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(54) **APPARATUSES AND METHODS FOR MODULATING FLUIDS USING ACOUSTICALLY OSCILLATING SOLID STRUCTURES**

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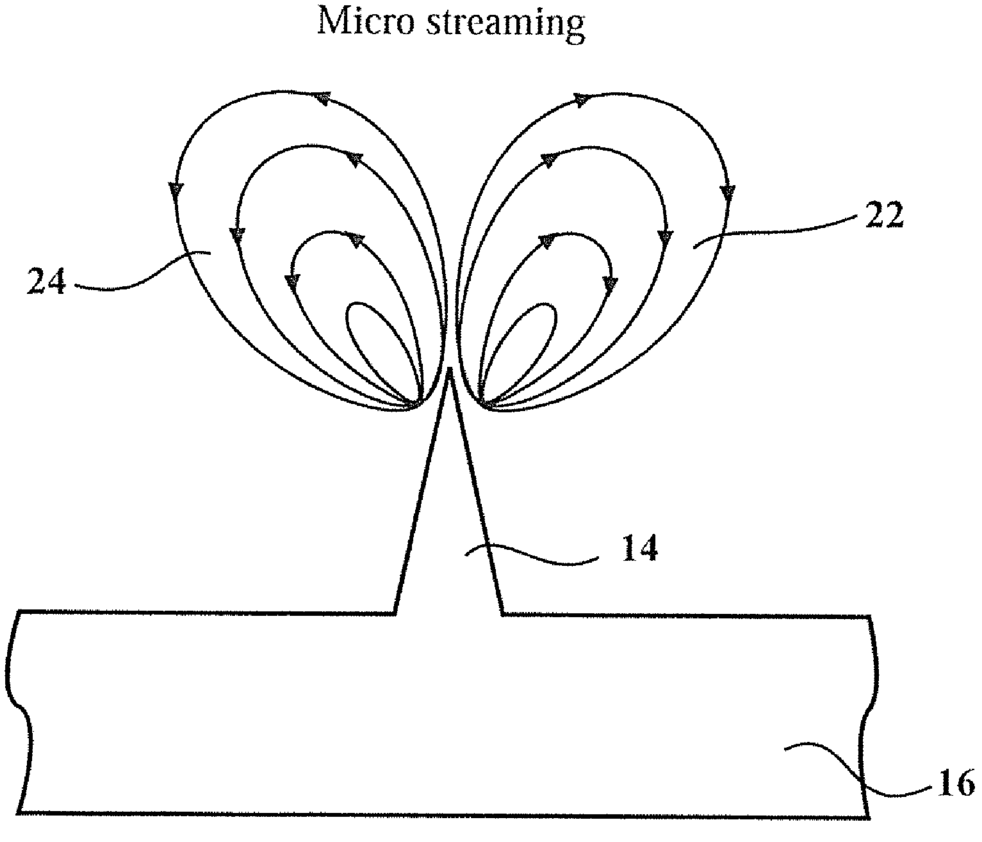
USPC **417/53**; 417/321; 417/410.1

(57) **ABSTRACT**

An acoustofluidic apparatus is provided that functions by the generation of microstreaming in fluid produced by the oscillation of oscillatory elements excited by an energy input such as acoustic energy. An acoustofluidic apparatus includes an oscillatory energy field generator in energetic contact with one or more oscillatory elements contained in a fluid passage. Oscillation of the oscillatory elements induces microstreaming in a fluid that can be used to mix laminar flows of differing fluids, as a micropump for the directional movement of fluid through a fluid passage, for the generation of waveforms in a fluid or plurality of fluids or for other purposes.



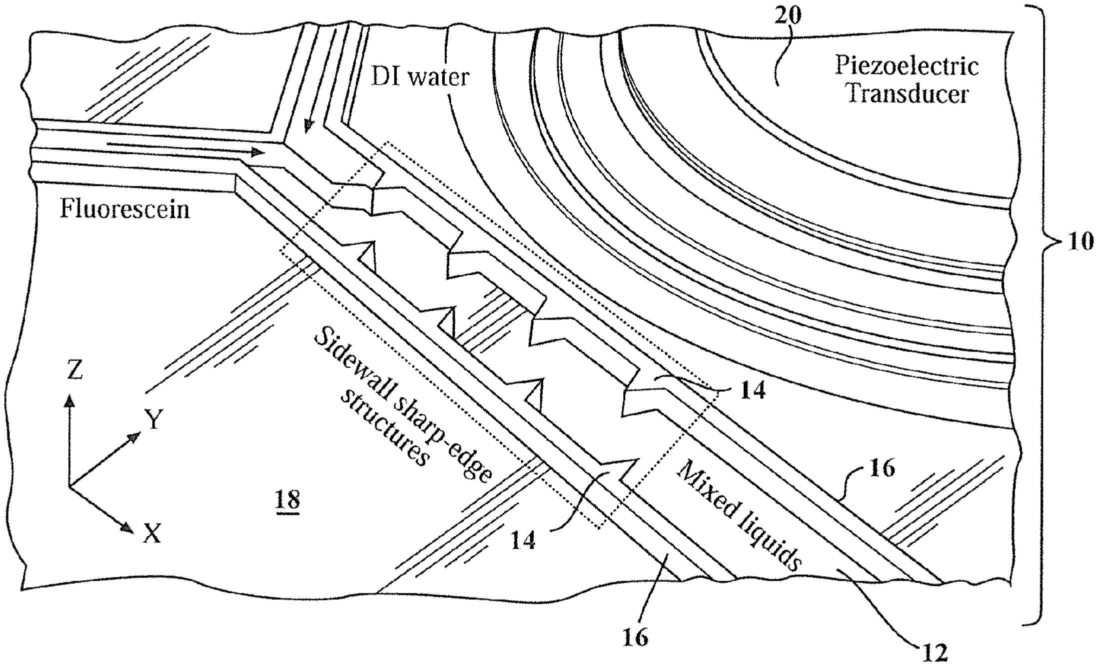

20
Piezoelectric Transducer
DI water
Fluorescein
10
14
Sidewall sharp-edge structures
Z
Y
X
18
14
16
Mixed liquids
16
12

FIG. 1A

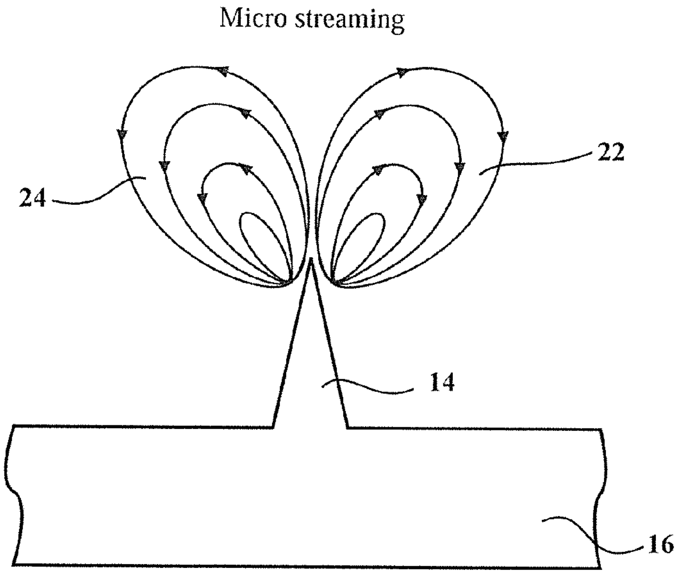

Micro streaming
24
22
14
16

FIG. 1B

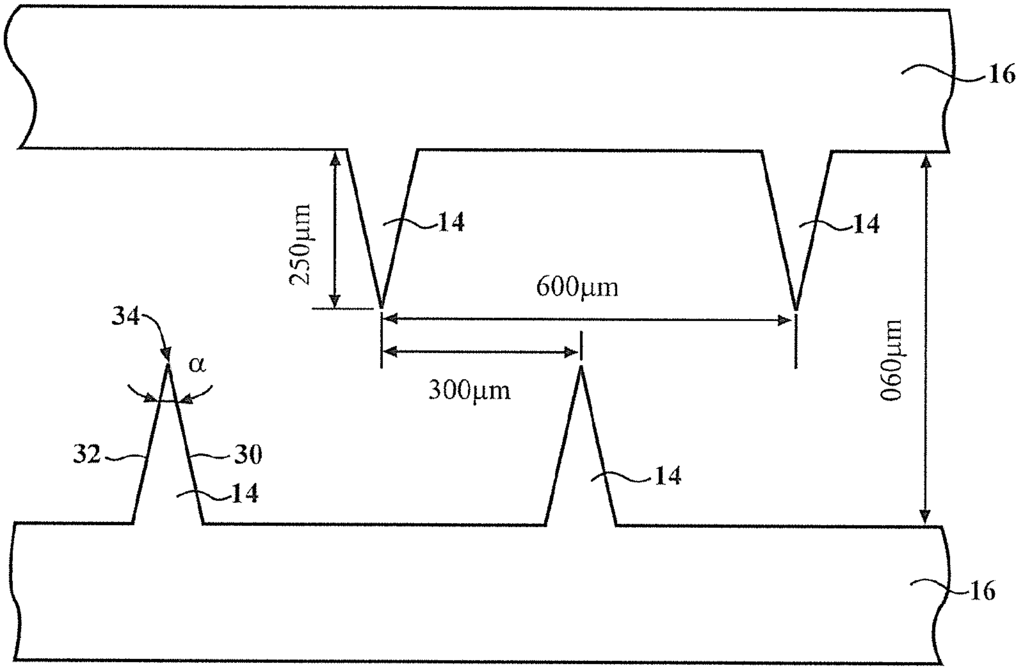

16
250μm
14
14
600μm
34
α
300μm
060μm
32
30
14
14
16

FIG. 1C

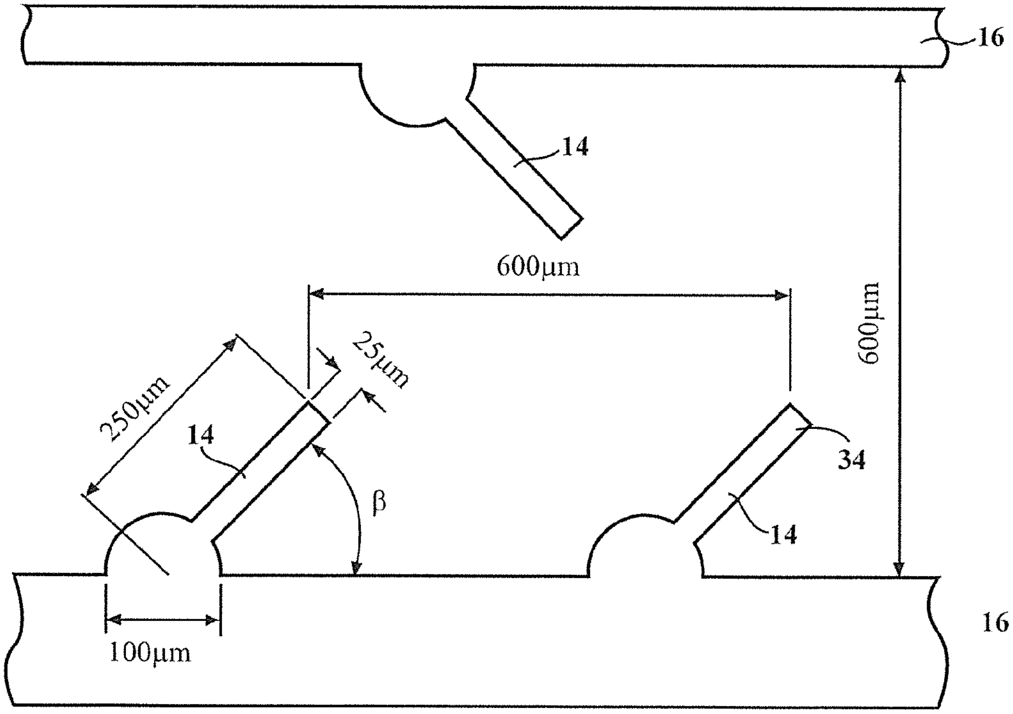

16
14
600μm
600μm
25μm
250μm
14
34
β
14
100μm
16

FIG. 1D

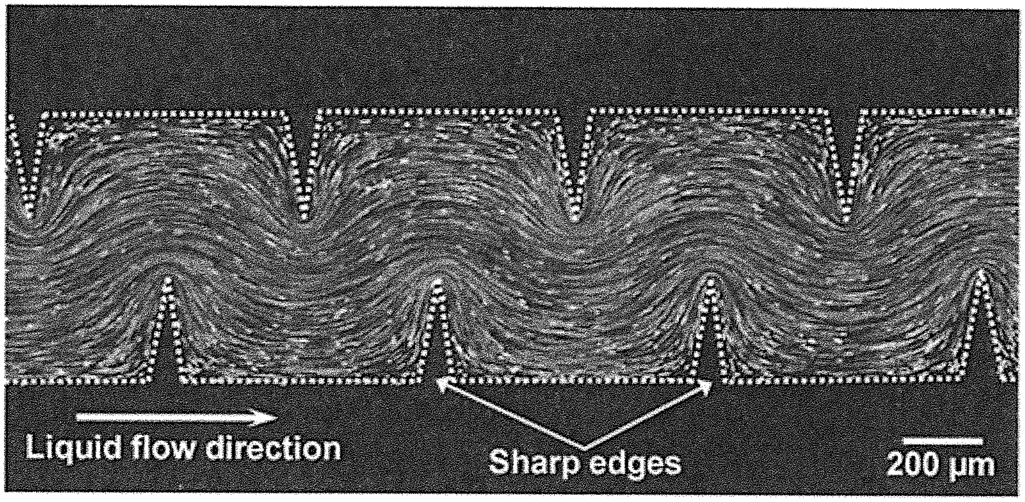


FIG. 2A

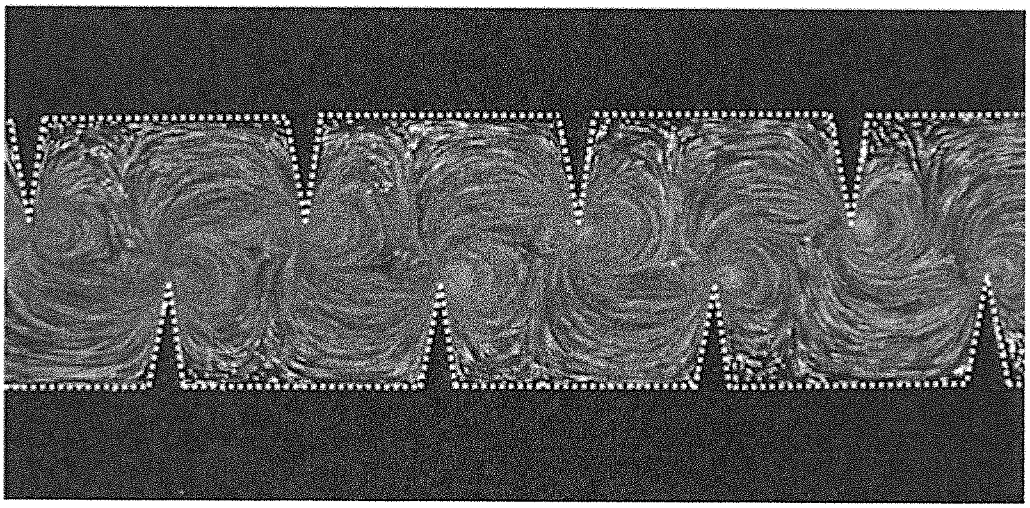


FIG. 2B

FIG. 3A

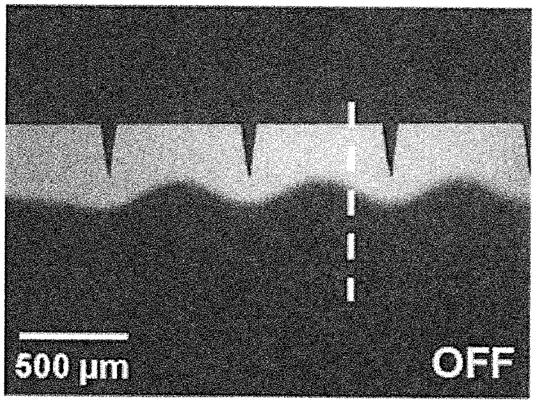
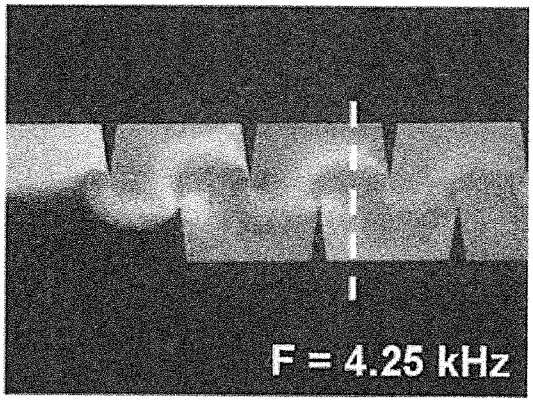


FIG. 3B



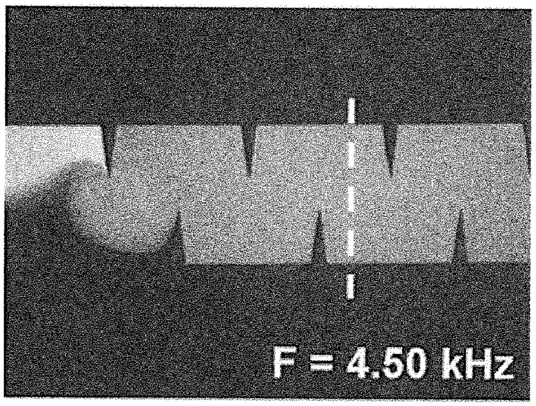


FIG. 3C

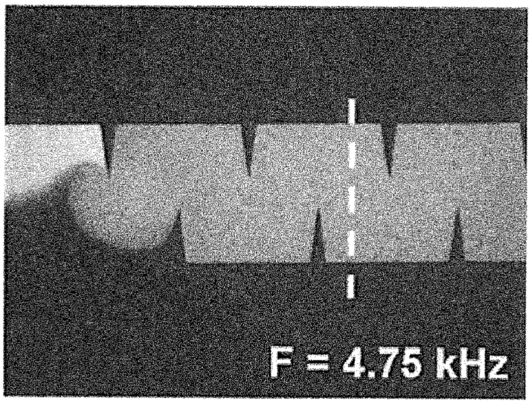


FIG. 3D

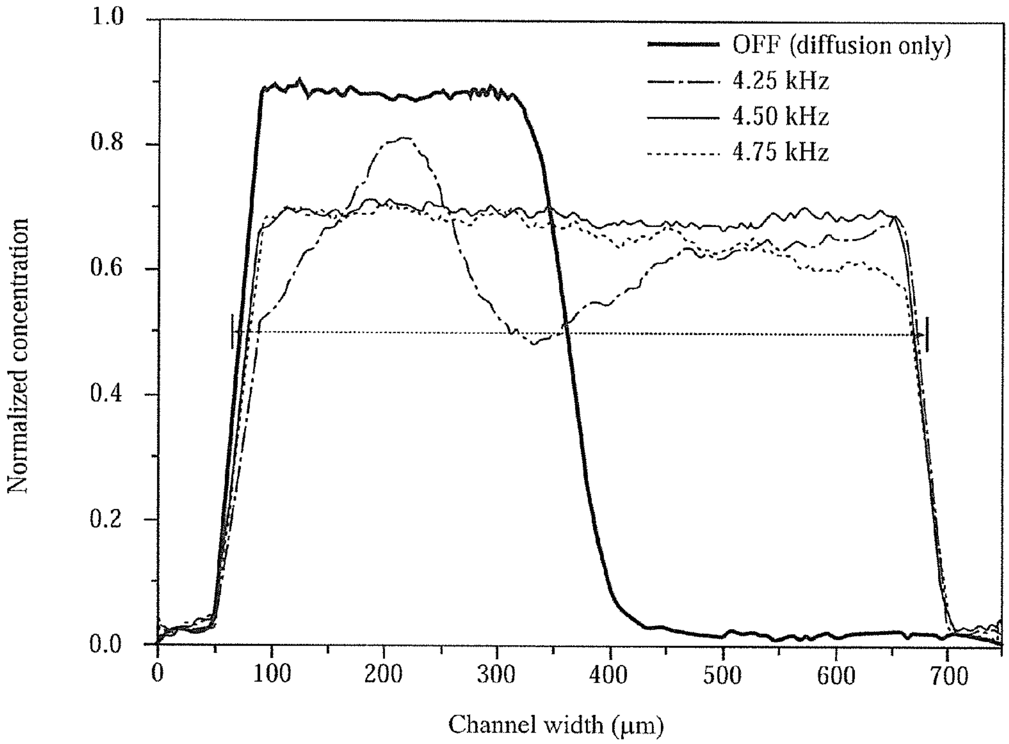

1.0
0.8
0.6
0.4
0.2
0.0
Normalized concentration
0
100
200
300
400
500
600
700
Channel width (μm)
OFF (diffusion only)
4.25 kHz
4.50 kHz
4.75 kHz

