LAB ON CHIP FOR MULTIPLE DISEASE DETECTION BASED ON ELECTROWETTING

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The Degree of Master of Technology

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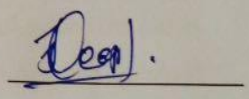
Declaration

I declare that this written submission represents my ideas in my own words, and where ideas or words of others have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be a cause for disciplinary action by the Institute and can also evoke penal action from the sources that have thus not been properly cited, or from whom proper permission has not been taken when needed



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Approval Sheet

This thesis entitled **Lab On Chip For Multiple Disease Detection Based On Electrowetting** by **Khandavalli Deep** is approved for the degree of Masters from IIT Hyderabad

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Dedicated to

This thesis is dedicated to my inspiring parents,
who have raised me to be the person I am today.
What I have and will accomplish, is only possible due to
their unconditional love, guidance, support and sacrifices.
Also, this thesis is dedicated to my teachers,
who have always been a great source of motivation
and inspiration. And to all my loving friends.

Abstract

Digital Microfluidics (DMF) is a new field of science and technology that introduces movement of nanoliter to microliter size droplets on patterned electrodes. Droplets can be moved, dispensed, merged, and split on devices. Sequential chemical reaction, and DNA extraction are examples of biological applications of DMF. Microfluidics-based biochips have varies applications like high throughput analyses, DNA sequencing, automated drug discovery, real time bio-molecular recognition, parallel immunoassays, single cell studies and protein RNA interaction and environmental toxicity monitoring. Discrete type flow uses array of electrodes, voltage to controlled droplet independently for the same application. The advantages of discrete type flow over continuous type are dynamic reconfigurability, reusable, control parameters are in electric domain, no mechanical parts and fault tolerance. The principle of electrowetting is used to deform and to actuate the droplet. The challenges with EWOD devices are the deciding threshold voltage, used for clinical diagnostic to protect the cell from damage, avoiding the cross talk between electrodes because of electrostatic effect to maintain droplet on proper path and the automated multiplexing technique use for switching the electrode voltage.

In this thesis, the design parameters, voltage and switching time is obtained by trial and error method to maintain the shape and path of the droplet. Fabrication of device shows the real time application. This data is farther used to simulate 2D mixing of dissimilar concentrated fluid.

Nomenclature

Symbol	Definition
μ	Dynamic viscosity
ρ	Density
γ_{SL}	Solid-liquid surface tension
γ_{LG}	Liquide-gas surface tension
γ_{SG}	Solid-gas surface tension
d	Dielectric layer thickens
U	Velocity scale
u	Vector velocity
p	Pressure
∇	Surface gradient operator
C	Capacitance
θ	Contact angle
n	Fluid concentration
M	Mesh flux
t	Time scale
D	Diffusivity
c	Diffusion constant

Contents

Chapter 1

Introduction

1.1 General

Advancement in micro-fabrication processes has led the concept of Electrowetting on dielectric (EWOD), one of the ideal paradigm for lab-on-a-chip systems based upon micromanipulation of discrete droplets. It enables control over fluid shape and flow by electrical signals alone, which is viable by effective utilization of the excess charge accumulation at the interface between the droplet and the dielectric surface, also by polarization of line tension at the three-phase line.

EWOD helps in discretizing the flow and hence it is called as Digital Microfluidic (DMF). DMF technology offers a platform for Lab-on-a-chip (LOC), which is concerned with the design of micro total analysis system (μTAS) for chemical and biological applications with the advantages of portability, higher sensitivity, reagent volume reduction, faster analysis, increased automation, low power consumption, compatibility with mass manufacturing, and high throughput. This emerging technology combines electronics with biology to open new application areas such as point-of-care diagnosis, on-chip DNA analysis, and automated drug discovery.

Recent year a great deal of scientific research is being committed to automate, integrate and miniature Microfluidic devices [1], [2], so that, it can be widely applicable and accessible. Various techniques such as Thermocapillary [21] Electrocapillarity [8] Electro-osmosis [9], Acoustics [10], Electric forces [11], Electromagnetic [12], Electrophoresis [13], Electrocapillary/Electrowetting [22]–[25]) have been reported to advance the Microfluidic devices, but among all EWOD is unique, because it is a direct way of controlling the surface tension of a fluid [17]. However Lab-on-chip (LOC) is such a device capable of handling a complete chemical or biological analysis protocol. One of the most prominent lines of research to attain this target consists in

manipulating discrete volumes of fluids called droplets rather than forcing continuous phase flow, thus Microfluidic based on Electrowetting is often referred to as Digital Microfluidics [2]-[6].

It has been reported by Center for Drug Research that, "The development of new pharmaceuticals is extremely expensive and time consuming. It takes an average of 13 years and more than 1 billion USD to develop a new drug. The single biggest factor driving this cost is the rate of failure, which about 90% of drug discovery project failing in the late clinical phases of development" [14].
Also the Research in the field of chemistry, biology and medicine (clinical and drug delivery) are lagging where the hundreds of reaction parallel need to take place simultaneously. These are the challenges that researchers are facing currently.

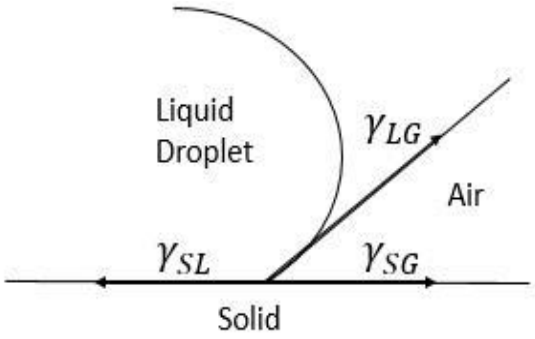


Figure 1: Three phase interphase line of solid, liquid and gas.

The challenges with EWOD devices are the deciding threshold voltage, used for clinical diagnostic to protect the cell from damage [15], avoiding the cross talk between electrodes because of electrostatic effect to maintain droplet on proper track and the automated multiplexing technique use for switching the electrode voltage.

In this paper, we efforts to address the challenges with DMF system to enhance the system performance and also for transporting and merging application, using multiphase simulation modules of COMSOL Multiphysics and Fabrication of the device. 2-D arrangement of all-in-single plane employed for simulation, resultant with ±0.2 deviation of anticipated result.

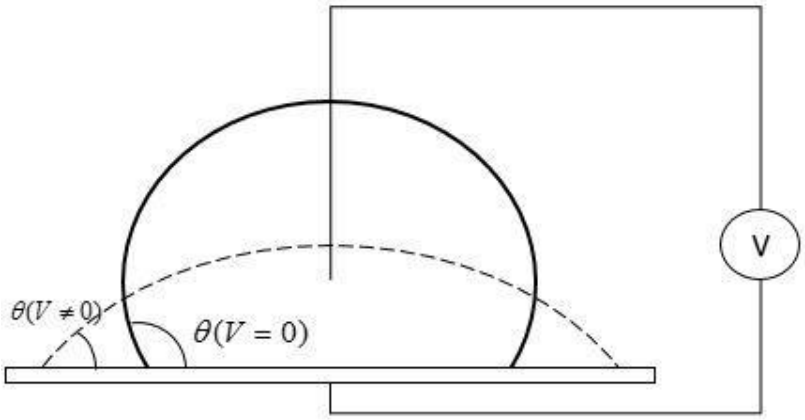


Figure 2: Electrowetting Principal.

1.2 Literature review

Shawn W. Walker, Benjamin Shapiro et al. (2009), work on the modeling and simulation of planar electrowetting on dielectric devices that move fluid droplets by modulating surface tension effects. They modeled fluid dynamic by Hele-Shaw type equation with a focus on inducing the relevant boundary phenomena. Also they include contact angle saturation and a contact line force threshold model that can account for hysteresis and pinning effects. Without them the simulations cab predict droplet motion that is much faster than in experiments (up to 10-20 times faster).To handle surface tension, conservation of mass and the nonlinear contact line pinning in a straightforward and numerically robust way they present a variational method and a corresponding finite element discretization. Finally they compare the simulation data to available experimental data for different cases of droplet motion like splitting and joining. As a result they obtain

Moving glycerin droplet, the applied voltage (50V) switches between the left and right electrodes every 2 s. Each electrode is square with a side length of 1.5mm. The large time scale because glycerin is highly viscous.Splitting water droplet, the three adjacent electrodes in a single row have the activation voltages of 25, 0 and 25V. Each electrodes is approximately square with a side length of 1.4 mm.Splitting glycerin droplet, the applied voltage (65V on the left and right electrodes) causes the droplet to be pulled apart and eventually split. Each electrodes is approximately square with a side length of 1.5 mm. The relaxation time after pinchoff is 1100ms.

