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White Blood Cell Analysis Using Watershed and Circular Hough Transform Technique

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Abstract **- In medical diagnosis, blood cell counts play very important role. The major issue in clinical laboratory is to produce a precise result for every test especially in the area of blood cell count. The number of blood cell is very important to detect as well as to follow the treatment of many diseases like anemia, leukemia etc. Blood cell count gives the vital information that helps in the diagnose of many of the patient's sickness. The old conventional method of Blood cell counting under microscope gives an unreliable and inaccurate result depending on clinical laboratory and technician skill. This paper presents complete and fully automatic method for White Blood Cell (WBC) analysis from microscopic blood cell images. The whole work has been developed using MATLAB environment.**

Keywords - Microscopic Blood cells image, Segmentation, Watershed Algorithm, Circular Hough Transform, WBC count.

I. INTRODUCTION

Cancer is one of the most feared diseases by human. Leukemia occurs when a lot of abnormal white blood cells are produced by bone marrow. Leukemia is a type of blood cancer, and it is detected late, it will result in death. Abnormal increase in WBC disrupts the balance of the blood system. For someone who has leukemia, bone marrow produces abnormal WBC. A large number of medical images in digital format are enerated by hospitals and medical institutions every day. The main purpose of the blood cell image analysis is differentiating the components of blood and counting of Red Blood Corpuscles (RBCs), White Blood Corpuscles (WBCs) and platelets by observing blood cell and also detecting various diseases. For example an image may contain up to 100 red cells and only 1 to 3 white cells. Abnormal high or low counts may indicate the presence of many form of disease, since blood counts are amongst the most commonly performed blood test in medicine. WBC reveals important diagnostic information about the patients. In blood cancer diagnosis, automatic detecting of WBC for manually locating, identifying and counting cells is an important role. Various types of cells in normal human blood are Red Blood Corpuscles, White Blood Corpuscles and Blood platelets. RBCs are simple and similar. WBC contains nucleus and cytoplasm. By identifying the WBCs, it also provided some information about the abnormal condition in our body. The proposed method provides a software-based cost effective and an efficient alternative in detecting and counting WBCs. Image processing techniques help to count the cells in the human blood and, at the same time, provide information of the cell morphology. In this proposed work, ten microscopic human blood cell images have been analyzed and the number of WBC counted accurately for detecting abnormalities in human health. The rest of this paper is organized as follows: Literature review is discussed in Section II, proposed system with block diagram is discussed in Section III, which is followed by test results and discussions in Section IV, and the conclusion in Section v.

II. LITERATURE REVIEW

Hiremath [1] used different techniques for segmentation i.e. segmentation using k-mean clustering followed by Expectation Maximization algorithm.. Farnoosh sadeghian et al. [2] has proposed a framework for white blood cell segmentation using active contour, snake algorithm and zack thresholding to extract nucleus and cytoplasm region in white blood cells. Yujie, Yuhki and Kohei [3] have proposed morphological methods to detect cancer cells. Vinod and kimbahune, neleh [4] has proposed counting and segmenting for various blood cells. Poomeokrakl and Neatpisamvanit [5] have proposed red blood cell counting and extraction. Fatin A.Dawood [6] has presented an unsupervised segmentation of microscopic white blood cells images using histogram equalization technique to segment nucleus. Navi D Jambhekar [7] has proposed Red Blood cell classification using Support vector machine. Nasrul and Muhammad [8] identify the red blood cells using Circular Hough transform technique. Heidi Berge [9] has proposed the segmentation of red blood cells in a thin blood smear image which is based on the Zack's Method. Guitao et al. [10] proposed the hough transform in detecting and extracting the red blood cells in the urine micrograph. Kendall preston [11] discussed the state of

