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(54) Title: CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

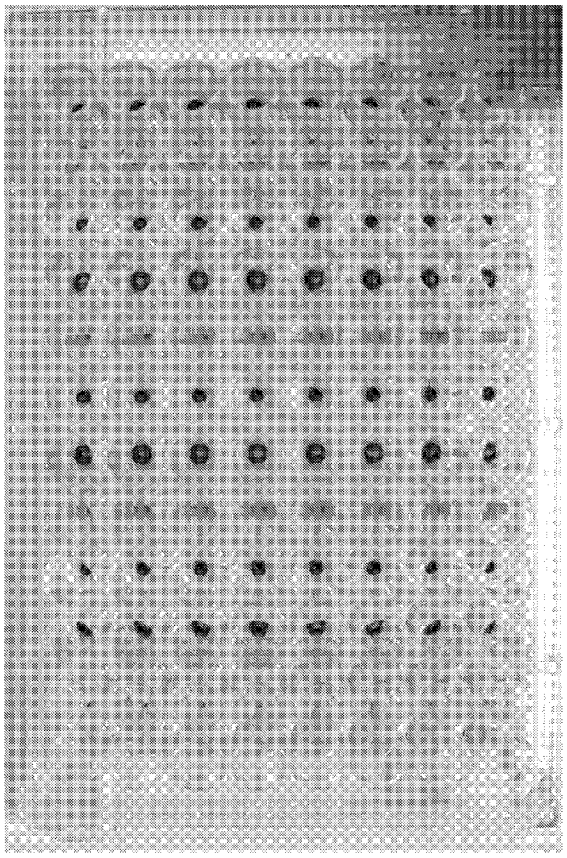

FIG. 1

(57) Abstract: A number of novel improved microfluidic configurations and systems and methods of manufacture and operation. In one embodiment, three wells are used for independent cell culture systems in a cell culture array. In a second aspect, artificial sinusoids with artificial epithelial barriers are provided with just one (optionally shared or multiplexed) fluidic inlet and one (optionally shared or multiplexed) fluidic output, where the medium output also functions as a cellular input. A pneumatic cell loader combined with other components provides a fully automated cell culture system. Magnetic alignment of plate molds provides advantages and ease of molded manufacture.

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CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from provisional patent application 61/037,297 filed 03-17-2008 and from 61/018,882 filed 01-03-2008, each incorporated herein by reference.

[0002] This application discusses technology related to U.S. 11/994,997, filed 08-11-2008, which is a National Stage Entry of PCT/US06/26364, filed 07-06-2006 and which claims priority from provisional patent application 60/773,467 filed 14 February 2006 and from provisional patent application 60/697,449 filed 7 July 2005.

[0003] This application discusses technology related to U.S. Application 12/019,857, filed 01/25/2008, which claims priority to U.S. Provisional Patent Application No. 60/900,651 filed on Feb. 8, 2007.

[0004] This application discusses technology related to U.S. Application Number: 11/648207, filed 12/29/2006, which claims priority to U.S. Provisional Patent Application U.S. provisional patent application No. 60/756,399 filed on Jan. 4, 2006. All of these applications are incorporated herein by reference for all purposes.

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FIELD OF THE INVENTION

[0006] The invention in various embodiments relates to handling of micro-objects, such as cells or micro-fabricated particles are beads, using a microfluidic system and particularly is directed to a configuration that can be used with various standard automated handling systems. In particular embodiments, the invention involves an automated system for cell culture.

BACKGROUND OF THE INVENTION

[0007] The discussion of any work, publications, sales, or activity anywhere in this submission, including in any documents submitted with this application, shall not be taken as an

admission that any such work constitutes prior art. The discussion of any activity, work, or publication herein is not an admission that such activity, work, or publication existed or was known in any particular jurisdiction.

[0008] Microfluidic cell culture is a promising technology for applications in the drug screening industry. Key benefits include improved biological function, higher-quality cell-based data, reduced reagent consumption, and lower cost. High quality molecular and cellular sample preparations are important for various clinical, research, and other applications. *In vitro* samples that closely represent their *in vivo* characteristics can potentially benefit a wide range of molecular and cellular applications. Handling, characterization, culturing, and visualization of cells or other biologically or chemically active materials (such as beads coated with various biological molecules) has become increasingly valued in the fields of drug discovery, disease diagnoses and analysis, and a variety of other therapeutic and experimental work.

[0009] Mammalian cell culture is particularly challenging, particularly for maintaining effective solid aggregates of cells in culture. Advances have been made by adapting various microfabrication and microfluidic technologies to cell culture, though there remains an ongoing need for a device that can be economically manufactured and used to provide effective cell culture.

[0010] Publications and/or patent documents that discuss various strategies related to cell culture using microfluidic systems and related activities include the following U.S. patent applications and non-patent literature, which, along with all citations therein, are incorporated herein by reference for all purposes. A listing of these references here does not indicate the references constitute prior art.

[0011] Cytoplex, Inc. 6,653,124 "Array-based microenvironment for cell culturing, cell monitoring and drug-target validation."

[0012] Cellomics, Inc. 6,548,263 "Miniaturized cell array methods and apparatus for cell-based screening."

[0013] Fluidigm, Inc. Published Application 20040229349 (Nov 18, 2004) "Microfluidic particle-analysis systems."

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[0014] Earlier work and patent applications as cited above involving at least one of the present inventors discuss various configurations, methods, and systems related to microfluidic cell culture and that work is incorporated herein by reference.

SUMMARY

[0015] The present invention involves various components, systems, and methods related to improved microfluidic cell culture systems. In one aspect, the invention involves novel microfluidic cell culture systems and methods that have advantages over previously proposed microfluidic structures. In another aspect, the invention involves novel structures and methods for integrating multiple microfluidic cell culture systems to a microtiter well plate structure, such as a standard culture-well plate formats (e.g., a 96-well SBS culture plate). In a further aspect, the invention involves novel fabrication methods for creating an array of microfluidic cell culture areas suitable for integration with a well plate. In another aspect, the invention involves novel systems, methods, and components for an improved automated high-throughput cell culture and/or screening system using microfluidic cell cultures.

[0016] In particular embodiments, key design features include the elimination of tubing and connectors to the plates themselves, the ability to maintain long-term continuous perfusion cell culture using a passive gravity-driven flow, and direct analysis on the outlet wells and/or cellular observation wells of the microfluidic plate.

[0017] For purposes of clarity, this discussion refers to devices, methods, and concepts in terms of specific examples. However, the invention and aspects thereof may have applications to a variety of types of devices and systems. It is therefore intended that the invention not be limited except as provided in the attached claims and equivalents.

[0018] Furthermore, it is well known in the art that systems and methods such as described herein can include a variety of different components and different functions in a modular fashion. Different embodiments of the invention can include different mixtures of elements and functions and may group various functions as parts of various elements. For purposes of clarity, the invention is described in terms of systems that include many different innovative components and innovative combinations of innovative components and known components. No inference should be taken to limit the invention to combinations containing all of the innovative components listed in any illustrative embodiment in this specification.

[0019] In some of the drawings and detailed descriptions below, the present invention is described in terms of the important independent embodiment of a complete, fully automated, cellular culture system and components thereof. This should not be taken to limit various novel aspects of the invention, which, using the teachings provided herein, can be applied to a number

of other situations. In some of the drawings and descriptions below, the present invention is described in terms of a number of specific example embodiments including specific parameters related to dimensions of structures, pressures or volumes of liquids, temperatures, electrical values, and the like. Except where so provided in the attached claims, these parameters are provided as examples and do not limit the invention to other devices or systems with different dimensions. For purposes of providing an more illuminating description, particular known fabrication steps, cell handling steps, reagents, chemical or mechanical process, and other known components that may be included to make a system or manufacture a device according to specific embodiments of the invention are given as examples. It will be understood to those of skill in the art that except were specifically noted herein otherwise, various known substitutions can be made in the processes described herein.

[0020] All references, publications, patents, and patent applications cited in this submission are hereby incorporated by reference in their entirety for all purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a top view of an example array of cell culture units according to specific embodiments of the invention. In this example, 32 culture units are provided on a 96-well plate (such as the Society for Biomolecular Screening (SBS) standard microfluidic bioreactor array schematic), with wells arranged in 12 columns (shown vertically) by 8 rows. In this example, each cell culture unit occupies three wells, one for use as a medium inlet, one for use as a cell inlet/medium outlet, and one for use for cell imaging (which appears as a dark rectangle in the wells in the figure) and/or for providing air passages to a cell culture area. In specific embodiments, each unit can be used as an independent biomimetic cell.

FIG. 2 is an underside view showing one culture unit occupying three wells according to specific embodiments of the invention.

FIG. 3 is a close-up underside view illustrating details of the microfluidic cell culture areas described above according to specific embodiments of the invention. In this figure, the cell inlet/media outlet is to the left, and the media inlet is to the right.

FIG. 4 is a close up micrograph of a cell culture area illustrating two large air holes at the left of the figure each connected to an air passage that is placed between the blocks, each block having four cell culture sinusoids according to specific embodiments of the invention. In this figure, the cell inlet/media outlet is to the right, and the media inlet is to the left. Also visible in the photo, are a media multiplexor structures to the left in each block, and an optional cell inlet multiplexor to the right in each block.

FIG. 5 is a close up micrograph of a cell culture area illustrating two large air holes at the left of the figure each connected to an air passage that is placed between the blocks, each block having eight cell culture sinusoids according to specific embodiments of the invention.

FIG. 6 illustrates high aspect ratio channels surrounding cell culture areas wherein channels between solid structures are approximately 4μm wide and 40μm tall to prevent cells from growing out. The channels in this example are separated by approximately 40μm.

FIG. 7A illustrates a cell inlet/media outlet of a modified cell culture area with a large rectangular cell inlet to provide for easier cell loading and with a cell loading perfusion area and a solid wall cell culture area. The arrows from the right indicate cell-loading direction.

FIG. 7B illustrates the media inlet/cell culture area of a modified microfluidic cell culture system according to specific embodiments of the invention. In this example, cell loading is from the right and media flow, as indicated by the arrows, is from the left.

FIG. 8 is a schematic showing three blocks of four long cell culture sinusoids, where the long cell sinusoids extend across two wells, and further shows a rectangular cell inlet region / flow outlet region, and four air holes connecting to four air channels.

FIG. 9A-B are simplified schematic diagrams illustrating in three dimensions the components of a multi cell (e.g., 3) microfluidic system including a representation of the well frame according to specific embodiments of the invention.

FIG. 10 is a simplified side view showing a structure according to specific embodiments of the invention illustrating two wells that are used in cell flow and fluid flow.

FIG. 11 is a close-up micrograph showing cells loaded in five sinusoid cell culture regions with four sinusoid channels between according to specific embodiments of the invention.

FIG. 12 shows four close-up micrographs showing cells loaded in four different sized sinusoid cell culture regions according to specific embodiments of the invention.

FIG. 13 is a schematic diagram showing steps from an empty culture region to performing a cell assay according to specific embodiments of the invention.

FIG. 14A-C shows a top view, side view, and plan view of a schematic of an example manifold according to specific embodiments of the invention. In this example, the eight tubing lines to the right are for compressed air, and each is configured to provide pressure to a column of cell inlet wells in a microfluidic array. The left-most line in the figure is for vacuum and connects to an outer vacuum ring around the manifold. Each column of wells is generally connected to a single pressure line with wells above imaging regions skipped.

FIG. 15 is a graph illustrating an example of flow rate difference between a surface tension mechanism and a gravity driven mechanism according to specific embodiments of the invention.

FIG. 16 is a graph illustrating an example of the extent to which gravity perfusion rate is responsive to the liquid level difference between the two upper reservoir wells according to specific embodiments of the invention.

FIG. 17A-B illustrate a tilting platform that can be used to control the liquid height difference between the inlet/outlet wells in a device or system according to specific embodiments of the invention and an example of flow rate versus plate tilt angle.

FIG. 18 illustrates a top view schematic of an example cell culture automation system according to specific embodiments of the invention.

FIG. 19 is a photograph of an example automated microfluidic perfusion array system according to specific embodiments of the invention.

FIG. 20 is a flow chart illustrating the process flow of typical operation steps.

FIG. 21 illustrates four microfluidic culture areas from an example array plate prepared in the example system described above using primary rat hepatocytes.

FIG. 22 illustrates a portion of a microfluidic culture area from an example array plate prepared in the example system described above using primary human hepatocytes.

FIG. 23 illustrates a layout of another type of cell culture array designed for general cell culture automation according to specific embodiments of the invention.

FIG. 24 illustrates an operation schematic for performing automated cell culture and immunostaining on a microfluidic array with gravity cell loading as described above. Example applications include stem cell culture and primary cell culture with immunofluorescence staining and microscopy.

FIG. 25 illustrates an alternative example SBS (Society for Biomolecular Screening) standard microfluidic bioreactor array schematic.

FIG. 26A-B illustrate an alternative cellular culture system assembly according to specific embodiments of the present invention showing (A) an example schematic microfluidics design for three cell units; (B) a soft lithography fabrication of this design with laser machining of four openings per culture unit.

FIG. 27 illustrates operation steps of a less automated or prototype system according to specific embodiments of the invention.

FIG. 28 illustrates a microfluidic mold fixed on glass plate with magnets according to specific embodiments of the invention. In one example embodiments, each magnet used is: 3/4"

Diameter x 1/16" Thick fabricated, e.g., from sintered Neodymium-Iron-Boron (NdFeB) with a Plating/Coating of Ni-Cu-Ni (Nickel) and a Grade: N40 with a Pull Force of: 4.0 lbs (1814 g).

FIG. 29 illustrates a stack of molds held together with magnetic clamps (e.g., Stack of self-aligned microfluidic molds) for forming polymer micro-molded structures according to specific embodiments of the invention.

FIG. 30 is a block diagram showing an example direct soft molding process according to specific embodiments of the invention.

FIG. 31A illustrates the two pieces in position before mounting to glass and coating. Because the top piece is injection molded, the bottom of the wells can be flat, rounded or tapered. One particular desired feature is that the bottom of the top piece, which covers the microfluidic structures, should be flat to ensure uniform molding across the array. This top piece can be either a proprietary top piece with wells as shown or, alternatively, can be a standard SBS multi-well plate.

FIG. 31B illustrates an example wherein the bottom of the top piece is chemically modified by a reagent (Sylgard Primecoat) so the soft polymers adhere to the bottom of the top piece after the molding process and illustrates an appropriate amount of soft polymer poured onto the center of the mold (usually a few milliliters, depending on the area to be covered as well as the thickness of the soft polymer after molding).

FIG. 31C illustrates an example wherein the top piece and the mold are sandwiched between two pieces of flat surfaces (usually glass plates) with clamping mechanisms (in this case, magnets) and the clamping mechanism holds the top piece and the mold together with alignment marks fitted to each other.

FIG. 31D illustrates an example wherein after detaching the molded microfluidic cell culture array with the top piece, a laser cutter is used to create fluidic connections between the microfluidic structures and the wells at specific locations (cell/reagent inlets/outlets).

FIG. 31E illustrates an example wherein the microfluidic cell culture array is bonded to a piece of rectangular glass. The glass and/or array may be subjected to oxygen plasma treatment before the bonding.

FIG. 31F illustrates an example wherein using a liquid dispenser, the microfluidic cell culture array is filled with priming solutions to maintain its modified surface chemistry. If bubbles appear to be trapped inside the array, additional vacuum steps are used to eliminate the bubbles.

FIG. 31G illustrates an example wherein to prevent liquid evaporation, the array is sealed with a tape.

FIG. 31H illustrates an example wherein the array is optionally fit into a frame so the finished array can be treated like a standard microtiter plate with the correct outside dimensions. In the case where the top piece is a standard microtiter well plate, this step may be unnecessary.

FIG. 32A-D illustrate four components of a direct soft molding process according to specific embodiments of the invention.

FIG. 33A illustrates a step wherein an appropriate amount of soft polymer is poured onto the center of the mold (usually a few milliliters, depending on the area to be covered as well as the spacer thickness) For example, for a mold 6" in diameter and a 150 micron spacer, the minimum amount required is π x 7.62cm x 7.62cm x 0.015cm ~ 2.75mL).

FIG. 33B illustrates a step wherein the acrylic sheet is sandwiched between two pieces of the glass plates so the magnets will press the acrylic sheet (with primer modified surface facing the mold) against the mold until the acrylic sheet hits the spacer. The soft polymer will then fill the space between the acrylic sheet and the mold to replicate the microfluidic structures. In particular embodiments, the magnet-assisted clamping mechanism holds the pieces together while the soft polymer is cured at elevated temperature (60 degreeC) for at least 2 hours.

FIG. 33C illustrates that after cooling the compartments down to approximately room temperature, the acrylic sheet with the soft polymer is detached from the mold. The microfluidic cell culture array is truthfully molded onto the soft polymer. To protect the soft polymer surface from contaminations from following processes, a surface protection tape (Ultron Blue Adhesive Plastic Film 80micron) may be applied to the top of the surface of the elastomer by a roller.

FIG. 33D illustrates that after separation from the mold a CO2 laser cutter (VersaLaser, 25W model) is used to create fluidic connections between the microfluidic structures and the injection molded wells (cell inlet and medium inlet). Since the soft polymer used in the process is gas permeable, "air holes" may be cut near the cell culture areas to promote air diffusion for better cell culture. The circular top piece is may be trimmed to rectangular shape at this stage.

FIG. 33E illustrates that after the surface protection tape is removed and the array is optionally ultrasonically cleaned (or water-jet cleaned) to shake off any dust created by the laser cutting step and optionally a new surface protection tape is applied, the microfluidic cell culture array is glued to the injection molded plate with an ultra-violet (UV) curable glue which is also bio-compatible (Loctite 3301). The plate with the microfluidic cell culture array is cured in a UV chamber for 30 minutes. After removal of the surface protection tape, both a glass substrate (e.g., White Float Glass) and the microfluidic cell culture array undergo oxygen plasma

treatment to activate the surface and the glass substrate encloses the microfluidic cell culture array through covalent bonding, as shown in FIG. 33F.

FIG. 33G illustrates that using a liquid dispenser, the microfluidic cell culture array is filled with priming solutions, as bubbles may be inside the array; and the array may be placed inside a vacuum chamber for bubbles removal and may also be placed inside a UV/Ozone chamber (Novascan) for sterilization.

FIG. 33H illustrates that to prevent liquid evaporation, the array is sealed with a tape (Excel Scientific, AlumaSeal).

FIG. 34 is a block diagram showing a representative example logic device in which various aspects of the present invention may be embodied.

FIG. 35 (Table 1) illustrates an example of diseases, conditions, or states that can evaluated or for which drugs or other therapies can be tested according to specific embodiments of the present invention.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

1. OVERVIEW

Definitions

[0021] A "particle" refers to biological cells, such as mammalian or bacterial cells, viral particles, or liposomal or other particles that may be subject to assay in accordance with the invention. Such particles have minimum dimensions between about 50-100 nm, and may be as large as 20 microns or more. When used to describe a cell assay in accordance with the invention, the terms "particles" and "cells" may be used interchangeably.

[0022] A "microwell" refers to a micro-scale chamber able to accommodate a plurality of particles. A microwell is typically cylindrical in shape and has diameter and depth dimensions in a preferred embodiment of between 100 and 1500 microns, and 10 and 500 microns, respectively. When used to refer to a microwell within the microwell array device of the invention, the term "well" and "microwell" are used interchangeably.

[0023] A "microchannel" refers to a micron-scale channel used for connecting a station in the device of the invention with a microwell, or a station and a valve associated with the microwell. A microchannel typically has a rectangular, e.g., square cross-section, with side and depth dimensions in a preferred embodiment of between 10 and 500 microns, and 10 and 500 microns, respectively. Fluids flowing in the microchannels may exhibit microfluidic behavior. When used to refer to a microchannel within the microwell array device of the invention, the term "microchannel" and "channel" are used interchangeably.

[0024] A "microfluidics device" refers to a device having various station or wells connected by micron-scale microchannels in which fluids will exhibit microfluidic behavior in their flow through the channels.

[0025] A "microwell array" refers to an array of two or more microwells formed on a substrate.

[0026] A "device" is a term widely used in the art and encompasses a broad range of meaning. For example, at its most basic and least elaborated level, "device" may signify simply a substrate with features such as channels, chambers and ports. At increasing levels of elaboration, the "device" may further comprise a substrate enclosing said features, or other layers having microfluidic features that operate in concert or independently. At its most elaborated level, the "device" may comprise a fully functional substrate mated with an object that facilitates interaction between the external world and the microfluidic features of the substrate. Such an object may variously be termed a holder, enclosure, housing, or similar term, as discussed below. As used herein, the term "device" refers to any of these embodiments or levels of elaboration that the context may indicate.

[0027] Microfluidic systems provide a powerful tool to conduct biological experiments. Recently, elastomer-based microfluidics has especially gained popularity because of its optical transparency, gas permeability and simple fabrication methods. However, the interface with the end-users requires labor-intensive hole punching through the elastomer, and additional steps of tubing and syringe pump connection.

[0028] The present invention involves integrated elastomer-based microfluidics on standard well plates, with special focus on hepatocyte culture applications. The invention further involves methods of manufacture of such plates and components and a system for automating cell culture using such plates. Advantages of specific embodiments include use of a standard microtiter plate format, tubing free cell culture, and a biomimetic liver microenvironment.

[0029] A system according to specific embodiments of the invention (for example, using 96-well standard plates) can be operated using standard techniques and equipment for handling standard microtiter plates, as are well known in the art. For example, liquid dispensing is achieved with standard pipette mechanics, and cell culture and analysis can be made compatible with existing incubators and plate readers.

[0030] According to further embodiments of the invention, a novel cell loading system uses a pneumatic manifold and pneumatic pressure to place cells in the micro culture area. With the addition of this cell loading system, microfluidic cell culture and analysis can be fully automated using other automated equipment that exists for handling standard titer plates.

[0031] In further embodiments, the gravity driven flow culture configuration utilizes the medium level difference between the inlet and outlet well as well as engineering the fluidic resistances to achieve the desirable flow rate in nL/min regime. This provides the significant advantage of being able to "passively" flow culture medium for long periods of time (up to 4 days) without the use of bulky external pumps or tubes.

[0032] In further embodiments, the invention involves a microfluidic system to allow control of the cell culture environment for long-term time-lapse microscopy of adherent cells. As the trend towards "systems biology" continues, it will become increasingly important to study dynamic behavior in individual live cells as well as to improve the functionality and economics of high throughput live cell screening. According to specific embodiments of the invention, the invention provides a multiplexed microfluidic flow chamber allowing for time-lapse microscopy experimentation among other assays. The microfluidic chamber uses an artificial endothelial barrier to separate cells from flow channels. The device is formatted to a standard well plate, allowing liquid and cell samples to be directly pipetted into the appropriate inlet reservoirs using standard equipment. A custom pneumatic flow controller is then used to load the cells into the culture regions as well as to switch between different exposure solutions. A digital software interface can be used to allow a user to program specific inputs (pulses, ramps, etc.) over time to expose the cells to complex functions during time-lapse imaging.

[0033] Dynamic responses in living cells are the foundation for phenomena such as biological signal processing, gene expression regulation, differentiation, and cell division. In specific embodiments, the invention involves a system capable of controlling the cellular micro-environment in a multiplexed format compatible with current cell culture methods. Cell response can be quantified using high magnification fluorescence microscopy to derive kinetic information with sub-cellular resolution. This capability has broad applications in cellular systems biology where dynamic single cell response experiments are not currently practical.

2. Microfluidic culture system and array

[0034] The application referenced above (U.S. 11/994,997) discussed a variety of different cell culture configurations and fabrication techniques. Portions of the operation of the cell culture areas and materials are useful as background to the present discussion. In some examples therein, one or more micro culture areas are connected to a medium or reagent channel via a grid of fluidic passages (or diffusion inlets or conduits), wherein the grid comprises a plurality of intersection micro high fluidic resistance passages. In one discussed example, passages in the grid are about 1 to 4 μm in height, 25 to 50 μm in length and 5 to 10 μm in width, the grid allowing for more even diffusion between medium or reagent channels and the culture area and

allowing for easier manufacturing and more even diffusion. The earlier application further discussed that the high fluidic resistance ratio between the microchamber and the perfusion/diffusion passages or grid (e.g., ratios in the range of about 10:1, 20:1 to 30:1) offers many advantages for cell culture such as: (1) size exclusion of cells; (2) localization of cells inside a microchamber; (3) promoting a uniform fluidic environment for cell growth; (4) ability to configure arrays of microchambers or culture areas; (4) ease of fabrication, and (5) manipulation of reagents without an extensive valve network. Examples were illustrated wherein a grid-like perfusion barrier can be much shorter than the culture area or can be near to or at the same height, according to specific embodiments of the invention and further wherein various configurations for culture devices were illustrated. The application also discussed a CAD drawing of a proposed 96-unit microfluidic bioreactor wherein each well was an SBS standard size (3.5 mm in diameter) in order to be compatible with existing robotic liquid handling systems and plate readers. The application also discussed several different configurations for an artificial sinusoid using both cut passages and grids and with a flow-around perfusion design.

[0035] FIG. 1 is a top view of an example array of cell culture units according to specific embodiments of the invention. In this example, 32 culture units are provided on a 96-well plate (such as the Society for Biomolecular Screening (SBS) standard microfluidic bioreactor array schematic), with wells arranged in 12 columns (shown vertically) by 8 rows. In this example, each cell culture unit occupies three wells, one for use as a medium inlet, one for use as a cell inlet/medium outlet, and one for use for cell imaging (which appears as a dark rectangle in the wells in the figure) and/or for providing air passages to a cell culture area. In specific embodiments, each unit can be used as an independent biomimetic cell..

[0036] FIG. 2 is an underside view showing one culture unit occupying three wells according to specific embodiments of the invention. In this example, the cell culture portion visible in the middle well is divided into four blocks, with each block having four separated cell culture channels surrounded by medium channels used for medium fluidic passage. In particular embodiments, these four separated cell culture channels may be referred to as sinusoids or artificial sinusoids, regardless of whether the far end of the areas has a rounded shape. Separation into four blocks facilitates air diffusion through the material that defines the microfluidic channels (such as silicone elastomer polydime-thylsiloxane (PDMS)) structure into the culture areas. Six air holes to facilitate air passage are shown.

[0037] FIG. 3 is a close-up underside view illustrating details of the microfluidic cell culture areas described above according to specific embodiments of the invention.

[0038] FIG. 4 is a close up micrograph of a cell culture area illustrating two large air holes at the left of the figure each connected to an air passage that is placed between the blocks, each block having four cell culture sinusoids according to specific embodiments of the invention.

