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A Comparative Study of White Blood cells Segmentation using Otsu Threshold and Watershed Transformation

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ABSTRACT

The aim of white blood cells (WBC) segmentation is to separate leukocytes from other different components in the blood peripheral image. In this paper, a method to segment white blood cells from microscopic images is proposed. The proposed method consists of three stages; Pre-processing, segmentation, and finally post-processing. In the pre-processing step; the color correction is used to enhance the image. In the segmentation step; two techniques have been used which are Otsu threshold and watershed marker-controlled followed by feature extraction. Shape features are used to differentiate between single and grouped cells. Artifacts are removed in the post-processing step. Experimental results show that the accuracy is 99.3% and 93.3% for the watershed based and the Otsu threshold based methods respectively. Experiments demonstrate that watershed marker-controlled outperforms Otsu threshold in the segmentation of WBC.

Keywords: White blood cells, Leukaemia, segmentation, Otsu threshold, watershed, feature extraction.

1 Introduction

Leukaemia results from the overproduction of immature White Blood Cells (WBC). WBCs are originally produced in the bone marrow. When the bone marrows are incapable of generating normal cells, the definition of leukaemia is located. It occurs in all ages. Normal or undiagnosed cells are called leukocytes while immature white cells are called blasts. Acute Leukaemia is classified according to the French-American-British (FAB) classification into: Acute Lymphocytic Leukaemia (ALL) and Acute Myelogenous Leukaemia (AML). The early detection and diagnosis of the leukaemia type will assist in the treatment for the identified type [1].

The detection and diagnosis process used to be performed by experienced operators. This manual procedure is slow and it relies on the experience of the operator. It is required to automate the process of detection and diagnosis of different leukaemia types. The automatic process needs an image, not a blood sample and suitable for low-cost, standard-accurate, and remote screening systems [2].

Many researchers were interested in the detection and classification of white blood cells. Research in this area includes cell identification, segmentation, feature extraction, and classification. Most of the proposed methods start with a pre-processing step, then segmentation, and finally the classification

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step. The accuracy of the feature extraction and classification depends on the segmentation of white blood cells.

Regarding the pre-processing of microscopic images, many techniques have been used in the literature as an enhancement step. Local contrast stretching (LCS) and median filter are applied on original acute lymphoblastic leukaemia image [3, 4]. Otsu's global thresholding method is used to automatically perform histogram shape-based image thresholding [2, 3]. While bimodal threshold followed by membrane boundary tracing (dilation and region filling) is performed in [5]. Contrast stretching technique is implemented in [1, 6, 7]. The triangle method or Zack algorithm is used in the pre-processing step in [8] to find a threshold value to separate cells from the background.

The detection of leukaemia has been proposed in [9, 10]. In the work proposed in [9], the RGB image is converted into HSI (hue, saturation, intensity) color model. The saturated component is extracted for further processing. Then, the gradient magnitude for the saturation components is obtained. Moreover, Sobel, Canny, Prewitt operators are used for the edge detection. After extracting the white cells from the image and elimination of the background and red blood cells (RBcs), dilation or erosion process is carried out. Finally, a watershed transform is carried out to separate the connected cell.

WBCs are segmented from color microscopic images in [2, 5, 8, 10, 11]. The active contour is used to segment the nucleus in the work presented in [11]. Watershed algorithm is used in [8, 12, 13]. A comparative analysis of edge-based segmentation and watershed segmentation on images of the red blood cells is reported in [13].

A differentiation between using HSI and RGB color space to segment ALL is reported in [3]. Segmentation based on HSI color space was chosen as it produced the highest ALL segmentation rate. Morphological operations as segmentation of WBCs are used in [2, 12].

White blood cells were classified into different categories related to the type of leukaemia [1, 4, 5, 12, 14]. These techniques are based on feature extraction and then applying a classifier. Feature extraction means to transfer the input data into different set of features. In [11], the roundness value of 0.8 is chosen to describe as a single leukocyte. While, the convex hull is used in [15]. Geometric features are extracted in [1, 6, 8, 12].

Different classifiers have been used such as: K-nearest neighbor (kNN) [1], Supported Vector Machine (SVM) [4, 8, 12], the Artificial Neural Network (ANN) [16].

From the three main components (RBC, WBC, and platelets) of the blood, this paper concentrates on the WBC segmentation from microscopic images. This paper is organized as follows; Section 2 describes the proposed method. Section 3 presents experimental results and conclusions are given in Section 4.

