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**Beebe et al.**

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(54) **PLATFORM FOR MULTI-VARIABLE SCREENING**

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See application file for complete search history.

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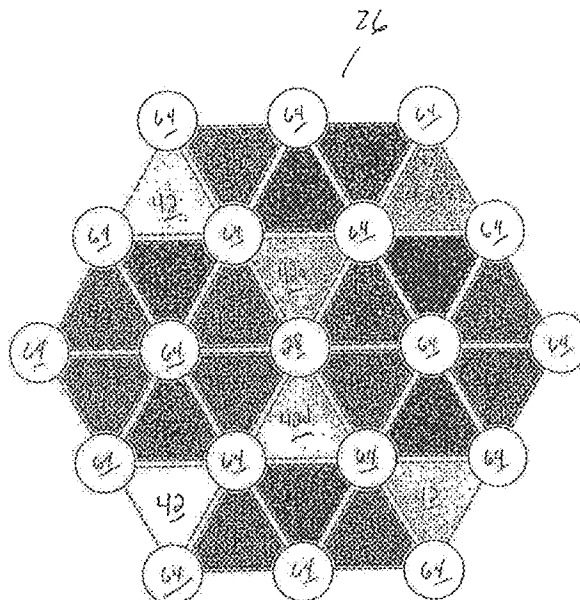
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(57) **ABSTRACT**

A platform and method for conducting multi-variable combinational interactions are provided. An array of multiplexing chambers is formed in a body. The body also includes a common well communicating with each multiplexing chamber of the array of multiplexing chambers and a plurality of variable wells. Each of variable wells communicates with at least one multiplexing chamber of the array of multiplexing chambers. The common well is loaded with a first variable and different variables are loaded in each of the plurality of variable wells. The interaction of the first variable with at least one of the different variables in each multiplexing chamber of the array of multiplexing chambers is observed.

**8 Claims, 7 Drawing Sheets**





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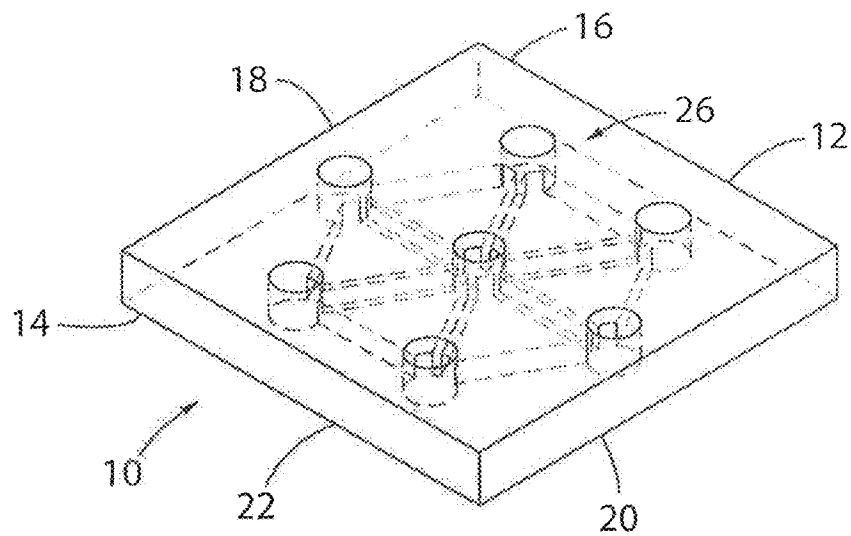


