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

(72) Inventors; and

(75) Inventors/Applicants (for US only): **WEITZ, David, A.** [US/US]; 213 Green Road, Bolton, MA 01740 (US). **ABATE, Adam, R.** [US/US]; 24 Berkeley Street, Apt. #1, Somerville, MA 02143 (US).

(74) Agent: **OYER, Timothy, J.**; WOLF, GREENFIELD & SACKS, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

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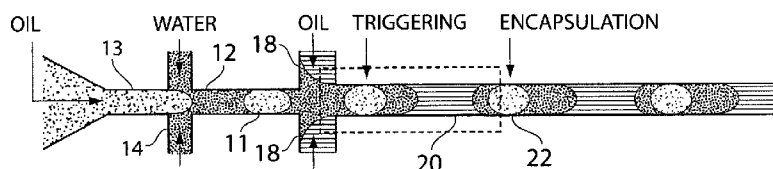


Fig. 1A

(57) Abstract: The present invention generally relates to systems and methods for creating emulsions, including multiple emulsions. In some cases, emulsions, including multiple emulsions, may be created through a "triggering" process, where a fluidic droplet or other entity is used to create one or more nestings of droplets containing the fluidic droplet or other entity. In such a manner, multiple emulsions may be formed in some cases, e.g., triple emulsions, quadruple emulsions, quintuple emulsions, etc. In certain embodiments, a first droplet (or other entity) is used to "plug" a channel; fluid pooling behind the droplet pushes the droplet through the channel to form the emulsion. This process may be repeated to create multiple emulsions in some cases. Other aspects of the present invention generally relate to systems for producing such emulsions, methods of using such emulsions, methods of promoting such emulsions, or the like.

METHOD FOR THE CONTROLLED CREATION OF EMULSIONS,
INCLUDING MULTIPLE EMULSIONS

FEDERALLY SPONSORED RESEARCH

5 Research leading to various aspects of the present invention were sponsored, at least in part, by the National Science Foundation, Grant Nos. DBI-0649865 and DMR-0602684. The United States Government has certain rights in the invention.

RELATED APPLICATIONS

 This application claims the benefit of U.S. Provisional Patent Application Serial
10 No. 61/160,020, filed March 13, 2009, entitled "Controlled Creation of Emulsions, Including Multiple Emulsions," by Weitz, *et al.* which is incorporated herein by reference.

FIELD OF INVENTION

 The present invention generally relates to systems and methods for creating
15 emulsions, including multiple emulsions.

BACKGROUND

 An emulsion is a fluidic state which exists when a first fluid is dispersed in a second fluid that is typically immiscible with the first fluid. Examples of common emulsions are oil in water and water in oil emulsions. Multiple emulsions are emulsions
20 that are formed with more than two fluids, or two or more fluids arranged in a more complex manner than a typical two-fluid emulsion. For example, a multiple emulsion may be oil-in-water-in-oil ("o/w/o"), or water-in-oil-in-water ("w/o/w"). Multiple emulsions are of particular interest because of current and potential applications in fields such as pharmaceutical delivery, paints and coatings, food and beverage, chemical
25 separations, and health and beauty aids.

 Typically, multiple emulsions of a droplet inside another droplet are made using a two-stage emulsification technique, such as by applying shear forces through mixing to reduce the size of droplets formed during the emulsification process. Other methods such as membrane emulsification techniques using, for example, a porous glass
30 membrane, have also been used to produce water-in-oil-in-water emulsions. Microfluidic techniques have also been used to produce droplets inside of droplets using a procedure including two or more steps. For example, see International Patent

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Application No. PCT/US2004/010903, filed April 9, 2004, entitled "Formation and Control of Fluidic Species," by Link, *et al.*, published as WO 2004/091763 on October 28, 2004; or International Patent Application No. PCT/US03/20542, filed June 30, 2003, entitled "Method and Apparatus for Fluid Dispersion," by Stone, *et al.*, published as WO
5 2004/002627 on January 8, 2004, each of which is incorporated herein by reference.

Multiple emulsions and the products that can be made from them can be used to produce a variety of products useful in the food, coatings, cosmetic, chemical, or pharmaceutical industries, for example. Methods for producing multiple emulsions providing consistent droplet sizes, consistent droplet counts, consistent thicknesses,
10 and/or improved control would make commercial implementation of these products more viable.

SUMMARY OF THE INVENTION

The present invention generally relates to systems and methods for creating emulsions, including multiple emulsions. The subject matter of the present invention
15 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 directed to a method. In one set of embodiments, the method includes acts of causing a deformable entity contained within a first fluid contained within a first channel to flow within the first channel towards an
20 intersection with a second channel and an outlet channel, the second channel containing a second fluid; and causing the deformable entity to plug the outlet channel, wherein the second fluid pools behind the deformable entity and the first fluid after the deformable entity plugs the outlet channel until the second fluid pushes the deformable entity into the outlet channel, thereby creating an emulsion in which the deformable entity is
25 contained within a droplet of the first fluid which is contained within the second fluid.

In another set of embodiments, the method providing a channel containing a deformable entity, a first fluid upstream of the deformable entity, and a second fluid upstream of the first fluid; causing the deformable entity within the channel to plug the channel; and applying a force to the deformable entity to unplug the channel such that an
30 emulsion is created in which the deformable entity is contained within the first fluid which is contained within the second fluid.

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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. In cases where the present specification and a document incorporated by reference include
5 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.

BRIEF DESCRIPTION OF THE DRAWINGS

10 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
15 embodiment of the invention shown where illustration is not necessary to allow those of ordinary skill in the art to understand the invention. In the figures:

Figs. 1A-1B illustrate the production of emulsions from two immiscible fluids, in one embodiment of the invention;

Figs. 2A-2C illustrate the triggering of droplets under certain conditions,
20 according to another embodiment of the invention;

Figs. 3A-3E illustrate the production of a monodisperse quintuple emulsion, in yet another embodiment of the invention;

Figs. 4A-4E illustrate the encapsulation of polyacrylamide gel particles, according to still another embodiment of the invention;

25 Figs. 5A-5B illustrate a device able to encapsulate droplets or other entities, in another embodiment of the invention;

Figs. 6A-6D illustrates the ordering of certain droplets produced in another embodiment of the invention;

Figs. 7A-7D illustrates encapsulation efficiencies in yet another embodiment of
30 the invention;

Figs. 8A-8C illustrate control of particle number and/or drop size, according to still another embodiment of the invention; and

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Figs. 9A-9E illustrate various multiple emulsions, in yet other embodiments of the invention

DETAILED DESCRIPTION

The present invention generally relates to systems and methods for creating emulsions, including multiple emulsions. In some cases, emulsions, including multiple emulsions, may be created through a “triggering” process, where a fluidic droplet or other entity is used to create one or more nestings of droplets containing the fluidic droplet or other entity. In such a manner, multiple emulsions may be formed in some cases, e.g., triple emulsions, quadruple emulsions, quintuple emulsions, etc. In certain embodiments, a first droplet (or other entity) is used to “plug” a channel; fluid pooling behind the droplet pushes the droplet through the channel to form the emulsion. This process may be repeated to create multiple emulsions in some cases. Other aspects of the present invention generally relate to systems for producing such emulsions, methods of using such emulsions, methods of promoting such emulsions, or the like.

Thus, in certain embodiments, the present invention generally relates to emulsions, including multiple emulsions, and to methods and apparatuses for making such emulsions. A “multiple emulsion,” as used herein, describes larger droplets that contain one or more smaller droplets therein. The larger droplets may be suspended in a third fluid. In certain embodiments, larger degrees of nesting within the multiple emulsion are possible. For example, an emulsion may contain droplets containing smaller droplets therein, where at least some of the smaller droplets contain even smaller droplets therein, etc. Multiple emulsions can be useful for encapsulating species such as pharmaceutical agents, cells, chemicals, or the like. As described below, multiple emulsions can be formed in certain embodiments with generally precise repeatability.

Fields in which emulsions or multiple emulsions may prove useful include, for example, food, beverage, health and beauty aids, paints and coatings, and drugs and drug delivery. For instance, a precise quantity of a drug, pharmaceutical, or other agent can be contained within an emulsion, or in some instances, cells can be contained within a droplet, and the cells can be stored and/or delivered. Other species that can be stored and/or delivered include, for example, biochemical species such as nucleic acids such as siRNA, RNAi and DNA, proteins, peptides, or enzymes, or the like. Additional species that can be incorporated within an emulsion of the invention include, but are not limited

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to, nanoparticles, quantum dots, fragrances, proteins, indicators, dyes, fluorescent species, chemicals, or the like. An emulsion can also serve as a reaction vessel in certain cases, such as for controlling chemical reactions, or for *in vitro* transcription and translation, e.g., for directed evolution technology.

5 Using the methods and devices described herein, in some embodiments, an emulsion having a consistent size and/or number of droplets can be produced, and/or a consistent ratio of size and/or number of outer droplets to inner droplets (or other such ratios) can be produced for cases involving multiple emulsions. For example, in some cases, a single droplet within an outer droplet of predictable size can be used to provide a
10 specific quantity of a drug. In addition, combinations of compounds or drugs may be stored, transported, and/or delivered in a droplet. For instance, hydrophobic and hydrophilic species can be delivered in a single, multiple emulsion droplet, as the droplet can include both hydrophilic and hydrophobic portions. The amount and concentration of each of these portions can be consistently controlled according to certain
15 embodiments of the invention, which can provide for a predictable and consistent ratio of two or more species in a multiple emulsion droplet.

 The following documents are incorporated herein by reference: U.S. Patent Application Serial No. 12/058,628, filed March 28, 2008, entitled "Emulsions and Techniques for Formation," by Chu, *et al.*, published as U.S. Patent Application
20 Publication No. 2009-0012187 on January 8, 2009; and International Patent Application No. PCT/US2006/007772, filed March 3, 2006, entitled "Method and Apparatus for Forming Multiple Emulsions," by Weitz, *et al.*, published as WO 2006/096571 on September 14, 2006. Also incorporated herein by reference are U.S. Provisional Application Serial No. 61/160,184, filed on March 13, 2009, entitled "Scale-up of
25 Microfluidic Devices," by Romanowsky, *et al.*; and U.S. Provisional Patent Application Serial No. 61/160,020, filed March 13, 2009, entitled "Controlled Creation of Emulsions, Including Multiple Emulsions," by Weitz, *et al.*

 In one aspect, an emulsion may be created through a "triggering" process, where a droplet or other entity is used to create one or more nestings of fluidic droplets
30 containing the droplet or other entity. An example of this process may be seen with reference to Fig. 1A. In this figure, a fluidic droplet 11 contained within a first channel 12 is formed at the intersection of an oil channel 13 and a water channel 14. It should be

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understood that oil and water are presented in this example for illustrative purposes only; as discussed below, the fluids contained within the channels, and the number and/or positioning of the channels, may vary in other embodiments. Also, other entities besides fluidic droplets, for instance, cells, particles, or gel particles, may also be used in certain
5 embodiments.

Fluidic droplet 11 within first channel 12 flows towards an intersection of the first channel and second channel 18, containing an oil in this example (which may or may not be the same as the oil within oil channel 13). Exiting this intersection is an outlet channel 20. However, in this example, fluidic droplet 11 has a volume such that it
10 has a cross-sectional area that is larger than the cross-sectional area of outlet channel 20. Thus, fluidic droplet 11 may “plug” outlet channel 20, i.e., since another fluid, e.g., water, cannot flow into outlet channel 20 while fluidic droplet 11 blocks the entrance to outlet channel 20 from the intersection.

Since water cannot enter outlet channel 20 without pushing fluidic droplet 11 into
15 outlet channel 20, the water may “pool” behind the fluidic droplet until it has sufficient pressure to be able to cause fluidic droplet 11 to deform and enter into outlet channel 20. This pooling and/or pressurization of the water may also cause a portion of the water to break off and form a fluidic droplet surrounding the fluidic droplet 11, thereby creating an emulsion in which fluidic droplet 11 is contained within a droplet of water, which in
20 turn is contained within oil, as is shown by encapsulated droplet 22 in Fig. 1A. By repeating this process using a plurality of fluidic droplets 11, a corresponding number of encapsulated droplets may be formed. In addition, since droplet formation typically will not occur without initial fluidic droplet 11, i.e., a droplet of water contained in oil will not typically form unless the water droplet also contains an internal oil droplet, the
25 emulsion droplets may be formed with a high degree of uniformity and a low “error” rate.

Accordingly, more generally, various aspects of the invention are directed to systems and methods for creating emulsions, including multiple emulsions, using a process in which a deformable entity, such as a fluidic droplet or a gel, at least partially
30 plugs an outlet channel, where the creation of a droplet containing the deformable entity is “triggered” by pushing the deformable entity into the outlet channel. The outlet channel may be, for instance, a microfluidic channel, as is discussed below. Typically,

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droplet formation cannot occur without this partial plugging (although there may be a relatively low “error” rate in some embodiments), and so the formation of the droplet is said to be “triggered” by creating and releasing the partial plug of the deformable entity into the outlet channel.

5 As used herein, a “deformable entity” is any entity able to at least partially plug an outlet channel, where a carrying fluid containing the deformable entity cannot flow past the deformable entity into the outlet channel while the deformable entity at least partially plugs the outlet channel. In some cases, the “plugging” may be complete, i.e., viewing the outlet channel in cross-section, it is not possible for a molecule of the
10 carrying fluid to flow through the outlet channel without crossing the deformable entity. However, in other cases, the plugging may be partial, such that it is theoretically possible for a molecule to enter into the outlet channel without crossing the deformable entity, although the carrying fluid may still be prevented from entering into the outlet channel due to effects such as viscosity, hydrophobic repulsion, charge repulsion, or the like.

15 In certain cases, the entity is deformable in that it can assume at least a first shape that is able to at least partially plug the outlet channel (e.g., a relatively spherical shape), and a second shape that is able to fit within and flow through the outlet channel (e.g., an elongated shape). Examples of deformable entities include, but are not limited to, fluidic droplets (e.g., liquids or gases), cells, gel particles, polymeric particles, or the like. As
20 used herein, the term “fluid” generally means a material in a liquid or gaseous state. Fluids, however, may also contain solids, such as suspended or colloidal particles, in certain instances.

