AUTOMATIC RED BLOOD CELL COUNTING BASED ON PARTICLE AREA

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ABSTRACT

In medical area, red blood cell (RBC) counting and analysis contributes important information in pathological diagnosis regarding disease such as anemia and low level of hemoglobin concentration. Normally the blood sample is processed in laboratory using hematology analyzer and blood smear is viewed under microscope. The analysis and counting process is conducted manually by pathologist. The task is very laborious, tedious, time-consuming and very dependent to the skill. This project applied image processing techniques to develop a computer-aided system for automated RBC counting. The RBC images will be pre-processed in early stage using morphological operations and thresholding to obtain the images with good quality. Then, the RBC images will be classified based on the particle area size into single and multi-overlap cells. For counting the total number of the RBC, mathematical numeric function is used. The accuracy of the result is determined by doing comparison with the ground truth data. The proposed method has been tested to the RBC images and performs a reliable system for counting RBC.

ABSTRAK

Dalam bidang perubatan, analisis dan pengiraan sel darah merah menyumbangkan informasi penting bagi diagnosis patologi berkenaan penyakit seperti anemia dan level kepekatan hemoglobin yang rendah. Kebiasaannya sampel darah diproses di dalam makmal menggunakan “hematology analyzer” (iaitu sejenis peralatan elektronik yang menguji bahan secara kimia) dan sampel darah diperhatikan di bawah mikroskop. Analisis dan proses pengiraan dijalankan secara manual oleh ahli patologi. Tugasan tersebut sangat rumit, memenatkan, mengambil masa dan sangat bergantung kepada kemahiran. Projek ini mengaplikasi teknik pemprosesan imej untuk menghasilkan suatu system berkomputer yang dapat mengira sel darah merah secara automatic. Imej sel darah merah akan diproses awal menggunakan “morphological operation” dan “threshold” untuk mendapatkan imej yang berkualiti baik. Kemudian imej akan dikelaskan mengikut saiz “particle area” kepada satu sel dan sel yang berganda. Bagi mengira jumlah sel darah merah, fungsi matematik digunakan. Ketepatan keputusan akan dinilai dan dibandingkan dengan data asal (kiraan sebenar). Kaedah yang dicadangkan ini telah diuji dan menunjukkan keberkesanannya untuk mengira sel darah merah.

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LIST OF SYMBOLS AND ABBREVIATIONS

RBC	-	Red Blood Cell
WBC	-	White Blood Cell
Hb	-	Hemoglobin
O_2	-	Oxygen
CO_2	-	Carbon dioxide
ME	-	Myalgic Encephalomyelities
MS	-	Multiple Sclerosis
MATLAB	-	Matrix Laboratory (A programming software)
Open CV	-	Open source Computer Vision (A programming software)
NI	-	National Instrument
AI	-	Automated Inspection
RGB	-	Red Green Blue (colour channel)
CHT	-	Circle Hough Transform
ANN	-	Artificial Neural Network
CCL	-	Connected Component Labelling
PCNN	-	Pulse Coupled Neural Network
MSE	-	Mean Squared Error

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CHAPTER 1

INTRODUCTION

This chapter will describe the background, problem statement and objectives of the research. It consist the definition of the terms that being studied such red blood cell and image processing. This research is applying knowledge of image processing for solving some issues that exist in medical area especially in the red blood cell analysis.

1.1 Research Background

Blood is a connective tissue consisting of red blood cells (RBC), white blood cells (WBC), and platelets suspended in plasma. As a medium of transportation for the whole body, blood composition is very vital to be monitored when it comes to medical inspection. RBC or erythrocytes are normally in round shape, biconcave and flattened, about 7 µm in diameter and 2.2 µm thick as shown in Figure 1.1. Unlike WBC, RBC lack of nuclei and contains of Hemoglobin (Hb) protein to carry oxygen (O_2) and carbon dioxide (CO_2) molecules in respiration system. The shape provides an increased surface area and size for diffusion process between blood and tissues [14].

