

Counting of RBC's and WBC's Using Image Processing Technique

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Abstract: The measure of WBC and RBC Cells are very important for the doctor to diagnose various diseases such as anaemia, leukaemia etc. So, precise counting of blood cells plays very important role. The old conventional method used in hospital laboratories involves manual counting of blood cells using a device called Haemocytometer. But this process is extremely monotonous, time consuming, and leads to inaccurate results. Even though hardware solutions such as the Automated Haematology Counter exists, they are very expensive machines and unaffordable in every hospital laboratory.

In order to overcome these problems, this paper presents an image processing technique to detect and to count the number of red blood & white blood cells in the blood sample image using circular Hough transform and thresholding techniques. Detection and counting of blood cells have been done on three microscopic blood images of each patient which resulted in accuracy of 93.1%. The use of image processing technique helps in improving the effectiveness of the analysis in term of accuracy and time consumption.

Keywords: WBC, RBC, Automated Haematology Counter, circular Hough transform, thresholding.

1. INTRODUCTION.

In medical analysis blood cell count plays vital role. Variations in the count of blood cells cause many diseases in the human body. For overall health assessment and diagnosis of many disorders complete blood count is required. Abnormal increase or decrease in cell count indicates that person has indispensable medical condition.

The Complete Blood Count (CBC) is a blood test, extensively used to check various disorders such as infections, allergies, problems with clotting, anaemia, leukaemia etc. In order to perform CBC test, the blood film is stained and then imaged with a transmission light microscope. Here the analysis of the blood sample is done manually in order to count number of blood cells and also to identify disorders in blood samples through a microscope. But it is a time consuming process and also leads to undesirable human error. In essence, the goal of this paper is to develop and validate the necessary image processing steps to count blood cells on blood smear slides. The current work aims to provide: mitigate problems posed by different conditions such as noisy and degraded images; detect the overlapping cells; to differentiate RBCs and WBCs which are present in a blood smear slide counting RBCs and WBCs.

1.1 Cellular elements of Blood

1.1.1 White blood cells or leukocytes

White blood cells are an important part of the body's immune system. They protect against certain bacteria, viruses, cancer cells, infectious diseases. The density of the leukocytes in the blood is 5000-7000 /mm³. Leukocytes are categorized in to 5 different types. They are Neutrophil, Eosinophil, Basophil, Monocyte, and Lymphocyte. Low WBC counts may indicate that a person is in risk of infection. High WBC counts might indicate an existing infection, tissue damage, or leukaemia

1.1.2 Red blood cells

Red blood cells, also known as Erythrocyte are the most important and numerous blood cells in the human body. Main function of RBCs is to carry oxygen to the cells in the body. They are minute disc shaped cells and contain a protein called hemoglobin which gives red color to blood. Decrease in level of RBC's may cause severe diseases including anemia, and leukemia.

1.1.3 Platelets: thrombocytes

A platelet is a cell fragment that circulates in the blood. A low platelet count can cause a person to bleed without their blood clotting . A high platelet count can increase the risk

of thrombosis (blood clots inside blood vessels), which stops blood from flowing properly.

2. CURRENT METHODS FOR BLOOD CELL COUNTING

2.1 Manual Method

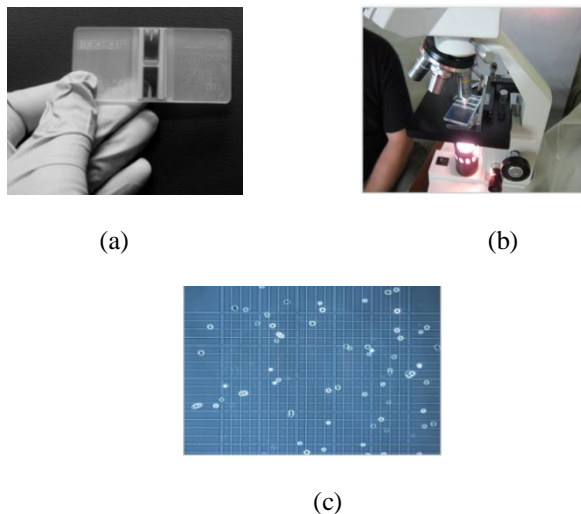


Fig 1(a) Haemocytometer (b) Haemocytometer in microscope (c) View of haemocytometer slide through the microscope

The conventional device used to count blood cells is the Haemocytometer. It consists of a thick glass microscope slide with a rectangular indentation creating a chamber of certain dimensions. This chamber is etched with a grid of perpendicular lines. It is possible to count the chamber of cells in a specific volume of fluid, and calculate the concentration of cells in the fluid. To count blood cell, physician must view haemocytometer through a microscope and count blood cells using hand tally counter.

