A Project Report On

AUTOMATED BLOOD CELLS SEGMENTATION & COUNTING

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University of Mumbai

CERTIFICATE

This is to certify that the project entitled "**Automated Blood Cell Segmentation & Counting"** is the bonafide work carried out by "**Siddique Sufiyan, Haidery Waqar Ahmed, Shaikh Waqas Ahmed, Sayyed Mohammed Rizwan**" students of B.E., Kalsekar Technical Campus, Panvel, during year 2014, in partial fulfilment of the requirements for the award of the Degree of B.E EXTC & that the project has not formed the basis for the award previously of any degree, diploma, associate ship, fellowship or any other similar titles.

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H.O.D

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(External)

Date :

DECLARATION

We hereby declare that the project entitled "**Automated Blood Cell Segmentation & Counting"** submitted for the B.E. Degree in Our original work the project has not formed the basis for the award of any degree, associate ship, fellowship or any other similar titles.

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ACKNOWLEDGEMENTS

It is with great enthusiasm and learning spirits that we are bringing out this project report. Before we get into the thick of things on this joyful occasion, we present our whole hearted complements with high regards and warm thanks to one and all that are the bond behind the sinews of this project. We give all glory and honor to Almighty God whose blessings made this Endeavour a success.

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ABSTRACT

Blood cell segmentation and identification are centered on the pre-processing of the image, segmentation of the Regions of Interest (ROI) and identification or classification of the cells. Recent studies have suggested different method for segmentation and identification of blood cells. Counting of blood cells (rbc or wbc) in blood cell images is very important to detect as well as to follow the process of treatment of many diseases like anaemia, leukaemia, lung nodule etc. However, locating, identifying and counting of blood cells manually are tedious and time consuming that could be simplified by means of automatic analysis, in which segmentation is a crucial step. We present an approach to automatic segmentation and counting of blood cells in microscopic blood cell images using Hough Transform. Morphological is a very powerful tool in image processing, and it is been used to segment and extract the blood cells (rbc or wbc) from the background and other cells. The algorithm used features such as shape of desired blood cells for counting process. The result presented here is based on images with normal blood cells.

The process is initiated by image acquisition and image enhancement process. Noise removal from the blood smear image is the first step. This removes the unwanted pixels from the image. Further the edges are preserved and binarization of the image is performed, separating the region of interest from the background. Further the edges are preserved and binarization of the image is performed. Then the task is to differentiate red blood cells from the various other components in the blood by the segmentation process. Morphological operations are applied on the blood image followed by RBC counting using Hough transform which is an efficient image segmentation technique. The primary goal of the proposed system is to detect and count all the RBC including the overlapping ones in the blood smear image.

Keywords: Red blood cell, SCILAB, Hough Transform, Morphological Image Processing, Oject detection and counting.

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Chapter 1

Introduction

1.1 Red Blood Cells

Red blood cells (RBCs), also called erythrocytes, are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (O2) to the body tissues via the blood flow through the system. They take up oxygen in the lungs or gills and release it into tissues while squeezing through the body's capillaries.

The cytoplasm of erythrocytes is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the red color of the cells. The cell membrane is composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deform-ability and stability while traversing the circulatory system and specifically the capillary network.

In humans, mature red blood cells are flexible and oval biconcave disks. They lack a cell nucleus and most organelles, in order to accommodate maximum space for hemoglobin. Approximately 2.4 million new erythrocytes are produced per second. The cells develop in the bone marrow and circulate for about 100120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells.

Red blood cells are also known as RBCs, red cells, red blood corpuscles, hematites, erythrocyte cells or packed red blood cells (pRBC) are red blood cells that have been donated, processed, and stored in a blood bank for blood transfusion.

One of the important information that doctors usually use to diagnose different diseases is the RBC count. The red blood cells are the most numerous blood cells in the human body, and it also called erythrocytes. The red blood cells are functioned to carry oxygen throughout our body. In health, the red blood cells vary relatively little in size and shape. In well-spread, dried and stained films the great majority of cells have round, smooth contours and diameters within the comparatively narrow range of 6.08.5m. According to American Cancer Society (2009), the normal red blood cell in our body is divided into four categories of ages, which are newborn, children, women and men. The effect of having high red blood cells in our blood

is it can be an indication of an undetected heart or lung problems. When any of these organs is not functioning properly, then blood oxygen levels go down.

Blood Cell Type	**Gender**	
	Men	**Women**
RBC	4.5-6.0 million/ microlitre	4.0-5.0 million/ microlitre
WBC	4.5-11 thousand/ microlitre	4.5-11 thousand/ microlitre
Platelet	150-450 thousand/ microlitre	150-450 thousand/ microlitre
Hematocrit	42% - 50%	36% - 45%
Hemoglobin	14-17 grams/100 mililitres	12-15 grams/100 mililitres

Table 1.1: Normal Blood Count Differentiate By Gender

Counting of red blood cells in a blood sample can give the pathologists valuable information regarding various hematological disorders. In the classical method for diagnosis of red blood examination in a blood sample, it is counted by manpower; hence it has deficiencies such as poor reliability, low efficiency and strong subjectivity. The diagnosis is the process of finding out what kind of disease a certain patient has and those diagnosed must always be accurate. A wrong diagnosis may lead the situation and condition of a patient become worst and some case, patient dies due to wrong dosage of drugs given. In order to overcome that weakness, some researchers have done some useful works especially in classifying blood cells from other cells, for example, classifying white blood cells from other cells such as red blood cells and platelets. Most of the researchers have concentrated on the classification of white blood cells since most of the diseases are easy to determine by analyzing the change in white blood cells. However, by counting the red blood cells, it also provides some information about the abnormal condition in our body. Analysis of microscopic images is used in many fields of technology and medicine. In some medical experiments, some drugs, with known effects in red blood cells membranes, are used to find out their activity.

1.2 Blood diseases involving the Red Blood Cells

Some blood diseases involving the red blood cells include:

- Anemias (or anemias) are diseases characterized by low oxygen transport capacity of the blood, because of low red cell count or some abnormality of the red blood cells or the hemoglobin.
- Sickle-cell disease is a genetic disease that results in abnormal hemoglobin molecules. When these release their oxygen load in the tissues, they become insoluble, leading to mis-shaped red blood cells. These sickle shaped red cells are less deformable and viscoelastic meaning that they have become rigid and can cause blood vessel blockage, pain, strokes, and other tissue damage.
- Thalassemia is a genetic disease that results in the production of an abnormal ratio of hemoglobin subunits.
- Spherocytosis is a genetic disease that causes a defect in the red blood cell's cytoskeleton, causing the red blood cells to be small, sphere-shaped, and fragile instead of donut-shaped and flexible.
- Pernicious anemia is an autoimmune disease wherein the body lacks intrinsic factor, required to absorb vitamin B12 from food. Vitamin B12 is needed for the production of hemoglobin.
- Aplastic anemia is caused by the inability of the bone marrow to produce blood cells.
- Pure red cell aplasia is caused by the inability of the bone marrow to produce only red blood cells.
- Hemolysis is the general term for excessive breakdown of red blood cells. It can have several causes and can result in hemolytic anemia.
- The malaria parasite spends part of its life-cycle in red blood cells, feeds on their hemoglobin and then breaks them apart, causing fever. Both sickle-cell disease and thalassemia are more common in malaria areas, because these mutations convey some protection against the parasite.
- Polycythemias (or erythrocytoses) are diseases characterized by a surplus of red blood cells. The increased viscosity of the blood can cause a number of symptoms.
- In polycythemia vera the increased number of red blood cells results from an abnormality in the bone marrow.

