Segmentation using Morphological Watershed Transformation for Counting Blood Cells

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ABSTRACT
Blood cell counting is a major field of study in biomedical engineering. In human blood cell segmentation cases, many methods are being studied and applied for obtaining better results. In this paper, we present an approach for counting different blood cells during blood smear test. The approach which is presented in this paper is by segmentation using morphological watershed transformation. Morphological operations are used for creating masks and marker-based watershed transform is used for segmentation of cells. Simulation results of counting red blood cells (RBCs), white blood cells (WBCs) and platelets in a blood smear test image are also presented. The simulations are done on MATLAB®.

Keywords
Biomedical image processing, Marker-based Watershed Segmentation, Morphological Image Processing, counting blood cells.

1. INTRODUCTION

The use of image processing helps to improve the image quality and analysis approach in different application. It improves the efficiency of the analysis in all dimensions. Processing images has a broad spectrum of application such as remote sensing via satellite and other spacecrafts, image transmission and storage, medical, radar, sonar and acoustic image processing, robotics and automated inspection of industrial products. As an important image processing technique, image segmentation plays a key role in practical applications such as medical science, industry and so on. The purpose of image segmentation is to segment images which have different characteristic tissues into different regions and extract interest objects.

For medical image segmentation, it is very important for the clinical diagnosis and quantitative analysis to segment the interesting medical image accurately. The complexity and diversity of medical images, uniform gray-scale intensity features, and the fact that image itself is also easily influenced by noise or other factors make medical image segmentation quite difficult; therefore, there is a need for a systematic medical image segmentation method. Many image segmentation techniques have been proposed. It is necessary to research medical image segmentation methods for medical applications area. Out of all those techniques, watershed transform serve the best because it guarantees the identification and formation of closed contour of objects and is best-known in gray images segmentation [9]. Current research is going on blood counting application in the image segmentation. It refers to an implementation of automated counting for blood cell which is manually done by hematocytometer by using counting chamber. Blood counting is synonym with the complete blood count or CBC which refers to compilation test of red blood cell (RBC), white blood cell (WBC), platelet, hemoglobin and hematocrit. Each of them has their role in the body system and the counting result is important to determine the capability or deficiency of the body system. In short, any abnormal reading of CBC can give a sign of infection or disease. For example, the present of bacterial infection is diagnosed from increasing WBC count. Plus, specific low vitamin may come from a decreased RBC and thrombocytopenia is referring to low platelet count. The result can influence physician to make the best response and monitor the drug effectiveness from the blood count. The implementation of image processing in blood cell image has bring a new idea how to decrease the cost for a clinical decision and at the same time give a reliable result. This is because it is only the software based improvement compared to other method which using highly cost hardware to perform blood cell counting [8].

In this paper, the counting of blood cells is done with the help of multiple image processing techniques. These techniques include spatial filtering, morphological operations and segmentation based on watershed transformation. The combination of Ycbcr color conversion and morphological operator produce segmented white blood cell (WBC) nucleus as well as platelets and hence can be segmented for counting. Then it is being used as a mask to remove WBC from the blood cell image and process of counting is initiated. Each technique is explained in different sections

In section II, complete blood count (CBC) is discussed. It includes the types of cells included in counting procedure, requirement of counting cells, and normal blood count for adults. In section III, an overview of various works in the field of blood cell image processing and counting is provided. Section IV provides a comprehensive knowledge of various morphological operators used in this paper. Section V is associated with the study of various techniques used for segmentation and their classification. It also includes information about the type of segmentation used in this paper i.e., watershed segmentation. We have also discussed the problem associated with this type of segmentation as well as the remedy. Section VI is all about the step by step methodology followed to count the blood cells. Section VII concludes our paper.

2. COMPLETE BLOOD COUNT
The Complete Blood Count (CBC) is a test that evaluates the cells that circulate in blood. Blood consists of three types of cells suspended in fluid called plasma: white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs) as

shown in figure 1. They are produced and mature primarily in the bone marrow and, under normal circumstances, are released into the bloodstream as needed.

A standard CBC includes the following [1]:

1) Evaluation of white blood cells: WBC count; may or may not include a WBC differential.

2) Evaluation of red blood cells: RBC count, hemoglobin (Hb), hematocrit (Hct) and RBC indices, which includes mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and sometimes red cell distribution width (RDW). The RBC evaluation may or may not include reticulocyte count.

3) Evaluation of platelets: Platelet count; may or may not include mean platelet volume (MPV) and/or platelet distribution width (PDW).

Additionally, WBC differential count typically includes counting the divisions of WBCs which are neutrophils, lymphocytes, monocytes, esosinophils, and basophiles. The results of a CBC can provide information about not only the number of cell types but also can give an indication of the physical characteristics of some of the cells.

The complete blood count (CBC) is often used as a broad screening test to determine an individual's general health status. It can be used to:

1) Screen for a wide range of conditions and diseases.

2) Monitor the condition and/or effectiveness of treatment after a diagnosis is established.

3) Monitor treatment that is known to affect blood cells.

