

**MODIFIED PIECEWISE LINEAR MAPPING
CONTRAST ENHANCEMENT AND LOCAL OTSU
SEGMENTATION METHODS FOR HEP-2 CELL
IMAGES**

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ENHANCEMENT AND LOCAL OTSU SEGMENTATION METHODS FOR
HEP-2 CELL IMAGES**

by

MOHAMAD SHAHRUL AFFENDI BIN BAHAROM

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LIST OF ABBREVIATIONS

2D	Two Dimension
AHE	Adaptive Histogram Equalization
ANA	Anti- Nuclear Antibody
BoW	Bag-of-Words
CAD	Computer Aided Diagnostic
CDC	Centre for Disease Control and Prevention
CDF	Continuous Distribution Function
CHE	Conventional Histogram Equalization
CLAHE	Contrast Limited Adaptive Histogram Equalization
CMYK	Cyan Magenta Yellow Black
ELISA	Enzyme-Linked ImmunoSorbent Assay
EM	Electromagnetic
ESIHE	Sub-Image Histogram Equalization
FCM	Fuzzy C-Means
GLCM	Grey Level Co-occurrence Matrix
GSC	Gaussian Scale Space
HE	Histogram Equalization
HEp-2	Human Epithelial Type 2
HIS	Hue Saturation Intensity
HSB	Hue Saturation Brightness
ICPR	International Conference on Pattern Recognition
IIF	Indirect Immunofluorescence
KM	K-Means

KNN	K-Nearest Neighbour
LBP	Local Binary Pattern
MATLAB	MATrix LABoratory
MD	Morphological Dilation
ME	Morphological Erosion
MHE	Modified Histogram Equalization
MIVIA	Machine Intelligence for Video, Image and Audio Processing
MO	Morphological Opening
MSE	Mean Square Error
MSSIM	Mean Structural Similarity
PDF	Probability Distribution Function
PSNR	Peak Signal-to-Noise-Ratio
RGB	Red Green Blue
ROI	Region of Interest
SE	Structuring Element
SIFT	Scale Invariant Feature Transformation
SLE	Systemic Lupus Erythematosus

LIST OF SYMBOLS

f	Original image
x	Horizontal axis of continuous spatial coordinate
y	Vertical axis of continuous spatial coordinate
(x,y)	Coordinate on the spatial domain vertical
μm	Micro meter
γ	Gamma correction variable value
Th	Threshold value
g	Segmented image
L	Total number of intensity levels
k	Intensity level of a pixel
n_k	Number of occurrences of a pixel with the level I_k
N	Total number of pixel in the input image
$f(k)$	Sigmoid function
L_{max}	Maximum number of intensity levels
L_{min}	Minimum number of intensity levels
C	Cut off value for sigmoid function
g	Gain value for sigmoid function
a	Control value for the gain coefficient in sigmoid function
$N \times N$	Size of square block pixels
$M \times N$	Size of image pixels
P_{nor}	Normalize PDF
P_c	Cumulative sums of normalize PDF
m_c	Cumulative mean

m_g	Global means
$B(x,y)$	Binary image
w	Watershed line
W	Number local windows
$l(x_i, y_i)$	Luminance function
$c(x_i, y_i)$	Contrast function
$s(x_i, y_i)$	Similarity function
TP	True Positive
TN	True Negative
FP	False Positive
FN	False Negative

KAEDAH PENINGKATAN KONTRAS PEMETAAN LELURUS SESECEBIS DAN PERUASAN OTSU TEMPATAN UNTUK IMEJ SEL HEP-2

ABSTRAK

Analisis Imej Immunofluorescence tidak langsung (IIF) untuk pengelasan Corak-Corak Epitelium Manusia (HEp-2) immunofluorescence ialah satu cara berkesan mengenal pasti kehadiran Antibodi Anti-Nuklear (ANA). Kebanyakan kerja sedia ada hanya tertumpu kepada klasifikasi sel HEp-2 dan hanya sedikit usaha telah ditumpukan untuk mendalami kepentingan teknik pra-pemprosesan dan segmentasi. Kajian ini menganalisis kepentingan kedua-dua cara, yang mungkin meningkatkan proses klasifikasi HEp-2. Pra-pemprosesan mampu memberikan sel kualiti lebih baik dalam satu imej melalui proses penambahbaikan kontras, manakala segmentasi yang mengasingkan informasi penting boleh meningkatkan keberkesanan klasifikasi. Disertasi ini membentangkan Kaedah Peningkatan Kontras Pemetaan Lelurus Sesecebis dan Perluasan Otsu Tempatan sebagai satu pendekatan pra-pemprosesan dan segmentasi untuk HEp-2 imej. Pra-pemprosesan yang dicadangkan memfokuskan kepada pengurangan kewujudan bendasing di latar belakang dan memperbaiki kontras sel menggunakan teknik Pemetaan Lelurus Sesecebis. Kemudian proses terus dengan proses segmentasi yang memperkenalkan satu segmentasi Otsu tempatan baru. Kaedah ini ialah satu kombinasi algoritma Otsu dan operasi morfologikal (termasuk legeh morfologikal). Peringkat ini membolehkan proses pengasingan informasi penting HEp-2 sel dan informasi bendasing dalam imej. Berdasarkan analisis kuantitatif dan kualitatif, mencadangkan kaedah pra-pemprosesan mampu meningkatkan kontras dan pada masa yang sama meminimumkan kewujudan bendasing. Bagaimanapun, beberapa imej terbeban dengan kehilangan sel tekstur. Sebaliknya, kaedah segmentasi yang dicadangkan berkebolehan untuk pembahagian sel dan mengasingkan daripada sel bergabung. Tetapi, sesetengah struktur sel secara tidak sengaja dibuang seperti sel kecil atau sel rosak. Konklusinya, walaupun terdapat kehilangan informasi sel, kedua-dua cara telah berjaya dalam meningkatkan kualiti HEp 2 imej dan menonjolkan maklumat sel yang penting.