Joining water droplets, the applied voltage 65V on the center electrode only cause the two droplets to flow together and eventually marge. Each electrodes is approximately square with a side length of 1.5 mm.

Tsung-Yu Ho, K. Chakrabarty et al. (2011) pointing the challenges facing biochips are similar to those faced by microelectronics some decade ago. To meet those challenges of increasing design complexity, they proposed computer-aided-design (CAD) tools for digital microfluidic biochip (DMFB). There paper provides an overview of DMFBs and describes emerging CAD tools for the automated synthesis and optimization of DMFB designs, from fluidic-level synthesis and chip-level design to testing. With the assistance of CAD tools, user can concentrate on the development and abstraction of nanoscale bioassays while leaving chip optimization and implementation details to CAD tools.

D. Chatterjee, B. Hetayothin et al. (2006), reported for the first time that it is possible to manipulate droplets of organic solvents, ionic liquids, and aqueous surfactant solutions in air by these mechanisms using only modest voltages (<100 V) and frequencies (<10 kHz). The feasibility of moving any liquid can be predicted empirically from its frequency-dependent complex permittivity, □ *. The threshold for droplet actuation in air with our two-plate device configuration is | □ *| > 8 6 10211.

Yen-Chen Lin, Kai-Cheng Chuang et al. (2006), are investigated on EWOD (electrowetting-on-dielectric) and LDEP (liquid dielectrophoresis) to provide digital and analog microfluidic functions on an integrated chip respectively. They succeed to integrate EWOD and LDEP on a single chip. EWOD is the diving mechanism for droplets in digital microfluidics. To provide sufficient voltage without electrolysis, 1 kHz signal was desirable for EWOD. EWOD actuation with different frequency, dielectric and surrounding media were tested. At 100 kHz, the EWOD actuation is less effective since the voltage across the dielectric is reduces. Moreover at 100 kHz, the expelling of satellite droplets was found. LDEP was found when manipulate liquid droplet in oil by applying 100 kHz. Three fundamental tools of integrated digital and analog microfluidics were developed in this paper, including a digital-to-analog microfluidic converter, an analog to digital microfluidic converter, and a valve.

Hyejin Moon and Sung Kwon Cho (2002), discusses and experimentally verifies how to lower the operating voltage that drives liquid droplets by the principle of electrowetting on dielectric (EWOD). Typically, much higher voltages (>100 V) are used to change the wettability of an electrolyte droplet on a dielectric layer compared with a conductive layer. Using very thin (700 Å) and high dielectric

constant (~180) materials, they achieved a significant contact angle change (120° to 80°) has been achieved with voltages as low as 15 V. A contact angle change of (120° to 80°) could be obtained at about 25 V for the SiO2 films. A high dielectric constant material can reduce the EWOD voltage even further. By incorporating BST (=180) as the main insulation layer, they succeeded in obtaining a contact angle change of (120° t0 80°) with only 15 V. To demonstrate the utility of this result, they applied the findings to the microfluidic device, successfully driving nanoliter-scale water droplets in a microchannel with only 15 V.

Roland Baviere, Jerome Boutet, et al. (2008), reports on the dynamics of droplets in the capillary regime induced by electrowetting-ondielectric actuation. They used side-view observations of stroboscopic recording techniques to measure and analyses droplet deformations as well as the front and rear apparent contact angles during motion. Secondly, the influence of viscosity on the droplet velocity as a function of the applied voltage was studied. Finally, the influence of the dielectric thickness on the droplet dynamics was studied. They studied Influence of viscosity on the velocity variation as a function of actuation voltage, for water/glycerol mixtures.

Real apparent contact angle measured at the transition between phages.Comparison for water droplet between apparent static and dynamic contact angles.Comparison of experimental data with Brochard's model (numerical parameters in the text) Comparison between static and dynamic electrowetting for a water droplet.Effect of dielectric and hydrophobic layer thickness on the velocity variation of a water droplet as a function of actuation voltage.

Liguo Chen, Xiawei Xu, at el. (2013), demanded the root reason for EWOD is the instantaneous pressure difference inside a droplet, which was obtained by means of numerical simulation. First, based upon the theory of EWOD, they design a graphical model of EWOD using VOF method. Next, driving that two kind of fluid which should follow the law of muss conservation and principle of momentum conservation. They conclude, In one period of motion, the highest pressure region inside a droplet will keep changing and transferring along with the driving time until a steady state of pressure difference is obtained, beside the much longer driving time is, the much larger pressure difference will be inside a droplet.

1.3 Objective and Scope

Based on the literature review, importance and challenges the objective of the project is as

- Change in droplet shape or contact angle by applying the electrical voltage to the dielectric coated electrode.
- Analysis of voltage distribution in EWOD system. To study the capacitance effect and velocity profile of droplets.
- Moving the droplet from one electrodes to others by switching the voltage from current electrode to adjacent electrode.
- Designing 2-d and array of electrodes platform for simulation.
- Selecting the suitable design parameter, material and methodology, so that the threshold voltage would be as minimum as possible to protect cells from damage. Avoiding the cross talk between electrodes to maintained droplets over proper track.
- Efficient switching addressing of the electrodes for multiplexing large number of switch by having less number of input pin
- And can be fabricated using micro/nano fabrication technology.
- Droplets can split, can be created by integrating reservoir with microfluidic platform.
- Numerous chemical and biological experiment can perform on thesameplatform

Chapter 2

Theory

2.1 Microfluidic biochips

Microfluidic biochips (also known as lab-on-a-chip) are an alternative to conventional biochemical laboratories, and are revolutionizing many application, such as molecular biology procedures, DNA analysis, proteomics (the study of proteins) and clinical pathology (diagnostic of diseases). They are becoming increasingly complex, with thousands of components, but are designed manually (called bottom-up full-custom design), which is extremely labor intensive and error prone. There are two type of biochips, microarray and microfluidic biochip. Microarray is again classified as DNA array and Protein array. Whereas microfluidic biochip are of continuous type and discrete type microfluidic. The continuous type flow can be carried out by using permanently etched channel, micro pump, micro valves and micro channels. On the other hand discrete type or digital microfluidic have all the control parameter in electrical domain, no mechanical component involvement in the process. Also each droplets controlled independently over the m×n array of electrodes. The advantages of discrete type flow over continuous flow are reusability, re-configurability and electrical domain parameters. The challenges facing biochips are similar to those faced by microelectronics some decades ago. A typical microprocessor today has over a billion transistors. Such a design complexity is possible because engineers are using Computer-Aided Design (CAD) tools, which, starting from a specification of the desired functionality, automatically build the best possible design (such a process is called top-down design). As in the microelectronics area, Comsol tools will reduce the development costs, increase the design productivity and yield, and are the key to the further growth and market penetration of biochips. My research vision is

to develop a design flow for biochips, which, starting from a system specification can automatically derive a physical biochip design.

