

Blood Cell Count using Digital Image Processing

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Abstract - The basic aim of this paper is to find the RBC and WBC count using Digital Image Processing (DIP) from blood smear images captured through a compound microscope. This paper presents a method to digitally analyze the image of blood cells and find the RBC and WBC count values from the blood smear microscopic images. Plane Extraction of the microscopic images is done followed by edge detection and morphological filling operation. Circular Hough transform is performed for RBC count, whereas boundary is detected for WBC. The obtained results of the experiment are compared with lab reports and an accuracy of 91% is achieved for RBC while an accuracy of 85% is obtained for WBC.

Keywords: *Digital Image Processing, Cell Counting, Automated Blood Cell Count System, Automated Analyzer, Morphological Filling*

Abbreviations: ABCCS - Automated Blood Cell Count System, RBC - Red Blood Cell, WBC - White Blood Cell, DIP - Digital Image Processing

I. INTRODUCTION

A developing era in the field of Computer Science Engineering is Digital Image Processing (DIP) and it has its branches in all the fields. One of the growing fields in it is the Medicinal part. DIP uses computer algorithms to perform image processing on digital images. The impact of digital images on modern society is so great, and image processing is a critical component in science. At present, the blood samples are taken to lab and processed with various substrates and the results are produced. Whereas this paper, a Biomedical – Computer Science based interdisciplinary work, applies the stain, makes the blood sample absorb the stain and then captures the image of it. Then it is digitally processed with software and the result is displayed immediately.

Haemocytometer is the device which is used in the labs to count the blood cells. A microscopic glass slide consists of a rectangular indentation which creates a chamber. A perpendicular line grid is etched in this chamber. It is possible to count the chamber of cells in a specific volume of fluid, and calculate the concentration of cells in the fluid. Physician views the haemocytometer using a microscope and count the blood cells using the hand counter.[9]

Drawbacks of using manual method for blood count is listed below

- Counting the cells manually is time consuming and laborious.
- Overlapping cells is a great overhead.
- Visual inception is not consistent all the time.

In case of an automated Blood Cell Counting, Complete blood count is performed by an automated analyzer. The blood is well mixed without shaking and placed on an analyzer rack. This instrument has many different components to analyze the different elements of the blood. The numbers and types of different cells within the blood are counted by the cell counting component. The results are printed out or sent to a computer for review.

Drawbacks of the automated method would be,

- Automated analyzer is not cheap.
- Variations in the shape and irregularities cannot be detected.

II. RELATED WORK

Morphological iterative threshold techniques were presented in the literature by Berge [1]. Segmentation has been performed on RBCs, along with clumped cells, and boundary curvatures were implemented to construct a Delaunay triangulation. Real microscopy images prepared in the laboratory were used in that experiment. This

method could not tolerate a high degree of overlapping cells. This iterative threshold method could not detect faded red cells.

In another work, a method to count the Red Blood Cells, White Blood Cells and Platelets was proposed by Khan [2]. This method involves several pre-processing steps for the conversion of image to binary (or) binary image. Optimal threshold value was calculated using histogram and it plays a major role in segmentation and cell counting. They were able to achieve 95.23% accuracy in comparison with manual counting and a hematology analyzer but this cannot detect overlapping cells. Hence, iterative thresholds lead to a high probability of losing valid information which is dangerous.

Distance transformation to solve the overlapping cells problem was introduced by Nguyen [3]. Clumped cells were concentrated in this method in a higher degree.

A randomized Hough Transformation method which detects circles and RHT is increased which is lesser efficient in complex images due to its probability usage problem was proposed by Chiu [4] which was fast enough.

Mahmood and Mansor [5] analyzed ten image samples of normal blood cells. Here, image was converted to the HSV color space and Saturation or "S" channel was selected to pursue with the image analysis. Morphological operators and thresholding method were used instead of S channel for cell segmentation. They used Circular Hough Transformation to detect or check the presence of circularity feature which indicates the red blood cells in order to perform counting and detecting process. Their proposed method achieved approximately 96.54% accuracy rate when compared with manual counting process.

Miss. Madhuri G. Bhamare and Prof. D.S.Patil [6] presented a preliminary study of automatic blood cell counting using digital image processing. A few pre-processing and post-processing techniques have been implemented on blood cells image in order to obtain a much clearer and clearer image.

