CHAPTER 1
INTRODUCTION

1.0 Introduction

Dielectrophoresis (DEP) is one of the nondestructive electrokinetic techniques that offer capability for manipulating nano-sized biomolecules like cancerous cell. There are wide ranges of bioparticles that have been investigated in DEP studies done by researchers (e.g Muhall *et al*, 2011; Pethig *et al*, 2010, Kadri, 2010; Fatayinbo, 2008). Numerous applications of DEP are used for the characterization and manipulation of viable mammalian, bacterial and plant cells, and for the sorting and separation of human cells of clinical significance. Hsiao *et al*, 2007 has stated, among these application, trapping and characterization of biological and biochemical particles is one of the fundamental procedures for further downstream processing such as cell culture, cell therapy, diagnostics, and hematology or morphological studies in clinical fields. This phenomenon has been used to investigate the electrical polarisability of a variety of biological cells and organelles (Jonathan et al, 1988).

The earliest studies listed were largely directed toward understanding how cells respond to DEP forces, and to what extent this could lead to a better understanding of their physicochemical properties. There is now, though, considerable effort being directed toward applying DEP for biomedical and biotechnological applications, such as cell sorting, tissue engineering, and biosensors.

Basically, the dielectrophoretic separation method operates on the basis that frequency ranges can be found where: (a) both viable and non-viable yeast cells exhibit positive DEP; and (b) viable cells exhibit positive DEP and non-viable cells negative DEP. In the DEP methods cell separation was achieved using the effect of positive dielectrophoresis created by means of a simple two-electrode system, and only poor

efficiency in cell separation was obtained. The high efficiency of separation obtained by us has resulted from two new features, namely the use of interdigitated microelectrode arrays and the controlled application of both positive and negative dielectrophoretic forces.

Polarisability depends partially on the reactance of the particle and medium and is therefore frequency dependent. Consequently, analysis of the dielectrophoretic frequency spectrum has long been used to study the dielectric properties of cells such as the conductivity and permittivity of the cell wall, membrane and cytoplasm. The simple design of the dot microsystem gives a rapid and simple method of the determination of particle polarisability DEP spectrum by using simple image processing techniques and without the need for superimposition of calculated electric field distributions.

1.2 Thesis Objectives

The objective of this research project is to improve dielectrophoretic data analysis algorithm in determining the relative polarisabality of dielectric particles.

1.3 Thesis structure

This thesis has been organised into five chapters. Each chapter begins with an introduction that briefly describes the objectives of the chapter.

Chapter 2 begins with a literature review on the historical development of cell separation using dielectrophoresis (DEP) and its theory. Overview on the cell polarisabality and analysis by using imaging techniques application. This chapter also illustrates on biophysical properties and mammalian cell function with also discussed about cancerous cell, K562 cells that used in this study.

In chapter 3, its document step of methodology involves in this research. This chapter illustrates methods that used to study on polarisabality on the DEP data collection. This chapter described about the image analysis and possible approach to evaluate the DEP polasabarity in data analysis.

Analysis of captured data and related result are discussed in chapter 4. Method of improvement are introduced to study polarisabality of K562 cells data captured by the previous system.

Finally, the overall conclusions of the thesis are presented in chapter 5. The main contributions extricated from the works are mentioned again briefly. Some suggestions for future works are also addressed in this chapter.

CHAPTER 2
BACKGROUND REVIEW

2.1 Introduction

This chapter will cover a review on DEP with the current research interest and its application by introducing the behind theory of DEP and biophysical properties. A review on mammalian cell structure and the cancer cell which is K562 that used in this study for data collection. The end of this chapter will review on image analysis algorithm and image enhancement technique by introducing the histogram equalization.

2.2 Dielectrophoresis (DEP)

The term dielectrophoresis (DEP) was first introduced by Pohl (Pohl, 1951) to describe the transnational motion of particles due to the application of non-uniform electrical fields. The dielectrophoretic motion is determined by the magnitude and polarity of the charges induced in a particle by the applied field. Usually, dielectrophoresis is performed under an alternating current (AC) field over a wide range of frequencies.

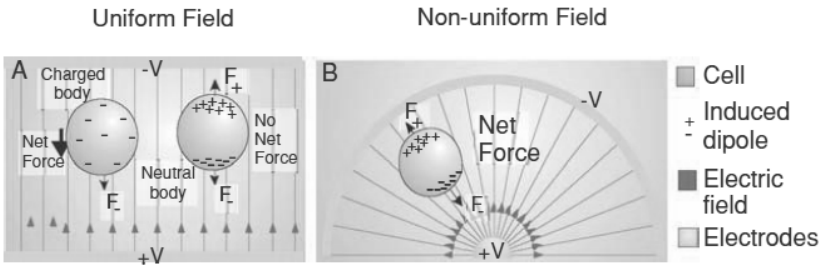


Figure 2.1: Dielectrophoresis. The left panel (A) shows the behavior of particles in uniform electric fields, while the right panel shows the net force experienced in a non-uniform electric field (B) (Voldman, 2001).

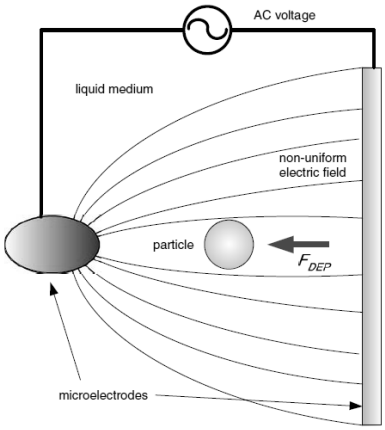


Figure 2.2 : Illustration of the Dielectrophoretic manipulation (Lai *et al*, 2008)

Dielectrophoresis is the motion of particles induced by polarisation effects in a non-uniform electric field. This motion is governed by the ratio of the polarisability between cells to that of the surrounding medium. It can either force cells to collect at the electrode edges when the particle is more polarisable than the surrounding medium (positive dielectrophoresis), or cause cells to collect away from the electrodes towards the region of lowest field strength when the medium is more polarisable than the cells (negative dielectrophoresis) (Hubner et al, 2008; pethig 2010; Gascoyne et al 1992).

DEP has been employed, among others, to separate live and dead cells, different strains of bacteria, viruses, DNA molecules, spores and algae. Other types of molecules have also been the subject of DEP characterisation studies, including nano-sized latex spheres and biopolymers (Kadri, 2010). Dielectrophoresis can be used to examine the effects of drugs on cells, to detect apoptosis and to separate viable from non-viable cells. Particles studied using dielectrophoresis include cancer cells, bacteria, virions, algae and red blood cells. To date, dielectrophoresis has been used most commonly to determine the particle properties by analysing the collection rate of particles at electrode edges (Hubner et al, 2005).

Dielectrophoresis is condition of being a force whose magnitude and direction are related to the particle's properties which is ideal for sorting systems, where populations of different particles are collected into homogeneous groups, either by providing a discriminating force-field through which mixtures are pumped by an external source, or by the induction of motion in the particles by the dielectrophoretic forces themselves.

