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APPLICANTS Rustem F. Ismagilov, Chicago, IL;				
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TITLE Device and method for pressure - driven plug transport				
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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

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INVENTOR(S)/APPLICANT(S)							
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TITLE OF INVENTION (280 characters max)							
DEVICE AND METHOD FOR PRESSURE - DRIVEN PLUG TRANSPORT							
CORRESPONDENCE ADDRESS							
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<input type="checkbox"/> A check or money order is enclosed to cover the Provisional filing fees.				PROVISIONAL			
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The invention was made by an agency of the United States Government or under a contract with an Agency of the United States Government.

- ☒ No.
☐ Yes, the name of the U.S. government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE:

TYPED OR PRINTED NAME: K. Shannon MrksichDate: May 9, 2002Registration No. 36,675
(if appropriate)

- ☐ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
APPLICATION FOR UNITED STATES LETTERS PATENT

INVENTOR:	RUSTEM F. ISMAGILOV
TITLE:	DEVICE AND METHOD FOR PRESSURE-DRIVEN PLUG TRANSORT
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DEVICE AND METHOD FOR PRESSURE-DRIVEN PLUG TRANSPORT

BACKGROUND OF THE INVENTION

Microfluidics deals with transport of fluids through networks of channels of micrometer dimensions. Microfluidic system (also called lab-on-a-chip) find applications for microscale chemical and biological analysis (micro-total-analysis systems). The main advantages of microfluidic systems are high speed and low consumption of reagents, therefore they are very promising for medical diagnostics and high-throughput screening. Highly parallel arrays of microfluidic systems are used for the synthesis of macroscopic quantities of chemical and biological compounds, *e.g.*, the destruction of chemical warfare agents and pharmaceuticals synthesis. Their advantage is improved control over mass and heat transport.

All microfluidic systems require a method of pumping fluids through the microchannels. In the two most common methods, the fluids are either driven by pressure or driven by electroosmotic flow (EOF). Flows driven by EOF are attractive because they can be easily controlled even in complicated networks. Because EOF-driven flows have flat, plug-like velocity profile, that is, the velocity of the fluid is the same near the walls of the channels and in the middle of the channel, and the transport is plug-like. Therefore, if small volumes of multiple analytes are injected sequentially into a channel, these plugs are transported as non-overlapping plugs (low dispersion), in which case the dispersion comes mostly from the diffusion between plugs. A main disadvantage of EOF is that it is generated by the motion of the double layer at the charge surfaces of the walls of the channel, and therefore it is highly sensitive to contamination of surfaces by charged impurities. This is not at issue when using channels with negative surface charge for the analysis and manipulation of DNA, which is uniformly negatively charged and does not adsorb to the walls. But this is a serious limitation for applications that involve proteins that are often charged and tend to adsorb on charged surfaces. In addition, high voltages are often not desirable or sources of high voltages may not be available, as in portable analyzers.

Flows driven by pressure are significantly less sensitive to surface chemistry than EOF. The main disadvantage of these flows is that they have parabolic flow profile instead of the flat profile of EOF. Solutes in the middle of the channel move

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much faster (about twice the average velocity of the flow) than solutes near the walls of the channels. Parabolic velocity profile is responsible for high dispersion in pressure-driven flows; a plug of solute injected into a channel is immediately distorted and stretched along the channel. This distortion is somewhat reduced by transport of the solute by diffusion from the middle of the channel towards the walls and back. The distortion is made worse by diffusion along the channel (the overall dispersion is known as Taylor dispersion).

Taylor dispersion broadens and dilutes sample plugs. Some of the sample is always left behind the plug as a tail. Overlap of these tails leads to cross-contamination of samples in different plugs. Therefore, samples are often introduced into the channels individually, separated by buffer washes. Interleaving samples with long buffer plugs, or washing the system with buffer between samples reduces the throughput of the system.

In EOF, flow transport is essentially linear, that is, if two reactants are introduced into a plug and transported by EOF, their residence time (and reaction time) can be calculated simply by dividing the distance traveled in the channel by the velocity. This linear transport allows precise control of residence times through the control of the lengths of the channels and the control of the flow rates. Dispersion in pressure-driven flow creates a broad range of residence times for a plug traveling in such flows, and this leads to reduced time control.

The issue of time control is important. Many chemical and biochemical processes happen on particular time scales, and measurement of reaction times can be indicative of concentrations of reagents or their reactivity. Stop-flow type instruments are typically used to perform these measurements. These instruments rely on turbulent flow to mix the reagents and transport them in a plug-like fashion. Turbulent flow occurs in tubes with larger diameter and at higher flow rates, and thus stop-flow instruments tend to use large volumes of reagents (on the order of ml/s). A microfluidic analog of stop-flow, which consumes smaller volumes of reagents (typically $\mu\text{l}/\text{min}$) could be immediately useful as a scientific instrument, and could find applications in diagnostics. So far, microfluidic devices have not been able to compete with stop-flow type instruments because EOF is usually too slow (although with less dispersion), and pressure-driven flows suffer from dispersion.

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In addition, mixing in microfluidic systems is often slow regardless of the method used to drive the fluid because flow is laminar in these systems (as opposed to turbulent in larger systems). Mixing in laminar flows relies on diffusion, and is especially slow for larger molecules such as DNA and proteins.

5 Particulates are often difficult to handle in microfluidic systems. Suspensions of cells in aqueous buffers can be handled because cells are isodense with these buffers. Particulates that are not isodense with the fluid are difficult to handle because they sediment to the bottom of the channel and eventually block the channel. Therefore, samples for analysis often require filtration to remove particulates.

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BRIEF SUMMARY OF THE INVENTION

The invention provides as method of conducting a reaction within a microfabricated substrate, comprising the steps of: introducing a carrier fluid into a first channel of the microfabricated substrate, introducing at least two different reagent fluids which together form a reaction and which are immiscible with the carrier fluid into the first channel from at least one or more second channel(s), and applying pressure to the first channel to induce a flow of the fluids in the microfabricated device to form plugs which comprise the reagent fluids, are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid and within which the reaction occurs.

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The invention also provides a method of conducting a reaction within a microfabricated substrate, comprising the steps of: introducing a carrier fluid into a first channel of the microfabricated substrate, introducing at least two different reagent fluids which are immiscible with the carrier fluid into the first channel from at least one or more second channel(s), and applying pressure to the first channel to induce a flow such as a laminar flow of the fluids in the microfabricated device to form plugs which comprise the reagent fluids, are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid and within which a reaction occurs.

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In addition, the invention is directed to a method of conducting a reaction within a microfabricated substrate, comprising the steps of: introducing a carrier fluid into a first and second channel of the microfabricated substrate, introducing a first

[Faint, illegible text from bleed-through]

reagent fluid which is immiscible with the carrier fluid into the first channel, introducing a second reagent fluid which is immiscible with the carrier fluid into the second channel, applying pressure to induce a continuous flow such as a continuous laminar flow of the fluids in the microfabricated device to form a first set of plugs comprising the first reagent fluid and a second set of plugs comprising the second reagent fluid; wherein the plugs in each set are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid, and merging the first and second channels into a single channel downstream of where the first and second set of plugs form, wherein the flow such as laminar flow within the microfabricated device forms merged plugs in which a reaction between the first and second reagent fluids can occur.

Moreover, the invention relates to a method of synthesizing a polymer comprising the steps of: introducing a carrier fluid into a first channel of a microfabricated substrate, introducing a first reagent fluid comprising a monomers together with a second reagent fluid comprising a polymerization catalyst into the first channel; wherein the first and second reagent fluids are immiscible with the carrier fluid, applying pressure to the first channel to induce a continuous flow such as a continuous laminar flow of the fluids in the microfabricated device to form plugs which comprise the reagent fluids, are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid and within which polymerization occurs.

The invention also provides a method of forming an unstable intermediate, comprising the steps of: introducing a carrier fluid into a first channel of a microfabricated substrate, introducing at least two different reagent fluids which together react to form an unstable intermediate and which are immiscible with the carrier fluid into the first channel, and applying pressure to the first channel to induce a continuous flow such as a continuous laminar flow of the fluids in the microfabricated device to form plugs which comprise the reagent fluids, are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid and within which the unstable intermediate is formed.

Further, the invention provides a device comprising one or more microfabricated substrates comprising: a first microchannel, at least one carrier fluid

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reservoir in fluid communication with the first microchannel, at least one second microchannel which is in fluid communication with the first microchannel, at least two reagent fluid reservoirs in fluid communication with the second microchannel, and a means for applying pressure, such as continuous pressure, to a fluid within the microchannel.

The present invention includes various non-limiting embodiments or modifications of the invention several of which are discussed in details below.

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE PHOTOGRAPHS

FIG. 1 shows a schematic diagram (top figure) and photographs (image below top figure) that depicts mixing in water/oil plugs and in laminar streams without oil.

FIG. 2 shows schematic diagrams (upper and lower left figures) and photographs (images to the right of the schematic diagrams) that depicts a stream of water/oil plugs in curved channels at flow rates of 0.5 $\mu\text{l}/\text{min}$ and 1.0 $\mu\text{l}/\text{min}$.

FIG. 3 shows a schematic diagram (figure at the upper left corner) and photographs (images to the left side of the figure) that shows the extent of mixing through light-induced fluorescence of the Ca^{+2} /Fluo-3 complex.

FIG. 4 shows a schematic diagram (top figure) and a photograph (image below the figure) that illustrates plug formation through the injection of oil and multiple reagent streams.

FIG. 5 shows photographs that illustrate the splitting of plugs and a time-controlled merging of plugs.

FIG. 6 shows a photograph that demonstrates the separation of aqueous- and oil-phase plugs into the divergent hydrophilic and hydrophobic channels.

FIG. 7 shows photographs that illustrate the merging of large and small plugs.

FIG. 8 shows a schematic diagram and photograph that illustrate a scheme for merging plugs using an arched or semi-circular channel.

FIG. 9 shows a schematic representation showing various points along a channel that correspond to different reaction or mixing times.

FIG. 10 shows a schematic representation that illustrates a channel designed to control the time intervals along the various points in a given image frame of the channel.

FIG. 11 shows schematic diagrams (upper and lower figures on the left side) and photographs (right side of schematic diagrams) that show mixing of plugs at flow rates of 0.5 $\mu\text{l}/\text{min}$ and 1.0 $\mu\text{l}/\text{min}$ using 90°-step channels.

FIG. 12 shows schematic diagrams (upper and lower figure on the left side) and photographs (right side of schematic diagrams) that show mixing in plugs at flow rates of 1.0 $\mu\text{l}/\text{min}$ and 0.5 $\mu\text{l}/\text{min}$ using 135°-step channels.

FIG. 13 shows photographs of particles clinging to the surface of a microchannel and particles encapsulated in and transported via plugs.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

The term “carrier fluid” refers to a fluid that is immiscible with a reagent fluid. The carrier fluid is introduced into a microfabricated substrate, chip, or device where it comes in contact with one or more reagent fluid at some point, region, area, or channel of the microfabricated substrate, chip, or device. The carrier fluid may also comprise a substance having both polar and non-polar groups or moieties, or it may contain one or more reagents.

The term "reagent fluid" refers to a fluid wherein a reaction can occur. Typically, the reagent fluid contains a reagent, although in some embodiments at least one reagent fluid may not. The fluid can be a solvent (such that the reagent dissolves) or carrier (where the reagent does not dissolve). The reagent fluid may contain a surfactant. At least two different reagent fluids are used in the present invention. When both reagent fluids contain reagent, the fluids are typically miscible, but can also be partially immiscible, so long as the reagents within each reagent fluid can react to form at least one product or intermediate.

The term “reagent” refers to a component of a reagent fluid which undergoes or participates (*e.g.*, by influencing the rate of a reaction) in at least one type of reaction with one or more components of other fluids in the microfabricated substrate, chip, or device to produce one or more reaction products or intermediates which may undergo a further reaction or series of reactions. A reagent contained in a reagent fluid may also undergo a reaction in which a stimulus such as radiation, heat, temperature or pressure change, ultrasonic waves, or a catalyst induces a reaction to

give rise to a transformation of the reagent to another reagent, intermediate, or product.

The term “plugs” refers to the droplets, which may be spherical or non-spherical, that form when at least one reagent fluid combine with a carrier fluid that is substantially immiscible with at least one of the reagent fluids and flow of the fluids is induced by pressure. The plugs may be in the form of droplets comprising an aqueous phase containing one or more reagents and/or one or more products formed from a reaction of the reagents, wherein the aqueous phase is surrounded by a non-polar or hydrophobic phase such as an oil. The plugs may also be in the form of droplets comprising mainly a non-polar or hydrophobic phase which is surrounded by an aqueous phase. The plugs may be encased by one or more layers of molecules that comprise both hydrophobic and hydrophilic groups or moieties. The term “plugs” also includes plugs comprising one or more smaller plugs, that is, plugs-within-plugs. The relative amounts of reagents and reaction products contained in the plugs at any given time depend on factors such as the extent of a reaction occurring within the plugs.

A “plug-forming region” is a junction between an inlet port and the first channel of a substrate, chip, or device of the invention. Preferably, the fluid introduced into the inlet port is “incompatible” (*i.e.*, immiscible) with the fluid in the first channel so that droplets of the fluid formed in the plug-forming region are entrained into the stream of fluid from the first channel.

An “outlet port” is an area of a microfabricated substrate, chip, or device that collects or dispenses the reagent fluid, carrier fluid, plugs or reaction product after detection, measurement, characterization, analysis, or sorting. A microfabricated substrate, chip, or device may contain more than one outlet port if desired.

The term “flow” means any movement of a solid or a fluid such as a liquid. For example, the movement of reagent fluid, carrier fluid, or a plug in a device or component of a device of the invention or in a device or component of a device involving a method of the invention, *e.g.* through channels of a microfluidic substrate, chip, or device of the invention, comprises a flow. The application of any force may be used to provide a flow, including without limitation: pressure, capillary action, electro-osmosis, electrophoresis, dielectrophoresis, optical tweezers, and combinations thereof, without regard for any particular theory or mechanism of action.

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particular cases, the critical length scale for novel properties and phenomena involving nanoparticles may be under 1 nm (*e.g.*, manipulation of atoms at approximately 0.1 nm) or it may be larger than 100 nm (*e.g.*, nanoparticle reinforced polymers have the unique feature at approximately 200-300 nm as a function of the local bridges or bonds between the nanoparticles and the polymer).


B. Methods of forming plugs

The present invention provides a method of conducting a reaction within a microfabricated substrate, comprising the steps of: introducing a carrier fluid into a first channel of the microfabricated substrate, introducing at least two different reagent fluids which together form a reaction and which are immiscible with the carrier fluid into the first channel from at least one or more second channel(s), and applying pressure to the first channel to induce a flow such as a laminar flow of the fluids in the microfabricated device to form plugs which comprise the reagent fluids, are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid and within which the reaction occurs.

The reagent fluids, which may comprise one or more reagents and/or solvents, may be introduced through one or more inlets. The solvent is preferably water. In a preferred embodiment, the reagent fluids do not significantly mix at or before the inlet. The reagents fluids may form distinct laminar streams at or before the inlet, and they may be separated by an additional fluid. Moreover, the reagent fluids may be introduced into the carrier fluid via inlets of differing size. The concentration of reagent fluids in the plugs may be adjusted by adjusting volumetric flow rates of the reagent fluids. Further, the diameters of the first channel and the second channel(s) may differ.

In one embodiment of the invention, at least one of the reagent fluids further comprises at least one surfactant. The carrier fluid may be an oil, preferably a fluorinated oil, and more preferably perfluorodecaline or perfluoroperhydrophenanthrene. Preferably, the refractive index of the carrier fluid and the reagent fluids are substantially similar.

The method may also comprise merging, mixing, separating, splitting, or monitoring the plugs or the reaction within the plugs. The reaction may be monitored at one or more positions along the channel of the microfabricated substrate. A



(Span 40), sorbitan monostearate (Span60) and sorbitan monooleate (Span80). Other non limiting examples of non ionic surfactants which may be used include polyoxyethylenated alkylphenols (for example, nonyl-, p-dodecyl , and dinonylphenols), polyoxyethylenated straight chain alcohols, polyoxyethylenated polyoxypropylene glycols, polyoxyethylenated mercaptans, long chain carboxylic acid esters (for example, glyceryl and polyglycerl esters of natural fatty acids, propylene glycol, sorbitol, polyoxyethylenated sorbitol esters, polyoxyethylene glycol esters, etc.) and alkanolamines (*e.g.*, diethanolamine fatty acid condensates and isopropanolamine fatty acid condensates). In addition, ionic surfactants such as sodium dodecyl sulfate (SDS) may also be used. However, certain surfactants are generally less preferably for many embodiments of the invention. For instance, in those embodiments where aqueous plugs are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials, a water soluble surfactant such as SDS may denature or inactivate the contents of the plug.

In one particularly preferred embodiment, the carrier fluid is an oil, such as a fluorinated oil. The carrier may contain between about 0-10%, preferably 0.2-5% by volume of a surfactant.

Manipulation is also dependent on permittivity (a dielectric property) of the plugs and/or particles with the suspending medium. Therefore, manipulation of individual molecules or particles can be accomplished in a fluid flow using dielectrophoresis. Dielectrophoresis is believed to produce movement of dielectric objects, which have no net charge, but have regions that are positively or negatively charged in relation to each other. Alternating, nonhomogeneous electric fields in the presence of plugs and/or particles, cause the plugs and/or particles to become electrically polarized and thus to experience dielectrophoretic forces. Depending on the dielectric polarizability of the particles and the suspending medium, dielectric particles will move either toward the regions of high field strength or low field strength. Using conventional semiconductor technologies, electrodes can be microfabricated onto a substrate to control the force fields in a micro fabricated sorting device of the invention. Dielectrophoresis is particularly suitable for moving objects that are electrical conductors. The use of AC current is preferred, to prevent permanent alignment of ions. Megahertz frequencies are suitable to provide a net

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alignment, attractive force, and motion over relatively long distances. See *e.g.* Benecke (21).

Radiation pressure can also be used in the invention to deflect and move objects (*e.g.*, plugs and particles contained therein) with focused beams of light such as lasers. Flow can also be obtained and controlled by providing a thermal or pressure differential or gradient between one or more channels of a device or in a method of the invention.

A plug forming liquid maybe an aqueous buffer solution, such as ultrapure water (*e.g.*, 18 mega ohm resistivity, obtained, for example by column chromatography), 10 mM Tris HCl and 1 mM EDTA (TE) buffer, phosphate buffer saline (PBS) or acetate buffer. Any liquid or buffer that is compatible with the reagents can be used. The fluid passing through the first channel is preferably immiscible with the plug forming fluid. Preferably, the fluid passing through the first channel is a non-polar solvent, most preferably an oil.

The fluids used in the invention may contain additives, such as agents which reduce surface tensions (*e.g.*, surfactants). Exemplary surfactants include Tween, Span, fluorinated oils, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing plug size, flow and uniformity, for example by reducing the shear force needed to extrude or inject plugs into an intersecting channel. This may affect plug volume and periodicity, or the rate or frequency at which plugs break off into an intersecting channel.

Microfluidic devices having rounded or non-rounded (*e.g.*, rectangular) channels may be used in accordance with the invention. Devices having rounded channels, are produced by heating the positive-relief mold to a sufficient temperature (*e.g.*, between 80-110 °C) so that the photoresist material flow, thereby giving the positive relief contours, which are normally rectangular, rounded edges.

In the particular devices described here, the inlet channel and the outlet channel had measured channel dimensions of approximately 50 μm wide by 50 μm high. However, the channels may taper to dimensions of approximately 25 μm wide by 50 μm high in the plug-forming region.

Fluids may be introduced into the microfluidic devices through pneumatically driven syringe reservoirs that contain either an aqueous solution (*i.e.*, water) or an oil. Various oils may be used in the devices, such as fluorinated oils. In each instance, the

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oil phase introduced into the device may also contain a surfactant with concentrations (vol/vol) of between about 0.5 - 10%. Water plugs may then be produced in the oil stream by modifying the relative oil and water pressures such that the water enter the plug-forming regions, shearing off into discrete plugs.

FIG. 1 shows a photograph that depicts mixing in water/oil plugs and in laminar streams comprising only aqueous fluids. The oil phase is introduced into a first channel (*i.e.*, the inlet port for the carrier fluid) of a microfabricated substrate while the three aqueous phases are introduced in a separate inlet port. A preferred scheme is one in which the aqueous streams initially coflow preferably along a short or minimal distance before coming in contact with the oil phase. In a preferred embodiment, the distance traversed by the one or more coflowing aqueous streams is approximately or substantially equal to the width of the channel.

The middle or second water phase in the top figure may be plain water, buffer, solvent, or a reagent fluid. The middle water phase would preferably initially separate the two other water streams before the aqueous streams come into contact with the oil phase. Thus, the intervening water phase would prevent, delay, or minimize the reaction or mixing of the two outer water phases before they come in contact with the oil phase. The water/oil plugs that form in the plug-forming region would then exit a channel, area, or region of the microfabricated substrate, chip, or device to enter a different channel, area, or region of the microfabricated substrate, chip, or device, or the plugs may exit the microfabricated substrate, chip, or device through an exit port. It can be seen in FIG. 1 that, in the absence of an oil phase, the water phases flow in laminar streams without significant mixing or with only partial mixing. In contrast, the reagents from the separate water phases mix substantially or completely in the water/oil plugs.

FIG. 2 shows a photograph that depicts a stream of water/oil plugs in curved channels at flow rates of 0.5 $\mu\text{l}/\text{min}$ and 1.0 $\mu\text{l}/\text{min}$. This scheme allows fast mixing of reagents in the plugs shown as elongated droplets flowing along a winding channel with smooth corners or curves. As in FIG. 1, the oil phase is introduced into an inlet port of a microfabricated substrate, chip, or device while the three aqueous phases are introduced in a separate inlet port. As in FIG. 1, a preferred scheme would be one in which the aqueous streams would initially coflow preferably along a short or minimal distance before coming in contact with the oil phase. In a preferred embodiment, the

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distance traversed by the one or more coflowing aqueous streams is approximately or substantially equal to the width of the channel. The middle or second water phase may comprise plain water, buffer, solvent, or a reagent fluid, and the middle water phase preferably initially separates the two other water streams before the aqueous streams come into contact with the carrier which, in this case, is the oil phase. Thus, the intervening water phase would prevent, delay, or minimize the reaction or mixing of the two outer water phases before they come in contact with the oil phase.

FIG. 3 shows a photograph that shows the extent of mixing through light-induced fluorescence of the Ca^{+2} /Fluo-3 complex. Fluo-3 is a long wavelength probe which is practically non-fluorescent by itself. Upon forming a complex with Ca^{+2} , however, the resulting fluorescence can dramatically increase up to more than a hundred-fold relative to that of Fluo-3 alone. The fluorescence depicted in the lower image in FIG. 3 was obtained after exposing the complex solution flowing through a channel with a mercury lamp. Instead of a lamp such as a mercury lamp, one may use an Ar laser to induce fluorescence. One may also use a microscope such as a confocal laser fluorescent microscope which normally comes equipped with Ar laser. The fluorescence image in FIG. 3 demonstrates substantial mixing, which can be quantitatively measured, of Ca^{+2} and Fluo-3 along the entire length of the channel, as evidenced by the fluorescence arising from the complex formed from the binding of Ca^{+2} with Fluo-3.