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the art in blood smear analysis automation and in other related areas including mulit-iresolution microscopy. Nicola Ritter [12] has proposed Otsu adaptive thresholding and watershed transform for segmenting and border identification of cells in image of peripheral blood smear slides. Roy Dimayuga et.al [13] used the histogram thresholding to distinguish the nucleus of the leukocyte or white blood cells from the rest of the cells in the image. Madhuri and Patil [14] has proposed the otsu adaptive thresholding in segmenting and extracting the WBC in the blood cell image based on thresholding. Sumeet [15] has proposed a method of RBC counting using watershed segmentation algorithm and determined the number of red blood cells using Circular Hough Transform technique. Nur Alom [16] used wavelet transformation for segmenting the different cells of blood and finally fuzzy inference system for final decision of blood cancer based on count of various cells. Fauziah kasmin et al. [17] has proposed detection of leukemia in microscopic image. Sharif J.M et al. [18] presented an approach for RBC segmentation using making and watershed algorithm to perform automated counting RBC. Sharif and Miswan [19] has proposed red blood cell segmentation using watershed and masking to segment the red cells. Dwi Anoraganingrum [20] described an automated cell tracking using median filter and morphological operations.

III. PROPOSED WORK

This work aims to apply image processing techniques to extracted white blood cells in blood smear microscopic images. Further automatically count the number of white blood cells. To identify and discover the blood cells from each other. The proposed method, the RGB images converted to gray level images. After getting gray scale image to classifying the image gray-level pixels may reduce and some image processing operations such as edge detection using sobel operator, image smoothing using median filtering, unsharp masking, and watershed are applied. Finally, morphological operations and Circular Hough Transform are used for final count. The Acquired blood cell image is preprocessed, enhanced, segmented and morphological operations are applied in order to count the WBCs. Figure 3.1 shows proposed method to count WBC in blood cell image

