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Analysis and Characterization of White Blood Cells

[1] Sonali Sonar, [2] Kanchan Bhagat

[1] North Maharashtra University,
Department of Electronics and Telecommunication Engineering,
J. T. Mahajan College of Engineering, Faizpur,India

[2] North Maharashtra University,
Department of Electronics and Telecommunication Engineering,
J. T. Mahajan College of Engineering, Faizpur, India

Abstract - Now days, blood testing is one of the most important clinical examinations. The characteristics (quantity, shape and color) of the white blood cell (WBC) can give vital information about a patient's health. But, the manual inspection is time-consuming and requires adequate technical knowledge. Therefore, automatic medical diagnosis systems are necessary to help physicians to diagnose diseases in a fast and competent way. The main aim of blood cell segmentation is to extract the cells from complicated background and to segment every cell into morphological components such as nucleus, cytoplasm, and some others. Accuracy of earlier algorithms depends momentously on the initial contrast of the image. This limitation leads to capturing of all objects with gray-levels close to that of the WBCs. To overcome this disadvantage we propose to use the nucleus minimum segment size as a constraint to eliminate the non-nucleus objects. The proposed algorithm used, reduces noise effect and enhances accuracy of segmentation. All previous methods use different techniques for segmentation which gives less efficiency compared to proposed method for nucleus and cytoplasm segmentation. In this paper, we propose a new method based on gray scale contrast enhancement and filtering. For removal of false objects minimum segment size is implemented. Near about 365 blood images will be tested for this technique. Each of the five normal white blood cell types can be evaluated to compare separate performance.

Keywords - *Blood cell, Dataset, Leucocyte, MATLAB Code, Segmentation, WBC.*

1. Introduction

Blood tests can investigate many diseases like cancer, HIV/AIDS, diabetes, anemia, and coronary heart disease [1] [2]. Therefore 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. Manual microscopic examination is a must when there is a suspicion of abnormality in the blood sample but it is tedious, time consuming, and subjective.

If the visual sample inspection is automated then it will help the pathologists to increase productivity and reduce costs. The automation process includes image acquisition, image processing, segmentation, feature extraction, and classification. Segmentation is considered the most important and critical step in the process as it affects the rest of the following steps [3]. In this paper main focus is on the segmentation step. We propose an efficient technique for white Blood cells (WBC) nuclei automatic segmentation. In this research, the algorithm proposed by Madhloom et al. [4] is modified to account for more general situations. The proposed modification is to reduce dependence on the image initial contrast. This contrast dependence leads to the capturing of all objects that have the same gray-level as of the WBCs. To overcome this disadvantage we propose to use some constraints to eliminate the false objects.

2. Blood Sample

Whole blood count i.e. total number of RBC, WBC and platelets in given blood sample is the first and most important requirement for the diagnosis of any disease. And if there are excess of any of these types or any of these is few in number then it assures the doctor that the person is not healthy for sure. Manual counting of them is very tedious task. First of all we will differentiate RBC and WBC.

Blood consists of mainly red blood cells (RBC), white blood cells (WBC), and platelets. Each has its own function in our body and posses equal importance. Blood contain three types of cells and cell fragments which are floating in liquid called plasma. These elements are given as follows:

- Red Blood Cells ("erythrocytes," "RBCs") - oxygen-carrying cells

- White Blood Cells ("leukocytes," "WBCs") - cells that help to protect our body against diseases and prepare the body's immune system
- Platelets ("thrombocytes") - fragments of cells that perform an important role in formation of blood clots

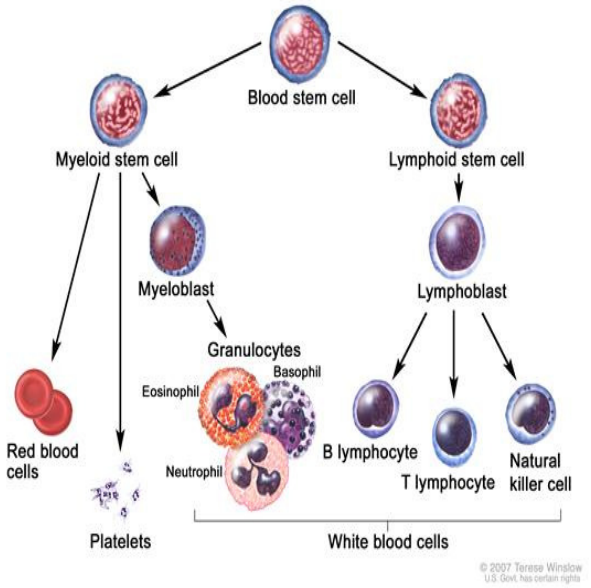


Fig. 1. Blood cell development

Both WBC and RBC have fixed count in our body. If their count is less than the ideal count then it gives signal that our body is not healthy. Therefore blood count assists in detecting many diseases in their initial stage.

