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Enhanced actuation and fabrication methods for integrated digital microfluidic systems

submitted by Mohamed Yafia Okba Salem in partial fulfilment of the requirements of the degree of Doctor of Philosophy.

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Abstract

Introducing a practical digital microfluidic (DMF) platform has been always an important goal that can facilitate and broaden the use of DMF systems in various applications on a regular basis. In particular, a desired DMF platform may be the one which is fabricated easily and is also affordable, portable, battery powered, and user friendly. It should perform DMF operations reliably, accept mass-produced replaceable chips and can be readily integrated into a post processing station as well. Accordingly, the development of a practical DMF platform with these characteristics is the main target of this research. Specifically, the research has enhanced DMF systems through i) improved and novel actuation mechanisms, ii) cost-effective rapid prototyping techniques, and iii) full system integration.

First, detailed experimental and numerical characterization of droplet morphology is performed to insinuate useful design and prototyping tips for enhanced DMF devices. Since droplet transport predominantly depends on the gap height, a major objective of the thesis is understanding the motion dynamics of a droplet at different gap heights. The characterization of the droplet behaviour in different regimes led to enhancing the actuation process in DMF systems. In addition, a variable gap size actuation (VGSA) mechanism is integrated into the DMF system to optimize DMF operations including droplet transport, splitting, dispensing and merging in real time. Consequently, the DMF operations can be performed reliably by adjusting the optimum operating conditions.

Second, for rapid prototyping DMF chips, a new fabrication method is presented in which the electrodes are generated using screen printing. As a batch fabrication technique, the proposed screen printing approach is advantageous to the widely reported DMF electrode fabrication methods in terms of fabrication volume, time, and cost. In addition, a laser scribing technique is introduced as a low-cost, rapid and facile method for fabricating laser scribed graphene electrodes for DMF systems.

Third, an ultraportable, low-cost, and modular DMF platform was successfully integrated with a smartphone that is used as a high-level controller. A colorimetric assay for pH value detection is introduced to demonstrate the performance of the device as a microscope and a post processing station.
Preface

The research introduced in this thesis is conducted in the UBC Advanced Control and Intelligent Systems (ACIS) laboratory under the supervision of Dr. Homayoun Najjaran. Production of this research has been made possible through financial contributions from Natural Sciences and Engineering Research Council (NSERC) Canada and Canadian Foundation Innovation (CFI).

The contributions of this thesis are highlighted as follows:

Chapter 1 includes an introduction about DMF systems. The motivation and the objectives of this work which includes the development of a practical digital microfluidics platforms based on enhancing the actuation, introducing new DMF operations, introducing new fabrication and rapid prototyping techniques, and performing full system integration. This chapter also includes an outline for the thesis and how it is organized.

Chapter 2 introduces the results of the detailed numerical and experimental study of necking phenomenon occurring during droplet transport in DMF systems which was published in one journal paper (Mohamed Yafia & Najjaran, 2015). In this work, previously proposed droplet transport models were critiqued and the limitations of those models depending on the system configuration and actuation forces were reported. The results and more accurate transport models will contribute to more effective control of DMF operations. I conducted all the testing and wrote most of the manuscript.

Chapter 3 introduces the concept of precise control of gap height in DMF devices and demonstrates its impact on DMF operations, experimentally. For this work, a sophisticated setup required for high precision motion control (<5 microns repeatability) of DMF plates was developed. The result of this work was published in Sensors and Actuators, Part B (Mohamed Yafia & Najjaran, 2013).

Chapters 4, 5 and 6 concentrate on practical aspects of DMF devices, specifically aiming to develop a practical and working DMF point of care testing (PCT) device. Chapter 4 introduces screen printing on paper as a cost-effective alternative approach for DMF electrode creation. This approach will promote low-cost disposable DMF chips that is essential for PCT. This work has been published in Journal of Micromechanics and Microengineering (Mohamed; Yafia, Shukla, & Najjaran, 2015).
Chapter 5 introduces another rapid prototyping technique for DMF chips using graphene. We used the laser scribing method to fabricate the chips directly from a DVD burner connected to a computer. This method was able to generate DMF platforms in open and closed DMF configurations. To show the capabilities of the fabricated systems, they were later used for single nucleotide DNA mismatch detection. We are about to submit the detailed results of this work to Biosensors and Bioelectronics.

Chapter 6 introduces our method to integrate all the components required for making a portable DMF device. We successfully introduced an ultraportable device that can be controlled through a smartphone that can act as a post processing station as well. A low voltage control circuit for the DMF that is well suited for portable PCT devices was designed and fabricated. The first prototype for the device is now ready and it is featured in several newspapers and the university website. This work has been published in Micromachines journal under a special edition entitled “Droplet Microfluidics: Techniques and Technologies” (Mohamed Yafia, Ahmadi, Hoorfar, & Najjaran, 2015).

List of publications

Journal Articles

Conference Proceedings

- Low cost graphene electrodes for performing digital microfluidic operations on a
- Droplet morphology changes induced by changing the actuation voltages in digital
- Low cost smartphone controlled digital microfluidic chip in a 3d-printed modular
  assembly with replacable glass and screen printed paper chips, M. Yafia, A. Ahmadi,
  K. Yesilcimen, M. Hoorfar and H. Najjaran, UTAS, Korea, 2015
- The effect of changing the gap height on droplet deformation during transport in
- Electrowetting on screen printed paper based substrate, M. Yafia, H. Najjaran,
- Screen printed paper based digital microfluidic platform, M. Yafia, H. Najjaran,
  CSME Toronto, June 2014, Toronto, ON, Canada.
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List of Symbols

*Ca*: Capillary number.

*h*: Gap height between the bottom and the top plates.

*L*: Electrode side length.

*P_x*, *P_y* and *P_a*: Droplet pressures at the activated right electrode, the non-activated middle electrode and the ambient pressure, respectively.

*R_x* and *R_y*: Radii of the droplet in the horizontal direction of the droplet motion at the activated right electrode and the non-activated middle electrode.

*r_x* and *r_y*: Radii of the droplet in the vertical direction to the droplet motion at the activated right electrode and the non-activated middle electrode.

*U*: Droplet velocity.

*We*_0: Electric Weber number.

*γ*: Surface tension.

*μ*: Droplet viscosity.

*ε_o*: Dielectric constant of the vacuum.

*ε_r*: Dielectric constant of the dielectric material.

*θ_t*, *θ_bx* and *θ_by*: Contact angles at the top plate, bottom plate of the activated right electrode and bottom plate of the non-activated middle electrode, respectively.
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It is a pleasure to thank the people who made this thesis possible. First and foremost, I would like to express my enduring gratitude to my supervisor, Dr. Homayoun Najjaran for his continuous support and guidance. He has inspired me throughout the progression of this research and provided me with invaluable advice, ideas, and answers for my endless questions. In addition, I would like to extend my enduring gratitude to Dr. Mina Hoorfar and Dr. Vladan Prodanovic for advising me and providing me with lots of great ideas throughout the research period.

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Dedication

To my dear parents, sister, grandparents and my wife.
Chapter 1 Introduction

1.1 Introduction to microfluidic systems

Microfluidics has revolutionized the field of biochemical analysis and clinical diagnosis in recent years (Haeberle & Zengerle, 2007). Microfluidic devices are now established as an efficient platform for fabricating lab on a chip (LOC) devices and micro total analysis (µTAS) systems with great capabilities in performing different chemical and biological applications (Kovarik et al., 2013; Malic, Brassard, Veres, & Tabrizian, 2010). Introduced after the traditional channel-based microfluidic devices, digital microfluidics (DMF) has emerged as a promising technology with several advantages (Mugele & Baret, 2005). First, DMF systems can manipulate individual micro-litre droplets on discrete arrays of electrodes omitting the need for microvalves or mechanical moving parts (M. Pollack, Shenderov, & Fair, 2002). Reconfigurability is another important advantage which allows performing various fluidic operations such as transport, merging, splitting and dispensing from reservoirs (S. Cho, Fan, Moon, & Kim, 2002) in a single fluidic platform.

Table 1.1 Comparison between the processes when traditional methods are used and microfluidics systems are used (Reprinted with permission from (Agresti et al., 2010). Copyright 2010 PNAS)

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<th>Microfluidic drops</th>
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<td>Total reactions</td>
<td>$5 \times 10^7$</td>
<td>$5 \times 10^7$</td>
</tr>
<tr>
<td>Reaction volume</td>
<td>100 µL</td>
<td>6 pl</td>
</tr>
<tr>
<td>Total volume</td>
<td>5,000 L</td>
<td>150 µL</td>
</tr>
<tr>
<td>Reactions/day</td>
<td>73,000</td>
<td>$1 \times 10^8$</td>
</tr>
<tr>
<td>Total time</td>
<td>~2 years</td>
<td>~7 h</td>
</tr>
<tr>
<td>Number of plates/devices</td>
<td>260,000</td>
<td>2</td>
</tr>
<tr>
<td>Cost of plates/devices</td>
<td>$520,000</td>
<td>$1.00</td>
</tr>
<tr>
<td>Cost of tips</td>
<td>$10 million</td>
<td>$0.30</td>
</tr>
<tr>
<td>Amortized cost of instruments</td>
<td>$280,000</td>
<td>$1.70</td>
</tr>
<tr>
<td>Substrate</td>
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<td>$0.25</td>
</tr>
<tr>
<td>Total cost</td>
<td>$15.81 million</td>
<td>$2.50</td>
</tr>
</tbody>
</table>

1.2 Motivation and objectives

The microfluidic technology has been implemented in various applications owing to its advantages over the conventional testing techniques (Samiei, Luka, Najjaran, & Hoorfar, 2016). Miniaturization, saving reagents, portability and reconfigurability are some of the main advantages that promote the potential of the DMF technology (Shen, Fan, Kim, & Yao, 2014). Researchers have been always trying to introduce new methods to enhance the actuation process for DMF systems (Rajabi &
Introducing a practical DMF platform has been always an important goal that can greatly help in increasing the use of DMF systems in many applications. However, the widespread use of the DMF platform is still limited due to several challenges including:

- For critical chemical and biological applications, the DMF operations have to be performed reliably. Increasing the reliability of the DMF operations depends on understanding all of the factors that can affect the droplet actuation process. One of these important factors that has not been yet explored is the effect of the gap height on the droplet dynamics. In particular, each operation in the four main DMF operations has its optimum gap height. Consequently, DMF operations have to be performed at different gap heights for having the most reliable operating conditions. However, a fixed gap height is maintained in all of the previously reported work in the literature. Since the gap height is very small (50-500 µm), researchers just use fixed spacers during the DMF operations.

- Fabricating the DMF chips is usually performed by advanced and complex techniques such as photolithography. Therefore, the fabrication process will require advanced facilities that are not readily available and the output DMF chips are going to be expensive. More importantly, current rapid prototyping techniques lack the mass production capability and simplicity required for fabricating cost effective DMF chips. In addition, DMF electrodes are usually made from gold, chrome, silver and copper. Accordingly, the cost of the current conductive electrode materials contributes in increasing the total cost of the DMF platform.

- The size of the DMF chip is very small and convenient for portable applications. However, performing DMF operations on a portable device is not a simple process since several peripherals need to be integrated to achieve successful DMF operations. DMF operations require high voltage signals. These signals are usually generated from bench top equipment, a signal generator and a high voltage power amplifier, which needs the main power supply at 220 V. In contrast, in portable platforms, these signals have to be
generated from a small power supply. The cost of the off-the-shelf electrical components that can generate these high voltage signals are high which increases the total cost of the device. Moreover, the portable device needs a controller that can send the commands for having sequential DMF operations. This controller needs an intuitive and robust way of communication with the user. After performing the DMF operation, a post processing station is required to analyze the results. A complete DMF-based devices may include: i) a microscope to monitor the processes, ii) a processor to analyze the images from the microscope, and iii) a communication link to send the test results to the user and send the commands to the controller for any further DMF operations needed. The size and the cost of each of the above mentioned peripherals is incompatible with the idea of having a low cost and portable handheld DMF platform. Moreover, the current power requirements of these peripherals is not small enough to be battery powered. Developing a small handheld DMF that can put these components together is very challenging.

Henceforth, developing a portable, reliable, cost effective and practical DMF platform will solve a lot of these problems. Enhancing the digital microfluidics operations accompanied by introducing new fabrication techniques where affordable DMF chips can be replaced easily on a handheld device can greatly increase the usage of DMF systems in many applications. Accordingly, the objectives of this work are as follows: Development of a practical DMF platform based on enhancing the DMF actuation process, introducing new DMF operations, introducing new fabrication and rapid prototyping techniques and performing full system integration. The methodology can be summarized as follows:

- One of the main objectives of this research is understanding all of the factors that can affect the droplet actuation process. This will help in increasing the reliability of the DMF operations. Since the DMF systems predominantly rely on the droplet transport process, researchers have shown particular interest in understanding the motion dynamics of the droplet. This thesis entails detailed experimental and numerical characterization of droplet morphology to insinuate useful design and prototyping tips for enhanced DMF devices. Such
deformation, elongation and necking corroborate that the existing analytical models may be inadequate for certain gap heights as they typically assume a circular shape for the droplet during motion. A variable gap size actuation (VGSA) mechanism is integrated into the DMF system. The VGSA mechanism serves to optimize the aspect ratio during performing different microfluidic operations by changing the gap height between the top and bottom plates. This in effect will have a direct impact on the four main DMF operations including droplet transport, splitting, dispensing and merging as they are greatly affected by changing the aspect ratio. Experimental results demonstrate that the VGSA mechanism significantly enhances the principal DMF operations by retaining the appropriate gap height for each operation.

- To solve the fabrication problems, new fabrication methods characterized by mass production capabilities have to be introduced. The fabrication method will have to allow for the use of inexpensive material such as paper. This will help reduce the overall cost of DMF devices. The fabrication steps in the new method have to be simple as well and can be performed without using advanced facilities and expensive equipment. In this work, a new fabrication method is presented in which the electrodes are generated using screen printing. The proposed screen printing approach, as a batch fabrication technique, is advantageous to the widely reported DMF fabrication methods in terms of fabrication time, cost and capability of mass production. Screen printing provides effective means for printing different types of conductive materials on a variety of substrates. Specifically, screen printing of conductive silver and carbon based inks is performed on paper, glass and wax paper. As a result, the fabricated DMF devices are flexible, disposable and incinerable. Hence, the main advantage of screen printing carbon based inks on paper substrates is more pronounced for point-of-care testing (POCT) applications that require a large number of low cost DMF chips, and laboratory setups that lack sophisticated microfabrication facilities. The resolution of the printed DMF electrodes generated by this technique is examined for proof of concept
using manual screen printing, but higher resolution screens and automated machines are available off-the-shelf, if needed. Another contribution of this research is the improved actuation techniques that facilitate droplet transport in electrode configurations with relatively large electrode spacing to alleviate the disadvantage of lower resolution screens. Thus, the fabrication cost was reduced significantly without compromising the DMF performance. In addition, a laser scribing technique is introduced as a low-cost, rapid and facile method for fabricating laser scribed graphene electrodes for DMF systems. At last, to make the DMF system portable and more accessible at low cost, this research reports the development of an integrated ultraportable, low-cost, and modular DMF system and its successful integration with a smartphone used as a high-level controller and post processing station. The main novelty of this work is the introduction of a series of technologies that together offer a fully integrated lab-on-a-chip device. Low cost, high voltage, and battery powered circuits are designed to generate the signals required for DMF operations. The low power rating of DMF systems allows replacing the high power bench top equipment with high voltage and low current electric circuitry. We will have to minimize the power consumption of the high voltage circuits, control circuit and switching circuit. A compact microcontroller will be used for controlling the high voltage switching algorithm. The smartphone controls the whole operation via a Bluetooth module, which is directly connected to the microcontroller. Finally, the integration of a microscopic lens with the smartphone will be demonstrated to examine the droplets sitting on this system and detect the color changes inside the droplet. This will prove that the smartphone can act as a microscope, a control and command station, and a post processing station with a user friendly interface.

All things together, all the thesis parts were contributing in introducing a full integrated DMF system based on enhancing the actuation and using new methods to fabricate the DMF chips at low cost with more accessible tools and equipment.
1.3 Literature review

1.3.1 Microfluidic systems

Microfluidic systems can be categorized according to the type of the flow from continuous flow systems to discrete droplet flow systems (Jean Berthier, 2012; Ni, Capecci, & Crane, 2016). Various applications are utilizing electrowetting on dielectric (EWOD) for droplets manipulation in DMF system (M Yafia & Najjaran, 2015). Several parameters can be involved in explaining the droplet transport process such as the contact angle of the droplet (Keshavarz-Motamed, Kadem, & Dolatabadi, 2010). Digital microfluidics is one of the EWOD platforms that outweigh some other platforms because of the benefits gained from manipulating microliter droplets without using mechanical valves or pumps at the microscale on horizontal and inclined surfaces (Datta, Das, & Das, 2016).

![Continuous microchannels system](image1)

![Closed DMF system](image2)

Figure 1.1 Continuous microchannels with fixed flow paths (Reprinted from (Junkin et al., 2016). Copyright 2016 Elsevier). The digital microfluidics system with reconfigurable droplet path: Reprinted with permission from (Barbulovic-Nad, Au, & Wheeler, 2010), Copyright 2010 Royal Society of Chemistry).
1.3.2 Digital microfluidics

One of the main advantages of the DMF system over the other microfluidic systems is the ability to move the droplets and route them to perform different applications in a reconfigurable manner where the droplets can be transported independently by discrete electrodes (M. Cho, Pan, & Member, 2008). Digital microfluidics (DMF) provides a promising platform for performing different chemical and biological applications (Jebrail, Bartsch, & Patel, 2012). Researchers nowadays are moving toward performing more tests on the DMF platform and everyday new applications appear to be applicable on the DMF chips. The small size of the DMF platform makes this technology favourable for portable devices since it can be implemented into a handheld device (J Gong, Fan, & Kim, 2004). For this reason, the DMF platform characteristics make it compatible to work as point of care and near-patient testing devices.

Among all possible actuation methods, electrowetting on dielectric (EWOD) has gained a lot of interest for actuating droplets in the microscale (Mugele & Baret, 2005). A lot of research has been done to improve droplet actuation on discrete electrodes of the DMF systems. In order to study the droplet motion and the forces involved in actuation in the DMF system more insight into the electrical and fluid dynamics properties that affect the droplet motion is needed. Several EWOD-based configurations have been used to manipulate droplets on a single plate (Fouillet & Achard, 2004; U. Yi & Kim, 2005) or between parallel plates (Mohamed Yafia & Najjaran, 2013) of DMF systems. Each configuration has some advantages and disadvantages.

Figure 1.2 The construction of closed DMF systems (left) and open DMF systems (right)

The main advantages of the open DMF systems is that it allows having direct access to the droplet, direct droplet dispensing, and lower friction by removing the upper plate. This in effect leads to higher droplet velocities. However, the uncontrolled evaporation and
incapability of these systems to split droplets are considered as the main disadvantages that limit the use of open systems in certain applications. The closed systems on the other hand, are more versatile as they can effectively handle all four basic and crucial microfluidic operations including transport, splitting, dispensing and merging (S. K. Cho, Moon, & Kim, 2003) with controlled evaporation using silicone oil as a filler medium (Hong Ren, Fair, Pollack, & Shaughnessy, 2002).

Figure 1.3 The 4 main DMF operations performed in DMF systems

Digital microfluidic devices are usually fabricated by the standard photolithography process. This process includes multiple steps including metal sputtering on glass substrates, ultraviolet light exposure, dry or wet etching for generating electrodes and spin-coating of photoresist, dielectric and hydrophobic materials. Therefore, introducing new methods to
facilitate and lower the cost of any of the fabrication steps can help expand the use of the DMF technology in different applications.

Recently, many research groups have demonstrated alternative methods and rapid prototyping techniques for fabricating DMF devices. They can be fabricated by micro-contact printing of electrodes on glass substrate (Watson et al., 2006). The micro-contact printing method eliminated the need for a cleanroom facility, but had several drawbacks such as the added complexity because of the additional chemical requirements.

![Figure 1.4 Rapid prototyping techniques for fabricating DMF chips. a) to d) show the process of ink jet printing of conductive ink on paper substrate (Reprinted with permission from (Ko, Lee, Kim, Lee, & Jung, 2014). Copyright 2014 John Wiley and Sons). e) Printing toner on PCB and then etching the copper substrate (Reprinted with permission from (M. Abdelgawad & Wheeler, 2007). Copyright 2007 John Wiley and Sons)](image)

Another rapid prototyping technique is to fabricate DMF devices on printed circuit board (PCB) copper substrates by patterning the spacing between the electrodes manually (Mohamed Abdelgawad & Wheeler, 2007). Fabricating electrodes by toner printing on
copper substrates has also been demonstrated (M. Abdelgawad & Wheeler, 2007). This method produced well-defined electrodes compared to the manually defined electrodes. However, this method added to the complexity, as it required additional chemical etching. The concept of all terrain droplet actuation (ATDA) was demonstrated, which allowed for droplet motion on flexible polymer surfaces (Mohamed Abdelgawad, Freire, Yang, & Wheeler, 2008). ATDA devices were also made by the process of photolithography and wet etching which is complicated, time consuming and costly. DMF electrodes printed on a paper substrate have been demonstrated using inkjet printing (Fobel, Kirby, Ng, Farnood, & Wheeler, 2014; Ko et al., 2014). This new approach can broaden the application of the digital microfluidic platform. However, inkjet printing of conductive inks in (Fobel et al., 2014) requires relatively expensive specialized equipment and cannot be easily used for mass production as the time of fabrication of one device can reach one minute. It is important to note that the major challenge and cost of fabrication is attributed to electrode fabrication. In view of a need for further improvement, this research has focused on introducing electrode fabrication techniques which are less labor, time and cost intensive.

The real value of the DMF platform is revealed when it is used in performing chemical and biological applications. Numerous applications were successfully implemented on the DMF platform. Droplets of several human physiological fluids were manipulated on a DMF platform to perform enzymatic glucose assay (Srinivasan, Pamula, & Fair, 2004). Applications for new born screening such as dried blood spot analysis has been performed on the DMF platform (Jebrail et al., 2011). Moreover, DNA sequencing of Candida parapsilosis was implemented on a PCB DMF chip with an optimized protocol to increase the signal over background (Boles et al., 2011). Hormone tests are important as they can be frequently required in clinical samples. Hence, estrogen assays were performed to quantify the estrogen hormone in breast tissue and blood on a DMF chip (Mousa et al., 2009). At last, advanced detection techniques can be integrated to the DMF platform such as surface plasmon resonance imaging where the output signal can be enhanced by 200% (Malic, Veres, & Tabrizian, 2011). A couple solid-phase microextraction (SPME) coupled with HPLC-MS was used to quantify the level of the free steroid hormones in urine on an open DMF system (Kihwan Choi et al., 2016). A summary for the applications that can be performed on a
portable DMF platform was recently introduced (Samiei et al., 2016). More advanced DMF platforms resulting from this research will increase their potential to implement more sophisticated fluidics operations and assays into DMF-based point-of-care or near-patient testing devices.