FIG. 4 shows a photograph that illustrates plug formation through the injection of oil and multiple reagent streams. Although FIG. 4 shows five separate reagent or solvent fluids, one may also separately introduce more than five reagent or solvent fluids into the microfabricated substrate, chip, or device. The reagent or solvent fluids may be different or some of them may be identical or similar. As in FIG. 1, the oil phase is introduced into an inlet port of a microfabricated substrate, chip, or device while the aqueous phases are introduced in a separate inlet port. A preferred scheme is one in which the aqueous streams would initially coflow preferably along a short or minimal distance before coming in contact with the oil phase. In a preferred embodiment, the distance traversed by the one or more coflowing aqueous streams is approximately or substantially equal to the width of the channel. One or more of the aqueous streams may comprise plain water, buffer, solvent, or a reagent fluid, and at least one aqueous phase would preferably initially separate at least two other aqueous

favorably with stop-flow instruments at slower time scales. In particular, the present invention can be advantageous for reactions that occur on a sub-second but slower than about 1 or about 10 ms time scale and where the primary concern is the solute volume required to perform a measurement.

5 Moreover, experiments involving complex chemical systems can be performed in accordance with the invention. For example, several unstable intermediates can be prepared in separate plugs, such as partially folded forms of proteins or RNA. The reactivity of the unstable intermediates can then be investigated when, for example, the plugs merge.

10 To illustrate an advantage offered by the invention over traditional techniques such as stop-flow methods, consider the following example. Two or more reagents, such as reagents A, B, C, are to be screened for the effects of different concentrations of reagents on some process, and the conditions under which an inhibitor can terminate the reaction of the enzyme with a substrate at various enzyme and substrate
15 concentrations is of interest. If A is an enzyme, B a substrate, and C an inhibitor, a device with 5 inlets such as A/water/B/water/C inlets can be used, and the flow rates at which A, B and C are pumped into the device can be varied. Preferably, the size of the plug is kept constant. Because different amounts of A, B, C are introduced, the concentrations of A, B, C in the plugs will vary. The concentrations of the starting
20 solutions need not be changed and one can rapidly screen all combinations of concentrations, as long as an enzymatic reaction or other reactions being screened can be detected or monitored. Because the solutions are flowing and the transport is linear, one can determine not only the presence or absence of an interaction or reaction, but also measure the rate at which a reaction occurs. Thus, not only
25 qualitative but also quantitative data can be obtained. The device need not be cleaned between runs since most, if not all, reagents are contained inside the plugs and leaves no residues.

30 To extend the range over which concentrations can be varied, one may use a combination of, say, reagents A, B, C, D, E and prepare a micromolar solution of A, a mM solution of B, and a M solution of C, and so on. This technique may be easier than controlling the flow rate over a factor of more than 10^6 . Using other known methods would be more difficult in this particular example because changing the ratio

This example may be adapted for studying, for example, RNA folding. Thus, one may monitor RNA folding in a solution in the presence of different concentrations of Mg^{2+} and H^+ . Previously, this was done using a stop-flow technique which is time consuming and requires a relatively large amount of RNA. Using the method of the invention, the entire phase space can be covered in approximately 15 minutes using 1 μ l/minute runs instead of the usual ml/shot runs.

Further, if a plug is generated with two reactive components, it can serve as a microreactor as the plug is transported down a microchannel, as demonstrated in Figs. 9 and 10. A plug's property, such as its optical property, can then be measured or monitored as a function of distance from a given point or region of a channel or the microfabricated substrate, chip, or device. When the plugs are transported at a constant flow rate, a reaction time can be directly determined from a given distance.

An advantage of the invention is that when plugs are formed continuously, intrinsically slow methods of observation can be used. For example, plugs flowing at a flow rate of 10 cm/s through a distance of 1 mm from a point of origin would be 10 ms old. In this case, the invention is particularly advantageous because it allows the use of a relatively slow detection method to repeatedly perform a measurement of, for example, 10 ms-old plugs for a virtually unlimited time. In contrast, to observe a reaction in a stop-flow experiment at a time, say, between 9 and 11 ms, one only has 2 ms to take data. Moreover, the present invention allows one to obtain information involving complex reactions several times, and simultaneously, simply by observing the channels at different distances from the point of origin.

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transported along multi-step channels instead of channels having round curves. An advantage of these multi-step configurations of channels is that they may provide further enhanced mixing of the substances within the plugs.

FIG. 13 shows a photograph that depicts particles clinging to the surface of a microchannel and particles encapsulated in and transported via plugs. Under certain circumstances or conditions, a reaction may occur in one or more channels or regions of the microfabricated substrate, chip, or device that give rise to particulates which then adhere to the walls of the channels. When they collect in sufficient number, the adhering particulates may thus lead or contribute to channel clogging or constriction. Particles encapsulated in the plugs of the invention would be prevented from adhering to the channel walls thereby eliminating or minimizing channel clogging or constriction. A further advantage offered by the invention is that the encapsulated particles may be more effectively prevented from interfering with desired reactions in one or more channels of the microfabricated substrate, chip, or device since the particulates would be prevented from directly coming into contact with substances outside the plugs containing the particulates.

C. Methods of merging plugs

The invention also provides a method of conducting a reaction within a microfabricated substrate, comprising the steps of: introducing a carrier fluid into a first and second channel of the microfabricated substrate, introducing a first reagent fluid which is immiscible with the carrier fluid into the first channel, introducing a second reagent fluid which is immiscible with the carrier fluid into the second channel, applying pressure to induce a continuous flow of the fluids in the microfabricated device to form a first set of plugs comprising the first reagent fluid and a second set of plugs comprising the second reagent fluid; wherein the plugs in each set are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid, and merging the first and second channels into a single channel downstream of where the first and second set of plugs form, wherein the flow within the microfabricated device forms merged plugs in which a reaction between the first and second reagent fluids can occur.

The first and second set of plugs may be substantially similar in size or they may have different relative velocities. In a preferred embodiment, at least one plug is

split such that a first portion of the plug passes into a second channel through an opening, wherein the second channel is downstream of where the plug is formed.

In another preferred embodiment, the method may further comprise splitting at least one merged plug such that a portion of the merged plug passes into a third channel through an opening, wherein the third channel is downstream of where the merged plug is formed.

FIG. 7 shows a photograph that illustrates a scheme for merging large and small plugs. As shown in FIG. 7, the large and small plugs flow along separate channels or channel branches towards a common channel where they flow as merged plugs or eventually form merged plugs. In a case where a large and a small plug do not converge at the same point at the same time, they eventually form a merged plug as the larger plug, which moves faster than the smaller plug, catches up with the small plug and merge with it. In the case where the larger and smaller plugs meet head on at the same point or region, they immediately combine to form a merged plug. The merged plugs may undergo splitting or further merging in other channels or channel regions, or they may be directed to other channels, channel branches, area, or region of the microfabricated device where they may undergo one or more "treatments" such as one or more types of characterizations, measurements, detection, sorting, or analysis.

FIG. 8 shows a photograph that illustrates another scheme for merging plugs. In this scheme, an arched, semi-circular, or circular channel provides a means for increasing the efficiency of plug merging. Thus, for example, a greater frequency of merging would occur within a more compact area or region of the microfabricated substrate, chip, or device. Using this scheme, plugs flowing along separate channels towards a common channel may merge within a shorter distance or a shorter period of time because the arched, semi-circular, or circular channel or channel branch converts or assists in converting initially out-of-phase plug pairs to in-phase plug pairs. Specifically, the arched, semi-circular, or circular channel or channel branch would allow a lagging plug to catch up and merge with a plug ahead of it, thereby increasing the number of merged plugs in a given period or a given area or region of a microfabricated substrate, chip, or device.

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D. Methods of splitting and/or sorting plugs

The present invention also provides a method of conducting a reaction within a microfabricated substrate, comprising the steps of introducing a carrier fluid into a first channel of the microfabricated substrate, introducing at least two different reagent fluids which together form a reaction and which are immiscible with the carrier fluid into the first channel from at least one or more second channel(s), applying pressure to the first channel to induce a flow such as a laminar flow of the fluids in the microfabricated device to form plugs which comprise the reagent fluids, are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid and within which the reaction occurs, and splitting at least one plug such that a first portion of the plug passes into a second channel through an opening, wherein the second channel is downstream of where the plug is formed.

The present invention also provides a method of conducting a reaction within a microfabricated substrate, comprising the steps of introducing a carrier fluid into a first and second channel of the microfabricated substrate, introducing a first reagent fluid which is immiscible with the carrier fluid into the first channel, introducing a second reagent fluid which is immiscible with the carrier fluid into the second channel, applying pressure to induce a continuous flow such as a continuous laminar flow of the fluids in the microfabricated device to form a first set of plugs comprising the first reagent fluid and a second set of plugs comprising the second reagent fluid; wherein the plugs in each set are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid, merging the first and second channels into a single channel downstream of where the first and second set of plugs form, wherein the flow such as a laminar flow within the microfabricated device forms merged plugs in which a reaction between the first and second reagent fluids can occur, and splitting at least one merged plug such that a portion of the merged plug passes into a third channel through an opening, wherein the third channel is downstream of where the merged plug is formed.

FIG. 5 shows a photograph that illustrates schemes for splitting plugs and time-controlled merging of plugs. As depicted in FIG. 5, the merging of the plugs can be achieved by controlling the arrival time of the plugs flowing in opposite directions

towards a common point, area, or region of the channel so that each pair of plugs arrive at the common point, area, or region of the channel at around the same time to form a single plug. FIG. 5 also shows a scheme for splitting plugs. As shown in FIG. 5, the plugs may be split by directing or allowing the plugs to pass through a T-shaped channel or a T-shaped region of a channel. In a preferred embodiment, the area or junction at which the plugs undergo splitting may be narrower or somewhat constricted relative to the diameter of the plugs a certain distance away from the junction. The resulting split plugs, as shown in FIG. 5, flow in separate channels or channel branches which may be perpendicular, as shown in FIG. 5, or nonperpendicular. The merged or split plugs may undergo further merging or splitting, or they may be directed to other channels, channel branches, area, or region of the microfabricated device where they may undergo one or more reactions or "treatments" such as one or more types of characterizations, measurements, detection, sorting, or analysis.

FIG. 6 shows a photograph that demonstrates the separation of aqueous- and oil-phase plugs into the divergent hydrophilic and hydrophobic channels. The channels are rendered hydrophilic or hydrophobic by pretreating a channel or region of a channel such that a channel or channel surface becomes predominantly hydrophilic or hydrophobic. As discussed in more detail below, microfabricated devices with hydrophilic channel surfaces may be fabricated using methods such as rapid prototyping in polydimethylsiloxane. The channel surface can be rendered hydrophobic either by silanization or heat treatment. For example, (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (United Chemical Technologies, Inc.) vapor may be applied to the inlets of the microfabricated substrate, chip, or device with dry nitrogen as a carrier gas to silanize the channel surface.

According to the invention, molecules, particles, or substances are sorted dynamically in a flow stream of microscopic dimensions based on the detection or measurement of a characteristic, marker, property, or reporter that is associated with the molecules, particles, or substances. More specifically, plugs of a solution (preferably an aqueous solution or buffer) containing a sample of molecules, particles, or substances are introduced through a plug-forming region into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the first channel. The

individual plugs are then analyzed and/or sorted in the flow stream, thereby sorting the molecules, particles, or substances contained within the plugs.

The flow stream in the first channel is typically, but not necessarily continuous and may be stopped and started, reversed or changed in speed. Prior to sorting, a liquid that does not contain sample molecules, particles, or substances can be introduced into a sample inlet port (such as an inlet well or channel) and directed through the plug-forming region, *e.g.*, by capillary action, to hydrate and prepare the device for use. Likewise, buffer or oil can also be introduced into a main inlet port that communicates directly with the first channel to purge the device (*e.g.*, of "dead" air) and prepare it for use. If desired, the pressure can be adjusted or equalized, for example, by adding buffer or oil to an outlet port.

The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug-forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs. Periodicity and plug volume may also depend on channel diameter the viscosity of the fluids, and shear pressure.

The concentration of molecules, particles, or substances in a plug can influence sorting efficiently and therefore is preferably optimized. Thus, in one embodiment of the invention, the sample concentration may be adjusted to be dilute enough that most of the plugs contain no more than a single molecule or particle, with only a small statistical chance that a plug will contain two or more molecules or particles. This is to ensure that for the large majority of measurements, the level of reporter measured in each plug as it passes through the detection region corresponds to a single molecule or particle and not to two or more molecules or particles.

A sample solution containing a mixture or population of molecule, particles, or substance in a suitable carrier fluid (such as a liquid or buffer described above) is supplied to the sample inlet port, and plugs of the sample solution are introduced, at the plug-forming region, into the flow passing through the first channel. The force and direction of flow can be controlled by any desired method for controlling flow,

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for example, by a pressure differential, by valve action or by electro osmotic flow (e.g., produced by electrodes at inlet and outlet channels). This permits the movement of the molecules, particles, or substances into one or more desired branch channels or outlet ports.

5 Preferably, both the fluid comprising the plugs and the fluid carrying the plugs (i.e., the aqueous and non polar fluids) have a relatively low Reynolds Number, for example 10^{-2} . The Reynolds Number represents an inverse relationship between the density and velocity of a fluid and its viscosity in a channel of given length. More
10 viscous, less dense, slower moving fluids over a shorter distance will have a lower Reynolds Number, and are easier to divert, stop, start, or reverse without turbulence. Because of the small sizes and slow velocities, microfabricated fluid systems are often in a low Reynolds number regime ($Re \ll 1$). In this regime, inertial effects, which cause turbulence and secondary flows, are negligible viscous effects dominate the dynamics. These conditions are advantageous for sorting, and are provided by
15 microfabricated devices of the invention. Accordingly the microfabricated devices of the invention are preferably operated at a low or very low Reynold's number.

E. Methods of detection

20 The detector can be any device or method for evaluating a physical characteristic of a fluid as it passes through the detection region. A preferred detector is an optical detector, such as a microscope, which may be coupled with a computer and/or other image processing or enhancement devices to process images or information produced by the microscope using known techniques. For example, molecules can be analyzed and/or sorted by size or molecular weight. Reactions can
25 be monitored by measuring the concentration of a product produced or the concentration of a reactant remaining at a given time. Enzymes can be analyzed and/or sorted by the extent to which they catalyze chemical reaction of a substrate (conversely, substrate can be analyzed and/or sorted by the level of chemical reactivity catalyzed by an enzyme). Cells and virions can be sorted according to
30 whether they contain or produce a particular protein, by using an optical detector to examine each cell or virion for an optical indication of the presence or amount of that protein. The molecule, particle, or substance may itself be detectable, for example by a characteristic fluorescence, or it may be labeled or associated with a reporter that

produces a detectable signal when, for example, the desired protein is present, or is present in at least a threshold amount. There is no limit to the kind or number of characteristics that can be identified or measured using the techniques of the invention, provided that the characteristic or characteristics of interest for sorting can be sufficiently identified and detected or measured to distinguish molecule, particles, or substances having the desired characteristic(s) from those which do not. For example, particulate size, hydrophobicity of the reagent versus carrier fluids, etc. can be used as a basis for sorting reagent fluids, reaction products or plugs.

In a preferred embodiment, the reagent fluids, reaction products or plugs are analyzed, detected, characterized and/or separated based on the intensity of a signal from an optically detectable compound associated with them as they pass through a detection window or "detection region" in the device. Reagent fluids, reaction products or plugs having an amount or level of the reporter at a selected threshold or within a selected range are diverted into a predetermined outlet or branch channel of the device. The reporter signal may be collected by a microscope and measured by a detector such as a photo multiplier tube (PMT). A computer digitizes the PMT signal and controls the flow via methods such as valve action or electro osmotic potentials. Alternatively, the signal can be recorded or quantified as a measure of the reporter and/or its corresponding characteristic or marker, *e.g.*, for the purpose of evaluation and without necessarily proceeding to sort the reagent fluids, reaction products or plugs.

To detect a molecule, particle, substance, or reporter or determine whether a molecule, particle, substance, or reporter has a desired characteristic, the detection region may include an apparatus for stimulating a molecule, particle, substance, or reporter for that characteristic to emit measurable light energy, *e.g.*, a light source such as a laser, laser diode, high intensity lamp, (*e.g.*, mercury lamp), and the like. In embodiments where a lamp is used, the channels are preferably shielded from light in all regions except the detection region. In embodiments where a laser is used, the laser can be set to scan across a set of detection regions from different analysis units. In addition, laser diodes maybe microfabricated into the same substrate, chip, or device that contains the analysis units. Alternatively, laser diodes may be incorporated into a second substrate, chip, or device (*i.e.*, a laser diode chip) that is

placed adjacent to the microfabricated analysis or sorter substrate, chip, or device such that the laser light from the diodes shines on the detection region(s).

In preferred embodiments, an integrated semiconductor laser and/or an integrated photodiode detector are included on the silicon wafer in the vicinity of the detection region. This design provides the advantages of compactness and a shorter optical path for exciting and/or emitted radiation, thus minimizing distortion.

As each plug passes into the detection region, it is examined for a predetermined characteristic or property (*i.e.*, using the detector) and a corresponding signal is produced, for example indicating that “yes” the characteristic is present, or “no” it is not. The signal may correspond to a characteristic qualitatively or quantitatively. That is, the amount of the signal can be measured and can correspond to the degree to which a characteristic is present. For example, the strength of the signal may indicate the size of a molecule, the amount of products(s) formed in a reaction, the amount of reactant(s) remaining, the potency or amount of an enzyme expressed by a cell, a positive or negative reaction such as binding or hybridization of one molecule to another, or a chemical reaction of a substrate, chip, or device catalyzed by an enzyme. In response to the signal, data can be collected and/or a flow control can be activated to divert a plug into one branch channel or another. Thus reactants, reaction products, or other substances within a plug at a discrimination region can be sorted into an appropriate branch channel according to a signal produced by the corresponding examination at a detection region. Optical detection of molecular, cellular or viral characteristics may be used, for example directly or by use of a reporter associated with a characteristic chosen for sorting. However, other detection techniques may also be employed.

In one embodiment of the invention, each detection region is formed from a portion of a channel of an analysis unit and includes a photodiode preferably located in the floor of the first channel. The detection region encompasses a receive field of the photodiode in the channel, which receive field has a circular shape. The volume of the detection region is the volume of a cylinder with a diameter equal to the receive field of the photodiode and a height equal to the depth of the channel above the photodiode.

The signals from the photodiodes can be carried to a processor via one or more lines, representing any form of electrical communication (including *e.g.* wires,

conductive lines etched in the substrate, etc.). The processor acts on the signals, for example by processing them into values for comparison with a predetermined set of values for sorting the molecules, particles, or substances. In one embodiment, the values correspond to the amount of optically detectable signal emitted from a cell, which is indicative of a particular type or characteristic of a molecule, particle, or substance giving rise to the signal. The processor uses this information (*i.e.*, the values) to control active elements in the discrimination region to determine how to sort the molecules, particles, or substances (*e.g.*, electro osmotic switching or valve action).

When more than one detection region is used, the photodiodes in the laser diode substrate, chip, or device are preferably spaced apart relative to the spacing of the detection regions in the analysis unit. That is, for more accurate detection, the photodiodes are placed apart at the same spacing as the spacing of the detection region.

The processor can be integrated into the same substrate, chip, or device that contains the analysis unit(s), or it can be separate, *e.g.*, an independent microchip connected to the analysis unit containing substrate, chip, or device via electronic leads that connect to the detection region(s) and/or to the discrimination region(s), such as by a photodiode. The processor can be a computer or microprocessor, and is typically connected to a data storage unit, such as computer memory, hard disk, or the like, and/or a data output unit, such as a display monitor, printer and/or plotter.

The types and numbers of molecules, particles, or substances based on detection of, for example, a reporter associated with or bound to the molecule, particle, or substance passing through the detection region, can be calculated or determined, and the data obtained can be stored in the data storage unit. This information can then be further processed or routed to the data outlet unit for presentation, *e.g.* histograms, of the types of molecules, particles, or substances or more specifically, levels of a protein, saccharide, or some other characteristic on the cell surface in the sample. The data can also be presented in real time as the sample is flowing through the device.

In an embodiment of the invention, the photodiode is larger in diameter than the width of the channel, forming a detection region that is longer (along the length of channel) than it is wide. The volume of such a detection region is approximately

equal to the cross sectional area of the channel above the diode multiplied by the diameter of the diode.

If desired, the device may contain a plurality of analysis units, *i.e.*, more than one detection and discrimination region, and a plurality of branch channels which are in fluid communication with and branch out from the discrimination regions. It will be appreciated that the position and fate of the molecules, particles, or substances in the discrimination region can be monitored by additional detection regions installed, for example, immediately upstream of the discrimination region and/or within the branch channels immediately downstream of the branch point. The information obtained by the additional detection regions can be used by a processor to continuously revise estimates of the velocity of the molecules, particles, or substances in the channels and to confirm that molecules, particles, and substances having a selected characteristic enter the desired branch channel.

A group of manifolds (a region consisting of several channels which lead to or from a common channel) can be included to facilitate movement of the sample containing molecules, particles, or substances from the different analysis units, through the plurality of branch channels and to the appropriate solution outlet. Manifolds are preferably microfabricated into the substrate, chip, or device at different levels of depth. Thus, devices of the invention having a plurality of analysis units can collect the solution from associated branch channels of each unit into a manifold, which routes the flow of solution to an outlet. The outlet can be adapted for receiving, for example, a segment of tubing or a sample tube, such as a standard 1.5 ml centrifuge tube. Collection can also be done using micropipettes.

F. Methods of synthesis

A method of conducting a reaction within a microfabricated substrate, comprising the steps of introducing a carrier fluid into a first channel of the microfabricated substrate, introducing at least two different reagent fluids which are immiscible with the carrier fluid into the first channel from at least one or more second channel(s), and applying pressure to the first channel to induce a flow such as a laminar flow of the fluids in the microfabricated device to form plugs which comprise the reagent fluids, are substantially similar in size, are surrounded by carrier

fluid, and are separated by substantially identical amounts of carrier fluid and within which a reaction occurs.

In the inventive method, when the reagent fluids may both comprise a reagent and solvent such that mixing of the reagent fluids results in formation of a reaction product. In another embodiment, one of the reagent fluids may be reagent free and simply contain fluid. In this embodiment, mixing of the reagent fluids will allow the concentration of the reagent in the plug to be manipulated.

In another embodiment, the method of the present invention comprising the steps of introducing a carrier fluid into a first and second channel of the microfabricated substrate, introducing a first reagent fluid which is immiscible with the carrier fluid into the first channel, introducing a second reagent fluid which is immiscible with the carrier fluid into the second channel, applying pressure to induce a continuous flow such as a laminar flow of the fluids in the microfabricated device to form a first set of plugs comprising the first reagent fluid and a second set of plugs comprising the second reagent fluid; wherein the plugs in each set are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid, and merging the first and second channels into a single channel downstream of where the first and second set of plugs form, wherein the flow such as the laminar flow within the microfabricated device forms merged plugs in which a reaction between the first and second reagent fluids can occur.

When plugs are merged to form merged plugs, the first and second set of plugs may be substantially similar in size or differing in size. Further, the first and second set of plugs may have different relative velocities.

The above method can be used for synthesizing a polymer. In this embodiment a first reagent fluid contains monomer and a second reagent fluid contains a polymerization catalyst. When the reagent fluids are combined, polymerization occurs. The method can be adapted to form block copolymers by merging plugs with different monomers downstream of where the first monomer is introduced.

The methods of the present invention can be used for synthesizing unstable intermediates. In this embodiment, at least two different reagent fluids which together react to form an unstable intermediate are used. As the unstable intermediates form along the flow path of the device, kinetic information of the reaction can be obtained.