2.2 Droplet actuation

There are several technique has been proposed for actuating the droplet like Electric forces, Electromagnetic, Electrocapillarity, Electro-osmosis,

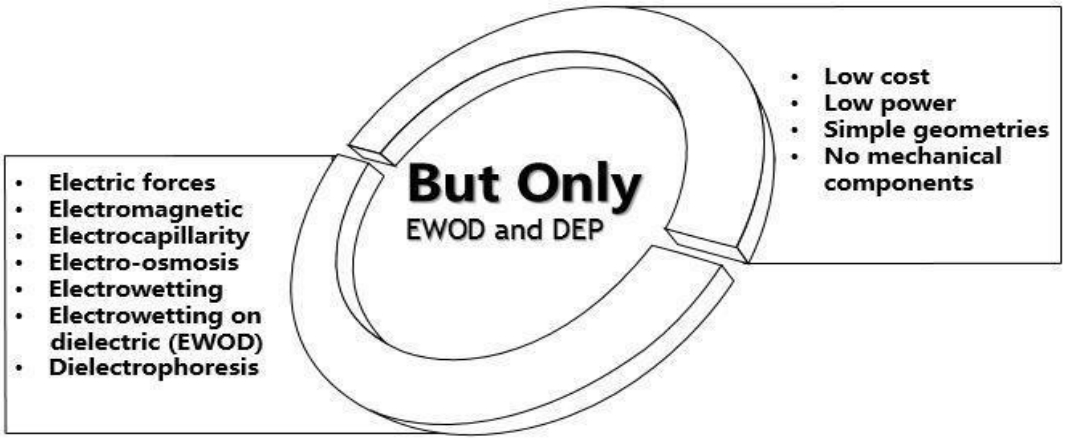


Figure 3: Available technique and there advantages.

Electrowetting, Electrowetting on dielectric (EWOD) and Dielectrophoresis (DEP) but among all the EWOD is unique, because of Low cost, Low power, Simple geometries and No mechanical components. The Electrical methods for droplet actuation are DEP and EWOD. DEP uses High frequency ac & high amplitude voltages Ac voltage of 230-300Vrms at 50-200 kHz. Whereas EWOD uses Low frequency (<1 kHz) Low amplitude of DC voltages. The advantages of EWOD over DEP are almost negligible Joule heating effect, direct way of controlling the surface tension Helping the multiple droplets to act like small micro reactors.

2.3 The Principle of EWOD

Lippmann proposed that by applying external voltage across the capillary, an electrostatic field will be generated and because of electrostatic charge interfacial tension between liquid-gas and liquid-substrate will changed [16], eventually resulting in deformation of a liquid droplet (Figure 2), This phenomenon of droplet deformation due to change in contact angle effected by applied voltage is referred to as electrowetting on dielectric (EWOD). Because of applied voltage two phenomenon may occurs, one is droplet will change its shape and deform if the voltage applied to the same electrode, or else droplet will deform at the same time will move to adjacent electrodes if applied voltage

is to the next or adjacent electrode. Because of electric voltage there will be electric field, and there is chance that droplet will undergo to electrolysis if the applied voltage is high. So to protect the droplet from electrolysis the dielectric layer is used on the top of the electrodes so that the electric field will be less and joule heating effect. Also the top of the microfluidic platform need to be hydrophobic to sustain the droplet shape.

2.4 Droplet Deformation

When a potential is applied, an electric field will generate (because of excess charge accumulation at three phase line tension [14]-[17], [25] or polarization of dielectric layer [7]), which lead the change in interfacial tension or surface tension between the liquid-gas and liquid-solid, resulting as change in contact angle or droplet deformation. The solid-liquid interfacial tension (γ_{SL}) (Figure 1) can be controlled by the electrical potential across the interface, and the result is expressed by using the Lippmann's Eq. (1) [16]

$$\gamma_{SL}(V) = \gamma_{SL}(0) - \frac{1}{2} CV^2$$

Where C (F/m2) is the capacitance of the dielectric layer, V is the applied voltage and $\gamma_{SL}(0)$ is the solid-liquid interfacial tension at potential zero.

The contact angle (θ) of droplet at the interface of dielectric layer can be measure by Young's Eq. (2) [19]

$$\cos\theta = (\gamma_{SG} - \gamma_{SL}) / \gamma_{LG}$$

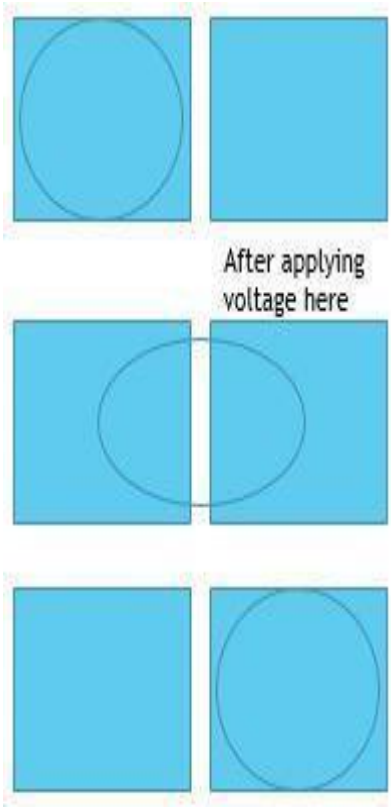
Where γ_{SG} and γ_{SL} are the interfacial tension of solid-gas and liquid-gas respectively. Incorporating the Young's Eq. to the Lippmann's Eq. (3), the resulting the change in contact angle $\theta(V)$ due to applied potential (V), and hence called Lippmann-Young equation

$$\cos\theta(V) = \cos\theta(0) + \frac{\varepsilon_0 \varepsilon}{2\gamma_{LG} d} V^2$$

Here, $\theta(V)$ is the resting contact angle i.e. without any applied potential. ε ($8.85 \times 10\text{-}12$F/m) is the permittivity of the free space, ε is the dielectric constant of dielectric layer, and d is the thickness of the dielectric film. Note that γ_{SG} and γ_{LG} assumed to be constant i.e. independent of applied potential [20]

2.5 Droplet Transport

A droplet can be transported by switching the voltage from current electrode to adjacent electrode, this cause asymmetrical change in the three phase line tension. No matter which method use for changing the interfacial tension (e.g., Electrocapillarity [8] Electro-osmosis [9], Acoustics [10], Electric forces [11], Electromagnetic [12], Electrophoresis [13] Thermocapillary [21] or Electrocapillary/Electrowetting[22]–[25]), the asymmetrical change in the interfacial tension induces an asymmetrical deformation of the liquid meniscus, which establishes a pressure difference inside liquid, gives a bulk fluid movement [26], which can be predicts using Brochard's model Eq. (4) [22], [27].



$$U = \frac{\varepsilon_0 \varepsilon \,(1-\cos\ \theta\ (V\ ))}{6\ \mu d l \sin\theta(V)}\ V^{\,2}$$

Where μ and l are the viscosity of the droplet and an empirical factor respectively.

Chapter 3

Modeling

The modeling of geometry in 2-D.We designed the 2-D geometry of single droplet on the single electrode platform to calculate and find out the suitable design parameters. Then we continue the same with droplet packet transformation on a single droplet on single line planer four electrodes

In this section, we discuss geometry design, Fluidic flow pattern and Boundary condition, COMSOL Physics used for simulation and electrode addressing.

3.1 Geometry (2-D)

The principal of various fluidic-operation is droplet manipulation. Although various geometry with driving factor has been proposed [28], [29]. The EWOD chips have much more attention because of portability, higher sensitivity, reagent volume reduction, faster analysis, increased automation, low power consumption, compatibility with mass manufacturing, and high throughput [25], [30] and also for direct way of controlling the surface tension of a fluid [17]. Figure 4(left) is used for performing the Lippmann-Young equation principal i.e. change in contact angel. The Brochard's model has been implemented on Figure 4(right). The critical and suitable geometry parameter has been derived using both the Figure 4(right and left). Figure 5(right) represent the schematic view of the EWOD platform. 'T' shape platform (Figure 5(left)) is designed for testing and simulation of droplet transport and mixing. The simulation for change in contact angle has been carried out on Figure 4(left) platform whereas Figure 4(right) geometry has used for finding the saturation voltage.. The principle of Lippmann-Young equation and Brochard's model has been implemented to deformation and transportation respectively. The width and length of the electrodes are 5µm and 5.5µm respectively for 1-6 electrodes, for 8-11 electrodes it is 6.7µm and 7µm respectively and for 7 it is 5µm and 7µm respectively. The different size of electrodes are chosen as the fluid volume is different at different time and places.

