US008945486B2

(12) **United States Patent**
Warrick et al.

(10) **Patent No.: US 8,945,486 B2**
(45) **Date of Patent: Feb. 3, 2015**

(54) **MICROWELL DEVICE**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/790,917**

(22) Filed: **Mar. 8, 2013**

(65) **Prior Publication Data**

US 2014/0255276 A1 Sep. 11, 2014

(51) **Int. Cl.**
G01N 31/16 (2006.01)
B01L 3/00 (2006.01)

(52) **U.S. Cl.**
CPC ***B01L 3/5085*** (2013.01); *Y10S 435/809* (2013.01)
USPC **422/552**; 422/400; 422/401; 422/407; 422/412; 422/500; 422/547; 422/551; 422/553; 422/559; 422/560; 422/561; 422/942; 422/946; 422/948; 435/3; 435/285.1; 435/286.1; 435/286.2; 435/287.2; 435/288.1; 435/288.4; 435/288.7; 435/303.1; 435/34; 435/374; 435/809; 435/6.14; 435/7.1; 435/91.2

(58) **Field of Classification Search**
USPC 422/407, 408, 551, 552, 553, 400, 401, 422/412, 500, 547, 559, 560, 561, 942, 946, 422/948; 435/3, 285.1, 286.1, 286.2, 287.2, 435/288.1, 288.4, 288.7, 303.1, 34, 374, 6, 435/6.14, 7.1, 91.2, 809; 436/809
See application file for complete search history.

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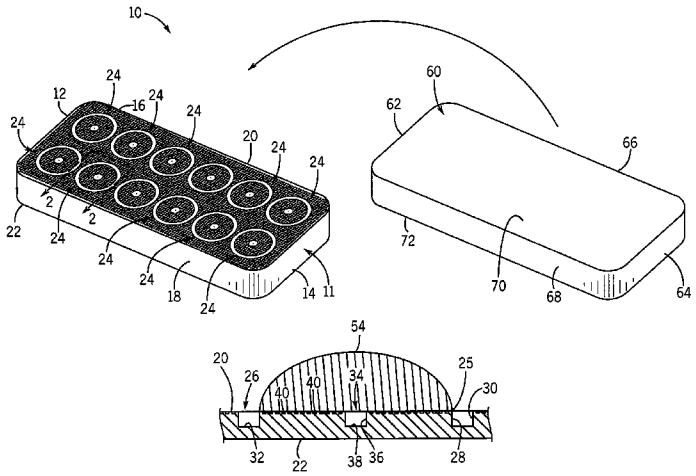
(57) **ABSTRACT**

A microwell device is provided. The device includes a plate having a upper surface. The upper surface has first and second recesses formed therein. Each recess has an outer periphery. First and second portions of microwells are formed in upper surface of the plate. The first portion of microwells are spaced about the outer periphery of the first recess and the second portion of microwells spaced about the outer periphery of the first recess. A first barrier is about a first portions of the microwells for fluidicly isolating the first portion of the microwells and a second barrier about a second portions of microwells for fluidicly isolating the second portion of the microwells.

