# Automated Detection of White Blood Cells (Leukocytes) in Digital Microscopic Image


**Final Year Project Report**

**Presented**

**by**

**Waleed Ahmed**
CIIT/FA18-BCE-065/ISB

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**Hasnain Ahmad**
CIIT/FA18-BCE-068/ISB

**In Partial Fulfillment**

**of the Requirement for the Degree of**

***Bachelor of Science in Electrical (Computer) Engineering***

**DEPARTMENT OF ELECTRICAL AND COMPUTER ENGINEERING**

# COMSATS UNIVERSITY ISLAMABAD

## June 2022

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# *Declaration*

*We, hereby declare that this project neither as a whole nor as a part there of has been copied out from any source. It is further declared that we have developed this project and the accompanied report entirely on the basis of our personal efforts made under the sincere guidance of our supervisor. No portion of the work presented in this report has been submitted in the support of any other degree or qualification of this or any other University or Institute of learning, if found we shall stand responsible.*

**Signature:_____**
**Name Waleed Ahmed**

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**Name Muhammad Haris Siddiqui**

**Signature:_____**
**Name Hasnain Ahmad**

COMSATS UNIVERSITY ISLAMABAD
June 2022

# Automated Detection of White Blood Cells (Leukocytes) in Digital Microscopic Image

An Undergraduate Final Year Project Report submitted to the

Department of
**ELECTRICAL AND COMPUTER ENGINEERING**

**As a Partial Fulfillment for the Award of Degree**

**Bachelor of Science in Electrical (Computer) Engineering**

*by*


| **Name** | **Registration Number** |
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| **Waleed Ahmed** | CIIT/FA18-BCE-065/ISB |
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**Supervised by**


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Assistant Professor
Department of Electrical and Computer Engineering
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COMSATS UNIVERSITY ISLAMABAD
June 2022

# *Final Approval*

*This Project Titled*
*Automated Detection of White Blood Cells (Leukocytes) in Digital Microscopic Image*

*Submitted for the Degree of*
**Bachelor of Science in Electrical (Computer) Engineering**

*by*

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*has been approved for*

## COMSATS UNIVERSITY ISLAMABAD

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*Designation*

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*Department of Electrical and Computer Engineering*

# *Dedication*

*First and foremost, We have to thank our parents for their love and support throughout our life. Thanks to them for giving us the strength to reach for the stars and chase our dreams.*

*We would like to thank our supervisor Dr. Ahsan Khawaja, for his guidance and support throughout this study and especially for his confidence on us. We would also like to thank Dr. Abrar and Mr. Muhammad Bilal Qasim for serving as members of our examiner committee. Their comments and questions were very beneficial in the completion of the manuscript.*

*To all our friends, thank you for your understanding and encouragement in many, many moments of crises. Your friendship has made our life a wonderful experience. We cannot list all the names here, but you will always be on our mind.*

*Thanks, Almighty Allah for always being there for us.*

*This Thesis is only a beginning of our journey*

# *Acknowledgments*

*Thoroughly thankful to Almighty Allah, who gave us the strength and patience to complete our Project. We would like to pay the debt of gratitude to Dr. Ahsan Khawaja being our supervisor for this work and for their precious time. He gave us the golden opportunity to do this project. His encouragement and guidance made it possible to complete our Final Year Project.*

*We would love to show our respects to the Electrical and Computer Engineering Department students who literally helped us understand the true ethics of lab norms.*

*We deem it a great honor to express our sincere sense of respect to our parents, family, and teachers for their encouragement, genuine prayers, and good wishes for the successful completion of our project.*

*We would also like to mention our friends for making our life at COMSATS the most memorable one.*

*In the end, we extend our thanks to the academic staff of the ECE for their kindness.*

***Waleed Ahmed***

***Muhammad Haris Siddiqui***

***Hasnain Ahmad***

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# *Abstract*

White blood cells that are also known as Leukocytes are very significant in defending the human body against several bacterial and viral infections. Identification of different kinds of white blood cells can assist in gathering valuable information for the diagnosis and treatment of different kinds of infections. The white blood cell identification can help to detect viral and bacterial infections within the body and alert the people who are responsible to take appropriate actions timely against these medical conditions, such as autoimmune diseases, immune deficiencies, and blood disorders. It is a very difficult and time and effort consuming process to manually identifying and counting of these cellular structures.

So, an automated system can be designed to identify different kinds of white blood cells on the basis of the features of their appearance e.g., color, geometric shape etc. Such a system can not only make the whole process fast and cost efficient but will also minimize the chances of human error. This project will be entirely focused on identifying different kinds of white blood cells and estimate their count in a sample microscopic blood smear image. Different image processing techniques will be used on publicly available datasets with expert marked ground-truths.

The desired outcome will be the comparison of efficacy and shortcomings of each of these methods for the leukocyte identification and enumeration. Also, the to make appropriate changes in different subsections and tuning parameters of the proposed method and analyze the effect of change and ultimately to improve the performance measures of overall method.

# Chapter 1

# Introduction

Blood is the most essential fluid of human body as it constitutes almost 8% of the total human body's weight. Blood has many components from which Red Blood Cells, White Blood Cells and Platelets are major components. White Blood cells contain nucleus and cytoplasm which can be distributed into four major categories basophils, monocytes, lymphocytes and neutrophils. Since White blood cells are major part of Immune system so, collecting quantitative and qualitive information about White Blood Cells can help pathologists in detecting and diagnosing several diseases. [1]

It's a difficult and time and effort consuming process to manually identify and count the white blood cells in microscope. So, there is a vital space for an automated system that identify different kinds of white blood cells based on their features of their appearance e.g., color, geometric shape, etc. This project will be entirely focused on identifying different kinds of white blood cells and estimate their count in a sample microscopic blood smear image. Segmentation has always been remained the major step in microscopic image analysis.

This project focusses more on the pre-processing techniques used to improve the overall contrast of image before segmentation. Objective for this project would be to change the methods and parameters of existing methods to analyze the effect of change and then setting those parameters to achieve maximum possible results. Then different image processing techniques are used on publicly available datasets with expert marked ground-truths. The desired outcome will be the comparison of the efficacy and shortcomings of each of these methods for leukocyte identification and enumeration.

A white blood cell count has a great significance in medical field as it can provide important information regarding the hidden infections within the human body that can be used to alert doctors to undiagnosed medical conditions, such as autoimmune diseases, immune deficiencies, and blood disorders. It can also help doctors in monitoring the effectiveness of chemotherapy or radiation treatment in cancer patients.

# 1.1 Background

## 1.1.1 Structure Of White blood Cell

WBCs are formed from the bone marrow inside the joints of bones and tracked down in the blood and lymphatic framework. Every White blood cell contains a nucleus, which is frequently huge and lobed, and it also assists with differentiating WBC from the other platelets. Every WBC structure comprises of a core, cytoplasm and cell divider

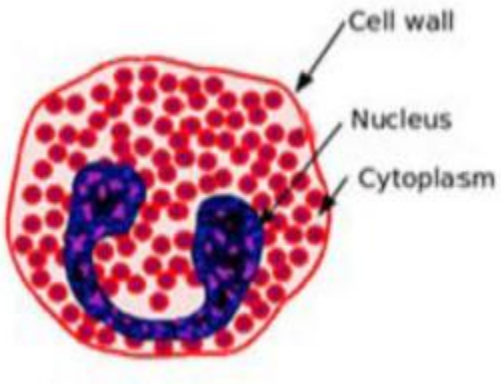


*Figure 1. Structure of White Blood Cell*

## 1.1.2 Types and subtypes of White Blood Cells

The cores of WBC have various shapes, surface and sizes and could introduce at least one projections in light of the response of their particular granules with a staining cycle as displayed in Fig.3. The most valuable shape, size and surface data for cell division and characterization comes from the cores of the WBCs [2]. To give a concise survey and viewpoint, the highlights and elements of types and sub-kinds of WBCs and data about WBC cores shape are made sense of as follows.

• Granulocytes are phagocytes, which can ingest infections, microbes and different parasites. They have apparent granules or grains in their cytoplasm and have enormous stretched or lobed cores. The width of cell gauges around from (12 - 20) μ, and their nucleoli shouldn't be visible. They represent roughly 60% of our WBCs. The sub-sorts of granulocytes are neutrophil, basophil and eosinophil [5].

• Neutrophils are a piece of the intrinsic invulnerable framework and a fundamental line of protection against microbes. The state of core is like a "U" or a twisted bar before division. They are otherwise called "band neutrophils". The width typically goes between (10-18) μ. The cytoplasm is moderate to bountiful with a couple of vague granules.

Neutrophils represent roughly (1% - 3%) of the fringe WBCs. The distance across of a sectioned neutrophil cell typically runs between (9 - 16) μ. They have a multi-lobed core (three or four projections regularly), and these flaps might cover or contort [6]. The quantity of flaps can build as indicated by the cell age. For instance, a hyper divided neutrophil cell has seven projections in mature stage. The intra-cell granules are apparent in the cytoplasm (Giemsa-stained, high amplification) [5].

• Basophils emit anticoagulant substances and antibodies that can battle against excessive touchiness responses in the circulatory system. They are the littlest coursing granulocytes. The basophilic granules in this cell are enormous and exceptionally various, so they frequently cover the core. The core is frequently bilobed or unsegmented and it is seldom isolated into three or four projections. The typical width ranges between around (10 - 15) μ.

• Eosinophils can let poisons out of their granules for killing microbes, like parasites and worms. They are effortlessly perceived in stained spreads by their enormous granules. The core of the eosinophil has frequently two projections associated by a band of atomic material. The measurement typically runs between (9 - 15) μ. They account between (1% - 4%) of the fringe WBCs [2, 5]. Microscopy Science: Last Approaches on Educational Programs and Applied Research (Enrique Torres-Hergueta and A. Méndez-Vilas, Eds.) 18.

Pathologists implement tiny blood smear examination to analyze different hematological infections. Assessment of platelets uncovers the wellbeing status of an individual. A blood problem is thought of as generally risky among sicknesses that can prompt demise. A large number of these blood illnesses are related with white platelets.

