Automatic Red Blood Cell Counting using Watershed Segmentation

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Abstract—The major issue in clinical laboratory is to produce a precise result for every test especially in the area of Red Blood Cell (RBC) count. The number of red blood cell is very important to detect as well as to follow the treatment of many diseases like anaemia, leukaemia etc. Red blood cell count gives the vital information that help diagnosis many of the patient’s sickness. The old conventional method of RBC counting under microscope gives an unreliable and inaccurate result depends on clinical laboratory technician skill. This method puts a lot of strain on the technician. Another method for RBC counting uses the automatic hematology analyzer, this machine is very costlier. So it is not possible all the hospital’s clinical laboratory implement such an expensive machine to count the blood cell in their laboratory. This paper introduces an efficient and cost effective computer vision system for automatic red blood cell counting using image based analysis.

Keywords—Health care applications, Red Blood Cell count, MATLAB, Morphological operations, Hough Transform.

I. INTRODUCTION

Content-based image indexing and retrieval has been an important research area in computer science for the last few decades. Many digital images are being captured and stored such as medical images, architectural, advertising, design and fashion images, etc. As a result large image databases are being created and being used in many applications. In this work, the focus of our study is on medical images. A large number of medical images in digital format are generated by hospitals and medical institutions every day. Consequently, how to make use of this huge amount of images effectively becomes a challenging problem [1]. In the field of biomeedicine, because of cell’s complex nature, it still remains a challenging task to segment cells from its background and count them automatically [2-5]. Among all of the body’s tissues, blood is unique due to its existence as the only fluid tissue. A blood cell can be any type of cell normally found in blood which falls into four categories which are red blood cell (RBC), white blood cell (WBC), platelet and plasma [6]. The differences between these groups lie on the texture, color, size and morphology of nucleus and cytoplasm. In blood smear, number of red cells is many more than white blood cells. For example an image may contain up to 100 red cells and only 1 to 3 white cells. Platelets are small particles and are not clinically important [7]. Blood cells form in the bone marrow, the soft material in the center of most bones. Leukocytes or WBCare cells involved in defending the body against infective organisms and foreign substances. Leukocytes cells containing granules are called granulocytes (composed of neutrophil, basophil, eosinophil). Cells without granules are called agranulocytes (lymphocyte and monocyte) [6]. These cells provide major defense against infections in organisms and their specific concentrations can help specialists to discriminate the presence or the absence of very important families of pathologies [8]. When infection occurs, the production of WBCs increases [6]. Abnormal high or low counts may indicate the presence of many forms of disease, since blood counts are amongst the most commonly performed blood test in medicine.

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 count 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 [14].

CBC consists of several counting of themain 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 1: Normal Blood Count Differentiate by Gender

<table>
<thead>
<tr>
<th>Blood Cell Types</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBX</td>
<td>5.4-6.0</td>
<td>4.0-5.0</td>
</tr>
<tr>
<td>Million/micro liter</td>
<td></td>
<td>Million/micro liter</td>
</tr>
<tr>
<td>WBC</td>
<td>4.5-11</td>
<td>4.5-11</td>
</tr>
<tr>
<td>Thousand/micro liter</td>
<td></td>
<td>Thousand/micro liter</td>
</tr>
<tr>
<td>Platelet</td>
<td>150-450</td>
<td>15.0-45</td>
</tr>
<tr>
<td>Thousand/micro liter</td>
<td></td>
<td>Thousand/micro liter</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>42%-50%</td>
<td>36%-45%</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>14-17</td>
<td>12-15</td>
</tr>
<tr>
<td>Grams/100 milli liters</td>
<td></td>
<td>Grams/100 milli liters</td>
</tr>
</tbody>
</table>

Jianhua et al. [21] stated that in the case of cell segmentation for blood, edge detection performs poorly on cell images because not all boundaries are sharp and it is difficult to get all edge information and locate cells accurately. They developed an iterative Otsu’s approach based on circular histogram for the leukocyte segmentation. Otsu’s approach is generalized on the base of least square method. R. Sukesh Kumar et al. [22] discussed about an approach for color image segmentation using higher order entropy as a textural feature for determination of thresholds over a twodimensional image histogram. Two basic models for color images are the RGB (Red, Green, Blue) color model and the HSL (Hue, intensity, saturation) color model. Two methods of color image segmentation used RGB space as the standard processing space. These techniques might be used in blood cell image segmentation. Color images are very rich source of information, because they provide a better description of a scene as compared to grayscale images. Hence, color segmentation becomes a very important issue [22]. Khoo Boon et al. [23] performed comparisons between nine image segmentation which is gray level thresholding, pattern matching, morphological operators, filtering operators, gradient-in method, edge detection operators, RGB color thresholding, color matching and HSL (hue, saturation, lightness) and color thresholding techniques on RBC and concluded that there is no single method can be considered good for RBC segmentation [23].

