

# METHODOLOGY FOR COUNTING OF BLOOD CELLS AND DETECTION OF DISEASE BY USING BLOB DETECTION AND GLCM

<sup>1</sup>CHUNDURI NAVEEN KUMAR, <sup>2</sup>Dr S ARUNA, <sup>3</sup>Dr K Srinivas Naik

<sup>1</sup> PG Scholar, Dept. of ECE(Biomedical), Andhra University College of Engineering, Vizag, AP, INDIA

<sup>2</sup>Associate Professor, Dept. of ECE, Andhra University College of Engineering, Vizag, AP, INDIA

<sup>3</sup>Associate Professor, Dept. of ECE, Vignan's Institute of Information Technology, Vizag, AP, INDIA

mr.naveenchunduri@gmail.com

## Abstract

Blood cells both white and red are important part of the immune system. These cells help fight infections by attacking bacteria, viruses, and germs that invade the body. In this project, a Blob detection algorithm is used for the segmentation and counting of WBCs and RBCs. The separation of WBCs from RBCs was achieved by thresholding, and specific pre-processing steps were developed for each cell type. Counting was performed for each image using the proposed method based on blob detection, which automatically counted the cells. The Blob detection works exactly like human eye, which differentiates on the basis of various parameters like colour, shape, size, distance etc. The disease is been identified by extracting the features using GLCM. The Experimental results are performed using matlab tool

## Keywords

WBC, RBC, Blob Detection, GLCM

## 1. Introduction

Platelets are one of the blood cells that stops the bleeding in the body from blood clotting. Platelets can detect if any blood vessels are damaged. Red blood cells are also tiny blood cells that is also important in the health of human through carrying fresh oxygen throughout the body whereas white blood cells helps protect the body from infections. Complete Blood Count (CBC) involves blood testing to determine the healthiness of the major components of blood which are platelets, red blood cells and white blood cells. Abnormalities of result based from references of normal count of cells may indicate an underlying medical condition that needs further evaluation.

For this past few years, CBC counting is one of the most studied area of research due to accuracy problem. Laboratories in the hospital in the Philippines are still using the traditional method of counting blood cells. This was done in either manual method through hemocytometer or by automated method through flow-cytometer. In this study, it uses images of the blood to calculate the number of cells since research on

medical images is new technology. Image processing is a method which involves signal processing and mathematical procedure to change the image into another form of desired image. Image analysis is the extraction of significant information from an image. Hence, this paper does not involve image processing only but analysis as well.

Nowadays, there are many ways of image processing and analysis of blood cell images. However, the quest for the highest accuracy is still one of the aims of the researchers. With so many studies, the researchers will present another way of counting blood cells through the use of strong level of algorithm with the help of Matlab programming language. This study used colour filtering to keep a specific hue while de-saturating the rest of the image. It also involves image segmentation to convert the image into multiple parts to identify which of the cells are platelets, red blood cells or white blood cells. Blob detection plays important role in this study which primarily detects the differences of each blood cells before the cells will be counted.

## 2. Related Work

Platelet count is one of the blood tests involved in the process of CBC to determine if the patient suffers from anemia, leukemia and etc. Plate counting is usually done manually but a recent study showed that this process can be done through Circular Hough Transform in a microscopic blood cell images. This process presented an accuracy rate of 96% compared with traditional manual counting [1]. Traditional white blood cell counting is a long process and contributes some inaccuracy. If more accuracy in white blood cell counting would like to obtain, an expensive haematological analysing machine is needed. Hence, a study about microscopic images of blood stained peripheral blood film for leukemia and normal condition was presented. It involves color space conversion, colorthresholding, filtering, marker controlled watershed and morphological operations which got an accuracy of 88.57% [2].

Detection and counting of white blood cells in blood samples were also presented through computer-aided and mobile-cloud-assisted blood analysis. The paper propose a smartphone-based cloud-assisted resource aware framework for localization of WBCs within microscopic blood smear images using a trained multi-class ensemble classification mechanism in the cloud. Its algorithm includes segmentation, extraction of texture, statistical, and wavelet features and then categorized into five classes: basophil, eosinophil, neutrophil, lymphocyte, and monocyte.

Counting each type of cells was then accomplished [3]. Abnormalities in white blood cells were also studied by researchers through digital image processing. The study presented is fast and inexpensive that can detect kind of diseases like Chronic Obstructive Pulmonary Disease, Immune system disorders, Neutropenia, HIV/AIDS, Lymphocytopenia, leukemia etc. There are two proposed framework presented in

the paper. The first framework determined the types of nucleus in WBC and the second framework is the counting of WBC and abnormal nucleus in the WBC. The result showed more than 85% accuracy [4].

