# QUANTITATIVE BIOCHEMICAL ASSAY ON A SURFACE MICROFLUIDIC DEVICE

Ravi Prakash, Dipankar Chugh and Karan V. I. S. Kaler
*Department of Electrical and Computer Engineering, Schulich School of Engineering, University of Calgary*
*2500 University Drive NW, Calgary, (T2N 1N4), Canada*

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Abstract: Quantitative analysis of chemical and biochemical molecules is an important requirement in many biochemical assays and can be a challenging task in microfluidic systems due to the smaller sample volumes. In the present study, we report on the detection and quantification of nucleic acid samples contained in nanoliter (nL) and picoliter (pL) droplets, formed by employing a DEP based surface microfluidic system. This surface microfluidic system utilizes non-uniform AC electric fields for dispensing multiple, nanoliter (and picoliter) sized aliquots of samples and reagents, which can furthermore be individually addressed, transported and mixed on-chip in a controlled and parallel fashion. Quantification of dsDNA samples is carried out using a fluorescence based Quant-IT™ PicoGreen® assay, performed on the surface microfluidic chip, while the low-level fluorescence emissions are quantified using a photo-multiplier tube. Our findings show that sample DNA concentrations remain uniform across the dispensed droplets, although the volume of droplets can be varied as per requirements. Experimental results furthermore prove that our DEP based and electric field assisted on-chip mixing methodology is at par with conventional mixing strategies such as vortexing, stirring etc. and more readily achieved compared to conventional closed channel microfluidic systems.

## 1 INTRODUCTION

Advances in microfabrication of microfluidic devices has given rise to the capability of performing biochemical assays and analysis using small amount of samples, giving rise to the concept of lab-on-a-chip (LOC), in which sample pre-treatment, transportation, reaction, separation and detection can be achieved on a common platform. These microfluidic LOC devices provide significant advantages over conventional approaches in handling, processing and sample analysis. The miniaturization specifically provides low consumption of reagents and samples, portability, low power consumption, high throughput screening, disposability, low cost and potential for automated and remote operation as a result of further system level integration.

Until recently, such microfluidic devices have employed microchannels etched in glass, silicon or other polymeric materials, to handle and process low amounts of fluidic samples, aided by valves and pumps to transport the sample or reagents (Hong and Quake 2003). In contrast, a new class of surface microfluidic (SMF) devices have emerged, which have circumvented the need for microchannels to confine fluids and furthermore require no external or on-chip pumping or valving. These SMF devices employ AC electric fields, typically in the low to mid-frequency regions of the frequency spectrum, to manipulate aqueous dielectric media, in form of droplets, on top of suitably tailored hydrophobic surfaces. The actuation and manipulation of liquids and droplets in these devices specifically leverage the motive forces of dielectrophoresis (DEP) or Electrowetting (EW) to affect sample handling and offer an attractive alternative to conventional closed channel microfluidic devices, by providing high speed dispensing of multiple, equi-volume, nanoliter sized sample droplets (Pollack et al. 2002, Jones 2001).

More recently, Chugh and Kaler have shown the integration of Liquid DEP (L-DEP) based droplet dispensing with the Droplet DEP (D-DEP) conveyance scheme to demonstrate automated binary mixing of two different equi-volume liquid sample droplets at specific reaction sites (Chugh and



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Kaler 2008, 2009). In this article we report on further enhancements to the L-DEP sample handling and manipulation scheme, including the ability of dispensing different quantities of reagents, by employing a tapered L-DEP electrode structure. The tapered L-DEP electrodes, detailed in later sections, were designed to facilitate automated dispensing of different sets of daughter droplets from the same parent but differing in volume. This automated variable volume sample droplet dispensing scheme is especially beneficial for chip based biochemical assays that require different amounts of sample or reagents to be mixed or titrated.

The utility and capabilities of the variable volume droplet dispensing is further demonstrated by performing a DNA-PicoGreen® assay, facilitated by the judicious integration of three sets of fishbone shaped electrodes, for transporting the dispensed sample and reagents droplets to specific reaction sites. The effectiveness of mixing of the DNA and PicoGreen (PG) sample droplets was quantified by detecting the fluorescence signal emitted as a result of DNA-PG binding using a photomultiplier tube (PMT). Quantitative gradient of DNA sample was clearly reflected in fluorescent intensity gradient in different mixed droplets. Further details of the SMF devices and measurement systems used to validate the variable volume dispensing and subsequent manipulation of the daughter droplets is provided in the materials and methods sections.