2.1 Basis of Differentiation

We can differentiate RBC and WBC with respect to their size. This parameter is helpful to differentiate them and then count them separately. By having a view on above picture we can say that RBC are commonly round and WBC are hardly round. Also we can predict that how many pixels are there in the diameter of a cell. Threshold value can be set for diameter or area of the cells. If the cell is larger than that specific area or diameter then it is WBC else RBC.

RBC count will be detailed with the help of following image:

Here it can be clearly seen that largely stained purple bodies are WBC. And also they are much larger in size than RBC.

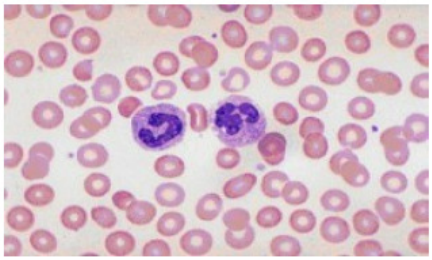


Fig. 2 Model Image

3. White Blood Cells and Their Types

White blood cells play an important role in your immune system. They are also called as leukocytes or sometimes WBCs by doctors. White blood cells flow in your blood stream, attacking invading bacteria, parasites and any other cells and objects that are not meant to be floating around inside your blood. White blood cells are not all equal. Actually there are six different types of white blood cells, each of which has a somewhat different role to play in your immune defence. The six types of WBCs are split in to two major parts, granulocytes and granulocytes.

3.1 Neutrophils

Neutrophils are the most commonly occurring type of white blood cell. They account for about 65% of all white blood cells, and are also known as polymorphonuclear leukocytes or a PMN for short. They are the primary defenders when bacterial and fungal infection occurred. Neutrophils can be thought of as the "first responders" in an invasion of foreign bacteria or fungi.

3.2 Basophils

Basophils are the least common type of white blood cell. Only 1% of your white blood cells consist of basophils. The primary function of a basophil is to release a chemical known as histamine in response an infection. Histamine is a chemical that has many functions, but it is primarily responsible for initiating an inflammatory reaction.

3.3 Eosinophils

Their share is about 4% of total leukocytes. These white blood cells perform two major functions as: Primary defenders against parasitic infections and elevators in cases of allergic reactions, such as hives, or even asthma related to allergies.

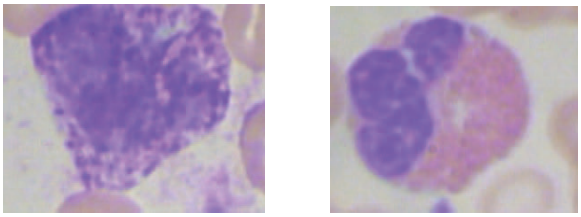
3.4 Lymphocytes

The first of the agranulocytes are lymphocytes. Like all agranulocytes they lack the membrane-bound granules found in the other category of white blood cells. Lyphocytes account for 25% of white blood cells. There are actually three different types of lymphocytes; B cells, T cells, and Natural Killer Cells. All three types have minutely varying functions.

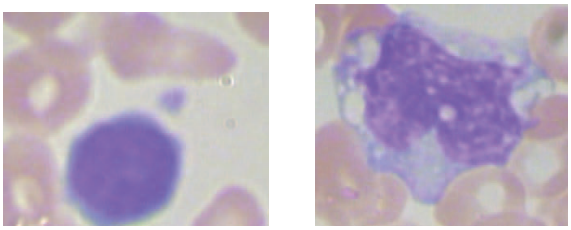
3.5 Monocytes

Monocytes share about 6% of white blood cells and have a somewhat unique and interesting role to play in your immune system. Monocytes are rather long lived compared to other white blood cells. They travel around in your blood, looking for bacteria, viruses and other "waste" that needs removal. When they find something that needs cleaning up, they swallow the offending particle in a process known as "phagocytosis". After swallowing these bits, the monocyte will break the invader in to smaller pieces and present them on its cell surface so that passing T cells can "learn" more about the chemical make-up of the invader and make it easier to kill more of them.