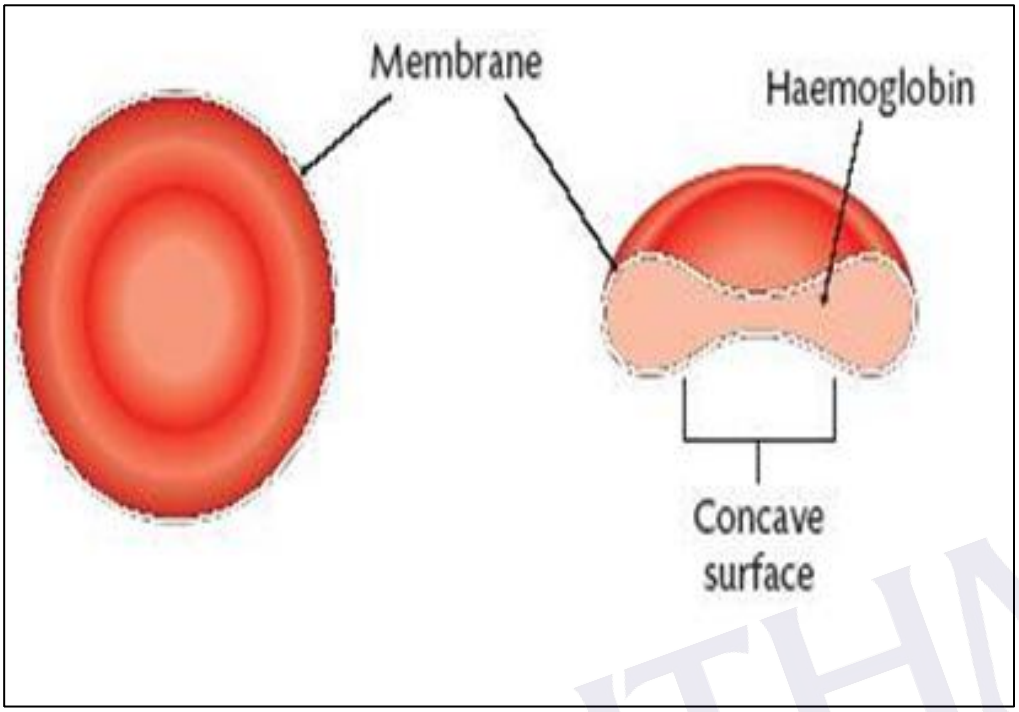

Figure 1.1: Red blood cell shape [22]

RBC can be found in normal and abnormal or sickle shape (Figure 1.2). Normal RBC is disc-shaped and looks like a doughnut without a hole in the center. It can live about 120 days. Sickle cells contain abnormal hemoglobin called sickled hemoglobin or hemoglobin S (HbS). Sickle cells usually indicate anemia disease and the shape is like a crescent. It tends to block the blood flow in small capillary and lead to severe pain and organ damage as well as risk of infection [26].

In medical area, RBC analysis contributes information of pathological diseases and condition. It helps doctors to determine the appropriate treatment to the patient. Any condition which there is an abnormally low of hemoglobin concentration or red blood cell count is indicating to anemia [14], low of specific vitamin [7] and might be secondary effect of several other disorders [24]. The shape of RBC and its deformability or abnormality has connection to the relevant disease such as Huntington's desease, Myalgic Encephalomyelities (ME) and Multiple Sclerosis (MS) [14].

However some factors should be taken into consideration when perform the RBC counting, including level age of people (which is children and adult, younger and older) and strenuous physical activity [24]. From that, the accurate diagnosis is very important to determine the exact disease and prepare the correct treatment to the patient.