## 2 BACKGROUND

Dielectrophoresis (DEP) is an electromechanical phenomenon, manifested as result of the interaction of the spatially non-uniform electric fields with polarizable materials (Pohl 1978). Non-uniform electric fields act on polar molecules, impelling them to regions of higher field strength. Although the origins of DEP can be dated back to the late 19$^{th}$ century (Pellat 1895), it is more recently that DEP has been usefully employed in SMF devices as a means of manipulating and transporting polarizable liquids on-chip (Ahmed and Jones 2006). The liquid and droplet DEP actuation methodology, underlying physical principles and electrode design parameters have been reported previously (Jones et al. 2001, Gunji et al. 2004). In the present study, a tapered L-DEP electrode structure was utilized for dispensing variable volume droplets as shown in Figure 2.1 (Chugh and Kaler 2009). These L-DEP electrodes, serve as a fluidic track for the 'parent' sample droplet pipetted at one end of the structure. On applying an AC voltage across the coplanar

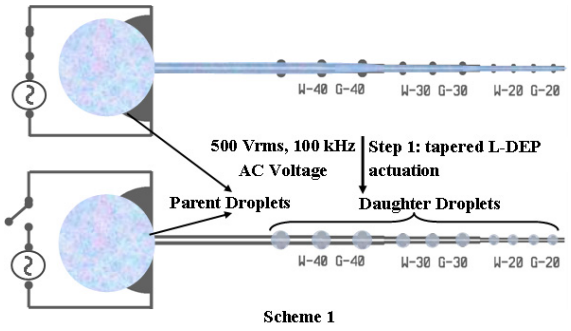


Figure 2.1: Tapered L-DEP actuation scheme 1.

electrodes, a liquid jet is formed from the sample droplet and conveyed rapidly towards the opposite end, covering regions of high field intensity. Once the jet has reached the opposite end, the applied voltage is removed and the jet disintegrates into smaller sized 'daughter' droplets at the semi-circular bump sites, as shown in Figure 2.1. The spacing between the bumps and jet break-up is governed by Rayleigh's instability criteria (Lord Rayleigh 1879). Further manipulation and transport of these dispensed daughter droplets was achieved through droplet-DEP actuation, developed by (Gunji et al. 2004).

In the present study, we have leveraged liquid and droplet DEP sample actuation schemes to dispense, transport, mix nano/picoliter droplets and execute biochemical assays on our DEP based SMF device.

## 3 EXPERIMENTAL

### 3.1 Device Fabrication

Surface microfluidic devices, used in the present study, were fabricated using commercially available 4" silicon wafers passivated by 5µm film of $SiO_2$. The device consists of L-DEP and D-DEP microelectrode structures, fabricated in two different metal layers (Aluminum) separated by a dielectric film (600nm thick $Si_3N_4$), sandwiched between them for electrical isolation. For the 'bottom' layer electrodes, a 200nm thick layer of Al was sputtered on the passivated silicon substrate. Microelectrode structures were patterned using standard photolithography procedure (Thirukumaran and Kaler 2007). Thereafter, a thin layer of silicon nitride (600 nm) was deposited on top of the Al microelectrodes using plasma enhanced chemical vapor deposition (PECVD) technique. A second layer of Al (200nm) was sputter deposited and

patterned to obtain the 'top' layer microelectrode structures, followed by a second $Si_3N_4$ deposition (400 nm) using PECVD. Finally, the top surface of the microfluidic chip was rendered hydrophobic by spin coating a thin layer (~ 0.1 µm) of Teflon® AF 2400 (DuPont Inc., USA), which is critical for reliable D-DEP actuation (Gunji et al. 2004).

