DEVELOPMENT OF AN IMPROVED IMAGE ANALYSIS FOR OPTICAL- BASED AC ELECTROKINETICS DATA

ADILAH BINTI HASHIM

RESEARCH REPORT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF ENGINEERING (BIOMEDICAL)

FACULTY OF ENGINEERING

UNIVERSITY OF MALAYA

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ABSTRACT

The availability of dielectrophoresis (DEP) method which apart of AC Electrokinetics for cell separation and manipulation are currently becoming highly purposeful due to its function variation and becoming possible. Commonly used in cancer cell detection and drug screening assessment, DEP are able to manipulate polarisable particles with the application of non-uniform electric considering the fact that movement will be induced onto the polarisable particles when placed in the electric fields. The application of DEP are used to be restricted in research for academic prospects only, rather than in manufacturing applications due to the image analyzing processes involved. Thus, this project aims to develop of an improved image analysis for optical based AC Electrokinetics data, which consists of image processing for purpose of development process. In order to perform a process, various image processing techniques have been applied on images that have been given from AC Electrokinetics data. The images are processed with various image processing techniques such as contrast enhancement and image segmentation. There are four contrast enhancement techniques. After that, the image is segmented by applying the image segmentation. The combination between contrast enhancement and image segmentation hopefully will giving a good result. Overall, the development of an image analysis for optical-based AC Electrokinetics data that has been developed. The development that provides an efficient alternative in analyzing the images. .

ABSTRAK

Ketersediaan kaedah dielectrophoresis (DEP) yang selain syarikat AC elektrokenetika pemisahan sel dan manipulasi sedang menjadi sangat bertujuan disebabkan oleh perubahan fungsi dan menjadi yang mungkin. Biasa digunakan dalam mengesan sel kanser dan penilaian saringan dadah, DEP mampu untuk memanipulasi partikel polarisable dengan permohonan bukan-seragam elektrik menimbangkan hakikat bahawa pergerakan akan didorong ke zarah polarisable apabila diletakkan dalam medan elektrik. Permohonan DEP digunakan dihadkan dalam penyelidikan bagi prospek akademik sahaja, bukannya dalam aplikasi pembuatan kerana imej menganalisis proses yang terlibat. Oleh itu, projek ini bertujuan untuk membangunkan analisis imej yang lebih baik untuk AC berasaskan optik elektrokenetika data, yang terdiri daripada pemprosesan imej bagi tujuan proses pembangunan. Yang diperlukan untuk melaksanakan sesuatu proses, pelbagai teknik pemprosesan imej telah digunakan pada imej-imej yang telah diberikan dari AC elektrokenetika data. Imej-imej yang diproses dengan pelbagai teknik pemprosesan imej seperti peningkatan kontras dan segmentasi imej. Terdapat empat teknik penambahbaikan Sebaliknya. Selepas itu, imej dibahagikan dengan menggunakan segmentasi imej. Gabungan antara peningkatan kontras dan segmentasi imej itu diharap akan memberikan hasil yang baik. Secara keseluruhannya, pembangunan analisis imej bagi optik berasaskan AC elektrokenetika data yang telah dibangunkan. Pembangunan yang menyediakan satu alternatif yang cekap dalam menganalisis imej.

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ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **ADILAH BINTI HASHIM** (I.C/Passport No: **810105-02-5154**)
Registration/Matric No: **KGL 100031**
Name of Degree: **Master of Engineering (Biomedical)**

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DEVELOPMENT OF AN IMPROVED IMAGE ANALYSIS FOR OPTICAL-BASED AC ELECTROKINETICS DATA

Field of Study: **DIELECTROPHORESIS**

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ACKNOWLEDGEMENT

In the name of God the Most Gracious and Most Merciful. First of all, I would like to thank God to always keep me in His Guidance throughout this life. Without all the strength given to me, this project might not have been completed.

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Adilah Hashim, May 2012

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DEP	-	Dielectrophoresis
AC	-	Alternating current

CHAPTER 1

INTRODUCTION

1.1 Overview

Technological innovation has yielded truly remarkable advances in health care during the last three decades. In just the last several years, breakthroughs in biotechnology, biomaterials, surgical techniques, and computer technology have helped to improve health care delivery and patient outcomes. The instruments that have been used in medical fields have helped experts to solve medical problems. Technological developments have given a new inspiration to researchers who have been struggling to obtain new knowledge to apply into new medical invention.

Based on these facts, the requirement for fast analysis of cancer images is of paramount importance in the healthcare industry. Cancer can be cured if it is detected and treated at the early stage which the procedure for early detection.

Dielectrophoresis is the phenomena where interactions between polarized particles with non-uniform electric fields exert motion onto the particles. It can be used and adopted to manipulate, separate, and analyze the cellular and viral particles responses (Hubner *et al.*, 2007; Hoettges *et al.*, 2008; Pethig, 2010). The induced motion and the manipulation of particles which have been polarized after being suspended in a non-uniform alternating electric field can cause repulsion

or attraction of cells with regards to the electrodes.DEP data have image which can include the image to analyzing with image processing. It can process for cancer data.

DEP has become recognizable since its introduction which defines DEP as "the motion of suspension particles relative to that of the solvent resulting from polarization forces produced by an inhomogeneous electric field" (Pohl, 1951). One of the advantages of DEP is that it does not need chemical markers, biochemical labels or bioengineered tags and at the same time does not exert any contact to any surfaces of cells making this technique preferable compared to chemical method such as fluorescent labels which are highly invasive and cell destructive (Hoettges *et al.*, 2008; Pethig, 2010).

There are a few major contributions of DEP-based technique especially in distinguishing dead and viable cells by separating and sorting them according to its conductivity of the cytoplasm, surface charge and the capacitance of the membrane. This is proven by a study done by Gascoyne *et al.* (1992) which demonstrate the separation of cancer cells on an electrode array due to the differences in terms of frequency shown between normal, leukemic and differentiation-induced leukemic mouse erythrocytes (Gascoyne *et al.*, 1992). This is further being supported by Becker *et al.* (1994 & 1995) who found and exploited the differences in the dielectric properties of metastatic human breast cancer cell from those of erythrocytes and T-lymphocytes resulting in the separation of breast cancer cells from the normal blood cells (Becker *et al.*, 1995; Becker *et al.*, 1994).

The current study has proposed an alternative solution to this problem through cost-effective and efficient software based application in recognizing and analyzing cancer cells based on images samples. This research aims to provide the development system that consists of image processing and feature extraction facilities. With the proposed intelligent screening system for acute leukaemia, it would assist patients and their family to plan the treatment option and budget finances.

1.2 Objective

The main objective of this project is the development of an improved image analysis for optical-based AC Electrokinetics data that can be used for enhance and segment of image. This main objective also covers the following sub-objectives:

a) To propose a contrast enhancement procedure that can be used for obtaining a fully enhance images.
b) To propose an image segmentation procedure that can be used for obtaining a fully segmented image.
c) To develop of an improved image analysis for optical-based AC Electrokinetics data system using the proposed techniques.

1.3 Scope the Study

Medical images are primarily visual in nature. The major strength in the application

of computers to medical imaging lies in the potential use of image processing and computer vision techniques for quantitative or objects analysis.

The use of images particularly in field of medical image processing is still limited. Thus, this research aims to develop of an improved image analysis for optical-based AC electrokinetics data system for images that consists of image processing facilities.

1.4 Organization of Project

This thesis consists of five chapters. These chapters discuss about the introduction, literature review, and development of an improved image analysis for optical-based AC electrokinetics, results and discussions, and conclusions with recommendations for future study.

Chapter 1 discusses briefly about introduction of the project. This chapter also presents the objectives, scope of the study and finally the organization of the project.

Chapter 2 presents the literature review which consists of the introduction, the DEP, the image processing and the development of an improved image analysis for optical-based AC Electrokinetics data system.

Chapter 3 describes the development of an improved image analysis for

optical-based AC electrokinetics data. This chapter consists of four main sections. The first section introduces the overview of the proposed develop system followed by the second section which describes the image acquisition. Section 3 describes the procedures for applying various image processing techniques such as contrast enhancement and image segmentation on image. Finally, the last section describes the data sample and evaluation methods of the enhancement and segmentation system.

Chapter 4 deals with the experimental results, provides the analysis for the finding and continue with the discussion. This chapter consists of five main sections. Section 1 presents the introduction of the chapter followed by the Section 2 which about data acquisition and image selected. Section 3 covers the results for contrast enhancement technique. Section 4 covers the results obtained the image segmentation. Finally, the overviews of the proposed development of an improved image analysis for optical-based AC Electrokinetics data system are described in the last section.

Chapter 5 put forwards the conclusion made based on the present study. The conclusions and contributions of the project are written based on the findings reported in the Chapter 4. Recommendations for future studies are presented due to their significance with the current research.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter discuss on two main topics that are Dielectrophoresis (DEP) and image processing.

2.2 Dielectrophoresis (DEP)

Dielectrophoresis (DEP), along with electrorotation (ROT) (Hughes, 2002; Lee *et al.*, 2004; Gascoyne *et al.*, 2006) is frequently grouped as a member of AC-electrokinetic technique which manipulates the mobility of cells in an electric field with non-uniformity and time-dependent properties in order to explore the electrophysiological properties of cells (Hughes, 2002; Jones *et al.*, 2003; Jones, 2003; Jones, 1995; Pethig *et al.*, 1997; Pohl *et al.*, 1981). Important information can be extracted from the electrophysiological properties especially at the cellular level. DEP method is known to be inevitably nondestructive and noninvasive, while being a label- free cell characterization technique when compared to the chemical methods which results in invasion of cellular trafficking or compared to electrical measurement method as such patch clamp which is highly destructive to cells (Labeed *et al.*, 2003).

The idea of dielectrophoresis was first discovered by Hatfield when he tried to separate the valuable mineral cassiterite from a large excess of quartz material. Hatfield realized the problem in electromagnetic separation was the generation of strong non-uniform electrical field even approaching the weakest electromagnetic field without caused any of dielectric breakdowns. Then, this idea was continued by Pohl who applied this theory to solve problem regarding of removing carbon-black filler from polyvinyl chloride samples. Then, he proceed his efforts in the development of methods and theories for dielectrophoresis characterization and separation of biological cells.

Over past 10 years, there are more than thousands research have been conducted in dielectrophoresis field (Pethig, 2010). This area is expected to face a huge growth due to its ability to integrate with advanced technologies like the used of thin film techniques or CMOS technology in fabricating the electrode. Besides, advanced in development of sophisticated electronic design has enhanced dielectrophoresis system by including some optical sensor or even the used of microcontroller for monitoring and manipulating purpose respectively. The existences of new materials like silicone polymer, silica glass or even indium tin oxide have given more choices for researchers in development of DEP system.

In general, dielectrophoresis (DEP) is a promising method for the separation and classification of biological cells in a miniaturized format. This technology allows cells to be manipulated electronically while suspended in a micro fluidic channel. Several dielectrophoretic configurations have been designed and fabricated using

micro-electro- mechanical-systems (MEMs).

If in the early age, the application of dielectrophoresis was more towards industrial application such as mineral sorting, assembling of micro component and manipulating fluid droplets as mentioned before, now the focus is diverge into biomedical application and it is expecting keep growing in future.

2.2.1 Theory of DEP

According to Pohl in 1951, dielectropherisis can be defined as "the motion of suspensoid particles relative to that of the solvent resulting from polarization forces produced by an inhomogeneous electric field". Polarization forces produced known as DEP force, which play an important role for manipulating and separating of target cells.

Particles can either moved toward or away from higher electrical gradient area. There are two factors that influenced the effectiveness of particle movement in DEP which are polarisability of the particle and its medium. These factors are differing from the electrophoresis phenomenon where mobility of ion depends on their total charge, size and shape.

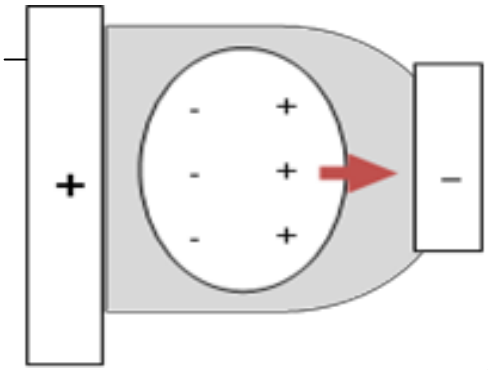


Figure 2.1: A schematic diagram of positive DEP (pDEP)

Based on Figure 2.1 above, positive DEP (pDEP) occurs when the particles are more polarisable than the surrounding medium, the induced dipoles align with the electric field and the particles move towards regions of higher field. Meanwhile, negative DEP (nDEP) occurs the particles are less polarisable than the surrounding medium, the induced dipoles will align against the applied field, causing the particles to move to regions of lower field strength. The DEP force can be described by the following question below (Hughes, 2002);

$$F_{DEP} = 2\pi r^3 \varepsilon_m \, Re[K(\omega)] \nabla E^2 \qquad (2.1)$$

where r is the cell radius, ε_m is the permittivity of the medium surrounding the cell, K(ω) is the complex Clausius- Mossotti factor, ∇ is the Del vector operator and E is the electric field strength in route means square (RMS) value.

Clausius- Mossotti factor is used to analyze the effective polarisability of the particles. According to Jones (1995), when Re [K (ω)] > 0 pDEP phenomenon will occur meanwhile when Re [K (ω)] < 0, nDEP phenomenon will occur. Furthermore, normally for spherical particles, the interval range of Re [K (ω)] is between −0.5 and 1. Clausius-Mossotti factor can be defined as equation below (Hughes, 2002);

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* - 2\varepsilon_m^*} \tag{2.2}$$

where ε_p^* and ε_m^* are the complex permittivity of the particles and medium respectively. Furthermore, ε^*can be determined using the equation below (Kadri, 2010);

$$\varepsilon^* = \varepsilon - \frac{j\sigma}{\omega} \tag{2.3}$$

where σ is conductivity, ε is permittivity and ω is the angular frequency of the applied AC electric field.

Frequency applied by AC input will affect the polarisability of the particles (Pethig, 2010). By looking at Equation 2.3, if the conductivity of particles predicted to be increased by a given frequency, the complex permittivity will be decrease as well as Clausius-Mossotti factors. This frequency exploitation strategy has played an important

role in particles separation where it linked to the different of polarisability of particle in the population.

Other than that, frequency has also play an important role in electromagnetic field produced. This condition has been mentioned by Muller *et al.* (1996) where the strength of electric field increased at higher frequency range up to MHz.

In the F_{DEP} Equation from (Eq. 1), the existence of Clausius-Mossotti factor, which is a frequency-dependent element, ensures that the F_{DEP} also varies with the frequency of the applied electric field. The factor relies upon the strength of the relative polarisability between the particle and the surrounding medium. If the factor is positive, it reflects that the particle is more polarized than the medium, thus effective motion will results in moving towards the area with the highest gradient of electric field. Opposing to this, when a negative DEP befall and the medium is more polarisable than the particle, the effective motion will result in repelling of particle towards the lower gradient of electric field. This will happen when the Clausius-Mossotti factor brings out a negative value.

In order to enable the dielectric properties to be directly calculated and correlated in a useful manner, the above analytical expressions are needed to be expanded. There are complexities faced while trying to do so even though the analytical expressions may have explained thoroughly the DEP behaviour of spheres in a medium. By performing the best-fit numerical analysis, an estimation of electrophysiological properties can be made to correlate the relevant cellular electrical

parameters with DEP behaviour.

The estimation method has also shown to be very useful in characterizing multiple cell populations within a heterogeneous cell sample (Chin *et al.*, 2006; Huang *et al.*, 1996; Broche *et al.*, 2005). A DEP spectrum has proven to be very beneficial in the extraction of electrophysiological properties because it also provides the estimation of the state and environment surrounding the cells.

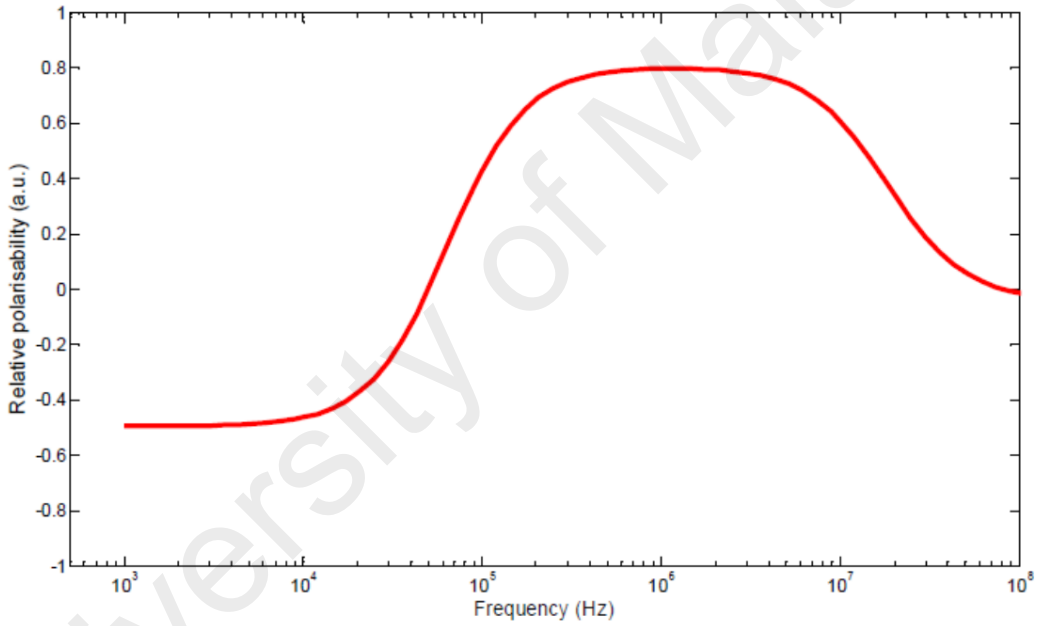


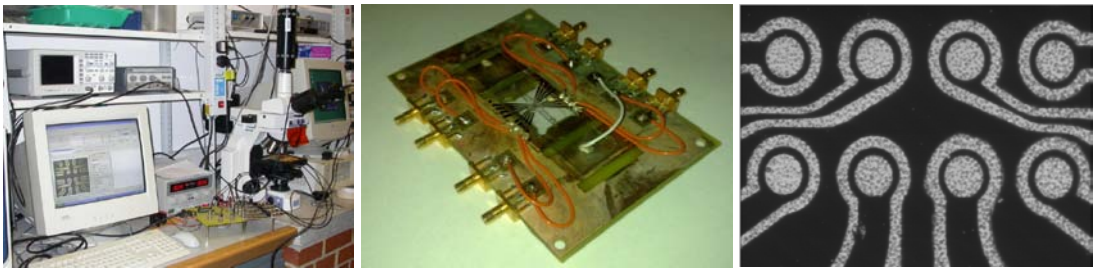
Figure 2.2: A typical shape of a DEP spectrum

Figure above shows the typical shape of the DEP spectrum while it can be shifted both ways, either to the right or to the left, depending on a particular influence that an external reagent has on the Clausius-Mossotti factor. This is due to the changes in components of Eq. 2 which consist of conductivity, permittivity, and angular frequency. A close example of this effect would be when an ionophore

molecules were attached to the cellular membrane, higher level of ions were allowed to pass through the membrane which ultimately disturb the conductivity thus disturbed the ionic distribution. This will in turn disturb the Clausius-Mossotti factor value and result in a frequency shift of the DEP spectra.

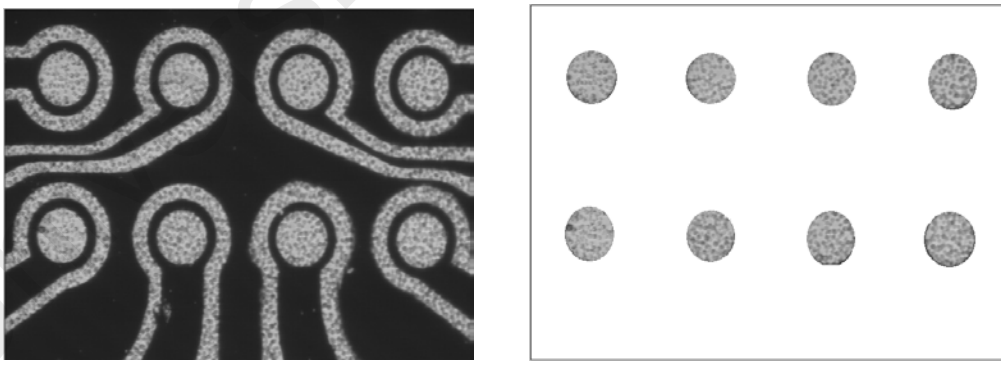
2.2.2 DEP data

The data was collected from the DEP experiment. The experiment was involved doing the hardware and the software. The hardware was created to the sample will operation when the sample was inside in the hardware, from that the circuit component also involve. The circuit had to connect with power supply. When the experiment was doing, computer will connect with digital microscope. The digital microscope is which have a camera also. They are much related connection. That functions is to looking the phenomena DEP which will display into computer and the software from the camera can to record the phenomena. The phenomena recorded will display in image data. Figure below is about the experiment and the image display. In this project the image of DEP data are already given, so that the doing experiment of this DEP is not involved. All image data have taken from Kadri,N.A, 2010.



(a) (b) (c)

Figure 2.3(a) the experimental set-up is shown containing the developed instrumentation and image acquisition device connected to a PC. Figure 2.3 (b) the hardware which is Electrode cartridge mounted with DEP-Dot system for up to 8 signals applied in parallel; Figure 2.3(c) the image data display after record which is DEP-Dot (some μm diameter) surface of a 4-by-2 microarray with interdot ground plane, in addition to the ITO counter electrode oppositely facing the microarray.(Adapted from Henry et.all, 2009).