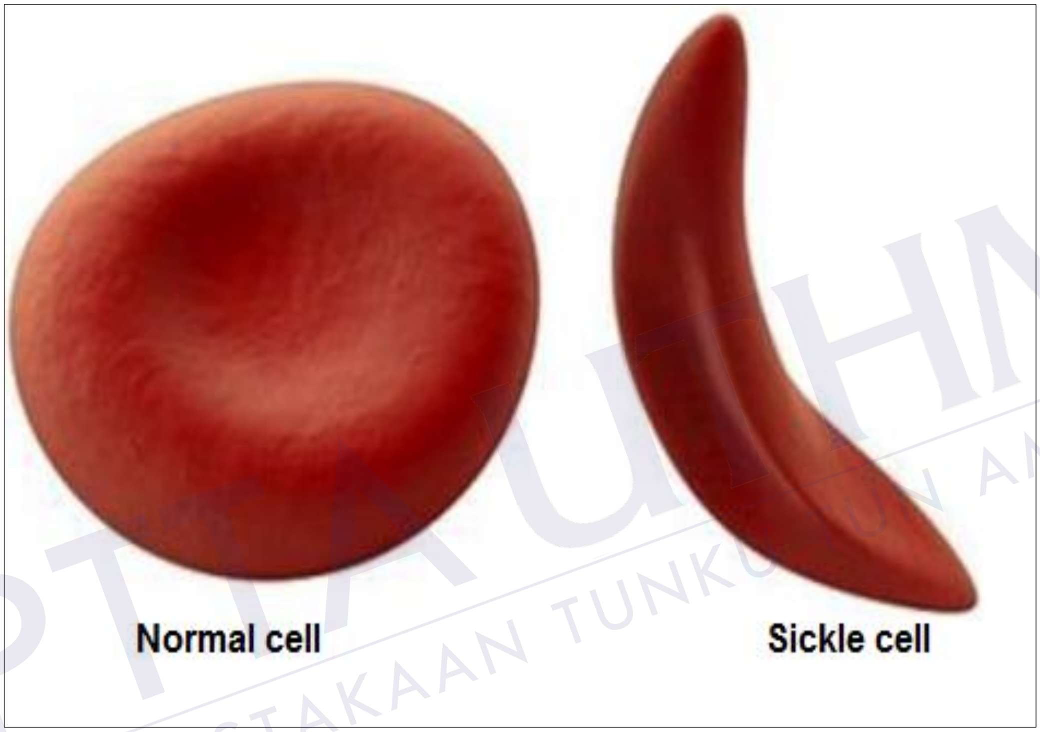

Figure 1.2: Normal and sickle RBC [26]

Normally the blood sample is taken and processed in the laboratory by using chemical electronic devices such as hemocytometer or hematology analyzer. This task is manually done by lab technologist. It is tedious and very dependent to their skill especially to count and analysis the cell through microscope [7]. The counting and analysis process is difficult when the cells are overlapped, usually such a finding neglected, and sometimes other particles such as platelet and WBC interfere too (Figure 1.3). The conventional method is time-consuming to complete and the counting task is laborious [5].

Image processing is very useful and widely used nowadays as alternative for improving the way of RBC counting and analysis. It improves the effectiveness of the analysis in term of accuracy, time consuming and cost. Such a procedure has been used in medical diagnosis for processing blood cell image especially for segmenting RBC from its complex environment in the blood. Segmentation of cell image is the key of medical image processing that directly impact the analysis and accuracy of the work [13]. From the analysis and counting, it will lead the doctor to make a decision of a patient health status. Various software provide tool for image processing such as MATLAB [7], Microsoft Visual Studio and Open CV [1], Lab VIEW [2], telemetry system [3] and in this project, National Instrument (NI) Vision Builder Automated for Inspection (AI). Most of the techniques can be found in the software, the importance is the understanding of how to apply it to obtain the desired outcome.