FIG. 1

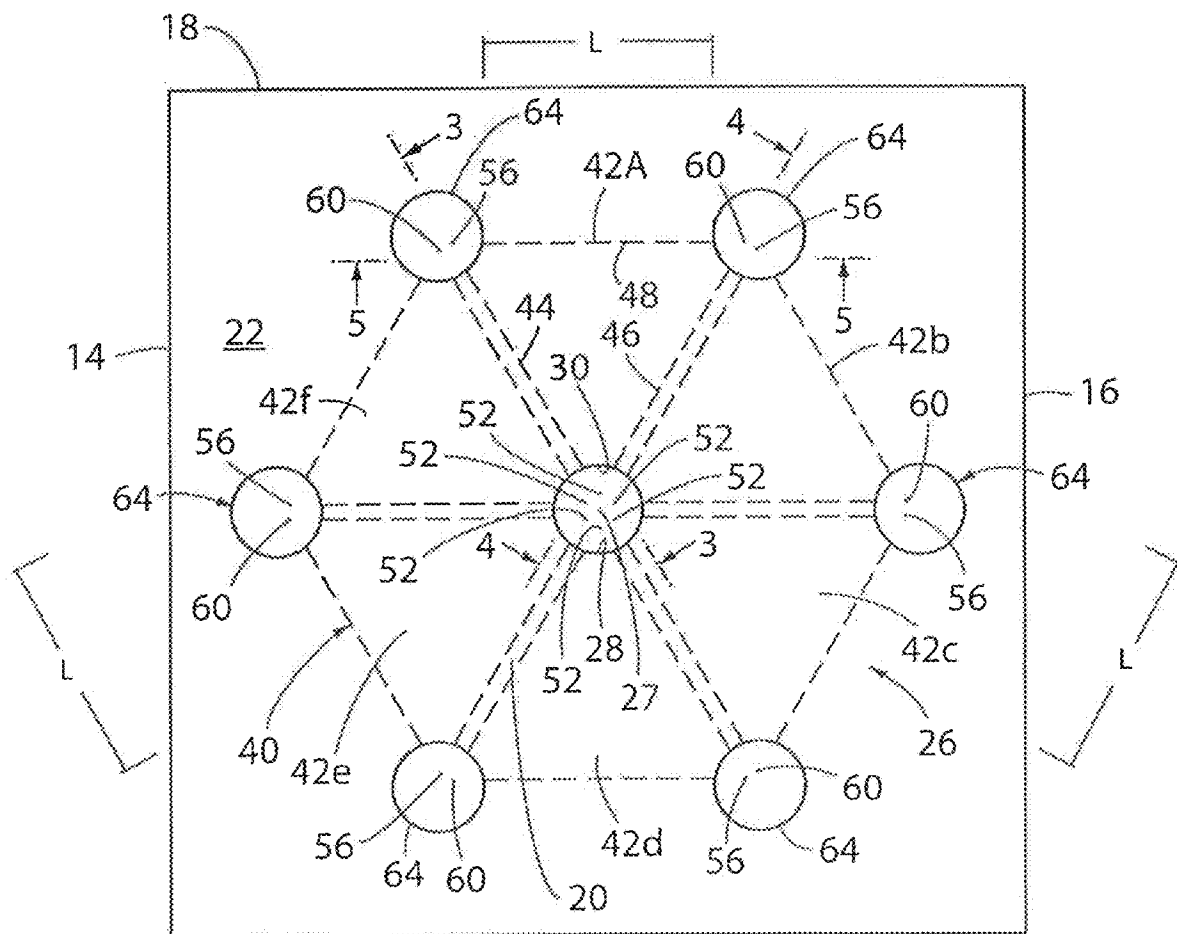


FIG. 2



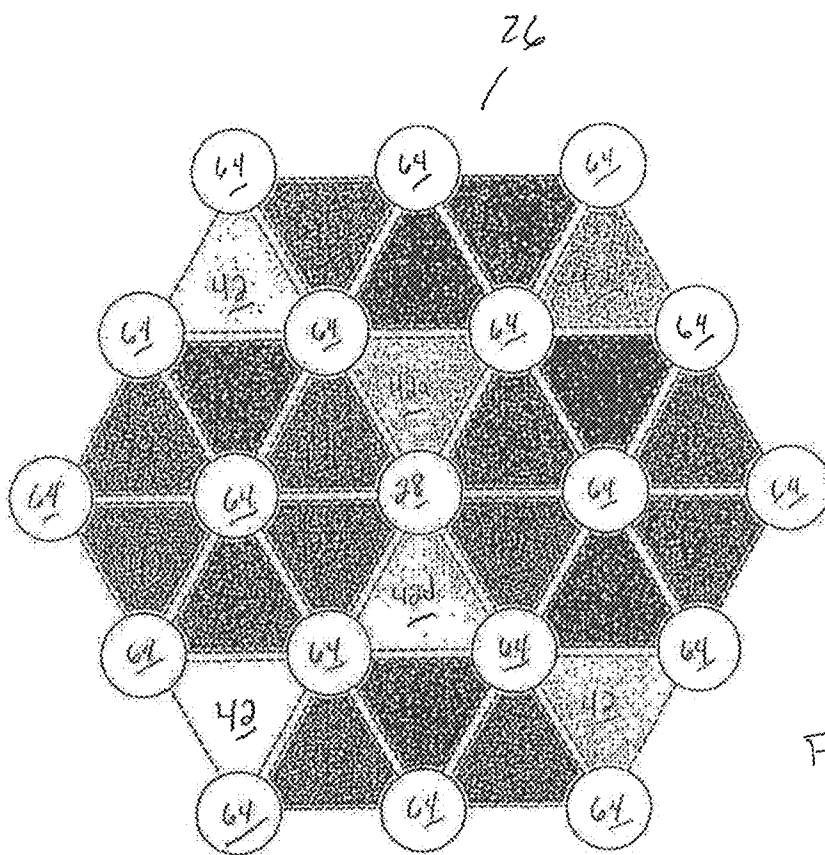


Fig. 2a

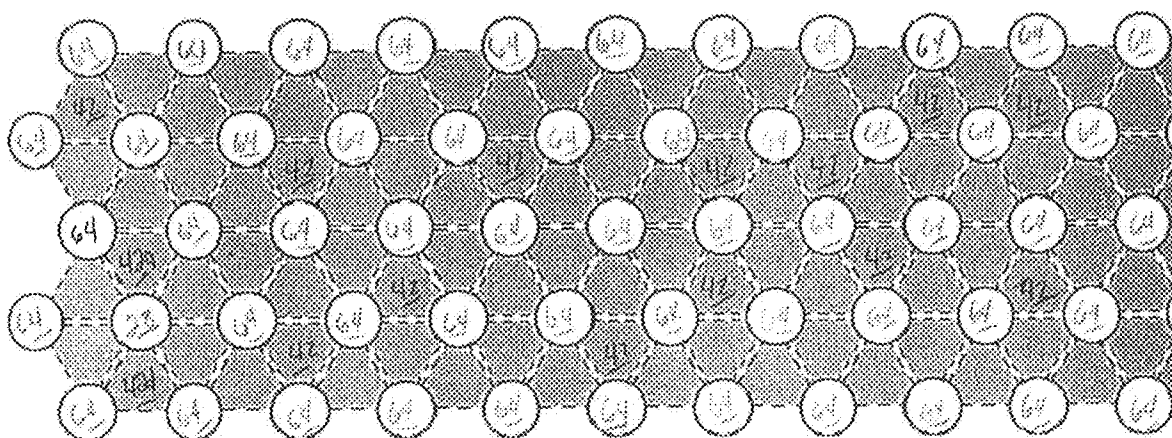


Fig. 2b



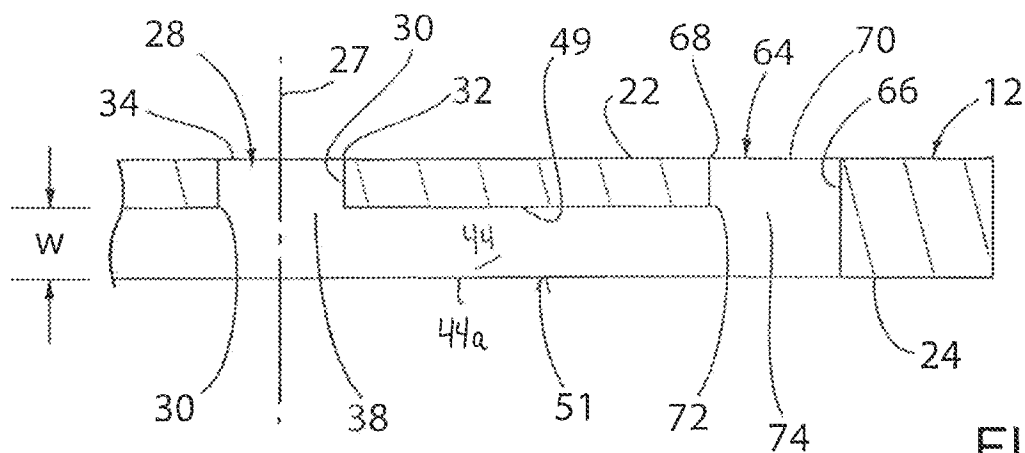


FIG. 3

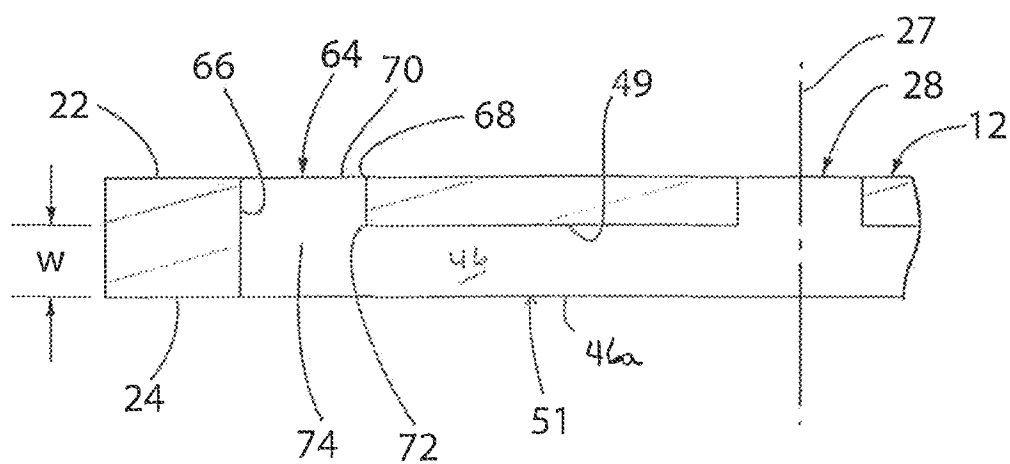


