MICROFLUIDIC BASED DIELECTROPHORESIS CELL SORTER

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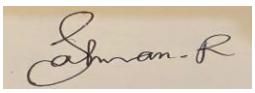

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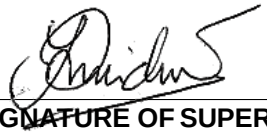

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A report submitted in partial fulfillment of the
requirements for the award of the degree of
Bachelor of Engineering (Electrical-Mechatronics)

School of Electrical Engineering

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Universiti Teknologi Malaysia

JULY 2020

DECLARATION

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DEDICATION

To all the engineers, doctors and people from all the fraternities, who work relentlessly to research and find cures for cancer to help and save other humans. To my family, especially my father who has devoted his life to give me a life full of privileges and taught me the true essence of rationality and logic. To all the people in all phases of my life who have believed in me, told me I could do it and aided in the process of who I have become. To my nephew Afraaz, who conquers the biggest part of my heart.

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ABSTRACT

Cancer is known to be the second leading cause of human death. It is a deadly disease that affects people throughout the world of any gender and any age. Mortality due to cancer is mainly due to late diagnosis and detection. Currently, there are few methods to detect cancer such as blood tests and biopsy. These methods come with subtle drawbacks which share similar disadvantages like huge time consumption to give a result of detecting stages of cancer. Dielectrophoresis (DEP) is a label-free method caused due to the non-uniform electric field. DEP is based on the parameters of cell properties such as size, capacitance, conductivity, and permittivity. The circulating tumor cells (CTCs) and red blood cells (RBCs) are different in size. So, the effect of the DEP force on them is different. This causes them to be located at different heights and flow rates, causing them to travel to different outlets. The study was carried out on simulation platform COMSOL Multiphysics by which the study on characterization of the design in terms of electric potential, pressure, fluid flow of the particles was carried out. The results contain two proposed designs of a microfluidic chip with array electrodes, consisting of two inlets and two outlets connected directly to the main channel. With the aid of dielectrophoresis field-flow-fractionation (DEP-FFF) using a low voltage of 10 Volts, the device was successful to separate cancerous cells from a mixture of CTCs and RBCs with an efficiency of 87% in 108 seconds.

ABSTRAK

Barah dikenali sebagai penyebab kedua utama kematian manusia. Ia adalah penyakit maut yang menyerang manusia di seluruh dunia dari sebarang jantina dan usia. Kematian akibat barah terutamanya disebabkan oleh diagnosis dan pengesanan yang lewat. Pada masa ini, terdapat beberapa kaedah untuk mengesan barah seperti ujian darah dan biopsi. Kaedah-kaedah ini mempunyai kekurangan yang mengandungi kelemahan yang serupa seperti penggunaan masa yang besar untuk memberikan hasil pengesanan tahap barah. Dielektroforesis (DEP) adalah kaedah bebas label yang disebabkan oleh medan elektrik yang tidak seragam. DEP didasarkan pada parameter sifat sel seperti ukuran, kemuatan, kekonduksian, dan ketelusan. Sel tumor beredar (CTCs) dan sel darah merah (RBCs) mempunyai ukuran yang berbeza. Jadi, kesan daya DEP terhadap kedua-dua jenis sel ini adalah berbeza. Ini menyebabkan kedua-dua jenis sel ini berada pada ketinggian dan kadar aliran yang berbeza, menyebabkan kedua-dua jenis sel ini bergerak ke jalan keluar yang berbeza. Kajian ini dilakukan pada platform simulasi COMSOL Multiphysics di mana kajian mengenai pencirian reka bentuk dari segi potensi elektrik, tekanan, aliran bendalir partikel dilakukan. Hasil kajian mengandungi dua reka bentuk cip mikrofluidik dengan tatasusunan elektrod, yang terdiri daripada dua saluran masuk dan dua saluran keluar yang disambungkan terus ke saluran utama. Dengan bantuan *dielectrophoresis field-flow-fractionation* (DEP-FFF) menggunakan voltan rendah 10 Volt, alat ini berjaya memisahkan sel barah dari campuran CTCs dan RBCs dengan kecekapan 87% dalam 108 saat.

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LIST OF ABBREVIATIONS

DEP	–	Dielectrophoresis
FFF	–	Field Flow Fractionation
CTC	–	Circulating Tumor Cells
RBC	–	Red Blood Cells
FACS	–	Fluorescence-Activated System
MACS	–	Magnetic activated system
EP	–	Electric Potential
Vpp	–	Voltage peak-to-peak
fCM	–	Classius Mossotti Frequency

LIST OF SYMBOLS

µm	–	Micrometer
µm/s	–	micrometer per second
cm	–	centimeter per second
kHz	–	kiloHertz
V	–	Volt
m/s	–	meter per second
εm	–	permittivity of material
Rms	–	root mean square
Pa	–	Pascal
kg/m^3	–	kilogram per meter cube
nm	–	nanometer

CHAPTER 1

INTRODUCTION

1.1 Problem Background

The medical sector has advanced in the past few centuries and is considered to be at its peak. Unfortunately, there are major issues that are still needed to be solved and cancer diagnosis is one of them. A statistics carried out by the National Cancer Institute suggests that the number of cancer cases by 2020 will be 1.9 million per year and death due to cancer to rise until 630,000 per year from 575,000 per year in 2010 [1].

Only in Malaysia, there were around forty-four thousand patients diagnosed with cancer and in more than half of the cases, early death was the result [2]. It has been reported that around 100,000 people are diagnosed with cancer at one specific time. It also has been estimated that one in four Malaysians will develop cancer by the age of 75.

The significant difference between a cancerous cell and a normal cell is that the cancerous cell tends to replicate itself and grow abnormally and uncontrollably. Due to this uncontrolled growth, it can migrate to other organs from the initial location or organ, which is known as metastasis [3]. Metastasis occurs at a late stage of cancer and is mostly incurable.

The early detection of cancer is considered to be most vital which can reduce mortality due to cancer. Early detection can be divided into two components :

1. Early Diagnosis
2. Screening

Early diagnosis is considered when prompt actions are taken based on the early signs diagnosed such as lumps, sores that fail to heal, abnormal bleeding, persistent indigestion, and chronic hoarseness. Screening is more of a general medical testing as a measure of precaution of general public who are otherwise considered healthy or do not show severe symptoms [4].

1.2 Problem Statement

Although there are many methods currently being practiced by the medical sector to detect cancer, these methods come with subtle drawbacks [5]. These methods range from conventional methods like a blood test, a biopsy to image processing to many different microfluidic based methods. In almost all the existing methods, time to get a final result, and the detection of which stage the cancer is in are major issues [6].

Among the aforementioned diagnostic methods, microfluidic has become quite popular in recent days due to its the ability of cellular level separation and analysis with high precision and instantaneity [6]. It has been a proven technology that came in the limelight in the past few years mostly due to its the inclusion of miniaturized devices, which as a result provides not only faster isolation speeds but also great efficiency and the reduction of equipment size [7,8]. Thus this project focuses mainly on designing a suitable microfluidic-based DEP cell sorter that can be used to separate cancerous cells from normal, healthy cells which can eventually help to detect cancer in an earlier stage. There are currently few prolific methods microfluidic-based methods used for operating cell separation namely Microfluidic Fluorescence-Activated System (FACS), Magnetic Activated System (MACS), Acuoustofluidic Cell Sorting but they come with their disadvantages like challenges to fabricate and also it can be a hard task to impose interfacing while dealing with biological samples such as cells [9-13]. The separation that is founded based on dielectrophoretic (DEP) forces can give faster process time and keep the purity and

efficiency of particles high after the separation is done and separation [14, 15]. Thus this study focuses mainly on designing a suitable microfluidic-based DEP cell sorter that can be used to separate cancerous cells from normal, healthy RBC cells which can eventually help to detect cancer in an earlier stage.

1.3 Project Objectives

1. To design and optimize a microfluidic chip based on Dielectrophoresis (DEP) that will give real time data about cancerous cells.
2. To characterize the microfluidic chip from electrical and pressure perspectives.
3. To implement separation of CTCs (Circulating Tumor Cells) from Red Blood Cells for early detection of cancer.

1.4 Project Scopes

Using DEP-based microfluidic chip can be a feasible solution to separate and detect the characteristics of a cancerous cell but due to the absence of the real chip and the required cell samples the study is limited to certain constraints:

1. Focused solely on simulation with COMSOL Multiphysics.
2. No physical experiment is involved.
3. Samples that will be mimicked during the simulation are Circulating Tumor Cells (CTCs) and Red Blood Cells (RBCs).
4. The proposed miniature-sized device only work with cells size less than 10 micrometer.

1.5 Report Organization

The Report is categorized into five chapters. All the chapters are in chronological order.

Chapter 1

This chapter discusses about the prime interest and motivation behind designing a microfluidic-based dielectrophoresis chip for cell sorting. The problem background, problem statement, poject objectives and scopes are thoroughly discussed in the chapter too. In the problem background the severity of cancer and the importance of the immediate actions are discussed. Based on the problem statement the objectives have been set to solve the faced problems. The scope defines the functionalities and constraints of the project.