 Thus, in one embodiment, the deformable entity is a fluidic droplet. A “droplet,” as used herein, is an isolated portion of a first fluid that is surrounded by a second fluid.
25 It is to be noted that a droplet is not necessarily spherical, but may assume other shapes as well, for example, depending on the external environment. In one embodiment, the droplet has a minimum cross-sectional dimension that is substantially equal to the largest dimension of the channel perpendicular to fluid flow in which the droplet is located. In some cases, the droplets within the first channel will have a homogenous distribution of
30 diameters, i.e., the droplets may have a distribution of diameters such that no more than about 10%, about 5%, about 3%, about 1%, about 0.03%, or about 0.01% of the droplets have an average diameter greater than about 10%, about 5%, about 3%, about 1%, about

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0.03%, or about 0.01% of the average diameter of the droplets, and correspondingly, droplets within the outlet channel may have the same, or similar, distribution of diameters.

The deformable entity may also be a cell or a gel in other embodiments. Non-limiting examples of cells include bacterium or other single-celled organisms, a eukaryotic cell, a plant cell, or an animal cell. For instance, if the cell is from an animal, the cell may be a cardiac cell, a fibroblast, a keratinocyte, a heptaocyte, a muscle cell, a blood cell, an endothelial cell, an immune cell (e.g., a T-cell, a B-cell, a macrophage, a neutrophil, a basophil, a mast cell, an eosinophil), etc. Non-limiting examples of gels include polyacrylamide gels or agarose gels, or hydrogels such as polyacrylates, polyvinyl alcohols, polyethylene glycols, polyethylene imines, polyanhydrides, or similar materials with suitable gelling compositions.

The deformable entity may flow through a first channel to an intersection with a second channel and an outlet channel. The intersection may be defined where a first channel meets a second channel and an outlet channel, where fluid flows in through the first and second channels and out through the outlet channels. In some cases, there may be more than one first channel and/or more than one second channel present. For instance, as a non-limiting example, Fig. 1A illustrates a system containing one first channel 12 and two second channels 18 connecting at an intersection with outlet channel 20. One or more fluidic channels may be microfluidic channels. Also as shown in Fig. 1A, the channels are depicted at intersecting generally perpendicularly to each other. However, in other embodiments, one or more of the channels may intersect at a non-perpendicular angle.

The deformable entity may be created within the first channel using any suitable technique, for instance, using flow-focusing techniques, by shaking or stirring a liquid to form individual droplets, creating a suspension or an emulsion containing individual droplets, or forming the droplets through pipetting techniques, needles, or the like. Other non-limiting examples of systems and methods for creating droplets and other entities within channels are disclosed in U.S. Patent Application Serial No. 11/024,228, filed December 28, 2004, entitled "Method and Apparatus for Fluid Dispersion," by Stone, *et al.*, published as U.S. Patent Application Publication No. 2005/0172476 on August 11, 2005; U.S. Patent Application Serial No. 11/246,911, filed October 7, 2005, entitled

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“Formation and Control of Fluidic Species,” by Link, *et al.*, published as U.S. Patent Application Publication No. 2006/0163385 on July 27, 2006; U.S. Patent Application Serial No. 11/360,845, filed February 23, 2006, entitled “Electronic Control of Fluidic Species,” by Link, *et al.*, published as U.S. Patent Application Publication No.

5 2007/0003442 on January 4, 2007; or International Patent Application No. PCT/US2008/007941, filed June 26, 2008, entitled “Methods and Apparatus for Manipulation of Fluidic Species,” each incorporated herein by reference in their entireties.

The fluid contained within the first channel may, in some cases, be immiscible
10 with the deformable entity if the deformable entity is a fluid. As used herein, 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, the fluid and the entity may be selected to be immiscible within the time frame of the formation of the entity (e.g., fluidic droplets)
15 within the fluid.

The first channel may intersect a second channel and/or an outlet at an intersection. The second channel may contain a second fluid that is, in some cases, immiscible with the first fluid, and may be miscible or immiscible with the deformable entity, if the deformable entity is a fluid. Thus, there may be three (or more) phases or
20 components present: a deformable entity (which may be a solid, a liquid, a gas, a gel, etc.), a first fluid, and a second fluid, which may be used to create a multiple emulsion in various embodiments. Examples of fluids potentially suitable for these phases are discussed in detail below.

As mentioned, the deformable entity may be sized so as to at least partially plug
25 the outlet channel when the deformable entity comes into contact with the entrance of the outlet channel at the intersection with the first channel. For instance, the deformable entity may have a volume such that, if the deformable entity were a perfect sphere, the radius of the sphere would be larger than the largest cross-sectional dimension of the outlet channel. In other cases, the deformable entity may be shaped such that it will not
30 fit within the outlet channel without needing to deform the entity in some fashion, e.g., by changing its shape, compressing it, shrinking it, or the like, such that the deformable

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entity may at least partially plug the outlet channel when first coming into contact with the outlet channel.

Once the deformable entity has at least partially plugged the outlet channel, fluids entering the intersection (e.g., the first and/or second fluids, or other fluids) may not be able to enter the outlet channel unless the deformable entity is deformed in some way. For instance, the deformable entity may be deformed so as to fit within the outlet channel, and thereby flows into the outlet channel, ceasing to plug the outlet channel. This process may, in some cases, be facilitated by the pooling and/or pressurization of the fluids behind the deformable entity; the increased pressure may force the movement or deformation of the deformable entity into the outlet channel. In some cases, additional forces may be applied to the deformable entity to facilitate its movement or deformation into the outlet channel, for instance, a gravitation force, a centrifugal force, a magnetic force (e.g., if the deformable entity, or a portion thereof, is magnetically susceptible), an electric force, etc.

In some embodiments, this process may also cause the first fluid (from the first channel) to pinch off to form a droplet surrounding the deformable entity, where the droplet is contained within the second fluid. Thus, in one embodiment, an emulsion may be created in which the deformable entity is contained within a droplet of the first fluid, which in turn is contained within the second fluid. As noted, in some cases, the deformable entity may be a fluid, thereby creating a triple emulsion. In other cases, the emulsion may be an emulsion of droplets containing cells, particles, gel particles, etc.

Thus, in one embodiment, a triple emulsion is produced, i.e., an emulsion containing a first fluid, surrounded by a second fluid, which in turn is surrounded by a third fluid. In some cases, the third fluid and the first fluid may be the same. These fluids can be referred to as an inner fluid (IF), a middle fluid (MF) and an outer fluid (OF), respectively, and are often of varying miscibilities due to differences in hydrophobicity. For example, the inner fluid may be water soluble, the middle fluid oil soluble, and the outer fluid water soluble. This arrangement is often referred to as a w/o/w multiple emulsion ("water/oil/water"). Another multiple emulsion may include an inner fluid that is oil soluble, a middle fluid that is water soluble, and an outer fluid that is oil soluble. This type of multiple emulsion is often referred to as an o/w/o multiple emulsion ("oil/water/oil"). It should be noted that the term "oil" in the above

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terminology merely refers to a fluid that is generally more hydrophobic and not miscible in water, as is known in the art. Thus, the oil may be a hydrocarbon in some embodiments, but in other embodiments, the oil may comprise other hydrophobic fluids. It should also be understood that the water need not be pure; it may be an aqueous solution, for example, a buffer solution, a solution containing a dissolved salt, or the like.

More than two fluids may be used in other embodiments of the invention. For example, in Fig. 9A-9E, multiple fluids are introduced at various intersections to create multiple emulsions of various nestings: single emulsions, double emulsions, triple emulsions, quadruple emulsions, quintuple emulsions, etc. Thus, certain embodiments of the present invention are generally directed to multiple emulsions, which include larger fluidic droplets that contain one or more smaller droplets therein which, in some cases, can contain even smaller droplets therein, etc. Any number of nested fluids can be produced, and accordingly, additional third, fourth, fifth, sixth, etc. fluids may be added in some embodiments of the invention to produce increasingly complex droplets within droplets. It should be understood that not all of these fluids necessarily need to be distinguishable; for example, a quadruple emulsion containing oil/water/oil/water or water/oil/water/oil may be prepared, where the two oil phases have the same composition and/or the two water phases have the same composition.

In one set of embodiments, a monodisperse emulsion may be produced, for example, if the deformable entities are monodisperse. Thus, the fluidic droplets (in any nesting level, in the case of a multiple emulsion) may each be substantially the same shape and/or size. The shape and/or size of the fluidic droplets can be determined, for example, by measuring the average diameter or other characteristic dimension of the droplets. The "average diameter" of a plurality or series of droplets is the arithmetic average of the average diameters of each of the droplets. Those of ordinary skill in the art will be able to determine the average diameter (or other characteristic dimension) of a plurality or series of droplets, for example, using laser light scattering, microscopic examination, or other known techniques. The average diameter of a single droplet, in a non-spherical droplet, is the diameter of a perfect sphere having the same volume as the non-spherical droplet. The average diameter of a droplet (and/or of a plurality or series of droplets) may be, for example, less than about 1 mm, less than about 500 micrometers, less than about 200 micrometers, less than about 100 micrometers, less than about 75

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micrometers, less than about 50 micrometers, less than about 25 micrometers, less than about 10 micrometers, or less than about 5 micrometers in some cases. The average diameter may also be at least about 1 micrometer, at least about 2 micrometers, at least about 3 micrometers, at least about 5 micrometers, at least about 10 micrometers, at least about 15 micrometers, or at least about 20 micrometers in certain cases.

The term “determining,” as used herein, generally refers to the analysis or measurement of a species, for example, quantitatively or qualitatively, and/or the detection of the presence or absence of the species. “Determining” may also refer to the analysis or measurement of an interaction between two or more species, for example, quantitatively or qualitatively, or by detecting the presence or absence of the interaction. Examples of suitable techniques include, but are not limited to, spectroscopy such as infrared, absorption, fluorescence, UV/visible, FTIR (“Fourier Transform Infrared Spectroscopy”), or Raman; gravimetric techniques; ellipsometry; piezoelectric measurements; immunoassays; electrochemical measurements; optical measurements such as optical density measurements; circular dichroism; light scattering measurements such as quasioelectric light scattering; polarimetry; refractometry; or turbidity measurements.

As the dispersity and/or size of the droplets can be narrowly controlled, emulsions can be formed that include a specific number of species or particles per droplet, in some embodiments of the invention. For instance, a single droplet may contain 1, 2, 3, 4, or more species. The emulsions can be formed with low polydispersity so that greater than about 75%, about 85%, about 90%, about 95%, or about 99% of the droplets formed contain the same number of species. In certain instances, the invention provides for the production of droplets consisting essentially of a substantially uniform number of entities of a species therein (i.e., molecules, cells, particles, inner droplets, etc.). For example, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%, or more of a plurality or series of droplets may each contain at least one entity, and/or may contain the same number of entities of a particular species. For instance, a substantial number of fluidic droplets produced, e.g., as described above, may each contain 1 entity, 2 entities, 3 entities, 4 entities, 5 entities, 7 entities, 10 entities, 15 entities, 20 entities, 25 entities, 30

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entities, 40 entities, 50 entities, 60 entities, 70 entities, 80 entities, 90 entities, 100 entities, etc., where the entities are molecules or macromolecules, cells, particles, inner droplets, etc. In some cases, the droplets may each independently contain a range of entities, for example, less than 20 entities, less than 15 entities, less than 10 entities, less than 7 entities, less than 5 entities, or less than 3 entities in some cases. For example, an emulsion can be formed in which greater than about 95% of the droplets formed contain a single cell at the point of droplet production, without a need to separate or otherwise purify the emulsion in order to obtain this level of dispersity. Typically, the fluid supporting the cell is the innermost fluid and is aqueous based. The surrounding fluid may be a non-aqueous fluid and other fluids, e.g., within the emulsion, may be aqueous or non-aqueous.

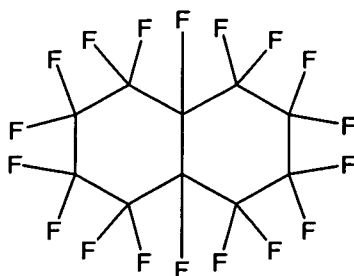
The rate of production of droplets may be determined by the droplet formation frequency, which under many conditions can vary between approximately 100 Hz and 5000 Hz. In some cases, the rate of droplet production may be at least about 200 Hz, at least about 300 Hz, at least about 500 Hz, at least about 750 Hz, at least about 1,000 Hz, at least about 2,000 Hz, at least about 3,000 Hz, at least about 4,000 Hz, or at least about 5,000 Hz, etc. In addition, production of large quantities of droplets can be facilitated by the parallel use of multiple devices in some instances. In some cases, relatively large numbers of devices may be used in parallel, for example at least about 10 devices, at least about 30 devices, at least about 50 devices, at least about 75 devices, at least about 100 devices, at least about 200 devices, at least about 300 devices, at least about 500 devices, at least about 750 devices, or at least about 1,000 devices or more may be operated in parallel. The devices may comprise different channels, orifices, microfluidics, etc. In some cases, an array of such devices may be formed by stacking the devices horizontally and/or vertically. The devices may be commonly controlled, or separately controlled, and can be provided with common or separate sources of fluids, depending on the application.

In some embodiments, the fluids used to form a multiple emulsion may be the same, or different. For example, in some cases, two or more fluids may be used to create a multiple emulsion, and in certain instances, some or all of these fluids may be immiscible. In some embodiments, two fluids used to form a multiple emulsion are compatible, or miscible, while a middle fluid contained between the two fluids is

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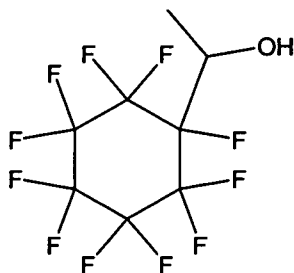
incompatible or immiscible with these two fluids. In other embodiments, however, all three fluids may be mutually immiscible, and in certain cases, all of the fluids do not all necessarily have to be water soluble. In still other embodiments, additional fourth, fifth, sixth, etc. fluids may be added to produce increasingly complex droplets within droplets, e.g., a first fluid may be surrounded by a second fluid, which may in turn be surrounded by a third fluid, which in turn may be surrounded by a fourth fluid, etc.

The fluids may be chosen such that the various droplets remain discrete, relative to their surroundings. As non-limiting examples, an emulsion may be created containing a first fluid, surrounded by a second fluid, which in turn is surrounded by a third fluid. In some cases, the first fluid and the third fluid may be identical or substantially identical, or the first fluid and the third fluid may be miscible; however, in other cases, the first fluid, the second fluid, and the third fluid may be chosen to be essentially mutually immiscible. One non-limiting example of a system involving three essentially mutually immiscible fluids is a silicone oil, a mineral oil, and an aqueous solution (i.e., water, or water containing one or more other species that are dissolved and/or suspended therein, for example, a salt solution, a saline solution, a suspension of water containing particles or cells, or the like). Another example of a system is a silicone oil, a fluorocarbon oil, and an aqueous solution. Yet another example of a system is a hydrocarbon oil (e.g., hexadecane), a fluorocarbon oil, and an aqueous solution. Non-limiting examples of suitable fluorocarbon oils include octadecafluorodecahydronaphthalene:



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or 1-(1,2,2,3,3,4,4,5,5,6,6-undecafluorocyclohexyl)ethanol:



In the descriptions herein, multiple emulsions are often described with reference to a three phase system, i.e., having a first fluid, a second fluid, and a third fluid.

