

DETERMINATION OF TYPES AND NUMBER OF LEUKOCYTES IN BLOOD SAMPLES

Ali PELGUR¹

Başkent University Research and Teaching Assistant

ABSTRACT

In this paper, a software solution developed to determine leukocytes in a random blood sample image, to find the number of them and to classify them by coloured circles is presented. Leukocytes are using in the determination of some health problems. Their types and amount in blood change in specific health problems and they become more significant. If we can use a computer vision system to determine and discriminate them we will have a fast and cheap tool to decide the problem in the body and hemotolog's diagnosis duration may become shorter. The software determines types and number of leukocytes in an image of blood sample painted by Wright's Stain. The determination of types of leukocytes is provided by shape of nucleus and the ratio of nucleus region to cytoplasm region of leukocyte. In output image the types of leukocytes are highlighted by circles in different colours. The study is evaluated in MATLAB R14 environment.

Keywords: White Blood Cell, Segmentation, Region Detection, Biomedical Image Processing

ÖZET

Bu makalede, herhangi bir kan örneği görüntüsü içindeki beyaz kan hücrelerini tespit etmek, sayısını bulmak ve türlerine göre ayrı renklerde çerçeve içine alarak belirginleştirmek üzere geliştirilen bir yazılım çözümü sunulmaktadır. Yazılım, "Wright's Stain" adlı boya ile boyanmış kan örneğinin görüntüsü içinden beyaz kan hücrelerini tespit etmekte ve her beyaz kan hücresinin türünü, çekirdek şekli ve çekirdeğin hücre sitoplazmasına oranı ile bulmakta ve tespit edilen her beyaz kan hücresini saymakta, toplam hücre sayısını bildirmekte ve türlerine göre hücrelerin sınırlarına halkalar çizerek kullanıcıya türlerini daha belirgin hale getirmektedir.

Anahtar Sözcükler: Beyaz Kan Hücresi, Bölütleme, Bölge Belirleme, Biyomedikal Görüntü İşleme

I. INTRODUCTION

The number and types of Leukocytes -White Blood Cells- (WBC) determination is an important topic in hematology since its role in the determination of some health problems. These cells are produced in large numbers when human body receives bacterial intruders, viruses or chemical stimulants. Leukocytes are very important indicators in determination of these intruders.[1] The types of WBC are a lot because they are produced reactively to illness typically. The population of some types of them increases in specific problems but the population of some of them does not. The procedure of this determination is an important part of the test known as "complete differential blood cell count (CBC)". Routinely, the differential count is done "by hand" (i.e., through the microscope) in smaller labs, and by automated methods in larger facilities. Erythrocytes (Red Blood Cells) and leukocytes in blood are viewed same through the microscope but leukocytes can be dicriminated when they are painted by "Wright's Stain". In WBC count, a healthy person has segmented neutrophils in 62%,lymphocytes in 25%,monocytes in 8%,eosinophils in 3%,basophils in

¹ Başkent Üniversitesi Biyomedikal Müh. Bölümü, 06530,Etimesgut- Ankara.
e-mail:apelgur@baskent.edu.tr

2%. For WBC count, the blood sample is painted with Wright's Stain by the observer and purple coloured leukocytes are classified according to nucleus types and dimensions and the ratio of nucleus dimension to cell dimension. The solution which uses this observation process is presented in the following.

II.METHODS

In the study, a few image processing algorithms are applied to blood sample images. A sample blood painted with "Wright's Stain" image is in Figure 1. Images are in RGB bitmap format and illumination remains same for all. Images are processed by RGB to gray, discrimination by threshold, segmentation, clustering, edge detection and morphological methods. However the method is useful, different light and exposure times give different colourful blood smear images. In the study, illumination and exposure time settled in specific points.

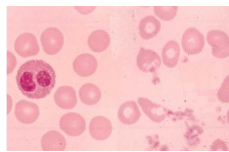


Figure 1: Sample blood painted with "Wright's Stain" image

RGB-to-Gray

As mentioned before, input image is in RGB 24-Bit bitmap format. Colour images are converted to gray-toned images by averaging the red, green, blue layer values for every pixel. The obtained image has 256 tones in gray from black to white. Input colour image and RGB-to-Gray converted image are in Figure 2a,2b.

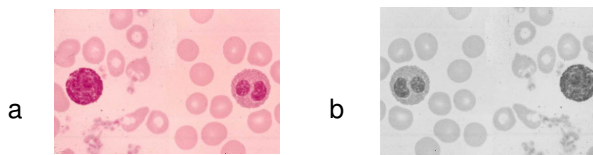


Figure 2: a- Input colour image, b- Converted to gray image

Discriminate Leukocytes By Threshold Value

Since mature erythrocyte cytoplasm has not got a nucleus they can be determined by eye in the painted blood sample easily. In the study, painted leukocytes' nucleuses will become more purple and cytoplasm of some of them will become more pink-purple coloured. Illumination is same for all images. After the RGB-to-Gray process, some of pixels which are belonging to leukocytes will become darker. Threshold level has been chosen as gray-180 for this study. Statistically, the chosen threshold value is found as same as all same illuminated and painted sample images. In discrimination of leukocytes by threshold process, the matrix which has image's gray pixels as values from 0 to 256 will be changed to 0s for pixel values under threshold. At the end of this process we will obtain leukocytes and platelets in the image as white points or in the matrix as gray 255 values. Platelets are remainder of dead blood cells and they are also painted with "Wright's Stain". The RGB-to-Gray image and threshold process image are in Figure 3a,3b.

