2013 First International Conference on Artificial Intelligence, Modelling & Simulation

Automated Red Blood Cells Counting in Peripheral Blood Smear Image Using Circular Hough Transform

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***Abstract*—In medical field, the number of red blood cells (RBC) are used as an indicator to detect the type of diseases such as malaria, anemia, leukemia and etc. The problems using manual counting of RBC under the microscope is tend to give inaccurate result and errors. This paper proposed a method to count a total number of RBC in peripheral blood smear image by using circular Hough transform (CHT) method. The process involves preprocessing and segmentation a single cell image of RBC after cropping it to get the minimum and maximum radius of cell. Then, CHT method is applied to detect and count the number of RBC based on the range radius of cells. The results show that from ten samples of peripheral blood smear image, the accuracy using CHT method is 91.87%.**

Keywords- Red blood cells; peripheral blood smear image; circular hough transform; pre-processing; segmentation

I. INTRODUCTION

The main functions of red blood cells (RBC) or erythrocytes is carrying oxygen from lung to tissues elsewhere and transport carbon dioxide from tissues to the lung [1]. The shape of RBC is biconcave diskettes with the diameter around 7 to 8.5 µm and non nucleus [2]. Anemia, thalassemia and malaria are the types of diseases that related to RBC. Anemia is defined as the concentration of hemoglobin in the blood less than135 g/dl in adult male and less than 11.5 g/dl in females it has many symptoms such as weakness, lethargy and palpitation [3]. This is a disease cause low oxygen transport capacity of the blood, because of low red cell count or some abnormality of the RBC or the hemoglobin. Malaria is one of the serious parasitic infections of human. The malaria parasite spends part of its life-cycle in RBC, feeds on their hemoglobin and then breaks them apart, causing fever [4]. Thalassemia also a genetic disease that is caused by the duration of the short life of RBC [5].

Image processing is used to modify the pictures to improve the image quality. Hence, it can be analyzed in many applications such as in the result accuracy and time consuming [6]. The major steps in image analysis are preprocessing, image segmentation, feature extraction and counting. The most important and challenging step is image segmentation because the feature extraction and counting depends on the correct segmentation of RBC. Besides that, the uncertainties inherent in the microscope image to identify whether it is a cell or foreign bodies such as dust can interfere in the image analysis process [7].

In this paper, we propose an automated of RBC counting in microscopic image using circular Hough transform (CHT). The peripheral blood smear slides are obtained from the hospital is capture using a camera that had been attached to the eyepiece of the microscope. Then, the images is analyzed and counted using CHT method.

II. METHOD

A. *Samples Images*

The peripheral blood films or microscopic blood image is obtained from the KPJ Specialist hospital is used as the blood sample images. These images are captured in RGB format with the resolution 1280x1024 pixels and magnification of the images 40x10 pixels using Motic camera that attached to a microscope.

B. *Experimental setup*

The system is operated by placing the peripheral blood smear slide under the microscope. The camera attaches to the eyepiece of the microscope and adjusts the magnification until a clear image is obtained. The blood cell image is captured automatically and analyzed to determine the total number of RBC by applying CHT algorithm.

C. *Algorithm 1 (Measure minimum and maximum radius of RBC)*

Fig. 1 shows the flow chart to measure the minimum and maximum radius of RBC. Matlab R2012a software is used to analyze the features of RBC.

978-1-4799-3251-1/13 $31.00 © 2013 IEEE
DOI 10.1109/AIMS.2013.59

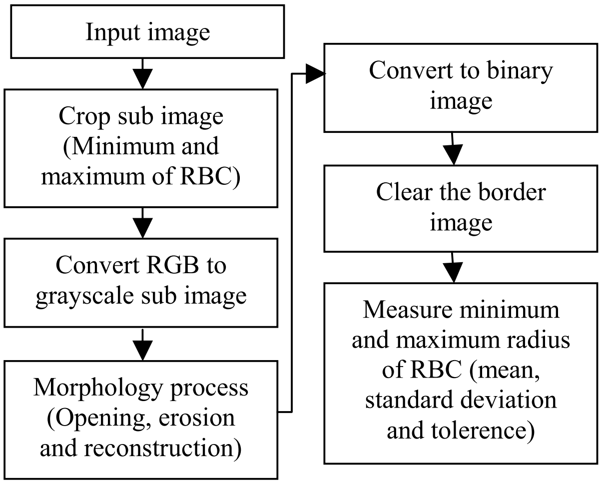


Figure 1. Flow chart of measurement minimum and maximum radius of red blood cells.

