CHAPTER 1

INTRODUCTION

1.1 WHITE BLOOD CELLS

White Blood Cells (WBCs) are also called leukocytes or leucocytes. These are cells of the immune system that are involved in the body against both infectious disease and foreign materials. These cells help fight infections by attacking bacteria, viruses, and germs that invade the body. All leukocytes are produced and derived from a multipotent cell. This is also known as a hematopoietic stem cell. They live for about three to four days in the human body. The segmentation has to be done to extract the nucleus of white blood cell image. A Scanning electron microscope image of a human blood is shown in Figure 1.1.

Figure 1.1 A Scanning electron microscope image of a human blood
The main task of the segmentation is to extract the objects from an image. During segmentation, the size and shape of the nucleus should be maintained. Many segmentation algorithms have been developed for various applications. So it may use the modified versions of moving K-Means algorithm called adaptive fuzzy moving K-Means and algorithm of Standard Fuzzy C-Means and Standard Fuzzy Possibilistic C-Means may be used. To overcome from the problem of image shaping, it may use the classification of RVM. This study gives a technique for WBC detection based on Relevance Vector Machine (RVM). This proposed method effectively works for WBC detection, and effectively reduces the computational time and preserve the shapes and edges of the image. The main objective is to segment the WBC from the blood smear image to detect an immature cell.

Clustering in image segmentation is defined as the process of identifying groups of similar objects. Clustering techniques can be classified into supervised and unsupervised clustering. Segmentation is the process of partitioning a digital image into multiple segments based on pixels. The result of image segmentation is a collection of segments which combine to form the entire image. Many different clustering techniques have been used for segmenting an image.

The process of K-Means, fuzzy C-Means algorithm may also explain here. Red Blood Cells (RBCs), White Blood Cells (WBCs) and blood platelets are the three different types of cells in a human body. In these three types, WBC is used for the automated detection. This type of method is used for automated detection of the diseases. A numbers of diseases are automatically detected by WBC such as lymphoblastic Leukaemia.

The diseases that are not detected very much accurate by microscope also needs some hematologists to manually count and classify the cells.
1.2 TYPES OF WHITE BLOOD CELLS

To improve the load work, by this type of automated detection method is used for diagnosis. So this method helps to remove the red blood cells and platelets from the background. There are five types of white blood cells or leukocytes in human blood, Shown in the Figure 1.2.

- neutrophils
- lymphocytes
- eosinophils
- monocytes
- basophils

![White Blood Cells](image)

**Figure 1.2 Sample White Blood Cells**

- **Neutrophils**: They kill, digest bacteria and fungi. They are the most numerous type of white blood cell and the first line of defense when infection strikes.

- **Lymphocytes**: They create antibodies to defend against bacteria, viruses, and other potentially harmful invaders.

- **Eosinophils**: They attack and kill parasites, destroy cancer cells, and help with allergic responses.

- **Monocytes**: They have a longer lifespan than many white blood cells and help to break down bacteria.
• **Basophils:** These small cells appear to sound an alarm when infectious agents invade our blood. They secrete chemicals such as histamine, a marker of allergic disease, that help control the body's immune response.

### 1.3 WHITE CELL FUNCTION DISORDERS

White blood cells (leukocytes) are an important part of the body's defense against infectious organisms and foreign substances. To defend the body adequately, a sufficient number of white blood cells must receive a message that an infectious organism or foreign substance has invaded the body, get to where they are needed, and then kill and digest the harmful organism or substance.

Like all blood cells, white blood cells are produced in the bone marrow. They develop from stem (precursor) cells that mature over time into one of the five major types of white blood cells called neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Normally, people produce about 100 billion white blood cells a day. The number of white blood cells in a given volume of blood is expressed as cells per micro liter of blood. The total white blood cell count normally ranges between 4,000 and 11,000 cells per micro liter. The proportion of each of the five major types of white blood cells and the total number of cells of each type can also be determined in a given volume of blood.

