# MEASURING PLATELET COUNT BY USING CONTOUR AWARE SEGMENTATION

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**ABSTRACT**

**Platelet count is a very important diagnosis test to identify Diseases like Dengue, Malaria, Yellow fever, and others. For dengue patientmonitoring platelet count is often needed. A suspected dengue patient needs a very quick diagnosis to give an accurate result of howcritical is the condition of the patient. In most laboratories, Leishman's-stained blood slides are used to count platelets. However, these manual platelets counting requires expert lab technician and the overhead increases manifold when huge blood samples are to be checked by lab technicians that make the entire process time-consuming. So, we can extract platelets from the microscopic image of blood cells, and that makes platelet counting task easy. Microscopic images of stained blood slides are captured using a light microscope.**

**Then using color-based segmentation and morphological operation, Platelets can be extracted. A comparative study between the platelet counts obtained before and after segmentation along with calculation of the efficiency of the system has shown this method to be robust and effective for automation of platelet count system.**

**Keywords:** MATLAB, **Microscopic Image, Morphological analysis,Image Processing**.

## INTRODUCTION

Red blood cells (RBCs), also called erythrocytes, constitute most of the blood giving it red color due to the presence of hemoglobin and they are the vertebrate organism's principal means of deliveringoxygen (O2) to the body tissues via the blood flow through the circulatory system. They take up oxygen in the lungs or gills and release it into the tissues while squeezing through the body's capillaries.

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

In humans, mature red blood cells are flexible and oval biconcave disks. They lack a cell nucleus and most organelles, in order to accommodate maximum space for hemoglobin. Approximately 2.4 million new erythrocytes are produced per second. The cells develop in the bone marrow and circulate for about 100-120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells. Red blood cells are also known as RBCs, Red Cells, Red Blood Corpuscles, Hematins, Erythroid Cells or Packed Red Blood Cells (PRBC) are red blood cells that have been donated, processed, and stored in a blood bank for blood transfusion.

One of the important information that doctors usually use to diagnose different diseases is the RBC count. The red blood cells are the most numerous blood cells in the human body, and it also called erythrocytes. The red blood cells are functioned to carry oxygen throughout our body. In health, the red blood cells vary relatively little in size and shape. In well- spread, dried and stained films thegreat majority of cells have round, smooth contours and diameterswithin the comparatively narrow range of 6.2-8.2 um.

## 1. RELATED WORKS

Before going implement the proposed work, in the part of related works, the following research papers have been referred considering their contents.

Nasrul Humaimi Mahmood and Muhammad Asraf Mansor [1] ("Red Blood Cells Estimation Using Hough Transform Technique") has given the details of Counting of RBC in a blood sample can givethe pathologists valuable information regarding various hematological disorders. There are four steps involved in estimating the RBC. These are acquisition, segmentation, feature extraction and estimating. The acquisition step used the existing blood sample images. Next the segmentation and feature extraction is done by using a morphological technique in order to distinguish the RBC form background and other cells. The last step is estimating the number of RBC by using Hough Transform. This technique is able to detect and estimate the number of RBC by determining the center point of the circle and then using IMDISTLINE.

M.Habibzadeh, A.Krzyak, T.Fevens [2] ("Counting of RBCs and WBCs in noisy normal blood smear microscopic images") it presents an accurate, robust mechanism to determine the distribution of blood smear particles. This work does not address issues such as deformed RBC shapes (teardrops, crescents, needles, or a variety of other forms), infected RBCs and extra overlapping cells which can be found with certain types of diseases. A set of blood smear test images are used to show that their proposed framework is more accurate in comparison with some classical methods, and also is much more robust for degraded images which are blurry and/or noisy. The first comparison method de-noises the image with a median filter with a 3x3 mask function and then uses Otsu binarization to create an intermediate image. Then a second intermediate image is created using canny edge detection. These two intermediate images are merged into the final image, objects with closed boundaries are filled.pixel wise, and the number of objects is counted using watershed segmentation. The second comparison method performs the same procedure using Otsu binarization but skips the canny edge detection step.

Alaa Hamouda, Ahmed Y. Khedr [3] ("Automated Red Blood Cell Counting") has given the details about an image involves some unwanted particles (noise). Therefore, some pre-processing is needed which is called image preparation phase. This phase consists of two steps which are histogram equalization and segmentation. In the first step, histogram equalization, the intensity value of the given image is adjusted using image intensity transformation. In the second step, segmentation, blood cell are detected by differentiating them from the background in terms of contrast. Changes in contrast can be detected by image processing operators that calculate the gradient of an image. Then a threshold can be applied to create a binary mask containing the segmented cell. The edge detection is done by using the Sobel operator. In the second phase, blood cells extraction, different methods are applied looking after highest accuracy as well as less complexity.

