# AN AUTOMATED WHITE BLOOD CELLS (WBCS) NUCLEUS LOCALIZATION AND SEGMENTATION VIA OTSU THRESHOLDING TECHNIQUE

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***ABSTRACT:*** *In the human body, distinctive kinds of illnesses may be observed even by analyzing samples of blood. In this paper, the principal purpose is to stumble on WBC from human blood. Those WBC defend the human against infectious illnesses. If there is any change within the blood count number, it indicates some issues within the body bio-mechanism. Segmentation is the primary and most critical step in automated blood WBCs classification. For this purpose, in advance, many hematological specialists tested blood samples, using clinical instruments which include, cytometer used to detect what kind of disorder is present within the body. As, the segmentation is practically a completely soporific, tiresome and mistakes inclined technique, specialists thus, prefer to apply automated systems for the production of leukocytes segmentation; specifically wanted for classification. In this research, the thresholding approach has been used for segmenting leukocytes by means of the usage of Otsu thresholding. After that, the mathematical morphing was used for eliminating all interfering additives which no longer behave like WBCs.*

**Keywords: -** WBC, Otsu thresholding, Blood disorders, segmentation.

## 1. INTRODUCTION

White blood cells (WBCs) in peripheral blood and bone marrow play a substantial function within the prognosis of diverse illnesses, which includes AIDS, leukemia, and different blood associated illnesses[1]. Their count number, additionally called the differential blood count, is a hallmark of diseases. In differential blood count, medical examiners rely on a large number of WBCs on blood slides and therefore estimate the proportion incidence of every kind of white blood cell [2]. Conventional counting techniques that depended on the microscope are time to ingest, complex, and vulnerable to mistakes.

In the meantime, automated popularity techniques make use of a flow cytometry and a blood cell analyzer. Those techniques are particularly hired for ordinary blood analysis as opposed to blood cell detection[3]. But, continually appoint blood smears from patients and a microscope to examine the form of blood cells for the scientific analysis of blood illnesses in sufferers[4]. As such, improvement of an automated cellular reputation device primarily based on picture processing and sample reputation to update manual reputation and counting has been the modern fashion[5].

Blood carries distinctive cell strains, the most crucial of which might be the white blood cell, platelet, and Red blood cell. White blood cells, which can be additionally referred to as immune cells, can assist the body to fight disease[6]. Accumulated photo samples comprise White blood cells, thereby influencing the processing and choice of White blood cells[7]. On this regard, the White blood cells segmentation algorithm must appropriately paintings on peripheral blood in picture processing for segmented. Numerous techniques were evolved for White blood cells segmentation [8]. Those techniques are typically primarily based on color area and mathematical morphology operations. *Putzu et al* proposed a technique primarily based at the cyan, magenta, yellow, and key plate colour area to split White blood cells due to the fact those cells lack the Y element[9]. Dividing the nucleus most effective in a peripheral blood photo is straightforward and speedy and will yield stepped forward segmentation. Those techniques are easy however incapable for as it should be segmenting the nucleus of WBCs whilst value White blood cells of the nucleus is near the cytoplasm[10]. Those techniques require a substantial quantity of time and can't cope with the hassle of overlapping WBCs.

Previous research especially used energetic contour fashions and mathematical morphology to section overlapping WBCs. but, immoderate segmentation, low accuracy of cell division, and different demanding situations continue to be and have to be triumph over[11]. The prevailing study specifically targets to develop a proper algorithm for segmentation of overlapping WBCs in peripheral blood pictures. Otsu's thresholding and mathematical morphing are hired to enhance segmentation.

## 2. Methodology

On this research, leishman stain approach was used for staining samples of blood smear pictures where all of the WBCs are stained in the blue shade and the dimensions of pics are (480×640)[12]. Picture data of those microscopic blood picture had been obtained from hospital and laboratories in Iraq. This studies painting is executed at the MATLAB R2014a picture processing toolbox. As visualized in figure (1) firstly, photo preprocessing implemented to the input picture as achieved in, which the nucleus region accompanied via Otsu's thresholding segmentation and morphological operations[13].

## 3. Proposed Methodology

In this study, the segmentation of WBCs was done based on the mathematical operations, thresholding approach and mathematical morphing were applied to obtain smoothing image.