Too few or too many white blood cells indicate a disorder. Leukopenia, a decrease in the number of white blood cells to fewer than 4,000 cells per microliter of blood, makes people more susceptible to infections. Leukocytosis, an increase in the number of white blood cells to more than 11,000 cells per microliter of blood, may result from the normal response of
the body to help fight an infection. However, an increase in the number of white blood cells can also result when the regulation of white blood cell development is disrupted and immature or abnormal cells are released into the blood.

Some white blood cell disorders involve only one of the five types of white blood cells. Other disorders may involve a few types together or all five types. Disorders of neutrophils and disorders of lymphocytes are the most common. Disorders that involve monocytes and eosinophils are less common, and disorders involving basophils are rare. White blood cells are an important component of our blood system, which is also made up of red blood cells, platelets, and plasma, and it shows in the below Figure 1.3.

![Figure 1.3 Components of the blood system](image)

Although white blood cells account for only about 1 percent of the blood, their impact is significant. White blood cells, also called leukocytes, are essential for good health and protection against illness and disease. Think of white blood cells as our immunity cells. In a sense, they are continually at war. They flow through our bloodstream to battle viruses, bacteria, and other
foreign invaders that threaten our health. When the body is in distress and a particular area is under attack, white blood cells rush in to help destroy the harmful substance and prevent illness.

White blood cells are produced inside the bone marrow and stored in our blood and lymphatic tissues. Because some white blood cells have a short lifespan of one to three days, our bone marrow is constantly producing them as shown in the Figure 1.4.

![Figure 1.4 White Blood Cells are protected in bone](image)

There are several different types of white blood cells. A major feature of some leukocytes is the presence of granules. White blood cells are often characterized as granulocytes or agranulocytes

- **Granulocytes** (polymorphonuclear leukocytes): leukocytes are characterized by the presence of granules. These granules (usually lysozymes) are membrane-bound enzymes that act primarily in the digestion of endocytosed particles. There are three types of granulocytes: neutrophils, basophils, and eosinophils.
- Neutrophils: It is very good at phagocytosing smaller chunks of material, like bacteria. They can also secrete chemicals to enhance an inflammatory response. They are also targeted to the bacteria they destroy by the immune system.

- Basophils: These cells secrete both histamine and heparin. Both of these chemicals promote the inflammatory response, but in different ways. Histamine draws blood into the damaged area, while heparin slows clotting so that more and more blood can still enter the damaged area.

- Eosinophils: These cells are particularly good at fighting off parasitic invasions. They, like neutrophils, are also targeted to bacteria by the immune system. They also secrete chemicals in allergic reactions.

- **Agranulocytes** (mononuclear leukocytes): leukocytes characterized by the apparent absence of granules in their cytoplasm. Although the name implies a lack of granules these cells do contain non-specific azurophilic granules, which are lysosomes. The cells include lymphocytes, monocytes, and macrophages.

- Monocytes: It is only found in the blood. As soon as they use diapedesis to enter tissues, they are called macrophages. These cells (that you learned about in the connective tissue chapter) crawl around and phagocytize all sorts of things whether are big or small. They are the ones that pick up cellular and tissue debris.
Lymphocyte: There are different types of lymphocytes. Some secrete toxic chemicals; others are more directly involved in an immune response. These are discussed in the immune system unit.

1.4 IMAGE SEGMENTATION

There are various segmentation methods depending upon the nature of segments, diversified backgrounds, contrast, etc. The most common segmentation methods are edge and border detection, region growing, filtering, mathematical morphology, watershed clustering, thresholding, nearest neighbourhood graphs, mean shift procedure and deformable models, colour image segmentation. In this work, they have used the HSV segmentation method. The primitive step of segmentation is to convert the RGB image to HSV image.