III. PROPOSED METHODOLOGY

Sometimes, inaccurate results are produced through manual counting method and it adds stress to the technician while submitting the report. Therefore, counting overlapping cells is a major problem. To address this problem, this paper uses digital image processing technique to minimize the errors and reduce the stress overload. This method of counting RBC and WBC helps in diagnosing various diseases such as anaemia, polycythemia etc. This paper introduces a cost effective

automatic blood cell counting method using image analysis techniques and specifically aims at improving the results using Plane extraction and Counting techniques. The proposed system of Automated Blood Cell Count System is shown in Figure 1.

Blood sample Acquisition

In this process, the slides are cleaned with ethanol using cotton. The fingers are cleaned with ethanol and are pricked with the help of Lancets. The blood drop is dropped onto the slide, smeared with another clean glass slide with an angle of 45 degrees inclination. Now the slide is kept for drying for five minutes. Leishman's stain is applied over the smeared blood and kept aside for drying. The stained slide is then washed with double distilled water to remove the excess stain. Nikon E 100 is used for getting the microscopic images. Adjustments are made to get the clear images. Nikon 21 Pixel Camera is used to obtain the images with the help of proper lighting and finer adjustments.

Image Processing

The image obtained from the preprocessing step will be in RGB scale. For counting the Red blood cells, the red plane of the image will be extracted and the counting process is done. [7] Similarly, for counting the White blood cells, the blue plane of the image is extracted and the counting process is applied. [8]

Noise Removal

This is a pre-processing step of an image sequence before feeding into the Segmentation process.[7] The image is first converted to its corresponding grey scale images. A pre-processing step is required to design a system that may be used under different conditions such as different blood staining techniques, types of chemical materials used, microscope types, illumination conditions, human errors, etc.,

Certain noises which are found in the images include

- Salt and Pepper Noise: random occurrences of both black and white intensity values
- Impulse Noise: Random occurrences of white intensity values
- Gaussian Noise: Impulse noise

After observing various sample images it was found that the median filter would be the best noise removal filter.

Median Filter

Each pixel value is replaced with the median of the gray scale values in the region of the pixel (i, j). A 3 x 3 region centered around pixel (i,j) has been taken and sorted (in ascending order) according to the intensity values of the pixels in the region. The middle value among those values is selected as the intensity of the current pixel. This is the very effective in removing salt and pepper or impulsive

noise while preserving the image detail.

Edge Detection

Edge preservation is an image processing technique to recover degraded and blurred images resulted while reducing the negative effect of noise in images. It can be a preliminary step toward better binarization and object segmentation.

In this paper, Canny edge detection algorithm is used over on the noise removed image to mark the edges of the cells. Canny edge detection algorithm detected the edges of the cells with maximal accuracy in case of sharp images whereas the accuracy of cell detection reduced in blurry images.[7] Since the cells are circular in shape, the edge detection was circular and complete. But in the case of practical images, after applying edge detection certain edges were incomplete.

On the whole the result of edge detection stage was an image in which there were two types of edges, one was the image where the cells were completely edge detected and another was the images where the cells edges were broken. To deal with these two different types of images and produce accurate results, a process called Morphological Filling was applied over the two sets of images which are explained below.

Morphological Filling

Morphological image processing is based on a strong mathematical concept which has been used to change the size, shape, structure and connectivity of objects in the image. It involves binary erosion, dilation, opening, closing and reconstruction. [7]

The erosion plays the role to shrinks and thins objects in image while dilation is used to grow and thicken the objects in image. Next, morphological opening is a combinational process of erosion and dilation while morphological closing is using the concept of dilation and continued by erosion. In other words, the functions of morphological opening are to remove, break and diminish the connection or objects which do not contain the structural elements. In contrary, morphological closing functions are used to join, fill and build connection and objects in the image. Using the closing image transformation with defined Structure Element (SE) unwanted edges, typically generated, are removed. The input to this block is the edge detected image. Morphological operation of filling is applied in the edge detected image. The output of the morphological filling operation is an image where the objects which was detected by Canny is highlighted with lesser pixel and the background is completely dark which explicitly differentiates the objects that is cells - Red blood cells in case of red plane extracted image and White blood cells in case of blue plane extracted image.

