132a and/or 132b may also assist the released target cells 110 to flow to the outlet 106 of the channel 102. As shown in Figure 1B, the target cells 110 flow at a delayed period after the extraneous carrier fluid 130 and/or non-target cells 114 flow to the outlet 106 of the channel 102.

[00041] In one embodiment, the energy source 132a is a discharge fluid that provides a fluid pressure to dislodge the captured target cells 110 from the indentations 116. In one embodiment, the flow rate of the discharge fluid is about 0.75 $\mu\text{L}/\text{min}$ to about 25 $\mu\text{L}/\text{min}$, or about 5 $\mu\text{L}/\text{min}$ to about 100 $\mu\text{L}/\text{min}$, about 15 $\mu\text{L}/\text{min}$ to about 500 $\mu\text{L}/\text{min}$, as illustrative examples. The velocity of the discharge fluid is about 50 $\mu\text{m}/\text{s}$ to about 400 $\mu\text{m}/\text{s}$, or about 100 $\mu\text{m}/\text{s}$ to about 800 $\mu\text{m}/\text{s}$, as illustrative examples. The pressure of the discharge fluid is about 0.1 mPa to about 1 mPa, or about 0.1 mPa to about 0.8 mPa, or about 0.1 mPa to about 0.5 mPa, as illustrative examples. In one embodiment, the shear stress of the discharge fluid can be about $2.5 \times 10^{-3} \text{ dyn}/\text{cm}^2$ to about $6 \times 10^{-2} \text{ dyn}/\text{cm}^2$, or about $3.5 \times 10^{-3} \text{ dyn}/\text{cm}^2$, as illustrative examples.

[00042] In one embodiment, the discharge fluid 132a may be applied as a pulse of fluid having enough pressure to dislodge the captured target cells 110. In one embodiment, the fluid pressure is sufficient to overcome functional group interactions between the target cells 110 and the indentations 116. The amount of fluid pressure is such that the shear stress will be below a level at which target cells 110 contained in the

indentations 116 would be damaged, and may vary depending on the type of target cell 110. Note that even though the figures show only initial and extraneous carrier fluids 112, 130 and discharge fluid 132a, the channel 102 may actually be continuously flowing a minimal amount of carrier fluid or other fluid so that the target cells 110 continue to thrive even when they are captured by the indentations 116. The continuous flow of a minimal amount of carrier fluid or other fluid has a fluid pressure that is sufficiently low enough not to dislodge the captured target cells 110.

[00043] In another embodiment, the energy source 132b is a stimulus that initiates a photo-chemical response in the indentations 116 to release the captured target cells 110, as will be described further below. The stimulus serves to make the indentations 116 less accessible to capture target cells 110, thus facilitating release of the target cells 110 to flow to outlet 106 of channel 102.

[00044] Figure 1C shows the captured target cells 110 being released from the indentations 116 and allowed to flow to the outlet 106 of the channel 102. In embodiments where the first energy source is discharge fluid 132a, the discharge fluid 132a can then carry the released target cells 110 to the outlet 106 of the channel 102. Further, even in embodiments where the first energy source is a stimulus 132b, discharge fluid 132a may still be flowed through channel 102 to assist in moving the target cells 110 toward the outlet 106 of the channel 102.

[00045] The detector 124 can detect when the extraneous carrier fluid 130 and/or non-target cells 114 reach the outlet 106. In one embodiment, a plurality of sample containers 126a through 126e are positioned ready to collect discharge from the outlet 106. In one embodiment, the plurality of sample containers 126a through 126e are located on a revolving track (not shown). When the detector 124 detects the extraneous carrier fluid 130, the revolving track can begin to operate so that at least one sample container 126a

through 126e is positioned under the outlet 106 to collect the extraneous carrier fluid 130. For example, Figure 1C shows sample container 126b positioned to collect the extraneous carrier fluid 130 and/or non-target cells 114.

[00046] Likewise, the detector 124 can detect when the target cells 110 and/or
5 discharge fluid 132a reach the outlet 106. Figure 1D shows that when the detector 124 detects the target cells 110, the revolving track can operate so that a different sampling container 126d is positioned under the outlet 106 to collect target cells 110 and/or discharge fluid 132a.

[00047] The foregoing shows that the target cells 110 can thus be captured and
10 retained by the indentations 116 of the flow surface 108 to delay the flow of target cells 110, thus separating the target cells 110 from the extraneous carrier fluid 130. As used herein, the term “extraneous carrier fluid” generally means the initial carrier fluid 112 less whatever was captured by the indentations 116. It is possible that the extraneous carrier fluid 130 includes at least some target cells 110 that were not captured by the indentations
15 116. In addition, it is possible that one or more non-target cells 114 were captured by one or more indentations 116, especially where a non-target cell 114 may have a portion having a smaller diameter than the diameter of the indentations 116. In these embodiments, the separation of the target cells 110 from the extraneous carrier fluid 130 should be viewed as increasing a concentration of the target cells 110 in a resulting
20 collection sample. Or, the separation of the target cells 110 from the extraneous carrier fluid can be one step to increasing the purity of the target cells 110 in a resulting sample, where an additional step will be applied (such as another pass through the cell separator apparatus disclosed herein, filtration, centrifugation, antibody process, beads), to remove the remaining non-target cells 114.

[00048] Figure 1E is an illustrative method for separating target cells using, for example, a cell separator. At 150, the method includes passing an initial carrier fluid containing target cells through an inlet of a channel to pass over a flow surface of the channel. The flow surface has indentations sized with a diameter that substantially
5 matches a diameter of the target cells. At 152, the indentations on the flow surface capture target cells as the initial carrier fluid passes over the flow surface, leaving extraneous carrier fluid to flow to the outlet of the channel.

[00049] At 154, a first energy source is applied to initiate release of the captured target cells from the indentations such that the released target cells flow to the outlet of the
10 channel. In one embodiment, the first energy source is applied at a particular time that causes the released target cells to flow to the outlet of the channel at a delayed period after the extraneous carrier fluid flows to the outlet of the channel. In one embodiment, the first energy source is a discharge fluid being applied to the inlet of the channel. In another embodiment, the first energy source is a stimuli, such as an optical device, being
15 applied to the flow surface.

[00050] At 156, the extraneous carrier fluid is collected. At 158, the target cells are collected separately from the extraneous carrier fluid.

Forming Flow Surfaces Having Indentations

[00051] Figures 2A through 2C show various illustrative embodiments of flow
20 surfaces, and particularly illustrative embodiments of indentations, that can be used for the targeted separation of cultured cells disclosed herein.

[00052] Figure 2A shows one embodiment of a portion of a flow surface 200A located on a portion of a channel wall 202. The channel wall 202 serves as a substrate for the flow surface 200A. The materials of the channel wall 202 can be, but are not limited to,
25 glass, quartz, polycarbonate, plastic, silicon, aluminum, stainless steel, ceramic, and the

like. Other materials for the channel wall 202 will be readily apparent to those skilled in the art, thus further description will not be provided to avoid obscuring the concepts herein.

[00053] The flow surface 200A includes a plurality of indentations 204. Each
5 indentation 204 is sized to receive at least a portion of a target cell 206. In one embodiment, the indentations 204 are rounded such that they conform to at least a portion of the target cell 206. In another embodiment, the indentations 204 may be cubical, triangular, or other polygonal three-dimensional shape. The height, h , of each indentation can be about 50 nm to about 500 μm , or about 500 nm to about 50 μm , or about 1000 nm
10 to about 25 μm , as illustrative examples. The diameter, d , of each indentation can be about 50 nm to about 500 μm , or about 500 nm to about 50 μm , or about 1000 nm to about 25 μm , as illustrative examples.

[00054] In one embodiment, the indentations 204 are arranged in a plurality of rows to form an array of indentations. As shown in Figure 2A, each row can have a distance, x ,
15 between indentations of about 10 nm to about 500 μm , or about 100 nm to about 50 μm , or about 200 nm to about 25 μm , as illustrative examples. In one embodiment, the rows of indentations align with the indentations the other rows. In another embodiment, the rows of indentations are offset from the indentations from adjacent rows. In one embodiment, the flow surface 200A includes an array of about 10 to about 1000
20 indentations by about 10,000 to about 1,000,000 indentations. Other embodiments of arranging an array of indentations are possible within the scope of this disclosure.

[00055] In one embodiment, the indentations 204 capture the target cells 206 using a physical interaction based on the indentations 204 having a diameter that is substantially similar to but not smaller than a diameter of a portion of the target cell 206. Various
25 methods are possible for manufacturing indentations having these characteristics.

[00056] In one embodiment, inclined UV lithography can be used to form a pattern of indentations in a layer of spin-coated negative photo resist, such as SU-8, such as using methods taught in *Simple Fabrication Process for Single Cell Analysis chip composed of Embedded Microchannels and Orifices*, T. Suzuki et al., The 2nd International Symposium on Micro & Nano Technology (ISMNT-2), Hsinchu, Taiwan, pp. 222-225, which is incorporated herein by reference.

[00057] In one embodiment, a mold can be fabricated using negative photoresists (such as SU-8 50 and SU-8 2002). The mold has a desired number and size of protrusions that match the desired number and size of indentations desired of the flow surface. A layer of poly-dimethylsiloxane (PDMS) can be poured onto the mold, cured, then separated from the mold to provide a layer of PDMS layer having a pattern of indentations formed therein. Additional details regarding this method are found in *Dynamic Single Cell Culture Array*, Di Carlo et al., Lab Chip, 2006, 6, 1445-1449, which is incorporated herein by reference.

[00058] In another embodiment, capillary force lithography can be used to form indentations in a poly(ethylene glycol) dimethacrylate (PEG-DMA) film layered on a silicone/glass substrate. A PDMS stamp or mold contains protrusions and is laid on the PEG-DMA film, which forms indentations in the PEG-DMA film and exposes the substrate. The exposed substrate in the indentations can promote cell adsorption. Additional details regarding this method are found in *A Simple Soft Lithographic Route to Fabrication of Poly(Ethylene Glycol) Microstructures for Protein and Cell Patterning*, Suh et al., Biomaterials 25 (2004) 557-563, which is incorporated herein by reference.

[00059] In still another embodiment, indentations can be formed on a surface of photo-responsive polymer (such as an azobenzene-functionalized polyurethane film) using an optical near field generated around polystyrene spheres that have substantially the same

diameter as the desired diameter of the indentation. The polystyrene spheres can be laid on a film of polyurethane polymer and irradiated with a linearly polarized Ar⁺ laser at 488 nm. Without being tied to one theory, it is believed that the indentations form by a process of self-assembly resulting from interactions (e.g., dipole-dipole and/or hydrogen bonding) between the polystyrene spheres and the photo-responsive polymer. The polystyrene spheres can then be removed by washing them from the polyurethane polymer, leaving an array of indentations. A description of how the optical near field conforms azobenzene moieties from a cis to a trans state to cause the polyurethane to conform to the shape of the sphere is found in *Topographical Nanostructure Patterning on the Surface of a Thin Film of Polyurethane Containing Azobenzene Moiety Using the Optical Near Field around Polystyrene Spheres*, Hasegawa et al., *Macromolecules*, 2001, 34 (21), 7471-7476, which is incorporated herein by reference.