To extract the cell portion, hue plane alone is considered. Based on observation, the hue value of a white blood cell was found to lie between 0.7 and 0.98. The image is thresholded based on the observed value which yields the result as shown. Then, dispensable regions such as platelets are eliminated by area thresholding. The area threshold value is fixed on experimental basis to obtain the entire region of cell. The next step is to extract the nucleus portion. For this, the saturation plane alone is considered and the nucleus is found to occupy a saturation level above 0.40. The chain of steps results in the binarized images of nucleus and cell region. Subsequently, binary image of nucleus is subtracted from cell image to extract cytoplasmic region. The cell segmentation images are shown in Figure 1.5. Then the resultant images are shown in the Figure 1.6.
Figure 1.5 Process of cell segmentation

- Segmentation divides an image into its constituent regions or objects

- Segmentation of images is a difficult task in image processing. It is still under research

- Segmentation allows to extract objects in images

- Segmentation is unsupervised learning

- Model based object extraction, e.g., template matching, is supervised learning.
Figure 1.6  (a) RGB image (b) HSV image (c) Hue plane image (d) Extraction of WBC using the threshold of Hue (e) Extraction of WBC using the threshold of total number of pixels (f) Saturation plane image (g) Extraction of WBC nucleus using the threshold of saturation (h) Extraction of nucleus using the threshold of total number of pixels (i) cytoplasm image obtained by subtracting nucleus from cell image

1.4.1  Segmentation Process

- Segmentation algorithms are based on one of two basic properties of colour, gray values, or texture: discontinuity and similarity

- First category is to partition an image based on abrupt changes in intensity, such as edges in an image

- Second category is based on partitioning an image into regions that are similar according to predefined criteria. Histogram
thresholding approach falls under this category. Thus the segmentation process is shown in Figure 1.7.

![Original picture](image1.png) ![Segmented picture](image2.png)

**Figure 1.7 Segmentation Process**

### 1.4.2 Segmentation Algorithms

- Suppose that the gray-level histogram corresponds to an image \( f(x,y) \) composed of dark objects on the light background, in such a way that object and background pixels have gray levels grouped into two dominant modes. One obvious way to extract the objects from the background is to select a threshold \( T \) that separates these modes.

- Then any point \((x,y)\) for which \( f(x,y) < T \) is called an object point, otherwise, the point is called a background point.

### 1.5 WBC SEGMENTATION

Ramesh et al (2012), gives image segmentation is a critical step in many image analysis problems. The segmentation step is crucial because the accuracy of the subsequent feature extraction and classification step depends
on correct segmentation of WBCs. The architecture of the WBC segmentation is shown in the Figure 1.8.

![WBC Segmentation Diagram](image)

**Figure 1.8 WBC segmentation**

It is also a difficult and challenging problem due to the complex appearance of these cells, uncertainty and inconsistencies in the microscopic image with variations in illumination. Improvement of cell segmentation has been a common direction in many research efforts. Many automatic segmentation methods have been proposed, most of them based on local image information such as histogram of regions, pixel intensity, discontinuity, and clustering techniques. The examples and Flowchart for WBC segmentations are shown in Figures 1.9 and 1.10.
Figure 1.9 Examples of WBC with (a) segmented and (b) nonsegmented nucleus

Cropped WBC

Threshold intensity image, eliminate small region

Threshold (Red-Blue) image

Y=Origina-RBC-background

Y=Fill Holes in Y

Morphological opening of Y

Boundary detection of the WBC

Threshold detection WBC

Nucleus image

WBC-Nucleus = Cytoplasm

Figure 1.10 Flowchart for WBC segmentation
Many of the segmentation algorithms introduced are based on the edge information present in images. As proposed by Ongun et al. (2001) WBCs were segmented using active contour models (snakes and balloons) which were initialized using morphological operators. This method works well only if the WBCs have dark cytoplasm and are distinctly separate from adjacent RBCs. They defined a new edge operator, the teager energy operator, to highlight the nucleus boundary which is very effective for segmenting the nuclei in cell images. They also used a simple morphological method to segment the cytoplasm from the background and the RBCs. The cytoplasm segmentation works well when the RBCs and WBCs are not close to each other. In contrast, our method works well even with a complicated background.

