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(71) Applicant: **PRESIDENT AND FELLOWS OF HARVARD COLLEGE** [US/US]; 17 Quincy Street, Cambridge, MA 02138 (US).

(72) Inventors: **WEITZ, David A.**; 213 Green Road, Bolton, MA 01740 (US). **DELAHANTY, Aaron Brace**; 180 N. Beacon Street, Brighton, MA 02135 (US). **DIDIER, Jonathan E.**; 24 Bay State Road #9, Cambridge, MA 02138 (US). **NOVAK, Richard**; 42 Rockview Street, Apt. 4, Jamaica Plain, MA 02130 (US). **XIN, Feng**; Tianjin University, Building 22, New Garden Village, Tianjin, 300072

(CN). **NAWAR, Saraf**; 894 Main Street, Unit 1, Wakefield, MA 01880 (US). **INGRAM, Miles**; 26 Inman Street, Cambridge, MA 02139 (US). **PITTI, Carlos Ng**; 82 S. Huntington Avenue, Apt. 12, Jamaica Plain, MA 02130 (US). **CALAMARI, Elizabeth**; 15 Upland Road, Ashland, MA 01721 (US). **NESTOR, Bret Andrew**; 1506 Lakeridge Drive N, Regina, Saskatchewan, S4X4L5 (CA). **GROEVER, Benedikt**; 45 Harrison Street, Somerville, MA 02143 (US).

(74) Agent: **CHEN, Tani**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

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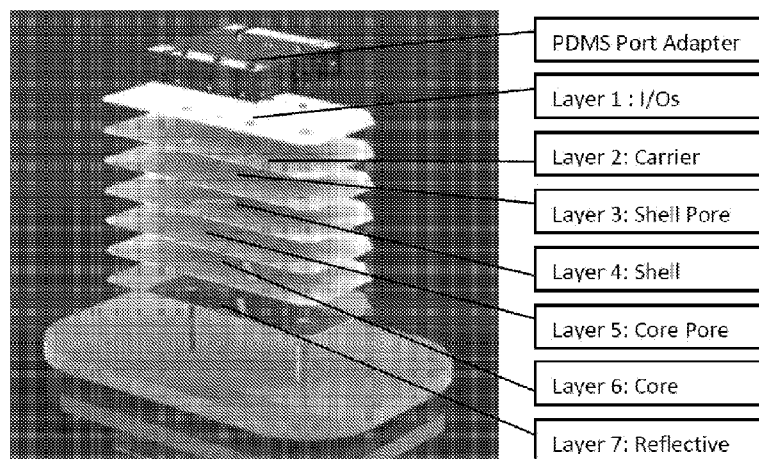


FIG. 1

(57) Abstract: The present invention generally relates to microfluidic devices, and techniques for making and using such devices. In some embodiments, the microfluidic device may be formed from two or more layers or plates, which can be assembled together. For example, the layers may include various channels that pass between different layers or plates. In some cases, the layers may be treated (e.g., with different coatings), for example, prior to assembly. This may allow for relatively precise control of different surface characteristics (e.g., hydrophilicity) within different portions of the microfluidic device. Such devices can be used in some embodiments to control or manipulate fluids, for example, to form droplets, or for other applications. Still other embodiments are generally directed to systems and methods for making and using such devices, kits including such devices, or the like.



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LAYERED MICROFLUIDIC DEVICES AND TECHNIQUES**RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Patent Application Serial No. 62/637,622, filed March 2, 2018, entitled "Layered Microfluidic Devices and Techniques," by Weitz, *et al.*, incorporated herein by reference.

FIELD

The present invention generally relates to microfluidic devices, and techniques for making and using such devices.

BACKGROUND

Microdroplets or microcapsules with controlled size, shape, chemistry, and/or mechanical properties have great value for multiple applications ranging from, e.g., controlled drug delivery to cosmetics. Thus, microfluidic devices have been developed to discretize fluids (including liquids, gases, colloids, and suspensions) in controlled, low-dispersity dimension volumes. Droplet generators have been used to partition liquid into droplets inside an immiscible carrier fluid such as oil. Higher-order emulsions, such as double emulsions, have been made using a similar approach, but often include additional fluidic connections and microfluidic junctions. While these devices have been demonstrated to work, scalability of such devices may be an issue. In order to facilitate commercialization of fluid partitioning applications, including double emulsions, manufacturable or robust devices having easily integrated surface modification would be desirable.

SUMMARY

The present invention generally relates to microfluidic devices, and techniques for making and using such devices. The subject matter of the present invention involves, in some cases, interrelated products, alternative solutions to a particular problem, and/or a plurality of different uses of one or more systems and/or articles.

In one aspect, the present invention is generally directed to a microfluidic apparatus. In one set of embodiments, the microfluidic apparatus, comprises a first lateral microfluidic channel defined by a first layer; a second lateral microfluidic channel, positioned substantially parallel to the first channel, defined by a second layer; a third lateral microfluidic channel, positioned substantially parallel to the first channel and the second channel, defined by a third layer; a first transverse microfluidic channel, defined by at least the first layer and the second layer, in fluid communication with the first lateral microfluidic channel and the second lateral microfluidic channel; and a second transverse microfluidic

channel, defined by at least the second layer and the third layer, in fluid communication with the second lateral microfluidic channel and the third lateral microfluidic channel.

The microfluidic apparatus, in accordance with another set of embodiments, comprises a first substantially planar layer, defining a first lateral microfluidic channel; and a second substantially planar layer, defining a second lateral microfluidic channel. In some cases, the second layer has a substantially identical composition to the first layer and is positioned substantially parallel to the first layer. In certain embodiments, the first lateral microfluidic channel has a first surface characteristic and the second lateral microfluidic channel has a second surface characteristic different from the first hydrophobicity. The apparatus may also comprise a transverse microfluidic channel, defined by and fluidly communicating between the first layer and the second layer, in fluid communication with the first lateral microfluidic channel and the second lateral microfluidic channel.

In one set of embodiments, the microfluidic apparatus comprises a plurality of at least three substantially planar layers, positioned in liquid-tight contact with each other. In some cases, the plurality of layers defines a junction of first, second, third, and fourth microfluidic channels. In certain embodiments, the first microfluidic channel and the second microfluidic channels are defined by a first layer, and the third microfluidic channel and the fourth microfluidic channel are each defined by and fluidly communicate between the first layer and at least one additional layer.

The microfluidic apparatus, in another set of embodiments, comprises a plurality of at least four substantially planar layers, positioned in liquid-tight contact with each other. In certain instances, at least one microfluidic channel passes substantially orthogonally through at least three of the planar layers.

In yet another set of embodiments, the microfluidic apparatus comprises a first substantially planar layer, defining a first lateral microfluidic channel; a second substantially planar layer, positioned substantially parallel to the first layer, defining a second lateral microfluidic channel; a third substantially planar layer, positioned substantially parallel to the first layer and the second layer, defining a third lateral microfluidic channel; a first transverse microfluidic channel, defined by and fluidly communicating between the first layer and the second layer, in fluid communication with the first lateral microfluidic channel and the second lateral microfluidic channel; and a second transverse microfluidic channel, defined by and fluidly communicating between the second layer and the third layer, in fluid communication with the second lateral microfluidic channel and the third lateral microfluidic channel.

In another aspect, the present invention is generally directed to a method. In one set of embodiments, the method includes flowing a first fluid through a first lateral microfluidic channel defined by a first substantially planar layer; flowing the first fluid into a transverse microfluidic channel, defined by and fluidly communicating between the first layer and a second substantially planar layer, wherein the second layer is positioned substantially parallel to the first layer; flowing a second fluid, through a second lateral microfluidic channel defined by the second layer, to contact the first fluid in the transverse microfluidic channel; and forming droplets of the first fluid, contained within the second fluid.

In another set of embodiments, the method comprises flowing a first fluid through a first lateral microfluidic channel defined by a first substantially planar layer; flowing the first fluid into a first transverse microfluidic channel, defined by and fluidly communicating between the first layer and a second substantially planar layer, wherein the second layer is positioned substantially parallel to the first layer; flowing a second fluid, through a second lateral microfluidic channel defined by the second layer, to contact the first fluid in the first transverse microfluidic channel; forming droplets of the first fluid, contained within the second fluid; flowing the droplets and the second fluid into a second transverse microfluidic channel, defined by and fluidly communicating between the second layer and a third substantially planar layer, wherein the third layer is positioned substantially parallel to the first layer and the second layer; flowing a third fluid, through a third lateral microfluidic channel defined by the third layer, to contact the second fluid in the second transverse microfluidic channel; and forming double emulsion droplets, comprising droplets of the first fluid contained within droplets of the second fluid, contained within the third fluid.

The method, in yet another set of embodiments, includes flowing a first fluid through a first microfluidic channel defined by a first substantially planar layer; flowing the first fluid through a transverse face of the first layer into the transverse face of a second substantially planar layer, wherein the second layer is positioned substantially parallel to the first layer; flowing a second fluid, through a second microfluidic channel defined by the second layer, to contact the first fluid; and forming droplets of the first fluid, contained within the second fluid.

In another aspect, the present invention encompasses methods of making one or more of the embodiments described herein, for example, a microfluidic device, such as a layered microfluidic device. In still another aspect, the present invention encompasses methods of using one or more of the embodiments described herein, for example, a microfluidic device, such as a layered microfluidic device.

Other advantages and novel features of the present invention will become apparent from the following detailed description of various non-limiting embodiments of the invention when considered in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Non-limiting embodiments of the present invention will be described by way of example with reference to the accompanying figures, which are schematic and are not intended to be drawn to scale. In the figures, each identical or nearly identical component illustrated is typically represented by a single numeral. For purposes of clarity, not every component is labeled in every figure, nor is every component of each embodiment of the
10 invention shown where illustration is not necessary to allow those of ordinary skill in the art to understand the invention. In the figures:

Figs. 1 and 2 illustrate a device in accordance with one embodiment of the invention;

Figs. 3 and 4 are schematic diagrams of certain devices according to various
embodiments of the invention, which can be used to produce double emulsion droplets
15 comprising a core fluid and a shell fluid;

Figs. 5A-5C illustrate various double emulsion droplets produced using certain
embodiments of the invention;

Figs. 6A-6B illustrate different channels produced in layers in accordance with some
embodiments of the invention;

20 Figs. 7A-7G illustrate other devices according to various embodiments of the
invention;

Fig. 8 illustrates one embodiment of the invention in which an array of droplet
generators are contained within a single device;

Fig. 9 are schematic diagrams of a device according to another embodiment, which
25 can be used to produce double emulsion droplets; and

Figs. 10A-10C a device for forming double emulsion droplets, in still another
embodiment of the invention.

DETAILED DESCRIPTION

30 The present invention generally relates to microfluidic devices, and techniques for
making and using such devices. In some embodiments, the microfluidic device may be
formed from two or more layers, which can be assembled together. For example, the layers
may include various channels that pass between different layers. In some cases, the layers
may be treated (e.g., with different coatings), for example, prior to assembly. This may allow
for relatively precise control of different surface characteristics (e.g., hydrophilicity) within

different portions of the microfluidic device. Such devices can be used in some embodiments to control or manipulate fluids, for example, to form droplets, or for other applications. Still other embodiments are generally directed to systems and methods for making and using such devices, kits including such devices, or the like.

5 One aspect of the invention is now described with reference to Fig. 7A. In this figure, microfluidic device 10 is composed of various layers. Although five layers are shown in this figure, it should be understood that this is by way of example only, and that in other embodiments, more or fewer layers may be used. In this figure, a second layer 21 is shown having a first lateral channel 22, while a fourth layer 41 has a second lateral channel 42. In
10 addition, between second layer 21 and fourth layer 41 is a first transverse channel or pore 32, which is partially defined within the second layer, the third layer, and the fourth layer. Similarly, between second layer 21 and fifth layer 51 is second transverse channel 52, partially defined in the second layer, the third layer, the fourth layer, and the fifth layer.

 It should be understood that channels defined within a layer need not necessarily be
15 defined completely internally within the layer. For example, as is shown in Fig. 7B, a channel may be defined partially within a layer, such that, when a first layer 11 is positioned next to a second layer 21, a channel 12 is defined. Thus, for example, channel 12 in first layer 11 may be defined in an outer surface of the layer. In some embodiments, the second layer may also have a complementary channel in it, but in some cases, no complementary
20 channel is present as is shown in this figure, e.g., the second layer may be substantially flat, such that upon assembly, a substantially closed channel can be formed.

 It will be noted that between each layer, the transverse channels may be aligned to allow fluid communication to occur between the various layers. For example, in Fig. 7A, first lateral channel 52 in layer 21, the first transverse channel 32 in layer 31, and the second
25 lateral channel 53 in layer 41 in Fig. 7A may be substantially aligned to allow fluid flow to occur. In addition, although first lateral channel 52 and second lateral channel 53 are shown to be substantially aligned in this figure, e.g., forming a continuous channel that spans all five layers 11, 21, 31, 41, and 51 this is by way of example only, and that different channels may span some or all of the layers, without necessarily being all aligned with each other. Any
30 combination of lateral, transverse, and/or other channels may be present within the device.

 The various layers within the device may be bonded together, e.g., in liquid-tight contact so as to prevent dripping of fluid from the channels, for instance, through the seams between the layers. Techniques for creating such liquid-tight contacts include applying pressure, bonding the layers together with an adhesive, or other techniques such as those

described herein. The layers may be formed from polymers such as cyclic olefin copolymers, polyethylene terephthalate, polymethyl methacrylate or the like, e.g., as discussed below. In some cases, the layers may be substantially transparent. The layers may also be substantially flat or planar, e.g., as shown in this figure, although in some cases, the layers are non-planar, but can have complementary surfaces that can be assembled in liquid-tight contact with each other.

In one set of embodiments, the layers may be treated in some fashion, e.g., prior to assembly. This may allow for relatively precise control of various surface characteristics of the microfluidic channels within the device. For example, first lateral channel 52 may have a first surface characteristic within first layer 11 (for example, a hydrophobic surface), but a second surface characteristic within layer 31 (for example, a hydrophilic surface). Other surface characteristics include applied coatings, surface roughness, or the like, e.g., as discussed in more detail below. In addition, it should be understood that one of the possible surface characteristics in the discussions herein may simply be the normal surface characteristics of the material forming the layer, i.e., without any treatment to change those surface characteristics.

