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Automated Cell Quantification of Blood Smears Using Digital Image Processing.

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ABSTRACT

To quantify the cells present in the sample blood smear and to find the irregularities in cell count and, thereby, deducing the disease. Our method of Cell quantification involves the counting of cells in an automated way using LabVIEW software. We have formulated an algorithm in order to reduce the time needed to process the image. And this method also aims to find out the irregularities in blood cell count or irregularities in their shape. Here the task is to detect immature cell using different image processing techniques and count total number of cells. So we need to use the technology that identifies different types of blood cells within short duration of time in times of emergency. Furthermore it is vital to study in detail how to differentiate different cell types and recognize it as an immature cell or mature cell and according to it, detect the disease. For this there are a series of steps involved:

1. Image capture
2. Image enhancement
3. Image segmentation
4. Feature extraction

A blood smear is a blood test used to look for abnormalities in blood cells. The test provides information on the number and shape of these cells, which can help doctors diagnose certain blood disorders or other medical conditions. Irregularities in the number of cells present per unit or irregularities in their shape are indicative of certain type of diseases. Many laboratories still use traditional method of counting - a tedious, expensive process prone to inter-observer variability. The manual counting process can be automated for fast and precise data gathering and reduced gross errors and speedy diagnosis. We present a method to segment and quantify the cells using image analysis techniques. Automated counting is more accurate and are faster than manual counting for multiple-color stained cells, especially when large numbers of cells need to be quantified (>500 cells). The problems that generally occur in traditional analysis of cell images such as: uneven background, overlapping cell images and cell detection with multiple stains can be reduced by a great quantity by this method. This method can be used in laboratories to save time, effort and cost.

Keywords: Automation, Blood disease detection, Blood smear, Cancer, Cell count, Diagnosis, Digital Image Processing, Histogram equalization, Thresholding, Quantification.

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INTRODUCTION

Common diseases like Malaria, Typhoid and Jaundice are marked by irregularities the shape of the blood. In the case of blood cancer- Leukemia and Lymphoma, the disease is characterized by the irregularity in the number of cells. The traditional blood test aims to find these irregularities or to analyze the components of the blood. The mentioned traditional method of diagnosis generally consists of series of various tests which includes a regular blood smear test for which the results may take a few hours to be conclusive. We aim to reduce the disadvantage of this traditional method by adapting Digital image processing of the blood cultures..

METHODS

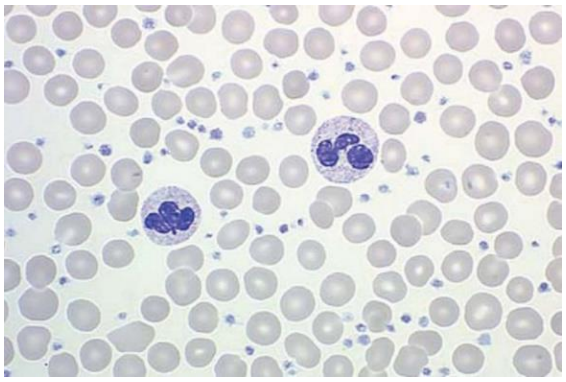
Quantification of RBC, WBC and platelets:

Pre- Processing:

Preprocessing images commonly involves removing low-frequency background noise, normalizing the intensity of the individual particles in the images, removing reflections, and masking. It is the process of enhancing data images prior to computational processing.

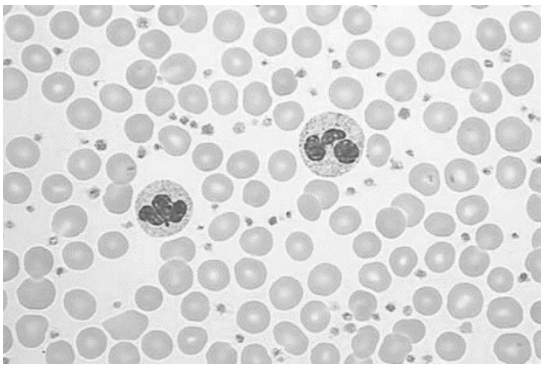
The image of the Blood smear was first obtained (See fig. 1A), and it was converted to a grey scale image from the RGB scale.

