B.E. PROJECT ON

PLATELET COUNT USING IMAGE PROCESSING TECHNIQUES

Submitted in Partial Fulfilment of the Requirements
for the Degree of
Bachelor of Engineering (Computer Engineering)

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CERTIFICATE

This is to certify that the report entitled **"PLATELET COUNT USING IMAGE PROCESSING "** being submitted by Rakshita Tandon, Rohan Sharma and Sahil Goel of the Department of Computer Engineering, Netaji Subhas Institute of Technology, New Delhi, for the award of the degree of "Bachelor of Engineering" is a bona fide record of the work carried out by them. They have worked under my guidance and supervision and have fulfilled the requirements for the submission of this report, which has reached the requisite standard.

It is further certified that the results contained in this report have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.

Dated: 2nd June, 2014.

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ABSTRACT

Platelet counting is one of the vital tests performed in primary health care centers for a variety of reasons such as if a disease or toxicity is suspected, if a medical condition is improving or worsening, to measure the success or failure of a medication or treatment plan or it may be ordered for professional or legal reasons. These tests are typically performed manually, which is labor intensive and requires an experienced laboratory technician. In addition the high costs and time requirements imposed by these tests compromise with the urgency in critical scenarios.

To address this challenge, we have developed an automated platelet counter for primary health care and resource-poor settings. The technology is based on a conventional microscope equipped with a digital camera linked to a personal computer, which can capture and analyze microscope images of blood samples. We, then apply image processing techniques, particular morphological analysis, to estimate the platelet count from these blood slides.

To evaluate the accuracy of our technique, the results were compared to platelet counts performed manually by an experienced laboratory technician. Our experiments prove that the technique is faster and less expensive than the conventional techniques, and gives a range within which the platelet count may lie. If the range happens to lie within the critical boundaries, then the user can use the manual techniques to get an exact count. Although, for majority of the applications the range is self sufficient in indicating the presence of any disorder.

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CHAPTER 1

INTRODUCTION

1.1 What are Platelets?

Platelets are one of the three cellular elements of the blood, whose function (along with the coagulation factors) is to stop bleeding. Platelets have no nucleus: they are fragments of cytoplasm which are derived from the megakaryocytes of the bone marrow, and then enter the circulation.

These inactivated platelets are biconvex discoid structures shaped like a lens, 2–4 μm in greatest diameter. Platelets are found only in mammals, an adaptation that may have evolved to offset the risk of death from hemorrhage at childbirth – a risk unique to mammals.

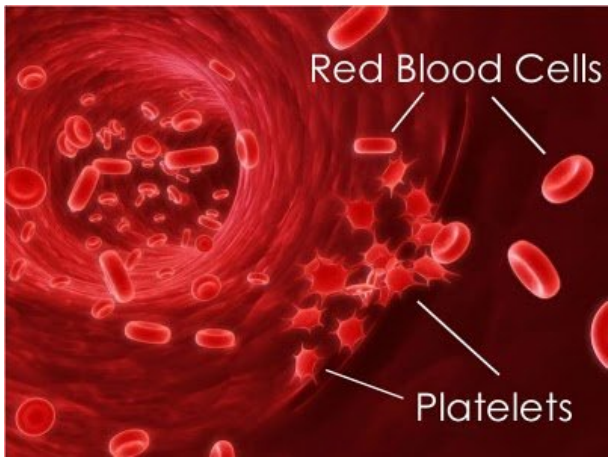


Figure 1. Platelets

1.2 Importance of Platelets

The main function of platelets is to contribute to hemostasis: the process of stopping bleeding at the site of interrupted endothelium. They gather at the site and unless the interruption is physically too large, they plug the hole.

First, platelets attach to substances outside the interrupted endothelium: adhesion.

Second, they change shape, turn on receptors and secrete chemical messengers: activation.

Third, they connect to each other through receptor bridges: aggregation. Formation of this platelet plug (primary hemostasis) is associated with activation of the coagulation cascade with resultant fibrin deposition and linking (secondary hemostasis).

These processes may overlap: the spectrum is from a predominantly platelet plug, or "white clot" to a predominantly fibrin clot, or "red clot" or the more typical mixture. The final result is the clot. Some would add the subsequent clot retraction and platelet inhibition as fourth and fifth steps to the completion of the process and still others a sixth step wound repair.

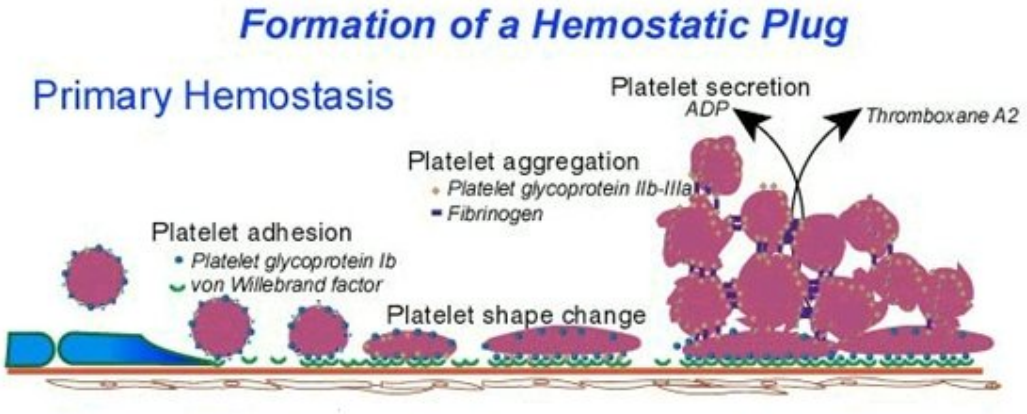


Figure 2. Formation of Hemostatic Plug

1.3 Healthy Platelet Count

A normal platelet count ranges from 150,000 to 450,000 platelets per microliter of blood. Having more than 450,000 platelets is a condition called thrombocytosis; having less than 150,000 is known as thrombocytopenia. You get your platelet number from a routine blood test called a complete blood count (CBC).

1.3.1 Thrombocytosis

Thrombocytosis (or thrombocythemia) is the presence of high platelet counts in the blood, and can be either primary (also termed essential and caused by a myeloproliferative disease) or reactive (also termed secondary). Although often symptomless (particularly when it is a secondary reaction), it can predispose to thrombosis in some patients.

In a healthy individual, a normal platelet count ranges from 150,000 and 450,000 per mm^3 (or microlitre) (150–450 x 109/L). These limits, however, are determined by the 2.5th lower and upper percentile, and a deviation does not necessary imply any form of disease. Nevertheless, counts over 750,000 (and

especially over a million) are considered serious enough to warrant investigation and intervention.

There are two types of Thrombocytosis:

- Primary or essential thrombocytosis – Abnormal cells in the bone marrow cause an increase in platelets, but the reason is unknown
- Secondary thrombocytosis – The same condition as primary thrombocytosis, but may be caused by an ongoing condition or disease such as anemia, cancer, inflammation, or infection

Signs and symptoms

High platelet levels do not necessarily signal any clinical problems, and are picked up on a routine full blood count. However, it is important that a full medical history be elicited to ensure that the increased platelet count is not due to a secondary process. Often, it occurs in tandem with an inflammatory disease, as the principal stimulants of platelet production (e.g. thrombopoietin) are elevated in these clinical states as part of the acute phase reaction.

High platelet counts can occur in patients with polycythemiavera (high red blood cell counts), and is an additional risk factor for complications.A very small segment of patients report symptoms of erythromelalgia, a burning sensation and redness of the extremities that resolves with cooling and/or aspirin use.

Scientific literature sometimes excludes thrombocytosis from the scope of thrombophilia by definition, but practically, by the definition of thrombophilia as an increased predisposition to thrombosis, thrombocytosis (especially primary thrombocytosis) is a potential cause of thrombophilia. Conversely, secondary thrombocytosis very rarely causes thrombotic complications.

When there are symptoms, they include spontaneous blood clots in the arms and legs, which if untreated can lead to heart attack and stroke. In severe cases, the patient might have to undergo a procedure called a platelet pheresis. This lowers the platelet count by removing the blood, separating out the platelets, and returning the red blood cells back to the body.

With secondary thrombocytosis, the symptoms are usually related to the associated condition. For example, if you have an infection or anemia, you treat those conditions and the platelet count comes down.

Diagnosis

Laboratory tests might include: full blood count, liver enzymes, renal function and erythrocyte sedimentation rate.

If the cause for the high platelet count remains unclear, bone marrow biopsy is often undertaken, to differentiate whether the high platelet count is reactive or essential.

Causes

Increase platelet counts can be due to a number of disease processes:

- Essential (primary)
  - Essential thrombocytosis (a form of myeloproliferative disease)
  - Other myeloproliferative disorders such as chronic myelogenousleukemia, polycythemiavera, myelofibrosis
- Reactive (secondary)
  - Inflammation
  - Surgery (which leads to an inflammatory state)
  - Hyposplenism (decreased breakdown due to decreased function of the spleen)
  - Splenectomy
  - Asplenia (absence of normal spleen function)
  - Iron deficiency anemia or hemorrhage
- Over-medication with drugs that treat thrombocytopenia, such as eltrombopag or romiplostim, may also result in thrombocytosis. Other causes include the following
  - Kawasaki disease
  - Soft tissue sarcoma
  - Osteosarcoma
  - Dermatitis (rarely)
  - Inflammatory bowel disease
  - Rheumatoid arthritis
  - Nephritis
  - Nephrotic syndrome
  - Bacterial diseases, including pneumonia, sepsis, meningitis, urinary tract infections, and septic arthritis.

The vast majority of causes of thrombocytosis are acquired disorders, but in a few cases, they may be congenital, such as thrombocytosis due to congenital asplenia.

Treatment

Often, no treatment is required or necessary for reactive thrombocytosis. In cases of reactive thrombocytosis of more than 1,000x109/L, it may be

considered to administer daily low dose aspirin (such as 65 mg) to minimize the risk of stroke or thrombosis.
However, in primary thrombocytosis, if platelet counts are over 750,000 or 1,000,000, and especially if there are other risk factors for thrombosis, treatment may be needed. Selective use of aspirin at low doses is thought to be protective. Extremely high platelet counts in primary thrombocytosis can be treated with hydroxyurea (a cytoreducing agent) or anagrelide (Agrylin).

1.3.2 Thrombocytopenia

The terms thrombocytopenia and thrombopenia, (British:-thrombocytopaenia and thrombopoaenia, respectively), refer to a relative decrease of platelets in blood.
A normal human platelet count ranges from 150,000 to 450,000 platelets per microlitre of blood. These limits are determined by the 2.5th lower and upper percentile, so values outside this range do not necessarily indicate disease. One common definition of thrombocytopenia is a platelet count below 50,000 per microlitre.

