**Abstract:** Medical diagnosis plays an important role in detecting and analyzing the clinical status of a particular disease. Most of the clinical diagnosis is done by collecting blood sample. The blood sample provides valuable information of particular illness such as sickle cell anemia, leukemia etc. The cells are differentiated based on various morphological structure of the nucleus. The blood smear is observed under light microscope and the cells (WBC/RBC) are counted or studied to determine certain clinical diseases. In order to maintain patient’s clinical history the cell image of blood sample is captured under light microscope by using high resolution webcam. Using these images it is possible to efficiently and automatically count the cells by using computer application. Thus image processing has turned out to be an important tool for automatic counting of total cells and also is capable of differentiating cells based on morphological structure simultaneously.

By using MATLAB image processing tool, the cells of white blood cells are differentiated into different segments and also the total count of the cells is also done based on the cell size and shape.

**Keywords:** White Blood cells, Light Microscope, Webcam, Image segmentation, Morphological studies.
1. Introduction:

There are various samples that can be used to detect or diagnose the disease or medical status of particular disease. Generally blood, urine, sputum, sperms or any part of the tissues are used to study the status of the patients disorder. Blood is the most important sample which gives complete details about the particular disease or disorder. The antibodies are mainly responsible in inducing immunity to fight against the particular pathogenic microbe or any toxic analytes. In humans most of the disease analysis is done by studying the blood sample. Antibodies are nothing but White blood cells.

The White blood cells generally identified based on granular and agranular nucleus. The granulocytes include white blood cells also called as leukocytes are used to study the abnormalities in the blood. Conventional clinical laboratory methods using Hemocytometer is available which is economically cheap but has certain disadvantages. The process requires lot of concentration. The laboratory technician requires lot of concentration and undergoes lot of strain at the time of counting cells.

This method also may pose certain manual errors. If any break occurs at the time of counting, then the counting of cells should be repeated again from the beginning. Hence it is time consuming. Although a hardware digital differentiating cell counter is available as an alternative that can count the number of blood cells (RBC, WBC, Platelets) automatically, there is an disadvantage with this method. It can count the number of cells in blood smear but cannot identify or differentiate the abnormal or mutated cells. Thus Image processing using Matlab tool is used to count and also morphologically separate the white blood cells.

2. Literature Review:

Anton Von Leeuwenhoek was first to isolate an count the blood cells using chicken blood sample. He used capillary with marking and microscope to study the red blood cells (RBC). Later other method has been developed for dilution of blood sample. The blood cells have been detected in 1658. Since then various methods have been practiced in blood cell counting. The main components of the blood are Red blood cells (RBC), White blood cells (WBC) and platelets. The RBC’s consist of erythrocytes which are approximately 4-6 millions in number in normal humans. The platelets are mainly responsible in blood clotting. These are around 150,000 in normal humans. White blood cells are mainly classified into
granulocytes and agranulocytes based on presence or absence of granules in the nucleus. Granulocytes are composed of neutrophiles, basophiles and Eosinophils. Agranulocytes are monocytes, lymphocytes and macrophages. Lymphocytes produce antibodies and neutrophiles play role of defense system. Lymphocytes increase during viral attack and neutrophiles increase drastically at the time of bacterial attack.

<table>
<thead>
<tr>
<th>HUMANS</th>
<th>WBC COUNT</th>
</tr>
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<tbody>
<tr>
<td>ADULTS</td>
<td>4,000-11,000mm³</td>
</tr>
<tr>
<td>CHILDREN</td>
<td>6,000-11,000mm³</td>
</tr>
<tr>
<td>NEW BORN</td>
<td>9000-30,000mm³</td>
</tr>
</tbody>
</table>

*Table 1: WBC count in Humans*

3. **Hemocytometer:**

*Figure 1: Hemocytometer*

It was Louis Charles who invented this special thick slide made of glass which consists of grooves which are deeply etched into lines which appear like grid. The deep grooves are etched with the help of diamond or laser. The white blood cells are counted at the chambers present to the edges of the grooves while red blood cells are counted located in the center of the grooves and are in turn subdivided into further smaller units. The slide is made up of thick glass which is carved at the center to form grid like chambers.
Each chamber has 1 X 1 mm squared dimensions. The total area of the chamber is divided into nine squares of equal dimensions. These chambers are further divided into 0.0625mm², 0.05mm² and 0.04mm² units. Now the central part of the chamber is further divided into finer grooves of 0.0025mm².

4. METHODOLOGY:

The clinical procedure involves the collection of blood sample from the patient and studied for analyzing the morphological status. The steps involved in the process are:

a. First the tip of the index finger has to sterilize with 80% ethanol.

b. With the help of sterile lancet the sterilized area is pierced and 0.5 ml of blood is collected with the help of WBC pipette.

c. Immediately WBC diluting fluid is sucked up to 11 marked on the pipette.

d. The Blood sample and the diluting fluid should be mixed thoroughly for 2-3 minutes.

e. Haemocytometer is used to study the WBC /RBC count.

f. Cover slip is placed over the hemocytometer in center position.

g. Blood sample collected is introduced into the hemocytometer at the end point where cover slip is placed.

h. Set-up is allowed to settle for around 20 minutes. Then this is observed under light microscope using 10x objective lens.