### 3.1 image Pre-processing

Photo preprocessing may be very critical in clinical analysis so that it will get excessive great medical picture, but many factors have effect on its acquisition despite the fact that photo processing cannot offer new facts for prognosis, it can enhance the visible effect to diagnose appropriately; so that the consequent picture is higher suited for machine interpretation . This degree consists of the authentic photo conversion which the colour pics (RGB) are transformed to the grayscale. In this segment, our consciousness is at the mathematics operation carried out to the photograph a good way to be easily segmented; to begin with convert the input picture to grayscale, then make two copies of grayscale photo, in a single replica histogram equalization H(i,j) and contrast starching C(i,j)[14].

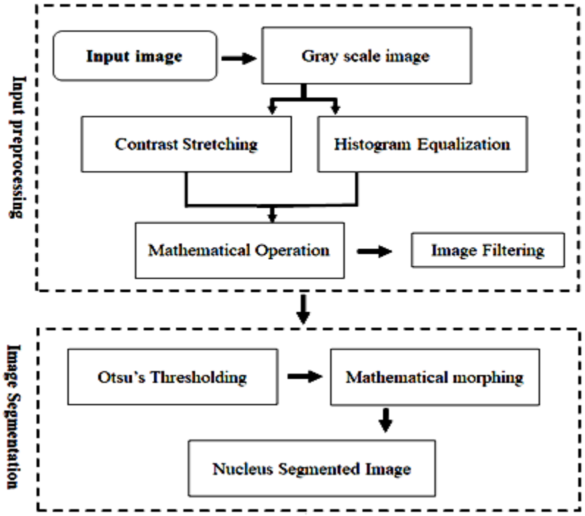


**Figure (1): Block Diagram of Proposed System**

Then, mathematical operations like addition is applied on the two images *C* (i,j) and *H* (i,j) and the output is image R1(i,j) as shown in equation (1) which highlight nucleus of leukocytes and brightens all other blood components image and then image subtraction is done with R1 (i,j) and H (i,j), which highlighted all of the objects in image. The output of subtraction operation is image R2(i,j) as shown in equation (2)

$$R1(i,j) = C(i,j) + H(i,j) \qquad (1)$$
$$R2(i,j) = R1(i,j) - H(i,j) \qquad (2)$$

Finally, combining both the images R1( i,j) and R2( i,j) to get image R3( i,j) as shown in equation(3), that results in the minimum effect of distortion in the nucleus

$$R3(i,j) = R1(i,j) + R3(i,j) \qquad (3)$$

**3.2 Segmentation using Otsu's Thresholding**

At this stage, WBC images are segmented to produce a number of regions; each region represents one cell from a smear of blood. Thresholding process is the handiest approach for segmenting specific picture; it's foremost purpose to partition all pixels of the picture into the foreground and the historic beyond primarily based on the intensity of gray or textures level. We've distinctive type thresholding strategies together with international, variable and more than one thresholding. In the global thresholding, the suitable threshold fee *T* is ready for the complete picture and on the idea of photo[15].

$$I_{bin}(i,j) = \begin{cases} 1 & I(x,y) \geq T \\ 0 & \text{otherwise} \end{cases}$$

Threshold value *T* changeover image relies upon on whether or not local or adaptive thresholding. In local thresholding, *T* relies upon a community of each pixel (x,y). Meanwhile, adaptive thresholding, *T* is a feature of the pixel (x, y). A couple of thresholding targets to discover more than one threshold values to split multiple items.

$$I_{bin}(i,j) = \begin{cases} a, & \text{if } I_{gray}(x,y) > T_2 \\ b, & \text{if } I_{gray}(x,y) < T_2 \\ c, & \text{if } I_{gray}(x,y) \leq T_1 \end{cases}$$

In our studies work, Otsu's global thresholding approach was used, which it is limiting the weighted within-elegance variance the threshold foreground and historical past pixels and operate at gray-level bimodular histogram or picture to establish a choicest threshold *T*, then used to binaries the photo [16].