FIG. 3E

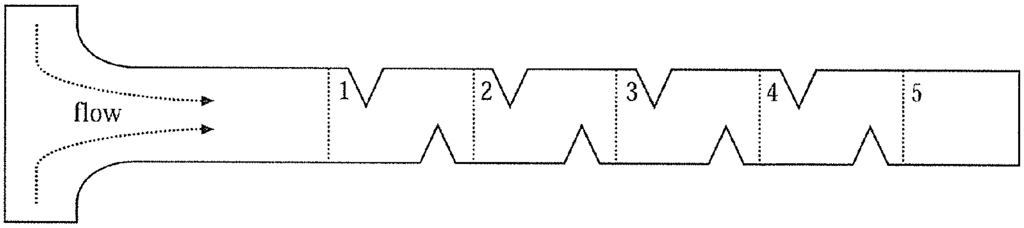

flow
1
2
3
4
5

FIG. 4A

FIG. 4B

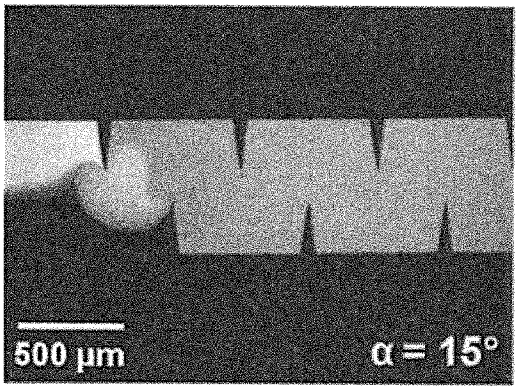
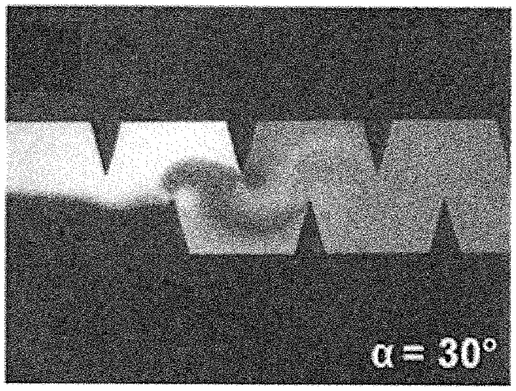


FIG. 4C



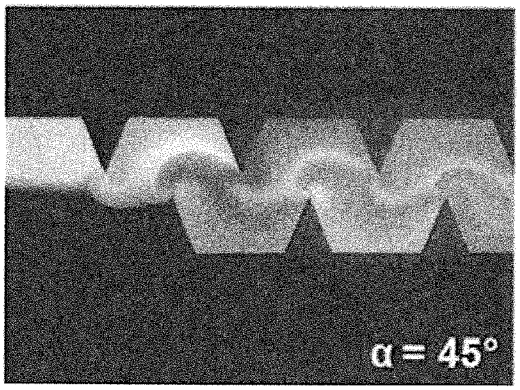


FIG. 4D

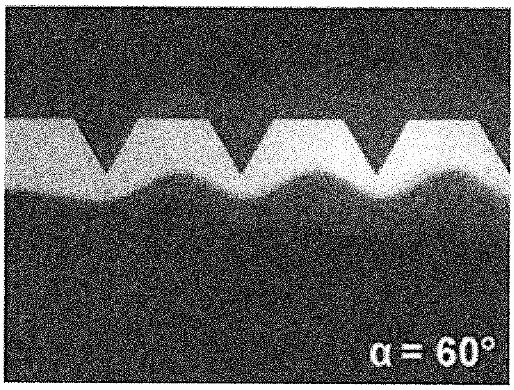


FIG. 4E

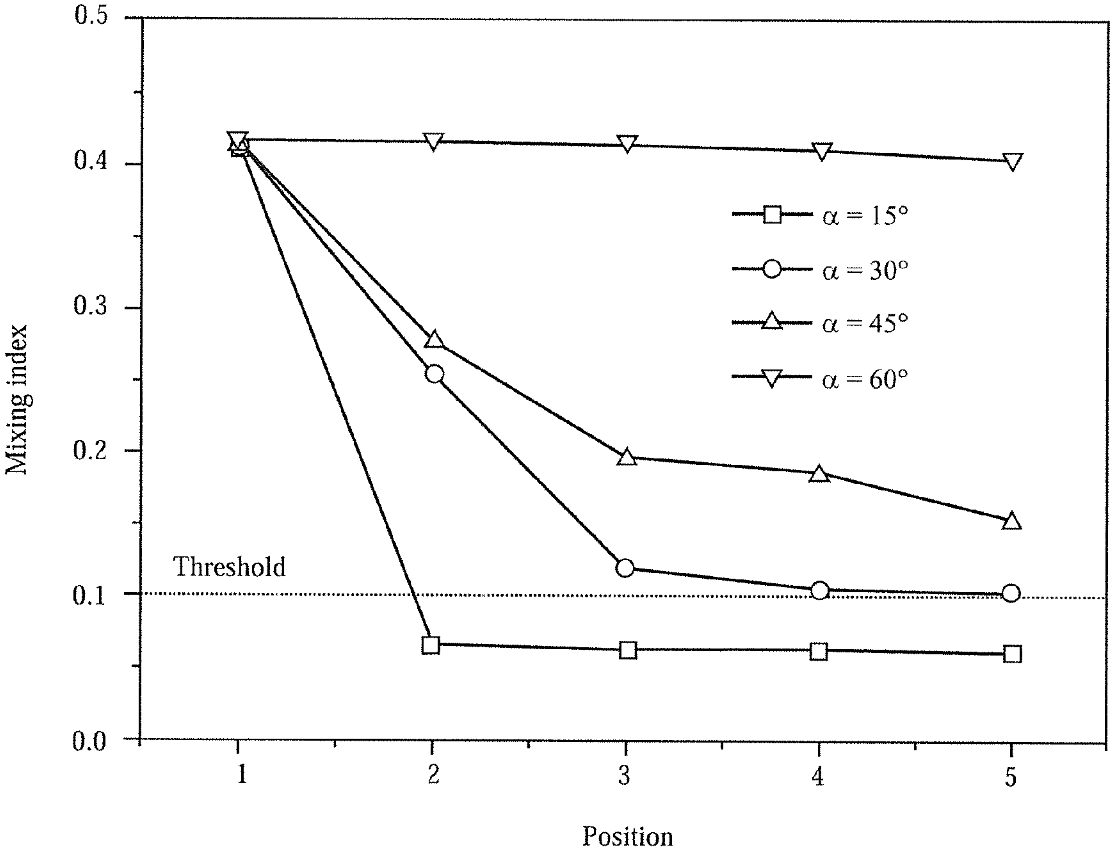

0.5
0.4
0.3
0.2
0.1
0.0
Mixing index
Threshold
1
2
3
4
5
Position
α = 15°
α = 30°
α = 45°
α = 60°

FIG. 4F

FIG. 5A

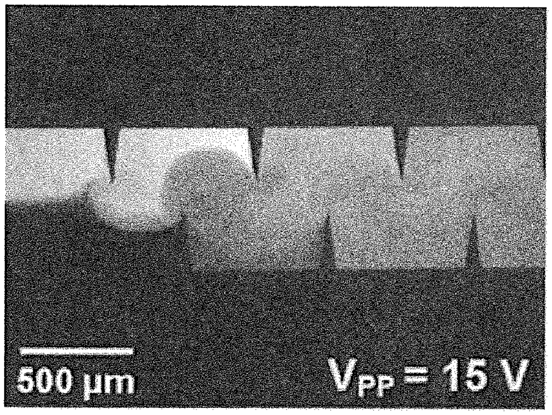
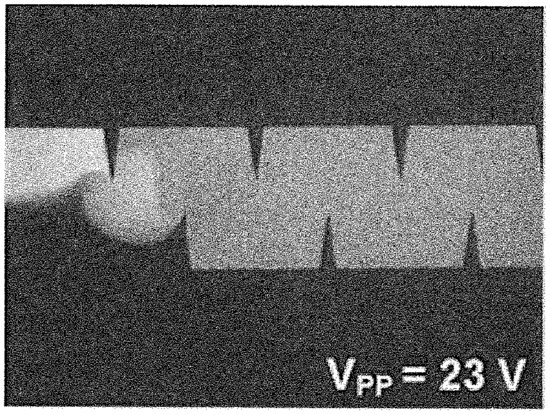


FIG. 5B



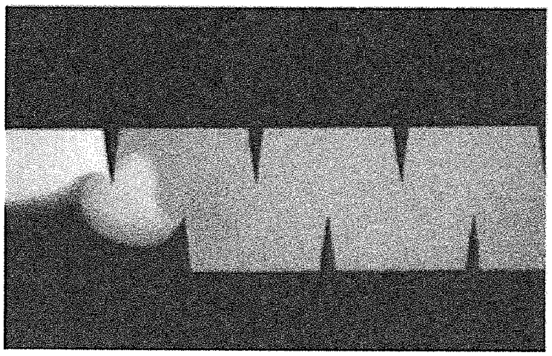


FIG. 5C

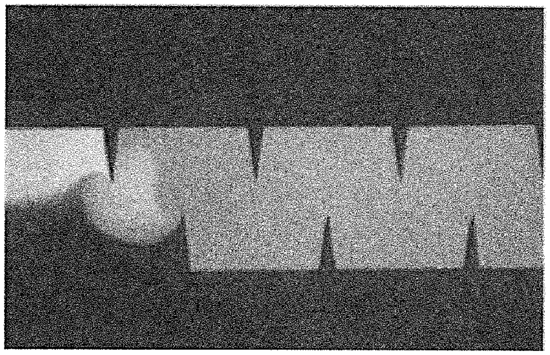


FIG. 5D

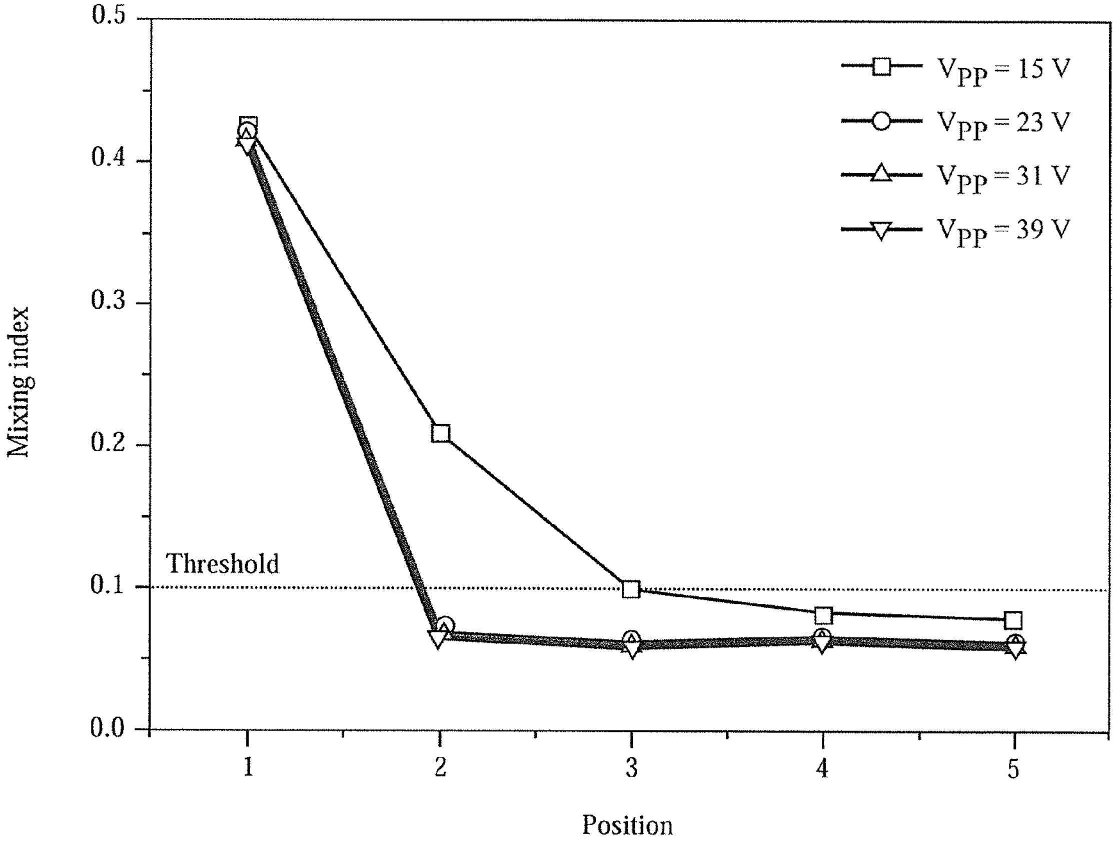

0.5
0.4
0.3
0.2
0.1
0.0
Mixing index
Threshold
1
2
3
4
5
Position
V_{PP} = 15 V
V_{PP} = 23 V
V_{PP} = 31 V
V_{PP} = 39 V

FIG. 5E

FIG. 6A

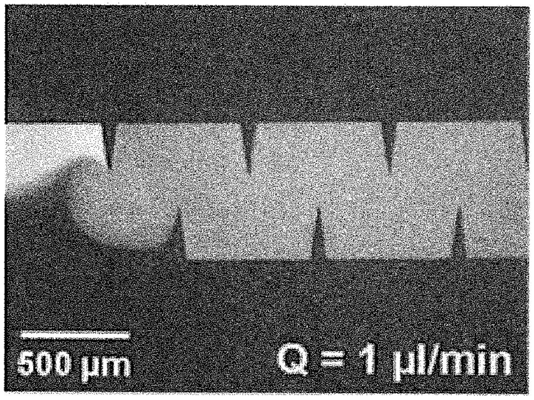
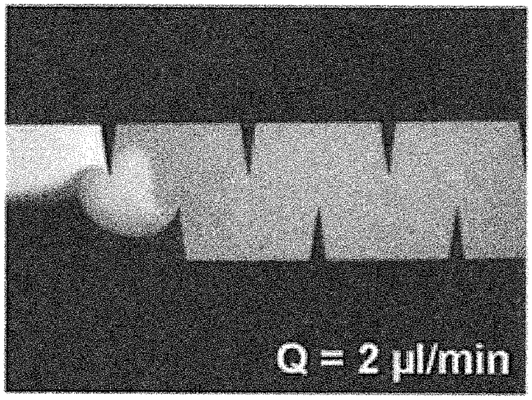


FIG. 6B



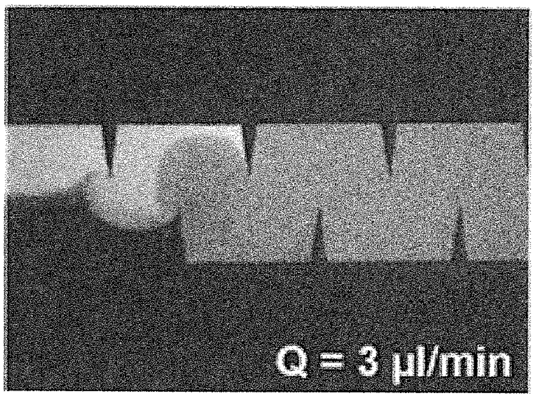


FIG. 6C

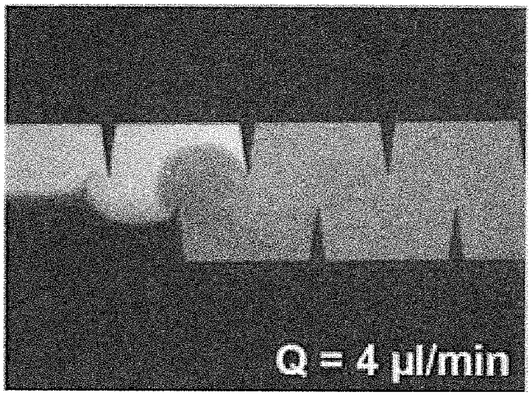


FIG. 6D

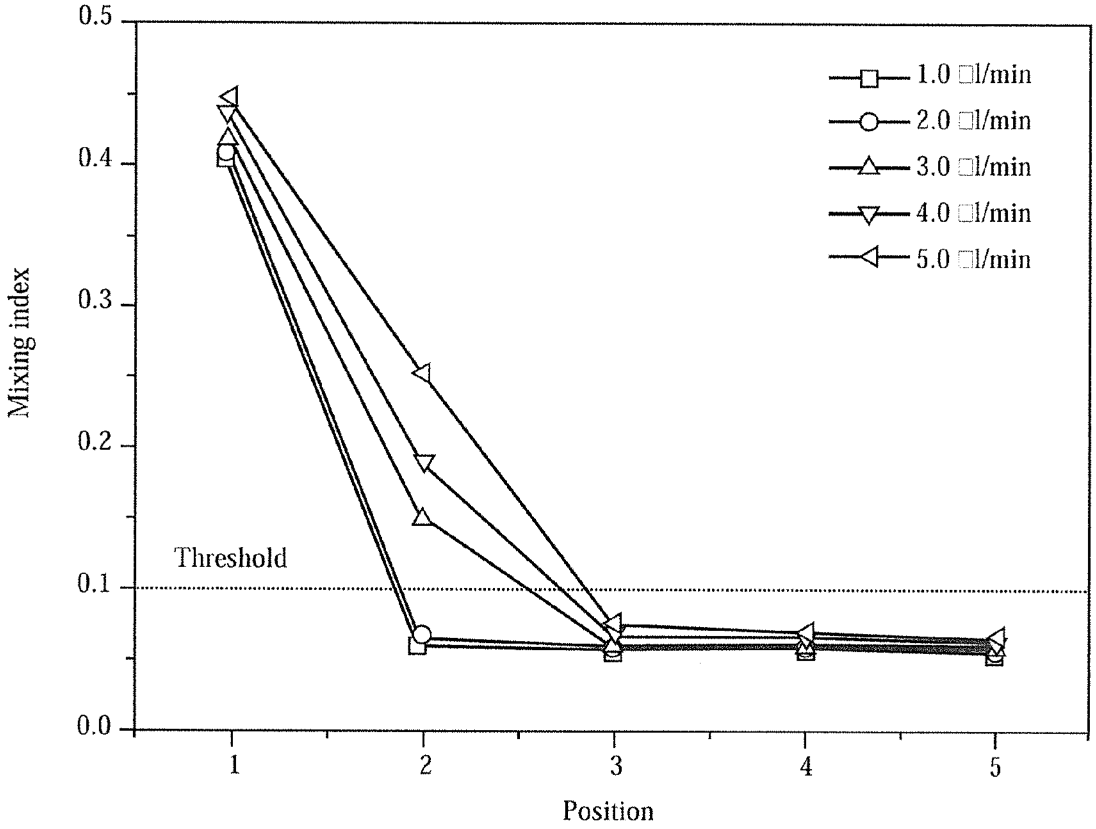
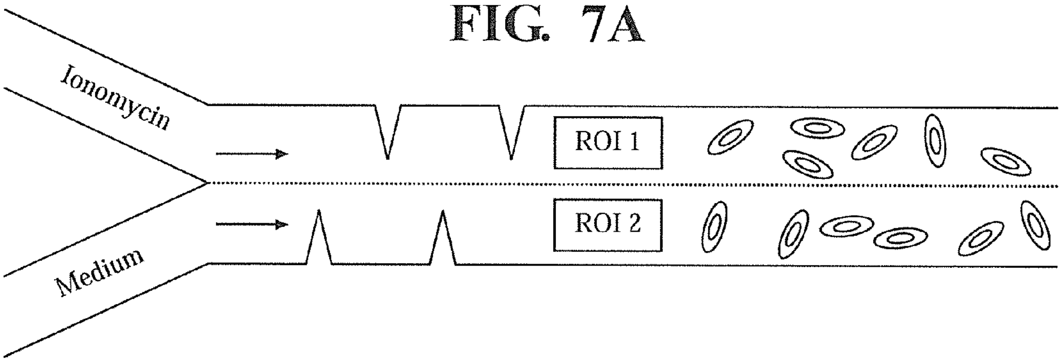