FIG. 4

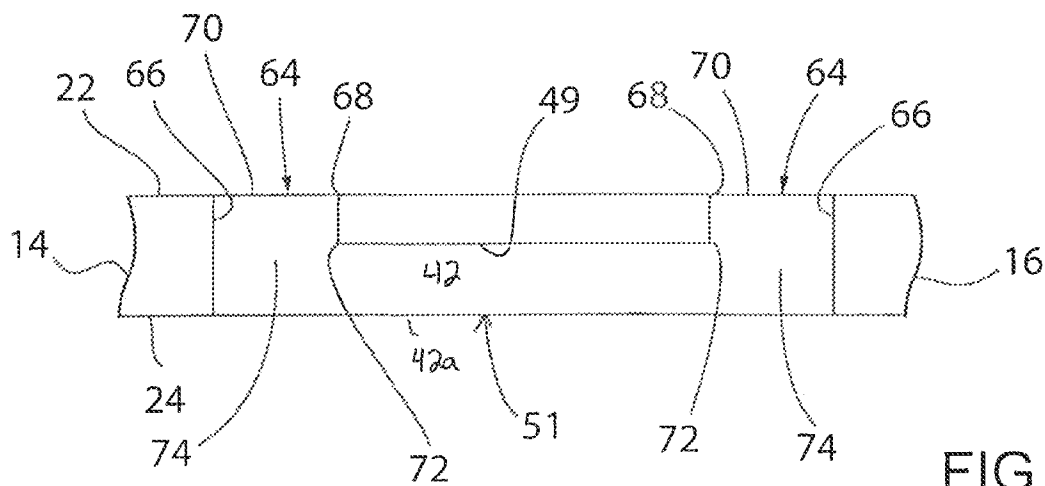
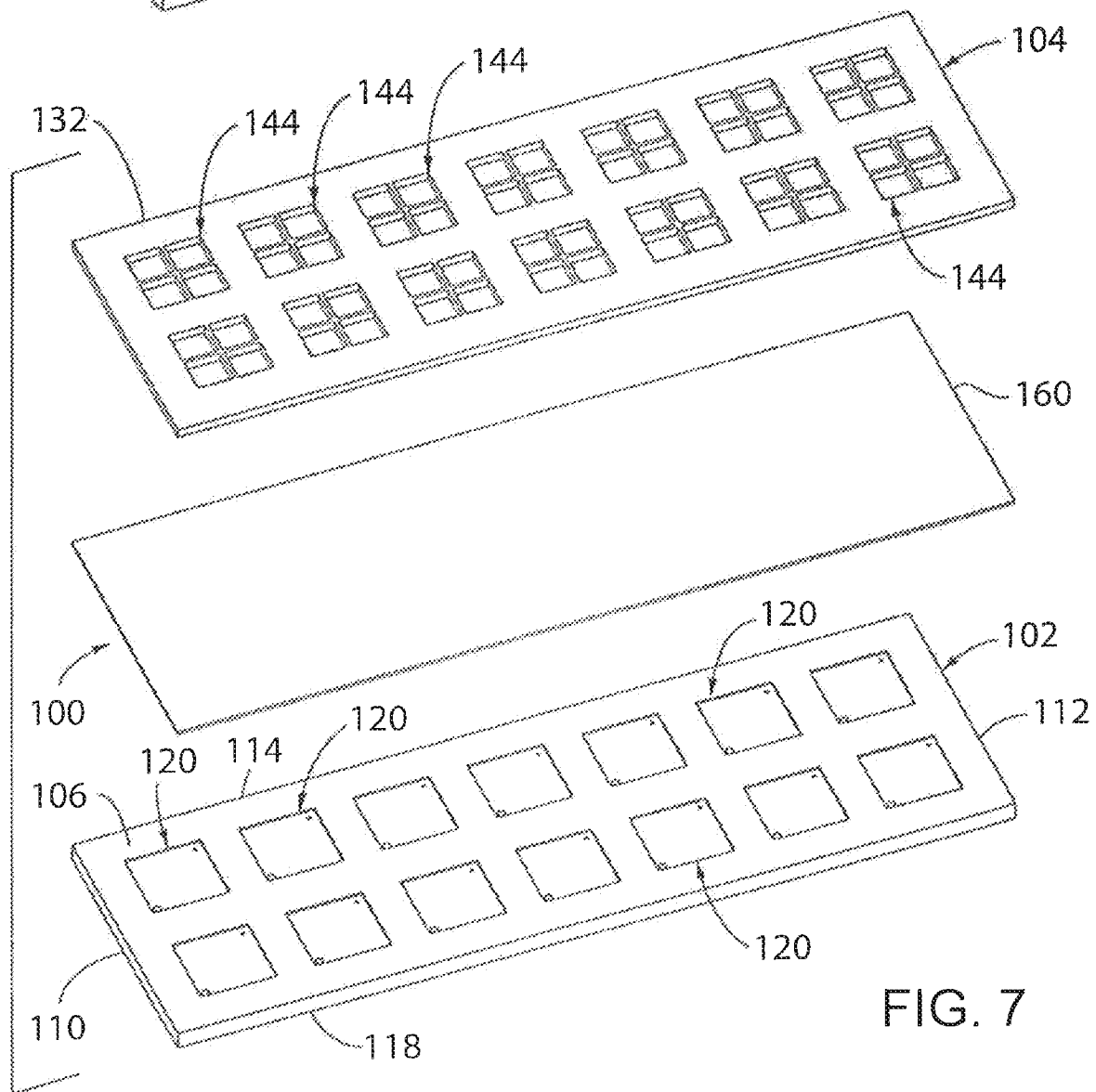
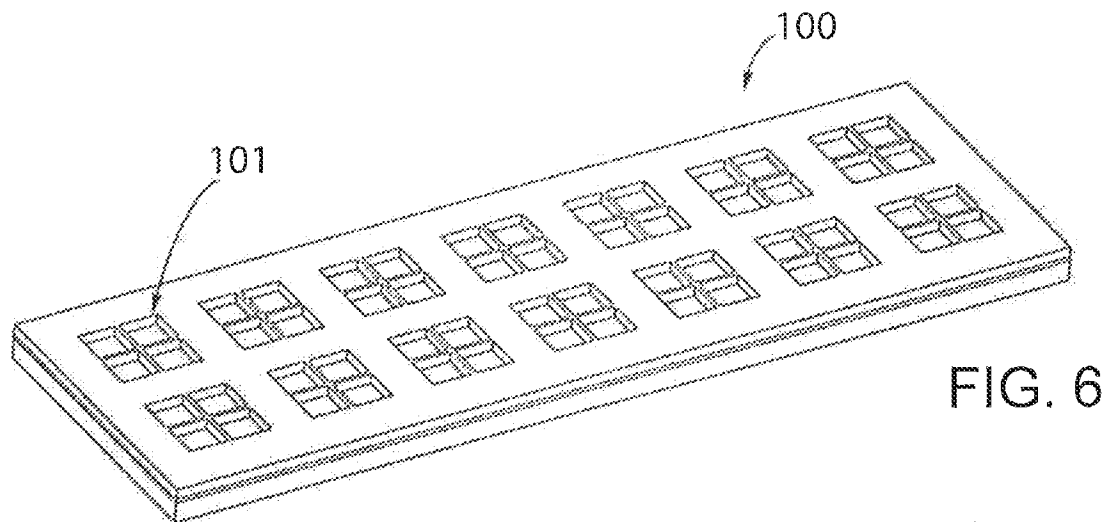
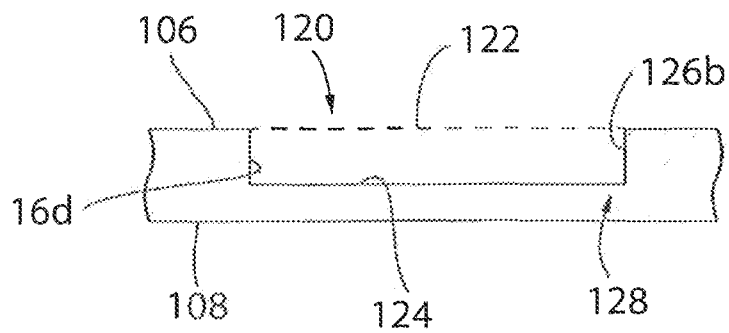
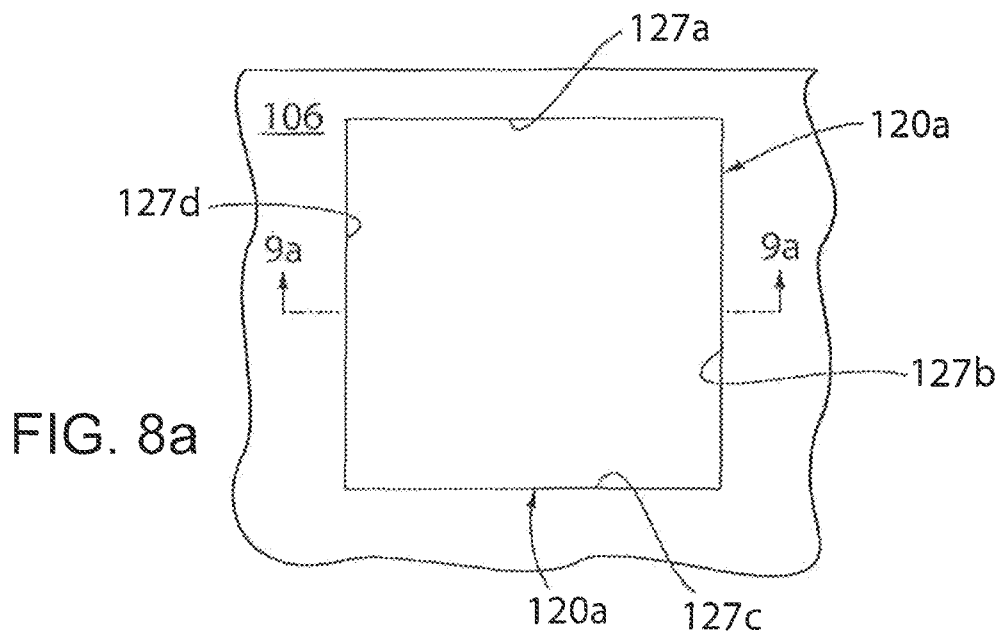
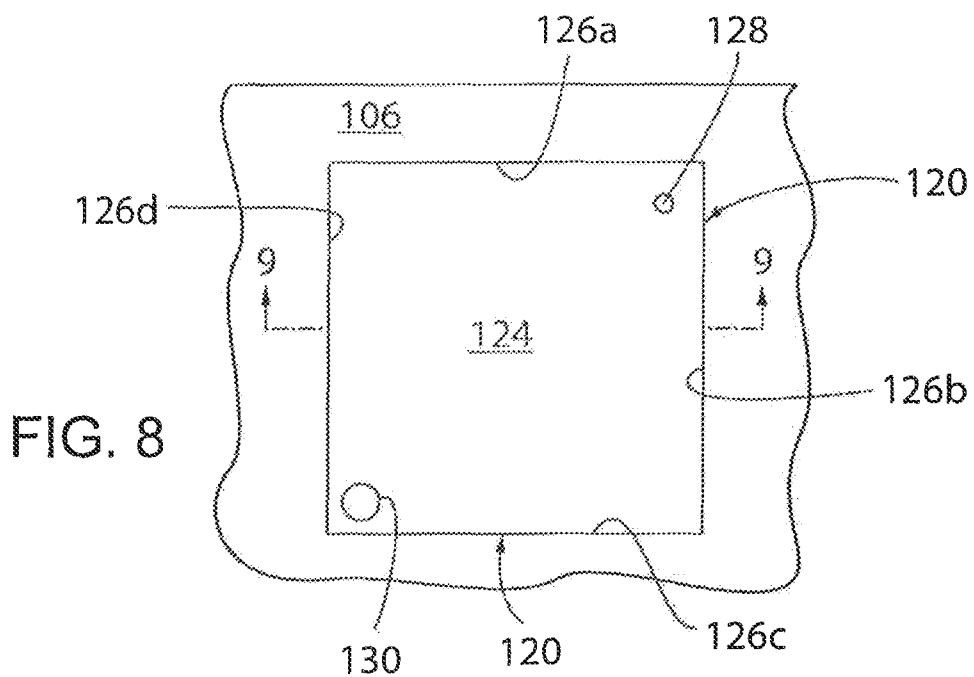


