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Automated Blood Cell Counting and Classification Using Image Processing

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ABSTRACT: In this paper, we are proposing a method in which we incorporate the image analysis & classification algorithm with the manual counting method of blood cell which gives results comparable with the very sophisticated automated blood cell counters.Image acquiringis done with the help ofmanual blood counting slide, USB compatible microscope and store it in the system or a computer for further processing. The stored image is processed through a software to enhance the quality of the image for accurate output data, which we compare with our known values and give an efficiency of the acquired output. We have used MATLAB [1] and Scilab [2] as the image processing software.

KEYWORDS: Hemocytometer, Image Pre-processing, Image segmentation, Feature Extraction, Erythrocytes, Leukocytes, Thrombocytes.

I.INTRODUCTION

In the human body, the bloodis a composite of plasma, Red Blood Corpuscles (RBCs), White Blood Corpuscles (WBCs)& platelets. Plasma contributes to 54.3%, RBC contributes to 45% and the rest is <1% i.e. WBC's and platelets [3].The three types of peripheral blood cells namely Erythrocytes (RBC), Leukocytes (WBC) and Thrombocytes (platelets)[4].

Blood cell has a different morphological characteristic as given below:
Erythrocytes (Red blood cell): They are the most abundant in blood with a diameter of 6 - 8µm and a thickness of 2µm. It is biconcave in shape and their cytoplasm is rich in hemoglobindue to which its red in color [5].
Leukocytes (White Blood cell): They are the first fighting mechanism of the body from a fungal infection, bacteria, attacking parasites & releases chemical histamine for allergic and antigen response. It varies from 8 µm to 30 µm.
Thrombocytes (Platelets): They are small cell fragment without a nucleus, have a diameter of 2-3µm and it helps in clothing injuries.

In themedicalfield, the blood cell count plays a vital role to determine humanhealth condition. If there is a variation of the blood cells, it can cause anemia to the human body so the blood count should fall in that range. The blood count plays an important role in the field of medical and education sectors. In medical it is used to study about new diseases by which how the blood cell vary and change its characteristic and research and invention of new biomedical machinery for understanding the change of the blood cell shape & structure and in education it is used for the student to educate about the blood cell in various streams related to medical. For male and female, the count differs as shown in the table below [6]:

Blood cell types	Gender	
	Male	Women
RBC	4.5 – 6.0million/microliter	4.0 – 5.0 million/microliter
WBC	4.5 – 11 thousand/microliter	4.5 – 11 thousand/microliter
Platelets	150 – 450 thousand/microliter	150-450 thousand/microliter

Table 1 Normal CBC counts

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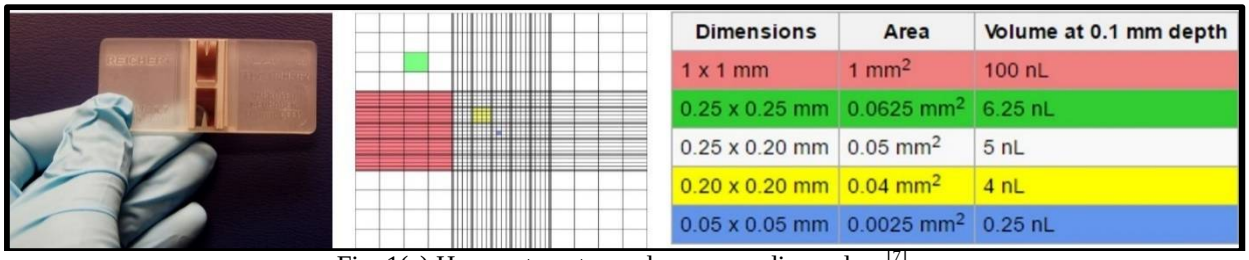
Thecount of WBCs, RBCs and Platelet is known as complete blood count. There are mainly two methods of blood counting.Earlier there was manual counting method, which was time-consuming, and the only method available. We need a hemocytometer, diluting fluid, pipette tube and a simple microscope.In this method, blood was drawn from thepatient and mixed with diluting fluid andwith the help of a pipette tube the blood is slowly poured onto hemocytometer. It consists of a thick glass microscope slide with a rectangular indentation that creates a chamber. The device is carefully crafted so that the area bounded by the lines is known, and the depth of the chamber is also known so it is possible to count the number of cells.Manual counting is useful in cases where automated analyzers cannot reliably count abnormal cells, such as those cells that are not present in normal patients and are only seen in peripheral blood with certain hematological conditions. Manual counting is subject to sampling error because so few cells are counted compared with automated analysis. A manual count will also give information about other cells that are not normally present in peripheral blood but may be released in certain disease processes.The Fig. 1(a) show hemocytometer and corresponding values.



