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Isolation and counting of cells in blood samples through image processing and fuzzy logic techniques

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ABSTRACT

In the medical world, especially in Iran, the blood cells counting and the diagnosis of a healthy or diseased cell occurs through the eye, not without defects. The most important defects of this work are waste of time and human error. We can do this automatically. This research counts cells automatically in order to determine the exact extent of the disease. Thus, we process the images of the cells, called the cell colony, identify the cells and separate from the colony. We store each of these cells as an image after separation. We process the stored images, calculate and examine their disease. We divided the tested images into three categories based on quality: Easy, Medium and Hard. We processed the images of each category, which have different results.

Keywords: *ANOMALY, IMAGE PROCESSING, Platelet Detection, Cell Isolation, Fuzzy Logic, BLOOD SAMPLES*

1. Introduction

Cell counting is very important in diagnosing diseases. The number of cells more or less than a normal range can indicate the presence of some infections. One way to determine infection (if any) is to count the white blood cells (WBC) or the red blood cells (RBC). A large number of white blood cells can be a sign of appendicitis, pneumonia, meningitis, leukemia, tonsillitis, chicken pox, etc., and a small number of white blood cells can be a sign of measles, typhoid, the flu, and so on.

In order to automatically count cells and diagnose diseased cells, there are several methods and most of them are based on binary image processing. In fact, in these methods, the programmer limits himself to classical logic. Previous work has used the Otsu method to select the appropriate Treshold and the Watershed algorithm to isolate and count cells. This method has used only the RGB image red channel for greater clarity. Some researchers have also used neural networks to classify cells. Their research's focus is more on detecting and counting red blood cells.

In this article, we have tried to use the color content of the images. The ultimate goal after detecting and counting cells is to calculate the percentage of cell health; we have used fuzzy logic to achieve it. In this article, the extraction channel fits the image itself. In fact, after the cell separation step, for determining the health of the cell, we use the color content of the images by fuzzy logic. This method makes it more flexible in determining cell health.

The next sections include the database, the stages, the laboratory results and the conclusion.

2. Data base

The database of blood samples includes images and types of blood samples with different qualities. We divided images into three categories in terms of processing complexity: Easy, Medium and Hard. The number of them is 19, 32 and 91 images, respectively, 142 images in total. McGill University medical and bioinformatics researchers prepared all images under experiment and provided to the authors of this article. See examples of these images in the pictures below:

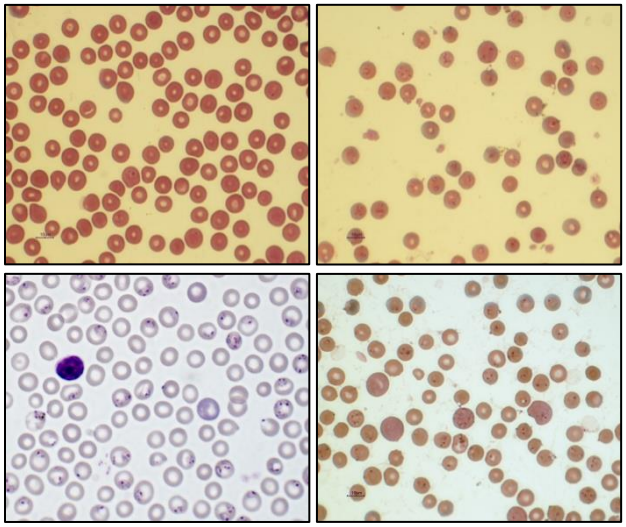


Figure 1: Easy group images with different quality

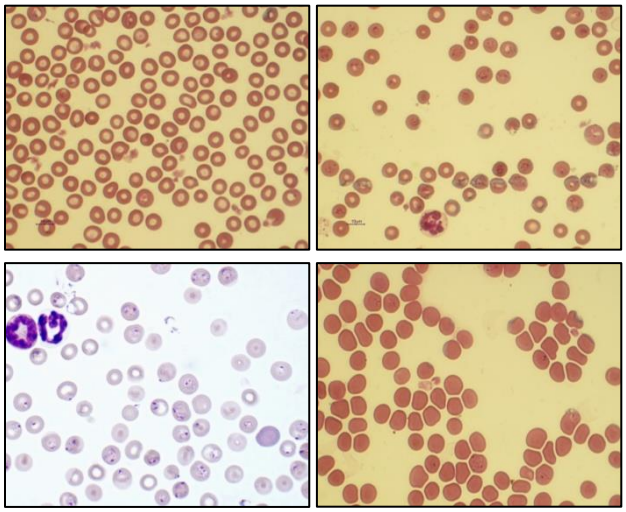
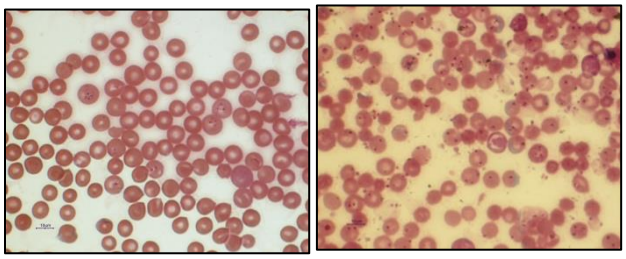


Figure 2: Medium group images with different quality



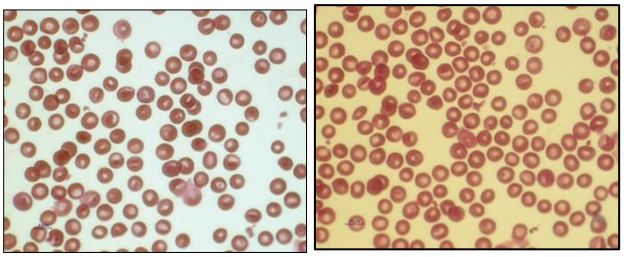


Figure 3: Images of Hard group with different quality

3. Methodology and procedure

According to Figure 4, we examine in detail the stages of preprocessing, cell isolation, cell count, and their health.

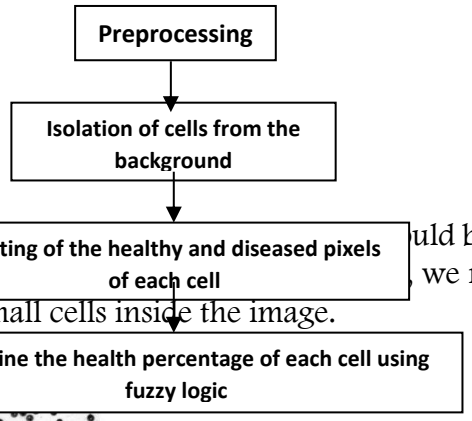
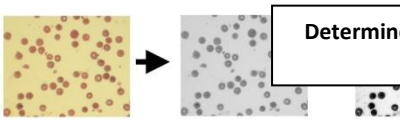


Figure 4: All stages of

3-1. Pre-processing: At uld be improved and the noises in the image should be ren , we must keep in mind that noise removal should not remove small cells inside the image.



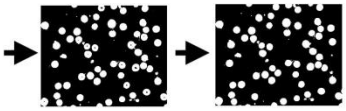


Figure 5: Pre-processing operations

3-2. Isolation of cells: In this stage, we scrolled the image from the upper left corner, poured into the stack the white part of the image concerning the cells, and stored the beginning and end of each cell in the table. We isolate each cell from the cell colony and stored as an image.

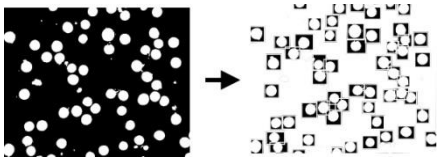


Figure 6: Cell isolation

1) Isolation of defective cells, platelets and overlapping cells from identified cells: In the blood samples, in addition to red and white blood cells, there are platelets that are not for processing, so they should be removed. For removing platelets, we first calculate the average area of cells in each blood sample; then we examined the area of each cell in the blood sample image to detect platelets. If the cell area is less than average, it is identified as platelets and is deleted from the image; if the cell area is larger than the average area, it is detected as an overlapping cell and processed for isolation.