AMT Nasution, EK Suryaningtyas [4] ("Automated morphological processing for counting the number of red blood cell") Morphological image processing is an image processing method that deals with recognition of an object, which is based on pre-defined criteria on their morphologies. The acquired image is a 24-bit RGB image, witha dimension of 640 x 480 pixels. For an ease of image binarization, the RGB image need to be grey-scaled image. The intensity level is still kept to be proportional, with a 256 scale of shade from total darkness to white. The produced grey-scaled image needs to be enhanced by using contrast-limited adaptive histogram equalization (CLHAE). The morphological operation to the RBCs is intended for recognizing RBCs, before the number of cells to be counted. At this stage, clumped cells which are commonly found in microscopy - need to be further separated. The watershed segmentation algorithm is then implemented to get clearer boundaries among clumped cells. This algorithm consists of several steps, i.e. determination of gradient magnitude, foreground markers, and of background markers. This algorithm is terminated by determination of watershed ridge lines. Region of Interest (ROI) is used for determination of which groups of blood cells which will be separated. The adjoining RBCs can be recognized by determining the morphological properties of all related cells. The separation can then be based on parameter of cell diameter. Cell splitting process is accomplished by implemented algorithm, which can be ordered as follows. First the edges of the adjoining RBCs will be detected, which results the cells with two consecutive boundaries, i.e. inner and outer boundaries. The next step is devoted to remove these outer boundaries, and image of separated RBCs will be produced.

J.M. Sharif, M. F. Miswan, M. A. Ngadi [5] ("Red Blood Cell Segmentation Using Masking and Watershed Algorithm")The methods used are Yebercolor conversion, masking, morphological operators and watershed algorithm. The combination of Yebercolor conversion and morphological operator produce segmented white blood cell nucleus. Then it is being used as a mask to remove WBC from the blood cell image. Morphological operators involve binary erosion to diminish small objects like platelet. The resulted RBCsegmentation is passed through marker controlled watershed algorithm which handles overlapping cells. Improvement needs to be done for both segmentation and overlapped cell handling to obtain better results in the future.

J.He,Q. D. M. Do, A. C. Downton ("A Comparison of Binarization Methods for Historical Archive Documents") Previous research has claimed that Sauvolas algorithm has superior performance to Niblacks. The experimental work with archive documents suggests this is not always true. One of the important advantages of Sauvolas algorithm is that it generates much less noise than Niblacks. For evaluation data, both Niblacks and adaptive Niblacks algorithm minimize noise to the same low level as Sauvolasalgorithm. Under these circumstances, Niblacks algorithm achieves superior performance than Sauvolas when both must have fixed parameters but deal with varying background images. This phenomenon indicates that Sauvolas algorithm is more sensitive to the change of background than Niblacks, and is hence more difficult to tune to varying backgrounds over a complete dataset. The backgrounds of evaluation data vary significantly image by image. InSauvolas evaluation data, the background of images didn't appear to vary so much. Although the new adaptive version of Sauvolas algorithm can improve the performance, it is still worse than Niblacks. However, Sauvolas algorithm has other advantages: it costs less computationally, as a smaller SW is used than

Niblacks algorithm. Conclusion is that the choice of algorithm isreally case dependent.

Meng-Ling Feng, Yap-Peng Tan ("Adaptive Binarization Method for Document Image Analysis") This paper proposed an adaptive binarization method, based on a criterion of maximizing local contrast. The proposed method overcomes, to a large extent the general problems of poor quality images. The common problems in poor quality text images include variable back-ground intensity due to non-uniform illumination, low contrast and large amount of random noise due to limited sensitivity of camera. Therefore, to have an accurate analysis, a versatile binarization method, which can correctly remove noise and unnecessary background and reliably keep all useful information, becomes indispensable. Raman Maini Dr. Himanshu Aggarwal [8] ("Study and provides filter have a major drawback of being very sensitive to noise. The size of the kernel filter and coefficients are fixed and cannot be adapted to a given image. An adaptive edge- detection algorithm is necessaryto provide a robust solution that is adaptable to the varying noise levels of the images to help distinguish valid image contents from visual artefacts introduced by noise. The performance of the canny algorithm depends heavily on the adjustable parameters, which is the standard deviation for the Gaussian filter, and the threshold values, T1 and T2 also control the size of the Gaussian filter. The bigger the value, the larger the size of the Gaussian filter becomes. This implies more blurring, necessary for noisy images, as well as detecting larger edges. However, the larger the scale of the Gaussian, the less accurate is the localization of the edge. Smaller values imply a smaller Gaussian filter which limits the amount of blurring, maintaining finer edges in the image. The user can tailor the algorithm by adjusting these parameters to adapt to different environments. Canny edge detection algorithm is computationally more expensive compared to Sobel, Prewitt and Roberts operator. However, the canny edge detection algorithm performs better than all these operators under almost all scenarios. Evaluation of the images showed that under noisy conditions, Canny, Log, Sobel, Prewitt, Roberts exhibit better performance, respectively.

Ngoc-Tung Nguyen, Anh-Duc Duong ("Cell Splitting with HighDegree of Overlapping in Peripheral Blood Smear") this study presents a method for extracting overlapped cells into individual cell. The proposed method is mainly focused on rapidly detecting centralpoint using the distance transform value. Additionally, a boundary covering degree of each center point is applied to select the best potential center points. Single cell extraction is employed in order to estimate the average size of cell. Finally, an effective algorithm is designed to split correctly and speedily. The robustness andeffectiveness of this method is assessed through the comparison with more than 400 images labelled manually by experts and exhibiting various clumped cell. As the result, the F-measure generally reaches 93.5 percent and more than 82 percent overlapped cells can be tolerated in the condition of non-distorted images.

