Nanotechnology Science and Technology

Microfluidics

Control, Manipulation and Behavioral Applications

Stefano Panzarella
Walter Maroni
Editors

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MICROFLUIDICS

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PREFACE

In this book, the authors discuss the control, manipulation and behavioral applications of microfluidics. Topics include dielectrophoresis based droplet microfluidic devices for on-chip bioassays; microfluidic biosensor systems for cell biology and drug discovery; controllable microfluidic generation of monodisperse multiple emulsion droplets; an alternative method to predict wettability for microfluidics; integration of capacitive micromachined ultrasound transducers to microfluidic devices; selection of easily accessible PCR-and bio-compatible materials for microfluidic chips; and electrokinetic manipulation of biomolecules.

Chapter 1 – Dielectrophoresis (DEP) is one of the principle electrokinetic methods of handling and processing micro-sized dielectric particles and biological cells immersed in aqueous media.

Example applications include the utility of DEP to affect separation of cells based on polarization, cell trapping/concentration, cell aggregation, etc. More recently the DEP phenomena/effect has been exploited at microscopic scales and successfully demonstrated to be capable of electro-actuating aqueous polarizable liquids on top of surfaces. This liquid DEP actuation capability has been utilized for novel dispensing, handling/manipulation of ultra-low volume aqueous suspension droplets in a rapid and automated fashion. This DEP assisted droplet dispensing capability and its integration with subsequent downstream manipulation capability suggests numerous means of applying it for lab-on-a-chip based biological/bio-chemical assays, employing ultralow sample/reagents.

The aim of this book chapter is to provide a concise review of the DEP phenomena, focusing primarily on its application to the actuation of polarizable aqueous media. In the first chapter section, the authors examine from a theoretical standpoint, the fundamental requirements of DEP assisted liquid actuation and the underlying concepts behind its various applications for lab-on-a-chip (LOC). The authors furthermore discuss how the dispensed daughter droplets, using another set of coplanar electrodes and AC excitation frequencies, can be individually transported to specific locations on the surface. Since the proposed utility of the devices focuses on bio-chemical samples and reagents, the authors specifically examine the impact of sample electrical and fluidic properties on the L-DEP actuation, droplet dispensing and transport.

This will be followed by a discussion of various approaches available for the fabrication of DEP surface microfluidic devices for reliable and repeatable operation.

Having laid a foundation for DEP based microfluidic devices, the article focuses on demonstrating various capabilities of the ultrafine droplet dispensing and manipulation

scheme. The versatility of the scheme is illustrated by dispensing homogeneous and multilayered droplets (emulsions/vesicles) as well as controlled dispensing of functionalized micro-beads and nanoparticles. Some frequently used bioassays, such as DNA quantification, selective DNA hybridization, synthesis of bilayer vesicle, membrane based bio-detection assay and 'bead based' DNA/RNA hybridization detection assay, are demonstrated leveraging DEP surface microfluidic technology to facilitate miniaturized chip based bio-detection/analysis. Other novel technology capabilities include the precision dispensing of monolayer/bilayer vesicles which can serve as scaffolds for the assembly of artificial cells. The concluding section examines the proposed improvements and future development and application of the DEP droplet technology.

Chapter 2 – The ability to translate drug-target interactions into cell phenotypic signatures has made label-free biosensors become a powerful platform for the molecular delineation of receptor signaling and drug pharmacology in native cells. Microfluidics enables miniaturization of assays, increases experimental throughput, and allows precise control of cell phenotypes, molecular concentrations and drug-target interactions. Integration of microfluidics with label-free biosensors would offer additional dimensions for investigating the cellular and molecular mechanisms of receptor signaling and drug actions down to single cell level. This chapter first reviews key aspects of microfluidics for studying cell biology, and discusses the recent advances and applications in cell biology and drug discovery of microfluidic biosensor devices.