## 3.2 Sample Preparation

Detection and quantification of dsDNA samples was carried out using a fluorescence based Quant-IT™ PicoGreen® assay (Molecular Probes™, Invitrogen, USA). PicoGreen® (PG) is a well-known fluorescent nucleic acid stain that selectively binds to dsDNA (Zipper et al. 2004). PG has a large enhancement in its fluorescence emission on binding to dsDNA, with excitation and emission maxima at 488nm and 520nm, while unbound PG has virtually no measurable fluorescence. A 200ng/µL stock DNA sample was prepared by suspending lyophilized plasmid DNA samples of pUC57 (GenScript, USA) in 1mM Tris solution, prepared fresh in de-ionized (DI) water on the day of the experiment. The pH of the Tris solution was adjusted to 7.5 using a 50mM MES solution. Dilutions of the stock DNA sample were made in Tris-MES solution. Final plasmid DNA concentrations were measured using a NanoDrop UV-Visible spectrophotometer (Thermo Scientific, USA). PG reagent was supplied as a 1mL concentrated solution in dimethylsulfoxide (DMSO). Based on the supplier's protocol and concentration of DNA sample used for on-chip assays, a working sample of the dye was prepared by making a 4 fold dilution of the concentrated dye (in DMSO) in Tris-MES solution. A small amount of Tween® 20 was added to all samples to minimize surface adsorption.

## 3.3 Experimental Setup

The experimental setup is comprised of a reflected fluorescence microscope system (BX51, Olympus, Japan), optically coupled to a CCD color camera and a Photomultiplier Tube (H7468-01, Hamamatsu, Japan) to facilitate fluorescence intensity measurements. A block diagram of the optical setup is schematically illustrated in Figure 3.3.1. The CCD camera was replaced by a high-speed camera (MS70K, *Mega Speed*, Canadian Photonics Inc.) for recording Liquid and Droplet-DEP actuations, when required. Electrical connections to the SMF chip were enabled using spring loaded contact pins and high voltage relays (9104 series, COTO Technology, USA) assembled on a printed circuit board (PCB). The PCB was mounted on-top of the SMF chip and

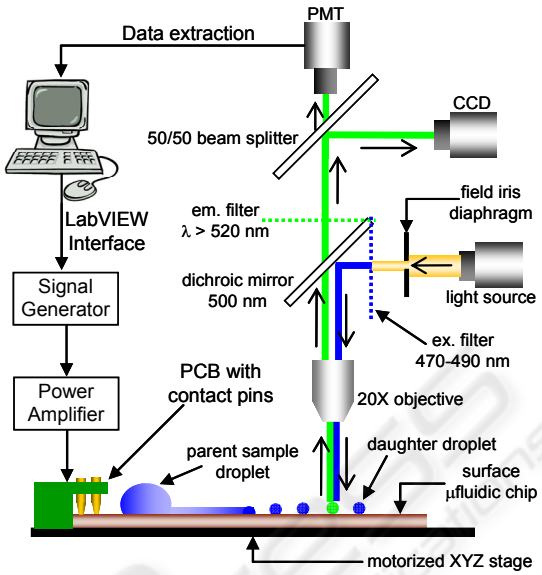


Figure 3.3.1: Schematic of the experimental setup.

the completed assembly was housed on a motorized microscope stage (Optiscan® ES103, Prior Scientific, USA).

A signal generator (TGA 1244, TTi, UK) and high-voltage amplifier (Precision Power Amplifier 5205A, Fluke) provided the AC voltages required for liquid and droplet actuations. Furthermore, a software driver, developed in LabVIEW (National Instruments, USA) was utilized to program the signal generator for timed and sequential application of AC voltages and controlling liquid and droplet DEP actuations.

## 3.4 Device Operation

In the present study, two different sets of experiments were performed to investigate (a) the variations in DNA (pUC57) concentration, in the individual daughter droplets, dispensed using the tapered L-DEP electrode structure (scheme 1) and (b) on-chip droplet dispensing and mixing of DNA and PG daughter droplets, together with fluorescence intensity measurements (scheme 2).