## 2.MATERIALS AND METHODS

### PRE-PROCESSING:

In Pre-processing step, the image is adjusted suitably for the next of the process. Here In Pre-processing step, the input image must be RBG.

### Color Model:

The purpose of a color model (also called color space or color system) is to facilitate the specification of colors in some standard, generally accepted way. In essence, a color model is a specification of a coordinate system and a subspace within that system where each color is represented by a single point.

**RGB Color Model**:

In the RGB model, each color appears in its primary spectral components of red, green, and blue. This model is based on a Cartesian coordinate system.

The color subspace of interest is the cube shown in Figure in which RGB values are at three corners;cyan, magenta, and yellow are at three other corners; black is at the origin; and white is at the corner farthest from the origin. In this model, the gray scale (points of equal RGB values) extends from black to white along the line joining these two points.

The different colors in this model arc points on or inside the cube, and are definedby vectors extending from the origin. For convenience, the assumption is that all color values have been normalized so that the cube shown in Figure is the unit cube.

That is, all values of R, G.and B are assumed to be in the range [0, 1].

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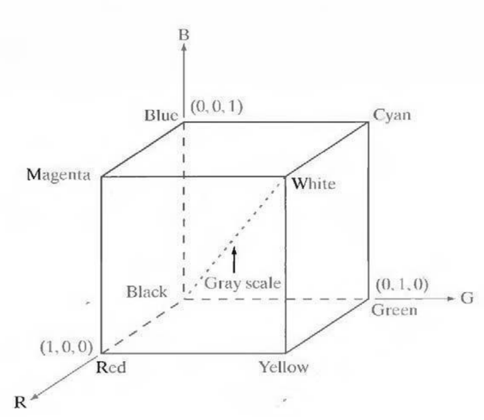



Figure : Schematic of the RGB color cube

Images represented in the RGB color model consist of three component images, one for each primary color. When fed into an RGB monitor, these three images combine on the phosphor screen to produce a composite color image.

Consider an RGB image in which each of the red, green, and blue images is an 8-bit image. Under these conditions each RGB color pixel [that is, a triplet of values (R, G, B)] is said to have a depth of 24 bits C image planes times the number of bits per plane). The termfull-color image is used often to denote a 24-bit RGB color image. The total number of colors in a 24-bit RGB image is $(2^8)^3$ = 16,777,216.

RGB is ideal for image color generation (as in image capture by a color camera or image display in a monitor screen), but its use for color description is much more limited.