White platelets give insusceptibility against infections by gulping unfamiliar bodies and creating antibodies. They are of five kinds named lymphocytes, monocytes, basophils, eosinophils, and neutrophils with differing sizes and geometry. They assume a critical part in the checking of various infections like leukemia, invulnerable lack disorder, hypersensitive responses, etc. Thus, separating data about white platelets from blood smears can help pathologists in determination. Checking arranged blood tests by pathologists for order is slow and tedious. A computerized platelet arrangement framework is expected to help pathologists.

The objective of the Leukocytes categorization is to separate cells from blood smear, take away the foundation and section every single piece of the cell-like core, cytoplasm, and so on. This study will zero in on programmed leukocytes nucleus categorization utilizing a system created by the image processing.

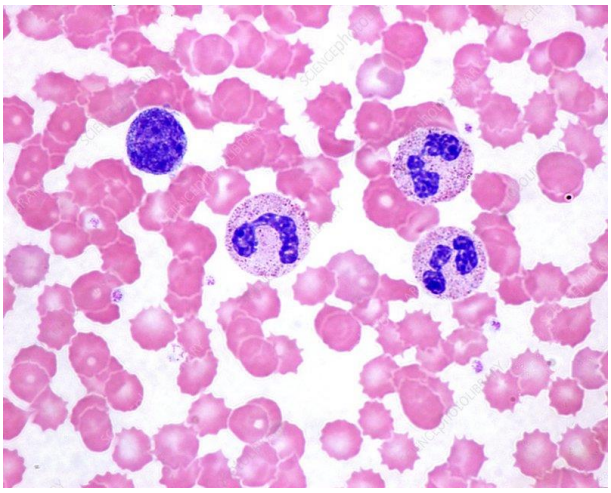

*Figure 2. Microscopic image of blood smear*

(WBCs) or leukocytes characterization is a fundamental process since it can assist hematologists in diagnosing many blood issues, like leukemia, a few immunological problems, and particular kinds of disease. The examination methodology can be finished via programmed and manual techniques to count and order white platelet. Manual arrangement of WBC has numerous clinical challenges, remembering mistakes for the exactness of results because of inspecting blunders and likelihood and unfortunate responsiveness, particularity, and prescient qualities [2]. Additionally, a few programmed approaches in the research centers have utilized instruments, for example, stream cytometry and programmed counting

machine to distinguish and arrange white platelets. These instruments don't use picture handling methods, and they can count and arrange white platelets quantitatively not subjective.

Blood tests can research numerous sicknesses like malignant growth, HIV/AIDS, diabetes, paleness, and coronary illness [2]. Subsequently, blood tests are of high significance for determination of numerous sicknesses and furthermore to examine elements of body organs like kidney, liver, thyroid, and heart. Manual minuscule assessment is a must when there is a doubt of irregularity in the blood test, however it is dreary, tedious, and abstract.

In the event that the visual example examination is computerized, it will assist the pathologists with expanding efficiency and lessen costs. The mechanization interaction incorporates picture securing, picture handling, division, include extraction, and arrangement. Division is viewed as the most significant and basic advance in the process as it influences the other following advances [3].

Along these lines, it is important to plan a programmed framework that incorporates picture handling, signal handling, design acknowledgment, or profound learning methods to give a subjective and quantitative assessment, exact outcomes, and quick handling. A mechanized characterization of the White platelet type framework comprises of six stages, as displayed in Figure 2) picture procurement, 2) picture pre-handling, 3) division, 4) highlights extraction and portrayals, 5) cell arrangement, and 6) the assessment cycle.

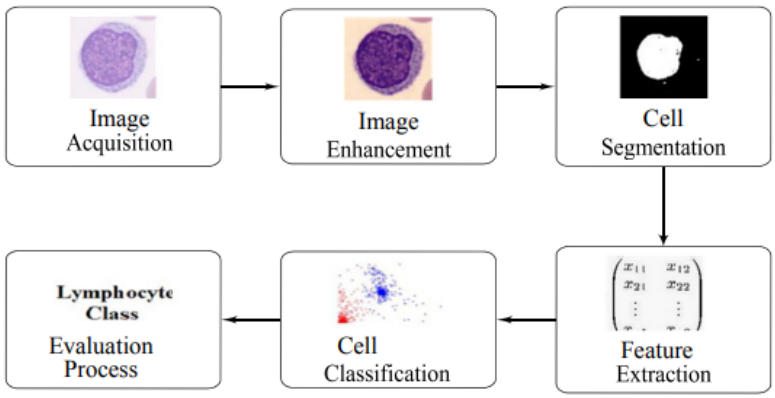


*Figure 3. Steps for Automated Detection of WBC*

## 1.2 Related Diseases

### 1.2.1 HIV & AIDS

HIV (human immunodeficiency Virus) is an infection which assaults cells that assist the body with tackling contamination, making an individual more exposed against different viruses and illnesses. It is spread by contact with specific organic liquids of an individual with HIV, most usually during unprotected sex or through sharing illegal drugs by means of injection. Whenever left untreated, HIV can prompt the illness AIDS (AIDS). The human body can't dispose of HIV and no viable HIV fix exists. In this way, when you have HIV, you have it forever.

Notwithstanding, by taking HIV medication (called antiretroviral treatment or ART), individuals with HIV can carry on with long and sound lives and forestall communicating HIV to their sexual accomplices. Furthermore, there are powerful strategies to forestall getting HIV.First distinguished in 1981, HIV is the reason for one of humankind's deadliest and most tireless scourges.. Around the world, 37.7 million [30.2-45.1 million] individuals were living with HIV toward the finish of 2020. Of these, 36 million were grown-ups and 1.7 million were youngsters matured 0-14 years. The greater part (53%) were ladies and young ladies [4].

| | People living with HIV in 2020 | People acquiring HIV in 2020 | People dying from HIV-related causes in 2020 |
|---|---|---|---|
| Total | 37.7 million [30.2–45.1 million] | 1.5 million [1.0–2.0 million] | 680 000 [480 000–1.0 million] |
| Adults (15+ years) | 36.0 million [28.9–43.2 million] | 1.3 million [910 000–1.8 million] | 580 000 [400 000–850 000] |
| Women (15+ years) | 19.3 million [15.5–23.1 million] | 660 000 [450 000–920 000] | 240 000 [170 000–360 000] |
| Men (15+ years) | 16.7 million [13.3–20.1 million] | 640 000 [460 000–890 000] | 340 000 [230 000–490 000] |
| Children (<15 years) | 1.7 million [1.2–2.2 million] | 150 000 [100 000–240 000] | 99 000 [68 000–160 000] |

*Figure 4. Summary of Global HIV epidemic from WHO*

### 1.2.2 Leukemia

Leukemia is a type of blood cancer. In layman terms, it can be defined as rapid and uncontrolled growth of abnormal white blood cells. Cancer can be formed anywhere in the body. In leukemia, this out-of-control growth of abnormal cells takes place in the bone marrow in joints and bones.

These abnormal cells are then spilled into the blood. Unlike the other cancers, leukemia often doesn't take a form of tumor that can be seen in imaging tests, such as X-rays.[5] The strict monitoring of four types of white blood cells named lymphocytes, monocytes, basophils and neutrophils with varying size and geometry can contribute in keeping track of leukemia.

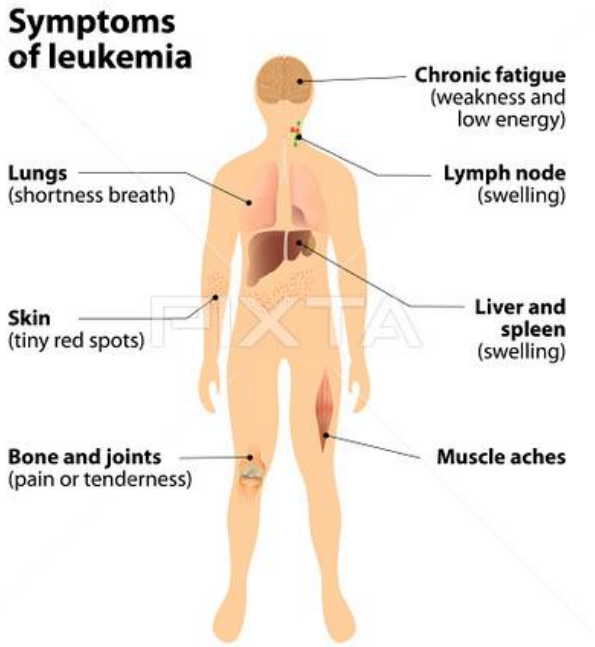


*Figure 5. Symptoms of leukemia*

Following chart shows the WHO's year wise data of the people affected by leukemia and the total number of deaths caused by leukemia. It shows how dangerous this disease is.

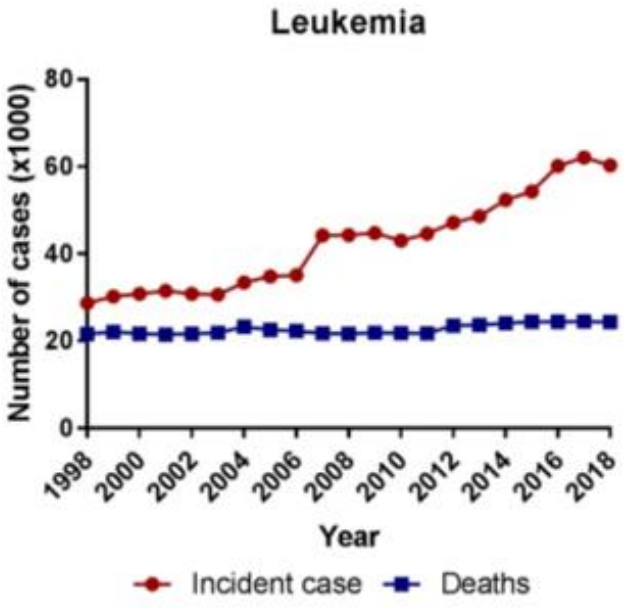


*Figure 6. Year-wise Leukemia Cases*

## 1.3 Motivation

Progresses in advanced imaging innovations and expanded independence in information control (handling, storing, and transformation), have made ready for mechanized analysis and treatment of sicknesses. Such frameworks permit much better asset the board concerning infection finding, forecast, counteraction, and treatment. Quicker and precise understanding of biomedical pictures and monitoring sickness movement over the long run make such Computer-Aided Di (CAD) framework a fundamental component representing things to come of medical services.