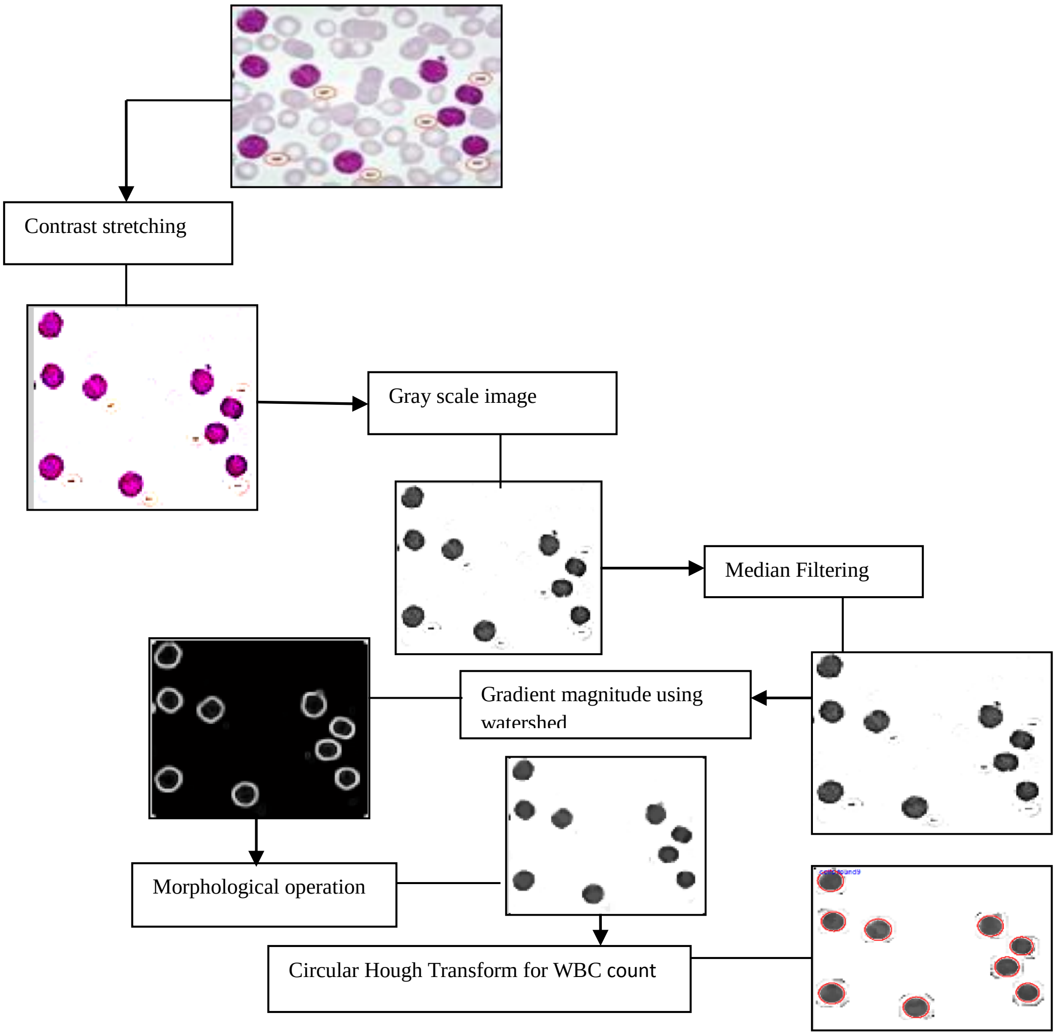


Figure 3.1 Proposed methods to count WBC in Blood Cell Image

A. Image Acquisition

The first step in the process is image acquisition i.e., to acquire a digital image. It requires an image sensor and the capability to digitize the signal produced by the sensor. Figure 3.1 shows the acquired blood cell image for further processing and analysis at a resolution of 120 × 90.

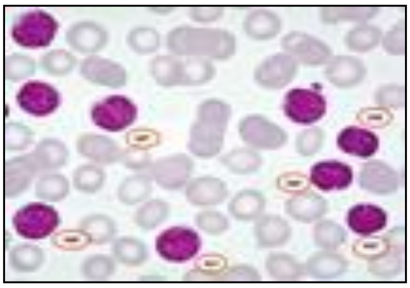


Figure 3.1 Blood smear image

B. Image Preprocessing

After the digital image has been obtained, the next step deals with pre-processing of that image. In the acquired image, the color of the entire blood element and the background color appear to be similar. Further RBC and WBC are grouped with blood platelets in the presence of noise and stain in blood slides. Hence preprocessing helps to overcome or reduce noise in the image. The gray level intensity of WBC is darker compared to the RBC. The blood plasma and dust particles in blood smear image are cleaned by removing all other blood particles. Figure 3.2, shows contrast stretching on blood cell image

To change the contrast or brightness of an image, the adjust contrast tool performs contrast stretching. In this process, pixel values below a specified threshold value are displayed as black, pixel values above a specified value are displayed as white, and pixel values in between these two values are displayed as shades of gray. The result is a linear mapping of a subset of pixel values to the entire range of grays, from black to white, producing an image of higher contrast.

Original blood cells images are in color. After contrast stretching, the image is converted into Grayscale image. Grayscale represents the intensity of the image, while converting to grayscale image to ease the process of ratio determination so the contrast image converted into grayscale image. Grayscale image by eliminating the hue and saturation information while retaining the luminance. The gray level intensity in WBC is darker when compared to the RBC. In blood smear image are cleaned by removing other object such as plasma and blood particles. Figure 3.3 shows Grayscale image.

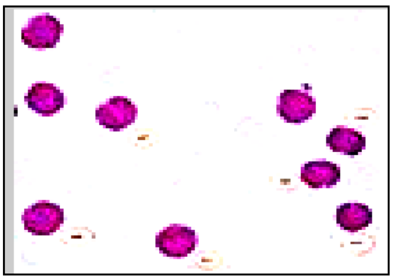


Figure 3 .2 Contrast Stretching

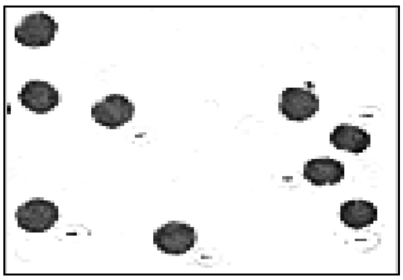


Figure 3.3 Gray-Scale image

C. Image Enhancement

After pre-processing, image enhancement is done. Image enhancement operations improve the quality of an image. They can be used to improve image's contrast intensification and brightness characteristics, reduce its noise cleaning or smoothing, content or sharpen and edge sharpening or crispening its details. Various image enhancement techniques are median filtering, wiener filtering, image negation, histogram plotting and image subtraction.