The weighted inside-elegance variance is given by

$$\sigma_w^2(T) = v_1(T)\sigma_1^2(T) + v_2(T)\sigma_2^2(T)$$

Wherein $\sigma_w$ is the within-variance of elegance and the magnificence variance of foreground or heritage and class probabilities are calculated from the histogram

$$v_1(T) = \sum_{i=1}^{T} P(i) \qquad v_2(T) = \sum_{i=T+1}^{L} P(i)$$

where*(i)* is the frequency *i*, I *L* is the quantity of gray in pithe cture. The elegance manner of foreground and historical past are estimated through the next formula

$$\mu_1(T) = \sum_{i=1}^{T} \frac{iP(i)}{q_1(T)} \qquad \mu_2(T) = \sum_{i=T+1}^{L} \frac{iP(i)}{q_2(T)}$$

The foreground variance and historical past pixels are given through

$$\sigma_1^2(T) = \frac{1}{v_1(T)} \sum_{i=1}^{t} (i-\mu_1)^2 P(i)$$

$$\sigma_2^2(T) = \frac{1}{v_2(T)} \sum_{i=T+1}^{L} (i-\mu_2)^2 P(i)$$

Now, we forestall and discover the value of *T* from (1,256), in order that weighted sum of within-elegance variance is minimal.

**3.3 Mathematical Morphing**

In photo processing every now and then to offer enhancement, segmentation, convex hull, recuperation, facet detection, texture analysis, shape, size analysis [16]. Mathematical morphological operations are a nonlinear, translation invariant adjustments photo analysis method which extracts objects from image through describing their geometric structure.

As we keep in mind only binary images so right here we handiest supply detail of binary morphological operations. In our research, we focus on the photograph morphing as it gets rid of the unwanted gadgets like RBCs and platelets from the photograph. In most of the studies work, mathematical morphing is used to clean the vicinity of hobby. There are four critical operators of mathematical morphing, erosion, dilation, starting, and ultimate.

The 2 primary operators or mathematical morphing are the erosion and dilations and at the mixture of them, other operators may be shaped [17]. Suppose we have photograph r and structuring element s, then the operations are denoted as:

Erosion $$R \Theta S = \bigcap_{s \in S} R_{-s}$$

Dilation $$R \oplus S = \bigcup_{r \in R} S_r$$

Commencing and the closing morphology operations are derived from the mixture of abrasion (erosion) and the dilation. Beginning used to smoothest the contour of gadgets; breaks slim isthmuses, and eliminate skinny protrusions through doing away with small objects from foreground. Closing is likewise used to easy contours through doing away with the small holes, fuses narrow breaks, long skinny gulfs, and fill the gaps in contour.

Opening $$R \circ S = \bigcup_{S \subseteq R} B_x$$

Closing $$R \bullet S = (R \oplus S) \ominus S$$

## 4. RESULTS AND DISCUSSION

A regular peripheral blood sample changed into taken and stained. Regular light microscope turned into used to acquire virtual pix from the blood slide using a 100x. An analog rate-coupled device (CCD) shade camera is connected to the microscope to capture shade photographs (i.e. 640x480 pixels). Total of (104) photographs had been used on this take a look at with the subsequent 5 cell kind's distributions (Table 1):

**Table (1):- Types and number of WBCs**

| WBC type | Number of images |
|---|---|
| Eosinophil | 10 |
| Basophile | 11 |
| Neutrophil | 62 |
| Monocyte | 7 |
| Lymphocyte | 14 |
| **Total** | **104** |

The nucleus segmentation sequence of Eosinophil has been shown in Figure (2). At the first step, after inputting the image, the original color of the image changed into the gray color, that leads to form two copies, one for contrast stretching and one for histogram equalizations after the mathematical

operation, the preprocessing step is ended. Otsu thresholding and morphological operation were done to complete segmented the image and obtain cell image with segmented nucleus with bi-lobed granules in shape.

For Basophil, bi- or tri-lobed with granules could be seen after segmented of the nucleus as seen in Figure (3).