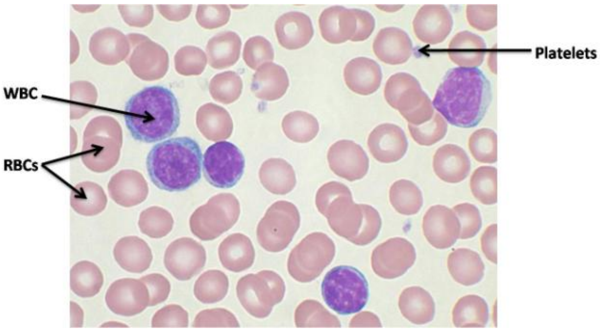


Fig 1: An image of blood smears showing Red Blood Cells, White Blood Cells and Platelets.

Normal complete blood count results for adults [2] are shown in table 1.

Table 1. NORMAL COMPLETE BLOOD COUNT RESULTS FOR ADULTS

Cell Type	Gender	
	Male	Female
RBC	4.32-5.72 million cells/uL	3.90-5.03 million cells/uL
Hemoglobin	135-175 grams/L	120-155 grams/L
Hematocrit	38.8-50.0 percent	34.9-44.5 percent
WBC	3,500 to 10,500 cells/uL	
Platelet	150,000 to 450,000 cells/uL	

uL- microliters

3. PROCESSING BLOOD CELL IMAGE

Several works have been done in the field of processing the blood cell image and counting methods. Recent works on this area are mainly focus in segmenting WBC, RBC and platelet. From the segmentation, some studies will use it to perform blood cell counting and classify abnormalities in the cell.

Use of morphological approach for 3D blood cell visualization can be seen in [7]. The 3D plotted image is obtained from 2D image by combining 2D image space with the intensity value presented as third axis. 2D image detection techniques are commonly applied for blood cell analysis on image taken from microscopy or scanner sample which normally could be affected by noise. In order to enhance the detection of blood cell for 2D image and to obtain the clearest view of the blood cell, 3D image detection, which is more flexible and accurate, is proposed. This approach helps to obtain a clear image for disease analysis. A color image taken from microscopy or scanner has to gone through such image analysis using intensity, and edge operator to detect the blood cell disease, before 3D visualization can be done.

Active contours [3] have been used extensively for medical image registration as they display good smoothing properties which allow accounting for the discrete nature of the image and noise from the acquisition process. However, contours are generally difficult to initialize around the region of interest (ROI). The marker based watershed segmentation can segment unique boundaries from an image or stack of images, however it has no smoothing/generalization properties. Combining the two approaches results in a segmentation method which both solves the contour initialization and generalization problem.

A similar technique is illustrated in [8]for segmentation of red blood cells using masking and watershed algorithm. This paper presents an approach for red blood cell (RBC) segmentation which is a part of study to perform automated counting for RBC. The methods involve are Ycbcr color conversion, masking, morphological operators and watershed algorithm.

In WBC segmentation, current technique used is Gradient Vector Flow (GVF) snake algorithm [4] to segment the nucleus and Zack Thresholding to segment the cytoplasm. Fuzzy approach is also being proposed for classified pixel to Region of Interest (ROI). In advanced, another work is using Fuzzy C-Means (FCM) clustering repeatedly for sub image component. The same work by using sub image component for feature space clustering is done. It is able to have accuracy of 98.9% for nucleus segmentation and 95.3% for cytoplasm segmentation. Another advance work also is done to classify WBC automatically by computing the WBC images in term of area, major axis length over minor axis length, perimeter, circularity and ratio of areas between nucleus and cytoplasm. For RBC segmentation, recent study use neural network training to extract RBC from the blood cell image. Almost the same method is applied by using Pulse-Coupled Neural Network (PCNN) with auto wave characteristic to improve the segmentation. Neural network is continued to be used as a method in one study to compare Artificial Neural Network (ANN) Back projection with morphological processing of Connected Component Labeling to perform RBC counting.

4. MORPHOLOGICAL IMAGE PROCESSING

Morphology is a broad set of image processing operations that process images based on shapes. Morphological operations apply a structuring element to an input image, creating an output image of the same size. In a morphological operation, the value of each pixel in the output image is based on a comparison of the corresponding pixel in the input image with its neighbors. The number of pixels added or removed from the objects in an image depends on the size and shape of the structuring element used to process the image. All morphological processing operations are based on two simple ideas, hit and fit. Fit stands for the condition when all pixels in the structuring element cover on pixels in the image whereas hit signifies the condition when any of the pixels in the structuring element covers on a pixel in the image. The different morphological operators used are discussed below.

4.1 Dilation

Dilation of an image F by structuring element S is given by F⊕S. The structuring element S is positioned with its origin at (x, y) and the new pixel value is determined using the rule:

$$g(x,y) = \begin{cases} 1 \ if\ S\ hits\ F \\ 0\ otherwise \end{cases} \qquad (1)$$

Dilation adds pixels to the boundaries of objects in an image. It can be used to repair breaks or intrusions i.e., it is used to enlarge objects. An illustration of dilation is shown in figure 2.