For the first set of experiments, utilizing scheme 1, a DNA+PG sample was prepared off-chip, in micro-centrifuge tube and vortexed to thoroughly mix the two components. A 1 µL droplet of this mixed sample was manually pipetted at one end of the tapered L-DEP electrode as shown in Figure 2.1. An AC voltage (500 Vrms @ 100 kHz) is briefly (40–100 ms) applied across the L-DEP electrodes to dispense nano and picoliter sized 'daughter' droplets. The amount of DNA (moles) in each daughter droplet can be co-related to the intensity of

fluorescence emissions observed from the daughter droplet. Thus to study DNA concentration uniformity in the dispensed daughter droplets of different volume, each droplet was individually observed using the optical set-up illustrated in Figure 3.3.1. Fluorescent emission from each daughter droplet is detected and quantified using a PMT. To ensure consistency and measure low-level fluorescent emissions from the daughter droplets, the PMT is operated at a constant high gain value ($0.7\times10^6$) with a fixed optical path and constant light intensity. A field iris diaphragm was used to restrict the diameter of the incident light beam illuminating the daughter droplets, which excludes extraneous light from entering the objective, thereby improving signal to noise ratio (S/N). The iris aperture was adjusted to circumscribe the largest daughter droplet (formed on the $w = g = 40$ µm L-DEP electrode) and kept same for all other smaller daughter droplets.

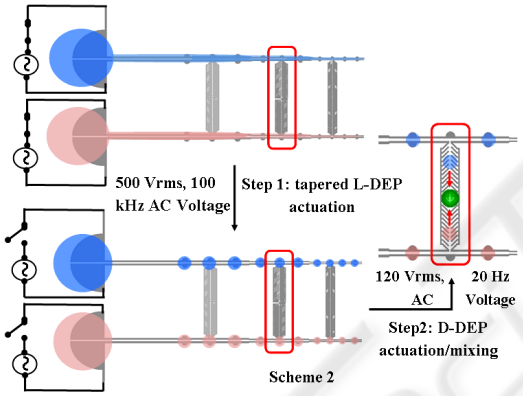


Figure 3.4.1: 1*x*1 matrix of tapered L-DEP and D-DEP structures for on-chip assay (Scheme 2).

In the second set of experiments, utilizing scheme 2, unmixed sample droplets (1 µL) of DNA and PG are individually pipetted over L-DEP structure, as shown schematically in Figure 3.4.1. For executing on-chip sample and reagent mixing, fishbone shaped D-DEP electrodes are integrated with the L-DEP electrodes. Daughter droplets of both DNA and PG are formed by employing the tapered L-DEP electrode, as mentioned previously. For mixing the DNA and PG daughter droplets, a lower frequency AC voltage (120 Vrms @ 20 Hz) is applied across the D-DEP electrodes, which transports the daughter droplets from semi-circular bumps. Selected video frames showing the integrated liquid and droplet actuation scheme are illustrated in Figure 4.4.1. All experiments were performed under a low viscosity silicone oil bath (200® FLUID, 5CST, Dow Corning) to prevent rapid evaporation of daughter droplets.

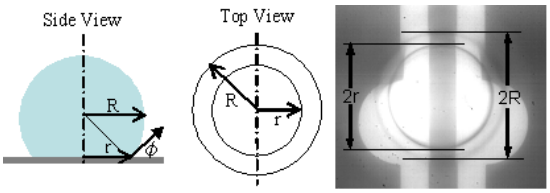


Figure 3.4.2: Volumetric measurement of daughter droplets.

Volumetric measurement for the dispensed daughter droplets was required in order to correlate the PMT output ($I_P$) with the DNA concentration. Since the top surface of the SMF chip is coated with Teflon®, resulting in a highly hydrophobic surface, droplets dispensed on the surface assume a nearly spherical shape (contact angle ~110°). However, droplet's contact angles have strong dependence on biomolecular species (such as enzymes, proteins, cells etc.), which can get adsorbed on the surface and reduce the contact angle (Prakash and Kaler 2008). Thus in order to estimate daughter droplet volumes, a visual inspection of droplets under high magnification objective (20X) was performed. A visual inspection helped distinguish and measure the two different radii of curvature of the daughter droplets (r: radius of curvature at the plane where the droplet contacts the surface and R: actual radius of the spherical droplet) as shown in Figure 3.4.2. The measured radii values were used to formulate and quantify the volume (V) of the daughter droplets using Eqn. 1.

