RED BLOOD CELLS EXTRACTION AND COUNTING

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ABSTRACT

Blood cell counting by laboratory task utilizes hemocytometer and microscope. The conventional task depends on physician skill. It is laborious. This paper shows the effectiveness of an automatic image processing method to detect normal red blood cells (RBCs) by peripheral blood smear microscope image. When single RBCs were extracted from sickle RBCs and white blood cells (WBCs) component, its images were analyzed and classified by neural network. Next RBCs were counted and displayed. This study found the method proposed system has sensitivity 0.86, specificity 0.76 and accuracy 0.74.

1. INTRODUCTION

Blood is a connective tissue consisting of cells suspended in plasma. Blood's major functions are to transport various agents such as oxygen, carbon dioxide, nutrients, wastes, and hormones. Blood cells are composed of erythrocytes (red blood cells, RBCs), leukocytes (white blood cells, WBCs) and thrombocytes (platelets). The most abundant small reddish cells are erythrocytes and called red blood cell. An erythrocyte is a discoid cell with a thick rim and a thin sunken center [1]. RBCs' two principal functions are to move oxygen from lung to tissues elsewhere and transport carbon dioxide from tissues to the lung. Whereas, the Leukocytes or white blood cells are part of the immune system.

The conventional device used to count blood cells is the hemocytometer. 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 [2,3]. To count blood cell, physician must view hemocytometer through a microscope and count blood cells using hand tally counter. The overlapped blood cells on the top-side and right-side of hemocytometer are not counted. Normally, the counting task is time-consuming and laborious. Several attempts have been made to mimic the procedure of cell recognition from image. The major application of neural networks was devoted to the WBCs classification via extracted morphologic parameters [4-6].

Some red blood cell classification task using neural network was adapted for Thalassemia diagnostic tool [7]. Many commercially available products have been developed to automatically count RBCs or WBCs [8]. Their advantages include automatic cell counting cells without hand tally counter, no requirement of messy washing and no associated biohazard. However, these products are expensive.

2. EXPERIMENT

This work aims to apply image processing to extract the blood image taken from blood smear microscope, then automatically counting red blood cells. This work can help release physicians from tedious and laborious blood cell counting task. The images of blood cell was digitized by the optical microscope. The composition of blood image consists of red blood cells, white blood cells and sickle red blood cells. The image was analyzed by manually looking for red blood cells. After that, the red blood cells were counted using the proposed red blood cell counting method, automatically. The proposed method consists of three steps. The first step is to apply an image processing to delete incompleted blood cells that overlap on the boundary of the image. Then, single blood cells were extracted from the image using edge detection algorithm [6] and each single blood cells image scale to 31x30 pixel. Finally, each single blood cells were analyzed by using a neural network to search for red blood cells and count them. An overall procedure is shown in Fig. 1.

Digital image processing was extensively used in this work. It is the key performance index to establish the ability of the proposed method [9].

2.1. Image processing

The main image processing tasks consists of enhancing the image's qualities and deleting overlapped blood cells in the boundary area of the image. Both tasks can be subdivided into smaller tasks as shown in Fig. 2 illustrating the main steps and examples of input/output images.

2.1.1 Histogram equalization

This process adjusts intensity values of the image by performing histogram equalization involving intensity transformation, so that the histogram of the output image approximately matches a predefined histogram.