Another method in counting and classification of white blood cells was also introduced using Artificial Neural Network (ANN). This method decreases the time of executing the segmentation and classification of WBC. Nucleus enhancement by finding the intensity maxima improves the detection and classification of Leukocytes and then classified based on various features extracted from segmented images. ANN was used to classify and confirm the white blood cells. The steps involved image acquisition, image segmentation, feature extraction and classification using ANN and counting. It was concluded that this new method was better in terms of accuracy and efficiency while considering the time and cost in dealing with this process [5].

Morphological features of cells make the counting of white blood cells easy. The extraction of nucleus of WBC can provide information about different kinds of diseases. After contrast stretching and histogram equalization of image, segmentation of nucleus from blood smear images using Otsu's thresholding technique was applied. It was then followed by minimum filter for reducing noise and increasing brightness of nucleus through mathematical morphological procedure [6]. Separation and counting of blood cells using geometrical features and distance transformed watershed was also introduced in a study. The proposed method operates on binary images taken from initial segmentation and consists of several detailed steps. Canny edge detection, the most popular edge detection, was done in the image. Morphological operations, the connectedness of the pixels in the image, were then performed. After that, the feature was extracted. After extraction, classification was done. Then clumped cells were separated. Finally, cell counting was established [7].

RBC microscopic images and Matlab are employed as a method in counting RBC. The mask of the RBC was used to count the number of RBC in a blood smear. It involves separation of color components in the image, then histogram was performed. Thresholding was done after the histogram. If object area is greater than the desired value, area from the image corresponding to the triple line square is then extracted. Finally, number of RBCs will be counted from the extracted area of the image. Further, blood smears are stained with Leishman stain. Images are acquired using light microscope connected to digital camera. Images are converted to their three colors components. Initial Segmentation is based on green component. Final RBCs segmentation and counting is based on the histogram of objects areas in the image [8].

Red blood cells can be counted based on other classification algorithms. In segmenting blood cell image, spectral angle mappings (SAMs) and support vector machines (SVMs) can be used. Identifying RBCs can be identified using standard RBC model based on SAM classification algorithm. This process obtained an accuracy of 93%. To improve this accuracy, SVM classification was performed and it resulted

to 98% after applying the additional process [9]. Overlapping cells can now be counted using morphological watershed transformation and regional maxima computation. Matlab was used in simulation of counting red blood cells, white blood cells and platelets. In the study, microscopic blood cell image must be obtained with high resolution. The image must be smoothened. Then the pre-processed image is converted to YCbCr and Grayscale image formats. After which, WBC and Platelets mask was done by applying morphological operations on Cb component. The RBC mask was obtained by applying morphological operations and thresholding operation. Then, computation was done for regional maxima points. Finally, it performed watershed segmentation on masks and compute count values. The minimum efficiency for the dataset was found to be 94.44%. [10].

A paper was presented to segment and count overlapped red blood cells image. It uses the combination of Pulse Coupled Neural Network (PCNN) and template matching algorithm. The isolated area was considered as a template. The steps done are converting the image from RGB to gray, segmentation using PCNN, eliminating unwanted objects, segmentation using template matching, count isolated blood cells, count overlapped cells and final counting. The researchers concluded that their algorithm performs better in counting overlapped red blood cells in comparison with the existing counting system [11]. Clinical decision support system for cells counting and classification is existing nowadays. A computer aided system can simulate a human visual inspection to automate process of detection and determination of WBCs and RBCs from blood sugar smears. This method has been tested on public datasets of blood cell images and demonstrated a reliable and efficient system for differential counting. The result obtained accuracy value of 99.2% for WBC and 98% for RBC [12].

The image processing techniques were applied to extract the needed feature (e.g. size,color, texture features etc...) to analyse different types of WBC [14]. The camera then took a snap shot of the blood smear sample and sent it to the computer in order to analyse and pre-classify the WBC using ANN. The classification accuracy percentage is 89% [15].In addition, the University of Kaiserslautern and the Westpfalz-Klinikum initiated research to automate the diagnosis process of WBC differentials [16]. Their system automatically analyzed the stained blood and bone marrow slides under the microscope. The main objective was to build a device that could count the different lineage and maturity levels of WBC [17] to determine the occurrence of leukemia and other diseases. Only segmentation of WBC is attempted in this research using image processing tool boxes in Matlab. Sabino et al [18] calculated four textural features of WBC based on Gray Level Co-occurrence Matrices (GLCM) (energy, entropy, inertia and local homogeneity) for Lymphocytes. WBC cell differentiation test these features to distinguish leukocyte cells. Their Experimental results verified that texture parameters were critical to distinguish between the five types of usual leukocytes and chronic lymphocytic leukemia. The input to their automatic sample recognition classifier was quantitative features generated from GLCM which proven useful and given 96%

classification accuracy. Zheng, Milthorpe and Johnes [19] attempted to classify manually cropped image data for WBC.