WBC segmentation scheme is drawn by combining scale-space filtering and watershed clustering. Scale space filtering is used to obtain the nucleus from the subimage. Watershed clustering in 3-D HSV (Hue, Saturation, Value) histogram is processed to extract the cytoplasm. This method may not be sufficient in the case of high density of cells, where the RBCs are clustered close to the WBCs. To overcome the difficulty associated with the high density of cells, some simple morphological operators are used to explore the scale-space properties of a toggle operator to improve the segmentation accuracy in the WBCs. To avoid leaking, a common problem in cell images due to the low contrast between nucleus, cytoplasm and background, they used a scale-space toggle operator is used for contour regularization.

In this method, cytoplasm segmentation presents a few limitations: The RBCs touching the WBC are also detected as part of the cytoplasm. In our method, they eliminate the RBCs before detecting the WBCs. The above mentioned algorithms are based on edge information. Edge detection methods
do not work very well when not all cell details are sharp. But these methods work well if the contrast between the background and the gray internal membrane of the cell is stretched using a contrast stretching filter. However, the problem of touching RBCs and WBCs remain unresolved. They used simple thresholding method for cytoplasm segmentation, which is based on the fact that the colour intensity of RBC in a blood image is quite different from that of cytoplasm. Nuclear segmentation was performed using a GVF (gradient vector flow) snake. A few approaches that were introduced recently dealt only with the segmentation of the nucleus of the WBC. The successful segmentation of the cytoplasm along with the nucleus segmentation aids in the automatic classification of the WBCs.

1.6 WBC CLASSIFICATION

WBCs are classified according to the characteristics of their cytoplasm and nucleus by Ramesh et al (2012). Pathologists traditionally report normal WBCs as classified into five classes, i.e., monocytes, lymphocytes, neutrophils, eosinophils, and basophils. Since the chosen features affect the classifier performance, deciding which features must be used in a specific data classification problem is as important as the classifier itself. Hematology experts examine the cell shape, size, colour, and texture in combination with the nuclear features. It is important to reflect the rules and heuristics used by the hematology experts in selection of the features. Several researchers have previously proposed features to differentiate WBCs. The WBC classifications are shown in Figure 1.11.
The several types of features such as intensity and colour-based features, texture-based features, and shape-based features are utilized for a robust representation of WBCs. Classification methods used in this work include k-Nearest Neighbors, Learning Vector Quantization, MultiLayer Perceptron, and Support Vector Machine. Then they evaluated the binary images of the cytoplasm and nucleus to characterize the feature set. The standard set of features like area, perimeter, convex areas, solidity, major axis length, orientation, filled area, eccentricity were separately evaluated for the nucleus and the cytoplasm.

In addition features like the ratio between the nucleus and the cell areas, the nuclear "rectangularity" (ratio between the perimeter of the tightest bounding rectangle and the nuclear perimeter), the cell "circularity" (ratio between the perimeter of the tightest bounding circle and the cell perimeter), number of lobes in nucleus, area, and mean gray-level intensity of the cytoplasm were computed. Their system was evaluated using 10-fold
cross-validation. The performance was compared using different classifiers like nearest neighbor classifiers, feed-forward neural network, radial basis function neural network, and parallel classifier built with feed-forward neural network. In this method, a preliminary classification of the WBC based on the number of lobes (single or multi-lobed) in the nucleus along with the feature set is suggested to achieve better classification rate for each of the WBC subtypes.

This approach aims at achieving a method to identify WBCs from manually prepared, Wright-stained WSI. The manually prepared Wright-stained peripheral smear is easily and routinely performed at low cost. A manual differential count is routinely performed in many laboratories when a standard WBC has an abnormal value or when requested by a clinician. The manual differential is performed by a lab technician or a hematopathologist. The personnel time associated with performing a 100 cell count can range from 1-2 minutes per cell. However, considering the volume of peripheral smears reviewed in a day, the time dedicated to cell counting can be considerable.

In addition, the technologist’s time per slide can be considerably greater when the white cell count is very low. By utilizing our oldest method whole slide imaging technology is sought to investigate the feasibility of automating the identification of WBCs from digital images. This is a preliminary investigation in which they sought to determine if these techniques were flexible enough to use without performing additional stains or cell preparations, adding additional steps, or interrupting the current procedures or workflow.