(a) Image from DEP data (b) Image after cropping and selected

Figure 2.4: (a) Image from DEP data and (b) Image after cropping and selected. (Adapted from Kadri, 2009)

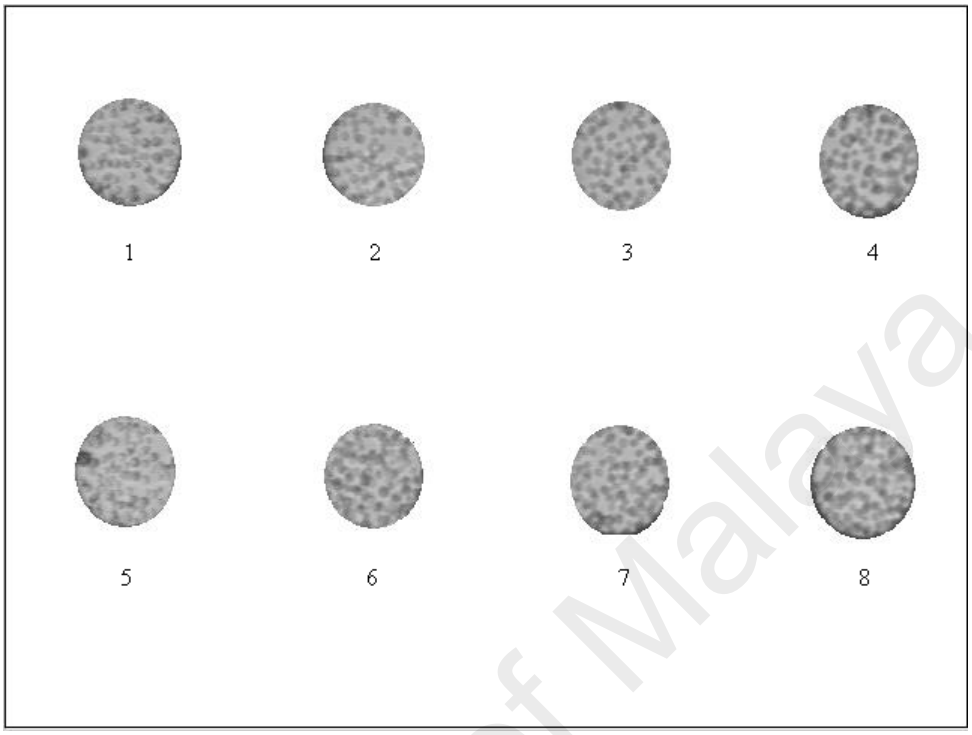


Figure 2.5: The number to know how to read data from experiment the image of DEP

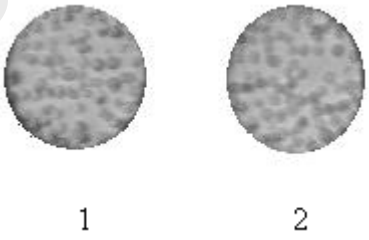


Figure 2.6: The selected image that want to process

Figure 2.3 is about overall experiment. Figure 2.4 (a) is about image from DEP data and Figure 2.4 (b) Image after cropping and selected. Figure 2.5 is about the

number to know how to read data from experiment the image of DEP. Lastly, Figure 2.6 is about the selected image that want to process.

2.3 Image Processing

An image is a representation of a real scene, either in black and white or colour, and either in print form or digital form. Digital images are represented by a number of rows and columns of cells called pixels. Each pixel contains the colour or gray level information for its respective spatial position in the image (Jain, Kasturi, & Schunck, 1995). For monochrome image, each pixel is represented by a gray level value. Meanwhile, each pixel in colour image is represented by red, green and blue values. Digital images can be created through the process of digitization. Digitization is the process of transforming image, text or sound from analog media into digital data (Jain, Kasturi, & Schunck, 1995). This process is important because computer can only process the digital data. The field of digital image processing is the study of algorithms for transformation of digital image.

Digital image processing is a well-developed field. This will give a positive impact for other application of digital image processing such as in medical imaging, satellite imaging, graphics and animations, robotics and photo enhancement. Image processing usually refers to digital image processing. As normally be defined, image processing is about the conversion of one image into another. In terms of medical field application, image processing is highly applicable for MRI SCAN (Magnetic Resonance Imaging) and CT SCAN (Computer Tomography).

2.3.1 Contrast Enhancement for Images

Contrast enhancement technique is widely used to increase the visual image quality. In general, there are two requirements to be fulfilled for colour image enhancement (Chatterji & Murthy, 1997). The first one is to keep the colour structure of the original image. The second requirement is to present as much information as the original. Contrast of the image is one of the factors that may influence the accuracy of interpretation by haematologists. Exposure of the microscope also influences the quality of captured images. Overexposure setting will lead in producing bright image, while underexposure setting will produce a dark image. Due to the low quality of the image, it will be hard to visualize and analyze the blood cell morphological features on the system for further image processing.

Thus, contrast enhancement at the pre-processing stage becomes the most important process for a successful feature extraction and diagnosis of image. The resulting enhanced medical images will provide clearer and cleaner images for better and easier disease process by doctor. Among the contrast enhancement techniques that have been developed for improving the image quality are partial contrast, bright stretching dark stretching and linear contrast techniques.

2.3.1.1 Partial Contrast Technique

Partial contrast is a linear mapping function that is used to increase the contrast level and brightness level of the image. The technique is based on the original

brightness and contrast level of the image to be adjusted. First, the system will find the range of where the majority input pixels converge for each colour space. Since the input image is in RGB colour space, so it is necessary to find the pixels range between the red, green and blue intensities. Then, the average of these three colour space will be calculated to obtain the upper and lower colour values by using the following formula in Equation 2.4 And Equation 2.5.(Weeks, 1996):

$$maxTH = (maxRed + maxGreen + maxBlue)/3 \quad (2.4)$$

$$minTH = (min\ Red + minGreen + min\ Blue)/3 \quad (2.5)$$

maxRed, *maxGreen* and *maxBlue* are the maximum colour level while *minRed*, *minGreen* and *minBlue* are the minimum colour level for each colour palette respectively. *maxTH* and *minTH* are the average number of maximum and minimum RGB colour space. *maxTH* and *minTH* will be used as the desired colour ranges for all three colour palette. Next is to start with the mapping function is given in Equation 2.6 (Weeks, 1996).

$$P_k = \frac{(max - min)}{(f_{max} - f_{min})}(q_k - f_{min}) + min \quad (2.6)$$

Where,

P_K : Colour level of the output pixel

q_K : Colour level of the input pixel

$f\ max$: Maximum colour level values in the input image

$f\ min$: Minimum colour level values in the input image

min : Desired minimum colour levels in the output image

max : Desired maximum colour levels in the output image

For partial contrast, the function in Equation 2.7 used for the pixels transformation which is based on the concept of linear mapping function shown in Equation 2.6.

$$out(x,y)=\begin{cases}\dfrac{in(x,y)}{\min TH}*N\min TH & for \quad in(x,y)>\min TH \\ \left[\dfrac{(NMaxTH-NMinTH)}{\max TH-\min TH}*(in(x,y)-f\min)\right]+\min & for \quad \min TH<in(x,y)<\max TH \\ \dfrac{in(x,y)}{\max TH}*N\max TH & for \quad in(x,y)<\max TH\end{cases} \tag{2.7}$$

Where,

In(x,y) : Colour level for the input pixel

Out(x,y) : Colour level for the output pixel

minTH : Lower threshold value

maxTH : Upper threshold value

NminTH : New lower stretching value

NmaxTH : New upper stretching value

By applying this technique, the pixel within the range of *minTH* and *maxTH* will be mapped to a new range and stretched to a wider range within *NmaxTH* and *NminTH*. The remaining pixel will experience compression. Figure 2.7 illustrates the stretching and compression process for partial contrast technique.

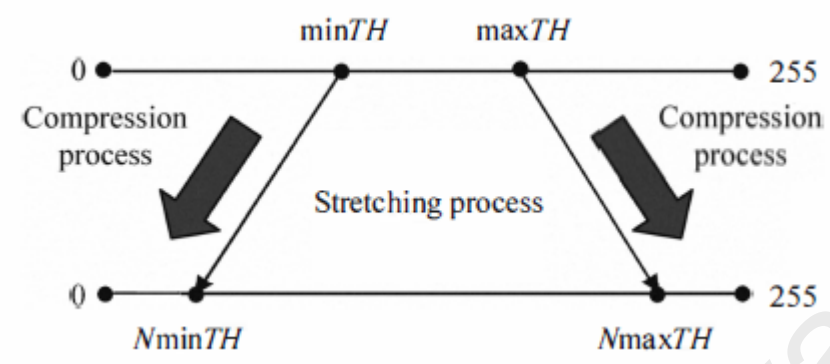


Figure 2.7: The stretching and compression process for partial contrast technique

2.3.1.2 Bright Stretching Technique

Bright stretching technique is based on linear mapping function shown in Equation 2.8. This method is normally used to enhance the brighter part of the image. For the bright stretching method, Equation 2.6 will be interpreted as follows (Weeks, 1996):

$$out(x,y)=\begin{cases}\dfrac{in(x,y)}{TH}*SFb & for \quad in(x,y)<TH\\ \left[\dfrac{(in(x,y)-TH)}{255-TH}*(255-SFb)\right]+SFb & for \quad in(x,y)>TH\end{cases} \tag{2.8}$$

TH and *SFB* are the threshold value and the bright stretching factor, respectively. The *SF* value should be smaller than TH. Figure 2.8 illustrates the stretching and compression process for bright stretching technique. Referring to

Figure 2.8, the pixel values which are less than threshold value will be compressed while the pixel values which are greater than the threshold value will be stretched.

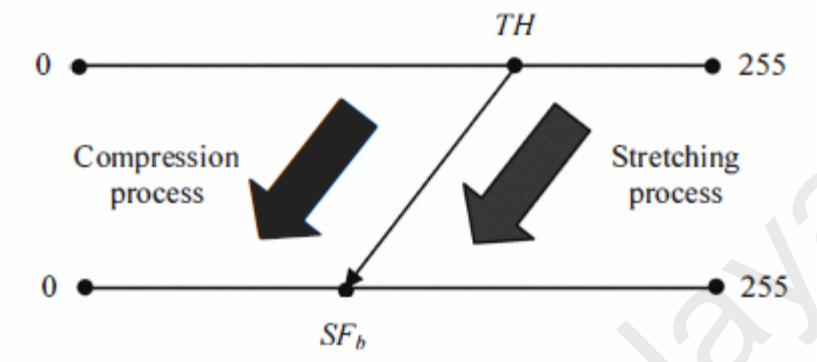


Figure 2.8: The bright stretching

2.3.1.3 Dark Stretching Technique

Dark stretching technique is a reverse process of bright stretching technique. Similar to bright stretching, dark stretching technique is also based on Equation 2.6 which involves linear mapping function. The equation for dark stretching is defined in Equation 2.9 (Weeks, 1996).

$$out(x,y) = \begin{cases} \dfrac{in(x,y) - TH}{255 - TH} * SFd & for \quad in(x,y) < TH \\ \left[\dfrac{(in(x,y) - TH)}{255 - TH} * (255 - SFd)\right] + SFd & for \quad in(x,y) > TH \end{cases} \quad (2.9)$$

TH and SF are the threshold value and the dark stretching factor, respectively. The SF value should be greater than TH. Figure 2.9 illustrates the stretching and

compression process for dark stretching technique. Referring to Figure 2.9, the pixel values which are less than threshold value will be stretched while the pixel values which are greater than the threshold value will be compressed.

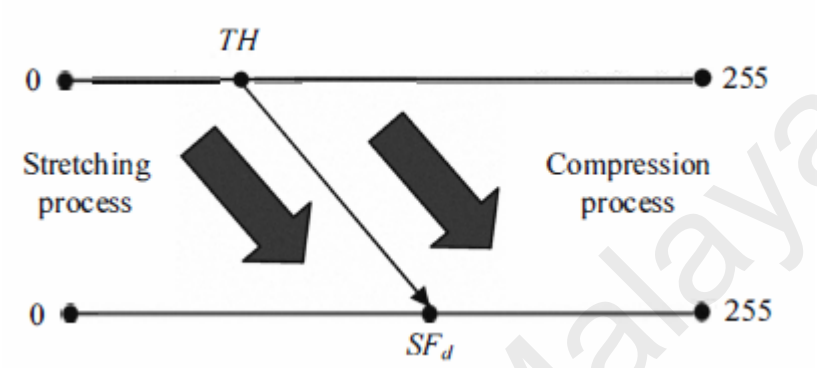


Figure 2.9: The dark stretching technique

2.3.1.4 Linear Contrast Technique

Similar to partial contrast, linear contrast technique is used to increase the contrast level and brightness level of the image. The technique is based on the original brightness and contrast level of the images to be adjusted. The equation of linear contrast algorithm is defined in Equation 2.10. (Ngah *et al*, 2000)

$$I_{output}(x,y) = 255.\frac{\left[I_{input}(x,y) - \min\right]}{(\max - \min)} \quad (2.10)$$

Where,

I input: The original RGB value of the pixel

I output: The new RGB value of the pixel

Min : Minimum RGB value

Max : Maximum RGB value

Based on Equation 2.10, linear contrast technique will consider each range of R, G and B colour space in the image. Thus, the range of each colour space will be used for contrast stretching process to represent each range of colour. This will give each colour space a set of minimum and maximum values. By applying this algorithm, each R, G and B colour space will be distributed linearly over the whole histogram so that the dynamic range of the histogram is fulfill (0 to 255).

2.3.1.5 Applications of Contrast Enhancement in Medical Field

Several previous studies have proved that contrast enhancement technique is capable of improving the medical image quality for visualization by suppressing the unwanted noises and increasing the visibility of low contrast image features.

2.3.2 Image Segmentation for Blood Images

Segmentation of an image refers to the separation of regions with similar characteristics. Image segmentation is the most important step in image analysis as it will directly affect the post-processing (Aus *et al*., 1987).

Image segmentation techniques can be classified into for main categories: thresholding, boundary-based, region based segmentation and hybrid techniques that combine and region criteria (Rangayyan, 2005). Some of these have been combined in order to segment the region of interest in medical images.

2.3.2.1 K-Mean

In data mining, *k*-means clustering is a method of cluster analysis which aims to partition *n* observations into *k* clusters in which each observation belongs to the cluster with the nearest mean. This results into a partitioning of the data space into Voronoi cells.

The problem is computationally difficult (NP-hard), however there are efficient heuristic algorithms that are commonly employed and converge fast to a local optimum. These are usually similar to the expectation-maximization algorithm for mixtures of Gaussian distributions via an iterative refinement approach employed by both algorithms. Additionally, they both use cluster centers to model the data, however *k*-means clustering tends to find clusters of comparable spatial extent, while the expectation-maximization mechanism allows clusters to have different shapes.

Given a set of observations (x_1, x_2, …, x_n), where each observation is a *d*-dimensional real vector, *k*-means clustering aims to partition the *n* observations into *k* sets ($k \leq n$) S = {S_1, S_2, …, S_k} so as to minimize the within-cluster sum of squares (WCSS):

$$\arg\min_{\mathbf{S}} \sum_{i=1}^{k} \sum_{\mathbf{x}_j \in S_i} \|\mathbf{x}_j - \boldsymbol{\mu}_i\|^2 \tag{2.11}$$

where μ_i is the mean of points in S_i.

2.3.2.2 MCM

Moving k-mean is the same name for moving c-mean (MCM). MCM is application from K-mean. The MKM clustering algorithm will then be used to determine the final value of each centre that will be referred as *CN*, *CC* and *CB* for A, B, and C respectively. Based on the Euclidean distance concept, the threshold value, *βNC* and *βCB* will be calculated by using Equation 2.12.

$$\beta_{NC} = \frac{C_N + C_C}{2}$$
$$\beta_{CB} = \frac{C_C + C_B}{2} \tag{2.12}$$

where,

β_{NC} - the threshold value to differentiate the A-B area

β_{CB} - the threshold value to differentiate the B-C area

2.3.2.3 FCM

Fuzzy c-means (FCM) is a method of clustering which allows one piece of data to belong to two or more clusters. This method (developed by Dunn in 1973 and improved by Bezdek in 1981) is frequently used in pattern recognition. It is based on minimization of the following objective function:

$$J_m = \sum_{i=1}^{N} \sum_{j=1}^{C} u_{ij}^{m} \left\| x_i - c_j \right\|^2 , \quad 1 \le m < \infty \qquad (2.13)$$

where m is any real number greater than 1, u_{ij} is the degree of membership of x_i in the cluster j, x_i is the ith of d-dimensional measured data, c_j is the d-dimension center of the cluster, and ||*|| is any norm expressing the similarity between any measured data and the center. Fuzzy partitioning is carried out through an iterative optimization of the objective function shown above, with the update of membership u_{ij} and the cluster centers c_j by:

$$u_{ij} = \frac{1}{\sum_{k=1}^{C} \left(\frac{\|x_i - c_j\|}{\|x_i - c_k\|} \right)^{\frac{2}{m-1}}} \quad , \quad c_j = \frac{\sum_{i=1}^{N} u_{ij}^{m} \cdot x_i}{\sum_{i=1}^{N} u_{ij}^{m}} \tag{2.14}$$

This iteration will stop when $\max_{ij} \left(\left| u_{ij}^{(k+1)} - u_{ij}^{(k)} \right| \right) < \varepsilon$, where ε is a termination criterion between 0 and 1, whereas *k* are the iteration steps. This procedure converges to a local minimum or a saddle point of J_m.

2.4 Summary

Reviews of the literature that necessitates the scope of the present work are briefly reported. This chapter has been started with a brief description introduction. Discussions are continued with DEP and image processing.

CHAPTER 3

DEVELOPMENT OF AN IMPROVED IMAGE ANALYSIS FOR OPTICAL –BASED AC ELECTROKINETICS DATA

3.1 Introduction

This chapter describes various applications of image processing techniques that will be applied on DEP images for the purpose of development system process. All the information steps for each technique that will be used in this project will be described in detail.

In order to perform the screening process, the actions to be taken will include 2 main steps, starting with the image acquisition, and image processing. Here the development system for images is fully developed by using MATLAB R2010a and Borland C++ Builder 6.0 software. A personal computer which runs on a Intel Core 2 Duo CPU 3.17 GHz processor with 3.48 GB RAM (Random Access Memory) that operates in Microsoft Windows XP Professional is used for developing the system. The procedures used to develop system for image are illustrated by the flow chart in Figure 3.1.

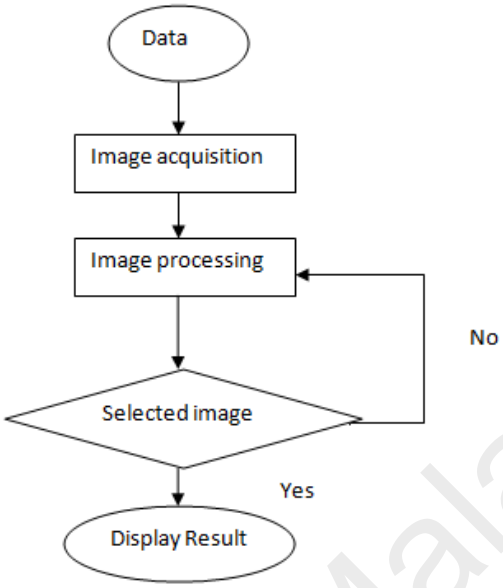
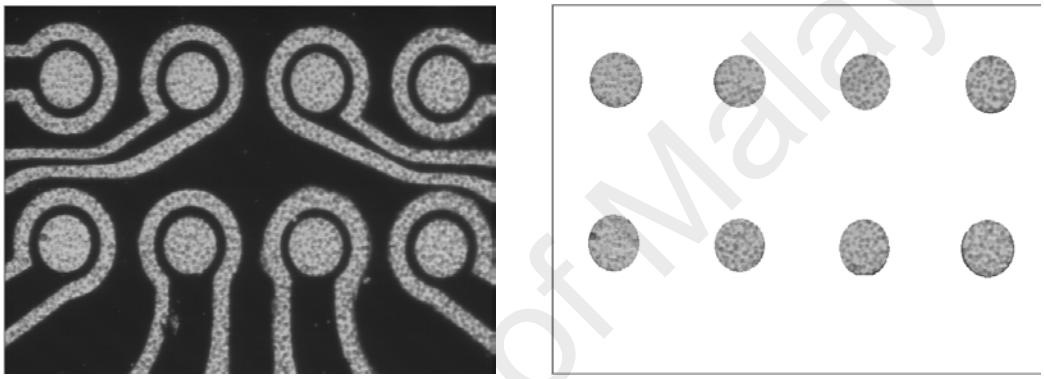


Figure 3.1: The development of an improved image analysis for optical-based AC electrokinetics data system.

During the image processing step, the blood image will be processed with various image processing techniques in order to obtain a fully segmented. The processes include image enhancement and image segmentation. Here, there are four contrast enhancement techniques that have been applied for enhancing the image namely partial contrast, bright stretching, dark stretching and linear contrast techniques. After the image has been enhanced, the image will be segmented by applying the image segmentation based on K-mean, MCM and FCM. Further details for the image acquisition and image processing are discussed in the following section.

3.2 Image Acquisition

Image acquisition is from the given data. Figure 3.2 (a) is about image from DEP data and Figure 3.2 (b) Image after cropping and selected. Figure 3.3 is about the number to know how to read data from experiment the image of DEP. Lastly, Figure 3.4 is about the selected image that want to process.



(a) Image from DEP data (b) Image after cropping and selected

Figure 3.2: (a) Image from DEP data and (b) Image after cropping and selected

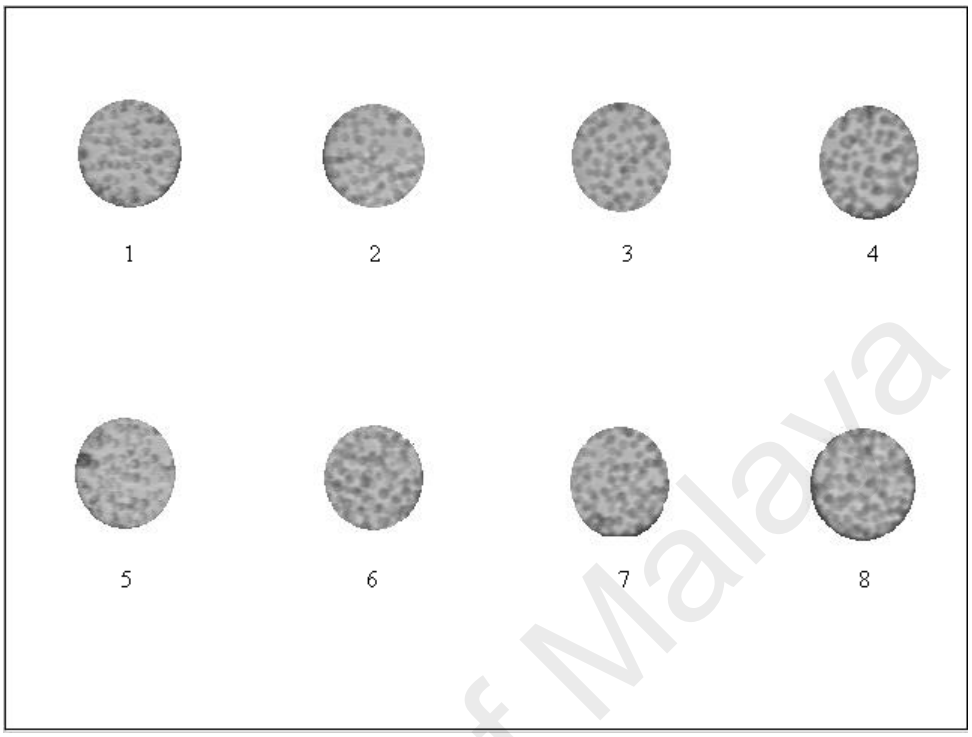


Figure 3.3: The number to know how to read data from experiment the image of DEP

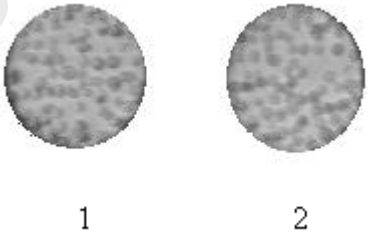


Figure 3.4: The selected image that want to process

They are have eight image, from the image the selected to process are number 1 and number 2 only. Something the different criteria have to learn between them until the image can tell the result is normal or cancer based on several of analysis. For that in this project, the image want to process to more clear hopefully which will also

related to get a good result of analysis. So, in this project, the image analysis is about image processing. The image processing is one to enhance and another one is to segment are applied. This is to still a looking before to choose a good result. In this project also done for image analysis with is looking at image from intensity histogram. Different between image number 1 and 2, is at the strength of value from the intensity value. More numbers of image from image 1 to image 8 will looking the strength of value are increase. This project is want to doing a first step before to go more. Thus, image processing is done.