In another non-limiting example, as is shown in Fig. 7C, transverse channel 18 may be defined in and pass between layers 11, 21, and 31, where layer 21 has been treated in some fashion to alter its surface characteristics, relative to layers 11 and 31. For instance, layer 21 may have a coating 17 that allows it to have different surface characteristics. As an example, coating 17 may include a species comprising a protein, an antibody, a nucleic acid, or another binding species that can bind to a species contained within fluid flowing through transverse channel 18. Due to the use of different layers in this example, the region where the binding species is present within the channel can be precisely controlled (for example, by applying the species to the channel before fabrication of the channel), e.g., to a small or relatively well-defined portion of the channel. In some embodiments, this may allow the control of a coating within a channel to a degree that is much more precise than can be achieved by simply flowing a species throughout the entire channel.

Such devices can be used in a variety of applications, e.g., involving microfluidic channels, some of which are discussed in more detail below. As an example, one embodiment of the invention is now described for the creation of droplets. Referring again to Fig. 7A, droplets 16 may be created by introducing a first fluid 15 from a suitable source of fluid into first lateral channel 22, and a second fluid 25 from a suitable source of fluid into second lateral channel 42. In this example, first fluid 15 may be substantially immiscible

with second fluid 25, and may be used to form droplets 16 within the second fluid, e.g., at intersection 33.

First fluid 15 may flow into one or more first lateral channels 22 within second layer 21, eventually reaching intersection 23 with transverse pore or channel 32 and flowing into
5 fourth layer 41. First fluid 15 encounters second fluid 25 flowing through second lateral channel 42, meeting at intersection 33. At that intersection, first fluid 15 may form droplets 16, contained within second fluid 25, e.g., due to the immiscibility between first fluid 15 and second fluid 25. Droplets 16, contained within the second fluid, may then exit through second lateral channel 42, entering into fifth layer 51, and exiting the device by transverse
10 channel 53.

A similar system may also be used to create double-emulsion droplets, as is illustrated in the example shown in Fig. 7D. In this figure, in addition to first fluid 15 and second fluid 25, a third fluid 35 is introduced from a suitable source of fluid into third lateral channel 62. Third fluid 35 may be substantially immiscible with second fluid 25, and may be the same or
15 different than first fluid 15. In this figure, double-emulsion droplets may be created as second fluid 25 (which may be in coaxial flow with the first fluid 16) enters intersection 63 from second lateral channel 42 and contact third fluid 35 entering from third lateral channel 62. Due to the immiscibility between second fluid 25 and third fluid 35, droplets 26 of second fluid 25 are formed within third fluid 35. The droplets 26 may contain droplets of
20 first fluid 15 as the co-axial flow 16 of the second fluid may break up simultaneously with fluid 25 at intersection 63, forming double-emulsion droplets (i.e., droplet-within-a-droplet), e.g., as is shown in this figure. As another example, by varying composition of fluid 15 and fluid 25 and relative flowrates, initial droplets of fluid 15 may form within fluid 25 at junction 43, these droplets may then be present singularly or in a plurality within droplet 26,
25 forming double-emulsion or multi-core emulsion droplets.

In addition, it should be understood that, depending on factors such as the microfluidic channel sizes and the relative flowrates of the first, second, and third fluids, different efficiencies, and rate of production of droplets and/or double-emulsion droplets may be formed. For example, some or all of the droplets that are produced may be single-
30 emulsion or double-emulsion droplets, some of the double-emulsion droplets may contain one or more than one droplet of the first fluid therein, etc.

The above discussion are various non-limiting examples of certain embodiments of the present invention. However, other embodiments are also possible in addition to those. Accordingly, more generally, various aspects of the invention are directed to various systems

and methods for microfluidic devices, especially layered microfluidic devices, including various techniques for making and using such devices.

For instance, one aspect of the present invention is generally directed to microfluidic devices that may be formed from one or more layers, some or all of which may contain
5 therein one or more microfluidic channels. The microfluidic channels may flow largely within a single layer (a lateral microfluidic channel), or may go between two or more layers (a transverse microfluidic channel).

In some cases, a microfluidic device may be fabricated from a plurality of layers. For example, the device may include any number of layers, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more
10 layers that are assembled together to produce the microfluidic device. In many prior art devices, microfluidic channels are defined within one or only a few layers, and do not pass between multiple layers of the device. For example, a device such as is shown in Fig. 7B may comprise a first layer 11 containing a microfluidic channel 12, and a second layer 21 defining the top of those channels. However, second layer 21 does not contain any
15 microfluidic channels, and simply defines the top of microfluidic channel 12 when placed in contact with first layer 11. Accordingly, microfluidic channel 12 is laterally defined within first layer 11, but does not pass into second layer 12. In contrast, in certain embodiments of the invention, one or more microfluidic channels within the device may pass through two, three, or more layers, for instance, as is shown in Figs. 3 or 4. It should be understood,
20 however, that not all of the microfluidic channels within the device have to pass through two or more layers; for instance, one or more channels may be laterally positioned within a single layer within the device.

In one set of embodiments, one or more of the layers may be planar, or at least substantially planar. For example, a substantially planar plate or layer may be generally box-
25 shaped or shaped like a rectangular solid. In one set of embodiments, at least one surface of the layer is substantially planar. In some cases, one of the dimensions of the layer may be smaller than the other orthogonal dimensions. For example, the smallest dimension may be less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, or less than 3% of the smallest of the other two orthogonal dimensions.

In some embodiments, the smallest dimension (e.g., the cross-sectional dimension or the thickness) may be less than 10 cm, less than 8 cm, less than 6 cm, less than 5 cm, less
30 than 4 cm, less than 3 cm, less than 2 cm, less than 1 cm, less than 9 mm, less than 8 mm, less than 7 mm, less than 6 mm, less than 5 mm, less than 4 mm, less than 3 mm, less than 2 mm, less than 1 mm, less than 900 micrometers, less than 800 micrometers, less than 700

micrometers, less than 600 micrometers, less than 500 micrometers, less than 400 micrometers, less than 300 micrometers, less than 200 micrometers, less than 100 micrometers, less than 10 micrometers, etc. In addition, in some cases, the smallest dimension may be at least about 10 micrometers, at least about 100 micrometers, at least about 200 micrometers, at least about 300 micrometers, at least about 400 micrometers, at least about 500 micrometers, at least about 600 micrometers, at least about 700 micrometers, at least about 800 micrometers, at least about 900 micrometers, at least about 1 mm, at least about 2 mm, at least about 3 mm, at least about 4 mm, at least about 5 mm, at least about 6 mm, at least about 7 mm, at least about 8 mm, at least about 9 mm, at least about 1 cm, at least about 2 cm, at least about 3 cm, at least about 4 cm, at least about 5 cm, at least about 6 cm, at least about 8 cm, at least about 10 cm, etc. In addition, the dimension may fall within any combination of these, e.g., the layer may have a thickness of between 100 micrometers and 500 micrometers, between 1 mm and 2 mm, between 1 cm and 3 cm, etc.

The two other dimensions may independently be the same or different. For example, the two dimensions may be substantially equal (i.e., forming a square), or the aspect ratio between the dimensions may be 1:2, 2:3, 3:4, etc. In addition, in some cases, one or both of the larger orthogonal dimension of the layer (e.g., its length or width) may be at least 1 cm, at least 2 cm, at least 3 cm, at least 4 cm, at least 5 cm, at least 6 cm, at least 8 cm, at least 9 cm, at least 10 cm, at least 15 cm, at least 20 cm, at least 25 cm, etc. In some embodiments, one or both of the larger orthogonal dimension of the layer may be less than 25 cm, less than 20 cm, less than 15 cm, less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 6 cm, less than 5 cm, less than 4 cm, less than 3 cm, less than 2 cm, etc. Additionally, combinations of any of these are also possible; for example, each of larger orthogonal dimensions may independently be between 5 cm and 15 cm, or between 20 cm and 25 cm, etc.

As mentioned, a plurality of layers may be present. The layers may all independently have the same, or different sizes. For example, various embodiments are generally directed to a microfluidic device comprising a plurality of substantially planar layers, e.g., assembled together as discussed herein. It should be understood, however, that the layers within the device need not be substantially planar or box-shaped. As non-limiting examples, the layers may be substantially curved, and/or have various protrusions or indentations, etc., although the layers may be able to be conformably assembled together, e.g., in liquid-tight fashion, at least proximate the region surrounding the channel.

The layers may be formed from any suitable material, and different layers within a device may independently be formed from the same or different materials. Non-limiting examples of suitable materials include metals (e.g., stainless steel), glass, or polymers or plastics, such as polydimethylsiloxane, cyclic olefin copolymers, or polyethylene terephthalate. In addition, in some cases, one or more of layers may each independently be transparent, translucent, or opaque. In some cases, some or all of the layers may each independently be coated, for example, with a reflective coating, metallic coating, an opaque coating, or the like.

In one set of embodiments, one or more of the layers may have defined therein a channel, such as a microfluidic channel. The microfluidic channel may be completely defined within the channel, or only partially defined within the channel. For example, in Fig. 7A, channel 12 runs through the interior of layer 11, while in Fig. 7B, channel 12 is an open channel that is defined on an outer surface of layer 11. In addition, as shown in this figure, channel 12 may also be defined by adding layer 21 to layer 11, as a portion of layer 21 will also define channel 12. It should be noted that in this figure, layer 21 does not contain a channel complementary to channel 12; the planar surface of layer 21 is sufficient to define the channel. In other embodiments, however, layer 21 may also contain a complementary channel to channel 12.

As mentioned, in some, but not all embodiments, the systems and methods described herein may include one or more microfluidic components, for example, one or more microfluidic channels. A microfluidic channel may have a cross-sectional dimension of less than 2 mm or 1 mm, and a ratio of length to largest cross-sectional dimension of at least 3:1. The “cross-sectional dimension” of the channel is measured perpendicular to the direction of fluid flow within the channel. Thus, some or all of the fluid channels in microfluidic embodiments of the invention may have maximum cross-sectional dimensions less than 2 mm, and in certain cases, less than 1 mm. In one set of embodiments, all fluid channels containing embodiments of the invention are microfluidic or have a largest cross sectional dimension of no more than 2 mm or 1 mm. In certain embodiments, the fluid channels may be formed in part by a single component (e.g. an etched substrate or molded unit), as noted above. Of course, larger channels, tubes, chambers, reservoirs, etc. can be used to store fluids and/or deliver fluids to various components or systems of the invention. In one set of embodiments, the maximum cross-sectional dimension of the channel, the largest dimension perpendicular to fluid flow is less than 5 mm, less than 3 mm, less than 2 mm, less than 1 mm, or less than 500 micrometers, less than 300 micrometers, less than 200 micrometers, less

than 100 micrometers, less than 50 micrometers, less than 30 micrometers, less than 20 micrometers, less than 10 micrometers, less than 5 micrometers, less than 3 micrometers, less than 1 micrometer, less than 500 nm, less than 300 nm, less than 100 nm, less than 50 nm, less than 30 nm, or less than 10 nm. In some cases the dimensions of the channel may be chosen such that fluid is able to freely flow through the channel. The dimensions of the channel may also be chosen, for example, to allow a certain volumetric or linear flowrate of fluid in the channel. Of course, the number of channels and the shape of the channels can vary. For example, various microfluidic channels may independently stay within a single layer (a lateral microfluidic channel), or may go between two or more layers (a transverse microfluidic channel). Embodiments including various arrangements of channels, e.g., for the production of droplets, are discussed in more detail herein.

In one set of embodiments, the transverse channels may be arranged to allow increased fluid flow. For instance, the transverse channels may be positioned such that transverse channels downstream are larger (e.g., have a greater average cross-sectional area) than those that are upstream. Thus, for example, fluid may flow from a first transverse channel, to a second transverse channel, to a third transverse channel, etc., and the first transverse channel may have an average cross-sectional area that is the same size or smaller than the average cross-sectional area of the second transverse channel, which in turn may be the same size or smaller than the average cross-sectional area of the third transverse channel, etc.

As examples, the succeeding cross-sectional area of a transverse channel may be at least 1, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.8, at least 2, at least 2.5, at least 3, at least 3.5, at least 4 times, at least 5 times, at least 6 times, etc. of the previous transverse channel, and/or no more than 7, no more than 6, no more than 5, no more than 4, no more than 3.5, no more than 3, no more than 2.5, no more than 2, no more than 1.8, no more than 1.6, no more than 1.5, no more than 1.4, no more than 1.3, no more than 1.2, or no more than 1.1 times the area of the previous transverse channel. For instance, a transverse channel may be between 1.1 and 3 times the area of a succeeding transverse channel.

The channel may include features on or in an article (e.g., a layer) that at least partially directs flow of a fluid. The channel can have any cross-sectional shape (circular, oval, triangular, irregular, square or rectangular, or the like) and can be covered or uncovered. The channel may have a cross-sectional shape that varies with the length dimension as is the example of transverse channel 51 and channel 52 in Fig. 7D (e.g. a conical nozzle or

diffuser). In embodiments where it is completely covered, at least one portion of the channel can have a cross-section that is completely enclosed, or the entire channel may be completely enclosed along its entire length with the exception of its inlet(s) and/or outlet(s). A channel may also have an aspect ratio (length to average cross sectional dimension) of at least 2:1, more typically at least 3:1, 5:1, 10:1, 15:1, 20:1, or more. An open channel generally will include characteristics that facilitate control over fluid transport, e.g., structural characteristics (an elongated indentation) and/or physical or chemical characteristics (hydrophobicity vs. hydrophilicity) or other characteristics that can exert a force (e.g., a containing force) on a fluid. The fluid within the channel may partially or completely fill the channel. In some cases where an open channel is used, the fluid may be held within the channel, for example, using surface tension (i.e., a concave or convex meniscus).