Further, such unstable intermediates can be further reacted with another reagent by merging plugs containing the unstable intermediate with the another reagent fluid.

G. Devices

The device of the invention may comprise at least one microfabricated substrate, and at least one of the microfabricated substrates may comprise an array of connected microchannels. At least one of the microchannels may have at least 1 bend. In another embodiment, at least one of the microchannels has a plurality of bends. Further, at least one of the microchannels may comprise at least one bend greater than about 10°, 135°, 180°, 270°, or 360°.

In an embodiment of the invention, the device also has a detection region along a channel within the device. There may be a plurality of detection regions and detectors, working independently or together, *e.g.*, to analyze one or more properties of a sample.

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The device may have one or more outlet ports or inlet ports. Each of the outlet and inlet ports may also communicate with a well or reservoir. The inlet and outlet ports may be in fluid communication with the channels or reservoirs that they are connecting or may be contain one or more valves.

5 A variety of channels for sample flow and mixing can be microfabricated on the substrate and can be positioned at any location on the substrate, chip, or device as the detection and discrimination or sorting points, *e.g.*, for kinetic studies. A plurality of analysis units of the invention may be combined in one device.

10 A device of the invention can be microfabricated with a sample solution reservoir or well at the inlet port, which is typically in fluid communication with an inlet channel. A reservoir may facilitate introduction of molecules, particles, or substances into the device and into the sample inlet channel of each analysis unit. An inlet port may have an opening such as in the floor of the microfabricated substrate, chip, or device, to permit entry of the sample into the device. The inlet port may also
15 contain a connector adapted to receive a suitable piece of tubing, such as liquid chromatography or HPLC tubing, through which a sample may be supplied. Such an arrangement facilitates introducing the sample solution under positive pressure in order to achieve a desired pressure at the plug-forming region.

20 A device of the invention may have an additional inlet port, in direct communication with the first channel at a location upstream of the plug-forming region, through which a pressurized stream or fluid flow is introduced into the first channel. Preferably, this fluid is one which is not miscible with the solvent or sample fluid. For example, most preferably the fluid is a non polar solvent, such as decane (*e.g.*, tetradecane or hexadecane), and the sample (*e.g.*, of molecules, particles, or
25 substances) is dissolved or suspended in an aqueous solution so that aqueous plugs of the sample are formed upon coming in contact with a stream of non-polar solvent at the plug-forming region.

30 A typical analysis unit of the invention comprises a main inlet that is part of and feeds or communicates directly with a first channel, along with one or more sample inlets in communication with the first channel at a plug-forming region situated downstream from the main inlet (each different sample inlet preferably communicates with the first channel at a different plug-forming region). The plug-forming region generally comprises a junction between the sample inlet and the first

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channel such that a prepared solution of a sample is introduced to the first channel in plugs. Preferably, the sample inlet intersects the first channel such that the pressurized sample solution is introduced into the first channel at an angle perpendicular to a stream of fluid passing through the first channel. For example, in preferred embodiments, the sample inlet and first channel intercept at a T-shaped junction; *i.e.*, such that the sample inlet is perpendicular (90 degrees) to the first channel. However, the sample inlet may intercept the first channel at any angle, and need not introduce the sample fluid to the first channel at an angle that is perpendicular to that flow. In exemplary embodiments the angle between intersecting channels is in the range of from about 60 to about 120 degrees. Particular exemplary angles are 45, 60, 90, and 120 degrees.

The first channel in turn communicates with two or more branch channels at another junction or "branch point", forming, for example, a T-shape or a Y-shape. Other shapes and channel geometries may be used as desired. In sorting embodiments, the region at or surrounding the junction can also be referred to as a discrimination region. Precise boundaries for the discrimination region are not required, but are preferred.

A detection region is within, communicating or coincident with a portion of the first channel at or downstream of the plug-forming region and, in sorting embodiments, at or upstream of the discrimination region or branch point. Precise boundaries for the detection region are not required, but are preferred. The discrimination region may be located immediately downstream of the detection region or it may be separated by a suitable distance consistent with the size of the molecules, the channel dimensions and the detection system. It will be appreciated that the channels may have any suitable shape or cross section (for example, tubular or grooved), and can be arranged in any suitable manner so long as flow can be directed from inlet to outlet and from one channel into another.

The channels of the invention are microfabricated, for example by etching a silicon substrate, chip, or device using conventional photolithography techniques, or using a micromachining technology called "soft lithography", developed in the late 1990's. These and other microfabrication methods may be used to provide inexpensive miniaturized devices, and in the case of soft lithography, can provide robust devices having beneficial properties such as improved flexibility, stability, and

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mechanical strength. When optical detection is employed, the invention also provides minimal light scatter from molecule or cell (including virion) suspension and chamber material. Devices according to the invention are relatively inexpensive and easy to set up.

5 A microfabricated device of the invention is preferably fabricated from a silicon microchip or silicon elastomer. The dimensions of the substrate, chip, or device are those of typical microchips, ranging between about 0.5 cm to about 5 cm per side and about 1 micron to about 1 cm in thickness.

10 The device may also contain one or more inlet or outlet ports which are fluid connect to the microchannels. The inlet and outlet ports may be in fluid communication with the microchannels or may be connected via a valve.

 In another embodiment the inventive device may contain a plurality of plug-forming regions. The different plug-forming regions may each be connected to the same or different channels of the device.

15 The device may comprise a first channel through which a pressurized stream or flow of a first fluid (*e.g.*, oil) is passed, and two or more inlet channels which intersect the first channel at plug-forming regions, respectively. Preferably, these inlet channels are parallel to each other and each intercept the first channel at a right angle. In specific embodiments wherein the plugs introduced through the different
20 plug forming regions are mixed, the inlet channels are preferably close together along the first channel. For example, the first channel will typically have a diameter of 60 μm , that tapers to 30 μm at or near the plug-forming regions. The inlet channels also preferably have a diameter of about 30 μm and, in embodiments where plug mixing is preferred, are separated by a distance along the first channel equal to approximately
25 the diameter of the inlet channel (*i.e.*, about 30 μm).

 Although the exemplary embodiment described here releases plugs of the first reactant upstream from the plugs of the second reactant, plugs of the different fluid or solutions may be released in any order. Thus, for example, an aqueous solution containing a first reactant may be released through the first inlet channel at the first
30 plug-forming region, and plugs of an aqueous solution containing a second reactant may be released through the second inlet channel at the second plug-forming region.

 A silicon substrate containing the microfabricated flow channels and other components is preferably covered and sealed, most preferably with a transparent

cover, *e.g.*, thin glass or quartz, although other clear or opaque cover materials may be used. When external radiation sources or detectors are employed, the detection region is covered with a clear cover material to allow optical access to the molecules, particles, or substances. For example, anodic bonding to a PYREX[®] cover slip can be accomplished by washing both components in an aqueous H₂SO₄/H₂O₂ bath, rinsing in water, and then, for example, heating to about 350°C while applying a voltage of 450V.

Analytical devices having microscale flow channels, valves and other elements can be designed and fabricated from a solid substrate material. Silicon is a preferred substrate material due to well developed technology permitting its precise and efficient fabrication, but other materials may be used, including polymers such as polytetrafluoroethylenes. Micromachining methods well known in the art include film deposition processes, such as spin coating and chemical vapor deposition, laser fabrication or photolithographic techniques, or etching methods, which maybe performed by either wet chemical or plasma processes.

The invention is further described below, by way of the following example. It will be appreciated by persons of ordinary skill in the art that this example is one of many embodiments and is merely illustrative. In particular, the device and method described in this example (including the channel architectures, valves, switching and flow control devices and methods) may be readily adapted to a multi phased device so that plugs may be analyzed, characterized, monitored, and/or sorted as desired by a user.

EXAMPLE

Microfluidic devices with hydrophilic channel surfaces were fabricated using rapid prototyping in polydimethylsiloxane. The channel surface were rendered hydrophobic either by silanization or heat treatment. To silanize the surfaces of channels, (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (United Chemical Technologies, Inc.) vapor was applied to the inlets of a device with dry nitrogen as a carrier gas at 40-60 mm Hg above 1 atm pressure. Vacuum was simultaneously applied to the outlet of the device at 650 mm Hg below atmospheric pressure. The silane vapor was applied for 1-3 hours. To treat the channels using heat, a device was placed in an oven at 120°C for approximately three hours. Alternatively, a device can

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be heated in a Panasonic "The Genius" 1300 Watt microwave oven at power level 10 for ten minutes.

Oils and aqueous solutions were pumped through devices using a kdScientific syringe pump (Model 200). Hamilton Co. GASTIGHT syringes were used (10-250 μ l) and Hamilton Co. 30 gauge Teflon[®] needles were used to attach the syringes to the devices. Oils and aqueous solutions were pumped through devices at volumetric flow rates ranging from 0.10 μ l/min to 10.0 μ l/min.

Aqueous solutions were colored using Crayola Original Formula Markers or Ferroin Indicator (0.025 M, Fisher Scientific). Oils that were used comprised perfluorodecalin (mixture of cis and trans, 95%, Acros Organics), perfluoroperhydrophenanthrene (tech., Alfa-Aesar), or 1H,1H,2H,2H-perfluorooctanol (98%, Alfa-Aesar). The experiments were typically performed using 9:1 mixtures of perfluorodecalin: 1H,1H,2H,2H-perfluorooctanol.

The experiments were monitored using a Lica MZFLIII stereoscope with Eostec (Schott-Fostec, LLC) Modulamps. Photographs of the experiments were taken with a Spot Insight Color Camera, Model # 3.2.0 (Diagnostic Instruments, Inc.). Spot Application version 3.4.0.0 was used to take the photographs with the camera.

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We claim:

1. A method of conducting a reaction within a microfabricated substrate, comprising the steps of:
 - introducing a carrier fluid into a first channel of the microfabricated substrate,
 - 5 introducing at least two different reagent fluids which together form a reaction and which are immiscible with the carrier fluid into the first channel from at least one or more second channel(s), and
 - applying pressure to the first channel to induce a flow of the fluids in the microfabricated device to form plugs which comprise the reagent fluids, are
 - 10 substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid and within which the reaction occurs.
2. The method of claim 1, wherein the reagent fluids are introduced through one or more inlets.
3. The method of claim 1, further comprising adjusting the concentration of
- 15 reagent fluids in the plugs by adjusting volumetric flow rates of the reagent fluids.
4. The method of claim 2, wherein the reagent fluids are introduced through a single inlet.
5. The method of claim 4, wherein the reagent fluids do not significantly mix at or before the inlet.
- 20 6. The method of claim 4, wherein the reagents fluids are distinct laminar streams at or before the inlet.
7. The method of claim 6, where the reagents fluids are separated by a an additional, fluid.
8. The method of claim 2, wherein the reagent fluids are introduced into the
- 25 carrier fluid via inlets of differing size.
9. The method of claim 1, wherein the diameters of the first channel and the second channel(s) differ.
10. The method of claim 1, wherein the reagent fluids comprise reagent and solvent.
- 30 11. The method of claim 10, wherein at least one of the reagent fluids further comprises surfactant.
12. The method of claim 10, wherein the solvent is water.
13. The method of claim 1, wherein the carrier fluid is an oil

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substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid and within which a reaction occurs.

29. The method of claim 27, wherein another reagent fluid consists essentially of solvent.

31. A method of conducting a reaction within a microfabricated substrate, comprising the steps of:

introducing a first reagent fluid which is immiscible with the carrier fluid into the first channel,

applying pressure to induce a continuous flow of the fluids in the microfabricated device to form a first set of plugs comprising the first reagent fluid and a second set of plugs comprising the second reagent fluid; wherein the plugs in each set are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid, and

32. The method of claim 31, wherein the first and second set of plugs are substantially similar in size.

34. The method of claim 31, further comprising splitting at least one plug such that a first portion of the plug passes into a second channel through an opening, wherein the second channel is downstream of where the plug is formed.

35. The method of claim 31, further comprising splitting at least one merged plug such that a portion of the merged plug passes into a third channel through an opening, wherein the third channel is downstream of where the merged plug is formed.

introducing a carrier fluid into a first channel of a microfabricated substrate, introducing a first reagent fluid comprising a monomers together with a second reagent fluid comprising a polymerization catalyst into the first channel; wherein the first and second reagent fluids are immiscible with the carrier fluid, applying pressure to the first channel to induce a continuous flow of the fluids in the microfabricated device to form plugs which comprise the reagent fluids, are substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid and within which polymerization occurs.

38. A method of forming an unstable intermediate, comprising the steps of:
introducing a carrier fluid into a first channel of a microfabricated substrate,
introducing at least two different reagent fluids which together react to form
an unstable intermediate and which are immiscible with the carrier fluid into the first
channel, and

39. The method of claim 38, further comprising:
introducing a second reagent fluid which is immiscible with the carrier fluid
into a second channel.

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merging the first and second channels into a single channel downstream of where the first and second set of plugs form, wherein the flow within the microfabricated device forms merged plugs in which a reaction between the unstable intermediate and second reagent fluid can occur.

5 40. The method of claim 38, further comprising employing a number of
microfabricated devices in parallel.

41. A device comprising one or more microfabricated substrates comprising:
a first microchannel,

at least one carrier fluid reservoir in fluid communication with the first
10 microchannel,

at least one second microchannel which is in fluid communication with the first microchannel,

at least two reagent fluid reservoirs in fluid communication with the second microchannel, and

15 a means for applying continuous pressure to a fluid within the microchannel.

42. The device of claim 41, comprising one microfabricated substrate.

43. The device of claim 41, wherein at least one of the microfabricated substrates comprises an array of connected microchannels.

20 44. The device of claim 41, wherein one of the microchannels has at least 1 bend.

45. The device of claim 41, wherein one of the microchannels has a plurality of bends.

46. The device of claim 41, wherein at least one of the microchannels comprises at least one bend of greater than about 10°.

25 47. The device of claim 41, wherein at least one of the microchannels comprises at least one bend of greater than about 90°.

48. The device of claim 41, wherein at least one of the microchannels comprises at least one bend of greater than about 135°.

49. The device of claim 41, wherein at least one of the microchannels comprises at least one bend of greater than about 180°.

50. The device of claim 41, wherein at least one of the microchannels comprises at least one bend of greater than about 270°.

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51. The device of claim 41, wherein at least one of the microchannels comprises at least one bend of less than about 360°.

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A method of conducting a reaction within a microfabricated substrate, comprising introducing at least two different reagent fluids which together form a reaction and which are immiscible with the carrier fluid into the first channel from at least one or more second channel(s), and applying pressure to the first channel to induce a flow of the fluids in the microfabricated device to form plugs which comprise the reagent fluids. The plugs may be substantially similar in size, are surrounded by carrier fluid, and are separated by substantially identical amounts of carrier fluid and within which a reaction occurs. Moreover, the invention relates to a method of synthesizing a polymer and an unstable intermediate. The invention also provides a device comprising one or more microfabricated substrates comprising at least two reagent fluid reservoirs in fluid communication with a microchannel.

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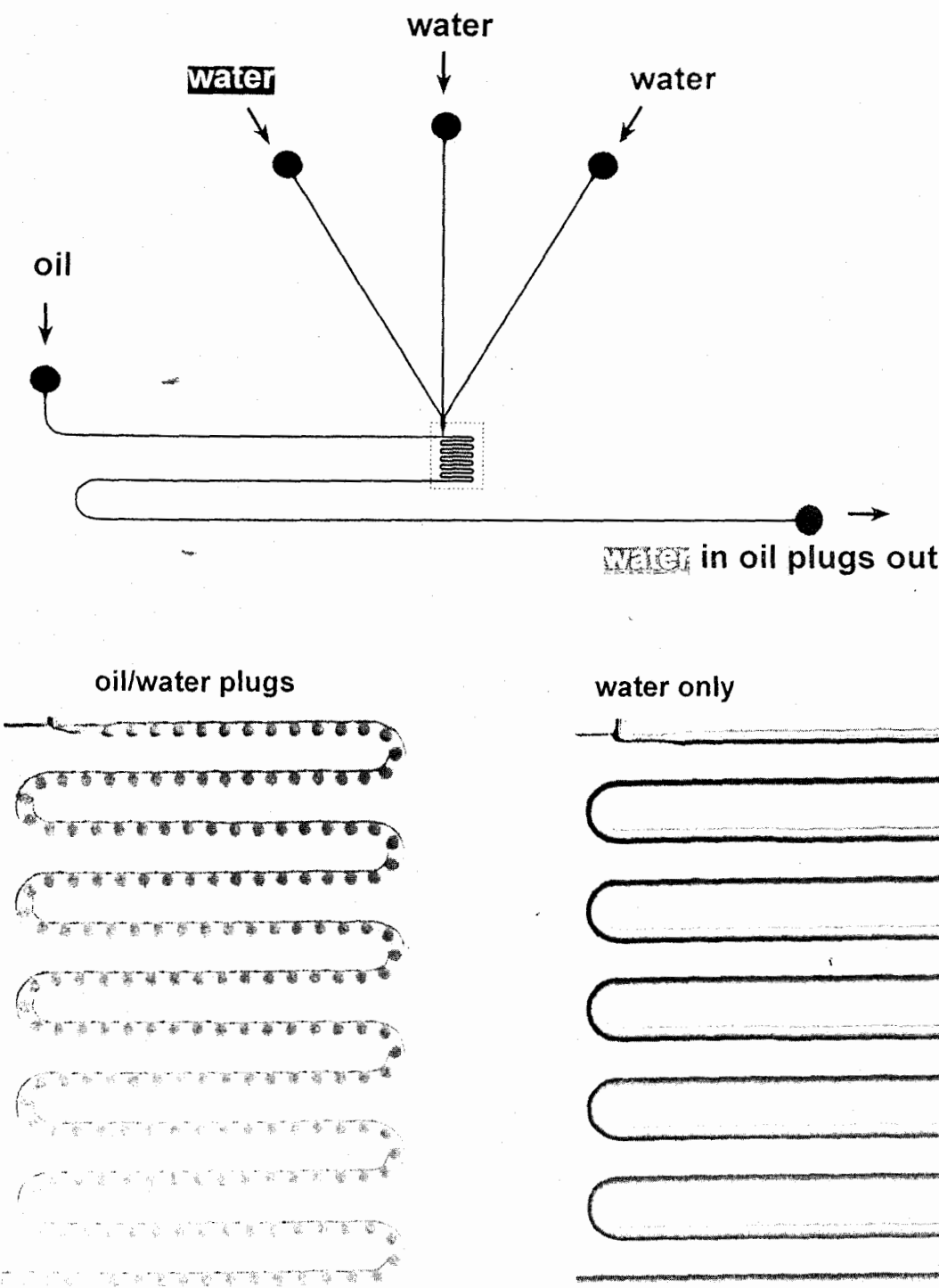
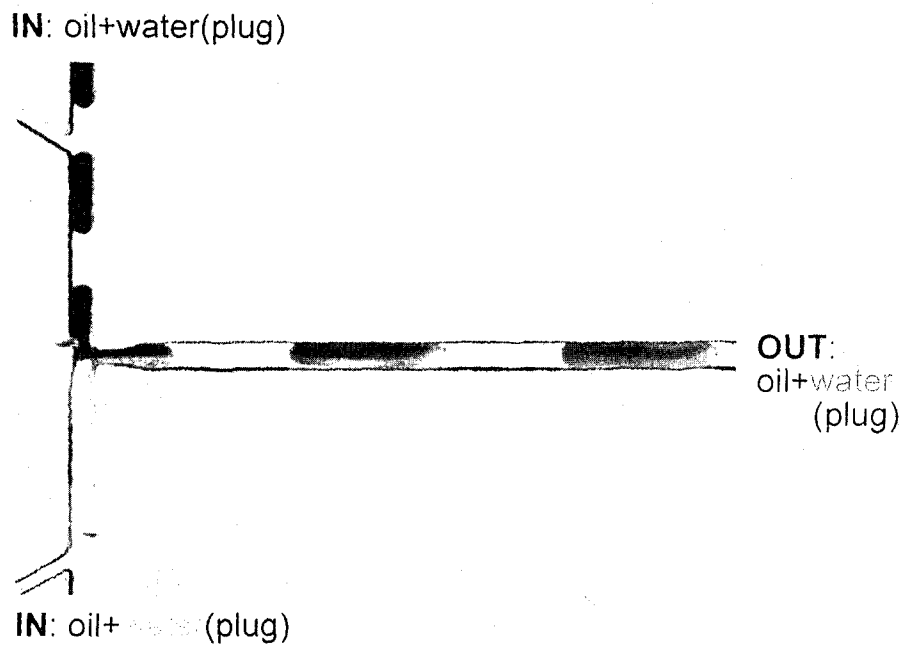


Fig. 1

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Aqueous plugs can be split

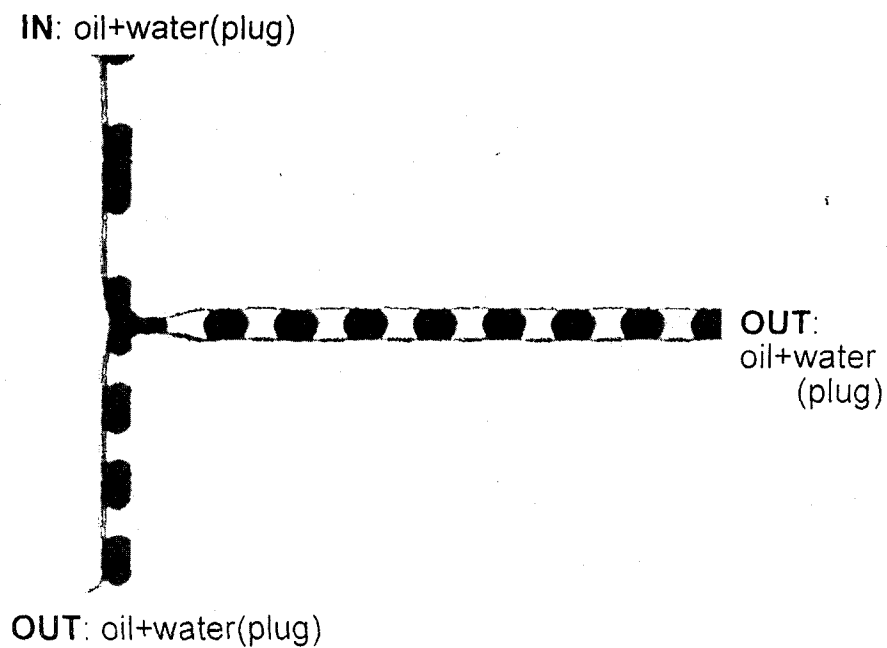
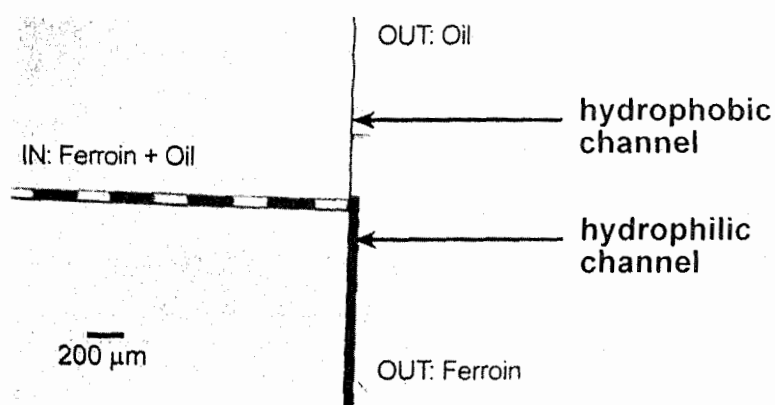


Fig. 5

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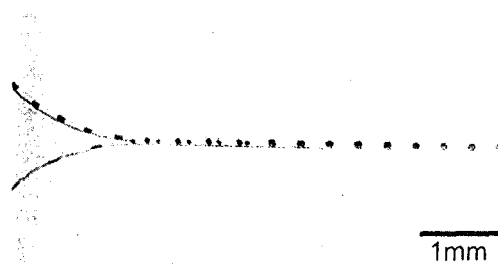
Aqueous plugs prefer to enter hydrophilic channels.