A major part of any white blood cell detection algorithm comprises of segmentation part. A series of methods and techniques like Otsu's Thresholding, K-Means Clustering, Watershed Segmentation and Neural Network based segmentation had been developed for better and efficient segmentation of white blood cells.

But there is a fine gap between the image pre-processing phase and segmentation phase. As pre-processing part of the algorithm is application oriented and subjective that's why there is not much work done in this regard. There are many already existing techniques for image pre-processing phase like histogram equalization, linear Contrast Stretching.

## 1.4 Contribution

The purpose of this project is to implement an already proposed technique of white blood cells segmentation and try to improve the performance measure of this technique by changing certain parts and performing appropriate optimizations in the methodology. In phase 1 of the working, the methodology of paper "An efficient technique for white blood cell nuclei automatic segmentation" by Mostafa Mahmood and Amr Guaily[] was implemented without any changes. The Technique was implemented on Matlab 2021 with systems specifications as under

- Intel Core i3 7$^{th}$ Gen Processor
- 12 GB RAM

In the 2$^{nd}$ phase of implementation, the color domain of the original image was converted from RGB to CMYK domain before applying the further steps of pre-processing or segmentation. In this phase sensitivity was improved with a handsome amount of 0.075, accuracy was decreased with a small amount of 0.0003 and specificity decreased with an amount of 0.02. In this phase of implementation, the filtering step was optimized. Just after the thresholding, the binary image was convoluted with a minimum filter of 2x2 filter which was 3x3 in initial implementation. Also, the iterations of application of filter was changed to 6 instead of 3.

In this phase morphological closing was introduced along with the morphological opening. In the initial methodology, only morphological opening was implemented. So, after introducing opening a further increase in sensitivity was achieved by an amount of 0.024. The accuracy and specificity were slightly decreased**.**

# Chapter 2

# Literature Review

In this chapter we are going to review different image segmentation techniques and methods that are already used by the researchers and also, we are going to find out their advantages and disadvantages for some particular applications as well as their overall performance. We will also review the accuracy of the results of these techniques and methods.

## 2.1 Problem Statement

The manual identification and counting of different types of white blood cells proved to be a time and effort taking process for the pathologists. So, there is a need of an automated system than can detect and count the white blood cells with such an accuracy that doctors and medical scientist can rely on its results.

The difficulties regarding WBC segmentation are its number of variety of shapes and sizes. The goal of WBC segmentation is to extract the cells from complicated background. WBC segmentation primarily extracts the cells from complicated background and segments every cell into morphological components such as nucleus, cytoplasm, and some others.

### 2.1.1 Review of Already Proposed WBC Segmentation Techniques

Hedge [6] suggested that blend of arithmetical and morphological tasks with dynamic form can section cores even if there should arise an occurrence of brightening varieties and revealed normal dice score of 0.965. For mechanization of WBCs cores discovery, a ton of endeavors have been made, yet at the same time there is a need of hearty and more exact technique which can likewise deal with power varieties.

Wang [7] proposed leukocyte cores division in view of part contrast in GGB variety space by switching pictures from RGB over completely to GGB. B-G values sectioned the cores and platelets and platelets are prohibited by applying limit.

Ongun [8] set forward division calculation in view of dynamic form models (snakes and inflatables) and utilized shape and morphological administrators. for the arrangement of WBCs. The calculation was made utilizing the investigations of

twelve classes of WBCs. Prinyakupt [9] divided WBC by contrasting straight promotion innocent Bayes classifier and got dice likeness of around 0.98 and 0.91 and rectification pace of around 98% and 94% in division, individually.

Li [10] suggested double edge strategy for WBC division which is blend of math activities alongside middle channels and the precision of proposed calculation was 97.85%. HSV variety portrayal close by and XOR consistent activities to section leukocyte and detailed precision was 97.7% for 10 pictures. Chu [11] proposed a technique for the divisions of WBCs with Dice Score of 0.95, Sensitivity of 0.93 and FPR of 0.0009.

In 2009, Sadeghian et al. [12] set forward a division calculation to portion WBCs as well as their cores and a division method to isolate the cytoplasm of the WBCs cell. His structure to section WBC is isolated into two sections. Right off the bat, Nucleus Segmentation-Based on morphological examination gives 92% precision while Cytoplasm Segmentation-Based on pixel-force thresholding giving 70% exactness.

His strategy incorporates a red, green, and blue (RGB) picture changed over completely to dark picture then vigilant edge discovery is applied trailed by inclination vector stream to associate the limit of the core then an opening filling procedure is applied to continually get the core. Additionally, Zack calculation is likewise applied into the dim picture to get the twofold picture to remove the cytoplasm of the cell by taking away the double picture from the dark picture.

Kose [13] Blood issue is thought of as generally perilous among sicknesses which can prompt passing. A large number of these blood illnesses are related with white platelets. White platelets give insusceptibility against infections by gulping the unfamiliar bodies and creating antibodies. Andrade [14] thought about various leukocyte division techniques and showed two strategies which gave most noteworthy division exactness of 97% however sectioned just 58.44% pictures.

In 2015 Madhloom et al. [15] proposed a division procedure to fragment WBCs and their core. The propose of the calculation was to mechanize the course of discovery and characterization of leukocytes. In particular, the white platelets are perceived and characterized into different unmistakable subtypes. The alteration was done to

diminish reliance on the picture starting differentiation. This difference reliance prompts the distinguish every one of the components that have a similar dim level as of the WBCs.

In the first place, it portions the WBCs in view of the variety change as well as a few morphological administrators. Also, the covered cells are isolated through marker-control watershed method. Besides, the core of the cells is sectioned however the cultivated area developing procedure. The aftereffect of their methodology is assessed utilizing relative extreme estimation exactness and misclassification mistake to gauge the precision and it accomplishes 96% for WBCs division and 94% for core division.

Vromen and McCane [16] set forward a model in view of shape following way to deal with tackle the issue a Scanning Electron Microscope picture of red platelets which is naturally portioning. Second request polynomial model was utilized and a basic Bayesian formula for dealing with guaranteed smooth border. Ghane [17] fragmented and removed WBC cores from blood smear utilizing K-implies bunching and watershed calculation

Deshmukh [18] fragmented cores by utilizing the blend of math activities, SVM (Support Vector Machine) classifier and K-implies bunching. Ramoser et al. [19] proposed a completely computerized approach for division of leukocyte that is vigorous concerning cell appearance and picture resolution. Nucleus and Cytoplasm characteristics are addressed by a bunch of highlights and pairwise SVM grouping separates between various cell types. Assessment on 1166 pictures (13 classes) brought about 95% right divisions and 75% to almost 100% right characterization.

Dhanachandra [20] Segmentation is achieved by utilizing K-means bunching calculation which will isolate the gathering of articles in the given picture. It will limit the amount of square distances between all places and group focuses.Angulo and Flandrin [21] set forward a model in view of consequently recognize the working reigon of fringe blood spreads stained with May-Grünwuald Giemsa.

The ideal locale is characterized by the very much spread piece of the smear. The calculation comprises of two phases. In first stage, Erythrocytes are extricated by applying picture investigation utilizing numerical morphology. Also, in second stage,

three sorts of particles are counted from the quantity of associated parts and the coefficient of spreading and the coefficient of covering are determined. Safuan [22] carried out thresholding procedures on various variety space portrayals like RGB, CMYK and HSV to fragment WBC.

# Chapter 3

# Working and Methodology

## 3.1 Flow Chart

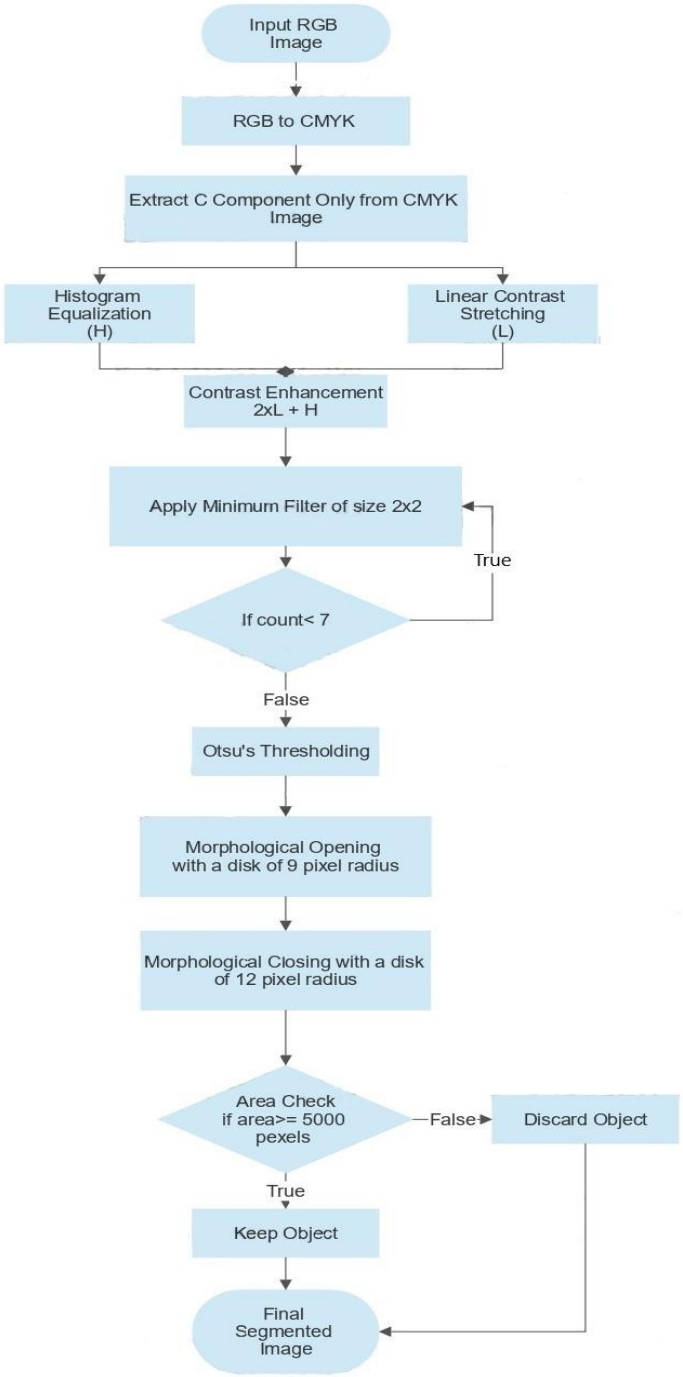


*Figure 7. Flow chart of Proposed Method*

## 3.2 Changing the Color Domain

The idea was developed on noticing that the color of cell nucleus is very much similar to cyan color which is a lot different from the color of cytoplasm. If we change from RGB (RED, GREEN, BLUE) color domain to CMYK (CYAN, MAGENTA, YELLOW, BLACK) and then separate the cyan component and apply other contrast enhancement techniques then most of the undesired objects or the noise from image would be automatically removed.

