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Paper Authors

***G. NAGA RAJU, DR P. V. RAMA RAJU, M. S. D. MAHESH, M. N. V. D. RAMYA, L. SRUJANA, K. N. V. S. SUKESH NAIDU.**

* Dept of ECE, SRKR Engineering College.

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AN APPROACH FOR WHITE BLOOD CELLS SEGMENTATION AND ITS CLASSIFICATION BASED ONGEOMETRICAL FEATURES

[1]G. NAGA RAJU, [2]DR P. V. RAMA RAJU, [3]M. S. D. MAHESH, [4]M. N. V. D. RAMYA, [5]L. SRUJANA, [6]K. N. V. S. SUKESH NAIDU

[1]Asst. Professor, [2] Professor & HOD, [3, 4, 5, 6] B. E Students

[1, 2,3,4,5,6] Dept of ECE, SRKR Engineering College (A), Bhimavaram, India.

ABSTRACT—Today, Identification of white blood cells is the first step to diagnose some particular disease such as acquired immune deficiency syndrome, leukemia and other blood-related diseases that are usually done by pathologists using the optical microscope. Manually this process is time-consuming and expensive to segment the nucleus and classification of the cell. So, an automatic system is preferable which reduces the times of segmentation and classification. This research is focused on segmentation of nucleus from blood smear images using Otsu's thresholding technique applied after contrast stretching and histogram equalization of image followed by filtration for reducing noise and increasing the brightness of nucleus, mathematical morphological opening is applied to remove the components which are not WBCs, then shape-based features are extracted on the basis of that classification rule is applied to classify them in their five category. The five categories are Lymphocytes, Monocytes, Basophils, Neutrophils, Eosinophils. The classification of a nucleus is necessary as they are used to identify different kind of diseases which are related to each type of white blood cells and also help in differential blood count of cells. For segmentation and classification, MATLAB software is used.

Keywords—white blood cells; mathematical morphing; differential blood count; segmentation; classification.

I. INTRODUCTION

Blood smear images from a microscope provide important information for diagnosing and predicting diseases in a hematological analysis.Blood tests are of high importance for diagnosis of many diseases and also to investigate functions of body organs such as kidney, liver, thyroid, and heart. Examples of the diseases that blood tests can investigate are: cancer, HIV/AIDS, diabetes, anemia, and coronary heart disease [1] [2]. Blood samples are prepared and sent to a blood cell counter for calculating each type of cell. If hematologist's find anunusual number of cells in any type, they will investigate further by looking into the microscopic blood smear, recount the number of cells and check their morphology in more detail. Any blood cells with irregular shapes or characteristics may trigger a presence of severe diseases. The visual inspection by hematologist's is quite tedious and time-consuming. Therefore, an automating process is highly desirable to accelerate the process.

Here firstly segmentation should be done very accurately if it is not done accurately then further analysis may not have effective results. This may lead to wrong medication to the patient. So, a perfect automating process should have the minimum error than the manual process. Many types of research taking place in making this process fast and error free. In the manual process the segmentation and classification of subtypes [3], firstly we have to crop the nucleus form microscopic blood image and then further analysis is done but here the main problem is the price of the equipment is very high which may not available in some clinics and hospitals [4].

White blood cells contain nuclei with DNA, the shape depends on the type of cell Certain WBCs produce antibodies. Lifespan is from 24 hours to several years. Size is 8-20 micrometers. There are five different types of WBC's Neutrophils, Eosinophils, Basophils, Lymphocytes, Monocytes [5].Different types of white blood cells count may lead to different kind of diseases such as Acquired Immuno Deficiency Syndrome (AIDS), Leukemia or cancer[6][7]. When the number of white blood cells increased the disease is known as Polycythemias and when there is the decrease in their differential count the disease is known as Anemia.

Neutrophils are high in number during bacterial infections and inflammation. Eosinophils are high in number with parasitic infections and allergic reactions. Basophils are high in number during allergic reactions and inflammatory reactions. Monocyte is high in number during bacterial and viral infections. Lymphocytes are high in number during viral infections and a typical form of infectious mononucleosis.

Generally, there are two protocols for blood countingused in the diagnosis of various types of hematological diseases used by the experts, one is complete blood count (CBC) and other is differential blood count (DBC).