The DEP force is dependent on several parameters: the dielectric properties and size of the particle, the frequency of the applied field and the electrical properties (conductivity and permittivity) of the medium (Mulhall et al, 2011; Hugness, 2002). Therefore, if is desired to achieve a good particle manipulation say cell separation, detailed analysis and careful selection need to be done in order to obtain the desired results. Future potential medical application by discriminating DEP is by possibility of offering a system for diagnostics cell sorting, cell sorting, enrichment of cell populations and cell counting.

2.2.1 Theory

Dielectrophoresis is a phenomenon in which a force is exerted on a dielectric particle when it is subjected to a non-uniform electric field. The magnitude of force can be expressed as:

$$\langle F_{\mathrm{DEP}} \rangle = 2\pi r^3 \varepsilon_m \mathrm{Re}\left\{ \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right\} \nabla \left| \vec{E}_{rms} \right|^2 \quad \text{..... (Eq. 1)}$$

Where r is the radius of the particle, εm is the medium permittivity, ∇ is the Del operator (gradient) on the applied electric field E and The factor in curly brackets is

known as the complex Clausius-Mossotti (CM) function, where εp* and εm* are the complex permittivities of the particle and medium, respectively. Depending on the value of the CM factor, the particles can either be attracted to or repelled from the strong electric field, which is called positive DEP or negative DEP.

Homogenous dielectric particles have a single dielectric dispersion caused by the accumulation of charges at the interface between the suspending medium and the particle. The DEP force expression (Eq. 1) holds true for the dielectrophoretic force of a homogeneous sphere. A biological cell is not homogenous; it is an anisotropic dielectric consisting of a membrane and a cytoplasm, each of which has discrete polarisabilities.

2.2.2 Biophysical properties

In the past few years, there has been an extensive research in the manipulation and analysis of biological cells at the micro scale. There is an increase interest in applying microelectromechanical systems for selective trapping, manipulation and separation of bioparticles. Although there is a huge demand of automated single-cell manipulation and analysis in immunology, developmental biology and tumor biology calling for the development of suitable microsystems, the approaches currently available to meet those needs are limited.

Biological particles are complex, heterogeneous structures with multiple layers, each possessing distinct electrical properties (Jones, 2003). Reliable dielectric models for such particles are crucial in biological dielectrophoresis. Consider the concentric, dielectric shell subjected to an electric field in Figure 2.3 (a). As before, assume that the non-uniformity of this field is modest on the scale of the particle's dimensions. It may be shown that the induced electrostatic potential outside the particle, that is, $|r| > R1$, is indistinguishable from that of the equivalent, homogeneous sphere of radius (R1) with permittivity (ε) shown in Figure 2.3 (b). As long as the physical scale of the

nonuniformity of the imposed field is much larger than the particle radius R1, the external field of the equivalent particle is indistinguishable from that of the heterogeneous particle.

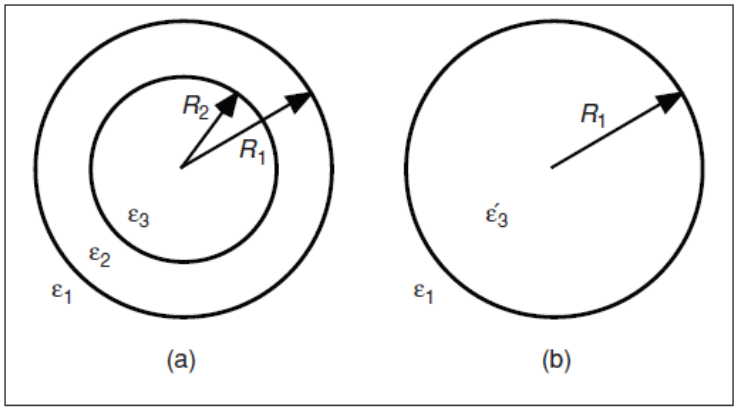


Figure 2.3: Multilayered shells: (a) spherical particle with one concentric shell; (b) equivalent homogeneous particle (Jones, 2003).

According to Jones, 2002, accurate models for viable, biological cells suspended in media must account for ionic charge conduction mechanisms within and without the cell and, for frequencies above 1 MHz, dielectric losses. The simplest way to represent ionic charge transport is to employ an ohmic model. Largely because of their structure and because of the high internal, electrical conductivity, this approximation usually suffices within the cell. On the other hand, an ohmic model is less successful in representing the aqueous, electrolytic media in which cells are commonly suspended. The problem is that double-layer phenomena introduce the complication of mobile space charge outside but directly adjacent to the cell wall.

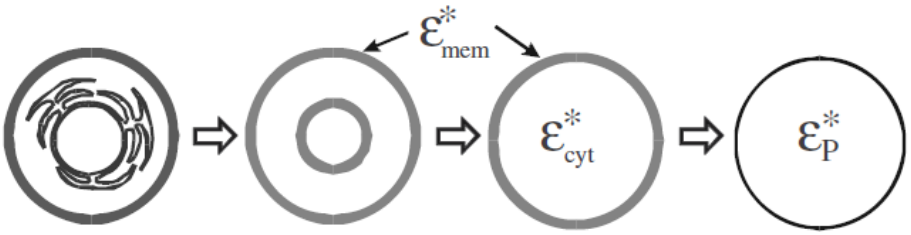


Figure 2.4: schematic representation a nucleated cell can progressively be simplified (Pethig, 2010)

Figure 2.4 illustrates schematic representation of how a nucleated cell can progressively be simplified to a homogeneous sphere of effective permittivity εp* that mimics the dielectric properties of the nucleated cell. The first step in simplification shown here is to represent the endoplasmic reticulum as a topographical feature that increases the effective capacitance of the nuclear envelope. The penultimate step represents the cell as a smeared-out cytoplasm surrounded by a membrane of complex permittivities εcyt* and εmem* respectively.

DEP can be used to non-invasively determine electrophysiological parameters such as conductivity (defined as the ability to carry electrical charge) and permittivity (the ability to store electrical charge) of the cytoplasm and membrane of cells by monitoring the frequency-dependent DEP collection rate (Broche et al, 2007).

2.2.3 Polarisabality

Dielectrophoresis can be further classified by direction (Figure 2.3). If the force is in the direction of increasing field gradient, it is termed positive dielectrophoresis. Its opposite effect, negative dielectrophoresis, acts to repel the particle from regions of high electric field, moving it “down” the field gradient. Whether a particle experiences positive or negative dielectrophoresis is dependent on its polarizability relative to its

surrounding medium; differences in the quantity of induced charge at the interface between particle and medium lead to dipoles oriented counter to the applied field (and hence positive dielectrophoresis) where the polarizability of particle is more than that of the medium, and in the same direction as the applied field (and hence negative dielectrophoresis) where it is less (Hugnes, 2002).