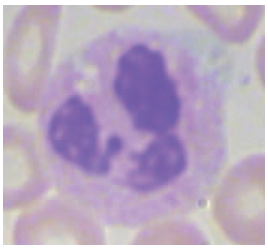
Here more concern is about classifying the WBC images because each cell type has different shape and colour as shown in Figure and which affects the classification accuracy



(a) Basophil (b) Eosinophil



(c) Lymphocyte (d) Monocyte



(e) Neutrophil

Fig. 3. White blood cells

4. Literature Review

Blood cell segmentation primarily extracts the cells from complicated background and segments every cell into morphological components such as nucleus, cytoplasm, and some others. The algorithm proposed by Ongun et al. [5] segments the WBCs using active contour models (snakes and balloons). These shape based and texture based features are utilized for the classification task. Near about twelve classes of WBCs are studied for this algorithm. Adollah et al. [6] present a comprehensive survey about segmentation methods. The main objective of his study is to develop an automated system on blood cell

classification. His work summarizes the most popular and accepted methodologies useful for the evaluation of image analysis prominently in the segmentation method.

Theerapattanakul et al. [7] use segmentation by using active contours. He uses double thresholding and then by scanning the binary image to find WBCs whose intensity of nucleus exceeds thresholding value. Initially circular shape (snake) is placed on the nucleus locations found and finally, active contour model is used with gradient flow vector force as a force to drive the snake contour fitting the WBC to be extracted. F. Sadeghian et al. [8] demonstrate a framework to segment WBC in two parts as:

Nucleus segmentation-Based on morphological analysis and gives 92% accuracy Cytoplasm segmentation-Based on pixel-intensity thresholding giving 78% accuracy

In case of above mentioned framework the major limitation is that for easy implementation, framework has done on sub-images. Madhloom et al. [4] suggests an algorithm to automate the process of detection and classification of leukocytes into various distinct subtypes. Here mainly white blood cell recognition and classification into various distinct subtypes is focussed. Here mainly focus is given on white blood cell nucleus segmentation which differentiate nucleus from the whole cell body by using a combination of automatic contrast stretching with the aid of image arithmetic operation, minimum filter and global threshold techniques. This proposed method gives accuracy between 85-98%. Markiewicz et al. [9] proposed an automatic system for blood cell recognition. The recognition is mainly carried out on the basis of the bone marrow images. He apply the morphological pre-processing of the image for individual cell extraction, generation and selection of the diagnostic features and the recognition system using Gaussian kernel Support Vector Machine (SVM) automatically and yields 87% accuracy.

In an automatic segmentation technique developed by Theera-Umpon [10], he uses the fuzzy C-means (FCM) algorithm to overly segment each cell image to form patches and mathematical morphology and nucleus smoothing and small patches removal. Angulo and Flandrin [11] investigate a technique to automatically detect the working area of peripheral blood smears stained with May- Grünwuald Giemsa. The optimal area is defined by the well spread part of the smear. In this algorithm two stages are present. In first stage, an image analysis is carried out using mathematical morphology to extract the erythrocytes. And in case of second, the number of connected components from the three kinds of particles is counted and the coefficient of spreading and the coefficient of overlapping are calculated.

Cao et al. [12] present an algorithm that focuses on the detection of red blood cells in urine image. For detection of red blood cells, at start he pre-processed urine image by an improved Sobel operator and localized RBCs using Hough Transform. Extraction and selection of features is carried out with the help of Principal Component Analysis (PCA) and then classification is done with LDA (Linear Discriminant Analysis).

Ramoser et al. [13] present a fully automated system for leukocyte segmentation which is robust with respect to cell appearance and image quality. Here set of features describes cytoplasm and nucleus properties and pairwise SVM classification discriminates between different cell types. Evaluation on a set of 1166 images (13 classes) resulted in 95% correct segmentations and 75% to 99% correct classification. Vromen and McCane [14] describe a model based contour tracing approach to tackle with the problem of automatically segmenting a Scanning Electron Microscope image of red blood cells. They use a second order polynomial model and a simple Bayesian approach to ensure smooth boundaries.