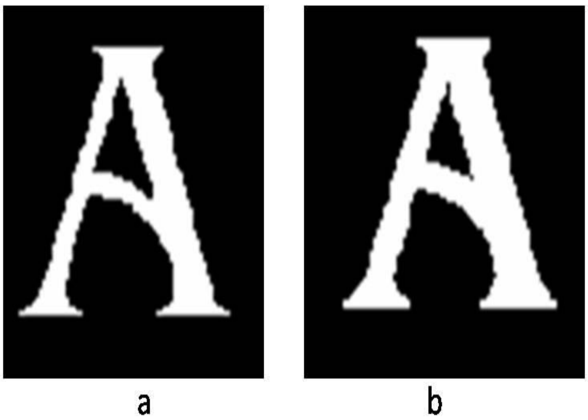


**Fig 2: An Illustration of Morphological Dilation.
a) Original image b) Dilation by 3*3 square structuring element**

4.2 Erosion

Erosion of an image F by structuring element S is given by F⊖S. The structuring element s is positioned with its origin at (x, y) and the new pixel value is determined using the rule:

$$g(x,y) = \begin{cases} 1 \ if\ S\ fits\ F \\ 0\ otherwise \end{cases} \qquad (2)$$

Erosion removes pixels on object boundaries in an image. It can be used to split apart joined objects or strip away extrusions i.e., it is used to shrink objects. An illustration of erosion is shown in figure 3.

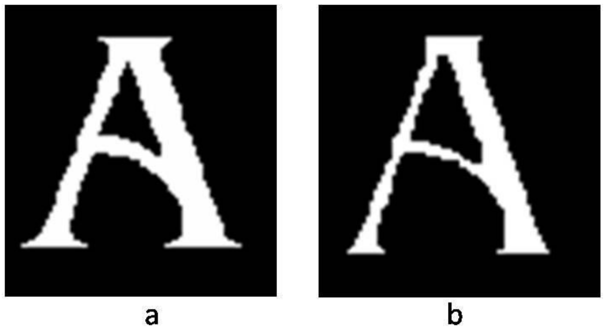


**Fig 3: An Illustration of Morphological Erosion.
a) Original image b) Erosion by 3*3 square structuring element**

4.3 Opening

The opening of image F by structuring element S denoted by F ○ S is simply erosion followed by dilation.

$$F \circ S = (F \ominus S) \oplus S \qquad (3)$$

Opening can be used for eliminating protrusions, breaking necks and smoothening contours. An illustration of opening is shown in figure 4.

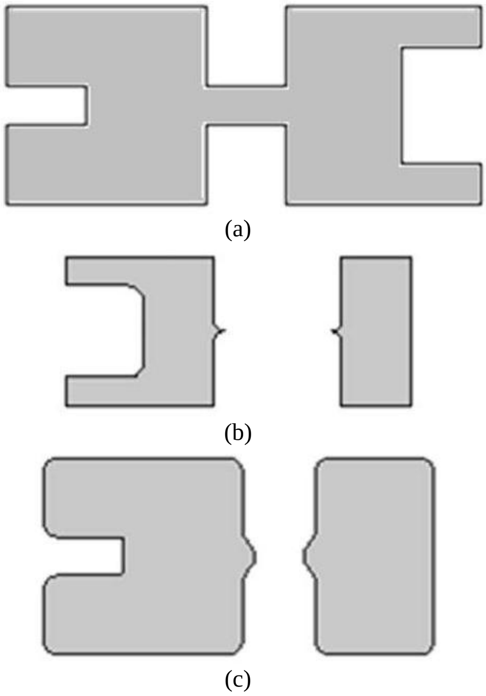


**Fig 4: An Illustration of Morphological Opening.
a) Original image. b) After Erosion. c) After Dilation.**

4.4 Closing

The closing of image F by structuring element S, denoted by F S is simply dilation followed by erosion.

$$F \bullet S = (F \oplus S) \ominus S \qquad (4)$$

Closing can be used for fusing narrow breaks and long thin gulfs, eliminating small holes, filling gaps in the contour and smoothening contours. An illustration of closing is shown in figure 5.

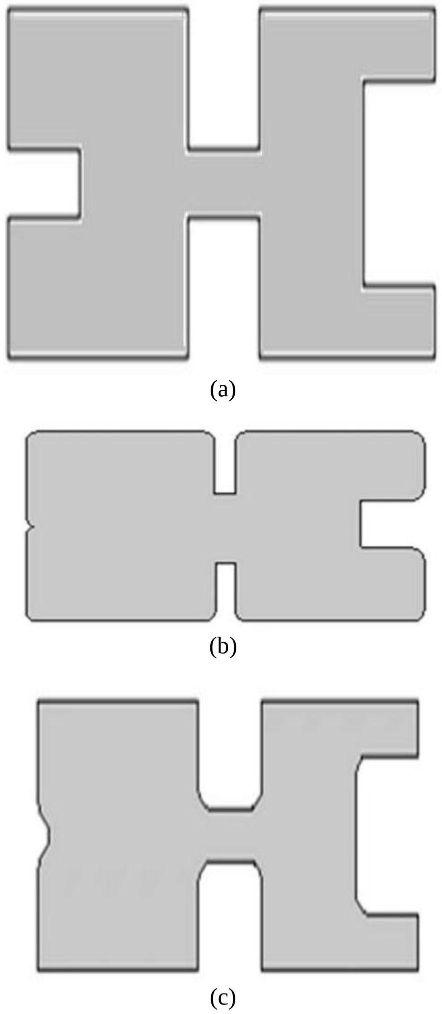


Fig 5: An Illustration of Morphological Closing. a) Original image. b) After Dilation. c) After Erosion.