In complete blood count the blood cells counting is done automatically by using the instrument cytometer, but in comparison with CBC the differential blood count is the method which is highly used in diagnosis of blood-related diseases even though it is highly complex, tedious and timeconsuming task and it uses the flow-cytometry for differential blood count of peripheral blood. It is used to calculate the percentage of occurrence of all the types of white blood cells in blood smears image where counting is done on 100 leukocytes. After the leukocytes classification, the diagnosis of particular diseases related to different type of white blood cells namely neutrophil, leukocyte, monocyte, basophil and eosinophil can be identified. Since in these manual methods besides being time-consuming require the experienced and knowledgeable expert for providing the good quality of DBC making. So, nowadays automatic system is preferable and also many researchers are working in this area for providing the fast and accurate segmentation of white blood cells so that the classification can be done very easily.

Many researchers have used different types of segmentation and classification techniques for identification of white blood cells. Lorenzo Putzu, Cecilia Di Ruberto in their research uses

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the CMYK color model for identification of leukocytes as in all the other component except white blood cells have some amount of yellow color present in them, whereas leukocytes show a good contrast in the Y component of CMYK color model and redistribution of image gray levels is done for making the segmentation process easier.

In their segmentation process, they make use of automatic threshold value based on triangle method or Zack algorithm and classification model used is SVM. P.S. Hiremath, Parashuram Bannigidad and Sai Geeta [8] used the color-based segmentation method using HSV model for the extraction of the nucleus and on the basis of the geometric features of the extracted nucleus are used to classify the different types of white blood cells. Nipon Theera-Upton in his research used the Fuzzy C-mean clustering to segment the white blood cells into two different parts, one in the nucleus and other in the cytoplasm.

Wang Shitong and Wang Min used the segmentation using the thresholding technique which is then followed by mathematical morphology (TSMM), and a new detection algorithm (NDA) based on fuzzy cellular neural networks. Nasrul Humaimi Mahmood in their research used the color-based segmentation in which the input image is converted from RBG format to International

Commission on Illumination L*a*b* (CIELAB) color space for segmentation of WBCs. Madhumala Ghosh used the statistical pattern analysis for the classification of white blood cells.

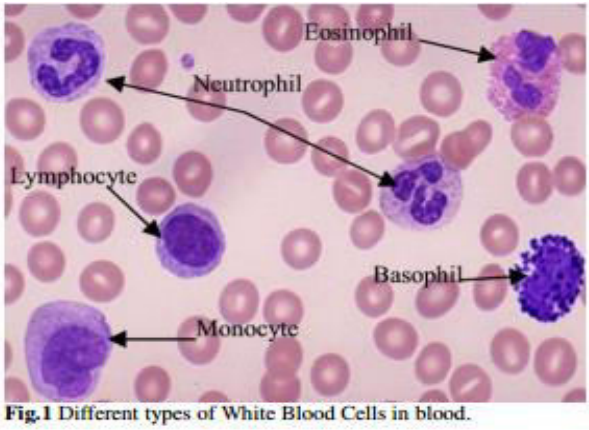

Fig.1 Different types of White Blood Cells in blood.

For segmentation of WBC nuclei Marker, controlled watershed segmentation is embedded with a morphological operator. Where they compute one cellular and eight nuclei based geometric features where only four features are fed to Na'ive Bayes classifier for classification. Aimi Abdul Nasir, Mohd Yusoff Mashor, and Rosline Hassan [9] in his research work used multilayer perceptron and simplified fuzzy ARTMAP Neural Networks for the Classification of acute leukaemia cells. Seyed Hamid Rezatofighi, Kosar Khaksari, Hamid Soltanian-Zadeh for segmenting nucleus and cytoplasm, they used Gram-Schmidt orthogonalization method and a snake algorithm. They extract three kinds of features from the segmented areas and also extract two groups of textural features by evaluating Local Binary Pattern (LBP) and co-occurrence matrix. But the best features only selected by using a Sequential Forward Selection (SFS) algorithm and compared the performance of two classifiers, ANN and SVM. But the best result they obtained from LBP and SVM. Bakht Azam, Rashid Jalal Qureshi, Zahoor Jan, and Taj Ali Khattak in their research they quantize the blood smear images for segmentation of WBC's, where they remove all the components

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which WBC are not, after that they convert image in binary format and labelled each segment, and after that if image contain some noise artifacts then they are eliminated by area filtering and morphological operations. After that, they counted the result of segmented leukocytes, which show the results closest to that of hematological experts. Leyza Baldo Dorini, Rodrigo Minetto, and Neucimar Jer'onimo Leite [10] proposed the method to segment the nucleus by using watershed transform and Level-Set methods for those methodsimage preprocessing need to done by using SMMT is essential and for localization of cytoplasm, they use granulometric analysis and morphological transformation scheme to give satisfactory results.

