

Red Blood Cell Segmentation Using Masking and Watershed Algorithm: A Preliminary Study

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Abstract—Image segmentation is the most important step and a key technology in image processing which directly affect the next processing. In human blood cell segmentation cases, many methods were applied for obtaining better results. It is basically an improved visualization to observe blood cell under blood smear process. This paper will present an approach for red blood cell (RBC) segmentation which is a part of study to perform automated counting for RBC. The methods involve are Ycbr color conversion, masking, morphological operators and watershed algorithm. The combination of Ycbr color conversion and morphological operator produce segmented white blood cell (WBC) nucleus. Then it is being used as a mask to remove WBC from the blood cell image. Morphological operators involve binary erosion diminish small object like platelet. The resulted RBC segmentation is passing through marker controlled watershed algorithm which handles overlapping cells. The improvement need to be done for both segmentation and overlapped cell handling to obtain better result in the future.

Keywords—Segmentation; blood cell counting; analyzing method; counting application; blood cell disorder.

I. INTRODUCTION

The use of image processing helps to improve the image quality and analysis approach from different application. It improves the effectiveness of the analysis in term of accuracy, time consuming and so on. The application area which has been covered so far are remote sensing, biometric, security and medical. One of the most important part in image processing is segmentation. It has been utilized to get specific identification from the image. One of the application area is blood cell image which used for medical diagnosis purpose. Many researches has been done blood cell image processing and it is efficiently cost and time consuming compared to flow cytometric method or other methods. Plus, it is more accurate in specifying and indentifying any abnormality or parasite from the segmented image.

Current research is doing on blood counting application in the image segmentation. It is an implementation of automated counting for blood cell which manually done by hematocytometer by using counting chamber. Blood counting is synonym with the complete blood count or CBC which refers to compilation test of red blood cell (RBC), white blood cell (WBC), platelet, hemoglobin and hematocrit. Each of them has their role in the body system and the counting result is important to determine the capability or deficiency of the body

system. In short, any abnormal reading of CBC can give a sign of infection or disease. For example, the present of bacterial infection is diagnosed from increasing WBC count. Plus, specific low vitamin may come from a decreased RBC and thrombocytopenia is referring to low platelet count. The result can influence physician to make the best response and monitor the drug effectiveness from the blood count [1].

II. COMPLETE BLOOD COUNT (CBC)

As mention earlier, CBC consists of several counting of the main component in the blood cell. Each of them has a standard quantity range as a reference for a healthy women and man. Any counting value out of the range is considered abnormal and physician will interpret the result for further action. In addition, differential count also include in the measurement of CBC as a division of WBC count for five different types of WBC. 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. Table 1 shows the standard CBC for the healthy person divided by gender.

TABLE I. NORMAL BLOOD COUNT DIFFERENTIATE BY GENDER

Blood cell 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

Several works on the image processing for the blood cell image and counting methods will be reviewed to give a clear view about the recent trends and methods in the area. From the review, one conclusion will be made based on the pro and contra of the methods and also any justification regarding the topic. The conclusion from this paper is hoped to be an initiated work to build an algorithm for image processing as the primary method to perform blood cell counting.

III. BLOOD CELL IMAGE PROCESSING

The implementation of image processing in blood cell image has bring a new idea how to decrease the cost for a clinical decision and at the same time give a reliable result. This is because it is only the software based improvement compared to other method which using highly cost hardware to perform blood cell counting. One work to do with Lohitha software development for automated blood counting is especially done to make an efficient method which costly effective for an undeveloped country like Sri Lanka [6]. Plus, there are also work on visualizing three dimensional (3D) from two dimensional (2D) images. From one study, 2D edge image from enhancement and edge detection process is converted to binary image for noise reduction [8]. 3D visualization is formed from a continuous step of zero order interpolation to represent surface algorithm and plotted three surfaces.

Recent works on this area are mainly focus in segmenting WBC, RBC and platelet. From the segmentation, some studies will use it to perform blood cell counting and classify abnormalities in the cell. In WBC segmentation, current technique used is Gradient Vector Flow (GVF) snake algorithm to segment the nucleus and Zack Thresholding to segment the cytoplasm [9]. Fuzzy approach is also being proposed for classified pixel to Region of Interest (ROI) [10]. In advanced, another work is using Fuzzy C-Means (FCM) clustering repeatedly for sub image component [11]. The same work by using sub image component for feature space clustering is done [12]. It is able to have accuracy of 98.9% for nucleus segmentation and 95.3% for cytoplasm segmentation. Another advance work also is done to classify WBC automatically by computing the WBC images in term of area, major axis length over minor axis length, perimeter, circularity and ratio of areas between nucleus and cytoplasm [13].

