AUTOMATED SEGMENTATION METHODS FOR MICROSCOPIC BLOOD CELLS

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ABSTRACT
In haematology, developing an automated blood cells types identifications based on microscopic examination could serve as a software tool for archiving haematology images, for automatic classification and it could help widely the clinicians in their laboratory tasks. This paper presents basic algorithms used in automated blood cell identifications based on microscopic image processing techniques. White blood cell segmentation method, red blood cell segmentation method and classification of basic blood cell types are presented. The promising results are compared with the manual investigation results obtained by the haematologists and experts of the field. A ratio of white blood cells over red blood cells is computed and gives an idea of the clinical diagnosis of the blood smear examined and all the examined samples matched the real clinical diagnostic.

KEY WORDS
Microscopic image processing, Adaptive Threshold, Red Blood Cells, White Blood Cells, Anemia, Nucleus and Cytoplasm segmentation, haematology.

1. Introduction

Haematology is referred to the study of the blood. The components of blood include Red Blood Cells (RBC) also called Erythrocytes, White Blood Cells (WBC) also called Leukocytes, platelets, and plasma. There are three general types of white blood cells: Lymphocyte, Monocyte and activated Lymphocyte [1].
In general, White Blood Cells (WBC) and Red Blood Cells (RBC) segmentation and identification have been widely dealt in image processing and pattern recognition techniques.
In [2], active contours have been used to segment the boundaries of the white blood cells. Nevertheless this method was limited due to its inability to handle accurately occluded cells. Other methods such as region growing segmentation have been proposed by Lezoray [3] and they reach 94.5 % average success rate for the nucleus segmentation and 93% for the cytoplasm.

Table 1
Blood Cells Properties

Property	Lymphocyte	Monocyte	Activated Lymphocyte
Size	1-3 RBC	> 3RBC	>3RBC
Nucleus Shape	Circle	Irregular	Irregular
NCR	High	Low to moderate	Low to moderate

However, there was no study based on haemotogical flowchart in white blood segmentation and anemia detection. This paper presents an automated algorithm based on a clinical flowchart proposed by the haematologist in order to differentiate between the different types of white blood cells. Three basic characteristic features have been extracted such as the size, the nucleus shape, the Nucleus to Cytoplasm ratio (NCR) and the existence of granularities in the cytoplasm. These characteristics (size, nucleus shape, NCR and the existing of granularities) for the three types of white blood cells, the lymphocytes, the monocytes and the activated lymphocytes could be resumed in Table 1. Monocytes are characterized by having an area greater than three times the area of the red blood cell, they also have a low to moderate NCR and there is no granular in their cytoplasm.
Lymphocytes are characterized by having an area ranging from 1 to 3 times the area of a red blood cell, they also have a high NCR and there is no granular in their cytoplasm.
Activated Lymphocytes are characterized by the fact that they have differently staining granules in their cytoplasm. After meeting and discussing with haematologists and specialized laboratory doctors and based on their clinical flowchart, the automated segmentation was based on the clinical flowchart presented in Figure 1 on which the classification of the white blood cells is based.
As it has been described in the clinical flowchart in order to identify the white blood cell, the first parameter to check is the granularity of the cytoplasm as described below. If the granularity is found then it is an activated

