Determine count of Red Blood Cells, White Blood Cells and Platelets from the image of a peripheral blood smear

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***Abstract*— A blood test recommended by any physician that includes quantification or counting of Red Blood Cells, White Blood Cells and Platelets in the body can provide useful insights related to a patient's heath. The manual counting of these blood cells is a slow and tedious task and the accuracy of such tests totally depend on the expertise of the lab technicians. Hence, we have developed a digital image processing technique in MATLAB that is capable of counting the Red Blood Cells (RBCs), White Blood Cells (WBCs) and Platelets respectively from a colored image of a peripheral blood smear/ film (PBF). And on the basis of the count of these blood cells we can determine some sort of health risks.**

Keywords*—Peripheral Blood Smear, RBC, WBC, Platelet, Image Segmentation, Preprocessing, Feature Extraction, Edge Detection, Circular Hough Transform.*

I. INTRODUCTION

Peripheral Blood Smear is prepared to find out whether red blood cells, white blood cells and platelets are normal in appearance or number and to distinguish them and to determine their relative percentage in the blood [2]. The normal count of theses blood cells is determined by per microliter of blood. The normal count of these blood components are enlisted below:

Sr. No.	Cell Type	Count per microliter
1.	Red Blood Cell	3.5-5.5 million
2.	White Blood Cell	4000-11000
3.	Platelet	150000-450000

These cell count can reveal a lot about the patient's health. For example: a high RBC count can be due to polycythemia and a low count indicates anemia. Or a high WBC count means that there may be any sort of infection in the body or a low count may be due to malaria or fever. Similarly, low platelet count can be reason of thrombocytopenia and a high count can be a result of injury or bleeding. Deriving this information from blood smear requires a well-prepared and well stained blood smear and some basic skills in the assessment method. Red blood cells (RBCs), also known as erythrocytes, are the most abundant cells in the blood. RBCs give blood its characteristic red color. RBCs constitute about 40 to 45 percent of the cells in blood. Hence, this percentage of RBCs can even be used to measure the percentage of others cells also, keeping the value/ count of RBCs as a reference. The ratio of RBCs, WBCs and Platelets are in such a way that there are 600 Red Blood Cells for 1 White Blood cell and 40 Platelets [7]. Examination of a blood smear is pivotal since quantitation of the different types of cells components can help in diagnosis of certain diseases.

II. METHOD

A. Image Acquisition

The peripheral blood film images or microscopic blood smear images were obtained from JNMC College Sawangi, Wardha. These images are captured in RGB at a magnification of 100x and 400x objective zoom using a camera connected to a microscope.

B. Preprocessing

The image preprocessing involves application of filters in-order to remove noise from a digital image to get accurate results in image segmentation and edge-detection. Digital images are prone to various types of noise. Noise is the result of errors in the image acquisition process that result in pixel values that do not reflect the true intensities of the real scene [6]. We have used Median Filter to extract noise from the input image, reason being that it is most effective in removing 'salt and pepper noise' [8] whilst preserving the edges of the foreground objects. Consider the image below, which involves application of Averaging and Median filter to the same image.

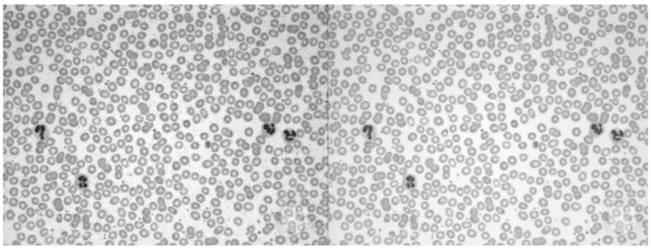

Figure 1: Averaging filter VS Median Filter

The Median filter is a nonlinear digital filtering technique, it is a robust filter. Median filters are widely used as smoothers for image processing, as well as in signal processing and time series processing.

C. Image Segmentation

The Image Segmentation involves two major steps that includes detection of WBC and determine their count using morphological transformations.

WBC Count: WBCs are white in color hence it is difficult to detect their outer region using edge detection. We are detecting WBCs on the basis of their nucleus which is dark blue in color. The adopted method works for all types of WBCs whether it is a Monocyte, Lymphocytes, Neutrophils, Basophiles or Eosinophils. The steps for detecting and determining the count of WBCs are as follows:

Step 1: Subtract the Red and Green channel from the Blue Channel. Multiplication factor of 0.5 for both Blue and Green Channel is used.