In one aspect, the channels within the device may have various surface characteristics, which may be defined by the materials forming the channels (e.g., the characteristics of the materials forming the layer containing channels), and/or the channels may be modified in some fashion to control or alter their surface characteristics. For example, the channels may be treated chemically, mechanically, etc. Non-limiting examples of surface characteristics include hydrophilicity or hydrophobicity, surface roughness, color, concentrations of chemical agents or chemical groups, or the like. In addition, in some cases, only a portion of a channel may have a specific surface characteristic, e.g., as is shown in Fig. 7C.

For example, one or more of the channels within a layer may be treated, for example, with an agent, to alter their hydrophilicity, hydrophobicity, organophilicity, etc. In some embodiments, for instance, a microfluidic channel may be treated such that it exhibits a contact angle of less than 90° (e.g., to render it hydrophilic) or greater than 90° (e.g., to render it hydrophobic). Non-limiting examples of agents that can be applied to a channel to render it more hydrophobic include silanes, Aquapel®, metal oxides, silicone, or the like. Non-limiting examples of agents that can be applied to a channel to render it more hydrophilic include polyethylene glycol, polypropylene glycol, silica nanoparticles, or the like.

Examples of silanes include, but are not limited to, a fluorosilane (i.e., a silane containing at least one fluorine atom) such as heptadecafluorosilane or heptadecafluorooctylsilane, or other silanes such as methyltriethoxy silane (MTES) or a silane containing one or more lipid chains, such as octadecylsilane or other $\text{CH}_3(\text{CH}_2)_n$ -silanes, where n can be any suitable integer. For instance, n may be greater than 1, 5, or 10, and less than about 20, 25, or 30. The silanes may also optionally include other groups, such as alkoxide groups, for instance, octadecyltrimethoxysilane. Other examples of suitable

silanes include alkoxysilanes such as ethoxysilane or methoxysilane, halosilanes such as chlorosilanes, or other silicon-containing compounds containing hydrolyzable moieties on the silicon atom, such as hydroxide moieties. In some cases, the silanes may contain other groups, for example, groups such as amines, which would make the coating more

5 hydrophilic. Non-limiting examples include diamine silane, triamine silane, or *N*-[3-(trimethoxysilyl)propyl]ethylenediamine silane. In some cases, more than one silane may be present. In addition, in some cases, one or more of the silanes may be hydrolyzed to form the corresponding silanol. Additional examples of silanes include perfluorosilanes, perfluorinated silanes, alkyl silanes (e.g., octadecyltrichlorosilanes), and the like. See, e.g.,
10 Int. Pat. Apl. Pub. No. WO 2009/120254, incorporated herein by reference.

Examples of metal oxides include, but are not limited to, aluminum, antimony, barium, bismuth, cadmium, calcium, cerium, cesium, chromium, cobalt, copper, dysprosium, erbium, gadolinium, germanium, hafnium, holmium, indium, iridium, iron, lanthanum, lead, lithium, lutetium, magnesium, manganese, molybdenum, neodymium, nickel, niobium,
15 palladium, potassium, praseodymium, rhodium, rubidium, ruthenium, samarium, scandium, silicon, silver, sodium, strontium, tantalum, terbium, thallium, tin, titanium, tungsten, vanadium, ytterbium, yttrium, or zirconium oxide. Additional examples include SiO₂, vanadia (V₂O₅), titania (TiO₂), and/or alumina (Al₂O₃). See, e.g., Int. Pat. Apl. Pub. No. WO 2009/020633, incorporated herein by reference.

20 In addition, in other embodiments, other coatings may be applied to a channel in a layer. For example, materials such as a polymer, a metal, chemical species, or a biological species may be coated onto a surface of a channel. Non-limiting examples of biological species include proteins, peptides, antibodies, enzymes, nucleic acids (e.g., DNA, RNA, etc.), carbohydrates, or the like may be coated onto a channel. In addition, in some embodiments,
25 the coating may be applied to a channel to expose a certain type of chemical group to the interior of the channel, for example, carboxylic acid groups, or hydroxide groups.

It should also be understood that the surface characteristics of a channel may be altered without necessarily coating an agent to the surface. For example, the channel can be plasma-treated, corona-treated, ozone-treated, etc. to alter its hydrophilicity or other surface characteristics. In addition, in some cases, the channel may be mechanically altered, e.g., by
30 applying abrasive to the channels to increase or decrease their surface roughness.

After the layers are prepared (which may in some cases be treated as discussed above), they may be assembled together to form a device, in accordance with certain aspects of the invention. The layers may be bonded together in liquid-tight contact with each other,

e.g., such that fluid does not leak through the seams between each layer during normal operation of the device. An entire layer, or only a portion thereof, may be bonded in liquid-tight contact with another layer. Each layer within the device may be bonded in liquid-tight contact to another layer, although in some cases, one or more of the layers may not

5 necessarily be in liquid-tight contact with another layer.

A variety of techniques may be used to bond the layers together, including thermal-bonding, hot embossing, solvent-bonding, microwave welding, plasma bonding (e.g., with oxygen plasma), anodic bonding, use of an adhesive, or the like. Examples of adhesives include, but are not limited to, cyanoacrylates, or APTES (3-aminopropyl-triethoxysilanes).

10 In addition, in some cases, alignment of the layers may be facilitated by mechanical guides to keep the layers aligned, e.g., while bonding of the layers occur. For example, the layers may include one or more holes that can be aligned together, e.g., through the use of alignment pins or screws that pass through the holes when the layers are properly aligned, for instance, as is shown in Figs. 1 and 2. These may pass through one or more, or all, of the
15 layers to be aligned. Additionally, one or more layers may be aligned and bonded prior to formation of holes or other features. For example, the device illustrated in Fig. 7D may be partially or fully assembled prior to formation of the concentrically aligned channels 51, 52, and 53.

In certain embodiments, a force may be applied to the device, e.g., to keep the layers
20 together. For example, a force may be applied using screws (e.g., driven through holes in one or more of the layers, clamps, or the like.

Fluids may be delivered into channels within a device via one or more fluid sources. Any suitable source of fluid can be used, and in some cases, more than one source of fluid is used. For example, a pump, gravity, capillary action, surface tension, electroosmosis,
25 centrifugal forces, etc. may be used to deliver a fluid from a fluid source into one or more channels in the device. A vacuum (e.g., from a vacuum pump or other suitable vacuum source) can also be used in some embodiments. Non-limiting examples of pumps include syringe pumps, peristaltic pumps, pressurized fluid sources, or the like. The device can have any number of fluid sources associated with it, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc., or
30 more fluid sources. The fluid sources need not be used to deliver fluid into the same channel, e.g., a first fluid source can deliver a first fluid to a first channel while a second fluid source can deliver a second fluid to a second channel, etc. However, in some cases, the same fluid source may be used to deliver fluid to more than one layer, e.g., a first layer and a third layer. In some cases, two or more channels are arranged to intersect at one or more intersections.

There may be any number of fluidic channel intersections within the device, for example, 2, 3, 4, 5, 6, etc., or more intersections.

As a non-limiting example, certain aspects of the invention are generally directed to systems and methods for making droplets, including single droplets, double emulsion
5 droplets (e.g., water-in-oil-in-water or oil-in-water-in-oil), or higher order droplets.

One embodiment of the invention is shown in Fig. 7A. In this figure, the device may comprise a second layer defining a first lateral microfluidic channel, in which a first fluid flows, which ultimately will form droplets within a second fluid. One or more than one such lateral channel may be present within the layer. It should be noted that the first fluid need not
10 originate from the second layer. For instance, the first fluid may pass through one or more other layers before entering the first lateral channel.

The first fluid may then pass through one or more layer, e.g., through a transverse channel between the first layer and the third layer. The transverse channel may be straight or it may have any number of turns, bends, chambers, or other features between the first channel
15 and the second channel, e.g., as is shown in Fig. 7E. In addition, in some cases, additional channels (not shown in this figure) may be present, e.g., which may add or remove additional material to or from the first fluid.

The layer which the first fluid enters through an intersection may be a layer which contains a second fluid. The second fluid may pass through a second lateral channel. One,
20 two, three, four, or more such channels may be present. It should be understood, however, that the second fluid need not originate from the second lateral channel; for example, the second fluid may pass through one or more other channels before entering the second layer.

An intersection between any two or more channels may allow a first fluid from a transverse channel and the second fluid from one or more second lateral channels to come
25 into contact with each other. The channels defining the intersection may meet orthogonally, or at any other suitable angles. At the intersection, the first fluid may form co-axially flow with the second fluid (as shown in Fig. 7D), or may form droplets contained within the second fluid (as shown in Fig. 7A). Upon formation, the droplets may exit the channel through an exiting channel. The exiting channel may also be present within the layer
30 containing the lateral channel, or the exiting channel may be a second transverse channel that exits the layer into one or more other layers. Accordingly, exiting the intersection may be a plurality of droplets of first fluid contained within the second fluid.

This can be extended to create higher-order droplets, e.g., droplets-within-droplets. For example, as shown in Fig. 7D, the second transverse channel may extend into another

layer, e.g., containing an intersection with a third fluid, e.g., in a third lateral channel. The channels defining the intersection may be meet orthogonally, or at any other suitable angles. There may also be one or more than one third lateral channel present within the layer, and the transverse channel may be straight or it may have any number of turns, bends, chambers, or the like. The second and third lateral channels may be adjacent or there may be any number of layers between the second and third lateral channels, and this number may be the same or different than the number of layers between the first and second lateral channels. At the intersection with the third fluid, the second fluid may be caused to form droplets, some or all of which may contain droplets of first fluid therein to form a double-emulsion droplet. The droplets exiting the intersection may then pass into an exiting channel, which may be laterally contained within the layer, or extend transversally into yet another layer within the device. In some embodiments, this pattern may also be repeated any number of additional times (e.g., with additional layers and fluids, etc. to create higher-order triple, quadruple, etc. droplets).

A non-limiting example of such a device for producing double emulsion droplets can be seen in Fig. 9, in accordance with another embodiment of the invention. In this figure, a plurality of substantially planar layers forms the device. Layer 0 is the bottommost layer, while the double emulsion droplets are collected from layer 7. Layer 1 is a first layer defining a first lateral microfluidic channel containing a first fluid (which becomes the core fluid of the double emulsion droplet), layer 3 is a second layer defining a second lateral microfluidic channel containing a second fluid (which becomes the shell fluid), and layer 5 is a third layer defining a third lateral microfluidic channel containing a third fluid (which contains the double emulsion of the first and second fluids).

In this example, the first fluid flows through the first layer to the first transverse channel in layer 2, where it flows up and intersects with the second fluid in layer 3 (shaded for ease of identification in this figure). It should be understood that although the first fluid can form droplets in other embodiments, the first fluid need not form droplets as shown here, and instead, the first and second fluids may flow alongside each other. In this case, the fluids travel through a second transverse channel in layer 4, intersecting the third fluid in layer 5. The second transverse channel may, in some cases, have an average cross-sectional area greater than the first transverse channel, e.g., to allow more fluid flow to occur. The third fluid and the first fluid may be the same or different fluids. The fluids may then pass through an exit transverse channel in layer 6 into layer 7. In some cases, the exit transverse channel may have an average cross-sectional area greater than the second transverse channel. At that point, the fluids may then form a double emulsion droplet within layer 7 (with a droplet of the

first fluid contained within a droplet of the second fluid, which in turn is contained within the third fluid). The double emulsion droplet may be collected for subsequent use.

In most, but not all embodiments, the droplets and the fluid containing the droplets are substantially immiscible. In some cases, however, they may be miscible. In some cases, a hydrophilic liquid may be suspended in a hydrophobic liquid, a hydrophobic liquid may be suspended in a hydrophilic liquid, a gas bubble may be suspended in a liquid, etc. Typically, a hydrophobic liquid and a hydrophilic liquid are substantially immiscible with respect to each other, where the hydrophilic liquid has a greater affinity to water than does the hydrophobic liquid. Examples of hydrophilic liquids include, but are not limited to, water and other aqueous solutions comprising water, such as cell or biological media, ethanol, salt solutions, etc. Examples of hydrophobic liquids include, but are not limited to, oils such as hydrocarbons, silicon oils, fluorocarbon oils, organic solvents etc. In some cases, two fluids can be selected to be substantially immiscible within the time frame of formation of a stream of fluids. Those of ordinary skill in the art can select suitable substantially immiscible fluids, using contact angle measurements or the like.

In certain aspects, one or more of the fluids may be one that is high-viscosity, or at least has a viscosity greater than water. For example the viscosity of a fluid may be at least 1 cP, at least 2 cP, at least 3 cP, at least 5 cP, at least 10 cP, at least 20 cP, at least 30 cP, at least 50 cP, at least 100 cP, at least 200 cP, at least 300 cP, at least 500 cP, at least 1,000 cP, at least 2,000 cP, at least 3,000 cP, at least 5,000 cP, at least 10,000 cP, etc.

Generally, two fluids are immiscible, or not miscible, with each other when one is not soluble in the other to a level of at least 10% by weight at the temperature and under the conditions at which the emulsion is produced. For instance, two or more fluids may be selected to be immiscible within the time frame of the formation of the fluidic droplets. Typically, a fluid is substantially immiscible with an adjacent fluid, although fluids that are not adjacent need not be immiscible, and may be miscible (or even identical) in some cases. However, it should be understood that immiscibility is not necessarily required in all embodiments; in some cases, two adjacent fluids are not immiscible, but may retain separation in other ways, e.g., kinetically or through short exposure times. In some embodiments, two fluids (e.g., the third fluid and the first fluid of a double emulsion droplet system) are compatible, or miscible, while the intervening fluid (e.g., the second fluid of a double emulsion droplet) is incompatible or immiscible with one or both. In other embodiments, however, all three (or more) fluids may be mutually immiscible, and in certain cases, all of the fluids do not all necessarily have to be water soluble. In still other

embodiments, as mentioned, additional fourth, fifth, sixth, etc. fluids may be added to produce increasingly complex droplets within droplets. In addition, the physical properties of each nesting layer of fluidic droplets may each be independently controlled, e.g., by control over the composition of each nesting level, for example, by controlling the fluids entering
5 each layer of the device.