3.3 Image Processing

The second of stage of performing development process is image processing. There are several image processing methods that have been proposed image recognition of some of the proposed segmentation techniques. There are several techniques will be applied during the image processing. Among the techniques are contrast enhancement, and image segmentation,. The procedures used to develop the image processing for images are illustrated by the flow chart in Figures 3.5.

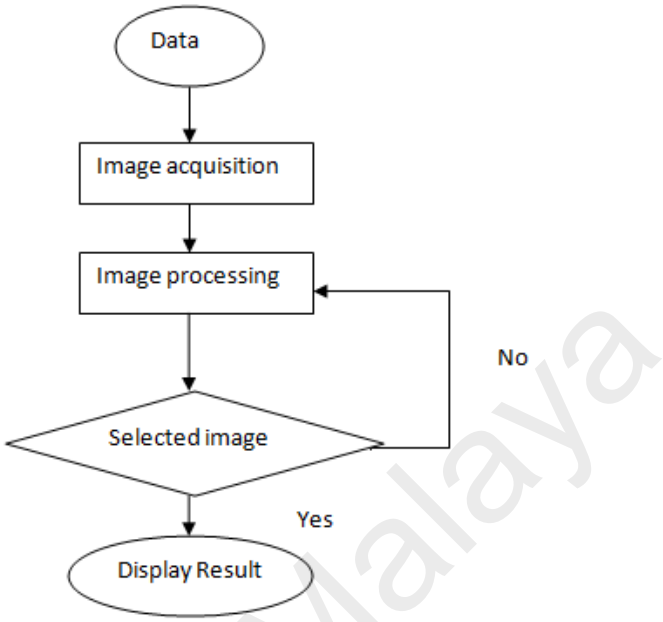


Figure 3.5: The proposed image processing steps for images.

Based on the flow chart Figure 3.5, in order to obtain the segmented image will first applied with contrast enhancement and image segmentation techniques.

3.3.1 Contrast Enhancement Techniques for Image

Contrast of the image is one of the factors that may influence the accuracy of interpretation. In this research, contrast enhancement technique has been applied on images for the two main purposes. Firstly is to improve the image quality. The images captured through the microscope may have their own weakness such as blurred or low contrast. Thus, contrast enhancement technique plays an important role in enhancing the quality and contrast of image. Secondly is to enhance the area of interest in image for easing the

segmentation process. In this research, the goal of image enhancement is to enhance the contrast of features of interest by using the contrast enhancement techniques.

Intensity histogram can be used to distinguish between the 1, 2 and 3 regions of image. Figure 3.4 represents the intensity histogram from an original image. Based on this histogram could be divided into three separate regions, indicated by (a), (b) and (c), respectively.

In this research, there are four contrast enhancement techniques that have been applied on images namely partial contrast, bright stretching, dark stretching and linear techniques. The full description about these four contrast enhancement techniques can be obtained from Chapter 2 In this project, the procedures used to develop the contrast enhancement techniques are as follows:

1. Develop the intensity histogram of the original image.
2. Select the threshold value and stretching factor by referring to the peak of 1, 2 and 3 regions of the histogram. Here, the selection of threshold value and stretching factor are based on the intensity histogram.
3. Apply the four contrast enhancement techniques by applying the threshold and stretching value on the original image.
4. Develop the intensity histogram from the resultant image in order to recognize the significance of the enhancement techniques on original image.

3.3.2 Image Segmentation

The colour image segmentation for image is performed based on K-mean, MCM, and FCM. It is the same procedure above is doing in this image segmentation.

The image segmentation techniques procedures for example:

1. The process begins with segmentation process that partitions the image cell into three main regions; A, B and C of the cell.
2. Grey level histogram of the cell image will be analyzed to obtain the initial centre of each region; *CNo*, *CCo* and *CBo*, for A, B and C respectively.
3. Each of regions has different range of grey level that start with A that has the lowest value, followed by B and C.

3.4 Development of an improved image analysis for optical-based AC electrokinetics data system

The development of improved image segmentation for optical-based AC electrokinetics data system for image is doing. It is display at desktop computer for easy to look the process result of enhancement and segmentation.

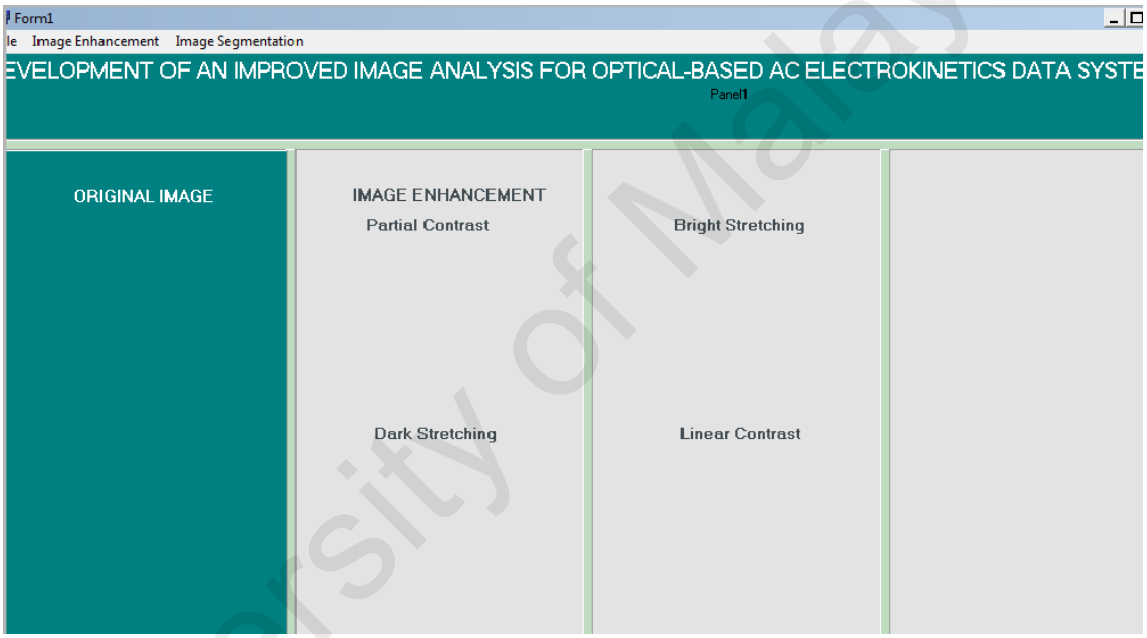


Figure 3.6. The development of an improved image analysis for optical-based AC electrokinetics data system.

3.5 Summary

This chapter described the proceding of using the proposed techniques. The successful of the development system for image to be used for the looking of image sample depends merely on the image processing that has been developed. During the image

processing process, there are four contrast enhancement namely partial contrast, bright strecthing, dark stretching and linear contrast that have been proposed for enhancing the contrast of images. Then, the enhanced images will be segmented by using the image segmentation based on K-mean, MCM, and FCM. The development of an improved image analysis for optical-based AC electrokinetics data system also has done.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

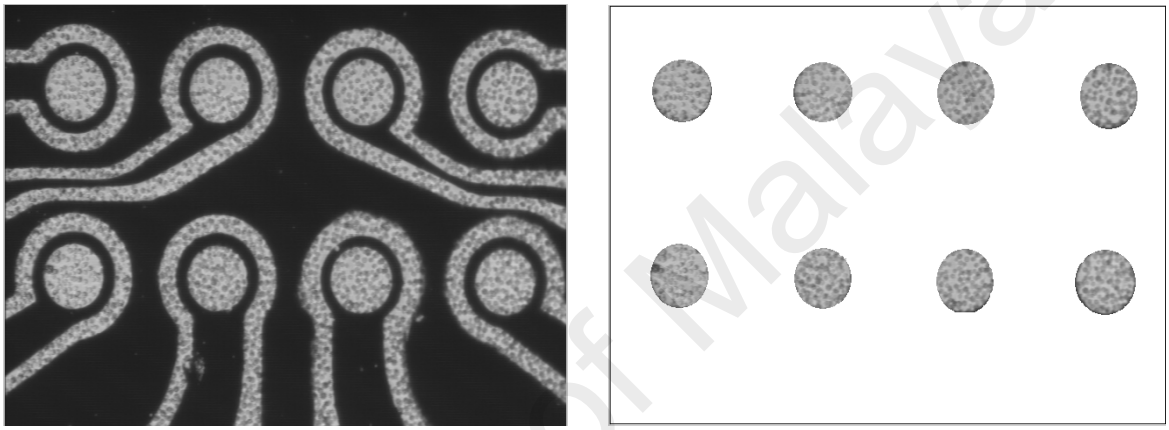
This chapter provides the results that have been obtained based on the application of image processing techniques that have been discussed in Chapter 2. The first section of this chapter will discuss the data acquisition. The second section will discuss the results obtained after applying the contrast enhancement techniques on DEP images. The results obtained after enhancing the images with the proposed contrast enhancement techniques will be shown and discussed. The third section of this chapter will discuss the result of image segmentation using K-Mean, FCM and MCM on DEP images. Finally, the conclusion of all image processing techniques that have been applied on images will be made at the end of this section.

4.2 Data acquisition

Image acquisition is from the given data. Figure 4.1 (a) is about image from DEP data and Figure 4.1 (b) Image after cropping and selected. Figure 4.2 is about the number to know how to read data from experiment the image of DEP. Lastly, Figure 4.3 is about the selected image that want to process.

They are have eight image, from the image the selected to process are number 1 and number 2 only. Something the different criteria have to learn between them until

the image can tell the result is normal or cancer based on several of analysis. For that in this project, the image want to process to more clear hopefully which will also related to get a good result of analysis. So, in this project, the image analysis is about image processing. The image processing is one to enhance and another one is to segment are applied. This is to still a looking before to choose a good result.



(a) Image DEP (b) Image DEP after cropping and select

Figure 4.1: Original image of DEP and image which is cropping and select

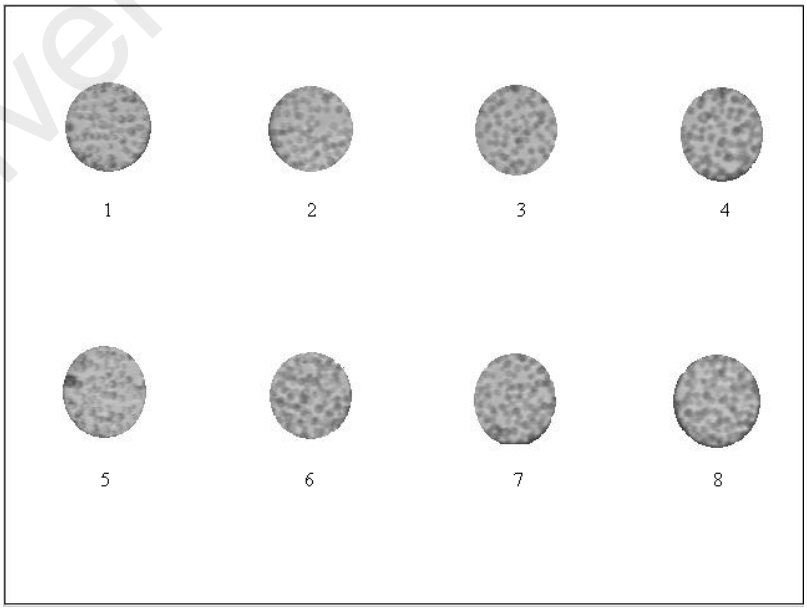


Figure 4.2: The number to know the reading data from experiment the Image of DEP

Figure 4.3: The selected image which wants to process

4.3 Contrast Enhancement Techniques

This section provides the results of the three contrast enhancement techniques namely partial contrast, dark stretching and linear contrast techniques that have been applied on images. These techniques have been chosen because number 1 and 2 can be used to improve the overall contrast of images. The quality of images can be determined based on human usual interpretation.

It also determined based on human visual interpretation as well as the intensity histogram plot. The resultant intensity histogram is used to measure the effect of stretching and compression process on the images. If the images have good contrast, the morphological features inside the images can be easily seen and detected.

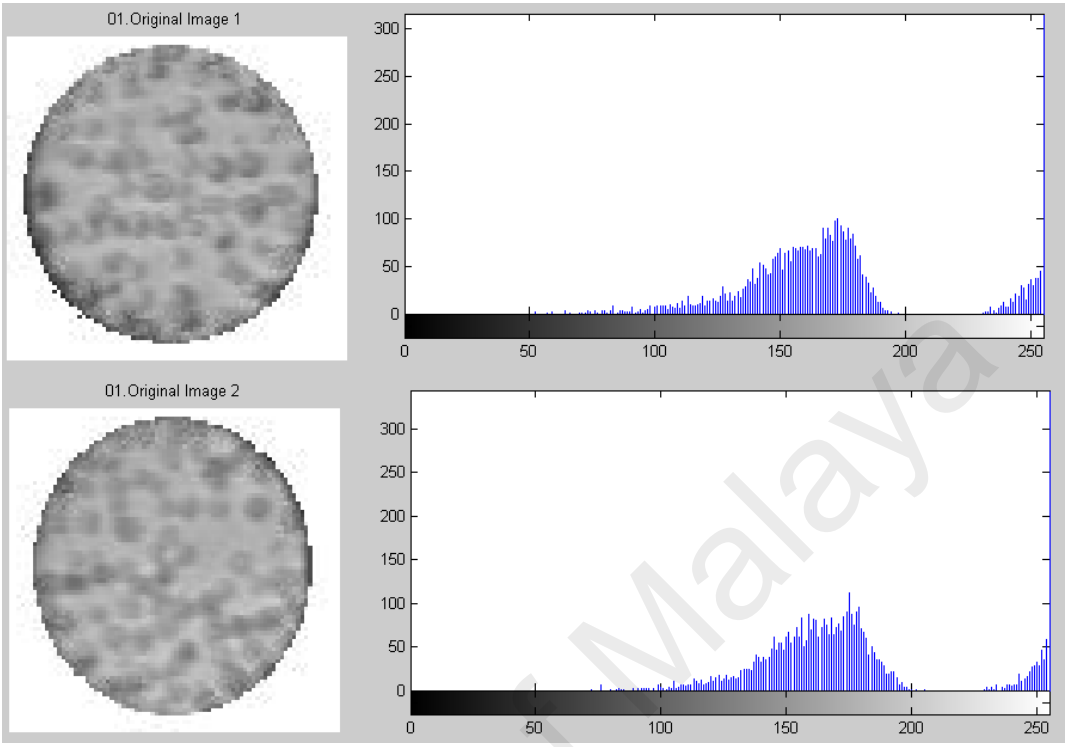


Figure 4.4: Intensity histogram DEP images for number 1 and 2

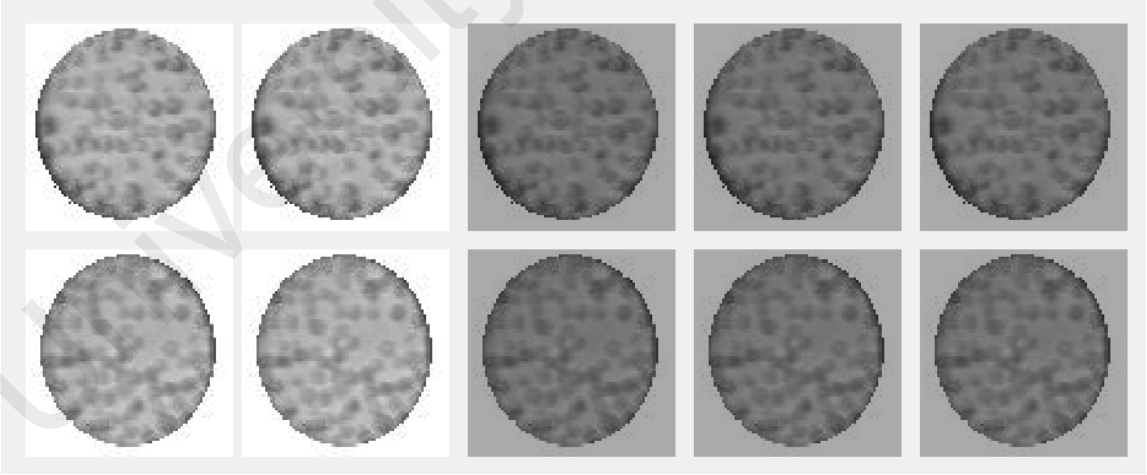


Figure 4.5: RGB of DEP images for number 1 and 2

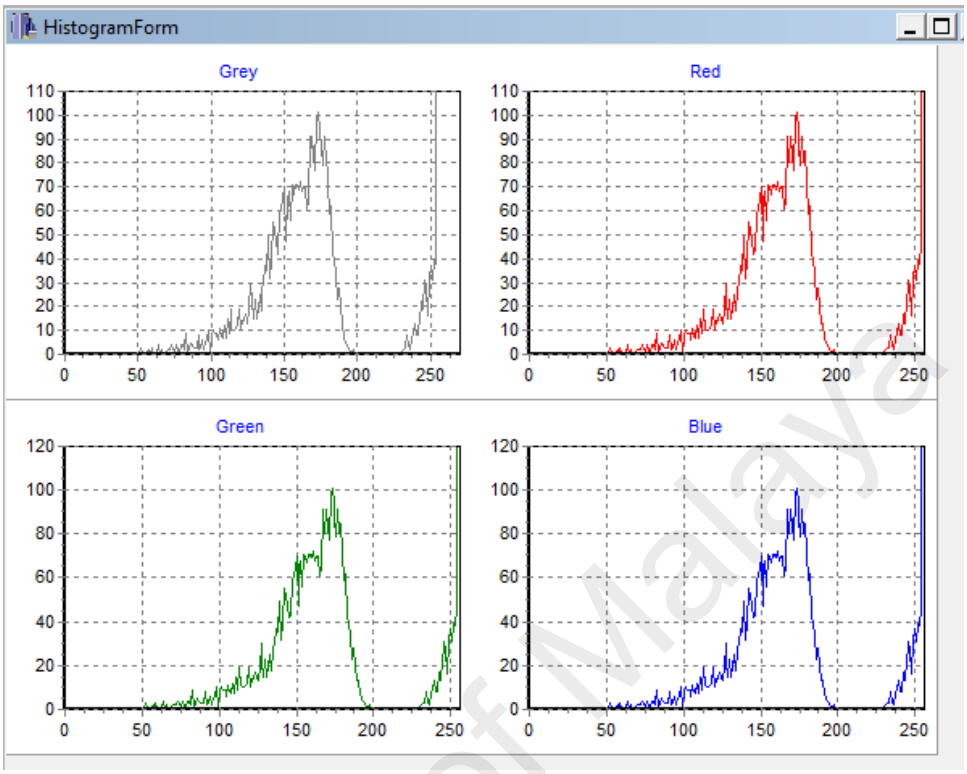


Figure 4.6: Intensity histogram based on original image, red image, green image and blue image.

In this section, the three contrast enhancement techniques have been applied on 1 and 2 images. Figure 4.4 shows the original images of number 1 and number 2 and the corresponding intensity histograms for them. Figure 4.5 represents the corresponding red, green, and blue (RGB) and RGB histograms for number 1 images in Figure 4.6.

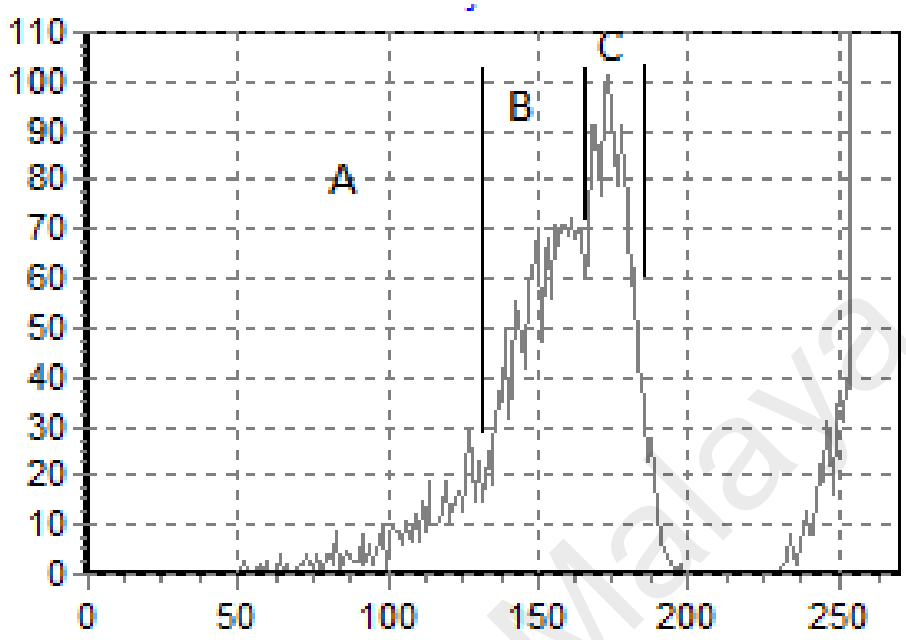


Figure 4.7: Intensity histogram that classifies A, B, and C regions.

Histogram can be used to identify whether the image are bright or dark. An image with its pixel value clustered at the right side of the histogram corresponds to bright image. While if its pixels at the left side corresponds to dark image. Based on the intensity histogram in Figure 4.7, the histogram could be divided into 3 separate regions, indicated by (A), (B) and (C).

