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DEVICE AND METHOD FOR PRESSURE - DRIVEN PLUG TRANSPORT							
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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.

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Respectfully submitted,

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(if appropriate)



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

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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 systems (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, so 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. EOF-driven flows have flat, plug-like velocity profile, that is, the velocity of the fluid is the same near the walls and in the middle of the channel. 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 an issue when using channels with negative surface charge for DNA analysis and manipulation because DNA 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 undesirable 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 pressure-driven flows is that they have parabolic flow profile instead of the flat profile of EOF. Solutes in the middle of the

channel move 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 solute transport via diffusion from the middle of the channel towards the walls and back, but 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. Thus, samples are often introduced into the channels individually, separated by buffer washes. On the other hand, 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 a proper adjustment of the channel lengths and flow rates. Dispersion in pressure-driven flow creates a broad range of residence times for a plug traveling in such flows, and this diminishes 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 large diameter and at high flow rates. 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, *e.g.*, as a diagnostic instrument. So far, microfluidic devices have not been able to compete with stop-flow type instruments because EOF is usually very slow (although with less dispersion) while pressure-driven flows suffer from dispersion.

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.

Particulates are often difficult to handle in microfluidic systems. While suspensions of cells in aqueous buffers can be relatively easy to handle because cells are isodense with these buffers, particulates that are not isodense with the fluid are difficult to handle because they tend to settle at the bottom of the channel and eventually block the channel. Therefore, samples for analysis often require filtration to remove particulates.

#### BRIEF SUMMARY OF THE INVENTION

The 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 of the fluids in the microfabricated substrate 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 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 substrate 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 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 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 substrate 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 substrate 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 substrate 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 substrate 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 substrate comprising one or more microfabricated substrates comprising: a first microchannel, at least one carrier fluid 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 THE DRAWINGS AND PHOTOGRAPHS

FIG. 1a is a schematic diagram contrasting laminar flow transport and laminar plug (LAMP) transport.

FIG. 1b 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 flow 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. 3a shows a schematic diagram (figure at the upper left corner) and photographs (images to the left side of the schematic diagram) that shows the extent of mixing through light-induced fluorescence of the  $\text{Ca}^{+2}$ /Fluo-3 complex.

FIG. 3b shows schematic diagrams, photographs, and plots that illustrate the extent of mixing of  $\text{Ca}^{+2}$  and Fluo-4.

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

FIG. 5 shows a schematic representation showing various points along a channel that correspond to different reaction or mixing times.

FIG. 6 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. 7 shows schematic diagrams (upper and lower figures on the left side) and photographs (images on the right side of the 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. 8 shows schematic diagrams (upper and lower figures 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. 9 shows photographs of particulates clinging to the surface of a microchannel and particulates encapsulated in and transported via plugs.

FIG. 10 shows photographs that illustrate the merging of large and small plugs.

FIG. 11 shows a schematic diagram and photograph that illustrate a scheme for merging plugs using an arched or semi-circular channel.

FIG. 12 shows photographs that illustrate the splitting of plugs and a time-controlled merging of plugs.

FIG. 13 shows a photograph that demonstrates the separation of aqueous and oil plugs into the divergent hydrophilic and hydrophobic channels.

FIGS. 14a-c are top views of channels that show the formation of uniform plugs using multiple aqueous streams and comparison of LAMP transport with laminar flow.

FIGS. 15a-d are top views of channels that show spontaneous merging of pairs of plugs into single plugs.

FIGS. 16a-c are top views of channels that show the spontaneous splitting of plugs at a branching point in a microchannel.

FIGS. 17a-c are top views of channels that show the spontaneous splitting of plugs at a branching point in a microchannel in the absence of a constriction.

## DETAILED DESCRIPTION OF THE INVENTION

### A. Definitions

The term “microfabricated substrate” or “substrate” refers to a layer or piece of material from which microfabricated devices or chips are prepared or manufactured. The term “microfabricated substrate” or “substrate” also refers either to an entire microfabricated device or chip or to a portion, area, or section of a



microfabricated device or chip which may or may not be removable or detachable from the main body of the device or chip. The substrate may be prepared from one or more materials such as glass, silicon elastomer, and polymers including, but not limited to, polypropylene or polyethylene.

5           The term “microfabricated device” refers to a device fabricated or manufactured using techniques such as wet or dry etching and/or conventional lithographic techniques or a micromachining technology such as soft lithography. A microfabricated device of the invention may have dimensions between about 0.3 cm to about 7 cm per side and between about 1 micron to about 1 cm thick, but the  
10           dimensions of the device may also lie outside these ranges.

          The term “carrier fluid” refers to a fluid that is immiscible with a reagent fluid. The carrier fluid may comprise a substance having both polar and non-polar groups or moieties.

          The term “reagent fluid” refers to a fluid wherein a reaction can occur.  
15           Typically, the reagent fluid contains a solvent and a reagent although in some embodiments at least one reagent fluid may not contain a reagent. The reagent may be soluble or insoluble in the solvent. The reagent fluid may contain a surfactant. At least two different reagent fluids are used in the present invention. When both reagent fluids contain reagents, the fluids are typically miscible, but can also be partially  
20           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 reagent fluids or a reagent-containing  
25           carrier fluid in the microfabricated substrate 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 wave, or a catalyst induces a reaction to give rise to a transformation of the reagent to another reagent,  
30           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 reagent fluid containing one or more reagents and/or one or more products formed from a reaction of the reagents, wherein the aqueous reagent fluid is surrounded by a non-polar or hydrophobic fluid such as an oil. The plugs may also be in the form of droplets comprising mainly a non-polar or hydrophobic fluid which is surrounded by an aqueous fluid. 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 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 that collects or dispenses the reagent fluid, carrier fluid, plugs or reaction product. A microfabricated substrate 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 substrate, or component of a substrate of the invention, or in a substrate or component of a substrate involving a method of the invention, *e.g.*, through channels of a microfluidic substrate 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.

The term “downstream” refers to a position relative to an initial position which is reached after the fluid flows past the initial point. In a circulating flow device, downstream refers to a position farther along the flow path of the fluid before it crosses the initial point again. “Upstream” refers to a point in the flow path of a fluid that the fluid reaches or passes before it reaches or passes a given initial point in a substrate or device.

An “inlet port” is an area of a microfabricated substrate that receives reagent fluids. The inlet port may contain an inlet channel, a well or reservoir, an opening, and other features that facilitate the entry of molecules, particles, or substances into the substrate. A microfabricated substrate may contain more than one inlet port if desired. The inlet port can be in fluid communication with a microchannel or separated from the microchannel by a valve.

The term “polymer” means any substance or compound that is composed of two or more building blocks (‘mers’) that are repetitively linked to each other. For example, a “dimer” is a compound in which two building blocks have been joined together. Polymers include both condensation and addition polymers. Typical examples of condensation polymers include polyamide, polyester, protein, wool, silk, polyurethane, cellulose, and polysiloxane. Examples of addition polymers are polyethylene, polyisobutylene, polyacrylonitrile, poly(vinyl chloride), and polystyrene. Other examples include polymers having enhanced electrical or optical properties (*e.g.*, a nonlinear optical property) such as electroconductive or photorefractive polymers.

The term “particles” means any discrete form or unit of matter. The term “particle” or “particles” includes atoms, molecules, ions, dimers, polymers, or biomolecules.

The term “particulate” refers to a cluster or agglomeration of particles such as atoms, molecules, ions, dimers, polymers, or biomolecules. Particulates may comprise solid matter or be substantially solid, but they may also be porous or partially hollow. They may contain a liquid or gas. In addition, particulates may be homogeneous or heterogeneous, that is, they may comprise one or more substances or materials.

The term “nanoparticles” refers to atomic, molecular or macromolecular particles typically in the length scale of approximately 1 - 100 nanometer range. Typically, the novel and differentiating properties and functions of nanoparticles are observed or developed at a critical length scale of matter typically under 100 nm. Nanoparticles may be used in constructing nanoscale structures and they may be integrated into larger material components, systems and architectures. In some 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 substrate 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 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 reaction of the first and second reagent fluids may form an insoluble reaction product within at least one plug. In addition, a reaction of the first and second reagent fluids may form a soluble reaction product within at least one plug. Monitoring may be performed by measuring an optical property of the reaction.

The method may additionally comprise controlling the flow velocity of a plug by adjusting reagent viscosity, plug size, plug shape, or microchannel geometry, or increasing the mixing rate of the reagent fluids in the plugs by adjusting the microchannel geometry. In a preferred embodiment, the method of the invention comprises separating the reagent fluids from the carrier fluid.

In one preferred embodiment, plugs conform to the size and shape of the channels while maintaining their respective volumes. Thus, as plugs move from a wider channel to a narrower channel they become longer and thinner, and vice versa. In a preferred embodiment, the plugs are at least about four times as long as they are wide. This plug configuration, which can be envisioned as a lozenge shape, flows smoothly and well through the channels. Longer plugs, produced in narrower

channels, provides greaterer shear, meaning the plugs can more easily be sheared or broken off from a flow, *i.e.*, with less force. Plugs may also tend to adhere to channel surfaces, which can slow or block the flow, or produce turbulence. Plug adherence is overcome when the plug is massive enough in relation to the channel size to break free. Thus, plugs of varying size, if present, may combine to form uniform plugs having a so-called critical mass or volume that results in smooth or laminar plug flow. In an exemplary embodiment with about 50 micron channels, a typical free flowing plug is about 50 microns wide and between about 50-200 microns long. Plug dimensions and flow characteristics can be influenced as desired, in part by changing the channel shape or dimensions such as the channel width.

More preferably, however, the microfabricated substrates of this invention generate round, monodisperse plugs. Monodisperse plugs may be particularly preferred such as in high throughput devices and other embodiments where it is desirable to generate plugs at high frequency.

The reagent fluids may be introduced through one or more inlets. Specifically, fluids may be introduced into the microfabricated substrate through pneumatically driven syringe reservoirs that contain either the reagent fluid or carrier fluid. In each instance, the plugs may be produced in the carrier fluid stream by modifying the relative pressures such that the reagent fluids enter the plug-forming regions, shearing off into discrete plugs.

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.

Suitable solvents for use in the invention, such as for solvents in reagent fluids, include organic solvents, aqueous solvents, oils, or mixtures of the same or different types of solvents, *e.g.* methanol and ethanol, or methanol and water. The solvents of the invention include polar and non-polar solvents, including those of intermediate polarity relative to polar and non-polar solvents. Preferably, the solvent is aqueous.

In a preferred embodiment, the solvent may be 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 or acetate buffer. Other solvents that are compatible with the reagents may also be used.

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

The carrier fluid, reagent fluid, or solvent 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. In one embodiment of the invention, at least one of the reagent fluids further comprises at least one surfactant.

Preferred surfactants that may be used include, but are not limited to, surfactants such as sorbitan-based carboxylic acid esters (*e.g.*, the “Span” surfactants, Fluka Chemika), including sorbitan monolaurate (Span20), sorbitan monopalmitate (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 polyglycerol 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 certain 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.

When the reaction mixture is being monitored by optical measurements, preferably, the refractive index of the carrier fluid and the reagent fluids are substantially similar.

FIG. 1a is a schematic diagram contrasting laminar flow transport and laminar plug (LAMP) transport in a microchannel. In the lower figure which depicts LAMP transport, two aqueous reagents (marked in red and blue) form laminar streams which are separated by a “divider” aqueous stream. The three streams enter a microchannel with flowing oil, at which point droplets (plugs) form and reagents come in contact. In a LAMP transport, rapid mixing of the reagents occurs within the plugs. In contrast, in a laminar flow transport, reagent mixing occurs slowly, and with high dispersion, as shown in the upper figure.

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

The middle or second aqueous reagent fluid in the top figure may be plain water, buffer, solvent, or a reagent fluid. The middle aqueous reagent fluid would preferably initially separate the two other aqueous reagent fluids before the aqueous fluids come into contact with the carrier fluid. Thus, the intervening aqueous reagent fluid would prevent, delay, or minimize the reaction or mixing of the two outer aqueous reagent fluids before they come in contact with the carrier fluid. The water/oil plugs that form in the plug-forming region would then exit a channel, area, or region of the microfabricated substrate to enter a different channel, area, or region of the microfabricated substrate or the plugs may exit the microfabricated through an exit port. It can be seen in FIG. 1 that, in the absence of an oil, the aqueous reagent

fluids flow in laminar streams without significant mixing or with only partial mixing. In contrast, the reagents from the separate aqueous reagent fluids mix substantially or completely in the water/oil plugs.

FIG. 2 shows photographs and schematic diagrams that depict a stream of plugs from an aqueous reagent fluid and an oil (carrier fluid) 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 carrier fluid is introduced into an inlet port of a microfabricated substrate while the three aqueous reagent fluids are introduced in a separate inlet port. As in FIG. 1, a preferred scheme would be one in which the aqueous reagent fluids would initially coflow preferably along a short or minimal distance before coming in contact with the carrier fluid. In a preferred embodiment, the distance traversed by the one or more coflowing aqueous reagent fluids is approximately or substantially equal to the width of the channel. The middle or second aqueous reagent fluid may comprise plain water, buffer, solvent, or a reagent fluid, and the middle aqueous reagent fluid preferably initially separates the two other aqueous reagent fluids before the aqueous reagent fluids come into contact with the carrier fluid which, in this case, is an oil. Thus, the intervening aqueous reagent fluid would prevent, delay, or minimize the reaction or mixing of the two outer aqueous reagent fluids before they come in contact with the oil (or carrier fluid).

FIG. 3a shows photographs and schematic diagram that show the extent of mixing through light-induced fluorescence of the  $\text{Ca}^{+2}$ /Fluo-3 complex along a sinuous channel. Fluo-3 is a long wavelength probe which is practically non-fluorescent by itself. Upon forming a complex with  $\text{Ca}^{+2}$ , 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  $\text{Ca}^{+2}$  and Fluo-3 along the entire length of the channel, as evidenced by the fluorescence arising from the complex formed from the binding of  $\text{Ca}^{+2}$  with Fluo-3.