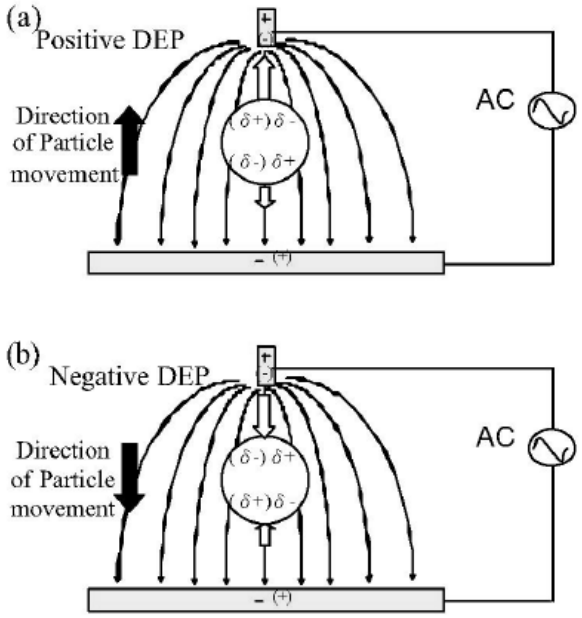


Figure 2.5: Direction of Particle Movement Depending on DEP Response (a) positive DEP particle (b) negative DEP particle (Hugnes, 2002)

When a particle is suspended in an alternating electric field which contains either a magnitude or phase gradient, a force is induced on the particle which acts either in the direction of the gradient or opposes it, according to whether or not the particle is more or less polarizable than the medium in which it is suspended. Here, a particle experiences force due to a non-uniform electric field (magnitude gradient) as illustrated in Figure 2.6 below.

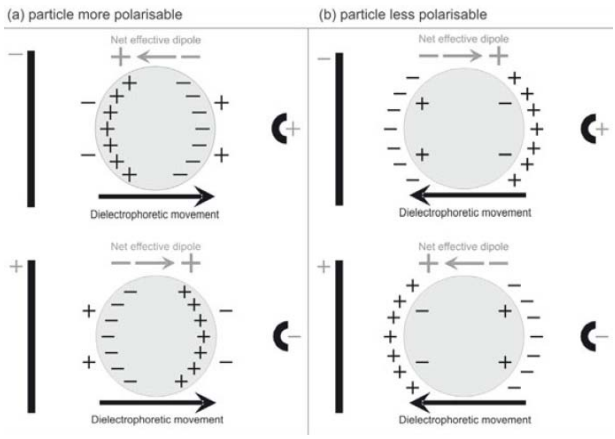


Figure 2.6: Schematic diagram of the polarisation and the induced dipole produced for the two cases: (a) particle more polarisable than the medium, with the induced dipole parallel to the field and (b) particle less polarisable than the medium, with the induced dipole anti-parallel to the field (Green N.G.,2011).

Polarisability depends partially on the reactance of the particle and medium and is therefore frequency dependent. Consequently, analysis of the dielectrophoretic frequency spectrum has long been used to study the dielectric properties of cells such as the conductivity and permittivity of the cell wall, membrane and cytoplasm. Figure 2.7 below shows a typical shape of the DEP spectrum based on the single shell mode.

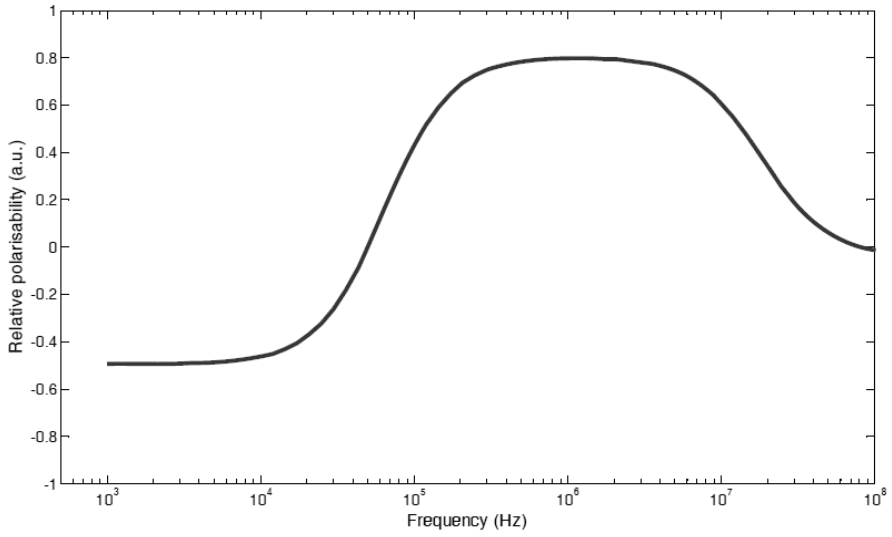


Figure 2.7: Typical shape of the DEP spectrum based on the single shell model (Kadri N.A, 2010)

Multi-shelled models are used to interpret dielectric spectroscopy of cells. The most common approach divides the model into concentric spheres with increasing radii.

Each sphere is a homogenous particle suspended in a medium that has the properties of the next biggest sphere. The simplest form of the model, a ‘singleshell’ model, is an approximation of the structure of a mammalian cell whereby the cell is divided into a homogenous core, representing the cytoplasm, surrounded by a shell representing the plasma membrane. (Mulhall et al, 2011)

2.3 Biological and K562 Cell

The cell is the functional basic unit of life. There are two types of cells which is eukaryotic and prokaryotic. The major difference between prokaryotes and eukaryotes is that eukaryotic cells contain membrane-bound compartments in which specific metabolic activities take place. A typical mammalian eukaryotic cell structure contains three primary components, namely plasma membrane, cytoplasm and nucleus as illustrates in Figure 2.7 and Figure 2.8.

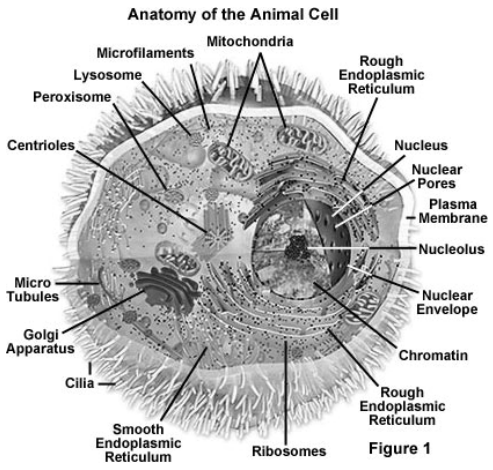


Figure 2.7: Mammalian Cell

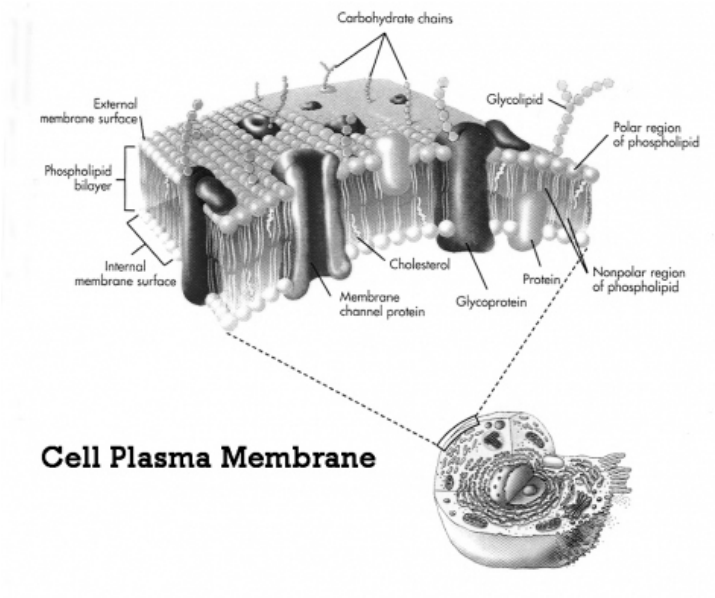


Figure 2.9: Plasma Membrane

The cell membrane, otherwise known as the plasma membrane is a semi-permeable structure consisting mainly of phospholipid (fat) molecules and proteins. They are structured in a fluid mosaic model, where a double layer of phospholipid molecules provide a barrier accompanied by proteins. The cell membrane surrounds the cytoplasm of a cell and, in animal cells, physically separates the intracellular components from the extracellular environment. Cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signaling and serve as the attachment surface for several extracellular structures, including the cell wall.