It is important to distinguish between their areas. From that it also tries to look a different for the red, green and blue histogram. But it will be difficult to select the threshold value (TH) based on the separate red, green and blue histogram. The TH that will be used for the image enhancement processes are selected based on the intensity histogram. The criteria of 3 separate regions are still search on what is mean. But for this project, from the separation finding to easy to process image based on intensity histogram.

4.3.1 Partial Contrast Techniques

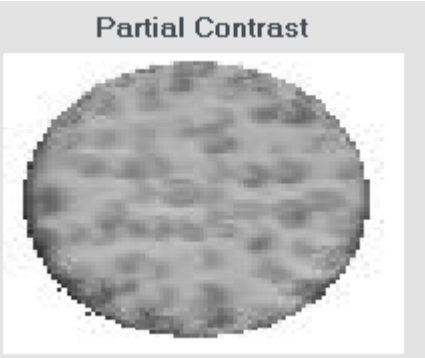


Figure 4.8: Results of images of partial contrast technique for number 1 images

The results obtained after applying the partial contrast technique on images are shown in Figure 4.8. The threshold value that have been used are different for every image. The reason is each image may have its own minimum and maximum value. This technique produces image with good contrast performance compares to the original image.

4.3.2 Bright Stretching Techniques

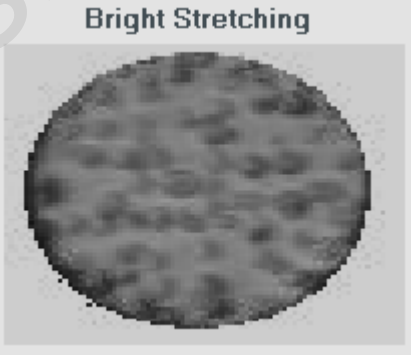


Figure 4.9: Results of images of bright stretching technique for number 1 image.

The results obtained after applying the technique on image are shown in Figure 4.9

4.3.3 Dark Stretching Techniques

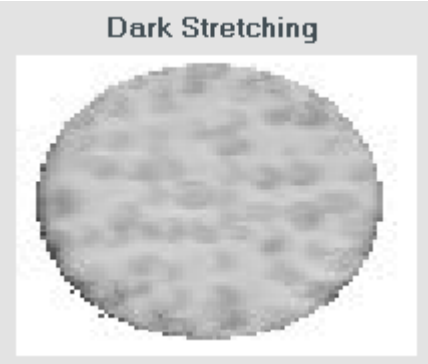


Figure 4.10: Results of images of dark stretching technique for number 1 image.

The results obtained after applying the technique on image are shown in Figure 4.10

4.3.4 Linear Contrast Techniques

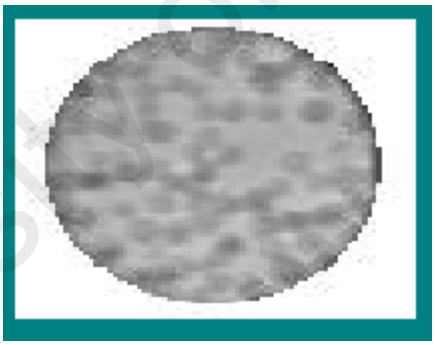


Figure 4.11: Results of images of linear Contrast technique for number 1 image

The result obtained after applying the technique an image is shown in Figure 4.11.

4.4 Image Segmentation Technique

Segmentation of an image refers to the separation of regions with similar characteristics. Image segmentation is the most important step in image analysis as it will directly affect the post-processing (Aus *et al.*, 1987).Some of these have been combined in order to segment the region of interest in medical images. The result for image segmentation technique as are below.

4.4.1. K-Mean

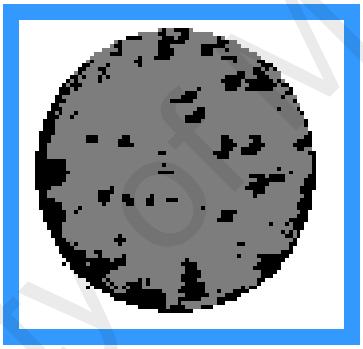


Figure 4.12: Results of images K-Mean

The result obtained after applying the technique an image is shown in Figure 4.12.

4.4.2. MCM

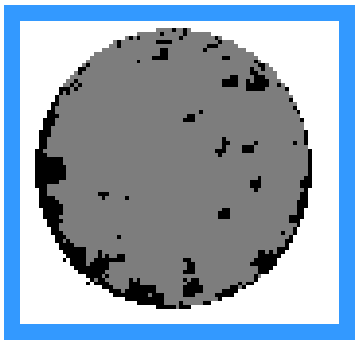


Figure 4.13: Results of images MCM

The result obtained after applying the technique an image is shown in Figure 4.13.

4.4.3. FCM

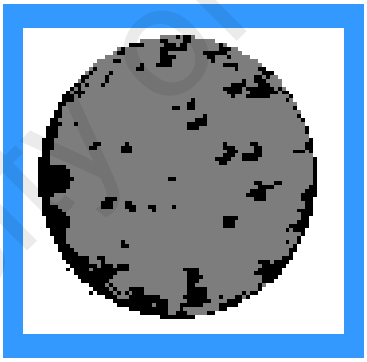


Figure 4.14: Results of images FCM

The result obtained after applying the technique an image is shown in Figure 4.14.

4.5 The proposed development of an improved image analysis for optical-based AC electrokinetics data system

This process has developed of an improved image analysis for optical-based AC Electrokinetics data system based on images. The proposed system consists of image procesiong such as image enhancement and image segmentation.

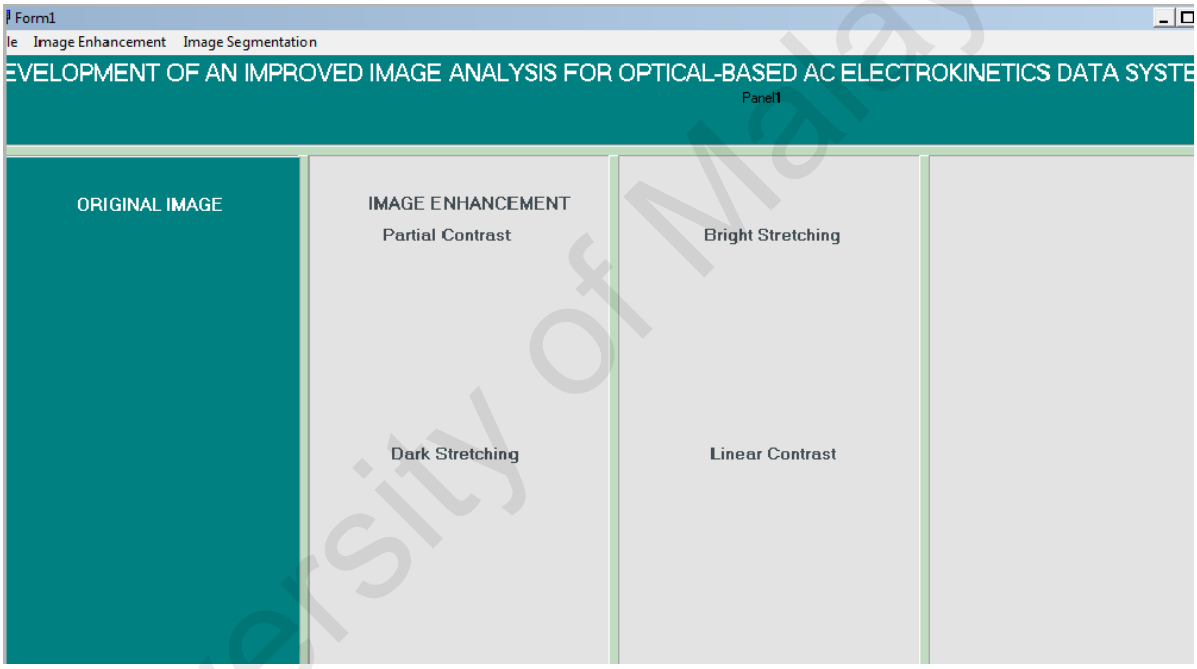


Figure 4.15(a): The main menu of development of an improved image analyisi for optical-based AC Electrokinetics data system for images.

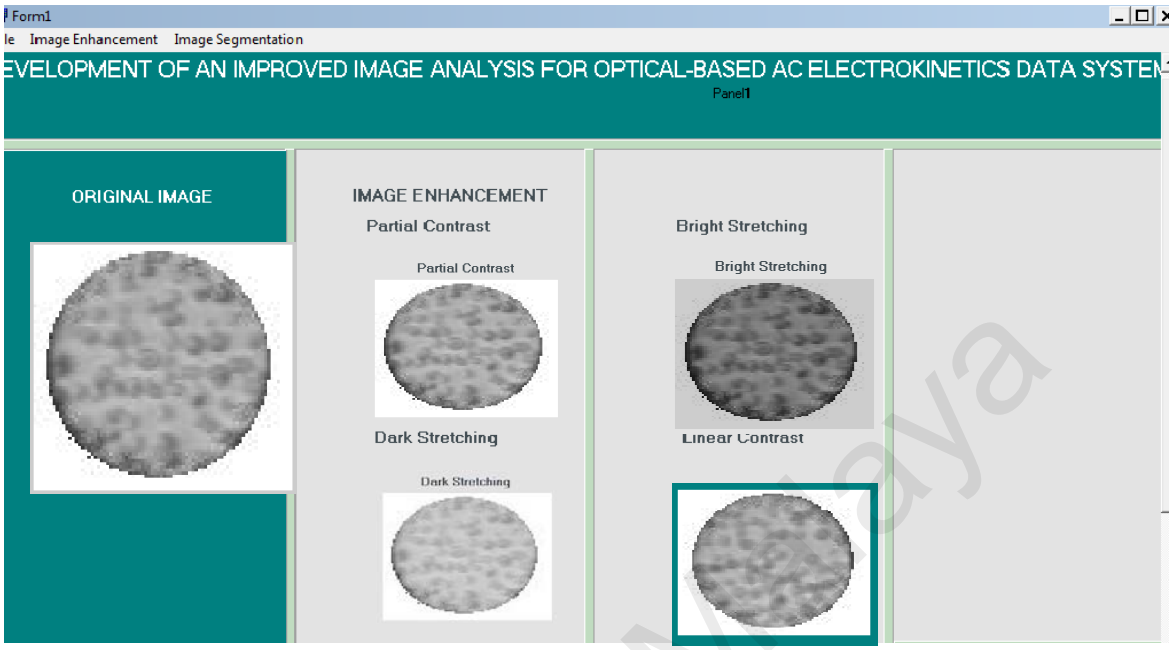


Figure 4.15(b): The image enhancement facility

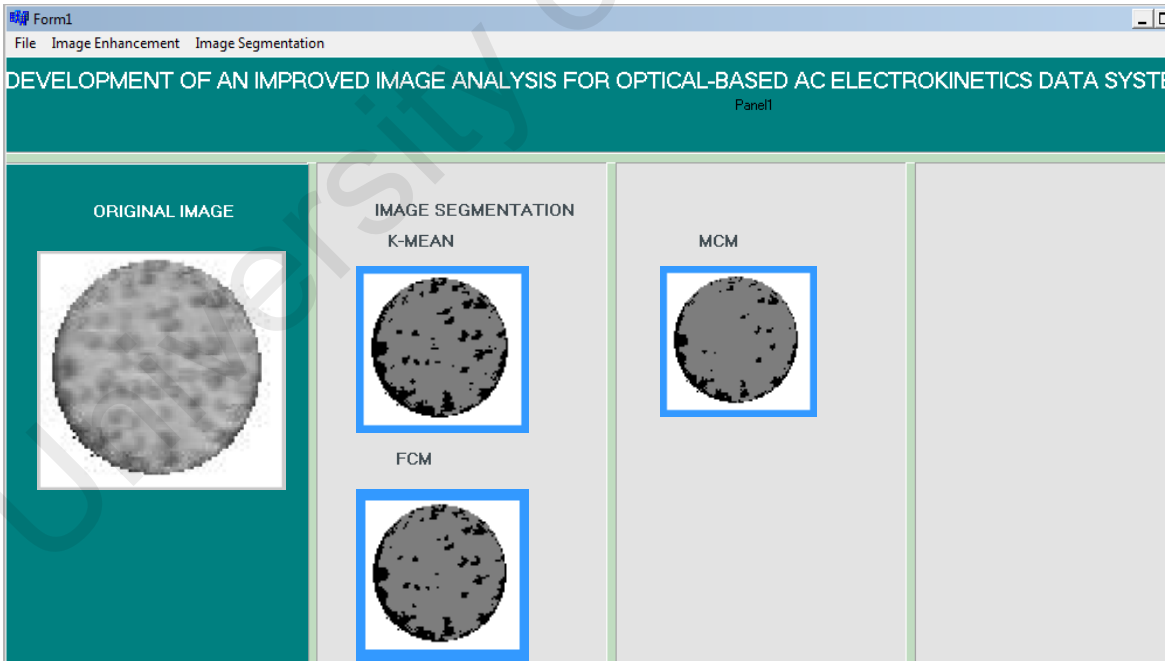


Figure 4.15(c): The image segmentation facility

Figure 4.15(a) represents the main menu of development of an improved image analyisi for optical-based AC Electrokinetics data system for images. Figure 4.15(b) represents the image enhancement facility which consists four contrast enhancement techniques namely partial contrast, bright stretching, dark stretching and linear contrast techniques. These techniques can be used to improve the image quality and enhance the image 1. Figure 4.15(c). The image segmentation facility for obtaining a fully segmented image. Based on these facilities, the proposed development of an improved image analysis for optical-based AC Electrokinetics data system could become a useful tool in medical field and is expected to help classify and investigate the process of image processing.

4.6 Conclusion

In this project, there are four contrast enhancement techniques that have been applied on image namely, partial contrast, bright stretching, dark stretching, and linear contrast techniques. There are three segmentation methods that have been proposed. The method is segmentation based on k-mean, MCM and FCM. The proposed development of an improved image analysis for optical-based AC Electrokinetics data system is capable to classify image processing easily.

CHAPTER 5

CONCLUSION

5.1 Summary

In this project, the development of an improved image analysis for optical-based AC Electrokinetics data system for image which consists of image processing for the purpose of system process bias been developed.

In this project, there are four contrast enhancement techniques that have been applied on images which are partial contrast, bright stretching, dark stretching and linear contrast techniques. The results produced by the proposed techniques are acceptable in terms of visual quality. Three segmentation techniques namely k-mean, MCM, and FCM have been to its good segmentation performance in obtaining a fully segmented of images hopefully.

The proposed system of the development of an improved image analysis for optical-based AC Electrokinetics data is capable to classify image processing with good performances.

5.2 Limitation

There are limitations of this project such as time. For process of to do programming must have more time. The getting all of result maybe will to be better

if have more time. The comparison for the data to choose a better image analysis cannot done to do because the limited time.

5.3 Future Recommendations

For future work, there are a few recommendations that can be adopted in order to improve the current work. Most importantly, the analysis has much process and a good research to explore more. It can do a lot of thing from the data collecting.

For example, we maybe can do as below:

1) Test region A, B, and C for PC, BS, DS and LC for image

	PC	BS	DS	LC
A				
B				
C				

Figure 5.1: Example of result

2) From that we are can select the good result of image to do image segmentation like K-Mean, MCM and FCM and then we can do the Sensitivity and Specificity to prove them.

The sensitivity and specificity are defined as below (Demir & Yener, 2005):

$$\text{Sensitivity} = \frac{\text{Number of TP}}{\text{Number of TP + Number of FN}} \times 100 \qquad (5.1)$$

$$\text{Specificity} = \frac{\text{Number of TN}}{\text{Number of TN + Number of FP}} \times 100$$

Where TP, TN, FP and FN are the true positive, true negative, false positive and false negative, respectively (Demir & Yener, 2005).

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ABSTRACT

The availability of dielectrophoresis (DEP) method which apart of AC Electrokinetics for cell separation and manipulation are currently becoming highly purposeful due to its function variation and becoming possible. Commonly used in cancer cell detection and drug screening assessment, DEP are able to manipulate polarisable particles with the application of non-uniform electric considering the fact that movement will be induced onto the polarisable particles when placed in the electric fields. The application of DEP are used to be restricted in research for academic prospects only, rather than in manufacturing applications due to the image analyzing processes involved. Thus, this project aims to develop of an improved image analysis for optical based AC Electrokinetics data, which consists of image processing for purpose of development process. In order to perform a process, various image processing techniques have been applied on images that have been given from AC Electrokinetics data. The images are processed with various image processing techniques such as contrast enhancement and image segmentation. There are four contrast enhancement techniques. After that, the image is segmented by applying the image segmentation. The combination between contrast enhancement and image segmentation hopefully will giving a good result. Overall, the development of an image analysis for optical-based AC Electrokinetics data that has been developed. The development that provides an efficient alternative in analyzing the images. .

ABSTRAK

Ketersediaan kaedah dielectrophoresis (DEP) yang selain syarikat AC elektrokenetika pemisahan sel dan manipulasi sedang menjadi sangat bertujuan disebabkan oleh perubahan fungsi dan menjadi yang mungkin. Biasa digunakan dalam mengesan sel kanser dan penilaian saringan dadah, DEP mampu untuk memanipulasi partikel polarisable dengan permohonan bukan-seragam elektrik menimbangkan hakikat bahawa pergerakan akan didorong ke zarah polarisable apabila diletakkan dalam medan elektrik. Permohonan DEP digunakan dihadkan dalam penyelidikan bagi prospek akademik sahaja, bukannya dalam aplikasi pembuatan kerana imej menganalisis proses yang terlibat. Oleh itu, projek ini bertujuan untuk membangunkan analisis imej yang lebih baik untuk AC berasaskan optik elektrokenetika data, yang terdiri daripada pemprosesan imej bagi tujuan proses pembangunan. Yang diperlukan untuk melaksanakan sesuatu proses, pelbagai teknik pemprosesan imej telah digunakan pada imej-imej yang telah diberikan dari AC elektrokenetika data. Imej-imej yang diproses dengan pelbagai teknik pemprosesan imej seperti peningkatan kontras dan segmentasi imej. Terdapat empat teknik penambahbaikan Sebaliknya. Selepas itu, imej dibahagikan dengan menggunakan segmentasi imej. Gabungan antara peningkatan kontras dan segmentasi imej itu diharap akan memberikan hasil yang baik. Secara keseluruhannya, pembangunan analisis imej bagi optik berasaskan AC elektrokenetika data yang telah dibangunkan. Pembangunan yang menyediakan satu alternatif yang cekap dalam menganalisis imej.

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **ADILAH BINTI HASHIM**

(I.C/Passport No: **810105-02-5154**)

Registration/Matric No: **KGL 100031**

Name of Degree: **Master of Engineering (Biomedical)**

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

DEVELOPMENT OF AN IMPROVED IMAGE ANALYSIS FOR OPTICAL-BASED AC ELECTROKINETICS DATA

Field of Study: **DIELECTROPHORESIS**

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ACKNOWLEDGEMENT

In the name of God the Most Gracious and Most Merciful. First of all, I would like to thank God to always keep me in His Guidance throughout this life. Without all the strength given to me, this project might not have been completed.

I would like to express my deepest appreciation to Dr Nahrizul Adib Kadri for accepting me as his project student and for his guidance, support, great ideas and suggestion. He also helped me to recognize my project to helo me in completion of this project successfully.

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Adilah Hashim, May 2012

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DEP	-	Dielectrophoresis
AC	-	Alternating current

CHAPTER 1

INTRODUCTION

1.1 Overview

Technological innovation has yielded truly remarkable advances in health care during the last three decades. In just the last several years, breakthroughs in biotechnology, biomaterials, surgical techniques, and computer technology have helped to improve health care delivery and patient outcomes. The instruments that have been used in medical fields have helped experts to solve medical problems. Technological developments have given a new inspiration to researchers who have been struggling to obtain new knowledge to apply into new medical invention.

Based on these facts, the requirement for fast analysis of cancer images is of paramount importance in the healthcare industry. Cancer can be cured if it is detected and treated at the early stage which the procedure for early detection.

Dielectrophoresis is the phenomena where interactions between polarized particles with non-uniform electric fields exert motion onto the particles. It can be used and adopted to manipulate, separate, and analyze the cellular and viral particles responses (Hubner *et al.*, 2007; Hoettges *et al.*, 2008; Pethig, 2010). The induced motion and the manipulation of particles which have been polarized after being suspended

in a non-uniform alternating electric field can cause repulsion or attraction of cells with regards to the electrodes.DEP data have image which can include the image to analyzing with image processing. It can process for cancer data.

DEP has become recognizable since its introduction which defines DEP as “the motion of suspension particles relative to that of the solvent resulting from polarization forces produced by an inhomogeneous electric field” (Pohl, 1951). One of the advantages of DEP is that it does not need chemical markers, biochemical labels or bioengineered tags and at the same time does not exert any contact to any surfaces of cells making this technique preferable compared to chemical method such as fluorescent labels which are highly invasive and cell destructive (Hoettges *et al.*, 2008; Pethig, 2010).

There are a few major contributions of DEP-based technique especially in distinguishing dead and viable cells by separating and sorting them according to its conductivity of the cytoplasm, surface charge and the capacitance of the membrane. This is proven by a study done by Gascoyne *et al*. (1992) which demonstrate the separation of cancer cells on an electrode array due to the differences in terms of frequency shown between normal, leukemic and differentiation-induced leukemic mouse erythrocytes (Gascoyne *et al.*, 1992). This is further being supported by Becker *et al*. (1994 & 1995) who found and exploited the differences in the dielectric properties of metastatic human breast cancer cell from those of erythrocytes and T-lymphocytes resulting in the separation of breast cancer cells from the normal blood cells (Becker *et al.*, 1995; Becker *et al.*, 1994).

The current study has proposed an alternative solution to this problem through cost-effective and efficient software based application in recognizing and analyzing cancer cells based on images samples. This research aims to provide the development system that consists of image processing and feature extraction facilities. With the proposed intelligent screening system for acute leukaemia, it would assist patients and their family to plan the treatment option and budget finances.

1.2 Objective

The main objective of this project is the development of an improved image analysis for optical-based AC Electrokinetics data that can be used for enhance and segment of image. This main objective also covers the following sub-objectives:

a) To propose a contrast enhancement procedure that can be used for obtaining a fully enhance images.
b) To propose an image segmentation procedure that can be used for obtaining a fully segmented image.
c) To develop of an improved image analysis for optical-based AC Electrokinetics data system using the proposed techniques.