The conversion from RGB to CMYK is quite simple. First, we normalized the intensity value of each R, G and B component by dividing them with max value of their range and then implementing following formula.

$$\begin{bmatrix} C \\ M \\ Y \end{bmatrix} = \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} - \begin{bmatrix} R \\ G \\ B \end{bmatrix}$$

CMYK K is the fourth color, black; because equal amount of CMY produces a muddy-looking black, as black is the predominant color we have to produce true or pure black

This could really help in clear segmentation of cell from rest of the image. Also applying this technique before any contrast enhancement technique will provide an advantage as contrast enhancement technique would not affect the noise components as they would have been minimized earlier.

## 3.3 Contrast Enhancement

Enhancing image contrast has always remained one of the most essential issues in the digital image processing history. Contrast enhancement has the most effective impact on image quality among all other image processing methods. Many methods and techniques have been proposed to enhance the contrast of the image but there seems to be no universal technique that fits perfectly in all applications, simply because contrast of the image is a subjective matter which purely depends on the application. In our project we have proposed the following method for enhancing the contrast of White blood cells in the images.

$$EI = 2 \times L + H$$

where EI is our enhanced image, L is the image resulted after applying the Linear Contrast Stretching method on the image converted from RGB to gray scale and H is the image resulted after applying Histogram Equalization

### 3.3.1 Linear Contrast Stretching

Contrast Stretching is one of the easiest piecewise defined linear functions. Poor lightning, lack of dynamic range in imaging sensor can be the causes of poor contrast in an image. Even the false setting of aperture of a lens in image acquisition can result in low contrast image.

In contrast stretching process the actual range of intensity levels in an image is expanded over the entire intensity levels range of the image storing medium or display device. As it is a piecewise defined function so we can take many breakpoints for deciding the range for which we want to increase or decrease the image contrast for a given range.

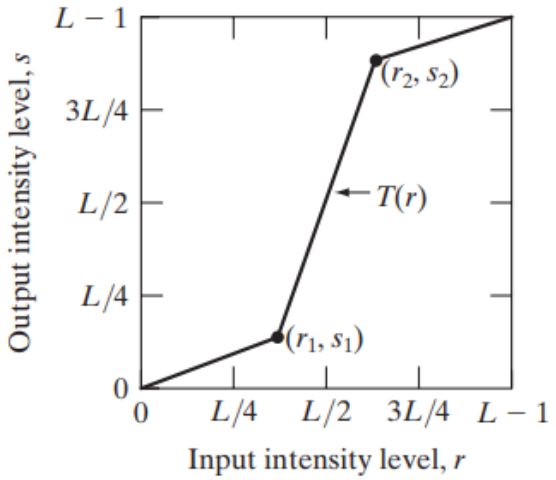


*Figure 8. Graphical representation of piece-wise Linear Contrast Stretching*

Where a1, a2 and a3 are the scaling parameters that control the results of process. If a1, a2 and a3 are 1, then gray levels would remain as it is. If a1 and a3 are 0 and r1 is equal to r2 then T(*) would become a thresholding function, and the resulted image would be a binary image.

$$s = T(r) = \begin{cases} a1r, & 0 \le r < r1 \\ a2(r - r1) + s1, & r1 \le r < r2 \\ a3(r - r2) + s2, & \end{cases} \qquad \begin{matrix} s1 = T(r1) \\ s2 = T(r2) \\ r2 \le r \le (L-1) \end{matrix}$$

The shape of the transformation function is controlled by the position of these points. If r1 is equal to r2 and r2 is equal to s2 then the transformation becomes a linear function which has no effect on the intensity levels. If $r1 = s1,\ s1 = 0$ and $s1 = L - 1$ then the transformation is simply a thresholding method that results in a binary image. Various extents of spread in the intensity levels of the output image are produce by intermediate values, thus affecting the image contrast. In general, it is assumed that $r1 \le r2$ and $s1 \le s2$, so that the function is monotonically rising and is single valued. This condition successfully maintains the order of intensity levels, thus try to prevent the creation of intensity patterns in the resulted image.

*Figure 9. Effect of Linear Contrast Stretching on gray scale image of cell*

## 3.3.2 Histogram Equalization

### 3.3.2.1 Histogram

The histogram of any given RGB or grayscale digital picture containing intensity values in the range of [0 , L-1] is a discrete function $h(rk)$ which gives the number of pixels lying on each gray scale value where $r_k$ is the intensity value of $k^{th}$ pixel and $n_k$ is the total pixel count in the image having the intensity $r_k$, In digital image processing it had been a common practice to normalize a histogram by dividing the intensity value of each pixel by the total number of pixels in the image. Total number of pixels can be obtained the product of total number of rows and total number of columns in the image, where. Thus, a normalized histogram can be given by following formula $p(rk\ /MN)$ for k = 1, 2, 3, 4, ……, L-1. In layman terms, it is an estimate of the probability of occurrence of a gray scale level in an image.

The summation of intensity values of all the pixels of a normalized histogram is equal to 1. Many spatial domain image processing techniques consider histograms as their basis for calculations and manipulations. Histogram manipulation had been used for enhancing the image in history, as shown in this section. In addition to provide some useful statistics for the image, the information driven from histograms is very useful in some other image processing methods e.g. image compression and classification.

### 3.3.2.2 Histogram Equalization

Histogram equalization is a method in which we rearrange the grey levels of the image in order to distribute the pixels equally on entire range of gray levels. This can much enhance details in the image. As full grey level range is used the contrast of the image is automatically improved.

Suppose f be any given picture that is addressed as a mr by mc lattice having pixel powers going from 0 to L − 1. L is the all out number of conceivable force values, frequently 256. Let p addresses the standardized histogram of the f with a canister for every conceivable power. Along these lines,

$$pn = \frac{number\ of\ pixels\ of\ intensity\ n}{total\ number\ of\ pixels} \quad n = 0,1,2,3, \ldots L\text{-}1$$

The image after histogram equalization can be calculated from the following formula.

$$g_{i,j} = floor(L-1)\sum_{n=0}^{fi,j} pn$$

Floor function is used to round down the intensity value to nearest integer.

$$T(k) = floor(L-1)\sum_{n=0}^{k} pn$$

This function results after thinking of the pixel intensity values of f and g as continuous and random variables X, Y on [0, L − 1] with Y defined by

$$Y = T(X) = (L-1)\int_0^x p \times (x)dx$$

where pX is the PDF of *f*. T is the cumulative CDF of X multiplied by the number of intensity levels -1. Y defined by T(X) is uniformly speeded on the range [0, L − 1], namely that $P_Y(y) = \frac{1}{L-1}$

$$\int_0^y PY(z)dz = probability\ of\ 0 \le Y \le y$$

$$= probability\ of\ 0 \leq X \leq T^{-1}(y)$$

$$= \int_0^{T^{-1}(y)} px(w)dw$$

$$\frac{d}{dy}(\int_0^y pY(y) = probability\ that\ 0 \leq Y \leq y$$

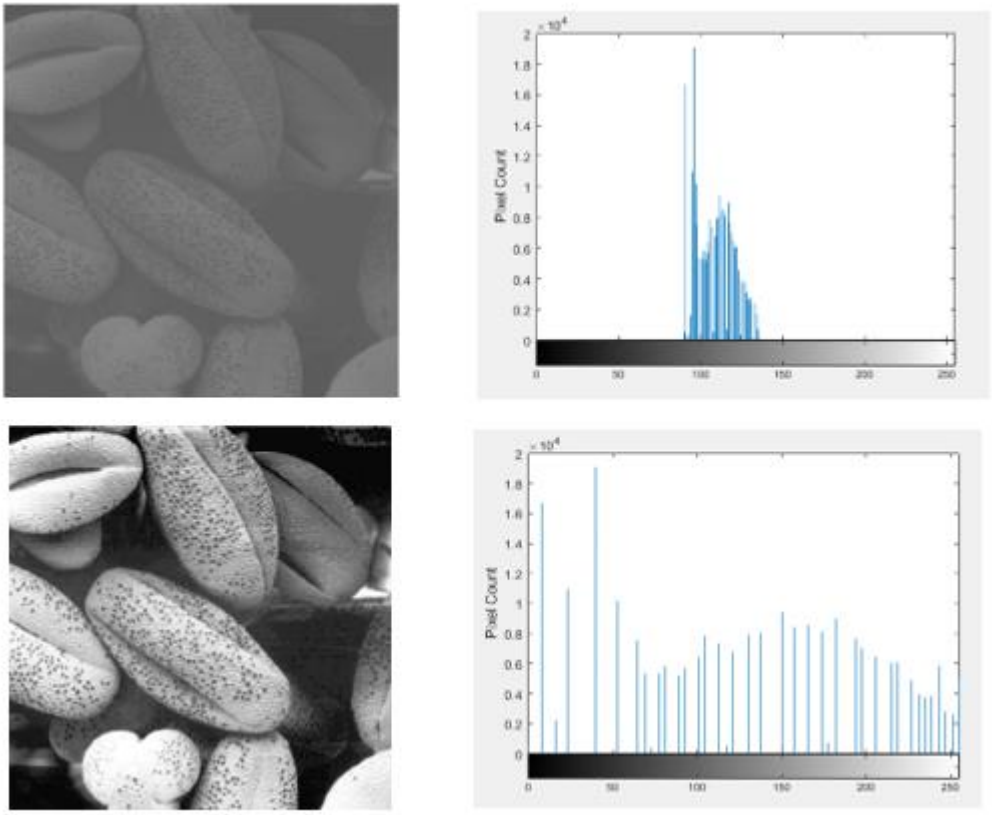


*Figure 10 Comparison of Images Before and after Histogram Equalization*

It can easily be observed from the figure 8 that in the histogram of original low contrast image the pixels density is concentrated in a few gray levels in the center of x-axis. Which represents that all the pixels are distributed among a very low number of gray levels. After applying histogram equalization in figure 6 the

pixels are now distributed on all grays levels of the image resulting in the enhancement of contrast

*Figure 11 Effect of Histogram Equalization on cell grayscale image*

## 3.4 Otsu's Thresholding

Image thresholding is a technique that uses pixel intensities to create a binary image. A grey scale image and a threshold are the most common inputs to a thresholding method. A binary image is the result. White(foreground) is assigned to output pixels in which the input image's intensity is higher than the threshold, while black is assigned to output image's intensity less than or equal to the threshold.

