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I, Ananda Banerjee, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Electrical Engineering.

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TOWARDS RECONFIGURABLE LAB-ON-CHIP USING VIRTUAL ELECTROWETTING CHANNELS

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TOWARDS RECONFIGURABLE LAB-ON-CHIP USING VIRTUAL ELECTROWETTING CHANNELS

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Lab-on-a-chip systems rely on several microfluidic paradigms. The first uses a fixed layout of continuous microfluidic channels. Such lab-on-a-chip systems are almost always application specific and far from a true “laboratory.” The second involves electrowetting droplet movement (digital microfluidics), and allows two-dimensional computer control of fluidic transport and mixing. Integrating the two paradigms in the form of programmable electrowetting channels would take advantage of both the “continuous” functionality of rigid channels, which are used in numerous applications, and the “programmable” functionality of digital microfluidics which permits electrical control of on-chip functions. In this dissertation, for the first time, such programmable virtual microfluidic channels were demonstrated using an electrowetting platform. These “wall-less” virtual channels can be formed reliably and rapidly, with propagation rates of 3.5-3.8 mm/s. Pressure driven transport in these virtual channels at flow rates up to 100 µL/min can be achieved without distortion of the channel shape. Further, these channels can be split into segments or droplets of precise volumes, with accuracies exceeding 99%. Critical parameters for such deterministic splitting of liquid samples have been identified with the aid of numerical models and confirmed experimentally. A number of reconfigurable operations were demonstrated, including interactions between continuously-flowing channels and programmed droplet motion. This approach was used to develop a programmable heterogeneous immunoassay protocol, showing a significant improvement in performance by using continuous flow washing as compared to the droplet-based washing in conventional digital microfluidics. The limit of
detection obtained with this technique for a streptavidin–biotin binding assay was 10 nM, illustrating potential for improved performance with other heterogeneous immunoassays. The first demonstration of reconfigurable electrowetting channels in this dissertation and their seamless integration with digital microfluidics makes this approach applicable to a wide range of clinical or pharmaceutical applications.
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CHAPTER 1

INTRODUCTION

Microfluidics permits transport, storage, and fluid manipulation in small volumes, and is the driving force behind the ‘lab-on-a-chip’ technologies. Conventional lab-on-a-chip platforms use structured microfluidic channel networks for transporting and confining liquids [1]. Glass and polymers are common substrate materials in these microfluidic devices, with fabrication procedures well established [2, 3]. While numerous applications in medical diagnostics, chemical sensing, and environmental monitoring have been demonstrated, any change in the design of these devices, regardless of how minute, requires a repeat of the entire fabrication cycle. A more generic approach to lab-on-a-chip involves programmable devices that can be adapted for a variety of applications. Electrowetting-based programmable devices provide such an opportunity.

Electrowetting is modulation of the macroscopic contact angle of small liquid volumes on a surface by altering interfacial tension through application of an external electric field [4]. This affords the means for actuating fluids reversibly without mechanical parts. Systems have been developed using this technology for processing droplets and these systems are popularly referred to as digital microfluidics (DMF) [5, 6]. Conventionally, DMF is a subset of droplet microfluidics where fluid manipulation is strictly limited to discrete volumes of fluids [7, 8]. There are some disagreements about nomenclature, but here the term DMF is used to refer to integrated systems in which droplets are manipulated on arrays of electrodes, rather than devices
where droplets are processed in enclosed microchannels. The latter format is a useful and popular paradigm and has been studied extensively by several groups [9-11]; in this dissertation the focus is on the former type of system. Such systems have been demonstrated in a variety of lab-on-a-chip applications using electrowetting transport [4, 12-14], dielectrophoresis [15, 16] and electrohydrodynamic forces [17-19]. To dispense with any ambiguity, here electrowetting based DMF systems is the focus.

In DMF systems picoliter to microliter sample volumes are independently manipulated on open arrays of insulated hydrophobic electrodes. By applying potentials in a series of programmed steps droplets can be made to split, merge, mix and dispense from reservoirs. Common to conventional microfluidics, these systems also benefit from small reagent consumption and easy integration with other analytical techniques. The feature that makes these systems unique and advantageous is that each droplet is individually controlled without the need for channel networks, pumps and valves. Various processes can therefore be simultaneously performed in a compact and simple design. Since droplet operations are performed on a generic array of electrodes, the sequence of steps is reconfigurable from one experiment to the next.

**Digital microfluidics**

DMF is a versatile fluid handling tool with several unique and useful attributes and it has been applied to a large number of applications in a wide variety of fields. Programmable microfluidic devices enable automation in lab-on-a-chip applications [4, 7, 20, 21]. A lot of interest has been directed recently towards miniaturized biochemical analysis on such devices [2, 22, 23]. DMF devices have been used to demonstrate a number of lab-on-a-chip applications [5, 6, 13, 14, 24-26]. A brief review of these applications is presented here.

Homogeneous chemical and enzymatic reactions are one of the fundamental applications
of DMF. These applications typically rely on dispensing droplets of precisely metered volumes and their subsequent mixing in isolated micro-reactors. The popular ambient medium in DMF systems is oil, but in some applications requiring organic solvents miscible with oil [27] this is not suitable. An open-air format is used in such cases making it applicable to a wider range of liquids [28]. Chemical and enzymatic reactions in DMF have been implemented to evaluate substances of interest, to synthesize compounds and to study reaction kinetics. Srinivas et al. developed and optimized a colorimetric glucose detection method involving a two enzyme reaction. The clinical applicability of the work was tested by using human physiological samples (whole blood, saliva, urine and serum)[25, 26]. Similarly, a chemical assay for detection of TNT was developed by Pamula et al. [29]. In the study of enzyme kinetics, Wheeler and Miller [30] developed the first oil a DMF device to study alkaline phosphatase activity. Nichols and Gardeniers [31] designed a DMF device to study the kinetic of tyrosine phosphatase. In the synthesis of new compounds DMF devices have been used for demonstrating microparticle synthesis [32] and artificial Golgi organelle synthesis [33].

Another fundamental area where DMF is popular is immunoassays. The primary objective in these assays is to detect an analyte of interest by exploiting specific antigen-antibody interactions. At least five different groups have reported immunoassays relying on antibodies bound on solid supports (heterogeneous immunoassays) using DMF. These include immunoassays for immunoglobulin G and ricin [34], insulin and interleukin-6 [35, 36] and immunoglobulin E [37]. These immunoassays used micro-beads as a solid support for antibodies. Alternatively, antibodies can be immobilized directly on the device surface. The first surface based immunoassay on DMF devices was demonstrated by Miller et al. [38].

The characterization of DNA samples and their manipulation is a critical process in
pharmaceutical research, forensics and medical diagnostics. These processes are a natural fit for microfluidics due small precious samples and a highly multiplexed execution. A great deal of interest has been shown for using DMF for developing DNA based applications. Specifically DMF devices have been used for DNA extraction and purification [35, 39], DNA hybridization assays [40], generation of recombinant DNA by ligation [41, 42], polymerase chain reaction (PCR) [35, 43, 44] and pyro-sequencing [45].

Proteomics is another area where DMF devices have been used to carry out experiments that require tedious multistep sample processing before analysis by mass spectrometers and other detectors. Methods have been developed for the purification of proteins and peptides from complex mixtures [46-49]. The efficiency of these methods is comparable to conventional techniques. Additionally, they do not require centrifugation and require less processing time. In many of these applications sample preparation by DMF has been combined with in-situ detection techniques.

Cell-based applications also have been demonstrated on DMF devices. These applications are challenging to implement with DMF because cells are extremely fragile and they must be transported without altering viability or function. Moreover, it is likely that cells and proteins gets adsorbed on hydrophobic surfaces making droplet motion difficult on DMF devices. However, a number of groups have demonstrated cell-based assays, cell sorting and cell culture [50-53]. The applications discussed so far utilize DMF as an automated fluid handling tool to carry out highly multiplexed and otherwise labor intensive techniques critical different applications.

Continuous channels and digital microfluidics

While droplet manipulation is a critical capability, it cannot replace the functionalities
provided by continuous microfluidics which enables processing of large sample volumes at high throughput. Attempts to demonstrate programmable continuous microchannels have been recently reported using surface energy [54] and microvalves [55, 56]. While these early devices provide continuous flow, they offer very limited re-configurability. A truly reconfigurable continuous microfluidic system, in essence the merging of the two paradigms of continuous and digital microfluidics, would permit wide range of microfluidic functions in a wide range of applications ranging from clinical diagnostics in resource-limited environments to rapid system prototyping to high throughput pharmaceutical applications.

The brief review of digital microfluidic applications presented in this chapter highlights the versatile attribute of programmable DMF devices. While this format is well-suited for nanoliter sample manipulation and reaction sequencing, conventional microfluidic channels are a more suitable for processing larger sample volumes and high-throughput applications.

Integration of digital microfluidics with conventional rigid channels has been attempted for harnessing the advantages of both formats. [22] However, the rigid microchannels lead to loss of reconfiguration, making devices application-specific. In this work the gap between conventional DMF devices and continuous rigid channel based microfluidics is bridged. It can be envisioned that, merging the paradigms will lead to high throughput fluid handling capabilities and lead to more versatile DMF applications.

**Scope of work**

In this dissertation, electrowetting was used to develop microfluidic devices capable of on-demand fluid manipulation in the form of discrete droplets as well as continuously-flowing channels. The structural and mass-transfer properties of the electrowetted were examined using experimentally-verified theoretical models and numerical simulations. Specifically, the
mechanisms behind forming channels, splitting them into precise portions, measuring the volume
of dispensed channels and droplets, and integrating channel and droplet functionality were
investigated. The processes and designs necessary for the fabrication of the microfluidic devices
were developed in a series of incremental developmental steps starting from the basic proof-of-
concept leading up to devices capable of computer controlled automation of microfluidic
functions. The implementation of heterogeneous immunoassay protocols using these devices was
demonstrated.

**Chapter summaries**

Microchannels of desired lengths and shapes can also be formed in a programmable
manner employing electrowetting as will be demonstrated in this dissertation. The physics
behind the formation of reconfigurable electrowetting microchannels is explored in Chapter 2. It
is demonstrated that electrowetting channels can support conventional pressure driven flows.
This is followed by new techniques that explore the critical conditions necessary for
reconfiguration of geometries as well as improved methods for creation of metered sample
volumes and their manipulation in the form of channels and droplets in Chapter 3. These two
chapters form the basis for the most critical functions in electrowetting which are transport,
splitting and merging. While these concepts have been explored from the perspective of
conventional DMF devices by other groups, here continuous fluid handling capabilities are
incorporated, thus improving DMF device technology. It is demonstrated that electrowetting
channels can be manipulated digitally to form droplets, measure and portion fluids and form
programmable liquid pathways for various lab-on-chip applications. Towards this end advanced
fluid handling with both droplets and channels is demonstrated in Chapter 4. In each step, the
devices are incrementally improved in design. In Chapter 5, heterogeneous immunoassay
protocols have been investigated to improve on the existing DMF based assay protocols by
simplifying the washing steps with continuous washing techniques. This is expected to lead to
better signal to noise ratio and better limits of detection for immunoassays and other bio-
chemical applications. Finally, in Chapter 6 the major conclusions are summarized and the
present challenges and future directions are discussed.
CHAPTER 2

PROGRAMMABLE ELECTROWETTING CHANNELS

Introduction

Programmable microfluidic devices have the potential to be reconfigured during operation. Digital microfluidic systems exhibit such functionality and are able to manipulate on-demand discrete sample volumes (droplets). Such systems have been demonstrated in a variety of lab-on-a-chip applications using electrowetting transport [4, 12-14], dielectrophoresis [15, 16] and electrohydrodynamic forces [17-19]. However, while droplet manipulation is a critical capability, it cannot replace the functionalities provided by continuous microfluidics which enables processing of large sample volumes at high throughput. Attempts to demonstrate programmable continuous microchannels have been recently reported using surface energy [54] and microvalves [55, 56]. While these early devices provide continuous flow, they offer very limited re-configurability. A truly reconfigurable continuous microfluidic system, in essence the merging of the two paradigms of continuous and digital microfluidics, would permit wide range of microfluidic functions in a wide range of applications ranging from clinical diagnostics in resource-limited environments to rapid system prototyping to high throughput pharmaceutical applications.

In this dissertation, for the first time, programmable formation of virtual microfluidic channels, as well as their continuous operation and reconfiguration with pressure driven flows
using an electrowetting platform is demonstrated. In earlier attempts, directional formation of virtual channels bounded by polymer post arrays was shown [57] and it was ascertained that electrowetting channels can be made to retain their geometry in the absence of external stimulus using Laplace barriers [58]. Figure 1 illustrates the overall concept of the platform. It consists of an electrically-programmable two-dimensional array of insulated electrodes on the bottom plate. The transparent top plate is conductive and it is separated from the bottom plate by a spacer layer. This arrangement is used to form a sealed cavity with inlet and outlet ports.

The capability of selectively applying potential to each electrode combined with sample injection using syringe pumps at the inlets makes it possible to demonstrate a variety of basic fluid manipulation and handling capabilities including, splitting and merging of microfluidic channels, mixing and sample transport in a programmable manner. The platform relies on electrowetting contact angle modulation of conducting aqueous samples in oil ambient over insulated electrodes, to define channel boundaries. Thus, in this work, first biologically-relevant

**Figure 1.** Illustration of microfluidic platform based on electrowetting, integrating continuous and digital paradigms. Insets show sample transport, splitting and merging of channels and mixing of samples. Transparent conducting front plate (not shown) is grounded.
electrolytes at various pH values were considered for the formation of reconfigurable channels. Using the data obtained from these tests, a theoretical model for electrowetting channel formation is presented, supported by experiments and numerical simulations. The knowledge of channel formation is further extended to demonstrate reconfiguration of virtual electrowetting channels by incorporating electrically-controlled functions such as splitting and merging in this platform. While digital microfluidics [13] relies only on droplet manipulation, this platform permits channel formation of desired geometries ‘on-demand’ that can sustain pressure driven flows.