Chapter 2

In this chapter current state-of-art methods that are used for cancer detection are reviewed. Researchers' works from journals, book chapters have been categorized into traditional and microfluidic-based methods. The limitations for each of the mehods have been mentioned in tabular form. A brief introduction of the Ciculating Tumor Cells, Microfluidics and Dielectrophoresis: Field Flow Fractionation have also been made.

Chapter 3

In this chapter the methodology of the project is discussed. The overall system architecture of the microfluidic device, equations involved in the proposed solution and the simulation configurations have been discussed.

Chapter 4

Chapter 4 is designated for the results and analysis. In this chapter all the results data are presented and relevant analysis were made. There resutls were based on the sustainability of the proposed designs, the effect of the DEP force, required voltage and frequency, efficiency and time taken by the chip to complete cell separation.

Chapter 5

This chapter describes the Gantt chart and finance required for the whole project

Chapter 6

In this chapter the summary of the whole project based on the all the other chapters in the report is presented. The things that were achieved throughout the project are mentioned. The future scopes have also suggested in this chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, all the major elements and methods will be discussed thoroughly to correlate the drawbacks of previous works and give a comprehensive overview of the topic. Various techniques used for a microfluidic-based cell sorting will be also discussed to eliminate the need for further explanation in the coming chapters.

2.1.1 Circulating Tumor Cells

Thomas Ashworth, an Australian physician was the first researcher to observe the circulating tumor cells, while he made a breakthrough conclusion that cells identical to the tumor itself were found in the blood of a patient with metastatic disease [16]. That means it is closely related to the degree of cancer cell metastasis and the stages of cancer development. The study has also shown that the monitoring of CTCs can be used throughout the therapy of a patient as well as before the initiation of the treatment itself [17].

2.1.2 Microfluidic

Microfluidics comprises the science of fluid manipulation on a very small scale which can go up to micro and nanometer scale [18]. This technology offers a growing set of tools for manipulating small volumes of fluids to control chemical, biological, and physical processes that are relevant to sensing.

The microfluidic laboratory-on-a-chip (LOC), a type of miniaturized devices mostly produced by the technique of microfabrication, has developed rapidly in the last few decades since its introduction in the 1990s. This leads to the possibility of carrying out analysis with a very limited amount of samples, making most bioanalysis very little invasive and possible in point-of-care centers that are not specialized [19]. Microfluidic devices have been used in the biomedical and cell engineering, largely because of the ability of microfluidic devices to mimic the physiological conditions such as pressure, temperature, and flow rate [20]. A typical Microfluidic device consisting of one inlet and an outlet is shown in Figure 2.1 and advanced Microfluidic device for cell sorting is shown in Figure 2.2**.**

The pioneering experiments of microfluidics-based antibody-antigen reactions were observed by Du et al. [21]. They followed a method where specific antibodies for endometrial cancers were used to cover the surface of the microchannel and the mixture fluid which consisted of the cancer cells were passed through the channel. The efficiency observed from the experiment was quite low, around 30%. Later, an efficiency of 50% was achieved when Wang et al. [22], made the surface of the microchannel relatively rugged using nano-cylinders, which increases the surface-volume ratio significantly. Although it was a better efficiency achieved, the number is still low.

Figure 2.1: Typical Microfluidic device consisting of one inlet and an outlet [8]

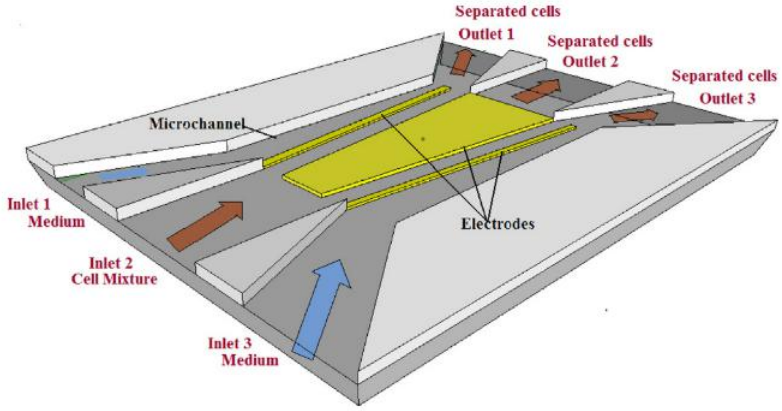

Figure 2.2: A microfluidic-based chip used for cell separation [24]

2.1.3 Dielectrophoresis- Field-Flow Fractionation Theory (DEP-FFF)

DEP is at its fundamental was first coined by H.A. Pohl, to define a force that is caused when neutral particles in a medium possessing different and varied dielectric properties are exposed into a non-uniform electric field [23]. As a result it polarizes the specific particles based on the nature of their dielectric properties [24]. The DEP force can be classified into two categories based on their correlations of dielectric properties of both particles and medium, in this case, the fluid [25]. As illustrated in In Figure 2.3, the positive DEP force (p-DEP) attracts the particles toward the higher electric gradient, on the other hand, the negative DEP force (n-DEP) repels the particles away from the higher electric gradient.

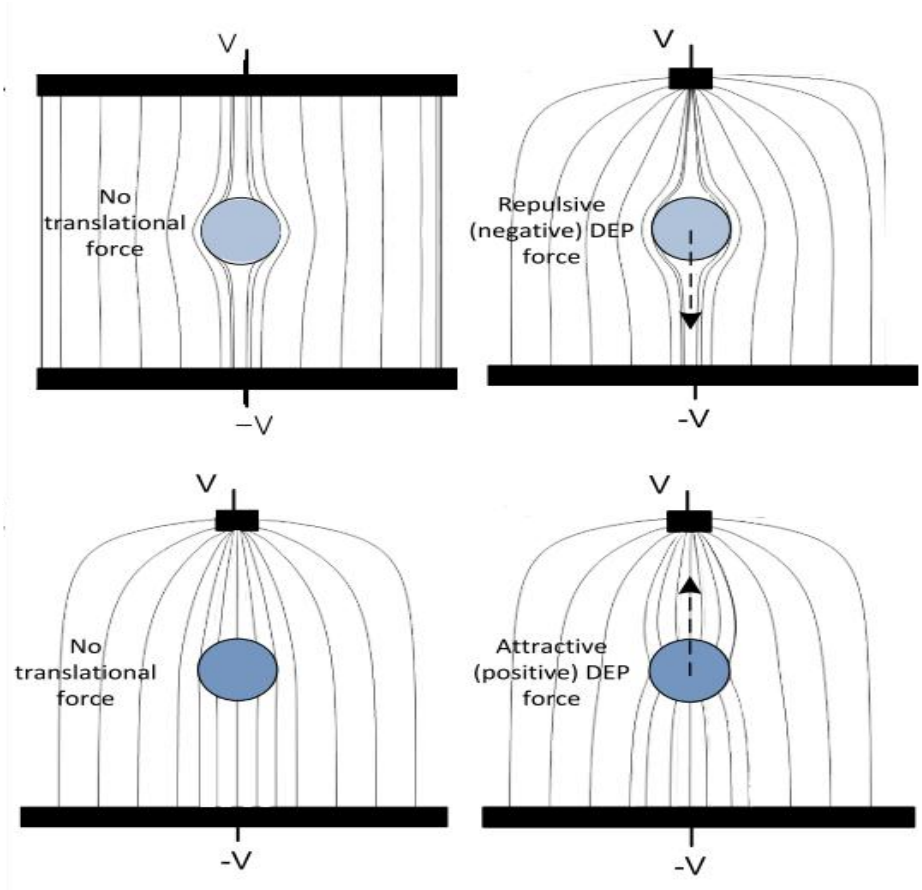

Figure 2.3: Illustration of p-DEP and n-DEP force on particles [8]

The DEP force can be expressed by the following equation (1) :

$$F_{DEP} = 2\pi\varepsilon m\ \varepsilon o\ Re[fcm]\ \nabla|Erms|/2 \qquad (1)$$

Where,

a: radius of the particle,

εm: permittivity of suspending medium,

εp: permittivity of the particle

E: electric field strength,

Re[fcm]: Real part of the Clausius–Mossotti factor.

2.2 Related works

To date, there are quite a few methods that can be used to detect cancer. These methods can be sub divided into two categories as illustrated in Figure 2.5.

