Liquid dielectrophoresis and surface microfluidics

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Liquid dielectrophoresis (L-DEP), when deployed at microscopic scales on top of hydrophobic surfaces, offers novel ways of rapid and automated manipulation of very small amounts of polar aqueous samples for microfluidic applications and development of laboratory-on-a-chip devices. In this article we highlight some of the more recent 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. We highlighted the utility of these devices for 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. © 2010 American Institute of Physics. [doi:10.1063/1.3411003]

I. INTRODUCTION

Development of micrototal analysis systems (μ -TAS) or also frequently referred to as laboratory-on-a-chip (LOC) is of increasing interest among the research and industrial community. Such “LOC” technology offers new prospects for routine chemical analysis, drug testing, bioassay, health care delivery, and diagnostic devices including noninvasive and early detection of cancers.

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 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 suitable sensors or detectors to monitor and analyze the contents of fluidic microchannel.^{1–4} Although good progress has been demonstrated along these lines, such systems tend to be complex and require complicated processing and furthermore costly to implement.

In this article we highlight an alternate microfluidic technology that leverages liquid dielectrophoresis (L-DEP) to affect the 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. The salient features of L-DEP phenomena are highlighted in Sec. III followed by a discussion on its on-chip sample manipulation capabilities that have been leveraged in the design and development of novel L-DEP based SMF devices. We also present some recent examples of model biochemical assays implemented on such SMF devices to further demonstrate the utility of these devices in providing chip-based bioassays.

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FIG. 1. Top and sectional views of various L-DEP based SMF devices.

II. FABRICATION

DEP based SMF devices are fabricated in a clean-room environment using conventional microfabrication techniques. Typically, these devices are comprised of an array of microelectrode structures in aluminum or indium tin oxide, patterned on an insulated substrate such as glass or silicon using standard photolithography and wet chemical etching procedures. These microelectrode structures, when electrically 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 are electrically insulated by depositing a thin layer of dielectric material (e.g., silicon nitride, silicon oxide, polydimethylsiloxane (PDMS), or commonly used photoresists such as SU-8), which otherwise will lead to sample electrolysis during actuation. The top surface of the SMF device is rendered hydrophobic by spin coating a thin layer of hydrophobic polymeric materials (typically Teflon®). A hydrophobic surface imparts a high contact angle to sample droplets and minimizes sample adhesion, found to be necessary for sample actuations.

In DEP based SMF systems, sample droplets may be manipulated in air, in an oil bath, or under an oil cover. In the latter, each sessile microdroplet is individually encapsulated in a separate oil covering, formed through the DEP actuation of emulsion jets,⁵ as illustrated in Sec. IV. Unless desired, sample droplets are dispensed and actuated under either an oil cover or oil bath in order to prevent evaporative losses and minimize sample contamination.

Large-scale integration of coplanar 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. An alternate approach implemented to overcome electrode density restriction is through the fabrication of electrodes in two separate metal layers, separated by an insulating dielectric layer. Figure 1 schematically illustrates a single and double metal layer L-DEP SMF. In the latter, a set of L-DEP electrodes is fabricated (photolithography) in bottom and top metal layers and can be oriented in any desired configuration, such as a matrix like geometry which can be used for high-throughput combinatorial biochemical assays.⁶

III. LIQUID DIELECTROPHORESIS

A. Physical phenomenon and a brief historical perspective

L-DEP is an electromechanical phenomenon, describing the motion of polar liquids induced by spatially nonuniform electric fields.⁷ Polarizable liquids, when subjected to a nonuniform

FIG. 2. Initial demonstration of the DEP effect and actuation of nonaqueous dielectric fluids.

electric field, are actuated, conveyed and collected in regions of high electric field intensity. The phenomenon was first investigated by Pellat in 1895, wherein he demonstrated the effect of the ponderomotive DEP forces on dielectric liquids (insulating oil).⁸ Pellat's experimental setup, as shown schematically in Fig. 2(a), is comprised of a pair of parallel electrode 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 nonuniform field effects, rises vertically upward (covering regions of higher electric field) and attains a new equilibrium height h given by

$$h \approx (\epsilon - \epsilon_o)E^2/2\rho g, \quad (1)$$

where $E \sim V/s$, $g (=9.81 \text{ m/s}^2)$ is the acceleration due to gravity, and ϵ_o is the permittivity of free space. In 1971, Melcher and co-workers⁹ 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 ($s=3 \text{ mm}$).⁹ 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 at 400 Hz⁹] is applied across the electrodes, the dielectric liquid (from the upper reservoir) can be raised to a height h_u , at which point the hydrostatic pressure difference between the two reservoirs establishes and sustains the liquid flow between them.