4.5 Reconstruction

Morphological reconstruction is a useful but little-known method for extracting meaningful information about shapes in an image. It involves two images and a structuring element (instead of a single image and structuring element). One image, the marker, is the starting point for the transformation. The other image, the mask, constrains the transformation. If G is the mask and F is the marker, the reconstruction of G from F, denoted $R_G(F)$, is defined by the following iterative procedure:

1. Initialize h1 to be the marker image, F.
2. Create the structuring element: B = ones(3).
3. Repeat: $h_{k+1} = (h_k \oplus B) \cap G$ until $h_{k+1} = h_k$.
4. $R_G(F) = h_{k+1}$. (Marker F must be a subset of G.)

Morphological reconstruction is used along with erosion to give an improved opening procedure, opening by reconstruction, which is used to restore the original shapes of the objects that remain after erosion.

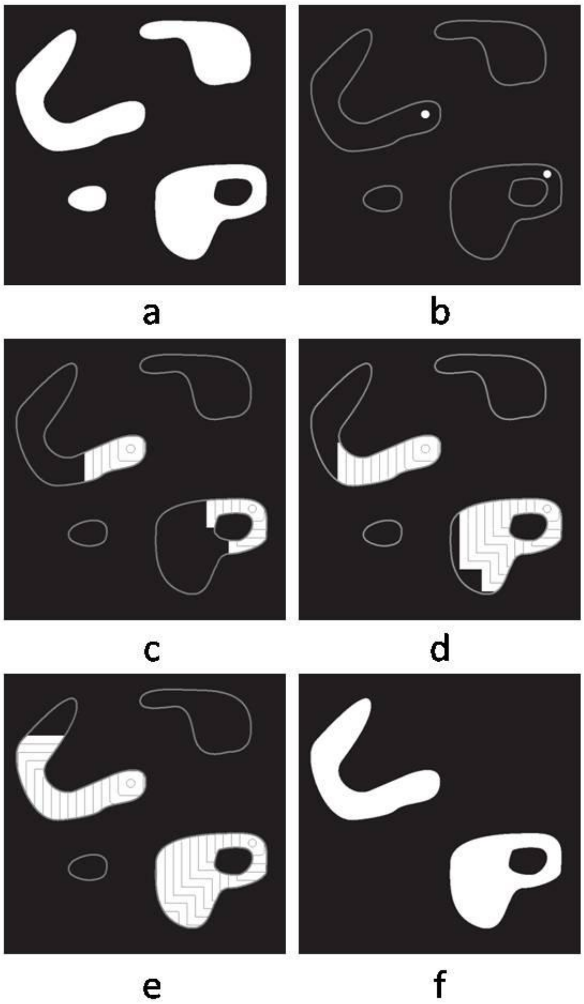


Fig 6: An Illustration of Morphological Reconstruction. a) Mask. b) Marker. c) Intermediate Result (100 iterations). d) Intermediate Result (200 iterations). e) Intermediate Result (300 iterations). f) Final Result.

5. IMAGE SEGMENTATION

Image segmentation is an essential process for most subsequent image analysis tasks. In particular, many of the existing techniques for image description and recognition, image visualization, and object based image compression highly depend on the segmentation results.

The general segmentation problem involves the partitioning of a given image into a number of homogeneous segments (spatially connected groups of pixels), such that the union of any two neighboring segments yields a heterogeneous segment. Alternatively, segmentation can be considered as a pixel labeling process in the sense that all pixels that belong to the same homogeneous region are assigned the same label. There are several ways to define homogeneity of a region based on the particular objective of the segmentation process. However, independently of the homogeneity criteria, the noise corrupting almost all acquired images is likely to prohibit the generation of error-free image partitions. Many techniques have been proposed to deal with the image segmentation problem. They can be broadly grouped into the following categories.

Histogram-Based Techniques: The image is assumed to be composed of a number of constant intensity objects in a well-separated background. The image histogram is usually considered as being the sample probability density function of a Gaussian mixture and, thus, the segmentation problem is reformulated as one of parameter estimation followed by pixel classification. However, these methods work well only under very strict conditions, such as small noise variance or few and nearly equal size regions. Another problem is the determination of the number of classes, which is usually assumed to be known. Better results have been obtained by the application of spatial smoothness constraints.