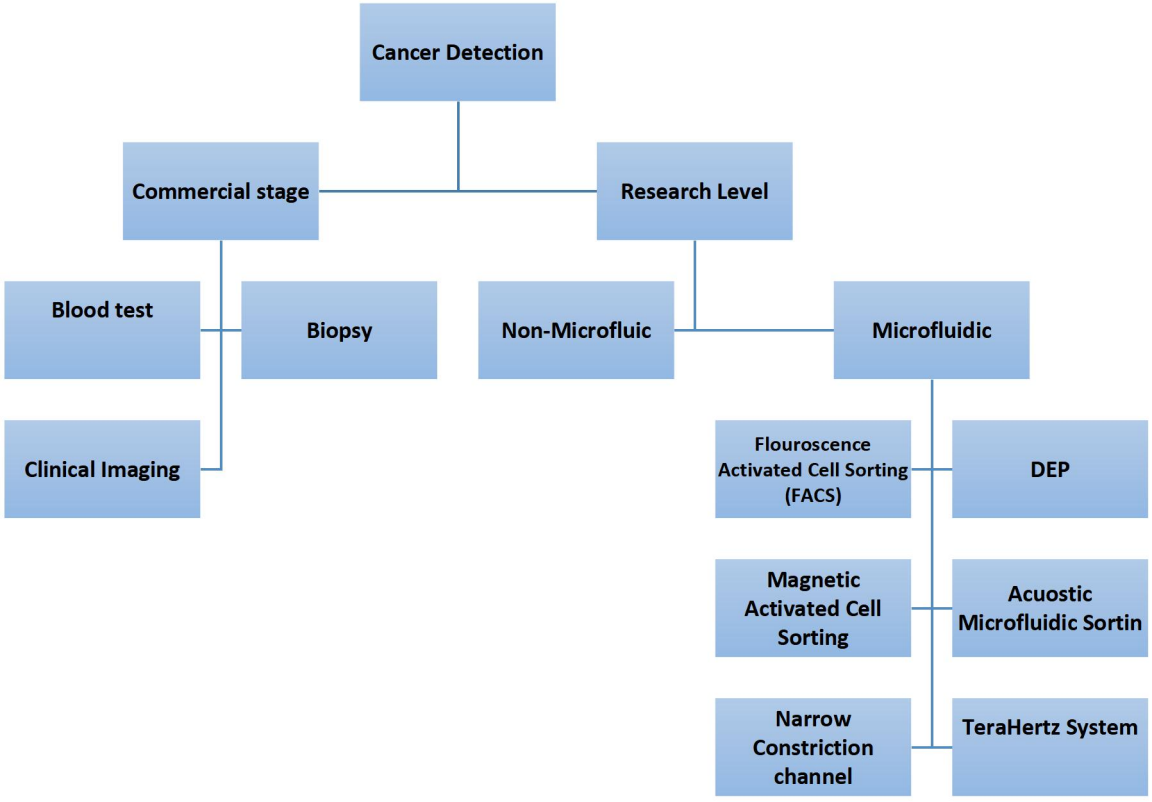

Figure 2.5: Breakdown of methods involving cancer detection

2.2.1 Conventional Methods

Some of the conventional and commercial methods are blood tests, clinical imaging and biopsies [28]. Implications of image processing and machine learning for optimization of cancer data and cancer diagnosis are relatively new approaches that are researched on.

During a blood test, the amount of various types of blood cells in the sample are measured, while in a biopsy test a sample of abnormal tissue from the body is extracted. Although these methods are currently being practiced by the medical sector to detect cancer, these methods come with subtle drawbacks shown in Table 2.1[5].

For instance, the blood test is incapable of giving a final result. It is also unable to provide detailed information about cancer detection [29]. They only provide some clues by identifying the cancer markers (biomarkers). This shows that the blood test can only provide qualitative data that leads to insufficient data to detect cancerous cells. Next, for clinical imaging, usually, the results are lagging behind the tumor progression. Research shows that specific screening test such as mammography takes around three days to weeks depending on the critical stage of cancer [30], while for the analysis of biopsies, it takes more than 10 days to give the result. Then, the clinical imaging method uses high technologies such as radiation and high magnetization force, so this leads to bigger investment and higher costs for the patient to pay [31]. Tissue biopsy is still considered to be the gold standard of methods for cancer diagnosis [32]. Although for this method, it is also been observed in many cases it can make an alternative path to spread and may hike the risk of bleeding, inflammation or even dissemination of cancer cells [33]. Also, it is a method that only outputs snapshots of static tumors of a particular time point rather than depicting dynamic changes that take place during the whole cancer treatment [34].

Table 2.1: Conventional cancer detection methods

Method	Author/Year	Research Title	Limitations
Blood Test	Lee D, Hwang B, Kim B. 2016	'The potential of dielectrophoresis activated cell sorter (DACS) as a next-generation cell sorter.	Cannot give the final result and cannot give confirmation.
Clinical Imaging	He M, Zeng. 2016	'Microfluidic Exosome Analysis toward Liquid Biopsy for Cancer', Biomicrofluidics.	Leads to bigger investment and higher cost for the patient to pay
Biopsy	Gascoyne P, Xiao-Bo Wang, Ying Huang, Becker F. 1997	'Dielectrophoretic Separation of Cancer Cells from Blood', Biomicrofludics.	✓ Time consuming ✓ Risk of bleeding, dissementation of cancer cells.

2.2.2 Cancer Detection through Image Processing

Although huge progress has been made in cancer diagnosis through image processing and machine learning over the years, the scope is still very small in terms of applications. It has been observed by S. Gokhale that around 15% of the images can go missing while trying to capture images through mammography [35]. This approach is also quite time-consuming and long since the whole process requires a lot of pre-processing of images and de-noising as illustrated in Figure 2.6 [36].

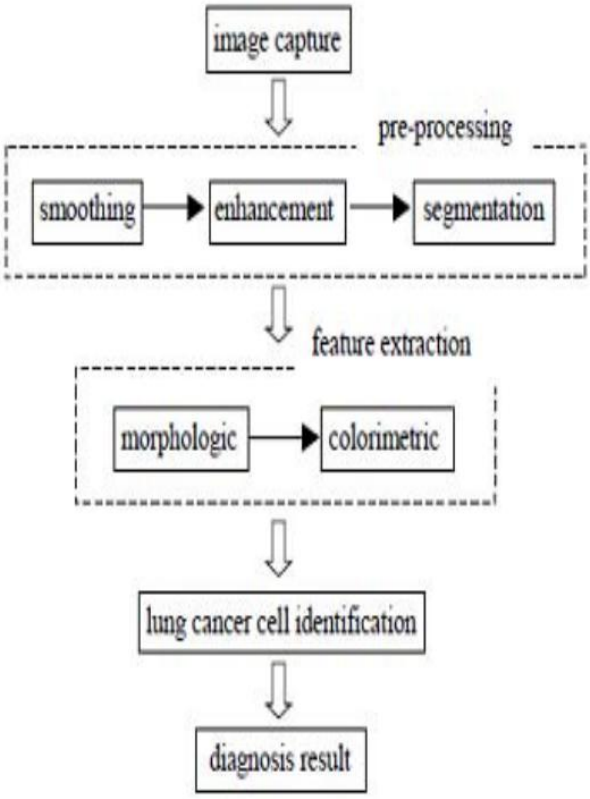

Figure 2.6: Entire process of cancer detection through Image Processing [36]

2.2.3 Microfluidic Fluorescence-Activated System (FACS)

The FACS system is a famous method of cell sorting in the current world. It has the ability to separate a selected population of cells from the heterogeneous mixture based on fluorescence and light scattering characteristics of each particle or cell as presented in Figure 2.7. Although this system is chosen by researchers for cell separation and sorting due to its compatibility, sensitivity, and high throughput but to do an experiment with FACS a large amount of material, samples are needed and in the medical world sample amount is a big concern due to its scarcity [37].

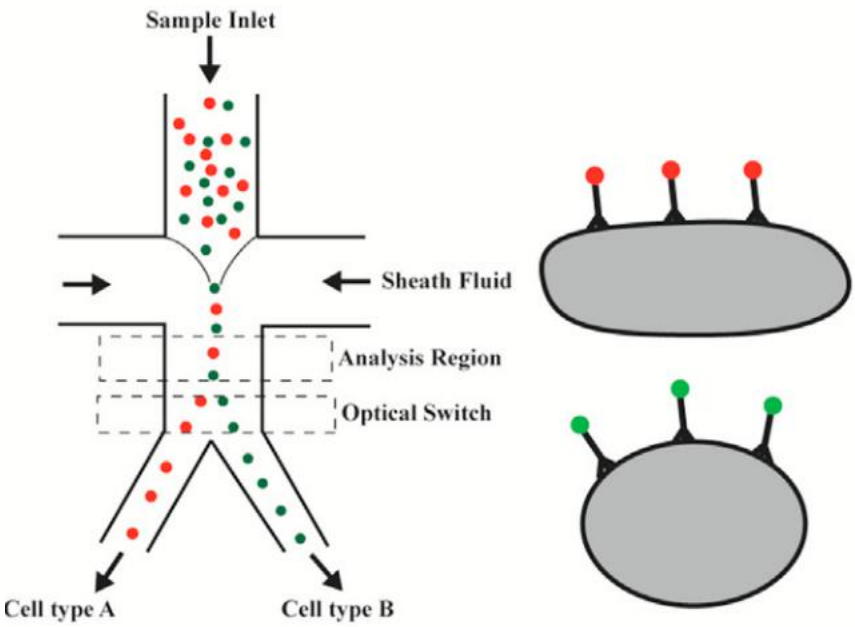

Figure 2.7 : Schematic representation of FACS [37]

2.2.4 Microfluidic Magnetic activated system (MACS)

By using marker specific antibodies conjugated to magnetic particles, MACS system separates the mixtures of cells as reffered by the schematic diagram in Figure 2.8. The system works by making the antibodies to be exposed to high affinity towards a particular cell surface markers. By using centrifugation and magnetophoresis in a single micro MACS configuration Kirby et al [38], have successfully isolated breast adenocarcinoma cells and HIV epitopes from a sample of blood. The sensitivity for both cases were found to be 1 cell/mL and had a capture rate efficiency of 88% and 92%, respectively. Besides that, the challenging part of the system was to remove antigen and label from the cell surface.