Median Filtering

Median filtering is more effective in image smoothening and preserve edges while removing noise. In this proposed work, median filtering techniques has been applying using the formula

$$v\,(m, n) = median\,\{y\,(m-k, n-l),\ (k, l)\ \in W\} \qquad (1)$$

Where W is a suitably chosen window. The algorithm for median filtering requires arranging the pixel values in the window in increasing or decreasing order and picking the middle value. N_w is even, then the median is taken as the average of the two values in the middle. N_w is odd, specify the window size.

2D median filtering example using a 3*3 sampling window:

Border values not changed

Sorted: 0 0 1 1 **1** 2 2 4 4

Input

1	4	0	1	3	1
2	2	4	2	2	3
1	0	1	0	1	0
1	2	1	0	2	2
2	5	3	1	2	5
1	1	4	2	3	0

Output

1	4	0	1	3	1
2	1	1	1	1	3
1	1	1	1	2	0
1	1	1	1	1	2
2	2	2	2	2	5
1	1	4	2	3	0

Steps for 2D-median filtering:

1. construct a window of size [m , n]
2. sort the values in the window
3. find the median value
4. keep the border values unchanged
5. replace the remaining values in the window with median value
6. slide the window

Figure 3.4 shows the image enhancement after applied median filtering.

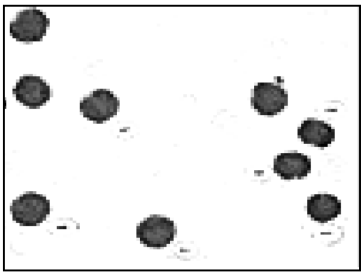


Figure 3.4 Median Filtering

D. Image segmentation

Image Segmentation partitions an input image into its constituent parts or objects. Various techniques used for segmentation are histogram, K-means clustering, Gram-Schmidt orthogonalization and snake algorithm. Different segmentation techniques are used by individual authors such as, combination of the watershed technique and a parametric deformable model, Hough transform techniques and so on. These methods are more complex and require more processing time in comparison with other methods. The advantage of this method is to provide more accurate result for segmentation.

Jianhua et al. [20] identified the cell segmentation for blood, edge detection performs poorly on cell images because not all boundaries are sharp and it is difficult to get all edge information and locate cells accurately. So, they developed an iterative Otsu's approach based on circular histogram for the leukocyte segmentation. Dwi Anoragaingrum [21] has proposed cell segmenting using median filtering and morphological operations are used. In this proposed work different techniques are used for segmentation i.e. segmentation using gradient magnitude. After applying gradient magnitude the segmentation and edge detection based on sobel operator. The borders of white blood cells are enhanced. The gradient is high at the borders of the objects and low (mostly) inside the objects. The unsharp masking technique is used to crispen the edges. A signal proportional to the unsharp or low-pass filtered, version of the image is subtracted from the gray image. This is equivalent to adding the gradient, or a high-pass signal, to the image. After applying gradient magnitude, marker controlled watershed algorithm is used to avoid over segmentation issue. When the operation of masking is applied, the masked image has diminished the red blood cells.

Unsharp masking operation can be represented as,

$$V(m, n) = u(m, n) + g\lambda(m, n) \quad (2)$$

Where $\lambda>0$ and g(m, n) is a suitably defined gradient magnitude at (m, n). Figure 3.5 shows the result obtained after applying segmentation algorithm.