[0039] FIG. 5 is a close up micrograph of a cell culture area illustrating two large air holes at the left of the figure each connected to an air passage that is placed between the blocks, each block having eight cell culture sinusoids according to specific embodiments of the invention.

[0040] FIG. 6 illustrates high aspect ratio channels surrounding cell culture areas wherein channels between solid structures are approximately 4μm wide and 40μm tall to prevent cells from growing out. The channels in this example are separated by approximately 40μm.

[0041] FIG. 7A illustrates a cell inlet/media outlet of a modified cell culture area with a large rectangular cell inlet to provide for easier cell loading and with a cell loading perfusion area and a solid wall cell culture area.

[0042] FIG. 7B illustrates the media inlet/cell culture area of a modified microfluidic cell culture system according to specific embodiments of the invention. In this example, cell loading is from the right and media flow, as indicated by the arrows, is from the left. A further difference in the modified design is that perfusion passages are absent in a portion of the cell culture channel (or artificial sinusoid). This has been found to more easily locate cells at the end of the cell culture channel in the cell culture area. Optionally, a portion of the cell culture channel near the fluid outlet has perfusion passages to ensure fluid flow after cells have aggregated at the culture end. The improved design provides for easier cell loading and a longer cell culture areas and cell culture channels to culture more cells and more uniform flow of nutrients. It has been found that in operation cells localize/stick to the areas of the culture channel that are immediately next to the perfusion passages. The segment of the cell culture channel between the main culture area region and the other set of perfusion passages near the cell inlet is devoid of cells, because the flow profile carries them out, particularly during cell loading. Thus, this modified design prevents the cells from spreading into the "flow" channels after a few days and stop the flow. In the modified design, the flow remains unhindered since the cells cannot spread past the long cell culture channel segment (where there are no perfusion passages). In an example system, up to about 2,500 liver cells may be cultured in each area as shown in FIG. 7 and FIG. 8.

[0043] FIG. 8 is a schematic showing three blocks of four long cell culture sinusoids, where the long cell sinusoids extend across two wells, and further shows a rectangular cell inlet region / flow outlet region, and four air holes connecting to four air channels.

[0044] FIG. 9A-B are simplified schematic diagrams illustrating in three dimensions the components of a multi cell (e.g., 3) microfluidic system including a representation of the well frame according to specific embodiments of the invention.

[0045] FIG. 10 is a simplified side view showing a structure according to specific embodiments of the invention illustrating two wells that are used in cell flow and fluid flow.

[0046] FIG. 11 is a close-up micrograph showing cells loaded in five sinusoid cell culture regions with four sinusoid channels between according to specific embodiments of the invention.

[0047] FIG. 12 shows four close-up micrographs showing cells loaded in four different sized sinusoid cell culture regions according to specific embodiments of the invention.

[0048] Thus, the present invention according to specific embodiments of the invention provides a number of novel improved microfluidic configurations. In a first aspect, three wells are used for each otherwise independent cell culture system. In a second aspect, artificial sinusoids with artificial epithelial barriers are provided with just one (optionally shared or multiplexed) fluidic inlet and one (optionally shared or multiplexed) fluidic output, where the medium output also functions as a cellular input. In a third aspect, artificial sinusoids with artificial epithelial barriers with just one fluidic inlet and one fluidic output are divided into blocks with air channels provided between blocks. In a fourth aspect, air holes are provided in the well chamber above the cell culture area of a microfluidic cellular culture array, where the medium output also functions as a cellular input. In a fifth aspect, a multiplexed medium inlet structure and multiplexed cellular input structure are provided to connect inputs and outputs to blocks of artificial sinusoids. In a sixth aspect, a multiplexed medium inlet structure and larger shared cellular input structure are provided to connect inputs and outputs to blocks of artificial sinusoids. In a seventh aspect, artificial sinusoids are configured with non-open portions of an epithelial barrier to better localize cells, and with perfusions inlets surrounding a cell culture area and optionally also present near a cell inlet area of the sinusoid. In an eighth aspect, longer artificial sinusoid chambers are provided.

[0049] As discussed elsewhere, various modifications may be made to the cell-culture area as described above. Various configurations are possible for the epithelial barrier, such as a grid-like passage structure. Other variations will be suggested to those of skill in the art having the teachings provided herein.

[0050] The structures disclosed above can also be adapted to systems using more or fewer wells on a standard microtiter well plate, such as those described in referenced documents and in other examples herein.

3. Example Device Operation

[0051] FIG. 13 is a schematic diagram showing steps from an empty culture region to performing a cell assay according to specific embodiments of the invention. Various novel aspects according to specific embodiments of the invention simplify these steps and allow them to be automated.

Cell Loading

[0052] Cell loading in specific embodiments of the invention can utilize the rapid surface tension flow between the cell inlet and the flow inlet. In this method, the cell inlet reservoir (upper and lower) is aspirated of its priming solution. Then, the flow inlet upper reservoir is aspirated. An amount (e.g., Five microliters) of cell suspension (e.g., trypsinized HeLa human cancer cell line, 5x10^5 cells/ml) is dispensed into the cell inlet lower reservoir. The flow inlet lower reservoir is aspirated, causing liquid to flow from cell inlet to flow inlet via surface tension/capillary force. Cell loading in various configurations can be completed in approximately 2-5 minutes. The cell loading reservoir is then washed with medium (e.g., Dulbecco's Modified Eagle's Medium, DMEM) and filled with e.g., 50-100 microliters of clean medium. At this state, the plate is was placed in a controlled culture environment for a period (e.g., 37C, 5% CO_2 incubator for 2-4 hours) to allow for cell attachment.

[0053] While such loading is effective for some microfluidic cell culture devices, in a presently preferred embodiment, a proprietary pneumatic manifold, as described elsewhere herein, is mated to the plate and pneumatic pressure is applied to the cell inlet area for more effective cell loading. For particular cell systems, it has been found that overall cell culture area design can be made more effective when it is not necessary to allow for passive cell loading.

[0054] FIG. 14A-C shows a top view, side view, and plan view of a schematic of an example manifold according to specific embodiments of the invention. In this example, the eight tubing lines to the right are for compressed air, and each is configured to provide pressure to a column of cell inlet wells in a microfluidic array. The left-most line in the figure is for vacuum and connects to an outer vacuum ring around the manifold. Each column of wells is generally connected to a single pressure line with wells above imaging regions skipped. The manifold is placed on top of a standard well plate. A rubber gasket lies between the plate and manifold, with holes matching the manifold (not shown). The vacuum line creates a vacuum in the cavities between the wells, holding the plate and manifold together. Pressure is applied to the wells to drive liquid into the microfluidic channels (not shown). A typical pressure of 1 psi is used, therefore the vacuum strength is sufficient to maintain an air-tight seal. In one example there are 9 tubing lines to the pressure controller: 8 lines are for compressed air and 1 line is for vacuum

(leftmost). In specific example embodiments, each column is connected to a single pressure line. Columns above the cell imaging regions are skipped.

[0055] Pressurized cell loading in a system according to specific embodiments of the invention has been found to be particularly effective in preparing cultures of aggregating cells (e.g., solid tumor, liver, muscle, etc.). Pressurized cell loading also allows structures with elongated culture regions, e.g., as shown in FIG. 7 and FIG. 8, to be effectively loaded. Use of a pressurized manifold for cell loading and passive flow for perfusion operations allows the invention to utilize a fairly simple two inlet design, without the need for additional inlet wells and/or valves as used in other designs.

Fluid Flow and Operation: Gravity and Surface Tension flow

[0056] The format of the microfluidic plate design allows two automation-friendly flow modalities dependent on the extent of dispensing/aspiration. The first is surface tension mediated flow. In this case, when the lower reservoir is aspirated in either one of the wells, the capillary force of the fluid/air interface along with the wetted surfaces (glass, silicone, acrylic) will rapidly draw liquid in from the opposing well until the lower reservoir is filled (or in equilibrium with the opposing lower reservoir). This effect is useful for microfluidic flows as it is only evident when the reservoir diameter is small and the flow volumes are small. In an example array design, the lower reservoir wells are 1-2 mm in diameter, and with a total flow volume of approximately 3-5 microliters. Since the microfluidic channel volume is only 0.2 microliters, this mechanism is well suited for cell loading and cell exposures.

[0057] The second mechanism is gravity driven perfusion, which is well suited for longer term flows, as this is dependent on the liquid level difference and not the reservoir dimensions. According to specific embodiments of the invention, this may be accomplished by adding more liquid into one reservoir (typically filling near the top of the upper reservoir). The fluidic resistance through the microfluidic channels will determine how long (e.g., 24 hours) to reach equilibrium between the wells and thus determine how often wells should be refilled.

[0058] FIG. 15 shows the flow rate difference between the surface tension mechanism and the gravity driven mechanism. For the surface tension flow, in an example, 5 microliters was dispensed into the lower reservoir followed by aspiration of the opposing lower reservoir. For the gravity flow, a liquid level difference of 2.5 mm was used, with both wells filled into the upper reservoir portion.

Changing gravity flow rate via liquid level

[0059] The gravity perfusion rate is also responsive to the liquid level difference between the two upper reservoir wells as illustrated in FIG. 16. This fact allows an automated

dispenser/aspirator to control and maintain a given perfusion flow rate over a 10-fold range during culture. Here, different liquid level differences were produced via dispensing volumes and measured for volumetric flow rate.

Controlling gravity perfusion rate via plate tilt angle

[0060] According to specific embodiments of the invention, the liquid height difference between the inlet/outlet wells across the plate can also be precisely controlled using a mechanical tilting platform. In this implementation, it is possible to maintain a constant flow rate over time, as well as back-and-forth flow with different forward and reverse times (i.e. blood flow). In the example illustrated in FIG. 17, both inlet and outlet reservoirs were filled with 50 microliters of solution. On a flat surface, there is no flow through the channels, and as the angle is increased, so is the flow rate. The photo shows a prototype controlled tilting platform, consisting of a mechanical platform, and an electronic switch.

[0061] In an example system, perfusion cell culture can be initiated by filling the flow inlet reservoir with 200-300 microliters of fresh medium (e.g., DMEM supplemented with 10% fetal bovine serum) and aspirating the cell inlet upper reservoir. The liquid level difference between the flow inlet and cell inlet wells will then cause a continuous gravity driven flow through the attached cells. For sustained culture, the flow inlet well is refilled and the cell inlet well aspirated during a period depending on fluidic resistance and reservoir volumes (e.g., every 24 hours).

Cell Assay and/or observation

[0062] Cell assay can be performed directly on the microfluidic cell culture using standard optically based reagent kits (e.g. fluorescence, absorbance, luminescence, etc.). For example a cell viability assay utilizing conversion of a substrate to a fluorescent molecule by live cells has been demonstrated (CellTiter Blue reagent by Promega Corporation). The reagent is dispensed into the flow inlet reservoir and exposed to the cells via gravity perfusion over a period of time (e.g., 21 hours). For faster introduction of a reagent or other fluid, the new fluid can be added to the flow inlet reservoir followed by aspiration of the cell inlet reservoir.

[0063] Data can be collected directly on the cells/liquid in the microfluidic plate, such as placing the plate into a standard fluorescence plate reader (e.g., Biotek Instruments Synergy 2 model). In some reactions, the substrate may diffuse into the outlet medium, and therefore be easily detected in the cell inlet reservoir. For cell imaging assays, the plate can be placed on a scanning microscope or high content system. For example, an automated Olympus IX71 inverted microscope station can be used to capture viability of cultured liver cells with a 20X objective lens.

[0064] By repeatedly filling/aspirating the wells, cells can be maintained for long periods of time with minimal effort (e.g. compared to standard "bioreactors" which require extensive sterile preparation of large fluid reservoirs that cannot be easily swapped out during operation).

4. Automated Systems

[0065] FIG. 18 illustrates a top view schematic of an example cell culture automation system according to specific embodiments of the invention. Because the plates are designed to be handled using SBS compliant instruments, various "off-the-shelf" machines can be used to create an automated system. This schematic shows an example of how this is accomplished. A robotic arm (plate handler) moves the microfluidic plates from station to station. An automated incubator stores the plates at the proper temperature and gas environment for long term perfusion via gravity flow. The pipettor dispenses liquids (media, drugs, assay reagents, etc.) to the inlet wells and removes liquid from the outlet wells. A plate reader is used for assay. The cell loader is optionally used to introduce the cells to the microfluidic arrays at the beginning of the experiment. The cell loader in particular is generally not "off-the-shelf" and operates by applying pneumatic pressure to specified wells of the array plate to induce flow. Standard or custom computer software is available to integrate operations.

[0066] FIG. 19 is a photograph of an example automated microfluidic perfusion array system according to specific embodiments of the invention. The basic process includes: 1) removing the plate from the incubator, 2) removing liquid from the outlet wells via the pipettor, 3) moving a media/drug storage plate from the "plate stacks," 4) transferring liquid from the media/drug plate to the microfluidic plate via the pipettor, 5) placing the microfluidic plate into the incubator, 6) repeat for each plate, 7) repeat after specified time interval (e.g. 24 hours).

[0067] FIG. 20 is a flow chart illustrating the process flow of typical operation steps. This figure illustrates, as an example, automated process steps an indicates an automated device that is used to perform such a step. A standard automated pipettor is used for an optional surface coating, to add cells in suspension, to add media or drugs or reagents, and to change media. Known automated pipettors can individually deliver or withdraw fluids from specified wells. In a specific embodiment, a proprietary cell loader is used to pressurize the cell inlet wells for cell loading. After a period in an incubator designed for optimal cell attachment, the cell loader can again be used to wash fluid and unattached cells from the microfluidic culture areas. One or more reading or analysis devices is used to assay the cells.

[0068] FIG. 21 illustrates four microfluidic culture areas from an example array plate prepared in the example system described above using primary rat hepatocytes. The cells were

cultured for 1 week with medium changed at a rate of 150ul per unit, twice a day. Cells were assayed at the end of 7 days for viability using the Cell Titer Blue Kit from Promega, and read on an automated fluorescence plate reader (Biotek Synergy). In this figure, an example microfluidic culture area uses a grid flow-through epithelial walls.

[0069] FIG. 22 illustrates a portion of a microfluidic culture area from an example array plate prepared in the example system described above using primary human hepatocytes. The cells were cultured in the microfluidic array according to specific embodiments of the invention, showing (a) phase contrast of freshly isolated human hepatocytes cultured in the microfluidic device for 13 days. (b) Viability of human hepatocytes cultured in the microfluidic device and in a 96-well dish measured by the CellTiter Blue assay (Promega, Inc.). (c) P450 CYP3A4 activity of cultured hepatocytes in the microfluidic device and 96-well dish measured via the P450-Glow assay (Promega, Inc.).

[0070] FIG. 23 illustrates a layout of another type of cell culture array designed for general cell culture automation according to specific embodiments of the invention. In this design, each culture unit consists of 4 well positions. The first well is for perfusion medium, the second well is for cell inlet, the third well is for imaging the microfluidic chamber, and the fourth well is the outlet. A cell barrier/perfusion barrier localizes cells to the cell area and improves nutrient transport during continuous perfusion culture. The low fluidic resistance of the cell inlet to outlet path enables cells to be rapidly loaded via gravity or surface tension methods without an external cell loading mechanism. The high fluidic resistance of the perfusion inlet channels allows long term continuous perfusion of medium via gravity flow without any external pump mechanism.

[0071] FIG. 24 illustrates an operation schematic for performing automated cell culture and immunostaining on a microfluidic array with gravity cell loading as described above. Example applications include stem cell culture and primary cell culture with immunofluorescence staining and microscopy.

[0072] FIG. 25 illustrates an alternative example SBS (Society for Biomolecular Screening) standard microfluidic bioreactor array schematic. A 16-unit microfluidic cell culture array filled with colored dyes so that microfluidic channels are visible. In this example, each unit occupies five wells, which from left to right are medium inlet, cell inlet, cell outlet, cell imaging, and medium outlet.

[0073] FIG. 26A-B illustrate an alternative cellular culture system assembly according to specific embodiments of the present invention showing (A) an example schematic microfluidics design for three cell units; (B) a soft lithography fabrication of this design with laser machining

of four openings per culture unit. This design is attached to a microplate with wells for receiving medium and cells as described herein.

[0074] FIG. 27 illustrates operation steps of a less automated or prototype system according to specific embodiments of the invention. The 96-well plate standard allows the microfluidic system to be operated using standard techniques and equipment. For example, liquid dispensing is achieved with standard pipette mechanics, and cell culture and analysis is compatible with existing incubators and plate readers. A custom built cell loading system can be used to load the cells using air pressure as described above. The gravity driven flow culture configuration utilizes the medium level difference between the inlet and outlet well as well as engineering the fluidic resistances to achieve the desirable flow rate in nL/min regime. This provides the significant advantage of being able to "passively" flow culture medium for long periods of time (for example, up to 4 days) without the use of bulky external pumps.

FABRICATION TECHNIQUES

5. Example 1

[0075] FIG. 30 is a block diagram illustrating two components of a direct soft molding process according to specific embodiments of the invention. The two components illustrated are: (1) An injection molded top piece made of acrylic containing at least alignment marks (to be assembled to the microfluidic mold) and well structures generally complying with standard microtiter plate formats. (Alternatively, a standard well plate may be used.) (2) A microfluidic mold fabricated using semiconductor technologies on a 6" silicon wafer containing the microfluidic cell culture arrays made of epoxy or electroplated metals, as well as the alignment marks so the well structures aligned to the microfluidic structures during the molding process. An injection molded top piece is made of acrylic or any similar suitable material and contains well structures that preferably comply with standard microtiter plate formats as will be discussed more herein. On the right is shown a microfluidic mold fabricated using any known semiconductor and/or microfabrication technologies on, for example, a 6" silicon wafer. The mold contains an impression the microfluidic cell culture arrays and can include components made of epoxy or electroplated metals, as well as the alignment marks so the well structures aligned to the microfluidic structures during the molding process. Generally, before further processing, the mold is coated with fluoropolymer to reduce stiction of the soft polymer to the mold.

[0076] Because the top piece containing the well structures is injection molded, the bottom of the wells can be flat, rounded or tapered. One particular desired feature is that the bottom of the top piece, which covers the microfluidic structures, is as flat as practically to assist uniform

molding across the array. According to specific embodiments of the invention, the bottom of the top piece can be chemically or mechanically or otherwise modified or primed by a reagent (such as Sylgard Primecoat) or an abrasive surface (sanding) or laser so the soft polymers adhere to the bottom of the top piece after the molding process. This treatment of the surface is indicated by the heavy line.

[0077] FIG. 31B illustrates an example wherein an appropriate amount of soft polymer is poured onto the center of the mold (usually a few milliliters, depending on the area to be covered as well as the thickness of the soft polymer after molding). The top piece and the mold are sandwiched between two pieces of flat surfaces (usually glass plates) with clamping mechanisms (in this case, magnets).

[0078] FIG. 31C illustrates an example wherein the clamping mechanism holds the top piece and the mold together with alignment marks fitted to each other. The soft polymer is then cured, for example by temperature or UV light of otherwise so the microfluidic cell culture array is truthfully molded onto the soft polymer. As an example, at elevated temperature (usually 60 C) for at least 2 hours.

[0079] FIG. 31D illustrates an example wherein after detaching the molded microfluidic cell culture array with the top piece, a laser cutter is used to create fluidic connections between the microfluidic structures and the wells at specific locations (cell/reagent inlets/outlets). The circular top piece is trimmed to rectangular shape at this stage. (Note that in this image, the structure is inverted.) Before enclosing the bottom of the microfluidic cell culture array, the molded piece is ultrasonically cleaned to shake off any dust created by the laser cutting step. A top piece may be trimmed to a rectangular shape at this stage. The cross section shown in through each of the fluidic connections for illustration purposes, though the laser only makes holes in the material and does not cut the wells apart. At this state, before enclosing the bottom of the microfluidic cell culture array, the molded piece is preferably ultrasonically or otherwise cleaned to shake off any dust created by the laser cutting step.

[0080] FIG. 31E illustrates an example wherein the microfluidic cell culture array undergoes oxygen plasma treatment and is bonded to a piece of rectangular glass.

[0081] FIG. 31F illustrates an example wherein using a liquid dispenser, the microfluidic cell culture array is filled with priming solutions to maintain its modified surface chemistry. If bubbles appear to be trapped inside the array, placement in a vacuum chamber may be used to eliminate the bubbles.

[0082] FIG. 31G illustrates an example wherein to prevent liquid evaporation, the array is sealed with a tape. FIG. 31H illustrates an example wherein the array is fit into a frame so the finished array can be treated like a standard microtiter plate with the correct outside dimensions.

6. Example 2

[0083] FIG. 32A-C illustrate three components of a direct soft molding process according to specific embodiments of the invention. In the figure, (A) shows an injection molded top piece that includes well structures complying with standard microtiter plate formats. As discussed above, rather than the injection molded top piece shown, a standard microtiter plate may be used as the top piece that includes well structures complying with standard microtiter plate formats., (B) Illustrates a 1.5mm thick acrylic circular sheet (6" in diameter), and (C) illustrates a microfluidic mold fabricated on a 6" silicon wafer containing microfluidic cell culture units (in this example 8 x 4 units) in an arrays made of epoxy, etched silicon, or electroplated metals, as well as a spacer to control the minimum thickness of the soft polymer after molding. The mold is coated with fluoropolymer to reduce stiction of the soft polymer to the mold. As shown in the figure, the microfluidic mold is glued to a 1mm thick soda lime glass (7" x 7") with four magnets (e.g., 15mm in diameter and 1.5mm in thickness) glued to the four corners of the glass. The other piece of the 1mm thick soda lime glass (7" x 7") with complementary magnets is prepared in similar fashion (FIG. 32D) with the magnets of opposite polarity glued on the four corners so the magnets will self-align onto the magnets in FIG. 32C. One side of the acrylic sheet is chemically modified by a reagent (e.g., Sylgard Primecoat) to induce the strong adhesion between the acrylic and the soft polymer (e.g., Sylgard 184) to be used during the molding process.

[0084] FIG. 33A illustrates a step wherein an appropriate amount of soft polymer is poured onto the center of the mold (usually a few milliliters, depending on the area to be covered as well as the spacer thickness) For example, for a mold 6" in diameter and a 150micron spacer, the minimum amount required is π x 7.62cm x 7.62cm x 0.015cm ~ 2.75mL).

[0085] FIG. 33B illustrates a step wherein the acrylic sheet is sandwiched between two pieces of the glass plates so the magnets will press the acrylic sheet (with primer modified surface facing the mold) against the mold until the acrylic sheet hit the spacer. The soft polymer will then fill the space between the acrylic sheet and the mold to replicate the microfluidic structures. In particular embodiments, the magnet-assisted clamping mechanism holds the pieces together while the soft polymer is cured at elevated temperature (60 degreeC) for at least 2 hours.

[0086] After cooling the compartments down to approximately room temperature, the acrylic sheet with the soft polymer is detached from the mold and a microfluidic cell culture array as described herein is truthfully molded onto the soft polymer.

[0087] To protect the soft polymer surface from contaminations from following processes, a surface protection tape (e.g., Ultron Blue Adhesive Plastic Film 80micron) is optionally applied to the top of the surface of the elastomer by a roller.

[0088] FIG. 33D illustrates a step wherein a CO2 laser cutter (VersaLaser, 25W model) is used to create fluidic connections between the microfluidic structures and the injection molded wells (cell inlet and medium inlet). Since the soft polymer used in the process is gas permeable, "air holes" are cut near the cell culture areas to promote air diffusion for better cell culture. The circular top piece may be trimmed to rectangular shape at this stage. The surface protection tape is removed and the array is ultrasonically cleaned (or water-jet cleaned) to shake off any dust created by the laser cutting step and a new surface protection tape is applied. In FIG. 33E, the microfluidic cell culture array is glued to the injection molded plate or a standard-well plate with an ultra-violet (UV) curable glue which is also bio-compatible (Loctite 3301). The plate with the microfluidic cell culture array is cured in a UV chamber for 30 minutes. After removal of the surface protection tape, both a glass substrate (White Float Glass) and the microfluidic cell culture array undergo oxygen plasma treatment to activate the surface. The glass substrate encloses the microfluidic cell culture array through covalent bonding, as shown in FIG. 33F. Using a liquid dispenser, the microfluidic cell culture array is filled with priming solutions as shown in FIG. 33G. Because bubbles may be present inside the array; the array is generally placed inside a vacuum chamber for bubbles removal. The plate may also be placed inside a UV/Ozone chamber (Novascan) for sterilization. To prevent liquid evaporation, the array is sealed with a tape (e.g., Excel Scientific, AlumaSeal) as shown in FIG. 33H.

INTEGRATED SYSTEMS

[0022] Integrated systems for the collection and analysis of cellular and other data as well as for the compilation, storage and access of the databases of the invention, typically include a digital computer with software including an instruction set for sequence searching and/or analysis, and, optionally, one or more of high-throughput sample control software, image analysis software, collected data interpretation software, a robotic control armature for transferring solutions from a source to a destination (such as a detection device) operably linked to the digital computer, an input device (e.g., a computer keyboard) for entering subject data to the digital computer, or to control analysis operations or high throughput sample transfer by the robotic control armature. Optionally, the integrated system further comprises valves,

concentration gradients, fluidic multiplexors and/or other microfluidic structures for interfacing to a microchamber as described.

[0023] Readily available computational hardware resources using standard operating systems can be employed and modified according to the teachings provided herein, e.g., a PC (Intel x86 or Pentium chip- compatible DOS,™ OS2,™ WINDOWS,™ WINDOWS NT,™ WINDOWS95,™ WINDOWS98,™ LINUX, or even Macintosh, Sun or PCs will suffice) for use in the integrated systems of the invention. Current art in software technology is adequate to allow implementation of the methods taught herein on a computer system. Thus, in specific embodiments, the present invention can comprise a set of logic instructions (either software, or hardware encoded instructions) for performing one or more of the methods as taught herein. For example, software for providing the data and/or statistical analysis can be constructed by one of skill using a standard programming language such as Visual Basic, Fortran, Basic, Java, or the like. Such software can also be constructed utilizing a variety of statistical programming languages, toolkits, or libraries.

[0024] FIG. 34 shows an information appliance (or digital device) 700 that may be understood as a logical apparatus that can read instructions from media 717 and/or network port 719, which can optionally be connected to server 720 having fixed media 722. Apparatus 700 can thereafter use those instructions to direct server or client logic, as understood in the art, to embody aspects of the invention. One type of logical apparatus that may embody the invention is a computer system as illustrated in 700, containing CPU 707, optional input devices 709 and 711, disk drives 715 and optional monitor 705. Fixed media 717, or fixed media 722 over port 719, may be used to program such a system and may represent a disk-type optical or magnetic media, magnetic tape, solid state dynamic or static memory, etc. In specific embodiments, the invention may be embodied in whole or in part as software recorded on this fixed media. Communication port 719 may also be used to initially receive instructions that are used to program such a system and may represent any type of communication connection.