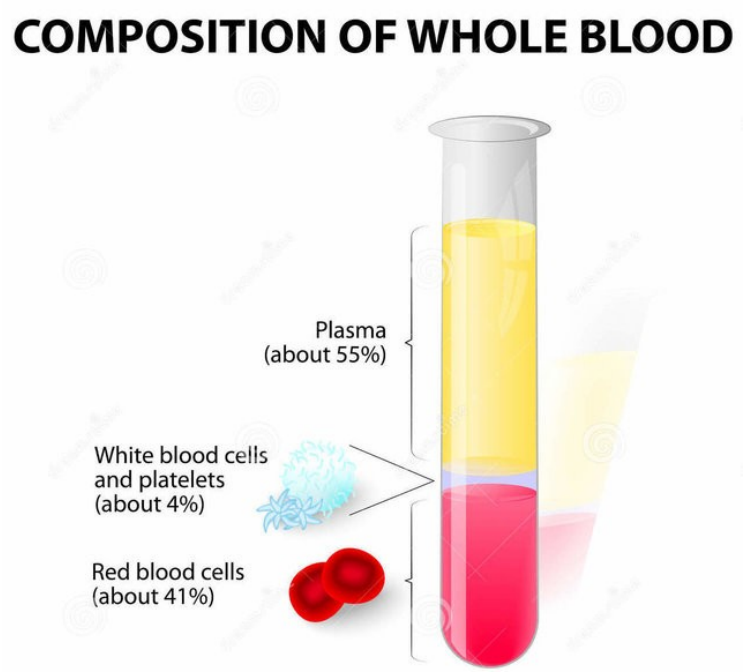

Fig 1.1 Composition of blood

1.3 Complete Blood Count

A complete blood count (CBC), also known as full blood count (FBC) or full blood exam (FBE) or blood panel, is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood. A scientist or lab technician performs the requested testing and provides the requesting medical professional with the results of the CBC.

The cells that circulate in the bloodstream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes). Abnormally high or low counts may indicate the presence of many forms of disease, and hence blood counts are amongst the most commonly performed blood tests in medicine, as they can provide an overview of a patient's general health status. A CBC is routinely performed during annual physical examinations in some jurisdictions.

Chapter 2

Literature Survey

- **M.Habibzadeh, A. Krzyak, T.Fevens, A.Sadr, Counting of RBCs and WBCs in noisy normal blood smear microscopic images, Proc. of SPIE Vol. 7963 79633I-1, 05 July 2011.**

  This paper presents an accurate, robust mechanism to determine the distribution of blood smear particles. This work does not address issues such as deformed RBC shapes (teardrops, crescents, needles, or a variety of other forms), infected RBCs and extra overlapping cells which can be found with certain types of diseases. A set of blood smear test images are used to show that their proposed framework is more accurate in comparison with some classical methods, and also is much more robust for degraded images which are blurry and/or noisy. The first comparison method de-noises the image with a median filter with a 3x3 mask function and then uses Otsu binarization to create an intermediate image. Then a second intermediate image is created using Canny edge detection. The second comparison method performs the same procedure using Otsu binarization but skips the Canny edge detection step.

- **Alaa Hamouda, Ahmed Y. Khedr, and Rabie A. Ramadan, Automated Red Blood Cell Counting, INTERNATIONAL JOURNAL OF COMPUTING SCIENCE, VOL. 1, NO. 2, FEBRUARY, 2012.**

  An image involves some unwanted particles (noise). Therefore, some preprocessing is needed which is called image preparation phase. This phase consists of two steps which are histogram equalization and segmentation. In the first step, histogram equalization, the intensity value of the given image is adjusted using image intensity transformation. In the second step, segmentation, blood cell are detected by differentiate them from the background in terms of contrast. Changes in contrast can be detected by image processing operators that calculate the gradient of an image. Then a threshold can be applied to create a binary mask containing the segmented cell. The edge detection is done by using the Sobel operator. In the second phase,

blood cells extraction, they have applied different methods looking after the highest accuracy as well as less complexity.

- **J.M. Sharif, M. F. Miswan, M. A. Ngadi, Md Sah Hj Salam, Red Blood Cell Segmentation Using Masking and Watershed Algorithm: A Preliminary Study, 2012 International Conference on Biomedical Engineering (ICoBE),Penang,Malaysia,27-28 February 2012.**

  The methods used are Yeber color conversion, masking, morphological operators and watershed algorithm. The combination of Yeber color conversion and morphological operator produce segmented white blood cell nucleus. Then it is being used as a mask to remove WBC from the blood cell image. Morphological operators involve binary erosion to diminish small objects like platelet. The resulted RBC segmentation is passed through marker controlled watershed algorithm which handles overlapping cells. Improvement needs to be done for both segmentation and overlapped cell handling to obtain better results in the future.

- **Meng-Ling Feng and Yap-Peng Tan, Adaptive Binarization Method for Document Image Analysis, 2004 IEEE International Conference on Multimedia and Expo (ICME).**

  This paper proposed an adaptive binarization method, based on a criterion of maximizing local contrast. The proposed method overcomes, to a large extent, the general problems of poor quality images. . The common problems in poor quality text images include variable background intensity due to non-uniform illumination, low contrast and large amount of random noise due to limited sensitivity of camera. Therefore, to have an accurate analysis of the, a versatile binarization method, which can correctly remove noise and unnecessary background and reliably keep all useful information, becomes indispensable.

- **Raman Maini Dr. Himanshu Aggarwal, Study and Comparison of Various Image Edge Detection Techniques, International Journal of Image Processing (IJIP), Volume (3): Issue (1).**

  Gradient-based algorithms such as the Prewitt filter have a major drawback of being very sensitive to noise. The size of the kernel filter and coefficients are fixed and cannot be adapted to a given image. An adaptive edge-detection algorithm is necessary to provide a robust solution that is adaptable to the varying noise

levels of the images to help distinguish valid image contents from visual artefacts introduced by noise. The performance of the Canny algorithm depends heavily on the adjustable parameters, which is the standard deviation for the Gaussian filter, and the threshold values, T1 and T2. also controls the size of the Gaussian filter. The bigger the value for, the larger the size of the Gaussian filter becomes. This implies more blurring, necessary for noisy images, as well as detecting larger edges. As expected, however, the larger the scale of the Gaussian, the less accurate is the localization of the edge. Smaller values of imply a smaller Gaussian filter which limits the amount of blurring, maintaining finer edges in the image. The user can tailor the algorithm by adjusting these parameters to adapt to different environments. Canny's edge detection algorithm is computationally more expensive compared to Sobel, Prewitt and Roberts operator. However, the Cannys edge detection algorithm performs better than all these operators under almost all scenarios. Evaluation of the images showed that under noisy conditions, Canny, LoG, Sobel, exhibit better performance, respectively.