They propose a simple segmentation scheme based on the difference in colour channels and morphological operations to segment the WBCs. The algorithm has low computational cost but good accuracy.
Two-step classification with the aid of a comprehensive feature set is helpful in realizing better accuracy rates in the classification of WBCs. In this initial work, our slide data was aggregated and analyzed as a group. They validate our approach on 320 images with 1938 cells. For comparison they implemented the segmentation algorithm and the features selected for classification were based on the features implemented.

1.7 DETECTION AND SEGMENTATION OF WHITE BLOOD CELLS FROM PERIPHERAL BLOODSMEAR

WBC counting is performed by pathologists only in thin areas of the blood smear. Similarly, they manually selected optimal regions adjacent to feathered edge and stored the images corresponding to the thin sections of the blood smear. While regions were manually selected in this preliminary study, additional work is being done to automate this process.

Each of these images has 2-15 WBCs. For easy identification of the WBCs, the whole blood slides are stained with Wright's stain, which produces a dark intensity in nuclei of WBCs. They convert the images from RGB (Red, Green, Blue) to HSV (Hue, Saturation, Value) space. First, using the saturation image, they threshold it to approximately identify the nuclei. A threshold value of 0.55 is used in our experiment (saturation range = 0-1). They eliminate extremely small regions based on their area. The advantage of using the saturation image for thresholding is to eliminate variations in illumination that occur. Finally, they crop the part around the nucleus making sure that the whole WBC is captured. If the distance between two nuclei is less than 5.52 μm, then they belong to the same WBC.

The advantage of using only the cropped image is that the regions containing mostly RBCs (which lack nuclei) are eliminated. The process of all the images and the detected WBC are recorded. The advantage of this method
is that they do not have any false negatives in the detection of the WBCs. They do capture some redundant cells (false positives) which have been stained, but they eliminate them in further processing by manually assigning them to a noise class. The following Figure 1.12 depicts the flowchart for WBC detection.

Figure 1.12 Flowchart for WBC detection

1.8 BLOOD

The functions and types of blood are discussed in the following section.

Blood is traditionally classified as a specialized form of connective tissue. To appreciate the basic unity of blood and other varieties of connective tissue, consider the following:
All the several blood cell types originate in the connective tissue of bone marrow.

Certain white blood cells, notably lymphocytes and monocytes, move freely back and forth between blood and other connective tissues.

The chemical composition of plasma is very similar to that of interstitial fluid in ordinary connective tissue.

All connective tissue consists of cells embedded in a matrix that consists of ground substance and fibers. Blood may thus be described as connective tissue whose matrix consists of free-flowing ground substance (plasma) with no fibers.

One highly specialized cell type, the Red Blood Cell (RBC, erythrocyte), normally occurs only in blood. Other blood cells, the so-called White Blood Cells (WBCs, leukocytes), are found in other connective tissues as well.

There are three types of blood cells, they are:

1. Red Blood Cells
2. White Blood Cells
3. Platelets

These types of blood cells are shown in Table 1.1 and the location of the bone marrow in bones are shown in Figure 1.13.
### Table 1.1 Different Blood Cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Cytoplasm</th>
<th>Nucleus</th>
<th>Granules</th>
<th>Central Granules</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red Blood Cell</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>Orange to pink to rose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>White Blood Cell Types</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Light blue</td>
<td>Deep blue to violet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
<td>Orange to pink</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>Pale gray to blue</td>
<td>Deep bluish to purple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Pale pink</td>
<td>Deep blue to violet</td>
<td>Purple to lilac</td>
<td></td>
</tr>
<tr>
<td>Basophiles</td>
<td></td>
<td></td>
<td>Deep blue to violet</td>
<td></td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
<td></td>
<td>Red to purple surrounded by light blue</td>
</tr>
</tbody>
</table>

Figure 1.13 Location of the bone marrow in bones and types of blood cells produced
1.8.1 Red Blood Cell

Red blood cells (abbreviated RBCs, also called erythrocytes from erythro= red + cyte= cell) are continually produced in bone marrow and recycled in spleen. In mature form they lack nuclei and most cytoplasmic structures; they are little more than discoid, flexible bags of hemoglobin.

