RED BLOOD CELL SEGMENTATION AND CLASSIFICATION METHOD USING MATLAB

ABDULLAH SALEH ALI ALGAMILI

A thesis submitted in partial
fulfillment of the requirement for the award of the
Degree of Master of Electrical Engineering

Faculty of Electrical and Electronic Engineering

Universiti Tun Hussein Onn Malaysia

DECEMBER 2016

DEDICATION

To my beloved father 'Saleh Ali', beloved mothers, darling wife, sons, beloved sisters and brothers and to all my inspiring friends**,** who have encouraged and guided me throughout my journey of education.

ACKNOWLEDGEMENT

Alhamdulillah, I am grateful to ALLAH SWT on his blessing and mercy for making this project successful.

First of all, I would like to express my heartiest appreciation to my supervisor, Prof. Muhammad Mahadi Abdul Jamil for his effort, guidance and support throughout this project. Without his advices, suggestions and guidance, the project would have not been successful in achieving the objectives.

My deepest appreciation also dedicated to my family, who are always there when it matters most.

Last but not least, I would like to appreciate all my lecturers who have taught me during three years period, many thanks for the lessons that have been delivered. Not forgetting Nayf Alduais, Wadhah Waheeb, Abdurahman Amer, and Bashar Ali as my friends and colleague in this project that giving me ideas, sharing sources of information and encouragement during the development of the project until it has been successfully completed.

ABSTRACT

Red blood cells (RBCs) are the most important kind of blood cell. Its diagnosis is very important process for early detection of related disease such as malaria and anemia before suitable follow up treatment can be proceed. Some of the human disease can be showed by counting the number of red blood cells. Red blood cell count gives the vital information that help diagnosis many of the patient's sickness. Conventional method under blood smears RBC diagnosis is applying light microscope conducted by pathologist. This method is time-consuming and laborious. In this project an automated RBC counting is proposed to speed up the time consumption and to reduce the potential of the wrongly identified RBC. Initially the RBC goes for image pre-processing which involved global thresholding. Then it continues with RBCs counting by using two different algorithms which are the watershed segmentation based on distance transform, and the second one is the artificial neural network (ANN) classification with fitting application depend on regression method. Before applying ANN classification there are step needed to get feature extraction data that are the data extraction using moment invariant. There are still weaknesses and constraints due to the image itself such as color similarity, weak edge boundary, overlapping condition, and image quality. Thus, more study must be done to handle those matters to produce strong analysis approach for medical diagnosis purpose. This project build a better solution and help to improve the current methods so that it can be more capable, robust, and effective whenever any sample of blood cell is analyzed. At the end of this project it conducted comparison between 20 images of blood samples taken from the medical electronic laboratory in Universiti Tun Hussein Onn Malaysia (UTHM). The proposed method has been tested on blood cell images and the effectiveness and reliability of each of the counting method has been demonstrated.

ABSTARK

Sel darah merah merupakan sel darah yang sangat penting. Ia merupakan diagnosis yang amat penting dalam melakukan proses awalan untuk mengesan penyakit seperti demam malaria dan anemia sebelum rawatan selanjutnya di berikan kepada pesakit. Sesetengah manusia penyakit boleh di kesan melalui kiraan bilangan sel darah merah. Kiraan sel darah merah memberi informasi penting dalam membantu diagnosis terhadap ramai pesakit. Kaedah konvensyen terhadap diagnosis sapuan darah RBC ialah dengan menggunakan microskop yang dikendalikan oleh patologi. Kaedah ini memerlukan proses dalam tempoh masa yang lama serta kajian dan penyelidikan. Dalam projek ini kiraan RBC automatik mencadangkan untuk melajukan tempoh masa dan mengurangkan potensi berlakunya kesilapan untuk mengenal pasti RBC. Pertama sekali RBC akan melalui pra-proses gambar dimana ia melibatkan global pengambangan. Seterusnya ia di teruskan dengan kiraan RBC dengan menggukan dua algoritma yang berbeza dimana titik perubahan pengsengmanan adalah perdasarkan jarak pertukaran, dan kedua adalah (rangkaian saraf buatan) “artificial neural network” (ANN) dengan memasukkan applikasi bergandung dengan kaedah regrasi.Sebelum menggunakan ANN klasifikasi terhadap langkah-langkah adalah diperlukan untuk mendapatkan data seterusnya dengan menggunakan pergerakan pegun. Masih terdapat kelamahan dan kekangan terhadap gambar berkenaan seperti ketepatan warna, butiran kurang tepat, pertindihan kondisi dan kualiti gambar.Oleh itu banyak kajian perlu di jalankan untuk mengendalikan masalah untuk menghasilkan analisis yang kuat untuk mencapai diagnosis untuk kegunan perubatan. Projek ini di laksanakan dengan penyelesaian yang baik dan membantu untuk menambah baikkan kaedah semasa supaya ia dapat memperbanyakkan berkebolehan, berkeupayaan tinggi dan berkesan. Akhir sekali projek ini akan di laksanakan dengan membuat perbandingan sekurang-kurangnya 20 keping contoh gambar darah yang di ambil daripada makmal perubatan Electronic di Universiti Tun Hussein Onn Malaysia (UTHM). Kaedah yang di cadangkan telah pun di uji terhadap gambar darah dan keberkesanan dan kebolehpercayaan terhadap kaedah kiraan telah pun di buktikan dengan demonstrasi.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMPOLES AND ABBREVIATIONS

RBC	Red Blood Cells
WBC	White Blood Cells
CBC	Complete blood count"
CAD	Computer-aided diagnosis
MCV	mean corpuscular volume
MCH	mean corpuscular hemoglobin
MSE	mean square error
PCNN	pulse coupled neural network
HT	Hough Transform
CHT	Circular Hough Transform
SEM	scanning electron microscope
ANN	Artificial Neural Network
CCL	connected-component labelling
GUI	Graphical user interfaces
GUIDE	GUI development environment

CHAPTER 1

INTRODUCTION

1.1 Project Background

In recent years, there has been an increasing interest in development of various algorithms for automated analysis of medical images in conjunction with advanced artificial intelligent, image processing and computer graphics techniques [1]. Blood cells are classified as erythrocytes (Red Blood Cells), leukocytes (White Blood Cells) and platelets (not considered real cells). The resultant count is the total number of erythrocytes and leukocytes expressed in a volume of blood [2]. Image segmentation and classification in natural and medical images is one of the most important topics in medical diagnosis [3]. As consequences, several automatic medical diagnosis systems have been developed to help doctors to diagnose disease particularly in red blood (RBC) and white blood (WBC) cells of human that provides valuable information to pathologists [1]. RBCs make up about 40% by blood volume. WBCs are fewer but larger in size than RBCs. Platelets are cell-like particles which are smaller than RBCs and WBCs [4]. Red blood cells are the most important kind of blood cell. Human beings use red blood cells as the main carriers that send oxygen to the all the human body's tissues via the blood flow tin the human being's circulatory system. Some of the human disease can be showed by counting the number of red blood cells, some of the human disease can be found by the measurement of the size of the cell or by the analysis of red blood cell's shape. The counting of the number of red blood cells is also an important item in normal blood examination [5].

Automatic counting systems have been available in the medical laboratories for the last 30 years. A low red blood cell count is the indicative for various diseases including anemia, blood loss, leukemia and malnutrition in People who have a less number of red blood cells.