Chapter 3 – Multiple emulsions, or "emulsions of emulsions", are complex nested liquid systems, in which dispersed droplets contain smaller droplets inside. They are widely used to encapsulate active ingredients in myriad applications, including drug delivery systems, foods, cosmetics, chemical separations, and syntheses of microspheres and microcapsules. Accurate control of size monodispersity and internal structure are critical for the versatility of such emulsions, because these attributes allow precise manipulation of the encapsulation of substances and the structure of synthesized materials. Compared with other emulsification techniques, such as multistep bulk emulsification and membrane emulsification, recently developed microfluidic technique shows much greater power in controlled generation of monodisperse multiple emulsion droplets. In this Chapter, droplet microfluidic device with highly scalability for generating monodisperse multiple emulsion droplets are firstly introduced, from which the readers can see the precise control on both the size and number of the inner droplets in multiple emulsions; then, based on this device, novel devices for controllable encapsulating droplets with different components in each level of multiple emulsions are introduced; the size, number, and ratio of the multicomponent inner droplets are under excellent control, which largely extend the emulsion structures and benefit the application of multiple emulsions.

Finally, the application of these multiple emulsions are demonstrated by template synthesis of functional microcapsules with highly monodispersity, controlled inner structures and encapsulation characteristics.

Chapter 4 – Wettability properties inside the microchannels of microfluidic devices have received increasing attention over the past few years due to their important roles in controlling liquid flow. Conventionally, the wetting properties of a surface are determined by contact angle measurement where a liquid drop is placed on the surface and the angle between the solid surface and liquid-vapour interface is measured. The size of the liquid drop on the surface is often larger than the size of components or features in microfluidic systems.

This renders conventional methods for measuring contact angle incapable of delivering the information required. This chapter presents a brief review of the authors' recent research in the development of an indirect approach for predicting contact angle by the use of surface analytical methods such as time-of flight secondary ion mass spectrometry (ToF-SIMS) and x-ray photoelectron spectroscopy (XPS). Two examples have been shown here. One is to predict micron-scale surface wettability maps of substrates by exploitation of surface chemical imaging using SIMS. Using the surface sensitive and chemically specific spectroscopy available with SIMS, it was possible to obtain information on species that are related to wetting behavior and their distribution over the surface. Another example has demonstrated that XPS, similar to SIMS, has the potential to give precise surface chemical information and provide insights into the quantitative assessment of the surface functional groups, allowing the prediction of surface wettability. The alternative method described here is of direct benefit to industries where wettability is crucial but contact angle measurements cannot be directly acquired on more complicated or flexible surfaces.

Chapter 5 – The design and manufacturing flexibility of capacitive micromachined ultrasound transducers (CMUT) makes them attractive option for integration with microfluidic devices both for sensing and fluid manipulation. CMUT concept is introduced here by presenting simplified equivalent circuit model; while finite element analysis (FEA) and near-field CMUT operation experiments were used as the main methods of research. The transient time FEA was used to simulate the transmission and reception of the short pulse and then analysis of the CMUT membrane motion in the time and frequency domains was made. 200 nm finite element spacing and 0.2 ns sampling time was used during the simulations for increased precision and reliability of the results. Surface micromachined CMUT devices with two-phase interdigital arrangement of the transducer elements were fabricated for experiments. The microchannels of different heights were modeled by attaching the adhesive tape of various thicknesses at the sides of the CMUT devices to form the bead for the liquid. The drop of the transformer oil was put in the microchannel before attaching the upper microchannel wall. Afterwards the electromechanical impedance of CMUT was measured with the network analyzer to evaluate the CMUT operating regime.

The finite element analysis showed that when the microchannel cross-section dimensions are comparable with the CMUT cell dimensions, the transducer is subjected to the pressure waves bouncing between the microchannel walls and comparatively compliant CMUT membranes. This situation causes the distortion of the CMUT operating regime and has to be accounted during the design and use of CMUT-coupled microfluidic devices. Corrections for the amount of the near-field distortions are to be made already during the design phase.

The authors found significant relationship between the CMUT membrane motion characteristics and the microchannel height. For example: CMUT having 5 MHz central frequency in immersion exhibits the broad band frequency spectra when in normal operating conditions, but the central frequency decreases by 60-70 % with decreasing the microchannel height from 400 microns to 2 microns, and the frequency band becomes narrower. It is also shown here how the simulated results can be experimentally verified.