Thresholding techniques is a common pre-processing technique in a wide range of fields. For example if you want to find a tumor in mammography or locate a natural catastrophe in satellite photos, you may use this technique.

One of the most typical image processing steps is the conversion of a grayscale image to black and white. Nobuyuki Otsu's technique, after his family name, is one of the numerous ways to binarization.

For all the possible threshold values, Otsu's thresholding method includes an iterating method. And after calculating a measure of spread for the pixel levels on each side of the threshold. For example, the pixels that either fall in the foreground or background. Finding the point where the spread of the foreground and background are equal to their smallest combined value is the goal of this exercise.

In order to demonstrate the procedure, the following 6x6 picture will be used: The picture's histogram may be seen directly to the right of the image. There are just six grayscale levels in this picture in order to keep it simple enough to read.

The Otsu technique is often used because of its simplicity of usage. It is referred known as Otsu. Repetitively cycling through all possible threshold values in order to assess the pixel level spread in either object or background is how it does this. If we are going to achieve our primary aim in lowering the amount of class variance, we need to find the optimal threshold value for clustering. Because the object under examination has such a tiny surface area in comparison to the background, this technique works well for isolating WBCs.

It can be seen in the image that a graph is given for every possible threshold value from 0 to 5. Foreground pixels are those with a level more than or equal to 3, whereas background pixels are those with a level lower than 3. The table show how effective this threshold is, as shown by the evidence.

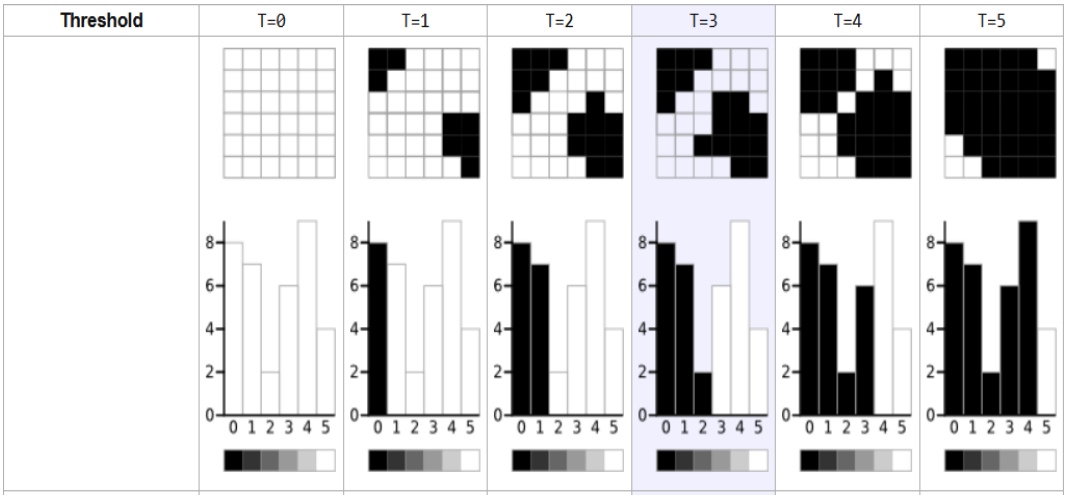


*Figure 12. Demonstration of Thresholding*

1. The image area is the total amount of non-zero pixels in the image region.

2. To measure a pixel's perimeter, measure the distance between two consecutive pixels on its border.

3. Thickness is a third factor. The regularity of an item may be gauged using its ratio.

$$T = \frac{4\pi A}{2P}$$

The object's surface area is denoted by the letter A, and its perimeter by the letter P. The greatest value of a circle is one. Things with a higher thinness ratio are more prevalent than irregularly shaped ones.

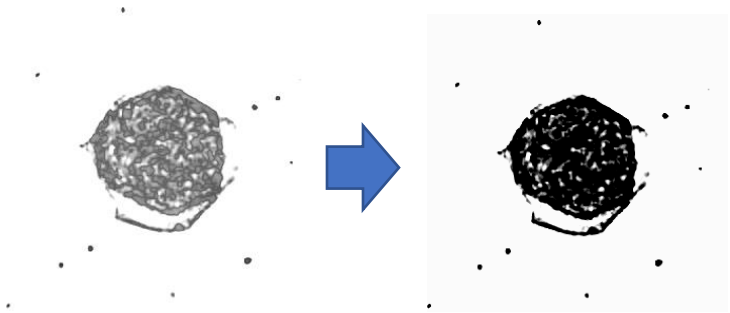

*Figure 13. Effect of thresholding on grayscale image*

### 3.4.1 Limitations of Otsu's Thresholding

A bimodal histogram with a deep and sharp valley in between the two peaks is ideal for using Otsu's method. All global thresholding algorithms fail under extreme noise, small object size, and inhomogeneous lighting. Otsu's method is no exception. Otsu method have proposed the local adaptation for those cases.

It is also important to note that the theoretical basis of Otsu's method is two Normal distributions with equal variance and equal size being blended together. No matter how well the working assumptions are met, statistical tests (to which the Otsu technique is closely related) may nevertheless operate properly even if they are not completely satisfied, and this is also true for Otsu's thresholding. Otsu's methods have been modified to accept more severe deviations of these assumptions, such as the Kittler-Illingworth technique[28].

## 3.5 Minimum Filtering

### 3.5.1 Filter in Digital Image Processing

Filtering in image processing means removing the unwanted and noisy elements from the image. As filtering is subjective topic so it can have different effects on the output image as per the application. Most often, filters are used to diminish or enhance the picture's high or low frequencies, such as smoothing or detecting edges. Filtering can be performed in spatial as well as frequency domain in image processing.

There are many types of filters that are known for there specific applications. For example Laplacian filters are well known for edges enhancement and sharpening, Gaussian filters are famous for smoothing operations and Median filters are effective in reducing the salt and pepper noise from the image.

We have used minimum filter after the thresholding operation to remove small unwanted white objects (noise elements) from the binary image. A minimum filter of window size 2x2 is used iteratively for 6 iterations.

### 3.5.2 Working of Minimum Filter

A minimum filter selects a minimum value from all the pixel values inside the window and places it in the center. In minimum filtering 2 parameters are really important i.e. window size and number of iterations for the filter traversal. In this way the small objects in the image having high intensity values are omitted from the image.

$$F(x,y) = \min_{(s,t)\in Sxy}\{g(s,t)\}$$

Following are the binary images to observe the effect of application of minimum filter on the binary image resulted after thresholding.

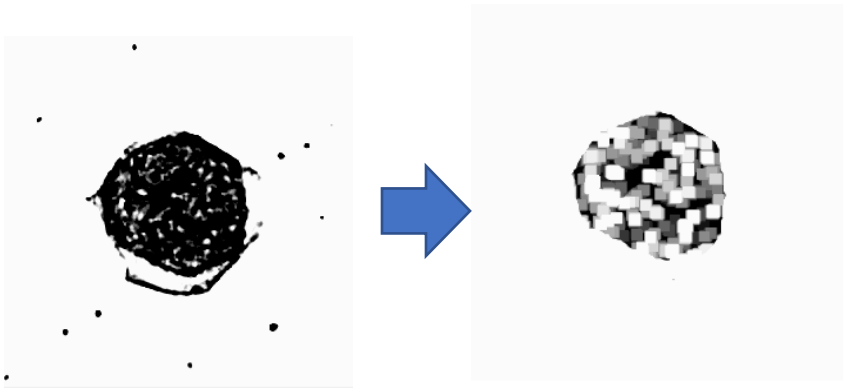

*Figure 14 Effect of minimum filtering on Binary image*

As it can be seen from the images that after applying the minimum filter iteratively on the image, small white objects (noise elements) other than white blood cells are omitted from the image. This is the exact effect that we wanted.

It can be seen that the small black features inside the white blood cells are also enhanced in an undesired manner. To remove this unwanted effect we will perform morphological opening and closing operations in the next step.

## 3.6 Morphological Operations

Morphological operations are a sets of image processing methods that depends on shapes processing image [15]. Morphological techniques apply structuring element on the given input binary image. Which then deliver a resultant picture of similar size and pixels. In a morphological operation, the value of every pixel in the resultant binary image depends on a comparison of the comparing pixel in the given image with its neighbor.

Morphological operations depend on overall order of pixel values. That does not include the numerically obtained value. This then subsequently are particularly helpful for handling with the high contrast pictures. Morphological techniques can likewise be applied to greyscale image to such an extent that they have unknown values for their light transfer functions. Therefore, their absolute pixels intensity value has practically the zero significance.

Morphological operations examine the images using small-shaped element or patterns called structuring elements. This structuring elements are placed at all potential areas in the

image. Then are compared with the corresponding pixel neighborhood. Some operations test whether an element "fits" into the area. While other test whether it "falls in" or meets the area. Morphological filtering of black and white images is performed by treating compound operations which includes the opening and closing as filters. They can go about as shape filters. For example, opening with a disc structuring element can smooth the corner from within.