**Device fabrication**

The construction of the device is schematically represented in Figure 2. Electrowetting devices were fabricated using glass slides as the bottom substrate. A thin 200 nm layer of gold over 10 nm tungsten seed layer was evaporated over a glass slide. The metal layer was then patterned using photolithography to define the electrodes and the electrical connections. The metal electrodes were then coated with a 1 µm thick Parylene-C (Speciality Coating Systems) layer. A spacer layer was patterned using a negative photoresist (Dupont PerMX 3050). This defined the height of the virtual channels (100 µm) and boundaries of the inlet and outlet ports. The inlet and outlet ports were confined at the back end and at the front end respectively to restrict the conducting fluid from moving into undesired areas of the chip. A glass slide with a conductive layer of ITO was used as a top cover. The top cover is drilled at the inlet and outlet locations using diamond coated drill bits. Both the bottom and the top substrates were coated with a thin (50 nm) layer of hydrophobic fluoropolymer (Cytonix Fluoropel 1601V). This ensured that the conducting liquid has a contact angle (Young’s angle) of 180º without an applied potential. Next, the top and bottom substrates are bonded together using an UV-curable
epoxy, ensuring that the device is sealed on all sides except at the inlet and the outlet. Ferrules are epoxy-glued over the inlet/outlet holes on the top plate to attach tubing that carries ambient oil (OS-30, Dow Corning) and the conducting fluid sample.

This general construction is maintained for all devices used in this work. Design modifications were made for upgrading the functionality of devices and will be presented in subsequent chapters. In Chapter 3 and Chapter 5 the same fabrication procedure is followed as is described here. In Chapter 4 arrayed electrowetting devices were fabricated using a significantly modified procedure developed in collaboration with University of Tennessee.

**Electrowetting characterization of electrolytes**

Electrolyte solutions and buffers of various ionic strengths and pH are widely used in biochemical analyses. Droplet microfluidics has been used to demonstrate diagnostic lab-on-chip, capable of processing biological samples including saliva, urine, blood and buffer solutions [25, 26]. Ultimately, the use of this platform in similar applications is envisioned. Thus, first several of the commonly used electrolytes including phosphates, acetates and chlorides were
considered for preparing the conductive fluid. The experimental setup used to characterize these biologically-relevant electrolyte solutions is illustrated in Figure 3. The metal layer was connected to a DC power supply and the setup was immersed in an oil bath filled with OS-30 oil. A droplet of electrolyte solution was carefully placed over the hydrophobic dielectric surface and a thin tungsten wire was inserted in it to provide the ground connection. Electrowetting contact angle modulation was performed by varying the applied voltage at the metal layer. The applied voltage \( V \) is related to the apparent contact angle \( \theta_V \) \cite{59, 60} by the Young-Lippmann equation \cite{4, 61}

\[
\cos \theta_V = \cos \theta_Y + V^2 (\epsilon_0 \epsilon / 2 \gamma_{cl} t) \tag{1}
\]

where \( \theta_Y \) is the contact angle at \( V = 0 \) V, \( \epsilon_0 = 8.854 \times 10^{-12} \) is the permittivity of free space, \( \epsilon = 3.15 \) is the relative permittivity of the Parylene C dielectric layer \cite{62, 63}, \( t \) is dielectric layer...
thickness, and $\gamma_{ci}$ is the interfacial tension between the conducting and insulating fluids. On a hydrophobic dielectric, where the Young’s angle $\theta_Y$ is approximately 180°, the contact angle can be modulated down to 45–60° by applying voltage suitably, before contact angle saturation occurs.

Electrowetting contact angle modulation was characterized for several biologically relevant electrolytes including, monosodium phosphate (NaH$_2$PO$_4$·7H$_2$O), sodium acetate (CH$_3$COONa) and sodium chloride (NaCl) at near neutral pH values (6-8). The data from these experiments are presented in Figure 4. Using these data, the value of the interfacial tension between the electrolyte droplet and the insulating oil ($\gamma_{ci}$) was estimated in each case and the Young-Lippmann equation (equation 1) was used to plot the theoretical trend. This trend analysis was performed for all three electrolytes at a concentration of 100 mM. The experimental data agree with the Young-Lippmann curves within ±3°, until contact angle saturation occurs. The Young-Lippmann model does not account for this saturation [64] and the physical origins of this phenomenon have not yet been explained successfully. The experimental data for electrowetting characterization of sodium acetate solutions at near neutral pH values indicates that pH does not have a significant effect on electrowetting modulation, which is in agreement with the recent work on contact angle saturation [64].

As indicated by the data, in this electrowetting setup all of the tested electrolytes start exhibiting contact angle saturation at or below 70 V. This value was chosen as the operating point for all the experiments described in this chapter to ensure that all the tested samples undergo maximum contact angle modulation in the devices. Phosphate electrolyte solution of 100 mM concentration was used as the conducting fluid in this work because of its lower saturation potential as compared with other electrolytes.
Figure 4. Electrowetting characteristic for 100 mM (a) sodium phosphate, (b) sodium acetate, and (c) sodium chloride solutions. Dashed lines represent theoretical values obtained from the Young-Lippmann equation (interfacial tension between conducting and insulating liquids (\(\gamma_{ci}\)) is estimated as indicated). Solid lines show the experimental data. (d) Electrowetting characteristic of sodium acetate electrolyte solution at pH 6, 7 and 8. Contact angle saturation occurs below 70 V for all samples.
Model of virtual channel formation

Theoretical analysis

To form a virtual channel on an electrowetting on dielectric (EWOD) platform, electric potential must be applied to adjacently placed, insulated electrodes such that the activated electrodes form a straight path of defined length and width. A microchannel is drawn over the electrodes when an electrolyte is injected through the inlet port over the first voltage-activated electrode. The conducting electrolyte electrowets the surface of the dielectric wherever potential is applied which lowers the apparent contact angle between the dielectric surface and the conducting medium. If the electrolyte is pumped into a device initially filled with insulating oil, it will conform to the shape of the activated electrodes and displace an equal volume of oil, thus forming a virtual channel.

The voltage-activated electrodes define a continuous path between the inlet and the outlet, over which the injected electrolyte propagates. Figure 5 illustrates the propagating meniscus of such a virtual microchannel with side panels illustrating top and cross-sectional views. If the Laplace pressure [4] on the front of the meniscus is assumed to be the same as pressure on the side of the propagating channel, the advancing meniscus pressure can be calculated as

\[ \Delta p = \gamma_{ci} \left( \frac{1}{R_h} + \frac{1}{R_v} \right) \] (2)
Figure 5. Schematic illustration of a virtual channel. (a) Conducting liquid forming a channel over an electrode-strip. (b) Top-view showing horizontal radius of curvature of the front of the meniscus and straight side-boundaries of the virtual channel. (c) Y-section depicting the horizontal radius of curvature and top and bottom contact angles of the front of the meniscus. (d) X-section showing the vertical radius of curvature of the side boundaries of the channel and the top and bottom contact angles of the meniscus on the channel sides.
where $R_h$ and $R_v$ represent the horizontal and vertical radii of curvature of the front meniscus.

From the channel geometry, the horizontal radius of curvature is assumed to be approximately half the width ($w$) of the channel ($R_h = w/2$). For the case of confinement between the top and bottom plates separated by a height ($h$), the vertical radius of curvature is given by

$$R_v = -h/(\cos \theta_t + \cos \theta_b)$$

(3)

where $\theta_t$ and $\theta_b$ are the contact angles on the top and bottom plates respectively. Although the results (Figure 4) show contact angle $\sim 170^\circ$ at an applied voltage of 0 V, the microscopic contact angle, which is not observable in this setup is expected to be closer to $180^\circ$ (the Young’s angle) since the top plate is coated with a hydrophobic fluoropolymer and is grounded. For simplicity of analysis it is assumed that this angle is $\theta_t \sim 180^\circ$. On the bottom plate, the contact angle is modulated to $\theta_b \sim 60^\circ$ by the applied voltage. Using these parameters, the Laplace pressure at the front of the meniscus can then be expressed as

$$\Delta p_{front} = \gamma_c(2/w + 1/2h)$$

(4)

For the sides of the channel, the vertical radius of curvature is denoted as $R_s$ and the contact angle on the bottom is represented by $\theta_s$ (Figure 5d). Looking from the top, the side of the channel is straight; therefore the horizontal radius of curvature is infinite. Using these parameters, the expression for the Laplace pressure for the side can be obtained from equation 2 and the expression for the vertical radius of curvature of the sides of the channel from equation 3 as
\[ \Delta p_{\text{side}} = \gamma c i \left(\frac{1}{R_s}\right) \] (5)

and

\[ R_s = \frac{h}{1 - \cos \theta_s} \] (6)

Since the Laplace pressure on the sides of the microchannel must equate the Laplace pressure at the front, equating equation 4 and 5 yields the value for \( R_s \) as

\[ R_s = \frac{2hw}{4h + w} \] (7)

The corresponding value of \( \theta_s \) can be determined from equation 6 as

\[ \theta_s = \cos^{-1} \left( \frac{w-4h}{2w} \right) \] (8)

In the experiments, the width of the electrode was \( w = 500 \mu\text{m} \) and the height of the channel (separation between top and bottom substrates) was \( h = 100 \mu\text{m} \). For these conditions, the side radius of curvature is calculated to be \( R_s \sim 111 \mu\text{m} \), while the side contact angle at the bottom is calculated to be \( \theta_s \sim 84^\circ \).

From the above theoretical discussion it may be observed that the bottom plate contact angle at the sidewalls of the virtual microchannel is larger than the contact angle at the front of the meniscus (\( \theta_s > \theta_b \)), which indicates bulging at the sidewalls. When a small volume of electrolyte is pumped into the channel its boundary bulges slightly on the sides, resulting in a change in the apparent contact angle at the bottom plate. This disrupts the equilibrium condition
such that the Laplace pressure from the sides becomes larger than the Laplace pressure at the front of the channel. To equilibrate pressures and relieve the sidewall bulge, the front meniscus of the channel must propagate forward over the electrode. In this manner, the liquid forms a continuous virtual channel by propagating over available activated electrodes, as long as the fluid is introduced at the inlet.

For a device consisting of a single inlet and a single outlet, once a microchannel is established by propagation over an activated path from the inlet to the outlet, the electrolyte can be continuously pumped at the desired flow rate. This continuous operation of these virtual “wall-less” channels with a syringe pump is similar to pressure driven flows in microfluidic channels with rigid channel boundaries. However, the geometry of the channel in this case is programmable and may be changed during the operation of the device, simply by applying potential to electrodes.

**Numerical analysis**

The theoretical model of channel formation was confirmed using numerical simulations in CFD-ACE+ (ESI Inc.). The modeled straight channel was 500 µm in length, 100 µm in height and 500 µm in width. Flow module and free surface module (VOF) were used to introduce a secondary liquid into a primary liquid, to realize an immiscible fluid-fluid interface. The primary liquid was defined as OS-30 oil and the secondary liquid was defined to be an electrolyte solution. The activated electrode was defined by a static wetting angle of 60º, while all the deactivated areas had a wetting angle of 180º. The choice of these values is consistent with the electrowetting characteristics of phosphate electrolyte solution of 100 mM concentration. In CFD-ACE+, the surface reconstruction method determines how the secondary fluid flows into the primary fluid. In this case, Piecewise Linear Interface Construction (PLIC) was used. Auto
time step was used in the simulation, with the initial time step set as 1 µs to accommodate the minimum cell size and reach a satisfactory convergence of all residuals to less than $10^{-4}$. Figure 6 illustrates the simulation results. The shape of the front meniscus and its radius of curvature match the previously described theoretical model closely. The horizontal radius curvature of the front meniscus was predicted to be $R_h \sim 250 \, \mu m$ (Figure 6b), confirming the assumption in the theoretical analysis. The vertical radius of curvature was measured from the simulated model to be $R_v \sim 200 \, \mu m$ and the bottom contact angle at the front meniscus to be $\theta_b \sim 60^\circ$ (Figure 6c). The simulation also predicted the contact angle at the side to be $\theta_s \sim 81.6^\circ$ and the sidewall radius curvature, to be $R_s \sim 116 \, \mu m$. These results confirm the theoretical model within tolerable error limits.

Figure 6. (a) CFD-ACE+ model of a propagating electrolyte meniscus (virtual channel) in oil. (b) Top view of simulated model indicating the horizontal radius of curvature of the front meniscus. (c) Y-section view of the simulated virtual microchannel. The simulated values of the vertical radius of curvature and bottom contact angle at the front of the meniscus are shown. (d) X-section view illustrating the sides of the channel indicating the radius of curvature of the sidewalls and the bottom contact angle at the sides.
Demonstration of virtual channel formation

Next, the formation of virtual microchannels is experimentally demonstrated. A device consisting of electrodes configured to form a virtual channel 3 cm in length is illustrated in Figure 7a. A power-supply set to provide square wave output at 70 V (RMS) at 100 Hz was connected through a switch box. The ITO layer at the bottom of the top plate was grounded as indicated. Once the device was completely filled with oil, the voltage was turned on and the device was ready to receive the electrolyte. The electrolyte solution (100 mM sodium phosphate) was colored red using pigment dispersion (Sun Chemical). This fluid was pumped in at a continuous flow rate of 10 µL/min.