**Dataset**

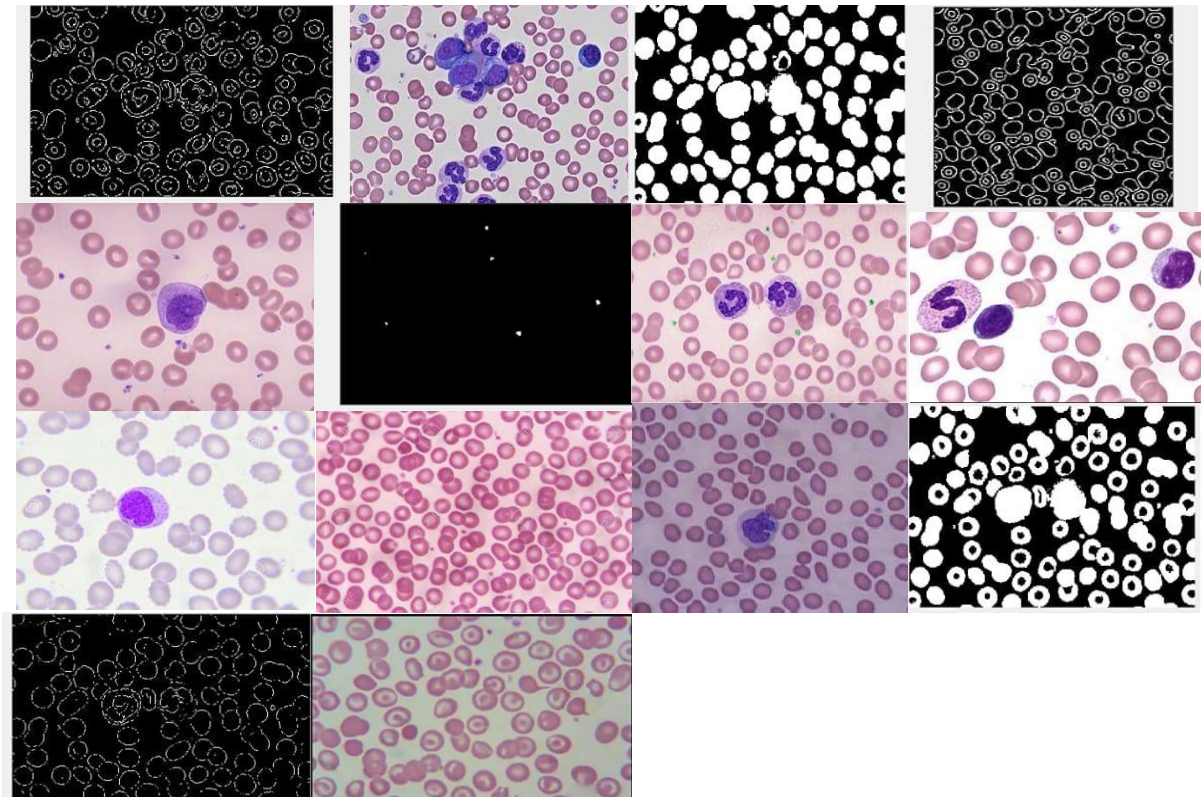


Fig :Different Digital Microscopic Image

**IMAGE SEGMENTATION:**

The goal of image segmentation is to simply and change the representation of an image into something that is more meaningful and easier to analyze. Image segmentation is typically used to locate objects and boundaries such as lines, curves, etc in images. The simplest method of image segmentation is called thresholding method. The key of this method is to select the threshold values or values when multiple-levels are selected.

The result of image segmentation is a set of segments that collectively cover the entire image, or a set of contours extracted from the image(edge detection). Each of the pixels in a region are similar with respect to some characteristics such as color, intensity or texture. Adjacent regions are significantly different color respect to same characteristics . Image segmentation is the process of assigninga label to every pixel in an image such that pixels with same label share certain characteristics.

**Morphological operations :**

Morphological is a broad set of image processing operations that process images based on shapes. Morphological operations apply a structuring element to input image ,creating an output image of the same size. In a morphological operations, the value of each pixelin the output image is based on a comparison of the corresponding pixel in the input image with its neighbors.

**Mathematical Morphological Operation:**

Morphological image processing is based on a strong mathematical concept which been used to change the size, shape, structure and connectivity of objects in the image. It involves binary erosion, dilation, opening, closing and reconstruction. Erosion plays the role to shrinks and thins objects in image. While dilation used to grows and thickens objects in image. Next, Morphological Opening isthe combination process of erosion and continued by dilation. WhileMorphological Closing is using the concept of dilation and continuedby erosion. In other words, the functions of morphological opening are to removes, break and diminished the connection or objects which not contain the structure elements. In contrary, morphological closing functions to join, fill and build connection and objects in the image. Using the closing image transformation with defined SE (Structure Element) unwanted edges, typically generated, are removed.

The input to this block is the edge detected image. Morphological operation of filling is applied in the edge detected image. The edges which are complete are filled from within. While the edges which are incomplete are left as it is. Disk shaped structuring element (se) isused for better results.

**Rules for dilation and erosion**:

**Dilation:**

The value of output pixel is the maximum value of all pixels in the neighborhood. In a binary image pixel set to 1 if any of the neighboring pixels have the value 1. Morphological dilation makes objects more visible and fills in the small holes in objects. Lines appear thicker, and filled shapes appear larger.

**Erosion:**

The value of the output pixel is the minimum value of all the pixels in the neighborhood . In a binary image a pixel is set to 0 if any of the neighboring pixels have the value 0. morphological erosion removes the floating pixel and thin lines so that substantive objects remain. Remaining lines appear thinner and shapes appear smaller.

**Contour aware segmentation:**

Blood cells exhibit wide variations of cell morphology and size that make them difficult to be segmented accurately especially due to overlapping. Contour aware conventional network generate multi- scale feature representation to deal with large variations and overlapped cells. Segmentation of touching and overlapped cells could be enhanced by using contextual features and CNN withauxiliary supervision. Thus , leveraging contextual information could improve the segmentation performance by recognizing its structure. In this work, we have used multilevel contextual feature representation that include contextual information.