Figure 1.5 I) and II) Coupling the DMF system with ionization mass spectrometry (Reprinted with permission from (Shih et al., 2012). Copyright American Chemical Society). III) Surface plasmon resonance imaging on a droplet enclosed in a closed DMF system (Reprinted with permission from (Malic et al., 2011). Copyright 2011 Elsevier).

1.4 Thesis outline and contributions

The thesis outline and contributions are organized as follows:

Chapter 1 presents the motivations and objectives of this research, a brief literature review on topics related to the focus of the thesis, and contributions of the research. It also
serves as an introduction to clarify the relationship among different aspects of the research towards advancement of digital microfluidic (DMF) systems introduced in the following chapters.

Chapter 2 presents the efforts aiming to enhance DMF actuation in different geometrical and droplet configurations for having a practical DMF platform. It also presents detailed experimental and numerical characterization of droplet morphology to insinuate useful design and prototyping tips for enhanced DMF devices. Such deformation, elongation and necking corroborate that the existing analytical models may be inadequate for certain gap heights as they typically assume a circular shape for the droplet during motion.

Chapter 3 presents a variable gap size actuation (VGSA) mechanism which is successfully integrated into the DMF system. The VGSA mechanism serves to optimize the aspect ratio during performing different microfluidic operations by changing the gap height between the top and bottom plates. This in effect will have a direct impact on the four main DMF operations including droplet transport, splitting, dispensing and merging as they are greatly affected by changing the aspect ratio that is the gap height divided by the DMF electrode width. Experimental results demonstrate that the VGSA mechanism significantly enhances the principal DMF operations by retaining the appropriate gap height for each operation.

Cost effective and rapid fabrication are other important aspects of DMF research to promote its widespread use. Chapter 4 presents a new fabrication method in which the electrodes are generated using the screen printing technique. The proposed screen printing approach, as a batch printing technique, is advantageous to the widely reported DMF fabrication methods in terms of fabrication time, cost and capability of mass production.
Chapter 5 presents a laser scribing technique as a low-cost, rapid and facile method for fabricating laser scribed graphene electrodes for DMF systems. Laser scribed graphene (LSG) electrodes are directly synthesized on flexible substrates to pattern the electrode arrays. The proposed method significantly facilitates the DMF electrode fabrication process by eliminating many steps of microfabrication as the electrodes are printed directly from the computer design to the chip. Specifically, this will eliminate the need for photolithography mask, etching equipment and a cleanroom.

Chapter 6 presents the development of an integrated ultraportable, low-cost, and modular DMF system and its successful integration with a smartphone used as a high-level controller and post processing station. Low power and cost effective electronic circuits are designed to generate the high voltages required for DMF operations in both open and closed configurations (from 100 to 800 V). The smartphone in turn commands a microcontroller that manipulate the voltage signals required for droplet actuation in the DMF chip and
communicates wirelessly with the microcontroller via Bluetooth module. Moreover, the smartphone acts as a detection and image analysis station with an attached microscopic lens.

Figure 1.7 A portable DMF platform that can be controlled and operated by a smartphone. The 3D design of the platform is shown from a) to c). d) The 3D printed platform with the smartphone attached to it. e) A schematic that shows the different components that are combined in this portable platform.
Chapter 2 Characterizing the droplet necking and the morphology variations induced by changing the gap height during transport

2.1 Overview

This chapter shows that the droplet morphology varies significantly during the transport process in digital microfluidic (DMF) systems depending on the gap height between the top and bottom plates. The experimental results elucidate the effect of changing the gap height on the initiation and progression of droplet motion. For example, extreme necking is observed during transport from one electrode to another at low gap heights. In essence, this necking is unlike the previously reported necking occurring during the splitting process over three electrodes. Pronounced elongation of droplets at certain stages during the droplet motion was examined. The effect of changing the gap height on the droplet velocity profile is illustrated. We found that prolonged actuation time is needed to transport the droplet successfully at low gap heights. This study entails detailed experimental and numerical characterization of droplet morphology to insinuate useful design and prototyping tips for enhanced DMF devices. Such deformation, elongation and necking corroborate that the existing analytical models may be inadequate for certain gap heights as they typically assume a circular shape for the droplet during motion.

Since the DMF systems predominantly rely on the droplet transport process, researchers have shown particular interest in understanding the motion dynamics of the droplet. Simple displacement and velocity profiles have been introduced during the transport process in open and closed systems (Bavière, Boutet, & Fouillet, 2008; M. Pollack et al., 2002).
Several theories have been introduced to describe the forces acting upon the droplet in an EWOD-based DMF system. These forces can be classified under two categories: the electrohydrodynamic driving forces and the mechanical forces opposing the droplet motion (Jean Berthier, 2012). Changing the dimensions of channels in continuous microfluidics systems affects the flow behaviour (Baroud, Gallaire, & Dangla, 2010). Similarly, this work will demonstrate and characterize how the electrode dimensions and the gap height used can greatly affect the droplet behaviour during motion in DMF systems. The objective of this work is to distinguish between the droplet transport hydrodynamics for a wide range of gap heights. One of the important findings of this work is demonstrating that necking occurs during droplet motion from one electrode to another at very low aspect ratios in addition to the necking that occurs during splitting. This study will cover the change of the droplet velocity, deformation and motion patterns among wide range of gap heights and aspect ratios. This work will also show how the motion of the droplet at low aspect ratios is more
prone to be affected by the surface conditions and pinning. Experimental and numerical results will confirm the effect of changing the gap height on the motion mechanism of the droplet and show that necking only occurs at a certain range of aspect ratios.

![Figure 2.2 Pressure measuring points, x & y, on the activated and non-activated part of the droplet during the transport process](image)

### 2.2 Theoretical analysis

DMF operations are mainly based on performing droplet transport, splitting, dispensing and mixing efficiently and reliably. Once the DMF chip is fabricated, the utilized electrode size will be fixed while performing these DMF operations. However, the droplet volume can vary during performing these operations due to several reasons such as splitting, mixing or evaporation. Therefore, adjusting the appropriate gap height for each operation will enhance the DMF performance significantly (Mohamed Yafia & Najjaran, 2013). Specifically, adjusting the gap height enables us to transport the droplets more reliably by adjusting the droplet footprint area based on its current volume. Accurate droplet positioning can be achieved when the gap height is adjusted and this will enhance symmetric splitting. In addition, adjusting the appropriate gap height helps in controlling the volume of the dispensed droplets from reservoirs, enhancing the droplet mixing rates, utilizing the
leftover droplets generated from splitting, or partial evaporation, and positioning the droplets more accurately for further processing. The process of precision control of the gap height was demonstrated in (Mohamed Yafia & Najjaran, 2013). Observation of the significant impact of gap height control on droplet transport motivated us to investigate droplet morphology in detail and elaborate its dependence on the aspect ratio, which is the ratio of the gap height to the electrode size. More precisely, this work will demonstrate the interaction of droplet actuation forces and explain their effects on droplet morphology and dynamics as the gap height is reduced from a relatively large to extremely small values. Thus, different droplet transport regimes were analysed both numerically and experimentally.

A pressure difference between the leading and the trailing edge of the droplet is needed to induce the motion of the droplet toward the activated electrode. Cho et al. demonstrated the relation between the radii of curvature and pressure at the droplet leading edge and the sides of droplet during the splitting process (S. K. Cho et al., 2003). In the splitting process, the droplet is attracted and stretched equally from both sides by two activated electrodes. The splitting process develops through different stages where necking is considered as one of the final stages that occur before the droplet breakup. Another work has used similar equations to create straight virtual micro-channels without any necking at the sides of the channel (A. Banerjee, Kreit, Liu, Heikenfeld, & Papautsky, 2012). They have also studied the effect of using this electrode in decreasing the variation of droplets volume after splitting (A. Banerjee, Liu, Heikenfeld, & Papautsky, 2012). Approximate pressure based equations were also used to describe the splitting and dispensing process (J. Berthier et al., 2006).

In the present work, necking is observed when only one electrode is used for actuation during the transport process at certain aspect ratios. On the contrary, necking was only observed in the splitting process (Bhattacharjee, 2012; S. Cho et al., 2002) where two electrodes are activated. Pressure based equations accompanied by related dimensionless numbers are going to be used to demonstrate the effect of changing the gap height and the electrode dimensions on the necking that happen during transport. Also CFD simulations will show that the necking happens at certain aspect ratios.
during transport. The relation between the pressures inside the droplet at the leading edge and the neck area can be expressed by the following equations (S. K. Cho et al., 2003):

\[
P_x - P_a = \gamma \left( \frac{1}{r_x} + \frac{1}{R_x} \right), \quad P_y - P_a = \gamma \left( \frac{1}{r_y} + \frac{1}{R_y} \right)
\]

\[
r_y = \frac{-h}{\cos \theta_t + \cos \theta_{by}}, \quad r_x = \frac{-h}{\cos \theta_t + \cos \theta_{bx}}
\]

where \(P_x, P_y,\) and \(P_a\) are the droplet pressures at the activated right electrode, the non-activated middle electrode and the ambient pressure, respectively. \(\gamma\) is the surface tension. \(\theta_t, \theta_{bx}, \theta_{by}\) are the contact angles at the top plate, bottom plate of the activated right electrode and bottom plate of the non-activated middle electrode, respectively. \(R_x\) and \(R_y\) are the radii of the droplet in the horizontal direction of the droplet motion at the activated right electrode and the non-activated middle electrode as shown in Figure 2.2. \(r_x\) and \(r_y\) are the radii of the droplet in the vertical direction to the droplet motion at the activated right electrode and the non-activated middle electrode. In this analytical model, using Eqs. (2.1) and (2.2), the necking radius \((R_y)\) can be calculated as follows:

\[
R_y = \frac{P_y - P_x}{\gamma} + \frac{1}{R_x} + \frac{\cos \theta_{by} - \cos \theta_{bx}}{h}
\]

The pressure change from the necking area to the leading edge \((P_y - P_x)\) varies throughout the different stages of the motion. However, based on the simulation results and calculations, the magnitude of this change is very small. This pressure change decreases significantly further by increasing the gap height. In this work, the effect of this pressure change is neglected as the shape of the following 3D curves will be only slightly affected by this small pressure difference. Moreover, the trend remained the same when the pressure terms are added to the equation. Under the assumption that the two pressures are equal, i.e. \(P_x = P_y\), the equation can be reduced as follows:
The horizontal radius at the side of the droplet \((R_y)\) can carry positive or negative values. \(R_y\) value becomes positive when the radius of curvature is pointing outward of the center of the droplet which happens when the droplet retains its nearly circular shape. The negative value of \(R_y\) indicates that the radius of curvature is pointing inward to the center of the droplet which happens during necking.

\[
R_y = \frac{R_x}{1 - \left(\cos\theta_{he} - \cos\theta_{ho}\right)\left(\frac{h}{R_y}\right)}
\] (2.4)

Figure 2.3 The horizontal radius at the non-activated part of the droplet \((R_y)\) on the z-axis plotted against the gap height \((h)\) and the horizontal radius at the activated part of the droplet \((R_x)\). The direction of necking radius \(R_y\) is demonstrated by the sign of the \(R_y\). The absolute value of \(R_y\) determines the degree of necking at the left hand side of the figure while the absolute value of \(R_y\) determines the radius of
curvature of the droplet at the right hand side of the figure. The absolute value of Ry decreases in the
direction of the white arrow.

The degree of necking is dependent on the absolute value of this radius of
curvature $|R_y|$. The degree of necking can be estimated by analysing $R_y$ values in
Figure 2.3 and Figure 2.4. $R_y$ holds a negative sign in the left side of Figure 2.3 and the
upper left side of Figure 2.4 (b) and (d). Lower absolute values for $R_y$, where $R_y$ holds
originally a negative sign, indicate more necking as the radius of curvature decreases.
Oppositely, higher absolute values for $R_y$ indicate less necking where the radius of
curvature increases. By observing the $R_y$ values, it can be noticed that necking is
encouraged to occur at low gap heights and high values of $R_x$. Positive values of $R_y$
indicate no necking at all. This can be observed at the right hand side of Figure 2.3 and
Figure 2.4 (b) and (d). The sign of $R_y$ depends on the value of ($h/R_x$) and ($\cos \theta_{bx} - \cos \theta_{by}$). $R_x$ can be related to the electrode length as $R_x = L/2$ when a circular droplet
occupies the electrode. Therefore, the ratio ($h/R_x$) in (3) can be related to the aspect
ratio which is calculated as the ratio between the gap height and the electrode side
length ($h/L$). The effect of the value ($\cos \theta_{bx} - \cos \theta_{by}$) on the necking can be observed
when the applied voltage changes. This in effect will change the value of $\cos \theta_{bx}$ as the
voltage changes according to Young-Lippman equation. In the present work, the
contact angle was measured by a side view camera as $109^\circ$ when the electrode is not
activated ($\theta_{by}$) and calculated from Young-Lippman equation as $82^\circ$ when the
electrode is activated ($\theta_{bx}$). The electrode side length used in this work is 2 mm and
the gap height is varied from 50 µm to 500 µm.

Eq. (4) is plotted in detail to gain an insight into the possible droplet
deformation modes by the EWOD effect. Figure 2.3 portrays the relationship between
$R_x$ and $R_y$ at different gap heights $h$. These parameters are plotted in a three
dimensional form in order to investigate how the electrode side length and the gap
height can greatly affect necking during transport (in terms of $R_y$). Severe necking
occurs when the absolute value of $R_y$ decreases which occurs at the left hand side of
Figure 2.3. Therefore, necking is more induced to occur when the gap height decreases
or $R_x$ increases as shown in Figure 2.3.
Figure 2.4 Three dimensional plots and their top view plot show the effect of changing the gap height and the radius at the leading edge ($R_x$) on the radius at the side of the droplet ($R_y$) represented on the colour
bar when the contact angle $\theta_{bx}$ changes from 82° (a and b) to 70° (c and d). The absolute value of $R_y$ decreases with the direction of the white arrow.

Figure 2.4 demonstrates the effect of changing the contact angle $\theta_{bx}$ on $R_y$. The contact angle $\theta_{bx}$ is 82° in Figure 2.4 (a) & (b) and 70° in Figure 2.4 (c) & (d). It can be noticed that the necking area, which corresponds to the negative values of $R_y$, increases or decreases according to the contact angle $\theta_{bx}$ value. $\theta_{bx}$ decreases when the applied voltage increases according to Young-Lippmann equation. When the contact angle $\theta_{bx}$ decreases the area of the negative values of $R_y$ increases. Therefore, this area is larger in Figure 2.4 (d) compared to Figure 2.4 (b). Figure 2.4 shows also that the absolute value of $R_y$ decreases when $\theta_{bx}$ decreases for a given $h$ and $R_x$. This indicates that necking is more likely to occur when $\theta_{bx}$ decreases while using the same aspect ratio.

2.3 Experimental setup

Figure 2.5 shows the experimental setup used in this work. It consists of a feedback control positioning system that can precisely set the gap height (i.e., the distance between the top and the bottom plate). Further details of our customized droplet analysis machine and its ability to enhance the performance of the DMF operations by varying the gap height in real time were discussed in this previous work (Mohamed Yafia & Najjaran, 2013). However, in this study our focus is on studying the changes in the droplet morphology during droplet transport, so the gap height is maintained constant during each experiment. The gap height is adjusted at various positions for running several experiments from 50 µm to 500 µm. DMF chips have been designed and fabricated using conventional photolithography. First, a thin Copper layer of 50 nm is deposited on a glass substrate by a sputtering deposition system (NEXDEP depositing system, Angstrom engineering, Kitchener, Ontario). Second, electrode patterns are formed on the glass substrate by photolithography and etching the copper coating. Finally, S1813 is spin coated on the chip to create a 1.5 µm dielectric layer which will be topped by a 50 nm Teflon AF1600 3% to create a hydrophobic layer on top of the dielectric layer. The fluid used in this experiment is DI water. The electrical signal used in droplets actuation is 200 V_{PP} (70.71 VRMS)
sinusoidal wave signal with frequency of 1 kHz. DC signals were avoided during experiments as noticeable degradation and electrolysis were observed during repetitive actuation on the same electrode over time.

![Figure 2.5 A schematic and a side view for the experimental setup used in this work.](image)

### 2.4 Results and discussion

Droplet motion development stages are demonstrated among different gap heights to illustrate the deformation patterns that occur at each gap height. This study will cover the gap heights from 50 to 500 µm with steps of 50 µm for running each
experiment. An additional experiment has been done at 75 µm to investigate the extreme necking and elongation at low gap heights. The electrode size used in the experiments is 2 mm × 2 mm. The effect of changing the gap height can be more significantly represented using the aspect ratio $h/L$ as discussed in Eq. (4).

![Figure 2.6](image)

Figure 2.6 The droplet morphology changes during motion at higher gap heights. The droplet motion when the droplet leading edge in (a) & (f) at 0 mm, (b) & (g) at 0.5 mm, (c) & (h) at 1 mm, (d) & (i) at 1.5 mm and (e) & (j) at 2 mm with 500µm and 300µm gap height, respectively.

2.4.1 Droplet morphology

The droplet motion is experimentally analysed in order to characterize the droplet transport process at different gap heights. Figure 2.6 and Figure 2.7 show the development stages of the droplet motion at different gap heights. Different droplet volumes where used in order to make sure that the droplet footprint area matches with
the electrode size at different gap heights. The droplet meniscus becomes more visible at higher gap heights due to the larger curvature in the vertical direction at the droplet edges. We noticed that the droplet meniscus becomes less visible at lower gap heights compared to higher gap heights. Therefore, a dashed line on the droplet meniscus is added to all the following figures in order to easily identify the droplet edges. The consecutives frames in Figure 2.6 and Figure 2.7 are taken during the droplet transport when the droplet leading edge is displaced toward the activated electrode by the following distances: 0 mm, 0.5 mm, 1 mm (middle of the activated electrode), 1.5 mm and 2 mm, respectively.

Figure 2.7 The droplet morphology changes during motion at lower gap heights. The droplet motion when the droplet leading edge in (a) & (f) at 0 mm, (b) & (g) at 0.5 mm, (c) & (h) at 1 mm, (d) & (i) at 1.5 mm and (e) & (j) at 2 mm with 100µm and 50µm gap height, respectively.
The characteristics of droplet motion and the changes in the droplet morphology at relatively high and low gap heights and aspect ratios can be observed in details in Figure 2.6 and Figure 2.7. At high aspect ratios, shown in Figure 2.6, the droplet remains almost in a circular shape without noticeable deformation during motion. On the contrary, Figure 2.7 shows that indeed the droplet loses its circular shape at very low aspect ratios throughout the different stages of motion. The droplet deformation will be discussed and defined quantitatively in the following section.

2.4.2 Droplet necking

The droplet deformation, necking and elongation has been measured by ImageJ (Schneider, Rasband, & Eliceiri, 2012). The droplet diameter is approximately occupying the electrode side length ($R_x=L/2$) when the droplet preserves its circular footprint on a single electrode. Figure 2.7 (b) and (g) show that the droplet leading edge starts moving when a small protruding radius $R_x$ is attracted toward the activated electrode. This small radius starts to increase and the droplet starts filling the activated electrode till the droplet fully occupy the whole electrode side length and $R_x$ become half of the electrode side length. Therefore, $R_x$ is continuously increasing and does not remain constant during the droplet motion development at low aspect ratios. Figure 2.8 shows that necking is more likely to occur at higher $R_x$ values and lower gap heights as discussed earlier. Therefore, droplet necking is more obvious at higher $R_x$ values and lower gap heights during the droplet motion development as shown in Figure 2.7 (c) and (h). Decreasing the gap height and increasing $R_x$ resembles decreasing the aspect ratio. Therefore, in order to be more specific, necking occurs mainly at low aspect ratios. This necking can be also demonstrated by the electric Weber number (Ha & Yang, 1998; Moukengué Imano & Beroual, 2006; Singh & Aubry, 2007). The electric Weber number defines the ratio between the deforming electric forces and the restoring interfacial forces due to surface tension as follows:

$$ We_0 = \frac{\varepsilon_0 \varepsilon_r E^2 R_x}{\gamma} = \frac{\varepsilon_0 \varepsilon_r V^2 R_x}{\gamma h^2} = \frac{\varepsilon_0 \varepsilon_r V^2}{2\gamma \frac{h^2}{L}} $$

(2.5)

where $We_0$ is the electric Weber number and $E$ is the electric field. High values of the electric Weber number ($We_0>1$) represent the increase in the electric potential or the decrease in the aspect ratio. When the electric Weber number increases at lower gap
heights, the localized deforming forces will overcome the effect of the restoring interfacial surface tension of the droplet and the droplet will become more prone to be stretched and increased necking will occur as shown in Figure 2.7. On the other hand, when the electric Weber number decreases at higher gap heights, the droplet surface tension effect increases and the droplet tends to move in bulk motion instead of being affected by the localized forces. Figure 2.8 demonstrates the relation between the degree of necking, the aspect ratio and the electric Weber number. The neck width is measured when the droplet leading edge reaches the middle of the activated electrode (at 1 mm displacement) to investigate the extreme necking that happens during transport especially at low aspect ratios.

Figure 2.8 The droplet necking that occurs when the droplet reaches 1mm of the activated electrode at (a) 50 µm, (b) 100 µm, (c) 200 µm, (d) 300 µm, (e) 400 µm and (f) 500 µm.
Figure 2.8 shows that necking increases by decreasing the gap height which decreases the aspect ratio and increases the electric Weber number. The neck width decreases significantly to less than half of the electrode side length when the gap height reaches 50 µm as shown in Figure 2.8 (a). This is realized by extreme localized forces applied to the leading edge when very high electric field tries to pull only the portion of the droplet near the activated electrode. Therefore, the droplet cannot retain its circular shape and tends to be attracted to the activated part especially at the low gap heights characterized by high electric Weber number where the electrical acting forces exceed the surface tension forces.

Figure 2.9 The droplet elongation during motion when the droplet reaches 1.5 mm of the activated electrode at (a) 50 µm, (b) 100 µm, (c) 200 µm, (d) 300 µm, (e) 400 µm and (f) 500 µm.
This deformation pattern occurred in both the experimental results and the simulations results that will be discussed later when the gap height is below 150 microns (with $\text{We}_0$ values greater than 1 above the green line in Figure 2.8). This necking reduces the area of the flow that leads to lower flow rate toward the activated electrode. This reduction in the flow rate will increase the transport time and the electrode has to be actuated for longer time in order to insure the completion of the droplet transport to the activated electrode. This longer actuation time will insure the sequential motion of the droplet from one electrode to another as the droplet leading edge should reach the end of the activated electrode. On the other hand, the necking starts to decrease when the aspect ratio increases and the electric Weber number decreases as shown in Figure 2.8.