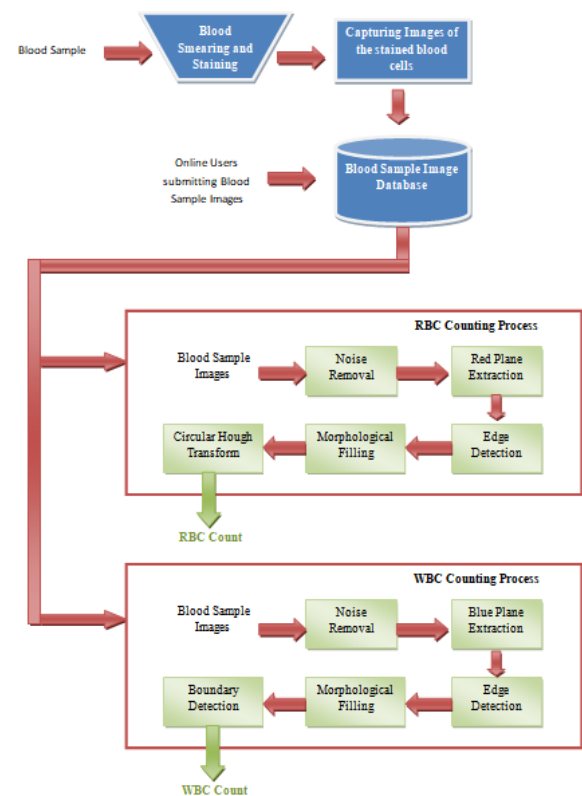


Figure 1 Proposed Automatic Blood Cell Counting System (ABCCS) Architecture

$$\text{Total RBC per cumm} = \frac{\text{RBC Counted by Hough Transform}}{\left[\frac{\text{Input Image Area}}{(\text{magnification} \times \text{magnification}) \times \text{film thickness}} \right] \times \text{Dilution Factor}} \quad (1)$$

$$\text{Total WBC per cumm} = \frac{\text{WBC Count}}{\left[\frac{\text{Input Image Area}}{(\text{magnification} \times \text{magnification}) \times \text{film thickness}} \right] \times \text{Dilution Factor}} \quad (2)$$

Circular Hough Transform

The circular Hough transform is then applied on the Red plane extracted image to count the Red blood cells as red blood cells are spherical that is circular in shape. This transform searches for the blood cells in the image and then detects them.[7] Detection process for circular hough transform is easier as the input image is morphologically filled. The function “draw circle” draws circles around the detected circular objects. Even the overlapped circles are detected. This method of counting cannot be used for white blood cells as they are not circular in shape.

Boundary Detection and counting

As the method of counting Red blood cells cannot be used for counting White blood cells, the method of Boundary detection and counting is used.[8] The input of this operation is morphologically filled Blue plane extracted image. A boundary around the filled objects is drawn and counting the number of cells drawn gives the total number

of White blood cells in the image.

Blood Cell Calculation

The total number of Red Blood Cells in the body given the number of cells in the image with prescribed magnification and size is calculated using the formula with the unit - million cells per cubic millimeter of blood is mentioned in [Eq 1] for RBC's and [Eq 2] for WBC's blood count.

IV. PERFORMANCE ANALYSIS

The ABCCS method of counting blood cells was experimented from the initial stage of collecting blood samples for about 63 subjects in real-time. A drop of blood was collected from the subject and was placed in one corner of the glass slide cleaned with ethanol and was smeared using another clean glass slide. After the process of drying, the blood cells were stained using Leishman's stain. The slide was dried and cleaned with double distilled water to remove the excess stain. The stained blood cells were oil immersed using cedar wood oil and viewed under a compound microscope under 100x magnification. The images of the oil immersed blood cells were captured using a digital camera. A sample image is shown in the Fig 2.

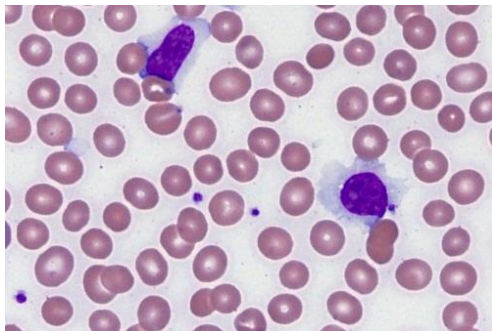


Figure 2 Sample Blood Smeared Image obtained under 100x microscope after staining