However, practically feasible applications of L-DEP necessitate that the dominating effect of macrolevel forces such as gravity and viscosity be subdued, and actuation voltages reduced to more nominal values. This was achieved by Jones in 2001 by implementing L-DEP at a microscale.¹⁰ Some of the important changes introduced in their work were (1) implementing L-DEP on a planar surface, (2) fabrication of microelectrode structures on an insulated substrate (glass by utilizing standard microfabrication techniques), silicon, and (3) deposition of thin insulating dielectric layers on top of electrodes, which facilitated the actuation of aqueous and conductive media.

B. L-DEP and droplet dispensing

While the benefits of favorable scaling laws at microscopic scales have been exemplified by Jones¹⁰ and Jones *et al.*¹¹ in 2001, growing interests in microfluidics during the past decade have opened new avenues for leveraging L-DEP as a sample manipulation methodology for microfluidic applications. One of the important applications of 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 picoliter volume droplets from a given larger parent sample droplet, as detailed in Sec. III C.

Figure 3(a) schematically illustrates the microelectrode arrangement of a simple L-DEP based droplet dispenser, consisting of a pair of coplanar electrodes of width w , separated by a gap g and an array of semicircular bumps (radius R), which function as droplet collection sites. A “parent sample droplet” ($1\text{--}2\ \mu\text{l}$) is manually pipetted at one end of the structure, as shown in Figs. 3(a) and 3(b). If a sufficiently large ac voltage ($\sim 300\text{--}450\ \text{V}_{\text{rms}}$ at $100\ \text{kHz}$) is applied across the L-DEP electrodes, DEP forces overcome sample surface tension and viscosity to form 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 ceases as the polarized liquid establishes a new hydrostatic equilibrium with the externally applied electric field. Subsequently, upon removal of the applied voltage, the liquid jet instantaneously (within $1\text{--}2\ \text{ms}$) disintegrates into multiple, equivolume “daughter” droplets, which are collected at semicircular bumps. Figure 3(b) (iv) illustrates an array of 19 droplets formed over L-DEP structure of dimensions $w=15\ \mu\text{m}$ and $g=15\ \mu\text{m}$ (and length $=6\ \text{mm}$). The breakup 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.¹² This effect, also referred to as Rayleigh’s instability criteria, is further illustrated in Fig. 4, 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 criterion, 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 (λ),¹² where

$$\lambda = 9R = 9(w + g/2). \quad (2)$$

Furthermore, the semicircular bumps patterned along the electrodes function more than just a passive droplet collection site. Upon removal of the applied ac voltage, these bumps essentially provide a spatially harmonic initial condition [since they are patterned at regular intervals at a spacing of λ given by Eq. (2)] that governs the trend of the ensuing instability in the liquid jet and thereby ensure a uniform breakup of the jet.^{11,13} The breakup of the liquid jet on L-DEP structures devoid of such bumps has also been studied and found to be nonuniform, leading to significant volumetric variations in the daughter droplets thus formed.

C. Volume of droplets

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 are either estimated theoretically, as described by Kanagasabapathi *et al.*¹⁴ and Ahmed *et al.*,¹³ or measured analytically, as shown by Kaler and co-workers.^{15,16} In theoretical estimations volume of each droplet is essentially equal to the liquid inventory held between two consecutive points of jet breakup (illustrated in Fig. 4) and depends on the profile of the liquid jet. Furthermore, theoretical calculation of the dispensed 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. On the other hand, droplet volumes can also be determined ana-

FIG. 3. (a) Typical electrode configuration and arrangement for DEP liquid actuation and droplet dispensing. (b) Video frames captured at different times: (i) $t=0$, (ii) $t=18\text{ ms}$ —liquid finger ejected from parent sample, (iii) $t=40\text{ ms}$ —liquid finger covers the entire electrode structure, and (iv) $t=42\text{ ms}$ —voltage turned off and droplets dispensed at the electrode bump sites. Electrode dimensions: $w=g=15\text{ }\mu\text{m}$, length=6 mm.

