Α

Project Report

On

"Classification Of RBC's & WBC's From Peripheral Blood Smear Using KNN Classifier"

Ву

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Certificate

This is to certify that

VARAD GAONKAR SAYLI KADU RISHIKUMAR SINGH SUSHANT ZUTSHI

have successfully completed the project titled

"Classification Of RBC's & WBC's From Peripheral Blood Smear Using KNN Classifier"

towards the fulfilment of the course of

Bachelors of Engineering

in

Instrumentation Engineering

as laid by the

University of Mumbai

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ABSTRACT

The main objective of this project is to present the blood cells classification method to develop an automatic differential blood count system. Blood is main circulating fluid in human body. The counting and analysis of blood cells provides a huge amount of information to the pathologist, for finding out various diseases, their causes and its treatment.

The proposed system consists of two automatic steps: segmentation of blood cells and classification. For classification, feature vector is created by extraction of various features from the segmented blood cells.

Experimental results have shown the effectiveness of the system.

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CHAPTER 1

Project Overview

- Introduction
- Aim of project
- Why MATLAB and not other programming languages?
- Advantages
- Disadvantages

• Introduction:

The process of life is maintained by blood, which is a specialized body fluid consisting of plasma and blood corpuscles. The blood corpuscles can be classified as erythrocytes, leukocytes and thrombocytes. To perform proper diagnosis of different diseases, identification of the blood cells and their relative quantity in the blood samples must be known. Different techniques are used in Pathological Lab for obtaining the Blood Report. But now with the help of MATLAB programming & Image Processing we have obtained the same report. The steps are: Image Acquisition, Image Pre-Processing, Image Segmentation, Feature Extraction & Classification.

For the image acquisition, the blood smear slides are taken and by connecting a high resolution digital camera to microscope, images are captured by adjusting microscope magnification to get good resolution. For identifying different types of blood cells and for counting their quantity in blood smear, image processing is used on various blood smear images. The image is preprocessed, so as to remove noise, and then conversion to gray level and contrast enhancement is done. Also the algorithm used for classification of various blood cells i.e, k-NN algorithm has been studied. For separating out various cells, binarization, morphological operations followed by watershed segmentation and labeling of segmented cells is performed. Different features of cells such as their area, circularity, presence of nucleus, etc are then extracted from the labeled image. From this features, database is created and with the help of this database, set of test images and KNN classifier, classification of various cells in an image is done.

• Aim of project

In this new generation, technological revolution is fast reducing the need of humans to operate machines. To promise the delivery of the product on time high tech production is essential. The objective of this project is to design, develop a simulation based on classification of WBC & RBC.

The notable thing about this project is the high level of flexibility & portability.

This project mainly focuses of the use of MATLAB for classifying WBC & RBC. It is dependent on the programming how to classify WBC & RBC.

We have designed the entire program on MATLAB R2017a (version). Here we have used GUI for better handling of the project to the user and k-NN Classifier for obtaining the output.

• Why MATLAB and not other programming languages?

MATLAB (matrix laboratory) is a multi-paradigm numerical computing environment and proprietary programming language. MATLAB allows matrix manipulations, plotting of functions and data, implementation of algorithms, creation of user interfaces, and interfacing with programs written in other languages.

MATLAB has several advantages over other methods or languages: Its basic data element is the matrix. Several mathematical operations that work on arrays or matrices are built-in to the MATLAB environment.

MATLAB is a general purpose programming language. When it is used to process images one generally writes function files, or script files to perform the operations. These files form a formal record of the processing used and ensures that the final results can be tested and replicated.

MATLAB provides many functions for image processing. Most of these functions are written in the MATLAB language and are publicly readable as plain text files. Thus the implementation details of these functions are accessible and open. One can examine the processing used in complete detail, and any challenges raised can be resolved easily with the library and help option provided. This makes MATLAB very different from other applications.

MATLAB® 7Programming



Advantages

- 1) Affordable This automatic differential blood count system doesn't require lot of machinery as compared to the traditional laboratory method.
- 2) Less equipments required Basic components required in this project is a PC with MATLAB software and images of blood samples.
- 3) Easy to handle and operate Since there are very less equipments it is easy to handle the project.
- 4) Requires low maintenance Only image samples and PC is involved it requires very less maintenance as compared to the equipments used in the laboratory.
- 5) Low human error According to the laboratory method, blood cells are classified and counted only by watching them under microscope and further by theoretical calculations. So there are a lot of chances of human error. But in this project we only used microscope for obtaining the images. Further every calculation and results are shown practically. So there is less human error.
- 6) No chemicals and chemical processes required only MATLAB programming is involved so there is no such use of chemicals as that of the laboratory method.
- 7) Time saving The results are obtained within minutes instead of consuming hours for chemical process.
- 8) More efficient.

• Disadvantages

1) The images used for the process should be clear and there must be less overlapping of the cells. If overlapping is more then it will be difficult to separate those cells and hence there will be an error for the further steps and calculations. So the images which will be used should have much separate cells if possible.