**MODIFIED PIECEWISE LINEAR MAPPING CONTRAST
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ABSTRACT

Indirect Immunofluorescence (IIF) image analysis for classification of Human Epithelial (HEp-2) immunofluorescence patterns is an effective way to identify the presence of Anti- Nuclear Antibody (ANA). Most existing works focussed on HEp-2 cell classification and very few efforts have been dedicated to study the importance of the pre-processing and segmentation techniques. This study analyses the importance of both methods, which could possibly improve the HEp-2 classification process. The pre-processing is capable to provide a better quality cell features in an image through contrast enhancement process, while segmentation which segregates important features could improve the classification performances. This dissertation presents a Modified Piecewise Linear Mapping and Local Otsu Segmentation as a pre-processing and segmentation approach for the HEp-2 images. Proposed pre-processing focus on minimizing the existence of noise in the background and stretching the cell's contrast by using piecewise linear mapping function technique. Then the process continues with segmentation process, which introduce a new local Otsu segmentation. This method is a combination of Otsu algorithm and morphological operation (including morphological watershed). This stage perform a segregation process to isolate an important information and distinguish an unwanted information about HEp-2 cell in the image. According to the qualitative and quantitative analysis, proposed pre-processing method is able to enhance the contrast and at the same time minimize the existences of the noise. However, some of the image suffers from texture's cell loss. On the other hand, the proposed segmentation method capable to segment cell and isolate most of the combined cell. But, some structure of unnecessary cell accidentally be removed such as small cell or damaged cell. As a conclusion, although there are some losses in cell information, both methods were successful in enhancing the quality of the HEp-2 image and highlighting important cell information.

CHAPTER ONE

INTRODUCTION

1.1 Research Background

Autoimmune disease is becoming a common threat in nation worldwide. Approximately 5% and 7% of the world's population suffers from one of more than 60 known autoimmune diseases (Davidson *et al.*, 2001). This disease happens when antibodies that developed by immune system occasionally fail to detect a healthy native body cells and tissues as foreign invaders (e.g. viruses and bacteria) for destruction. When antibodies mistakenly attack and destroy a healthy cell in the human body, they are called autoantibodies. One of the autoantibodies is the antinuclear antibodies (ANA), which refer to a specific group of autoantibodies that target the nucleus of a cell. Human beings have autoantibodies, but in the smaller quantity. The existence of a large quantity of ANAs can indicate an autoimmune disease. The effect of the disease is chronic and leads to seriously damaging tissue and organs (Elbischger *et al.*, 2009). This disease often targets certain organs in thyroiditis or involves a particular tissue in different places (e.g. the basement membrane in both lung and kidney). Examples of autoimmune disease are systemic lupus erythematosus (SLE), rheumatoid arthritis, multiple sclerosis, and diabetes mellitus type 1. The treatment of autoimmune disease is typically with immunosuppression that is a medication, which decreases the immune response.

One of the common autoimmune diseases in Malaysia is SLE. The prototypical autoimmune disease is characterized by autoantibody production, complement activation, and immune complex deposition leading to diverse clinical manifestations and target tissue damage. The prevalence of SLE is estimated to be between 40 and 400 cases per 100,000 individuals (Helmick *et al.*, 2008). In Asian, the prevalence of SLE generally falls within 20-30/100,000 individuals. SLE is more frequent (among

Chinese communities) in Asia than it is in India and Tropical Africa (Frank, 1980). Since Malaysia is a multiracial country (that consist of Malays (55.1%), Chinese (24.3%), and Indians (7.4%) which represent the largest ethnic groups), a prevalence of 43/100,000 individuals have been reported (Osio-Salido and Manapat-Reyes, 2010; Wang *et al.*, 1997). Likewise, the Chinese have the highest prevalence of SLE in Malaysia (57/100,000), followed by Malays (33/100,000) and Indians (14/100,000) (Chua *et al.*, 2008; S.N.Yap *et al.*, 1999). The overall 5-year and 10-year survival rates were reported as 82% and 70%, respectively (Wang *et al.*, 1997), whereas the overall mortality rate was 20.2% (Yeap *et al.*, 2001). Renal autoimmune diseases recorded by (Wang *et al.*, 1997) as the highest autoimmune diseases that affect the Malaysian patients. However, the major cause of death in Malaysia is the patients that infected with SLE as reported by (Yeap *et al.*, 2001).

The ANA test is commonly used by specialist or doctor to identify the existences of Connective Tissue Disease such as SLE, sjogren's syndrome, and rheumatoid arthritis (Meroni and Schur, 2010). The purpose of applying this test is to screen the patients with autoantibodies. During the test, specialist is able to link the autoantibody patterns with individual diseases for guidance of specific method, which help to screen out patients who have a negative test and monitor patients during treatment. An applicable ANA tests that utilized screening of a great variety of autoimmune diseases consist of Indirect Immunofluorescence (IIF) method (based on Human Epithelial type 2 (HEp-2) cells) and Enzyme-Linked ImmunoSorbent Assay (ELISA). Even though IIF is the old method for screening the autoantibodies, these test remains the standard protocol because of the high sensitivity and the large range expressions of antigens (Meroni and Schur, 2010; Wiik *et al.*, 2010). In addition, the American College of Rheumatology issued a position statement (an expression of how

a given service fills a particular doctor or researcher need in a way that its competitors don't.) highlighting that the IIF method based on HEp-2 cells for ANA analyse remains the gold standard (Rheumatology, 2011).