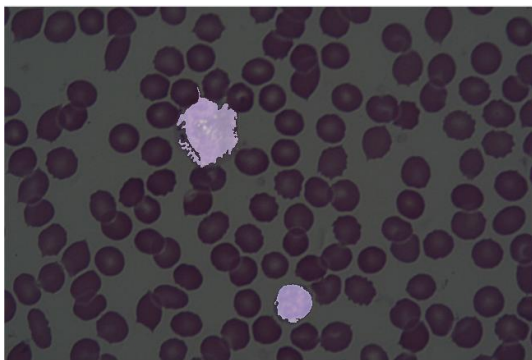


Figure 3 Blue plane Extracted after noise removal

The images obtained from the microscope under 100x magnification were subjected to Digital Image Processing. The unnecessary noises in the images were removed using Median Filter [8]. The Noise Removed Images were next

subjected to the process of Plane extraction. Red plane was extracted in order to count the RBC as depicted in Figure 1 and Blue plane was extracted for the WBC counting as depicted in Figure 1. One sample image where the Blue plane was extracted for WBC Counting process is shown in the Figure 3.

Once the required plane is extracted, the basic DIP operation that is, Edge Detection [8] and Morphological Filling [8] is done to clearly distinguish the required plane objects in the image. A sample image after the application of the above two DIP operations is shown in the Figure 4.

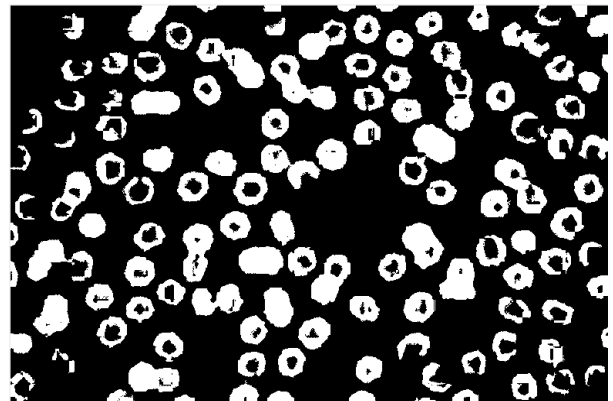


Figure 4 Image after Morphological filling operation

Now, two different methods are employed for counting RBC and WBC. RBCs are circular in shape and hence, they can be detected and counted using Circular Hough Transform. An image under RBC counting process after the application of Circular Hough Transform is shown in the Figure 5. For WBC, Circular Hough transform cannot be used as WBC is shapeless and therefore, boundary is detected for any object in the blue plane extracted image and thereby the objects are counted. The boundary detected blue plane extracted image is shown in the Figure 6 which is under WBC Counting process.

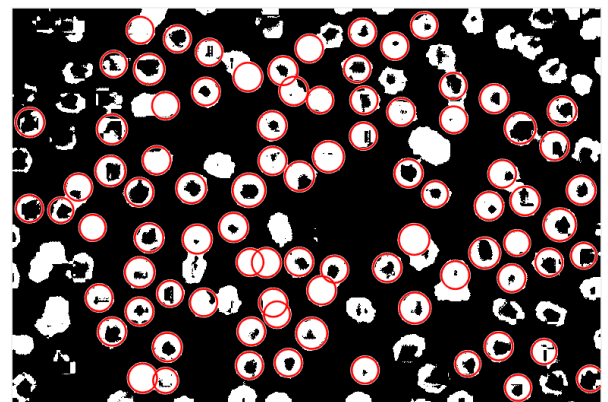


Figure 5 Image after application of Circular Hough Transform Operation for RBC

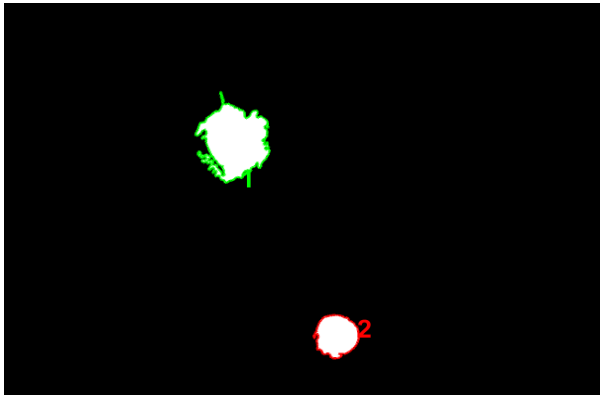


Figure 6 Image after Boundary Detection for RBC

A snapshot of the MATLAB command window displaying the count results of RBC and WBC in an image of prescribed area and 100x magnification by the ABCCS method is shown in the Figure 7. These MATLAB results are employed in the formulae Eq1 and Eq2 to obtain the total RBC and WBC count in the body of the subject respectively.

```

Command Window
New to MATLAB? See resources for Getting Started.

In wbc (line 18)
Warning: Image is too big to fit on screen; displaying at 17%
> In images.internal.initSize (line 71)
In imshow (line 305)
In wbc (line 52)

2
fx >>
    
```

```

Command Window
New to MATLAB? See resources for Getting Started.

>>
>>
>> zbc
    85

fx >>
    
```