### 2.4.3 Droplet elongation

Determining the droplet elongation is considered as an additional measure to quantify the droplet deformation during the transport process. Figure 2.9 demonstrates the elongated length, which is the distance between the leading and the trailing edge of the droplet, at different gap heights. The total length of the droplet is measured when the droplet leading edge reaches 1.5 mm on the activated electrode (75 % of the total displacement). Increased elongation that can reach 77% more than the original droplet length can be observed when the gap height is below the 100 µm range. This is accompanied by extreme necking that happens during transport as shown previously in Figure 2.8. On the other hand, the droplet elongation decreases below the range of 35 % when the gap height is more than 100 µm (See Figure 2.9 (c)-(f)).

### 2.4.4 Droplet velocity and momentum

The differences in the droplet transport modes among several gap heights are depicted by the displacement and velocity diagrams. They are measured using a position tracking software called Tracker (Brown, 2013). The displacements and the velocities measured from the experimental data belong to the droplet leading edge (blue) and the droplet trailing edge (red).
Figure 2.10 The velocity during motion when the gap height is 50 µm, 75 µm, 100 µm, 150 µm, 200 µm, 250 µm, 300 µm, 350 µm, 400 µm, 450 µm and 500 µm, respectively.
Determining the droplet leading edge position and velocity is important as the droplet sequential motion from one electrode to another is dependent on the ability of the droplet leading edge to reach the vicinity of the next electrode that is going to be activated. In other words, the droplet leading edge velocity determines the maximum droplet motion frequency that can be achieved. Figure 2.10 shows the droplet displacement and velocity versus the transport time at different gap heights (from 50 µm to 500 µm). The difference between the droplet leading edge position and the droplet trailing edge position indicates the droplet elongation at any instant during motion. This difference between the droplet leading edge and the droplet trailing edge increases at lower gap heights as shown on the displacement curves of Figure 2.10. This difference also depicts the increase in the electric Weber number and the decreased influence of the surface tension as the droplet cannot easily remain intact in a circular shape.

The droplet leading edge velocity, colored in blue on the velocity curves in Figure 2.10, passes through three phases during motion. The first phase is characterized by having an initial high velocity peak and ends when the velocity goes below 50 mm/s. The second phase starts by a gradual increase in the velocity till it reaches a second high velocity peak. In the third phase, the velocity decreases till the droplet comes to rest. The initial high velocity peak can be explained as follows: the motion starts by bulging of a small portion of the leading edge of the droplet when it is attracted by the driving forces as shown in Figure 2.7 (b). This small portion is affected by the localized driving force and impeded by a small viscous shear stress as the opposing shear stress is directly proportional to the droplet moving area touching the upper and the lower plates. After this initial motion, the shear stress starts to increase when a larger droplet portion moves toward the activated electrode as shown in Figure 2.10 (a). This leads to a highly impeded droplet motion till the end of the activated electrode without any droplet leading edge second high velocity peak at low gap heights. On the contrary, the droplet momentum and the low opposing shear stress leads to faster rolling at higher gap heights. The droplet decelerates with a very fast rate at higher gap heights as shown in the velocity diagrams in in the right side of Figure 2.10. This can be observed also from the
increased duration of phase 3 at lower gap heights compared to higher gap heights in Figure 2.10. The activated electrode will pull the droplet backward if it starts to overshoot and the droplet will come to rest when the electrical driving forces become balanced around the droplet. This behaviour occurs at higher gap heights and can be observed during the droplet motion at 500 µm. The velocity diagrams show the transition in the number of the velocity peaks of the droplet leading edge at different gap heights. This number changes from having a single high velocity peak at lower gap heights (occurs at the beginning of the motion) to having two velocity peaks at higher gap heights (The first occurs at the beginning of the motion and the second occurs when the droplet leading edge cover 1.5 mm or 75% of the activated electrode). Figure 2.10 (k) shows clearly that the droplet leading edge has two velocity peaks when the gap height is 500 µm. However, when the gap height decreases to 50 µm, only one velocity peak can be observed at the beginning of the motion and the second peak disappears (see Figure 2.10 (a)). When this second peak disappears, it is replaced by a constant damped velocity till the end of the motion. Therefore, phase 2 does not exist at extremely low gap heights as the velocity does not increase till the end of the motion (see Figure 2.10 (a) and (b)). Figure 2.10 also demonstrates that the droplet trailing edge velocity peak, in red, occurs nearly at the same time of the second droplet leading edge velocity peak where the droplet reaches its maximum momentum.

To sum up, the droplet leading edge second velocity peak decreases as the gap height decreases till the second peak completely vanish at extremely low gap heights as shown in Figure 2.10. Meanwhile, the droplet trailing edge has only one main velocity peak.

2.4.5 Initially overlapping droplet with the activated electrode

This section illustrates the differences between the droplet motion development when a portion of the droplet is initially overlapping and when it is not initially overlapping with the activated electrode. Noticeable necking during transport can be observed when the leading edge of the droplet meniscus is almost touching and not initially overlapping with the neighbouring activated electrode before the beginning of the motion. However,
necking can be barely noticed if a portion of the droplet is initially overlapping with the activated electrode before the beginning of the motion as shown in Figure 2.11 (a). This figure shows the experiment that is performed at 50 µm gap height on an initially overlapping droplet (Figure 2.11 (a) to (c)) and initially non overlapping droplet (Figure 2.11 (c) to (e)).

Figure 2.11 Initially overlapping droplet motion at 50 µm gap height when the leading edge of the droplet is (a) at the beginning of the motion, (b) half the of the activated electrode and (c) the end of the activated electrode (The middle electrode is the activated one).followed by initially non overlapping motion in (c), (d) and (e) (The right electrode is the activated one).
Slight necking can barely be noticed in Figure 2.11 (b) when a considerable portion of the droplet is initially overlapping with the electrode before starting the actuation. In this case, the actuation forces are distributed along the initially overlapping portion of the droplet and the droplet is dragged along the overlapping portion length. On the contrary, necking occurs when the droplet is initially non-overlapping and almost touching the activated electrode where the localized forces are acting on a small portion of the droplet leading edge. The droplet touching without overlapping situation occurs in the case of sequential forward motion at low gap heights when the droplet leading edge stops exactly at the end of the activated electrode without overshooting. This can be noticed at the end of the droplet motion in Figure 2.11 (c) and (e). Therefore, necking occurred only during the consecutive forward motion at extremely low aspect ratios when the droplet is almost touching the neighbouring electrode.

The total transport time is another difference that can be noticed between the motion of an initially overlapping droplet and a non-overlapping droplet. The total transport time is considerably higher in the case of non-overlapping droplet due to the extreme necking that happens. This necking restricts the area of the flow. Consequently, the flow rate decreases and higher transport time can be observed as discussed earlier.

It is worth to mention that smaller gap heights will pronounce two distinct effects. First, more localized actuation forces acting on the leading edge than the trailing edge can cause elongation. Second, it is noted that small pinning and lagging occurs during motion at low gap heights only at the trailing edge. This led to a small scale stretching at the trailing edge which can be noticed in Figure 2.12 (b). This lag in the tailing edge can be attributed to other non-linearities such as more pronounced effect of pinning due to the surface imperfections at lower gap heights. This pinning can be noticed clearly in the droplet motion shown in Fig. 2.12 (b). Figure 2.12 shows three consecutive frames that are taken at two different stages of the droplet motion when the gap height is 50 µm. Figure 2.12 (a), (b) and (c) show how the droplet is pinned when the droplet is stretched and elongated at the left corner of its trailing
edge. This pinning can also be observed from the sudden displacements and velocity spikes of the droplet trailing edge at lower gap heights as shown in the left side curves of Figure 2.10. This pinning does not occur when the gap height increases as the large velocity spikes of the droplet trailing edge disappear in the right side curves of Figure 2.10.

![Image of droplet motion and pinning](image)

**Figure 2.12** Effect of surface conditions and imperfections on contact line pinning during motion at low gap heights.

### 2.5 Flow simulation

Analytical models cannot handle the deformable non-circular shape of the droplet during motion at certain aspect ratios. Therefore, another approach should be considered to simulate the droplet behaviour at different stages of the motion.
development. Computational fluid dynamics (CFD) simulations have been performed to demonstrate the droplet morphology and deformation patterns at different gap heights and different geometrical and electrical configurations. FLOW-3D has been used as a CFD simulation tool in several microfluidics studies (Bhattacharjee, 2012; Chandorkar & Palit, 2009; Howell & Li, 2010). Fluid interfaces and free surfaces are modelled in FLOW-3D using volume of fluid technique. Volume of fluid (VOF) method is used to approximate the fluid boundaries by fractional volumes (Hirt & Nichols, 1981). VOF has been previously used in simulating droplets motion in DMF systems (Dolatabadi, Mohseni, Arzpeyma, & Dolatabadi, 2002; Jang et al., 2007). The VOF technique is based on tracking the fluid interface when the fluid is moving through the computational grid. This technique calculates the fractional volume in each cell according to the following equation:

$$\frac{\partial F}{\partial t} + \vec{V} \cdot \nabla F = 0$$  \hspace{1cm} (2.6)

where \( t \) is the time, \( \vec{V} \) is the velocity vector and \( F \) is the fractional volume of the fluid inside the computational cell. The value of the \( F \) is unity when the cell is fully occupied by the fluid and it becomes zero when the cell is empty. Therefore, there will be a fluid interface when the \( F \) value lies between 0 and 1. Next, Flow 3D uses the following equations for calculating the electric potential and electric field distributions (“FLOW-3D user manual,” 2008):

$$\nabla \cdot (K \nabla \Phi) = -\frac{\rho_e}{\varepsilon_0}$$  \hspace{1cm} (2.7)

$$E = -\nabla \Phi$$  \hspace{1cm} (2.8)

where \( K \) is the spatially variant dielectric constant, \( \rho_e \) is the free charge density, \( E \) is the electric field and \( \Phi \) is the electric potential. Afterwards, the electrohydrodynamic forces are coupled with the fluid flow module and Navier-Stokes equations. The electrical forces are calculated using the Maxwell stress tensor (C.W. Hirt, 2004):

$$F_i = \nabla_k T_{ik}$$  \hspace{1cm} (2.9)

$$T_{ik} = \varepsilon E_i E_k - 0.5 \delta_{ik} \varepsilon E^2$$  \hspace{1cm} (2.10)
where $T_{ik}$ is the Maxwell stress tensor and $\delta_{ik}$ is the Kronecker delta. For choosing the appropriate grid density, Jang et al. (Jang et al., 2007) compared the difference in the pressure between the right interface of droplet and the left one against the number of cells used in the simulation. They found that the pressure difference became stable when the number of cells was increased to 74,286 cells/mm$^3$ (which corresponds to 23.7 µm for cell side length in case of using cubic cells). In our study, the smallest mesh size used is 15 µm and the largest mesh size used is 20 µm which is still smaller than their recommended value. The spacing between the electrodes (20 µm) is neglected for simplifying the mesh construction. The thickness of the hydrophobic layer was ignored because it is considered very small (50 nm) compared to the dielectric layer thickness (1.5 µm) and gap height thickness (50 µm to 500 µm). The simulations did not account for the effect of surface roughness and contact line friction. The DMF system is completely symmetric. Accordingly, only half of the geometry was modelled to reduce the computational time.

**Table 2.1 Cases studied by CFD simulations.**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gap height</th>
<th>Electrode side length</th>
<th>Actuation voltage</th>
<th>Deformation status</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)Verified by experimental results</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.1</td>
<td>50 µm</td>
<td>2 mm</td>
<td>70.71 V</td>
<td>Cross validation and comparison between numerical and experimental results.</td>
</tr>
<tr>
<td>a.2</td>
<td>75 µm</td>
<td>2 mm</td>
<td>70.71 V</td>
<td></td>
</tr>
<tr>
<td>a.3</td>
<td>100 µm</td>
<td>2 mm</td>
<td>70.71 V</td>
<td></td>
</tr>
<tr>
<td>a.4</td>
<td>500 µm</td>
<td>2 mm</td>
<td>70.71 V</td>
<td></td>
</tr>
<tr>
<td>(b)Varying the electrode dimensions</td>
<td></td>
<td></td>
<td></td>
<td>Decreased necking by 44% compared to case a.1</td>
</tr>
<tr>
<td>b.1</td>
<td>50 µm</td>
<td>1 mm</td>
<td>70.71 V</td>
<td></td>
</tr>
<tr>
<td>(c)Varying the Voltage</td>
<td></td>
<td></td>
<td></td>
<td>Increased necking by 24% compared to case a.1</td>
</tr>
<tr>
<td>c.1</td>
<td>50 µm</td>
<td>2 mm</td>
<td>80 V</td>
<td></td>
</tr>
<tr>
<td>c.2</td>
<td>50 µm</td>
<td>2 mm</td>
<td>60 V</td>
<td>Decreased necking by 29% compared to case a.1</td>
</tr>
</tbody>
</table>

The simulation results exhibit the developing stages of the droplet motion toward the activated electrode. Table 1 summarizes the simulation cases that were performed to confirm the effect of changing the geometrical and electrical constraints on the necking phenomenon. Cases from a.1 to a.4 were performed to verify that the output of the CFD simulation matches with the experimental results. In these four cases, the voltage and electrode dimensions used are the same ones used in the
experiments (70.71 V and 2 mm × 2mm) and only the gap height is changed to the same gap heights used in the experiments. The degree of necking and droplet elongation in the numerical results match the experimental results, as it is shown in the comparison between them in Figure 2.13 and Figure 2.14. The red colour in the CFD simulations figure represents the voltage surrounding the activated electrode. Case b.1 represents the effect of changing the electrode side length and keeping the same gap height and actuation voltage (50 µm and 70.71 V). The decrease in the electrode side length will increase the aspect ratio. This in effect will decrease the electric Weber number which will lead to less necking than case a.1.

![Figure 2.13 CFD simulation results for droplet motion at different gap heights when the droplet leading edge reaches (a) 0.5 mm, (b) 1 mm and (c) 1.5 mm. at 50 µm, 75 µm, 100 µm and 500 µm.](image)

When the applied voltage increases, the contact angle $\theta_{bx}$ decreases. We can notice from Figure 2.4 that this will decrease the absolute values of $R_y$ while using the same aspect ratio which will increase the degree of necking. Moreover, when the electric potential increases, the electric Weber number increases and the surface tension influence decreases. In case c.1 the effect of increasing the actuation voltage was studied. Meanwhile, the dimensions were kept the same as case a.1. We found
that the degree of necking has increased compared to case a.1. Conversely, when the voltage was decreased in case c.2, the value of $\theta_{bx}$ has increased. This in effect has increased the absolute values of $R_y$ and has decreased the degree of necking. Additional simulation cases were performed to investigate the effect of changing the viscosity on the droplet morphology at low gap heights. It is worth to note that changing the viscosity (increasing it up to 50 folds compared to the DI water viscosity) did not change the droplet morphology and the deformation patterns.

Figure 2.14 Verifying the simulation results against the experimental results at different gap heights.

This can be explained by calculating the capillary number that describes the relative importance of viscous and surface tension forces. The capillary number is given by the following equation:
\[ Ca = \frac{\mu U}{\gamma} \] (2.11)

where \( Ca \) is the capillary number, \( \mu \) is the viscosity and \( U \) is the velocity. In this study, the capillary number is very small \((Ca \approx 10^{-4})\). Meaning that the effect of the viscous forces is very small compared to the effect of the restoring surface tension forces that resist the deformation. Elevated viscosities significantly decreases the droplet velocity, which will in turn increase the simulation time. However, higher viscosities have little impact on droplet deformation in our simulations. In addition, the increase in the viscosity will increase the viscous shear. This in effect will increase the friction during motion without affecting the deformation patterns.

2.6 Summary

This chapter demonstrates how the aspect ratio, in terms of gap height and electrode side length, has a great effect on the droplet shape, droplet dynamics and how the droplet motion develops during transport. Severe necking accompanied by elongation and stretching in the total length of the droplet were observed during the droplet transport at extremely low aspect ratios.

Several experiments were introduced to show how the droplet velocity is affected by changing the gap height. Two velocity peaks can be noticed at higher gap heights for the droplet leading edge. The value of the second velocity peak starts to decrease when the gap height decreases until it totally disappears. Only one velocity peak for the droplet trailing edge can be observed. The position of this velocity peak changes according to the value of the gap height. Sudden velocity spikes occur at the droplet trailing edge because of the droplet pinning at low aspect ratios. On the contrary, droplet motion at higher gap heights does not show any contact line pinning where it moves freely with a nearly circular footprint. Moreover, droplet motion at higher gap heights can overshoot at the end of motion while the motion is damped at very low gap heights. At extremely low gap heights, the electrode should be activated for longer time to insure that the droplet will be transported completely to the activated electrode. The electric Weber number can give an additional explanation to the necking as the electric Weber number increases by either decreasing the aspect
ratio or increasing the applied voltage. This in effect will decrease the surface tension influence compared to the electrical potential and the localized forces applied and will lead to more necking and further changes in the droplet morphology.

Computational fluid dynamics (CFD) simulations were performed and their results match with the experiments. Analytical models are considered invalid when the droplet becomes deformed and non-circular at certain aspect ratios. As a result, numerical simulations were used instead of analytical models to more adequately demonstrate the droplet behaviour observed. Additional cases were tested to show the effect of changing the aspect ratio in terms of the electrode side length. Moreover, further cases were performed to investigate the effect of changing the applied voltage on the droplet morphology during transition. A comparison has been made between these different cases to summarize the effect of each parameter on the droplet morphology during transport. This work would also serve to further stimulate theoretical work in EWOD to extend the analytical models for handling the irregular droplet shapes during different stages of motion.
Chapter 3  High precision control of gap height for enhancing principal digital microfluidics operations

3.1 Variable gap size actuation (VGSA) mechanism

A variable gap size actuation (VGSA) mechanism is integrated into the digital microfluidic (DMF) system. The VGSA mechanism serves to optimize the aspect ratio during performing different microfluidic operations by changing the gap height between the top and bottom plates. This in effect will have a direct impact on the four main DMF operations including droplet transport, splitting, dispensing and merging as they are greatly affected by changing the aspect ratio. Experimental results demonstrate that the VGSA mechanism significantly enhances the principal DMF operations by retaining the appropriate gap height for each operation which is also dependent on droplets volumes when using fixed electrode size. Specifically, varying the gap height precisely between the two plates will enable us to transport the droplets more reliably, control the volume of the dispensed droplet, carry out splitting and merging more effectively, facilitate motion of residual droplets resulting from splitting or partial evaporation, enhance mixing at faster rates, achieve accurate positioning of droplets regardless of their volume, and minimize evaporation without complicating the DMF system with the use of a filler medium. The proposed mechanism is realized by accurate positioning of the top plate over the fixed bottom plate, instead of maintaining a fixed gap height during the operation. In this work, an experimental setup is constructed for the proof of concept to meet precise alignment requirements of the two parallel plates using a feedback-controlled positioning system. Three different methods viz., visual, capacitance-based and encoder values, are used to measure the gap height between the two plates precisely. For practical lab-on-chip devices, micro-actuators in conjunction with capacitance measurement feedback can be used to position the top plate during the operation. In this way, the proposed VGSA mechanism will introduce a mean for optimizing the parameters controlling the DMF operations.

Different approaches have been adopted in the published research to improve these operations, for example, by improving the electrode shape design (Rajabi & Dolatabadi, 2010), use of different dielectric materials in fabrication (Liu, Dharmatileke, Maurya, & Tay, 2009), varying electrode configurations in open systems (M Abdelgawad, Park, &
Wheeler, 2009), pre-charging for reducing the threshold voltage for actuation (Kyungyong Choi, Im, Choi, & Choi, 2011), active matrix actuation to decrease the number of addressing connections (Noh, Noh, Kreit, Heikenfeld, & Rack, 2012), and manipulating droplets on large array of small electrodes of thin film transistors (TFT) (Hadwen et al., 2012).

In this work, a mechanism for controlling the gap height between the top and the bottom plate is introduced by integrating a $xyz$ feedback controlled micro-stage to demonstrate a new technique for enhancing the four basic microfluidic operations. The same technique can also be used to switch between an open and closed DMF system to benefit from the advantages of both on a single platform. The effect of the gap height on the microfluidic operations have been investigated in the past, but a mechanized gap height manipulation has not been examined as a way of improving the DMF performance and applicability to date. For example, Chen et al. (C.-H. Chen, Tsai, Chen, & Jang, 2011) used aluminium foil as a flexible spacer between the plates to study the effect of changing the gap height within a small range (16 to 32 microns). However, this change can not be used as an integral feature of the DMF system. Moreover, larger gap height changes are required to have a noticeable difference on the DMF operations and this might be the reason why no complete droplet motion has been achieved in their work.

3.2 Effect of changing the gap height on the DMF operations

Manipulating the droplets between two parallel plates is affected by several geometrical and electrical aspects. In the literature, the gap height is defined by the thickness of the spacers used and it remains constant while performing the microfluidic operations. The aspect ratio ($h/L$), where $h$ is the gap height between the two parallel plates and $L$ is the side length of the electrode, is considered as one of the important parameters that affect droplets actuation. The size of the electrode cannot be modified once the chip is fabricated. However, modifying the aspect ratio can be feasible by changing the gap height. Meanwhile, there is a preferable gap height range for performing each operation. Accordingly, the following section will illustrate the strong relation between the gap height and the different DMF operations.
3.2.1 Transport

DMF system relies mainly on moving the droplets on discrete electrodes. Successive droplet motion is attainable as long as the droplet meniscus can reach the vicinity of the neighbouring electrodes. There is no limit on the maximum droplet volume that can be transported as the droplet can be stretched on more than one electrode. However, there is a minimum droplet footprint area required for having sequential motion of the droplets. This area is determined by the aspect ratio and the droplet volume. Though, as long as the aspect ratio and the gap height are defined by fixed spacers, there will be a limited capability to operate on droplets with defined volume and footprint area lower than a certain limit (where the diameter of the droplet is smaller than the electrode side length). Some techniques are used for increasing the overlap area between two electrodes by modifying the electrode design such as using inter-digitated electrode design (M. Pollack et al., 2002).

Major forces acting on the droplet during motion will change significantly by changing the gap height and footprint surface area of the droplet. The shear force from the two parallel plates is one of the opposing forces that will increase by decreasing the gap height. This force can be accounted by the following equation (Bahadur & Garimella, 2006):

\[ F_s = (2\pi r^2) \frac{6\mu U}{h} \]  

where \( r \) is the radius of the droplet, \( \mu \) is the viscosity of the droplet, \( U \) is the velocity of the droplet and \( h \) is the gap height between the two parallel plates. The friction force can increase by decreasing the gap height due to the increase in the droplet radius. This force can be calculated by the following equation (Bahadur & Garimella, 2006; Hong Ren et al., 2002):

\[ F_f = 4\pi r U \zeta \]  

where \( \zeta \) is the friction factor. Likewise, the driving force on the non-conductive droplet will increase by decreasing the gap height as shown in Figure 3.1. This force is calculated by using the electromechanical model at different gap heights (Chatterjee, Shepherd, & Garrell, 2009). The electromechanical model is used as it accounts for the changes in the driving force when the gap height is modified in terms of the change in the droplet capacitance. The parameters used in generating this model are listed in Table 3.1.
According to the previous equations, changing the gap height and the droplet footprint area, in terms of the droplet radius, are going to affect the major forces acting on the droplet.

