# DEVELOPMENT OF PARTICLE MANIPULATION AND ANALYSIS TOOL FOR MICROFLUIDIC DEVICE USING DIELECTROPHORESIS

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# DEVELOPMENT OF PARTICLE MANIPULATION AND ANALYSIS TOOL FOR MICROFLUIDIC DEVICE USING DIELECTROPHORESIS

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**DEVELOPMENT OF PARTICLE MANIPULATION AND ANALYSIS TOOL FOR MICROFLUIDIC DEVICE USING DIELECTROPHORESIS**

## ABSTRACT

Manipulation and analysis of microparticles in a microfluidic device finds wide application in numerous fields such as application in microbiology, drug and medicine assessment, point-of-care for disease diagnosis and microengineering. Different microfluidic devices were designed and developed to meet the needs for every application based on different engineering practices. The principle of electro-kinetics has had great impact in application to particle manipulation as it attempts to move, assembly, rotate, or separate different types of particles by changing their electrical fields. Many types of electro-kinetics are used in particle manipulation such as electrolysis, electro-osmosis, capillary osmosis, diffusiophoresis, dielectrophoresis, and sedimentation potential. In this study, I focus on the application of dielectrophoresis in manipulation of microparticles on a microfluidic device. Microfluidic chips based on a dielectrophoresis (DEP) technique hold several advantages for microparticle manipulation, such as fast result processing, instant deployment of parameters involved, a small amount of sample required, high spatial resolution, and high accuracy of target selection. Conventional dielectrophoresis techniques such as travelling wave DEP, insulative DEP and 2-dimension DEP were studied and compared with the current study in terms of the ease of fabrication, materials used and ease of access. However, there is an unmet need to develop DEP microfluidic chips on different substrates for different applications in a low cost, facile, and rapid way. For example, most existing DEP microfluidic chip fabrication methods are limited to certain substrate materials such as the photolithography technique, which applies primarily to glass, rather than being adaptable. Further, disposable DEP microfluidic devices are preferred due to the potential issues of electrode damage and sample

contamination as induced by the electrolyte electrolysis process on the electrode surface in the DEP procedure. This study develops a new facile and low cost method based on a screen-printing technique for fabrication of electrodes of DEP chips on both solid/soft and transparent/non-transparent substrates (*i.e.*, polymethyl-methacrylate (PMMA), poly(ethylene terephthalate) (PET) and A4 paper intended to provide a good base to enhance electro-kinetic devices as research continues. The fabricated PMMA-based DEP microfluidic chip was selected as an example and successfully used to trap and align polystyrene (PS) microparticles in a suspension and cardiac fibroblasts in a cell culture solution, proving the feasibility of the fabricated DEP microfluidic chip for both microparticle and biological cell trapping . The capability of the developed electrode fabrication method shows its compatibility with different kinds of DEP substrates, which could expand the future application field of DEP microfluidic chips, including new forms of point-of-care diagnostics and trapping circulating tumor cells.

Keywords: Microfluidic, microparticles manipulation, dielectrophoresis (DEP), cell trapping and microchip.

**PEMBANGUNAN KAEDAH MANIPULASI DAN ANALISIS ZARAH UNTUK PERANTI MIKROFLUIDIK BERDASARKAN DIELECTROPHORESIS**

## ABSTRAK

Manipulasi dan analisis mikro partikel dalam peranti microfluidic mendapati penggunaan yang luas dalam pelbagai bidang seperti aplikasi dalam bidang mikrobiologi, penilaian dadah dalam perubatan, 'point-of-care' untuk diagnosa penyakit dan mikro-kejuruteraan. Pelbagai Peranti microfluidic telah direka dan diciptakan untuk memenuhi keperluan bagi setiap aplikasi berdasarkan amalan kejuruteraan yang berbeza. Prinsip elektro-kinetik mendapati pengaruh yang kuat dalam aplikasi manipulasi zarah kerana ia berfungsi dalam menggerak, menghimpun, memutar, atau mengasingkan beberape jenis zarah dengan penukaran tapak elektrik. Banyak jenis prinsip elektro-kinetik terlibat dalam manipulasi zarah seperti elektrolisis, elektro-osmosis, kapilari osmosis, diffusiophoresis, dielectrophoresis, dan potensi pemendapan. Dalam kajian ini, tumpuan difokus pada penggunaan dielectrophoresis dalam manipulasi mikro zarah pada peranti microfluidic. Cip microfluidic berdasarkan teknik dielectrophoresis (DEP) memiliki beberapa kelebihan untuk manipulasi microparticles, seperti pemprosesan hasil secara segera, serta-merta pengubahsuaian parameter yang terlibat, sejumlah kecil sampel yang diperlukan, resolusi spatial yang tinggi, dan ketepatan yang tinggi dalam pemilihan sasaran. Teknik dielectrophoresis konvensional seperti DEP gelombang-menjalar, DEP insulative dan DEP 2-dimensi telah dikaji dan dibandingkan dengan kajian semasa dari segi memudahkan teknik fabrikasi, bahan yang digunakan dan memudahkan akses. Walau bagaimanapun, masih muncul keperluan yang tidak dipenuhi untuk penghasilan cip microfluidic DEP pada substrat yang berbeza untuk aplikasi yang berbeza dalam kos yang rendah, facile, dan cara yang pesat. Sebagai contoh, kaedah fabrikasi DEP microfluidic cip yang sedia ada adalah terhad kepada bahan-bahan substrat tertentu seperti teknik photolithography yang dilamarkan pada kaca, tidak sesuai untuk bahan

mentah yang lain. Selain itu, peranti microfluidic DEP yang dapat dimusnahkan dapat sokongan yang kuat disebabkan oleh isu-isu yang berpotensi kerosakan elektrod dan pencemaran sampel yang disebabkan oleh proses elektrolisis elektrolit di permukaan elektrod dalam prosedur DEP itu. Kajian ini mengaji potensi terhadap kaedah yang baru secara facile dan kos yang rendah berdasarkan teknik skrin percetakan untuk pembuatan elektrod cip DEP di kedua-dua substrat pepejal / lembut dan lutsinar / tidak lutsinar (iaitu, polymethyl-methacrylate (PMMA), poli (etilena terephthalate) (PET) dan kertas A4 bertujuan untuk menyediakan asas yang baik untuk meningkatkan peranti elektro-kinetik penyelidikan berterusan. Plat PMMA DEP cip microfluidic telah dipilih sebagai contoh dan berjaya digunakan untuk memerangkap dan menyelaraskan polistirena (PS) mikro zarah dalam suspensi dan fibroblas jantung dalam larutan kultur sel, membuktikan kecekapan DEP cip microfluidic yang direka untuk pemerangkapan kedua-dua sampel iaitu mikro zarah dan biologikal sel. Keupayaan bagi membangunkan kaedah fabrikasi elektrod menunjukkan keserasian dengan pelbagai jenis substrat DEP, yang boleh digunakan dalam aplikasi bidang cip microfluidic DEP pada masa depan, termasuk bentuk baru ‘point-of-care’ diagnostik dan pemerangkapan sel-sel tumor yang beredar.

Kata kunci: Microfluidic, manipusi mikro zarah, dielectrophoresis (DEP), sel pemerangkapan dan mikrocip.

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## LIST OF SYMBOLS AND ABBREVIATIONS

| | | |
|---|---|---|
| AC | : | alternating current |
| AC-DEP | : | alternating current dielectrophoresis |
| $AgNO_3$ | : | silver nitrate |
| CNC | : | Computer numerical control |
| $CO_2$ | : | carbon dioxide |
| DC | : | direct current |
| DF | : | dengue fever |
| DMEM | : | Dulbecco's modified eagle medium |
| DENV | : | dengue virus |
| DEP | : | dielectrophoresis |
| DDI $H_2O$ | : | distilled/deionized water |
| DFC | : | dielectrical field cage |
| EP | : | Electrophoresis |
| FEA | : | Finite element analysis |
| IDEA | : | Interdigitated Electrode Array |
| IPA | : | 2-isopropanol |
| ITO | : | indium tin oxide |
| MAC-ELISA | : | IgM antibody capture enzyme linked immunosorbent assay |
| MEMS | : | Micro electro-mechanical system |
| MZI | : | Mach-Zehnder interferometer |
| EDC | : | N'-(3-dimethylaminopropyl) carbodiimide hydrochloride |
| FBS | : | fetal bovine serum |
| $N_2$ | : | nitrogen gas |

| | | |
|---|---|---|
| OC | : | open channel |
| OD | : | optical density |
| PS | : | poly-styrene |
| PBS | : | phosphate buffered saline |
| PDMS | : | polydimethylsiloxane |
| PET | : | polyethylene-glycol-terephthalate |
| PMMA | : | polymethyl-methacrylate |
| POC | : | point-of-care |
| SPSS | : | Statistical Package for the Social Sciences |
| TWDEP | : | traveling wave dielectrophoresis |
| UV | : | Ultraviolet |

# CHAPTER 1: INTRODUCTION

## 1.1 Overview

In the past decade, interest in particle manipulation and analysis of complex biological systems such as living cells using microfluidic systems or microfluidics chips has attracted increased attention. Different techniques have been applied in microfluidic for particle manipulation research and applications, such as covalent binding, optical-induced dielectrophoresis, and the application of the principle of electrokinetics. The ability to manipulate microscale particles and biological cells through operations such as trapping, focusing, characterizing, pairing and separating target particles plays an important role in many biological and colloidal science applications.

Current trends for development of Microfluidics-based Systems in biological and colloidal science applications are the implantation of microchip systems in sensor (integrated electrodes), combining microsensors with fluidic components (pumps, flow sensors, heaters, reservoirs) into systems; and the applications, which had a much greater impact, focused on the miniaturization of analytical chemical methods, in particular separations, fluid handling machines, and cell separators, also known as lab-on-a-chip.

Amongst the range of available lab-on-a-chip compatible devices, point-of-care (POC) diagnostics offer great potential for diagnosis and monitoring of infectious diseases in resource-limited settings. POC techniques offer several advantages including disposability which might reduce the risk of contamination, lower production cost, user friendliness, ease of use and portability, which is most important for a patient monitoring system.(Huckle, 2008).

Trapping of microparticles (*e.g.*, cells (VanDelinder & Groisman, 2007), bacteria (Koklu *et al.*, 2010), protozoa (Antia *et al.*, 2007), and polystyrene (Dash & Mohanty, 2014)) has found widespread application in numerous fields, such as point-of-care diagnostics (Temiz *et al.*, 2014), high throughput screening (Gossett *et al.*, 2010), and

drug analysis (Labeed *et al.*, 2003). For instance, trapping of Malaria-infected red blood cells from a mixture consisting of healthy and infected cells is a very important step in the preparation of blood samples for the clinical diagnosis of Malaria (Nam *et al.*, 2013). For these applications, it is important to trap target microparticles on a testing platform to carry out detection tests with a high resolution and sensitivity.

## 1.2 Microfluidics

Microfluidics technologies are powerful methods able to address many of the challenges faced by conventional diagnostic devices (Chin *et al.*, 2007; Myers & Lee, 2008). Generally, microfluidics can be defined as the study of fluid flow in and around microscale objects. Microfluidics-based biological Microelectromechanical (Bio-MEM) Systems provide a device with compatibility with standard laboratory equipment and features which both to prevent leakage and discriminate between cells. These advances in microfluidic technology enable on-chip POC diagnosis and real-time monitoring of infectious diseases from a small volume of bodily fluids (El-Ali *et al.*, 2006). These technologies can also be used to integrate various assays into a single device (Dittrich & Manz, 2006; West *et al.*, 2008) and to deliver target samples to specific reaction chambers in a controlled manner (Bhattacharyya & Klapperich, 2007; Whitesides, 2006). In addition, with these features, microfluidic devices have been used for sample preparation, such as continuous blood flow fractionation (VanDelinder & Groisman, 2007), nucleic acid extraction (Kokoris *et al.*, 2005) and purification of small molecules (Helton & Yager, 2007).

The major target of microfluidics-based Bio-MEM Systems devices for POC diagnostics is able to analyze small volumes of bodily fluids such as blood, saliva and urine. Microfluidic-based devices offer several advantages for bio-microparticle manipulation, including fast result processing, a small amount of sample required, high

spatial resolution, user friendly operation, and low fabrication cost (Čemažar *et al.*, 2013; Dou *et al.*, 2014; Whitesides, 2006). For example, a continuous-flow dielectrophoretic microfluidic device has been developed and applied to high throughput isolation and recovery of cancerous cells from blood samples (Gupta *et al.*, 2012). Due to the contagious nature of these samples, the designed devices should be disposable to protect the users from exposure to biohazardous waste or any virus/bacterial infection. In addition, the disposable POC devices can avoid some otherwise necessary steps, such as cleaning processes between sample preparations, which thus makes the devices easier to use.

Various microfluidic platforms have been developed for trapping microparticle based on various principles, such as electrokinetics (electrophoresis(EP), dielectrophoresis (DEP) (P. Y. Chiou *et al.*, 2005; Samiei *et al.*, 2015)), optical tweezers (Chiou *et al.*, 2003), magnetophoresis (Kirby *et al.*, 2012), electrical means, and mechanical filtration *etc.* (Prince *et al.*, 2007). Amongst these, DEP, which is concerned with the force experienced by a polarizable particle when subjected to a non-uniform electric field, has attracted special attention due to its high accuracy of target selection (Sebastian *et al.*, 2007), its being label free, the simple instrumentation and its scalability (Joel. Voldman, 2006). For instance, nanometer-sized beads and stem cells were trapped and separated from a mixture in a continuous-flow microfluidic system with a local dielectrophoretic force on multiple strip electrodes (M. W. Wang, 2009). Further discussion of the application of DEP to micro particle manipulation will be presented in the following chapter.