Clinical procedure for an ANA test using IIF method based on HEp-2 cells is carried out by firstly taking the blood serum of the patient. Then, the patient's sample is incubated with the slides containing fixed HEp-2 cells. In this mixing moment, the antibodies in the blood serum that are diluted with a fluorescent antibody reagent react and bind selectively to various components within the nucleus of HEp-2 cells. Then, it form a different patterns of stained cells depending on types of autoimmune diseases. At the end of the procedure, highly qualified and skilful specialists perform an inspection on the fluorescence slide under the fluorescence microscope and captured an image of HEp-2 cell (as known as IIF image) to reveal HEp-2 cells. Generally, specialist will fulfil the following requirements in capturing HEp-2 cell images for classification process:

1. Image consist at least one or two fluorescence mitotic cells for supporting the judgement of the classification of the pattern.
2. Only specific fluorescence intensities of an image will be selected by the specialist to be classified and the rest is saved for a patient's record. There are three levels of fluorescence intensity as visualized in Figure 1-1 based on guidelines by the Centre for Disease Control and Prevention (CDC) (Control, 1996) as follows:
 - Positive: This level of intensities have a bright fluorescence stain (with different stain for each pattern) spread out the entire nucleus of interphase and mitotic cells. These intensities are the ideal brightness and contrast image to be analysed.

- Intermediate: Intermediate intensity samples usually exhibit low contrast with fluorescence stain in the entire nucleus of interphase and mitotic cells. In some cases, the colour of the intermediate intensity is shown by the fluorescence with reddish stain on the cells. This level still can be considered as an image that could possibly be classified.
 - Negative: There is no significant fluorescence stain pattern on the cell and staining with reddish colour. This level indicates that the patient has a negative result and will not be included in classification step for cell's pattern.
3. Further classification of the IIF images with intermediate and positive fluorescence intensity levels will be done based on staining pattern. The different staining patterns can be classified into six main groups (as visualized in Figure 1-2) as follows (Hiemann *et al.*, 2009; Meroni and Schur, 2010; Soda and Iannello, 2009; Wiik *et al.*, 2010):
- Centromere: Discrete uniform speckles through the nucleus and the number of speckles correspond to a multiple of the normal chromosome number.
 - Homogeneous: Diffuse staining of the whole nucleus, with or without apparent masking of the nucleoli.
 - Nucleolar: Fluorescent staining of the nucleoli within the nucleus, sharply separated from the unstained nucleoplasm,
 - Coarse Speckled: Coarse fluorescent aggregates at the nucleus.
 - Fine Speckled: Fine fluorescent aggregates throughout the nucleus.
 - Cytoplasmatic: Granular fluorescence in the cytoplasm

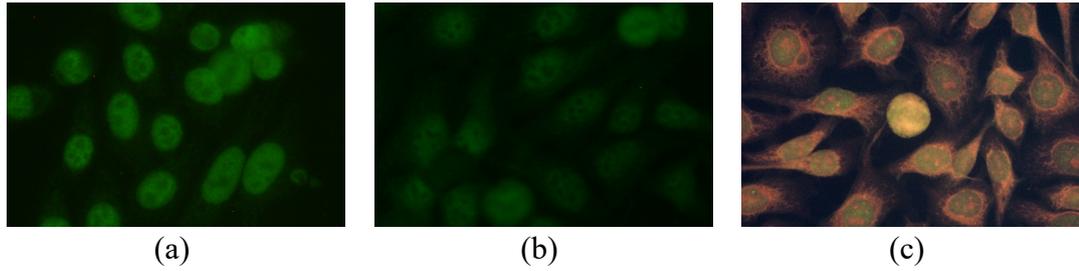


Figure 1-1: Differences fluorescence intensity level of HEp-2 cell images, (a) positive, (b) intermediate and (c) negative (Labratories, 2016; MIVIA, 2010).

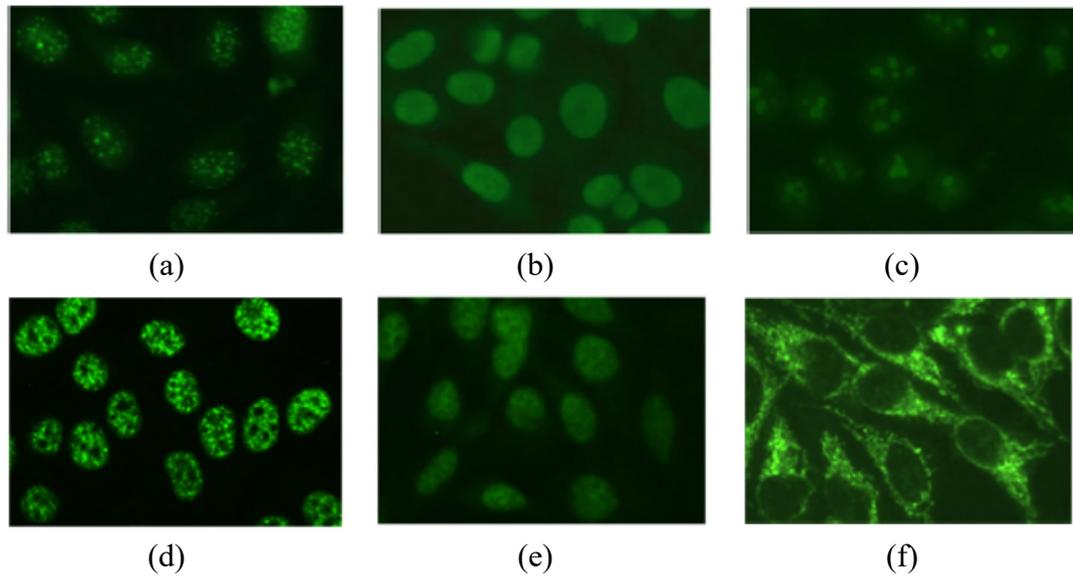


Figure 1-2: Six type of positive HEp-2 cell staining pattern, (a)Centromere, (b)Homogeneous, (c) Nucleolar, (d) Coarse Speckled, (e) Fine Speckled and (f) Cytoplasmatic (Labratories, 2016).