14 Claims, 3 Drawing Sheets



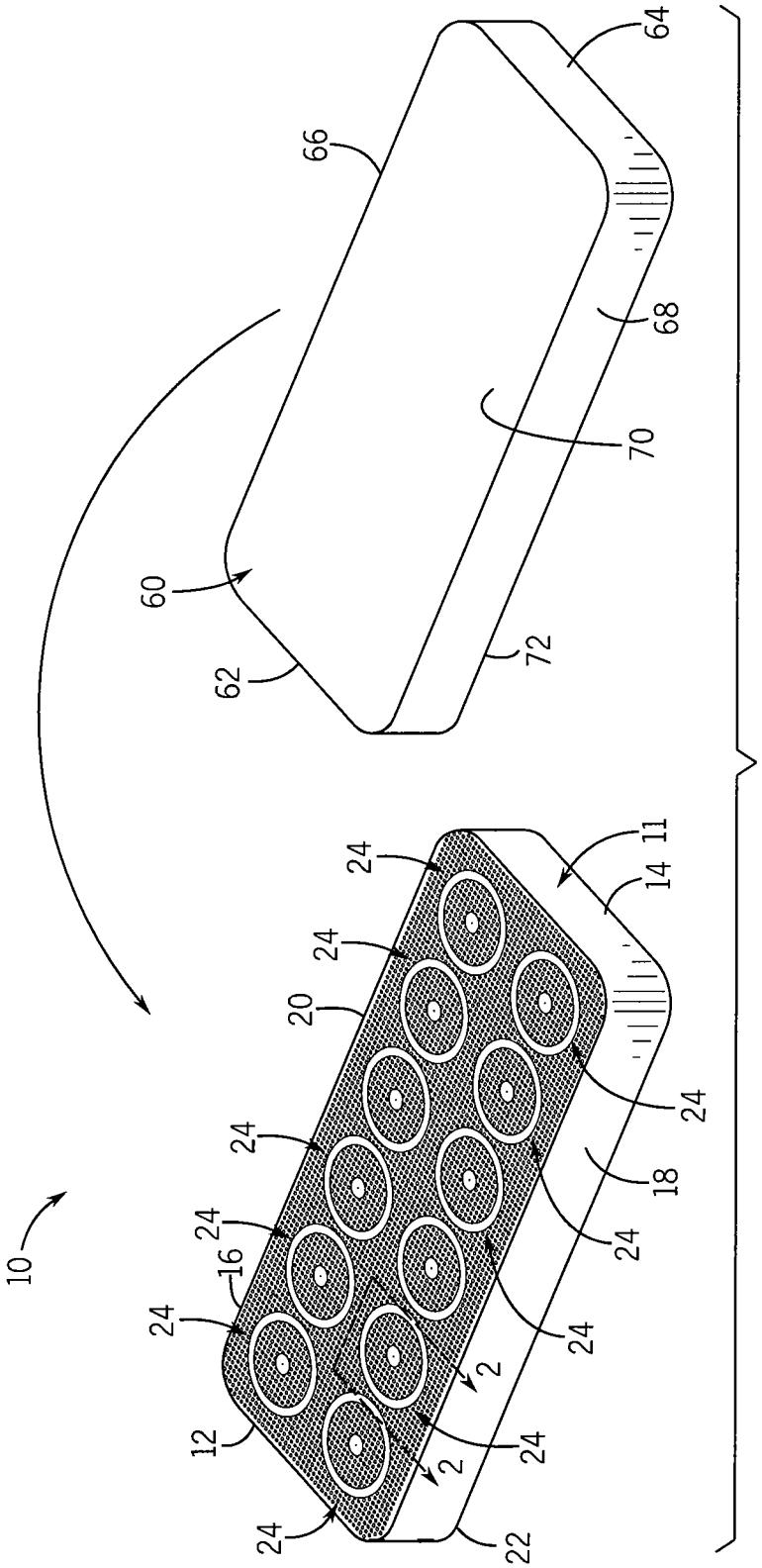


FIG. 1

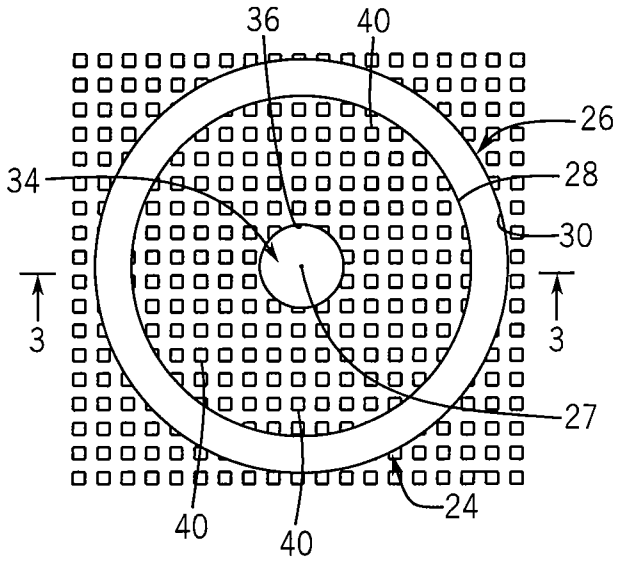


FIG. 2

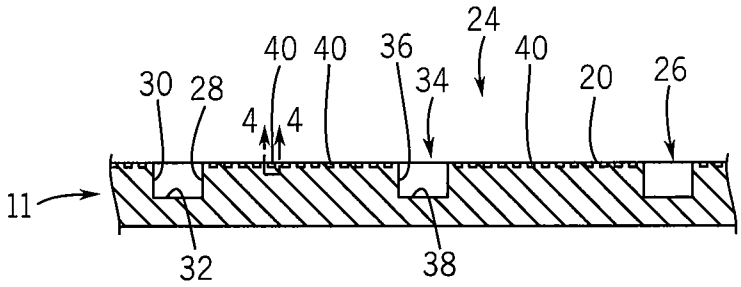


FIG. 3

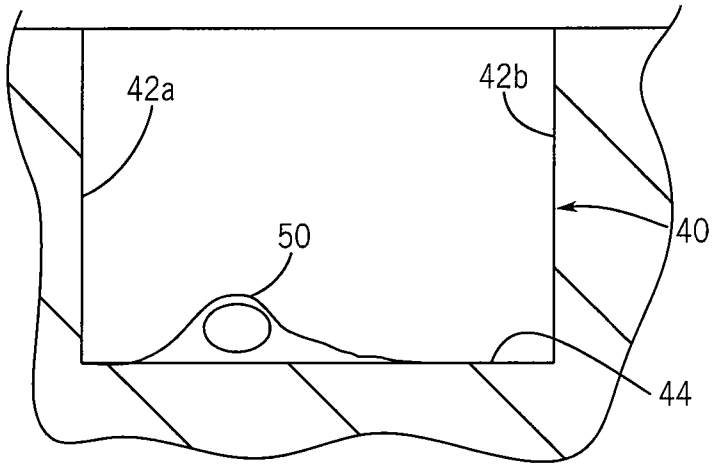


FIG. 4

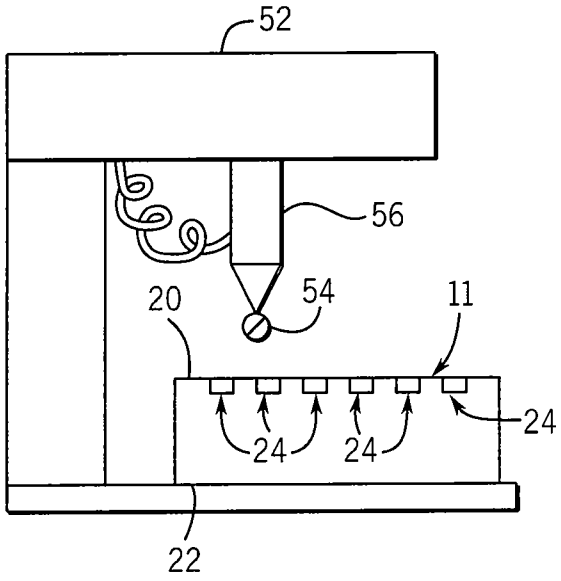


FIG. 5

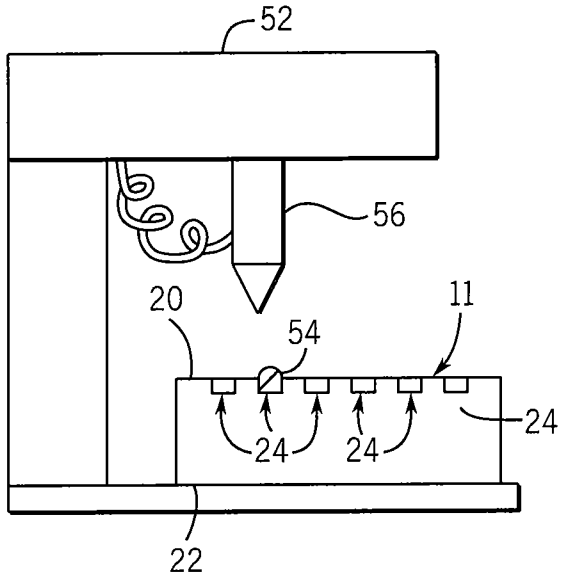


FIG. 6

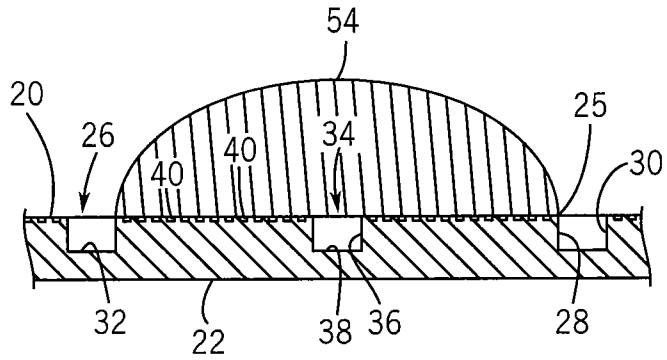


FIG. 7

MICROWELL DEVICE

REFERENCE TO GOVERNMENT GRANT

This invention was made with government support under RR023167 and AI091646 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

This invention relates generally to microfluidic devices, and in particular, to a microwell device for isolating a fluid, such as an analyte, into very small volumes.

BACKGROUND AND SUMMARY OF THE INVENTION

Techniques for studying single cells have become indispensable in cell biology for their ability to identify characteristics and behaviors that would otherwise be hidden using population averaged measures. As single-cell techniques continue to develop, these techniques have the potential to significantly impact many different areas of study. For example, the study of virus infections and virus-host interactions are particularly well-suited for such techniques. Virus infections are generally rapid and dynamic events that exhibit high levels of heterogeneity stemming from multiple sources. Thus, at any given time during an infection, different cells can respond with phenotypically different behavior and progress at different times and rates, making it difficult to use average readouts to make inferences concerning the sequence or timing of infection events or for relating changes in one biological measure to another.