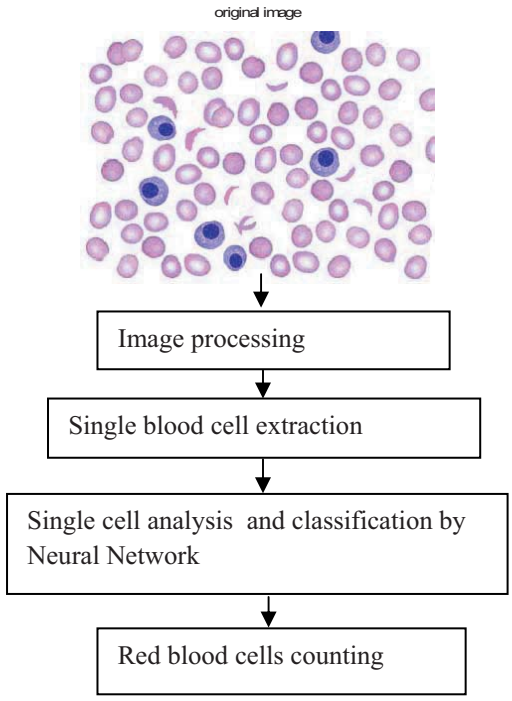


Figure 1. A proposed red blood cell counting procedure

2.1.2 Contrast and brightness adjustment

To adjust brightness of an image, an histogram of the interested image is used to determine data and display ranges of the image. The data range is the range of intensity values actually used in the image. The display range is the black-to-white mapping used to display the image determined by the image class. Contrast adjustment is done by manipulating the display range of the histogram while the data range of the image remains constant.

2.1.3 Cell detection

The major challenge of red blood cell detection is the incomplete or overlapped blood cells around the boundary area of an image. The objective of blood cell detection is to detect cells which differentiate themselves from the background in terms of contrast. Changes in contrast can be detected by image processing operators that calculate the gradient of an image. Then a threshold can be applied to create a binary mask containing the segmented cell.

The edge detection is done by using the Sobel operator. The broader of the blood membranes were enhanced. An edge enhanced gray level image is thus produced. Next, the Canny method is applied to search for edges by looking for local maxima of the gradient. The gradient is calculated using the Gaussian kernel. The proposed method applies two threshold values, to detect strong and weak edges, and includes the weak edges in the output only if they are connected to the strong edges.

2.1.4 Image dilation

The first step is to apply morphological operator to create a structuring element specified by interested shapes, disk-shaped structuring element. Depending on shape structures, disk-shaped approximation are suitable for computing granulometrics. Disk-shaped structuring element is approximated by specifies radius from the origin of granule. The structuring element members, binary gradient mask, consist of all pixels whose no greater than radius away from the origin. Then the binary gradient mask is dilated using the vertical structuring element followed by the horizontal structuring element. The dilation morphological operator has been used to better connect separated points of the membrane [6].

2.1.5 Interior gap filling

The dilated gradient mask shows the outline of the blood cell quite nicely, but there are still holes in the interior of the cell. Filling internal holds of the connected element get the biggest area in the processed image.

2.1.6 Object smoothening (Erosion)

All of blood cells of interest has been successfully segmented. Finally, in order to make the segmented object look better, the objects in the processed image can be smoothed by eroding the image. This step reduces the spur elements along the membrane edges. Figure 3 shows the outline of the resulting smoothed image.

2.2. Single blood cell extraction

This method extracts the single blood cell from the derived binary image to obtain cell's position. The single blood cells extraction involves several steps as described in the following topics.

2.2.1 Border padding

As the neighborhood operator block slides over the entire image, some of the pixels around the border may be missing, if the center pixel is on the border of the image. The missing pixels will be padded using 0 value (black) to complete the image.

2.2.2 Centroid finding

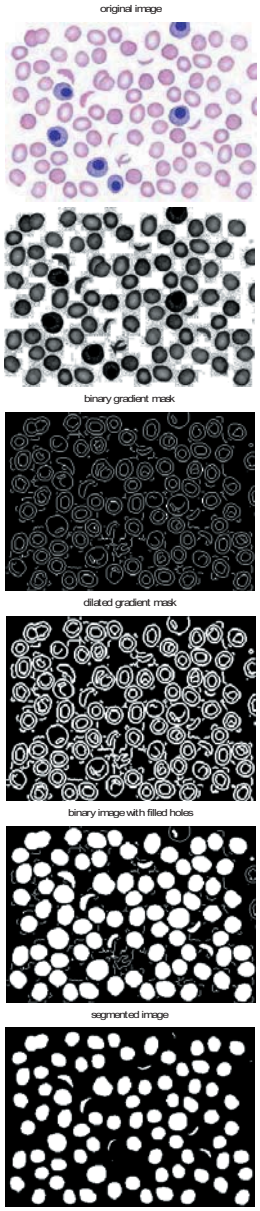
The centroid of the converted binary image is measured by finding the center of mass of the binary image region. The centroid coordinates are definded as x-coordinate and y-coordinate. All other elements of centroid are in order of dimension.