Edge-Based Techniques: The image edges are detected and then grouped (linked) into contours/surfaces that represent the boundaries of image objects. Most techniques use a differentiation filter in order to approximate the first order image gradient or the image Laplacian. Then, candidate edges are extracted by the gradient threshold or Laplacian magnitude. During the edge grouping stage, the detected edge pixels are grouped in order to form continuous, one-pixel wide contours as expected. A very successful method was proposed by Canny according to which the image is first convolved by the Gaussian derivatives, the candidate edge pixels are isolated by the method of non-maximum suppression and then they are grouped by hysteresis thresholding. The method has been accelerated by the use of recursive filtering and extended successfully to 3D images. However, the edge grouping process presents serious difficulties in producing connected, one-pixel wide contours/surfaces.

Region-Based Techniques: The goal is the detection of regions (connected sets of pixels) that satisfy certain predefined homogeneity criteria. In region-growing or merging techniques, the input image is first tessellated into a set of homogeneous primitive regions. Then, using an iterative merging process, similar neighboring regions are merged according to a certain decision rule. In splitting techniques, the entire image is initially considered as one rectangular region. In each step, each heterogeneous image region of the image is divided into four rectangular segments and the process is terminated when all regions are homogeneous. In split-and-merge techniques, after the splitting stage a merging process is applied for unifying the resulting similar neighboring regions. However, the splitting technique tends to produce boundaries consisting of long horizontal and vertical segments (i.e., distorted boundaries). The heart of the above techniques is the region homogeneity test, usually formulated as a hypothesis testing problem.

Markov Random Field-Based Techniques: The true image is assumed to be a realization of a Markov or Gibbs random field with a distribution that captures the spatial context of the scene. Given the prior distribution of the true image and the observed noisy one, the segmentation problem is formulated as an optimization problem. The commonly used estimation principles are maximum a posteriori (MAP) estimation, maximization of the marginal probabilities (ICM) and maximization of the posterior marginals. However, these methods require fairly accurate knowledge of the prior true image distribution and most of them are quite computationally expensive.

Hybrid Techniques: The aim here is offering an improved solution to the segmentation problem by combining techniques of the previous categories. Most of them are based on the integration of edge- and region-based methods. In, the image is initially partitioned into regions using surface curvature sign and, then, a variable-order surface fitting iterative region merging process is initiated. In, the image is initially segmented using the region-based split-and-merge technique and, then, the detected contours are refined using edge information. In, an initial image partition is obtained by detecting ridges and troughs in the gradient magnitude image through maximum gradient paths connecting singular points. Then, region merging is applied through the elimination of ridges and troughs via similarity/dissimilarity measures.

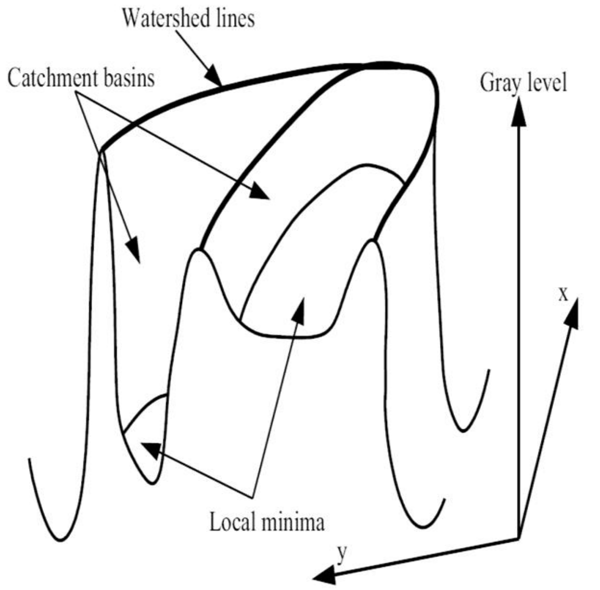
The algorithm proposed in this paper belongs to the category of hybrid techniques, since it results from the integration of multiple techniques through the morphological watershed transform. Many morphological segmentation approaches using the watershed transform have been proposed in the literature [23], [24]. When watershed transform is applied to any gradient image, image tessellation with a large number of primitive regions is produced. This initial over segmentation is due to the high sensitivity of the watershed algorithm to the gradient image intensity variations, and, consequently, depends on the performance of the noise reduction algorithm. Over segmentation is reduced by marker controlled watershed transform.