[0025] Various programming methods and algorithms, including genetic algorithms and neural networks, can be used to perform aspects of the data collection, correlation, and storage functions, as well as other desirable functions, as described herein. In addition, digital or analog systems such as digital or analog computer systems can control a variety of other functions such as the display and/or control of input and output files. Software for performing the electrical analysis methods of the invention are also included in the computer systems of the invention.

Other Embodiments

[0026] Although the present invention has been described in terms of various specific embodiments, it is not intended that the invention be limited to these embodiments. Modification within the spirit of the invention will be apparent to those skilled in the art.

[0027] It is understood that the examples and embodiments described herein are for illustrative purposes and that various modifications or changes in light thereof will be suggested by the teachings herein to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the claims.

[0028] All publications, patents, and patent applications cited herein or filed with this submission, including any references filed as part of an Information Disclosure Statement, are incorporated by reference in their entirety.

WHAT IS CLAIMED:

1. A microfluidic cell culture system comprising:

a medium inlet reservoir fluidically connected to a plurality of microfluidic medium channels;

a plurality of cell culture areas each located at an end of a cell culture channel;

said cell culture channel having a cell inlet opening fluidically connected to a cell inlet reservoir for introducing cells to said plurality of cell culture areas;

a perfusion barrier between said cell culture areas and said plurality of microfluidic medium channels, said perfusion barrier containing a plurality of perfusion passages;

a media outlet reservoir fluidically connected to said cell culture channels;

such that fluid introduced into said medium inlet reservoir must pass through a part of said cell culture channels before reaching said media outlet reservoir.

2. The system of any of the above claims further wherein said system is integrated into a standard well plate.

3. The system of any of the above claims further wherein said system is integrated into a standard well plate and further wherein each independent device occupies three well positions, a first well acting as a medium inlet, a second well acting as a medium outlet/cell inlet, and a middle well acting as a viewing/cell culture area.

4. The system of any of the above claims further wherein said cell culture channels include a first portion near said cell inlet that contains solid barriers walls with no perfusion passages to prevent cell adhesion.

5. The system of any of the above claims further wherein said cell culture channels include a second portion nearer to said cell inlet than said first portion, said second portion having a plurality of perfusion passages to facilitate media flow out of said cell culture region.

6. The system of any of the above claims further wherein said standard well plate is handled by robotic equipment allowing for automated cell culture and analysis, said robotic equipment being in part equipment designed for use with standard well plates.

7. The system of any of the above claims further wherein said standard well plate is configured to pneumatically couple with a pneumatic manifold, said manifold providing pneumatic pressure to drive cells into said cell culture areas.

8. The system of any of the above claims further wherein:

said cell culture areas are separated into at least three blocks, each block containing at least two cell culture areas; each block having an air channel adjacent thereto.

9. The system of any of the above claims further wherein:
cells are introduced into said cell culture areas using pneumatic pressure, and thereafter, cells are maintained by perfusion of media using a passive gravity driven fluid flow.

10. The system of any of the above claims further wherein:
a plurality of said perfusion passages are substantially narrower than cells to be cultured.

11. The system of any of the above claims further wherein:
a plurality of said perfusion passages are substantially narrower than about 6 microns.

12. The system of any of the above claims further comprising:
a large fluidic opening directly connected to said cell culture channels to allow for easier cell flow.

13. The system of any of the above claims further comprising:
a fluidic multiplexor connecting said media inlet reservoir to said microfluidic medium channels.

14. The system of any of the above claims further wherein:
a plurality of said cell culture channels comprise a cell culture area with perfusion passages to said media channels and a non perfusion cell channel portion for better localizing cells introduced under pneumatic pressure.

15. The system of any of the above claims further wherein:
a plurality of said cell culture channels comprise a cell culture area with perfusion passages to said media channels and a non perfusion cell channel portion for better localizing cells introduced under pneumatic pressure and an outlet portion with perfusion passages near to an inlet of said cell culture channel.

16. The system of any of the above claims further wherein:
said cell culture areas are elongated areas mimetic of a liver sinusoid;
said cell culture areas are loaded with cells from said cell inlet reservoir;
after cell loading and settling, media is loaded from said media inlet and removed from said cell inlet/medium outlet reservoir.

17. A microfluidic cell culture device comprising:

a medium inlet reservoir and a channel for introducing liquid media to a plurality of cell culture areas;

a cell inlet/medium outlet reservoir for introducing cells to said plurality of cell culture areas;

wherein said cell culture areas are elongated areas mimetic of a liver sinusoid;

wherein said cell culture areas are loaded with cells from said cell inlet/medium outlet reservoir;

wherein after cell loading and settling, media is loaded from said media inlet and received and/or removed from said cell inlet/medium outlet reservoir;

such that a portion of said media passes through cells in said culture area.

18. The device of claim 17 further wherein said device is integrated into a standard well plate.

19. The device of claim 17 further wherein said device is integrated into a standard well plate and further wherein each independent device occupies three well positions, a first well acting as a medium inlet, a second well acting as a medium outlet/cell inlet, and a middle well acting as a viewing/cell culture area.

20. The device of claim 17 further wherein said elongated cell culture areas include a first portion near said cell inlet that contains solid walls with no perfusion passages to prevent cell adhesion.

21. The device of claim 20 further wherein said elongated cell culture areas include a second portion nearer to said cell inlet than said first portion that contains walls with some perfusion passages to facilitate media flow out of said cell culture region.

22. The device of claim 19 further wherein said standard well plate is handled by robotic equipment allowing for automated cell culture and analysis, said robotic equipment being in part equipment designed for use with standard well plates.

23. The device of claim 19 further wherein said standard well plate is configured to pneumatically couple with a pneumatic manifold, said manifold providing pneumatic pressure to drive cells into said cell culture areas.

24. The device of claim 17 further wherein:

said cell culture areas are separated into at least three blocks, each block containing at least two cell culture areas; each block having an air channel adjacent thereto.

25. A method of culturing cells comprising:

placing cells into a well of a standard well plate, said well fluidically connected to a plurality of microfluidic channels, said microfluidic channels connected to a plurality of cell culture areas;

placing media into a different well of said standard well plate, said well fluidically connected to a plurality of microfluidic perfusion passages, said microfluidic perfusion passages providing perfusion passages through said micro cell culture areas;

allowing said cells to culture for an appropriate time;

observing and/or assaying cells and or media in said standard well plate.

26. The method of claim 25 further comprising:

interfacing said standard well plate with a pneumatic manifold for providing air pressure to drive said cells into said cell culture areas, said pneumatic manifold providing sufficient pressure to form cell aggregates in said cell culture areas.

27. The method of claim 26 further wherein said pneumatic manifold interfaces with said well plate using a vacuum seal.

28. The method of claim 26 further comprising:

using a pipette to introduce and/or remove fluids from said reservoirs, said fluids passively perfusing through said cell culture areas as a result of gravity and/or differential fluid levels; and or surface tensions.

29. The method of claims 25 through 28 further wherein said steps are performed by fully automated robotic equipment.

30. The method of claims 25 through 28 further wherein said steps are performed by fully automated robotic equipment and further wherein said combination of pneumatic cell loading and passive perfusion allows simultaneous automatic culture of a large number of plates using one pneumatic manifold and one automated pipettor because plates do not need to be attached to any equipment during perfusion fluid flow.

31. A method of fabricating a structure with micron-scale areas comprising:

fixing a mold to a first plate, said mold including negative impressions of one or more desired micro features;

said mold further comprising at least one magnetic region;

placing a formable substance between said first plate and a second plate, said second plate, further comprising at least one magnetic region complementary said at least one magnetic region of said first plate;

positioning said first plate and said second plate so that the magnetic attraction between said magnetic regions hold the plates together with sufficient pressure to mold said formable substance;

allowing said formable substance to cure to a desired hardness; and

removing hardened formable substance with micron-scale structures.

32. The method of claim 31 further wherein:

said magnetic attraction aligns said plates; and

said aligning and holding are accomplished without need for mechanical clamps.

33. The method of claim 31 further comprising:

fixing a first plurality of magnets to said first plate;

fixing a second plurality of magnets to said second plate;

said second plurality of magnets being complementary to said first plurality.

plate and first magnets.

34. The method of claim 33 further wherein:

said first and second pluralities each comprise at least four magnets.

35. The method of claim 33 further wherein:

said first and second pluralities of magnets remain fixed to said plates through repeated molding operations.

36. The method of claim 33 further wherein:

said plates can each comprise any suitable combination of any desirably rigid material, such as glass, plastic, acrylic, metal, ceramic, and may have any suitable coating;

said mold may be formed using any know micro-fabrication technique of any suitable materials, including silicon, any metals, ceramics, suitably hardened plastics, etc.

37. A microfluidic cell culture device comprising:

at least two fluidic reservoirs;

a microfluidic passage with at least two fluidic access ports, each in connection with one or more of said fluidic reservoirs;

wherein a portion of at least one of the passages has a height and/or width smaller than the diameter of cells to be cultured in said reservoirs;

thereby providing a cell culture area allowing continuous perfusion between said fluidic reservoirs.

38. The cell culture device of claim 37 further wherein:

said passages have a height and/or width smaller than the diameter of cells, wherein said height is between 20-60 microns and said width is between 2-8 microns.

39. The cell culture device of claim 37 further wherein:

said passages have a height and/or width smaller than the diameter of cells, wherein said height is about 40 microns and said width is about 4 microns.

40. The cell culture device of claim 37 further comprising:

a plurality of cell culture units;

each of said units comprising at least two fluidic reservoirs; and

a plurality of said units being fluidically addressable in a cell culture array.

41. The device of claim 40 where each unit of the array is fluidically independent.

42. The device of claim 37 where said fluidic reservoirs are open to the air.

43. The device of claim 37 where said fluidic reservoirs are accessible via liquid dispensing methods.

44. The device of claim 40 wherein the array complies with SBS microtiter plate standards.

45. The device of claim 40 wherein the array contains more than 16 addressable units.

46. The device of claim 40 wherein the array density is more than 2 units per square centimeter.

47. The device of claim 40 wherein the reservoir spacing is less than 5 mm.

48. The device of claim 37 wherein flow is driven by pneumatic pressure.

49. The device of claim 37 wherein flow is driven by a liquid height difference between one or more of the reservoirs.

50. The device of claim 37 wherein flow is driven by surface tension and/or capillary forces.

51. The device of claim 37 wherein the cells are loaded using gravity driven flows.

52. The device of claim 37 wherein the cells are loaded by surface tension driven flows.

53. The device of claim 37 wherein the cells are loaded by positive pneumatic pressure.

54. The device of claim 37 wherein the fluidic access ports are created using a laser.

55. The device of claim 37 wherein the flow rate is controlled by the liquid level difference between said fluidic reservoirs.

56. The device of claim 37 wherein the fluidic reservoirs and the microfluidic cell culture device are manufactured separately and then integrated afterwards.

57. The device of claim 37 wherein the cells are cultured in the proximity of the fluidic access ports.

58. The device of claim 37 wherein the cells are cultured in a 3D matrix.

59. The device of claim 37 wherein the microfluidic passages are coated with extracellular matrix.

60. The device of claim 37 wherein the microfluidic passages are made of gas permeable materials.

61. The device of claim 37 wherein at least some portions of the microfluidic passages and the fluidic reservoirs are made of the same material.

62. The device of claim 37 furthering comprising:

one or more particles, beads, or other nano/micro fabricated materials introduced into the culture unit.

63. The device of claim 37 where the flow rate in one or more channels is largely determined by a high fluidic resistance region.

64. An automated system for controlling fluid flows in a microfluidic array comprising:

at least two fluidic reservoirs;

a microfluidic passage with at least two fluidic access ports, each in connection with one or more of said fluidic reservoirs;

wherein a portion of at least one of the passages has a height smaller than the diameter of the cells; and

wherein cells are cultured under continuous perfusion between said fluidic reservoirs

65. The system in claim 64 where flow control is operated by pneumatic pressure.

66. The system in claim 64 where flow control is operated by robotic dispensing/removal of liquid from said fluidic reservoirs

67. The system in claim 64 where cells are loaded by pneumatic pressure while media flow is controlled by height of fluids in said fluidic reservoirs.

68. The system in claim 64 where fluid in contact with the microchannels is switched at least once.

69. The system in claim 64 where two or more inlet reservoirs are controlled simultaneously to produce a dynamically changing mixture.

70. The system in claim 64 where two or more inlet reservoirs are flowed in series to create a time-varying output condition.

71. The system in claim 64 where the flow methodology does not interfere with optical analysis of the microfluidic array.

72. The system in claim 64 where the flow methodology does not interfere with cell culture in a standard incubation chamber.

73. The system in claim 64 such that multiple independent culture units are controlled simultaneously.

74. A microfluidic structure comprising:
two or more inlet channels in communication with a downstream channel or chamber;
a fluidic resistance in at least one of the inlet channels is significantly larger than the fluidic resistance in the outlet channel;
such that the resulting flow when one or more of the inlets is pressurized is dominated by transport to the downstream channel or chamber and not through any of the other inlet channels.

75. The structure in claim 74 whereby the fluidic resistance ratio is greater than 10.

76. The structure in claim 74 whereby the fluidic resistance ratio is greater than 50.

77. The structure in claim 75 where the high fluidic resistance is caused by a region of the inlet channel with significantly different height than the downstream channel.

78. The structure in claim 74 applied upstream of a microfluidic cell culture unit or array.

79. The structure in claim 74 whereby flow between the inlet channels is countered by the liquid head pressure in the inlet reservoir.

80. The structure in claim 74 where the inlet channel is of sufficient volume such that any backflow from the other inlets is completely contained during the duration of use.

81. A microfluidic structure according to one or more of the figures, descriptions, and appendices herein.

82. A cell culture structure according to one or more of the figures, descriptions, and appendices herein.

83. A cell culture system according to one or more of the figures, descriptions, and appendices herein.

84. A method of culturing cells according to one or more of the figures, descriptions, and appendices herein.

85. A method of fabricating a microfluidic structure according to one or more of the figures, descriptions, and appendices herein.

86. A method of operating a microfluidic structure system according to one or more of the figures, descriptions, and appendices herein.

87. A method of operating a cellular culture system according to one or more of the figures, descriptions, and appendices herein.

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

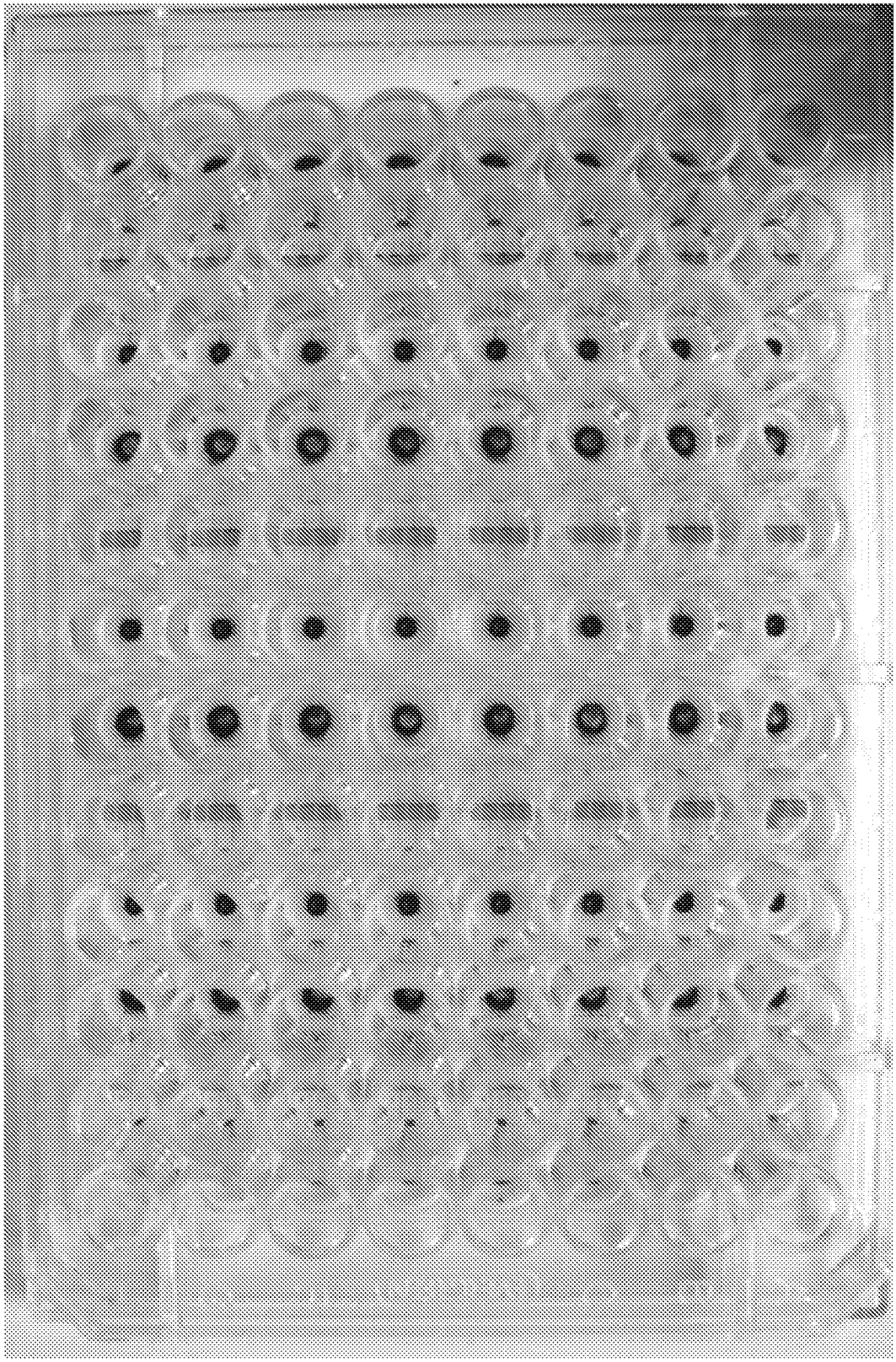

FIG. 1

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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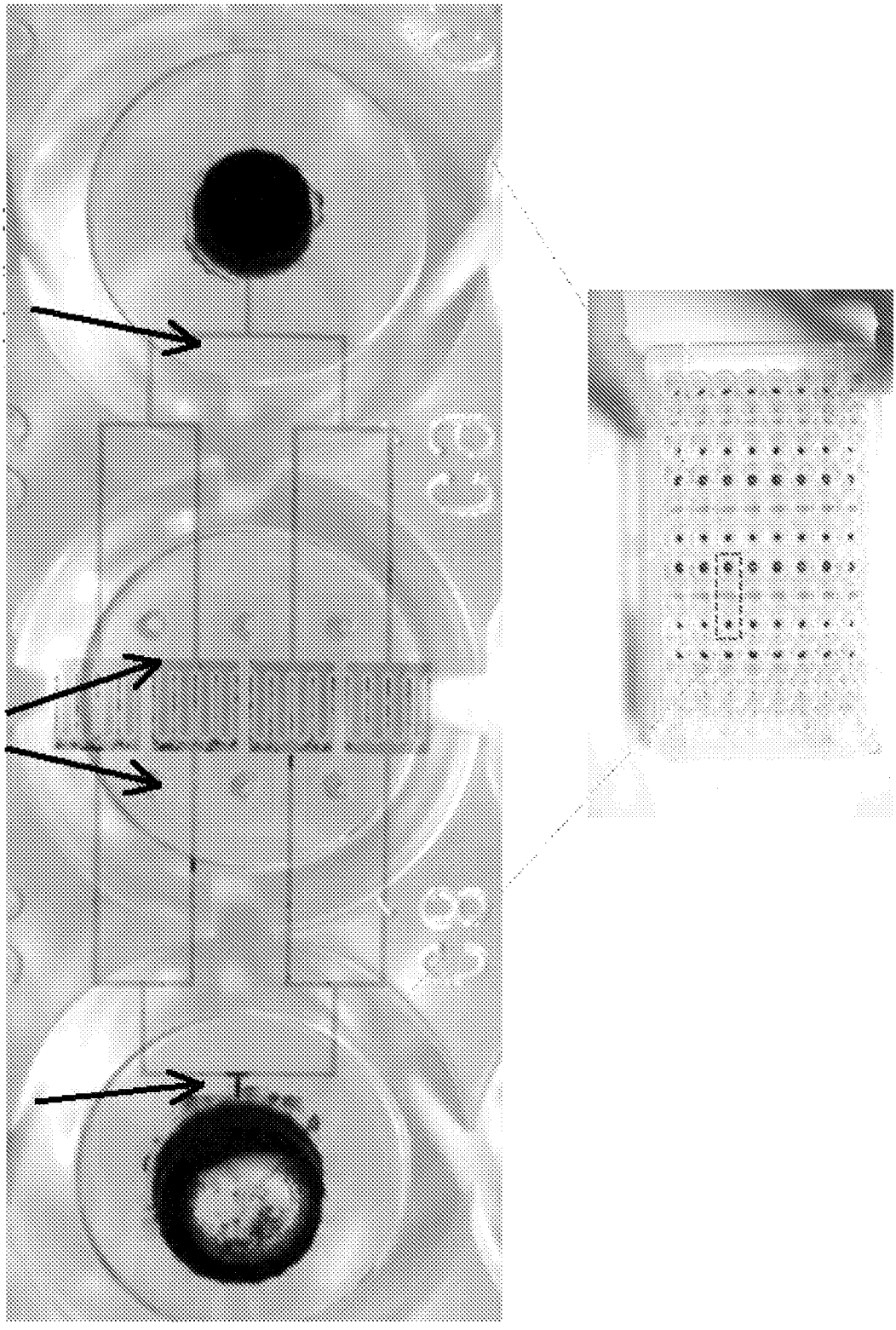

FIG. 2

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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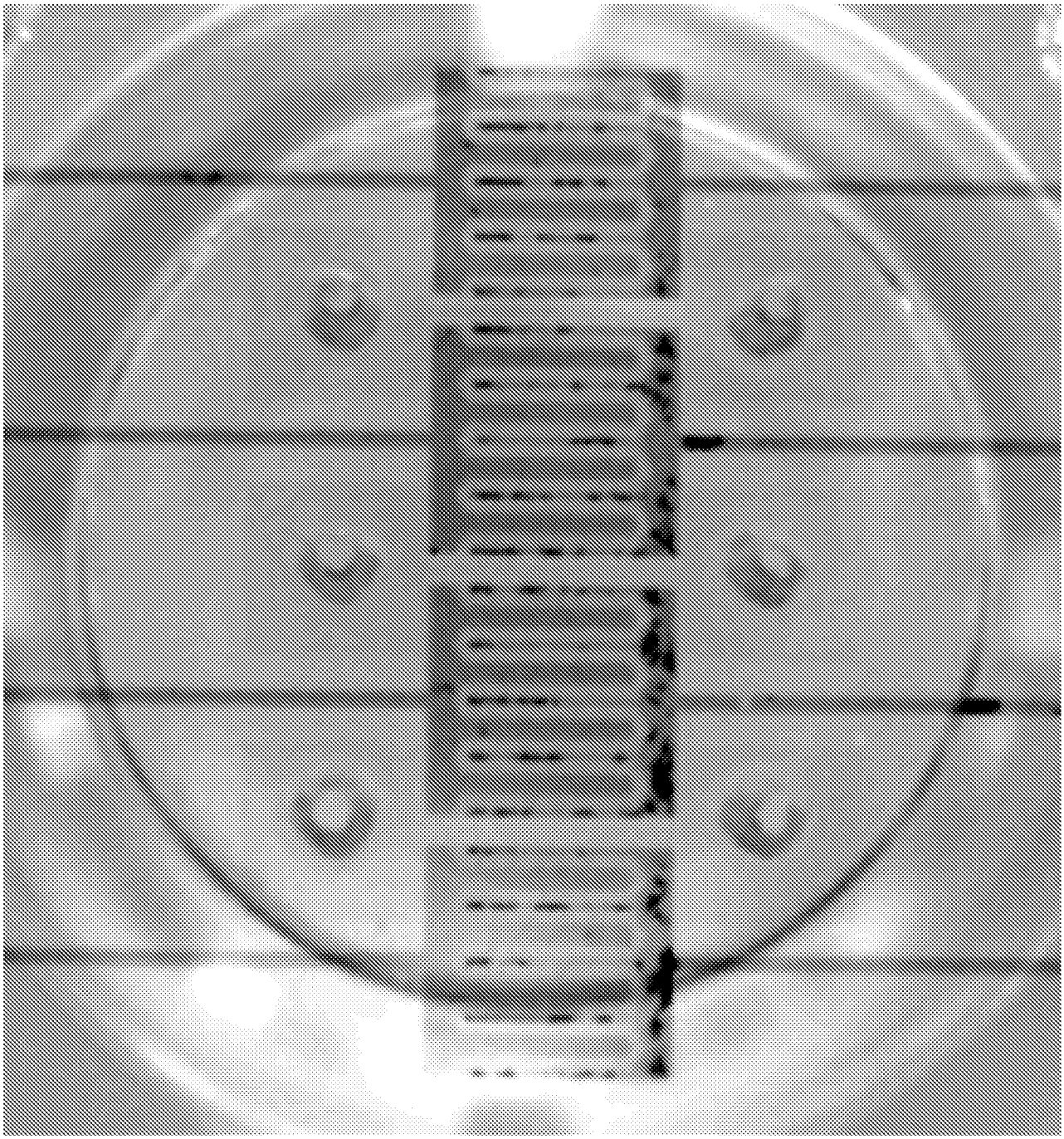

