Controlled Dispensing of Ultrafine, Variable Volume, Emulsion Droplets

ABSTRACT

Embodiments of the present invention comprise a surface microfluidic system with varying coplanar electrode structure capable of precision dispensing of an array of variable volume droplets in a rapid, controlled and automated fashion. Furthermore, the invention provides surface microfluidic methods and systems for the creation and transport of emulsion droplets, including vesicles and cells.
FIG. 1

(A) Single layer L-DEP SMF device

(B) Two layer L-DEP SMF device

- Substrate: SiO₂ on Si: 0.5 nm
- Electrodes: Al or ITO: 100 nm
- Dielectric 1: Si₃N₄: 600 nm
- Dielectric 2: Si₃N₄: 500 nm
- Hydrophobic coating
- Teflon®: 100 nm

Top view

Cross-sectional view
Step 1: Clean Si or glass wafers with Piranha.

Step 2: Deposit Aluminum (thickness ~ 200nm).

Step 3: Spin-coat photoresist (HPR 304; thickness ~ 1.2μm).

Step 4: UV exposure through photomask.

FIG. 2a
Step 5: Develop the resist (354 developing solution for HPR 504)

Step 6: Wet chemical etching to pattern aluminum

Step 7: Strip the remaining photoresist

Step 8: Deposit dielectric (Si₃N₄) (thickness ~ 500nm)

Substrate  Aluminum  Photore sist (HPR 504)

Dielectric, Si₃N₄

FIG. 2b
Parent sample droplet (DI water 1-2 μL)

Liquid jet

$V_s @ 100$ kHz

(a) semi-circular bumps

FIG. 4a
FIG. 4b
Continuous tapered L-DEP electrode

Parent sample droplet (0.8 µL)

Actuated tapered aqueous jet

FGS. 7a-7b

FIGS. 7a-7b

Actuated jet length (µm)

Force (N)

$F_{DEP}$ (Uniform jet)

$F_{Y}$ (Uniform jet)

$F_{\mu}$ (Uniform jet)

$F_{\mu}$ (Tapered jet)

$F_{Y}$ (Tapered jet)

$F_{DEP}$ (Tapered jet)

FIG. 8
(a) Scheme 1
(b) Scheme 2
(c) Scheme 3
(d) Scheme 4
(e) Scheme 5

Non-symmetric
Electro-Mechanical Pinch
Symmetric

(f)

FIGS. 9a-9f
FIGS. 10a-10d

\begin{align*}
\lambda &= 207\,\mu\text{m} \\
\delta &= 400\,\mu\text{m} \\
\delta &= 15\,\mu\text{m}
\end{align*}
Scheme 1
Emulsion daughter droplets

Scheme 2

Parent aqueous droplet  Oil droplet

FIG. 11
FIG. 13
FIG. 14a

FIG. 14b
(i) L-DEP actuation

(ii) D-DEP actuation

(iii) Array of daughter droplets

(iv) D-DEP actuation

(v)

AC voltage
L-DEP: 450 V_{RMS} @ 100 kHz
D-DEP: 120 V_{RMS} @ 30 Hz

Ground
Switch

FIG. 15
An L-DEP based SMF chip for variable volume droplet array dispensing

Contact Pads 2x2 multiplexed reaction chambers

A DEP based multiplexed reaction chip for combinatorial biochemical assays

Various advanced L-DEP electrode designs

FIG. 16a

FIG. 16b
FIGS. 25a-25i
FIGS. 26a-26f

FIG. 27
POPC (1-palmitol-2-oleoyl-sn-glycero-3-phosphocholine) 10 mg/mL stock in chloroform

10 µL of POPC stock

1 µL of NBD-PC stock

Evaporated under air flow
thin, dry lipid film obtained

10 mL of Mineral Oil
(Crystal Plus 70 fg obtained added to create dispersion 4 minutes

After Sonication, the lipid dispersion was left overnight to ensure uniform dispersion of lipid molecules in mineral oil before emulsion droplet dispensing.

FIG. 28

(a)

Teflon®

Dielectric (Si,Ni)

Sample droplet

Step 1: L-DEP actuation at 500 Vrms, 100 kHz AC Voltage

Aqueous parent droplet

dispersion media

(b)

Formed Emulsion droplets

(f)

(g)

(d)

Lipid Molecule

Aqueous solution

Mineral/Silicone oil

Dispensed droplet

FIG. 29

Bilayer/Vesicles formed

Monolayer formed
L-DEP actuation. Formation of Emulsion jet

Jet Break-up and formation of precision emulsion droplets

(b) Emulsion droplet (bright)

(c) Emulsion droplet (Fluorescent)

(d) Lipid Monolayer (Bright)

(e) Lipid Monolayer (Fluorescent)

(f) Lipid Bilayer (Bright)

(g) Lipid Bilayer (Fluorescent)

(h)

(i)

(j)

FIGS. 30a-30j
Step 1: L-DEP actuation (480 Vpp, 100 kHz AC voltage) of DEP chip in a mineral oil bath.

Schematic of a continuous tapered, pinched DEP electrode pair.

Step 2: Suspension of variable volume single emulsion (SE) droplets with lipid monolayer at the aqueous-oil interface.

Dispensed single emulsion droplets with lipid monolayer.

Step 3: Spontaneous assembly of the second lipid monolayer by introducing aqueous phase on top of the encapsulating oil bath.

Dispensed bilayer lipid vesicles on the chip surface.

Figure 31

Legend symbols:
- Lipid molecule
- Droplet
- Aqueous solution
- Mineral/Silicone oil
CONTROLLED DISPENSING OF ULTRAFINE, VARIABLE VOLUME, EMULSION DROPLETS

CROSS-REFERENCE TO RELATED APPLICATIONS

0001. This application claims priority to U.S. Provisional Application No. 61/361,854 filed Jul. 6, 2010, the entire contents of which is specifically incorporated by reference herein without disclaimer.

BACKGROUND OF THE INVENTION

0002. 1. Field of the Invention

0003. This invention relates to a surface microfluidic system and more particularly relates to a system and method for emulsion droplet dispensing technology.

0004. 2. Description of the Related Art

0005. Development of miniaturized total analysis systems (μ-TAS) or also frequently referred to as lab-on-a-chip (LOC) is of increasing interest among the research and industrial community. Such ‘laboratory-on-a-chip’ technology offers new prospects for routine chemical analysis, drug testing, bioassay, healthcare delivery, diagnostic devices including non-invasive and potentially the early detection of cancers.

0006. The initial development of such systems has in the past deployed ‘closed channel’ microfluidic devices, incorporating valves and pumps or other means to confine and manipulate aqueous samples. Typically, such microchannels are either etched or formed in glass, silicon or polymeric substrate using conventional microfabrication techniques. However, in order to truly miniaturize and automate such closed channel microfluidic systems, capable of hand-held operation, it requires on-chip pumping to transport the fluidic media and valving for fluidic steering and sample-reagent isolation as well as the integration of suitable sensors or detectors to monitor and analyze the contents of the fluidic microchannel. Although good progress has been demonstrated along these lines, such systems tend to be complex and require complicated processing and furthermore costly to implement.

0007. The referenced shortcomings are not intended to be exhaustive, but rather are among many that tend to impair the effectiveness of previously known techniques in microfluidic transport; however, those mentioned here are provided to demonstrate that the methodologies appearing in the art have not been satisfactory and that a significant need exists for the techniques described and claimed in this disclosure.

SUMMARY OF THE INVENTION

0008. From the foregoing discussion, it should be apparent that a need exists for a system and method for the precise handling and processing of small amounts of aqueous samples in the form of subnanoliter droplets on tops of surfaces employing “surface microfluidic” (SMF) devices that are devoid of any microchannels.

0009. Thus, in accordance with certain aspects of the present invention, there is provided a surface microfluidic system, comprising: a solid substrate; a first electrode structure defining a first surface fluidic flow path along a gap between the electrodes of the first electrode structure, the first electrode structure varying along the length of the first electrode structure such that variable-sized droplets are dispensed along the first surface fluidic flow path during use; and an electrical source coupled to the first electrode structure. The electrical source may be an Alternating Current (AC) electrical source. In certain embodiments, the system further comprises a control unit configured to control the electrical source, such as a computer-implemented control unit. In preferable embodiments, the system is further defined as a dielectrophoretic actuator, i.e., an actuator using dielectrophoretic force.

0010. In a further embodiment, the system comprises a second electrode structure, the second electrode structure defining a second surface fluidic flow path along a gap between the electrodes of the second electrode structure and intersecting with the first electrode structure, the second surface fluidic flow path initiating from a predefined site on the first surface fluidic flow path. In specific embodiments, the second electrode structure is fishbone-shaped or comb-shaped.

0011. The system may be used for dispensing or transport of emulsion droplets, thus obviating the need to use an oil bath to prevent sample evaporation. Thus, in certain aspects, the surface of the substrate or the system is not immersed in oil. One or more emulsion droplet reservoirs may be coupled to the first electrode structure in certain aspects.

0012. Embodiments of methods of moving an emulsion droplet are also presented. In one embodiment, the method includes providing one or more sample droplets. The method may also include applying a liquid dielectrophoretic actuation force to the one or more sample droplets to form an emulsion jet along the gap between electrodes of a first electrode structure. Additionally, the method may include removing the liquid dielectrophoretic actuation force to dispense emulsion droplets from disintegration of the emulsion jet. Also, the method may include applying a droplet actuation force to at least one of the emulsion droplets to cause the at least one of the emulsion droplets to transport along the gap between electrodes of a second electrode structure.

0013. A “droplet actuation force,” as used herein, refers to a combination of liquid dielectrophoresis and electrowetting configured such that a droplet propagates along a path.

0014. “An emulsion droplet,” as used herein, refers to a small volume (for example in the scale of nanoliter or less) of mixture of two or more immiscible (unblendable) liquids, including a vesicle, a microscopic particle or a cell. A vesicle like a liposome can be defined as an enveloped structure of at least a liquid layer within at least another liquid layer immiscible from the former liquid layer. The emulsion droplets may be single-layered or multi-layered.

0015. As used herein, the term “daughter droplet” refers to the one or more droplets formed along predefined points of the dielectrophoresis electrodes. For example, daughter droplets may be formed at semicircular bumps in the electrodes. In another embodiment, daughter droplets may be formed between pinched portions of the electrodes. In some embodiments, a daughter droplet may include an emulsion droplet.

0016. In certain aspects, the sample droplets are in the form of emulsion. In alternative embodiments, the emulsion jet may be formed by mixing at least two dielectrophoretically-actuated liquid jets. The method may further comprise mixing the transported daughter emulsion droplet with a different droplet at a predefined site. In further aspects, the different droplet may be transported by dielectrophoretic force to the predefined site.

0017. In additional aspects, the first and/or second electrode structures may be comprised in a surface microfluidic
system. The surface microfluidic system may comprise a substrate layer, an electrode layer, an electrically insulating dielectric layer, and a hydrophobic layer on the top of the system. The surface microfluidic system may further comprise a first electrical source coupled to the first electrode structure and a second electrical source coupled to the second electrode structure. The first and second electrical sources may be an AC electrical source. More specifically the first electrical source for forming an emulsion jet may be at an electric frequency of at least or about 50, 100, 200, 250, 300, 350, 400 kHz or any intermediate ranges. The second electrical source for transporting the emulsion droplet may be at an electric frequency of at least or about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200 Hz or any intermediate ranges. In further embodiments, the surface microfluidic system further comprises a control unit that is configured to control the first electrical source and/or the second electrical source.  

[0018] For dispensing variable-sized droplets, the first electrode structure used in the method or system as described herein may vary along the length of the first electrode structure such that the droplets (e.g., emulsion droplets) dispensed are variable in size. In particular aspects, the first electrode structure may be tapered, more specifically, continuously tapered. For example, the first electrode structure may be comprised of individual electrode segments of width (w) and gap (g) varying from at least 40 μm at one end to at most 10 μm at the opposite end along the length of the first electrode structure.  

[0019] The precision dispensing of the fluid droplet control for example, the first electrode structure used in the method or system as described herein comprises a plurality of semicircular electrode sites spaced at predefined intervals, also known as bumps. The first electrode structure may comprise indentations at predefined locations for precision dispensing of sample droplets, also known as pinches.  

[0020] Embodiments discussed in the context of methods and/or compositions of the invention may be employed with respect to any other method or composition described herein. Thus, an embodiment pertaining to one method or composition may be applied to other methods and compositions of the invention as well.  

[0021] “Comprising” is used in its inclusive sense and does not exclude other elements being present.  

[0022] The word “particle” includes any substance, including an inorganic material, liquid droplet, molecule such as DNA, RNA or other subcellular components, or intact cells that is capable of being affected by a dielectrophoretic force field.  

[0023] “Open” means free from lateral constraint by solid objects except for constraint by a single supporting surface that provides lateral constraint in one direction.  

[0024] As herein the specification, “a” or “an” may mean one or more. As herein used in the claim(s), when used in conjunction with the word “comprising”, the words “a” or “an” may mean one or more than one.  

[0025] The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or”. As used herein “another” may mean at least a second or more.  

[0026] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.  

[0027] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.  

BRIEF DESCRIPTION OF THE DRAWINGS  

[0028] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.  

[0029] FIG. 1: Top and sectional views of examples of various L-DEP based SMD devices.  


[0031] FIG. 3: An example of a DRIE processed silicon master.  

[0032] FIGS. 4a-4b: FIG. 4a: An example of electrode configuration and arrangement for DEP liquid actuation and droplet dispensing. FIG. 4b: Video frames captured at different times: (i) t=0, (ii) t=18 ms—liquid finger ejected from parent sample, (iii) t=40 ms—liquid finger covers the entire electrode structure, and (iv) t=42 ms—voltage turned off and droplets dispersed at the electrode bump sites. Electrode dimensions: w=g=15 μm, length=6 mm.  

[0033] FIGS. 5a-5d: L-DEP actuation recorded through a high-speed camera at 2000 fps illustrating the breakup of liquid jet. Scale bar in (a) is 100 μm. L-DEP electrode dimensions: w=40 μm; g=40 μm; λ=540 μm.  

[0034] FIG. 6: An example of experimental setup utilized for recording high-speed L-DEP actuations, fluorescence imaging, quantitative and volumetric analysis of dispersed droplets.  

[0035] FIGS. 7a-7b: FIG. 7a: A 3d schematic diagram of a tapered aqueous jet protruding from the parent sample droplet and propagating along the continuously tapered L-DEP electrode length; FIG. 7b: Top view of the model.  

[0036] FIG. 8: Plots showing comparison between the various forces involved in L-DEP actuation (500 V_{pp max}, AC voltage at 100 kHz), for a uniform L-DEP actuation (w=g=40 μm; electrode length=4000 μm) and a continuous tapered L-DEP actuation (tapering from w=g=40 μm to 10 μm with electrode length=4000 μm; see Scheme 2 in FIG. 9b).  

[0037] FIGS. 9a-9f: Schematic images illustrating the various L-DEP electrode designs used during experimentations.  

[0038] FIGS. 10a-10d: Micro-droplets dispensed by L-DEP actuations on structures with different electrode architectures. (FIG. 10a) uniform width/gap L-DEP electrode; (FIG. 10b) uniform width/gap L-DEP electrode pinched at lambda; (FIG. 10c) continuous tapering width/gap L-DEP electrode pinched at a fixed separation 1; and (FIG. 10d) stepped tapering L-DEP electrode with uniform width/gap electrode sections.  

[0039] FIG. 11: Emulsion droplet dispensing schemes leveraging L-DEP.
FIGS. 12a-12d. Micrographs comparing dispensed single and double emulsion droplets. FIG. 12a: Section of an array of aqueous glycerol solution (25% by volume)-in-5 cSt silicone oil single emulsion droplets. FIG. 12b: A single emulsion droplet observed under 50x magnification. FIG. 12c: Section of an array of aqueous glycerol solution (25% by volume)-in-5 cSt silicone oil-in-aqueous glycerol solution (25% by volume) double emulsion droplets. FIG. 12d: A double emulsion droplet observed under 50x magnification (water-in-oil-in-water type double emulsion).

FIG. 13: A schematic diagram illustrating DEP actuation of a liquid jet, emanating from a parent sessile droplet upon AC voltage excitation of the coplanar electrodes.

FIGS. 14a-14b: FIG. 14a: A schematic of an integrated L-DEP and D-DEP electrode structure that can be used for mixing nanoliter-sized daughter droplets and performing biochemical assays. FIG. 14b: Schematic representation of D-DEP electrode structure. Note the fishbone-shaped electrode array and the pitch illustrated in the magnified view on the left. A sample droplet is manually pipetted at one end of the structure and, on application of low-frequency AC voltage, the droplet is transported to the other end of the structure.

FIGS. 15a-15: Exemplary embodiments of surface microfluidic systems for combinatorial biochemical assays on a 2x2 matrix.

FIGS. 16a-16b: FIG. 16a: Block diagram of the surface microfluidic system utilized to actuate samples and droplet dispensing. FIG. 16b: Examples of surface microfluidic (SMF) chips for various lab-on-a-chip applications.

FIGS. 17a-17c: Photograph illustrating the profiles of different homogeneous liquid jets; FIG. 17a: sample 1: 0% glycerol; FIG. 17b: sample 2: 12.5% glycerol; FIG. 17c: sample 3: 25% glycerol by volume; actuated on w=25 micron L-DEP electrode.

FIGS. 18: Plots showing the experimental and theoretical dynamics of L-DEP actuated homogeneous liquid jets; sample 1: DI water actuated at 400V; sample 2: 12.5% glycerol actuated at 400V, sample 3: 25% glycerol actuated at 450V and sample 4: 50% glycerol (% by vol.) actuated at 1500V.

FIGS. 19: L-DEP actuation of DI water in air and in submerged baths of 1 cSt silicon oil and 5 cSt oil; all actuations at 400V.

FIGS. 20a-20b: Photographs of emulsion jets and subsequently dispensed daughter droplets of various samples; FIG. 20a sample 2-5 cSt oil jet and (FIG. 20b) daughter droplets; (FIG. 20c) emulsion jet of sample 3-5 cSt oil and (FIG. 20d) formed daughter droplets; (FIG. 20e) emulsion jet of sample 4-5 cSt oil; and (FIG. 20f) formed daughter droplets; (FIG. 20g) emulsion jet of DI water (sample 1)-5 cSt oil on w=30 micron structure and (FIG. 20h) emulsion jet of DI-20 cSt oil on w=15 micron structure.