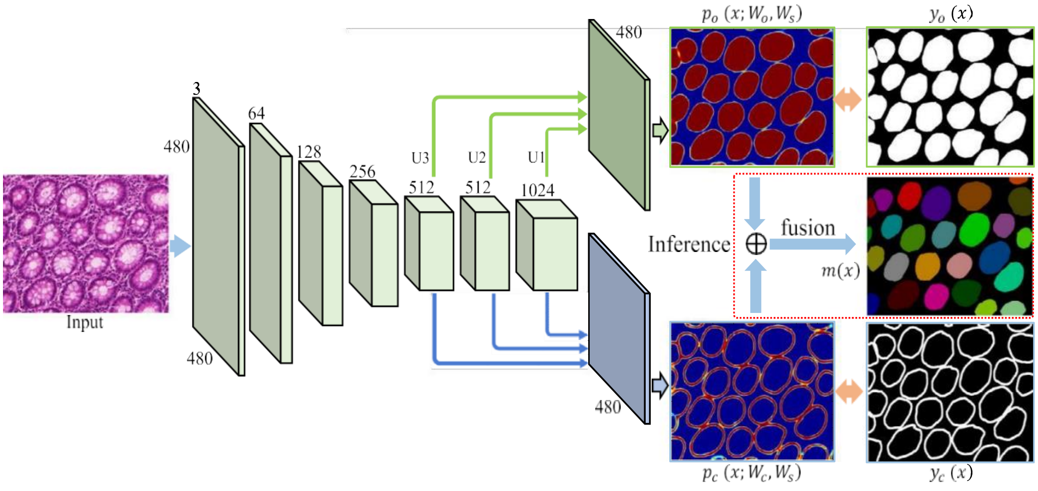



Fig : Overview Of Contour Aware Segmentation

**Platelet counting algorithm:**

Step1: Start

Step2: Blood seamer image data sets are collected

Step3: To perform the image pre-processing method

Step4: To perform image segmentation

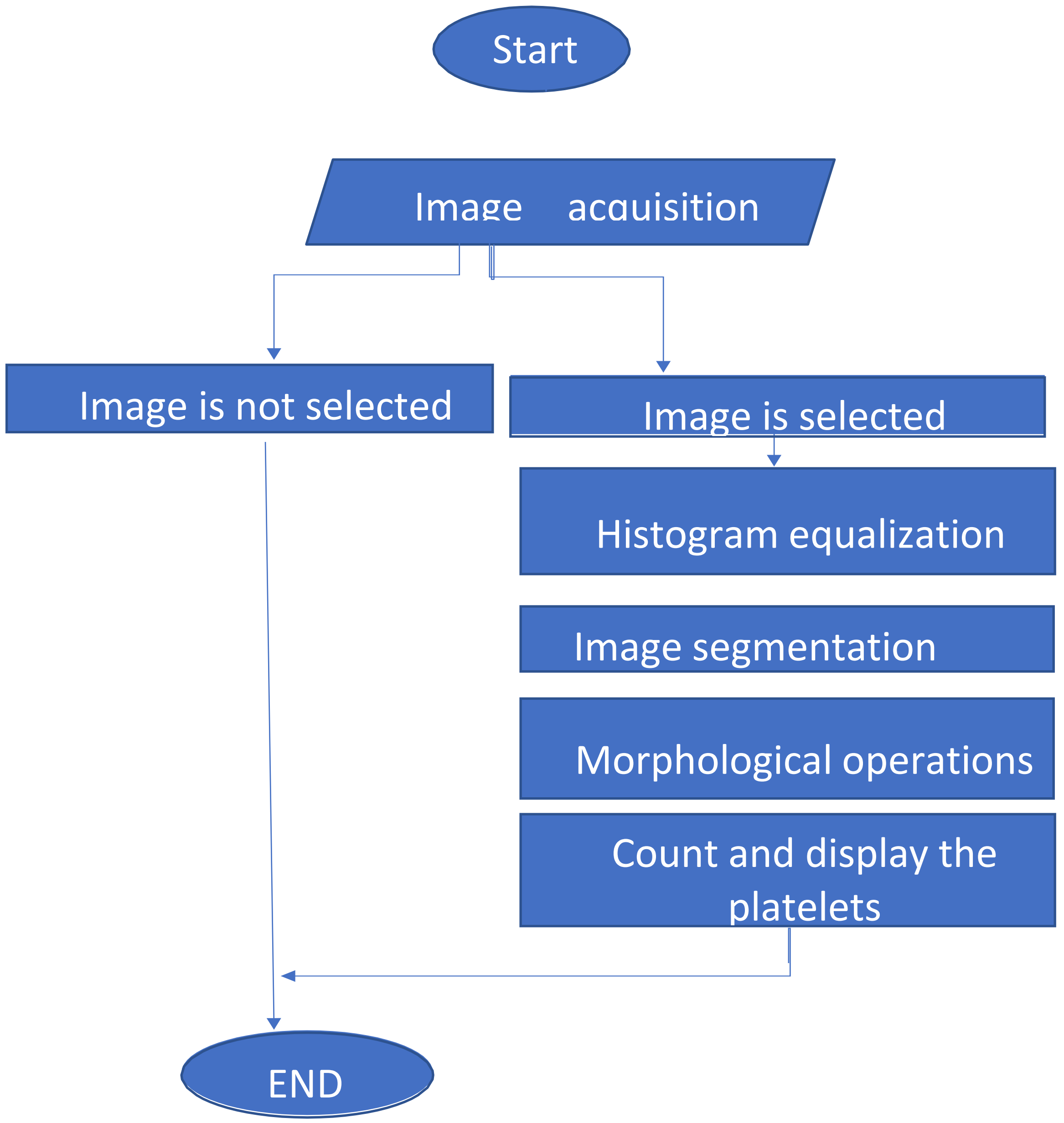
Step5: To perform morphological operations

Step6: Counting the platelets

Step7: Display the count of platelets.