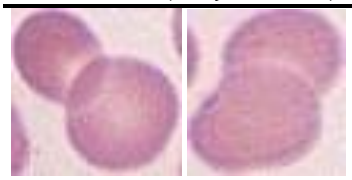


Figure 7: Sample of overlapping cells

Before applying the overlapping cell isolation algorithm, for achieving better conclusion, we flatten the surface of the binary image and eliminate image noise. For this purpose, we used the smoothing algorithm, which eliminates noise and flattens the edges of the image. First, we define four filters as follows:

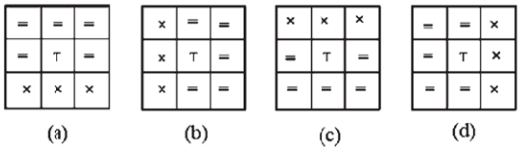


Figure 8: Four filters of smooth algorithm

First, we scroll the pixels of the image from the bottom right corner, examine each pixel with its 8 neighbors, and value them based on pixels with an equal sign. Neighbors marked with an equal sign indicate the target pixel value (zero or one), and we ignore the neighbors marked with a cross. Each filter is applied as long as it does not cause any change in the image.

After flattening and removing the noise, to make the image horizontal, we have to rotate the image in the direction of its axis of least inertia so that it touches the horizontal axis. Thus, we have to obtain the image covariance matrix. We should calculate the eigenvector corresponding to the least eigenvalue and the tangent of this vector, and rotate our image negatively.

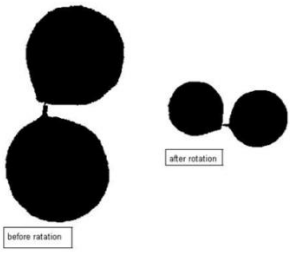


Figure 9: Image before rotation and after rotation based on eigenvector

After rotating the image and applying a Gaussian filter on the image, we get the top and bottom contour of the image using the top and bottom profiles.

Figure 10: shows the bottom profile (left) and top (right) profile images of the cell.

Figure 11: Left to right contours, lower half of the image (left) and upper half of the image (right)
Then we scroll the top and bottom contours of the image separately from right to left and vice versa, and keep the points with an angle between 30 and 90 degrees. After obtaining the breakpoints, we isolate the cells, rotate the image in the opposite direction and rotate it again.
Table 1: Comparison of detection rates of overlapping cells

Group of images under experiment	Detected number of program	Number of overlapping cells in the images	detection percentage	Mean diagnosis
Easy	37	39	94	68.10
Medium	81	99	81	
Hard	171	282	60	

2) Identifying white blood cells and removing them from the image: Blood contains platelets, white blood cells and red blood cells. Characteristics of the isolation of red blood cells from white include their color and size. Normal white blood cells are about two to three times the size of red blood cells, and their percentage in the blood is 2 to 100 or 1 to 100. After isolating the cells and removing the overlap, we obtain the average size of the cells and separate the largest ones, and after examining its color characteristics, we diagnose the cells.
3-3. Counting the healthy and diseased pixels of each cell using fuzzy logic: To calculate the health and disease of a cell, we cannot use classical logic. Thus, we use fuzzy logic in two parts of the program. We examine each one below:
3-3-1. Using Fuzzy Logic to Count Pixels Belonging to Anomalies and Calculate Cell Disease Percentage: First, we need to extract the appropriate color channel according to the image; we should specify most of cell image pixels belong to which RGB color channel. After specifying the extraction color channel, we can see that most of the pixels in the Anomaly section have a lower amount of color related to their extraction channel than the pixels of the healthy sections (these pixels are more colorful). Therefore, we form two fuzzy sets:
a) Set of pixels belonging to healthy sections: The range of this set is defined between 150 and 200. We consider the membership function of the set to be a trapezoid and assume that pixels with a color value between 170 and 200 are completely members of the healthy set.
b) Pixel set belonging to defective sections: The range of this set is defined between 0 and 170. We consider the membership function of the set to be a trapezoid and assume that pixels with a color value between 0 and 150 are completely members of the defective set.