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Chapter 3

Problem Statement & Objective

3.1 Problem Definition

Currently the complete blood count is done using automated methods such as flow cytometry. Such methods give accurate results, but are costly. Also these methods give only the count of the cells in the blood. They cannot be used to detect irregularities or variation in the shape and size of the cells. Counting using the hemocytometer is manual and prone to human errors during counting. The pathologist has to differentiate between various types of cells in the blood while analyzing multiple blood samples can become strenuous. Counting overlapping blood cells is also a major problem. These methods require state of the art biomedical instruments which are costly and require trained personnel to operate.

We will try to develop a system which will overcome the above drawbacks. The proposed system will take a magnified image of the blood smear and apply various image processing techniques to count the number of red blood cells in it. White blood cells, platelets will automatically be removed from the image. This reduces the region of interest. Overlapping cells can be easily detected. The user of the system does not have to be trained in any particular way. Any person with basic computing skill can easily operate the system. The system can be installed on basic computer configuration. Hence it will have widespread use and ease the method of counting.

3.2 Existing System

Counting the cells in a patient's blood was performed manually, by viewing a slide prepared with a sample of the patient's blood under a microscope. Nowadays, this process is generally automated by use of an automated analyzer. The hemocytometer or hemocytometer is a device originally designed for the counting of blood cells. It is now also used to count other types of cells as well as other microscopic particles. The hemocytometer was invented by Louis-Charles Malassez and consists of a thick glass microscope slide with a rectangular indentation that creates a chamber. This chamber is engraved with a laser-etched grid of perpendicular

lines. The device is carefully crafted so that the area bounded by the lines is known, and the depth of the chamber is also known. It is therefore possible to count the number of cells or particles in a specific volume of fluid, and thereby calculate the concentration of cells in the fluid overall. Complete blood count performed by an automated analyzer. The blood is well mixed (though not shaken) and placed on a rack in the analyzer. This instrument has many different components to analyze 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.

Fig 3.1: Hematology analyzers

Blood counting machines aspirate a very small amount of the specimen through narrow tubing. Sensors count the number of cells passing through the tubing, and can identify the type of cell; this is flow cytometry. The two main sensors used are light detectors and electrical impedance. Because an automated cell counter samples and counts so many cells, the results are very precise. However, certain abnormal cells in the blood may not be identified correctly, requiring manual review of the instrument's results and identification of any abnormal cells the instrument could not categorize. In addition to counting, measuring and analyzing red blood cells, white blood cells and platelets,automated hematology analyzers also measure the amount of hemoglobin in the blood and within each red blood cell. This information can be very helpful to a physician who, for example, is trying to identify the cause of a patient's anaemia. If the red cells are smaller or larger than normal, or if there is a lot of variation in the size of the red cells, this data can help guide the direction of further testing and expedite the diagnostic process so patients can get the treatment they need quickly.

3.3 Drawbacks of Existing System

Manual counting is useful in cases where automated analyzers cannot reliably count abnormal cells, such as those cells that are not present in normal patients and are only seen in peripheral blood with certain hematological conditions. Manual counting is subject to sampling error because so few cells are counted compared with automated analysis.

Medical technologists examine blood film via a microscope for some CBCs, not only to find abnormal white cells, but also because variation in the shape of red cells is an important diagnostic tool. Although automated analysers give fast, reliable results regarding the number, average size, and variation in size of red blood cells, they do not detect cells' shapes. Also, some normal patients' platelets will clump in anti-coagulated blood, which causes automatic analyses to give a falsely low platelet count. The technician viewing the slide in these cases will see clumps of platelets and can estimate if there are low, normal, or high numbers of platelets.

3.4 Aims & Objectives

A single blood smear image can be processed multiple times for various detections of blood components unlike an original blood sample. The aim of this project and related work is to

- Automate the process of red blood cell counting.
- Develop and validate the necessary image processing steps to quantify and count peripheral blood particles on blood smear slides.
- Ease the working of the pathologist.
- To help the doctor make a better diagnosis.
- To implement the system in an open source environment which helps to reduce the cost of the system.

The objectives are to differentiate RBCs and WBCs which are present in a blood smear slide, average size estimation of the RBC particles, detect the overlapping RBC, mitigate problems posed by different conditions such as noisy and degraded images, differing blood staining techniques, various types of microscope illumination, overlapping and adjacent cells, to get an efficient result, to get an accurate result.

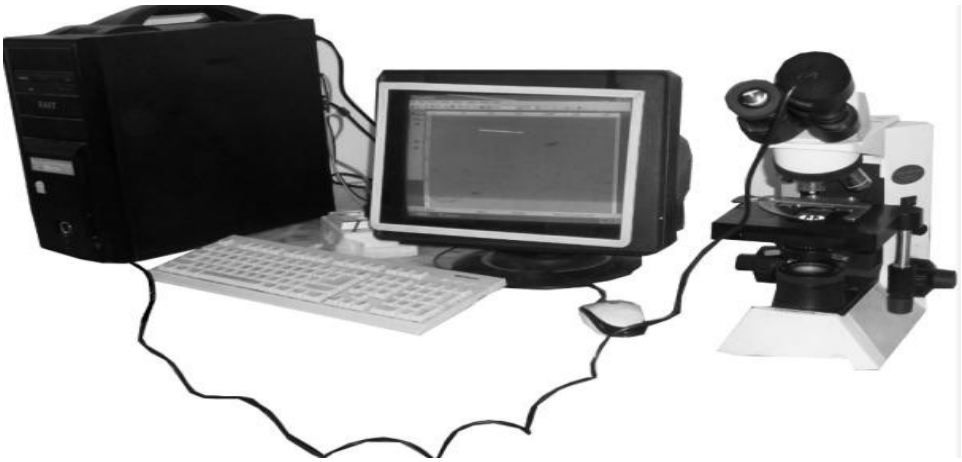


Fig 3.2: Complete system for cell counting

3.5 Scope

Automation of red blood cell counting is not widespread in our country. Practitioners are reluctant to perform newer methods and change their ways. This is due to reduced knowledge of the system as well as the cost of the automated analyzers. Our proposed system will be easy to operate. It can be used in the pathological laboratories. Personnel with even basic knowledge of computers will be able to operate the system. The system will reduce the time taken to count the RBC. This will increase the amount of samples that can be analyzed in unit time and hence give better results. It will also remove the errors induced due to human limitations. Hence the improved results will result in a better diagnosis of diseases related to the count of RBC.