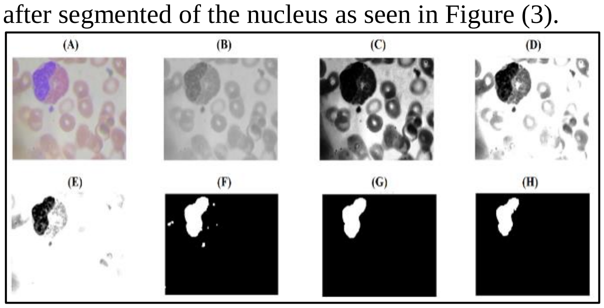


**Figure (2) Segmentation of Eosinophil cell(A) Original image (B) gray scale (C) Histogram equalization (D) R1 = C + H (E) R1 +R2 (F) Otsu threshold (G) Mathematical morphing operation (H) Segmented nucleus image**

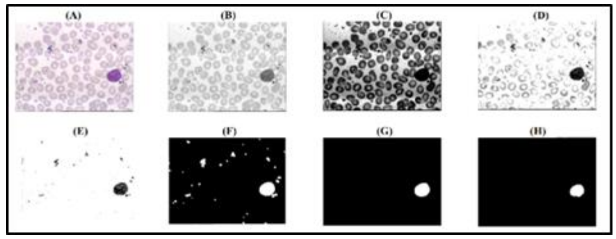


**Figure (3) Segmentation of Basophile cell (A) Original image (B) gray scale (C) Histogram equalization (D) R1 = C + H (E) R1 +R2 (F) Otsu threshold (G) Mathematical morphing operation (H) Segmented nucleus image**

While for Neutrophil, their nucleus is two to five-lobed with granules. The lobes are connected by a thin strand after segmented of the nucleus as seen in Figure (4).

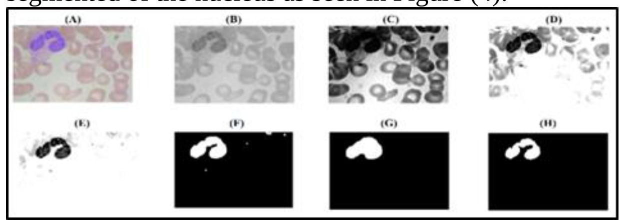


**Figure (4) Segmentation of Neutrophil cell (A) Original image (B) grayscale (C) Histogram equalization (D) R1 = C + H (E) R1 +R2 (F) Otsu threshold (G) Mathematical morphing operation (H) Segmented nucleus image**

Meanwhile, for Monocyte, They have the largest white blood cell shaped with nucleus a granules after segmented of the nucleus as seen in Figure (5).

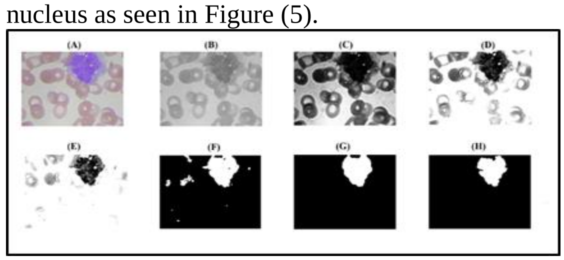


**Figure (5) Segmentation of Monocyte cell (A) Original image (B) grayscale (C) Histogram equalization (D) R1 = C + H (E) R1 +R2 (F) Otsu threshold (G) Mathematical morphing operation (H) Segmented nucleus image**

A lymphocyte is distinguished by having a deeply staining nucleus that may be eccentric in a location with granules, and a relatively small amount of cytoplasm after segmented of the nucleus as seen in Figure (6).

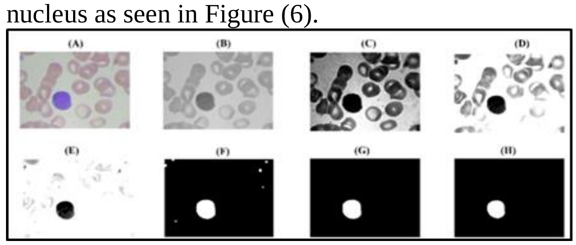


**Figure (6) Segmentation of Lymphocyte cell (A) Original image (B) gray scale (C) Histogram equalization (D) R1 = C + H (E) R1 +R2 (F) Otsu threshold (G) Mathematical morphing operation (H) Segmented nucleus image**

The results demonstrated the benefits of this method in preference to others[18]. In nucleus segmentation, the utilization algorithm that isn't always related to the color of nucleoli because there are the various colors of the nucleus in an exclusive type of white blood cells. So, it offers excessive accuracy bring about segmenting nucleus in any type of White blood cells and in any size illumination that causes the distinct color area in images. Additionally, in a White blood cells segmentation method in which used the thresholding approach. The nucleus is completely segmented from different components. This method is quite simple with excessive pace and trustable accuracy.