Poomcokrak and Neatpisarnvanit [15] detailed a method which is used to detect normal red blood cells (RBCs).Here neural network is used for classification. This study found that the proposed method gives sensitivity 0.86, specificity 0.76 and accuracy 0.74.
N. Sharma and L. M.Aggarwal [16] proposed a paper which details techniques for automated medical image segmentation. These methods are specifically discussed in the context of CT and MR images. The approaches explained in this review can be ordered according to applicability, suitability, performance, and computational cost. Performance of segmentation techniques as thresholding, and region based techniques can be enhananced by integrating them with artificial intelligence techniques. Techniques based on textural features utilizing atlas or look-up-table have excellent results on medical image segmentation. However it is difficult to correctly select and label data, segment complex structure with variable shape, size, and properties with atlas based technique. In this case we can use unsupervised methods such as fuzzy means algorithm. Many neural network-based algorithms are also available for texture-based segmentation and classification having good accuracy. But they need extensive supervision and training.

G. Lebrun, C. Charrier [21] proposed a fast and efficient segmentation scheme for cell microscopic image. This scheme mainly concentrates on how to reduce the complexity of decision functions produced by support vector machines (SVM) while preserving recognition rate. Vector quantization is used to reduce the inherent redundancy present in huge pixel databases. Hybrid color

space design is also used in order to improve data set size reduction rate and recognition rate. A new decision function quality criterion is presented to select good trade-off between recognition rate and processing time of pixel decision function.Then a new segmentation scheme using probabilistic pixel classification with several free parameters and an automatic selection is developed. Another important contribution here is the definition of a new quality criterion for evaluation of cell segmentation. The results conclude that the selection of free parameters of the segmentation scheme by optimisation of the new quality cell segmentation criterion produces efficient cell segmentation.

S. Chinwaraphat1, A. Sanpanich [22] detailed a scheme of modified fuzzy clustering for white blood cell segmentation. In this study first of all the segmentation is carried out by using a standard FCM clustering technique to classify the image of blood sample slide into 4 primary groups as white blood cell nucleus, white blood cell cytoplasm, plasma and red blood cell. Then FCM is modified to eliminate a scattering or false clustering which was present due to an unclear or color pixel similarity between cytoplasm and plasma background was implemented again literately until those errors were minimized. The minimization in each iteration loop was carried out by using a neighboring color pixel of its scattering as a reference. Finally the output shows that the modified method is able to extract nucleus and cytoplasm region more efficient than normal FCM.

Zhaozheng Yin, Ryoma Bise, Mei Chen and Takeo Kanade [23] proposed a method for cell segmentation in microscopy imagery using a bag of local Bayesian classifiers. In microscopy imagery cell segmentation is important for many bioimage applications such as cell tracking. For segmentation of cells from the background accurately, a pixel classification approach which is independent of cell type or imaging modality is presented. This method trained a set of Bayesian classifiers from clustered local training image patches. Each Bayesian classifier is an expert to make decision in its particular domain. The decision from the mixture of experts determines how likely a new pixel is a cell pixel. The proposed method details the effectiveness of this approach on four cell types with diverse morphologies under different microscopy imaging modalities.

Marco Antonio Garcia de Carvalho, Tiago William Pinto, Roberto Marcondes C´esar J´unior [24] presented a technique for Image Segmentation Using Watershed and Normalized Cut. This technique proposes an image segmentation strategy which uses two ways to convert images into graphs: Pixel affinity and watershed transform. Both ways provide us result as a similarity matrix that is used to calculate the spectral graph properties (eigenvalues and eigenvectors)

Proposed algorithm reduces noise and enhances accuracy compared to one proposed by Madhloom et al. [4].By comparing Madhloom et al. [4] with proposed algorithm by visual inspection using a test image we can easily predict that the relative size test is very important to get rid of all the non nucleus objects which ultimately implies superiority of proposed algorithm.

5. Proposed Algorithm

The available techniques for image segmentation can be divided into two main groups as follows [16]: methods based on gray level (e.g.: thresholding, Edge based segmentation), and methods based on image texture. Here, the algorithm proposed in [4] is modified to reduce noise and enhance accuracy. The main disadvantage of the algorithm proposed in [4] is that its accuracy depends momentously on the initial contrast of the image. This limitation leads to capturing of all objects with gray-levels close to that of the WBCs, as will be displayed later using a test image. To overcome this disadvantage we propose to use the nucleus minimum segment size as a constraint to eliminate the non-nucleus objects. A nucleus segment minimum size limit is chosen to be half the RBC average size.

This value is selected by experiment. Also morphological opening is executed to remove small pixel groups.