Chapter 4

Software Tools

4.1 Scilab

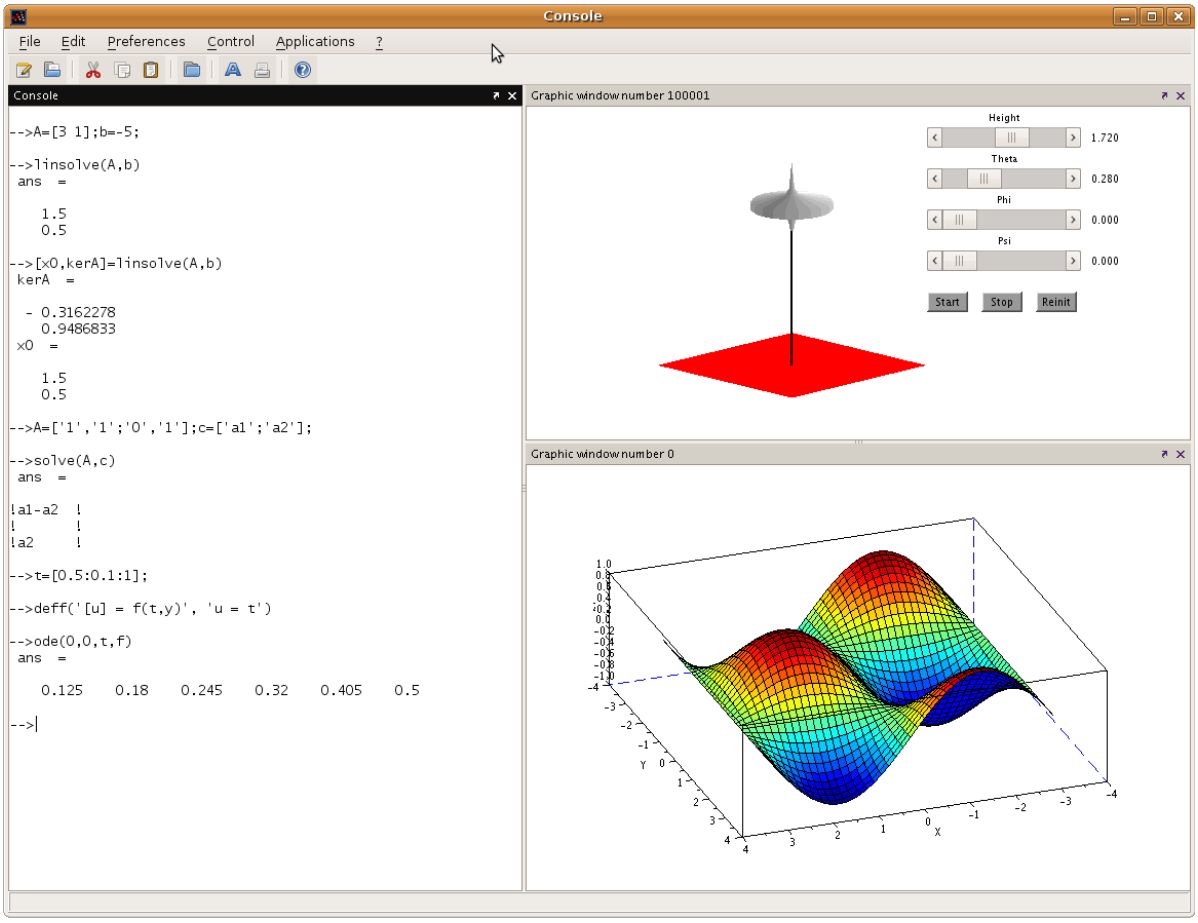


Fig 4.1: Scilab console window

4.1.1 Overview of Scilab

Scilab is a programming language associated with a rich collection of numerical algorithms covering many aspects of scientific computing problems. From the software point of view, Scilab is an interpreted language. This generally allows to get faster development processes, because the user directly accesses a high-level language, with a rich set of features provided by the library. The Scilab language is meant to be extended so that user-defined data types can be defined with possibly overloaded operations. Scilab users can develop their own modules

so that they can solve their particular problems. The Scilab language allows to dynamically compile and link other languages such as Fortran and C: this way, external libraries can be used as if they were a part of Scilab built-in features. Scilab also interfaces LabVIEW, a platform and development environment for a visual programming language from National Instruments.

From the license point of view, Scilab is a free software in the sense that the user does not pay for it and Scilab is an open source software, provided under the Cecill license. The software is distributed with source code, so that the user has an access to Scilab's most internal aspects. Most of the time, the user downloads and installs a binary version of Scilab, since the Scilab consortium provides Windows,Linux and Mac OS executable versions. Online help is provided in many local languages.

4.1.2 Image Processing using Scilab

Scilab can be used for image processing and its analysis by using toolbox which can be externally installed by Atoms. Atoms is a external module management for Scilab, which contributed Scilab development by writing external modules that extend Scilab capabilities in specific fields. Since Scilab has many contributed toolboxes for different tasks, the toolboxes which is used by our system is,

- Scilab Image Processing Toolbox (SIP)
- Image Processing Design Toolbox (IPD)

4.1.3 Software Specification

General

Software Name	: Scilab
Version	: 5.5
Category	: Math Software

System Requirements

Operatin System	: Ubuntu 14.04, Linux Mint
File Size	: 170MB

4.1.3 Mini Tutorial on Scilab

- **Reading an image**

  To read an image into a Scilab variable, one must type, for example:

  – > im = imread(’myimage.png’);

  The file format is automatically detected. From now on the image is in memory, stored as an array of pixels. We call gray-scale those images represented as a 2D array with values from 0 to 2^16 - 1 (65535). True-color images are made of three gray-scale images, one for each channel (Red, Green and Blue), represented in a 3D array (N × M × 3).

- **Viewing an image**

  To show a true-color or gray-scale image:

  – > imshow(im);

- **Writing a matrix as an image file**

  – > imwrite(im,’foo.jpeg’);

  or

  – > imwrite(im, map,’foo.jpeg’);