3.2.2 Splitting

Droplet splitting is considered as one of the critical DMF operations as there are strict electrical and geometrical considerations have to be addressed for performing successful splitting. The splitting process is used for different purposes such as dilution and changing the solution concentration (Jian Gong & Kim, 2008), and purification after particle separation (Fan, Huang, Wang, & Peng, 2008).

Previous studies showed the effect of the gap height on having successful splitting and mentioned that the gap height should be lower than a certain value to perform successful splitting (S. Cho et al., 2002). Another study explained the splitting stages such as pinching, necking and cutting and verified that these steps occur at different aspect ratios (S. K. Cho et al., 2002).
al., 2003). Furthermore, the minimum voltage required for splitting must be exceeded in order to have two equal splitted volumes (J. H. Song, Evans, Lin, Hsu, & Fair, 2008). An approximate estimation for the maximum gap height required for splitting was found to be (J. Berthier et al., 2006):

\[ h < -L \cos \theta_o \]  

(3.3)

where \( \theta_o \) is the contact angle when there is no applied voltage. Similarly, the electrical constraint for splitting can be defined by a static splitting model for 3 electrode splitting scheme shown in the following equation (J. H. Song et al., 2008):

\[ V^2 - V_T^2 = \frac{2ht\gamma_{lg}}{\varepsilon_o \varepsilon_r L} \]  

(3.4)

where \( \varepsilon_o \) and \( \varepsilon_r \) are the dielectric constants for vacuum and the dielectric layer respectively, \( t \) is the dielectric layer thickness, \( \gamma_{lg} \) is the surface tension of the air-liquid interface and \( V_T \) is the threshold voltage for actuation.

The effect of the gap height \( h \) is shown clearly in the previous geometrical and electrical constraints equations. Therefore, the gap height is considered as one of the dominant parameters for having successful splitting operation.

### 3.2.3 Dispensing

Micro total analysis systems (µTAS) are one of the promising applications that can be performed on DMF chips. In these applications controlling the volume of the dispensed droplet plays an important role since precise metering of the samples and reagents is required. The dispensing accuracy is acceptable in the range of ±5% and can be considered convenient for many applications (Rose, 1999).

Dispensing can be described as a cutting process for generating daughter droplets. The dispensing process can be categorized according to the place of the liquid reservoir. The liquid reservoir can be either located on the chip or at an external supply (M. Pollack et al., 2002). In case of an off-chip reservoir, an external dispensing system which has connections to the DMF chip is supplying the fluid. On the other hand, the daughter droplets are
generated from a reservoir droplet sitting on a larger electrode in case of on-chip dispensing systems.

The dispensing process can be controlled by a feedback signal coming from capacitance measurement (H Ren, Fair, & Pollack, 2004). Moreover, adding a PID controller has enhanced the dispensing process (Jian Gong & Kim, 2008). The long-term reproducibility and reliability was investigated for having consistent dispensing (Elvira, Leatherbarrow, Edel, & DeMello, 2012). The dispensing reliability has shown improvement when the gap height was decreased from 120 to 76 µm which decreased the volume variation from 10% to 6%. They also found that when the reservoir droplet volume matches the electrode size the dispensing becomes more accurate with lower volume variation. This can be easily adjusted once we have control on the gap height in order to make the reservoir droplet footprint always matches the reservoir electrode area when dispensing is initiated.

The maximum height for dispensing the droplets can be approximated by the dispensing model shown in eqn.(5) (J Berthier et al., 2005). They have also mentioned that the maximum gap height for dispensing is limited by geometrical and electrical constraints. The gap height condition for dispensing can be extracted from their model with some modifications in order to adapt it to the normal dispensing process as they used a special design with a wall between the reservoir and the working electrodes. The generalized equation without this wall can be shown by the following equation:

\[ h < \frac{(\cos(\theta_s) - \cos(\theta_p))}{\frac{2}{L} + \frac{1}{R_s}} \]  \hspace{1cm} (3.5)

where \( \theta_s \) is the saturation angle and \( R_s \) is the reservoir droplet radius. Another model can be used to determine the voltage required for dispensing (J. H. Song et al., 2008):

\[ \nu^2 - \nu_T^2 > \frac{8h\gamma}{\lg} \sqrt{\frac{e \cdot \varepsilon_r \cdot L(N)^2 + 1}{e_o}} \]  \hspace{1cm} (3.6)
where $N'$ is the number of electrodes where necking occurs ($N' \geq 1$). Consequently, all these evidences indicates the effect of the gap height on the dispensing process.

### 3.2.4 Merging and Mixing

The last operation in the four main operations is merging that is followed by mixing the droplets completely. Mixing the droplets is required for coalescing different chemical and biological samples and reagents. Therefore, a rapid complete mixing is required in order to have a homogenous droplet and insure the completion of chemical reactions.

Several techniques were used to improve mixing. An algorithm was developed to handle the different ways of mixing the reagents and how to use properly the waste droplets (Hsieh, Ho, & Chakrabarty, 2012). A dilution mechanism is proposed based on 1:X cutting ratio which can enable faster dilution (Jian Gong & Kim, 2008). A rolling based model was introduced to show that the mixing diffusion time will drastically decrease by increasing the number of rolls (Fowler, Moon, & Kim, 2002). The patterns of rolling without diffusion have shown that the number of interface layers is exponentially dependent on the number of rolls. Mixing on several electrodes was faster and more reliable due to the decrease in the flow reversibility compared to the two electrode mixing scheme (Paik, Pamula, Pollack, & Fair, 2003).

The effect of the gap height on the mixing time was investigated (Paik, Pamula, & Fair, 2003). The authors reported that the optimum aspect ratio for mixing on a 4-electrode scheme is equal to 0.4. They also noticed that decreasing the gap height reduced the circulation and mixing flow in the vertical direction, and therefore this confirms the gap height influence on the mixing process.

### 3.3 Experimental setup

#### 3.3.1 Equipment

The experimental setup has been designed to meet the precise alignment requirements as the top and the bottom plate in our setup are not going to be physically connected. Precise tilting stages for adjusting and aligning the plates parallel to each other are used for adding fine alignment ability to the setup. The top plate is mounted on a 1 axis tilting platform (TGN80, Newport) with 2 arc.sec. sensitivity. This tilting platform is attached to XYZ electromechanical feedback controlled positioning system consisting of 3 linear servo motors.
(Two MX80L for X&Y directions and one 404 XR for the Z direction, Parker) with motion accuracy of 5 microns.

The bottom plate is mounted on a linear stage (M-460P, Newport). This linear stage is fixed on a 2 Axis Tilt & Rotation Platform with 2 arc.sec. sensitivity (M-37, Newport).

Three microscopic cameras were integrated to the system for imaging the top and the two side views. A card edge connector (1-5147490-0, TE connectivity) was used for addressing the connection pads on the chip. The bottom glass plate was grinded at the side edge near the connection pads in order to fit it easily inside the card edge connector. The design of the mask used in fabrication is shown in Figure 3.2 where the bottom connection pads that are designated for the card edge connector can be seen.

The electrical signals required for actuation are produced from a function generator (AFG3021B, Tektronix) coupled with high voltage amplifier (PZD700A, Trek). An opto-isolator (MOC3063) coupled with a triac (BT136) are used for AC signal switching. The signals are distributed and addressed for each connection by using a microcontroller (Arduino Mega 2560).

A 50 µm spacer is fixed on the two sides of the bottom plate. This spacer is added as a physical lower limit to prevent squeezing the droplets between the two plates and protect the surfaces of the top and the bottom plates from touching each other and deteriorating the surfaces. Additional limit is added to the actuator software when the top plate reaches the spacers on the left and the right sides of the bottom plate which is monitored by the two side views cameras simultaneously. This limit is added to prevent the excessive non intentional contact between the top and the bottom plate. In the experiments, the gap height was monitored through the Z-axis actuator software with 500 nm measurement resolution. The liquid used in the experiments is DI water generated from a water purification system (PURELAB Option-R15 BP, ELGA) with resistivity of 18 MΩ.cm.

Two image analysis programs were used. The first software is ImageJ (Schneider et al., 2012) which is used for calculating the surface area of the droplets. The second software is Tracker (Brown, 2013) which is an open source video analysis program that is used for calculating the velocity and the acceleration of the droplet during motion.
3.3.2 Fabrication of the DMF chip

The DMF chip is fabricated on a 75 mm x 50 mm glass substrate with 1 mm thickness. A 50 nm copper layer is deposited on the substrate by a sputtering deposition system (NEXDEP deposition system, Angstrom Engineering). The required patterns on the chip are generated by photolithography.

On the lower plate, a 1.5 μm layer of photoresist (S1813) is spin coated and used as a dielectric layer. A hydrophobic layer is added by spin coating of 50 nm Teflon AF1600 3% on the upper and the lower plates.

3.4 New actuation mechanisms

After demonstrating the effect of the aspect ratio with different cases, the only way to modify the aspect ratio during DMF operations is by changing the gap height since the electrode size is fixed. The proposed variable gap size actuation mechanism will replace the use of fixed spacers. Consequently, this will enable carrying out each DMF operation effectively as will be demonstrated later in this work.

The proposed method to enhance the actuation mechanisms is achieved by moving the top plate and fixing the bottom plate of the closed DMF system. This motion is controlled with motion accuracy of 5 microns during performing DMF operations. Meanwhile, the top and the bottom plate will always be kept parallel and aligned to each other.

Figure 3.2 The experimental setup and a schematic drawing showing the degrees of freedom for each component in the setup. (a) 4 degrees of freedom are available for the top plate. (b) 4 degrees of freedom
are available for the bottom plate. (c) The side and back lighting sources for imaging. (d) Nano droplet dispenser attached to the setup. (e) One top view camera and two side view cameras. (f) Top view of the mask used. (g) 2 degrees of freedom are available for the side view camera.

3.5 Results and discussions

3.5.1 Effects on DMF operations

Changing the gap height during the DMF operations is going to add an additional degree of freedom and flexibility for all the operations as it will facilitate working within the favourable gap height range for each operation. Specific gap height can be adjusted in order to transport different droplets volumes reliably on the same electrode size. Another gap height can be used for having successful splitting. Controlling the volume of the dispensed droplet can be achieved by adjusting the appropriate gap height according to the droplet volume required in any following processes. In addition, the gap height can be adjusted for the optimum mixing aspect ratio to minimize the mixing time. Therefore, every process has a favourable gap height range which will be demonstrated in this section.

3.5.2 Transport

Figure 3.3 shows a sequential transport process for a droplet of volume 1.61 µL on several electrodes. Meanwhile, the gap height is modified by moving the top plate with velocity of 50 µm/s. The droplet footprint area is inversely proportional to the gap height. When the gap height is decreased, the droplet footprint area becomes large and it tends to occupy larger area on the neighbouring electrodes. We noticed that the droplet tends to keep its circular footprint at higher gap heights and stays at the same position on the new electrode even after deactivating the electrode as shown in Figure 3.3 (a), (d) and (e). On the contrary, when the droplet footprint area is increased after decreasing the gap height, the droplet tends to lose its complete circular shape as shown in Figure 3.3 (b) and (c). Moreover, the meniscus of the droplet at the leading edge starts to retract and the footprint shape of the droplet at the final resting position becomes unpredictable after deactivating the electrical signal. This situation is not preferable as the droplet meniscus should reach and overlap with the next neighbouring electrode even after deactivating the electrode for having sequential motion. Retraction can occur in low gap heights due to the surface tension force acting on the droplet when it is stretched on more than one electrode and loses its circular shape. This
phenomenon is also affected by the excessive shear stress at lower gap heights, contact angle hysteresis and contact line pinning which prevent the contact line from moving freely, and it is also related to the surface conditions (S. K. Cho et al., 2003). Figure 3.3 shows that the sequential transport at higher gap heights is more reliable and the positioning of the droplet becomes more accurate. In this case, the most accurate positioning occurs when the gap height is increased to 450 µm as shown in Figure 3.3 (a). Increasing the gap height allows the transport of larger droplets volume on the same footprint area and electrode size. By managing this properly, the DMF chip will become more efficient for handling larger droplets volumes. A vibration of very small order of magnitude was noticed at the bottom plate and did not affect the operation. It can only be noticed on the droplet meniscus when the gap height is decreased.

The velocity and acceleration of the droplet at 500 µm, 250 µm, and 150 µm spacing is studied in order to analyse the difference in the droplet transport modes between high and low gap heights. The leading edge of the droplet was tracked and the images used in this analysis were taken at 1700 frames per second with resolution of 4.77 µm/pixel. This high speed allowed observing specific details on the transport mode especially the velocity and the acceleration at the beginning of motion. These details could not be noticed at lower imaging speeds due to aliasing problem.

At higher gap heights the velocity curve exhibited two peaks at the beginning and at the middle of the displacement. On the contrary, at lower gap heights only one peak can be found at the beginning of motion and the velocity tends to decrease till the end. Figure 3.4 demonstrates that as the gap height decreases, the velocity at the first peak increases. Conversely, the velocity at the second peak decreases. This result shows that the droplet is impeded by high shear force from the plates at lower gap heights.

Similarly, the acceleration of the droplet was found to be higher at the beginning of the transport at lower gap heights. This result confirms that higher driving forces are generated lower gap heights. The total transport time from one electrode to another was not greatly affected by changing the gap height as it ranged from 36 ms to 47 ms for the tests shown in Figure 3.4. This can be explained by noticing that the variation in the driving force
versus the impeding shear force is opposite to each other during changing the gap height at fixed voltage and frequency.

The ability to transport some types of liquids is dependent on the gap height as motion has not been achieved for some liquids at any voltage or frequency until the gap height was decreased (Chatterjee, Hetayothin, Wheeler, King, & Garrell, 2006). The authors observed in their study that manipulating some liquids is feasible at any gap height while

**Figure 3.3 Variable gap transport with different footprints.**

The electrode is energized

The electrode is de-energized
manipulating other types can not be achieved unless the gap height is low. By using the VGSA, moving those liquids can be achieved by lowering the gap height when needed.

Adjusting the gap height can minimize contact area between the droplet and the two parallel plates. Reducing this area can help in decreasing the adsorption of the biomolecules on the surface of the DMF chip. By decreasing the gap height, reliable motion can be achieved for the residual droplets that are generated from splitting, dispensing or even after partial evaporation of the droplets as the VGSA mechanism can ensure the droplet overlap with the neighbouring electrodes.

Figure 3.4 The velocity and the acceleration versus the displacement of the droplet when the gap height equals to 500 µm, 250 µm and 150 µm.

3.5.3 Splitting

Splitting is the most sensitive operation that can be performed on DMF chips. In order to achieve successful splitting, certain electrical and geometrical requirements have to be fulfilled as mentioned earlier.
Figure 3.5 shows the sequence of performing several operations at their appropriate gap heights. At the beginning, a 0.65 µL droplet is manipulated easily across the electrodes while its footprint is adjusted. Despite the facile motion at higher gap heights, the splitting process cannot be achieved. For this reason, the gap height is reduced to 105 µm for starting the splitting process.

In case of ideal symmetrical splitting, the volume and footprint area of the splitted droplets will be only dependant on the volume of the mother droplet. In case of non-ideal splitting, the volume of the two sister droplets may not be the same as a result of several factors that can affect the splitting process. For example, if the initial position of the mother droplet before splitting is not exactly centred between the two electrodes it can result in unequal splitting, and if there is surface imperfection at any of the two side electrodes. Therefore, in non-ideal splitting the two splitted droplets may not have the same volume and one droplet may become larger than the other and this will increase the probability of having smaller droplets that cannot be transported reliably after splitting. Accordingly, the gap height has to be reduced again for the following operations.

Larger difference between the volumes of the two splitted droplets was noticed in our experiments if the mother droplet is sitting on one side more than the other like Figure 3.3 (e). It should be noted that in order to position the centre of the droplet exactly on the centre of the square electrode, the gap height should be adjusted to make the footprint diameter nearly equal or slightly larger than the electrode side length. If the droplet footprint remains large at lower gap heights, then the droplet cannot be placed exactly at the centre of the middle electrode. So the proposed VGSA mechanism can be used to place the droplets accurately at the required position before starting the splitting process or any other process that require accurate positioning of the droplets.

In this experiment, the volumes of the droplets on the left and right hand sides are 0.31µL and 0.33 µL respectively even after centring and positioning the mother droplet at higher gap height before decreasing it for splitting. The specified volume and footprint area of the smaller droplets after splitting cannot ensure the droplet overlap with the next electrode at 105 µm gap height. Consequently, the gap height is reduced again to 65 µm in order to facilitate the motion of the two smaller droplets, and then both of them were moved
to the left direction at this modified lower gap height. Afterwards, the two droplets were merged to form a larger droplet. At this low gap height it is noticed that the droplet can not relax to the circular footprint shape and this is going to affect the sequential reliable transport of large footprint droplets at low gap heights as demonstrated before. For this purpose, the gap height was increased again to have more reliable transport.

![Diagram](image)

**Figure 3.5 Steps of the VGSA splitting merging technique:** (a) Transport of the mother droplet. (b) Reducing the gap height to achieve splitting by moving the top plate downward. (c) The droplet is splitted and the smaller droplets can not be transferred reliably to the neighbouring electrodes. (d) The droplets footprint is adjusted by reducing the gap height again. (e) The splitted droplets are manipulated individually at modified lower aspect ratio. (f) Merging the two droplets. (g) Optimized transport at higher aspect ratio.
3.5.4 Dispensing

One of the major advantages of varying the gap height is controlling the volume of the dispensed droplets. The dispensing system that uses fixed spacer at certain gap height will dispense fixed volume that is determined by the geometrical parameters.

The process of variable volume droplet dispensing is demonstrated in Figure 3.6. Initially, the gap height is lowered in order to start the dispensing process as dispensing cannot be started at higher gap heights as discussed earlier. Secondly, the dispensing process starts by dispensing the first droplet of volume of 1.08 µL at 220 µm gap height. Afterwards, the gap height was decreased to 170 µm followed by dispensing the second droplet of volume 0.92 µl. The change in the dispensed volumes and the gap height is noticeable in Figure 3.6 and there is a significant change in the footprint area of the dispensed droplet number 1 as shown in Figure 3.6 (b) and (c).

The footprint area of the reservoir droplet can affect the dispensing consistency as it was noted that the reliability increased when the reservoir droplet area equals to the reservoir electrode area (Elvira et al., 2012). The dispensing reproducibility was investigated at different aspect ratios for off-chip dispensing (H Ren et al., 2004) where they provided a preferable aspect ratio for dispensing (aspect ratio of 2.5 for 1 mm electrode side length). Therefore, the gap height can be adjusted for having more reliable dispensing by matching the optimum dispensing parameters for each electrode size and for changing the volume of the dispensed droplets.

Figure 3.6 (a) Dispensing cannot be achieved at high aspect ratios. (b) The aspect ratio is decreased in order to start the dispensing process. (c) The aspect ratio is decreased again to dispense smaller droplet volume.
3.5.5 Merging and Mixing

Several mixing techniques were used for achieving more efficient mixing at shorter mixing time. The VGSA mechanism can enhance the mixing process by adjusting the gap height for higher mixing rate as good mixing is hindered at low aspect ratio and there is an optimum gap height for mixing efficiently (Paik, Pamula, & Fair, 2003).

Figure 3.5 shows the difference in the footprint area before and after merging the two droplets at low and high gap heights. We will have the advantage of having better mixing and increased circulating flow in the vertical direction by merging then increasing the gap height followed by rolling at higher aspect ratio. The VGSA mechanism can allow merging of more than two droplets together and utilize them efficiently at different gap height for any later DMF operations.

3.5.6 Additional advantages of changing the gap height

The position of the top plate can be adjusted in order to change the DMF system from open to closed system and vice versa. As mentioned earlier, some operations like splitting and dispensing cannot be achieved in the open system. By using the VGSA, on-chip dispensing and splitting can be done for droplets sitting on the open system. This will enable taking the advantages of both closed and open systems simultaneously by rapid positioning of the top plate.

Increasing the footprint area can increase the detectability of the droplet for any sensing mechanism. For instance, increasing the footprint surface area can increase the illuminated portion for photodiode detection techniques. Another advantage can be gained by decreasing the gap height is the increase in the capacitive sensing sensitivity during the measurement as the capacitance of any liquid droplet increases at lower gap heights.

Varying the gap height will enable us to control the evaporation rate and the concentration as changing the gap height is going to affect the evaporation rate (Schertzer, Gubarenko, Ben Mrad, & Sullivan, 2009). For a confined droplet with low gap height, the diffusion is the only controlling mechanism (Clément & Leng, 2004) and the change in the volume during evaporation can be calculated by the following equation:
\[
\frac{\partial V}{\partial \tau} = \frac{D n_s^2 v}{R_0 \ln \left( \frac{R_o}{R_s} \right)} (3.7)
\]

where \( J \) is evaporation flux, \( D \) is the diffusion coefficient, \( n_s \) is the gas density number at the edge of the droplet (saturated water vapour), \( R_o \) is the instantaneous radius of the droplet, \( R_s \) is the outer radius of the covered plates and \( V \) is the molecular volume of the liquid. It is noticed from eqn. (7) that there is a direct relationship between the evaporation rate and gap height as the evaporation rate \( \propto \text{const} \cdot f(h, R_o) \). Accordingly, the droplets at lower gap heights will evaporate at slower rate. This has been demonstrated experimentally for two gap heights (Schertzer et al., 2009). By using this mechanism, it is feasible to control the evaporation rate without using a filler liquid like silicon oil which is commonly used for decreasing the evaporation.

### 3.6 Gap height measurement

Measuring the gap height is essential for providing a feedback signal to any kind of micro actuator that can be used to change the gap height. There are several methods to measure the gap height between the top and the bottom plate. Some of these methods can be complicated and expensive like Laser interferometry and continuous detection of the droplet area by image analysis during changing the gap height. However, these methods cannot be easily added to the DMF system.

Capacitance measurement can provide a built in tool inside the DMF system that can be easily integrated on the chip with simple electronic circuits. In the DMF literature, capacitance measurement has been used for several purposes (Jian Gong & Kim, 2008; Murran & Najjaran, 2012; Schertzer, Ben-Mrad, & Sullivan, 2010).

A Hex Schmitt trigger circuit is used for measuring the change in the droplet capacitance during changing the gap height. A dedicated circular shape side electrode is used for measuring the capacitance as shown in Figure 3.2. The relation between the droplet capacitance and the gap height is inversely proportional. Similarly, the change in the frequency generated from the Hex Schmitt circuit is inversely proportional to the measured capacitance. Therefore, the change in the frequency generated is directly proportional to the
change in the gap height. The trend line in Figure 3.7 confirms the direct linearity relationship between the gap height and the frequency generated.