[00060] Thus far, methods for forming the flow surface with indentations have provided embodiments of indentations that capture the target cells using mechanical size-matching principles. In another embodiment, the indentations 204 also use chemical interactions to capture and retain target cells 206. In one embodiment, the indentations 204 have functional groups that interact with particular functional groups located on the target cells 206. Thus, target cells 206 can be selected based on whether they contain one or more functional groups that can interact with the functional groups of the indentations 204.

[00061] Figure 2B shows an illustrative example of indentations having functional groups. Figure 2B shows another illustrative example of a flow surface 200B bonded to channel wall 202. The flow surface 200B includes indentations 204 that include one or more functional groups 208 configured to interact with one or more functional groups 210 of the target cell 206. When a non-target target cell 206 has too large of a diameter

and/or does not have functional groups complementary to the functional groups 208 of the indentations 104 (or may have one or more functional groups 214 that cannot interact with the functional groups 208 of the indentation 204), then the indentation 204 is not able to capture the non-target target cell 206. Note that the functional groups 214 and
5 functional groups 210 are schematically representations of chemical functional groups only. The schematic shapes simply represent that the functional groups 214 and functional groups 210 are configured to be complementary to each other.

[00062] The functional groups 208 of indentations 204 may be generated as part of the process of making indentations 204, such as by using a polymer materials as the flow
10 surface 200B that already has the desired functional groups 208.

[00063] In yet another embodiment, the surface of the indentations 204 may be imprinted complementarily with the target cells 206. This can include using the target cells 206 to actually form the indentations 204 (including forming functional groups 208) in a flow surface 200B. Thus, the flow surface 200B can be specialized to recognize
15 certain target cells 206 due to imprinting. Even when non-target target cell 206 of the same or smaller size are contained in the initial carrier fluid, the indentations 204 do not capture the non-target target cell 206 because the functional groups 208 of the indentations 204 do not interact with the non-target cells 114.

[00064] In one embodiment, a method for forming indentations 204 having functional
20 groups 208 is shown in Figure 2C. The substrate 202 is at least partially coated with a photo-responsive polymer 216. In one embodiment, the photo-responsive polymer 216 is a diazo compound, such as the diazo compound shown in Figure 2D, which can be synthesized according to Watanabe et al., Chem., 1996, 6, 1487, incorporated herein by reference. The photo-responsive polymer film 216 can be formed by preparing a 8 wt %
25 pyridine solutions of photo-responsive polymer. This solution is passed through a 0.20

mm filter (Millipore) to remove impurities before spin-coating. An optically clear film of photo-responsive polymer 216 is then spin-coated onto a substrate 202 (e.g., glass substrate). The resultant photo-responsive polymer film 216 is placed in a vacuum oven at 130 °C for over 24 hrs to remove any solvent.

5 **[00065]** To form the indentations, an aqueous solution of imprinting cells 218 held in saline, PBS, or other culture medium, is poured onto the photo-responsive polymer film 216. The imprinting cells 218 were allowed to arrange themselves on the photo-responsive polymer film 216 by a self-organization process. In one embodiment, the imprinting cells 218 contain the same functional groups 210 as the target cells. In one
10 embodiment, the imprint cells 218 are the same type of cell as the target cells. The functional groups 210 assist to imprint a specific recognition site 208 into the indentation.

[00066] An optical device irradiates light onto the photo-responsive polymer film 216. Various light sources may be used, such as, but not limited to, , a mercury-vapor lamp, a xenon-mercury lamp, a high intensity discharge lamp, an LED lamp, a laser (e.g., an
15 argon laser, and the like. The light source can be radiated at normal incidence to the surface of the photo-responsive polymer film 216. The photo-responsive polymer film 216 can also be held vertically to avoid the effects of gravity. The range of wavelength from the optical device can be from about 400 nm to about 550 nm, from about 480 nm to about 490 nm, or about 488 nm, as illustrative examples. The irradiation time may range
20 from 30 seconds to 10 minutes, or about 1 minute to about 5 minutes, or about 5 minutes, as illustrative examples. The intensity of the irradiation may range from about 70 W/cm² to about 280 W/cm².

[00067] Resultant distribution of intensity of the light irradiated on the imprinting cells 218 and on the photo-responsive polymer film creates indentations beneath the imprinting
25 cells 218 due to a volume change between bright parts and dark parts of the photo-

responsive polymer film similar to that taught in Hasegawa et al., Macromolecules, 34, 7471 (2001)), incorporated by reference. In other words, the imprinting cells 218 block the irradiation to create shade, which creates concaves in the photo-responsive polymer film. Without being tied to one theory, it is believed that the indentations form by a process of self-assembly resulting from interactions (e.g., dipole-dipole and/or hydrogen bonding) between the polystyrene spheres and the photo-responsive polymer.

[00068] The photo-responsive polymer film 216 was then washed with benzene for 3 days in order to remove the imprinting cells 218 from photo-responsive polymer 216 to reveal the indentations 220 formed therein. The photo-responsive polymer film 216 having indentations 220 was then subsequently dried in vacuo at 25 °C for 2 days.

[00069] In still another embodiment, Figure 2E shows another embodiment of a flow surface 200C in which at least a portion of a surface of the indentations 204 can be coated with a support molecule. Figure 2E shows a schematic of an illustrative support molecule, a chemical operation of the support molecule upon irradiation by an optical energy source, and an interaction between a cell and the support molecule when the polarity of the support molecule changes. The support molecules 250 shown on the left has a first region 252 configured to bind to a surface of the indentation 204 and a photo-responsive region 256 on the opposing end of the first region 252. The support molecule 250' on the left is shown in a first conformation. The support molecule 250' on the right has a first region 252 bound to the indentation 204, but a photo-responsive region 256' in a second conformation due to a first optical energy source 260 irradiating the support molecule 250. The numerals for support molecule 250 and support molecule 250' are used to indicate that the conformation of the support molecule has changed.

[00070] The photo-responsive region 256 is configured to change conformation between at least a first polarization 258 (indicated by the dashed area on the left) and a

second polarization 258' (indicated by the dashed area on the right) upon irradiation from at least the first optical energy source 260. The photo-responsive region 256/256' includes one or more polar groups that alter polarity from the first polarization 258 to the second polarization 258', and vice versa. In some embodiments, the support molecule
5 may have additional functional groups that have other functionalities.

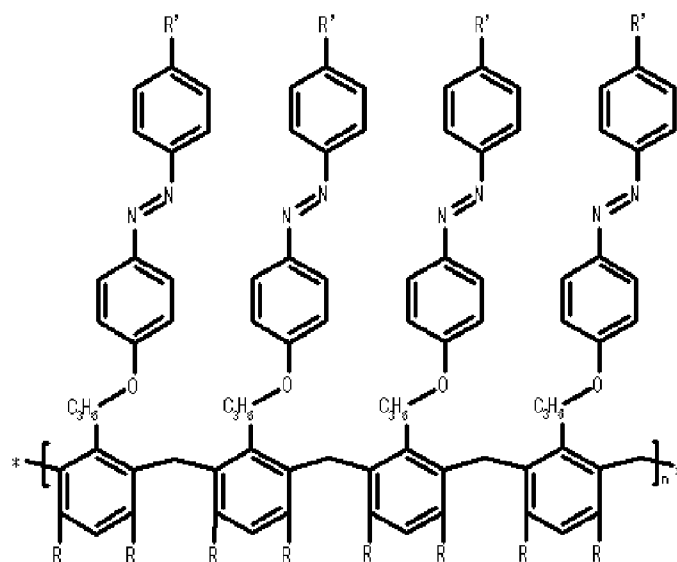
[00071] When the photo-responsive region 256 has the first polarization 258, the photo-responsive region 256 has a first conformation that is accessible to and/or capable of adhering to target cells 206. This is shown in Figure 2E on the left by a cell 206 that is adhered to the photo-responsive region 256 having the first conformation. When the
10 photo-responsive region 256' has the second conformation, the photo-responsive region 256' is more hydrophilic than the first polarization 256, making the photo-responsive region less accessible and/or less conducive to adhering to target cells 206. This is shown in Figure 2E on the right by the photo-responsive region 256' being more likely to bond to water molecules 264 and less likely to adhere to cells so that the target cell 206 being
15 unadhered, detached, or disconnected from the photo-responsive region 256' having the second polarization 258'.

[00072] Embodiments herein contemplate that when the photo-responsive region 256 of the support molecules is in the first conformation that is able to adhere to target cell 206, the first polarization 258 is more hydrophobic than the second polarization 258',
20 thus encouraging cells to adhere thereto. When the photo-responsive region 256' conforms to the second conformation, the second polarization 258' is more hydrophilic than the first polarization 258, thus being more attracted to water than to hydrophobic lipid cellular walls. It may be the case that the first polarization 258 is actually somewhat hydrophilic, in which case, the second polarization 258' can be characterized as more
25 hydrophilic than the first polarization 258. In some embodiments, the photo-responsive

region may go through more than two conformations, with each conformation having a different polarization, phases, or grades of polarization, with some grades being more hydrophobic and some grades being more hydrophilic.

[00073] It may be the case that the first conformational state is actually somewhat
5 hydrophilic, in which case, the second conformational state can be characterized as more hydrophilic than the first polarization. In some embodiments, the photo-responsive region may go through more than two conformations, with each conformation having a different polarization or phases or grades of polarization, with some grades being more hydrophobic and some grades being more hydrophilic. The terms “hydrophobic” and
10 “hydrophilic” are used broadly in conjunction with terms like “more” and “less” so that it is clear that the disclosure herein is not limited to the first conformational state being hydrophobic *per se* or the second conformational state being hydrophilic *per se*. Rather, the second conformational state is generally “more hydrophilic” than the first conformational state. Or in other terms, the second conformational state is generally “less
15 hydrophobic” than the first conformational state.

[00074] The embodiment of Figure 2E shows one example of altering polarity of the photo-responsive region 256/256' using a cis-to-trans transformation of the support molecule 250/250'. The support molecule 250/250' shown in Figure 2E is reproduced below:



In one embodiment, R is C_8H_{17} or C_mH_{2m+1} where m is 3 to 18 and R' is OCH_2COOH , OCH_2CH_2COOH , $OCH_2CH_2OCH_2COOH$, or $OCH_2CH_2OCH_2CH_2COOH$. The diazo compound is a crown conformer of ocarboxymethylated calix[4]resorcinarene (CRA-CM) bearing four p-octylazobenzene residues at one of the rims of the cyclic skeleton. CRA-CM is a macrocyclic amphiphile (including the R group) forms the first region 252 that binds to the indentations 204. The azobenzene moieties (including the R' group) form the photo-responsive region 256/256'. The CRA-CM form a rigid cylindrical macrocycle that tethers the azobenzene moieties and can be densely packed into a single-molecule support layer.

[00075] The azobenzene moieties (i.e., the photo-responsive region 256/256') isomerizes upon irradiation of a certain wavelength of an optic energy source. The azobenzene moieties have polar groups R' that switch polarity from a first polarization 258 to a second polarization 258' upon irradiation by the optic energy source.

[00076] In one embodiment, the indentations 204 having support molecules 250 of an azobenzene compound may be prepared by immersing the flow surface 200C formed using any of the methods above in a dilute solution of CRA-CM, yielding a robust

monolayer of support molecules 250 with dense packing over the flow surface and coating the indentations 204.