5 However, it should be noted that this is by way of example only, and that in other systems, additional fluids may be present within the multiple emulsion droplet.

Accordingly, it should be understood that the descriptions of the first fluid, the second fluid, and the third fluid are by way of ease of presentation, and that the descriptions herein are readily extendable to systems involving additional fluids, e.g., quadruple

10 emulsions, quintuple emulsions, sextuple emulsions, septuple emulsions, etc.

As fluid viscosity can affect droplet formation, in some cases the viscosity of any of the fluids in the fluidic droplets may be adjusted by adding or removing components, such as diluents, that can aid in adjusting viscosity. For example, in some embodiments, the viscosity of a first fluid and a second fluid are equal or substantially equal. This may

15 aid in, for example, an equivalent frequency or rate of droplet formation in the first and second fluids. In other embodiments, the viscosity of the first fluid may be equal or substantially equal to the viscosity of the second fluid, and/or the viscosity of the first fluid may be equal or substantially equal to the viscosity of the second fluid. In yet another embodiment, the third fluid may exhibit a viscosity that is substantially different

20 from the first fluid and/or the second fluid. A substantial difference in viscosity means that the difference in viscosity between the two fluids can be measured on a statistically significant basis. For example, the viscosities may differ by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, etc., relative to the smaller of the two viscosities being compared. Other distributions of fluid viscosities

25 within the droplets are also possible. For example, the second fluid may have a viscosity greater than or less than the viscosity of the first fluid, the first fluid may have a viscosity that is greater than or less than the viscosity of the third fluid, the second fluid may have a viscosity that is greater than or less than the viscosity of the third fluid, etc. It should

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also be noted that, in higher-order droplets, e.g., containing four, five, six, or more fluids, the viscosities may also be independently selected as desired, depending on the particular application.

In certain embodiments of the invention, the fluidic droplets (or a portion thereof) may contain additional entities or species, for example, other chemical, biochemical, or biological entities (e.g., dissolved or suspended in the fluid), cells, particles, gases, molecules, pharmaceutical agents, drugs, DNA, RNA, proteins, fragrance, reactive agents, biocides, fungicides, preservatives, chemicals, or the like. Cells, for example, can be suspended in a fluid emulsion, or contained in a gel. Thus, the species may be any substance that can be contained in any portion of an emulsion. The species may be present in any fluidic droplet, and/or any portion of an emulsion, for example, within an inner droplet, within an outer droplet, within a deformable entity, etc. For instance, one or more cells and/or one or more cell types can be contained in a droplet or in an emulsion.

In one aspect of the present invention, multiple emulsions are formed by flowing two, three, or more fluids through a system of conduits or channels. One or more of the channels may be microfluidic. "Microfluidic," as used herein, refers to a device, apparatus or system including at least one fluid channel having a cross-sectional dimension of less than about 1 millimeter (mm), and in some cases, a ratio of length to largest cross-sectional dimension of at least 3:1. One or more channels of the system may be a capillary tube. In some cases, multiple channels are provided. The channels may be in the microfluidic size range and may have, for example, average inner diameters, or portions having an inner diameter, of less than about 1 millimeter, less than about 300 micrometers, less than about 100 micrometers, less than about 30 micrometers, less than about 10 micrometers, less than about 3 micrometers, or less than about 1 micrometer, thereby providing droplets having comparable average diameters. One or more of the channels may (but not necessarily), in cross section, have a height that is substantially the same as a width at the same point. Channels may include an orifice that may be smaller, larger, or the same size as the average diameter of the channel. For example, channel orifices may have diameters of less than about 1 mm, less than about 500 micrometers, less than about 300 micrometers, less than about 200 micrometers, less than about 100 micrometers, less than about 50 micrometers, less than

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about 30 micrometers, less than about 20 micrometers, less than about 10 micrometers, less than about 3 micrometers, etc. In cross-section, the channels may be rectangular or substantially non-rectangular, such as circular or elliptical.

A "channel," as used herein, means a feature on or in an article (substrate) 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. 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).

The channel may be of any size, for example, having a largest dimension perpendicular to fluid flow of less than about 5 mm or 2 mm, or less than about 1 mm, or less than about 500 microns, less than about 200 microns, less than about 100 microns, less than about 60 microns, less than about 50 microns, less than about 40 microns, less than about 30 microns, less than about 25 microns, less than about 10 microns, less than about 3 microns, less than about 1 micron, less than about 300 nm, less than about 100 nm, less than about 30 nm, or less than about 10 nm. In some cases the dimensions of the channel may be chosen such that fluid is able to freely flow through the article or substrate. 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 be varied by any method known to those of ordinary skill in the art. In some cases, more than one channel or capillary may be used. For example, two or more channels may be used, where they are positioned inside each other, positioned adjacent to each other, positioned to intersect with each other, etc.

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A variety of materials and methods, according to certain aspects of the invention, can be used to form systems (such as those described above) able to produce the multiple droplets described herein. In some cases, the various materials selected lend themselves to various methods. For example, some components of the invention can be formed
5 from solid materials, in which the channels can be formed via micromachining, film deposition processes such as spin coating and chemical vapor deposition, laser fabrication, photolithographic techniques, etching methods including wet chemical or plasma processes, and the like. See, for example, *Scientific American*, 248:44-55, 1983 (Angell, *et al*). In one embodiment, at least a portion of the fluidic system is formed of
10 silicon by etching features in a silicon chip. Technologies for precise and efficient fabrication of certain fluidic systems and devices of the invention from silicon are known. In another embodiment, various components of the systems and devices of the invention can be formed of a polymer, for example, an elastomeric polymer such as polydimethylsiloxane ("PDMS"), polytetrafluoroethylene ("PTFE" or Teflon[®]), or the
15 like.

Different components can be fabricated of different materials. For example, a base portion including a bottom wall and side walls can be fabricated from an opaque material such as silicon or PDMS, and a top portion can be fabricated from a transparent or at least partially transparent material, such as glass or a transparent polymer, for
20 observation and/or control of the fluidic process. Components can be coated so as to expose a desired chemical functionality to fluids that contact interior channel walls, where the base supporting material does not have a precise, desired functionality. For example, components can be fabricated as illustrated, with interior channel walls coated with another material. Material used to fabricate various components of the systems and
25 devices of the invention, e.g., materials used to coat interior walls of fluid channels, may desirably be selected from among those materials that will not adversely affect or be affected by fluid flowing through the fluidic system, e.g., material(s) that is chemically inert in the presence of fluids to be used within the device. A non-limiting example of such a coating is disclosed below; additional examples are disclosed in U.S. Provisional
30 Application No. 61/160,184, filed on March 13, 2009, entitled "Scale-up of Microfluidic Devices," by Romanowsky, *et al.*; U.S. Provisional Application Serial No. 61/040,442, filed on March 28, 2008, entitled "Surfaces, Including Microfluidic Channels, With

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Controlled Wetting Properties,” by Weitz, *et al.*; and Int. Pat. Apl. Pub. No. WO 2009/120254, published October 1, 2009, filed on February 11, 2009 as PCT/US2009/000850, entitled “Surfaces, Including Microfluidic Channels, With Controlled Wetting Properties,” by Abate, *et al.*, each of which incorporated herein by
5 reference in their entirety.

In one embodiment, various components of the invention are fabricated from polymeric and/or flexible and/or elastomeric materials, and can be conveniently formed of a hardenable fluid, facilitating fabrication via molding (e.g. replica molding, injection molding, cast molding, etc.). The hardenable fluid can be essentially any fluid that can
10 be induced to solidify, or that spontaneously solidifies, into a solid capable of containing and/or transporting fluids contemplated for use in and with the fluidic network. In one embodiment, the hardenable fluid comprises a polymeric liquid or a liquid polymeric precursor (i.e. a “prepolymer”). Suitable polymeric liquids can include, for example, thermoplastic polymers, thermoset polymers, or mixture of such polymers heated above
15 their melting point. As another example, a suitable polymeric liquid may include a solution of one or more polymers in a suitable solvent, which solution forms a solid polymeric material upon removal of the solvent, for example, by evaporation. Such polymeric materials, which can be solidified from, for example, a melt state or by solvent evaporation, are well known to those of ordinary skill in the art. A variety of polymeric
20 materials, many of which are elastomeric, are suitable, and are also suitable for forming molds or mold masters, for embodiments where one or both of the mold masters is composed of an elastomeric material. A non-limiting list of examples of such polymers includes polymers of the general classes of silicone polymers, epoxy polymers, and acrylate polymers. Epoxy polymers are characterized by the presence of a three-
25 membered cyclic ether group commonly referred to as an epoxy group, 1,2-epoxide, or oxirane. For example, diglycidyl ethers of bisphenol A can be used, in addition to compounds based on aromatic amine, triazine, and cycloaliphatic backbones. Another example includes the well-known Novolac polymers. Non-limiting examples of silicone elastomers suitable for use according to some embodiments of the invention include
30 those formed from precursors including the chlorosilanes such as methylchlorosilanes, ethylchlorosilanes, phenylchlorosilanes, etc.

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Silicone polymers are preferred in one set of embodiments, for example, the silicone elastomer polydimethylsiloxane. Non-limiting examples of PDMS polymers include those sold under the trademark Sylgard by Dow Chemical Co., Midland, MI, and particularly Sylgard 182, Sylgard 184, and Sylgard 186. Silicone polymers including
5 PDMS have several beneficial properties simplifying fabrication of the microfluidic structures of some embodiments of the invention. For instance, such materials are inexpensive, readily available, and can be solidified from a prepolymeric liquid via curing with heat. For example, PDMSs are typically curable by exposure of the prepolymeric liquid to temperatures of about, for example, about 65 °C to about 75 °C
10 for exposure times of, for example, about an hour. Also, silicone polymers, such as PDMS, can be elastomeric, and thus may be useful for forming very small features with relatively high aspect ratios, necessary in certain embodiments of the invention. Flexible (e.g., elastomeric) molds or masters can be advantageous in this regard.

One advantage of forming structures such as microfluidic structures of certain
15 embodiments of the invention from silicone polymers, such as PDMS, is the ability of such polymers to be oxidized, for example by exposure to an oxygen-containing plasma such as an air plasma, so that the oxidized structures contain, at their surface, chemical groups capable of cross-linking to other oxidized silicone polymer surfaces or to the oxidized surfaces of a variety of other polymeric and non-polymeric materials. Thus,
20 components can be fabricated and then oxidized and essentially irreversibly sealed to other silicone polymer surfaces, or to the surfaces of other substrates reactive with the oxidized silicone polymer surfaces, without the need for separate adhesives or other sealing means. In most cases, sealing can be completed simply by contacting an oxidized silicone surface to another surface without the need to apply auxiliary pressure
25 to form the seal. That is, the pre-oxidized silicone surface acts as a contact adhesive against suitable mating surfaces. Specifically, in addition to being irreversibly sealable to itself, oxidized silicone such as oxidized PDMS can also be sealed irreversibly to a range of oxidized materials other than itself including, for example, glass, silicon, silicon oxide, quartz, silicon nitride, polyethylene, polystyrene, glassy carbon, and epoxy
30 polymers, which have been oxidized in a similar fashion to the PDMS surface (for example, via exposure to an oxygen-containing plasma). Oxidation and sealing methods useful in the context of the present invention, as well as overall molding techniques, are

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described in the art, for example, in an article entitled "Rapid Prototyping of Microfluidic Systems and Polydimethylsiloxane," *Anal. Chem.*, 70:474-480, 1998 (Duffy, *et al.*), incorporated herein by reference.

In some embodiments, certain microfluidic structures of the invention (or
5 interior, fluid-contacting surfaces) may be formed from certain oxidized silicone polymers. Such surfaces may be more hydrophilic than the surface of an elastomeric polymer. Such hydrophilic channel surfaces can thus be more easily filled and wetted with aqueous solutions.

In one embodiment, a bottom wall of a microfluidic device is formed of a
10 material different from one or more side walls or a top wall, or other components. For example, the interior surface of a bottom wall can comprise the surface of a silicon wafer or microchip, or other substrate. Other components can, as described above, be sealed to such alternative substrates. Where it is desired to seal a component comprising a silicone polymer (e.g. PDMS) to a substrate (bottom wall) of different material, the substrate may
15 be selected from the group of materials to which oxidized silicone polymer is able to irreversibly seal (e.g., glass, silicon, silicon oxide, quartz, silicon nitride, polyethylene, polystyrene, epoxy polymers, and glassy carbon surfaces which have been oxidized). Alternatively, other sealing techniques can be used, as would be apparent to those of ordinary skill in the art, including, but not limited to, the use of separate adhesives,
20 bonding, solvent bonding, ultrasonic welding, etc.

In one aspect of the invention, a surface of a microfluidic channel may be modified to facilitate the production of emulsions such as multiple emulsions. In some cases, the surface may be modified by coating a sol-gel onto at least a portion of a microfluidic channel. As is known to those of ordinary skill in the art, a sol-gel is a
25 material that can be in a sol or a gel state, and typically includes polymers. The gel state typically contains a polymeric network containing a liquid phase, and can be produced from the sol state by removing solvent from the sol, e.g., via drying or heating techniques. In some cases, as discussed below, the sol may be pretreated before being used, for instance, by causing some polymerization to occur within the sol.

30 In some embodiments, the sol-gel coating may be chosen to have certain properties, for example, having a certain hydrophobicity. The properties of the coating may be controlled by controlling the composition of the sol-gel (for example, by using

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certain materials or polymers within the sol-gel), and/or by modifying the coating, for instance, by exposing the coating to a polymerization reaction to react a polymer to the sol-gel coating, as discussed below.