CHAPTER 2

Literature Study

- Traditional Laboratory Method
- Differentiation of Blood Cells
- MATLAB Image Processing
- k-NN Classifier

• Traditional laboratory method

In pathological labs, mostly 2 methods are used for classification and counting of blood cells. They are:

- 1) Using Haemocytometer
- 2) Fields Staining Method

1)Using Haemocytometer-

In this method a thick slide is used. This slide has a square gap in it. Blood drop of patient is taken out and placed inside this square gap and a cover piece is placed on it. This slide is then placed under compound microscope(8x OR 10x magnification).

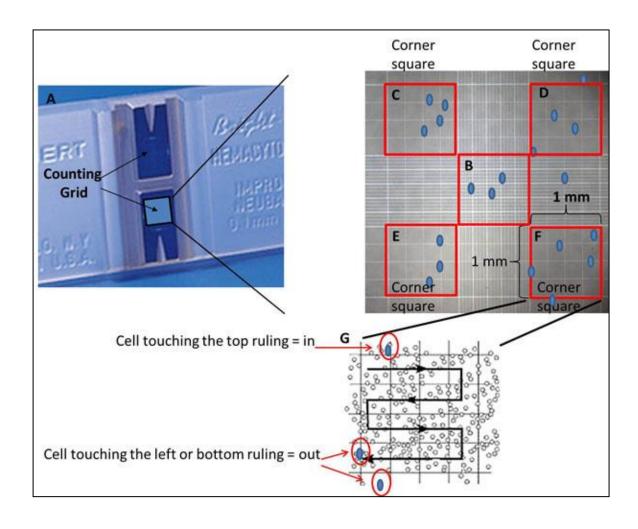
This slide is then watched through microscope and an arbitrary number (random count of cells) is removed.

The thick slide which is used have different chambers for easy rectification of cells. It has 4 WBC chambers and 2 RBC chambers. RBC chamber is divided into 16x16 and WBC chamber is divided into 4x4. In those specific chambers we see only that particular type of blood cells.

Suppose if by any chance there is any WBC present in RBC chamber then it is floated and circulated using different chemicals and moved to WBC chamber.

Then counting of one WBC chamber is done and it is multiplied by 4. RBC is directly counted from the chamber. Then by using formula, number of cells is calculated. The formula has different parameters like area of slide, volume of slide, thickness of slide, amount of cells present in 1ml of blood sample, etc.

Usually total volume of sample blood is generally taken as 1ml.



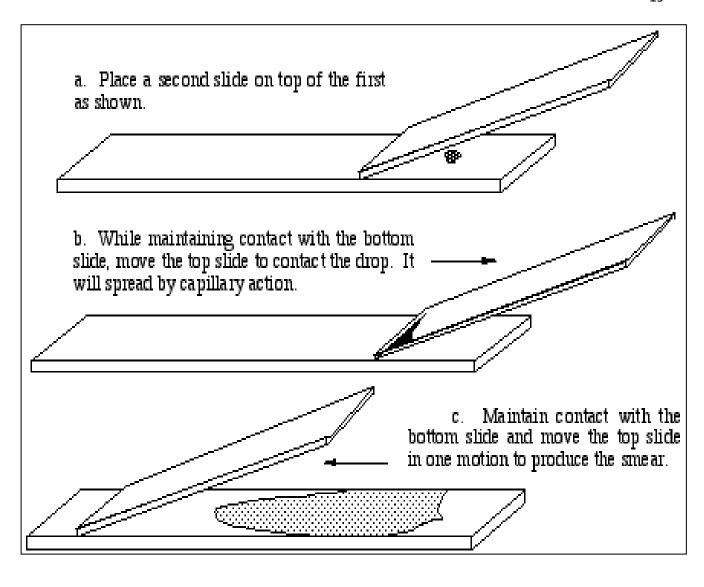


2) Using Fields Staining Method-

In this method one drop of blood is placed on one slide and with the help of another slide it is pulled completely around the slide. Then a cover piece is placed on it and this slide is watched using compound microscope (10x magnification).

Then the blood cells present on the slides are directly observed under microscope and total number of cells present on the slide is removed. Then by using formula total number of cells is calculated.

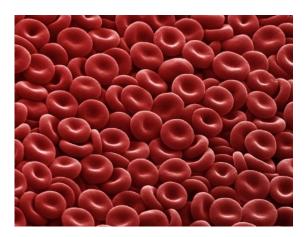
Arbitrary number of cells is calculated by just observing the cells, so for better vision different chemicals and dyes are used. In this method WBC and RBC are differentiated with the help of presence of nucleus



Differentiation of Blood Cells

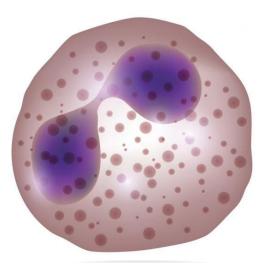
In the peripheral blood, blood cells are classified as: Erythrocytes - Red Blood Cells (RBC's), leukocytes - White Blood Cells (WBC's), and thrombocytes - platelets. Different categories of blood cell exhibit different morphologic characteristics that can be used, to identify and classify different cells.