5.1 Watershed Transform

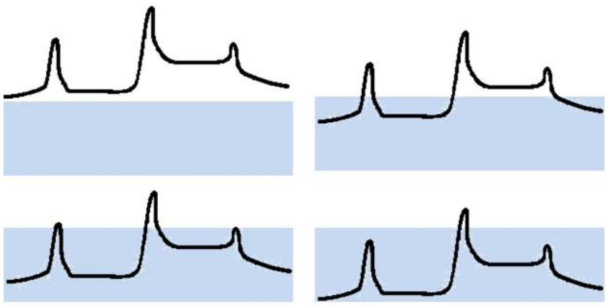
In grey scale mathematical morphology the watershed transform, originally proposed by Digabel and Lantuéjoul and later improved by Beucher and Lantuéjoul, is the method of choice for image segmentation [25]. The watershed transform can be classified as a region-based segmentation approach. The intuitive idea underlying this method comes from geography: it is that of a landscape or topographic relief which is flooded by water, watersheds being the divide lines of the domains of attraction of rain falling over the region. An alternative approach is to imagine the landscape being immersed in a lake, with holes pierced in local minima. Basins (also called `catchment basins') will fill up with water starting at these local minima, and, at points where water coming from different basins would meet, dams are built. When the water level has reached the highest peak in the landscape, the process is stopped. As a result, the landscape is partitioned into regions or basins separated by dams, called watershed lines or simply watersheds. An example of surface model for watershed transform is shown in figure 7 (a) and simulation of immersion showing the flooding of basins is also shown in figure 7 (b).

Watershed transform is defined and explained comprehensively in [25]. Watershed transform may be defined for continuous case as well as discrete case. For discrete case, watershed can be defined on the basis of immersion technique of topographical distance.

The advantages of watershed segmentation are threefold. Firstly, the results are connected regions with enclosed boundaries of single pixel wide, different from the traditional edge-based approaches generating disconnected contours. Secondly, the region contours adhere well to the real object boundaries. Furthermore, the combination of regions produced by watershed segmentation is equal to the entire image. Whereas, it is clear that some important drawbacks also exist. Watershed segmentation is sensitive to noise, the main reason leading to over-segmentation shown in figure 8. Besides, it doesn't work well in detection of thin structures and significant areas with low contrast boundaries.



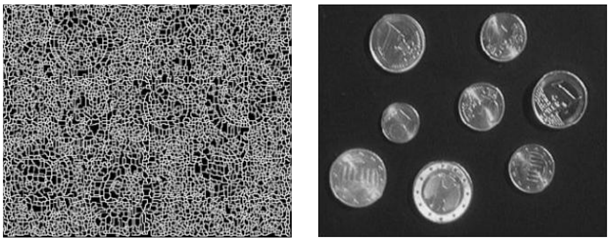
(a)



(b)

Fig 7: a) Surface model for watershed transforms. b) Immersion simulation of surface model.

Several approaches exist to remedy the over-segmentation problem, such as region merging algorithm, modified gradient algorithms, marker-controlled methods, multi-scale segmentation, and hierarchical segmentation. The main advantage of the marker controlled watershed method over other previously developed remedies in segmentation method is that it allows segmentation of particular objects and thus is ideal for counting applications.



(a) (b)

Fig 8: Problem of over segmentation.

Marker Based Watershed Segmentation

As already stated, watershed segmentation suffers from the problem of over segmentation. Markers based segmentation servers as an ideal solution to this problem. A marker is connected component belonging to an image. Selection of markers comprises of two steps. First the image is preprocessed and then a set a criterion bust be defined to select a marker. A common method for marker selection can be a morphological threshold operator. It can be achieved by a morphologic reconstruction with recursive erosion from the regional minima.

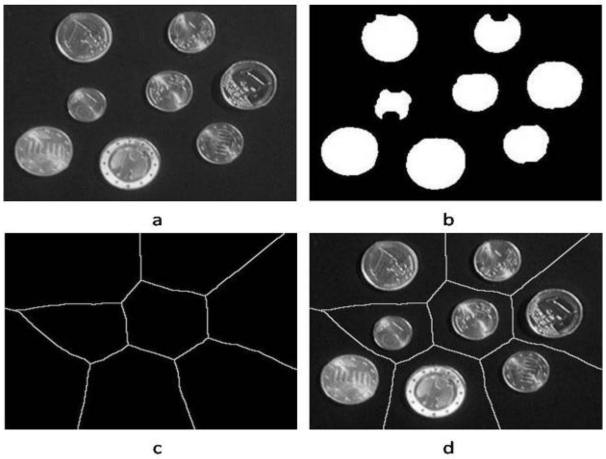


Fig 9: An illustration of marker controlled watershed segmentation.
a) Original Image. b) Marker image. c) Watershed lines computed using mask. d) Final result.

This technique is derived from the fact that a part of the problem that led to over segmentation was the large number of potential minima. Most of these minima can be eliminated due to their small size. To minimize the effect of small spatial details, two methods can be used. Image can be filtered with a smoothening filter such as Gaussian filter. However, morphological operators can achieve better results and are used in this paper. Finally, after creating the marker image, the watershed segmentation of the binary image showing the watershed lines can be super imposed on the original image.

6. METHODOLOGY

In this section, the algorithm for actual blood cell segmentation is proposed. Highly magnified image of blood smear is used of segmentation purpose shown in figure 9. The algorithm is implemented in MATLAB software. The flow diagram for the whole process is shown in figure 10. The image is processed using various techniques discussed in subsequent sections for segmentation and counting purpose.