It then performs closing filter with a disc structuring element which can smooth corner from the outside. However, these operations can also filter out any details in the image that are smaller than structuring elements, e.g., Opening is filtering the black and white images at a scale determined by the size of the structuring elements. The filter only passes portions of the pixels that match structural elements. Smaller objects are excluded from the resultant images. The size of the structuring elements is generally significant for eliminating noisy details, but not for destroying objects of interest.

### 3.6.1 Structuring Element

A Structuring element is a small binary image or a template which can be of many shapes. The small matrix of pixels has a value of zero or one:

- The dimension of the matrix defines the size of the structuring elements.

- The pattern of 1s and 0s defines the shapes of the structuring elements.
- The origin of a structuring element is usually one of its pixels, although generally the origin can often be outside the structuring elements.

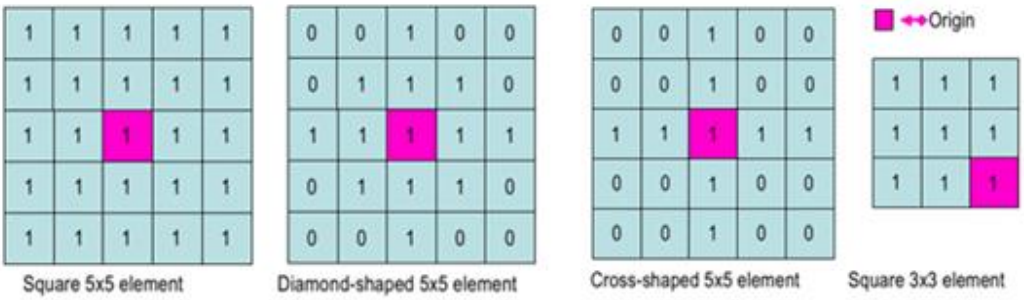


*Figure 15 Different types of Structuring Element*

## 3.6.2 Morphological Opening

Morphological openings can be useful for eliminating little articles from the paired picture. Be that as it may, it keeps up with the shape and size of greater articles in the picture. The initial activity obscures the picture, and afterward the obscured picture is extended involving the equivalent organizing component for the two tasks. It is accomplished by first eroding an image and then diluting it. Opening eliminates any narrow connections and lines between two area. Openings eliminate any narrow connections between cells and the lines between the two areas. Opening operation first perform Erosion and then Dilation.[23].

$$A \circ B = (A \ominus B) \oplus B.$$

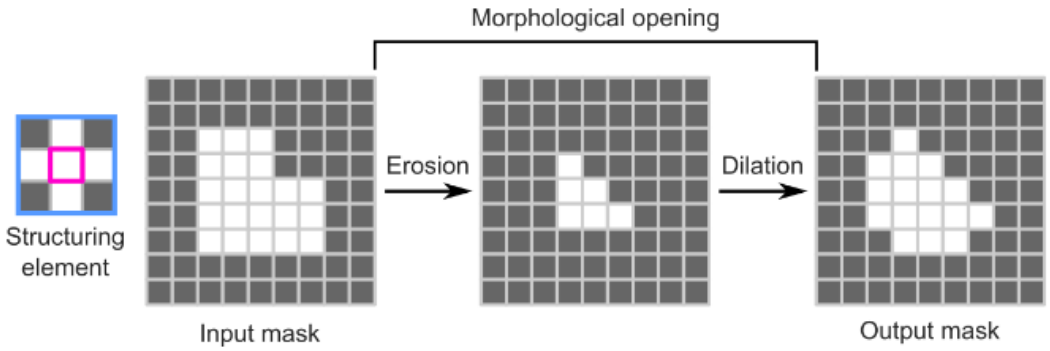


*Figure 16. Demonstration of Opening Effect*

### 3.6.2.1 Erosion

The relegated organizing component are utilized to investigate and lessen the shapes contained in the given info pictures. In unambiguous cases it approaches a neighborhood minima channel. Also, organizing components pack a picture by killing a layer of pixels from the internal and external limits of an area. Utilizing disintegration, we can eliminate openings and holes between various regions for bigger and more modest subtleties.

That is, the resultant pixel esteem is the base worth of all pixels in the area. In a paired picture, on the off chance that any contiguous pixel has a worth of 0, a

pixel itself has a worth of 0. Morphological disintegration takes out island and little article, so just fundamental items remain.

$$A \ominus B = \{x : B_x \subseteq A\}.$$


*Figure 17. Effect of the Structuring element size in erosion*

### 3.6.2.2 Dilation

Dilation expands pixels in an image or add the pixels to the edges of an object. First, we move the structuring element onto the image object to perform the dilation operation. The resultant pixel value has the maximum value of all pixels in the region. In a black and white image, if any adjacent pixel has a value of 1, that pixel is assigned the value 1. The dilation filter makes object more visible through the image and fills the small holes in between the object. Lines appear thicker and patches appear larger.

$$A \oplus B = \{x : \hat{B}_x \cap A \neq \emptyset\}.$$

*Figure 18. Effect of the Structuring element size in Dilation*

### 3.6.3 Morphological Closing

Morphological closure can be helpful to fill little openings in an image while keeping the shape and size of elements in the image. The closing operation is basically inverse of the opening operation. First dilation is performed followed by erosion. The Closing operation is useful for closing little openings inside foreground object or small black spots on objects. The Closing operation expands the image, then uses the same structuring elements for the two operations to blur the expanded image.

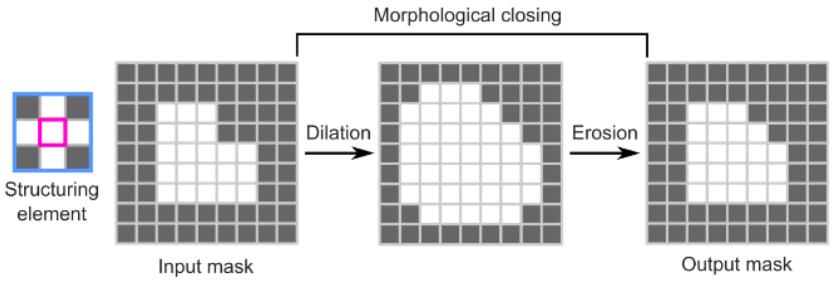


*Figure 19 Demonstration of Closing Effect*

After going through thresholding's operations and algorithm, the time to apply the two morphological operations which are discussed briefly above is considered. The morphological opening operation in our project is performed with a 9 pixel-diameter disk which is followed by morphological closing operation with a 12 pixel-diameter disk to clear the boundaries of White Blood Cells and fill the hole to improve the segmentations results.

These values are taken after some research and testing and are also defined on the resolution of the input images. It then ignores objects smaller than 5000 pixels to remove redundant objects which is also called an area check. This threshold is based on the minimum size of White Blood Cells.[19]

## 3.7 Minimum Area Check

The estimating unit for the picture is the quantity of pixels. For instance, the picture region for a given molecule may be 100 pixels, and the periphery length may be 50 pixels, and so forth. The genuine estimating unit is millimeters, consequently a change from pixels to millimeters is required. At the end of the day, the size of mm/pixel not entirely set in stone.

Imaging process the twofold picture of the example Quarter utilized for adjustment, and the picture of the rest two examples will show up in the comparative design. Note that the circle shows up in olive shape brought about by non-square pixel shape.[26]Least square procedure utilized in displaying the most extreme pixel number and genuine measurement can be checked on as follows,

$$P = D\beta + \epsilon$$

where P is the greatest estimated (or checked) pixel number in x and y bearing inside the example of interest, P= [pl p2 p3 """ pn] T, n=27; D is the real example measurement in millimeter (mm), D = [dl d2 d3 ... dn] T, n=27; f} is the halfway relapse coefficient; 6 is the irregular mistake with zero mean and change, e = [el e2 es "." en] T, n=27; and "T" indicates grid render. The complementary of the incline of every straight line can be taken so the ideal scaling component of millimeter/pixel. The outcomes are 0.8802 millimeter/pixel in the level bearing, and 0.6551 mm/pixel in the upward heading.[16]

Preceding adjustment, the article region is estimated in number of pixels. Utilizing the scale factors got beforehand, the deliberate region as far as square millimeter can be gained. above condition shows the discoveries against the comparing real regions, and the deliberate region information focuses are bend fitted by a straight line utilizing least square technique which is actually the first-request fitting lines don't go through the beginning.

The fitting might exhibit the disparity between the deliberate regions and their relating genuine regions quantitatively as well as graphically in general. Note, that the worth on the abscissa is genuine region, and this prompts the connection between the genuine region and the deliberate region. This capacity was viewed as