• Erythrocytes - (RBC's): The erythrocytes are the cells which are highest in numbers. It does not have nucleus. Their cytoplasm consists of hemoglobin that gives a typical red color of the cells. Its primary Function is to transport oxygen from the lungs to the cells of the body & assist with CO2 removal. RBC's are circular, biconcave, non-nucleated cells. The size is about 7um in diameter and 2.5um in thickness. There are about 5.1 to 5.8 million RBCs per cubic mm of blood in adult males and 4.3 to 5.2 million per cubic mm in adult female. The average life span is about 120 days. RBCs maintain blood pH as hemoglobin acts as a buffer. They also maintain the viscosity of blood.

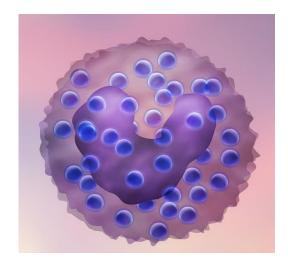


• <u>Leukocytes - (WBC's):</u> Leucocytes are colourless, nucleated, amoeboid and phagocytic cells. The size is about 8 to 15um. There are about 5000 to 9000 WBCs pr cubic mm of blood. The average life span is about 3 to 4 days. In terms of the size and shape of the nucleus, the colour of the cytoplasmic staining, and percentage ratio of nucleus to cytoplasm, leukocytes can be classified into5 major types as: Eosinophils, Basophills, Neutrophils, Monocytes & Lymphocytes.

• Eosinophils: The nucleus of eosinophil is frequently bi-lobed and its cytoplasm consists of orange to red stained granules. They constitute about 3% of total WBCs. They are non-phagocytic and their number increases during allergic conditions. They show anti histamine property. The cytoplasmic granules are stained with acidic dye such as eosin.



• **Basophils:** They are very less in blood. The nucleus of basophil is frequently twisted. They constitute about 0.5% of total WBCs. After staining, the cytoplasm is full of large, deep-bluish to purple granules. The cytoplasmic granules are stained with basic dyes such as methylene blue. They are non-phagocytic. They release heparin and histamine.



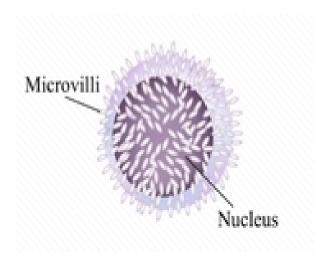
•Neutrophils: The nucleus of neutrophil is frequently multilobed hence they are called polymorphonuclear and its cytoplasm has very tiny faintly pink stained granules with low visibility. The cytoplasmic granules are stained with neutral dyes. They constitute about 70% of total WBCs. They are phagocytic in function and engulf micro organisms.



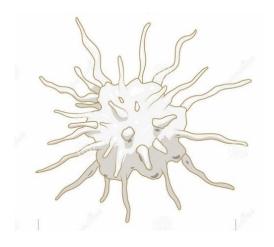
•Monocytes: Monocytes are the largest of all WBCs, circulating, white blood cells having the kidney shaped nucleus. The cytoplasm is of light blue colour. They constitute about 5% of total WBCs. They are phagocytic in function. At the site of infection monocytes enlarge and differentiate into macrophages which engulf micro organisms and remove cell debris. Hence, they are also called as scavenger cells.



• Lymphocytes: Lymphocytes are of 2 main types, B cells and T cells. It has a large, dark-staining nucleus with a small cytoplasm to nucleus ratio. They produce antibodies and are responsible immune response of the body.



• Thrombocytes –(blood platelets): The thrombocytes are colorless, disc-shaped, biconvex cell fragment without a nucleus. They are the smallest elements of blood measuring about 2.5 to 5 um in diameter. They are about 2.5 lakhs per cubic mm of blood. Their life span is about 5 to 10 days. They are fragments formed from large cells called megakaryocytes of bone marrow. Formation of platelets is called thrombopoisis. Its main function is blood clotting in the area of wound.

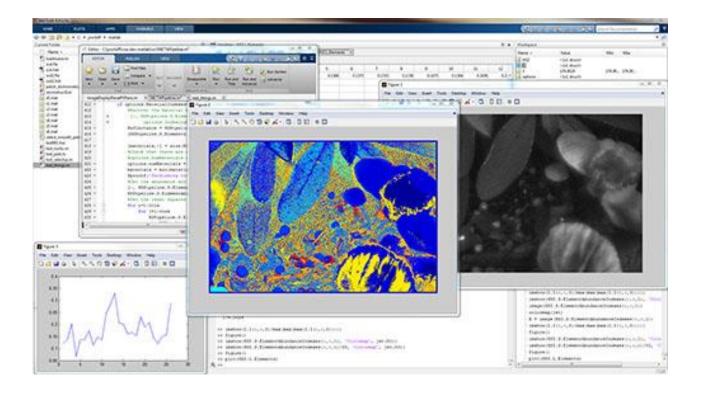


• MATLAB Image Processing:

Image processing is a method to convert an image into digital form and perform some operations on it, in order to get an enhanced image or to extract some useful information from it. It is the analysis and manipulation of a digitalized image especially to improve its quality. It is a type of signal dispensation in which input is image, like video frame or photograph and output may be image or characteristics associated with that image. Usually **Image Processing** system includes treating images as two dimensional signals while applying already set signal processing methods to them.