## 1.3 Motivation

The cost of diagnostics and the production cost of microfluidic systems are important parameters for biological and colloidal science applications (Huckle, 2006). Current DEP

microfluidic devices are usually fabricated through expensive microfabrication techniques (*e.g.*, thin-film deposition, sputtering, chemical vapor deposition (Cetin *et al.*, 2014)), which are complicated, time-consuming and not suitable for mass production (Martinez-Duarte, 2012). To reduce the cost of device set up, a few criteria should be considered: minimal use of expensive reagents such as titanium, inexpensive manufacturing for mass production, effective quality control, and miniaturization of the size of device (Yager *et al.*, 2008). A few types of material, such as polymethyl-methacrylate (PMMA) (Muck *et al.*, 2004), silicon, glass (Harrison *et al.*, 1992), poly(dimethylsiloxane) (PDMS) (McDonald & Whitesides, 2002) and paper *etc.* (Martinez *et al.*, 2008; Martinez *et al.*, 2009), have been chosen as substrates of microfluidic chips to meet the demands of different applications (Nge *et al.*, 2013; Sackmann *et al.*, 2014). However, most existing fabrication methods are limited to certain substrate materials, rather than being adaptable. Furthermore, several aspects need to be considered for clinical use of microfluidics-based-biological Microelectromechanical Systems devices in resource-limited settings and environmental conditions, such as insufficient water, unreliable electricity, high temperatures (35–45 °C), and humidity (Petti *et al.*, 2006). Thus, disposable DEP microfluidic devices are preferred due to the potential issue of electrode damage and sample contamination as induced by the electrolyte electrolysis process on the electrode surface in the DEP procedure (Gallo-Villanueva *et al.*, 2009). Therefore, it is important to develop a low-cost, facile, and rapid method to fabricate disposable DEP microfluidic chips with different substrates for different applications.

## 1.4 The scope of research and objectives

The research proposed will focus on DEP device improvement especially for new electrode fabrication techniques and micro particle including bio-particle and polybead

trapping. Besides, the research will also continue the study from previous research done in the BioMEMs field. The previous research successfully proved the functionality of DEP devices for rare cell separation. Since particle separation from a mixture solution and particle assessment are major concerns in tissue engineering and clinical research, development of better DEP device with low production cost and user friendly features could help in both. The DEP device is based on cellular dielectric properties, and will be designed and fabricated based on criteria acquired from clinical users in order to achieve early stage detection for tropical disease.

This study aims to provide a functional particle manipulation and analysis tool with better efficiency and ease of operation. Research targets micro-particle manipulation with a shorter process time and better accuracy than existing solutions, whilst using a method based on target particle dielectric properties. In this research, material uses of the DEP device, properties of DEP for the target particle, functional frequency used and some environment conditions will all be treated as factors assessed for effect on the final result. Different experimental protocols will be designed to identify the limitations of the research.

The main objective of this study is to develop a micro-particle manipulation microchip based on the theory of DEP. Theoretical and experimental work are combined to investigate the performance of this fabricated microchip. In particular, the specific aims of this study are as follows:

i. To develop a simple low cost dielectrophoresis (DEP) micro-particle manipulation microchip based on a screen printing technique.

Through a low cost and, facile screen printing technique, to fabricate a DEP electrode on a polymethyl-methacrylate (PMMA) substrate and used as a micro-particle manipulation microfluidic chip.

ii. To develop a portable microfluidic system for bio-particles manipulation based on the theory of DEP.

A DEP electrode is to be fabricated to manipulate target bio-particles such as acting as a fibroblast trapping device, combined with a hardware case to form a portable bio-particle manipulation microchip which can trap targets and analyze them based on the imaging.

iii. To design a method for particle manipulation on a polymer-based POC diagnostic platform.

This is to be simple, low cost and needing no advanced equipment to operate. Current devices using polymers for diagnosis include blood test tubes and glucometers, both of which are made from polydimethylsiloxane and PMMA. They are in high demand in resource-limited places and developing countries. Thus, it is very important to be able to have a target particle manipulation tool attached to polymer-based diagnosis devices.

## 1.5 Conclusion

This Chapter has provided a synopsis of the thesis by highlighting some of the previous research on particle manipulation techniques and the combination of these techniques with microfluidic device in different applications and studies. In doing so the Chapter briefly stated how this thesis will contribute to the study of low cost, facile micro-particle manipulation tool development. The Chapter also introduces the motivations that I faced in my study and how these challenges

served as the instigating factor for this research project. In Chapter 2 a detailed background for the study and discussion of theoretical framework used in this study will be discussed.

# CHAPTER 2: LITERATURE REVIEW

This section will cover relevant background information that relates to the primary aspects of the project, the methods used for particle manipulation, comparison for the methods, the different types of DEP and the background studies of DEP, factors that influenced the DEP experiment and development of simulation.

The review will start with a brief outline of common techniques used in particle manipulation and comparison; this is followed by the brief description of DEP, along with a concise review of the relevant literature. A short review of the forces that exist in the microchannel that affect particle movement will be subsequently included. A brief discussion of the simulation software, COMSOL will be included in the last part of the chapter.

## 2.1 Methods of particle manipulation

Many cell or particle manipulation techniques have been discussed and their functionalities have been proved by several experiments (Joel. Voldman, 2006). Amongst the techniques which have been proposed, the most straightforward technique is to traps and separates the target cell or particle by covalently binding and tethering it to a surface or a larger particle. The target particle can be separated from the unwanted particle mechanically at the end of the process (Lakowicz, 2006). The advantage of using this method is that the particle can bind to the desired subject in the position that is easy to manipulate. The disadvantages of this technique are first that the use a of tether could impair the biological activities of the molecule and secondly that the binding location on the molecule is not always well known, as most relevant biomolecules have more than one site for covalent bonding. There might be unknown interactions which occur on the biomolecules such as hydrophilicity.

The second technique major that been widely used in particle manipulation experiments is the optical trapping. An example is optoelectronic tweezer (Burke, 2003; Chiou *et al.*, 2003). By using a laser beam to induce a dielectrophoretic mechanism between two photo conductive layers, a force is exerted as the result of radiation pressure with the geometry of the force being related to the dielectric mismatch of the solution and the cell or particle, thus inducing and trapping the particle in the DEP field (Ashkin, 1970; Perkins, 2009). The size of particle trapped is dependant on the irradiance of the laser beam which is inversely proportional to the cube of the particle's radius. Because of that, for biomolecules with nano-sizes, high intensity light needed to trap. The irradiance would be high enough to either rapidly photo bleach a fluorescent label (sample stops photons) or might damage the particle (cause the biological function to be impaired). The technique of optical trapping will be further discussed in the following section.

Electrokinetic refers to the movements of particles under the electric field by the effects of any or all of electro-osmosis, electrophoretic, or dielectrophoretic (King, 2009). Electro-osmosis can be defined as a flow induced in an ionic solution due to an applied electric field (Wong *et al.*, 2004). The generated forces on ions in electric double layers consist of inner and outer layers next to the solid surface of the fluid chamber, and affect the fluid velocity. The inner layer is the interfacial region of the solid that is charged at the surface and the fluid. The outer layer refers to the solution that contains the mobile ions. Because of the electrostatic forces, the fluid will be immobile at the inner layer interfaces. When a direct current been applied, an electric field is produced, and the free ions will experience a force, dragging the surrounding liquid by viscoelastic forces.

Electrophoresis can be defined as the movements of charged molecules, particles or cells in either a direct current (DC) or a low-frequency alternating current field. The subject under the effects of electrophoresis will be accelerated toward the terminal with a

velocity affected by the viscosity of the medium and the hydrodynamic radius of the subject (Desai & Armstrong, 2003; Mehrishi & Bauer, 2002). The charge on the subject and strength of the electric field will also affect the speed and movement of the subject towards the terminal. Electro-osmosis has similar properties to electrophoresis. The speed of particles moved by the induced electric field is directly proportional to the strength of the applied electric field at the terminal of electrodes (Mehrishi & Bauer, 2002). Furthermore, both the electro-osmosis and electrophoresis techniques require a uniform electric field, which must exist in any device fabricated. Both mechanisms will normally react simultaneously during the process of particles separation.

Similar to electrophoresis, DEP describes the movement of particles with induced charges. The major difference between electrophoresis and DEP is that DEP happen under a non-uniform electric field. The induced charges are not uniformly distributed over the surface of the particle and the charges thus form a macroscopic dipole that interacts with the non-uniform electric field. As a result, the forces on a particle scale with the cube of its radius and small particles require very high electric field gradients or the field strengths (J. Voldman *et al.*, 2001). More about DEP will be discussed in the following section.

In DEP, a force is induced by a non-uniform electric field to cause movement along the field (Pohl & Crane, 1971). A dielectric is a material which has the capacity to polarise when subject to an applied electric field (Morgan H, Green NG 2003). When a dielectric particle been induced under a non-uniform electric field, the particle will be polarized and electric charges on the surface of the particle will interact with the electric field (Hughes *et al.*, 2002). The DEP force exists because of the interaction of electric charges on the particle surface and the applied electric field. If the electric field strength is spatially uniform, the force will be equally cancelled. Thus, the cell will remain in its original position. When the electric field strength is non-uniform, there will be a force difference

which will cause a motion of particle through the field. The factors that affect the strength of the force, the direction of the particle movement, and the velocity of the cell movement, are the electrical properties of the particle and suspending medium, the frequency of the applied electric field and the particle shape and sizes (Joel. Voldman, 2006). The electrophysiological spectrum or 'fingerprint' of the cell can be obtained by determine cell movement over the selected frequencies. DEP can also be used to measure the ability of cell to conduct electric charge, which can be defined as the cell's conductivity and the capacitance of cell which is its ability to store electric charge (Mulhall *et al.*, 2011).

## 2.2 Comparison with conventional techniques

DEP has a few advantages over electrophoresis. Movement by electrophoresis (EP) is determined by a net intrinsic electrical charge carried by that particle. To utilize the movement of the electrically-charged particles in a homogeneous electrical field due to the Coulombic body force (electrophoretic force), the particle needs to be charged and the applied electric field needs to be constant or direct current (DC). However, the movement of particles due to DEP is determined by the magnitude and polarity of charges induced in the particle by an applied field. It occurs in a DC field as well as in an AC field over a wide range of frequencies. The electric field has to be non-homogeneous in this case (Pysher & Hayes, 2007).

## 2.3 Different types of DEP for particle manipulation device

This section will discuss several types of DEP techniques which were chose to study and apply in particle manipulation research.

### 2.3.1 Travelling Wave DEP

Travelling wave DEP device which is application for the changing of phase of applied electric field to generate the electric field gradient and cause a force for linear motion of the particle that experience the electric filed. Interdigitated array electrodes have been used for traveling- wave DEP to manipulate bioparticles. The electrodes are in line shape arrangement as shown in the Figure 2.1. These electrodes are independently controlled with different electric field phases, and particles are levitated against gravity owing to negative DEP. Fractionation can be achieved by varying the electric field phases to push the particles transverse to the direction of flow at different velocities.

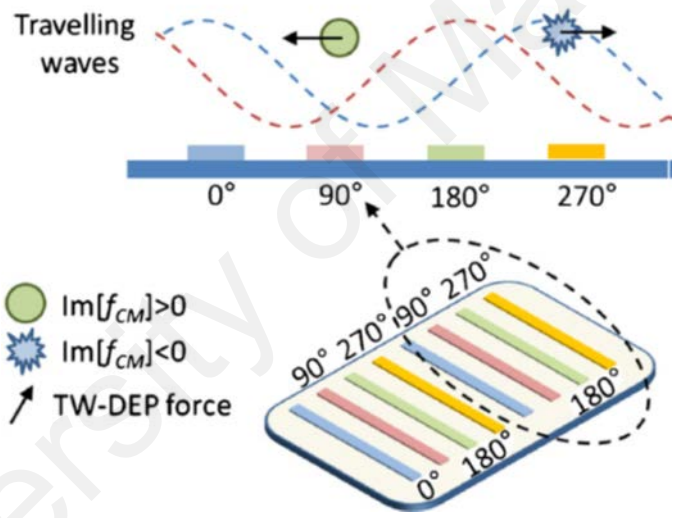



**Figure 2.1: The separation of particle through travelling wave DEP (adapted from (Khoshmanesh *et al.*, 2011)).**

The particle subjected to the traveling-wave field will move along or against the direction of field travel. In 1992, Huang established the model of traveling wave dielectrophoresis as:

$$F_{TWDEP} = \frac{-4\pi^2 r^3 \varepsilon_m Im[f_{cm}] E^2}{\lambda} \quad (2.1)$$

where λ is the wavelength of the electric field. It's equal to the distance between electrodes of the same phase.

Cui and Morgan explained the design and fabrication of a travelling wave DEP device and demonstrated particle motion using polystyrene latex particles (Cui & Morgan, 2000). The main advantage of travelling wave DEP is that fractionation may be achieved based on the particles' differing velocities alone. Positive DEP is no needed in the process which normally used to drive the fluid flow or to trap the particles. As an example, when the electric field phase was changed in a sequence of 0°, 90°, 180°, 270° along a parallel electrode in a line like a travelling wave as shown in Figure 2.1, the interaction between the electric field and the induced dipole produces a force parallel to the electrode and move the particles along the electrode. Basically, this is phenomenon of applied travelling wave dielectrophoresis (TWDEP) for particle separation. The common electrode designs for this method are the spiral electrode design (X. B. Wang *et al.*, 1997) (Goater *et al.*, 1997) and the parallel electrode design (Hagedorn *et al.*, 1992) as suggested by many researchers. These structures can be easily fabricated by depositing a thin film multi-metal electrode such as Cr/Au, or Ti/Pt on a substrate through classical photolithography technology (Tay *et al.*, 2009).

### 2.3.2 Insulative DEP

An insulative DEP technique requires constrictions or expansions in channel geometry to produce a non-uniform electric field so that is can deflect or trap bio-particles through negative DEP (Pratt *et al.*, 2011). The non-uniform electric field can be produced by designing the geometrical constrictions in an insulating substrate such as quartz, glass, plastics, or polymer, instead of metallic microelectrodes (Chou & Zenhausern, 2003). For insulative DEP devices, an inhomogeneous electric field is generated by spatially non-uniform insulating structures between electrodes (Insulating DEP). This method was first

reported in tests on cell fusion at the field constriction by Masuda (Masuda *et al.*, 1989). Insulative DEP devices have also been successfully performed in cell manipulation, virus assessment, DNA separation, and polystyrene particle manipulation by many researchers (Chou & Zenhausern, 2003; Cummings & Singh, 2003; Lapizco-Encinas *et al.*, 2005). Iliescu's group proposed a novel 3-D dielectrophoretic chip which using two planar electrodes that made from a stainless steel mesh, and bonded on both sides of a glass frame which is filled with round silica beads (Iliescu *et al.*, 2007). The silica beads present inside the chamber built up a non-uniform of media to generate an electric field gradient when an AC electric field was applied to the mesh electrodes as shown in the Figure 2.2.