The number and the morphological status of the WBC can be studied under the microscope manually. Since this method may result in manual errors, the best tool that can be used in Blood count is image-processing by using MATLAB. For this purpose clear images are captured using High resolution webcam.
Figure 3: Complete Setup For Counting

The image capturing sensor was carefully separated from the webcam setup and then this webcam was attached to the microscope in the place of eyepiece. Before this the size of the cylinder of the microscope was increased slightly using plastic mould. Now the whole setup of both webcam lens and the cylindrical tube are completely sealed with cardboard paper to keep it intact and also to reduce the effect if intensity of white light. Now again it is wrapped properly with piles of papers. This Webcam is connected to light microscope and clear images are captured. The hemocytometer with the sample was placed on the center point of the stage of the microscope and images were capture using low magnification lens 10X.

5. Objective:
The main objective is to implement a means to count and also identify the size, shape of the WBC using image processing. This application is done by using function regionprops in MATLAB. Mapping functions usually have one or more parameters that need to be specified. A useful tool for establishing suitable values for these parameters is the intensity histogram, which lists the frequency (number of occurrences) of each intensity value in the image. The image processing tool box, in MATLAB, extents the MATLAB technical computing environment with functions for acquiring image enhancement and segmentation.

6. Proposed Work:
After Webcam is connected to light microscope clear images are captured. The images captured are stored in standard format such as JPEG (Joint Photographic Expert Group). Then these images are studied using various functions in MATLAB like RGB, grayscale, morphological analysis which include erosion, dilation, line thickening, line thinning etc.
Remote
Blood sample collection

Direct
Diluting blood with WBC diluting fluid

Cover slip and hemocytometer

Slide with blood sample is observed under

Images captured

Acquisition

Pre-processing the image

Enhance the image

Segmentation process

A

Drop of EDTA is added if sample is from remote area
7. Software:
The MATLAB version 6.0 or later is suitable to perform special biological functions. MATLAB provides extensive image processing toolbox library suitable for our work. Region props function available in the toolbox can perform all almost all image metrics calculations.

8. Result And Explanation:
Around 450 images of different samples were captured to study and analyze the morphological features and also total number of white blood cells present. After images are captured, these images are stored in standard format JPEG (Joint Photographic Expert group). Now different series of steps of Image processing are applied on these images. Initially, the first process in image processing i.e., image acquisition is considered to be most important step because if the images captured are not perfect or clear then even after applying enhancement or segmentation process on the images will not produce accurate and reliable result. The most important difficulty in capturing the blood cells is illumination effect. The white blood cells have capability to produce illumination which will interfere with the capturing process.
In addition these illuminations may also result in producing certain amount of noise during image capturing process because which the images captured will be fuzzy and not clear to
process. Thus the image acquisition is most important step and for this reason high resolution (16X) webcam is preferred to capture images.

Then the images are preprocessed before other algorithms are applied. In this process the extraction of regions that are of interest and also removal of artifacts from the images is done. Here the RGB images are converted into grey scale image. This step is carried out because the memory required to store the information regarding the grey scale images is small compared to RGB images. We are interested in morphological observation and total count of white blood cells that are present in the given blood sample, thus color images is not an important criteria to consider. Then enhancement of image is done by carrying out contrast adjustment process. In this step the binary images are reversed, that is when the color image is converted into grayscale image initially the binary images formed will appear black with white background and on applying contrast adjustment i.e. histogram equalization with binary thresholding the images will be white with black background.

![Figure 7: Original Image](image1)

![Figure 8: Gray Image](image2)

![Figure 9: First Adjustment Image](image3)
Further image segmentation is done in order to find the image and also identify its boundaries so that it would be easy to analyze the objects of interest in the image with even more clarity.

At this point morphological operators like dilation and erosion are applied so as to repair the boundaries of the objects and also for restoring the proper information of the object. A special function Regionprops in Matlab is applied to the images. Using this special function various properties of the objects can be calculated. The properties that are calculated include finding length of the object and also bounding box of the respective objects. Finally the objects which share similar number of pixels is labeled as one group are represented with certain color.
Now the white blood cell analysis and counting is done. From the result evaluation of the condition of the patient that is whether the person whose sample tested is normal or suffering with any abnormalities can be done. We also collected few images from internet that are available in various links and applied our algorithm.
We have applied our algorithm on both our images and also already existing images in internet and could succesfully run the algorithm. The accuracy of our images is claimed to be around 93% and on existing images is nearly 95%.

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<th>AUTOMATED COUNT</th>
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9. Conclusion:
This is a real time application where we can not only count the cells but also differentiate them based on their size and shape. This helps us to identify the presence of any abnormal cells that are present in the sample. Based on which it is possible to estimate whether the patient is suffering from any disease (Malaria, typhoid) or any abnormalities (leukemia, sickle cell) or any other clinical problems.
References:


4. "Masanori Sugisaka ,Object Detection using Circular Hough Transform, Department of Electrical and Electronics Engineering, Faculty of Engineering Oita University, 700 Dannoharu, Oita-shi, 870-1192 Japan".