FIG. 3

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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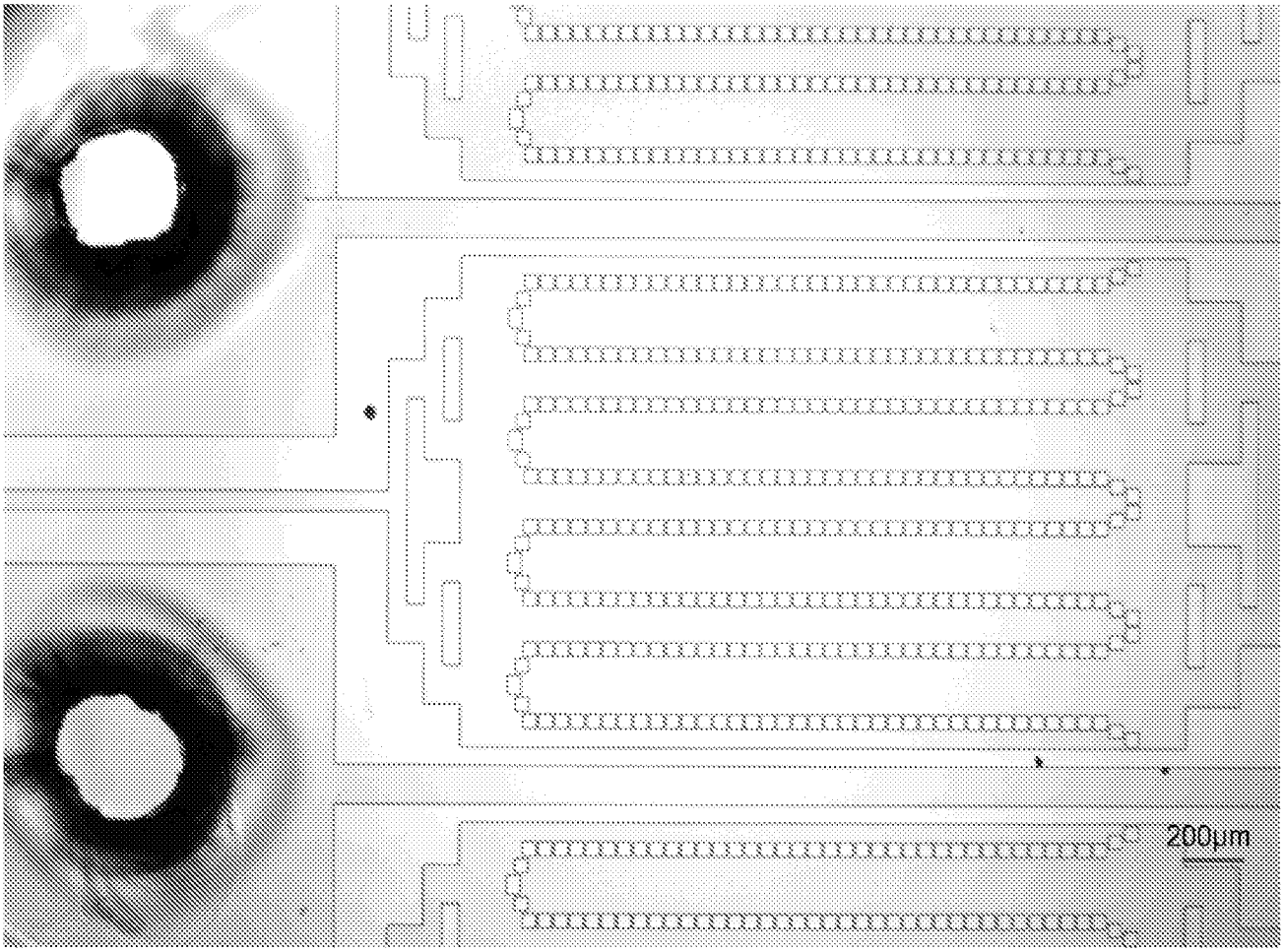

FIG. 4

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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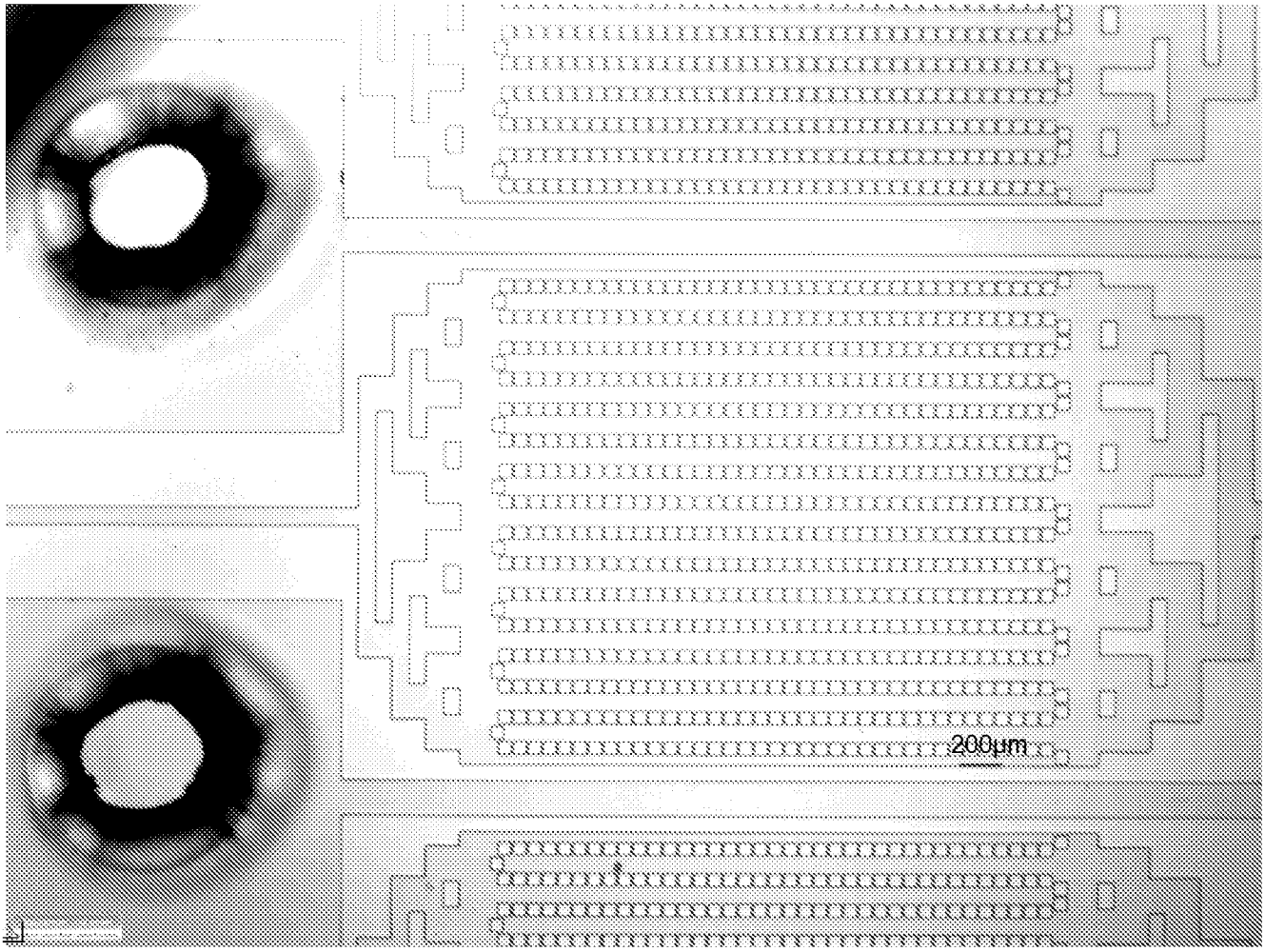
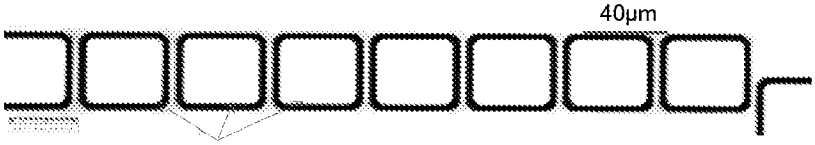


FIG. 5

Cells are cultured in this area



4µm wide and 40µm tall channels prevent cells from growing out.

FIG. 6

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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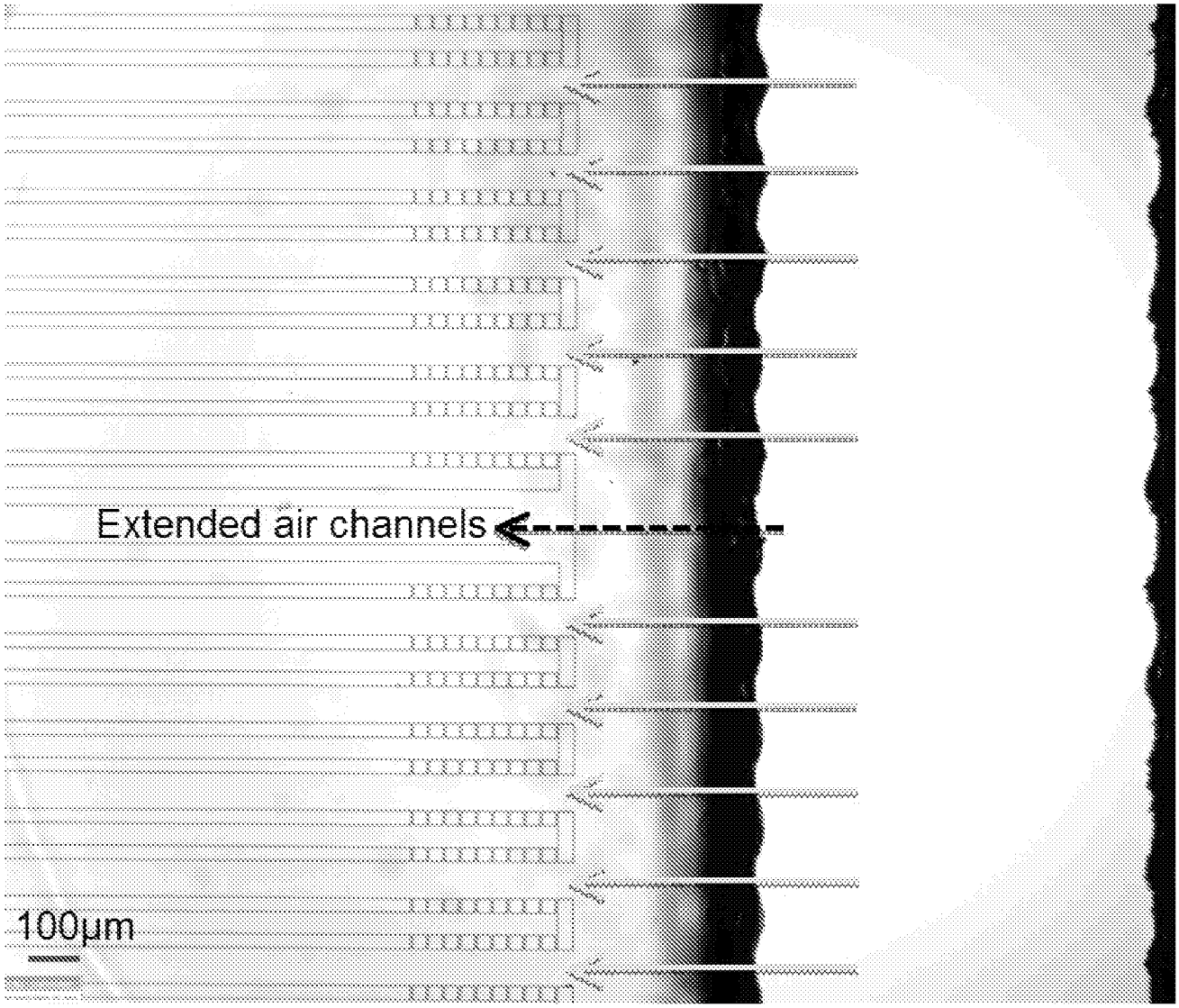

FIG. 7A

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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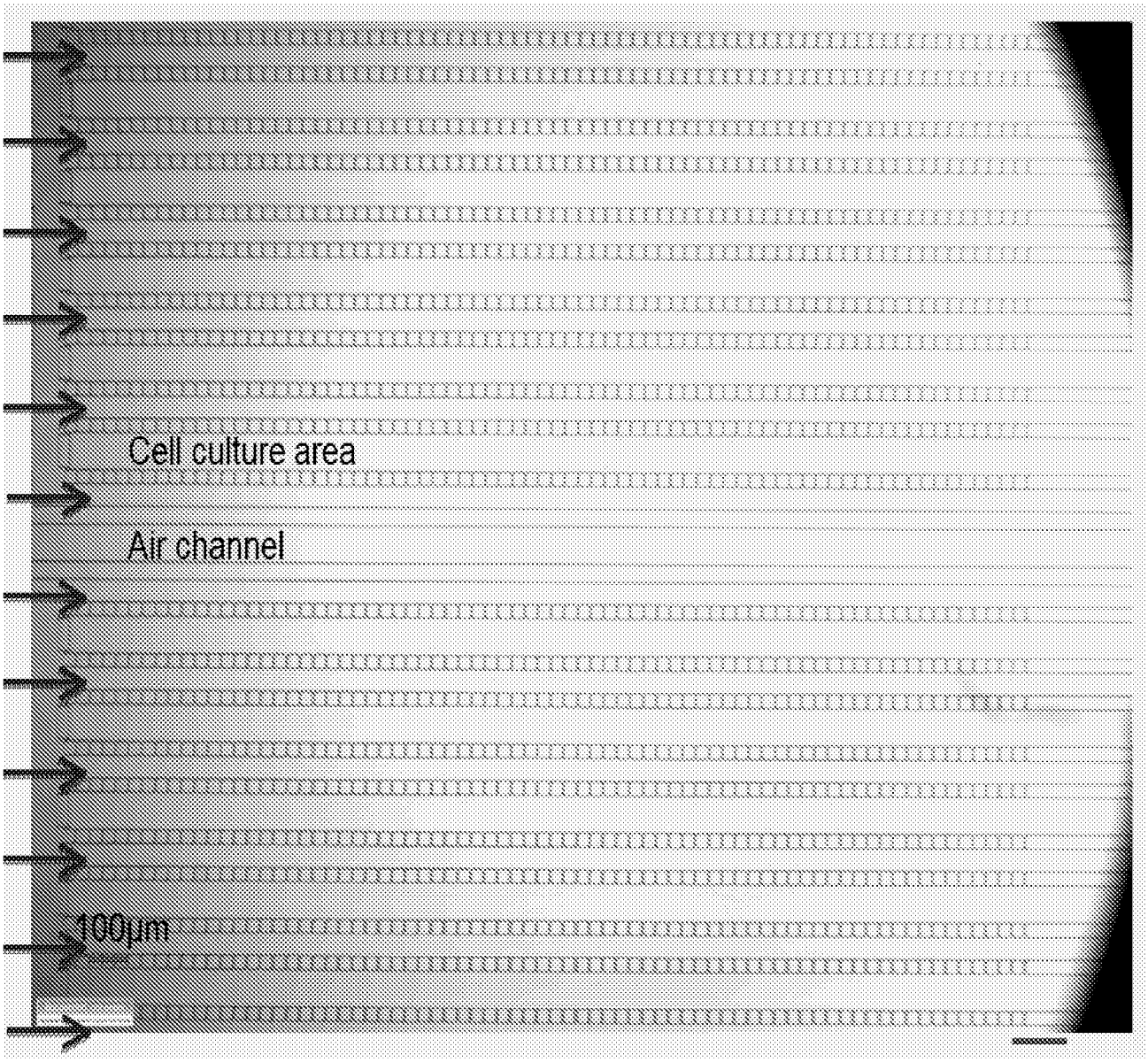

FIG. 7B

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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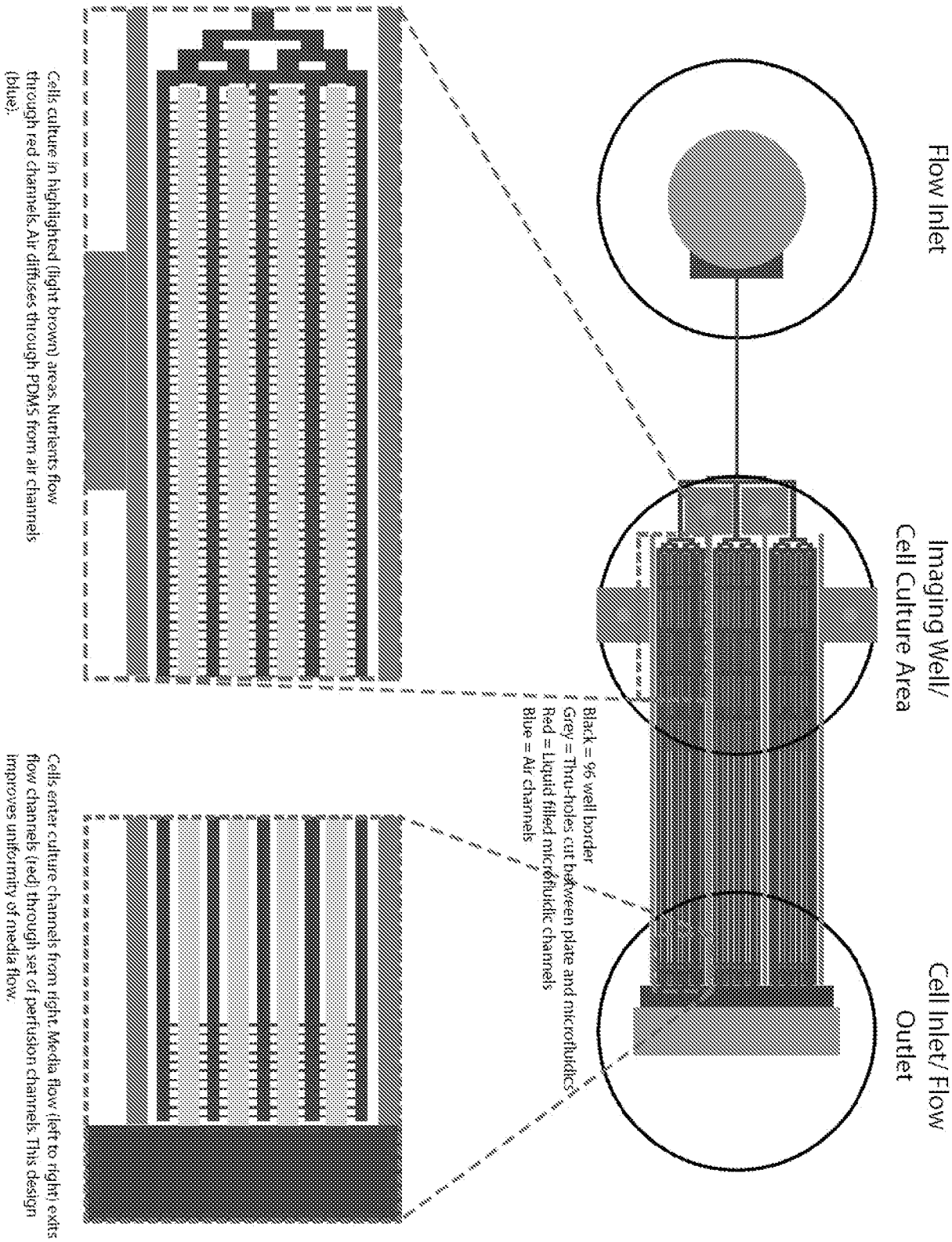

FIG. 8 E.G., 2,500 CELLS IN EACH SINUSOID

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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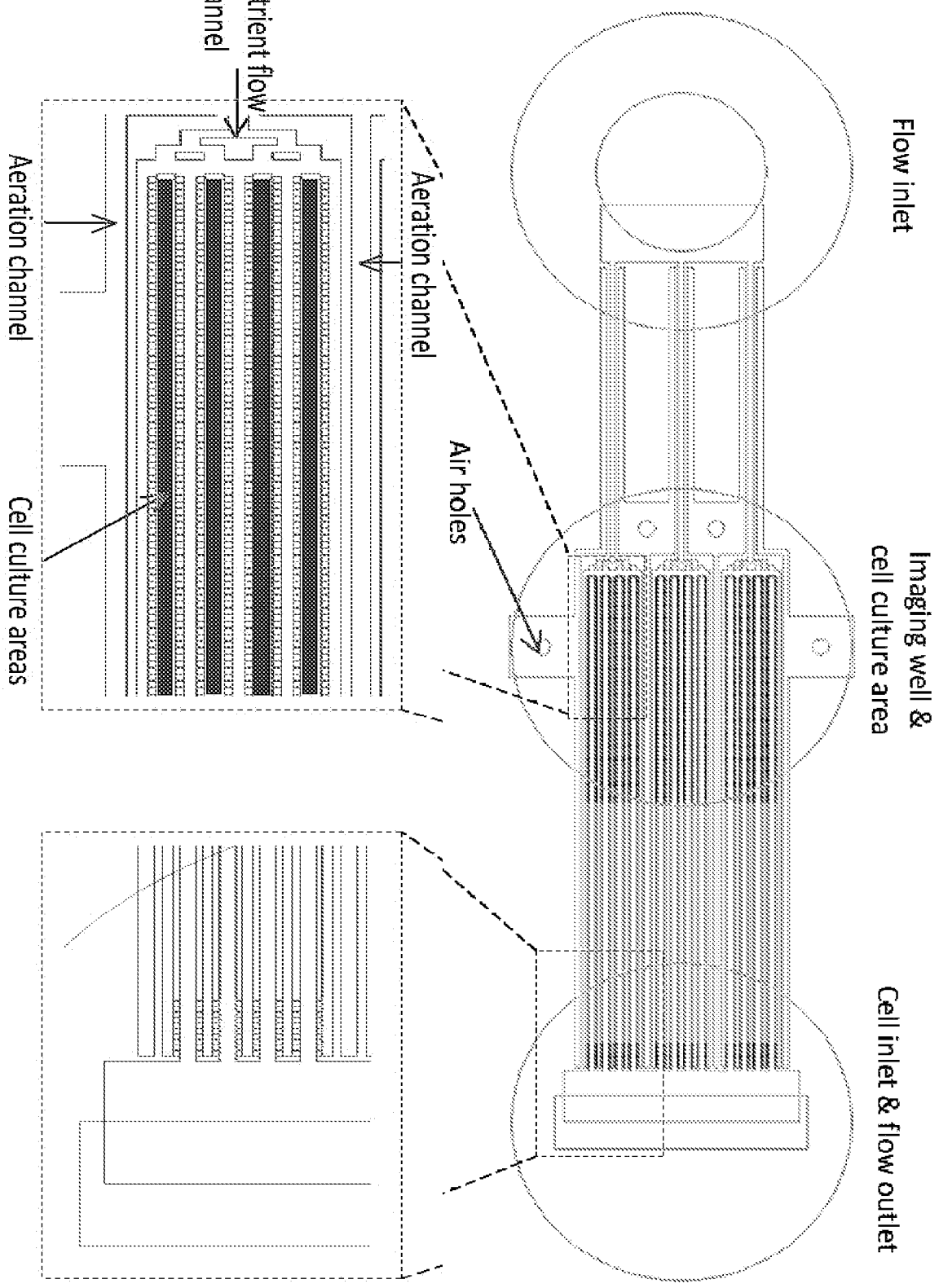

FIG. 8

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
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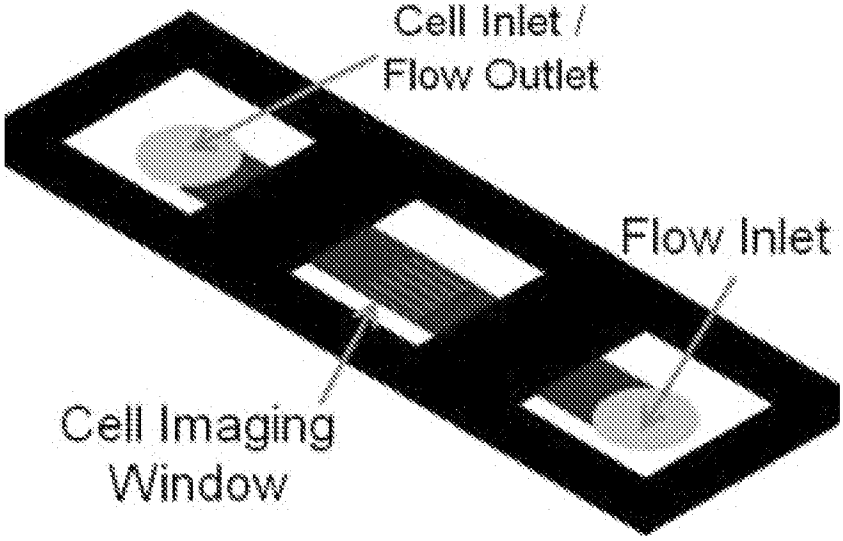

FIG. 9A

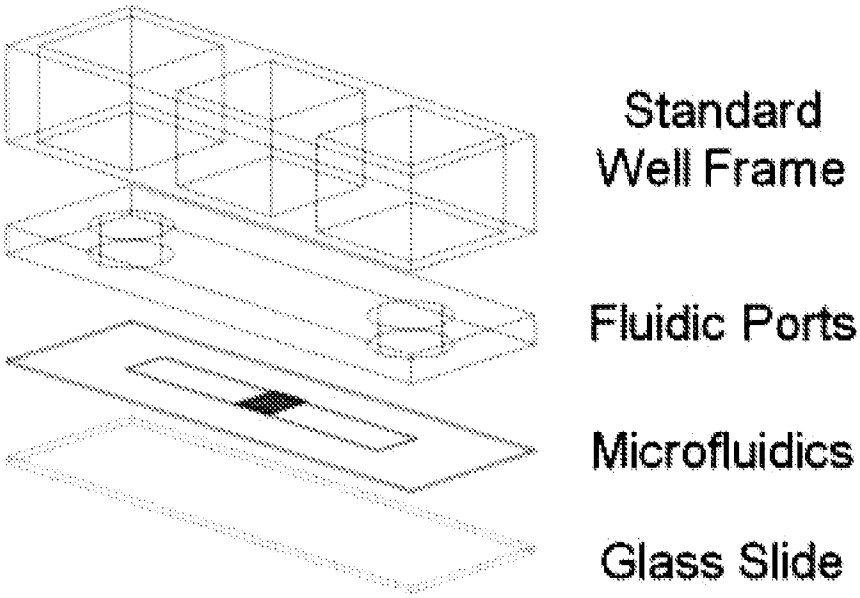

FIG. 9B

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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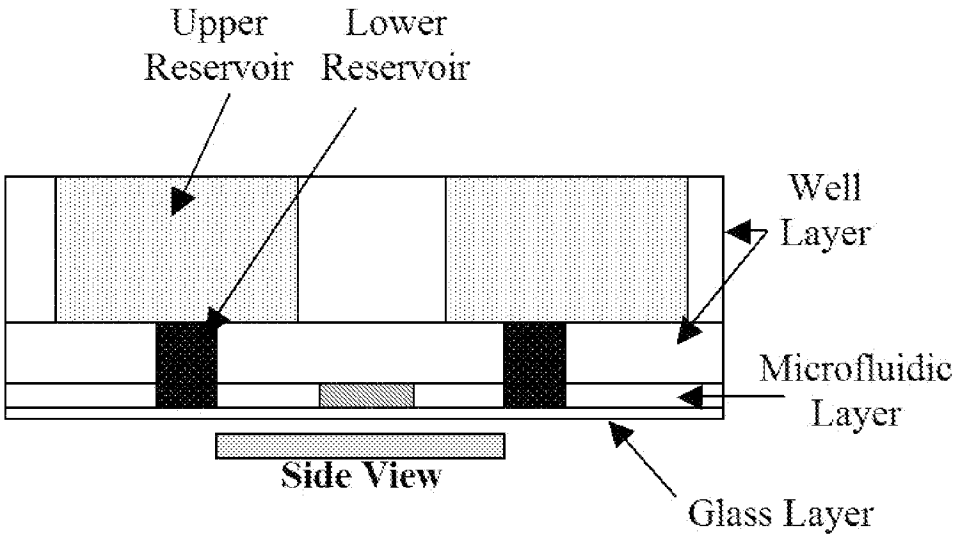