Thus, as a non-limiting example, in a double emulsion droplet, the first fluid (innermost fluid) may be an aqueous or hydrophilic fluid (a “water” phase), the second fluid (middle fluid) may be a lipophilic or hydrophobic or “oil” phase that is substantially immiscible with the aqueous fluid, the third fluid (or outer fluid) may be an aqueous fluid (a
10 “water” fluid) that is substantially immiscible with the second fluid. This is sometimes generally referred to as a W/O/W double emulsion droplet (for water/oil/water), although it should be understood that this is mainly for the sake of convenience; for instance, the first fluid can be any suitable aqueous fluid, and it need not be pure water. For example, the aqueous fluid may be water, saline, an aqueous solution, ethanol, or the like, or any other
15 fluid miscible in water. The oil, in contrast, may be immiscible in water, at least when left undisturbed under ambient conditions. In similar fashion, an O/W/O double emulsion droplet may be similarly defined. Furthermore, these principles may be extended to higher-order multiple emulsions droplets, e.g., as discussed herein. For example, a triple emulsion droplet may comprise a first fluid, surrounded by a second fluid, surrounded by a third fluid,
20 contained in a fourth fluid, etc. In addition, it should be understood that in some cases, more than one droplet of a fluid may be present, e.g., within another droplet. Thus, for example, a double emulsion droplet may contain two, three, or more droplets of first fluid, contained within a droplet of a second fluid, that in turn is contained within a third fluid.

Still additional non-limiting examples are provided in Figs. 7F and 7G. Fig. 7F shows
25 an example in which at least some of the channels are not completely defined as completely passing through a layer. Fig. 7G illustrates another embodiment for producing double emulsion droplets. These embodiments comprise a variety of layers 11, 21, 31, 41 and channels passing through one or more of these layers to produce various intersections, where various fluids can interact, for example, to mix, react, form droplets as shown in these
30 examples, or the like.

U.S. Pat. Nos. 8,337,778; 8,765,485; 8,802,027; 8,883,291; 9,038,919; 9,789,482; and 9,878,325 are each incorporated herein by reference in their entireties.

In addition, U.S. Provisional Patent Application Serial No. 62/637,622, filed March 2, 2018, entitled "Layered Microfluidic Devices and Techniques," by Weitz, *et al.*, is incorporated herein by reference in its entirety.

5 The following examples are intended to illustrate certain embodiments of the present invention, but do not exemplify the full scope of the invention.

EXAMPLE 1

Double emulsions are used widely in both industry and academia, but devices used to produce such double emulsions tend to be made of either expensive or hard to manufacture-at-volume materials. However, the device described in this example offers an inexpensive,
10 easily manufactured, reconfigurable, and scalable method of producing microfluidic droplets.

The device described in this example is manufactured from low-cost thin polymer films or layers, and can be assembled easily, for example, by non-expert users, or through automated manufacturing. In some cases, various surface properties can be precisely controlled. For example, localized surface treatments (e.g., hydrophilic, hydrophobic,
15 organophilic, etc.) can be applied to individual discrete surfaces of layers, entire layers (or several sub-assembled layers), and in some cases, such layers can be pre-treated prior to full assembly.

Issues of alignment and concentricity of fluid flows can also be handled. For example, pores or holes within a layer can readily be made concentric by various alignment
20 methods, and/or by fabricating multiple pores or holes at once after partially or fully assembling multiple layers. The concentric design may allow well-defined sheath flows, and/or prevent certain fluids from contacting side walls within the device. The hole and channel dimensions can be easily controlled, e.g., to produce a variety of droplet sizes. Furthermore, the device in some cases may be well suited to scale-up of droplet throughput,
25 e.g., via multiple devices, multiple droplet-making junctions in a single device, or the like.

The device in this example is composed of seven 150-micrometer thick polymer layers. Materials such as polycarbonate (PC), cyclic-olefin-copolymer (COC), polymethyl methacrylate (PMMA) and polyethylene terephthalate (PET) can be used (PET, for example, can be easily laser-cut). The thin polymer layers were aligned by two 2-mm alignment pins
30 fixed to a solid base comprised of a rigid substrate such as polymethyl methacrylate (PMMA) or aluminum. Polymer layers may be, for example, solvent, thermal or adhesive bonded. Functional devices have been developed using a polypropylene-silicone adhesive to bond layers. In order to interface with fluid inlets/outlets, a PDMS block was attached to the top

layer with 1-mm punched holes for use with 18 gauge pin connectors. This device is shown in Fig. 1 as an exploded view of a complete layered double emulsion device

The device was attached to inlet and outlet reagents via gauge-pins and tubing. Flow was provided via peristaltic, diaphragm pumps, pressure-driven and volumetrically-driven commercially available pumps. The port configuration is shown in Fig. 2. In this figure, 1 and 6 are inlets for the core fluid, 3 and 4 are inlets for the shell fluid, 5 is the carrier inlet, and 2 is the device outlet.

The central portion of the device features the pores and a T-junction, dimensions of which are used to control droplet breakup. A cross-section view of this region and a functional schematic are shown in Fig. 3.

One feature of the device is the co-axial flow between the core and shell phase combined with the T-junction orthogonal flow of the carrier phase. Droplet break-up may occur as the core enters the shell phase, and is believed to be driven by a balance between shear-elongation and interfacial tension. Depending on the balance of these forces core phase may enter a state of co-axial flow with the shell phase. While the formation of the final double emulsion may include “squeezing” or “plugging” due to obstruction or partial-obstruction of the top carrier channel. See, e.g., Fig. 4, showing a schematic of double emulsion droplet generation. In some cases, formation of the final double emulsion may again be a result of a balance between viscous shear elongation and interfacial tension depending on the surfactant chemistry and flowrates used.

Examples of double emulsions may be seen in Figs. 5A-5C, with double emulsion outer shell diameters ranging from about 50 micrometers to about 200 micrometers (although smaller or larger diameters are also possible). Different thickness shells can be seen in Fig. 5. Due to the transparent nature of the device, droplet imaging can be done *in situ*, which may allow for real-time analysis of droplet quality, dimensions, etc. This may be useful, for example, in the context of high-volume manufacturing of microcapsules.

Furthermore, due to the fact that droplet generation occurs orthogonal to individual layers each individual layer may contain a plurality of droplet generators. These droplet generators may have independent and isolated fluidic channels (e.g., as shown in Fig. 8) or may have common lateral supply channels.

Because the device in this example is assembled from individual layers and the flow path is orthogonal to the plane of each layer, it is possible to have distinct and opposing surface treatments in close physical proximity. For example, in the case of water/oil/water emulsions, the layers associated with the core phase and the carrier phase could be plasma,

corona, or otherwise treated to be hydrophilic, while the layers associated with the oil phase could be silane-treated or otherwise made hydrophobic, fluorophilic, or organophilic.

The same laser-cutting process used to cut the outline and channels of each layer can also be used to drill the holes. By adjusting the laser speed or power, hole diameters of various sizes, e.g., ranging from 50 to 150 micrometers in diameter, can be produced (Figs. 6A and 6B). However, other methods may be used to create pores or channels including microetching, radiation exposure, hot embossing, injection molding, by hot pin, or the like. Fig. 6B shows an example of hole sizes prepared as a function of laser power and speed.

EXAMPLE 2

This example illustrates a layered device prepared in accordance with another embodiment of the invention. The layered device was used to produce double emulsion droplets of an inner fluid, contained within a middle fluid, contained within an outer phase fluid.

The inner and outer phases each were water with 5 wt% PVA (polyvinyl alcohol) (Sigma Aldrich 363170, dissolved at 60 °C for 24 hours, then filtered). The middle phase was substantially immiscible with the inner and outer phases. The middle fluid contained 98 wt% mineral oil (Sigma Aldrich 330779), 2 wt% SPAN 80, and 0.01 mg/ml red Nile (to make it easier to identify) (Sigma Aldrich 72485).

Fig. 9 shows a cross section of the device. The device in this example is made of sequentially stacked layers which are individually cut prior assembly. The double emulsions (capsules) are formed at a single junction out of three different phases: the inner, middle and outer phase flow inside the channel cut into layers 1, 3, and 5 respectively. The pores inside layer 2 and 3 extrude the inner and middle phase. The pore in layer 6 is used for droplet break-up.

This device was assembled as outlined below:

PET: Grafix 0.005 inches (0.127 mm) Clear DuraLar

3M9960: 3M, 3M9960

Acrylic: Clear Cast Acrylic Sheets, 1/16 inches thick (1.5875 mm), McMaster-Carr 8560K174

Adhesive: Adhesive Research, AR90880

Layer 0: PET (total thickness: 125 micrometers)

Layer 1: Adhesive + PET + Adhesive (375 micrometers)

Layer 2: PET (125 micrometers)

Layer 3: Adhesive (125 micrometers)

Layer 4: 3M9960 (125 micrometers)

Layer 5: Adhesive + PET + Adhesive (375 micrometers)

5 Layer 6: PET (125 micrometers)

Layer 7: Adhesive + Acrylic (>1 mm)

The device was prepared as follows. First, channels were laser cut in the hydrophobic, hydrophilic, acrylic, and adhesive films. Then pores in the device were CNC
10 microdrilled in the device. These had dimensions of 200 micrometers (layer 2), 360 micrometers (layer 4), and 900 micrometers (layer 6). The layers without pores were then stacked (passively) using pin alignment (alignment pins: low tolerance metric gauge pins, McMaster-Carr 2281A2), while the layers with pores were stacked using an active alignment stage. After each new layer was applied, a hydraulic press was used to press the layers
15 together (up to 18 bar). Stainless steel dispensing needles with Luer lock connections were used for the inlets on top of the device (McMaster-Carr 75165A675, OD 1.27 mm) (Fig. 10B). The tips of stainless steel dispensing needles were used for the outlets on the top layer of the device (McMaster-Carr 75165A245, OD 2.1 mm) (Fig. 10C).

Fig. 10B is an image of four devices on the same chip with stainless steel dispensing
20 needles for inlets and outlets (McMaster-Carr 75165A675, OD 1.27 mm). Each device has a total of 3 inlets (inner, middle, and outer phase) plus 1 outlet, each with a corresponding needle. For each incoming phase, the acrylic manifold divided the fluid flow into a left-coming and a right-coming flow as shown in Fig. 9.

Fig. 10C shows an alignment station for stacking layers passively using pin alignment
25 (alignment pins: low tolerance metric gauge pins, McMaster-Carr 2281A2). The holes for each pin are drilled with a high precision CNC machine.

The device was able to produce double emulsion droplets having an average size (diameter) of about 600 micrometers. Images of the double emulsions in the outlet channel inside the manifold can be seen in Fig. 10A. It should be understood, however, that this is by
30 way of example only, and other size emulsion droplets (including smaller droplets) can be produced in other embodiments of the invention.

Fig. 10A illustrates the formation of such droplets (looking in through the exit transverse channel). This microscope image shows double emulsion droplets produced using

the device described in Fig. 9. There can be seen a clear distinction between the inner (water + 5% PVA), middle (mineral oil), and outer phases (water + 5% PVA).

For testing, 3 syringe pumps were used for each of the three phases into their respective channels, with an acrylic manifold on the top layer (which could also be made of stainless steel or aluminum) that distributed the liquids into the device.

While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

In cases where the present specification and a document incorporated by reference include conflicting and/or inconsistent disclosure, the present specification shall control. If two or more documents incorporated by reference include conflicting and/or inconsistent disclosure with respect to each other, then the document having the later effective date shall control.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.”

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one,

optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

When the word “about” is used herein in reference to a number, it should be understood that still another embodiment of the invention includes that number not modified by the presence of the word “about.”

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

CLAIMS

What is claimed is:

1. A microfluidic apparatus, comprising:
 - a first lateral microfluidic channel defined by a first layer;
 - 5 a second lateral microfluidic channel, positioned substantially parallel to the first channel, defined by a second layer;
 - a third lateral microfluidic channel, positioned substantially parallel to the first channel and the second channel, defined by a third layer;
 - a first transverse microfluidic channel, defined by at least the first layer and
10 the second layer, in fluid communication with the first lateral microfluidic channel and the second lateral microfluidic channel; and
 - a second transverse microfluidic channel, defined by at least the second layer and the third layer, in fluid communication with the second lateral microfluidic channel and the third lateral microfluidic channel.
- 15 2. The microfluidic apparatus of claim 1, wherein the first transverse microfluidic channel and the first lateral microfluidic channel are substantially orthogonal.
3. The microfluidic apparatus of any one of claims 1 or 2, wherein the second transverse
20 microfluidic channel and the first lateral microfluidic channel are substantially orthogonal.
4. The microfluidic apparatus of any one of claims 1-3, wherein the second transverse
25 microfluidic channel and the second lateral microfluidic channel are substantially orthogonal.
5. The microfluidic apparatus of any one of claims 1-4, wherein the second transverse
30 microfluidic channel and the third lateral microfluidic channel are substantially orthogonal.
6. The microfluidic apparatus of any one of claims 1-5, wherein the first transverse microfluidic channel and the first lateral microfluidic channel intersect.

7. The microfluidic apparatus of any one of claims 1-6, wherein the second transverse microfluidic channel and the first lateral microfluidic channel intersect.
- 5 8. The microfluidic apparatus of any one of claims 1-7, wherein the second transverse microfluidic channel and the second lateral microfluidic channel intersect.
9. The microfluidic apparatus of any one of claims 1-8, wherein the second transverse microfluidic channel and the third lateral microfluidic channel intersect.
- 10 10. The microfluidic apparatus of any one of claims 1-9, wherein the first transverse microfluidic channel, the second microfluidic lateral channel, and the second transverse microfluidic channel each intersect.
11. The microfluidic apparatus of any one of claims 1-10, wherein the first layer defines
15 at least two first lateral microfluidic channels that intersect the first transverse channel.
12. The microfluidic apparatus of any one of claims 1-11, wherein the first layer defines
20 at least two first lateral microfluidic channels that simultaneously intersect the first transverse channel.
13. The microfluidic apparatus of any one of claims 1-12, wherein the second layer
25 defines at least two second lateral microfluidic channels that intersect the first transverse channel.
14. The microfluidic apparatus of any one of claims 1-13, wherein the second layer
defines at least two second lateral microfluidic channels that simultaneously intersect
the first transverse channel.
- 30 15. The microfluidic apparatus of any one of claims 1-14, wherein the second layer
defines at least two second lateral microfluidic channels that intersect the second
transverse channel.