The K562 cells is cancerous cells line was originally derived from a 53 year old female patient with chronic myeloid leukemia (Kadri, 2010). Many DEP-based studies used the cell line to study apoptosis mechanisms and reaction to anti-cancer treatments (e.g. Gascoyne et al., 1993; Burt et al., 1990).

2.4 Imaging Techniques in DEP Data Analysis Algorithm

Previously, in order to extract the relative polarisability under the DEP influence, an image analysis program was developed based on the Cumulative Modal Intensity Shift (CMIS) algorithm for the relative polarisability. This method relied on the modal value of the histogram of the captured images. Generally, the algorithm will first detect the modal value by finding the peak of the histogram, and subsequently will sum the total number of pixels starting from this point onwards to the maximum light intensity value of the histogram.

2.4.1 Histogram Equalization

Therefore, in an image, a great deal of extra information may be contained in the color, and this extra information can then be used to simplify image analysis (Sharma et al, 2011). The goal of this study is to process an image so that the outcome is more suitable than the original image for any specific application. This can be done adjusting the background illumination according to better human vision and by preserving the color and the contrast. The technique not only improves the visual interpretability for human viewers but also increase the acuity of information contained within the image (Xiao D. Et al, 2007; Sharma et al, 2011).

Contrast enhancement technique is widely used to increase the visual image quality. The histogram of a discrete gray-level image represents the frequency of occurrence of all gray-levels in the image. Histogram equalization (HE) is a technique commonly used for image contrast enhancement, since HE is computationally fast and simple to implement. It works by flattening the histogram and stretching the dynamic range of the gray-levels by using the cumulative density function of the image.

The goal of histogram equalization is to improve the contrast of an image. The idea is to transform an image in such a way that the transformed image has a nearly

uniform distribution of pixel values (Eq. 2). Histogram equalization is performed by a homogenous point operator:

$$f: \mathbf{I}(\mathbf{p}) \rightarrow \mathbf{I}_{eq}(\mathbf{p})$$

$$f(a) = \sum_{r=1}^{a} \frac{n(r)}{N} \quad \text{.....Eq.(2)}$$

Where ***n(r)*** is the number of pixels with intensity ***r*** in image ***I***, and ***N*** is the total number of pixels. Image enhancement processes consist of a collection of techniques that seek to improve the visual appearance of an image or to convert the image to a form better suited for analysis by a human or machine.

CHAPTER 3
MATERIALS AND METHODOLOGY

3.1 Introduction

This chapter documents the methodology involved in this research project. Section 3.2 covers on the system design overview by previous researcher in obtaining corresponding DEP data that will be used for further analysis. . Then, this chapter continues with the documentation on the techniques used in analysing existent data in section 3.3.

3.2 DEP System Design

Dielectrophoresis has been using as cell trapping, levitation, separation, and sorting on an electrode array by varying electrode shape and arrangement (Hsiao *et al*, 2007). Generally, the develop system involved the multiple output waveform generator, the microelectrode device, the image capturing device (i.e. digital video camera with at least one frame per second capture rate), and the computer program to analyse the captured images prior to the production of DEP (Figure 3-1). The electrode design would be a planar electrode type, with circular dots to create the chamber for the DEP effects to be observed and recorded. The experiments were conducted within the range of 10 kHz to 1 MHz, supplied at 8 Vp-p for 20 seconds, using parallel microelectrode with 200 μm diameter dots which was suited for cells having diameters of about 15-20 μm. Basically, the main part this research project is focusing in developing program that capable to analyse the captured images stored by existent data and to improve the data analysis algorithm.

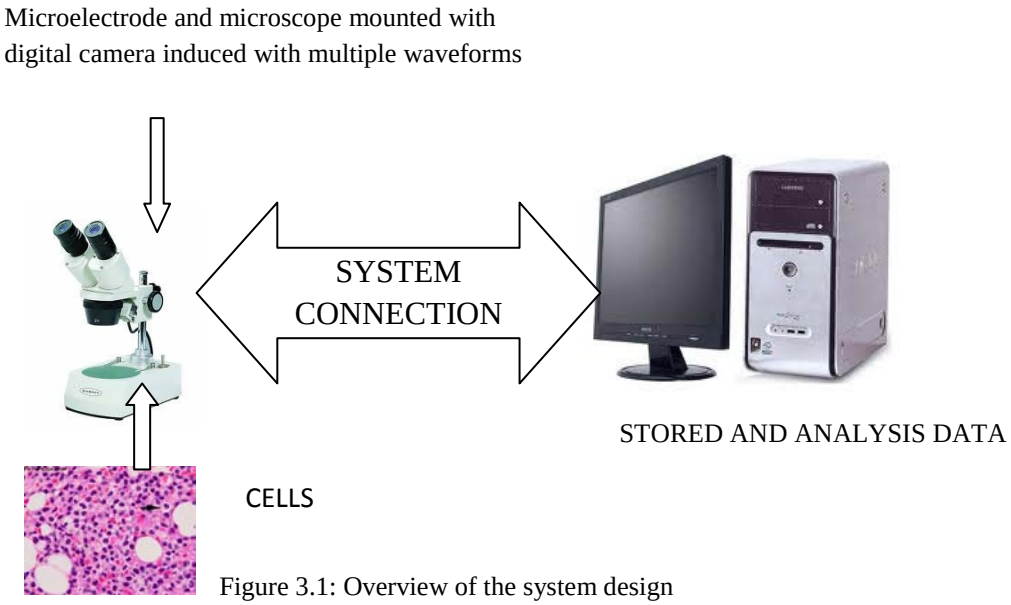


Figure 3.1: Overview of the system design

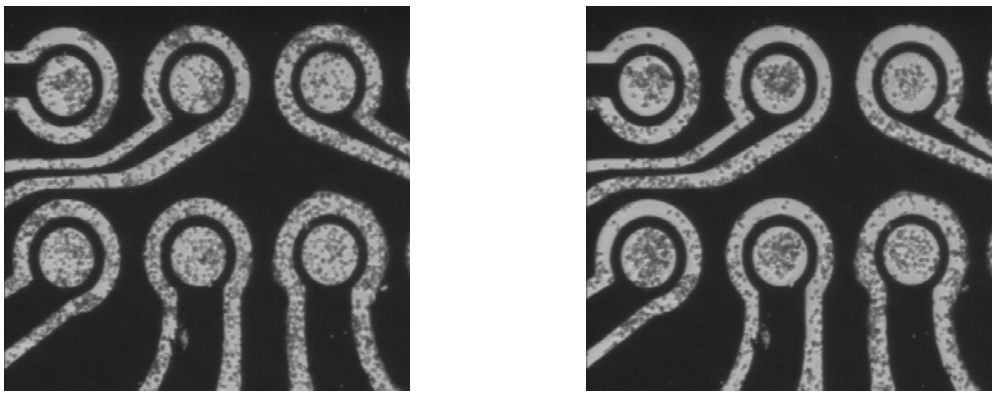
3.3 Data Acquisition

This research project analysis was based on collected data by previous researcher (Kadri, 2010). The experiments were conducted using the multiple waveform generator and microelectrode device developed. The experimental protocol for conducting a DEP experiment by previous researcher in recording specified data is presented in Appendix A. Kadri, 2010 has stated in his thesis, there are few determining factors in achieving an optimal condition in which to conduct the experiments which are an estimation of the cell count, cell size, medium conductivity, dot microarray diameter, and the amplitude of the AC signal input.