Dimensions	Area	Volume at 0.1 mm depth
1 x 1 mm	1 mm^2	100 nL
0.25 x 0.25 mm	0.0625 mm^2	6.25 nL
0.25 x 0.20 mm	0.05 mm^2	5 nL
0.20 x 0.20 mm	0.04 mm^2	4 nL
0.05 x 0.05 mm	0.0025 mm^2	0.25 nL

Fig. 1(a) Hemocytometer and corresponding values[7]

Nowadays there are automated counters, which arehighly accurate and reliable but costly. It involves passing a dilute solution of the blood through an aperture across which an electrical current is flowing. The passage of cells through the current changes the impedance between the terminals. The Fig. 2(b) show the working principle of automated counters.

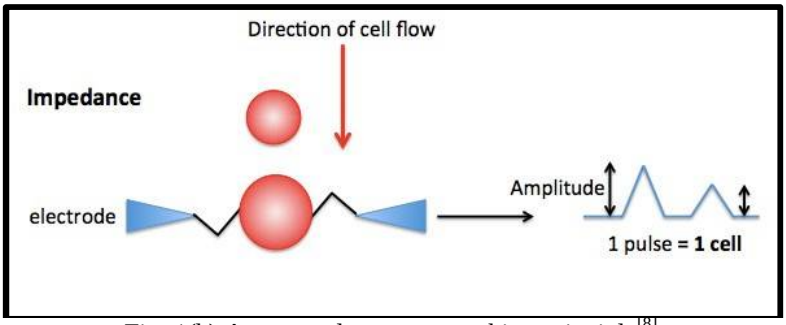


Fig. 1(b) Automated counters working principle[8]

Automated counters are easy to use, and they greatly reduce the amount of human effort required to count cells. They are both precise and reliable, but may have difficulty obtaining accurate measurements of cells that are highly irregularly shaped, are extremely small, or are in cell suspensions that are extremely dilute or contain a large variety of cells that need to be distinguished, as it cannot differentiate between dust particles with thesame characteristic as blood cell.

As stated, both these techniques are still used for the educational and medical purpose.

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II.METHODOLOGY

In this paper, there is a re-performingof the manual method with the help of open source simulation software Scilab to process the image and give thecount of the blood cells and we also use a proprietary image processing software know as MATLAB to do the same process as in Scilab. Hemocytometer (counting chambers that hold a specified volume of diluted blood and divide it with grid lines) are used to calculate the number of blood cells per unit liter of blood. The blood is mixed with adiluent and with the help of pipette tube, blood is placedin the counting chamber.By manually counting the cells in the grid and using a formula show below. In the formula the number of cells counted are the cells counted manually in its area of visibility and the dilution means how many times it has been diluted and the productsaredivided by the volume of blood poured on the hemocytometer, this method canalso give the complete body blood cell count. In this paper, a simple hemocytometer and a microscope with inbuilt USB port are used. The captured imageswere stored in a system or computer so that we could process through our software to give a count and then compared with the blood reports.We have asimple protocol which reduces most of pre-processing like blood cell sorting[9-13] and staining of cells[14-16].

$$\text{No of blood cell per } (\mu l) = \frac{\text{No of cells counted x Dilution}}{\text{Volume of fluid}}$$ [18]

We are using simulation software namely MATLAB & Scilab to process the image by which we remove the unwanted data from the image give the count of the blood cell namely RBC, WBC& Platelets. In this paper, we are using analgorithm based on image processing comprising of six stages as shown below:

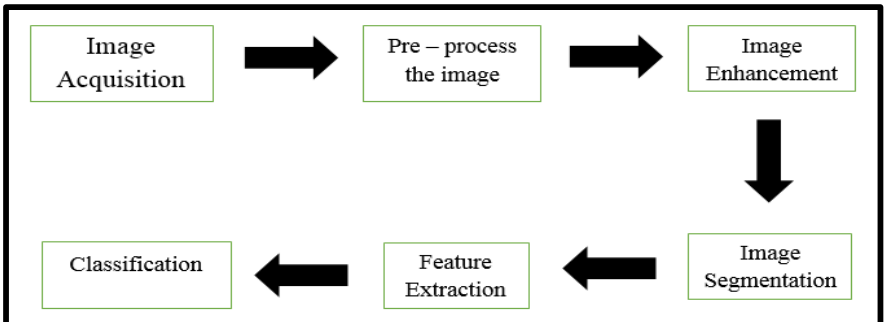

Fig. 2 Algorithm of Image processing

Step 1: Images were acquired using microscope and staining was done with field stain A and B.Staining is done on the image so that it would be convened for visuallyanalyzing the different blood cell. The blood was diluted 20 times; this is done to separate the cells from one another so that we are able to acquire a proper image for our process. This is our input image as shown in Fig. 3 below.

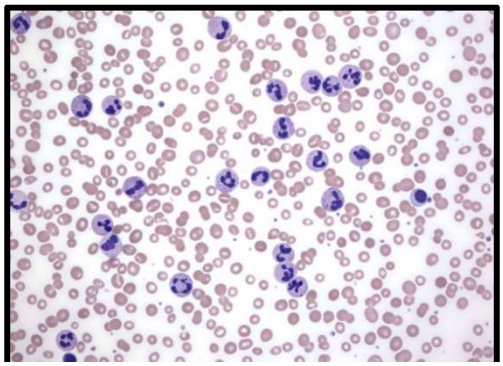

Fig. 3 Input Image

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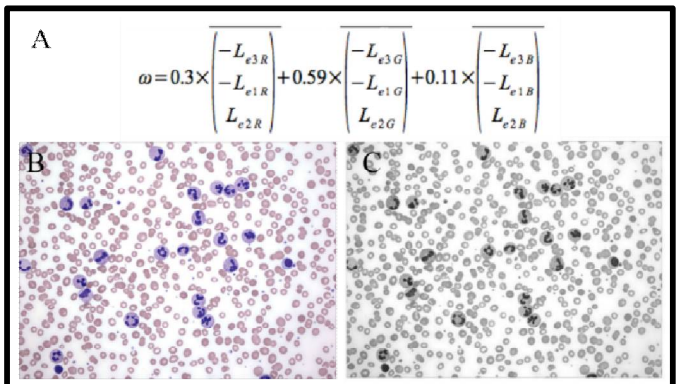

Fig. 4 RGB to grayscale image

Step 2: Image pre-processing step. In this step, the imagegets converted from RGB to grayscale using theformula given in Fig. 4 (A) and sample input (fig. 4 (B)) and output (fig. 4 (C)) images are shown above. In grayscale, each pixel is stored as a byte and each pixel value varies from 0 (black) to 255 (white). Therefore, every pixel has a different intensity information. Fig. 4 (A) Formula for conversion from RGB to Grayscale. (B) Original Input Image. (C) Grayscale converted image.

Pre-processing also involves removal of noise using amedian filter (also known as rank filtering). In this, the pixels are ranked and the output pixel is determined by the median of the neighborhood pixel or the mean and it is applied to every pixel, as it filtering does not affect the sharpness of the image. Fig. 5 shows method of calculating median and snippet shows the results of median filtering. Median filtering is done in order to avoid salt and paper noise, which is generally a problem with CCD cameras.