A magnified image of the propagating meniscus of the conducting (red) fluid during virtual channel formation is shown in Figure 7b. The horizontal radius of curvature of this

Figure 7. (a) A virtual microfluidic device, illustrating formation of a 30 mm long straight channel. Conducting liquid is phosphate electrolyte solution (100 mM) colored with red pigment dispersion. Flow rate of 10 µL/min was maintained at the inlet using a syringe pump. (b) Magnified image of the propagating meniscus, showing relatively straight side walls (single line) and gradient at the front of the meniscus. (c) Time Lapsed images of channel formation. (d) Rise of liquid meniscus in a capillary tube connected at outlet shows continuous transport through the virtual channel.
meniscus is clearly half channel width, measured be $R_h \sim 250 \, \mu m$ which agrees with modeling results and the assumption made in the theoretical analysis. The front of the meniscus exhibits visible fading when viewed from the top, indicating a thinner fluid due to electrowetting of the meniscus at the bottom down to $\theta_b \sim 60^\circ$. The sidewalls of the channel appear relatively sharp and straight, which is expected since the sidewall contact angle $\theta_s$ is quite vertical, 82-84$^\circ$ from the theoretical analysis and the numerical model. These results substantiate the theoretical analysis of virtual channel formation.

The straight virtual channel forms over the activated electrode at a continuous flow rate as indicated by the time lapsed images in Figure 7c. As more fluid is pumped in, the excess volume of oil inside the sealed cavity of the device drains out through the outlet. The rate of formation of the channel was measured from the time lapsed images to be $\sim 3.5 \, mm/s$, which is slightly less than the simulated prediction (3.8 mm/s). The propagation rate can also be estimated from the input flow rate ($Q = 10 \, \mu L/min$) and the hydraulic diameter $D_h$ of the virtual channel ($D_h = 4A/P$, where $A$ is the cross-section area and $P$ is the perimeter). From the cross-sectional parameters calculated by equations 7 and 8, the hydraulic diameter of the virtual channel is $D_h \sim 162 \, \mu m$, which yields the channel propagation velocity of $\sim 3.7 \, mm/s$. Overall, the experimental results are in reasonable agreement with numerical and theoretical predictions. Figure 7d illustrates continuous pumping through a capillary tube connected at the outlet.

Continuous operation of virtual channels is a two-step process. The device by itself is a sealed cavity with operational inlets and outlets. The first step is to form the virtual channel which proceeds with the introduction of conducting fluid through the inlet and the consequent displacement of oil through the outlet. The velocity of channel formation is a function of the flow rate at which fluid is introduced at the inlet, and the channel dimensions. The flow rate can
be adjusted to obtain the desired velocity for a given channel dimension. However, it was observed that high rate of formation of virtual channels leads to unpredictable bulging of the channel near the inlet and spreading of the conducting fluid into undesired areas of the chip.

The second step is continuous pressure driven transport through the formed virtual channel. After successfully connecting a conducting liquid conduit from the inlet to the outlet, the oil has no path to exit the device because of the edge seal. This ensures that the device can be continuously operated at high flow rates without deforming the boundaries of the channel. The maximum rate at which a virtual channel can be formed is smaller than the rate at which the channels can be continuously operated. Figure 7d illustrates continuous pumping through a capillary tube connected at the outlet. The meniscus rises at a constant rate. Flow rates as high as 100 µL/min have been tested successfully without deforming the boundaries of the channel.

**Demonstration of reconfiguration of virtual channels**

The idea of forming straight channels by activating electrodes in a straight path can be extended to form channels with non-linear geometry. However, to reconfigure the geometry of the flow path it is necessary to first establish the capability of splitting and merging electrowetting channels. The mechanism is similar to that of droplet splitting and merging [14]. This operation is demonstrated here using a three electrode configuration, where the middle electrode is switched off to achieve splitting. Devices were fabricated using the same procedure as described earlier. Instead of a single electrode however, several closely-spaced electrodes were adjacently laid out and activated to form a straight liquid conduit, by pumping electrolyte from an inlet. The flow was stopped when the channel had formed completely connecting the inlet to the outlet. To achieve splitting of the channel, a 4 mm long electrode segment was deactivated. Figure 8a illustrates splitting of a virtual channel. In this case, the deactivated strip
was preceded by a longer fluid conduit (towards the inlet). The conduit of fluid succeeding it is shorter in length. It was observed that necking starts to occur on the non-energized strip near the succeeding end, so that the larger fluid conduit retains most of fluid when the splitting is complete. Importantly, both fluid conduits gain a certain volume of fluid that was earlier occupying the electrode strip. This leads to a certain amount of bulging. Since the Laplace pressure at the sidewalls of both fluid segments must be equal at the instance of the splitting, it occurs in a way such that both conduits of fluid bulge by the same amount and the longer conduit of fluid can accommodate a larger volume of fluid by undergoing the same amount of bulge at its side walls as the smaller conduit of fluid.

Merging of two liquid conduits has also been demonstrated using the same experimental setup. Figure 8b illustrates two approaching menisci. The same electrode that was de-activated to effect splitting was turned back on, which resulted in the liquid being pulled back into the activated electrode. The menisci approach and merge to reform a single conduit.

A combination of the functions of formation, transport, splitting and merging can be used to define channels of desired geometry connecting several inlets to several outlets. Virtual

![Figure 8](image). (a) Photograph of device, showing splitting. Close-up of deactivated electrode shows necking (b) Merging of two approaching menisci. White lines are used to mark the boundaries of the electrodes.
microfluidic channels designed for a particular function can be formed in a programmable fashion, by appropriately biasing electrodes and maintaining a supply of conducting fluid. In conventional microfluidic devices with channels of fixed geometries, active or passive valves are used for switching fluidic pathways. In contrast, virtual microfluidic channels can be re-routed or reconfigured to select one or more of several possible fluidic pathways for transport between inlets and outlets. The functionality provided by active or passive valves on a microfluidic channel of fixed geometry, is achieved by splitting and merging channels in a programmable fashion by appropriately activating or deactivating certain electrodes.

As a proof of this concept of programmable channel formation and reconfiguration of fluidic pathways, a device with a pair of inlets and outlets in the form of an ‘X’ channel is demonstrated. Figure 9 illustrates the various fluidic pathways that were tested. One or both inlets (‘A’ and ‘B’) could be selectively connected through a straight channel to one or both outlets (‘C’ and ‘D’). The flow rate at each inlet was maintained at 5 µL/min. The virtual channel was first formed between inlet ‘B’ and outlet ‘D’ by activating corresponding electrodes. The straight electrode strip in middle always stays on. Then, the electrode corresponding to outlet ‘D’ was switched off and the electrode corresponding to outlet ‘C’ was switched on. This resulted in a splitting of the channel over ‘D’, followed by formation of a new channel over ‘C’, defining a new path. The splitting in this case occurs close to the outlet reservoir of ‘D’. This is because the outlet reservoir is completely filled and has no room to bulge. The straight channel in the middle is the larger body of fluid and can accommodate the volume previously occupied by the channel over ‘D’. When the electrode corresponding to ‘C’ is turned on, the bulge in the straight channel is relieved by the propagation of the meniscus over the electrode. In a similar fashion the channel could be reconfigured to obtain the different fluidic pathways as illustrated. The switching of
fluidic pathways between outlets could be performed while fluid was still being pumped. When a particular inlet needed to be activated or deactivated, the syringe pump connected to it had to be turned on or off appropriately, accompanied by the application of the voltage at the necessary electrodes.

**Summary**

In this chapter, a model of virtual channel formation has been developed and the most critical basic functions such as pressure driven transport and switching of programmable fluidic pathways has been demonstrated. At this stage metal electrodes have been used to define specific geometries. An array of smaller, individually-addressable electrodes, such as active matrix drive, to achieve any desired geometry would provide for a more versatile platform. In the subsequent chapters a more detailed investigation of splitting of channels and reconfigurability will be
presented. Such programmability would move us beyond today’s ‘instrument’-on-chip, and lead to a new class of ‘laboratory’-on-a-chip systems. Such an agile lab-on-chip would allow access to a large library of laboratory tasks, but with portability and user-control similar to that of a field-programmable gate array (FPGA), an integrated circuit designed to be configured by the customer or OEM designer after manufacturing, and perhaps ultimately similar to a smart phone.
CHAPTER 3

SPLITTING OF FLUIDS ON ELECTROWETTING DEVICES

Introduction

Splitting and merging of droplets is a basic function in digital microfluidics, and most applications are highly sensitive to variations in sample volume [65]. Yet, droplet generation approaches often exhibit ~10% variability in the dispensed volume [66, 67]. Splitting a droplet typically involves abrupt de-activation of an energized electrode and thus de-wetting migration of fluid to adjoining energized electrodes. Variations in the generated droplet volume have been attributed to hydrodynamic instabilities arising in the splitting region and decrease in volume of the source reservoir [14]. Syringe pumps have been used for dispensing droplets in attempts to address this issue [68, 69] but without significant success. A precise method of volume control using capacitive and resistive feedback has recently been reported [70]. However, this approach adds complexity because of the necessary signal processing and may not be readily adaptable to electrowetting channels.

In this chapter, a simple approach for deterministic and accurate splitting of electrowetted fluid volumes is demonstrated. This method of splitting is applicable for smaller droplets as well as larger fluid segments and does not require complex signal processing. In this approach, deactivation of electrodes is performed by gradually ramping down voltage, so that splitting occurs with a deterministic de-wetting migration of fluids. It is shown that hydrodynamic instabilities responsible for splitting of fluid volumes can be controlled to produce predictable
fluid volumes. The time necessary for this gradual ramping was optimized using numerical models and confirmed experimentally. The findings indicate that sample volumes generated by controlling potential at the splitting region yield sample volumes with <1% variation. Several key aspects of splitting fluids have been investigated including the length and position of the deactivated region and its effect on dewetting fluid migration. It was found that migration of sample in the splitting region is proportional to the lengths of the split segments in an electrowetting microchannel.

**Device design**

The devices used in this portion of the dissertation were fabricated with the same process discussed in chapter 2. The devices consisted of 500 µm wide electrodes in the shape of a straight microchannel. The devices used for splitting required a larger surface area and hence a larger 50 mm × 50 mm glass substrate was used. The design of the electrodes specifically used for splitting is described here. Splitting electrodes of different lengths (3 mm, 4 mm, 5 mm and 6 mm) were placed at the center of the channel for testing centered splitting with changing area of the splitting region as shown in Figure 10a. Splitting electrodes were also placed at different positions along the microchannel to test the effect of channel length on dewetting migration of fluid from the splitting region. Channel electrodes were placed adjacent to the splitting electrode connecting the inlet and outlet ports as illustrated in Figure 10c. A visual length marker was patterned adjacent to the splitting electrode for observing fluid position during splitting. In addition, a width marker consisted of parallel 10-µm wide lines and 10-µm spacing, was patterned next to the channel electrodes for observing changes in volume (bulging) during the splitting process. The splitting electrode was electrified through two variable 1 MΩ resistors.
Figure 10. (a) Electrowetting based device for demonstrating deterministic splitting and volume generation. The increase in volume (bulging) due to fluid redistribution can be measured using the included width markers. Length markers along the splitting electrode can be used for observing the position of splitting. (b) Cross-section of electrowetting based device. The voltage applied to the splitting electrode is controlled by suitably varying the resistances R1 and R2 connected to it. (c) Formation of 500 µm wide, 25 mm long microfluidic channel.
so that applied potential could be precisely controlled by varying a second resistor in series (Figure 10b). The applied potential was monitored with a voltmeter across the second resistor. A transparent conductive top plate (100 nm ITO over glass) provided electrical ground, and contained drilled inlet and outlet ports. The height of the channel was defined by using a 100 µm thick spacer layer (PERMX) between the top and bottom substrates.

A virtual microfluidic channel was formed over all three voltage-activated electrodes with the introduction of the aqueous conducting liquid at the inlet as illustrated in Figure 10c. The aqueous conducting liquid was 100 mM sodium phosphate solution mixed with red aqueous pigment dispersion (Sun Chemical). The cavity of the device was filled with oil before the aqueous sample was introduced. Excess oil left the cavity of the device through the outlet. As characterized in chapter 2, saturation potential for this aqueous conducting liquid is <70 V with the saturation contact angle of ~ 60° [71]. After the channel was fully formed, the inlet and outlet reservoirs were balanced and completely filled with aqueous conducting liquid. This allowed us to overcome possible issues with splitting associated with variation in reservoir volume [14].

**Model of splitting and fluid migration**

**Theoretical analysis**

Laplace pressure across the aqueous-oil interface is a function of horizontal \((R_h)\) and vertical \((R_v)\) radii of curvatures of the meniscus and the interfacial tension \(\gamma_{ci}\) between the conducting aqueous liquid and the insulating oil. This was described in chapter 2, equation 1. It was also shown in chapter 2 that the sidewall vertical radius of curvature \((R_s)\) differs from the front of the meniscus. The \(R_s\) and the bottom contact angle at the sidewalls \((\theta_s)\) were defined by equating the Laplace pressures at the front of the advancing meniscus with the Laplace pressure
at the sidewalls [71] in equations 7 and 8. In a more general form the same expressions can be expressed as

\[
R_s = \frac{hw}{2h-w(\cos \theta_0 + \cos \theta_{sat})}
\]  

(9)

and

\[
\theta_s = \cos^{-1}\left(\cos \theta_{sat} - \frac{2h}{w}\right)
\]  

(10)

where \(h\) is the height of the channel, \(w\) is the width of the channel, \(\theta_0\) is the contact angle when no voltage is applied, \(\theta_{sat}\) is the saturated contact angle at an applied voltage such that increasing voltage does not change this value. For this system, \(\theta_0=180˚\) and \(\theta_{sat}=60˚\). The channel dimensions \(h\) (spacer thickness) and \(w\) (electrode width) are 100 \(\mu\)m and 500 \(\mu\)m respectively. Using equations 9 and 10 along with these parameters, the sidewall radius of curvature, \(R_s\approx 111 \mu \text{m}\) and bottom contact angle at the sidewalls is \(\theta_s\approx 84˚\). At equilibrium, the area of the channel cross-section \(A\) is given by:

\[
A = hw - R_s \sin \theta_s (h + R_s) + \frac{\theta_s}{180} \pi R_s^2
\]  

(11)

Hence for these devices at equilibrium, equation 11 yields the area of cross-section \(\approx 44,775 \mu \text{m}^2\). The initial volume of this 25 mm long microchannel is \(\approx 1.12 \mu \text{L}\). When voltage at the center-splitting electrode is turned off (or reduced), the fluid over the splitting region de-wets as compared to the adjacent energized electrodes. Fluid from the splitting region migrates to the adjoining electrowetted regions, causing the microchannel to shrink over the splitting electrode.
This also causes bulging over the adjacent channel electrodes, which now accommodates the excess fluid and expands from the original equilibrium shape (Figure 11). This increase in the cross-section of fluid segments is accompanied by a reduction in the sidewall radius of curvature $R_s$, and a consequent increase in the sidewall contact angle $\theta_s$ at the bottom.