In our research work, we focus on accurate segmentation of white blood cells so that the further operations such as feature extraction and classification on those segmented images can be done easily and produce the desired output. Segmentation is done by using Otsu's global thresholding method which calculates the one threshold value for the entire image and that the features are extracted from the segmented image which helps in classification.

II. METHODOLOGY

A. Proposed Segmentation technique

The proposed segmentation is based on gray scaling, thresholding and morphological operations. Imagepre-processing A. Gray-scale Image Initially, the source image that is taken from the microscope camera is a color image, to make the image processing reliable for the proposed method development, the original colored input images will be converted into grey level images.

B. Contrast and brightness adjustment

This process is applied to that grayscale image to improve the quality and the dynamic range of the image.

C. Histogram equalization

By performing histogram equalization adjusts intensity values of the image that involve intensity transformation, so that the histogram of the output image from brightness adjustment approximately matches a pre-defined histogram known as histogram matching or specification. After Image preprocessing addition process will brighten most of the part except the nucleus part by performing the image addition. In the next step, subtraction process will highlight all the objects and its borders in the image including the cell nuclei in the final addition the process will remove almost all the other blood components while retaining the nuclei with minimum effect of distortion on the nuclei part of the white blood cells.

D. Filtering

The filter works in the same way as the median filter, however instead of changing the pixel intensity with the median intensity value, in minimum filters, the pixel intensity is replaced with the minimum intensity value. This step is repeated 3 times for best filtering results which were evident by trials.

E. Thresholding and Binarization

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Using the threshold from Otsu's technique we could convert the image to its binary version [11].

F. Morphological Opening

It is done by applying erosion followed by dilation [12]. Erosion is applying a structuring element B on a binary image A as shown by the equation.

$$A \ominus B = \{z \in E \mid B_z \subseteq A\}$$

Where the structuring element B_z is defined by

$$B_Z = \{b + z \mid b \in B\}, \forall z \in E$$

where E is an integer grid. Similarly, erosion is defined by

$$A \oplus B = \{z \in E \mid (B^s)Z \cap A \bullet \emptyset\}$$

B^s is given by

$$B^s = \{x \in E \mid -x \in B\}$$

So, the morphological opening is defined as

$$A \circ B = (A \ominus B) \oplus B$$

The last step is to check the relative size (area) of each object with respect to average RBC area. The 50% value is used as a minimum nucleus segment threshold. This value was chosen by trials which gave the best accuracy of segmentation

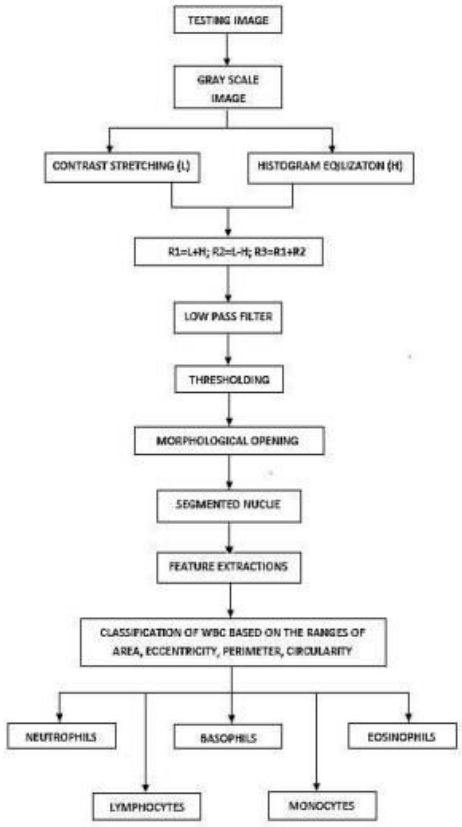

Fig. 2 Block Diagram

Proposed Classification technique

The proposed classification is based on feature extraction.

On the segmented nucleus, features are extracted which help in the classification of white blood cells. The features which we have used in this experiment are circularity, perimeter, eccentricity and area When all the features of segmented nuclei are extracted, then some values for each feature which is maximum and minimum, extracted for every class of white blood class and saved for further operations. While testing the segmented image of white blood cells if the value of features for particular nucleus lies between the maximum

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and the minimum value of features values stored for particular class then the segmented nucleus belongs to that class.

Algorithm

1. Input the testing image.

2. Convert input image to grayscale.

3.Apply contrast stretching and histogram equalization on the grayscale image.

4. Perform the mathematical operation on an image obtained from step 3 for removing almost all other blood components except nucleus.

5. After that apply a minimum 3-by-3 filter for removing noise and increase the darkness of nuclei.

6. Apply the Otsu's global thresholding technique to get the binary image.

7. Apply mathematical morphing to smooth the components and remove the those which are not WBC and also remove WBC on the boundary.