FIG. 5











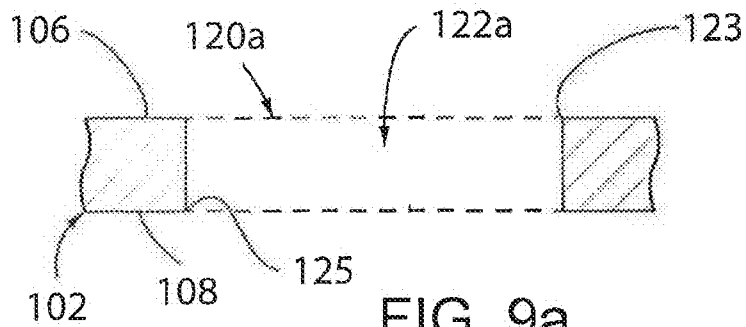


FIG. 9a

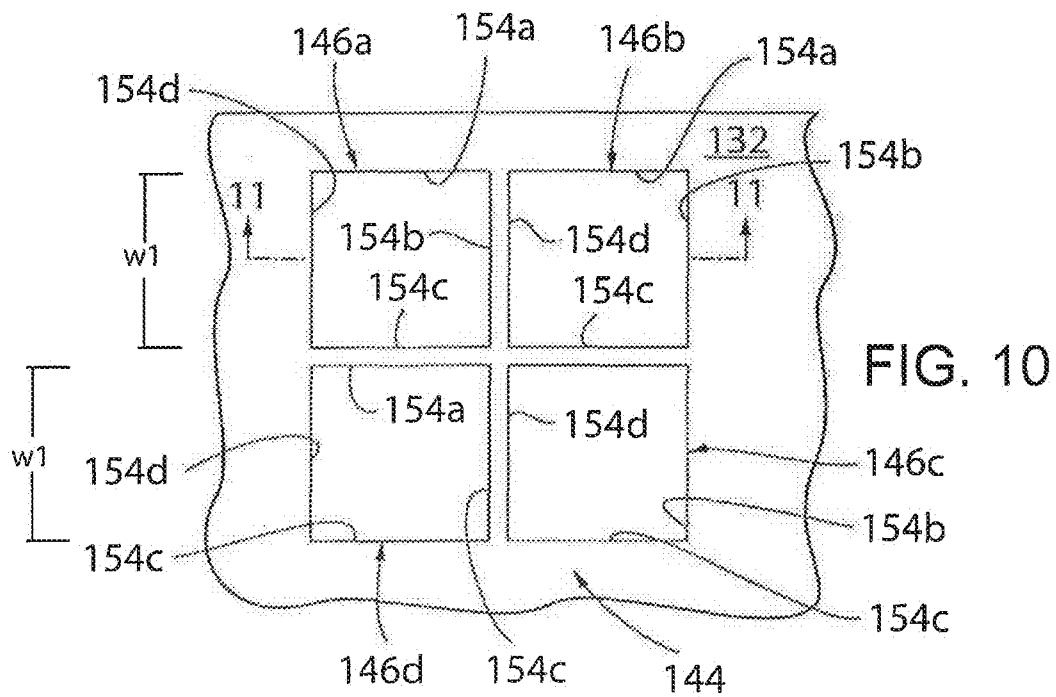


FIG. 10

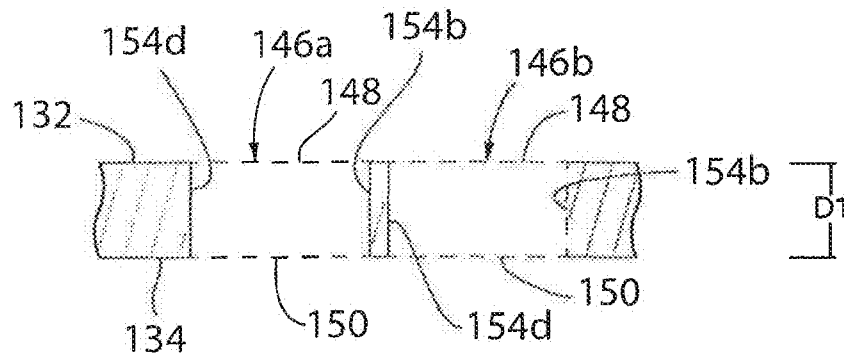
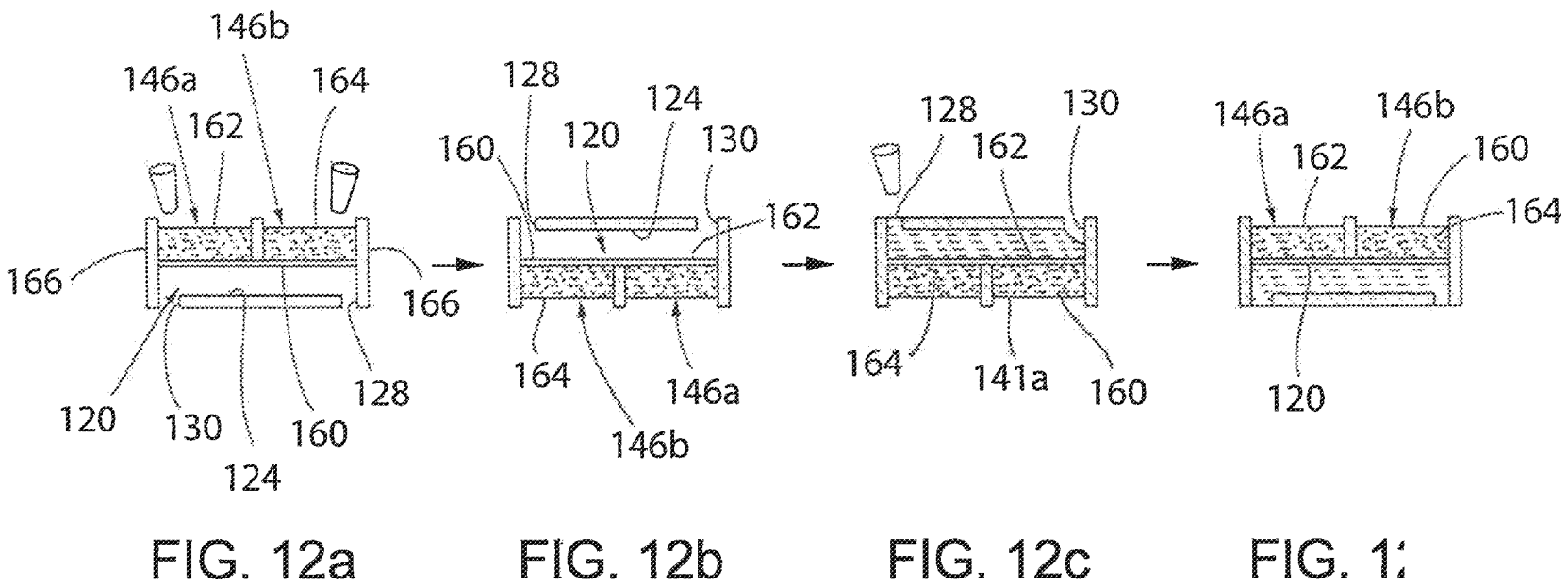


FIG. 11







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## PLATFORM FOR MULTI-VARIABLE SCREENING

### FIELD OF THE INVENTION

The invention relates generally to microbiology, and in particular, to a platform and method for multi-variable combinatorial screening with sufficient throughput to allow for large scale screening of microbial community interactions at the microscale.

### BACKGROUND AND SUMMARY OF THE INVENTION

Early microbiology studies are primarily based on adaptations of a one microbe, one disease hypothesis, known as Koch's postulates, introduced in the late 1800s. Koch's postulates states that a disease causing microbe should have the following four criteria: 1) the microbe must be found in all organisms affected from the disease, but not in healthy organisms; 2) the microbe must be isolatable from the disease affected organism and also grown in pure culture; 3) the pure cultured microbe should be able to cause the same disease when introduced into a healthy organism; and 4) the microbe must be able to be isolated from the infected organism and be identical to the original isolated strain. More recently, however, researchers have realized that microbes do not exist as single entities, but as complex multispecies communities in the majority of environments ranging from soil to the gastrointestinal tract of animals. These multi-microbial species interactions play a significant role in human health and pathology. For instance, the human gut alone has an estimated 500 to 1000 different microbial species. Different members within the microbial community interact locally in ways that can affect the dynamics and stability of the population as a whole. The role of these interactions in human health and pathology has recently emerged, with various studies showing that microbial communities inhabiting the gut play a critical role in human pathologies like diabetes, obesity, Parkinson's, Alzheimer's, and even cancers.

Recent advances in both throughput and cost reduction in metagenomic sequencing technologies have enabled the study of microbiomes and their participating members with unprecedented throughput and offer insight into a more systems-level understanding of the identities of microbial community members. Despite progress in these areas, much remains to be understood regarding how the individual members of a community interact with their adjacent neighbors and the physiological functions that arise from these interactions within their microenvironment. Multi-variable combinatorial screening is a universal critical step in the development of combinatorial drug treatments, stem cell differentiation, studying cell-cell/microbe interactions, among many other applications involving multi-parameter experimental optimization.

Current standard technologies for studying microbial communities include 16S ribosomal RNA (rRNA) sequencing, fluorescence in situ hybridization (FISH), macroscale (mostly pairwise) co-cultures, and computer-based modeling. 16S rRNA sequencing can provide valuable information regarding which microbial species are present within a community, but cannot elucidate what interactions occur between which members of the community. FISH can provide information regarding the spatial distribution of different microbial species within a sample, but cannot infer functional readouts, is challenging to perform and is also

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limited to the number of fluorescent probes and wavelengths available. Pairwise co-cultures are generally performed in conventional laboratory culture vessels like multi-well plates, tubes, and solid agar plates. Although suitable for small scale interaction screens for a handful of communities, the process is ill suited for taking on the colossal task of screening and re-assembling the vast parameter space of interactions for even small numbers of microbial species. Additionally, macroscale liquid cultures are prone to disturbance by convective flow, causing rapid mixing and dilution of localized diffusible factors. As a result, valuable local interaction phenotypes could be lost. Computer based modeling is useful for gaining understanding of how competition and cooperation within a microbial community can maintain stability, survival, and biodiversity, such as "rock-paper-scissors" interaction dynamics, and fills some of the gaps in current experimental limitations in screening multi-species interactions. However, the results obtained from modeling biological systems is highly hypothetical and often not translatable to real-world conditions.