Step 2: Remove the pixels that have the value of more than 50.

Step 3: Apply morphological transformation to the resultant image for removing the connected components using **'bwareaopen ()'** function and apply morphological operations i.e. *opening*, to remove noise from the background of binary image and *closing* to fill black holes in the foreground objects.

Step 4: Use **'bwlabel ()'** function to label these resultant binary objects and print their number.

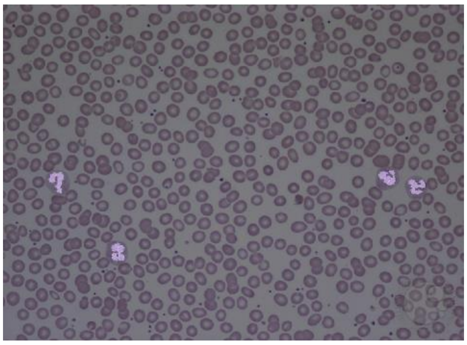

Figure 2. (a): WBC Detection on the basis of their nucleus

Figure 2. (b): WBC nucleus conversion to Black & White

The count of WBC in this case is **4**. We have multiplication factor of 7,500 hence the result is **30,000** which is much larger value than the threshold value of 4,000-11,000. Which means that there is a risk of severe-infection or leukemia in the body.

The multiplication factor is found through following way:

As we mentioned earlier that there are 600 RBCs for every 1 WBC and 40 platelets. The average normal count of RBC is around 4.5 million/ microliter. Hence,

$$\frac{600}{1} = \frac{4500000}{x}$$

Therefore, x = 7500. Similarly we can find the multiplication factor for platelets using the same method.

D. Feature Extraction

The Circular Hough Transform (CHT) processes a set of feature points in the image space into a set of accumulated votes in a parameter space [4]. Then, for each feature point, votes are then gathered in an accumulator array for all parameter combinations. The array elements that contain the highest number of votes indicate the presence of the shape [1]. CHT is a feature extraction techniques used in image analysis to detect the circular shapes in an image.

RBC Count: The RBC count is determined using Circular Hough Transform along with **'imfindcircle ()'** function. We have 5 parameters here, they are:

- Image: The image will be a binary image.
- Object Polarity: Object Polarity is 'dark', since the circular objects are darker than the background.
- Radius Ratio: The radius ratio is [10 -20].
- Sensitivity: **0.82** in this case.
- Method: 'Two Stage'.

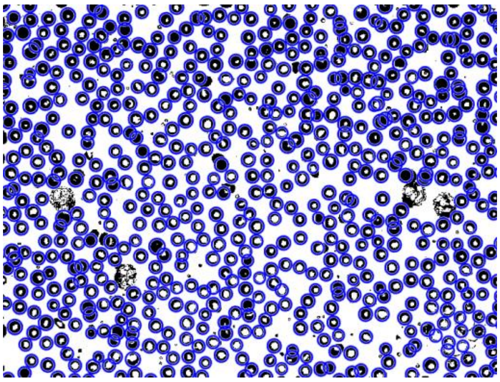

Figure 3: Red Blood Cell Count

RBC Count is **652** for above image. Total RBC count per microliter is **4,920,000** which is a normal count and within range of 3.5 – 5.5 million.

Platelet Count: The Platelet count is determined using Circular Hough Transform as well. The number of parameters are same except the value of radius ratio, that differs from RBC Count i.e. is **1- 2**. There are several steps to detect circles in an image using CHT. Firstly, any edge detection technique can used such as Prewitt, Canny, Sobel or morphological operation to detect all edge in the image [3]. Then, draw a circle on the desired radius at each edge point in the parameter space. But in our case we have used **'imfindicircle ()'** function along with CHT to count RBCs and Platelets as well.