For RBC segmentation, recent study use neural network training to extract RBC from the blood cell image. Almost the same method is applied by using Pulse-Coupled Neural Network (PCNN) with autowave characteristic to improve the segmentation [7]. Neural network is continued to be used as a method in one study to compare Artificial Neural Network (ANN) Backprojection with morphological processing of Connected Component Labeling to perform RBC counting [14]. The result shows that the Connected Component Labeling has higher accuracy of 87.74 % compare to backprojection of ANN with 86.97 % accuracy with hematology analyzer Sysmex KX-21 as benchmark reading result.

A. Classify blood cell disorder

In classifying abnormalities, both red and white-blood cell segmentation is applied with various feature extraction algorithms. From there, feature selection or classifier is used to make a complete abnormal classification. One work is using neural network model together with Principal Component Analysis (PCA). Inner and outer edge information is used to classify RBC abnormalities while nucleus and cytoplasm is the features extracted from WBC [15]. Further work from WBC segmentation can be used to identify four different types of leukemia. They are Acute lymphoblastic leukemia (ALL), Acute myelogenous leukemia (AML), Chronic lymphocytic leukemia (CLL), and Chronic myelogenous leukemia (CML).

Feature extraction is being used together with other methods to classify them like bimodal thresholding, dilation, region filling, boundary tracing and others [16]. From RBC segmentation, parasitemia can be classified by using feature extraction and Standard support vector machines (SVM) [17]. It is done after preprocessing, edge detection and segmentation process by using Otsu thresholding and watershed algorithm to handle overlapping cells.

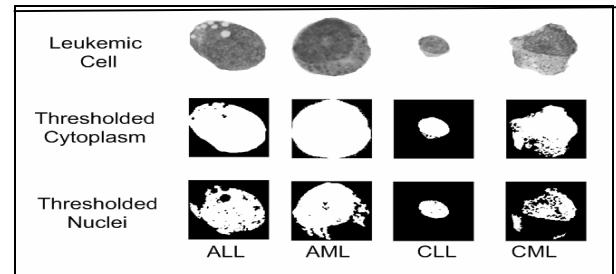


Figure 1. Four different forms of Leukemia undergo Biomodal thresholding.

Gloria Diaz has reviewed some methods to segment RBC and each of the method has their own advantages and disadvantages [18]. One of them is Otsu algorithm for green histogram together with k-nearest neighbour (k-nn) classifier to use the RGB color space for pixel based application. It is applied to decreases segmentation time and classified the image into background, RBC and parasites. Other methods exist including active contour, Universidade Nova de Lisboa, Portugal (UNLP) descriptor, two stages of ANN classifier and two stages of SVM classifier to detect parasites and any abnormalities.

IV. METHODOLOGY

One experiment is conducted to segment RBC from a microscopic image of blood cell for 40 times objective. The process involves few image processing steps including filtering, image enhancement, color conversion and segmentation process. The programming part is being done by using MATLAB. 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 substracted WBC and platelet. Figure 2 shows step by step methods the approach technique used in this study and figure 3 shows the blood cell image for 40X objective