In view of the foregoing, it is evident that there exists a significant need for practical microscale co-culture tools with sufficient throughput to allow for large scale screening of microbial community interactions at the microscale. This is a critical piece of the puzzle to enable scientists to gain functional insight and understanding of how different community members interact and drive community behavior within the microenvironment. Development of microscale co-culture tools with sufficient throughput to allow for large scale screening of microbial community interactions at the microscale could lead to important insights into future clinical treatment strategies. For example, studies have shown that microbes in co-cultures can exhibit differing responses to antibiotic treatment compared to those in isolation, in which the microbial members in cohabitation can either antagonize or potentiate antibiotic susceptibility to the whole community. Hence, in the context of polymicrobial infection, interplay between two or more invading pathogens is likely clinically significant and unfortunately, unaccounted for with current methods.

Heretofore, patients found to have an infection undergo antibiotic sensitivity testing performed on their specific bacterial pathogens. This type of testing helps clinicians select antibiotic therapy in a patient-specific manner. Unfortunately, antibiotic sensitivity testing can be a poor predictor of patient outcome. For example, in the phase 3 clinical trial for cefotaxime, a "sensitive" test result, in the best case scenario, meant 93% of patients would respond. Importantly, 64% of patients who had a "resistant" test result also responded to the antibiotic therapy. Both sources of error are clinically significant. Clinicians want to treat patients with antibiotics that are effective and also provide good antimicrobial stewardship by avoiding jumping to backup/reserve antibiotics if other, more frequently used options, would also be effective.

There are a number of factors that contribute to the poor predictive value of state of the art antibiotic sensitivity tests, most of which come back to the fact that the assay is oversimplified; it is performed on pure bacterial isolates. It neglects the role of both the patient's immune system and the role other nearby pathogens may play. In the context of polymicrobial infection, interplay between two or more invading pathogens is likely clinically significant and unfortunately, unaccounted for with current methods.

Therefore, it is a primary object and feature of the present invention to provide a platform and method for multi-variable combinatorial screening testing that has sufficient



throughput to allow for large scale screening of microbial community interactions at the microscale.

It is a further object and feature of the present invention to provide a platform and method for multi-variable combinational screening testing that allows individual microbes of a microbial community to interact with their adjacent neighbors.

It is a further object and feature of the present invention to provide a platform and method for multi-variable combinational screening testing that allows for the observation of the physiological functions that arise from the interaction of individual microbes of a microbial community and their adjacent neighbors.

It is a further object and feature of the present invention to provide a platform and method for multi-variable combinational screening testing that is simple and straightforward to utilize.

In accordance with the present invention, a platform for multi-variable combinational screening is provided. The platform includes a body defining a first multiplexing chamber having an outer periphery. A plurality of first wells are spaced about the outer periphery of the first multiplexing chamber. Each first well is spaced from an adjacent first well by a uniform distance.

The body further defines a second multiplexing chamber having an outer periphery. The second multiplexing chamber communicates with at least one of the plurality of first wells along the outer periphery of the second multiplexing chamber. A second well may also communicate with the second multiplexing chamber along the outer periphery of the second multiplexing chamber. The first and second multiplexing chambers have generally triangular configurations defined by three sides of generally equal length. Each side of the triangular configuration intersects an adjacent side at a corresponding vertex. Each of the plurality of first wells are provided at a corresponding vertex of the triangular configuration of the first multiplexing chamber. The first and second first wells of the plurality of first wells communicate with the second multiplexing chamber along corresponding vertices along a first side of the second multiplexing chamber.

In accordance with a further aspect of the present invention, a platform for multi-variable combinational screening is provided. The platform includes a body defining a central well and a plurality of multiplexing chambers extend radially from the central well. Each of the multiplexing chambers has an identical configuration.

It is contemplated for each of the plurality of multiplexing chambers to have a triangular configuration. In such configuration, each of the plurality of multiplexing chambers is defined by first, second and third sides and first, second and third vertices. The central well defines the first vertex of each of the multiplexing chambers. The body also includes corner wells defining the second vertex of each of the multiplexing chambers and the third vertex of an adjacent multiplexing chamber. Each of the first, second and third sides of each of the multiplexing chambers have lengths. The lengths of the first, second and third sides of each of the multiplexing chambers are generally equal. Hence, each of the plurality of multiplexing chambers may be considered to have an equilateral triangular configuration. The body may define six multiplexing chambers. Each multiplexing chamber includes first, second and third vertices. The central well is located at the first vertex of each of the multiplexing chambers. Corner wells are located at the second vertex of each of the multiplexing chambers and the third vertex of a multiplexing chamber adjacent thereto.

In accordance with a still further aspect of the present invention, a method for conducting multi-variable combinational interactions is provided. The method includes the step of providing an array of multiplexing chambers in a body. The body includes a common well and a plurality of variable wells. The common well communicates with each multiplexing chamber of the array of multiplexing chambers. Each of variable wells communicates with at least one multiplexing chamber of the array of multiplexing chambers. The common well is loaded with a first variable and different variables are loaded in each of the plurality of variable wells. The interaction of the first variable with at least one of the different variables in each multiplexing chamber of the array of multiplexing chambers is observed.

Each of the multiplexing chambers of the array of multiplexing chambers extends radially from the common well and have an identical configuration. For example, each of the multiplexing chambers of the array of multiplexing chambers may have a triangular configuration. Each of the multiplexing chambers of the array of multiplexing chambers is defined by first, second and third sides and first, second and third vertices. The common well is located at the first vertex of each of the multiplexing chambers of the array of multiplexing chambers. Each variable well is located at the second vertex of each of the multiplexing chambers of the array of multiplexing chambers and the third vertex of an adjacent multiplexing chamber of the array of multiplexing chambers such that each variable well communicates with one of the multiplexing chambers of the array of multiplexing chambers and with the adjacent multiplexing chamber of the array of multiplexing chambers.

Each of the first, second and third sides of each of the multiplexing chambers of the array of multiplexing chambers have lengths. The lengths of the first, second and third sides of each of the multiplexing chambers of the array of multiplexing chambers are generally equal. As such, each of the multiplexing chambers of the array of multiplexing chambers may have an equilateral triangular configuration.

It is contemplated for the array of multiplexing chambers to be defined by six multiplexing chambers. Each multiplexing chamber includes first, second and third vertices. The common well is located at the first vertex of each of the multiplexing chambers. Each variable well is located at the second vertex of each of the multiplexing chambers and at the third vertex of an adjacent multiplexing chamber such that each variable well communicates with a corresponding one of the multiplexing chambers and with the adjacent multiplexing chamber.

The first variable may be one of a microbe, a cell, a drug, an antibiotic and a soluble factor and each of different variables may be one of a microbe, a cell, a drug, an antibiotic and a soluble factor that is different from the first variable and the other different variables. Each multiplexing chamber of the array of multiplexing chambers communicates with the first variable and two different variables. The combination of the first variable and two different variables communicating with each multiplexing chamber is different than the combination of the first variable and two different variables communicating with each of the other multiplexing chambers of the array of multiplexing chambers.

#### 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



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others which will be readily understood from the following description of the illustrated embodiment.

In the drawings:

FIG. 1 is an isometric view of a microfluidic device including a platform for effectuating a method in accordance with the present invention;

FIG. 2 is a top plan view of the platform of FIG. 1;

FIG. 2a is a schematic, top plan view of the microfluidic device of FIG. 1 expanded to include an increased higher number of multiplexing chambers;

FIG. 2b is a schematic, top plan view of the microfluidic device of FIG. 1 expanded in a single direction to include an increased higher number of multiplexing chambers;

FIG. 3 is a cross-sectional view of the platform taken along line 3-3 of FIG. 2;

FIG. 4 is a cross-sectional view of the platform taken along line 4-4 of FIG. 2;

FIG. 5 is a cross-sectional view of the platform taken along line 5-5 of FIG. 2;

FIG. 6 is an isometric view of an alternate embodiment of a microfluidic device including a plurality of platforms for effectuating a method in accordance with the present invention;

FIG. 7 is an exploded view of the microfluidic device of FIG. 6;

FIG. 8 is a top plan view of a first layer of the microfluidic device of FIG. 6;

FIG. 8a is a top plan view of an alternate construction of a first layer of the microfluidic device of FIG. 6;

FIG. 9 is a cross-sectional view of the first layer of the microfluidic device taken along line 9-9 of FIG. 8;

FIG. 9a is a cross-sectional view of the first layer of the microfluidic device taken along line 9a-9a of FIG. 8a;

FIG. 10 is a top plan view of a second layer of the microfluidic device of FIG. 6;

FIG. 11 is a cross-sectional view of the second layer of the microfluidic device taken along line 11-11 of FIG. 10; and

FIGS. 12a-12d are a plurality of schematic views showing sequential steps performed with the platform of FIG. 6 to effectuate the methodology of the present invention.

#### DETAILED DESCRIPTION OF THE DRAWINGS

Referring to FIGS. 1-2, a microfluidic device in accordance with the present invention is generally designated by the reference numeral 10. Microfluidic device 10 may be formed from polystyrene (PS) or polydimethylsiloxane (PDMS), however, other materials are contemplated as being within the scope of the present invention. In the depicted embodiment, microfluidic device 10 includes base 12 having first and second ends 14 and 16, respectively, and first and second sides 18 and 20, respectively. First and second ends 14 and 16, respectively, and first and second sides 18 and 20, respectively, of base 12 define the outer periphery of base 12. The outer periphery of base 12 interconnects upper and lower surfaces 22 and 24, respectively.