Fig. 6

Case 1:15-cv-00152-RGA Document 74-3 Filed 09/01/16 Page 54 of 91 PageID #: 5338

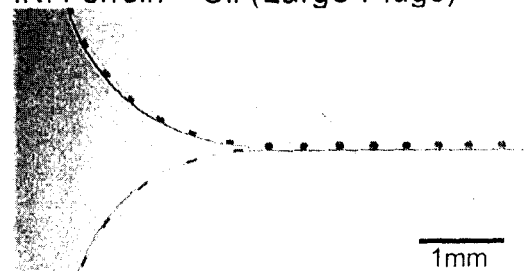
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IN: Ferroin + Oil (Large Plugs)



IN: Ferroin + Oil (Small Plugs)

IN: Ferroin + Oil (Large Plugs)

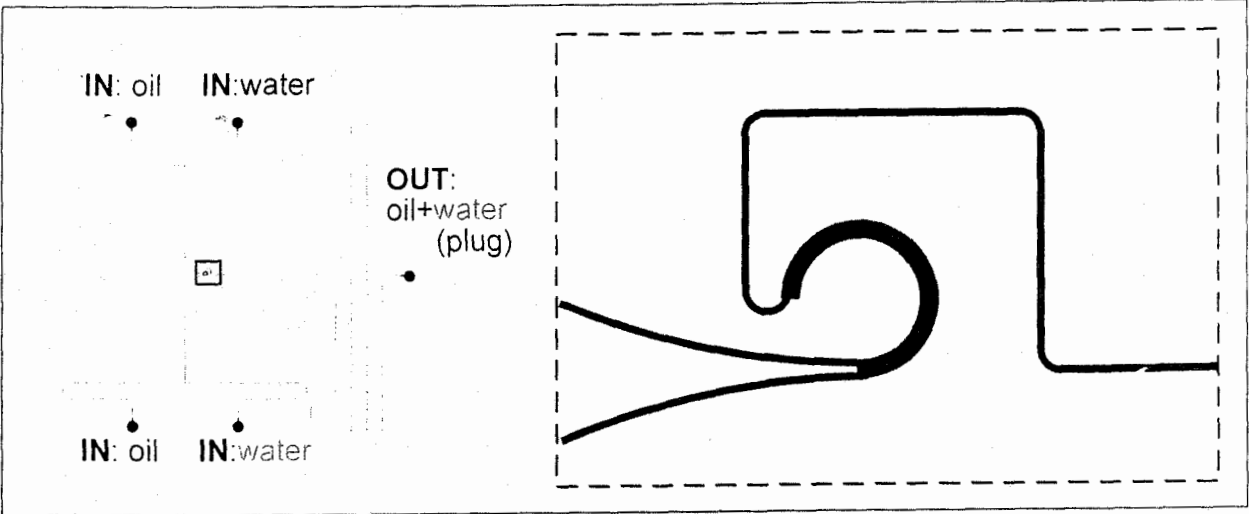


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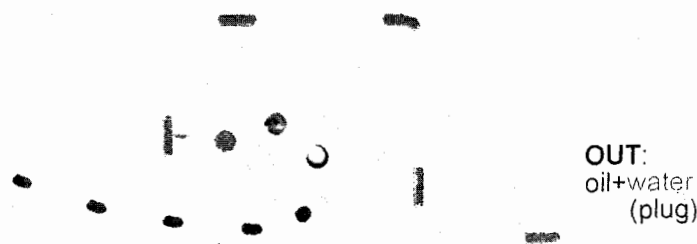
Fig. 7

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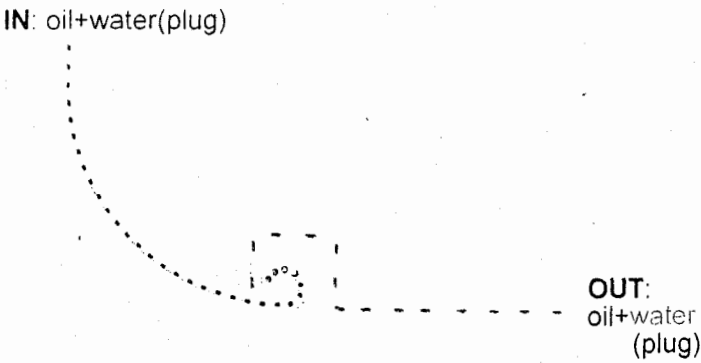
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IN: oil+water(plug)



IN: oil+ (plug)



IN: oil+ (plug)

Fig.8

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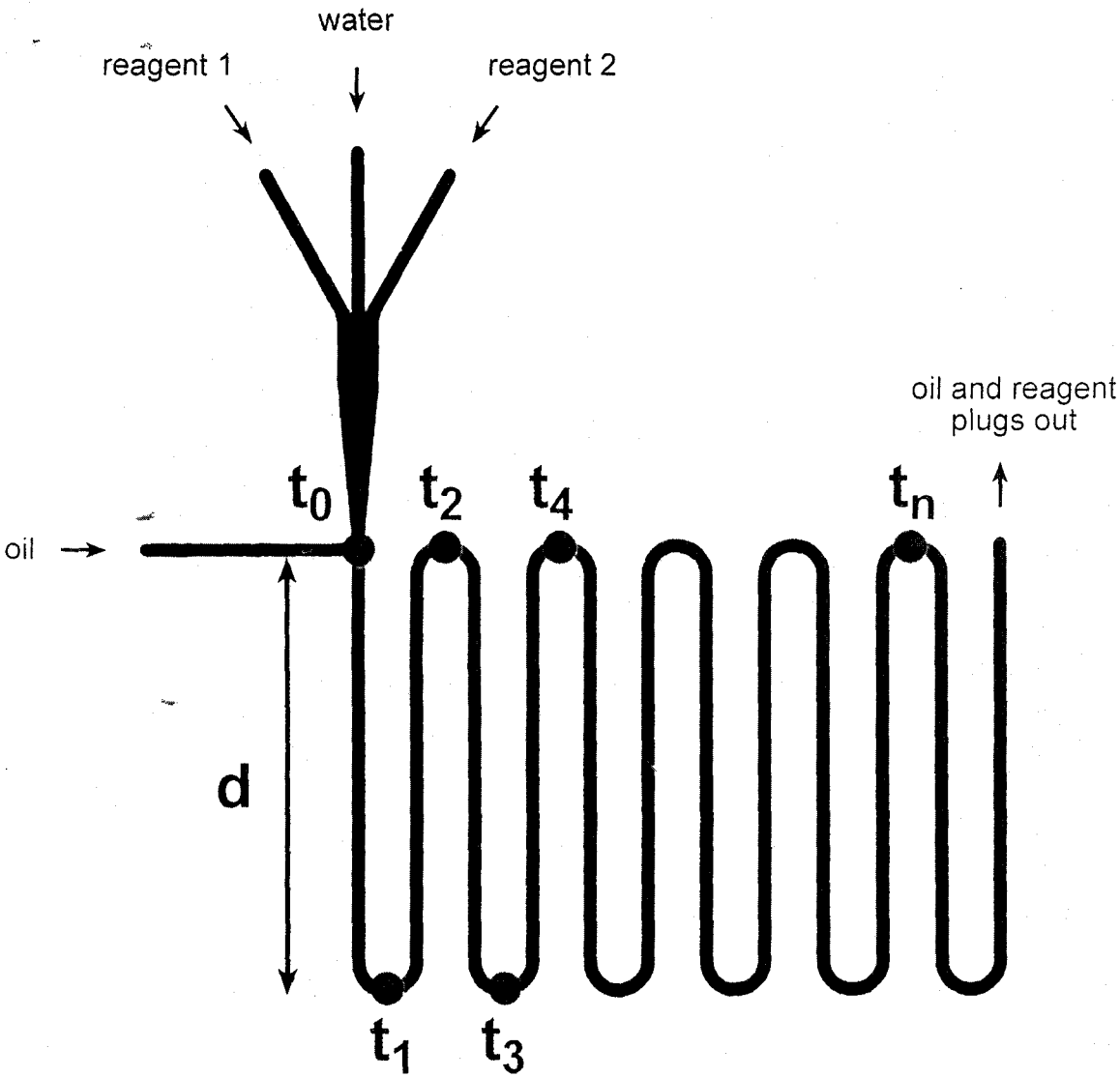


Fig. 9

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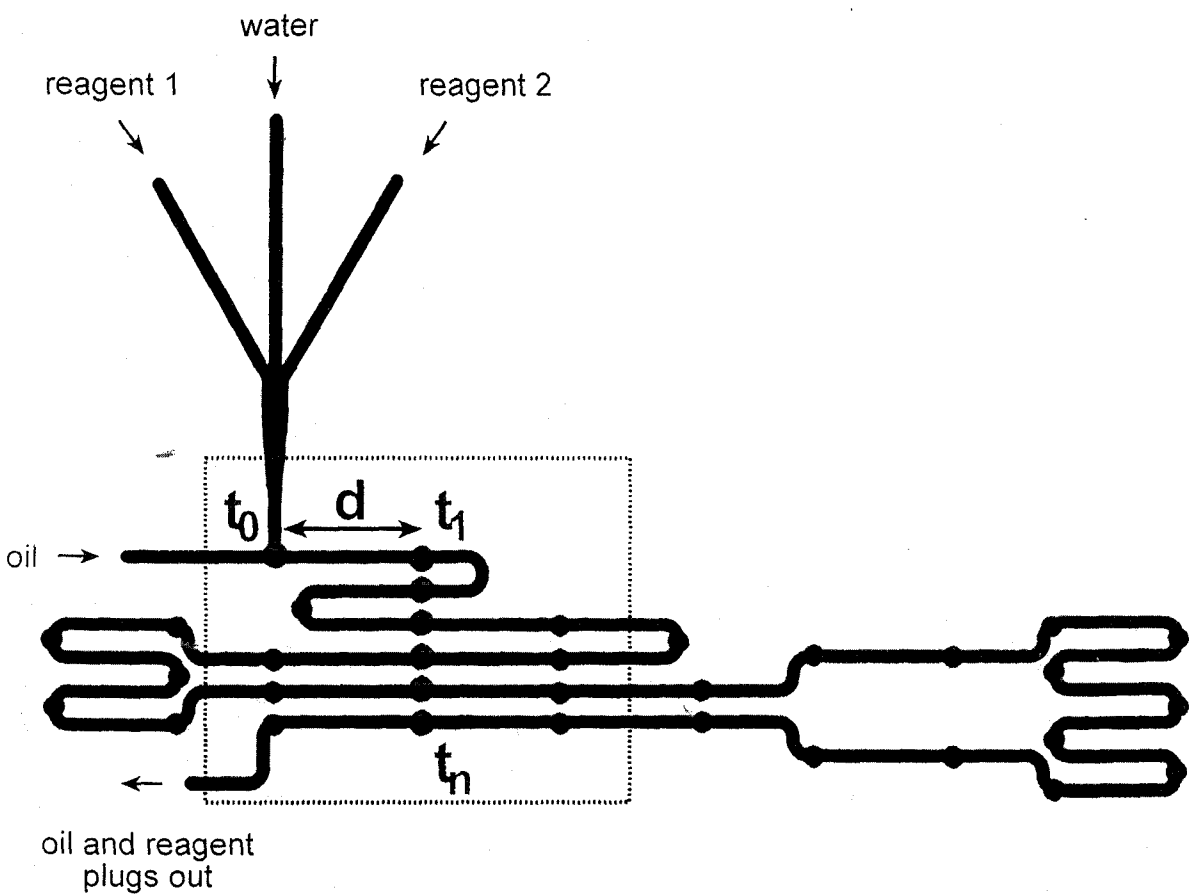


Fig. 10

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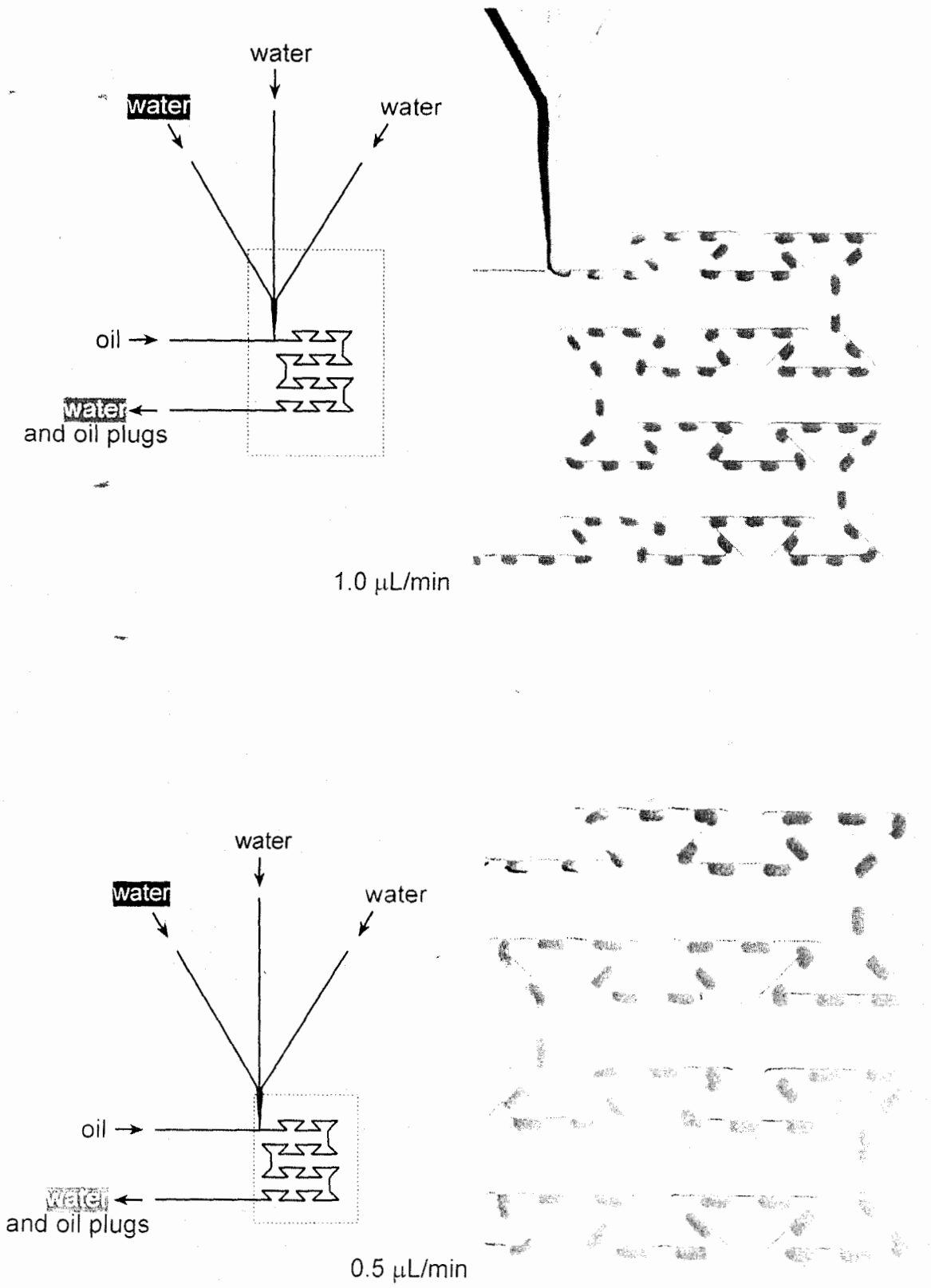


Fig. 12

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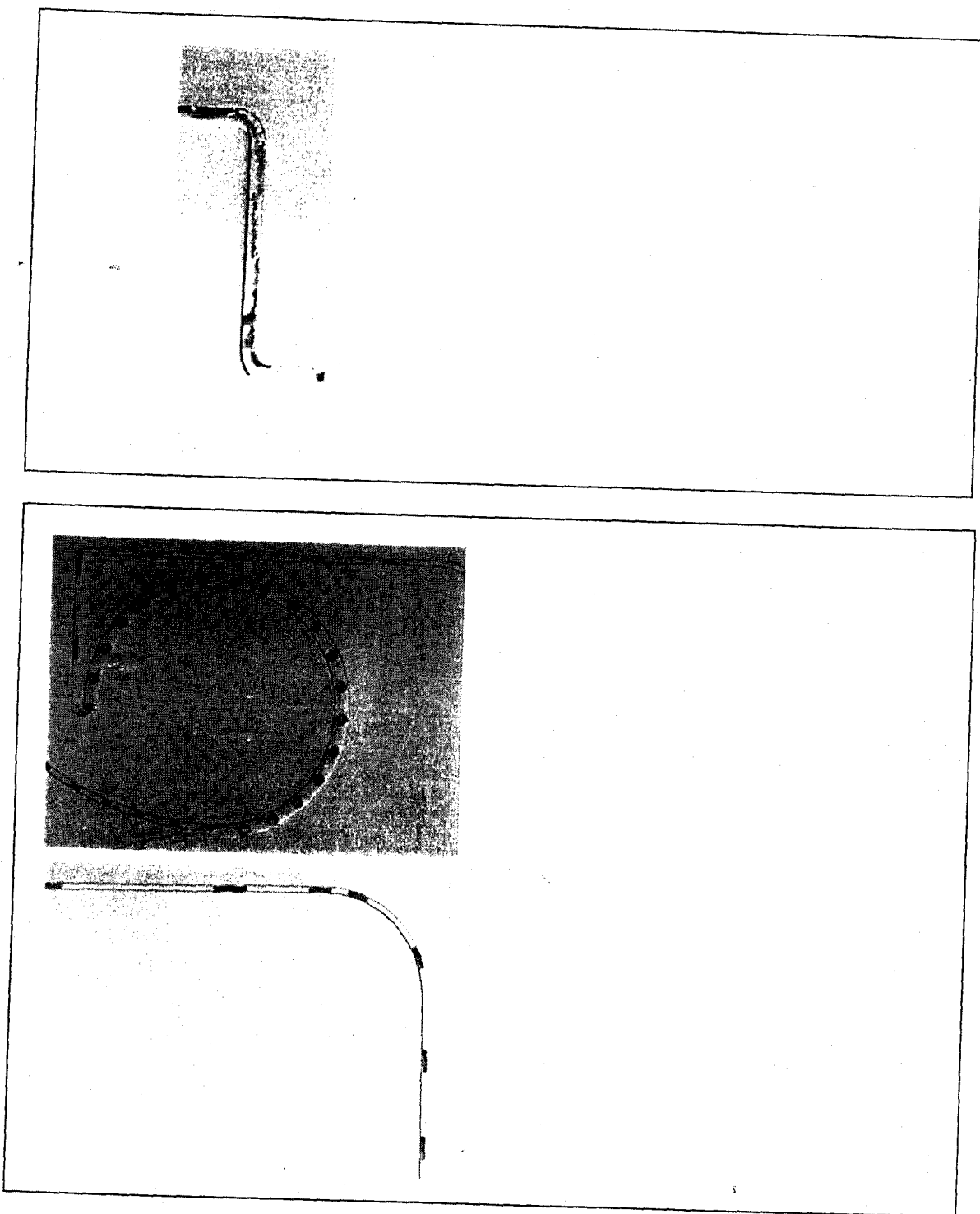


Fig. 13

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US008329407B2

(12) United States Patent

Ismagilov et al.

(16) Patent No.: US 8,329,407 B2**(22) Date of Patent: *Dec 11, 2012**

(54) METHOD FOR CONDUCTING REACTIONS INVOLVING BIOLOGICAL MOLECULES IN PLUGS IN A MICROFLUIDIC SYSTEM

(75) Inventors: Rustem F Ismagilov, Chicago, IL (US); Joshua David Tice, Webster, NY (US); Cory John Gerdt, Chicago, IL (US); Bo Zheng, Chicago, IL (US)

(73) Assignee: The University of Chicago, Chicago, IL (US)

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

This patent is subject to a terminal disclaimer.

(21) Appl. No.: 13/024,145

(22) Filed: Feb. 9, 2011

(65) Prior Publication Data

US 2011/0177494 A1 • Jul. 21, 2011

Related U.S. Application Data

(63) Continuation of application No. 12/777,099, filed on May 10, 2010, which is a continuation of application No. 10/765,718, filed on Jan. 26, 2004, now Pat. No. 7,901,939, which is a continuation-in-part of application No. 10/434,970, filed on May 9, 2003, now Pat. No. 7,129,091.

(60) Provisional application No. 60/394,544, filed on Jul. 8, 2002, provisional application No. 60/379,927, filed on May 9, 2002.

(51) Int. Cl.
C12Q 1/68

(2006.01)

(52) U.S. Cl. 435/6.12; 435/6.19; 436/535; 436/164; 436/172

(58) Field of Classification Search None
See application file for complete search history.

(56) References Cited**U.S. PATENT DOCUMENTS**

3,960,187 A	6/1976	Stock et al.	
4,853,336 A	8/1989	Saros et al.	
4,963,498 A	10/1990	Hillman et al.	
5,185,099 A	2/1993	Delpuech et al.	
5,739,036 A	4/1998	Parris	
5,872,010 A	2/1999	Karger et al.	
5,887,755 A	3/1999	Hood, III	
5,997,636 A	12/1999	Gamarnik et al.	117/70
6,118,849 A	9/2000	Tanimori et al.	
6,130,098 A	10/2000	Handique et al.	
6,140,053 A	10/2000	Köster	435/6

(Continued)

FOREIGN PATENT DOCUMENTS

CN 2482070 3/2002

(Continued)

OTHER PUBLICATIONS

Pollack et al. "Electrowetting-based actuation of droplets for integrated microfluidics", Lab Chip, 2002, v. 2, pp. 96-101.*

(Continued)

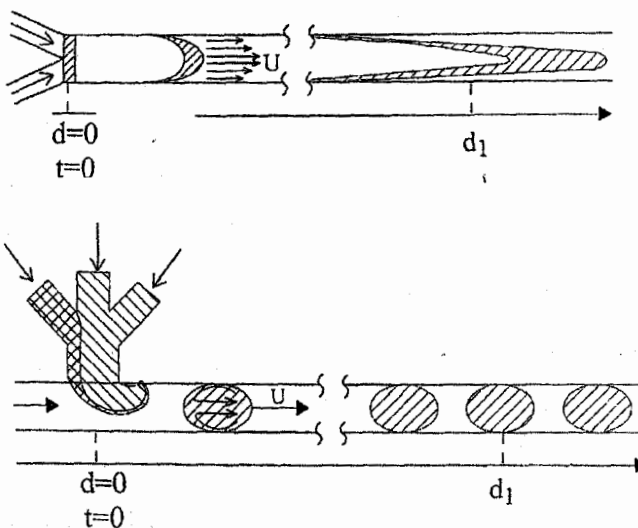
Primary Examiner — Yelena G Gakh

(74) Attorney, Agent, or Firm — Brinks Hofer Gilson & Lione

(57) ABSTRACT

The present invention provides microfabricated substrates and methods of conducting reactions within these substrates. The reactions occur in plugs transported in the flow of a carrier-fluid.