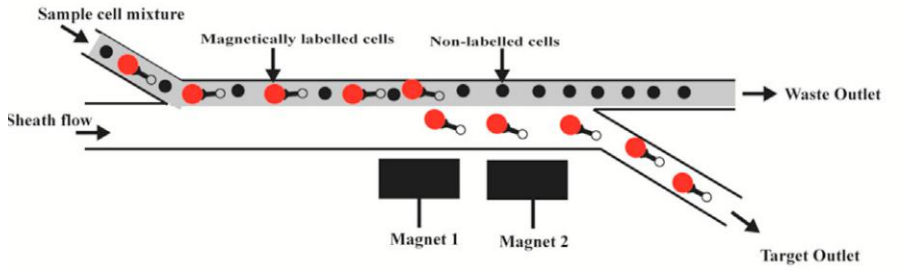

Figure 2.8 : Schematic representation of MACS [38]

2.2.5 Acuoustofluidic Cell Sorting

The separation in acoustofluidic occurs when there is an interaction between electro-mechanically non-spontaneous acoustic waves and fluids and inclusions within the fluids. There are many ways to generate acoustic waves but using piezoelectric transducers for cell sorting is quite widespread [39].

The separation in acoustofluidic technique happens as a result of the interplay between acoustic streaming induced drag forces and Acoustic Radiation Force (ARFs) as shown in Figure 2.9. For operating all the forces and to provide the acoustic waves, the device is required to be operated at high voltage resulting in enormous heatand which eventually affects the viability of the cell [40]. The Table 2.2 summarizes all the microfluidic based methods.

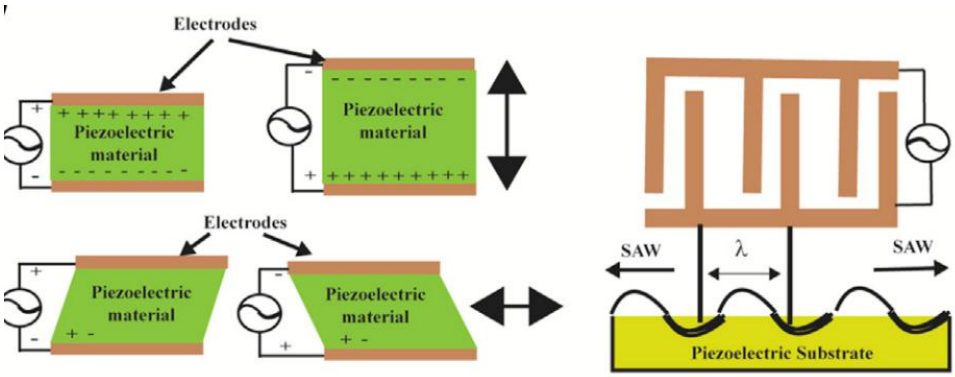

Figure 2.9 : Schematic representation of Acuostofluidic [39]

Table 2.2: Microfluidic based cell separation methods

Method	Author/Year	Research Title	Limitations
FACS (Flourosence Activated Cell Sorting)	Gross A., Schoendube J., Zimmermann S., Steeb M., Zengerle R., Koltay P. 2015	Technologies for single-cell isolation.	Large amount of material, samples needed
MACS (Magnetic Activated Cell Sorting)	Sivaramakrishnan, Muthusaravanan,Kothandan et. al. 2020.	Active microfluidic systems for cell sorting and separation.	Removing antigen and label from the cell surface is challenging
Acoustofluidic	Sivaramakrishnan, Muthusaravanan,Kothandan et. al. 2020.	Active microfluidic systems for cell sorting and separation.	Operated at high voltage resulting in enormous heat which affects cell viability.

2.2.6 Microfluidic device with a Narrow Constriction Straight Channel and Two Reservoirs

Hesam Babahosseini and others conducted an experiment to use the microfluidic chip to detect cancer cells. The whole experiment was based on the elasticity comparisons between the normal and cancerous cell [41]. The microchip has a mechanism to deliver, trap, and pass the cells continuously through a constriction channel while cell transit time is measured by processing the video images obtained shown in Figure 2.10. Flow is established in the delivery channel by a difference in the level of a solution in the reservoirs at the inlet and outlet of the channel. Single cells are trapped at the entrance and passed through the constriction channel of the device. Once a cell is captured into the trap and is traveling through the constriction channel, another cell does not come in. Although, the results in terms of transient time for the experiment for two different cell types were impressive, the experiment was inconclusive since it still could not be determined how different positioning of the electrodes or other design factors would affect the results and timing.

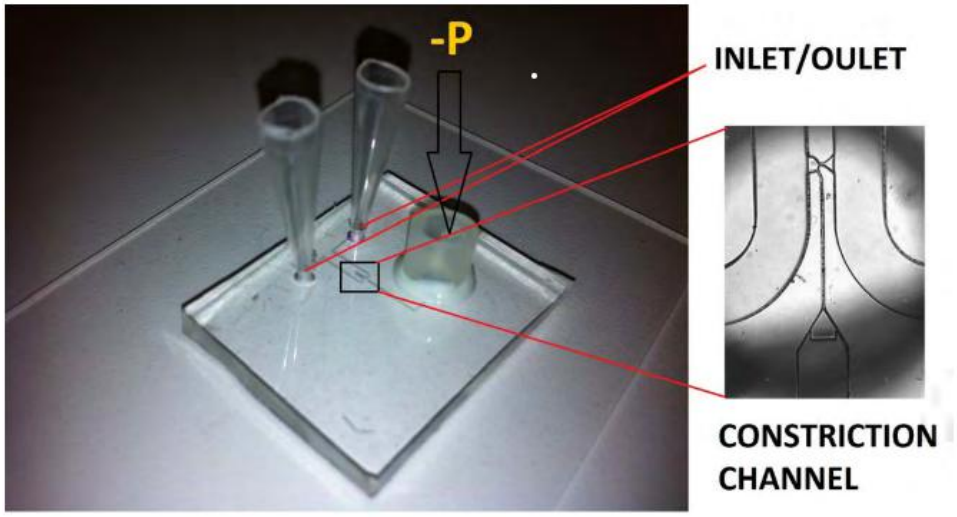

Figure 2.10: Configuration of Microfluidic device with a narrow constriction straight channel and two reservoirs [41]

2.2.7 Cell Detection in Microfluidic System by Terahertz Technique

In another experiment by Sung-Yen Pao and others where microfluidic technology was used along with Terahertz technique conducted as shown in Figure 2.11, it was tried to find how different materials differ in the results of separation of the cell. Using DEP as a buffer, which is yet to be confirmed to be the ideal the experiments concluded what material or substance is more suitable to be filled in, in the microchannel of the microfluidic device [42]. The results in the experiment do not provide any insight on the time consumption and also the variation of the device itself similar to the aforementioned work.

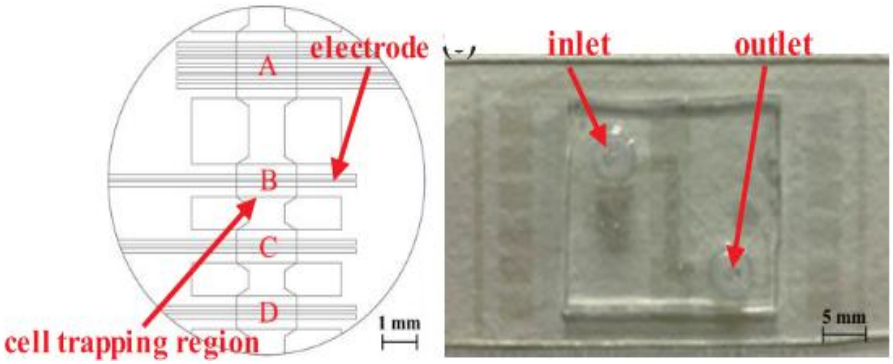

Figure 2.11: Configuration of Microfluidic System by Terahertz Technique [42]

2.3 Research Gaps

Based on the limitations and constraints from the methods that were reviewed the Research gaps are such:

1. Hard to fabricate
2. Challenging to interface with the biological samples
3. Cannot detect stages of cancer

2.4 Summary

It is necessary to find a new method, which can give higher accuracy of quantitative data instead of qualitative data [29]. It is also fundamental to develop a method that can give real-time notification to detect cancer at an early stage.
To summarize the research gap that combine all the methods is as follows : -

1. Most of them are label based methods
2. Challenging to fabricate
3. Quite hard to impose interfacing while dealing with biological samples such as cells.
4. Cannot detect the stage of cancer.

CHAPTER 3

METHODOLOGY

Included in this section is the details of the proposed work starting from the working principle of the DEP-based microfluidic chip to the approach taken to complete this study. In this chapter, the details of the project phases is briefly discussed too.