Chapter 5

System Design

5.1Flow Chart

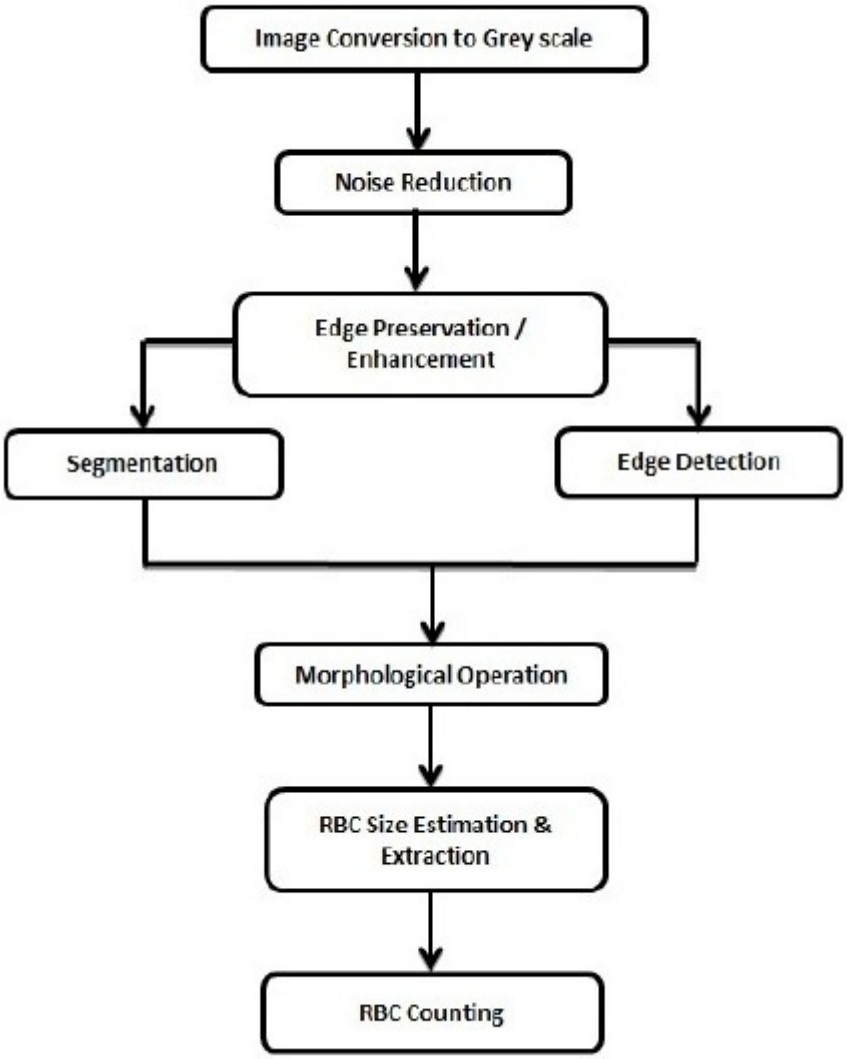


Fig 5.1: Detailed Block Diagram of System

5.1.1 Image Acquisition

Figure shows the flowchart of the red blood cells counting process. In data acquisition, we used sample images from an online medical library as an input image. These images can be acquired by the user through a simple user interface, from their computers. As these images contain noise and are not fit for direct processing they need to be enhanced for further analysis.

5.1.2 Noise Removal

This is a preprocess of an image sequence before feeding into the segmentation process. The image is first converted to its corresponding grey level images. To design a reliable system that maybe used under different conditions such as different blood staining techniques, types of chemical materials used, microscope types, illumination conditions, human errors, etc. a pre processing step is required. we will apply any Digital filter in it appropriately the best.

Some Type of Noise Found in Image Processing:

- Salt and pepper noise: random occurrences of both black and white intensity values
- Impulse noise: random occurrences of white intensity values
- Gaussian noise: impulse noise but its intensity.

5.1.3 Edge Preservation

Edge preservation is an image processing technique to recover degraded and blurred images resulted while reducing the negative effect of noise in images. It can be a preliminary step toward better binarization and object segmentation. In our project Canny edge detection algorithm, on the noise removed image, to mark the edges of the cells. It was observed that edge detection produced best results in case of sharp images whereas in blurry images the accuracy of edge detected is reduced. Since the cells are circular in shape we expected the edge detected to be circular and complete. But in the case of practical images, after applying edge detection we found certain edges were incomplete. On the whole the result of edge detection stage was an image, in which there were two types of edges,

- The complete ones complete cell was enclosed in the boundary;
- The broken ones the entire edge was not detected.

5.1.4 Mathematical Morphological Operation

Mathematical morphology will be used to segment RBC based on elimination WBC appearance. Morphological image processing is based on a strong mathematical concept which been used to change the size, shape, structure and connectivity of objects in the image. It involves binary erosion, dilation, opening, closing and reconstruction. The technique also extended the use in greyscale image. Erosion plays the role to shrinks and thins objects in image while dilation used to grows and thickens objects in image. Next, morphological opening is the combination process of erosion and continued by dilation while morphological closing is using the concept of dilation and continued by erosion. In other words, the functions of morphological opening are to removes, break and diminished the connection or objects which not contain the structure elements. In contrary, morphological closing functions to join, fill and build connection and objects in the image. Using the closing image transformation with defined SE (Structure Element) unwanted edges, typically generated, are removed.

5.1.5 Red Blood Cell Extraction

A normal blood cell is primarily one of two major particles: a RBC with a normal Probability Distribution Function (PDF) around 6.08.5 m or a WBC (7-18 m) which includes a nucleus and cytoplasm is about 1-3 times bigger than normal and mature RBCs. Moreover, WBCs are classified into five main shape groups with varying degrees of non-convexity. We use size characteristics as an effective factor to distinguish between the two main types of RBC and WBC cells. After morphological closing on the image we have an image with some broken edges and filled cells. We remove the broken edges from the image so that we are left with the filled cells only. Now the actual size extraction algorithm starts. We find the area of all the regions that are filled and then find the statistical parameters like mean, median, standard deviation etc. Since most of the regions are single cells it is expected that the mean value of the regions will give an idea of size of single cells in an image. Using this concept we have made three ranges, Single Cell Size = (Mean Standard Deviation) to (Mean + Standard Deviation) Double Cell size = (Mean + Standard Deviation +1) to (2*Mean + Standard Deviation) Triple Cell Size = (2*Mean + Standard Deviation+1) to (3*Mean+ Standard Deviation) We then find out number of regions falling in each range.

5.1.6 Red Blood Cell Counting

From the above we can count easily by

- No. of Single cells = No of regions in single cell image x 1
- No. of Double cells = No of regions in double cell image x 2

- No. of Triple cells = No of regions in triple cell image x 3
- Total Count = No. of Single cells + No. of Double cells + No. of Triple cells.
- Hence we will get the best result in counting.

Using the above method some cells whose edge is not completely detected are left out.

5.1.7 Hough Transform Method

The Hough transform is a feature extraction technique used in image analysis, computer vision, and digital image processing. The purpose of the technique is to find imperfect instances of objects within a certain class of shapes by a voting procedure. This voting procedure is carried out in a parameter space, from which object candidates are obtained as local maxima in a so-called accumulator space that is explicitly constructed by the algorithm for computing the Hough transform.

Circle Detection Process

The process of identifying possible circular objects in Hough space is relatively simple,

- First we create our accumulator space which is made up of a cell for each pixel, initially each of these will be set to 0.
- For each (edge point in image(i, j)): Increment all cells which according to the equation of a circle could be the center of a circle, these cells are represented by the letter 'a' in the equation.
- For all possible value of a found in the previous step, find all possible values of b which satisfy the equation.
- Search for the local maxima cells, these are any cells whose value is greater than every other cell in it's neighbourhood. These cells are the one with the highest probability of being the location of the circle(s) we are trying to locate.

Note that in most problems we will know the radius of the circle we are trying to locate beforehand, however if this is not the case we can use a 3 dimensional accumulator space, this is much more computationally expensive. This method can also detect circles that are partially outside of the accumulator space if enough of its area is still present within it. The Hough transform can be used to determine the parameters of a circle when a number of points that fall on the perimeter are known. A circle with radius R and center (a, b) can be described with the parametric equations.

$$(\text{i-a})^2 + (j-b)^2 = r^2$$

When the angle sweeps through the full 360 degree range the points (x, y) trace the perimeter of a circle. If an image contains many points, some of which fall on perimeters of circles, then the job of the search program is to nd parameter triplets (a, b, R) to describe each circle. The fact that the parameter space is 3D makes a direct implementation of the Hough technique more expensive in computer memory and time. If the circles in an image are of known radius R, then the search can be reduced to 2D.The objective is to nd the (a, b) coordinates of the centers.

$$x = a + R \cos(\theta)$$

$$y = b + R \sin(\theta)$$

The locus of (a, b) points in the parameter space fall on a circle of radius R centered at (x,y). The true center point will be common to all parameter circles, and can be found with a Hough accumulation array.