Signs and symptoms
Often, low platelet levels do not lead to clinical problems; rather, they are picked up on a routine full blood count (or complete blood count). Occasionally, there may be bruising, particularly purpura in the forearms, petechia (pinpoint hemorrhages on skin and mucous membranes), nosebleeds, and/or bleeding gums.
Eliciting a full medical history is vital to ensure the low platelet count is not due to a secondary process. It is also important to ensure that the other blood cell types, such as red blood cells and white blood cells, are not also suppressed. Painless, round and pinpoint (1 to 3 mm in diameter) petechiae usually appear and fade, and sometimes group to form ecchymoses. Larger than petechiae, ecchymoses are purple, blue or yellow-green bruises that vary in size and shape. They can occur anywhere on the body.
A person with this disease may also complain of malaise, fatigue, and general weakness (with or without accompanying blood loss). In acquired thrombocytopenia, the patient's history may include the use of one or several offending drugs.
Inspection typically reveals evidence of bleeding (petechiae or ecchymoses), along with slow, continuous bleeding from any injuries or wounds. Adults may have large, blood-filled bullae in the mouth. If the person's platelet count is between 30,000 and 50,000/mm3, bruising with minor trauma may be expected; if it is between 15,000 and 30,000/mm3, spontaneous bruising will be seen (mostly on the arms and legs).

Causes

Decreased platelet counts can be due to a number of disease processes:

- Decreased production
  - Vitamin B12 or folic acid deficiency
  - Leukemia or myelodysplastic syndrome
  - Decreased production of thrombopoietin by the liver in liver failure
  - Sepsis, systemic viral or bacterial infection
  - Dengue fever can cause thrombocytopenia by direct infection of bone marrow megakaryocytes, as well as immunological shortened platelet survival.
  - Hereditary syndromes
  - Congenital amegakaryocytic thrombocytopenia
  - Thrombocytopenia absent radius syndrome
  - Fanconianemia
  - Bernard-Soulier syndrome, associated with large platelets
  - May-Hegglin anomaly, the combination of thrombocytopenia, pale-blue leuckocyte inclusions, and giant platelets
  - Grey platelet syndrome
  - Alport syndrome
  - Wiskott–Aldrich syndrome

- Increased destruction
  - Idiopathic thrombocytopenic purpura
  - Thrombotic thrombocytopenic purpura
  - Hemolytic-uremic syndrome
  - Disseminated intravascular coagulation
  - Paroxysmal nocturnal hemoglobinuria
  - Antiphospholipid syndrome
  - Systemic lupus erythematosus
  - Post-transfusion purpura
  - Neonatal alloimmune thrombocytopenia
  - Splenic sequestration of platelets due to hypersplenism
  - Dengue fever has been shown to cause shortened platelet survival and immunological platelet destruction.
  - HIV-associated thrombocytopenia
  - Gaucher's disease

- Medication-induced

  Thrombocytopenia-inducing medications include:
  - Direct myelosuppression
  - Valproic acid
  - Methotrexate
  - Carboplatin
  - Interferon
  - Isotretinoin
  - Panobinostat
  - Other chemotherapy drugs
  - Singulair (montelukast sodium)
  - H2 blockers and proton-pump inhibitors have shown increased thrombocytopenia symptoms, such as red dots near the bottom of the legs.[5]
  - Immunological platelet destruction
  - A drug molecule binds to the Fab portion of an antibody: A classic example is the quinidine group of drugs. The Fc portion of the antibody molecule is not involved in the binding process.
  - A drug molecule binds to the Fc antibody moiety, with the drug/antibody complex subsequently binding and activating the platelets: Heparin-induced thrombocytopenia (HIT) is a classic example, in which the heparin-antibody-platelet factor 4 (PF4) complex binds to the Fc receptors on the surface of the platelet. Since the Fc moiety is now unavailable to the Fc receptors of the reticuloendothelial cells, the normally occurring destruction of the platelets is prevented. This may explain why severe thrombocytopenia is not a common feature of HIT.
  - Abciximab-induced thrombocytopenia.

- Other causes
  - Snakebites, particularly by pit vipers.[7]
  - Onyalai, a disease of unknown etiology, is seen only in parts of Africa, but suspected of being caused by poor nutrition or consumption of tainted food.[8]
  - Excess consumption of oils containing erucic acid, such as Lorenzo's oil or mustard oil; see the side effects of taking Lorenzo's oil.

- Niacin Toxicity - Reversible thrombocytopenia has been observed in patients with niacin toxicity, particularly when large doses (3000mg/day) have been prescribed in patients with impaired renal function. The toxicity in this situation has been known to manifest itself in the form of increased renal impairment and declining platelet count.
- Pseudothrombocytopenia

Diagnosis

Laboratory tests might include: full blood count, liver enzymes, renal function, vitamin B12 levels, folic acid levels, erythrocyte sedimentation rate, and peripheral blood smear.

If the cause for the low platelet count remains unclear, a bone marrow biopsy is usually recommended, to differentiate whether the low platelet count is due to decreased production or peripheral destruction.

Thrombocytopenia in hospitalized alcoholics may be caused by splenomegaly, folate deficiency, and, most frequently, a direct toxic effect of alcohol on production, survival time, and function of platelets. Platelet count begins to rise after 2 to 5 days' abstinence from alcohol. The condition is generally benign, and clinically significant hemorrhage is rare.

Lab tests to determine the platelet count and clotting function may also be done. In severe thrombocytopenia, a bone marrow study can determine the number, size and maturity of the megakaryocytes (the bone marrow cells that release mature platelets). This information may identify ineffective platelet production as the cause of thrombocytopenia and rule out a malignant disease process at the same time.

Treatment

Treatment is guided by etiology and disease severity. The main concept in treating thrombocytopenia is to eliminate the underlying problem, whether that means discontinuing suspected drugs that cause thrombocytopenia, or treating underlying sepsis. Diagnosis and treatment of serious thrombocytopenia is usually directed by a hematologist.

Corticosteroids may be used to increase platelet production. Lithium carbonate or folate may also be used to stimulate the bone marrow production of platelets. Platelet transfusions may be used to stop episodic abnormal bleeding caused by a low platelet count. However, if platelet destruction results from an immune disorder, platelet infusions may have only a minimal effect and may be reserved for life-threatening bleeding.

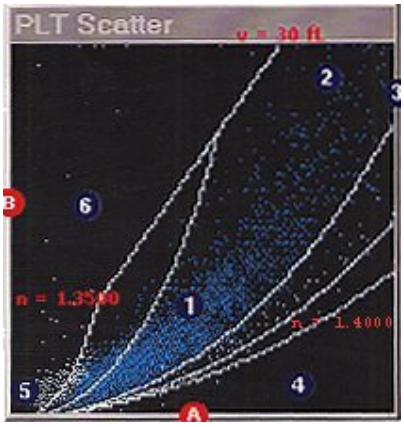
Specific treatment plans often depend on the underlying etiology of the thrombocytopenia.

CHAPTER 2

PLATELET COUNT DETERMINATION

Platelet counts can be done manualy using a hemocytometer or with an automated analyzer. Counts can also be estimated during blood smear examination. Since many laboratories use instruments that count platelets, red cells and leukocytes concurrently, a platelet count is a routinely reported result on complete or automatdhemograms.

2.1 Automated platelet counts with the ADVIA



1. Platelets
2. Large platelets
3. Red blood cells
4. RBC fragments
5. Debris
6. Ghosts

Figure 3. ADVIA

The ADVIA measures platelets by flow cytometry based on principles of light scattering. Platelets are identified by their size (< 30 fL) and refractive index (n = 1.35 to n = 1.40). The platelet cytogram on the left is a graphical representation of how the Advia counts platelets. Low light scatter (refractive index or internal complexity) is plotted against the X axis and high light scatter (cell size) is plotted against the Y axis (B). Platelets are detected in the region labeled 1. Large platelets (section 2) are identified on the basis of size (> 20 fL) and refractive index (which distinguishes them from red cells). In camelids with iron deficiency anemia or hemolyzed samples from any animal, small or lysed red blood cells may be erroneously counted as platelets, falsely increasing the platelet count.

All blood smears are also examined for the presence of platelet clumps (see image to the right below), which will affect the accuracy of the platelet count.

Platelet clumps decrease the platelet count obtained by any method. Thus any provided count should be considered a minimum platelet count in the sample. In some samples with severe platelet clumps (many blood samples from cats), the platelet count is totally invalid and a count will not be provided. Under these circumstances (or any other situations resulting in erroneous counts, such as an iron deficient camelid), a platelet count will not be provided and the smear estimate of platelet numbers (increased, adequate, low? or low) given with our hemograms should be used as a guide as to the actual platelet count.
Cats are notorious for platelet clumping (their platelets are activated at the slightest provocation) and it is difficult to obtain accurate counts in this species. Platelet clumping is usually due to a sample collection problem and can be minimized by collecting blood from a large peripheral vein (cephalic or jugular), such that blood flows smoothly into the vacutainer or syringe, and using a 22 or 23 g needle (in a dog or cat). The blood should be mixed with the anticoagulant as soon as possible after collection, by gentle rotation or inversion. Platelet clumping increases with time, so platelet counts should be done as soon as possible after collection to maintain accuracy.

2.2 Manual Platelet Counts

Platelet counts can be done manually with a commercial diluting system, hemocytometer, and a microscope. These counts are less accurate than automated counts, because platelets can be difficult to distinguish from debris. Platelet clumping will also decrease the hemocytometer platelet count.
In a platelet count, 50 µl EDTA blood (collected with an Eppendorf pipette) is mixed in 950 µl dilution solution (collected with an Eppendorf pipette). This results in a dilution of 1:20. The mixture must stand approximately 5 minutes so that the erythrocytes are completely lysed. Then the suspension is mixed and put into the counting chamber. The chamber is left in a moist environment for 20-30 minutes so the platelets can settle without the chamber drying. Like the erythrocyte count, 80 small squares are counted.
Calculation of the platelet count is achieved by using the formula below using these factors:

1. the number of platelets (Tc) counted in the small squares,
2. the dilution of the cell solution,
3. the number of counted squares,
4. the volume above a square, and
5. the conversion factor which is necessary in order to come to the volume of one litre.

Manual count method has the following characteristics –

- Laborious

- Time Intensive
- Subjective
- High Inter- observer CVs of 10-25 %

2.3 Platelet Count Estimates from a Blood Smear Examination

In a well-prepared smear, platelets are estimated by counting the average number of platelets seen per 100x oil immersion field in the monolayer. In general, 10 oil immersion fields are counted and the results averaged (this accounts for uneven dispersal of platelets in the smear). Then the following formula is applied:
Estimated platelet count/µL = average count in 10 fields x 15,000.
For example, if an average field contains 7 platelets, an estimate of 105,000/µL would be appropriate. This value would then be compared to the normal range for the species in question ... 105K would be "low" (below reference intervals) for a dog, but "adeqate" (or within reference intervals) for a horse

Problems of Peripheral Smear Platelet Check

- Platelet Clumps
- Platelet Satellitism on WBCs
- Poor Smearing
- Highly subjective

2.4 Platelet Counting by the RBC/Platelet Ratio Method

An EDTA-anticoagulated blood specimen is prediluted in a sterile buffered solution. The platelets then are stained with specific fluorescent antibodies. The stained platelets in solution are diluted to the counting concentration, and the platelets and RBCs are counted on a flow cytometer with thresholds set to discriminate platelets from RBCs on the basis of fluorescence amplitude and scatter amplitude. The RBC/PLT ratio is determined, and the platelet count is calculated from an accurate RBC count of the sample, obtained using a cell counter that meets previous ICSH specifications.

For platelet and RBC enumeration, a fluorescent flow cytometer with hydrodynamic focusing and the capability of measuring forward light scatter and fluorescence is used. The instrument should have sufficient sensitivity to scattered and fluorescein fluorescent light to reliably count fluorescein isothiocyanate-labeled spherical particles of 2 µm in diameter.