[00077] When the photo-responsive region 256' is more hydrophilic, the support molecules are more likely to bond to water molecules 264 than to the cells. This is also referred to as "wettability" of the indentations 204. This is due to a change in conformation of the photo-responsive region 256', which causes the photo-responsive region 256' to become less or more hydrophobic/hydrophilic depending on the conformation when manipulated by an optical energy source. When the the photo-responsive region 256' is less hydrophilic, the the photo-responsive region 256' is more able to adhere to cells. When the the photo-responsive region 256' is more hydrophilic, the hydrophobic portions of the support molecules are less available and less susceptible to bonding with the membranes of the cultured cells and the hydrophilic portions of the photo-responsive region 256' is more likely to bond to water than to the cells. This wettability of the indentations 204 eases separation of the target cells 206 because the target cells 206 become naturally unadhered, detached, or disconnected to the indentation 204 in a noninvasive manner.

[00078] In some embodiments, a conformation of the photo-responsive region 256/256' not only affects the conformational state, but may also have a steric effect that contributes to cell adherence. When the photo-responsive region 256 is in the first conformation that is more conducive to cell adherence than the second conformation, the first conformation may also have a chemical structure that is more accessible to the cultured cells. This is shown in Figure 2E by the diazo functional group $N=N$ being located on top of the support molecule 250 and exposed to be accessible to a target cell 206. When the photo-responsive region 256' is in the second conformation, the photo-responsive region 256' may have a second chemical structure where sites to which the

target cell 206 would normally adhere are now less accessible, making it less conducive for the target cell 206 to adhere. This is shown in Figure 2E by the polar groups R' being on the top of the support molecule 250' and exposed to be accessible to water molecules 264, while the diazo functional group N=N is now part of the cyclic tethering chain.

5 Further, the support molecules 250 are densely packed in a monolayer over the indentations 204 so the N=N diazo groups are surrounded by other cyclic chains, preventing access by target cell 206 to the diazo groups.

[00079] In one embodiment, the flow surface 200C and/or bottom of the channel wall 202 is at least partially transparent to allow light to pass through the bottom of the flow surface 200C. In another embodiment, the channel wall may have a top opening or be at least partially transparent allowing light to reach the flow surface 200C from above.

[00080] Turning back to Figures 1A through 1D, the support molecules 250 are operable in the indentations 116 to assist the capture and release of target cells 110. As shown in Figures 1A through 1D, the stimuli 132b may be an optical device that is operable to selectively radiate optical energy onto at least a portion of the flow surface 108 at the desired time. In one embodiment, the optic device irradiates a light in a wavelength of about 400 nm to about 500 nm, or about 420 nm to about 470 nm, or about 430 nm to about 440 nm, as illustrative examples. In one embodiment, the optic device irradiates a light having a power of about 1 mW/cm² to about 100,000 mW/cm², or about 10 mW/cm² to about 10,000 mW/cm², or about 100 mW/cm² to about 1,000 mW/cm², as illustrative examples. In one embodiment, the optic device 114 irradiates a light for about 0.1 sec to about 10,000 sec (2.75 days), or about 1 sec to about 1,000 sec (about 16 minutes), or about 10 sec to about 100 sec, as illustrative examples. In one embodiment, the optic device irradiates a light at a distance from the flow surface 108 of about 1 cm to about 200 cm, or about 5 cm to about 100 cm, or about 25 cm to about 50 cm, as

illustrative examples. In each of these examples, the entire flow surface 108 does not have to be irradiated; rather an optic element can irradiate only a portion of the flow surface 108 at the wavelength, power, time period, and/or distance as illustratively described above. Further various combinations of wavelength, power, time period, and/or distance from the cell separator may be applied to trigger the photo-responsive region to conform from the first polarization to the second polarization.

[00081] In some embodiments, the photo-responsive region is activated using an optic device including, but not limited to, a mercury-vapor lamp, a xenon-mercury lamp, a high intensity discharge lamp, an LED lamp, and the like. Various optic devices are commercially sold and can be readily adapted to be included in an apparatus of this disclosure.

[00082] The foregoing provides efficient, cost-effective ways of separating and/or concentrating target cells from a carrier fluid that may have other non-target cells. While single target cells have been shown in the above illustrative embodiments, the term “target cell” also contemplates cell aggregations in which it is desired to separate and/or concentrate cell aggregations. Cells such as stem cells typically aggregate in a cell culture. Indentations can be created with an appropriate size of the aggregation based on the disclosure herein. Other variations will also be apparent based on the disclosure herein.

[00083] The present disclosure is not to be limited in terms of the particular embodiments described in this application, which are intended as illustrations of various aspects. Many modifications and variations can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the disclosure, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such

modifications and variations are intended to fall within the scope of the appended claims. The present disclosure is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled. It is to be understood that this disclosure is not limited to particular methods, reagents, compounds
5 compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[00084] In an illustrative embodiment, any of the operations, processes, etc. described herein can be implemented as computer-readable instructions stored on a computer-
10 readable medium. The computer-readable instructions can be executed by a processor of a mobile unit, a network element, and/or any other computing device.

[00085] There is little distinction left between hardware and software implementations of aspects of systems; the use of hardware or software is generally (but not always, in that in certain contexts the choice between hardware and software can become significant) a
15 design choice representing cost vs. efficiency tradeoffs. There are various vehicles by which processes and/or systems and/or other technologies described herein can be effected (e.g., hardware, software, and/or firmware), and that the preferred vehicle will vary with the context in which the processes and/or systems and/or other technologies are deployed. For example, if an implementer determines that speed and accuracy are
20 paramount, the implementer may opt for a mainly hardware and/or firmware vehicle; if flexibility is paramount, the implementer may opt for a mainly software implementation; or, yet again alternatively, the implementer may opt for some combination of hardware, software, and/or firmware.

[00086] The foregoing detailed description has set forth various embodiments of the
25 devices and/or processes via the use of block diagrams, flowcharts, and/or examples.

Insofar as such block diagrams, flowcharts, and/or examples contain one or more functions and/or operations, it will be understood by those within the art that each function and/or operation within such block diagrams, flowcharts, or examples can be implemented, individually and/or collectively, by a wide range of hardware, software, 5 firmware, or virtually any combination thereof. In one embodiment, several portions of the subject matter described herein may be implemented via Application Specific Integrated Circuits (ASICs), Field Programmable Gate Arrays (FPGAs), digital signal processors (DSPs), or other integrated formats. However, those skilled in the art will recognize that some aspects of the embodiments disclosed herein, in whole or in part, can be equivalently implemented in integrated circuits, as one or more computer programs 10 running on one or more computers (e.g., as one or more programs running on one or more computer systems), as one or more programs running on one or more processors (e.g., as one or more programs running on one or more microprocessors), as firmware, or as virtually any combination thereof, and that designing the circuitry and/or writing the code for the software and or firmware would be well within the skill of one of skill in the art in 15 light of this disclosure. In addition, those skilled in the art will appreciate that the mechanisms of the subject matter described herein are capable of being distributed as a program product in a variety of forms, and that an illustrative embodiment of the subject matter described herein applies regardless of the particular type of signal bearing medium used to actually carry out the distribution. Examples of a signal bearing medium include, 20 but are not limited to, the following: a recordable type medium such as a floppy disk, a hard disk drive, a CD, a DVD, a digital tape, a computer memory, etc.; and a transmission type medium such as a digital and/or an analog communication medium (e.g., a fiber optic cable, a waveguide, a wired communications link, a wireless communication link, etc.). 25

[00087] Those skilled in the art will recognize that it is common within the art to describe devices and/or processes in the fashion set forth herein, and thereafter use engineering practices to integrate such described devices and/or processes into data processing systems. That is, at least a portion of the devices and/or processes described
5 herein can be integrated into a data processing system via a reasonable amount of experimentation. Those having skill in the art will recognize that a typical data processing system generally includes one or more of a system unit housing, a video display device, a memory such as volatile and non-volatile memory, processors such as microprocessors and digital signal processors, computational entities such as operating
10 systems, drivers, graphical user interfaces, and applications programs, one or more interaction devices, such as a touch pad or screen, and/or control systems including feedback loops and control motors (e.g., feedback for sensing position and/or velocity; control motors for moving and/or adjusting components and/or quantities). A typical data processing system may be implemented utilizing any suitable commercially available
15 components, such as those typically found in data computing/communication and/or network computing/communication systems.

[00088] The herein described subject matter sometimes illustrates different components contained within, or connected with, different other components. It is to be understood that such depicted architectures are merely exemplary, and that in fact many
20 other architectures can be implemented which achieve the same functionality. In a conceptual sense, any arrangement of components to achieve the same functionality is effectively "associated" such that the desired functionality is achieved. Hence, any two components herein combined to achieve a particular functionality can be seen as "associated with" each other such that the desired functionality is achieved, irrespective of
25 architectures or intermedial components. Likewise, any two components so associated

can also be viewed as being "operably connected", or "operably coupled", to each other to achieve the desired functionality, and any two components capable of being so associated can also be viewed as being "operably couplable", to each other to achieve the desired functionality. Specific examples of operably couplable include but are not limited to
5 physically mateable and/or physically interacting components and/or wirelessly interactable and/or wirelessly interacting components and/or logically interacting and/or logically interactable components.

[00089] With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the
10 singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity.

[00090] It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (*e.g.*, bodies of the appended claims) are generally intended as "open" terms (*e.g.*, the term "including" should be interpreted as "including
15 but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to
20 understanding, the following appended claims may contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles "a" or "an" limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even
25 when the same claim includes the introductory phrases "one or more" or "at least one"

and indefinite articles such as "a" or "an" (*e.g.*, "a" and/or "an" should be interpreted to mean "at least one" or "one or more"); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that

5 such recitation should be interpreted to mean at least the recited number (*e.g.*, the bare recitation of "two recitations," without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to "at least one of A, B, and C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (*e.g.*, "a system

10 having at least one of A, B, and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where a convention analogous to "at least one of A, B, or C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (*e.g.*, "a system having at least

15 one of A, B, or C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of

20 including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B."

[00091] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the

25 Markush group.

[00092] As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being
5 broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as “up to,” “at least,” and the like include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed
10 above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

[00093] From the foregoing, it will be appreciated that various embodiments of the
15 present disclosure have been described herein for purposes of illustration, and that various modifications may be made without departing from the scope and spirit of the present disclosure. Accordingly, the various embodiments disclosed herein are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

CLAIMS

1. An apparatus for cell separation, the apparatus comprising:
a channel having an inlet and an outlet, the inlet configured to receive an
initial carrier fluid containing at least a first cell; and
5 a flow surface extending between the inlet and the outlet of the channel,
wherein the flow surface is configured to selectively capture at least the first cell
and to permit the carrier fluid to flow therethrough.
2. The apparatus as recited in claim 1, wherein the flow surface includes one
or more indentations sized with a diameter substantially similar to a diameter of at least a
10 portion of the first cell, wherein the one or more indentations are configured to capture
one or more target cells from the initial carrier fluid and release captured one or more
target cells responsive to a first energy source to allow the released one or more target
cells to flow to the outlet of the channel.
3. The apparatus as recited in one of claims 1-2, further comprising a first
15 container configured to collect the extraneous carrier fluid flowing from the outlet of the
channel and a second container configured to collect the released one or more target cells
flowing out of the channel separately from the extraneous carrier fluid.
4. The apparatus as recited in one of claims 1-3, further comprising a detector
in communication with the outlet of the channel and configured to perform one or more
20 of:
detect flow of extraneous carrier fluid flowing from the outlet of the
channel; and
detect flow of one or more target cells flowing from the outlet of the
channel.