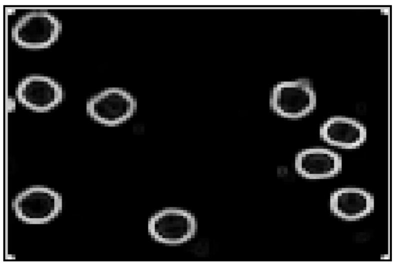
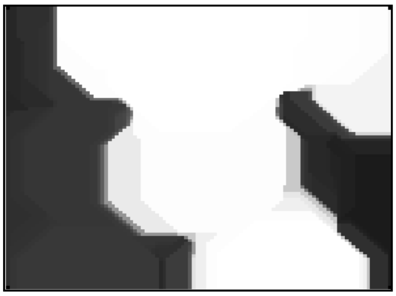


Fig. 3.5 Gradient Magnitude

D. Morphological operations

A morphological operation helps to detect exact shape of object. The data should be represented as a boundary or as a complete region. It refers to certain operations where an object is hit with a structuring element and thereby reduced to more revealing shape. To create a structuring element specified by a shapes structures like disk, disk-shaped approximation are suitable for computing cells. Disk-Shaped structuring element is approximated by specified radius from the origin of cells. Morphological operation includes erosion, dilation, filtering, and granulometry. In this proposed work morphological erosion and dilation had been applied to this image to eliminate small unwanted pixel and image smoothing. The erosion operation uniformly reduces the size of objects in relation to their background and dilation expands the size of objects. Besides dilation and erosion secondary operations like opening (erosion followed by dilation) and closing (dilation followed by erosion) can be applied on the image. Opening used to smooth the contours of cells and parasites; and closing used to fill the holes and gaps.

Figure 3.7 and figure 3.9 shows the results obtained after a series of morphological operations namely erosion and dilation. It is eliminate small unwanted pixel and for image smoothening. Perform the morphological area closing on the lower pixel image to fill the hole and the unwanted small pixels are eliminated. The dilation and area closing have been applied on higher pixel image.



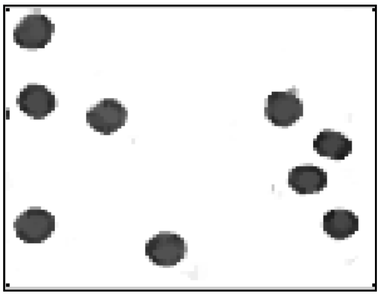
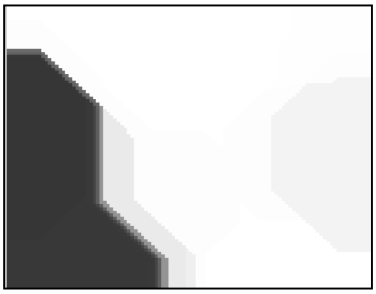


Figure 3.6 Opening

Figure 3.7 Erosion

Figure 3.6 and Figure 3.8 shows the area closing on the lower pixel image and higher pixel image using after morphological operation involving erosion and dilation, the WBC can be viewed accordingly



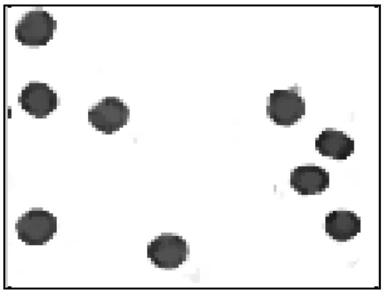


Figure 3.8 Closing

Figure 3.9 Dilation

E. WBC count

In blood cell analysis differential counts of RBCs and WBCs also have importance in order to diagnose of various diseases. In general, leukemia is thought to occur when some blood cells acquire mutations in their DNA. Certain abnormalities cause the cell to grow and divide more rapidly and to continue living when normal cells would die. Over time, these abnormal cells can crowd out healthy blood cells in the bone marrow, leading to fewer healthy blood cells and causing the signs and symptoms of leukemia. Myelogenous leukemia affects the myeloid cells.

Myeloid cells give rise to red blood cells, white blood cells and platelet-producing cells. The myelogenous leukemia cells grow more quickly. In this proposed work, to estimate the number of white blood cells in the blood smear image obtained after morphological operations is used as an input for Region of Interest (ROI) and Circular Hough transform process.