Image Processing forms core research area within engineering and computer science disciplines too.

In this project image processing is the base of the project. It is used for converting image into gray scale, removal of noise i.e. noise reduction, contrast enhancement, binarization, erase holes, remove border touching cells, watershed segmentation, feature extraction and classification.



k-NN classifier :

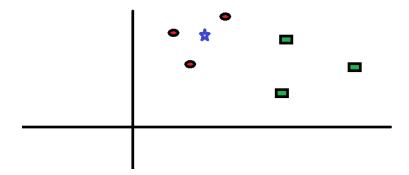
In pattern recognition, the k-nearest neighbors algorithm (k-NN) is a non-parametric method used for classification and regression. In both cases, the input consists of the k closest training examples in the feature space. The output depends on whether k-NN is used for classification or regression:

- In k-NN classification, the output is a class membership. An object is classified by a majority vote of its neighbors, with the object being assigned to the class most common among its *k*nearestneighbors (*k* is a positive integer, typically small). If *k* = 1, then the object is simply assigned to the class of that single nearest neighbor.
- In k-NN regression, the output is the property value for the object. This value is the average of the values of its *k* nearest neighbors.

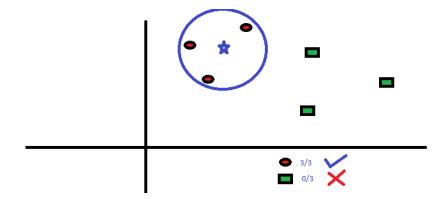
k-NN is a type of instance-based learning, or lazy learning, where the function is only approximated locally and all computation is deferred until classification. The *k*-NN algorithm is among the simplest of all machine learning algorithms.

How does the KNN algorithm work?

Let's take a simple case to understand this algorithm. Following is a spread of red circles (RC) and green squares (GS):



You intend to find out the class of the blue star (BS) . BS can either be RC or GS and nothing else. The "K" is KNN algorithm is the nearest neighbours we wish to take vote from. Let's say K=3. Hence, we will now make a circle with BS as centre just as big as to enclose only three data points on the plane. Refer to following diagram for more details:



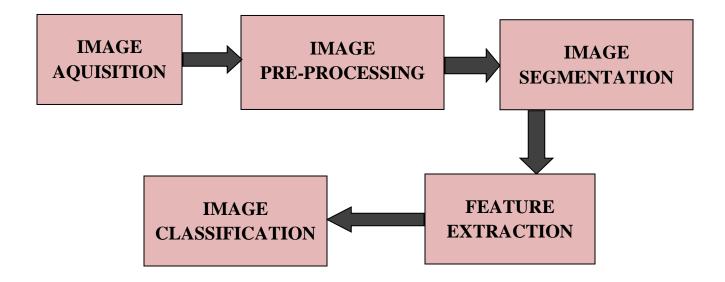
The three closest points to BS is all RC. Hence, with good confidence level we can say that the BS should belong to the class RC.

CHAPTER 3

Methodology

- Flow of work
- Image Processing Techniques

• Flow of work



• Image processing techniques

Image Acquisition: The images from blood smear slides are captured by connecting high resolution digital camera to a microscope by adjusting microscope magnification to get good resolution.

Image Pre-processing: The pre-processing stage includes noise reduction and contrast enhancement of acquired image and is performed to prepare the image for the further stages. For processing the image it is converted into gray scale image, to avoid being influenced by dye colour. A typical peripheral blood smear image consists of four components, namely background, erythrocytes, leukocytes, and thrombocytes. To segment the desired object from the background, the green channel is used, as it is found that the green component of the RGB input image gives the best contrast between the background and the foreground which consists of blood cells.

Image Segmentation: In image processing, segmentation is the process of partitioning a digital image into multiple segments. For blood cells classification, segmentation of cells is required. So, for segmentation of blood cells, the segmentation process based on thresholding, morphology, and watershed segmentation is used. It is performed to cover every element in the blood slide in a distinct area.

Feature Extraction: In pattern recognition and in image processing, feature extraction is a special form of dimensionality reduction. To analyse the individual objects of interest from the background in the segmentation process, border touching cells obtained in binary images are removed and then labelling of the objects within the segmented image is performed. The features of the blood cells such as area, histogram, circularity, cytoplasm ratio, colour of cytoplasm are extracted from a set of images to prepare a database, required for classification of cells.

Image Classification: There are various techniques to classify images. The algorithm used here is k-NN. In pattern recognition, the k-nearest neighbour algorithm is a method for classifying objects based on closest training examples in the feature space. k-NN is a type of instance based learning, where the function is only approximated locally and all computation is deferred until classification.

CHAPTER 4

- MATLAB Processing Stages
- Implementation
- Working

The Blood Sample of Infected Person whose blood report is to be created is extracted and placed on a microscopic slide. This blood on slide is known as Blood smear.

