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RESEARCH ARTICLE

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WHITE BLOOD CELL ANALYSIS AND CLASSIFICATION USING DIFFERENT SEGMENTATION METHODS ON BLOOD IMAGE SIMPLIFIED BY SMMT OPERATOR

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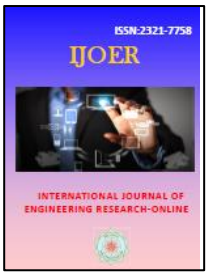

ABSTRACT

White Blood Cell (WBC) counting plays a major role in the determination of the patient's health for different stages, such as diagnosis and treatment. The traditional differential counting method for WBC count is tedious and time consuming. In Indian scenario, major of pathologists use manual methods, for counting the blood cell using microscopic blood image. In this direction for automation, researchers and engineers used image processing algorithms such as image segmentation, thresholding, histogram equalization etc. where each technique is found tohave some advantages along with some drawbacks at each stage. In this direction, this paper presents WBC segmentation using different image segmentation methods such as watershed transform and level set method for nucleus segmentation and Mathematical Morphology(MM) operator and Granulometric analysis for cytoplasm segmentation where Self-dual Multiscale Morphological Toggle (SMMT) operator is used as preprocessing algorithm for image simplification. Features such as area, Length, solidity, & circularity are excited from segmented image to classify WBC into five types viz.; basophil, eosinophil, lymphocyte, neutrophil & monocyte. Further WBC classification and counting has been carried out as it is helpful for identifying some diseases. Classification is carried out by adopting simple conditions checking algorithm. It has been found that, watershed transform provides better segmentation and counting results amongst tested four segmentation methods. It gives ~87% accuracy for WBC segmentation for non-overlapping WBC images and ~50% accuracy for overlapping WBC images. Watershed provides ~70% of accuracy for WBC counting.

Keywords—WBC; Watershed transform; level set method; MM operator; Granulometric analysis; WBC classification; WBC count..

I. INTRODUCTION

Human blood contains number of cells like Red Blood Cells (RBC), WBC of five types, platelets etc. WBC count variation is an important metric in monitoring diseases like cancer, AIDS. So WBC counting is necessary, and in order to achieve this we need to segment WBCs from overall blood cell image where contrast between the nucleus, cytoplasm and background is usually low and hence making segmentation is a challenging process.

Traditional WBC counts and classification methods are manual which includes a Hem cytometer charged with the diluted blood, and counting nuclei manually in the appropriate areas of the grid using a light microscope. The manual WBC is a reasonable test since it is not time and labor-intensive and besides it gives acceptably accurate results. Automated cell counters are machines that automatically count cells, this automated cell counter uses optical or electrical impedance sensors to count number of cells go through the tube. As against this the current researchers have focused on WBC analysis using Pixel and Region based segmentation, Segmentation using thresholding, Histogram equalization, Morphological Granulometric Features of Nucleus, Mathematical morphology and scale space algorithm.

Many automated techniques were proposed to overcome the time consuming task of human experts in counting and classifying WBC as mentioned below. Rosenfeld discusses two forms of segmentation [1]. Pixel based image segmentation and region based segmentation. In which pixels are classified independently. In Region based segmentation, the goal is to split the image into distinct connected regions. Both forms of segmentation require some experimentation to develop a good semantic model that can be used to split or merge regions.

Sonka, Halavac and Boyle nominate thresholding as the simplest segmentation process, this is used by Mosthefa Mohamed, BehrouzFar[2] and reported that thresholding can be useful in the case of segmenting images of microscopic blood cells where cytoplasm, nucleus and background each have their own distinctive grey levels. This technique has problems where the lighting level varies from one image to another.

Fatin A. Dawood has introduced the segmentation process based on using histogram equalization to detect every element in the blood slide and cut out the WBC segment from all other elements by color concentration [3]. This method was found to be more appropriate than thresholding method especially to overcome the time-consumed in counting and segmenting WBC.

