



(51) International Patent Classification:
B81B 7/00 (2006.01) **B81C 1/00** (2006.01)

[US/US]; 77 Massachusetts Ave., Cambridge, MA 02139 (US).

(21) International Application Number:
PCT/US2009/053574

(72) Inventors; and

(22) International Filing Date:
12 August 2009 (12.08.2009)

(75) Inventors/Applicants (for US only): **BAZANT, Martin, Z.** [US/US]; 40 Pleasant St. #1, Arlington, MA 02476 (US). **LEVITAN, Jeremy** [US/US]; 195 Binney Street #1303, Cambridge, MA 02142 (US).

(25) Filing Language: English

(74) Agent: **COHEN, Mark, S.**; Pearl Cohen Zedek Latzer, LLP, 1500 Broadway, 12th Floor, New York, NY 10036 (US).

(26) Publication Language: English

(30) Priority Data:
61/088,160 12 August 2008 (12.08.2008) US

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,

(71) Applicant (for all designated States except US): **MAS-SACHUSETTS INSTITUTE OF TECHNOLOGY**

[Continued on next page]

(54) Title: INDUCED-CHARGE ELECTRO-OSMOTIC MICROFLUIDIC DEVICES

FIGURE 1A

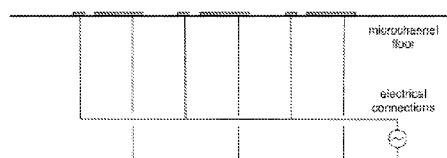


FIGURE 1B

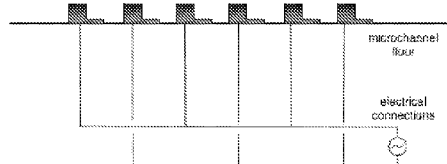


FIGURE 1C

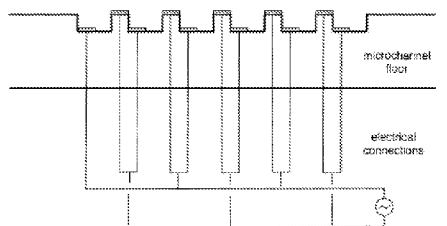
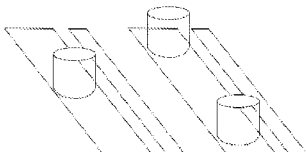


FIGURE 1D



(57) Abstract: This invention provides devices and apparatuses comprising the same, for efficient mixing of relatively small volumes of fluid and materials suspended thereby. Such devices utilize nonlinear electrokinetics as a primary mechanism for driving fluid flow, and such flow is modulated to promote chaotic mixing. Methods of cellular analysis and high-throughput, multi-step product formation using devices of this invention are described.





HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ,

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

INDUCED-CHARGE ELECTRO-OSMOTIC MICROFLUIDIC DEVICES

FIELD OF THE INVENTION

[001] The invention relates to the fields of microfluidics, micro-total-analysis systems (μ TAS) and micro-electro-mechanical systems (MEMS), in particular modulated microfluidic mixers driven by induced-charge electro-osmosis.

BACKGROUND OF THE INVENTION

[002] The ability to mix fluids in micron-sized channels is essential for many emerging technologies, such as in vivo drug delivery devices, micro-electro-mechanical systems (MEMS), and micro-total-analysis systems (μ TAS). Methods for the rapid mixing of non-homogeneous fluids in micron-scale devices are required, since the absence of turbulent mixing on these small length scales implies that mixing occurs by molecular diffusion alone. This typically takes from seconds to minutes—far too slow for envisioned applications. New technologies are thus required for the manipulation, transport and mixing of fluids on these small length scales.

[003] Microfluidics is a growing area of science and technology with important applications in biomedical devices and portable electronics. Traditional pressure-driven flows do not scale well with miniaturization, due to large viscous stresses, so other pumping techniques have been explored. An attractive alternative is electro-osmosis, the effective slip of a liquid electrolyte past a solid surface in response to an applied electric field, since it does not involve any moving parts, is unaffected by miniaturization, and integrates well with standard microelectronics and fabrication methods. The standard technique of (capillary) electro-osmosis involves a DC electric field applied down a microchannel made of insulating material to generate a plug flow. The electric field acts on the equilibrium surface charge in the diffuse-part of the double layer, and the resulting electro-osmotic flow is linear in the applied field.

[004] Various methods have been described to alter the surface charge in linear electro-osmosis to allow some degree of local flow control. For example, one method applies “field-effect electro-osmosis” to control capillary electro-osmosis by applying voltages at secondary electrodes just outside the channel surface, to alter the equilibrium surface charge (or “zeta potential”) driving steady flow at the insulating channel wall and another controls liquid flow down a traditional insulating capillary by the same effect.

[005] Capillary electro-osmosis, with or without field-effect flow control, however, is not ideal for certain microfluidic applications, since the electric field is applied down the channel, a large voltage is required, e.g. 100 Volts across a 1cm device to generate typical fields of 100 V/cm. Since electro-osmosis is linear in the applied field, a direct current must be sustained through Faradaic electrochemical reactions at the electrodes generating the field, which can produce gas bubbles, electrode

degradation/dissolution, hydrodynamic instability, and sample contamination. Further, the typical fluid velocity is fairly small (e.g. 100 micron/sec for a 100 Volt/cm field) and only increases linearly with the voltage.

[006] Microfluidic devices based on nonlinear electro-osmotic flow have also been developed. For example, nonlinear electro-osmotic flow, varying as the square of the applied voltage, termed “AC electro-osmosis” (ACEO), over a pair of flat, parallel-stripe microelectrodes on a flat insulating surface, has been described.

[007] While existing ACEO pumps operate at much lower power (mA) and lower voltage (Volt) than microfluidic pumps based on linear electro-osmosis, nonetheless, current ACEO devices are somewhat inefficient for long-range pumping, and more general flow topologies for simultaneous mixing and pumping have not been developed.

[008] Existing ACEO designs are suboptimal for effective mixing in a fixed volume or cavity of a microfluidic device. Reliance on molecular diffusion, which is the hallmark for the existing ACEO devices, can be too slow for many potential applications.

SUMMARY OF THE INVENTION

[009] In one embodiment, this invention provides a device comprising at least one microfluidic chamber for mixing an electrolyte fluid, said chamber comprising:

- a plurality of electrodes proximal to, positioned on, or comprising at least one surface of said chamber arranged in at least two series; and
- a source, providing an electric field in said chamber, wherein said source applies voltage selectively to said first series such that said voltage is not simultaneously or commensurately applied to second series of electrodes of said plurality;

whereby an electroosmotic flow trajectory created by application of voltage to said first series varies from an electroosmotic flow trajectory created by application of voltage to said second series.

[0010] In one embodiment, the at least two series are positioned such that an electroosmotic flow trajectory created by a first series is in a direction opposite to an electroosmotic flow trajectory created by a second series of said at least two series. In some embodiments, the first series is so positioned such that an electroosmotic flow trajectory created thereby is parallel to a long axis of said device and said second series is so positioned such that an electroosmotic flow trajectory created thereby is perpendicular thereto, or vice versa. In some embodiments, the at least two series are positioned on opposing surfaces of said chamber.

[0011] In some embodiment, the series are arranged asymmetrically with respect to a central axis in the chamber and in another embodiment, the electrodes are arranged in a symmetric pattern in the chamber, while in another embodiment, the electrodes are arranged in an asymmetric pattern in the chamber. In another embodiment, the electrodes are arranged in a gradient pattern in the chamber.

5 [0012] In some embodiments, the source modulates the magnitude or frequency of the voltages applied to the series of electrodes, and in some embodiments, the magnitude or direction of electroosmotic flow is changed thereby. In some embodiments, the changed electroosmotic flow is slower than electroosmotic flow in said chamber prior to modulation of said magnitude or frequency.

[0013] In one embodiment, the electric field is comprised of a DC electric field, or in another
10 embodiment, the electric field is comprised of an AC or pulsed AC electric field.

[0014] In one embodiment,

[0015] In one embodiment, at least one of said plurality of electrodes or a first portion thereof is varied by at least 1% in height, in surface area or in vertical positioning within said chamber, or a combination thereof, with respect to another of said plurality of electrodes or a second portion of said at least one of
15 said plurality of electrodes. In some embodiments, each series of said plurality of electrodes comprises at least one electrode, or a portion thereof, which is raised with respect to another electrode, or another portion of said at least one electrode in said series, or in another embodiment, which is lowered with respect to another electrode, or another portion of said at least one electrode in said series.

[0016] In another embodiment, each series of said plurality of electrodes comprises at least one electrode
20 or at least a portion thereof having a height or depth which is varied proportionally to a width of another electrode, another portion of said at least one electrode, or a combination thereof, in said series. In one embodiment, each series of said plurality of electrodes comprises at least one electrode, or portions thereof, having height or depth variations from about 1% to about 1000% of:

- a width of another electrode in said series, another portion of said at least one electrode
25 in said series, or a combination thereof;
- a gap between said at least one electrode and another electrode in said series;
- or a combination thereof.

[0017] In another embodiment, at least one electrode is not flat and in another embodiment, positioning
30 of the electrodes in the chamber is varied with respect to gaps between the electrodes, spacing of the electrodes, or a combination thereof. In one embodiment, the gaps are between about 1 micron and about 50 microns and in another embodiment, gaps between the electrodes, spacing of the electrodes, height of the electrodes or portions thereof, shapes of the electrodes or portions thereof, surface area of the

electrodes or portions thereof, volume of the electrodes or portions thereof, vertical positioning of the electrodes or portions thereof within said chamber or a combination thereof is unequal. In one embodiment, gaps between the electrodes, spacing of the electrodes, or a combination thereof is equal. In another embodiment, electrode widths are between about 0.1 microns and about 50 microns. In another embodiment, at least one electrode of the plurality of electrodes comprises at least one raised portion of the electrode in the form of a cylinder of arbitrary cross section. In another embodiment, at least one electrode of the plurality of electrodes comprises an exposed surface, which is flat, and not coplanar with another exposed surface of the electrode or of another electrode in the series.

[0018] In another embodiment, the chamber is comprised of a transparent material, which in one embodiment is a plastic or in another embodiment is a polymer. In one embodiment, the device is comprised of a material, which is transparent at a given wavelength corresponding to excitation and emission of a fluorophor. In one embodiment, the device is comprised of a material, which is transmissive at a certain wavelength, or in some embodiments, at a wavelength that corresponds to excitation/emission of a compound or reagent. In another embodiment, the microfluidic channels and/or devices comprising the same are comprised of a material functionalized via SAM, or in another embodiment, comprising an adhesion layer, as described herein.

[0019] In another embodiment, the electrodes are arranged in a gradient pattern in the chamber.

[0020] In another embodiment, the source applies a peak to peak AC voltage of between about 0.1 and about 10 Volts. In another embodiment, the AC frequency is between about 1 Hz and about 100 kHz.

[0021] In another embodiment, this invention provides an apparatus comprising a device of this invention.

[0022] In another embodiment, this invention provides a method of mixing a fluid, said method comprising

- i. applying a fluid to a device of this invention; and
- ii. selectively applying voltage to the at least two series, such that voltage is not simultaneously or commensurately applied to the at least two series;

whereby upon selective application of voltage to the series, electro-osmotic flows with varied trajectories is generated in a region proximal to each of the series, resulting in mixing of the applied fluid.

[0023] In one embodiment, the method further comprises assay or analysis of the fluid, and in one embodiment, the analysis is a method of cellular analysis. In another embodiment, the method comprises the steps of:

- a. introducing a buffered suspension comprising cells and a reagent for cellular analysis into the microfluidic chamber; and

- b. analyzing at least one parameter affected by contact between the suspension and the reagent.

[0024] In one embodiment, the reagent is an antibody, a nucleic acid, an enzyme, a substrate, a ligand, or a combination thereof. In one embodiment, the reagent is coupled to a detectable marker. In one
5 embodiment, the marker is a fluorescent compound, and in another embodiment the device is coupled to a fluorimeter or fluorescent microscope.

[0025] In another embodiment, the method further comprises the step of introducing a cellular lysis agent to the device. In another embodiment, the reagent specifically interacts or detects an intracellular compound. In another embodiment, the assay or analysis of the fluid is a method of analyte detection or
10 assay.

[0026] In one embodiment, the method further comprises the steps of:

- a. introducing an analyte to the device;
- b. introducing a reagent to the device; and
- c. detecting, analyzing, or a combination thereof, of the analyte.

[0027] In one embodiment, mixing reconstitutes a compound in the device, upon application of the fluid, and in one embodiment, the compound is solubilized slowly in fluids. In one embodiment, the mixing results in high-throughput, multi-step product formation.

[0028] In one embodiment, according to this aspect of the invention, the method further comprises the steps of:

- a. introducing a precursor to the device;
- b. introducing a reagent, catalyst, reactant, cofactor, or combination thereof to the device;
- c. providing conditions whereby the precursor is converted to a product; and
- d. optionally, collecting the product from the device.

[0029] In one embodiment, the method further comprises carrying out iterative introductions of the reagent, catalyst, reactant, cofactor, or combination thereof in (b), to the device.

[0030] In one embodiment, the reagent is an antibody, a nucleic acid, an enzyme, a substrate, a ligand, a reactant or a combination thereof. In one embodiment, the mixing results in drug processing and delivery. According to this aspect and in one embodiment, the method further comprises the steps of:

- i. introducing a drug and a liquid comprising a buffer, a catalyst, or combination thereof to the device;
- ii. providing conditions whereby the drug is processed or otherwise prepared for delivery to a subject; and

- iii. collecting the drug, delivering the drug to a subject, or a combination thereof.

[0031] In one embodiment, the method further comprises the step of carrying out iterative introductions of liquid to the device. In one embodiment, introduction of the liquid serves to dilute the drug to a desired concentration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] The subject matter regarded as the invention is particularly pointed out and distinctly claimed in the concluding portion of the specification. The invention, however, both as to organization and method of operation, together with objects, features, and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanying drawings in which:

[0033] Figure 1 schematically depicts embodiments of elements of the devices of this invention. Four examples of arrays of microelectrodes for incorporation within the devices of this invention are depicted, wherein each array is capable of driving nonlinear electrokinetic flow in one dominant direction upon the application of a spatially periodic AC voltage: 1A. asymmetric planar electrodes; 1B. non-planar, stepped 3D electrodes; 1C. 3D stepped electrodes with non-polarizable side walls; and 1D. general 3D electrodes with raised metal or non-polarizable structures on an underlying pattern.

[0034] Figure 2 shows embodiments of the invention capable of rapid fluid mixing. Two electrode arrays, arbitrarily referred to as "A" (2-10) and "B" (2-20) are positioned in a chamber (2-30), driving nonlinear electrokinetic flows in different directions. Rapid mixing by chaotic advection is achieved by modulating the AC voltage of each array and switching between on and off states of each array: A. two arrays are positioned on opposite walls of the chamber and switch back and forth in dominance; B. two arrays are positioned on the same wall leaving the opposite wall available for other uses, for example and in some embodiments, providing detection sites for particles, molecules, or cells suspended in the fluid. The voltage of only one array is modulated in time.

[0035] Figure 3 shows an embodiment of the invention with four electrode arrays (3-10), (3-20), (3-30), (3-40) in a confined microchamber (3-50). Four different flow patterns are illustrated, representing certain embodiments of this invention, which may arise as a result of modulating application of voltage, etc., in different combinations, for the respective arrays. In each flow state, the fluid content can be trapped in a convection cell, such that, for example, suspended particles may be separated into different convection cells, which then act as traps. Such traps are illustrated by the dotted line boundary demarcation, which effectively divide the region into separate convection cells. , By alternating between two or more of these states, rapid chaotic mixing can be achieved.

[0036] Figure 4 depicts an embodiment of the invention directed to mixing in a continuous flow via positioning and activating specific arrays in time to drive flows transverse to the channel axis. Short arrows indicate flow direction. Two opposite walls of channel 4-50, are lined with four electrode arrays (A represents 4-10), (B represents 4-20), (C represents 4-30), (D represents 4-40) in figure 4A for mixing.

5 Other geometries for the electrodes are envisioned for this embodiment, as well. Figure 4B, depicts a channel with a larger cross section than figure 4A, which in turn results in slower propulsion across the chamber and longer mixing times therefore.

[0037] Figure 5 depicts an embodiment of the devices of the invention, which selectively traps, pumps, and mixes a fluid in a microchannel. A periodic pattern of four electrode arrays (5-10), (5-20), is
10 positioned along one wall of a microchannel (5-30). Each array can be turned on separately, and each array may drive flows in alternating directions, when voltage is applied thereto. For example, when two interlaced sets of arrays, A (5-10) and B (5-20) are positioned in a chamber, which pump in opposite directions, when both A and B are activated at once, the opposing flows form closed convection cells to trap fluid and/or position particles above each array (5A). Figure 5B depicts activating a single set "A" (5-
15 10), driving a net flow in a single direction, which for example, may disperse and/or transport trapped fluid or particles therein. Figure 5C depicts activating set "B" (5-20) driving a similar flow in the opposite direction. By temporally modulating the sets of electrodes, e.g. by switching between states 5A, 5B, and/or 5C, rapid chaotic mixing can be achieved. Net pumping in a desired direction can be achieved at the same time as mixing by tuning the fraction of the time and/or magnitude of the flow (via the voltage)
20 from set A vs. set B, etc. In other embodiments, temporal modulation of one or more of these states is superimposed on a system with a background pressure-driven flow, which would also suffice for rapid active mixing of the fluid as it is pumped through the channel.

[0038] Figure 6 depicts another embodiment of a device of this invention, wherein arrays comprising two sets of electrodes ("A" being 6-10 and "B" being 6-20) drive flows in the same direction. Each set
25 includes a shifted mirror image pattern on the opposite wall of the channel (i.e. 6-30 corresponds to 6-20, in terms of orientation, and 6-40 corresponds to 6-10). When all electrodes are activated, a uniform plug flow is formed which can be used to transport coherent localized volumes of fluid or particles. With temporal modulation of the two sets of electrodes, various pumping strengths and flow recirculation patterns are generated. For example, temporal modulation of the two sets of electrodes A and B, by
30 alternating the strength of pumping by each in time, causes recirculating flow patterns superimposed on the plug flow to cause rapid mixing in the downstream direction. The modulation time period is comparable to the mean advection time across each individual electrode array, in some embodiments, a scenario which efficiently promotes chaotic mixing.