### 3. Proposed Methodology

The algorithm used in this study includes five (5) steps: Image Uploading, Color Filtering, Image Segmentation, Blob Detection and the Cell Counting and features extraction.

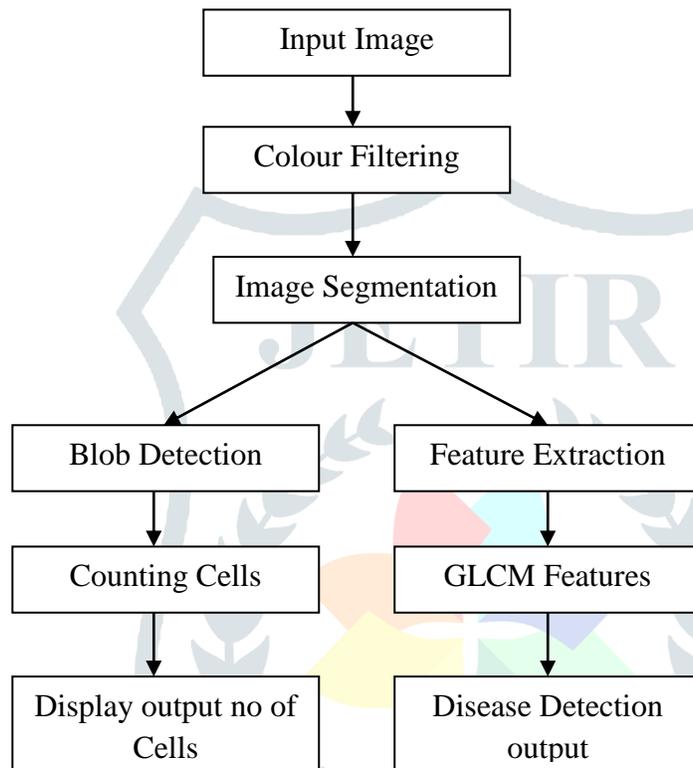


Fig1. Proposed Methodology

The proposed methodology process flow is shown in Fig 1. Further we discuss about the colour filtering and flowed by segmentation process in identifying the disease and number count of the blood cells.

#### A. Image Uploading

The ten (10) square subdivision images of the of theblood specimen were uploaded in the matlabbased program, processed and analyzed. Images used in both WBC and RBC programs were samples captured using 40x magnification setting of a microscope while in Platelet program, 100x magnification images were used. High magnification was necessary for platelet counting since among the three cells, platelet cells are the smallest as shown in Fig2.

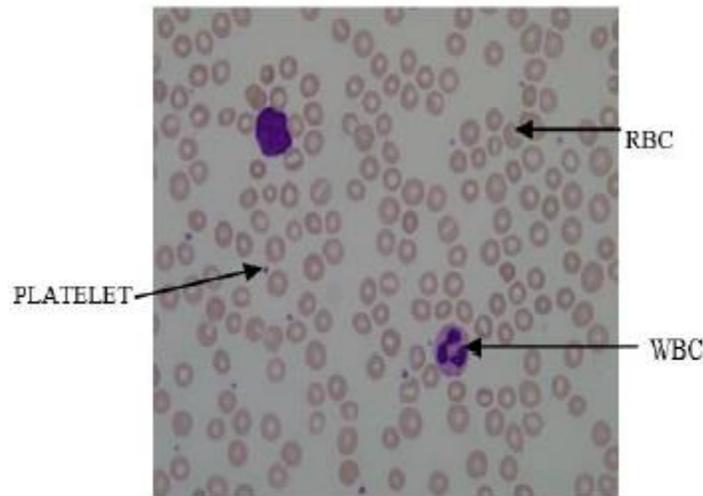


Fig2. Blood Cell Specimen

### B. Color Filtering

In Color Filtering process, color determination was done to distinctively characterize the WBC, RBC and Platelet cells from each other. Specific color pixel values were identified and were converted from BGR to HSV in order to get the correctly processed the setting of upper and lower bounds as the range of color values only needed for image segmentation. Sample result for color filtration.

### C. Image Segmentation

In Image segmentation process, we first masked out the resulting HSV image to separate objects from the background using a pixel feature value. In our study, we used Otsu's binarization technique for thresholding purpose. In this technique, it automatically calculates threshold values from the two peaks of the histogram of a bimodal image using the formula shown below in equation 1. It actually finds a value of  $t$  which lies in between two peaks such that variances to both classes are minimum.

$$\sigma_w^2(t) = q_1(t)\sigma_1^2(t) + q_2(t)\sigma_2^2(t) \quad (1)$$

Where  $\sigma$  is the variance,  $\sigma_w^2(t)$  weighted variance and  $q_1(t), q_2(t)$  are the probabilities of the two classes divided by a threshold  $t$ , which is within the range from 0 to 255 respectively.