FIGS. 21a-20c: Video frame sequence showing breakup of DI water-5 cSt silicone oil emulsion jet and the formation of picoliter emulsion daughter droplets upon removal of the L-DEP actuation voltage at frame (FIG. 21b).

FIGS. 22a-22b: (FIG. 22a) Bright field and (FIG. 22b) Fluorescent images of an array of 30 picoliter sample 2 (glycerol 12.5% by vol. in DI) emulsion droplets dispensed using L-DEP actuation scheme on w=15 micron electrode structure; observed under 10x magnification.

FIGS. 23a-23b: Photographs showing emulsion droplets of 60 pl and 200 pl formed over (FIG. 23a) w=20 micron and (FIG. 23b) w=30 micron electrodes; observed under 50x magnification.

FIG. 24: Effect of glycerol concentration on the stability (sample volume and droplet radius) of sub-nanoliter droplets of sample 1: DI water; sample 2: 12.5% glycerol, sample 3: 25% glycerol and sample 4: 50% glycerol (% by vol.)

FIGS. 25a-25i: Micrographs showing shapes of stable DI water jet, breaking jet and the dispersed array of daughter droplets for the three different tapered electrode designs used. (FIG. 25a) continuous tapered DI water jet; (FIG. 25b) non-reliable break-up of the continuous tapered jet; (FIG. 25c) dispersed uniform daughter droplet array; (FIG. 25d) tapered DI water jet on continuous tapered electrode with weighted bump spacing (weighted λ); (FIG. 25e) reliable break-up of the tapered jet; (FIG. 25f) dispersed and precisely positioned daughter droplet array; (FIG. 25g) tapered DI water jet on continuous tapered electrode with fixed, independent bump spacing of 500 μm and suitably incorporated pinches; (FIG. 25h) user-controlled break-up of the tapered jet and; (FIG. 25i) dispersed precision daughter droplet array.

FIGS. 26a-26f: Micrograph images showing actuation and droplet dispensing over a uniform (w=30 μm) L-DEP electrode with various arrangements of bumps and pinches for a comparative analysis of their contribution in controlling the jet break-up. (FIG. 26a) Actuated DI water jet over one end section of L-DEP electrode containing λ/4, λ, and 2λ bump separations and specifically indicating placement of one asymmetric pinch within the first 2λ part; (FIG. 26b) Break-up of the modified jet which is strongly controlled by the position of various pinches; (FIG. 26c) Precision droplet formed at 2λ spacing but not in λ/4 spacing whereas also showing the override in droplet positioning due to the asymmetrically placed pinch (between the λ and 2λ regions); (FIG. 26d) Actuated DI water jet over the alternate end section of L-DEP electrode scheme containing fixed L and 2λ bump separations and specifically indicating placement of one asymmetric pinch between the fixed L and the 2λ part; (FIG. 26e) Break-up of the modified jet and (FIG. 26f) Precision droplet formed at 2λ and fixed L spacing whereas also showing the override in droplet positioning due to the asymmetrically placed pinch (between the fixed L and 2λ regions).

FIG. 27: Plot showing comparison between the controls over size of the dispersed daughter droplets for the various tapered L-DEP electrode schemes shown in FIGS. 9a-9f. This plot also compares the actual droplet volumes of the dispersed droplets with the theoretically estimated droplet volumes, calculated using Eqs. 34 and Eqs. 35 in Example 2.

FIG. 28: Schematic representation of lipid sample preparation method. The stock lipid samples used were purchased from Avanti Polar Lipids as 99% pure chlorofom stock solutions and the mineral oil from STE Oil Company.

FIGS. 29a-29f: Schematic diagrams showing the experimental steps: (FIG. 29a) X-sectional view of the SMF device; (FIG. 29b) L-DEP actuation and formation of single emulsion droplets; (FIG. 29c) Dispersed emulsion droplets with lipid monolayer (Diameter: 40-60 μm); (FIG. 29d) Lipid monolayer droplets formed in mineral/silicone oil bath; (FIG. 29e) 50% by vol. aqueous glycerol solution sinks in the lighter oil bath; (FIG. 29f) Lipid bilayer/vesicles formed.
FIGS. 30a-30i: Micrographs showing the formed lipid membranes and vesicles (captured using BX51 Olympus Fluorescent Microscope under 20x magnification); (FIG. 30a) formation of emulsion jet and dispensed array of single emulsion droplets over an exemplary L-DEP electrode scheme; (FIGS. 30b-30c) Bright field and fluorescent image of dispensed single emulsion droplet (monolayer at interface); (FIGS. 30d-30e) Bright field and fluorescent image of stable lipid monolayer in oil bath; (FIGS. 30f/30g) Bright field and fluorescent image of formed lipid bilayer separating the two aqueous mediums; (FIGS. 30h-30j) Bright field and fluorescent images of red fluorescent beads (size 1.5 μm) encapsulated by supported lipid membrane. Note the green fluorescence from the red fluorescent beads confirming presence of a bio-functional lipid membrane encapsulating the beads which are in turn encapsulated inside the vesicle.

FIG. 31: Schematic illustrating chip-based assembly of variable volume lipid bilayer vesicles.

FIGS. 32a-32c: Micrographs showing the formation of array of aqueous-oil bilayer vesicles on a continuous tapered, pinched L-DEP electrode scheme with semi-circular bumps; (FIG. 32a) A continuous tapered L-DEP electrode scheme with semi-circular bumps and electromechanical pinches (w=g vary from 40 μm (wide end) to 10 μm (narrow end)); (FIG. 32b) A single emulsion water-in-mineral oil tapered liquid jet formed during L-DEP actuation; (FIG. 32c) array of dispensed variable volume, single emulsion water-in-oil type daughter droplets. Volume of encapsulated aqueous sample range from 1.5 nl to 50 pl.

FIGS. 33a-33c: Micrographs showing the formation of array of homogeneous sample/reagent droplets on a continuous tapered, pinched L-DEP electrode scheme without semi-circular bumps; (FIG. 33a) A continuous tapered L-DEP electrode scheme with electromechanical pinches (w=g vary from 40 μm (wide end) to 10 μm (narrow end)); (FIG. 33b) A homogeneous aqueous tapered liquid jet formed during L-DEP actuation; (FIG. 33c) array of dispensed variable volume, aqueous daughter droplets. Volume of the dispersed droplets range from 1.5 nl to 50 pl.

FIGS. 34a-34c: Micrographs showing the formation of array of aqueous-oil bilayer vesicles on a continuous tapered, pinched L-DEP electrode scheme without semi-circular bumps; (FIG. 34a) A continuous tapered L-DEP electrode scheme with electromechanical pinches (w=g vary from 40 μm (wide end) to 10 μm (narrow end)); (FIG. 34b) A single emulsion water-in-mineral oil tapered liquid jet formed during L-DEP actuation (less perturbation in jet shape due in absence of semi-circular bumps (see FIG. 32b)); (FIG. 34c) array of dispensed variable volume, single emulsion water-in-oil type daughter droplets (more uniform aqueous-to-oil ratio in the dispensed variable droplet). Volume of encapsulated aqueous sample range from 1.5 nl to 50 pl.

FIGS. 35a-35j: Micrographs showing an integrated electrode scheme that comprises of two variable volume, L-DEP based dispensing schemes and a single surface electrowetting based move-and-mix-all droplet transport scheme for dispensing and large scale maneuvering of homogeneous droplets and vesicles. (FIG. 35a) Image showing the integrated electrode scheme; (FIG. 35b) two continuous tapered homogeneous liquid jets during L-DEP actuation under an oil bath; (FIG. 35c) two arrays of variable volume sample and reagent daughter droplets; (FIG. 35d) Electrowetting based droplet transport and mixing (3 out of 4 pairs mixed).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

I. INTRODUCTION

Liquid dielectrophoresis (L-DEP), when deployed at microscopic scales on top of non-wetting (hydrophobic) surfaces, offers methods of rapid, automated manipulation of very small amounts of polar aqueous samples for microfluidic applications and development of Lab On a Chip (LOC) devices. In certain aspects of this invention, there have been provided developments and applications of L-DEP in handling and processing of various types of aqueous samples and reagents of biological relevance including emulsions, using such microchip based surface microfluidic (SMF) devices. Specifically the utility of these devices may include on-chip bioassays, including nucleic acid analysis. Furthermore, the parallel sample processing capabilities of these SMF devices together with suitable on or off-chip detection capabilities, suggest numerous applications and utility in conducting automated multiplexed assays, a capability much sought after in the high throughput diagnostic and screening assays.

II. FABRICATION

The microfluidic system or device in certain aspects of the invention may comprise a substrate material (silicon, glass, etc) housing a pair of coplanar electrodes. In a particular embodiment, the coplanar electrodes may assume a tapered geometry. In a further embodiment, the electrodes may be passivated by a thin dielectric layer and furthermore a thin layer of hydrophobic polymeric materials (e.g., Teflon®) applied to render the surface hydrophobic.

DEP based SMF devices may be fabricated in cleanroom environments using the conventional microfabrication techniques. These devices may comprise an array of microelectrode structures in aluminum or indium tin oxide (ITO), patterned on an insulated substrate such as a glass or silicon using standard photolithography and wet chemical etching procedures. These microelectrode structures, when suitably energized, serve as fluidic tracks or an open microchannel over which aqueous samples, in the form of sessile microscopic sized droplets can be conveyed (to an on-chip reaction site), mixed (with other sessile reagent droplets) or partitioned into smaller droplets (from a larger parent aliquot).

For facilitating actuation of aqueous and conductive samples, the metal electrodes may have electrical isolation from the fluidic sample, achieved by depositing a thin layer of dielectric material (e.g. silicon nitride, silicon dioxide, PDMS or commonly used photo-resists such as SU-8), which otherwise may lead to sample electrolysis during actuation. The top surface of the SMF device may also be rendered hydrophobic by spin-coating a thin layer of hydrophobic polymeric materials (e.g., Teflon®). A hydrophobic surface imparts a high contact angle to sample droplets and minimizes sample adhesion, found to be important for sample actuations.

In DEP based surface microfluidic systems, sample droplets may be manipulated in air, in an oil bath, or under an oil cover. Unless desired, sample droplets may be dispensed and actuated in an oil bath or under an oil cover in order to prevent evaporative losses and minimize sample contamination. In a particular embodiment, each sessile micro-droplet is
individually encapsulated in a separate oil covering, formed through the DEP actuation of emulsion jets.

FIG. 1 schematically illustrates a single and double electrode layer L-DEP SMF. In certain embodiments, a surface microfluidic system comprising a single metal layer may be used. Such a system may comprise a substrate material such as glass or silicon, a layer of electrodes such as metal electrodes like aluminum or indium tin oxide (ITO), wherein the electrodes may be patterned on the substrate, for example, by standard photolithography and wet chemical etching procedures. The system may further comprise a dielectric layer deposited on the electrodes and furthermore a hydrophobic layer to render the surface hydrophobic.

Large-scale integration of co-planar electrodes on a substrate is usually constrained by the ability to address the innermost (or central) electrodes in an array. Placement of contact pads in the center of an array for facilitating electrical connections (inaccessible otherwise) is least desirable as it adversely affects the quality of surface coatings and is also often limited by available space. To overcome electrode density restriction, the fabrication of electrodes in two separate metal layers, separated by an insulating dielectric layer, may be implemented in certain embodiments. As illustrated by the two layer L-DEP SMF device in FIG. 1, a set of L-DEP electrodes may be fabricated (photolithography) in bottom and top metal layers and may be oriented in any desired configuration, such as a matrix like geometry which can be used for high-throughput combinatorial biochemical assays.

A Device Fabrication Procedure

In the example shown in FIG. 2, the process flow for fabrication procedure of a two layer L-DEP SMF device includes the following steps:

1) Fabrication may begin with a substrate 201 like silicon wafer, such as commercially available 4" silicon wafers (SQI, U.S.A). The silicon wafer may be further more insulated with a 5 μm thick SiO2 layer or 4° Borofloat® glass wafers. The substrate 201 may be cleaned with piranha solution (3 parts H2SO4:1 part H2O2) for 10-15 min and subsequently rinsed with DI water.

2) A thin layer of metal 202 (such as Aluminum (Al) or ITO, nearly 0.2 μm thick), may be sputter-deposited onto the substrate 201 (Kurt J. Lesker Magnetron sputtering system) for fabricating microelectrode structures.

3) The wafer 201 may be spin-coated (Sollice Spinner, CA, USA) with a HPR 504 203, a commonly used positive photoresist in photolithography. The wafer 201 may be subsequently soft baked at 110°C. (90 sec for Si wafers and 30 min for glass wafers).

4) UV exposure (2.5-3.0 seconds, 356 nm) of the spin-coated substrate may be performed in a mask alignment system (ABM Inc., CA, USA) with selectively distributed photomask 204. UV exposure causes a depolymerization reaction in positive photoresist 203 in the absence of photomask 204. Exposed areas can now be selectively developed.

5) After UV exposure the photoresist may be developed in developer 354 (8-10 sec). The wafer 201 may be immediately rinsed with DI water to prevent the resist from over developing.

6) The pattern obtained in the photoresist layer 203 may be then transferred to the metal layer 202, by wet-chemical etching (Al etchant for Aluminum and 50% by V HCl-DI water solution for ITO.

7) The remaining photoresist may be washed off from the wafer, using acetone.

8) The Al microelectrodes may be electrically passivated by depositing a 0.5-0.7 μm thick layer of dielectric 205 such as silicon nitride (Si3N4) using plasma enhanced chemical vapor deposition (PECVD) technique (Orion III PECVD System, Trion Technologies, U.S.A).

9) The wafer may be once again be spin-coated with HPR 504 as described in step 3.

10) The photoresist may be exposed and developed, same as steps 4 and 5.

11) The silicon nitride layer may be selectively etched from certain areas of the chip to expose the underlying bonding pads, for making electrical connections. For this, reactive ion etching (RIE) of the nitride layer may be carried out in an RIE chamber (Phantom III RIE System, Trion Technologies, U.S.A).

12) Finally, the top surface of the microfluidic chip may be made hydrophobic by spin coating a thin layer (~0.1 μm) of Teflon® AF (DuPont Inc., USA).

B. Fabrication Procedure for Polymer Cartridge for Device Packaging

In certain embodiments, there may be provided methods for packaging the surface microfluidic devices or systems described herein. Polymer cartridges may be fabricated for such packaging and the fabrication procedure may involve two major steps: (1) Fabrication of a silicon master using Deep Reactive Ion Etching (DRIE) technique, and (2) Stamping microstructure in polymer substrates using the Hot Embossing procedure.

1. Fabrication of a Silicon Master

A silicon master may be fabricated using the Deep Reactive Ion Etching (DRIE) process. The wafer processing steps are detailed below. The master may be used further for fabricating polymer cartridges using the Hot Embossing procedure.

a. The design (or layout) for polymer cartridges may be drawn in L-EDIT v8.0 (MEMS Pro, MEMS CAP, CA, USA) and transferred to a sodalime glass mask using a pattern generator (DWL 200, Heidelberg Instruments, CA, USA).

b. Fabrication begins with commercially available 1 mm thick 4° silicon wafers (SQI, U.S.A), which serves as the substrate. The substrate may be cleaned with piranha solution (3 parts H2SO4:1 part H2O2) for 10-15 min and subsequently rinsed with DI water.

c. A thin layer of aluminum (Al, nearly 0.2 μm thick), may be sputter deposited onto the substrate (Kurt J. Lesker sputtering system). The Al may serve as a hard mask for the DRIE step to facilitate selective etching of silicon wafer.

d. The aluminum layer may be patterned using standard photolithographic procedure. The wafer may be spin-coated (Solice Spinner, CA, USA) with AZ 4620, a commonly used positive photoresist in micro-fabrication procedures. The wafer may be subsequently soft baked at 110°C and left for hydration at room temperature for 2 hours. UV exposure (12 seconds, 356 nm) of the spin-coated substrate may be performed in a mask alignment system (ABM Inc., CA, USA). After UV exposure the photoresist may be developed in AZ400K (90 sec). The wafer may be then immediately rinsed with DI (de-ionized) water to prevent the resist from over
developing. The patterned obtained in the photoresist layer may be then transferred to the metal layer, by wet-chemical etching. The remaining photoresist may be washed off from the wafer, using acetone.

The substrate could be then ready for the DRIE step. In general, DRIE provides anisotropic etching of the Silicon and facilitates fabrication of microstructures with high-aspect ratio. The patterned Si wafer may be etched using DRIE facility at NanoFab. In the first step, nearly 350 microns of the silicon substrate may be etched. Thereafter, steps ‘c’ and ‘d’ may be repeated again with a different photomask, complementing the feature etched in the previous steps. A second DRIE step may be performed, and another 350 micron of Silicon may be selectively etched.

An example of the DRIE processed silicon master is shown in FIG. 3. Hot Embossing and Fabrication of Polymer Cartridges

Hot embossing is an economical and straightforward method for precision micromachining of polymer substrates. Hot embossing may be the stamping of a pattern into a polymer softened by raising the temperature of the polymer just above its glass transition temperature. The stamp used to define the pattern in the polymer may be made in a variety of ways including micromachining from silicon, LIGA, and machining using a CNC tool (for making large features). A wide variety of polymers have been successfully hot embossed with micron-scale (and below) size features, including polycarbonate and PMMA. This technique is used primarily for defining micro-channels and wells for fluidic devices. The benefits of this approach are the ability to take advantage of the wide range of properties of polymers, as well as the potential to economically mass produce parts with micron-scale features.

For example, the silicon masters (fabricated as described above) may be utilized for stamping microstructures in polymer substrates. The process may involve three major steps, which are (a) heating the polymer substrate to a temperature slightly higher (≈20°C) than the glass transition temperature, (b) bringing the polymer substrate and silicon master in contact and application of pressure for indentation, and (c) cooling the substrate below glass transition temperature and releasing the substrate and master. For example, Jenoptik HEXO2 may be used, which is an automated hot embosser, available at NanoFab. Hot embossing in two different polymers may be attempted, which are polyethylene-tetraphthalate glycol (PETG) and cyclic olefin copolymer (COC), commonly used materials for fabrication of microstructures. Commercially available polymer sheets of PETG and COC may be procured and cut in to 5”x5” square pieces and utilized as substrates without any further processing. The embossing process parameters corresponding to the above mentioned polymers are summarized in Table 1.