As hereinafter described, microfluidic device 10 defines platform 26 for effectuating a method of multi-variable combinational screening in accordance with the present invention. It is noted that microfluidic device 10 may include additional platforms 26 provided therein for effectuating the methodology in after described, without deviation from the scope of the present invention. Referring to FIGS. 2-4, in the depicted embodiment, platform 26 includes a vertical common well 28 extending along a corresponding axis 27 into base 12 from upper surface 22 thereof. Common well 28 is

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defined by a generally cylindrical side wall 30 having an upper edge 32 intersecting upper surface 22 of base 12 so as to define opening 34. Lower edge 36 of side wall 30 defines outlet 38 of common well 28.

Platform 26 further includes an array of multiplexing chambers 40 extending radially outward from axis 27 and communicating with exit 38 of common well 28. In the depicted embodiment, six (6), generally triangular multiplexing chambers 42a-42f are provided in base 12. As hereinafter described, each multiplexing chamber 42a-42f is identical in structure and configuration. As such, the following description of multiplexing chamber 42a is understood to describe multiplexing chambers 42b-42f as if fully described herein.

Multiplexing chamber 42a is generally triangular in shape and is defined by first sidewall 44 lying in a first plane, a second sidewall 46 lying in a second plane and third sidewall 48 lying in a third plane. First, second and third sidewalls 44, 46 and 48, respectively, depend from upper, generally planar, chamber surface 49 and intersect lower surface 24 of base 12 at corresponding lower terminal edges 44a, 46a and 48a, respectively. It can be appreciated that lower edges 44a, 46a and 48a of first, second and third sidewalls 44, 46 and 48, respectively, lie in a common plane with lower surface 24 of base 12 and define opening 51. First, second and third sidewall 44, 46 and 48, respectively, have a common width and, for reasons hereinafter described, first, second and third sidewall 44, 46 and 48, respectively, have a generally equal length L. Hence, multiplexing chamber 42a has an equilateral triangular configuration. The first and second planes, respectively, are provided at an angle of approximately 60 degrees to each other and intersect each other along a line, thereby defining first vertex 52 of the triangular configuration of multiplexing chamber 42a. It can be appreciated that first vertex 52 is adjacent to axis 27. Similarly, the first and third planes, respectively, are provided at an angle of approximately 60 degrees to each other and intersect each other along a line, thereby defining second vertex 56 of the triangular configuration of multiplexing chamber 42a. Second and third planes, respectively, are provided at an angle of approximately 60 degrees to each other and intersect each other along a line, thereby defining third vertex 60 of the triangular configuration of multiplexing chamber 42a.

As best seen in FIG. 2, it is understood that second vertex 56 of each multiplexing chamber 42a-42f is adjacent to third vertex 60 of an adjacent multiplexing chamber 42a-42f. In other words, second vertex 56 of multiplexing chamber 42a is adjacent to third vertex 60 of adjacent multiplexing chamber 42f; second vertex 56 of multiplexing chamber 42f is adjacent to third vertex 60 of adjacent multiplexing chamber 42e; second vertex 56 of multiplexing chamber 42e is adjacent to third vertex 60 of adjacent multiplexing chamber 42d; second vertex 56 of multiplexing chamber 42d is adjacent to third vertex 60 of adjacent multiplexing chamber 42c; second vertex 56 of multiplexing chamber 42c is adjacent to third vertex 60 of adjacent multiplexing chamber 42b; and second vertex 56 of multiplexing chamber 42b is adjacent to third vertex 60 of adjacent multiplexing chamber 42a.

Platform 26 further includes a plurality of variable wells 64 provided in base 12. Variable wells 64 extend into base 12 from upper surface 22 thereof along corresponding axes at a location which overlaps second vertices 56 of each multiplexing chamber 42a-42f (e.g. multiplexing chamber 42a) and third vertices 60 of the multiplexing chamber 42a-42f adjacent thereto (e.g. multiplexing chamber 42f). As best seen in FIGS. 3-5, each variable well 64 is defined by



a generally cylindrical side wall **66** having an upper edge **68** intersecting upper surface **22** of base **12** so as to define opening **70**. Lower edge **72** of side wall **66** of each variable well **64** defines an outlet **74** having sufficient diameter to communicate with two adjacent multiplexing chambers **42a-42f** (e.g. multiplexing chambers **42a** and **42f**). It is contemplated to provide a semi-permeable membrane between outlets **74** of variable wells **64** and corresponding multiplexing chambers **42a-42f** to prevent direct liquid mixing/crosstalk between via convective flow between variable wells **64** and corresponding multiplexing chambers **42a-42f**.

In operation, it is intended for platform **26** to be utilized for the multiplex analysis of the interaction of three variables within a single chamber (e.g., multiplexing chambers **42a-42f**). By way of example, multiplexing chambers **42a-42f** may be filled through opening **51** with a desired media, such as a solution, gel, or the like. A fixed or first variable "experimentee" is loaded in common well **28** so as to pass into each multiplexing chamber **42a-42f** through outlet **38** thereof. The fixed variable "experimentee" may take the form of a microbe, a cell species, a drug or antibiotic, a soluble factor or another factor. In addition, each variable well **64** may be loaded with a different "experimenting" variable which passes into the two multiplexing chambers **42a-42f** with which outlet **74** of variable well **64** communicates. The different "experimenting" variables may take the form of various microbes, cells, drugs, antibiotics, soluble factors or other factors. In this way, a user may simply and easily observe the interaction of the experimentee and experimenting variables within each of the multiplexing chambers **42a-42f**. It can be appreciated that platform **26** enables high-throughput screening of three interacting variables within a single multiplexing chamber **42a-42f** at a time with simplicity in readout.

Further, it is noted that due to configuration of the plurality of multiplexing chambers **42a-42f**, namely, the equilateral triangular configuration, the first, second and third vertices **52**, **56** and **60**, respectively, of each of the plurality of multiplexing chambers **42a-42f** are an equal distance apart, thereby allowing for the variables loaded into common well **28** and variable wells **64** to contribute equally in terms of interaction distance therebetween. Since the triangle is the most basic unit that can define a planar surface, the interaction network may be scaled out in both the x and y dimensions, as compared to a linear 2-way interaction network which is confined to only one dimension at a time in terms of geometrical design. Further, by utilizing a triangular configuration, a multiplexing chamber **42a-42f** may be joined to an adjacent multiplexing chamber **42a-42f** along a single side thereof which, in turn, allows the two adjacent multiplexing chambers **42a-42f** to share two wells, but have a single well isolated from the adjacent multiplexing chamber. This allows for a 3-factorial but single variable comparison between any two (2) neighboring triangular multiplexing chambers. It can be appreciated that microfluidic device **10** may be used to screen drug combinations that exert highest potency, find transcription factor combinations that show the highest stem cell differentiation efficiency, elucidate cell-cell or microbe interactions mechanisms, optimize chemical factors for cell culture, and multi-cell/organ drug metabolism.

By way of example, it can be understood that device **10** is amenable to solid culture and liquid culture or a combination of both. More specifically, for solid culture of prokaryotic cells (such as bacteria), variable wells **64** and multiplexing chambers **42a-42f** may be filled with a warm agar solution including culture media (for example, Luria

Broth (LB), Tryptic Soy Broth (TSB), Mueller Hinton Broth (MHB), or the like). The warm agar solution is allowed to solidify at room temperature or lower, e.g. 4° C. Device **10** filled with the agar solution can be stored for an extended period of time before use. Thereafter, bacteria may be inoculated by pipetting a bacteria solution onto the solid agar surface at opening **51** of a corresponding multiplexing chamber **42a-42f** and thereafter cultured therein with device **10** facing either up or down. The experimenting variables such as drugs, antibiotics, other soluble factors or cells are added to device **10** via pipetting the solution on the solid agar surface at openings **70** of variable wells **64** and allowing the solution to absorb/diffuse therein.

For solid culture of eukaryotic cells (such as mammalian cells), variable wells **64** and multiplexing chambers **42a-42f** can be filled as heretofore described with a hydrogel solution (such as collagen, matrigel, polyethylene glycol (PEG) gels, or the like) including culture media (such as Dulbecco's Modified Eagle's medium (DMEM), Roswell Park Memorial Institute medium (RPMI), or the like) and allowed to solidify to form a gel matrix. Cells may be premixed with the gel and loaded together into device **10** or seeded on top of the solidified gel matrix at opening **51** of a corresponding multiplexing chamber **42a-42f** and/or openings **70** of variable wells **64**.

For a mixed culture of eukaryotic cells and prokaryotic cells, a first portion of variable wells **64** may be loaded with eukaryotic cell-compatible gels such as collagen, matrigel, PEG gels, or the like, while a second portion of variable wells **64** may be loaded with prokaryotic cell-compatible gels such as agar. It is noted that for this "mixed gel" type of culture, one gel has to be fully solidified prior to adding the other gel to prevent the mixing thereof.