For example, the sol-gel coating may be made more hydrophobic by
5 incorporating a hydrophobic polymer in the sol-gel. For instance, the sol-gel may contain one or more silanes, for example, a fluorosilane (i.e., a silane containing at least one fluorine atom) such as heptafluorosilane, 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
10 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. In general, most silanes can be used in the sol-gel, with the particular silane being chosen on the basis of desired properties such as hydrophobicity. Other silanes (e.g., having shorter or longer chain lengths) may also be chosen in other
15 embodiments of the invention, depending on factors such as the relative hydrophobicity or hydrophilicity desired. In some cases, the silanes may contain other groups, for example, groups such as amines, which would make the sol-gel more hydrophilic. Non-limiting examples include diamine silane, triamine silane, or *N*-[3-(trimethoxysilyl)propyl] ethylene diamine silane. The silanes may be reacted to form
20 oligomers or polymers within the sol-gel, and the degree of polymerization (e.g., the lengths of the oligomers or polymers) may be controlled by controlling the reaction conditions, for example by controlling the temperature, amount of acid present, or the like. In some cases, more than one silane may be present in the sol-gel. For instance, the sol-gel may include fluorosilanes to cause the resulting sol-gel to exhibit greater
25 hydrophobicity, and other silanes (or other compounds) that facilitate the production of polymers. In some cases, materials able to produce SiO_2 compounds to facilitate polymerization may be present, for example, TEOS (tetraethyl orthosilicate).

It should be understood that the sol-gel is not limited to containing only silanes, and other materials may be present in addition to, or in place of, the silanes. For
30 instance, the coating may include one or more metal oxides, such as SiO_2 , vanadia (V_2O_5), titania (TiO_2), and/or alumina (Al_2O_3).

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In some instances, the microfluidic channel is constructed from a material suitable to receive the sol-gel, for example, glass, metal oxides, or polymers such as polydimethylsiloxane (PDMS) and other siloxane polymers. For example, in some cases, the microfluidic channel may be one in which contains silicon atoms, and in
5 certain instances, the microfluidic channel may be chosen such that it contains silanol (Si-OH) groups, or can be modified to have silanol groups. For instance, the microfluidic channel may be exposed to an oxygen plasma, an oxidant, or a strong acid cause the formation of silanol groups on the microfluidic channel.

The sol-gel may be present as a coating on the microfluidic channel, and the
10 coating may have any suitable thickness. For instance, the coating may have a thickness of no more than about 100 micrometers, no more than about 30 micrometers, no more than about 10 micrometers, no more than about 3 micrometers, or no more than about 1 micrometer. Thicker coatings may be desirable in some cases, for instance, in applications in which higher chemical resistance is desired. However, thinner coatings
15 may be desirable in other applications, for instance, within relatively small microfluidic channels.

In one set of embodiments, the hydrophobicity of the sol-gel coating can be controlled, for instance, such that a first portion of the sol-gel coating is relatively hydrophobic, and a second portion of the sol-gel coating is relatively hydrophobic. The
20 hydrophobicity of the coating can be determined using techniques known to those of ordinary skill in the art, for example, using contact angle measurements such as those discussed below. For instance, in some cases, a first portion of a microfluidic channel may have a hydrophobicity that favors an organic solvent to water, while a second portion may have a hydrophobicity that favors water to the organic solvent.

25 The hydrophobicity of the sol-gel coating can be modified, for instance, by exposing at least a portion of the sol-gel coating to a polymerization reaction to react a polymer to the sol-gel coating. The polymer reacted to the sol-gel coating may be any suitable polymer, and may be chosen to have certain hydrophobicity properties. For instance, the polymer may be chosen to be more hydrophobic or more hydrophilic than
30 the microfluidic channel and/or the sol-gel coating. As an example, a hydrophilic polymer that could be used is poly(acrylic acid).

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The polymer may be added to the sol-gel coating by supplying the polymer in monomeric (or oligomeric) form to the sol-gel coating (e.g., in solution), and causing a polymerization reaction to occur between the polymer and the sol-gel. For instance, free radical polymerization may be used to cause bonding of the polymer to the sol-gel coating. In some embodiments, a reaction such as free radical polymerization may be initiated by exposing the reactants to heat and/or light, such as ultraviolet (UV) light, optionally in the presence of a photoinitiator able to produce free radicals (e.g., via molecular cleavage) upon exposure to light. Those of ordinary skill in the art will be aware of many such photoinitiators, many of which are commercially available, such as Irgacur 2959 (Ciba Specialty Chemicals) or 2-hydroxy-4-(3-triethoxysilylpropoxy)-diphenylketone (SIH6200.0, ABCR GmbH & Co. KG).

The photoinitiator may be included with the polymer added to the sol-gel coating, or in some cases, the photoinitiator may be present within the sol-gel coating. For instance, a photoinitiator may be contained within the sol-gel coating, and activated upon exposure to light. The photoinitiator may also be conjugated or bonded to a component of the sol-gel coating, for example, to a silane. As an example, a photoinitiator such as Irgacur 2959 may be conjugated to a silane-isocyanate via a urethane bond, where a primary alcohol on the photoinitiator may participate in nucleophilic addition with the isocyanate group, which may produce a urethane bond.

It should be noted that only a portion of the sol-gel coating may be reacted with a polymer, in some embodiments of the invention. For instance, the monomer and/or the photoinitiator may be exposed to only a portion of the microfluidic channel, or the polymerization reaction may be initiated in only a portion of the microfluidic channel. As a particular example, a portion of the microfluidic channel may be exposed to light, while other portions are prevented from being exposed to light, for instance, by the use of masks or filters. Accordingly, different portions of the microfluidic channel may exhibit different hydrophobicities, as polymerization does not occur everywhere on the microfluidic channel. As another example, the microfluidic channel may be exposed to UV light by projecting a de-magnified image of an exposure pattern onto the microfluidic channel. In some cases, small resolutions (e.g., 1 micrometer, or less) may be achieved by projection techniques.

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Another aspect of the present invention is generally directed at systems and methods for coating such a sol-gel onto at least a portion of a microfluidic channel. In one set of embodiments, a microfluidic channel is exposed to a sol, which is then treated to form a sol-gel coating. In some cases, the sol can also be pretreated to cause partial polymerization to occur. Extra sol-gel coating may optionally be removed from the microfluidic channel. In some cases, as discussed, a portion of the coating may be treated to alter its hydrophobicity (or other properties), for instance, by exposing the coating to a solution containing a monomer and/or an oligomer, and causing polymerization of the monomer and/or oligomer to occur with the coating.

The sol may be contained within a solvent, which can also contain other compounds such as photoinitiators including those described above. In some cases, the sol may also comprise one or more silane compounds. The sol may be treated to form a gel using any suitable technique, for example, by removing the solvent using chemical or physical techniques, such as heat. For instance, the sol may be exposed to a temperature of at least about 150 °C, at least about 200 °C, or at least about 250 °C, which may be used to drive off or vaporize at least some of the solvent. As a specific example, the sol may be exposed to a hotplate set to reach a temperature of at least about 200 °C or at least about 250 °C, and exposure of the sol to the hotplate may cause at least some of the solvent to be driven off or vaporized. In some cases, however, the sol-gel reaction may proceed even in the absence of heat, e.g., at room temperature. Thus, for instance, the sol may be left alone for a while (e.g., about an hour, about a day, etc.), and/or air or other gases may be passed over the sol, to allow the sol-gel reaction to proceed.

In some cases, any ungelled sol that is still present may be removed from the microfluidic channel. The ungelled sol may be actively removed, e.g., physically, by the application of pressure or the addition of a compound to the microfluidic channel, etc., or the ungelled sol may be removed passively in some cases. For instance, in some embodiments, a sol present within a microfluidic channel may be heated to vaporize solvent, which builds up in a gaseous state within the microfluidic channels, thereby increasing pressure within the microfluidic channels. The pressure, in some cases, may be enough to cause at least some of the ungelled sol to be removed or “blown” out of the microfluidic channels.

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In certain embodiments, the sol is pretreated to cause partial polymerization to occur, prior to exposure to the microfluidic channel. For instance, the sol may be treated such that partial polymerization occurs within the sol. The sol may be treated, for example, by exposing the sol to an acid or temperatures that are sufficient to cause at least some gellation to occur. In some cases, the temperature may be less than the temperature the sol will be exposed to when added to the microfluidic channel. Some polymerization of the sol may occur, but the polymerization may be stopped before reaching completion, for instance, by reducing the temperature. Thus, within the sol, some oligomers may form (which may not necessarily be well-characterized in terms of length), although full polymerization has not yet occurred. The partially treated sol may then be added to the microfluidic channel, as discussed above.

In certain embodiments, a portion of the coating may be treated to alter its hydrophobicity (or other properties) after the coating has been introduced to the microfluidic channel. In some cases, the coating is exposed to a solution containing a monomer and/or an oligomer, which is then polymerized to bond to the coating, as discussed above. For instance, a portion of the coating may be exposed to heat or to light such as ultraviolet light, which may be used to initiate a free radical polymerization reaction to cause polymerization to occur. Optionally, a photoinitiator may be present, e.g., within the sol-gel coating, to facilitate this reaction.

Additional details of such coatings and other systems may be seen in U.S. Provisional Patent Application Serial No. 61/040,442, filed March 28, 2008, entitled "Surfaces, Including Microfluidic Channels, With Controlled Wetting Properties," by Abate, *et al.*; and an International Patent Application filed February 11, 2009, entitled "Surfaces, Including Microfluidic Channels, With Controlled Wetting Properties," by Abate, *et al.*, each incorporated herein by reference in their entireties.

In another aspect, the present invention provides systems and techniques for parallel use of microfluidic methods and devices for focusing and/or forming discontinuous sections of similar or dissimilar size in a fluid, including emulsions and multiple emulsions such as those described above. In one aspect, a fluid distribution article is used to distribute fluid from one input to a plurality of outputs. Using the disclosed methods and articles, a plurality of microfluidic devices may be connected in three dimensions. Microfluidic systems and the techniques are described in which, in

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some cases, it can be important to control back pressure and flow rate such that a microfluidic process, such as droplet formation, can be carried out reproducibly and consistently across a variety of similar or identical process locations. In some cases, channel dimensions are chosen that allow pressure variations within parallel devices to
5 be substantially reduced. In some embodiments, the present invention involves devices and techniques associated with manipulation of emulsions such as those described herein.

In one set of embodiments, the present invention involves formation of drops of a dispersed phase within a dispersant, of controlled size and size distribution, in a flow
10 system free of moving parts to create drop formation. That is, at the location or locations at which drops of desired size are formed, the device is free of components that move relative to the device as a whole to affect drop formation or size. For example, where drops of controlled size are formed, they are formed without parts that move relative to other parts of the device that define a channel within the drops flow. This can be referred
15 to as “passive control” of drop size, or “passive breakup” where a first set of drops are broken up into smaller drops.

Parallel microfluidic devices can be used to produce large-scale quantities of product by integrating many individual devices onto the same monolithic chip. In some cases, a parallel microfluidic device can generate emulsions in quantities of liters per day
20 per integrated chip, or even greater. In some embodiments, parallel scale-up is accompanied by a fluid distribution article for inputting fluids to, and collecting product from, an array of devices. As described in more detail below, the fluid distribution article and array of devices can be fabricated using known methods. The fluid distribution article can be used to operate an arbitrary number of microfluidic devices
25 with a minimum number of interfaces to external fluid supplies and collectors, connect a high density array of devices, and promote a long functioning lifetime of the integrated device through system redundancy.

In some embodiments, the fluid distribution article includes one or more layers of fluidic channels stacked above the layer(s) of microfluidic devices. The fluid
30 distribution article can serve one-dimensional (1-D), two-dimensional (2-D), and/or three-dimensional (3-D) arrays of devices in a scalable, parallel configuration. For example, a 1-D linear array of devices may be served by a single set of fluidic channels,

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where a 1-D array of microfluidic devices in fluid communication with a series of channels placed directly over the corresponding inlet or outlet of every device in the array. In some embodiments, the fluid distribution article channels have at least one aperture each on the top side of the channel for supplying fluid to the corresponding
5 channels and/or collecting product from the corresponding channels. Similar designs may be used for more complicated arrays, such as 2-D arrays.

In some embodiments, the distribution channels in each set of distribution channels are incorporated into a single layer. Thus, a 2-D array can be constructed by fabricating devices in a first layer, distribution channels in a second layer on top of the
10 first layer, and distribution channels in a third layer on top of the second layer. Those skilled in the art will recognize that the order of assembly may be different.

In some cases, a 3-D array is constructed by connecting units of 2-D arrays. In some embodiments, a set of distribution channels are used to fluidically connect units of 2-D arrays. A 3-D array may be constructed in a variety of conformations, for example
15 by stacking 2-D arrays, placing 2-D arrays side-by-side, etc. An array may be operated with a single set of inputs and/or outputs.

A fluid distribution article can be used to interface with an array of many independent microfluidic devices, thereby allowing an assembly comprising an arbitrary number of devices to be served with a single set of inlets and outlets. In some
20 embodiments, the methods and articles of the present invention allow scaling to at least about 100 devices, at least about 1000 devices, at least about 10000 devices, at least about 100000 devices, or even more.

In one embodiment, an article of the invention is may be constructed containing a plurality of devices arranged in three dimensions (e.g. a cube-like structure). For
25 example, such an article may contain at least 50, 100, 200, 400, 600, or even 10000 devices. In certain instances, an article containing at least such numbers of devices may occupy a volume of less than 5 cm³.

Other examples may be seen in U.S. Provisional Application Serial No. 61/160,184, filed on March 13, 2009, entitled "Scale-up of Microfluidic Devices," by
30 Romanowsky, *et al.*, incorporated herein by reference.