Mixing index
0.5
0.4
0.3
0.2
0.1
0.0
Position
1
2
3
4
5
Threshold
1.0 □l/min
2.0 □l/min
3.0 □l/min
4.0 □l/min
5.0 □l/min

FIG. 6E

FIG. 7A



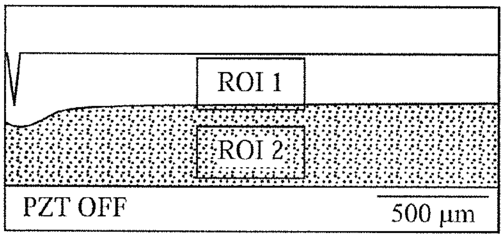


FIG. 7B

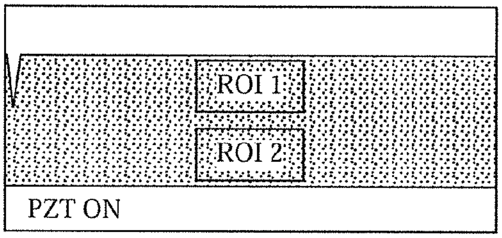


FIG. 7C

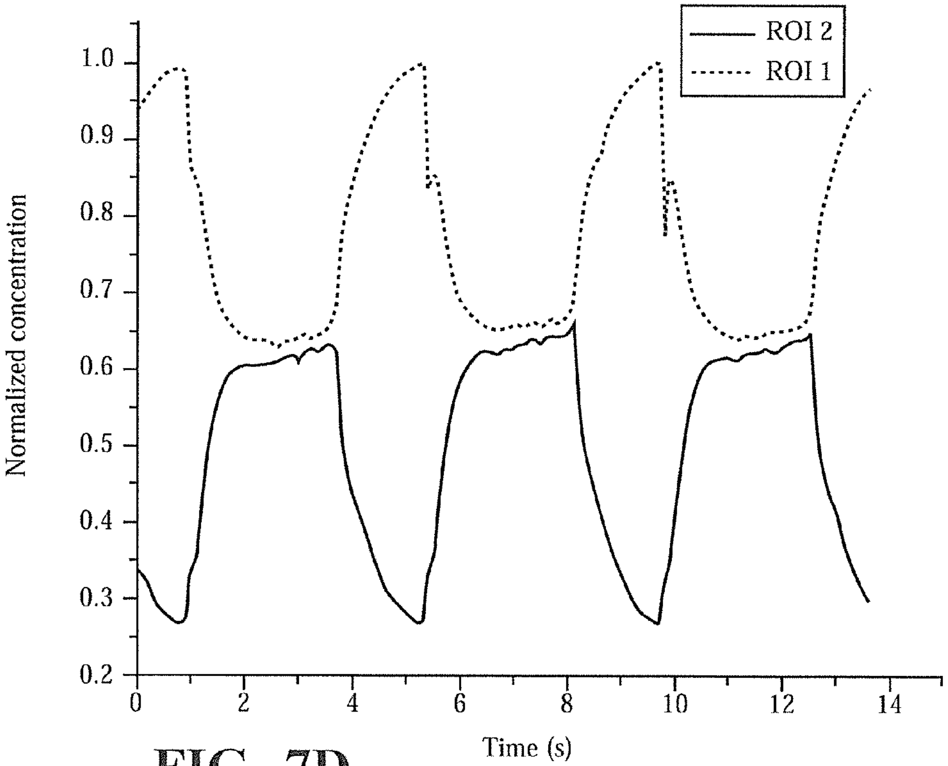


FIG. 7D

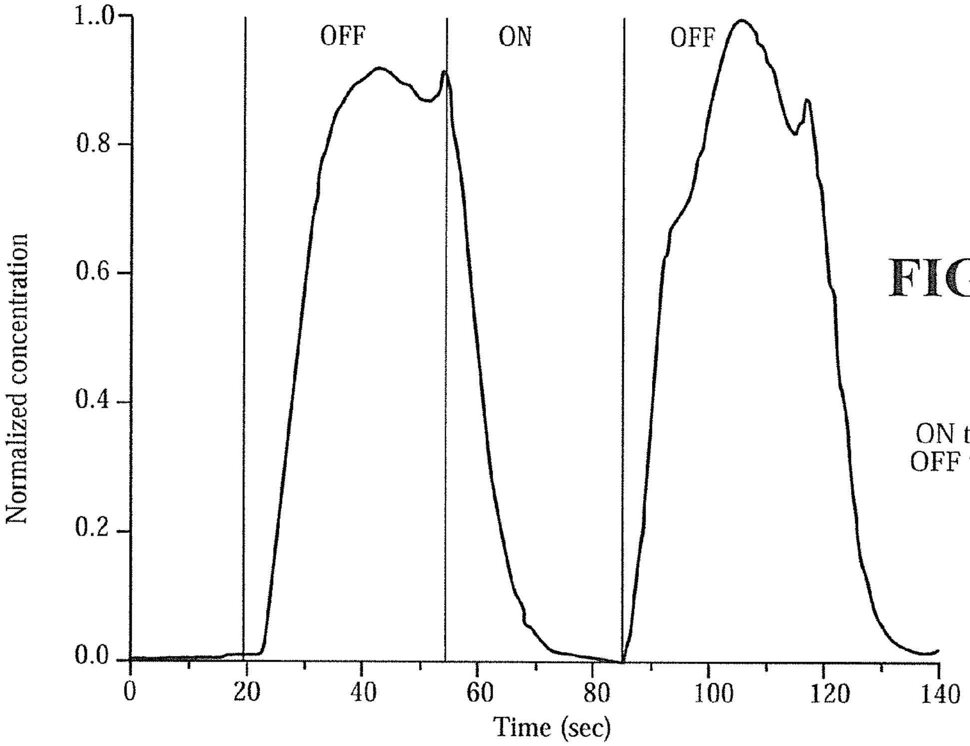


FIG. 8A

ON time: 30 sec
OFF time: 30 sec

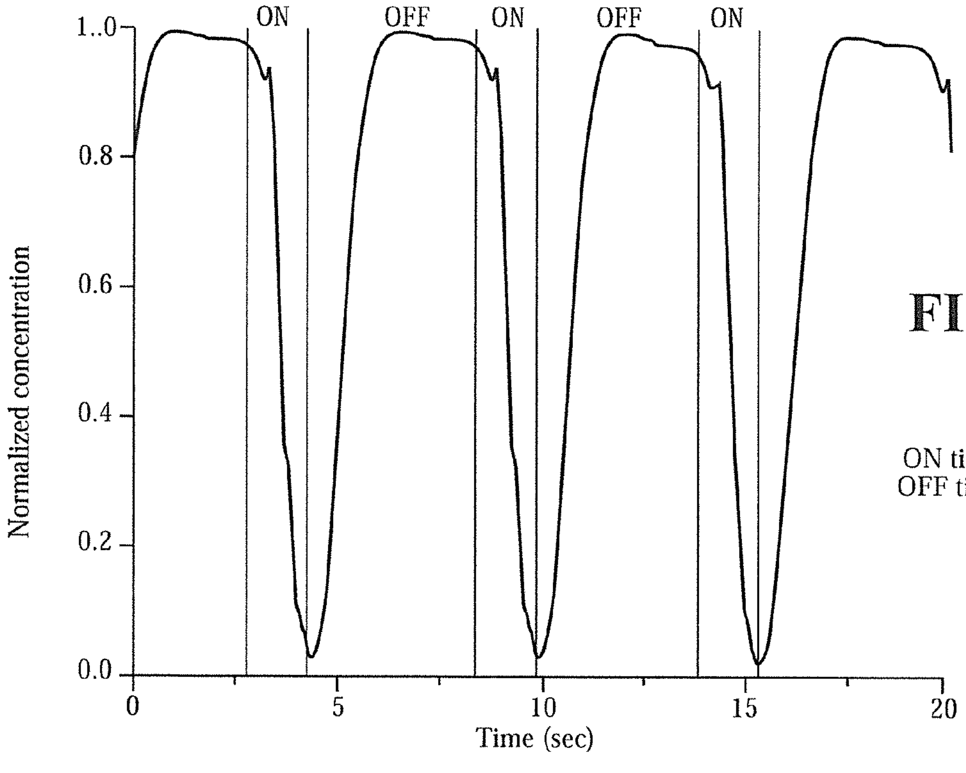


FIG. 8B

ON time: 1.0 sec
OFF time: 4.0 sec

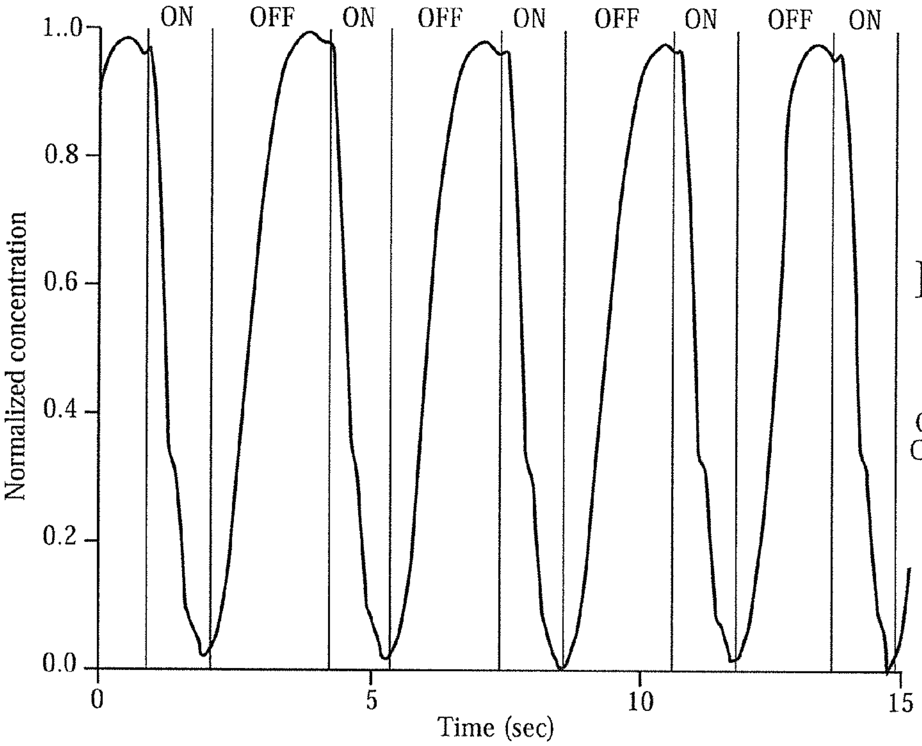


FIG. 8C

ON time: 1.0 sec
OFF time: 2.0 sec

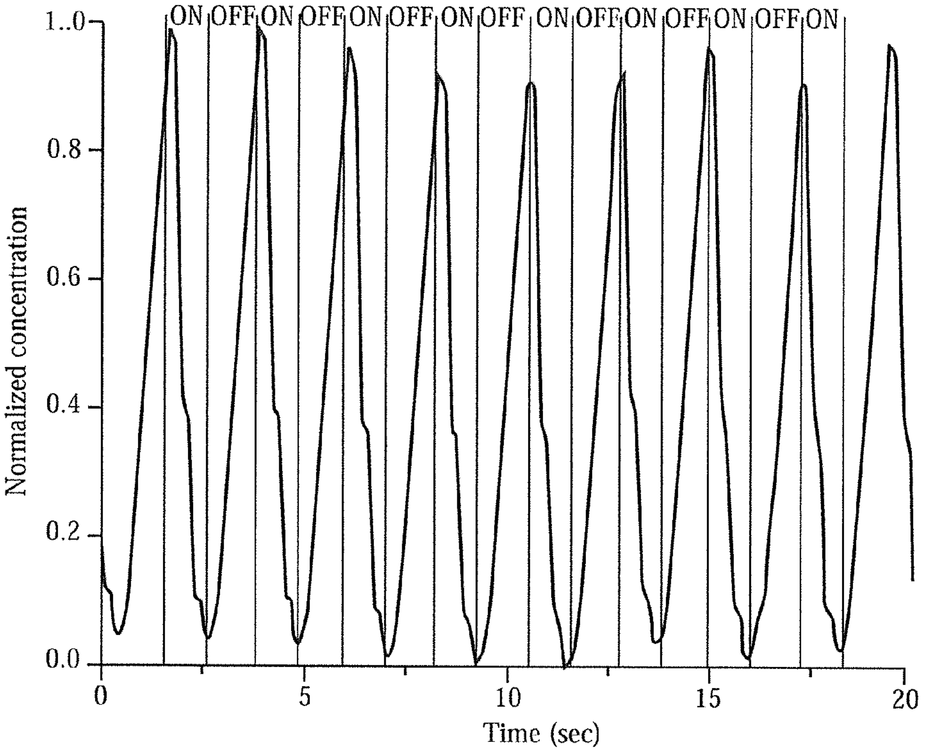


FIG. 8D

ON time: 0.5 sec
OFF time: 0.5 sec

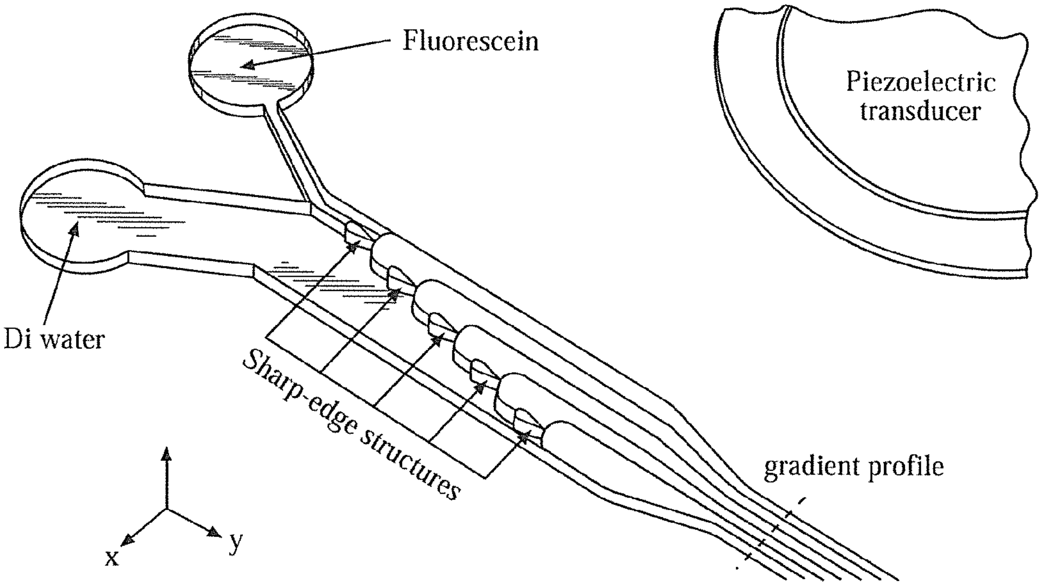

Fluorescein
Piezoelectric transducer
Di water
Sharp-edge structures
gradient profile
x
y

FIG. 9A

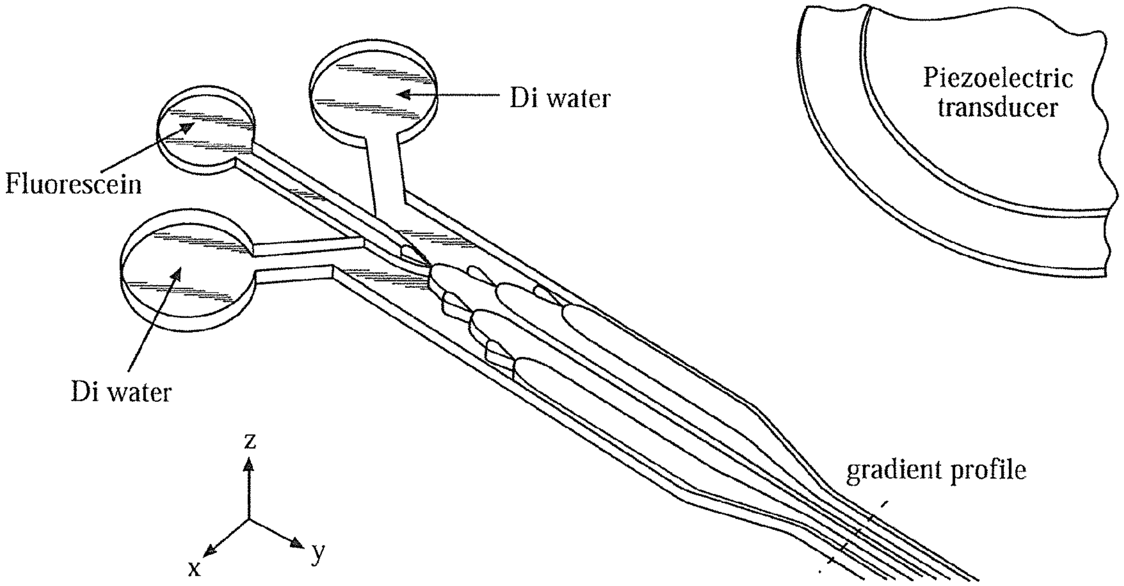

Di water
Piezoelectric transducer
Fluorescein
Di water
z
gradient profile
x
y

FIG. 9B

FIG. 10A

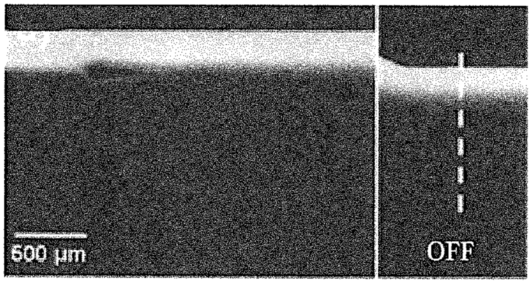
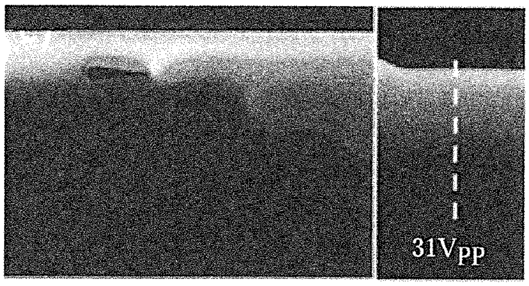