1.2 Research Motivation

Nowadays, there is a huge demand for HEp-2 cell image analysis due to its effectiveness and high quality for identifying autoimmune diseases. Despite its advantages, the method still does not achieve its target to becoming the best method

of identifying autoimmune disease because this method is subjective, low level of standardization, time consuming and lack of automated solution (Bizzaro *et al.*, 1998; Pham *et al.*, 2005). Each ANA specimen must be examined under a fluorescence microscope with minimum two specialists, and the quality of diagnosis depends on their experience and expertise. This issues renders the test result to become more subjective as interpretation might be vary between specialists. Thus, the result gives low reproducibility and large inter-/intra- personnel/laboratory variability (Hiemann *et al.*, 2009; Soda and Iannello, 2009). Other than that, poor standardization such as the fluorescence intensity level and cell-staining pattern leads to incomparability and difficult interpretation.

In addition, low-level standardization limits the communication between the clinic units and affects the reproducibility of IIF reading. Time consuming during applying IIF method is one of the issue happens when the method is performed by manual interpretation. Human evaluation takes a lot of time to classify the pattern and suffer from very high variability. As a demand for applying IIF method in diagnosis of autoimmune disease is increasing, a new method should be developed from the manual interpretation to automated interpretation. This development could possibly reduce errors due to subjective misinterpretation for pattern and reduce fatigue of the specialist. The process of the classification can be speeded up and could produce a standard result of IIF reading.

1.3 Problem Statement

To address subjective misinterpretation issues mentioned in Section 1.2, it is possible to use Computer Aided Diagnostic (CAD) systems which automatically determine the HEp-2 pattern in the given cell images of a specimen (Cordelli and Soda,

2011; Hiemann *et al.*, 2009; Hsieh *et al.*, 2009b; Perner *et al.*, 2002; Soda and Iannello, 2009; Strandmark *et al.*, 2012b). Most of the existing CAD systems have a common trend: they use carefully handpicked features, which may only work in a particular laboratory environment and/or microscope configuration. Also, several approaches employ a large number of features and apply an automated feature selection process (Hiemann *et al.*, 2009). With the increasing interest in employing image analysis techniques for various routine clinical pathology tests, there remains a need for novel approaches to allow efficient interpretation of automatically classifying a HEp-2 cell pattern (e.g. speckled, homogeneous, nucleolar, centromere). The results produced by these techniques can be combined with subjective analysis done by scientist, leading to test results being more reliable and consistent across laboratories. These days, several techniques have been proposed in the current research for all the major stages of the IIF diagnostic procedure. The techniques that have been involved in the CAD system are image acquisition (Soda *et al.*, 2006), pre-processing (Qi *et al.*, 2015), segmentation (Percannella *et al.*, 2012; Perner *et al.*, 2002), mitotic cell recognition (Foggia *et al.*, 2010; Iannello *et al.*, 2014), fluorescence intensity classification (Soda and Iannello, 2006; Soda *et al.*, 2008) and staining pattern classification (Hiemann *et al.*, 2009; Hiemann *et al.*, 2007; Soda and Iannello, 2009).

The above mentioned techniques are important for HEp-2 cell classification process, but only a few efforts have been committed to study the benefits of the pre-processing and segmentation techniques. The reason of this technique is not fully applied to this medical image because it can only give a minor effect in the classification process. Thus, most of the researchers that involved in this field just overlook these techniques and focus on the classifier techniques. Still, applying pre-processing and segmentation method could possibly improve the classification. The

pre-processing is capable to provide a better quality cell features in image through image enhancement process, while segmentation which segregates important features could improve the classification performances. Recently, researchers have found three significant features of HEP-2 cell image that could affect the classification accuracy. These three features are fluorescence intensity, shape and texture of HEP-2 cell (Di Cataldo *et al.*, 2014; Foggia *et al.*, 2013; Qi *et al.*, 2015; Wiik *et al.*, 2010).

Previously, specialists have analysed the variability between a set of classification on fluorescence intensity, which is divided into three classes (i.e. negative, intermediate and positive HEP-2 image) as mentioned in Section 1.1. However, certain specialist gives a different intensity labels on the same images in spite of their comparable experience (Foggia *et al.*, 2013). Since intermediate intensity samples usually exhibit low contrast, experts in this field have difficulties to classify the fluorescence intensity, which can affect the performance of the subsequent phase. Di Cataldo have highlighted the under segmentation of foreground cell happen because of the non-uniformity of the HEP-2 image (Di Cataldo *et al.*, 2014). Usually, each foreground cell in the image does not have the same fluorescence intensity value. This difference could affect the size of the cell during the segmentation process. Some of the foreground cells could be bigger and some of them could be smaller, which depend on the fluorescence intensity on the cell. Oversize and undersize of the foreground cell can give a false information (e.g. texture and shape) during features extraction. HEP-2 pattern consists of texture or random fluorescent staining pattern either inside or outside of the cell (nucleoli), such as border, chromatin, non-chromatin, nucleoli, nucleolar and chromosome staining pattern (Wiik *et al.*, 2010). But, along with the staining, a lot of noise is probably located within the texture of the image (Qi *et al.*, 2015). Strong noise exists, especially on the HEP-2 intermediate images. The

existences of the noise as additional texture on the cell could cause the misclassification of the staining pattern.

Identification of HEp-2 staining pattern as mentioned in Section 1.1 requires the classifier uses three information from an image normally; positive or intermediate intensity, presence of mitotic cell and staining pattern for interphase cell. However, mitotic cells have received little attention in previous works even though it plays an important part in CAD growth, as a part to assist the IIF diagnostic procedure. Modern HEp-2 recognition only focused on the staining pattern of interphase cells instead of using both of interphase and mitotic cells in identifying ANA (Iannello *et al.*, 2014). Consequently, previous recognition algorithms produced a low classification accuracy since this algorithm did not reconsider the existence of the mitotic cell as a valuable information to identify the staining pattern.