The following applications are each incorporated herein by reference: U.S. Patent Application Serial No. 08/131,841, filed October 4, 1993, entitled "Formation of

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Microstamped Patterns on Surfaces and Derivative Articles,” by Kumar, *et al.*, now U.S. Patent No. 5,512,131, issued April 30, 1996; U.S. Patent Application Serial No. 09/004,583, filed January 8, 1998, entitled “Method of Forming Articles including Waveguides via Capillary Micromolding and Microtransfer Molding,” by Kim, *et al.*,
5 now U.S. Patent No. 6,355,198, issued March 12, 2002; International Patent Application No. PCT/US96/03073, filed March 1, 1996, entitled “Microcontact Printing on Surfaces and Derivative Articles,” by Whitesides, *et al.*, published as WO 96/29629 on June 26, 1996; International Patent Application No.: PCT/US01/16973, filed May 25, 2001, entitled “Microfluidic Systems including Three-Dimensionally Arrayed Channel
10 Networks,” by Anderson, *et al.*, published as WO 01/89787 on November 29, 2001; U.S. Patent Application Serial No. 11/246,911, filed October 7, 2005, entitled “Formation and Control of Fluidic Species,” by Link, *et al.*, published as U.S. Patent Application Publication No. 2006/0163385 on July 27, 2006; U.S. Patent Application Serial No. 11/024,228, filed December 28, 2004, entitled “Method and Apparatus for
15 Fluid Dispersion,” by Stone, *et al.*, published as U.S. Patent Application Publication No. 2005/0172476 on August 11, 2005; International Patent Application No. PCT/US2006/007772, filed March 3, 2006, entitled “Method and Apparatus for Forming Multiple Emulsions,” by Weitz, *et al.*, published as WO 2006/096571 on September 14, 2006; U.S. Patent Application Serial No. 11/360,845, filed February 23, 2006, entitled
20 “Electronic Control of Fluidic Species,” by Link, *et al.*, published as U.S. Patent Application Publication No. 2007/000342 on January 4, 2007; and U.S. Patent Application Serial No. 11/368,263, filed March 3, 2006, entitled “Systems and Methods of Forming Particles,” by Garstecki, *et al.* Also incorporated herein by reference are U.S. Provisional Patent Application Serial No. 60/920,574, filed March 28, 2007,
25 entitled “Multiple Emulsions and Techniques for Formation,” by Chu, *et al.*

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

The controlled encapsulation of objects inside of drops is important for a variety
30 of applications in microfluidics, including for the screening and evolution of cells and enzymes, and for the synthesis of colloids with structured interiors. However, in standard techniques, the encapsulation of the objects is poorly controlled: whereas drops

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are formed at a regular rate, objects are encapsulated at random intervals, so that only a small fraction of the drops contain the proper number of objects. This problem is greatly magnified with the encapsulation of multiple objects. For example, in biological and chemical assays, it is often necessary to encapsulate cells, enzymes, beads, and other
5 substrates and reagents, inside of the drops. However, with multiple objects, the encapsulation errors multiply, so that only a miniscule fraction of the drops are usable, and the vast majority must be discarded; this can negate the speed and efficiency of droplet microfluidics for these processes. Similarly, the formation of multiple emulsions requires the controlled encapsulation of several objects, in this case, drops. The drops
10 must be formed in a sequential array of drop makers and controllably encapsulated to assemble the multiple emulsions. If the encapsulation is poorly controlled, the multiple emulsions will not be properly assembled, resulting in polydispersity. An optimal system for encapsulating objects would allow the formation of the drop to be coupled to the encapsulation of the object; this would increase encapsulation efficiency and would
15 make the devices more useful.

This example shows a system that affords control over encapsulation. The object to be encapsulated is used to trigger the formation of the drop. This allows the encapsulation to synchronize with the drop formation, at kilohertz speeds, and without active adjustment of flow rates. The object in this example was selected to be of a
20 similar size to the drop formation nozzle; this allows the object to plug the nozzle, increasing its hydrodynamic resistance, and thereby triggering the formation of the drop. The triggering makes the devices more controllable and can be implemented over a range of flow rates, and for a variety of channel scales, geometries, and aspect ratios. As a demonstration of this, triggering was used to synchronize an array of five drop makers,
25 to produce highly monodisperse quintuple emulsions. Triggering is also applicable to the encapsulation of solid particles. As a demonstration of this, this example used polyacrylamide hydrogel particles to trigger drop formation, to achieve near-perfect encapsulation efficiency of the particles.

Double emulsions of drops of two immiscible fluids can assemble into a core-shell structure. To form a double emulsion, the two fluids may be emulsified in two
30 sequential steps on the microfluidic device. For this, two sequential drop makers functionalized to have opposite wettability are used, as illustrated in Fig. 1A, which is a

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schematic of device utilizing triggering to produce monodisperse double emulsions. To fabricate the device, soft-lithography in PDMS was used. To spatially control wettability, necessary to form the double emulsions, a photoreactive sol-gel coating was used; the first drop maker was hydrophilic and the second hydrophobic. The flow rates
5 was set to 200 microliters/h for the inner phase, 550 microliters/h for the middle, and 400 microliters/h for the continuous phase. The first drop maker produced oil drops dispersed in water; the oil drops flowed into the second drop maker where they triggered the formation of the outer water drops, producing oil/water/oil double emulsions, as illustrated in Fig. 1A. To observe the triggering, the second drop maker was imaged at
10 the region demarcated by the box in Fig. 1A. To visualize the flows in the middle phase, 1 micrometer polystyrene particles were dispersed in the water to act as tracers. The device was imaged with a 40X objective having a 1 micrometer depth of field. Movies were recorded of the double emulsion formation at 25,000 fps using a Phantom V7 camera. From the movies, the locations of the inner drop and the tracer particles were
15 tracked. From the trajectories, the velocity vector fields were calculated, as measured in the reference frame of the inner drop.

The formation of a double emulsion in the second drop maker is shown in Fig. 1B; the inner drop is just visible to the left of the junction at $t = 0$ s, and then a little to the right at $t = 0.24$ s. Before the drop enters the nozzle, the nozzle's hydrodynamic
20 resistance is low, so that the water middle phase floods in ahead of the oil drop, as shown by the large forward velocity in the vector fields at $t = 0$ s. To fit in the nozzle, the oil drop must deform into a "sausage" shape, as shown at $t = 0.48$ s and $t = 0.64$ s. The oil drop obstructed the nozzle, increasing its hydrodynamic resistance. Because the fluids were supplied at constant volumetric flow rate using syringe pumps, the increased
25 resistance leads to an increase in pressure behind the oil drop. This pressure propels the drop forward and triggers the formation of the outer drop. As the oil drop moved forward, space was opened up behind it, into which the newly injected fluids flow. Because the channel walls were hydrophobic, the water avoided the walls and flowed through a cylindrical column about the center of the channel. By contrast, the oil
30 continuous phase wetted the walls and flows through a ring about edges of the channel, as shown at $t = 0.88$ s in Fig 1b. The Laplace pressure of the inner drop held the water interface outwards towards the channel walls, as shown at $t = 0.88$ s in the figure.

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Triggering is thus a consequence of this flow geometry. As the inner drop traveled forward, the volume behind it was divided into two regions: a central region, into which the water flows, and an outer region, into which the oil continuous phase flows. The volume made available to each is proportional to their respective cross-sectional areas, times the speed of the drop,

$$U_{cent}' = A_{cent} * v,$$

$$U_{out}' = A_{out} * v,$$

where v is the speed of the drop, U_{cent}' and U_{out}' are the volume growth rates of the two regions, and A_{cent} and A_{out} are the cross-sectional areas. Dividing the equations, the volume fraction of the fluids that can be accommodated behind the drop is:

$$U_{out}' / U_{cent}' = A_{out} / A_{cent}.$$

The actual volume fraction was fixed by the pumps. For the water drop to form, oil must collect in the nozzle to pinch-off the water phase. The volume fraction supplied by the pumps must therefore exceed that which can be accommodated behind the drop:

$$U_{out} / (U_{mid} + U_{in}) > U_{out}' / U_{cent}',$$

$$U_{out} / (U_{mid} + U_{in}) > A_{out} / A_{cent},$$

where U_{out} , U_{mid} , and U_{in} , are the flow rates of the outer, middle, and inner phases, respectively. This is the condition for triggering. The flow rate ratio may be fixed to $U_{out} / (U_{mid} + U_{in}) = 0.533$ using the pumps. From the images and the known channel dimensions, the area ratio can be calculated to be $A_{out} / A_{cent} = 0.377$. At these flow rates, triggering occurs, as demonstrated by the formation of the neck in the middle phase, $t = 0.88$ s, its narrowing, $t = 1.00$ s, and collapse at $t = 1.16$ s.

Triggering is possible because the inner drop plugs the nozzle. To show that the size of the drop is of critical importance for plugging the nozzle and inducing triggering, the inner drop size may be varied at fixed flow rate, and the effect on triggering can be observed. For this, valve-based flow focusing may be used: valves placed on the first

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drop maker and can be actuated to constrict the nozzle; this causes the drop maker to produce smaller drops, without changing the flow rates. To enable triggering, the flow rates were set to 33 microliters/h for the inner, 33 microliters/h for the middle, and 200 microliters/h for the outer phases. When the inner drops are small, they do not plug the nozzle, and no triggering was observed, as demonstrated by the encapsulation of several inner drops in Fig 2A, showing photomicrographs of double emulsions with inner drops of different size. In this regime, encapsulation of the inner drops was decoupled from production of the outer drops. This allowed the number encapsulated to be controlled by actuating the valve without changing the size of the outer drops, as shown for $D/W < 0.97$ in Figs. 2B and 2C. In particular, Fig. 2C illustrates the diameter of the double emulsions, as a function of the core drop diameter divided by the nozzle width. When the core is smaller than the nozzle, triggering does not occur and encapsulation is decoupled from double emulsion production. By contrast, when the core is of a similar size to the nozzle, the core triggers the production of the double emulsion, so that only one core is encapsulated per drop.

Because flow rates were unchanged with the valve actuation, the number encapsulated is equal to the volume of inner phase supplied over the drop formation interval, divided by the volume of the inner drops:

$$N = U_{in} / (U_{in} + U_{out}) * [D_{dbl} / D_{in}]^3,$$

as demonstrated by direct comparison with the experimental measurements in Fig 2B.

By contrast, in the triggering regime, the formation of the outer drop appeared to be strongly coupled to the encapsulation of the inner drop, so that the number encapsulated is always one, irrespective of flow rate and valve actuation. Instead, changing the inner drop size changes the outer drop size, as demonstrated in the images for $D/W \geq 0.97$ in Fig 2A, and quantified by the measurements in Figs. 2B and 2C. This distinctive behavior can be understood in terms of the synchronization induced by triggering. The outer drop was only formed when the inner drop was present for encapsulation. If the inner drops were larger, then they were made more slowly, because they take longer to fill in the first drop maker. Therefore, the outer drops were also larger, because they were triggered more slowly in the second drop maker. This lead to a

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simple linear prediction for the size of the inner drop and the size of the double emulsion:

$$D_{dbl} = (U_{in} + U_{mid}) / U_{in} * D_{in},$$

5

where the slope of the line is the volume fraction of the double emulsions, and is set by the volume fraction of the fluids supplied by the pumps, as shown by comparison with the experimental data in Fig 2C. This makes it simple to control the dimensions of the double emulsions in this system: one simply varies the volume fractions supplied by the pumps.

10

EXAMPLE 2

One feature of triggering is that coupled drop makers become synchronized. This is very useful for forming higher order multiple emulsions, such as triple or quadruple emulsions, which require the use of large arrays of drop makers. To demonstrate the utility of triggering for synchronization, in this example, an array of five drop makers was synchronized to produce monodisperse quintuple emulsions, as shown in Figs. 3A-3C. To implement triggering, each nozzle was designed to be slightly smaller than the drop from the previous stage. This allowed the drop, whether it is a single, double, or multiple emulsion, etc., to plug the nozzle, to induce triggering. The flow rates of the device were set to 200 microliters/h for the inner, and 400, 600, 800, 1400, and 2500 microliters/h for the subsequent stages. The first drop maker thus produced monodisperse single emulsion drops at 1 kHz. The drops flowed into the downstream array of drop makers, where they triggered a cascade of encapsulations: the single triggers the formation of a double in the second stage, which triggers the formation of a triple in a third, which triggers a quadruple in the fourth, culminating with the quadruple emulsion triggering the formation of a quintuple emulsion in the fifth stage, as shown in Fig 3A.

25

In particular, Fig. 3A is a photomicrograph of quintuple emulsion maker consisting of five sequentially arrayed drop makers. Fig. 3B illustrates intensity time traces of the core drops as they move through each of the five drop makers. Each peak in the intensity corresponds to a core drop moving through a drop maker. Fig. 3C shows normalized pair cross-correlation of the intensity time traces; the darkest curve

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corresponds to the cross-correlation between the single and double emulsion drop makers, whereas the lightest curve corresponds to the quadruple and the quintuple emulsion drop makers.

To analyze the synchronization of the drop makers, drop motion was tracked through the quintuple emulsion maker. Movies of the formation were recorded at 9,000
5 fps using a fast camera. The images were processed so that the innermost “core” drops appeared as bright spots on a black background. The intensity as a function of time was then measured in the processed images at five specific locations: at the entrances to each of the five nozzles. The intensity time traces for each of the drop makers were plotted as
10 separate curves in Fig. 3D; each peak in the curves corresponds to a core drop moving past a measurement location. The arrow drawn over the curves follows a core drop as it is encapsulated four times to produce a quintuple emulsion. The core drop was first observed in the first drop maker just after being formed, as shown by the peak at the tail of the arrow in Fig 3D. As it traveled downstream, it was observed again at a later time
15 in the second drop maker, as shown by the dark gray curve in Fig 3D. The core then moves into the third, fourth, and fifth stages, where it is again was observed along the arrow at later times, Fig 3D. The arrow can be shifted by one period to follow the formation of the next quintuple emulsion; this is possible because the drop formation is periodic and synchronized by triggering. To quantify the synchronization, the pair-wise
20 cross-correlation for adjacent drop makers could be calculated, Fig. 3E. The cross-correlations were averages over the entire operation of the device, and thus is representative its long-time performance and synchronization. Like the individual time tracers, the cross-correlations were locked in phase and frequency, because the drop makers were synchronized and triggered periodically by the drip of the first drop maker,
25 as shown in Fig 3E.

EXAMPLE 3

Triggering can also be applied to the encapsulation of particles to achieve very high encapsulation efficiency. To illustrate this, triggering was used to encapsulate solid particles inside of drops. For the particles, 30 micrometers polyacrylamide gels
30 synthesized with microfluidic emulsion polymerization were used. The particles were flowed into a flow-focus junction where they triggered drop formation, as shown by the encapsulation in Figs. 4A and 4C. Just as with drops, if the particles were smaller then

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the nozzle they did not trigger drop formation, as shown in Figs. 4B and 4D. The encapsulation efficiency was quantified by counting the number of particles in each drop for devices with different nozzle widths. From the counts, the standard error was computed, plotted in Fig 4E. Just as with drops, triggering afforded control over the encapsulation of particles and required that they be nearly as large as the nozzle, as shown in Fig 4E.

Fig. 4 illustrates drop formation being triggered by the encapsulation of solid particles. Photomicrograph of microfluidic devices with 25 micrometers (Fig. 4A) and 60 micrometers (Fig. 4B) nozzles; the images in Figs. 4C and 4D show magnified views of the drop formation nozzles. The particles were 30 micrometers in diameter. The scale bars denote 100 micrometers. Fig. 4E illustrates encapsulation error as a function of the particle diameter divided by the nozzle width. When $D/W \sim 1$, triggering occurs, and encapsulation errors are rare; however, when $D/W < 1$, triggering does not occur, and encapsulation errors are common.

EXAMPLE 4

Droplets are useful for chemical and biological assays performed in microfluidic devices. The droplets can serve, for example, as picoliter reactor volumes in which to perform the assays. The droplets can be formed, merged, and sorted, with control and at high speeds. This combination of speed, control, and containment of the reactions, is useful for many applications, including high-throughput screening, combinatorial chemistry, genetic sequencing, and the directed evolution of cells, organisms, and enzymes.