FIG. 4. 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: $w=40\text{ }\mu\text{m}$; $g=40\text{ }\mu\text{m}$; $\lambda=540\text{ }\mu\text{m}$.

lytically 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° , in the top view, each droplet appears as a concentric ring, as shown in Fig. 5. The inner and outer radii r_D and R_D for each droplet are measured through any image analysis software, and are further used for calculating droplet volumes by using

$$V = \int_{\phi=0}^{\pi-\phi} \int_{\theta=0}^{2\pi} \int_{r=0}^{R_D} r^2 \sin \phi d\phi d\theta dr = \frac{2}{3} \pi R_D^3 \left(1 + \frac{\sqrt{R_D^2 - r_D^2}}{R_D} \right) \quad (3)$$

(where $\sin \phi = r_D/R_D$).

FIG. 5. Experimental setup utilized for recording high-speed L-DEP actuations, fluorescence imaging, and volumetric analysis of dispensed droplets.

FIG. 6. Microdroplets dispensed by L-DEP actuations on structures with different electrode architectures: (a) uniform width/gap L-DEP electrode; (b) uniform width/gap L-DEP electrode pinched at λ ; (c) continuous tapering width/gap L-DEP electrode pinched at a fixed separation l ; and (d) stepped tapering L-DEP electrode with uniform width/gap electrode sections.

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° . This is often the case in L-DEP actuations where the surface of the SMF device is made hydrophobic and the sessile microdroplets are nearly spherical in shape.

D. Taper L-DEP

So far, L-DEP actuations have been shown to create and dispense an array of equivolume droplets. As described above, the formation of equivolume 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. Recently we have reported on a tapering L-DEP scheme and demonstrated the formation of an array of droplets of varying volume.¹⁷ In a tapering L-DEP structure, shown in Figs. 6(c) and 6(d) and Fig. 7, the electrode width and gap are varied, either discretely or in a continuous fashion, along the electrode length. In such a scenario, the liquid jet emanated from the parent droplet 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 sizes and volumes, as illustrated in Fig. 6(d). Droplet volumes, determined analytically, are summarized in Table I along with electrode dimensions. 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, Fig. 7 illustrates an array of different volume droplets formed by actuating a DNA-PicoGreenTM (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).¹⁸ The fluorescence emissions from each droplet were individually detected and quantified (as photocurrent; I_p) using a photomultiplier tube (PMT) (a block diagram of the experimental setup is schematically illustrated in Fig. 5) and are shown plotted in Fig. 7(b), where the I_p values provide us 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 biomolecules (proteins, enzymes, or cells) may be readily achieved, which can then be mixed (on-chip) with another set of buffer droplets (using droplet actuation schemes described in Sec. V) 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. Such and other applications for the tapered L-DEP structures are currently being investigated.

(a)

(b)

FIG. 7. (a) Bright field and fluorescence images of variable volume Pico green labeled DNA sample droplets. (b) The PMT current confirms the uniform dispensing of DNA sample in equivolume (black) and variable volume dispensing arrangement (red).

E. Jet breakup

The breakup of the liquid jet into microdroplets, as discussed and illustrated in Secs. III B–III D, 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 us with necessary formulations to configure precise droplet formation sites by determining the spacing between semicircular bumps. This, however, poses an important limitation on the density of the droplet array, i.e., the droplets can only be formed at regular intervals spaced by a distance $\lambda(=9R)$. In certain applications, such as those involving large scale integration (LSI) (discussed in Sec. V), it may be desired to have a more proactive control over liquid jet breakup and spacing between consecutive droplets. Furthermore, the semicircular bumps placed along the L-DEP electrodes, which provide the static harmonic initial condition, governing the course of

TABLE I. L-DEP electrode dimensions and typical values of associated droplet volumes. The droplet volumes were determined analytically as per Eq. (3) and described in Sec. III C.