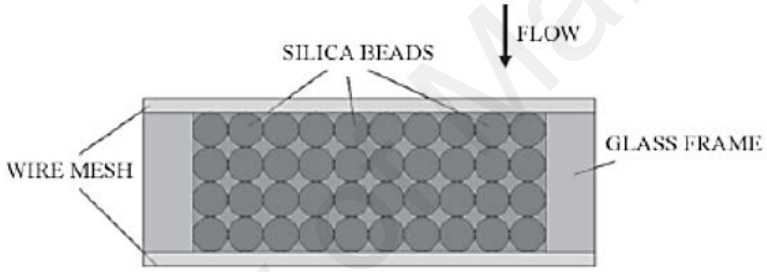



**Figure 2.2: A schematic of 3-D DEP filter using silica beads (adapted from (Tay et al., 2009))**

Recently, a DEP device which can focus and continually cell separation process using opposite of two DEP force fields that generated by a geometrical arrangement of lateral metal electrodes and a patterned insulator was reported by Demierre's group. By using different frequencies, two inhomogeneous DEP force fields were generated and it can be used to determine the different position of equilibrium for particles that have different dielectric properties (Demierre *et al.*, 2008).

### 2.3.3 DEP Devices with Planar Electrodes (2-D DEP)

In the last decade, most of the researches who worked up on DEP studies were using planar electrode for their research. The planar electrodes can be either made using a thin

film metal layer deposited on the glass surface (Kadri *et al.*, 2012) or on the silicon substrate (Docoslis *et al.*, 1997) to form the electrodes as shown in Figure 2.3.

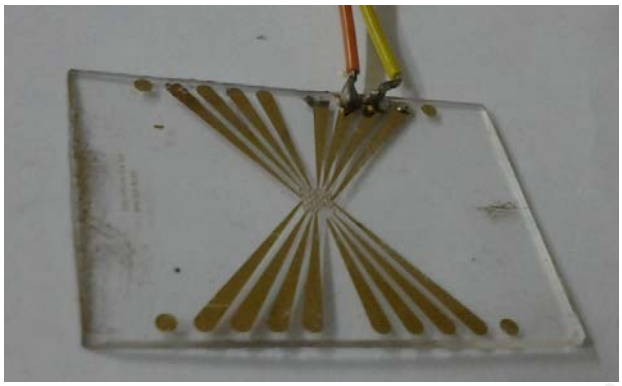


**Figure 2.3: The gold coated planar electrode.**

The thin electrodes are generally made of Chromium with gold (Asbury *et al.*, 2002), Titanium with gold (Morgan *et al.*, 1999) , and Titanium with platinum (Li *et al.*, 2005). The thicknesses of the planar electrodes are in the region of 0.1 mm to 2 mm. In order to make sure the gold and platinum stick on the glass, chromium and titanium are necessary to improve the adhesiveness of gold and platinum on the glass surface. Micro channels or micro chambers which were formed by plastic gasket or polyresin gasket bonded to glass was ensured the flow of the particle on the surface of electrode (Wee *et al.*, 2012). Such devices have been successfully applied to trap cells, bacteria, viruses, proteins, DNA and even nano structures such as carbon nanotubes and nanowires (Kadri *et al.*, 2012; Tay *et al.*, 2009). In the study conducted by researchers, Prof. Hua Dong and his group, they developed a method to fabricate 2-D electrode based on screen printing technique (Zhu *et al.*, 2015). This technique has overcome the challenges that the 2-D planar electrodes are fabricated via complex, time-consuming and relatively expensive microfabrication techniques which are not suitable for mass production (Martinez-Duarte, 2012) and using cost effective material, carbon paste as the material to 'print' out the interdigitated electrode which not only reduces dramatically the chip cost but also increases particle trapping efficiency.

### 2.3.4 DEP Devices with Bi-layer Electrodes

Although planar DEP devices have been successfully developed for a wide range of bio-particles separation, they still have a few limitations to prevent this technique from being used in practical applications in biological and medical fields. The main reason is because DEP effect will decrease when the distance of the particle from the planar electrodes increase and caused only a few targets can be trapped. These phenomena showed that planar DEP devices are not sufficient for high throughput practical application (Honegger & Peyrade, 2013).

In order to improve the efficiency of planar DEP devices, a system consists of dual layer of electrodes array has been developed. The main concern for this design is the accurate alignment of the top and bottom electrodes. The unmatched position of electrodes might cause the adverse impact of the holding force (X. Wong & Rosales, 2008). Thus, Wang designed a bi-layer electrode with appropriate array and demonstrated the solution for the problem of unmatched electrode position in order to trap and separate the target stem cells with attaching antibody-immobilized nanobeads on the surface out from the fluid mixture as shown in the Figure 2.4 (M. W. Wang, 2009).

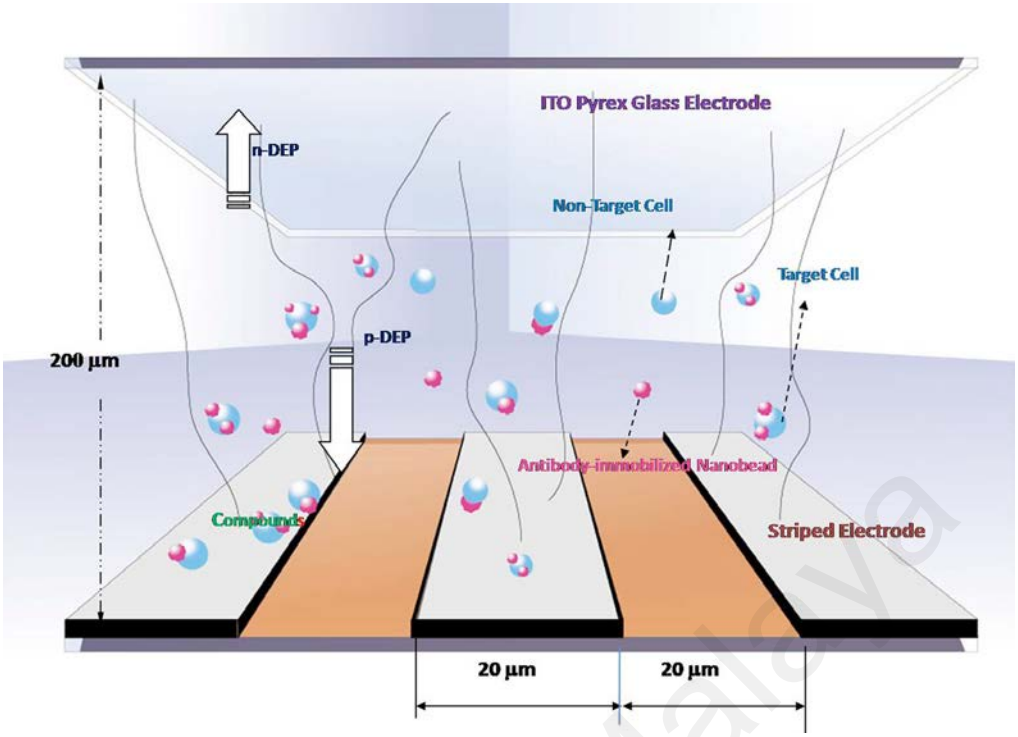



**Figure 2.4: The mixture of normal cells and nanobeads attached cells were subjected to DEP field between bi-layer electroce formed by ITO Pyrex glass and striped electrode. Target cells were trapped on stripped electrode and separated out from the mixture (reproduced from Wang, 2009).**

Previously, Schnelle's group (Schnelle *et al.*, 2000) designed a 3-D microelectrode system, called a dielectrical field cage (DFC). The system consisted of two layers of electrode structures separated by a thick polymer spacer, or ceramics forming a flow channel. The dielectrical field cage was formed by eight electrodes symmetrically arranged on the top and bottom glass slides.

Suehiro's group (Suehiro & Pethig, 1998) reported a similar structure where the electrode system consisted of two glass plates, on which parallel strip electrodes were fabricated, placed together with a spacer between them so that their electrodes face each other and cross at right angles to form the grid. The similar design of electrode system also been reported in Qian's group research. The design is shown in the Figure 2.5. The limitation of these designs are the difficulties of control the trapping forces and the crossing angles that ensure the highest efficiency.

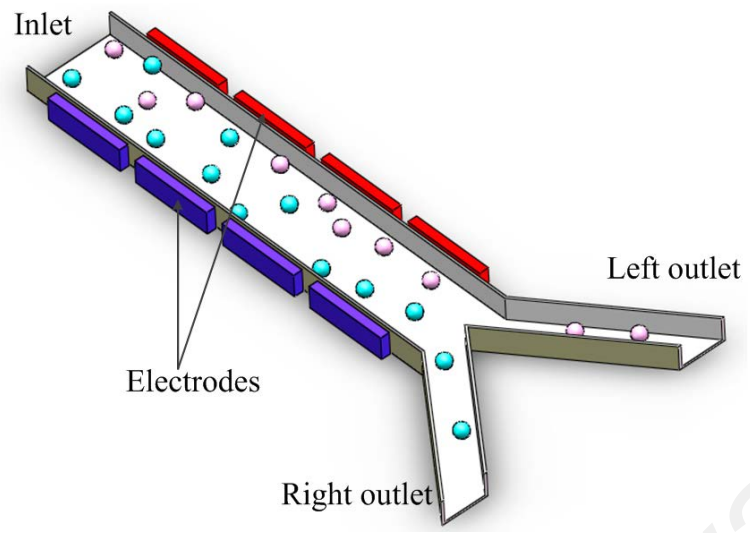



**Figure 2.5: Schematic diagram of the microchip for particle separation based on bi-layer electrodes (reproduced from (Qian *et al.*, 2014))**

### 2.3.5 Optically induced DEP

Using light to excite a photoconductive layer and create an electric field gradient in the sample medium to perform particles sorting (Fan & White, 2011; Hwang & Park, 2011), separation (Hwang & Park, 2009), assembly (Hwang *et al.*, 2009), DNA manipulation (C.-H. Wang *et al.*, 2014), and isolation specify target (Perkins, 2009) has create a new field in DEP research. The Figure 2.4 showed an example of light DEP device for particles manipulation. The sample suspension is placed in between a conductive layer and a photoconductive glass; the photoconductive glass is on top of another conductive layer as shown in Figure 2.6.

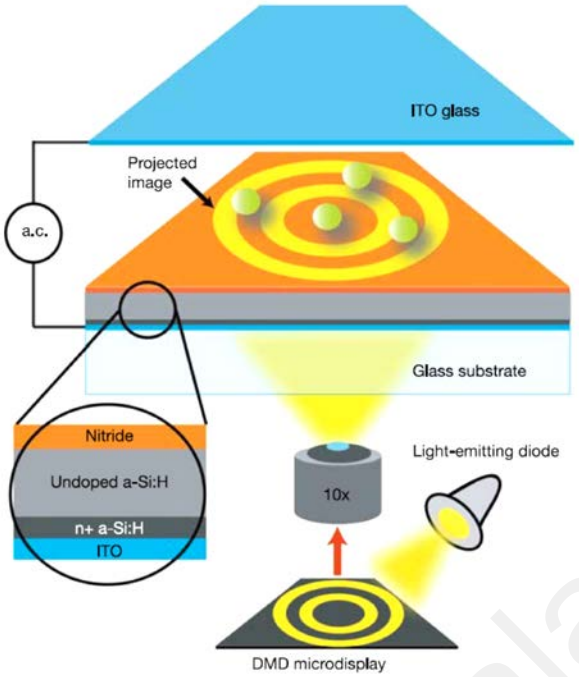



**Figure 2.6: Device structure used in optoelectronic tweezers. Liquid that contains microscopic particles is sandwiched between the top ITO glass and the bottom photosensitive surface consisting of ITO-coated glass topped with multiple featureless layers (adapted from (Pei Yu Chiou *et al.*, 2005)**

Different light patterns can be designed and applied to implement a variety of functions. A line can be formed along the manipulation area to selectively drag particles around; a ring can be made smaller or bigger for the same purpose; a dot or an array of dot can be arrange to draw the targets to certain focus areas (Honegger & Peyrade, 2013). The possibilities are endless since any electrode pattern, given by the illuminating light, can be generated. The fabrication process of these devices, in the case where the conductive and photoconductive layers are not patterned, is quite straightforward since only layer deposition is needed, not photolithography or etching. Thus, the potential for mass fabrication of device using this technique is high (Fan & White, 2011). The fabrication infrastructure needed in this case is minimal although it can be expensive. Wide surfaces can be coated with the appropriate layers and latter diced to specific size depending on the application. However, ITO-coated substrates and gold coated transparent substrates are relatively expensive and disposable devices may not be as feasible (Jamshidi *et al.*, 2008). Besides, the significant cost when using this technique is

on the optical and illumination systems needed to create the virtual electrodes. As an example, a spot size ~1.5 μm, which can be achieved using a micromirror array and basic optics, allows for the fabrication of quite small virtual electrodes and narrow gaps in between them which can be quite useful when manipulating nanometer-sized particles (Hwang & Park, 2011). The throughput of the device may not be high since the virtual electrodes generated using this technique are still a surface effect. Furthermore, the field of view of the optical system which in this case represents the area for particle manipulation is limited in size to a couple of centimeters square. On the other hand, the illumination system could be mounted on a motorized platform to enable wider coverage and the quick addressing of any area of the device (Martinez-Duarte, 2012). Building upon the results from previous applications, the implementation of light induced electrodes on the ceiling or walls of the channel represents the addition of more optical and illumination systems rendering the system complex and will double up the cost of fabrication. The optically induced DEP is perfectly suited for single cell manipulation due to the high degree of control over targeted cells and the endless list of trajectories one can implement. However, this technique is not suitable to implement in complex microfluidic device and cannot use for multiple targets manipulation microfluidic device (Martinez-Duarte, 2012).

## 2.4 Polarisation & Principle of DEP

Polarisation can be defined as the induced redistribution of charges bound within a material, whereby charges of the same magnitude and opposite polarity are displaced to different locations within the material.

When the electric field has been removed, the material will back to its initiate state. Since the polarisation is not instantaneous, after the removal of the electric field

surrounding charges need some time to be distributed and to achieve an equilibrium state. The period of time named as relaxation time ($\tau$).