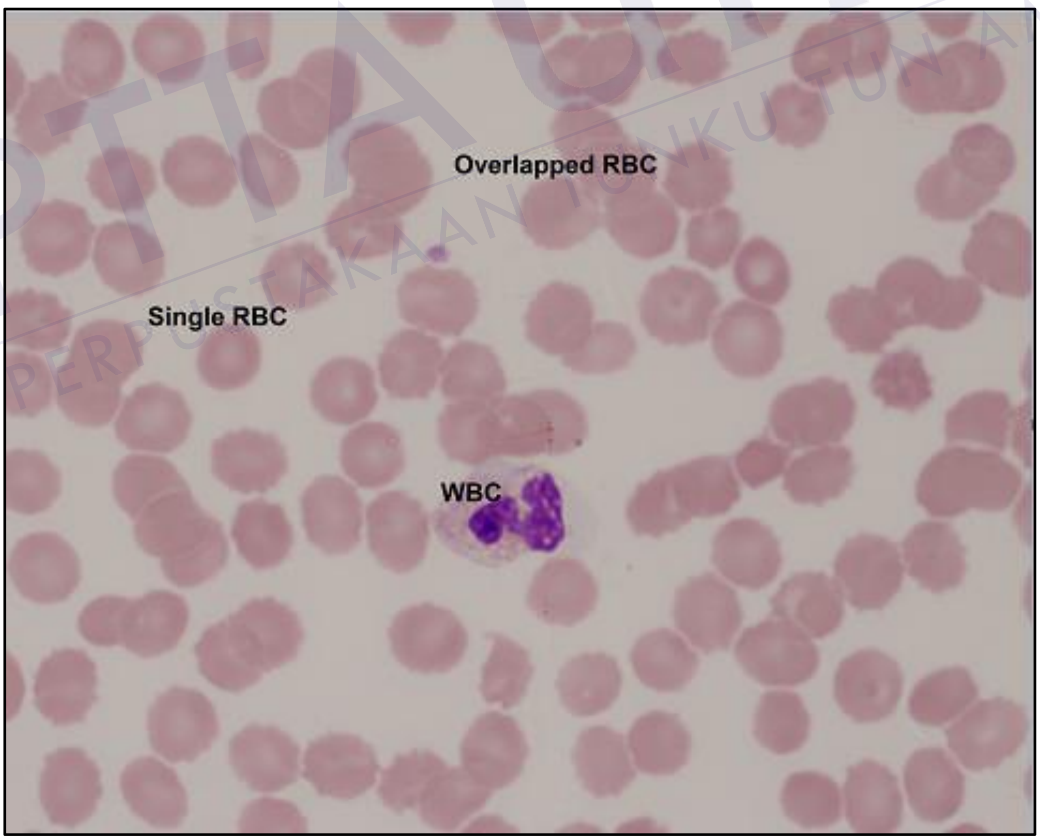

Figure 1.3: Image of Blood Smear

1.2 Problem Statement

Current method to count and analysis RBC is using a laboratory device known as hematocytometer. It consists of a thick glass microscope slide with a rectangular indentation creating a chamber of certain dimensions. This chamber is etched with a grid of perpendicular lines. It is possible to count the chamber of cells in a specific volume of fluid and calculate the concentration of cells in the fluid [20]. To count blood cells, lab technologist must view hemocytometer through a microscope and count blood cells using hand tally counter. However, the overlapped blood cells cannot be counted by using hemocytometer and be neglected. This method is laborious, tedious and time consuming to complete it. Knowledge, skill and experience will help to identify and count the cells.

RBCs are not suspended alone in the blood volume thus the issue of how to distinguish and segment RBCs from other components of blood such as WBCs, platelet and plasma is always being raised. Besides, those cells sometimes are found overlapping and clumping to each other. The complicated and high degree of overlapping condition is very difficult to solve. There is also possibility of having irregular and normal shape of cells that mix and stick together.

Development of automated system for analysis and counting RBCs in the blood sample is very useful. It will improve the existing method and help the pathologist and doctors to diagnose the patients accurately.

1.3 Aim and Objectives

The aim of this project is to count the total number of red blood cell in blood samples since medical diagnosis concern about that pretty much. The accuracy of the result for the RBC number in a sample image and fast performance of operation will also be the aims of this project. From the total count of RBCs, it can help the doctors determine the disease of the patient. To support the aims, there are objectives that will be formulated and focused in this project.

The objectives of this project are:

1. To develop a method for automatically segment out RBC region in various environment condition.
2. To construct a procedure for counting the RBC number in overlapping and non-overlapping condition.
3. To access the performance of the develop method in real condition.

1.4 Scope of the Project

This project will be focusing in the boundary that related to the stated objectives. It will involve the image processing techniques and come out with the algorithm for counting the RBC numbers.