**Figure 11.** Fluid redistribution during ramped splitting causes microchannel in the splitting region to shrink and the adjacent regions to bulge from their original equilibrium shape. Equilibrium cross-section is dark red, with lighter shades indicating splitting progression. The projection of the bulged sidewall can be viewed from top and used to calculate volume.

Redistribution of volume from the splitting region to the adjoining hydrophilic regions was examined by observing the change in the sidewall bulge. When seen from the top, the bulge in the sidewalls appears to form a uniformly advancing front as illustrated in Figure 11. The projection of the bulge, $w_p$ can be related to the radius of curvature of the sidewalls, $R_s$ and the sidewall contact angle of the at the bottom, $\theta_s$ by the following relation:

$$w_p = 2R_s \sin^2 \left( \frac{\theta_s - 90}{2} \right)$$  \hspace{1cm} (12)
The projection width, \( w_p \) relates the observable bulging of the sidewalls to the area of cross-section of the fluid segment.

Changes in projection width with increasing bottom contact angle (bulging) are illustrated in Figure 12. The precise volume of fluid segments of known length created by the process of splitting can be calculated by measuring the projection width experimentally and finding the corresponding value of the bottom contact angle and the associated area of cross-section from the graph (Figure 12). The final volume of the channel is the product of the length of the channel and its cross-section. The process of visual volume measurement was aided by the width markers lining the channel electrodes. This method of volume calculation is generally applicable to any electrowetting on dielectric based system and fluid combination as long as the values of \( \theta_0 \) and \( \theta_{sat} \) are known.

Under equilibrium conditions, migration of fluid to the adjoining electrowetted segments should be governed by the maintenance of uniform Laplace pressures at all regions of the oil-

![Figure 12](image_url)

**Figure 12.** Microchannel sidewalls bulge to accommodate migrating fluid from the splitting region. (a) Plot shows the decrease in the sidewall radius of curvature and the increase in area of cross-section with increasing bottom contact angle \( \theta_s \). (b) The projection of the bulged sidewalls seen from the top can be used as measure of the area of cross-section of the microchannel. The projection width \( w_p \) increases with increase in bulging. Each value of \( w_p \) corresponds to a particular value of \( \theta_s \) and an associated area of cross-section.
water interface. This implies that fluid migration to either side of the splitting region would be determined by the ability of the adjoining electrowetted segments to accommodate the excess volume through bulging of sidewalls. This is consistent with the mechanisms of droplet splitting described in literature [14, 72]. The lengths of the electrowetted segments on either side of the splitting region ultimately determine the amount of volume that can be accommodated. If the voltage at the splitting electrode is switched off abruptly, the fluid volume occupying this region becomes hydrodynamically-unstable without any confining forces to hold it in place. Migration of fluid from this region to the adjoining voltage-activated segments becomes unpredictable because of the short time during which mass transfer occurs. The nature of this unpredictability lies in the proportion of fluid migrating to either side, causing deviation in volume of droplets in electrowetting digital microfluidic devices.

**Numerical analysis**

A more deterministic approach for dispensing electrowetted fluid volumes must account for the time required for mass-transfer from the splitting region to the adjoining segments. Fluid migration from the splitting region to the adjacently-located existing fluid can be controlled in a more predictable manner if voltage applied at the splitting electrode is gradually reduced. As the voltage is reduced the aqueous conducting liquid migrates towards either side of the splitting region. The time needed for the gradual voltage ramping was optimized and estimated from simulations in ESI CFD ACE+. The numerical simulation was also used to confirm the theoretical model. For the simulation the length of the splitting region was more than twice the width of the channel as illustrated in Figure 13. The adjacent electrodes were of unequal length with the right electrode 3× in length compared to the one on the left.
The modeled straight fluid slug was 1125 µm in length, 20 µm in height and 100 µm in width. The splitting electrode was 225 µm long, with an electrode of 300 µm long on the left side and an electrode of 900 µm on the right side. The original device dimensions were scaled down to obtain these values. Scaling was necessary for keeping the size of the simulation within manageable limits. Flow module and free surface module (VOF) were used to define a secondary liquid inside of a primary liquid, to realize an immiscible fluid-fluid interface. The primary liquid was defined as OS-30 oil and the secondary liquid was defined to be an electrolyte solution. The activated electrode was defined by a static wetting angle of 60°, while all the deactivated areas had a wetting angle of 180°. The choice of these values is consistent with the electrowetting characteristics of phosphate electrolyte solution of 100 mM concentration. In CFD-ACE+, the surface reconstruction method determines how the secondary fluid flows into the primary fluid. In this case Piecewise Linear Interface Construction (PLIC) was used. Auto time step was used in the simulation, with the initial time step set as 1 ms to accommodate the minimum cell size and reach a satisfactory convergence of all residuals to less than $10^{-5}$.

To follow deterministic splitting, fluids would have to redistribute from the splitting region such that 25% of the fluid migrates to the left and 75% to the right. In other words, proportional distribution of fluid maintains equal Laplace pressures on either side of the splitting region which is the desirable parameter for deterministic splitting [71]. It was found that for a splitting region length of 200 µm in a 100 µm wide channel, the minimum time required for deterministic splitting is 160 ms with a uniform ramp from 70V down to 30 V. Figure 13 illustrates this result. For a slower ramp or abrupt splitting the fluid migration was predicted not to follow a proportional distribution indicating hydrodynamic instability conditions. This is
shown as the unpredictable splitting region in the graph (Figure 13). When the ramping time was gradual, proportional distribution of fluids was predicted by the simulation, confirming the theoretical model. It was assumed that for longer splitting lengths and wider channels mass transfer would take longer and hence require more gradual ramps. To keep all parameters constant for experiments all voltage ramps were greater than 2s ensuring enough time for mass transfer from the splitting region.

**Demonstration of optimized splitting and critical splitting parameters**

**Abrupt splitting vs. ramped splitting**

Deterministic splitting with gradually reducing voltage at the splitting region was tested by conducting sets of comparative experiments. The experiments involved splitting a 25mm long
fluid segment at the center, over splitting electrodes of 3mm length. The volume of the entire microchannel was 1.12 µL. In the first set of experiments, the voltage to the splitting electrode was abruptly switched off after the channel was formed to split the channel into two halves. In the latter set, the same experiment was repeated by gradually ramped down the voltage applied to the splitting electrode by changing the resistance of the network through which the splitting electrode was connected. The volume of the split segments on both sides of the splitting region was observed in both cases and calculated by measuring the projection width of the bulge on either side of the splitting region, as described previously.

When voltage was abruptly turned off at the splitting electrode, it was observed that the fluid migration to either side of the splitting region was unpredictable. The projection widths on both sides of the splitting region were unequal. A representative result is shown in Figure 14b, illustrating that most of the volume over the splitting region migrated to one side causing it to bulge more. The initial width of the virtual channel, \( w \) is the same as the electrode width (500 µm). The projection width of the bulged channel is measured by first obtaining the entire width of the bulged channel \((w + 2w_p)\) and then subtracting the width of the channel from this. In this case, the projection width was \(~26 \mu m\) on one side and \(~5 \mu m\) on the other. The volume of the segment was calculated to be 586 nL, with the volume of the other segment 534 nL. This is a deviation of \(\pm 5\%\) from the targeted volume of 0.558µL. The standard deviation in volume was found to be 2 nL \((n = 5)\). The accuracy of the method of measurement was confirmed by
Figure 14. (a) Experimental results showing both deterministic and unpredictable splitting at centered electrode. For the gradual voltage ramp, a symmetric radius of curvature is observed. (b) The final bulge on both sides is equal ($w_p \sim 13.5 \ \mu m$ – indicated with dashed white lines) for gradually ramped splitting. Abruptly turning off voltage leads to hydrodynamic instabilities and disproportional fluid distribution as indicated by the unequal bulging on two sides.
the fact that the sum of the individually-measured/calculated split volumes added up to the initial volume of the microchannel.

Gradually decreasing voltage at the splitting electrode yielded two split fluid segments of equal volume (Figure 14a). The splitting progressed with the formation of symmetric radii of curvature on the sidewalls over the splitting electrode. Fluid migrated to the adjoining voltage activated regions causing them to bulge. When splitting was completed, the projection width of the bulged segments was measured using the 10 µm width markers patterned on either side. The projection width was found to be ~13.5 µm on either side of the splitting region yielding a volume of 558 nL on either side. The experiments were repeated 5 times and the measurements were found to be within 1% maximum variation. The standard deviation in volume was found to be 0.001 µL (n = 5). The volumes created on both sides of the splitting region were equal confirming the hypothesis. Figure 15 summarizes the comparative results of both experiments. The data shows a clear improvement in the uniformity of split volumes when a ramped splitting

Figure 15. The target volume on each side was ~ 0.56 µL. 5 sets of experiments each with ramped splitting (red) and abrupt splitting (blue) are shown. The volume variations from the target ranged <1% for ramped splitting and +/-5% for abrupt splitting.
approach was used.

**Effect of size of the splitting region**

Reconfiguration of channel geometries on electrowetting devices can be described as a series of programmed transitional steps involving splitting, merging and transport of fluid segments. These transitional steps often involve splitting of fluid volumes into correctly portioned electrowetted segments to arrive at the new channel geometry. Not only does it require a programmable user interface, it is equally important to correctly estimate the volume of each segment in each transitional steps between two geometries for accurate rendition of desired geometries. Here this aspect of programmed reconfiguration is explored. Based on the theory developed earlier in this chapter it is possible to estimate the fluid transfer from a de-activated segment to adjoining electrowetted fluid volumes based entirely on their length and cross-sectional area. However, the splitting region needs to be within certain dimensional limits for deterministic migration of fluids. To explore these limits splitting regions of different lengths were investigated in the next set of experiments.

From theoretical considerations, the limits for the length of the splitting region for 25 mm long channel lie between 500 µm and 5mm without reaching the maximum bulging conditions. Beyond 5 mm, the amount of fluid migration required to split the channel into two equal halves would be beyond the capacity of the adjoining channels which are each 10 mm in length. The reason for this is that, the bottom contact angle reaches its maximum limit of 180° similar to the non-electrowetted state of the fluid, thus losing confinement. In this set of experiments a ramped splitting approach was used to effect a more deterministic fluid migration as already shown in the previous discussion. The objective was to find out if centered ramped splitting could divide fluid into exactly two halves when the area of the splitting region is increased incrementally. In
addition, experimental confirmation of the theoretical upper limit of the splitting region length would further validate the model.

As voltage to the splitting electrode was ramped down, fluid migration was observed by monitoring bulging of the adjacent segments which accommodate the influx of fluid. The splitting electrode length in these experiments ranged from 3mm to 6 mm in 1 mm increments. The total length of the channel in this case was also 25 mm. Experiments with 5mm and 6 mm splitting lengths are illustrated in Figure 16a. This bulging was measured as the projection width of the channel \( w_p \) in each case as illustrated in the representative images in Figure 16b. These images show an incremental increase in \( w_p \) of the adjoining segments with the increase in the length of the splitting region and a consequent decrease in the available length of the adjoining segments. For example, for a 3mm long splitting electrode the adjacent segments on each side are 11 mm in length. When the splitting region length is increased to 5 mm, the adjacent segments are only 10 mm in length but have to accommodate additional fluid migrating from the splitting region.

For these experiments, 3 mm splitting electrodes yielded very accurate results, dividing the channel into two equal halves. The larger splitting lengths performed less accurately in comparison. The average projection widths for 3 mm, 4 mm and 5 mm splitting segments were found to be 13.5 ± 0.01 µm, 26.6 ± 0.31 µm and 41.5 ± 0.76 µm respectively (\( n = 5 \)). Figure 16c summarizes these results. Since the splitting regions were centered, it was expected that the two split segments would yield accurately the same volume. This was confirmed within tolerable experimental limits, with the average volume error in the 0.33 – 1.3% range. These errors can be attributed to the larger mass transfer and still existing hydrodynamic instabilities for the longer necking during the final stages of the splitting process, which are more significant in longer
channels. However, the maximum error we observed was ~1.3% indicating the accuracy of this technique. To reaffirm these results, the length of the splitting region can be chosen to yield splitting accuracies greater than 99%.