If the experiment is been conducted under alternating current to generate the electric field, the process of polarisation is frequency dependent. Conductivity or the ability to redistribute the free charges will be the dominant mechanism under the low frequency electric field because polarisation realised its maximum potential before the direction of electric field changes. On the other hand, perturbation of bound charges or the permittivity of charges dominates the polarisation during high frequency applied. It is because of the higher rate of direction changes in the electric field rather than the time needed to realise the maximum potential. The difference between the two stages is termed dielectric dispersion.

The dielectric force happened between separated charges on each sides of induced dipole and applied electric field. If the current applied to form the uniform electric field, the dielectric particle in the suspended medium will experience a same magnitude forces from opposite direction. Thus, the resultant force on the particle is zero and it will remain on its original position.

However, when a non-uniform electric field applied, a gradient of electric field strength exists. Thus, the particle located on one pole of the dipole is greater than on the other. Therefore, the difference of Coulomb forces imparted on the induced dipole. The particle will move due to the force gradient existed. The direction of movement is depended on the polarisability of particle and medium. When the particle is more polarisable than the suspending medium, the particle will move in the direction of high field strength regions. This phenomenon can be defined as positive DEP (+DEP). If the suspending medium is more polarisable than the particle, the particle will move to the region which has low field strength. The phenomena named negative DEP (–DEP). When

the particle and suspending medium have the same polarisability, the particle will stay stationary at its original position. The frequency for this phenomenon happened was declared as crossover frequency ($F_{cross}$).

### 2.4.1 Equation of DEP

The magnitude of particle displacement is proportional to the force exerted on the particle. The DEP for is given by the Equation (2.2):

$$F_{DEP} = 2\pi r^3 \varepsilon_m Re\left[K(\omega)\right]\nabla E_{rms}^2 \tag{2.2}$$

where r is the radius of the particle, $\varepsilon_m$ is the medium permittivity, $\nabla$ is the Del operator (gradient) on the applied electric field E and $Re[K(\omega)]$ is the real part of the Clausius-Mossotti factor. The Clausius-Mossotti factor is given by Equation (2.3):

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \tag{2.3}$$

where $\varepsilon_p^*$ and $\varepsilon_m^*$ are the complex permittivity of the particle and medium, respectively. The complex permittivity is given by Equation (2.4).

$$\varepsilon^* = \varepsilon - \frac{j\sigma}{\omega} \tag{2.4}$$

where ε is the permittivity, ω is the angular frequency, $j$ is the square root of -1 and σ is the conductivity of the particle. In order to determine the permittivity and conductivity of the particle, derivation of the measurement of cell motion as a function of frequency can be used.

However, when a particle has a non-spherical shape (e.g., ellipsoidal shape) equation (2.2) cannot be used. The following equation gives the DEP force for a more general field aligned ellipsoid of radius, *r* and length, *l* (Jones & Jones, 2005). This equation can be used to study the dielectrophoretic response of biological particle such as red blood cells, yeast and E.coli and Nano-particles such as carbon nanotubes.

$$F_{DEP} = \frac{\pi r^2 l}{3} \varepsilon_m Re\left[K(\omega)\right] \nabla E_{rms}^2 \quad (2.5)$$

## 2.5 Forces in microfluidic device

Besides the DEP force take action of the particles in the DEP-based microfluidic device, some other forces will also affect the directions and magnitudes of particles movement on the platform. Thus, in order to achieve the manipulation of the particle through DEP forces, I have to study the other forces and minimize their reaction on target particles. These forces, depending on their formation, will cause the particles to travel in different pathway, or remain static in initial position. These forces include Brownian forces, Coulomb forces, gravitational forces, hydrodynamic forces, electro-thermal forces and capillary reaction.

The main external influence on a particle suspended in a fluid is gravitational force. The gravitational force or sedimentation forces resulted from the gravity to pull down the particle toward the floor of microfluidic channel. The levels of levitation of particles in a microfluidic channel under the influence of gravitational force and other forces are usually used for mechanically separate the micro particles (X. B. Wang *et al.*, 1997).

In a suspension of microparticles, the second force that will take action on the particle might be the Brownian motion. The Brownian motion is the random movement of particles due to the unbalanced molecular impacts on colloidal particles. However, Brownian motion can be negligible for the particles in many cases when the target has a size larger than 1 μm for microfluidic applications (Castellanos *et al.*, 2003).

Coulomb forces reacted on particles and the charges of the medium are caused by the distortion of the molecular charge density and polarization of each particle when explore to an external electrical field(Zeng, 2011). It happened because of the electrical

interaction of charges on particles with each other. When the frequency changed, the Coulomb forces may dominate and caused the dielectrophoretic force been counteracted.

By the principle of hydrodynamic, the flow within the solution in a channel applies forces to the particles and inversely the motion of particles perturbs the solution. Thus, the motion of one particle generates long range, nearly instantaneous hydrodynamic interactions that transfer the forces to surrounding. The hydrodynamic forces involve in the flow consist of drag force and lift force. The equilibrium positions of particles inside the channel are determined by the competition between hydrodynamic (drag and lift) forces acting on the particles flow in microfluidic channel (Sun *et al.*, 2013).

Electro-thermal force is a large power density generated around the electrodes, which will rise the temperature of fluid medium and electrode due to heat conduction, causing the localized of Joule heating (Gunda *et al.*, 2012). The increase of temperature will change the electrode conductivity and permittivity and generates the electro-thermal force. The magnitude and direction of electro-thermal force depend on the strength of the electric field and the frequency of the AC-current supplied. When certain sections of the fluid medium have different temperatures, natural convection occurs(Cetin & Li, 2008). The electro-thermal fluid flow will cause undesirable forces on the particles (Castellanos *et al.*, 2003). However, the electro-thermal force can be negligible in certain cases with the correct frequency selected and proper flow rate of the medium through the channel (Chen & Du, 2006).

## 2.6 COMSOL Multiphysics

COMSOL Multiphysics (previously called FEMLAB) is Finite Element Analysis (FEA) solver and simulation software which contains different modules for various applications. COMSOL Multiphysics is compatible with MATLAB (high performance language for mathematical computing) and its toolboxes help compile different physics

equations to simulate a real world system. It can be applied for numerous physical and engineering applications where a large variety of programming, preprocessing and post processing protocol have to be done. The modules are cross-platform and in addition to conventional physics-based user interfaces, COMSOL Multiphysics also allows for entering coupled systems of Partial Differential Equations (PDEs). The PDEs can be entered directly or using the weak forms. In this study, COMSOL was used to understand the underlying concepts of AC-DEP.

## 2.7 Summary

Manipulation of micro-particles especially bio-particle in microfluidic chips based on the technique of AC-DEP including separation, sorting, trapping, concentrating and pairing of cells, is important for a variety of drug testing, diagnostic and clinical applications. However, techniques of fabrication of DEP electrodes for use in microfluidic chip have a lot of challenges such as time consuming and complicated microfabrication techniques, relatively expensive material (ITO, gold and titanium) and fouling of sample due to electrolysis process.

In this study, I report a relatively simple and cost effective screen printing technique in fabrication of DEP electrode. The material used for my study is silver ink which is cheaper in cost compare to ITO, gold and titanium. The complete process of electrode fabrication can be done within 3 hours. The details of the development of AC-DEP microfluidic chip will be discussed in the following chapters.

## CHAPTER 3: COMPUTATIONAL MODELLING FOR DEP FORCE

In this chapter, the design specifications of electrode for microfluidic chip were studied and computational modelling for DEP force existed on the target particle would be demonstrated to prove the capability of particle manipulation. The flow chart in this chapter illustrating the design specifications and validation process for the electrode of the DEP microfluidic chip.

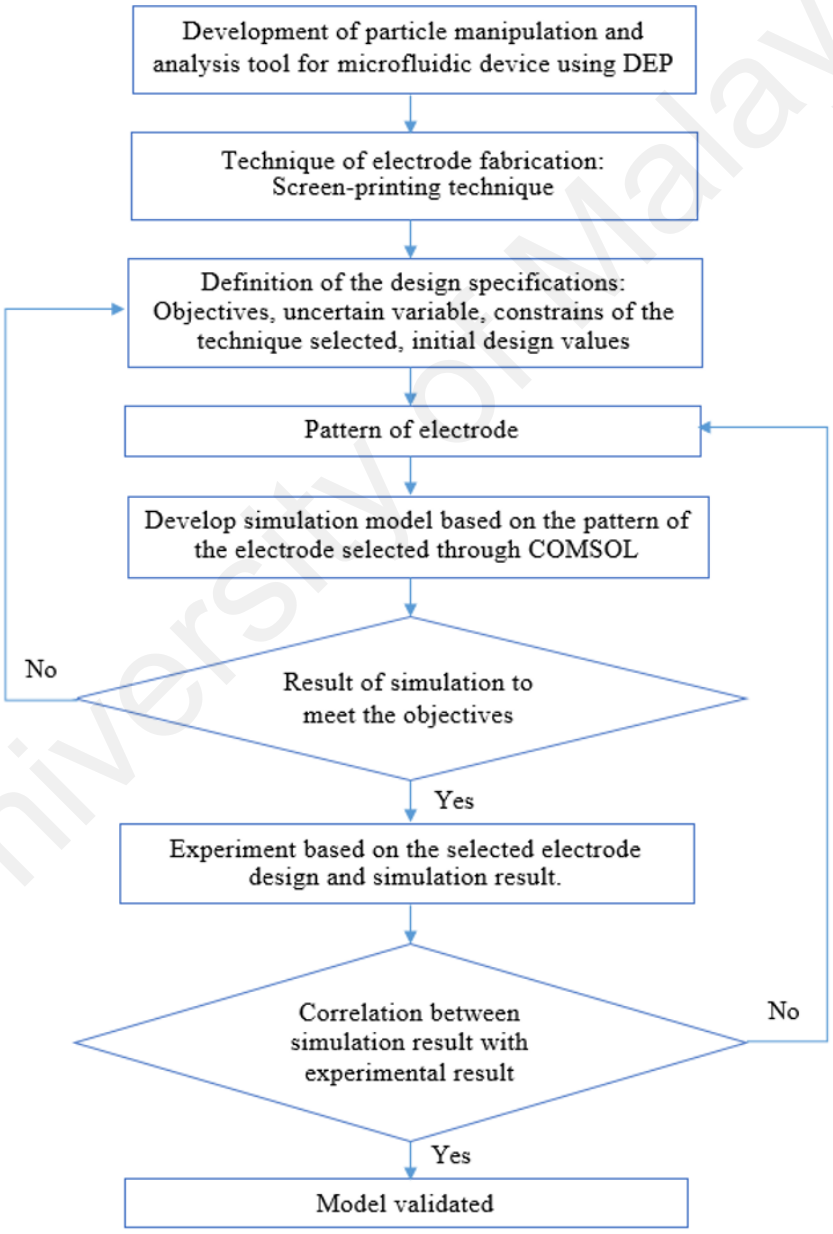



**Figure 3.1: The flow chart illustrating the design specifications and validation process for the electrode of the DEP microfluidic chip.**

Analysis and illustrations using COMSOL modeling have been performed to demonstrate the consequences of parameter changes in the DEP studies. Different 2D and 3D models have been developed to study the factors affecting the DEP force through parametric studies.

The modeling part includes defining the parameters and variables, defining the geometry, applying and configuring the necessary physics, finding the results and plotting the graphs. Analysis of all the parameters is done using mesh plots, point graphs, surface plots and arrow diagrams. Further analysis is done using MS Excel and SPSS to study the relationships between each of the factors and how these relationships affect the DEP force.

**Table 3.1: Parameters used for the DEP Model. ****

| Name | Expression | Value | Description |
|---|---|---|---|
| $V_0$ | 10 [V] | 10 V | Potential different of positive electrodes |
| $V_1$ | -10 [V] | -10 V | Potential different of ground electrodes |
| f0 | 100 [kHz] | 100 kHz | Frequency of the electric field |
| sigma_f | 55 [mS/m] | 55 $mSm^{-1}$ | Fluid medium conductivity |
| Sigma_f1 | 0.3-0.6 [S/m] | 0.3 $Sm^{-1}$ | Conductivity of culture medium |
| epsilon_f | 80 | 80 | Fluid relative permittivity |
| rho_f | 1000 [kg/m^3] | 1000 $kgm^{-3}$ | Fluid density |
| mu_f | 1E-3 [Pa*s] | 1000 Pa·s | Fluid dynamic viscosity |
| rho_p | 1050 [kg/m^3] | 1050 $kgm^{-3}$ | Particle density (RBC) |
| dp1 | 1.8 [μm] | 1.8 μm | Particle diameter |
| dp2 | 5.0 [μm] | 5.0 μm | Particle diameter |
| sigma_p1 | 0.25 [S/m] | 0.25 $Sm^{-1}$ | Shell electrical conductivity for the first particle |
| sigma_p2 | 0.31 [S/m] | 0.31 $Sm^{-1}$ | Shell electrical conductivity for the second particle |

**Table 3.1 continued**

| | | | |
|---|---|---|---|
| epsilon_s1 | 50 | 50 | Shell relative permittivity for the first particle |
| epsilon_s2 | 59 | 59 | Shell relative permittivity for the second particle |
| th_s1 | 8 [nm] | 8 nm | Shell thickness for the first particle |
| th_s2 | 9 [nm] | 9nm | Shell thickness for the second particle |
| per_poly | 2.25E-11 | 22.5 p | Permittivity of Polystyrene beads |

**All parameters listed are changeable to suite with different situation and setting.

At lower frequencies the polarizability of the particles increases due to the presence of the ionic double layer resulting in an increase in the DEP force on the particle. It is important to consider this double layer when working with DEP force as it affects the conductivity of the particle.

## 3.1 Geometry

The model to determine the DEP force includes both inlet and outlet of the channel, positive and negative electrodes with a gap for insulation in between them, and the wall of the channel. After the validity of the model is established, parameters and equations used are checked. The cover layer and the medium region are then made according to the specified dimensions.