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

# 4 RESULTS AND DISCUSSION

## 4.1 Sub-nanoliter Droplet Dispensing and Bio-sample Quantification

A tapered L-DEP structure (refer scheme1) was used to dispense droplets of mixed DNA-PG sample with volumes 2.4 nL, 0.9 nL and 0.25 nL, as shown in Figure 4.1.1. The droplet volumes were estimated using equation 1, described above. In order to confirm that sample concentration remains invariant for all the dispensed droplets irrespective of the droplet volume, photocurrent values corresponding to different sized daughter droplets and 4 different DNA concentrations were measured and are summarized in Table 1.

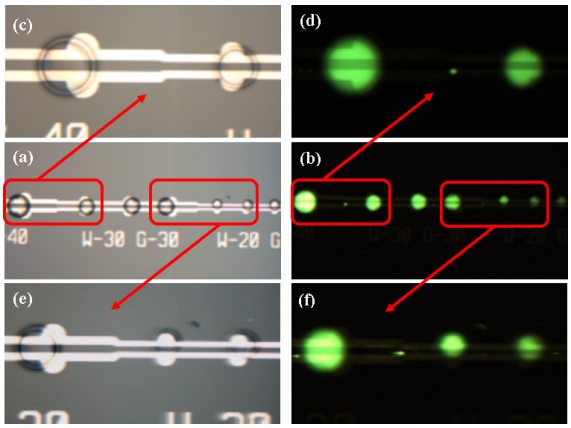


Figure 4.1.1: Bright field and fluorescent images of different sized daughter droplets containing DNA-PG complex; (a, b) Bright field and fluorescent image of all the three sections of tapered L-DEP electrode; (c, d) Bright field and fluorescent image of 40-30 tapered electrode section; (e, f) Bright field and fluorescent image of 30-20 tapered electrode section.

In general, fluorescence emission from the DNA-PG complex is reported to be linearly proportional to the quantity of DNA (Singer et al. 1997). Therefore, since $I_P$ is directly related to the amount of DNA (or number of moles) in daughter droplets, the ratio $I_P$/vol. provides a direct measure of sample DNA molar concentration for each daughter droplet.

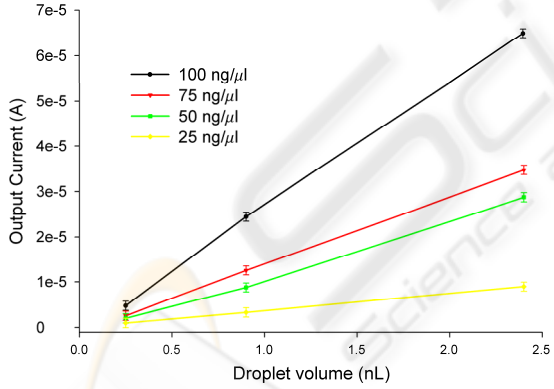


Figure 4.1.2: Plots showing PMT output for different sized daughter droplets of different conc. DNA-PG solutions.

These values, along with the ratio of PMT photocurrent ($I_P$) and droplet volume are tabulated in Table 1. We found that the ratio ($I_P$/vol.) remains constant for all the different sized daughter droplets (corresponding to a specific DNA concentration) confirming that the DNA concentration in each of these daughter droplets remains invariant. This was further evident from the plot of output current ($I_P$) vs. measured droplet volume, shown in Figure 4.1.2,

Table 1: Experimental results extracted using scheme 1.

| Sample | w = g (micron) | $I_P$ (μA) | Vo (nL) | $I_P$/vol. | Correlation factor |
|---|---|---|---|---|---|
| 25 ng/μL DNA with 50X PG | 40-40 | 10.97 | 2.4 | 4.57 | 5.47 |
| | 30-30 | 3.99 | 0.9 | 4.43 | 5.65 |
| | 20-20 | 1.19 | 0.25 | 4.77 | 5.24 |
| 50 ng/μL DNA with 50X PG | 40-40 | 22.77 | 2.5 | 9.11 | 5.49 |
| | 30-30 | 8.76 | 0.9 | 9.73 | 5.14 |
| | 20-20 | 2.21 | 0.25 | 8.83 | 5.66 |
| 75 ng/μL DNA with 50X PG | 40-40 | 33.71 | 2.4 | 14.05 | 5.34 |
| | 30-30 | 12.57 | 0.9 | 13.96 | 5.37 |
| | 20-20 | 3.24 | 0.25 | 12.98 | 5.78 |
| 100 ng/μL DNA with 50X PG | 40-40 | 43.68 | 2.4 | 18.20 | 5.49 |
| | 30-30 | 16.53 | 0.9 | 18.36 | 5.45 |
| | 20-20 | 4.63 | 0.25 | 18.52 | 5.40 |
| Variance | | | | | 0.04 |