3.1 System Architecture

Figure 3.1 illustrates the system architecture where the mixture of RBC and CTC is injected in inlet A along with hydrodynamic forces in B, the cells pass to travel all along the main channel and experiences sedimentation and levitation caused by gravity and repulsion from DEP force. As long as the DEP force is greater in magnitude than gravity, particles will ravel diagonally due to hydrodynamic force. After the cells experience the repulsive and attractive force, it will then be separated into Branch C (an outlet for healthy cell) and Branch D (cancerous cell).

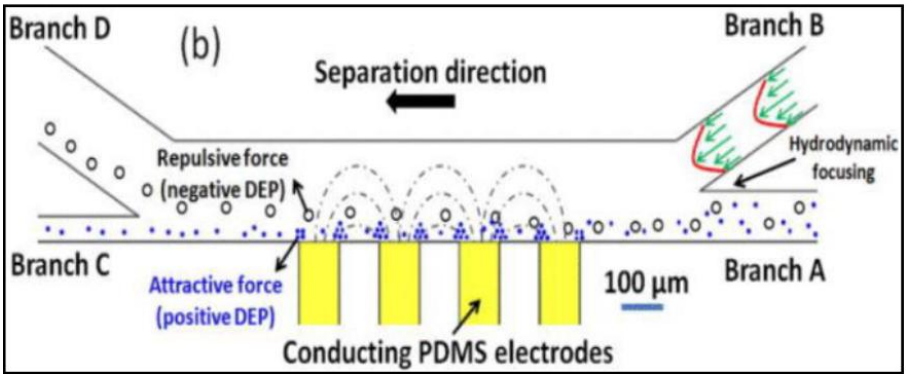

Figure 3.1: The working principle of Dielectrophoresis [25]

3.2 Project Flow Chart

The work-flow of the project shown in Figure 3.3, illustrates the flow that was initiated with studying the problem background in detail and based on that set up the objectives. While going through all the processes of a literature review by reading similar and related works to understand the theoretical basis. Upon comprehending certain research gaps, the methodology has been set. The microfluidic phenomenon was studied and the best method among the microfluidic for cell sorting was studied and studied upon how it could solve the research gap. After the preliminary results were found, it was checked through feedback to check if the objectives were met. Until the objectives were met, the processes were redone.

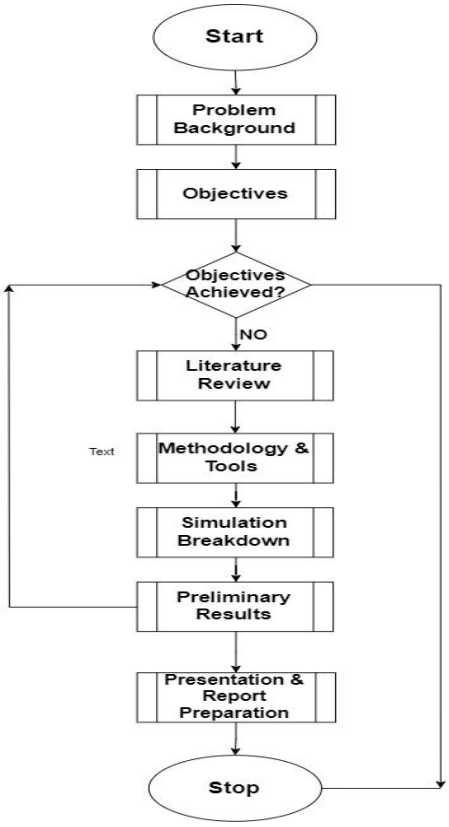

Figure 3.2: Project Flow Chart for FYP.

3.3 Working principle of the DEP-Microfluidic device in cell sorting

The height of the position of the cell depends on the balance of DEP force and the sedimentation of the particles, meaning gravitational force. The cells of different sizes would therefore be located at different heights and will be subject to different flow rates, thereby inevitably raveling to different outlets as shown in Figure 3.3.

Through analyzing the intrinsic dielectric properties of both the particles and the medium, the magnitude and direction of the DEP force can be determined [26,27].

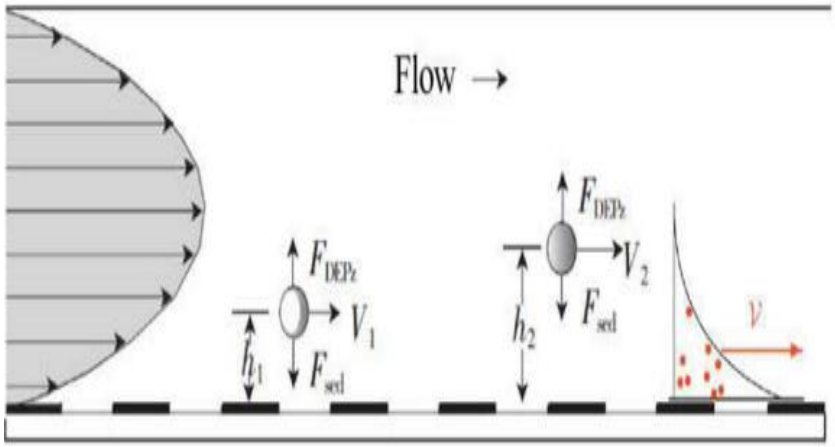

Figure 3.3: Working Principle of separation of CTC and RBC DEP-Microfluidic device [26,27]

3.4 COMSOL simulation Configurations

Table 3.1 illustrates all the parameters that were studied and the constraints of the experiment. These data are very important because DEP force acts differently to particles that are different in size and other dielectric properties. Based on these differences and the effect of DEP accordingly, this study has been designed.

Table 3.3 : Parameters of CTC and RBC [43,44] .

Particle Parameters	**Circulating Tumor Cells**	**Red Blood Cells**	**Unit**
Diameter	2.4	5	μm
Conductivity	0.25	0.31	S/m
Relative permittivity	50	59	
Shell electrical conductivity	1.00E-06	1.00E-06	S/m
Shel relative permittivity	6	4.44	
Shell thickness	8	9	nm

In Table 3.2, the combined input parameters used in the simulation is summarized. It can be observed that the sizes of the particles, the CTC and the RBC is in nanometer which makes use of microfluidic very compatible with these biological samples. The input parameters are set while the mixture of the cells were injected through the inlet. Through the parameters, the nature of electric field and hence DEP force can be manipulated.

Table 3.4: Parameters of the combination of particles during the simulation [43,44]

Variables	**Circulating Tumor Cells and Red Blood Cells**	**Unit**
Frequency of electrical field	100	kHz
Fluid medium conductivity	55	mS/m
Fluid relative permittivity	80	
Fluid density	1000	kg/m^3
Fluid dynamic viscosity	1.00E-03	Pa*s
Particle density	1050	kg/m^3

3.5 Equations involved in the working principle

The DEP force (F_{DEP}) exerting it effects on a particle which has a spherical shape and has a radius of *r* dispersed in a fluid is given as Eq. (1) as stated in [45-46]:

$$F_{DEP} = 2\pi^3 \varepsilon_m \, Re[fcm] \, \nabla |E^2 rms| \qquad (1)$$

where Re[fcm] represents real number of the Clausius–Mossotti (CM) factor and Erms is the root-mean-square of the applied electric field. The CM factor (*fcm*) is a numerical representation of how effectively a particle is polarized. It can vary with the sorrounding medium and the complex part of the dielectric properties. The

complex CM factor is represented as Eq. (2):

$$\left[\frac{\varepsilon_{P}^{*}-\varepsilon_{M}^{*}}{\varepsilon_{P}^{*}+2\varepsilon_{M}^{*}}\right] \qquad (2)$$

ε_{p}^{*} and ε_{m}^{*} represent the complex permittivities of the particle and medium, respectively. This parameter depends on the conductivity σ and frequency *f*, as shown in Eq. (3):

$$\varepsilon = \varepsilon - \frac{j\sigma}{2\pi f} \qquad (3)$$

The CM factor when expanded as illustrated in Eq.(4):

$$\mathrm{Re}(f_{cm}) = \frac{(\sigma_p-\sigma_m)(\sigma_p+2\sigma_m)+4\pi^2 f^2(\varepsilon_p-\varepsilon_m)(\varepsilon_p+2\varepsilon_m)}{(\sigma p+2\sigma m)2+4\pi 2 f 2(\varepsilon_p+2\varepsilon_m)2} \qquad (4)$$

Observation of Eq. (4) depicts that the sign of the CM factor depends on the electrical conductivities of the particle and the medium at low frequencies and the permittivities at high frequencies. THe polarity of the DEP force usually depends on the polarity of the real part of CM factor, Re[*fcm*]. If the Re[*fcm*] is positive the particles in the region is attracted toward a region which has higher electric field gradient and when Re[*fcm*] is negative, there is a repulsion of the particles that moves them away from a high electric field gradient. Given the small size of CTCs, the particles might not deviate enough to be collected in the outlets.

In order to solve for this challenge the repulsion on the cells due to nDEP force is utilized in this study along with a support of hydrodynamic force to ravel the CTC particles to an outlet since they might not have enough deviation.