For the whole blood RBC count, a semiautomated, single-channel, aperture-impedance particle counter is used. The instrument should have an orifice diameter of 80 to 100 μm and a length 70% to 100% of the diameter, and the volume displaced during the counting period must be known to within an accuracy of 1%, traceable to a national or international metrologic standard.

2.5 Impedance Platelet Counts

The method draws on a hydrodynamic focusing flow in an impedance chamber. On the basis of impedance, platelet size is estimated and a histogram of platelet volume is prepared. Platelet number and mean platelet volume are estimated onthe basis of the PLT histogram surface, limited by the lower andupper volume discriminator.

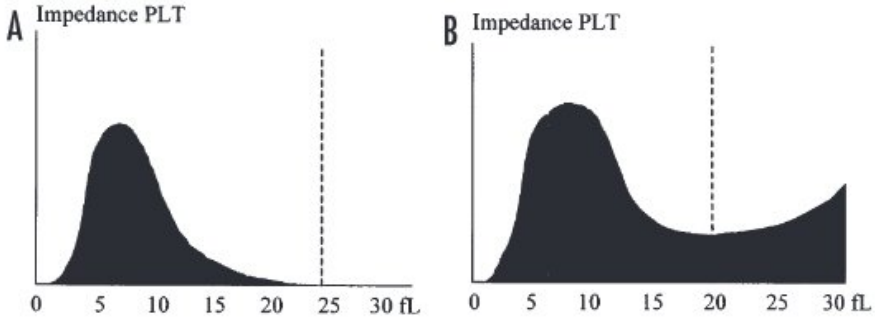


Figure 4. Platelet Volume Histogram : (A) Normal (B) With hindered separation due to Microcytes

Characteristics of Impedance Count Method –

- Coulter Principle or Resistance detection method
- Cells suspended in an elecrolyte solution
- Change in electric impedance -impedance signal
- Impedance signal- Directly proportional to the volume of the cell

2.6 Optical Platelet Counts

To measure platelet count, we apply multi-angle polarized light scatter separation (MAPSS). Atwo-dimensional analysis estimates the complexity and optical density of the platelets, represented as a cytogram of the light intensity at 7- and 90-degree angles. Three moving discrimination lines create a window separating PLT from other elements.
Two angles of laser light scatter are measured:

- Light Scatter- 2-3°C- volume (plt size)
- Light Scatter- 5-15°C- refractive index (plt density)

RBC fragments have a different RI as compared to platelets and hence can be separated.RBC fragments do not contain RNA while giant platelets and immature forms contain RNA and are called reticulated platelets. These are easily separated from microcytic RBCs and fragments.

CHAPTER 3

OUR NOVEL APPROACH

3.1 Introduction

In this chapter we discuss the implementation of our algorithm for platelet estimation. We have devised a new technique in order to reduce the cost and time associated with the conventional techniques. In this approach, we use images of blood slides collected with the help of a digital camera attached to a computer and after application of morphological image processing techniques, we estimate the platelet count. The accuracy of our technique is verified by comparing these results with the manual count. Hence, our technique is a major improvement over others in terms of :

i. Cost
ii. Time

We can use our technique to give a range within which the platelet count may lie. If the range happens to lie within the critical boundaries, then the user can use the manual techniques to get an exact count. Although, for majority of the applications the range is self sufficient in indicating the presence of any disorder. In the subsequent sections, we have summarized the technique that has been adopted by us.

3.2 Technique

3.2.1. Dataset Collection

The dataset used in this project consisted of actual microscopic images of blood samples. The images were captured with an optical laboratory Olympus microscope coupled with a Canon Power Shot G5 camera. All of the images are in JPG format with 24-bit color depth and a resolution of 720 × 570 pixels. The images were taken at magnification factor of 100x.

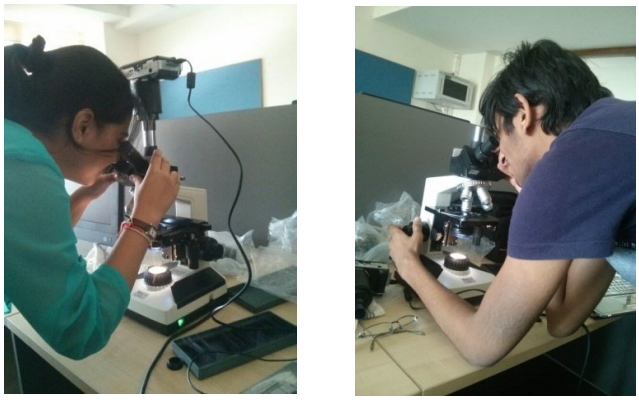


Figure 5. Dataset Collection

3.2.2 Algorithm

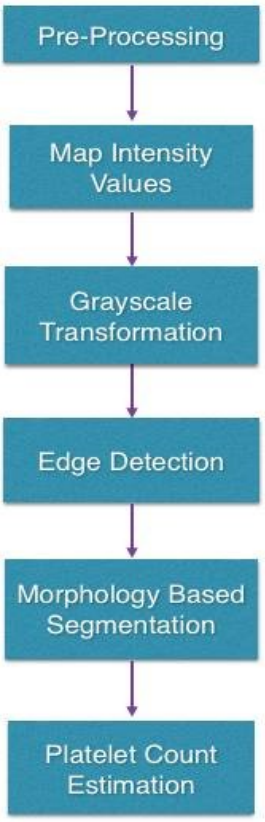


Figure 6. Proposed Algorithm

Our proposed system is depicted in Fig 6. It consists of various functional modules. The input image of blood slide is fed to the system. As the image consists of several elements, only the platelets are separated using morphological characteristics such as their size. The entire implemented code is illustrated in Appendix A.1. The major functional modules are described in detailed as follows.

3.2.2.1 Pre- Processing

In the pre-processing stage we apply the rotationally symmetric Gaussian low pass filter to the whole image to reduce high frequency noise. Gaussian filters have the properties of having no overshoot to a step function input while minimizing the rise and fall time. This behavior is closely connected to the fact that the Gaussian filter has the minimum possible group delay. It is considered the ideal time domain filter.

In one dimension, the Gaussian function is:

$$g(x) = \frac{1}{\sqrt{2\pi\sigma^2}} \cdot e^{-\frac{x^2}{2\sigma^2}}$$

where σ is the standard deviation of the distribution.

In two dimensions, it is the product of two such Gaussians, one per direction:

$$g(x,y) = \frac{1}{2\pi\sigma^2} \cdot e^{-\frac{x^2+y^2}{2\sigma^2}}$$

Shown graphically, we see the familiar bell shaped Gaussian distribution.

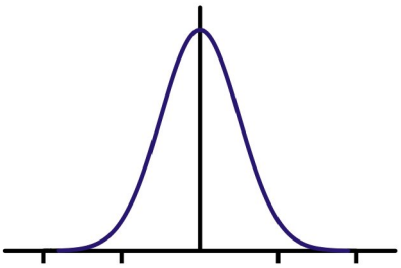
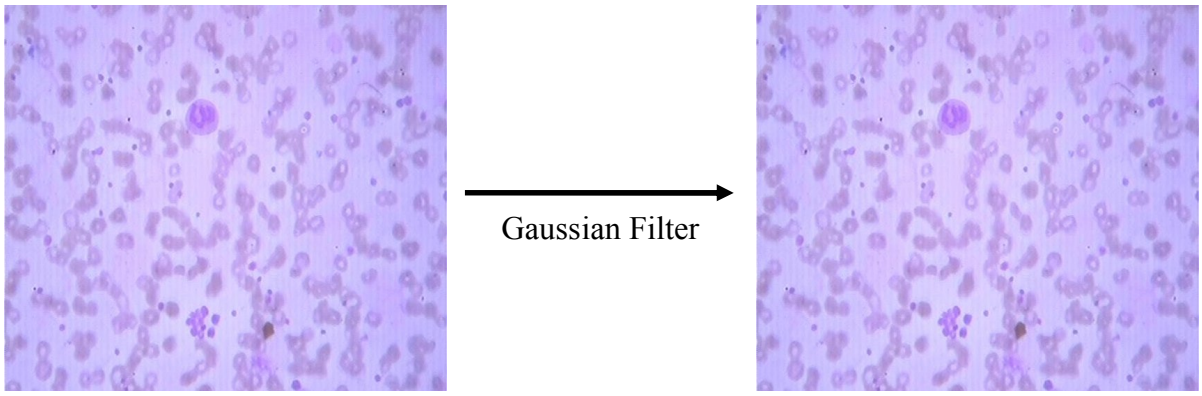


Figure 7. Gaussian Curve

The effect of the pre-processing stage is depicted in Figure 8.



Original Image

Pre- Processed Image

Difference between Original & Pre-Processed Image

Figure 8. Pre- Processing Stage

3.2.2.2 Map Intensity Values

Intensity adjustment is a technique for mapping an image's intensity values to a new range.To enhance the contrast of the image and detect platelets more effectively, we map the intensity values from original color domain to the domain in which platelets intensity value lie.

Imadjust inbuilt function in MATLAB was used to map the intensity range to a new domain. The general syntax of this function is represented as follows :

J = imadjust (I, [low_in; high_in], [low_out; high_out], gamma)

maps the values in I to new values in J, where gamma specifies the shape of the curve describing the relationship between the values in I and J. If gamma is less than 1, the mapping is weighted toward higher (brighter) output values. If gamma is greater than 1, the mapping is weighted toward lower (darker) output values. If you omit the argument, gamma defaults to 1 (linear mapping). Appendix A.2 gives a detailed description of Imadjust Code used in MATLAB. The tranformation from the pre-processed image to an intensity mapped image is represented in Figure 9.