This blood smear is then placed under a digital microscope. The details of digital microscope are given below:

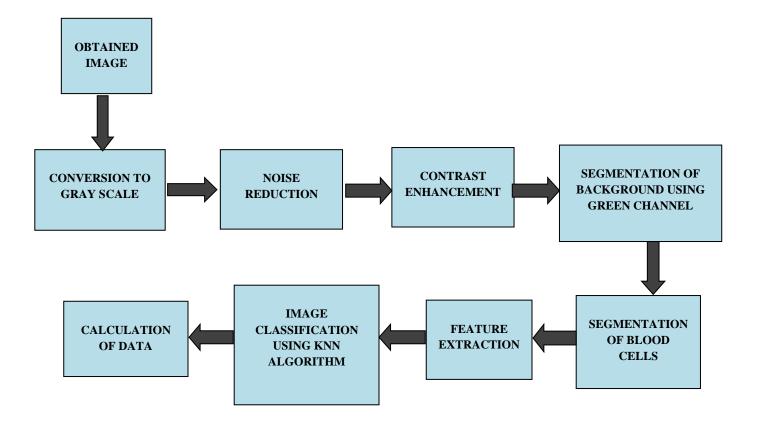
- Ocular dioper adjustable on both eye tubes; Nosepiece: revolving quadruple; Stage: mechanical double layer size: 4-1/2inchx 4-15/16inch (115mm x 125mm); Stage x-y stroke (travel range): 2-13/16inch x 1-3/16inch (70mm x 30mm); Condenser and diaphragm: NA1.25 Abbe condenser with iris diaphragm.
- Transmitted (lower) illuminator: LED light, intensity adjustable; Focus adjustment: Coaxial coarse and fine knobs on both sides; All metal mechanical components; Power supply: AC/DC adapter, 7.5V/7.5W (UL approved) Input: 100-240V.
- Digital camera: true color 640 x 480 pixels 0.45X reduction lens to get larger field of view - Software compatible with Windows XP/Vista/7/8/10, and Mac OS -Capturing microscope images.

The required images of blood smear are then captured with the help of digital microscope and then transferred for MATLAB Processing.

MATLAB Processing includes following steps:

- Conversion to gray scale
- Noise reduction
- Contrast enhancement
- Binarization
- Segmentation
- Feature extraction
- Classification
- Calculation

The above processes are represented in flowchart for better understanding and visualization.



Implementation

MATLAB environment:

To implement the new algorithm, the "MATLAB" tool is used. MATLAB has an Image-processing toolbox, which contains all functions that are used to analyze the image such as reading, enhancement, converting from one image type to another, segmentation, labeling and many more.

The functions that used to run the algorithm are described below.

1 Reading Image (Imread):

MATLAB deals with the image as a two dimensional matrix. Imread reads the image into MATLAB's environment as a matrix.

The basic syntax:

Image=imread ('file-name');

MATLAB reads the image file "file-name" into an array image. Imread can read many different formats such as JPG, PNG, GIF and TIF.

2 Showing Image (Imshow):

Imshow displays images on the MATLAB desktop.

The basic syntax:

Imshow (I)

MATLAB displays the image I in a graphics figure, where I is a grayscale, RGB (truecolor), or binary image. For binary images, imshow displays pixels with the value 0 (zero) as black and 1 as white.

3 Converting Colored Image to Gray (rgb2gray):

There are four types of image:

- 1- RGB image
- 2- Gray Scale images
- 3- Binary image "Black and white"
- 4- Indexed images

Rgb2gray converts the image from RGB into a gray-scale image [5].

Basic syntax:

I =rgb2gray (RGB);

MATLAB converts the true color image RGB to the grayscale intensity image I. The rgb2gray function converts RGB images to grayscale by eliminating the hue and saturation information while retaining the luminance. If you have Parallel Computing ToolboxTM installed, rgb2gray can perform this conversion on a GPU.

4 Calculating Threshold and Converting Gray Image to Binary (im2bw):

Converting the image from RGB into a binary image first requires computing the threshold of the gray image using the "graythresh" function.

Basic Syntax: Level = graythresh (RGB);

MATLAB computes a global threshold (Level) that can be used to convert an intensity image to a binary image with im2bw. Level is a normalized intensity value that lies in the range [0, 1]. The graythresh function uses Otsu's method, which chooses the threshold to minimize the intraclass variance of the black and white pixels. Then this threshold converts the image to black and white using "im2bw".

Basic Syntax: BW = im2bw (Grayimage, Level);

MATLAB converts the grayscale image Grayimage to a binary image. The output image BW replaces all pixels in the input image with luminance greater than Level with the value 1 (white) and replaces all other pixels with the value 0 (black). It specifies a Level in the range [0,1]. This range is relative to the signal levels possible for the image's class.

Therefore, a Level value of 0.5 is midway between black and white, regardless of class. If you do not specify Level, im2bw uses the value 0.5.

5 Filling Images Holes (Imfill):

Imfill fills image regions and holes.

Basic syntax:

Filled = imfill (BW, 'holes');

MATLAB fills holes in the input binary image BW. In this syntax, a hole is a set of background pixels that cannot be reached by filling in the background from the edge of the image .

6 Clearing the Border (Imclearborder):

This function clears any objects that touch the image borders.