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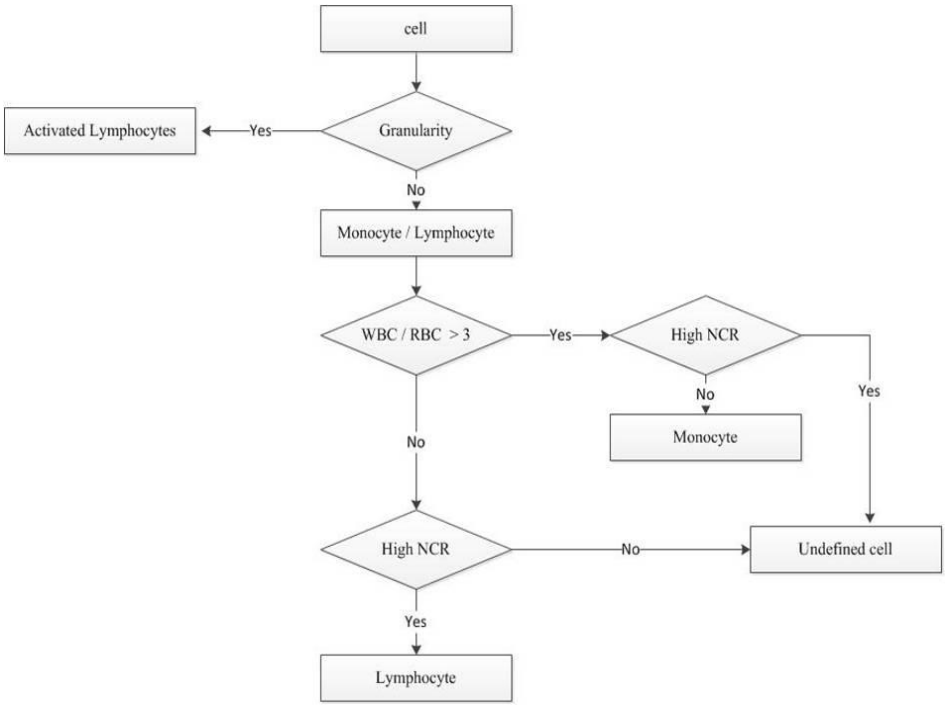

Figure 1. Clinical flowchart of blood cells differentiation

lymphocyte, if not then we compare the WBC area to the RBC area.

If the ratio WBC over RBC is bigger than 3 based on blood cells properties shown in table 1 then the NCR is tested, if the NCR is smaller than 0.75, empirical clinical value proposed by the clinician, then the cell is classified to be a monocyte, if not then the cell is unclassified. The unclassified is generally considered as a cell in maturation phase, not completed differentiated.

In this paper, we present five implemented algorithms.

1) The first one consists in the segmentation of the nucleus of the white blood cells, determining their centroid in the microscopic image, their area and their different characteristics. And finally, this algorithm counts the number of white blood cells depicted in the image.
2) The second algorithm segments the cellular cytoplasm and the nucleus, this algorithm allows to detect the area of the nucleus and the area of the cytoplasm detected plus the nucleus to cytoplasm ratio. Moreover, the computation of the circularity and of the shape of the nucleus have been extracted.
3) The third algorithm detects the granular particles in the cytoplasm region and it allows to define if the cell is among the granulocytes cells or not
4) The fourth algorithm developed comes to complement the previous already developed on the WBC, it segments the red blood cell and allows to detect their number, their area and other characteristics.
5) The detection of the red blood cells has been improved using the circular Hough transform.

All these algorithms have been implemented using Matlab from MATHWORKS©.

2. Materials and Methods

2.1 White Blood Cell Segmentation

The detection of white blood cells in our system is based on the classification of morphological features. Figure 2 presents the flowchart of segmentation of white blood cells based on the green component in the RGB image since it shows more contrast in the nucleus.

A linear contrast stretching [4] is used as shown in equation (1) and then histogram equalization is done for contrast enhancement.

$$I_p(x,y) = \frac{255 \quad * \quad [\, I_0(x,y) \quad - \quad \min\,]}{(\max \quad - \quad \min)} \tag{1}$$

Where: Ip (x,y) is the color level for the output pixel(x, y) after the contrast stretching process.

$I_0(x,y)$: is the color level input for data the pixel(x, y).

max: is the maximum value for color level in the input image.

min: is the minimum value for color level in the input image.

After contrast enhancement of the nuclei, an adaptive threshold using Otsu algorithm [5] (Figure 3) has been

applied. Morphological opening is used to remove small pixels group. The procedure of the morphological opening is carried with a disk structuring element having an optimal value, with a radius of 9 for best opening results. Knowing that 9 pixels is relatively very small comparing the size of the cells of interest. Hence, all connected components with size less than 10% (value chosen by experimentations over multiple trials) of the WBC average area are eliminated (Figure 4). Finally, morphological erosion is used to separate overlapping white blood cells. Upon trial experiences, our disk-structuring element should have a radius of 11, this radius will not affect the size and the shape of the nucleus, for obtaining optimum separation.