Previous experiment involved eight individually programmed input signals, thus opening up a real possibility of conducting real-time DEP experiments by capturing cell images within 21 seconds during conducting an experiment which is 22 images was captured and stored in *.*mat* file and .

3.4 Analysis of Data

Particularly, Analysis of DEP data was achieved using a MATLAB program version 2010b. In this project, the analysis involved to improve DEP data analysis algorithm for determining the relative polarisabality of dielectric particles is based on image analysis techniques.



(a) (b)

Figure 3.2: Example of Collection data of K562 cell in captured images at t=0 sec (before (a)) and at t=12 sec (after (b))

System will captured a frame (Figure 3.2) movement of K562 cells with specified induced frequency on each dot within 21 seconds with induced frequency range within10 kHz to 1 MHz. The polarisabality was observed within the circular dots depending on the specified induced frequency. The images captured are segmented according to regions of interest, a circular dots. A general picture of analysis on captured images shows in figure 3.3 below.

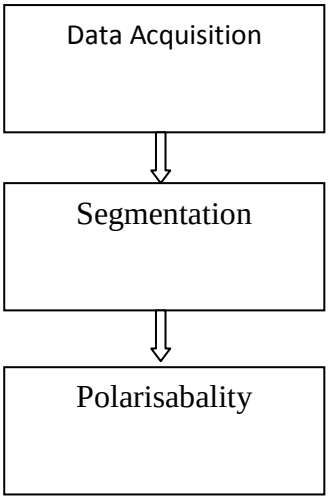


Figure 3.3: Step of data analysis

The electrophysiological properties of the cell membrane were extracted from the DEP spectra by the fitting of the plot to the single-shell model data. Data were required from previous study by Kadri, 2010. In this process, the digital image analysis techniques offer a great value and allows for rapid characterization.

DEP can be used to non-invasively determine electrophysiological parameters such as conductivity (defined as the ability to carry electrical charge) and permittivity (the ability to store electrical charge) of the cytoplasm and membrane of cells by monitoring the frequency-dependent DEP collection rate (Broche *et al*, 2007).

CHAPTER 4
RESULT AND ANALYSIS

4.1 Introduction

This chapter document origin of data acquisition and how the data was collected. Section 4.3 have observed the negative and positive DEP on the cells pattern movement. The next section document on result on analysis data and the analysis step algorithm apply in this project. This section shows application technique on histogram equalization offer better images in this analysis.

4.2 Data Acquisition

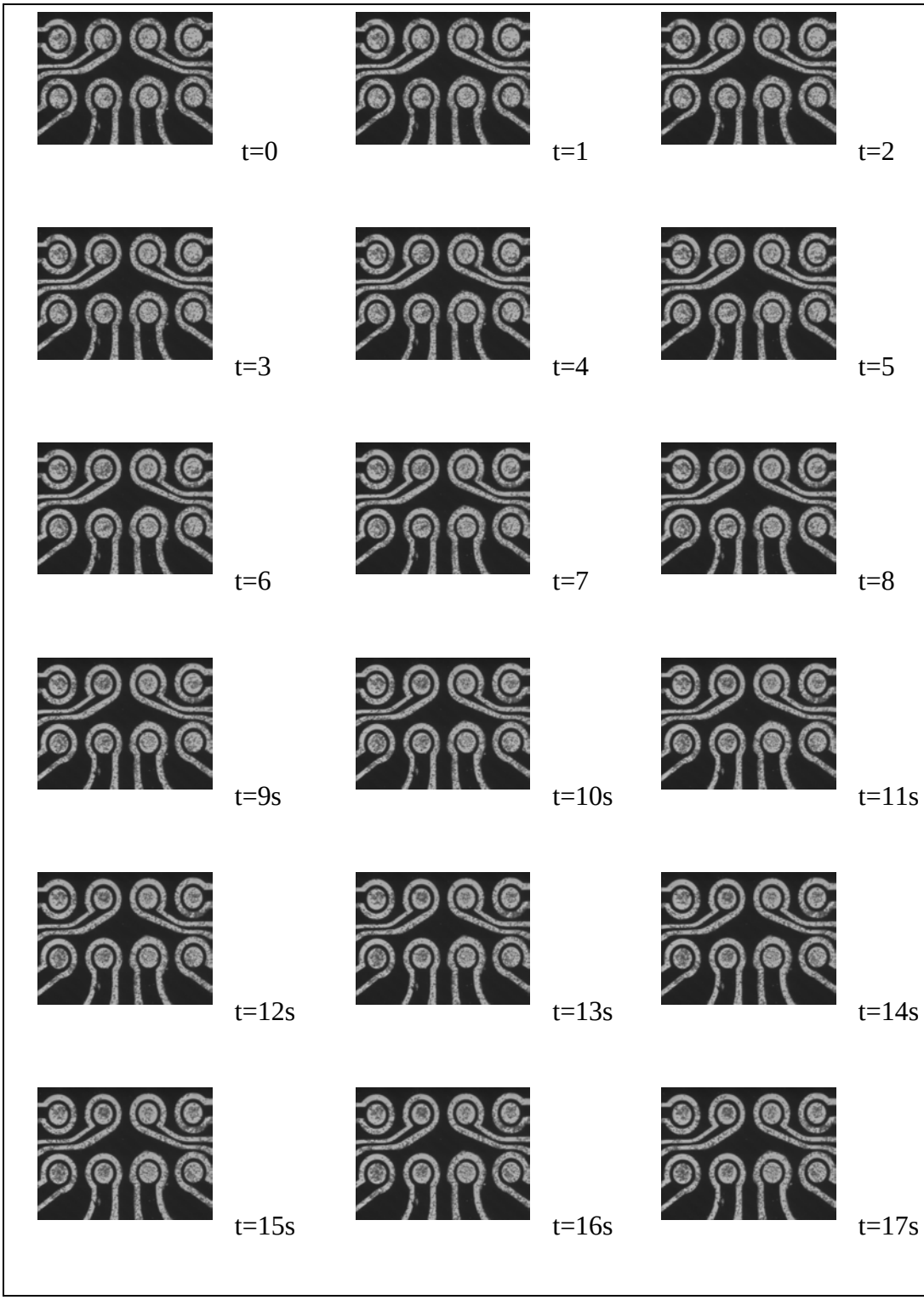
This study based on 10 set of collection data for 10 experiments conducted by previous researcher (Kadri, 2010) group at University of Surrey. The experiments were conducted within the range of 10 kHz to 1 MHz, supplied at 8 Vp-p for 21 seconds with K562 cells. Each set of collected data consist 22 images captured by the system using microscope mounted with digital camera as capturing device with specified induced frequency on each circular dots. Colour images with dimension of 480 x 640 pixels was captured, other related data such as frequency induced and time by system stored as *.*mat* file. Further analysis on previous collected data was conducted via Matlab version 2010ab .