[0039] Figure 7 depicts an embodiment of modulating the voltage of two or more electrodes on opposite sides of a microchannel. The microchannel contains an array of polarizable (typically metallic or metal coated) posts (7-50; 7-60), which may have symmetric and/or asymmetric geometry and orientation. The device can be subject to time-modulated AC voltages. The dominant AC voltage can be applied alternately to electrode pair A (7-10) and C (7-30) and to electrode pair B (7-20) and D (7-40). An AC voltage applied to each electrode, (7-10)-(7-40) gives each a tunable phase shift relative to the others. By switching the AC fields, rapid chaotic mixing can be achieved in the channel around the posts. In some embodiments, broken symmetry in the geometry of the post array leads to time-modulated pumping through the channel, which is in addition to the time-modulated mixing as described.

[0040] Figure 8 depicts an embodiment of a device of the invention where polarizable posts (8-50) and (8-60) are placed in a microfluidic chamber with at least four electrodes placed on different walls, to allow application of dominant electric fields in orthogonal directions. Left/right using (8-20) and (8-40) electrodes, or up/down using (8-10) and (8-30) electrodes. Time modulation strategies can be used to achieve chaotic mixing in the chamber, as described herein. In some embodiments, switching time is comparable to convection time for the dominant fluid vortices amongst the array of posts.

[0041] It will be appreciated that for simplicity and clarity of illustration, elements shown in the figures have not necessarily been drawn to scale. For example, the dimensions of some of the elements may be exaggerated relative to other elements for clarity. Further, where considered appropriate, reference numerals may be repeated among the figures to indicate corresponding or analogous elements.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0042] In the following detailed description, numerous specific details are set forth in order to provide a thorough understanding of the invention. However, it will be understood by those skilled in the art that the present invention may be practiced without these specific details. In other instances, well-known methods, procedures, and components have not been described in detail so as not to obscure the present invention.

[0043] This invention provides, in some embodiments, devices and apparatuses comprising the same, for the mixing and/or pumping of relatively small volumes of fluid. Such devices utilize nonlinear electrokinetics as a primary mechanism for driving fluid flow.

[0044] In some embodiments, the term “nonlinear electrokinetic flow” refers to any electrically driven fluid flow which scales nonlinearly with the applied voltage or electric field. Examples include induced-charge electro-osmotic (ICEO) flow around polarizable surfaces, AC electro-osmotic (ACEO) flow over electrode arrays, and AC electrothermal flows. In some embodiments the invention describes how such

time-dependent modulation of a set of such nonlinear electrokinetic flows can be exploited for mixing, pumping, and trapping fluid volumes in microfluidic devices.

[0045] In some embodiments, this invention provides a device comprising at least one microfluidic chamber for mixing an electrolyte fluid, the chamber comprising:

- 5 ➤ a plurality of electrodes proximal to, positioned on, or comprising at least one surface of the chamber arranged in at least two series; and
- a source, providing an electric field in said chamber, wherein the source applies voltage selectively to the first series such that said voltage is not simultaneously or commensurately applied to the second series of electrodes of said plurality;

10 whereby an electroosmotic flow trajectory created by application of voltage to the first series varies from an electroosmotic flow trajectory created by application of voltage to the second series.

[0046] Figure 1 exemplifies an embodiment of a device of this invention, depicting multiple periodic arrays of microelectrodes, which drive nonlinear electrokinetic flow which pumps fluid over the array in one direction (e.g. from left to right, or vice versa). In one embodiment the plurality of electrodes as depicted in Figure 1A comprises an asymmetric planar array of flat electrodes of different widths and gaps within each period in the array. The figure depicts one example for the positioning of the plurality of electrodes and the electrical connections for AC forcing in the device. The skilled artisan will readily appreciate other manner of positioning of electrodes within the device and electrical connections, which may be applied thereto to arrive at the devices of this invention.

20 [0047] Another embodiment of a device of this invention is depicted in Figure 1B. In this aspect, and others, the means for AC forcing may comprise a power supply, a voltage source, a function generator or a combination thereof. According to this aspect, as depicted in side-view in Figure 1B, the plurality of electrodes form an array of non-planar stepped electrodes. In some embodiments the plurality of electrodes form an array wherein each electrode is broken into two horizontal surfaces at different heights on dielectric steps (Figure 1C). In some embodiments the plurality of electrodes form a planar array with three-dimensional metal or dielectric structures on the electrodes to further shape the flow by contributing to the directional pumping and/or providing more complex local flows over the array (Figure 1D). In some embodiments, the devices of this invention may comprise combinations of arrays of electrodes as described herein, such that a device may comprise a series of electrodes positioned such that the trajectories of fluid flow from each series differs, however the electrodes comprising each series are similar in geometry, and dimension, and in some embodiments, in orientation. In some embodiments, additional arrays may be incorporated in such devices, which comprise electrodes varying in heights, or comprise electrodes having a portion of such electrode varying in height, referred to herein, in some

embodiments as "stepped electrodes". In some embodiments, the devices comprise stepped and planar electrodes, wherein flow trajectories of such electrodes are altered with respect to each other, or in some embodiments, are in a similar direction, however, their activation may be modulated in time. In some embodiments, such devices may further comprise conducting posts, as described herein. It is to be understood that any combination of arrays or series of electrodes may be positioned within the device, in any order, orientation and/or patterns, to achieve modulated flows as described herein and is to be considered as part of this invention.

[0048] In some embodiments traveling-wave voltages are applied to the plurality of electrodes or to specific series of electrodes, by appropriate electrical connections. In some embodiments the voltage source produces a four-phase pulse in the electrode array. It is to be understood that various embodiments regarding the design of the plurality of electrodes and arrays depicted herein are for illustrative purposes alone, and are not to be considered as limiting the scope of what is envisioned regarding positioning, orientation and embodied electrodes for use in the design and development of the devices of this invention. The devices of this invention are not to be construed as limited by the embodiments presented, but rather are to be understood to encompass the full scope of what the skilled artisan would appreciate the invention encompasses, based on the description provided herein.

[0049] In some embodiments the plurality of electrodes form a three-dimensional array wherein each electrode comprises portions, such that each portion comprises a horizontal surface at different heights on dielectric steps. In some embodiments the devices comprise three-dimensional metal or dielectric structures on the electrodes to further shape the flow by contributing to the directional pumping and/or providing more complex local flows over the array.

[0050] In one embodiment, this invention makes use of ACEO-based devices which pump and/or mix fluid, by a mechanism which utilizes nonlinear electrokinetic pumps and mixers involving three-dimensional structures in microchannels. The driving principle in these devices is termed "induced-charge electro-osmosis" (ICEO), which, in one embodiment refers to nonlinear (voltage-squared) electro-osmotic flow, which results when an electric field acts on its own induced charge at a polarizable (metal or dielectric) surface.

[0051] In one embodiment, this invention provides a device comprising at least one microfluidic chamber for mixing an electrolyte fluid, the chamber comprising:

- a plurality of electrodes proximal to, positioned on, or comprising at least one surface of the chamber, the electrodes or portions thereof are varied in height or in surface area or in vertical location by at least 1%, wherein:
 - the plurality of electrodes are arranged in at least two series, with each series varying in terms of an electroosmotic flow trajectory created by the series upon

application of voltage thereto, from at least a series proximally located thereto on the at least one surface; and

- a source, providing an electric field in the chamber, wherein the source applies voltage selectively to the series such that the voltage is not simultaneously or commensurately applied to all series of electrodes of the plurality.

[0052] In one embodiment, this invention provides a microfluidic device comprising:

- at least one port for fluid entry into, egress from, or a combination thereof of the device; and
- at least one microfluidic channel in fluid communication with the ports, wherein the microfluidic channel comprises:
 - a passageway for transmitting an electrolyte fluid; and
 - a plurality of electrodes connected to a source, providing an electric field in the microchannel; wherein the electrodes are parallel-positioned or interdigitated and are co-axial, with respect to each other, in at most one dimension, whereby the electric field produces a dominant electro-osmotic flow across the microfluidic channel.

[0053] In one embodiment, the devices of this invention further comprise at least one inlet port, at least one outlet port and at least one microfluidic channel in fluid communication with the ports.

[0054] In one embodiment, the microfluidic device comprises placement of the elements on a substrate, or in another embodiment, the microfluidic chamber is contiguous with the substrate.

[0055] In one embodiment, the term “a” refers to at least one, which in some embodiments, is one, or in some embodiments two or more, or in some embodiments, pairs of, or in some embodiments, a series of, or in some embodiments, any multiplicity as desired and applicable for the indicated application.

[0056] In one embodiment, the substrate and/or other components of the device can be made from a wide variety of materials including, but not limited to, silicon, silicon dioxide, silicon nitride, glass and fused silica, gallium arsenide, indium phosphide, III-V materials, PDMS, silicone rubber, aluminum, ceramics, polyimide, quartz, plastics, resins and polymers including polymethylmethacrylate (PMMA), acrylics, polyethylene, polyethylene terephthalate, polycarbonate, polystyrene and other styrene copolymers, polypropylene, polytetrafluoroethylene, superalloys, zircaloy, steel, gold, silver, copper, tungsten, molybdenum, tantalum, KOVAR, KEVLAR, KAPTON, MYLAR, teflon, brass, sapphire, other plastics, or other flexible plastics (polyimide), ceramics, etc., or a combination thereof.

[0057] In some embodiments, the devices will comprise at least one bubble trap or at least one gas permeable membrane proximal to a microfluidic channel, which in turn may facilitate filling of such channel with a fluid as described herein.

[0058] The substrate may be ground or processed flat. High quality glasses such as high melting borosilicate or fused silicas may be used, in some embodiments, for their UV transmission properties when any of the sample manipulation and/or detection steps require light based technologies. In addition, as outlined herein, portions of the internal and/or external surfaces of the device may be coated with a variety of coatings as needed, to facilitate the manipulation or detection technique performed, to enhance flow, to promote mixing, or combinations thereof.

[0059] In one embodiment, the substrate comprises a metal-bilayer. In some embodiments, such substrates comprise adhesive or bonding layers such as titanium or chrome or other appropriate metal, which is patterned or placed between the electrode surface and another component of the device substrate, for example, between a distal gold electrode and an underlying glass or plastic substrate.

[0060] In one embodiment, the metal-bilayer is such that a metal is attached directly to an electrode, which comprises, or is attached to another component of the substrate.

[0061] In another embodiment, the substrate comprises an adhesive layer between, for example underlying glass or plastic substrate and an electrode such as a polymer, a monolayer, a multilayer, a metal or a metal oxide, comprising iron, molybdenum, copper, vanadium, tin, tungsten, gold, aluminum, tantalum, niobium, titanium, zirconium, nickel, cobalt, silver, chromium or any combination thereof. In another embodiment the substrate comprises electrodes of zinc, gold, copper, magnesium, silver, aluminum, iron, carbon or metal alloys such as zinc, copper, aluminum, magnesium, which may serve as anodes, and alloys of silver, copper, gold as cathodes.

[0062] In another embodiment, the substrate comprises electrode couples including, but not limited to, zinc-copper, magnesium-copper, zinc-silver, zinc-gold, magnesium-gold, aluminum-gold, magnesium-silver, magnesium-gold, aluminum-copper, aluminum-silver, copper-silver, iron-copper, iron-silver, iron-conductive carbon, zinc-conductive carbon, copper-conductive carbon, magnesium-conductive carbon, and aluminum-conductive carbon. In some embodiments electrodes within a single array are comprised of the same material, or in some embodiments, of a different material. In some embodiments, electrodes within a single device, but within separate arrays or series of electrodes are comprised of the same material, or in some embodiments, of a different material. In one embodiment an electrode as described herein may be comprised of any conductive material, for example, and in some embodiments, gold, copper, gold-palladium, nickel, or any other suitable material, as will be appreciated by the skilled artisan. In some embodiments, an electrode as described herein may be comprised of any suitable

material as herein described. In some embodiments electrodes as described herein may be coated. In some embodiments electrodes as described herein may be plated.

[0063] In some embodiments, the substrate may be further coated with a dielectric and/or a self-assembled monolayer (SAM), to provide specific functionality to the surface of the device to which the material is applied.

[0064] In one embodiment, the term “chambers” “channels” and/or “microchannels” are interchangeable, and refer to a cavity of any size or geometry, which accommodates at least the indicated components and is suitable for the indicated task and/or application.

[0065] In some embodiments such channels comprise the same materials as the substrate, or in another embodiment, are comprised of a suitable material which prevents adhesion to the channels, or in another embodiment, are comprised of a material which promotes adhesion of certain material to the channels, or combinations thereof. In some embodiments, such materials may be deposited according to a desired pattern to facilitate a particular application.

[0066] In another embodiment, the substrate and/or chambers of the devices of this invention comprise a material which is functionalized to minimize, reduce or prevent adherence of materials introduced into the device. For example, in one embodiment, the functionalization comprises coating with extracellular matrix protein/s, amino acids, PEG, or PEG functionalized SAM's or is slightly charged to prevent adhesion of cells or cellular material to the surface. In another embodiment, functionalization comprises treatment of a surface to minimize, reduce or prevent background fluorescence. Such functionalization may comprise, for example, inclusion of anti-quenching materials, as are known in the art. In another embodiment, the functionalization may comprise treatment with specific materials to alter flow properties of the material through the device. In another embodiment, such functionalization may be in discrete regions, randomly, or may entirely functionalize an exposed surface of a device of this invention.

[0067] In one embodiment, the invention provides for a microchip comprising the devices of this invention. In one embodiment, the microchip may be made of a wide variety of materials and can be configured in a large number of ways, as described and exemplified herein, in some embodiments and other embodiments will be apparent to one of skill in the art.

[0068] The composition of the substrate will depend on a variety of factors, including the techniques used to create the device, the use of the device, the composition of the sample, the molecules to be assayed, the type of analysis conducted following assay, the size of internal structures, the placement of electronic components, etc. In some embodiments, the devices of the invention will be sterilizable as well, in some embodiments, this is not required. In some embodiments, the devices are disposable or, in another embodiment, re-usable.

[0069] Microfluidic chips used in the methods and devices of this invention may be fabricated using a variety of techniques, including, but not limited to, hot embossing, such as described in H. Becker, et al., Sensors and Materials, 11, 297, (1999), hereby incorporated by reference, molding of elastomers, such as described in D.C. Duffy, et. al., Anal. Chem., 70, 4974, (1998), hereby incorporated by reference, injection molding, LIGA, soft lithography, silicon fabrication and related thin film processing techniques, as known in the art, photolithography and reactive ion etching techniques, as exemplified herein. In one embodiment, glass etching and diffusion bonding of fused silica substrates may be used to prepare microfluidic chips.

[0070] In one embodiment, microfabrication technology, or microtechnology or MEMS, applies the tools and processes of semiconductor fabrication to the formation of, for example, physical structures. Microfabrication technology allows one, in one embodiment, to precisely design features (e.g., reservoirs, wells, channels) with dimensions in the range of $<1\ \mu\text{m}$ to several centimeters on chips made, in other embodiments, of silicon, glass, or plastics. Such technology may be used to construct the microchannels of the devices of this invention, in one embodiment.

[0071] In one embodiment, fabrication of the device may be accomplished as follows: first, a glass substrate is metallized. The choice of metal can be made with respect to a variety of desired design specifications, including resistance to oxidation, compatibility with biological materials, compatibility with substrates, etc. The metallization layer may be deposited in a specific pattern (i.e. through adhesive or shadow-masked metal evaporation or sputtering), in one embodiment, or, in another embodiment, it may be etched subsequent to deposition. Metals can include, but are not limited to gold, copper, silver, platinum, rhodium, chromium, etc. In some embodiments, the substrate may be coated with an initial layer of a thin metal, which promotes adhesion of another metal to the substrate. In some embodiments, metals may also be adhered to the substrate via adhesive. In some embodiments, the substrate is ground flat to promote adhesion. In some embodiments, the substrate is roughened to promote metal adhesion.

[0072] According to this aspect of the invention, and in one embodiment, the deposited metal may either be deposited in the final topology (i.e. through a mask) or, in another embodiment, patterned post-deposition. According to the latter embodiment, a variety of methods may be used to create the final pattern, as will be understood by one skilled in the art, including *inter-alia*, etching and laser ablation. Mechanical forms of removal (milling, etc.) may be used, in other embodiments.

[0073] In one embodiment, gold is deposited on chromium and the gold is etched using a photoresist mask and a wet gold etchant. The chromium remains a uniform film, providing electrical connection for subsequent electrodeposition (forming the anode connection). In another embodiment, gold is deposited via electron-beam evaporation onto an adhesion layer of titanium. The gold is patterned using a wet etchant and photoresist mask. The titanium is left undisturbed for subsequent electrodeposition.

[0074] In another embodiment, the metal may be patterned prior to deposition. A shadow mask can be utilized in one embodiment. The desired shape is etched or machined through a thin metal pattern or other substrate. The etched substrate is then held parallel to the base substrate and the material is deposited via evaporation or sputtering through the mask onto the substrate. In some embodiments, this method is desirable in that it reduces the number of etch steps.

[0075] In another embodiment, the patterned surface is formed by transferring a pre-etched or stamped metal film with adhesive onto the substrate. In one embodiment, the various devices on the layer have a common electrical connection enabling subsequent electrodeposition, and are deposited strategically so that release and dicing results in proper electrical isolation.

[0076] In another embodiment, a rigid stamp is used to puncture a thin metal film on a relatively pliable elastic (plastic) substrate. The rigid stamp can have, in some embodiments sharp or blunt edges.

[0077] In some embodiments, the thickness of deposited metals is tailored to specific applications. In one embodiment, thin metal is deposited onto the surface of the wafer and patterned. According to this aspect of the invention, and in one embodiment, the patterned surface forms a common anodic connection for electroplating into a mold.