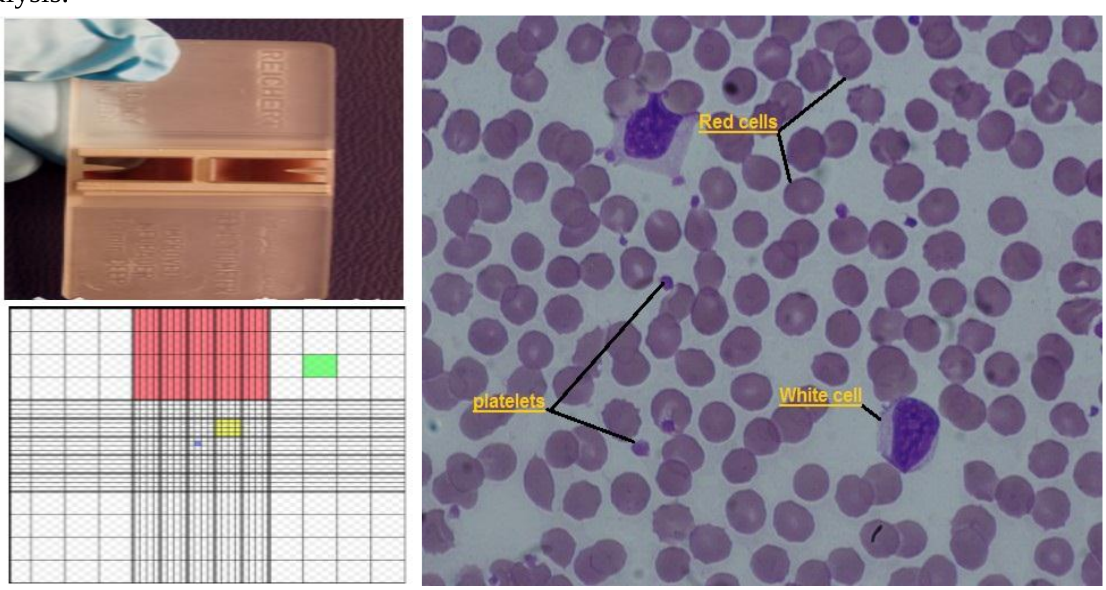
Pathologists can get valuable information regarding various blood oriented disorders through red blood cells counting in a blood sample. In the conventional method of red blood examination in a blood sample done by manpower; hence it has deficiencies such as less accuracy, poor reliability, and strong subjectivity. The diagnosis is defined as the process of finding out what kind of disease a certain patient has and those diagnosed must always be accurate. Sometimes a wrong diagnosis may lead to situation that wrong dosage of drugs given to the patient, some cases it may lead to loss of patient life [6]. The conventional method is not suitable for telemedicine system, In order to overcome these kind of situations. Nowadays, research in image processing have developed a wide variety of segmentation algorithms. Image segmentation technology separate image regions by using techniques that include edge detection and tracking, image gray-value threshold of the image, region growth and separation, fitting statistic models, pixel clustering, classification using neural networks, and so on [5]. some research have done some useful works especially in classifying blood cells from other cells, for example, classifying red blood cells from other cells such as white blood cells and platelets. Most of the researches have concentrated on the classification of white blood cells since most of the diseases are easy to determine by analyzing the change in white blood cells. However, red blood cells also provides some information about the abnormal condition in our body [6].

In this project, there are four steps involved in counting the red blood cells, image acquisition, segmentation, morphological operations, and classification. The acquisition step used the existing blood sample images. Next, the image segmentation and feature extraction is done by using a morphological technique in order to distinguish the red blood cells from background and other cells. Then the image classified into every single RBCs. The purpose of all is to count the number of red blood cells.

1.2 Problem Statement

The conventional device used to count blood cell is the haemocytometer. It was originally designed for the counting of blood cells. In order to count blood cell, physician must view haemocytometer through a microscope and count blood cells using hand tally counter. The overlapped blood cells cannot be counted by using haemocytometer. Furthermore, other cells besides normal RBC such as WBC, irregular shape of RBC and platelet are also elements that interfere during RBC counting as shown in figure 1.1. Normally, the counting task is time-consuming and laborious [7].

This project investigates automated counting of red blood cells and describes a method to classify the different shapes of clustered RBCs using the image processing techniques. Development of automated system that can identify or classify each of single clustered cells in the blood samples will help to overcome the burden of a manual process. This automated system will really helpful for hematologists or medical practitioner. Any abnormal reading of complete blood count (CBC) can give a sign of infection or disease. The result can influence physician to make the best response and monitor the drug effectiveness from the blood analysis.



(a) (b)

Figure 1.1: (a) Haemocytometer, and (b) blood cells type image in blood sample

1.3 Aim and Objectives

The aim of this project is to automate the counting process of Red Blood Cells using Segmentation and Classification methods, ease the working of the pathologist, to help the doctor make a better diagnosis. The following are the formulated objectives:

i. To develop automated RBCs counting system using segmentation and classification method.

ii. To test the segmentation and classification performance for system accuracy and reliability towards the RBCs count process.

1.4 Scope of works

This project is concerned with the scopes as following:

i. To develop RBC pre-processing image, measuring and feature extraction of red blood cells.

ii. To test the RBC image segmentation and classification system performance.

iii. To produce the summary of analyses for the comparison of the segmentation and classification performance for system accuracy and suitability judgment towards the RBCs count project.

1.5 Outline of thesis

This project is divided into five chapters. The scope of each chapter is explained as below: First chapter gives the background of the thesis, problem statement, aim and objective, scopes of works and outline of the thesis.

Chapter II is about the literature review, in which previous studies and theories related to this project are discussed and reviewed. It is also describe about RBC image segmentation and classification using several methods such as neural network and nearest mean. Literature review provides a background of this project and also gives and direction in this project.

Chapter III deals with a project methodology. It describes the detailed methods that have been used to conduct this project. This chapter proposes the method that involved in this project including image pre-processing using morphological, WBCs separation, threshold method, and feature extraction. The segmentation method that is proposed is watershed algorithm depends on Distance transform. The classification method that is proposed is ANN classification depend on regression fitting application in Matlab.

Chapter IV is for the results and discussion. This chapter will highlight the result of each method that is proposed in this project also the each of the performance evaluation conducted to find the most suitable method that provides by Matlab that suits with this RBC counting project.

Chapter V concludes this project. It also describes the next step that need to be done in the future works.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

RBC analysis through image processing method has been subject of interest of many researchers recently because of conventional method using haemocytometer is quite tedious and time consuming. Most of the previous work using MATLAB image processing toolbox as their main tool for analyses the RBC image because of its convenient for evaluating newly developed algorithm [7].

Complete blood count "CBC "process can be automated by computerized techniques which are more reliable and economic. Therefore, there is always a need to develop systems to provide assistance to hematologists and to alleviate the suffering or repetitive work of physicians. Computer-aided diagnosis (CAD) will establish methods for precise, accurate, robust and reproducible measurements of blood smear particles status while reducing human error and diminish the cost of instruments and material used. Afterwards, software provides the capabilities of upgrading and measurement variability without major changes and extra burdens. Distance transform watershed algorithm can avoid over segmentation issue and it has being used to handle overlapping cell. The main idea in this project is the using of masking and morphological operation function to eliminate unwanted objects [8]. The computerized steps into automated blood examination refers to a work done by Bentley and Lewis [9] in 1975. In this early work, authors used of colour information analysis to obtain integrated data on erythrocytes size in a numbers of normal and abnormal red blood cells. This project went after to address the correlation between

MCV (mean corpuscular volume) refers to the size of erythrocyte and MCH (mean corpuscular hemoglobin) refers to the concentration of hemoglobin in red blood cells. One decade after, the first fully automated processing of blood smear slides was introduced by Rowan [10] in 1986.

Many combination of method has been tested in the RBC image processing. The main challenge of such process is identification of normal RBC in the blood sample since the variety in shape of such cell may exist. It will become worse if the cell is clump and overlap in a group. Such a problem is a main challenge for a researcher to identify or to classify single RBC in this region.

Some of the previous works just ignore the overlapped RBC [11][12] and some of it proposed a method to classify/separate the overlapped cell [13][14][15] by using sophisticated image processing methodology. In this project, the focus is on an automated method for RBC counting including overlapping condition. In following section, explanation of various methods available to overcome such an issue is given in detail.