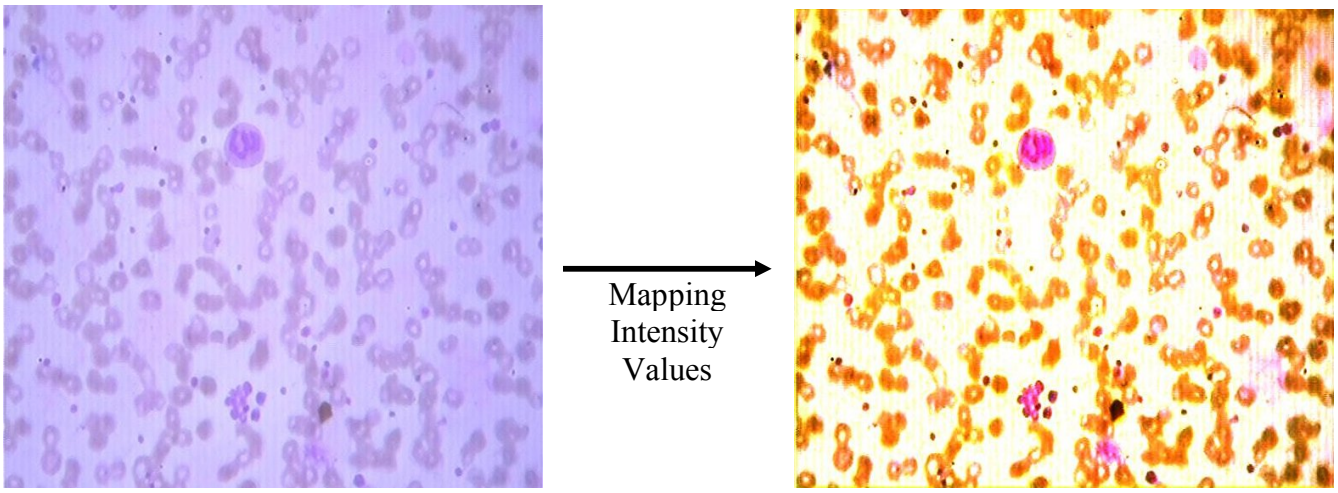


Figure 9. Mapping Intensity Values to a New Domain

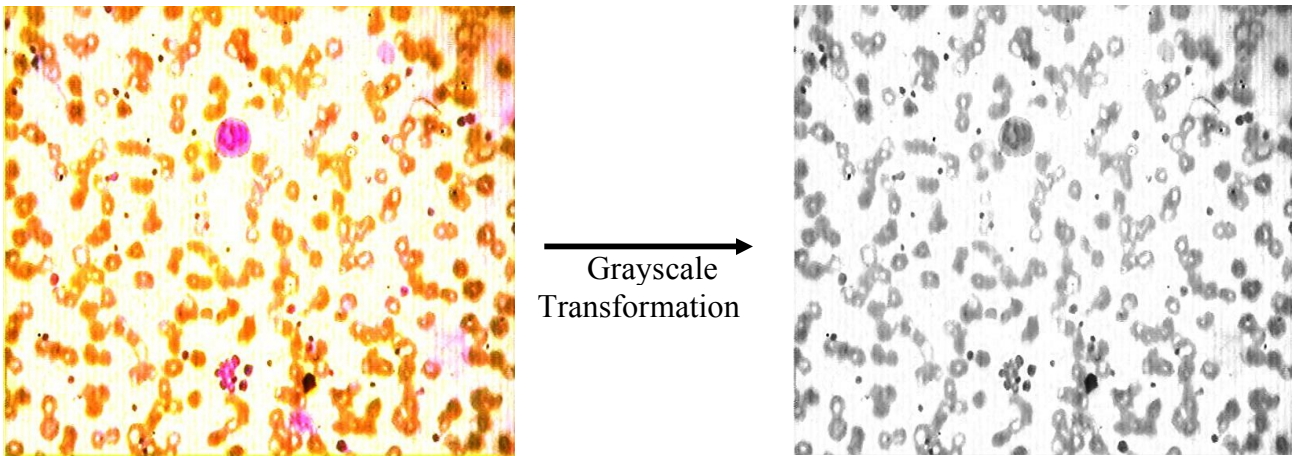
3.2.2.3 Grayscale transformation

Gray scale images are often the result of measuring the intensity of light at each pixel in a single band of the electromagnetic spectrum (e.g. infrared, visible light, ultraviolet, etc.) and in such cases they are monochromatic proper when only a given frequency is captured. They can also be synthesized from a full color image. Grayscale images have many shades of gray in between. Grayscale images are also called monochromatic, denoting the presence of only one (mono) color (chrome).

After using the color information of the pixels and enhancing the contrast of the image in the previous step, we transform the image to grayscale image (8 bit depth) by eliminating the hue and saaturation information while retaining the luminance. To transform it into grayscale image we used the Matlab function RGB2GRAY(I) where I is an input image. Appendix A.3 gives a detailed description of the rgb2gray inbuilt function in MATLAB.This function uses the following formula to transform color image to grayscale image.

Gray(I,J) = 0.2989 * Red(I,J) + 0.5870 * G(I,J) + 0.1140 * B(I,J)

The resulting image will be two dimensional. The value 0 represents black and the value 255 represents white. The range will be between black and white values.
Figure 10 depicts how a color image is transformed to grayscale image.



Intensity Mapped Image Gray Scale Image

Figure 10. Transformation to Grayscale Image

3.2.2.4 Edge Detection

Edge detection is the name for a set of mathematical methods which aim at identifying points in a digital image at which the image brightness changes sharply or, more formally, has discontinuities. The points at which image brightness changes sharply are typically organized into a set of curved line segments termed edges. Edge detection is used for image segmentation and data extraction in areas such as image processing, computer vision, and machine vision.

We have used a Sobel Filter for the purpose of edge detection. It is a discrete differentiation operator, computing an approximation of the gradient of the image intensity function. At each point in the image, the result of the Sobel operator is either the corresponding gradient vector or the norm of this vector. The Sobel operator is based on convolving the image with a small, separable, and integer valued filter in horizontal and vertical direction and is therefore relatively inexpensive in terms of computations.

The operator uses two 3×3 kernels which are convolved with the original image to calculate approximations of the derivatives - one for horizontal changes, and one for vertical. If we define A as the source image, and G_x and G_y are two images which at each point contain the horizontal and vertical derivative approximations, the computations are as follows:

$$\mathbf{G}_x \quad \begin{bmatrix} -1 & 0 & +1 \\ -2 & 0 & +2 \\ -1 & 0 & +1 \end{bmatrix} * \mathbf{A} \quad \text{and} \quad \mathbf{G}_y \quad \begin{bmatrix} +1 & +2 & +1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{bmatrix} * \mathbf{A}$$

where $*$ here denotes the 2-dimensional convolution operation.

The x-coordinate is defined here as increasing in the "right"-direction, and the y-coordinate is defined as increasing in the "down"-direction. At each point in the image, the resulting gradient approximations can be combined to give the gradient magnitude, using:

$$\mathbf{G} = \sqrt{\mathbf{G}_x^2 + \mathbf{G}_y^2}$$

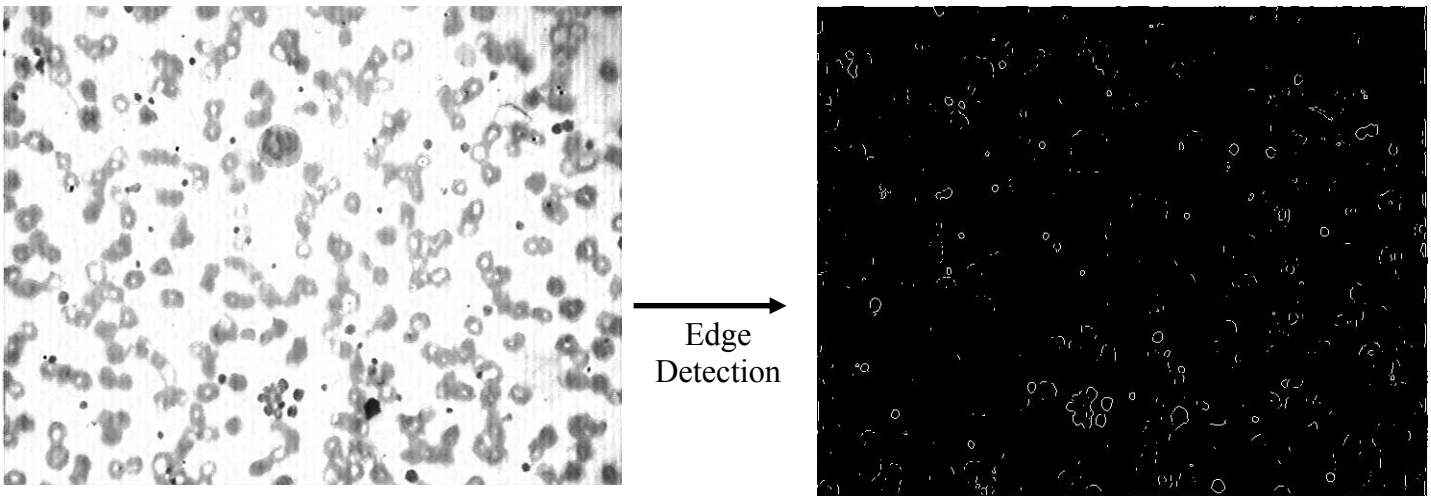
Using this information, we can also calculate the gradient's direction:

$$\mathbf{\Theta} = \operatorname{atan2}(\mathbf{G}_y, \mathbf{G}_x)$$

where, for example, Θ is 0 for a vertical edge which is darker on the right side.

MATLAB uses the following function for detecting edges from an input image using the Sobel Filter.
BW = edge(I, 'sobel', thresh)

which takes a grayscale image I as its input, and returns a binary image BW of the same size as I, with 1's where the function finds edges in I and 0's elsewhere. It also specifies the sensitivity threshold for the Sobel method. It ignores all edges that are not stronger than thresh. If you do not specify thresh, or if thresh is empty ([]), edge chooses the value automatically. Appendix A.4 gives a detailed description of the edge inbuilt function in MATLAB.
Figure 11 depicts how edges are depicted from the grayscale image using the sobel filter.



Gray Scale Image Edge Detected Image

Figure 11. Edge Detection using Sobel Filter

3.2.2.5 Morphology Based Segmentation

Morphological image processing is a collection of non-linear operations related to the shape or morphology of features in an image. Morphological operations rely only on the relative ordering of pixel values, not on their numerical values, and therefore are especially suited to the processing of binary images. Morphological operations can also be applied to gray scale images such that their light transfer functions are unknown and therefore their absolute pixel values are of no or minor interest.

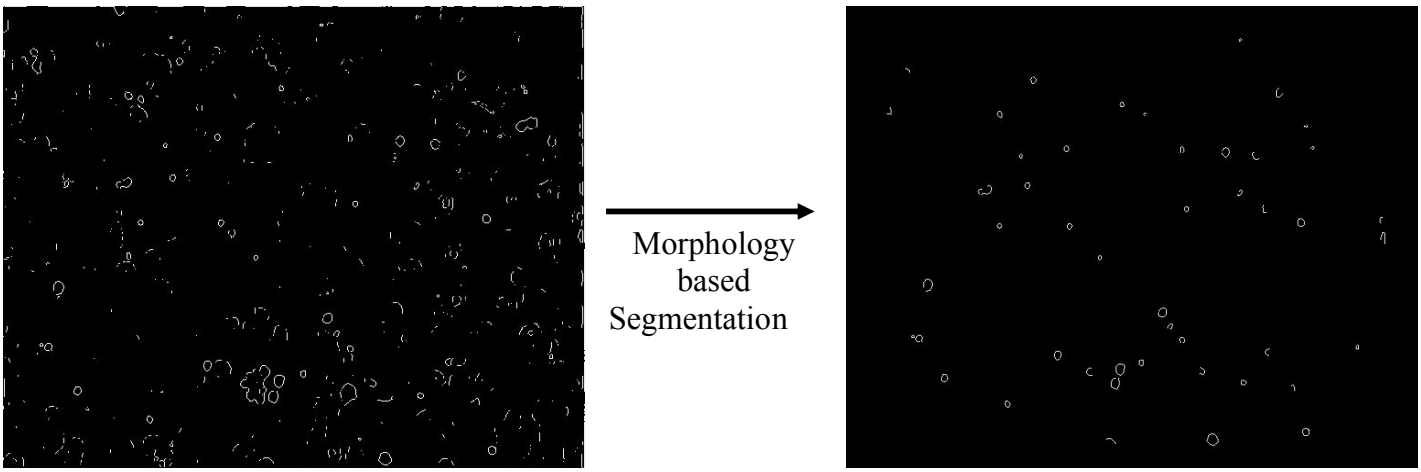
As can be seen, there are many false edges obtained in the last Image in addition to the RBC's and WBC's boundaries, hence we need to filter the image and

delete all the non-required edges and boundaries.