### D. Blob Detection

A Blob is a group of connected pixels in an image that share some common property (e.g. grayscale value). The goal of blob detection is to identify and mark these regions.

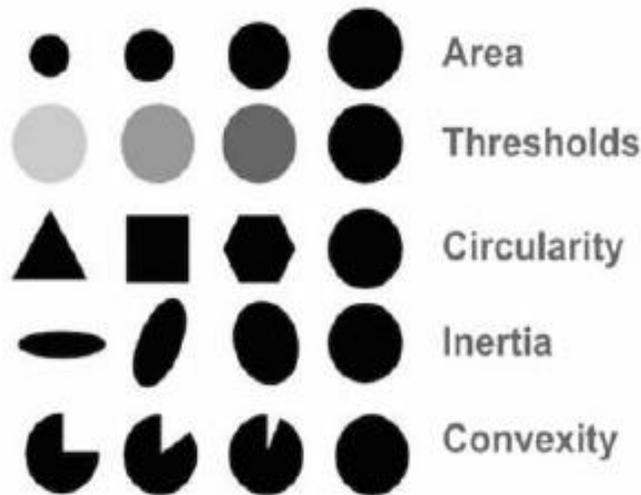


Fig3. Blob Detection Parameters

Blob detection provides methods for segregating those samples by thresholding, grouping, merging and radius calculation. Thresholding converts the source images to several binary images by applying the source images the threshold from minimum to maximum threshold. Grouping is identifying binary images connected with pixels or binary blobs. Merging is computing the center of the binary blob located closer than minimum distant between blobs and the last radius calculation by computing radii of the new merge blobs.

### E. Cell Counting

Having successfully isolated the cells for RBC, WBC and Platelet cell counter, each of the 10 images were process separately. The number of cells per image is summed up and was accordingly configured to get the correct results which are expected to achieve close to the expected text results if not the same.

In WBC Counter, the total sum of the cell counts from the ten images needs to be multiplied with 0.1 to get the final WBC test results as shown in equation 2. Whereas for RBC Counter, the total sum of the cell counts from the ten images needs to be multiplied with 0.001 to get the final RBC test results as shown in equation 3. Finally, for platelet count is shown in equation 4.

$$WBC_T = \sum(\text{CellCount}(1), \text{CellCount}(2) \dots \text{CellCount}(10)) \times 0.1 \quad (2)$$

$$RBC_T = \sum(\text{CellCount}(1), \text{CellCount}(2) \dots \text{CellCount}(10)) \times 0.001 \quad (3)$$

$$PLT_T = \sum(\text{CellCount}(1), \text{CellCount}(2) \dots \text{CellCount}(10)) \quad (4)$$

### F. Feature Extraction

After the segmentation is performed on lung region, the segmented nodules are used for feature extraction. Feature extraction is one of the most important steps in this system. A feature is a significant piece of information extracted from an image which provides more detailed understanding of the image. A feature is defined as a function of one or more measurements, the values of some quantifiable property of an object, computed so that it quantifies some significant characteristics of the object.

## GLCM Features

A gray level co-occurrence matrix is a second order statistical measure. GLCM is the gray-level co-occurrence matrix (GLCM), also known as the gray level spatial dependence matrix. The Gray-Level Co-occurrence Matrix (GLCM) is based on the extraction of a gray-scale image. The GLCM functions characterize the texture of an image by calculating how often pairs of pixel with specific values and in a specified spatial relationship occur in an image, creating a GLCM, and then extracting statistical measures from this matrix. Statistical parameters calculated from GLCM values are as follows: The matlab function `GLCM = graycomatrix(I)` creates a gray-level co-occurrence matrix (GLCM) from image I. `graycomatrix` creates the GLCM by calculating how often a pixel with gray-level (grayscale intensity) value  $i$  occurs horizontally adjacent to a pixel with the value  $j$ . (You can specify other pixel spatial relationships using the 'Offsets' parameter -- see Parameters.) Each element  $(i,j)$  in GLCM specifies the number of times that the pixel with value  $i$  occurred horizontally adjacent to a pixel with value  $j$ . The matlab function `graycomatrix` calculates the GLCM from a scaled version of the image. By default, if I is a binary image, `graycomatrix` scales the image to two gray-levels. If I is an intensity image, `graycomatrix` scales the image to eight gray-levels.

Along with GLCM following features is extracted i.e. mean, standard deviation, entropy, RMS, Variance, kurtosis, skewness for identifying the disease.

## 4. Results and Discussion

In this paper we identify the white blood cells count, red blood cells count and platelets count and also identify the disease based on the platelets count.