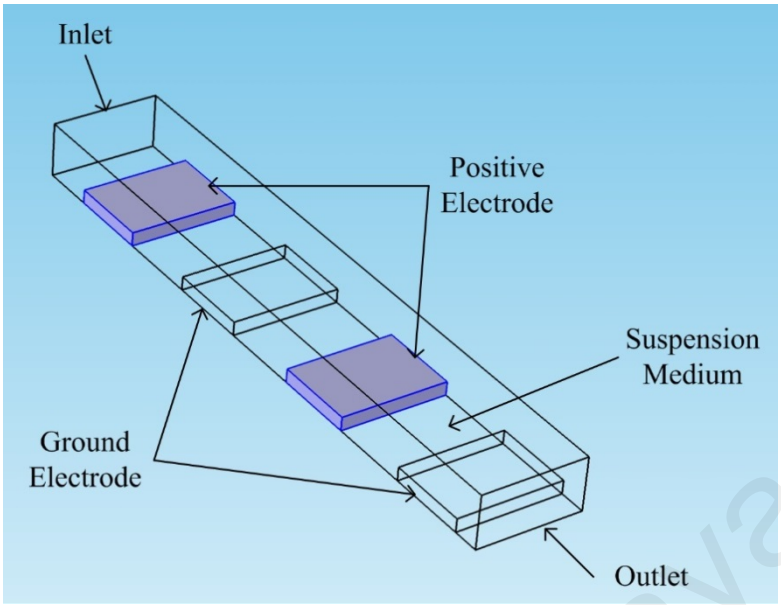



**Figure 3.2: 3D model of the electrode setup used in determine the factors.**

The other parameters used in making the model are defined under parameters and variables in Table 3.1. The shape and dimensions of the electrodes are important factors that determine the positioning and separation of particles using DEP. COMSOL allows the user to add and couple different physics to understand the mechanisms behind any process. For our study the AC/DC and electrostatics physics were used to study the phenomena of DEP.

The materials used for the different components of the electrodes are crucial as they determine the DEP force. The electrodes are made of Ag conductive ink and cover layer is placed over them to form a microchannel (Wei Hong *et al.*, 2015). Deionized water is the medium used to suspend the particles in this model.

A positive potential is given to the positive electrodes and the other electrodes are grounded, as shown in Figure 3.1. The input potential is given as a parameter so that different values can be substituted if required. The electric field is the negative gradient of the electric potential. Thus, the electrostatics physics is bound by the following equations:

$$D = \varepsilon_o E + P \tag{3.1}$$

where, $D$ is the electric displacement, $E$ is the external electric field and $P$ is the polarization. When this is expressed, I can use Gauss law. Because of polarization Gauss law is given as the following:

$$\nabla \cdot D = \rho_v \tag{3.2}$$

where $\rho_v$ is the free volume charge density. Also, I know that the electric field is the negative gradient of the electric potential.

$$E = -\nabla V \tag{3.3}$$

For some analyses (e.g., twDEP models) time dependent inputs are given. In these cases the voltage is defined as a sine wave with the adjacent electrodes having a phase lag. This helps to analyze the change of forces with time.

## 3.2 COMSOL Simulation

Parametric sweeps are performed to analyze the different parameters which affect the DEP forces acting on the subject particles. Four different parametric sweeps are done. The first one is for electric potential varying from 5V to 20V peak to peak. From the equation of DEP, $F_{DEP} = 2\pi r^3 \varepsilon_m Re\left[K(\omega)\right]\nabla E_{rms}^2$ (Equation (2.2)) and $E = -\nabla V$ (Equation (3.3)), it is known that the voltage applied directly affects the DEP force and reacts with the particle subjected to the DEP field.

The second sweep is the conductivity of the suspension medium used in the simulation. The change is made for the value of conductivity of deionized water to the conductivity of cell cultured medium. As discussed in section 2.4, the Clausius-Mossotti factor, $K(\omega)$ is key for determining the direction of the DEP force. The CM factor not only modifies the strength and imposes a direction on the DEP force, it also translates the difference in polarization between the particle and the medium (Morganti *et al.*, 2011). The equation for calculating the Re[$K(\omega)$] varies with the geometry of the particle. Hence it has to be

derived for each shape of particle. The analytical derivation of the Re[$K(\omega)$] for a spherical particle is done for this research. Substituting Equation (2.4) in Equation (2.3), I get the following equation:

$$K(\omega) = \frac{\left(\varepsilon_p - i\left(\frac{\varepsilon_p}{\omega}\right) - \varepsilon_m + i\left(\frac{\sigma_m}{\omega}\right)\right)}{\left(\varepsilon_p - i\left(\frac{\sigma_p}{\omega}\right) + 2\varepsilon_m - 2i\left(\frac{\sigma_m}{\omega}\right)\right)} = \frac{\omega(\varepsilon_p - \varepsilon_m) - i(\sigma_p - \sigma_m)}{\omega(\varepsilon_p + 2\varepsilon_m) - i(\sigma_p + 2\sigma_m)} \quad (3.4)$$

After multiply the conjugate of the equation above, I got

$$K(\omega) = \frac{\omega^2(\varepsilon_p - \varepsilon_m)(\varepsilon_p + 2\varepsilon_m) + (\sigma_p - \sigma_m)(\sigma_p + 2\sigma_m) - i\omega(\sigma_p - \sigma_m)(\varepsilon_p + 2\varepsilon_m) - (\sigma_p + 2\sigma_m)(\varepsilon_p - \varepsilon_m)}{\omega^2(\varepsilon_p + 2\varepsilon_m)^2 + (\sigma_p + 2\sigma_m)^2} \quad (3.5)$$

From the equation, I will have a real part which is

$$\mathrm{Re}\left[K(\omega)\right] = \frac{\omega^2(\varepsilon_p - \varepsilon_m)(\varepsilon_p + \varepsilon_m) + (\sigma_p - \sigma_m)(\sigma_p + 2\sigma_m)}{\omega^2(\varepsilon_p + 2\varepsilon_m)^2 + (\sigma_p + 2\sigma_m)^2} \quad (3.6)$$

and the imaginary part which is,

$$\mathrm{Im}\left[K(\omega)\right] = \frac{-i\omega(\sigma_p - \sigma_m)(\varepsilon_p + 2\varepsilon_m) - (\sigma_p + 2\sigma_m)(\varepsilon_p - \varepsilon_m)}{\omega^2(\varepsilon_p + 2\varepsilon_m)^2 + (\sigma_p + 2\sigma_m)^2} \quad (3.7)$$

The real part is used to calculate the DEP force. It determines the direction of particle movement and the crossover frequency.

The third parameter is the frequency of voltage supplied which is swept for a wide range of values. The equation for force is then plotted and the magnitude of force was verified, in turn verifying the creditability of the model. And the frequency affects the polarization as stated in the equation of DEP. Finally the radius of the polystyrene beads and subject bio-particles are swept for different values and also analysis on the effect of

radius on conductivity is studied. The parametric sweeps showed the trend of change of force with these parameters.

In some cases it is necessary to use two changes in the same study. Both frequency and radius affect the CM factor which in turn affects the DEP force directly. The sign of the CM factor determines if the particle is going to move towards the electrode or away from the electrode. The results from COMSOL simulation are obtained and showed the comparison when the parameters were changed. Particle tracing study is done to determine where the particles will end up when a particular factor in the model is changed. Results of these models helped illustrate the consequences of the problems in the literature.

## 3.3 Result of DEP simulation

This section will discuss on the DEP simulation results obtained when DEP field were supplied and related physical parameters are changed such as the channel height, target particle, and frequencies applied.

### 3.3.1 Change of physical parameter

In the figures below, the particle manipulation simulation process will be shown. Two straight forward reaction of DEP effect can be seen in the figures which are the particle accumulation or particle trapping and also the particle been pushed away from the electrodes on the channel.

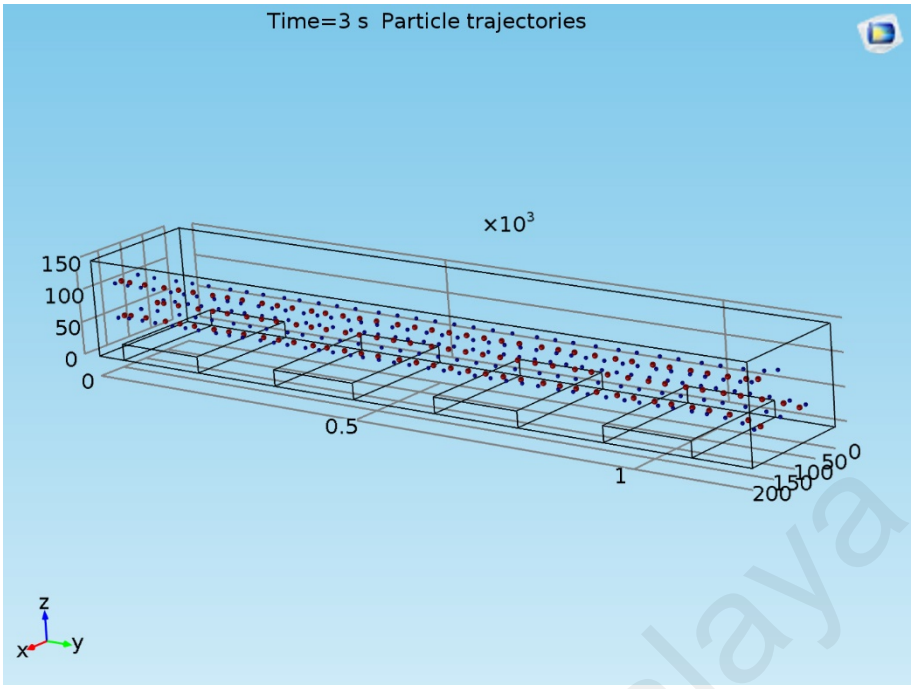
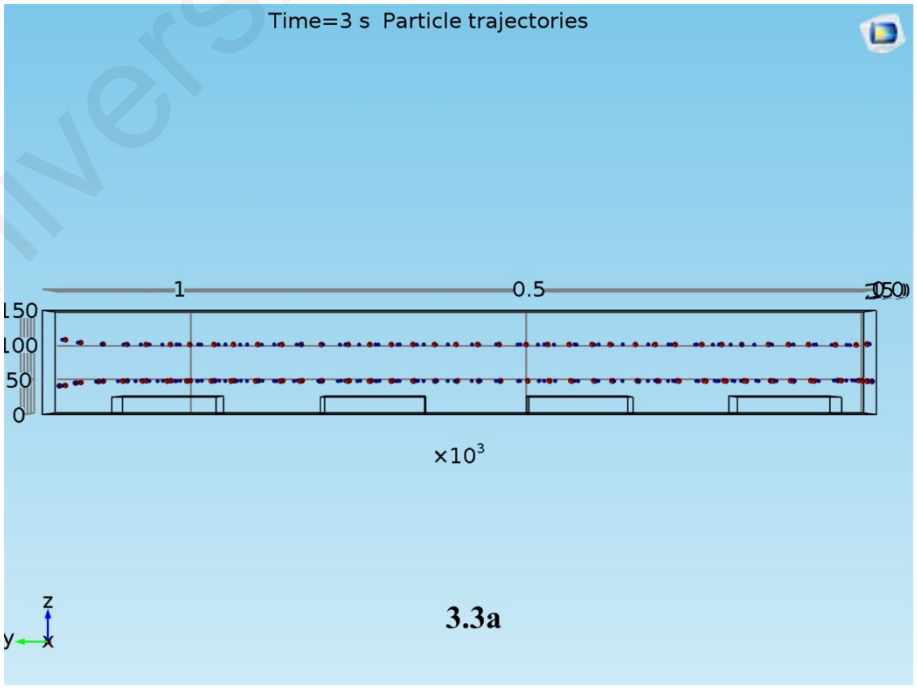



**Figure 3.3: Figure showed the distribution of particle flowing in the micro channel with the DEP deactivated. Red dots represent the red blood cells while blue dot represent the platelets.**

Figure 3.2 clearly shows that the samples (red blood cells (RBCs) and platelets) are normally distributed along the channel. It is a very important message to show that the particle is not affected by other force beside the gravitational and drag/lift force.