The table depicts that the size of a platelet is much smaller than WBC's and RBC's, this property can thus be used to filter out platelets from other blood components.

Type of Blood Cell	Size[μm]
White Blood cell	10-20
Red Blood cell	6-10
Platelets	2-4

All false positives are eliminated using the size as one of the factor. Also, the shape of the platelets will be circular or nearly circular. This property further helps in eliminating false positives such as straight line edges. Appendix A.1 contains the entire code which uses the size of the platelet as an important morphological parameter to separate the platelets from other elements.
Figure 12 illustrates how platelets are extracted from the edge detected image.



Edge Detected Image Final Extracted Platelets

Figure 12. Platelet Extraction using Morphology Based Segmentation

3.2.2.6 Platelet Count Estimation

Once we have obtained the final image depicting all the platelets in the given blood slide, the total number of platelets must be estimated. This is done by labelling all 8-connected components and counting distinct entities. This gives the platelet count for one image. To estimate the platelet count for one entire sample, following steps are taken:

1. The estimate is made by counting the average number of platelets seen per 100x oil immersion field in the monolayer of a well-spread smear.
2. This number multiplied by 15,000 equals the approximate platelet count/µL.

3. This value would then be compared to the reference interval for the species in question. For the estimate, an actual count is not provided but platelets are designated into specific categories:

- "Increased" - the platelet count is estimated to be above the reference interval.
- "Adequate" - the platelet count is estimated to be within the reference interval.
- "Low?" - the platelet count is estimated to be mildly decreased or within low "normal limits".
- "Low" - the platelet count is estimated to be below the reference interval.
- "Very low - the platelet count is estimated to be <30,000/µL.

For example, if an average field contains 7 platelets, an estimate of 105,000/µL would be appropriate. This value would be "low" (below the reference interval) for a dog, but "Adequate" (within the reference interval) for a horse (horses tend to have lower platelet counts that other species).

Often, a useful judgement about platelet adequacy can be made in this manner even if platelets are too clumped to allow an actual count by manual or automated methods. Our technique is hence beneficial in predicting an approximate count of the platelets in a given sample without sacrificing on the cost and time bounds. If the range predicted by our technique is found close to the critical boundaries, then a manual count can be initiated for that sample to accurately predict the platelet count. However, for most applications, the range predicted by our technique should suffice.

Therefore, our technique is a major improvement over other conventional methods that are followed till date. The time and cost constraints imposed by the conventional techniques may delay the entire process and can prove to be fatal in critical situations. Our method, if adopted, can predict an estimate in a very short span of time and hence, the subject may also be started with some initial medication, till the accurate count is obtained using conventional methods.

CHAPTER 4

EXPERIMENTS AND RESULTS

In this section, we perform several experiments on the different samples and compare the experimental platelet count with the manual count (as specified by the Lab Technician). Our results prove that the experimental values are very close to the actual count and hence, serve as an accurate estimate in general scenarios. Our results have also shown that the time required for estimation is highly improved and the costs are also diminished. The time required is 15 seconds for each blood sample and the only cost incurred only includes the cost of dataset collection and processing cost. The following section presents a detailed analysis of the experiments performed followed by a brief summary of the important results obtained.

4.1 Experiments

We have performed our experiments on 15 samples collected by us using a digital camera connected to the microscope. We have compared our results with 2 other approaches to estimate platelet count, namely :

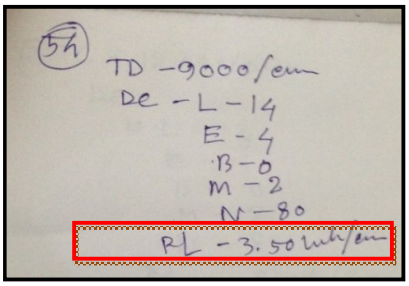
- Manual Count : It is estimated by an experienced technician by observing the blood slide under the microscope.
- Machine Count : It is estimated using ADVIA using principles of light scattering.

4.1.1 Sample 1

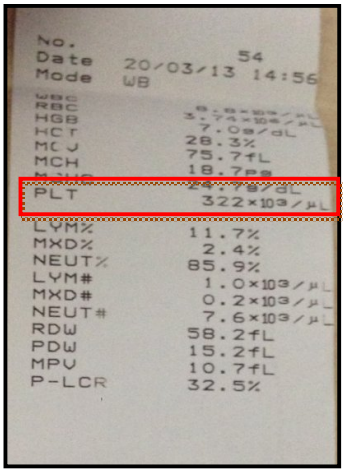
Result comparison for Sample 1 is summarised in the following table.

Manual Count (/µl)	Machine Count(/µl)	Proposed Technique(/µl)
3,50,000	3,22,000	3,10,000

It is clearly observed that our proposed method is working and the results are very close to the actual platelet count. Also, this estimate gives a rough figure and can easily be classified in "Adequate" range. Figure 13 shows the machine and manual count for Sample 1.



Manual Count



Machine Count

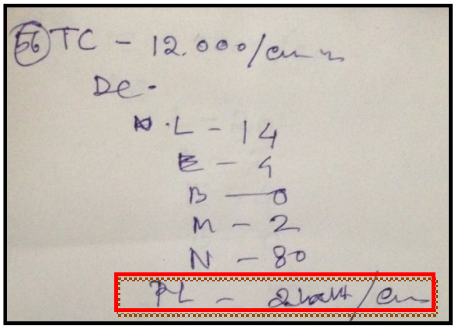
Figure 13. Manual And Machine Count For Sample 1

4.1.2 Sample 2

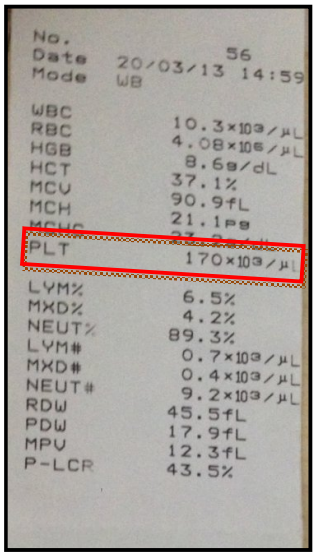
Result comparison for Sample 2 is summarised in the following table.

Manual Count (/μl)	Machine Count(/μl)	Proposed Technique(/μl)
2,00,000	1,70,000	1,50,000

It is clearly observed that our proposed method is working and the results are very close to the actual platelet count. Also, this estimate gives a rough figure and can easily be classified in "Low?" range. Figure 14 shows the machine and manual count for Sample 2.



Manual Count



Machine Count

Figure 14. Manual and Machine Count for Sample 2

4.1.3 Combined Analysis

The combined analysis for 5 other samples is depicted in Figure 15.

This graph clearly depicts that our proposed technique is consistent in predicting the range where the platelet count may lie. Such a range can be extremely useful in predicting whether the whether the platelet count lies in the critical range and if so, a detailed machine count may be initiated for that blood sample. In the majority of cases, the range would be self- sufficient in predicting a disorder.

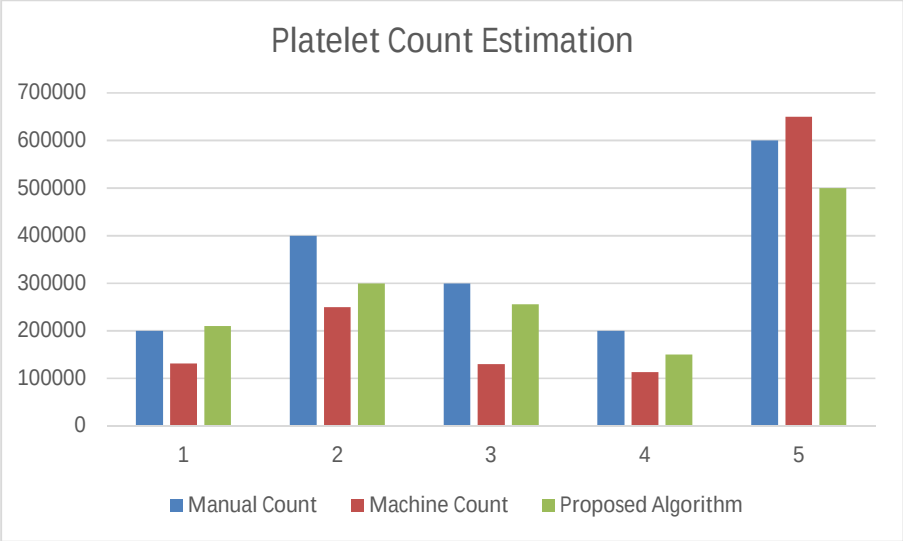


Figure 15. Comparison of Different Techniques

4.2 Results

Our experiments have demonstrated that the proposed method of cell counting is fast, cost effective and accurate to produce blood cell report. It gives up to 95% accurate results for platelet count which is an important criteria to diagnose diseases like dengue at initial stages. The time and cost limitations associated by conventional techniques are overcome in our method, as well as an estimate of the range in which the platelet count may lie is accurately predicted. Hence, our technique is extremely suitable in the modern day scenario where 100's of samples are collected each day and hence, the time and cost factors are crucial while predicting platelet estimates.

CHAPTER 5

LIMITATIONS, CONCLUSIONS, FUTURE SCOPE AND REFERENCES

5.1 Limitations

Some of the limitations of the proposed technique can be summarised as :

- The procedure can fail if the quality of image degrades.
- The procedure requires data acquisition task which should be done carefully so as to cover maximum cross-sections of the slide.
- This technique cannot give an accurate count as compared to manual count, so if the estimate lies in critical range, manual counting method should be followed.

5.2 Conclusion

In this project we have developed a software based solution for blood cell count for underdeveloped and developing countries like Pakistan, India etc. which are resource less to produce and provide expensive Optical Machine for Platelet counter in every hospital laboratory or primary health centres of the country.

Proposed method of cell counting is fast, cost effective and accurate to produce blood cell report. It can categorize 95-100% blood samples accurately and takes about 15 seconds of processing time per sample.

We can use our technique to give a range within which the platelet count may lie. If the range happens to lie within the critical boundaries, then the user can use the manual techniques to get an exact count. Although, for majority of the applications the range is self sufficient in indicating the presence of any disorder.

5.3 Future Scope

Our proposed technique has been used to estimate the total number of platelets in a given sample. This technique can further be extended to analyse the shape and dimensions of platelets using morphological analysis and further use these results to predict any abnormality.

The technique can also be refined to identify WBC and RBC in the given sample as well as predict any sort of deformity upon detailed analysis of these components.

5.4 References

[1] Lazuardi, Lutfan, et al. "Automatic platelets counter for supporting dengue case detection in primary health care in indonesia." *Studies in health technology and informatics* 192 (2012): 585-588.

[2] Mathur, Atin, Ardhendu S. Tripathi, and Manohar Kuse. "Scalable system for classification of white blood cells from Leishman stained blood stain images."*Journal of pathology informatics* 4.Suppl (2013).

[3] Joshi, M.S Minal and A.H Karode. "Detection of Acute Leukemia using White Blood Cells Segmentation based on Blood Samples." *international journal of electronics* (2013).

[4] Khan, Sanaullah, et al. "An Accurate and Cost Effective Approach to Blood Cell Count." *International Journal of Computer Applications* 50 (2012).