FIG. 3b shows photographs and plots that illustrate the mixing process in a microchannel. Reactions were conducted to quantify the extent of mixing in a channel. Chemical reactions were performed using the laminar plug transport (LAMP) system. Fig. 3b shows a top view of a microchannel having a curvilinear portion through which rapid mixing inside plugs occurs. The curvilinear portion of the microchannel network had a diameter of about 29  $\mu\text{m}$  while the straight portions had a diameter of 50  $\mu\text{m}$ . The period (measured from one crest to the next or from one trough to the next) of the curvilinear portion of the microchannel network was about 146  $\mu\text{m}$ .

In the top photograph in Fig. 3b, a solution of deep red  $\text{Fe}(\text{SCN})_x^{(3-x)}$  complexes ( $x \sim 3$ ,  $\lambda_{\text{max}} \sim 480 \text{ nm}$ ,  $\epsilon \sim 5 \times 10^3 \text{ cm}^{-1} \text{ M}^{-1}$ ), which was prepared by mixing 0.067 M  $\text{Fe}(\text{NO}_3)_3$  with 0.2 M KSCN, was used to dye one of the aqueous streams.  $\text{KNO}_3$  (0.2M) was also added in the aqueous streams. The photograph was taken using an exposure time of 5 microseconds. As shown in the top photograph, in the first two and a half periods or so along the curvilinear channel, the plugs exhibited streaks of white and red which demonstrate incomplete mixing within the plugs. Plugs farther away showed a uniform red color which indicates a more or less complete mixing within the plugs.

The photographs in the middle shows quantitative mixing as can be seen from the fluorescence intensity due to the binding ( $k = 7.1 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ ) of  $\text{Ca}^{2+}$  ions to a calcium-sensitive dye, fluo-4, which is non-fluorescent in the absence of calcium. An 80 mM solution of  $\text{CaCl}_2$ , 54 mM solution of Fluo-4, and 20mM MOPS buffer (3-(N-morpholino)propanesulfonic acid) were used in the experiment. The exposure time used in this case was 2 seconds.

The two plots shown in Fig. 3b show the relative intensity of the  $\text{Ca}^{2+}$ /Fluo-4 complex as a function of distance ( $\mu\text{m}$ ) and time (millisecond, ms). The ratios in the relative intensity versus distance plot correspond to the total aqueous solution flow rate relative to the oil flow rate, while the quantities following the ratios (e.g., 33 mm/s) correspond to the total flow rate of the aqueous and oil streams. The curves in the plot of relative intensity versus distance show that, regardless of the flow rate, the relative intensity reaches a maximum and levels off at around 550  $\mu\text{m}$  from the point of mixing. This indicates a more or less complete mixing at this point. The weak dependence of the mixing distances on the flow rate may be attributed to chaotic

mixing of the fluid which occurs as the fluid is transported inside a plug through the curvilinear portion of the microchannel.

On the other hand, the relative intensity-versus-time plot reveals that the curves corresponding to the two slowest flow rates (33 mm/s and 69 mm/s) exhibit a linear behavior over the time range shown and do not reach more than 80% relative intensity even after 4 ms. In contrast, the three other curves with faster flow rates attain more than 80% relative intensity at around 2.5 ms. These results indicate that faster flow rates give rise to faster mixing inside the plugs. Thus, based on the plots shown in FIG. 3b, the time required for mixing can be reduced simply by using faster flow rates.

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

FIG. 5 shows a schematic representation of an image showing various points along a channel that correspond to different reaction or mixing times while FIG. 6 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. In FIG. 5, given a constant fluid flow rate  $u$ , one may determine a reaction time corresponding to the various times  $t_1, t_2, t_3, \dots, t_n$  along the channel. Thus, if the distance between

each pair of points  $n$  and  $n-1$ , which correspond to time  $t_n$  and  $t_{n-1}$ , are the same for a given value of  $n$ , then the reaction time corresponding to point  $n$  along the channel may be calculated from  $t_n = nd/u$ . Thus, the set-up shown in FIG. 5 allows one to conveniently and repeatedly monitor a reaction at any given time  $t_n$ . In principle, the scheme illustrated in FIG. 5 allows one to cover a greater time period for monitoring a reaction by simply extending the length of the channel that is to be monitored at a given flow rate or by decreasing the flow rate over a given channel distance. The same principle applies to the scheme in FIG. 6 except that the distance corresponding to a point  $n$  from a common point of origin along the channel differs from that corresponding to another channel by a power or multiples of 2.

Time control is important in many chemical and biochemical processes. Stop-flow type instruments are typically used to measure reaction kinetics. These type of instruments rely on turbulent flow to mix the reagents and transport them in a plug-like fashion. Because turbulent flow occurs in tubes with relatively large diameters and at high flow rates, stop-flow instruments tend to use large volumes of reagents (*e.g.*, on the order of ml/s). A microfluidic analog of a stop-flow instrument that consumes small volumes of reagents, *e.g.*, on the order of  $\mu\text{l}/\text{min}$ , would be useful in various applications such as diagnostics. Thus far, microfluidic devices have not been able to compete with stop-flow instruments because EOF is usually too slow (although it has less dispersion), and pressure-driven flows suffer from dispersion. In addition, mixing is usually very slow in both systems.

Stop-flow instruments have sub-millisecond mixing, and could be useful for experiments where such fast mixing is required. The present invention compares 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 millisecond (ms) time scale and where the primary concern is the solute volume required to perform a measurement.

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. 5 and 6. 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. 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.

FIG. 7 shows photographs and schematics that illustrate fast mixing at flow rates of 0.5  $\mu\text{l}/\text{min}$  and 1.0  $\mu\text{l}/\text{min}$  using 90°-step channels while FIG. 8 illustrates fast mixing at flow rates of 1.0  $\mu\text{l}/\text{min}$  and 0.5  $\mu\text{l}/\text{min}$  using 135°-step channels. As can be seen in FIG. 7 and FIG. 8, the plugs are 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. 9 shows photographs that depict particulates clinging to the surface of a microchannel and particulates 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 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. Particulates 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 particulates may be more effectively prevented from interfering with desired reactions in one or more channels of the microfabricated substrate 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 involving merging of plugs 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 substrate 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 substrate 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. 10 shows photographs that illustrate a scheme for merging large and small plugs. As shown in FIG. 10, 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 substrate where they may undergo one or more “treatments” such as one or more types of characterizations, measurements, detection, sorting, or analysis.

FIG. 11 shows a photograph and schematic diagram that illustrate 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. 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.

#### **D. Methods of splitting and/or sorting plugs**

The present invention also provides a method of conducting a reaction involving splitting of plugs 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 substrate 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 substrate 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 substrate 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. 12 shows photographs that illustrate schemes for splitting plugs and time-controlled merging of plugs. As depicted in FIG. 12, 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. 12 also shows a scheme for splitting plugs. As shown in FIG. 12, 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. 12, flow in separate channels or channel branches which may be perpendicular, as shown in FIG. 12, 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 substrate where they may undergo one or more reactions or "treatments" such as one or more types of characterizations, measurements, detection, sorting, or analysis.

FIG. 13 shows a photograph that demonstrates the separation of aqueous and oil 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 substrates 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 with dry nitrogen as a carrier gas to silanize the channel surface.

In the invention, 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, 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.

In preferred embodiments of the invention, the molecules, particles, or substances are detected, analyzed, characterized, or sorted dynamically in a flow stream of microscopic dimensions based on the detection or measurement of a characteristic, marker, property, or tag 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 substrate (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 tag 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.

Manipulation of individual molecules or particles can be accomplished in a fluid flow using methods or techniques such as 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 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 substrate or in a method of the invention.

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 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 microfabricated substrates of the invention. Accordingly the microfabricated substrates of the invention are preferably operated at a low or very low Reynold's number.

#### **E. Methods of detection**

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 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). Biological particles or molecules such as cells

and virions can be sorted according to 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 tag that produces a detectable signal when, for example, a 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 group, moiety, or compound (referred to here as “tag”) 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 tag at a selected threshold or within a selected range are diverted into a predetermined outlet or branch channel of the substrate. The tag 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 tag 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 tag or determine whether a molecule, particle, substance, or tag has a desired characteristic, the detection region may include an apparatus for stimulating a molecule, particle, substance, or tag 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 that contains the analysis units. Alternatively, laser diodes may be incorporated into a second substrate (*i.e.*, a laser diode chip) that is placed adjacent to the microfabricated analysis or sorter substrate 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.*, a corresponding signal produced by the plug, or the molecules, particles, or substances contained in the plugs, are detected and measured to determine whether or not a given characteristic or property is present. 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 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 characteristics may be used directly or by the use of a tag associated with a characteristic or property 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

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 molecule, particle, or substance 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).

The processor can be integrated into the same substrate that contains the analysis unit(s), or it can be separate, *e.g.*, an independent microchip connected to the analysis unit containing substrate 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.

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 substrate 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 at different levels of depth. Thus, substrates 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**

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 substrate 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.

5 In the inventive method, the reagent fluids may both comprise a reagent and solvent such that mixing of the reagent fluids results in the 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.

10 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  
15 a continuous flow such as a laminar flow of the fluids in the microfabricated substrate 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  
20 single channel downstream of where the first and second set of plugs form, wherein the flow such as the laminar flow within the microfabricated substrate forms merged plugs in which a reaction between the first and second reagent fluids can occur.

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

30 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 substrate, information regarding, for example, the reaction kinetics can be obtained. Such unstable intermediates can be further reacted with another reagent by merging plugs containing the unstable intermediate with the another reagent fluid.

All of the above synthesis methods of the present invention can be used to form quantities of reaction product by running multiple reactions in parallel.

Experiments involving complex chemical systems can also 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.

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 concentrations is of interest. If A is an enzyme, B a substrate, and C an inhibitor, a substrate 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 substrate 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 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 qualitative but also quantitative data can be obtained. The substrate need not be cleaned between runs since most, if not all, reagents are contained inside the plugs and leave no residue.

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 of reagents inside the plug requires changing the size of the plugs, which makes merging complicated.

5 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  
10 minutes using 1  $\mu$ l/minute runs instead of the usual ml/shot runs.

This particular example highlights the usefulness of the invention in, for example, the study of protein/protein interaction mediation by small molecules, protein/RNA/DNA interaction mediation by small molecules, or a protein/ several small molecule binding events. Other interactions involving several components at  
15 different concentrations may also be studied using the method of the invention.

#### G. Devices

The invention also provides a device comprising one or more microfabricated substrates comprising: a first microchannel, at least one carrier fluid reservoir in fluid  
20 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 continuous pressure to a fluid within the microchannel.

The device of the invention may comprise at least one microfabricated  
25 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  $10^\circ$ ,  $135^\circ$ ,  $180^\circ$ ,  $270^\circ$ , or  $360^\circ$ .

30 A device according to the invention comprises at least one inlet port in communication with a first channel at or near a plug-forming region, a detection region within or coincident with all or a portion of the first channel or plug-forming region, and a detector associated with the detection region. In certain embodiments

the device may have two or more plug-forming regions. For example, embodiments are provided in which the analysis unit has a first inlet port in communication with the first channel at a first plug-forming region, a second inlet port in communication with the first channel at a second plug-forming region (preferably downstream from the first plug-forming region), and so forth.

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.

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.

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. Substrates 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.

A substrate 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 to permit entry of the sample into the device. The inlet port may also 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.

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). A plurality of analysis units of the invention may be combined in one device. The plug-forming region generally comprises a junction between the sample inlet and the first 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 (*i.e.* at an angle of 90°) 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°. Particular exemplary angles are 45°, 60°, 90°, and 120°.

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 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.

A microfabricated substrate of the invention is preferably fabricated from materials such as glass, polymers, silicon microchip, or silicon elastomer. The dimensions of the substrate typically range between about 0.3 cm to about 7 cm per side and about 1 micron to about 1 cm in thickness.

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.

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 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  $\mu\text{m}$ , that tapers to 30  $\mu\text{m}$  at or near the plug-forming regions. The inlet channels also preferably have a diameter of about 30  $\mu\text{m}$  and, in embodiments where plug mixing is

preferred, are separated by a distance along the first channel equal to approximately the diameter of the inlet channel (*i.e.*, about 30  $\mu\text{m}$ ).

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 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 preferably covered with a clear cover material to allow optical access to the molecules, particles, or substances. For example, anodic bonding to a PYREX<sup>®</sup> cover slip can be accomplished by washing both components in an aqueous  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$  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 may be 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, *e.g.*, used in conjunction with one or more devices or methods, so that plugs may be analyzed, characterized, monitored, and/or sorted as desired by a user.

## EXAMPLE

### Example 1

The extent of mixing over a given period in a sinusoidal microchannel was measured using two different oil/water volumetric ratios (see FIGS. 14a-b). An experiment was also performed to compare LAMP transport and laminar flow (see FIGS. 14a-c). Three separate water inlets were used to introduce water into the microchannels. The figures in the first column each shows a top view of the microchannel network. The photographs in the middle column show the formation and transport of plugs. The photographs in the third column are magnified views of the plug-forming region at different time intervals. All microchannels had 50 x 50  $\mu\text{m}^2$  cross-sections.

The total flow rates in the main channel were 7.5  $\mu\text{L}/\text{min}$  (50 mm/s average flow velocity) in the three experiments. The oil stream contained PFD which is a 10 : 1 mixture of perfluorodecaline and 1H,1H,2H,2H-Perfluorooctanol. Microfluidic networks in PDMS were fabricated using rapid prototyping and volumetric flow rates through each channel were controlled using syringe pumps. The pressures in the system were allowed to vary to accommodate changes in the volumetric flow rates.

FIG. 14a shows the formation of small plugs and their rapid mixing at high oil (6.0  $\mu\text{L}/\text{min}$ ) to water (0.5  $\mu\text{L}/\text{min}$ ) flow rate ratio. The corresponding magnified views over a period between time  $t=0$  ms to  $t=6$  ms and at time  $t=30$ ms show details of the plug formation process. As shown by the magnified views, the water stream marked in red formed a hook-like structure as it hit the oil stream. The end-portion of the hook was the plug in its embryonic form which grew in time to adopt an egg-shaped or ovoid structure. At around  $t=6$  ms, the budding plug attained a diameter roughly matching the microchannel internal diameter and began to break away from what in effect would be the budding plug's umbilical cord (*i.e.*, the thin stream of water connecting the plug to the parent water stream).