Basic Syntax: Cleared = imclearborder (Filled);

MATLAB suppresses structures that are lighter than their surroundings and that are connected to the image border. Use this function to clear the image border. Filled can be a grayscale or binary image. For grayscale images, imclearborder tends to reduce the overall intensity level in addition to suppressing border structures. The output image, Cleared, is grayscale or binary, depending on the input. The default connectivity is 8 for two dimensions and 26 for three dimensions, and

7 Deleting Small Objects (Bwareaopen):

This function deletes any object thats area is less than a specific value.

Basic Syntax:

Deleted = bwareaopen (Cleared, Value);

MATLAB removes all connected components (objects) that have fewer Value pixels from the binary image Cleared, producing another binary image, Deleted. This operation is known as an area opening.

8 Labelling the Objects (Bwlabel):

This function labels all objects in the binary image [5].

Basic Syntax:

Labeled = bwlabel (BW);

MATLAB returns the label matrix Labeled that contains labels for the 8-connected objects found in BW. The label matrix, Labeled, is the same size as BW.

9 Calculating Region Properties (Regionprops):

Regionprops calculates the proprieties of image objects. Some examples of proprieties:

- 1- Centroid: return the center of the object
- 2- Boundary box: return the smallest rectangle containing the object
- 3- Major axis length: return the length of the major axis of the ellipse
- 4- Minor axis length: return the length of the minor axis of the ellipse [5].

Basic Syntax:

Stats = regionprops (BW, properties)

MATLAB returns measurements for the set of properties specified by properties for each connected component (object) in the binary image, BW. Stats is struct array containing a struct for each object in the image. You can use regionprops on contiguous regions and discontiguous regions.

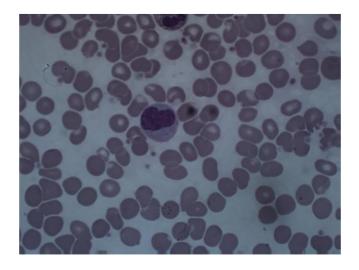
Working

Blood sample of patient is placed on microscopic slide and this blood smear is placed under digital microscope and image is captured. This image is then transferred to PC connected with the digital microscope.

Next step is MATLAB processing. MATLAB processing includes processes like reading the image, converting it to gray scale, noise reduction, contrast enhancement, binarization, fill holes, remove border touching cells, watershed segmentation, bounding cells, feature extraction, creating database and calculation.

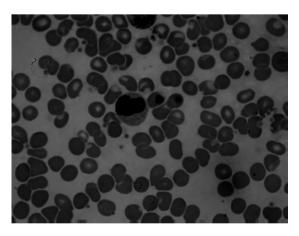
Step 1: Image is called.

Command: imread(img.jpg)



Step 2: It is converted from RGB to gray. The blood sample image is a colored image. It should be converted to gray scale for use in coming steps that handles 2-D images.

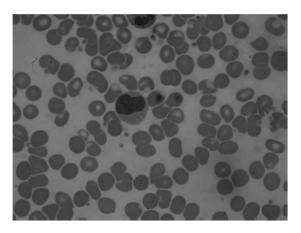
Command: rgb2gray(img)



Step 3: Removing noise from the image. The unwanted parts are removed from the image.

Command: strel('disk',20)

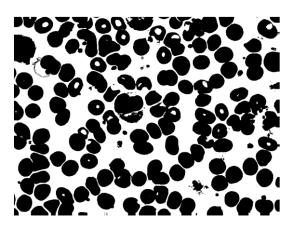
Imerode(bw,se)



Step4: Binarization. The image needs to be a binary image to analyze it, so it needs to be converted from gray scale to a binary image.

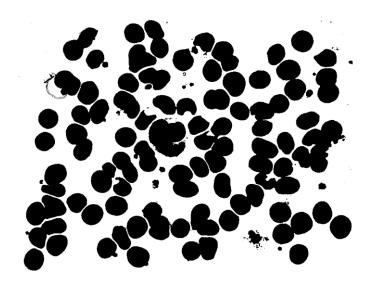
Command: adaptthresh(g,0.55)

imbinarize(g,t)



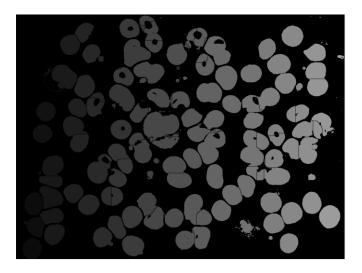
Step 5: Clear border touching cells. The cells touching to the border are removed as it cannot be involved in further process.

Command: imclearborder(d)



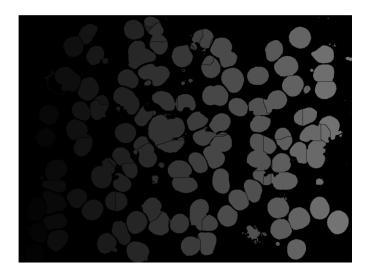
Step 6: Watershed segmentation. Some cells might be overlapped. So to remove the overlapping watershed segmentation is used.