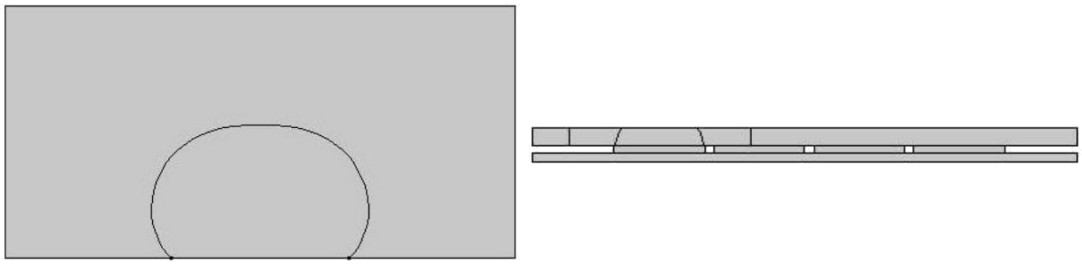


Figure 4: 2-D geometry for contact angle simulation and droplet packet transform.

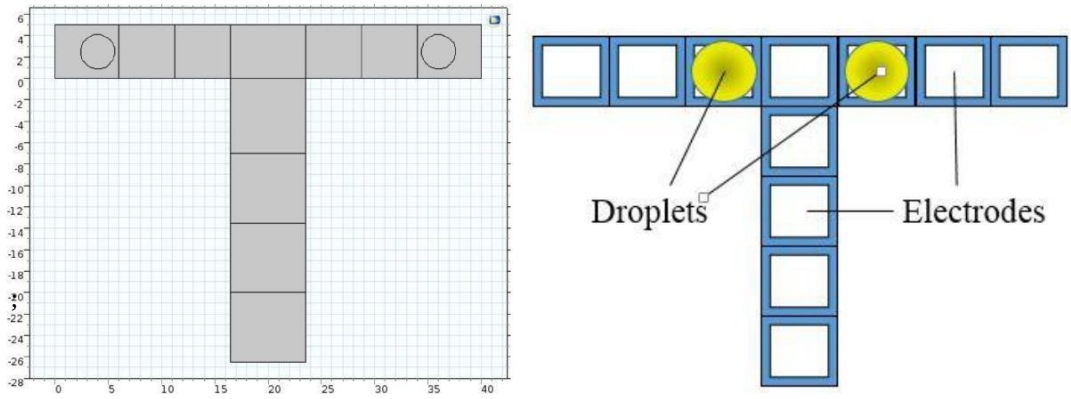


Figure 5: 2-D geometry for mixing application

3.2 Fluidic flow pattern and Boundary condition

Assuming fluid flow as Incompressible flow and also neglecting the inertial term (stokes flow), the Navier–Stokes equation can be expressed as Eq. 5 where, U is the fluid velocity, ρ is the fluid density, p is the pressure, μ is the dynamic viscosity, F is the represents body forces per unit volume and ∇ is the vector operator

$$\rho \frac{\partial U}{\partial t} + \rho(U \cdot \nabla)U = -\nabla p + \mu \nabla^2 U + F$$

$$\nabla \cdot U = 0 \qquad (5)$$

Convection Diffusion equation has been used for mixing two different concentrated fluid. The droplets have the concentration 0 and 1 mol/m^3 respectively.

$$d_a \frac{\partial con}{\partial t} + \nabla \cdot (-c\nabla con) + \beta \cdot \nabla con = f$$

$$\nabla = [\frac{\partial}{\partial x}, \frac{\partial}{\partial y}] \qquad (6)$$

Where d_a is the damping coefficient, β is the convection coefficient, c is the diffusion coefficient, and *con* is the concentration, f is the source term. All the values of coefficient are one.

The liquid-air interface can be defined for time dependent solution as Eq. (7)

$$u_1 = u_{2,1}\, n_1 \cdot T_1 = \sigma(\nabla_t \cdot n_1) n_1 - \nabla_t \sigma$$

$$u_1 = u_2 + M_f \left(\frac{1}{\rho_1} + \frac{1}{\rho_2}\right) n_1 \qquad (7)$$

$$u_{mesh} = (u_1 \cdot n_1) - \frac{M_f}{\rho_1} n_1$$

Where ρ_1 and ρ_2 are the density of the fluid and air. u_1 and u_2 are the velocity of fluid and surrounding air respectively. n_1 and n_2 are the concentration of respective fluids. M_f is the mass flux, which we chosen zero, then the Eq 7 can be simplified as

$$u_1 = u_2$$

$$u_{mesh} = (u_1 \cdot n_1)$$

The fluid and dielectric interface defined $\theta_c = \theta_w$, where θ_w is represented in as Eq. 3

The Navier–Stokes equation is used for calculating surface velocity profile of the droplet, then that velocity coupled with level set method and convection diffusion equation for transporting the droplet with deformation and mixing application. The level method expression for time dependent and incompressible fluid is as follows.

$$\rho \frac{\partial u}{\partial t} + \rho(u.\nabla)u = \nabla \cdot [-pI + \mu(\nabla u + (\nabla u)_T)] + f$$

$$\nabla \cdot u = 0$$

3.3 Parameter and Meshing

The droplet is chosen as water droplet having the dynamic viscosity of 1.002 Pa-s. The electrodes material chosen as copper and top hydrophobic material as ITO. The meshing element, grid and degree of freedom is different for different geometry. The geometry of single electrodes change in contact angle have the total number of meshing element are 15657and edge element are 671. The packet transform geometry has total number of element 27601. The 3-D geometry of change in contact angle of 43962 element. Whereas for packet transform of 3-D

geometry has 455181 element. The 2-D convection diffusion mixing geometry have the total number of meshing element of 67526. The selective meshing has been done for all geometry. The selective meshing have the certain advent of less number of meshing element will be less, less simulation time, good resolution andgoodcontrastetc.

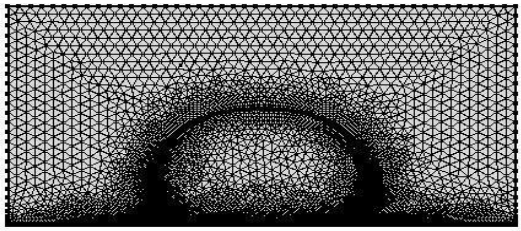


Figure 6: 2-D meshing for change in contact angle

Figure 7: 2-D meshing for droplet packet transform.

3.4 Electrode Adressing

It is a method to apply and switch the potential from current electrode to adjacent electrode. The efficient switching mechanism is required for mixing number of fluids. The efficient mixing can be possible by varying the space and time parameter. The Lippmann-Young Eq. can been simulated only by applying and removing potential from the same electrode with respect to time parameter [32], whereas droplet transport and mixing has been simulated by switching the potential from current electrode to adjacent electrode with respect to space and time parameter (Figure 14). The time delay for switching and the electrodes activation time is very crucial parameter for application such as mixing and transport. The matalab interphase is coupled with comsol for switching the electrode voltage. Each and every electrode is allocated with specific parameter. Those parameter are called for activation and deactivation of the electrodes. The activation time is 0 Sec and delay/pause time is 0.07 Sec. By various testing and simulation we have obtained the effective delay of 0.07 s.