2 Methodology

In this section, the segmentation method is discussed. Figure 1 shows a block diagram of the proposed method. It consists of three main steps; pre-processing, segmentation and post processing.

2.1 Pre-processing

In this step, the color of the test images is corrected due to the different illumination of capturing images. Color correction is used in the pre-processing. Mean (desired) intensity is extracted from the

mean histograms of red, green and blue channels. Then, it is applied to the image as an enhancement step. Figure 2 shows the input test image (RGB) and the color corrected image. Figure 3 illustrates the three components of the RGB (red - green - blue) image and the corresponding histograms. For this test image, the desired mean intensity is calculated and equal to 141.7128.

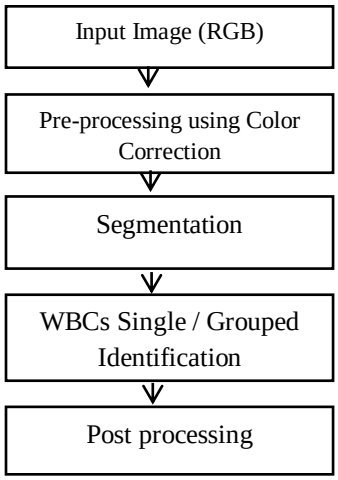


Figure 1: Block diagram of the segmentation steps.

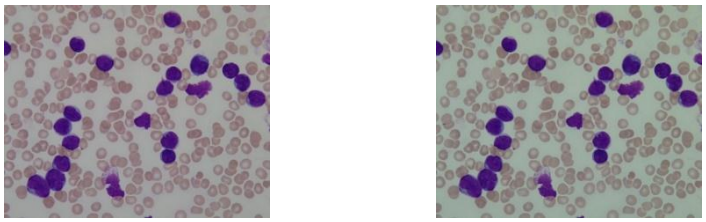


Figure 2: (a) Input image and (b) Color corrected image.

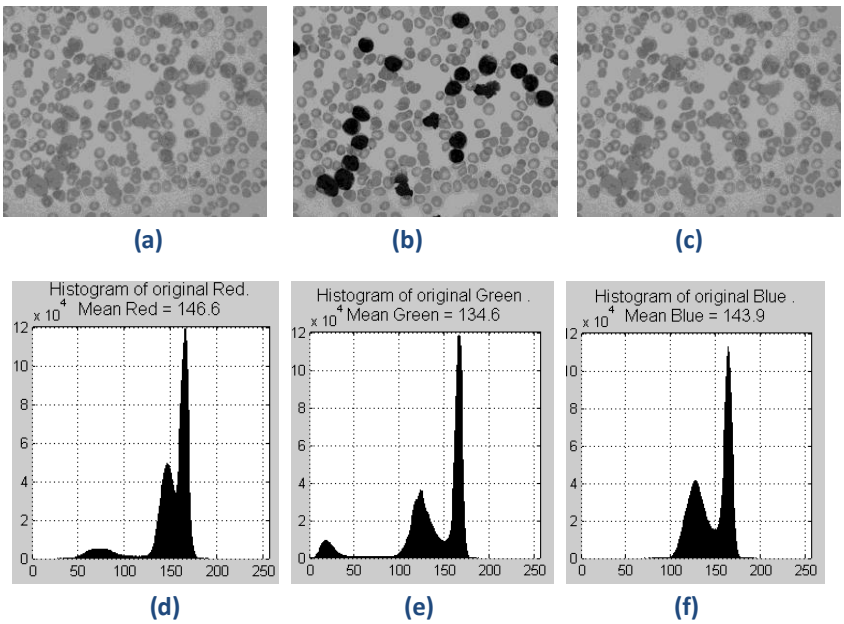


Figure 3: (a) Red component, (b) Green component, (c) Blue component, and (d, e, f) histograms of red, green and blue components respectively.

2.2 Segmentation

Segmentation is the process of partitioning an image into a set of objects [17]. Many color spaces have been used, such as RGB, HSI, and LAB. The RGB format is most straightforward because it deals directly with the red, green, and blue image that are closely associated with the human visual system. The HSI format, hue and saturation can best be described by the use of a color circle. The hue of a color refers to the spectral wavelength that it most closely matches. The saturation is the radius of the point from the origin of the color circle and represents the purity of the color. The RGB and HSI formats can be easily converted from one to the other. A color image can be converted to a monochrome image by averaging the RGB components together, which discards all chrominance information during the conversion [18].