$$\boldsymbol{At = \frac{Am + 7.989}{0.9742}}$$

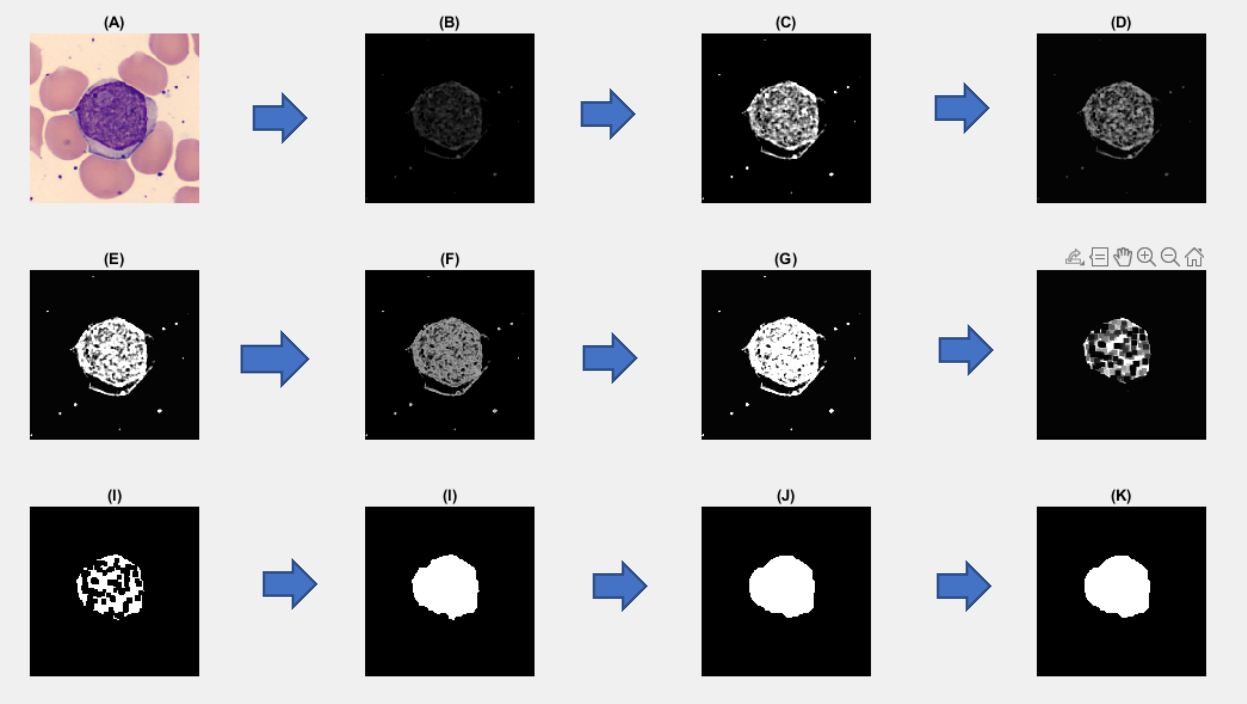


*Figure 20. (A). Orignal RGB Image (B).C Component of CMYK Domain (C).Image after linear contrast Streching (D). Image after Histogram Equalization (E). L+H image (F). E image - Hist (G) 2*Linear+Hist (H).Image After Minimum Filtering (I). Image after opening (J). Image after closing (K). Image after Area Check*

# Chapter 4

# Experiments and Results

With the passage of time the digital image processing is witnessing new ideas and techniques is various fields that are more advanced and accurate than their existing counter parts. In this kind of scenario, it is necessary for any researcher that his/her idea or product should produce better results in terms of both accuracy and performance compared to the existing ideas.

In the way to automated white blood cells detection, there are many factors that paly a role of hurdle such as different shapes and sizes of different types of white blood cells i.e. basophils, monocytes, lymphocytes and neutrophils. An improved white blood cells detection technique can play an important role in white blood cells segmentation and counting which can help pathologist to work more efficiently with less effort.

This chapter focusses on comparing the results of proposed method with the existing methods. Also, it presents a comprehensive analysis on the achieved results. It will also cover a brief comparison of advantages and shortcoming of proposed method and already existing methods.

## 4.1 Dataset

Dataset that is used for this proposed method is a publicly available BCCD dataset. This dataset was used by ‘’An efficient technique for white blood cell nuclie automatic segmentation” by Mostafa Mahmood and Amr Guaily[]. This dataset was gathered by a normal peripheral blood sample which was stained using standard Gismo-Right method. A light microscope at 100X zoom was used to capture digital images. 100 out of 365 images are taken for implementation of the functionality. Each image has a resolution of 300x300.

## 4.2 Evaluation Criteria

In the evaluation of proposed technique, the image resulted after the segmentation is compared with its corresponding ground truth image. The accuracy of the proposed method depends on how it differentiates the white blood cells from the background. The segmented binary image is compared with the ground truth image pixel by pixel. This comparison results in the four categories of pixels count. First one is “True Positive”, these are the cell pixels that are correctly segmented in the resultant image compared to the ground truth. Second category is “True Negative”, these are the background pixels that are correctly

segmented. Third category is the "False Positives", these are the cell pixels that are wrongly segmented i.e., they exist in segmented image but not exists in ground truth. Fourth category is the "False Negatives", these are the background pixels that are wrongly segmented i.e., they exist in segmented image, but they do not exist in ground truth.

In the phrase "True Positive", second word "positive" points towards the pixel lying on a foreground i.e. white blood cell and "negative" points towards the pixel lying on the background. The first word suggests that weather pixel was segmented correctly or not with respect to the ground truth.

## 4.3 Results

### 4.3.1 Phase 1 (Implementation without any change)

In phase 1 of the working, the methodology of paper "An efficient technique for white blood cell nuclei automatic segmentation" by Mostafa Mahmood and Amr Guaily was implemented without any changes. Following table shows the results of initial implementation without any optimizations.

### 4.3.2 Phase 2 (Changing the Color Domain)

In the 2nd phase of implementation, the color domain of the original image was converted from RGB to CMYK domain before applying the further steps of pre-processing or segmentation. In this phase sensitivity was improved with a handsome amount of 0.075, accuracy was decreased with a small amount of 0.0003 and specificity decreased with an amount of 0.02.

### 4.3.3 Phase 3 (Changing filter size in minimum filtering)

In this phase of implementation, the filtering step was optimized. Just after the thresholding, the binary image was convoluted with a minimum filter of 2x2 filter which was 3x3 in initial implementation. Also, the iterations of application of filter was changed to 6 instead of 3.

### 4.3.4 Phase 4 (Adding Morphological Opening step)

In this phase morphological closing was introduced along with the morphological opening. In the initial methodology, only morphological opening was implemented. So, after introducing opening a further increase in sensitivity was achieved by an amount of 0.024. The accuracy and specificity were slightly decreased**.**

## 4.4 Phase-Wise Tabulated Results

Table 1. Performance measures in Original Paper

| Average Accuracy | Average Sensitivity | Average Specificity |
|---|---|---|
| 0.979601134 | 0.874141382 | 0.995129388 |

Table 2. Performance measures after changing RGB Color Domain to CMYK Color Domain

| **Parameters** | **RGB Color Domain** | **CMYK Color Domain** |
|---|---|---|
| Average Accuracy | 0.979601134 | 0.976753175 |
| Average Specificity | 0.995129388 | 0.979220785 |
| Average Sensitivity | 0.874141382 | 0.949528978 |

Table 3. Performance measures after changing 3x3 Min Filter to 2x2 Min Filter

| **Parameters** | **3x3 Min Filter** | **2x2 Min Filter** |
|---|---|---|
| Average Accuracy | 0.976753175 | 0.975609864 |
| Average Specificity | 0.979220785 | 0.978573548 |
| Average Sensitivity | 0.949528978 | 0.94317967 |

Table 4. Performance measures after applying Morphological Closing along with Morphological Opening

| **Parameters** | **Morphological Opening** | **Morphological Opening & closing** |
|---|---|---|
| Average Accuracy | 0.975609864 | 0.971635147 |
| Average Specificity | 0.978573548 | 0.972264606 |
| Average Sensitivity | 0.94317967 | 0.970664008 |

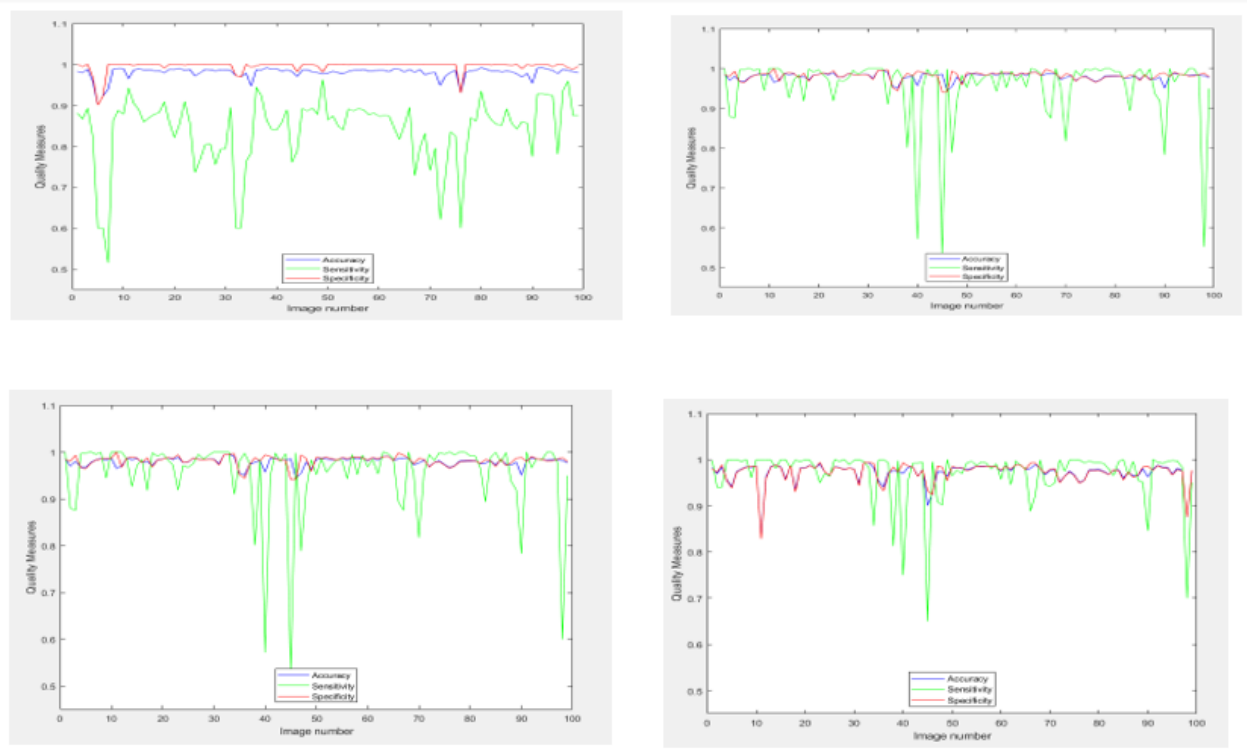


*Figure 21. Comparison of Results after operations performed in tables (1)(2)(3)(4) in Graphical Representation*

## 4.5 Comparison with state-of-the-art

Table 5. Comparison Table

| Type | Method | Year | Accuracy % | Specificity % | Sensitivity % |
|---|---|---|---|---|---|
| Unsupervised | Madhloom et al. [4]. | 2015 | 95.7978 | 98.9475 | 96.8925 |
| | Ghane N, Vard A, Talebi A, Nematollahy[1] | 2017 | 98.4708 | 98.6014 | 93.8764 |
| | J. Wu, P. Zeng, Y. Zhou and C. Olivier[3] | 2006 | 94.4325 | 97.4907 | 91.3214 |
| | Hegde, R. B., Prasad, K., Hebbar[7] | 2019 | 99.4764 | 97.0856 | 94.6675 |
| | Proposed Method | 2022 | 97.1343 | 97.2745 | 97.0853 |

# Chapter 5

# Conclusion and Future Work

## 5.1 Conclusion

In this project, our goal was to first implement the proposed technique "An Efficient Technique for White Blood Cells Nuclei Automatic Segmentation" [24]. We have made several optimizations in the proposed methods. In the initial phase of implementation, same methodology was implemented without any change and the results for accuracy, Specificity and Sensitivity were recorded.