[5]Tulsani, Hemant. "Segmentation using morphological watershed transformation for counting blood cells." *IJCAIT* 2.3 (2013): 28-36.

[6] Bennett, JH. The Employment of the Microscope inMedical Studies. Stewart, Edinburgh, 1841.

[7] https://ahdc.vet.cornell.edu/sects/clinpath/modules/hemogram/plt_sm.htm

[8] https://ahdc.vet.cornell.edu/sects/clinpath/modules/hemogram/plt_ct.htm

[9] http://en.wikipedia.org/wiki/Platelet

[10] http://en.wikipedia.org/wiki/Complete_blood_count

APPENDIX A

A.1: High Level Implementation Code

```
function [pl,cnt,p] = plat(f)
%mapping to different color domain
tc=imadjust(f,[.3922 0 130/255;1 .40 1],[],4);
imshow(tc);
p=rgb2gray(tc);
figure,imshow(p),figure,imshow(tc),figure;
pl=edge(p,.10);
imshow(pl),figure;
pl=bwlabel(pl);

for i=1:max(pl(:))

[x y]=find(pl==i);
[minimumx,mini]=min(x);
[minimumy,minj]=min(y);
[maximumx,maxi]=max(x);
[maximumy,maxj]=max(y);

if(maximumx - minimumx < 7 || maximumx - minimumx >50 || maximumy - minimumy <7 || maximumy -
minimumy >50 || (mini==maxj || minj==maxi) || (mini==minj || maxi==maxj)  )
z=find(pl==i);
pl(z)=0;
end
end
pl=bwlabel(pl);
cnt=max(pl(:));
cnt
end
```

```
function [ average ] = plat_cnt(sno,max)
tic
sum=0;
for i=1:max
str=strcat('C:\users\sahil\desktop\Old Slides\',num2str(sno),'\',num2str(i),'.jpg');
temp=imread(str);
[p,cnt]=plat(temp);
sum=sum+cnt;
end
average=(sum/max);
platelets=average*15000
toc
end
```

A.2: Imadjust Function

```
function out = imadjust(varargin)
[img,imageType,lowIn,highIn,lowOut,highOut,gamma] = ...
```

```
parseInputs(varargin{:});

validateLowHigh(lowIn,highIn,lowOut,highOut);
gamma = validateGamma(gamma,imageType);

if ~isfloat(img) && numel(img) > 65536
% integer data type image with more than 65536 elements
out = adjustWithLUT(img,lowIn,highIn,lowOut,highOut,gamma);

else
classin = class(img);
classChanged = false;
if ~isa(img,'double')
classChanged = true;
img = im2double(img);
end

if strcmp(imageType, 'intensity')
out = adjustGrayscaleImage(img,lowIn,highIn,lowOut,highOut,gamma);
elseif strcmp(imageType, 'indexed')
out = adjustColormap(img,lowIn,highIn,lowOut,highOut,gamma);
else
out = adjustTruecolorImage(img,lowIn,highIn,lowOut,highOut,gamma);
end

if classChanged
out = changeclass(classin,out);
end

end


function out = adjustWithLUT(img,lowIn,highIn,lowOut,highOut,gamma)

imgClass = class(img);
out = zeros(size(img),imgClass);

%initialize for lut

switch imgClass
case'uint8'
lutLength = 256;
conversionFcn = @im2uint8;
case'uint16'
lutLength = 65536;
conversionFcn = @im2uint16;
case'int16'
lutLength = 65536;
conversionFcn = @im2int16;
otherwise
eid = sprintf('Images:%s:internalError',mfilename);
msg = 'Internal error: invalid class type.';
error(eid,'%s',msg);
end

for p = 1:size(img,3)
lut = linspace(0,1,lutLength);
scalingFactor = 1;
   lut = adjustArray(lut,lowIn(p),highIn(p),lowOut(p),highOut(p), ...
gamma(p),scalingFactor);
```

```
lut = conversionFcn(lut);
out(:,:,p) = intlut(img(:,:,p),lut);
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
function out = adjustColormap(cmap,lIn,hIn,lOut,hOut,g)

% expansion factor that can expand a 1-by-3 range to the size of cmap.
expansionFactor = ones(size(cmap,1), 1);
out = adjustArray(cmap, lIn, hIn, lOut, hOut, g, expansionFactor);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
function out = adjustGrayscaleImage(img,lIn,hIn,lOut,hOut,g)

expansionFactor = 1;
out = adjustArray(img, lIn, hIn, lOut, hOut, g, expansionFactor);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
function out = adjustTruecolorImage(rgb,lIn,hIn,lOut,hOut,g)

out = zeros(size(rgb), class(rgb));
expansionFactor = 1;
for p = 1 : 3
    out(:,:,p) = adjustArray(rgb(:,:,p), lIn(p),hIn(p), lOut(p), ...
hOut(p), g(p), expansionFactor);
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
function out = adjustArray(img,lIn,hIn,lOut,hOut,g,d)

%make sure img is in the range [lIn;hIn]
img(:) =  max(lIn(d,:), min(hIn(d,:),img));

out = ( (img - lIn(d,:)) ./ (hIn(d,:) - lIn(d,:)) ) .^ (g(d,:));
out(:) = out .* (hOut(d,:) - lOut(d,:)) + lOut(d,:);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
function [img,imageType,low_in,high_in,low_out,high_out,gamma] = ...
parseInputs(varargin)

iptchecknargin(1,4,nargin,mfilename);
img = varargin{1};


% Default values
lowhigh_in  = [0; 1];
lowhigh_out = [0; 1];
gamma = 1;

if nargin == 1
% IMADJUST(I)
if ndims(img) ~= 2
eid = sprintf('Images:%s:oneArgOnlyGrayscale',mfilename);
error(eid, ...
'IMADJUST(I) is only supported for 2-D grayscale images.');
```

```
end
iptcheckinput(img, {'double''uint8''uint16''int16''single'}, ...
        {'2d'}, mfilename, 'I', 1);

    s = warning('off','Images:imhistc:inputHasNaNs');
    lowhigh_in = stretchlim(img);
warning(s)

else
if nargin == 2
if ~isempty(varargin{2})
        lowhigh_in = varargin{2};
end

elseif nargin == 3
)

if ~isempty(varargin{2})
            lowhigh_in = varargin{2};
end
if ~isempty(varargin{3})
            lowhigh_out = varargin{3};
end
else
if ~isempty(varargin{2})
            lowhigh_in = varargin{2};
end
if ~isempty(varargin{3})
            lowhigh_out = varargin{3};
end
if ~isempty(varargin{4})
gamma = varargin{4};
end
end
imageType = findImageType(img, lowhigh_in, lowhigh_out);
checkRange(lowhigh_in, imageType, 2,'[LOW_IN; HIGH_IN]');
checkRange(lowhigh_out, imageType, 3,'[LOW_OUT; HIGH_OUT]');
end

[low_in high_in]   = splitRange(lowhigh_in, imageType);
[low_out high_out] = splitRange(lowhigh_out, imageType);

function imageType = findImageType(img, lowhigh_in, lowhigh_out)

if (ndims(img)==3 && size(img,3)==3)
% RGB image
iptcheckinput(img, {'double''uint8''uint16''int16''single'}, ...
        {}, mfilename, 'RGB1', 1);
imageType = 'truecolor';

elseif (numel(lowhigh_in) == 2 && numel(lowhigh_out) == 2) || ...
size(img,2) ~= 3

iptcheckinput(img, {'double''uint8''uint16''int16''single'}, ...
        {'2d'}, mfilename, 'I', 1);
imageType = 'intensity';

else
%Colormap
iptcheckmap(img,mfilename,'MAP',1);
```

```
imageType = 'indexed';
end

function checkRange(range, imageType, argumentPosition, variableName)

if strcmp(imageType, 'intensity')
if numel(range) ~= 2
eid = sprintf('Images:%s:InputMustBe2ElVec',mfilename);
error(eid, ...
'Function %s expected its %s input argument, %s\n%s', ...
mfilename, iptnum2ordinal(argumentPosition), variableName, ...
'to be a two-element vector.');
end
else
if ~(numel(range) == 2 || isequal(size(range), [2 3]))
eid = sprintf('Images:%s:InputMustBe2ElVecOr2by3Matrix',mfilename);
error(eid, ...
'Function %s expected its %s input argument, %s\n%s', ...
mfilename, iptnum2ordinal(argumentPosition), variableName, ...
'to be a two-element vector or a 2-by-3 matrix.');
end
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
function [rangeMin rangeMax] = splitRange(range, imageType)

if numel(range) == 2
if strcmp(imageType, 'intensity')
rangeMin = range(1);
rangeMax = range(2);
else
% Create triples for RGB image or Colormap
rangeMin = range(1) * ones(1,3);
rangeMax = range(2) * ones(1,3);
end
else
% range is a 2 by 3 array
rangeMin = range(1,:);
rangeMax = range(2,:);
end


%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
function validateLowHigh(lowIn,highIn,lowOut,highOut)

if any(lowIn >= highIn)
eid = sprintf('Images:%s:lowMustBeSmallerThanHigh',mfilename);
error(eid, '%s: LOW_IN must be less than HIGH_IN.',...
upper(mfilename));
end

if isInvalidRange(lowIn) || isInvalidRange(highIn) ...
        || isInvalidRange(lowOut) || isInvalidRange(highOut)
eid = sprintf('Images:%s:parametersAreOutOfRange',mfilename);
error(eid, '%s: LOW_IN, HIGH_IN, LOW_OUT and HIGH_OUT %s',...
upper(mfilename), 'must be in the range [0.0, 1.0].');
end
```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%
function isInvalid = isInvalidRange(range)

isInvalid = min(range) < 0 || max(range) > 1;


%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%
function gamma = validateGamma(gamma,image_type)

if strcmp(image_type,'intensity')
iptcheckinput(gamma,{'double'},{'scalar', 'nonnegative'}, ...
mfilename, 'GAMMA', 4)
else
iptcheckinput(gamma,{'double'},{'nonnegative','2d'},...
mfilename, 'GAMMA', 4)
if numel(gamma) == 1,
gamma = gamma*ones(1,3);
end
end
```

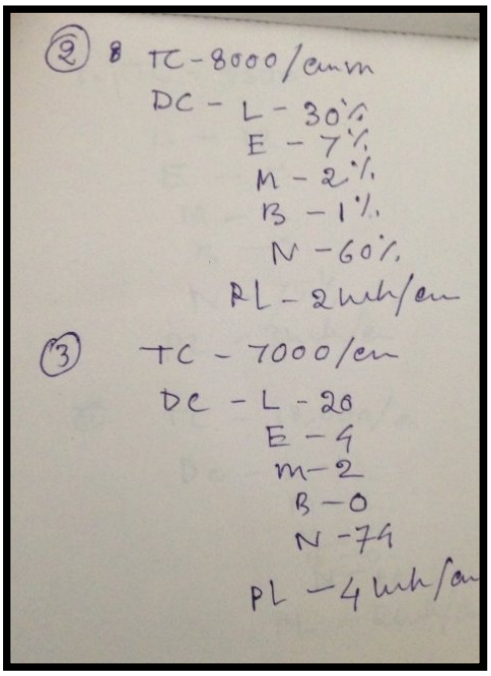
A.3:Rgb2Gray Function

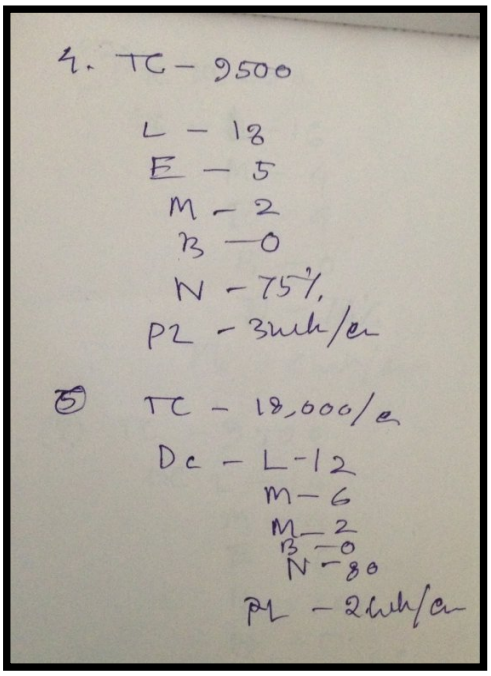
```
function I = rgb2gray(varargin)

