

# An Efficient Automatic Segmentation Method For Leukocytes

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## Abstract

Blood tests are of the most important and counting of leukocytes in peripheral blood is commonly used in basic clinical diagnosis. A major requirement for this paper is an efficient method to segment cell images. This work presents an accurate segmentation method for automatic count of white blood cells. First a simple thresholding approach is applied to give initial labels to pixels in the blood cell images. The algorithm is based on information about blood smear images, and then the labels are adjusted with a shape detection method based on large regional context information to produce meaningful results. This approach makes use of knowledge of blood cell structure, the experimental result shows that this method is more powerful than traditional methods that use only local context information. It can perform accurate segmentation of white blood cells even if they have unsharp boundaries.

**Keywords:** Leukocytes, Thresholding, Pixels, Peripheral Blood, Segmentation.

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## 1. INTRODUCTION

There are many different classes of white blood cells present in blood smear images. Differential count of these various types of cells gives valuable information and plays an important role in the diagnosis of differential diseases. It's a tedious task to count class of cells manually. An automatic counter using computer vision helps this medical test rapidly and accurately. The first step of this automatic analysis is a segmentation of blood cells images, which differentiates meaningful objects from the background. In these case attempting to identify white blood cells with an accurate segmentation methods.

This step is crucial because the result is the basis of subsequent analysis. The success of classification depends mainly on the correct segmentation of images. It's also difficult and challenging problem due to the complex nature of the cells and uncertainty in the microscopic images. Cells often overlap each other and have variation of different size and shape. The contrast between the cells boundary and the background varies according to illumination in consistency.

Many segmentation methods of blood cell images have been proposed. Histogram thresholding, edge detection and regional growing methods are often used. Thresholding techniques always can't produce meaningful result since no spatial information is used during the selection of the segmentation threshold. They are often combined with mathematical morphology operation. Edge detection method perform poorly on cell image because not all cells boundaries are sharp, so it's difficult to get all the edge information and locate the cells

Most of these mentioned methods are on short decision processes and often make wrong crisp decisions. Relaxation methods are proposed to avoid it. A fuzzy patch label relaxation algorithm used patches that provide more useful and meaningful context information that compact local context to obtain better segmentation results. But sometimes the information of a few neighbour patches still not enough to make correct decision. For example, too bright illumination often leads

to bright gaps inside cytoplasm, and then a part of cytoplasm may be labeled as red blood cells since it is separate from the other parts of the cytoplasm and is similar to a red blood cell in position relationship. The neighbour patches play poorly on recovering this mistake. Hence a shape detection method is proposed to provide fast and accurate segmentation by using large regional context information. Since more information is used to help make decision, the approach is more robust and efficient.

In this paper presents a white blood cell segmentation approach that consists of three steps. First is simple thresholding approach combined with mathematical morphology operation is applied to give initial labels to pixel in the blood cell images. The algorithm is based on priori information about blood smear images derived from a learning process. Next the labels are adjusted with a shape detection method based on large regional context information, which makes use of knowledge of blood cell structure. At last the regions of white blood cells are marked and the shapes of the regions are arranged to form rounded boundaries. The following sections give a detailed explanation of the proposed method along with some experiments results.

## **2. PROPOSED METHOD**

### **2.1 Initial Segmentation**

The goal of initial segmentation is to separate four different regions roughly, background, red cells, cytoplasm and nucleus. Information of colours, brightness and gradients are used in thresholding to create initial labels of the pixels. The bright white or yellow regions correspond to background. The dark regions correspond to nucleus. The regions that have intermediate brightness and small gradients correspond to red cells. Other regions are labeled as cytoplasm temporarily. Most thresholds are derived from priori information of blood smear images. But some sensitive thresholds should be selected automatically to adapt to the illumination variation, which greatly influence the segmentation result.

To select an optimal threshold, we change it from a smaller value to larger one by a small step and segment the image with it. If the segmentation is correct with this threshold, the edges of the segmented image should have relatively large gradients, which correspond the real edges of different regions. So we calculate the number of the edge points that have relatively large gradients while the threshold changes and select the threshold that leads to the largest number of edge points to serve as the optimal threshold.

The regions labeled cytoplasm after thresholding are not real cytoplasm regions. They are repartitioned to three different parts according to their connection status with other components. Regions connected with only red cell regions are labeled as red cell. Regions connected only with nucleus and regions connected with both are relabeled separately.

Every time the labels of pixels change, the different types of regions are smoothed with mathematical morphology operation to get connected regions and eliminate single points and lines caused by noises.