2.2 Complete blood count (CBC)

CBC consists of several counts of the major components in the blood cells. Everyone has a standard quantitative range as a reference for healthy women and men. Any counting value out of the range is considered as abnormal and physician will take the result for further action. In addition, the difference count also includes the division of WBC counts as five different types of WBCs in the measurement of CBC. They are neutrophils, lymphocytes, monocytes, eosinophils and basophils. The standard count for them is 60%, 30%, 5%, 4% and below 1% respectively from the total WBC counts. The below table 1.1 shows the standard CBC for the healthy person divided by gender [8].

Table 1.1: Normal blood count differentiated by gender [8]

Blood cell Gender types	**Gender**	
	Men	**Women**
RBC	4.5 - 6.0 million/microliter	4.0 - 5.0 million/microliter
WBC	4.5 - 11 thousand/ microliter	4.5 - 11 thousand/ microliter
platelet	150 - 450 thousand/ microliter	150 - 450 thousand/ microliter
Hematocrit	42% to 50%	36% to 45%
Hemoglobin	14 - 17 grams/100 milliliters	12 - 15 grams/100 milliliters

2.3 Image Acquisition and Enhancement

As shown in Figure 2.1, the image is taken from an optical microscope and the process of capturing the image will involve a blood smear process on the prepared sample. A blood smear is the process of preparing a blood sample on a microscope slide for observation. The process for displaying the RBC image will involve digitization of image from the optical image with 40 times (40X) objective which equal to approximately 400 magnification [3].

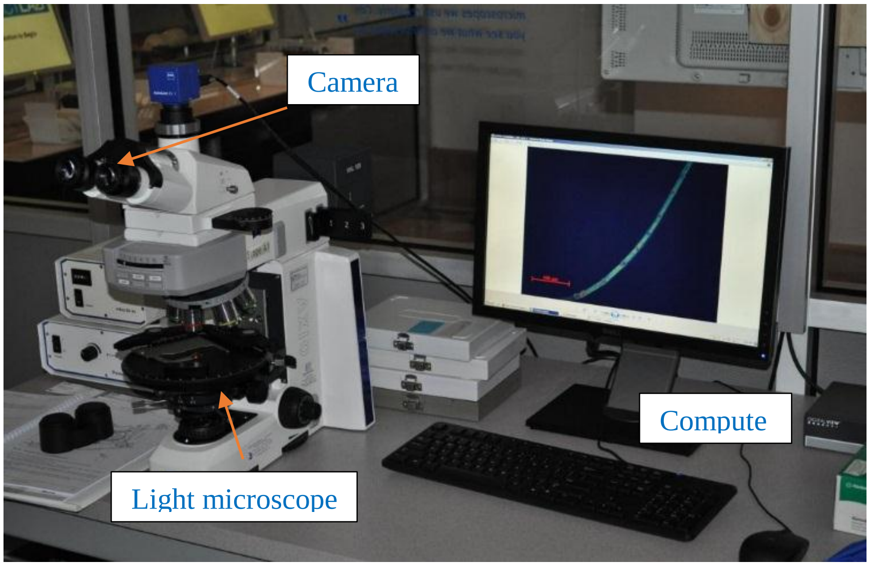


Figure 2.1: Image Acquisition Equipment [3]

The images are then being filtered to reduce or minimize noise. Several filtration techniques are used such as average filter and median filter [11][16][17]. Average filter is a linear spatial domain filter and function to decrease all noises in sample. It uses a defined filter mask to average grey level pixel in the neighborhood.

While median filter is a nonlinear spatial filter that changes the gray value at the center pixel with median value of the gray value of the pixel group. Edge detection reduces amount of data, filters useless information and preserves important structural details. Histogram equalization is used to adjust intensity value of image [18][14][19]. Contrast and brightness adjustment is a step that has been used in image processing. Both adjustments used histogram of interested image to display the range of intensity value of image [7].

2.4 Image Conversion

Previous method has been done on image conversion from original image to gray image [20][21][16][22]. The classification of images by gray-level pixels can reduce and simplify some image processing operations such as edge detection, edge smoothing, feature extraction, image processing and image registration [21].

RGB image conversion into binary image also has been done on previous work [23][30]. Conversion to binary image is helpful to identify foreground and background of the image. Since the binary image is in the black-and-white mode, the image can be easily recognized. This process will continue through the threshold method, the threshold will be used as a reference to identify the object and its background.

2.5 Cell Detection

Cell detection is one of the methods to identify the perimeter or boundary cell. One of the most popular cell detection that been used is edge detection method. Most edge detection methods such as Canny or Sobel edge detectors use image intensity gradient size to identify object boundaries in an image [12][14]. Edges in images are regions with very high contrast in intensity of pixels; detection of edges reduces the amount of data, filters useless information and preserves important structural details. This method is multi-step procedure; it first finds edges by looking for local maxima of the gradient of image.

The gradient is calculated using the derivative of a Gaussian filter which smooth the image in order to reduce noise and unwanted details as well as textures [25]. Edge detection does not work well between two overlapping cell. This is due to the change of intensity between two overlapping cells is very slow. That’s why it not suitable for detection of inter-cell-boundaries.

2.6 Feature Extraction

An image feature is a distinguishing primitive characteristic or attribute of an image. Some features are natural in the sense that such features are defined by the visual appearance of an image, while other, artificial features result from specific manipulations of an image. Natural features include the luminance of a region of pixels and gray scale textural regions. Image amplitude histograms and spatial frequency spectra are examples of artificial features. Image features are of major importance in the isolation of regions of common property within an image and subsequent identification or labeling of such regions [25].

2.7 Morphological Operation

Morphology is a broad set of image processing operations that process images based on shapes [22]. Morphological operations apply a structuring element to an input image, creating an output image of the same size. In a morphological operation, the value of each pixel in the output image is based on a comparison of the corresponding pixel in the input image with its neighbors. The number of pixels added or removed from the objects in an image depends on the size and shape of the structuring element used to process the image [14][24]. This adding and removing object is also called 'dilation' and 'erosion'.

Morphological operation is used to separate overlapped image. But there is only certain condition where morphological operation can be used. It is normally works to separate minor overlapped image. This method is not suitable to separate overlapped image.

All morphological processing operations are based on two simple ideas, hit and fit. Fit stands for the condition when all pixels in the structuring element cover on pixels in the image whereas hit signifies the condition when any of the pixels in the structuring element covers on a pixel in the image.

Next, morphological opening is the combination process of erosion and continued by dilation while morphological closing is using the concept of dilation and continued by erosion. In other words, the functions of morphological opening are to removes, break and diminished the connection or objects which not contain the structure elements. In contrary, morphological closing functions to join, fill and build connection and objects in the image [8].

However both of opening and closing operation have similar task which is smoothen the object contours but in different ways. For reconstruction, it using two images which is marker and mask as initial point of transformation and transformation inducer. The structuring element will act as connectivity element.. Reconstruction also can be applied for filling holes and clearing border objects with the combination of marker and mask function [8].