1.3 Scope the Study

Medical images are primarily visual in nature. The major strength in the application of computers to medical imaging lies in the potential use of image processing and computer vision techniques for quantitative or objects analysis.

The use of images particularly in field of medical image processing is still limited. Thus, this research aims to develop of an improved image analysis for optical-based AC electrokinetics data system for images that consists of image processing facilities.

1.4 Organization of Project

This thesis consists of five chapters. These chapters discuss about the introduction, literature review, and development of an improved image analysis for optical-based AC electrokinetics, results and discussions, and conclusions with recommendations for future study.

Chapter 1 discusses briefly about introduction of the project. This chapter also presents the objectives, scope of the study and finally the organization of the project.

Chapter 2 presents the literature review which consists of the introduction, the DEP, the image processing and the development of an improved image analysis for optical-based AC Electrokinetics data system.

Chapter 3 describes the development of an improved image analysis for optical-

based AC electrokinetics data. This chapter consists of four main sections. The first section introduces the overview of the proposed develop system followed by the second section which describes the image acquisition. Section 3 describes the procedures for applying various image processing techniques such as contrast enhancement and image segmentation on image. Finally, the last section describes the data sample and evaluation methods of the enhancement and segmentation system.

Chapter 4 deals with the experimental results, provides the analysis for the finding and continue with the discussion. This chapter consists of five main sections. Section 1 presents the introduction of the chapter followed by the Section 2 which about data acquisition and image selected. Section 3 covers the results for contrast enhancement technique. Section 4 covers the results obtained the image segmentation. Finally, the overviews of the proposed development of an improved image analysis for optical-based AC Electrokinetics data system are described in the last section.

Chapter 5 put forwards the conclusion made based on the present study. The conclusions and contributions of the project are written based on the findings reported in the Chapter 4. Recommendations for future studies are presented due to their significance with the current research.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter discuss on two main topics that are Dielectrophoresis (DEP) and image processing.

2.2 Dielectrophoresis (DEP)

Dielectrophoresis (DEP), along with electrorotation (ROT) (Hughes, 2002; Lee *et al.*, 2004; Gascoyne *et al.*, 2006) is frequently grouped as a member of AC- electrokinetic technique which manipulates the mobility of cells in an electric field with non-uniformity and time-dependent properties in order to explore the electrophysiological properties of cells (Hughes, 2002; Jones *et al.*, 2003; Jones, 2003; Jones, 1995; Pethig *et al.*, 1997; Pohl *et al.*, 1981). Important information can be extracted from the electrophysiological properties especially at the cellular level. DEP method is known to be inevitably nondestructive and noninvasive, while being a label- free cell characterization technique when compared to the chemical methods which results in invasion of cellular trafficking or compared to electrical measurement method as such patch clamp which is highly destructive to cells (Labeed *et al.*, 2003).

The idea of dielectrophoresis was first discovered by Hatfield when he tried to separate the valuable mineral cassiterite from a large excess of quartz material. Hatfield realized the problem in electromagnetic separation was the generation of strong non-uniform electrical field even approaching the weakest electromagnetic field without caused any of dielectric breakdowns. Then, this idea was continued by Pohl who applied this theory to solve problem regarding of removing carbon-black filler from polyvinyl chloride samples. Then, he proceed his efforts in the development of methods and theories for dielectrophoresis characterization and separation of biological cells.

Over past 10 years, there are more than thousands research have been conducted in dielectrophoresis field (Pethig, 2010). This area is expected to face a huge growth due to its ability to integrate with advanced technologies like the used of thin film techniques or CMOS technology in fabricating the electrode. Besides, advanced in development of sophisticated electronic design has enhanced dielectrophoresis system by including some optical sensor or even the used of microcontroller for monitoring and manipulating purpose respectively. The existences of new materials like silicone polymer, silica glass or even indium tin oxide have given more choices for researchers in development of DEP system.

In general, dielectrophoresis (DEP) is a promising method for the separation and classification of biological cells in a miniaturized format. This technology allows cells to be manipulated electronically while suspended in a micro fluidic channel. Several dielectrophoretic configurations have been designed and fabricated using micro-electro-

mechanical-systems (MEMs).

If in the early age, the application of dielectrophoresis was more towards industrial application such as mineral sorting, assembling of micro component and manipulating fluid droplets as mentioned before, now the focus is diverge into biomedical application and it is expecting keep growing in future.

2.2.1 Theory of DEP

According to Pohl in 1951, dielectropherisis can be defined as "the motion of suspensoid particles relative to that of the solvent resulting from polarization forces produced by an inhomogeneous electric field". Polarization forces produced known as DEP force, which play an important role for manipulating and separating of target cells.

Particles can either moved toward or away from higher electrical gradient area. There are two factors that influenced the effectiveness of particle movement in DEP which are polarisability of the particle and its medium. These factors are differing from the electrophoresis phenomenon where mobility of ion depends on their total charge, size and shape.

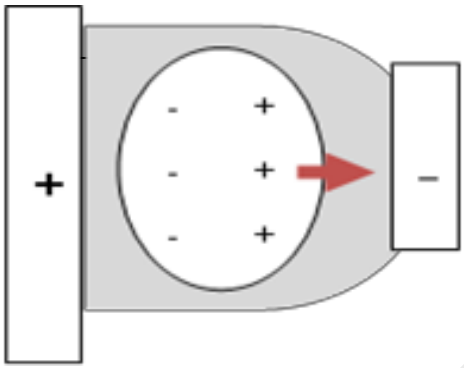


Figure 2.1: A schematic diagram of positive DEP (pDEP)

Based on Figure 2.1 above, positive DEP (pDEP) occurs when the particles are more polarisable than the surrounding medium, the induced dipoles align with the electric field and the particles move towards regions of higher field. Meanwhile, negative DEP (nDEP) occurs the particles are less polarisable than the surrounding medium, the induced dipoles will align against the applied field, causing the particles to move to regions of lower field strength. The DEP force can be described by the following question below (Hughes, 2002);

$$F_{DEP} = 2\pi r^3 \varepsilon_m \, Re[K(\omega)] \nabla E^2 \qquad (2.1)$$

where r is the cell radius, ε_m is the permittivity of the medium surrounding the cell, K(ω) is the complex Clausius- Mossotti factor, ∇ is the Del vector operator and E is the electric field strength in route means square (RMS) value.

Clausius- Mossotti factor is used to analyze the effective polarisability of the particles. According to Jones (1995), when Re [K (ω)] > 0 pDEP phenomenon will occur meanwhile when Re [K (ω)] < 0, nDEP phenomenon will occur. Furthermore, normally for spherical particles, the interval range of Re [K (ω)] is between −0.5 and 1. Clausius- Mossotti factor can be defined as equation below (Hughes, 2002);

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* - 2\varepsilon_m^*} \tag{2.2}$$

where ε_p^* and ε_m^* are the complex permittivity of the particles and medium respectively. Furthermore, ε^*can be determined using the equation below (Kadri, 2010);

$$\varepsilon^* = \varepsilon - \frac{j\sigma}{\omega} \tag{2.3}$$

where σ is conductivity, ε is permittivity and ω is the angular frequency of the applied AC electric field.

Frequency applied by AC input will affect the polarisability of the particles (Pethig, 2010). By looking at Equation 2.3, if the conductivity of particles predicted to be increased by a given frequency, the complex permittivity will be decrease as well as Clausius-Mossotti factors. This frequency exploitation strategy has played an important role in particles

separation where it linked to the different of polarisability of particle in the population.

Other than that, frequency has also play an important role in electromagnetic field produced. This condition has been mentioned by Muller *et al.* (1996) where the strength of electric field increased at higher frequency range up to MHz.

In the F_{DEP} Equation from (Eq. 1), the existence of Clausius-Mossotti factor, which is a frequency-dependent element, ensures that the F_{DEP} also varies with the frequency of the applied electric field. The factor relies upon the strength of the relative polarisability between the particle and the surrounding medium. If the factor is positive, it reflects that the particle is more polarized than the medium, thus effective motion will results in moving towards the area with the highest gradient of electric field. Opposing to this, when a negative DEP befall and the medium is more polarisable than the particle, the effective motion will result in repelling of particle towards the lower gradient of electric field. This will happen when the Clausius-Mossotti factor brings out a negative value.

In order to enable the dielectric properties to be directly calculated and correlated in a useful manner, the above analytical expressions are needed to be expanded. There are complexities faced while trying to do so even though the analytical expressions may have explained thoroughly the DEP behaviour of spheres in a medium. By performing the best-fit numerical analysis, an estimation of electrophysiological properties can be made to correlate the relevant cellular electrical parameters with DEP behaviour.

The estimation method has also shown to be very useful in characterizing multiple cell populations within a heterogeneous cell sample (Chin *et al.*, 2006; Huang *et al.*, 1996; Broche *et al.*, 2005). A DEP spectrum has proven to be very beneficial in the extraction of electrophysiological properties because it also provides the estimation of the state and environment surrounding the cells.

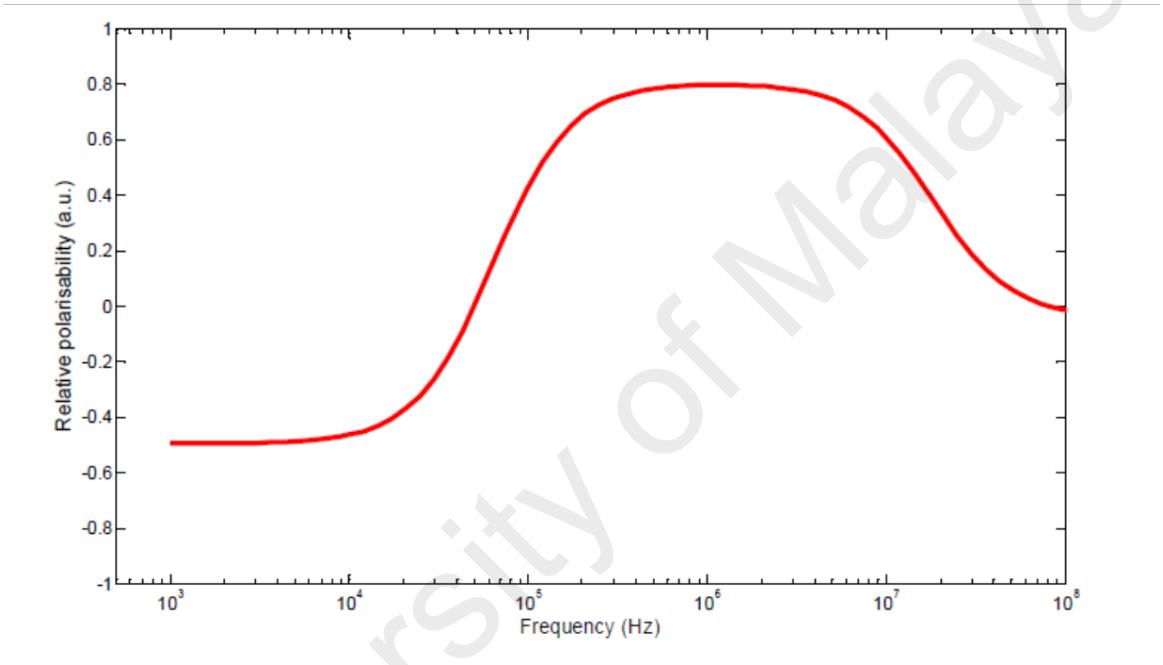


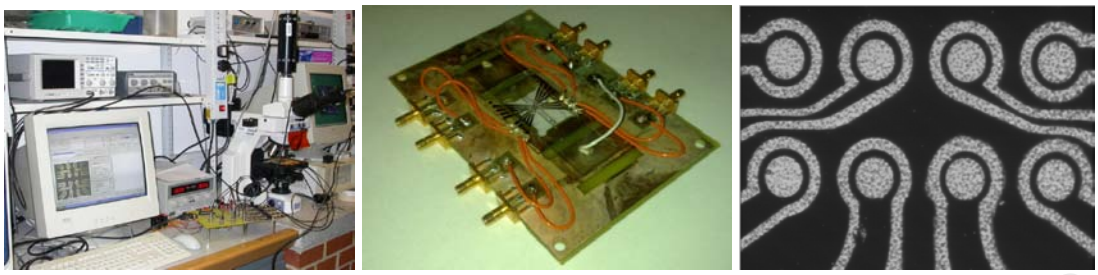
Figure 2.2: A typical shape of a DEP spectrum

Figure above shows the typical shape of the DEP spectrum while it can be shifted both ways, either to the right or to the left, depending on a particular influence that an external reagent has on the Clausius-Mossotti factor. This is due to the changes in components of Eq. 2 which consist of conductivity, permittivity, and angular frequency. A close example of this effect would be when an ionophore molecules were attached to the cellular membrane, higher level of ions were allowed to pass through the membrane

which ultimately disturb the conductivity thus disturbed the ionic distribution. This will in turn disturb the Clausius-Mossotti factor value and result in a frequency shift of the DEP spectra.

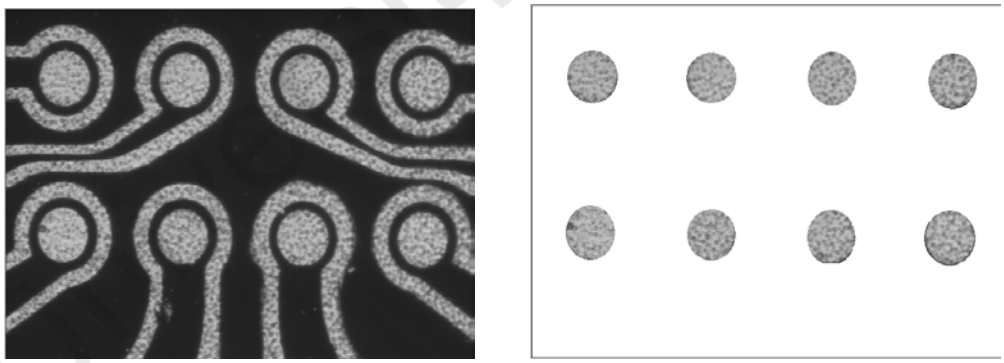
2.2.2 DEP data

The data was collected from the DEP experiment. The experiment was involved doing the hardware and the software. The hardware was created to the sample will operation when the sample was inside in the hardware, from that the circuit component also involve. The circuit had to connect with power supply. When the experiment was doing, computer will connect with digital microscope. The digital microscope is which have a camera also. They are much related connection. That functions is to looking the phenomena DEP which will display into computer and the software from the camera can to record the phenomena. The phenomena recorded will display in image data. Figure below is about the experiment and the image display. In this project the image of DEP data are already given, so that the doing experiment of this DEP is not involved. All image data have taken from Kadri,N.A, 2010.



(a) (b) (c)

Figure 2.3(a) the experimental set-up is shown containing the developed instrumentation and image acquisition device connected to a PC. Figure 2.3 (b) the hardware which is Electrode cartridge mounted with DEP-Dot system for up to 8 signals applied in parallel; Figure 2.3(c) the image data display after record which is DEP-Dot (some μm diameter) surface of a 4-by-2 microarray with interdot ground plane, in addition to the ITO counter electrode oppositely facing the microarray.(Adapted from Henry et.all, 2009).



(a) Image from DEP data (b) Image after cropping and selected

Figure 2.4: (a) Image from DEP data and (b) Image after cropping and selected. (Adapted from Kadri, 2009)

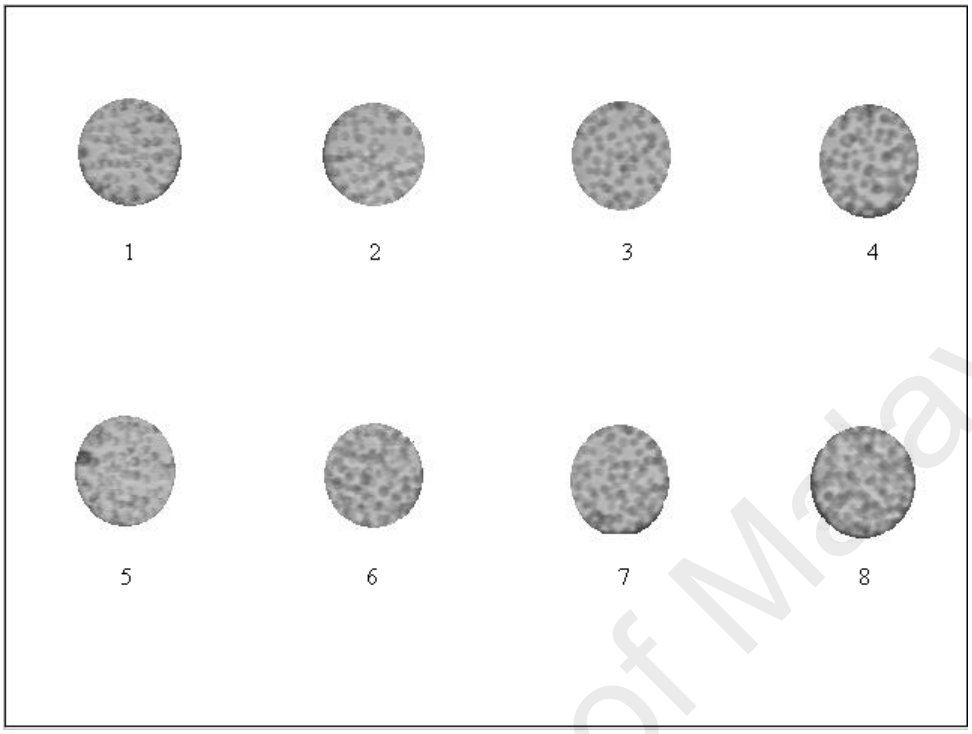


Figure 2.5: The number to know how to read data from experiment the image of DEP

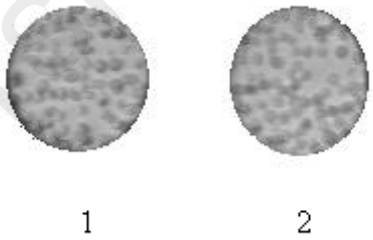


Figure 2.6: The selected image that want to process

Figure 2.3 is about overall experiment. Figure 2.4 (a) is about image from DEP data and Figure 2.4 (b) Image after cropping and selected. Figure 2.5 is about the number to

know how to read data from experiment the image of DEP. Lastly, Figure 2.6 is about the selected image that want to process.

2.3 Image Processing

An image is a representation of a real scene, either in black and white or colour, and either in print form or digital form. Digital images are represented by a number of rows and columns of cells called pixels. Each pixel contains the colour or gray level information for its respective spatial position in the image (Jain, Kasturi, & Schunck, 1995). For monochrome image, each pixel is represented by a gray level value. Meanwhile, each pixel in colour image is represented by red, green and blue values. Digital images can be created through the process of digitization. Digitization is the process of transforming image, text or sound from analog media into digital data (Jain, Kasturi, & Schunck, 1995). This process is important because computer can only process the digital data. The field of digital image processing is the study of algorithms for transformation of digital image.

Digital image processing is a well-developed field. This will give a positive impact for other application of digital image processing such as in medical imaging, satellite imaging, graphics and animations, robotics and photo enhancement. Image processing usually refers to digital image processing. As normally be defined, image processing is about the conversion of one image into another. In terms of medical field application, image processing is highly applicable for MRI SCAN (Magnetic Resonance Imaging) and CT SCAN (Computer Tomography).

2.3.1 Contrast Enhancement for Images

Contrast enhancement technique is widely used to increase the visual image quality. In general, there are two requirements to be fulfilled for colour image enhancement (Chatterji & Murthy, 1997). The first one is to keep the colour structure of the original image. The second requirement is to present as much information as the original. Contrast of the image is one of the factors that may influence the accuracy of interpretation by haematologists. Exposure of the microscope also influences the quality of captured images. Overexposure setting will lead in producing bright image, while underexposure setting will produce a dark image. Due to the low quality of the image, it will be hard to visualize and analyze the blood cell morphological features on the system for further image processing.

Thus, contrast enhancement at the pre-processing stage becomes the most important process for a successful feature extraction and diagnosis of image. The resulting enhanced medical images will provide clearer and cleaner images for better and easier disease process by doctor. Among the contrast enhancement techniques that have been developed for improving the image quality are partial contrast, bright stretching dark stretching and linear contrast techniques.

2.3.1.1 Partial Contrast Technique

Partial contrast is a linear mapping function that is used to increase the contrast level and brightness level of the image. The technique is based on the original brightness and

contrast level of the image to be adjusted. First, the system will find the range of where the majority input pixels converge for each colour space. Since the input image is in RGB colour space, so it is necessary to find the pixels range between the red, green and blue intensities. Then, the average of these three colour space will be calculated to obtain the upper and lower colour values by using the following formula in Equation 2.4 And Equation 2.5.(Weeks, 1996):

$$maxTH = (maxRed + maxGreen + maxBlue)/3 \quad (2.4)$$

$$minTH = (min\ Red + minGreen + min\ Blue)/3 \quad (2.5)$$

maxRed, *maxGreen* and *maxBlue* are the maximum colour level while *minRed*, *minGreen* and *minBlue* are the minimum colour level for each colour palette respectively. *maxTH* and *minTH* are the average number of maximum and minimum RGB colour space. *maxTH* and *minTH* will be used as the desired colour ranges for all three colour palette. Next is to start with the mapping function is given in Equation 2.6 (Weeks, 1996).

$$P_k = \frac{(max - min)}{(f_{max} - f_{min})}\left(q_k - f_{min}\right) + min \quad (2.6)$$

Where,

P_K : Colour level of the output pixel

q_K : Colour level of the input pixel

$f\ max$: Maximum colour level values in the input image

$f\ min$: Minimum colour level values in the input image

min : Desired minimum colour levels in the output image

max : Desired maximum colour levels in the output image

For partial contrast, the function in Equation 2.7 used for the pixels transformation which is based on the concept of linear mapping function shown in Equation 2.6.

$$out(x,y)=\begin{cases}\dfrac{in(x,y)}{\min TH}*N\min TH & for \quad in(x,y)>\min TH \\ \left[\dfrac{(NMaxTH-NMinTH)}{\max TH-\min TH}*(in(x,y)-f\min)\right]+\min & for \quad \min TH<in(x,y)<\max TH \\ \dfrac{in(x,y)}{\max TH}*N\max TH & for \quad in(x,y)<\max TH\end{cases} \tag{2.7}$$

Where,

In(x,y) : Colour level for the input pixel

Out(x,y) : Colour level for the output pixel

minTH : Lower threshold value

maxTH : Upper threshold value

NminTH : New lower stretching value

NmaxTH : New upper stretching value

By applying this technique, the pixel within the range of *minTH* and *maxTH* will be mapped to a new range and stretched to a wider range within *NmaxTH* and *NminTH*. The remaining pixel will experience compression. Figure 2.7 illustrates the stretching and compression process for partial contrast technique.