After initial labeling with thresholding method and morphology operation, the blood smear images are segmented roughly into four regions. Pixels that are sure enough to belong to background or nucleus are determined and other labels of pixels will be adjusted in the following steps.

### **2.2 Label Connection with Shape Detection**

Now the initial nucleus regions along with cytoplasm regions around them make up the initial leukocyte regions. They are not real leukocyte regions since there are many other regions in them such as red cell and stain regions which may be connected with real leukocyte regions. Some of these false regions can be eliminated by a scan method.

Real leukocyte regions are connected regions with convex shapes. False regions may be connected with them, but there still will have some relatively large gaps between them. So when we scan the images in a certain direction, back ground regions will be detected between false

regions and real regions. Separate regions in this direction can be eliminated. That is although the false regions are connected with real leukocyte regions somewhere; they are separate in certain directions and can be detected by scanning in these directions. Fuzzy C-Means is a clustering method which allows single data belong to more than one clusters. This method (Dunn, 1973) is a pattern recognition based on minimization of the following objective function given as Equation (2) below:

The FCM algorithm attempts to partition a finite collection of  $N$  elements  $X=\{X_1, X_2, \dots, X_n\}$  into a collection of  $c$  fuzzy clusters with respect to some given criterion. Given a finite set of data, the algorithm returns a list of  $c$  cluster centres  $C=\{C_1, C_2, \dots, C_n\}$  and a partition matrix

Like the K-means clustering, the FCM aims to minimize an objective function:

$$J_m = \sum_{i=1}^N \sum_{j=1}^c u_{ij}^m \|x_i - c_j\|^2 \quad \text{-----(1)}$$

where:

$$u_{ij} = \frac{1}{\sum_{k=1}^c \left( \frac{\|x_i - c_j\|}{\|x_i - c_k\|} \right)^{\frac{2}{m-1}}} \quad \text{-----(2)}$$

$$c_j = \frac{\sum_{i=1}^N u_{ij}^m \cdot x_i}{\sum_{i=1}^N u_{ij}^m} \quad \text{-----(3)}$$

where each element  $u_{ij}$  tells the degree to which element  $x_i$  belongs to cluster  $C_j$ .

This differs from the k-means objective function by the addition of the membership values  $u_{ij}$  and the fuzzifier  $m$ , with  $m \geq 1$ . The fuzzifier  $m$  determines the level of cluster fuzziness. A large  $m$  results in smaller memberships  $||^*$  is any norm expressing the similarity between any measured data and the center.

The algorithm is composed of the following steps:

1. Initialize  $U=[u_{ij}]$  matrix,  $U(0)$
2. At  $k$ -step: calculate the centers vectors  $C(k)=[c_j]$  with  $U(k)$

$$c_j = \frac{\sum_{i=1}^N u_{ij}^m \cdot x_i}{\sum_{i=1}^N u_{ij}^m}$$

3. Update  $U(k)$ ,  $U(k+1)$

$$u_{ij} = \frac{1}{\sum_{k=1}^c \left( \frac{\|x_i - c_j\|}{\|x_i - c_k\|} \right)^{\frac{2}{m-1}}}$$

4. If  $\|U(k+1) - U(k)\| < \epsilon$  then STOP; otherwise return to step 2.

After the scan process a large region containing several white blood cells has been segmented out. Then we'll give accurate segmentation in smaller regions each containing a single white blood cell. Nucleus regions are always in the centre of the leukocyte. There are many "cytoplasm" regions around them, which may be labeled incorrectly. The real cytoplasm regions can be picked out with a regularity detection method. The distance from a boundary point of the cell to center

point of the nucleus is calculated to serve as the radius. If a real leukocyte region is missed, a sharp decrease can be detected while we check the radiuses of boundary points in turn. If a false region is added into the leukocyte region, a sharp increase will be detected. These two cases both lead to great variation in radius. So the cytoplasm regions that together form a region with the least variation of the radius should be labeled real leukocyte regions.

### 2.3 Shape Arrangement

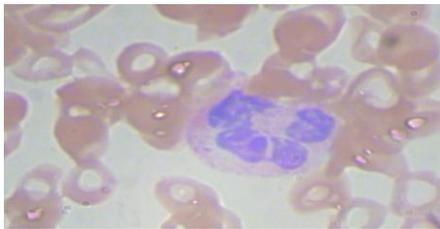
Most pixels have been correctly labeled after the previous processes. But the unsharp boundaries between the cells and the background often lead to small regions around the white blood cells, which may be edge regions of the cells and influence the shapes of the cells. The labels of these small regions can be determined according to their positions.