13 Claims, 63 Drawing Sheets



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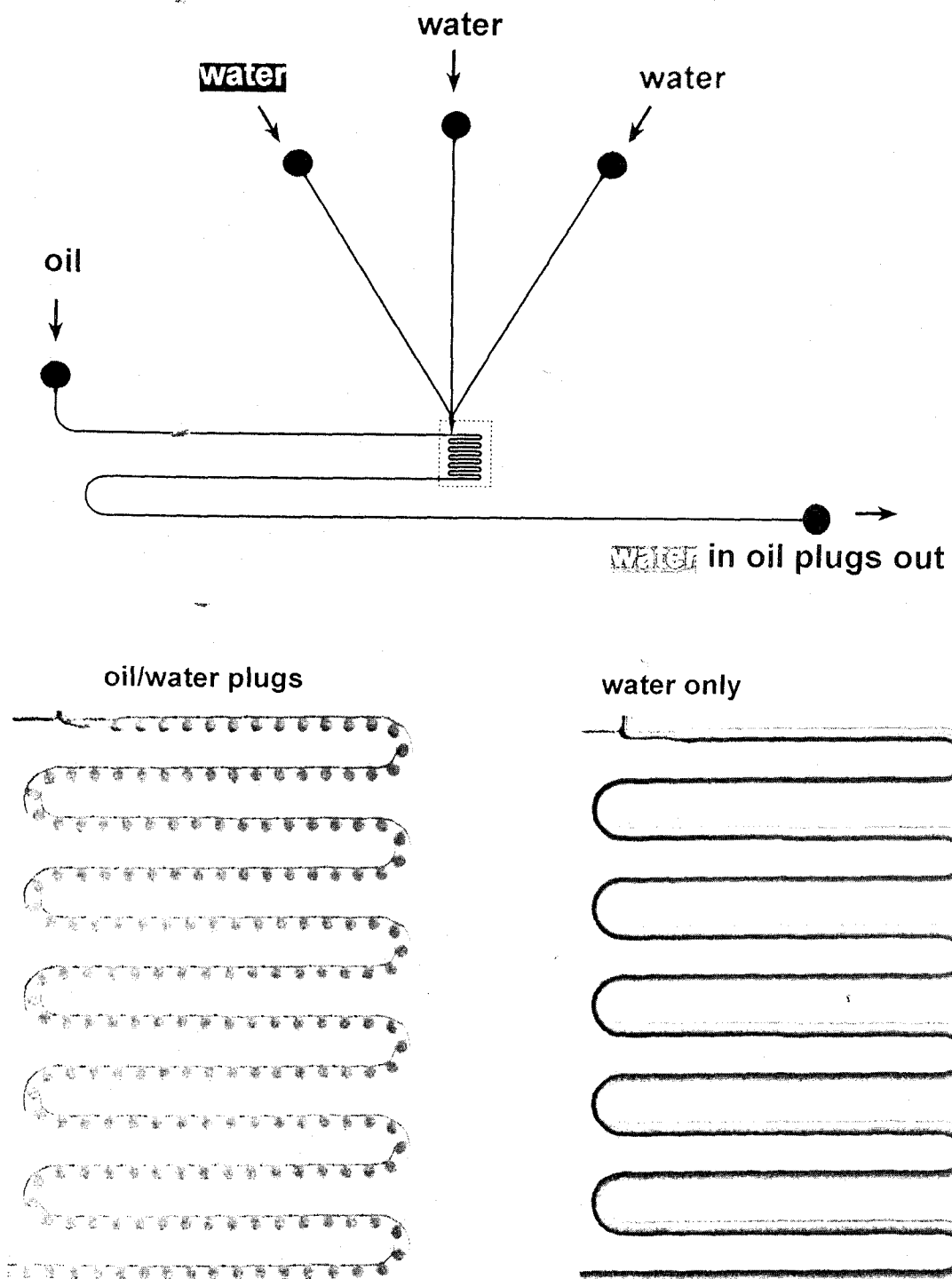


Fig. 1

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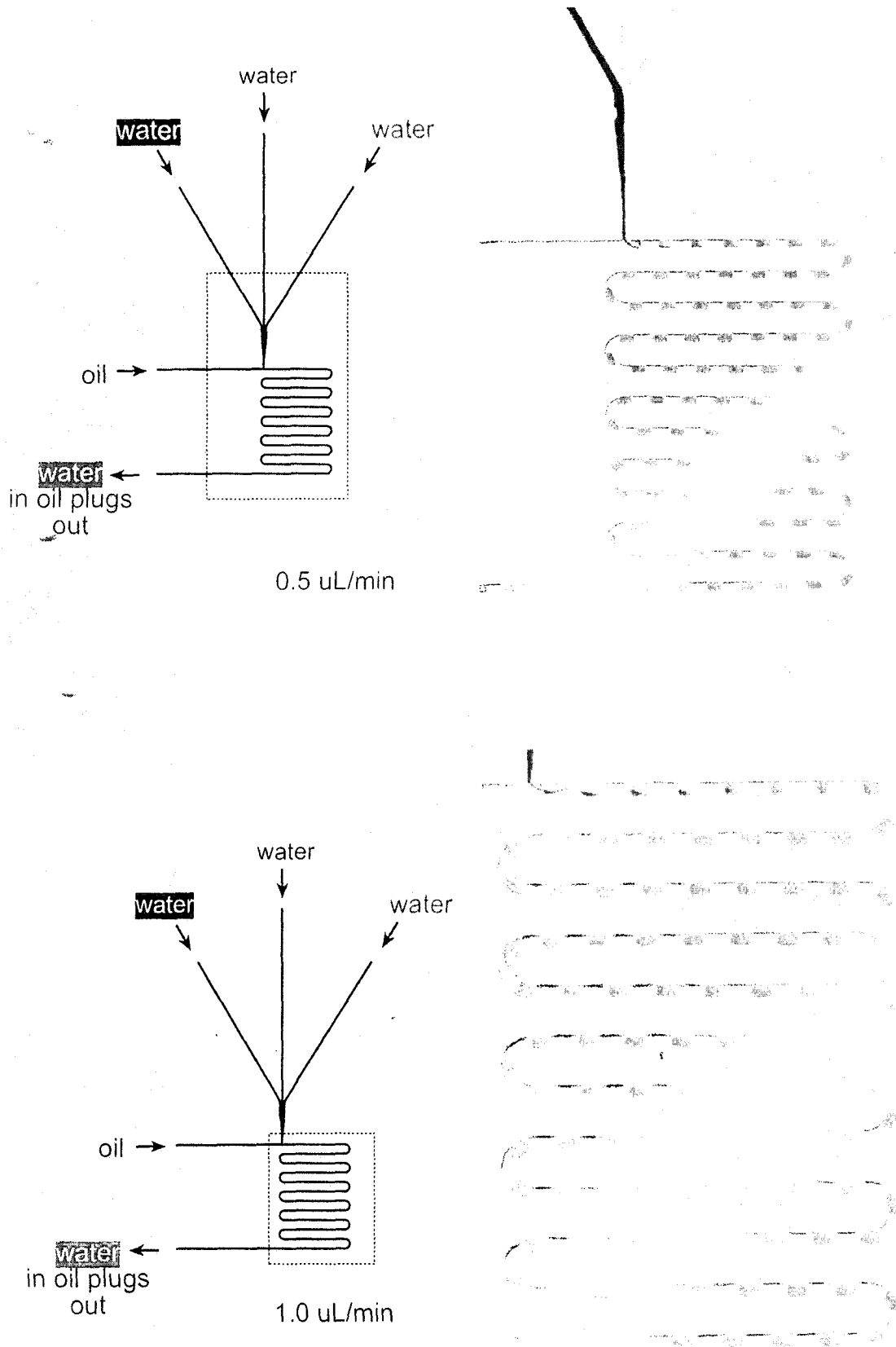
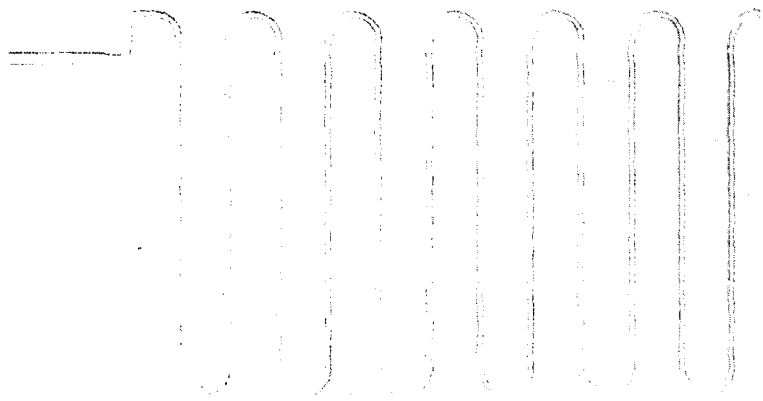
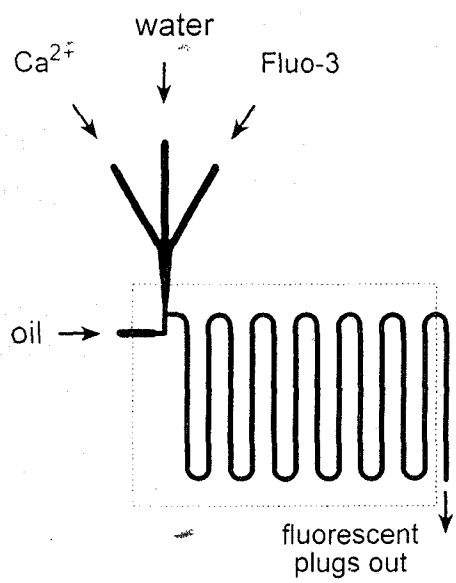


Fig. 2

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Bright field image

Fluorescence image

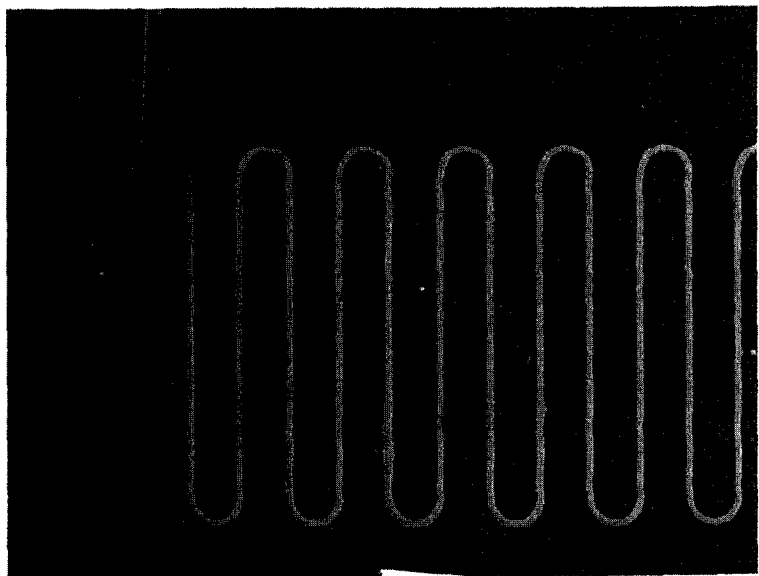


Fig. 3

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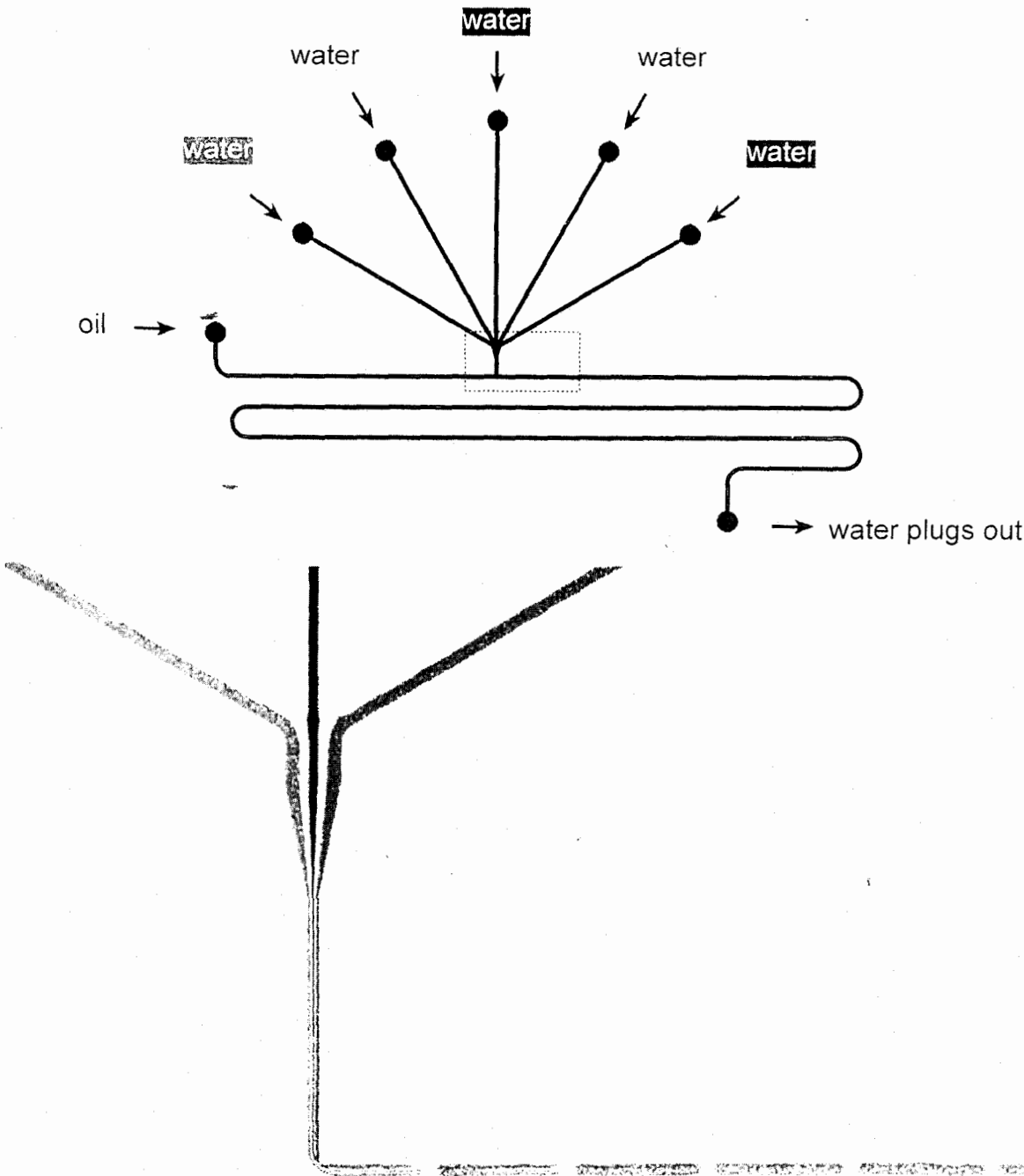
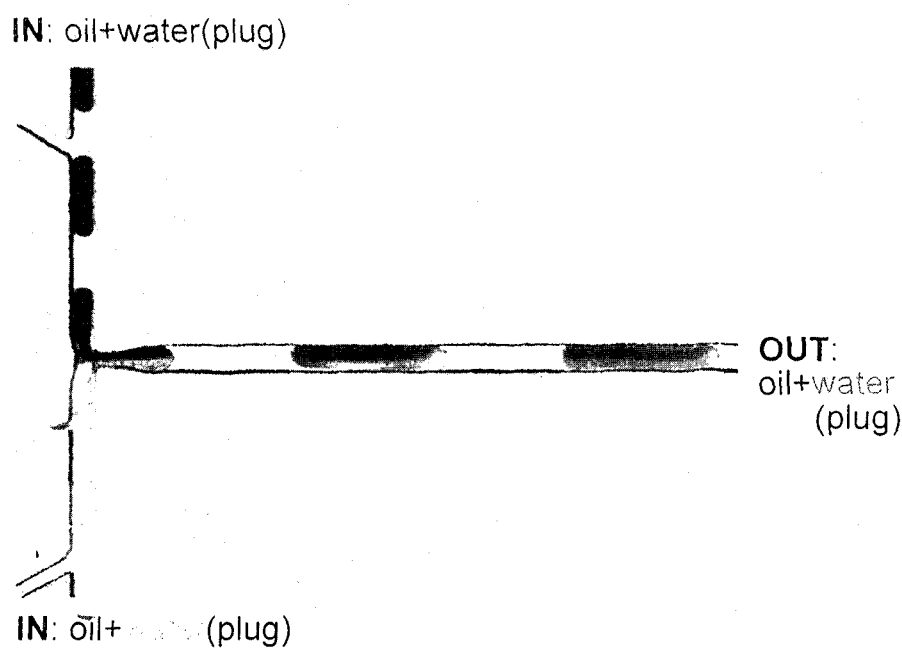


Fig. 4

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Aqueous plugs can be split

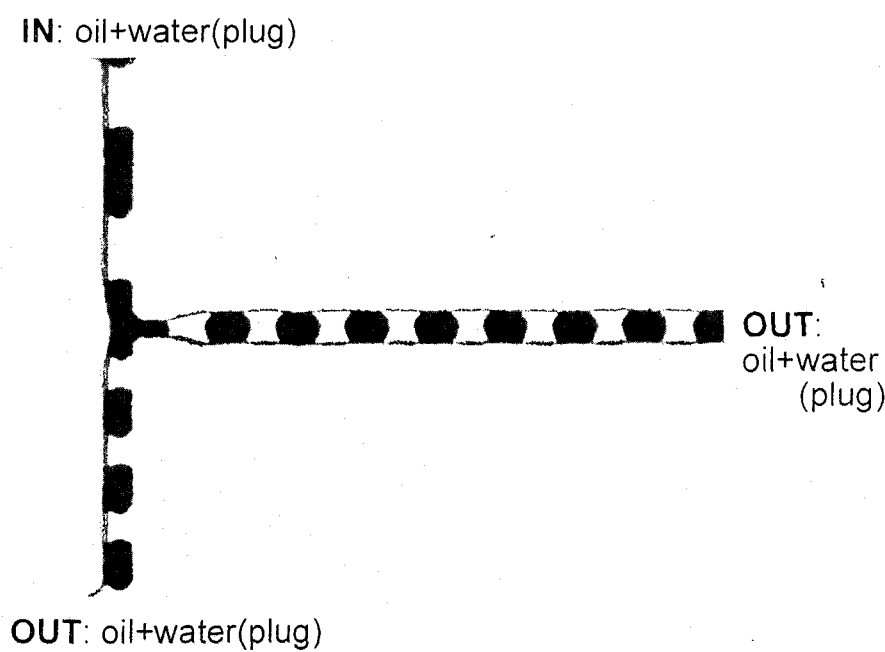
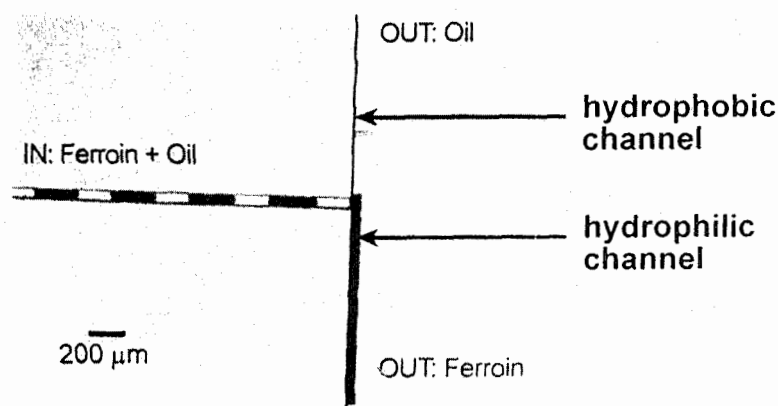


Fig. 5

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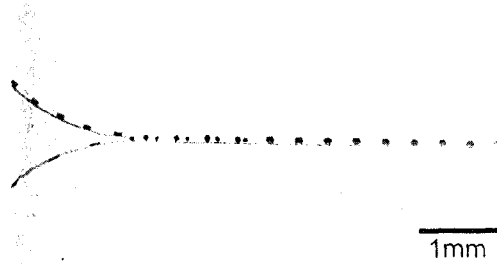


Aqueous plugs prefer to enter hydrophilic channels.