III. LIQUID DIELECTROPHORESIS (L-DEP)

Microfluidic sample manipulation methods on planar substrates utilizing electric fields are an attractive alternative to conventional closed channel microfluidic systems and open a whole new domain of surface microfluidics. Individual sessile microdroplets that are aliquots of sample and reagents become the new discrete fluid processing units in surface microfluidic systems, replacing the conventional closed channel microfluidic systems. A number of techniques for manipulating liquid droplets on planar substrates have been reported in the past, such as electrowetting on dielectric (EWOD), L-DEP, thermocapillary actuation, light-induced actuation or opto-electrowetting. Such techniques rely on altering the surface tension of fluids at a liquid-solid interface (except for L-DEP) through electric fields, chemical and thermal gradients or optical means. Sample actuation mediated by electric fields is relatively straightforward compared to other strategies listed above as the electric field strength, frequency and its spatial orientation can be readily configured by applying suitable voltage signals across metal electrodes, patterned photolithographically on an insulated substrate. Thus L-DEP and EWOD are popular methodologies for sample manipulation in surface microfluidic systems, where the droplets being manipulated by electric fields are housed on top of a pair of co-planar metal electrodes, patterned on an insulated substrate, or alternatively, are sandwiched between the two electrodes, respectively.

In certain aspects of the invention, dielectrophoresis may be employed for actuation and precision dispensing of variable volume droplets or emulsion droplets. For example, a tapered coplanar electrode structure may be used for such purpose by leveraging L-DEP.

A. Physical Phenomenon and a Brief Historical Perspective

Liquid Dielectrophoresis (L-DEP) is an electromechanical phenomenon, describing the motion of polar liquids induced by spatially non-uniform electric fields (Pohl 1978). Polarizable liquids when subjected to a non-uniform electric field are actuated, conveyed and collect in regions of high electric field intensity. The phenomenon was first investigated by Pellat in 1894, wherein he demonstrated the effect of the ponderomotive DEP forces on dielectric liquids (insulating oil). Pellat’s experimental set-up is comprised of a pair of parallel electrodes plates separated by a gap s, partially immersed in a reservoir of dielectric liquid of density ρ and permittivity ε. Upon the application of a voltage V across the electrodes, the dielectric liquid between the electrodes, due to non-uniform field effects, rises vertically upwards (covering regions of higher electric field) and attains a new equilibrium height h, given by

\[ h = \frac{V^2 s^2 \rho}{2 \varepsilon_0} \]  

where, \( F = 9.81 \text{ m/s}^2 \) is the acceleration due to gravity and \( \varepsilon_0 \) is the permittivity of free space. In 1971 Melcher and co-workers developed a dielectric siphon, shown in FIG. 2(b), which comprised of an upper (filled with dielectric liquid) and lower (initially empty) reservoir, connected through a pair of electrodes (s=3 mm). The working principle of the dielectric siphon is derived from the L-DEP phenomenon, wherein electric fields were utilized for initiating liquid flow between the two reservoirs. When a sufficiently large voltage (20-30 kV @ 400 Hz) is applied across the electrodes, the dielectric liquid (from the upper reservoir) can be raised to a

<table>
<thead>
<tr>
<th>Polymer Substrate</th>
<th>Glass transition temp.</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>Pressure (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PETG</td>
<td>80°C</td>
<td>110°C</td>
<td>20 min</td>
<td>3.4 MPa</td>
</tr>
<tr>
<td>COC</td>
<td>65°C</td>
<td>80°C</td>
<td>15 min</td>
<td>3.4 MPa</td>
</tr>
</tbody>
</table>

TABLE 1

Hot embossing parameters for polymer substrates.
height \( h_0 \), at which point the hydrostatic pressure difference between the two reservoirs establishes and sustains the liquid flow between them.

[0102] However, practically feasible applications of L-DEP necessitate that the dominating effect of macro-level forces such as gravity and viscosity be subdued, and actuation voltages reduced to more nominal values. This may be achieved by implementing L-DEP at a microscale. For example, the system of the present invention may comprise one or more of the following features: (1) implementing L-DEP on a planar surface, (2) fabrication of planar micro-electrode structure by utilizing standard microfabrication techniques, on an insulated substrate (e.g., glass) and, (3) deposition of thin insulating dielectric layers on top of electrodes, which facilitated the actuation of aqueous and conductive media.

[0103] B. L-DEP and Droplet Dispensing

[0104] Growing interests in microfluidics during the last decade has opened new avenues for leveraging L-DEP as a sample manipulation methodology for microfluidic applications. One of the important application the L-DEP phenomenon in this domain has been the development of a L-DEP based droplet dispensing tool for the automated dispensing of multiple nano to pico-liter volume droplets from a given large parent sample droplet, as detailed in the following section.

[0105] FIG. 4a schematically illustrates the microelectrode arrangement of an exemplary L-DEP based surface microfluidic system. This example shows an embodiment for equi-volume droplet dispensing scheme. A surface microfluidic system may comprise a pair of coplanar electrodes of width \( w \), separated by a gap \( g \). The system may comprise an array of semi-circular bumps (radius \( R \)), which function as droplet collection sites or do not comprise bumps.

[0106] For example, a "parent sample droplet" (e.g., 1-2 \( \mu \)L) may be deposited at one end of the structure, as shown in FIGS. 4a-4b). The droplet may be placed by manually pipetting or automatic deposition. A sufficiently large AC voltage (for example, \( 300-450 \) V \( V_{\text{peak}} @ 100 \) kHz) may be applied across the L-DEP electrodes, and therefore DEP forces may overcome sample surface tension and viscosity to form and actuate a liquid jet from the parent droplet. The liquid jet is rapidly conveyed along the electrodes, covering regions of higher electric field strength, as explained previously. Once the jet has covered the entire length of the electrodes, its motion seizes as the polarized fluid establishes a new hydrostatic equilibrium with the externally applied electric field. Subsequently, upon removal of the applied voltage, the liquid jet instantaneously (within 1-2 ms) disintegrates into multiple, equal volume 'daughter' droplets, which are collected at semi-circular bumps. FIG. 4b(iv), illustrates an array of 19 identical volume droplets formed over L-DEP structure of dimensions \( w=15 \) \( \mu \)m and \( g=15 \) \( \mu \)m (and length=6 mm).

[0107] The break-up of the liquid jet is governed and explained by the capillary instabilities (induced by surface forces at solid-liquid and liquid-medium interface) that develop rapidly along the jet once the stabilizing influence of the electric field is lost. This effect, also referred to as Rayleigh's instability criteria is further illustrated in FIG. 5, which shows the development of instabilities through necking of the liquid jet at points of maximum hydrodynamic instability upon removal of externally applied AC voltage. According to Rayleigh's instability criteria, for a liquid rivulet of radius \( R = (w+g/2) \), the hydrodynamic instabilities are maximum, and located periodically at distances governed by the Rayleigh wavelength \( \lambda \), where

\[
\lambda = 2\pi R = \frac{9}{2} g \frac{w + g}{2}
\]

[0108] Furthermore, the semi-circular bumps patterned along the electrodes function more than just passive droplet collection sites. For example, after the removal of AC voltage, the bumps could provide a spatially harmonic (since they may be patterned at regular intervals at a spacing of \( \lambda \), given by equation 2) initial condition that governs the trend of ensuring instability in the liquid jet and thereby ensure a uniform break-up of the jet. The break-up of the liquid jet on utilizing electrode structures devoid of such bumps has been studied and found to yield non-uniform droplet placement, and furthermore lead to significant volumetric variations in the daughter droplets thus dispensed.

[0109] C. Characterization of Volume of Droplets

[0110] Accurate knowledge of droplet volumes (formed via L-DEP actuation) is critical in many applications, especially those involving quantitative analysis of biochemical assays. Due to the extremely small size of the droplets and associated low volumes, droplet volumes may be either estimated theoretically or measured analytically.

[0111] In one embodiment, droplet volumes may be theoretically estimated. In theoretical estimations volume of each droplet is essentially equal to the liquid inventory held between two consecutive points of jet break-up (illustrated in FIGS. 5a-d) and depends on the profile of the liquid jet. Furthermore, theoretical estimation of droplet volumes is based on several assumptions pertaining to the profile of the liquid jet, which is governed by factors such as L-DEP electrode dimensions, sample surface tension, sample conductivity, surface hydrophobicity, strength of electric field etc. However, many of these factors are unaccounted for in the assumptions, thereby leading to inaccuracies in droplet volume calculations.

[0112] In other embodiments, droplet volumes can also be determined analytically once they have been dispensed. The analytical methodology involves visual inspection of each of the droplets individually (from top, facilitated by a reflected microscope system). If the droplet contact angle is greater than 90\(^\circ\), in the top-view, each droplet appears as a concentric ring, as shown in FIG. 6. The inner and outer radii \( r_i \) and \( r_o \) for each droplet is measured through any image analysis software, and are further used for calculating droplet volumes by using equation 3:

\[
V = \int_{\theta=0}^{\pi} \int_{r_{\text{in}}}^{r_{\text{out}}} r^2 \sin \theta \, dr \, d\theta
\]

\[
= \frac{2}{3} \pi \left( R_i^3 - R_o^3 \right)
\]

(3)

where, \( \sin \theta = \frac{r_i}{R_o} \).

[0113] The analytical methodology, although laborious, is more accurate than any other technique presented so far, however it can only be used when the droplet's contact angle is greater than 90\(^\circ\). This is often the case in L-DEP actuations where the surface of the SMF device is made hydrophobic and sessile micro-droplets are nearly spherical in shape.

IV. ACTUATION AND PRECISION DISPENSING OF VARIABLE VOLUME DROPLETS

[0114] In certain embodiments, there may be provided a liquid dielectrophoresis L-DEP based droplet dispensing...
scheme, which utilizes a pair of tapered co-planar electrodes to automatically and simultaneously dispense an array of daughter droplets of varying volume. The dispersed droplets may be furthermore precisely positioned at specific locations on the chip. The characteristic features of the tapered electrode scheme is examined in detail both from a theoretical and experimental view point and compared with the actuation and droplet dispensing feature of the uniform electrode arrangement. There may be further provided methods and systems for the precision dispensing of variable volume droplets using the tapered electrodes, aided by the judicious use of bumps and pinch arrangements on the actuation electrodes.

Miniaturized droplet based surface microfluidic (SMF) devices offer simple, cost effective and reliable means of handling very small fluidic volumes of samples and reagents in the form of discrete droplets for various types of chemical and biochemical assays. Droplet-based chemistry permits reagents to be quantized in discrete packets and mixed in strictly stoichiometric ratios. Furthermore, droplet based microfluidic systems overcome the limitations of diffusion based, laminar mixing of chemicals and biosamples in conventional closed channel microfluidic systems due to the inherent low Reynolds number and perfectly laminar flow profiles. Droplet based devices, in addition, offer greater versatility than the conventional microchannel devices since individual droplet volumes can be precisely controlled when dispersed and further manipulated in a rapid fashion. For example droplet based microarrays may be used for high-throughput assays. However, to achieve this goal, it would be beneficial to achieve simultaneous and automated dispensing of a multitude of samples/reagents in a rapid fashion.

Controlled volume dispensing and transport in these devices is achieved in variety of ways utilizing either active or passive schemes. The active schemes primarily utilize electrical forces at microscales to control individual droplet motion. Examples of some such active schemes include electrocapillarity, electro-osmosis, electroconvection and electro-wetting, electrostatics and liquid dielectrophoresis (L-DEP). The passive approaches essentially leverage externally generated flows that are tailored locally by the microchannel geometry or by surface wetting patterns, to manipulate micro scale droplets. The passive schemes, although highly efficient in droplet generation, are far less effective in precision controlling of the droplet volumes as well as more static and less suitable for applications such as large scale integration (LSI) and high throughput screening (HTS) for which elevated droplet manipulation speeds are important.

Droplet based microfluidic systems, in recent years have demonstrated numerous advantages and exciting potential for lab-on-a-chip applications. In order to fully realize the potential benefits of this technology, precision dispensing and manipulation of droplets of known volume and sample concentration in a rapid and controlled manner would be important. So far L-DEP actuations have been used to create and disperse an array of equi-volume droplets. As described above, the formation of equi-volume droplets can be attributed to the uniform profile of liquid jet (i.e. its physical dimensions) governed primarily by the L-DEP electrode dimensions characterized by width (w) and gap (g) that are kept constant throughout the electrode length. This restriction to equi-volume droplets as well as accompanying restriction in size and spacing of the dispersed droplets by Rayleigh’s instability could be overcome by the use of a tapered electrode structure by leveraging the phenomena of liquid dielectrophoresis (L-DEP). The use of a tapered electrode structure may provide the rapid and controlled microactuation of aqueous samples and subsequent dispensing of variable volume droplets in nanoliter to picoliter regime.

The transient behavior of the tapered liquid jet departs significantly from that of a uniform liquid jet case and is not adequately explained in terms of a simplified lumped capacitance model as in the case of the uniform jet, during the L-DEP actuation. A more generalized numerical model is developed for the tapered actuation scheme to account for the experimental observations.

It has furthermore been demonstrated that the density of the dispersed droplets can be proactively controlled by the judicious placement of electrode bumps and pinches in the electrode structure thus overcoming the limitations imposed by Rayleigh’s instability criterion. Such droplet dispensing schemes in certain aspects of the present invention are superior to existing L-DEP based dispensing schemes which are restricted in size and spacing of the dispersed droplets by Rayleigh’s instability criteria and furthermore mostly restricted to equi-volume droplets.

A Theory

Dielectrophoresis (DEP) is a term coined by H. A. Pohl to describe an electrokinetic phenomenon arising from the interaction of polarizable materials (such as dielectric fluids) when subjected to a spatially non-uniform electric field. Dielectric fluids, under the influence of such non uniform fields, tend to preferentially redistribute over regions of high electric field. This electrokinetic field effect in practice is opposed by the fluidic surface tension (F_s) and viscous damping force (F_d). At sufficiently high electric field strengths, this electrically induced DEP force can overcome the opposing fluidic forces, resulting in the microactuation of liquids. In practice the DEP actuation of liquids is facilitated by applying an AC voltage across a pair of coplanar metal electrodes of width w and gap g, coated with a thin insulating layer (e.g., SiO_2 or Si_3N_4). In such a case, when a parent sample droplet is placed at one end of the electrode structure and electrodes energized by an AC voltage (V), a liquid is actuated and rapidly propagates over the insulated electrode pair, covering the entire length electrode within few milliseconds. A uniform width-gap electrode arrangement results in formation of a uniform and nearly hemi-cylindrical jet. Dynamics of such a uniform liquid jet actuation has been analyzed in terms of a lumped parameter model.

In this model, for a uniform electrode pair (w-g) and assuming that the liquid jet is nearly uniform and symmetrically covering the electrode structure, the force-momentum equation for transient behavior of liquid jet is given as below:

$$\frac{\pi \rho R^2}{2} \frac{d \gamma}{dt} \left[ \frac{d \gamma}{dt} \right] = F_{\text{DEP}} - F_s - F_d$$

where $z$ is the actuated jet length. In this equation, the DEP force ($F_{\text{DEP}}$), modeled as a lumped expression for the uniform electrode case is

$$F_{\text{DEP}} = \frac{\pi \rho R^2}{2} \frac{d \gamma}{dt} \left[ \frac{d \gamma}{dt} \right] = \frac{(K_m - 1) \epsilon_0^2 \omega_0 V^2}{2(\epsilon_0 + 3\epsilon_0) \epsilon_0 + 2(\epsilon_0)}.$$
Here, \( c_d \) is capacitance per unit length of the dielectric layer (SiN), \( c_{hyp} \) is capacitance per unit length of a hypothetical hemi-cylindrical jet of radius \( R = w + g/2 \) and expressed as:

\[
c_d = \frac{K_{hyp} w}{d}, \quad c_{hyp} = \varepsilon_0 \frac{K(1-k^2)}{2K(k^2)} \quad \text{and} \quad k = \frac{g}{2R}.
\]

[0124] The term \( K(k^2) \) is the complete elliptical integral of the first kind \( K \), and \( K_e \) are the relative dielectric constants for the dielectric layer (SiN) and the aqueous sample respectively.

[0125] Furthermore, the surface tension force is written as:

\[
F_n = nRT
\]

[0126] Since the liquid jet is assumed to be hemi-cylindrical with a semi-circular cross-section, the viscous force is represented as follows:

\[
F_\mu = 2RT\tau_\mu \quad \text{where} \quad \tau_\mu = \frac{\partial \tau}{\partial x} \frac{D_\mu}{R} \frac{dz}{dt}.
\]

[0127] \( \tau_\mu \) is the shear stress due to viscosity and is correlated to average jet velocity by the viscosity coefficient \( \mu \). Using the force expressions in Eqn. 7, the governing differential equation for this case of uniform electrode-gap dimensions can be easily linearized as:

\[
\frac{d^2 z^2}{dt^2} + \frac{1}{T_\mu} \frac{dz^2}{dt} = \frac{F_{DEP} - F_\mu}{\mu R^3/4}.
\]

\[
T_\mu = \frac{\mu R^3}{4D_t}
\]

is a characteristic time that delineates the boundary between viscous and inertial dominant behavior. Solving with initial conditions, \( z(t=0)=0 \) yields the following transient response of the DEP actuated liquid jet,

\[
z(t) = \sqrt{\frac{T_\mu(F_{DEP} - F_\mu)}{\mu R^3/4}} \left(1 + \frac{1}{T_\mu} \left(\frac{v_t}{\mu R^3/4} - 1\right)\right)
\]

[0128] For the case where \( t \gg T_\mu \), the above general expression simplifies to:

\[
z(t) = \frac{2}{R} \sqrt{\frac{T_\mu(F_{DEP} - F_\mu)}{\mu \pi}} \left(1 + \frac{\mu R^3/4}{4}\right) t.
\]

[0129] This \( t^{0.5} \) behavior of liquid jets for a uniform electrode structures has been experimentally verified for both homogenous and emulsion sample jets with varying fluidic and dielectric properties.