FIG. 14b shows the formation of medium-sized plugs and their slower mixing at intermediate oil (3.0  $\mu\text{L}/\text{min}$ ) to water (1.3  $\mu\text{L}/\text{min}$ ) ratio. The plug formation process was similar to that shown in FIG. 14a except that here the plug appeared to grow faster than in FIG. 14a (compare for example the period between  $t=4$  ms to  $t=6$  ms in FIG. 14a and the period between  $t=2$  ms to  $t=3$  ms in FIG. 14b). Considering

only the greater oil-to-water flow rate ratio used in the experiment depicted in FIG. 14a, this would seem surprising.

FIG. 14c shows that the mixing is slow in laminar streams in the absence of oil. Here, the water flow rates were 0.3  $\mu\text{L}/\text{min}$  and 2.4  $\mu\text{L}/\text{min}$ . The magnified view of microchannel sections at around time  $t=6$  ms shows that the water streams initially remained segregated. At around  $t=270$  ms, the water streams can be seen to have largely coalesced.

### Example 2

Experiments were conducted to investigate the merging of plugs using different channel junctions (T- or Y-shaped), cross-sections, and flow rates (see FIG. 15a-d). The figures on the left side of FIGS. 15a-d show top views of microfluidic networks that comprise channels having either uniform or nonuniform dimension (*e.g.*, the same or different channel diameters). The corresponding figures on the right are microphotographs that include a magnified view of two plug streams (from the two separate channels portions of which form the branches of the Y-shaped junction) that merges into a common microchannel.

In FIG. 15a, the oil-to-water volumetric ratio was 4:1 in each pair of oil and water inlets. The flow rates of the combined oil/water stream past the junction where the oil and water meet was 8.6 mm/s. The channels, which were rectangular, had dimensions of 50 (width)  $\times$  50 (height)  $\mu\text{m}^2$ . As shown in FIG 15a, plugs that flow in uniform-sized channels typically merged only when they simultaneously arrived at the T-junction. Thus, plug merging in these channels occur infrequently. In addition, lagging plugs were typically not able to catch up with leading plugs along the common channel.

FIG. 15b illustrates plug merging occurring between plugs arriving at different times at the Y-shaped junction (magnified view shown). In FIG. 15b, the flow rates for the combined oil/water fluid past the junction where the oil and water meet were 6.9 mm/s for the 50  $\times$  50  $\mu\text{m}^2$  channel and 8.6 mm/s for the 25  $\times$  50  $\mu\text{m}^2$  channel. The oil-to-water volumetric ratio was 4:1 in each pair of oil and water inlets. The two channels (the branch channels) merged into a common channel that had a 100  $\times$  50  $\mu\text{m}^2$  cross-section. As shown in the figure, the larger plugs from the bigger channel are able to merge with the smaller plugs from the narrower channel even when they

do not arrive at the junction at the same time. This is because lagging larger plugs are able to catch up with the leading smaller plugs once the plugs are in the common channel.

FIG. 15c depicts in-phase merging (*i.e.*, plug merging upon simultaneous arrival of at least two plugs at a junction) of plugs of different sizes generated using different oil/water ratios at the two pairs of inlets. The flow rate corresponding to the fluid stream resulting from a 1:1 oil-to-water volumetric ratio was 4.0 mm/s, while that corresponding to the 4:1 oil-to-water volumetric ratio was 6.9 mm/s. Each branch channel of the Y-shaped portion of the network (magnified view shown) had a dimension of  $50 \times 50 \mu\text{m}^2$  while the common channel (the channel to which the branch channels merge) was  $125 \times 50 \mu\text{m}^2$ .

FIG. 15d illustrates defects (*i.e.*, plugs that fail to undergo merging when they would normally merge under typical or ideal conditions) produced by fluctuations in the relative velocity of the two incoming streams of plugs. In this experiment, the flow rate corresponding to the fluid resulting from a 1:1 oil-to-water volumetric ratio was 4.0 mm/s, while that corresponding to the 4:1 oil-to-water volumetric ratio was 6.9 mm/s. Each branch channel that formed one of the two branches of the Y-shaped intersection (magnified view shown) was  $50 \times 50 \mu\text{m}^2$  while the common channel (the channel to which the two branch channels merge) is  $125 \times 50 \mu\text{m}^2$ .

### Example 3

The splitting of plugs was investigated using a microchannel network with a constricted junction. In this case, the plugs split and flowed past the junction into two separate branch channels (in this case, branch channels are the channels to which a junction branches out) that are at a  $180^\circ$ -angle to each other (see FIGS. 16a-c each of which show a microchannel network viewed from the top). In these experiments, the outlet pressures,  $P_1$  and  $P_2$ , past the constricted junction were varied such that either  $P_1 \approx P_2$  (FIG. 16b) or  $P_1 < P_2$  (FIG. 16c). Here, the relative pressures were varied by adjusting the relative heights of the channels that were under pressures  $P_1$  and  $P_2$ . Since longer plugs tend to split more reliably, this branching point (or junction) was made narrower than the microchannel to elongate the plugs. FIG. 16a shows a schematic diagram of the microchannel network used in the experiment. The oil-to-



water ratio was 4:1 while the flow rate past the junction where the oil and water meet was 4.3 mm/s.

FIG. 16b is a microphotograph showing the splitting of plugs into plugs of approximately one-half the size of the initial plugs. The channels, which were rectangular, had a cross-section that measured  $50 \times 50 \mu\text{m}^2$ . The constricted section of the channel right next to the branching point measured  $25 \times 50 \mu\text{m}^2$ . The outlet pressures,  $P_1$  and  $P_2$ , were about the same in both branch channels. Here, the plugs split into plugs of approximately the same sizes.

FIG. 16c is a microphotograph showing the asymmetric splitting of plugs (*i.e.*, the splitting of plugs into plugs of different sizes or lengths) which occurred when  $P_1 < P_2$ . The microphotograph shows that larger plugs (somewhat rectangular in shape) flowed along the channel with the lower pressure  $P_1$ , while smaller plugs (spherical in shape) flowed along the channel with the higher pressure  $P_2$ . As in FIG. 16b, each of the channel cross-section measured  $50 \times 50 \mu\text{m}^2$ . The constricted section of the channel at the junction measured  $25 \times 50 \mu\text{m}^2$ .

#### Example 4

The splitting of plugs was investigated using a microchannel network without a constriction such as the one shown in FIGS. 16b-c. In this case, the plugs split and flowed past the junction in two separate channels at a  $90^\circ$ -angle to each other. FIGS. 17a-c, which represent top views of a microchannel network, show that plugs behave differently compared to the plugs in Example 3 when they flow past a junction in the absence of a channel constriction, such as a constriction shown in FIGS. 16b-c. As FIG. 17c shows, when  $P_1 < P_2$ , the plugs remained intact after passing through the junction. Further, the plugs traveled along the channel that had the lower pressure ( $P_1$  in FIG. 17c) while the intervening oil stream split at the junction. The splitting of the oil stream at the junction gives rise to a shorter separation between plugs flowing along the channel with pressure  $P_1$  compared to the separation between plugs in the channel upstream of the branching point or junction.

#### Example 5

Microfluidic devices with hydrophilic channel surfaces were fabricated using rapid prototyping in polydimethylsiloxane. The channel surfaces 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 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  $\mu$ l) and Hamilton Co. 30 gauge Teflon<sup>®</sup> 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  $\mu$ l/min to 10.0  $\mu$ 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 Fostec (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.

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 substrate 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.
6. The method of claim 4, wherein the reagents fluids are distinct laminar
  - 20 streams at or before the inlet.
7. The method of claim 6, where the reagents fluids are separated by 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.
11. The method of claim 10, wherein at least one of the reagent fluids further
  - 30 comprises a 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.

14. The method of claim 1, wherein the carrier fluid is a fluorinated oil.
15. The method of claim 1, wherein the carrier fluid is perfluorodecaline or perfluoroperhydrophenanthrene.
16. The method of claim 1, further comprising merging, mixing, separating,  
5 splitting, or monitoring the plugs.
17. The method of claim 1, further comprising monitoring the reaction within the plugs.
18. The method of claim 17, wherein monitoring comprises measuring the optical properties of the reaction.
- 10 19. The method of claim 17, wherein the refractive index of the carrier fluid and the reagent fluids are substantially similar.
20. The method of claim 17, wherein the reaction is monitored at one or more positions along the channel of the microfabricated substrate.
- 15 21. The method of claim 1, further comprising separating the reagent fluids from the carrier fluid.
22. The method of claim 1, further comprising controlling the flow velocity of a plug by adjusting reagent viscosity, plug size, plug shape, or microchannel geometry.
23. The method of claim 1, further comprising increasing the mixing rate of the reagent fluids in the plugs by adjusting the microchannel geometry **or flow rate**.
- 20 24. The method of claim 1, wherein the reaction of the first and second reagent fluids forms an insoluble reaction product within at least one plug.
25. The method of claim 24, wherein the insoluble reaction product is a nanoparticle.
26. The method of claim 1, wherein the reaction of the first and second  
25 reagent fluids forms a soluble reaction product within at least one plug.
27. 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  
30 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 substrate 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.

28. The method of claim 27, wherein at least one of the reagent fluids comprises a reagent and solvent.

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

30. The method of claim 27, wherein another reagent fluid consists essentially of solvent and surfactant.

10 31. 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,

15 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 substrate 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

20 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 substrate forms merged plugs in which a reaction between the first and second reagent fluids can occur.

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

33. The method of claim 31, wherein the first and second set of plugs have different relative velocities.

30 34. The method of claim 31, wherein portions of the first channel, the second channel, and the single channel resulting from the merging of the first and second channels form a Y-shaped junction.

35. The method of claim 34, wherein portions of the first and second channels that form a pair of branches of the Y-shaped junction are at a 180°-angle to each other.

36. The method of claim 34, wherein a cross-section dimension of the first channel differs from a cross-section dimension of the second channel.

37. The method of claim 34, wherein a cross-section dimension of the first channel is approximately equal to a cross-section dimension of the second channel.

38. 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.

39. 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.

40. The method of claims 38 or 39, wherein the splitting of the at least one plug or the at least one merged plug is achieved by directing the at least one plug or the at least one merged plug into a junction that branches out into two or more branch channels that are at different pressures.

41. The method of claim 40, wherein the pressures in a first branch channel and a second branch channel are fixed by adjusting a relative height or relative orientation of the first branch channel and the second branch channel.

42. The method of claim 40, wherein a first branch channel is at an angle of about 90° relative to a second branch channel.

43. The method of claim 40, wherein a first branch channel is at an angle of about 180° relative to a second branch channel.

44. The method of claim 40, wherein a plurality of plugs that flow in a first branch channel and produced from the splitting of the at least one plug or the at least one merged plug are larger or longer than the plugs that flow in a second branch channel.

45. The method of claim 40, wherein a constriction exists at or near the junction.

46. 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 of the fluids in the microfabricated substrate 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.

47. The method of claim 46, further comprising employing a number of microfabricated substrates in parallel.

48. 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 of the fluids in the microfabricated substrate 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.

49. The method of claim 48, further comprising:

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

applying pressure to induce a continuous flow of the fluids in the microfabricated substrate to form 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 substrate forms merged plugs in which a reaction between the unstable intermediate and second reagent fluid can occur.

50. The method of claim 48, further comprising employing a number of microfabricated substrates in parallel.

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

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

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

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

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

52. The device of claim 51, comprising one microfabricated substrate.

53. The device of claim 51, wherein at least one of the microfabricated substrates  
10 comprises an array of connected microchannels.

54. The device of claim 51, wherein one of the microchannels has at least 1 bend.

55. The device of claim 51, wherein one of the microchannels has a plurality of bends.

56. The device of claim 51, wherein at least one of the microchannels comprises at  
15 least one bend of greater than about 10°.

57. The device of claim 51, wherein at least one of the microchannels comprises at least one bend of greater than about 90°.

58. The device of claim 51, wherein at least one of the microchannels comprises at least one bend of greater than about 135°.

59. The device of claim 51, wherein at least one of the microchannels comprises at  
20 least one bend of greater than about 180°.

60. The device of claim 51, wherein at least one of the microchannels comprises at least one bend of greater than about 270°.

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



# ABSTRACT OF THE DISCLOSURE

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 substrate 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.

Patent Application for: Device and Method For Pressure-  
Driven Plug Transport  
Inventor(s): Rustem Ismagilov  
Attorney Docket No. and Serial No. 7814

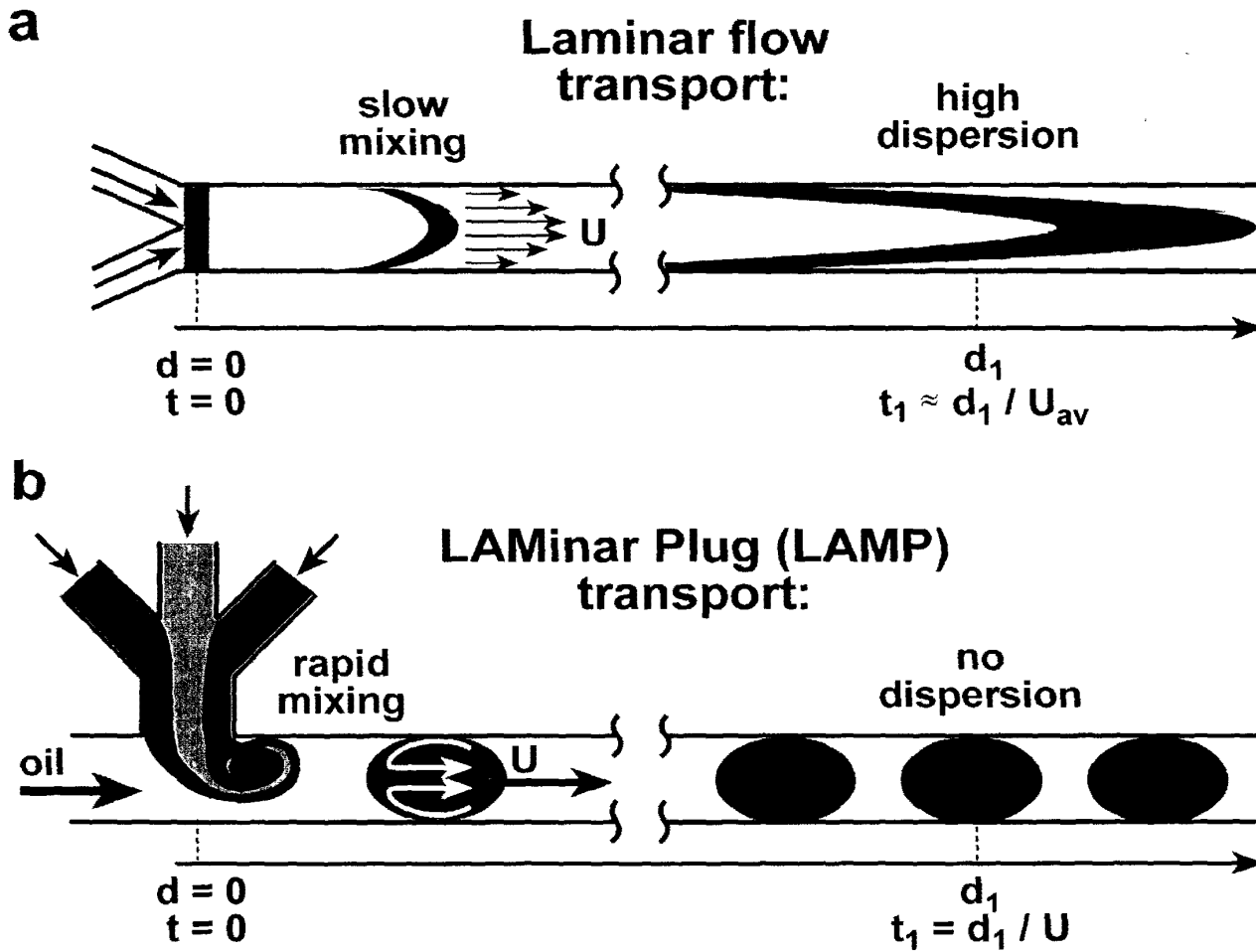


FIG. 1a

## Faster mixing inside oil/water plugs versus slower mixing in laminar flows (same flow rate of 7 $\mu\text{L}/\text{min}$ )