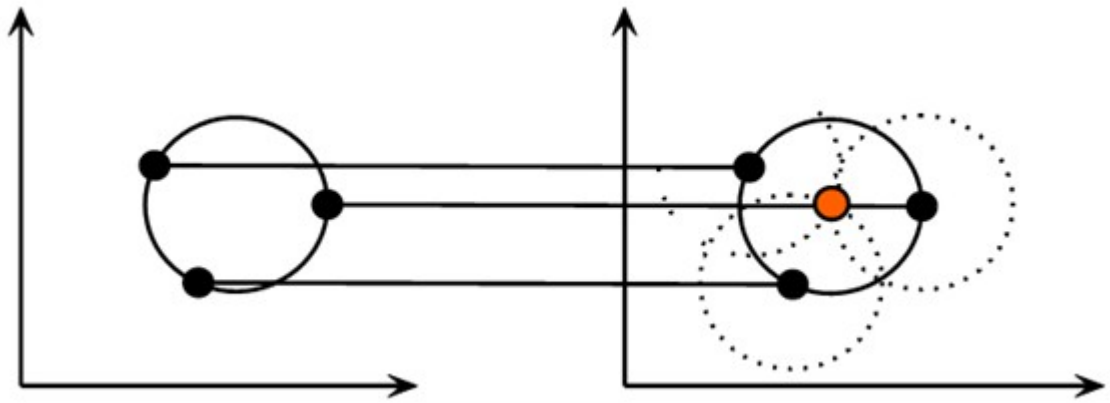


Fig 5.2: Circular Hough Transform

5.1.8 RBC Count Algorithm

Step 1: Start

Step 2: If file is selected in Image acquisition, go to step4.

Step 3: If file is not selected, go to step 9.

Step 4: Noise removal will be performed.

Step 5: Edge detection method by Canny Edge Detection Method.

Step 6: Morphological operation will be performed.

Step 7: RBC size extraction.

Step 8: RBC will be counted i.e. RBC Count.

Step 9: End.

Chapter 6

Implementation and Result

6.1 GUI (User Interface)

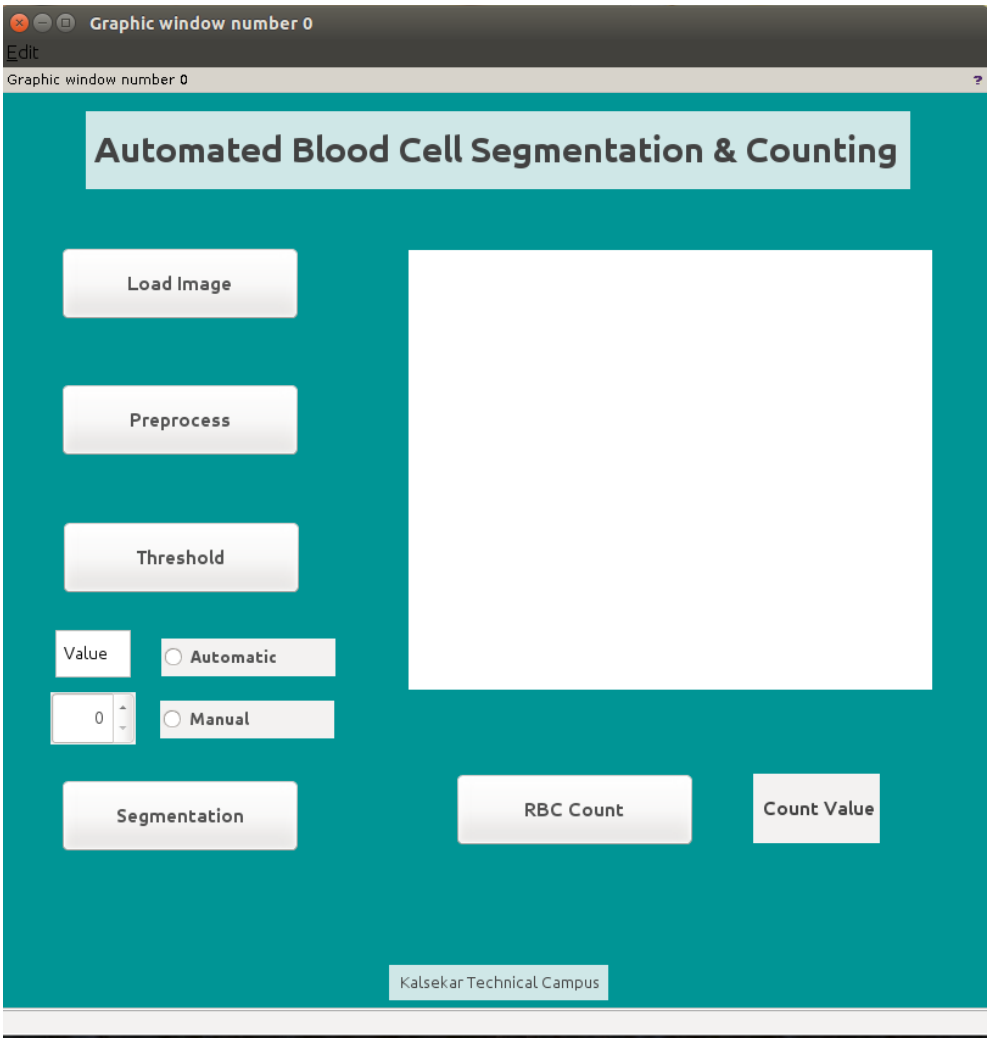


Figure 6.1: Graphical User Interface of the Automated RBC Count

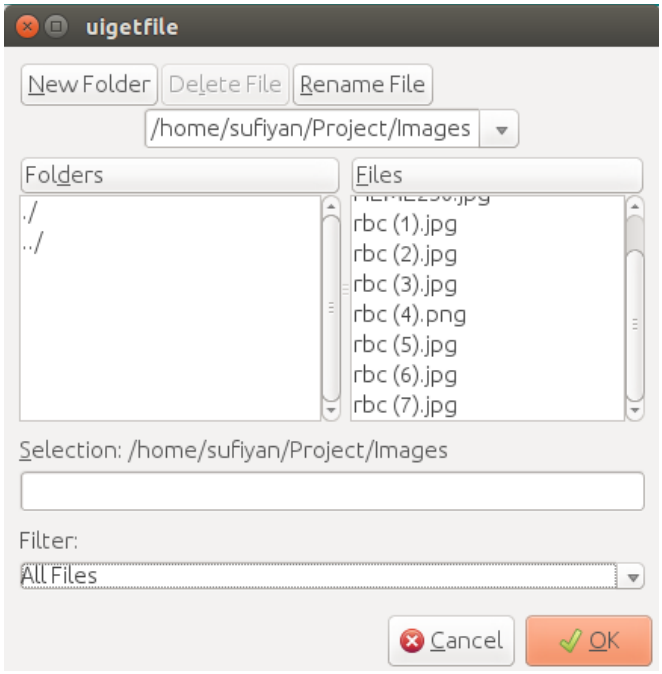


Fig 6.2: Image browsing through Interface.