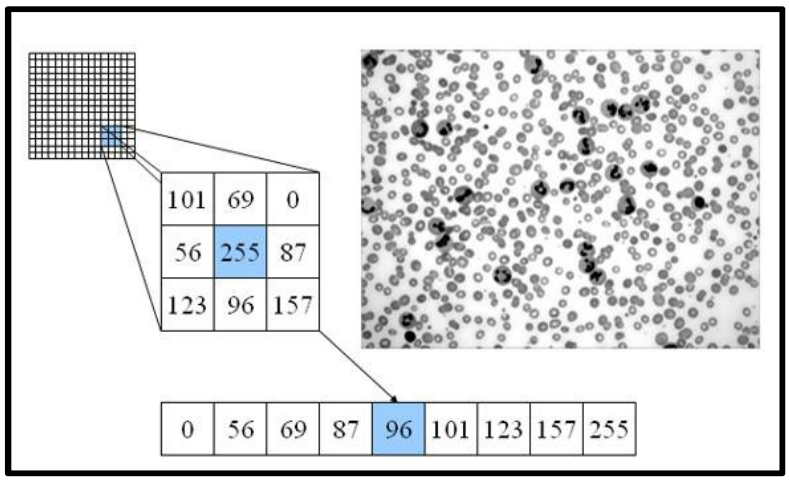

Fig. 5 Matrix show the method of calculating themedian value. Snippet shows median filtered image.

Step 3: Image enhancement involves histogram stretching which is known to increase the dynamic range. In this method, the basic shape of the histogram is not altered, but we spread it so as to cover the entire dynamic range. Fig. 6 shows histogram before and after stretching and the enhance image. In the below image the grey level of the original image is 84 minimum and 153 maximum. After applying histogram, stretching the grey level range has occupied the entire dynamic range as shown in sketched image 0 minimum to 255 maximum.

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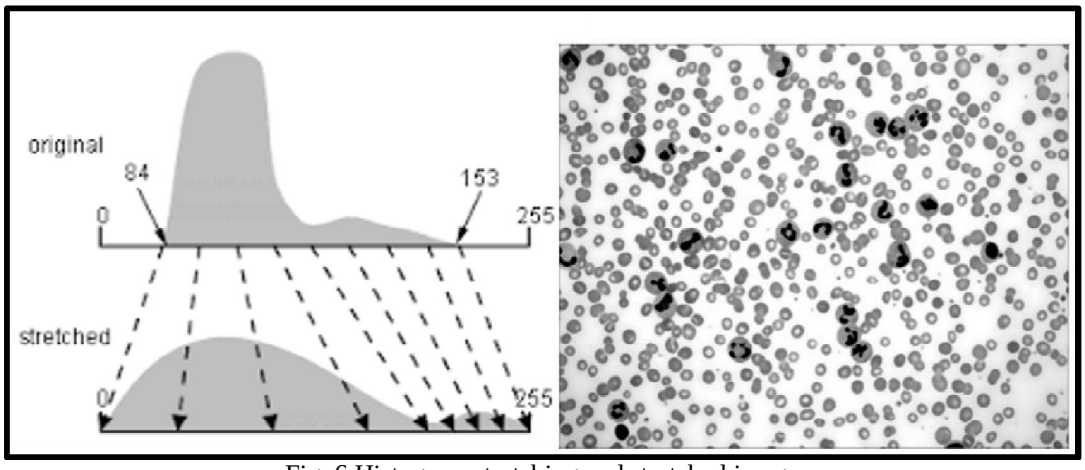

Fig. 6 Histogram stretching and stretched image

Step 4: After enhancing theimage, the next step is segmenting the object as per their size and shape for generating ageneralized algorithm to give CBC counts. For this purpose, three different algorithms were tried namely Canny, LoG and Sobel as shown in the fig. 7. This algorithm is known as edge detection, which is a very important part of image segmentation. An edge detection helps in finding pixels that form a boundary between two distinct regions. For the blood cell to clearly bevisible,all the blood cell images were tested with thedifferent algorithm by varying the threshold. So we have decided on visual evidence Canny [17]is the best-suited filter. In it, the image is first smoothened using a Gaussian low pass filter and then take the first derivative. The algorithm of thecanny filter is as given in the fig. 8.