### Case1.

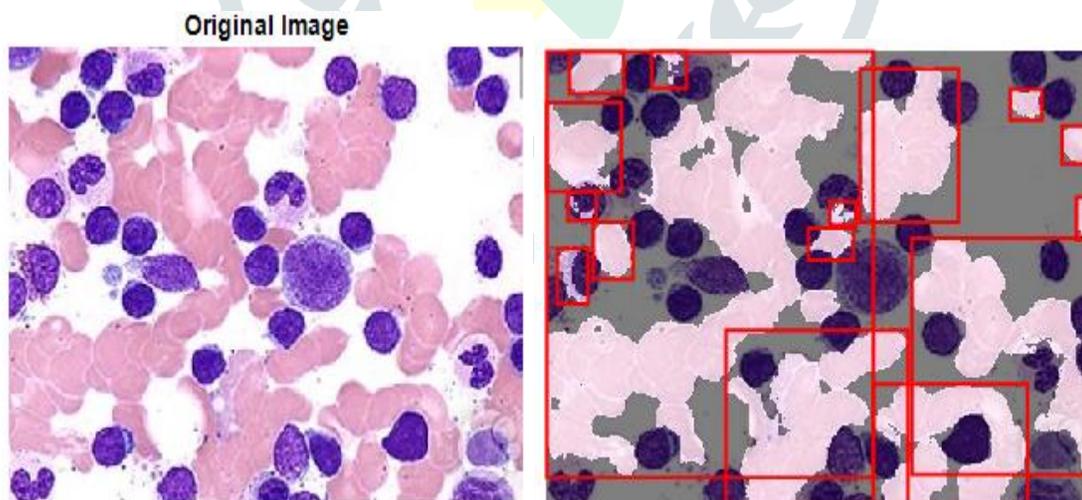


Fig4. Input Image.

Fig5. Detection of RBC

The input image which is given for processing and performing experimental evaluation is shown in figure 4. Figure 4 consists of blood cells. In figure 5 we can see the detection of RBC cells which is one part our proposed processing. Based on the detection of cells the number of cell count is identified and final disease prediction is observed.

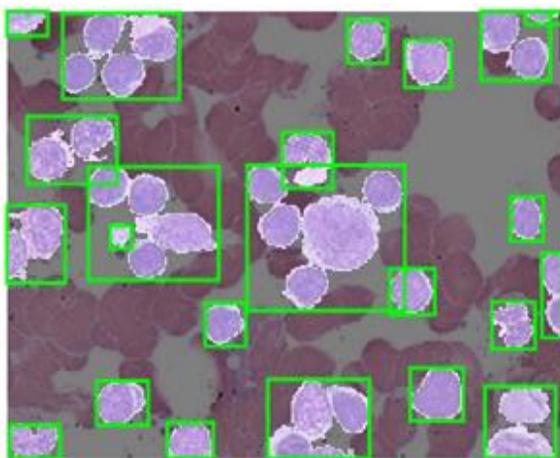


Fig6. Detection of WBC.

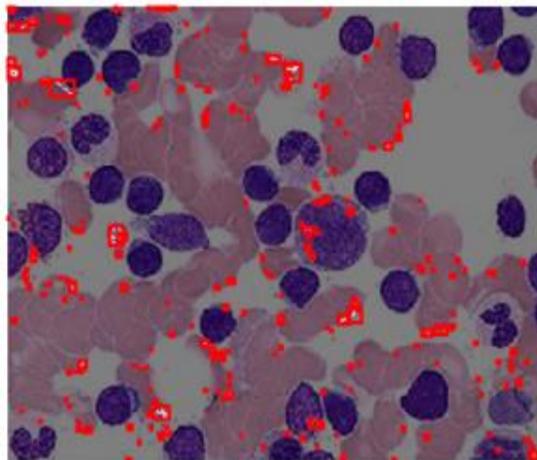
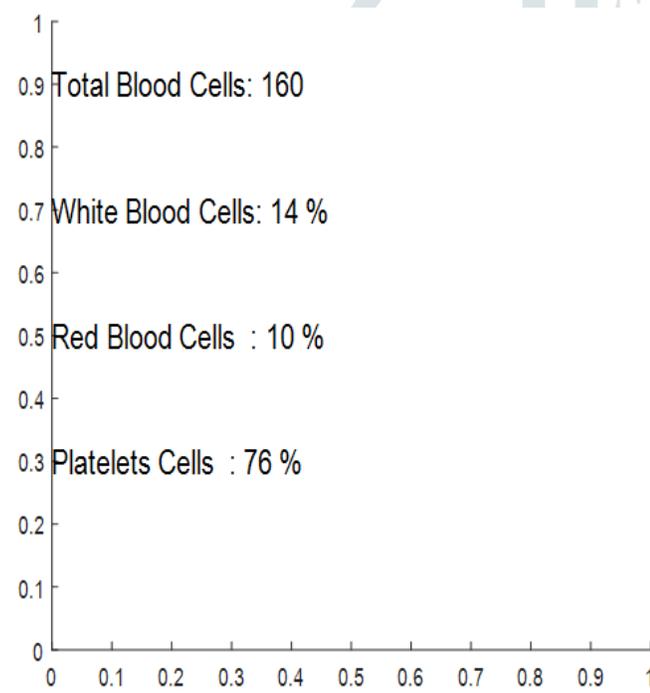