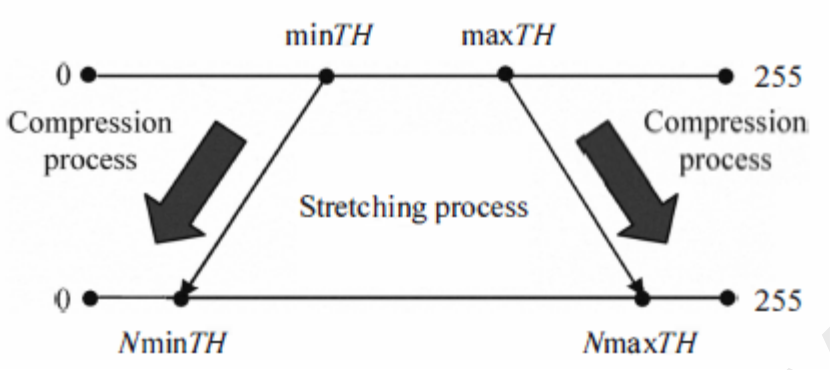


Figure 2.7: The stretching and compression process for partial contrast technique

2.3.1.2 Bright Stretching Technique

Bright stretching technique is based on linear mapping function shown in Equation 2.8. This method is normally used to enhance the brighter part of the image. For the bright stretching method, Equation 2.6 will be interpreted as follows (Weeks, 1996):

$$out(x,y)=\begin{cases}\dfrac{in(x,y)}{TH}*SFb & for \quad in(x,y)<TH \\ \left[\dfrac{(in(x,y)-TH)}{255-TH}*(255-SFb)\right]+SFb & for \quad in(x,y)>TH\end{cases} \tag{2.8}$$

TH and *SFB* are the threshold value and the bright stretching factor, respectively. The *SF* value should be smaller than TH. Figure 2.8 illustrates the stretching and compression process for bright stretching technique. Referring to Figure 2.8, the pixel values which are

less than threshold value will be compressed while the pixel values which are greater than the threshold value will be stretched.

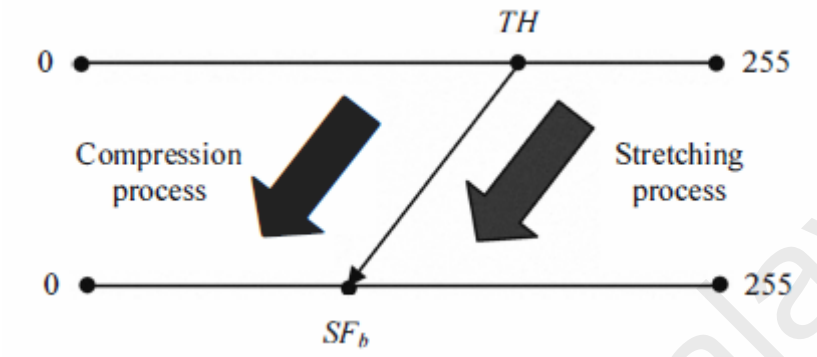


Figure 2.8: The bright stretching

2.3.1.3 Dark Stretching Technique

Dark stretching technique is a reverse process of bright stretching technique. Similar to bright stretching, dark stretching technique is also based on Equation 2.6 which involves linear mapping function. The equation for dark stretching is defined in Equation 2.9 (Weeks, 1996).

$$out(x,y) = \begin{cases} \dfrac{in(x,y) - TH}{255 - TH} * SFd & for \quad in(x,y) < TH \\ \left[\dfrac{(in(x,y) - TH)}{255 - TH} * (255 - SFd)\right] + SFd & for \quad in(x,y) > TH \end{cases} \quad ..(2.9)$$

TH and SF are the threshold value and the dark stretching factor, respectively. The SF value should be greater than TH. Figure 2.9 illustrates the stretching and compression process for dark stretching technique. Referring to Figure 2.9, the pixel values which are less than threshold value will be stretched while the pixel values which are greater than the threshold value will be compressed.

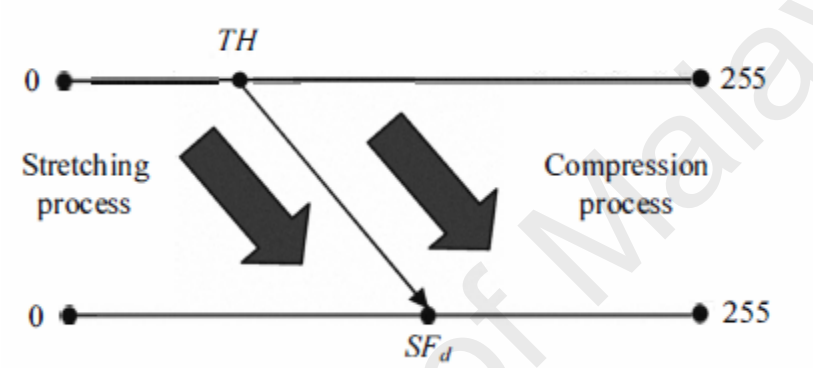


Figure 2.9: The dark stretching technique

2.3.1.4 Linear Contrast Technique

Similar to partial contrast, linear contrast technique is used to increase the contrast level and brightness level of the image. The technique is based on the original brightness and contrast level of the images to be adjusted. The equation of linear contrast algorithm is defined in Equation 2.10. (Ngah *et al*, 2000)

$$I_{output}(x,y) = 255.\frac{\left|I_{input}(x,y) - \min\right|}{(\max - \min)} \qquad (2.10)$$

Where,

I input: The original RGB value of the pixel

I output: The new RGB value of the pixel

Min : Minimum RGB value

Max : Maximum RGB value

Based on Equation 2.10, linear contrast technique will consider each range of R, G and B colour space in the image. Thus, the range of each colour space will be used for contrast stretching process to represent each range of colour. This will give each colour space a set of minimum and maximum values. By applying this algorithm, each R, G and B colour space will be distributed linearly over the whole histogram so that the dynamic range of the histogram is fulfill (0 to 255).

2.3.1.5 Applications of Contrast Enhancement in Medical Field

Several previous studies have proved that contrast enhancement technique is capable of improving the medical image quality for visualization by suppressing the unwanted noises and increasing the visibility of low contrast image features.

2.3.2 Image Segmentation for Blood Images

Segmentation of an image refers to the separation of regions with similar characteristics. Image segmentation is the most important step in image analysis as it will directly affect the post-processing (Aus *et al*., 1987).

Image segmentation techniques can be classified into for main categories: thresholding, boundary-based, region based segmentation and hybrid techniques that combine and region criteria (Rangayyan, 2005). Some of these have been combined in order to segment the region of interest in medical images.

2.3.2.1 K-Mean

In data mining, *k*-means clustering is a method of cluster analysis which aims to partition *n* observations into *k* clusters in which each observation belongs to the cluster with the nearest mean. This results into a partitioning of the data space into Voronoi cells.

The problem is computationally difficult (NP-hard), however there are efficient heuristic algorithms that are commonly employed and converge fast to a local optimum. These are usually similar to the expectation-maximization algorithm for mixtures of Gaussian distributions via an iterative refinement approach employed by both algorithms. Additionally, they both use cluster centers to model the data, however *k*-means clustering tends to find clusters of comparable

spatial extent, while the expectation-maximization mechanism allows clusters to have different shapes.

Given a set of observations (x_1, x_2, …, x_n), where each observation is a *d*-dimensional real vector, *k*-means clustering aims to partition the *n* observations into *k* sets ($k \leq n$) S = {S_1, S_2, …, S_k} so as to minimize the within-cluster sum of squares (WCSS):

$$\underset{\mathbf{S}}{\arg\min} \sum_{i=1}^{k} \sum_{\mathbf{x}_j \in S_i} \|\mathbf{x}_j - \boldsymbol{\mu}_i\|^2 \qquad (2.11)$$

where μ_i is the mean of points in S_i.

2.3.2.2 MCM

Moving k-mean is the same name for moving c-mean (MCM). MCM is application from K-mean. The MKM clustering algorithm will then be used to determine the final value of each centre that will be referred as *CN*, *CC* and *CB* for A, B, and C respectively. Based on the Euclidean distance concept, the threshold value, *βNC* and *βCB* will be calculated by using Equation 2.12.

$$\beta_{NC} = \frac{C_N + C_C}{2}$$
$$\beta_{CB} = \frac{C_C + C_B}{2} \qquad (2.12)$$

where,

β_{NC} - the threshold value to differentiate the A-B area

β_{CB} - the threshold value to differentiate the B-C area

2.3.2.3 FCM

Fuzzy c-means (FCM) is a method of clustering which allows one piece of data to belong to two or more clusters. This method (developed by Dunn in 1973 and improved by Bezdek in 1981) is frequently used in pattern recognition. It is based on minimization of the following objective function:

$$J_m = \sum_{i=1}^{N} \sum_{j=1}^{C} u_{ij}^{m} \left\| x_i - c_j \right\|^2 , \quad 1 \le m < \infty \qquad (2.13)$$

where m is any real number greater than 1, u_{ij} is the degree of membership of x_i in the cluster j, x_i is the ith of d-dimensional measured data, c_j is the d-dimension center of the cluster, and ||*|| is any norm expressing the similarity between any measured data and the center. Fuzzy partitioning is carried out through an iterative optimization of the objective function shown above, with the update of membership u_{ij} and the cluster centers c_j by:

$$u_{ij} = \frac{1}{\sum_{k=1}^{C} \left(\frac{\|x_i - c_j\|}{\|x_i - c_k\|} \right)^{\frac{2}{m-1}}}, \qquad c_j = \frac{\sum_{i=1}^{N} u_{ij}^m \cdot x_i}{\sum_{i=1}^{N} u_{ij}^m} \tag{2.14}$$

This iteration will stop when $\max_{ij} \left\{ \left| u_{ij}^{(k+1)} - u_{ij}^{(k)} \right| \right\} < \varepsilon$, where ε is a termination criterion between 0 and 1, whereas *k* are the iteration steps. This procedure converges to a local minimum or a saddle point of J_m.

2.4 Summary

Reviews of the literature that necessitates the scope of the present work are briefly reported. This chapter has been started with a brief description introduction. Discussions are continued with DEP and image processing.

CHAPTER 3

DEVELOPMENT OF AN IMPROVED IMAGE ANALYSIS FOR OPTICAL –BASED AC ELECTROKINETICS DATA

3.1 Introduction

This chapter describes various applications of image processing techniques that will be applied on DEP images for the purpose of development system process. All the information steps for each technique that will be used in this project will be described in detail.

In order to perform the screening process, the actions to be taken will include 2 main steps, starting with the image acquisition, and image processing. Here the development system for images is fully developed by using MATLAB R2010a and Borland C++ Builder 6.0 software. A personal computer which runs on a Intel Core 2 Duo CPU 3.17 GHz processor with 3.48 GB RAM (Random Access Memory) that operates in Microsoft Windows XP Professional is used for developing the system. The procedures used to develop system for image are illustrated by the flow chart in Figure 3.1.

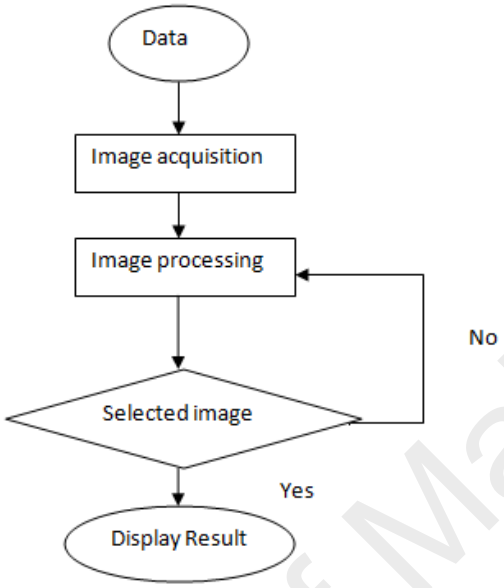
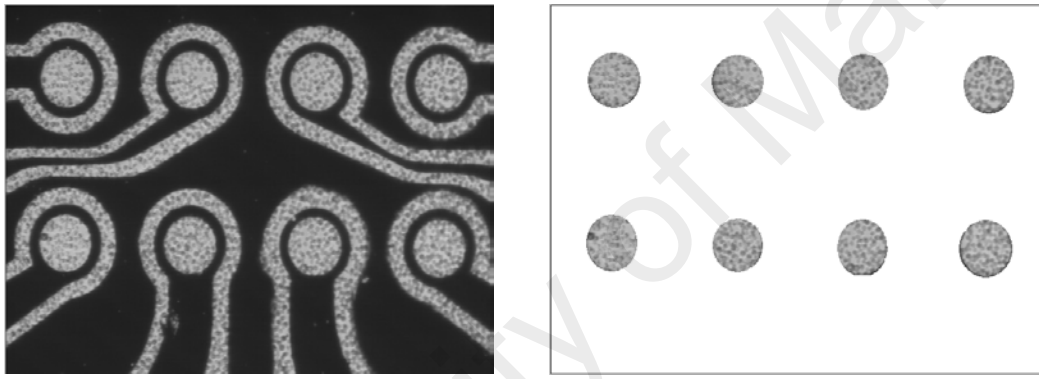


Figure 3.1: The development of an improved image analysis for optical-based AC electrokinetics data system.

During the image processing step, the blood image will be processed with various image processing techniques in order to obtain a fully segmented. The processes include image enhancement and image segmentation. Here, there are four contrast enhancement techniques that have been applied for enhancing the image namely partial contrast, bright stretching, dark stretching and linear contrast techniques. After the image has been enhanced, the image will be segmented by applying the image segmentation based on K-mean, MCM and FCM. Further details for the image acquisition and image processing are discussed in the following section.

3.2 Image Acquisition

Image acquisition is from the given data. Figure 3.2 (a) is about image from DEP data and Figure 3.2 (b) Image after cropping and selected. Figure 3.3 is about the number to know how to read data from experiment the image of DEP. Lastly, Figure 3.4 is about the selected image that want to process.



(a) Image from DEP data (b) Image after cropping and selected

Figure 3.2: (a) Image from DEP data and (b) Image after cropping and selected

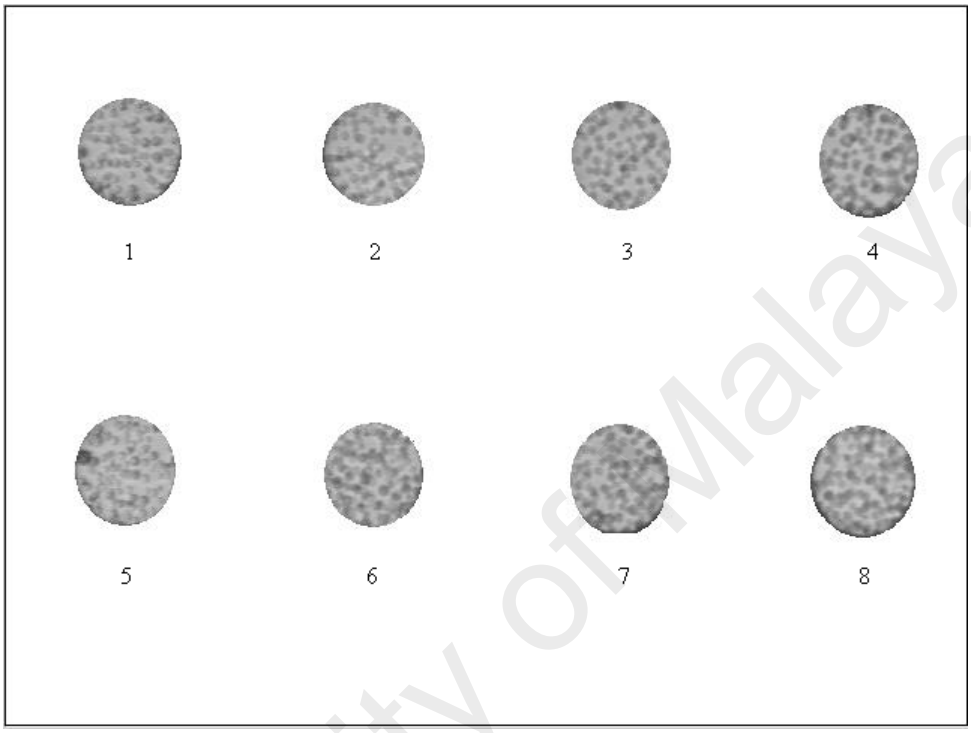


Figure 3.3: The number to know how to read data from experiment the image of DEP

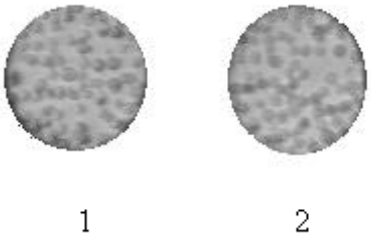


Figure 3.4: The selected image that want to process

They are have eight image, from the image the selected to process are number 1 and number 2 only. Something the different criteria have to learn between them until the image

can tell the result is normal or cancer based on several of analysis. For that in this project, the image want to process to more clear hopefully which will also related to get a good result of analysis. So, in this project, the image analysis is about image processing. The image processing is one to enhance and another one is to segment are applied. This is to still a looking before to choose a good result. In this project also done for image analysis with is looking at image from intensity histogram. Different between image number 1 and 2, is at the strength of value from the intensity value. More numbers of image from image 1 to image 8 will looking the strength of value are increase. This project is want to doing a first step before to go more. Thus, image processing is done.

3.3 Image Processing

The second of stage of performing development process is image processing. There are several image processing methods that have been proposed image recognition of some of the proposed segmentation techniques. There are several techniques will be applied during the image processing. Among the techniques are contrast enhancement, and image segmentation,. The procedures used to develop the image processing for images are illustrated by the flow chart in Figures 3.5.

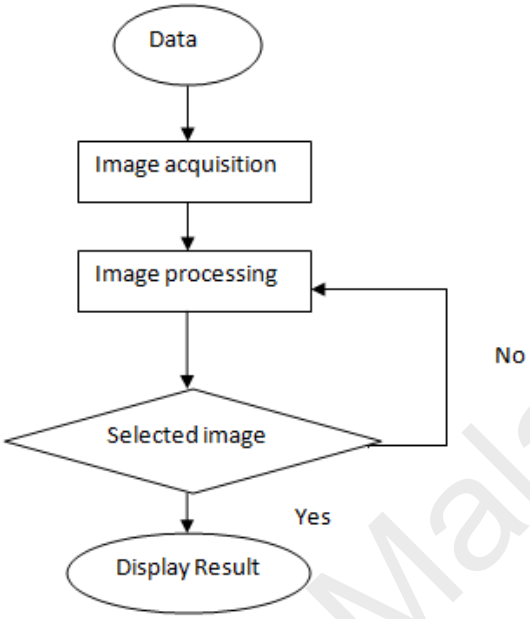


Figure 3.5: The proposed image processing steps for images.

Based on the flow chart Figure 3.5, in order to obtain the segmented image will first applied with contrast enhancement and image segmentation techniques.

3.3.1 Contrast Enhancement Techniques for Image

Contrast of the image is one of the factors that may influence the accuracy of interpretation. In this research, contrast enhancement technique has been applied on images for the two main purposes. Firstly is to improve the image quality. The images captured through the microscope may have their own weakness such as blurred or low contrast. Thus, contrast enhancement technique plays an important role in enhancing the quality and contrast of image. Secondly is to enhance the area of interest in image for easing the segmentation process. In this

research, the goal of image enhancement is to enhance the contrast of features of interest by using the contrast enhancement techniques.

Intensity histogram can be used to distinguish between the 1, 2 and 3 regions of image. Figure 3.4 represents the intensity histogram from an original image. Based on this histogram could be divided into three separate regions, indicated by (a), (b) and (c), respectively.

In this research, there are four contrast enhancement techniques that have been applied on images namely partial contrast, bright stretching, dark stretching and linear techniques. The full description about these four contrast enhancement techniques can be obtained from Chapter 2 In this project, the procedures used to develop the contrast enhancement techniques are as follows:

1. Develop the intensity histogram of the original image.
2. Select the threshold value and stretching factor by referring to the peak of 1, 2 and 3 regions of the histogram. Here, the selection of threshold value and stretching factor are based on the intensity histogram.
3. Apply the four contrast enhancement techniques by applying the threshold and stretching value on the original image.
4. Develop the intensity histogram from the resultant image in order to recognize the significance of the enhancement techniques on original image.

3.3.2 Image Segmentation

The colour image segmentation for image is performed based on K-mean, MCM, and FCM. It is the same procedure above is doing in this image segmentation.

The image segmentation techniques procedures for example:

1. The process begins with segmentation process that partitions the image cell into three main regions; A, B and C of the cell.
2. Grey level histogram of the cell image will be analyzed to obtain the initial centre of each region; *CNo*, *CCo* and *CBo*, for A, B and C respectively.
3. Each of regions has different range of grey level that start with A that has the lowest value, followed by B and C.

3.4 Development of an improved image analysis for optical-based AC electrokinetics data system

The development of improved image segmentation for optical-based AC electrokinetics data system for image is doing. It is display at desktop computer for easy to look the process result of enhancement and segmentation.

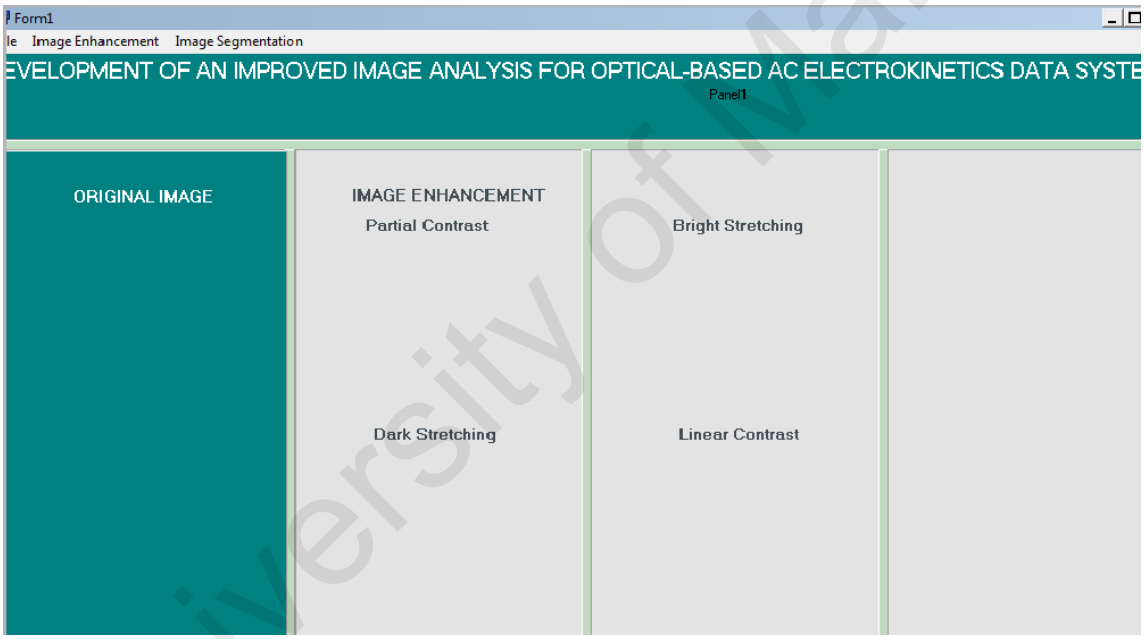


Figure 3.6. The development of an improved image analysis for optical-based AC electrokinetics data system.