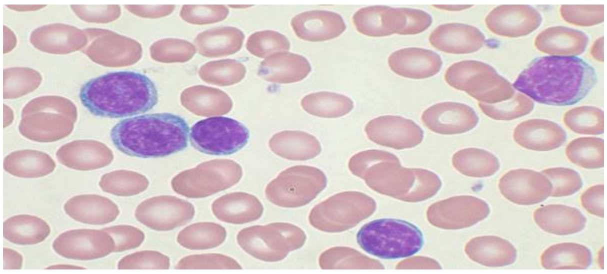


Fig 9: Image used for segmentation purpose.

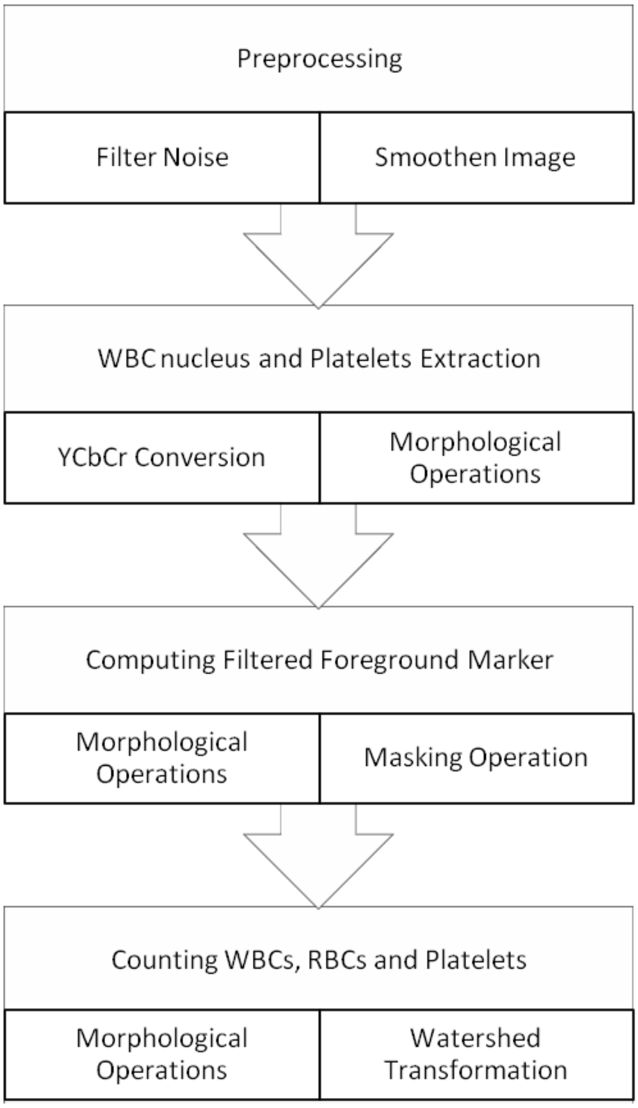


Fig 10: Flowchart of proposed algorithm.

6.1 Preprocessing

In image processing step, the image is enhanced in term of quality level to be prepared for the next process. Average filter is applied on the image to remove any random noise and smoothen it. General equation to filter M X N image f(x,y) with filter size of m x n, w(s,t) is given below.

$$g(x,y) = \frac{\sum_{s=-a}^{a} \sum_{t=-b}^{b} w(s,t) f(x+s, y+t)}{\sum_{s=-a}^{a} \sum_{t=-b}^{b} w(s,t)} \qquad (5)$$

A 3x3 average filter has been used as it is the smallest mask and it can filter any random noise in small area. This will diminish non important detail from the image.

6.2 Color Conversion

YCbCr format is the most common format used in video and digital photography systems. Y is the luminance component, Cb is the blue difference and Cr is the red difference chrominance component. RGB color format is converted to Ycbcr format will be based on the formula on the equation 2.0. The Ycbcr color representation can be used to overcome the illumination issue which often occurs in blood cell microscopic image. The second component i.e., Cb component of the Ycbcr color has been chosen and it shows the clear appearance of the WBC nucleus and platelets as shown in figure 11.

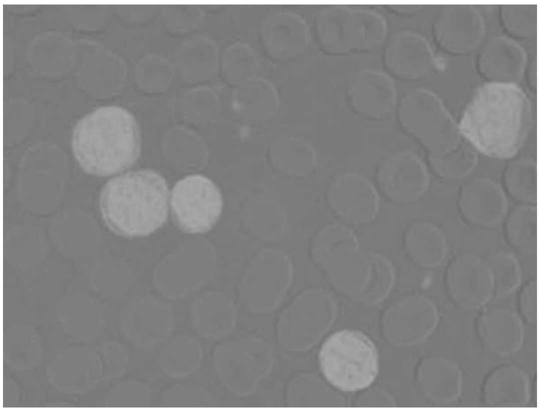


Fig 11: Second component of YCbCr image.