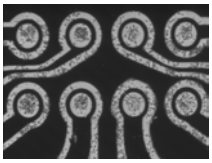
4.2 Positive and Negative DEP of K562 Cells

In this project, computerized image acquisition system allow to measure the rate of motion of cells as they moved under the influence of a non-uniform alternating electric field produced by an microelectrode array in specified time of experiment.

Example of the DEP of K562 cells images captured in for one set of experiment experienced negative DEP are shown in Figure 4.1.

Figure 4.1: Captured DEP images data





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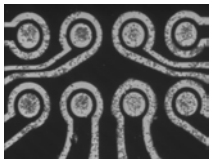

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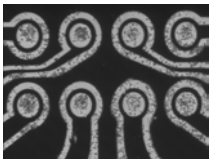
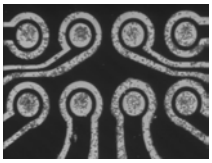


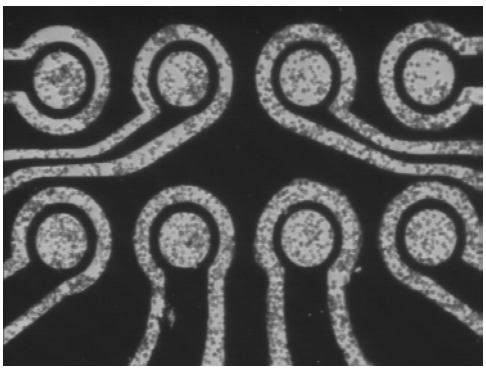
t=20s



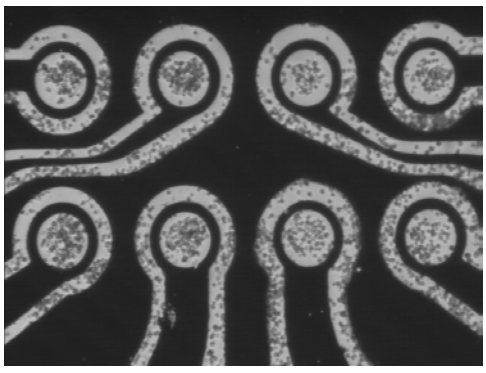
t=21s

Figure 4.1: Captured DEP images data (continued)

Based on the collected data, the positive dielectrophoresis may cause the cells to collect at the electrode edges when the particle is more polarisable than the surrounding medium and negative dielectrophoresis cause cells to collect away from the electrodes towards the region of lowest field strength when the medium is more polarisable than the cells as showns in Figure 4.2 and Figure 4.3 respectively. Cells collected at highly inhomogeneous electric field regions of the array when the electrical polarizability of cells exceeded that of the suspending medium or away from such regions when their polarizability was less than that of their medium.

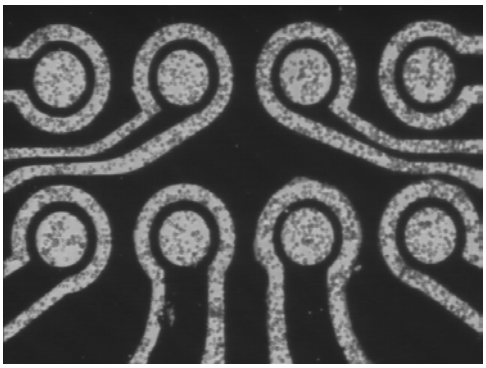


Cells at t=0s experienced negative DEP

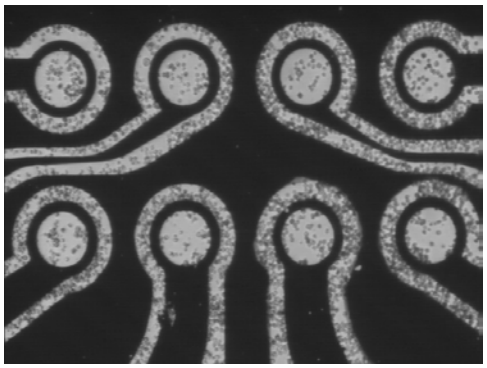


Cells at t=20s experienced negative DEP

Figure 4.2: Pattern of collected cells with experienced negative DEP



Cells at t=0s experienced positive DEP

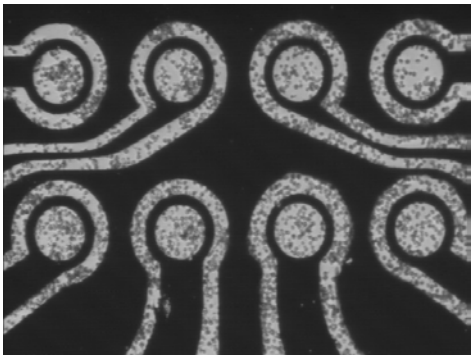


Cells at t=20s experienced positive DEP

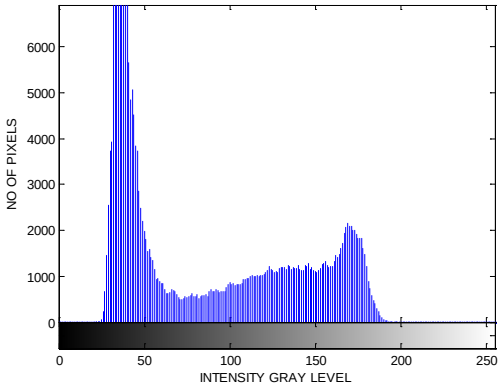
Figure 4.3: Pattern of collected cells experienced with positive DEP

4.3 Analysis Data

An image may be defined as a two-dimensional function, ***f (x, y)***, where x and y are spatial coordinates, and the amplitude of f at any pair of coordinates ***(x, y)*** is called the intensity or gray level of the image at that point. Figure 4.4 below shows the interest images captured for further analysis and distribution of intensity of grey level for captured image.



(a)



(b)

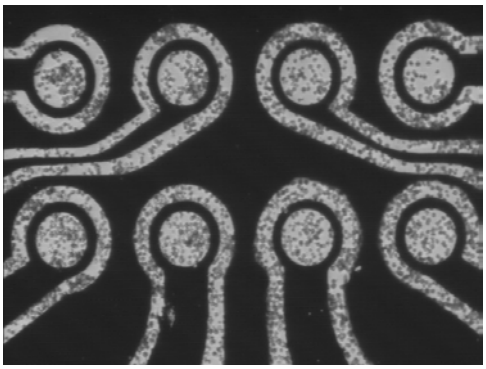
Figure 4.4: Distribution of intensity grey level (a) of the whole original images (a)

In histogram, the frequency of the appearance of each gray level is calculated in an intensity histogram over the entire image. The maxima and minima (peaks and

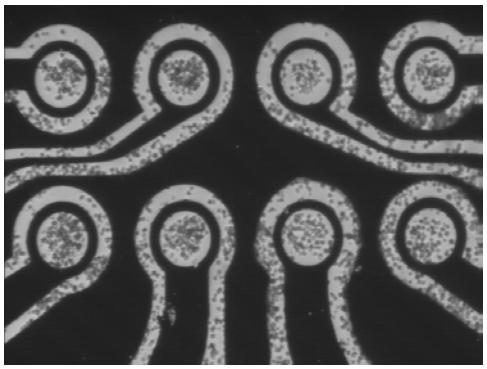
valley) are determined in the function given by frequencies in the histogram. Above figure demonstrates the laying intensities of cell in circular dot in between value 80 to 190. This value is valid for other images.