Fig7. Detection of Platelets

After detection of RBC cell, from figure 6 the detection of WBC cells are shown. Based on the detection of WBC cell, the count of cells will be evaluated. Finally from figure 7 shows the detection of platelets. Depending on the platelets count we can identify whether the patient is suffering from dengue or not.



Entropy	:	0.097193
Autocorrelation	:	1.019556
Contrast	:	0.014126
Correlation	:	0.196817
Dissimilarity	:	0.014126
Energy	:	0.968485
Homogeneity	:	0.992937
Variance	:	0.584781
Report	:	POTENTIAL LEUKEMIA DETECTED
Report	:	No DENGUE

Fig8. Overall Report

Overall report obtained by using the proposed methodology is shown in fig 8. Various GLCM features are evaluated and their values are shown. For the given input image, based on the features there is no dengue detected. The count of various cells has been calculated.

**Case2.**

Another input image is considered to perform the experimental evaluation.

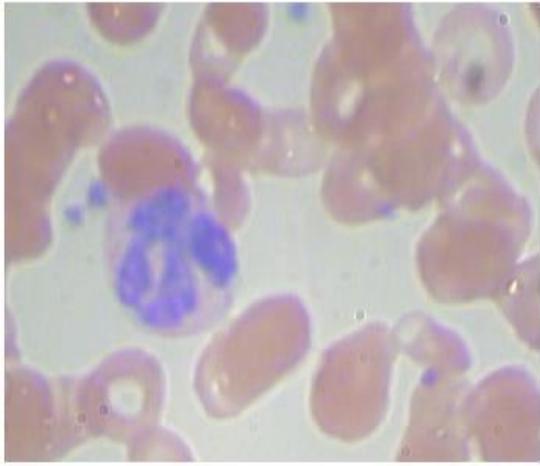


Fig9. Input Image.

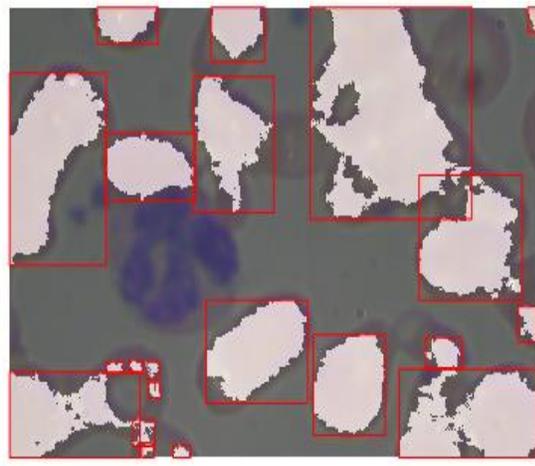


Fig10. Detection of RBC

The input image which is given for processing and performing experimental evaluation is shown in figure 9. Figure 9 consists of blood cells. In figure 10 we can see the detection of RBC cells which is one part our proposed processing. Based on the detection of cells the number of cell count is identified and final disease prediction is observed.

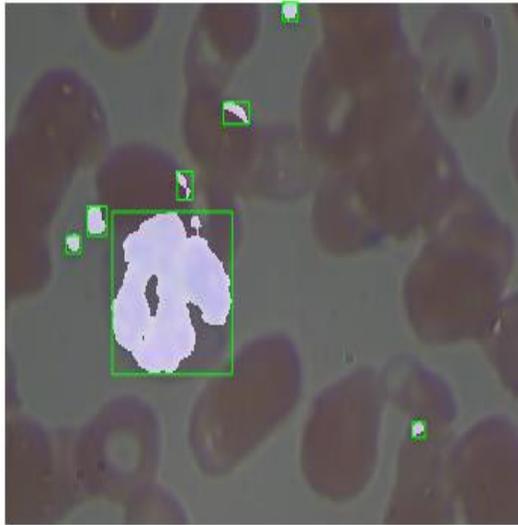


Fig11. Detection of WBC.