When the splitting region length is higher than 5 mm a saturation in the capacity of the adjoining segments was observed. A representative result with splitting region length of 6mm is illustrated in Figure 16a. Instead of splitting into two segments, the creation of a third droplet over the splitting electrode was observed. The adjoining segments in this case were 9.5 mm in length and were saturated (bottom contact angle ~ 180˚) during ramped fluid migration, with excess fluid still remaining over the splitting electrode. Once this condition is reached it is no longer favorable for the remaining fluid in the splitting region to migrate to the adjoining segments leading to the creation of an undesirable droplet and over-bulged boundaries of the adjacent segments. Beyond saturation of the capacity of an electrowetted segment, the fluid no longer conforms exactly to the shape of the energized electrodes and resorts towards a more unpredictable and more natural circular shape wherever possible. The model of visual volume calculation does not account for these deformities beyond saturation and hence volume error measurements for the 6 mm long splitting region are not included.
Figure 16. (a) Ramped splitting with different splitting region lengths is illustrated. 5mm splitting region results in splitting with symmetric necking of fluid near the center line. For a 6 mm splitting region droplet formation due to excess volume was observed. (b) Projection widths of split segments, with splitting region of different lengths located at the center of the channel (same target volume). (c) The projection widths of split volumes resulting from ramped centered splitting with different splitting lengths is plotted. The errors increase as the splitting region length increases.
Effect of position of the splitting region

The location of the splitting region in a microchannel or the ratio of the lengths of the microchannels on either side of the splitting region determines the volume of fluid that migrates to each side during the splitting process. As described earlier, de-wetting migration of fluid occurs such that the Laplace pressure across the whole oil-water interface remains uniform. This implies that more fluid will migrate from the splitting region to a longer adjoining channel. The ratio with which the fluid distributes from the splitting region is dependent on the lengths of the adjoining segments. The volume of fluid migrating to one side (left side for example) can be expressed as

\[ \text{Vol. migrating to left} = \frac{\text{left electrode length}}{(\text{left+right) electrode length}} \times \text{splitting electrode vol.} \]  \hspace{1cm} (12)

The CFD ACE+ simulations were used to confirm this. Experimental validation of this idea was carried out by placing the splitting region at different offset distances from the center of the channel. Zero offset or centered splitting electrode has already been explored in the previous section. The splitting electrode used in this case was 3.5 mm in length with offset distances of 0 mm (centered) to 6 mm in 1.5 mm increments. Similar to the previous experiments, the total channel length was 25 mm and the channel width was 500 µm. These electrode arrangements are illustrated in Figure 17a. The length of the splitting electrode was chosen such that deterministic splitting can proceed with predictable fluid migration when voltage to the splitting electrode is ramped down.

The ratio of the lengths of the adjoining channels changed with every offset distance. When ramped splitting was implemented, the bulging of these adjoining channels was used as a
measure of the proportion with which the fluid from the splitting region distributed to the two unequal adjoining channels. The most important observable in this experiment was the projection width of the two unequal segments on either side after the completion of the splitting process. According to the model the projection width \( w_p \) on two sides should be equal after fluid distribution. A representative result is illustrated in Figure 17b. The bulging of the channels of unequal length closely matched for all the cases. However, as the offset distance was increased, there were slight deviations in projection width (bulging). This may be attributed to the larger proportion of fluid migrating to one side. This is similar to the errors observed with larger splitting electrode lengths as described in the previous section.

The results of offset splitting experiments are summarized in Figure 17c. The projection widths on either side of the splitting region were observed for each offset distance. The plot shows that the projection widths on were within a maximum deviation of 1\% of each other. These projection widths were then used to calculate the volume of the split segments as described in the theoretical model. As illustrated in Fig. 17d, the volume errors were within the 0.1 \(-\) 1.09\% range. Similar to centered splitting these errors show a consistent increase with increase in offset distance. The distribution of fluid from the splitting region follows the ratio of lengths of the adjoining segments. For centered splitting this ratio is 1:1 and volumes split were shown to be equal. For offset splitting, the split volumes follow the ratio of lengths.

The split volumes were then analyzed to determine the ratio of fluid distribution. For example, for an offset distance of 1.5 mm the left segment was 9.25 mm and the right electrode was 12.25 mm in length such that the ratio of the left electrode to the combined total of both electrodes is \(~43\%\). The initial volume of the segment on the left \((w_p = 0 \mu m)\) was 414 nL. After splitting, this segment bulges with a projection width of 19.8 \mu m, yielding a volume of 481 nL.
Figure 17. (a) Splitting experiments with a 3.5 mm splitting electrode at different offset distances from the center of the 25 mm long channel. (b) Representative result of offset splitting illustrating a splitting region at 4.5 mm towards the left of the center of the microchannel. Ramped splitting clearly shows necking occurring towards the left suggesting greater volume migration towards the right (longer channel). The bulging on both sides was measured to be the same. (c) The experiments were repeated 5 times with the splitting regions located at different offset distances from the center of the channel. The projection widths for offset splitting are plotted. A deviation of ~1% was observed for larger offset distances. (d) Table of metrics associated with offset splitting shows the electrode lengths for each experiment and the average volume errors.
Hence the excess volume accommodated was 68 nL. The total initial volume on the 3.5 mm long splitting electrode was 156 nL. The ratio of fluid that migrated from this region to the left electrode was 0.067/0.156 ~ 43%. Comparing this to the ratio of the electrode sizes confirms the theory conclusively.

**Summary**

Splitting finite volumes with accuracy is one of the fundamental functions of digital microfluidic platforms. These experiments provide a conclusive guideline towards the design of reconfigurable devices, and that is to decrease the length of the splitting region to reduce the errors in generated volumes. Deactivation of larger electrowetted microfluidic networks should proceed stepwise by gradually turning off smaller segments at a time. This will allow for a more predictable means of reconfiguring electrowetting microfluidic devices. Further, the transitions between steps should be designed such that splitting operations are centered whenever possible to minimize complications with fluid unequal fluid migration.

The theoretical models and experiments presented thus far serve to validate the physical principles and operations that govern the basic microfluidic functions on the electrowetting device. In subsequent chapters these concepts will be used to demonstrate more elaborate application oriented functionalities of the device.
CHAPTER 4

COMBINING ELECTROWETTING CHANNELS AND DROPLETS

Introduction

Automation of microfluidic functions such as transport, storage and fluid manipulation in small volumes is critical towards successful implementation of applications on ‘lab-on-a-chip’ platforms. In a bio-analytical laboratory the challenges associated with repetitive and labor intensive processes can be addressed using programmable liquid handling. Significant effort has been directed towards the development of such systems for miniaturized analysis in biological and chemical applications [2, 5, 6, 73, 74]. Programmable microfluidic devices are reconfigurable and hence more versatile for on-demand liquid handling [75]. Digital microfluidic systems exhibit such functionality and are able to manipulate discrete sample volumes (droplets). Despite the importance of droplet manipulation, the unique functionalities provided by continuous microfluidics in processing of large sample volumes at high throughput cannot be easily reproduced using droplets.

In this chapter, programmed interaction of both continuous as well as discrete microfluidic functions is demonstrated for the first time. In earlier chapters electrowetting channels were discussed in detail [71]. It was also demonstrated that electrowetted fluid segments can be portioned into precisely metered volumes using voltage ramping [76]. The possibility of programmed fluid manipulation on arrayed electrodes has been shown by Rack et al. [77]. This work is extended to combine pressure driven continuous channel formation,
programmed transition between complex microfluidic structures and interaction between continuous microfluidic structures through automated droplet manipulation.

The overall concept of the arrayed device is illustrated in Figure 18. The device relies on electrowetting contact angle modulation of conducting aqueous samples in oil ambient over insulated electrodes, to define the boundaries of electrowetted channels or droplets. It consists of an electrically-programmable two-dimensional array of 360 insulated electrodes on the bottom plate. The transparent top plate is conductive and it is separated from the bottom plate by a spacer layer. This arrangement is used to form a sealed cavity with inlet and outlet ports. A computer user interface lets the user define the desired channel geometry and droplet microfluidic functions to be carried out. The capability of selectively applying potential to each electrode combined with sample injection using syringe pumps at the inlets makes it possible to

![Figure 18](image.png)

**Figure 18.** Microfluidic platform based on electrowetting, integrating continuous and digital paradigms. Inset shows arrayed electrodes of size 1mm$^2$. Sample introduced through inlet forms a channel which can be split into droplets of various sizes. Transparent conducting front plate (not shown) is grounded.
demonstrate a variety of fluid manipulation and handling capabilities including, continuous channel formation, splitting and merging of microfluidic channels, mixing of portioned droplets and sample transport in a programmable manner. The specifics of fluid handling and associated volumes of channels and droplets are drawn from theoretical models described in previous work [71, 76]. Several demonstrations of automated liquid handling included in this dissertation make it relevant for applications in biochemical analysis. As a proof of concept a glucose assay was tested on this device and all associated reagents were characterized for electrowetting.

**Device design and fabrication**

The devices that we used in this study were different in construction than the ones described in Chapters 2 and 3. The fabrication procedure for these devices was developed in collaboration with The University of Tennessee. The devices consisted of an array of interdigitated electrodes as opposed to purpose designed electrodes for specific experiments. The array was laid out as 5 × 8 blocks of 40 addressable electrodes. Each interdigitated electrode measures 1 mm × 1 mm with a gap of 40 μm between inter-digitations as illustrated in Figure 18. The device used here has arrayed electrodes. To make a large array, 9 unit arrays (total 360 electrodes) were duplicated and connected by inter-connection layer. Figure 19a shows the cross-section of the electrowetting device. A 500 nm buffer SiO₂ coated silicon wafer was used as the bottom substrate. Chromium (Cr) inter-connection layer (150 nm) was sputter deposited wafer and subsequently lithographically patterned and wet chemically etched with a standard Cr wet etch solution (9% (NH₄)₂Ce(NO₃)₆ + 6% (HClO₄) + H₂O). After a SiO₂ inter-metal dielectric layer (400 nm) was deposited at 350°C via plasma enhanced chemical deposition (PECVD), 80 μm square via holes for electrical contact were formed by photolithography and dry etch process. Cr electrowetting electrodes (150 nm) were deposited by sputtering and subsequently
As an electrowetting dielectric layer, PECVD SiO$_2$ (300 nm) was deposited at 350°C. The top plate used was an indium-tin oxide (ITO) coated glass. To make all the surfaces hydrophobic, the bottom and top plates were dip-coated in Cytonix FluoroPel 1601V solution and baked for 20 min at 140 °C.

A photo resist film (Dupont PerMX 5050) was used to create the requisite gap of 100 µm between the top and bottom plate. This photoresist layer was patterned to include inlet and outlet reservoirs for fluid introduction and elution. Holes were drilled on the top plate corresponding to these reservoirs. The top and bottom plates were sealed together with UV epoxy (Dymax, OP-30). Silicone oil (Dow Corning OS-30 oil) was introduced as an insulating ambient medium into device through the inlet port on the top plates. Figure 19b shows a photograph of a complete

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*Figure 19.* (a) Cross-section of electrowetting device. Aqueous fluid introduced through inlet conforms to the shape of the activated electrodes forming electrowetted structures. (b) Photograph of completed device with inset showing arrayed electrodes. The electrical interface at the edges of the device were interfaced to a power supply and controlled using a computer interface. (c) LabView based computer interface. A single unit array consisting of 40 electrodes could be programmed to carry out any number of steps at specific time intervals. The functions are replicated on all 9 unit arrays connected in parallel.
device. Electrical connections between electrowetting array and data acquisition (DAQ) card were made using a customized test clip with spring loaded pins. The DAQ card was controlled by a signal generating program using LabView software.

The user interface in LabView (Figure 19c) allows the user to control the device and turn the electrodes in the device on or off in any desired pattern for the necessary durations of time. Aqueous fluid introduced through the inlet port using a syringe pump, (Figure 19a) conforms to the shape of the activated electrodes. Channels and droplets could be formed and manipulated in this manner. Transport, sample metering into droplets and transition between different complex geometries could all be accomplished using similar principles but involves more elaborate program sequences to be supplied by the user through the user interface. The current capability limits us to program a $5 \times 8$ unit array of individual electrodes. 9 such unit arrays connected in parallel allows us to control a set of 360 electrodes, with each unit array replicating the user input in tandem.

**Electrowetting characterization**

Each aqueous fluid used on the device has its own characteristic interfacial tension $\gamma_{ci}$, and hence it was important to determine the voltages at which they saturate. Using the contact angle at saturation it is possible to determine the area of cross-section and an estimation of the volume of the electrowetted segments, as described in Chapters 2 and 3. Specifically, an aqueous red dye (Sun Chemical) used for all generic demonstrations of electrowetting functionality has been characterized. The reagents associated with a colorimetric glucose assay (Cayman Chemical) have also been characterized for electrowetting contact angle modulation. These included a Phosphate buffer at pH 7.2, glucose standard solution (50 mg/dL) and enzyme mixture. The results of these experiments are presented in Figure 20. As indicated by the data, all
Figure 20. Electrowetting characterization of aqueous conducting fluids. The saturation angle varied between 60° ~ 75°. The dashed lines show the predicted values using Lippmann equation and the solid line indicates experimental observations. The maximum voltage for saturation was 40V. Since all the reagents saturate below this voltage, the operating point was chosen at 40V for all experiments. The dashed lines show the predicted values using Lippmann equation and the solid line indicates experimental observations.
of the tested solutions start exhibiting contact angle saturation at or below 40 V. This value was chosen as the operating point for all the experiments to ensure that all the tested samples undergo maximum contact angle modulation in the devices. Using these data, we estimated the value of the interfacial tension between the aqueous droplet and the insulating oil ($\gamma_{ci}$) in each case and used the Young-Lippmann equation (equation 1) to plot the theoretical trend. The experimental data agree with the Young-Lippmann curves within ±3º, until contact angle saturation occurs.

The data from these experiments were used estimate the volumes of these reagents over a single 1 mm × 1mm electrode using equations 9,10 and 11 for device height $h = 100 \mu$m. The volume was then obtained by multiplying the area of cross-section with the lengths of the electrowetted fluid segments. This analysis is summarized in Table 1. The data shows that the volumes are within ~2.5 nL of each other. Although this is a small value compared to the total volume accommodated over a single electrode ~92 nL, these minute variations in volume can be important in biochemical analysis. The volumes of each reagent over a unit electrode could be used to estimate the volumes of larger electrowetted segments by counting the number of electrodes.