Mature RBCs are nucleated in non-mammalian vertebrates (fish, birds, reptiles, and amphibians) but are anucleate in all mammals (although there is some interspecies variation in cell shape). A common misconception (easily found on the Internet) holds that camels are an exception to this rule. This mistake may have originated in the early years of histology (1800s), from observations of camels' ovoid RBCs which are thickest in the center. The example of RBC is shown in the Figure 1.14 given below.

![Image of normal RBCs](image)

**Figure 1.14 Example of normal RBCs**

The red blood cells here are normal, happy RBC's. They have a zone of central pallor about 1/3 the size of the RBC. The RBC's demonstrate minimal variation in size (anisocytosis) and shape (poikilocytosis). A few
small fuzzy blue platelets are seen. In the center of the field, there is a band neutrophil on the left and a segmented neutrophil on the right.

1.8.2 White Blood Cell

White blood cells (abbreviated WBCs, also called leukocytes from *leuko*= white + *cyte*= cell) comprise several distinct cell types, neutrophils, eosinophils, basophils, lymphocytes, and monocytes. Certain developmental and morphological similarities permit these first three cells to be usefully grouped together as granulocytes or polymorphonuclear leukocytes. The latter two types are then categorized as mononuclear leukocytes. Example of normal WBC is shown in the Figure 1.15.

![Image of White Blood Cells](image)

**Figure 1.15 Example of normal WBCs**

1.8.3 Platelets

Platelets are biconvex discs, fragments of cytoplasm 2–3 µm in diameter, found only in the blood of mammals. Platelets form by budding off from megakaryocytes in the bone marrow, and then entering the circulation. They help stop bleeding.
The main function of platelets is to contribute to hemostasis: the process of stopping bleeding at the site of interrupted endothelium. First, platelets stick to substances outside the interrupted endothelium: adhesion. Second, they change shape, turn on receptors and secrete chemical messengers: activation. Third, they stick to each other: aggregation. Formation of this platelet plug (primary hemostasis) is followed by 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.

1.9 PROBLEMS AFFECTING WHITE BLOOD CELLS

White blood cell count can be low for a number of reasons - when something is destroying the cells more quickly than the body can replenish them or when the bone marrow stops making enough white blood cells to keep one healthy. When the white blood cell count is low, one becomes extremely susceptible to any illness or infection, which can spiral into a serious health threat.

The health care provider can see whether white blood cell count is normal through a blood test known as the complete blood count. If the count is too low or too high, one may have a white blood cell disorder.

A number of diseases and conditions may influence white blood cell levels:

- **Weakened immune system**: This is often caused by illnesses such as Human Immunodeficiency Virus (HIV/AIDS) or by treatments related to cancer. Cancer treatments such as chemotherapy or radiation therapy can destroy white blood cells and leave one vulnerable to infection.
- **Infection:** A higher-than-normal white blood cell count usually indicates some type of infection - white blood cells are multiplying to destroy an enemy, such as bacteria or a virus.

- **Myelodysplastic syndrome:** This condition causes abnormal bone marrow cell production.

- **Cancer of the blood:** Cancers including leukemia and lymphoma can cause uncontrolled growth of an abnormal type of blood cell in the bone marrow, resulting in a greatly increased risk for infection and or serious bleeding.

- **Myeloproliferative disorder:** This disorder refers to various conditions that trigger the excessive production of immature blood cells. This can result in an unhealthy balance of all types of blood cells in the bone marrow and too many or too few white blood cells in the blood.