CHAPTER 2

LITERATURE REVIEW

This chapter will discuss the previous researches that have been done within the same area of the topic. Literature review is important to tell whether the research is relevant and feasible to be studied. It also can be a guidance and boundary so that the research is reasonable and can produce some useful findings. The review is done on the techniques used for processing image and counting the RBC.

2.1 Image Processing in RBC Segmentation and Counting

Image processing is a successive tool to improve the capability of human to identify and extract the important information or data from images or video by using a machine language [7]. Image can be defined as a matrix of light intensity level that can be manipulated or operated by using computer algorithm [10]. Image processing provides many techniques to increase the effectiveness of decision making, shorten the consumed time and reduce the cost [4, 18].

The use of image processing for RBC analysis has been produced in previous studies. Many techniques and algorithm have been proposed to achieve the good findings. Recent studies on this area mainly focus in segmenting and classification of the cells for counting purpose.

Software based for developing automatic system to analysis RBC can reduce cost compared to expensive hardware that being used nowadays. There are several examples of software that commonly used to perform image processing such as MATLAB, Open CV, and Lab View. This project uses a part of embedded system of NI Lab View which is Vision Builder. Image processing techniques mostly were utilized to perform pre-processing, segmentation of the image, classification or identification and counting the cells.

The images basically are obtained from the samples of blood that being captured using light microscope which the process involve the preparation of blood smear in the lab [1, 7]. Blood smear is a process to put the blood specimen on the slide that being observed under the microscope. The image then will be filtered to reduce or minimize the noise such as using average or median filter. The conversion of colour from RGB into other channels helped in solving illumination problem [7] and as a preparation for the next stage of image processing.

According to Ventalakshmi in 2013 [3] histogram thresholding and morphological operator is used to segment the image as well to extract or differentiate RBC from other cells (WBC and platelets) and its background. For RBC counting process, Circle Hough Transform (CHT) technique is used to find a circle characterized by a center point and a known radius (Figure 2.1). CHT is modified version from classic Hough Transform that detect parametric curve in images. Another paper is by Wei *et. al* in 2009 [16] that adopting extended HT for cover different sizes of co-center circles and multiple radii called Multi-Scale Circular Hough Transform (MSCHT).

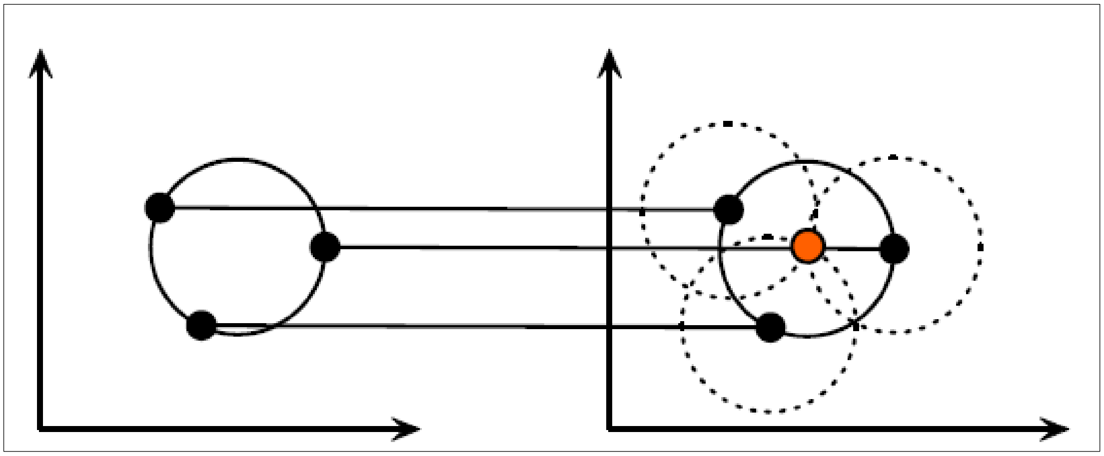

Figure 2.1 : Circle Hough Transform accumulation array [23]

Study by Sharif *et. al* in 2012 [7] proposed mathematical morphological operator to segment the image based on elimination of WBC. The step involved erosion, dilation, opening, closing and reconstruction. To separate the overlapped cells, marker controlled watershed algorithm is used where it based on the distance of overlapping cells (Figure 2.2). However, it still has weaknesses where it cannot handle the area that contains important information.