![Figure 3.7 The relation between the droplet capacitance and the gap height on the designated sensing electrode.](image)

3.7 Summary

In this chapter, a variable gap size actuation (VGSA) mechanism has been introduced and successfully integrated into digital microfluidic (DMF) chips to control precisely the position of the top plate of the closed parallel plate DMF system. A comprehensive experimental study has shown the added benefits of using VGSA. Specifically, this research demonstrates how controlling the gap height allows the DMF system to assume an appropriate aspect ratio for each operation. We also demonstrate how the sequential adjustment of the gap height during a multi-operation process can guarantee successful progress of the process until completion. For instance, VGSA can provide real-time modification of the droplet footprint area using programmed automation and synchronization between the DMF electrical signals and the top-plate actuator used to facilitate the droplet motion before and after merging and splitting that may otherwise result in immovable leftover droplets.
The effect of VGSA on droplet transport was investigated for a range of different gap heights. The optimum transport is achieved when the top plate is at the highest position as long as the droplet can still touch the neighbouring electrodes. It is noted that high gap heights can also facilitate positioning of the droplet e.g., when it is important to centre a droplet on an electrode before starting an operation. A high gap height is also preferable because it can prevent droplet retraction that may halt droplet sequential motion on a DMF device. Retraction can occur in low gap heights due to the surface tension force acting on the droplet when it is stretched on more than one electrode and loses its circular shape. Clearly, increasing the gap height is limited by the droplet volume since the footprint area must be large enough for the droplet to touch a neighbouring electrode to remain active. Lower gap heights can also be preferable at times. For example, the lower the gap height the less the droplet side surface area and hence the less the contamination or evaporation rates.

The VGSA mechanism significantly reduces the residual sample waste volume of a reservoir. It is shown that by reducing the gap height it is possible to dispense a droplet from a reservoir which, with the initial aspect ratio, has an insufficient sample volume to generate a movable droplet. It is also shown that with varying the gap height one can control the volume of the dispensed droplets from a reservoir on a chip with a fixed electrode size. The VGSA mechanism can facilitate splitting of a droplet. For this purpose, first the gap height is increased to position the droplet at the centre of the electrode accurately, and then the top plate is lowered to reach a gap height that makes the splitting process possible at a given voltage. VGSA can also be used to mobilize the residual droplets that have reduced to immovable droplets as a result of splitting or partial evaporation.

It is worth to add that the VGSA allows for adjustment of the gap height (and hence the aspect ratio) if there was a certain optimum gap height that could optimize all of the DMF operations. However, the optimum gap height that should be used differs from one operation to another. In essence, a certain gap height may be favourable to one operation but can hinder successful, efficient and reliable completion of other operations. Hence, the proposed protocol for real-life applications is to use the VGSA to set and reset the most suitable gap height associated with a single ongoing operation, or the most critical operation (e.g., splitting) among several parallel operations.
Finally, the experimental setup used in this research is for proof of concept and may not be a practical solution, especially for portable devices. Naturally, the integration of a micro-actuator into a handheld digital microfluidic device is an interesting next step for this work. Considering that the positioning of the top plate is an integral part of a feedback controlled micro-actuator, this chapter also reported the successful use of capacitance measurement for estimating the gap height in this chapter.
Chapter 4  Fabrication of digital microfluidic devices on flexible paper-based and rigid substrates via screen printing

4.1  Overview on screen printing for DMF devices

In this work, a new fabrication method is presented for digital microfluidic (DMF) devices in which the electrodes are generated using screen printing technique. This method is applicable to both rigid and flexible substrates. The proposed screen printing approach, as a batch printing technique, is advantageous to the widely reported DMF fabrication methods in terms of fabrication time, cost and capability of mass production. Screen printing provides an effective mean for printing different types of conductive materials on a variety of substrates. Specifically, screen printing of conductive silver and carbon based inks is performed on paper, glass and wax paper. As a result, the fabricated DMF devices are characterized by being flexible, disposable and incinerable. Hence, the main advantage of screen printing carbon based inks on paper substrates is more pronounced for point-of-care applications that require a large number of low cost DMF chips, and laboratory setups that lack sophisticated microfabrication facilities. The resolution of the printed DMF electrodes generated by this technique is examined for proof of concept using manual screen printing, but higher resolution screens and automated machines are available off-the-shelf, if needed. Another contribution of this research is the improved actuation techniques that facilitate droplet transport in electrode configurations with relatively large electrode spacing to alleviate the disadvantage of lower resolution screens. Thus, the cost of fabrication can be reduced significantly without compromising the DMF performance. The paper-based devices have already shown to be effective in continuous microfluidics domain, so the investigation of their applicability in DMF systems is worthwhile. With this in mind, successful integration of a paper-based microchannel with paper-based digital microfluidic chip is demonstrated in this work.

Here, screen printing is introduced for fabricating digital microfluidic electrodes for printing diverse conductive materials on different kinds of substrates. The DMF electrodes are fabricated by screen printing conductive ink on rigid and flexible substrates, for instance, glass and paper substrates. Screen printing is a preferred technique in arts and industry because of its low cost and mass production capability. It is one of the printing techniques
mainly used for commercial printing such as printing patterns on clothes and mass production of posters. For scientific purposes, screen printing is used in fabricating paper-based sensors for electrochemical detection (Dungchai, Chailapakul, & Henry, 2009). Screen printing has also been used for producing electrical conductive textiles (Kazani, Hertleer, Mey, Schwarz, & Guxho, 2012).

Our goal is to assess the possibility of a mass production printing technique used in the printed electronic industry in fabrication of DMF chips. Screen printing, inkjet printing, flexo printing and offset-gravure printing are commonly used in batch wise production and roll-to-roll processing (Suganuma, 2014). Some of these techniques need high initial cost investment and a well-equipped facility. Similarly, screen printing can be performed on a large scale using automated equipment. However, screen printing can also be performed manually on a small scale with lower cost equipment.

Thus, the advantages of screen printing compared to the reported DMF fabrication techniques can be summarized as follows:

- The fabrication procedure is simple and cost effective as it requires low cost fabrication facilities that are readily available. More precisely, screen printing can combine the simplicity and precision at the same time without using any specific automated or even electrically powered equipment.
- Screen printing is one of the techniques that are well known for their mass production capability.
- It is also characterized by reduced production time of DMF electrodes compared to the current DMF fabrication techniques as large number of electrodes can be printed instantaneously.
- This technique can be used for printing different conductive materials for fabricating the DMF electrodes.
- Furthermore, it can generate the DMF electrodes on various kinds of flexible and rigid substrates. Paper substrates are considered as one of the promising substrates to be used as demonstrated later in this work.

DMF systems can be categorized as open and closed systems. The droplet volume utilized in the open system is usually higher than the closed system. The droplet volume
range can vary from 3 µL to 17 µL (M Abdelgawad et al., 2009; A. N. Banerjee, Qian, & Joo, 2011). On the other hand, the droplet volume in the closed system can be as small as several picoliters (Welch, Lin, Madison, & Fair, 2011).

Finally, methods of integrating paper based DMF to continuous paper microchannel are investigated in order to combine the applications that have been performed on these two microfluidic systems on one simple and cost effective platform. Micro paper analytical devices (µPAD) provide a low cost platform for several chemical applications (Carrilho, Martinez, & Whitesides, 2009). Paper based substrates can be easily perforated, cut and shaped to the required pattern. Moreover, the light weight, flexibility and white color of the paper promote its usage in many applications such as colorimetric assays (Dungchai, Chailapakul, & Henry, 2010). Other devices developed to perform diagnostic functions include ELISA on paper and detecting glucose from urine (Cheng et al., 2010; Lankelma, Nie, Carrilho, & Whitesides, 2012). Generally, paper-based devices are termed as “Zero Cost Diagnosis Devices” in the scientific community as they are more affordable and easier to fabricate on a larger scale.

Integrating digital microfluidics into paper platforms can be done by several techniques as different methods are available for printing conductive inks on paper substrates (Russo et al., 2011; Siegel et al., 2010; Windmiller et al., 2012; Wood, Hrehorova, & Joyce, 2005). Microchannels patterning on paper can be formed by wax channels and photoresist channels (Carrilho et al., 2009; Martinez, Phillips, Butte, & Whitesides, 2007). Complete hydrophobic barriers fabricated by the previous methods can be used to make reaction chamber, channels or reservoirs. Micro channel patterns can be drawn on the paper beforehand using a printer, and can later be painted by wax pen to get a more precise geometry (Lu, Shi, Jiang, Qin, & Lin, 2009). 3D channels can also be constructed on several paper layers (Martinez, Phillips, & Whitesides, 2008). Laser engraving and wax dipping can be used to generate microchannel on wax paper (Songjaroen, Dungchai, Chailapakul, & Laiwattanapaisal, 2011). In addition, screen printing can also be used to generate the wax hydrophobic microchannels (Dungchai, Chailapakul, & Henry, 2011).
As the fluid wicks through paper, it eliminates the need to pump the fluid inside the system. This property further reduces the cost of these devices. The successful integration between this platform and the DMF platform is going to be demonstrated later in this work.

![Diagram of screen mesh preparation steps](image)

**Figure 4.1** The preparation steps required for patterning the electrodes on the screen mesh are demonstrated from a) to e). The screen printing process where the ink is deposited on the substrate is demonstrated in steps g) and h).

### 4.2 Methods/Experimental section

#### 4.2.1 Screen printing

Figure 4.1 demonstrates the steps required for patterning the electrodes design on the screen. First, a blank stainless steel screen is stretched on a surrounding frame with a defined tension. Next, the emulsion is deposited on the screen to cover it completely, and the screen is left to dry. A transparent mask is designed and printed according to the electrode patterns. Afterwards, the mask is fixed on the emulsion side of the screen and the screen is exposed to ultraviolet light. Finally, the screen is water sprayed to remove the unexposed emulsion. The removed emulsion leaves uncovered pores where the ink can pass through them. In order to deposit the screen printing ink through the mesh, a complete contact should occur between
the screen and the substrate where the ink is deposited. This is done when the squeegee pushes the screen directly on the substrate, and the emulsion blocks the excess ink. The emulsion exposure process required for processing the patterns on the screen can be done by several techniques. Some emulsions require ultraviolet light to be fully exposed, and other emulsions can be exposed using the sunlight only. The emulsion deposition on the screen with the required thickness and the exposure process has been done by Sefar Inc., Buffalo, NY. Further screen printing instructions and procedures can be found in (Dubey, 1974; Faine, 1991). The total time required for preparing the patterns on the screen is approximately 20 minutes. At this stage, the screen printing process can start generating large number of DMF chips instantaneously once the screen is fabricated according to the previous steps.

Three conductive screen printable inks were tested. The first ink is silver based ink Dupont® 5025 (Dupont®, Wilmington, Delaware). The second ink is carbon based ink Dupont® 7102 and the third ink is Bare conductive® ink (Bare conductive®, London, UK). The screens are fabricated either from polyester or stainless steel. The polyester screens are flexible and resilient but they are more prone to absorb moisture and swelling. The stainless steel screens are stronger and can handle more abrasion with reduced swelling which can help in increasing the lifetime (Groza, Shackelford, Lavernia, & Powers, 2010). In addition, several screen printing parameters mentioned above can affect the lifetime of the screen. Reducing the mechanical stress by decreasing the screen tension, and increasing the off-contact distance can increase the screen lifetime (Horvath, Harsanyi, Henap, & Torok, 2012). The screens used in this work are stainless steel screens stretched on a 12”×12” (304.8 mm×304.8 mm) cast aluminum frame. The mesh count is 400 wires/inch with mesh opening of 0.0018 inch (45.72 µm) and open area of 51 %. The mesh angle used is 22° and wires of diameter of 0.0007” (17.78 µm) are used with weave thickness of 0.001” (25.4 µm). The screen tension used is 21 N/cm. The emulsion type used is E-80 and the emulsion thickness used is 10 µm. The off-contact distance, which is the adjusted gap between the screen and the substrate, is kept at 3 mm. Calendared mesh wires were chosen to decrease the thickness of the deposited ink during printing the electrode. The theoretical wet printed thickness can be calculated by the following equation:
where $T_w$ is the wet printed ink thickness, $T_e$ is the emulsion thickness, $T_m$ is the weave thickness $A_o$ is the percentage of open area. The previous screen printing parameters are kept the same for all the inks and substrates used during the printing process. After the printing process, the silver based ink required curing at 120 C° for 5 minutes and the other two carbon based inks are left for air drying at room temperature. If the printing process stops and the ink moisture evaporates, the ink can dry out and block the screen pores especially with patterns of critical dimensions. This effect can be minimized by supplying fresh ink continuously to avoid the drying out of the ink on the screen. The automated screen printing machines are able to account for this effect during batch printing. Furthermore, the use of water soluble inks that can be easily washed will facilitate resorting the screens even if the screens are blocked by the dried ink. The silver based ink Dupont® 5025 and the carbon based ink Dupont® 7102 are not water soluble as they required specific chemical solvents for cleaning the screens after the printing process. Based on our observations, the dried ink and the residue generated from the previous two inks cannot be totally removed and cleaned from the screens even after using their specific chemical solvent. On the other hand, the Bare conductive® ink was easier to clean from the screens as it is water soluble and does not require any specific chemicals during removal. Therefore, the configuration of Bare conductive ink printed on a paper substrate is preferred and used in all the experimental DMF tests demonstrated in this work.

4.2.2 Digital microfluidics fabrication

After printing the electrodes on the substrate, adding a dielectric layer and hydrophobic layer is required to functionalize the electrodes for performing DMF operations. Parafilm is used as an interchangeable sacrificial dielectric layer that can be easily applied and replaced on all the substrates (Yang, Luk, Abdelgawad, Barbulovic-Nad, & Wheeler, 2008). The stretching steps of the Parafilm layer are shown in Figure 4.2. The Parafilm layer has an original thickness of 120 µm before stretching. A rectangle of 5 mm × 2 mm was stretched in the horizontal direction along the 5 mm side gradually to 20 mm × 2 mm. The stretched piece was cut to four pieces as it is shown in Figure 4.4 (c). The thickness of the dielectric layer can be controlled by additional stretching along the 2 mm side. Next, the
Parafilm layer was pressed gently on the top of the chip. Accordingly, the previous steps can introduce a dielectric layer that can be easily applied to the system without using any additional equipment. This simple stretching is subject to some variability if it is performed manually. Typically, the application of the dielectric layer in the existing techniques is through a chemical vapour depositing system for depositing Parylene-C. However, the use of the Parafilm alternative has enabled droplet motion without using other dielectric materials that require additional time consuming processes and expensive equipment. The sequence of the previous DMF fabrication steps is the same for all the substrates except one additional step while using wax paper. Before applying the Parafilm layer, the wax paper was fixed on a rigid substrate in order to avoid the wrinkling that may occur to the substrate. Lastly, a water repellent (Rain-X®) was used to increase the contact angle of the droplet on the surface.

A function generator (AFG3021B, Tektronix) connected with a high voltage amplifier (PZD700A, Trek) were used to generate the high voltage signals required for actuation. The chip is inserted to a card edge connector (3-5147490-0, TE connectivity) that is attached to a microcontroller for routing the droplets (Arduino Mega 2560) and solid state relays were used for high voltage switching.

4.2.3 Paper microchannels fabrication

The paper microchannels used in this work are generated on filter paper using wax as a hydrophobic barrier. The paper used in this microchannel is Whatman™ 1 filter paper. The wax patterns are printed using Xerox® ColorQube™ 9201. Lastly, the printed microchannel patterns are heated on a hot plate at 150°C for 120 seconds.

4.3 Results and Discussions

This section summarizes all the tests that were performed to assess the quality of the fabricated chips and determine if the fabricated chips are capable of performing successful DMF operations.
4.3.1 Screen printing

Printing the electrodes with clearly defined spacing is important to avoid the malfunction of the DMF system. Therefore, the quality of the printed electrodes fabricated by this method is examined using SEM images. In the following experiments, the same printing procedures and parameters mentioned earlier was followed with all the inks and substrates used. The thickness of the printed electrode remains nearly constant when different inks are printed on different types of substrates as shown in Figure 4.2 e), f), g) and h). According to the parameters mentioned in eqn. 1, the theoretical wet printed thickness of the electrode is 22 µm. This thickness decreases after the moisture content of the ink evaporates. The actual measurements by the SEM images in Figure 4.2 demonstrates that the final ink thickness is around 10 µm.
Figure 4.3. Details of the output resolution of DMF electrodes produced by screen printing using a 400 wires/inch mesh size. Variable connection line widths (from 50 µm to 400 µm) are printed with variable electrode spacings (from 50 µm to 300 µm).

The electrode spacing has been examined from the top view by SEM images to determine the effect of the ink spreading on the substrates used. A 300 µm electrode spacing was patterned on the screen mesh and the actual spacing between the printed electrodes was examined when carbon is printed on normal paper and wax paper as shown in Figure 4.2 (a) and (b), respectively. We can notice that the electrode spacing decreases from the nominal 300 µm spacing to 275 µm on the normal paper as the ink spreads on a larger surface area because of the paper hydrophilicity and absorptivity. On the other hand, the ink tends to shrink, and the spacing increases to 338 µm when carbon ink is printed on wax paper. This increase occurs because the wax paper surface is hydrophobic and moisture resistant. The surface of the connection lines has been also examined by SEM to insure the connection reliability. Figure 4.2 (c) and (d) depict the continuity of the connection lines when carbon is printed on wax paper and when silver is printed on normal paper, respectively.
Figure 4.4 Screen printed electrodes are demonstrated on a) paper substrate, b) glass substrate and c). Wax paper substrate, d) several DMF chips printed on a paper substrate. e) Dielectric layer (Parafilm) stretching steps. f) A droplet sitting on DMF chip printed on wax paper substrate where the wax paper is kept flat by fixing it on top of a glass substrate with silver ink and parafilm dielectric layer.

The output resolution of the electrodes generated can be observed in Figure 4.3. The figure shows the contrast and the clarity of the printed connection lines with thicknesses of 50 µm till 400 µm. The 50 µm printed connection line width was not consistent and showed
discontinuities. Lines with thickness of 100 µm showed better consistency and lines with thickness of 200 µm are recommended for increasing the connection reliability. Next, the resolution of the line spacing between the patterned electrodes was tested. The line spacing between the electrodes is clearly defined when it is greater than 150 µm. However, small sparks were observed when the line spacing was reduced to 150 µm. These sparks occurred when high voltages are applied (600 V in open system configuration). Therefore, the paper becomes more prone to breakdown when the spacing decreases as small scattered dots of ink were observed between the electrodes. To avoid this problem, it is recommended either to apply a thinner and stronger dielectric layer or increase the line spacing between the electrodes. However, applying thinner and stronger dielectric layer such as Parylene-C requires chemical vapour deposition system that may increase the cost and the complexity of the fabrication process. More reliable operation at high voltages without any sparks was observed when the electrode spacing was increased to 200 µm. Figure 4.3 shows the resolutions that can be achieved using manual screen printing to meet the goal of fabricating the DMF electrodes with readily available tools without using precise automated screen printing machines and sophisticated equipment. The achieved resolution is adequate for performing digital microfluidic operations, as it is shown later in this work. It is worth to note that more precise patterns with high resolutions can be generated using screen printing starting from 6 µm (“Kuroda Electric (http://www.kuroda-electric.eu/ultra-fine-pattern-screen-printing),” 2014), 50 µm (Robertson, Shipton, & Gray, 1999) and 100 µm (Parashkov, Becker, Riedl, Johannes, & Kowalsky, 2005). However, the initial cost of the screen printing mesh increases for higher resolutions. In order to assess the resolution of the screen printed electrodes used in performing DMF operations, it has to be compared with the resolution reported by the current DMF fabrication techniques. The printing resolution of the photolithographic techniques can start from few microns to reach the submicron scale (Welch et al., 2011). The micro contact printing resolution ranges from 50 to 70 µm (Watson et al., 2006) while the toner printing technique can reach 200 µm (M. Abdelgawad & Wheeler, 2007). The Piezo inkjet printing resolution can reach 30 µm, but it is more reliable when it is increased to 60-90 µm (Fobel et al., 2014), and the desktop ink jet printing can reach 500 µm (Ko et al., 2014). While the screen printing resolution is comparable to the existing
techniques, the main advantage of screen printing would be more pronounced when the cost and time of fabrication is also taken into account.

Generating the electrodes on one chip can take several hours using the conventional photolithographic techniques. Reduced fabrication time can be achieved using rapid prototyping techniques. Micro contact printing reduced the fabrication time to 150 min per 6 chips (25 min/chip) (Watson et al., 2006). In addition, Piezo inkjet printing can generate one chip in one minute approximately (Fobel et al., 2014), and a desktop laser printer can generate 80 chips of size 2 cm × 2 cm in less than 10 min (M. Abdelgawad & Wheeler, 2007) (7.5 second/chip of 2 cm × 2 cm size). Screen printing speed can vary from 6 in/sec (152.4 mm/s) to 12 in/sec (300 mm/s) [39]. Therefore, the screen printing average speed can generate a sheet of 180 mm × 150 mm size with 9 designs of size 5 cm × 4 cm shown in Figure 4.4 (d) in less than 2 seconds. These benchmark numbers reveal that screen printing method is better in terms of fabrication time (less than 0.22 second/chip of 5 cm × 4 cm size). All the electrodes in Figure 4.4 d) are printed instantaneously when the squeegee passes on the screen and this demonstrates the mass production capability once the screen is ready for printing.

In order to compare the total fabrication time for the DMF chip, the additional processing time required before and after the printing process should be considered. The fabrication time is mainly distributed among the following three processes: pre-printing preparation process, actual printing process and post-printing preparation steps required to enable DMF operations on the chip. The post-printing steps includes depositing a dielectric layer and a hydrophobic layer on top of the printed substrate. The time required for adding these two layers is the same for any fabrication technique. Some techniques require time consuming pre-printing preparation processes to print just one chip such as photolithography. Screen printing requires pre-printing preparation processes for patterning the screen mesh before being able to print large number of chips. On the other hand, other techniques do not require any pre-printing preparation steps such as Inkjet printing. However, the inkjet printing process itself can be time consuming compared to the batch printing techniques. Afterwards, the ability of performing screen printing on different substrates was tested. Figure 4.4 demonstrates how the silver ink Dupont® 5025 is printed on a paper substrate,
glass substrate and wax paper substrate using the same printing parameters mentioned earlier. Wax paper was tested as it is very flexible, has a smoother surface than the normal paper and has higher moisture resistance compared to the normal paper. Figure 4.4 f) demonstrates how the wax paper is fixed to a glass substrate in order to apply the Parafilm layer and avoid wrinkling of the substrate. Figure 4.4 d) demonstrates also the ability of the screen printing technique to produce large number of screen printed electrodes.