For a mixed solid and liquid co-culture system, either variable wells **64** or multiplexing chambers **42a-42f** may be preloaded as heretofore described with a solid gel matrix prior to loading a liquid media in order to prevent direct liquid convection between variable wells **64**. In other words, either variable wells **64** or multiplexing chambers **42a-42f** can receive the solid gel matrix, but the solid gel matrix has to be loaded first and allowed to solidify prior to loading the liquid media. For example, for a solid prokaryotic cell co-culture with eukaryotic cells in liquid, variable wells **64** are first loaded with a solid agar gel, followed by adding liquid eukaryotic cell culture media into multiplexing chambers **42a-42f**. The eukaryotic cells are then seeded into multiplexing chambers **42a-42f** including liquid cell culture media, while the prokaryotic cells are seeded on top of the solid agar matrix at openings **70** in variable wells **64** and allowed to adhere.

It is noted that the experimenting variables such as drugs, antibiotics, other soluble factors can be added to variable wells **64** in a variety of ways. As noted above, if variable wells **64** are preloaded with a solid culture gel such as agar or collagen, then the experimenting variable can be added on top of the solid culture gel at opening **70** of a corresponding variable well **64** and allowed to absorb or diffuse into the solid culture gel. Alternatively, an experimenting variable or variables may be mixed with the liquid gel solution prior to loading in a corresponding variable well **64**. Thereafter, the mixture may be loaded into the corresponding variable well **64**. The experimenting variable-infused gels can be stored for an extended period of time without cross-contamination/mixing as long as the corresponding multiplexing chambers **42a-42f** are left empty and not filled with liquid or solid media. In this manner, diffusion of the experimenting variable into the corresponding multiplexing chamber **42a-42f** is



only initiated upon the adding of liquid/solid media into the corresponding multiplexing chamber 42a-42f.

If variable wells 64 are filled with a liquid media/reagent such as phosphate-buffered saline (PBS), LB or DMEM, the experimenting variables may be added to a corresponding variable well 64 by pipetting the experimenting variables into the liquid media. However, such liquid media are less amenable to long term storage and transportation when received with device 10. In order to overcome this limitation, the liquid media and/or the experimenting variables may be dried by desiccation or lyophilization inside device 10 after loading. After drying, the dried liquid media inside variable wells 64 can be stored for an extended period of time without cross-contamination/mixing therebetween as long as corresponding multiplexing chambers 42a-42f are left empty and not filled with a liquid or a solid media. To "re-activate" the dried liquid media, water or other liquid/solid media solutions may be added to a corresponding variable well 64 to re-dissolve the liquid media therein. As described above, diffusion into multiplexing chambers 42a-42f is only initiated upon the filing of multiplexing chambers 42a-42f with a liquid/solid media.

It is contemplated to affix a removable membrane to upper surface 22 of base 12 which overlaps openings 70 to variable wells 64 to isolate the media inside variable wells 64 from the external embodiment during storage. Similarly, a removable membrane may be affixed to lower surface 24 of base 12 which overlaps openings 51 to multiplexing chambers 42a-42f to further isolate the dried liquid media inside variable wells 64 from the external embodiment during storage.

Referring to FIGS. 2a-2b, using the design principles of the microfluidic device 10 described above, it can be appreciated that the scale of platform 26 may be expanded to include a higher number of variable wells 64 and multiplexing chambers, generally designated by the reference numeral 42. By way of example, platform 26 may be expanded to provide for a scaled-out symmetrical hexagonal design, FIG. 2a or scaled-out in a single direction, FIG. 2b. It can be appreciated that multiplexing chambers 42 are identical in structure to microfluidic chambers 42a-42f, and as such, the prior description of microfluidic chamber 42a is understood describe multiplexing chamber 42 as if fully described herein. The choice of the configuration depends on the scale of the experiment (number of combinations required) and the distance of interactions in question.

Referring to FIG. 7, an alternate embodiment of a microfluidic device in accordance with the present invention is generally designated by the reference numeral 100. As hereinafter described, microfluidic device 100 incorporates multiple platforms 101 for testing the antibiotic sensitivity of a polymicrobial infection. Microfluidic device 100 includes first and second layers 102 and 104, respectively, FIG. 7. Referring to FIGS. 7-9, first layer 102 is formed from a polymeric material (e.g., polystyrene) and includes upper and lower surfaces 106 and 108, respectively, interconnected by first and second ends 110 and 112, respectively, and first and second sides 114 and 118, respectively. A plurality of wells 120 are provided in upper surface 106. In the depicted embodiment, the plurality of wells 120 are arranged in two rows and seven columns. However, the number and arrangement of the plurality of wells 120 in upper surface 106 of first layer 102 may be varied without deviating from the scope of the present invention.

Each of the plurality of wells 120 includes an opening 122 communicating with upper surface 106 of first layer 102 and is defined by a generally planer lower surface 124 spaced

from upper surface 106 of first layer 102 by a sidewalls 126a-126d and generally parallel to lower surface 108 of first layer 102. In the depicted embodiment, sidewalls 126a-126d have identical depths D and identical widths W. However, the depths and widths of sidewalls 126a-126d may be varied without deviating from the scope of the present invention. In addition, sidewall 126a and sidewall 126c are generally parallel to each other and perpendicular to sidewalls 126b and 126d. Similarly, sidewall 126b and sidewall 126d are generally parallel to each other and perpendicular to sidewalls 126a and 126c. As described, well 120 has a generally square configuration in cross-section. A media inlet 128 extends between lower surface 124 of each of the plurality of wells 120 and lower surface 108 of first layer 102 at a location adjacent the intersection of sidewalls 126a and 126b. In addition, an air outlet 130 extends between lower surface 124 of the plurality of wells 120 and lower surface 108 of first layer 102 at a location adjacent the intersection of sidewalls 126a and 126b.

Referring to FIG. 9a, an alternate construction of the plurality of wells in first layer 102 is generally designated by the reference numeral 120a. Each of the plurality of wells 120a includes an opening 122a extending through first layer 102. Opening 122a has a first end 123 communicating with upper surface 106 of first layer 102 and a second end 125 communicating with lower surface 108 of first layer 102. Sidewalls 127a-127d define opening 122a and are generally perpendicular to upper and lower surfaces 106 and 108, respectively, of first layer 102. It is contemplated for sidewalls 127a-127d have identical depths D and identical widths W. However, the depths and widths of sidewalls 127a-127d may be varied without deviating from the scope of the present invention. In addition, sidewall 127a and sidewall 127c are generally parallel to each other and perpendicular to sidewalls 127b and 127d. Similarly, sidewall 127b and sidewall 127d are generally parallel to each other and perpendicular to sidewalls 127a and 127c. As described, each of the plurality of wells 120a has a generally square configuration in cross-section.

Referring to FIGS. 7 and 10-11, second layer 104 is formed from a polymeric material (e.g., polystyrene) and includes upper and lower surfaces 132 and 134, respectively, interconnected by first and second ends 136 and 138, respectively, and first and second sides 140 and 142, respectively. A plurality of sets 144 of chambers 146a-146d extend through second layer 104 between the upper and lower surfaces 132 and 134, respectively, thereof. It is intended for the number of the plurality of sets 144 of chambers 146a-146d in second layer 104 to correspond to the number of the plurality of wells 120 in upper surface 106 of first layer 102. As such, in the depicted embodiment, the plurality of sets 144 of chambers 146a-146d are arranged in two rows and seven columns.

In the depicted embodiment, each set 144 of chambers 146a-146d includes four chambers 146a-146d of identical configuration and proportion arranged in two rows and two columns. However, the number and configuration of chambers 146a-146d may be varied, as desired. Further, in view of the foregoing, it can be understood that the description of chamber 146a hereinafter provided describes chambers 146b-146d as if fully described herein. Chamber 146a includes an upper opening 148 communicating with upper surface 132 of second layer 104 and a lower opening 150 communication with lower surface 134 of second layer 104. Sidewalls 154a-154d extending between upper surface 132 and lower surface 134 of second layer 104 so as to define chamber 146a. Sidewalls 154a-154d have identical depths D



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and identical widths W1. In addition, sidewall 154a and sidewall 154c are generally parallel to each other and perpendicular to sidewalls 154b and 154d. Similarly, sidewall 154b and sidewall 154d are generally parallel to each other and perpendicular to sidewalls 154a and 154c.

In order to construct platform 101 of microfluidic device 100, first and second layers 102 and 104, respectively, are positioned such that lower surface 134 of second layer 104 is directed at upper surface 106 of first layer 102, FIG. 7. Permeable membrane 160 is positioned between lower surface 134 of second layer 104 is directed at upper surface 106 of first layer 102. By way of example, permeable membrane 160 may take the form of a 0.2 micrometer (μm) porous polycarbonate membrane. Thereafter, first and second layers 102 and 104, respectively, are bonded together in any conventional manner such that first and second ends 136 and 138, respectively, and first and second sides 140 and 142, respectively, of second layer 104 are aligned with first and second ends 110 and 112, respectively, and first and second sides 114 and 118, respectively, of first layer 102, thereby capturing permeable member 160 therebetween.