FIG. 10B



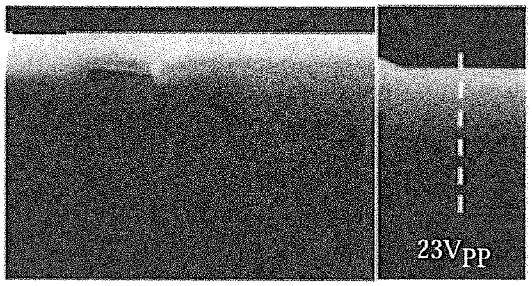


FIG. 10C

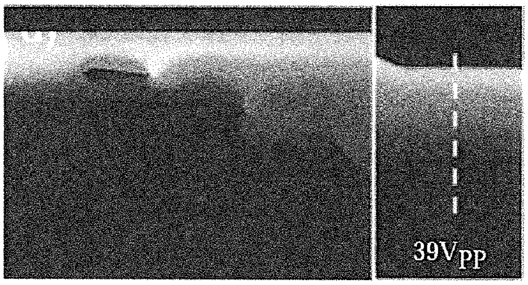


FIG. 10D

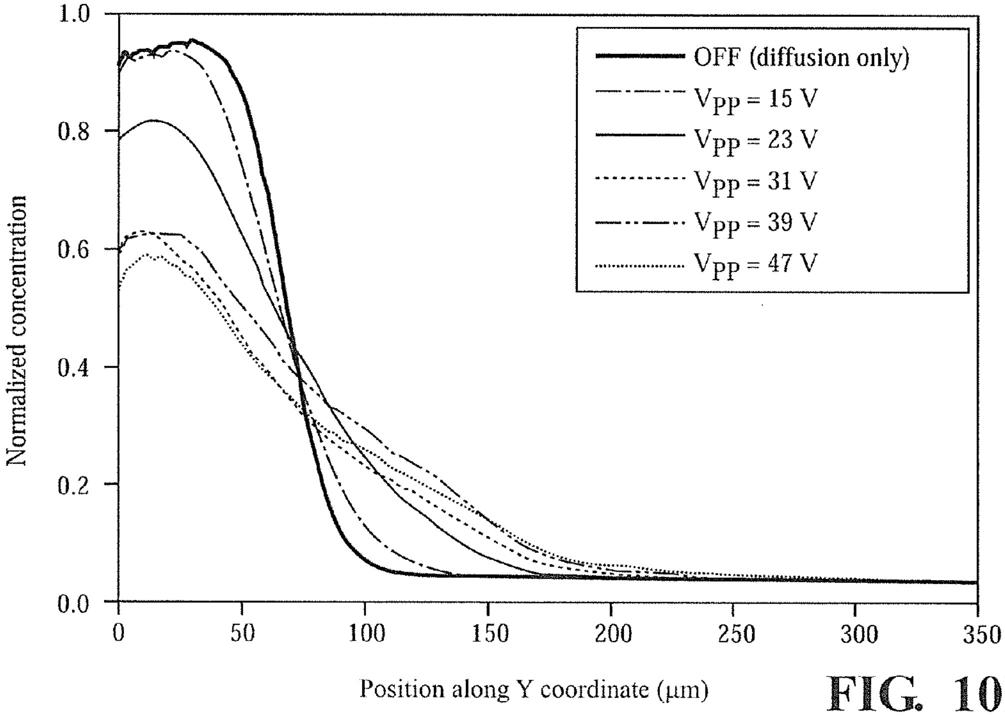


FIG. 10E

FIG. 11A

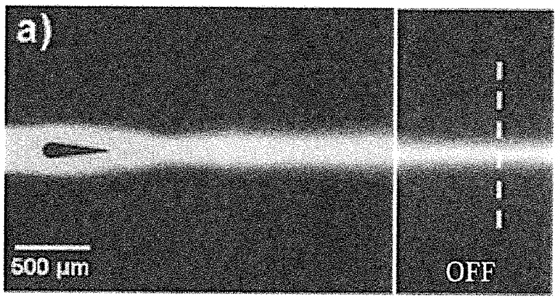
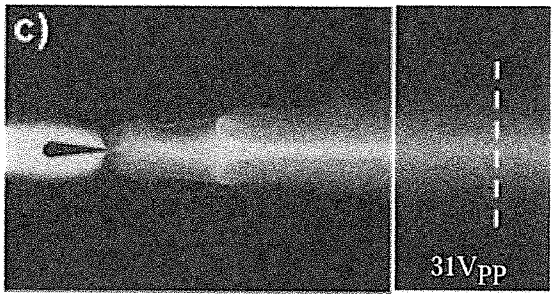


FIG. 11B



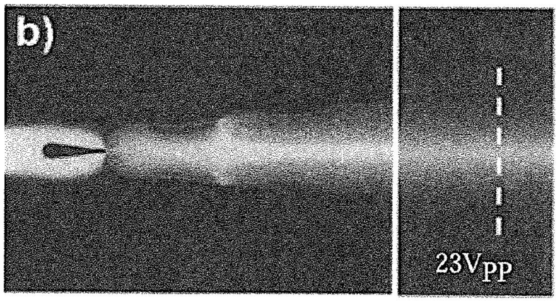


FIG. 11C

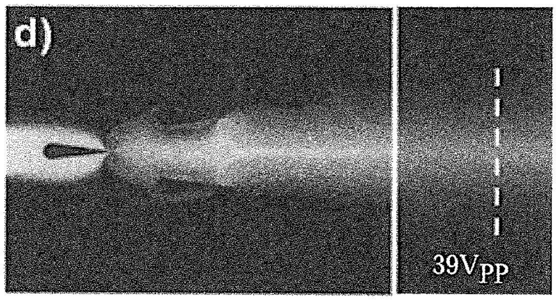


FIG. 11D

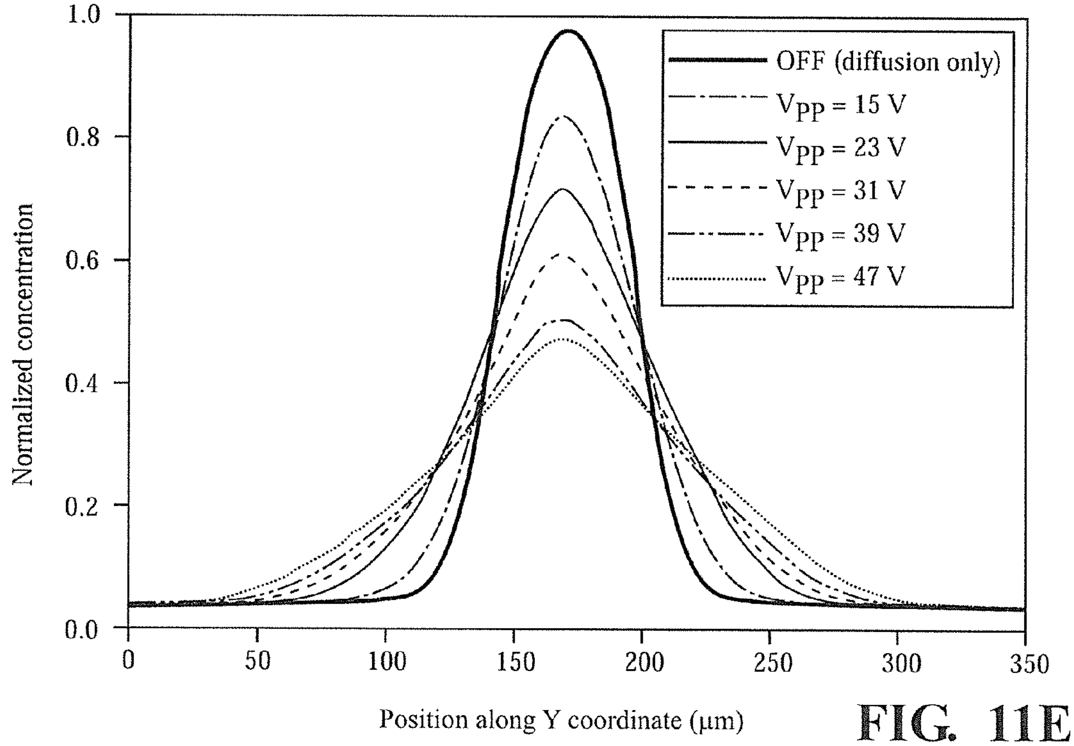
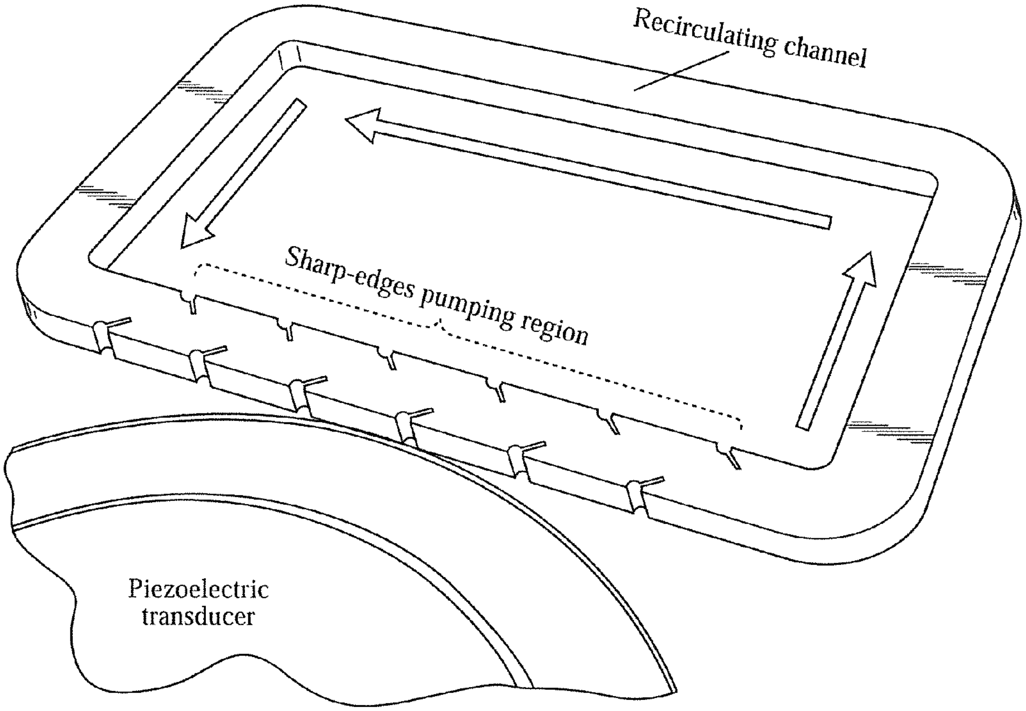


FIG. 11E

FIG. 12A



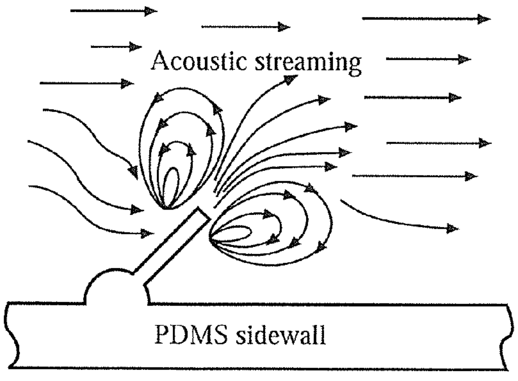


FIG. 12B

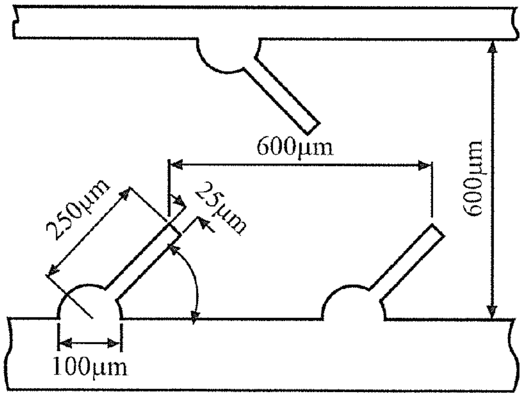


FIG. 12C

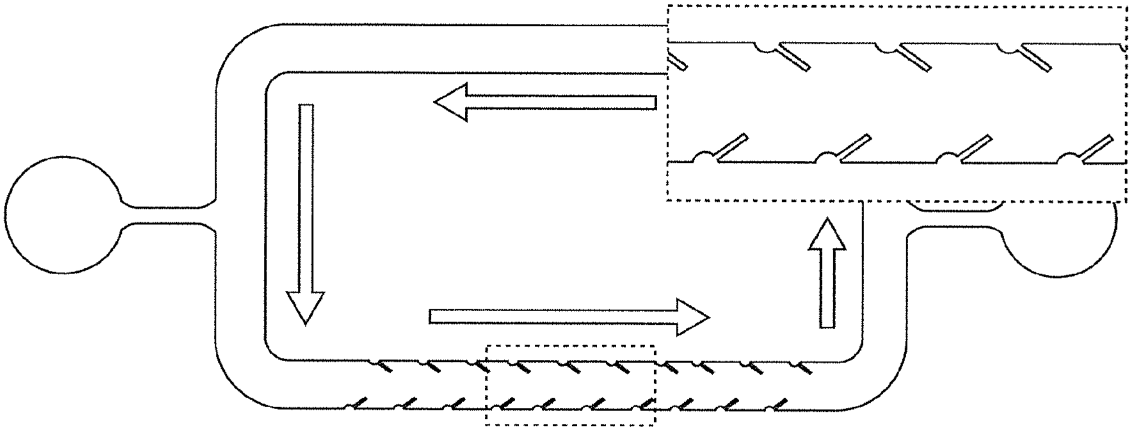


FIG. 13A

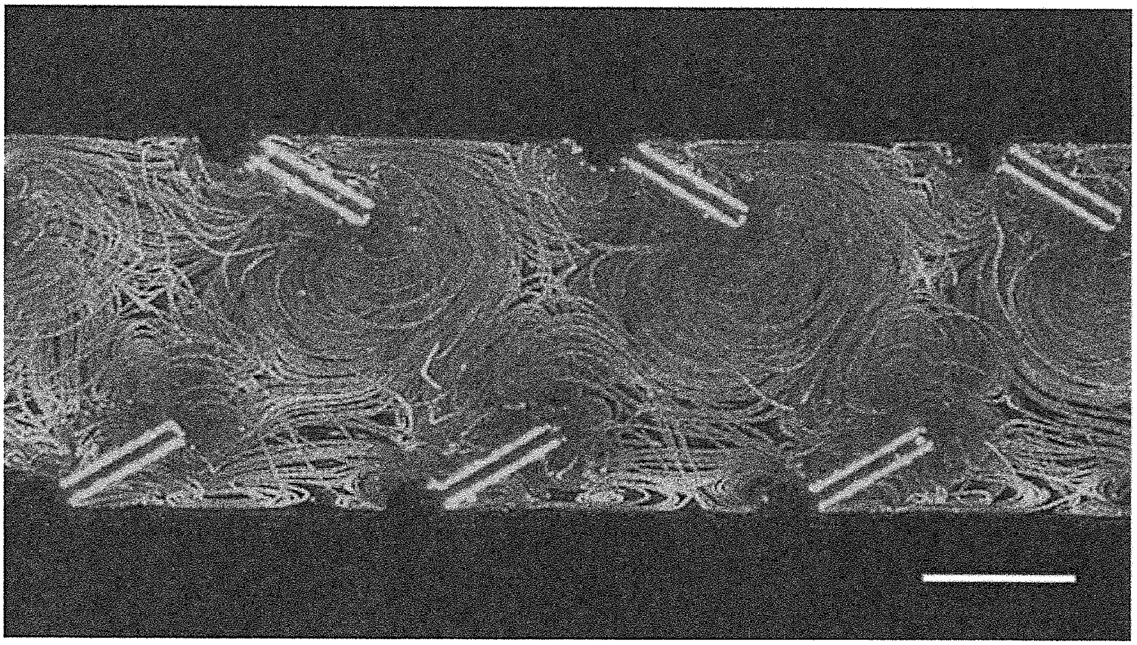


FIG. 13B

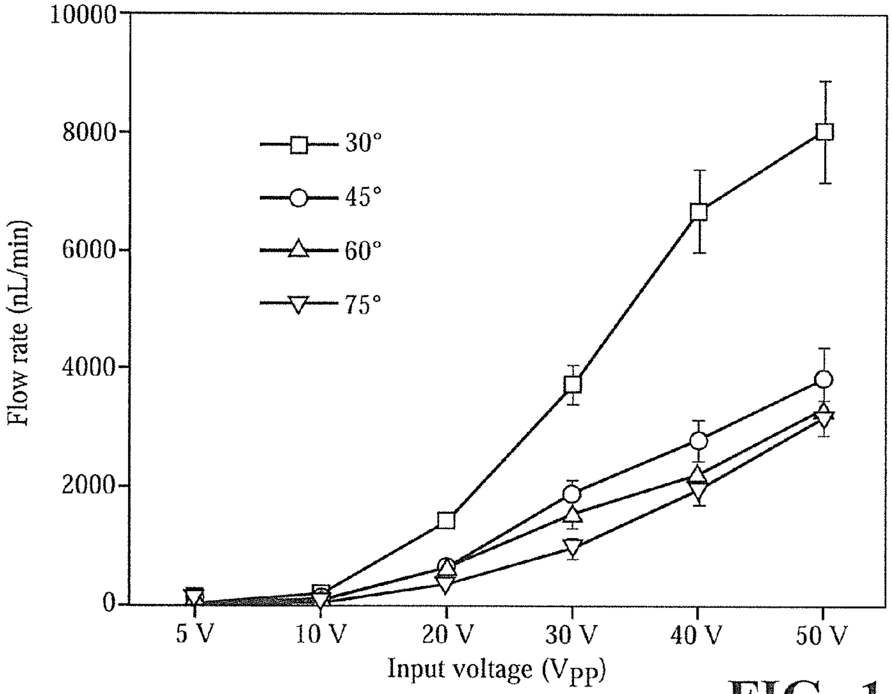


FIG. 14A

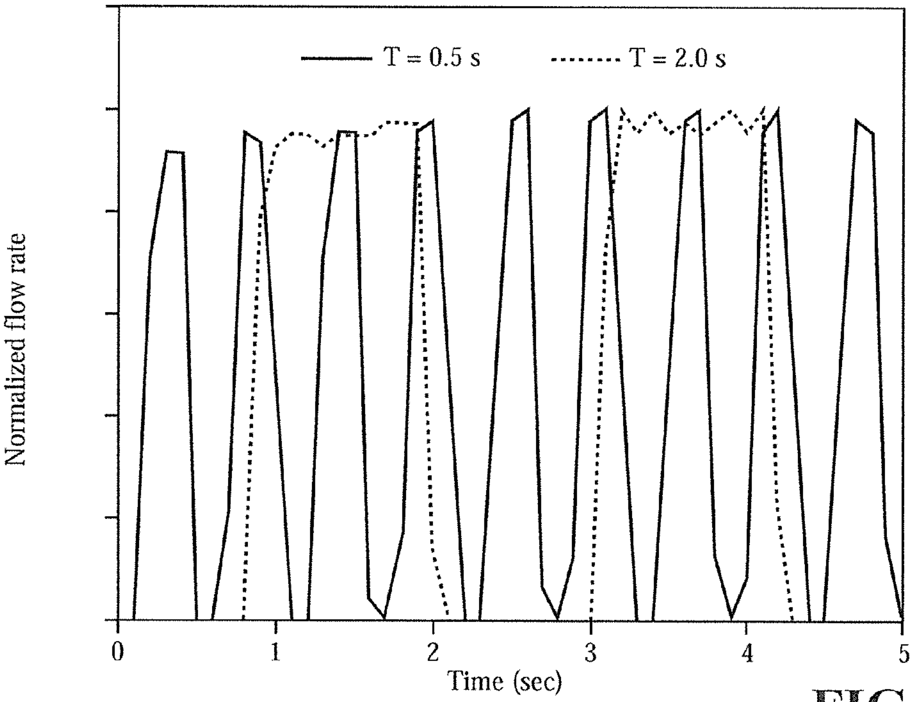


FIG. 14B

APPARATUSES AND METHODS FOR MODULATING FLUIDS USING ACOUSTICALLY OSCILLATING SOLID STRUCTURES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application depends from and claims priority to U.S. Patent Application No: 61/884,907 filed Jul. 11, 2013, the entire contents of which are incorporated herein by reference.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under Grant No. ECCS0824183, awarded by the National Science Foundation and Grant No. OD007209 awarded by the National Institutes of Health. The Government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] Modulation of fluid flow in a microfluidic channel is demonstrated via the acoustic microstreaming phenomenon induced by the oscillation of oscillatory elements. By optimizing the design of the oscillatory elements, excellent fluid modulation performance can be achieved in a simple device, making the acustofluidic apparatus a promising candidate for a wide variety of applications.