6.2 Processing and Analysis

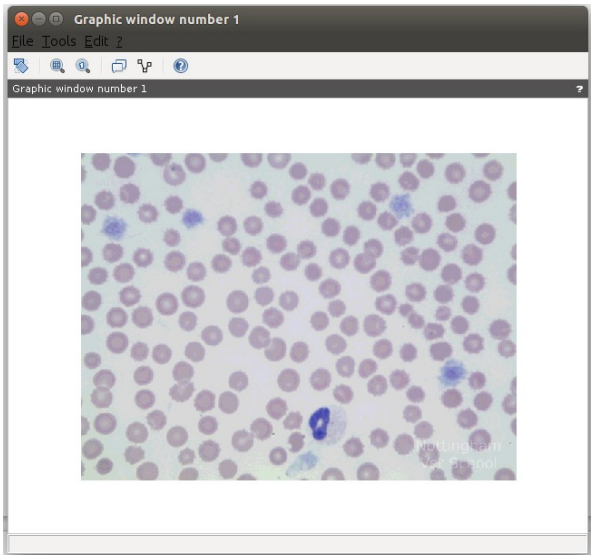


Fig 6.3:Original blood smear image as input

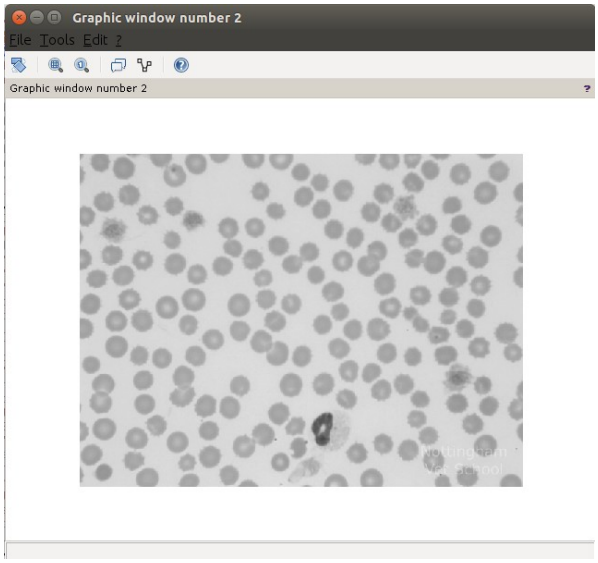


Fig 6.4: Grayscale of the Original Image

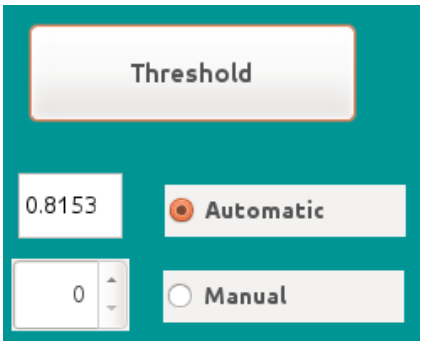


Fig 6.5: Gray threshold inf function to minimize the intra-class variance of black and white pixels

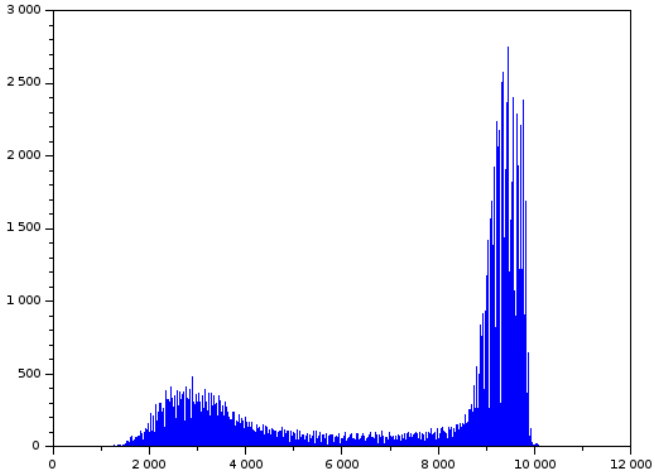


Fig 6.6: Histogram for thresholding

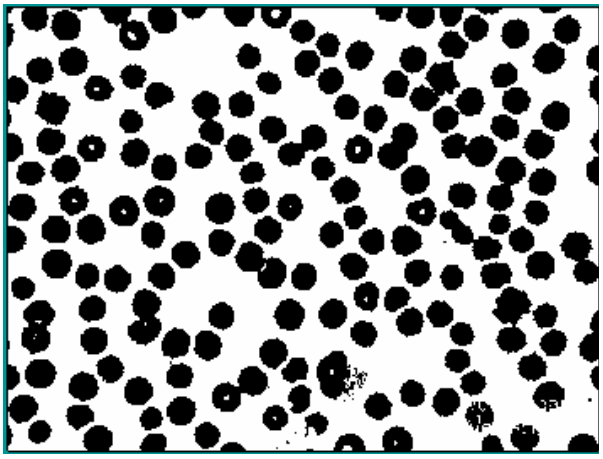


Fig 6.7: Binary image with automatic thresholding of 81%

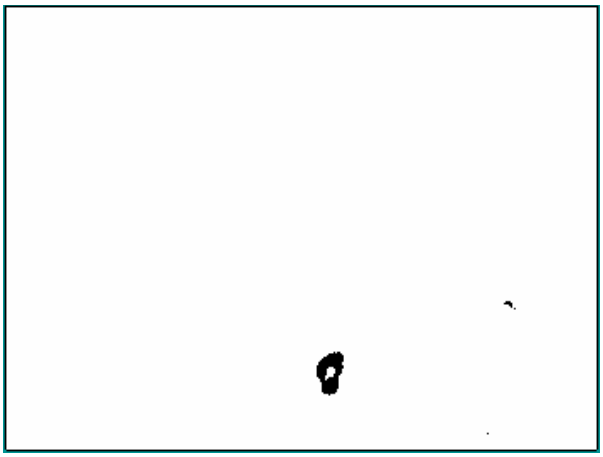


Fig 6.8: Binary image with manual thresholding of 51%

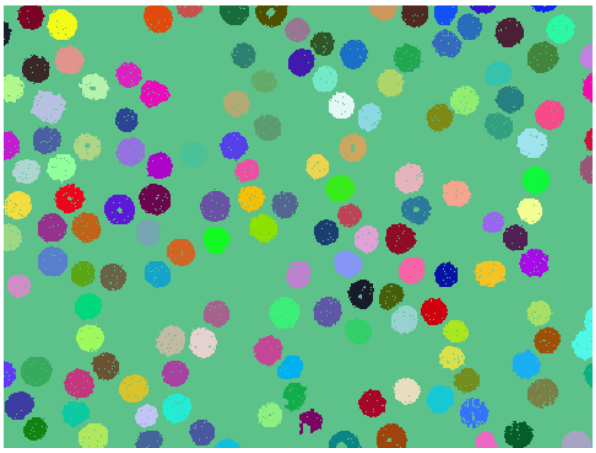


Fig 6.9: Segmented image with salt and pepper noise

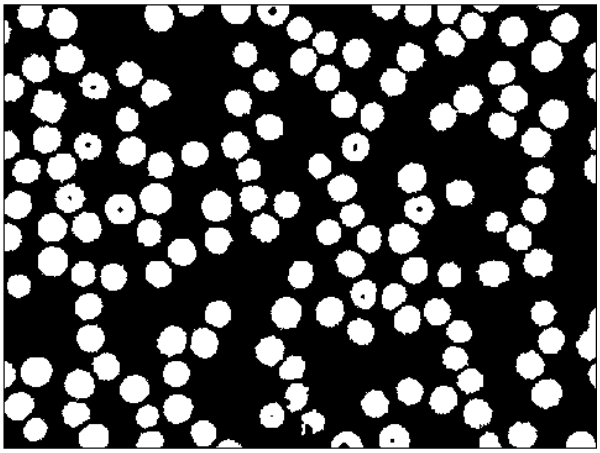


Fig 6.10: Filtered Image with WBC rejected

Fig 6.11: RBC count value on GUI

6.3 White Box Testing

White-Box testing sometimes called glass-box testing ,is test case design philosophy that uses the control structure described as part of component-level design to derive test cases.