where a constant slope ($I_P$/vol.) was observed for each of the four different DNA sample concentrations (25, 50, 75 and 100 ng/μL).
From this plot and Table 1, we then estimate a correlation factor to normalize $I_P$/vol. ratio against different DNA concentrations, the inverse of which provides a measure of photo-current per picogram of DNA sample. The above findings suggest that using tapered L-DEP scheme, we can dispense multiple sample droplets with different amounts (moles) of DNA sample, while keeping the concentration same as the parent sample. DNA concentrations were quantified in the dispensed daughter droplets ranged from 2.4 nL to 0.25 nL, a capability that is not readily achieved by today's microfluidic devices.

## 4.2 Reliability of the L-DEP Scheme for Multiplexed Applications

Having shown that DNA concentrations are invariant for daughter droplets dispensed using L-DEP actuation, we further assess the reliability and repeatability of DEP based droplet dispensing scheme. For this, we conducted several experiments on simple L-DEP dispensing scheme (scheme 1) with the same parent sample conc. (50 ng/μL DNA-PG) to see the reliability and repeatability of our scheme in dispensing uniform conc. daughter droplets.

The results of 6 different set of experiments with the same parent DNA sample droplet over different but, identical L-DEP electrode structures are shown plotted in Figure 4.2.1. The results clearly show a

reliable dispensing of 6-8 daughter droplets with equal concentration (confidence of fit ~92.5%).

The high speed of L-DEP actuation (40 msec for dispensing an array of sub-nanoliter droplets) and the hydrophobic Teflon® coated surface minimizes surface adsorption (Prakash and Kaler 2008) for biological sample and the accuracy in the size and concentration of dispensed daughter droplets makes the scheme suitable for use in an on-chip multiplexed biochemical assay device.

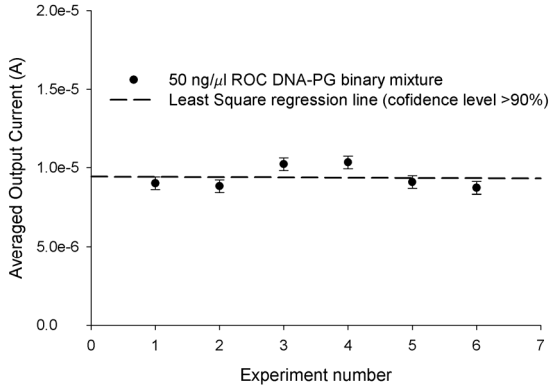


Figure 4.2.1: Plot showing repeatability of L-DEP based sub-nanoliter droplet dispensing scheme.

## 4.3 On-chip Mixing of Constant Volume Sample and Reagent Droplets using D-DEP

One of the objectives of our DEP based SMF device is to extend the idea of mixing to implement chip-based multiplexed assays where several different samples and reagents are mixed in all possible combinations in a parallel, automated fashion. Effective mixing of samples and reagents is an essential step involved in all chemical and biochemical applications. Mixing on-chip is not as readily achieved compared to macroscopic mixing of samples, where vortexing or stirring actions could be easily exploited. This is due to the small size, low sample/reagent volumes and laminar flow characteristics of the conventional closed-channel microfluidic devices resulting in a slow and diffusion limited mixing. In contrast, SMF devices are capable of mixing sub-nanoliter volumes of sample and reagent droplets more efficiently (Fair et al. 2003). We use D-DEP actuation, where electric field mediated stirring action (electroconvective effects) takes place during droplet transportation and facilitates mixing once the droplets come in contact and merge. To demonstrate this, we used an integrated Liquid and Droplet-DEP structure (shown boxed in Figure 3.4.1) which was elaborated in the experimental section. Three different concentrations of the DNA sample along with PG dye were used. Roughly 0.6 nL volume of DNA sample and the PG dye was first dispensed using the L-DEP dispensing scheme. The DNA sample and PG daughter droplets thus formed were then moved towards the reaction/mixing site using D-DEP actuation. Fluorescence emissions were recorded in real-time as the two daughter droplets mixed and are shown in Figure 4.3.1. We observed a steady increase in the PMT output which indicates that all the three different conc. of DNA sample demonstrate a nearly similar mixing or, ligand binding kinetics (evident from the identical slope of PMT current vs. time curves).