Fig. 6

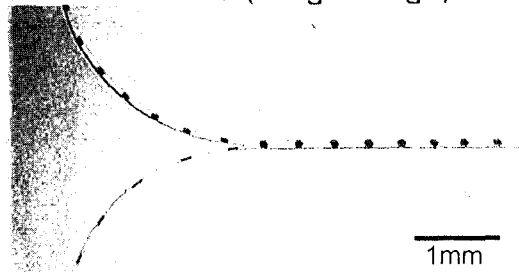
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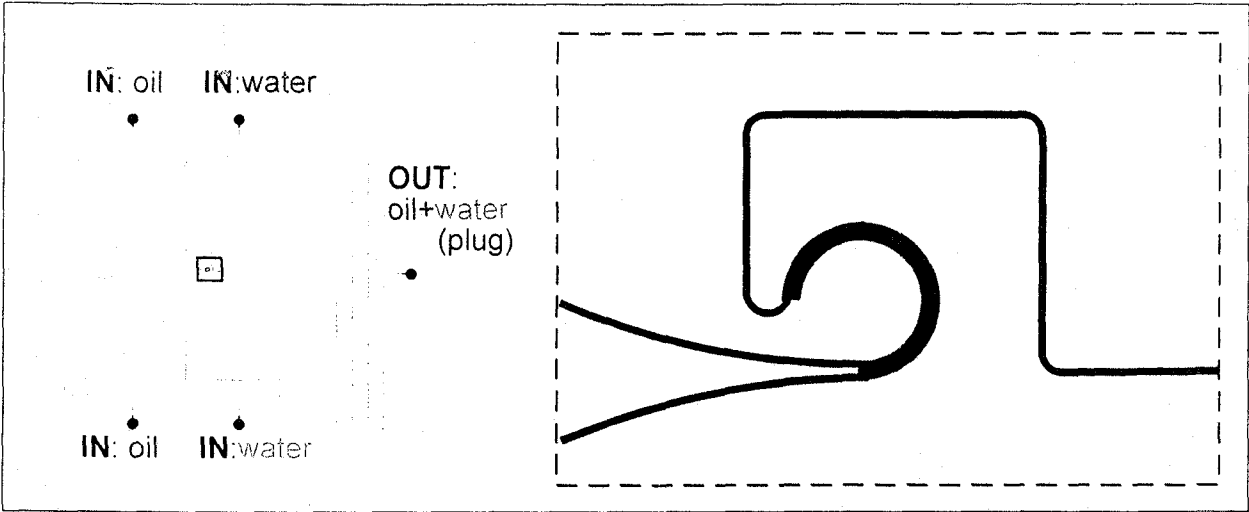
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Fig. 7

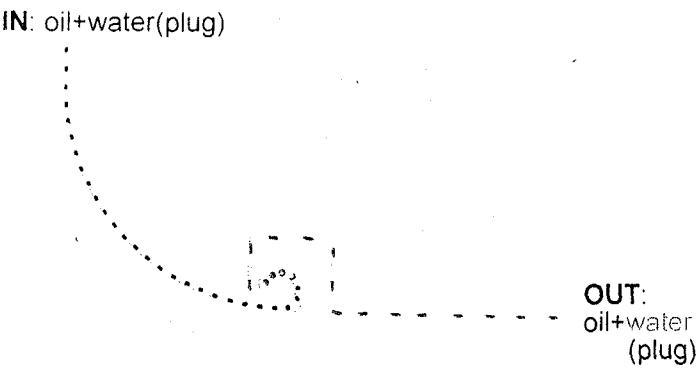
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IN: oil+water(plug)



IN: oil+ (plug)



IN: oil+ (plug)

Fig.8

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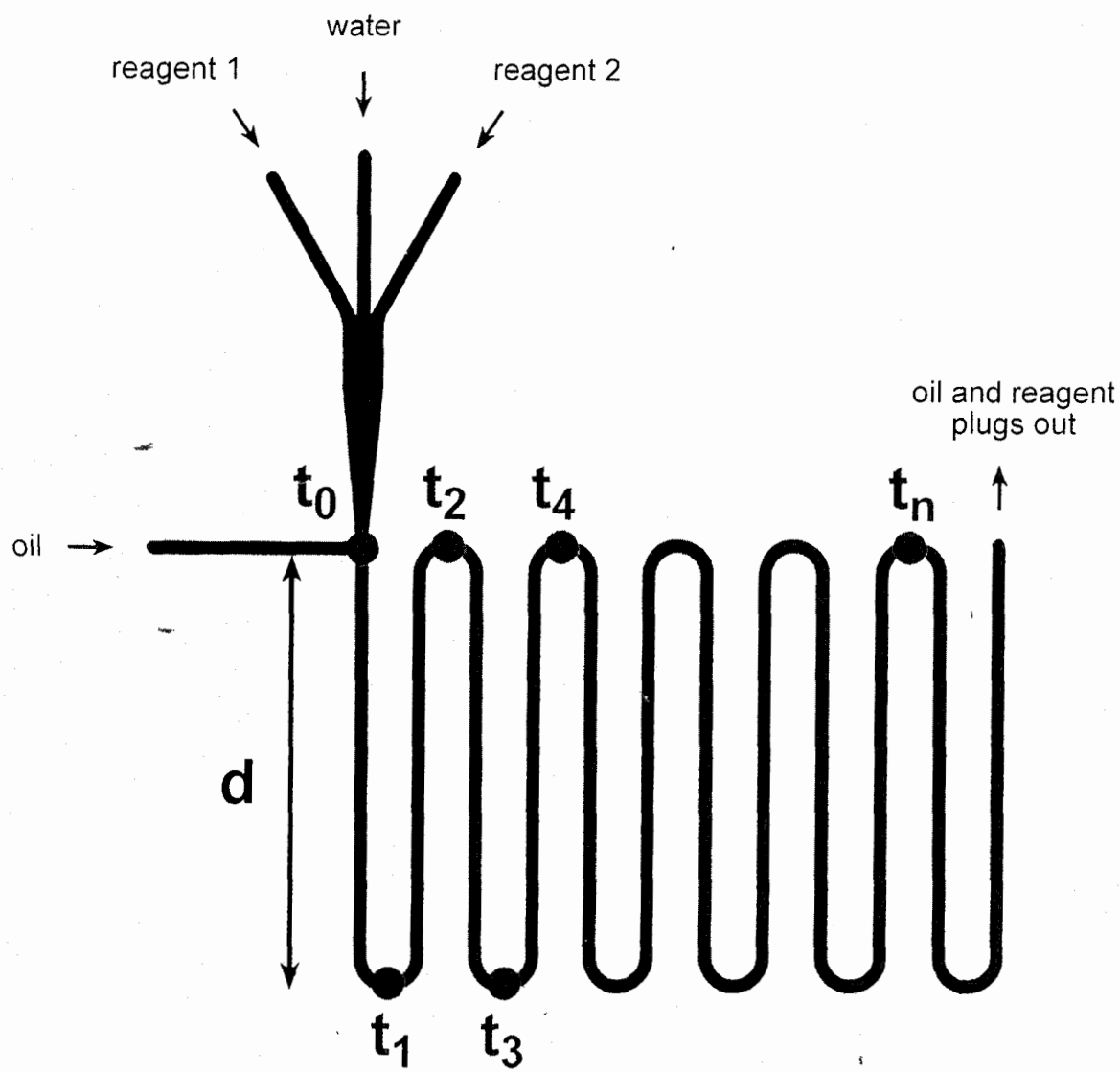


Fig. 9

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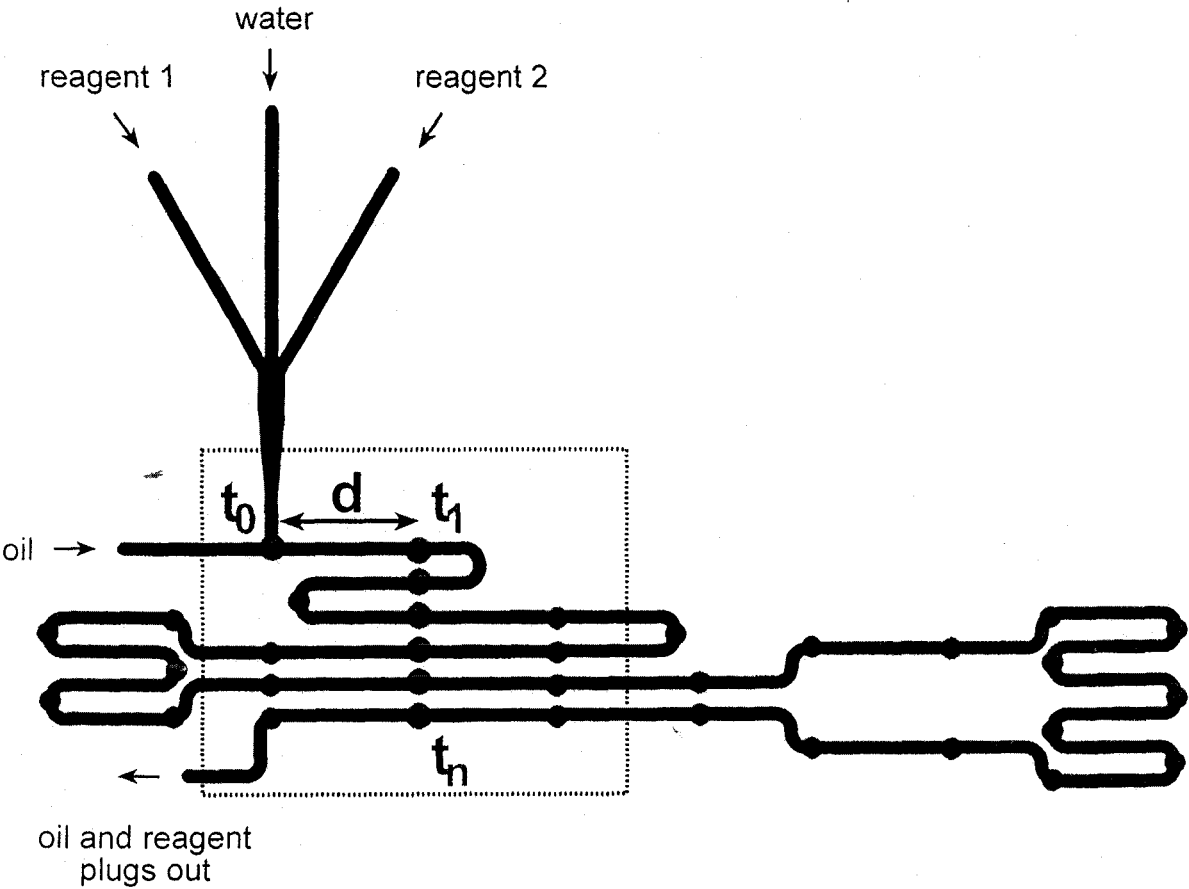


Fig. 10

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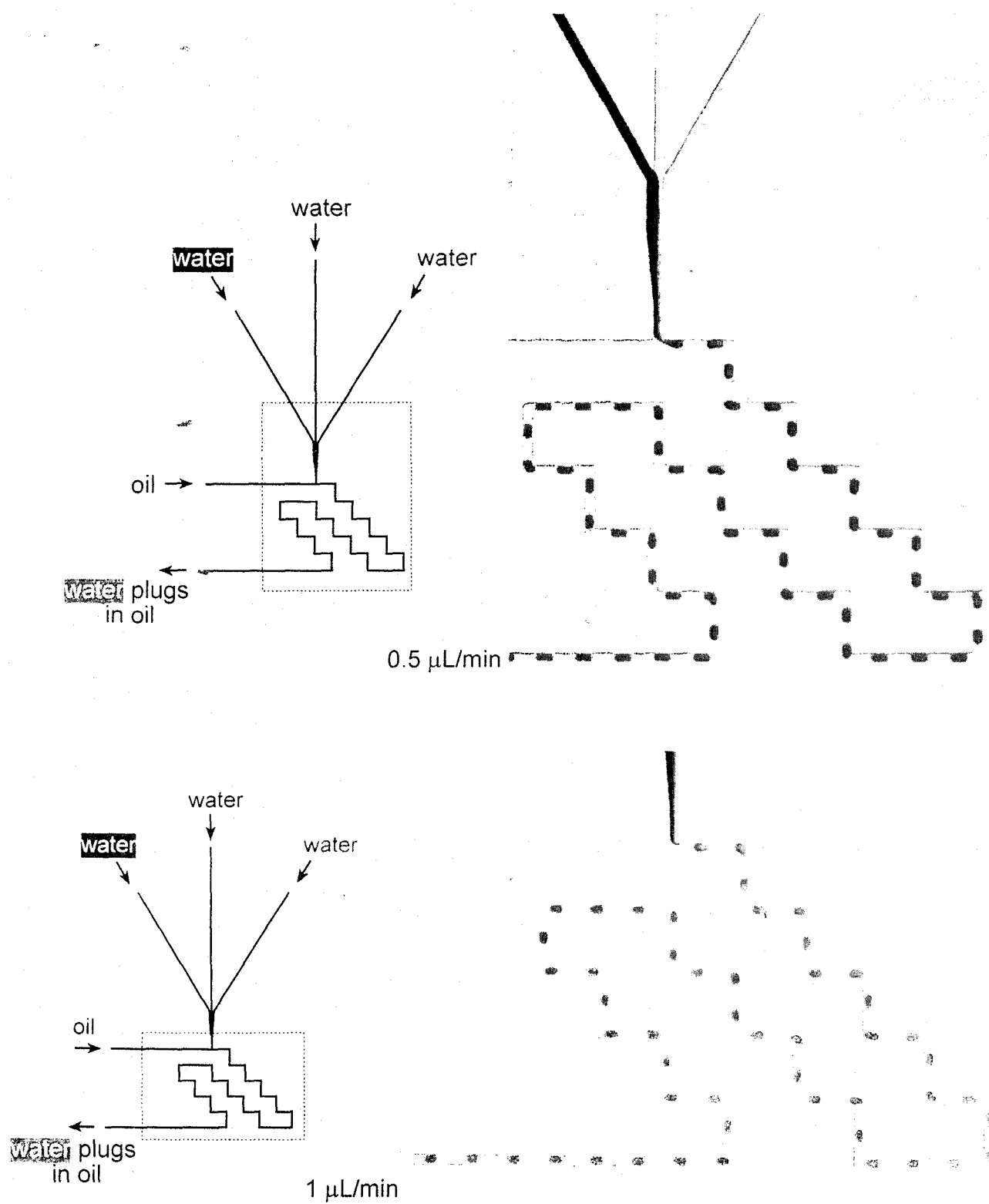


Fig. 11

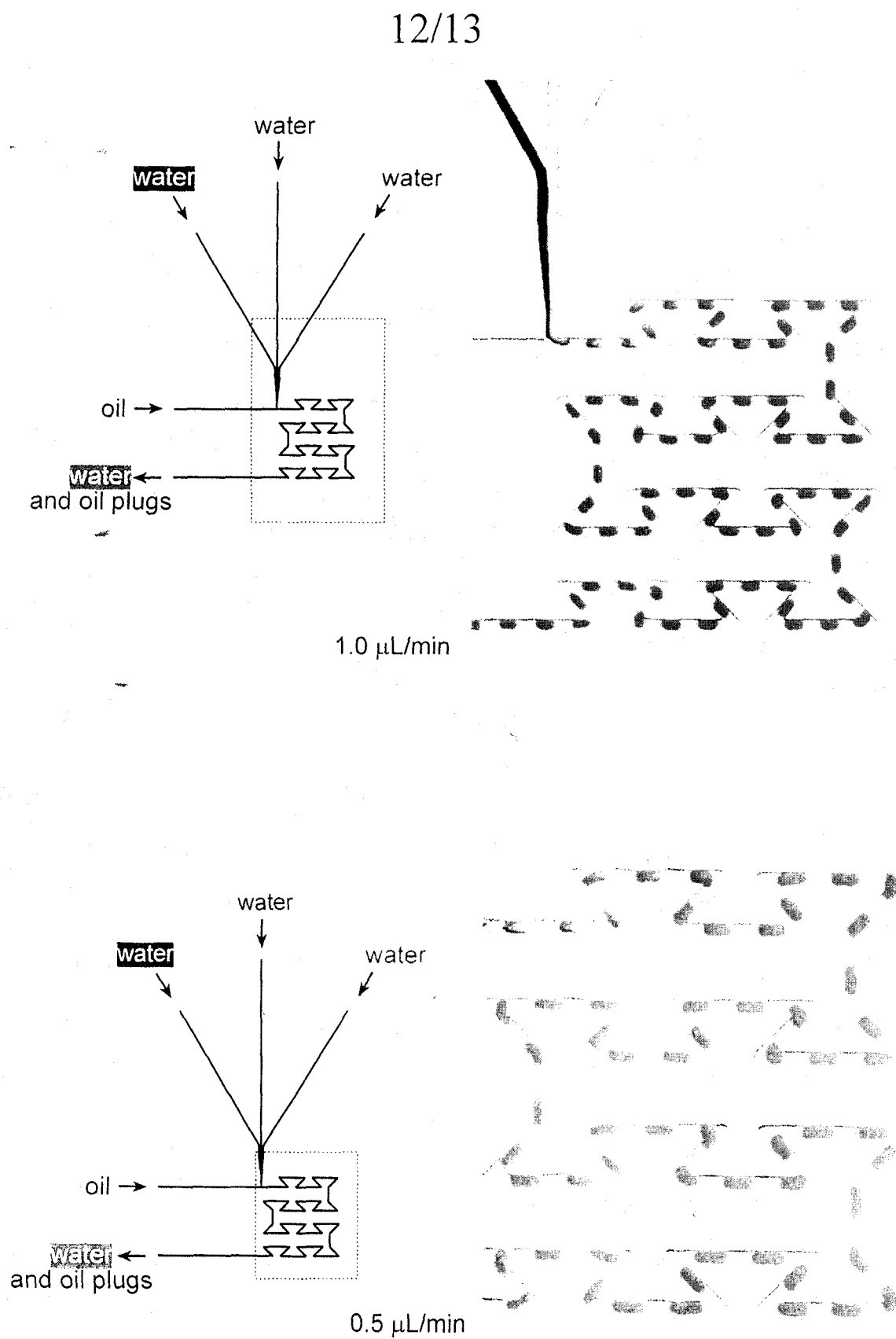


Fig. 12

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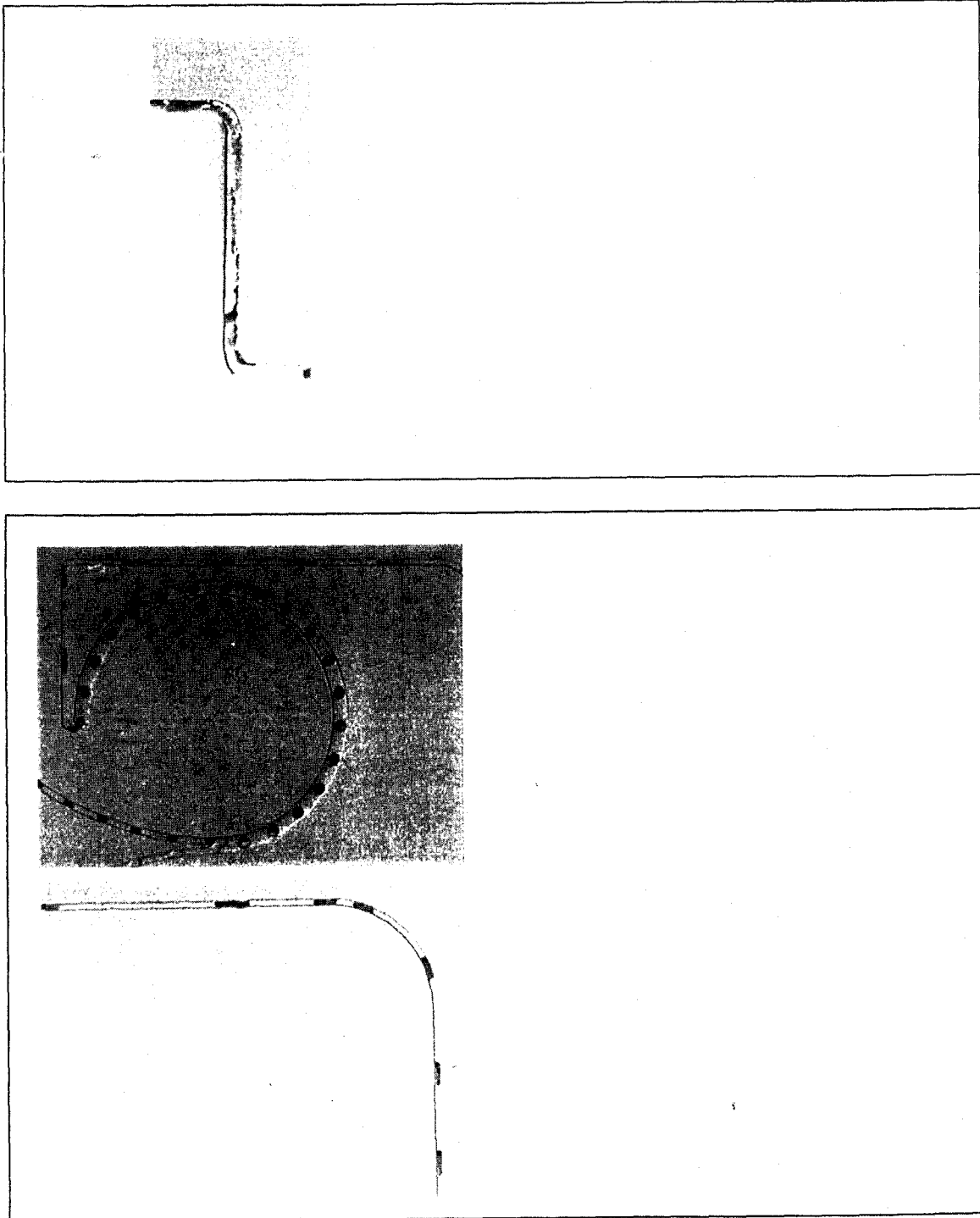


Fig. 13

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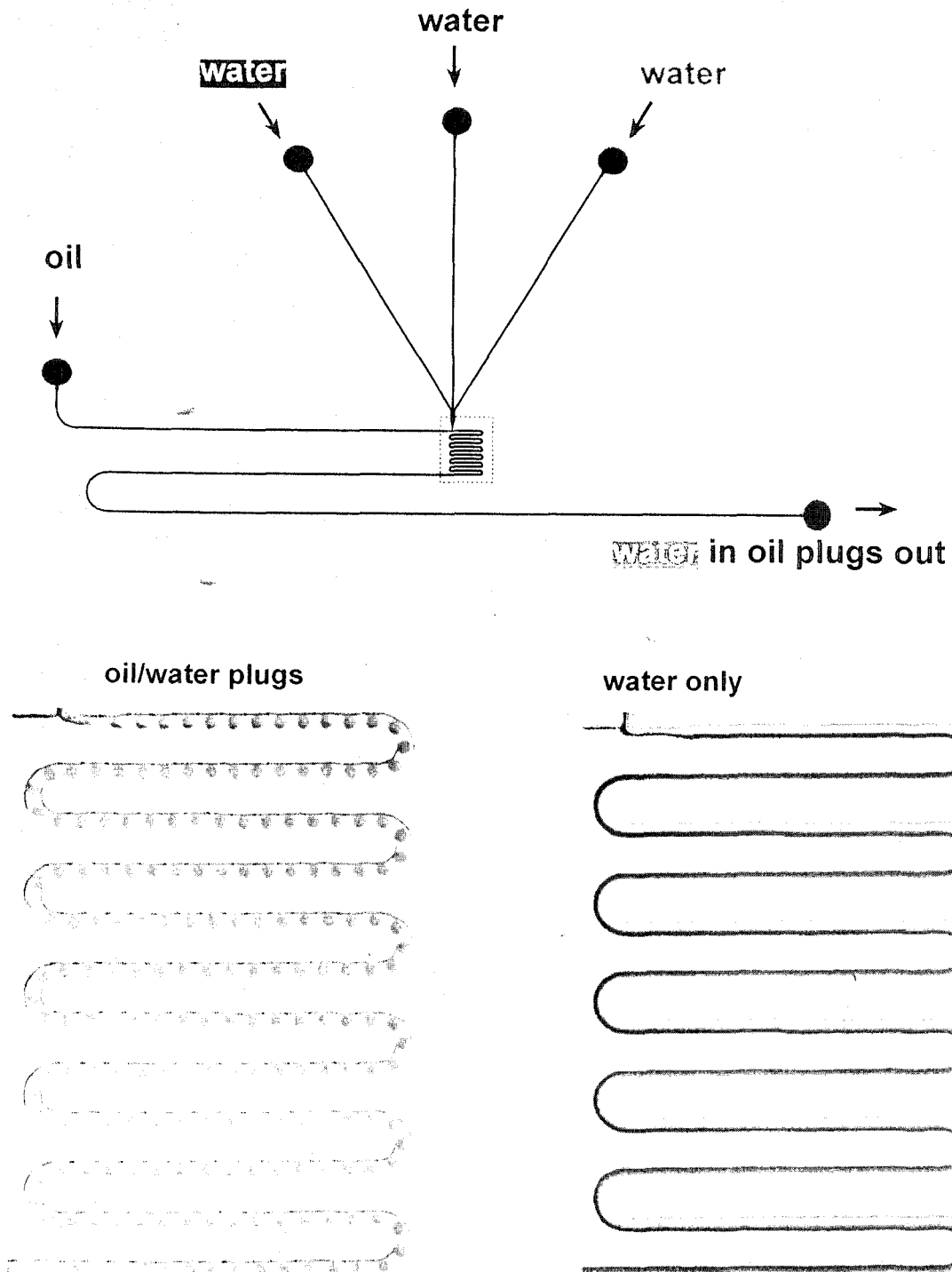