Chapter 6 – Conventional fabrication of microfluidic chip is a complicated and time, effort and material consuming process. Consequently, due to high expenses, it has poor applicability for performing mass biological analysis by microfluidics. In this study, the authors report several measures to make simple, low-cost and rapidly fabricated two-dimensional (2D) and three-dimensional (3D) microfluidic chips for biological analysis. The

authors employed various easily accessible and polymerase chain reaction (PCR) compatibility verified materials in the process of design, patterning and bonding. Firstly, they established a simple method for evaluating the PCR compatibility of various common materials, as DNA and proteins are common components or targets in biological analysis. These materials employed in fabricating microfluidic chips, include silicon, several kinds of silicon oxide, glasses, plastics, wax, and adhesives. Two-temperature PCR was performed with these materials to determine their PCR-inhibitory effect. In most cases, the addition of bovine serum albumin (BSA) effectively improved the reaction yield. The authors also studied the individual PCR components from the standpoint of adsorption. Most of the materials did not inhibit the DNA, although they noticeably interacted with the polymerase. The authors' results provide an overview of materials that are PCR-friendly for fabricating microfluidic devices. The PCR reaction, without any additives, performed best with pyrex glass, and it performed worst with PMMA. Then, based on the results of the PCR-compatible screening test, the authors concentrated on fabricating microfluidic chips using easily accessible materials, such as paper and wax. The authors successfully shortened the whole fabrication process for complicated 2D and 3D microfluidic chips from the usual several days into several minutes by using easily accessible materials and methods. They demonstrated that these types of chips have good bio-compatibility enabling their applications in many biological analyses such as PCR and DNA capillary electrophoresis. The authors also dramatically simplified the process of fabricating complicated 3D chips by using cyanoacrylate-based resin or adhesive wax as the bonding material to integrate chip materials such as paper, glass slides or other polymer films. With this process, a 3D microfluidic chip is achievable by vacuating and venting the chip. And the bio-compatibility and applicability of the paper-based 3D microfluidic chip was verified in the applications such as PCR, *HeLa* cell electroporation and the chemotaxis of *E. coli*. In all, the authors' PCR-compatibility test provides good hints as to the selection of materials for microfluidic chips employed in biological analysis. Base on this, the authors succeeded in fabricating complicated microfluidic chips using easily accessible materials that may make it much more applicable than conventional methods.

Chapter 7 – Electrokinetics in microfluidic system provides effective molecular manipulation technique in micro/nano domain, which matches the length scale of biomolecules. A lot of demonstrations have been reported and shown the manipulation of different biomolecules using different kinds of electrokinetic phenomena. This chapter reviewed the most commonly used electrokinetic phenomena including DC electrophoresis, AC electrophoresis, DC electroosmosis, AC electroosmosis, electrowetting, and opto-electrically induced electrokinetics. The principle behind and recent developments on the electrokinetic manipulation will be discussed. An updated and systematic in-depth discussion is provided in the field of electrokinetic manipulation.

CONTENTS

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Chapter 1

DIELECTROPHORESIS BASED DROPLET MICROFLUIDIC DEVICES FOR ON-CHIP BIOASSAYS

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ABSTRACT

Dielectrophoresis (DEP) is one of the principle electrokinetic methods of handling and processing micro-sized dielectric particles and biological cells immersed in aqueous media.

Example applications include the utility of DEP to affect separation of cells based on polarization, cell trapping/concentration, cell aggregation, etc. More recently the DEP phenomena/effect has been exploited at microscopic scales and successfully demonstrated to be capable of electro-actuating aqueous polarizable liquids on top of surfaces. This liquid DEP actuation capability has been utilized for novel dispensing, handling/manipulation of ultra-low volume aqueous suspension droplets in a rapid and automated fashion. This DEP assisted droplet dispensing capability and its integration with subsequent downstream manipulation capability suggests numerous means of applying it for lab-on-a-chip based biological/bio-chemical assays, employing ultralow sample/reagents.

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1. INTRODUCTION

Microfluidic systems handle sample volumes in range of microliter and lower while utilizing structures and geometries in micron/sub-micro region. A microfluidic system contains a combination of generic components to achieve the most essential of laboratory functionalities on the miniaturized scale. These components include:

- Method for introducing samples and reagents, i.e. Dispensing and sample loading
- Method for moving samples to targeted regions, i.e. transport/manipulation
- Method for combining or mixing the samples/reagent
- Method for detection, sample purification/pre-screening etc…