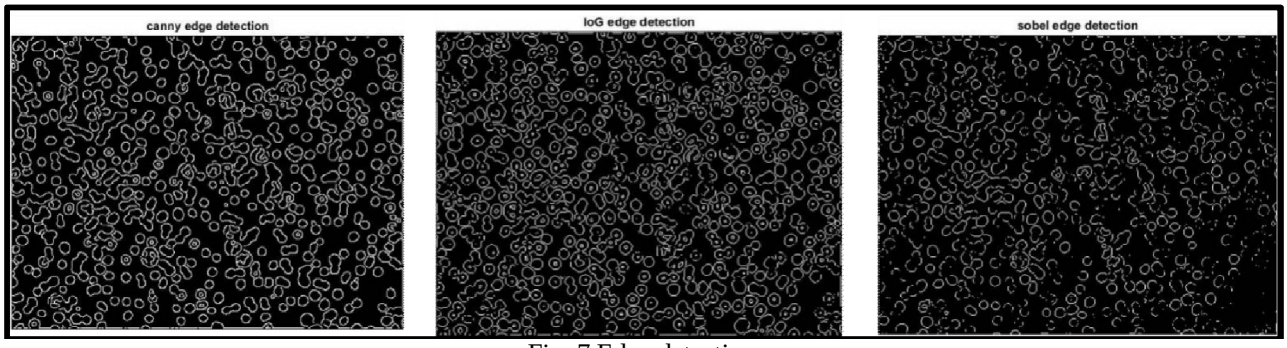

Fig. 7 Edge detection

The second part of segmentation algorithm involves segmentation using Otsu`s algorithm as shown in figure 8. The image processing software automatically performs the thresholding by selecting an optimized threshold value.In this algorithm the image, consist of two sets of pixels namely foreground pixel that relates to the object and background pixel that relates to the background, it then calculates an optimal threshold, which separates the two sets of pixels to minimize the intraclass variance of the threshold as shown below.

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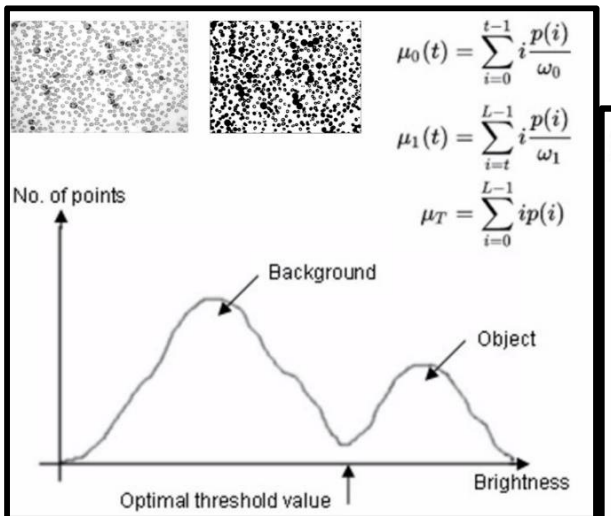

Fig. 8 Otsu's algorithm for thresholding

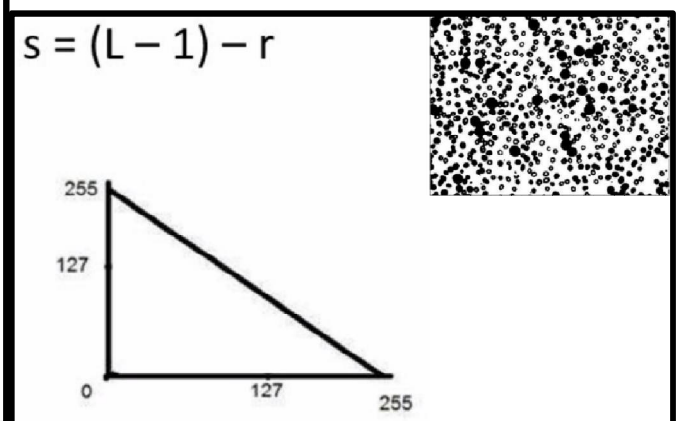

Fig. 9 Negative operation

Since our object was darker from the background, we needed to carry out image negative operation as shown in fig. 10. Figure 10 shows input image after thresholding.

Finally, for segmentation, we logically ORed the results from Canny and thresholding. Fig. 11 shows input to OR operation. In this operation, both the image pixels are compared and the higher value pixel or pixel with value 1 is considered and with only edge detection every data is not possible to acquire so we also take Otsu's thresholding data and OR both operation so that every pixel or information is acquired.