Engaging the automated structure, ten images were investigated to drive out the accuracy of the system. The initiation of the test was triggered with manual enumeration of the white blood cells in the original picture.

$$accuracy = \frac{Number\ of\ WBC\ in\ AutoCount}{Number\ of\ WBC\ in\ manual\ count} \quad (2)$$

An average accuracy over the 10 images tested is obtained around 95.6 %. The nucleus detected correspond perfectly to the white blood cells nucleus as shown in Figure 5. On the binary image obtained in Figure 4-b, the centroid of each cell is computed and it is used in order to isolate the cell.

2.2 Cellular Cytoplasma and Nucleus Segmentation

Segmentation of the white blood cell is started using the previous method to detect and isolate the nucleus of the white blood cells. The centroid of each nucleus is used in order to isolate every leukocyte in a rectangular box with a certain size from the gray-scale original image as shown in Figure 6. The size depends on the magnitude of the original image to allow the cropped image to contain all the leukocyte. The next step is the leukocyte membrane selection as shown in Figure 6. In this step we start by applying the adaptive canny edge detection on the image segmented before. This filter is commonly known in edge detection for its efficiency in compensating the lighting and the contrast variations. Sobel Edge Enhancing is reapplied to reconstruct and to increase the thickness of borders of the membranes.

Next, the dilation, with a structural element as a disk with size 4, has been employed to better connect disunited points of the membrane border, and to form the perimeter of the cell as a connected item (thicker more than one pixel). Then a hole filling procedure based on morphological reconstruction [6] is applied. Moreover, an erosion operator has been done in order to separate the leukocyte cell at the middle from other red blood cells at the border. Afterward, a dilation operator with a structural element disk size 4, has been employed to better connect disunited points of the membrane border, and to form the perimeter of the cell as a connected item (thicker more than one pixel). Finally we make sure to clear all the borders by using morphological operations to suppress light structures connected to image border.

Furthermore, in order to obtain a more accurate result in the thresholding while applying Otsu algorithm [5], the image has been cropped based on the major axis direction of the cells in order to compute a local adaptive threshold. The isolated cell is then multiplied by the leukocyte membrane selection as a mask afterward an Otsu threshold is applied for cytoplasm detection. Finally, the nucleus of the leukocyte is detected by applying XOR on cytoplasm and leukocyte images. Based on these, the NCR is computed knowing the area of the nucleus over the area of the cytoplasm.