Much of the work to date has focused on technology that can demonstrate some or all of the aforementioned capabilities. A microfluidic device that achieves all the above mentioned functionalities is essentially a miniaturized replacement to the conventional Laboratory and is often deemed as "Lab on a Chip (LOC)" device [1, 2]. Based on the methodology implemented to achieve one or more of the targeted applications, such devices have been classified as follows:

1.1. Close Channel Microfluidic Device or, Analog Microfluidic Device

These are continuous flow devices that leverage micro-channels, high pressure micro-valves, pumps and tubing arrangement to facilitate LOC functionalities [3]. The micro-channels and micro-valves are usually fabricated in PDMS using soft-lithography and assembled with the off-chip pumping/tubing arrangement to feed the liquid sample at the required high pressure [4, 5]. Since flow in micro-channels is mostly laminar given that viscosity dominates over inertia at these scales, it results in a non-turbulent mixing scheme which occurs as a result of diffusion of molecules across the interface between the fluids. So, the mixing achieved in micro-channels requires long channel lengths.

Also, the requirement of bulky off-chip components hinders the possibility of multiplexing and microfluidic large scale integration (MF-LSI). Large scale integration (LSI) refers to the ability to integrate numerous functional components on one device to achieve

complex tasks. In closed channel microfluidics, MF-LSI refers to microfluidic chips with numerous micro-mechanical valves that can transport and manipulate numeral fluidic samples in parallel and automated fashion [6]. MF-LSI and high throughput screening (HTS) [7, 8] are two more recent advancements in close-channel microfluidics. Different liquid handling techniques have been attempted to overcome certain drawbacks of the conventional close channel method. Quake and co-workers have demonstrated design and fabrication protocols for multiplexed arrays of monolithic valves which are addressed in a controlled, multiplexed fashion, enabling MF-LSI and HTS [8, 9]. This is achieved by using multilayer soft-lithography technique in two overlapping layers. The 'flow' layer contains the microfluidic channels and the 'control' layer harbors the channels required to actuate the valves and control the flow. In another recent approach, centrifuge-based close channel microfluidic platforms, also known as "Lab on a Disc" are devised where the underlying physical principles of centrifugal pumping is utilized in context of microfluidic systems and the various centrifuge fluidic functions, such as valving, mixing, sample splitting and separation, are introduced [10]. But, even with the advent of multiplexing in close-channel microfluidic, sensor integration and bulky off-chip component remain as major unaddressed drawbacks of this technology.

1.2. Surface Microfluidic Device or, Digital Microfluidic Device

In the case of surface microfluidic (SMF) devices, precision sample droplets, controlled over patterned surfaces are used to dispense and manipulate various samples and reagents. Several droplet actuation and manipulation schemes have been utilized in recent years. Some of these schemes such as: a) droplet manipulation by controlling the surface wettability (surfaces coated with self-assembled monolayer) [11]; b) use of surface temperature gradients for sample droplet manipulations [12] are deemed as passive since there is no active switching of external fields. Passive droplet transport schemes are slow and restrictive and hence they have mostly been replaced by more common active digital microfluidic technologies where the electric field is actively controlled to achieve droplet manipulations. One of these schemes is based on Surface acoustic waves (SAW), where patterned, inter-digitated (IDT) electrodes are used to create controlled surface acoustic vibrations which control the transport of individual sample droplets [13]. Additional schemes have also been proposed where droplets are manipulated using piezo-electric effects and by using acoustic waves [14], but the two more popular SMF actuation approaches are based on use of patterned, programmable electrodes which are suitably energized using an external AC/DC supply to overcome the fluidic resistance to achieve droplet manipulation. The two popular schemes are: Electrowetting (EW) or, Electrowetting-on-Dielectric (EWOD) and Dielectrophoresis (DEP).

Electrocapillarity, first demonstrated by Gabriel Lippmann in 1875 [15], is quoted as the basis for the modern day electrowetting devices (EW). In such EW devices, low voltage (0-100 Vpp), low frequency (DC-1 kHz) electric field are implemented to perturb the interfacial equilibrium of the liquid-surface or liquid-liquid interface and hence control the shape of a sample/reagent droplets. Suitably designed and fabricated electrode arrays can be utilized to facilitate dispensing, splitting, transport, merging and multiplexed screening of sample/ reagent droplets and henceforth achieving most of the requirements of a LOC devices [16, 17,

18]. Although, EW or, EWOD actuation schemes provide more flexible droplet handling capabilities, its speed and proficiency is controlled by their fluidic properties.