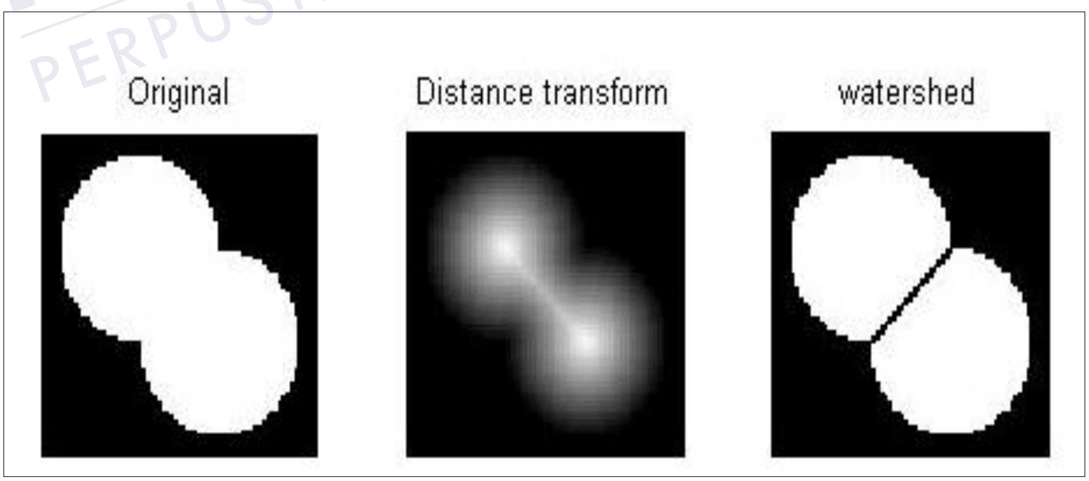

Figure 2.2: Separating overlapped objects using distance transform and watershed [redrawn using MATLAB]

In 2013, Tulsani *et. al* [6] also implemented the similar method of morphological watershed transform algorithm for segmentation of the cells (Figure 2.3). The paper also proposed a technique to eliminate WBC and platelet by using color conversion in YCbCr format. The Cb component has been chosen as it showed the clear appearance of WBC and platelets.

An improvement has been made before that by Huang in 2010 [13] that combined the watershed algorithm with mathematical morphology using corrosion and expansion algorithm.

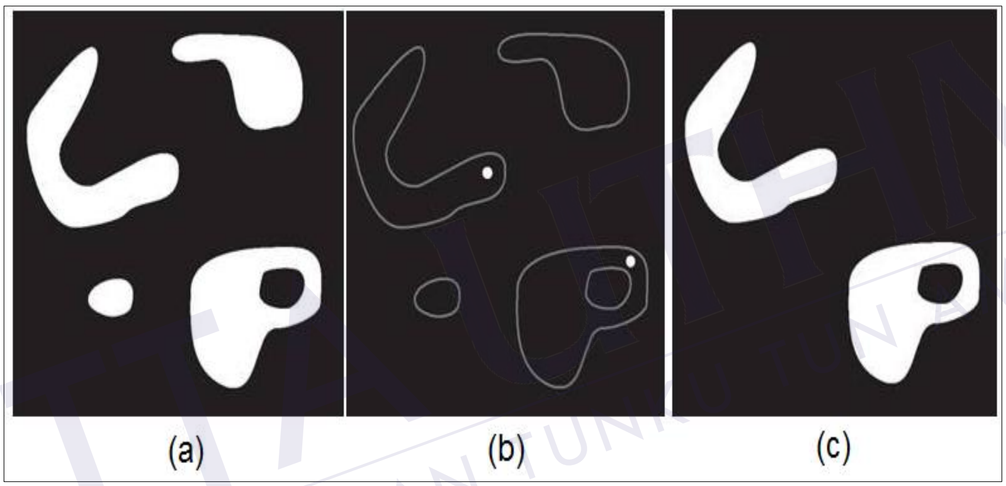

Figure 2.3: Morphological reconstruction to remove unwanted object (a) Mask (b) Marker (c) Result [redrawn from [6]]