3.5 Summary

This chapter described the proceding of using the proposed techniques. The successful of

the development system for image to be used for the looking of image sample depends merely on the image processing that has been developed. During the image processing process, there are four contrast enhancement namely partial contrast, bright strecthing, dark stretching and linear contrast that have been proposed for enhancing the contrast of images. Then, the enhanced images will be segmented by using the image segmentation based on K-mean, MCM, and FCM. The development of an improved image analysis for optical-based AC electrokinetics data system also has done.

CHAPTER 4

RESULT AND DISCUSSION

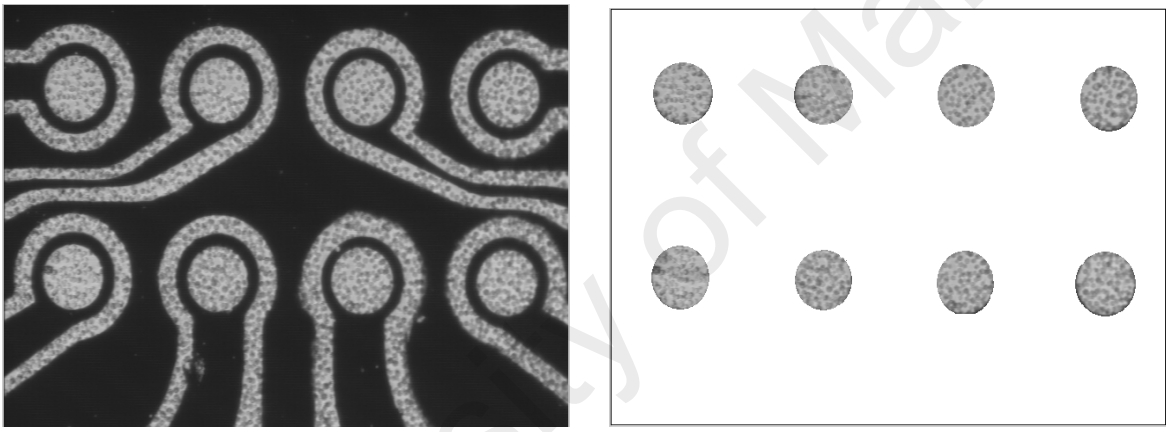
4.1 Introduction

This chapter provides the results that have been obtained based on the application of image processing techniques that have been discussed in Chapter 2. The first section of this chapter will discuss the data acquisition. The second section will discuss the results obtained after applying the contrast enhancement techniques on DEP images. The results obtained after enhancing the images with the proposed contrast enhancement techniques will be shown and discussed. The third section of this chapter will discuss the result of image segmentation using K-Mean, FCM and MCM on DEP images. Finally, the conclusion of all image processing techniques that have been applied on images will be made at the end of this section.

4.2 Data acquisition

Image acquisition is from the given data. Figure 4.1 (a) is about image from DEP data and Figure 4.1 (b) Image after cropping and selected. Figure 4.2 is about the number to know how to read data from experiment the image of DEP. Lastly, Figure 4.3 is about the selected image that want to process.

They are have eight image, from the image the selected to process are number 1 and number 2 only. Something the different criteria have to learn between them until the image can tell the result is normal or cancer based on several of analysis. For that in this project, the image want to process to more clear hopefully which will also related to get a good result of analysis. So, in this project, the image analysis is about image processing. The image processing is one to enhance and another one is to segment are applied. This is to still a looking before to choose a good result.



(a) Image DEP (b) Image DEP after cropping and select

Figure 4.1: Original image of DEP and image which is cropping and select

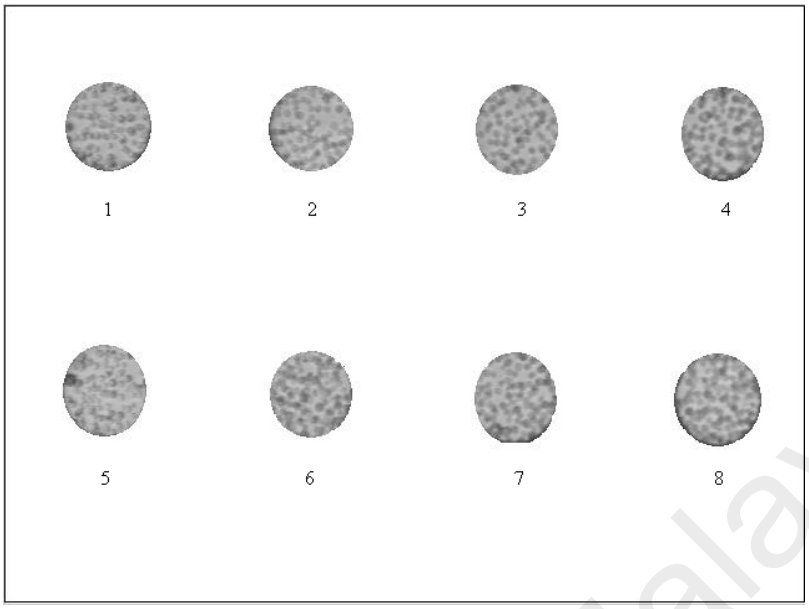


Figure 4.2: The number to know the reading data from experiment the Image of DEP

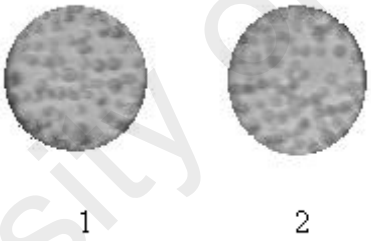


Figure 4.3: The selected image which wants to process

4.3 Contrast Enhancement Techniques

This section provides the results of the three contrast enhancement techniques namely partial contrast, dark stretching and linear contrast techniques that have been applied on images. These techniques have been chosen because number 1 and 2 can be used to improve the overall contrast of images. The quality of images can be determined based on human usual interpretation.

It also determined based on human visual interpretation as well as the intensity histogram plot. The resultant intensity histogram is used to measure the effect of stretching and compression process on the images. If the images have good contrast, the morphological features inside the images can be easily seen and detected.

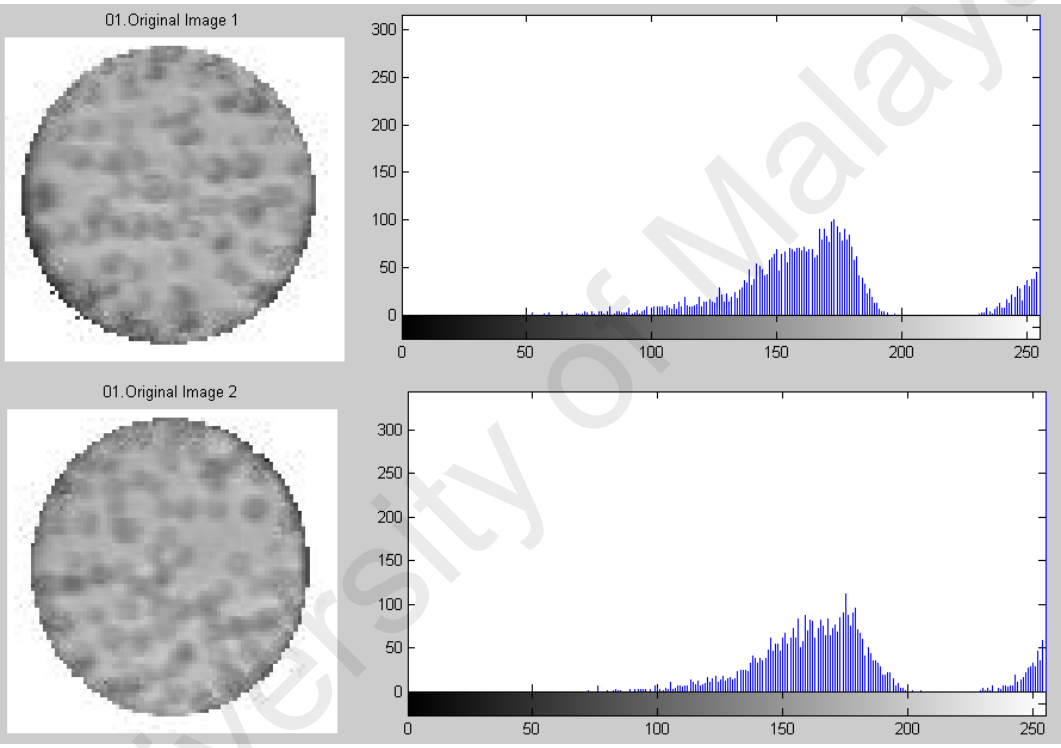


Figure 4.4: Intensity histogram DEP images for number 1 and 2

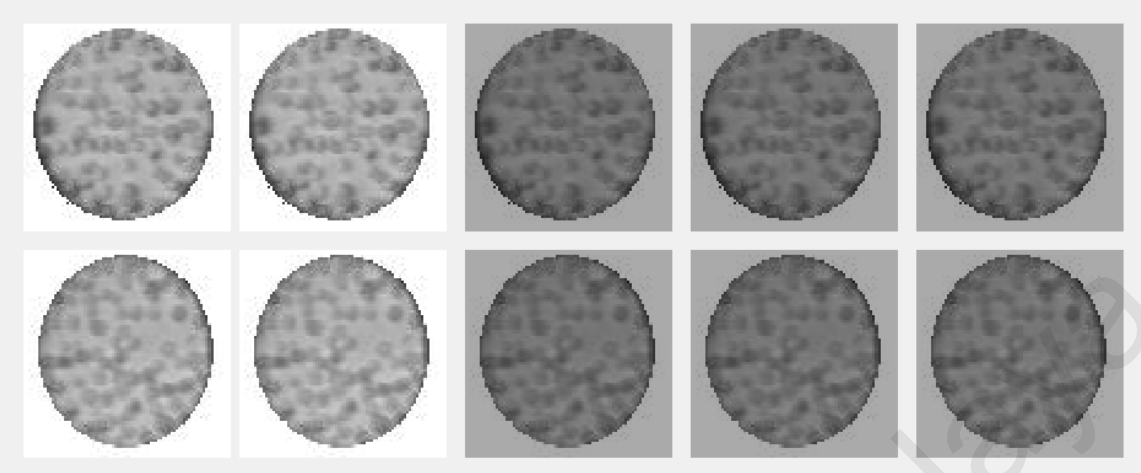


Figure 4.5: RGB of DEP images for number 1 and 2

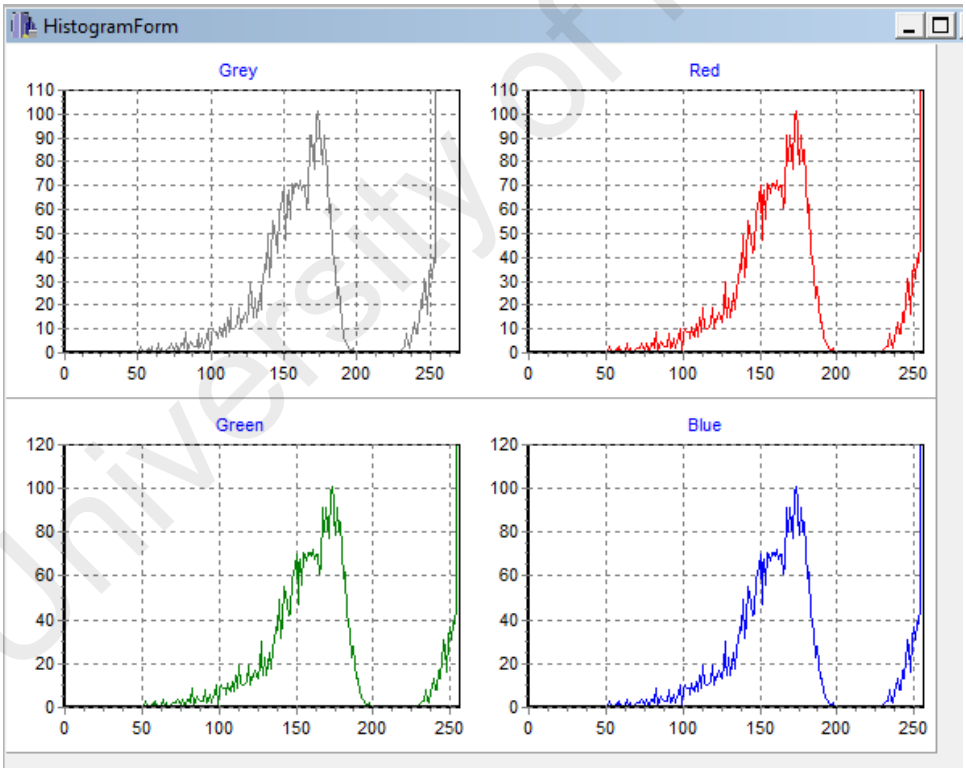


Figure 4.6: Intensity histogram based on original image, red image, green image and blue image.

In this section, the three contrast enhancement techniques have been applied on 1 and 2 images. Figure 4.4 shows the original images of number 1 and number 2 and the corresponding intensity histograms for them. Figure 4.5 represents the corresponding red, green, and blue (RGB) and RGB histograms for number 1 images in Figure 4.6.

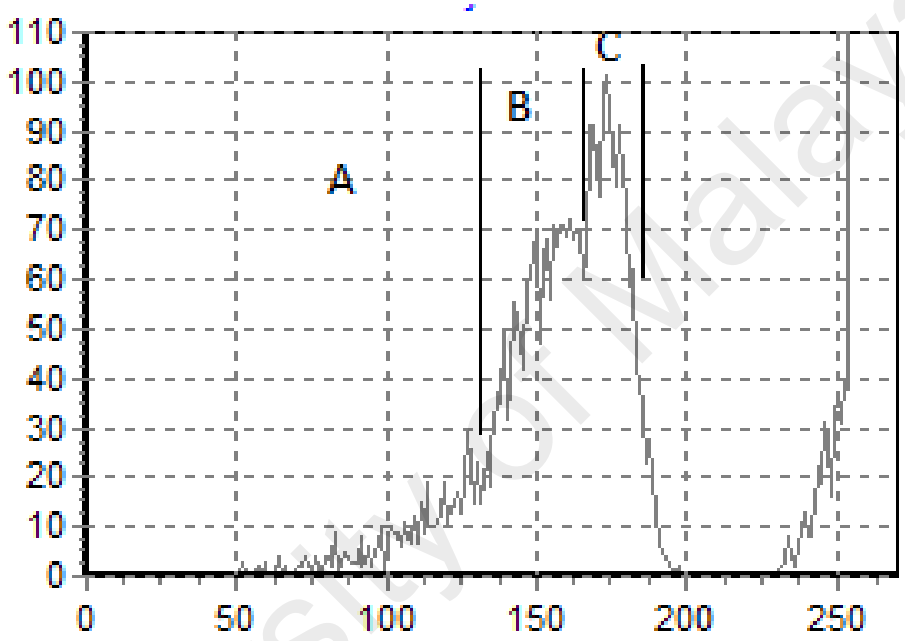


Figure 4.7: Intensity histogram that classifies A, B, and C regions.

Histogram can be used to identify whether the image are bright or dark. An image with its pixel value clustered at the right side of the histogram corresponds to bright image. While if its pixels at the left side corresponds to dark image. Based on the intensity histogram in Figure 4.7, the histogram could be divided into 3 separate regions, indicated by (A), (B) and (C).

It is important to distinguish between their areas. From that it also tries to look a different for the red, green and blue histogram. But it will be difficult to select the threshold value (TH)

based on the separate red, green and blue histogram. The TH that will be used for the image enhancement processes are selected based on the intensity histogram. The criteria of 3 separate regions are still search on what is mean. But for this project, from the separation finding to easy to process image based on intensity histogram.

4.3.1 Partial Contrast Techniques

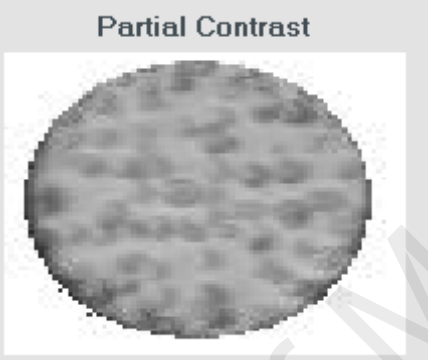


Figure 4.8: Results of images of partial contrast technique for number 1 images

The results obtained after applying the partial contrast technique on images are shown in Figure 4.8. The threshold value that have been used are different for every image. The reason is each image may have its own minimum and maximum value. This technique produces image with good contrast performance compares to the original image.

4.3.2 Bright Stretching Techniques

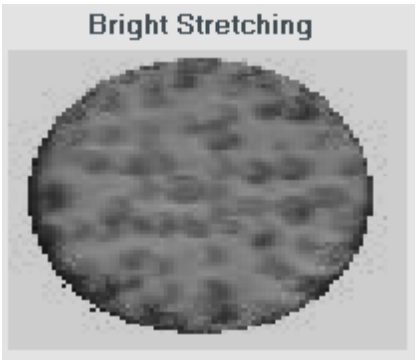


Figure 4.9: Results of images of bright stretching technique for number 1 image.

The results obtained after applying the technique on image are shown in Figure 4.9

4.3.3 Dark Stretching Techniques

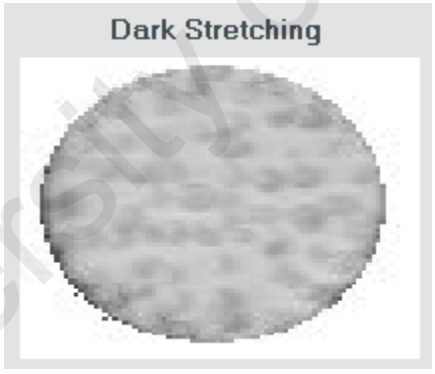


Figure 4.10: Results of images of dark stretching technique for number 1 image.

The results obtained after applying the technique on image are shown in Figure 4.10

4.3.4 Linear Contrast Techniques

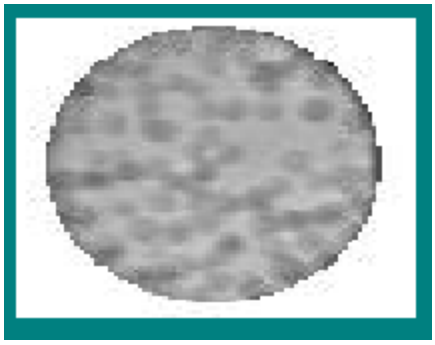


Figure 4.11: Results of images of linear Contrast technique for number 1 image

The result obtained after applying the technique an image is shown in Figure 4.11.

4.4 Image Segmentation Technique

Segmentation of an image refers to the separation of regions with similar characteristics. Image segmentation is the most important step in image analysis as it will directly affect the post-processing (Aus *et al*., 1987).Some of these have been combined in order to segment the region of interest in medical images. The result for image segmentation technique as are below.

4.4.1. K-Mean

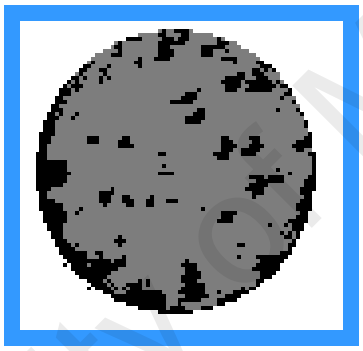


Figure 4.12: Results of images K-Mean

The result obtained after applying the technique an image is shown in Figure 4.12.

4.4.2. MCM

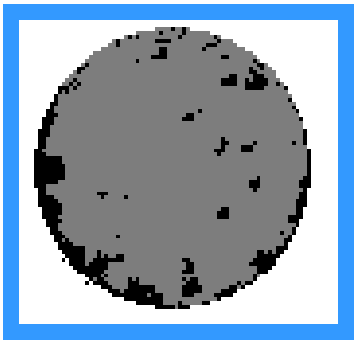


Figure 4.13: Results of images MCM

The result obtained after applying the technique an image is shown in Figure 4.13.

4.4.3. FCM

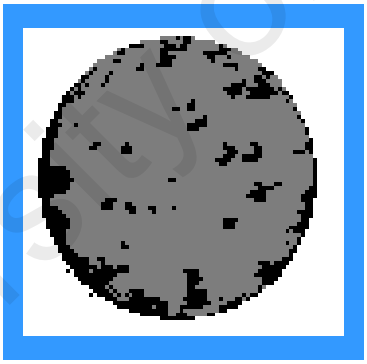


Figure 4.14: Results of images FCM

The result obtained after applying the technique an image is shown in Figure 4.14.

4.5 The proposed development of an improved image analysis for optical-based AC electrokinetics data system

This process has developed of an improved image analysis for optical-based AC Electrokinetics data system based on images. The proposed system consists of image procesiong such as image enhancement and image segmentation.