The most basic advantage of single cell data for addressing these challenges is the ability to categorize a heterogenous group of individual cells into cohorts or subpopulations with similar individual characteristics to explore the potential relationship of those characteristics to heterogenous outcomes. In other words, the heterogeneous system behavior can be leveraged to learn more about important cellular characteristics. The most prominent example of this is the use of flow cytometry, where multiple fluorescent tags or reporters can be simultaneously quantified for each cell in a population of thousands to provide exquisite, quantitative insight into the presence and nature of subpopulations. However, this powerful tool is often difficult to apply in the area of virology given the danger of contamination and production or aerosolized virus on shared flow cytometry equipment. Flow cytometry is also typically limited to endpoint analysis. Although many other single cell techniques have been developed such as droplet-based microfluidics and microfluidic flow traps, sandwiched microwells (SMAs) offer an attractive alternative with respect to flexibility, throughput, cost, and required expertise for operation. Further, SMAs offer the capability to observe single cell behavior over time.

As is known, a SMA is a sandwiched structure that is formed from a first plate with an array of microwells formed therein and a second plate that acts as a lid. When sandwiched together, the microwells and the lid create sealed chambers in which a screening reaction can be carried out. It can be appreciated that the use of microwells (wells on the order of ~1-200 μm) is prevalent in microscale device design primarily to help isolate analytes into very small volumes. By doing this, assays can be made vastly more sensitive and can be massively parallelized. Although microwells can be used to isolate small volumes of liquid for screening, they are extremely useful for isolating individual or small numbers of

particles or molecules suspended in that liquid or fluid for independent analysis. These types of advantages drive much of the current research in the area of microfluidics in general. The reduced volumes of the analytes allow for more sensitive detection of proteins and other molecules given that when these molecules are produced in a microwell (100×100×100 μm=1 nano liter) during a reaction or cell culture, they are diluted into much less volume than that of a more standard reaction or culture vessel, such as a 96 well plate (200 micro liters). Consequently, a 200,000 fold reduction in volume produces a 200,000 fold increase in the concentration of the produced molecule. The increase in the concentration of the produced molecule greatly increases the ability to detect such production.

Heretofore, however, methods to interface with and leverage microwells with these types of dimensions have been limited. Current embodiments of SMAs allow only a single experimental condition to be examined per chip, thereby making it difficult to control for chip-to-chip differences. Further, current methods for loading and treating the microwells, although generally easy, are relatively difficult to control and standardize.

Therefore, it is primary object and feature of the present invention to provide a microwell device for isolating a fluid, such as an analyte, into very small volumes.