[0130] In certain embodiments of the invention, a more rigorous numerical and analytic approach to formulate the behavior of a non-uniform tapered jet has been provided, obtained by L-DEP actuation over a continuous tapered electrode structure, shown in FIGS. 7a-7b. In a non-uniform tapered electrode design (as shown in FIGS. 7a-7b), the width and gap of the electrode pair is tapered while maintaining, \( w = g \) along the entire electrode length. In order to practically implement such a tapered electrode system with experimentally significant electrode length, one has to use low taper angles (-1°). For this continuous tapered electrode scheme, the DEP and surface tension forces cannot be represented by constant lumped expressions. These force expressions have been developed and the resulting non-linear differential equation (NLDE) has been solved in order to describe the dynamics of a liquid jet, actuated over a tapered electrode structure.

[0131] The force-momentum equation governing a tapered jet actuation is as follows:

\[
\frac{dP}{dt} = F_{DEP} - F_\mu.
\]

[0132] In this equation, \( dP/dt \) is the rate of momentum change, which is equated to the net force acting on the jet at any instant \( t \), during the jet actuation. The time and shape dependent momentum term is derived and expressed in terms of indefinite integration as follows:

\[
\rho(z, t) = \frac{\mu R^3}{2} \left(\int_0^t \left(1 - \frac{z^2}{L^2}\right) dz\right) \frac{dz}{dt}
\]

where, \( R(z) = R_0 \left(1 - \frac{z}{L_0}\right) \)

is the radius of the tapered jet at a position \( z \) and time \( t \), along the electrode with \( R_0 \) being the jet radius at the wider electrode end.

\[
L_0 = \frac{w_1 L}{w_1 - w_t}
\]

is the hypothetical length at which the tapered electrode arrangement will converge to a point (i.e. \( w = g = 0 \)) (see FIGS. 7a-7b) while \( w_t \) and \( w_t \) are electrode widths at the wide and narrow ends respectively. In the entire analysis, this hypothetical length is used to normalize the actuated jet length as;

\[
z_0 = \frac{z}{L_0}
\]

The analysis yields the following expression for rate of change of momentum,

\[
\frac{dP}{dt} = \alpha(t) \frac{d^2 z}{dt^2} + \alpha_1(t) \left(\frac{dz^2}{dt}\right)
\]
is a polynomial expression that depends on \( z \) and hence on \( t \), the DEP force term is now formulated using the concept of virtual work as:

\[
F_{\text{DEP}} = \frac{V^2}{2} \frac{dC(z)}{dz}.
\]

(14)

\( C(z) \) is the total system capacitance and can be written as:

\[
C(z) = C_{\text{drop}} + C_{\text{air}} + C_{\text{cap}}.
\]

(15)

Here \( C_{\text{drop}} \) is the capacitance term due to the parent droplet, \( C_{\text{air}} \) corresponds to the capacitance of the system comprising part of electrode which is covered by the fluidic jet and \( C_{\text{cap}} \) is the capacitance of the uncovered electrode segment. Because of the tapered electrode arrangement, \( C_{\text{air}} \) and \( C_{\text{cap}} \) are again expressed as indefinite integral functions of \( z \) (or, \( R(z) \)). The formulae for these capacitances and the formulated DEP force are as follows:

\[
C_{\text{air}} = \int_0^Z \frac{R(z)}{c_1 + c_2 R(z)} \, dz
\]

\[
C_{\text{cap}} = \int_0^Z \frac{R(z)}{c_1 + c_2^2 R(z)} \, dz - \int_0^Z \frac{R(z)}{c_1 + c_2 R(z)} \, dz
\]

\[
F_{\text{DEP}} = \frac{V^2}{2} \left( C(z) - 1 \right) \frac{dR(z)}{dz}.
\]

(16)

where \( T_{\text{dep}} = \frac{\mu R^2}{4\pi d} \).

\[
F(z) = \frac{2}{\mu \pi d R^2} \left( \frac{V^2 (1 - z)}{c_1 + c_2 R(z)} (c_1 + c_2 R(z)) + \frac{\pi R^2}{c_1 + c_2 R(z)} \right).
\]

(17)

(18)

In order to carry out the numerical analysis the coefficient terms may be regrouped and a more compact notation for the NLDE may be used, which is given as follows:

\[
z_0 (1 - \frac{z^2}{2}) \left( \frac{d^2 z_0}{dt^2} + \frac{d z_0}{dt} \frac{d^2 z_0}{dt} + \frac{z_0}{T_{\text{dep}}} \frac{d z_0}{dt} \right) = F(z)
\]

(19)

where \( T_{\text{dep}} = \frac{\mu R^2}{4\pi d} \).

The variation of the three forces with actuated jet length, for the continuous tapered jet compared to the uniform jet, as shown in FIG. 8, provides valuable insight about the subtle differences in their dynamics. Both \( F_{\text{DEP}} \) and \( F_{\mu} \) are forces with constant magnitude for the case of the uniform jet actuation whereas \( F_{\mu} \) is the damping force that initially increases with increased projection area of the moving jet and saturates as the jet propagates. In case of tapered jet actuation, both \( F_{\text{DEP}} \) and \( F_{\mu} \) decrease in magnitude along with the viscous force which indicates that there is a limit on actuation lengths for tapered jets when they are actuated from the wider side of the electrode structure. This comparison also suggests that the dynamics of a tapered jet is anticipated to be significantly different than that of a uniform jet.

In order to carry out the numerical analysis the coefficient terms may be regrouped and a more compact notation for the NLDE may be used, which is given as follows:

\[
z_0 (1 - \frac{z^2}{2}) \left( \frac{d^2 z_0}{dt^2} + \frac{d z_0}{dt} \frac{d^2 z_0}{dt} + \frac{z_0}{T_{\text{dep}}} \frac{d z_0}{dt} \right) = F(z)
\]

(19)

\[
z_0 (1 - z_0) (1 - \frac{z_0}{2}) \left( \frac{d^2 z_0}{dt^2} + \frac{d z_0}{dt} \frac{d^2 z_0}{dt} + \frac{z_0}{T_{\text{dep}}} \frac{d z_0}{dt} \right) = F(z)
\]

(20)

<table>
<thead>
<tr>
<th>Variables and Coefficients</th>
</tr>
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<td>( a = \frac{V^2 (1 - z)}{c_1 + c_2 R(z)} (c_1 + c_2 R(z)) + \frac{\pi R^2}{c_1 + c_2 R(z)} )</td>
</tr>
<tr>
<td>( b = \frac{3K_{\text{cap}}}{K_{\text{cap}} R_0^2} )</td>
</tr>
<tr>
<td>( c = \frac{3K_{\text{cap}}}{K_{\text{cap}} R_0^2} )</td>
</tr>
<tr>
<td>( d = \frac{3}{\mu \pi d R_0^2} )</td>
</tr>
<tr>
<td>( e = \frac{3}{\mu \pi d R_0^2} )</td>
</tr>
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</table>

(21)

Behavior of a tapered jet for the case of small \( z \) (or small \( t \)): If analyzing the initial phase of a tapered jet actuation, under the assumption of a low taper angle, it can be approximated that:

\[
r(z) = R_0 \left( 1 - \frac{z}{L_a} \right) \approx R_0, \text{ as } z \to L_a.
\]

(22)
Thus, the R.H.S term in Eqn. 18 reduces to a constant value:

$$F(b) = \left( \frac{V^2(K_w - 1)C_{air}}{2(\varepsilon + 2K_wC_{air}/(\varepsilon C_{air}))} - \pi R_b^2 \right) \frac{2}{\rho \pi R_b^2}$$  \hspace{1cm} (21)

Whereas the L.H.S of the Eqn. 18 simplifies to:

$$\frac{d^2 z}{dt^2} - \left( \frac{dz}{dt} \right)^2 \frac{dz}{dt}$$

(22)

[0140] With further rearrangement, this equation is identical to the governing DE of a uniform electrode scheme with $R_e$ as the uniform jet radius. As a result of this analysis, it can be expected that the finger dynamics would show a $t^{-3/2}$ behavior for very small actuation lengths.

[0141] The NLDE was solved numerically using Wolfram Mathematica (version 7.0) and the resulting solution together with its comparison to the experimental observations is presented in Example 2.

[0142] B. Varying Electrode Structure

[0143] The surface microfluidic system may have a varying electrode structure to create variable droplet dispensing. For example, the electrode structure may be varied in dimensions of width (w) and gap (g) along the length of the electrode structure. Gap (g), as described herein, refers to the width of the gap between the electrodes that forms a flow path unless otherwise specified.

[0144] There may be provided non-limiting examples of various electrode structures suitable for creating variable-sized droplet dispensing during use as illustrated in FIGS. 9a-9f. For example, FIG. 9a illustrates Scheme 1 as a step tapered electrode (electrode length may be 4500 μm) with segments, e.g., having w=g=35 μm, 30 μm, 25 μm, 20 μm, 15 μm and 10 μm. FIG. 9b illustrates Scheme 2 as a continuous tapered electrode design (electrode length=4000 μm) with no bumps or pinches, e.g., tapered from w=g=40 μm to 10 μm; FIG. 9c illustrates Scheme 3 as a continuous tapered design dimensionally similar to FIG. 9b but with bumps placed at weighted λ (Rayleigh wavelength). FIG. 9d illustrates Scheme 4 as a continuous tapered design incorporating electro-mechanical pinches placed at a fixed separation (λ) and tapered, e.g., from w=g=40 μm to 10 μm. FIG. 9e illustrates Scheme 5 as a uniform L-DEP electrode (e.g., w=g=30 μm) with specifically positioned bumps and pinches to create variable separation droplet dispensing. FIG. 9f illustrates an example of an electromechanical pinch design.

[0145] Upon removal of the applied voltage, the liquid jet formed by the DEP forces may break up into multiple daughter droplets, which may be collected at the semicircular bumps. The break-up of the liquid jet into micro-droplets, as discussed and illustrated previously is a manifestation of an important and well-known physical phenomenon of capillary based hydrodynamic instability pertaining to cylindrical jets. Rayleigh’s criterion furthermore provides formulations to configure precise droplet formation sites by determining the spacing between semi-circular bumps. However, this poses an important limitation on the density of the droplet array, i.e. droplet can only be, or have been shown to form at regular intervals spaced by a distance λ (~3R).

In certain applications, such as those involving large scale integration (discussed in the following sections), it may be desired to have a more proactive control over liquid jet break-up and spacing between consecutive droplets. Furthermore, the semi-circular bumps placed along the L-DEP electrodes, which provide the static harmonic initial condition, governing the course of instability, by itself, is insufficient for such a task. This implies that arbitrary positioning of the bumps along L-DEP electrodes cannot provide precise control over the break-up of liquid-jet. For example, in L-DEP actuations on structures wherein the spacing between bumps was fixed at λ/2 and 2λ (λ=3R being the ideal value, equation 2), the jet break-up was highly non-uniform for bump spacing λ/2, while in the latter case, the jet break-up was observed to be imperfect (bump spacing 2λ) resulting in the formation of undesired smaller sized satellite droplets spaced at regular intervals of λ.

[0146] It has been discovered that such imperfections in the break-up of the liquid jet can be overcome by incorporating structural modifications in the form of ‘pinches’ along the L-DEP electrodes. A pinch 901 as illustrated in FIG. 9c (also shown in FIGS. 10b-10c), is an indentation in an electrode structure. The pinch structure may be incorporated in the L-DEP electrode at locations where the jet break-up is desired. In terms of functionality, a pinch essentially promotes capillary instability and in turn redefines the points of maximum hydrodynamic instability (for the liquid jet), compared to those arising in a homogenous semi-cylindrical liquid jets. The efficacy of pinch structures is demonstrated through the micrographs presented in FIGS. 10b-10d, which shows an array of droplets formed over two distinct L-DEP electrode structures. In FIG. 10b, an array of droplets is generated with periodicity 2λ (instead of λ) by controlling the break-up of the liquid jet through pinched structures, furthermore ensuring nearly perfect break-up of the jet without the formation of satellite droplets. In another embodiment, the L-DEP electrode shown in FIG. 10c may have a pair of continuously tapering electrodes (e.g., w and g from 40 μm to 15 μm), laden with pinch structures, which may be utilized for controlling droplet spacing. In other embodiments, there may also be provided tapering L-DEP electrodes (devoid of any pinch indentations) as shown in FIG. 10d, wherein the spacing between the bumps is a function of electrode dimensions w and g (represented by equation 2). However, in the continuously tapering structure, the spacing between the bumps and droplets is kept constant (~400 μm) throughout the structure, which does not conform to equation 2, which demonstrates that the break-up of the liquid jet can be proactively controlled through judicious structural modifications in the L-DEP electrodes.

[0147] In a varying electrode structure such as a tapered L-DEP structure, for example as shown in FIGS. 10c-10d, the electrode width and gap may be varied, either discretely or in a continuous fashion, along the electrode length. In such a scenario, the liquid jet emanated from the parent droplet and assumes the tapering profile of the L-DEP electrodes and thus provides a simplistic way of controlling liquid inventory held between two consecutive bumps. Subsequently, when the externally applied voltage is removed the tapering liquid jet breaks into droplets of different size and volume, as illustrated in FIG. 10d.

[0148] The tapering actuation scheme can serve as a valuable droplet dispensing tool in applications which involve the creation of concentration, mass or pH gradients in biochemical assays. For example, an array of different volume droplets may be formed by actuating a DNA-PicoGreen™ (PG) complex (prepared off-chip) on a tapering L-DEP structure. The DNA-PG complex produces a bright green fluorescence
emission ($\lambda_{em} = 520$ nm) when excited by light at suitable wavelengths ($\lambda_{ex} = 488$ nm). The fluorescence emissions from each droplet were individually detected and quantified (as photocurrent; $I_p$) using a photomultiplier tube (a block diagram of the experimental set-up is schematically illustrated in FIG. 6) and could be plotted, where the $I_p$ values provide a direct measure of the amount of DNA in each of the droplets. Furthermore, the linear relation between $I_p$ and droplet volume implies that the DNA concentration in each of droplets remains constant. In a similar way, a mass gradient of other bio-molecules (proteins, enzymes or cells) may be readily achieved, which can then be mixed (on-chip) with another set of buffer droplets (for example, using droplet actuation schemes described in section VI) to equalize (or normalize) droplet volumes (i.e. a smaller droplet may be mixed with a bigger one and vice-versa) and establish on-chip concentration gradients.

V. DISPENSING AND TRANSPORT OF EMULSIONS

[0149] By definition, an emulsion is a mixture of at least two immiscible liquid phases. There exist a variety of techniques for producing emulsions in the micro to nano-meter size ranges. For example, such emulsions may be formed by using dispersion or high-energy emulsification techniques requiring high-shear stirring, high-pressure homogenizers and ultrasonicators. Ultrafine emulsions may also be produced using Phase Inversion Temperature (PIT) method or alternatively using microchannel technology.

[0150] Liquid Dielectrophoresis (L-DEP) has been successfully leveraged at microscopic scales and shown to provide a controllable means of on-chip precision dispensing and manipulation of sub-nanoliter single emulsion droplets. Certain aspects of the invention provide methods and systems related to dynamics of DEP actuated emulsion jets. Furthermore, features and aspects of these emulsion jets, their break-up and formation of sub-nanoliter emulsion droplets may be described. Applications of the such methods and systems in dispensing encapsulated sub-nanoliter droplets is contemplated in various field including micro-TAS, on-chip handling and storage of intact viable cells and other biological samples for longer duration in controlled environment as well as solving the more general encapsulation issues in surface microfluidic devices. Scalability of these methods and systems is shown by producing controlled sample-oil single emulsion droplets (aqueous samples in oil) in range of 50-400 picoliters. Scaling laws, as it applies to liquid DEP, further suggests that even smaller droplets, approaching the femtoliter volume scales are achievable by judiciously scaling down the device geometry.

[0151] Surface microfluidic devices have been successfully implemented in producing lab-on-chip systems, capable of providing miniaturized biochemical assays and combinatorial chemistry applications on a portable, low cost and miniaturized technology footprint. Frequently such devices employ schemes that judiciously utilize dielectrophoresis and/or electrowetting phenomena at microscopic scales to manipulate nanoliter scale volumes of biochemical samples. For example, integrated DEP based droplet dispensing and transport functions may be used in performing on-chip analysis of fluidic biological/biochemical samples. In practice, such picoliter manipulations of aqueous samples are only usefully exploited if they are conducted on surfaces that are immersed in an oil bath. This although satisfactory in circumventing evaporation of the nanoliter sample/reagent droplets, introduces several other issues including sample contamination through the oil bath and perturbations of the oil bath resulting in the previously dispensed droplets being displaced from their specific on-chip locations. This can adversely impact the reliability and repeatability of subsequent manipulations of the droplets.

[0152] The capabilities and limitations of the prior technologies are summarized as follows:

[0153] a) None of these technologies are readily scaled to enable on-chip droplet level emulsion formation, a key component for on-chip sample preparation;  
[0154] b) Most of these techniques require bulk quantities of samples and cumbersome technologies that would make them quite expensive;  
[0155] c) The techniques employ fairly high concentrations of surfactants to stabilize and control the size range of produced emulsion droplets. These surfactants are not suitable for many biochemical applications since they tend to denature or even destroy the biochemical sample (e.g. proteins, antibodies and cells) present in the dispersed phase.

[0156] In view of the above mentioned drawbacks, certain aspects of the invention provide an alternative approach to dispense the sample droplets, in the form of an emulsion droplet. For example, in the emulsion droplet, the liquid sample may be individually encapsulated inside a slightly larger sized oil droplet, such as various viscosity silicon oil packets. Thus, if the reagent sample could be dispensed as nanoliter emulsion droplets with a protective oil cover, it would overcome many of the drawbacks of the existing experimental schemes. This droplet dispensing scheme not only solves the encapsulation problem, but it also opens immense opportunities for applicability in field of storing bio-samples in miniaturized quantities; samples such as gene pool, embryonic cells or even plant seeds.

[0157] A. Production of Emulsion Jets

[0158] The ideology behind leveraging DEP for precision emulsion dispensing is based on the simplicity and accuracy of L-DEP based droplet dispensing methodology. The presented schemes allow to extend the droplet dispensing mechanism to single and even multi-layered emulsion droplets.

[0159] FIG. 11 illustrates two possible ways that one can implement L-DEP to produce an aqueous-oil emulsion jet. Scheme 1 involves a parent droplet in form of a microliter liquid sample droplet covered with a comparable oil droplet. This water-oil parent droplet arrangement when subjected to L-DEP actuation ejects an emulsion jet and subsequent breakup of this emulsion jet, upon removal of the applied electric field generates multiple precisely single emulsion droplets of sample volumes as low as 30 picoliter at specific bump sites. The break-up of different aqueous solution (e.g., DI water-glycerol mixture)-in-oil emulsion jets may result in formation of arrays of single emulsion droplets.