1.4 Research Objectives

To overcome the after mentioned limitations, this research focuses on the following objectives:

- To develop a new pre-processing technique based on piecewise linear mapping for contrast enhancement of HEp-2 cell images.
- To develop a new segmentation technique based on Otsu thresholding for determination of significant features of HEp-2 immunofluorescence staining patterns.

1.5 Research Scope

The scope of this research is to support the growth of automated classification of IIF images by the development of new pre-processing and segmentation method for detection of mitotic cell in HEP-2 cell images. Both methods will be developed to have the following criteria:

- The proposed pre-processing method is able to enhance the low quality of the HEP-2 images. The method will assist both segmentation and feature extraction process. This method will also be able to enhance the contrast and also reduce noise of an image.
- The proposed segmentation method should be able to segment cell in the low quality HEP-2 image. This method is developed to solve the over segmented cell and separate the combined or overlapping cells.

It is worthy to have a pre-processing and segmentation process with an ability to detect both mitotic and interphase cells that could help to develop systems for automatic slide analysis of IIF. Since this study uses a MIVIA public dataset, the availability of HEP-2 images provided is the main constraint of these research in image analysis. Thus, the limitations of this study are based on the dataset that have been provided, which is dataset of positive and intermediate HEP-2 image. Other than that, HEP-2 cells only consist of six different patterns such as centromere, homogeneous, nucleolar, coarse speckled, fine speckled and cytoplasmatic. The development and analysis of proposed new pre-processing and segmentation algorithm for detection of mitotic cell in HEP-2 cell images is achieved using MATrix LABORatory (MATLAB) R2014b and worked in a computer workstation with an AMD Turion(tm) X2 Dual-Core Mobile RM-74 2.20GHz processor, 4.00 GB RAM and running Microsoft Windows 8 32-bit operating system.

1.6 Thesis Outline

This thesis is divided into five chapters. Chapter 1 (Introduction) describes briefly the topic of research with the reasons for performing detection of mitotic cell of HEp-2 image. Furthermore, this chapter also contains background study, research motivation, problem statement, research objectives, scope of research and thesis outline of this research.

Chapter 2 (Literature Review) presents literature reviews on the research areas that related to this thesis. This includes an overview of the fundamental concepts of digital image, image pre-processing, image segmentation and classification of staining pattern IFF image. In addition, it also reviews histogram equalization image enhancement and Otsu segmentation methods, which are used in this study. General overview of autoimmune diseases is also discussed in this chapter. Comparisons between several image pre-processing and image segmentation in previous studies are carried out in order to improvise the techniques for detection of mitotic cell. The advantages and limitations of these state-of-the-art methods are presented as well.

Chapter 3 (Methodology) presents the methods that are implemented in this project and explains in detail the processes involved. The information about data samples employed in this study is provided as well. Type of performance analysis and metric is also discussed.

In Chapter 4 (Results and Discussion), the simulated experimental results of the proposed pre-processing and segmentation methods and other state-of-the-art methods are compared and presented in this chapter. Discussion on the result obtained is provided in order to show and prove that the proposed method could achieve the objective of this study.

Chapter 5 (Conclusion) summaries this study and highlights the significances and contributions of this research work. In addition, future developments are also recommended in order to improve the performance of the developed system.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter presents an overview of the related fundamental research studies on digital image processing techniques. In addition, an analysis and classification of HEP-2 cell images in previous research are reviewed as well. The study focused on the pre-processing, segmentation and mitotic recognition for staining pattern classification of HEP-2 cells.

2.2 Digital Image

Visual information is the most vital type of information received, processed and translated by the human brain. One third of the cortical area of the human brain is able to process this visual information. Normally, the human eye is an image's translator, which is capable of altering a specific wavelength in the light spectrum into colours, which then simplified it to be digested by the brain. The range of electromagnetic (EM) wavelengths that can be captured by the human eye is called visible light. Human beings principally rely heavily on vision to make sense of the world. Humans look at things not only to identify and classify, but able to scan for differences, and acquire an overall rough feeling for a scene with a fast scan.

Human eye is imperfect. It's only capable to sense and digested a limited range of EM spectrum i.e. $0.43\mu\text{m}$ to $0.79\mu\text{m}$. Unlike human eye, which are limited to the visual band of the EM, imaging machines such as ultrasound, electron microscopy, and computer generated image are capable to use almost the entire EM spectrum (e.g. ranging from gamma to radio waves) (Gonzalez *et al.*, 2011). Thus, imaging machines

can operate on images generated by the sources that humans are not familiar and covers a wide and varied field application. The digital image is the visual information acquired by the computer-based technology with advanced digital imaging acquisition devices. Digital image may be defined as a two-dimension function by using amplitude at any pair of spatial coordinates, which called the intensity or grey level of the image at that point or pixel. It plays an increasingly important role in various aspects of our daily life, as well as in a wide variety of disciplines and fields in science and technology. An example of the applications such as photography, television, robotics, medical diagnosis and industrial inspection.