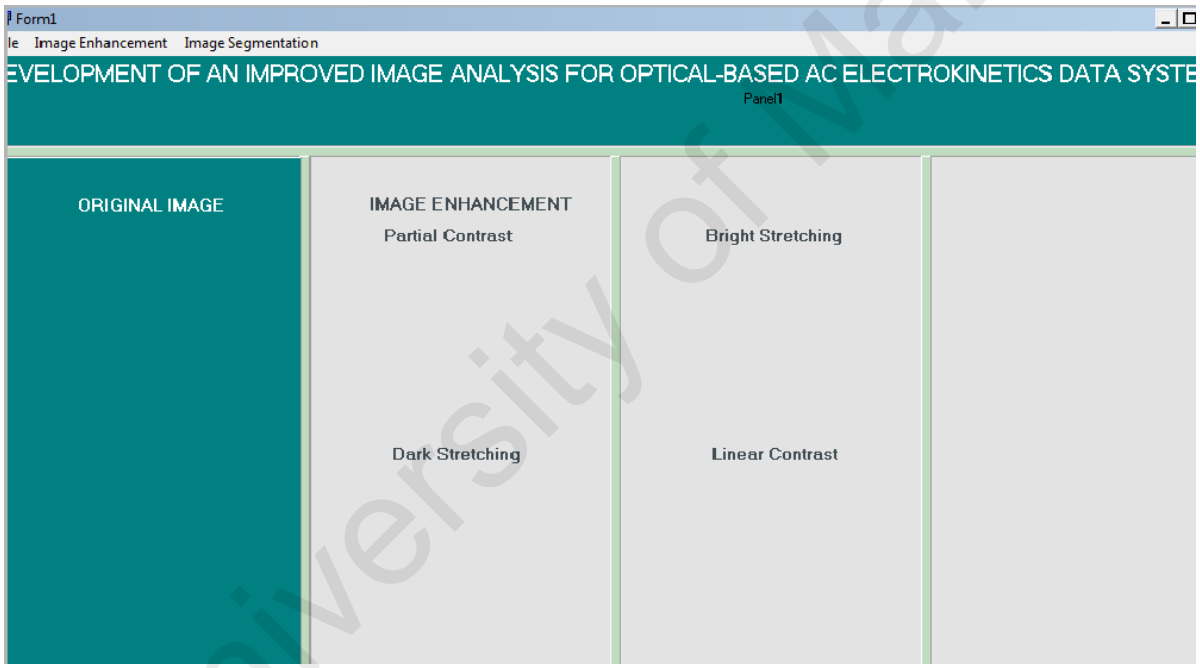


Figure 4.15(a): The main menu of development of an improved image analyisi for optical-based AC Electrokinetics data system for images.

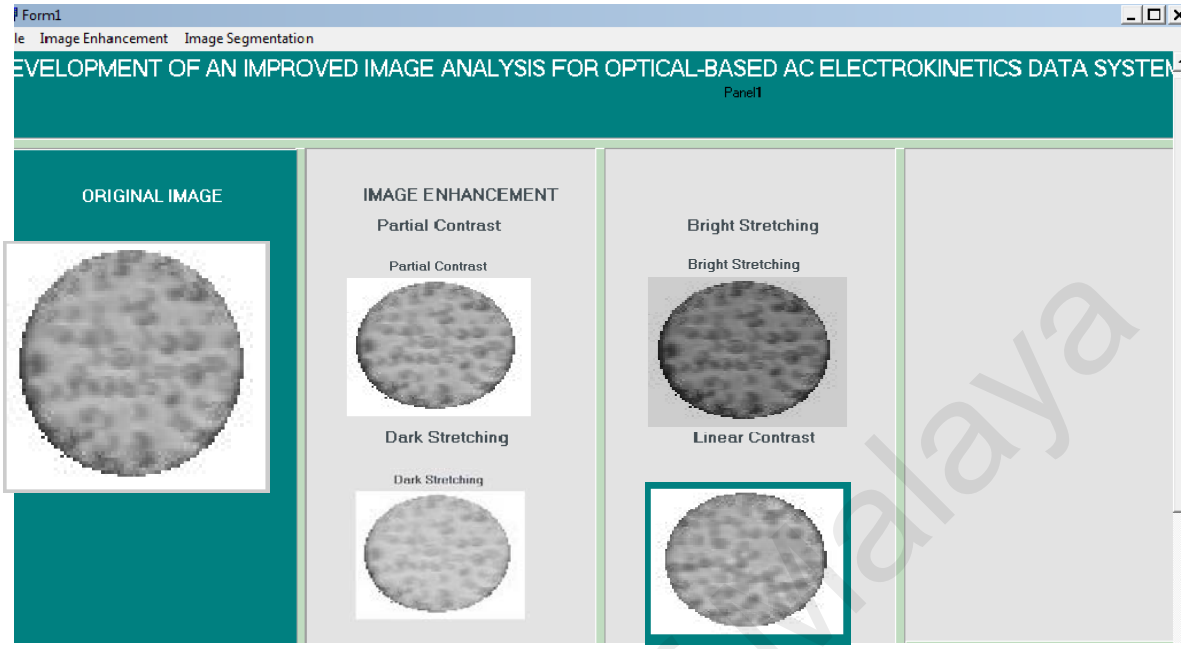


Figure 4.15(b): The image enhancement facility

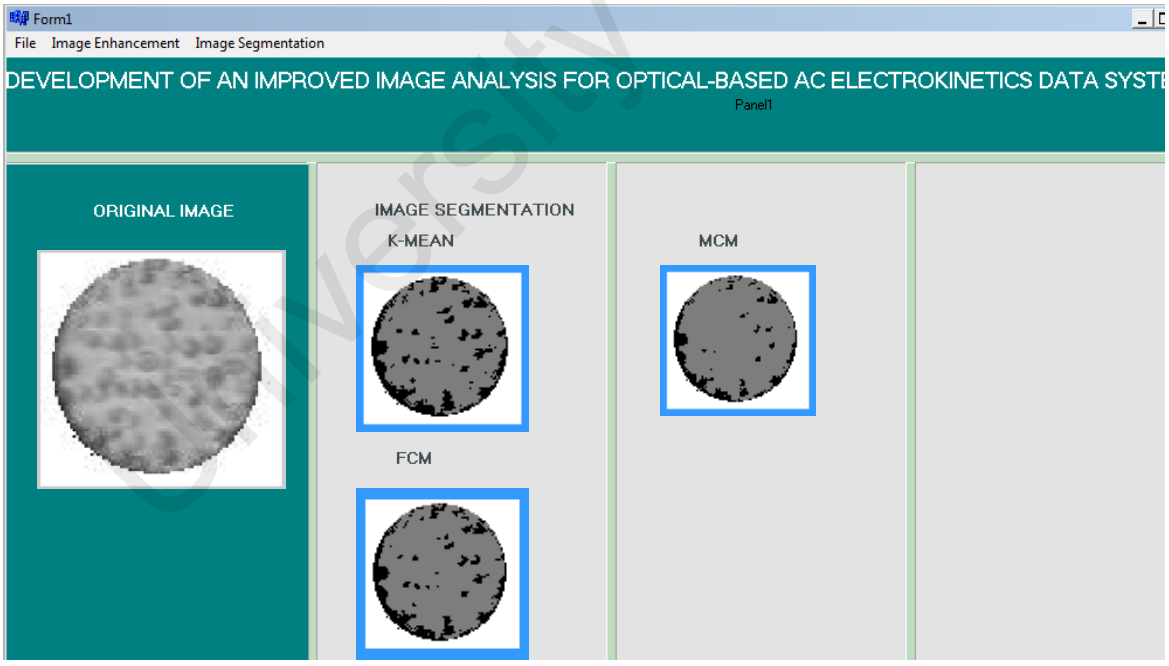


Figure 4.15(c): The image segmentation facility

Figure 4.15(a) represents the main menu of development of an improved image analyisi for optical-based AC Electrokinetics data system for images. Figure 4.15(b) represents the image enhancement facility which consists four contrast enhancement techniques namely partial contrast, bright stretching, dark stretching and linear contrast techniques. These techniques can be used to improve the image quality and enhance the image 1. Figure 4.15(c). The image segmentation facility for obtaining a fully segmented image. Based on these facilities, the proposed development of an improved image analysis for optical-based AC Electrokinetics data system could become a useful tool in medical field and is expected to help classify and investigate the process of image processing.

4.6 Conclusion

In this project, there are four contrast enhancement techniques that have been applied on image namely, partial contrast, bright stretching, dark stretching, and linear contrast techniques. There are three segmentation methods that have been proposed. The method is segmentation based on k-mean, MCM and FCM. The proposed development of an improved image analysis for optical-based AC Electrokinetics data system is capable to classify image processing easily.

CHAPTER 5

CONCLUSION

5.1 Summary

In this project, the development of an improved image analysis for optical-based AC Electrokinetics data system for image which consists of image processing for the purpose of system process bias been developed.

In this project, there are four contrast enhancement techniques that have been applied on images which are partial contrast, bright stretching, dark stretching and linear contrast techniques. The results produced by the proposed techniques are acceptable in terms of visual quality. Three segmentation techniques namely k-mean, MCM, and FCM have been to its good segmentation performance in obtaining a fully segmented of images hopefully.

The proposed system of the development of an improved image analysis for optical-based AC Electrokinetics data is capable to classify image processing with good performances.

5.2 Limitation

There are limitations of this project such as time. For process of to do programming must have more time. The getting all of result maybe will to be better if have more time. The comparison for the data to choose a better image analysis cannot done to do because the limited time.

5.3 Future Recommendations

For future work, there are a few recommendations that can be adopted in order to improve the current work. Most importantly, the analysis has much process and a good research to explore more. It can do a lot of thing from the data collecting.

For example, we maybe can do as below:

1) Test region A, B, and C for PC, BS, DS and LC for image

	PC	BS	DS	LC
A				
B				
C				

Figure 5.1: Example of result

2) From that we are can select the good result of image to do image segmentation like K-Mean, MCM and FCM and then we can do the Sensitivity and Specificity to prove them.

The sensitivity and specificity are defined as below (Demir & Yener, 2005):

$$\text{Sensitivity} = \frac{\text{Number of TP}}{\text{Number of TP} + \text{Number of FN}} \times 100 \qquad (5.1)$$

$$\text{Specificity} = \frac{\text{Number of TN}}{\text{Number of TN} + \text{Number of FP}} \times 100$$

Where TP, TN, FP and FN are the true positive, true negative, false positive and false negative, respectively (Demir & Yener, 2005).

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APPENDIXES

Appendixe A

Matlab code: (Example)

```
load Combined_Dots_8p_K562-101114C_Exp_1_14-Nov-2010_.mat;

imshow (uint8(DDS_control.IMAGES {1,1}(:,:,1)));

-----------------------------------------------------------------------------------------------------------

RGB = imread('1.jpg');

R=RGB(:,:,1);

G=RGB(:,:,2);

B=RGB(:,:,3);

figure, imshow(RGB);

figure,imshow(R);

figure, imshow(G);

figure, imshow(B);

figure,imhist(RGB);
```

Appendixe B

Borland C++ Code: (Example)

```
//---------------------------------------------------------------------------
```

```
void __fastcall TForm1::DarkStretching1Click(TObject *Sender)
{
 float cf,sf,maxgray;
 float merahst,birust,hijaust;
 maxgray = 100;//StrToFloat(LMDEdit1->Text);
 sf =150;//StrToFloat (LMDEdit2->Text);
 cf = 255.0 - sf;
 //----
 //int minr,maxr,ming,maxg,minb,maxb;
 minmerah =0;//255;
 maxr =255;// 0;
 minhijau =0;// 255;
 maxg =255;// 0;
 minbiru =0;// 255;
 maxb =255;// 0;
 //----
//----DS bermula -----
 for(int y=0;y<609;y++)
 {
  for(int x=0;x<809;x++)
  {
   if (merah[x][y]<maxgray)
   {
   merahst = ((float)merah[x][y]-(float)minmerah)/(maxgray-(float)minmerah)*sf;
   }
   else
   {
   merahst = (((float)merah[x][y]-maxgray)/ (255.0 - maxgray) *cf)+ sf;
   }
   //------

   if (hijau[x][y]<maxgray)
   {
   hijaust = ((float)hijau[x][y]-(float)minhijau)/(maxgray-(float)minhijau)*sf;
   }
   else
   {
   hijaust = (((float)hijau[x][y]-maxgray)/(255.0-maxgray)* cf)+ sf;
   }

   if (biru[x][y]<maxgray)
   {
   birust = ((float)biru[x][y]-(float)minbiru)/(maxgray-(float)minbiru)*sf;
   }
   else
   {
   birust = (((float)biru[x][y]-maxgray)/(255.0-maxgray) * cf)+ sf;
   }

  Form1->temp->Canvas->Pixels[x][y]=(TColor)RGB(merahst,hijaust,birust);

  merah[x][y]=merahst;
  hijau[x][y]=hijaust;
  biru[x][y]=birust;
  }

  Form1->ImageDS->Picture= Form1->temp->Picture;
  Form1->ImageDS->Visible=true;
  Form1->ImageDS->Stretch=true;
 }
}

//---------------------------------------------------------------------------
void __fastcall TForm1::BrightStretching1Click(TObject *Sender)
{
 float cf,sf,mingray;
```

```
 float NewTH;
 float merahst,birust,hijaust;
 mingray = 100;//StrToFloat(LMDEdit3->Text);//TH
 NewTH = 50;//StrToFloat (LMDEdit4->Text);//BS factor
 //sf = 255.0 - temp;
 sf = 255.0 - mingray;

//---
 Form1->temp1->Picture= Form1->Image1->Picture;
 for(int y=0;y<609;y++)
 {
  for(int x=0;x<809;x++)
  {
  int pixelcolor = (DWORD)Form1->temp1->Canvas->Pixels[x][y];

  merah[x][y] =GetRValue(pixelcolor);
  biru[x][y] =GetBValue(pixelcolor);
  hijau[x][y]=GetGValue(pixelcolor);

  if(merah[x][y]>maxr) { maxr =merah[x][y];}
  if(merah[x][y]<minmerah) { minmerah =merah[x][y];}
  if(hijau[x][y]>maxg) { maxg =hijau[x][y];}
  if(hijau[x][y]<minhijau) { minhijau =hijau[x][y];}
  if(biru[x][y]>maxb) { maxb =biru[x][y];}
  if(biru[x][y]<minbiru) { minbiru =biru[x][y];}
  }
 }
//---

 for(int y=0;y<609;y++)
 {
  for(int x=0;x<809;x++)
  {
  if ((float)merah[x][y]>mingray)
  {
  merahst = (((float)merah[x][y]-mingray)/(255.0-mingray)*sf) + NewTH;
  }
  else
  {
  merahst = ((float)merah[x][y]-(float)minmerah)/ (mingray)*NewTH;
  //merahst = ((float)merah[x][y]-mingray)
  }
  //---------

  if ((float)hijau[x][y]>mingray)
  {
  hijaust = (((float)hijau[x][y]-mingray)/(255.0-mingray)*sf )+ NewTH;
  }
  else
  {
  hijaust = ((float)hijau[x][y]-(float)minhijau)/ (mingray)* NewTH;
  }
  //---------
  if ((float)biru[x][y]>mingray)
  {
  birust = (((float)biru[x][y]-mingray)/(255.0-mingray)*sf) +NewTH;
  }
  else
  {
  birust = ((float)biru[x][y]-(float)minbiru)/(mingray)* NewTH;
  }
  Form1->temp1->Canvas->Pixels[x][y]=(TColor)RGB(merahst,hijaust,birust);

  merah[x][y]=merahst;
  hijau[x][y]=hijaust;
  biru[x][y]=birust;
```

```
 }
 Form1->ImageBS->Picture= Form1->temp1->Picture;
 Form1->ImageBS->Visible=true;
 Form1->ImageBS->Stretch=true;
 }
}

//---------------------------------------------------------------------------
void __fastcall TForm1::PartialContrast1Click(TObject *Sender)
{
 int minM,minH,minB,maxM,maxH,maxB;
 maxB=maxH=maxM=0;
 minB=minH=minM=0;
 int k;

 int old_min,Newmin,old_max,Newmax;
 old_min =10;//StrToFloat(LMDEdit5->Text);
 Newmin=50;//StrToFloat(LMDEdit6->Text);
 old_max =25;//StrToFloat(LMDEdit7->Text);
 Newmax =225; //StrToFloat(LMDEdit8->Text);

 Form1->temp2->Picture= Form1->Image1->Picture;
 for(k=0;k<256;k++)//Clearkan histogram
 {
  BilPixR[k]=0;
  BilPixG[k]=0;
  BilPixB[k]=0;
 }

 for(int y=0;y<609;y++)
 {
  for(int x=0;x<809;x++)
  {
   int pixelcolor = (DWORD)Form1->temp2->Canvas->Pixels[x][y];
   red[x][y] = GetRValue(pixelcolor);
   blue [x][y] = GetBValue(pixelcolor);
   green[x][y] = GetGValue(pixelcolor);
  }
 }
 for(y=0;y<609;y++)
 {
  for(x=0;x<809;x++)
  {
   cMerah=red[x][y];
   cHijau =green[x][y];
   cBiru =blue[x][y];

   BilPixR[cMerah]++;
   BilPixG[cHijau]++;
   BilPixB[cBiru]++;
  }
 }
 int totalpixm=0;
 for(x=0;x<256;x++)
 {
  totalpixm = totalpixm + BilPixR[x];
 }

 int totalpixh=0;
 for(x=0;x<256;x++)
 {
  totalpixh = totalpixh + BilPixG[x];
 }

 int totalpixb=0;
 for(x=0;x<256;x++)
```

```
{
 totalpixb = totalpixb + BilPixB[x];
}

k=0;
cHijau=0,cMerah=0;cBiru=0;;
float sumR=0,sumR1=0;
float sumB=0,sumB1=0;
float sumG=0,sumG1=0;
float percentR=0,percentG=0,percentB=0;
float percentR1=0,percentG1=0,percentB1=0;

for(k=0;k<256;k++)
{
sumR+=BilPixR[k];
percentR= ((float)sumR/(float)totalpixm)*100;

 if (percentR>=old_min)
 {
  minM=k ;
  break;
 }
}

sumR=0;
for(k=0;k<256;k++)
{
 sumR+=BilPixR[k];
 percentR= ((float)sumR/(float)totalpixm)*100;

  if (percentR>=Newmin)
  {
   THR=k ;
   break;
  }
}

//sumG=0;
for(k=0;k<256;k++)
{
 sumG+=BilPixG[k];
 percentG= ((float)sumG/(float)totalpixh)*100;

  if (percentG>=old_min)
  {
   minH=k;
   break;
  }
}

sumG=0;
for(k=0;k<256;k++)
{
sumG+=BilPixG[k];
percentG= ((float)sumG/(float)totalpixh)*100;

 if (percentG>=Newmin)
 {
  THG=k ;
  break;
 }
}

for(k=0;k<256;k++)
{
 sumB+=BilPixB[k];//sumB=sumB + BilPixB[k]
```

```
 percentB= ((float)sumB)/((float)totalpixb)*100;
 if (percentB>=old_min)//lebih 15(16-255) dan a=0
 {
 minB=k;
 break ;
 }
}

sumB=0;
for(k=0;k<256;k++)
{
 sumB+=BilPixB[k];//sumB=sumB + BilPixB[k]
 percentB= ((float)sumB)/((float)totalpixb)*100;
 if (percentB>=Newmin)//lebih 15(16-255) dan a=0
 {
  THB=k;
  break;
 }
}

for(maxM=255;maxM>=0;maxM--)
{
 sumR1+=BilPixR[maxM];
 percentR1= ((float)sumR1/(float)totalpixm)*100;
 if (percentR1>=old_max)
 break;
}

for(maxH=255;maxH>=0;maxH--)
{
 sumG1+=BilPixG[maxH];
 percentG1= ((float)sumG1/(float)totalpixh)*100;
 if (percentG1>=old_max)
 break;
}

for(maxB=255;maxB>=0;maxB--)
{
 sumB1+=BilPixB[maxB];
 percentB1= ((float)sumB1/(float)totalpixb)*100;
 if (percentB1>=old_max)
 break;
}

float THmin=float((float)THR+(float)THB+(float)THG)/3;
float temp =THmin;
float sf=Newmax;//Upper threshold
float cf=255-sf;//Lower threshold
//sf = 255-temp;
float biruC,merahC,hijauC;

float max=float((float)maxM+(float)maxB+(float)maxH)/3;
/*ListBox1->Items->Add("max="+(AnsiString)max);
ListBox1->Items->Add("maxM="+(AnsiString)maxM);
ListBox1->Items->Add("maxB="+(AnsiString)maxB);
ListBox1->Items->Add("maxH="+(AnsiString)maxH);*/
//ListBox1->Items->Add("merahC="+(AnsiString)merahC);
float min=float((float)minM+(float)minB+(float)minH)/3;
/* ListBox1->Items->Add("min="+(AnsiString)min);
ListBox1->Items->Add("minM="+(AnsiString)minM);
ListBox1->Items->Add("minB="+(AnsiString)minB);
ListBox1->Items->Add("minH="+(AnsiString)minH); */
{
 for(y=0;y<609;y++)
 for(x=0;x<809;x++)
 {
```

```
if (THR>=255&&THG>=255&&THB>=255 )
{
 merahC=red[x][y];
 hijauC=green[x][y];
 biruC=blue[x][y];
}

else
{// if (min<red[x][y]<max)//max value for new threshold value
 if (red[x][y]<=max && red[x][y]>=min)
 { //use formula  o/p=((max-min:new range)/(max-min:oriRange)*(in(x,y)-min))+10
 merahC=((float)red[x][y]-min)/((max-min))*(sf-temp)+(temp);
 }

 else if(red[x][y]>max)
 {
 merahC = (((float)red[x][y]-max)/ (255 - max) *cf)+ sf;
 }

 else
 {
 merahC = ((float)red[x][y])/((float)min)*temp;
 }

 //if (min<green[x][y]<max)
 if (green[x][y]<=max&&green[x][y]>=min)
 {
 //hijauC=((float)(max-min)/(maxM-minM)*green[x][y])+min;
 hijauC=((float)green[x][y]-min)/((max-min))*(sf-temp)+(temp);
 }

 else if (green[x][y]>max)
 {
 hijauC = (((float)green[x][y]-max)/ (255 - max) *cf)+ sf;
 }

 else
 {
 hijauC = ((float)green[x][y])/((float)min)*temp;
 }
 //if (min<blue[x][y]<max)
 if (blue[x][y]<=max&&blue[x][y]>=min)
 {
 //biruC=((float)(max-min)/(maxM-minM)*blue[x][y])+min   ;
 biruC=((float)blue[x][y]-min)/((max-min))*(sf-temp)+(temp);
 }

 else if (blue[x][y]>max)
 {
 biruC = (((float)blue[x][y]-max)/ (255 - max) *cf)+ sf;
 }

 else
 {//biruC = ((float)blue[x][y])*(temp)/ (min);
 biruC = ((float)blue[x][y])/((float)min)*temp;
 }

 if(merahC>255)
 {merahC=255;};
 if(merahC<0)
 {merahC=0;};
 if(hijauC>255)
 {hijauC=255;};
 if(hijauC<0)
 {hijauC=0;} ;
 if(biruC>255)
```

```
 {biruC=255;};
 if(biruC<0)
 {biruC=0;};

 red[x][y]=merahC;
 green[x][y]=hijauC;
 blue[x][y]=biruC;
 }
Form1->temp2->Canvas->Pixels[x][y]=(TColor)RGB(merahC,hijauC,biruC);
}
```

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