**Table 1. Volumes of the reagents over a single 1mm × 1mm electrode were calculated using the saturation contact angle $\theta_{sat}$ and eq. 2-4.**

<table>
<thead>
<tr>
<th>Fluid</th>
<th>$\theta_{sat}$</th>
<th>$R_s$ µm</th>
<th>$\theta_s$</th>
<th>$A$ µm²</th>
<th>Volume over one electrode nL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye</td>
<td>70.0</td>
<td>116.6</td>
<td>81.8</td>
<td>94418.8</td>
<td>94.4</td>
</tr>
<tr>
<td>Buffer</td>
<td>60.0</td>
<td>142.9</td>
<td>72.5</td>
<td>92743.1</td>
<td>92.7</td>
</tr>
<tr>
<td>Glucose</td>
<td>75.0</td>
<td>106.2</td>
<td>86.6</td>
<td>95192.3</td>
<td>95.2</td>
</tr>
<tr>
<td>Enzyme</td>
<td>60.0</td>
<td>142.9</td>
<td>72.5</td>
<td>92743.1</td>
<td>92.7</td>
</tr>
</tbody>
</table>

**Demonstration of channels and droplets**

Fluid introduction into electrowetting devices has been approached in different ways. In a
two plate electrowetting system, the popular techniques in digital microfluidic devices include “smashing”, pipetting through a hole on the top plate and more recently, syringe pump based fluid introduction. The smash method involves suspending a measured volume of aqueous solution in a larger droplet of oil on the bottom substrate and placing the top plate over this arrangement to smash the fluids. While this method is suitable for some droplet based demonstrations, it cannot be used reliably for multiple reagents and continuous channels. In the second approach, the device is prefilled with the ambient medium (oil or air) and aqueous reagents are pipetted through individual holes drilled on the top plate. These reagents are subsequently dispensed as smaller droplets by splitting from these larger reservoirs. Although this method is used widely, the finite nature of the reservoirs leads to changing droplet volumes as more droplets are dispensed from the same reservoir. The third and the more recent approach uses a syringe pump to introduce fluids through inlet ports drilled on the top plate. This technique is more versatile of the three because of its ability to dispense controlled volumes as well as supporting continuous flows and has been used in this work.

In earlier chapters electrodes shaped in the form of long channels were used for demonstrating the formation and programmed reconfiguration of continuous channels [71]. Here arrays of smaller 1mm2 electrodes have been used. The advantage to using this arrangement is that it gives access to a larger variety of geometries that can be programmed. Channel formation and their continuous pressure driven operation were tested on the device to confirm that it is similar in behavior with earlier devices. The device was programmed by activating a series of 24 electrodes connecting one inlet to one outlet in a straight line. Subsequently, a syringe pump was used to dispense red fluid through the inlet (Figure 21a) at a continuous rate of 5µL/min. The fluid meniscus showed a ratcheting motion from electrode to electrode as it moved forward. We
attribute this ratcheting motion to partial overlap at the boundary of each electrode. When each electrode electrowets the incoming fluid it takes time to completely fill and populate the gap to the next electrode during which the meniscus appears static. As soon as the meniscus touches the next electrode it shoots forward. A 24 mm long channel was formed in this way between the inlet and outlet ports in ~24s. The total channel volume from inlet to outlet (not including the ports) was 2.3 µL as roughly estimated from the dispensed volume reading on the syringe pump. This was confirmed from earlier electrowetting characterization of the red dye for which the area of cross-section was 94420 µm². For a 24 mm long channel the volume can be simply estimated by multiplying the cross-section with the length, which yields ~2.27 µL.

After channel formation, the long channel was split into smaller droplets of different sizes. Figure 21b shows three droplets covering 2, 3 and 4 electrodes each. These droplets were transported repeatedly, moving each droplet at a speed of 1mm every second as indicated by the time lapsed sequence. Droplet movement was achieved by activating electrodes adjacent to the droplets and then turning off the electrodes over which the droplets resided. This sequence was programmed into the labView interface for each droplet such that the droplets could be moved quickly and uniformly. The speed with which the droplets moved could be varied by changing the intervals between activation and deactivation of electrodes. As speed was increased, it was observed that larger droplets (3 electrodes or more in size) could not be moved easily at higher speeds. This is because mass transfer takes longer for higher volumes.
Larger electrowetted structures such as channels can also be transported in a similar manner as droplets. To demonstrate this we formed a straight channel 2 mm wide. Once fully formed this electrowetted segment was moved laterally across the rows of the arrayed device. Electrodes adjacent to the channel were activated in a row and then the row over which the channel resided was turned off to effect its motion as illustrated in Figure 21c. The time required for such a migration is much larger compared to the motion of smaller droplets. For shifting the channel by 1mm laterally it took about 5s. It was observed that both in the case of larger droplets as well as elongated electrowetted segments transport of fluid does not take place simultaneously over all the activated rows. Typically the fluid starts to migrate to the adjacent activated row at one spot and the whole fluid follows (refer to ESI video for a more elaborate demonstration).

The transport of larger electrowetting structures may be important in various applications where...
the ratio of mixing between two reagents is large. Conventionally this is carried out by transporting multiple droplets of one reagent and mixing with one droplet of another reagent in digital microfluidics. However, this method is prone to errors because of the elaborate nature of the fluid handling protocols. Methods shown here on the other hand allow the user to estimate the volume of the structure in a single step and then transport it as a single body of fluid to its destination on the chip. Programmed transport of a wide range of volumes can be critical to the execution of complex biochemical analysis such as assays.

The behavior of conventional microfluidic applications is heavily dependent on the geometry of the microfluidic structures. Programmable reconfiguration of electrowetted structures allows users to change the behavior of the device and to fine-tune its functionality without having to redesign the system. It also allows the user to carry out functions such as agitated mixing of two or more samples and directing droplets or continuous flows into different paths. The programmed transition between different shapes of electrowetted structures is demonstrated here. 6 of the 9 unit arrays on the device were loaded with equal volumes of red dye by forming channels through an inlet and then splitting the channel and suitably transporting the volumes to the individual unit arrays. Figure 22 illustrates the scheme of the transitions. Electrodes are turned on in a “U” shape and then transitioned through a 2-step sequence (not shown) to a “C” shape over 2 seconds. In a similar manner transitions were repeatedly carried out to get the “T” shape and back to the “U” shape. These letters represent the collaborative efforts of The University of Cincinnati (UC) and The University of Tennessee (UT), Knoxville on this project. Most importantly these transitions demonstrate a proof of concept for on-demand formation of complex microfluidic structures and their programmed reconfiguration.
The focus of this work is to widen the applicability of an electrowetting platform beyond processing droplets by incorporating larger volume electrowetting structures in the form of channels and complex geometries on the same platform. To demonstrate interactions between channels and droplets an experiment was devised where droplets were transferred between two channels. First, two channels were formed as shown in Figure 23a. Channel 1 was connected to the inlet and supplied by a syringe pump. The two channels were each 2 mm wide and were spaced apart by a gap spanning 4 electrodes. Next, three droplets were drawn from Channel 1 by activating three electrodes. The excess fluid required for this was supplied from the syringe pump (~0.3 µL). Another set of three electrodes were turned on to elongate the liquid fingers projecting from Channel 1. To split the three droplets the electrodes adjacent to the channel were deactivated. Subsequently, the three droplets were transported towards Channel 2 and merged with it.

The volumes associated with the process of droplet transfer were closely monitored to understand the interaction of the droplets and the channels. Earlier we estimated the volume of red liquid over a single electrode to be ~ 94.4 nL and suggested that the volumes of larger structures could be estimated by counting the number of electrodes they occupy. For this
experiment these estimations were verified in two ways. First the dispensed fluid from the syringe pump was monitored and this provided an approximate volume for the channels as well as the droplets. Second, the channel length was imaged and the theoretical model of channel cross-section was applied to calculate the volumes of each channel for a constant length for each step. The data from these calculations is presented in Figure 23b. As shown in the plot, the volume of Channel 1 increases by ~0.3µL as it is supplied by a syringe pump to generate the liquid fingers.

As the droplets separate from Channel 1 there is a drop in volume greater than the volume of the separated droplets and a subsequent equilibration to the original volume of the channel. This is attributed to the sudden retraction of the liquid fingers over the deactivated electrodes effecting splitting and the resulting elution of fluid through the inlet. The volume of Channel 1 quickly returns to its equilibrium after the dip in volume without any additional

**Figure 23.** (a) Two straight channels were formed. Three droplets were split from Channel 1 by supplying red fluid through inlet and then transported to merge with Channel 2 (b) The changes in volumes of the two channels were monitored. Channel 1 gains volume as it is supplied with excess volume. The volume of Channel 1 suddenly drops as the three droplets are split from it. Channel 2 gains the same volume. Finally, both channels equilibrate slowly towards their original volumes.
dispensing of fluid suggesting a damped oscillatory behavior. Channel 2 was not connected to an inlet/outlet and therefore for consistency we measured its volume over a constant length. The volume of Channel 2 increases as it receives the three droplets and this increase was ~0.3 µL verifying the earlier estimation. The length of the channel increases slightly to compensate for the excess volume returning the channel shape to its original equilibrium. Not accounting for this increase a dip in volume over the measured length was observed, suggesting the same oscillatory behavior as observed for Channel 1. This behavior suggests that electrowetted structures have a natural tendency to return to their equilibrium condition.

This aspect of electrowetting structures can be very useful for maintaining precision in dispensed volumes of droplets. Restricted electrowetting structures have a tendency to bulge for accommodating excess fluids as discussed in chapter 4 [76]. Although this effect is measurable, it may be difficult to precisely control it for practical applications. On the other hand allowing these structures to maintain equilibrium allows the user to reliably form metered volumes of droplets or larger electrowetted shapes.

**Demonstration of bioassay**

The techniques demonstrated in this work are generic in nature and can be applied to various applications on electrowetting platforms. These techniques were used to test a simple colorimetric glucose assay. A glucose assay was chosen because of its popularity and wide applicability. Determination of glucose levels in blood is critical in the control of diabetes. In general, glucose assays are of two types, enzymatic or non-enzymatic. While non-enzymatic assays reduce all sugars, enzymatic assays are specific to glucose allowing for more accurate quantification. An enzymatic assay kit from Cayman Chemical that uses glucose oxidase-peroxide reaction for determination of glucose concentration was chosen for this study.
Three different unit arrays on the chip were loaded with enzyme mixture (Cayman Chemical) from the glucose assay kit by forming a channel and then dividing it into equal segments and suitably transporting the segments. Next the glucose samples premixed with assay buffer were introduced through another inlet. Droplets of three different glucose concentrations (0, 20, and 50 mg/dL) were transported and merged with the enzyme. The merged electrowetted segments were then incubated by agitation with sequential activation and deactivation of electrodes. The samples developed color according to their concentration as shown in Figure 24.

**Figure 24.** (a) Colorimetric glucose assay demonstration on-chip. Three different concentrations of glucose are shown here. (b) The concentrations were quantized using CIE color space analysis.

We have quantized the values of concentrations by analyzing the colorimetric results in CIE color-space [78] as shown in Figure 24b. However, the reflective nature of the Silicon substrate limited this analysis. The outcome of this experiment was demonstration showing a color gradient between three different concentrations of glucose as indicated by the data in Figure 25b. Although the same kind glucose assay has been repeatedly demonstrated in digital microfluidics, here it serves as a qualitative demonstration of automated fluid handling techniques.
Summary

Digital microfluidic devices have gained popularity due to their inherent ability to adapt to different applications without fundamental changes in device design. The incorporation of continuous fluid handling with pressure driven flows makes these programmable devices more widely applicable for the ubiquitous goal of lab-on-a-chip. Continuous rigid channel have been incorporated with digital microfluidic platforms [22, 79], but in that approach, the continuous channels were only used for fluid delivery to the digital microfluidic device. While discrete droplets can be successfully manipulated and serially processed, continuous channels and the programmable functionality of larger electrowetting structures including complex geometries provide an opportunity to widen the functions and to simplify the operations of the digital microfluidic devices. As demonstrated, electrowetting channels allow the user to precisely control larger volumes without the inherent complexity and errors involved with multiple smaller droplets. The optimization of fluid handling protocols and their automation has been demonstrated as proof of concept. The following chapter deals with the use of these fluid handling capabilities for the implementation of heterogeneous immunoassays using magnetic particles.
CHAPTER 5

TOWARDS HETEROGENOUS IMMUNOASSAY ON-CHIP

Introduction

Immunoassay is one of the most powerful and sensitive bio-analytical techniques used in numerous applications that range from the diagnosis of malaria [80] to the detection of biological warfare agents [81, 82]. Since its conception in the 1960s, the demand for immunoassays have grown with the increased pace of discovery of new biomarkers, driving the development of fully automated immunoassay analyzers by 1980s [83]. Immunoassays rely on the specificity of reactions between an antibody and an antigen for measuring relevant analytes in various applications. The conventional implementation of immunoassay most commonly involves a 96 well plate format and an automated fluorescence analyzer (fluorometer). While this is the current gold standard for immunodiagnostics, the need for relatively large sample volume and instrumental costs limit effectiveness of this approach. Using microfluidics can offer a potential solution to these issues [84]. Smaller dimensions of microfluidic devices reduce reagent consumption and save time. While most of the microfluidic assays use continuous flows [85] or droplets [8] within rigid networks of micrometer dimensions, an alternative approach with digital microfluidics has been growing in popularity [5, 20, 24-26].

Among the various available formats for immunoassays, heterogeneous immunoassays are the most popular due to their high sensitivity and lower limits of detection. In this format, the antigen-antibody complex is typically immobilized on a solid phase such as micro-beads or well-
plate. This allows the removal of unbound molecules in the sample matrix to be washed away. Subsequently detection is performed using a direct fluorescent label on a secondary antibody or alternatively an enzyme labeled secondary antibody (ELISA). At least five different research groups have reported immunoassays relying on antibodies bound on solid supports (heterogeneous immunoassays) using DMF. These include immunoassays for immunoglobulin G and ricin [34], insulin and interleukin-6 [35, 36] and immunoglobulin E [37]. These immunoassays used micro-beads as a solid support for antibodies. Alternatively, antibodies can be immobilized directly on the device surface. The first surface based immunoassay on DMF devices was demonstrated by Miller et al. [38].