[0160] In other aspects of the invention, Scheme 2 as shown in FIG. 11 may be used to actuate micro-liquid sample located at one end of the electrodes and another parent oil droplet placed at the opposite end of the electrode structure. In this scheme, the mechanism of formation of emulsion jet may be a two step process. In step 1 the homogenous aqueous jet forms and spreads over the electrode length and in step 2 the oil redistributes itself over the already formed homogenous jet, forming a water-in-oil emulsion jet. The electric
field may be now removed and the emulsion jet may break up, forming emulsion daughter droplets at specific bump sites.

[0161] In further embodiments, the concept of forming emulsion droplets includes multi-layered emulsions. To achieve this, Scheme 1 and Scheme 2 as illustrated in FIG. 11 may be combined in such a way that a double or higher order emulsion jet is formed. The break-up of such a multi-layered emulsion jet may result in formation of multi-layered emulsion droplets. Aqueous-in-oil-in-aqueous double emulsion jet and double emulsion droplets have been successfully formed by actuating a parent droplet in form of a microliter aqueous sample droplet covered with a comparable oil droplet from one side and a different aqueous sample droplet from the other side with a time delay. The time delay is to allow the formation of the aqueous solution-in-oil jet before actuating the second aqueous jet from the opposite end. The process results in the water jet overlapping with the already formed single emulsion jet and forming a double emulsion jet. Micrographs illustrating the dispersed single and double emulsion droplets are shown in FIGS. 12a-12f.

[0162] Application of this emulsion dispensing scheme is envisioned in various fields including micro-TAS, on-chip handling and storage of cells and various other biological samples for extended periods in controlled environment, in solving the encapsulation issues in surface microfluidic devices, forming laser fusion targets, cryogenics on a chip, targeted drug delivery mechanism.

[0163] B. Actuation of Emulsion Jets

[0164] Dielectrophoresis (DEP) is an electromechanical phenomenon that manifests itself as a direct consequence of the interaction of a non-uniform electric field with polarizable matter. This non-uniform electric field mediated interaction, in the case of polarizable liquids, acts on the polar molecules to impel them to region of higher field strength.

[0165] As illustrated in FIG. 13, at sufficiently high field strength, the DEP force induced by application of a voltage 1301 can overcome the liquid cohesive forces and actuate a liquid jet (i.e., a liquid rivulet) 1303 from a parent sessile drop 1302. The non-uniform electric field, important for such liquid DEP actuation, is facilitated by a pair of coplanar electrodes 1305 of width w and spacing (i.e., gap) g. On applying an AC voltage 1301 (e.g., -450 V_{RMS} at 1kHz) across the electrodes 1305, a liquid jet 1303 is actuated from the parent sample 1302, and travels rapidly along a flow path defined by a gap between electrodes 1305, covering regions of high electric field intensity. Once the rivulet 1303 has covered the full length of the electrodes 1305, its motion ceases, as the rivulet establishes a hydro-electrostatic equilibrium with the non-uniform electric field.

[0166] The applied AC voltage 1301 may be removed, as a result of which the stabilizing influence of the electric field may be lost and the liquid rivulet 1303 may instantaneously break up into discrete ‘daughter’ droplets influenced by the Rayleigh’s instability criteria. The Rayleigh’s theory may predict that the most unstable wavelength (\lambda) for a liquid rivulet to be 9.016\sqrt{\text{radius}} (R) of the liquid rivulet, where R-w^2/g and w and g are the width and gap between the electrodes, respectively. In certain embodiments, to enhance the formation of uniformly sized droplets and/or to increase capillary instability, semi-circular bumps 1304 of radius R_{cump} may be placed along the length of the electrodes 1305 at predetermined locations to serve as collection sites. For example, bumps 1304 may be placed at locations that are spaced by integer multiples of Rayleigh’s unstable wavelength (\lambda).

[0167] In certain aspects, a surface microfluidic system such as the L-DEP chip may comprise a pair of coplanar electrodes 1305 patterned on an insulating substrate 1308 (e.g., Si wafer with 5 µm thick SiO\textsubscript{2} passivation). These electrodes may be metal electrodes, such as aluminum (Al). The electrodes may serve as a track or an open channel over which a liquid rivulet 1303 is conveyed and subsequently dispensed into multiple droplets.

[0168] The electrodes 1305 may be insulated to prevent sample electrolysis and minimize conductive losses to liquid sample during actuation: for example, the electrodes 1305 may be coated with a thin insulating dielectric layer 1306 (e.g., SiO\textsubscript{2} or Si\textsubscript{3}N\textsubscript{4}).

[0169] The system may further comprise a surface coating 1307, which use any material that prevents electrolysis during actuation and/or imparting the contact angle (>90°) to the sample droplets placed on the substrate for reliable L-DEP actuation and daughter droplet formation. Also, surface treatments could be used to reduce wetting hysteresis. For example, Teflon® AF Resin or polytetrafluoroethylene (PTFE), SU-8® (a negative photoresist, widely used in microfabrication), polydimethylsiloxane (PDMS), or Parylene® (a variety of chemical vapor deposited poly(p-xylylene) polymers). For example, the entire system may be spin coated with a hydrophobic layer 1307 to render the surface hydrophobic. The hydrophobic coating 1307 prevents the parent and daughter droplets formed during stages of actuation from collapsing, for example, due to the high contact angle (120°) of water on Teflon®. It may furthermore help in the case of protein suspension samples to minimize sample loss due to surface adsorption.

[0170] Although designed for open channel use, nonetheless, a surface microfluidic device or system according to certain aspects of the invention may use packaging technology to reduce possible sample contamination and evaporation in certain aspects. A cover made for example from PDMS (polydimethylsiloxane) may be used. Heat dissipation may also be maximized to deal with Joule heating effects, especially in aqueous biological media containing significant ions. Heat dissipation may be achieved through supporting the electrodes on a metal base with an insulator between the electrodes and the metal base. The metal base, for example made of aluminum, assists in achieving heat dissipation.

[0171] Heat dissipation may also be achieved through coating the surface of the device with transformer oil or other relatively viscous oil, when aqueous media is used as the motive liquid. The water rivulet extends through the oil, underneath it, and the oil assists in heat dissipation from the liquid rivulet. In preferably aspects, the surface may not be coated or immersed in oil. To prevent evaporation, emulsion droplets may be applied instead of oil bath, therefore avoid the problems associated with oil bath or oil coating of the whole surface. The low-high voltage switches may be housed on the system. Care may also be taken to avoid bio-fouling problems.

[0172] To model the actuation and subsequent dynamics of the DEP actuated advancing liquid jet, a lumped parameter model may be used. In this model, application of AC voltage V induces a DEP force F_{DEP} that is sufficient in magnitude to overcome the surface tension (F_s) and viscous (F_v) forces, resulting in a thin liquid jet to protrudes from the parent
sessile droplet and be rapidly conveyed along the electrodes until it covers the entire electrode structure, thus establishing a new equilibrium.

Assuming the liquid jet is uniform, cylindrical in shape, and symmetrically covering the entire width and gap of electrode structure, the force-momentum equation for transient behavior of liquid jet is obtained,

\[ \frac{\partial p R^2}{\partial t} \left( \frac{dZ}{dt} \right) = \vec{F}_{\text{DEP}} - \vec{F}_g - \vec{F}_a \]

where \( Z \) is the length of liquid jet along the electrode length at any time \( t \); \( R \) represents the radius of semi-circular jet cross-section ( \( R = \sqrt{w^2 + g/2} \) ) and \( \rho \) is the mass density of the liquid. Under the assumption that the jet profile remains fixed during actuation (hemicylindrical), the total capacitance of the L-DEP actuation system at time \( t \) is given as

\[ C(Z) = \frac{Z}{\varepsilon_0 + \frac{1}{2} \varepsilon_d + \frac{1}{2} \varepsilon_{\text{air}} + \frac{1}{2} \varepsilon_{\text{cap}}} \]

where \( L \) is the electrode length, \( k_\text{w} \) is dielectric constant of aqueous sample, \( \varepsilon_d \) is capacitance per unit length of the dielectric layer (Si, N), \( \varepsilon_{\text{cap}} \) is the capacitance per unit length of a hypothetical hemi-cylindrical jet with \( R = \sqrt{w^2 + g/2} \). The capacitances per unit length used are given by

\[ c_d = \frac{k_\text{w} \varepsilon_0 w}{d} \quad \text{and} \quad c_{\text{cap}} = \varepsilon_0 \frac{K(1 - k^2)}{2k^2} \]

Now using the principal of virtual work, the DEP force can be expressed as

\[ F_{\text{DEP}} = \frac{\mathbf{V}^2}{2} \frac{dC}{dZ} \]

Substituting values of capacitance per unit length in \( C(Z) \) expression and then solving eqn. 25 provides the DEP force for a homogeneous liquid jet as represented in eqn. 26

\[ F_{\text{DEP}} = \frac{(k_\text{w} - 1) \varepsilon_0 k \varepsilon_0 V^2}{2(\varepsilon_d + 3\varepsilon_\text{air})(\varepsilon_d + 3\varepsilon_{\text{cap}})} \]

where, \( V \) is the applied actuation voltage. The capillary and viscous forces can be expressed as

\[ F_{\gamma} = \rho \gamma \]

and

\[ F_{\eta} = (2\rho Z) \tau_\eta = \rho \frac{\partial v}{\partial x} = \frac{D \rho dZ}{R d\tau} \]

Substituting eqn. 27 and 28 into eqn. 23 and rearranging yields

\[ \frac{d^2 Z}{dt^2} + \frac{1}{T_\mu} \frac{dZ}{dt} = \frac{F_{\text{DEP}} - F_g}{\rho R^2} \]

The solution yields transient behavior of a homogeneous liquid jet,

\[ Z(t) = \frac{T_\mu (F_{\text{DEP}} - F_g)}{\rho R^2} \left( 1 + T_\mu e^{-\frac{T_\mu}{\mu}} \right) \]

where, \( T_\mu = \frac{\rho R^2}{4D} \)

is a characteristic time, that delineates the boundary between viscous and inertia dominated behaviors.

For a microscopic sized liquid jet, the fluid viscosity predominates over inertial effects (\( \tau >> 1 \)), and thus in such a case a transient response, exhibiting a \( \tau^{0.5} \), time dependence is anticipated, where

\[ Z(t) = \frac{2}{R} \sqrt{\frac{T_\mu (F_{\text{DEP}} - F_g)}{\rho \gamma}} \]

The suitability of the above lumped model in predicting the dynamic behavior of emulsion jets (water, water-glycerol) solutions, in oil cover has been investigated. It has been observed that the dynamic behavior of DEP actuated aqueous jet in air and under oil bath exhibit very similar characteristic features.

In contrast, the transient response of water-oil emulsion jets, although exhibiting a similar \( \tau^{0.5} \) dependence, exhibits a slower response and furthermore varying the viscosity of covering oil media only marginally impacts the jet dynamics and associated velocities. The slower response of the emulsion jets compared to the homogenous jets may be accounted for and attributed to a reduction in the effective capacitance of the emulsion jet compared to the homogenous jet case.

To investigate this model for different emulsion jets, the total system capacitance is modified as:

\[ C(Z) = \frac{Z}{\varepsilon_0 + \frac{1}{2} \varepsilon_d + \frac{1}{2} \varepsilon_{\text{air}} + \frac{1}{2} \varepsilon_{\text{cap}} + C_{\text{eff}}} \]

where \( C_{\text{eff}} \) is the effective capacitance per unit length of the emulsion jet. Using eqn 25, the modified \( F_{\text{DEP}} \) can be written in terms of this effective jet capacitance (\( C_{\text{eff}} \); \( \varepsilon_d \), \( \varepsilon_{\text{air}} \) and \( V \) (actuation voltage). Further grouping the constants (\( \varepsilon_d \) and \( \varepsilon_{\text{cap}} \)) allows to observe the effect of dielectric properties of the emulsion jet on \( F_{\text{DEP}} \). The rearranged equation can be written as:
is the capacitance per unit length for the part of the electrodes not covered by the emulsion jet.

The term effective capacitance \( C_{e_{\text{eff}}} \) have been used essentially to point out the alteration in \( F_{\text{DEP}} \) resulting from capacitive coupling of emulsion liquid jet. For a homogeneous jet, effective capacitance \( C_{e_{\text{eff}}} \) is simply \( k_e C_{\text{em}} \) and eqn. 33 reduces to eqn. 26, whereas, for an emulsion jet it is controlled by the composition, shape and dielectric constants of the constituting jets (oil and aqueous solution). By suitably incorporating this effective capacitance value extracted from the experimental data plots for a particular emulsion jet, the changed dynamics of the emulsion sample jets could be modeled. A more detailed account of experimental findings and comparisons with the lumped model is presented in the Example 1.

**VI. INTEGRATED LIQUID DEP MICROCHIP TECHNOLOGY**

One of the key advantages of microfluidic devices (and for executing on-chip biochemical analysis) in general, is the ability to transport and mix different samples in a controlled, sequential and pre-determined fashion. While in closed channel microfluidic devices this is achieved by controlling liquid flow through micro-valves and pumps, in SMF devices or systems, individual micro-droplets may be manipulated (split, merged, mixed) by means of electric fields, surface acoustic waves or spatial thermal gradients. In a further embodiment to advance the sample manipulation capabilities of SMF systems, there may be provided the integration of an electrostatic droplet actuation methodology with the L-DEP droplet dispensing scheme. Such an integration may facilitate the transport and mixing of nano and pico-liter droplets (formed by L-DEP actuation), and may open new avenues for the development of D-DEP based high-throughput SMF devices.

In certain embodiments, the electrostatic droplet actuation scheme utilizes low frequency electric fields (configured through a pair of co-planar electrodes) for moving micro-droplets on planar substrates. A particular microfluidic device or system of the present invention may combine and integrate two elements: liquid dielelectrophoretic actuation-based microfluidic system and an electrostatic droplet-based transport subsystem, which may be based on the same phenomenon of DEP as droplet DEP (D-DEP), but with different electrode structure, e.g., a fishbone-like structure.

FIG. 14 presents a schematic example of an integrated L-DEP and droplet actuation (D-DEP) electrode structure. Two sets of L-DEP electrodes 1401-1402, arranged in parallel, may be utilized for creating nanoliter-sized aliquots from different parent sample/reagent droplets (‘A’ 1403 and ‘B’ 1404), simultaneously. These electrodes may be furtherly integrated with D-DEP electrodes 1405 at semi-circular bumps 1406 that act as a bridge between them and allow subsequent transportation and mixing of the two different sample daughter droplets 1407 and 1408. The width and gap of the L-DEP electrodes may be chosen to be 30 or 40 μm, while the D-DEP electrodes may be suitably scaled for actuating nanoliter-sized daughter droplets. The pitch 1411 of the fishbone D-DEP electrode structure 1405 may be decided by the size to be actuated over the electrode structure. As an electrode design consideration, the pitch (p) of the fishbone-shaped DEP electrodes 1405 could be considerably smaller compared to the droplet size (p=\%th of droplet diameter).

For example, L-DEP electrodes 1401 or 1402 may have width and/or gap of at least or about 20, 30, 40, 50, 60 μm or any intermediate number or ranges. The L-DEP electrodes may have bump sites with bump spaced at \( \lambda \) (=9R), wherein bump size (R) is at least or about 20, 30, 40, 50, 60 μm or any intermediate number or ranges. D-DEP electrodes 1405 may be fish-boned, and may have a width of at least or about 80, 90, 100, 110, 120, 130, 140, 150 μm or any intermediate number or ranges. The gap of the D-DEP electrodes may be much shorter, such as at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μm or any intermediate number or ranges. The L-DEP electrodes may have bump sites with bump spaced at \( \lambda \) (=9R), wherein bump size (R) is at least or about 20, 30, 40, 50, 60 μm or any intermediate number or ranges.

Frequency of the AC voltage used for D-DEP actuation has a strong dependence on the viscosity of the surrounding media (in addition to other sample properties such as droplet’s contact angle with the surface, sample viscosity, etc.). Higher viscosity media dampens the motion of the droplet. Although experiments in the initial study on D-DEP were done in air with 1 μL DI water sample droplets, manipulation of nanoliter-sized daughter droplets necessitates the use of an oil cover to prevent their rapid evaporation if homogenous droplets or samples are used. Thus, an example of AC voltage for droplet actuation may be 30 Hz for 5 cSt silicone oil. Whereas, D-DEP actuation in air could be achieved up to 110 Hz.

After the formation of daughter droplets by L-DEP actuation, manipulation and transport of the nanoliter daughter droplets may be achieved through the droplet actuation methodology like D-DEP. For example, a low-frequency of AC voltage 1410 (e.g., 90-120 V/μm at 30 Hz) may be applied across the D-DEP electrodes 1405, which transports daughter droplets along the D-DEP electrodes from semi-circular bumps 1406.

The fishbone shaped electrode architecture 1405 for droplet actuations may be suitably incorporated with L-DEP electrodes at droplet formation sites 1406 (i.e. the semi-circular bumps). Once an array of droplets is obtained, a low frequency AC voltage (10-30 Hz) may be selectively applied across the fishbone electrodes to transport daughter droplets to an on-chip location, where they can be mixed with other sample or reagent droplets. The integrated liquid DEP and droplet actuation scheme can be furthermore leveraged for the design and development of high-throughput surface microfluidic systems through large scale integration.

Similar to L-DEP, the D-DEP actuation scheme also utilizes a pair of coplanar metal electrodes, patterned on an insulated substrate. FIG. 14b shows a schematic representation of the planar D-DEP electrode geometry, which comprises an array of diagonal fishbone-shaped electrodes 1405. The electrodes 1405 may be electrically insulated from the sample by depositing thin films of dielectric materials, while the top surface may be made hydrophobic by spin-coating a thin layer of hydrophobic material such as amorphous Teflon®. Teflon® coatings impart a high initial contact angle
(-110°) to the sample droplet, which has been found to be critical for droplet motion. A sample droplet (~1-2 μL, DI water) may be manually dispensed at one end of the electrode structure. On application of a low-frequency AC voltage 1410 (for example in the range 10-110 Hz) across the electrodes 1405, the sample droplet undergoes periodic deformations (spreading and restoration of the spherical shape, induced by AC electric field) and the entire droplet may be transported along the electrodes towards the opposite end.