Fig. 1

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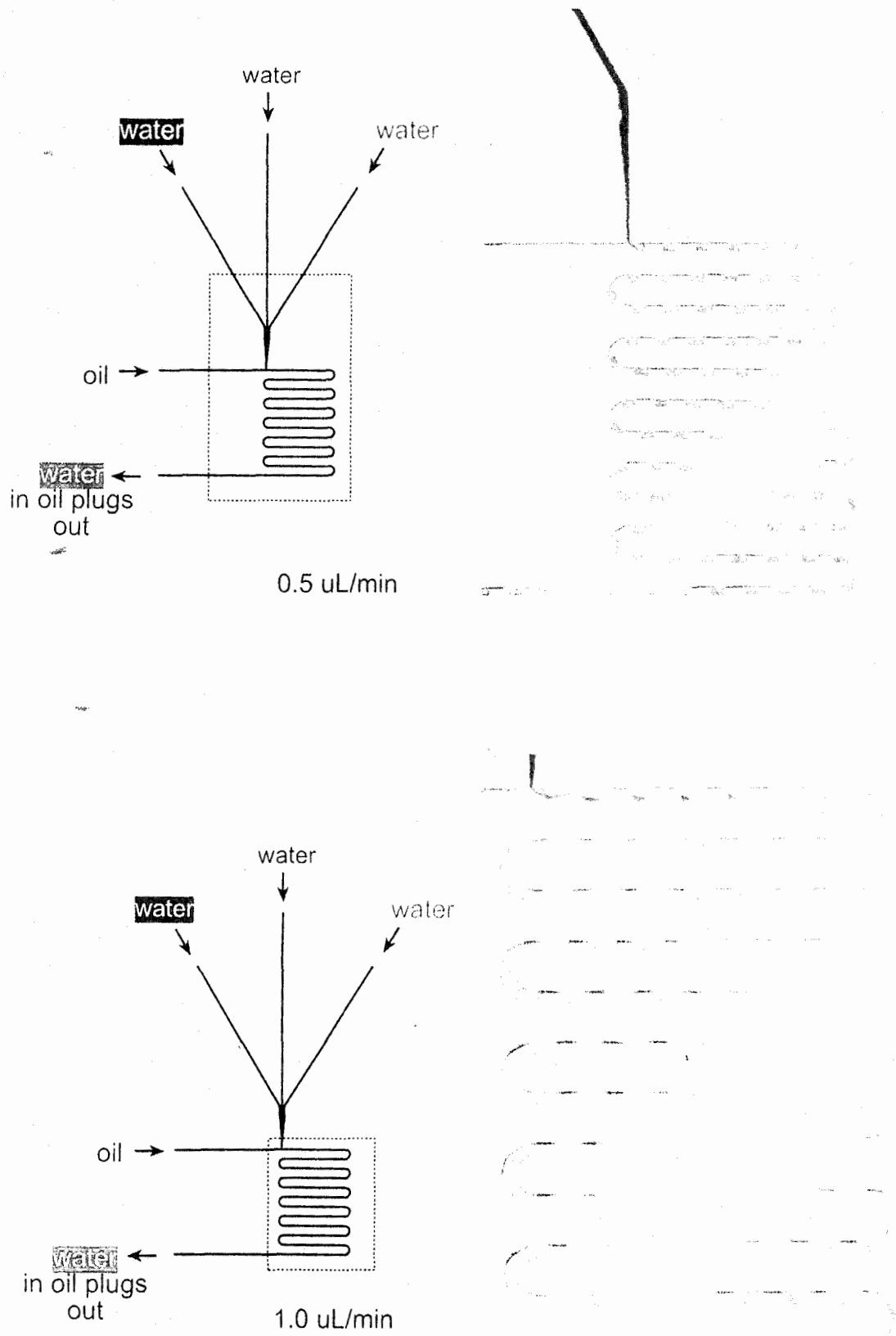
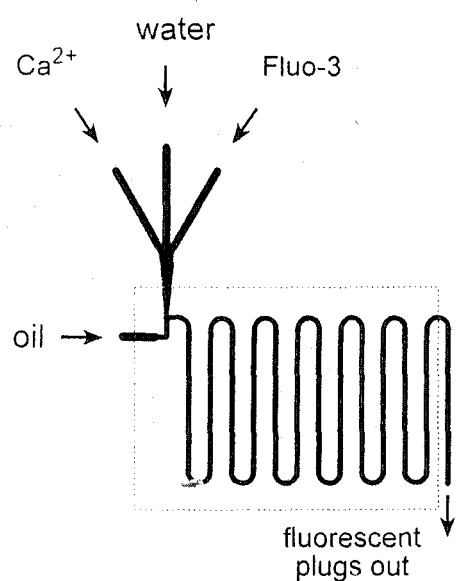
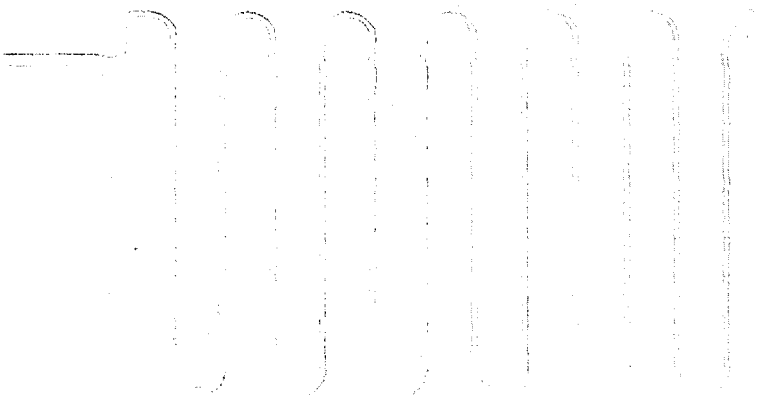


Fig. 2

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Bright field image



Fluorescence image

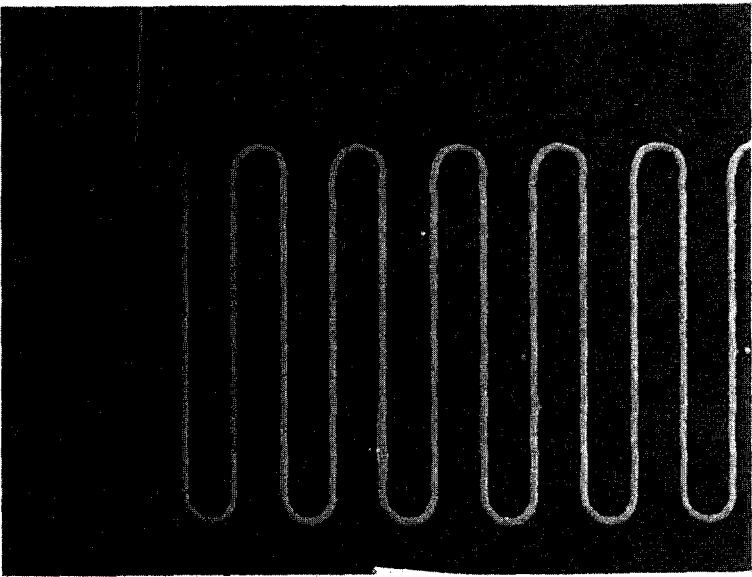


Fig. 3

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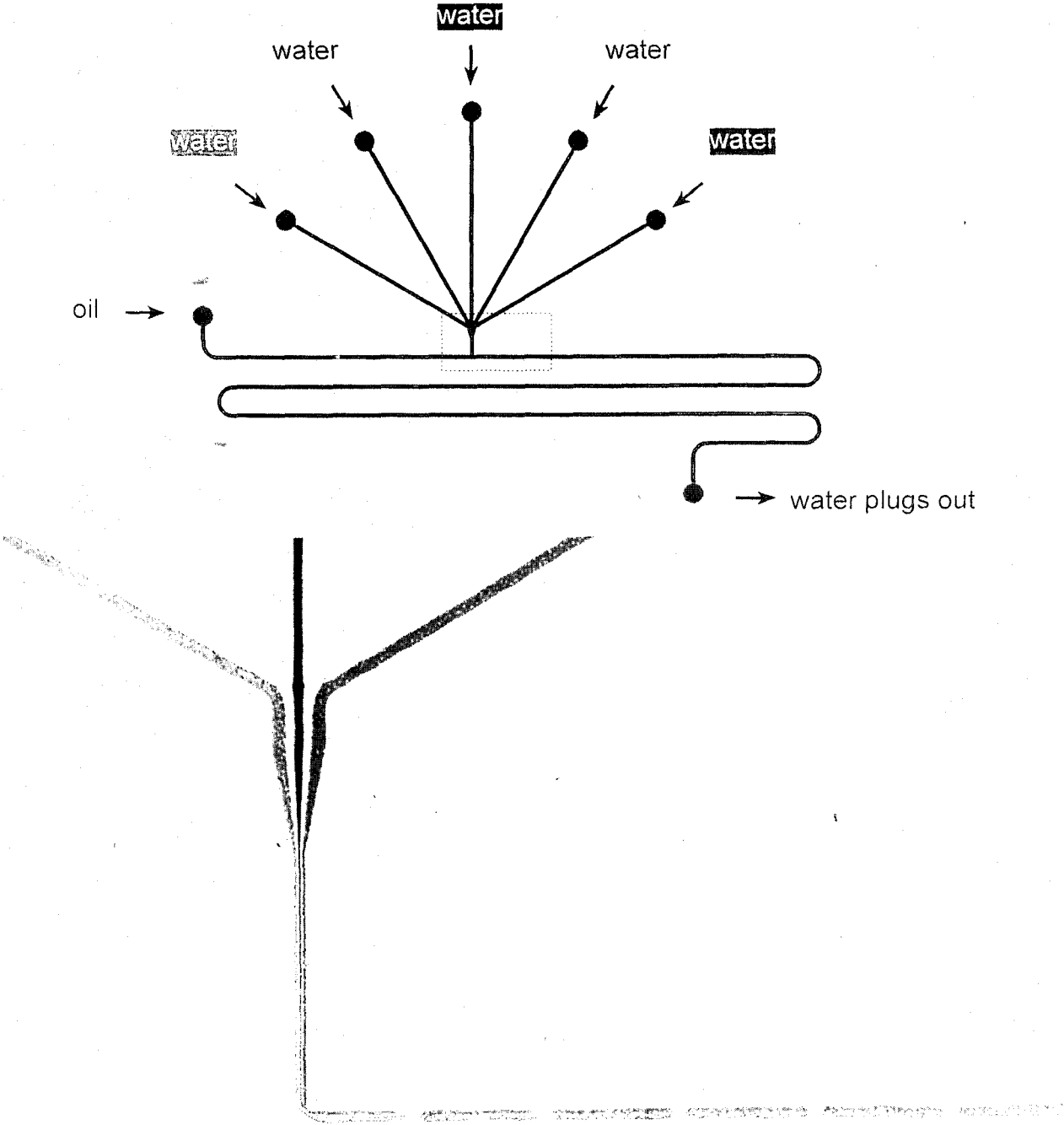
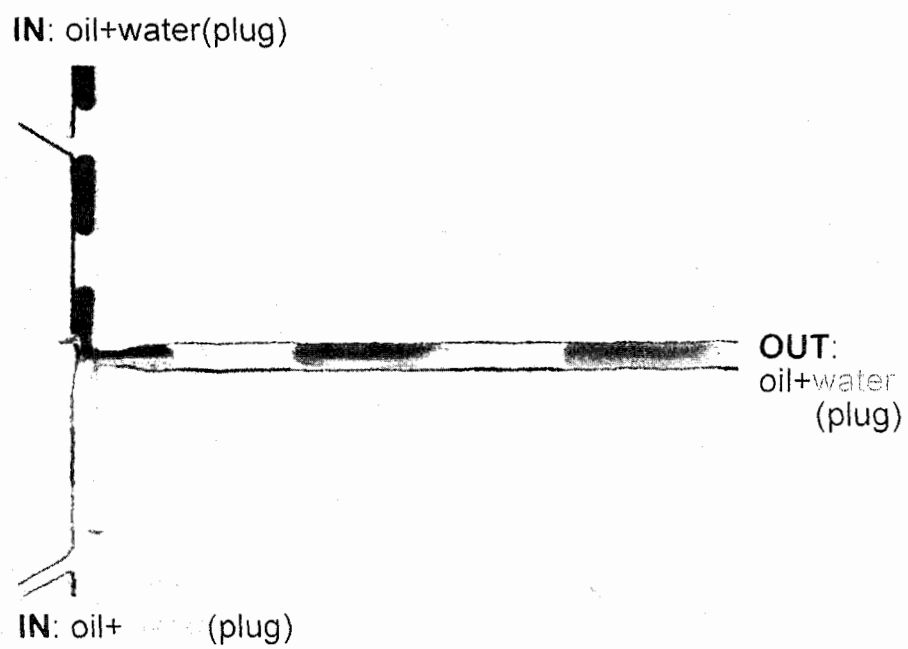


Fig. 4

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Aqueous plugs can be split

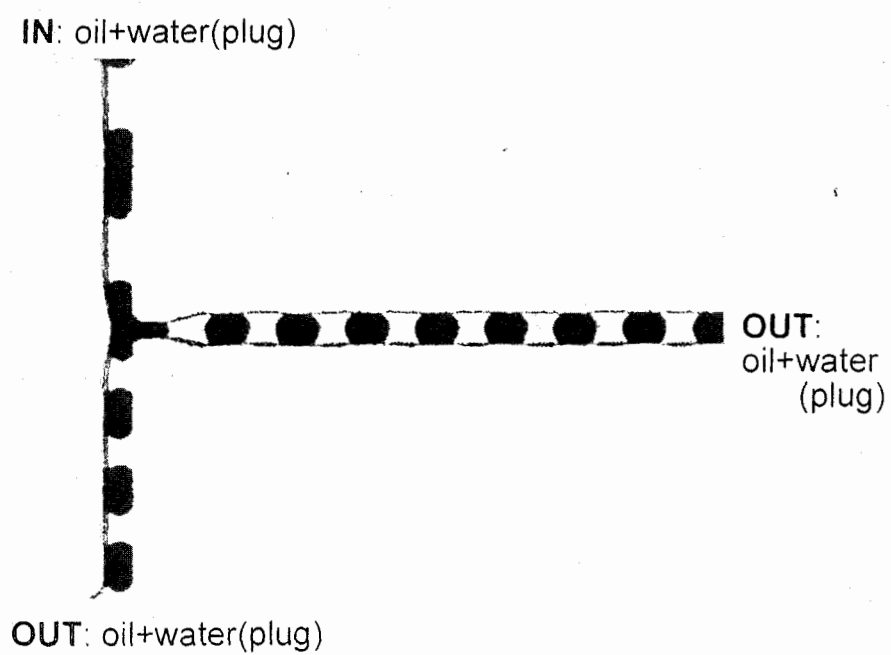
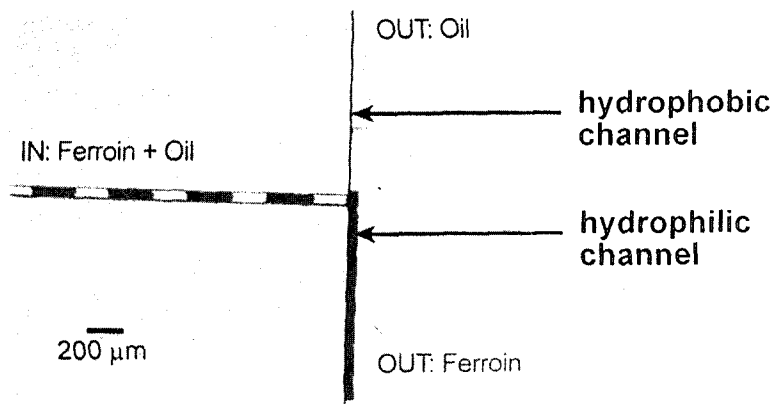


Fig. 5

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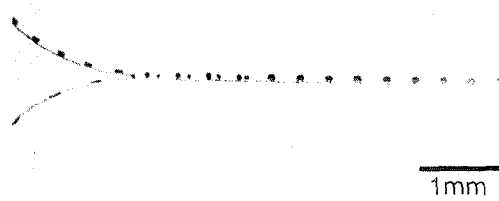


Aqueous plugs prefer to enter hydrophilic channels.

Fig. 6

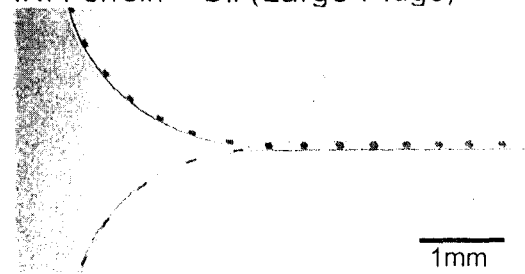
7/13

IN: Ferroin + Oil (Large Plugs)



IN: Ferroin + Oil (Small Plugs)

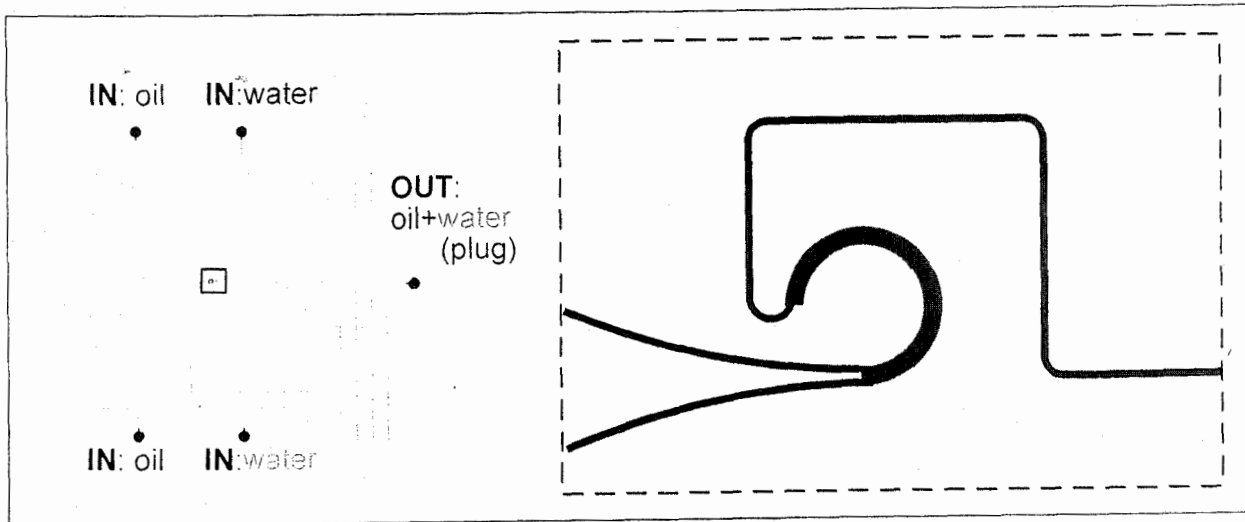
IN: Ferroin + Oil (Large Plugs)



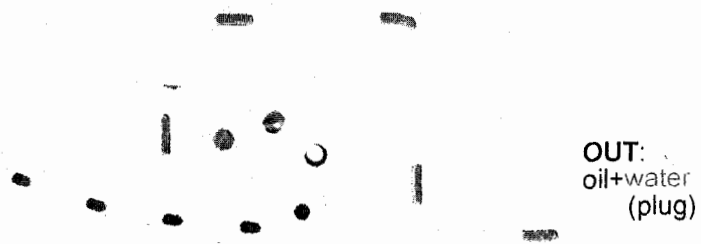
IN: Ferroin + Oil (Small Plugs)

Fig. 7

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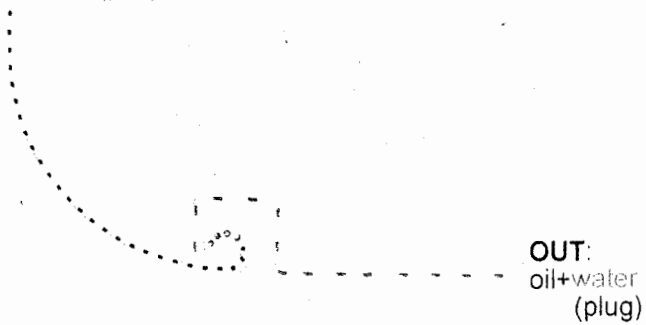


IN: oil+water(plug)



IN: oil+ (plug)

IN: oil+water(plug)



IN: oil+ (plug)

Fig.8

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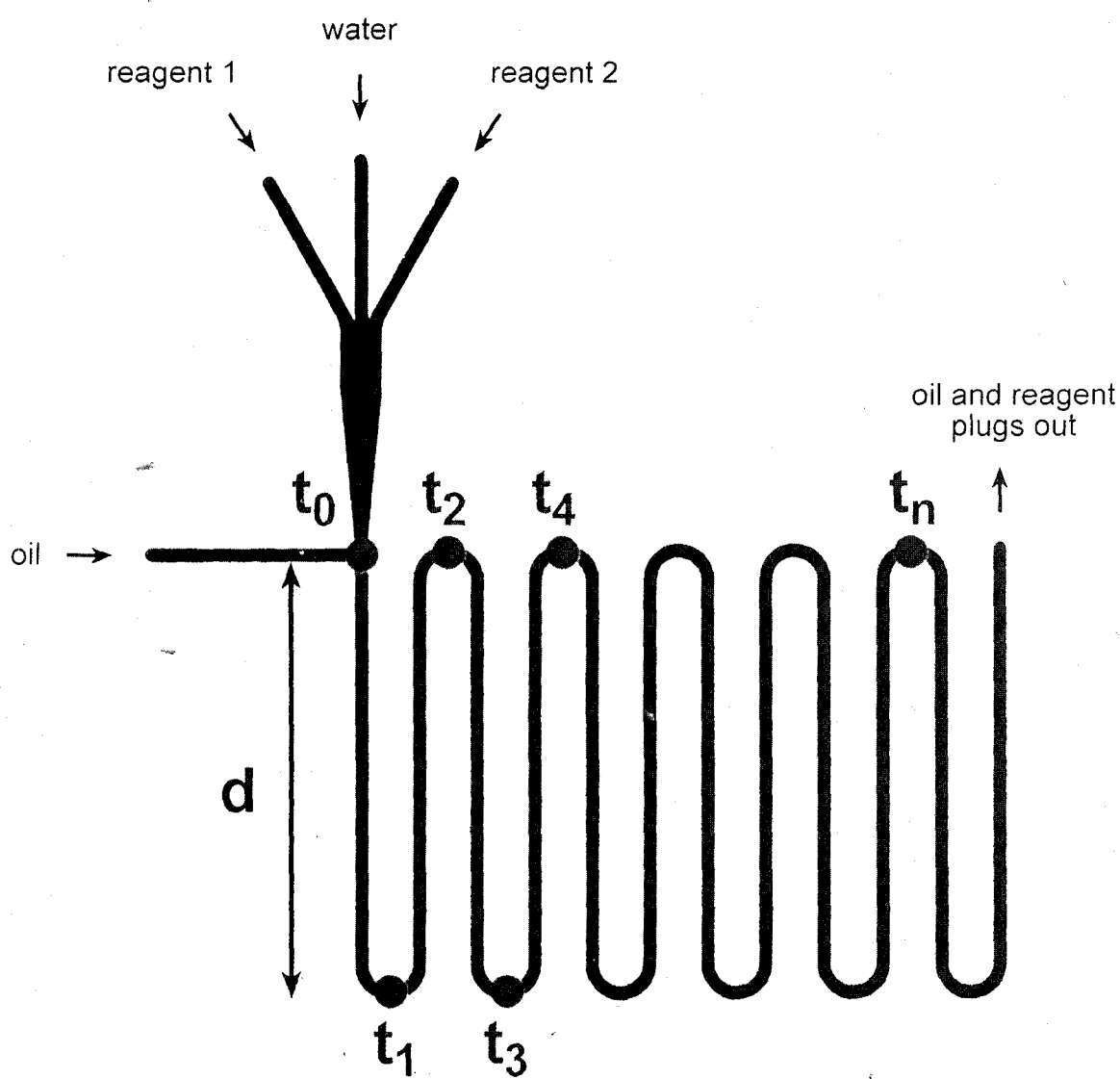