Figure 7 Snapshot of the results obtained in MATLAB Command Window

The ABCCS method was experimented for 63 subjects in real time and the results of them by ABCCS method was validated or cross checked by the method of manual count using Haemocytometer. The RBC and WBC results given by the ABCCS method and by the Haemocytometer of a randomly selected ten subjects out of 63 are compared in the graph which is depicted in the Figure 8 and Figure 9 respectively. The Accuracy Percentage of the RBC and WBC Count of the same ten subjects given by the ABCCS method was measured in comparison with the count given by the Haemocytometer and the same is depicted in the Figure 10.

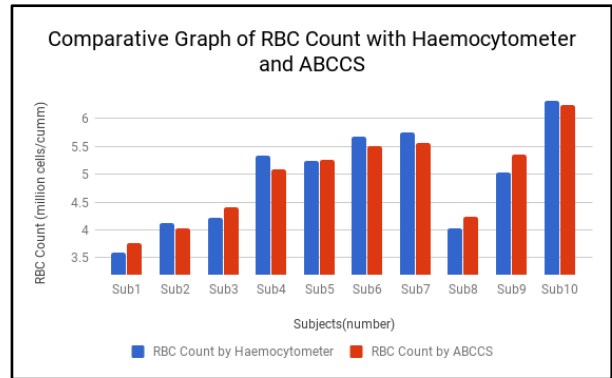


Figure 8 Comparative Graph of RBC Count with Haemocytometer and ABCCS

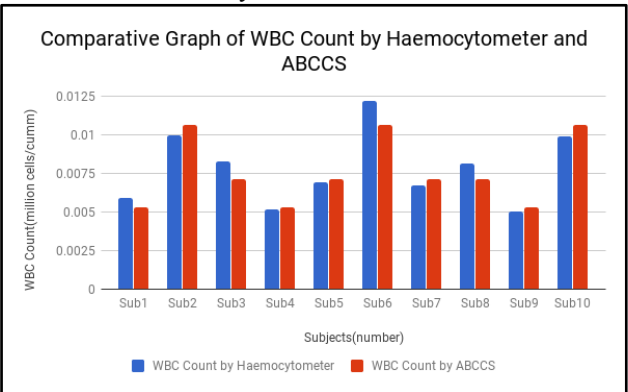


Figure 9 Comparative Graph of WBC Count of Haemocytometer and ABCCS

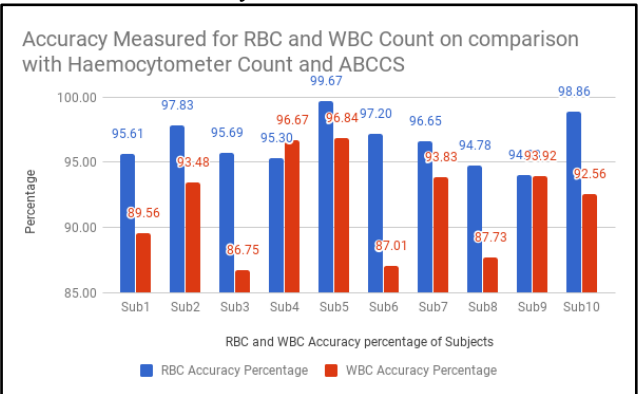


Figure 10 Accuracy Graph of RBC and WBC Count on Comparison with Haemocytometer and ABCCS

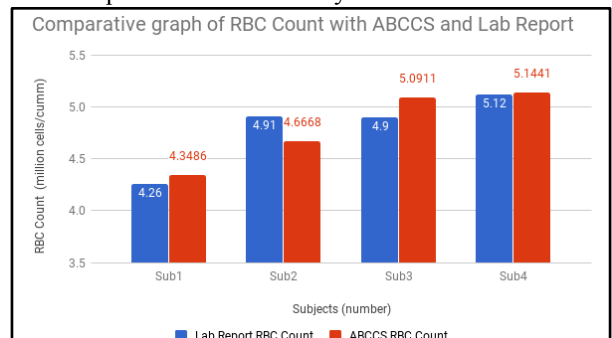


Figure 11 Comparative graph of RBC Count of ABCCS and Lab Report

As a second level of validation, the results of a few subjects out of the 63 subjects were cross checked with Lab Reports. The results of the subjects given by the ABCCS method and the Lab and individual accuracy of the subjects' RBC and WBC count is clearly tabulated in the Table 1.