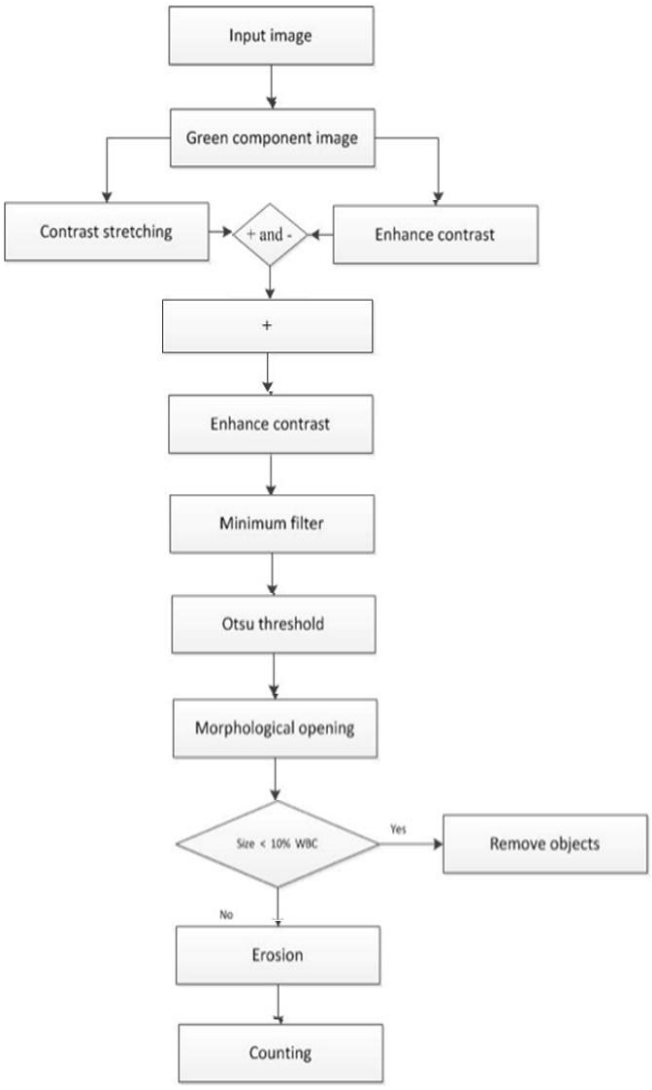

Figure 2. Flowchart of the white blood segmentation

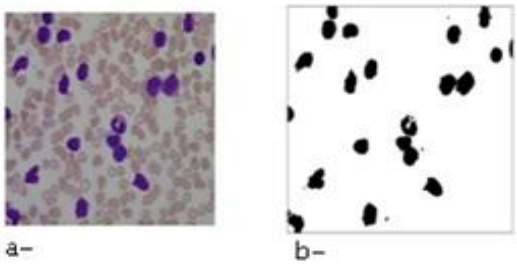

Figure 3. a- Original RGB microscopic image showing in magenta the white blood cells. b- Binary image after Otsu algorithm

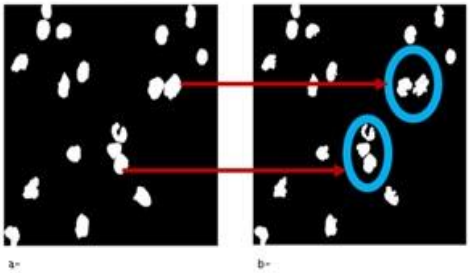

Figure 4. a- Binary image after removing connected components with area less than 10% of the average area of the white blood cell. b- Separation of connected white blood cells after erosion

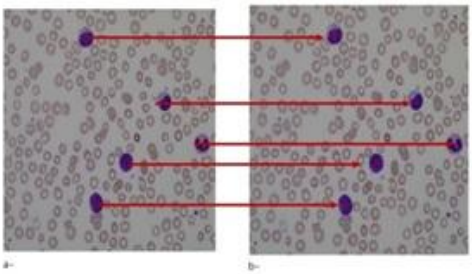

Figure 5. Depicted centroids of white blood cells

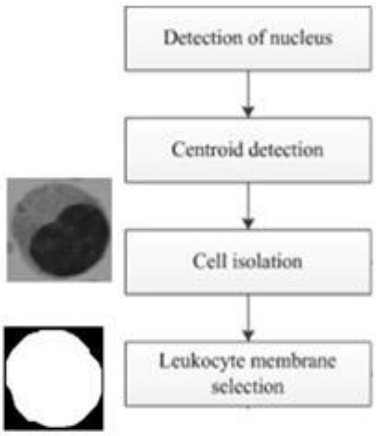

Figure 6. Flowchart of the leukocyte membrane selection

2.3 Granularities Detection

The granular cells are very small comparing to nucleus. They surround the nucleus in the cytoplasm. Hence, the detection of granular cells helps to classify the white blood cells as shown in Figure 7. The granularities in the cytoplasm region indicate that the white blood cells could be neutrophils, basophils or eosinophils. If there is no granularities in the cytoplasm, the white blood cell could be a lymphocyte or a monocyte.

2.4 RBC Segmentation

As illustrated in Figure 8, the Segmentation and the extraction processes are done in the hue-saturation value color space (HSV). The input image is converted to HSV. S component is then extracted before Otsu threshold is used to determine the threshold value, that is applied on the S component image to obtain the binary image. To eliminate all the black holes in the white blood cells and platelets, the morphological operators are applied. A second operation is required in order to sort RBC, that's why Otsu Threshold is applied on the green component of the original image to In order to eliminate all the black holes in the white blood cells, red blood cells and platelets, morphological operators are applied. An example of the centroids of the RBCs detected are illustrated in Figure 9. To evaluate our method, ten images were investigated to drive out the accuracy, a total of 90% average total accuracy has been obtained over the red blood cells depicted in the 10 images. The errors come from the overlapping of two or more cells, which the algorithm did not detect them. The algorithm weakness was the inability to detect the border cells showing in the figure less than their size. In order to improve the RBC segmentation accuracy, the RBC segmentation using circular Hough transform [7] comes to overcome this weaknesses.