[0190] The DEP actuation principle and droplet motion can be explained as a combinatorial effect of DEP and electrowetting. The geometrically asymmetric fishbone-shaped electrodes may be used to configure spatially non-uniform electric fields, while the low-frequency electric fields may be simultaneously utilized for modulating the solid-liquid interfacial energy of the sample droplet, thereby increasing the sample’s surface wetting ability.

[0191] A Large Scale Integration (LSI) system may be provided that comprises small, elementary cells/blocks which are patterned with symmetrical repetition to generate higher order array schemes. In case of DEP based SMF devices, the building block is a simple integrated L-DEP and droplet actuation electrode. This simple integration is incorporated into a matrix design where more than one set of sample/reagents can be manipulated and mixed in parallel, automated fashion. Since the goal is to achieve a compact HTS system, overlap of electrical wiring and electrodes is anticipated in most instances. One way to avoid such overlap is to provide insulation between the overlapping wires/electrodes such that there are no unnecessary electrical intersections.

[0192] In certain aspects to achieve such LSI schemes, there may be provided a two layer fabrication process where microelectrode structures are fabricated in two metal layers, separated by a thin dielectric layer sandwich between them for electrical isolation, as described previously and shown in FIG. 1. Using a two layer fabrication scheme, a 2x2 matrix SMF system has been successfully designed and demonstrated, which could dispense arrays of daughter droplets using four different sample/reagents and furthermore mix them at specific reaction sites in all possible binary combinations. One such matrix is shown in FIG. 15. A higher order matrix could also be designed on similar grounds demonstrating the inherent concept that with appropriate number of electrical bonding pads and suitably placed integrated L-DEP and droplet actuation electrodes, one could easily generate an N x M array that would work as a microfluidic platform for achieving any set of combinatorial biochemical assays involving M samples and N reagents.

[0193] Such an SMF system may be used for DNA hybridization analysis. As illustrated in FIG. 15, a 2x2 SMF system comprises four sets of L-DEP electrodes arranged in a matrix like geometry along with droplet actuation electrodes integrated at suitable locations. The 2x2 SMF system may facilitate the actuation and mixing of four different oligonucleotide samples, labeled with different reporter and quencher fluorochromes, and allowed use to study base pair complementarities using the principles of fluorescence resonance energy transfer method (FRET). Other prominent examples of DNA assays demonstrated on surface microfluidic platforms (which utilize the widely popular phenomena of electrowetting for sample manipulation) include DNA ligation, nucleic acid extraction purification and DNA amplification through PCR, and patterning substrates (referred as biochips) with an array of oligonucleotides. The biochips may be tailored for use in standard bench-top surface plasma resonance imaging systems for DNA hybridization studies.

VII. APPLICATIONS

[0194] The innovation resulting from various aspects of the present invention provides a new class of fluidic Microsystems, which find numerous applications in biological, biotechnology and clinical laboratories that rely or benefit from rapid, reliable and non-invasive dispensing, transport, or mixture of biological materials in emulsion.

[0195] There may be provided some examples and demonstrations of biochemical assays that could be implemented on DEP based surface microfluidic systems. These assays demonstrate the efficacy of DEP based SMF devices in handling biological samples for LOC related applications. One of the major challenges in this realm, concerning specifically L-DEP (and not the electrostatic droplet actuations) is the actuation of samples having a considerable electrical conductivity, such as salt solutions and buffers. For dielectric samples having finite electrical conductivity, the DEP force is not only frequency dependent, but L-DEP actuation of samples with high electrical conductivity (σ>30×10^{-4} Sm^{-1}) at such radio frequencies (100 kHz) and associated high voltages often results in boiling of samples due to Joule’s heating. To ameliorate this, biological samples (nucleic acids and proteins) are mostly prepared in non-ionic buffers such as HEPES and Tris, with low salt concentrations, and pH in the physiological range (7.4-7.6) to help maintain sample stability.

[0196] Another problem plaguing surface microfluidic actuation schemes in general is the nonspecific and often irreversible adsorption of proteins on hydrophobic surfaces (such as Teflon®), also referred as biofouling. Biofouling often results in increased sample stiction to the surface, exhibited by a significant drop in droplet contact angles that adversely affects sample actuation and device performance. In addition to this, biofouling followed by uncontrolled and unrestricted desorption of proteins become a source of cross-contamination, which limits device re-usability. There may be intricate strategies to address and overcome the problem of biofouling, most of which are based on superficial modifications of surfaces by depositing self-assembled monolayers or other polymeric materials which respond to optical, thermal, chemical (pH) or electrical signals to control interfacial interactions. However, the applicability of these strategies is severely limited by the nature of application and device format. It has also been shown that the extent of biofouling can be limited, if not completely overcome, by the use of water immiscible silicone and fluorinated oils.

[0197] In certain aspects of the present invention, the presence of low viscosity and low surface tension oils encapsulate sample droplets and minimizes contact between the sample and surface. Encapsulation by immiscible oil is furthermore desired as it aids in controlling the rapid evaporation of nanoliter droplets. For example, biocompatible Polyethylene glycol (PEG) based additives in protein solutions may be used to overcome biofouling on Teflon® coated surfaces in electrowetting based microfluidic and actuation of concentrated protein solutions (up to 50 mg/mL; e.g., BSA, fibrinogen) may be greatly facilitated (in air) by adding a small amount of PEG in protein solutions.

[0198] Detection and quantification of nucleic acids is performed routinely in life sciences research and is an integral part of a wide range of biochemical assays such as (quantifi-
fying nucleic acids post) polymerase chain reactions or nucleic acid extractions from cells, DNA hybridization studies and quantifying DNA fragments in cDNA library production. Most common method for quantifying DNA is through spectroscopic analysis as DNA molecules are known to absorb UV light at 260 nm. For more sensitive analysis (i.e. measuring ultra low concentrations), especially in microfluidic studies, where sample volumes are typically in the range of nano-liters to pico-liters, fluorescence based analysis can be utilized. In such fluorescence based analysis, nucleic acids are stained with selective fluorochromes, which upon binding to nucleic acid molecules produce characteristic fluorescence emissions, when excited by light of suitable wavelengths.

[0199] The integrated liquid DEP and droplet actuation scheme may be used for fluorescence based DNA quantification on a SMF device. For example, aliquots of ds DNA (pUC57) and a well known nucleic acid stain, PicoGreen® (Molecular Probes, Invitrogen) were individually actuated on a pair of L-DEP electrodes. The nano-liter droplets of both the sample and reagent thus formed were subsequently actuated using the integrated fishbone shaped droplet actuation electrodes and mixed on-chip. The intensity of the fluorescence emission emanating from the mixed droplets was furthermore quantified using a photo-multiplier tube (PMT), which provided a direct measure of the amount of DNA present in each droplet.

[0200] Additional applications may be contemplated regarding the ultralinear, variable volume emulsion droplet dispensing technology. Aspects of the current invention allow a user to dispense controlled volumes of single or multi-layered droplets at specific on-chip locations and furthermore individually address these multi-phase droplets. Following are some of the applications that such a technology can provide.

[0201] This technology can dispense single and multi-layered emulsion droplet arrays where chemical or biochemical samples, in range of pico to femto grams, are individually encapsulated within very stable oil covers resulting in an on-chip storage mechanism that can be utilized to design specific cryogenic chips which can store cells, DNA/RNA samples, protein samples and even embryo samples at fairly low temperatures for long extended periods. Cryopreservation of micro-biological species is often achieved in glycerol solutions at very low temperatures to prevent cell damages during freezing process (−140°C). (Li et al., 2010).

[0202] As described in Example 3, it has been shown that when a lipid dispersion media (lipid dispersion in mineral oil) replaces the normal silicone oil in the actuation scheme, the actuation results in formation of precise and stable lipid mono-layers which can be transformed into lipid bilayers, resulting in formation of variable but controlled sized vesicles. The formed vesicles can contain polysytren or streptavadin coated bead sample, cell or other bio-particle and provide a controlled environment for various experimental studies such as observing growth micro-organism on-chip. In addition, the technology may result in formation of solid-supported membranes by depositing lipid bilayers onto polymer micro-beads and hence creating bio-functionalized surfaces. These bio-functional surfaces have several applications and can be deployed as bio-sensors and detectors.

[0203] By using co-transfection, DNA strands can be attached to the membrane surfaces and used to achieve artificial assemblies of biological compartments that can be fused or lysed for experimentation (Hadorn and Hotz, 2009).

[0204] The precision emulsion dispensing scheme can also be implemented for producing laser targets as it can dispense highly concentric single and multi-layered emulsion droplets which would be suitable for forming concentric foam shells used in laser targets (Reichelt, 1985).

[0205] In further aspects, these solid-supported membranes may realize the potential to replicate cell structures and provide users with systems such as cell-on-a-chip.

[0206] In still further aspects, these precision emulsion droplet arrays may be used to generate very accurate nuclear fusion targets, in a rapid, automated fashion.

VIII. EXAMPLES

[0207] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques developed to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

DEP Actuation of Emulsion Jets and Dispensing of Sub-Nanoliter Emulsion Droplets

[0208] Prior to experimentation, the L-DEP chip was secured on to a printed circuit board (PCB) platform, where pogo pins and wires were used to provide external connections to the electrodes. The PCB platform housing the L-DEP chip was mounted on the microscope (Olympus BS-S1) and monitored with a high speed camera (Mega speed), as shown in the block diagram (FIG. 16a). The L-DEP actuation was controlled by a control unit 1601 such as a computer and facilitated by data acquisition hardware/software interface (LabVIEW 8.2, National Instruments NI, USA). The video data from the camera was captured at 1500-2500 fps and subsequently digitized and analyzed to obtain images and also to extract the transient response of the liquid and emulsion jets described herein. A DEP based multiplexed reaction chip for combinatorial biochemical assays is shown in FIG. 16b.

[0209] For example, a parent sample (1-2 μL) is manually pipetted and placed at one end of the L-DEP electrode structure. On application of 15 volts (for example in the range 300-500V@100 kHz) across the electrode pair, a thin liquid jet protrudes from the parent sample drop and moves along the electrode. The jet assumes a semi-circular cross-section and it covers entire length of electrode track establishing a new equilibrium under the influence of induced DEP, surface tension and viscous forces. When the applied voltage is turned off, this condition of equilibrium is disrupted and thus the liquid jet is subject to hydrodynamic instability. Under such a situation, Rayleigh’s criteria governs the breakup of the unstable liquid jet into equi-spaced sub-nanoliter droplets (referred to as daughter droplets), according to which the points of maximum instability of the liquid jet are separated by a characteristic distance known as Rayleigh’s wavelength (λ=R0). To facilitate droplet collection upon breakup of the liquid jet, bump sites may be located midway between the initial points of maximum instability, separated by Rayleigh’s
wavelength. The dispensed daughter droplets may be spherically shaped and ~60 μm in diameter (~60 nL) and they may be periodically positioned at the electrode bumps.

Furthermore, in certain aspects of the invention, electrodes (width, spacing, as well as size and location of electrode bumps) may be specifically designed to generate sub-nanoliter emulsion droplets which are helpful in encapsulating the daughter droplets in comparable sized oil covers at specific sites. This is achieved by actuating a sample-oil emulsion jet from a parent sample (oil covered water drop) which upon breakup generates emulsion droplets in sub-nanoliter regime. The term emulsion jet is used for these actuated liquid jets as they comprise a uniform hemi-cylindrical sample jet encapsulated in an oil cover which upon breakup results into formation of sub-nanoliter emulsion droplets.

1. L-DEP Actuation of Homogenous Liquids

Several aspects of L-DEP actuation have been studied in the recent years including effect of electrode dimension, dielectric materials and their thickness, cut-off frequency for L-DEP actuation and hydrophobicity of surface coatings. This Example has been specifically focused on the impact of various fluidic properties (surface tension, viscosity and dielectric constant) on L-DEP actuation.

L-DEP actuation of different liquid samples was experimentally investigated by varying the concentration (% by volume) of glycerol in the parent glycerol-DI water samples (sample 1: DI water, sample 2: 12.5% glycerol, sample 3: 25% glycerol and sample 4: 50% glycerol). The transient behavior of L-DEP actuated liquid jets has been observed and recorded for various types of parent samples including DI water, DI water-glycerol solutions, both in air and in an oil bath.

FIGS. 17a-17c shows images of the liquid jet for DI water and glycerol samples of varying concentration. It is apparent from the images that liquid jet of a DI water sample (FIG. 17a) is very nearly uniform and is confined over the electrode area, except for the minor perturbation at the electrode bumps.

In contrast, the glycerol sample jets, with increasing glycerol concentration (FIGS. 17b-17c) are less well confined and furthermore cover the electrode bump sites. One plausible reason for this difference in the profile of glycerol jets is attributed to a decrease in surface tension of the liquid with increasing glycerol concentration thus the jet although still confined by electric field tends to cover more of the bump area and hence results in a distorted hemi-cylindrical jet.

FIG. 18 shows on the same plot the experimental data (liquid jet displacement (Z) versus time) for L-DEP actuations conducted in air and the corresponding theoretical fit for homogenous liquid samples, as predicted by the lumped model (eqn. 31). The experimental values of fluidic properties of liquid samples (surface tension and viscosity) used and relevant to these experiments were measured and referred to separately. The model predictions were obtained from an iterative curve fitting process using eqn. 31, where surface tension and viscosity of liquid samples were used as adjustable parameters. The fitting exercise, summarized in Table 2, yields good agreement between the experimental values and the lumped parameter values of surface tension and viscosity, extracted utilizing the lumped model. The fits in FIG. 18 have confidence values close to 90%. The fitted theoretical curve was also used to extract values of effective jet capacitance (ε_e|k_e|c_μ) of these homogeneous liquid jets.

### Table 2

| Sample No. | % by vol. (Gly.) | Expt. Jet Cap. (ε_e|k_e|c_μ) | Expt. Viscosity (cSt)* | Th. Viscosity (cSt)* | Expt. ST dyne/cm** | Th. ST dyne/cm** |
|------------|-----------------|-----------------------------|------------------------|---------------------|-------------------|-----------------|
| 1          | 0               | 553.6                       | 1                      | 73                  | 73                | 73              |
| 2          | 12.5            | 539.8                       | 2                      | 71                  | 69                | 69              |
| 3          | 25              | 519                         | 4                      | 68                  | 66                | 66              |
| 4          | 50              | 477.5                       | 8                      | 65                  | 65                | 65              |

*Expt. viscosity values in centistokes (cSt) were measured using SVM (Stabinger Viscometer)

The experimental data for the homogenous sample jets appears to be in good agreement with theoretical plots extracted using the lumped parameter model, confirming that the lumped model can successfully account for and accommodate the variation in the fluidic properties in predicting the response of L-DEP actuated jets.

2. L-DEP Actuation of Emulsions

When the liquid samples were actuated under an oil cover, the transient behavior of the emulsion jet exhibited similar dependence; however the liquid jet velocities were significantly lower than those observed in air.

To compare the emulsion jet with an oil bath scenario, where the liquid jet pushes through the bulk oil medium as the liquid jet develops over the electrode pair, the same experiments under 1 cSt and 5 cSt silicon oil bath was conducted.

The results of these experiments, shown plotted in FIG. 19, suggests that the transient behavior of the liquid jet remains fairly similar in character, under different viscosity oil baths and furthermore very similar to that observed in air. Interestingly, the average jet velocities of liquid jets actuated in air and in an oil bath are very similar in magnitude (6-10 cm/sec) but significantly higher for the emulsion jets under similar conditions (3-4 cm/sec) (FIG. 19). These findings suggested that the dynamics of jet motion is only marginally impacted by the viscosity of the oil bath.

A similar set of DEP actuation experiments were conducted on various oil covered DI water parent droplets. In such experiments, n delay (5-10 msec) in the emergence of
the emulsion jet from the oil covered parent droplet was observed. This delay is due to the fact that initially, on application of the actuation voltage, the liquid jet is developed and is conveyed under an oil cover (oil droplet), before it protrudes through the outer boundary of parent oil droplet. The fitted values of surface tension and viscosity (theoretical values in Table 3) are agreeably different from their corresponding experimental values as they are resultant of the specific combination of aqueous sample and silicone oil.

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>1-1 Cst oil</td>
<td>0 (DI)</td>
<td>334.1</td>
<td>359.1</td>
<td>1</td>
<td>1</td>
<td>73</td>
<td>70</td>
</tr>
<tr>
<td>1-5 Cst oil</td>
<td>0 (DI)</td>
<td>333.5</td>
<td>339.1</td>
<td>1</td>
<td>2</td>
<td>73</td>
<td>70</td>
</tr>
<tr>
<td>1-20 Cst oil</td>
<td>0 (DI)</td>
<td>330.8</td>
<td>332.2</td>
<td>2</td>
<td>8</td>
<td>73</td>
<td>70</td>
</tr>
<tr>
<td>2-1 Cst oil</td>
<td>12.5</td>
<td>307.9</td>
<td>311.4</td>
<td>2</td>
<td>4</td>
<td>71</td>
<td>68</td>
</tr>
<tr>
<td>2-5 Cst oil</td>
<td>12.5</td>
<td>307.3</td>
<td>311.4</td>
<td>2</td>
<td>4</td>
<td>71</td>
<td>68</td>
</tr>
<tr>
<td>2-20 Cst oil</td>
<td>12.5</td>
<td>304.5</td>
<td>304.5</td>
<td>4</td>
<td>6</td>
<td>68</td>
<td>65</td>
</tr>
<tr>
<td>3-1 Cst oil</td>
<td>25</td>
<td>299.6</td>
<td>304.4</td>
<td>4</td>
<td>7</td>
<td>68</td>
<td>65</td>
</tr>
<tr>
<td>3-5 Cst oil</td>
<td>25</td>
<td>297.6</td>
<td>304.4</td>
<td>4</td>
<td>7</td>
<td>68</td>
<td>65</td>
</tr>
<tr>
<td>3-20 Cst oil</td>
<td>25</td>
<td>294.8</td>
<td>297.6</td>
<td>4</td>
<td>11</td>
<td>68</td>
<td>64</td>
</tr>
</tbody>
</table>

*Expt. viscosity values were measured using SVM (Stabinger Viscometer)

[0227] In addition to the transient behavior of the DEP actuated jets, the profile of the various sample jets in equilibrium was furthermore investigated, prior to the removal of the actuation voltage.