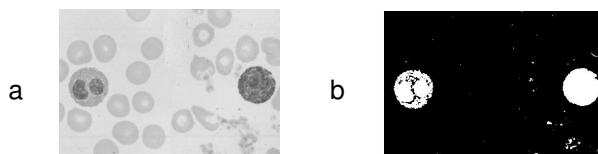


Figure 3: a- RGB-to-Gray image, b- Image after discrimination by threshold level

Clearance Of Platelets

Platelets can be painted by “Wright’s Stain” like leukocytes. So, they can be viewed as white spots in Figure 4b. To clear these spots, an erosion algorithm is used. [2,3] The erosion algorithm which can be also found image processing toolbox of MATLAB uses 8-connected neighbourhood. White pixel areas of platelets are smaller than leukocytes so the erosion algorithm clear platelets with this method. Previous step image and clearance of platelets image are in Figure 4a,4b.

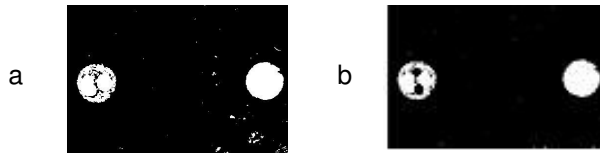


Figure 4: a- Image with Platelets, b- Clearance of Platelets

To Number Leukocyte Pixels

In previous process the image with leukocytes only was obtained. These cell pixels are white areas on black background in two coloured black-white image. Because of the threshold process, some pixels which are belonging to cytoplasm become black pixels. Every black pixel in cell white area is filled with white pixel and the algorithm with 8-connected neighbourhood is used at this point.[4] The previous image and the result image is in Figure 5.

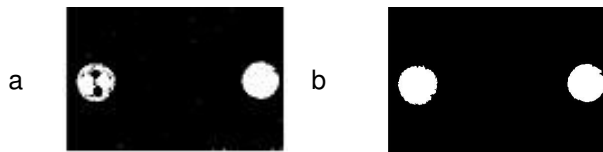


Figure 5: a-Clearance of Platelets, b- Filling spaces in cell areas

The next step of this process is labelling the cells in black-white image for every white independent areas. Every leukocyte is found in white regions. An algorithm is used in labelling and that method is one of useful MATLAB tools called as “bwlabel”. [2] In this method, 1 to n values which are according to one of leukocytes are placed in a 0s matrice parallel to image matrice. For example, for first leukocyte pixels which are in 8-connected neighbourhood, 1s are placed in matrice, for second leukocyte pixels, 2s are placed in matrice, for n’t h leukocyte pixels, n’s are placed in matrice. Since the parallel matrice have been numbered one can evaluate every leukocyte independently and also this information is used to find leukocyte numbers in sample image. A slice of the matrice values is sampled in Figure 6.

```

:
0011111111111111111000...00222222222222222220000
000111111111111111100000...0002222222222222200000
00000000000000000000...00000000000000000000000
:

```

Figure 6: Numbering the leukocytes

Every leukocyte is evaluated independently after this point. In next part of the paper and in the next figures two types of leukocytes are evaluated in a parallel way.

Clusterization Of Nucleus And Cytoplasm Pixels Of Leukocytes

Every pixel is described in red, green, blue matrix layers. The RGB values for pixels form vectors in RGB colour space cube. "K-Means Clustering Algorithm" is used in clusterization of nucleus and cytoplasm. "K-Means Clustering Algorithm" is an algorithm for partitioning (or clustering) N data points –pixels in here- into K disjoint subsets S_j containing N_j data points so as to minimize the sum-of-squares criterion

$$J = \sum_{j=1}^K \sum_{x \in S_j} |x_n - \mu_j|^2, \quad (1)$$

where x_n is a vector representing the n'th data point and μ_j is the geometric centroid of the data points in S_j . [5] In general, the algorithm does not achieve a global minimum of over the assignments. In fact, since the algorithm uses discrete assignment rather than a set of continuous parameters, the "minimum" it reaches cannot even be properly called a local minimum. Despite these limitations, the algorithm is used fairly frequently as a result of its ease of implementation.

The algorithm consists of a simple re-estimation procedure as follows. Initially, the data points are assigned at random to the K sets. For step 1, the centroid is computed for each set. In step 2, every point is assigned to the cluster whose centroid is closest to that point. These two steps are alternated until a stopping criterion is met, i.e., when there is no further change in the assignment of the data points.

In this study, centroids for nucleus, cytoplasm and background plasm pixels are not assigned random but they are assigned to chosen values. These values are found statistically before the solution. For that illumination and exposure time, the geometric centroids found statistically are described as RGB=(180,80,100) for nucleus pixels, RGB=(255,140,150) for cytoplasm and RGB=(255,200,200) for background plasm in first assignment.