2.5 RBC Segmentation using Circular Hough Transform

Based on the previous algorithm, we consider as red blood cell all detected connected components with circularity higher than 0.75. All cells with circularities less than 0.75 could be connected blocs of RBCs, a flat irregular RBC due to the sliding of the strips or due to the bulb air existence between the strips.

Therefore, circular Hough transform has been applied to detect the red blood cells as shown in Figure 10 to increase the accuracy of red blood cell segmentation to 96.3%.

Established on the significant average accuracy, we will extract the equivalent average for the red blood cells calculation. The average of the average area of red blood cells is around 3798 in Pixels Square and correspond to 38.48 in μm^2 knowing that the range of area of red blood cells 28.5-50.2 μm^2.

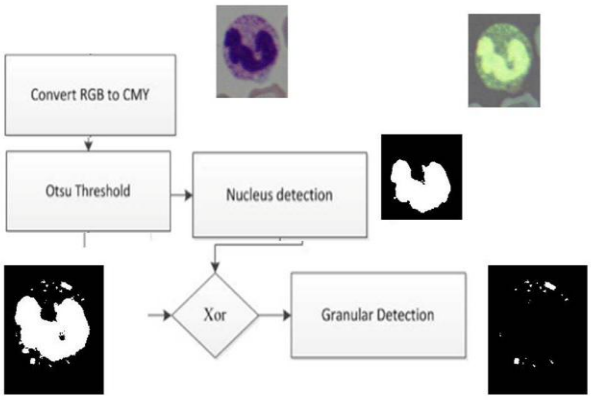

Figure 7. Granularities detection

3. Results and Analysis

Based on the algorithms cited above, we computed the average area of the white blood cells over the average area of the red blood cell (WBC/RBC), the nucleus circularity described in (3) and the NCR (4).

$$circularity = \frac{Perimeter^2}{2.\pi Area} \quad (3)$$

$$NCR = \frac{Nucleus\ Area}{Cytoplasm\ Area} \quad (4)$$

Figure 10 shows examples of classified white blood cells based on the feature characteristics cited in Table 1. After applying the clinical flowchart described in Figure 1 on different samples, 14 over 15 of the lymphocytes have been detected, and 15 over 16 of monocytes, and 2 over 2 of the activated lymphocytes matched the laboratory results. Consequently, approximately 94% of the monocytes matched the manual identification of the hematologist, 93.5% of the lymphocytes and 100% of the granulocytes. an average of 95.8 % of these results matched the laboratory examinations and 4.2 % were inaccurate.

The computation of number of WBCs and the number of RBCs could be used to compute the ratio as shown below in order to recognize if the sample of blood refers to a leucopenia diagnosis or leukocytosis diagnosis.

$$ratio = \frac{Number\ of\ WBC}{Number\ of\ RBC} \quad (5)$$

If the ratio is lower then 0.010, then the person is diagnosed with leucopenia. If the ratio is higher than 0.050, then the person is diagnosed with leukocytosis. If the ratio is in between 0.010 and 0.050, the person is considered normal. Figure 12 shows the ratio WBC/RBC, 100% true, for 10 samples of blood microscopic images.