2.2.1 Binary and Grayscale Image

Digital image may be defined as a two-dimension (2D) function. This 2D function uses amplitude at any pair of spatial coordinates, which called the intensity or grey level of the image. Most of digital image processing operations are carried out using intensity images. An intensity image is a data matrix, whose values have been scaled to represent intensities. The elements of an intensity image, usually have an integer value in the range of 0 to 255. There are four types of images, i.e. binary, grayscale, true colour (as known as Red Green Blue (RGB)) and indexed. In this section, both binary and grayscale will be explained in detail as they will be used in this research. Binary image is represented by two intensities that are black and white. Each pixel can be either black (with 0 of intensity value) or white (with 1 of intensity value). Since there are only two possible values for each pixel, each pixel only consist of one bit. This type of images can therefore be very useful in terms of storage and image processing. The images such as text (printed or handwriting), fingerprints, or

architectural plans with a binary representation may be suitable for analysis process. Binary images often implemented in digital image processing as masks or as the output of specific operations such as segmentation, thresholding, and etc. (Gonzalez *et al.*, 2011; Solomon and Breckon, 2011; Sonka *et al.*, 2014). An example of binary image is shown in Figure 2-1(b). Grayscale image can be represented with each pixel by a shade of grey, usually in the range of 0 to 255 of intensities values. This range means that each pixel can be characterized by eight bits, or exactly one byte. This is a regular range for image file handling. In image processing, the grayscale can be calculated through rational numbers and image's pixel is stored in binary form. It is useful for programming because a single pixel can occupy a single byte. However, the accuracy provided in this format is barely enough to avoid visible artifacts. An example of grayscale HEP-2 image is shown in Figure 2-19(c).

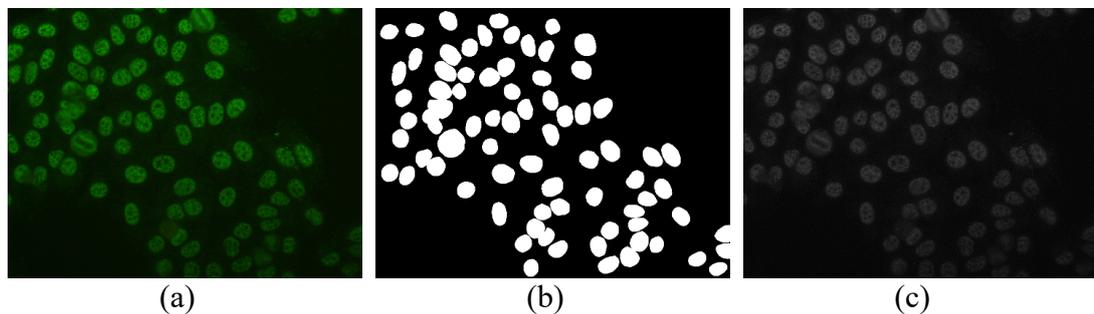


Figure 2-1: Differences type of HEP-2 cell images (a) original RGB image, (b) binary image and (c) grayscale image (MIVIA, 2010).

2.2.2 Colour Image

The digital image can also be presented as true colour images. The visual data representation of these image contains more than one channel that defines colour of a particular pixel. For visual standard results, it is necessary to provide with minimum

three colour channels for each pixel, which are translated as coordinates in colour space. The red, green and blue (RGB) colour space is one of the examples and commonly used in computer displays. A colour image that has three channels per pixel contains of the interpreted of both the chrominance and intensity of light. Moreover, the actual visual information stored in the digital image is the brightness information in each spectral band. For colour images, they contain three or more channels and can be introduced based on variety of colour spaces such as RGB, Hue Saturation Intensity (HSI), Cyan Magenta Yellow Black (CMYK) and etc. (Gonzalez *et al.*, 2011; Solomon and Breckon, 2011). In this section, both RGB and HSI will be explained in detail as they are used in this research

The RGB colour model is a colour model based on three basic primary colours namely red, green and blue. These basic colours are combined together in various ways to replicate a wide array of colours. At the early age of electronic technology, the RGB colour model already had a solid theory behind it. The theory is based on Young–Helmholtz theory of trichromatic colour vision that introduced by Thomas Young and Hermann Helmholtz in the nineteenth century (Hunt, 2005). Zero intensity for each channel gives the black colour in pixel, and full intensity of each channel gives a white colour in pixel. When the intensities for all the channels are the same, the pixel becomes black or a shade of grey. The result is a colourized hue when the intensities are different. It became more or less saturated, depending on the intensities of the primary colours engaged. An example of RGB model is shown in **Figure 2-2(a)**. The main function of these colour models is to display an image in electronic systems, such as computers and etc.

The HSI colour model is an attractive colour model for image processing applications since it represents colours similarly how the human eyes observe colours.

It comes from the three main colours namely hue, saturation and intensity. Hue channel is a colour, describes a pure apparent colour of the spectrum (i.e. red, green, yellow, orange, blue, and etc.), which represent a particular wavelength frequency. Saturation is the purity of the colour, could be more or less saturated colours, which refers to the amount of white light mixed with the hue's colours. Pastels are one of the examples of less saturated colours in saturation channel. Intensity refers to the value of brightness of light present. When intensities at its uppermost brightness, colours will become bright, and at its least brightness, colours become dim (Gonzalez and Woods, 2006). An example diagram of comparison between RGB and HSI coordinates model is shown Figure 2-2 below. The intensity axis represents the luminance information. The hue and saturation axis are coordinates on the rectangular plane of intensity. Hue is the angle, specified such that blue at 240 degrees, green at 120 degrees, and red is at zero (Davidson *et al.*, 2016). Hue thus indicates what humans simply understand as colour. Saturation is the magnitude of the colour vector estimated in the rectangular plane to intensity, and so indicate the difference between low saturation colours and high saturation colours.