It is a further object and feature of the present invention to provide a microwell device for isolating a fluid into very small volumes which is simple to utilize and inexpensive to manufacture.

It is a still further object and feature of the present invention to provide a microwell device for isolating a fluid into very small volumes which may be used in combination with conventional micropipetting equipment.

In accordance with the present invention, a microwell device is provided. The device includes a plate having a upper surface with a plurality of microwells formed therein. The microwells are adapted for receiving a fluid therein. A barrier extends about a first portion of the microwells. The barrier prevents fluid deposited on the first portion of the microwells from flowing therepast.

A recess formed in the upper surface of the plate within the barrier. The recess has an outer periphery and the first portion of microwells are spaced about the outer periphery of the recess. The recess has a volume and each of the microwells also has a volume. The volume of the recess is greater than the volumes of the microwells.

By way of example, the barrier may be a channel formed in the upper surface of the plate. The channel has a volume which is greater than the volumes of the microwells. The barrier is generally circular. The barrier may be a first barrier and the device may also includes a second barrier extending about a second portion of the microwells. The second barrier prevents fluid deposited on the second portion of the microwells from flowing therepast.

In accordance with a further aspect of the present invention, a microwell device is provided. The device includes a plate having a upper surface with a plurality of microwells formed therein. The microwells are adapted for receiving a fluid therein. First and second recesses may also be formed in the upper surface of the plate. Each recess has an outer periphery. A first portion of microwells are spaced about the outer periphery of the first recess and a second portion of microwells are spaced about the outer periphery of the second recess.

A first barrier may be positioned between the first and second portions of microwells for fluidicly isolating the first portion of the microwells from the second portion of microwells. In addition, a second barrier may also be positioned

between the first and second portions of microwells for fluidicly isolating the second portion of the microwells from the first portion of microwells. The first barrier may take the form of a first channel in upper surface of the plate that extends about the first portion of microwells. The first channel may have a generally circular configuration. It is contemplated for the first channel to have a volume and for each of the first portion of microwells has a volume. The volume of the first channel is greater than the volumes of each of the first portion of microwells. The second barrier may take the form of a second channel in upper surface of the plate that extends about the second portion of microwells.

It is intended for the first and second recesses to have volumes and for each of the first and second portions of microwells to have a volume. The volume of the first recess is greater than the volumes of each of the first portion of microwells and the volume of the second recess is greater than the volumes of each of the second portion of microwells.

In accordance with a still further aspect of the present invention, a microwell device is provided. The device includes a plate having a upper surface. The upper surface includes first and second recesses formed in the upper surface of the plate. Each recess has an outer periphery. A first portion of microwells is formed therein in the upper surface of the plate. The first portion of microwells is spaced about the outer periphery of the first recess. A second portion of microwells is also formed in the upper surface of the plate. The second portion of microwells spaced about the outer periphery of the first recess. A first barrier extends about the first portion of the microwells for fluidicly isolating the first portion of the microwells and a second barrier extends about the second portions of microwells for fluidicly isolating the second portion of the microwells.

The first barrier includes a first channel extending about the first portion of microwells. The first channel has a generally circular configuration and a volume. Each of the first portion of microwells also has a volume. The volume of the first channel is greater than the volumes of each of the first portion of microwells. The second barrier includes a second channel extending about the second portion of microwells. The first and second recesses have volumes and each of the first and second portions of microwells have a volume. The volume of the first recess is greater than the volumes of each of the first portion of microwells and the volume of the second recess is greater than the volumes of each of the second portion of microwells. A lid having a surface may also be provided. The lid is moveable between a first position wherein the surface of the lid is spaced from the upper surface of the plate and a second position wherein the surface of the lid is in engagement with the upper surface of the plate.

BRIEF DESCRIPTION OF THE DRAWINGS

The drawings furnished herewith illustrate a preferred construction of the present invention in which the above advantages and features are clearly disclosed as well as others which will be readily understood from the following description of the illustrated embodiment.