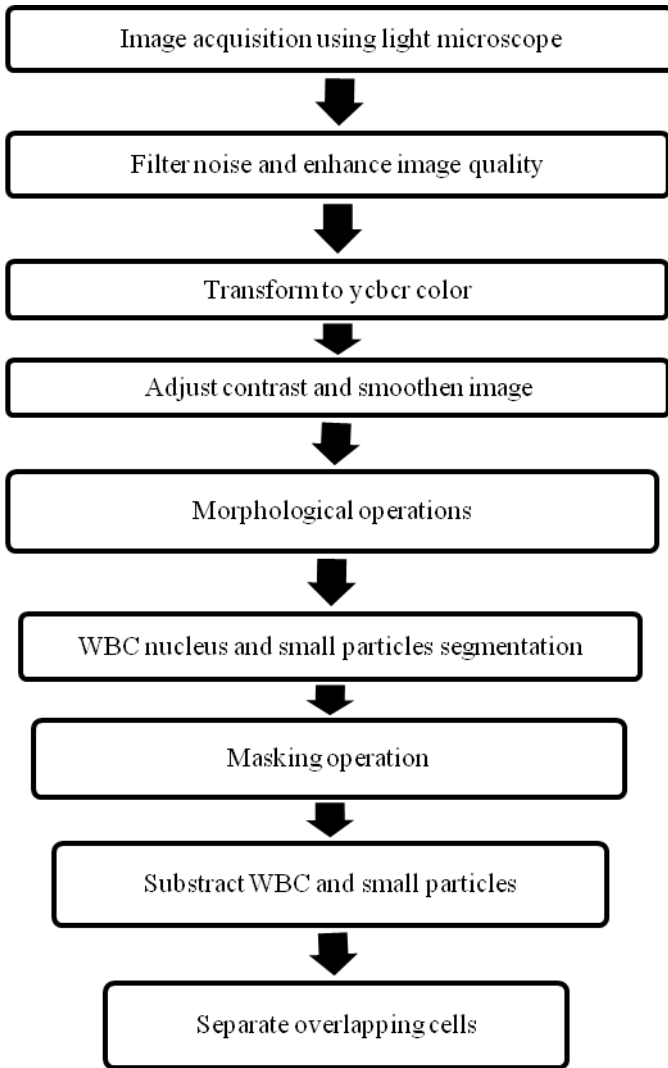


Figure 2. Segmentation steps for RBC

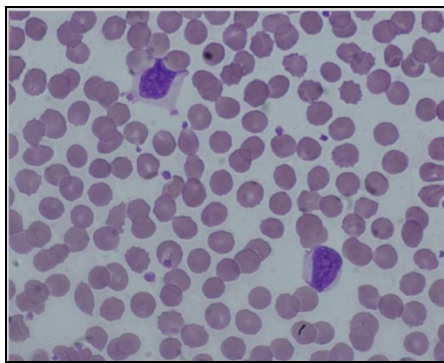


Figure 3. Original image of blood for 40X objective

A. Image preprocessing

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.

By using light microscope, twenty images are produced with 40 times objective which equal to 400 times magnification. At this level of magnification, the appearance of each cell can be observed clearly. Average filter will be used in the beginning of the process to filter a random noise in the image. It will be based on the equation 1.0 to filter M X N image with filter size of m x n.

$$g(x, y) = \frac{\sum_{s=-a}^a \sum_{t=-b}^b w(s, t) f(x + s, y + t)}{\sum_{s=-a}^a \sum_{t=-b}^b w(s, t)}$$

Equation 1. Average filter formula where x=0,1,2,...,M-1 and y=0,1,2,...,N-1 and also a=(m-1)/2 and b=(n-1)/2.

In this step, 3x3 average filter has been used as it is the smallest mask and it can filter any random noise in small area. The filtering process makes the image getting blur and less detailed. This will diminished non important detail from the image. Next process, image contrast is being adjusted until it give a clear view of the cell from the background.

B. Color conversion

The conversion from RGB color to Ycbcr will be based on the formula on the equation 2.0. The Ycbcr color representation can be used to overcome the illumination issue which often occurs in blood cell microscopic image. The second component of the Ycbcr color has been chosen and it shows the clear appearance of the WBC nucleus and platelet. Contrast and brightness adjustment continue to be used in the next process to give a clear view of the color representation image. The used of median filter is to filter any noise without require any blurring process. This will further enhance the appearance of the image.

$$\begin{bmatrix} Y \\ Cb \\ Cr \end{bmatrix} = \begin{bmatrix} 16 \\ 128 \\ 128 \end{bmatrix} + \begin{bmatrix} 65.481 & 128.553 & 24.966 \\ -37.797 & -74.203 & 112.00 \\ 112.000 & -93.786 & -18.214 \end{bmatrix} \begin{bmatrix} R \\ G \\ B \end{bmatrix}$$

Equation 2. Ycbcr color conversion

C. Morphological image processing

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. 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.

However both of opening and closing operation have similar task which is smoothen the object countours 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. One of the technique is opening by reconstruction which restored the exact shape of the object which is been eroded while the normal opening technique will depends on the similarity between objects and structuring element. Reconstruction also can be applied for filling holes and clearing border objects with the combination of marker and mask function.

D. Separate overlapping cell

Watershed algorithm is applied distance image for an overlapping cell. However, it cannot handle when the overlapping area contain important information. Geometrical function on the distance function has been introduced to overcome this problem and it improves the segmentation accuracy and reducing the computational cost. The other recent common methods used for overlapping and clumped cells are concavity analysis and template matching. 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.

V. RESULT AND DISCUSSION

This chapter will discuss further the implementation of the method to get signifiant result for segmentation of RBC. As mention earlier, the process involves few image processing steps like filtering, image enhancement, color conversion and segmentation process. After filtering and image adjustment process, the image is tranformed to Ycbr color conversion. The conversion can help to avoid illumination in the image and the difference of of the cell can be observed from the second component of Ycbr color. Plus, contrast/brightness adjustment and median filtering are enhancing the morphological view of the cell. Morphological operators which involve reconstruction, fill holes, erosion and area opening are used to segment the WBC nucleus. Figure 4 shows a segmented nucleus of WBC.