The RBC and WBC Count given by the ABCCS method and the RBC and WBC Count given by the Lab is compared in the graphs and is shown in the Figure 11 and Figure 12. The Accuracy of the results for each subject in depicted in the Figure 13.

Table 1 Comparative Results of Lab Reports with ABCCS

SUBJECT	RBC COUNT – LAB REPORT	WBC COUNT- LAB REPORT	RBC COUNT BY ABCCS	WBC COUNT BY ABCCS	ACCURACY OF RBC COUNT	ACCURACY OF WBC COUNT
PERSON 1	5.12	0.009580	5.1441	0.010606	99.52%	89.3%
PERSON 2	4.26	0.009950	4.3486	0.010606	97.93%	89.3%
PERSON 3	4.91	0.007850	4.6668	0.00714	95.04%	90.95%
PERSON 4	4.90	0.007850	5.0911	0.00714	96.1%	90.95%

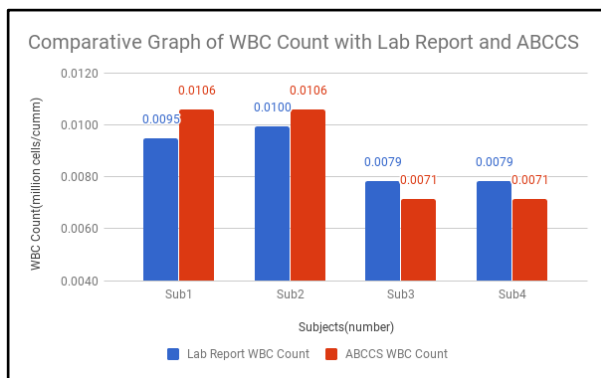


Figure 12 Comparative graph of WBC Count with Lab Report and ABCCS

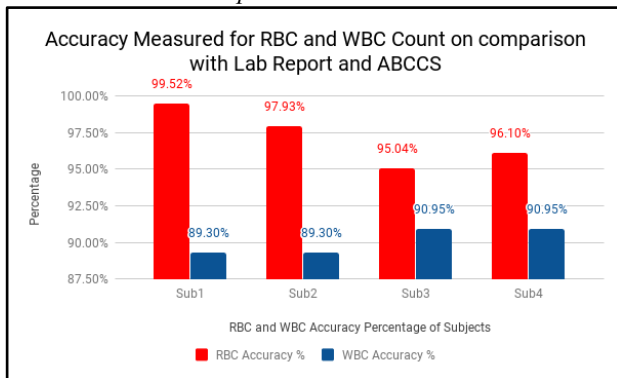


Figure 13 Accuracy Measured for RBC and WBC on comparison with Lab Report and ABCCS

The ABCCS method of counting the RBC and WBC was experimented initially with 150 blood smear images along with ground truth values which were obtained after requisition through proper channel from the medical website biosigdata.com. The RBC and WBC count results of the 150 blood smear images given by the ABCCS method was tabulated and accuracy for each of the 150 subjects were calculated. A comparative graph between the RBC count results of ABCCS method and the ground truth values of the biosigdata.com of randomly selected

ten subjects is shown in the Figure 14. An accuracy of 95% was achieved by the ABCCS method in the case of RBC count on an average.