Step8: End

**Flow Chart**



**System Architecture:**

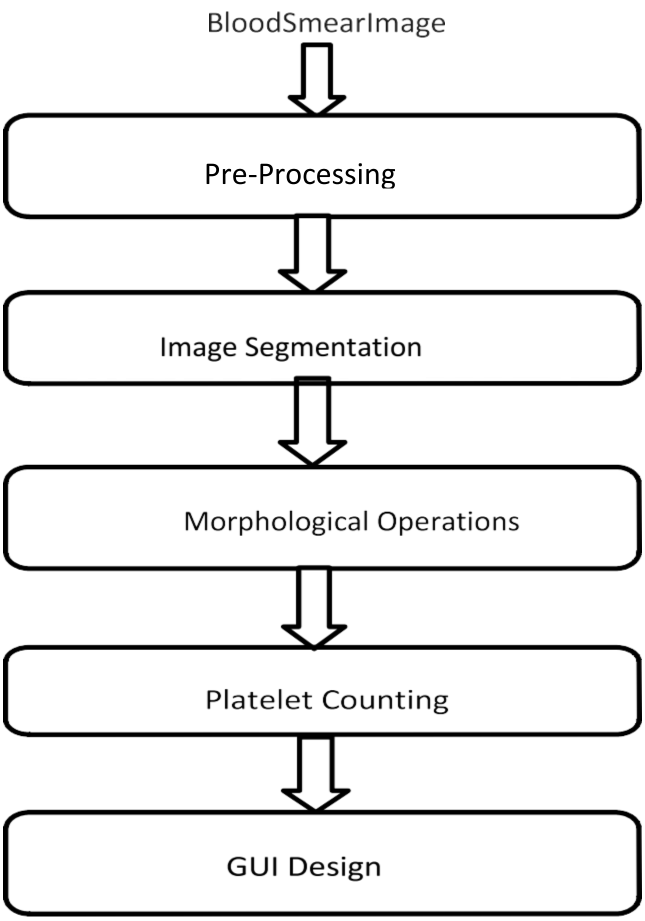



Fig : System Architecture

The images are used in data acquisition stage are taken from online medical library. The input image needs to be pre-processed for further analysis. Segmentation and extraction is the process of differentiate red blood cell background and another cells. The final stage is counting the number of red blood cell using Hough transform technique.

**Different approaches and kinds of techniques for image segmentation**, we can start discussing the specifics. Following are the primary types of image segmentation techniques:

1. Thresholding Segmentation
2. Edge-Based Segmentation
3. Region-Based Segmentation
4. Neural Networks for Segmentation

Let's discuss each one of these techniques in detail to understand their properties, benefits, and limitations:

### 1. Thresholding Segmentation

The simplest method for segmentation in image processing is the threshold method. It divides the pixels in an image by comparing the pixel's intensity with a specified value (threshold). It is useful when the required object has a higher intensity than the background (unnecessary parts).

You can consider the threshold value (T) to be a constant but it would only work if the image has very little noise (unnecessary information and data). You can keep the threshold value constant or dynamic according to your requirements.

The thresholding method converts a grey-scale image into a binary image by dividing it into two segments (required and not required sections).

According to the different threshold values, we can classify thresholding segmentation in the following categories:

**Otsu's Binarization**

In simple thresholding, you picked a constant threshold value and used it to perform image segmentation. However, how do you determine that the value you chose was the right one? While the straightforward method for this is to test different values and choose one, it is not the most efficient one.

Take an image with a histogram having two peaks, one for the foreground and one for the background. By using Otsu binarization, you can take the approximate value of the middle of those peaks as your threshold value.

In Otsu binarization, you calculate the threshold value from the image's histogram if the image is bimodal.

This process is quite popular for scanning documents, recognizing patterns, and removing unnecessary colours from a file. However, it has many limitations. You can't use it for images that are not bimodal (images whose histograms have multiple peaks).

**Adaptive Thresholding**

Having one constant threshold value might not be a suitable approach to take with every image. Different images have different backgrounds and conditions which affect their properties.

Thus, instead of using one constant threshold value for performing segmentation on the entire image, you can keep the threshold value variable. In this technique, you'll keep different threshold values for different sections of an image.

This method works well with images that have varying lighting conditions. You'll need to use an algorithm that segments the image into smaller sections and calculates the threshold value for each of them.

### 2. Edge-Based Segmentation

Edge-based segmentation is one of the most popular implementations of segmentation in image processing. It focuses on identifying the edges of different objects in an image. This is a crucial step as it helps you find the features of the various objects present in the image as edges contain a lot of information you can use.

Edge detection is widely popular because it helps you in removing unwanted and unnecessary information from the image. It reduces the image's size considerably, making it easier to analyse the same.

Algorithms used in edge-based segmentation identify edges in an image according to the differences in texture, contrast, grey level, colour, saturation, and other properties. You can improve the quality of your results by connecting all the edges into edge chains that match the image borders more accurately.

There are many edge-based segmentation methods available. We can divide them into two categories:

**Search-Based Edge Detection**

Search-based edge detection methods focus on computing a measure of edge strength and look for local directional maxima of the gradient magnitude through a computed estimate of the edge's local orientation.

**Zero-Crossing Based Edge Detection**

Zero-crossing based edge detection methods look for zero crossings in a derivative expression retrieved from the image to find the edges.

Typically, you'll have to pre-process the image to remove unwanted noise and make it easier to detect edges. Canny, Prewitt, Deriche, and Roberts cross are some of the most popular edge detection operators. They make it easier to detect discontinuities and find the edges.

In edge-based detection, your goal is to get a partial segmentation minimum where you can group all the local edges into a binary image. In your newly created binary image, the edge chains must match the existing components of the image in question.