5 5. The apparatus as recited in claim 4, wherein the initial carrier fluid includes one or more non-target cells that are not captured by the one or more indentations when passing over the flow surface, wherein the detector is further configured to detect flow of the one or more target cells flowing from the outlet of the channel.

6. The apparatus as recited in one of claims 1-5, further comprising a flow regulator in fluid communication with the inlet of the channel and configured to regulate a flow of the initial carrier fluid into the inlet of the channel.

10 7. The apparatus as recited in claim 6, wherein the flow regulator is configured to regulate a flow of discharge fluid into the inlet of the channel, the discharge fluid configured to provide a first energy source to release one or more target cells when captured in the one or more indentations.

8. The apparatus as recited in one of claims 6-7, further comprising a reservoir connected to the flow regulator and configured to hold the initial carrier fluid.

15 9. The apparatus as recited in claim 8, wherein the reservoir is configured to hold discharge fluid.

20 10. The apparatus as recited in one of claims 1-9, wherein the one or more first target cells are osteocytes, epidermal cells, organ cells, epithelial cells, ciliated epithelial cell, chondrocytes osteoblasts, osteocytes, muscle cells, glia cells, schwan cells, myelin sheath cells, hematopoietic cells, mast cells, hepatocytes, hepatic parenchymal cells, bone marrow cells, embryonic stem cells (ESCs), pluripotent stem cells, induced pluripotent stem cells, embryonic stem cells, adult stem cells, and multipotent and induced multipotent stem cells, blood cells, or a combination thereof.

25 11. The apparatus as recited in one of claims 1-10, wherein the one or more non-target cells are feeder cells, stimulating cells, immune cells, or a combination thereof.

12. The apparatus as recited in one of claims 1-11, wherein the flow surface comprises one or more of a negative photoresist, poly-dimethylsiloxane, poly(ethylene glycol) dimethacrylate, an azobenzene-functionalized polyurethane, or a combination thereof.

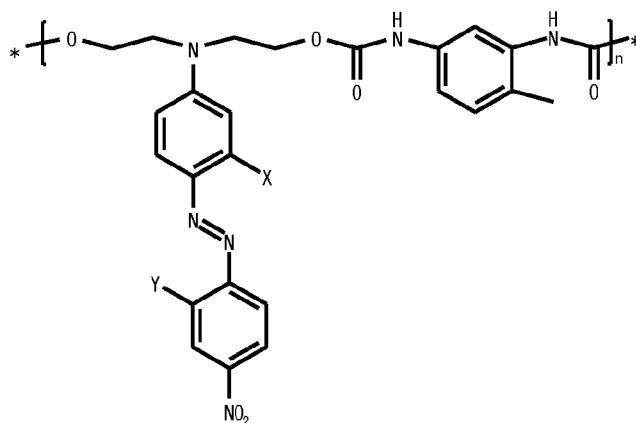
5 13. The apparatus as recited in one of claims 1-12, wherein the flow surface includes a $-\text{NO}_2$ or $-\text{NHCOO}$ functional group configured to interact with a first target cell having $-\text{OH}$, $-\text{NH}_2$, or $-\text{COOH}$ functional groups.

14. The apparatus as recited in one of claims 1-13, wherein the flow surface is at least partially coated with one or more photo-responsive molecules having a first
10 region configured to bind to the flow surface of the indentation and having a photo-responsive region configured to change conformation between a first more hydrophobic conformation and a second more hydrophilic conformation responsive to irradiation from at least an optical energy source,

wherein the first conformation increases the accessibility of the at least one
15 indentation to receive and interact with the first target cell, and

wherein the second conformation decreases the accessibility of the at least one indentation to receive and interact with the first target cell.

15. The apparatus as recited in claim 14, wherein the one or more photo-responsive molecules include an azobenzene having a formula of:



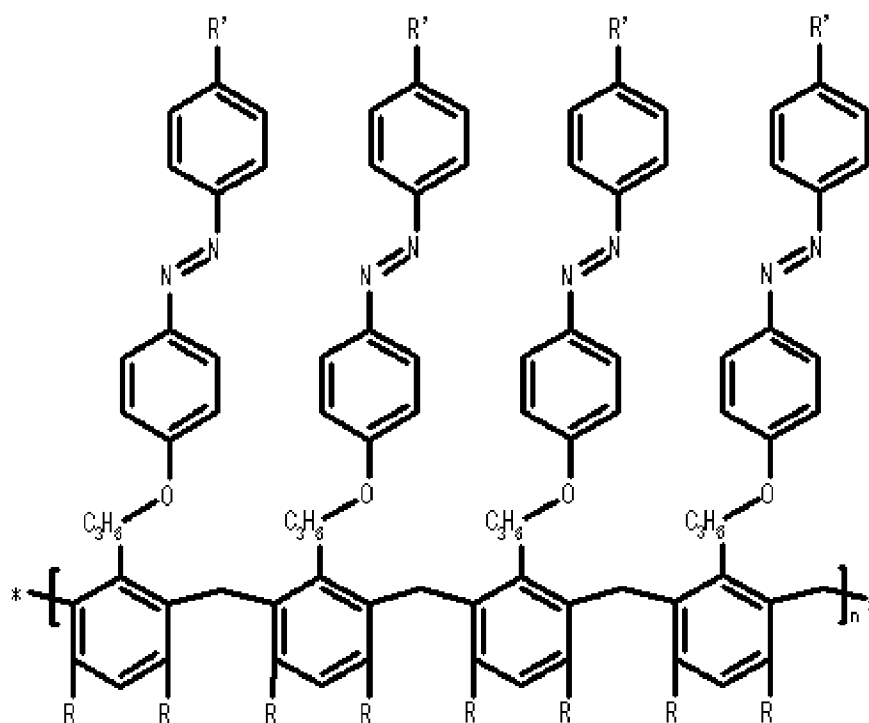
where X = H, CH₃, or C_mH_{2m+1} where m is integer between 1 and 12;

where Y = H, CH₃, or C_mH_{2m+1} where m is integer between 1 and 12; and

5 n = 5 to 200.

16. The apparatus as recited in claim 14, wherein the first conformation of the molecules is a more hydrophobic state and the second conformation of the molecules is a more hydrophilic state.

17. The apparatus as recited in claim 14, wherein the one or more photo-
10 responsive molecules include an azobenzene having a formula of:



wherein R is C₈H₁₇ or C_mH_{2m+1} where m is 3 to 18 and R' is OCH₂COOH, OCH₂CH₂COOH, OCH₂CH₂OCH₂COOH, or OCH₂CH₂OCH₂CH₂COOH.

18. The apparatus as recited in one of claims 1-17, further comprising an optical device configured to irradiate the photo-responsive molecules.

19. An apparatus for separating or concentrating target cells, the apparatus comprising:

a channel having an inlet and an outlet, the inlet configured to receive an initial carrier fluid containing a plurality of first target cells; and

10 a flow surface extending between the inlet and the outlet of the channel, the flow surface configured to include one or more indentations configured to capture and subsequently selectively release one or more of the plurality of first target cells when the initial carrier fluid passes over the flow surface, leaving extraneous carrier fluid to flow to the outlet of the channel.

20. The apparatus as recited in claim 19, wherein the initial carrier fluid includes one or more non-target cells that are not captured by the one or more indentations when passing over the flow surface.

21. The apparatus as recited in one of claims 19-20, wherein the flow surface
5 comprises one or more of a negative photoresist, poly-dimethylsiloxane, poly(ethylene glycol) dimethacrylate, an azobenzene-functionalized polyurethane, or a combination thereof.

22. The apparatus as recited in one of claims 19-21, wherein the one or more indentations are further at least a partially coated with one or more photo-responsive
10 molecules having a first region configured to bind to a surface of the indentation and having a photo-responsive region configured to change conformation between a first conformation and a second conformation responsive to irradiation from at least an optical energy source,

wherein the first conformation increases the accessibility of the at least one
15 indentation to receive and interact with the first target cell, and

wherein the second conformation decreases the accessibility of the at least one indentation to receive and interact with the first target cell.

23. The apparatus as recited in one of claims 19-22, wherein the one or more indentations are configured to release captured one or more target cells responsive to a
20 first energy source to allow the released one or more target cells to flow to the outlet of the channel.

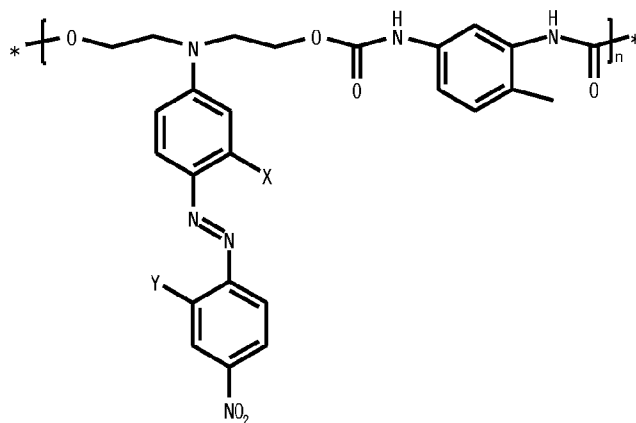
24. The apparatus as recited in one of claims 19-23, wherein the one or more first target cells are osteocytes, epidermal cells, organ cells, epithelial cells, ciliated epithelial cell, chondrocytes osteoblasts, osteocytes, muscle cells, glia cells, schwan cells,
25 myelin sheath cells, hematopoietic cells, mast cells, hepatocytes, hepatic parenchymal

cells, bone marrow cells, embryonic stem cells (ESCs), pluripotent stem cells, induced pluripotent stem cells, embryonic stem cells, adult stem cells, and multipotent and induced multipotent stem cells, blood cells, or a combination thereof.

25. The apparatus as recited in one of claims 19-24, wherein the one or more
5 non-target cells are feeder cells, stimulating cells, immune cells, or a combination thereof.

26. The apparatus as recited in one of claims 19-25, wherein the one or more indentations include a $-\text{NO}_2$ or $-\text{NHCOO}$ functional group configured to interact with a first target cell having $-\text{OH}$, $-\text{NH}_2$, or $-\text{COOH}$ functional groups.