L-DEP electrode dimensions ($w=g$; μm)	Radius of liquid finger ($R=w+g/2$; μm)	Rayleigh's wavelength ($\lambda=9R$; μm)	Volume of daughter droplet $V=\pi\lambda R^2/2$ (theoretical)	Volume of daughter droplet $V=2/3\pi R_D^3(1+\sqrt{R_D^2-r_D^2}/R_D)$
10	15	135	47 pl	15–20 pl
15	22.5	202.5	161 pl	90–100 pl
20	30	270	380 nl	230–250 pl
30	45	405	1.28 nl	1.2–1.4 nl
40	60	540	3.05 nl	2.2–2.4 nl

instability (as delineated Sec. III B), 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 breakup of liquid jet. In a recent study (further validating the above conjecture), Ahmed *et al.* showed L-DEP actuations on structures wherein the spacing between bumps was fixed at $\lambda/2$ and 2λ [$\lambda=9R$ being the ideal value, Eq. (2)].¹³ They reported the jet breakup to be highly nonuniform for bump spacing $\lambda/2$, while in the latter case, the jet breakup was observed to be imperfect (bump spacing 2λ) resulting in the formation of undesired satellite droplets at regular intervals of λ .

Our recent investigations have revealed that such imperfections in the breakup of the liquid jet can be overcome by incorporating structural modifications in the form of “pinches” along the L-DEP electrodes. A pinch, depicted in Fig. 6(c), is an indentation in the L-DEP electrode that can be incorporated at locations where the jet breakup is desired. In terms of functionality, a pinch essentially promotes capillary instability and in turn redefines points of maximum hydrodynamic instability (for the liquid jet), compared with those arising in a homogenous semicylindrical liquid jets. The efficacy of pinch structures is demonstrated through the micrographs presented in Fig. 6, which shows an array of droplets formed over two distinct L-DEP electrode structures. In Fig.

FIG. 8. Emulsion droplet dispensing schemes leveraging L-DEP.

FIG. 9. Photographs of emulsion jets and subsequently dispensed daughter droplets of various samples: (a) sample2-5cSt oil jet and (b) daughter droplets; (c) emulsion jet of sample3-5cSt oil and (d) formed daughter droplets; (e) emulsion jet of sample4-5cSt oil; and (f) formed daughter droplets; (g) emulsion jet of DI water(sample1)-5cSt oil on $w=g=30\text{ }\mu\text{m}$ structure; and (h) emulsion jet of DI-20cSt oil on $w=g=15\text{ }\mu\text{m}$ structure. Reprinted with permission from R. Prakash and K. V. I. S. Kaler, *Lab Chip*, 9, 2836 (2009). Copyright 2009, The Royal Society of Chemistry.

6(b), an array of droplets is generated with periodicity 2λ (instead of λ) by controlling the breakup of the liquid jet through pinched structures, furthermore ensuring nearly perfect breakup of the jet without the formation of satellite droplets. In addition to this, the L-DEP electrode shown in Fig. 6(c) presents a more interesting case, where a pair of continuously tapering electrodes (w and g from 40 to $15\text{ }\mu\text{m}$), laden with pinch structures, was utilized for controlling droplet spacing. Note that in tapering L-DEP electrodes (devoid of any pinch indentations), also shown in Fig. 6(d), the spacing between the bumps is a function of electrode dimensions w and g [represented by Eq. (2)]. However, in the continuously tapering structure, the spacing between the bumps and droplets is kept constant ($=400\text{ }\mu\text{m}$) throughout that does not conform to Eq. (2), which clearly demonstrates that the breakup of the liquid jet can be proactively controlled through structural modifications in the L-DEP electrodes.¹⁹ A further understanding of the pinch structures is a subject of present investigations, where we are trying to uncover its limitations in controlling jet breakup.

IV. L-DEP AND EMULSIONS

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 us to extend the droplet dispensing mechanism to single and multilayered emulsion droplets.

There are two possible ways that one can implement L-DEP to produce an aqueous-oil emulsion droplet, as shown in Fig. 8. Scheme 1 involves a parent droplet in form of a microliter aqueous 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 positioned single emulsion droplets at specific bump sites. The dynamics and breakup of such an emulsion jet has been explored and furthermore compared with the behavior of a homogeneous liquid jet.⁵ The breakup of different aqueous solution [de-ionized (DI)-glycerol mixture]-in-oil emulsion jets results in formation of arrays of single emulsion droplets, as shown in Fig. 9. Furthermore, stability

FIG. 10. Micrographs showing (a) bright field and (b) fluorescent images of an array of 30 pL sample2 (glycerol 12.5% by volume in DI) emulsion droplets dispensed using L-DEP actuation scheme on $w=g=15\text{ }\mu\text{m}$ electrode structure; observed under 10x magnification; a typical single emulsion droplet observed under 50x magnification. Reprinted with permission from R. Prakash and K. V. I. S. Kaler, *Lab Chip*, 9, 2836 (2009). Copyright 2009, The Royal Society of Chemistry.

and reliability of this emulsion dispensing mechanism were observed in.⁵ Figure 10 demonstrates such an array of single emulsion droplets (volume=30 pL) dispensed using $w=g=20\text{ }\mu\text{m}$ electrode structure and a magnified image of individual emulsion droplet.