Dielectrophoresis (DEP) was originally defined by H. A. Pohl in 1958 [19] as the underlying concept for manipulating dielectric media under the influence of spatially non-uniform electric field. However, the first experimental demonstration of DEP goes further back to Pellat's demonstration of wall-less flow systems in 1894 [20]. Melcher's dielectric siphon in 1971 [21] was another experiment implementing the non-uniform electric field to manipulate fluidic dielectric samples. Both these observations, which are summarized in the theory section, leveraged DEP on macroscale and required extremely high actuation voltages (~ 30 kVrms) but when the dimensions are scaled down to micron or sub-micron scale as in case of microfluidics, the required actuation voltages can be substantially lowered due to a favorable scaling relation (see Theory section) [22]. DEP has now emerged as a successful tool to manipulate samples for on-chip, microfluidic applications.

The phenomenon of DEP has since been implemented in flow separation [23], classification and segregation of bio-particles in various fluidic media (particle-DEP) [24, 25, 26] and for dispensing and subsequent manipulation of ultrafine (in nL – pL volume range), sample/reagent droplets [27, 28, 29], commonly known as Liquid-DEP (L-DEP). This chapter focuses on the various characteristic attributes of L-DEP and covers its advancement as a suitable microfluidic tool for chip-based bio-applications.

Electrostatic droplet actuation or, D-DEP: The electrostatic droplet actuation scheme, also termed as droplet-DEP (D-DEP) [29, 30, 31] has been used to achieve subsequent manipulation of L-DEP actuated droplets in the reported work. Electrostatic droplet actuation scheme was first demonstrated by Washizu and co-workers [30] in 2004.

The actuation scheme uses fishbone shaped electrodes, energized at low frequency AC voltage/or voltage pulse in order to manipulate droplets on open surfaces. The underlying concept of this actuation methodology is explained in a later section.

2. THEORY

2.1. Liquid Dielectrophoresis- The Phenomena and Its Characteristic Features

L-DEP- Theoretical background

As described earlier, DEP is an electrokinetic phenomenon that dictates the influence of an external, spatially non-uniform electric field, on a dielectric medium. In case of fluidic samples, the DEP force acts on the polar fluidic molecules and tends to redistribute the fluidic mass towards the regions of high field intensity. This electrokinetic re-distribution is in practice opposed by the fluidic surface tension (F_γ) and the viscous damping force (F_μ) and was termed as liquid-dielectrophoresis (L-DEP) by Jones and co-workers [27, 32]. Essentially, in case of L-DEP actuation, polarizable liquid is manipulated by this ever changing hydrostatic equilibrium of the DEP force and the fluidic resistance and is well contained and furthermore shaped by the spatially non-uniform electric field.Even before the phenomenon of L-DEP was officially termed and defined, there were intuitive demonstrations where electric field based ponderomotive force was used to demonstrate electrocapillarity

effect. This includes Pellat's classical demonstration in 1894 [20] which provides a basis for wall-less flow of dielectric liquids. In his experiment (shown in Figure 1(a)), Pellat used two plane parallel electrodes, oriented vertically at a separation s and partially immersed in a dielectric liquid (density ρ and permittivity ε). When a voltage V is applied across the electrodes, in order to attain a new hydrostatic equilibrium, liquid between the two electrodes rises to a height h given by,

$$h \approx \frac{(\varepsilon - \varepsilon_0)V^2}{2s^2 \rho g} \tag{1}$$

where, g = 9.81 m/s^2 is the acceleration due to gravity and ε_0 is the permittivity of space.

Another example of wall-less flow structure is the dielectric siphon [21] which was demonstrated by Melcher and co-workers in 1971 and is shown in Figure 1(b). In this set-up, they had two closely spaced (s < 1 cm) parallel electrodes running between two reservoirs placed at different levels and containing dielectric liquids.

At sufficiently high voltage (30 kVrms at 400 Hz), a fluidic connection was successfully established between the two reservoirs. Since both the demonstrations required impractically high voltages in order to achieve fluidic manipulation at macroscale, the use of DEP was less favored as compared to conventional hydraulic and pneumatic components (valves and pressure pumps). However, in more recent attempts by Jones and co-workers (1975), where they used DEP to maneuver dielectric fluids at miniaturized scales [21, 22], it was successfully demonstrated that excessive voltage requirements and issue of electrical break-down can be avoided at microscale applications mostly because of the favorable scaling laws.