FIG. 10

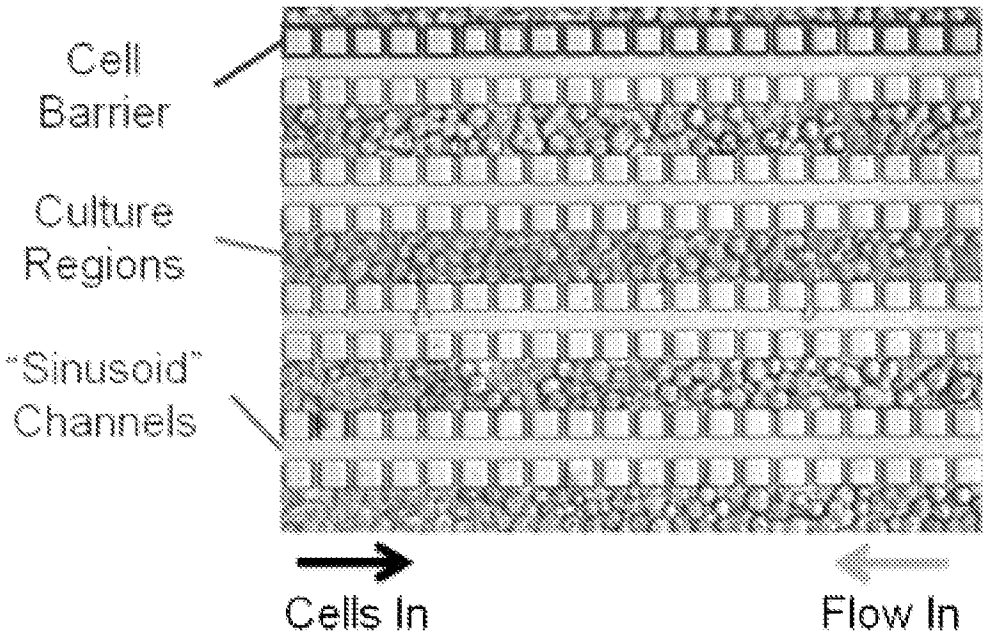

FIG. 11

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
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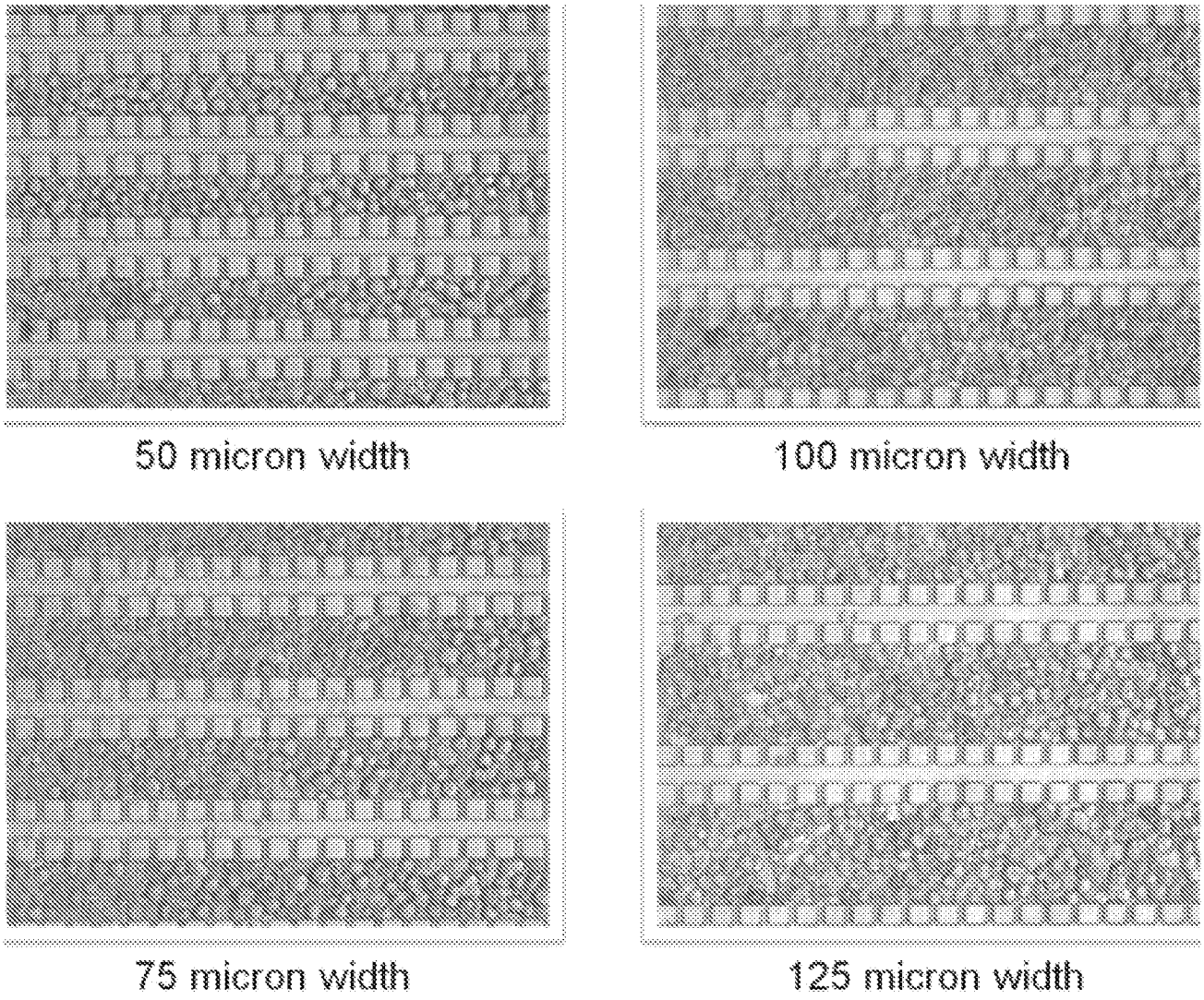

FIG. 12

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

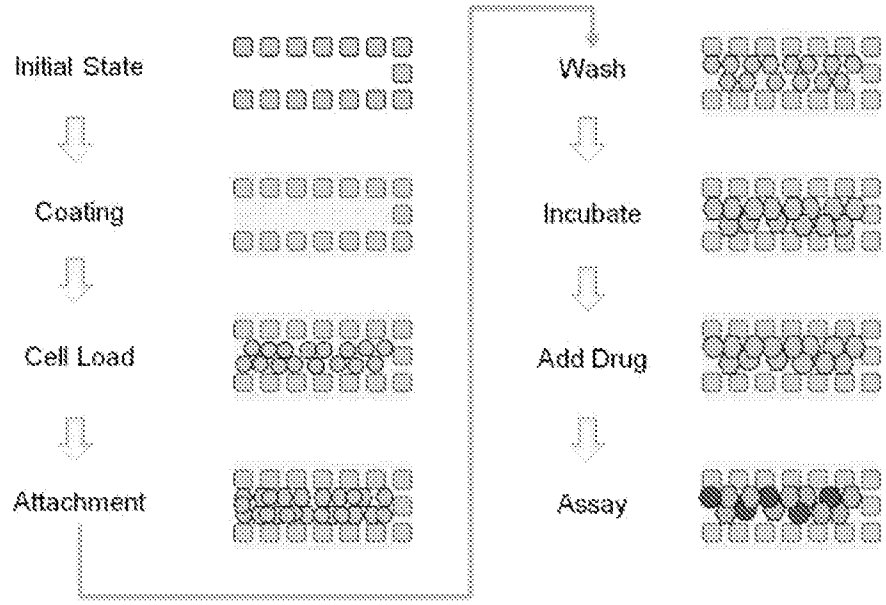

FIG. 13

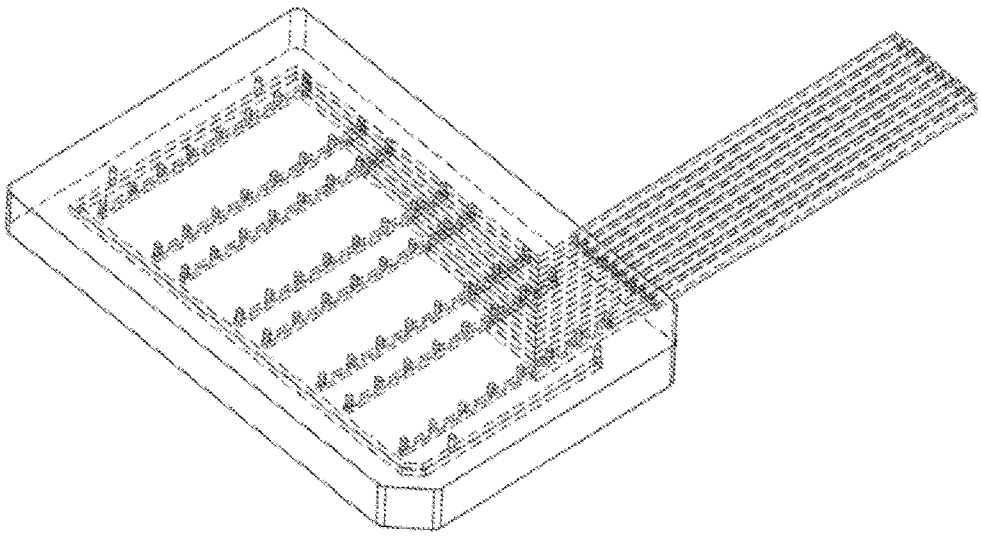

FIG. 14B

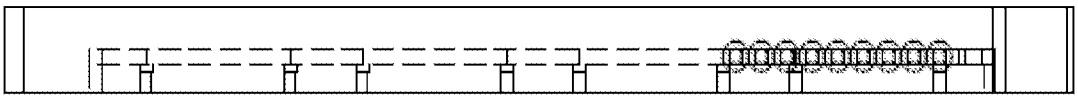

FIG. 14C

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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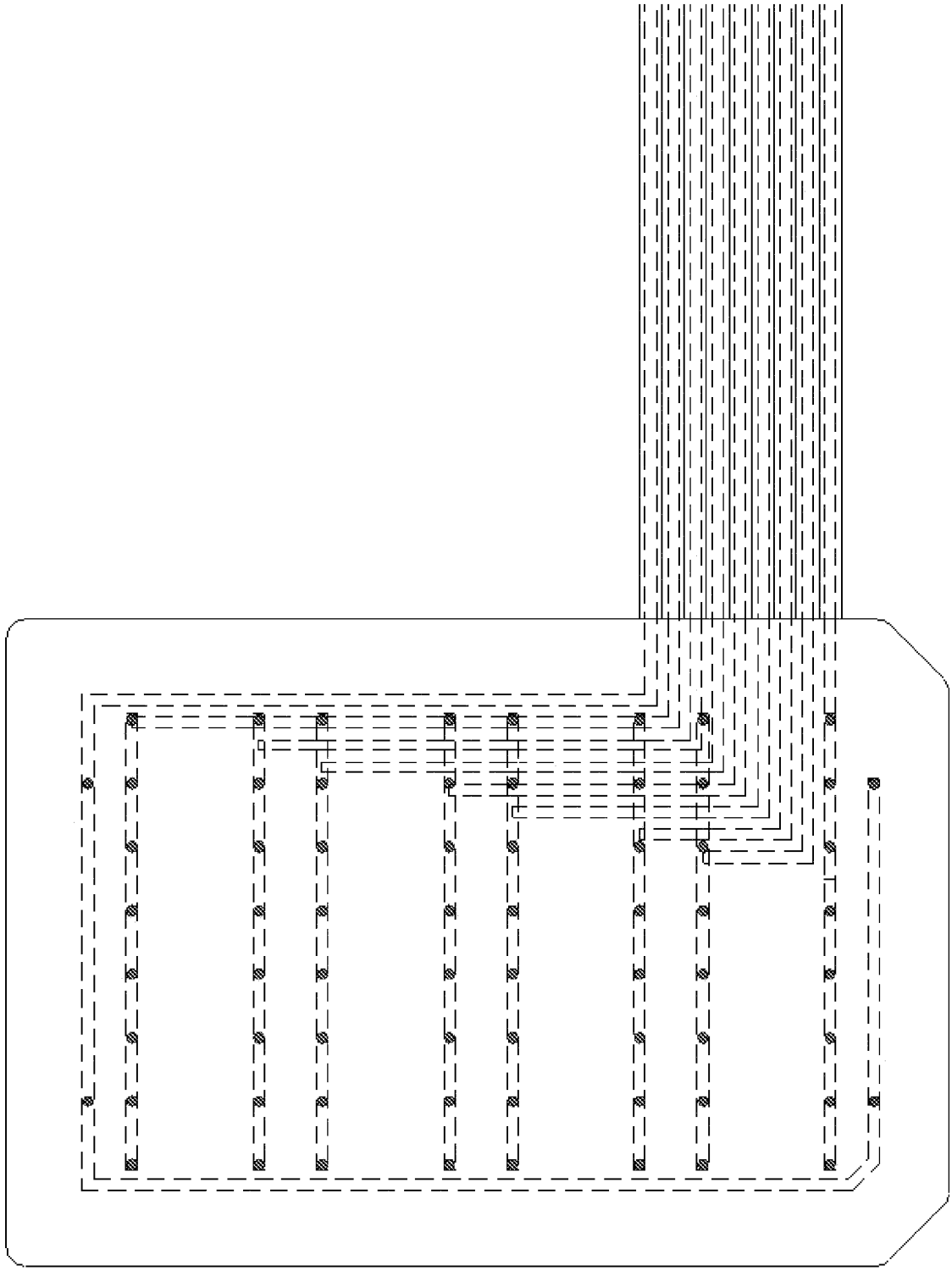