2.8 Image Segmentation

Segmentation is one of the most crucial tasks in image processing and computer vision [29]. As mentioned earlier blood cell contains RBC, WBC, platelet and sickle RBC. To identify each of this item, there are several method of image processing has been done.
Initial success on segmentation of medical imaging and blood segmentation was obtained with graph theory [26 [27] [28] which was used to navigate around edge pixels in an available image. However this approach has involved images of single objects manually located in an image. Further, it does not address the problems of multiple objects in the image. Therefore, object location, removal of extraneous edges (internal to the cell), or the selection of suitable starting and ending points for the graph search are the initial steps which are should considered. These arguments rely too heavily on quantitative analysis of manual aforementioned preprocessing steps where it is always an inconsistency with this argument. There is no consensus among researchers regarding what method can be applied for different conditions, and there is no general agreement about these initial steps.
Due to complexity of the problem at hand some of the papers are limited to image-based comparisons based on red cells segmented either manually [30] [31], or semi-automatically[32] [33] [34] [35] they proposed a framework with three steps to identify rolling leukocytes in microscopic images. This work profits gradient inverse coefficients of variation (GICOV) to discriminate leukocytes in-vivo environment. Authors first build a set of arbitrary number of ellipses by varying radii and orientation. Local maximum in gradient inverse coefficients of varying value denote presence of white blood cell in a close-by ellipse area where ellipses corresponding to locally maximum GICOV will be relaxed to flexible contours by active B-spline curves. Some of them used a method to estimate circularity ratio of cells [36]. Watershed algorithm is a method used to segment RBC in overlapping area [11][12][22]. However, it cannot handle when the overlapping area contain important information and it is hard to ensure the accuracy of segmentation due to the large error. The improvement has been done by combining mathematical morphology using corrosion and expansion algorithm with the principle of watershed

algorithm [13].The line operator with 20 line segments in various directions over a global Otsu threshold image has been applied. One of the previous work employed a K-means classification to detect of leukocyte and then counting RBC was addressed using watershed [40].

The distance transform is a useful tool employed in conjunction with the watershed transform. It computes the distance from every pixel to the nearest non-zero valued pixel. On previous work, distance transform is used combined with watershed for splitting clumped cells. The main function of distance transform is it detects the cell central point. Thus if the image is inconsistent in shape or overlapped, by using this method it can detect cell image based on the central point's [20].

The other recent common methods used for overlapping and clumped cells are concavity analysis and template matching [11]. On the other hand, concavity analysis is used to measure split lines for an overlapped cells. Nevertheless, it is only applicable for a pair of cells but useless against multiple overlapping cells. Plus, a very accurate segmentation is needed to apply this method. Other technique template matching which uses a template of RBC or clumping area to be matched to the object in the image able to separate small cell in shape and size. However, it is computationally expensive.

In a research, template matching method was combined with pulse coupled neural network (PCNN) since PCNN cannot cope with overlapped cells. However the accuracy decreases whenever the RBCs are overlapped totally because the area of one cell is considered as a template and the algorithm works only in 100x microscope scale [21].

Hough transform method also been used in previous work. The Hough Transform (HT) has been recognized as a very powerful tool for the detection of parametric curves in images [15][16][37]. It implements a voting process that maps image edge points into manifolds in an appropriately defined parameter space. The Circular Hough Transform (CHT) is one of the modified versions of the HT. The CHT concentrates to find circular patterns within an image. The Circle Hough Transform is designed to find a circle characterized by a center point.

Contour tracing approach has been used to segment scanning electron microscope (SEM) images. The method views contour detection and negotiating perceived problem areas one at a time but it still has lack when facing overlapping cells. It applies Bayesian tracking framework [38]. Consequently, the RBC segmentation of SEM image utilized shape reconstruction and multi-scale surface based on shape from shading technique combined with linear approximation [17]. Other than that, classification of RBC has been done through depth map and surface feature for different surface shapes [39].

2.8.1 Watershed transform

The algorithm introduced by Luc Vincent and Pierre Soille is based on the concept of "immersion". Each local minima of a gray-scale image I which can be regarded as a surface has a hole and the surface is immersed out into water. Then, starting from the minima of lowest intensity value, the water will progressively fill up different catchment basins of image (surface) I. Conceptually, the algorithm then builds a dam to avoid a situation that the water coming from two or more different local minima would be merged. At the end of this immersion process, each local minimum is totally enclosed by dams corresponding to watersheds of image (surface) Figure 2.2 shows this procedure graphically [22].

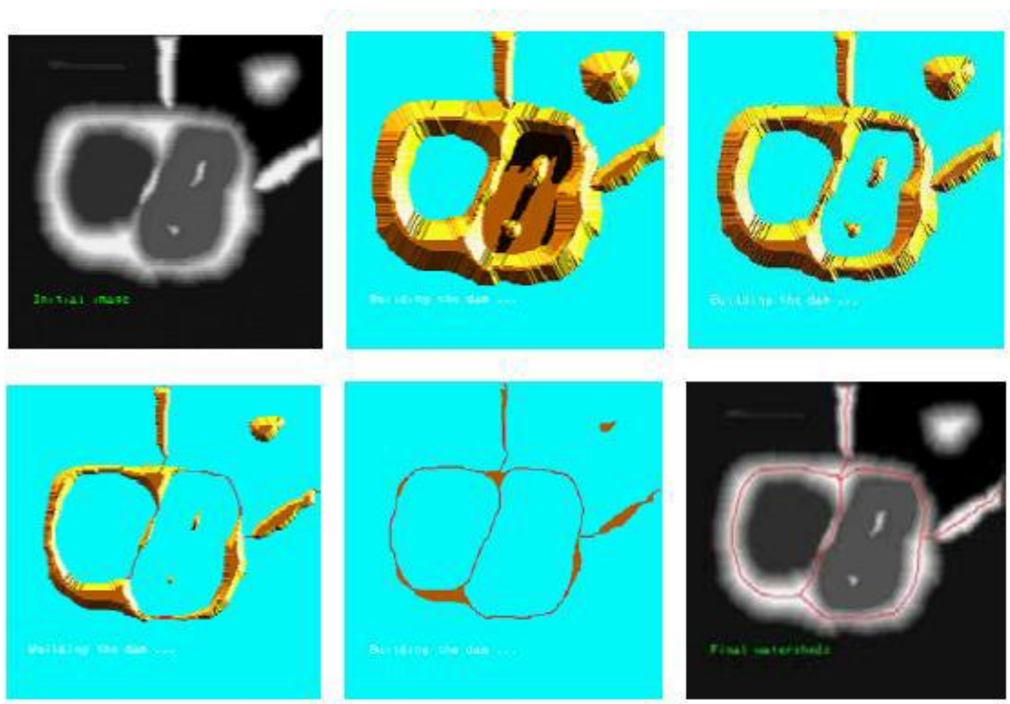


Fig 2.2: Flooding process in watershed transform [22]

The watershed transform has been widely used in many fields of image processing, including medical image segmentation, due to the number of advantages that it possesses: it is a simple intuitive method, it is fast and can be parallelized and an almost linear speedup was reported for a number of processors up to 64) and it produces a complete division of the image in separated regions even if the contrast is poor, thus avoiding the need for any kind of contour joining. It is appropriate to use this method to segment the high-resolution remote sensing image [22].

Watershed segmentation is one of the most potential algorithms that have been used for cell segmentation because of its adaptability to segment complex images [11]. The performance of watershed segmentation depends on how the correct markers are chosen, otherwise it suffers from over segmentation. The main advantage of the marker controlled watershed method over other previously developed remedies in segmentation methods is that it allows segmentation of particular objects and is thus applicable for counting applications [15].

Hemant et.al [15] used marker controlled watershed segmentation for counting of WBCs, RBCs and platelets. The blood images were pre-processed to filter noise, and then converted in to binary for colour distinction between the cells and to create a marker, finally watershed transform was applied. Although the blood cells were segmented, the overlapped RBCs were considered as one cell in their work. The accuracy of the segmentation is hence decreased. Karunakar [18] proposed an algorithm to automatically count RBCs using windows based applications in mobile phones. The basic segmentation algorithm is based on Otsu's threshold, morphological operations like opening, closing and filtering and also marker controlled watershed segmentation. They obtained a minimum accuracy of 80%; however the overlapping cells were not considered. Sharif et.al [24] proposed an algorithm to segment RBCs, by removing WBCs and platelets using masking and morphological operations, and then applying marker controlled watershed segmentation. The algorithm is capable of handling only touched cells but not overlapping cells.