27. The apparatus as recited in one of claims 19-26, wherein the one or more
10 indentations include an azobenzene have a formula of:



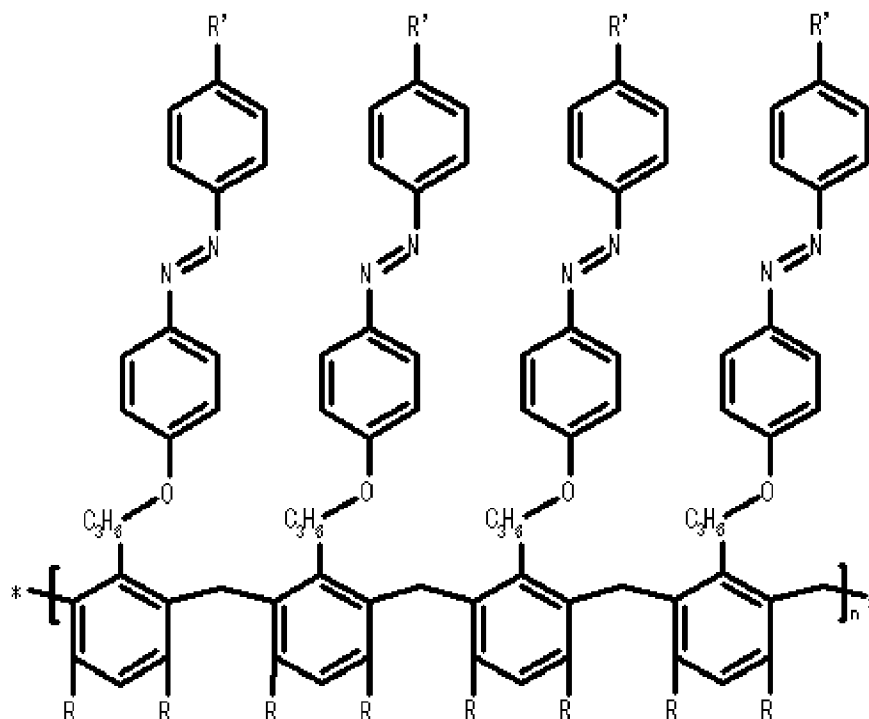
where $X = \text{H}$, CH_3 , or $\text{C}_m\text{H}_{2m+1}$ where m is integer between 1 and 12;

15 where $Y = \text{H}$, CH_3 , or $\text{C}_m\text{H}_{2m+1}$ where m is integer between 1 and 12; and

$n = 5$ to 200

28. The apparatus as recited in one of claims 22-27, wherein the first conformation of the molecules is a more hydrophobic state and the second conformation of the molecules is a more hydrophilic state.

29. The apparatus as recited in one of claims 22-28, wherein the one or more photo-responsive molecules include an azobenzene having a formula of:



wherein R is C_8H_{17} or C_mH_{2m+1} where m is 3 to 18 and R' is OCH_2COOH ,
 5 OCH_2CH_2COOH , $OCH_2CH_2OCH_2COOH$, or $OCH_2CH_2OCH_2CH_2COOH$.

30. The apparatus as recited in claim 19-29, further comprising an optical device configured to irradiate the photo-responsive region of at least some of the molecules with the first optical energy source.

31. A method for separating target cells from a carrier fluid, the method
 10 comprising:

passing an initial carrier fluid containing a plurality of first target cells through an inlet of a channel to pass over a flow surface of the channel, the flow surface having at least one indentation sized with a diameter that substantially matches a diameter of at least a portion of the first target cell to capture one or more of the plurality of first target
 15 cells as the initial carrier fluid passes over the flow surface, leaving extraneous carrier

fluid to flow to an outlet of the channel; and

applying a first energy source to initiate release of the captured one or more target cells from the at least one indentation such that the released one or more target cells flow to the outlet of the channel.

5 32. The method as recited in claim 31, further comprising

collecting the extraneous carrier fluid; and

collecting the at least one target cell separately from the extraneous carrier fluid.

10 33. The method as recited in one of claims 31-32, further comprising detecting when the extraneous carrier fluid have been collected so as to determine when to apply the first energy source onto the flow surface.

34. The method as recited inn one of claims 31-33, wherein the at least one indentation is at least partially coated with photo-responsive molecules having a first region configured to bind to a surface of the at least one indentation and having a photo-responsive region having a first conformation, further comprising

15 radiating a first optical energy onto at least some of the photo-responsive molecules so that the photo-responsive region changes to a second conformation that decreases the accessibility of the at least one indentation to receive and interact with the first target cell so that the at least one indentation releases the at least one target cell.

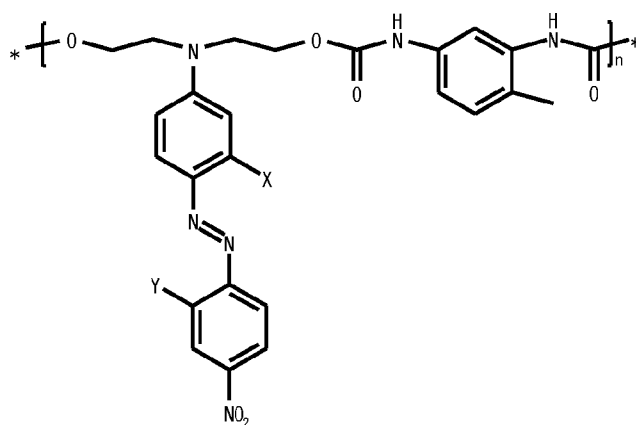
20 35. The method as recited in one of claims 31-34, further comprising regulating a flow of the initial carrier fluid over the flow surface and revolving a plurality of sampling containers at a speed to match the flow of the initial carrier fluid so that the extraneous carrier fluid are collected in separate sampling containers from the at least one target cell.

36. The apparatus as recited in one of claims 31-35, wherein the one or more first target cells are osteocytes, epidermal cells, organ cells, epithelial cells, ciliated epithelial cell, chondrocytes osteoblasts, osteocytes, muscle cells, glia cells, schwan cells, myelin sheath cells, hematopoietic cells, mast cells, hepatocytes, hepatic parenchymal cells, bone marrow cells, embryonic stem cells (ESCs), pluripotent stem cells, induced pluripotent stem cells, embryonic stem cells, adult stem cells, and multipotent and induced multipotent stem cells, blood cells, or a combination thereof.

37. The method as recited in one of claims 31-36, wherein the at least one indentation includes a $-\text{NO}_2$ or $-\text{NHCOO}$ functional group configured to interact with the plurality of target cells having $-\text{OH}$, $-\text{NH}_2$, or $-\text{COOH}$ functional groups.

38. The method as recited in one of claims 34-37, wherein the first conformation of the molecules is a more hydrophobic state and the second conformation of the molecules is a more hydrophilic state.

39. The method as recited in one of claims 34-38, wherein the one or more indentations include an azobenzene have a formula of:



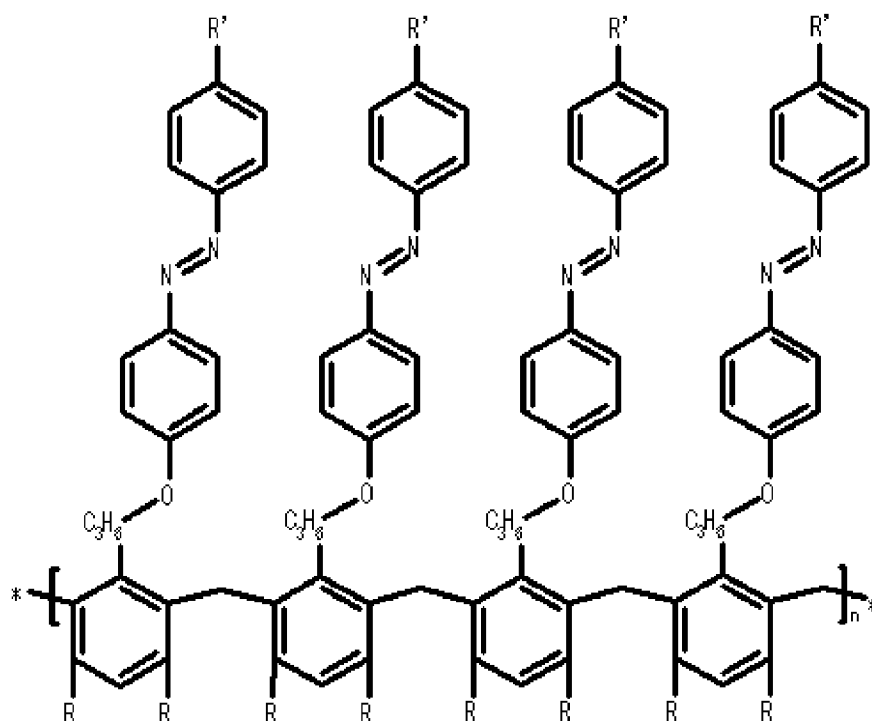
where $X = \text{H}$, CH_3 , or $\text{C}_m\text{H}_{2m+1}$ where m is integer between 1 and 12;

where $Y = \text{H}$, CH_3 , or $\text{C}_m\text{H}_{2m+1}$ where m is integer between 1 and 12; and

n = 5 to 200.

40. The method as recited in one of claims 34-39, wherein the first conformation of the molecules is a more hydrophobic state and the second conformation of the molecules is a more hydrophilic state.

5 41. The method as recited in one of claims 34-40, wherein the one or more photo-responsive molecules include an azobenzene having a formula of:



wherein R is C_8H_{17} or C_mH_{2m+1} where m is 3 to 18 and R' is OCH_2COOH , OCH_2CH_2COOH , $OCH_2CH_2OCH_2COOH$, or $OCH_2CH_2OCH_2CH_2COOH$.

10 42. A method for manufacturing a device for separating target cells from a carrier fluid, the method comprising:

forming a photo-responsive polymer layer, the photo-responsive polymer having a diazo moiety;

placing one or more imprinting cells onto the photo-responsive polymer layer;

15 irradiating the photo-responsive polymer layer with a first optical energy source;

and

removing the one or more imprinting cells to expose one or more indentations formed on photo-responsive polymer layer.

43. The method as recited in claim 42, wherein the one or more imprint cells
5 are one or more of stem cells, osteocyte cells, epidermal cells, organ cells, tissue engineering cells, pluripotencial cells, pluripotencial stem cells, multipotencial stem cells, hematopoietic stem cells, mesenchymal stem cells, epithelial stem cells, bone marrow stem cells, germ stem cells, somatic stem cells, modified stem cells, or a combination thereof.

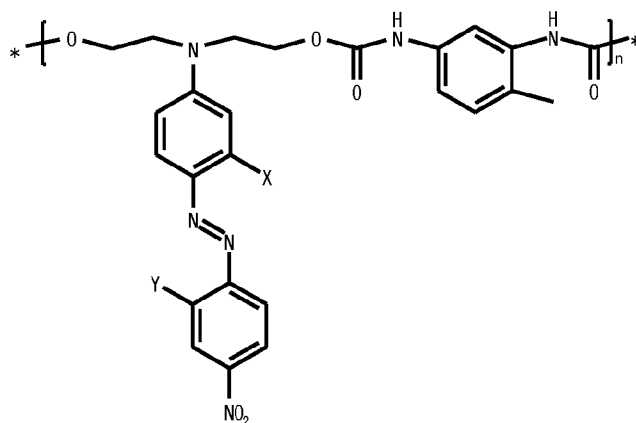
10 44. The method as recited in one of claims 42-43, wherein the imprinting is configured for a particular cell aggregation size.

45. The method as recited in one of claims 42-44, wherein the photo-responsive polymer has a first region configured to bind to the flow surface of the indentation and having a photo-responsive region configured to change conformation
15 between a first more hydrophobic conformation and a second more hydrophilic conformation responsive to irradiation from at least an optical energy source,

wherein the first conformation increases the accessibility of the at least one indentation to receive and interact with the first target cell, and

wherein the second conformation decreases the accessibility of the at least
20 one indentation to receive and interact with the first target cell.