Command: watershed(bw)

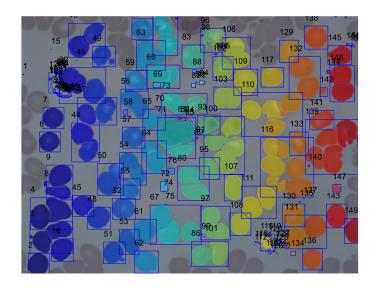


Step 7: Fill the holes

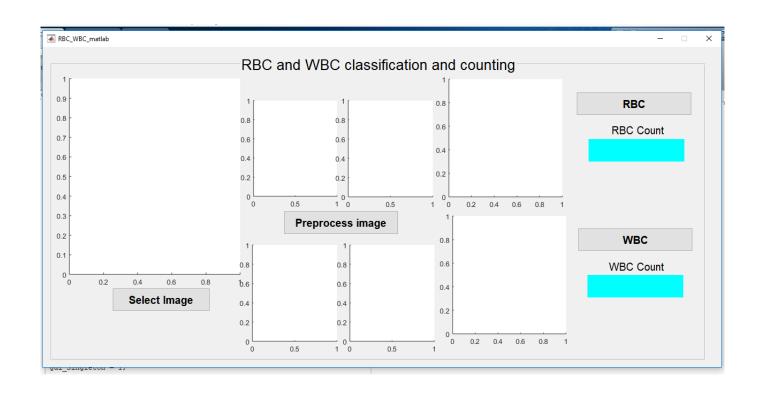
Command: imfill(bw,holes)

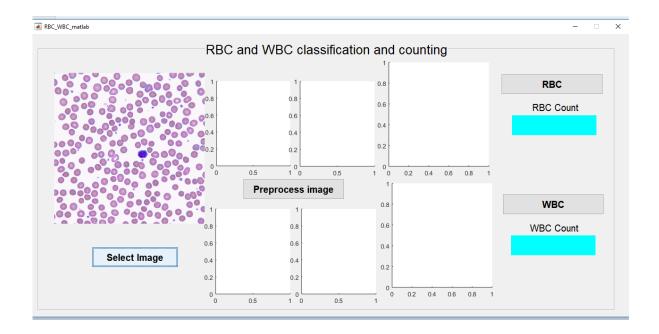


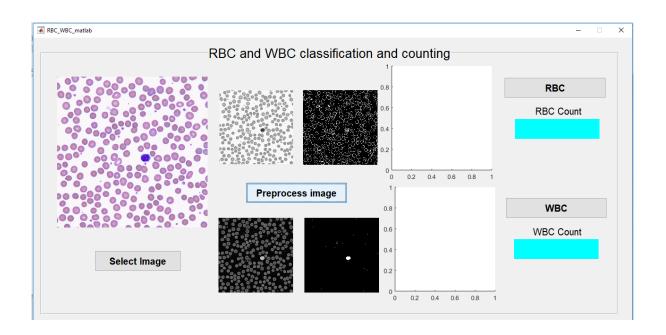
Step 8: Calculate cells using bounding box property. The following property is used to draw a rectangle around the blood cells to show the results. Command: regionprops(table,bw)

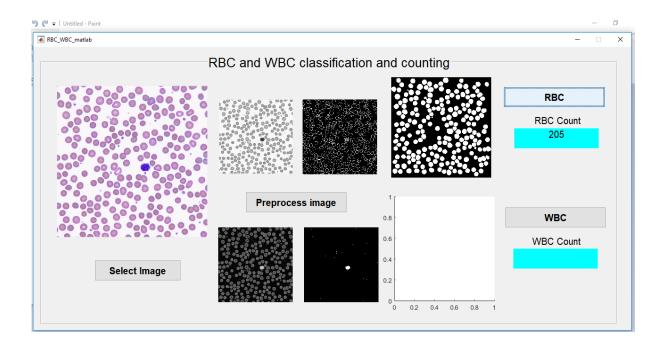


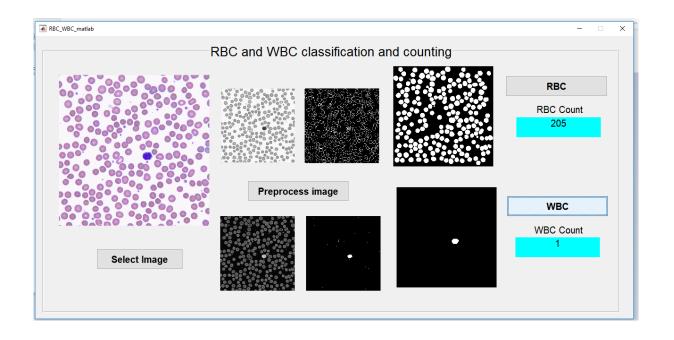
Step 9: Calculating different features of cells and creating its database and GUI.











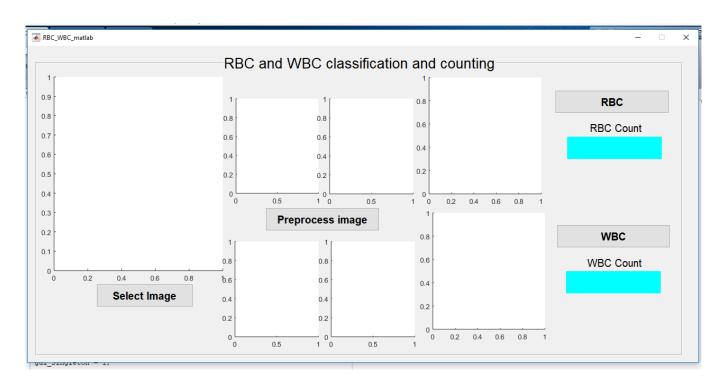
CHAPTER 5

- Results
- Conclusion

Results

From the images of blood samples which were collected we have obtained the total number of count of erythrocytes (RBCs) & leucocytes (WBCs) successfully.