Fig 1A: Original Image



This was done in order to make the processing easier and more defined. The process was carried out using IMAQ create, IMAQ read and IMAQ write function.

Fig 1B: Grey scale image



The resultant image was then given to linear contrast operator in order to enhance the quality of the image and parameters were set in accordance with the image.

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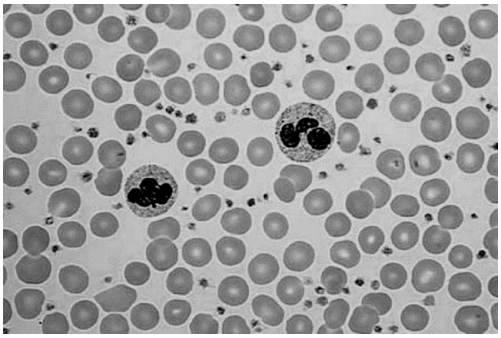
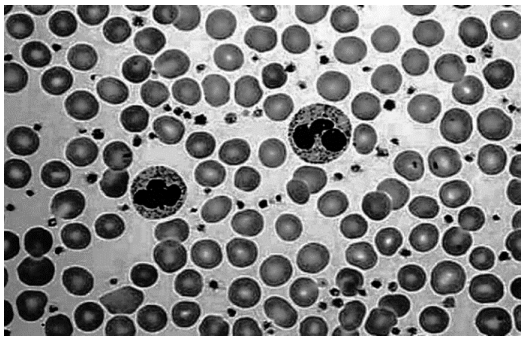
Fig 1C: Contrast enhancement



This image was further altered by using a histogram equalization operator. **Histogram equalization** is one of the methods for enhancing the contrast of images. In this method, the histogram of the original image is transformed by using its normalized cumulative sum. Then the intensity values of the original image are mapped to new intensity to give a uniform histogram of intensity values.

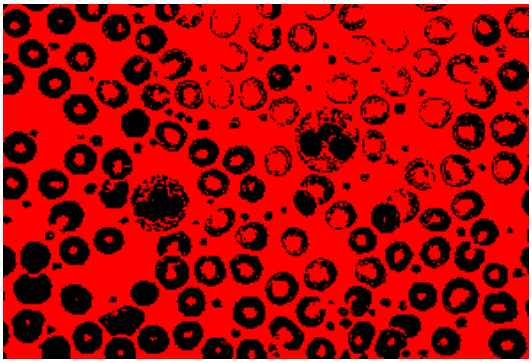
Fig 1D: Histogram equalization



Processing and methodology

After the preprocessing, the image is then subjected to a series of image processing techniques. They are Thresholding, removal of particles, removal of boundary and filling holes, edge detection, and computing the number of cells in the image, based on the circumference/radius of the required cell taken into consideration. The Image thresholding is a simple, yet effective, way of partitioning an image into a foreground and background. This image analysis technique is a type of image segmentation that isolates objects by converting grayscale images into binary images. Image thresholding is most effective in images with high levels of contrast. It is the simplest method of image segmentation. From a grayscale image, thresholding is used to create binary images