In the drawings:

FIG. **1** is an exploded, isometric view of a microwell device in accordance with the present invention in an initial configuration;

FIG. **2** is an enlarged, top plan view of the microwell device of the present invention taken along line **2-2** of FIG. **1**;

FIG. **3** is a cross-sectional view of the device of the present invention taken along line **3-3** of FIG. **2**;

FIG. **4** is an enlarged view of the microwell device of the present invention taken along line **4-4** of FIG. **3**;

FIG. **5** is a first, side elevational view of the microwell device of the present invention positioned on a micropipetting station;

FIG. **6** is a second, side elevational view of the microwell device of the present invention positioned on a micropipetting station; and

FIG. **7** is an enlarged, cross-sectional view of the microwell device of the present invention, similar to FIG. **3**, with a drop of fluid deposited thereon.

DETAILED DESCRIPTION OF THE DRAWINGS

Referring to FIG. **1**, a microwell device for use in the method of the present invention is generally designated by the reference numeral **10**. In the depicted embodiment, microwell device **10** includes plate **11** defined by first and second ends **12** and **14**, respectively; first and second sides **16** and **18**, respectively; and upper and lower surfaces **20** and **22**, respectively. It can be appreciated that plate **11** of microwell device **10** may have other configurations without deviating from the scope of the present invention. Further, it is contemplated for plate **11** to be fabricated from a gas permeable material so as to facilitate cellular growth and development, as hereinafter described. However, other materials are contemplated as being with the scope of the present invention.

Upper surface **20** of plate **11** includes a plurality of microwell regions **24** formed therein. Each of the microwell regions **24** are identical in structure, and as such, the following description is understood to describe each of the microfluidic regions. Each microwell region **24** is a defined by a barrier. By way of example, the barrier may take the form of a generally circular channel, designated by the reference numeral **26**, extending about center **27**. FIG. **2**. It can be appreciated that channel **26** can have other configurations without deviating from the scope of the present invention. As best seen in FIGS. **2-3**, channel **26** is defined by generally circular, radially inner wall **28** and generally circular, outer wall **30**, which are generally perpendicular to upper surface **20**. Inner and outer walls **28** and **30**, respectively, are interconnected by lower wall **32** extending between the lower ends thereof. It is contemplated for channel **26** to have a depth preferably in the range of 200 to 1000 micrometers and a volume in the range of 2 to 75 microliters.

Microwell region **24** further includes recess **34** centered at center **27**. In the depicted embodiment, recess **34** has a generally circular cross section. However, it can be appreciated that recess **34** can have other configurations without deviating from the scope of the present invention. By way of example, recess **34** is defined by a generally circular wall **36**. Wall **36** is generally perpendicular to upper surface **20** and is radially spaced from center **27**. Recess **34** terminates at lower wall **38** such that recess **34** has a depth in the range of 200 to 1000 micrometers and a volume in the range of 0.025 to 3.5 microliters.

Microwell region **24** further includes a plurality of rows of circumferentially spaced microwells, generally designated by the reference numeral **40**. The rows of microwells **40** are radially spaced between wall **36** of recess **34** and inner wall **28** of channel **26**. In the depicted embodiment, each microwall **40** has a generally cubic configuration. However, it can be appreciated that microwells **40** can have other configurations without deviating from the scope of the present invention. Referring to FIG. **4**, each microwell **40** is partially defined by sidewalls **42***a*-**42***b* extending generally perpendicular to upper surface **20**. Sidewalls **42***a*-**42***b* are interconnected by

lower wall **44** extending between the lower ends thereof. It is contemplated for each microwell **40** to have a depth of approximately 50 micrometers and a volume of approximately 0.1 nanoliter.

In operation, it is contemplated to culture desired cells, generally designated by the reference numeral **50**, in microwells **40** of one or more microwell regions **24** of plate **11**. In order to deliver the desired cells to each microwell **40** of a selected microwell region **24**, a robotic micropipetting station **52** is provided, FIG. **5**. As is known, modern high-throughput systems, such as robotic micropipetting station **52**, are robotic systems designed solely to position a tray (i.e. plate **11** of microwell device **10**) and to dispense or withdraw microliter drops into or out of that tray at user desired locations (i.e. microwell regions **24** of plate **11**) with a high degree of speed, precision, and repeatability.