BACKGROUND OF THE INVENTION

[0004] The ability to achieve rapid and homogeneous mixing of chemical/biological species enables a wide variety of applications, such as chemical kinetic studies[1,2] and nanomaterial synthesis.[3-7] While microfluidic devices seem to be an excellent platform for carrying out these studies due to their short reaction times, high throughput, and reduced reagent consumption, effectively mixing fluids at the microscale is not a trivial process.[8-16] Due to inherently small channel dimensions, the flow of fluid in microfluidic devices is usually laminar. Under laminar flow conditions viscous forces dominate over inertial forces and fluids are not easily mixed. In order to enable microfluidic applications that require mixing, a number of micromixing methods have been reported. These methods include: chaotic advection,[17-21] hydrodynamic focusing,[22-25] electrokinetically driven mixing,[26-31] 3D combinatorial mixing,[32,33] meandering channels as well as magnetically,[34,35] thermally,[36] and optically[37] induced mixing.

[0005] Recently, acoustic-based micromixers have attracted significant attention due to their non-invasive nature[38-42] and simple mixing mechanism. In acoustic-based mixers, acoustic waves propagate into fluid media and induce pressure fluctuations, resulting in the disturbance of the laminar-flow pattern to facilitate mixing.[43-49] The mixing performance of acoustic-based mixers can be further improved through the use of bubbles in the microfluidic channel. When bubbles are coupled with an acoustic wave, the acoustic streaming phenomenon[50] is developed. This phenomenon results in a more prominent perturbation of the surrounding fluids, greatly facilitating the mass transport of fluids. Thus far, bubble-based acoustic mixers[51-54] have been used for characterizing enzyme reactions,[2] enhancing DNA hybridization,[51,55] generating chemical gradients,[56] and developing advanced optofluidic devices.[57] Although acoustically driven, bubble-based micromixers have shown tremendous potential in a wide variety of applications, there are many concerns regarding bubble instability,[53,57] heat generation,[48] and inconvenient bubble-trapping processes. To take advantage of acoustic streaming without the drawbacks of microbubbles,[58-61] there is a need to explore alternative methods that can effectively and conveniently generate acoustic micromixing.

[0006] In addition to mixing of fluids on the microscale level, significant efforts have been made towards developing reliable, robust microfluidic pumps. Prior pumps have been characterized as either active or passive. Passive pumps such as surface-tension based microfluidic pumps offer several advantages including their simple operation, low cost and semi-automation; however, they are vulnerable to evaporation caused by environmental changes, and to a flow rate changing with time. Active pumps that use mechanical or electrical systems to initiate fluid pumping may potentially provide solutions to the challenges that passive pumps encounter. Active pumps offer the advantages of flexibility in terms of temporal control of pumping behaviour and adjustable flow rates. Most prior active pump systems require sophisticated fabrication processes for patterning electrodes and for making multi-layer devices, complicated optical setups, or well-trained personnel to operate the devices, limiting active pumps from being developed and integrated as an “on-chip” pumping unit for handheld, portable platforms.

[0007] Overall, there is a great need for mechanisms that can be used for modulating fluids on the microscale level that are robust, simple to manufacture and operate, and have excellent flexibility for a broad range of applications such as inducing mixing, generating concentration or temporal gradient profiles, generating fluid flow such as in a pumping apparatus, producing activity in rapid on/off format for usefulness in waveform control, and other possible uses.

SUMMARY OF THE INVENTION

[0008] The following summary of the invention is provided to facilitate an understanding of some of the innovative features unique to the present invention and is not intended to be a full description. A full appreciation of the various aspects of the invention can be gained by taking the entire specification, claims, drawings, and abstract as a whole.

[0009] Novel apparatuses and methods are provided to control a chemical microenvironment using oscillatory elements capable of oscillating in an acoustic field where the oscillation is useful to produce arbitrary temporal waveforms in a flow system such as buffer solutions. Examples of the invention include using an acoustically activated, oscillatory element based microfluidic system for generating arbitrary temporal chemical waveforms (both digital and analog) by mixing the stimuli and buffer solutions in a time-dependent fashion. This approach permits continuous modulation of the signal characteristics including shape, frequency, amplitude, and duty cycle, with frequencies reaching up to 30 Hz, and in some examples frequencies greater than 30 Hz.

[0010] By incorporating oscillatory structures in ladder-like, parabolic, or other arrangements into a single fluid channel, both static and pulsatile chemical gradients are achievable. With its advantages in functionality and versatility, the chemical waveform generation and switching methods presented herein are powerful tools that may be used in many biological and chemical applications.

[0011] As such, it is a first object of the invention to provide an apparatus useful for inducing microstreaming in a fluid. An apparatus is provided that is useful for many such processes including a fluid passage, said fluid passage comprising at least one sidewall, at least one inlet, and at least one outlet; an oscillatory element comprising a tip extending into said fluid passage; and an acoustic source in acoustic contact with said oscillatory element, said acoustic source operable to vibrate said oscillatory element or a portion thereof, so as to create microstreaming in a fluid within said fluid passage. The oscillatory element optionally includes two adjacent surfaces extending between said sidewall and said tip, said adjacent surfaces being disposed at a tip angle with respect to each other, the tip angle being less than 180 degrees, optionally less than or equal to 45 degrees, optionally less than or equal to 30 degrees, optionally less than or equal to 15 degrees. I some embodiments, the surface is not in the form or a point, but instead has a surface with a width and a length, the length is optionally but not required to be equal to the height of said fluid channel. Optionally, the width is from 5 to 100 micrometers. In some embodiments, the oscillatory element is extending at a tilting angle relative to the length of said sidewall, said tilting angle being 90 degrees or less, optionally any angle in either direction from 0 degrees to 70 degrees. In some embodiments, the apparatus includes a plurality of oscillatory elements with two or more of said oscillatory elements having different oscillating frequencies. An acoustic source is optionally a piezoelectric transducer. In some embodiments, an acoustic source is operable to vibrate the oscillatory element at a frequency in the range of 2 Hz to 900 MHz, optionally at least 1 kHz. It is appreciated that any of the claimed elements can be combined in ways other than explicitly recited herein.

[0012] It is another object of the invention to provided methods of inducing microstreaming in a fluid. Provided are processes of inducing microstreaming in a fluid including contacting a first fluid in a fluid passage with a oscillatory element; and acoustically oscillating said oscillatory element with an acoustic source in acoustic contact with said oscillatory element so as to induce microstreaming in said first fluid. In some embodiments, the oscillatory element comprises a tip angle of 45 degrees or less. Optionally, the oscillatory element is oriented relative to an edge of said fluid passage by a tilting angle from 0 degrees to 70 degrees. Optionally, the oscillatory element oscillates at a frequency in the range of 2 Hz to 900 MHz, optionally 1 kHz, optionally more than 1 kHz. In some embodiments, the microstreaming induces mixing of said first fluid and a second fluid, and optionally a third fluid, within said fluid channel. In some embodiments, the microstreaming induces flow of the fluid in the fluid channel by the microstreaming being directionally oriented. Optionally, a process induces both pumping (e.g. fluid flow) and mixing of a first fluid and a second fluid. The process optionally produces a concentration gradient of a component of said first fluid, said second fluid, or both. Optionally, the process further includes contacting said first fluid and said second fluid with a plurality of oscillatory elements off set and generating a gradient profile between said first fluid and said second fluid. Optionally, the process further includes producing a concentration gradient of a component of said first fluid, said second fluid, optionally said third fluid, or any combination thereof. The plurality of oscillatory elements are optionally arranged in an angled ladder configuration, a parabolic configuration, or a V form. A process optionally includes alternating said acoustic source from an on state to an off state to produce a temporal gradient waveform of concentration of said first fluid and said second fluid. As such, the microstreaming is optionally operable to produce fine or gross gradients of concentration between two or more fluids in a flow passage that is useful for many assays such as cellular or other bio assays, binding of particular element, or many other uses. Fluid can be simultaneously mixed and pumped in a channel, or either mixed or pumped at one or other locations. Some embodiments induce mixing. Some embodiments, inducing pumping of fluid. Some embodiments induce both mixing and pumping. Rapid control over on/off status of the acoustic source allows both temporal and special control of element concentrations in a region of interest allowing generation of chemical waveforms that can be used for many types of studies.

[0013] It is another object of the invention to provide processes for pumping a fluid through a channel, optionally a microchannel, including contacting a fluid in a fluid passage with an oscillatory element, said oscillatory element, or oscillating portion thereof, oriented relative to an edge of said fluid passage by a tilting angle from 0 degrees to 180 degrees; and acoustically oscillating said oscillatory element with an acoustic source in acoustic contact with said oscillatory element so as to induce directional microstreaming in said fluid. In the processes, the oscillatory element(s) are optionally at a tilting angle of 0 degrees to 70 degrees in either direction relative to the edge of a flow passage. Optionally, the oscillatory element oscillates at a frequency in the range of 2 Hz to 900 MHz, optionally at least 1 kHz. Pumping is induced by the oscillation of the fluid passages. Pumping is optionally tightly controlled in time and space by controlling the off/on status of the acoustic source so as to be capable of generating temporal waveforms of fluid flow at desired locations in a flow passage. The pumping action is optionally used with a mixing action between two or more fluids containing different chemical (optionally biological) components.

[0014] Overall, the apparatuses and processes provided address many issues with prior devices including simple and low cost manufacture, tight regulation of activity, rapid on/off responses, and the ability to generate fine chemical gradient profiles.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. **1**A illustrates an acoustofluidic apparatus according to one embodiment;

[0016] FIG. **1**B is a schematic of exemplary microstreaming produced by oscillation of a oscillatory element acoustofluidic apparatus according to one embodiment;

[0017] FIG. **1**C illustrates the dimensions and spacing of a fluid passage and oscillatory elements in an acoustofluidic apparatus according to one embodiment;

[0018] FIG. **1**D illustrates the dimensions and spacing of a fluid passage and oscillatory elements arranged at a tilting angle in an acoustofluidic apparatus configured as a micropump according to one embodiment;

[0019] FIG. **2**A illustrates laminar flow of fluid in a fluid passage when an exemplary acustofluidic device is not activated;

[0020] FIG. **2**A illustrates laminar flow of fluid in a fluid passage when an acustofluidic device is activated to cause oscillation of the oscillatory elements in the fluid passage producing microstreaming of the fluid about the oscillatory elements;

[0021] FIG. **3**A illustrates laminar flow of two adjacent fluids introduced by two separate inlets into a fluid passage when an exemplary acustofluidic device is not activated with the dashed line depicting a region of interest for the quantification of mixing of the two flows;

[0022] FIG. **3**B illustrates incomplete mixing of two adjacent fluids introduced by two separate inlets into a fluid passage when an exemplary acustofluidic device is activated with an acoustic frequency of 4.25 kHz;

[0023] FIG. **3**C illustrates complete mixing of two adjacent fluids introduced by two separate inlets into a fluid passage when an exemplary acustofluidic device is activated with an acoustic frequency of 4.5 kHz;

[0024] FIG. **3**D illustrates complete mixing of two adjacent fluids introduced by two separate inlets into a fluid passage when an exemplary acustofluidic device is activated with an acoustic frequency of 4.75 kHz;

[0025] FIG. **3**E illustrates the normalized dye concentration profile across the flow passage width for the three driving frequencies of 4.25 kHz, 4.50 kHz, and 4.75 kHz;

[0026] FIG. **4**A illustrates an acoustofluidic apparatus according to one embodiment used to quantitatively characterize the mixing performance along the entire length of the flow passage with 5 regions of interest indicated as **1**, **2**, **3**, **4**, and **5**;

[0027] FIG. **4**B illustrates mixing with a tip angle of 15 degrees demonstrating complete mixing by position **2**;

[0028] FIG. **4**C illustrates mixing with a tip angle of 30 degrees demonstrating complete mixing by position **5**;

[0029] FIG. **4**D illustrates mixing with a tip angle of 45 degrees;

[0030] FIG. **4**E illustrates no observable mixing with a tip angle of 60 degrees;

[0031] FIG. **4**F illustrates the mixing performance of an exemplary acoustofluidic apparatus dependent on tip angle;

[0032] FIG. **5**A illustrates mixing in an acoustofluidic device with a tip angle of 15 degrees oscillated by a transducer with a driving voltage of 15V;

[0033] FIG. **5**B illustrates mixing in an acoustofluidic device with a tip angle of 15 degrees oscillated by a transducer with a driving voltage of 23V;

[0034] FIG. **5**C illustrates mixing in an acoustofluidic device with a tip angle of 15 degrees oscillated by a transducer with a driving voltage of 31 V;

[0035] FIG. **5**D illustrates mixing in an acoustofluidic device with a tip angle of 15 degrees oscillated by a transducer with a driving voltage of 39V;

[0036] FIG. **5**E illustrates the mixing performance of an exemplary acoustofluidic apparatus dependent on driving voltage;

[0037] FIG. **6**A illustrates mixing in an acoustofluidic device with a tip angle of 15 degrees and a flow rate of 1 μl/min with a driving frequency of 4.50 kHz and a driving voltage of 31 Vpp;

[0038] FIG. **6**B illustrates mixing in an acoustofluidic device with a tip angle of 15 degrees and a flow rate of 2 μl/min with a driving frequency of 4.50 kHz and a driving voltage of 31 Vpp;

[0039] FIG. **6**C illustrates mixing in an acoustofluidic device with a tip angle of 15 degrees and a flow rate of 3 μl/min with a driving frequency of 4.50 kHz and a driving voltage of 31 Vpp;

[0040] FIG. **6**D illustrates mixing in an acoustofluidic device with a tip angle of 15 degrees and a flow rate of 4 μl/min with a driving frequency of 4.50 kHz and a driving voltage of 31 Vpp;

[0041] FIG. **6**E illustrates the mixing performance of an exemplary acoustofluidic apparatus dependent on fluid flow rate;

[0042] FIG. **7**A is a schematic of an exemplary acoustofluidic device and two regions of interest (ROI) as used for determining calcium responses of U251 cells due to differing concentrations of agonist as generated by oscillation of the oscillatory elements;

[0043] FIG. **7**B illustrates laminar flow of two fluids when the device of FIG. **7**A is inactive;

[0044] FIG. **7**C illustrates mixing of the two fluids when the device of FIG. **7**A is activated generating an intermediate concentration of agonist at ROI**1** and ROI**2**.

[0045] FIG. **7**D illustrates temporal control over mixing achieving different chemical signal profiles at two the ROIs;

[0046] FIG. **8**A illustrates normalized concentration of agonist at the two ROIs under exemplary temporal control;

[0047] FIG. **8**B illustrates normalized concentration of agonist at the two ROIs under exemplary temporal control;

[0048] FIG. **8**C illustrates normalized concentration of agonist at the two ROIs under exemplary temporal control;

[0049] FIG. **8**D illustrates normalized concentration of agonist at the two ROIs under exemplary temporal control;

[0050] FIG. **9**A illustrates an acoustofluidic apparatus for fine gradient production according to one embodiment;

[0051] FIG. **9**B illustrates an acoustofluidic apparatus for fine gradient production according to one embodiment;

[0052] FIG. **10**A illustrates side-by-side laminar flow of the fluids in the embodiment of FIG. **9**A when the apparatus is not activated;

[0053] FIG. **10**B illustrates mixing of fluids when the apparatus of FIG. **9**A is activated at 23 Vpp;

[0054] FIG. **10**C illustrates mixing of fluids when the apparatus of FIG. **9**A is activated at 31 Vpp;

[0055] FIG. **10**D illustrates mixing of fluids when the apparatus of FIG. **9**A is activated at 39 Vpp;

[0056] FIG. **10**E illustrates the gradient profiles achieved transversely across the fluid passage at various voltages;

[0057] FIG. **11**A illustrates side-by-side laminar flow of the fluids in the embodiment of FIG. **9**B when the apparatus is not activated;

[0058] FIG. **11**B illustrates mixing of fluids when the apparatus of FIG. **9**B is activated at 23 Vpp;

[0059] FIG. **11**C illustrates mixing of fluids when the apparatus of FIG. **9**B is activated at 31 Vpp;

[0060] FIG. **11**D illustrates mixing of fluids when the apparatus of FIG. **9**B is activated at 39 Vpp;

[0061] FIG. **11**E illustrates the gradient profiles achieved transversely across the fluid passage at various voltages;

[0062] FIG. **12**A illustrates a schematic of an acustofluidic micropump according to one embodiment with oscillatory elements arranged at a tilting angle according to one embodiment;

[0063] FIG. **12**B illustrates exemplary directional microstreaming achieved as a result of activation of oscillatory elements at a tilting angle less than 90 degrees;

[0064] FIG. **12**C illustrates exemplary dimensions of elements in the pumping region of a micropump according to one embodiment;

[0065] FIG. **13**A illustrates an exemplary micropump with a fluid passage designed to produce fluid pumping in a counter-clockwise direction employing 30° tilted oscillatory elements as shown in greater detail in the inset;

[0066] FIG. **13**B illustrates microstreaming patterns around the tips of oscillatory elements in the pumping region when the piezoelectric transducer was activated at 6.5 kHz;

[0067] FIG. **14**A illustrates the pumping performance of the four different tilting angles of oscillatory elements under different input voltages using the apparatus of FIG. **12**A; and

[0068] FIG. **14**B illustrates alternately pulsatile fluid pumping achieved by switching the piezoelectric transducer to ON and OFF at various burst frequencies, 0.5 Hz (T=2 sec) and 2 Hz (T=0.5 sec) using the apparatus of FIG. **12**A.

DETAILED DESCRIPTION OF THE INVENTION

[0069] The following description of particular embodiment (s) is merely exemplary in nature and is in no way intended to limit the scope of the invention, its application, or uses, which may, of course, vary. The invention is described with relation to the non-limiting definitions and terminology included herein. These definitions and terminology are not designed to function as a limitation on the scope or practice of the invention but are presented for illustrative and descriptive purposes only. While the processes or compositions are described as an order of individual steps or using specific materials, it is appreciated that steps or materials may be interchangeable such that the description of the invention may include multiple parts or steps arranged in many ways as is readily appreciated by one of skill in the art.

[0070] It will be understood that when an element is referred to as being “on” another element, it can be directly on the other element or intervening elements may be present therebetween. In contrast, when an element is referred to as being “directly on” another element, there are no intervening elements present.

[0071] It will be understood that, although the terms “first,” “second,” “third” etc. may be used herein to describe various elements, components, regions, layers, and/or sections, these elements, components, regions, layers, and/or sections should not be limited by these terms. These terms are only used to distinguish one element, component, region, layer, or section from another element, component, region, layer, or section. Thus, “a first element,” “component,” “region,” “layer,” or “section” discussed below could be termed a second (or other) element, component, region, layer, or section without departing from the teachings herein.

[0072] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used herein, the singular forms “a,” “an,” and “the” are intended to include the plural forms, including “at least one,” unless the content clearly indicates otherwise. “Or” means “and/or.” As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items. It will be further understood that the terms “comprises” and/or “comprising,” or “includes” and/or “including” when used in this specification, specify the presence of stated features, regions, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, regions, integers, steps, operations, elements, components, and/or groups thereof. The term “or a combination thereof” means a combination including at least one of the foregoing elements.

[0073] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. It will be further understood that terms such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art and the present disclosure, and will not be interpreted in an idealized or overly formal sense unless expressly so defined herein.