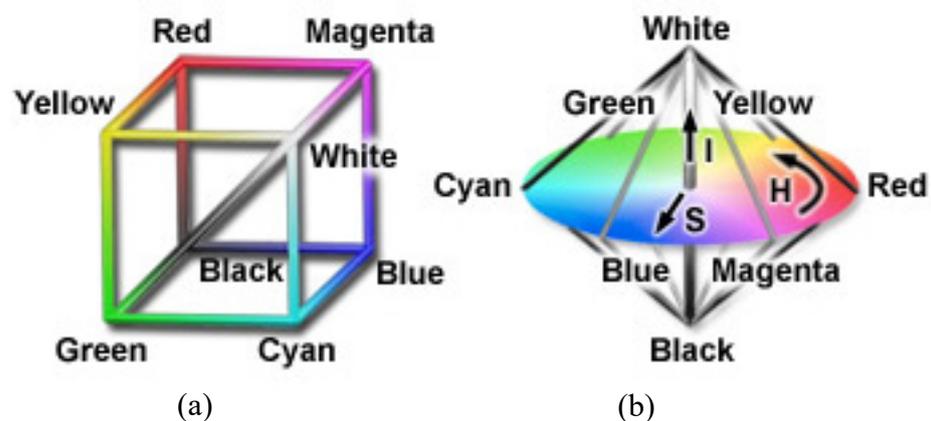


Figure 2-2: Colour model a) RGB and (b) HSI (Davidson *et al.*, 2016)

2.3 Image Processing

Image processing is the use of computer computation to perform a process to an image in order to enhance or to extract some useful information from it (Sonka *et al.*, 2014; Sridhar, 2011). Image processing can be classified into two types, which are digital and analogue image processing. Digital image processing refers to the alteration of the input digital images that performing a series of mathematical operations by using a digital devices. In contrast, analogue image processing refers to the adjustment to a signal that is the source of an analogue image by using an analogue device (Gonzalez and Woods, 2006). In recent years, digital image processing has become very popular. It gives a lot of advantages over analogue image processing as it gives a continuous development of algorithms in processing the input data, which is capable to avoid complications, e.g. the build-up of noise and signal distortion during processing. Moreover, digital image processing is among rapidly growing knowledges in forms of core research area within engineering and computer science disciplines.

In particular real-world task, digital image processing includes image acquisition, pre-processing, segmentation, representation and description, and recognition and interpretation. Firstly, acquiring image is applied to produce a digital image by using either a camera, or scanner, or any device which is capable to capture a digital image. The next step is pre-processing, the step taken before the main image processing task been applied to digital images. The task is to perform some basic processes in order to solidify the captured image to be more suitable for the next processing task. In this case, it may improve the contrast, removing noise, and identifying regions, and etc. After having a good image, image segmentation is applied to segregate a digital image into several regions and then sorting the separation of image into regions of the same characteristic. Representation and description refer to

extracting the specific features, which clarify the difference between objects. This task involves a process to detect curves, holes and corners which are able to distinguish the different objects. Lastly, recognition and interpretation refer to allocating labels to objects based on their descriptors from the previous task, and transfer meanings to those labels. This task can identify and interpret an individual object (McAndrew, 2004). The next sub-topic will be discussed in detail for both of contrast enhancement and segmentation as references for this research.

2.3.1 Image Pre-Processing

The use of digital processing techniques for pre-processing has received much awareness with the publicity given to applications especially in medical research. Image enhancement is one of the interactive pre-processing that consist of a collection of techniques. These techniques seek to improve the visual appearance of the poor quality image or to convert it to a better form suited for analysis by a human or a machine (Pratt, 2006). Examples include contrast and edge enhancement, pseudo colouring, noise filtering, sharpening, and magnifying. This process can be helpful in features extraction, image analysis, and visual information display. The enhancement process itself might not increase the characteristic information content in the image, but the process is capable to emphasize specified image characteristics. Generally, the main reason of low quality image are due to the improper process during acquisition process and poor quality of image acquisition devices (e.g. transmitting through a noisy channel, memory hardware in storage damaged, insufficient lighting, interruptions atmospheric disturbances and etc.), which cause an image suffer from poor contrast, unwanted noises present, loss of image detail, non-uniform illumination, blurring and incorrect colour balance (Cordelli and Soda, 2011; Iqbal *et al.*, 2010;

Miros *et al.*, 2015; Sheet *et al.*, 2010; Tang and NAM Isa, 2014). A poor image quality could affect the performance of the pre-processing, in extracting features from digital images and may harvest wrong information during the process. Therefore, it is important to enhance the quality of the original image in order to increase the performance of digital image processing.

Image enhancement approaches can be classified into two broad categories, i.e. spatial and frequency domain methods. The term spatial domain refers to the image plane itself, and approaches in this category are based on direct manipulation of pixels in an image. Frequency domain processing techniques are based on modifying the Fourier transform of an image. To be clear, only the spatial domain processing will be highlighted as it will be used in this research. The direct manipulation of spatial domain processing basically involves an operator which defines over some neighbourhood of pixels (x, y) of an image. An operator uses a rectangular sub-image or window area centred at that pixel and moving from pixel to pixel which cover every pixel in the image. The operator is applied at each pixel to produce the output at the same pixel and uses only the pixels in the area of the image spanned by the neighbourhood (Gonzalez and Woods, 2006; Gonzalez *et al.*, 2011; Solomon and Breckon, 2011).

Among all image enhancement techniques, grey level transformation function is one of the simplest techniques, which shows three fundamental functions used frequently for image enhancement, i.e. linear (negative and identity transformations), logarithmic (log and inverse-log transformations), and power-law (n^{th} power and n^{th} root transformations) (Gonzalez and Woods, 2006; Gonzalez *et al.*, 2011). The grey level transformation map plot shown in Figure 2-3 depicts several curves that fall into the above three types of enhancement techniques. The transformation results in

reversing of the grey level intensities of the image, thereby producing a negative like image, e.g. changing a picture of the pattern in the panda's body with inverted pattern. The negative image with grey levels in the range $(0, L-1)$ is obtained by the negative transformation as shown in Figure 2-3. The logarithmic function expresses high gradient in quarter of four of the functions and low gradient in the rest of the functions. This transform is used to expand the values of bright pixels and compress values of dark pixels. The opposite of this applies for inverse-log transform. The log function is able to compress the dynamic range of images with large variations in pixel values. Figure 2-3 illustrates logarithmic and inverse logarithmic functions. The last transformation function is also known as gamma correction. For various values of γ , different levels of enhancements can be obtained. Both log-transformation and power-law functions are presented in exactly same function curve, but the difference between these two is that using the power-law function has more possible transformation curve. Gamma correction is important in displaying an accurate image on a computer screen. Images that are not corrected by gamma correction with proper adjustment can look too dark. In addition, power-law transformations are useful for general-purpose contrast manipulation and highlighting an image.