As best seen in FIGS. **5-6**, micropipetting station **52** includes micropipette **56** for depositing drop **54** of a fluid, e.g. a reagent or a cell suspension, on the selected microwell region **24**. More specifically, micropipette **56** is axially aligned with center **27** of the selected microwell region **24**, FIG. **5**. Thereafter, micropipette **56** deposits drop **54** (e.g. a preselected cell suspension) on recess **34** of the selected microwell region **24**. With drop **54** deposited on the selected microwell region **24**, the outer periphery of drop **54** pins at radially inner edge **25** of channel **26**, FIG. **7**, thereby preventing the fluid of drop **54** from flowing therepast. It can be appreciated that in the event the outer periphery of drop **54** fails to pin at radially inner edge **25** of channel **26**, channel **26** acts to accommodate the overflow of fluid from drop **54** and to prevent such fluid from flowing to an adjacent microwell region **24**. As a result, the selected microwell region **24** is isolated from adjacent microwell regions of plate **11** of microwell device **10**. As such, the cell suspension may be selectively deposited on a single microwell region **24** without contaminating adjacent regions. The cells **50** in the drop **54** are allowed to settle in microwells **40** of microwell region **24**. Thereafter, any excess fluid provided on the selected microwell region **24** is aspirated.

It is understood that recess **34** allows for the complete aspiration of any excess fluid provided on the selected microwell region **24** without the excessive flows or shear normally associated therewith. More specifically, the excess portion of drop **54** deposited on the selected microwell region **24** may be aspirated at recess **34** without losing cells **50** being cultured in microwells **40** of the selected microwell region **24**. Further, it is noted that after aspiration of the excess fluid of drop **54**, the fluid within each microwell **40** in the selected microwell region **24** is substantially flush with upper surface **20** of plate **11**, thereby allowing for the efficient washing and treatment of the cells **50** therein.

Once the excess fluid is aspirated from the selected microwell region **24**, micropipette **56** of micropipetting station **52** may be used to deposit a second drop **54** (e.g. a desired analyte, a second cell suspension or the like) on recess **34** of the selected microwell region **24**. Recess **34** acts to minimize the excessive flows or shear on cells **50** being cultured in microwells **40** of the selected microwell region **24**. By minimizing the excessive flows or shear associated with the depositing of drop **54** on the selected microwell region **54**, it is intended to prevent cells **50** being cultured in microwells **40** of the selected microwell region **24** from becoming dislodged. Thereafter, any excess fluid provided on the selected microwell region **24** may aspirated. It can be appreciated that the process heretofore described may be repeated for the treating, labeling, washing and/or conducting of experiments on cell **50**, thereby allowing such steps to be conducted using a micropipette, eliminating the need to address each well individually using prohibitively expensive sub-nanoliter dispensing technologies or complicated droplet microfluidic systems.

It is further contemplated to apply lid **60** onto plate **11** of microwell device **10** to trap the cells, particles and/or fluids within microwells **40**. By way of example, in the depicted embodiment, lid **60** is defined by first and second ends **62** and **64**, respectively; first and second sides **66** and **68**, respectively; and first and second surfaces **70** and **72**, respectively. It can be appreciated that lid **60** may have other configurations without deviating from the scope of the present invention.

In operation, lid **60** is moved between a first position wherein lid **60** is spaced from plate **11** of microwell device **10** and a second position wherein first surface **70** of lid **60** is brought into contact with upper surface **20** of plate **11**, thereby trapping the cells and/or fluids within microwells **40**. It is noted that as lower surface **70** of lid **60** is brought into contact with upper surface **20** of plate **11**, any small volumes of fluid provided on upper surface **20** of plate **11** are squished and spread along upper surface **20** within microwell regions **24**. It can be appreciated that each channel **26** about a corresponding microwell region **24** is adapted to receive any excess fluid that spreads along upper surface **20** within microwell region **24**, thereby preventing the fluid from flowing into adjacent microwell regions. As a result, each channel **26** about a corresponding microwell region **24** acts as a barrier during application of lid **60** to prevent fluid on upper surface **20** of one of the microwell regions **24** from flowing into and contaminating the other microwell regions **24** provided on plate **11**. In view of the foregoing, it can be appreciated that channels **26** about microwell regions **24** allow a user to maintain different conditions on each microwell region **24** of plate **11**.