### 3. Region-Based Segmentation

Region-based segmentation algorithms divide the image into sections with similar features. These regions are only a group of pixels and the algorithm find these groups by first locating a seed point which could be a small section or a large portion of the input image.

After finding the seed points, a region-based segmentation algorithm would either add more pixels to them or shrink them so it can merge them with other seed points.

Based on these two methods, we can classify region-based segmentation into the following categories:

**Region Growing**

In this method, you start with a small set of pixels and then start iteratively merging more pixels according to particular similarity conditions. A region growing algorithm would pick an arbitrary seed pixel in the image, compare it with the neighbouring pixels and start increasing the region by finding matches to the seed point.

When a particular region can't grow further, the algorithm will pick another seed pixel which might not belong to any existing region. One region can have too many attributes causing it to take over most of the image. To avoid such an error, region growing algorithms grow multiple regions at the same time.

You should use region growing algorithms for images that have a lot of noise as the noise would make it difficult to find edges or use thresholding algorithms.

**Region Splitting and Merging**

As the name suggests, a region splitting and merging focused method would perform two actions together – splitting and merging portions of the image.

It would first the image into regions that have similar attributes and merge the adjacent portions which are similar to one another. In region splitting, the algorithm considers the entire image while in region growth, the algorithm would focus on a particular point. The region splitting and merging method follows a divide and conquer methodology. It divides the image into different portions and then matches them according to its predetermined conditions. Another name for the algorithms that perform this task is split-merge algorithms.

### 4. Neural Networks for Segmentation

Perhaps you don't want to do everything by yourself. Perhaps you want to have an AI do most of your tasks, which you can certainly do with neural networks for image segmentation.

You'd use AI to analyse an image and identify its different components such as faces, objects, text, etc. Convolutional Neural Networks are quite popular for image segmentation because they can identify and process image data much quickly and efficiently. The experts at Facebook AI Research (FAIR) created a deep learning architecture called Mask R-CNN which can make a pixel-wise mask for every object present in an image. It is an enhanced version of the Faster R-CNN object detection architecture. The Faster R-CNN uses two pieces of data for every object in an image, the bounding box coordinates and the class of the object. With

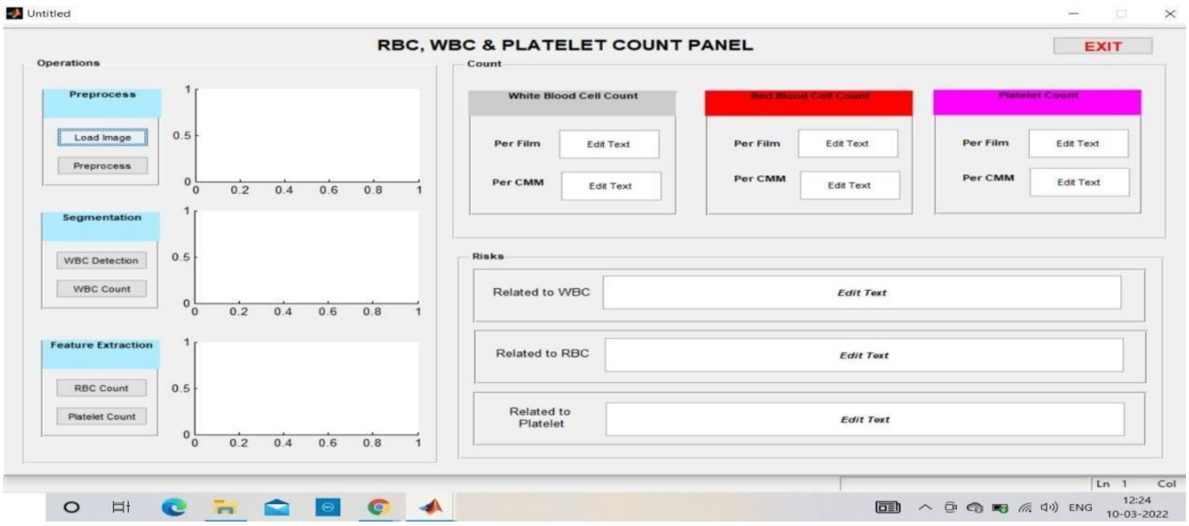
Mask R-CNN, you get an additional section in this process. Mask R-CNN outputs the object mask after performing the segmentation.

A convolutional neural network (CNN) is a class of deep neural networks used in image recognition problems Coming to how CNN works, the images given as input must be recognized by computers and converted into a format that can be processed. For this reason, images are first converted to matrix format. The system determines which image belongs to which label based on the differences in images and therefore in matrices. It learns the effects of these differences on the label during the training phase and then makes predictions for new images using them. CNN consists of three different layers that are a convolutional layer, pooling layer, and fully connected layer to performthese operations effectively. The feature extraction process takes place in both convolutional and pooling layers. On theother hand, the classification process occurs in fully connected layer. These layers are examined sequentially in the following.