FIG. 14A

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

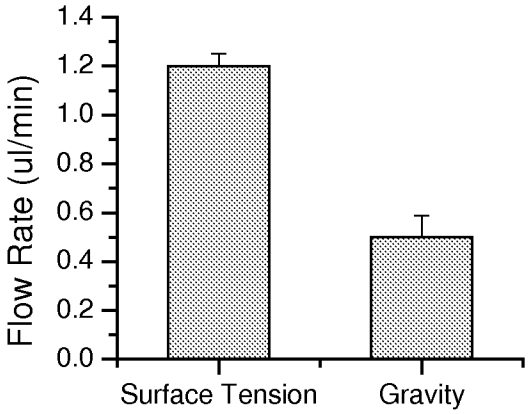

FIG. 15

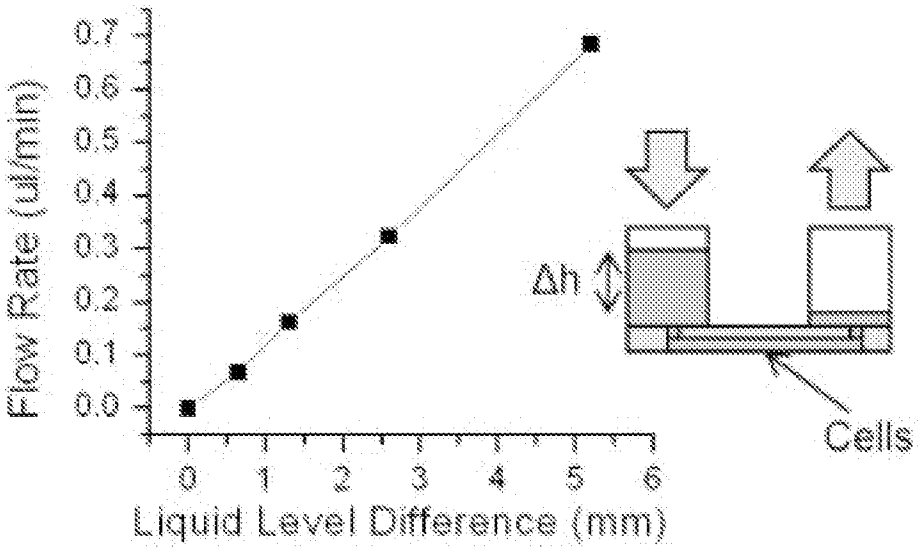

FIG. 16

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

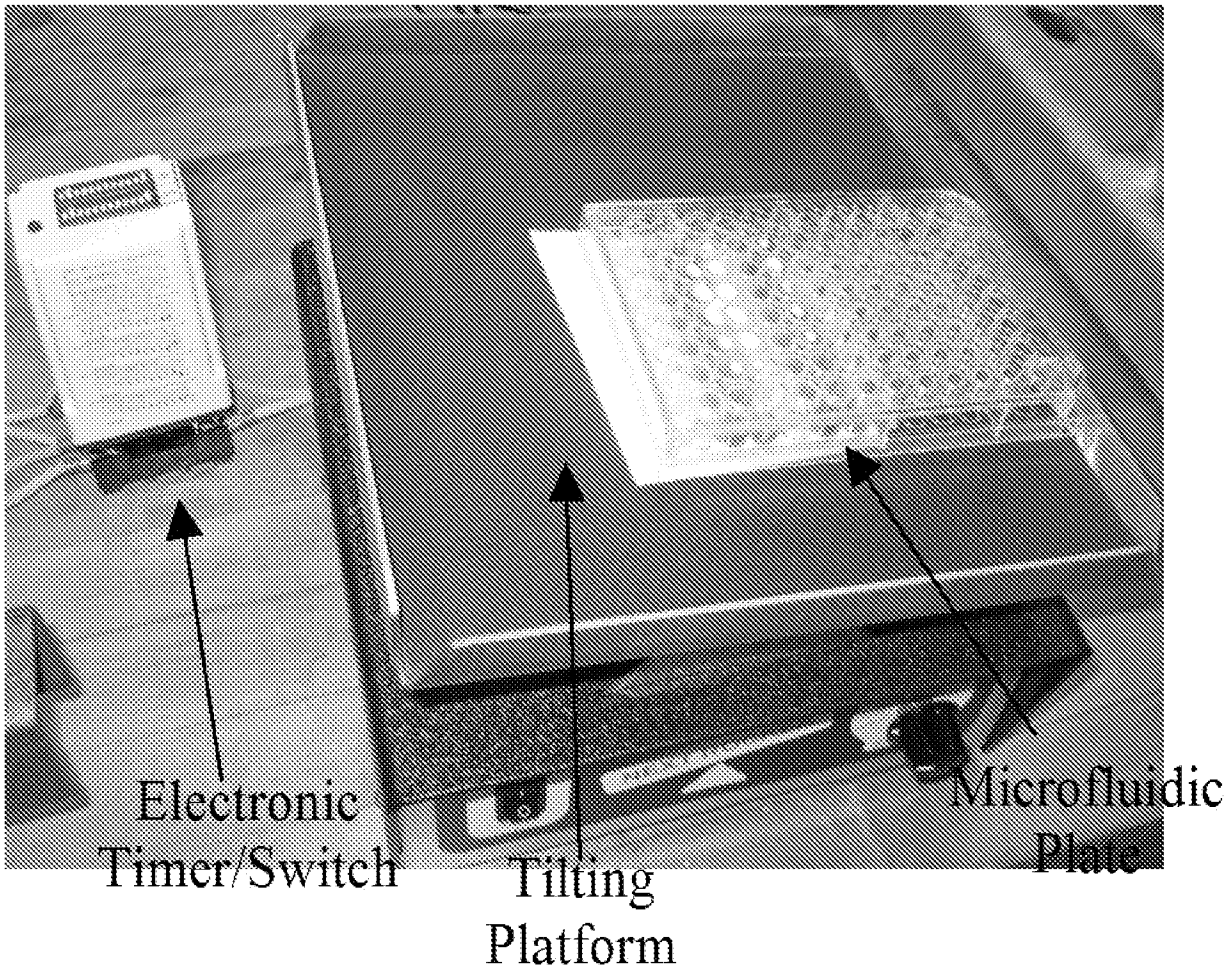

FIG. 17A

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

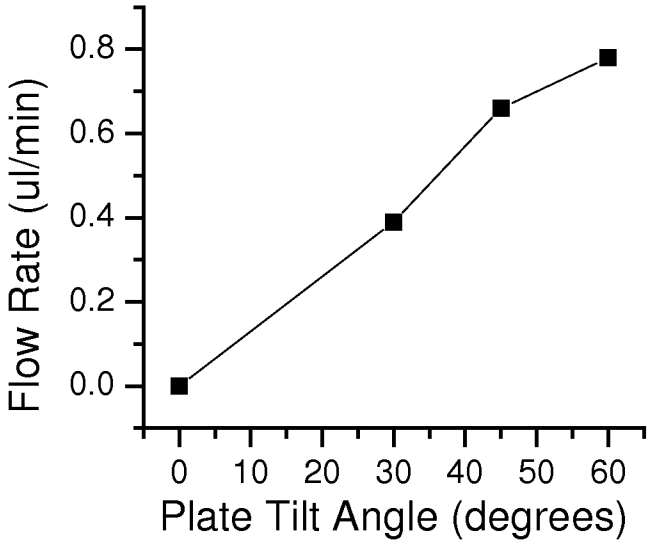

FIG. 17B

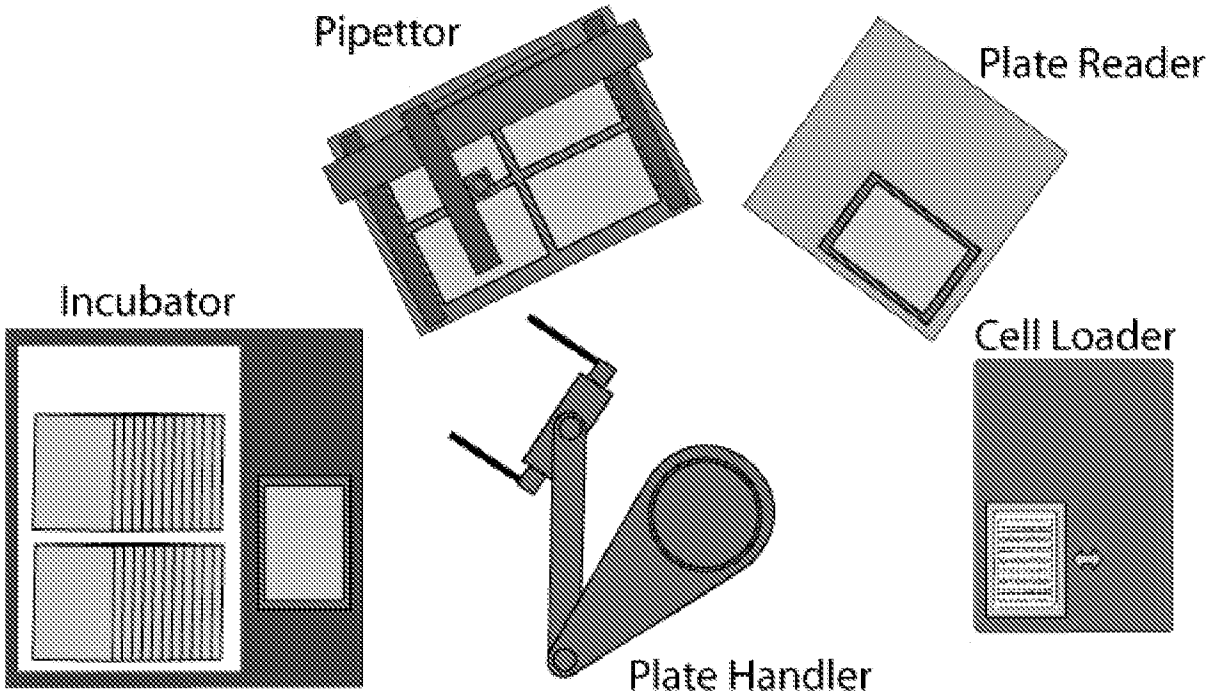

FIG. 18

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

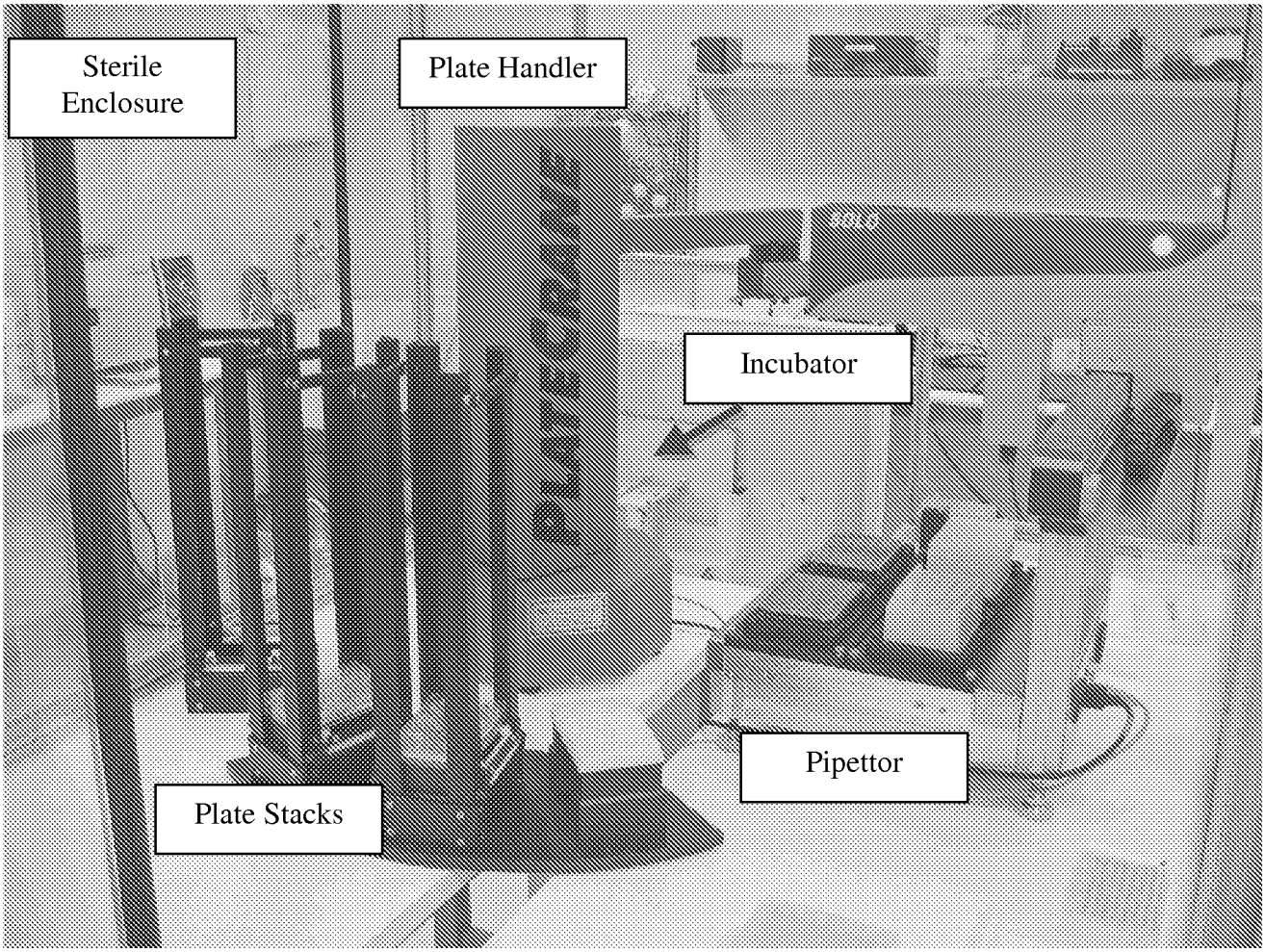

FIG. 19

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

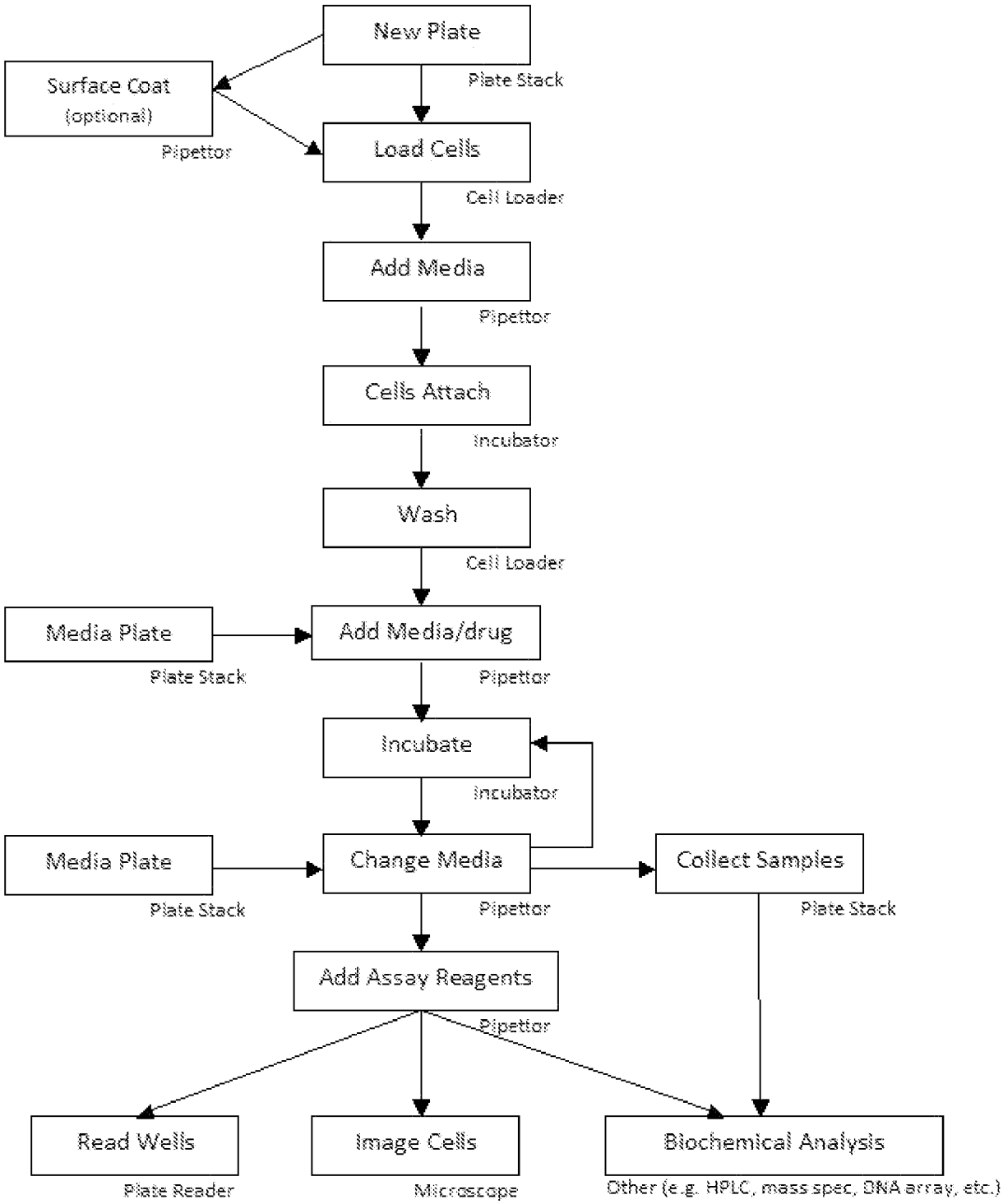
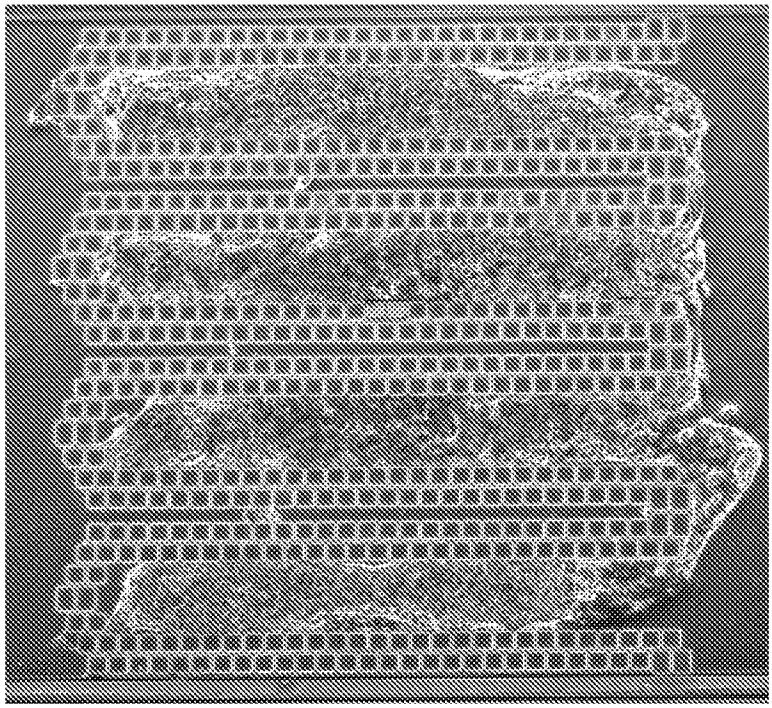


FIG. 20

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871



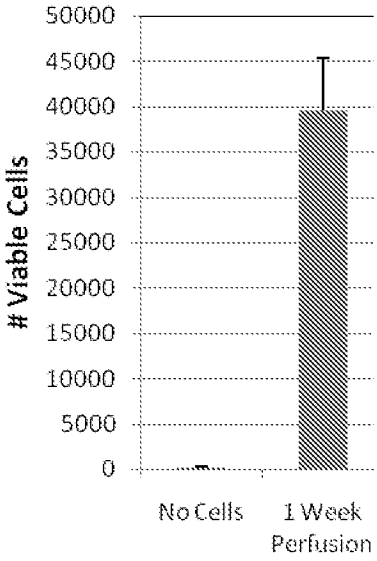


FIG. 21

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
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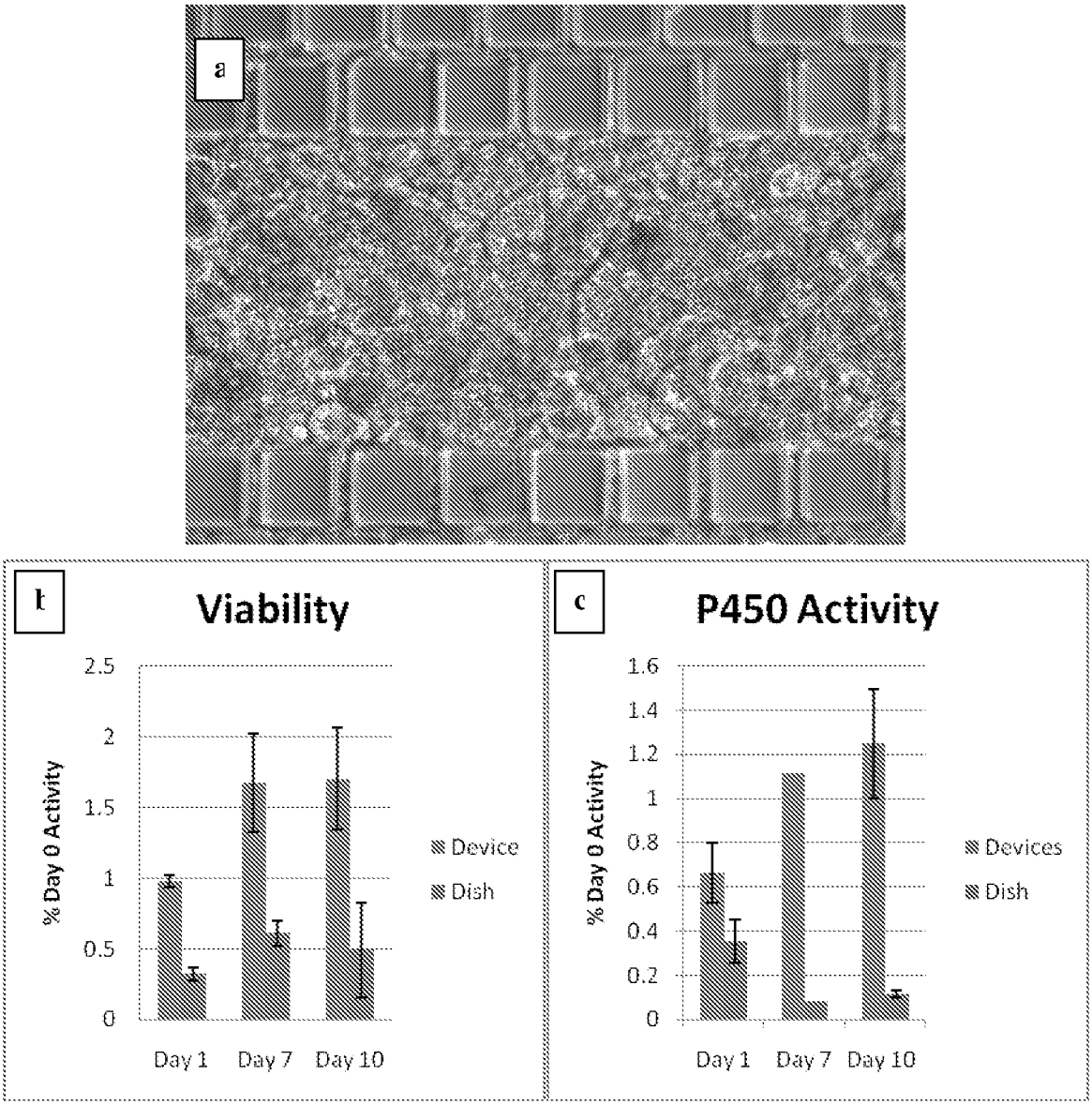

FIG. 22

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

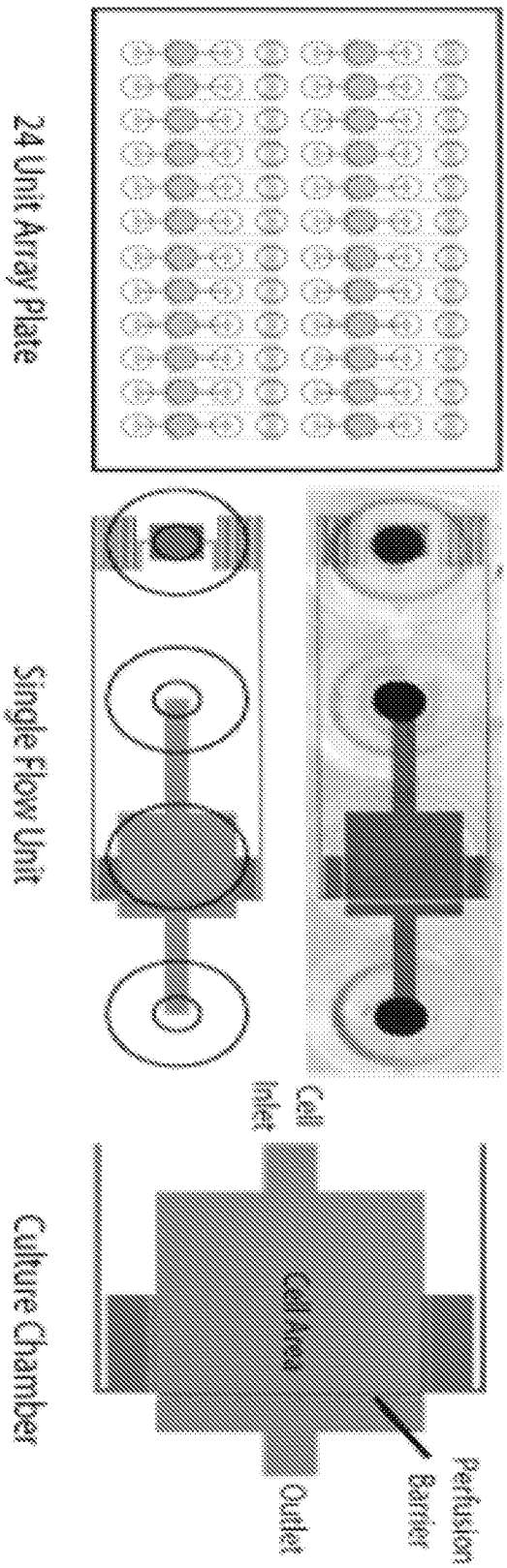

FIG. 23

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

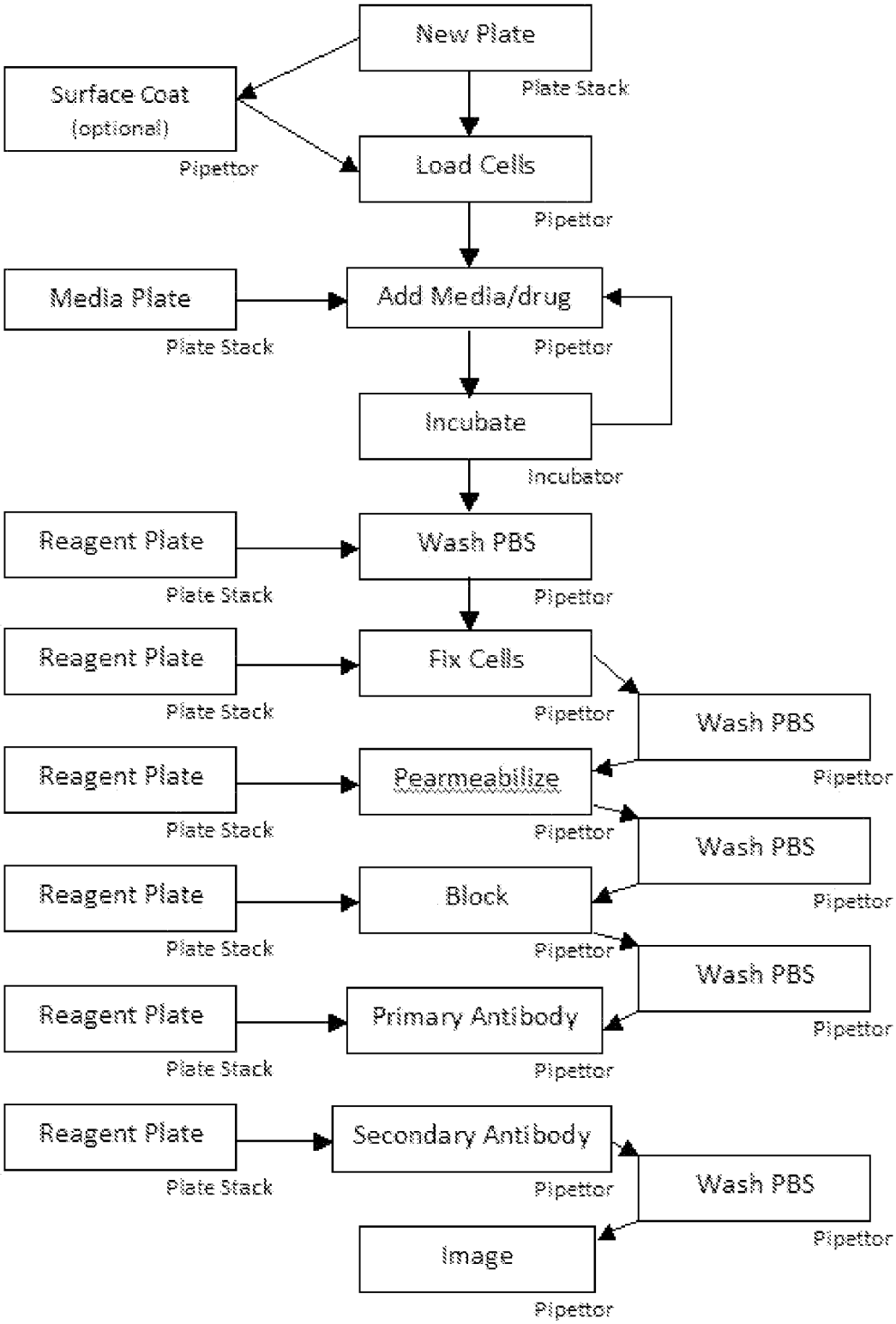

FIG. 24

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

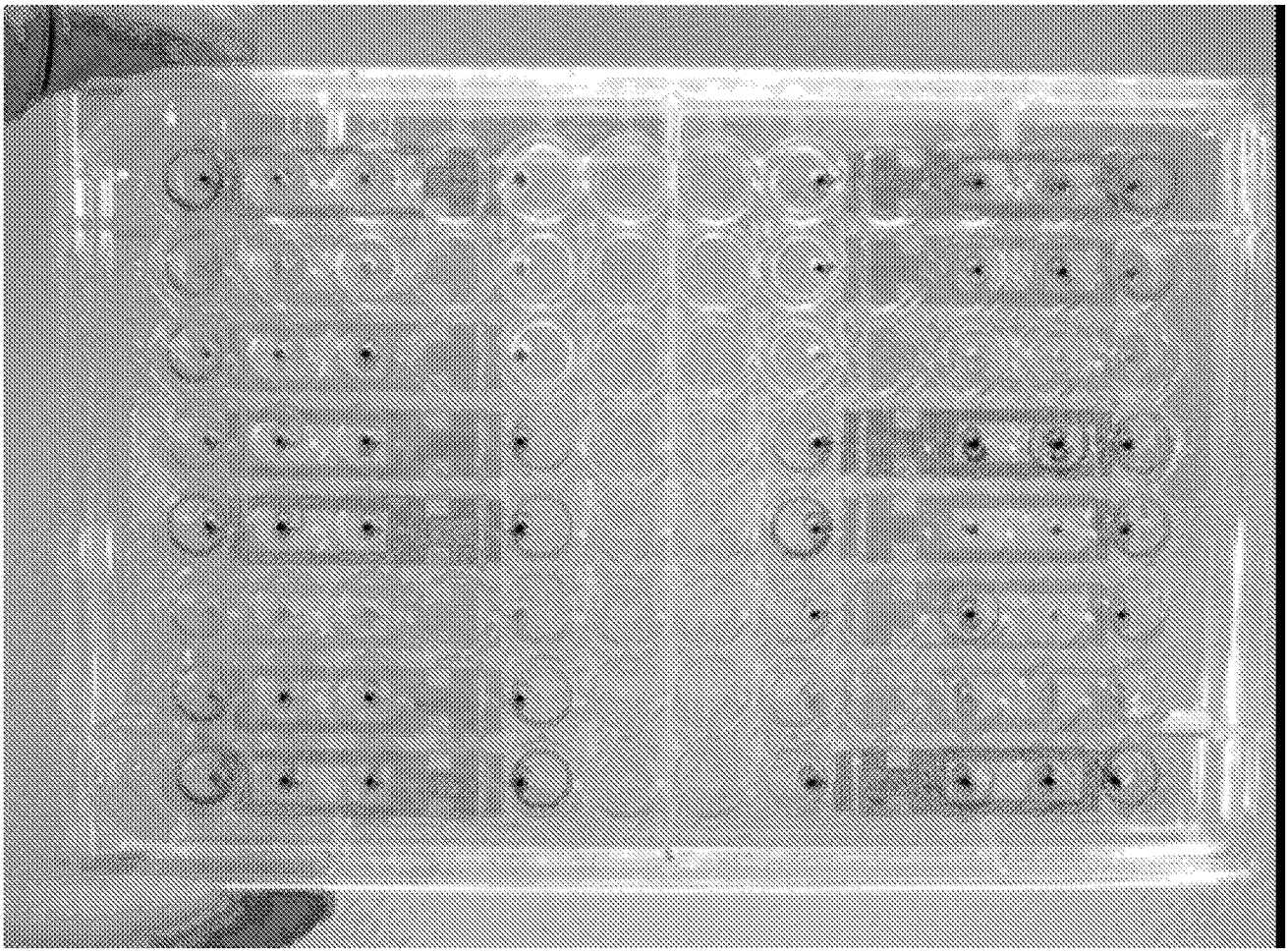

FIG. 25

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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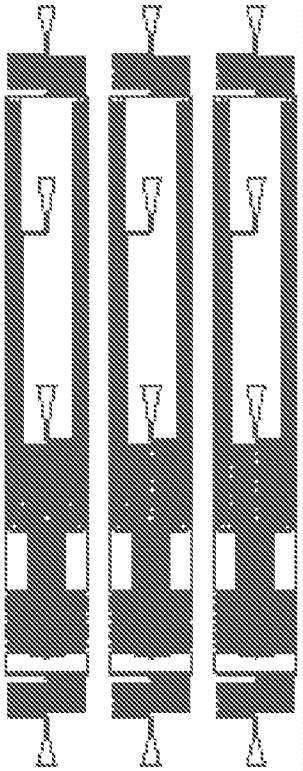
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871



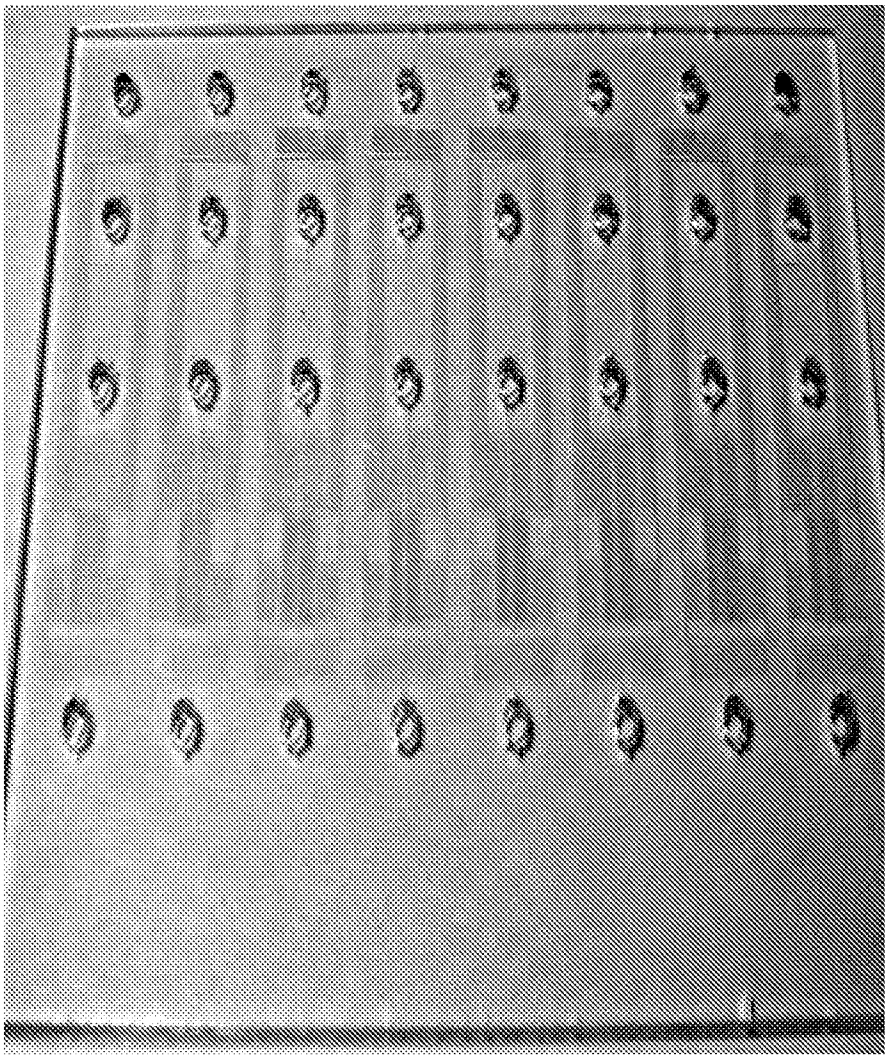


FIG. 26A ***FIG. 26B***

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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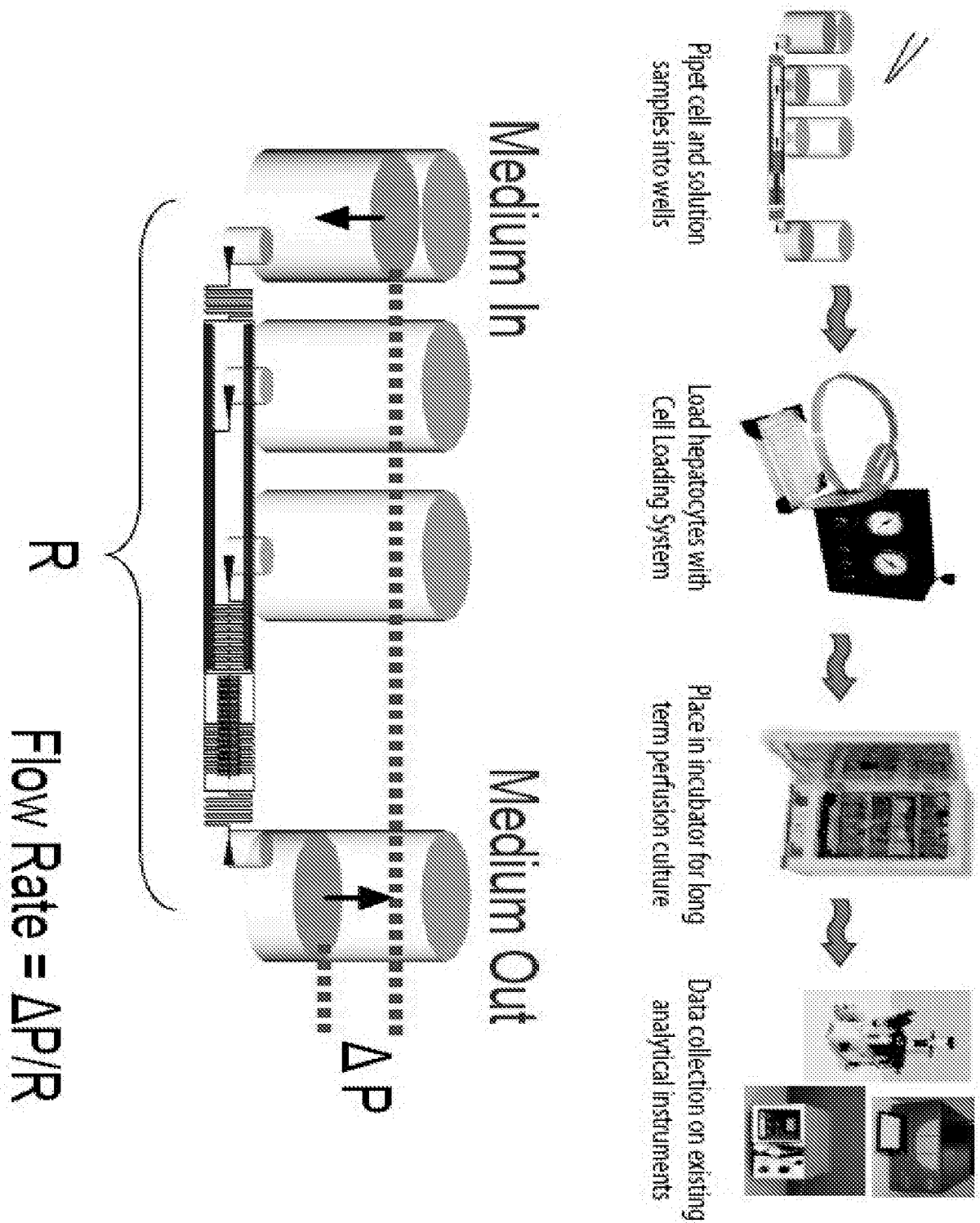