Fig. 2 shows the examples of input and output RBC images. The process starts by reading the captured image from the microscope in RGB format using camera automatically as shown in Fig. 2(a). Then, the users select and cropped the smallest and greatest cell contained in the blood sample image and convert into grayscale images using 'rgb2gray' function in Matlab This process is called thresholding. It continues by morphology process. Opening, closing and reconstruction are applied to remove the background noise and filled internal holes of cells to obtain better image before converting into a binary image as shown in Fig. 2(b). Otsu method is applied to set the threshold level and convert the gray level image into a segmented binary image as shown in Fig. 2(c). This process separates the cell image from the background. Next, 'imclear' function used to clear the border image to obtain a single cell as shown in Fig. 2(d).

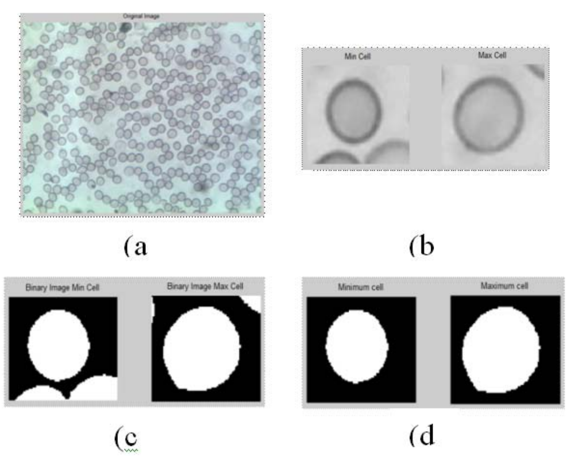


Figure 2. Sample image 1 (a) Microscopic image, (b) Cropped sub image in gray scale image (minimum and maximum of RBC), (c) binary image and (d) image after the border is cleared.

The segmented image is labeled and 'regionprops' function is used to measure the radius of the cell. Then, the mean, standard deviation and tolerence are calculated based on the minimum and maximum radius to find the radius range of cell. Equations (1), (2) and (3) are used to calculate the mean, standard deviation (StdDeviation) and tolerence respectively. Tolerence is the radius range of a cell that will be used in CHT techniques to draw the circle of cell.

$$Mean, X = \frac{MinRadius + MaxRadius}{2} \tag{1}$$

$$StdDeviation = \sqrt{([MinRadius - X)\hat{}2 + (MaxRadius - X)\hat{}2])} \tag{2}$$

$$Tolerence = \pm StdDeviation \tag{3}$$

D. Algorithm 2 (Counting the RBC using CHT technique)

CHT is a feature extraction techniques used in image analysis to detect the images in a circular shape [8,9].

Equation 4 is the equation of a circle parametric form. Where a and b are the center of the circle in the *x* and *y* direction respectively and *r* is the radius.

$$(x - a)^2 + (y - b)^2 = r^2 \tag{4}$$

The circle is describe by two following (5) and (6)

$$x = a + r\cos\theta \tag{5}$$

$$y = b + r\sin\theta \tag{6}$$

There are several steps to detect circles in an image using CHT. Firstly, any edge detection technique are used such as prewit, canny, sobel or morphological operation to detect all edge in the image [10]. Then, draw a circle on the desired radius at each edge point in the parameter space as shown in Fig. 3. Fig. 3 is the edge point in the image space and parameter space with constant or known radius. The highest number of circle intersect correspond to the center of the circle in the image [10].

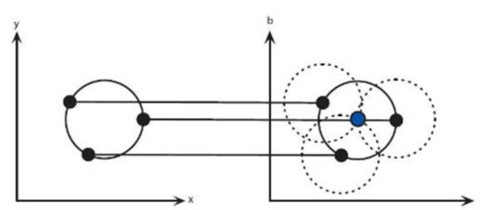
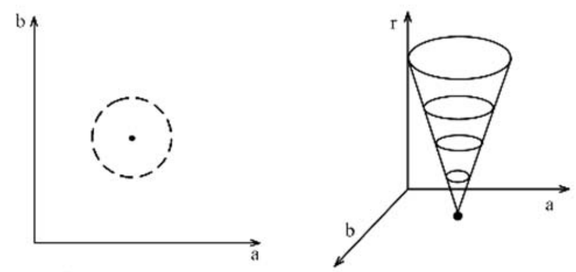


Figure 3. The points in image space and parameter space with constant radius.[10]

CHT have two cases which is circle detection with known radius and unknown radius. Circle detection with known radius, the CHT can be applied to find the center point (a,b) of the circle if we known a number of point that fall on the perimeter [11].