Hough transformation is applied to find a circle in the image of edge points, which corresponds to the rough region of the cell. Small regions over this circle are added to the cell region and the small gaps inside cell regions are filled to form an orbicular shape of the cell.

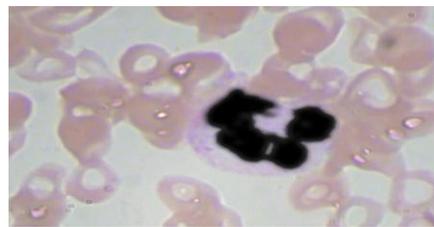
Although we adjust labels of points at every step to avoid making false segmentation, there is no guarantee that the segmentation results are correct all the time. So we identify the regularity of the final boundary of cells by calculating the variation of the radius. Great variation corresponding to an irregular shape means possible incorrect segmentation. The irregular edges are marked to remind users that these edges should be checked manually to avoid mistakes

## 3. EXPERIMENTAL RESULTS

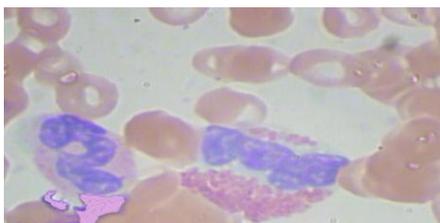
The method has been applied to 25 slides and 128 white blood cells have been segmented from background and overlapping red cells successfully. The boundaries of them have been traced out and irregular shapes are marked to avoid mistakes



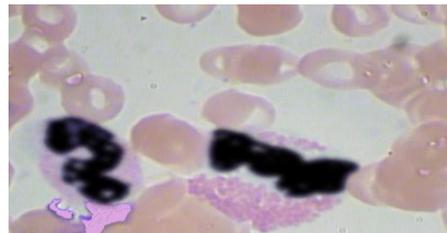
**FIGURE (1.a.1):** Original Image.



**FIGURE (1.a.2):** Segmented Image.



**FIGURE (1.b.1):** Original Image.



**FIGURE (1.b.2):** Segmented Image.

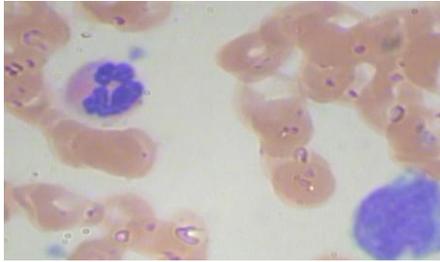


FIGURE (1.c.1): Original Image.