Nipon Theera et al. have introduced the method which is based on Morphological Granulometric Features of Nucleus and mainly invented for blood in bone marrow [4]. Previously mentioned methods for WBC segmentation are applicable only on peripheral blood, where particularly these researchers have worked on bone marrow images. In this the author has classified WBCs with the help of different classifiers such as Bay's classifier and artificial neural network where input to this classifier was nucleus based features namely area of nucleus, location of its pattern, and two granulometric moments.

Tenn Francis Chen used Level-set methods combine global smoothness with the flexibility of topology changes [5]. This helps significantly statistical classification over conventional. However, level-set methods suffer from heavy computational burden because of a lot of iterations. To overcome this fast level-set framework based on the watershed algorithm for the segmentation of complicated structures from a volumetric data set like MRA image is represented in[6].

Leyza Baldo Dorini et al. used Self-dual Multiscale Morphological Toggle (SMMT) technique proposed by Witkin [7]. The approach [8] and [9] is a novel multiscale approach, named scale space. This method constitutes the monotonicity property, which states that, the number of features must necessarily be a monotonic decreasing function of scale. The properties of SMMT operator is used to improve the quality of the image to be used in the segmentation algorithms. By choosing the appropriate scale, only the features of interest are preserved. When varying the number of iterations, the edge-preserving region merging conduces to a contour regularization and consequently to an improved gradient image, thus yielding better segmentation results. After image simplification using SMMT operator, the cell nucleus was extracted using the watershed transform. Then, based on the size distribution information of the red blood cells (RBC), the cytoplasm was segmented using basic image processing operations such as, thresholding and morphological opening. However there is no report on automated WBC counting after segmentation.

To fill this gap in this paper we have presented an automatic method to count WBCs after segmentation of nucleus by watershed transform and level set method and cytoplasm by MM operator and granulometric analysis on SMMT operated image. Then comparison of our results has been carried out with pathologist results[11]. The theoretical background on SMMT, segmentation methods, classification and counting is presented in the following section.

II. Therotical Background

In this section mathematical analysis on SMMT operator for image preprocessing, Watershed transform, Level set method, MM operator and granulometric Analysis is presented which are used in our implemented work.

1)***SMMT operator:*** SMMT Operator is used as a pre-processing algorithm as it simplifies the image for accurate contour recognition [10]. SMMT operator extract the specific features of interest SMMT operator defined as,

$$(f \oslash g_\sigma)^k(x) = \begin{cases} \emptyset_1^k(x) & \text{if} \emptyset_1^k(x) - f(x) < f(x) - \emptyset_2^k(x) \\ f(x) & \text{if} \emptyset_1^k(x) - f(x) = f(x) - \emptyset_2^k(x) \\ \emptyset_2^k(x) & \text{otherwise} \end{cases} \quad (1)$$

Where,

$$\emptyset_1^k(x) = (f \oplus g_\sigma)^k(x) \quad \cdots \text{dilation} \quad (2)$$

$$\emptyset_2^k(x) = (f \ominus g_\sigma)^k(x) \quad \cdots \text{erosion} \quad (3)$$

Nucleus segmentation has been carried out by two different approaches namely watershed transform and Level set method and cytoplasm segmentation has been carried out by MM operator and granulometric analysis.

2) ***Watershed Transform:*** Watershed Transform is based on visualizing an image in three dimensions: two spatial coordinates versus intensity. In such topographic interpretation, three points are taken into consideration: (a)points belongs to regional minima (b)points at which a drop of water, if placed at the location of any of those points, would fall with certainty to a single minimum, and (c)points at which water would fall to more than one such minimum. For particular regional minimum, the set of points satisfying condition (b) is called the catchment basin or watershed of that minimum. The points satisfying condition (c) form crest lines on the topographic surface and are termed divide lines or watershed lines.

Watershed segmentation algorithm:

Let M1, M2,……..Mg sets denoting the coordinates of the points regional minima of an image g(x, y).