When the operation of masking is applied, the masked image has diminished the WBC nucleus morphological view. After morphological operation involving binary erosion and filling holes, the RBC can be viewed accordingly. In this study, masking has been used to remove WBC and platelet is substracted by morphological operators. The left one will be RBC which represent the RBC segmentation. Figure 4 shows the result of the RBC segmentation from the elimination of WBC nucleus and small particles including platelets.

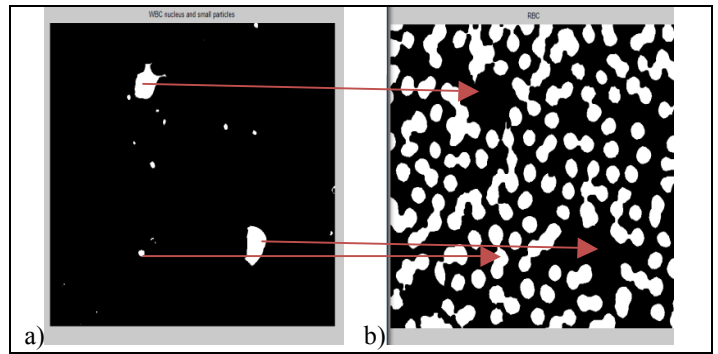


Figure 4. a) Segmented WBC nucleus b) RBC segmentation from the elimination of WBC nucleus and small particles.

To handle overlapping RBC, it involves Laplace of Gaussian (LoG) edge detection, morphological operation, gradient magnitude and marker controlled watershed algorithm. The result from erosion on RBC segmentation result and gradient magnitude has being used together as mask with watershed algorithm to form marker controlled watershed algorithm. This could avoid oversegmentation which often occurs for watershed algorithm. After dilation, it being used together with LoG edge detection on the Ycbr second component of the image as mask and segmented RBC as marker. Lastly, it being superimpose to the original image. The result of the applied method is present in the figure 5.

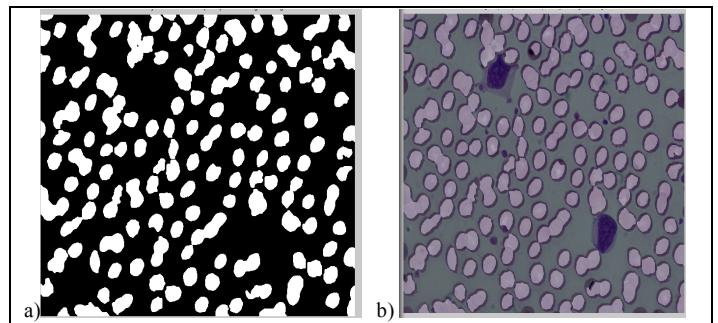


Figure 5. a) Separation of overlapping cell b) Superimpose on original image.

This method has been applied to twenty images and it shows a quite similar result for all of the images. It could be observed that the segmentation of WBC and small particles like platelet play a big role to segment RBC by using masking operation. The result from the segmentation further used to be separated to identify each component of the RBC. However, there are several weaknesses in this segmentation where roughly it can be seen that RBC is not perfectly segmented and separated. One of the problems is there is some debris and irrelevant objects appear in the image which results in decreasing in accuracy. This is due to improper condition of the microscope which might have dust and also any mistake during the slide image preparation. Plus, the method only capable to extract the nucleus part of WBC and to improve the method, the masking operation should take the cytoplasm region of WBC as a mask also to completely remove WBC from the image. Moreover, the border identification of weak

edge boundary of RBC must be improved especially to handle the overlapping cells. The technique only capable on handling touched or small overlap in the image but unsuccessful for a big overlap.

VI. CONCLUSION

RBC is the dominant composition among the blood cells and the information extracted from its analysis can give a valuable data for a response index. A mask of WBC nucleus has been used in this study as it can easily be segmented through a color conversion, contrast adjustment and morphological approach. Thus, RBC is the object component left in the image when WBC subtracted through masking operation and platelet is diminished through morphological operation. Marker controlled watershed algorithm can avoid oversegmentation issue and it has being used to handle overlapping cell. The main idea in this research is the using of masking and morphological operation function to eliminate unwanted objects. This research has used the technique from the combination of pixel based, region based and morphological segmentation and it is hoped that a better mix methods can be developed from a variety of methods especially reviewed in this paper and from a further study to improve the segmentation accuracy.

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