8. Compute geometric shape-based features of WBC.

9. Apply the classification rule if feature fi where i=1,2,3,4 lies between [fcimax, fcimin] then nucleus belong to that class c and classify the WBC as any of neutrophil, basophil, eosinophil, lymphocyte and monocyte.

III. RESULTS

In our experiment, the image which we take gets converted into a data set of 480*640*3 images [13]. The segmentation algorithm applied to these images to get the nucleus present in the image. The Fig.3 shows the RBG image gets converted into grey scale image. The Fig.4 is the image obtained after I3 which we get after doing various mathematical operations, this shows various white blood cells present in the image. After obtaining this image thresholding is applied by using Otsu's thresholding method then we get the image which is shown in Fig.5. Final segmentation image Fig.6 is obtained after Mathematical Morphing which fills the holes present in WBC and the cells are removed which are present on the boundary as they may not give the desiredresult as they have a geometric size less compared to the required size. For the classification of WBC in any one of the five categories i.e. neutrophil, basophil, eosinophil, monocyte and lymphocyte we have to calculate area, perimeter, eccentricity and circularity.

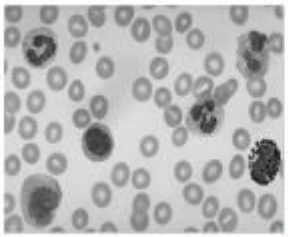

Fig. 3 Grayscale Image

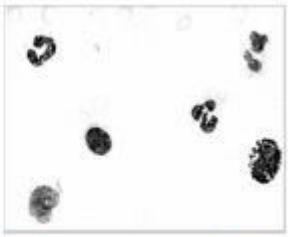

Fig. 4 Image obtained after I_3

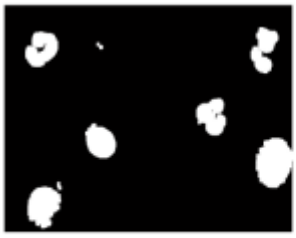

Fig. 5 After Otsu's Thresholding after Mathematical Morphing

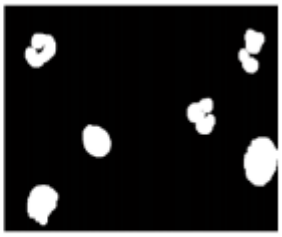

Fig. 6 Final Segmented image

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Here in fig. 7 and fig. 8 shows the final output with individual steps of images of lymphocytes and neutrophil respectively.

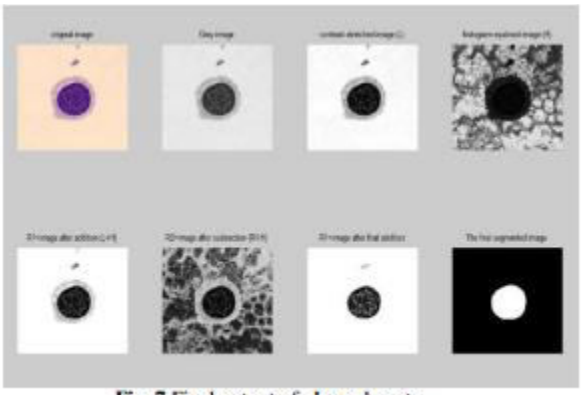

Fig. 7 Final output of Lymphocyte

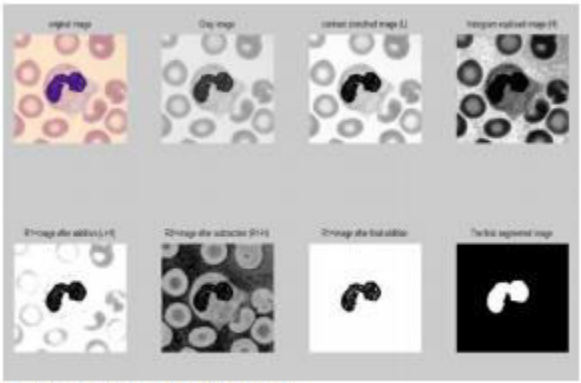

Fig. 8 Final output of Neutrophil

TABLE I.NUMBER OF USED WHITE BLOOD CELL IMAGES IN TESTING.