In many of these applications, it is desirable to have efficient loading of the drops with the cells, beads, gel particles, and the other substrates that are needed in the assays. However, in standard techniques, the loading may be poorly controlled: the objects may be diluted down to very low concentrations and are encapsulated into the drops at random, a process that is governed by Poisson statistics. The result is that while some of the drops do contain a single object, many, or even a majority, of the drops are empty. Nevertheless, by exploiting the speed of the devices and the ease with which the drops can be made, such inefficiency can be overcome by over-encapsulating the objects and simply discarding the empty drops. This is possible for assays that require the use of a single object, but becomes impractical for assays that require the encapsulation of

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multiple objects. In the case of multiple encapsulations, the error probabilities multiply so that, for a random process, only a miniscule fraction of the drops contain the proper number of objects. Such vast inefficiency can reduce or negate the speed and efficiency of the droplet microfluidic processes.

5 Fig. 5A is a schematic diagram showing an encapsulating device as used in this example, while Fig. 5B is a photomicrograph of the encapsulation junction. The particles were injected into the junction at a very high volume fraction through the upper inlet; additional water was flowed in through the first set of side inlets. By tuning the particle and water flow rates, the particle frequency and particle/water volume fraction
10 could be controlled independently. The oil was flowed into the junction through the second set of inlets, where the drops are formed.

 Fig. 6 illustrates the geometrical ordering and periodicity achieved with this device. Fig. 6A is a photomicrograph of a compacted gel particle suspension being flowed into a microfluidic device. The high volume fraction of the suspension caused
15 the particles to order into a regular spacing. This regularity gave rise to the periodic ejection of a particle from the spacer junction. To quantify the periodicity of the particle ejection, image analysis was used to measure the grayscale intensity of the particles as they flowed past the detection window drawn in the image. Fig. 6B illustrates grayscale intensity of the gel particles measured in the detection window as a function of time;
20 each particle was observed as a peak in the intensity. The peaks were observed periodically because the particles pass the detection window periodically. To quantify this periodicity, the power spectra of the intensity time trace was measured, as is shown in Fig. 6C. The power spectra have peaks at the first and second harmonics of the average particle frequency of 1.6 kHz. Fig. 6D shows the measured droplet frequency as
25 a function of time for 30 particles.

 Similarly, Fig. 7 illustrates encapsulation efficiency with geometrical ordering and triggering. In particular, Fig. 7A is a photomicrograph of an encapsulation device in which particles order, are spaced out with water, and then were encapsulated into drops. The width of the nozzle, shown in the middle of the image, was equal to the diameter of
30 the particles; this allowed the particle to trigger drop formation and can be used to compensate for slight frequency variations. In contrast, Fig. 7B is a photomicrograph of an encapsulation device in which the nozzle was much wider than the particle diameter

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so that particles could not trigger drop formation. To achieve high encapsulation efficiency with this device, the particle ejection frequency and drop formation frequencies must be carefully matched by adjusting flow rate. Fig. 7C is a photomicrograph of device in which 10 micrometer particles were encapsulated. The smaller particles were polydisperse and did not form a regular ordering prior to encapsulation; as a result, encapsulation efficiency was significantly reduced.

To quantify the encapsulation efficiency, the number of particles encapsulated into each drop could be counted and the probability distributions plotted. In Fig. 7D, the device in which particles first ordered and then triggered drop formation (T, O) produced the highest encapsulation efficiency, about 97%. The device with ordering but no triggering (O), produced lower, but still very high encapsulation efficiency. A device with limited ordering and no triggering (O, squares) produced only low encapsulation efficiencies. A Poisson distribution (P) with an average of one is also plotted for comparison.

Fig. 8 illustrates control of particle number and drop size. Fig. 8A illustrates the number of particles encapsulated as a function of flow rate. The reasonable agreement with a linear fit demonstrates that increasing the particle number did not significantly affect the nature of the drop formation. Fig. 8B illustrates drop diameter as a function of middle phase flow rate. Adding more fluid into the inner phase increased the size of the drop, as illustrated by the fit to a cube root. Fig. 8C illustrates a series of photomicrographs of drops containing gel particles in which the number and drop size were independently controlled by controlling the inner and middle phases, respectively.

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.

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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
5 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
10 mutually inconsistent, is included within the scope of the present invention.

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
15 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.
20 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
25 “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
30 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,

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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
5 (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.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

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
10 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
15 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
20 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.

It should also be understood that, unless clearly indicated to the contrary, in any
25 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,”
30 “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,

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respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

What is claimed is:

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CLAIMS

1. A method, comprising:
 - causing a deformable entity contained within a first fluid contained within
 - 5 a first channel to flow within the first channel towards an intersection with a
 - second channel and an outlet channel, the second channel containing a second
 - fluid; and
 - causing the deformable entity to plug the outlet channel, wherein the
 - second fluid pools behind the deformable entity and the first fluid after the
 - 10 deformable entity plugs the outlet channel until the second fluid pushes the
 - deformable entity into the outlet channel, thereby creating an emulsion in which
 - the deformable entity is contained within a droplet of the first fluid which is
 - contained within the second fluid.
- 15 2. The method of claim 1, wherein the deformable entity is a fluidic droplet.
3. The method of claim 1, wherein the deformable entity is a cell.
4. The method of claim 1, wherein the deformable entity is a gel droplet.
- 20 5. The method of claim 1, wherein, in the absence of the deformable entity, the first
- fluid and the second fluid do not form an emulsion within the outlet channel.
6. The method of claim 1, wherein the deformable entity, when contained within the
- 25 first channel, substantially plugs the first channel.
7. The method of claim 1, wherein the intersection is an intersection of the first
- channel, two second channels, and an outlet channel.
8. The method of claim 1, wherein the first fluid is immiscible in the second fluid.
- 30 9. The method of claim 1, wherein the first fluid is aqueous.

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10. The method of claim 1, wherein the second fluid is aqueous.
11. The method of claim 1, further comprising flowing a plurality of deformable
5 entities within the first channel.
12. The method of claim 11, further comprising creating an emulsion containing a
plurality of emulsified droplets such that at least about 75% of the deformable
entities are each contained within a separate droplet of the first fluid contained
10 within the second fluid.
13. The method of claim 12, wherein at least about 85% of the deformable entities
are each contained within a separate droplet of the first fluid contained within the
second fluid.
- 15 14. The method of claim 13, wherein at least about 90% of the deformable entities
are each contained within a separate droplet of the first fluid contained within the
second fluid.
- 20 15. The method of claim 14, wherein at least about 95% of the deformable entities
are each contained within a separate droplet of the first fluid contained within the
second fluid.
- 25 16. The method of claim 15, wherein at least about 99% of the deformable entities
are each contained within a separate droplet of the first fluid contained within the
second fluid.
17. The method of claim 1, wherein the deformable entity in the outlet channel has an
average diameter of no more than about 1 mm.
- 30 18. The method of claim 1, wherein the viscosity of the first fluid is substantially
equal to the viscosity of the second fluid.

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19. The method of claim 1, wherein at least some of the second fluidic droplets contain a species therein.
- 5 20. The method of claim 1, wherein the species is selected from the group consisting of cells, drugs, nucleic acids, proteins, fragrances, nanopmethods, or quantum dots.
21. The method of claim 19, wherein at least about 90% of the second fluidic
10 droplets each contain the species.
22. The method of claim 19, wherein at least about 99% of the second fluidic droplets each contain the species.
- 15 23. A method, comprising:
providing a channel containing a deformable entity, a first fluid upstream of the deformable entity, and a second fluid upstream of the first fluid;
causing the deformable entity within the channel to plug the channel; and
applying a force to the deformable entity to unplug the channel such that
20 an emulsion is created in which the deformable entity is contained within the first fluid which is contained within the second fluid.
24. The method of claim 23, wherein the deformable entity is a fluidic droplet.
- 25 25. The method of claim 23, wherein the deformable entity is a cell.
26. The method of claim 23, wherein the deformable entity is a gel droplet.
27. The method of claim 23, wherein the first fluid is immiscible in the second fluid.
30
28. The method of claim 23, wherein the first fluid is aqueous.

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29. The method of claim 23, wherein the second fluid is aqueous.
30. The method of claim 23, further comprising flowing a plurality of deformable entities within the channel.
- 5 31. The method of claim 30, further comprising creating an emulsion containing a plurality of emulsified droplets such that at least about 75% of the deformable entities are each contained within a separate droplet of the first fluid contained within the second fluid.
- 10 32. The method of claim 31, wherein at least about 85% of the deformable entities are each contained within a separate droplet of the first fluid contained within the second fluid.
- 15 33. The method of claim 32, wherein at least about 90% of the deformable entities are each contained within a separate droplet of the first fluid contained within the second fluid.
- 20 34. The method of claim 33, wherein at least about 95% of the deformable entities are each contained within a separate droplet of the first fluid contained within the second fluid.
- 25 35. The method of claim 34, wherein at least about 99% of the deformable entities are each contained within a separate droplet of the first fluid contained within the second fluid.
36. The method of claim 23, wherein the deformable entity in the outlet channel has an average diameter of no more than about 1 mm.
- 30 37. The method of claim 23, wherein the viscosity of the first fluid is substantially equal to the viscosity of the second fluid.

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38. The method of claim 23, wherein at least some of the second fluidic droplets contain a species therein.
39. The method of claim 38, wherein the species is selected from the group
5 consisting of cells, drugs, nucleic acids, proteins, fragrances, nanopmethods, or quantum dots.
40. The method of claim 38, wherein at least about 90% of the second fluidic droplets each contain the species.
10
41. The method of claim 38, wherein at least about 99% of the second fluidic droplets each contain the species.

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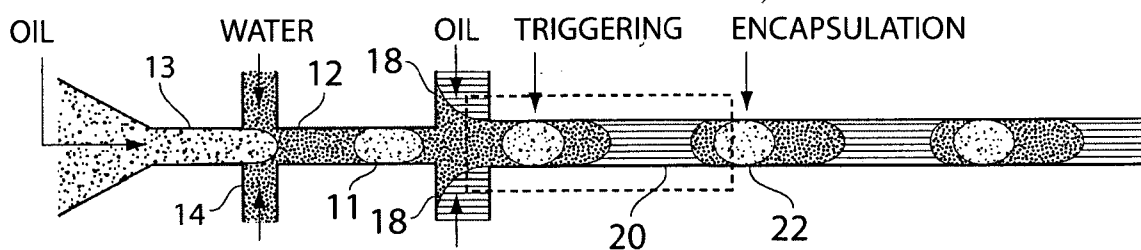


Fig. 1A

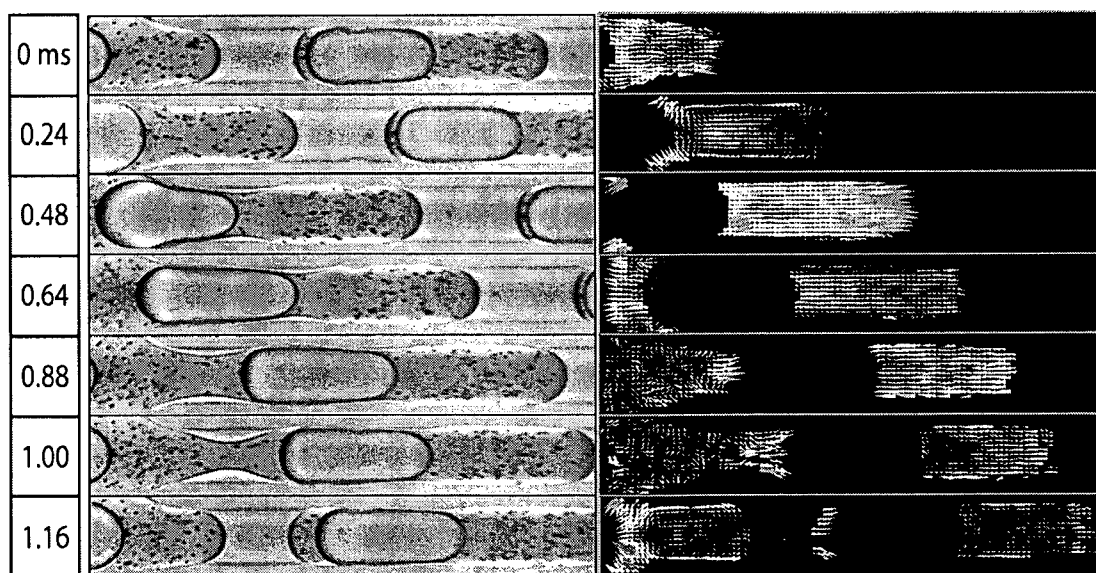


Fig. 1B

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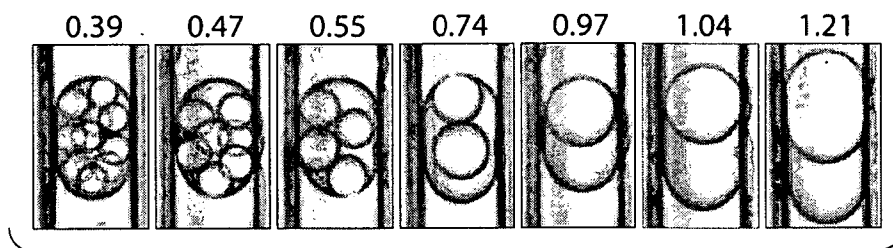