[0074] Provided are acustofluidic apparatuses that can be configured for many purposes including mixing of fluids, actively pumping fluid flow, generating chemical/biochemical gradient, creating tuneable chemical distribution, or many other uses. Embodiments include methods and apparatuses using one or more oscillatory elements driven by an energy field (optionally acoustic) to provide a unique and versatile method to generate prescribed temporal chemical gradient waveforms by mixing two or more fluids, such as first and second liquid flows (e.g. stimulus and buffer solutions), optionally in a time-dependent manner, or to move fluid through a channel. This approach is capable of generating not only digital chemical waveforms, but also analog waveforms whose characteristics, including shape, frequency, amplitude, and duty cycle, can be modulated by controlling the oscillation of one or more oscillatory elements within a flow channel

[0075] In some embodiments, an apparatus is in the form of a micromixer operable to intermix fluids that addresses issues with prior micromixers in that the micromixer does not require bubbles for operation and effectively causes tuneable mixing of a fluid or other material contained within a fluid passage. The apparatus functions by the incorporation of one or more oscillatory elements that terminate in or otherwise include a tip that will oscillate when in acoustic contact with one or more acoustic sources so as to produce microstreaming in fluid or other material.

[0076] As such, an acustofluidic apparatus is operable for generating a chemical gradient in a fluid flow, or for actively pumping a fluidic chemical material within a fluid passage. As used herein the word “chemical” is understood to include both chemical and biological such as in the case of cells or other multichemical living or non-living system. An apparatus includes a fluid passage, the fluid passage including at least one inlet. Optionally a fluid passage includes a first inlet configured to introduce a first fluid flow into the fluid passage, and a second inlet configured to introduce a second fluid flow into the flow channel. The fluid passage also includes at least one outlet where one or more fluids in either a mixed or non-mixed state may be discharged from the fluid passage. An apparatus includes one or more oscillatory elements that each include a tip extending into the fluid passage. In communication with the oscillatory element is an oscillatory energy field generator, optionally an acoustic source, the generator operable to produce oscillation in the oscillatory element. The oscillation of the oscillatory element induces microstreaming in one or more fluid flows optionally generating a chemical gradient having a time-dependence controllable using the oscillatory energy field generator. The result is a mixing at the chemical level of the one or more fluids where the parameters of the mixing are controlled or controllable by the energy field produced by the energy field generator. The result of the apparatus is the ability to tightly control all parameters of mixing or pumping of one or more fluids within the flow channel thereby allowing the creation of gradients tuned for a desired concentration, time, or area in the flow.

[0077] An acoustofluidic apparatus includes: a channel defining a fluid passage, the channel having at least one sidewall, at least one inlet and at least one outlet; an oscillatory element comprising a tip extending into the fluid passage; and an acoustic source in acoustic contact with the oscillatory element, the acoustic source operable to vibrate the oscillatory element so as to create microstreaming in a fluid within said fluid passage.

[0078] A fluid passage is a portion of or an entire channel capable of containing a fluid, optionally a flowing fluid. A fluid passage is optionally formed of transparent or opaque material. In many embodiments, a fluid passage is located on a substrate. The substrate is optionally formed of the same of different material as the fluid passage. The fluid passage is optionally formed from a solid material. A fluid passage is optionally formed from polymer such as polydimethylsiloxane (PDMS), polypropylene (PP), polyethylene terephthalate (PET), polybutylene terephthalate (PBT), polycarbonates (PC), polyethylene (PE), polylactic acid (PLA), nylon, PET copolymers, acrylics, Surlyn™, polyethylene naphthalate (PEN), polyamides, polycarbonate co-polymers, elastomeric polymers—thermoplastic elastomers, thermoplastic urethanes, poly urethanes, acrylic co-polymers, acrylonitrile butadiene styrene, or other thermoplastics, glass such as borosilicate glass or other glass material, quartz, steel optionally stainless steel, gold, combinations thereof, or other material known in the art and suitable for such a purpose. A fluid passage optionally has a surface roughness that is sufficiently smooth to allow laminar flow of the fluid moving within the flow channel.

[0079] The fluid passage(s) optionally has a cross-sectional shape that is circular, oval, rectangular, square, trapezoidal, triangular, irregular, or other shape. Optionally, the shape of the fluid passage varies with linear distance along the flow direction, or intended flow direction, of the fluid. A fluid passage has length longitudinal to the fluid flow that is optionally linear or generally linear, curved, angled, irregular or other desired shape. An exemplary cross-sectional dimension of a fluid passage is in the range of 1 μm to 30 mm or greater, or any value or range therebetween. A cross-sectional dimension of a fluid passage is optionally 1 μm to 10 mm, optionally 1 μm to 1 mm, optionally 10 μm to 1 mm, optionally 100 μm to 500 μm. The cross-sectional dimension is optionally configured to correspond to the type of fluid passing through the fluid passage taking into account considerations of viscosity, chemical or biological content, or other necessary parameters.

[0080] A fluid passage includes one or more inlets and one or more outlets. An inlet represents an opening through which a fluid may pass to enter the fluid passage or portion thereof. An outlet is an opening through which a fluid may pass to exit the fluid passage or portion thereof. In a simplified, non-limiting embodiment, two inlets are present and one outlet is present. Typically, the number of inlets corresponds to the number of differing fluids to pass into the fluid passage during operation of the apparatus. In some embodiments, the outlet is of larger cross sectional dimension that an inlet or other portion of the flow channel.

[0081] A fluid passage is optionally a microchannel. A microchannel is a fluid passage with a cross-sectional dimension on the order of micrometers or less. A fluid passage optionally has one side or edge defined by the substrate material. A fluid passage optionally has a width and length parallel to the plane of a substrate. A fluid passage also has a height that extends in a direction perpendicular (i.e. normal) to a substrate. The height of a fluid passage is optionally from 1 μm to 10 mm or greater. A height of a fluid passage is optionally 1 μm to 1 mm, optionally 5 μm to 1 mm, optionally 10 μm to 1 mm, optionally 100 μm to 500 μm. A width of a fluid passage in a direction parallel to a substrate surface or perpendicular to a fluid flow direction is any width suitable for containing the number of fluids to be flowed through the passage.

[0082] A fluid passage contains a fluid during operation of the apparatus. Optionally, a fluid passage surrounds a fluid. A fluid is optionally a liquid at testing temperatures and pressures. A fluid is optionally a biologically compatible media such as water or buffered liquid illustratively including phosphate, tris(hydroxymethyl)aminomethane (tris), citrate, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), or other buffering system. A fluid is optionally water, saline, an organic liquid, or other desired flowable material. A fluid is optionally a gel. A fluid is optionally a suspension of one or more types of suspended particles, cells or other substance. A fluid optionally contains one or more test substances. A test substance is any chemical or biological material that is desired for testing. A fluid has a test substance concentration. Optionally, a first fluid and a second fluid contain the same or different test substances or concentrations depending on the desired outcome of the system. Optionally, a first fluid and a second fluid are different types of fluids illustratively but not limited to an organic and an aqueous fluid respectively, or vice versa. The fluid type in many embodiments is non-limiting other than the fluid is capable of moving through the flow channel.

[0083] A fluid passage is optionally presented on a substrate either by the substrate being adjacent to the fluid passage or integrated with the fluid passage as an edge or wall portion. A substrate is any material of suitable shape and dimension to support a fluid passage, and optionally any structure located within the fluid passage. A substrate is optionally suitable to conduct or transfer energy from an oscillatory energy field generator so as to transfer the energy to the oscillating structure thereby providing the desired oscillation of the oscillating structure. A substrate is optionally made form a polymeric material, illustratively polypropylene (PP), polyethylene terephthalate (PET), polybutylene terephthalate (PBT), polycarbonates (PC), polyethylene (PE), polylactic acid (PLA), nylon, PET copolymers, acrylics, Surlyn™, polyethylene naphthalate (PEN), polyamides, polycarbonate co-polymers, elastomeric polymers—thermoplastic elastomers, thermoplastic urethanes, poly urethanes, acrylic co-polymers, acrylonitrile butadiene styrene, or other thermoplastics, glass such as borosilicate glass or other glass material, quartz, steel optionally stainless steel, silicon, aluminum, gold, combinations thereof, or other material known in the art and suitable for such a purpose.

[0084] A fluid passage includes one or more oscillatory elements. An oscillatory element includes a tip extending into the fluid passage. In some embodiments, the entire oscillatory element is located within the fluid passage such that laminar flow around the oscillatory element may occur in the absence of oscillation of the oscillatory element(s). The overall structure of the oscillatory element includes at least a portion of the oscillatory element that has an oscillating structure whereby the oscillating structure is operable to oscillate at an oscillatory frequency when contacted by acoustic or other energy capable of inducing oscillation in the oscillatory element. An oscillatory element is optionally in the form of a sharp edge, a narrow edge or point is present at the tip of the oscillatory element. The tip extends into the fluid passage relative to a wall or other surface of the fluid passage. Optionally, the tip extends from the substrate to the opposing surface of the fluid passage, or near enough thereto so as to substantially produce microstreaming in the fluid in the height of the fluid passage. Optionally, an oscillatory element is substantially in the form of a rod or a needle.

[0085] A fluid passage optionally includes a single oscillatory element, optionally two oscillatory elements. The number of oscillatory elements is limited only by the length of the fluid passage, the dimensions of the oscillatory element(s), and the desired use of the apparatus. Optionally, a fluid passage includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more oscillatory elements. In some embodiments, oscillatory elements are dispersed on a single wall of a fluid passage. Optionally, oscillatory elements are dispersed on two opposing sides of a fluid passage. When oscillatory elements are present on opposing sides, the arrangement of the oscillatory elements on a first edge is optionally offset or directly across from oscillatory elements on the opposing or second edge. A plurality of oscillatory elements is optionally uniformly distributed, randomly distributed, or combinations thereof. Optionally, oscillatory elements serving different purposes are dispersed within other oscillatory elements serving disparate purposes. For example, in an exemplary embodiment, a plurality of oscillatory elements suitable for pumping fluid are arranged upstream from oscillatory elements suitable for producing fluid intermixing or other purpose. In an alternate exemplary embodiment, a plurality of oscillatory elements suitable for pumping fluid are arranged interspersed with oscillatory elements suitable for producing fluid intermixing or other purpose.

[0086] Oscillatory elements are optionally dispersed at a distance relative to an adjacent oscillatory element. A distance is optionally 5 mm or less, optionally 3 mm, 900 μm, 800 μm, 700 μm, 600 μm, 500 μm, 400 μm, 300 μm, 200 μm, or less. In some embodiments, the distance of the oscillatory element is 30% to 500% the width of the fluid passage. In some embodiments, the distance is equal to the fluid passage width.

[0087] An oscillatory element is of suitable composition and configuration so as to be capable of oscillating, or a portion thereof capable of oscillating, when exposed to energy from an acoustic source, as one example. An oscillatory element is or includes a structure that is capable of oscillation, but does not need to be oscillating at all times. Oscillation is defined as movement about a central parameter such as movement side to side or other direction, by movement due to flexing of an outer dimension of an oscillating structure, or by other recognized oscillatory movement.

[0088] An oscillatory element terminates in a tip oriented on a position of the oscillatory element distal from a base associated with a surface of a fluid passage. A tip is optionally in the form of a sharp angle defined by the terminus of two adjacent surfaces oriented at an angle relative to each other. Adjacent surfaces extend between a sidewall or other surface of a fluid passage and the tip. The angle of the adjacent surfaces a is optionally less than 180 degrees. The angle α is optionally less than or equal to 60 degrees, optionally less than or equal to 30 degrees, optionally less than or equal to 15 degrees.

[0089] An oscillatory element is oriented relative to the longitudinal direction of the fluid passage in the region of the oscillatory element at a tilting angle (β). The tilting angle is optionally used to generate microstreaming that is directionally oriented, optionally to cause a change in fluid flow. The change in fluid flow is optionally used to pump the fluid in a desired direction within the fluid passage. A tilting angle is optionally from 0 degrees to 180 degrees or any value or range therebetween. Optionally, a tilting angle is 0 degrees, 30 degrees, optionally 45 degrees, optionally 60 degrees, optionally 70 degrees, optionally 90 degrees, optionally 140 degrees.

[0090] An oscillatory element has a length and a width. A length is optionally 5 mm or less, optionally 500 μm or less, optionally 400 μm or less, optionally 300 μm or less, optionally 200 μm or less. The length of an oscillatory element is governed by the width of the fluid passage such that the length cannot be too long so as to substantially impede fluid flow through the channel. An oscillatory element optionally has a length that is less than half the width of a flow channel, but it is appreciated that the oscillatory element is optionally longer than half the width of the flow channel optionally in the case where the oscillatory element is not oriented with a length that is normal to the surface from which it extends.

[0091] An oscillatory element has a width. The width of an oscillatory element defined as substantially perpendicular to the length of the oscillatory element, where the width is optionally uniform or variable along the length of the oscillatory element. When an oscillatory element is in the form of a sharp edge, a portion of the oscillatory element is wider at the portion near the base of the oscillatory element relative to the width near the tip.

[0092] In some embodiments the adjacent edges are parallel or terminate in a tip prior to the adjacent edges forming an angle such that the tip is a blunt surface relative to an angle. A blunt surface has a width. The width is optionally from 1 μm to 5 mm or any value or range therebetween.

[0093] An apparatus includes an oscillatory energy field generator operable to produce oscillation in the oscillatory element(s). An oscillatory energy field generator is any device capable of producing energy that will impart oscillation in an oscillating particle. Energy is optionally acoustic, electrical, optical, magnetic, or other energy. Devices capable of generating such energies are known in the art. The exemplary embodiments herein describe an acoustic source that creates acoustic energy that is felt or received by the oscillatory element causing oscillation in the oscillatory element or portion thereof when the energy is of the correct parameters to produce such oscillation. It is appreciated that acoustic energy and acoustic energy generators are presented herein for exemplary purposes alone and not as a limitation on the present invention. An oscillatory energy field generator is optionally a piezoelectric transducer. Acoustic-based oscillating structure manipulation methods are excellent alternatives to conventional methods. Compared to their optical, electrical, or magnetic counterparts, acoustic-based methods are relatively non-invasive to biological objects and work for most microparticles regardless of their optical, electrical, or magnetic properties.

[0094] An oscillatory energy field generator is optionally a chirp interdigital transducer (IDT) or other acoustic energy generating device. An oscillatory energy field generator is formed or attached to the substrate and when energized by an input signal creates a vibration in the substrate. This vibration passes into the oscillating structure directly or indirectly via an intermediate structure to produce oscillation in the oscillatory element(s). An electronic control circuit is optionally wired to the oscillatory energy field generator to produce the input signal thereby producing the energy field. This circuit may take a variety of forms as is known in the art.

[0095] The energy field produces an oscillation of the oscillatory element or portion thereof that is in physical contact with one or more fluids in the flow channel (when present). The oscillation of the oscillating structure induces a mixing of the fluid by creating microstreaming in the fluid. The resulting microstreaming optionally generates a chemical gradient between two or more fluids, induces a pumping action on a fluid, or combinations thereof. A chemical gradient or pumping action has a time-dependence, spatial dependence, concentration dependence, or composition dependence controllable by the oscillatory energy field generator.

[0096] An apparatus can be manufactured by several processes useful for forming microchannel apparatuses. Illustrative examples include, but are not limited to laser cutting, waterjet cutting, injection molding, photolithography, soft lithography, chemical etching, CNC micromachining, vacuum molding, stamping, among others, or combinations thereof. Illustrative methods such as photolithography and micromachining are commonly used in the semiconductor manufacturing arts are useful for forming an acustofluidic apparatus. In some embodiments, individual elements are separately manufactures and bonded together to form an apparatus using bonding materials as recognized in the art.

[0097] An exemplary embodiment of an acoustofluidic apparatus is illustrated in FIG. **1**A depicting an experimental setup of a sharp-edge-based acoustofluidic micromixer **10**. A single-layer fluid passage **12**, optionally formed from polydimethylsiloxane (PDMS), has eight oscillatory elements illustrated as a sharp edge **14** on its sidewalls **16** (four edges on each side). The illustrated embodiment is fabricated and bonded onto a glass slide **18**. An acoustic source illustrated as a piezoelectric transducer **20** (model no. 273-073, RadioShack Corp.) is then attached adjacent to the fluid channel **12** by using epoxy (PermaPoxy™ 5 Minute General Purpose, Permatex). Upon the actuation of the piezoelectric transducer, the sharp-edges **14** acoustically oscillate to generate a pair of counter-rotating vortices **22**, **24** (double-ring recirculating flows, micromixing) in the fluid around the tip of each sharp-edge **14**, as shown in FIG. **1**B. The double-ring recirculating flows drastically enhance the mass transport across the channel width by breaking the interface of laminar fluids and optionally causing a mixing between two or more laminar fluid flows.

[0098] While the sharp edges are illustrated as pointed elements having a triangular shape with two flat surfaces, other shapes may be used for alternative embodiments. In one example, oscillatory elements take the form of thin rectangular elements, having generally parallel side surfaces and a short end surface. This results in two "tip angles" of 90 degrees. Further alternatives include structures with two side surfaces that curve or angle in near the outer end to define a pointed tip. This may have a shape similar to a sharpened pencil as viewed in a side view. Further alternatives may also be used, as long as sufficient acoustic streaming results, allowing acceptable levels of mixing.

[0099] The illustrated structures are very small and are two dimensional. That is, they have a constant cross section in the z-dimension. The channel has a top to bottom dimension (perpendicular to the page of the drawing) and the illustrated structures extend from the top to the bottom with a uniform shape. Alternatively, three dimensional shapes may be used, such as a cone, post or needle that extends into the fluid passage. Other shapes will be clear to those of skill in the art.

[0100] Though not illustrated, the height of the oscillatory elements may be varied. In the illustrated embodiment, the oscillatory elements extend from each side wall and are interdigitated. The combined height of the oscillatory elements on opposite sides is 500 μm (250 μm each) while the channel has a total width of 600 μm. Put another way, the channel may be said to have only 100 μm of clear space. As such, the oscillatory elements extend more than 80% of the width of the channel (more than 40% from each side). The height may be varied depending on the desired performance characteristics. Also, in some embodiments, oscillatory elements may extend from only a single side. However, it is preferred that the oscillatory elements extend across a total of at least 10% of the channel and that oscillatory elements extend from opposed sides of the channel to provide this combined total. Oscillatory elements, such as extending 25% or more across the channel may be used in some embodiments. Greater than 75% may be used in some applications.

[0101] FIG. **1**C shows an exemplary design of an acustofluidic micromixer with sidewall sharp-edges serving as an oscillating structure. The length, width and depth of the illustrated microchannel are 1 cm, 600 μm, and 50 μm, respectively. Each sharp-edge oscillatory element is designed to be of a constant height of 250 μm and tip angle (α), optionally variable. The sharp-edge may also be referred to as an oscillating structure optionally serving as an oscillatory element or pumping element. In the illustrated embodiment, the oscillatory element is a pointed element having two surfaces **30** and **32** extending away from the pointed tip **34**, with the surfaces **30** and **32** defining the tip angle α therebetween. While the surfaces **30** and **32** are illustrated as flat, other shapes are possible that are regular or irregular. While sharp-edges are attached to the sidewall in the illustrated embodiment, they should also work when they are in other parts of the channel. Five different tip angles (180° illustrating a blunt end surface, 15°, 30°, 45° and 60°) were chosen to investigate the resulting acoustic streaming effect and determine the optimal angle for best mixing performance.

[0102] FIG. **1**D schematically shows an exemplary design and working mechanism of an acoustofluidic apparatus functional as an acoustofluidic pump. The acoustofluidic pump, briefly, is made by bonding a single-layer PDMS fluid passage **16** with a piezoelectric transducer (Part no. 81-7BB-27-4L0, Murata Electronics) attached adjacent to it onto a single glass slide using a thin layer of epoxy (PermaPoxy™ 5 Minute General Purpose, Permatex). The pumping region illustrated in FIG. **1**D, is designed with twenty tilted oscillatory elements **14** on the sidewalls of the fluid passage (ten on each side). Acoustically oscillated by the activation of the piezoelectric transducer, the tilted oscillatory elements generate a tilted micromixing pattern around the tip **34**, thereby producing a net force pointing towards the direction that oscillatory element structure is orientated. As a result, fluid pumping occurs because the generated net forces push the bulk fluid to flow forward. In this exemplary design the microchannel has a width of 600 μm and a depth of 100 μm, and oscillatory elements are identical. Different tilting angles (β) of oscillatory elements, including 30°, 45°, 60° and 70°, are created to investigate the resulting pumping behaviour and determine optimal angle for best pumping performance.