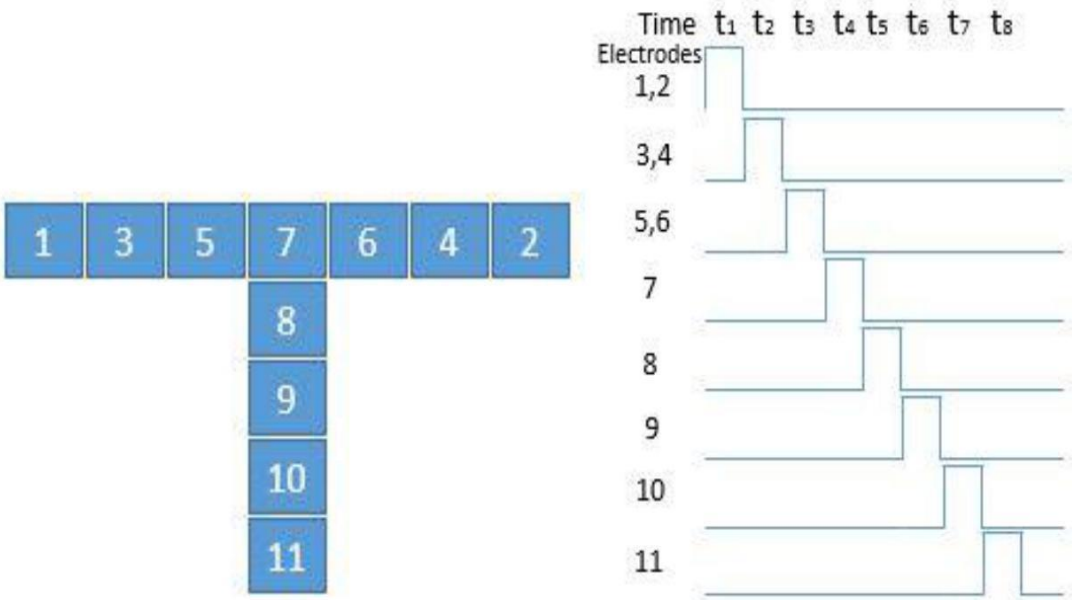


Fig.8: Diagramatic View of eletrodes wetting

```
%-- connect to the COMSOL a = function retval ('COM5.0')

 %-- specify pin mode a.pinMode(1-10,'input');
 a.pinMode(11,'output');

 %-- digital i/o

Elec1 = '1,2';

switch(elec1)

 a.digitalRead(1,2) % read pin 1,2 a.digitalWrite(13,0)
 % write 0 to pin 13 case 'A'

 Activate (1,2)

Deactivate (3,4)

Fprintf (1 & 2 are activated);1

Pause(0.07) %in seconds
```

Table 1: Electrode addressing

Electrodes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
	1	1	0	0	×	×	×	×	×	×	×
	0	0	1	1	0	0	×	×	×	×	×
Activation	×	×	0	0	1	1	0	0	×	×	×
Sequence	×	×	×	×	×	0	1	0	×	×	×
	×	×	×	×	×	×	0	1	0	×	×
	×	×	×	×	×	×	×	0	1	0	×
	×	×	×	×	×	×	×	×	0	1	0
	×	×	×	×	×	×	×	×	×	0	1

```
switch(elec4)
 a.digitalRead(7) % read pin 7
 a.digitalWrite(11,0) % write 0 to pin 11
 case 'A'

 Activate (7)

Deactivate (5,6,8)

Fprintf (7 are activated);

Pause(0.07) %in seconds


Elec5 = '8';

switch(elec5)
 a.digitalRead(8) % read pin 8
 a.digitalWrite(13,0) % write 0 to pin 13
 case 'A'

 Activate (8)

Deactivate (7,9)

Fprintf (8 are activated);

Pause(0.07) %in seconds


Elec6 = '9';
```

```
switch(elec6)
a.digitalRead(9) % read pin 9
a.digitalWrite(11,0) % write 0 to pin 11

  case ‘A’
 Activate (9)

Deactivate (8,10)

Fprintf (9 are activated);

Pause(0.07) %in seconds
Elec7 = '10';

switch(elec7)
a.digitalRead(10) % read pin 10
case ‘A’

Activate(10)

Deactivate (9,11)

Fprintf (10 are activated);

Pause(0.07) %in seconds

Elec8 = '11';

switch(elec8)
a.digitalRead(11) % read pin 11
a.digitalWrite(11,0) % write 0 to pin 11
case ‘A’

Activate (11)

Deactivate (10)

Fprintf (11 are activated);

Pause(0.07) %in seconds

%-- close session delete(a)
```

digitalWrite(11,0) % write 0 to pin 11

The total switching time is of 0.49 Sec for mixing two fluid. While activating some electrode the adjacent electrodes must be deactivated and are in don't care condition.

3.5 COMSOL Physics for simulation

AC/DC, Fluid Flow and Chemical Species Transport physics are used for simulation in COMSOL platform. In particular we use Electric Current (ec), Laminar Two-Phase Flow, Moving Mesh (tpfmm) for simulation of Lippmann-Young equation i.e change in contact angle for droplet deformation, additionally Level Set (tpf) with Electric Current (ec) for simulation of 2D droplet transport. This physics are used for 2-D. The surface velocity is calculated by Laminar Two-Phase Flow, Moving Mesh (tpfmm) using navier stokes equation then that coupled with level set method and convection diffusion equation for transporting and mixing droplets respectively.

Chapter 4

Result and Discussion

The change in contact angle and transportation of a single droplet has been simulated to find the proper set of design parameter and the voltage criteria. It has been studied that the critical voltage is of 12.7 V whereas the saturation voltage is 17.5 V. Also we studied for other intermediate voltages. Above 17.5 V the droplet shape is gating distorted. Element size scale factor for meshing the simulation of Lippmann-Young equation was chosen as 0.2, whereas the scale factor chosen for the study of droplet transport was 0.5. Navier Slip boundary condition was implemented to represent the liquid-solid interface for both the cases of simulation. The prescribed mesh displacement property applied to the left and right boundary for the simulation of Lippmann-Young equation.

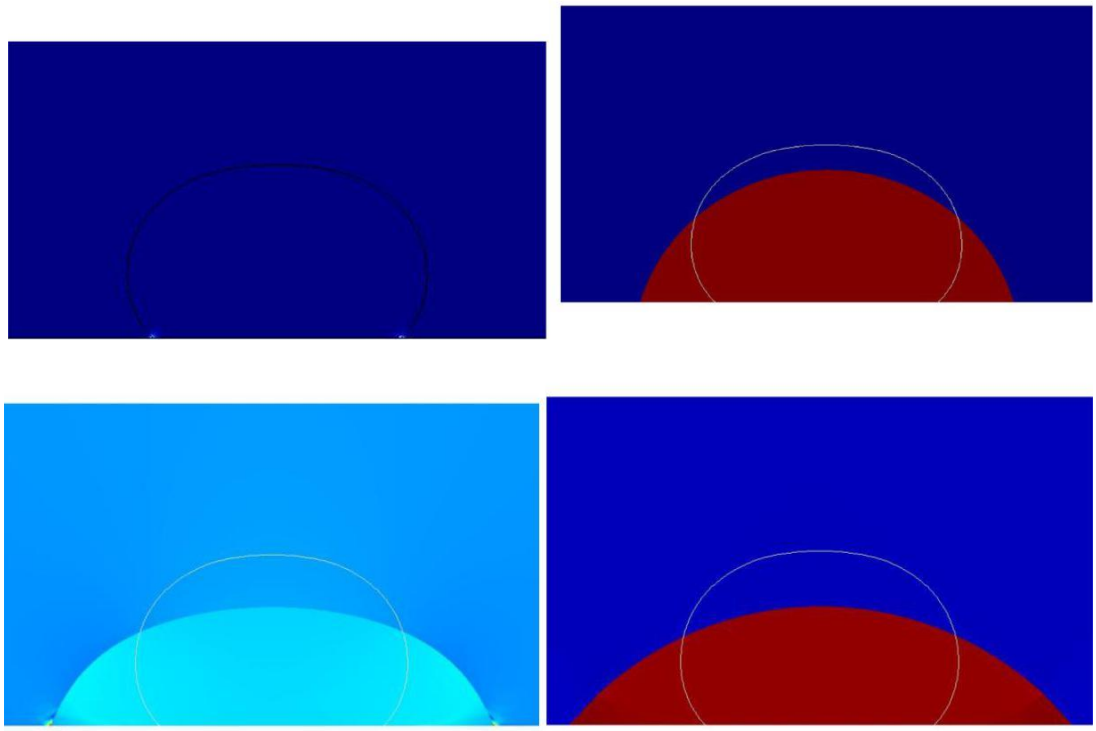


Figure 9: Contact angle with respect to 0, 12.7, 15 and 17.5 V respectively.

The below colors indicating the electric field distribution across the fluid. Result shows effective change in contact angle taken place from 12.7 V to 17.5 V, behind the 17.5 V there is no change in the droplet shape i.e. saturated but because of high electric voltage stronger electric field distribution.