Fig. 2A

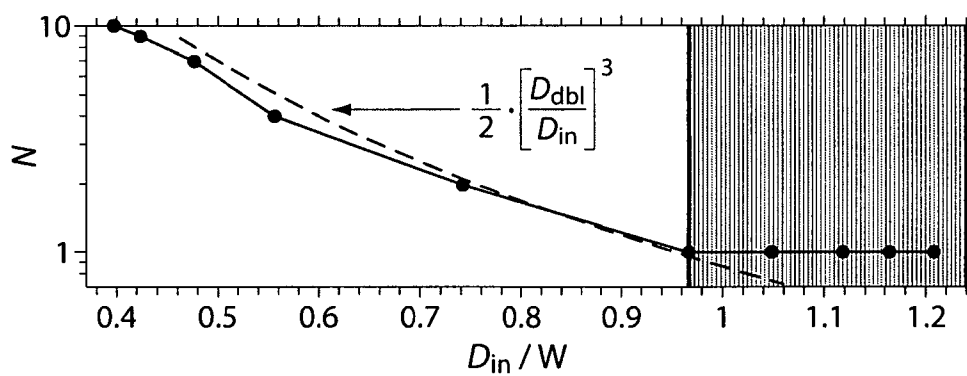


Fig. 2B

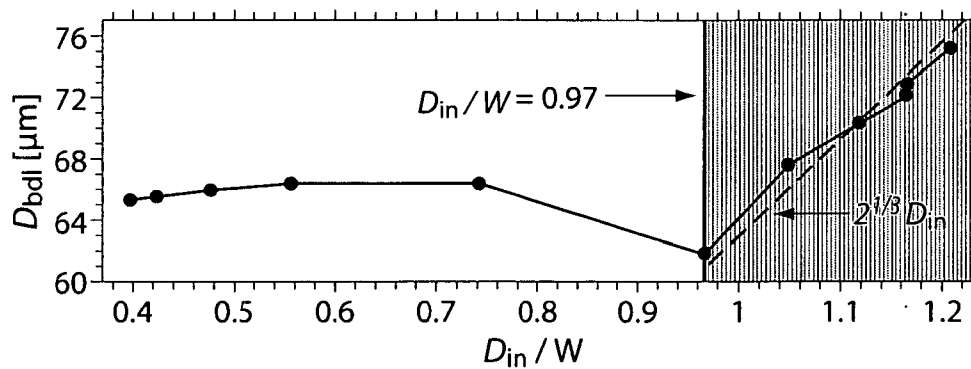


Fig. 2C

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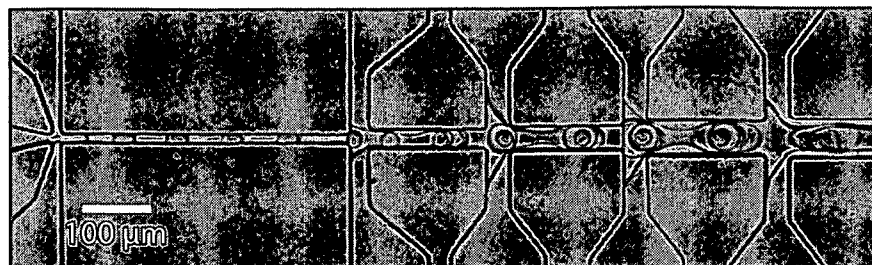


Fig. 3A

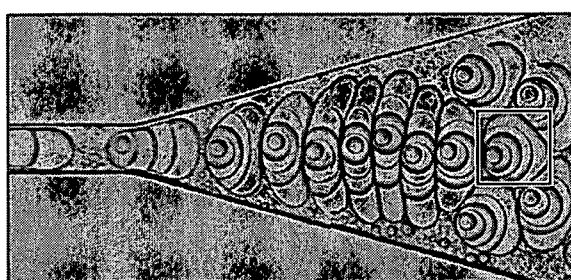


Fig. 3B

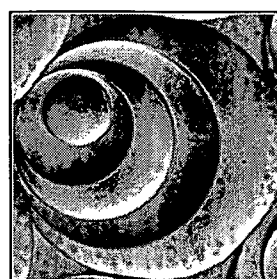


Fig. 3C

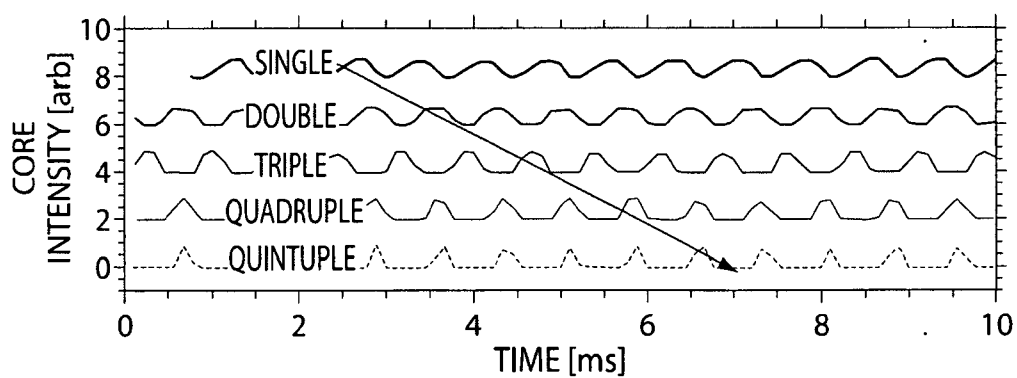


Fig. 3D

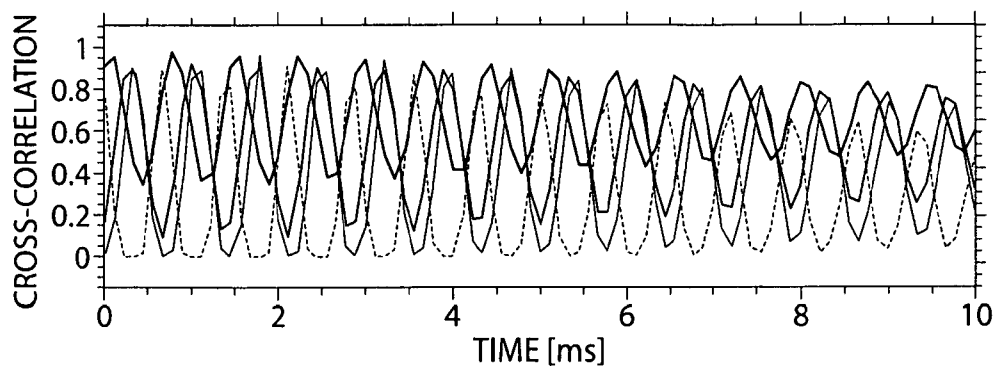


Fig. 3E

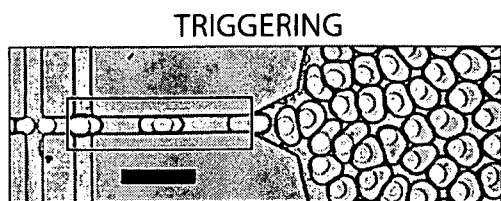


Fig. 4A

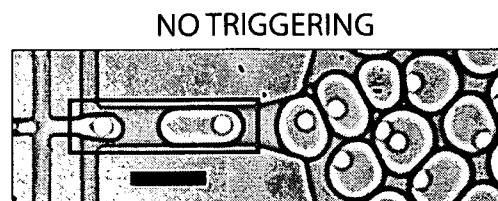


Fig. 4B

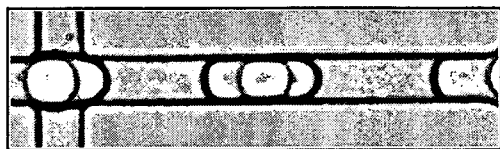


Fig. 4C



Fig. 4D

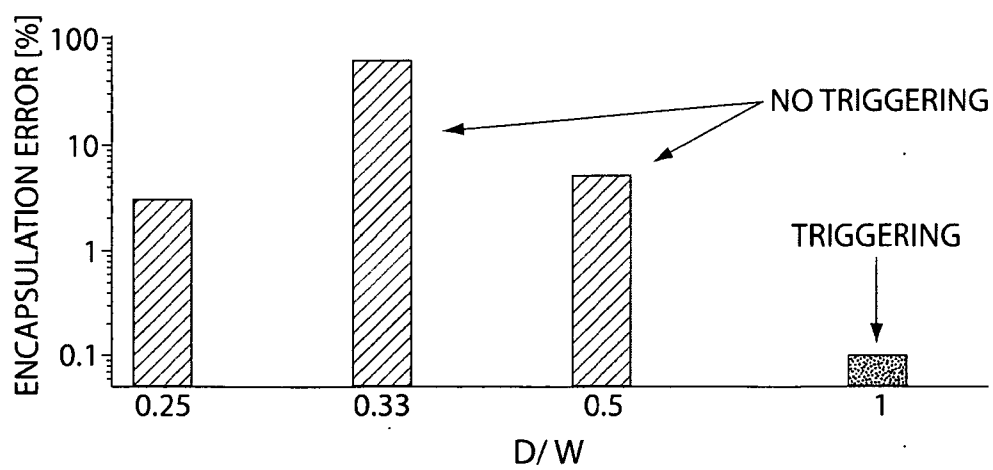


Fig. 4E

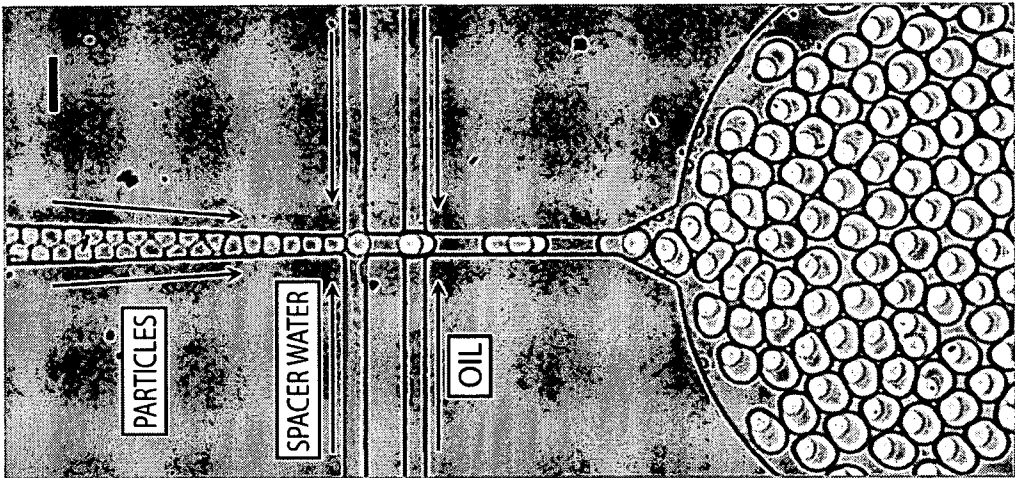


Fig. 5B

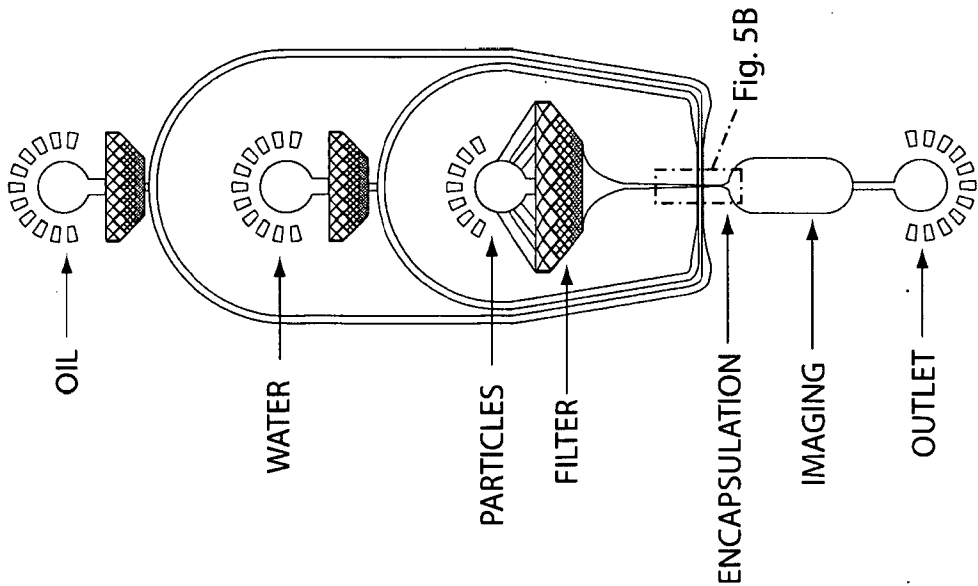


Fig. 5A

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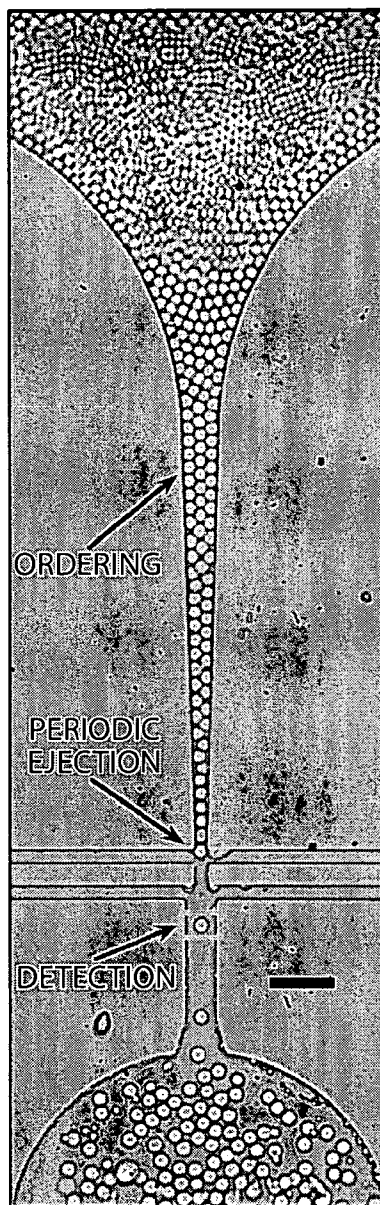