46. The method as recited in claim 45, wherein the photo-responsive polymer includes an azobenzene have a formula of:



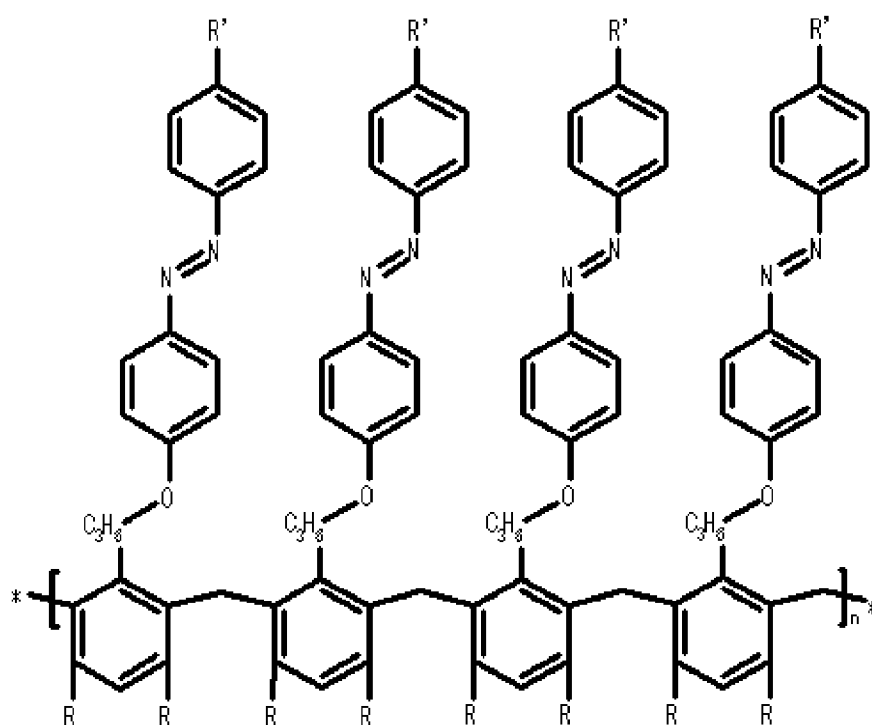
where X = H, CH₃, or C_mH_{2m+1} where m is integer between 1 and 12;

where Y = H, CH₃, or C_mH_{2m+1} where m is integer between 1 and 12; and

5 n = 5 to 200.

47. The method as recited in claim 45, wherein the first conformation of the molecules is a more hydrophobic state and the second conformation of the molecules is a more hydrophilic state.

48. The method as recited in claim 45, wherein the one or more photo-
10 responsive molecules include an azobenzene having a formula of:



wherein R is C₈H₁₇ or C_mH_{2m+1} where m is 3 to 18 and R' is OCH₂COOH, OCH₂CH₂COOH, OCH₂CH₂OCH₂COOH, or OCH₂CH₂OCH₂CH₂COOH.

49. The method as recited in one of claims 42-48, further comprising coupling
 5 an optical device configured to irradiate the photo-responsive molecules to the device.