Alternatively, one can use scheme 2 where we actuate microliter liquid sample located at one end of the electrodes and have another parent oil droplet placed at the opposite end of the electrode structure. The mechanism of formation of an emulsion jet is a two step process. In step 1 the homogeneous aqueous jet forms and spreads over the electrode length and in step 2 the oil redistributes itself over the already formed homogeneous jet, forming a water-in-oil emulsion jet. The electric field is now removed and the emulsion jet breaks up, forming emulsion daughter droplets at specific bump sites.

More recently, we have extended the concept of forming emulsion droplets to multilayered emulsions. To achieve this, one can combine scheme 1 and scheme 2 in such a way that a double or higher order emulsion jet is formed. The breakup of such a multilayered emulsion jet results in the formation of multilayered emulsion droplets. We have successfully formed aqueous-in-oil-in-aqueous double emulsion jet and double emulsion droplets 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. This process results in the water jet overlapping and goes over the already formed single emulsion jet and forming a double emulsion jet. Micrographs illustrating the dispensed single and double emulsion droplets are shown in Fig. 11.¹⁹

Application of this emulsion dispensing scheme is envisioned in various fields including microTAS, on-chip handling and storage of cells, and various other biological samples for extended periods in controlled environment, in solving the encapsulation issues in SMF devices, forming laser fusion targets, cryogenics on a chip, targeted drug delivery mechanism.²⁰

V. INTEGRATED LIQUID DEP MICROCHIP TECHNOLOGY

One of the key requirements 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,

FIG. 11. Micrographs comparing dispensed single and double emulsion droplets. (a) Section of an array of aqueous glycerol solution (25% by volume)-in-5cSt silicone oil single emulsion droplets. (b) A single emulsion droplet observed under 50x magnification. (c) Section of an array of aqueous glycerol solution (25% by volume)-in-5cSt silicone oil-in-aqueous glycerol solution (25% by volume) double emulsion droplets. (d) A double emulsion droplet observed under 50x magnification (water-in-oil-in-water-type double emulsion).

and predetermined fashion. While in closed channel microfluidic devices this is achieved by controlling liquid flow through microvalves and pumps, in SMF devices, individual microdroplets are manipulated (split, merged, mixed) by means of electric fields^{11,21,22} surface acoustic waves^{23,24} or spatial thermal gradients.²⁵ To further advance the sample manipulation capabilities of DEP based SMF devices, we have recently demonstrated the integration of an electrostatic droplet actuation methodology with the L-DEP droplet dispensing scheme.^{17,26} Such an integration has been shown to facilitate the transport and mixing of nano- and picoliter droplets (formed by L-DEP actuation), and has opened new avenues for the development of DEP based high-throughput SMF devices.

The electrostatic droplet actuation scheme, developed initially by Gunji *et al.*,²⁷ utilizes low frequency electric fields (configured through a pair of coplanar electrodes) for moving microdroplets on planar substrates. Figure 12 presents an integrated L-DEP and droplet actuation structure and schematically illustrates the fishbone shaped electrode architecture for droplet actuations, suitably incorporated with L-DEP electrodes at droplet formation sites (i.e., the semicircular bumps). Once an array of droplets is obtained, a low frequency ac voltage (10–30 Hz) is selectively applied across the fishbone electrodes to transport daughter droplets to an on-chip location,

FIG. 12. Integrated L-DEP and droplet actuation structures. Once an array of droplets is dispensed via L-DEP actuation, a low frequency (20 Hz) ac voltage is applied across fishbone-shaped electrodes for droplet transport.

where they can be mixed with other sample or reagent droplets, as shown in Fig. 12. The integrated liquid DEP and droplet actuation scheme can be furthermore leveraged for the design and development of high-throughput SMF systems through LSI.