FIG. 27

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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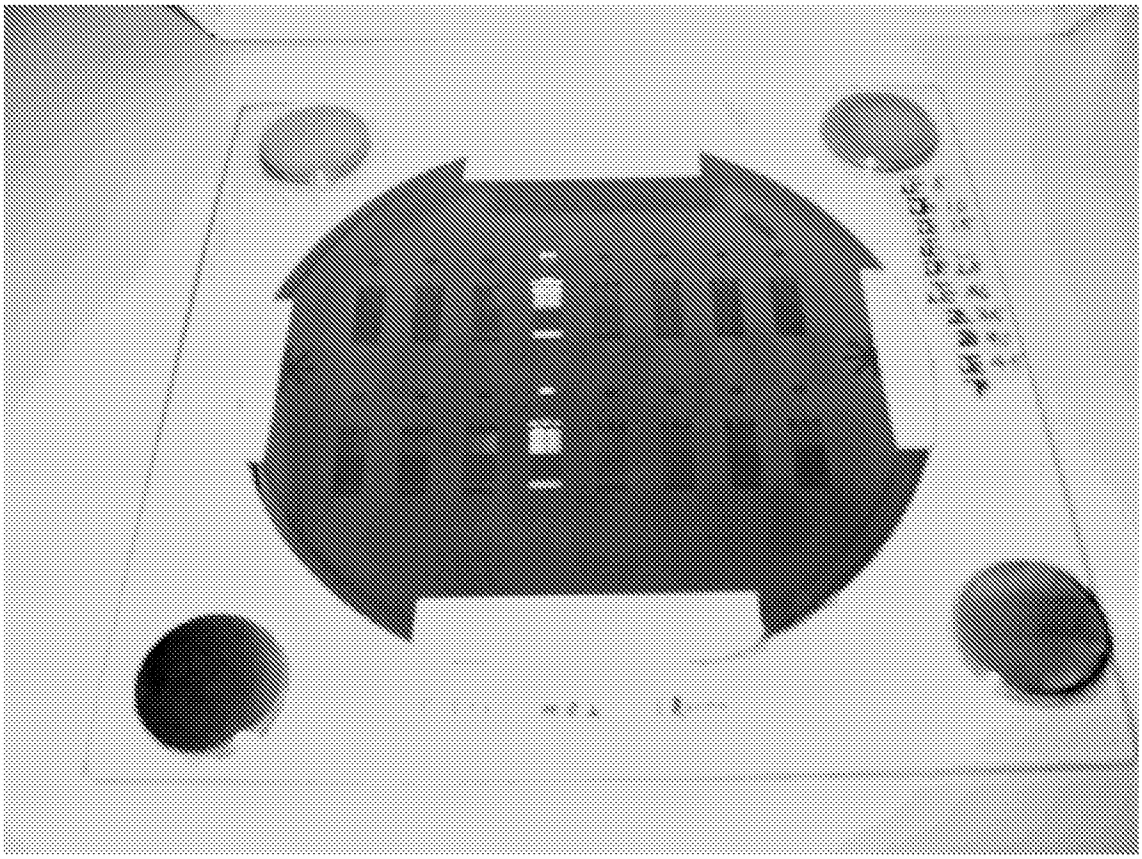

FIG. 28

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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FIG. 29

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

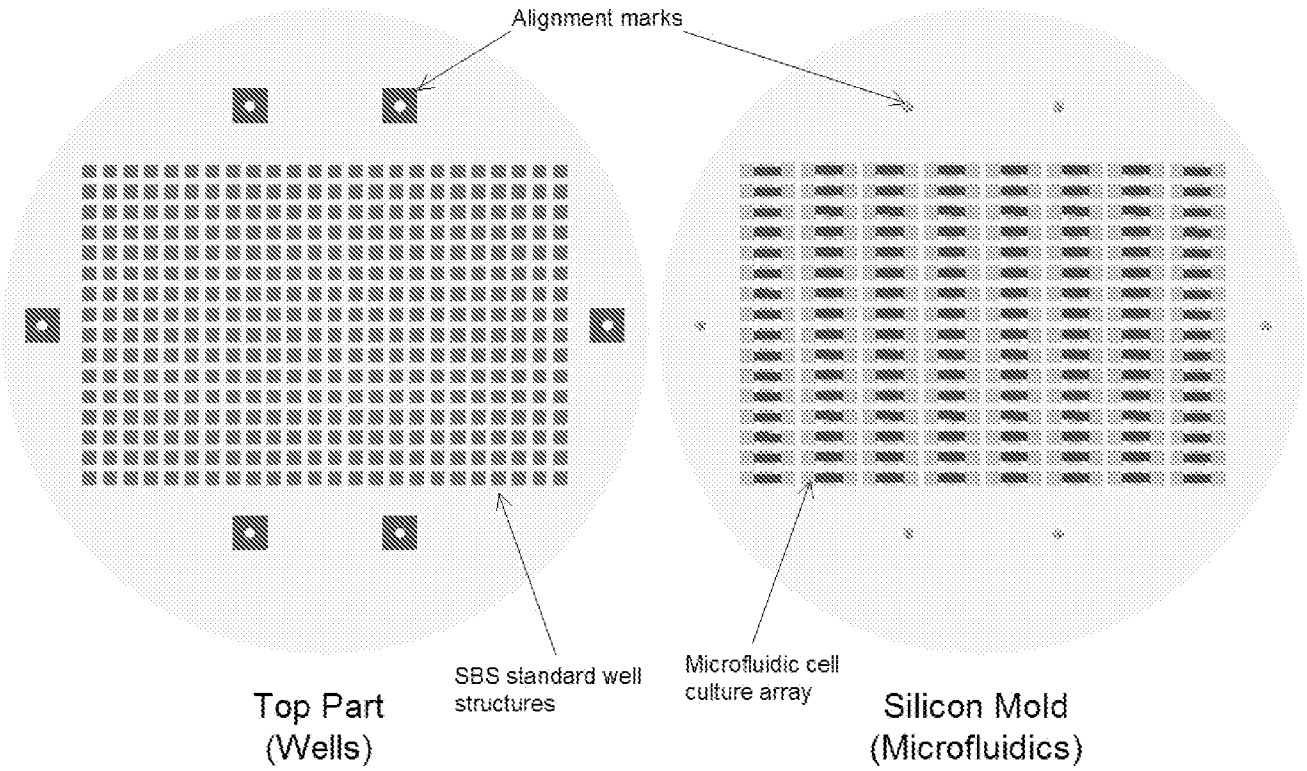

FIG. 30

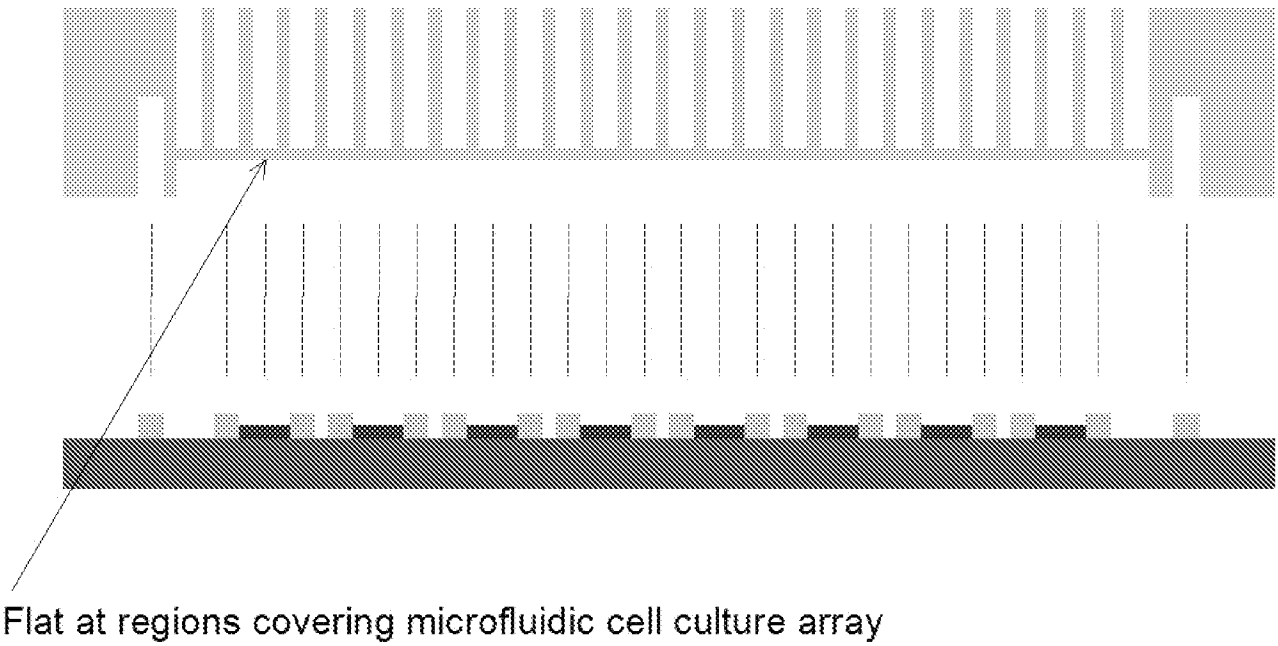

FIG. 31A

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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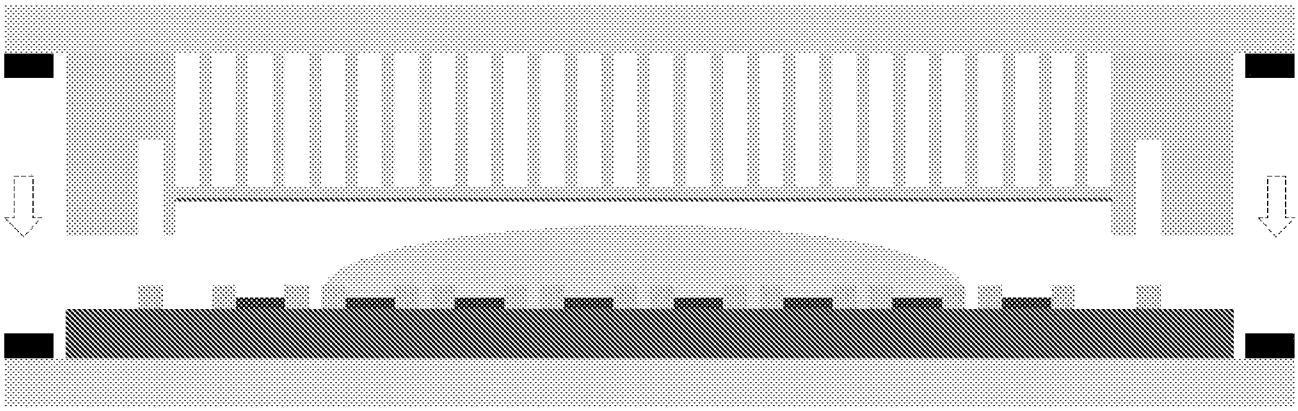

FIG. 31B

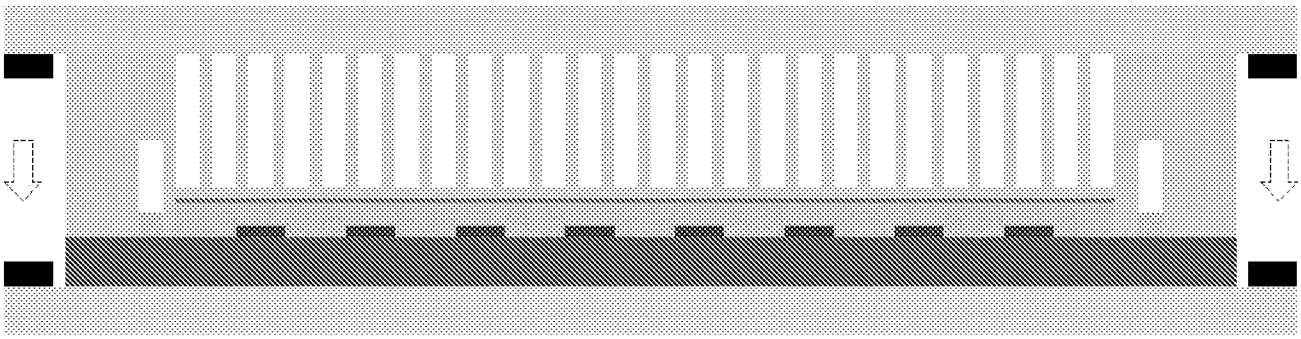

FIG. 31C

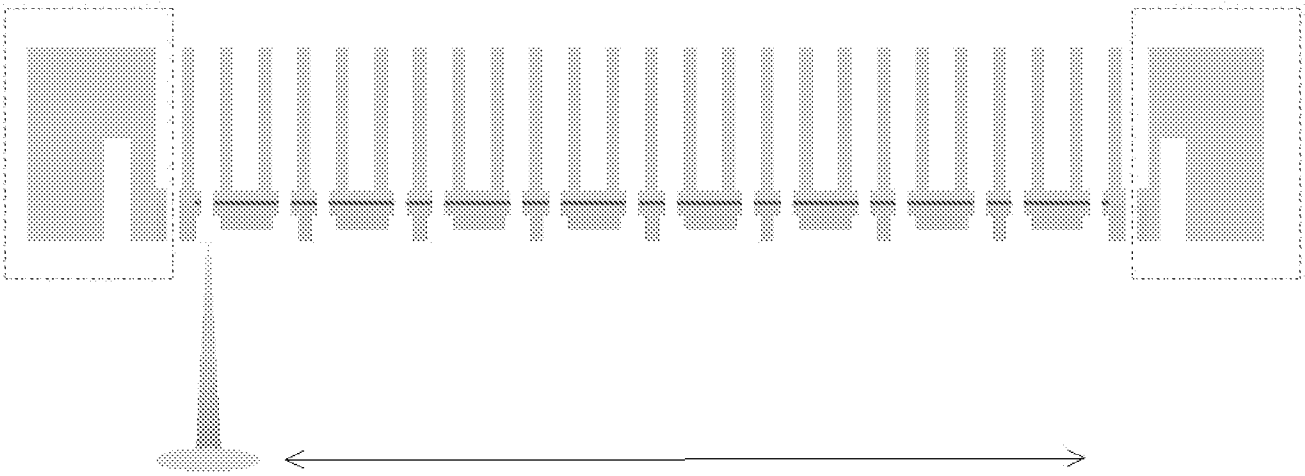

FIG. 31D

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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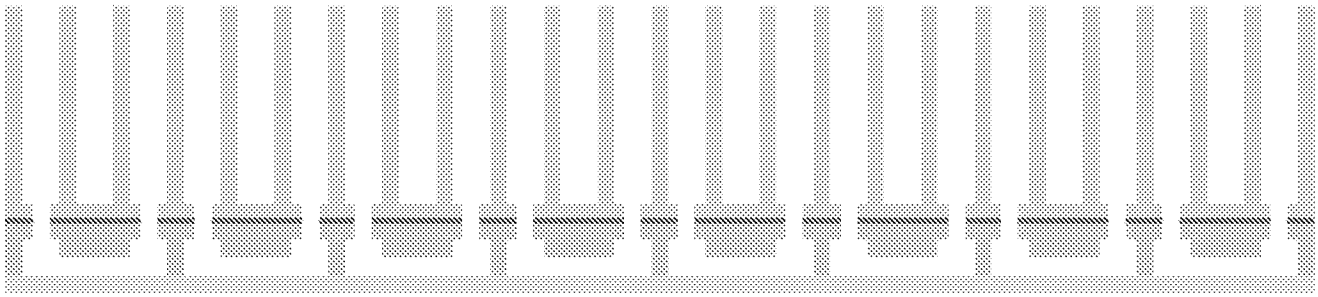

FIG. 31E

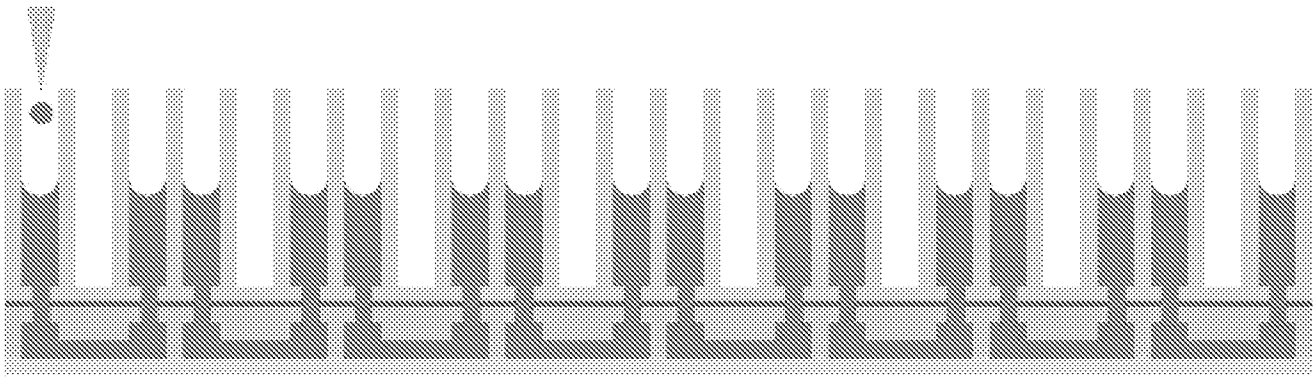

FIG. 31F

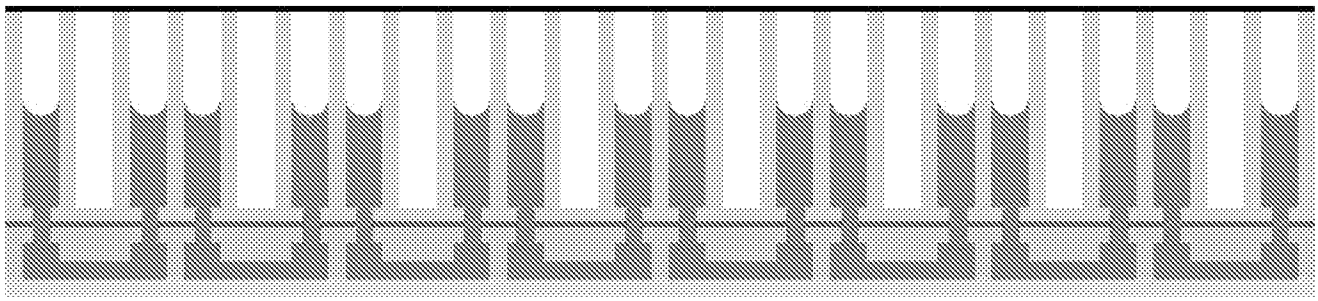

FIG. 31G

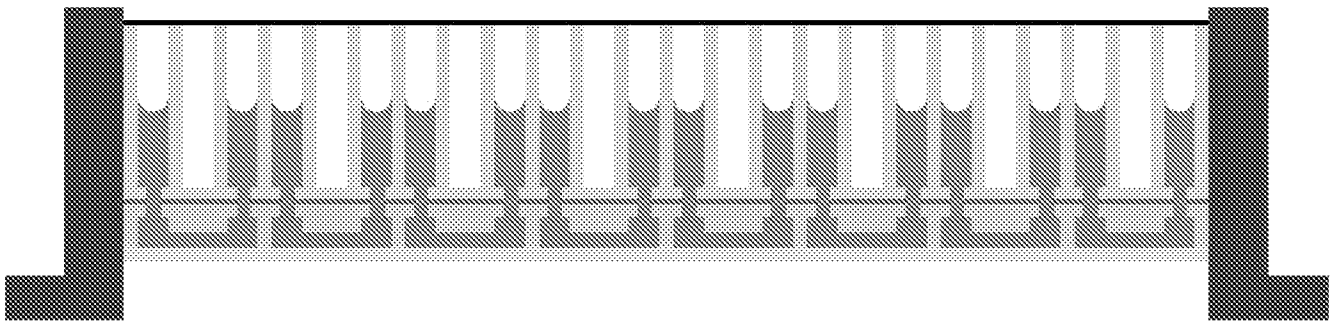

FIG. 31H

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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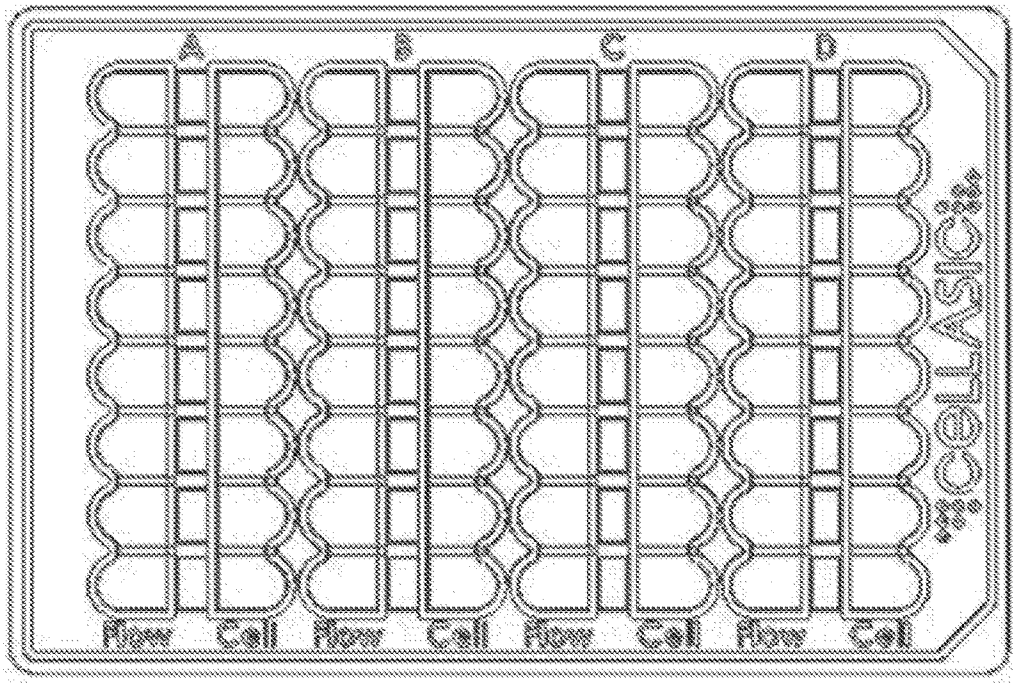