Drawbacks of the manual method

- Manual counting task is time-consuming and laborious.
- Counting overlapping blood cells is a major problem.
- Difficult to get consistent results from visual inspection.

2.2 Automated method

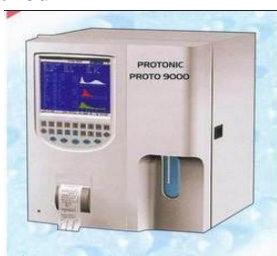


Fig 2: Automated cell counter

Complete blood count is performed by an automated analyser. The blood is well mixed (though not shaken) and placed on a rack in the analyser. This instrument has many different components to analyse different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood. The results are printed out or sent to a computer for review.

Drawbacks of automated method

- Automated analyser is Costly
- Cannot detect irregularities or variation in the shape and size of the cells.

3. RELATED WORK

Berge et al. [1] presented an approach which is based on a morphological method and iterative threshold techniques. Segmentation was performed on red blood cells, which included clumped cells, and boundary curvatures were used to construct a Delaunay triangulation. They used real microscopy images prepared in the laboratory. Their method was not tolerated with a high degree of overlapping cells. Additionally, the iterative threshold method was unable to detect faded red cells. Khan et al. [2] proposed a method to count WBCs, RBCs, and platelets. It requires several pre-processing steps before converting the image to binary. Segmentation and cell counting were performed based on the optimal threshold value, which was determined from a histogram. They achieved 95% accuracy with their proposed method compared to manual counting and a haematology analyzer. Drawback of this method is that, it is unable to detect overlapping cells. When using iterative thresholds, the probability of losing useful information from the image is high; this decreases the accuracy of segmentation. Nguyen et al. [3] used distance transform to solve the overlapping cells problem; they proposed a method that concentrated on clumped cells. Chiu et al. [4] presented a fast randomized Hough Transform method for detecting circles, to improve RHT which is less efficient in complex images due to its probability usage problem. Mahmood and Mansor et al. [5] examined 10 image samples of normal blood cells; image transformed to the HSV color space, and then Saturation or "S" channel was selected to proceed with image analysis. Morphological operators and thresholding method were used over S channel for cell segmentation. They used Circular Hough Transform to investigate the circularity feature of the red blood cells in order to perform detection and counting. Their proposed method achieved approximately 96% of accuracy rate in comparison to manual counting.

Venkatalakshmi and Thilagavathi et al.[6] have also applied circular Hough Transform method to count the RBCs from microscopic images after performing preprocessing steps such as HSV transformation, S channel extraction, histogram

thresholding morphological operations, XOR logical operation, and Canny edge detection. However, again, this proposed idea is less tolerant to any overlapping cells or irregular cells' shape. Maitra et al. [7] presented a composition method to extract red cell from five microscopic images; these steps include spatial smoothing and filtering, adaptive histogram equalization, and edge detection. Similar to, they used basic Circular Hough Transform to detect the red cells based on prior information such as size and shape features. Those methods employ classic Hough Transform method to detect the blood cells which inherits some drawbacks, for example, required more computation, high memory consumption, and less ability in detecting overlapping cells or irregular cells.

4. PROPOSED SYSTEM FRAME WORK

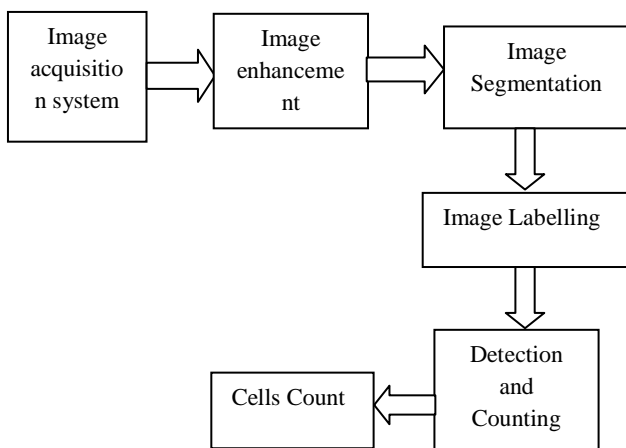


Fig 3: General methodology for the proposed method.