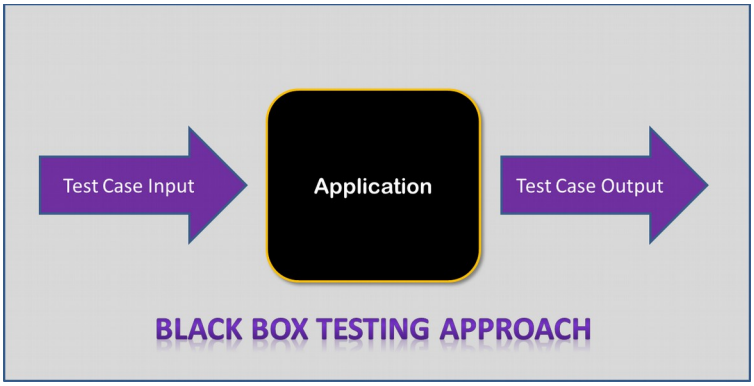
White-box testing:

- Knowing the internal workings of a product, test that all internal operations are performed according to specifications and all internal components have been exercised
- Involves tests that concentrate on close examination of procedural detail
- Logical paths through the software are tested
- Test cases exercise specific sets of conditions and loops

These Test Cases:

- Guarantee that all independent paths within a module have been exercised at least once
- Exercise all logical decisions on their true and false sides
- Execute all loops at their boundaries and within their operational bounds
- Exercise internal data structures to ensure their validity

6.4 Black Box Testing



Black-box testing, also called behavioral testing, focuses on the functional requirements of the software. That is, black-box testing enables the software engineer to Derive sets of input conditions that will fully exercise all functional requirements for program. Black-box testing is

not an alternative to white-box techniques. Rather, it is a complementary approach that is likely to uncover a different class of errors than white-box methods. Black-box testing attempts to find errors in the following categories:

(a) Incorrect or missing functions,

(b) Interface errors,

(c) Errors in data structures or external database access,

(d) Behavior or performance errors,

(e) Initialization and termination errors.

Unlike white-box testing, which is performed early in the testing process, black box testing tends to be applied during later stages of testing. Because black-box testing purposely disregards control structure, attention is focused on the information domain.

Tests are designed to answer the following questions:

- How is functional validity tested?
- How is system behavior and performance tested?
- What classes of input will make good test cases?
- Is the system particularly sensitive to certain input values?
- How are the boundaries of a data class isolated?
- What data rates and data volume can the system tolerate?
- What effect will specific combinations of data have on system operation?

By applying black-box techniques, we derive a set of test cases that satisfy the following Criteria:

(a) Test cases that reduce, by a count that is greater than one, the number of additional test cases that must be designed to achieve reasonable testing

(b) Test cases that tell us something about the presence or absence of classes of errors, rather than an error associated only with the specific test at hand.

6.5 Analysis

For reasons that are not completely clear, a greater number of errors tends to occur at the boundaries of the input domain rather than in the ”center.” It is for this reason that boundary value analysis (BVA) has been developed as a testing technique. Boundary value analysis leads

to a selection of test cases that exercise bounding values. Boundary value analysis is a test case design technique that complements equivalence partitioning. Rather than selecting any element of an equivalence class, BVA leads to the selection of test cases at the ”edges” of the class. Rather than focusing solely on input conditions, BVA derives test cases from the output domain as well.

Guidelines for BVA are similar in many respects to those provided for equivalence partitioning:
(a) If an input condition specifies a range bounded by values a and b, test cases should be designed with values a and b and just above and just below and b.
(b) If an input condition specifies a number of values, test cases should be developed that exercise the minimum and maximum numbers. Values just above and below minimum and maximum are also tested.
(c) Apply guidelines 1 and 2 to output conditions. For example, assume that a temperature vs. pressure table is required as output from an engineering analysis program. Test cases should be designed to create an output report that produces the maximum (and minimum) allowable number of table entries.

Test ID	**Input**	**Output**	**Output**	**RESULT**
TID_1	A properly stained blood smear image	Correct Total RBC Count	Correct Total RBC Count	Passed
TID_2	A blurred image	Very low RBC Count	Incorrect RBC Count	Passed
TID_3	An image which is not properly stained	Zero Count	Zero Count	Passed
TID_4	Inappropriate image	Zero Count	Displays few RBC Count	Failed

Chapter 7

Conclusion

7.1 Conclusion

As a conclusion, this research successfully uses various image processing techniques for Red Blood Cell Estimation.

- It utilizes morphological approaches for segmentation, extraction and estimation in order to solve problem in image processing of the red blood cells. The results of the image act as an accurate outcome of determining the number of red blood cells by using Hough transform technique.
- It proposes an image processing system that uses SCILAB software for blood cell counting. By using the SCILAB, all the importances aspects of a correct algorithm has been successfully produced.
- With a correct algorithm, the red blood cells can be detected and segmented as well as estimate the number of the red blood cells. It enables the study of the morphological features of RBC by the pathologist can determine whether the person is normal referring the amount of RBC in human blood.
- Actual volume of the blood sample is calculated with proper magnification factor. There is a need for fast and cost-effective production of blood cell count reports.
- This system includes an effective and efficient method in recognizing and counting blood cells as a practical alternative to the manual blood cell counting.
- Since it's an on-going study, more enhancements and improvement could be done in the future works. The system can be further improvised for detecting various diseases related to different blood cell morphologies.

7.2 Future Scope

A count for the WBC can also be conducted after thorough research. Further, classification of the different types of WBC present in the blood can be done by using various image processing techniques. Using the morphology various diseases that affect the shape and size of the cells can be detected by examining the cells for the particular variation. For example, certain red blood cells of a patient affected by anaemia turn to sickle shape. On examining the blood smear of a patient suspected of anaemia, the sickle shaped cells can be detected by edge detection techniques and then be counted. The RBC count only helps in partial diagnosis of blood; the counting of white blood cells will improve it further. Any development of finding hemoglobin concentration can be clubbed with this project to find

- Mean corpuscular volume (mcv)
- Packed cell volume (pcv)
- Mean cell hemoglobin (mch)
- Mean cell hemoglobin concentration (mchc) more the number of parameters that can be found from blood the more accurate the results will be.

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