[0078] In one embodiment, molding may be used. In one embodiment, molding comprises a variety of plastics, ceramics, or other material which is dissimilar to the base substrate. In one embodiment, the molding material is removed following electroplating. In some embodiments, the molding material is sacrificial.

[0079] In another embodiment, thick (greater than a few microns) metal is deposited and subsequently etched to form raised metal features.

[0080] In other embodiments, welding, assembly via SAMs, selective oxidation of thin metals (conversion of, for instance, aluminum to aluminum oxide) comprise some of the methods used to form insulating areas and provide electrical isolation.

[0081] In other embodiments, passivation of the metal surfaces with dielectric materials may be conducted, including, but not limited to, spin-on-glass, low temperature oxide deposition, plastics, photoresists, and other sputtered, evaporated, or vapor-deposited insulators.

[0082] In some embodiments, the microfluidic channels used in the devices and/or methods of this invention, which mix and optionally convey fluid, may be constructed of a material which renders it transparent or semitransparent, in order to image the materials being assayed, or in another embodiment, to ascertain the progress of the assay, etc. In some embodiments, the materials further have low conductivity and high chemical resistance to buffer solutions and/or mild organics. In other embodiments, the material is of a machinable or moldable polymeric material, and may comprise insulators, ceramics, metals or insulator-coated metals. In other embodiments, the channel may be

constructed from a polymer material that is resistant to alkaline aqueous solutions and mild organics. In another embodiment, the channel comprises at least one surface which is transparent or semi-transparent, such that, in one embodiment, imaging of the device is possible.

[0083] In some embodiments, the devices of this invention have at least one inlet and/or at least one outlet.

[0084] In one embodiment, the inlet, or in another embodiment, the outlet may comprise an area of the substrate in fluidic communication with one or more microfluidic channels, in one embodiment, and/or a sample reservoir, in another embodiment. Inlets and outlets may be fabricated in a wide variety of ways, depending upon, in one embodiment, on the substrate material utilized and/or in another embodiment, the dimensions used. In one embodiment inlets and/or outlets are formed using conventional tubing, which prevents sample leakage, when fluid is applied to the device, under pressure. In one embodiment inlets and/or outlets are formed of a material which withstands application of voltage, even high voltage, to the device. In one embodiment, the inlet may further comprise a means of applying a constant pressure, to generate pressure-driven flow in the device.

[0085] In one embodiment, a “device” or “apparatus” of this invention will comprise at least the elements as described herein. In one embodiment, the devices of this invention comprise at least one microchannel, which may be formed as described herein, or via using other microfabrication means known in the art. In one embodiment, the device may comprise a plurality of channels. In some embodiments, the devices of this invention will comprise a plurality of channels, or microchannels. In one embodiment, the phrase “a plurality of channels” refers to more than two channels, or, in another embodiment, channels patterned according to a desired application, which in some embodiments, refers to channels varying by several orders of magnitude, whether on the scale of tens, hundreds, thousands, etc., as will be appreciated by one skilled in the art.

[0086] In some embodiments, the device comprises microchannels connected to the chamber wherein mixing occurs, whereby the channels convey the mixed fluid from one area of the device to another, via the microchannels. In another embodiment, the channels convey mixed fluid out of the device. In another embodiment, the chamber is in fact a microchannel.

[0087] In one embodiment, the devices of this invention mix and optionally pump fluids using non-linear electroosmotic flow generated within the device.

[0088] In one embodiment, the devices of this invention comprise electrodes connected to a source providing an electric field in the microchannel, wherein the device comprises two or more parallel or interdigitated electrodes, which when in the presence of electrolyte fluids in the device and application of the field produce electro-osmotic flows so that the electrolyte fluid is driven across the microfluidic channels.

[0089] In some embodiments, the term “electrode” is to be understood to refer to the metal or other conducting material- electrode per se, as well as a substrate onto which such an electrode is affixed, or which comprises the electrode, or is proximal to the electrode.

[0090] The electrodes of the devices of this invention will have varied height, in some embodiments, or in other embodiments, will not be co-axial, with regard to Cartesian axes, in more than one dimension. It is to be understood that with reference to varied spatial apportionment of the electrodes, e.g. their height, that such reference is in terms of the vertical placement of the electrode, as well as the electrode placed on an underlying substrate. For example, this invention is to be understood to comprise a chamber comprising a pair of electrodes, wherein the electrodes have a comparable width and depth, however one electrode's height may be 10 micron with another being 40 microns, or with another also being 10 microns, however the electrode is positioned on a substrate of 30 microns in height.

[0091] It is to be understood that with reference to variance in height, such reference is to be understood to encompass distance normal or orthogonal to the surface on which the electrodes are placed, or in other embodiments, in the direction orthogonal to the mean plane of the surface while, for example, “horizontal” may refer to a direction coplanar with the mean plane of the surface.

[0092] In some embodiments, the arrangement of the electrodes is such so as to promote mixing of the materials in the microchannel, as will be appreciated by one skilled in the art, and as exemplified hereinbelow.

[0093] In some embodiments, the geometries of the electrodes are varied so as to promote mixing of the fluid in discrete regions of the channel, and/or conveyance of mixed material.

[0094] In some embodiments flat electrode pairs are arranged in an array on or as part of a substrate. In one embodiment in each pair of electrode, the electrodes are of a rectangular shape. In one embodiment in each pair of electrodes, the two rectangular electrodes have the same length and depth, but differ in width. In some embodiment such different in size and in surface area affects the electric field around each electrode, or between two such electrodes.

[0095] In some embodiments electrode pairs are of a stepped shape. In one embodiment the electrodes are L-shaped, with one area elevated higher than another area in the electrode. In some embodiment such asymmetric geometry of the electrodes affects the electric field around each electrode, or between two such electrodes.

[0096] In some embodiments the two electrodes forming an electrode pair are placed in different height with respect to the underlying substrate. In one embodiment one electrode is placed higher than another electrode. In some embodiment such asymmetric geometry of the electrodes affects the electric field around each electrode, or between two such electrodes. In some embodiment electrodes contain more than one conducting element. In one embodiment the conducting elements are in contact with each other.

In one embodiment an electrode in an electrode pair is made of a planar, rectangular element and a cylindrical element. In one embodiment the cylindrical element is positioned on the planar element and the two elements are in contact. In one embodiment a second electrode in a pair, comprises a similar but narrower planar element and lacks the cylindrical element. In one embodiment such arrangement of an electrode pair breaks the symmetry of the electric field around each electrode and between two electrodes. In some embodiments any geometry and shape of electrodes and any number of contacted conductive elements can be used. In one embodiment electrode elements or parts can be flat, thick, thin, cylindrical, spherical, tear-drop shaped, saw-tooth shaped, zig-zaged, wavy, porous, rectangular, square, ball-shaped, triangular, diamond-shaped, star-shaped or any combination thereof.

[0097] In some embodiments some electrode parts can be higher than others, deeper, thicker, co-axial, incorporated in, surrounding, placed on top of or overlap with, other parts of the electrodes.

[0098] In some embodiments electrodes are placed on one side of a four-sided chamber. In one embodiment the electrodes are placed on two, three or four sides of a four-sided chamber. In one embodiment electrodes are placed around the inner part of a cylindrical chamber or channel. In one embodiment electrodes and electrode arrays forms circles around the inner part of a channel or a chamber. In one embodiment electrodes form a C-shape which overlaps with the inner part of a cylindrical channel. In one embodiment one, two, three, four or any higher number of close to C-shaped or curved electrodes can align a cross section of a cylindrical channel, with gaps between them. In one embodiment four curved electrodes, each with a length that is smaller than a quarter of the circumference of the inner part of a cylindrical channel are placed around a cross-section of an inner part of the cylindrical chamber.

[0099] In some embodiment an array of electrodes is placed on the substrate. In one embodiment at least two arrays of electrodes are placed on the substrate. In one embodiment the at least two arrays comprises long rectangular shaped electrodes. In some embodiments the at least two arrays of electrodes are oriented perpendicular to each other with respect to the long axis of the electrodes in each array. In one embodiment perpendicular electrode arrays are placed on the same side of the chamber. In one embodiment the perpendicular arrays are placed on opposite sides of the chamber. In some embodiments perpendicular arrays are placed adjacent to each other.

[00100] In some embodiment each array can control fluid flow in a different direction through the modulated application of voltage. In one embodiment, when an electric field is applied to the electrode array, a fluid flow is induced perpendicular to the long axis of the electrodes in the array. In one embodiment this fluid flow is tangential to the substrate on to which the electrodes are fixed. In some embodiments at least two electrode arrays can be operated simultaneously or alternately by applying various electric fields to the arrays. In one embodiment switching the applied electric field in two

perpendicular electrode arrays can control, change, orient or modulate the direction of fluid flow in the chamber.

[00101] In one embodiment, two electrode arrays are placed on one side of a four-sided chamber. In one embodiment two additional electrode arrays are placed on the opposite side of the four-sided chamber. In one embodiment the electrodes are rectangular. In one embodiment applying an electric field to all four electrode arrays induces four fluid currents next to each array. In one embodiment the four fluid currents cause confinement of liquid portions in four areas adjacent to the four electrode arrays. In some embodiments such confinement can be used to trap particles, solutes, or cells in the fluid. In some embodiments such confinement can be used to pump or mix particles, solutes, or cells in the fluid. In some embodiments turning on and off the electric fields applied to each electrode array in a defined way, changes the kinetics of fluid circulation in the chamber, for example, with regard to regions of the chamber wherein fluid occupying such regions is more rigorously circulated within the region. In one embodiment, one, two, three, four or more fluid-circulating areas can be formed in a channel. In some embodiments the number of fluid circulation areas depends on the number and orientation of the electrode arrays to which electric field is applied.

[00102] In one embodiment an array of rectangular electrodes is placed on the inner part of a substrate forming a wall of a chamber. In one embodiment the long axis of the rectangular electrodes is parallel to the long axis of the chamber. In one embodiment the long axis of the electrodes is perpendicular to the long axis of the chamber. In one embodiment when the long axis of the electrode is parallel to the long axis of the channel or chamber, fluid flow is induced perpendicular to the main fluid flow along the channel. In some embodiments such fluid flow caused by applying an electric field to the electrodes, can cause, pumping or mixing of fluid and fluid contents. Such perpendicular fluid flow can slow down the flow in the main flow direction along the channel. Such fluid flow can prolong the time in which particles, solutes or cells spend within the channel or within a special area in the channel. Such perpendicular fluid flow can concentrate a species in a certain area along the channel. Such perpendicular fluid flow can eliminate particles or solutes from certain areas within the channel. Such perpendicular fluid flow can create concentration gradients within the channel. In some embodiments, arrays comprising a large number of electrodes can slow down the progression of a fluid in a more efficient way than arrays comprising smaller number of electrodes.

[00103] In one embodiment multiple arrays of rectangular electrodes are arranged in a row along the long axis of a microfluidic channel. In one embodiment, the arrays are defined as "A" and "B" arrays. In one embodiment a "B" array is placed after an "A" array along the long axis of the channel. In one embodiment the electrode array arrangement along the long axis of the channel has the pattern of ABABABABA, with respect to the names of the electrode arrays. In one embodiment, "A" and "B"

electrode arrays are independently electronically addressed. In one embodiment voltage can be applied to all of the arrays, and in other embodiments voltage can be applied to the "A" arrays or to the "B" arrays only.

[00104] In some embodiments, when voltage is appropriately applied to the "A" and "B" arrays, fluid mixing can be achieved. In one embodiment, fluid mixing adjacent to "A" arrays has an opposite fluid flow direction when compared to the fluid flow adjacent to a "B" array. In one embodiment, such mixing can trap or hold or confine particles or solutes in the fluid, to areas adjacent to "A" or "B" arrays.

[00105] In one embodiment applying an electric field to the "A" arrays or to the "B" arrays only, can cause fluid flow along alternating directions or opposing directions along the channel. In some embodiments such configuration is used to concentrate species in the fluid, to enhance or reduce chemical reactions between species in a fluid, and to facilitate detection of the fluid content.

[00106] In some embodiments, both set of electrodes ("A" and "B") drive flows in the same direction. Each set also optionally includes a shifted 'mirror image' pattern on the opposite wall of the channel. In that case, when all electrodes are on, there is a uniform plug flow with low hydrodynamic dispersion, which can be used to trap and transport coherent localized volumes of fluid or particles. With temporal modulation of the two sets of electrodes A and B, alternating the strength of pumping by each in time, recirculating flow patterns can be superimposed on the plug flow to cause rapid mixing in the downstream direction. In some embodiments, the modulation time period is comparable to the mean advection time across each individual electrode array to promote chaotic mixing.

[00107] In some embodiments, arrays of electrodes are placed on opposite sides of the inner walls of a channel. In one embodiment, two opposing electrode arrays define an area in the channel. In one embodiment, within this area inside the channel, one or more polarizable posts are placed. In one embodiment the polarizable posts affect, divert or change the local electric field. In some embodiments the polarizable posts divert, change or affect the fluid flow direction in the vicinity of the posts. In one embodiment, modulating the voltage of two or more electrode arrays on opposite sides of a microchannel containing one or more polarizable posts, controls the mixing pattern of the fluid in the channel. In one embodiment modulating the AC voltage between an opposing pair of electrodes confining the area of the posts cause mixing driven by induced-charge electro-osmotic flows around the polarizable posts. In some embodiments such flows can also cause transverse pumping due to broken opposing electrode symmetry.

[00108] In one embodiment, a device of this invention can be subject to time-modulated AC voltages. In one embodiment a four-electrode array configuration is used. In some embodiments four electrode arrays or four electrodes are positioned on two sides of a four-sided channel. In one embodiment the electrodes or arrays are referred to as "A", "B", "C", "D", electrodes, each is placed on one side of the channel. In one embodiment electrodes "A" and "B" are placed on one side and electrodes "C" and "D"

are placed on the opposing side. In one embodiment a dominant AC voltage can be applied to "A" and "C" electrodes or to "B" and "D" electrodes. In one embodiment, the dominant AC voltage can switch from being between A and C to B and D. One way to control such switching is to apply an AC voltage to each electrode, A-D, and give each a tunable phase shift relative to the others. In one embodiment, in one state A and B have zero phase shift (same AC voltage) while C and D have a half-period phase shift (same AC voltage, opposite of A and B). In a second state, A and C have zero phase shift, while B and D have a half-period phase shift. By switching between these two (or more) states, rapid chaotic mixing can be achieved in the channel around the posts. In some embodiments, the mixing can be superimposed on a background pressure-driven flow through the post array. In other embodiments, broken symmetry in the geometry of the post array leads to time-modulated pumping through the channel, along with the time-modulated mixing.

[00109] In one embodiment, a device of this invention can be subject to time-modulated AC voltages. In one embodiment a four-electrode array configuration is used. In some embodiments four electrode arrays or four electrodes are positioned on four sides of a four-sided channel. In one embodiment the electrodes or arrays are referred to as "A", "B", "C", "D", electrodes, each is placed on one side of the channel. In one embodiment electrodes "A" and "C" are placed on opposing sides and electrodes "B" and "D" are placed on opposing sides. In one embodiment, polarizable posts are placed in a microfluidic chamber with at least four electrodes placed on different walls as described above. In one embodiment, such arrangement allows the application of dominant electric fields in orthogonal directions, e.g. left/right using electrodes B and D, or up/down using electrodes A and B. The same sort of time modulation strategies described above can be used to achieve chaotic mixing in the chamber. The switching time should be comparable to the convection time for the dominant fluid vortices amongst the array of posts.

[00110] In some embodiments, the device is so constructed so as to promote mixing in certain channels and conveyance to other channels, which in turn may comprise additional steps, which require mixing, as described herein.

[00111] In some embodiments, the devices of this invention facilitate deposition of fluids at a site distal to the microchannels, for further processing, or other manipulations of the conveyed material.

[00112] In some embodiments, electroosmosis in the devices of this result in the creation of a dominant flow. The term "dominant flow" refers, in some embodiments, to propulsion of fluid in a desired direction (also referred to as "positive direction"), with minimal, or less propulsion of fluid in an undesired direction (also referred to as "negative direction"). In some embodiments, concurrent propulsion in both positive and negative directions may result in drastically reduced overall flow, which occurs with planar electrodes, which are approximately likewise proportioned in at least two of three

dimensions, for example, likewise in terms of height and depth, and varied at most in terms of width, in previous ACEO devices. Devices of this invention are likewise proportioned in at most only one of three dimensions, thus varied in terms of height and depth, of an electrode, or portions thereof. Thus, in some embodiments, electrodes in devices of this invention are likewise proportioned in terms of width, likewise proportioned in terms of their depth, however the height of each electrode, or in some embodiments, the height of portions of each electrode, or in some embodiments, the height of pairs of electrodes, or in some embodiments, the height of portions of electrode pairs are varied. In some embodiments, such height alterations may comprise raised or stepped electrode structures, or lowers or recessed electrode structures in a device to provide vertical differences in the electrode structure.

[00113] In some embodiments, the terms “height alterations” or “height variance” or other grammatical forms thereof, refer to differences in height, which exceed by at least 1.5%, or in some embodiments, 3%, or in some embodiments, 5%, or in some embodiments, 7.5%, or in some embodiments, 10%, or more the referenced electrode. For example, a planar electrode pair in an array may vary in height by up to 0.25 %, as a result, for example, of different deposition of material forming the electrodes on a surface of a channel in the device. In the devices of this invention, in contrast, height variances between at least two electrodes, or electrode pairs, or series in a given device, will be more pronounced, and not a reflection of undesired variance due to material deposition.

[00114] In some embodiments, the term “dominant flow” refers to electroosmotic flows, or flows as a result of application of an electric field in a chamber of the devices of this invention. It is to be understood that a dominant flow may be instituted that is less in magnitude, or varied in direction, for example, than other flows in the device, such as other background flows, pressure-driven flows for applying materials to the device, etc.

[00115] In some embodiments, the devices of this invention may cause flows for mixing or controlling flow rate (faster/slower/stopping/starting...) in a channel which also has a stronger more “dominant” background flow (e.g. pressure-driven from elsewhere), where the device's dominant effect is still smaller than the background flow, yet is nonetheless greater in magnitude than similar electroosmotic flows would be with the use of planar electrodes. “Dominant” in reference to flows caused by the devices/apparatuses/methods of this invention may be understood, in some embodiments, to specifically exclude background flow, or non-electroosmotic flow.