WBC type	*Number of images used*
Basophil	3
Eosinophil	11
Lymphocyte	59
Monocyte	32
Neutrophil	98
Total	**203**

TABLE II.FEATURES EXTRACTED AFTER TRAINING WBC IMAGES

No.	Dataset of White Blood Cells				
	Area	*Perimeter*	*Eccentricity*	*Circularity*	*Class (WBC)*
1	18801	671.3270	0.8807	1.907	Neutrophil
2	19746	763.8549	0.8463	2.3514	Neutrophil
3	19547	736.6833	0.8824	3.209	Neutrophil
4	27407	889.536	0.7987	2.2978	Eosinophil
5	33577	1006	0.848	2.3985	Eosinophil
6	10335	383.705	0.6871	1.336	Lymphocyte
7	25452	601.0854	0.6181	1.1296	Lymphocyte
8	50499	3.699	0.759	1532.1	Basophil
9	37407	870.2641	0.912	1.919	Monocyte
10	39690	870.2641	0.2826	1.5185	Monocyte

TABLE III. MAXIMUM AND MINIMUM VALUE OF FEATURES FOR EACH OF THE WBC

WBC	Features of White Blood Cells				
		Area	*Perimeter*	*Eccentricity*	*Circularity*
Neutrophil	Min	18801	671.327	0.562	1.907
	Max	37979	1079.5	0.904	3.01
Eosinophil	Min	24456	889.536	0.71	2.278
	Max	43884	1208.25	0.9056	3.2648
Basophil	Min	47180	1223.2	0.759	2.523
	Max	61981	1882	0.816	4.263
Monocyte	Min	19603	602.80	0.2862	1.3760
	Max	54521	1002.74	0.912	1.919
Lymphocyte	Min	10335	383.705	0.618	1.1296
	Max	45298	85.0489	0.837	1.345

Total of 203 images were used in this study with the following 5 cell types distributions as shown in TABLE I.TABLE IIshows the dataset of 10 white blood cells. TABLE III shows the maximum and minimum value of each features of different type of WBC classes. Most of the white blood cells are

identified as lymphocytes and neutrophil because they are present in a large number in human blood as compared to other white blood cells.

IV. CONCLUSION

In our experiment, we classified the white blood cells. This classification is done according to the features obtained from the nucleus of the cell based on segmentation and classification is based on the features of white blood cells. In this, the time required to differentiate a cell is less compared to manual methods. It shows better results if the segmentation of WBC is done accurately. Here we used 203 different white blood cells images which are 98, 11, 3, 32 and 59 for neutrophil, eosinophil, basophil, monocyte and lymphocyte. At the time of testing lymphocyte gives 100% accuracy and basophil have the lowest accuracy result and overall performance is 73% for 203images of white blood cells.

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ABOUT AUTHORS:

Dr. P. V. Rama Raju Presently working as a Professor and HOD of Department of Electronics and Communication Engineering, S.R.K.R. Engineering College, AP, India. His research interests include Biomedical-Signal WBC Features of White Blood Cells Area Perimeter Eccentricity Circularity Neutrophil Min 18801 671.327 0.562 1.907 Max 37979 1079.5 0.904 3.01 Eosinophil Min 24456 889.536 0.71 2.278 Max 43884 1208.25 0.9056 3.2648 Basophil Min 47180 1223.2 0.759 2.523 Max 61981 1882 0.816 4.263 Monocyte Min 19603 602.80 0.2862 1.3760 Max 54521 1002.74 0.912 1.919 Lymphocyte Min 10335 383.705 0.618 1.1296 Max 45298 85.0489 0.837 1.345 Processing, Signal Processing, Image Processing, VLSI Design, Antennas and Microwave Anechoic Chambers Design. He is author of several research studies published in national and international journals and conference proceedings.

G. Naga Raju Presently working as assistant professor in Dept. of ECE, S.R.K.R. Engineering College, Bhimavaram, AP, India. He received B.Tech degree from S.R.K.R Engineering College, Bhimavaram in 2012, and M.Tech degree in Computer electronics specialization from Govt. College of Engg., Pune university in 2004. His current research interests include Image processing, digital security systems, Signal processing, Biomedical Signal processing, and VLSI Design

M. S. D. Mahesh Presently pursuing Bachelorof Engineering degree in Electronics&Communicationenginee ring at S.R.K.R. Engineering College, AP, India.

M. N. V. D. Ramya Presently pursuing Bachelor of Engineering degree in Electronics&Communication engineering at S.R.K.R. Engineering College, AP, India.

L. Srujana Presently pursuing Bachelorof Engineering degree in Electronics&Communicationengine ering at S.R.K.R. Engineering College, AP, India.

K. N. V. S. Sukesh Naidu Presently pursuing Bachelorof Engineering degree in Electronics&Communicationengineering at S.R.K.R. Engineering College, AP, India.