In the proposed method, the segmentation step is performed using two different techniques which are the Otsu threshold and the watershed marker-controlled.

2.2.1 Otsu threshold

Firstly, the image is converted to HSI format, Then, S component is extracted to work on it. S component can provide almost similar pixel values and shape to original blasts [6]. Then, Otsu threshold is applied to segment the cells. The image contains artifacts which needed to be enhanced in order to count the cells. Objects of small number of pixels were removed using morphological opening (using a disk structuring element). Figure 4 illustrates the process applied on the S component.

To determine the single and the grouped cells (leukocytes), features are extracted. Four shape features for the cells are considered as the shape of the nucleus is important for blasts' identification [18]. These features are area, perimeter, thinness ratio, and solidity.

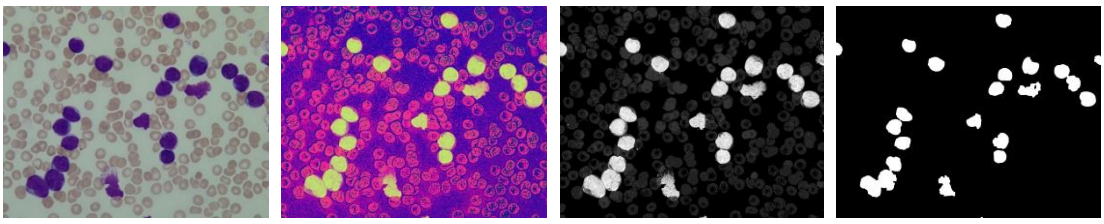
1. **Area:** the total number of none zero pixels within the image region.
2. **Perimeter:** the distance between successive boundary pixels.
3. **Thinness Ratio:** to define the regularity of an object.

$$T = 4\pi A/P^2 \quad (1)$$

Where A is the area, and P is the perimeter of an object. This measure takes a maximum value of 1 for a circle. Objects of regular shape have a higher thinness ratio than irregular ones.

4. **Solidity:** Area / Convex Area [18].

Figure 5 illustrates grouped and single cells (leukocytes). Single leukocytes are identified by small area and high thinness ratio. The grouped leukocytes are characterized by bigger area, small thinness ratio, and high solidity.



(a) (b) (c) (d)

Figure 4: (a) Input image, (b) HSI image, (c) S component, and (d) Output after Otsu threshold.

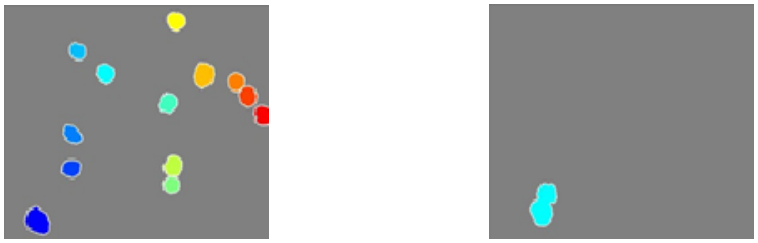
(a) (b)

Figure 5: (a) Single leukocytes, and (b) grouped leukocytes.

2.2.2 Watershed-marker controlled

Images can be effectively segmented using the watershed transformation. This includes region based approach [19]. It depends on: detection of discontinuities, thresholding, and region processing. Discontinuities of the image are detected using the image gradient. An image gradient is a directional change in the intensity or color in an image. Image gradients may be used to extract information from images. Marker extraction is one of the important steps in watershed transform segmentation. A marker must be placed inside every object that needs to be extracted. The regional maxima (minima) can be used as inner (outer) markers for watershed segmentation [18]. The result after the watershed transformation is an image containing the white blood cells (single and grouped cells).

Features such as: area, roundness and solidity are extracted to differentiate between single and grouped leukocytes. The resultant image is shown in Fig. 6 for single and grouped leukocytes.



(a) (b)

Figure 6: (a) Single leukocytes, and (b) grouped leukocytes.