To sum up, the previous comparisons demonstrate that some techniques (e.g., Photolithography) can generate high resolution patterns, but they can be sophisticated and more time consuming with low throughput. Other rapid prototyping techniques are less time consuming and can generate higher number of DMF chips (e.g., Inkjet printing). Furthermore, this work presents screen printing as one of the batch printing techniques that can offer higher throughput with adequate resolution for performing DMF processes.

4.3.2 Digital microfluidics operations

![Figure 4.5 Contact angle change versus the applied voltage.](image)

The performance of DMF systems mainly depends on the ability to manipulate the contact angle. The stronger the EWOD forces, the larger the change in the contact angle. Therefore, an electrowetting test has been performed to characterize the change in the contact
angle due to the applied voltage. This change can be expressed by Lippman Young equation (Mugele & Baret, 2005):

\[
\cos \theta_v = \cos \theta_0 + \frac{\varepsilon_d \varepsilon_0 V^2}{2 \gamma_{lg} d} \tag{4.2}
\]

Where \( \theta_v \) is the contact angle when voltage \( V \) is applied, \( \theta_0 \) is the contact angle at zero voltage, \( \varepsilon_d \) is the dielectric constant of the dielectric layer and \( \varepsilon_0 \) is the vacuum permittivity, \( d \) is the insulator thickness and \( \gamma_{lg} \) is the liquid gas interfacial surface tension. Electrowetting test has been performed to examine the capability of the screen printed electrodes with the Parafilm dielectric layer and Rain X, sprayed on to top to act as a hydrophobic layer, in modifying the droplet contact angle among different voltages. Figure 4.5 demonstrates the decrease in the contact angle when higher voltages are applied. The decrease in the contact angle continued until contact angle saturation occurred at 53° and applied voltage of 330 \( V_{rms} \). The wire inserted in the middle of the droplet will generate small error in the droplet contact angle measurement where the droplet contact will be different between the right and the left-hand side.

![Figure 4.6 a) Variation of the silver ink resistivity when it is printed on different substrates. b) Variation of the carbon based inks resistivity when they are printed on paper.](image)
The parafilm layer was successfully used in moving DI water droplets. Several testing need to be conducted to evaluate its performance when using other conductive and non-conductive droplets. Figure 4.6 demonstrates the change in the resistance of the three inks used in this work. The resistance of each ink was tested on 10 samples of rectangular electrodes with 15 mm × 1.6 mm size. Afterwards, the above dimensions and the printed ink thickness were used in order to calculate the resistivity of the printed inks regardless of any geometry used. Figure 4.6 shows that the resistance of the silver based ink is the lowest compared to the 7102 carbon based ink and the Bare conductive® ink. Figure 4.6 (a) also shows that the 5025 silver ink has the lowest resistance when it is printed on a glass substrate compared to the paper and the wax paper substrates. The printed silver on glass shows the lowest resistance variability and better consistency according to the error bars in Figure 4.6 (a). Figure 4.6 (b) shows that the resistance of the Bare conductive ink is less than the carbon ink with lower variability. We tried and analysed the performance of different screen printable conductive inks but eventually used the water soluble ink that is easily washable, Bare conductive, in all of our DMF related experiments. The DMF chip layers used in all the coming DMF experiments are constructed as follows (from the bottom to the top): Paper based substrate, Bare conductive ink electrodes, Parafilm dielectric layer and Rain-X hydrophobic layer.

Figure 4.7 The effect of changing the frequency on the droplet motion of a 10 µL droplet. a) Large amplitude oscillations were noticed at 10 Hz, b) droplet oscillations are reduced and noticeable spreading
of the droplet can be observed at 1 kHz. For a 20 µL droplet Vibrations can be noticed at 10 Hz in c) and droplet spreading toward the activated electrode without vibrations can be noticed in d) at 1 kHz.

The droplet transport process is considered one of the main operations that can be performed on the digital microfluidic platform. In this work, two parallel electrode arrays were designed to work in an open digital microfluidic system configuration. One of the two electrode arrays is for grounding and the other is for applying the high voltage needed in the actuation process (Mohamed Abdelgawad et al., 2008). A square wave AC signal of 600 Vpp (300 Vrms) was used. Several tests were performed to examine the droplet motion on this platform. In the first test, the electrical signal frequency was varied to investigate the frequency effect on the droplet motion in an open DMF system. The frequency was varied from 10 Hz to 10 kHz. Figure 4.7 demonstrates the droplet motion tests that have been performed on two droplets with volumes of 10 µL and 20 µL. The 10 µl droplet exhibited large amount of oscillation when the frequency was 10 Hz as shown in Figure 4.7 (a) and Figure 4.8. This oscillation occurred because of the nature of the applied square wave that kept the voltage level of the activated electrode oscillating between +600 V and -600 V. Meanwhile, the grounded electrode remained at 0 V. Therefore, the droplet experienced higher voltage toward the activated electrode during the first half of the cycle and higher voltage level toward the grounded electrode during the other half of the cycle. The degree of the oscillation decreased when the frequency increased to 100 Hz and turned to just a noticeable vibration on the droplet meniscus as shown in Figure 4.8. Steady motion toward the activated electrode was observed without any oscillations or vibrations when the frequency was increased to 1 kHz and the droplet motion was impeded at the end of the activated electrode. The oscillating motion that was observed for the 10 µL droplet at 10 Hz helped the droplet to overlap with neighbouring electrodes. Accordingly, oscillating the droplet at very low frequency (10 Hz) can facilitate the sequential motion of the droplets when there is relatively large spacing between the electrodes.

In the second test, the droplet volume was increased to 20 µL and the frequency was varied to investigate the droplet behaviour on the same electrode size (2 mm × 2mm). At 10 Hz, small oscillations were observed during the motion in Figure 4.7 (c). These oscillations have lower magnitude than the oscillations that were observed in the first experiment of the
10 µL droplet at 10 Hz. The increased droplet diameter and footprint of the 20 µL droplet had a great effect on limiting the droplet oscillation as both tests were performed on the same 2 mm electrode size. Lower amplitude oscillations were noticed when the frequency was increased to 100 Hz as shown in Figure 4.8. A video showing how the droplet reacts when different frequencies are applied can be found in the supplementary data of the following paper (Mohamed; Yafia et al., 2015). This video shows how the droplet spreads when high frequencies are applied (>1 kHz) and shows also the difference between large and small amplitude oscillations at low frequencies (<1 kHz).

Figure 4.8 The amplitude of the droplet oscillation for a 10 µL and 20 µL droplet at 10 Hz, 100Hz and 1 kHz.
In the previous tests, the droplet may not overlap with the neighbouring electrodes and this may hinder the consecutive motion of the droplets. Therefore, another test has been performed to examine the effect of separating the grounded electrodes instead of grounding all of them during the operation. Three tests were performed where the grounding configuration of the upper electrodes row is varied while keeping the same middle electrode in the bottom row activated. Figure 4.9 a) shows that the droplet overlap with neighbouring electrode on the left hand side if the top left electrode is grounded and the right one was kept floating. The droplet moved to the opposite direction when the top left electrode was kept floating and the top right electrode was grounded. Therefore, this test demonstrates that the droplet overlapping position can be controlled by changing the grounding configuration where the droplet can lean toward the left hand side, the right hand side according to the grounding and the floating electrodes configuration. This can help in achieving more reliable motion if it is used to adjust the droplet position and increase the droplet overlapping with the neighbouring electrode on the left or the right hand side.

![Figure 4.9 The effect of changing the grounding configuration on the droplet oscillation.](image)

### 4.4 Integration with Paper Microchannels

Several tests were performed to investigate the appropriate method of connecting continuous paper microchannels with DMF systems. The integration of continuous paper microchannel to screen printed paper based DMF platform is demonstrated in Figure 4.10. Microchannels of different widths and designs were generated to determine the integration effectiveness. The electrode beneath the entrance of the channel should be activated in order to move the droplet to the entrance of the microchannel. When the droplet reaches the entrance it wicks with the exposed part of the paper and the flow is initiated inside the channel as shown in Figure 4.10 (a.2), (b.2) and (c.2). The velocity of the flow inside the 250 µm channel was measured to be 0.27 mm/s in Figure 4.10 (a.3). The flow inside this
microchannel kept decelerating and stopped before reaching the end of the channel because the small flow rate could not compensate the loss in the flow due to evaporation. The small velocity and limited flow inside the microchannel was improved by increasing the width of the entrance of the channel to 1.5mm as shown in Figure 4.10 (b.1). The velocity of the flow is then measured starting from the flow position at Figure 4.10 (b.2). The increase in the width of the entrance of the channel to 1.5 mm has increased the velocity to 1.28 mm/s. Additional test was done to assess the effect of increasing the channel width on the flow velocity and the flow rate. The channel width was increased to 600 micron as shown in Figure 4.10 (c.3). Consequently, the velocity has increased to 9.4 mm/s. The integration between the screen printed paper-based digital microfluidic platform and the paper microchannels will produce a hybrid paper based system that has a great potential in many applications and can be easily fabricated and mass produced with low cost.

Figure 4.10 Different configurations of continuous microchannel integrated to DMF platform a) A continuous microchannel of 250 µm width with the same thickness at the entrance, b) A continuous microchannel of 250 µm width with wider entrance c) A continuous microchannel of 600 µm width.
4.5 Summary

In this chapter, a new technique for the fabrication of digital microfluidic electrodes using screen printing on both rigid and flexible substrates has been demonstrated. This work promotes screen printing, as one of the common batch printing methods, for fabricating the DMF chips. The proposed method outperforms the reported DMF rapid prototyping techniques in terms of fabrication steps, fabrication materials, fabrication time, fabrication cost, equipment availability and mass production capability. DMF electrodes were screen printed directly on paper, glass and wax paper substrates. Fabricating the DMF chips on paper based substrates by screen printing provides us with a promising tool to fabricate DMF devices that are disposable and flexible. Different electrode spacings and connection line thicknesses were examined to assess the output resolution of this printing technique when it is used for printing DMF chips. A Parafilm dielectric layer was tested because of its low cost and simplicity, which is in line with our general desire of fabricating DMF devices easily. An electrowetting test has been performed to investigate the change in the contact angle that occurs when the voltage is applied to the screen printed platform. Improved actuation methods were introduced to facilitate the droplet motion when the electrode spacing is relatively large. Screen printed DMF electrodes allowed the integration between the DMF system and paper analytical devices (µPADs) on an all paper-based system easily. Three different continuous microchannels with different designs were tested. Increasing both the entrance thickness and the microchannel width has increased the flow rate significantly. Successful droplet transport on this combined platform has been demonstrated in this work. Therefore, low-cost paper-based micro channels can also be used at the detection stage followed by high precision DMF operations in the same device.
Chapter 5  Low-cost graphene-based digital microfluidic system for single nucleotide mismatch discrimination

5.1  Laser scribed graphene DMF chips (LSG DMF chips)

In this work, the laser scribing technique is introduced as a low-cost, rapid and facile method for fabricating digital microfluidic (DMF) systems. Laser scribed graphene (LSG) electrodes are directly synthesized on flexible substrates to pattern the electrode arrays. Graphene is well known for its outstanding properties, and now the laser scribing technique is used for its low cost and versatility in creating different graphene patterns. The proposed method significantly facilitates the DMF electrode fabrication process by eliminating many steps of microfabrication as the electrodes are printed directly from the computer design to the chip. Specifically, this will eliminate the need for photolithography mask, etching equipment and a cleanroom. The fabricated LSG DMF chips are tested in both open and closed DMF systems where there is a great difference in the configuration of the electrodes and the voltage requirements. An electrowetting test has been performed to investigate the effectiveness of the laser-scribed graphene DMF electrodes in changing the contact angle of droplets. Different DMF operations are successfully performed using the proposed LSG DMF chips. Several tests are performed to assess the quality and output resolution of the proposed electrode fabrication technique and to determine the performance of such patterned electrodes in the DMF systems. To verify the efficacy of the proposed fabrication method, a one-step direct assay for detection of *L. pneumophila* DNA on an LSG chip is demonstrated without the need for any washing step. We were able to demonstrate high specificity of distinguishing single nucleotide mismatch and to detect as low as 1 nM target DNA concentrations. The proposed rapid and easy to manufacture LSG DMF chips are particularly suitable for portable one-step and highly specific for detecting one mismatch.

Introducing rapid prototyping techniques have increased the number of published papers in the area of droplet-based microfluidics (Chou, Lee, Yang, Huang, & Lin, 2015). Consequently, several attempts have been initiated to fabricate the DMF platform at low cost without using highly equipped facilities (Mohamed Abdelgawad & Wheeler, 2007; Fobel et al., 2014; Ko et al., 2014; Watson et al., 2006; Mohamed; Yafia et al., 2015). Photolithography is the most conventional fabrication technique for the DMF systems.
Although, this technique is able to generate very high-resolution features, it requires multiple step fabrication process and well-trained individuals as well as sophisticated equipment and facilities. Conversely, rapid prototyping techniques provide the opportunity to fabricate DMF chips easily with modest equipment and in a more cost effective manner. Some of these techniques provide the ability to print the DMF actuation electrodes on flexible substrates, modify easily the electrodes patterns and reprint them. This ability to reconfigure the electrode design is barely achievable with the most conventional techniques. For example, screen printing is a rapid prototyping technique that is suitable for low cost and mass production of DMF chips, but it requires several steps to prepare and pattern the electrode (Mohamed; Yafia et al., 2015).

In this work, a straightforward fabrication method is introduced where the electrodes are printed directly from a CAD drawing in the computer into the DMF chip in a single step using Graphene. Graphene is one of the promising materials that has shown great potential for many different applications (Novoselov et al., 2004). It is characterized by having outstanding crystal properties with a two-dimensional sheet structure that is only one atom thick (Geim & Novoselov, 2007). However, graphene generation is not a simple process (Novoselov et al., 2012). Reducing graphene oxide (GO) is one of the techniques capable of producing reduced graphene oxide (rGO) layers (Pei & Cheng, 2012). The methods used to reduce GO to graphene utilized photochemical, photo-thermal or laser reduction of GO where laser, infrared, ultraviolet and flash light sources were used (Cote, Cruz-Silva, & Huang, 2009; Zhang et al., 2014). Unfortunately, these techniques were not able to pattern and reconfigure the electrode design easily and accurately. For this reason, laser scribing technique has been introduced to reduce graphene to graphene oxide with readily available and simple equipment (Strong et al., 2012). The conductivity of the GO layer before the laser scribing process varies from $8.07 \times 10^{-4}$ S/m to $5.42 \times 10^{-3}$ S/m. This conductivity increases significantly when GO is reduced to graphene and it becomes $2.35 \times 10^{3}$ S/m (El-Kady & Kaner, 2013). Recently, many applications that require graphene are taking advantage of the laser scribing technique. One of the highest power densities for micro super capacitors has been introduced using the laser scribing technique with power density of 20 W/cm$^3$ (El-Kady, Strong, Dubin, & Kaner, 2012). In addition, graphene based electronic devices, such
as in-plane transistors, photodetectors, loudspeakers and flexible strain sensors have been fabricated using the laser scribing technique (Tian, Shu, et al., 2014; Tian, Yang, et al., 2014).

Electrowetting phenomena has been discovered by G. Lippmann in 1875 (Lippmann, 1875). Droplet manipulation and actuation has been later demonstrated using electrowetting-on-dielectric (EWOD) by adding a dielectric layer and a hydrophobic layer on top of the electrodes, respectively (Moon, Cho, Garrell, & Kim, 2002). Electrowetting-on-dielectric experiments have been performed on graphene sheets fabricated by chemical vapor depositing technique (X. Tan, Zhou, & Cheng, 2012). The graphene layers were then transferred to glass and PET substrates using advanced and sophisticated fabrication procedures. The authors performed an EWOD test without performing DMF operations and noticed that graphene exhibited higher capacitive impedance compared to gold. Their experiments showed that using graphene led to fewer defects and pinholes, reduced electrolysis and lower leakage current. Digital microfluidic systems are characterized by having discrete electrodes where EWOD is utilized to perform several operations on droplets in the micro-scale. Droplet transport, mixing, merging and dispensing have been demonstrated on the DMF platform (S. Cho et al., 2002; S. K. Cho et al., 2003). DMF systems can be classified under two main categories: open and closed systems. In the open system, the droplet sits on a single plate where both the ground and the activated electrodes are located (Cooney, Chen, Emerling, Nadim, & Sterling, 2006; U.-C. Yi & Kim, 2006). On the contrary, the droplet can be sandwiched between two plates in the closed system (Fair et al., 2001; Hong Ren et al., 2002). Several dielectric layers and hydrophobic layers can be added to the top and the bottom plates to functionalize the droplet motion in the DMF systems. The difference in the construction of these two systems can be observed in Figure 5.1.
Figure 5.1 Single plate (open) digital microfluidic system, on the left side, versus parallel plate (closed) digital microfluidic system, on the right side. Middle Left: Two fabricated open system DMF chips printed with a long electrode acting as a common ground and five discrete electrodes for droplet manipulation. Middle right: A closed system with 12 electrodes and four reservoirs. Bottom: The steps of the stretching process of the designed molecular probe during the process of the DNA mismatch detection.

Each of these systems has several advantages and disadvantages. Open systems are characterized by having easy access to the droplet, having higher droplet velocities and mixing rates. On the other hand, closed systems are characterized by having more controlled evaporation as the droplets are sandwiched between two plates where a filler medium can be
used to minimize the evaporation. High voltage electrical signals are usually used to transport the droplets on the electrode array. Open systems require higher voltages during operation (500-700 Volts) (M Abdelgawad et al., 2009; Mohamed Abdelgawad et al., 2008) compared to the closed systems (25-200 Volts) (Lin, Evans, & Welch, 2010). The graphene based DMF chips presented here are going to be able operate in these two configurations as it will be demonstrated later in this work.

Water-born disease outbreaks are a major concern in developed countries. *Legionella* is responsible for one third of these outbreaks. *Legionella* is found in almost all natural and man-made water systems such as showers, cooling towers, hot tub and air conditioners. Inhalation of the water aerosols containing these bacteria causes an acute form of pneumonia. Legionelosis outbreaks are associated with 15-20% mortality rates, which can even lead to up to 50%. To date, more than 52 species of *Legionella* have been identified and only half of these species are associated with human disease. Since in some cases there is only one or two nucleotides difference in their DNA sequence, developing a highly specific detection method capable of distinguishing single nucleotide mismatch is crucial. Here, a sophisticated molecular probe was designed for this purpose, which consists of a fluorescent and quencher at each end of molecular probe. In the absence of the target DNA, the fluorescent dye and quencher are in proximity of each other which results in no fluorescent signal. When the target DNA hybridizes to this molecular probe, its open and fluorescent dye and quencher are situated at distance from each other. By design of this molecular probe there is no need for a washing step as with normal DNA detector probes to wash away excess of these probes. This is particularly advantage for open DMF setups, in which the detection will happen in a single step by mixing the target DNA and molecular probe and results can be visualized with fluorescent microscopy.

To sum up, this research introduces a new simple method to fabricate graphene based DMF systems. This fabrication method allows us to generate graphene based electrodes directly using a laser scribing DVD burner. As graphene is well known for its outstanding properties, laser scribing technique is used for its low cost and versatility in creating different graphene patterns. The graphene electrodes are directly synthesized on flexible and bendable Polyethylene terephthalate (PET) substrates. This method is characterized by being simple
and cost effective compared to the other complex processes reported to fabricate graphene before. Moreover, this method provides the capability to change the designs and electrode patterns easily unlike the conventional photolithographic technique which requires high resolution masks for each design. This work demonstrates that the laser scribed graphene (LSG) DMF electrodes where used in successful electrowetting tests and for performing various digital microfluidic operations. Here, the detection of single nucleotide mismatch is successfully demonstrated for detection of *Legionella* DNA samples.

### 5.2 Experimental setup and fabrication

The graphene oxide solution is prepared according to Hummer’s method (Jr & Offeman, 1958). Sonication of the solution is performed for 1 hour using Ultrasonic cleaner Branson 3510 (Fisher scientific, Waltham, MA) to ensure the complete dispersion of the graphene oxide particles inside the solution. A 5 mg/ml GO solution is dispensed by a micro pipette to ensure that the concentration of dispensed GO volume per unit area is 0.57 µl/mm². The substrate is left to dry out for 24 hours to have a uniform layer of GO. Lightscribe CDs are different than normal CDs as they have water marks near to the center for accurate positioning of the CD during the laser scribing process (see Figure 5.1). Memorex™ lightscribe CDs are used in our experiments. Two softwares were tested to burn the required patterns on the CD. Nero Express 2014 (Nero AG, Karlsbad, Germany) and Lightscribe template labeler (Hewlett-Packard, Palo Alto, CA). Both softwares successfully generated the patterns required for normal labelling uses on the CD. Testing on a bare laser scribing CD, without attaching the PET with dried GO on top, showed that the 50 µm spacings between the electrodes were not visible and the electrodes were completely connected to each other when the Lightscribe template labeler software. Next, laser scribing the same patterns using Nero Express was performed where the 50 µm electrode spacing became visible and the overall contrast and sharpness has been improved. Moreover, Nero Express had more options to scale the drawings exactly using an on screen scale which gives us the ability to set exactly the outer diameter of the patterns. An additional step that has improved the darkness of the patterns significantly was to change the contrast settings inside the lightscribe control panel from the default factory settings to darker labels with longer
labelling time. A PET sheet of 100 µm thickness (3M) is used as a substrate that is fixed on top of the CD surface. The graphite oxide solution is added on top the PET surface by drop casting then fixed on the top of the CD surface by an adhesive spray (LePage Pres-tite Multi-purpose Spray Adhesive). After that, the CD that holds graphite oxide on top of the PET substrate is inserted to the lightscribe DVD drive where the patterns were laser scribed to generate conductive graphene. The laser follows the designed patterns to reduce the graphene oxide layer to conductive graphene as shown in Figure 5.2.

Figure 5.2 a) PET substrate is fixed on top of the DVD. b) GO solution is dispensed. c) The substrate is left to dry for 24 hours. d) The DVD in inserted in the laser scribing drive where the laser is used to form the required patterns. On the right hand side the steps of Drop casting graphene oxide on PET substrate affixed on top of a DVD. On the right hand side the PET substrate is cut and placed on a CD then the GO solution is drop casted and left to dry.

5.3 Molecular probes design and Hybridization assays:

5.3.1 Chemicals and reagents

Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA, U.S.A.). DNA capture probes (CP), complementary to L. pneumophila ‘s16s rRNA, were selected and designed using Proimose (Ashelford, Weightman, & Fry, 2002) and OligoArchitect Online from sigma-aldrich. For hybridization 10mM Tris-HCl (pH 8.0) solution with 1mM MgCl2 was used. A fluorescence microscope (Leica Z16 APO, Concord, Ontario) coupled with fluorescence illuminator (X-Cite® series 120 Q,
Excelitas Technologies Corp., Waltham, MA) was used for measurement of the fluorescence intensity of the droplets on the chip. All images were captured using a CCD camera (Leica DFC340 FX, Concord, Ontario) and analyzed by ImageJ (National Institutes of Health, Bethesda, MD). All measurements were subtracted by the intensity obtained from a negative control. The negative control droplet contained molecular beacons. The lower detection limit was defined as the smallest concentration of an analyte, calculated as the blank signal plus or minus three standard deviations. All data were expressed as the mean ± standard deviation.