[0103] Applications of the apparatus include microfluidic devices (as used here, this term includes nanofluidic devices), for chemical, biological (including molecular biology, cell migration, cytotoxicity), and biochemical (including enzyme, protein, DNA, RNA, proteomics, pathology, and the like) analysis, assay, detection, modification, interaction, preparation, treatment, or characterization applications. Applications also include a fluid mixing apparatus for any application, including chemical formulations, inkjet apparatus, chemical deposition, film formation, and the like, optofluidic devices (e.g. to obtain gradient refractive indices, for example for lens arrays), and the like. Specific applications include nanofluidic devices, chemical probing of cells, and programmable chemical waveform generation and switching using acoustically activated bubbles. Examples include apparatuses and methods for generating chemical concentration or physical (e.g. electrical and/or optical property) gradients that may be dynamically controlled by an electronic circuit, e.g. one providing a variable drive signal to a piezoelectric transducer.

[0104] Fluid flows are optionally liquids, suspensions, and the like. Flows may include suspended particles, such as biological structures illustratively including, but not limited to cells, platelets, or proteins, among others. Applications include characterization of particles such as cells, including cell chemotaxis, cell differentiation, and cell migration studies in a dynamic chemical environment.

[0105] Hence, spatial and temporal chemical gradient profiles are achieved using one or more acoustically driven oscillating oscillatory elements located within a fluid passage, for example using a single oscillatory element located within the fluid passage, or a plurality of oscillatory elements, for example positioned in a ladder-like formation within the flow channel. Changing the applied voltage of a drive signal applied to an acoustic transducer such as a piezoelectric transducer dynamically tunes the generated chemical gradient profiles, both spatially and temporally. More complex and abundant chemical profiles through changing location(s) of the oscillatory element, for example, may be made. The design of the ladder-like formation may be modified using different configurations of the oscillatory elements within the flow channel. Chemical gradients may be adjusted using flow rate control of inlet fluid flows in combination with drive signal modification.

[0106] Acoustofluidic-based methods and apparatus for generating chemical gradient can be used in many chemical and biological studies and applications, such as apparatuses and methods for investigating cell chemotaxis, differentiation, and migration in a dynamic chemical environment.

[0107] Using many embodiments of the apparatus, it is possible to measure the dynamics of receptor-mediated signaling and other cellular responses to small molecules. The device can also be used to study cellular processes that span a wide range of time scales, from milliseconds to hours. Generating waveforms in continuous flow also eliminates the abrupt changes in shear stress at the cell membrane in segmented flow devices, more closely mimicking the in-vivo chemical signals. These precisely controlled chemical waveforms can be used for measuring the kinetics of fast enzymatic reactions, explaining the specificity and efficiency of gene expression, and developing time-release drugs, among other applications. Chemical waveforms may have markedly different effects on cellular signaling pathways that receive, transmit, process, and implement directions from chemical stimuli, compared with constant signals, and arbitrary chemical waveforms can be determined.

[0108] Examples of the present invention further include apparatuses and methods for generating tuneable, pulsatile chemical gradient generation via acoustically driven oscillating bubbles.

[0109] A novel concept of generating both static and pulsatile chemical gradients using acoustically activated oscillatory elements was developed, in some examples using a ladder-like arrangement. These results show that the chemical gradient profiles can be effectively tuned by regulating the amplitude of the oscillatory element oscillation.

[0110] Pulsatile chemical gradients generated in microfluidic devices may be used for the characterization of dynamic biological and chemical processes. Spatial and temporal characteristics of chemical stimuli play an important role in cell signalling, and hence this may be investigated using described approaches.

[0111] Pulsatile chemical gradients may also be used in an apparatus and methods for high-throughput characterization of cellular processes such as directed migration, differentiation, and apoptosis. Apparatuses and methods allow dynamic temporal control of chemical gradients to be achieved.

[0112] Also provided are processes of inducing microstreaming in a fluid including contacting a fluid in a fluid passage with an oscillatory element and oscillating the oscillatory element with an energy source in energetic contact with said oscillatory element so as to induce microstreaming in said fluid. The energy is optionally acoustic energy and the energy source is optionally an acoustic source. Any device described herein and equivalents are operable to induce microstreaming in a fluid.

[0113] Microstreaming in a fluid directionally oriented is capable of inducing a pumping action in the fluid so as to move fluid through a fluid passage. As such, processes of pumping a fluid in a fluid passage are also provided. Processes include contacting a fluid in a fluid passage with an oscillatory element, the oscillatory element or oscillating portion thereof oriented relative to an the longitudinal direction of the fluid passage by a tilting angle from 0 degrees to 180 degrees, optionally 0 to 70 degrees, where 0 degrees is parallel to the longitudinal direction of the fluid passage at the location of the oscillatory element; and oscillating the oscillatory element with an energy source in energetic contact with the oscillatory element so as to induce directional microstreaming in the fluid. The energy is optionally acoustic energy and the energy source is optionally an acoustic source. Any device described herein and equivalents are operable to induce microstreaming in a fluid.

[0114] Various aspects of the present invention are illustrated by the following non-limiting examples. The examples are for illustrative purposes and are not a limitation on any practice of the present invention. It will be understood that variations and modifications can be made without departing from the spirit and scope of the invention.

EXAMPLES

Example 1

Sharp Edge Based Oscillatory Elements in a Micromixer

[0115] An exemplary single passage apparatus is illustrated in FIG. **1**A. A single-layer fluid passage **12** is formed from polydimethylsiloxane (PDMS) with eight oscillatory elements illustrated as a sharp edge **14** on its sidewalls **16** (four edges on each side). The single-layer PDMS microchannel was fabricated using soft lithography and the mold replica technique. A silicon mold for the microchannel was patterned in photoresist (Shipley 1827, MicroChem, Newton, Mass.) and etched with Deep Reactive Ion Etching (DRIE, Adixen, Hingham, Mass.). The mold was then coated with 1H,1H,2H, 2H-perfluorooctyl-trichlorosilane (Sigma Aldrich, St. Louis, Mo.) to reduce its surface energy and any subsequent damage to the PDMS channel during the demolding process. Sylgard™ 184 Silicone Elastomer Base and Sylgard™ 184 Silicone Elastomer Curing Agent (Dow Corning, Midland, Mich.) were mixed at a 10:1 weight ratio and cast onto the silicon mold. The uncured PDMS on the silicon mold was then degassed in a vacuum chamber for 2 h to remove any air bubbles and later cured at 65° C. for 45 min. After removing the cured PDMS from the mold, the inlets and the outlets were drilled into the PDMS using a silicon carbide drill bit (model 220/395, Dremel). The microfluidic flow channel was then bonded to a micro cover glass used as a substrate **18** that had been pre-treated with oxygen plasma. An acoustic source illustrated as a piezoelectric transducer **20** (model no. 273-073, RadioShack Corp.) is then attached adjacent to the fluid channel **12** by using epoxy (PermaPoxy™ 5 Minute General Purpose, Permatex).

[0116] To demonstrate and characterize the fluid flow pattern inside the above exemplary fluid passage due to the acoustic streaming, a solution containing 1.9 μm diameter DRAGON GREEN fluorescent beads (Bangs Laboratory) was first infused into the fluid passage. FIG. **2**A shows the flow pattern of fluorescent beads in the absence of acoustic activation (with the piezoelectric transducer OFF). In the presence of acoustic activation (with the piezoelectric transducer ON), oscillating sharp-edges induced a strong acoustic microstreaming effect (FIG. **2**B). The microstreaming greatly enhanced the mass transport of the two fluids by perturbing the bulk flow and breaking the interface of laminar flow, thereby enabling fast and homogeneous mixing.

[0117] The mixing performance of the exemplary sharp-edge-based micromixer was characterized by injecting DI water and fluorescent dye (fluorescein) into the fluid passage through two separate inlets (**24**, **26** in FIG. **1**A). The sharp-edges **14** were acoustically oscillated by the piezoelectric transducer **18** that was driven by an amplified sine-wave signal from a function generator and an amplifier. To determine the frequency at which the oscillating sharp-edges generate the strongest acoustic streaming effect, the device was tested with 15° tip angle sharp-edges, and swept the frequency with a 50 Hz increment from 1 kHz to 100 kHz. Experimental results indicated that the strongest acoustic streaming effect was generated when the sharp-edges were excited at the frequency of 4.50 kHz. FIG. **3**A shows the unmixed laminar flow profile at a flow rate of 1 μl/min with the piezoelectric transducer OFF, in which a clear fluid interface was observed. FIGS. **3**B-**3**D show the mixing results due to the presence of acoustic waves at frequencies of 4.25 kHz, 4.50 kHz, and 4.75 kHz, respectively. Homogeneous mixing of DI water and fluorescein was achieved when the sharp-edges were excited at frequencies of 4.50 kHz and 4.75 kHz, while incomplete mixing was observed at a frequency of 4.25 kHz. To further verify the mixing performance and identify the optimized driving frequency of the piezoelectric transducer, the cross-sectional dye concentration profiles (the dashed lines in FIG. **3**A-D) were plotted by measuring the grey scale value of the experimental images. FIG. **3**E shows the normalized dye concentration profile across the flow passage width for the three driving frequencies. The concentration profiles show that a uniform gray-scale value distribution across the channel width was observed at a frequency of 4.50 kHz, suggesting that 4.50 kHz is the proper driving frequency to develop the strongest acoustic streaming phenomenon and achieve optimized mixing performance for this apparatus at this flow rate. As a result, the frequency of 4.50 kHz was used in all the following experiments with this apparatus. It will be understood by those of skill in the art that other driving frequencies may be used in other configurations and/or for other fluids, flow rates, etc.

[0118] Once the driving frequency was determined, the effect of the tip angle of sharp-edges on the mixing performance was investigated. To quantitatively characterize the mixing performance along the entire length of the flow passage, the mixing index (M) of fluids was measured at five different positions (indicated as **1**, **2**, **3**, **4**, and **5** in FIG. **4**A) along the channel. The mixing index is defined as the standard deviation of normalized gray-scale values, which were extracted from the experimental images obtained. A mixing index of 0.5 indicates completely unmixed fluids, while a mixing index of 0.0 indicates completely mixed fluids. A mixing index of 0.1 was chosen as the upper-level threshold for acceptable mixing. FIG. **4**A-D shows the mixing efficiencies of the four different tip angles of sharp-edges at a flow rate of 2 μl/min (4 μl/min for the total flow rate of the two co-injected fluids), a driving frequency of 4.50 kHz, and a driving voltage of 31 V (peak to peak). With a tip angle of 15° a mixing index of 0.065 was achieved at position **2**, suggesting excellent mixing of DI water and fluorescein. For sharp-edges with a tip angle of 30°, complete mixing was observed at position **5** suggesting that a longer mixing distance was required. Incomplete mixing was observed for sharp-edges with a tip angle of 45° (FIG. **4**D). With a tip angle of 60°, a side-by-side laminar flow was observed due to the unmixed fluids (even in the presence of acoustic wave), and only negligible mixing, which was caused by diffusion, was observed at downstream positions (FIG. **4**E). The results showed that as the tip angle of sharp-edges decreased, the mixing performance significantly improved. The results can be explained by approximating the oscillation of sharp-edges as the cantilever vibration. For cantilever vibration, one can use the following equation,

$$k=F/\delta=Ewt^3/4L^3 \quad (1)$$

where k is the spring constant, E is the Young's modulus of material, w is the width of cantilever, t is the thickness of the cantilever, and L is the length of the cantilever. Sharp-edges with different tip angles in this study all have the same values for Young's modulus, equivalent widths (50 μm), and equivalent lengths (250 μm). The only variable that changes with varying tip angles is the thickness, which increases as the angle increases. Thus sharp-edges with a smaller tip angle should have a lower spring constant. If the input power is constant, a lower spring constant of the cantilever will cause a larger vibration amplitude at the free end of the cantilever. Treating each single sharp-edge as one cantilever, similarly, the sharp-edges with a tip angle of 15° should have the largest vibration amplitude because of its smallest spring constant. This explains why the sharp-edges with the tip angle of 15° induced stronger acoustic streaming effects than those with tip angles of 30°, 45°, or 60°.

[0119] In light of the above, tip angles of less than 30 degrees are preferred for some embodiments, while greater angles may be acceptable for other embodiments. For example, tip angles of 45 degrees or less may be used in some embodiments.

[0120] Mixing performance was further characterized by applying different driving voltages to the piezoelectric transducer. FIG. **5** shows the mixing performance with different driving voltages at a flow rate of 2 μl/min and a driving frequency of 4.50 kHz. The results show that as the driving voltage of the piezoelectric transducer increased, the mixing efficiency was improved, and acceptable mixing was observed starting from position **2** with driving voltages of 23 VPP, 31 VPP, and 39 VPP. With a driving voltage of 15 VPP, the acceptable mixing index was achieved at position **3**, suggesting that a lower driving voltage induced weaker acoustic streaming effects; therefore a longer mixing distance was required.

[0121] FIG. **6**A-D shows the mixing efficiency at different flow rates (1, 2, 3, 4, and 5 μl/min) with a driving frequency of 4.50 kHz and a driving voltage of 31 VPP. At lower flow rates (1 and 2 μl/min), acceptable mixing was achieved at position **2**, which suggests excellent mixing of the two fluids and shorter mixing distances were required for low flow rates (FIGS. **6**A and **6**B). For higher flow rates (3, 4, and 5 μl/min), the mixing index at position **2** was increased with an increase in flow rate, and acceptable mixing was only observed after passing position **3**. The results suggest that the mixing index increases as flow rate increases, since the ability to oscillate sharp-edges to induce acoustic streaming might be suppressed by high flow rates. The upper limit of flow rate, by which a mixing index less than 0.1 was achieved after passing position **2** (after the first pair of sharp-edges), was 2 μl/min (4 μl/min for the total flow rate of two coinjected fluids). Although mixing indices less than 0.1 were achieved with the flow rates higher than 2 μl/min, they were only observed after passing position **3**, suggesting a longer mixing distance.

[0122] The mixing time of the sharp-edge-based micromixer was also characterized. The average mixing time (Ts) was estimated using the following equation,

$$\tau_s = L_{mix}/V_{avg} \qquad (2)$$

where τ_s is the mixing time, L_{mix} is the distance from unmixed to completely mixed regions, and V_{avg} is the average fluid velocity. The mixing distance was measured to be approximately 400 μm from FIG. **6**B, and the average fluid velocity was calculated to be 2.2 mm/s by dividing the combined flow rate by the cross-sectional area of the channel (600 μm by 50 μm). The mixing time was thus calculated to be around 180 ms which is comparable to those of existing microfluidic mixers.[42-45,51,53] The inventors believe that the mixing time can be further shortened through the optimization of design parameters, such as the distance between consecutive single sharp-edge or the height of sharp-edges.

[0123] In conclusion, the present invention provides an acoustofluidic micromixer based on the acoustic streaming effects induced by oscillating sharp-edges. The recirculating flows induced by the oscillation of sharp-edges allow two fluids to interchange and thus enhances the mass transport across the channel, greatly improving the mixing efficiency. Experiments demonstrate that homogeneous mixing across the channel width can be achieved and the mixing time was calculated to be ~180 ms. The effects of the sharp-edge geometry, the driving frequency, the driving voltage, and the flow rates on mixing performance were investigated. The sharp-edge-based acoustofluidic micromixer has many desirable characteristics, such as its excellent mixing performance, simplicity, convenient and stable operation, fast mixing speed, and ability to be toggled on-and-off. These characteristics make it promising for a wide variety of lab-on-a-chip applications.

Example 2

Cellular Calcium Responses Measured with an Acustofluidic Micromixer

[0124] The apparatus of Example 1 was used to probe the calcium responses of U251 cells. Briefly, stimulus and buffer, were co-injected through two separate inlets, and they could be uniformly mixed due to the acoustic streaming effects that controlled by the actuation of PZT. The fluid passage was divided into two regions of interest (ROI) along the fluidic interface, ROI **1** and ROI **2** as illustrated in FIG. **7**A. When the PZT was inactivated, two ROIs were only filled with the stimulus and the buffer, respectively, while being filled with uniformly mixed solution, once the PZT was activated as shown in FIG. **7**B and C, respectively. By alternate actuation of the PZT, temporal control over mixing is achieved generating different chemical signal profiles at two the ROIs as illustrated in FIG. **7**D. This enables the study of cellular responses to stimuli with two different concentration amplitudes. Adjusting the burst frequency and the duty cycle of input signals of PZT allows generation of various pulsed chemical signal profiles using our mixing-based cell stimulator as illustrated in FIG. **8** with various on/off time periods.

[0125] The calcium response of U251 cells exposed to sustained and pulsed stimulations of ionomycin, a calcium ionophore was studied. Briefly, U251 cells were first loaded into the fluid passage, and randomly seeded in the two ROIs. After seeding, HBSS containing 2 μM Fluo4-AM was gently and slowly delivered into the channel to stain the cells, followed by washing with phosphate buffered saline (PBS). Then medium (PBS) and medium containing 2 μM ionomycin were injected into separate inputs and allowed to flow together through the fluid passage. U251 cells in ROI **1** and ROI **2** were subjected to differing concentration amplitudes of ionomycin between 2 to 1 μM and between 0 and 1 μM, respectively. With exposure to a sustained stimulus of 60 sec, U251 cells in ROI **1** showed a decayed fluorescence intensity over time, after that, they expressed an increased fluorescence intensity [FIG. **7**A]. By contrast, those in ROI **2** showed enhanced florescence intensity over time [FIG. **7**B]. The cells responded to the pulsed stimulus at a frequency of 0.0625 Hz. When exposed to a pulsed stimulus at higher burst frequency of signals, including 0.25 Hz and 1 Hz, U251 cells in both ROIs failed to respond faithfully to the oscillating concentrations of stimulus, rather, integrated the signal. The results suggest that the cells are actually acting as a low-pass filter.

Example 3

Production of Fine Gradient Profiles in an Acustofluidic Micromixer

[0126] An acoustofluidic micromixer is formed substantially as in Example 1 with a wide fluid passage including oscillatory elements in the form of sharp edges dispersed transversely from substrate to passage edge such that fluid is capable of flowing around both adjacent surfaces from base to tip. Two illustrative embodiments tested are presented in FIG. **9**A illustrating a linear pattern and FIG. **9**B illustrating a parabolic pattern of the oscillatory elements.

[0127] Fluorescein and PBS solutions are transferred into the fluid channel to characterize chemical gradients produced by the apparatuses. Upon the actuation of the piezoelectric transducer, the sharp edges are acoustically oscillated to generate acoustic streaming effects around the tip of each sharp-edge, and to mix the fluids from the first inlet containing a fluorescein composition and a second inlet containing water. Due to the step-like arrangement of sharp-edge oscillatory elements, the fluids are mixed in a step-wise fashion producing a fine gradient profile from one edge of the fluid passage to the opposing edge. A linearly varying gradient is generated by the arrangement of oscillatory elements of FIG. **9**A. A parabolic chemical gradient is generated by the arrangement of oscillatory elements in FIG. **9**B employing two water inlets and a central fluorescein containing inlet.

[0128] As illustrated in FIG. **10**A, using the apparatus of FIG. **9**A, when the piezoelectric transducer was OFF, a side-by-side laminar flow was observed due to the nature of low Reynolds number in microfludic channel. Once the piezoelectric transducer was actuated, acoustic microstreaming induced mixing in a step-wise fashion was observed, thereby generating concentration gradients of fluorescein as illustrated in FIGS. **10**B-D. To verify the gradients generated, the cross-sectional fluorescence intensity profiles at downstream position ROI (dashed line in FIG. **9**) were plotted as illustrated in FIG. **10**E. As expected, linear gradients were obtained. In addition, gradients can be tuned as upon altering the driving voltage of the piezoelectric transducer with increasing voltage producing gradients with finer profiles.