[0228] It is interesting to note that although the effective capacitance of emulsion jet affects the dynamics, however the actuation voltage to actuate the emulsion sample is primarily controlled by parent sample and only minimally impacted by oil cover. All the emulsion samples were successfully actuated at voltage similar in value to that used for homogeneous samples, although, more reliable actuations for the high viscosity oils (~20 Cst) would have slightly higher voltages.

[0229] Based on the experiments, actuation of high viscosity silicone oils (low dielectric constant), on Teflon® coated surfaces, would use very high voltages (600-800V) as compared to the 400-450V used for DI water (400V). So, while actuating a water-oil emulsion parent droplet, oil is not actuated. Experiments show that, upon application of 400V, 100 kHz AC signal across the pair of electrode, a liquid jet protrudes from the parent droplet (DI water). When this jet penetrates the external boundary of parent oil droplet, a pulling force is exerted by oil which now tends to redistribute itself over the water jet, thus forming an emulsion jet. This pulling force appears at the water-oil interface due to the phenomenal difference in their surface tension. Once the emulsion jet is formed, the dynamics is in good agreement with the effective capacitance lumped parameter model. So, although the DEP actuation voltages are controlled by parent sample droplet (DI water in this case), the dynamics of jet motion is controlled by the effective capacitance of the emulsion jet.


[0231] The break-up of homogenous liquid jets is explained using Rayleigh's instability of a cylindrical jet which is subject to hydrodynamic instability once voltage is removed.

[0232] Unlike the homogenous jet, the break-up of an emulsion jet may be influenced by the hydrodynamic instabilities of the constituting jets (FIG. 20). Now, water and glycerol solutions having a very high surface tension and contact angle on Teflon®, become highly unstable as the
stabilizing DEP force is removed. However, oil appears to respond slower to the destabilizing forces as one would expect since Teflon® is a wetting surface for oil (lower contact angle on Teflon®) and furthermore the surface tension of silicon oils is fairly low (~20 dyne/cm) as compared to the used aqueous solutions (65-73 dyne/cm). This behavior was observed and evidence when the break-up of emulsion jet, captured by a high speed camera at 1500 fps, with a time resolution of 0.67 msec as shown in (FIG. 21).

It has been observed that break-up of oil sheath occurs after the inside liquid jet has already broken into daughter droplets at the specific bumps. Break-up of oil sheath occurs over a longer time frame (10-15 msec; frames b-e) as compared to break-up of sample jet, which occurs in 1-2 msec and it is facilitated by the instability produced as a result of formation of sample daughter droplets inside the oil sheath. This was further supported as it has been observed that the break-up of a similar sized homogeneous oil jet is even more sluggish (~15 msec) and unpredictable, which results in non-uniform daughter droplets.

To generate emulsion droplets, in sub-nanoliter volume range, a finer electrode structure (w~g~15 micron, R_{sample} (bump radius)~23 micron) and parent droplet comprised of glycerol (12.5%) and 5% silicone oil were used. An example of the sub-nanoliter emulsion droplets dispensed using the above electrode structure where each emulsion droplet comprise glycerol sample of approximately 30 picoliter (based on measured radius of droplets), encapsulated by a 90 nm in diameter oil cover droplet. Green Fluorescent Beads (diameter: 1 micron) were used to observe the stability of these emulsion droplets produced from L-DEP actuation scheme. The beads were observed to be confined at the sample-oil interfacial boundary, showing the underlying symmetry in these sub-nanoliter emulsion droplets.

Using the above emulsion dispensing scheme and structures with different electrode dimensions emulsion droplets ranging in volume from 30 pl to 450 pl have been successfully and repeatedly dispensed. (see Table 4 and FIG. 22-23). To emphasize on the stability of these emulsion droplets, volume of these emulsion droplets were measured roughly an hour after dispensing.

FIG. 23 shows the scalability of the dispensing scheme as the electrode dimensions are changed from w~g~20 micron (FIG. 4.4b) to w~g~30 micron (FIG. 23b), the liquid sample contained in these emulsion droplets are 60 pl (picoliter) and 200 pl respectively. The image in Figure 23a shows emulsion droplet formed over w~g~30 micron electrode structure which is intersected by a fishbone shaped electrode arrangement which may be used to transport these emulsion droplets from their dispensing sites to specific on-chip locations.

| TABLE 4 |
| Size and scalability of the L-DEP emulsion droplet dispensing scheme |

<table>
<thead>
<tr>
<th>Sample2-oil</th>
<th>Sample3-oil</th>
<th>Sample4-oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode dimension (μm)</td>
<td>V_{sample} (pl)</td>
<td>R_{oil} (μm)</td>
</tr>
<tr>
<td>w = g = 15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>w = g = 20</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>w = g = 30</td>
<td>180</td>
<td>90</td>
</tr>
<tr>
<td>w = g = 40</td>
<td>450</td>
<td>120</td>
</tr>
</tbody>
</table>

Once the sample droplets are dispensed their lifetime and stability have been the focus. In addition to water droplets, samples with varying amounts of glycerol also experimented with. The rationale behind using glycerol-water solutions is that glycerol increases solution viscosity, which helps to reduce loss of solution. Besides, the hydrogen bonding between glycerol and water molecules makes it a stable solution as is evident from its application in storing biological samples such as blood samples. Furthermore, glycerol does not adversely affect or denature protein, DNA and other key ingredients of biological samples.

FIG. 24 shows experimental results, where the radius and volume of various sample droplets is shown plotted as a function of time. This data was extracted from videos captured right after dispensing homogeneous sub-nanoliter droplets of different samples using L-DEP actuation over w~g~20 micron electrode structure, demonstrating the stability of these sub-nanoliter daughter droplets after DEP dispensing. This droplet volume versus time data shows that the evaporation of pure DI water on Teflon® is in agreement with the (t0.96) behavior predicted by Shahidzadeh-Bonn et al. (2006), where t0 is the time at which the droplet disappears.

Unlike pure DI water sample, in the case of glycerol samples the rate of sample volume loss is more complex, since these samples are not pure solvents rather a homogenous mixture of two miscible liquids with different fluidic properties. In the case of glycerol droplets, the rate of volume loss is a function of time. This behavior is anticipated and explained by the increasing glycerol concentration in the daughter droplet due to loss of solvent water molecules. Sample volume loss decreases with increased glycerol concentration but, even with very high glycerol concentrations used (up to 50% by vol.), convective volume losses were still present and affected the stability of these homogeneous droplets.

In order to circumvent solvent sample loss due to convective effects, a major factor contributing to sample loss, there may be provided any suitable means of encapsulating the dispensed droplets. The L-DEP emulsion dispensing scheme facilitated the encapsulation of these sub-nanoliter sized liquid sample droplets in uniform and comparable sized oil covers. The presence of such an oil cover results in a substantial reduction in convective sample loss and a stable constant volume sample droplets are maintained for an extended period of time (see Table 4 for stable volume of emulsion droplets measured roughly an hour after dispensing).

Glycerol emulsion droplets are found to be the most stable at higher glycerol concentrations which helps to reduce evaporative solvent losses while the oil cover helps to minimize the convective losses of solvent to the surrounding environment as well as protecting the droplets from any external contaminants. Stability and prevention from external contamination is critically important when its applicability is contemplated in on-chip miniaturized storage of biological samples such as cells, embryos, blood samples, for extended period of time.

6. Conclusions

This Example, supported by the lumped parameter model, has focused on the utility of L-DEP to actuate liquid samples, employing both homogenous and emulsion parent sample droplets, to dispense precision picoliter sized daughter droplets on top of hydrophobic surfaces. The experimental finding in the Example concerning the temporal response of
the liquid jet ejected from the parent sample, as the first step in the droplet dispensing scheme, are generally in good agreement with the jet dynamics predicted by the lumped parameter model. Here the transient motion of both the homogenous and emulsion jets exhibit a similar temporal dependence ($t^m$), however, the magnitudes of the responses (actuation speed) differ significantly. This aspect of the emulsion jet behavior may be accounted for and attributed to a reduction in the effective capacitance of the emulsion jet as compared to the homogenous jet.

[0245] It has been further shown that the DEP actuated emulsion jet, once the applied voltage is switched off, breaks up and forms uniformly sized daughter emulsion droplets at the periodically spaced electrode bump locations. A more detailed examination of the emulsion droplet reveals that the destabilization and breakup of the jet is primarily controlled by the breakup of the aqueous phase of the jet, which in turn influences and controls the breakup of the oil encapsulating the aqueous phase.

[0246] In conclusion, the dispensing of both homogenous sample droplets as well as encapsulated single emulsion droplets in the picoliter volume range on top of surfaces may be achieved quite readily by implementing DEP. This dispensing capability provides alternative means of both dispensing and subsequent manipulation of sample droplets in the domain of microfluidics. The oil encapsulated single emulsion droplets formed using L-DEP can be transported by integrating the low frequency Droplet DEP actuation scheme as shown in the next Example.

Example 2

Liquid DEP Actuation and Precision Dispensing of Variable Volume Droplets

[0247] 1. Device Fabrication

[0248] The micro-fabricated SMF device, used for experimentation, comprises a pair of patterned planar metal electrodes coated with a thin insulating dielectric layers, deposited over a passivated silicon wafer (SiO$_2$ on Si). In this Example five different L-DEP electrode schemes were tested, as shown in FIG. 9. To fabricate these SMF devices, initially a 150-200 nm thick metal (Al or ITO) layer is deposited using a sputtering process and then patterned using standard lithography and wet etching procedures.

[0249] The silicon wafer housing the patterned electrodes were then further coated and passivated with a thin insulating dielectric (450 nm of Si$_3$N$_4$ or SiO$_2$), using plasma enhanced chemical vapor deposition (PECVD) method and subsequently patterned using a reactive ion etching (RIE) process. In the final fabrication step, the top surface of the SMF device is rendered hydrophobic by spin coating a thin layer (~0.1 µm) of Teflon® AF 2400 (Grade: 400S1-100-1, DuPont Inc., USA), to facilitate the L-DEP based droplet dispensing methodology.

[0250] 2. Experimental Setup and Procedure

[0251] A schematic diagram of the optoelectronic setup used for experimentation is illustrated in FIG. 6. The SMF device was securely placed with the help of spring loaded pogo pins, onto a PCB to provide electrical connection to the bonding pads that energize the L-DEP electrodes. This arrangement was placed on a reflection fluorescence microscope platform (BX51, Olympus, Japan) coupled to a high speed camera (Mega speed) and a photomultiplier tube (PMT) (H-7468-10, Hamamatsu, Japan) for measuring the fluorescence intensity and quantification of the dynamics of the DEP actuated liquid jet. A signal generator (TGA 1244, TTI, UK) and a high-voltage, high-frequency power amplifier (Precision Power Amplifier 5205A, Fluke) arrangement was used to provide the AC voltage to the electrode arrangement. The actuation process was controlled using a software driver developed using labview (NI Labview, USA) and the output data was recorded in form of high speed videos (original frame rates: 2000-2500 fps) using the high speed camera. The high speed videos were digitized and an image probing program (provided by Mega speed) was used to capture the temporal behavior of DEP actuated liquid jet.

[0252] A 0.8 µL parent droplet is placed onto the wider end of a taper electrode. Upon application of ~500 Vrms AC voltage at 100 kHz, a liquid jet protrudes from the parent droplet and propagates rapidly along the electrode length. Due to continuous tapered electrode design (as shown in scheme), the liquid jet narrows down along the length while rapidly covering the entire electrode area and upon subsequent removal of applied AC voltage, hydrodynamic instability breaks the tapered jet into small fragments. These small liquid fragments reshape to stabilize themselves, forming nano to picoliter sized daughter droplets. To prevent volumetric loss, experiments were carried out under a 5 cSt oil cover, either in form of a bath or using the emulsion production experimental scheme described previously. Break-up of a uniform cylindrical jet is governed by Rayleigh’s instability criteria which determines the points of maximum instability to be separated by a characteristic length, also known as Rayleigh’s wavelength and is related to the radius of the cylindrical jet as: $\lambda = \frac{2\pi}{k} R$.

[0253] 3. Numerical Solution for Dynamics of Tapered Jets

[0254] The NLDE (Eqs. 18 and 19) governs the dynamics of tapered liquid jets actuated using the L-DEP actuation methodology in certain aspects of the present invention. The goal is to obtain a solution for the differential equation that can account for the dynamics of tapered jets with different fluidic properties. The fluidic properties (dielectric constant, surface tension and viscosity) of the aqueous parent sample droplet were varied by using different compositions of gycerol and de-ionized (DI) water solutions. The various sample compositions and the corresponding fluidic properties are tabulated in Table 5. As mentioned earlier, Wolfram Mathematica software (version 7.0) was used to obtain numerical solution for the governing DE.

**TABLE 5**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>% by volume glycerol</th>
<th>Relative dielectric constant $K_0$</th>
<th>Surface tension $\sigma$ (dyne/cm)</th>
<th>Viscosity $\eta$ (cSt)</th>
<th>Density $\rho$ (kg/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>80</td>
<td>73</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>77</td>
<td>69</td>
<td>3</td>
<td>1050</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>74</td>
<td>66</td>
<td>5</td>
<td>1080</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>66</td>
<td>65</td>
<td>9</td>
<td>1100</td>
</tr>
</tbody>
</table>

*Relative dielectric constant values were measured using liquid test fixture (Agilent 1615A) and TDR (Agilent 4294A).
**Viscosity values were measured using a viscometer (Stabinger viscometer SVM 3000).
ing the values of the five coefficients (Eqn. 20) and $T_{gb}$, the numerical solutions for the various experimental fluid jets were generated.

[0256] These numerical solutions were then compared to the experimental dataset and plotted alongside to observe the agreement between the generated numerical solution and the experimental plots. This comparison shows that the numerical solution successfully captures the transient behavior of the actual liquid jets. An extensive curve-fitting exercise was furthermore implemented to obtain a numerical expression that suitably fits the numerical solution and compared it with the well-established $t^{0.5}$ behavior of uniform jets.

[0257] The curve fitting exercise was also conducted using Mathematica. An initial generic polynomial expression $(z_a-b_1+b_2t+b_3t^2+\ldots)$ displayed a very poor quality of fit (goodness of fit <70%). Since the theoretical analysis for small actuation times indicated a significant contribution of $t^{0.5}$ term, a separate curve fitting exercise using only $t^{0.5}$ was carried out. This exercise resulted in a very accurate fit (>99%) for the initial time scale but it failed to account for the remaining actuation time which indicated that the best fit could be a linear combination of the $t^{0.5}$ and a linear (and possibly higher order) polynomial terms. A final expression of form: $z_a-b_1t+b_2t^{0.5}+b_3t$, achieved a fairly good fit (>99.5%) during the entire actuation period.

[0258] The inclusion of higher order terms did not result in any significant improvement. This exercise along with the theoretical analysis indicate that the differences in dynamics of a uniform jet and a tapered jet. A tapered jet behaves very similar to uniform jets for the initial actuation period (small t). But, as the jet propagates, due to the modified flow terms, its dynamics exhibits linear time dependence, which is indicative of an inertia dominant behavior. Another interesting fact is that, upon extending the actuation times to very large magnitudes, a saturation zone is observed where the jet is almost stationary, indicating that it will never approach the theoretical limit of the tapered electrode (i.e. $L_s$).


[0260] The concept of Rayleigh’s instability has been extended to explain the break-up of nearly hemi-cylindrical jets formed over uniform L-DEP electrodes.

[0261] However, it has been previously demonstrated that break-up of such jets on planar surfaces, is not as reliable and uniform as predicted by the Rayleigh’s instability criteria. In other words, break-up of uniform jets result in formation of daughter droplet arrays with non-uniform size and spacing. To overcome this non-uniformity, semi-circular bumps are incorporated along the entire electrode length and separated by Rayleigh’s wavelengths ($\lambda$), which act as collection sites for the produced daughter droplets. In the aspects for a tapered liquid jet, Rayleigh’s wavelength may not be a fixed length but rather a continuously varying parameter which decreases as the electrodes taper down.

[0262] FIGS. 25a-25c demonstrates break-up of such a continuous tapered liquid jet formed by employing an electrode scheme which comprises a continuous tapered L-DEP electrode pair with no bumps, and the resulting array of dispensed daughter droplets. It has been observed that the size and spacing of daughter droplets doesn’t vary as a uniform continuous function, and the non-uniform spacing is similar to the case of a uniform jet break-up without bumps. FIGS. 25a-25c illustrates the break-up of a continuous tapered electrodes with bumps incorporated at specific bump separations that continuously decreases along the electrode length.

[0263] The bump separation was numerically calculated by using the radius of an equivalent uniform hemi-cylindrical jet corresponding to specific segments of the tapered cylindrical jet. It is evident in this case that the placement of dispensed daughter droplet is more precise and predictable as compared the case without bumps. But, it has also been observed that although the bumps play no active role in jet break-up and they are only incorporated to overcome any irregularities during the process when broken jet segments recollect to form daughter droplets, they do tend to create small distortion in the shape of the formed liquid jets, especially as the liquid surface tension is reduced (for example by using higher glycerol conc.).

[0264] An alternative to using bumps is to use electromechanical instability in form of pinches along the L-DEP electrode. These pinches are very narrow electrode regions (see FIG. 9) which actively control the electrical and fluid mechanical properties of jet propagation and jet break-up by modifying the shape of the tapered liquid jet. When suitably incorporated these pinched regions become specific regions/points of maximum instability, allowing the user to suitably modify $\lambda$ and thus create an array of daughter droplets with programmable droplet size and spacing. This was achieved by using electrode scheme as shown in FIGS. 9d-9f where the pinch regions are positioned separated by an arbitrary length L which does not correspond to $\lambda$. For any specific section of the formed tapered jet and bumps were placed symmetrically around these pinched electrode regions as droplet collection sites, at the fixed separation of L (=500 µm). Break-up of such a pinched tapered jet and dispensing of a controlled daughter droplet array is shown in FIGS. 25a-25c.