C(Mi) -a set denoting the coordinates of the points in the catchment basin associated with regional minimum Mi. notation min and max denote the minimum and maximum values of g(x, y). T[n] represent the set of coordinates (s,t) for which g(s,t) < n. that is,

$$T[n]=\{(s,t)\backslash g(s,t)<n\} \quad (4)$$

Where, T[n] is set of coordinates of points in g(x,y) lying below the plain g(x,y)=n, The topography will be flooded in integer flood instruments, from n=min+1 to n=max+1. For algorithm of watershed transform refer [10].

3) ***Level set Method***: Segmentation using Level set Method has the advantage to deal with topological changes or discontinuities that may arise during the evolution of the level zero curves. To prevent the leaking of the curves moving toward object edges, the following expression is introduced:

$$g_I(x, y) = \frac{1}{(1 + |\nabla(G\sigma * I(x,y))|)} \quad (5)$$

where, Gσ* I denotes the convolution of the image I with a Gaussian kernel of standard deviation σ. The term ∇(Gσ *I(x, y)) is essentially zero, except near significative variations of the gradient. Thus, g I(x, y) approaches one out of the edges and tends to zero near them.

4) ***MM operator:*** Mathematical morphology examines the geometrical structure of an image by probing it with small patterns, called 'structuring elements', of varying size and shape. This procedure is well-suited for exploring geometrical and topological structures such as nucleus & cytoplasm. A succession of such operators is applied to an image in order to make certain features apparent, distinguishing meaningful information

from irrelevant distortions, by reducing it to a sort of caricature.

These methods require the various MM operations, Bottom and top hat transform and flood fill operation. The Bottom and top hat transform and Flood fill operation theory is explained further.

Bottom hat: Bottom-hat transform is applied to emphasize brighter regions.

$$T_{hat}(f) = f - (f \circ b) \quad (13)$$

$$B_{hat}(f) = (f \cdot b) - f \quad (14)$$

Flood Fill Operation: The flood fill algorithm takes three parameters: a start node, a target node and a replacement color. The algorithm looks for all nodes in the array that are connected to start node by a path of target color and changes them to the replacement color.

5) ***Granulometric Analysis:*** Granulometric function helps to achieve size distribution information of each element in a microscopic blood image. The reason for calculating size of each element is to select size of structuring element which is based on size of Red blood cells (RBC's). This empirically computed size of RBCs can vary due to pathologies. However most of the blood cell part are near to7.5-8 micrometers in diameter..

III. database Preparation

Collection of database of microscopic blood cell image samples is carried out from internet. It consists of 56 samples. There was microscope having magnification (100X) resolution. Blood image database of 56 samples were prepared, The proposed scheme has been applied on 56 microscopic blood images obtained from internet. Sizes of these images range from 120 X 90 to 1584 X 1443 pixels. This database consists of two different kind of image sets viz.;46 Non-overlapping WBC images and 10 overlapping WBC images.

IV. Experimentation

Microscopic blood image is as shown in Fig. 1 which contains RBC and WBC with nucleus and cytoplasm. Figure 2 shows flow diagram for methodology of implementation for WBC segmentation classification and counting.

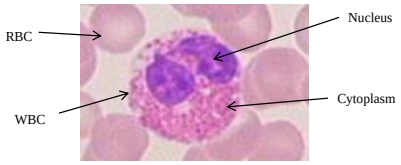

Fig. 1. Sample of Normal White Blood Cell Image

At the start image has been chosen from the database which is firstly simplified by SMMT operator. This SMMT operated image is then used for nucleus segmentation using watershed transform and level set method and for cytoplasm segmentation using MM operator and granulometric analysis method as explained further. These segmentation results are further used for feature extraction such as area, length, solidity and circularity of WBC in order to classify WBC in five types viz,; Basophil, Eosinophil, Lymphocyte, Neutrophil and monocyte. Classification results are used to obtain the WB count. Four algorithm flowchart used for segmentation are discussed in a) whereas classification and counting are explained in b)