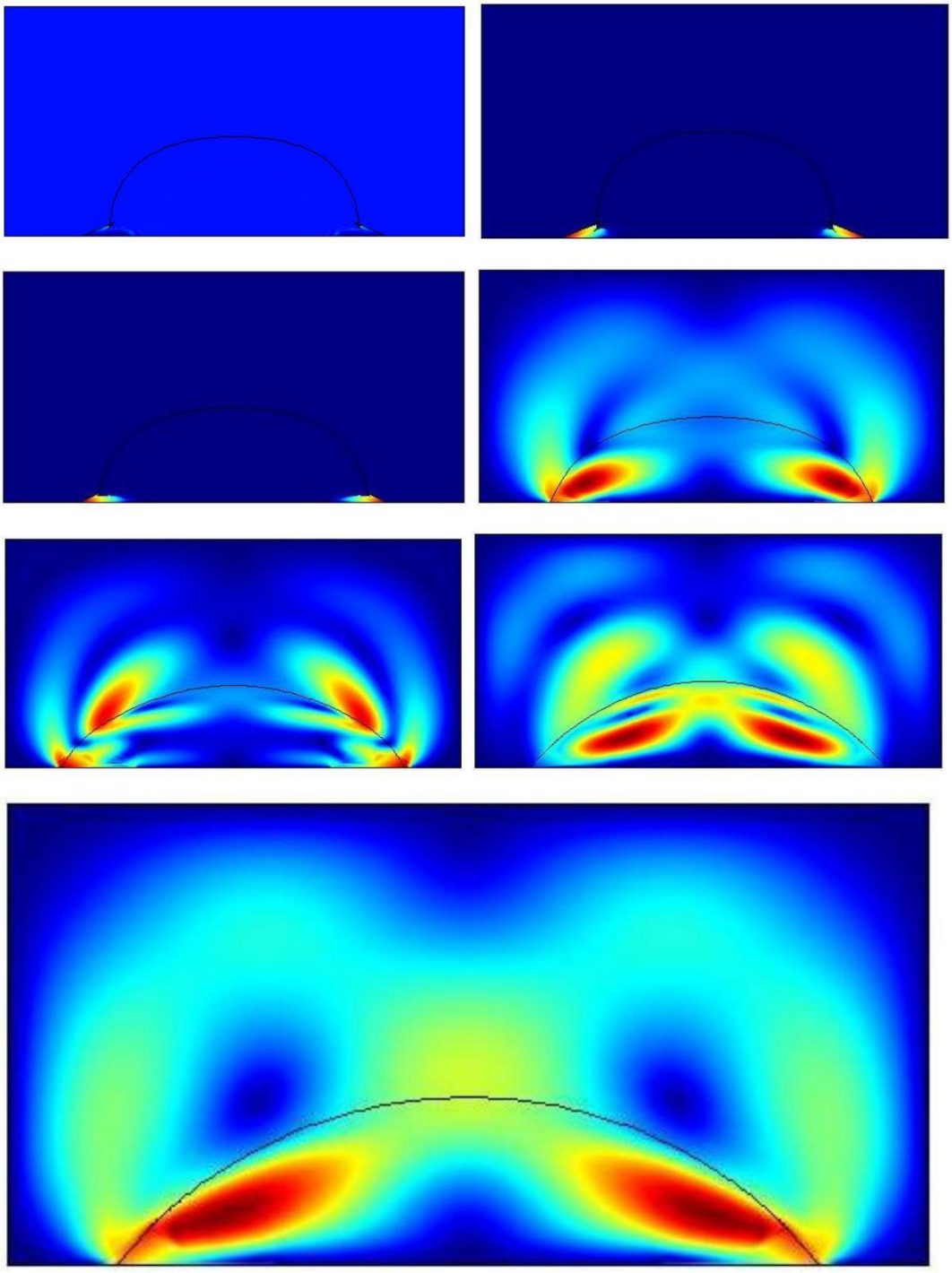


Figure 10: Electric field simulation. The droplet is getting saturated but still there is a change in electric field.

To maintain the shape of the droplet another simulation has been performed, where it studied that above certain voltage no more shape deformation, but if the voltage farther increase beyond certain value then shape of the droplet will get distorted. The saturation voltage is same as the 2-D contact angle simulation i.e. 17.5 V, but the maximum limit is of 22 V.

Figure 11: Droplet shape is getting distorted behond 22 V.

The change in contact angle with respect to 0, 12.7, 15 and 17.5 V is shown in figure 15. The study has been continued for 14.9 V and 15.3 V. The change in contact angle and the relative change in contact angle has been recorded and listed on below table.

Table 2: Relative Change in Contact angle with respect to voltages.

Voltage (V)	**Contact angle (θ)**	**Relative Change in contact angle (θ)**
0	130^{o}	0^{o}
12.7	126^{o}	4^{o}
14.3	114^{o}	10^{o}
15.9	90^{o}	24^{o}
17.5	51^{o}	39

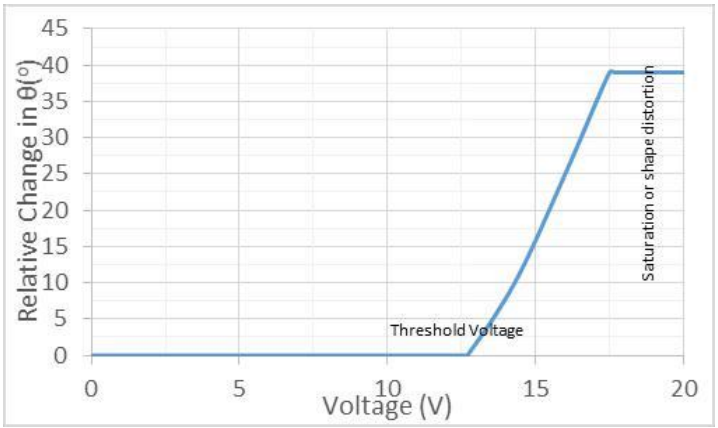


Figure 13: Contact angle modulation efficiency plot.

The graph is showing the relative change in contact angle Vs voltage. Operating voltage must lies on the slope line.

An arrangement containing four electrodes in line. The simulation results on single droplet transport and switching at different time in below Figure 19 (a), (b), (c), (d), (e), (f), (g) and (h) respectively by applying wide range of voltage. Droplet packet transform has been simulated and the switching delay time is 0.002 Sec. The experiment has been done for forward and reverse transform.