The procedure involves two significant phases.

5. PHASE 1: Total Blood Cells Count

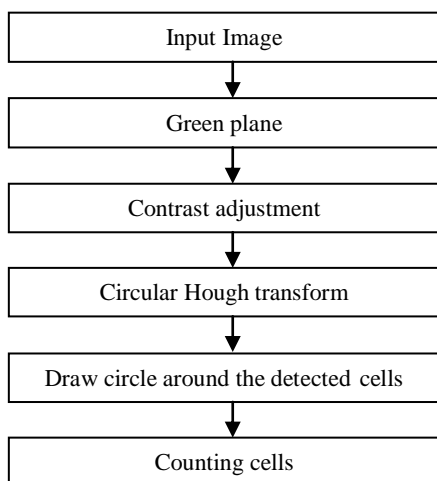


Fig 4: Flow chart for counting of blood cells

5.1 Image Acquisition

The digital microscope is interfaced to a computer and the microscopic images are obtained as digital images.

5.2 Image Enhancement

For better segmentation of the blood cells, the obtained image has to be enhanced.

➤ Green Plane Extraction:

The green plane is extracted from the imported blood cell image. The other planes such as red and blue are not considered because they contain less information about the image.

➤ Contrast Adjustment

To enhance the image, its contrast is adjusted by altering its histogram. The image's histogram is equalized.

5.3 Image Segmentation

This involves selecting only the region of interest in the image. Here only the blood cells are selected, because they are the areas of interest. When circular Hough transform is applied, not much of the image segmentation is needed because the applied transform looks only for the circular objects in the image.

5.4 Detection of Blood Cells

The circular Hough transform is then applied to the contrast adjusted image. This transform searches for the blood cells in the image and then detects them. The function "draw circle" draws circles around the detected cells. Even the overlapped circles are detected.

5.5 Counting of Blood Cells

Counting the number of cells drawn gives the total number of blood cells in the image.

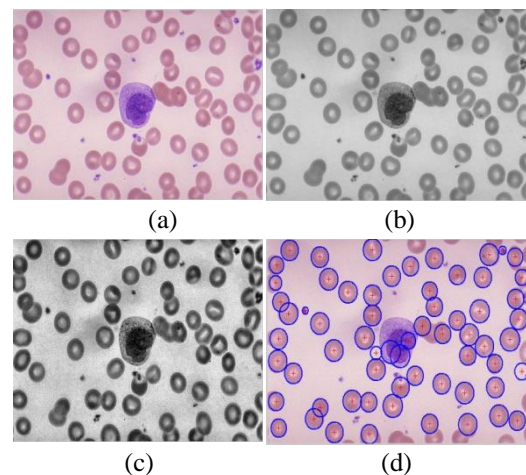


Fig 5:(a) Original blood sample image, (b) green plane of an image, (c) contrast adjusted image ,(d) detected blood cells

6. PHASE 2: WHITE BLOOD CELLS COUNTING

6.1 Image Acquisition

It is a process of acquiring a digital image.

6.2 RGB to Gray conversion

Image obtained by digital camera is in RGB format. But for post processing, this image need to be converted into gray scale format. This makes processing much simpler.

6.3 Developing Histograms

Histogram is a distribution of pixel intensity values which can be constructed by splitting the range of the data into equal-sized bins. For analysis purpose such that; to select the threshold value histogram is required.

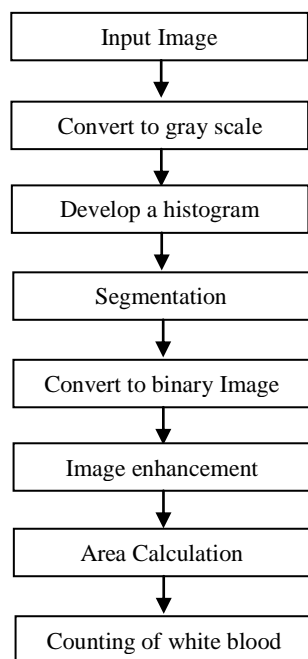


Fig 6: Flow chart for counting of white blood cells

6.4 Image Segmentation by gray thresholding

The gray thresholding function uses Ostu's method, which chooses threshold valve to minimize intra-class variance of the black and white pixels. Here only white blood cells are selected, because they are the areas of interest.