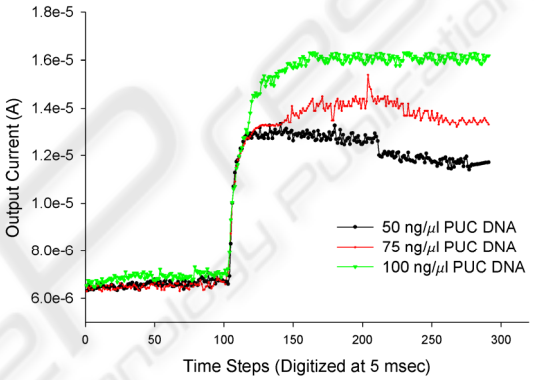


Figure 4.3.1: Plot showing time domain data extracted from PMT illustrating binary mixing of sample and reagent daughter droplets.

The PMT output finally saturates and remains constant, indicating complete and thorough mixing of DNA sample with the PG reagent. The entire mixing assay was complete within 4-5 sec from manual pipetting of the parent sample droplets.

These results clearly suggest that on-chip mixing can be more readily achieved using our DEP based SMF device as compared to conventional closed-channel microfluidic devices which require large sample volumes (in mL), longer (in mm) and wider mixing channels, larger mixing time and furthermore sophisticated pump and valve arrangements (Park et al. 2006).

## 4.4 On-chip Variable Volume, Multiplexed DNA-PicoGreen® Assay

Having demonstrated the capability of our SMF device in achieving some of the key sample handling requirements for an on-chip assay system including, (1) dispensing arrays of sub-nanoliter sample and

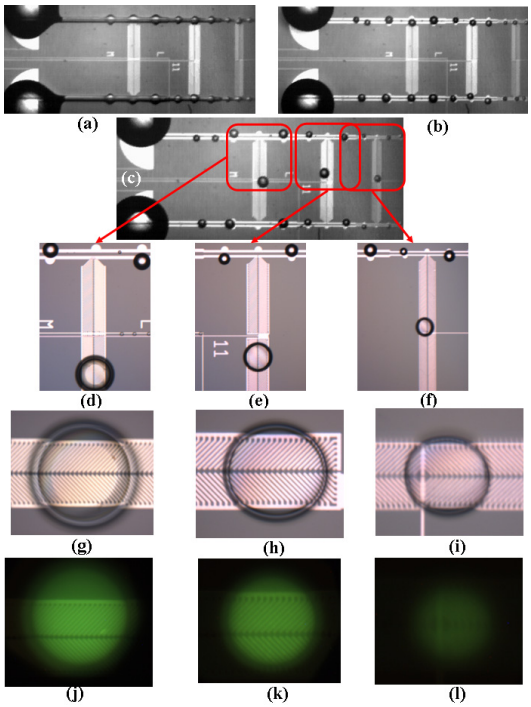


Figure 4.4.1: Micrographs demonstrating results of on-chip DNA quantification assay; (a, b)L-DEP actuation and droplet dispensing; (c) mixed daughter droplets; (d, e, f) Unmixed DNA sample droplets (along tapered L-DEP electrodes) and mixed DNA-PG droplets; (g-l) bright field and fluorescent images of the mixed DNA-PG droplets showing the fluorescent intensity gradient.

reagent droplets with controllable sample mass and uniform concentration, and (2) efficient mixing of a pair of dispensed sample and reagent droplets, we now demonstrate an on-chip nucleic acid assay by implementing a 1*x*1 matrix of tapered L-DEP and D-DEP electrode structures illustrated in scheme 2 (Figure 3.4.1).

Four different concentrations (25, 50, 75 and 100 ng/□L) of DNA sample were actuated over one of the two tapered L-DEP electrodes to dispense multiple daughter droplets with uniform concentrations but varying volumes and different moles of DNA. On the other tapered L-DEP electrode, PG sample was actuated in a similar fashion.