X = parse_inputs(varargin{:});
origSize = size(X);

% Determine if input includes a 3-D array
threeD = (ndims(X)==3);

% Calculate transformation matrix
T = inv([1.0 0.956 0.621; 1.0 -0.272 -0.647; 1.0 -1.106 1.703]);
coef = T(1,:)';

if threeD
%RGB
% Shape input matrix so that it is a n x 3 array and initialize output
% matrix
  X = reshape(X(:),origSize(1)*origSize(2),3);
sizeOutput = [origSize(1), origSize(2)];

% Do transformation
if isa(X, 'double') || isa(X, 'single')
    I = X*coef;
    I = min(max(I,0),1);
else
%uint8 or uint16
    I = imlincomb(coef(1),X(:,1),coef(2),X(:,2),coef(3),X(:,3), ...
class(X));
end
%Make sure that the output matrix has the right size
  I = reshape(I,sizeOutput);

else
  I = X * coef;
  I = min(max(I,0),1);
  I = [I,I,I];
end


%%%
%Parse Inputs
%%%
function X = parse_inputs(varargin)

iptchecknargin(1,1,nargin,mfilename);

if ndims(varargin{1})==2
if (size(varargin{1},2) ~=3 || size(varargin{1},1) < 1)
eid = sprintf('Images:%s:invalidSizeForColormap',mfilename);
msg = 'MAP must be a m x 3 array.';
error(eid,'%s',msg);
end
if ~isa(varargin{1},'double')
eid = sprintf('Images:%s:notAValidColormap',mfilename);
    msg1 = 'MAP should be a double m x 3 array with values in the';
    msg2 = ' range [0,1].Convert your map to double using IM2DOUBLE.';
error(eid,'%s %s',msg1,msg2);
end
```

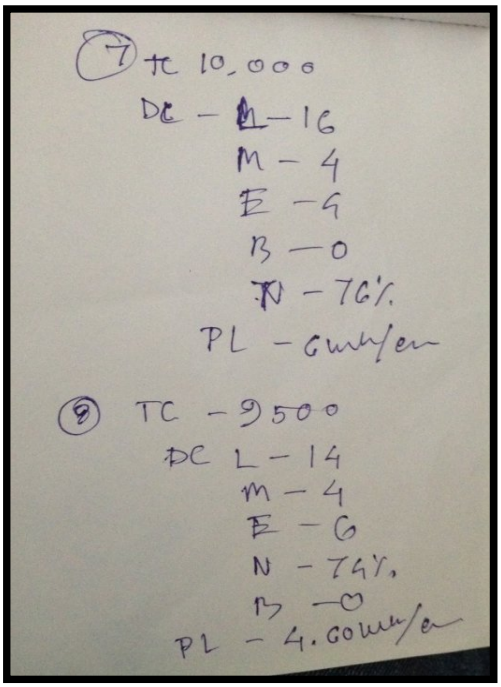
```
elseif (ndims(varargin{1})==3)
if ((size(varargin{1},3) ~= 3))
eid = sprintf('Images:%s:invalidInputSize',mfilename);
msg = 'RGB must be a m x n x 3 array.';
error(eid,'%s',msg);
end
else
eid = sprintf('Images:%s:invalidInputSize',mfilename);
  msg1 = 'RGB2GRAY only accepts a Mx3 matrix for MAP or a MxNx3 input for ';
  msg2 = 'RGB.';
error(eid,'%s %s',msg1,msg2);
end
X = varargin{1};