In the next phase of implementation, the color domain of the input image was shifted to other color domain i.e., RGB to CMYK color domain. The main reason behind the color domain shifting was that the color of cell nucleus was very much similar to the cyan component of the CMYK color domain. So, Separating the cyan component from the converted image can automatically eliminate the undesired objects from the image as they belong to a different component. Also, the cytoplasm of the cell has a different color from the nucleus so its edges can easily be differentiated by changing the color domain.

In the third phase of implementation, originally thresholding step was followed by a filtering step. Filter used here was a minimum filter with window size of 3x3. This filter was used iteratively 3 times. We have changed the window size of the filter from 3x3 to 2x2. This step was done by hit and trail approach. After taking many different window sizes it was observed that more control for the smaller noise object can be achieved by reducing the window size.

The number of iterations a filter is applied on the image effect the nucleus pixels in a negative way. If the iterations are increased by a very large number than the nucleus could have been blacked out completely. This is because the minimum filter chooses the minimum i.e., the darkest intensity value from its neighborhood and place it.

After the Application of minimum filter, the binary image was not worth evaluation, so some morphological operations were performed on the image. Initially in the method

proposed by Mostafa Mohamed, Behrouz Far, Amr Guaily [24] only a morphological opening was implemented. Morphological opening is comprised of two steps erosion and dilation. The order of the steps is what differentiates morphological opening and closing. In Opening the erosion is performed first and then dilation. Erosion has the effect of removing pixels from the boundaries of an object and hence reducing the size of object. Dilation has the effect of adding the pixels on the boundaries and hence increasing the size of the object. In this phase of implementation, a combination of morphological opening followed by morphological closing is implemented. The overall effect of this process was observed as to minimizing the incorrect effect of minimum filtering i.e. black spots formed inside the cell as a result of minimum filtering are reduced. And the small object connected to the surface of nucleus were also removed.

We showed a robotized strategy for recognition of cores and Leukocytes thinking about variety and enlightenment varieties. Division of cores were considered as beginning advance to recognize Leukocytes on the grounds that these are unmistakably dim articles in fringe blood smear images. We considered G'G'B portrayal of unique RGB picture which feature scores locales. We utilized number juggling activities and Zack's thresholding for discovery of cores and gotten normal DSC of 0.97 and awareness of 0.96.

Dynamic shapes strategy was utilized for discovery of leukocytes thinking about cores as cover. We considered cover of variable size in view of region and circularity of cores. This brought about by and large DSC of 0.976. [21]The proposed technique accomplished a normal awareness of 0.97 for Leukocyte recognition. Versatile approach to choosing covers for discovery of Leukocytes added to higher proficiency in Leukocyte identification. Platelet division assumes an essential part in mechanizing lab methodology.

A robotized technique should find the cells accurately independent of variety, enlightenment, brilliance also, staining varieties for additional handling like element extraction and arrangement. The proposed strategy tended to these varieties by presenting an original picture improvement technique. Further, a technique could be produced for partition of covered cells for exact location of leukocytes.[17]

## 5.2 Future Work

In future White Blood Cell segmentation can be further enhanced by supervised learning algorithms that can be run on newly developed and remotely available GPUs. These techniques can maximize the accuracy measures of the Segmentation because of their modern and robust computing abilities. Newly developed algorithms like convolutional neural networks and yolo v4 and v5 can be used in the detecting and classification of leukocytes.

These algorithms will be able to perform segmentation with high accuracies as these techniques use machine learning algorithms to train the system on very large image datasets. And also have capacity to improve the performance with the passage of time on further training on new images Soon these techniques become mature enough to gain the confidence of the medical professionals like pathologists and will be used in professional medical and pharmaceutical labs in near future.

# Bibliography

[1] Ghane N, Vard A, Talebi A, Nematollahy P. Segmentation of white blood cells from microscopic images using a novel combination of K-means clustering and modified watershed algorithm. J Med Sign Sens 2017;7:92-101.

[2] "What Are Blood Tests?" National Heart, Lung, and Blood Institute (NHLBI), online.

[3] J. Wu, P. Zeng, Y. Zhou and C. Olivier, "A novel color image segmentation method and its application to white blood cell image analysis," in 8th international Con. on Signal Processing, 2006.

[4] H. T. Madhloom, S. A. Kareem, H. Ariffin, A. A. Zaidan, H. O. Alanazi and B. B. Zaidan, "An Automated White Blood Cell Nucleus Localization and Segmentation using Image Arithmetic and Automatic Threshold," Journal of Applied Sciences, vol. 10, no. 11, pp. 959-966, 2010

[5] “Blood Diseases," The British Medical Journal, vol. 2, no. 3907, pp. 998 - 999, 1935.

[6] Leukemia & Lymphoma Society. Leukemia. (https://www.lls.org/leukemia) Accessed 11/10/19

[7] Hegde, R. B., Prasad, K., Hebbar, H., & Singh, B. M. K. (2019). Image processing approach for detection of leukocytes in peripheral blood smears. Journal of medical systems, 43(5), 114.

[8] Wang, Y., & Cao, Y. (2019). Quick leukocyte nucleus segmentation in leukocyte counting. Computational and mathematical methods in medicine, 2019

[9] G. Ongun, U. Halici, K. Leblebicioglu and V. Atala, "An automated differential blood count system," in Int. Conf. of the IEEE Engineering in Medicine and Biology Society, 2001, Istanbul.

[10] Prinyakupt, J., & Pluempitiwiriyawej, C. (2015). Segmentation of white blood cells and comparison of cell morphology by linear and naïve Bayes classifiers. Biomedical engineering online, 14(1), 63.

[11] Li, Y., Zhu, R., Mi, L., Cao, Y., & Yao, D. (2016). Segmentation of white blood cell from acute lymphoblastic leukemia images using dual-threshold method. Computational and mathematical methods in medicine, 2016.

[12] Chu, R., Zeng, X., Han, L., & Wang, M. (2015, June). Subimago segmentation in a single white blood cell image. In 2015 7th International Conference on Computational Intelligence, Communication Systems and Networks (pp. 152- 157). IEEE.

[13] Sadeghian F, Seman Z, Ramli AR, Abdul Kahar BH, Saripan M-I. A framework for white blood cell segmentation in microscopic blood images using digital image processing. Biol Proced Online 2009;11:196-206.

[14] Kose, K., Cetin-Atalay, R., & Cetin, A. E. (2014). Special issue on microscopic image processing.

[15] Andrade, A. R., Vogado, L. H., de MS Veras, R., Silva, R. R., Araujo, F. H., & Medeiros, F. N. (2019). Recent computational methods for white blood cell nuclei segmentation: A comparative study. Computer Methods and Programs in Biomedicine, 173, 1-14.

[16] H. T. Madhloom, S. A. Kareem and H. Ariffin. “Computer-aided acute leukemia blast cells segmentation in peripheral blood images”. Journal of Vibroengineering, vol. 17, pp. 4517-4532, 2015.

[17] J. Vromen and B. McCane, "Red Blood Cell Segmentation from SEM Images," in Image and Vision Computing (IVCNZ), New Zealand, 2009.

[18] Ghane, N., Vard, A., Talebi, A., & Nematollahy, P. (2017). Segmentation of white blood cells from microscopic images using a novel combination of K-means clustering and modified watershed algorithm. Journal of medical signals and sensors, 7(2), 92.

[19] Deshmukh, P., Jadhav, C. R., & Rani, N. U. (2018). Automatic White Blood Cell Segmentation for Detecting Leukemia. In Information and Communication Technology for Sustainable Development (pp. 385-392). Springer, Singapore.

[20] H. Ramoser, V. Laurain, H. Bischof and R. Ecker, "Leukocyte segmentation and classification in blood-smear images," in IEEE Engineering in Medicine and Biology, Shanghai, 2005.

[21] Dhanachandra, N., Manglem, K., & Chanu, Y. J. (2015). Image segmentation using K-means clustering algorithm and subtractive clustering algorithm. Procedia Computer Science, 54, 764-771.

[22] J. Angulo and G. Flandrin, "Automated detection of working area of peripheral blood smears using mathematical morphology," Analytical Cellular Pathology, pp. 37-49, 1 January 2003.

[23] Safuan, S. N. M., Tomari, M. R. M., & Zakaria, W. N. W. (2018). White blood cell (WBC) counting analysis in blood smear images using various color segmentation methods. Measurement, 116, 543-555.

[24] Dorini LB, Minetto R, Leite NJ, editors. White blood cell segmentation using morphological operators and scale-space analysis.XX Brazilian Symposium on Computer Graphics and Image Processing ( SIBGRAPI ); 2007: IEEE.

[25] Sadeghian F, Seman Z, Ramli AR, Kahar BHA, Saripan M-I. A framework for white blood cell segmentation in microscopic blood images using digital image processing. Biological procedures online. 2009;11(1):196

[26] Mostafa Mohamed, Behrouz Far, Amr Guaily. An Efficient Technique for White Blood Cells Nucle Automatic Segmentation. 2012 IEEE International Conference on Systems, Man, and Cybernetics October 14-17, 2012, COEX, Seoul, Korea.

[27] H. Maerz, Aggregate sizing and shape determination using digital image processing, International Center for Aggregate Research (ICAR) 6th Annual Symposium Proceedings, St. Louis, Missouri, April 19-20, 1998, 195-203.

[28] Ban, Y., #38; Yousif, O. A. (2012). Multitemporal Spaceborne SAR Data for Urban Change Detection in China. IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing 1087–1094.