6.5 Convert to binary image

Segmented image need to be converted to binary image in order to make further processing easier.

6.6 Image Enhancement

6.6.1 Canny edge detector

The goal of edge detection is to extract the important features like lines, corners, curves etc. from the edges of animas. The Canny Edge detector known as optimal edge detector aims to satisfy three main criteria: **Low error rate, Good localization, and Minimal response, Dilation** Once the edges are find out dilation operation need to be applied on edge detected image. Dilation adds pixels to the boundaries of objects in images.

6.6.2 Image labelling

Connected components labelling scans an image and groups its pixels into components based on pixel connectivity, *i.e.* all pixels in a connected component share similar pixel intensity values and are in some way connected with each other. Once all groups have been determined, each pixel is labelled with a graylevel or a color according to the component it was assigned to.

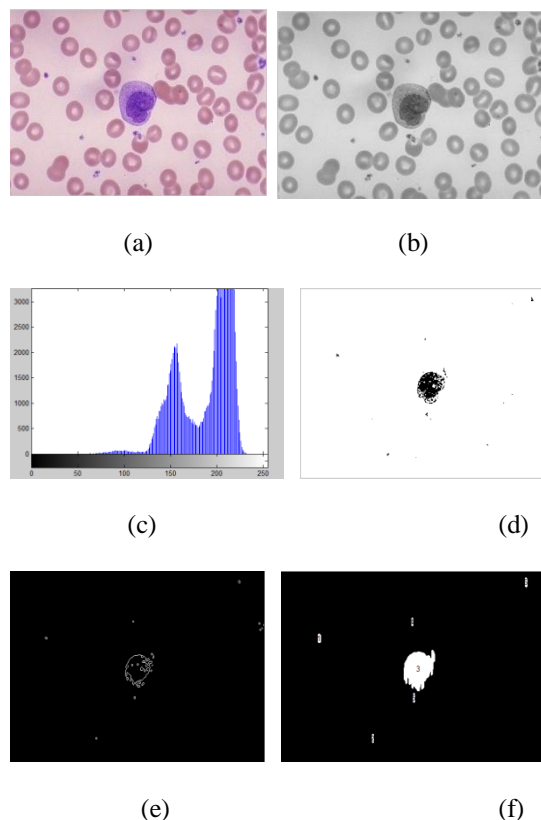


Fig 7 :(a) Original blood sample image, (b) Greyscale image, (c) Histogram, (d) binary image, (e) Edge detected image, (f) labelled image

6.7 Area Calculation

Area: Sum of pixels enclosed by cell boundary has to be calculated. Based on area and perimeter of each object white blood cells are counted.

7. EXPERIMENTATION

The experiment is done on 10 patients. Three blood sample images of each patient are taken for counting RBC & WBC. After counting the number of WBCs and RBCs, the counts are put into the equation 1 and 2 and the normalized counting is done.

Formula for counting RBC

$$N = \frac{C}{A} \times 10000 \dots \dots \dots (1)$$

Where N - RBC count in million cubic
 millimeter
 C - RBC Count in an image
 A - Input image area

Formula for counting WBC

$$N = C \times 3000 \dots \dots \dots (2)$$

Where N - WBC count in cubic millimeter
 C - Count of WBC in an image

Results of 2 patients;

Patient - I & Patient - II is tabulated.