FIG. 32A

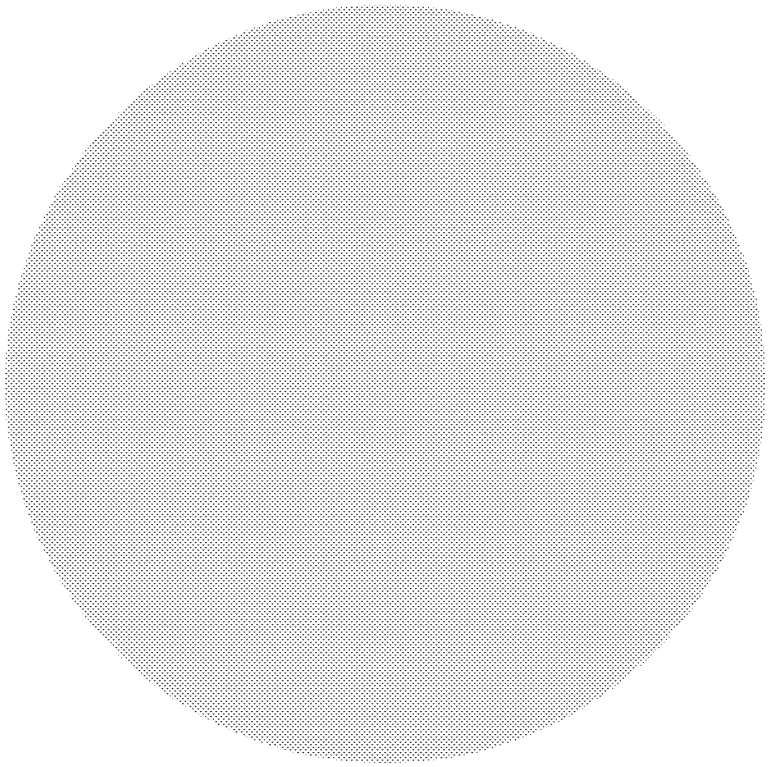

FIG. 32B

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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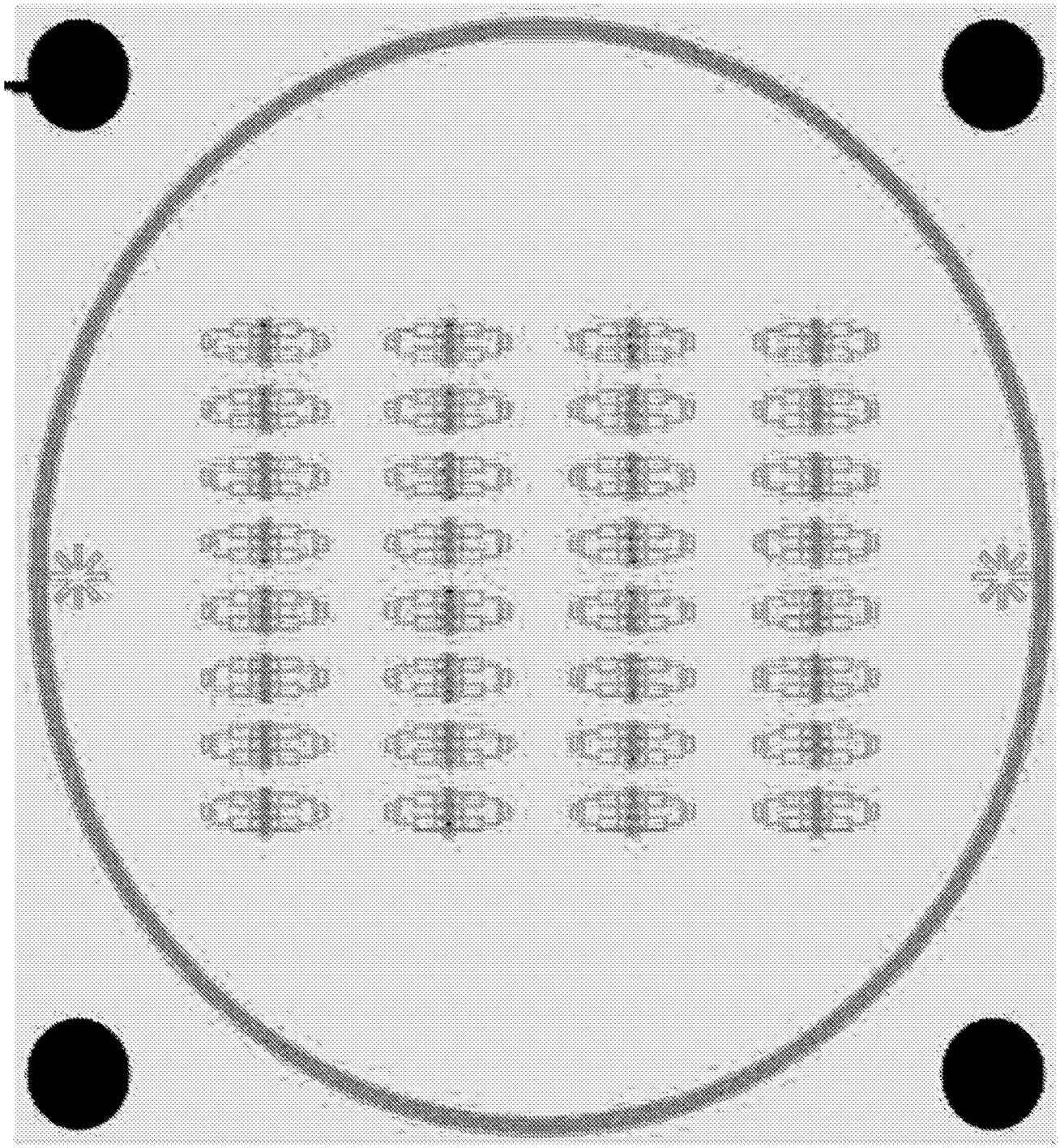

FIG. 32C

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

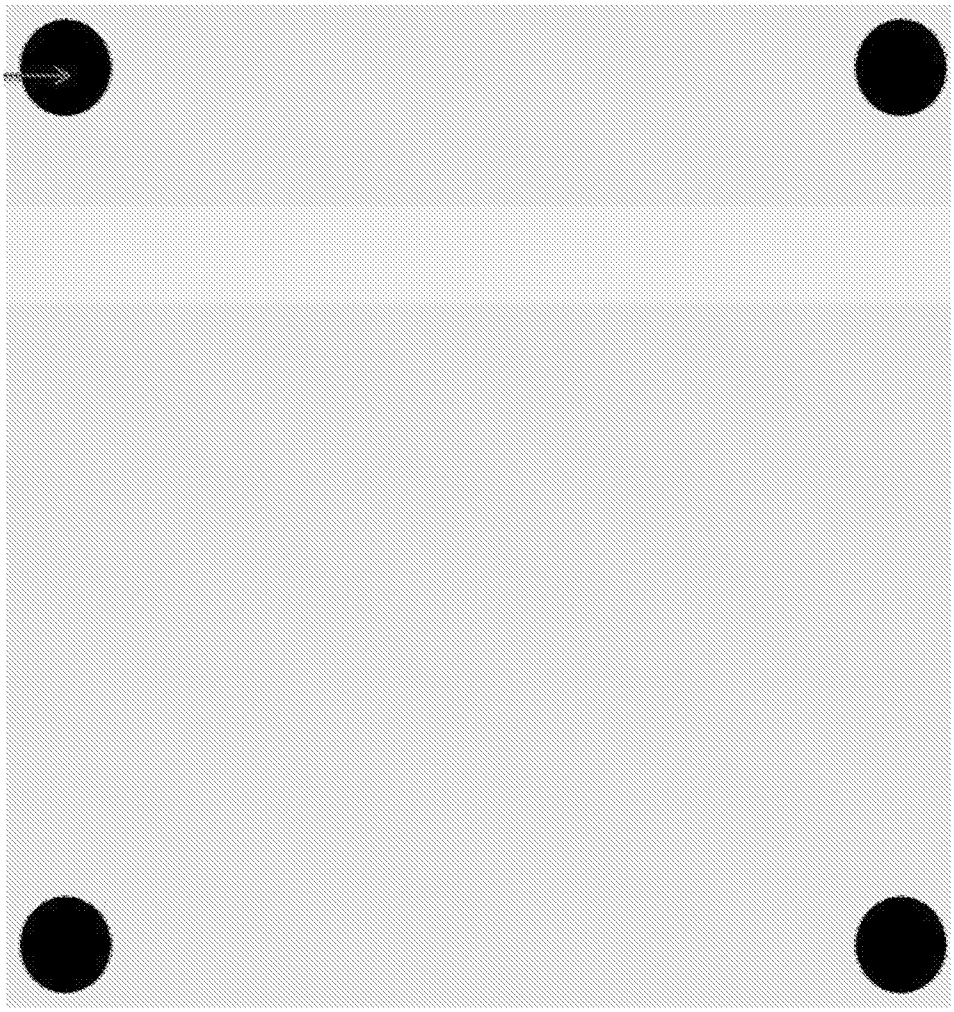

FIG. 32D

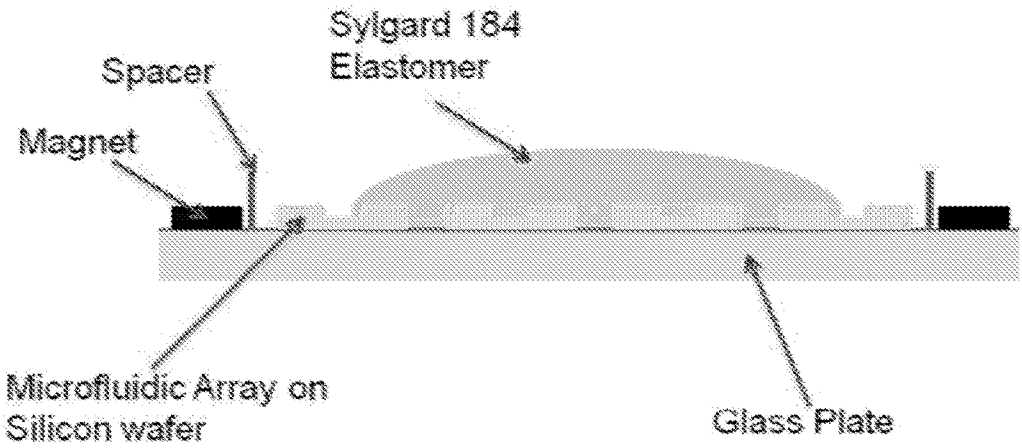

FIG. 33A

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

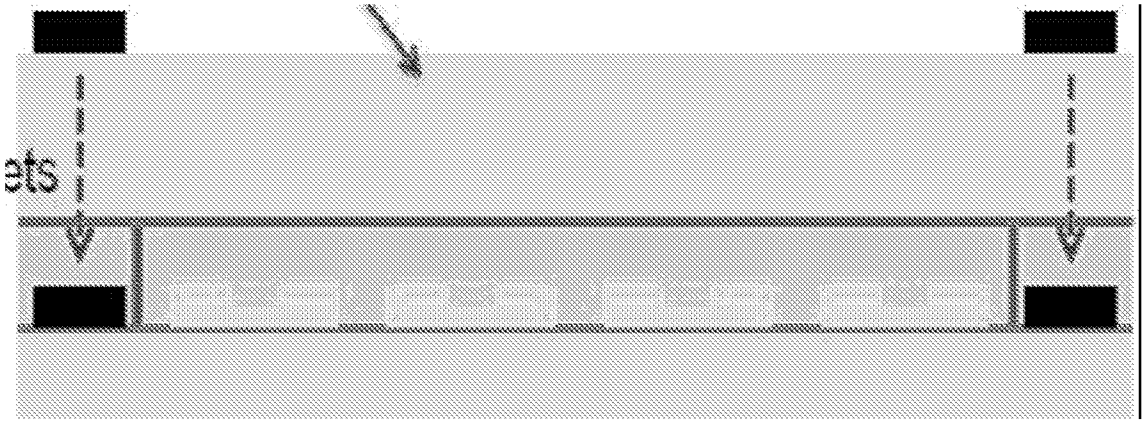

FIG. 33B

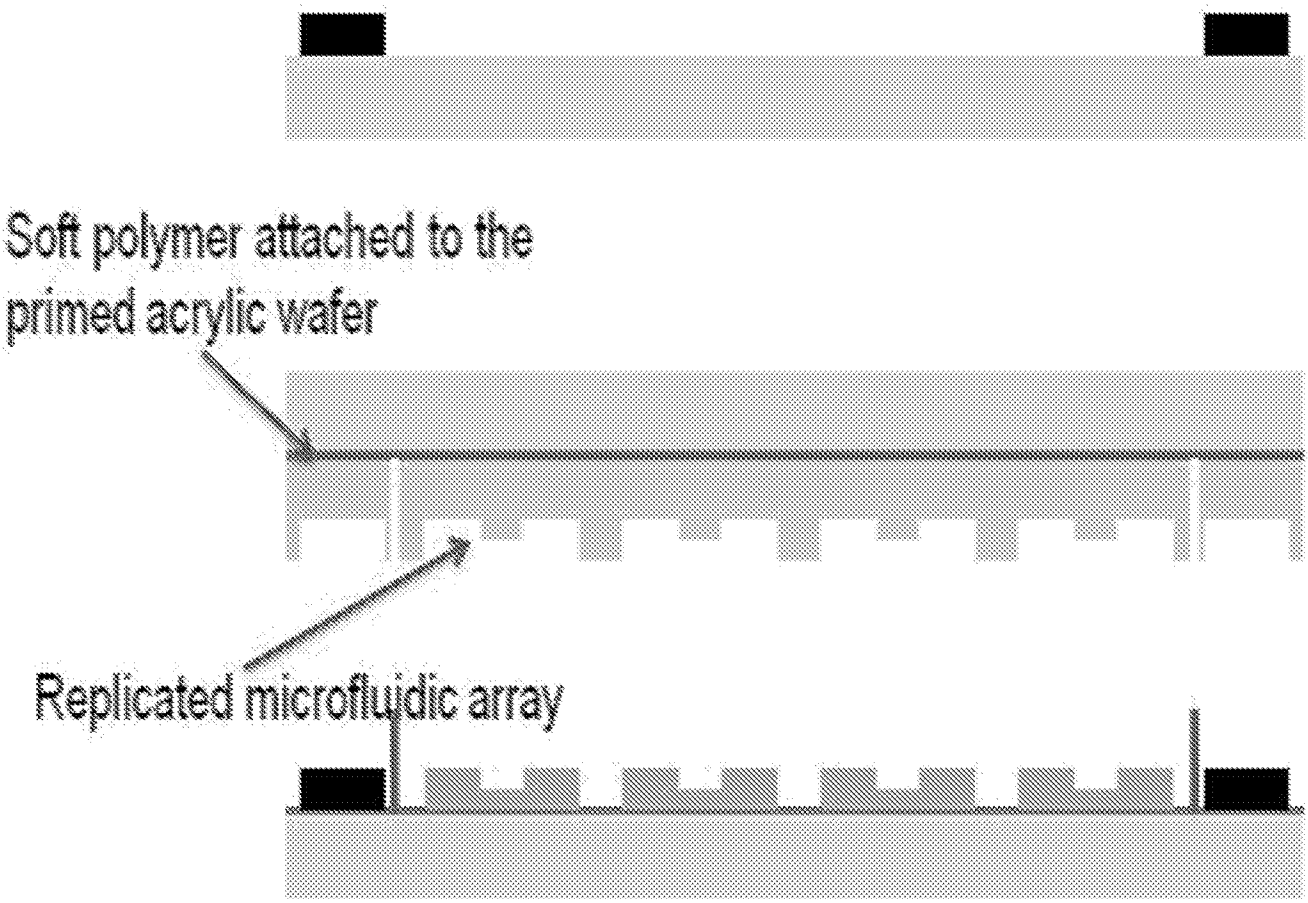

FIG. 33C

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

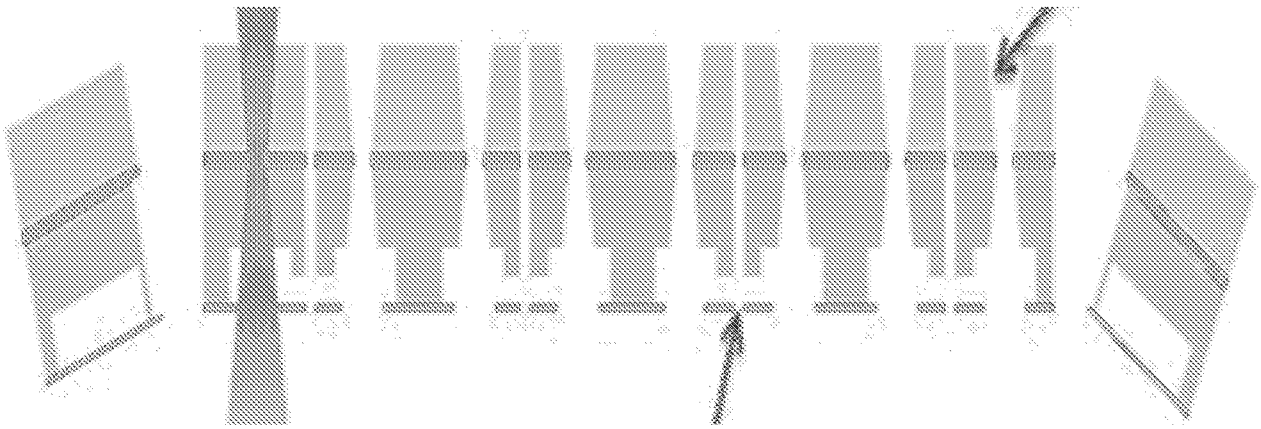

FIG. 33D

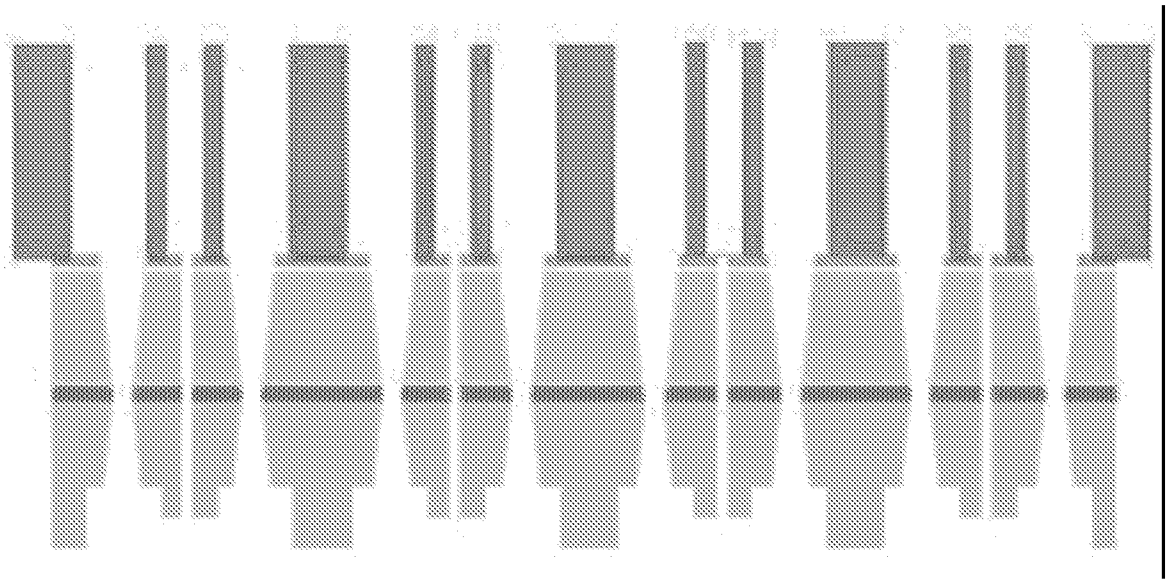

FIG. 33E

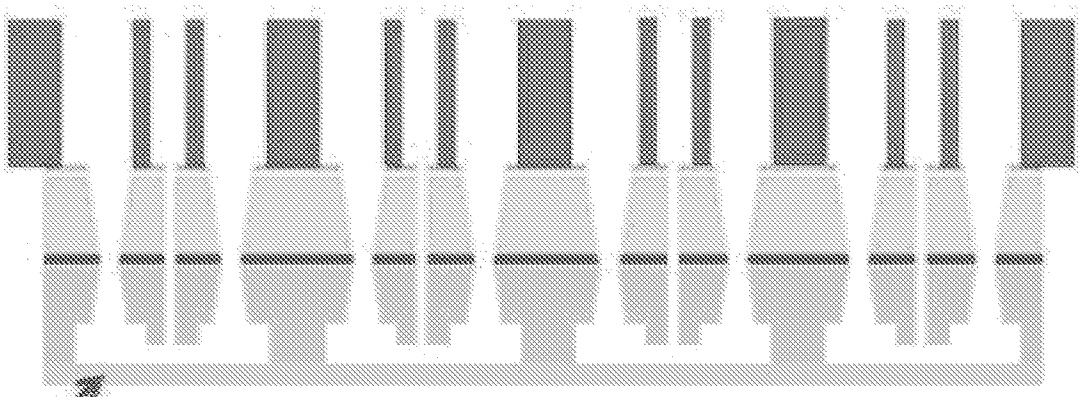

FIG. 33F

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

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QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

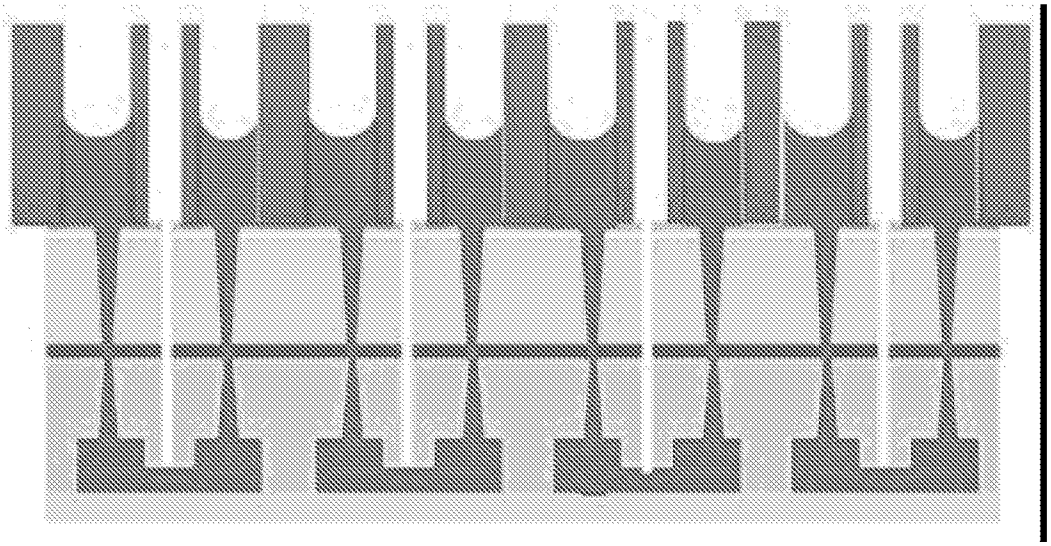

FIG. 33G

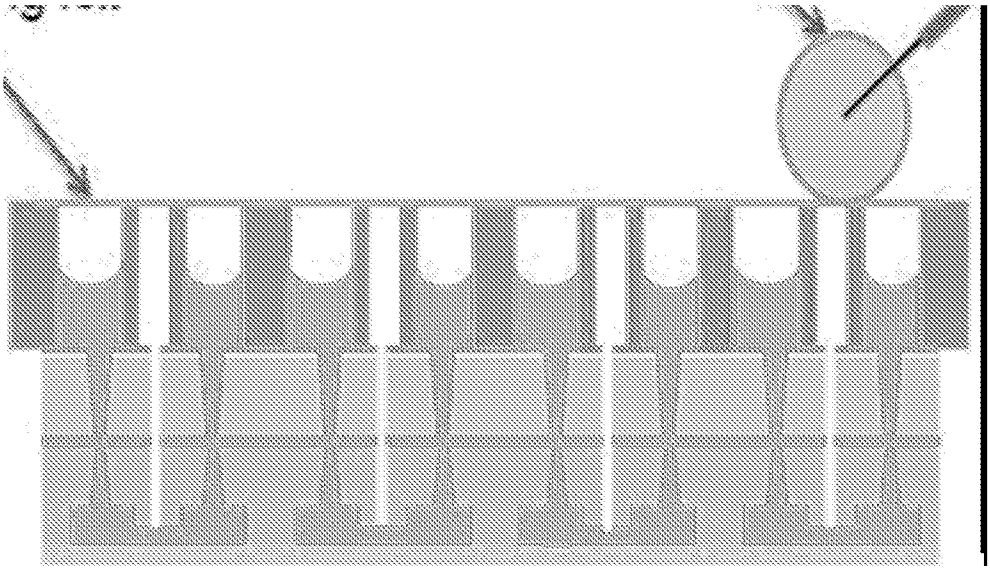

FIG. 33H

CELL CULTURE ARRAY SYSTEM FOR AUTOMATED ASSAYS AND METHODS OF OPERATION AND MANUFACTURE THEREOF

Paul J. Hung Filed 5-January-2009
QIPLG Attorney Docket No.: 549-000110PC; SJL Tel. No. 510-337-7871

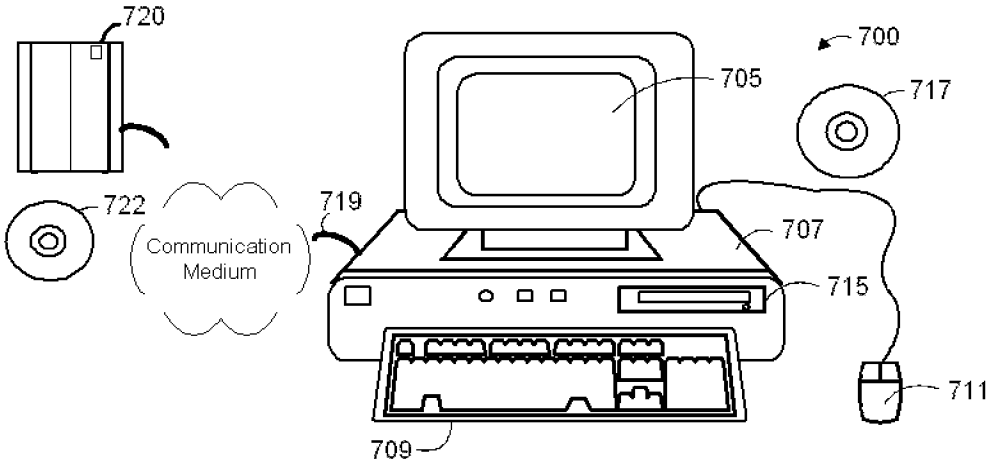

FIG. 34

Disease Classification	*Disease*
Cardiovascular Disease	Atherosclerosis; Unstable angina; Myocardial Infarction; Restenosis after angioplasty or other percutaneous intervention; Congestive Heart Failure; Myocarditis; Endocarditis; Endothelial Dysfunction; Cardiomyopathy
Endocrine Disease	Diabetes Mellitus I and II; Thyroiditis; Addisson's Disease
Infectious Disease	Hepatitis A, B, C, D, E; Malaria; Tuberculosis; HIV; Pneumocystis Carinii; Giardia; Toxoplasmosis; Lyme Disease; Rocky Mountain Spotted Fever; Cytomegalovirus; Epstein Barr Virus; Herpes Simplex Virus; Clostridium Dificile Colitis; Meningitis (all organisms); Pneumonia (all organisms); Urinary Tract Infection (all organisms); Infectious Diarrhea (all organisms)
Angiogenesis	Pathologic angiogenesis; Physiologic angiogenesis; Treatment induced angiogenesis
Inflammatory/Rheumatic Disease	Rheumatoid Arthritis; Systemic Lupus Erythematosis; Sjogrens Disease; CREST syndrome; Scleroderma; Ankylosing Spondylitis; Crohn's; Ulcerative Colitis; Primary Sclerosing Cholangitis; Appendicitis; Diverticulitis; Primary Biliary Sclerosis; Wegener's Granulomatosis; Polyarteritis nodosa; Whipple's Disease; Psoriasis; Microscopic Polyanngiitis; Takayasu's Disease; Kawasaki's Disease; Autoimmune hepatitis; Asthma; Churg-Strauss Disease; Beurger's Disease; Raynaud's Disease; Cholecystitis; Sarcoidosis; Asbestosis; Pneumoconioses
Transplant Rejection	Heart; Lung; Liver; Pancreas; Bowel; Bone Marrow; Stem Cell; Graft versus host disease; Transplant vasculopathy
Leukemia and Lymphoma	

FIG. 35. (TABLE 1)