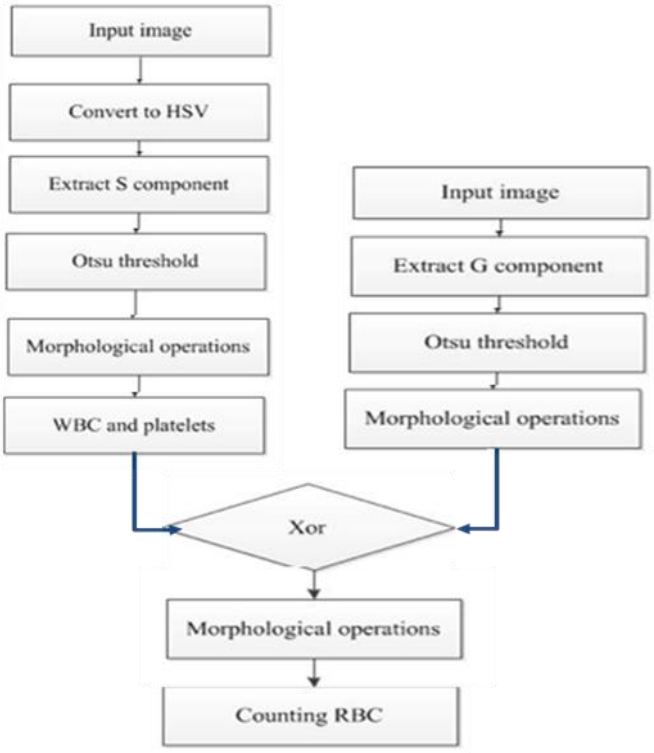

Figure 8. Flowchart of Red Blood Cells (RBC) segmentation

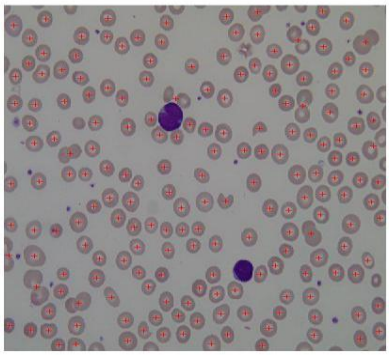

Figure 9. Centroids of Red Blood Cells depicted

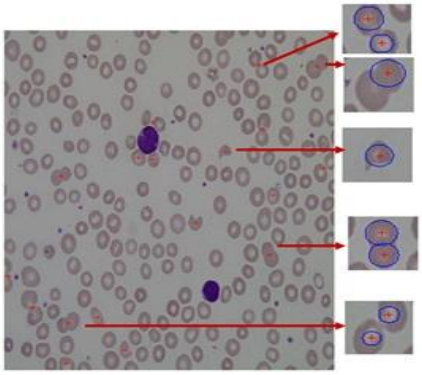

Figure 10. Overlapped RBCs detection using Circular Hough Transform

Images	WBC/ RBC	Nucleus Circularity	NCR	Granular	Type
	3.415	0.7664	0.596	NO	Mono
	2.733	0.7515	0.828	No	Lympho
	3.050	0.2916	0.458	Yes	Granulo

Figure 11. Examples for the white blood cells classification based on the feature characteristics cited in Table 1

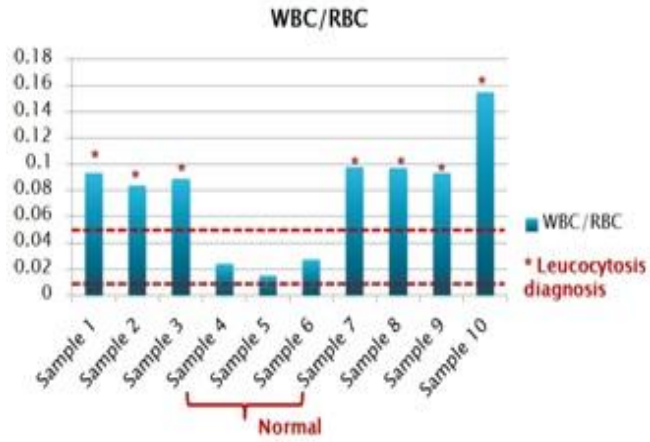

Figure 12. 10 samples diagnosis results that match the real clinical diagnosis of the each blood smear sample

4. Conclusion

The paper presents series of feature extraction methods to screen automatically a sample of blood smear from a patient. It allows to extract the WBC and the RBC in each microscopic image, to compute the ratio WBC/RBC and finally to classify the WBC according to some characteristics cited in the clinical flowchart as it has proposed by the hematologist.

The algorithms developed could be resumed as follow: detection and counting of nucleus, segmentation of cellular cytoplasm and nucleus and granular detection of WBC cells, finally with RBCs segmentation and counting.

The method presented in this paper shows promising results relatively to RBC counting and WBC segmentation and the clinical diagnosis of the anomalies in the blood smear.

Nevertheless, other features in white blood cells require investigation in order to classify the different types of white blood cells. The clinical flowchart should be extended to additional features and to allow the discrimination of the different types of white blood cells. The chromatin is an essential feature to be automatically analyzed, that until now, there is no automated reliable software that could be used and this feature still relies on the empirical investigation of the field expert.

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