16. The microfluidic apparatus of any one of claims 1-15, wherein the second layer defines at least two second lateral microfluidic channels that simultaneously intersect the second transverse channel.
- 5 17. The microfluidic apparatus of any one of claims 1-16, further comprising an exit microfluidic channel defined by the third layer, in fluid communication with the second transverse microfluidic channel and the third lateral microfluidic channel.
- 10 18. The microfluidic apparatus of any one of claims 1-17, further comprising:
a fourth layer; and
a third transverse microfluidic channel in fluid communication with the second transverse microfluidic channel and the third lateral microfluidic channel, defined by at least the third layer and the fourth layer.
- 15 19. The microfluidic apparatus of any one of claims 1-18, wherein the first layer and the second layer are in physical contact.
20. The microfluidic apparatus of any one of claims 1-19, wherein the second layer and the third layer are in physical contact.
- 20 21. The microfluidic apparatus of any one of claims 1-20, further comprising one or more substantially planar layers positioned between and substantially parallel to the first layer and the second layer.
- 25 22. The microfluidic apparatus of any one of claims 1-21, further comprising one or more substantially planar layers positioned between and substantially parallel to the second layer and the third layer.
- 30 23. The microfluidic apparatus of any one of claims 1-22, further comprising:
a source of a first fluid in fluid communication with the first lateral microfluidic channel;
a source of a second fluid in fluid communication with the second lateral microfluidic channel; and

a source of a third fluid in fluid communication with the third lateral microfluidic channel.

- 5 24. The microfluidic apparatus of claim 23, wherein the first fluid and the second fluid are substantially immiscible.
25. The microfluidic apparatus of any one of claims 23 or 24, wherein the second fluid and the third fluid are substantially immiscible.
- 10 26. The microfluidic apparatus of any one of claims 23-25, wherein the first fluid and the third fluid are substantially miscible.
27. The microfluidic apparatus of any one of claims 23-25, wherein the source of the first fluid is the source of the third fluid.
- 15 28. The microfluidic apparatus of any one of claims 23-27, wherein at least one source of fluid comprises a pump.
29. The microfluidic apparatus of any one of claims 23-28, wherein the first fluid has a viscosity of at least 2 cP.
- 20 30. The microfluidic apparatus of any one of claims 1-29, wherein the first layer and the second layer have substantially identical compositions.
- 25 31. The microfluidic apparatus of any one of claims 1-30, wherein the second layer and the third layer have substantially identical compositions.
32. The microfluidic apparatus of any one of claims 1-31, wherein the first lateral microfluidic channel and the second lateral microfluidic channel have different hydrophobicities.
- 30 33. The microfluidic apparatus of any one of claims 1-32, wherein the second lateral microfluidic channel and the third lateral microfluidic channel have different hydrophobicities.

34. The microfluidic apparatus of any one of claims 1-33, wherein at least one microfluidic channel of the microfluidic apparatus comprises a coating that increases the hydrophobicity of the at least one microfluidic channel, relative to the at least one microfluidic channel in the absence of the coating.

35. The microfluidic apparatus of any one of claims 1-34, wherein at least one microfluidic channel of the microfluidic apparatus comprises a coating that decreases the hydrophobicity of the at least one microfluidic channel, relative to the at least one microfluidic channel in the absence of the coating.

36. The microfluidic apparatus of any one of claims 1-35, wherein at least one microfluidic channel of the microfluidic apparatus comprises a sol-gel coating.

37. The microfluidic apparatus of any one of claims 1-36, wherein at least one microfluidic channel of the microfluidic apparatus comprises a silane coating.

38. The microfluidic apparatus of any one of claims 1-37, wherein at least one microfluidic channel of the microfluidic apparatus is plasma-treated.

39. The microfluidic apparatus of any one of claims 1-38, wherein at least one microfluidic channel of the microfluidic apparatus is corona-treated.

40. The microfluidic apparatus of any one of claims 1-39, wherein at least one microfluidic channel of the microfluidic apparatus is ozone-treated.

41. The microfluidic apparatus of any one of claims 1-40, wherein the first lateral microfluidic channel and the third lateral microfluidic channel have substantially identical hydrophobicities.

42. The microfluidic apparatus of any one of claims 1-41, wherein at least one of the first layer, the second layer, and the third layer comprises cyclic olefin copolymer.

43. The microfluidic apparatus of any one of claims 1-42, wherein each of the first layer, the second layer, and the third layer comprises cyclic olefin copolymer.
44. The microfluidic apparatus of any one of claims 1-43, wherein at least one of the first layer, the second layer, and the third layer comprises polyethylene terephthalate.
45. The microfluidic apparatus of any one of claims 1-44, wherein each of the first layer, the second layer, and the third layer comprises polyethylene terephthalate.
46. The microfluidic apparatus of any one of claims 1-45, wherein at least one of the first layer, the second layer, and the third layer comprises glass.
47. The microfluidic apparatus of any one of claims 1-46, wherein at least one of the first layer, the second layer, and the third layer has a cross-sectional dimension of at least about 100 micrometers.
48. The microfluidic apparatus of any one of claims 1-47, wherein at least one of the first layer, the second layer, and the third layer has a cross-sectional dimension of at least about 300 micrometers.
49. The microfluidic apparatus of any one of claims 1-48, wherein at least one of the first layer, the second layer, and the third layer has a cross-sectional dimension of at least about 1 mm.
50. The microfluidic apparatus of any one of claims 1-49, wherein each of the first layer, the second layer, and the third layer has a cross-sectional dimension of at least about 10 micrometers.
51. The microfluidic apparatus of any one of claims 1-50, wherein each of the first layer, the second layer, and the third layer has a cross-sectional dimension of at least about 100 micrometers.
52. The microfluidic apparatus of any one of claims 1-51, wherein at least two layers within the microfluidic apparatus are in liquid-tight contact with each other.

53. The microfluidic apparatus of any one of claims 1-52, wherein each of the layers within the microfluidic apparatus are in liquid-tight contact with each other.
- 5 54. The microfluidic apparatus of any one of claims 1-53, wherein at least two layers within the microfluidic apparatus are solvent-bonded to each other.
55. The microfluidic apparatus of any one of claims 1-54, wherein at least two layers within the microfluidic apparatus are thermally bonded to each other.
- 10 56. The microfluidic apparatus of any one of claims 1-55, wherein at least two layers within the microfluidic apparatus are bonded to each other via an adhesive.
57. The microfluidic apparatus of claim 56, wherein the adhesive is a UV-cured adhesive.
- 15 58. The microfluidic apparatus of any one of claims 1-57, further comprising a source of a force configured and arranged to force at least two layers within the microfluidic apparatus together.
- 20 59. The microfluidic apparatus of any one of claims 1-58, further comprising one or more alignment pins passing through at least one of the first layer, the second layer, and the third layer.
60. The microfluidic apparatus of any one of claims 1-59, further comprising one or more alignment pins passing through each of the first layer, the second layer, and the third layer.
- 25 61. The microfluidic apparatus of any one of claims 1-60, wherein at least one of the first layer, the second layer, and the third layer is transparent.
- 30 62. The microfluidic apparatus of any one of claims 1-61, wherein at least one of the first layer, the second layer, and the third layer is translucent.

63. The microfluidic apparatus of any one of claims 1-62, wherein at least one of the first layer, the second layer, and the third layer is opaque.
64. The microfluidic apparatus of any one of claims 1-63, wherein at least one of the transverse channels is conical.
65. A microfluidic apparatus, comprising:
a first substantially planar layer, defining a first lateral microfluidic channel;
and
a second substantially planar layer, defining a second lateral microfluidic channel, wherein the second layer has a substantially identical composition to the first layer and is positioned substantially parallel to the first layer, and wherein the first lateral microfluidic channel has a first surface characteristic and the second lateral microfluidic channel has a second surface characteristic different from the first hydrophobicity; and
a transverse microfluidic channel, defined by and fluidly communicating between the first layer and the second layer, in fluid communication with the first lateral microfluidic channel and the second lateral microfluidic channel.
66. The microfluidic apparatus of claim 65, wherein the surface characteristic is hydrophilicity.
67. The microfluidic apparatus of any one of claims 65 or 66, wherein the surface characteristic is roughness.
68. The microfluidic apparatus of any one of claims 65-67, wherein the surface characteristic is color.
69. The microfluidic apparatus of any one of claims 65-68, wherein the first surface characteristic is a first coating on the first layer and the second surface characteristic is a second coating or no coating on the second layer.
70. The microfluidic apparatus of claim 69, wherein the first coating comprises a metal.

71. The microfluidic apparatus of any one of claims 69 or 70, wherein the first coating comprises a protein or a peptide.
- 5 72. The microfluidic apparatus of any one of claims 69-71, wherein the first coating comprises an antibody.
73. The microfluidic apparatus of any one of claims 69-72, wherein the first coating comprises a nucleic acid.
- 10 74. The microfluidic apparatus of any one of claims 69-73, wherein the first coating comprises a hydrophilic coating.
75. The microfluidic apparatus of claim 74, wherein the hydrophilic coating comprises a carboxylic acid.
- 15 76. The microfluidic apparatus of any one of claims 69-75, wherein the first coating comprises a hydrophobic coating.
77. The microfluidic apparatus of claim 76, wherein the hydrophilic coating comprises a silicone.
- 20 78. The microfluidic apparatus of any one of claims 76 or 77, wherein the hydrophilic coating comprises a sol-gel coating.
- 25 79. The microfluidic apparatus of any one of claims 65-78, further comprising:
a third substantially planar layer, positioned substantially parallel to the first layer and the second layer, defining a third lateral microfluidic channel
- 30 80. The microfluidic apparatus of claim 79, further comprising a second transverse microfluidic channel, defined by and fluidly communicating between the second layer and the third layer, in fluid communication with the second lateral microfluidic channel and the third lateral microfluidic channel.

81. The microfluidic apparatus of claim 80, wherein the transverse microfluidic channel and the second transverse microfluidic channel are in fluid communication.
- 5 82. The microfluidic apparatus of any one of claims 79-81, wherein the transverse microfluidic channel is defined by and fluidly communicates between the first layer, the second layer, and the third layer.
- 10 83. The microfluidic apparatus of claim 82, wherein the transverse microfluidic channel is in fluid communication with the third lateral microfluidic channel.
84. The microfluidic apparatus of any one of claims 79-83, further comprising an exit microfluidic channel defined by the third layer, in fluid communication with the transverse microfluidic channel.
- 15 85. The microfluidic apparatus of any one of claims 79-84, further comprising a fourth substantially planar layer, positioned substantially parallel to the first layer, the second layer, and the third layer.
- 20 86. The microfluidic apparatus of claim 85, further comprising a third transverse microfluidic channel, defined by and fluidly communicating between the third layer and the fourth layer, in fluid communication with the transverse microfluidic channel.
87. A method, comprising:
- 25 flowing a first fluid through a first lateral microfluidic channel defined by a first substantially planar layer;
- flowing the first fluid into a transverse microfluidic channel, defined by and fluidly communicating between the first layer and a second substantially planar layer, wherein the second layer is positioned substantially parallel to the first layer;
- 30 flowing a second fluid, through a second lateral microfluidic channel defined by the second layer, to contact the first fluid in the transverse microfluidic channel; and
- forming droplets of the first fluid, contained within the second fluid.

88. A method, comprising:

flowing a first fluid through a first lateral microfluidic channel defined by a first substantially planar layer;

5 flowing the first fluid into a first transverse microfluidic channel, defined by and fluidly communicating between the first layer and a second substantially planar layer, wherein the second layer is positioned substantially parallel to the first layer;

flowing a second fluid, through a second lateral microfluidic channel defined by the second layer, to contact the first fluid in the first transverse microfluidic channel;

10 forming droplets of the first fluid, contained within the second fluid;

flowing the droplets and the second fluid into a second transverse microfluidic channel, defined by and fluidly communicating between the second layer and a third substantially planar layer, wherein the third layer is positioned substantially parallel to the first layer and the second layer;

15 flowing a third fluid, through a third lateral microfluidic channel defined by the third layer, to contact the second fluid in the second transverse microfluidic channel; and

forming double emulsion droplets, comprising droplets of the first fluid contained within droplets of the second fluid, contained within the third fluid.

20 89. A method, comprising:

flowing a first fluid through a first microfluidic channel defined by a first substantially planar layer;

25 flowing the first fluid through a transverse face of the first layer into the transverse face of a second substantially planar layer, wherein the second layer is positioned substantially parallel to the first layer;

flowing a second fluid, through a second microfluidic channel defined by the second layer, to contact the first fluid; and

forming droplets of the first fluid, contained within the second fluid.

30 90. A microfluidic apparatus, comprising:

a first lateral microfluidic channel defined by a first layer;

a second lateral microfluidic channel, positioned substantially parallel to the first channel, defined by a second layer;

a third lateral microfluidic channel, positioned substantially parallel to the first channel and the second channel, defined by a third layer;

a first transverse microfluidic channel in fluid communication with the first lateral microfluidic channel and the second lateral microfluidic channel; and

5 a second transverse microfluidic channel in fluid communication with the second lateral microfluidic channel and the third lateral microfluidic channel.

91. The microfluidic apparatus of claim 90, wherein the first transverse microfluidic channel is defined by at least a fourth layer positioned between and substantially
10 parallel to the first layer and the second layer.
92. The microfluidic apparatus of claim 91, wherein the fourth layer and the first layer are in physical contact.
- 15 93. The microfluidic apparatus of any one of claims 91 or 92, wherein the fourth layer and the second layer are in physical contact.
94. The microfluidic apparatus of claim 91, comprising more than one layer positioned between and substantially parallel to the first layer and the second layer.
20
95. The microfluidic apparatus of claim 90, wherein the second transverse microfluidic channel is defined by at least a fifth layer positioned between and substantially parallel to the second layer and the third layer.
- 25 96. The microfluidic apparatus of claim 95, wherein the fifth layer and the second layer are in physical contact.
97. The microfluidic apparatus of any one of claims 95 or 96, wherein the fifth layer and the third layer are in physical contact.
30
98. The microfluidic apparatus of claim 95, comprising one or more substantially planar layers positioned between and substantially parallel to the second layer and the third layer.