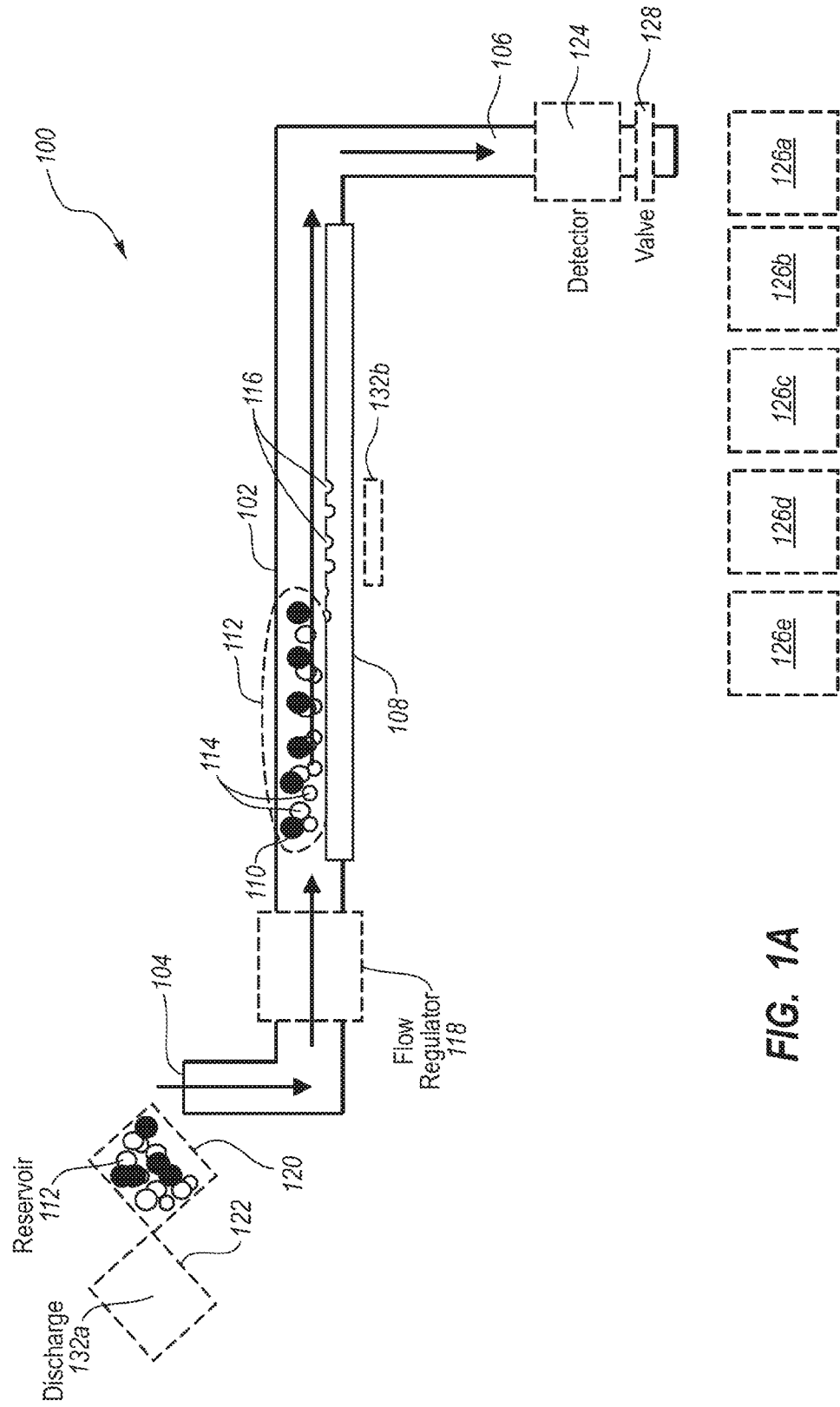


FIG. 1A

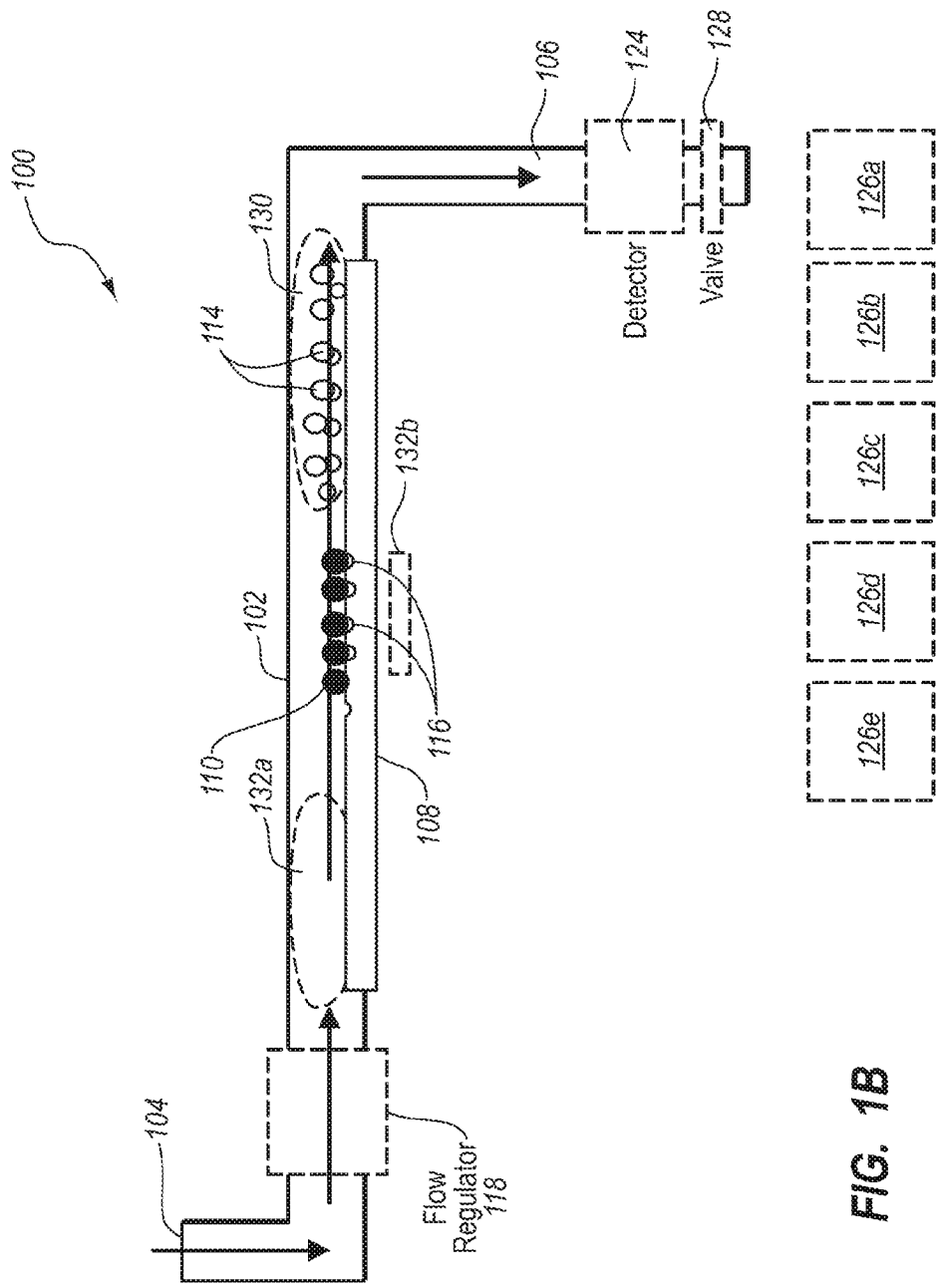


FIG. 1B

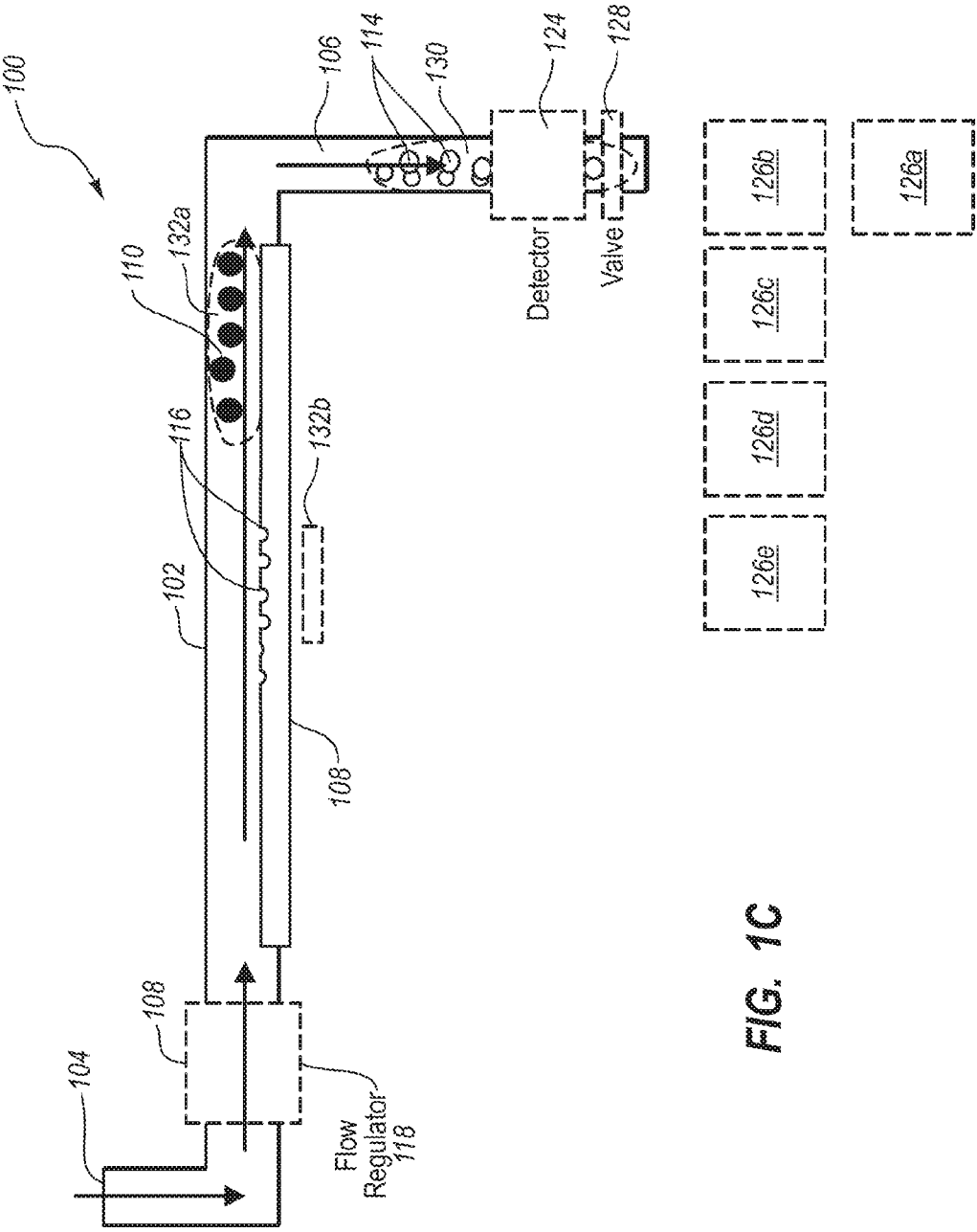


FIG. 1C

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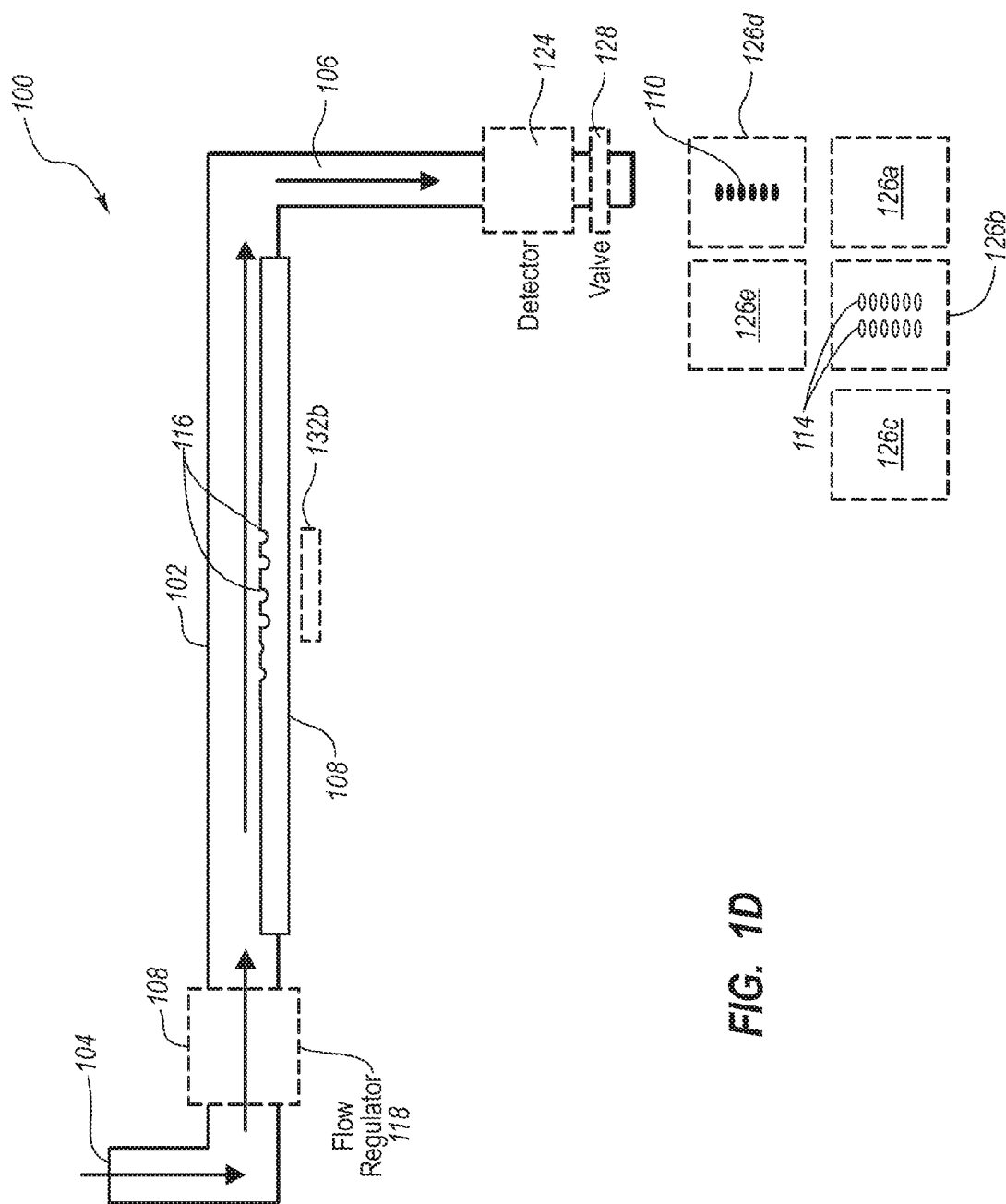