Fig 2A: Thresholding



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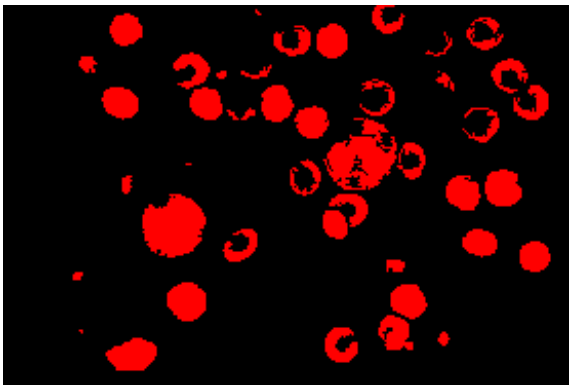
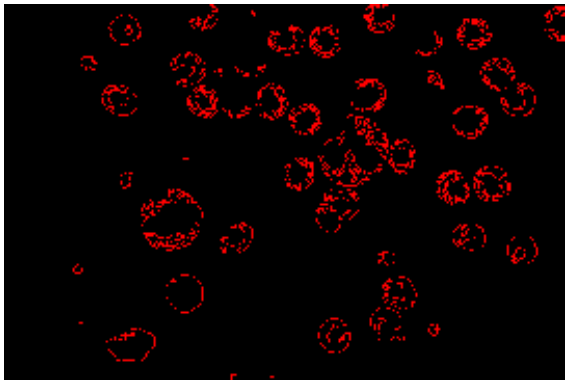
After thresholding the image is further processed, so as to remove the boundary and fill in the holes, so that the final image comprises of the cells alone without any noises or disturbances.

Fig 2B: Boundary removal and hole filling



This operation is then followed by edge detection of the cell. Edge detection is an image processing technique for finding the boundaries of objects within images. It works by detecting discontinuities in brightness. The Edge detection operator used in this paper is the differentiation operator. The operator has to be changed depending upon the image characteristics.

Fig 2C: Edge detection



By employing a function to count the number of cells in the given area the number of RBC are found. Then the threshold value is increased to isolate the WBC cells from the RBC's. This was extended in order to count the platelets also.

Fig 2D-1: Isolating the WBC from RBC

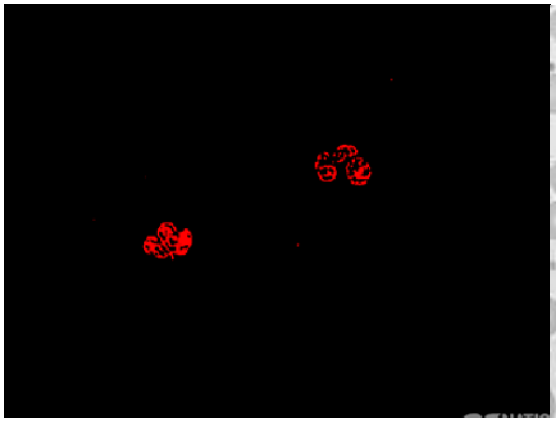
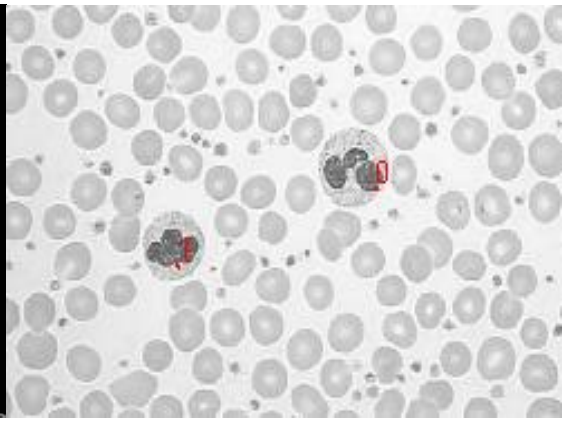