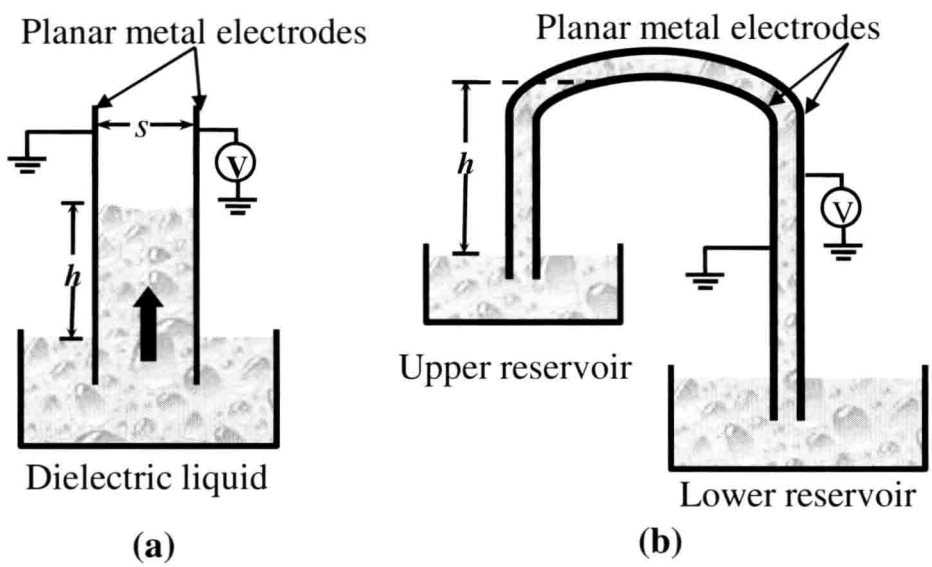

Figure 1. Schematics showing (a) Pellat's classic demonstration of a wall-less flow structure; (b) Dielectric siphon developed by Melcher and co-workers.

Table 1. Rheological and electrical properties of used liquid samples

Sample name	Composition (% by Vol. of glycerol)	Jet Capacitance (C_{eff} in pF)*	Viscosity (cSt)*	Surface Tension (dyne/cm)*	Conductivity (μS/cm)
1	0	553.60	1	73	0.65
2	12.5	539.76	2	71	2.4
3	25	519	4	68	6.0
4	50	477.48	8	65	15.5

* Jet capacitance, viscosity, surface tension and conductivity values referred from [35, 38].

The reason behind this can be better understood once the concepts of L-DEP are elaborated in the next section. In 2001, Jones and co-workers [32] developed an L-DEP based droplet dispensing scheme by using miniaturized co-planar metal electrodes which were micro-fabricated on silicon/glass substrates.

Once successfully implemented in fluidic manipulation at microscale, L-DEP based scheme have since been modified and state-of-art microfabrication techniques have been used to devise DEP based SMF LOC devices [29, 33, 34].

The two most common scenarios of DEP based dielectric manipulation are often classified as: particle-DEP, where a dielectric micro-particle is moved, screened or, sorted by means DEP forces and Liquid-DEP (L-DEP) where polarizable/dielectric liquids are dispensed and further manipulated using DEP forces. Due to its favourable scalability in the micro-dimension [22], DEP has developed into a suitable means of sample handling and processing in microfluidics where its applications range from particle/cell sorting [23, 24, 25], homogenous and uniform droplet dispensing [27], single emulsion droplet dispensing [35], and controlled volume dispensing [36], uniform deposition of macro-molecules and micro-sized particles in nanoliter-picoliter sample aliquots (daughter droplets) [37, 38].

In the next segment, fundamental concepts of L-DEP, Jones's simplified' lumped capacitance model and its modification to accommodate more complex liquid actuations are explained. Experimental results obtained from the various L-DEP based liquid actuation methods are utilized to validate the theoretical models. Aqueous samples used during these L-DEP actuations are reported in Table 1.