99. The microfluidic apparatus of any one of claims 90-98, wherein the first transverse microfluidic channel and the first lateral microfluidic channel are substantially orthogonal.
- 5 100. The microfluidic apparatus of any one of claims 90-99, wherein the second transverse microfluidic channel and the first lateral microfluidic channel are substantially orthogonal.
- 10 101. The microfluidic apparatus of any one of claims 90-100, wherein the second transverse microfluidic channel and the second lateral microfluidic channel are substantially orthogonal.
- 15 102. The microfluidic apparatus of any one of claims 90-101, wherein the second transverse microfluidic channel and the third lateral microfluidic channel are substantially orthogonal.
- 20 103. The microfluidic apparatus of any one of claims 90-102, wherein the first transverse microfluidic channel and the first lateral microfluidic channel intersect.
- 25 104. The microfluidic apparatus of any one of claims 90-103, wherein the second transverse microfluidic channel and the first lateral microfluidic channel intersect.
105. The microfluidic apparatus of any one of claims 90-104, wherein the second transverse microfluidic channel and the second lateral microfluidic channel intersect.
- 30 106. The microfluidic apparatus of any one of claims 90-105, wherein the second transverse microfluidic channel and the third lateral microfluidic channel intersect.
107. The microfluidic apparatus of any one of claims 90-106, wherein the first transverse microfluidic channel, the second microfluidic lateral channel, and the second transverse microfluidic channel each intersect.

108. The microfluidic apparatus of any one of claims 90-107, wherein the first transverse channel has a first average cross-sectional area, and the second transverse channel has a second average cross-sectional area.
- 5 109. The microfluidic apparatus of claim 108, wherein the second average cross-sectional area is greater than the first average cross-sectional area.
110. The microfluidic apparatus of any one of claims 109 or 110, wherein the second
10 average cross-sectional area is 1.1 to 3 times the area of the first average cross-sectional area.
111. The microfluidic apparatus of any one of claims 108-110, further comprising an exit microfluidic channel, in fluid communication with the second transverse microfluidic channel and the third lateral microfluidic channel.
- 15 112. The microfluidic apparatus of claim 111, wherein the exit microfluidic channel is defined by a sixth layer.
113. The microfluidic apparatus of claim 112, wherein the sixth layer and the third layer
20 are in physical contact.
114. The microfluidic apparatus of any one of claims 108-113, wherein the exit microfluidic channel has a third average cross-sectional area that is greater than the first average cross-sectional area and the second average cross-sectional area.
- 25 115. The microfluidic apparatus of any one of claims 108-114, wherein the third average cross-sectional area that is about 1.5 to about 5 times larger than the second average cross-sectional area.
- 30 116. The microfluidic apparatus of any one of claims 90-115, further comprising:
a source of a first fluid in fluid communication with the first lateral microfluidic channel;
a source of a second fluid in fluid communication with the second lateral microfluidic channel; and

a source of a third fluid in fluid communication with the third lateral microfluidic channel.

- 5 117. The microfluidic apparatus of claim 116, wherein the first fluid and the second fluid are substantially immiscible.
118. The microfluidic apparatus of any one of claims 116 or 117, wherein the second fluid and the third fluid are substantially immiscible.
- 10 119. The microfluidic apparatus of any one of claims 116-118, wherein the first fluid and the third fluid are substantially miscible.
120. The microfluidic apparatus of any one of claims 116-119, wherein the source of the first fluid is the source of the third fluid.
- 15 121. The microfluidic apparatus of any one of claims 116-120, wherein at least one source of fluid comprises a pump.
122. The microfluidic apparatus of any one of claims 116-121, wherein the first fluid has a viscosity of at least 2 cP.
- 20 123. The microfluidic apparatus of any one of claims 90-122, wherein the first layer and the second layer have substantially identical compositions.
- 25 124. The microfluidic apparatus of any one of claims 90-123, wherein the second layer and the third layer have substantially identical compositions.
125. The microfluidic apparatus of any one of claims 90-124, wherein the first lateral microfluidic channel and the second lateral microfluidic channel have different hydrophobicities.
- 30 126. The microfluidic apparatus of any one of claims 90-125, wherein the second lateral microfluidic channel and the third lateral microfluidic channel have different hydrophobicities.

127. The microfluidic apparatus of any one of claims 90-126, wherein at least one microfluidic channel of the microfluidic apparatus comprises a coating that increases the hydrophobicity of the at least one microfluidic channel, relative to the at least one microfluidic channel in the absence of the coating.

128. The microfluidic apparatus of any one of claims 90-127, wherein at least one microfluidic channel of the microfluidic apparatus comprises a coating that decreases the hydrophobicity of the at least one microfluidic channel, relative to the at least one microfluidic channel in the absence of the coating.

129. The microfluidic apparatus of any one of claims 90-128, wherein at least one microfluidic channel of the microfluidic apparatus comprises a sol-gel coating.

130. The microfluidic apparatus of any one of claims 90-129, wherein at least one microfluidic channel of the microfluidic apparatus comprises a silane coating.

131. The microfluidic apparatus of any one of claims 90-130, wherein at least one microfluidic channel of the microfluidic apparatus is plasma-treated.

132. The microfluidic apparatus of any one of claims 90-131, wherein at least one microfluidic channel of the microfluidic apparatus is corona-treated.

133. The microfluidic apparatus of any one of claims 90-132, wherein at least one microfluidic channel of the microfluidic apparatus is ozone-treated.

134. The microfluidic apparatus of any one of claims 90-133, wherein the first lateral microfluidic channel and the third lateral microfluidic channel have substantially identical hydrophobicities.

135. The microfluidic apparatus of any one of claims 90-134, wherein at least one of the first layer, the second layer, and the third layer comprises cyclic olefin copolymer.

136. The microfluidic apparatus of any one of claims 90-135, wherein each of the first layer, the second layer, and the third layer comprises cyclic olefin copolymer.
- 5 137. The microfluidic apparatus of any one of claims 90-136, wherein at least one of the first layer, the second layer, and the third layer comprises polyethylene terephthalate.
138. The microfluidic apparatus of any one of claims 90-137, wherein each of the first layer, the second layer, and the third layer comprises polyethylene terephthalate.
- 10 139. The microfluidic apparatus of any one of claims 90-138, wherein at least one of the first layer, the second layer, and the third layer comprises glass.
140. The microfluidic apparatus of any one of claims 90-139, wherein at least one of the first layer, the second layer, and the third layer has a cross-sectional dimension of at
15 least about 100 micrometers.
141. The microfluidic apparatus of any one of claims 90-140, wherein at least one of the first layer, the second layer, and the third layer has a cross-sectional dimension of at least about 300 micrometers.
- 20 142. The microfluidic apparatus of any one of claims 90-141, wherein at least one of the first layer, the second layer, and the third layer has a cross-sectional dimension of at least about 1 mm.
- 25 143. The microfluidic apparatus of any one of claims 90-142, wherein each of the first layer, the second layer, and the third layer has a cross-sectional dimension of at least about 10 micrometers.
144. The microfluidic apparatus of any one of claims 90-143, wherein each of the first
30 layer, the second layer, and the third layer has a cross-sectional dimension of at least about 100 micrometers.
145. The microfluidic apparatus of any one of claims 90-144, wherein at least two layers within the microfluidic apparatus are in liquid-tight contact with each other.

146. The microfluidic apparatus of any one of claims 90-145, wherein each of the layers within the microfluidic apparatus are in liquid-tight contact with each other.
- 5 147. The microfluidic apparatus of any one of claims 90-146, wherein at least two layers within the microfluidic apparatus are solvent-bonded to each other.
148. The microfluidic apparatus of any one of claims 90-147, wherein at least two layers within the microfluidic apparatus are thermally bonded to each other.
- 10 149. The microfluidic apparatus of any one of claims 90-148, wherein at least two layers within the microfluidic apparatus are bonded to each other via an adhesive.
150. The microfluidic apparatus of claim 149, wherein the adhesive is a UV-cured
15 adhesive.
151. The microfluidic apparatus of any one of claims 90-150, further comprising a source of a force configured and arranged to force at least two layers within the microfluidic apparatus together.
- 20 152. The microfluidic apparatus of any one of claims 90-151, further comprising one or more alignment pins passing through at least one of the first layer, the second layer, and the third layer.
- 25 153. The microfluidic apparatus of any one of claims 90-152, further comprising one or more alignment pins passing through each of the first layer, the second layer, and the third layer.
154. The microfluidic apparatus of any one of claims 90-153, wherein at least one of the
30 first layer, the second layer, and the third layer is transparent.
155. The microfluidic apparatus of any one of claims 90-154, wherein at least one of the first layer, the second layer, and the third layer is translucent.

156. The microfluidic apparatus of any one of claims 90-155, wherein at least one of the first layer, the second layer, and the third layer is opaque.