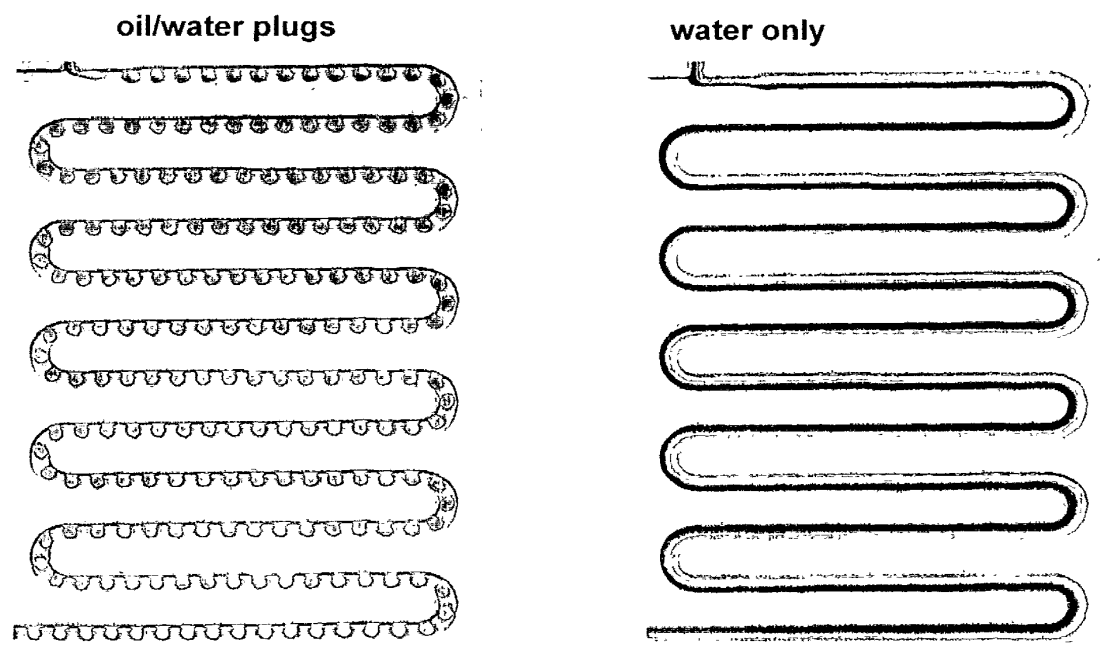
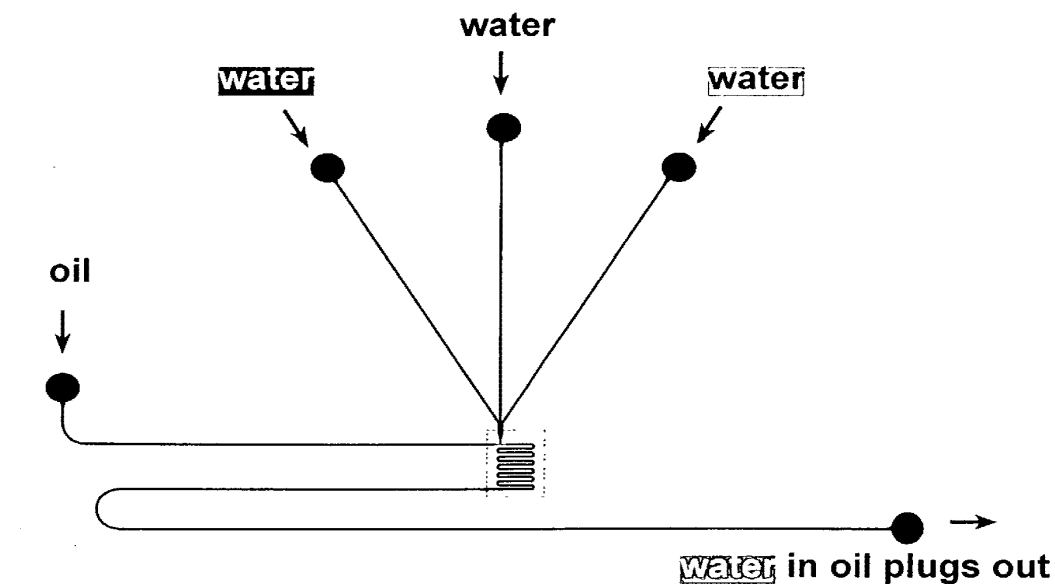


FIG. 1b

## Fast mixing of plugs in curved channels

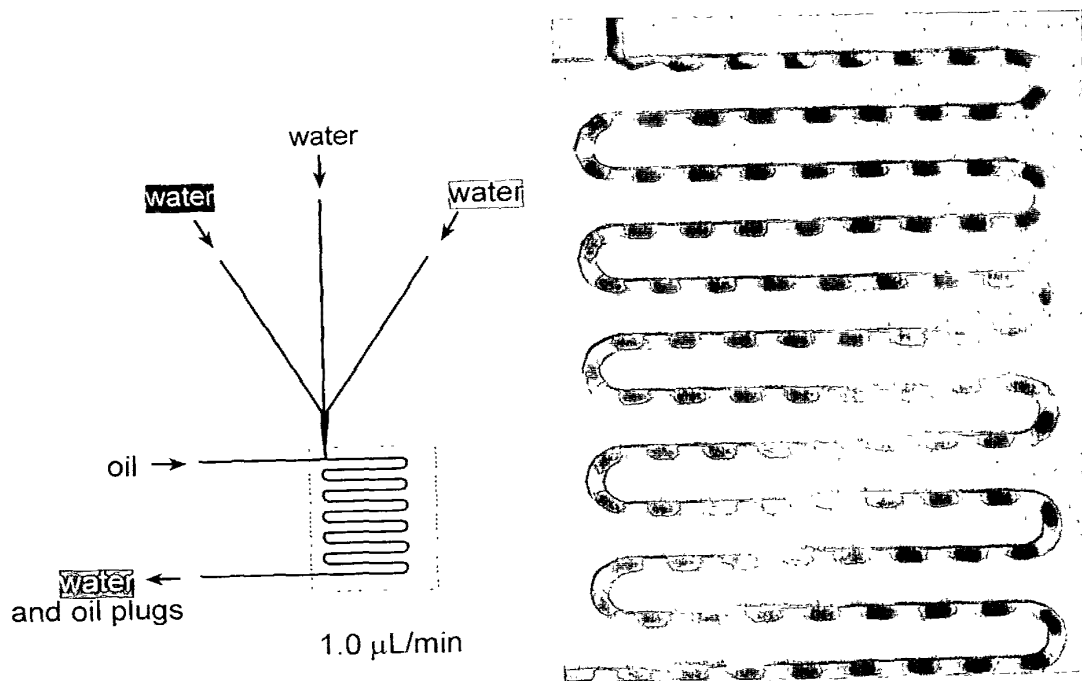
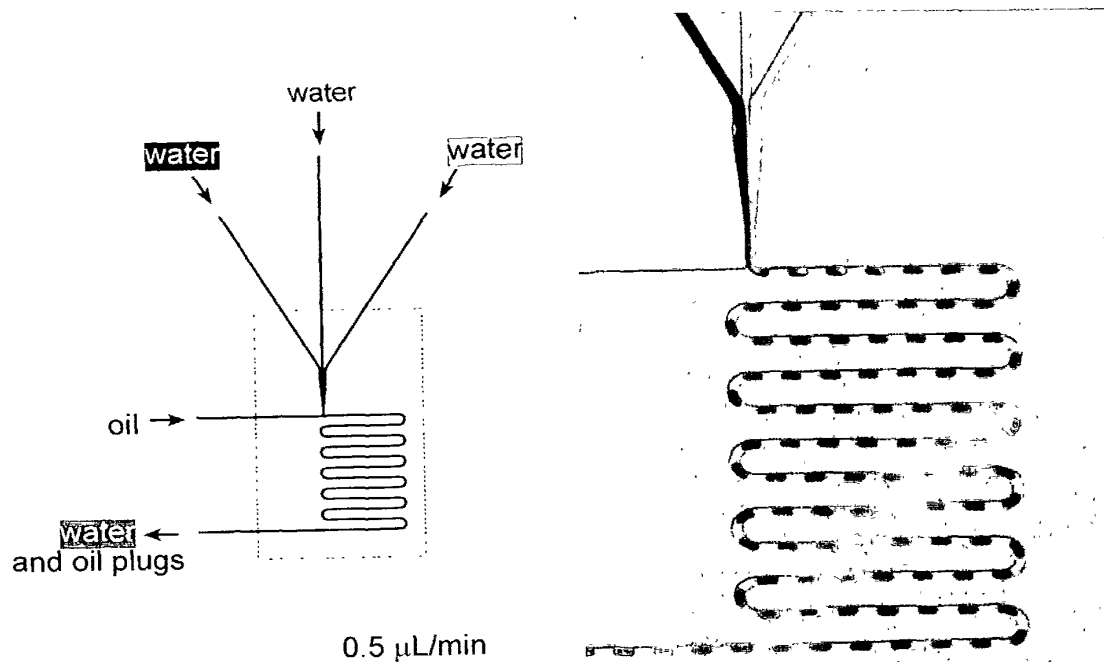
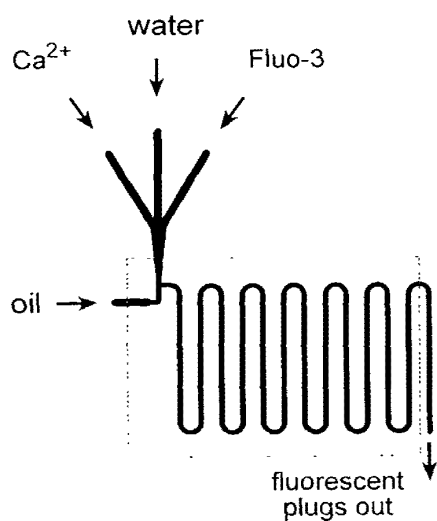


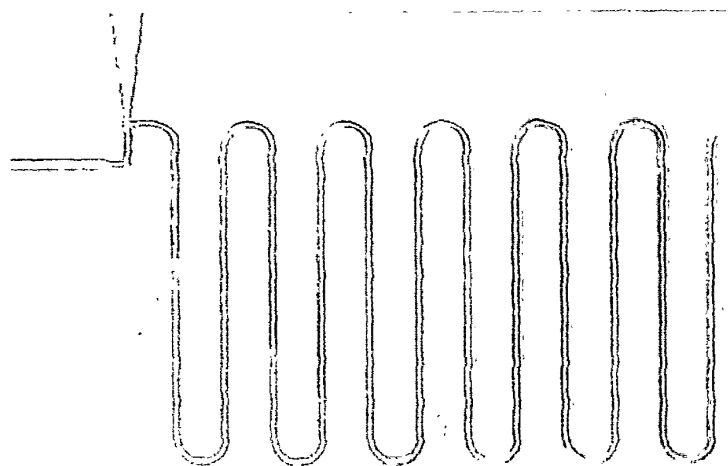
FIG. 2

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Attorney Docket No. and Serial No. 7814