With the help of this count later we can calculate the total number of RBCs and WBCs present in the patient whose sample is being collected.



Display of GUI

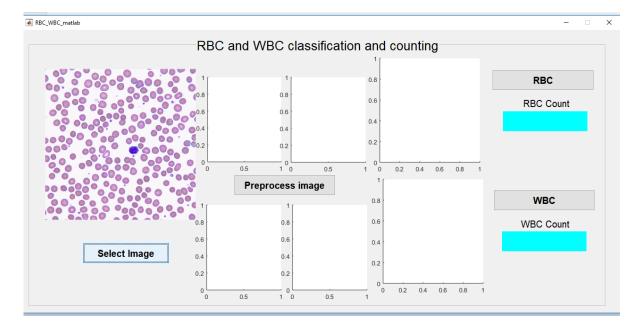


Image after pressing select image button

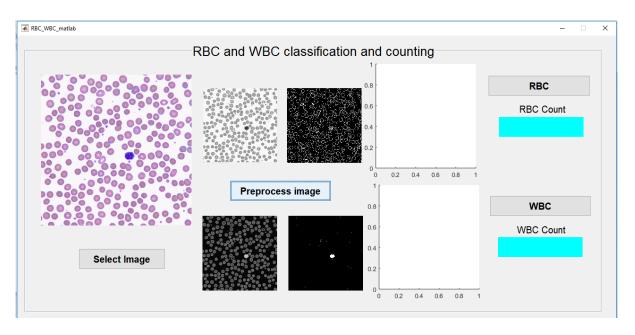


Image after pressing preprocess image

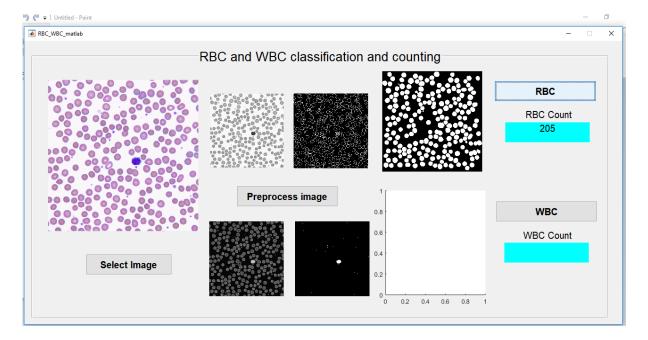


Image after pressing RBC

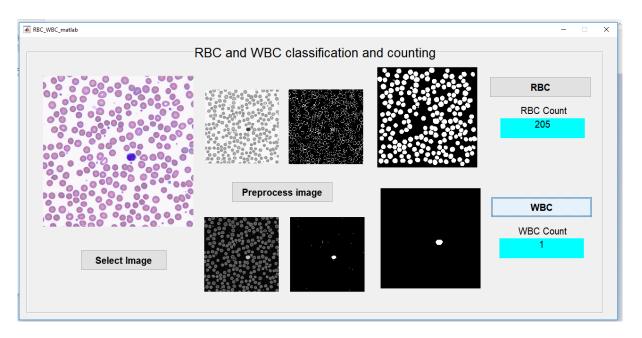


Image after pressing WBC

• Conclusion

Literature shows that traditional methods are very tedious, have less accuracy and depend on the patience of the technician in pathology labs.

Using image processing techniques is useful and better than existing techniques of medical diagnosis.

Experimental results have shown that the RBCs and WBCs count can be obtained with the help of MATLAB software.

This project introduced an algorithm to classify and count blood cells from image with the help of Image processing.

The classifier used here to classify the blood cells is k-NN classifier. The results have shown that this classifier has worked successfully and helped us to obtain required output.

CHAPTER 6

- Future Recommendation
- Application

• Future Scope

This project is the future of medical science. It shows that the new algorithm has high accuracy. The process used is quite efficient and easy to handle and thus in near future this method can be brought into practice.

Although we have better results for RBCs and WBCs the system should be further improved to detect the platelets.

This method can also be used to detect sickle blood cells. Sickle blood cells are certain type of RBCs whose shape gets changed due to inherited blood disorder. Normally RBCs are circular in shape but due to sickle cell disease the RBCs become crescent shaped which cause them to break apart easily. This results to anaemia. So the detection of sickle cells can be done using this method.

Since this method is efficient and time saving it has bright future in hospitals and pathological labs and also in those areas where pathological labs aren't available.

• Applications

- 1) It can be used in such rural areas where Pathological Labs are not easily available and affordable.
- 2) It can also be used in hospitals for easy and early outputs.
- 3) This modern technique can be used in pathological labs instead of traditional technique to obtain better results.
- 4) One of the important risk factor is infection and spreading of viruses, so this method can be easily brought into use since there are minimum chances of communication.

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