FIG. 1D

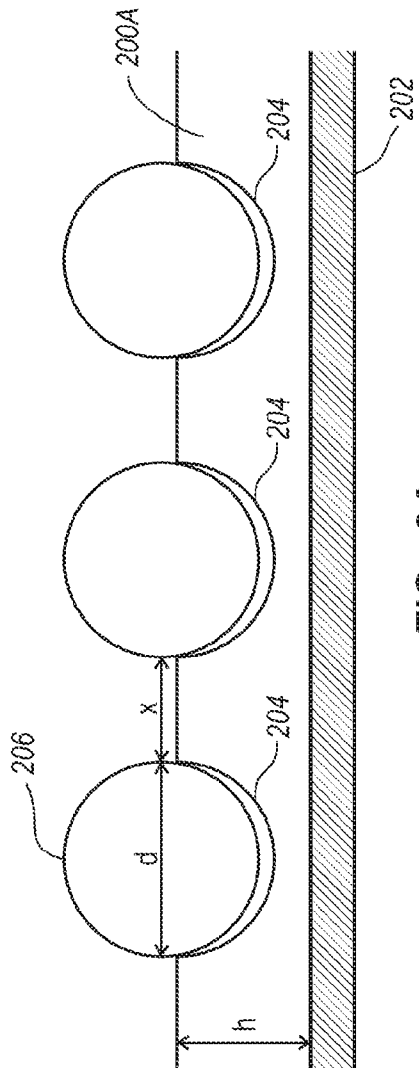


FIG. 2A

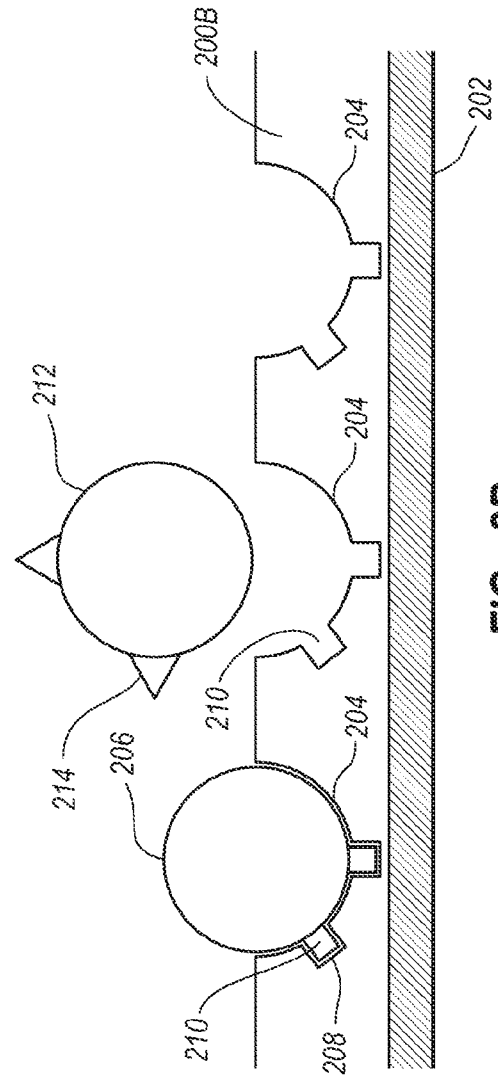


FIG. 2B

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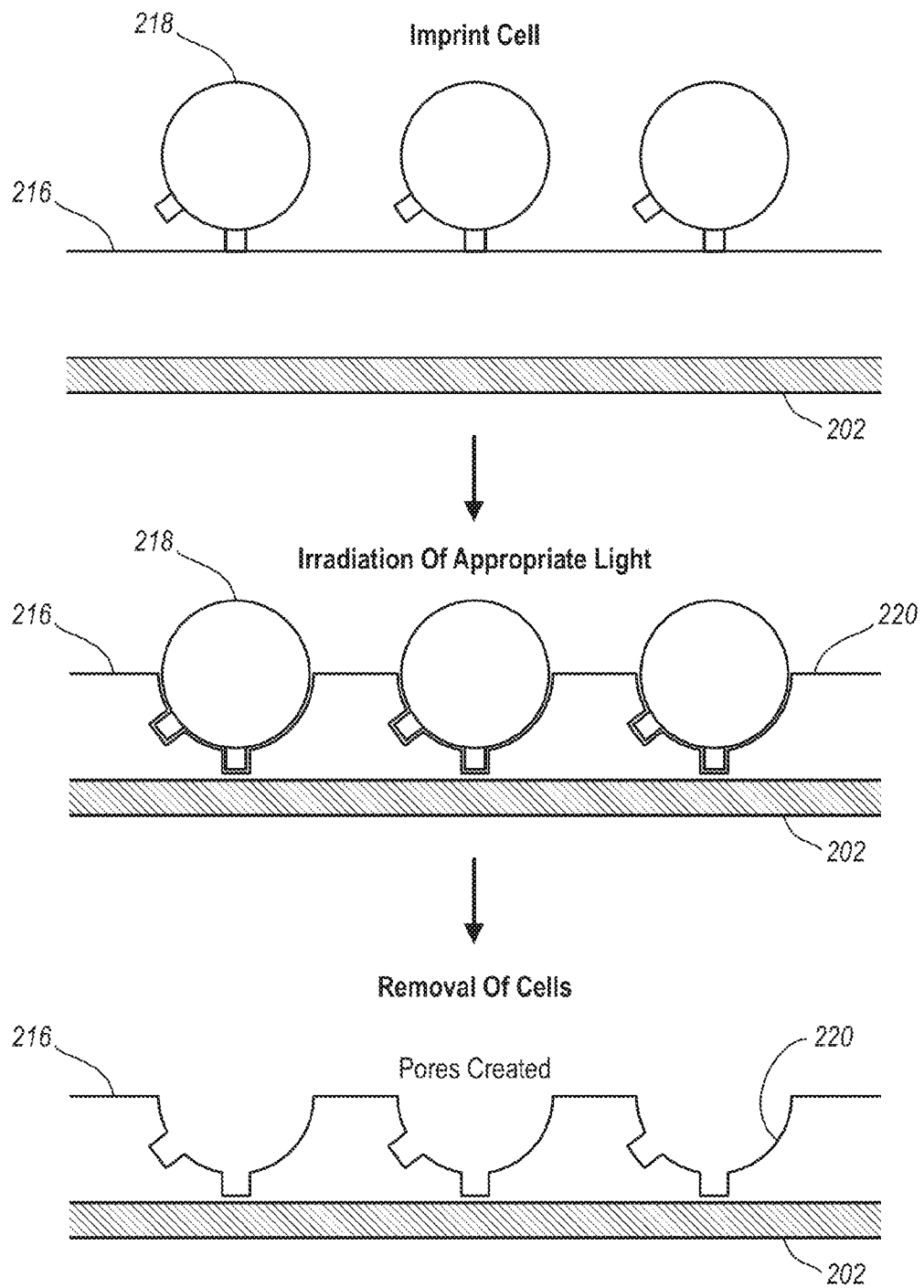


FIG. 2C

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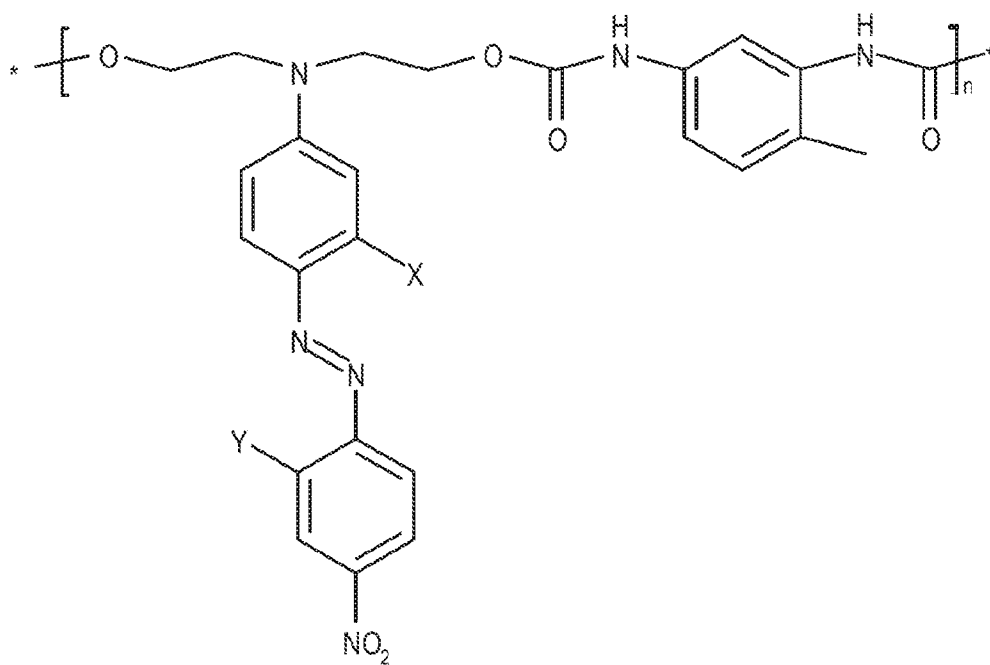


FIG. 2D

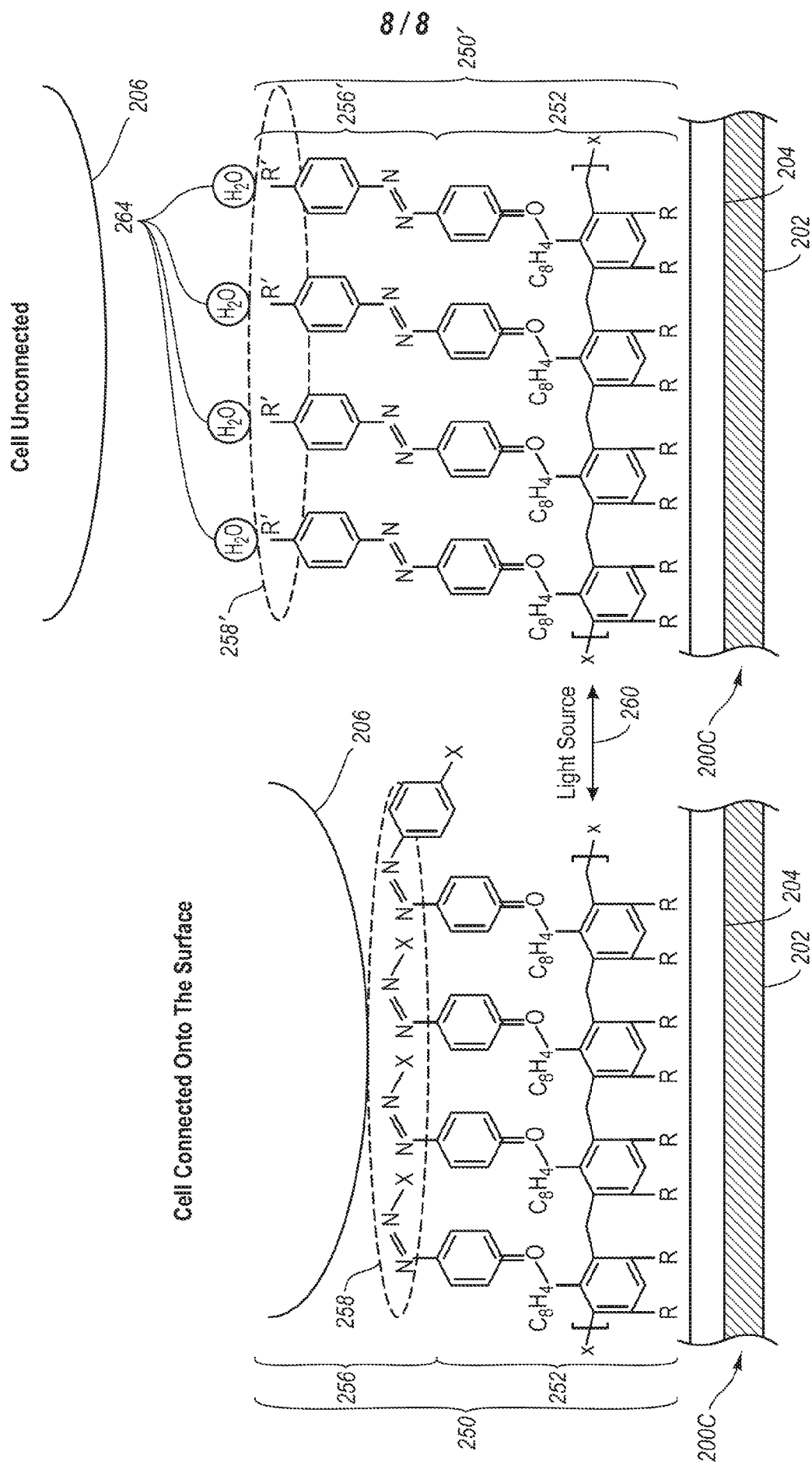


FIG. 2E

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2010/057749

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C12M 3/00 (2006.01)

G01N 33/48 (2006.01)

G03F 7/00 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

WPI, EPODOC, MEDLINE, HCA, BIOSIS (cell, sort, separate, azobenzene, diphenyldiazene, photoresponsive, photoisomer, photoswitch, photoinducible, photomodulate, optic switch, channel, indentation, microchannel, photolithography, capture, bind, anchor, adhesive, selective release, energy source, light, photo, wavelength, optic, irradiate, cis, trans, isomer, change, state, conformation, first, second, hydrophobic, hydrophilic, photoresist, poly dimethylsiloxane, polyethylene glycol dimethylacrylate, flow, fluid, liquid, microfluidic)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/133208 A2 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY et al.) 14 December 2006. See Abstract, Claims, pg. 4 lines 24 – 29, pg. 6 lines 4 – 26, pg's 10, 12 – 16, 18 – 20, Fig's 7A, 7B.	1 – 6, 8 – 12, 19 – 21, 23 – 25, 31 – 33, 35, 36
X	LIU, Y-J. et al. "A micropillar-integrated smart microfluidic device for specific capture and sorting of cells", Electrophoresis, 2007, Vol. 28, No. 24, pages 4713 – 4722. See Abstract, Materials and Methods, section 2.3.1, Figures 1 – 3.	1, 3 – 6, 8 – 11, 19, 20, 24, 25



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
24 March 2011

Date of mailing of the international search report
29 MAR 2011

Name and mailing address of the ISA/AU

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2010/057749

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PHILLIPS, J.A. et al. "Enrichment of cancer cells using aptamers immobilized on a microfluidic channel", Analytical Chemistry, 2009 (February), Vol. 81, No. 3, pages 1033 – 1039. See Abstract, Headings: 'Device Fabrication and Operation' and 'PDMS Microfluidic Device'.	1, 4 – 6, 8 – 12
A	KATAOKA, K. "Molecular Design of Materials for Cell Separation", Artificial Organs, 1988, Vol. 12, No. 6, pages 511 – 513. See whole document.	
A	GOROSTIZA, P & ISACOFF, E.Y. "Optical Switches for Remote and Noninvasive Control of Cell Signalling", Science, 2008, Vol. 322, No. 5900, pages 395 – 399. See whole document.	
A	NATANSOHN, A. & ROCHON, P. "Photoinduced Motions in Azo-Containing Polymers", Chemical Reviews, 2002, Vol. 102, No. 11, pages 4139 – 4175. See whole document.	
A	US 2003/0219889 A1 (SUMARU et al.) 27 November 2003. See whole document.	
A	WO 2005/060668 A2 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 7 July 2005. See whole document.	
A	EDAHIRO, J. et al. "In Situ Control of Cell Adhesion Using Photoresponsive Culture Surface", Biomacromolecules, 2005, Vol. 6, No. 2, pages 970 – 974. See whole document.	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2010/057749

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO 2006133208		AU 15925/01	CA 2390375
		US 6692952	US 2004166555
		WO 0135071	EP 1236032
US 2003219889		EP 1365017	US 2006128006
US 2003339373		JP 2003339373	
WO 2005060668		US 2007134420	
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			
END OF ANNEX			