[00116] This invention, in some embodiments, provides for the modulation of such electroosmotic flows, such that chaotic mixing of the fluid is accomplished. In some embodiments, such modulation may result in creating multiple dominant flows, sequentially, as a function of engagement of a particular series of electrodes.

[00117] For example, and in some embodiments, two or more series of electrokinetic pumps operating in different directions are turned on and off either at specific intervals, or in some embodiments, at set patterns, or in some embodiments, randomly to mix. The term “series” in some embodiment, refers to positioning and modulation of at least one or a group of electrodes as described herein, such that electroosmotic flows arising upon their engagement are in a comparable or similar direction, or in some embodiments, at a comparable or similar flow rate. In some embodiments, pumps in a series as described herein may encompass pumps located proximally along a Cartesian axis, wherein the electrodes/pumps have at least one surface of such structure abutting a common substrate. In some embodiments, pumps in a series as described herein may encompass pumps located proximally along a Cartesian axis, wherein the electrodes/pumps do not share a common substrate. In some embodiments, a series of pumps may be alternating with another series of pumps, such that for example a first series of pumps results in horizontal fluid flows, whereas the second series results in vertical fluid flows, and such series may alternate, such that overall flow may follow a pattern, for example, and in one embodiment, wherein flow is horizontal, then vertical, then horizontal and vertical again.

[00118] In some embodiments, the modulation of the voltage is slower than the operating AC frequency of each pump. According to this aspect, and in one embodiment, such control enables each pump the time to generate a quasi-steady flow in its particular direction prior to switching. Similarly, and representing additional embodiments of the invention, modulation of the voltage may be periodic and sinusoidal, at a lower frequency than the typical AC operating frequency of each pump.

[00119] In some embodiments, selective application of voltage to a particular series may result in the conveying of the mixed fluid to yet other arrays comprising pumping units so oriented so as to promote further mixing, or in some embodiments, the orientation is to convey the fluid elsewhere within the device structure, or out of the device.

[00120] According to this aspect of the invention, each series may be modulated such that the magnitude, frequency or combination thereof of the voltage applied to each series is varied to maximize chaotic mixing. For example, the voltage of series 1 can be lowered to turn it off, while the voltage of series 2 is raised to turn it on. In some embodiments, the frequency of series 1 can be lowered or raised out of the operating range, while the frequency of series 2 is brought into the operating range. It is to be understood that multiple series can be thus modulated, with each series being engaged in any desired pattern or timing, and that reference to 2 alternating series is not to be taken in any way to limit the invention, but rather as an exemplary of how any number of series may be modulated with respect to another in an embodiment of a device of this invention.

[00121] It will be appreciated by the skilled artisan that it may be desirable to have smooth transition between engagement of the respective series of electrodes. Such transition can be effected by any number

of means, for example via ensuring that the modulating waveform (which provides a sinusoidal envelope for the magnitude of the AC voltage at the operating frequency) is phase shifted by 90 degrees (1/4 period) between one pump and the other, so that one is effectively on while the other is off, with the ability to control, in some embodiments, that switching is a smooth transition from one pump to the other, and not sudden.

[00122] In some embodiments, the characteristic time scale for switching is comparable to the time for flow to circulate at least halfway around the vortex generated by the pump in the cavity. According to this aspect, and in one embodiment, the switching leads to stretching and folding in the two different pumping directions, which produces chaotic streamlines and very rapid mixing in the same way as the rolling of dough in a bakery.

[00123] In some embodiments, the devices/methods of this invention promote chaotic mixing, which in turn results in non-steady time-averaged flow (at the time scale of the applied AC voltage), the latter of which is not very effective for mixing in a fixed volume or cavity of the microfluidic device. In some embodiments, chaotic mixing as a function of the methods/devices of this invention outperform steady flow in a fixed volume, the latter of which mainly reduces the length needed for diffusion from the chamber size to the smallest dimension of the flow structure. Chaotic mixing, as achieved by the methods/devices of this invention, may reduce such length, or time for the flow to reach such structure, etc., as well as provide for active contact between the same.

[00124] In some embodiments, electrodes within a series may vary in terms of their height, width, shape, etc. In some embodiments, a series as described herein may be defined by the physical placement of the electrodes within the series, or in another embodiment, by the overall flow of fluid once the electrodes which comprise the series are engaged.

[00125] Another non-limiting embodiment of a modulated device comprising ICEO microfluidic pumps comprising with opposing flow direction is presented in Figure 5. .

[00126] According to this aspect, the device comprises a series of pumps, which if voltage is applied equally and simultaneously thereto would pump in opposing directions (5-10 versus 5-20). The device comprises each series of pumps being arranged on two opposing substrates (5-30 versus 5-40), such that pairs of pumps on opposing substrates ultimately participate cooperatively toward flow in a single direction, and specific.

[00127] Another embodiment of the device comprises a first series of electrode or electrode-array pumps so arranged such that when engaged, fluid is pumped in the direction of the series of pumps located adjacently on the same substrate, and the second series of pumps, similarly pumps in the direction of its neighbor. A third series of pumps located on a second substrate, however, pumps in a direction opposite to that of the first series of pumps, and the fourth series of pumps. Any number of patterns of

engagement of the pumps can be envisioned, which selectively engage a series of pumps in a desired order, to facilitate fluid flow, which results in a desired pattern for mixing fluid contained therein. Depending upon the desired fluid flow direction, selective engagement of one series can then direct flow to a desired location in the device.

5 [00128] In one embodiment, two or more electrokinetic pumps operating in different directions are turned on and off at specific intervals, or in another embodiment, at set patterns, or in another embodiment, randomly to mix. In another embodiment, two or more electrokinetic pumps pumping in opposing directions are turned on and off either at specific intervals, at set patterns, or randomly to mix. According to this aspect, and in one embodiment, the devices thus described may result in regions or
10 temporary interruption of flow, as a function of the equal and opposite flow initiated proximal to the oppositely positioned series of electrodes. Such temporary interruption in flow may, in some embodiments serve as a trap and release for material suspended in the flow, for examples, particles in flow, when two pumps are simultaneously engaged and hence fluid flow proximal to each is equal and opposite in direction.

15 [00129] In some embodiments, the devices of this invention include an alternating current electrical controller e.g., which is capable of generating a sine or square wave field, or other oscillating field, which allows for modulation of engagement of a particular series of electrodes, as described herein.

[00130] In some embodiments, the devices of this invention include a voltage controller that is capable of applying selectable voltage levels, simultaneously or sequentially, e.g., to a series of electrodes. Such a
20 voltage controller is optionally implemented using multiple voltage dividers and multiple relays to obtain the selectable voltage levels. In some embodiments, multiple independent voltage sources are used. In some embodiments, the voltage controller is as described in U.S. Pat. No. 5,800,690. In some embodiments, modulating voltages affects a desired fluid flow characteristic, e.g., continuous or discontinuous (e.g., a regularly pulsed field causing the sample to oscillate direction of travel), and/or
25 direction of such flow, thereby contributing to chaotic mixing as described herein.

[00131] In some embodiments, the devices of this invention provides for induced charge electroosmotic flow over entire arrays of electrodes, and mixing therein, over a large surface area of the chamber of the device.

[00132] In some embodiments, the electrodes and metal structures are all “flat” in the sense that the
30 primary exposed surfaces are co-planar and parallel to at least one surface of the channel, although the electrodes may be arranged at different heights and transverse positions in three-dimensional geometries. In other embodiments, the devices comprise periodic arrays of non-flat, three-dimensional electrodes, with raised and lowered sections (on a single electrode). In some embodiments, the series as described herein may comprise electrodes of differing heights their placement is varied, however the trajectories of

the flow generated thereby are in a comparable direction, or magnitude. In some embodiments, such variance defines different series.

[00133] In another embodiment, at least one electrode of the plurality of electrodes is not flat. In another embodiment, the plurality of electrodes comprises at least one electrode, which is raised with respect to another electrode. In another embodiment, the plurality of electrodes comprises at least one electrode, which is lowered with respect to another electrode. In another embodiment, the plurality of electrodes comprises at least one electrode having a height, which is proportional to a width of another electrode. In another embodiment, the plurality of electrodes comprises at least one electrode having a height, which varies by about 1% to about 100% of a width of another electrode. In another embodiment, the electrodes are not co-axial, with respect to each other, in any dimension. In another embodiment, the positioning of the electrodes in the microfluidic channel is varied with respect to gaps between the electrodes, spacing of the electrodes, or a combination thereof. In another embodiment, the electrodes are arranged in a symmetric pattern in the microfluidic channel, and in another embodiment, the gaps between the electrodes, the spacing of the electrodes, or a combination thereof is equal.

[00134] It is to be understood that any variance as described herein with reference to one electrode versus another in the plurality of the devices/apparatuses of this invention is to be taken to refer to portions of electrodes as well, where variance in shape, width, depth, height reflects such variance within a single electrode, in terms of portions of the electrode, different electrodes in the device and any combination thereof.

[00135] In another embodiment, the electrodes are arranged in an asymmetric pattern in the microfluidic channel, and in another embodiment, the gaps between the electrodes, the spacing of the electrodes, or a combination thereof is unequal.

[00136] In another embodiment, the electrodes are arranged in a gradient pattern in the microfluidic channel.

[00137] The term “gradient”, in some embodiments, refers to an arrangement which has gradual or gradated differences, for example in electrode height, from one terminus of such arrangement to another, or in some embodiments, gradual or gradated differences, for example in electrode width, gradual or gradated differences, for example in electrode depth, gradual or gradated differences, for example in electrode shape, gradual or gradated differences, for example in electrode circumference, gradual or gradated differences, for example in the angle at which each electrode is deposited in an array in a device of the invention, or gradual or gradated differences, in any combination thereof, or any desired parameter of the same. In some embodiments, the term gradual or gradated differences refers to differences, which are based on a pattern, in ascending or descending value, which may be consecutive or non-consecutive.

[00138] In some embodiments, the term “gradient” refers to any of parameter with regard to electrode geometry, which may vary by any defined/desired period, for example incrementally, or as a multiple or exponential scale, in one or more directions. For example, the layout (gaps, widths, heights, etc.) of each pair of electrodes in an interdigitated array could be rescaled to get larger (or smaller) with distance along the array in the direction of pumping so that the local pumping flow is slower (or faster).

[00139] In some embodiment, a species is defined by specific intervals in such a gradient arrangement. In some embodiments, each graduated change defines a series. In some embodiments, changes in flow as a function of placement within a gradient defines a species.

[00140] In some embodiment, the gradient may be a function of the gaps between electrodes, spacing of electrodes, height of electrodes or portions thereof, shapes of electrodes or portions thereof, or a combination thereof.

[00141] In some embodiments, a pair may define a series, or in some embodiments a series is defined by any desired number of electrodes.

[00142] In some embodiments, arrangement of electrodes which vary in at least 2 or 3 dimensions, in a series may be such that when a field is applied, one of the electrodes in the pair promotes fluid conductance in a particular direction, and another series promotes fluid conductance in another direction. In some embodiments, such electrodes may be constructed in particular geometries, as described herein, and as will be appreciated by one skilled in the art, such that fluid conductance in the desired direction, versus the alternate direction is optimized.

[00143] In some embodiments, a series of electrodes/pumping units are so positioned as described herein, which promote chaotic mixing, and such series are positioned proximal to another series or pair of series, which in turn, via the methods of modulation as herein described, promotes fluid flow in a dominant direction, such that mixing of the fluid is localized to the electrodes involved in chaotic mixing, and once mixing is sufficient, the fluid is then conveyed in a dominant direction by the latter electrode series. Various permutations of such arrangements to promote mixing and/or conveyance are readily apparent to one skilled in the art.

[00144] In some embodiments, the electrodes may be arranged in a series, with varying at least 2 of the 3 dimensions of at least one electrode in a given series. Such series may be odd- or even- in number. In some embodiments, the electrodes in a given series may vary in any way as described herein in terms of electrode geometry, patterning in the device, or a combination thereof, and the devices of this invention may comprise multiple series, which in turn may add to the complexity of the arrays of electrodes and capabilities of the devices of this invention.

[00145] In another embodiment, the gaps are between about 1 micron and about 50 microns, and in another embodiment, the electrode widths are between about 0.1 microns and about 50 microns.

[00146] In some embodiments, the term “dominant flow” refers to propulsion of fluid in alternating directions, which may be modulated, for example via varying the frequency or strength of the field applied, and/or varying or modulating the electrode heights, or portions thereof, resulting in a net conveyance of fluid in a desired direction at a specific time or condition. In some embodiments, the term “dominant flow” refers, to greater propulsion of fluid in a positive rather than negative direction. In some embodiments, the term “greater propulsion” refers to a net propulsion of 51%, or in another embodiment, 55%, or in another embodiment, 60%, or in another embodiment, 65%, or in another embodiment, 70%, or in another embodiment, 72%, or in another embodiment, 75%, or in another embodiment, 80%, or in another embodiment, 83%, or in another embodiment, 85%, or in another embodiment, 87%, or in another embodiment, 90%, or in another embodiment, 95% of the fluid being conveyed in a device of the invention, in a desired or positive direction. In some embodiments, the term “greater propulsion” reflects propulsion of the amount of fluid conveyed in a desired direction as a function of time, with propulsion being greater in a desired direction, predictably, in comparison to a similarly constructed device comprising electrodes of comparable, as opposed to varied height.

[00147] In some embodiments, the term “dominant flow” reflects propulsion of fluid conveyed in a desired direction, wherein such fluid is well mixed during, or prior to conveyance in a net desired direction.

[00148] The devices of this invention enable conveyance of a fluid, which is an electrolyte fluid. In one embodiment, the term “electrolyte fluid” refers to a solution, or in another embodiment, a suspension, or, in another embodiment, any liquid which will be conveyed upon the operation of a device of this invention. In one embodiment, such a fluid may comprise a liquid comprising salts or ionic species. In one embodiment, the ionic species may be present, at any concentration, which facilitates conduction through the devices of this invention. In one embodiment, the liquid is water, or in another embodiment, distilled deionized water, which has an ionic concentration ranging from about 10nM to about 0.1M. In one embodiment, a salt solution, ranging in concentration from about 10nM to about 0.1M is used.

[00149] In another embodiment, the fluid comprises solutions or buffered media for use suitable for the particular application of the device, for example, with regards to the method of cellular analysis, the buffer will be appropriate for the cells being assayed. In one embodiment, the fluid may comprise a medium in which the sample material is solubilized or suspended. In one embodiment, such a fluid may comprise bodily fluids such as, in some embodiments, blood, urine, serum, lymph, saliva, anal and vaginal secretions, perspiration and semen, or in another embodiment, homogenates of solid tissues, as described, such as, for example, liver, spleen, bone marrow, lung, muscle, nervous system tissue, etc., and may be obtained from virtually any organism, including, for example mammals,

rodents, bacteria, etc. In some embodiments, the solutions or buffered media may comprise environmental samples such as, for example, materials obtained from air, agricultural, water or soil sources, which are present in a fluid which can be subjected to the methods of this invention. In another embodiment, such samples may be biological warfare agent samples; research samples and
5 may comprise, for example, glycoproteins, biotoxins, purified proteins, etc. In another embodiment, such fluids may be diluted, so as to comprise a final electrolyte concentration which ranges from between about 10nM – 0.1M.

[00150] In one embodiment, the pH, ionic strength, temperature or combination thereof of the media/solution, etc., may be varied, to affect the assay conditions, as described herein, the rate of transit
10 through the device, mixing within the device, or combination thereof.

[00151] As will be appreciated by those in the art, virtually any experimental manipulation may have been done on the sample prior to its use in embodiments of the present invention. For example, a variety of manipulations may be performed to generate a liquid sample of sufficient quantity from a raw sample. In some embodiments, gas samples and aerosol samples are so processed to generate a liquid sample
15 containing molecules whose separation may be accomplished according to the methods of this invention.

[00152] In one embodiment, the devices of this invention comprise a series of electrodes, wherein each series comprises electrodes, which are not flat. In some embodiments, the electrodes are so constructed so as to comprise sections having at least two different vertical positions. In some embodiments, the transition between sections of different vertical heights is smooth, or in other
20 embodiments, step-wise. In some embodiments, the different vertical positions of the sections differ with respect to other sections in the same electrode, and in some embodiments, with other electrodes of which the series is comprised.

[00153] In some embodiments, the devices of this invention comprise electrodes, which are interlaced electrodes, which can be varied to adjust the mixing capability of the device and optionally the frequency
25 response and/or rate of fluid conductance.

[00154] In some embodiments, the elements of the device are so arranged so as to promote passage of mixed fluid over a sensor on, for example, a wall of the microchannel.

[00155] The design of electrodes which comprise sections which vary in terms of their vertical position may be readily accomplished by known means in the art. For example, the devices may be fabricated as
30 described herein, with successive electroplatings in order to alter the height, shape, etc. of the electrode. In some embodiments, such manufacture results in the production of electrodes with smooth transitions between the different vertical positions, and in other embodiments, with step-wise transitions, which vary in terms of the degree of drop between the different vertical positions. Positioning of these electrodes within the device, will, in some embodiments, be a reflection of a desired flow rate through the devices of

this invention. In some embodiments, construction of the devices with such pumping elements facilitates greater flow rate, as a function of a “conveyor-belt” phenomenon, as described and exemplified herein.

[00156] Some embodiments of arrays or electrode series as herein described, and polarity of the respective electrodes may be varied as a function of their placement in the device, as will be appreciated by one skilled in the art. In some embodiments, the electrodes are arranged with a variety of geometries, such as a square, hexagon, interlocking or inter-digitating designs, etc., as will be appreciated by one skilled in the art. Such orientation may be particularly useful in promoting mixing of the fluids used in the devices and methods of this invention.