2.2.3 Single blood cell isolation window.

Each type of blood cells (RBCs, WBCs, sickle RBCs) is isolated by using its centoid coordinates. The isolated blood cells are contained in windows of size 31x30. Window's corners positions are right top, left top, right bottom and left bottom.

2.2.4 Transfer window to original RGB image

Any position of each single blood cell windows were transferred to the original RGB image. Then we get single cell in the format 31x30 pixel RGB image. The RGB blood cells window are shown in Fig 4.



Original image.

Step 1. Equalizing image, Step 2 .Adjusting of an Image.

Step 3. Detecting entire cell

Step 4. Dilating an image

Step 5. Filling interior gaps

Step 6. Smoothening an object. (Erosion)

Figure 2. The main steps and examples of input/output images.

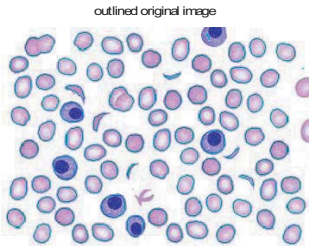
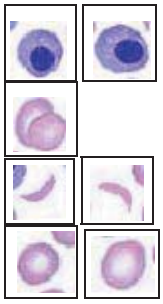


Figure 3. The outline of the result on the original image.



Single white blood cell window

Overlap red blood cell window

Single sickle red blood cell window

Single red blood cell window

Figure 4. The example of single blood cell window in RGB format.

2.3. Red blood cell separation and counting.

To separate red blood cells, the white blood cells are the first to be removed from the target image, then the sickle blood cells. There are several steps involved in white and sickle red blood cells removal as followings. The most important step is to apply neural network (NN) to classify and count only red blood cell in blood plasma that show in Fig 5.

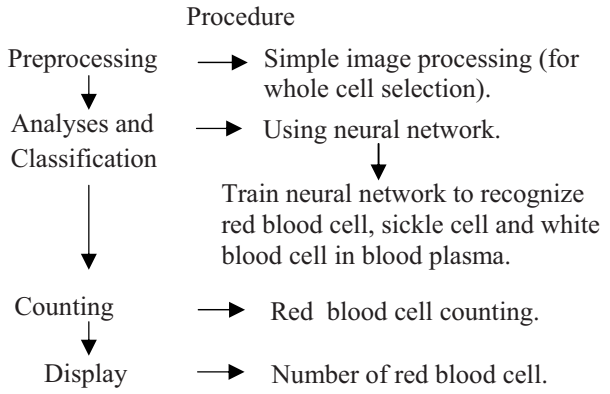


Figure 5. Procedure of red blood cell separation and counting.

From the RGB window of the single cell, the WBCs can be identified base on the blue level in the RGB(multidimensional matrix). The obtained mean values will be used to classify WBCs from other blood cells. The classification threshold level is 225 (0-255). The remaining blood cell after this process are RBC and sickle RBC. Red blood cells are identified and counted by neural network.

Neural networks have been applied very successfully in the identification and control of dynamic systems[10]. Neural networks are composed of simple elements operating in parallel. The network function is determined largely by the connections between elements. We can train a neural network to perform a particular function by adjusting the values of the connections (weights) between elements. Multilayer perceptron (MLP) neural networks using are adjusted or trained, so that a particular input leads to an output value of each blood cell window. Neural network consists of three fully connected layers as shown in Fig 6. There are 930 input nodes in the input layer, 10 hidden nodes in hidden layer, two bias constants, and one output in output layer. This is a simple structure which is fully connected. The number of hidden nodes was chosen on the basis of trials and errors with the training set to be as low as possible.