Watershed segmentation has also been used by Gonzalez and Ballarin [44] for segmentation of biomedical images. They proposed the use of K-means clustering to cluster objects of interest for automatic detection of markers and the applied watershed transform on them. Their algorithm was simple and robust. Sun and Luo [45] proposed an algorithm to segment overlapping binary particle images using distance transform and then applied watershed segmentation. The overlap parameter was defined and a criterion was built to merge the spurious local minima and hence over segmentation was avoided.

Other than watershed segmentation, other methods like clustering, contour tracing, Hough Transform and Distance Mapping have also been proposed for RBCs count. Nasution and Suryaningtyas [46] have tried to compare RBCs count using Connected Component Labelling and Backprojection of Artificial Neural Network and obtained an accuracy of 87.74% and 86.97% respectively. The image was first pre-processed, and then the watershed segmentation was applied next. Region of interest was determined for clumping and unclumping cells. Edge detection was applied for clumping cells, and then the outer boundaries were removed. The separated clumping and unclumping cells were superimposed for the counting process [46]. Maitra, Gupta and Mukherjee [47] presented to segment and count RBCs in microscopic blood images using Hough Transform. Hough transform was used as a feature extraction tool to extract RBCs based on their shapes and sizes by detecting circles in the image after the pre-processing step [47]. However, the result of Hough transform depended on the image quality and object shapes.

Habibzadeh et al. [2] proposed to count RBCs and WBCs in a noisy blood image. Their work used Bivariate wavelet to noise reduction, Kuwahara filter for edge preservation, merged Otsu and Niblack method for binarization, size estimations for

WBCs and RBCs separation. Cells in their work were finally counted using Immersion watershed algorithms. The methods still confront with the overlapping cells caused the estimation size of cells fail and then effects the accuracy results of counting cells. Li et al. [31] presented an algorithm to segment touching cell nuclei of zebra fish using gradient vector flow tracking and separated the image into smaller regions with nucleus and applied adaptive thresholding. The paper provided a good algorithm to depress over segmentation and under segmentation. Vromen and McCane [48] presented a model to segment Scanning Electron Microscope Image of RBCs using contour tracing approach. Their results were satisfactory but still needed some improvement in removing falsely detected contours for minimally occluded cells. Figure 2.3 shows the limitations of the previously proposed algorithms confront with overlapping cells.

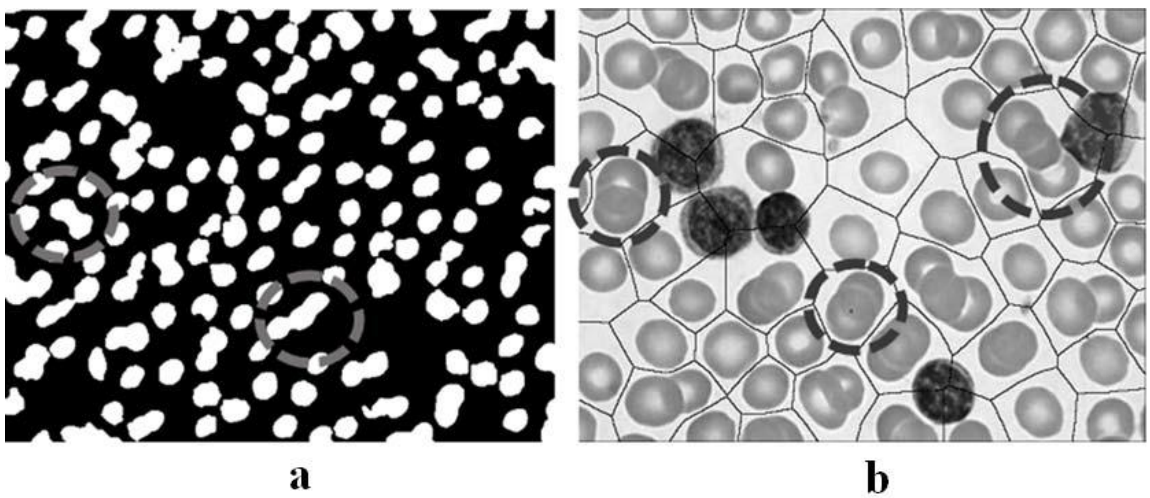


Figure 2.3: Example results from previously proposed methods which confront with overlapping cells (dash-line circle) (a) Masking and Watershed algorithm proposed in [24], (b) Morphological Watershed Transformation algorithm proposed in [15].

Khan and Maruf [49] presented a framework for cell segmentation by detection of centroids in microscopic images facilitated by computing template based pattern matching on the distance transform called distance mapping. The accuracy obtained was relatively higher than compared to other methods of segmentation. However this method over counted the cells since it had false positives. Nguyen, Duong and Vu [50] also employed distance transform for splitting clumped cells by detecting central points and estimating the cell size for cell count. In order to increase the accuracy of detecting central points, boundary-covering degree was applied to each point. Their algorithm had limitations on segmenting large number of overlapped cells.

2.9 Image Classification

A neural network is a computational structure inspired by the study of biological neural processing. There are many different types of neural networks, from relatively simple to very complex, just as there are many theories on how biological neural processing works. In image classification, Multilayer perceptron artificial neural network (ANN) is most common method used to identify and count RBC [18][14]. It performed by adjusting the value of the weight between the elements and the mean square error is calculated from there. The weight value is used to separate abnormal RBC and Normal RBC, thus the quantity of normal RBC is counted. In some other research, back projection of ANN compared with connected-component labelling (CCL) has been proved. Haematology analyser Sysmex KX-21 was used as benchmark for the comparison. The average accuracy of the CCL is 87.74% and the back projection ANN produced 86.97% of accuracy [41]. One of the previous study use method of Artificial Neural Network (ANN) classifier to classified RBCs as normal/abnormal it is Levenberg Marquardt algorithm with mean square error (MSE) cost function [3].

The images is recorded and converted into grayscale images for easy processing and given to the artificial neural network. The result found is the complete healthy RBC as well as incomplete non circular RBC i.e. sickled cells [25].

2.9.1 Artificial Neural Network (ANN) classifier

In order to be able to discriminate between RBCs clustering types in the image by using the selected features, a robust classifier should be used. The classification module is performed by using artificial neural network (ANN) classifier. The ANNs are a mathematical approximation of a biological brain, and have been identified as a useful framework for precise modelling of nonlinear response. It comprises a number of neurons connected together to form a network. The weights that linked between the neurons, i.e. *Wij* and *Wjk* are where the functionality of the network resides. Before the network can be useful, it needs to be trained. Basically the training session will alter the weights so that the error between the inputs and targets can be minimized. One of the fastest training approaches is Levenberg Marquardt algorithm with mean square error (MSE) cost function. Here we feed the data from the RBC features, i.e. [compactness and seven HU moments invariant], to the input neurons, and RBCs clustered types to the targets neuron during the training process. The networks setting is consider optimal for the highest recognition rate in both training and validation set [3].

2.10 Summary

RBC classification using image processing has been done in many previous works. As we know RBC analysis using image processing is not a new thing in medical diagnosis. Researchers focus on the improvement of the accuracy and promising result in their research by using many different methods. A framework for automatically classify the RBC into many type of cluster is proposed.

There are still weaknesses and constraints due to the image itself such as color similarity, weak edge boundary, overlapping condition, image quality, contrast, brightness, illumination and noise. Thus, more study must be done to handle those matters to produce strong analysis approach for medical diagnosis purpose. This project is hoped to can build a better solution and help to improve the current methods so that it can be more capable, robust, and effective whenever any sample of blood cell is analyzed.