Fig 2D-2: Count of WBC



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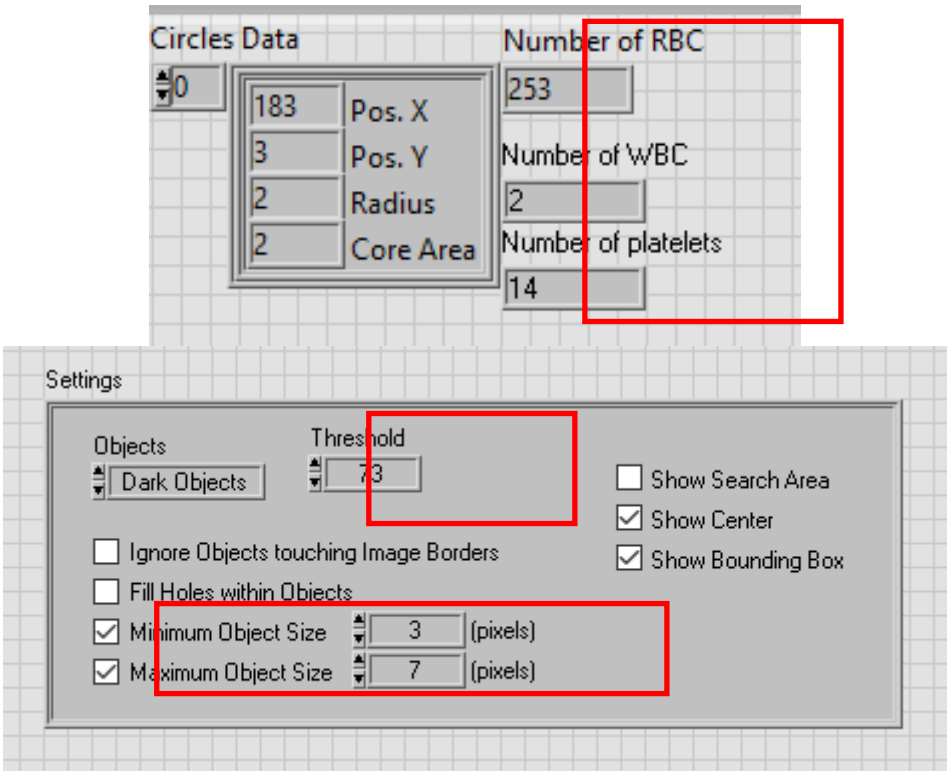
Then the Number of RBC, WBC and platelets in the given sample are counted separately and their count is obtained.

Fig 2E: Quantification Data



The above image shows the number of circles counted from the edge detected image..Hence this gives us the number of cells in the image and the cells were quantified.

The normal ratio of RBC and WBC in a human is given below

Table 1: Table showing the average no. of RBC and WBC in a normal human being:

	Red Cells per microlitre (µL) of blood	White Cells per microlitre (µL) of blood
Men	4.7 to 6.1 million	5,000 to 10,000
Women[2]	4.2 to 5.4 million	4,500 to 11,000
Children[3]	4.0 to 5.5 million	5,000 to 10,000

The ratio of white blood cell in our body is 1000:1. So if number of white blood cells increase remarkably in large number then the person is succumbed to suffer from the leukemia. It further falls into two type: acute and chronic. With respect to the white blood count, some physicians may choose to treat if the count is doubling rapidly, and at times just on the basis of the high white blood count (definitions of high differ greater than 100,000, 200,000, or250,000).

Detection of Malarial parasite in Blood smear

Another quantification methodology which is applied for detection of malarial parasite in a blood smear is as follows.

Malaria is caused by the presence of Plasmodium falciparum parasite. This parasite affects the red blood cells. Generally when RBC's in the human body mature, they lose their nucleus whereas the malaria affected RBC's contain nuclei. This is the key identification on which the below algorithm is based upon. Hence

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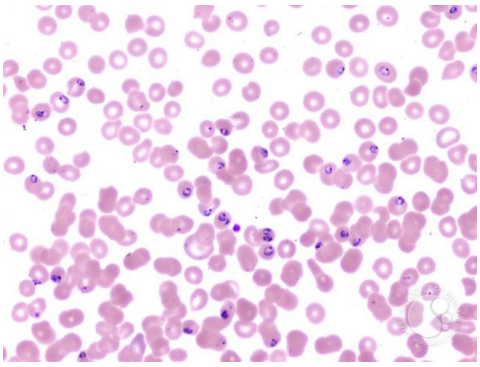
a blood test is done in order to identify the infected RBC's Initially the blood smear is stained with a purple dye.