DMF uses a programmable grid of addressable electrodes to effect electrowetting manipulation of sample droplets. This technique permits complex multistep assay protocols to be automated, while providing a generic platform form for performing a variety of assays, saving time and fabrication costs. However, digital microfluidic devices are limited by finite reagent volumes which result in variations of droplet sizes and errors in assay results [65]. The aim here is to develop an electrowetting based device for implementing immunoassays integrating the programmable functionality of digital microfluidic systems and the continuous flow operation of conventional microfluidic devices. The primary objective is to design and test the protocols for performing immunoassays. While it is obvious that a continuous source of reagents can eliminate such problems as limited wash buffer volume and droplet size variations [76], it is necessary to quantitatively optimize the protocols and demonstrate for the first time an optimized digital microfluidic device capable of performing assay protocols utilizing continuous flows. It is expected that this system will show improved immunoassay performance because of precise fluid handling characteristics. Once optimized, this system can help fuel the development of a
new class of automated immunoassay analyzers that require a very small amount of sample per test (few nL) while improving limits of detection.

Device design

The devices were designed to demonstrate assay protocols with a combination of precise droplet handling and continuous channel functionalities. To achieve this, a hybrid design consisting of both smaller electrodes for droplets as well as longer channel forming electrodes was used. The overall concept of the device is illustrated in Figure 25a. Devices were fabricated following the same procedure described in Chapter 2. A 50 mm × 50 mm glass substrate was used to pattern aluminum electrodes using standard photolithography. Longer channel forming electrodes connected the inlets and outlets to the reactor region located at the center of the device consisting of a number of smaller 500 µm × 500 µm electrodes. A cross-section of the various layers on the device is illustrated in Figure 25b. The aluminum electrodes were insulated using a 500 nm thick layer of Parylene-HT (Speciality Coating Systems). A 100 µm thick spacer layer was patterned on the bottom substrate forming the height of the device. The spacer layer consisted of patterned inlet/outlet ports. The top plate was ITO coated glass for electrical ground. Inlet and outlet ports were drilled on the top glass cover corresponding to the inlet outlet ports patterned on the spacer layer. All surfaces were coated with Fluoropel 1601V for hydrophobicity ensuring a contact angle ~180° when no voltage is applied for aqueous samples. The top and bottom plates were bonding together with a UV-curable epoxy (Dymax OP-30) ensuring that the device cavity is sealed except at the inlet and outlet ports.

The patterned electrodes on the device connected to metal traces leading to the electrical interface at the edges of the device. The electrical interface allowed the device to be connected to
Figure 25 (a) Schematic diagram illustrating hybrid electrowetting device for channel and droplet functionality. (b) Cross-section of electrowetting device. (c) Photograph of device showing the reactor in the center consisting of insulated electrodes. The electrodes were connected to a computer controlled interface through electrical connectors at the sides of the device.
a programmable computer interface capable of individually addressing all of the 40 electrodes on the devices. A completed device is illustrated in Figure 25c. The device cavity was initially filled with oil. The inlet/outlet ports could be connected to syringe pumps for supplying the necessary reagents. The reactor section at the center of the device was designed to handle droplets of various sizes with a minimum droplet volume of ~26 nL on a single activated electrode to longer channels with the activation of a series of electrodes with volumes in excess of 500 nL from inlet to outlet.

**Device operations**

The devices were tested to confirm their ability to perform the basic DMF functions of droplet creation, transport and mixing in addition to channel formation and continuous flows. These tests were similar to the ones described in Chapter 4, but were aimed towards the specific immunoassay protocols as will be presented below. Each device was connected to a power supply via the electrical interface. The power supply could be controlled through a graphical user interface (GUI) depicting each electrode on the device. Through this interface, the device could be programmed to carry out sequential or parallel microfluidic operations by switching on or off power to each of the 40 addressable electrodes on the device. The GUI also allowed for automated time sequences to be carried out repetitively and without user intervention. This enabled functions such as droplet transport and mixing to be automated. The loading of samples was achieved by connecting the inlet port with a syringe pump supplying aqueous fluid in measured amounts and the subsequent activation of channel and droplet electrodes to obtain the necessary functions. The microfluidic functions tested on the device are illustrated in Figure 26.
Two different inlet ports were used to supply the device with two different colored aqueous samples (blue and yellow). The use of channels as a continuous source of fluid for the creation of smaller droplets is an accurate method of volume metering for the generation precise sample volumes and we have discussed this in detail in Chapter 3. Here we apply those principles to create droplets. Droplets measuring ~ 80nL were dispensed from their respective source channels by continuously filling the electrodes using a syringe pump and subsequently deactivating an electrode to effect the splitting of the droplet from the source channel. These droplets were then transported by sequential activation and deactivation of electrodes to merge the droplets together. This was followed by agitation of the larger merged droplet by switching on and off electrodes in quick succession (0.1s) over which it resided. This led to the creation of

![Diagram of droplet generation and transport](image)

**Figure 26** Testing of device functionality with channels and droplets is illustrated. Droplets are generated from two different colored fluids are metered and generated from channels and mixed together. These droplets are then portioned into droplets of smaller sizes.
a uniformly mixed green droplet. This large droplet was then portioned first into two equal halves and then each half was further divided into smaller droplets. Subsequently all the smaller droplets were merged together again (not shown in Figure 26).

This demonstration of DMF functionality and the use of continuous channels on the same device outline the basic functionalities of the combined paradigm of digital microfluidics and continuous microfluidics. Immunoassays require a number of reagents to be mixed in precise quantities which, in concept is shown here. The use of a continuous channel serves another more important purpose of performing the washing steps in heterogeneous immunoassays which are crucial for high sensitivity. The continuous channels have been optimized for this purpose as will be demonstrated later.

**Macroscale heterogeneous immunoassay protocols**

An immunoassays consists of a highly-specific reaction between an antibody and an antigen. Several formats exist for achieving this reaction to identify and quantify the analyte of interest. The most common format requires immobilizing the antibody-antigen complex on a solid surface so that excess analyte in the sample matrix can be washed off. This lends a high degree of accuracy and low limits of detection to this set of techniques termed as heterogeneous immunoassay. The use of microparticles as a solid support is attractive as it offers a dramatic increase in surface to volume ratio and serves as a simple mechanism for reproducibly delivering antibodies into a microfluidic device. Paramagnetic particles respond to external magnetic fields and can be easily immobilized without the need for special microstructures for physical retention [86]. Magnetic forces exerted on paramagnetic particles can also be used to enhance mixing [87], and serve as detection mechanism for immunoassays [88]. Owing to these advantages magnetic microparticles of 1 μm diameter have been used for experiments.
The most common antigen-antibody complexes in immunoassays rely on biotin-avidin binding. Biotin is a vitamin that can either bind to streptavidin or avidin which are proteins. The natural attraction of these two proteins for one another is a property that has been used to facilitate coupling of indicator molecules to antigens or antibodies. The purpose of this work was to develop the assay protocols that could potentially be applied to any immunoassay on digital microfluidic platforms. Therefore instead of a specific assay a more generic binding system was chosen. Streptavidin covered magnetic particles served as antibodies immobilized on a solid support and biotinylated FITC served as the antigen (analyte). This pair represents a very generic configuration of specific binding used for immunoassays without the complexities of handling complex sample matrices. Biotin-streptavidin binding as such does not comprise of an immunoassay on its own. Normally both biotin and streptavidin are bound to other antigens or antibodies and the specificity of binding between biotin and streptavidin is exploited for detection. The devices here were optimized based on this specific binding.

The binding between streptavidin coated magnetic particles (Pierce) and biotinylated FITC (Sigma Aldrich) was carried out using conventional techniques. This provided a point of reference to compare and adapt the specifics of the conventional heterogeneous assay protocols to the process on the device. First, a series of biotinylated FITC solutions were made using Phosphate Buffered Saline (PBS) at pH 7.2. The concentration range of these solutions was between 1nM and 1mM of Biotin-FITC in PBS in increments of 10×. The streptavidin coated magnetic particles were suspended in PBS with a concentration of 0.1 mg/mL. A strong permanent magnet was used to concentrate the particles inside test-tubes while the supernatant fluid was discarded using a pipette. A same amount of fluid was used to re-suspend the particles in fresh buffer solution with subsequent sonication and agitation. This was repeated 5 times to
ensure that the particles were washed and free from impurities. The buffer used for washing the particles was PBS with 0.01 wt. % of Tween 20. This ensured that the particles did not stick to surfaces.

Next, separate test tubes were used to mix 100 µL of particle containing solution (0.1 mg/mL) with 100 µL of Biotin-FITC containing solutions. The solutions were allowed to incubate for 15 minutes before the washing steps were performed. The procedure is schematically illustrated in Figure 27. The incubation time allows the Streptavidin on the magnetic particles to bind with the Biotin-FITC in the solution. The binding correlates with the concentration of Biotin-FITC in the solutions which is the analyte in this case. The excess unbound Biotin FITC was removed using a series of 5 washing steps during which the bound particles are concentrated with a magnet and resuspended in fresh PBS. These washing steps ensure that no unbound Biotin-FITC remains in solution. After washing, the solutions were tested for fluorescence intensity using a 96 well plate reader (BioteK). The excitation and emission wavelengths were 490 nm and 525 nm respectively. This process was repeated 3 times and three sets of measurements were collected. The results of this experiment are illustrated in

**Figure 27.** Schematic illustration of assay protocol for 96 well plate implementation.
Figure 28. The data shows a clear and linear increase in fluorescence signal with increase in concentration of Biotin-FITC from 0.1 µM to 0.1 mM. At lower concentrations from 1 nM to 0.1 µM, the slope of the curve changes. This may be attributed to the relatively higher noise levels at lower concentrations of Biotin-FITC and auto-fluorescence from the 96 well plates. The nature of the data is in agreement with similar experiments done with streptavidin coated particles binding to biotin-4-fitc [89].

In practical applications of immunoassays another antigen binds to the streptavidin coated particles. Biotinylated-FITC is commonly used as a conjugate for quantifying the antigen using the fluorescence. This alters the characteristic curve such that the antigen is detected with the signal emitted from the biotinylated-FITC. The strength of the signal depends on the concentration of biotin-FITC which correlates to number of antigen molecules. However the number of biotin-FITC molecules on each streptavidin coated particle is generally greater than 1 with multiple streptavidin binding sites at which antigens attach. For this reason the...
characterized antigens have a lower limit of detection compared to the biotin-FITC conjugate itself which enables detection. For this study biotin-FITC was used as the antigen and therefore, concentrations of biotin-FITC were characterized directly vs. the fluorescence signal. The limits of detection for the biotin-FITC are indicative of the practical applications they can be used for. A 10nM fluorescence signal from Biotin FITC may correspond to a much smaller limit of detection for other antigens in practical applications using biotin-FITC for fluorescence characterization.

**Assay protocols for electrowetting device**

The implementation of heterogeneous immunoassays on DMF devices typically involves dispensing a droplet of magnetic particle (with antibodies on surface) solution and droplets of analyte solution. These droplets are mixed and incubated by transporting them to a common location. This is followed by immobilization of the particles with an externally applied magnetic field and the subsequent removal of the supernatant fluid containing the excess analytes. The leftover magnetic particles are then washed with buffer solution by transporting a series of buffer solution droplets over the immobilized magnetic particles. Typically the number of washing steps (droplets) is >5. The washed bound particles are then characterized for analyte concentration using a suitable fluorescent label or chemiluminescence [38]. The washing steps in this approach require the dispensing of precise volumes of droplets of wash buffer and multiple transport and mixing steps. The dispensing and washing of steps have been identified as the main source of errors in assay results using this approach [65].

To minimize these sources of errors and to reduce the complexities involved with multiple washing steps, a new and refined protocol was developed specifically for consistent droplet volume generation and washing of immobilized particles. All discrete droplets were
dispensed from a continuously sourced channel allowing for accurate droplet volumes. The details of this optimization have been discussed in detail in Chapter 3. The washing steps were carried out using a continuously flowing channel of wash buffer to eliminate the necessity for multi-step washing. This approach removes the complexities associated with the multi-step washing procedures in conventional DMF implementation by simplifying the procedure of washing to a single step process. Further, it ensures efficient removal of excess analyte, since the volume of wash buffer is not limited by the number of droplets. The combination of these two refinements can potentially improve the performance of immunoassay applications on DMF devices.

The washing of immobilized magnetic particles with continuous flows was optimized to determine the limits of flow rates that can be used without flowing away particles. This is critical, since the amount of particles directly correspond to the amount of analyte to be detected, any loss directly translates into errors in detected concentration. The volume of fluids used for the washing steps was determined by the amount of time for which the washing was done. This volume was $>100\times$ compared to the total volume of wash buffer used in typical droplet based washing. For these experiments a single 25 mm long, 500 µm wide straight channel was activated on the electrowetting device (chapter 3). An ND-42 grade cylindrical magnet with a diameter of 3/16th inch (KJ Magnetics) with a pull force of 0.75 lb. (surface field – 3661 Gauss) was placed underneath the glass substrate. Magnetic particle solution with a concentration of 0.1 mg/mL was used to form a straight channel. A volume of 500 nL was dispensed from the syringe pump through the inlet. This is illustrated in Figure 29a. As the magnetic particle solution formed the channel the magnetic particles were concentrated over the region underneath which the magnet was positioned. The inlet port was then disconnected from the magnetic particle.
source and a second wash buffer solution was connected. The wash buffer was pumped using a syringe pump at various flow rates to test for the retention of particles. The flow rates were tested in the 1-100 µL/min range. In each case, the flow rate was maintained for 3 min and a sample was collected from the outlet to check for the number of particles eluted with the flow. The number of particles was estimated using a haemocytometer. It was found that no particles eluted till the flow rate reached 30 µL/min. Beyond this flow rate, the rate of particle elution continued to rise. The results are illustrated in Figure 29b. The flow rate used washing with continuous flows for all subsequent experiments was chosen to be 10 µL/min, which was much lower than the minimum flow rate at which particles were observed to elute. All washing steps were done for 3 minutes or a volume of ~30 µL of wash buffer to ensure that immobilized magnetic particles are free from unbound analyte.