Fig. 6A

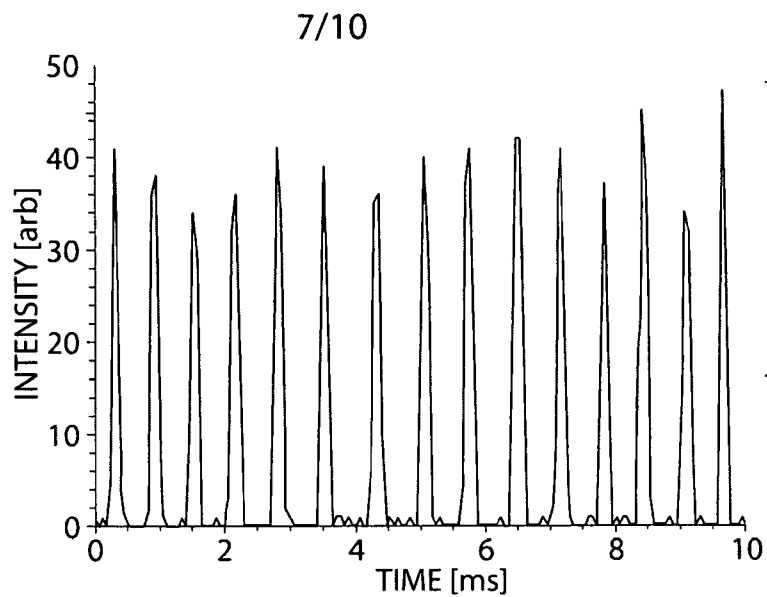


Fig. 6B

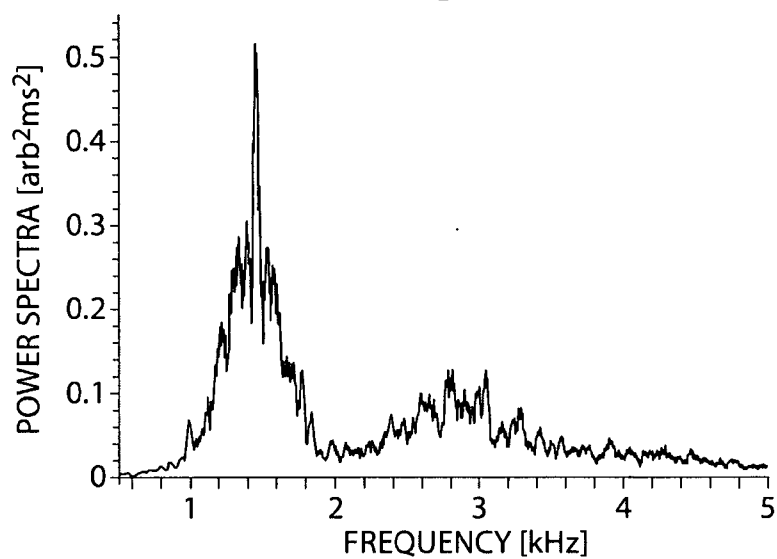


Fig. 6C

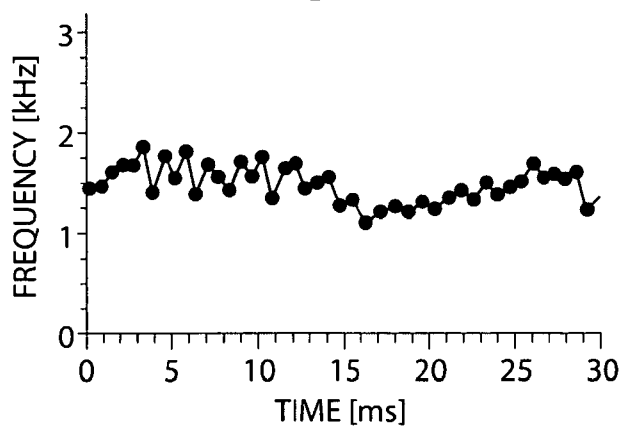


Fig. 6D

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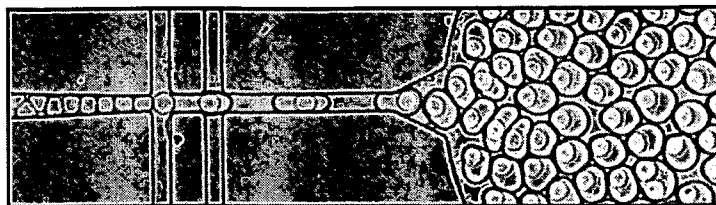


Fig. 7A

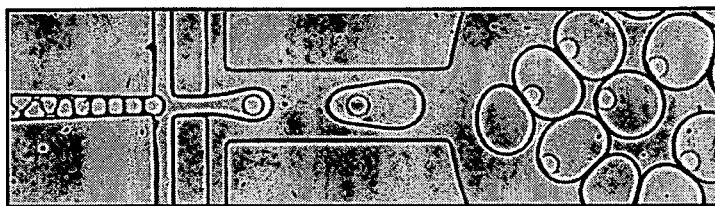


Fig. 7B

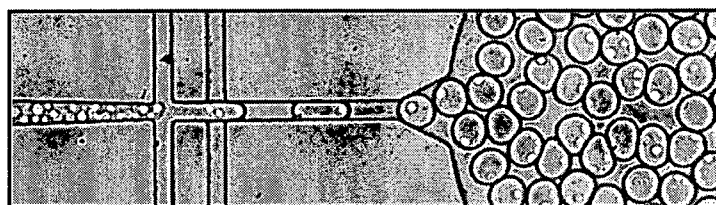


Fig. 7C

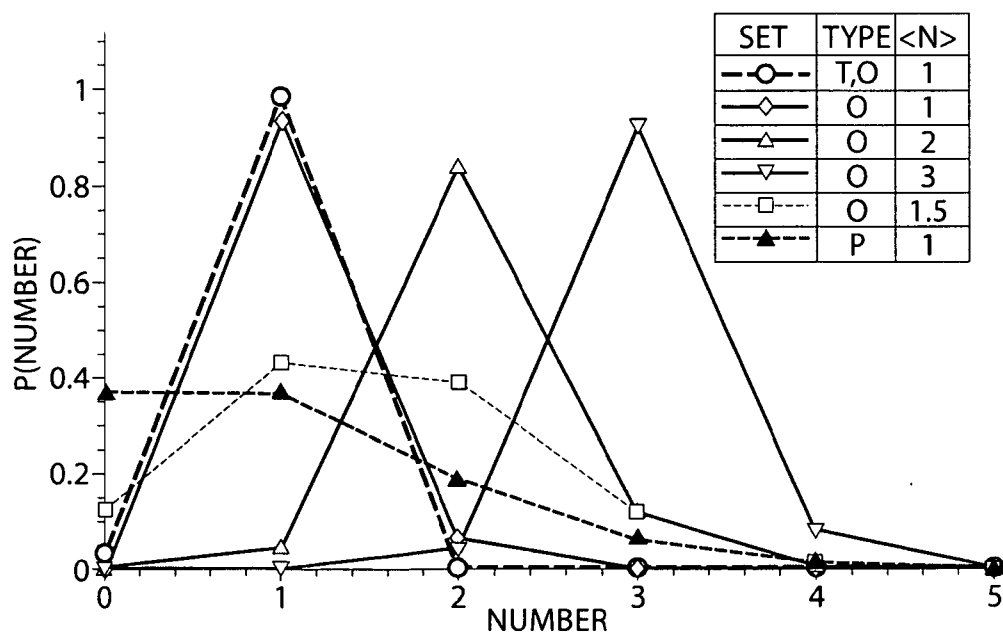


Fig. 7D

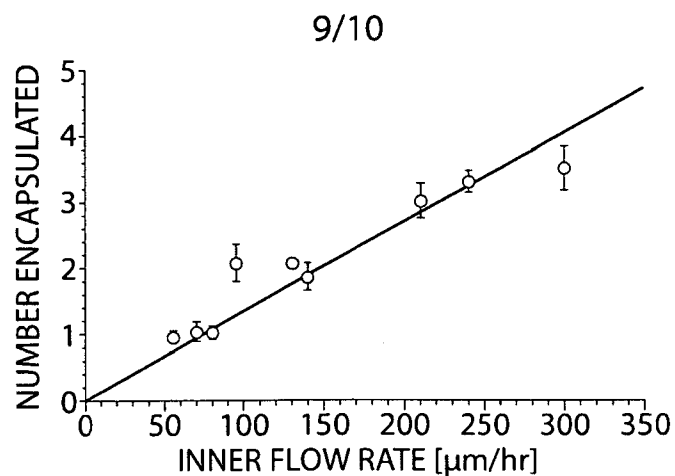


Fig. 8A

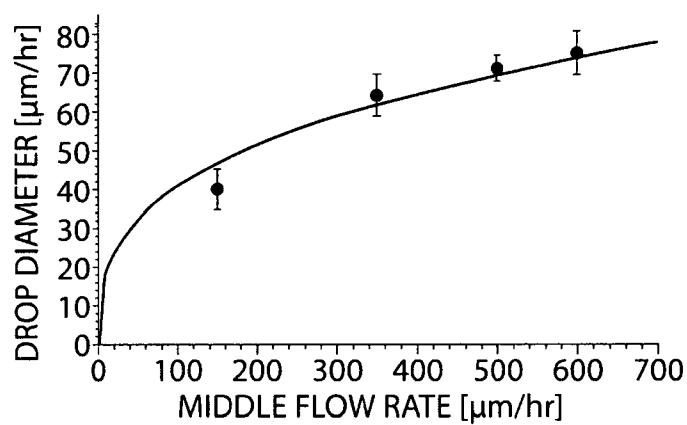


Fig. 8B

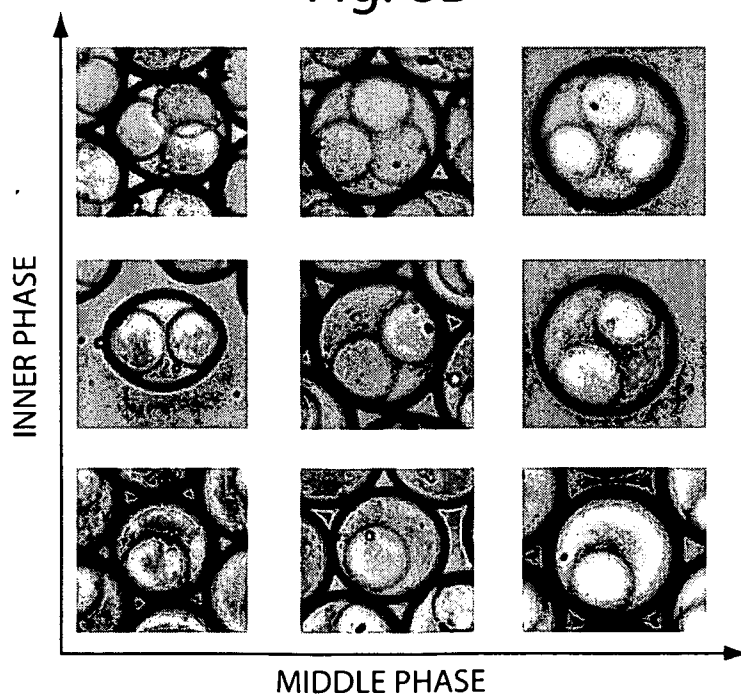
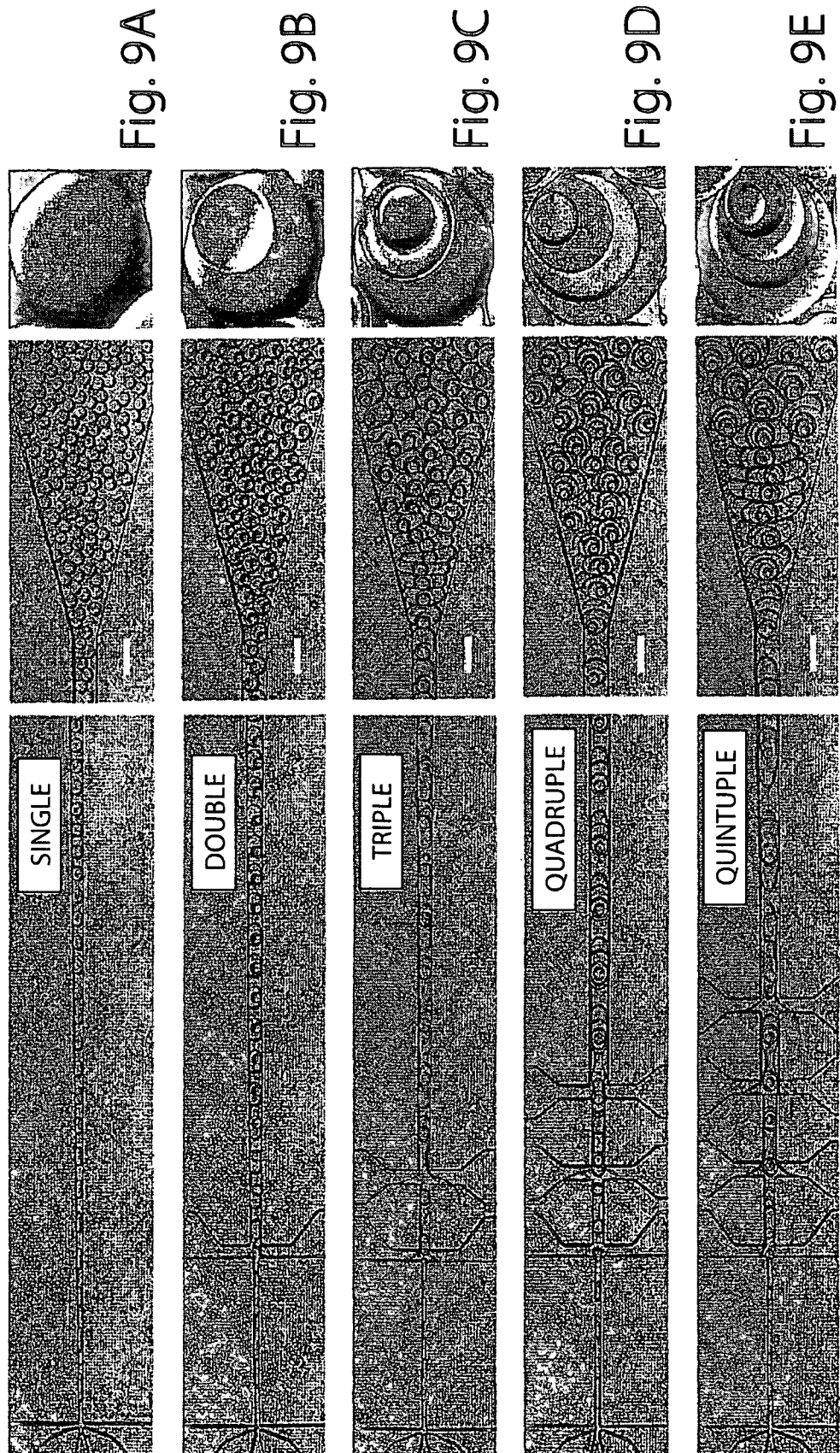


Fig. 8C

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/000763

A. CLASSIFICATION OF SUBJECT MATTER

INV. B01F3/08 B01F13/00 B01J13/04
ADD.

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)

B01F B01J

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FR 2 696 658 A1 (HOSPAL IND [FR]; INST NAT SANTE RECH MED) 15 April 1994 (1994-04-15) page 1, line 6 - line 10 page 5, line 24 - page 8, line 21 page 9, line 1 - page 10, line 19 figures 1-5	1-41
A	DE 10 2005 048259 A1 (LANDESSTIFTUNG BADEN WUERTTEMBERG [DE]) 19 April 2007 (2007-04-19) paragraph [0001] paragraph [0051] - paragraph [0054] figures 7a-7f	1-41
A	EP 1 757 357 A1 (JAPAN SCIENCE & TECH AGENCY [JP]) 28 February 2007 (2007-02-28) paragraph [0001] paragraph [0020] - paragraph [0022] figure 6	1-41

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"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

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Date of the actual completion of the international search

6 July 2010

Date of mailing of the international search report

20/07/2010

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NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
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Authorized officer

Real Cabrera, Rafael

INTERNATIONAL SEARCH REPORT

Information on patent family members

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