Pre-processing

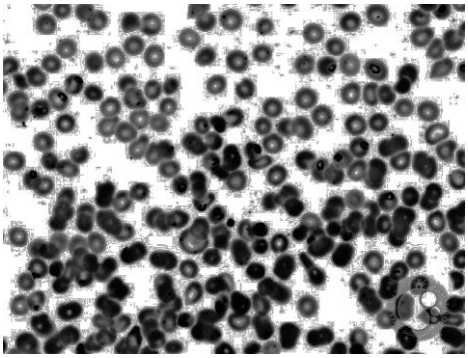
Fig 2F: Original image



Courtesy: Image bank, American society of Hematology

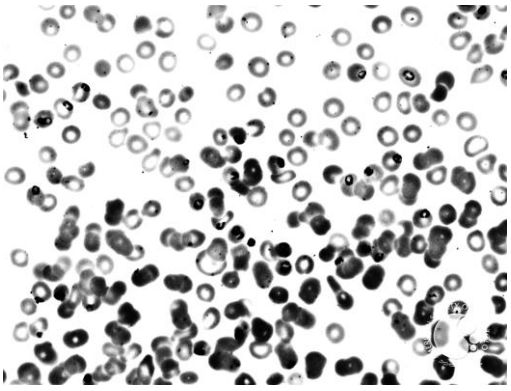
This is the blood smear which has the purple stained malarial parasite.

Fig 2G: Value component



The color planes of the sample is extracted, HSV (Hue, Saturation, Value).This is performed by using the Extract color planes tool. From the extracted planes only the value component is considered since it gives us a clearer view of the dark and bright areas in the blood smear. The image is then equalized and filtered to remove noise, blurring and poorly lit parts of the image.

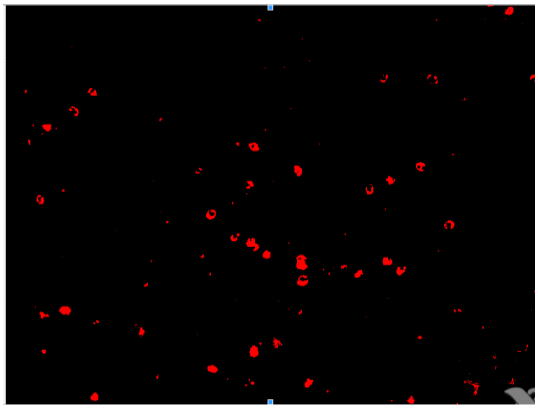
Fig 2H: Equalised Image



After equalizing, thresholding is done to separate the purple stained malarial parasites. Since the parasite infected areas of the blood smear is at a higher intensity level when compared to the rest of the smear, this allows it to be separated at a particular threshold value as shown below

Processing and methodology

Fig 2I: Threshold- Isolation of parasites



By adjusting the thresholding limit and several other parameters the parasite infected RBCs are isolated andby specifying the radius of the cell the number of malarial parasite infected RBCs can be counted.