## Rapid mixing can be quantified by fluorescence of $\text{Ca}^{2+}$ and Fluo-3

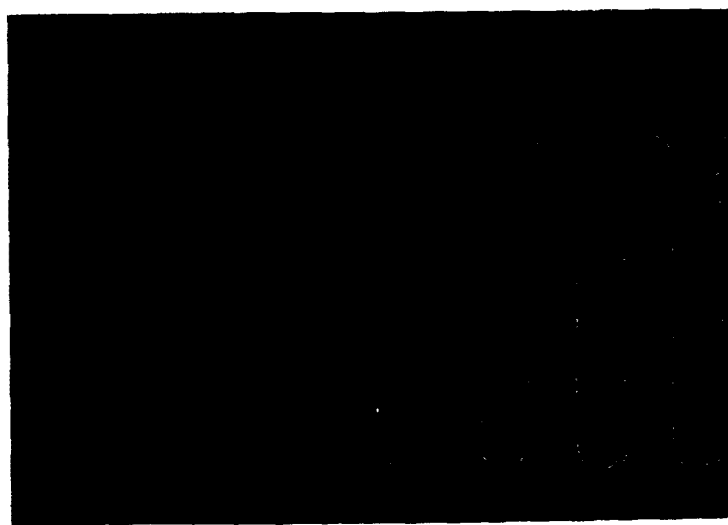


Bright field image



HS113\_BF

Fluorescence image



HS113\_33\_brightened

FIG. 3a

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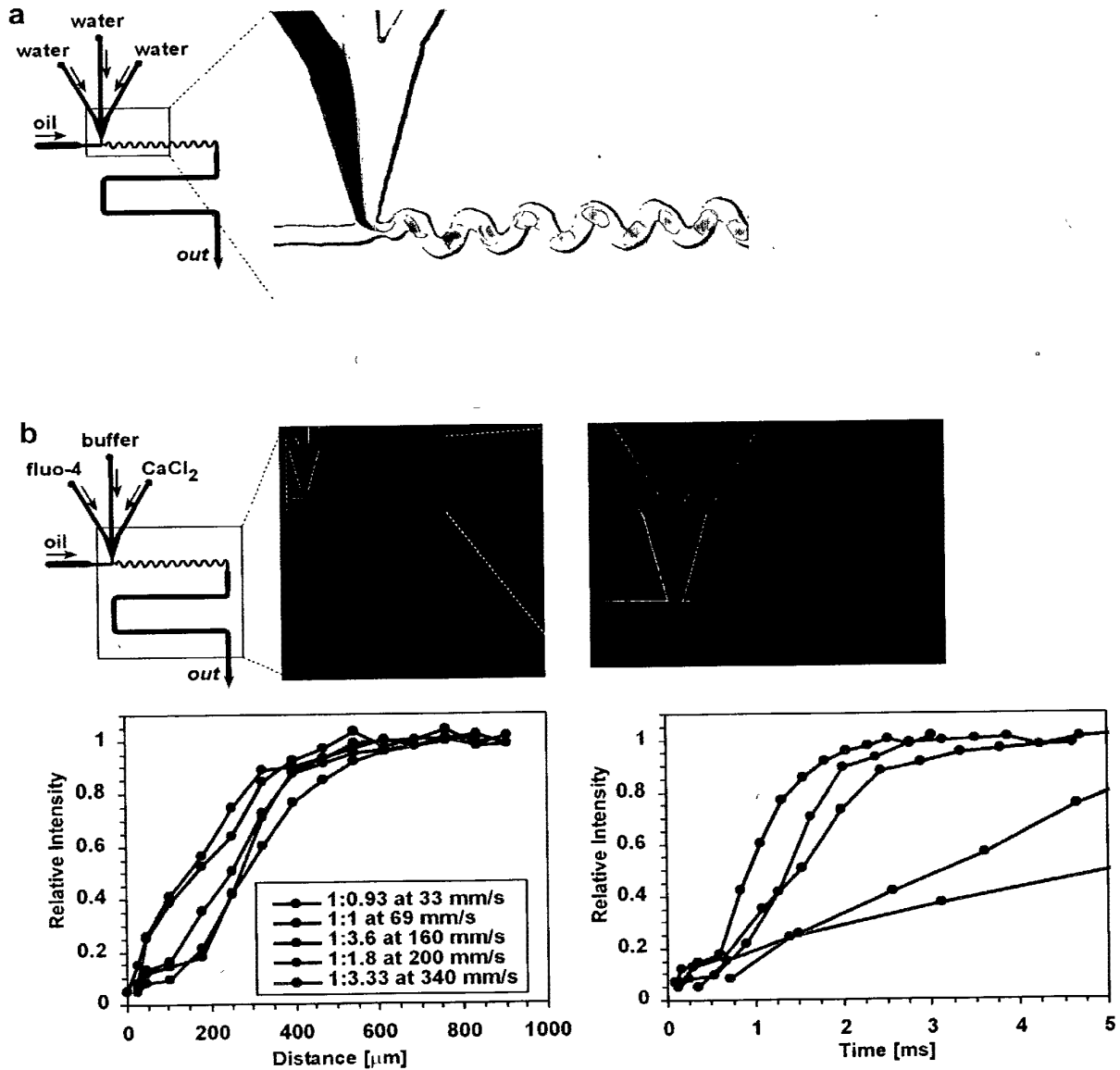


FIG. 3b

Patent Application for: Device and Method For Pressure-  
Driven Plug Transport  
Inventor(s): Rustem Ismagilov  
Attorney Docket No. and Serial No. 7814

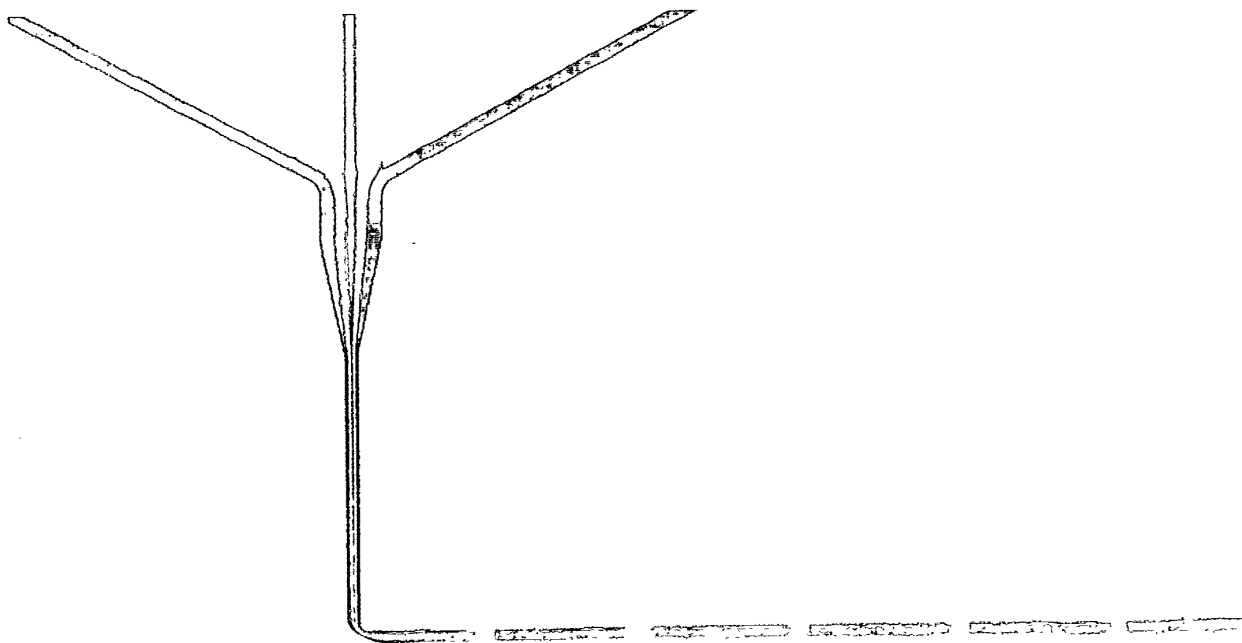
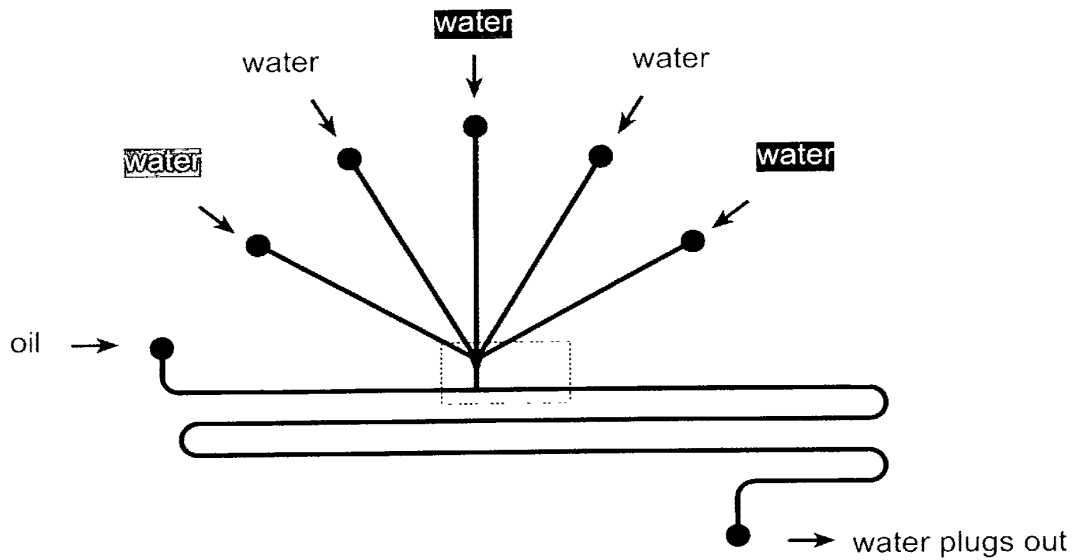
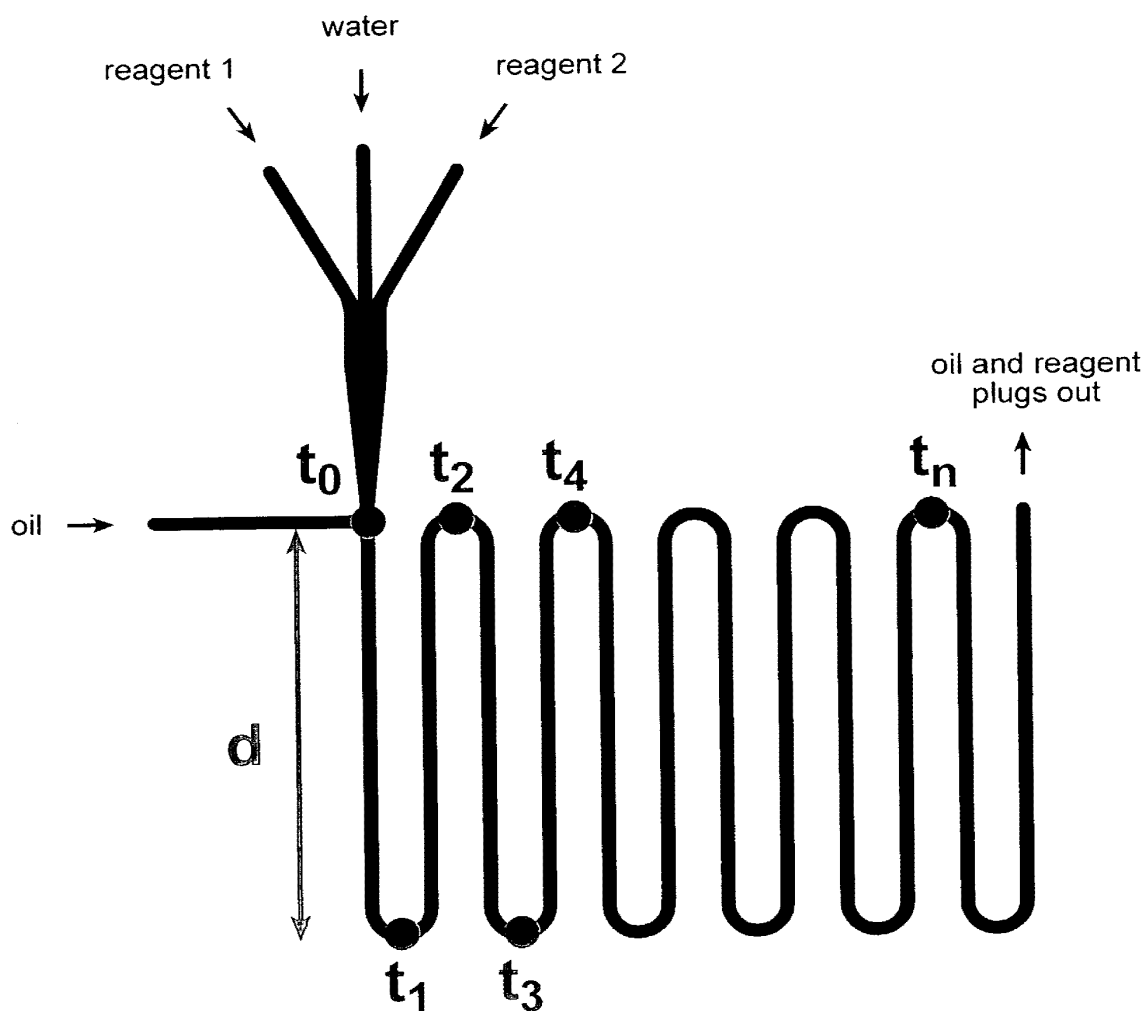


FIG. 4

## Multiple time-resolved data points can be obtained from a single image



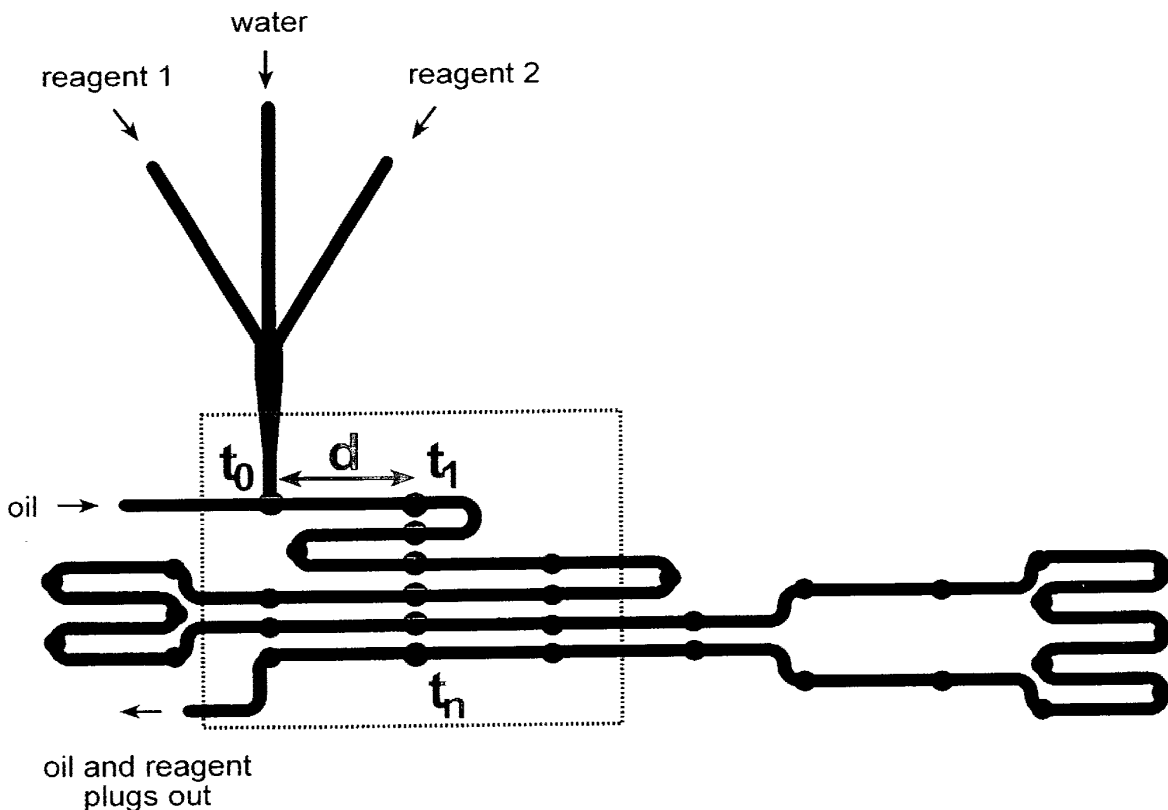
Let  $u$  = speed,  $d$  = distance,  $t$  = time  
where  $t_0 = 0$ ; then  
 $t_1 = d/u$ ,  
 $t_2 = 2d/u$ ,  
 $t_3 = 3d/u$ , etc.