[00157] In one embodiment, the term “mixing” as used herein refers to circulation of materials to promote their distribution in a volume of space, for example, a mixture of 2 species, in a device of this invention, refers, in one embodiment, to a random distribution of the 2 species within a given volume of space of the device, e.g., in a microchannel of the devices of this invention. In one embodiment, the term “circulation” and “mixing” are interchangeable. In one embodiment, mixing refers to a change in a particular distribution which is not accompanied by agitation of the sample, in one embodiment, or in another embodiment, minimal agitation and/or formation of “bubbles” in the liquid medium in which the species are conveyed.

[00158] In some embodiments, the pumping element will comprise electrodes fashioned to assume a variety of geometries, as described and exemplified herein, to reflect a consideration of a desired trajectory for conducting the fluid in the devices of this invention, to suit a particular application. In some embodiments, the geometry may approximate a checkerboard, interlocked “E” designs, designs as depicted herein, or one related thereto.

[00159] While the electrode and field polarities as “+” and “-” signs throughout, all fields can also be AC or DC corresponding to electrode polarities oscillating between + and -, giving rise to the same induced-charge electro-osmotic flow. Thus all of the devices of the invention can operate in AC or DC.

[00160] In some embodiments, the present invention provides for the operation of the device in AC with DC offset, as will be understood by one skilled in the art, for example, as described in U. S. Patent Number 5,907,155. In another embodiment, asymmetric driving signals may be used.

[00161] In some embodiments, this invention takes advantage of the fact that there is a competition between regions of oppositely directed electro-osmotic slip on the surfaces of interlaced electrodes of opposite polarity, which in turn results in net pumping over the surface. According to this aspect of the invention, by raising the surfaces pumping in the desired direction (and/or lowering those not pumping in the desired direction) one effectively “buries” the reverse convection rolls. If the height difference is comparable to the width of the buried electrodes, the reverse convection rolls turn over near the upper

surface and provide an effective “conveyor belt” for the primary pumping flow over the raised electrodes, as further described and exemplified hereinbelow.

[00162] In some embodiments, the pumping element has raised portions of the electrodes that pump in the dominant direction (up to stagnation points on each electrode), by a height varied proportionally to the width of the unraised region.

[00163] In some embodiments, the devices of this invention comprise raised electrodes, or in other embodiments, raised portions of electrodes, whose height is about proportional to the width of the unraised, recessed or combination thereof electrode, or portion of an electrode. In some embodiments the raised electrodes and/or raised portions of electrodes, have a height less than the width of the unraised electrode, or portion thereof. In some embodiments, the term “less than” in this context is by a value of about 1%, or about 5%, or about 8%, or about 10%, or about 15%, or about 17%, or about 20%, or about 25% or about 50%, as compared to the referenced value or parameter.

[00164] In some embodiments, the term “about” as used in this invention, is to be understood to encompass a value deviating by +/- 1%, or in another embodiment, by +/- 2.5%, or in another embodiment, by +/- 5%, or in another embodiment, by +/- 7.5%, or in another embodiment, by +/- 10%, or in another embodiment, by +/- 15%, or in another embodiment, by +/- 20%, or in another embodiment, by +/- 25%, with respect to the referenced value or parameter.

[00165] This invention provides, in some embodiments, specific designs for periodic three-dimensional electrode structures, which achieve much faster flows than existing ACEO devices by roughly an order of magnitude, for the same applied voltage and minimum feature size. In some embodiments, the design of the devices of this invention exploit the basic idea of positioning electrodes to recess reverse convection rolls as a “conveyor belt” to enhance the pumping flow driven by raised surfaces, and such positioning maximizes fluid mixing thereby.

[00166] In some embodiments, devices with multiple electrodes, may comprise electrodes which are all of the same shape, different shapes, different sizes, etc. In some embodiments, the electrodes are fashioned as steps, rounded steps, trapezoids, which are continuous along the y-axis, z-axis, or combinations thereof, or in some embodiments, are discontinuous along the y-axis, z-axis, or combinations thereof. The electrodes of which the devices of this invention are comprised are co-axial in at most one dimension. The term “co-axial” refers, in some embodiments, to sharing a Cartesian axis with the indicated element. In some embodiments, the electrodes in the devices of this invention share an x-axis, a y-axis, but not a z-axis. In some embodiments, the electrodes as positioned in the devices of this invention share an x-axis and not a y- or a z-axis. In some embodiments, the electrodes as positioned in the devices of this invention have a comparable overall geometry, which differs in overall scale, or in some embodiments, differ primarily in height.

[00167] In some embodiments, electrodes are raised or lowered above the mean level of the microchannel surface, on which such electrodes are patterned or affixed, or the surface of the substrate, which comprises the electrodes as part of the microchannel surface. Such raising or lowering, will exceed that of the normal thickness of a metal layer, for example, such raising or lowering will be greater than a typical 1 micron thickness of previous electrodes used in certain microfluidic devices, such that the devices of this invention are more truly 3 dimensional structures.

[00168] In some embodiments, such electrodes may be formed or arranged in any geometry, such that electrodes are coaxial in at most one dimension, when such electrodes result in fluid flows of opposing direction.

[00169] External circuitry can be used to control electrical connections and/or to fix the voltage/potential of any or all of the electrodes. Background electrode potential can be controlled relative to the pumping element electrodes in magnitude, frequency, and phase lag.

[00170] In some embodiments, the total charge on the electrodes can also be controlled. Charge can be controlled relative to the background electrodes in magnitude, frequency, and phase lag, as above.

[00171] In some embodiments, additional electrode geometries can include rounded portions, which can be fabricated for instance, by evaporating through a narrow slit, or by wet etching a vertical, electroplated electrode.

[00172] In some embodiments, the background electrodes can be arranged in a variety of geometries relative to the pumping electrode. The background electrodes can be parallel to one another and transverse to a background fluid flow, or in other embodiments, they can be parallel to one another and parallel to background fluid flow. In some embodiments, they can have an angle between them, resulting in some electric field gradients, which may enhance fluid mixing.

[00173] The electrical connections between electrodes and external circuitry can, in some embodiments, be as simple as planar wires connecting the center posts to the external circuits. The electrical connections can be electroplated, in some embodiments. The electrical connections can be buried beneath an insulating material, in some embodiments.

[00174] Driving and control electronics can be manufactured on-chip along with the electrodes, in some embodiments. The driving and control electronics can be a separate electronics module, in some embodiments, an external stand-alone unit or microfabricated electronics. The microfabricated electronics module, in some embodiments, can be wire-bonded to the chip containing the electrodes or can be flip-chip bonded.

[00175] Fluidic channels can be fabricated by a variety of means, including soft-lithographic molding of polymers on rigid or semi-rigid molds. Channels can also be fabricated in glass via wet etching, plasma etching or similar means. Channels can be formed in plastics via stamping, hot embossing, or

other similar machining processes. The channels can then be bonded to the substrate containing the electrode structures. Alignment marks can be incorporated onto the substrate to facilitate assembly. In some instances, metal surfaces can be exposed on substrate and channels to enable metal-to-metal bonding. Glass-to-glass bonding can be done at elevated temperatures and with applied potential.

5 Plastic-to-glass can be facilitated with cleaning of glass surfaces prior to bonding, or fabrication of the fluidic portion of the device can be accomplished by any means known in the art.

[00176] Raised supports of an insulating or semiconducting nature can be fabricated on the substrate as well, in some embodiments, on which the pumping electrodes and/or background electrode may be mounted, to provide for differences in height, for uses as described herein.

10 [00177] In some embodiments, this invention provides a device comprising a microfluidic loop. In some embodiments, the device will comprise ports and machinery such that fluid injected in one port can be recirculated across one or more regions of the device, for example to regions for the detection of materials, or in some embodiments, separation of material, or in some embodiments, mixing of materials, which may be effected by the micropumps of the devices of this invention, prior to ejection through
15 another port, in some embodiments, as described and exemplified herein.

[00178] In one embodiment, the device is adapted such that analysis of a species of interest may be conducted, in one embodiment, in the device, or in another embodiment, downstream of the device. In one embodiment, analysis downstream of the device refers to removal of the obtained product from the device, and placement in an appropriate setting for analysis, or in another embodiment, construction of a
20 conduit from the device, for example, from a collection port, which relays the material to an appropriate setting for analysis. In one embodiment, such analysis may comprise signal acquisition, and in another embodiment, a data processor. In one embodiment, the signal can be a photon, electrical current/impedance measurement or change in measurements. It is to be understood that the devices of this invention may be useful in various analytical systems, including bio-analysis micro-systems, due to
25 the simplicity, performance, robustness, and ability to be integrated to other separation and detection systems and any integration of the device into such a system is to be considered as part of this invention. In one embodiment, this invention provides an apparatus comprising a device of this invention, which in some embodiments, comprises the analytical modules as described herein.

[00179] In some embodiments the device comprises posts that are placed within the chamber. In some
30 embodiments electro-osmotic flow is generated at asymmetric conducting posts. In some embodiments a conductive post placed in an AC or a DC applied fields with broken fore-aft or left-right symmetry generally produce net electro-osmotic pumping along the direction of broken symmetry. Therefore, it is possible to produce linear channel pumps using conductive posts, which possess broken asymmetry. In one embodiment breaking symmetry with respect to the conducting array is achieved using triangular

conductive posts. In one embodiment, the applied field can either be along the direction of the channel or across the channel, perpendicular to it.

[00180] In some embodiments electro-osmotic flows are generated by posts with symmetry broken in the channel direction, and an AC or DC field directed along or across the microchannels. Other broken symmetry conducting posts, such as conducting posts having a cross-section of a tear-drop or triangle, dielectric or metallic partial coatings, zig-zag or wavy shape, can also be used. In the case of a broken fore-aft spatial symmetry, the sharpest point of the cross section of the post is directed opposite to the desired flow direction of induced-charge electro-osmotic pumping. In the case of a broken left-right spatial symmetry, the sharpest point of the cross section is directed in the desired direction of induced-charge electro-osmotic pumping. Another embodiment for preparing posts may be to simply place two or more wires of different cross sections against each other to approximate a triangle shape. In this way, an AC electro-osmotic linear-channel pump can be built out of ordinary metal micro-wires of circular cross-section.

[00181] In some embodiments asymmetric posts can be arranged in extended arrays to provide the distributed forcing needed to drive fluid quickly along lengthy channels.

[00182] In some embodiments, asymmetric conducting posts are of cylindrical shape and are covered with a dielectric or metallic coating. The coatings of the conducting posts are directed opposite the flow direction, in an AC or DC field directed along the microchannel. In some embodiments, the conducting posts produces flows that are directed in along the field axis and out perpendicular to the field axis, providing a unique mixing pattern. The asymmetric shape of the conductive posts provides the necessary force to pump fluid through a microchannel. In some embodiments any broken symmetry will facilitate the production of a pump/mixer.

[00183] In some embodiments the conductive posts are shaped as asymmetric metal ridges patterned on the walls of a microchannel between the electrodes. The electrodes allow reversing their polarities and producing AC or DC fields. The asymmetric ridges are designed to lean in the direction of the flow, in an AC or DC field directed along the microchannel. The surface of the asymmetric ridges is a grooved metallic surface, not connected in any way to the external circuit, which includes normal electrodes positioned in the channel walls on either side of the grooved surface.

[00184] In one embodiment, the conductive post or element is an array of conductive posts or elements, as will be appreciated by one skilled in the art. Some embodiments of arrays of such elements are described hereinabove. In some embodiments, the arrays may comprise a lattice, which may have a variety of geometries, such as a square, hexagon, etc., as will be appreciated by one skilled in the art. Such orientation or arrays may be particularly useful in the micromixers of this invention. In one embodiment, a single unit functions as both micropump and micromixer, as will be appreciated by one

skilled in the art. In one embodiment, the term “mixing” as used herein refers to circulation of materials to promote their distribution in a volume of space, for example, a mixture of 2 species, in a device of this invention, refers, in one embodiment, to a random distribution of the 2 species within a given volume of space of the device, e.g., in a microchannel of the devices of this invention. In one embodiment, the term “circulation” and “mixing” are interchangeable. In one embodiment, mixing refers to a change in a particular distribution which is not accompanied by agitation of the sample, in one embodiment, or in another embodiment, minimal agitation and/or formation of “bubbles” in the liquid medium in which the species are conveyed.

[00185] In some embodiments, the conductive post or element may be fashioned to assume a variety of geometries, as described and exemplified herein. In one embodiment, such design will reflect a consideration of a desired trajectory for a particular application. In some embodiments, the geometry may approximate an arrowhead, a teardrop, or elliptical shape, or one related thereto.

[00186] In another embodiment, this invention provides a method of mixing a fluid, the method comprising applying a fluid to a device of this invention and selectively applying voltage to the at least series of electrodes contained therein, such that the voltage is not simultaneously or commensurately applied to the two series whereby upon selective application of the voltage to the series, electro-osmotic flows with varied trajectories are generated in a region proximal to each of the series, resulting in mixing of the electrolyte fluid.

[00187] In some embodiments, a series of electrodes are so positioned, that when voltage is applied thereto, a dominant electroosmotic flow is generated, which drives the mixed electrolyte fluid across the chamber. According to this aspect of the invention, modulation of such application of voltage is such that electro-osmotic flows with varied trajectories result, which mix an introduced fluid, following which, via selective modulation of such electrodes, a dominant flow is created to convey the mixed fluid to a desired location within the device, or to exit the device.

[00188] In another embodiment, this invention provides a method of mixing a fluid, comprising applying a fluid to a device or an apparatus of this invention.

[00189] In some embodiments, the invention provides methods, devices and apparatuses for mixing or stirring fluid in a fixed chamber, and may optionally provide for long range pumping down a channel of a device of this invention. In some embodiments, such stirring may be applied in a multitude of applications, including any of the methods as described herein, or other applications, readily appreciated by one skilled in the art. For example, such methods, devices and apparatuses may find application in bioassays, and may, for example, impart greater speed or sensitivity to such assays. In some embodiments, such methods, devices and apparatuses may find application in the construction, probing or assay of DNA arrays, in a fixed chamber, or in another embodiment, in a microfluidic loop

arrangement and may, for example, impart greater speed or sensitivity to such assays, allow for smaller sample or probe quantities for such assay, or other advantages apparent to one in the art.

[00190] In some embodiments, the terms “mixing” or “circulating” are to be understood as interchangeable. In some embodiments, “circulating” or “mixing” capabilities of the methods, devices and apparatuses of this invention may involve arrangement of the electrodes such that flow over the electrodes impinges on a wall of the channel, resulting in greater mixing.

[00191] In some embodiments, “circulating” or “mixing” capabilities of the methods, devices and apparatuses of this invention may further promote increased diffusion of molecular species or decrease the distance over which diffusion must act, or in some embodiments, eliminate concentration variations in a fluid. Such an effect may reduce the rate of dispersion along the flow by carrying unit volumes of the fluid between fast and slow moving regions. In net effect, i.e., as the fluid progresses through the mixing apparatus, the mixing of the fluid or fluids is increased as the diffusion area is increased and, consequently, the time required to achieve mixing to a desired homogeneity is reduced.

[00192] In some embodiments, the methods, devices and apparatuses of this invention may circulate fluid in a “closed box” where fluid is injected into the device by any means known in the art and mixed therein.

[00193] In some embodiments, the term “mixing” refers to fluid in the devices/apparatuses of the invention having at least two varied trajectories, upon applying voltage to a respective series of electrodes. In some embodiments, the devices/apparatuses of the invention promote flow along at least one trajectory that effectively stirs the fluid, circulates the fluid, or a combination thereof.

[00194] In some embodiments, the invention provides devices/apparatuses/methods for circulating/mixing a fluid over a target surface with a bound reagent, or in other embodiments, circulates a fluid having a reagent that specifically fluorescently labels analytes that are bound to that surface, which may be assessed via optical means, or in some embodiments, the surface is so constructed so as to detect changes in gate voltage on a transistor structure when an analyte or reagent binds, and when binding creates electrical, conducting, or semiconducting connections between two electrodes on the surface. Such applications may find use in the methods of this invention, as described herein, and as will be appreciated by one skilled in the art.

[00195] In some embodiments, this invention provides for analysis, detection, concentration, processing, assay, production of any material in a microfluidic device, whose principle of operation comprises electro-osmotically driven fluid flow, for example, the incorporation of a source providing an electric field in a microchannel of the device, and provision of an electrokinetic means for generating fluid motion whereby interactions between the electric field and induced-charge produce electro-osmotic flows, and wherein the electric field is supplied as a function of application of voltage to a series of

electrodes arranged in the device, whereby flow in the region proximal to the series is such that flow proximal to a first series has a varied trajectory from that proximal to a second series. Such flows may in turn, find application in mixing of materials, and optionally fluid conductance, and any application which makes use of these principles is to be considered as part of this invention, representing an embodiment thereof.

[00196] In some embodiments, the invention provides methods for circulating fluid in a microfluidic cavity, comprising applying the fluid to a device comprising two or more series of electrodes connected to a source wherein each electrode in each series has stepped or recessed features, which in some embodiments, produces a flow, which has a nonzero component directed toward a boundary of a channel in the device. In some embodiments, such devices and methods of their use allow for the conveyance of, *inter alia*, cells, analytes, antibodies, antigens, DNA, polymers, proteins in solution, and others over a desired surface, for example, a detection surface.

[00197] According to this aspect, and in some embodiments, a capture antibody, or cross-linking agent, or enzyme in solution is applied to such device, and is conducted such that these reagents come into contact with the desired surface. In some embodiments, a portion of the device optically transparent, or facilitates optical detection of a label, which may be incorporated in the agents or reagents as described herein, to facilitate detection. For example, at least a portion of the device may be transparent at a wavelength corresponding to excitation and emission for a fluorescent tag, which may be coupled to a reagent or compound in the fluids applied to the device. In some embodiments, according to this aspect, the device may be constructed to comprise non-transparent sections, to minimize or abrogate photobleaching of sensitive reagents.

[00198] In one embodiment, the method further comprises assay or analysis of the fluid, and in one embodiment, the analysis is a method of cellular analysis. In another embodiment, the method comprises the steps of:

- a. introducing a buffered suspension comprising cells and a reagent for cellular analysis into the microfluidic chamber; and
- b. analyzing at least one parameter affected by contact between the suspension and the reagent.