The same procedure for the circle detection with unknown radius like circle detection with known radius. However, the process for unknown radius are quite challenging [10] because it used three dimensions to search space (a,b,r) where a and b are the center point of the circle and r is the radius [11]. Fig. 4 shows the point in image space and 3D parameter space.



a) Image space (b) Parameter space

Figure 4. The points in image space and parameter space with a constant radius. [11]

Fig. 5 shows the RBC counting process using CHT technique.

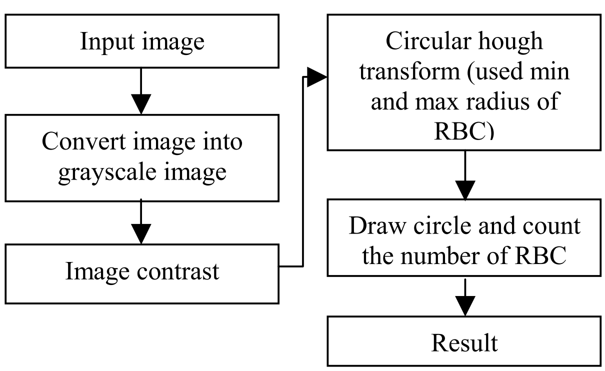


Figure 5. Flow chart of RBC counting process using CHT technique.

The input image is the image capture from the microscope using camera automatically. Next, the pre-processing technique is applied such as thresholding and image contrast to detect and extract the RBC from the images as shown in Fig. 6(a) and Fig. 6(b). Then, CHT is applied to the contrast image to analyze the RBC based on the minimum and maximum radius of RBC. Fig. 6(c) shows the result of detected the RBC after applying pre-processing and CHT process.

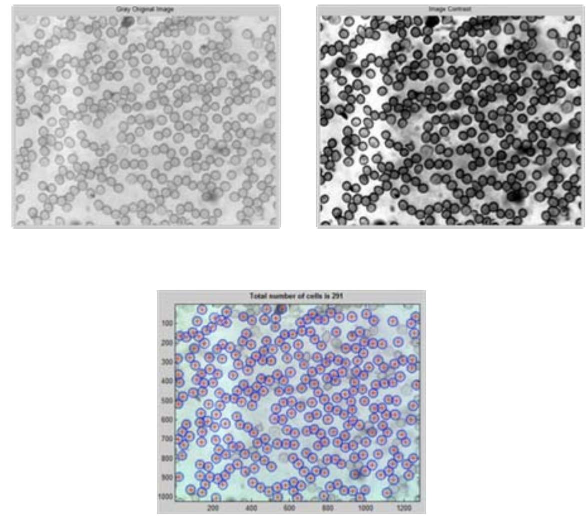


Figure 6. Sample image 1 (a) Grayscale image, (b) Image contrast and (c) Image after CHT

III. RESULTS AND DISCUSSIONS

It is important to count radius range in a number of RBC. Each sample image has difference radius range that needs to be determined before counting process. Table I presents the radius and tolerance for each 10 set of microscopic image.

TABLE I. RADIUS RANGE OF RBC

Sample image	Radius (pixel)		Tolerance (pixel)
	Minimum	Maximum	
1	23.35	26.78	± 4.85
2	22.17	25.65	± 4.92
3	21.89	23.94	± 2.89
4	23.52	26.28	± 3.9
5	21.02	24.14	± 4.42
6	21.09	24.90	± 5.38
7	22.45	25.92	± 4.9
8	20.54	23.99	± 4.85
9	22.69	26.56	± 5.48
10	22.10	24.98	± 4.07

The accuracy is measured based on the result of RBC counted using the CHT technique compared to the manual counting. Equation (7) is used to calculate the accuracy of the system.