In this study, the frequency was fixed to 100 kHz which resulted in experiencing of nDEP force by RBCs and CTCs but the effect of DEP force on the RBC is evidently greater than CTC which is caused due to the difference in size between the both particles. The total DEP force is comprised as the force provided due to the differential electrodes and friction working against it as shown in Eq. (5):

$$F_{TOT} = F_{DEP} - fv \qquad (5)$$

where υ refers to the velocity of the cells relative to the medium and the friction f can be quantified as

$$f = 6\pi\eta r \qquad (6)$$

Since the particle is spherical and is flowing in a medium that has viscosity η, the steady-state velocity of the cell due to dielectrophoresis is obtained when considering a net force on the cell equal to 0, and is given by the magnitude of the DEP force divided by the friction factor,

$$F_{DEP} \Big/ f \qquad (7)$$

and is therefore proportional to the square of the cell radius.

3.6 Summary

The methodology has been set to fulfill the objectives of this study. The working principle of DEP based microfluidic cell sorting method is utilized based on the particle types and nature of the study. After thorough planning, a feasible and reasonable project flow both in terms of steps and time distribution throughout the two semesters of FYP-1 and FYP-2 has been presented.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

The project consists of three main components: 1. Designing the microfluidic chip based on Dielectrophoresis, which includes the dimension of the main channel, inlets and outlets and electrodes. 2. Characterize and optimize the designed chip in terms of electrode position, sustainability. 3. Observing the time taken and efficiency of the process of separation of circulating tumor cells and red blood cells in two different outlets.

In this chapter, all processes and approaches taken toward reaching the results are discussed. The improvements that are required for the obtained results to be adequate for this study are also discussed.

4.2 Classifications of Results

Two microfluidic chip are designed and both have two inlets and two outlets as can be seen in Figure 4.1 and Figure 4.2. The dimensions of the chips are 5.6 cm by 3.2 cm and 5.8 cm by 3.4 cm, respectively. Two different configurations of liquid array electrodes were made and the results based on their electric potential distribution, pressure distribution, fluid flow velocity and separation of particles were observed as mentioned in Table 4.1 and Table 4.2.

The values of frequency and voltage of the electric field were varied in order to observe the optimum and minimum values required for the separation.

4.3 Design Specifications

The proposed devices in Figure 4.1a and Figure 4.1b are two-dimensional illustrations where both configurations consist of two inlet ports in which one consists of the mixture of particles (CTC and RBC), the other is used for hydrodynamic force or buffer solutions. Comprising of a resistor ladder network and interconnection channel electrodes, the outlets are connected at the far end of the main channel. The outlets are connected and have identical shapes and size but particles ravel up in different outlets after the separation process is done. It is designated in a miniature device with the dimension as illustrated in Figure 4.1a and Figure 4.1b, respectively. In configuration 1, the main channel is 560 μm long with 50μm of width. The inlets and outlets have an identical dimension of 200μm × 50μm. The array electrodes are placed on both sides of the main channel in parallel with a shift of 20μm between oppositely placed electrodes. In configuration 2, the main channel is 580 μm long with 50μm of width. The inlets and outlets have an identical dimension of 200μm × 50μm. The overall device has a dimension of 6 cm by 3.2 cm. The square shaped liquid array electrodes are placed on the upper part of the main channel which has 42 μm width and length and is placed at an angle of 5 degrees.

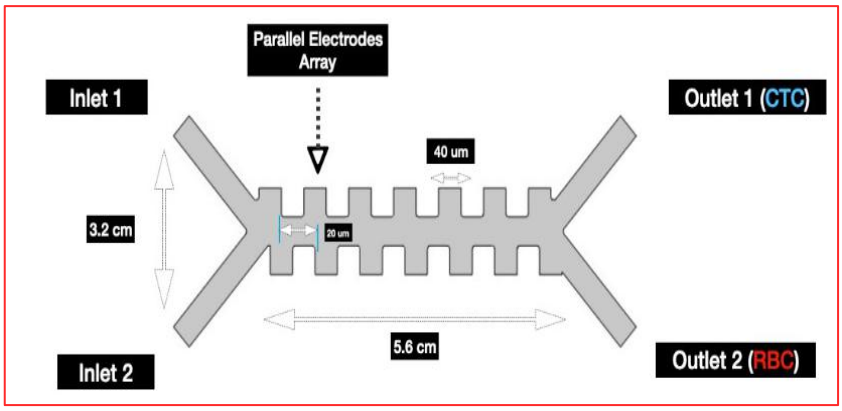

Figure 4.1a: Design of Configuration 1

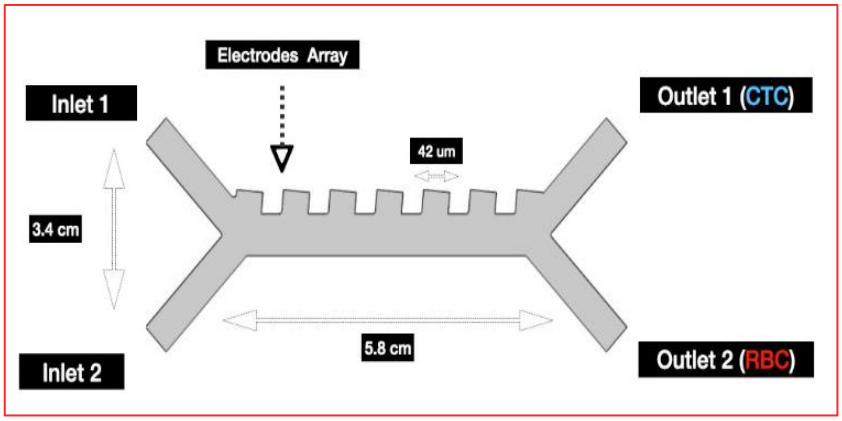

Figure 4.1b: Design of Configuration 2

4.4 Design Justification

Designs that had counter attributes were simulated as in Figure 4.2a and Figure 4.2b to justify the design that is suggested earlier. In Figure 4.2a, parallel electrode arrays were used on both sides of the channel and the test for separation was carried out. It was seen that both the RBC and CTC particles raveled to one single inlet. This is due to the DEP force being eliminated by each other.

In Figure 4.2b, design of one inlet was tested and was found out that both the particles ended up in the bottom outlet without any separation shown. This is because one inlet eradicates the hydrodynamic force which is exerted through the Inlet 2 at 850μm/s. Due to the absence of the hydrodynamic force that was used in the proposed design, the CTCs sink to the bottom outlet since they are not deviated enough due to their very small size.

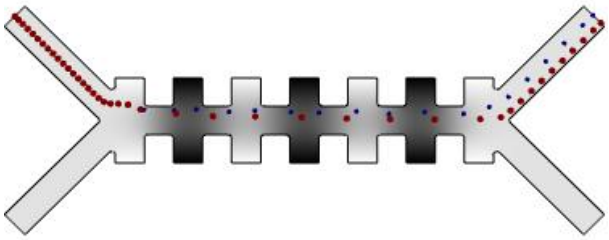

Figure 4.2a: Trial Design with electrodes with no shift

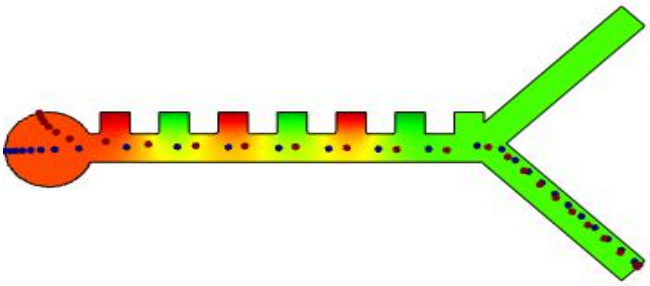

Figure 4.2b: Trial Design with one inlet

4.5 Findings

4.5.1 Sustainability Test: Electric Potential, Pressure, Fluid Flow Velocity

Both the designs were tested separately for the particle separation and also sustainability in terms of electric potential distribution, pressure distribution, fluid flow velocity. The voltage used was 10Vpp and the frequency provided was 100 kHz. The studies suggest that although both configurations could successfully separate the particles from a mixture put in inlet 1, their efficiency, sustainability factors differed at various levels.

Configuration	Electrical Potential distribution	Pressure	Fluid Flow Velocity
1. Oppositely facing array electrode	Inlets have around 3V. Outlets have -3V Figure 4.3a(i), Figure 4.3a(ii)	Design 1 can uphold maximum of 5 Pascal. Figure 4.3c(i)	The maximum Fluid flow of 0.0015 m/s velocity. Figure 4.3c(i)
2. One-sided equidistant array electrodes	Both inlets +3V and outlets have +5 V. Figure 4.3b(i), Figure 4.3b(ii)	Case 2: Design 2 can uphold maximum of 5.4 Pascal Figure 4.3c(ii)	The aximum Fluid flow of 0.0015 m/s velocity. Figure 4.3d(ii)
3. Conclusion	The overall distribution is same except design 1 has different outlet polarity than design 2.	Similar pressure distribution except design 2 can uphold higher liquid pressure.	Identical fluid velocity is observed in both designs.