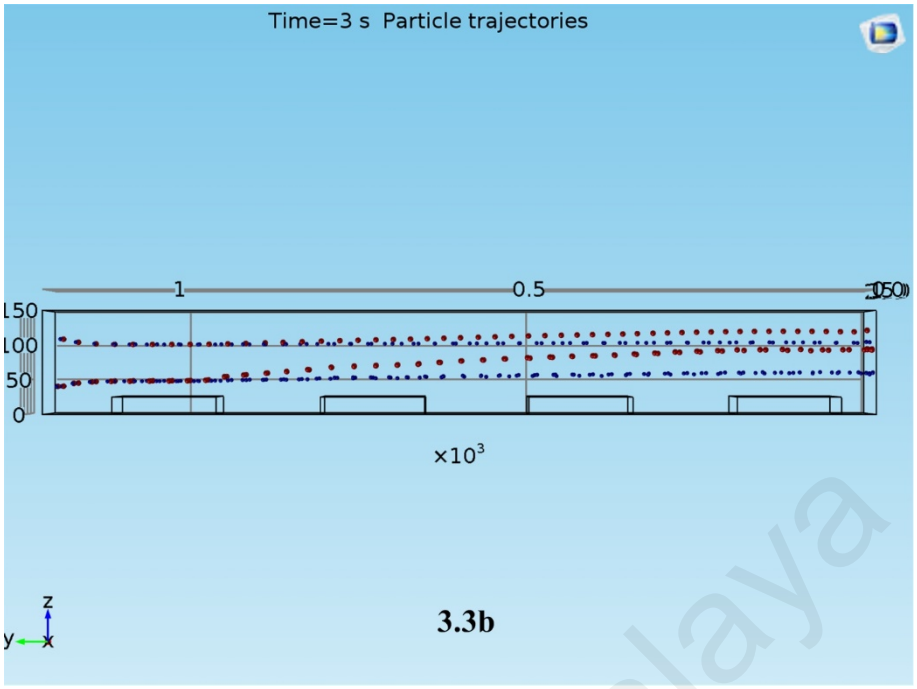


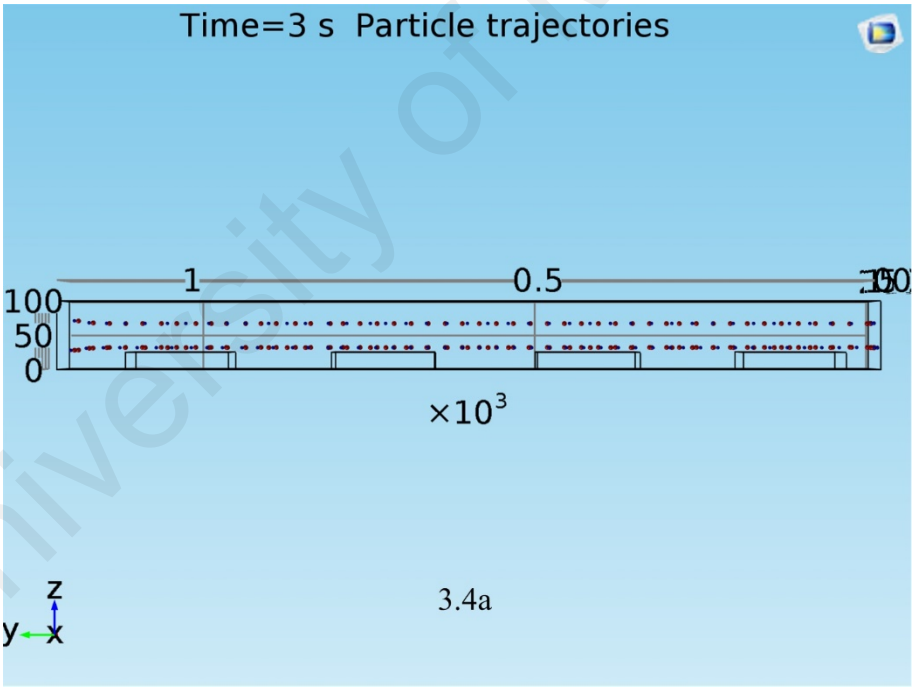

**Figure 3.4a and 3.3b show the particles changed before and after DEP force applied.**

When the voltage had been supplied to the electrodes, the RBCs and platelets are subjected to DEP force, with changes as shown in the Figure 3.3a and Figure 3.3b. In order to show the changes, Figure 3.3 is displayed in a y-z axis view. At the end of the channel, the RBCs and platelets are pushing upward from the electrode and the RBCs with larger diameter will experience a stronger DEP force. Due to the larger surface and higher conductivity level, the DEP force on RBCs is stronger than the platelets experience. The DEP force increases when the radius of the particle, r, increases. This can be explained using Equation(2.2). In this equation, the DEP force is directly proportional to the radius of particle. Thus, RBCs will experience stronger DEP force with their bigger cell radius compared to platelets. However, as the result shown in Figure 3.3, the trapping and separation did not really happen, and RBCs and platelets remained in a mixture at the end of the simulation.

The height of the channel was reduced in order to make sure the stronger DEP force can affect the cells and platelets. This can be explained in Equation (2.2). From the Equation (2.2),

$$F_{DEP} \propto \nabla E_{rms}^2 \quad (3.8)$$

As the electric field intensity is directly proportional to the distance between the electrodes and the cells. The electric field intensity is stronger when the cell is closer to the electrodes. Thus, the DEP force increases when the cells are moved closer to the electrodes. When the channel height was reduced from 150 μm to 100 μm, the RBCs are polarized and pulled toward the electrode due to the –ve DEP force. When the trapped RBCs reached the saturation rate, others RBCs will follow the flow to the next electrode and the +ve DEP force will push away the RBCs and platelets from the electrode (shown in Figure 3.4a and Figure 3.4b). The platelets from the same inlets will experience +ve DEP force which will push the platelets away from the electrode.




3.4a

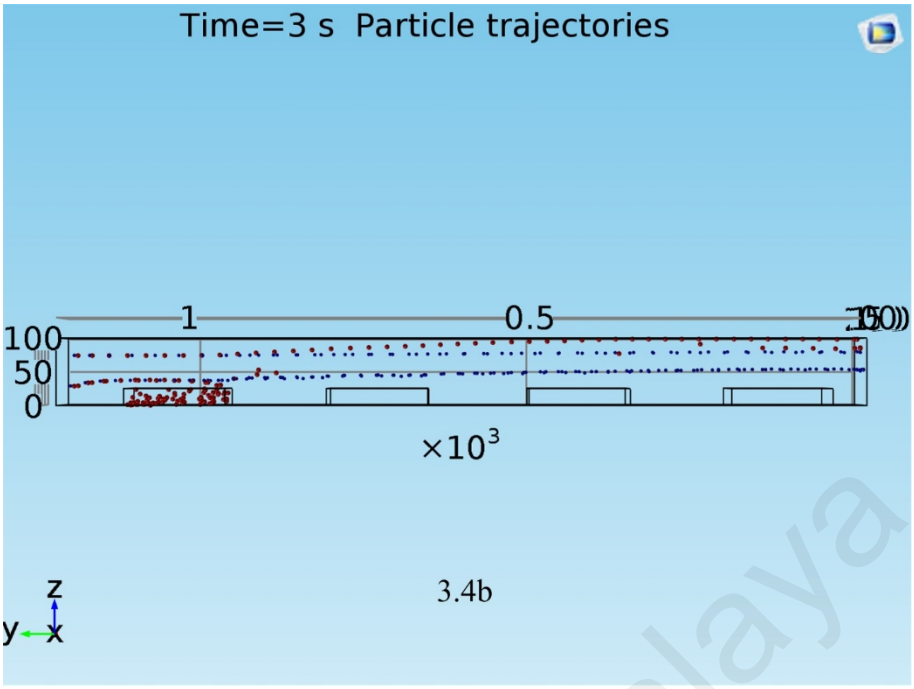



**Figure 3.5a and 3.4b show the movement of RBCs and platelets before and after the voltage been supplied.**

With the parameters set, RBCs have successfully been trapped at certain points of the electrode. The results show the settings and parameters which are required to run the real-time experiment for DEP microfluidic chip in order to achieve the particle manipulation process. A positive DEP effect showed on the RBCs in the simulation also provided an option for a particle manipulation process. The +ve DEP effect can be used to separate a target particle from a mixture by pushing them away from the bottom part of a microchannel (Pommer *et al.*, 2008). The setting and parameters will be used in the fabrication of DEP electrode and particle manipulation experiment.

# CHAPTER 4: FABRICATION OF DEP MICROFLUIDIC CHIP

This chapter describes the processes of a fabrication of DEP microfluidic chip from the hardware system (gasket) to the electrode and microchannel for the device. The fabrication processed involved technique of screen printing though carbon dioxide laser, a heat curing process and works using a computer numerical control (CNC) machine.

## 4.1 Gasket fabrication

A new hardware system was designed and fabricated to use with the DEP device. The result was good and proved the system which can be used for further research (Wee *et al.*, 2012).

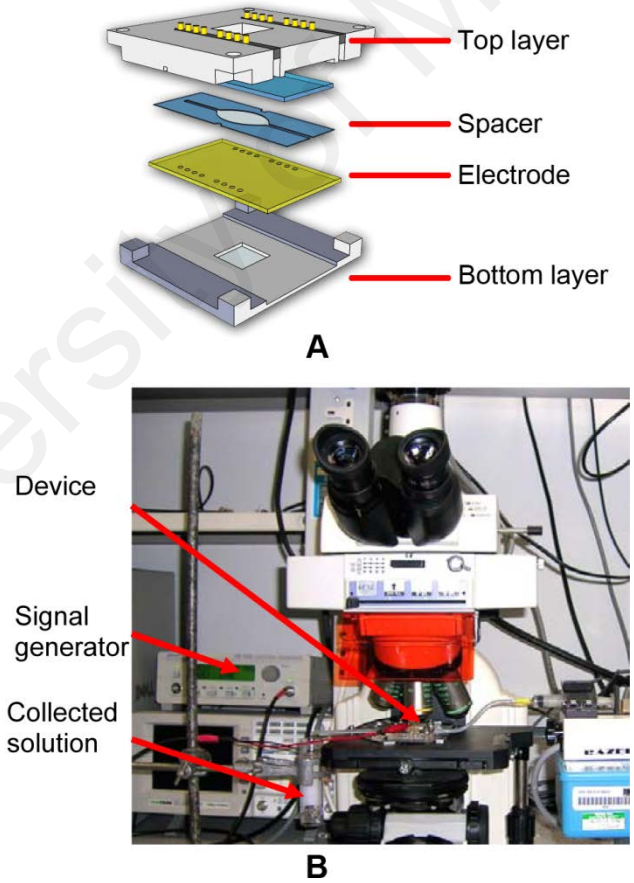



**Figure 4.1: a) Schematic diagram of the developed device; b) overall setup of components used in the experimental procedures.**

The design was fabricated using Perspex and PET as raw materials. The experiment was undertaken using the hardware systems combined with mechanical and electrical

parts from the bio-particle separation devices. The hardware system was designed based on a few criteria: to prevent the leakage of suspended sample due to the medium pressure, to ensure the light penetration at the center of the device, and specific materials used to ensure the potential for reuse of the system.

## 4.2 DEP electrode fabrication

I applied a screen printing technique in fabrication of DEP chip used in this study. The following protocol described the development of a simple process, with a low cost DEP chip being made consisting of micro channels and a DEP electrode.

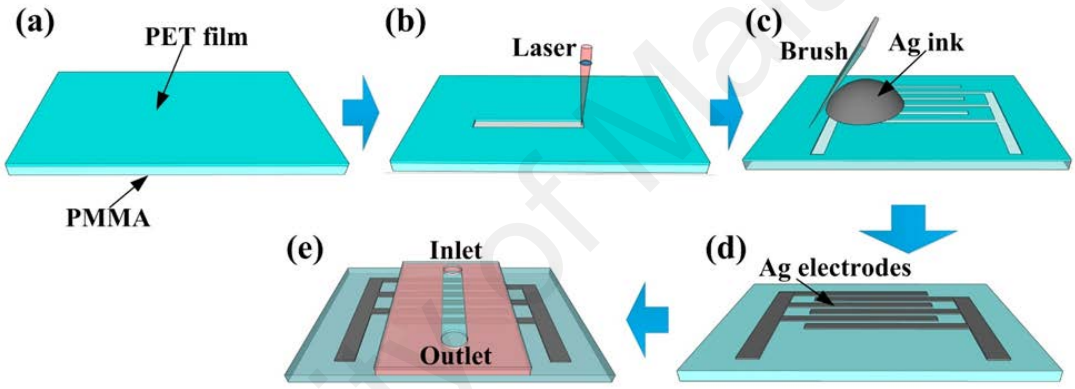



**Figure 4.2: Schematic of the fabrication process of a DEP microfluidic chip on a PMMA substrate**

Referring to Figure 4.2,

(a) A PET film was pasted on PMMA substrate.

(b) $CO_2$ laser was used to cut out the electrode pattern on PET.

(c) Silver conductive ink was applied on PET with brush.

(d) After curing process, electrode pattern formed on PMMA substrate.

(e) With the PMMA channel and cap cover on top of electrode, the DEP microfluidic chip is fully fabricated.

In this work, I selected conductive silver ink as the DEP electrode material based on its liquid state under room temperature, and thus suitability for screen printing. Using our

method, Ag conductive inks were screen-printed on three representative substrate materials of microfluidic chips, *i.e.*, PMMA, PET and paper, and the obtained DEP chips with Ag electrode patterns are as shown in Figure 4.3(a-c). In the figures, the Ag electrode patterns (with average width of 611 ± 17 μm and electrode gap width of 227 ± 34 μm) were uniformly formed on PMMA (Figure 4.3a), PET (Figure 4.3b) and A4 paper (Figure 4.3c), respectively. It proves the feasibility of our screen-printing method for fabricating the electrode pattern of DEP chip on different substrates, which could contribute to the further combination of the DEP technique with the microfluidic chips with different substrates.

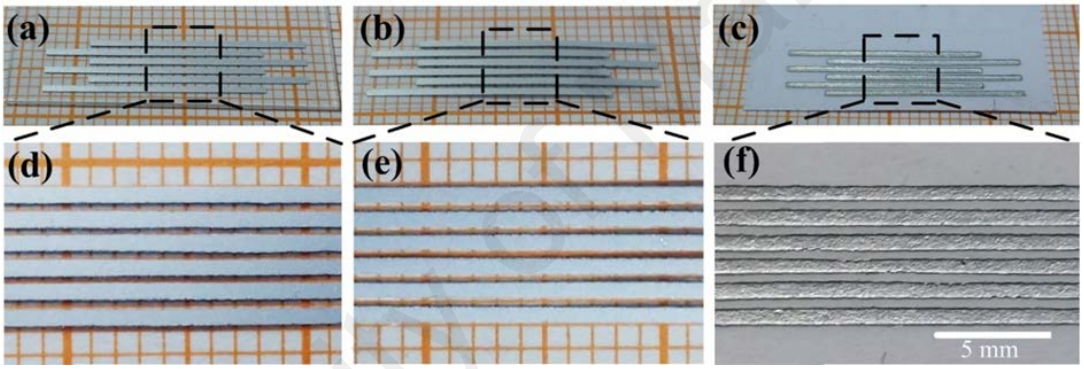



**Figure 4.3: (a-c) Photographs and (d-f) microscopic images of the Ag electrode patterns printed on different substrates: (a,d) a PMMA substrate, (b,e) a PET substrate, and (c,f) an A4 paper substrate. The background substrates are the graph papers with squares of 1 mm x 1 mm (length × width).**

From the microscopic images of the Ag electrode lines on these substrates shown in Figure 4.3(d-f), I observed that the Ag inks were well printed on these three substrates with uniform width and straight and obvious electrode boundary. The resistance of each fabricated Ag electrode was measured by a multimeter (UT58A, UNI-T, China) and found to be 15.02 ± 1.26 $\Omega \cdot cm^{-1}$, 19.58 ± 5.13 $\Omega \cdot cm^{-1}$ and 16.92 ± 3.04 $\Omega \cdot cm^{-1}$, respectively. Such good conductivities indicate the suitability of all substrates to be used as the electrodes of DEP microfluidic chips. Results were consistent among substrates, independent of hardness and translucency.

## 4.3 DEP microfluidic chip fabrication

The flow of fabrication of DEP microfluidic chip presented in the figure below.