[00199] In one embodiment, the reagent is an antibody, a nucleic acid, an enzyme, a substrate, a ligand, or a combination thereof. In one embodiment, the reagent is coupled to a detectable marker. In one embodiment, the marker is a fluorescent compound, and in another embodiment the device is coupled to a fluorimeter or fluorescent microscope.

[00200] In another embodiment, the method of mixing a fluid further comprises the step of introducing a cellular lysis agent to the device. In another embodiment, the reagent specifically interacts or detects an intracellular compound

[00201] In another embodiment, a secondary reagent may also be present in addition to the lysing agent for facilitating further analysis or manipulation of the cells.

[00202] In one embodiment, the surface of the microchannel may be functionalized to reduce or enhance adsorption of species of interest to the surface of the device. In another embodiment, the surface of the microchannel has been functionalized to enhance or reduce the operation efficiency of the device.

[00203] . In another embodiment, the assay or analysis of the fluid is a method of analyte detection or assay. According to this aspect and in one embodiment, the method further comprises the steps of:

- a. introducing an analyte to the device;
- b. introducing a reagent to the device; and
- c. detecting, analyzing, or a combination thereof, of the analyte

[00204] In one embodiment, mixing reconstitutes a compound in the device, upon application of the fluid, and in one embodiment, the compound is solubilized slowly in fluids.

[00205] In one embodiment, the device is further modified to contain an active agent in the microchannel, or in another embodiment, the active agent is introduced via an inlet into the device, or in another embodiment, a combination of the two is enacted. For example, and in one embodiment, the microchannel is coated with an enzyme at a region wherein molecules introduced in the inlet will be conveyed past, according to the methods of this invention. According to this aspect, the enzyme, such as, a protease, may come into contact with cellular contents, or a mixture of concentrated proteins, and digest them, which in another embodiment, allows for further assay of the digested species, for example, via introduction of a specific protease into an inlet which conveys the enzyme further downstream in the device, such that essentially digested material is then subjected to the activity of the specific protease. This is but one example, but it is apparent to one skilled in the art that any number of other reagents may be introduced, such as an antibody, nucleic acid probe, additional enzyme, substrate, etc.

[00206] In one embodiment, processed sample is conveyed to a separate analytical module. For example, in the protease digested material described hereinabove, the digestion products may, in another embodiment, be conveyed to a peptide analysis module, downstream of the device. The amino acid sequences of the digestion products may be determined and assembled to generate a sequence of the polypeptide. Prior to delivery to a peptide analysis module, the peptide may be conveyed to an interfacing module, which in turn, may perform one or more additional steps of separating, concentrating, and or focusing.

[00207] In another embodiment, the microchannel may be coated with a label, which in one embodiment is tagged, in order to identify a particular protein or peptide, or other molecule containing the recognized epitope, which may be a means of sensitive detection of a molecule in a large mixture, present at low concentration.

5 [00208] For example, in some embodiments, reagents may be incorporated in the buffers used in the methods and devices of this invention, to enable chemiluminescence detection. In some embodiments the method of detecting the labeled material includes, but is not limited to, optical absorbance, refractive index, fluorescence, phosphorescence, chemiluminescence, electrochemiluminescence, electrochemical detection, voltametry or conductivity. In some embodiments, detection occurs using laser-induced
10 fluorescence, as is known in the art.

[00209] In some embodiments, the labels may include, but are not limited to, fluorescent lanthanide complexes, including those of Europium and Terbium, fluorescein, fluorescamine, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue™, Texas Red, 1,1'-[1,3-propanediylbis[(dimethylimino-3,1-
15 propanediyl]]bis[4-[(3-methyl-2(3H)-benzoxazolylidene)methyl]]-,tetraioide, which is sold under the name YOYO-1, Cy and Alexa dyes, and others described in the 9th Edition of the Molecular Probes Handbook by Richard P. Haugland, hereby expressly incorporated by reference. Labels may be added to 'label' the desired molecule, prior to introduction into the devices of this invention, in some embodiments, and in some embodiments the label is supplied in a microfluidic chamber. In some
20 embodiments, the labels are attached covalently as is known in the art, or in other embodiments, via non-covalent attachment.

[00210] In some embodiments, photodiodes, confocal microscopes, CCD cameras, or photomultiplier tubes maybe used to image the labels thus incorporated, and may, in some embodiments, comprise the apparatus of the invention, representing, in some embodiments, a "lab on a chip"
25 mechanism.

[00211] In one embodiment, detection is accomplished using laser-induced fluorescence, as known in the art. In some embodiments, the apparatus may further comprise a light source, detector, and other optical components to direct light onto the microfluidic chamber/chip and thereby collect fluorescent radiation thus emitted. The light source may comprise a laser light source, such as, in some embodiments,
30 a laser diode, or in other embodiments, a violet or a red laser diode. In other embodiments, VCSELs, VECSELs, or diode-pumped solid state lasers may be similarly used. In some embodiments, a Brewster's angle laser induced fluorescence detector may used. In some embodiments, one or more beam steering mirrors may be used to direct the beam to a desired location for detection.

[0001] In one embodiment, a solution or buffered medium comprising the molecules for assay are used in the methods and for the devices of this invention. In one embodiment, such solutions or buffered media may comprise natural or synthetic compounds. In another embodiment, the solutions or buffered media may comprise supernatants or culture media, which in one embodiment, are harvested from cells, such as bacterial cultures, or in another embodiment, cultures of engineered cells, wherein in one embodiment, the cells express mutated proteins, or overexpress proteins, or other molecules of interest which may be thus applied. In another embodiment, the solutions or buffered media may comprise lysates or homogenates of cells or tissue, which in one embodiment, may be otherwise manipulated for example, wherein the lysates are subject to filtration, lipase or collagenase, etc., digestion, as will be understood by one skilled in the art. In one embodiment, such processing may be accomplished via introduction of the appropriate reagent into the device, via, coating of a specific channel, in one embodiment, or introduction via an inlet, in another embodiment.

[00212] It is to be understood that any complex mixture, comprising two or more molecules, whose assay is desired, may be used for the methods and in the devices of this invention, and represent an embodiment thereof.

[00213] In one embodiment, the mixing results in high-throughput, multi-step product formation.

[00214] In one embodiment, according to this aspect of the invention, the method of mixing further comprises the steps of:

- a. introducing a precursor to the device;
- b. introducing a reagent, catalyst, reactant, cofactor, or combination thereof to the device;
- c. providing conditions whereby the precursor is converted to a product; and
- d. optionally, collecting the product from the device.

[00215] In one embodiment, the method further comprises carrying out iterative introductions of the reagent, catalyst, reactant, cofactor, or combination thereof in (b), to the device.

[00216] In one embodiment, the reagent is an antibody, a nucleic acid, an enzyme, a substrate, a ligand, a reactant or a combination thereof.

[00217]

[00218] In one embodiment, the mixing results in drug processing and delivery. According to this aspect and in one embodiment, the method further comprises the steps of:

- i. introducing a drug and a liquid comprising a buffer, a catalyst, or combination thereof to the device;

- ii. providing conditions whereby the drug is processed or otherwise prepared for delivery to a subject; and
- iii. collecting the drug, delivering the drug to a subject, or a combination thereof.

[00219] In one embodiment, the method further comprises the step of carrying out iterative introductions of liquid to the device. In one embodiment, introduction of the liquid serves to dilute the drug to a desired concentration.

[00220] In some embodiments, the term “drug processing” refers to reconstitution of a drug, altering a drug, modifying a drug, or any preparation desired to prepare a drug or composition for administration to a subject.

[00221] In some embodiments, the invention provides devices preloaded with a compound, for example a lyophilized drug, which is packaged and distributed as such, under sterile conditions. In some embodiments, according to this aspect, a fluid is introduced into such a device, and the drug or other compound contained therewithin is reconstituted or diluted or processed, in some embodiments, just prior to delivery to a subject, or for any period of time, or for storage, etc.

[00222] Metabolic processes and other chemical processes can involve multiple steps of reactions of precursors with an enzyme, or catalyst, or mimetic, etc., in some embodiments, with or without the involvement of cofactors, in other embodiments, to obtain specific products, which in turn are reacted, to form additional products, etc., until a final desired product is obtained. In one embodiment, the devices and/or methods of this invention are used for such a purpose. In one embodiment, such methodology enables use of smaller quantities of reagents, or precursors, which may be limiting, in other embodiments, wherein such methodology enables isolation of highly reactive intermediates, which in turn may promote greater product formation. In another embodiment, such methodology enables greater sensitivity of detection, as well, and use of lesser quantity of compound and/or reagent, due to enhanced mixing of the same. It will be apparent to one skilled in the art that a means for stepwise, isolated or controlled synthesis provides many advantages, and is amenable to any number of permutations.

[00223] It is to be understood that any of the embodiments described herein, with regards to samples, reagents and device embodiments are applicable with regard to any method as described herein, representing embodiments thereof.

[00224] In another embodiment, the modulated induced-charge electroosmotic devices of this invention circulate solutions containing probe molecules over target surfaces. In one embodiment, the probe may be any molecule, which specifically interacts with a target molecule, such as, for example, a nucleic acid, an antibody, a ligand, a receptor, etc. In another embodiment, the probe will have a moiety which can be chemically cross-linked with the desired target molecule, with reasonable specificity, as will be appreciated by one skilled in the art. According to this aspect of the invention and in one

embodiment, a microchannel of the device may be coated with a mixture, lysate, sample, etc., comprising a target molecule of interest.

[00225] In one embodiment, such a device provides an advantage in terms of the time needed for assay, the higher sensitivity of detection, lower concentration of sample/reagents needed, since the sample may be recirculated over the target surface, or combination thereof.

[00226] In some embodiments, in devices for use in regulating drug delivery, the second liquid serves to dilute the drug to a desired concentration. In one embodiment, the device comprises valves, positioned to regulate fluid flow through the device, such as, for example, for regulating fluid flow through the outlet of the device, which in turn prevents depletion from the device, in one embodiment. In another embodiment, the positioning of valves provides an independent means of regulating fluid flow, apart from a relay from signals from the subject, which stimulate fluid flow through the device.

[00227] In another embodiment, this invention provides a device for use in drug delivery, wherein the device conveys fluid from a reservoir to an outlet port. In one embodiment, drug delivery according to this aspect of the invention, enables mixing of drug concentrations in the device, or altering the flow of the drug, or combination thereof, or in another embodiment, provides a means of continuous delivery. In one embodiment, such a device may be implanted in a subject, and provide drug delivery in situ. In one embodiment, such a device may be prepared so as to be suitable for transdermal drug delivery, as will be appreciated by one skilled in the art.

[00228] Although the present invention has been shown and described with respect to several preferred embodiments thereof, various changes, omissions and additions to the form and detail thereof, may be made therein, without departing from the spirit and scope of the invention.

EXAMPLES

EXAMPLE 1:

General Schematic for Devices Comprising

Modulated Induced-Charge Electro-Osmotically Driven

Microfluidic Pumps and mixers

[00229] In order to devise a means for fast chaotic mixing in a fixed volume of a microfluidic device, electrokinetic-based devices were modeled. According to this aspect, devices, including channels and electrodes were constructed, by standard methodology, whereby arrays of electrodes with broken symmetry are prepared, for example, as described in United States Patent Application Serial Number 11/700,949, filed on Feb. 1, 2007, fully incorporated herein by reference. The fast mixing is accomplished by modulating the action of two or more electrokinetic pumps in time.

[00230] In some embodiments, the invention involves nonlinear electrokinetic devices for rapid three-dimensional mixing by chaotic advection in a confined microchamber. The devices contain two or more

arrays of electrodes, for example, as designated by (1-10) in figure 1, which comprise electrode-array pumps, such as (but not limited to) those illustrated in Figure 1. Each electrode array is positioned so that it drives nonlinear electrokinetic flow in a different direction from at least one of the others. The set of arrays is thus capable of producing at least two different convection patterns within the microchamber.

5 [00231] In one embodiment of the invention, an array of electrodes is so positioned to give the desired effect. According to this aspect of the invention, the array of electrodes asymmetric planar electrodes in each period (Figure 1A), while in another embodiment, the array comprises modulating surface height of the electrodes, e.g. via secondary electroplating steps, etc. on even symmetric electrode arrays, or in some embodiments, stepped 3D electrode arrays enable efficient, unidirectional pumping over the array for a wide range of operating conditions (voltage, frequency, concentration, etc.) with non-planar, stepped 3D electrodes (Figure 1B), 3D stepped electrodes with non-polarizable side walls (Figure 1C) or general 3D electrodes with raised metal or non-polarizable structures on an underlying pattern (Figure 1D).

[00232] The nonlinear electrokinetic flows over the electrode arrays are modulated in time, in coordination with each other, so as to achieve efficient mixing of the bulk fluid and/or efficient transport of particles to a detection site or sites in the microchamber. The AC voltage (1-40 in figure 1) applied to each array to drive nonlinear electrokinetic flow (typically kHz to Mhz) is slowly modulated in time with period T (typically mHz to kHz) and not in-phase. Rapid mixing can be achieved by the fold-and-stretch route to chaos, if the period $T=U/L$ is set to be of order the time for a typical fluid element moving at speed U to traverse a convection roll of size L, set by the geometry of the chamber and electrode arrays.

20 [00233] Fast chaotic mixing by electrokinetic pump modulation, relies, in some embodiments, on: (i) the local time-averaged pumping velocity increasing with the magnitude of the applied voltage, typically as voltage squared.

[00234] In one embodiment, two or more electrokinetic pumps operating in different directions are turned on and off either at specific intervals, or in another embodiment, at set patterns, or in another embodiment, randomly to mix the fluids applied to devices comprising the electrokinetic pumps.

[00235] In the embodiment of the devices and methods of this invention, referring to for example, Figure 2A, the electrode arrays 2-10 and 2-20 cover opposite surfaces of the microchamber 2-30 and are oriented at right angles. The AC voltage to each array is modulated by out of phase signals, so that the system alternates between two flow states, 2-10 on / 2-20 off and 2-10 off / 2-20 on, as shown.

30 [00236] In the embodiment of the devices and methods of this invention, referring to for example, Figure 2B, the two electrode arrays 2-10 and 2-20 are placed on the same wall of the microchamber, again oriented in perpendicular directions, switching between the states 2-10_{on} / 2-20_{on} and 2-10_{off} / 2-20_{off}. In one aspect, Array 2-10 remains on at all times with a constant, unmodulated AC voltage, driving a steady convection roll in one direction, concurrent with array 2-20 being switched between on and off

states by modulation of its AC voltage, leading to different, spiral convection patterns during the “on” state. Since the electrode arrays only occupy one wall of the microchamber, the other walls, especially the large, flat opposite wall, can be used for other purposes, such as distributing surface sites for detecting particles or cells suspended in the fluid, e.g. in protein immunoassays or DNA microarrays.

5 [00237] In another embodiment of the invention, see for example, Figure 3, where four electrode arrays (3-10)-(3-40) are placed in a confined microchamber (3-50). Four different flow patterns with different convection cells are illustrated which result from turning on different combinations of the array pumps. By maintaining one of these flow states, the fluid and any suspended particles can be separated into different convection cells, which thus act as traps, indicated by dotted-line separatrices. By alternating
10 between two or more of these states, rapid chaotic mixing can be achieved.

[00238] In some embodiments, the fluid can be introduced into the microchamber from an inlet microchannel using nonlinear electrokinetic flow over one or more of the electrode series.

[00239] In some embodiments, this invention provides for temporal modulation of the magnitude or frequency of the voltages applied to each of two or more pumps (e.g. in a series) so as to effectively turn
15 them on and off at different times. In some embodiments, this modulation of the voltage is much slower than the operating AC frequency of each pump, so that each pump has time to generate a quasi-steady flow in its particular direction prior to switching. In some embodiments, the modulation may be random, or in some embodiments, the modulation may be accomplished via the use of a digital/sudden switch turning one off and the other on. In some embodiments, the modulation may be periodic and sinusoidal,
20 at a lower frequency than the typical AC operating frequency of each pump.

[00240] In some embodiments, the modulation which accomplishes the switching may involve the magnitude or frequency of the AC voltage applied to each of the two (or more) pumps. For example, the voltage of one can be lowered to turn it off, while the voltage of the other is raised to turn it on. In some
25 embodiments, the frequency of one can lowered or raised out of the operating range, while the frequency of the other is brought into the operating range.

[00241] According to this aspect of the invention and in one embodiment, the switching may be accomplished by driving each pump with a voltage consisting of a product of two sinusoidal waveforms, one at the operating frequency and the other at the modulating frequency.

[00242] According to this aspect, and in one embodiment, the modulating waveform (which provides a
30 sinusoidal envelope for the magnitude of the AC voltage at the operating frequency) is phase shifted by 90 degrees (1/4 period) between one pump and the other, so that one is effectively on while the other is off, with the ability to control, in some embodiments, that switching is a smooth transition from one pump to the other, and not sudden.

[00243] In some embodiments, the characteristic time scale for switching is comparable to the time for flow to circulate at least halfway around the vortex generated by the pump in the cavity. According to this aspect, and in one embodiment, the switching leads to stretching and folding in the two different pumping directions, which produces chaotic streamlines and very rapid mixing in the same way as the rolling of dough in a bakery.

[00244] In some embodiments, the device is used for mixing multiple fluids in the cavity, or for dispersing molecules or colloidal particles suspended in a fluid. In the latter case, the molecules can be efficiently and uniformly brought to detection sites on the non-pumping surfaces, as in DNA microarrays or other biological assays. The convection produced by the mixer could quickly and thoroughly pass all molecules in solution all detection spots in the microarray, thereby reducing the detection time and increasing the sensitivity of the device, compared to the usual case of no flow, where the detection relies on bulk diffusion alone for transport to the surface.

[00245] In some embodiments, advantages of using devices and apparatuses comprising the electrokinetic pumps of this invention include the ability to operate such devices at low, battery voltages (a few volts) and low power (milliwatts), while generating fast flows, up to mm/sec.

[00246] Another advantage of using devices and apparatuses comprising the electrokinetic pumps of this invention is that such pumps may be easily and cheaply constructed by a variety of microfabrication methods.

EXAMPLE 2:

Complex Modulated Devices Comprising ICEO Microfluidic Pumps

[00247] An embodiment of a modulated device comprising ICEO microfluidic pumps is shown in Figure 4.