Three different sets of paired fishbone electrodes were used to simultaneously transport sample and reagent droplets from each of the three different steps of the tapered L-DEP electrode structure to the corresponding reaction sites under the influence of an externally applied electric field, to facilitate DNA-PG sample mixing (Figure 3.4.1). The entire process was conducted within 2-4 seconds of actuation (Figure 3.4.1 and Figure 4.4.1).

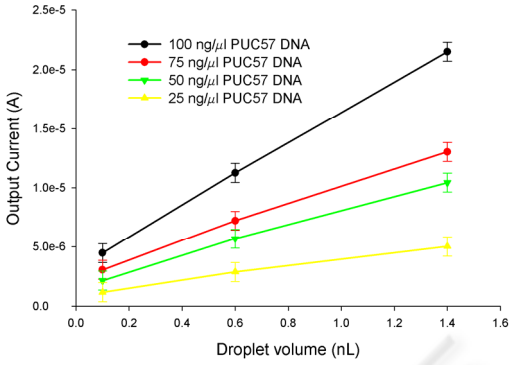


Figure 4.4.2: Plots showing PMT output for on-chip DNA-PG assay using experimental scheme 2, for different DNA sample concentrations.

Table 2: Results extracted from on-chip DNA-PG assay experiments employing scheme 2.

| DNA conc. | PG | w = g (micron) | $I_P$ (μA) | vol. (nL) | $I_P$/vol. | Correlation factor |
|---|---|---|---|---|---|---|
| 25 ng/μL | 50X | 40-40 | 5.96 | 1.4 | 4.26 | 5.87 |
| | | 30-30 | 2.78 | 0.6 | 4.63 | 5.40 |
| | | 20-20 | 1.13 | 0.1 | 4.51 | 5.54 |
| 50 ng/μL | | 40-40 | 12.44 | 1.4 | 8.88 | 5.63 |
| | | 30-30 | 5.49 | 0.6 | 9.15 | 5.46 |
| | | 20-20 | 2.15 | 0.1 | 8.60 | 5.81 |
| 75 ng/μL | | 40-40 | 17.85 | 1.4 | 12.7 | 5.88 |
| | | 30-30 | 7.71 | 0.6 | 12.8 | 5.83 |
| | | 20-20 | 3.25 | 0.1 | 13.0 | 5.77 |
| 100 ng/μL | | 40-40 | 24.50 | 1.4 | 17.5 | 5.71 |
| | | 30-30 | 11.26 | 0.6 | 18.7 | 5.33 |
| | | 20-20 | 4.47 | 0.1 | 17.8 | 5.59 |
| Variance | | | | | | 0.04 |

The PMT output current ($I_P$) corresponding to the mixed droplets was plotted against the volume of the individual DNA daughter droplets, measured prior to mixing and shown in Figure 4.4.2. The ratio $I_P$/vol. correlates to the DNA concentration in the dispensed daughter droplets. Constant slopes for each of the different DNA concentrations used, indicate that the ratio of output current ($I_P$) and DNA droplet volume and hence DNA concentration, remains constant for each of the mixed droplets. Ip/vol. values and the corresponding correlation factor for on-chip DNA-PG assay are reported in Table 2. These values of correlation factor reported in Table 2 are furthermore in good agreement with the values of correlation factor reported in Table 1 for the corresponding off-chip mixed DNA-PG droplets, suggesting that on-chip quantification assay is successfully achieved.

# 5 CONCLUSIONS

In this study, we have successfully demonstrated the utility of a tapered electrode structure to dielectrophoretically dispense variable volume nanoliter to sub-nanoliter sample droplets (2.4 nL to 0.25 nL) on top of hydrophobic surfaces, with precision. This tapered droplet scheme was furthermore interfaced with a fishbone droplet conveyance scheme to demonstrate its utility in performing a quantitative, multiplexed assay.
The fluidic sample handling capabilities of the SMF devices reported in this article may be potentially leveraged for several purposes including drug discovery, genomics and pathogen detection. This SMF scheme can also be multiplexed to an m×n matrix to achieve HTS capabilities as an alternative to the existing close channel technology (Thorsen et al. 2002). The oil bath submerged experimental setup can be replaced by using the sub-nanoliter emulsion dispensing scheme, reported by Prakash and Kaler (2009).

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