F. Circular Hough Transform

The Hough transform is able to detect analytically defined shapes such as straight line, circle, as well as the parameter curve. Circular Hough transform technique is able to detect and estimate the number of white blood cells by determining the center point and radius of circle is needed from original image and based on trails for each image. We found the radius by using Region of Interest. In this image the minimum radius is 2 and maximum radius is 15. Thus the Hough transform is applied when both radiuses are having determined and the result of this process is shown in Table. I.

Table I: Minimum and maximum radius based on 10 trails

Image	Region of Interest	
	Minimum Of Radius	Maximum Of Radius
Image 1	2	25
Image 3	2	15
Image 3	2	18
Image 4	2	15
Image 5	2	18
Image 6	1	35
Image 7	1	35
Image 8	2	15
Image 9	1	35
Image 10	1	35

IV. RESULT AND DISCUSSION

The European Leukemia Net is a publicly funded research network of excellence. Goal is cure of leukemia by integration of European leukemia research and spread of excellence. The website delivers information for physicians, patients (e.g. patient organizations in Europe), ongoing clinical trials and further information about the disease. This image set having 250 blood smear images samples. The proposed method was finally tested on 20 images of European Leukemia image set. Figure 4.1 shows the results of the blood cells images preprocessing applied to the blood cell image for blood cell counting. After getting grayscale image, applying thresholding method, and morphological operations are applied and the binary images of WBC for each blood cell images were obtained. Object pixels will have the value 1 and the background pixel will have the value of 0. There are small spots of objects appearing in the WBC background images.

The result of the image can be determining the number of blood cell by using Region of interest (ROI) and Circular Hough transform technique. The noise may be due to illumination or shadows that make region of interest (ROI) appear as blurred image region. After getting the binary image the final process is counting the number of white blood cells is to perform Circular Hough transform technique. White blood cells have been encircled by red circle and counting process refer to the number of red circles in the image

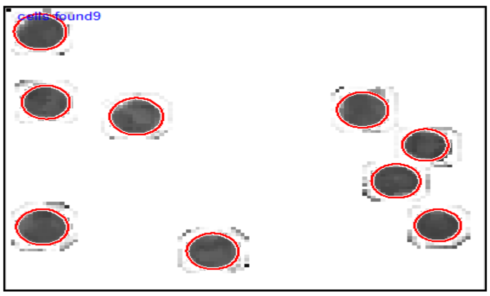


Figure 4.2 Counting of WBC

Table II: Performance of the proposed method

Image	Manual count	Auto count	Accuracy
Image 1	50	46	92
Image 2	9	9	100
Image 3	7	6	86
Image 4	7	7	100
Image 5	3	3	100
Image 6	23	17	74
Image 7	17	13	76
Image 8	13	13	100
Image 9	23	23	100
Image 10	14	14	100

The accuracy is measured based on final results produced by the algorithm to refer the manual counting process. Table II shows a comparison between manual counting and estimation by proposed method for 10 image samples. Figure (4[a-j]) shows the results of 10 samples of white blood cells.