Therefore,  
 $t_n = n d/u$ .

FIG. 5



## Time intervals within an image frame can be controlled by channel design



Let  $u$  = speed,  $d$  = distance and  $t_n$  = time at distance  $n$ .

Can gather data from a single image at short and long time intervals

For example, here:

$$t_n = \frac{2(n-1) \cdot d}{u}$$

Therefore, can gather data for  $n = 0, 1, 2, 4, 8, 16, 32 \dots$

FIG. 6

## Fast mixing of plugs in 90° step channels

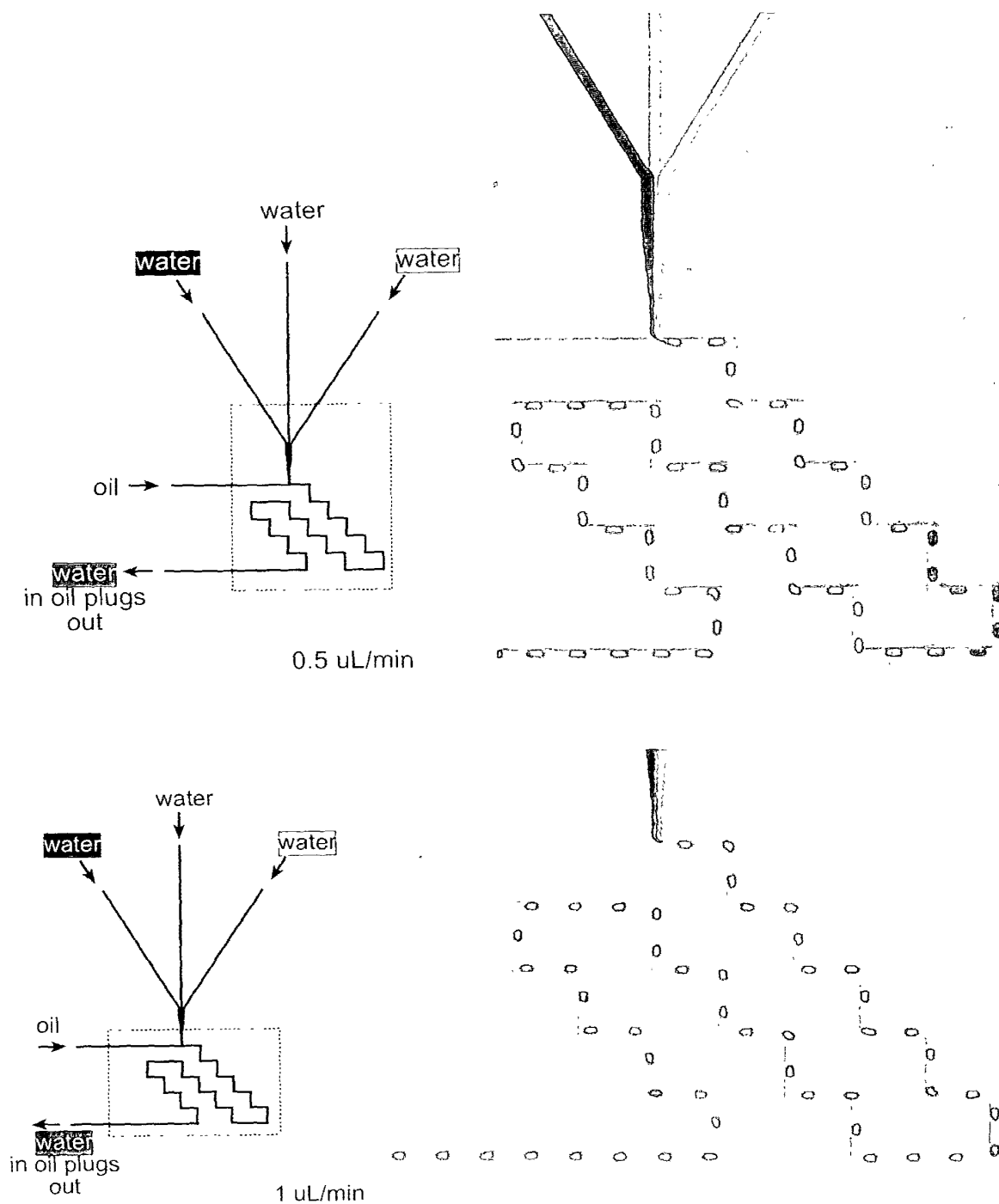


FIG. 7

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Driven Plug Transport  
Inventor(s): Rustem Ismagilov  
Attorney Docket No. and Serial No. 7814

# Fast mixing of plugs in 135° step channels

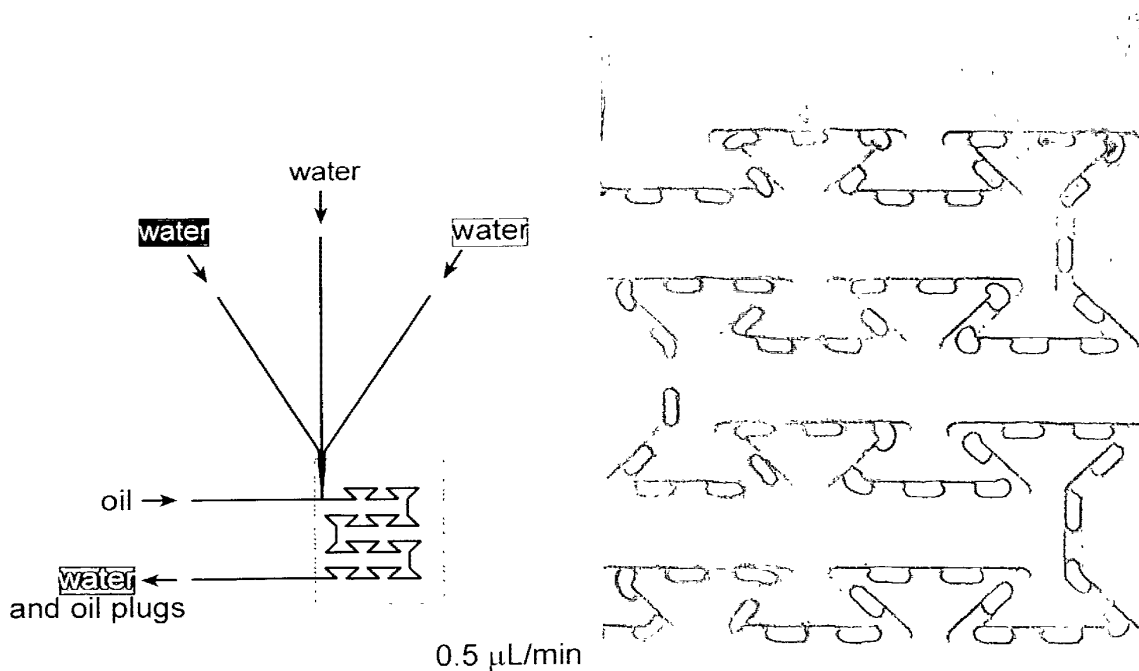
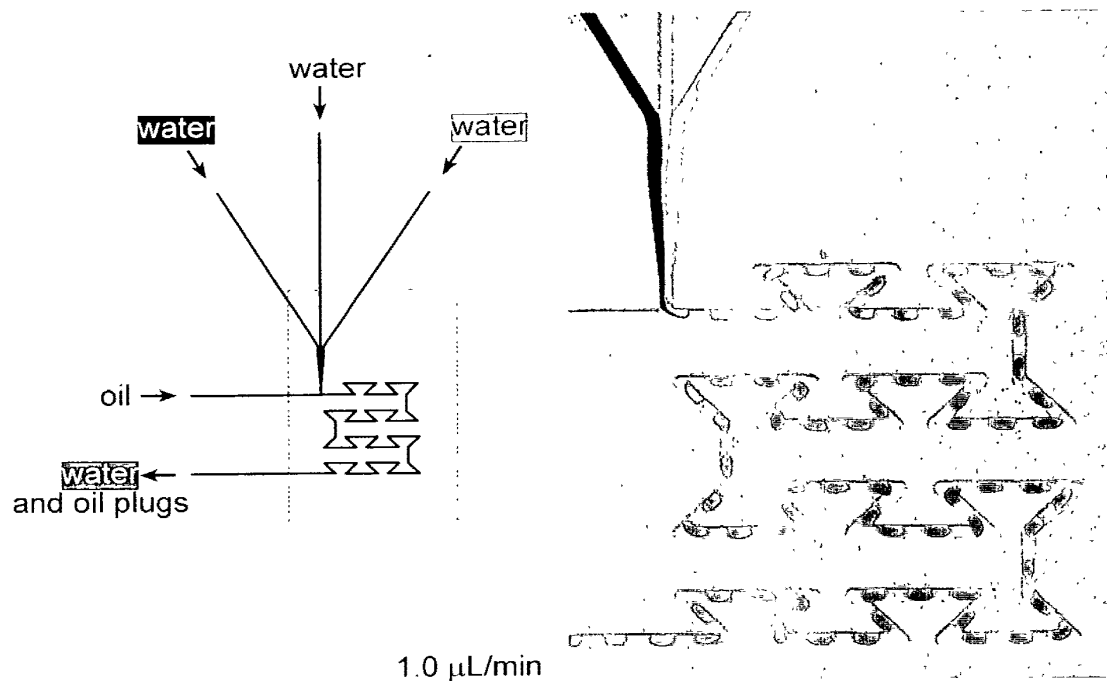


FIG. 8

# Transporting Particles in Microchannels with Plugs

Patent Application for: Device and Method For Pressure-

Driven Plug Transport

Inventor(s): Rustem Ismagilov

Attorney Docket No. and Serial No. 7814

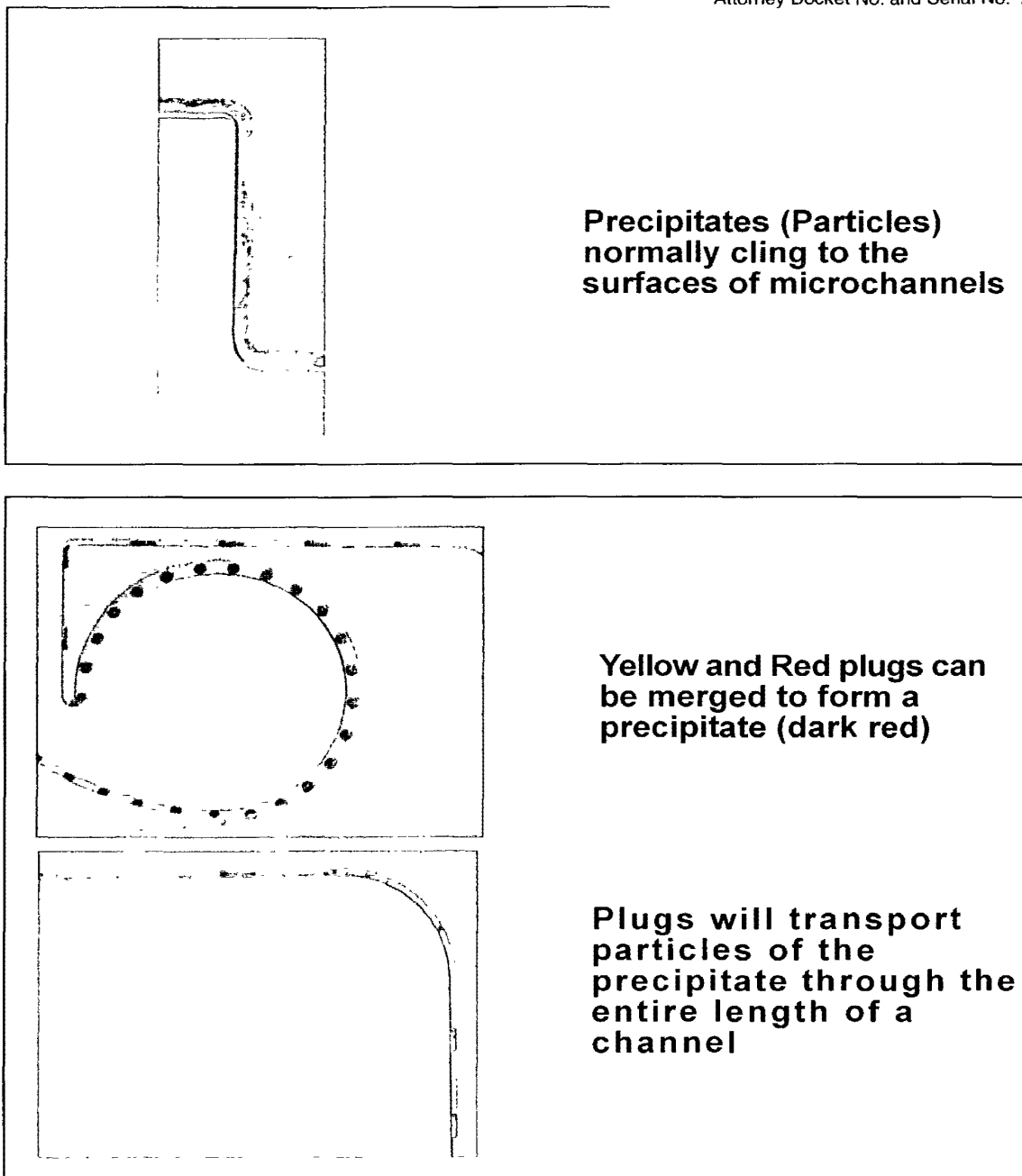
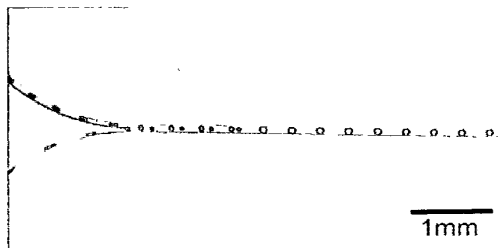