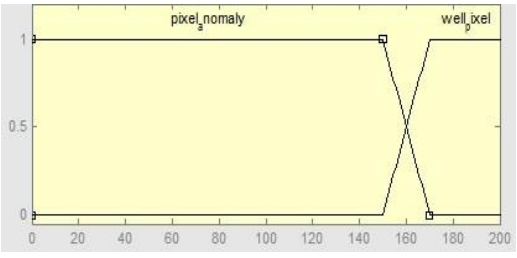


Figure 12: Fuzzy set of pixels
3-4. Using fuzzy logic to diagnose cell health and disease: Here we also form two fuzzy sets for healthy and defective cells. The following figure shows these two fuzzy sets:

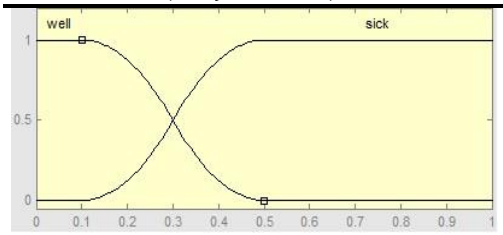


Figure 13: Fuzzy set for diseased and healthy cells

We have two fuzzy sets and for both sets, we use the SMF membership function:

a) Well: Cells with a probability of disease between 0 and 0.1 are 100% members of this group. If the probability of disease is more than 0.5, their degree of membership for this group is 0.

b) Sick: Cells with a probability of disease between 0.5 and 1 are 100% members of this group. If the probability of disease is less than 0.1, their degree of membership for this group is 0.

First, we have to calculate the probability of healthy or disease of the cell so that we can give it as input to the fuzzy set. We calculate the probability by converting the color image of the isolated cell to gray, calculating its Treshold, then improving this gray image and calculating its Treshold again, and converting the improved gray image to a binary image through the difference of these two Tresholds. We observe that the areas of the Anomaly of the color image are white in the binary image, but depending on the earlier mentioned color difference, a series of points in addition to the Anomaly points are white. For this reason, we consider points belonging to the Anomaly section whose equivalent points in their color image are points belonging to the defective sections in the fuzzy set. Through this method, we count the number of points in the Anomaly, we also count the total number of points inside the cell, get a ratio through these two values, and give it to the fuzzy set as input.

The following is the output of this set per cell with a disease probability of 0.75:

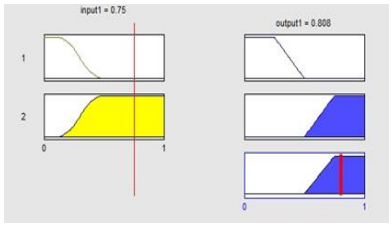


Figure 13: Cell health fuzzy set output per 0.75 input

As you can see in the figure, a cell with a disease probability of 0.75 falls completely under the diseased cell set.

4. Laboratory results

In this section, we will introduce the graphical interface of the program and display the test results in bulk.

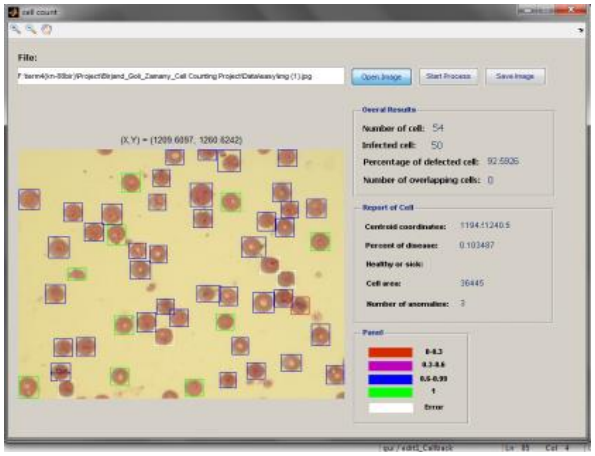


Figure 14: Graphic interface of user for data testing

After processing the image information, including the number of cells, number of diseased cells, detection percentage, and number of overlapping cells, we display information about the percentage of disease, number of cell anomalies, area and coordinates of the center of gravity of each cell display through clicking on it. We have used different colors to show the health of each cell. For example, green indicates the complete health of the cell and red indicates the health of the cell between 0 and 0.3. White indicates cells that have not received processing.