Table 4.1 : Sustainability findings from two different configurations

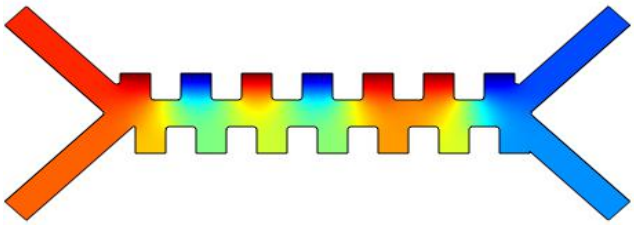

Figure 4.3a(i): EP distribution illustration for Design 1

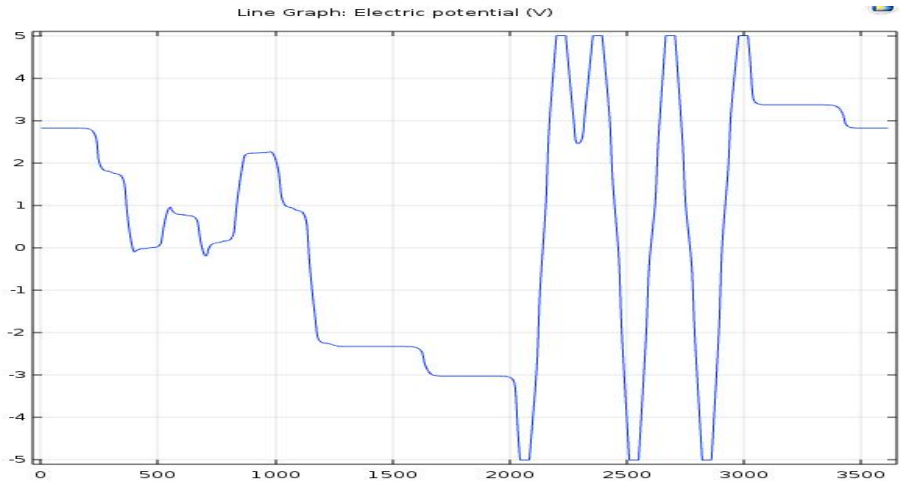

Figure 4.3a(ii): EP distribution graph over time for Design 1

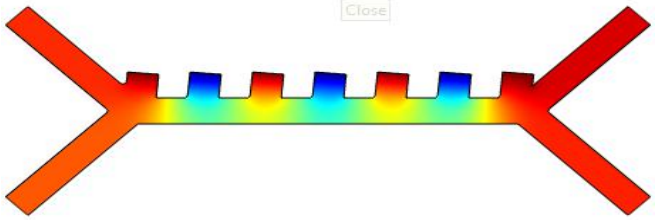

Figure 4.3b(i): EP distribution illustration for Design 2

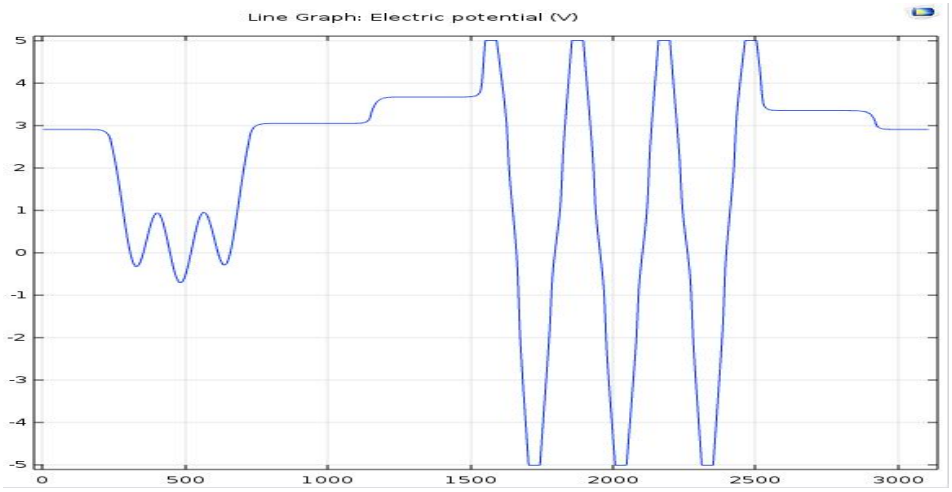

Figure 4.3b(ii): EP distribution graph over time for Design 2

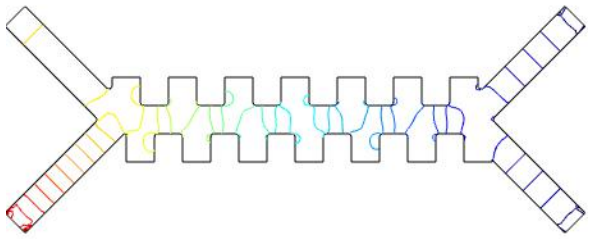

Figure 4.3c(i): Pressure distribution in Design 1

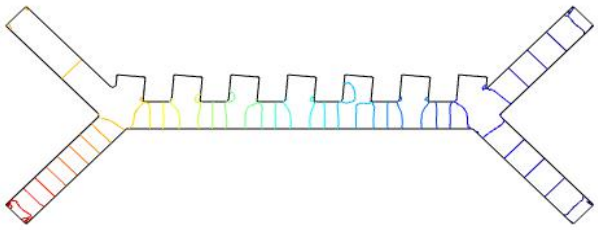

Figure 4.3c(ii): Pressure distribution in Design 2

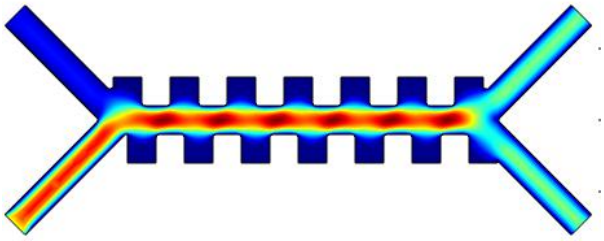

Figure 4.3d(i): Fluid flow illustration in Design 1

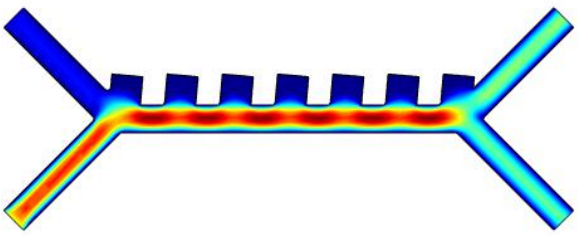

Figure 4.3d(ii): Fluid flow illustration in Design 2

4.5.2 Sustainability Test: Required Voltage and Frequency

Voltages were varied from a 4 Vpp to 40 Vpp in this study to find out the minimum voltage that was required for the proposed design to perfom separation. Frequency was varied from 10kHz to 110Khz and the separation time was observed. With any voltage over 30 Volt (peak-to-peak) applied the particles were seen to be damaged and the separation failed due to the phenomenon called Joule heat effect as shown in Figure 4.4a. [47]. The separation was seen to work the best with 100 kHz. Frequency above 100kHz would fail the separation process as seen in Figure 4.4b. It was found that 10 Volt (peak-to-peak) was the most optimum voltage that could be used with 100 kilo Hertz frequency.

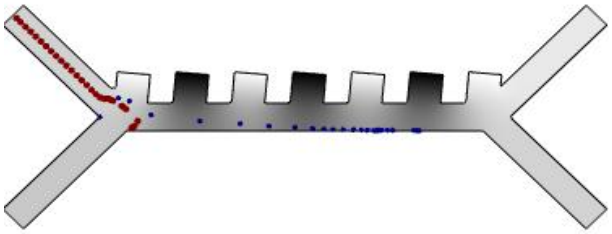

Figure 4.4a: Particles situation when voltage above 30 V is applied

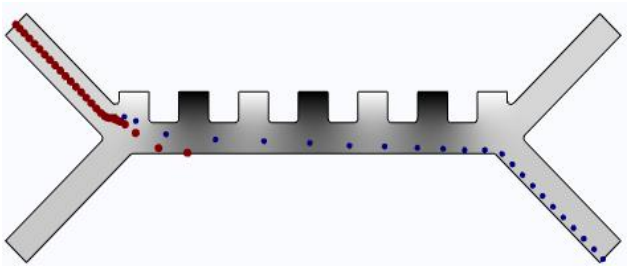

Figure 4.4b: Particles situation when frequency above 100kHz is applied

4.5.3 Effects of DEP force on particle separation

To observe the effects of DEP force on the particle separation, two studies were carried out where at first the particle trajectories without DEP force applied are presented in Figure 4.5a and Figure 4.5b. The blood cells were released at the same time and followed a similar path. In this simulation, the starting time is 0s and ended within 51 seconds. At this instance of applied DEP force = 0, it has been observed that none of the particles were separated and ended up in the inlet 1.

In the second instance when DEP force was applied, the particles were successfully separated. The CTC particles at outlet 1 and the RBC particles at outlet 2 were extracted.