The retention of magnetic particles during continuous washing was further tested by performing the binding of streptavidin coated magnetic particles to Biotin-FITC samples off-chip with washing steps as in the conventional implementation. These bound particles in solution were subsequently captured on chip and measurements were made for fluorescence under a microscope using a FITC filter cube. The measurements were repeated after washing the particles with continuous flow for 3 minutes at 10 µL/minute. This was repeated for Biotin FITC
Figure 29 (a) Frames showing particle washing experiments at different flow rates. It can be clearly seen that a small number of particles start to escape at 30 µL/minutes with a rising trend. (b) The particles eluted at the outlet were estimated using haemocytometer. The inlet count or estimated original concentration was $2 \times 10^9$ particles/mL.
samples in the concentration range between 0.1 µM and 0.1 mM concentration which was earlier observed to be the linear range of the 96 well plate implementation. The results of this experiment are summarized in Figure 30. The data shows that when bound particles are subjected to continuous washing, there is no loss in fluorescence signal intensity, confirming ~100% particle retention.

**On-chip implementation of optimized assay protocol**

The optimizations discussed above were combined together and tested on chip as a proof of concept demonstration. The streptavidin coated magnetic particles were introduced on the chip first with the ND-42 magnet placed over the reactor. The magnetic particle solution formed a channel with the particles concentrating near the magnet. The concentrated particles were separated from the source channel by deactivating one electrode and forming a 80 µL droplet of

![Graph showing fluorescence intensity vs. concentration of biotin](image)

**Figure 30** Results with off-chip binding are illustrated. Blue indicates data before washing and red indicates data after 3 minutes of washing on the same samples. There is little or no loss in signal strength indicating ~100% particle retention. Insets show fluorescent images captured for each concentration.
concentrated particles. Next, the Biotin-FITC solution was dispensed from another inlet to form a channel and another 80 µL droplet was formed and transported to the particle droplet and mixed together. The magnet was removed to allow the particles to resuspend in solution. The combined droplet was agitated to effect complete mixing and incubation for 5 minutes. Next the magnet was placed back over the droplet to concentrate the bound particles and the supernatant fluid containing the excess Biotin-FITC was removed by electrowetting transport leaving behind immobilized bound particles. These particles were then subjected to continuous washing using PBS introduced at one of the inlets and making a channel that flows over the immobilized particles to an outlet in the device. The washing was carried out for 3 min. The washed particles were then observed under a microscope and characterized for fluorescence. This procedure was repeated for the same concentration range of Biotin-FITC as in the 96 well plate conventional execution of the protocol to get an accurate comparison of performance.

The results of this experiment are illustrated in Figure 31. Assay protocol implemented on-chip yielded better results compared to the implementation of the conventional implementation of the assay protocol using 96 well plate. The linear range has been extended to 10 nM, as compared to 100 nM for the 96 well plate implementation. The limit of detection for on-chip implementation was limited by the background noise level. These experiments were repeated in triplicate and the protocol was found to yield consistent results. The data from these experiments compare favorably with heterogeneous immunoassay implementations on conventional DMF devices using only droplets. Although a direct comparison could not be found in literature, the deviations in signal are much lower when compared to the heterogeneous assays for immunoglobulin G and ricin [34], insulin and interleukin-6 [35, 36] and immunoglobulin E [37]. A similar microscale immunoassay was performed by Hayes et al. [90] where
complexation of anti-FITC immobilized on particles with FITC was carried out. The tested range was 2 - 46 µM of FITC, with a limit of detection at 10 µM based on background fluorescence. Compared to this our system shows a limit of detection at 10 nM based on background fluorescence. The same group demonstrated the detection of IL-5 using the same FITC mechanism where the detectable limit of IL-5 was 10 pg/mL [90]. As discussed previously, the actual concentration of FITC was higher than the detected antigen. This confirms that our results show a definite improvement in performance by lowering limits of detections.

**Summary**

DMF devices allow for versatile operation and multiple functions to be integrated on demand, making this tool desirable for complex analytical techniques such as immunoassays. However, the limitations imposed by the conventional DMF with processing only discrete
droplet volumes adds complexity to the system when precise volumes need to be generated or continuous processing steps need to be mimicked. The approach developed here for fluid handling improves on the existing capabilities of DMF devices by incorporating continuous functionalities. The demonstration of improved assay protocol is an example where this technique can be successfully applied for better result. The assay protocol chosen for demonstration represents a general technique used regularly for heterogeneous immunoassays. The results of the binding between streptavidin coated magnetic particles and biotin-FITC was better when implemented on the electrowetting device using the optimized fluid dispensing and washing with continuous flows.
CHAPTER 6

CONCLUSIONS

Summary

Geometries form the basis of many continuous microfluidic applications. True
programmability in a microfluidic platform can be realized when specific *geometries* can be
defined by the user, suiting the needs of a particular application. Modular microfluidic
breadboards[55] adopt a scheme where separate interconnections are manufactured for each
application. This allows for a certain level of abstraction to access specific microfluidic
functions. General purpose microfluidic chips have also been reported recently.[24] However,
one of these examples offer a truly general and reprogrammable fluid handling tool and need to
be redesigned to suit specific applications.

Droplet manipulation has been widely discussed in both rigid channels as well as in
electrowetting based multiphase fluidic systems. EWOD based microfluidic platforms[13, 14]
have become popular in the recent years due to their inherent capability to be programmable.
While digital microfluidics allows for a certain degree of computer control of functions, they are
limited by small sample volumes and non-continuous operation. The current state of the art does
not allow for the manipulation of continuous flows or larger microfluidic networks to be
implemented in a programmable manner. A microfluidic platform capable of on-demand
formation of microchannels of desired geometries can take advantage of the vast library of
applications that have been developed using continuous microfluidics over the years. Provision
for the processing of discrete sample volumes and precise control of sample metering should also be built into such a platform. Programmability in microfluidic channels allows wider access to multiple microfluidic functions. The integration of continuous microfluidic functions on an electrowetting based platform would lead to the merging of the two paradigms of continuous microfluidics and droplet based digital microfluidics, allowing for a much wider range of functionality and possible applications.

The work presented here bridges the gap between droplet based DMF and continuous flows by implementing continuous flows on electrowetting devices. Pressure driven flows have been demonstrated for the first time and theoretical and CFD models have been used to investigate the nature of these electrowetting channels. It was found that continuous flows as high as 100 µL/minutes could be supported on electrowetting based programmable channels without channel deformation even though the channels are not supported by any rigid walls. The flow rate was only limited by the quality of sealing the device at the inlet and outlet ports to insure finite volume operation. Sealing of devices is critical for supporting continuous flows. At higher flow rates interfacial tension at the channel walls does not provide sufficient force for confining the flows. The confinement depends entirely on finite volume operation such that the volume of oil enclosed in the device cavity remains intact throughout the flow operation. Any change in volume of oil (due to leakage) translates to a change in the volume of the aqueous fluid forming the channels which leads to a deformation in the shape of the channels.

The integration of continuous flows is an important step towards implementation of programmable microfluidic networks. However, it needs to be accompanied by appropriate procedures for metering precise fluid volumes. These fluid volumes cannot be limited to the production of droplets. The incorporation of longer channels necessitates creation of larger
volumes of fluid of arbitrary quantities and their reconfiguration in the form of microfluidic networks requires an understanding of mass-transfer during the reconfiguration and splitting process.

Splitting finite volumes with accuracy is one of the fundamental functions of digital microfluidic platforms. The experiments presented here provide a conclusive guideline towards the design of reconfigurable devices. Deactivation of larger electrowetted microfluidic networks should proceed stepwise by gradually turning off smaller segments at a time. This allows for a more predictable means of reconfiguring electrowetting microfluidic devices. Further, the transitions between steps should be designed such that splitting operations are centered whenever possible to minimize complications with fluid unequal fluid migration. The experiments also provide insights on the behavior of electrowetted fluid segments while accommodating excess fluid migrating to them while splitting. Fluid segments have a finite volume and this volume can expand within certain limits as demonstrated by observation on bulging of channels. When this capacity is exceeded the confinement of these segments is lost. Hence it is important to design the reconfiguration steps to account for it.

In the case of conventional droplet microfluidics, applications typically rely on dispensing, transporting, splitting and recombining precise sample volumes. The characterization of results in applications such as immunoassays depends on the concentration of analytes in droplets. Dispensing and splitting have been shown to be the main source of degradation in assay performance [73]. These results can be directly applied towards increasing the accuracy of such systems. The guidelines set for generating finite split volumes can be used for dispensing droplets of sample reagents and analytes such that consistent volumes are produced each time for reliable analysis and characterization.
Droplets occupying an electrode have certain dimensions so that they can be reliably transported. These droplets are normally split from a reservoir using an elongated neck roughly 5× compared to the pitch of the electrodes. With subsequent generation of multiple droplets the reservoir volume and consequently the effective dimensions decreases resulting in changing ratios in the lengths of the segments adjoining the splitting region. As shown in our results, this leads to a change in the volume migrating from splitting region. Thus droplet sizes would decrease with each generated droplet [66, 67]. A continuous source of fluid for replenishing the source reservoir is therefore necessary for precise dispensing of droplets. Feedback-based droplet dispensing systems with elaborate signal processing modules have been demonstrated with highly mono-disperse droplet characteristics [70] to circumvent this problem. In contrast, the system described herein is simple in design and can be used to generate and manipulate arbitrary sample volumes in the form of droplets or virtual channels with similar precision. The theoretical model used and validated here can be extended to exactly calculate the adjustments necessary for precise generation of sample volumes.

The major challenge in this approach is to be able to control the initial volume of the electrowetting channel. The larger volumes of these channels enable us to use syringe pumps to dispense a predetermined amount of sample for channel formation which reduces errors. However, when replenishing a channel with smaller amounts of sample dispensed from a syringe pump, the errors may be larger. This problem can be overcome by using a larger reservoir in between the syringe pump and the actual electrowetting channel. Incorporation of visual width markers provides a simple real-time monitoring system for characterizing sample volume. The concept of ramped splitting for dispensing finite sample volumes is a readily adaptable and can potentially benefit electrowetting applications requiring accurate sample dispensing. The work
was done with electrowetting channels but the same model can be extended to digital microfluidics and droplet dispensing.

The principles behind channel formation and precise volume generation led to the combination of the two paradigms and the combined functionalities were demonstrated on the arrayed electrowetting device consisting of 360 addressable electrodes. It was demonstrated that the shapes of the electrowetting channels have a natural tendency to equilibrate when not restricted to a limited number of electrodes or area of channels. This means that the volume per unit length of these structures remains constant. This feature has wide benefits for biochemical applications requiring precision in volume of reagents. Combined with accurate splitting of fluid segments an arrayed electrowetting device can process arbitrary sample volumes with easy programmability as was demonstrated by automated transitions between different electrowetting structures. The combination of electrowetting channels and droplets marks a significant step towards the development of elaborate biochemical analysis tools using electrowetting.

The methods developed for accurate fluid handling were used for demonstrating heterogeneous immunoassay protocols that were adapted and optimized for use with continuous channels. This lends a number of advantages to existing immunoassay implementations on DMF devices. First, a continuous source of fluid eliminates errors in dispensing volumes of reagents. This is critical for accurately rendering the correct ratios in which reagents have to be mixed for biochemical applications. Second and more importantly, the washing steps in heterogeneous immunoassays have been reduced to a single step process with the use of continuously flowing buffer solutions compared to the cumbersome multi-droplet washing approach in DMF. This new approach to removal of unbound analytes is not limited by the volume of wash buffer and
can potentially lead to a lower limit of detection without having to process and automate 10s of droplets in series.

**Challenges and future work**

Electrowetting based DMF devices require multi step fabrication procedures with several photolithography and deposition steps. This makes the devices costly to produce. However, if the devices are reliable and reusable the cost per use of device can be reduced drastically. The major challenge facing the devices demonstrated in this dissertation is the fact that the devices show poor reliability. This is attributed to the nature of the materials used for fabrication. Dielectric breakdown was observed for all our devices after 10-15 min of continuous operation. The use of more advanced dielectrics can help solve this issue in the future. The use of better and more rugged materials can also lead to the adaptability of this technology for a wider range of chemical biological reagents.

The second major challenge has to do with the nature of hydrophobic surfaces. Electrowetting devices used in this dissertation rely on hydrophobic coatings to effect electrowetting actuation and relaxation. But these same coatings are prone to attract proteins and enzymes on the surface for biochemical reactions. This was observed for the enzymatic reactions involving the glucose assay demonstrate in Chapter 4. This problem was partially solved by the use of Pluronic F-127, but this solution is not very general. The reusability of the devices are also affected by the deposition of proteins or enzymes on the surface. To tackle this problem, the dielectric layer may be removed and redeposited. This solution has its challenges, however, an adhesive removable hydrophobic dielectric layer may be used to solve this issue.

Finally, the techniques and process development demonstrated here are widely applicable for a large number of applications. However, the applications shown in this dissertation are
limited to the demonstrations of working principles. Immunoassays require multi step sample preparation and while all the tools exist on this device to handle such an eventuality, a more comprehensive testing of a real assay needs to be implemented to realize the versatility of this programmable device. It may be envisioned that, this technology can be adapted towards other applications by the incorporation of appropriate sensors and associated electronics in chemical sensing and environmental monitoring.
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