Lumped capacitance model for homogenous L-DEP actuation

A simple L-DEP micro actuation scheme consists of a pair of coplanar metal electrodes of widthw and gap g, insulated on top with a thin dielectric layer with a hydrophobic top surface coating, to prevent sample electrolysis and facilitate droplet dispensing and their subsequent manipulation (fabrication details in next section). When a parent sample droplet is placed on one end of the electrode pair and the electrodes are electrically energized by a sufficiently high AC voltage (typically ~ 200-500 Vpp at 50 kHz-1 MHz), a liquid jet is ejected from the parent droplet and is rapidly conveyed along the electrode pair, covering the entire electrode length within a few milliseconds (Figure 2).

In order to prevent evaporative sample loss, all liquid actuations are carried out in a low viscosity (5-10 cSt) silicone oil bath. The profile of the actuated liquid jet is actively controlled by the shape of the electrode arrangement, top surface coating and fluidic properties of the liquid sample. The dynamics of such L-DEP actuation of homogenous liquid jet has been analyzed based on a lumped parameter model [32].

Figure 2 shows the physical model for a liquid jet protruding from a parent sample droplet during uniform L-DEP actuation. In this model, the DEP force (F_{DEP}) is opposed by surface tension (F_γ) and viscous force (F_μ) and under the assumption of a uniform hemi-cylindrical jet, the force-momentum equation is described by:

$$\frac{dP}{dt} = F_{DEP} - F_\gamma - F_\mu \tag{2}$$

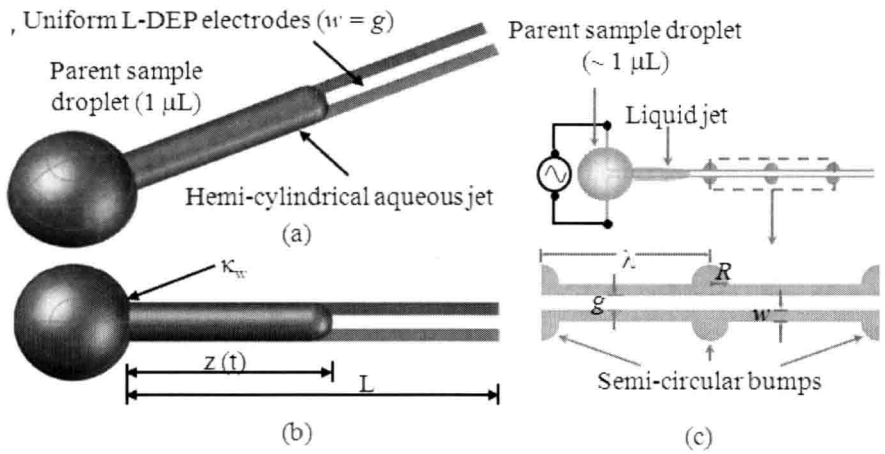

Figure 2. (a) 3-d model of a uniform L-DEP actuation; (b) Top view of the model; (c) Schematic details of a simple uniform L-DEP electrode scheme.

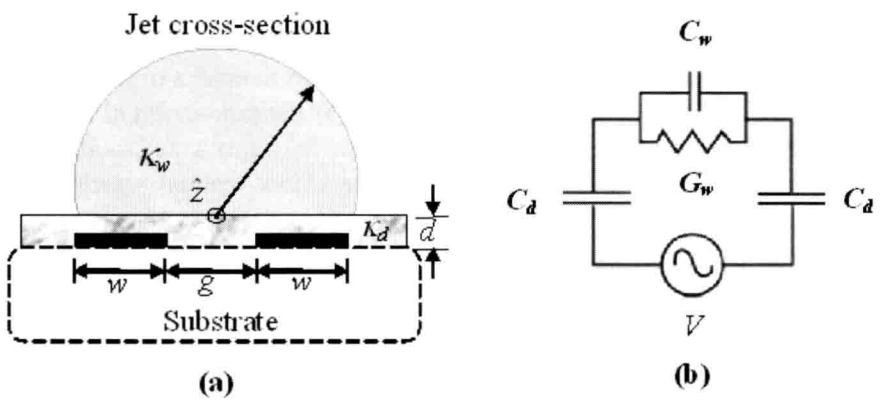

Figure 3. (a) X-sectional view of an aqueous jet during L-DEP actuation; (b) RC equivalent circuit for the capacitive coupled electrode/actuation scheme on the SMF chip.