## 5. CONCLUSION

In this research work, a white blood cell segmentation set of rules have been presented. This paper gives insights into White blood cells segmentation with the aid of obtaining cellular seeds and setting apart adhesive cells in peripheral blood images beneath one of a kind shade situation. The proposed technique affords an inexpensive processing time and offers accurate effects. The proposed technique exhibits strong efficiency with greater performance for white blood cell segmentation in peripheral blood image.

**Declaration of interest**

The authors declare that there are no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## REFERENCES


1. Terwilliger T, Abdul-Hay M: **Acute lymphoblastic leukemia: a comprehensive review and 2017 update**. *Blood cancer journal* 2017, **7**(6):e577.
2. Lassale C, Curtis A, Abete I, van der Schouw YT, Verschuren WM, Lu Y: **Elements of the complete blood count associated with cardiovascular disease incidence: Findings from the EPIC-NL cohort study**. *Scientific reports* 2018, **8**(1):3290.
3. Kahng J, Kim Y, Kim M, Oh E-J, Park Y-J, Han K: **Flow cytometric white blood cell differential using CytoDiff is excellent for counting blasts**. *Annals of laboratory medicine* 2015, **35**(1):28-34.
4. Adewoyin A: **Peripheral blood film-a review**. *Annals of Ibadan postgraduate medicine* 2014, **12**(2):71-79.
5. Cromey DW: **Digital images are data: and should be treated as such**. In: *Cell Imaging Techniques.* Springer; 2012: 1-27.
6. Roland L, Drillich M, Iwersen M: **Hematology as a diagnostic tool in bovine medicine**. *Journal of Veterinary Diagnostic Investigation* 2014, **26**(5):592-598.
7. Abi-Rached L, Jobin MJ, Kulkarni S, McWhinnie A, Dalva K, Gragert L, Babrzadeh F, Gharizadeh B, Luo M, Plummer FA: **The shaping of modern human immune systems by multiregional admixture with archaic humans**. *Science* 2011:1209202.
8. Sadeghian F, Seman Z, Ramli AR, Kahar BHA, Saripan M-I: **A framework for white blood cell segmentation in microscopic blood images using digital image processing**. *Biological procedures online* 2009, **11**(1):196.
9. Arslan S, Ozyurek E, Gunduz-Demir C: **A color and shape based algorithm for segmentation of white blood cells in peripheral blood and bone marrow images**. *Cytometry Part A* 2014, **85**(6):480-490.
10. Reed C: **Studies of Nucleon-Gold Collisions at 200 GeV per Nucleon Pair Using Tagged d+ Au Interactions**. *arXiv preprint arXiv:10034941* 2010.
11. Prinyakupt J, Pluempitiwiriyawej C: **Segmentation of white blood cells and comparison of cell morphology by linear and naïve Bayes classifiers**. *Biomedical engineering online* 2015, **14**(1):63.
12. Sathpathi S, Mohanty AK, Satpathi P, Mishra SK, Behera PK, Patel G, Dondorp AM: **Comparing Leishman and Giemsa staining for the assessment of peripheral blood smear preparations in a malaria-endemic region in India**. *Malaria journal* 2014, **13**(1):512.
13. Prodanov D, Verstreken K: **Automated segmentation and morphometry of cell and tissue structures. Selected algorithms in imageJ**. In: *Molecular Imaging.* InTech; 2012.
14. Gautam A, Singh P, Raman B, Bhadauria H: **Automatic classification of leukocytes using morphological features and naïve Bayes classifier**. In: *Region 10 Conference (TENCON), 2016 IEEE: 2016*. IEEE: 1023-1027.
15. Yang X, Shen X, Long J, Chen H: **An improved median-based Otsu image thresholding algorithm**. *Aasri Procedia* 2012, **3**:468-473.
16. Gautam A, Bhadauria H: **Classification of white blood cells based on morphological features**. In: *Advances in Computing, Communications and Informatics (ICACCI, 2014 International Conference on: 2014*. IEEE: 2363-2368.
17. Gonzales R, Woods E: **Digital Image Processing, 3, d edition**. In.: Prentice-Hall; 2007.
18. Huang Y, Wang S: **Multilevel thresholding methods for image segmentation with Otsu based on QPSO**. In: *Image and Signal Processing, 2008 CISP'08 Congress on: 2008*. IEEE: 701-705.


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