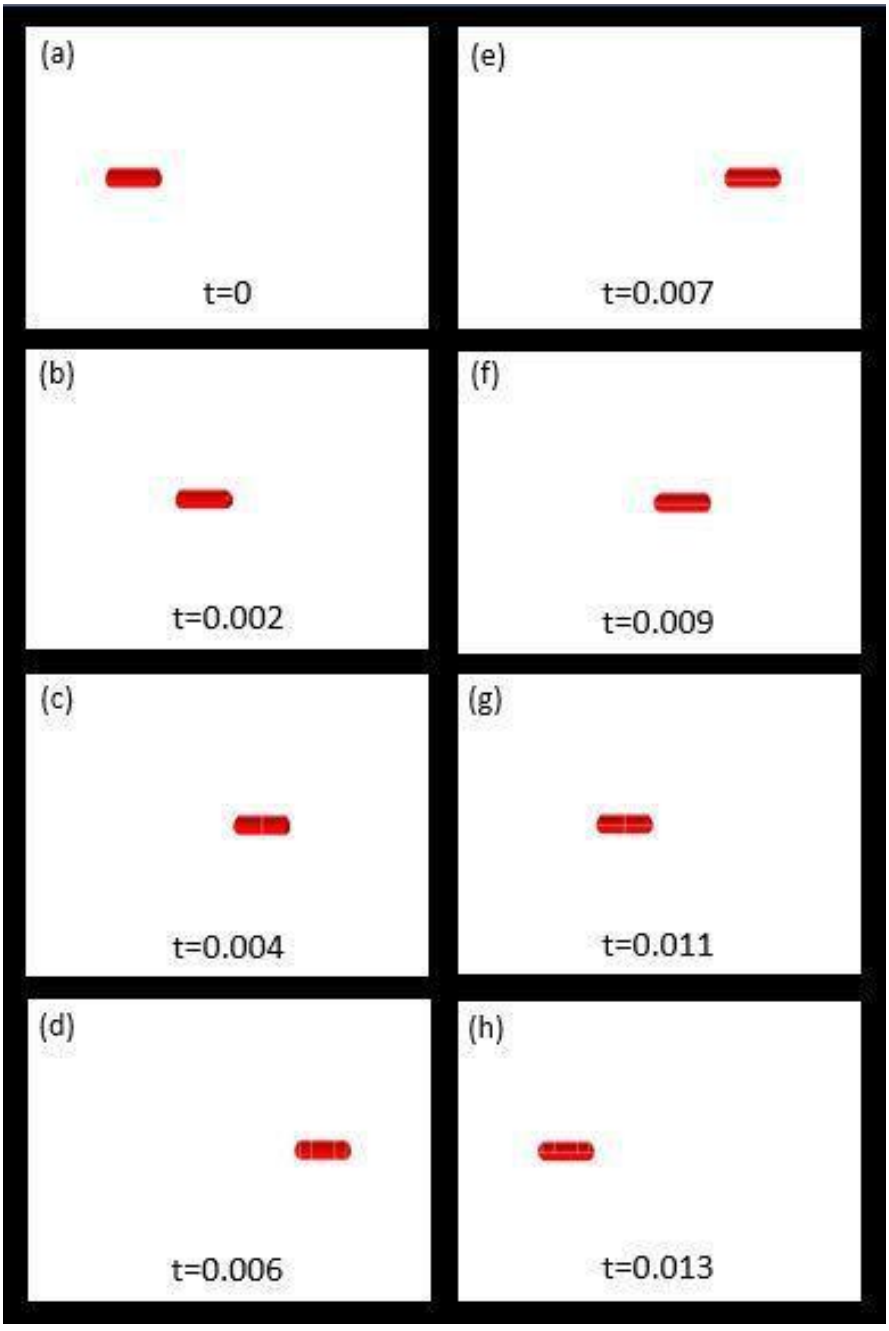


Figure 14: (a), (b), (c) and (d) are the position of the droplet when the compounding electrode voltage is 15V and others are at 0V.

Chapter 5

Fabrication

5.1. Photolithography

Digital Microfluidic devices are fabricated by photolithography, and etching in cleanroom facilities. Photolithography is a process, which is used for microfabrication. In this process you can selectively coat, or remove parts of thin films on substrates. During photolithography a pattern is transferred from a photo mask to the surface of the substrate. Steps of photolithography include:

- Wafer cleaning: Wafers are chemically cleaned in order to remove any extra, and unwanted fine dirt, and particles on the surface.
- Photoresist coating: photoresist is applied to the surface to make the surface photo sensitive.
- Soft baking: Essential step during which almost all the solvents are removed from the photoresist coating, and the photoresist becomes photosensitive, or imageable.
- Mask alignment: this step is also crucial in the patterning process of the substrate. A mask is a square plate that has the desired pattern of metal film on one side. The mask should be precisely aligned with the wafer so that the pattern is transferred onto the wafer surface.
- Light exposure: After aligning the mask with the wafer, the photoresist is exposed to ultraviolet light. This exposure takes place through the metal pattern on the mask.
- Development: Depending on the type of the photoresist being either positive, or negative, the part, which is exposed, and unexposed to the light remains, or is removed from the substrate respectively. Figure 5 explains the difference between positive and negative photoresist.
- Hard baking: Hard baking is the last step of photolithography. This step is also necessary and it hardens the photoresist, and improves the adhesion of the photoresist to the surface of the substrate

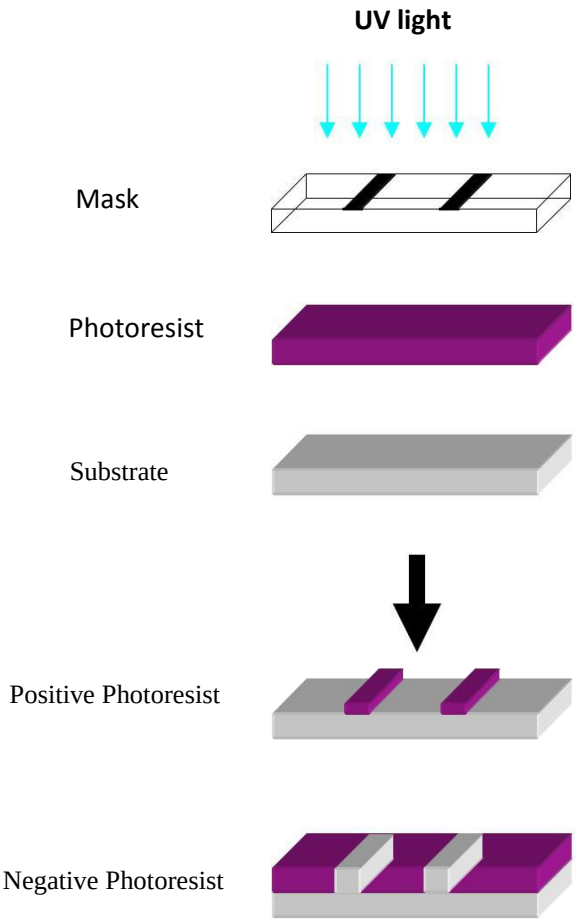


Figure 15: Positive and Negative photoresist comparison

5.2. Cleanroom Procedure

Devices for this experimental project were fabricated in IIT Hyderabad Nano-X lab where cleanroom facility is available.The procedure starts with cleaning the Quartz wafers in piranha solution for 10 min. Piranha solution was prepared as a 3:1 (*v/v*) mixture of sulfuric acid, and hydrogen peroxide. Then the wafers were coated with Chromium (270 *nm*) by electron beam deposition. Next step was to clean the wafers with rinsing in DI-water followed by nitrogen gun drying. Afterwards, the substrates were primed by spin coating with HMDS

(3000 *rpm*, 30 *s*). After priming, photoresist was coated on the wafer using again spin coating (3000 *rpm*, 30 *s*). Next step was to pre-bake the substrates on a hotplate at 100 °C for 2 *min*. Then we reach the step of UV light exposure. The wafers were aligned with the mask and exposed to UV radiation (35.5 $mW\ cm^{-2}$, 365 *nm*, 4 *s*) through a photo mask using mask aligner. Next step after the exposure was to develop the wafers in MF-321 for 3 *min*. Afterwards, the wafers had to be post-baked on a hot plate at 100 °C for 1 *min*. At this point the photolithography section of the fabrication is completed.

After completing photolithographic steps, the substrates were dipped in chromium etchant for 30 *s*. Then after etching the undesired chromium away, the remaining photo resist was stripped in AZ-300 by immersing the substrates in the solution for 10 *min*. Last step before coating the insulating and hydrophobic layers of the devices is cleaning them in piranha solution for 30 *s*. The schematic diagram of the photolithography procedure is shown in figure