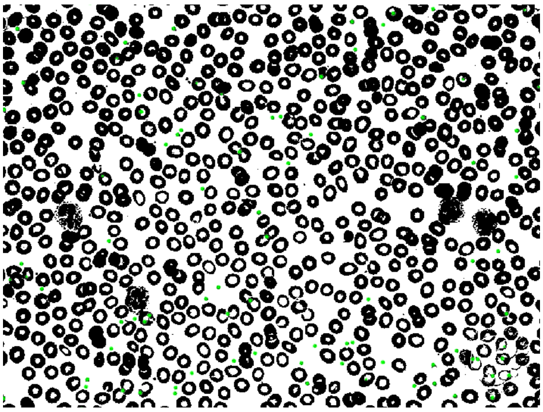

Figure 4: Platelet Count

Platelet count in this image is **97**, the total count will be **727,500** which is more than the normal range of 150,000-450,000. This can be due to bleeding, malaria or dengue.

Since, we have found the count of RBC and Platelets using Circular Hough Transform but the methodology differs slightly. Consider the flow chart given below, illustrating how the count of RBCs and Platelet was found. The counting of RBCs and Platelets work for 100x and 400x zoom images as well.

NOTE: Increasing the sensitivity factor can lead to false calculation of objects.

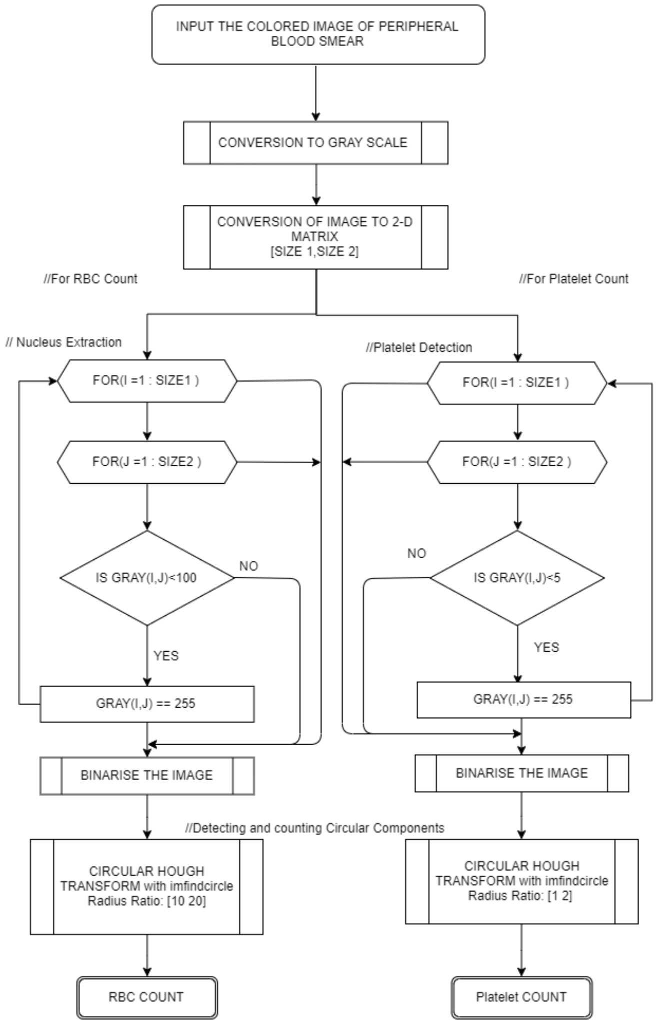

Figure 5: Flowchart for RBC and Platelet Count

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III. RESULT AND CONCLUSION

The processing was done on 10 images. Hence we are including count of WBC, RBCs and Platelet whose count accuracies are enlisted below, the accuracy of count is according to the blood smear film:

Sr. No.	RBC	WBC	Platelet
1.	99.23%	66.66%	81.25%
2.	99.38%	100%	93.18%
3.	98.37%	100%	64.70%
4.	97.20%	75%	74.193%
5.	99.53%	100%	91.935%
6.	90.69%	75%	68.88%
7.	98.60%	77.77%	64.44%
8.	88.59%	75%	63.54%
9.	98.05%	100%	79.66%
10.	98.15%	100%	88.04%

NOTE: Accuracy of WBC count can be 100% in some cases since they are least in numbers i.e. 0-5 per peripheral blood film. The formula for calculating the accuracy of count in percentage is [5]:

$$\text{Accuracy} = \frac{\text{Cell count using Proposed Algorithm}}{\text{Manual Count}} \times 100$$

Hence, the count accuracy of Red Blood Cells, White Blood Cells and Platelets is mentioned below:

Red Blood Cell: 96.83%

White Blood Cell: 86.94%

Platelet Count: 77.03%

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