Table 5.1 DNA sequences used in the experiments

<table>
<thead>
<tr>
<th>Names</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Probe</td>
<td>Fluorescein /CGAGCC ATTATCTGACCGTCCCA GGCTCG /</td>
</tr>
<tr>
<td></td>
<td>Iowa Black FQ</td>
</tr>
<tr>
<td>Perfect match</td>
<td>TGGGACGCGTCAGATAAT</td>
</tr>
<tr>
<td>1 MM</td>
<td>TGGGACGGACAGATAAT</td>
</tr>
<tr>
<td>2 MM</td>
<td>TGGGACGAAACAGATAAT</td>
</tr>
<tr>
<td>3 MM</td>
<td>TGGGACGAAATAGATAAT</td>
</tr>
</tbody>
</table>

5.4 Results and discussions

An electrowetting test has been performed to determine if the new fabricated system is capable of changing the droplet contact angle and to characterize the change in the contact angle at different voltages. Figure 5.3 shows that the fabricated chip (LSG graphene electrode covered with a 10 µm Parylene-C as a dielectric layer and Teflon AF1600 on top) is able to manipulate the contact angle at different voltage levels. In the open system, voltages up to 800 V where used to manipulate the droplets.

The GO solution usually covers the whole CD area to print small structures all over the CD printable area. However, DMF chips are relatively large and cannot be easily repeated compared to the previously reported structures such as transistors that can be easily printed repeatedly on the whole CD area (Tian, Yang, et al., 2014). Therefore, the GO solution was dispensed only on the designed DMF chip area to prevent wasting large amounts of unused GO area. Figure 5.1 shows the DMF platform after the DMF electrodes are fabricated using the laser scribing technique. In this figure, on chip reservoirs are arranged around the electrodes array for performing closed DMF operations where a top grounded plate of ITO
covered with a hydrophobic layer will be added. We can notice in Figure 5.1 that the GO solution was just added to the rectangular section of the chip instead of covering the whole CD surface compared to the previous works (El-Kady & Kaner, 2013; El-Kady et al., 2012; Strong et al., 2012). The amount of the dried GO on the surface depends on the dispensed volume of the GO solution and its concentration. In this work, the amount of the dried GO per unit area used is 0.57 mg/mm$^2$). Using PET substrates for fabricating DMF systems can enhance the capabilities of the chip where Figure 5.3 b) shows that the fabricated DMF chips are flexible and are easily bendable. This can allow the droplets to move on inclined, twisted and vertical direction (Mohamed Abdelgawad et al., 2008).

![Figure 5.3](image)

**Figure 5.3** a) The electrowetting test performed to characterize the change in the contact angle. b) The fabricated chips are bendable which can allow the droplets to move on inclined, twisted and vertical direction.

Several tests are performed to characterize the output resolution of the LSG DMF electrodes. It is important to determine the minimum attainable printing resolutions and investigate the limitations and the capabilities of the laser scribing technique as the printing resolution is critical for fabricating the electrodes required for DMF applications. During the laser scribing process, laser passes in circular patterns on the dried GO. Consequently, the graphene layers are not formed uniformly and concentric circular patterns due to the printing path can be seen clearly on the chip surface in Figure 5.4. In order to characterize the uniformity of the printed patterns, two tests were performed to discover how the printing in
circular manner can affect the spacings between the electrodes and the line thickness of the connection lines.

Figure 5.4 Printing concentric circles with resolutions increasing from 25 µm to 350 µm to assess and characterize the printing quality in all the directions and angles in a) and b). c) Printing resolution test on vertical and horizontal lines. d) Two long electrodes printed in the vertical and horizontal direction 15 × 1.6 mm which shows the effect of the printing direction on the measured resistance in f). e) DMF electrodes printed at different resolutions. f) The resistance of the two laser scribed graphene electrodes in the horizontal direction (parallel to the printing direction) and the vertical direction (perpendicular to the printing direction).

In the first test, concentric circles are printed with equal line thickness and spacings starting from 25 µm till 350 µm. Figure 5.4 a) and b) show that the lines are completely connected in the 25 µm range at the first circle and the spacing disappears completely. The
upper yellow marker in Figure 5.4 a) demonstrate that the 25 µm line spacing is not visible where the lines are connected. The line spacing at 50 and 75 µm starts to become visible and the 100 micron is well defined. However, in Figure 5.4 b) the 25, 50 and 75 µm lines are connected and the 100 µm is visible and starting from the 125 µm it becomes more defined. As has been noted above, the lines are completely connected at 25 µm and when the line spacing increases to 50 µm and 100 µm, obvious difference can be noticed in the printed lines in the horizontal direction compared to the vertical direction. Given these points, the printing process is not uniform in all directions and the printing resolution is affected by the position of the patterns placed and the printing direction.

In the second test, Figure 5.4 c) shows parallel graphene lines printed in the horizontal and the vertical direction to assess the printing resolution in these two main directions. The line spacings are increasing from 50 µm to 300 µm. Conversely, the line thicknesses are decreasing from 300 µm down to 50 µm in the opposite direction. In other words, the first 300 µm line thickness is followed by 50 µm spacing then the line thickness decreases and the line spacing increases with 50 µm steps. The horizontal lines are tangent to the circular printing direction and the vertical lines are perpendicular to the circular printing direction according to the printing direction shown in Figure 5.4 d). When the spacing is 50 µm between the first two vertical lines from the left, the figure shows that they become completely connected. On the other hand, the first two horizontal lines from the top are not completely connected where very small gaps start to appear at the same theoretical 50 µm spacing. At 150 µm spacing, the lines in the horizontal direction are more defined and separated compared to the lines in the vertical direction that are still connected at some points. The rest of the graphene lines and spacings at higher resolutions are not greatly affected. In like manner, the line widths are smooth and more defined in the horizontal lines compared to the corrugated and more connected vertical lines in Figure 5.4 c). Under these circumstances, the printing resolution is slightly better when the printed lines are parallel to the printing direction at printing resolutions higher than 200 µm.

Figure 5.4 e) shows the arrangement of the electrodes in an open system configuration. The line spacing between the electrodes was increased from 25 µm to 300 µm.
Similarly, the electrodes are completely connected at 25 µm and they start to separate when the spacing goes above 150 µm.

Figure 5.5 a) Droplet transport experiments with various droplet volumes in open DMF systems. b) Two droplet merged and mixed then the bigger droplet is transported. c) The peak and averaged droplet velocity measured from the experiments. d) and e) Transport, merging and mixing droplets on a laser scribed graphene based DMF systems in closed DMF configuration.

To mitigate all the previous irregularities in the line spacings, the dimensions of the DMF electrodes should be increased and a larger spacing is used, 250 µm, in the later experiments. In this previous work, DMF operations where performed when the line spacing between the electrodes went up to 300 µm (Mohamed; Yafia et al., 2015). The resistance of the vertical and the horizontal printed lines shown in Figure 5.4 d) have been measured to
investigate if the printing direction can affect the resistance on relatively large electrodes. Figure 5.4 f) shows that the resistance in the vertical direction is relatively higher than the resistance in the horizontal direction. This difference can be explained when we look closely at the printing patterns engraved on the two lines in Figure 5.4 d). The vertical line shows the small curves that are formed along printing direction where the transition between each curve results in larger resistance. On the other hand, the horizontal lines do not show these many curves and small corrugations along the long electrode in the horizontal direction which results in lower overall resistance. The resistance mainly affects the threshold voltage required for actuation. Specifically, the higher the resistance the higher the applied voltage. Moreover, the current consumed is in the µ ampere range so the consumed power will not be affected.

Various droplet volumes can be used in open and closed DMF systems as shown in Figure 5.5. The present configuration (2 mm electrode size and 250 µm electrode spacing) is able to move wide range of droplet volumes which starts from 10 µl up to 30 µl in open DMF system as shown in Figure 5.5 a). Smaller droplet volumes can be transported in open system configuration using a smaller electrode size if needed. In Figure 5.5 b), two droplets were merged and mixed and then the resultant big droplet was transported to show that the same electrode size can handle various range of droplet volumes. Figure 5.5 c) shows the average droplet velocity measured when the droplet moves from one electrode to another. The peak droplet velocity also is plotted against the voltage used (AC square wave signal with frequency of 10 kHz). The experiments show that the average and the peak velocity are within the range of the previous reported values that ranges from 5 mm/s to 250 mm/s. However, the velocities in the present work cannot be directly compared to their studies as they used different electrode size, shape and grounding configuration which can generate different actuation forces (M Abdelgawad et al., 2009).

A transparent ITO ground plate, coated with hydrophobic layer, is then added on top to test the LSG DMF electrodes in closed DMF system configuration where several tests and DMF operations were performed. In Figure 5.5 d), a 4 µl droplet is transported successfully using 250 V at frequency of 10 kHz. Furthermore, another test was performed to demonstrate the merging of two droplets, 2 µl each. After merging the droplets, they were mixed and then
transported as a one 4 µl droplet over the electrodes array in a squared shape mixing pattern as shown in the sequenced images in Figure 5.5 e).

![Graph showing normalized fluorescent intensity for perfect match, 1-3 mismatches, and control.](image)

**Figure 5.6 Normalized fluorescent intensity corresponding to perfect match, 1-3 mismatches and control.**

To demonstrate the applicability of the LSG in point-of-care applications, this work further demonstrates a one-step direct assay for detection of L. pneumophila DNA without the need for any washing step with high specificity for discriminating a single nucleotide mismatch in the DNA sequences shown in Table 5.1. In order to obtain high specificity, in addition to the considerations in designing of molecular beacons, the hybridization conditions in particular salt concentration is critical. Here, several salt concentrations were examined and 10mM Tris-HC solution with 1mM MgCl₂ resulted in highest specificity (Data not shown). In order to demonstrate the selectivity of the designed molecular beacon targeting *L. pneumophila*’s DNA, several DNA targets complementary to the molecular beacon with 1 to 3 mismatches were designed. As can be seen in Figure 5.7, there is a clear signal reduction by increasing the number of mismatches. This is due to the fact that by introducing mismatches, the stability of the molecular beacon-target duplex was reduced (due to lowering the duplex melting temperature) and this resulted in fewer molecular beacons in opened state and (quencher remains in proximity of the fluorophore) therefore less fluorescent signal. The control signal is the signal that occur in the absence of the target DNA.
Our results suggested that, with design of the molecular beacon and careful selection of hybridization buffer, a highly specific probe was introduced which can distinguish single nucleotide mismatch. In order to determine the working range of our detection system, several concentrations of the target DNA were hybridized with the molecular beacon. Shown in Figure 5.7, the limit of detection can reach as low as 1 nM target DNA concentrations. It is worth to note that a lower limit of detection would be possible if a lower concentration of the molecular beacon is used due to a lower background fluorescent.

![Figure 5.7](image)

Figure 5.7 Measured relative fluorescence intensity of Legionella DNA concentrations.

5.5 Summary

In this chapter, a new electrode fabrication method is introduced for digital microfluidic systems. The proposed method involves laser scribing of graphene (LSG) to generate DMF electrodes on both rigid and flexible substrates directly from a CAD design without the need for microfabrication cleanroom facility. Several tests were conducted first to characterize the LSG fabricated DMF chips and second to evaluate the performance of the LSG-based chips. The printing resolution of the LSG DMF electrodes has been characterized for both open and closed DMF systems. The resolution of the horizontal and vertical printed graphene lines may be slightly different due to the circular laser scribing paths of the device.
used. However, this limitation is due to the laser scribing technique used and not a limitation for the proposed LSG DMF chips. In our experiments, the printing resolution was constrained to 250 µm to make sure that there is uniform gap resolutions across the horizontal and vertical directions. The LSG DMF electrodes substantially changed the contact angle of the droplet using electrowetting-on-dielectric EWOD. Several DMF operations were introduced in both the closed and the open DMF to transport, merge and mix small droplet volumes starting from 2 µl in the closed system up to 30 µl in the open system. To demonstrate the applicability of the LSG in point-of-care applications, this chapter further demonstrates a one-step direct assay for detection of *L. pneumophila* DNA without the need for any washing step with high specificity discriminating a single nucleotide mismatch. This was achieved by designing a molecular beacon with fluorophore and quencher in proximity in the closed state. The portable, low cost and easy to manufacture DMF chips in combination with one-step and highly specific molecular probes, offers a great platform for point of care diagnostics.
Chapter 6  Performing full system integration by introducing an ultra-portable smartphone controlled DMF system in a 3D-printed modular assembly

Portable sensors and biomedical devices are influenced by the recent advances in microfluidics technologies, compact fabrication techniques, improved detection limits and enhanced analysis capabilities. This chapter reports the development of an integrated ultra-portable, low-cost, and modular digital microfluidic (DMF) system and its successful integration with a smartphone used as a high-level controller and post processing station. Low power and cost effective electronic circuits are designed to generate the high voltages required for DMF operations in both open and closed configurations (from 100 to 800 V). The smartphone in turn commands a microcontroller that manipulate the voltage signals required for droplet actuation in the DMF chip and communicates wirelessly with the microcontroller via Bluetooth module. Moreover, the smartphone acts as a detection and image analysis station with an attached microscopic lens. The holder assembly is fabricated using three-dimensional (3D) printing technology to facilitate rapid prototyping. The holder features a modular design that enables convenient attachment/detachment of a variety of DMF chips to/from an electrical busbar. The electrical circuits, controller and communication system are designed to minimize the power consumption in order to run the device on small lithium ion batteries. Successful controlled DMF operations and a basic colorimetric assay using the smartphone are demonstrated.

6.1 Portable devices and microfluidics

Recently, there has been a trend toward developing compact and portable devices with user friendly interface for numerous biomedical and chemical applications (Gao et al., 2011; Lee, Kim, Chung, Demirci, & Khademhosseini, 2010; Martinez, Phillips, Carrilho, et al., 2008; Yu et al., 2014). Microfluidics is one of the promising platforms for developing portable devices that allow real-time screening and on-chip diagnosis (Beebe, Mensing, & Walker, 2002; Whitesides, 2006). In particular, the reconfigurable architecture of digital microfluidic (DMF) systems permits the real-time change of fluidic protocols on the same chip, which cannot be achieved in conventional continuous flow systems (Jebrail et al., 2012;
Shen et al., 2014; Sista et al., 2008; Srinivasan et al., 2004; Su & Chakrabarty, 2008; Mohamed Yafia & Najjaran, 2013). Although several attempts have been made to introduce continuous microfluidic operations inside portable devices (Lagelly et al., 2004; Stedtfeld et al., 2012), few studies have focused on developing portable DMF platforms (J Gong, 2007; J Gong et al., 2004; Kim et al., 2011).

Modern electronic devices and fabrication technologies have enabled size reduction and miniaturization of the digital microfluidic systems without limiting their capabilities and functionalities. Gong et al. (J Gong et al., 2004) have introduced a packaged DMF system with time multiplexed driving scheme in order to minimize the number of the control channels. Another research group has introduced a more advanced packaging for the DMF system in a portable box that can be controlled by a handheld tablet (Kim et al., 2011). However, these attempts suffered from the complexity of the design and the high cost of the components used. Moreover, reproducing these devices is challenging and extensively time consuming.

One of the main challenges in developing portable and cost effective DMF systems is regarding the portability of the control modules, high voltage electrical components and imaging and post processing systems. On the other hand, to avoid cross contamination, and also to be used for different applications, the DMF chips must be easily replaced. Recent advances in the hardware and software capabilities of smartphones creates an opportunity for development of these portable DMF systems (Martinez, Phillips, Carrilho, et al., 2008). To address the above mentioned issues, three-dimensional (3D) printing can be used to integrate the smartphones with replaceable and modular DMF systems. Although smartphones and 3D printing technology have been used for numerous continuous microfluidic applications (B. Li et al., 2014; Park, Li, McCracken, & Yoon, 2013), their use have not been explored for DMF applications.

In this work, an ultra-portable low cost smartphone controlled DMF system in a 3D printed modular assembly is introduced. The main components of this system include holder assembly, battery-powered high voltage electronic circuitry and a smartphone controlling the digital microfluidic operations via Bluetooth connection. Overall, the developed assembly offers portability and modularity at lower cost with reduced complexity and easier
fabrication. Another important advantage of this device is the compatibility with any smartphone that has a built in camera and can communicate through a Bluetooth connection. Moreover, the assembled device does not require external peripherals such as high voltage amplifiers, external power source, microscopic camera, image analysis and post processing stations. The modular system reported here enhances the previous designs (J Gong, 2007; J Gong et al., 2004; Kim et al., 2011, 2013) as it is monitored and controlled by smartphones, and it is more compact, cost effective and can be easily reproduced.

6.2 Experimental Setup

A schematic hierarchy of the proposed ultra-compact DMF platform is shown in Figure 6.1. Low cost high voltage battery powered amplifier circuit is designed to generate the voltage required for closed and open DMF systems. A microcontroller is used to control the DMF droplet motion and routing configuration. A smartphone is then used to communicate and send commands to the microcontroller. For this purpose, a Bluetooth module is used to send and receive commands and establish the connection with the microcontroller. The smartphone is also used to monitor the droplet motion and acts as a post processing station as it can perform image processing and detect any color changes. In order to increase the magnification, a microscopic lens is attached to the smartphone camera. The microscopic lens also helps in the size reduction of the device by decreasing the focal distance from the chip to the smartphone to 1 cm only. In addition, 3D printing is used to fabricate modular holder assembly that allows the detachment of the DMF chip from the holder.
6.2.1 Chip Fabrication

Microfluidic chips can be categorized according to the type of the flow inside them such as continuous flow in closed microfluidic channels and discrete droplets motion on digital microfluidics systems. Several microfluidic operations can be performed in the continuous microfluidic systems such as droplet splitting (Yap et al., 2009), droplet merging (Luong, Nguyen, & Sposito, 2012), droplet dispensing (S. H. Tan, Maes, Semin, Vrignon, & Baret, 2014; S. H. Tan, Semin, & Baret, 2014). Transport, merging, splitting and dispensing
of discrete droplets have been demonstrated successfully on the DMF platform (Bavière et al., 2008; S. K. Cho et al., 2003; M. Pollack et al., 2002).

Digital microfluidic systems can manipulate discrete droplets using two main configurations: open and closed systems. Electrowetting on a dielectric (EWOD) is one of the actuation techniques used to manipulate the droplets in both open and closed DMF devices (Mugele & Baret, 2005). DMF chips can be fabricated by several methods such as photolithography (M. G. Pollack, Fair, & Shenderov, 2000), inkjet printing and rapid prototyping on paper substrates (Fobel et al., 2014; Ko et al., 2014; Mohamed; Yafia et al., 2015). Open and closed DMF systems may each offer some advantages and disadvantages for different applications. Using the open system enables dispensing the droplets easily by having direct access to the surface of the chip. Higher droplet velocities can be achieved by reducing the friction when the top plate is removed. However, open system require higher voltages during droplet actuation. On the other hand, the evaporation in the closed system can be more controlled particularly when oil is used as a filler medium. Finally, closed systems have lower voltage requirements. On the contrary, the closed systems have increased friction and lower droplet velocities with low mixing rates for the droplets.

In this work, EWOD-operated digital microfluidic chip is fabricated by conventional photolithographic technique. A Copper layer of 80 nm is deposited on a glass substrate by a sputtering deposition system (NEXDEP depositing system, Angstrom engineering, Kitchener, Ontario) followed by photoresist spin coating (S1813) at 2000 RPM for 26 sec. to create a layer with thickness of 1.5 µm. Then, the photoresist layer is covered by a mask and exposed to ultraviolet (UV) light according to the designed patterns. The photoresist is developed in MF-319, and then the copper is etched in ferric chloride solution. Finally, Parylene-C of 10 µm thickness is deposited using a chemical vapour depositioning system (PDS 2010 Labcoter® Parylene Deposition System, Specialty Coating Systems, Inc., Indianapolis, IN). The electrode size used in the experiments is 2 mm × 2 mm.

6.2.2 Holder assembly

Three dimensional (3D) printing is an additive manufacturing process that can provide a precise, compact and rapidly prototyped packaging for the portable DMF systems. 3D printing has been used in different biomedical and engineering applications (C. Chen,
Erkal, Gross, Lockwood, & Spence, 2014; Comina, Suska, & Filippini, 2015; Shallan, Smejkal, Corban, Guijt, & Breadmore, 2014). For instance, microfluidic lab on a chip devices are 3D printed for chemical synthesis (Kitson, Rosnes, Sans, Dragone, & Cronin, 2012) and for fabricating customizable chemical labware (Johnson, 2012). Biomedical and tissue engineering has also benefited from the 3D printing technique (Beyer, Bsoul, Ahmadi, & Walus, 2013; Butscher, Bohner, Hofmann, Gauckler, & Müller, 2011; Leukers et al., 2005; Miller, 2014). Moreover, 3D printing technique has been used recently for fabricating micro lithium ion batteries (Sun et al., 2013).

A 3D printer (Objet500 Connex, Stratasys) with a resolution of 14 µm is used to fabricate the DMF modular parts required for assembling the portable DMF device. The 3D printed holder assembly has the necessary components including chip frame and spacer, electrical connection holders, batteries and phone holders.

6.2.3 Electrical components

High voltages are usually required for actuating the electrodes in closed (typically 20 V to 300 V) and open (typically 100 V to 700 V) DMF systems. These voltage signals are usually generated by high cost and high power bench top equipment. However, the droplet actuation process requires low power and low current consumption (within the micro-watt range) (Fair et al., 2001). On the other hand, the high voltage amplifier circuit used in the literature (J Gong et al., 2004) utilized high cost commercial version of DC to DC converters that can reach 6000 V and the voltages required for DMF operations are typically lower than this value.

The main components of the electronic parts include low cost and low power high voltage amplifier, small and compact microcontroller, phone-to-microcontroller connections via Bluetooth, high voltage switching circuit, portable power supply (Lithium ion battery) and DMF chip-to-board connections.

A low power microcontroller (Arduino Micro) is chosen to control the high voltage switching circuit and route the droplets accordingly. A software program is developed for programming the microcontroller to route the droplets based on the required DMF operations. Moreover, this program is used for the communication between the smartphone
and the microcontroller using the Bluetooth module to send and receive commands in serial communication mode.