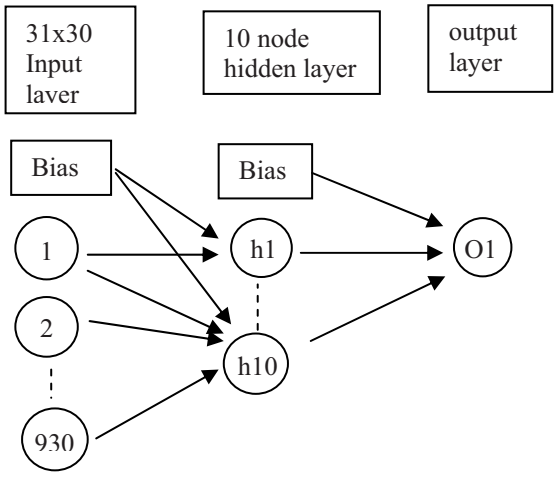


Figure 6. Structure of neural network in this study.

The training patterns were prepared and assessed, manually. They consist of 59 RBCs patterns and 59 non RBCs (sickle RBCs and WBCs) patterns. Each pattern was scaled to 31x30 pixel image. The images were converted to grayscale and inputted to train neural network with hyperbolic tangent sigmoid transfer function. The output from neural network are given the value between -0.9 to 0.9 with respect to target value -1 (non RBCs) and 1 (RBCs). The mean squared error was calculated when each pattern was presented. The weights in neural network were trained to obtain the 0.001 mean squared error with 5,000 epochs.

The values obtained from neural network step were used to separate sickle RBCs from normal RBCs. The (31x30 pixel) images from image processing step were validation set. The number of normal RBCs are counted using result from neural network.

3. RESULT AND DISCUSSION

The image was processed by image processing steps. The original image that shown in the task was very bright. The steps of equalizing and adjusting of an image could effect the contrast of blood cells from the background. Edges of blood cells wound be clearly shown. Next, detecting entire cell step caused binary image lines. The lines were formed by contrast in the image. Then dilating an image would thicken lines. Finally, filling interior gaps and smoothening an object would only show blood cells.

The procedure of single blood cells extraction gave blood cell windows (31x30 pixel). The parameters of the proposed windows were chosen from data set. In Fig 4, blood cells completely appeared in the windows but there were some parts of the neighbor blood cells. Results of final extraction RBCs and WBCs that overlap on the boundary of the image were not extracted. RBCs, WBCs and sickle RBCs collapsed on the other blood cell were not extracted. The RBCs counting achieves 59 out of 68 isolation task. The results were showed in table 1. Figure 7 shows the comparison between a case of incorrect isolation with correct isolation.

	Original image	Final extraction
Red blood cell overlapping the rim	2	0
Overlapped Red blood cell	2	0
Red blood cell	68	59
Sickle red blood cell	6	3
Overlapped sickle red blood cell	1	0
White blood cell overlapping the rim	1	0
White blood cell	5	1

Table 1. RBCs detection results

Sickle RBCs (incorrect) window

normal RBCs (correct) window

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Figure 7. Comparison between incorrect and correct RBCs isolation.

From the above figure, the sickle RBCs were separated as RBCs by the proposed algorithm. Because it is big when comparing to the contained window, like normal RBCs. Nevertheless, this problem could be solved by increasing training sickle RBCs patterns in neural network. The results were calculated by the following equation.

Sensitivity = TP/(TP+FN)

Specificity = TN/(TN+FP)

Accuracy = (TP+TN)/(TP+FP+FN+TN) (1)

When TP (True Positive) is RBCs counted. TN (True Negative) is non RBCs and is not counted. FP (False Positive) is non RBCs counted. FN (False Negative) is RBCs but it is not counted. From the result, the method has sensitivity = 0.86, specificity = 0.76 and accuracy = 0.74. The proposed algorithm offers medium-to-high accuracy, but it automates the RBCs counting.

4. CONCLUSION

This work aimed to study the possibility of red blood cells counting. Further more, study of the collapsed red blood cells should be done in order to get more accuracy. Results show that the automatic red blood cell extraction and counting start from image processing then single blood cell extracted and finally separating red blood cell offers 74% accuracy or better. Authors believe that the classifier (multi layer perceptron neural networks) is suitable for the RBCs counting application. Higher accuracy can be achieved when the number of sample training images is increased.

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