[00248] In this embodiment of the invention, mixing in a continuous flow along a microfluidic channel is achieved using electrode arrays positioned and activated in time as shown in Figure 3 to drive flows transverse to the channel axis. In Figure 4A, two opposite walls of the channel are lined with the four electrode arrays (4-10)-(4-40) for mixing in steady flow. In some embodiments of the invention, the electrodes need not be positioned in parallel strips, but form any desired pattern, for example, a wavy or herringbone pattern, to further enhance mixing. In one embodiment, for example as shown in Figure 4B, the mixing section of the channel has a larger cross section to slow down the fluid, causing it to spend more time inside the mixer before proceeding downstream.

[00249] In some embodiments, the devices/methods of this invention allow for trapping, pumping and mixing of materials in a fluid sample (Figure 5). According to this embodiment, selective trapping, pumping, and mixing of a fluid or suspension of particles may be accomplished. In one aspect, a periodic

pattern of electrode arrays (5-10), (5-20) is positioned along at least one wall of a microchannel (5-30), which drive flows in alternating directions when turned on. For example, there can be two interlaced sets of arrays, 5-10 and 5-20, pumping in opposite directions. When both sets 5-10 and 5-20 are activated at once as in Fig 5A, the opposing flows form closed convection cells to trap fluid and/or position particles above each array. When only set 5-10 is activated as in Fig 5B, a net flow is driven in one direction, which disperses and transports the trapped fluid or particles. (Hydrodynamic dispersion results from non-uniform slip flows.) In another embodiment, by activating set 5-20 as in Fig 5C, a similar flow is driven in the opposite direction. By temporally modulating the sets of electrodes, e.g. by switching between states 5A, 5B, and/or 5C, rapid chaotic mixing can be achieved. Net pumping can in a desired direction be achieved at the same time as mixing by tuning the fraction of the time and/or magnitude of the flow (via the voltage) from set 5-10 versus set 5-20. Note that temporal modulation of one or more of these states superimposed on a background pressure-driven flow would also suffice for rapid active mixing of the fluid as it is pumped through the channel.

[00250] According to this aspect of the invention, each series may be modulated such that the magnitude, frequency or combination thereof of the voltage applied to each series is varied to maximize chaotic mixing. For example, a signal generator may be used to operate the respective array of pumps at various AC.

[00251] Modulation may further comprise a step whereby once the material has been mixed for a desired period of time, the fluid is conveyed in a desired dominant direction.

[00252] In another embodiment (Figure 6), in a similar device to that described for Figure 5, both sets of electrodes may be so modulated so as to drive flows in the same direction.

[00253] In some embodiments, each set also may optionally include a shifted mirror image pattern on the opposite wall of the channel (e.g. array 6-10 vs. array 6-20 in figure 6). According to this aspect, when all electrodes are on, there is a uniform plug flow with low hydrodynamic dispersion, which can be used to transport coherent localized volumes of fluid or particles.

[00254] With temporal modulation of the two sets of electrodes 6-10 and 6-20, alternating the strength of pumping by each in time, re-circulating flow patterns can be superimposed on the plug flow to cause rapid mixing in the downstream direction. The modulation time period should be comparable to the mean advection time across each individual electrode array to promote chaotic mixing.

EXAMPLE 3:

Modulated Devices Comprising ICEO Microfluidic Pumps Comprising Polarizable surfaces

[00255] In some embodiments, this invention provides devices/methods including modulated devices comprising ICEO-driven microfluidic pumps comprising polarizable surfaces. Figure 7 shows the

general concept modulating the voltage of two or more electrodes on opposite sides of a microchannel containing an array of one or more polarizable (typically metallic or metal coated) posts, which may have symmetric and/or asymmetric arrangements and cross sections. Applying a steady AC voltage between an opposing pair of electrodes such as 7-10 and 7-30 or 7-20 and 7-40 to cause mixing driven by induced-charge electro-osmotic flows around the polarizable structures (such flows can also cause transverse pumping due to broken left-right symmetry) can be extended to incorporate time-modulated AC voltages. In one embodiment, the dominant AC voltage can switch from being between 7-10 and 7-30 to 7-20 and 7-40. One way to control such switching is to apply an AC voltage to each electrode, (7-10)-(7-40), and give each a tunable phase shift relative to the others. For example, in one state 7-10 and 7-20 have zero phase shift (same AC voltage) while 7-30 and 7-40 have a half-period phase shift (same AC voltage, opposite of 7-10 and 7-20); this is equivalent to having simply two larger electrodes on opposite sides of the channel applying a uniform transverse (up/down) AC field. In a second state, 7-10 and 7-30 have zero phase shift, while 7-30 and 7-40 have a half-period phase shift; this is equivalent to imposing a primarily longitudinal (left/right) AC field. By switching between these two (or more) states, as envisioned encompassing embodied aspects of this invention, rapid chaotic mixing can be achieved in the channel around the posts. In some embodiments, the mixing can be superimposed on a background pressure-driven flow through the post array. In other embodiments, broken symmetry in the geometry of the post array leads to time-modulated pumping through the channel, along with the time-modulated mixing. According to this aspect, the devices will comprise more than one polarizable post .

[00256] Figure 8 depicts another embodiment of the invention, where the polarizable posts (8-50) and (8-60) are placed in a microfluidic chamber with at least four electrodes placed on different walls, to allow for application of dominant electric fields in orthogonal directions, e.g. left/right using electrodes 8-20 and 8-40, or up/down using electrodes 8-10 and 8-30. According to this aspect, and in some embodiments, the same sort of time modulation strategies can be used to achieve chaotic mixing in the chamber. The switching time should be comparable to the convection time for the dominant fluid vortices amongst the array of posts.

[00257] It is to be understood with respect to the devices/methods of this invention that any number of patterns of engagement of the pumps can be envisioned, which selectively engage a series of pumps in a desired order, to facilitate fluid flow, which results in a desired pattern for mixing fluid contained therein.

[00258] According to this aspect of the invention, each series may be modulated such that the magnitude, frequency or combination thereof of the voltage applied to each series is to maximize chaotic mixing.

[00259] Modulation in this aspect, as well, may further comprise a step whereby once the material has been mixed for a desired period of time, the fluid is conveyed in a desired dominant direction.

[00260] While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications

5 and changes as fall within the true spirit of the invention.

CLAIMS

[00261] What is claimed is:

1. A device comprising at least one microfluidic chamber for mixing an electrolyte fluid, the chamber comprising:

- a plurality of electrodes proximal to, positioned on, or comprising at least one surface of said chamber arranged in at least two series; and
- a source, providing an electric field in said chamber, wherein said source applies voltage selectively to said first series such that said voltage is not simultaneously or commensurately applied to second series of electrodes of said plurality;

whereby an electroosmotic flow trajectory created by application of voltage to said first series varies from an electroosmotic flow trajectory created by application of voltage to said second series.

2. The device of claim 1, wherein said at least two series are positioned such that an electroosmotic flow trajectory created by a first series is in a direction opposite to an electroosmotic flow trajectory created by a second series of said at least two series.

3. The device of claim 1, wherein said first series is so positioned such that an electroosmotic flow trajectory created thereby is parallel to a long axis of said device and said second series is so positioned such that an electroosmotic flow trajectory created thereby is perpendicular thereto, or vice versa.

4. The device of claim 1, wherein said at least two series are positioned on opposing surfaces of said chamber.

5. The device of claim 1, wherein said source modulates the magnitude, the frequency or a combination thereof of the voltages applied to said series of electrodes.

6. The device of claim 5, wherein the magnitude or direction of electroosmotic flow is changed thereby.

7. The device of claim 6, wherein said changed electroosmotic flow is slower than electroosmotic flow in said chamber prior to modulation of said magnitude or frequency.

8. The device of claim 1, wherein said electric field is comprised of a DC electric field.

9. The device of claim 8, wherein said source modulates the magnitude of said DC electric field.

10. The device of claim 9, wherein the magnitude of said electric field varies with time.

11. The device of claim 1, wherein said electric field is comprised of an AC or pulsed AC electric field.
12. The device of claim 11, wherein the source modulates the amplitude or the frequency or a combination thereof of said AC electric field.
- 5 13. The device of claim 12, wherein the amplitude of said AC field varies with time.
14. The device of claim 1, wherein said electric field is comprised of an AC or pulsed AC electric field with a DC offset.
15. The device of claim 1, wherein said series are arranged asymmetrically with respect to a central axis in said pumping unit.
- 10 16. The device of claim 1, wherein said electrodes are arranged in a symmetric pattern in said chamber.
17. The device of claim 1, wherein said electrodes are arranged in an asymmetric pattern in said chamber.
18. The device of claim 1, wherein said electrodes are arranged in a gradient pattern in said
15 microfluidic channel.
19. The device of claim 1, wherein at least one of said plurality of electrodes or a first portion thereof is varied by at least 1% in height, in surface area or in vertical positioning within said chamber, or a combination thereof, with respect to another of said plurality of electrodes or a second portion of said at least one of said plurality of electrodes.
- 20 20. The device of claim 19, wherein each series of said plurality of electrodes comprises at least one electrode, or a portion thereof, which is raised with respect to another electrode, or another portion of said at least one electrode in said series.
21. The device of claim 19, wherein each series of said plurality of electrodes comprises at least one electrode, or a portion thereof, which is lowered with respect to another electrode, or another
25 portion of said at least one electrode in said series.
22. The device of claim 21, wherein each series of said plurality of electrodes comprises at least one electrode or at least a portion thereof having a height or depth which is varied proportionally to a width of another electrode, another portion of said at least one electrode, or a combination thereof, in said series.

23. The device of claim 19, wherein each series of said plurality of electrodes comprises at least one electrode, or portions thereof, having height or depth variations from about 1% to about 1000% of:

- a width of another electrode in said series, another portion of said at least one electrode in said series, or a combination thereof;
- a gap between said at least one electrode and another electrode in said series;
- or a combination thereof.

24. The device of claim 19, wherein said at least one electrode is not flat.

25. The device of claim 19, wherein positioning of said electrodes in said chamber is varied with respect to gaps between said electrodes, spacing of said electrodes, or a combination thereof.

26. The device of claim 25, wherein said gaps are between about 1 micron and about 50 microns.

27. The device of claim 25, wherein said gaps between said electrodes, said spacing of said electrodes, height of said electrodes or portions thereof, shapes or said electrodes or portions thereof, surface area of said electrodes or portions thereof, volume of said electrodes or portions thereof, vertical positioning of said electrodes or portions thereof within said chamber or a combination thereof is unequal.

28. The device of claim 25, wherein said gaps between said electrodes, said spacing of said electrodes, or a combination thereof is equal.

29. The device of claim 19, wherein said electrode widths are between about 0.1 microns and about 50 microns.

30. The device of claim 19, wherein at least one electrode of said plurality of electrodes comprises at least one raised portion of said electrode in the form of a cylinder of arbitrary cross section.

31. The device of claim 19, wherein at least one electrode of said plurality of electrodes comprises an exposed surface, which is flat, and not coplanar with another exposed surface of said electrode or of another electrode in said series.

32. The device of claim 1, wherein at least one electrode of said plurality of electrodes comprises an edge, which is straight and not parallel to another edge in said electrode or in another electrode.

33. The device of claim 31, wherein said electrode comprises an edge, which forms a chevron or sawtooth pattern, either vertically or horizontally.

34. The device of claim 1, wherein at least one electrode comprises an edge, which is curved.

35. The device of claim 33, wherein said electrode comprises an edge, which forms a wavy or arc-like pattern, vertically, horizontally, or a combination thereof.

36. The device of claim 1, wherein at least one electrode of said plurality of electrodes comprises an exposed surface, which is not flat, and arbitrarily curved in three dimensions.

5 37. The device of claim 1, wherein said source applies a peak to peak AC voltage of between about 0.1 and about 10 Volts.

38. The device of claim 1, further comprising conducting posts.

39. The device of claim 38, wherein said conducting posts are positioned between at least two electrodes.

10 40. The device of claim 38, wherein said conducting posts have a cylindrical shape.

41. The device of claim 38, wherein the shape of said posts approximates an arrowhead, teardrop, or ellipse along at least one axis.

42. The device of claim 38, wherein said conducting posts have at least one spherical, rectangular, triangular, or square face.

15 43. The device of claim 38, wherein said conducting posts are positioned with a gap having a size of between 1 and 500 microns.

44. The microfluidic device of 38, wherein said conducting posts are symmetric with respect to each other.

20 45. The device of claim 38, wherein at least one dimension of said conducting post ranges from about 5 to about 250 μm .

46. A method of mixing a fluid, said method comprising

i. applying a fluid comprising an electrolyte to the device of claim 1; and

ii. selectively applying voltage to said at least two series, such that said voltage is not simultaneously or commensurately applied to said at least two series;

25 whereby upon selective application of said voltage to said series, electro-osmotic flows with varied trajectories are generated in a region proximal to each of said series, resulting in mixing of said fluid.

30 47. The method of claim 46, wherein said at least two series are positioned such that an electroosmotic flow trajectory created by a first series is in a direction opposite to an electroosmotic flow trajectory created by a second series of said at least two series.

48. The method of claim 46, wherein said first series is so positioned such that an electroosmotic flow trajectory created thereby is parallel to a long axis of said device and said second series is so positioned such that an electroosmotic flow trajectory created thereby is perpendicular thereto, or vice versa.

5 49. The method of claim 46, wherein said at least two series are positioned on opposing surfaces of said chamber.

50. The method of claim 46, wherein the magnitude or the frequency or a combination thereof of the voltages applied to said series of electrodes is modulated.

51. The method of claim 50, wherein varying said magnitude or frequency or a combination thereof of
10 the voltages applied is via a smooth transition.

52. The method of claim 50, wherein the magnitude or direction of electroosmotic flow is changed thereby.

53. The method of claim 52, wherein said changed electroosmotic flow is slower than electroosmotic flow in said chamber prior to modulation of said magnitude or frequency.

15 54. The method of claim 46, wherein said modulated magnitude or frequency or a combination thereof of voltages applied results in a greater value applied to one of said series than another.

55. The method of claim 54, wherein said modulated magnitude or frequency or a combination thereof of voltages applied may be varied in terms of to which of said series said greater value is applied.

56. The method of claim 46, wherein multiple fluids may be introduced into said chamber such that
20 said method is useful for mixing multiple fluids.

57. The method of claim 46, wherein said electric field is comprised of a DC electric field.

58. The method of claim 46, wherein said electric field is comprised of an AC or pulsed AC electric field.

59. The method of claim 46, wherein said electric field is comprised of an AC or pulsed AC electric
25 field with a DC offset.

60. The method of claim 46, wherein said series are arranged asymmetrically with respect to a central axis in said pumping unit.

61. The method of claim 46, wherein each series of said plurality of electrodes comprises at least one electrode, or a portion thereof, which is raised with respect to another electrode, or another
30 portion of said at least one electrode in said series.

62. The method of claim 46, wherein each series of said plurality of electrodes comprises at least one electrode, or a portion thereof, which is lowered with respect to another electrode, or another portion of said at least one electrode in said series.

63. The method of claim 46, wherein said at least one electrode is not flat.

5 64. The method of claim 46, wherein at least one electrode of said plurality of electrodes comprises an exposed surface, which is flat, and not coplanar with another exposed surface of said electrode or of another electrode in said series.

65. The method of claim 46, wherein at least one electrode of said plurality of electrodes comprises an exposed surface, which is curved in three dimensions.

10 66. The method of claim 46, wherein said source applies a peak to peak AC voltage of between about 0.1 and about 10 Volts.

67. The method of claim 66, wherein said AC frequency is between about 1 Hz and about 100 kHz.

68. The method of claim 46, wherein said device comprises conductive posts.

15 69. The method of claim 68, wherein said device comprises conducting posts positioned between at least two electrodes.

70. The method of claim 68, wherein said conductive posts have a cylindrical shape.

71. The method of claim 68, wherein the shape of said posts approximates an arrowhead, teardrop, or ellipse along at least one axis.

20 72. The method of claim 68, wherein said conductive posts have at least one spherical, rectangular, triangular, or square face.

73. The method of claim 68, wherein a gap or a space exists between said posts and said electrodes.

74. The method of claim 68, wherein said gap size is on the micron scale.

75. The method of claim 68, wherein said gap size is between 1 and 500 microns.

76. The method of claim 68, wherein said posts change the direction of the applied electric field.

25 77. The method of claim 76, wherein said change in electric field causes a change in fluid flow direction in said device.

78. The method of claim 77, wherein said change in fluid flow direction causes mixing of said fluid.

79. The method of claim 46, wherein said method further comprises assay or analysis of said fluid.

80. The method of claim 79, wherein said analysis is a method of cellular analysis.

81. The method of claim 79, wherein said method further comprises the steps of:

- a. introducing a buffered suspension comprising cells and a reagent for cellular analysis into said microfluidic chamber; and
- b. analyzing at least one parameter affected by contact between said suspension and said reagent.

82. The method of claim 81, wherein said reagent is an antibody, a nucleic acid, an enzyme, a substrate, a ligand, or a combination thereof.

83. The method of claim 81, wherein said reagent is coupled to a detectable marker.

84. The method of claim 83, wherein said marker is a fluorescent compound.

85. The method of claim 84, wherein said device is coupled to a fluorimeter or fluorescent microscope.

86. The method of claim 81, further comprising the step of introducing a cellular lysis agent in said port.

87. The method of claim 81, wherein said reagent specifically interacts or detects an intracellular compound.

88. The method of claim 81, wherein said assay or analysis of said fluid is a method of analyte detection or assay.

89. The method of claim 79, further comprising the steps of:

- a. introducing an analyte to said device;
- b. introducing a reagent to said device; and
- c. detecting, analyzing, or a combination thereof, of said analyte.

90. The method of claim 46, wherein said mixing reconstitutes a compound in said device, upon application of said fluid.

91. The method of claim 90, wherein said compound is solubilized slowly in fluids.

92. The method of claim 46, wherein said mixing results in high-throughput, multi-step product formation.

93. The method of claim 92, further comprising the steps of:

- a. introducing a precursor to the device;
- b. introducing a reagent, catalyst, reactant, cofactor, or combination thereof to said device;
- c. providing conditions whereby said precursor is converted to a product; and

d. optionally, collecting said product from said device.