Final processing results:

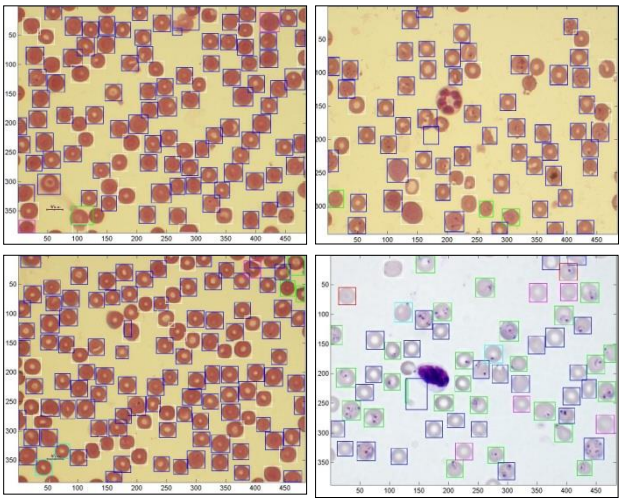
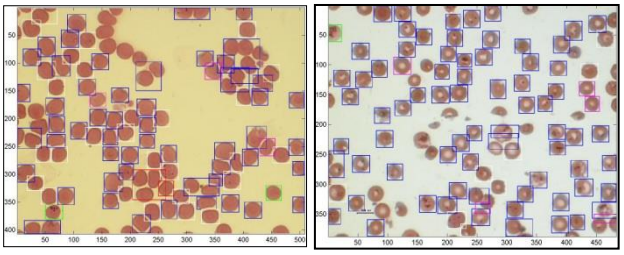


Figure 15: Final display of Easy group cell processing



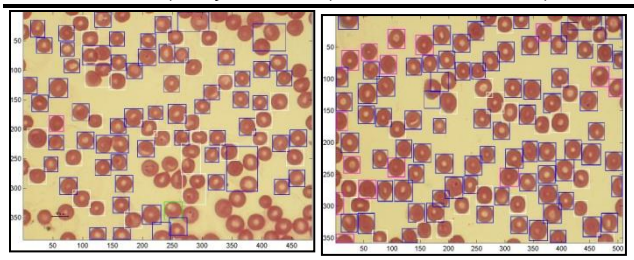


Figure 16: Final representation of Medium group cell processing

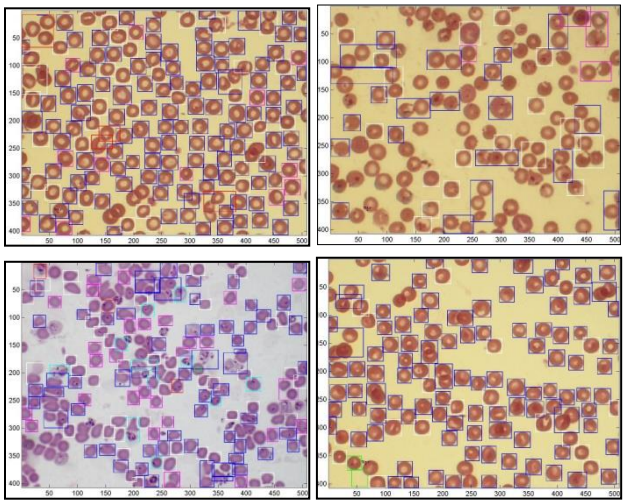


Figure 17: Final representation of Hard group cell processing

Figures 18 and 19 show examples of correct overlap detection. Some images are overlapping cell in color mode and sent to the isolation phase. In this phase, after being converted to a binary image and recognizing the connected components, they are stored separately and they no longer need to enter to the isolation section. Figure 20 shows an example of such a misdiagnosis.

Figure 18: Image of overlapping cells (middle image) and post-isolation images (sides)

Figure 19: Image of overlapping cells (middle image) and post-isolation images (sides)

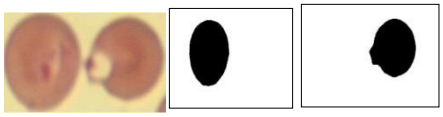


Figure 20: Cells that have been misidentified overlapping and the final stored images

5. Conclusion

Image quality is one of the most important principles in image processing. In the tested images, some images were less accurate due to poor quality. Although we improved the image quality in the preprocessing stage, in some cases the result was not very desirable. The positive point about

this article is its use of fuzzy logic instead of classical logic. By fuzzy logic, it is possible to use the color content of images during processing. The most important part of processing is the calculation of cell health, in which the use of color content plays an important role. After calculating the health of each cell using fuzzy logic, the program specifies the cells it has identified and processed with distinct colors for easier diagnosis of the disease. Moreover, the program makes possible to view more details of each cell, such as area, Gravity center in the colony of cells, and the rate of disease.

Appreciation

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