%no logical arrays
if islogical(X)
eid = sprintf('Images:%s:invalidType',mfilename);
msg = 'RGB2GRAY does not accept logical arrays as inputs.';
error(eid,'%s',msg);
end
```

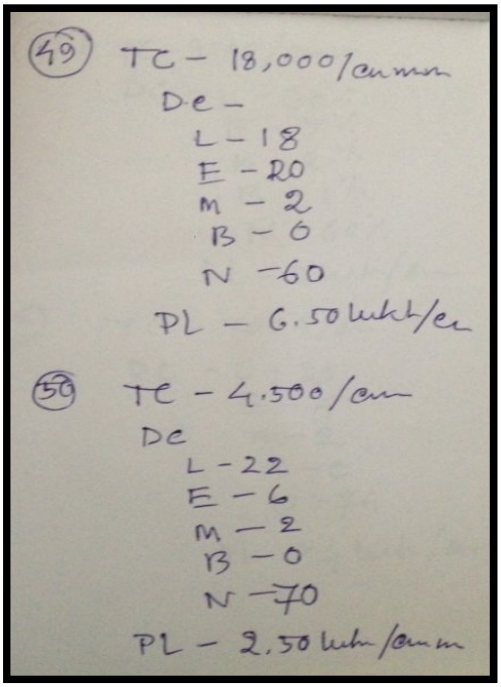
APPENDIX B

B.1 Manual Count Images









51
TC - 8500/cmm
DC
L - 16%
E - 4%
B - 0%
M - 2%
N - 78%
PL - 2.50 lakh/cmm

52 TC - 6000/cmm
DC
L - 20
E - 8
B - 0
M - 4
N - 68
PL - 2 lakh/cm

54
TD - 9000/cm
DC - L - 14
E - 4
B - 0
M - 2
N - 80
PL - 3.50 lakh/cm

55 TC - 20,000/cm
DC
L - 35
E - 5
M - 3
B - 0
N - 57
PL - 4.50 lakh/cm

56 TC - 12.000/cmm
DC -
L - 14
E - 4
B - 0
M - 2
N - 80
PL - 2 lakh/cm

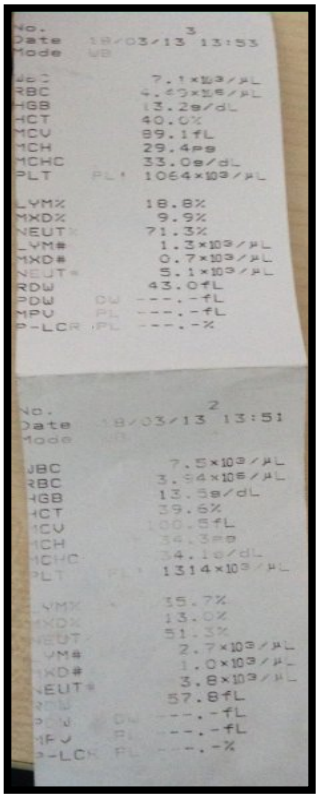
57 TC - 14000/cm
DC - L - 12
E - 3
M - 2
B - 0
N - 83
PL - 2 lakh/cm

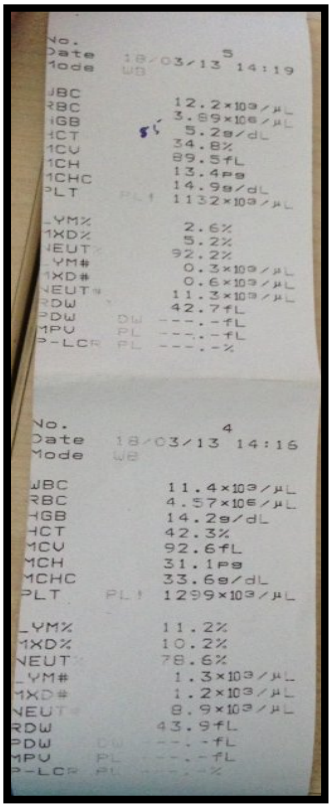
58 TC - 5000/cm
DC
L - 30
M - 4
E - 4
B - 0
N - 62
PL - 3.50 lakh/cm

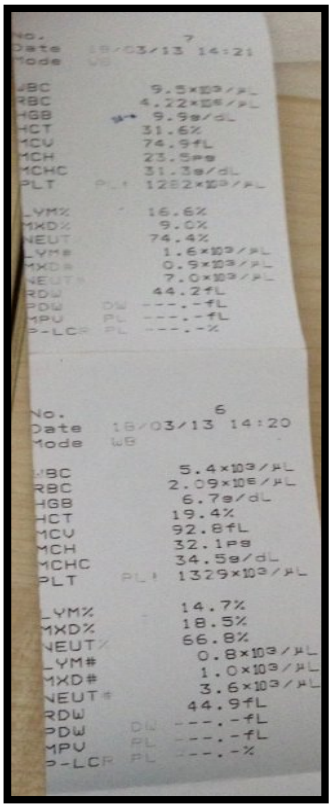
59 TC - 7000/cmm
DC
L - 20
E - 5
M - 2
B - 0
N - 73
PL - 2.30 lakh/cm

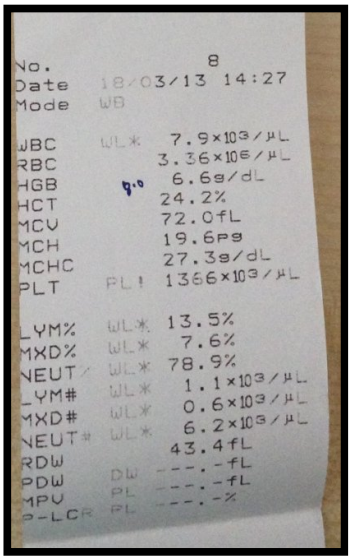
60 TC - 6000/cm
DC
L - 22
E - 4
M - 2
B - 0
N - 72
PL - 1.60 lakh/cm

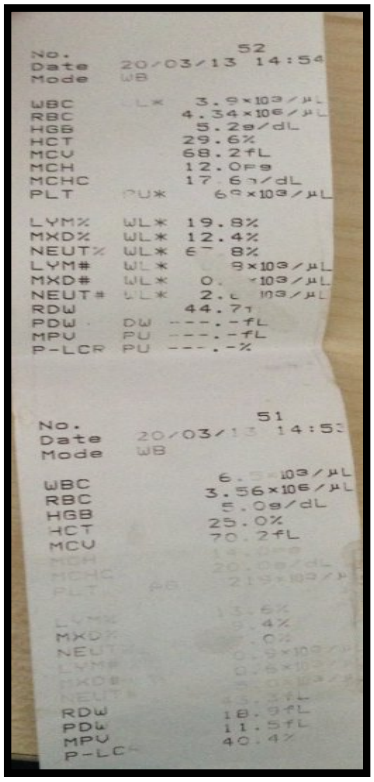
B.2 Machine Count Images









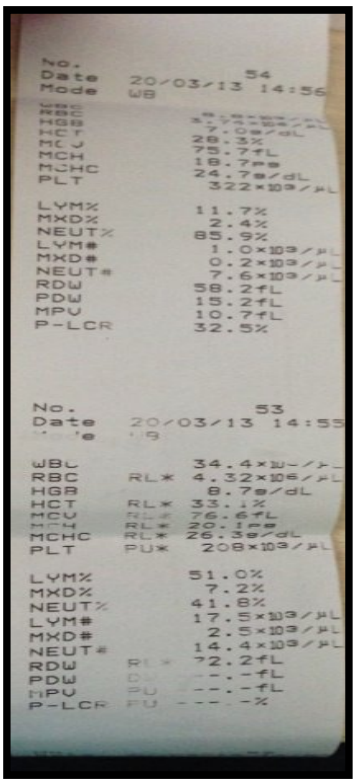


No. 52
Date 20/03/13 14:54
Mode WB

WBC	WL*	3.9×10³/μL
RBC		4.34×10⁶/μL
HGB		5.2g/dL
HCT		29.6%
MCV		68.2fL
MCH		12.0pg
MCHC		17.6g/dL
PLT	PU*	69×10³/μL
LYM%	WL*	19.8%
MXD%	WL*	12.4%
NEUT%	WL*	67.8%
LYM#	WL*	9×10³/μL
MXD#	WL*	0.[illegible]×10³/μL
NEUT#	WL*	2.6×10³/μL
RDW		44.7fL
PDW	DW	---.-fL
MPV	PU	---.-fL
P-LCR	PU	---.-%

No. 51
Date 20/03/13 14:52
Mode WB

WBC		6.[illegible]×10³/μL
RBC		3.56×10⁶/μL
HGB		5.0g/dL
HCT		25.0%
MCV		70.2fL
[illegible]		[illegible]
PLT	AG	219×10³/μL
[illegible]		13.6%
[illegible]		1.4%
MXD%		1.0%
NEUT%		0.9×10³/μL
LYM#		0.6×10³/μL
MXD#		0.1×10³/μL
NEUT#		0.2×10³/μL
RDW		18.9fL
PDW		11.5fL
MPV		40.4%
P-LCR		

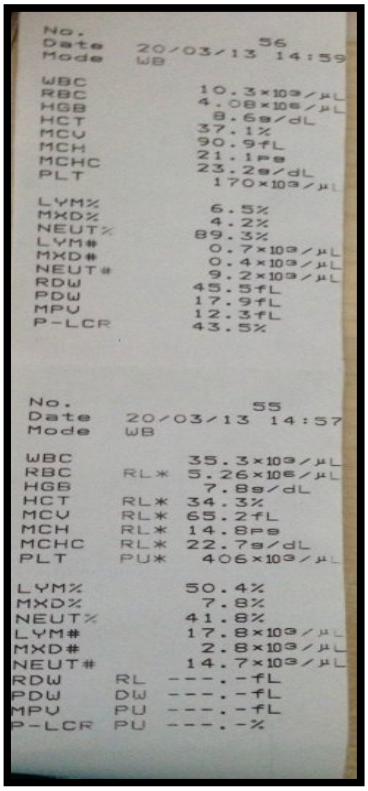


No. 54
Date 20/03/13 14:56
Mode WB

[illegible]		[illegible]
[illegible]		7.0g/dL
[illegible]		28.3%
[illegible]		79.7fL
[illegible]		18.7pg
[illegible]		24.7g/dL
PLT		322×10³/μL
LYM%		11.7%
MXD%		3.4%
NEUT%		85.9%
LYM#		1.0×10³/μL
MXD#		0.2×10³/μL
NEUT#		7.6×10³/μL
RDW		58.2fL
PDW		15.2fL
MPV		10.7fL
P-LCR		32.5%

No. 53
Date 20/03/13 14:55
Mode WB

WBC		34.4×10³/μL
RBC	RL*	4.32×10⁶/μL
HGB		8.7g/dL
HCT	RL*	33.1%
MCV	RL*	76.6fL
[illegible]	RL*	20.1pg
MCHC	RL*	26.3g/dL
PLT	PU*	208×10³/μL
LYM%		51.0%
MXD%		7.2%
NEUT%		41.8%
LYM#		17.5×10³/μL
MXD#		2.5×10³/μL
NEUT#		14.4×10³/μL
RDW	RL*	72.2fL
PDW	DW	---.-fL
MPV	PU	---.-fL
P-LCR	PU	---.-%

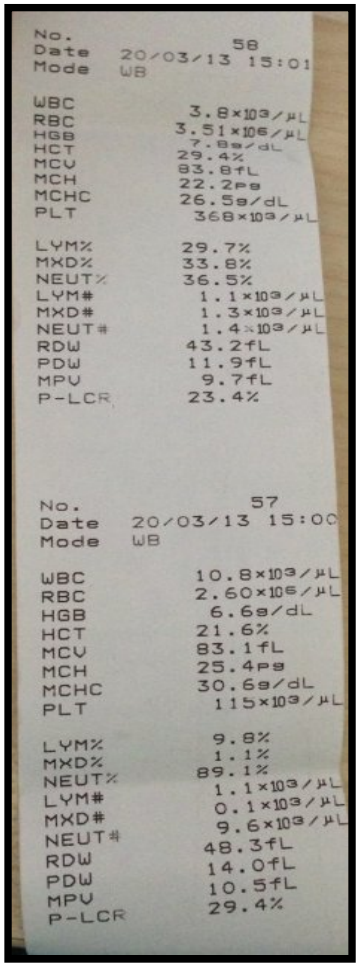


No. 56
Date 20/03/13 14:59
Mode WB

WBC		10.3×10³/μL
RBC		4.08×10⁶/μL
HGB		8.6g/dL
HCT		37.1%
MCV		90.9fL
MCH		21.1pg
MCHC		23.2g/dL
PLT		170×10³/μL
LYM%		6.5%
MXD%		4.2%
NEUT%		89.3%
LYM#		0.7×10³/μL
MXD#		0.4×10³/μL
NEUT#		9.2×10³/μL
RDW		45.5fL
PDW		17.9fL
MPV		12.3fL
P-LCR		43.5%

No. 55
Date 20/03/13 14:57
Mode WB

WBC		35.3×10³/μL
RBC	RL*	5.26×10⁶/μL
HGB		7.8g/dL
HCT	RL*	34.3%
MCV	RL*	65.2fL
MCH	RL*	14.8pg
MCHC	RL*	22.7g/dL
PLT	PU*	406×10³/μL
LYM%		50.4%
MXD%		7.8%
NEUT%		41.8%
LYM#		17.8×10³/μL
MXD#		2.8×10³/μL
NEUT#		14.7×10³/μL
RDW	RL	---.-fL
PDW	DW	---.-fL
MPV	PU	---.-fL
P-LCR	PU	---.-%

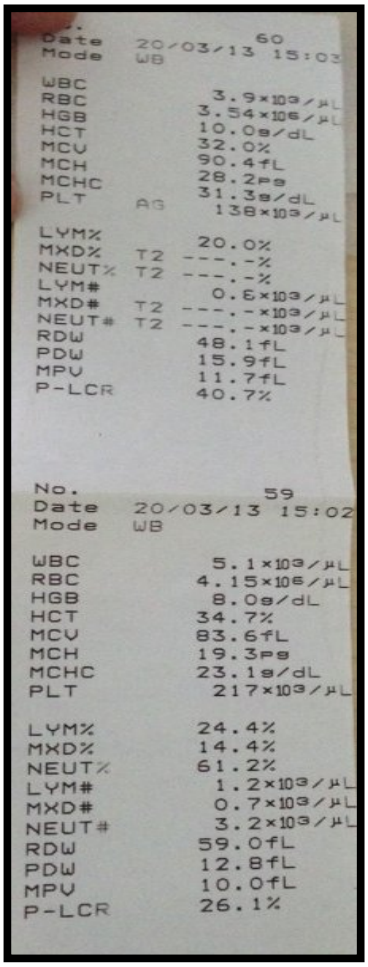


No. 58
Date 20/03/13 15:01
Mode WB

WBC		3.8×10³/μL
RBC		3.51×10⁶/μL
HGB		7.8g/dL
HCT		29.4%
MCV		83.8fL
MCH		22.2pg
MCHC		26.5g/dL
PLT		368×10³/μL
LYM%		29.7%
MXD%		33.8%
NEUT%		36.5%
LYM#		1.1×10³/μL
MXD#		1.3×10³/μL
NEUT#		1.4×10³/μL
RDW		43.2fL
PDW		11.9fL
MPV		9.7fL
P-LCR		23.4%

No. 57
Date 20/03/13 15:00
Mode WB

WBC		10.8×10³/μL
RBC		2.60×10⁶/μL
HGB		6.6g/dL
HCT		21.6%
MCV		83.1fL
MCH		25.4pg
MCHC		30.6g/dL
PLT		115×10³/μL
LYM%		9.8%
MXD%		1.1%
NEUT%		89.1%
LYM#		1.1×10³/μL
MXD#		0.1×10³/μL
NEUT#		9.6×10³/μL
RDW		48.3fL
PDW		14.0fL
MPV		10.5fL
P-LCR		29.4%



No. 60
Date 20/03/13 15:03
Mode WB

WBC		3.9×10³/μL
RBC		3.54×10⁶/μL
HGB		10.0g/dL
HCT		32.0%
MCV		90.4fL
MCH		28.2pg
MCHC		31.3g/dL
PLT	AG	138×10³/μL
LYM%		20.0%
MXD%	T2	---.-%
NEUT%	T2	---.-%
LYM#		0.8×10³/μL
MXD#	T2	---.-×10³/μL
NEUT#	T2	---.-×10³/μL
RDW		48.1fL
PDW		15.9fL
MPV		11.7fL
P-LCR		40.7%

No. 59
Date 20/03/13 15:02
Mode WB

WBC		5.1×10³/μL
RBC		4.15×10⁶/μL
HGB		8.0g/dL
HCT		34.7%
MCV		83.6fL
MCH		19.3pg
MCHC		23.1g/dL
PLT		217×10³/μL
LYM%		24.4%
MXD%		14.4%
NEUT%		61.2%
LYM#		1.2×10³/μL
MXD#		0.7×10³/μL
NEUT#		3.2×10³/μL
RDW		59.0fL
PDW		12.8fL
MPV		10.0fL
P-LCR		26.1%

B.3 : Observations for Different Samples

Sample No.	Manual Count(μL)	Machine Count(μL)	Proposed Method Count(μL)
2	2,00,000	1,31,000	1,80,000
3	4,00,000	6,64,000	4,40,000
4	3,00,000	3,20,000	2,50,000
5	2,00,000	1,70,000	2,10,000
7	6,00,000	7,50,000	6,50,000
8	4,60,000	3,50,000	5,00,000
49	6,50,000	8,00,000	6,00,000
50	2,50,000	1,83,500	3,00,000
51	2,50,000	2,19,000	2,22,000
52	2,00,000	1,68,000	1,80,000
54	3,50,000	3,22,000	3,25,000
55	4,50,000	4,06,000	4,25,500
56	2,00,000	1,70,000	1,85,000
57	2,00,000	1,15,000	2,50,000
58	3,50,000	3,68,000	3,40,000
59	30,000	18,000	10,000
60	80,000	92,000	70,000