2.11 Related Works

A Number of works have been conducted in the area of general segmentation and classification methods, Edge and border detection, region growing, filtering, mathematical morphology, and watershed clustering. Chen,Yang, and Petriu pointed out that the Watershed segmentation is an effective method for gray level image segmentation. Watershed algorithm is a method used to segment RBC in overlapping area [11][12][22]. However, it cannot handle when the overlapping area contain important information and it is hard to ensure the accuracy of segmentation due to the large error. The improvement has been done by combining mathematical morphology using corrosion and expansion algorithm with the principle of watershed algorithm [13] Thresholding is the simplest method of image segmentation. It can be used to create binary images from a grayscale image [23]. Marques stated that the Segmentation is one of the most crucial tasks in image processing and computer vision [29]. The major application of neural networks was devoted to the classification of RBCs is done by morphologic parameters [1] [41].

CHAPTER 3

METHODOLOGY

3.1 Introduction

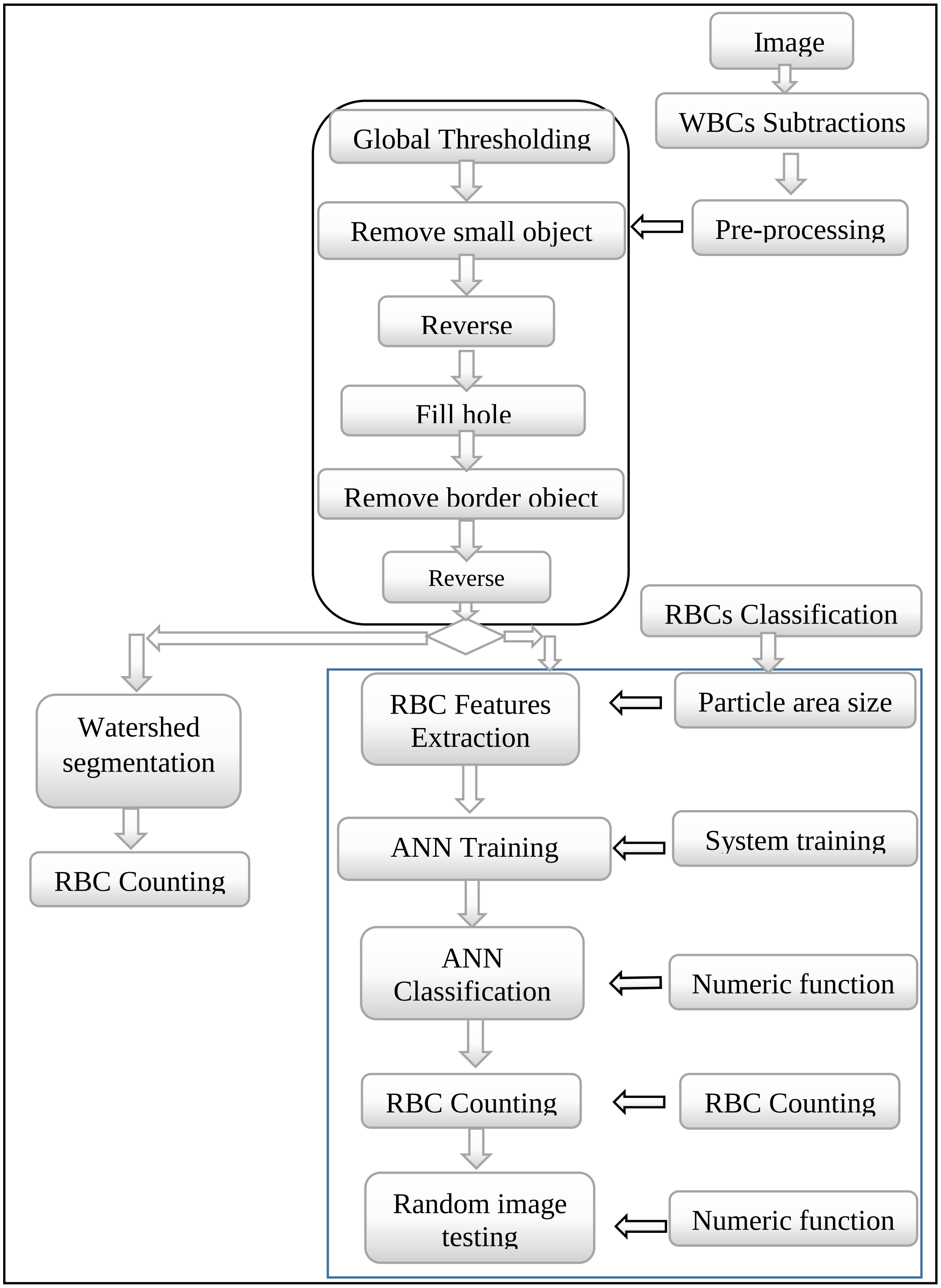


Figure 3.1: RBC counting flow diagram

There are several steps taken in for RBC counting system. The problem domain in this case is to count RBC from a blood cell image automatically. Whereby, the goal is to segment and classify RBC between many types of shape. The foundation methods that are taken in digital image processing will be similar one to another. Image pre-processing is not a one-step process: most solutions follow a sequential processing scheme whose main steps are described in this project. The complete RBCs counting flow diagram is shown in Figure 3.1.

3.2 Image acquisition

The blood cell image is taken from the medical electronic laboratory in Universiti Tun Hussein Onn Malaysia (UTHM). After preparing the slide of blood cell, it is been observed under conventional microscopy for 40 times (40X) objective which equal to approximately 400 magnification. The chosen image consists of all blood cell types and the aim of the study is to segment RBC from a subtracted WBC and platelet and classify it to get the exact counting number of all RBCs cell. Figure 3.2 shows the blood cell image for 40X objective.

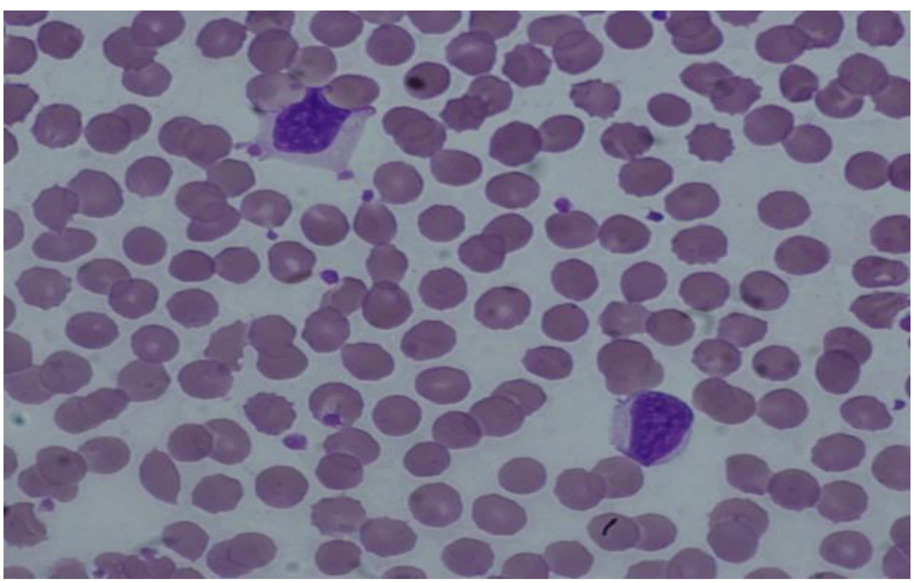


Figure 3.2: Original image of blood for 40X objective

3.3 Pre-processing

The goal of the pre-processing stage is to improve the quality of the acquired image. In image processing step the image is being enhance in term of quality level to be prepared for the next process. It is because the produced image may have some artifacts and illumination issue and this will be handled in the next process. As described earlier, blood cell contains RBC, WBC and platelet.

During pre-processing, unwanted image need to be removed. This process will remain RBC as the remaining object to be analyzed for next process. Possible algorithms to be employed during this stage including border image removal, removing small objects and filling holes of the RBC images.