157. The microfluidic apparatus of any one of claims 90-156, wherein at least one of the transverse channels is conical.

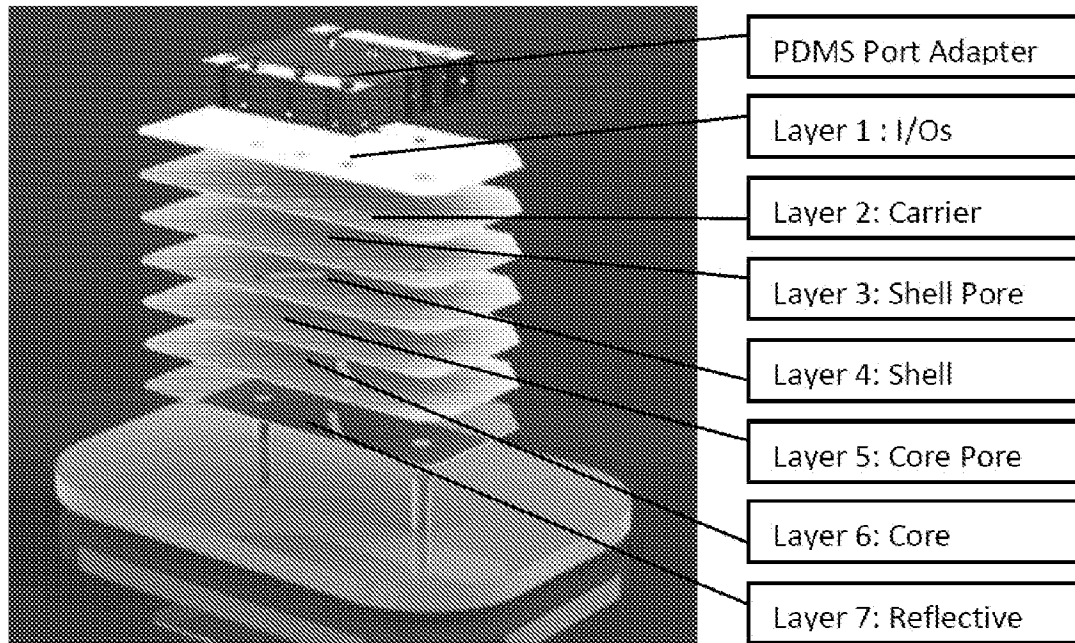


FIG. 1

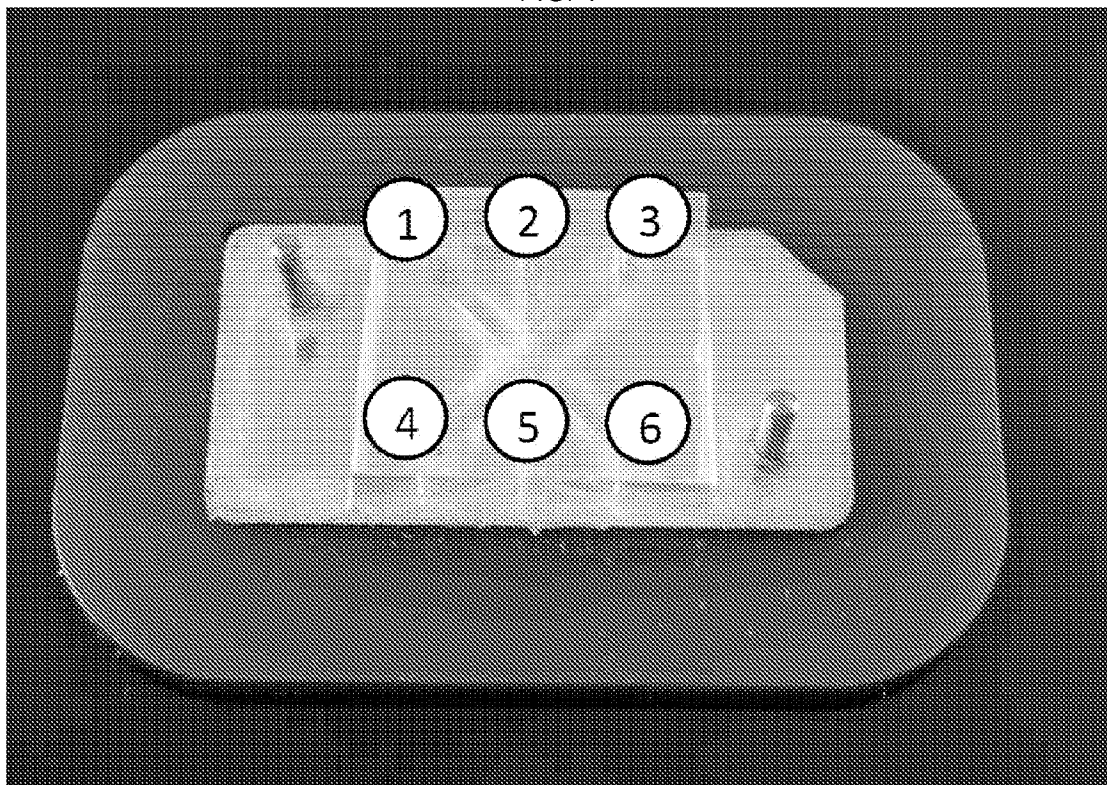


FIG. 2

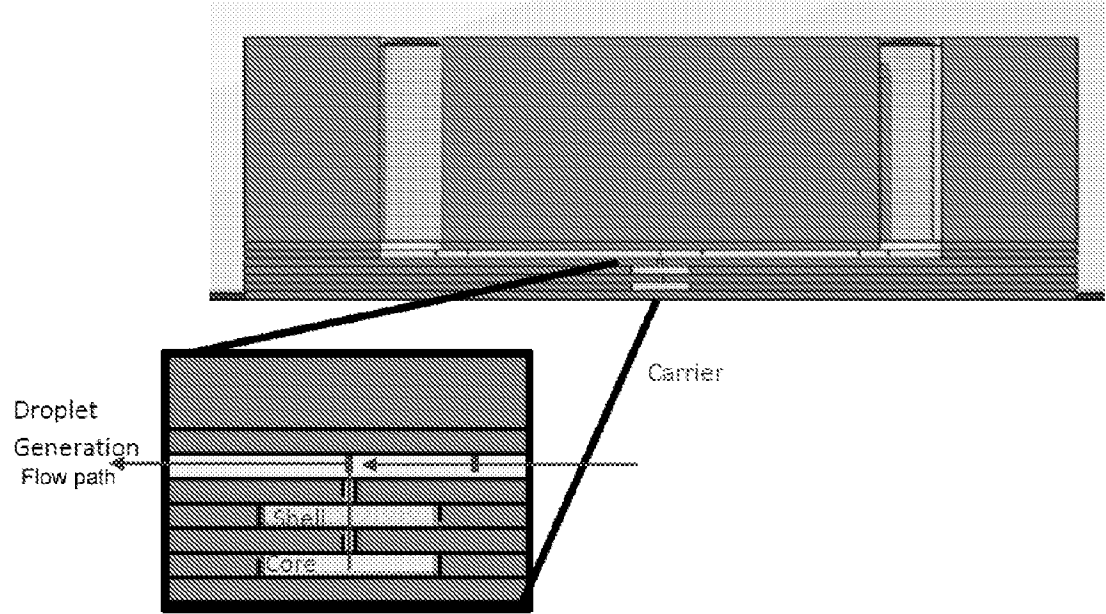


FIG. 3

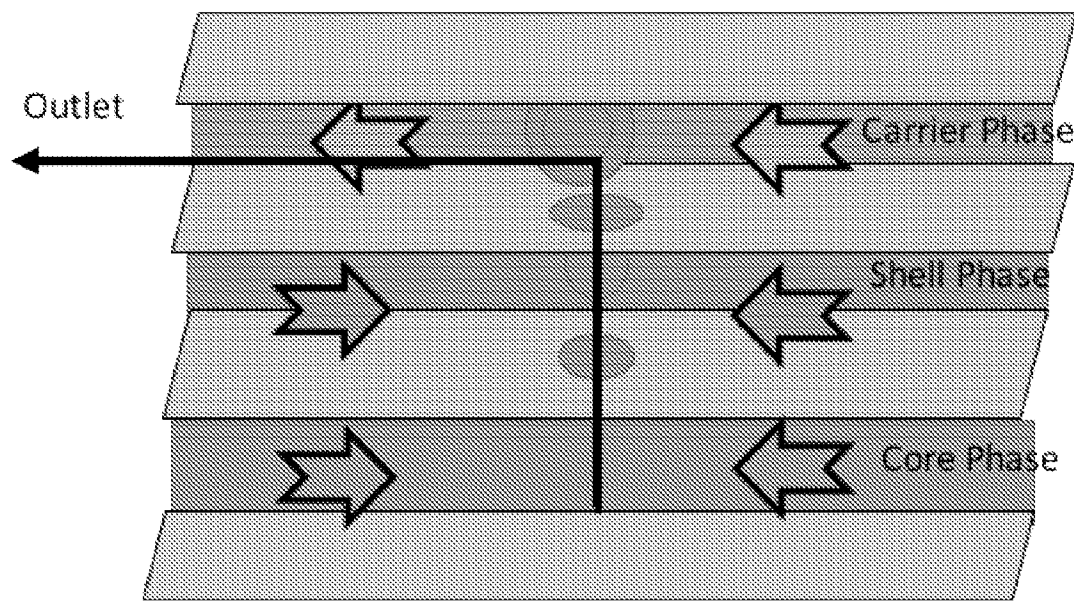


FIG. 4

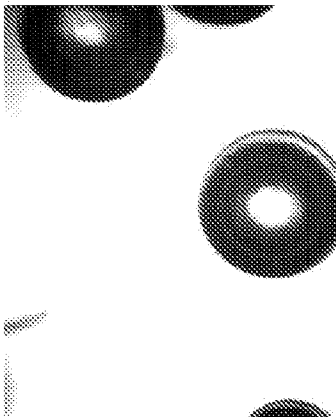


FIG. 5A

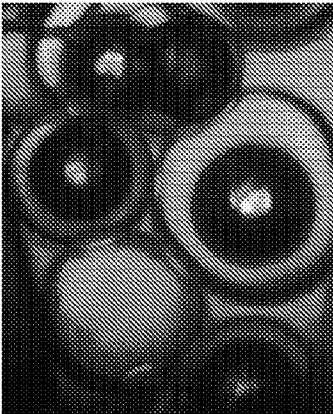


FIG. 5B



FIG. 5C

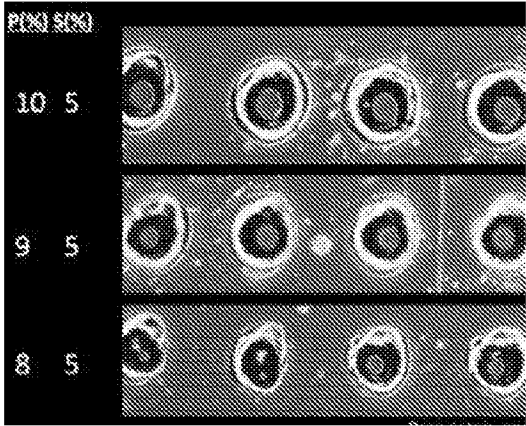


FIG. 6A

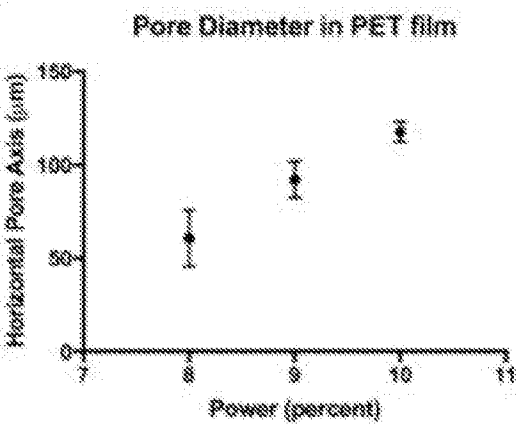


FIG. 6B

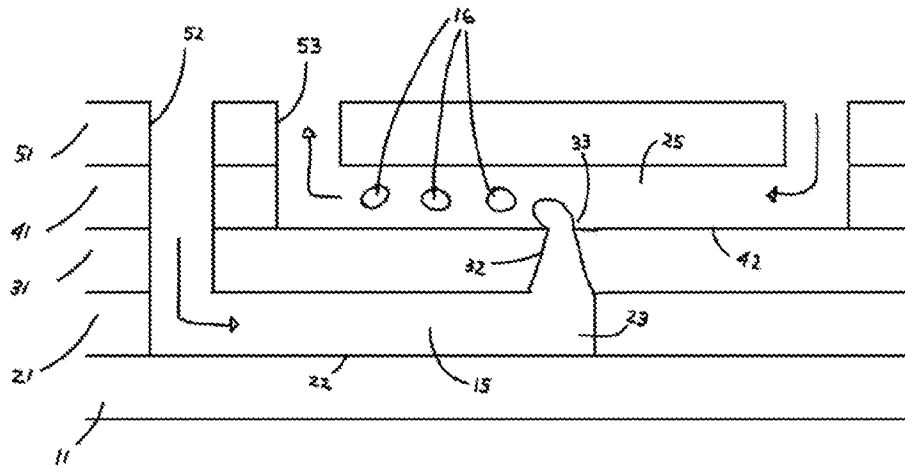


FIG. 7A

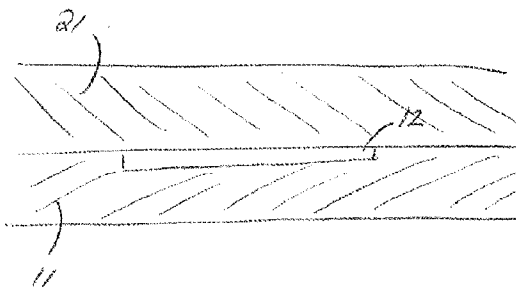


FIG. 7B

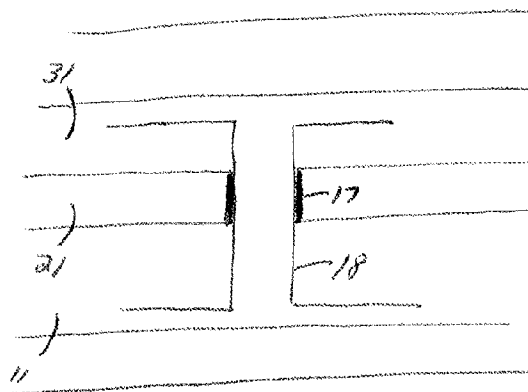


FIG. 7C

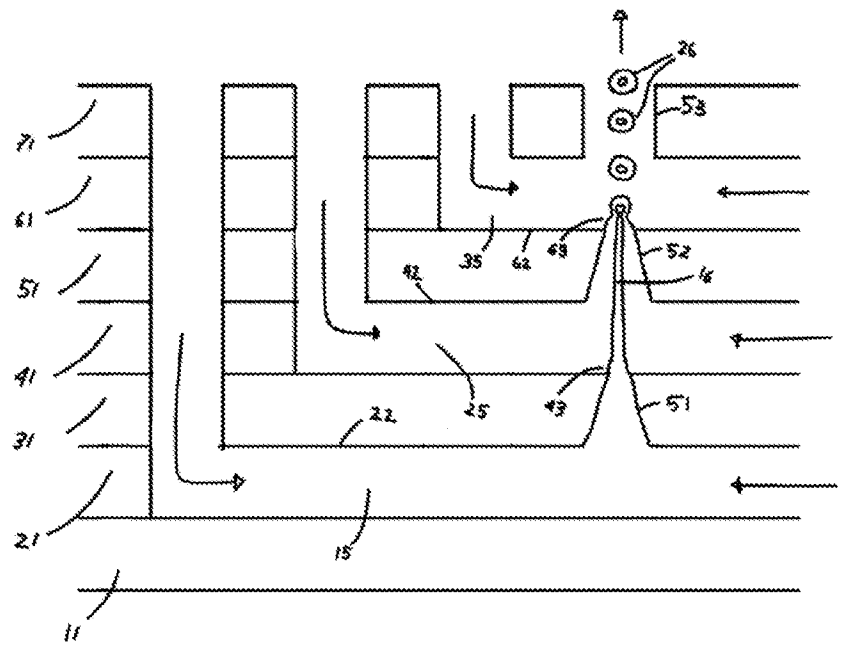


FIG. 7D

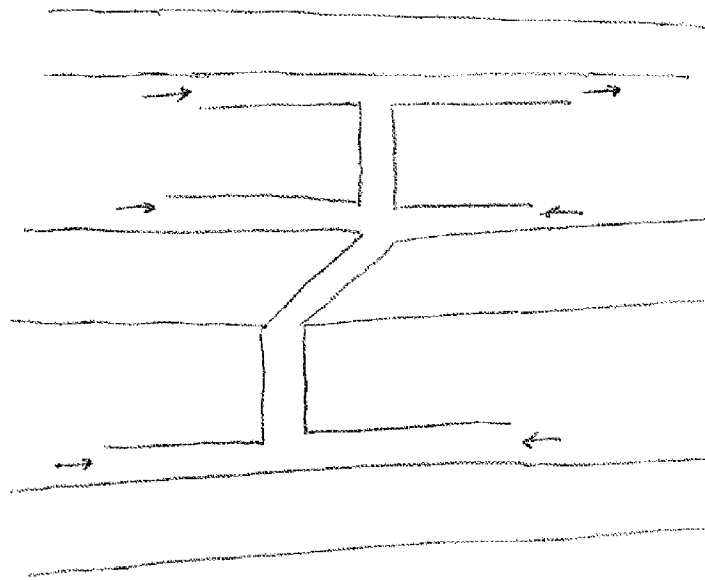


FIG. 7E

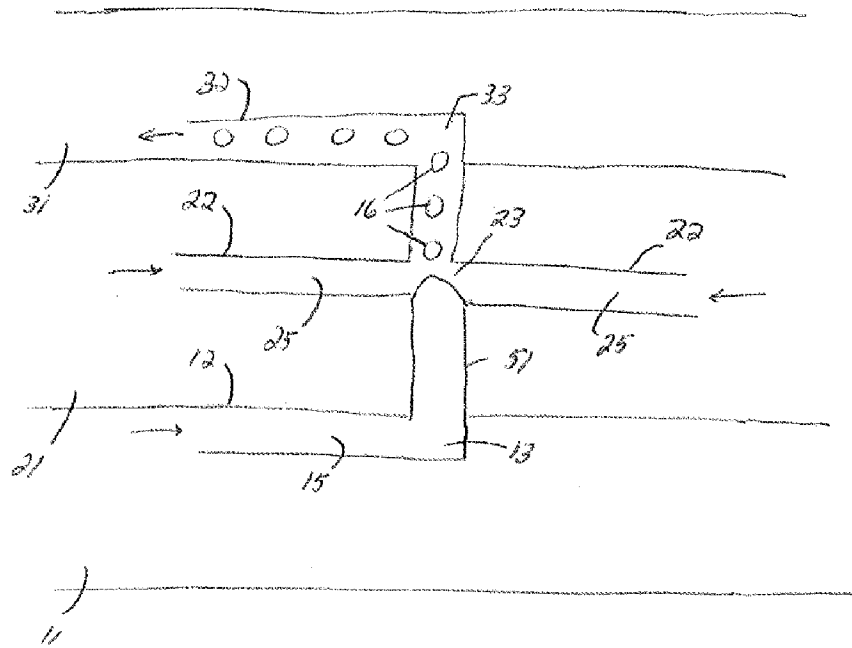


FIG. 7F

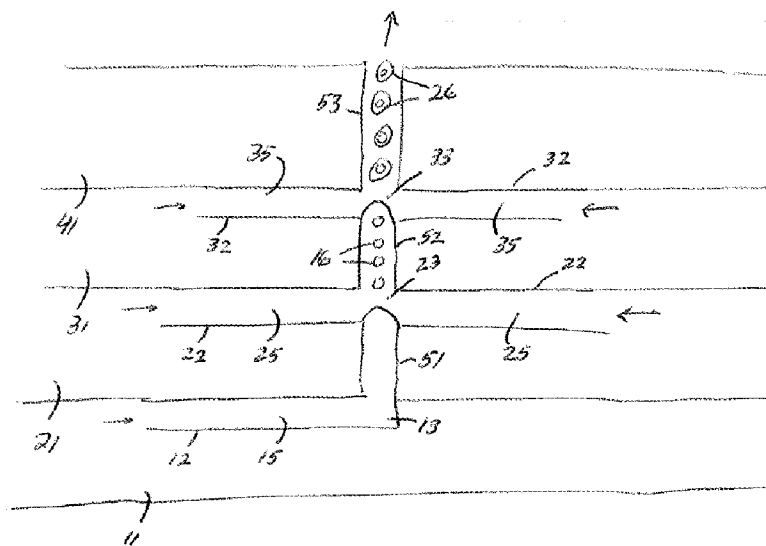


FIG. 7G

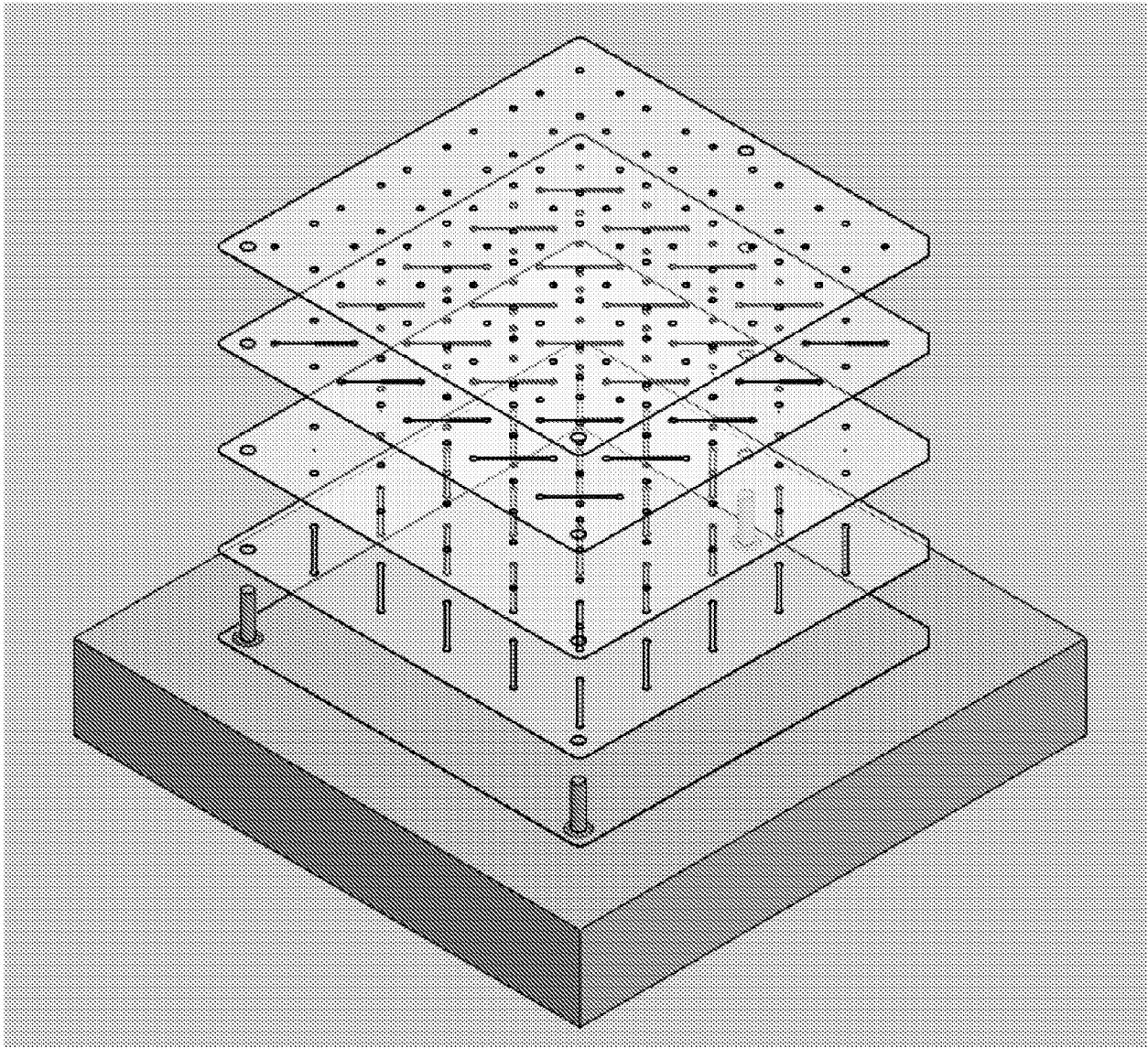


FIG. 8

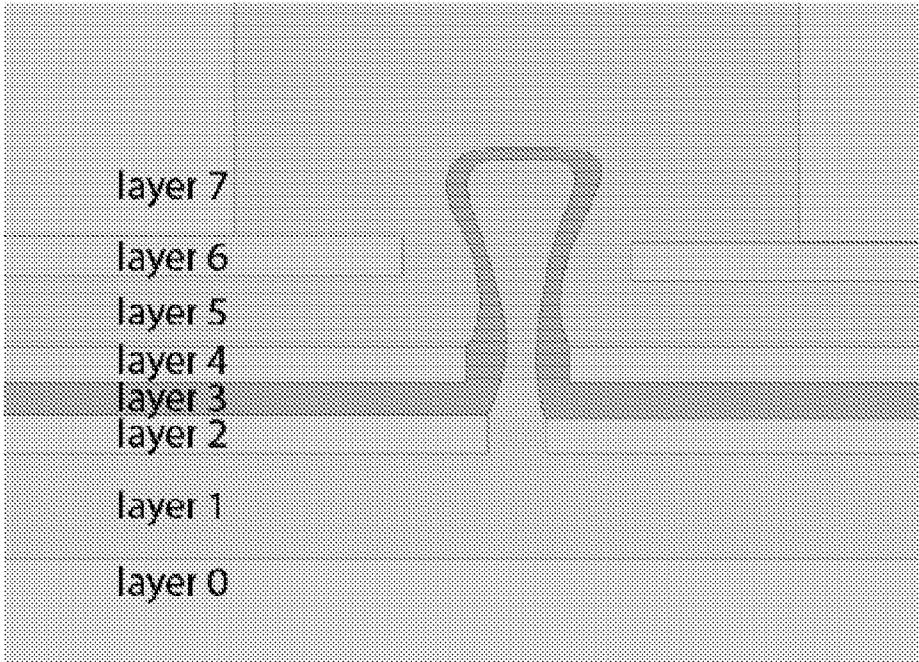


FIG. 9

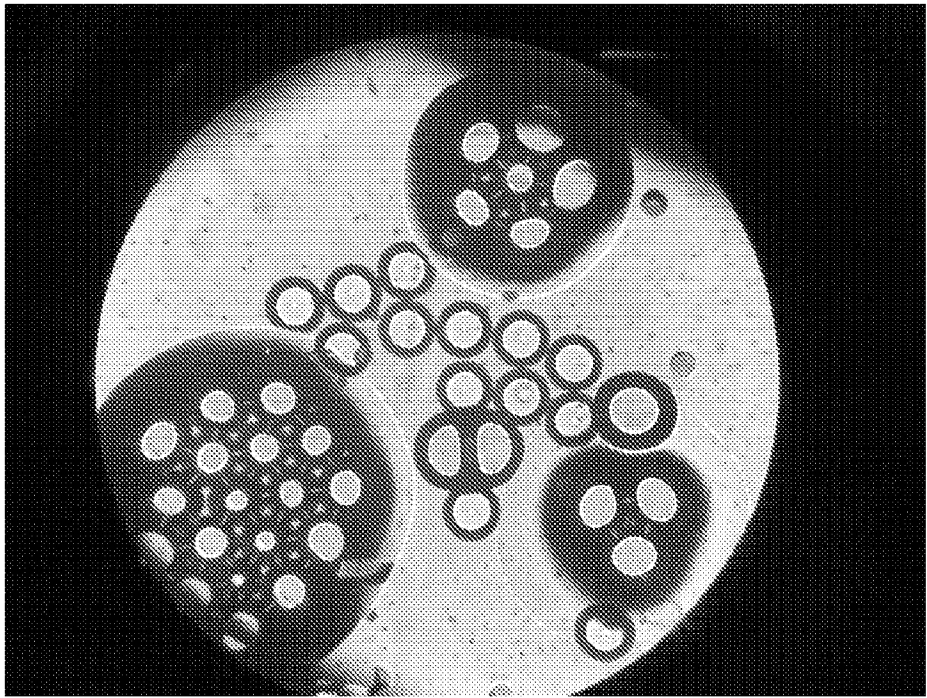


FIG. 10A

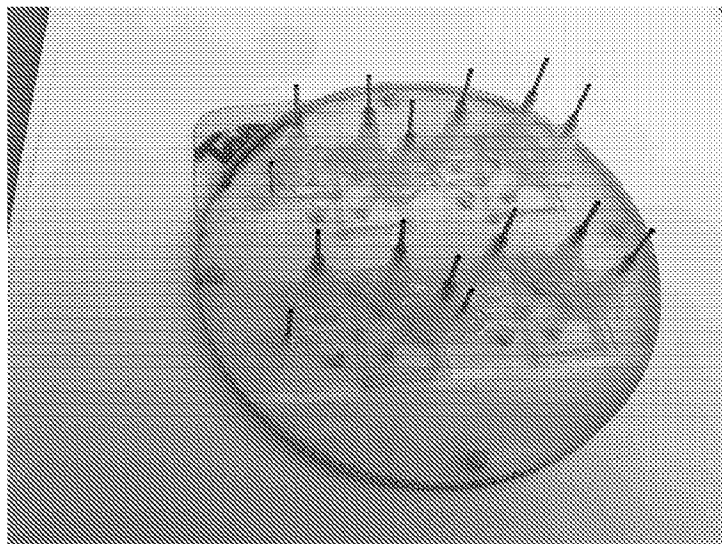


FIG. 10B

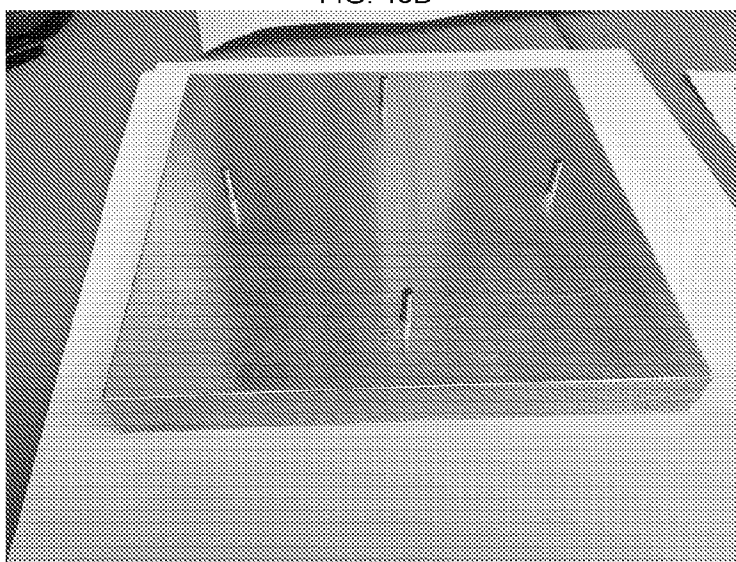


FIG. 10C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/19929

A. CLASSIFICATION OF SUBJECT MATTER

IPC - B01L 3/00; G01N 15/08, 33/483; F17D 1/00 (2019.01)