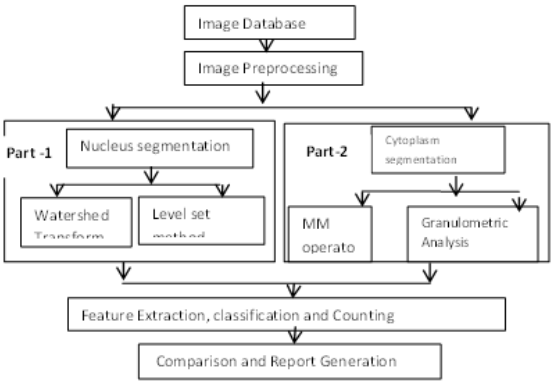

Fig. 2. Flow chart of WBC segmentation and counting

a) Segmentation of preprocessed image

i) WBC Segmentation: Watershed Transform:

Marker-controlled watershed segmentation follows basic steps as depicted Fig. 3. SMMT operator is applied on input image, I to create simplified image Is. Then segmentation is carried out on this image whose dark regions are the segmented objects. These are connected blobs of pixels within each of the objects that are taken as foreground markers. Background marker is computed based on pixels that are not part of any object. Segmentation function is modified so that it only has minima at the foreground and background marker locations.

At the last watershed transform is applied to obtain segmented nucleus along watershed lines.

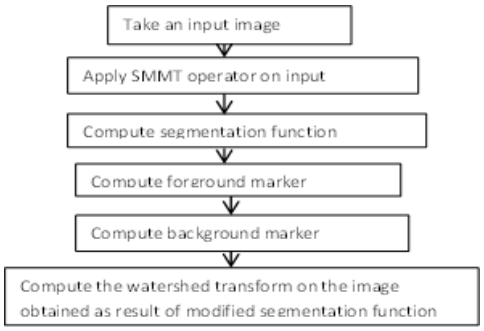

Fig. 3. Flow Chart of Watershed Transform

ii) WBC Segmentation: Level set Method

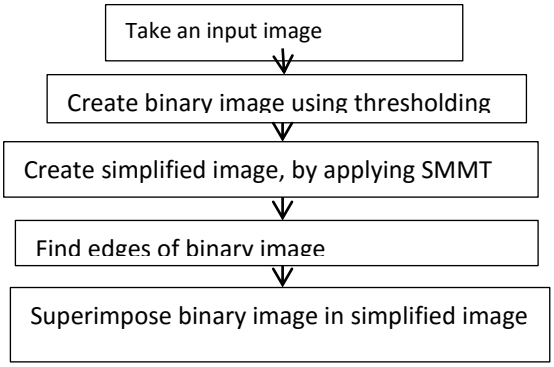

Fig. 4. Flow chart of Level set method

Segmentation by level set method follows the steps given in Fig. 4. Here, binary image Ib, is created by using appropriate thresholding on the input image, I. Image simplification is carried out by applying SMMT operator on image I. The Canny method was used to obtain an edge of binary image, Ib, which uses two thresholds to detect strong and weak edges. At the last, image Ib, is superimposed in simplified image, Is. This gives segmented nucleus of WBC. Results obtained from nucleus segmentation were used to compute WBC count. At Comparison stage, WBC count results were compared with results obtained from laboratory by experts on the same samples.

iii) Cytoplasm Segmentation: MM Operator

Figure 5 is subjected to MM operator to segment WBC cytoplasm. Here, SMMT simplified image is subjected to bottom hat transform to emphasize the cytoplasm region. Then by using thresholding binary image is created then flood fill operation was performed in order to fill small regions. Finally previously segmented nucleus is added into image obtained in fourth step, so that each and every WBC is taken into consideration.