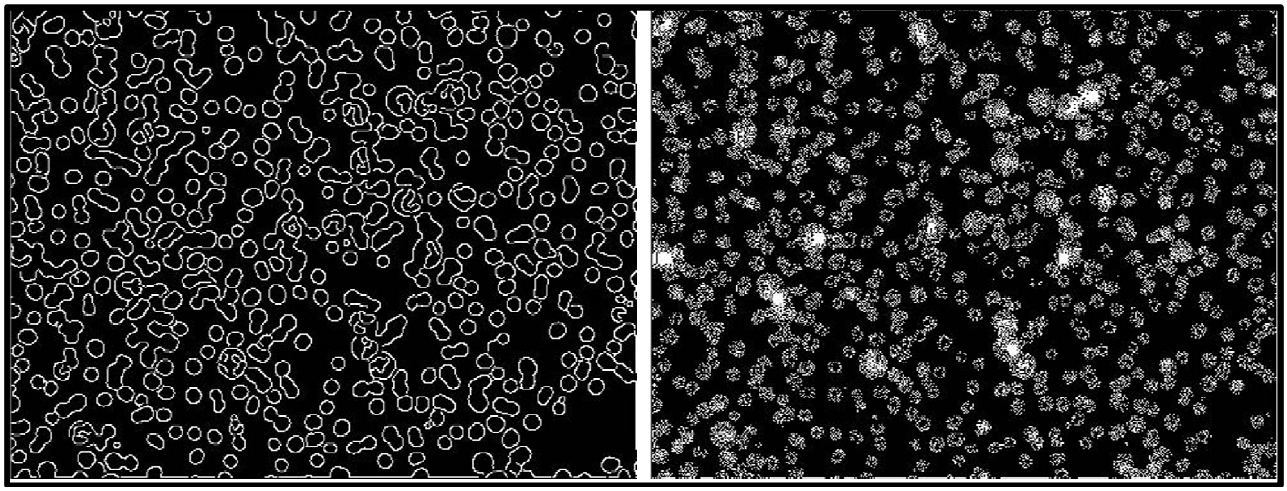

Fig. 11 OR operation

Step 5: Image features were extracted from thebinary image using four connected components. The main properties that were derived from the image processing software are Area, Bounding Box, and centroid. As area defines the actual number of pixel in the region, thebounding box is the smallest rectanglecontaining region and centroid specifies the center of the mass of the region. With the help of all these features, we are able to proceed to the next step that is the classification.

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Step 6: Classification was done on the blood cell using basic properties of blood cells. The cells were divided into three types namely RBCs, WBCs and Platelets. After classification, counts were generated and cross verified with the standard Sysmex automated counter systems.

III.RESULTS AND DISCUSSIONS

CCD camera was used acquired the images for our image processing software. It was found that any variation in luminance and any vibration of stage adversely affect the system performance. We took around 2ml of blood on to an EDTA tube which prevents the blood cell from rouleaux formation and platelet activation since we needed the isolated healthy cells in theimage.We took 10µl of blood and mix it with 200µl saline hence 20 times dilution was achieved. This was done in order to separate cells from each other. 20 µl of thediluted blood sample is poured on to simple hemocytometer. It was carefully placed under a microscope which has an inbuilt camera so the image can be stored onto a system. Here we use SISMAX 800i to count cells for verification. We conducted above-mentioned study 30 times with 20 different blood samples and results are given in the table below:

RBCs		WBCs		Platelets	
Report count	**Algorithm count**	**Report count**	**Algorithm count**	**Report count**	**Algorithm count**
Per µL in 10^6	**Per µL in 10^6**	**Per µL in 10^3**	**Per µL in 10^3**	**Per µL in 10^3**	**Per µL in 10^3**
2.15	2.1	6.77	9.3	234	212
2.15	2.27	6.77	0.6	234	301
1.44	1.42	5.07	5.1	498	490
1.44	1.47	5.07	5.1	498	205
1.27	1.25	5.1	3.6	70.8	59.4
1.27	1.27	5.1	2.6	70.8	59.4
4.2	4.43	10.4	15	892	808
4.2	4.52	10.4	19.8	892	938
3.09	3.05	11.4	10.2	656	742
3.09	3	11.4	10.8	656	547
3.42	3.18	9.4	9.3	698	718
3.42	3.48	9.4	8.4	698	515
1.17	1.15	4.7	7.2	271	194
1.17	1.19	4.7	4.8	271	191
0.91	0.84	2.3	1.8	102	82.8
0.91	0.864	2.3	0	102	113
0.63	0.638	4.04	3.9	76.1	46.8
0.63	0.636	4.04	0.6	76.1	171
0.29	0.264	5.07	4.8	64.6	45
0.29	0.314	5.07	0.3	64.6	18
Accuracy of RBCs	99.74%	Accuracy of WBCs	86.08%	Accuracy of PLTs	90.66%

The bar graphs shown below are the computed results of our algorithm with reports generated from automated counter.