Fig 2J: Count of parasites

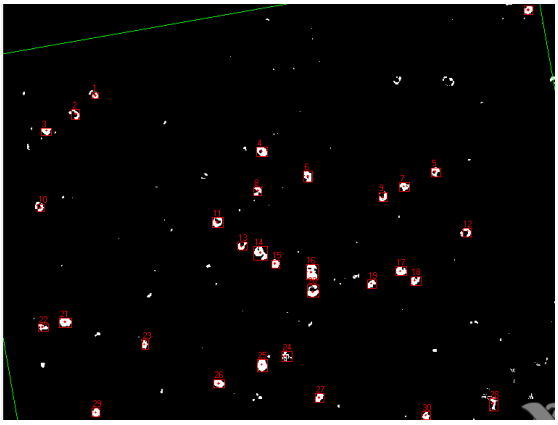


Fig 2K: Quantification of Parasite affected cells

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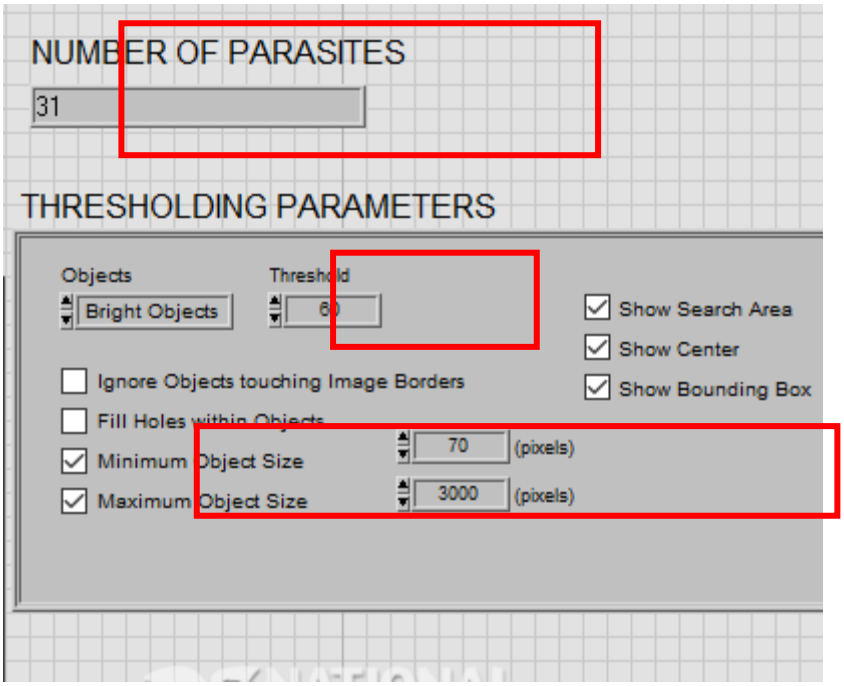

Parasitemia

Parasitemia is the presence of parasites in the blood. The parasite count indicates the severity of the disease. Hence 4%-5% of parasitemia or more is indicative of severe malarial infection. In this particular algorithm, the parasite infected cells is weighed against the total number of RBC's present in the blood smear sample. The number of RBC's are counted by using the previously mentioned algorithm.

(No of parasite infected RBC/Total no of RBC)*100
Percentage value >5% (malarial infection)
Diagonised result from algorithm
(31/347)*100 = 8.93%

Hence the parasitemia is higher than the cut-off value which is indicative of the severity of the malarial infection.

RESULTS AND DISCUSSION

By finding the count of RBC, WBC and Platelets, various diseases like Leukemia, lymphoma, anemia etc., can be diagnosed, while the parasite count (parasitemia) is helpful in determining the load of parasites in the blood sample. For example the ratio of RBC to WBC is 600:1, if the count of WBC exceeds this ratio, it may an indication of any infection and if it varies by a large ratio it maybe an indication of Leukemia. . To detect different types of geometrical shape of cells like basophils, eosinophil, lymphocytes, monocytes etc. shape based features are used and accordingly by counting immature cells, diseases can be diagnosed. This project can be in collaboration with a diagnostician in order to diagnose the diseases correctly.

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Fig 3A: Front panel of quantification of cells