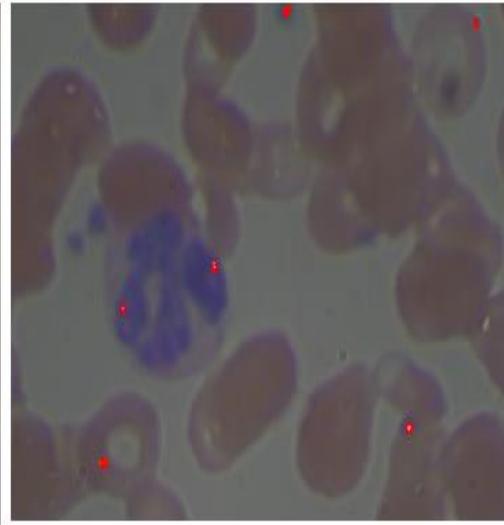


Fig12. Detection of platelets

After detection of RBC cell, from figure 11 the detection of WBC cells are shown. Based on the detection of WBC cell, the count of cells will be evaluated. Finally from figure 12 shows the detection of platelets. Depending on the platelets count we can identify whether the patient is suffering from dengue or not.

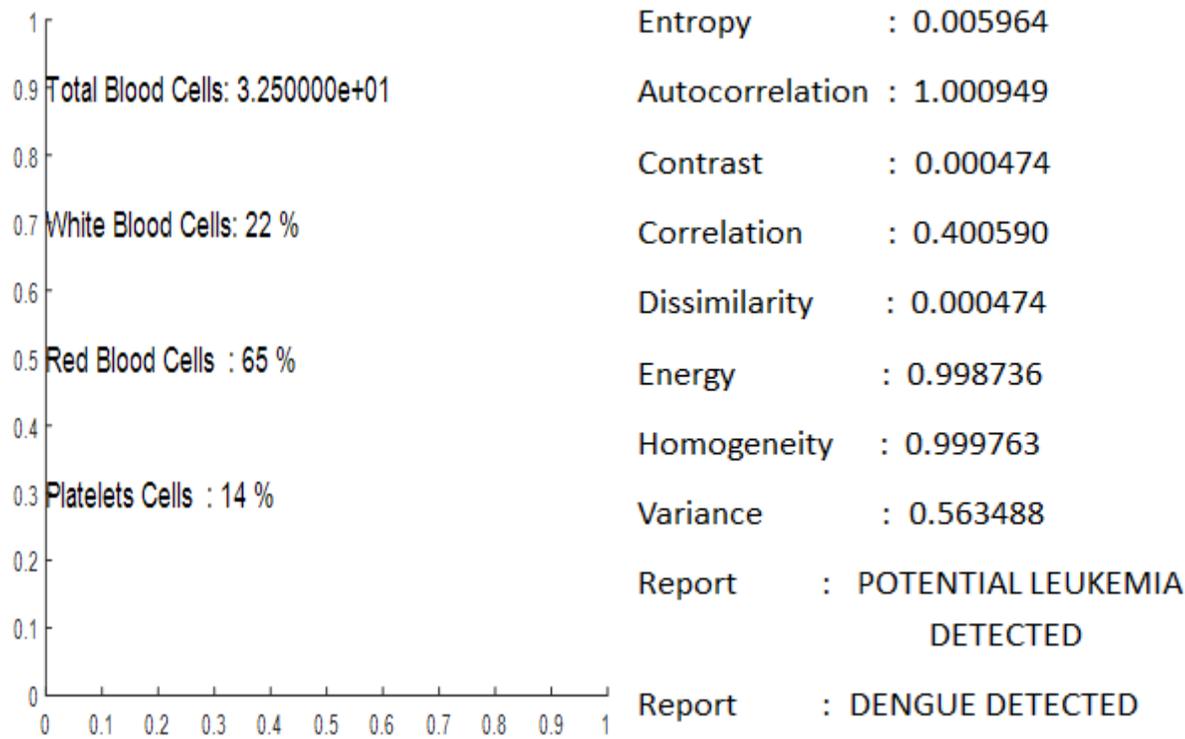


Fig13. Overall Report

Overall report obtained by using the proposed methodology is shown in fig 13. Various GLCM features are evaluated and their values are shown. For the given input image, based on the features there is dengue detected. The count of various cells are been calculated.

The rate of accuracy obtained using various techniques are shown in below table1.

Table1. Comparison of Accuracy

Method	WBC Accuracy	RBC Accuracy
Colour Based and Hough Transform[ref 1]	64.5%	81%
Morphological and watershed Algorithm[ref 2]	74.5%	93.5%
<b>Proposed Blob and GLCM extraction</b>	<b>90.1%</b>	<b>94.6%</b>

## 5. Conclusion

We manually selected and analysed set of blob detection in order to provide a system review about the current state of detectors and the potential areas of research. In this project we presented more accurate blood cell counting using a new algorithm with the help of matlab programming language. The implementation of image processing and analysis for the platelet, red blood and white blood cells was made possible and resulted to high level of accuracy. Proposed method provides an actual count of the RBCs and WBCs detected. Feature extraction is performed and GLCM features are calculated to diagnose the disease. The diseases like leukemia and dengue are identified.