FIG. 9

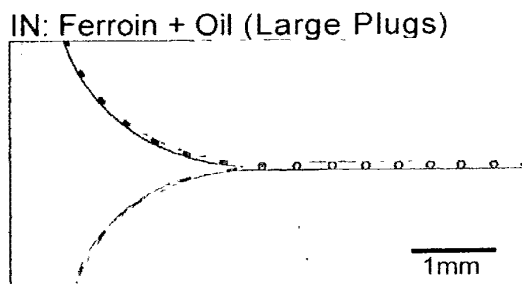
# Merging Plugs by Manipulating the Sizes of Plugs

IN: Ferroin + Oil (Large Plugs)



**Larger plugs move more quickly than smaller plugs and overtake the smaller plugs**

IN: Ferroin + Oil (Small Plugs)

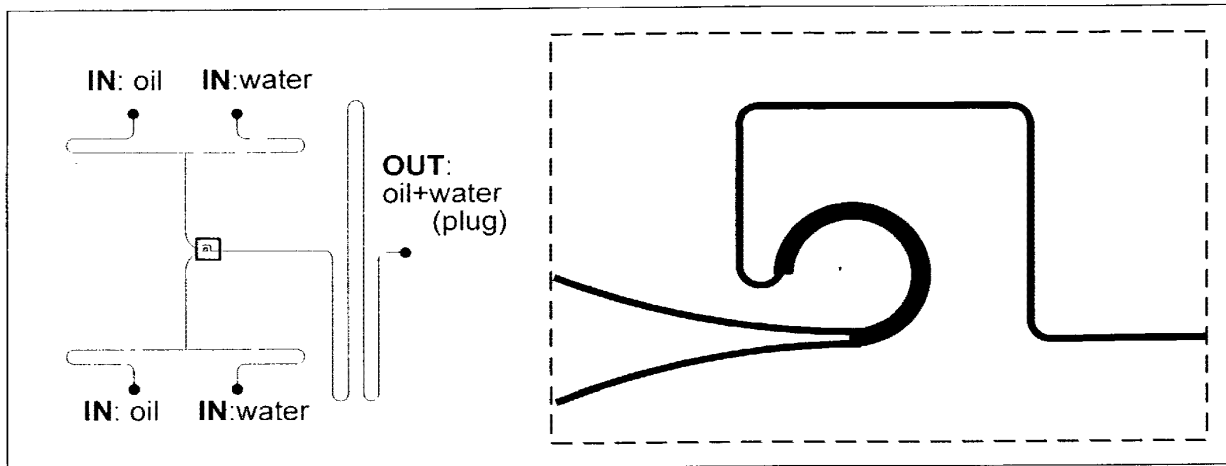


**If two plugs converge to the same point at the same time, they merge immediately**

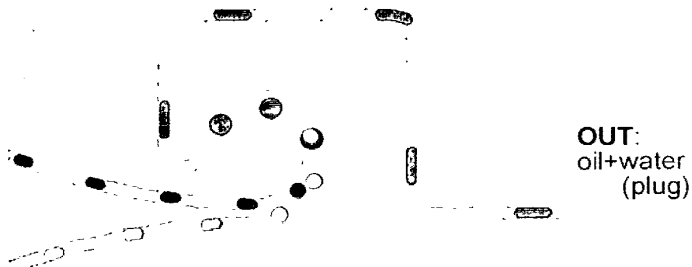
IN: Ferroin + Oil (Small Plugs)

FIG. 10

## Improved way to merge plugs

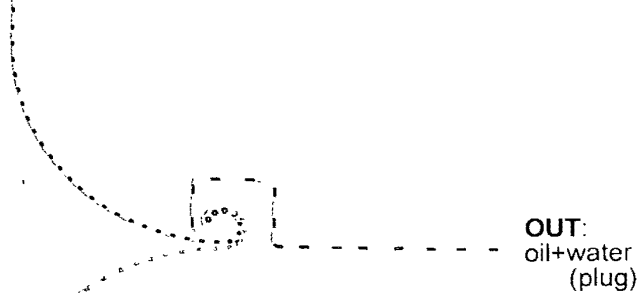


IN: oil+water(plug)



IN: oil+water(plug)

IN: oil+water(plug)



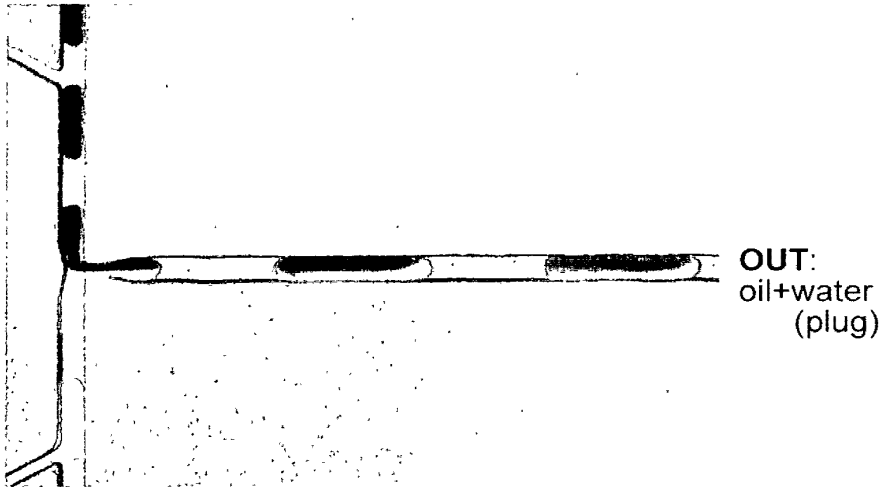
IN: oil+water(plug)

FIG. 11

## Arbitrary time-controlled fluidic networks can be constructed in this system because

### Aqueous plugs can be merged

IN: oil+water(plug)



IN: oil+water(plug)

### Aqueous plugs can be split

IN: oil+water(plug)

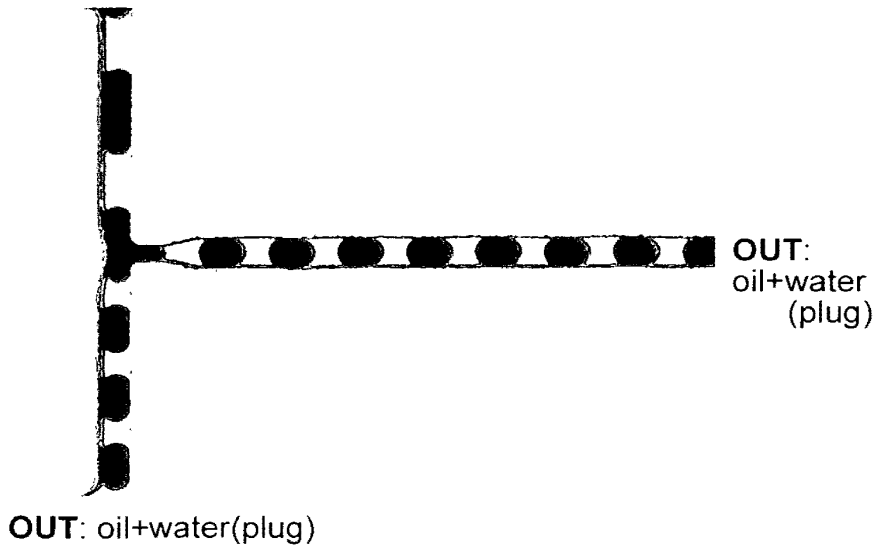
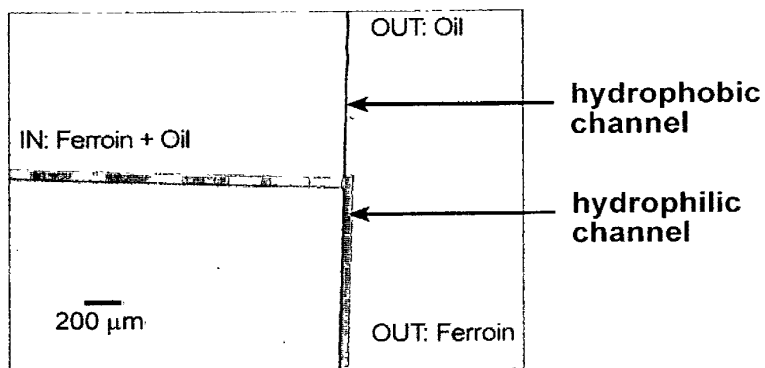