A LSI system essentially consists of 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. We incorporate this simple integration 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 High Throughput Screening (HTS) system, overlap of electrical wiring and electrodes is anticipated in most instances. One way to avoid such overlap is to fabricate wires and electrodes in two separate metal layers which are isolated from each other by a dielectric sandwich between them as discussed previously in Sec. II and shown in Fig. 1. Using a two layer fabrication scheme, we have successfully designed and demonstrated a 2×2 matrix that 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. 13 and its applicability in achieving complex biochemical assays is detailed in Sec. VI. 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 $M \times N$ array²⁸ that would work as a microfluidic platform for achieving any set of combinatorial biochemical assays involving M samples and N reagents.

VI. APPLICATIONS

In this section we present some recent examples and demonstrations of biochemical assays that have been reported and implemented on DEP based SMF 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 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,^{11,29} but L-DEP actuation of samples with high electrical conductivity ($\sigma > 30 \times 10^{-4} \text{ S m}^{-1}$) 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 nonionic 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. Another problem plaguing SMF actuation schemes in general is the nonspecific

FIG. 13. Combinatorial biochemical assay on a 2×2 matrix.

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.³⁰ In addition to this, biofouling followed by uncontrolled and unrestricted desorption of proteins becomes a source of cross contamination, which limits device reusability. Researchers have developed intricate strategies to address and overcome the problem of biofoul-

ing, 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.^{31–34} 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. In SMF devices the presence of low viscosity and low surface tension oils encapsulates 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. In a recent study, Luk *et al.*³⁵ reported on the use of biocompatible polyethylene glycol (PEG) based additives in protein solutions to overcome biofouling on Teflon[®] coated surfaces in electrowetting based microfluidic devices.³⁵ They showed that actuation of concentrated protein solutions (up to 50 mg/ml; bovine serum albumin (BSA), fibrinogen) is greatly facilitated (in air) by adding a small amount of PEG in protein solutions.

A. On-chip DNA analysis

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 quantifying 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 ultralow concentrations), especially required in microfluidic studies, where sample volumes are typically in the range of nanoliters to picoliters, 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.

Recently, we have reported on fluorescence based DNA quantification on a SMF device using the integrated liquid DEP and droplet actuation scheme presented in Sec. V and illustrated in Fig. 12.¹⁶ In this study, aliquots of dsDNA (pUC57) and a well known nucleic acid stain, PicoGreen[®] (Molecular Probes, Invitrogen) were individually actuated on a pair of L-DEP electrodes. The nanoliter 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 PMT, which provided a direct measure of the amount of DNA present in each droplet.

In another article, we have presented DNA hybridization analysis on a 2×2 SMF device,¹⁵ illustrated in Fig. 13, the 2×2 SMF devices consist of four sets of L-DEP electrodes arranged in a matrix like geometry along with droplet actuation electrodes integrated at suitable locations. The 2×2 SMF devices facilitate the actuation and mixing of four different oligonucleotide samples, labeled with different reporter and quencher fluorochromes, and allowed us to study base pair complementarities using the principles of fluorescence resonance energy transfer method. Other prominent examples of DNA assays demonstrated on SMF platforms (which utilize the widely popular phenomena of electrowetting for sample manipulation) include DNA ligation,³⁶ nucleic acid extraction and purification,³⁷ and DNA amplification through polymerase chain reaction (PCR).^{38,39} In a recent report, Malic *et al.*⁴⁰ presented an electrowetting based SMF system, configured for patterning substrates (referred as biochips) with an array of oligonucleotides. Their SMF device is comprised of a top and bottom plate,⁴¹ wherein the hydrophobic Teflon[®] layer of the top plate (which is also the biochip) was selectively patterned to expose the underlying gold layer (which also serves as the ground electrode) for the immobilization of oligonucleotides (tagged with thiol groups). Reagent and sample droplets containing oligonucleotides were transported to on-chip immobilization/binding sites (using electrowetting actuation) and were allowed to covalently attach to the exposed gold surface. The biochips were tailored for use in standard bench-top surface plasma resonance imaging systems for DNA hybridization studies.

VII. CONCLUSION

The ability to dispense and subsequently manipulate liquids in the form of droplets using L-DEP provides new and novel means of handling various types of biological samples and chemical agents and microscopic particles. By suitably integrating the droplet dispensing and transport schemes we have demonstrated the utility of such SMF devices in performing biochemical assays. The L-DEP device technology at present relies on off-chip detectors and sensors to analyze the contents of the dispensed and manipulated droplets. This limitation in the present deployment, however, can be overcome by hybrid integrating sensors/detectors and sources.

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