Fig. 9

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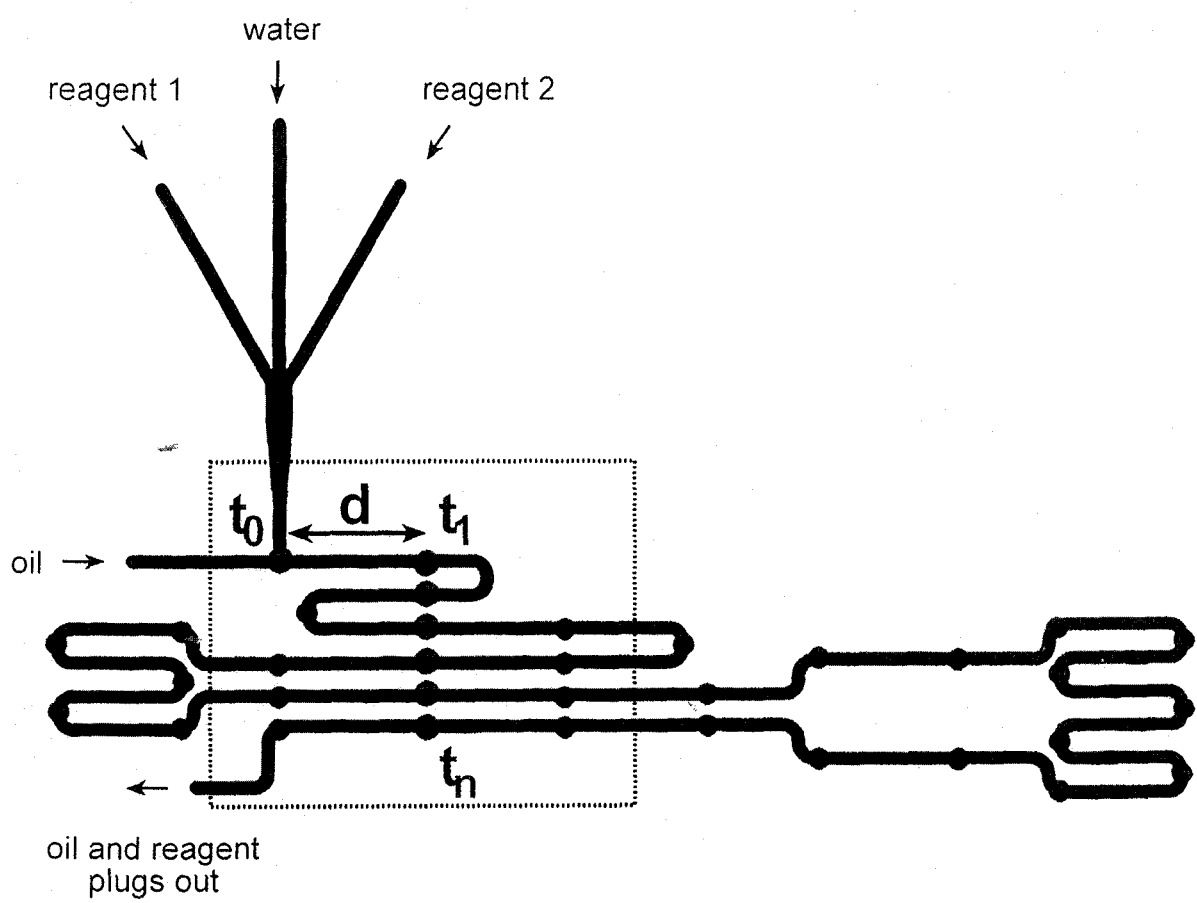


Fig. 10

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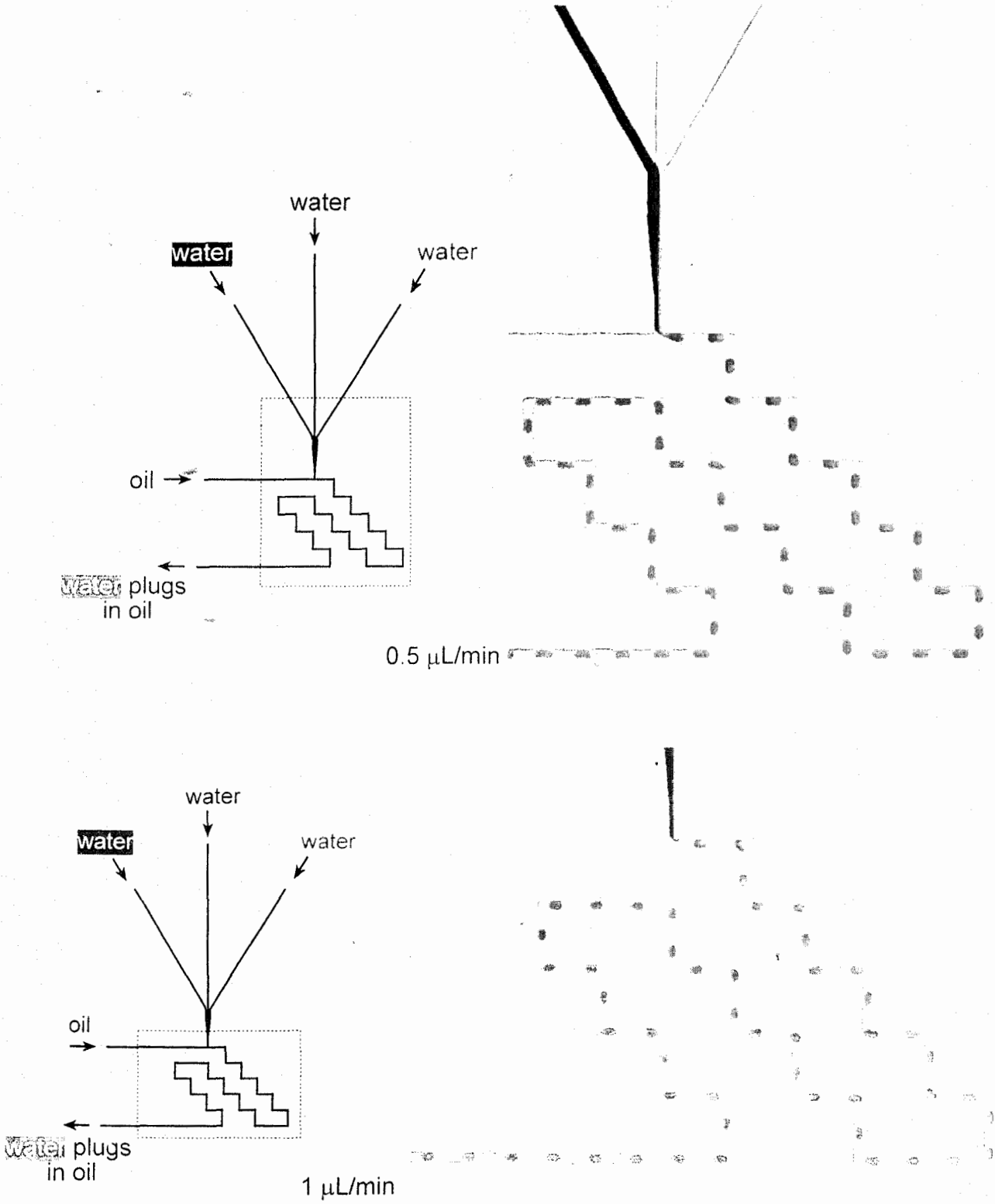
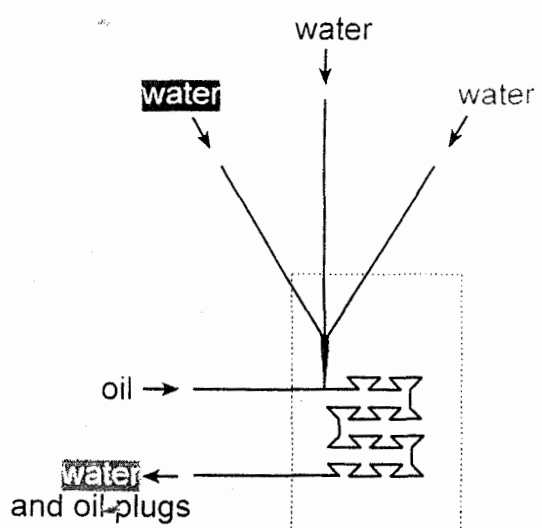
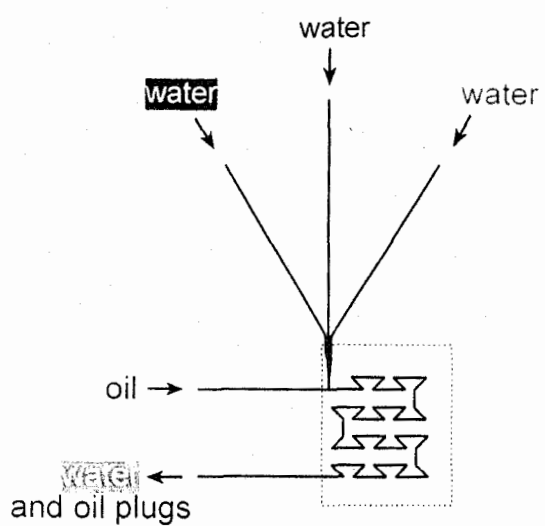
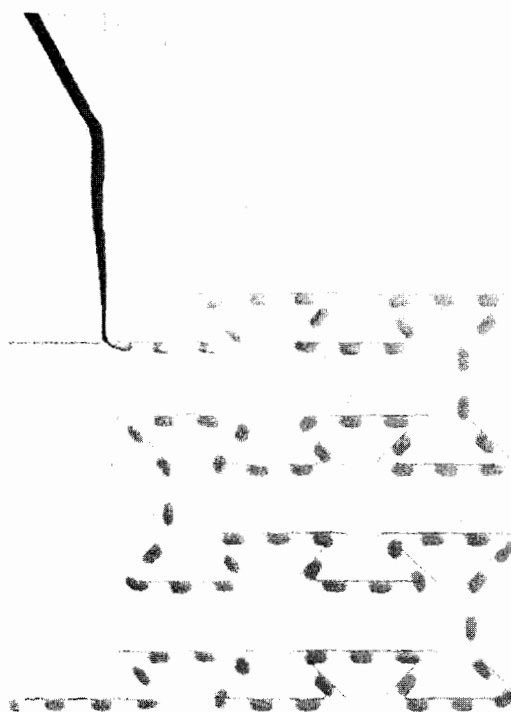


Fig. 11

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1.0 µL/min



0.5 µL/min

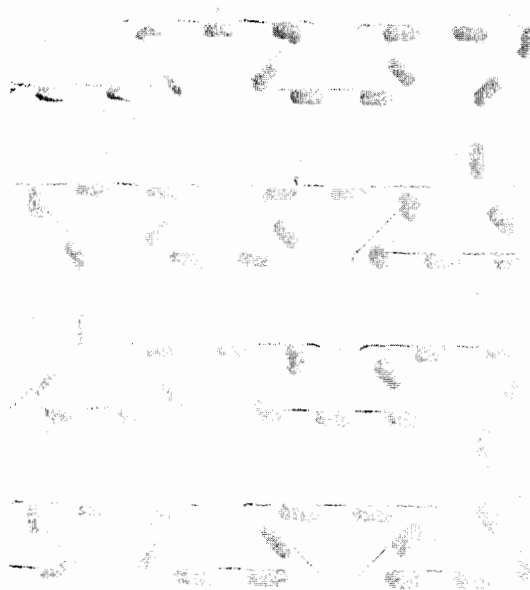


Fig. 12

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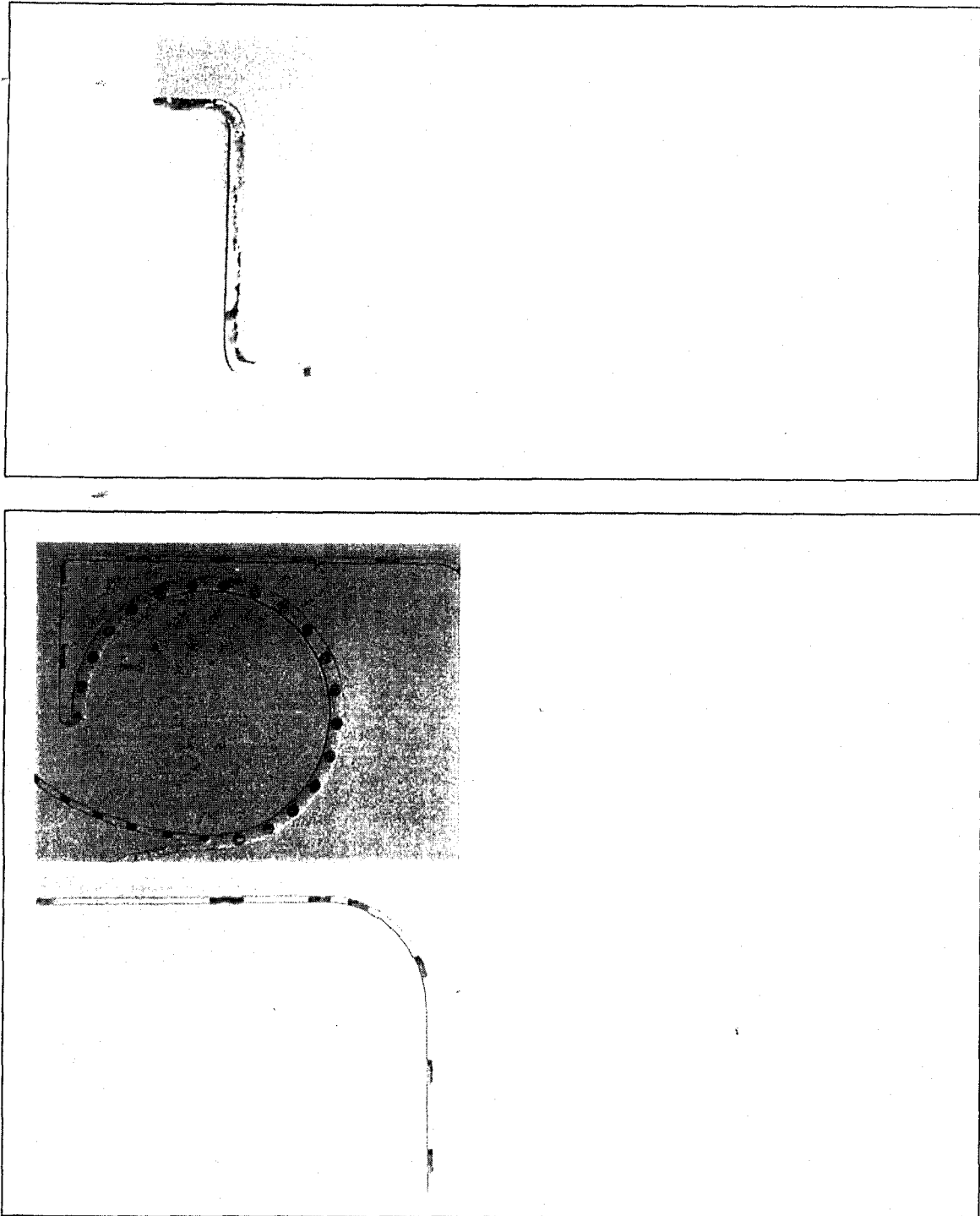


Fig. 13

PTO/SB/68 (02-10)

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

(10) **Patent No.:** **US 8,622,987 B2**
(45) **Date of Patent:** **Jan. 7, 2014**

(54) **CHEMISTRODE, A PLUG-BASED MICROFLUIDIC DEVICE AND METHOD FOR STIMULATION AND SAMPLING WITH HIGH TEMPORAL, SPATIAL, AND CHEMICAL RESOLUTION**

(58) **Field of Classification Search**
USPC 604/500; 600/573
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,853,336 A 8/1989 Saros et al.
4,963,498 A 10/1990 Hillman et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CN 2482070 3/2002
EP 0 816 837 1/1998

(Continued)

OTHER PUBLICATIONS

Tanaka, H. et al., "Ethanol Production from Starch by a Coimmobilized Mixed Culture System of *Aspergillus awamori* and *Zymomonas mobilis*", *Biotechnology and Bioengineering*, vol. XXVII, 1986, pp. 1761-1768.

(Continued)

Primary Examiner — Max Hindenburg

Assistant Examiner — Renee Danega

(74) *Attorney, Agent, or Firm* — Brinks Gilson & Lione

(57)

ABSTRACT

A method for sampling and/or introducing a matter to an environment comprises introducing a first array of plugs through a first microchannel of a device into an exchange region of the device in which mass transport between the environment and the plug fluid of at least one plug in the first array of plugs occurs and a second array of plugs is formed. The exchange region is in fluid communication with the first microchannel. The method further comprises directing the second array of plugs into a second microchannel downstream of and in fluid communication with the exchange region.

23 Claims, 7 Drawing Sheets

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(73) **Assignee:** **The University of Chicago**, Chicago, IL (US)

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 221 days.

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(22) **PCT Filed:** **Jun. 4, 2009**

(86) **PCT No.:** **PCT/US2009/046255**

§ 371 (c)(1),
(2), (4) **Date:** **Dec. 22, 2010**

(87) **PCT Pub. No.:** **WO2009/149257**

PCT Pub. Date: **Dec. 10, 2009**

(65) **Prior Publication Data**

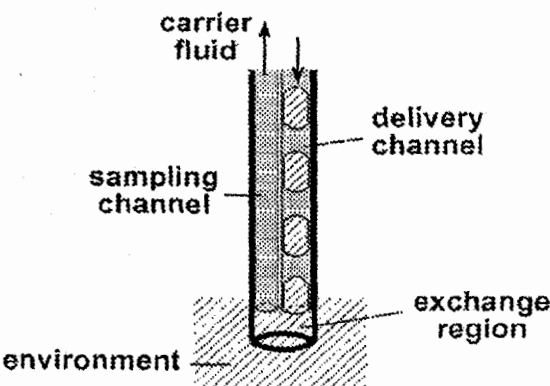
US 2011/0112503 A1 May 12, 2011

Related U.S. Application Data

(60) Provisional application No. 61/130,930, filed on Jun. 4, 2008.

(51) **Int. Cl.**
A61M 31/00 (2006.01)

(52) **U.S. Cl.**
USPC 604/500; 600/573; 436/34



US 8,622,987 B2

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**CHEMISTRODE, A PLUG-BASED
MICROFLUIDIC DEVICE AND METHOD
FOR STIMULATION AND SAMPLING WITH
HIGH TEMPORAL, SPATIAL, AND
CHEMICAL RESOLUTION**

This application is the national phase application of PCT Application No. PCT/US2009/046255, filed Jun. 4, 2009, which claims priority to U.S. Provisional patent application Ser. No. 61/130,930, filed Jun. 4, 2008, the entireties of both of which are hereby incorporated by reference.

The following are hereby incorporated by reference in their entireties for descriptions of uses, applications, and techniques for plug-based methods of analysis, manipulation of plugs, fluid handling, sorting of plugs, device materials, compositions, manufacturing techniques and the use and formation of holding components and loading components:

U.S. Pat. No. 7,129,091 to Rustem F. Ismagilov et al.;
U.S. Provisional Patent Application Ser. No. 60/881,012 to Rustem F. Ismagilov, entitled "Device and Method for Crystallization", filed Jan. 17, 2007;

U.S. Provisional Patent Application Ser. No. 60/875,856 to Delai Chen et al., entitled "Using Three-Phase Flow of Immiscible Liquids to Prevent Coalescence of Droplets in Microfluidic Channels: Criteria to Identify the Third Liquid and Validation with Protein Crystallization", filed Dec. 19, 2006;

U.S. Provisional Patent Application Ser. No. 60/763,574 to Rustem F. Ismagilov et al., entitled "Method and Apparatus for Assaying Blood Clotting", filed Jan. 31, 2006;

U.S. patent application Ser. No. 11/174,298 to Rustem F. Ismagilov et al., entitled "Microfluidic System", filed Jul. 1, 2005;

U.S. patent application Ser. No. 11/082,187 to Rustem F. Ismagilov et al., entitled "Microfluidic System", filed Mar. 16, 2005;

U.S. Provisional Patent Application Ser. No. 60/623,261 to Rustem F. Ismagilov et al., entitled "Microfluidic Devices", filed Oct. 29, 2004;

U.S. Provisional Patent Application Ser. No. 60/585,801 to Rustem F. Ismagilov, entitled "Applications of a Microfluidic System", filed Jul. 2, 2004;

U.S. patent application Ser. No. 10/765,718 to Rustem F. Ismagilov et al., entitled "Device and Method for Pressure-Driven Plug Transport and Reaction", filed Jan. 26, 2004;

U.S. patent application Ser. No. 11/589,700 to Rustem F. Ismagilov et al., entitled "Device and Method for Pressure-Driven Plug Transport and Reaction", filed Oct. 30, 2006;

U.S. Provisional Patent Application Ser. No. 60/394,544 to Rustem F. Ismagilov, entitled "Device and method for pressure-driven plug transport", filed Jul. 8, 2002;

U.S. Provisional Patent Application Ser. No. 60/379,927 to Rustem F. Ismagilov, entitled "Device and Method for Pressure-Driven Plug Transport", filed May 9, 2002;

U.S. Provisional Patent Application Ser. No. 61/080,541 to Timothy R. Kline et al., entitled "Microfluidic Blood Typing And Agglutination Assays", filed Jul. 14, 2008;

International Patent Application Publications WO09015296, WO09037361, WO08079274, WO08063227, WO07133710, WO07081387, WO07081386, WO07081385, WO09048673, WO08021123, WO09061372, WO09050512, WO09029229, WO09011808, WO07030501, WO08130623, WO09005680, and WO09015390; and

U.S. patent application Ser. Nos. 12/127,328, 12/172,186, 11/763,421, 12/058,628, 11/698,298, 11/360,845, 10/963,044, 11/643,151, and 11/633,849.

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This invention was made with government support under grant number 0526693 awarded by the National Science Foundation (NSF), grant number EB001903 awarded by the National Institutes of Health, and grant number OD003584 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

The present disclosure relates to microfluidic devices and methods. More particularly, the present disclosure relates to chemistries, plug-based microfluidic devices and methods for stimulation and sampling with high temporal, spatial, and chemical resolution.

BACKGROUND

Microelectrodes have revolutionized the understanding of the spatiotemporal dynamics of systems that generate or respond to electrical signals by enabling localized electrical stimulation and recording. However, such detailed understanding of systems that rely on chemical signals is substantially unavailable.

BRIEF SUMMARY

In one aspect, a method for sampling and/or introducing a matter to an environment comprises introducing a first array of plugs through a first microchannel of a device into an exchange region of the device in which mass exchange between the environment and the plug fluid of at least one plug in the first array of plugs occurs and a second array of plugs is formed. Without wishing to be bound by theory, it is thought that, typically, plugs form at low values of the capillary number. In certain embodiments, as carrier fluid and plug fluid enter into the second channel, the second array of plugs is formed due to the surface tension and immiscibility of the two fluids. The exchange region is in fluid communication with the first microchannel. The method further comprises directing the second array of plugs into a second microchannel downstream of and in fluid communication with the exchange region.

In another aspect, a method for sampling and/or introducing a matter to an environment comprises introducing a first array of plugs through a first microchannel of a device into an exchange region of the device in which mass transport between the environment and at least one plug in the first array of plugs occurs and a second array of plugs forms. The exchange region is in fluid communication with the first microchannel. The method further comprises storing and/or analyzing the contents of at least one plug in the second array of plugs after it passes the exchange region.

In yet another aspect, a method for sampling an aqueous environment comprises introducing a fluid through a first microchannel of a device, wherein the fluid is immiscible with water, into an exchange region of the device in which mass transport between the aqueous environment and the fluid occurs and an array of plugs is formed in the exchange region. The exchange region is in fluid communication with the first microchannel. The method further comprises directing the array of plugs into a second microchannel downstream of and in fluid communication with the exchange region; and storing, analyzing or both storing and analyzing the contents of at least one of the plugs.

In still another aspect, a method for introducing a matter to an environment comprises introducing an array of plugs comprising a carrier fluid and at least one plug, wherein the at least