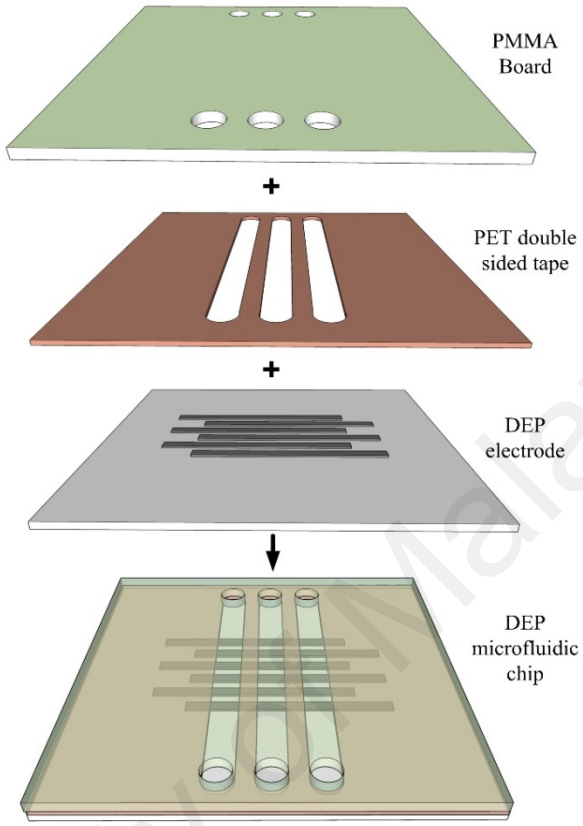



**Figure 4.4: The schematic diagram of the fabrication of DEP microfluidic chip**

A PMMA board with a thickness of 1 mm was cut out by a laser cutter (Versa LASER VLS3.50) to form a cover for DEP microfluidic chip. The channels with the size of 120 μm × 500 μm × 2500 μm were formed through the cutting on PET double sided tape with the same laser cutter. The PMMA board and PET tape were then pasted onto the integrated electrode pattern with a line width of 300 μm and gap width of 400 μm (through fabrication protocol discussed on section 4.2), which was designed by Corel Draw software. The final DEP microfluidic chip was put under a vacuum suction pump to ensure there was no air trapped between the PMMA cover with the DEP electrode. The preparation procedures for the PET-based and the A4 paper-based DEP microfluidic chips are similar to the above procedure, except changing the substrate to PET or A4 paper, respectively.

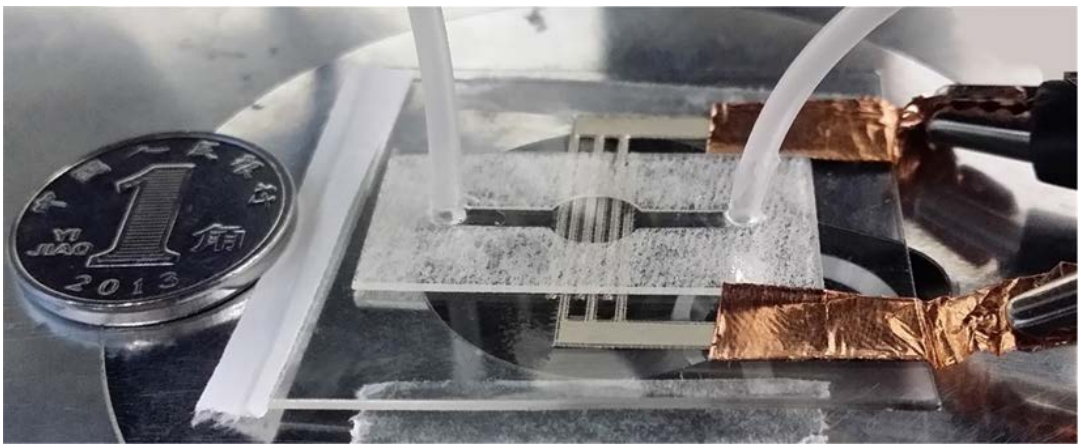



**Figure 4.5: A fabricated DEP microfluidic chip placed on microscope stage. A Chinese coin of 10 cent with diameter of 18 mm was placed besides the DEP chip for size comparison.**

The three straight line channels showed in the schematic diagram were changed to a single channel with a larger reaction area on the DEP electrode when fabricated in order to provide a bigger experimental visual view and better image capture. The PET double sided tape is semitransparent thus it will affect the image quality by partly blocking the light source of microscopy system. The channel was made in a symmetrical shape in order to provide smoother flow of the particle suspension moving from inlet towards the outlet. It has been found that an uneven flow will create a localized collection of particles around the edges of the chamber and cause an internal drag force effect on coming particles.

# CHAPTER 5: CHARACTERIZATION OF DEP MICROFLUIDIC CHIP

This section consists of three parts. The first is the sample preparation for the DEP experiment. Two types of samples: polystyrene beads with different diameters and cardiac fibroblast, were prepared for the particle manipulation test. The second part is an experimental study of sample crossover frequency under the effect of DEP. The result are compared with the theoretical values and used in the further analysis. The third part is an experimental setup which is based on the parameter from COMSOL simulation modeling and several experiments for evaluating the particle manipulation of DEP microfluidic chip. Pro and cons are discussed for DEP microfluidic chip.

## 5.1 Sample preparation

3, 5, and 10 μm diameter Polystyrene beads (Sigma) were chose as target samples in the particle manipulation of DEP. The aqueous microparticle suspensions of polystyrene particles were diluted with deionized (DI) water in volume ratios of 1:50, 1:40 and 1:20, respectively. The three suspensions were then ultrasonicated for 5 min to generate homogenous microparticle solution. In each experiment, 80-100 microliter of the particles suspension was injected into the micro channel through the input port.

Another sample used comprised cardiac fibroblasts with density of 106 cells $ml^{-1}$, in which the cardiac fibroblasts were isolated from the heart of neonatal Sprague-Dawley rats (1-3 day-old) and the sample solution was prepared according to the following protocol: First, the heart tissues of rats were excised following euthanasia. The heart tissues were washed with phosphate buffered saline (PBS) (pH = 7.4, Sigma-Aldrich, USA) and minced into small pieces. Tissue digestion was performed using 0.8% collagenase type II enzyme (MP Biomedicals, Aurora, USA) solution at 37°C with agitation. The pellets were resuspended in a cell culture medium composed of Dulbecco's Modified Eagle's medium (DMEM)/Ham F-12, 10% fetal bovine serum (FBS) and 1%

antibiotic/antimycotic (Gibco, New York, USA), and then plated for 45 minutes in a cell culture dish at 37°C and 5% $CO_2$ (Ruwhof *et al.*, 2000). Cardiac fibroblasts on day 2 after isolation were used for the DEP experiments. The following work was previously published in Journal of Micromechanic and Microengineering (Wei Hong *et al.*, 2015).

## 5.2 Characterization of DEP microfluidic chip on sample crossover frequency

I analyzed the DEP spectra with using PS beads as sample microparticles for the following particle trapping test. The deionized water used had a measured conductivity value of $2.0\times10^{-4}$ S m$^{-1}$ and a relative permittivity of 78.5. The overall conductivity of PS beads is defined as $\sigma_{\text{bulk}} + \frac{2K_s}{r}$ , where $\sigma_{\text{bulk}}$ is the bulk conductivity (negligible for polystyrene particles), $r$ is the average radius of PS beads, and $K_s$ is the total surface conductance (composed of the contributions of the Stern layer and diffuse layer formed around the particles). According to the product information, the relative permittivity of the PS beads is 2.55. To check the function of the fabricated DEP microfluidic chip for microparticle trapping, the crossover frequencies (*i.e.*, the change from positive DEP (p-DEP) to negative DEP (n-DEP)) of PS beads were measured by increasing the applied frequencies from lower values to higher ones. To reduce the probability of sudden electrolysis of electrolyte caused by the capacitive effect, the frequencies of AC signals were increased and decreased gradually during DEP experiments. At lower frequencies, the PS beads clearly exhibited a p-DEP effect and assembled to the positions close to the electrode. When the PS beads showed a n-DEP effect, they repelled from the electrode and assembled at the gap between electrodes. For the 3 μm-, 5 μm- and 10 μm-diameter PS beads, the crossover frequencies were 295 ± 30 kHz, 148 ± 12 kHz and 64 ± 7.1 kHz, respectively. Using the obtained crossover frequencies and Eq. 2, the overall conductivities of PS beads were calculated to be $1.6 \times 10^{-3}$ S·m$^{-1}$, $9.6 \times 10^{-4}$ S·m$^{-1}$ and $4.8 \times 10^{-4}$ S·m$^{-1}$ for the PS beads with diameters of 3 μm, 5 μm and 10 μm, respectively. Based on the above results, the relation between $Re[K(f_{cm})]$ and frequency is illustrated

in Figure 5.1. Considering that the cardiac fibroblasts I used have an approximately spherical shape and a diameter about 10 μm (similar to the 10 μm-diameter PS beads), I did not calculate their crossover frequency again. Since the single-shell model was used for the DEP experiments for trapping cardiac fibroblasts, the obtained crossover frequency of 10 μm-diameter PS beads was also applied for the cardiac fibroblasts to achieve the objective of cell trapping (Gagnon, 2011).

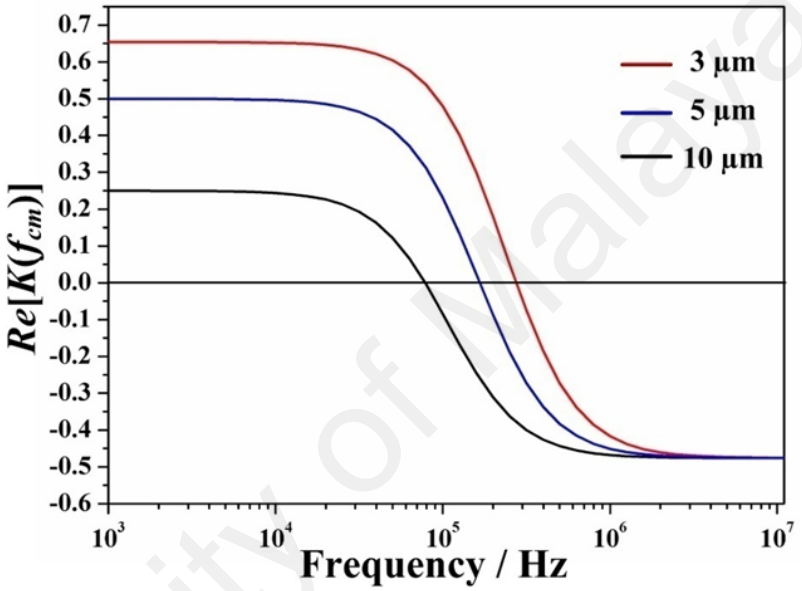



**Figure 5.1: Relationship of Re[K(fcm)] vs. Frequency for microparticles with diameters of 3 μm (red curve), 5 μm (blue curve) and 10 μm (black curve), showed in log graph respectively.**

## 5.3 Particle manipulation analysis

This section consists of two parts which are the discussion on manipulation of PS particle through the microfluidic AC-DEP chip and manipulation of bioparticle through the microfluidic AC-DEP chip.

### 5.3.1 Manipulation of micro polystyrene beads

To evaluate the performance of screen-printed microfluidic AC-DEP chip, different sizes of PS particles (3, 5, 10 μm, Sigma, China) and cardiac fibroblasts were mixed thoroughly and injected into the chip using a syringe pump at different flow rates (0.1 – 0.3 mL/h). A multiple output signal generator was used to generate the different

frequencies for particle trapping and manipulation from 100 Hz to 10 MHz. The movements of the PS beads and the cardiac fibroblasts on microfluidic chip were observed under microscope using the objective lens at 10×, 20× and 40× magnifications under bright field, and the images and videos were captured by a camera attached to the microscope. The values of various experimental parameters, such as microparticle size, biological cell type, applied frequency value, suspension medium and applied AC voltage, were carefully selected for the DEP experiments. After the experiment, the sample can be collected from the DEP chip outlet to a collecting tube for further analysis. The obtained DEP experimental results are shown in Figure 5.2. Before applying the sinusoidal signal to the DEP chip, the PS beads were homogenously and randomly distributed in the microchannel of the DEP chip (Figure 5.2a). When applying an AC voltage of 20 $V_{pp}$ with a frequency of 1 MHz to the two Ag electrodes, the 3 μm-diameter PS beads started to move and were repelled under the DEP force, and finally arranged in a chain-shape along the gap between the two electrodes after 5 minutes (Figure 5.2b), which indicates that the PS beads experienced a n-DEP force. The final trapping and alignment positions of these PS beads were close to the bottom electrode, which is due to the effect of the strength of the DEP force distribution leading to the alignment of PS beads at the positions of lower electrical field. After switching off the applied signals, the PS beads were released from the DEP force and spread away from the original chain (Figure 5.2c). When applying the same sinusoidal signals as in Figure 5.2b to the electrodes again, the PS beads were trapped and aligned at similar positions as the previous process (Figure 5.2d), proving the repeatability of the fabricated DEP device for trapping the PS microparticles.

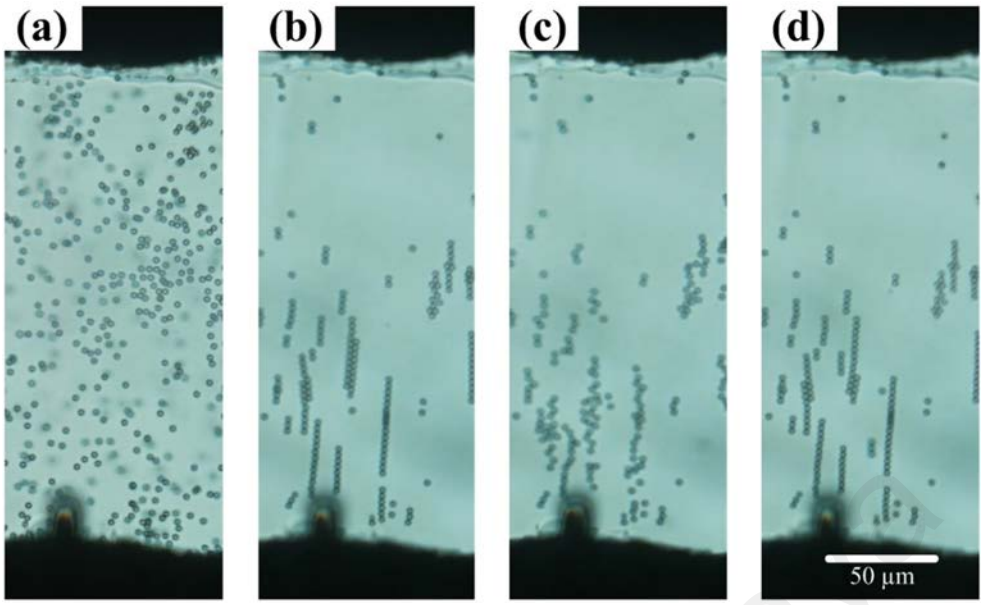



**Figure 5.2: Microscopic images of a 3 µm-diameter PS beads suspension in the microchannel of a fabricated PMMA-based DEP microfluidic chip (magnification of 40×). (a) Before applying a sinusoidal signal to the DEP chip; (b) Applying an AC voltage of 20 Vpp with frequency of 1 MHz to the two Ag electrodes of DEP chip for 5 minutes; (c) After switching off the applied signals; (d) Re-applying the sinusoidal signals to the electrodes.**

To further prove the capability of our fabricated DEP microfluidic chip to trap microparticles with bigger sizes and from mixed solution, a suspension with 5 µm- and 10 µm-diameter PS beads was used as the second sample solution. The same DEP experimental procedure was performed on the DEP microfluidic chip injected with the PS beads mixture and the obtained results are represented in Figure 5.3.