A paper from Nguyen in 2010 [12] proposed algorithm for splitting the clumped cells. It used Otsu's algorithm for getting threshold in order to separate background and foreground and for detection of cell central points, it applied Euclidean distance transform. The advantages of this method are it can reduce the disadvantage of concavity analysis methods and model-based approach, detect other distorted structures and independent cell size and the merge process is much simpler compared to watershed segmentation merge process. Concavity analysis is measuring split lines in overlapped cells but has the problem that cannot cope with multiple overlapping cells. However, there was still weakness regarding quality of the image such as blur and non-focused image.

In study by Tomari *et.al* in 2014 [1], the image was processed through three main stages which is segmentation, feature extraction and classification. Segmentation process was done by using adaptive global thresholding. The extracted features such as moment values, area and perimeter are used to identify overlapping and non-overlapping region. For classification, artificial neural network (ANN) classifier is used to train and test the data.

Multilayer perceptron artificial neural network is a method used to classify RBC as proposed by Jambhekar in 2011 [10]. In the paper too, segmentation process was performed using histogram thresholding. By removing the overlapped cells, it successively counted the number of RBC and WBC. The result also showed the complete health RBC and incomplete non-circular (sickled cells) of RBC. The accuracy was 81% and increased when training was increased.

Another paper by Poomcokrak in 2008 [18] also implemented ANN to classify and count RBC. The function was performed by adjusting the weight between the elements and the mean squared error (MSE) was calculated. The value obtained is used to separate sickle RBC from normal RBC and the number of normal RBC is counted. The result shows 74% accuracy of counting RBC.

Natsution in 2008 [17] reported a comparison of two methods for counting RBC. It used backprojection ANN and connected component labelling (CCL). The result showed the accuracy was higher (86.97%) by using ANN back projection than using CCL (87.74%).

Other method used for segmentation and counting process by Adagale in 2013 [4] was Pulse Coupled Neural Network (PCNN) combined with template matching algorithm since PCNN alone cannot cope with overlapped cells. PCNN consider overlapped cells as a single cell. Template matching uses a template of RBC to be matched to the object for separating small cell in shape and size. However the accuracy decreased whenever the RBCs are overlapped totally because the area of one cell was considered as a template and the algorithm works only in 100x microscope scale.

Contour tracing approach has been used to segment scanning electron microscope (SEM) images as reported by Vromen in 2009 [15]. The method viewed contour detection and negotiating perceived problem areas one at a time but it still has lack when facing overlapping cells. It applied Bayesian tracking framework. The contours were categorized into three fully correct, minimally obscured and incorrect. The result showed 95.7% were either unobscured or minimally obscured with 0.6% false negative and 4.3% false positive.

A study by Ajay in 2014 [2] used Lab View software with Vision Assistant to apply morphological operation for diagnosis blood cells in real time. It implemented pattern matching method to identify the abnormal RBC for anemia analysis. It also developed a man-machine interface so that the procedure could be in real time and user friendly.

Using Lab View software too, Goyal in 2006 [21] proposed an artificial intelligent method for diagnosis anemia disease using the concept of fuzzy logic. It has two parts; find the group of disease using fuzzy interferences and another one using knowledge base.

From the previous researches, the process that apparently important was segmentation or classification of the RBC from the blood image. To execute the process, most of the studies applied the similar method as stated above. They also improved the classic method for getting the better result or to complement their objective.

In this project, there are two methods will be applied to count RBC number. First, segmentation and object detection based on particle area and then mathematical function to count. This method is implemented using NI Vision Builder AI. Second, finding and counting connected objects in binary image using MATLAB.

2.2 Summary

RBC counting and analysis using image processing is not a new thing in medical diagnosis. It always goes through improvement as a new research is carried out. The researchers offered many different methods as long as it can be giving a better accuracy and promising results. However 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 studies must be done to handle those matters to produce strong analysis approach for medical diagnosis purpose. This project is hoped 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.

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