FIG. 12

# Alternating Aqueous Phase Plugs and Oil Plugs can be Separated

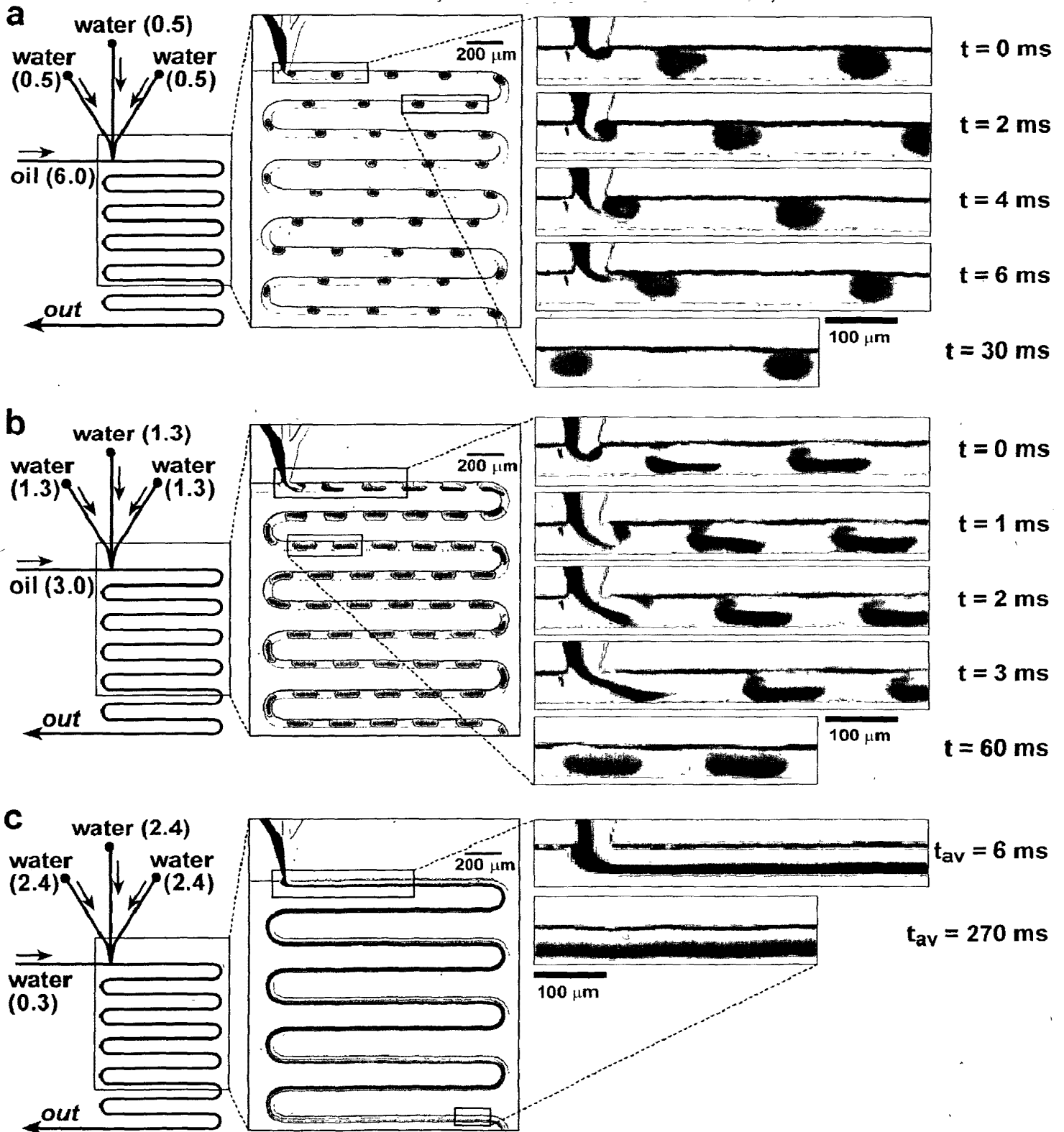


**Aqueous plugs prefer to enter  
hydrophilic channels.**

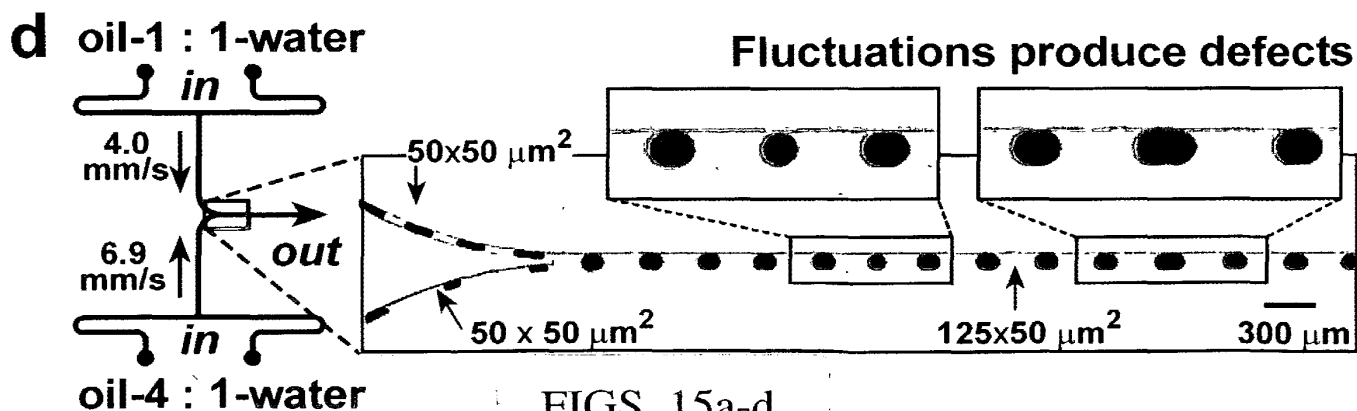
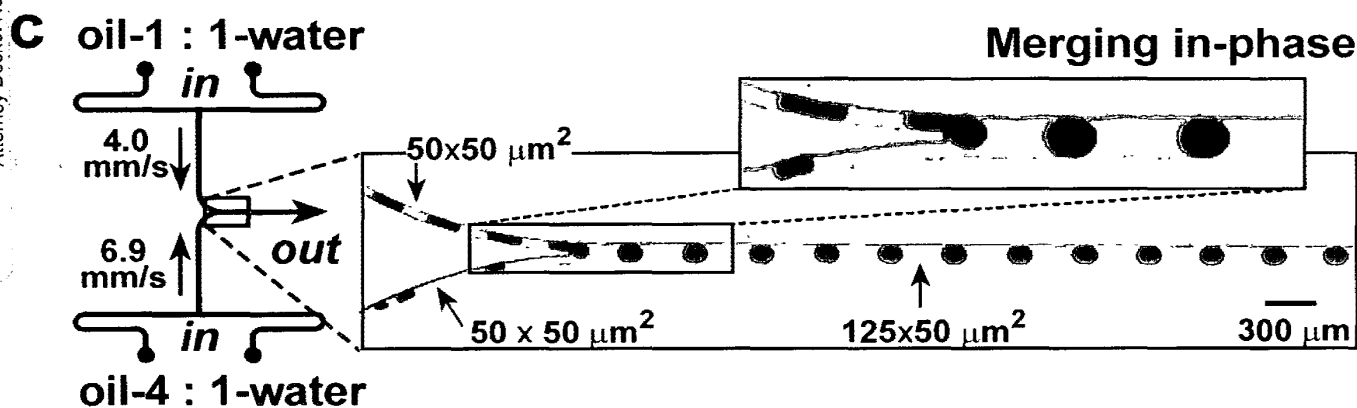
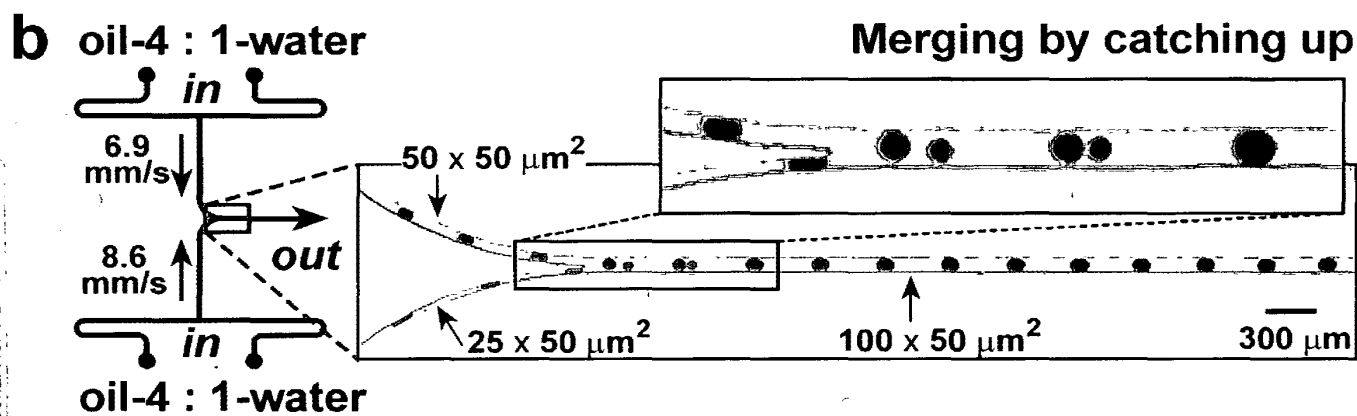
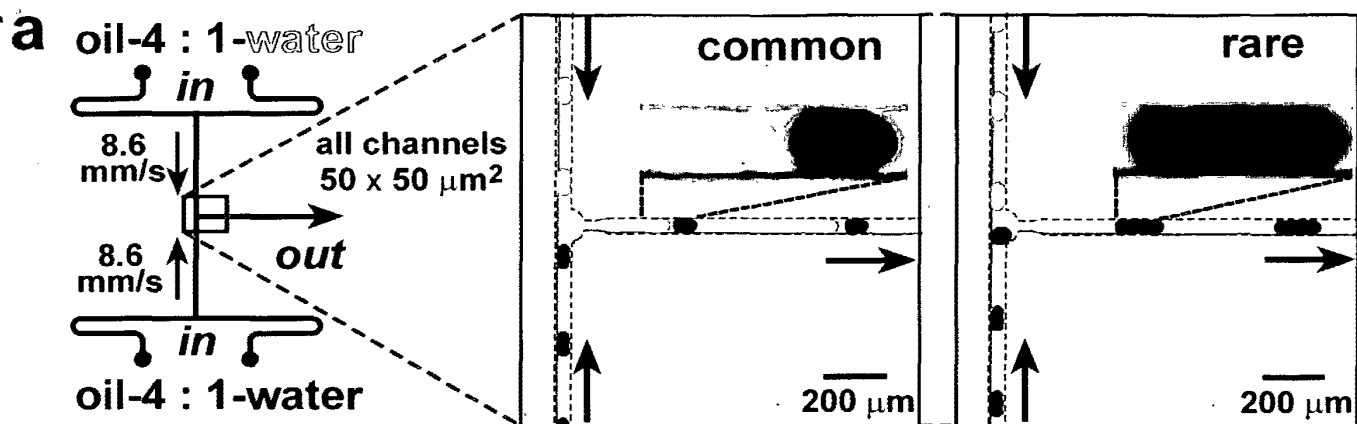
FIG. 13



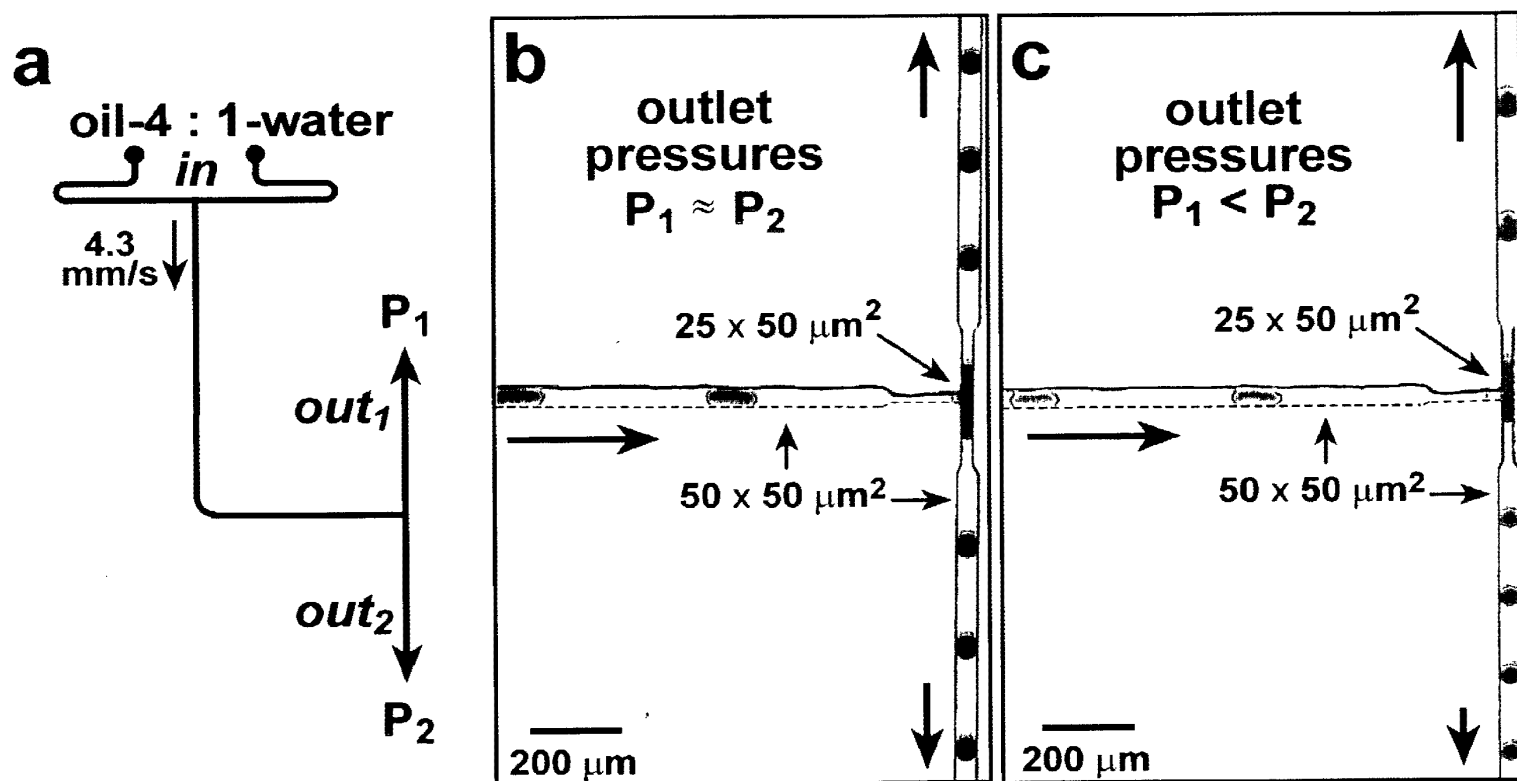
Patent Application for: Device and Method For Pressure-Driven Plug Transport  
 Inventor(s): Rustem Ismagilov  
 Attorney Docket No. and Serial No. 7814



FIGS. 14a-c

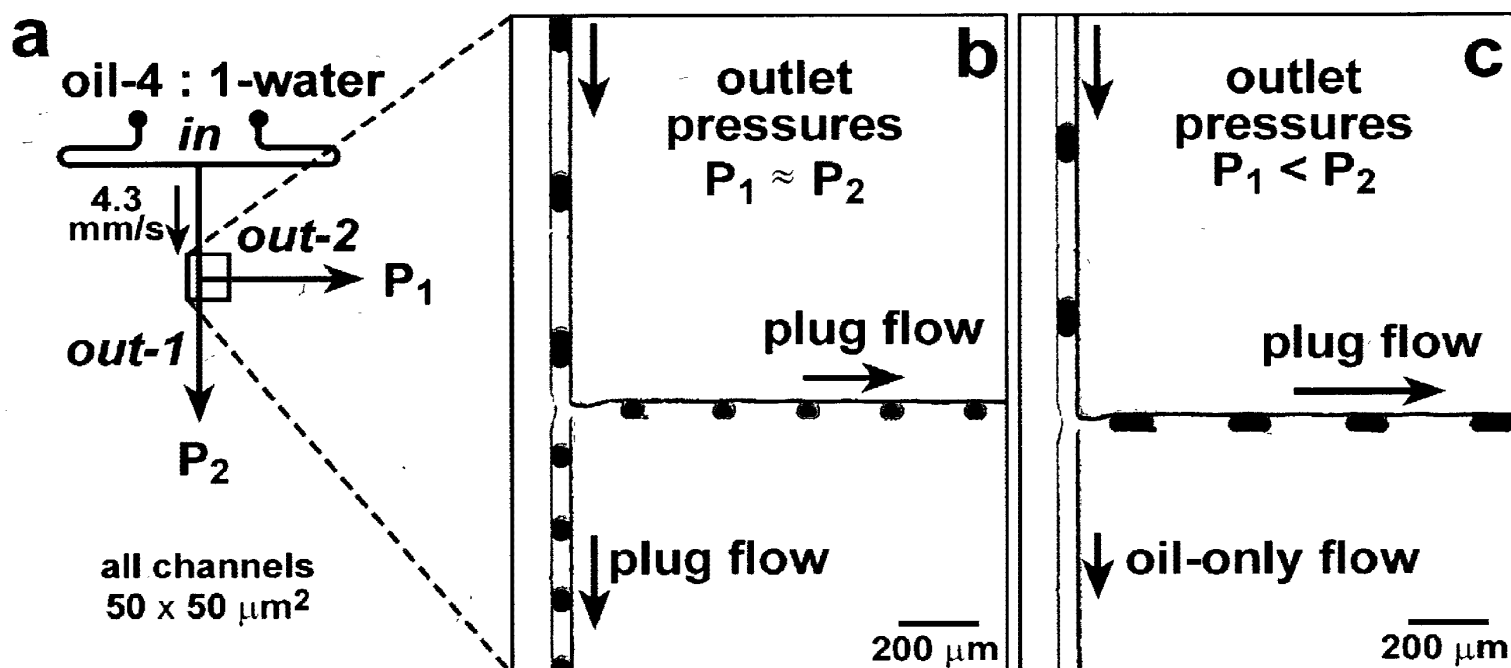


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Inventor(s): Rustem Ismagilov  
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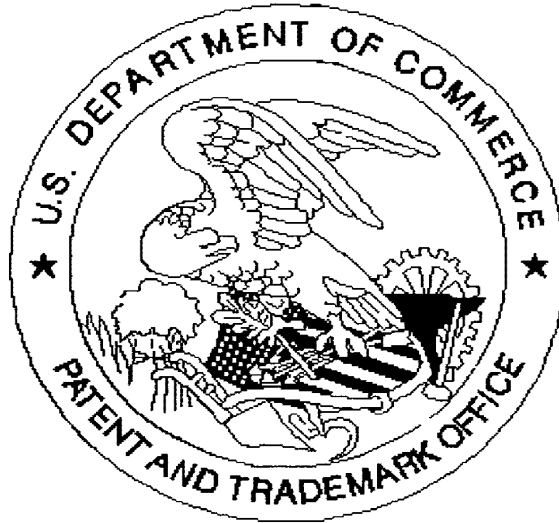
FIGS. 16a-c

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FIGS. 17a-c

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