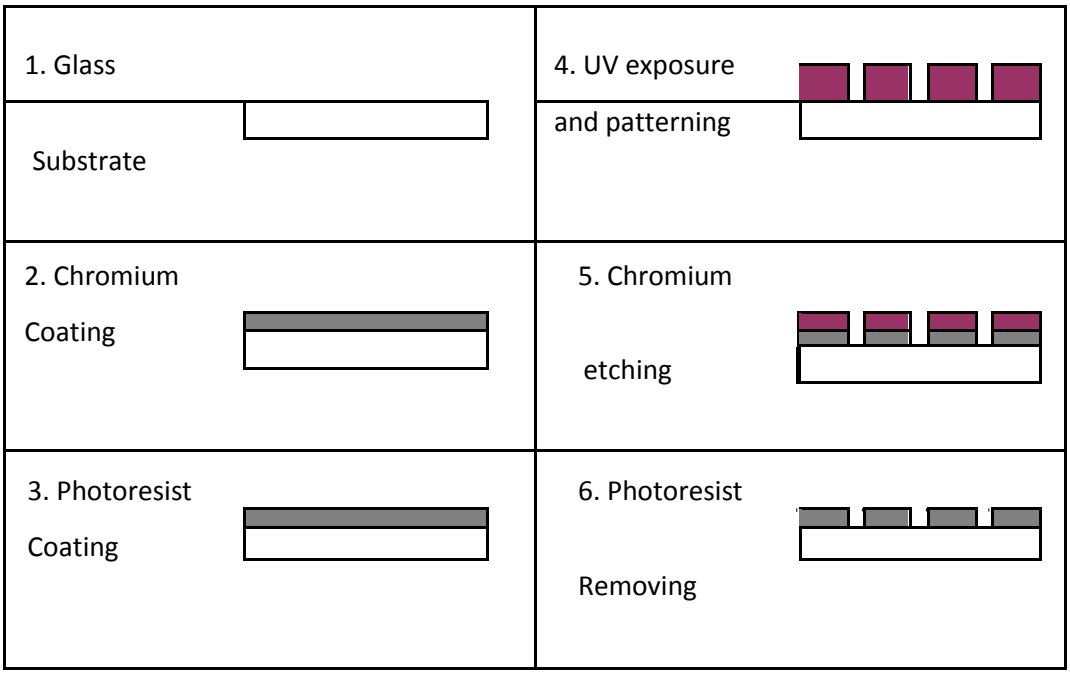


Figure16 : Cleanroom fabrication

5.3 Fabrication Procedure For Electrowetting

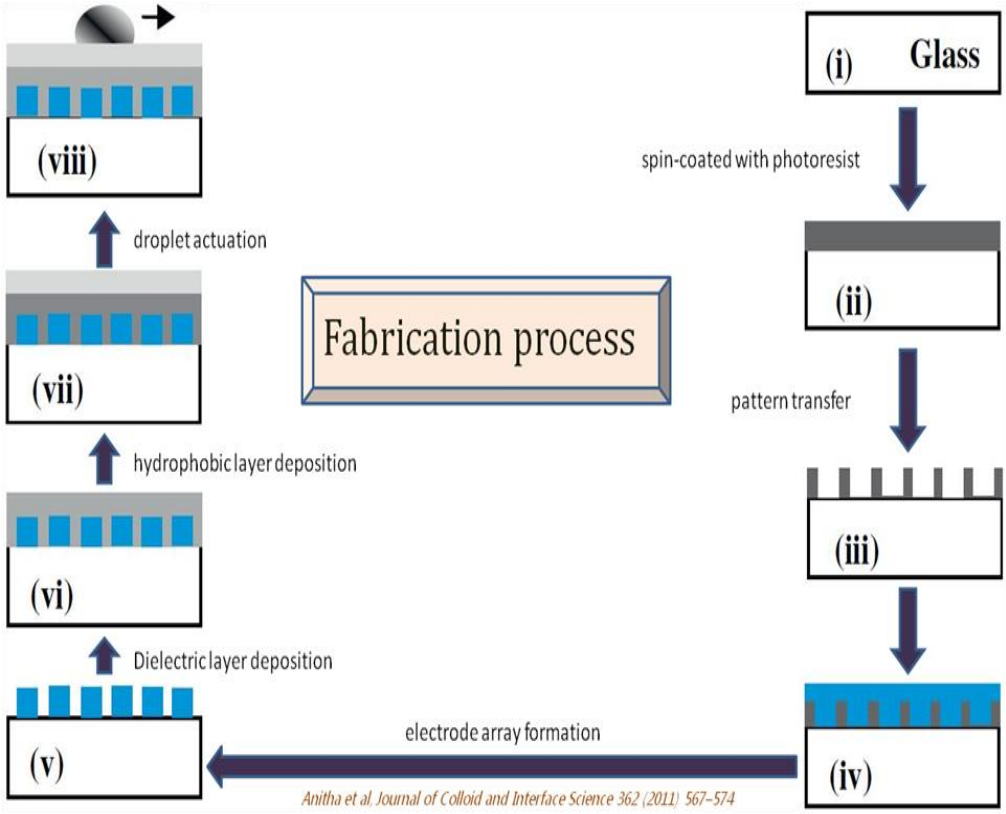
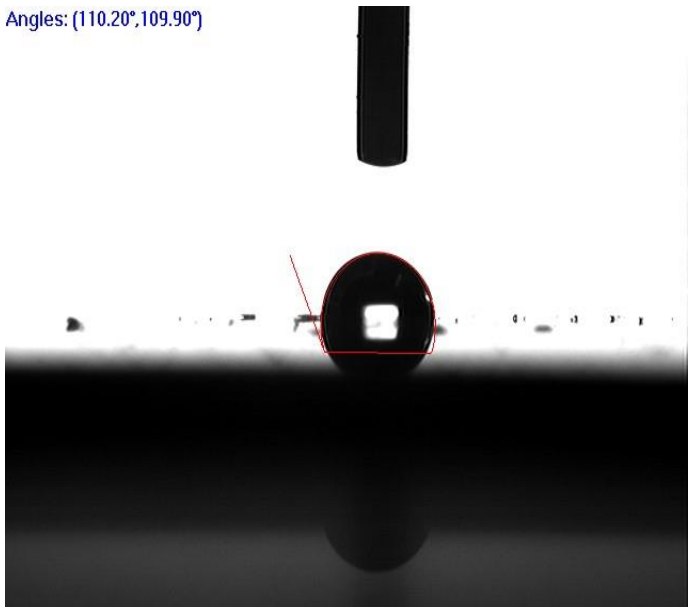
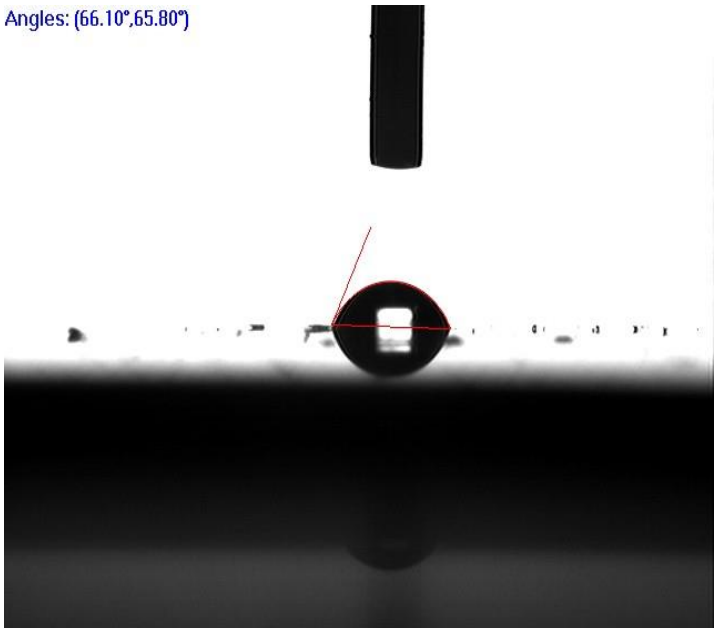


Fig 17. Fabrication Procedure For Electrowetti

5.4 **Contact angle change and Electrode**



Before Applying Voltage



After Applying Voltage

Fig 18. Contact angle change before and after applying voltage.

Chapter 6

Conclusion

In the present work, we have calculated the 2-D droplet change in contact angle with respect to voltage and time. Also we have simulated the time dependent transport of a single droplet over a one-dimensional electrode array with respect to time and space.. The efficient switching mechanism has obtained by comsol. Using the information gathered from the above simulation, the complex systems can be over simplified to address the challenges we mention in this paper.

The LoC does not involve with animal testing and human trial for pharmaceutical research which makes the process cost effective and time saving as well. The idea of home health care device can be extended for applications like RBC count for cancer patient.

The simulated result will also help for farther study, which enabling the integration of multiple subsystem modules into an automated NGS library sample preparation system. This emerging technology combines electronics with biology to open new application areas such as point-of-care diagnosis, on-chip DNA analysis, and automated drug discovery. Fabrication of the device gives various applications in research field which has many advantages.

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