CPC - B01L 3/00; C12M 23/16; G01N 15/08, 27/44791, 33/483; F17D 1/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2012/0328834 A1 (UNGER, MA et al.) 27 December 2012; figures 7A, 7B, 26B; paragraphs [0013], [0016], [0108], [0110], [0112], [0264]	1-2, 3/1-2, 65-66, 67/65-66, 87-92, 93/91-92, 94-96, 97/95-96, 98
A	US 2018/0031564 A1 (SU, WH) 1 February 2018; abstract; figure 6; paragraph [0064]	1-2, 3/1-2, 65-66, 67/65-66, 87-92, 93/91-92, 94-96, 97/95-96, 98
A	US 2016/0340631 A1 (RESEARCH FOUNDATION OF THE CITY UNIVERSITY OF NEW YORK, et al.) 24 November 2016; figure 1; paragraph [0026]	1-2, 3/1-2, 65-66, 67/65-66, 87-92, 93/91-92, 94-96, 97/95-96, 98
A	US 2013/0068310 A1 (UNIVERSITY OF WASHINGTON THROUGH ITS CENT) 21 March 2013; entire document	1-2, 3/1-2, 65-66, 67/65-66, 87-92, 93/91-92, 94-96, 97/95-96, 98
A	US 2016/0271609 A1 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 22 September 2016; entire document	1-2, 3/1-2, 65-66, 67/65-66, 87-92, 93/91-92, 94-96, 97/95-96, 98

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

12 April 2019 (12.04.2019)

Date of mailing of the international search report

03 MAY 2019

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/19929

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-64, 68-86, 99-157
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.