### 1.10 PURPOSE OF A WBC COUNT

It is normal for doctors to order a complete blood count and check WBC count during an annual physical examination. The doctor may recommend a WBC count if one complains of persistent body aches, fever, chills, or headaches. A WBC count can detect hidden infections within our body and alert doctors to undiagnosed medical conditions, such as autoimmune diseases, immune deficiencies, and blood disorders. This test also helps doctors monitor the effectiveness of chemotherapy or radiation treatment in cancer patients.
1.11 WHAT TO EXPECT FROM A WBC COUNT

A health-care provider or lab technician will draw blood to check WBC count. This blood sample is taken either from a vein in the arm or a vein on the back of the hand. It only takes a couple of minutes to draw blood and the patient may experience minor discomfort. The health-care provider will clean the needle site to kill any germs and then tie an elastic band around the upper section of one’s arm. This elastic band is used so that the blood can fill the vein making it easier for the blood to be drawn.

The health-care provider slowly inserts a needle into arm or hand, and collects the blood in an attached tube. The provider then removes the elastic band from around arm and slowly removes the needle. The technician applies gauze to the needle site to stop the bleeding. Health-care providers use a different technique when drawing blood from young children and infants. With these patients, providers first puncture the skin with a lancet (a pricking needle) and then use a test strip or a small vial to collect the blood.

Leukopenia is the medical term used to describe a low WBC count. Conditions or illnesses that can trigger a low number include:

- HIV
- autoimmune disorders
- bone marrow disorders/damage
- lymphoma
- severe infections
- liver and spleen diseases
- lupus
- radiation therapy
1.12 CLUSTERING TECHNIQUES

The Clustering is the process used in data mining and image processing mostly. They are used to group the objects based on their values or distance, etc. For Images the clustering work is to segment the required objects from other objects in an image. This plays a major role in medical image segmentation, satellite image segmentation and many others. In this study clustering technique is used for WBC segmentation from cell images using various fuzzy based techniques to achieve the best clustering technique for WBC segmentation.

1.13 FUZZY C-MEANS CLUSTERING

Clustering using Fuzzy C-Means (FCM) algorithm is an unsupervised clustering technique which is mostly used in image segmentation. FCM is able to preserve more information from the original image. In fuzzy clustering, every point has a degree of belonging to clusters, rather than belonging completely to just one cluster.

1.14 MODIFIED FCM ALGORITHM

A modified Fuzzy C-Means clustering algorithm is proposed for the segmentation of images of FCM. The modified Fuzzy C-Means clustering (FCM) algorithm includes both the local spatial information from neighbouring pixels, and the spatial Euclidian distance to the cluster's center of gravity.

1.15 FUZZY POSSIBILISTIC C-MEANS

Fuzzy Possibilistic C-Means algorithm was developed by Gomathi & Thangaraj (2010), to improve this weakness of FCM, and to produce memberships that have a good explanation for the degree of belonging for the
data, the constrained condition of the fuzzy C partition to obtain a Possibilistic type of membership function and propose of FCM to PCM for unsupervised clustering.

The clustering algorithms can be explained. Machine learning is a branch of artificial intelligence, concerns the construction and study of systems that can learn from data.

1.16 RELEVANCE VECTORE MACHINE

Relevance Vector Machines (RVM) has recently attracted much interest in the research community because they provide a number of advantages. They are based on a Bayesian formulation of a linear model with an appropriate prior that results in a sparse representation. As a consequence, they can generalize well and provide inferences at low computational cost. In this work, the basic theory of RVM is presented for regression and classification, followed by two examples illustrating the application of RVM is presented for object detection and classification. The first example is target detection in images and RVM is used in a regression context. The second example is detection and classification of microclassifications from mammograms and RVM is used in a classification framework.

- Bayesian alternative to support vector machine (SVM)

Limitations of the SVM

- two classes
- large number of kernels (in spite of sparsity)
- kernels must satisfy Mercer criterion
– cross-validation to set parameters $C$ (and $\varepsilon$)
– decisions at outputs instead of probabilities

The graphical model representation for regression and SVM Classification are shown in Figures 1.16 and 1.17.

![Graphical model representation (regression)](image)

**Figure 1.16 Graphical model representation (regression)**

- For classification sigmoid (or softmax for multi-class) outputs are used and noise node is omitted.

**Classification: SVM**

![Classification of SVM](image)

**Figure 1.17 Classification of SVM**
Properties of RVM

The comparison of Support Vector Regression (SVR) and Relevance Vector Regression (RVR) is shown in Figure 1.18.