94. The method of claim 93, further comprising carrying out iterative introductions of said reagent, catalyst, reactant, cofactor, or combination thereof in (b), to said device.

95. The method of claim 93, wherein said reagent is an antibody, a nucleic acid, an enzyme, a substrate, a ligand, a reactant or a combination thereof.

96. The method of claim 46, wherein said mixing results in drug processing and delivery.

97. The method of claim 96, wherein said method further comprises the steps of:

- i. introducing a drug and a liquid comprising a buffer, a catalyst, or combination thereof to the device;
- ii. providing conditions whereby said drug is processed or otherwise prepared for delivery to a subject; and
- iii. collecting said drug, delivering said drug to a subject, or a combination thereof.

98. The method of claim 97, further comprising carrying out iterative introductions of said liquid to said device.

99. The method of claim 97, wherein introduction of said liquid serves to dilute said drug to a desired concentration.

FIGURE 1A

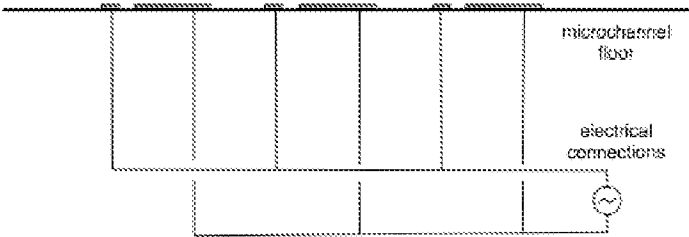


FIGURE 1B

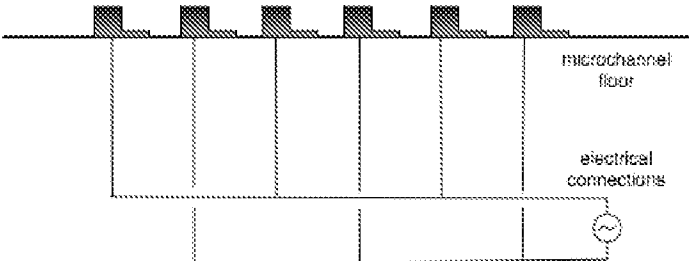


FIGURE 1C

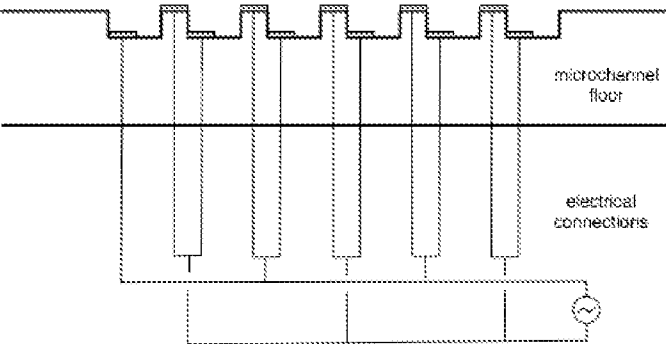


FIGURE 1D

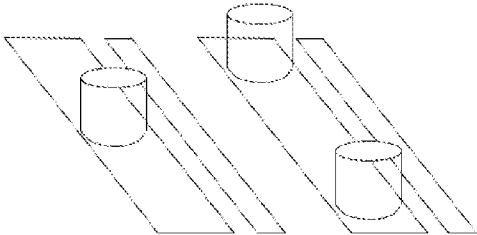


FIGURE 2A

2/6

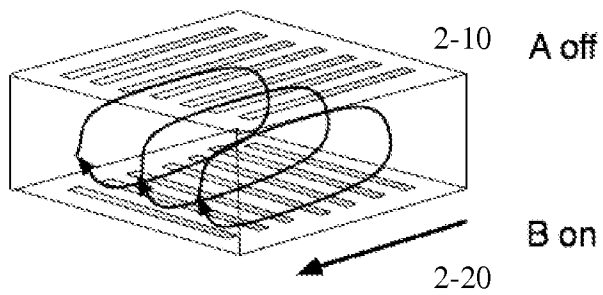
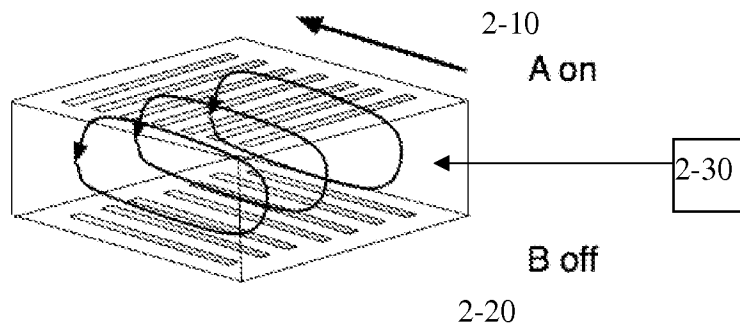
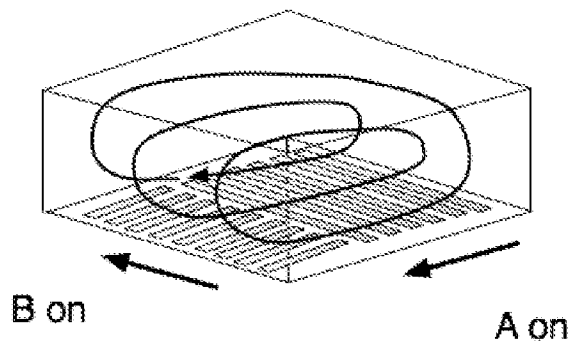
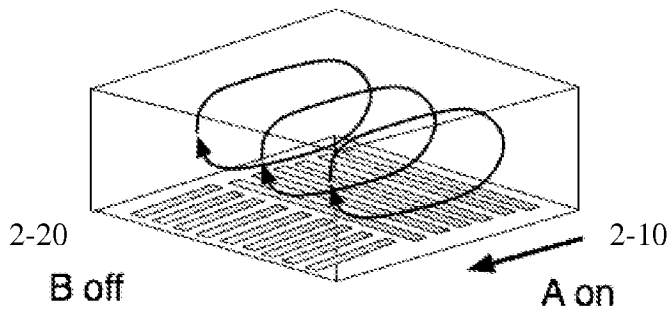


FIGURE 2B



3/6

FIGURE 3

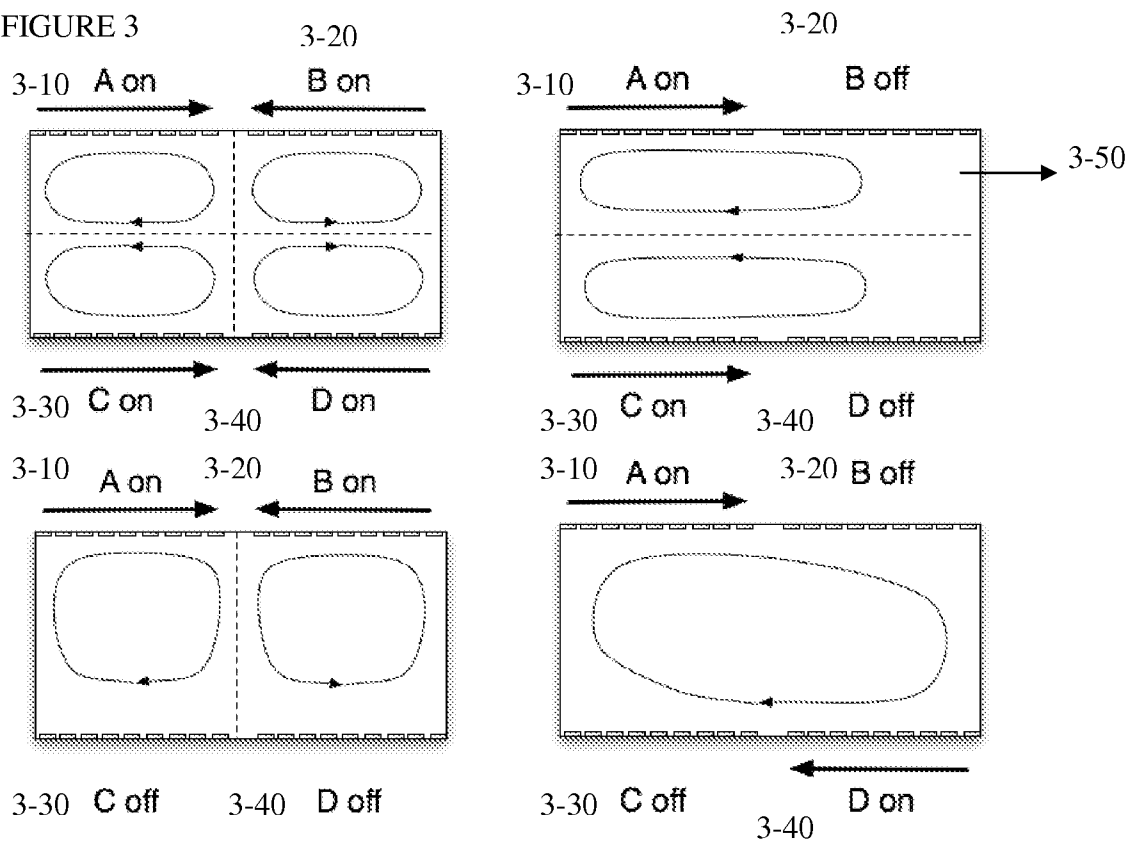
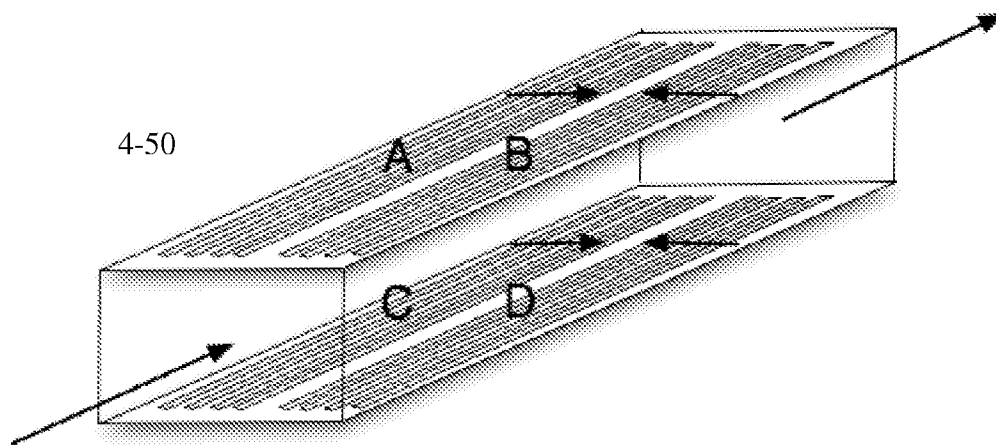


FIGURE 4A



4/6

FIGURE 4B

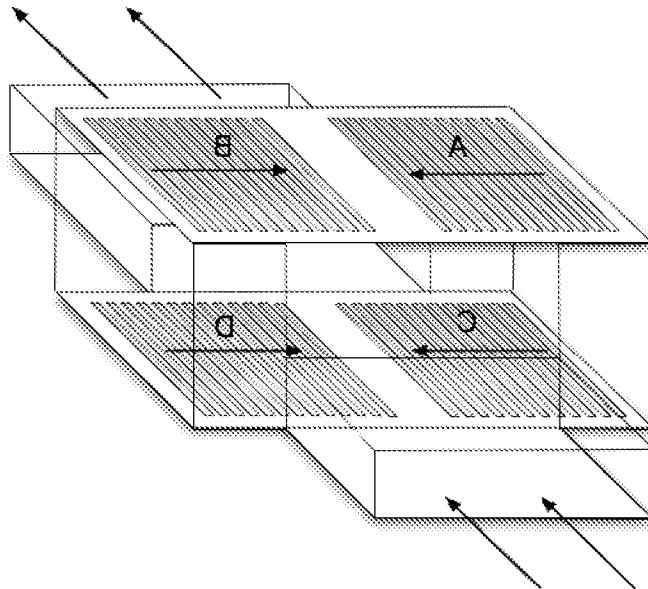


FIGURE 5A

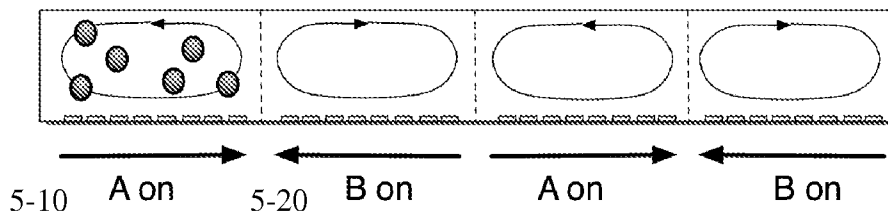


FIGURE 5B

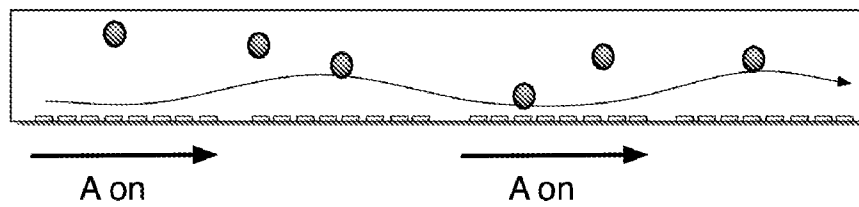
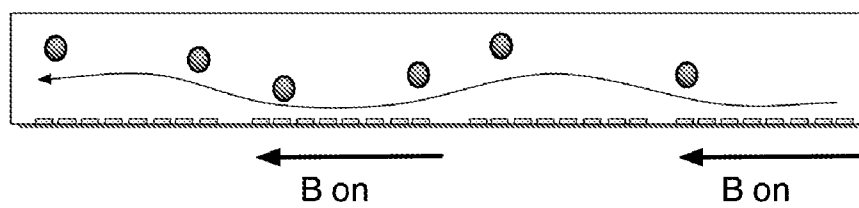


FIGURE 5C



5/6

FIGURE 6A

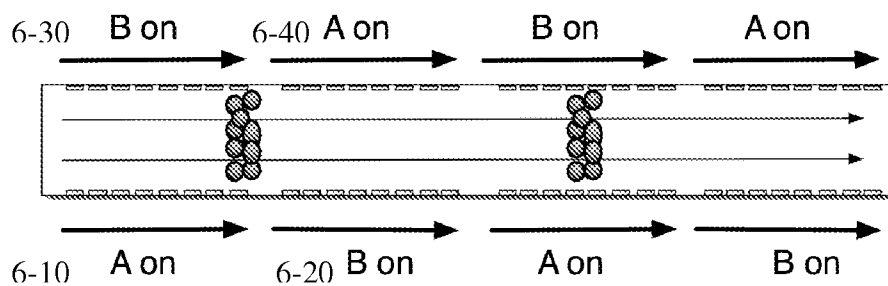


FIGURE 6B

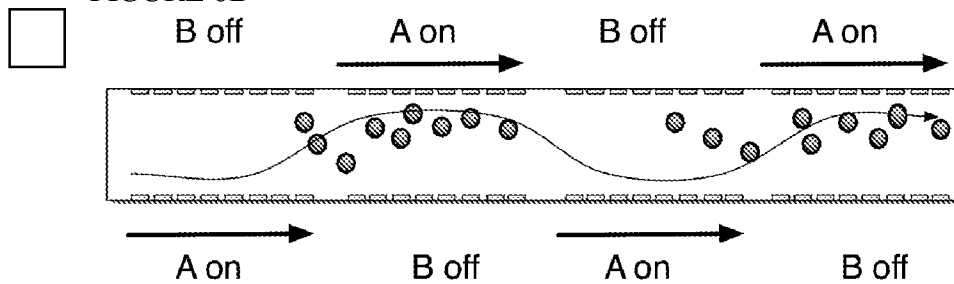


FIGURE 6C

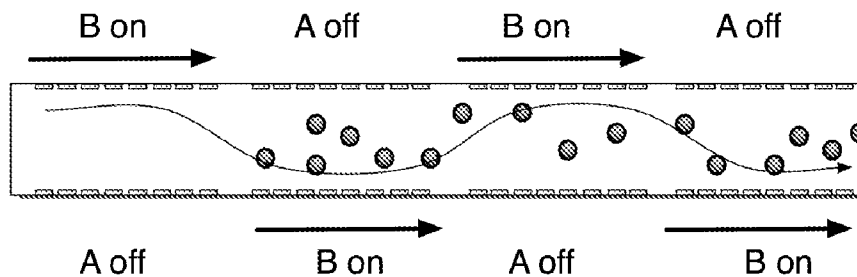


FIGURE 7

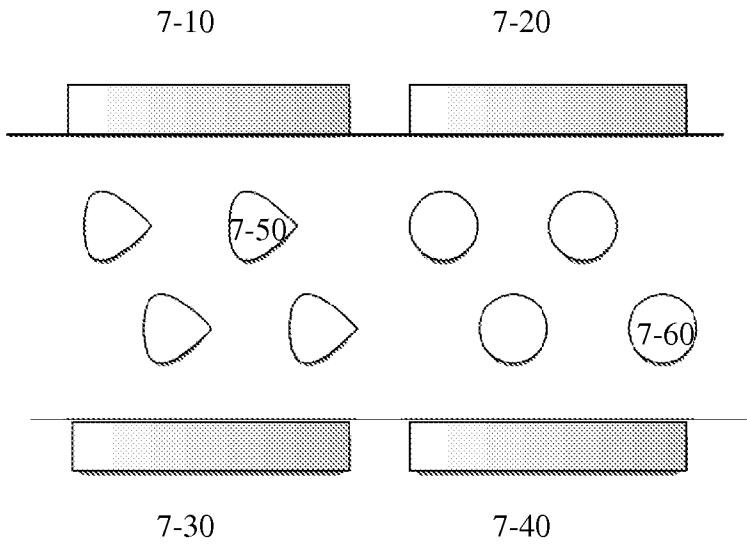


FIGURE 8