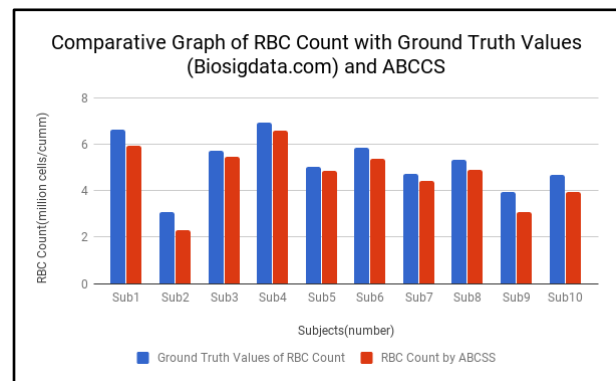


Figure 14 Graph comparing RBC Count with Ground truth values and ABCCS

Similarly, a comparative graph between the WBC count results of ABCCS method and the ground truth values of the biosigdata.com of randomly selected ten subjects is shown in the Figure 15. An accuracy of 89% was achieved by the ABCCS method in the case of WBC count on an average.

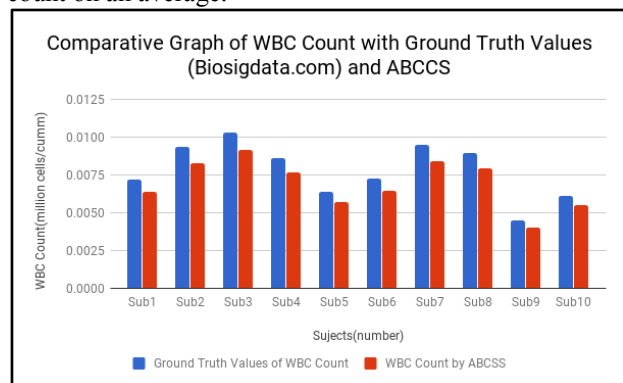


Figure 15 Comparative Graph of WBC Count with ground truth values and ABCCS

A web module is also developed for the user to enter his/her details and the microscopic image input. The calculated value is also shown and based on the normal count of blood cells, the system also gives us a feedback whether the given person is anaemic depending upon the value we get from the image. A screenshot of the output is shown in Figure 16.

Figure 16 Web Module for Online users

V. CONCLUSIONS AND FUTURE WORK

The ABCCS method solves the problem of counting RBC with an overall accuracy of 91% and WBC with an accuracy of 85%. By using the proposed technique in image processing, analysis of blood cell image is more accurate as well as this method is efficient in terms of time and cost compared to existing techniques of blood cell analysis. The proposed method performed the segmentation and counting of RBCs and WBCs well when results were compared with the ground truth (biosigdata.com), which was determined by experts.

This system could further be extended for counting platelets and using complex functions to find the different kind of WBC present in the smeared image and hence improving the accuracy of the proposed method.

REFERENCES

- [1]. H. Berge, D. Taylor, S. Krishnan, and T. S. Douglas, "Improved red blood cell counting in thin blood smears," in Proceedings of the 8th IEEE International Symposium on Biomedical Imaging: From Nano to Macro (ISBI '11), pp. 204–207, Chicago, Ill, USA, April 2011.
- [2]. S. Khan, A. Khan, and A. Naseem, "An accurate and cost effective approach to blood cell count," International Journal of Computer Applications, vol. 50, no. 1, pp. 18–24, 2012.
- [3]. N. Nguyen, A. Duong, and H. Vu, "Cell splitting with high degree of overlapping in peripheral blood smear," International Journal of Computer Theory and Engineering, vol. 3, no. 3, pp. 473–478, 2011.
- [4]. S.-H. Chiu, J.-J. Liaw, and K.-H. Lin, "A fast randomized Hough transform for circle/circular arc recognition," International Journal of Pattern Recognition and Artificial Intelligence, vol. 24, no. 3, pp. 457–474, 2010.
- [5]. N. H. Mahmood and M. A. Mansor, "Red blood cells estimation using Hough transform technique," Signal & Image Processing, vol. 3, no. 2, pp. 53–64, 2012.
- [6]. Miss. Madhuri G. Bhamare and Prof. D.S.Patil, "Automatic Blood Cell Analysis By Using Digital Image Processing: A Preliminary Study," International Journal of Engineering Research & Technology (IJERT), ISSN: 2278-0181, vol. 2 Issue 9, September - 2013.
- [7]. S.Sridhar, "Digital Image Processing", Oxford University Press, 2011.
- [8]. Rafael C.Gonzalez and Richard E.Woods, "Digital Image Processing", Third Edition, Pearson Education, 2009.
- [9]. Thejashwini M and M C Padma, "Counting of RBC's and WBC's Using Image Processing Technique", International Journal on Recent and Innovation Trends in Computing and Communication, vol. 3, no. 5, pp.2948 – 2953, 2015.