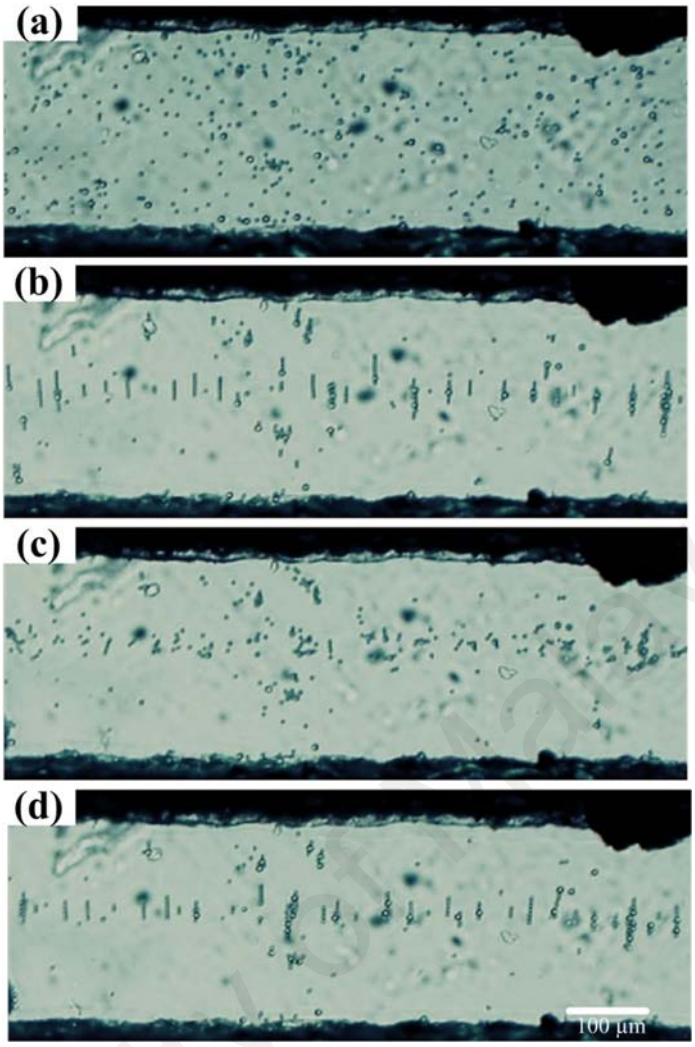



**Figure 5.3: Microscopic images of a suspension with a 5 µm- and 10 µm-diameter PS beads mixture in the microchannel of a fabricated PMMA-based DEP microfluidic chip (magnification of 10×). (a) Before applying a sinusoidal signal to the DEP chip; (b) Applying an AC voltage of 20 $V_{pp}$ with frequency of 1 MHz to the two Ag electrodes of DEP chip; (c) After switching off the applied signals; (d) Re-applying the sinusoidal signals to the electrodes.**

As shown in Figure 5.3b and Figure 5.3d, after applying an AC voltage of 20 $V_{pp}$ with frequency 1 MHz to the electrodes, both the 5 µm- and 10 µm-diameter PS beads moved along a central streamline in the gap between the two electrodes, indicating that the 5 µm- and 10 µm-diameter PS beads exhibited n-DEP response in the applied frequency range. But the alignment positions of these two kinds of PS beads were in the center of the gap between two electrodes, rather than at the vicinity of the bottom electrode (as observed in Figure 5.2b and Figure 5.2d). It could be due to the different particle sizes and weights leading to the different polarization forces applied to the particles. The 5 µm-diameter PS

beads were aligned and attached to the 10 μm-diameter PS beads. This may be due to a non-uniform electrical field generating a polarization region on the surface of the 10 μm-diameter PS beads with a larger surface area, which then attracts the smaller 5 μm-diameter beads to their surfaces.

The above DEP experimental results prove the feasibility of the fabricated DEP microfluidic chip with Ag electrodes for trapping microparticles with different sizes and from a mixture suspension. It is noted that the PS beads could also assemble on the Ag electrode surface due to a p-DEP force in our experiment. But this could not be observed under the inverted microscope due to the non-transparent Ag electrodes used in this case. In addition, the alignment of PS beads can be used to quantify the DEP force applied to the beads by assessing the light intensity change on the electrode before and after applying the signal to the DEP chip (Kadri *et al.*, 2012). To analyse the results from the experiment, a dielectrophoresis based algorithms will be studied and developed. Further study of the device will be decided following the analysis and discuss of the initial experimental results.

### 5.3.2 Manipulation of bioparticles

To prove the feasibility of our DEP microfluidic chip for manipulation of bioparticles, I selected cardiac fibroblasts as the target sample for the DEP experiment due to its approximately spherical shape and average cell diameter being close to 10 μm which is the average size of the day 2 cultured fibroblasts (Freitas, 1999). Before the DEP experiment, the cell viability and cell count were tested to ensure that the cells were alive. A cell density of $10^6$ cells $ml^{-1}$ in a suspension PBS medium with conductivity of 15.0 $\times 10^{-3}$ S $m^{-1}$ was used in the following DEP experiments. As shown in Figure 5.4a, after injecting the PBS medium containing a suspension of cardiac fibroblasts to the DEP microfluidic chip, the cells distributed randomly in the microchannel of the DEP chip.

After applying an AC voltage of 20 $V_{pp}$ with frequency of 1 MHz to the Ag electrodes, some of fibroblasts moved to assemble and aligned in a line-shape (Figure 5.4b), proving the feasibility of our DEP microfluidic chip to trap real cell samples. The alignment of the fibroblasts is not as obvious as of the PS beads observed above (Figure 5.2 and Figure 5.3), which could be due to the less conductive cell culture medium and the sticking of the adhesive fibroblast cells to the platform. As shown in Figure 5.4, the orthogonal chain of fibroblasts happened at the edge of electrode when voltage was supplied. The alignment of fibroblasts showed that of the gradient instantaneously produced on those particular surfaces; at right angles between the two electrodes.

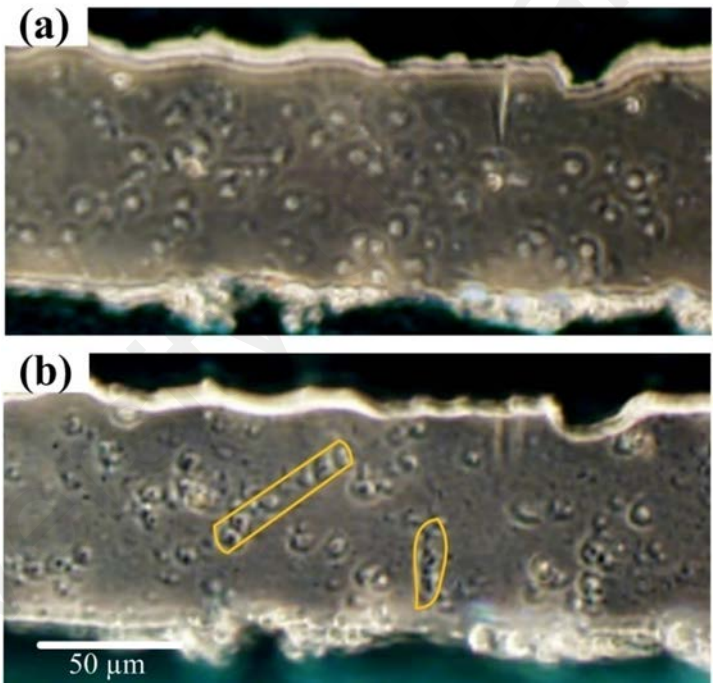



**Figure 5.4: Microscopic images of a cell culture solution with cardiac fibroblasts of density of 106 cells ml-1 in the microchannel of a fabricated PMMA-based DEP microfluidic chip (magnification of 20×). (a) Before applying a sinusoidal signal to the DEP chip; (b) Applying an AC voltage of 20 $V_{pp}$ with frequency of 1 MHz to the two Ag electrodes of DEP chip for 5 minutes.**

# CHAPTER 6: CONCLUSION AND FUTURE WORK

## 6.1 Conclusion

Microfluidics devices show great potential to meet technical requirements for particles manipulation especially with their low fabrication cost in resource limited settings. This study showed a facile and low cost microfluidic chip was developed through screen-printing technique and applied to fabricate silver electrodes on both solid/soft and transparent/non-transparent DEP substrates, including PMMA, PET and paper. However, one major challenge still remains: to develop these microfluidic device effectively with a high resolution of chip fabrication for improved manipulation work. From the research of my study, the electrode fabricated with screen printing technique can be potentially increased in resolution with a high accuracy laser cutting machine that can improve the efficiency of particle manipulation and the function of microfluidic chip. As shown in the literature, microfluidic technologies hold great promise to achieve standard detection sensitivity levels for POC through quantitative, proof-of principle studies in a fast, controlled, and high-throughput manner.

Furthermore, to get approval for clinical use of DEP microfluidic devices (e.g., regulatory demands by Food and Drug Administration (FDA) or National Institution of Health (NIH)), microfluidic technologies should also be capable of satisfying critical evaluation criteria, such as testing characteristics and factors: (i) test performance (sensitivity and specificity), (ii) ease of use, (iii) conditions of use and storage, and (iv) shelf life (Peeling *et al.*, 2008; Weigl *et al.*, 2008). Specifically, for commercialization, the efforts in this field should be made towards minimally instrumented POC diagnostics by developing platforms that can function without any peripherals (Weigl *et al.*, 2008).

The principles of DEP have been successfully integrated with current microfluidic devices for on-chip manipulation and assessment of bio-particle at resource-limited

settings. The DEP based microfluidic device which been developed in this study has significant advantages over conventional particle manipulation techniques such as reducing costs and increasing portability and disposability. The future trends of particle manipulation will focus on faster processing time, higher accuracy and lower fabrication cost. Hence, further development of current DEP microfluidic devices can reach the aims for better microfluidic devices. Here, I specify key requirements, such as disposability, cost-effectiveness, ease of use, and portability that can improve and further advance microfluidic device for a wider use at resource-limited settings.

## 6.2 Future work

As had been mentioned before, the analysis and modeling carried out in this thesis is in no way exhaustive. In view of this and the availability of numerous techniques of electrode fabrication, I have proposed several studies to be carried out in the future as a continuation of this work. Among others are:

- Based on screen printing technique, fabricate multiple formation of electrode arrays in order to perform different types of particle manipulation research such as particle separation, particle pairing and so on.
- By using laser machine with higher resolution, improve the resolution of the electrodes in order to generate stronger DEP field for particle manipulation.
- By using screen printing technique, fabricate the paper-based DEP electrode and perform bio-particle manipulation research on the electrode based on current experiment set up.

As the technology to manipulate particle on microfluidic device further, so does the availability of techniques of electrode fabrication. This development would certainly be a challenge to BioMEMS in resource limited setting. I hope to contribute to this endeavor by continuously attempting to a lower cost, more simple electrode fabrication technique

and hopefully, with the knowledge emerging from this technique, better microfluidic chip could be developed which would help guarantee the particle manipulation result and to be used in country under resource limited setting.

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## APPENDIX A LIST OF PUBLICATIONS AND PAPERS PRESENTED

**Conference proceeding**

Wee, W.H., Razak, M., Abdul, A., & Kadri, N. A. (2012). Dielectrophoretic K562 cell entrapment device using benchtop microfluidics fabrication. *Advanced Science Letters*, *15*(1), 1-4.

**Research article**

Wee WH, Li Z, Hu, Kadri NA, Xu Feng, Li F, Pingguan- Murphy B. (2015). Fabrication of dielectrophoretic microfluidic chips using a facile screen-printing technique for microparticle trapping. *Journal of Micromechanic and Microengineering*. (Accepted)

Wee, W.H., Razak, M., Abdul, A., & Kadri, N. A. (2012). Electrochemical Cell Entrapment Device for BioMEMS Applications Using Benchtop Fabrication Techniques. *Int. J. Electrochem. Sci.* (Accepted)

Li, Z., Li, F., Hu, J., Wee, WH., Han, Y., Pingguan-Murphy, B., Xu, F. (2015). Direct writing electrodes using ball pen for paper-based point-of-care testing. *Analyst*. (Accepted)

Zedong Li, Hao Liu, Cheng Ouyang, Wei Hong Wee, Xingye Cui, Belinda Pingguan-Murphy, Fei Li, Feng Xu. (2015). Recent advances in directly writing electronics and their emerging applications. *Advanced Functional Material*. (Accepted)

Jie Hu, Chee-Hong Takahiro Yew, Xiaoshuang Chen, Shangsheng Feng, Qu Yang, Wei-Hong Wee, Belinda Pingguan-Murphy, Tian Jian Lu, Feng Xu. (2015). Paper-based capacitive sensors for identification and quantification of chemicals at the point of care. *Talanta* (Accepted)

Chee Kuang Tang, Wei Hong Wee, Norita Mohd Zain, Belinda Pingguan-Murphy, Abdul Kariem Arof. (2015). Development of chitin fibre reinforced Chitosan composite doped with silver salt as antimicrobial membrane. *Digest Journal of Nanomaterials and Biostructures.* (Submitted)