- comparable error rates to SVM on new data
- no cross-validation to set complexity parameters
- applicable to wide choice of basis function
- multi-class classification
- probabilistic outputs
- dramatically fewer kernels (by an order of magnitude)
- slower to train than SVM.

![Support Vector Regression vs. Relevance Vector Regression](image)

**Figure 1.18 Comparisons of SVR and RVR**
1.17 PROBLEM STATEMENT

In laboratories, equipment is available for performing differential count of cells. But, in the case of a few diseases where the size and shape of a WBC has to be diagnosed, human intervention is needed. A trained laboratorian prepares blood smear and examines it through a microscope. Unfortunately, the accuracy of cell identification and classification is strongly dependent on the manual capability. As the number of samples increase, the identification process tends to be error-prone. The situation warrants the need for automating the system so that accurate diagnosis could be provided. Then WBC detection methods for microscopic cell images have been proposed, most of which are edge-based or region-based scheme. Edge-based scheme performs poorly on cell images because not all cell boundaries are sharp and hence it is difficult to get all the edge information and locate the cells accurately.

1.18 MOTIVATION

The main motivations are, the result for WBC is to be accrued and the processing time is to be less by using the cluster called Modified Fuzzy Possibilistic C-Means (MFPCM). It motivates that the image of WBC may not be poor, and the shapes and edges need to be perfect by using the algorithm of MFPCM. This proposed algorithm accounts for pixel spatial information which helps to keep continuity on neighbouring pixel values of the cells. The clustering technique depends on pixel levels and this objective function varies for different pixel levels in the image.

1.19 SCOPE OF THIS RESEARCH

Image processing techniques are necessary to exploit the information contained in medical images, to be successfully used in CAD
systems. In addition, computer-aided tools can analyze the volume image in a reasonable amount of time. These are valuable tools used for segmentation and detection of white blood cells. Image segmentation consists in parceling or delimiting the image into different regions according to some properties or features describing these regions.

1.20 OBJECTIVES OF THIS RESEARCH

Main objectives are:

- To identify the shapes and edges of the segmentation depending on the image
- To have smoothly shaped surface with clear gray level steps between different surfaces
- To use machine learning techniques for good image segmentation and detection
- To utilize the fuzzy clustering techniques for accurate results
- To improve the efficiency by using three-feature vectors like area, length, and perimeter in RVM.

1.21 ORGANIZATION OF THE THESIS

The thesis is organized as follows:

Chapter 1

Deals with the introduction of Clustering in image segmentation. The image segmentation is more simple and efficient.
Chapter 2

Reviews the previous work done in the areas of image segmentation and techniques that are improved using FPCM and MFPCM algorithm.

Chapter 3

Describes the first approach, “WBC Image Segmentation using Adaptive Fuzzy Moving K-Means Algorithm”.

Chapter 4

Discusses the second approach to improve the analyzing work stress namely, “WBC Image Segmentation Using Modified Fuzzy Possibilistic C-Means Algorithm”.

Chapter 5

Describes the classification namely, “WBC Image Segmentation and Classification using Relevance Vector Machine”.

Chapter 6

Presents the Observations and findings for the proposed methodologies.

The performance of the proposed approach is evaluated based on the parametric standards like accuracy, time, etc.

Chapter 7

Provides the concluding remarks of the proposed approaches.
The works of several researchers are quoted and used as evidence to support the concepts explained in the thesis. All such evidences used are listed in the reference section of the thesis.

1.22 SUMMARY

This chapter summarizes the most popular and accepted methodologies applicable to the evaluation of image analysis especially in the segmentation method that has been proposed. The ultimate goal of blood cell segmentation is to extract the image of blood cell from complicated background and to segment every cell into morphological components such as nucleus, cytoplasm, holes and some others. The main objective of this study is to develop an automated system on blood cell classification. The image processing using clustering algorithms is involved for segmenting the white blood cell image to detect the immature cell. Many different clustering techniques have been proposed for segmenting an image in a better way.