2.3 Post-processing

2.3.1 Exoskeleton

After having a separate image that contains the grouped cells (as in Fig. 5.b and Fig. 6.b). It is required to separate them into a set of single cells. A step is required to separate touching (adjacent) cells. This is achieved by performing '*exoskeleton*'. The exoskeleton is a binary morphological processing and it represents the skeleton of the background outside the objects. It is then followed by computing the logical operation AND of the original binary image and the inverted skeleton image [18]. Firstly, sub image of connected cells are cropped and thresholded using Otsu threshold. Morphological cleaning and erosion with a predetermined structuring element are performed. Finally, the exoskeleton is applied. Figure 7 illustrates sub-images to demonstrate procedures described in this sub-section.

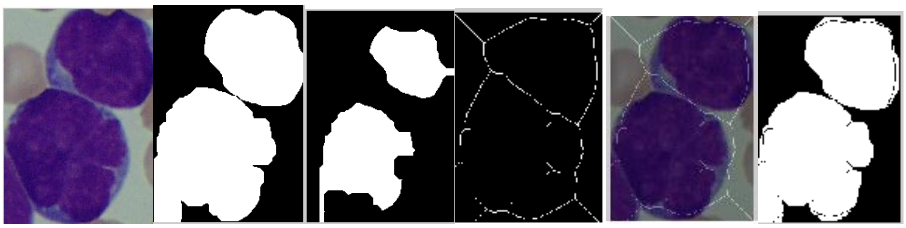


Figure 7: (a) Sub-image, (b) Otsu threshold, (c) Erosion, (d) Exoskeleton image, (e) Exoskeleton superimposed on original sub-image, and (f) Final binary image.

2.3.2 Image cleaning

To improve the final result, image cleaning is used to remove leukocytes on the border and random cells (artifacts). Area and convex area are used to calculate the solidity value [8]. All objects with solidity less than a predetermined value are discarded. Figure 8 shows the final results.

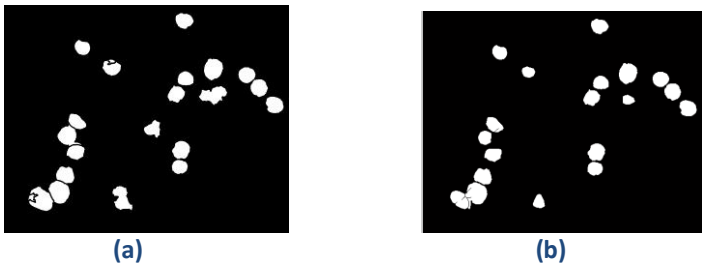


Figure 8: Output image: (a) before cleaning, and (b) after cleaning.

3 Experimental Results

3.1 Dataset

For performance evaluation and comparison, a publicly available dataset is used. The dataset has been captured with an optical laboratory microscope coupled with a Canon power shot G5 camera. All images are in JPG format with 24-bit color depth, resolution 2592 × 1944 [20]. The ALL-IDB database has two versions (ALL-IDB1 and ALL-IDB2). This dataset is composed of 108 images. It contains about 39000 blood elements. Images are taken with different magnifications of the microscope ranging from 300 to 500.

3.2 Results

Otsu threshold and watershed marker-controlled techniques are used to divide the test image into two sets of images; one contains the single leukocytes and the other contains the grouped cells.

Otsu threshold is able to segment white blood cells only based on the intensity values. On the other hand, the watershed algorithm can be used to segment both types of cells (white and red). As the main focus is to segment the WBC, then the final segmented image contains the WBC only based on the intensity values from the RGB color corrected image.

Figure 9 illustrates three test images from the ALL_IDB1 dataset and the corresponding segmented images using both techniques after the post processing and cleaning step.

3.3 Discussion

For performance evaluation, images from the ALL_IDB1 dataset are used and results are compared to another method in the literature [8]. In this work, a manual counting for the WBC was performed using a set of 33 test images and compared to the automatic counting.

In our experiments, the same 33 images were used for segmentation of the WBC using the Otsu and watershed. Then, the number of segmented WBCs are compared to the manual count and the method in [8]. Table 1 summarizes results obtained for counting WBCs.

For these images, Otsu threshold results in an accuracy of 66%, 70%, and 83% respectively, while the watershed technique is able to successfully segment WBCs.

Segmentation using the Otsu threshold results in an average accuracy of 93.3% while the watershed results in 99.3%. The method in [8] results in an average accuracy of 91.7%. Watershed marker-controlled method is better than the Otsu threshold in the detection and segmentation of WBCs.