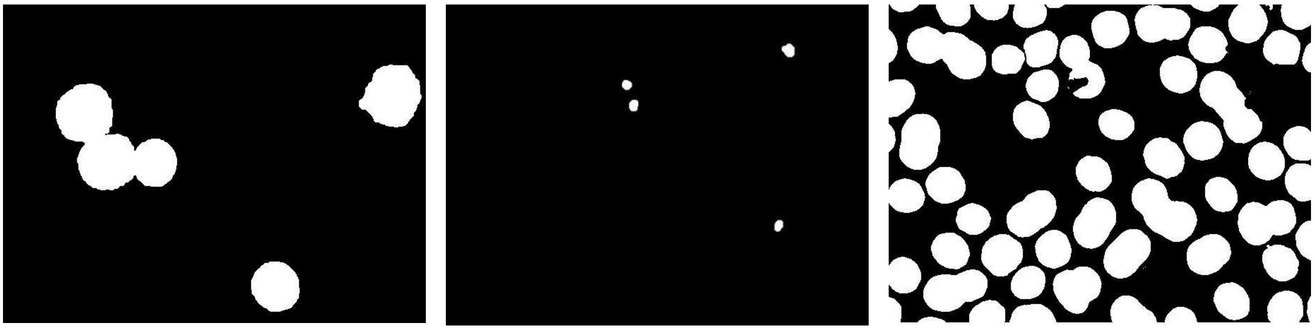
6.3 Morphological Operations

As already discussed, morphological image processing is based on a strong mathematical concept which is used to change the size, shape, structure and connectivity of objects in the image. It involves various binary and grayscale operations. The operations used are erosion, dilation, opening, closing and reconstruction. All of these have already been discussed in previous sections.

To obtain WBC mask, the image in figure 8 is converted to binary image on which the morphological opening operation is applied. The resulting mask containing both WBCs and platelets is obtained. Now, with another opening and image subtraction, individual images for WBCs and platelets can be obtained. It is to be noted here that the application of opening or closing operation on image may distort the original shape of the objects in the image. So, a slightly modified version of opening, called opening by reconstruction is used in this step.

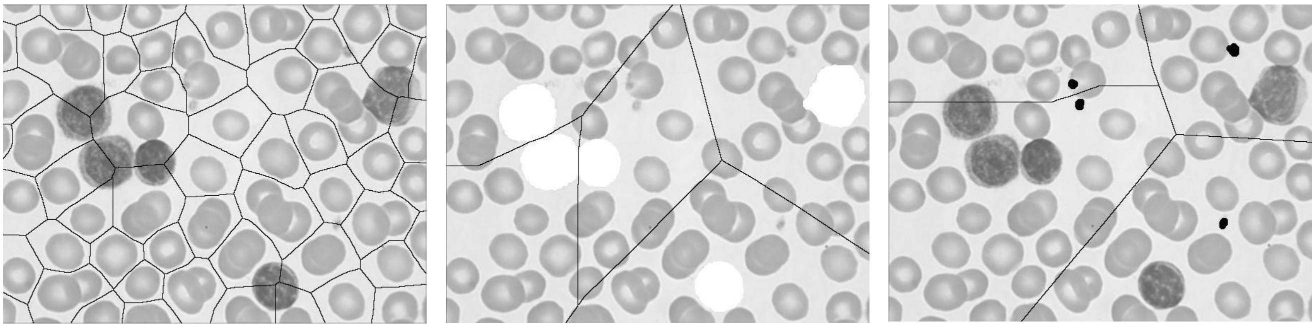
Next, the blood smear image in figure 9 is converted to grayscale and opening by reconstruction operation is carried out instead of conventional opening. In the first step, erosion is carried out and then instead of dilation, reconstruction operation is carried out. Similarly, closing by reconstruction of the image is carried out. Finally, the binary base image containing all the cells is obtained and the mask is subtracted from it to get RBC binary image.

Now, we have individual binary images each for RBCs, WBCs and platelets. Watershed transformation is applied on these images to obtain final count values.



a b c

Fig 11: a) WBC mask. b) Platelet mask. c) RBC mask.



a b c

Fig 12: Segmented images showing watershed lines. a) Segmented RBC image. b) Segmented WBC image. c) Segmented platelet image.

6.4 Segmentation

The binary images are segmented to separate each cell. Watershed transform is used for segmentation. Watershed segmentation is an effective approach in our case because the resulting boundaries form closed, connected regions, which are joined to form the entire image region. These boundaries known as watershed ridge lines are formed dividing the image into different regions and is superimposed on original image. The implementation of watershed segmentation using marker based approach, the problem of over segmentation is effectively eliminated. The results of segmentation are shown in figure 12.

7. CONCLUSION

In this paper, we have presented a highly efficient method for CBC. The masks for each type of cell can be efficiently obtained using color conversion and morphological operators. Marker based segmentation solves the problem of over segmentation associated with watershed transform. This method has been applied different images and it shows a quite similar result for all of the images. It could be observed that the segmentation of WBC and small particles like platelet play a big role to segment RBC by using masking operation. The WBC and platelets masks are themselves used for their segmentation. However, this technique is only capable of handling touched or small overlap in the image but unsuccessful for a big overlap. Further study in this field may improve the segmentation accuracy and provide better results.

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