5.1 Proposed Algorithm Steps

The blood image is processed as follows:

1) Convert the input image, A, to a gray scale image B.
2) Adjust the gray scale image, B, intensity values with a linear contrast stretching to get image L.
3) Enhance the contrast of the gray scale image, B, using histogram equalization to get image H.
4) Obtain the image R1=L+H.
5) Obtain the image R2=L-H.
6) Obtain the image R3=R1+R2.
7) Implement, three times, 3-by-3 minimums filter on the image R3.
8) Calculate a global threshold value using Otsu's method.
9) Convert R3 to binary image using the threshold from step 8.
10) Use morphological opening to remove small pixel groups. Use a disk structuring element with a radius of 9 pixels.
11) Connect the neighboring pixels to form objects.

12) Apply the size test to remove all objects that are less than 50% of average RBC area.

Figure 1 shows an overview of the proposed algorithm.

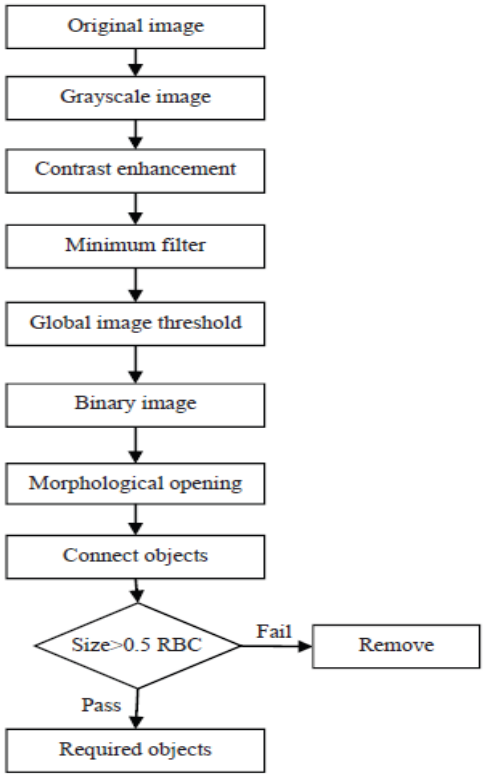


Fig. 4. Proposed algorithm to segment WBCs

5.2 The Details of the Proposed Algorithm

In step 1, we start by transforming the original smeared image to a gray-scale image, which presents the nuclei of the WBCs the darkest areas in the image. Then the gray scale image goes through a series of processes to enhance its contrast. In step 4, the addition process will brighten most of the details in the image except the nuclei since by performing the image addition, all the resultant pixels exceeding the intensity value of 225 is truncated to 255. While in step 5, the subtraction process will highlight all the objects and its borders in the image including the cell nuclei. Step 6, the addition 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. After enhancing the contrast, step 7 applies a minimum filter. 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 was evident by trials. In step 8, a thresholding technique (Otsu's method) is used. Using the threshold from step 8, we could convert the image R3 to its binary version. Then, in step 10, we use morphological opening to remove the small groups of pixels which can form false objects. Morphological opening [17]: is done by applying erosion followed by dilation. Erosion: is applying a structuring element B on a binary image A as show by the following equation:

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

Where the structuring element Bz 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

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

Bs is given by

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

So the morphological opening is defined as,

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

Then, in step 11, the algorithm presented in [18] will be used to count and locate the nuclei of the WBCs. The last (step 12) 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.

6. Classification of Leukemia Infected and Healthy Images Using Naive Bayes Classifier

The Leukemia infected and Healthy images are shown as figures below. Leukemia infected and healthy blood cells are classified using naïve Bayesian classifier in Matlab software. In experiment, Naive Bayes classifier, in training phase, train the Classifiers with features for blood cell dataset. Naïve Bayes classifier is used to find leukemia cell. We used 5 healthy blood cell images and 2 leukemia cells. Result of classification of Naive Bayes classifier has shown in Fig.6 and Fig7.

Naïve Bayes Classifier detected 90% of Leukemia images.

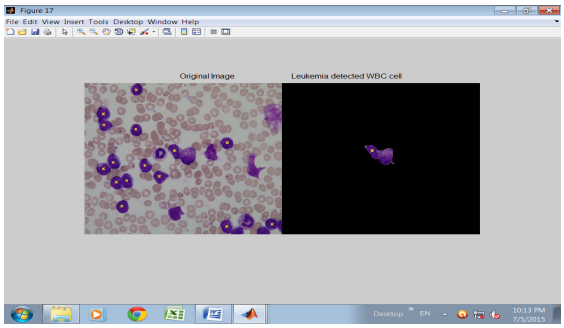


Fig. 5: Leukemia infected cell and segmented infected cell

7. Result

Segmentation accuracy results for each cell type using both the proposed algorithm and algorithm proposed by Madhloom et al. [4]. It shows that the superiority of proposed algorithm for each cell type and for the overall performance of 79.7% compared to 55.9%.