In image enhancement process, there are two common image processing techniques used in order to reduce the noise and at the same time to enhance the image. Figure 3 shows the flow process in enhancement processing which are analyses in hue-saturation value color space (HSV) and the green component image. For HSV, we proceed with analyses in saturation component, S, because S image show clearly the bright objects such as white blood cell and parasites, therefore, it's easy to distinguish the red blood cells with another cell.
of each pixel on an edge from the center. Heidi Berge [10] has proposed the segmentation red blood cells in a thin blood smear image which is based on the Zack’s Method [11]. This method is one of the approaches to determine the red blood cells tresholding where aline is drawn between the two peaks and between these two peaks, they used the point which isfarthest from the drawn line as a threshold for red blood cell. In the conclusion for this technique, the segmentation result is better to the blood smear which in case red blood cells is sparse and in the image. However, in images with high Red blood cell concentrations, large clumps may result and this method is less accurate. Guitao et. al [12] purposed the Hough transform in detecting and extracting the red blood cells in the urine micrograph. Based on Hough transform, Guitao has used the geometrical feature to detect the circle center in the image.

Haider Adnan Khan et al. presented a framework for cell segmentation and counting by detection of cell centroids in microscopic images [16]. Preprocessing is done with Contrast-Limited Adaptive Histogram Equalization to get enhanced image. Next, cells are separated from background using global thresholding. Then, distance transform of binary image is computed which converts background using global thresholding. Then, distance get enhanced image. Next, cells are separated from background and blood cells by using morphological opening and closing method. Last step of algorithm is the segmentation between background and blood cells by using feedforward backpropagation algorithm. For training neural network, the input is Hue, Saturation, Value and standard deviation. After deriving output from feedforward backpropagation network, the input is RGB to gray conversion. After that object counting becomes trivial. By observing results, it can be seen that, except for the case of merged objects, the method identifies the objects correctly in more than 90 % of the cases, and the number of falsepositives is always low. The overall deviation was 8 %; such a number falls to 2.5 % if the images with merged objects are not taken into account.

IV. RESULTS AND DISCUSSION

![Figure 4: Original Image of Blood for 40X object](image)

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 subtracted by morphological operators. The left one will be RBC which represent the RBC segmentation. Figure 5 showsthe result of the RBC segmentation from the elimination of WBC nucleus and small particles including platelets.

![Figure 5: a) Segmented WBC nucleus b) RBC segmentation from the elimination of WBC nucleus and small particles](image)

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 Ycbcr second component of the image as mask and segmented RBC as marker. Lastly, it being superimposed to the original image. The result of [19] is given in figure 6.

Figure 6: a) Separation of overlapping cell b) Superimpose on original image.

The circular hough transform is applied to the contrast adjusted image by some of researchers.

Figure 7(a) Microscopic Image and (b) Green Plane Extraction – I

Figure 7(c) Contrast adjustment-1 and (d) Accumulation array-1

Figure 7(e) Detected blood cells-1 and (f) Binary image-1

Figure 7(g) Holes filled-1 and (h) Borders cleared-1

Figure 7(i) Labelled Image

The results of the image can be used as good input in determining the number of red blood cells by using Hough transform technique. By using the MATLAB, all the importance’s aspects like correct algorithm and system has been successfully produced. With correct algorithm, the red blood cells can be detected and segmented as well as estimated number of the red blood cells. Through system created using MATLAB, it also enable the study of the morphological features of the red blood cells image, thus, can determine whether the person is normal or otherwise by referring amount of red blood cells in human blood [15].
Figure 8: results of 8 samples of red blood cells after performing Hough transform technique.

Object counting using image processing has huge applications where automation is to be introduced and time of counting is to be reduced. Some of the main applications of object counting in industrial systems are packaging, quality control, and so on. It is helpful in the research areas where objects are of very small size. Object counting algorithm can be also used to track and identify objects. The present methods can be extended to have counting system based on user selected attributes.

V. CONCLUSION

Image processing techniques are helpful for object counting and reduce the time of counting effectively. Proper recognition of the object is important for object counting. The accuracy of the algorithm depends on camera used, size of objects, whether or not objects touching and illumination conditions.

REFERENCES