Switched mode power supplies (SMPS) are used for converting electrical power from one form to another. SMPS are usually used to change voltage type and level in many electrical equipment including cell phone chargers. SMPS can be categorized according to the change that occurs in response to the output voltage type compared to the input voltage type. In this work, a boost DC to DC converter is used to yield the high voltages required for DMF operations from a DC battery source. Boost DC to DC converters are characterized by several advantages such as their compact size as small inductors can be used, they can operate at high efficiencies and the power consumption can be reduced to preserve the charge of the battery. Accordingly, boost DC to DC converters are used in this work to replace the highly powered, costly and bulky bench top equipment.

Figure 6.2 a) Circuit diagram of the boost DC to DC converter used to generate high voltages from the battery source. b) 555 timer circuit used to generate the pulses required for the switched mode power supply. c) The high voltage switching circuit used to control the droplet routing.
The circuit in Figure 6.2 is designed to amplify the battery voltage generated from the two small lithium ion batteries from 8 V approx. up to 800 V. The output voltage can be controlled by either changing the pulses switching frequency or the duty cycle. More details about the circuit details can be found in the appendix.

We developed a code to send and receive serial commands from the microcontroller to the smartphone and vice versa. The code is developed using Arduino 1.0.6 platform. The application used to send and receive commands from the smartphone is S2 Terminal for Bluetooth.

### 6.2.4 Imaging accessories

A microscopic lens (Micro Phone Lens, Olympia, Washington) is integrated to the platform to analyze the droplet motion and monitor the DMF operations. Phone camera is used for detection as it can identify any change that occurs in the droplet color. The microscopic lens is attached easily on any smartphone camera by just pressing it gently on the surface of the camera. The magnification of the lens is 15X with working distance of 1 cm. An open source app (Color Analyzer), available on the smartphone app store, is used for detecting the color. For more advanced image analysis, the OpenCV libraries can be used (Vazquez-Fernandez, Garcia-Pardo, Gonzalez-Jimenez, & Perez-Freire, 2011).

### 6.3 Results and Discussion

To demonstrate the effect of duty cycle on the output voltage, experiments were performed to change the duty cycle for the pulse frequency of 20 kHz. The current drawn by the circuit is also measured to determine the power consumption of the circuit. The duty cycle of the pulsating signal is the ratio between the time when the MOSFET is on and the total periodic time of the signal. The duty cycle of the signal controls the voltage gain ratio between the output and the input voltages. As the duty cycle increases, the voltage gain increases as shown in Figure 6.3 a) and Equation (1) in the appendix. In addition, the current drawn by the circuit also increases by increasing the duty cycle. The current drawn increases significantly beyond 100 mA when the duty cycle passes beyond 97.5%. It should be noted that current consumption must be reduced to preserve the battery power. Figure 6.3 b) shows the output voltage wave form and the input switching pulses going from the 555 timer to the MOSFET when the duty cycle is 97.5% and the frequency is 20 kHz. The experiment in
Figure 6.3 b) depicts that the 600 V output voltage wave form generated has a voltage ripple of ± 20 V which is around 3% of the total output voltage.

The output voltage can also be controlled by changing the switching frequency. Figure 6.4 a) shows the average output voltage generated across different frequencies when the duty cycle is kept constant at 50 %. As shown in Figure 6.4 a), the current drawn from the batteries is less than 50 mA across any tested frequencies which indicates the low power consumption at these operation modes. Figure 6.4 b) depicts the output voltage and the
switching pulses going from the 555 timer to the MOSFET when the duty cycle is 50% and the frequency is 20 kHz. The average output voltage is 200 V and the ripple in this case is ±5 V which is around 2.5 % of the total output voltage. It is worthy to note that using low frequencies is not preferable as the output voltage exhibits high voltage ripple at lower frequencies below the 10 kHz range. On the whole, the designed circuits are able to generate the high voltages required and take advantage of the low power consumption of the DMF system. A high voltage regulator circuit can be added to rectify the output signal generated. However, the current output wave form is adequate for DMF operations as it is shown later in this work.

Figure 6.4 a) The output voltage generated experimentally from the DC to DC boost converter and the current drawn versus the frequency when the duty cycle is 50%. b) The output voltage waveform (Dark Blue) and the switching pulses waveform (Cyan) when the duty cycle is 50 % and the frequency is 20 kHz
where the vertical scale for the output voltage waveform and the switching pulses waveform is 100 V and 5 V, respectively.

Afterwards, the high voltage output of the boost DC to DC converter is chopped with the full bridge circuit to an AC square wave signal, demonstrated in Figure 6.5, and then forwarded to the switching circuit in Figure 6.2 c). The sequential voltage control algorithm is programmed on the microcontroller which sends a 5 V signal to the switching circuit which in turn activate the desired electrode. The Bluetooth module integrated to the system is used to send and receive commands from the phone and communicate with the microcontroller. All these circuits are packaged in the device shown in Figure 6.6 and Figure 6.7. The total power consumption of the device with all the peripheral circuits has to be determined in order to assess the attainable operation time when batteries are used as a power source. The total current drawn by the system including the DC to DC converter, the 555 oscillator circuit, the high voltage switching circuit, the microcontroller and the Bluetooth module is 180 mA when these circuits are running simultaneously. Therefore, the total power consumption full power operation mode is around 1.47 watts. This amount of current can run the system in continuous operation mode for more than 22 hours on the two small lithium ion batteries with total capacity of 4000 mAh. This total current consumption of the system is measured when the system runs at full power. However, the sequence of the DMF operations and post processing do not require running all of them simultaneously. Therefore, additional running time can be achieved by switching off some circuits and putting them to sleep mode based on the DMF schedule of operation.

For rapid prototyping and repeatable fabrication, the device is 3D printed with taking account of the portability, modularity and smartphone compatibility according to the designed model shown in Figure 6.6. The model is divided to three main parts: top plate holder, bottom plate holder and peripheral electronic box. The bottom plate holder is designed to seat the DMF chip and connect the electrodes to the high voltage circuits directly. The top plate holder is designed to secure the top grounding indium tin oxide (ITO) plate on top of the bottom plate. The smartphone rests above the top plate holder where the thickness of the top plate is designed to adjust the focal distance between the smartphone camera and the droplets (1 cm approx.). Another 3D printed part is designed to enclose the
required electrical circuits, electrodes’ pin connections, control and communication systems and batteries. The top plate and bottom plate holders can be attached and detached easily from the circuits’ part. As a result, DMF chip part can be easily removed if further testing, analysis or post processing is needed directly on the chip. The measured total weight of the device is just 235 grams which demonstrates the device compactness and portability. The cost of the components used to construct the device are introduced in Table 1 to estimate the total cost of the device. The whole device costs 62 US$ approximately.

<table>
<thead>
<tr>
<th>Circuit</th>
<th>Components</th>
<th>Price in US$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boost DC to DC converter</td>
<td>10 mH inductor (Murata Power Solutions inductor, 85ma, Radial Leaded)</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Vishay Siliconix, MOSFET IRFBG30 Transistor, N Channel, 3.1 A, 1 KV</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td>Ceramic Capacitor 100PF</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Through Hole Metal Film Resistor, 10 Mohm</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>1N4937 Diode</td>
<td>0.032</td>
</tr>
<tr>
<td>Pulses generator circuit</td>
<td>Texas Instruments NE555P, Precision Timer, 500 KHZ, 16 V, DIP-8</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>2 capacitors and 2 resistors</td>
<td>0.4</td>
</tr>
<tr>
<td>Microcontroller</td>
<td>Arduino Micro</td>
<td>19</td>
</tr>
<tr>
<td>Bluetooth module</td>
<td>HC-06 Bluetooth module</td>
<td>7</td>
</tr>
<tr>
<td>Full bridge AC</td>
<td>4 MOSFETS and a full bridge driver</td>
<td>16</td>
</tr>
<tr>
<td>Micro lens</td>
<td>Micro Phone lens</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 6.1 The cost of the components of the device.

Total cost : 62 US$
Figure 6.5 The 600 V_{pp} output AC signal from the circuits used in moving the droplets

Figure 6.6 A 3D CAD model (SolidWorks, Dassault Systems) for the modular DMF platform. a) Front view showing how the phone is attached on top of the device, b) back view, c) exploded view showing the parts required for inserting the chip inside the device and d) shows how the DMF chip can be replaced and installed easily to the device
Figure 6.7 shows the fabricated 3D printed and modular DMF platform assembly. This figure exhibits the portability of the device as it shows the device when it is hand-held. The DMF chip frames are designed to be easily inserted and ejected from the chip-to-board connection holder. A smartphone which is within the same size as the platform is used to monitor the system and detect any color changes of the droplets. Furthermore, the smartphone can be used to control the routing algorithm for droplet motion based on the post processing results.

Figure 6.7 The final prototyped portable DMF modular platform.

Figure 6.8 demonstrates a successful experiment for droplet motion where the droplet is transported using the electrical signal generated in Figure 6.5. The smartphone is used to send the commands and control the motion sequence. A demonstrative video is added to the
supplementary data of the following paper (Mohamed Yafia et al., 2015) in order to show how the system works and how the smartphone can control the DMF operations using this device.

![Droplet Motion Direction](image)

**Figure 6.8 The smartphone controls a preprogrammed droplet motion on top of a DMF chip.**

Several tests have been performed for assessing the capability of the system colorimetric assays on the DMF chip. Some applications exhibit slight color difference and some difficulties may arise while distinguishing between the droplets color using image analysis techniques using the ordinary electrode design. Therefore, a new electrode design is proposed to detect the slight change in the color easily. This new design features a small window inside the electrode. The color is averaged on this window for having a consistent image analysis area on the chip. This design helps in eliminating the error that may arise from the electrodes reflection and the empty spacing between the electrodes when the color is averaged on the whole droplet footprint area. Therefore, the measurement on this window is not affected by the background colors. The difference between the ordinary electrode design and the new design with the dedicated image analysis section is demonstrated in Figure 6.9.

![Ordinary electrode design with background noise. b) The new design with the dedicated image analysis section.](image)

**Figure 6.9 a) Ordinary electrode design with background noise. b) The new design with the dedicated image analysis section.**

As a proof of concept application for the developed system, a basic colorimetric assay is performed. Solutions with phenolphthalein indicator at different pH values are used to
assess the combined capability of the lens and the phone camera in detecting slight difference in color changes on DMF chips. Figure 6.10 shows a droplet of pH 7 with transparent color on the left side and a droplet of pH 13 with pink color on the right hand side. The RGB values are measured by averaging the color on the image analysis section. Moreover, an additional grayscale indicator was calculated to determine the pH of the solution using the following equation (Saravanan, 2010; M. Song, Tao, Chen, Bu, & Yang, 2013):

$$\text{Grayscale} = 0.299 \times \text{Red} + 0.587 \times \text{Green} + 0.114 \times \text{Blue} \quad (6.1)$$

The RGB value combination determines the color of any object. However, those three color components do not have equal effect on color changes. As the color becomes darker, it can be noticed that the red and the blue components show smaller changes compared to the significant changes in the green component. In addition, the eye sensitivity is a function of wavelength and is the greatest at a wavelength of about $5.6 \times 10^{-7}$ m (yellow-green) (Faughn & Serway, 2003). Moreover, it can be noticed that the green color has the highest weight in
the gray scale equation. Accordingly, the green component is the most affected component when the color becomes darker. Therefore, when there is significant color changes, the green component has higher rate of change and it intersects the blue component when the color becomes darker. To investigate the effects of droplet volume on the color measurement, the averaged RGB values (over the designated region) were measured for different droplet volumes ranging from 5 µl to 20 µl. Shown in Figure 6.11, no significant change was observed in the measurement.

**Figure 6.11** The change in the volume of the droplet did not have a significant effect on the droplet color measurements when the droplet volume was varied from 5 µl to 20 µl.

Overall, the modular design, integration with smart phones, low (3D printing) fabrication cost and cost effective battery-powered electrical components extends the implementation of DMF technology to a wider range of applications.
6.4 Summary

In this chapter, a portable, smartphone controlled, battery powered and 3D printed modular DMF device has been introduced. The main novelty of this work is the introduction of a series of technologies that together offer a fully integrated lab-on-a-chip device. Compact and low cost circuits were designed to generate the high voltages required for performing DMF operations. The low power rating of DMF systems allowed replacing the high power bench top equipment with high voltage and low current electric circuitry. Minimizing the power consumption of the high voltage circuits, control circuit and switching circuit has been performed successfully. The designed system can be powered by small lithium ion batteries continuously for more than 22 hours with a single full charge. A compact microcontroller is used for controlling the high voltage switching algorithm. The smartphone controls the whole operation via a Bluetooth module, which is directly connected to the microcontroller. DMF operations have been performed successfully on the system. Finally, the integration of a microscopic lens with the smartphone has been demonstrated to examine the droplets sitting on this system and detect the color changes inside the droplet. We designed a chip with a dedicated region for color detection. In the new design, the color is measured only on this specific region which decreases the error, and makes the color detection independent on the droplet volume. To verify the independency, this chapter studied the effect of changing the droplet size from 5 µl to 20 µl on the measured RGB values.
Chapter 7 Conclusions and future work

This work improved the practicality of DMF platforms by addressing the challenging problems that hinder the widespread use of DMF systems in mainstream research or microfluidic-based commercial devices. Throughout the research and development process, several scientific contributions and engineering analyses were introduced to enhance the accessibility and the reliability of the DMF platform. All things considered, the DMF platform can be rapidly and easily prototyped at low cost using these new fabrications techniques. The DMF operations can be performed reliably by adjusting the optimum operating conditions. The small size of the platform has shown its compatibility with size of the smartphones that are commonly used nowadays. In this chapter, a summary of conclusions and recommendations for future work are presented.

7.1 Conclusions

To enhance the DMF operations, thorough characterization for the droplet morphology was performed at different conditions. This research demonstrated how the aspect ratio, in terms of gap height that is divided by the electrode side length, has a great effect on the droplet shape, droplet dynamics and how the droplet motion develops during transport. Severe necking accompanied by elongation and stretching in the total length of the droplet were observed during the droplet transport at extremely low aspect ratios. Several experiments were introduced to show how the droplet velocity is affected by changing the gap height. The electric Weber number can give an additional explanation to the necking as the electric Weber number increases by either decreasing the aspect ratio or increasing the applied voltage. Computational fluid dynamics (CFD) simulations, using FLOW 3D, were performed and their results match with the experiments. Analytical models are considered invalid when the droplet becomes deformed and non-circular at certain aspect ratios. As a result, numerical simulations, using Flow-3D, were used instead of analytical models to more adequately demonstrate the droplet behaviour observed.

In the second part of enhancing the DMF operations, a variable gap size actuation mechanism has been introduced and successfully integrated into digital microfluidic chips to control precisely the position of the top plate of the closed
parallel plate DMF system. A comprehensive experimental study has shown the added benefits of using VGSA. It is demonstrated how controlling the gap height allows the DMF system to assume an appropriate aspect ratio for each operation. It is also demonstrated how the sequential adjustment of the gap height during a multi-operation process can guarantee successful progress of the process until completion. The effect of VGSA on droplet transport was investigated for a range of different gap heights. The VGSA mechanism significantly reduces the residual sample waste volume of a reservoir. The VGSA mechanism can facilitate splitting of a droplet. For this purpose, first the gap height is increased to position the droplet at the centre of the electrode accurately, and then the top plate is lowered to reach a gap height that makes the splitting process possible at a given voltage. VGSA can also be used to mobilize the residual droplets that have reduced to immovable droplets as a result of splitting or partial evaporation. The experimental setup used in this research is for proof of concept and may not be a practical solution, especially for portable devices. Naturally, the integration of a micro-actuator into a handheld digital microfluidic device is an interesting next step for this work. Recently, a piezoelectric top plate control system was introduced (Y. Li, Baker, & Raad, 2016). The size of this mechanism is small compared to our electromechanical actuator. This small and compact size can be implemented easily on a portable device.

To facilitate the mass production of DMF chips in a simple and cost effective manner, a new technique for the fabrication of digital microfluidic electrodes using screen printing on both rigid and flexible substrates has been demonstrated. This work promotes screen printing, as one of the common batch printing methods, for fabricating the DMF chips. The proposed method outperforms the reported DMF rapid prototyping techniques in terms of fabrication steps, fabrication materials, fabrication time, fabrication cost, equipment availability and mass production capability. DMF electrodes were screen printed directly on paper, glass and wax paper substrates. Different electrode spacings and connection line thicknesses were examined to assess the output resolution of this printing technique when it is used for printing DMF chips. A Parafilm dielectric layer was tested because of its low cost and simplicity, which is
in line with our general desire of fabricating DMF devices easily. Improved actuation methods were introduced to facilitate the droplet motion when the electrode spacing is relatively large.

For the purpose of facilitating DMF fabrication, another electrode fabrication method is presented which involves laser scribing of graphene (LSG) to generate DMF electrodes on both rigid and flexible substrates directly from a CAD design without the need for the microfabrication cleanroom facility. Several tests were conducted first to characterize the LSG fabricated DMF chips and second to evaluate the performance of the LSG-based chips. The printing resolution of the LSG DMF electrodes has been characterized for both open and closed DMF systems. The resolution of the horizontal and vertical printed graphene lines may be slightly different due to the circular laser scribing paths of the device used. However, this limitation is due to the laser scribing technique used and not a limitation for the proposed LSG DMF chips. To demonstrate the applicability of the LSG in point-of-care applications, This research further demonstrates a one-step direct assay for detection of *L. pneumophila* DNA without the need for any washing step with high specificity discriminating a single nucleotide mismatch. This was achieved by designing a molecular beacon with fluorophore and quencher in proximity in the closed state. The portable, low cost and easy to manufacture DMF chips in combination with one-step and highly specific molecular probes, offers a great platform for point of care diagnostics.

Finally, the full integration of the practical DMF device has been performed successfully where a portable, smartphone controlled, battery powered and 3D printed modular DMF device has been introduced. Compact and low cost circuits were designed to generate the high voltages required for performing DMF operations. The low power rating of DMF systems allowed replacing the high power bench top equipment with high voltage and low current electric circuitry. Minimizing the power consumption of the high voltage circuits, control circuit and switching circuit has been performed successfully. The designed system can be powered by small lithium ion batteries continuously for more than 22 hours with a single full charge. A compact microcontroller is used for controlling the high voltage switching algorithm. The smartphone controls the whole operation via a Bluetooth module, which is directly connected to the microcontroller. Finally, the integration of a microscopic
lens with the smartphone has been demonstrated to examine the droplets sitting on this system and detect the color changes inside the droplet.

7.2 Recommendations for future work

We found some interesting topics during our research and development process that can be further inspected. We propose the following points for further investigations:

- During the droplet necking during motion, it can be noticed that droplet neck size decreases too much. This can lead to cutting of the droplet. If this phenomenon is characterized, it will establish the rules for having a splitting process just between 2 electrodes with only one activated electrode. This is unlike the normal splitting process that happens between 3 electrodes where two of them have to be activated at the same time. This can occur by decreasing the gap height more than the minimum reported value in this work, 50 µm, at 2 mm electrode size.

- The two-electrode splitting technique can also be realized by changing the voltage. Chapter 2 demonstrates that the electric weber number can greatly affect the necking process. A numerical simulation was performed to increase the voltage and it showed that the droplet cutting might occur just between the two electrodes. More simulations at different conditions are needed to study and analyze this phenomenon. This phenomenon should be verified and characterized experimentally to establish the splitting rules.

- Another method to perform the two-electrode splitting process is to change the electrode size. Increasing the electrode length at the same gap height will also help in achieving more necking conditions that can help in this proposed splitting process.

- This work would also serve to further stimulate theoretical work in EWOD to extend the analytical models for handling the irregular droplet shapes during different stages of motion.

- In this work, different fabrication techniques were proposed on different substrates using different kinds of inks. Currently, new fabrication techniques, substrate material and conductive inks are introduced. We foresee great potential in following the printed electronics industry and the recent innovations and make use of it in the field of digital microfluidics. For example, carbon nanoparticles and new graphene
fabrication techniques are still under development. Accordingly, DMF chips can be fabricated using these techniques if they become more affordable and has the mass production capability.

- The experimental setup used in this work for changing the gap height cannot be used in portable devices. Recently, a piezoelectric system was used to change the gap height on a smaller scale compared to the experimental setup used in this work (Y. Li et al., 2016). However, piezo actuators require certain operating signals and voltages that may increase the complexity of the system and limit its portability. More research is needed to find other simple micro-actuators that can change the gap height in a portable device easily.
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Appendix A: Electrical circuits details for the portable device

The circuit is chosen in order to meet the following design considerations: high voltage output with high gain ratio, low power consumption in order to be easily powered by batteries, few number of components, low cost, compact and rapidly prototyped. The boost DC (Direct Current) to DC converter can run in two modes: Continuous Conduction Mode (CCM) and Discontinuous Conduction Mode (DCM). It is easier to generate higher voltages with larger amplification factor in the DCM mode. The voltage amplification ratio between the input and the output voltage without accounting for the losses can be expressed as (Kazimierczuk, 2008):

$$\text{gain} = \frac{V_o}{V_i} = \frac{1 + \sqrt{1 + \frac{2D^2V_o}{f_sLI_o}}}{2},$$  \hspace{1cm} (1)

where $V_o$ and $V_i$ are the output and input voltages, respectively, $f_s$ is the switching frequency, $D$ is the duty cycle, $L$ is the inductance, and $I_o$ is the output current.

The circuit in Figure A.1. is designed to amplify the battery voltage generated from the two small lithium ion batteries from 8 V approximate up to 800 V. The output voltage can be controlled by either changing the pulses switching frequency or the duty cycle. The pulses required for switching the MOSFET are generated from a 555 oscillator circuit working in stable operation mode as it is shown in Figure A.2. The frequency and the duty cycle of the generated pulses are calculated as

$$f = \frac{1.44}{(R_1 + 2R_2)C}$$ \hspace{1cm} (2)

and

$$D = \frac{R_1 + R_2}{R_1 + 2R_2}$$ \hspace{1cm} (3)
Figure A.1 Circuit diagram of the boost DC to DC converter used to generate high voltages from the battery source.

Figure A.2 555 timer circuit used to generate the pulses required for the switched mode power supply.

The circuit is constructed using the five following components: IRFBG30 Power MOSFET, 100 pF capacitor, 10 MOhm resistor, 10 mH inductor and a fast recovery diode 1N4937. The DC signals generated by the boost circuit can be used directly in actuating the droplets. However, some problems occur with prolonged actuation with DC signals such as electrolysis and charge trapping. Using the AC (Alternating Current) signals can solve these problems and they are usually preferred over the DC signals for droplet actuation. Therefore, an additional DC to AC converter circuit is used. This circuit includes a full bridge driver and 4 MOSFETs for chopping the DC signal to a square wave AC signal. The pulses required for operating this circuit are generated from a 555 oscillator circuit working in astable operation mode. A solid state relay (AQV259) is used for controlling the high voltage signal in the
switching circuit. The system is powered by small lithium ion batteries (Pukcell 3.7 V 2000 mAh). A 5 V voltage regulator IC (UA7805) is used to provide a stabilized power source for operating the microcontroller and the Bluetooth module safely from the slightly higher voltage output of the lithium ion batteries.