Euclidean distance of every pixel's RGB vector to the centroids is calculated in image matrix masked for first leukocyte. The pixel will be assigned to the class in which centroid is closer than others. For example, the image matrix's 50,50 pixel in Figure 1 has a RGB=(255,199,203) which is closer to plasm background colour centroid.

After first clusterization of pixels, new centroids for nucleus, cytoplasm and plasm background are found with averaging the RGB values of pixels in the classes. The process is operated again and again till the previous and next centroids become same. First assignment of statistically centroids' RGB values make calculation and re-assignment time shorter.

All pixels of nucleus, cytoplasm and plasm background are discriminated exactly and they are placed in new matrices. At this point, nucleus and cytoplasm class pixel areas and ratios of them are found for every leukocyte by the method of labelling [2] which is mentioned before. This algorithm is easy to use and faster. Determination of nucleus and cytoplasm pixel areas of two leukocytes are shown in Figure 7a,7b, 7c and Figure 8a,8b,8c.



Figure 7: a- 1st leukocyte discriminate from all other cells and platelets in blood sample
b- Nucleus pixels which has granulas
c- Cytoplasm pixels are smaller since granulas of nucleus



Figure 8: a- 2nd leukocyte discriminate from all other cells and platelets in blood sample
 b- Nucleus pixels
 c- Cytoplasm pixels

Determination Of The Types Of Leukocytes In The Blood Sample

In hematology some features which are form of this solution's feature space are settled to determine the types of leukocytes. Neutrophils and Eosinophils are equal types with equal nucleus and cytoplasm pixel areas as a ratio of 1:1 for observer. Neutrophil's nucleus is in form of a band or connected lobes but eosinophils has got two nucleus parts like in Figure 8a. So they can be determined easily by eye but to determine whether it is eosinophil or neutrophil in the leukocyte image which has got equal nucleus and cytoplasm areas is evaluated with same labelling algorithm of Haralick et al. Monocytes have got one and great nucleus with a 5:1 nucleus to cytoplasm ratio and basophils have got granulated cytoplasm and so they may be viewed as full with nucleus. The ratio is greater than 20:1. Lymphocytes are greater than other leukocytes mentioned above. Their cell pixel areas are 3:2 times greater than others. All of them form the feature space of this study and types of leukocytes determined with them. After that, an edge detection algorithm [6] is operated for every leukocyte and the edges of pixel areas of leukocytes are different colour circled with respect to their types. At the end of all processes coloured circles which belong to specific types of leukocytes are merged with blood sample image and the output image will be viewed. The blood sample image and output image with colour circles are shown in Figure 9a,9b.

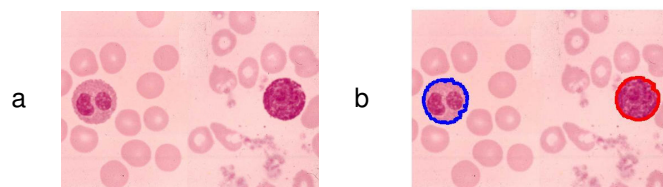


Figure 9: a- Blood sample image, b- Processed output image

Blue circle is for eosinophils and Red circle is for basophils. Other samples are shown in the presentation.

III. RESULTS

Discrimination of leukocytes in blood sample images and determination of the types and the number of leukocytes in blood sample images are provided by this solution. The solution is limited by the illumination and exposure time processes. It needs painting process as same as traditional way. First assignment of statistically centroids is also one of the limitations of the solution. The solution is proposed to hematologists and technicians make white blood cell count procedure shorter and easy by a computer vision system. This study may be a base for further studies which include neural networks for independent systems from human beings.

IV. REFERENCES

- [1] <http://www.psbcb.org/hematology>.
- [2] HARALICK, R.M., SHAPIRO, L.G., "*Computer and Robot Vision, Volume I*" Addison-Wesley, **1992**.
- [3] VAN DEN BOOMGAARD, VAN BALEN, "Image Transforms Using Bitmapped Binary Images," *Computer Vision, Graphics, and Image Processing: Graphical Models and Image Processing*, **Vol. 54,3**: 254-258, **1992**.
- [4] SOILLE, P., "*Morphological Image Analysis: Principles and Applications*", Springer-Verlag, **1999**.
- [5] HARTIGAN, J., WONG, M., "A K-means Clustering Algorithm", *Applied Statistics*, **1979**.
- [6] GONZALEZ, R.C., WOODS, R.E., "*Digital Image Processing Using Matlab*", Prentice Hall, **2004**.

BIOGRAPHY

Ali PELGUR was graduated from Başkent University, Türkiye as an Electrical and Electronics Engineer in 2002. He has started his academic career as a research and teaching assistant in Başkent University Biomedical Engineering Department after graduation. He is a Ph.D. candidate in METU Electrical and Electronics Engineering Graduate Program. He is interested in Inverse Problems in Electromagnetics, Biomedical Signal and Image Processing, Electromagnetic Wave Propagation Simulation and Analysis in various media.