Table 1: Segmentation accuracy results comparison

Technique	**WBC cell type**					
	Basophil	Eosinophil	Lymphocyte	Monocyte	Neutrophil	Total
Mad. [4]	0.643	0.448	0.589	0.574	0.57	0.559
Proposed	0.804	0.693	0.838	0.863	0.803	0.797

Table 2: Result Table for Healthy cells

Cell Type	Mean Red	Mean Green	Mean Blue	Diameter
Healthy Cell1	113.787	79.621	143.493	115
Healthy Cell2	114.253	81.451	141.047	113
Healthy Cell3	118.039	83.249	145.251	115
Healthy Cell4	117.427	83.370	146.120	112
Healthy Cell5	127.966	95.404	151.865	111
Average Value	118.294	84.619	145.555	113.2

Table 3: Result Table for Leukemia cells

Cell Type	Mean Red	Mean Green	Mean Blue	Diameter
Leukemia Cell1	174.498	152.176	190.529	189.000
Leukemia Cell2	190.715	167.450	228.900	299.000
Leukemia Cell3	189.465	168.150	215.169	141.000
Leukemia Cell4	177.293	164.219	191.723	163.000
Leukemia Cell5	175.701	167.286	192.330	141.000
Leukemia Cell6	164.561	143.925	161.942	141.000
Leukemia Cell7	193.213	174.658	205.303	506.000
Leukemia Cell8	162.088	142.252	185.775	141.000
Leukemia Cell9	176.991	157.623	187.492	322.000
Leukemia Cell10	200.272	174.703	210.793	141.000

From above two tables:
We can estimate that Leukemia cells always have -
1. Mean red > 118.294
2. Mean green > 84.619
3. Mean blue > 145.555
4. Diameter >113.2

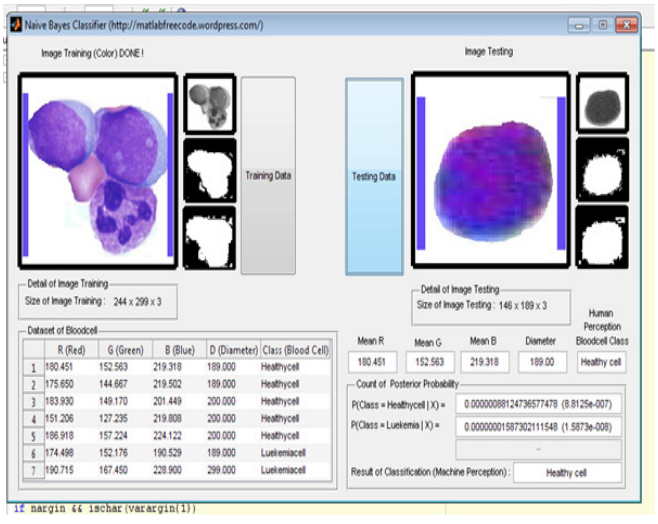


Fig. 6: Result of classification of Healthy cell

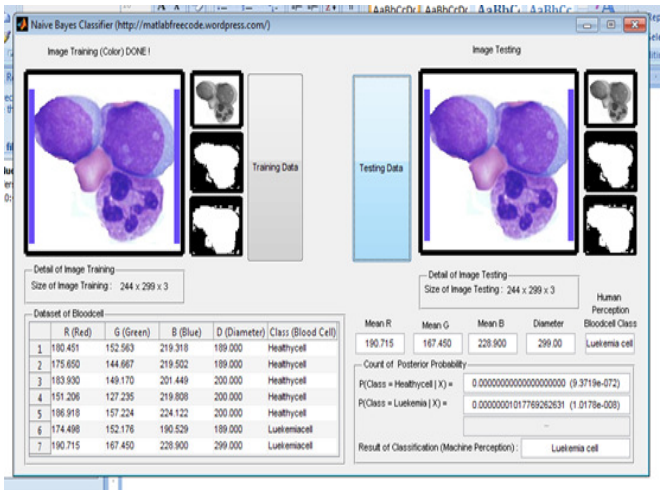


Fig. 7: Result of classification of Leukemia cell

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