3.3.1 Global Thresholding

The cells are separated from the background using global thresholding. The optimum threshold is calculated using the well-known Otsu's method. Otsu's method is a histogram based image thresholding method that separates the image pixels into two classes with minimal intra-class variance. In this project, global colour thresholding process is applied to convert the image from RGB to binary image.

3.3.2 Global Color Thresholding

Colour thresholding converts a colour image into a binary image. To threshold a colour image, specify a threshold interval for each of the three colour components. A pixel in the output image is set to 1 if and only if its colour components fall within the specified ranges. Otherwise, the pixel value is set to 0.

For a pixel in the colour image to be set to 1 in the binary image, its red value should lie between 130 and 200, its green value should lie between 100 and 150, and its blue value should lie between 55 and 115.

3.3.3 Binary Image

Binary images are encoded as a 2D array, typically using 1 bit per pixel, where a 0 usually means “black” and a 1 means “white” (although there is no universal agreement on that). The main advantage of this representation usually suitable for images containing simple graphics, text, or line art is its small size.

```
img1=imread('myrbcs11.jpg');
img2=rgb2gray(img1);
img3=im2bw(img2,graythresh(img1));
figure, imshow(img3)
```

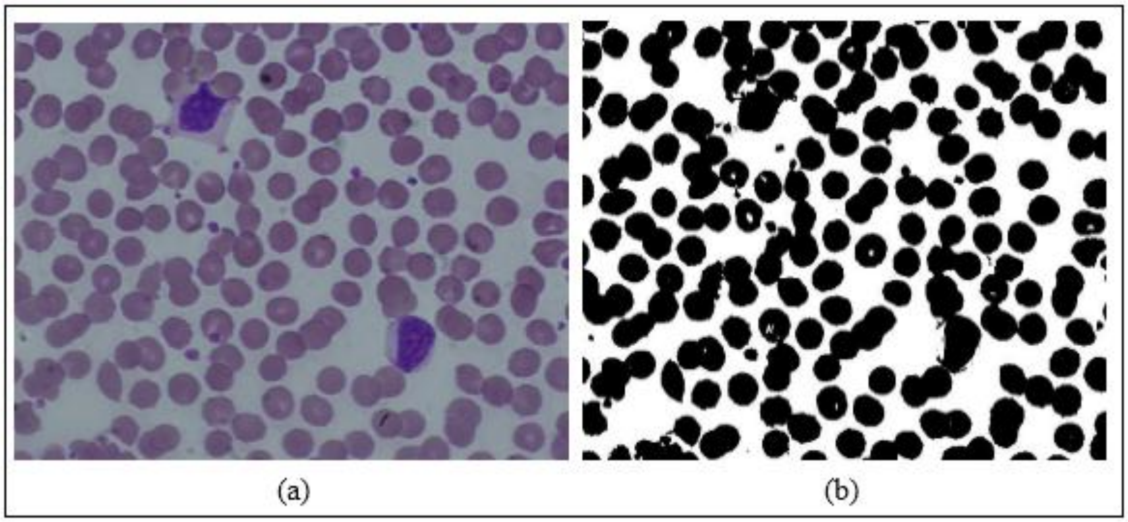


Figure 3.3: conversion from (a) RGB to (b) binary image

3.3.4 Morphological Operation

Mathematical morphological will be used to segment RBC based on elimination WBC appearance. Morphological image processing is based on a strong mathematical concept which been used to change the size, shape, structure and connectivity of objects in the image. It involves binary erosion, dilation, opening, closing and reconstruction. The technique also extended the use in grayscale image. Erosion plays the role to ‘shrinks’ and ‘thins’ objects in image while dilation used to ‘grows’ and ‘thickens’ objects in image.

REFERENCES

[1] Tomari, R., Zakaria, W. N. W., Jamil, M. M. A., Nor, F. M., & Fuad, N. F. N. (2014). Computer aided system for red blood cell classification in blood smear image. Procedia Computer Science, 42, 206-213.

[2] Habibzadeh, M. (2015). Automatic Segmentation and Classification of Red and White Blood cells in Thin Blood Smear Slides (Doctoral dissertation, Concordia University).

[3] Yang, L. (2009). Robust segmentation and object classification in natural and medical images. Rutgers University.

[4] Kareem, S., Morling, R. C., & Kale, I. (2011, May). A novel method to count the red blood cells in thin blood films. In Circuits and Systems (ISCAS), 2011 IEEE International Symposium on (pp. 1021-1024). IEEE.

[5] Cai, R., Wu, Q., Zhang, R., Fan, L., & Ruan, C. (2012, October). Red blood cell segmentation using Active Appearance Model. In Signal Processing (ICSP), 2012 IEEE 11th International Conference on (Vol. 3, pp. 1641-1644). IEEE.

[6] Venkatalakshmi, B., & Thilagavathi, K. (2013, April). Automatic red blood cell counting using Hough transform. In Information & Communication Technologies (ICT), 2013 IEEE Conference on (pp. 267-271). IEEE.

[7] Lias, J. (2015). Analysis of red blood cell (RBC) classification using Ni vision builder Ai (Doctoral dissertation, Universiti Tun Hussein Onn Malaysia).

[8] Sharif, J. M., Miswan, M. F., Ngadi, M. A., Salam, M. S. H., & Jamil, M. M. B. A. (2012, February). Red blood cell segmentation using masking and watershed algorithm: A preliminary study. In Biomedical Engineering (ICoBE), 2012 International Conference on (pp. 258-262). IEEE.

[9] Bentley, S. A., & Lewis, S. M. (1975). The use of an image analysing computer for the quantitation of red cell morphological characteristics. British journal of haematology, 29(1), 81-88.

[10] Rowan, R., & England, J. M. (1986). Automated examination of the peripheral blood smear. Automation and quality assurance in hematology, 129-177.

[11] Lepcha, P., Srisukkham, W., Zhang, L., & Hossain, A. (2014, December). Red Blood based disease screening using marker controlled watershed segmentation and post-processing. In Software, Knowledge, Information Management and Applications (SKIMA), 2014 8th International Conference on (pp. 1-7). IEEE.

[12] Karunakar, Y., & Kuwadekar, A. (2011, September). An unparagoned application for red blood cell counting using marker controlled watershed algorithm for Android mobile. In Next Generation Mobile Applications, Services and Technologies (NGMAST), 2011 5th International Conference on (pp. 100-104). IEEE.

[13] Huang, J. (2010, October). An improved algorithm of overlapping cell division. In Intelligent Computing and Integrated Systems (ICISS), 2010 International Conference on (pp. 687-691). IEEE.

[14] Poomcokrak, J., & Neatpisarnvanit, C. (2008). Red blood cells extraction and counting. In The 3rd International Symposium on Biomedical Engineering (pp. 199-203).

[15] Tulsani, H., Saxena, S., & Yadav, N. (2013). Segmentation using morphological watershed transformation for counting blood cells. IJCAIT, 2(3), 28-36.

[16] Mahmood, N. H., Lim, P. C., Mazalan, S. M., & Razak, M. A. A. (2013). Blood cells extraction using color based segmentation technique. International journal of life sciences biotechnology and pharma research, 2(2), 233-240.

[17] Wang, R., MacCane, B., & Fang, B. (2010, December). RBC image segmentation based on shape reconstruction and multi-scale surface fitting. In Information Science and Engineering (ISISE), 2010 International Symposium on (pp. 586-589). IEEE.

[18] Karunakar, Y., & Kuwadekar, A. (2011, September). An unparagoned application for red blood cell counting using marker controlled watershed algorithm for Android mobile. In Next Generation Mobile Applications, Services and Technologies (NGMAST), 2011 5th International Conference on (pp. 100-104). IEEE.

[19] Di Rubeto, C., Dempster, A., Khan, S., & Jarra, B. (2000). Segmentation of blood images using morphological operators. In Pattern Recognition, 2000. Proceedings. 15th International Conference on (Vol. 3, pp. 397-400). IEEE.