With first and second layers 102 and 104, respectively, bonded together as heretofore described, each set 144 of chambers 146a-146d is aligned with a corresponding one of the plurality of wells 120, FIGS. 6 and 12a-12d, or alternatively, with one of the plurality of wells 120a, FIGS. 8a and 9a. With each set 144 of chambers 146a-146d aligned with a corresponding one of the plurality of wells 120, sidewalls 154a of chambers 146a and 146b of each set 144 of chambers 146a-146d are generally co-planar with sidewall 126a of a corresponding well 120 of each of the plurality of wells 120; sidewalls 154b of chambers 146b and 146c of each set 144 of chambers 146a-146d are generally co-planar with sidewall 126b of a corresponding well 120 of each of the plurality of wells 120; sidewalls 154c of chambers 146c and 146d of each set 144 of chambers 146a-146d are generally co-planar with sidewall 126c of a corresponding well 120 of each of the plurality of wells 120; and sidewalls 154d of chambers 146d and 146a of each set 144 of chambers 146a-146d are generally co-planar with sidewall 126d of a corresponding well 120 of each of the plurality of wells 120. Permeable member 160 separates each set 144 of chambers 146a-146d for a corresponding well 120 of each of the plurality of wells 120.

Alternatively, with each set 144 of chambers 146a-146d is aligned with a corresponding one of the plurality of wells 120a, sidewalls 154a of chambers 146a and 146b of each set 144 of chambers 146a-146d are generally co-planar with sidewall 127a of a corresponding well 120a of each of the plurality of wells 120a; sidewalls 154b of chambers 146b and 146c of each set 144 of chambers 146a-146d are generally co-planar with sidewall 127b of a corresponding well 120a of each of the plurality of wells 120a; sidewalls 154c of chambers 146c and 146d of each set 144 of chambers 146a-146d are generally co-planar with sidewall 127c of a corresponding well 120a of each of the plurality of wells 120a; and sidewalls 154d of chambers 146d and 146a of each set 144 of chambers 146a-146d are generally co-planar with sidewall 127d of a corresponding well 120a of each of the plurality of wells 120a. Permeable member 160 separates each set 144 of chambers 146a-146d for a corresponding well 120a of each of the plurality of wells 120a.

In operation, different microbes are provided in each chamber 146a-146d of each set 144 of chambers 146a-146d. The microbes may take the form of bacteria, viruses, fungi, yeasts, parasites, antibiotics or a combination thereof. By

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way of example, different bacterial strains (e.g., bacterial strains 162 and 164 in FIGS. 12a-12d) may be provided or cultured in media 166 in each chamber 146a-146d of each set 144 of chambers 146a-146d. For example, a small volume (10 μL) of four samples of bacterial strains from the same patient may be provided or cultured individually in each chamber 146a-146d. Permeable membrane 160 is specifically chosen so that media 166 from chambers 146a-146d of each set 144 of chambers 146a-146d does not flow through permeable membrane 160 into a corresponding well 120 or 120a when the corresponding well 120 or 120a is empty (in other words, filled with air), FIGS. 12a and 12b. When utilizing the plurality of wells 120, each of the plurality of wells 120 may be filled through media inlets 128 with different medias, e.g., different antibiotic medias, collectively designated by the reference numeral 168, FIG. 12c. It can be appreciated that microfluidic device 100 may be flipped upside down to facilitate filling of the plurality of wells 120 given that the surface tension of media 166 in chambers 146a-146d of each set 144 of chambers 146a-146d retains media 166 therein. Alternatively, wherein utilizing the plurality of wells 120a, each of the plurality of wells 120a may be filled through second end 125 of opening 122a with different medias, e.g., different antibiotic medias, as heretofore described. It can be appreciated that microfluidic device 100 may be flipped upside down to facilitate filling of the plurality of wells 120a given that the surface tension of media 166 in chambers 146a-146d of each set 144 of chambers 146a-146d retains media 166 therein.

Once the plurality of wells 120 or 120a with different medias 168 (e.g., different antibiotic therapies), each of the plurality of wells 120 or 120a serves as a liquid pool that fluidically connects each of chambers 146a-146d of a corresponding set 144 of chambers 146a-146d through permeable membrane 160. For example, with well 120 filled with media 168, bacteria 162 and 164 in chambers 146a-146d of the corresponding set 144 of chambers 146a-146d will be in soluble factor contact through diffusion, FIG. 12d. The structure of permeable membrane 160 is intended to be efficient in preventing bacteria migration, while providing sufficiently fast diffusion. By analyzing the different media 168 in each of the plurality of wells 120 or 120a, the susceptibility of the combination of specific bacterial pathogens cultured in chambers 146a-146d to various antibiotic therapies provided in the plurality of wells 120 or 120a may be simply and easily assessed.

As described, microfluidic device 100 allows for the simultaneous testing of various antibiotic therapies to be performed on a combination of specific microbes provided in sets 144 of chambers 146a-146d. It can be understood that the number of chambers in each set 144 of chambers 146a-146d may be increased or decreased to correspond to the number of microbes in a desired combination. Further, it can be appreciated the open-microfluidic nature enables unique advantages in accessibility, allowing the microbes, e.g. bacteria 162 and 164, to be easily recollected for traditional antibiotic sensitivity or antibiotic susceptibility measures or biofilm assessment.

Further, it is contemplated to pre-load the plurality of wells 120 or 120a with a selected antibiotic so as to provide microfluidic device 100 as a pre-packaged kit to test different microbes, e.g., gram negative and positive bacteria, thereagainst. For example, the plurality of wells 120 or 120a may be preloaded with: penicillins, including amoxicillin+/-clavulanate, ampicillin+/-sulbactam, and piperacillin+/-tazobactam; cephalosporins, including cefepime, cefoxitin, cefazolin, and ceftriaxone; carbapenems, including meropen-



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nem and ertapenem; monobactams, including aztreonam; fluoroquinolones, including ciprofloxacin; aminoglycosides, including gentamicin; macrolides, including azithromycin; and others, including vancomycin, clindamycin, rifampin, trimethoprim+/-sulfamethoxazole and tetracycline. A removable membrane may be affixed lower surface 108 of first layer 102 of which overlaps openings 122a of the plurality of wells 120a to isolate the media within the plurality of wells 120a from the external embodiment during storage. Similarly, a removable membrane may be affixed to upper surface 132 of second layer 104 which overlaps openings 148 of chambers 146a-146d to further isolate the media inside the plurality of wells 120 or 120a from the external embodiment during storage. When using the micro-fluidic device 100, any removable membranes affixed thereto may be removed thereby allowing a user to load different microbes in each chamber 146a-146d of each set 144 of chambers 146a-146d, as heretofore described, to test the gram negative and positive bacteria against the pre-loaded antibiotic.

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 platform for multi-variable combinational screening, comprising:

a body defining:

a first multiplexing chamber having an outer periphery; and

a plurality of first multiplexing wells spaced about the outer periphery of the first multiplexing chamber, each first multiplexing well spaced from an adjacent first multiplexing well by a uniform distance

wherein:

the first multiplexing chamber has generally triangular configuration defined by three sides of generally equal length, each side of the first multiplexing chamber intersecting an adjacent side of the first multiplexing chamber at a corresponding vertex;

each of the plurality of first multiplexing wells are provided at a corresponding vertex of the triangular configuration of the first multiplexing chamber, and

the first multiplexing chamber is defined by:

a first segment extending axially along a first side of the triangular configuration and interconnecting a first well of the plurality of first multiplexing wells and a second well of the plurality of first multiplexing wells;

a second segment extending axially along a second side of the triangular configuration and interconnecting the second well of the plurality of first multiplexing wells and a third well of the plurality of first multiplexing wells; and

a third segment extending axially along a third side of the triangular configuration and interconnecting the first well of the plurality of first multiplexing wells and the third well of the plurality of first multiplexing wells.

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2. The platform of claim 1 wherein the body further defines a second multiplexing chamber having an outer periphery, the second multiplexing chamber communicating with at least one of the plurality of first multiplexing wells along the outer periphery of the second multiplexing chamber.

3. The platform of claim 2 wherein the second well of the plurality of first multiplexing wells communicates with the second multiplexing chamber along the outer periphery of the second multiplexing chamber.

4. The platform of claim 2 wherein the second multiplexing chamber has a generally triangular configuration defined by three sides of generally equal length, each side of the second multiplexing chamber intersecting an adjacent side of the second multiplexing chamber at a corresponding vertex.

5. The platform of claim 4 wherein the first and second wells of the plurality of first multiplexing wells and the first segment communicate with the second multiplexing chamber along corresponding vertices and a first side of the second multiplexing chamber.

6. A platform for multi-variable combinational screening, comprising:

a body defining:

a central well; and

a plurality of multiplexing chambers extending radially from the central well, each of the multiplexing chambers:

having an identical, generally triangular configurations;

is defined by first, second and third sides and first, second and third vertices, wherein the central well defines the first vertex of each of the multiplexing chambers;

corner wells defining the second and third vertices of each of the multiplexing chambers, the corner well at the third vertex of one of the multiplexing chambers defining the corner well at the second vertex of an adjacent one of the multiplexing chambers;

a first segment extending axially along a first side of the triangular configuration and interconnecting the central well at the first vertex and the corner well at the second vertex;

a second segment extending axially along a second side of the triangular configuration and interconnecting the central well at the first vertex and the corner well at the third vertex; and

a third segment extending axially along a third side of the triangular configuration and interconnecting the corner well at the second vertex and the corner well at the third vertex.

7. The platform of claim 6 wherein each of the first, second and third sides of each of the multiplexing chambers have lengths, the lengths of the first, second and third sides of each of the multiplexing chambers being generally equal.

8. The platform of claim 6 wherein each of the plurality of multiplexing chambers has an equilateral triangular configuration.

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