Table-1: Blood cells Counted by proposed method

Patient - I		
Image Samples	Blood cells Counted by proposed method	
	RBC in million cubic mm	WBC in cubic mm
1	4.6756	6000
2	4.6296	6000
3	4.757	6000
AVG	4.6874	6000
Patient - II		
1	3.8364	6000
2	3.5367	6000
3	3.7747	0
AVG	3.7159	4000

Patient - I	
Blood cells Counted by Automated Analyzer	
RBC in millions cubic mm	WBC in cubic mm
5.05	6000
Patient - II	
3.18	3800

Table-2: Blood cells Counted by Automated Analyzer

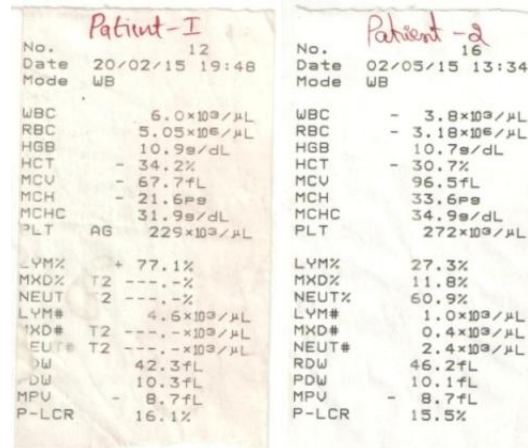


Fig 8. Pathology Lab reports of Patient I & II

The results are compared with the reports obtained by Automated Analyzer of Patient - I & Patient - II. If the blood cells having more irregularities, in such cases automated analyzer also fails to give accurate results. In these conditions doctors prefer manual counting to analyze results produced by automated analyzer. Means, if patient having anaemia symptoms the blood cells counted by automated analyzer is not matching with the symptoms then doctors prefers to do manual counting in order to diagnose the problem. So the proposed system is more efficient in terms of time consumption, cost and also user friendly. Hence Pathologist can get the result of blood cell test within 20 to 40 seconds as tested.

From the below graph it is observed that the results obtained by the proposed method offer a good conventionality with the automated counting method.

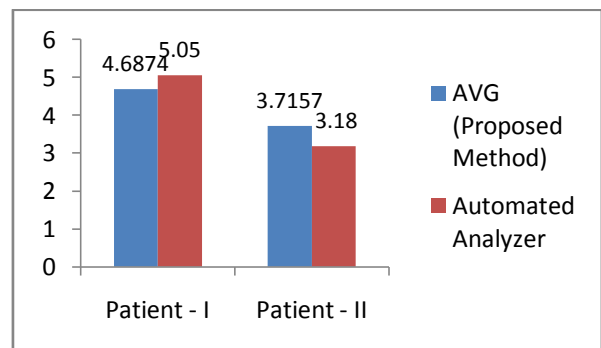


Fig 9: RBC in millions cubic mm

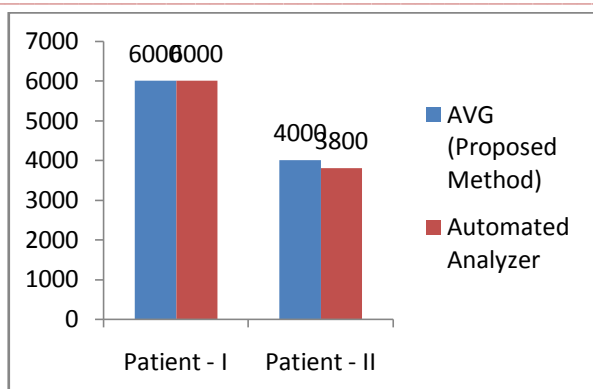


Fig 10: WBC in cubic mm

The results are presented and the graphical user interface (GUI) is developed to provide user friendly for analysis.

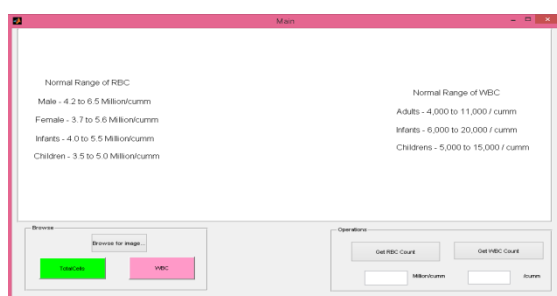


Fig 11: GUI for counting rbc's and wbc's

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CONCLUSION

This paper presents a software based solution for counting the blood cells. Proposed method of cell counting is fast, cost effective and produces accurate results. It can be easily implemented in medical facilities anywhere with minimal investment in infrastructure. This method can also recognise the overlapping cells and counts them separately. The average time required and the average accuracy of the proposed system is 20 seconds and 93.01% respectively.

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