## References

- [1] P. Kaur, V. Sharma, N. Garg, "Platelet Count using ImageProcessing", 2016 International Conference on Computing for Sustainable Global Development, 2016, pp. 2574-2577.

- [2] R. Ahasan, A.U. Ratul, A.S.M. Bakibillah, "White blood cells nucleus segmentation from microscopic images of strained peripheral blood film during leukemia and normal condition", 2016 5th International Conference on Informatics, Electronics and Vision, ICIEV 2016, pp. 361-366.
- [3] M. Sajjad, S. Khan, Z. Jan, K. Muhammad, H. Moon, J.T. Kwak, S. Rho, Seungmin, W. Sung I. Mehmood, "Leukocytes Classification and Segmentation in Microscopic Blood Smear: A Resource-Aware Healthcare Service in Smart Cities", IEEE Access 2017, pp. 3475-3489.
- [4] K.K. Jha, B.K. Das, H.S. Dutta, "Detection of abnormal blood cells on the basis of nucleus shape and counting of WBC", Proceeding of the IEEE International Conference on Green Computing, Communication and Electrical Engineering, ICGCCEE 2014.
- [5] S. Manik, Lalit, M. Saini, N. Vadera, "Counting and Classification of White Blood Cell using Artificial Neural Network (ANN)", 1st IEEE International Conference on Power Electronics, Intelligent Control and Energy Systems (ICPEICES-2016), pp. 1-5.
- [6] Gautam, Anjali, Bhaduria, Harvindra, "Classification of White Blood Cells Based on Morphological Features", 2014 International Conference on Advances in Computing, Communications and Informatics (ICACCI), pp. 2363-2368.
- [7] J. Hari, A.S. Prasad, S.K. Rao, "Separation and counting of blood cells using geometrical features and distance transformed watershed", Proceedings of the IEEE International Caracas Conference on Devices, Circuits and Systems, ICCDCS 2014 pp. 1-5.
- [8] S. Abbas, "Microscopic images dataset for automation of RBCs counting", Data in Brief 5 2015., pp. 35-40.
- [9] J. Lou, M. Zhou, Q. Li, C. Yuan, H. Liu, "An automatic red blood cell counting method based on spectral images", 2016 9th International Congress on Image and Signal Processing, BioMedical Engineering and Informatics (CISP-BMEI), pp. 1391-1396.
- [10] Tulsani, Hemant, Gupta, Rashmi, Kapoor, Rajiv, "An Improved Methodology for Blood Cell Counting", IEEE Impact 2013, pp. 88-92.
- [11] S.S. Adagale, S.S. Pawar, "Image Segmentation using PCNN and Template Matching for", IEEE 2013.
- [12] Loddo, Andrea, Putzu, Lorenzo, Ruberto, Cecilia DI, Fenu, Gianni, "A Computer-Aided System for Differential Count from Peripheral Blood Cell Images", Proceedings - 12th International Conference on Signal Image Technology and Internet-Based Systems, SITIS 2016, pp. 112-118.
- [13] W. V. d. Meer, "Blood Cell Morphology: Controversies and Alternatives", Thesis Radboud University Nijmegen, *The Netherlands*, ISBN 90-9020526-8, 2006.
- [14] K. S. Saladin, "Anatomy and physiology: The unity of form and function", McGraw-Hill, 4th, chap. 18, 680-696. 2007.

- [15] D. Anoragaingrum, "Cell segmentation with median filter and mathematical morphology operation", proceeding of the IEEE 10th International Conference on *Image Analysis and Processing (ICIAAP)*, 1043-1046, 1999.
- [16] H. Choi, and R. Baraniuk, "Multiscale Image segmentation using wavelet domain hidden Markov models", *IEEE Transaction on image processing*, 10(9), pp.1309-1321, 2001.
- [17] B. K. Das, K. K. Jha, and H. S. Dutta, "A New approach for Identification of Disease Affected Blood Cells", accepted ICICA 2014, will be published in *IEEE Xplore*
- [18] H. Harms and H. Magnus Aus, "Tissue Image Segmentation with Multicolor, Multifocal Algorithms", *Pattern Recognition Theory and Applications*, Edited by P.A. Devjver and J. Kittler, Springer-Verlag Berlin, Heidelberg Thesis *A.R.J. Kafz56*, 1987 .
- [19] S. J. Russell and P. Norvig, "Artificial Intelligence A Modern Approach", Prentice-Hall, Inc. *Upper Saddle River, New York*, 1995.