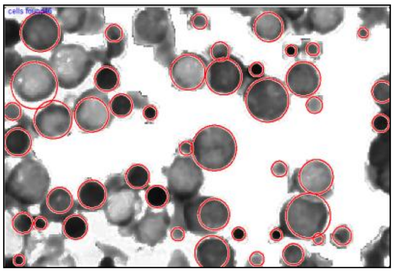


Figure (a) WBC count of image 1

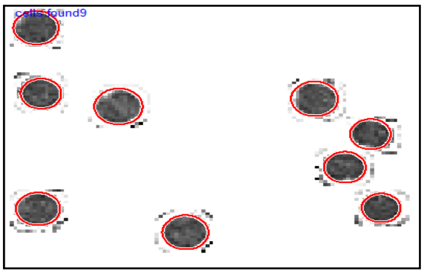


Figure (b) WBC count of image 2

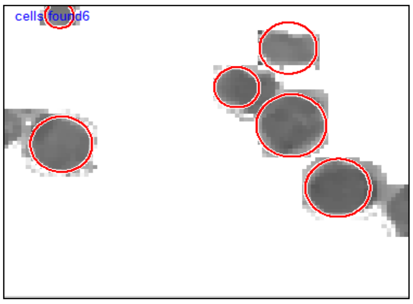


Figure © WBC count of image 3

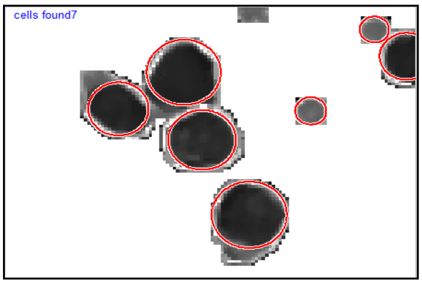


Figure (d) WBC count of image 4

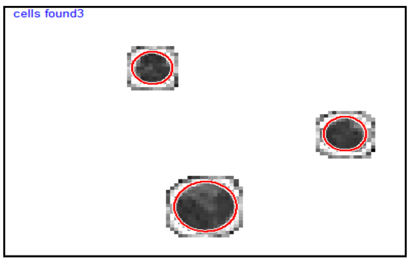


Figure (e) WBC count of image 5

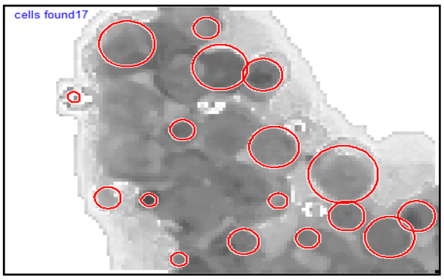
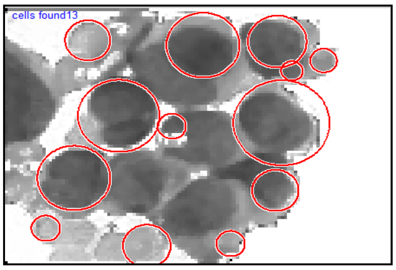


Figure (f) WBC count of image 6



Figure(g) WBC count of image 7

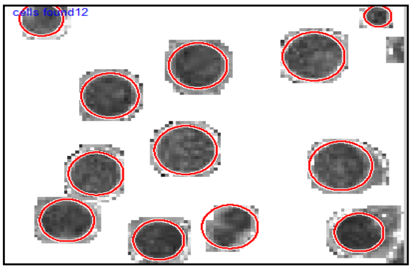


Figure (h) WBC count of image 8

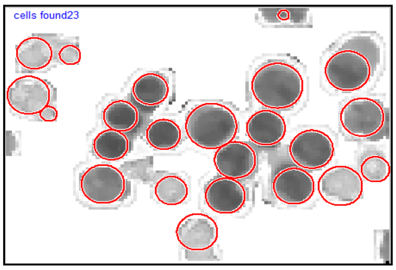


Figure (i) WBC count of image 9

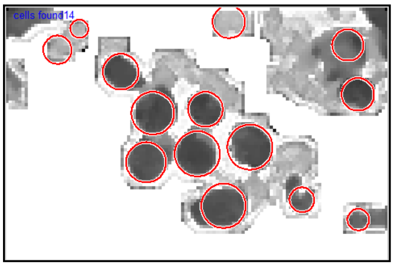


Figure (j) WBC count of image 10

Figure 4[a-j] Ten samples of the WBC count of Blood Cell images

V. CONCLUSION

By using digital image processing, analysis of blood cell image is more accurate. The proposed method is efficient in terms of cost and time consuming compared to existing techniques of blood cell analysis. Day by day research work is increasing in this field and various image processing techniques are implemented in order to get more accurate result. This research involves detecting the types of leukemia using Microscopic blood sample images. The proposed work helps to identify abnormality detection. For example in case of leukemia, counting the number of WBCs in the image and then comparing it to the normal count of WBCs can help us to detect abnormality in the blood cell from which we can conclude that the patient has leukemia or not.

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