**Convolutional Layer:**

Convolutional layer is the base layer of CNN. It is responsible for determining the features of the pattern. In this layer, the input image is passed through a filter. The values resulting from filtering consist of the feature map. This layer applies some kernels that slide through the pattern to extract low- and high-level features in the pattern [43]. The kernelis a 3x3 or 5x5 shaped matrix to be transformed with the input pattern matrix. Stride parameter is the number of steps tuned for shifting over input matrix**.**

**Result:**



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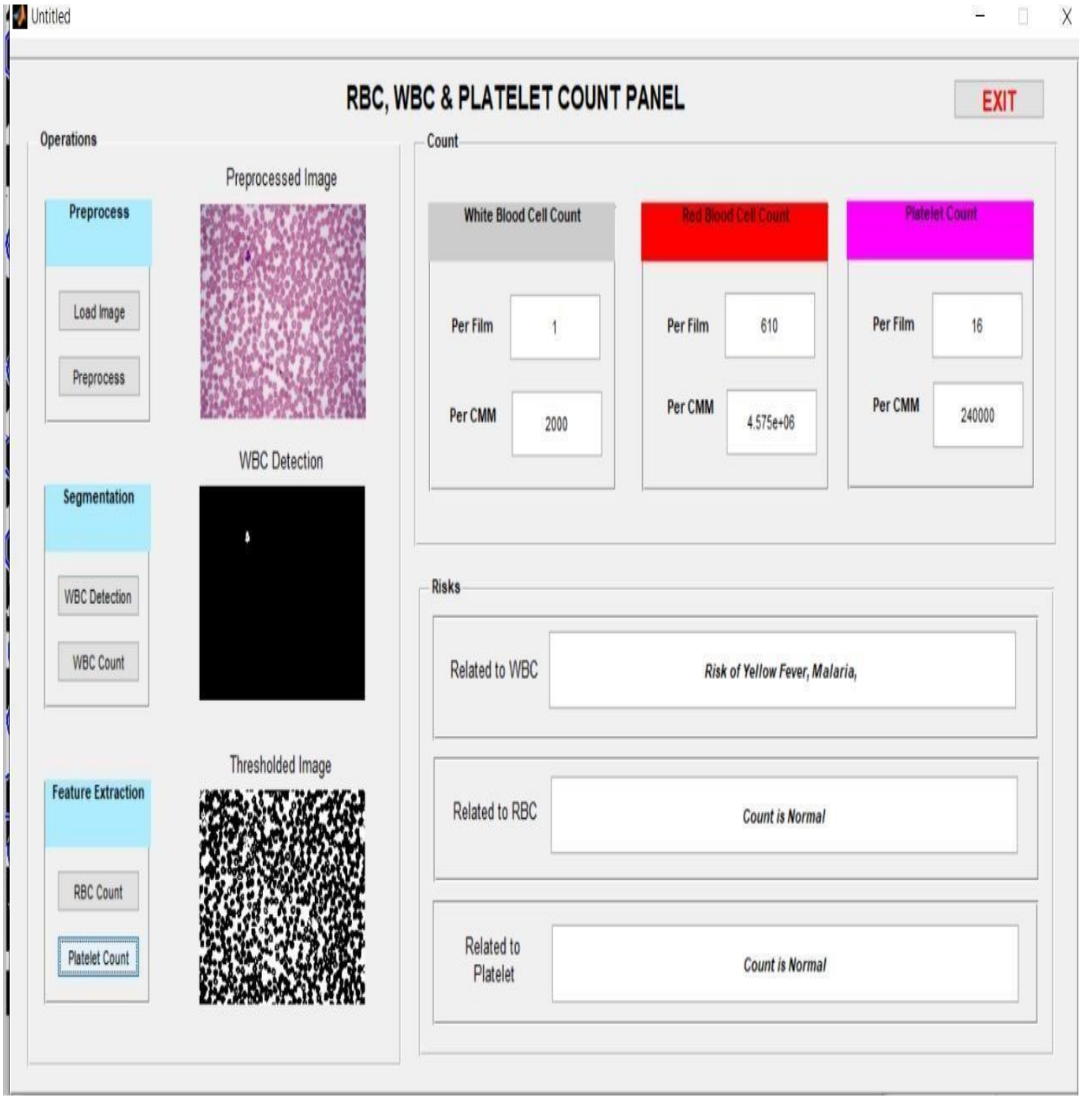


**CONCLUSION**

As a conclusion, this project successfully uses various image processing techniques for Red Blood Cell Detection. It utilizes morphological approaches for segmentation, extraction and detection in order to solve problem in image processing of the red blood cells. The results of the image act as an accurate outcome of determining the number of red blood cells by using Circular Hough transforms technique.

It proposes an image processing system that uses MATLAB software for blood cell counting. By using the MATLAB, all the importance's aspects of a correct algorithm has been successfully produced. With a correct algorithm, the red blood cells can be detected and segmented as well as estimate the number of the red blood cells. It enables the study of the morphological features of RBC by the pathologist can determine whether the person is normal referring the amount of RBC in human blood. Actual volume of the blood sample is calculated with proper magnification factor.

There is a need for fast and cost-effective production of blood cellcount reports. This system includes an effective and efficient methodin recognizing and counting blood cells as a practical alternative to the manual blood cell counting.

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