[0129] FIG. **11**A-D illustrates shows the concentration gradients that generated using the design in FIG. **9**B. Different concentration gradients were experimentally observed when different driving voltages of the piezoelectric transducer were applied FIG. **11**B-D. As illustrated in FIG. **11**E, varying the voltage of the piezoelectric transducer resulted in finer gradient profiles.

[0130] Generation of gradients with more complex profiles is possible by changing the driving voltages or the arrangement of oscillatory elements. In addition, temporally changing gradients can be generated by easily controlling the driving voltage and the actuation time of the piezoelectric transducer.

Example 4

An Acustofluidic Micropump

[0131] An acustofluidic micropump is formed substantially as described in Example 1 with a plurality of oscillatory elements arranged in a fluid passage at a tilting angle of less than 90 degrees causing a tilting of the oscillatory elements in the same direction. FIG. **12**A schematically shows the design and working mechanism of the acoustofluidic micropump according to this exemplary design. Briefly, the acoustofluidic micropump was made by bonding a single-layer PDMS fluid passage formed by the methods of Example 1 with a piezoelectric transducer (Part no. 81-7BB-27-4L0, Murata Electronics) attached adjacent to it onto a single glass slide using a thin layer of epoxy (PermaPoxy™ 5 Minute General Purpose, Permatex).

[0132] To demonstrate pumping behaviour, the flow channel was designed to be a rectangular recirculating channel composed of four interconnected channels—left-channel, right-channel, upper-channel and lower-channel. The lower channel includes the pumping region and was designed with twenty tilted oscillatory elements on its sidewalls (ten on each side), while all the other three channels were straight channels without any structure. The piezoelectric transducer, activated by amplified sine-wave signals from a function generator (AFG3011C, Tektronix) and an amplifier (25A250A, Amplifier Research), was used to acoustically oscillate the oscillatory elements to generate acoustic streaming effects. As illustrated in FIG. **12**B, when acoustically oscillated by the activation of the piezoelectric transducer, the tilted oscillatory elements generate a directional microstreaming pattern around the tip, thereby producing a net force pointing towards the direction that oscillatory element is orientated. Fluid pumping occurs as a result of the net forces pushing the bulk fluid to flow forward. FIG. **12**C illustrates the dimensions of the acoustofluidic micropump: the microchannel has a width of 600 μm and a depth of 100 μm, and each sharp-edge structure is identical. Different tilting angles (β) of oscillatory elements, including 30°, 45°, 60° and 70°, were chosen to investigate the resulting pumping behaviour and determine optimal angle for best pumping performance.

[0133] As shown in FIG. **13**A, the recirculating fluid passage was designed to produce fluid pumping in a counter-clockwise direction employing 30° tilted oscillatory elements, as shown by the inset. A solution containing DI water and 1.9 μm diameter DRAGON GREEN fluorescent beads (Bangs Laboratory) was injected into the channel to characterize the acoustic streaming patterns induced by the oscillation of tilted oscillatory elements. The acoustic frequency was swept from 1 kHz to 100 kHz 50 Hz increments. The microstreaming patterns, as shown in FIG. **13**B, were developed around the tips of oscillating oscillatory elements in the pumping region, when the piezoelectric transducer was activated at 6.5 kHz, the frequency at which the pumping occurs on account of the induced acoustic streaming effect. For an acoustic micropump of this exemplary design, 6.5 kHz was the working frequency for the piezoelectric transducer to activate the pumping action, and thus this frequency was used for all the following experiments.

[0134] To further investigate the influence of the tilting angle of oscillatory elements on the pumping performance, a mixture of DI water mixed with polystyrene beads of different diameters (20 μm and 0.9 μm) was injected into the channel. And the estimated average flow rate inside the channel was calculated by tracking average bead velocity in the upper channel, in which 50-100 beads were randomly selected and tracked for each independent experiment. In addition to the effect of tilting angle, the pumping performance under different input voltages of the piezoelectric transducer was also characterized. FIG. **14**A illustrates the pumping performance of the four different tilting angles of oscillatory elements under different input voltages. The results show that when the piezoelectric transducer was activated with voltages ranging from 5 Vpp to 50 Vpp, pumping occurred at all tilting angles. As the tilting angle was decreased, the generated pumping flow rate increased. Of the four different tilting angles, the device with 30° tilted sharp-edge structures, as shown in FIG. **14**A, generated significantly greater pumping flow rate, and with a voltage of 50 Vpp, it generated a flow rate as high as 8 μL/min, which corresponds to a calculated pumping pressure of 76 Pa. Lower pumping flow rates generated by 45°, 60° and 70° tilted oscillatory elements can be attributed to the fact that with larger the tilting angle, the generated net force points more towards the direction perpendicular to the flow direction, thereby weakening the force that could push the bulk fluid to flow forward. In addition, as the voltage was increased, the pumping flow rate also increased, indicating that the pumping flow rate could be controlled by adjusting the input voltages. Using the tilted oscillatory elements in the fluid channel, wide-range pumping flow rates, from nL/min to μL/min, were generated by adjusting the input voltage to the piezoelectric transducer. Aside from the function of continuous fluid pumping, FIG. **14**B demonstrates that alternately switching the piezoelectric transducer to ON and OFF at various burst frequencies, 0.5 Hz (T=2 sec) and 2 Hz (T=0.5 sec), that the function of pulsatile fluid pumping could be realized, thereby indicating that the profile of the pumping flow rate could be modulated by programming the input signal to the piezoelectric transducer.

[0135] Various modifications of the present invention, in addition to those shown and described herein, will be apparent to those skilled in the art of the above description. Such modifications are also intended to fall within the scope of the appended claims.

REFERENCE LIST

[0136] 1. L. Pollack, M. W. Tate, N. C. Darnton, J. B. Knight, S. M. Gruner, W. a Eaton, and R. H. Austin, *Proceedings of the National Academy of Sciences,* 1999, 96, 10115-10117.

[0137] 2. Y. Xie, D. Ahmed, M. I. Lapsley, S.-C. S. Lin, A. A. Nawaz, L. Wang, and T. J. Huang, *Analytical chemistry,* 2012, 84, 7495-501.

[0138] 3. J. DeMello and A. DeMello, *Lab on a Chip,* 2004, 4, 11N.

[0139] 4. L.-H. Hung, S.-Y. Teh, J. Jester, and A. P. Lee, *Lab on a chip,* 2010, 10, 1820-5.

[0140] 5. L. Capretto, W. Cheng, D. Carugo, O. L. Katsamenis, M. Hill, and X. Zhang, *Nanotechnology*, 2012, 23, 375602.

[0141] 6. G. Schabas, H. Yusuf, M. G. Moffitt, and D. Sinton, *Langmuir: the ACS journal of surfaces and colloids*, 2008, 24, 637-43.

[0142] 7. S. Yang, F. Guo, B. Kiraly, X. Mao, M. Lu, and T. J. Huang, *Lab on a chip,* 2012, 12, 2097-102.

[0143] 8. C. Ho and Y. Tai, *Journal of Fluids Engineering,* 1996, 118, 437-47.

[0144] 9. G. M. Whitesides, *Nature*, 2006, 442, 368-73.

[0145] 10. X. Mao, J. R. Waldeisen, B. K. Juluri, and T. J. Huang, *Lab on a chip,* 2007, 7, 1303-8.

[0146] 11. X. Mao, B. K. Juluri, M. I. Lapsley, Z. S. Stratton, and T. J. Huang, *Microfluidics and Nanofluidics,* 2009, 8, 139-44.

[0147] 12. N.-T. Nguyen and Z. Wu, *Journal of Micromechanics and Microengineering,* 2005, 15, R1-R16.

[0148] 13. L. Capretto, W. Cheng, M. Hill, and X. Zhang, *Microfluidics*, 2011, 27-68.

[0149] 14. P. Neuzil, S. Giselbrecht, K. Länge, T. J. Huang, and A. Manz, *Nature Reviews Drug Discovery,* 2012, 11, 620-32.

[0150] 15. X. Mao and T. J. Huang, *Lab on a Chip,* 2012, 12, 4006-9.

[0151] 16. X. Mao and T. J. Huang, *Lab on a Chip,* 2012, 12, 1412-6.

[0152] 17. F. Guo, M. I. Lapsley, A. A. Nawaz, Y. Zhao, S.-C. S. Lin, Y. Chen, S. Yang, X.-Z. Zhao, and T. J. Huang, *Analytical chemistry,* 2012, 84, 10745-9.

[0153] 18. H. Song and R. F. Ismagilov, *Journal of the American Chemical Society,* 2003, 125, 14613-9.

[0154] 19. E. M. Miller and A. R. Wheeler, *Analytical and bioanalytical chemistry,* 2009, 393, 419-26.

[0155] 20. C.-H. Hsu and A. Folch, *Applied Physics Letters,* 2006, 89, 144102.

[0156] 21. S. Hossain, M. A. Ansari, and K. Y. Kim, *Chemical Engineering Journal,* 2009, 150, 492-501.

[0157] 22. X. Mao, J. R. Waldeisen, and T. J. Huang, *Lab on a chip,* 2007, 7, 1260-2.

[0158] 23. X. Mao, S.-C. S. Lin, C. Dong, and T. J. Huang, *Lab on a chip,* 2009, 9, 1583-9.

[0159] 24. T.-H. Wang, Y. Peng, C. Zhang, P. K. Wong, and C.-M. Ho, *Journal of the American Chemical Society,* 2005, 127, 5354-9.

[0160] 25. J. Knight, A. Vishwanath, J. Brody, and R. Austin, *Physical Review Letters,* 1998, 80, 3863-66.

[0161] 26. C.-Y. Lee, G.-B. Lee, L.-M. Fu, K.-H. Lee, and R.-J. Yang, *Journal of Micromechanics and Microengineering,* 2004, 14, 1390-8.

[0162] 27. C. K. Harnett, J. Templeton, K. a Dunphy-Guzman, Y. M. Senousy, and M. P. Kanouff, *Lab on a chip,* 2008, 8, 565-72.

[0163] 28. M. Sigurdson, D. Wang, and C. D. Meinhart, *Lab on a chip,* 2005, 5, 1366-73.

[0164] 29. W. Y. Ng, S. Goh, Y. C. Lam, C. Yang, and I. Rodriguez, *Lab on a chip,* 2009, 9, 802-9.

[0165] 30. J. T. Coleman, J. McKechnie, and D. Sinton, *Lab on a chip,* 2006, 6, 1033-9.

[0166] 31. R.-J. Yang, C.-H. Wu, T.-I. Tseng, S.-B. Huang, and G.-B. Lee, *Japanese Journal of Applied Physics,* 2005, 44, 7634-7642.

[0167] 32. C. Neils, Z. Tyree, B. Finlayson, and A. Folch, *Lab on a chip,* 2004, 4, 342-50.

[0168] 33. T. W. Lim, Y. Son, Y. J. Jeong, D.-Y. Yang, H.-J. Kong, K.-S. Lee, and D.-P. Kim, *Lab on a chip,* 2011, 11, 100-3.

[0169] 34. K. S. Ryu, K. Shaikh, E. Goluch, Z. Fan, and C. Liu, *Lab on a chip,* 2004, 4, 608-13.

[0170] 35. G.-P. Zhu and N.-T. Nguyen, *Lab on a chip,* 2012, 12, 4772-80.

[0171] 36. J. Tsai and L. Lin, *Sensors and Actuators A: Physical,* 2002, 97-98, 665-671.

[0172] 37. A. N. Hellman, K. R. Rau, H. H. Yoon, S. Bae, J. F. Palmer, K. S. Phillips, N. L. Allbritton, and V. Venugopalan, *Analytical chemistry,* 2007, 79, 4484-92.

[0173] 38. S.-C. S. Lin, X. Mao, and T. J. Huang, *Lab on a chip,* 2012, 12, 2766-70.

[0174] 39. X. Ding, S.-C. S. Lin, B. Kiraly, H. Yue, S. Li, J. Shi, S. J. Benkovic, and T. J. Huang, *Proceeding of National Academy of Sciences of the United States of America* (*PNAS*), 2012, 109, 11105-9.

[0175] 40. J. Shi, X. Mao, D. Ahmed, A. Colletti, and T. J. Huang, *Lab on a chip,* 2008, 8, 221-3.

[0176] 41. A. R. Rezk, A. Qi, J. R. Friend, W. H. Li, and L. Y. Yeo, *Lab on a chip,* 2012, 12, 773-9.

[0177] 42. W.-K. Tseng, J.-L. Lin, W. C. Sung, S.-H. Chen, and G.-B. Lee, *Journal of Micromechanics and Microengineering,* 2006, 16, 539-548.

[0178] 43. A. Wixforth, C. Strobl, C. Gauer, A. Toegl, J. Scriba, and Z. v Guttenberg, *Analytical and bioanalytical chemistry,* 2004, 379, 982-91.

[0179] 44. T. Frommelt, M. Kostur, M. Wenzel-Schafer, P. Talkner, P. Hänggi, and A. Wixforth, *Physical Review Letters,* 2008, 100, 034502.

[0180] 45. G. G. Yaralioglu, I. O. Wygant, T. C. Marentis, and B. T. Khuri-Yakub, *Analytical chemistry,* 2004, 76, 3694-8.

[0181] 46. K. Sritharan, C. J. Strobl, M. F. Schneider, A. Wixforth, and Z. Guttenberg, *Applied Physics Letters,* 2006, 88, 054102.

[0182] 47. H. Yu, J. Kwon, and E. Kim, *Journal of Microelectromechanical Systems,* 2006, 15, 1015-1024.

[0183] 48. Z. Yang, S. Matsumoto, H. Goto, M. Matsumoto, and R. Maeda, *Sensors and Actuators A: Physical,* 2001, 93, 266-272.

[0184] 49. S. Oberti, A. Neild, and T. W. Ng, *Lab on a chip,* 2009, 9, 1435-8.

[0185] 50. P. Marmottant and S. Hilgenfeldt, *Nature,* 2003, 423, 153-156.

[0186] 51. R. H. Liu, J. Yang, M. Z. Pindera, M. Athavale, and P. Grodzinski, *Lab on a chip,* 2002, 2, 151-7.

[0187] 52. A. R. Tovar and A. P. Lee, *Lab on a chip,* 2009, 9, 41-3.

[0188] 53. D. Ahmed, X. Mao, J. Shi, B. K. Juluri, and T. J. Huang, *Lab on a chip,* 2009, 9, 2738-41.

[0189] 54. D. Ahmed, X. Mao, B. K. Juluri, and T. J. Huang, *Microfluidics and Nanofluidics,* 2009, 7, 727-731.

[0190] 55. K. Rodaree, T. Maturos, S. Chaotheing, T. Pogfay, N. Suwanakitti, C. Wongsombat, K. Jaruwongrungsee, A. Wisitsoraat, S. Kamchonwongpaisan, T. Lomas, and A. Tuantranont, *Lab on a chip,* 2011, 11, 1059-64.

[0191] 56. D. Ahmed, C. Y. Chan, S.-C. S. Lin, H. S. Muddana, N. Nama, S. J. Benkovic, and T. J. Huang, *Lab on a chip,* 2013, 13, 328-31.

[0192] 57. P.-H. Huang, M. I. Lapsley, D. Ahmed, Y. Chen, L. Wang, and T. Jun Huang, *Applied physics letters,* 2012, 101, 141101.

[0193] 58. A. Hashmi, G. Heiman, G. Yu, M. Lewis, H.-J. Kwon, and J. Xu, *Microfluidics and Nanofluidics,* 2012, 14, 591-596.

[0194] 59. A. Hashmi, G. Yu, M. Reilly-Collette, G. Heiman, and J. Xu, *Lab on a chip,* 2012, 12, 4216-27.

[0195] 60. Y. Xu, A. Hashmi, G. Yu, X. Lu, H.-J. Kwon, X. Chen, and J. Xu, *Applied Physics Letters,* 2013, 102, 023702.

[0196] 61. M. V Patel, A. R. Tovar, and A. P. Lee, *Lab on a chip,* 2012, 12, 139-45.

[0197] 62. H. Bruus, J. Dual, J. Hawkes, M Hill, T. Laurell, J. Nilsson, S. Radel, S. Sadhal, and M. Wiklund, *Lab on a chip,* 2011, 11, 3579-80.

[0198] 63. J. Shi, D. Ahmed, X. Mao, S.-C. S. Lin, A. Lawit and T. J. Huang, *Lab on a chip,* 2009, 9, 2890-5.

[0199] 64. J. Shi, H. Huang, Z. Stratton, Y. Huang, and T. J. Huang, *Lab on a chip,* 2009, 9, 3354-9.

[0200] Patents, publications, and applications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents, publications, and applications are incorporated herein by reference to the same extent as if each individual patent, publication, or application was specifically and individually incorporated herein by reference.

[0201] The foregoing description is illustrative of particular embodiments of the invention, but is not meant to be a limitation upon the practice thereof. The following claims, including all equivalents thereof, are intended to define the scope of the invention.

1. An acoustofluidic apparatus comprising:

a fluid passage, said fluid passage comprising at least one sidewall, at least one inlet, and at least one outlet;

an oscillatory element comprising a tip extending into said fluid passage; and

an acoustic source in acoustic contact with said oscillatory element, said acoustic source operable to vibrate said oscillatory element or a portion thereof, so as to create microstreaming in a fluid within said fluid passage.

2. The acoustofluidic apparatus of claim **1**, said oscillatory element comprising two adjacent surfaces extending between said sidewall and said tip, said adjacent surfaces being disposed at a tip angle with respect to each other, the tip angle being less than 180 degrees.

3. The acoustofluidic apparatus of claim **2**, wherein the tip angle is less than or equal to 45 degrees.

4. The acoustofluidic apparatus of claim **1**, said tip having a surface with a width and a length.

5. The acoustofluidic apparatus of claim **4**, said length equal to the height of said fluid channel.

6. The acoustofluidic apparatus of claim **4**, said width is from 5 to 100 micrometers.

7. The acoustofluidic apparatus of claim **1**, said oscillatory element extending at a tilting angle relative to the length of said sidewall, said tilting angle being 90 degrees or less.

8. The acoustofluidic apparatus of claim **7**, said tilting angle is from 0 degrees to 70 degrees.

9. The acoustofluidic apparatus of claim **1**, wherein the acoustic source is a piezoelectric transducer.

10. The acoustofluidic apparatus of claim **1**, wherein the acoustic source is operable to vibrate the oscillatory element at a frequency in the range of 2 Hz to 900 MHz.

11. A process of inducing microstreaming in a fluid comprising:

contacting a first fluid in a fluid passage with a oscillatory element; and

acoustically oscillating said oscillatory element with an acoustic source in acoustic contact with said oscillatory element so as to induce microstreaming in said first fluid.

12. The process of claim **11** wherein said oscillatory element comprises a tip angle of 45 degrees or less.

13. The process of claim **11** wherein said oscillatory element is oriented relative to an edge of said fluid passage by a tilting angle from 0 degrees to 70 degrees.

14. The process of claim **11** wherein said oscillatory element oscillates at a frequency in the range of 2 Hz to 900 MHz.

15. The process of claim **11** wherein said microstreaming induces mixing of said first fluid and a second fluid, and optionally a third fluid, within said fluid channel.

16. The process of claim **15** producing a concentration gradient of a component of said first fluid, said second fluid, optionally said third fluid, or any combination thereof.

17. The process of claim **15** further comprising contacting said first fluid and said second fluid with a plurality of oscillatory elements off set and generating a gradient profile between said first fluid and said second fluid, and optionally said third fluid.

18. The process of claim **15** further comprising alternating said acoustic source from an on state to an off state to produce a temporal gradient waveform of concentration of said first fluid and said second fluid.

19. A process of pumping fluid through a channel comprising:

contacting a fluid in a fluid passage with an oscillatory element, said oscillatory element, or oscillating portion thereof, oriented relative to an edge of said fluid passage by a tilting angle from 0 degrees to 180 degrees; and

acoustically oscillating said oscillatory element with an acoustic source in acoustic contact with said oscillatory element so as to induce directional microstreaming in said fluid.

20. The process of claim **19** wherein said tilting angle is from 0 degrees to 70 degrees.

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