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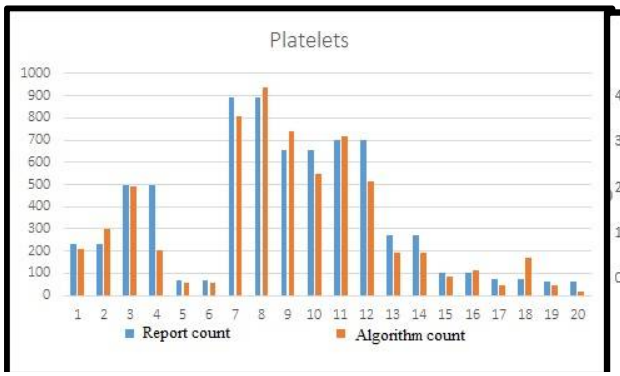

Fig. 12 Platelet results

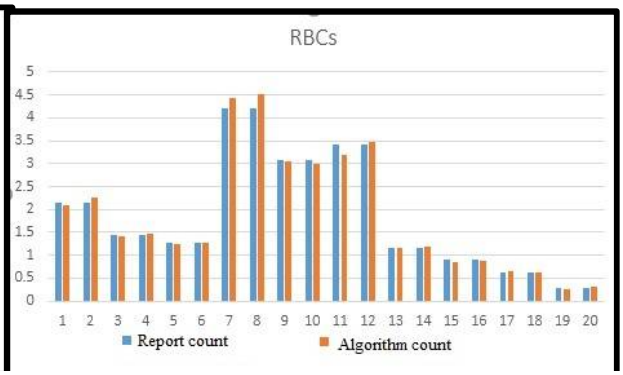

Fig. 13 RBC results

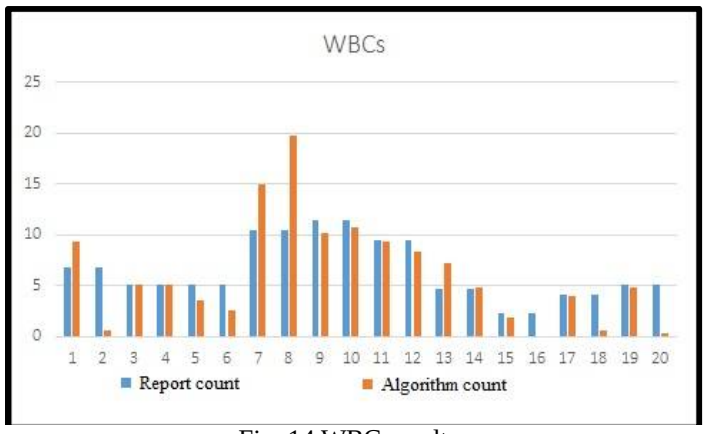

Fig. 14 WBC results

IV.APPLICATION & FUTURE SCOPE

The main area where it would be applicable is in themedical field for research of change of cell structure during different vaccination and when different infection which affect the human body and it can be also used for identifying different blood cells in the blood like eosinophil andbasophil.It can also be used for education purpose for comparing the counts with manual counting andwill have a better understanding of the cell structure. It can be incorporate for counting animal blood cell as cell characteristic changes for different type of animal[19] like arthropods and mollusks as a future scope.

V.CONCLUSIONS

It is found that the automated algorithm is agood system in the point-of-care scenarios where expert pathologist cannot reach all the time. Our software results were matching closely with the reports generated from the system. The algorithmhas achieved automated platelet, RBC and WBC counting with 90%, 99%, and 86% accuracy respectively. The accuracy of the overall system was found out to be 85% with 30 different images from 20 different blood samples.

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