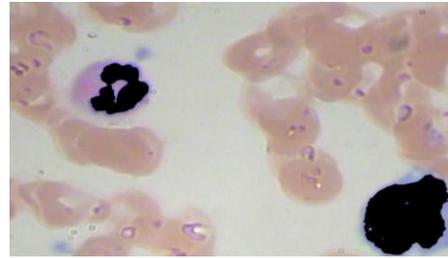
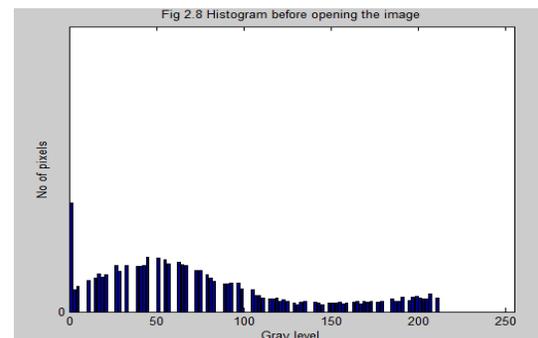
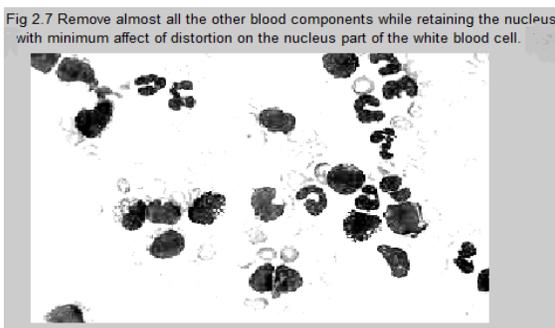
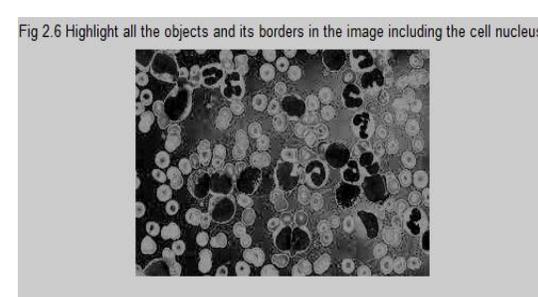
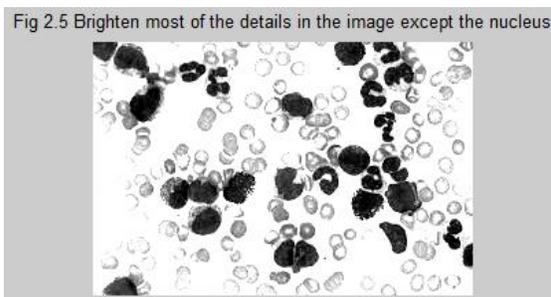
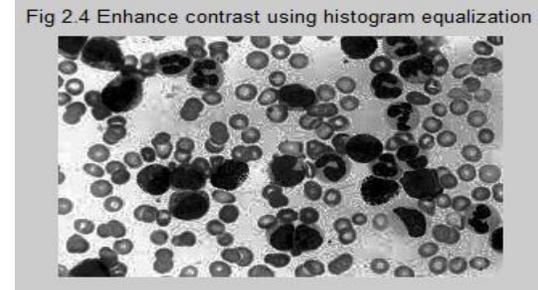
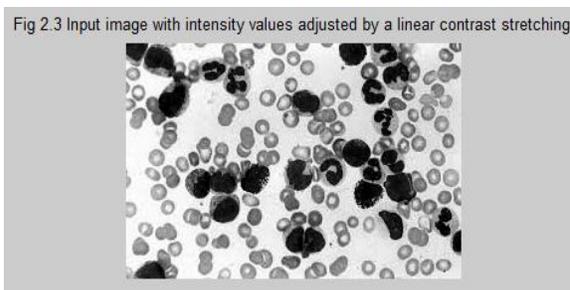
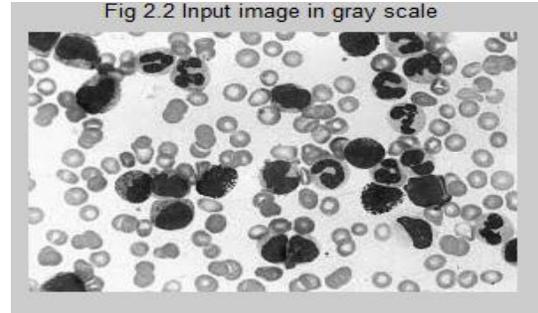
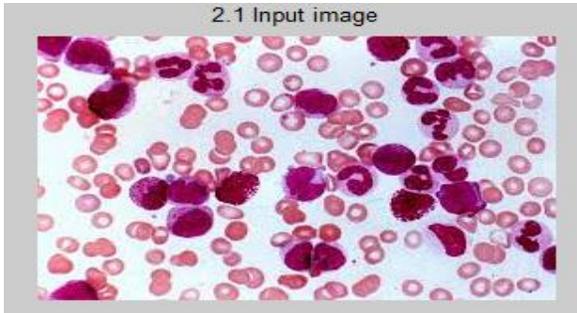
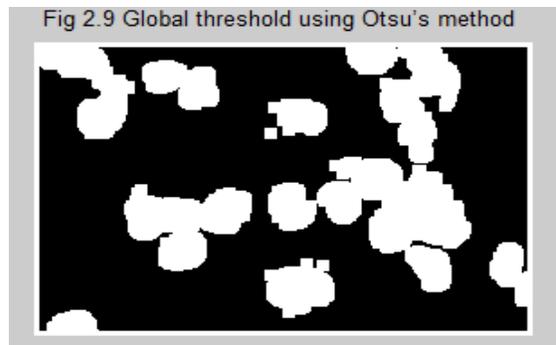


FIGURE (1.c.2): Segmented Image.





#### 4. CONCLUSION

The proposed shape detection method uses information from a large region that contains a whole white blood cell rather than information from a small local region or a few neighbour patches. Therefore it is powerful to obtain reliable and meaningful segmentation results and trace out accurate boundaries of white blood cells. Although the labels of the pixels are adjusted step by step to avoid wrong crisp decisions that are difficult to be reversed, it is not an iterative approach which makes it faster than most relaxation methods.

#### 5. FUTURE SCOPE OF WORK

The shape detection and segmentation of white blood cell can be approximated by a quasi circular form or a circular form. The automatic segmentation of WBC's in complicated and cluttered images can be approached by circle detection optimization methods.

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