[20] Nguyen, N. T., Duong, A. D., & Vu, H. Q. (2010, October). A new method for splitting clumped cells in red blood images. In Knowledge and Systems Engineering (KSE), 2010 Second International Conference on (pp. 3-8). IEEE.

[21] Adagale, S. S., & Pawar, S. S. (2013, December). Image segmentation using PCNN and template matching for blood cell counting. In Computational Intelligence and Computing Research (ICCIC), 2013 IEEE International Conference on (pp. 1-5). IEEE.

[22] Kaur, A. (2014). Aayushi.: Image segmentation using watershed transform. Int. J. Soft Comput. Eng.(IJSCE), 4(1), 5-8.

[23] B. Bobier and M. Wirth.(2008) Evaluation of binarization algorithms. Technical report,Department of Computing and Information Science, University of Guelph,Guelph, ON, 2008.

[24] Chen, Q., Yang, X., & Petriu, E. M. (2004, October). Watershed segmentation for binary images with different distance transforms. In Proceedings of the 3rd IEEE International Workshop on Haptic, Audio and Visual Environments and Their Applications (pp. 111-116).

[25] Jambhekar, N. D. (2011). Red blood cells classification using image processing. Science Research Reporter, 1(3), 151-154.

[26] Martelli, A. (1976). An application of heuristic search methods to edge and contour detection. Communications of the ACM, 19(2), 73-83.

[27] Osowski, S., Siroić, R., Markiewicz, T., & Siwek, K. (2009). Application of support vector machine and genetic algorithm for improved blood cell recognition. Instrumentation and Measurement, IEEE Transactions on, 58(7), 2159-2168.

[28] Fleagle, S. R., Johnson, M. R., Wilbricht, C. J., Skorton, D. J., Wilson, R. F., White, C. W., ... & Collins, S. M. (1989). Automated analysis of coronary arterial morphology in cineangiograms: geometric and physiologic validation in humans. Medical Imaging, IEEE Transactions on, 8(4), 387-400.

[29] Marques, O. (2011). Practical image and video processing using MATLAB. John Wiley & Sons.

[30] Li, G., Liu, T., Nie, J., Guo, L., Chen, J., Zhu, J., ... & Wong, S. T. C. (2008). Segmentation of touching cell nuclei using gradient flow tracking. Journal of Microscopy, 231(1), 47-58.

[31] Albertini, M. C., Teodori, L., Piatti, E., Piacentini, M. P., Accorsi, A., & Rocchi, M. B. (2003). Automated analysis of morphometric parameters for accurate definition of erythrocyte cell shape. Cytometry part A, 52(1), 12-18.

[32] Robinson, R. D., Benjamin, L. J., Cosgriff, J. M., Cox, C., Lapets, O. P., Rowley, P. T., ... & Wheeless, L. L. (1994). Textural differences between AA and SS blood specimens as detected by image analysis. Cytometry, 17(2), 167-172.

[33] Costin, H., Rotariu, C., Zbancioc, M., Costin, M., & Hanganu, E. (2001). Fuzzy rule-aided decision support for blood cell recognition. Fuzzy Systems & Artificial Intelligence, 7(1-3), 61-70.

[34] Gering, E., & Atkinson, C. T. (2004). A rapid method for counting nucleated erythrocytes on stained blood smears by digital image analysis. Journal of Parasitology, 90(4), 879-881.

[35] Dong, G., Ray, N., & Acton, S. T. (2005). Intravital leukocyte detection using the gradient inverse coefficient of variation. Medical Imaging, IEEE Transactions on, 24(7), 910-924.

[36] Rathore, S., Iftikhar, A., Ali, A., Hussain, M., & Jalil, A. (2012). Capture Largest Included Circles: An Approach for Counting Red Blood Cells. In

Emerging Trends and Applications in Information Communication Technologies (pp. 373-384). Springer Berlin Heidelberg.

[37] Xiong, W., Ong, S. H., Kang, C., Lim, J. H., Liu, J., Racoceanu, D., & Foong, K. (2009, October). Cell clumping quantification and automatic area classification in peripheral blood smear images. In Biomedical Engineering and Informatics, 2009. BMEI'09. 2nd International Conference on (pp. 1-5). IEEE.

[38] Vromen, J., & McCane, B. (2009, November). Red blood cell segmentation from SEM images. In 2009 24th International Conference Image and Vision Computing New Zealand. IEEE (pp. 44-49).

[39] Wang, R., & McCane, B. (2008, December). Red Blood Cell Classification through Depth Map and Surface Feature. In Computer Science and Computational Technology, 2008. ISCSCT'08. International Symposium on (Vol. 2, pp. 339-342). IEEE.

[40] Wei, X., Cao, Y., Fu, G., & Wang, Y. (2015). A counting method for complex overlapping erythrocytes-based microscopic imaging. Journal of Innovative Optical Health Sciences, 8(06), 1550033.

[41] Nasution, A. M. T., & Suryaningtyas, E. K. (2008, December). Comparison of red blood cells counting using two algorithms: Connected component labeling and backprojection of artificial neural network. In PhotonicsGlobal@ Singapore, 2008. IPGC 2008. IEEE (pp. 1-4). IEEE.

[42] Nasution, A. M. T., & Suryaningtyas, E. K. (2008, December). Comparison of red blood cells counting using two algorithms: Connected component labeling and backprojection of artificial neural network. In PhotonicsGlobal@ Singapore, 2008. IPGC 2008. IEEE (pp. 1-4). IEEE.

[43] Jha, K. K., Das, B. K., & Dutta, H. S. (2014, March). Detection of abnormal blood cells on the basis of nucleus shape and counting of WBC. In Green Computing Communication and Electrical Engineering (ICGCCEE), 2014 International Conference on (pp. 1-5). IEEE.

[44] Gonzalez, M. A., & Ballarin, V. L. (2009). Automatic marker determination algorithm for watershed segmentation using clustering. Latin American applied research, 39(3), 225-229.

[45] Sun, H. Q., & Luo, Y. J. (2009). Adaptive watershed segmentation of binary particle image. Journal of microscopy, 233(2), 326-330.

[46] Nasution, A. M. T., & Suryaningtyas, E. K. (2008, December). Comparison of red blood cells counting using two algorithms: Connected component labeling and backprojection of artificial neural network. In PhotonicsGlobal@ Singapore, 2008. IPGC 2008. IEEE (pp. 1-4). IEEE.

[47] Maitra, M., Gupta, R. K., & Mukherjee, M. (2012). Detection and counting of red blood cells in blood cell images using Hough transform. International journal of computer applications, 53(16).

[48] Vromen, J., & McCane, B. (2009, November). Red blood cell segmentation from SEM images. In 2009 24th International Conference Image and Vision Computing New Zealand. IEEE (pp. 44-49).

[49] Khan, H. A., & Maruf, G. M. (2013, May). Counting clustered cells using distance mapping. In Informatics, Electronics & Vision (ICIEV), 2013 International Conference on (pp. 1-6). IEEE.

[50] Nguyen, N. T., Duong, A. D., & Vu, H. Q. (2010, October). A new method for splitting clumped cells in red blood images. In Knowledge and Systems Engineering (KSE), 2010 Second International Conference on (pp. 3-8). IEEE.

[51] Rahmani, M. K. I., Pal, N., & Arora, K. (2014). Clustering of Image Data Using K-Means and Fuzzy K-Means. International Journal of Advanced Computer Science and Applications, 5(7), 160-163.

[52] Bradley, P. S., & Fayyad, U. M. (1998, July). Refining Initial Points for K-Means Clustering. In ICML (Vol. 98, pp. 91-99).

[53] Nandy, S., Sarkar, P. P., & Das, A. (2012). An Improved Gauss-Newtons Method based Back-propagation algorithm for fast convergence. arXiv preprint arXiv:1206.4329.