A histogram is graph that uses a linear scale of 256 levels, and shows the relative distribution of pixel color values in the image, from black to white and everything in between. Value of 0 at left side is solid black and where in the right side value of 255 is pure white.

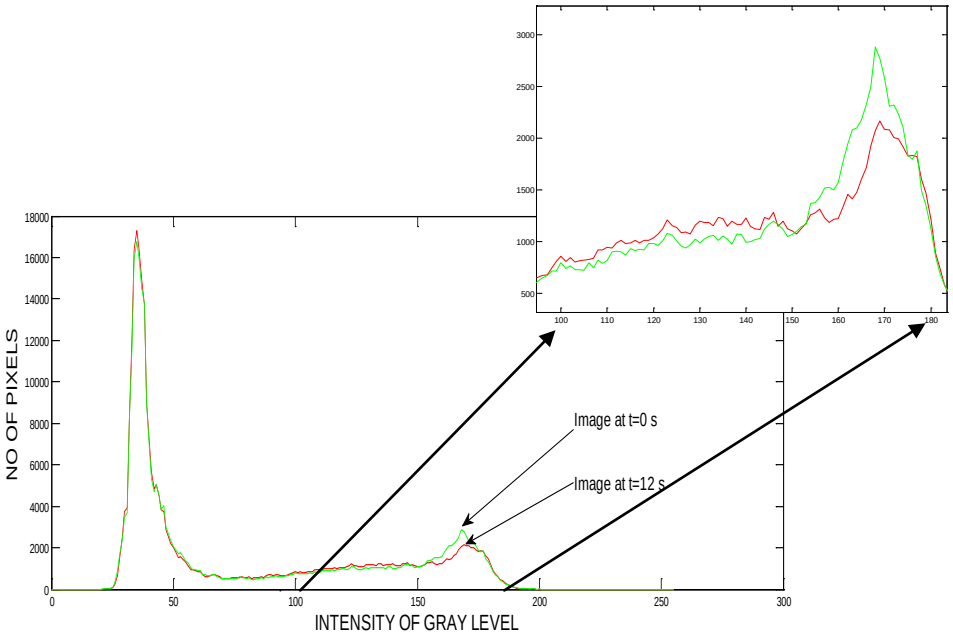
In order to differentiate histogram between the first images at t=0s captured with images at t=12s at specified circular range area, both of histogram are plotted in Figure 4.5, in order to show the intensity for both images capture. This figure illustrates that number of pixel lays between intensity value between 150 and 190 is low in images at t=12s compared to images at= 0s which means the distribution shows cells is much more distributed in the circular dot at t= 12s compared to distribution cells at t=0s.



(a) image captured at t=0s



(b) Image captured at t=12s



(c) Histogram comparison

Figure 4.5: Intensity of gray level for images captured at (a) t=0s (b) t= 12s and (c) Histogram comparison

Based on 50 sample of images the laying intensities of cells in circular dot in between value 90 to 190, which is in this case the intensity of cells is between value 80 to 90 and others values is belong to intensity of background within the circular dots. The reduction number of pixels value in intensity range 150 to 190 in figure 4.5 (c) causes by accumulation of cells within the circular dots.

In this analysis there are a few step involves in order to analysis the image and produce final output of polarisabality of DEP induced to cells. General step of analysis for this project is shows in figure 4.6.

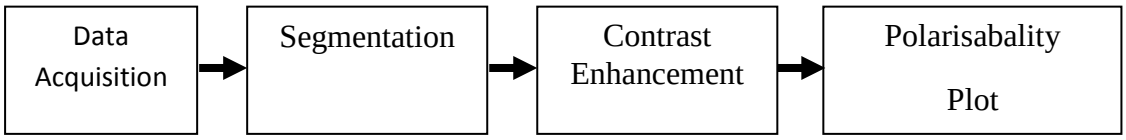


Figure 4.6: Step of analysis

In order to analyse the polarisabality, an essential steps before the captured images can be analysed and the corresponding DEP polarisabality, the captured images need to be segment on the circular dots within the image. The goal of segmentation is to simplify or to change the representation of an image into something that is more meaningful and easier to analyze (Papadopoulos *et al*, 2005).

DEP can be used to non-invasively determine electrophysiological parameters such as conductivity and permittivity of the cytoplasm and membrane of cells by monitoring the frequency-dependent DEP collection rate. In this study, the polarisabality is observed by the collection rate of cells within the circular dots. Before, the region can be further analyse segmentation of circular dots is essential.

The segmentation algorithm will generally conduct two main processes, namely the production of a layer mask to distinguish between the electrodes and the dots based on a chosen intensity threshold value and the detection of bona fide dots based on the similarities of the area sizes. The segmented images are used for further analysis in order to investigate the images of cell movements within each of the dots for further analysis in polarisabality of particles cells. The segmentation of images captured into circular dots from its background is illustrated in Figure 4.7 below.

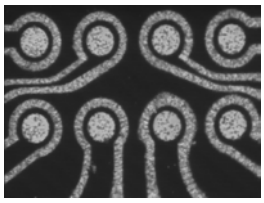
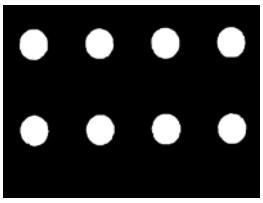
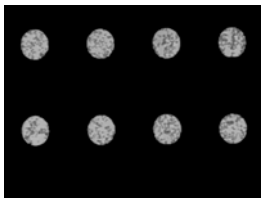
		
Original Image	Mask layer	Segmented Circular Dots

Figure 4.7: Segmentation of circular dots

In order to preserve a better images for further analysis, histogram equalization (HE) is applied after images was segmented into corresponding dots images. The main idea behind HE is to uniformly distribute the image pixels over the entire gray level range such that a better quality may be reached

HE is one of the most reliable, acceptable and extensively applied algorithms to perform image enhancement. HE also attends and stretches the dynamic range of image histogram and it results in overall image contrast enhancement. The main idea behind HE is to uniformly distribute the image pixels over the entire gray level range such that a better quality may be reached. HE is a spatial-domain enhancement technique and was proposed as an efficient technique for the enhancement of gray level images in real time applications. HE equalizes the contrast throughout the image and makes it easier to see the image detail in the regions that are originally very light or very dark. HE is an enhancement method performed on an image for locally adjusting each picture element value to improve the visualization of structures in both the darkest and lightest portions of the image at the same time (see in Figure 4.8). Effectively, in this research the HE is performing after dot was segmented into circular 8 dots in the analysis process.