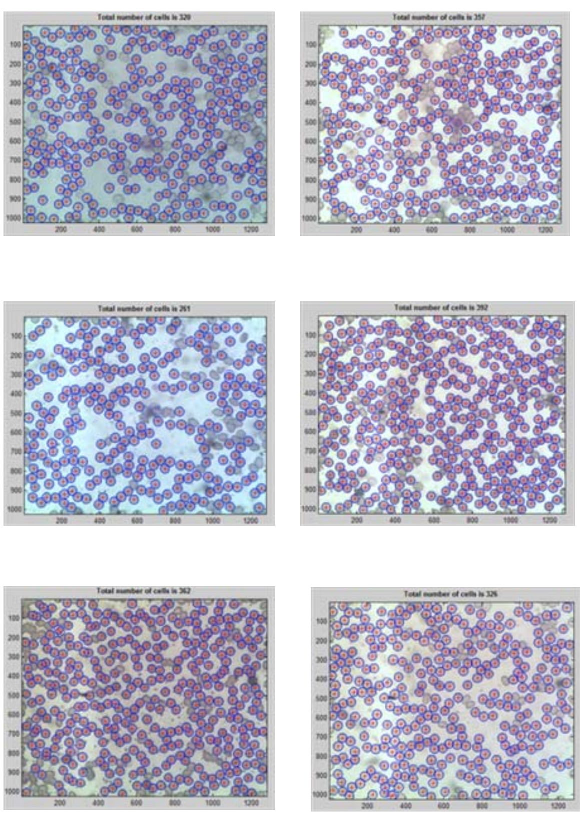
$$Accuracy = (\frac{RBCcountCHT}{Actual}) \times 100\% \qquad (7)$$

Based on these 10 samples of microscopic image, the average of accuracy 91.87% was achieved. Comparing the input image with the image after applying CHT, we could not achieve 100% of accuracy because some of the RBC is not counted properly due to incompleteness of circle drawing and out of range of the radius of cells that had been cropped by the user. Table II shows the result of RBC counted for 10 samples of microscopic image

TABLE II. RESULT OF RBC COUNTING USING CHT

Sample image	Number of Red Blood Cell (RBC)		Percentage of accuracy (%)
	Actual number	Counted using CHT	
1	322	291	90.37
2	360	320	88.89
3	397	357	89.90
4	296	261	88.18
5	413	392	94.91
6	408	362	88.73
7	348	326	93.68
8	400	387	96.75
9	310	286	92.26
10	363	345	95.04

To determine the accuracy of developed algorithm, different sample of microscopic image namely sample image 2, 3, 4, 5, 6, 7, 8, 9 and 10 were tested and the result presented in Fig. 7.



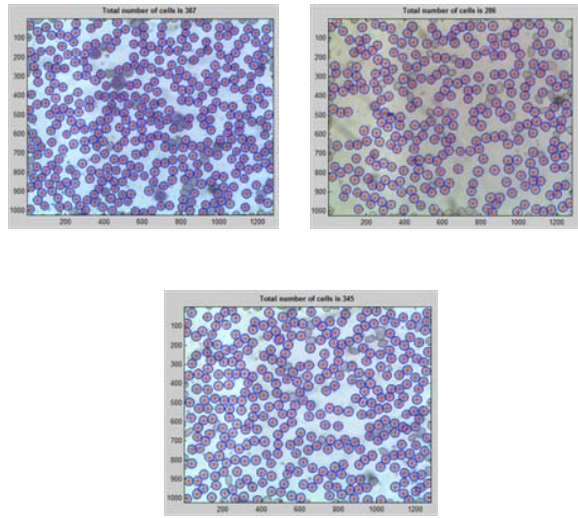


Figure 7. The result of 9 samples of RBC after performing CHT

IV. CONCLUSION

In this paper, we have proposed a method to detect and count the RBC in microscopic image using CHT automatically. This method can provide cost effective and alternative way to recognizing, analyzing and counting the circular cells. It also can identify and count separately the overlapping RBC. The result unable to get 100% accuracy was due to the out of range of the radius during the cropping process. This will be improved in future work.

ACKNOWLEDGMENT

The authors are grateful to Pathologist lab, KPJ Johor Specialist Hospital for providing peripheral blood film of blood cell. This work is supported by Research University Grant (GUP) of Universiti Teknologi Malaysia (UTM), PY/2012/00151, and Q.J130000.2623.05J58.

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