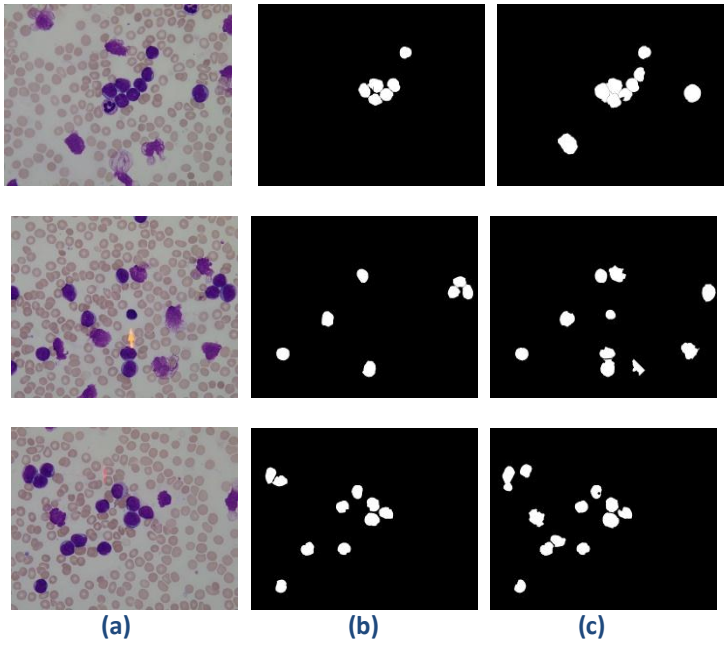


Figure 9: (a) Im001, Im002 and Im003 test images, (b) Otsu threshold, and (c) watershed marker-controlled.

4 Conclusions

In this paper, white blood cells were segmented from microscopic color images. The proposed method consists of three steps; pre-processing, segmentation, and post-processing. Color correction is used in the pre-processing to enhance color test images. In the segmentation step, two different techniques have been experienced. These techniques are the Otsu threshold and watershed marker-controlled. The exoskeleton is used to separate adjacent cells followed by image cleaning to remove artifacts and small size objects. A comparison between Otsu threshold and watershed technique is performed. The watershed marker-controlled step gives better results than the Otsu threshold.

Table 1 Segmentation results

	Putzu et al. [8]			Proposed			
Image no.	**Manual Count**	**Automatic count [8]**	**Accuracy**	**Otsu**	**Accuracy**	**Watershed Algorithm**	**Accuracy**
Image 1	9	5	55%	6	66%	9	100%
Image 2	10	10	100%	7	70%	10	100%
Image 3	12	11	91%	10	83%	12	100%
Image 4	7	4	57%	6	85%	7	100%
Image 5	24	19	79%	19	79%	24	100%
Image 6	18	18	100%	15	83%	18	100%
Image 7	7	7	100%	7	100%	7	100%
Image 8	17	16	94%	17	100%	17	100%
Image 9	7	7	100%	7	100%	7	100%
Image 10	12	12	100%	12	100%	12	100%
Image 11	15	12	80%	15	100%	15	100%
Image 12	12	12	100%	12	100%	12	100%
Image 13	10	7	70%	10	100%	10	100%
Image 14	5	3	60%	5	100%	3	80%
Image 15	17	17	100%	17	100%	17	100%
Image 16	16	16	100%	16	100%	16	100%
Image 17	3	3	100%	3	100%	3	100%
Image 18	8	8	100%	8	100%	8	100%
Image 19	12	12	100%	12	100%	12	100%
Image 20	2	2	100%	2	100%	2	100%
Image 21	3	3	100%	3	100%	3	100%
Image 22	5	5	100%	4	80%	5	100%
Image 23	6	6	100%	6	100%	6	100%
Image 24	4	4	100%	4	100%	4	100%
Image 25	3	3	100%	3	100%	3	100%
Image 26	5	5	100%	5	100%	5	100%
Image 27	3	3	100%	3	100%	3	100%
Image 28	2	2	100%	2	100%	2	100%
Image 29	4	4	100%	4	100%	4	100%
Image 30	3	3	100%	3	100%	3	100%
Image 31	2	2	100%	2	100%	2	100%
Image 32	2	2	100%	2	100%	2	100%
Image 33	2	2	100%	2	100%	2	100%
Summation of WBCs	267	245		249		265	
Total Accuracy			**91.7%**		**93.2%**		**99.2%**

***Note: manual count is done without counting cells on border**

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