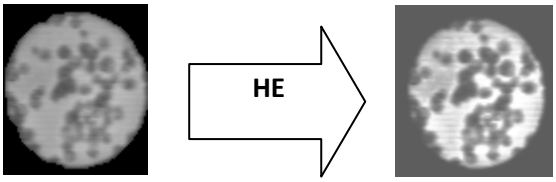


Figure 4.8: Example of performing Histogram Equalization on segmented dots

The relative polarisabality is as illustrates in Figure 4.9. This figure shows the relative Polarisabality of K562 cells with specified range of frequency induced by the system range 10KHz to 1 MHz. Polarisability depends partially on the reactance of the particle and medium and is therefore frequency dependent. This has been used as a key of study on dielectric properties of cells such as the conductivity and permittivity of the cell wall, membrane and cytoplasm.

The simple design of the dot microsystem gives a rapid and simple method of the determination of particle polarisability; the simplicity of the system enables rapid and accurate determination of the DEP spectrum using simple image processing techniques by comparing the change in light intensity against specified induced frequency and without the need for superimposition of calculated electric field distributions.

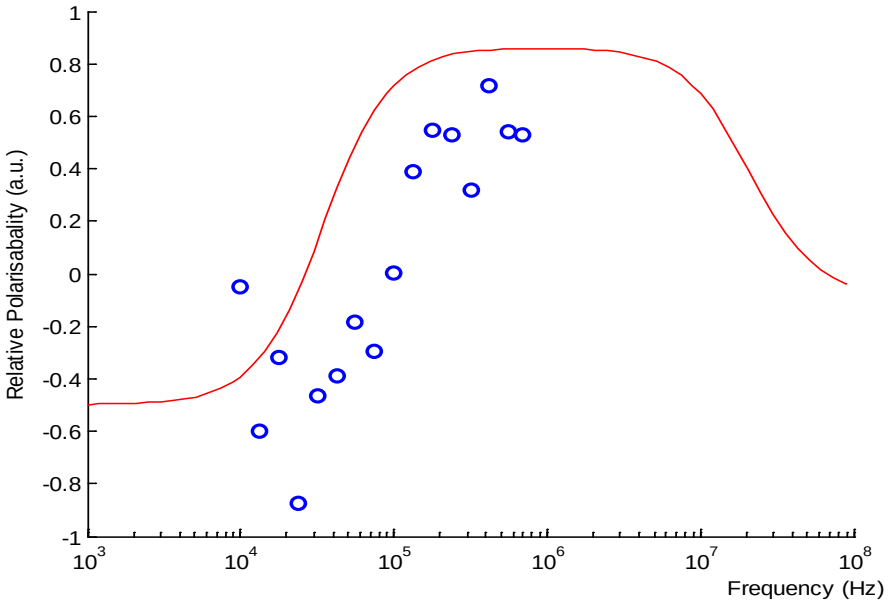


Figure 4.9: Relative Polarisabality

Image processing allows quantitative measurements to be made of the dielectrophoretically induced motion in viable mammalian cells. Image analysis appears to be a powerful tool for developing the potential of dielectrophoresis as a tool for studying mammalian cells and separating different cell types. Enhanced segmented image by using HE is applied as a step of improvement in the image analysis of polarisabality. Image contrast enhancement is very important for medical diagnosis. HE local contrast stretching methods have potential to be used for enhancing the K562 cells images captured images. As a result, the captured images have been applied with this technique appear to be clearer and achieved satisfactory.

CHAPTER 5
CONCLUSION

5.1 Conclusion

Based on the collected data, the positive dielectrophoresis cause the cells to be collected at the electrode edges when the particle is more polarisable than the surrounding medium and negative dielectrophoresis cause cells to collect away from the electrodes towards the region of lowest field strength when the medium is more polarisable than the cells.

Image analysis appears to be a powerful tool for developing the potential of dielectrophoresis as a tool for studying mammalian cells and separating different cell types. This study brings attention on analysis of image in order to improve analysis algorithm to study the polarisabality of cells. Effective polarizability-frequency spectrum measurement allow us to determine the membrane capacitance, the intrinsic dielectric dispersions, and also, membrane changes without the need for superimposition of calculated electric field distributions. This can be achieved by improving of dielectrophoretic data analysis algorithm in determining the relative polarisabality of dielectric particles.

5.2 Future Work

In future work, this element analysis in image analysis can be improve more by study on the other effect of image enhancement for example thresholding, Median filtering the processing, Pixel point processing and other types in order to produce better images data in preserving edges and images information. To note this study focusing in grey-level images, there is possibly to analyse and incorporate with colour, and texture in achieving better information to study polarisabality and finally capable to

produce powerful tool for developing the potential of dielectrophoresis as a tool for studying mammalian cells and can be accurately separating different type of cells . In fact, colour and texture are fundamental features in defining visual and stored more information compared the grey level images.

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APPENDIX A

The experiments were conducted using the multiple waveform generator and microelectrode device developed. The experimental protocol for conducting a DEP experiment by previous researcher in recording specified data is presented in Table below.

Step 1	Take 20 ml each of cultured cells from two T75 flasks (commonly the day after cell passaging to maximise cell viability)
Step 2	Place cell suspension in 50 ml tubes, and centrifuge each at 180 g for five minutes. Remove supernatant when completed.
Step 3	Add 10 ml of conductive medium (10 mS/m) to each tube, and gently mix the suspension using a pipettor.
Step 4	Centrifuge each tube at 115 g for five minutes. Remove supernatant when completed.
Step 5	Repeat Steps 3 and 4.
Step 6	Depending on the cell concentration required, appropriate amount of conductive medium is added. Typically, add 1 ml of medium to achieve 1×107 cells per ml.
Step 7	Pipette the solutions into two Eppendorf tubes: one each for control and drug-treated samples.
Step 8	Conduct cell counting and cell viability estimations from the control tube.
Step	Set up the microelectrode device (preferably completed prior to conducting an experiment): fabricate the gasket and attach it to the

9	electrode
Step 10	Set up the microscope: 4x magnification, and ensure uniform amount of light shining through the dots of the electrode.
Step 11	Set up the program: number of electrodes to be used (multiples of 4), start and end frequency, duration of signal and image capture, peak-to-peak voltage, dot diameter, and other information. The typical steps and values used for conducting an experiment are detailed below.
Step 12	Using a syringe, push a small amount of cell suspension through the inlet to completely cover the chamber created by the gasket. Avoid bubbles and/or over spilling of cell suspension out of the outlet.
Step 13	Start applying the signal.
Step 14	Re-suspend the cell suspension at the end of an experimental run, either by lightly tapping the electrode or pushing another small amount of cell suspension through the inlet.
Step 15	Repeat Steps 13 and 14 as necessary (routinely five) for control samples.
Step 16	Clean the microelectrode device thoroughly using 70% ethanol and deionised water.
Step 17	Add the required amount of drug to the sample tube. Start the stopwatch. Deliver a small sample of drug treated cells to the inlet of the device using a syringe.
Step 18	Start applying the signal. Record the stopwatch time at the moment that the signal is switched on from the waveform

	generator.
Step 19	Repeat Steps 13 and 14 as necessary (routinely for 60 minutes).

Experimental procedures for K562 cells (Kadri, 2010)