[0265] An interesting scenario would be where one can compare the active control provided by a well-positioned pinch to the passive control provided by the bumps. To facilitate such a comparison, a different uniform electrode scheme has been used (shown in FIG. 9e and FIG. 26) which has four different sections of bump separation $\lambda$/4, $\lambda$, $\lambda$, and an arbitrary separation length L. (L=$\lambda$, $\lambda$, $\lambda$). Some of the sections on this L-DEP electrode arrangement have bumps placed at $\lambda$, but pinched in between in a non-symmetric fashion (FIG. 9f and FIG. 26). As a result, upon jet break-up, the daughter droplets are not formed at the bumps separated by $\lambda$ but symmetrically around the pinched region (see FIG. 26). On another aspect of this scheme, very small sized pinched region have been placed, compared to $\lambda$, and in this case upon break-up, positioning of the daughter droplets is not controlled by the pinch. This confirms that only a pinch with suitable size can overcome or successfully alter Rayleigh’s wavelength and in turn allows the user to program and control droplet dispensing.

[0266] 5. Droplet Volume Measurement and Comparison of Various Dispensing Schemes

[0267] The volume of dispensed daughter droplets has been theoretically estimated and furthermore compared with the actual dispensed droplet volumes calculated experimentally using the experimental scheme shown in FIG. 6. Theoretical estimation of daughter droplet volume for a uniform L-DEP electrode structures has been studied. Previously, it has been established that break-up of a tapered liquid jet is influenced both by the position of bumps and pinches along the electrode length. In such cases, the jet disintegrates symmetrically between the positioned bumps and in case of a pinched electrode, it fragments precisely in the pinched region. The dispensed daughter droplet volumes have been estimated by calculating the volume of the sectional conical region ($V_{drop}$).
in each segment and for calculating the volume in case of the pinched taper design ($V_{\text{drop2}}$) the pinched region has been subtracted from the volume expression. The expressions for droplet volumes are as follows:

$$V_{\text{drop1}} = \frac{1}{3} \pi (R_1^2 L_1 - R_{1i}^2 L_{1i})$$

$$V_{\text{drop2}} = V_{\text{drop1}} - \frac{1}{2} \pi L_0 (R_{1i}^2 - R_{1i}^2).$$

[0268] Here, $R_1$ and $R_i$ is the jet radius at respectively the left and right end of the sectional conical region; $L_1$ and $L_{1i}$ is the axial length of the cone that incorporates the sectional conical region; $L_0$ is the pinch length; $R_{1i}$ and $R_{1i}$ are the inner and outer radii of the hemi-cylindrical pinched region that is not covered by the jet during actuation and break-up. [0269] The theoretically calculated droplet volumes for the various taper designs were compared to the actual dispersed droplet volumes, measured experimentally. The plots demonstrate good agreement between the actual dispersed droplet volumes and their theoretical estimation, except for the case of continuous taper scheme without bumps and pinches where inconsistency in volume and positioning of dispensed droplets has been observed. This also illustrates the sort of control that placement of a pinch provides by controlling the density of dispensed daughter droplets and making the scheme more suitable for large scale integration. [0270] The outcomes of this volume measurement and comparison exercise is summarized in Table 6, further indicating the suitability of these scheme for integrating with droplet transport schemes, for utility in Lab-On-a-Chip (LOC) based combinatorial chemistry applications.

### Table 6

<table>
<thead>
<tr>
<th>Electrode Scheme</th>
<th>No. of Droplets</th>
<th>Position of Droplets</th>
<th>Droplet spacing</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme 1</td>
<td>9</td>
<td>Positioned at step A</td>
<td>Reliable but unfavourably high packaging density for smaller droplets</td>
<td></td>
</tr>
<tr>
<td>Scheme 2</td>
<td>6-8</td>
<td>Uncontrolled</td>
<td>Dispensing not repeatable</td>
<td></td>
</tr>
<tr>
<td>Scheme 3</td>
<td>12</td>
<td>Positioned at weighted A</td>
<td>Reliable but unfavourably high packaging density for smaller droplets</td>
<td></td>
</tr>
<tr>
<td>Scheme 4</td>
<td>8</td>
<td>Positioned at fixed separation (500 μm)</td>
<td>Controlled dispensing in the entire volume range</td>
<td></td>
</tr>
</tbody>
</table>

[0271] Controlled and Reliable Dispensing of Variable Volume Droplets

[0272] Previously, the dynamics of tapered liquid jet actuation have been described and furthermore shown by suitable incorporation of pinches and bumps, one can achieve a user controlled droplet dispensing scheme. Methods were develop to devise a flexible dispensing methodology where size and positioning of the droplets is not restricted by Rayleigh’s criteria, for example, by using varying electrode structure.

[0273] Since such droplet dispensing schemes could become an active part of multiplexed, combinatorial, biochemical assay chips where multiple samples/reagents can be actuated and manipulated in parallel and automated fashion, to achieve combinatorial chemistry. The scheme provides user with leverage to improve the compactness of such multiplexed assay chips while also incorporating more fluidic sample manipulations such as active control on dispensed sample mass or sample concentration. Also this scheme of tapered L-DEP electrode is far superior to its predecessor, the step tapered electrode design (Fig. 9a) in several aspects as it provides better control over size and positioning of daughter droplets, in the dispensed droplet arrays. However, it may need to be established that the new taper electrode scheme is equally reliable when it comes to dispensing uniform cone.

[0274] droplet arrays of biological samples such as double strand (ds)-DNA plasmids. To demonstrate this, a binary mixture of 50x PicoGreen® (PG) dye (Molecular Probes™, Invitrogen, USA) and 50 ng/μL pUC57 ds-DNA (GenScript, USA) in a 1 mM Tris solution was used as the parent sample droplet.

[0275] Volume of these droplets was determined experimentally along with the photocurrent response ($I_p$) of the individual droplets. Since the photocurrent response is proportional to the fluorescence emission and hence the amount of DNA sample in the individual droplet, ratio $I_p$/vol. is correlated to DNA sample cone, in the dispersed daughter droplets. Fig. 27 shows a comparison between plots of $I_p$ Vs. the droplet volumes for the two electrode schemes. A linear plot with a constant overlapping slope is reported for both the step tapered and the new continuous tapered electrode scheme corresponding to identical parent sample compositions, confirming that the new scheme is capable of dispensing arrays of user controlled daughter droplets with uniform and known sample concentrations.

[0276] 7. Conclusions

[0277] The dispensing and subsequent manipulation of very small (sub-nanoliter range) and precise volumes of liquids in the form of droplets, provides new and means of handling aqueous biological samples and chemical agents that can be exploited by LOC devices in performing biochemical assays. In this Example, L-DEP and tapered electrode structures have been specifically leveraged to demonstrate the utility of SMF devices to actuate and dispense an array of droplets of varying and controlled volume, in a reliable fashion. Such a dispensing scheme is advancement of the existing L-DEP based dispensing scheme where droplet volumes and positioning are restricted by the inherent Rayleigh’s instability criteria. It has furthermore been shown that the density of the dispersed droplets can be enhanced by the judicious incorporation of pinches and bumps in the electrode
structure thus overcoming the limitations of jet breakup, as predicted by Rayleigh's instability criterion.

Example 3

Chip-Based Unilamellar Vesicle Formation and Dispensing using Dielectrophoresis [0278] Lipid vesicles are important bio-articles, serving as drug delivery agents, artificial cells, chemical bioreactors and potentially molecular biosensors. The Example focuses on the formation and dispensing of identically sized large unilamellar vesicles in a rapid and controlled fashion by leveraging dielectrophoretic forces, utilizing a surface microfluidic (SMF) device. This approach provides an alternative, less cumbersome scheme of forming vesicles in the micron size range, circumventing the need for on/off-chip pumping required in pulsed microfluidic jetting method.

[0279] The lipid samples used in this Example were prepared using the procedure outlined in FIG. 28. The SMF chip, comprised of patterned planar metal (150 nm Aluminum) electrodes coated on top with a dielectric layer (450 nm Si$_3$N$_4$), deposited on a passivated silicon wafer. The chip was rendered hydrophobic by spin coating (~0.1 μm) of Teflon® AF (FIG. 2(a)). To prepare the emulsion, Liquid-Dielectrophoresis (L-DEP) actuation methodology was used. A 1 μL aqueous parent sample (25% glycerol by volume), covered by a droplet of the dispersion media was placed on one end of the L-DEP electrodes (FIG. 29a). On application of an AC voltage (500 V/m (100 kHz)), an emulsion jet emerged from the parent droplet and rapidly (for example within ~5-10 msec) conveyed over the electrodes. Upon removal of the electric field, jet disintegrated to form sub-nanoliter sized emulsion daughter droplets. The formation and break-up of the jet is explained above. This resulted in precision emulsion droplets with a lipid monolayer at the water-oil interface (FIG. 29c, FIGS. 30b-30c). The outlined procedure of forming emulsion droplets is far more precise and controllable than any of the comparable on-chip or off-chip methodolgy, eliminating the need for sedimentation. The outer cover of these emulsion droplets was then diffused in a compatible viscosity dispersion media (doped with silicone oil) bath resulting in stable lipid monolayer formation which encapsulates the aqueous solution within the silicone oil bath (FIG. 29d, FIGS. 30d-30e). Finally, to assemble the second lipid layer, these droplets needed to be transferred through an interface oil-aqueous phase boundary. To achieve this, a high viscosity glycerol solution (50% by volume in de-ionized water) was placed on top of the mixed oil layer, forming a lipid layer at the interface which gradually sank towards the chip surface (FIGS. 30e-30f). Upon observing this arrangement after roughly 30 minutes, it was clear that the heavy aqueous media now contained the aqueous daughter droplets, confirming that a very stable lipid bilayer has been formed that prevents loss of shape/volume for these micro-sized droplets (FIG. 29f, FIGS. 30f/30g). The vesicles thus formed were simultaneously loaded with fluorescent polymer beads (diameter: 0.5 μm-5 μm) by adding them to the parent droplet which resulted in encapsulation of the beads by lipid membranes (FIG. 30h-30j) which are very stable, solid-supported membranes with bio-functionality.

[0280] The SMF DEP scheme successfully dispensed arrays of lipid vesicles, identical sized and precisely positioned, for subsequent bio-functionalization, targeted drug delivery, artificial cell models and bio-sensing utility.

Example 4

[0281] FIG. 31 illustrates one embodiment of a method for forming a variable volume lipid bilayer vesicle. Generally, the method includes forming a single emulsion droplet, forming a monolayer lipid vesicle from the single emulsion droplet, and forming a bilayer lipid vesicle from the monolayer lipid vesicle.

[0282] In one embodiment, the method includes dispensing variable volume Single Emulsion (SE) droplets with a lipid monolayer at an aqueous-oil interface as shown in Step 1 of FIG. 31. This step may be performed using an L-DEP device according to the present embodiments. An L-DEP actuation voltage of 480 V/m at a frequency of 100 kHz may be applied to the L-DEP device to form the SE droplets at the aqueous-oil interface. In a particular embodiment, the SE droplet may have a volume of 1.2 nL-30 pL.

[0283] The method may also include submerging the DEP chip in an oil bath. For example, the oil bath may include mineral oil. As shown in Step 2 of FIG. 31, submerging the DEP chip in an oil bath may form monolayer lipid vesicles from the variable volume SE droplets.

[0284] The method may further include forming bilayer lipid vesicles on the chip surface. For example, the bilayer lipid vesicles may be dispensed on the surface of the DEP chip as shown in Step 3 of FIG. 31. In a particular embodiment, the second lipid monolayer may be spontaneously assembled by introducing an aqueous phase on top of the encapsulating oil bath. For example, the second lipid layer may sink to the surface of the DEP chip as illustrated in FIG. 31.

Example 5

[0285] FIGS. 32-35 illustrate various alternative DEP electrode configurations that may be adapted for use with the present embodiments. For example, FIGS. 32-34 illustrate a comparison of a pinched L-DEP device having semi-circular bumps and a pinched L-DEP device without the semi-circular bumps. Additionally, FIG. 35 illustrates an alternative embodiment of a D-DEP electrode having a comb-shape.

[0286] FIGS. 32a-32c illustrate the formation of variable volume bilayer lipid vesicles on a pinched L-DEP electrode scheme with semi-circular bumps. An embodiment of the L-DEP electrode is illustrated in FIG. 32a. A single emulsion water-in-mineral oil tapered liquid jet may be formed on the L-DEP electrode by applying a voltage to the electrodes, as illustrated in FIG. 32b. When the voltage is removed from the electrodes, the jet may form an array of dispersed variable volume, single emulsion water-in-oil daughter droplets as illustrated in FIG. 32c. In a particular embodiment, the electrode may include electromechanical pinches as shown in FIG. 32d. In one embodiment, the electromechanical pinches may vary in width from 40 μm at the wide end to 10 μm at the narrow end. Such an embodiment may yield encapsulated aqueous samples ranging from 1.5 nL to 50 pL. In particular, such an embodiment may yield variable volume bilayer lipid vesicles having a diameter in the range of 30 μm to 150 μm.

[0287] By way of comparison, FIG. 35a-c illustrates a similar process yielding aqueous daughter droplets. As shown in FIG. 35c, the bilayer lipid vesicles of FIG. 35c are similar in size and position to the aqueous daughter droplets of FIG.
Additionally, Fig. 33c illustrates that a pinched L-DEP electrode scheme that does not include the semi-circular bumps may produce daughter droplets of roughly equivalent volume and uniformity.

Similarly, Fig. 34a-c show that the bilayer lipid vesicles may be formed with more uniformity on a L-DEP chip that does not include the semi-circular bumps, because removal of the bumps results in less perturbation of the jet shape. As shown in Fig. 34c, this L-DEP scheme may also yield encapsulated aqueous samples ranging from 1.5 nl to 50 nl.

Fig. 35a-d illustrate an alternative embodiment of the D-DEP electrode scheme. In one embodiment, the D-DEP electrode may include two columns of comb-shaped electrodes aligned opposite to each other as shown in Fig. 35a. In one embodiment, this configuration may provide a single surface electrowetting based move-and-mix-all droplet transport scheme for dispensing and large scale maneuvering of homogeneous droplets and vesicles. Fig. 35b illustrates two continuous tapered homogeneous liquid jets during L-DEP actuation under an oil bath. Fig. 35c illustrates two arrays of variable volume sample and reagent daughter droplets. Fig. 35d illustrates electrowetting based droplet transport and mixing, for example out of 4 pairs are mixed.

All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES


What is claimed is:

1. A surface microfluidic system, comprising:
   a solid substrate;
   a first electrode structure defining a first surface fluidic flow path along a gap between the electrodes of the first electrode structure, the first electrode structure varying along the length of the first electrode structure such that variable-sized droplets are dispensed along the first surface fluidic flow path during use; and
   an electrical source coupled to the first electrode structure.

2. The system of claim 1, further defined as a dielectrophoretic actuator.

3. The system of claim 1, further comprising a control unit configured to control the electrical source.

4. The system of claim 1, wherein the surface of the substrate is not immersed in oil.

5. The system of claim 1, further comprising one or more emulsion droplet reservoirs coupled to the first electrode structure.

6. The system of claim 1, wherein the first electrode structure is tapered.

7. The system of claim 6, wherein the first electrode structure is continuously tapered.

8. The system of claim 7, wherein the first electrode structure comprises individual electrode segments of width (w) and gap (g) varying from at least 40 μm at one end to at most 10 μm at the opposite end along the length of the first electrode structure.

9. The system of claim 1, wherein the first electrode structure comprises a plurality of semi-circular electrode sites spaced at predefined intervals.

10. The system of claim 1, wherein the first electrode structure comprises indentations at predefined locations for precision dispensing of sample droplets.

11. The system of claim 1, wherein the electrical source is an AC electrical source.

12. The system of claim 1, further comprising a second electrode structure, the second electrode structure defining a second surface fluidic flow path along a gap between the electrodes of the second electrode structure and intersecting with the first electrode structure, the second surface fluidic flow path initiating from a predefined site on the first surface fluidic flow path.

13. The system of claim 12, wherein the second electrode structure is fishbone-shaped.

14. The system of claim 12, wherein the second electrode structure is comb-shaped.

15. A method of moving an emulsion droplet, comprising:
   providing one or more sample droplets;
   applying a liquid dielectrophoretic actuation force to the one or more sample droplets to form an emulsion jet along the gap between electrodes of a first electrode structure;
   removing the liquid dielectrophoretic actuation force to dispense emulsion droplets from disintegration of the emulsion jet; and
   applying a droplet actuation force to at least one of the emulsion droplets to cause the at least one of the emulsion droplets to transport along the gap between electrodes of a second electrode structure.

16. The method of claim 15, wherein the emulsion droplets are single-layered.

17. The method of claim 15, wherein the emulsion droplets are multi-layered.

18. The method of claim 15, wherein the emulsion droplets comprise vesicles.

19. The method of claim 18, wherein the emulsion droplets comprise symmetrically assembled bi-layer vesicles.

20. The method of claim 18, wherein the emulsion droplets comprise asymmetrically assembled bi-layer vesicles.

21. The method of claim 15, wherein the emulsion droplets comprise microscopic particles or cells.

22. The method of claim 15, wherein the sample droplets are in the form of emulsion.
23. The method of claim 15, wherein the emulsion jet is formed by mixing at least two dielectrophoretically-actuated liquid jets.

24. The method of claim 15, wherein the first electrode structure varies along the length of the first electrode structure such that the emulsion droplets dispensed are variable in size.

25. The method of claim 24, wherein the first electrode structure is tapered.

26. The method of claim 25, wherein the first electrode structure is continuously tapered.

27. The method of claim 15, wherein the first electrode structure comprises a plurality of semi-circular electrode sites spaced at predefined intervals.

28. The method of claim 15, wherein the first electrode structure comprises indentations at predefined locations for precision dispensing of sample droplets.

29. The method of claim 15, wherein the second electrode structure is fishbone-shaped.

30. The method of claim 15, wherein the second electrode structure is comb-shaped.

31. The method of claim 15, wherein the first and second electrode structures are comprised in a surface microfluidic system.

32. The method of claim 31, wherein the surface microfluidic system comprises a substrate layer, an electrode layer, a dielectric layer, and a hydrophobic layer on the top of the system.

33. The method of claim 31, wherein the surface microfluidic system comprises a first electrical source coupled to the first electrode structure and a second electrical source coupled to the second electrode structure.

34. The method of claim 33, wherein the first and second electrical sources are an AC electrical source.

35. The method of claim 33, wherein the surface microfluidic system further comprises a control unit that is configured to control the first electrical source and/or the second electrical source.

36. The method of claim 15, further comprising mixing at least one of the emulsion droplets with a different droplet at a predefined site.

37. The method of claim 36, wherein the different droplet is transported by dielectrophoretic forces to the predefined site.

* * * * *