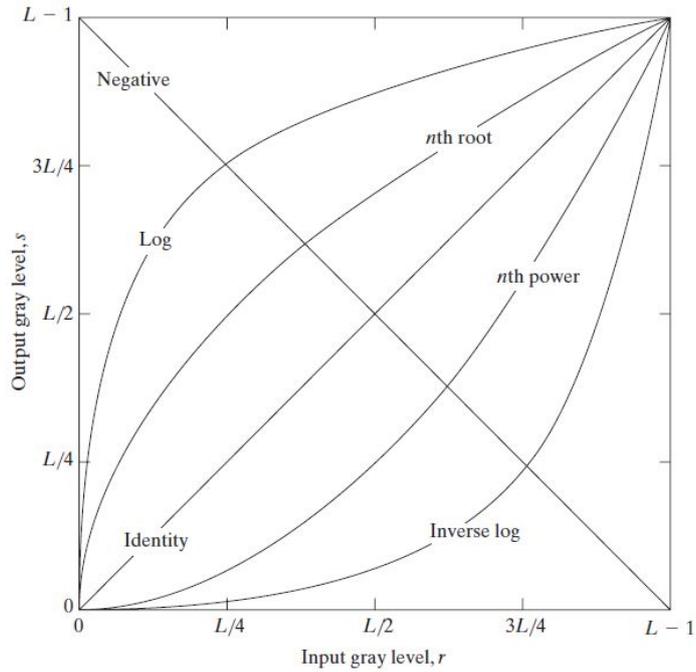


Figure 2-3: Grey level transformation map plot (Gonzalez and Woods, 2006; Gonzalez *et al.*, 2011)

Piecewise linear function is an alternative approach to the methods discussed for image enhancement before. The advantage of this function over others type of function as per discussed so far is that the shape functions can be more complex. This function has an advantage to perform the contrast adjustment to specific grey-level. Piecewise linear function can be categorized into three transformation functions namely contrast stretching, grey-level slicing and bit-plane slicing (Gonzalez and Woods, 2006; Patrascu, 2005; Russo, 2004). One of the well-known contrast-stretching transformation is the piecewise linear function. The theory behind contrast stretching is to expand the wide range of the grey levels in the image being processed. The transformation function can be multiple use depends on the algorithm. It can be a linear function (that produces a few changes to grey levels), thresholding function (that creates a binary image) and stretching function (that spread grey levels for contrast enhancement). Grey-level slicing is another transformation function that have the

capabilities to highlight any specific range of grey levels in an image. There are two common ways of doing level slicing. The first approach is to display a high or low value for all grey levels in the range of interest. This transformation, produces a binary image. The second approach is to brighten the desired range of grey levels, but preserves the background and grey-level tonalities in the image (Gonzalez and Woods, 2006; Pratt, 2006). Lastly, bit-level slicing by using the transformation function has the abilities to perform separation of grey-level based on bit planes. Assume that each pixel in an image is represented by 8 bits. The image is contained of eight bit planes, ranging from bit-plane 0 (the lowest bit) to bit-plane 7 (the highest bit). Usually the top four of the higher bit planes contains the important visual data. The other bit planes contribute to more deep details in the image. Advantage of separating a digital image into its bit planes is convenient for determining the comparative data played by each bit of the image. Also, this type of decomposition is beneficial for image compression process (Gonzalez and Woods, 2006)

Histogram Equalisation (HE) has also been recognized as histogram modelling and histogram manipulation. HE is a popular technique for pre-processing in contrast enhancement of an images (Gonzalez *et al.*, 2011; Natarajan and Ramesh, 2018; Tian and Cohen, 2018). It is the most frequently used methods due to its straightforwardness and comparatively better performance on almost all types of images. Histograms are the basis for numerous spatial domain processing techniques and it can be used effectively for image enhancement during digital image processing. It modifies the wide range of intensities which affecting the contrast of an image using non-linear and non-monotonic transfer function. This transfer function is able to remapping the intensity values in order to obtain a desired shape of the intensity histogram. HE performs its process by remapping the grey levels of the image based on the Probability

Distribution Function (PDF) and Continuous Distribution Function (CDF) of the input grey levels (Soong-Der and Ramli, 2003). Many researchers have already applied these HE into their contrast enhancement methods (Nithyananda and Ramachandra, 2016; Sheet *et al.*, 2010; Tang and Isa, 2014; Temiatse *et al.*, 2017). Normally, it can be classify into two principle categories, i.e. global and local contrast enhancement (Joung-Youn *et al.*, 2001).

Global method uses the histogram information of the entire input image for transformation function and it is suitable for overall enhancement. One of the methods is Conventional Histogram Equalization (CHE), which is the most widely used for image enhancement techniques as mentioned by (Tang and Isa, 2014). Despite of its advantages, this method suffered from shifting of mean brightness and over-enhancing on high frequency pixels of the image. This method alters the appearance of the resultant image and leads to loss of image details. Other than that, Modified Histogram Equalization (MHE), which proposed by (Abdullah-Al-Wadud, 2012). MHE technique manipulates the accumulation in the input histogram components before the equalization, which is capable to eliminate the possibility of the low histogram components to be compressed. This compression may cause some part of the image accidentally removed. Even though this global method is suitable for overall enhancement, it fails to handle the local brightness features of the input image. For example, if there are some grey levels in the image with very high frequencies, they dominate the other grey levels having lower frequencies and then causes significant contrast loss of other small ones.

Local method is a different version compared to the global technique which is able to overcome their contrast loss issues earlier. It uses a contextual region (tiles) that slides through every pixel of the image continuously and only the region of pixels