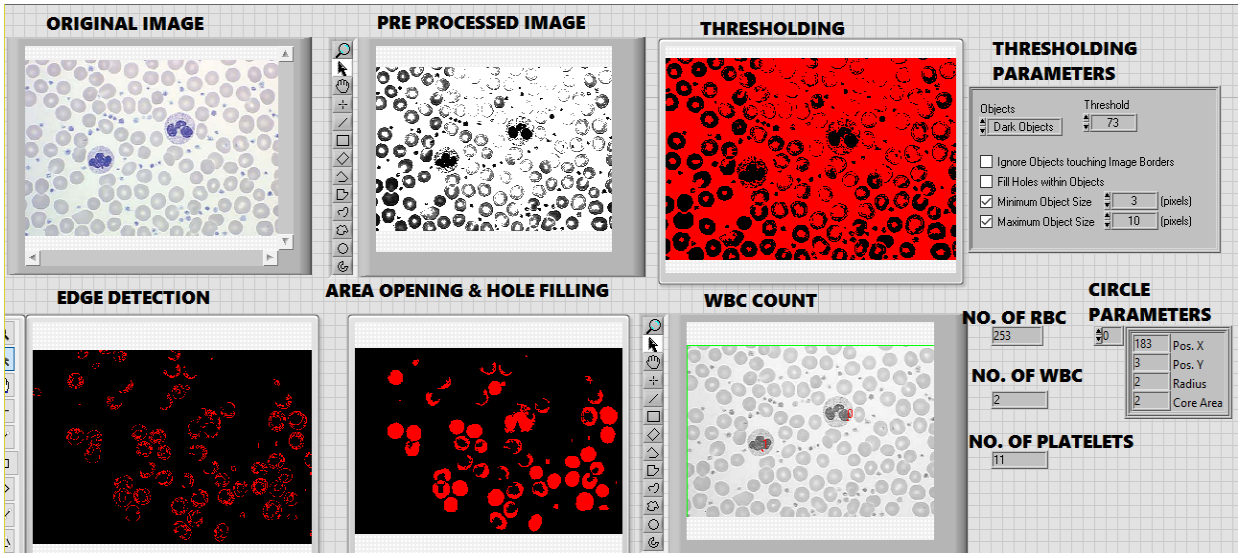
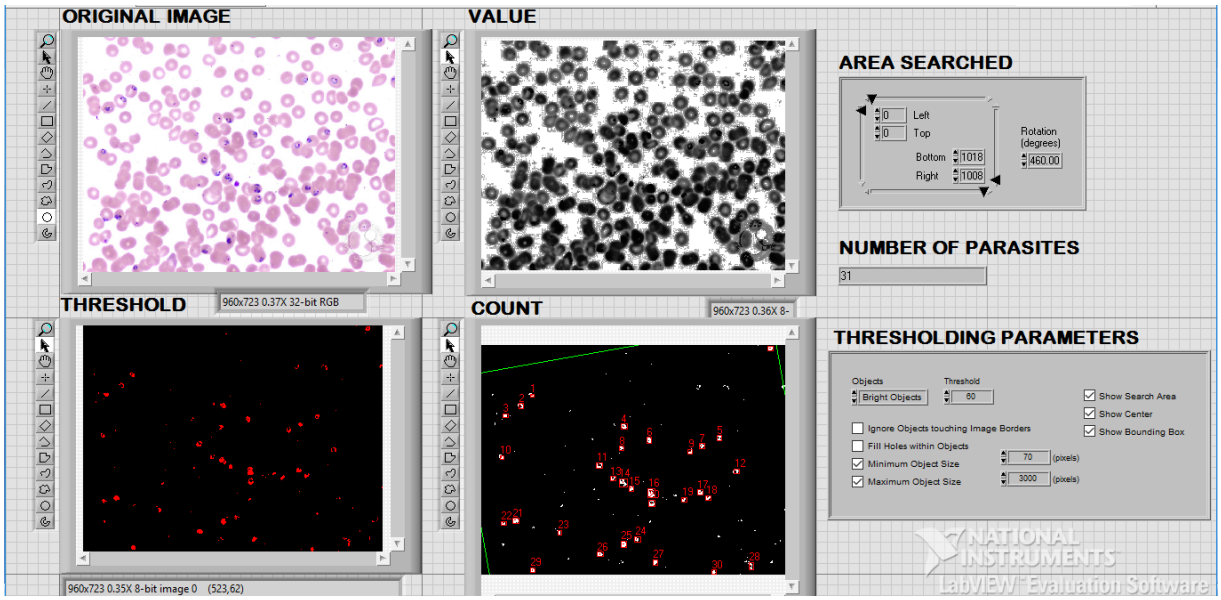


Fig 3B: Front panel of malarial parasite detection



CONCLUSION

Traditional methods of blood smear analysis was researched and found to be labor intensive. In order to automate it, algorithms have been formulated for the identification of irregularities in blood cells and quantification of blood cells in smears and the required infected cells were recognized. Although the methodologies exhibit basic functionality, further work is necessary in order to improve accuracy.

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