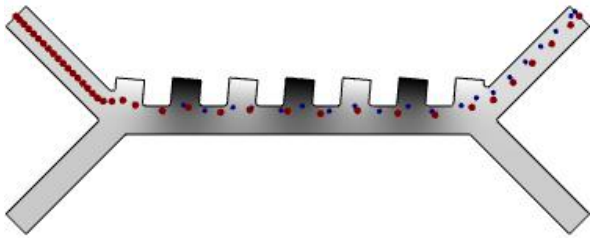

Figure 4.5a: Particle separation when DEP force = 0

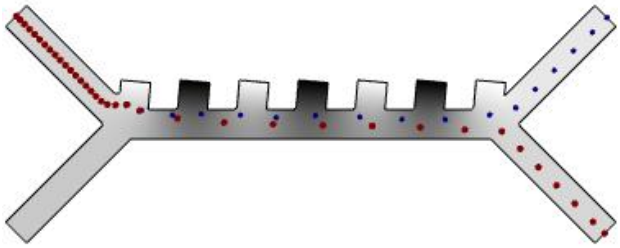

Figure 4.5b: Particle separation when DEP force applied

4.5.4 Particle Separation

In this study, the particles separation efficiency and time taken for each of the design to complete the separation process was observed. Although both of the designs could successfully operate the separation process as seen in Figure 4.6a and Figure 4.5b but they differed in terms of time and efficiency. Both of these attributes are vital for the designs to meet the objectives. Design 1 could manage a faster separation with 118 seconds but with an efficiency of 48%. On the contrary, although Design 2 took a long time with 128 seconds, it obtained an efficiency of 88%. 48 out of 52 CTC cells could be extracted at the outlet.

Table 4.2: Findings in terms of particle separation for both configurations

Configuration	**Particle Separation Efficiency**	**Time Taken**
One-sided equidistant array electrodes	Successfull with 48% efficiency recorded. Figure 4.6a(i), Figure 4.6a(ii).	108 seconds taken to complete the whole process.
Oppositely facing array electrode	Successfull with 88% efficiency recorded. Figure 4.6b	128 seconds taken to complete the whole process.
Conclusion	Design 2 is more efficient in terms of separation. More number of CTCs can be extracted without damage.	Design 1 can process faster compared to Design 2.

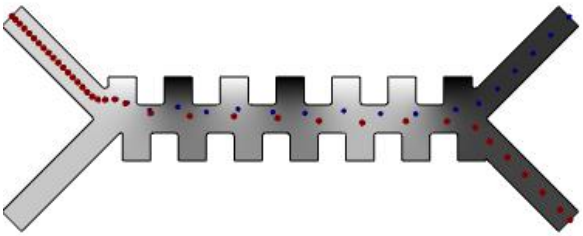

Figure 4.6a(i): Particles separation illustration for Design 1.

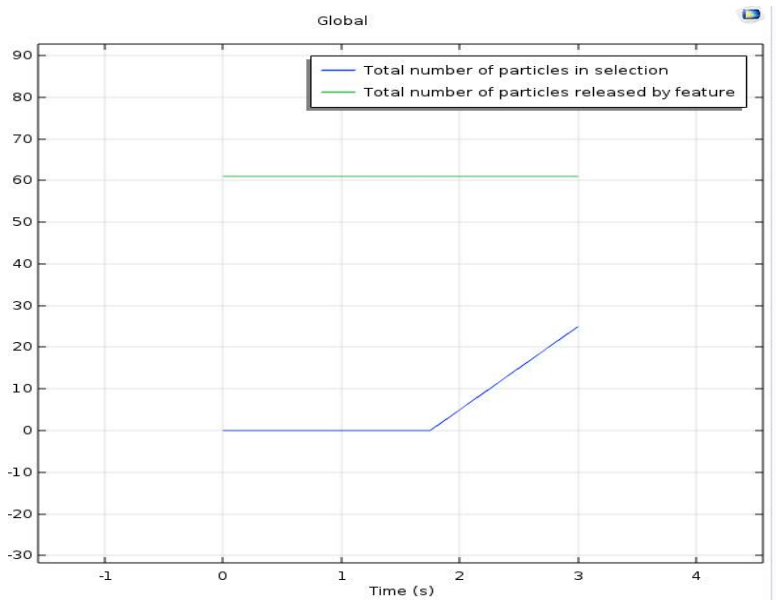

Figure 4.6a(ii): Particles separation efficiency graph for Design 1.

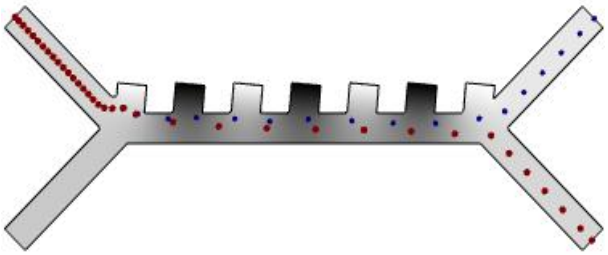

Figure 4.6b(i): Particles separation illustration for Design 2.

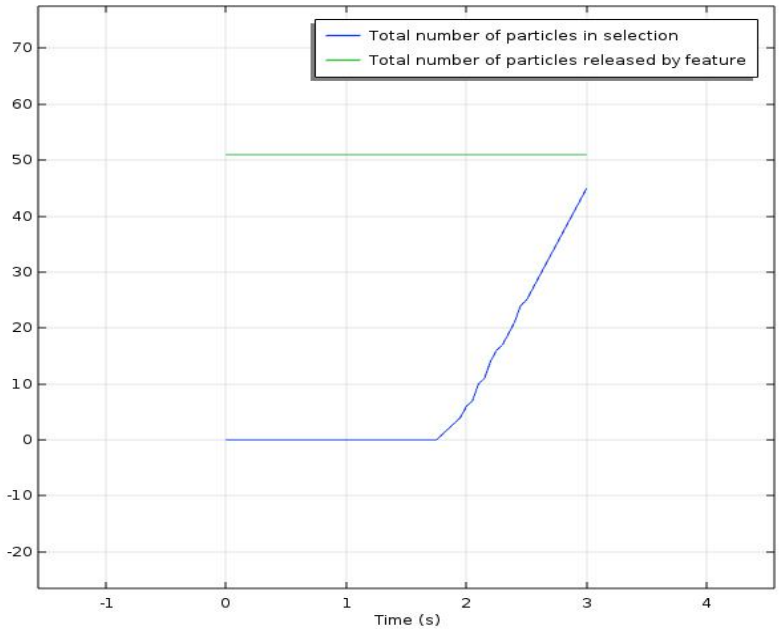

Figure 4.6b(ii): Particles separation efficiency graph for Design 2.

4.4 Summary

The results for two configurations with dimension 6 cm into 3 cm have been recorded in this study. The results illustrate that differences in the electrode configuration can affect the particle separation both in terms of success and time. The results in terms of particle separation also depend on the input parameters like the frequency and voltage provided for the non-uniform electric field. The electric potential distribution and fluid flow did not show a huge difference when the parameters were altered.

CHAPTER 5

PROJECT MANAGEMENT

5.1 Introduction

This chapter includes the project schedules and cost estimation of the project. The first part explains the project schedule and the second part is the cost estimation of the project. The price of the software is an approximation. Different levels of licencse can cost different amount.

5.2 Gantt Chart

Tables 5.1 and Tables 5.2 illustrates the timeline throughout the FYP-1 and FYP-2. The distribution was set based on the amount of work required. In FYP-1, setting up the methodology while simultaneously reviewing other authors' work took the most time. In FYP-2, working on the optimization of the chip and getting the sustainability study were the most time consuming.

Table 5.1: Gantt Chart - FYP1

Task Name	September	Ocotber	November	December
Problem Background				
Objectives				
Literature Review				
Methodology				
Preliminary Results				
Presentation preparation + Report				

Table 5.2: Gantt Chart - FYP2

Task Name	Feb	Mar	Apr	May	June
Design two more configurations of the chip					
Test electric potential distribution, fluid flow and particle separation for all configurations					
Work on optimization of the chip					
Write results, discussion, conclusion					
Prepare the final report					
Prepare presentation for FYP-2					

5.3 Cost Estimation

In this sub section, the project cost estimation is presented. Table 5.3 summarizes the price of the software license essential for the project.

Table 5.3: Project cost estimation

Cost Estimation	
Components	**Price**
Comsol Multiphysics	RM 16,000

CHAPTER 6

CONCLUSION AND FUTURE WORKS

This study throughout the Final Year Project presents the design and the simulation of a microfluidic device that can perform particle separation. The results include the designs of two functional microfluidic chip-based device, sustainabilty test and characterization of proposed designs in terms of electric potential, pressure and fluid flow velocity and optimization in terms of electrode positioning, channel dimensions, optimum voltage and frequency. One design achieved a 88% efficiency and the time taken for the separation process was about 128 seconds. The results show that the objectives that were set at the beginning of the project were achieved through this study based on simulation through the software COMSOL.

5.1 Future Works

Future scope for this project is to design more different configurations of the chip and compare the results each of the configuration provide. The chip can be optimized further and then should be proceeded to fabrication.

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