It is further contemplated to functionalize lower surface **70** of lid **60** with antibodies to enable capture of specific analytes for surface-based detection methods, such as antibody staining, sandwich-ELISA, or label-free detection methods like the LED-based IRIS. In addition, it can be appreciated that lid **60** can be removed from plate **11** without perturbing cells **50**, and thereafter, replaced to enable a variety of protocols.

Various modes of carrying out the invention are contemplated as being within the scope of the following claims particularly pointing out and distinctly claiming the subject matter, which is regarded as the invention.

We claim:

1. A microwell device, comprising:

a plate having a upper surface including a plurality of microwells formed therein, each of the microwells having a volume and being adapted for receiving a fluid therein; and

a barrier extending about a first portion of the microwells, the barrier preventing fluid deposited on the first portion of the microwells from flowing therepast, wherein the barrier is a channel formed in the upper surface of the plate; and

a recess formed in the upper surface of the plate within the barrier, the recess being fluidicly isolated from the barrier and having a volume greater than the volume of each of the microwells.

2. The device of claim **1** wherein the recess has an outer periphery and wherein the first portion of microwells are spaced about the outer periphery of the recess.

3. The device of claim **1** wherein the channel has a volume and wherein each of the microwells has a volume, the volume of the channel being greater than the volumes of the microwells.

4. The device of claim 1 wherein the barrier is generally circular.

5. The device of claim 1 wherein the barrier is a first barrier and wherein the device further comprises a second barrier extending about a second portion of the microwells, the second barrier preventing fluid deposited on the second portion of the microwells from flowing therepast.

6. A microwell device, comprising:

a plate having a upper surface including a plurality of microwells formed therein, each of the microwells having a volume and being adapted for receiving a fluid therein;

first and second recesses formed in the upper surface of the plate, each recess having a volume and an outer periphery;

a first barrier extending about the first recess and positioned between the first and second portions of microwells for fluidicly isolating the first portion of the microwells from the second portion of microwells, the first barrier being fluidicly isolated from the first recess, wherein the first barrier includes a first channel extending about the first portion of microwells in the upper surface of the plate;

a second barrier extending about the second recess and positioned between the first and second portions of microwells for fluidicly isolating the second portion of the microwells from the first portion of microwells, the second barrier being fluidicly isolated from the second recess; and

wherein:

the volume of each of the first and second recesses is greater than the volume of each of the microwells;

a first portion of microwells is spaced about the outer periphery of the first recess; and

a second portion of microwells is spaced about the outer periphery of the second recess.

7. The device of claim 6 wherein the first channel has a generally circular configuration.

8. The device of claim 6 wherein the first channel has a volume, the volume of the first channel being greater than the volumes of each of the first portion of microwells.

9. The device of claim 6 wherein the second barrier includes a second channel extending about the second portion of microwells in the upper surface of the plate.

10. A microwell device, comprising:

a plate having a upper surface, the upper surface including:

first and second recesses formed in the upper surface of the plate, each recess having an outer periphery and a volume;

a first portion of microwells formed therein, the first portion of microwells spaced about the outer periphery of the first recess and each of the first portion of microwells having a volume less than the volume of the first recess;

a second portion of microwells formed therein, the second portion of microwells spaced about the outer periphery of the second recess and each of the second portion of microwells having a volume less than the volume of the second recess;

a first barrier about the first recess and the first portion of the microwells for fluidicly isolating the first portion of the microwells, the first barrier being fluidicly isolated from the first recess, wherein the first barrier includes a first channel extending about the first portion of microwells; and

a second barrier about the second recess and the second portion of microwells for fluidicly isolating the second portion of the microwells, the second barrier being fluidicly isolated from the second recess.

11. The device of claim 10 wherein the first channel has a generally circular configuration.

12. The device of claim 10 wherein the first channel has a volume, the volume of the first channel being greater than the volumes of each of the first portion of microwells.

13. The device of claim 10 wherein the second barrier includes a second channel extending about the second portion of microwells.

14. The device of claim 10 further comprising a lid having a surface, the lid moveable between a first position wherein the surface of the lid is spaced from the upper surface of the plate and a second position wherein the surface of the lid is in engagement with the upper surface of the plate.

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