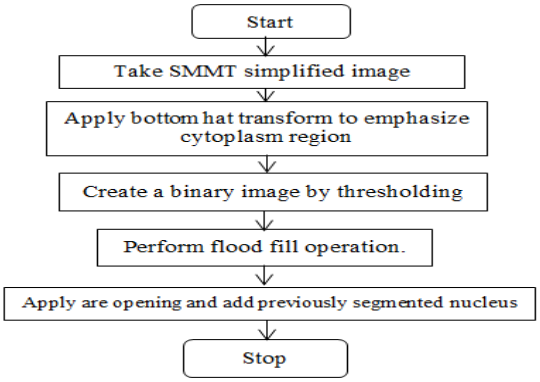

Fig. 5. Flow chart of MM operator

iii) Cytoplasm Segmentation: Granulometric Analysis

Granulometric analysis is the last segmentation method is used to segment the cytoplasm of WBC. As shown in Figure 6 first step in this method is take a microscopic blood image as input. Then second step is to find size of RBC using empirical method. For this at start use disc shaped structuring element with basic size. Apply structuring element to a image visualize the image for RBC removed. In case if RBC is not removed increase the structuring element size further and repeat the process until RBCs are removed. Third step is to apply thresholding to get binary image. Then obtained RBC size was considered as structuring element for opening process to remove unwanted parts from image.

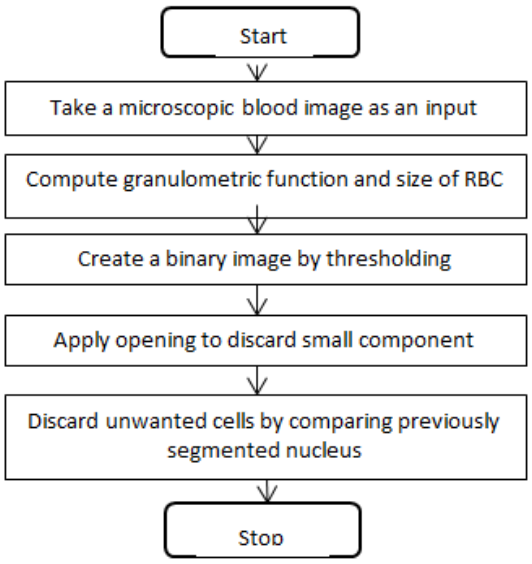

Fig. 6. Flow chart of Granuometric Analysis

b) Feature Extraction and classification: Feature extraction in image processing is a technique of redefining a large set of redundant data, into a set of features of reduced dimension. Since the chosen features affect the classifier performance much, deciding on which features to be used in a specific data classification problem is as important as the classifier itself. For classifying cells successfully, hematology experts examine the shape of the cells and nuclei. In every separate WBC image, it is necessary to analyse quantitatively certain morphological features. Characteristics to be analyzed are the nucleus area, length, solidity and circularity.

Area: For nucleus area measurement we assigned the pixels in region of interest (ROI) equal to 1 in binary image of leukocyte.

Solidity: Solidity which is obtained when the nucleus part expressed in pixels divided by area of smallest area. This value is relevant to the classification since it represents the measurement of the nucleus fullness.

Circularity: Circularity parameter explains complexity of the nucleus structure. It is obtained by dividing nucleus area by the square of the nucleus circumference. The value obtained in this way is maximum for circular shape of the nucleus. As structure complexity of nucleus decreases the circularity decreases

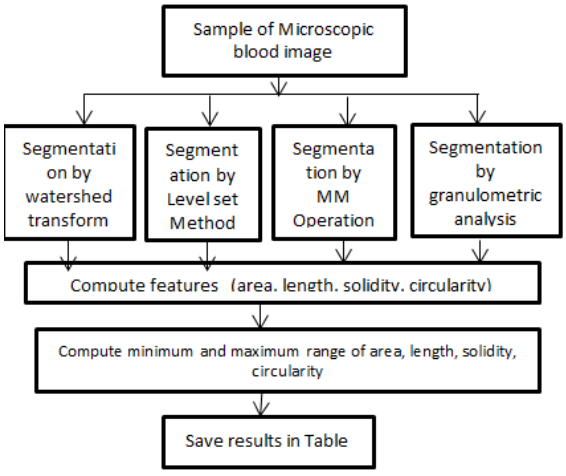

Fig. 7. Flow chart for WBC segmentation, Feature extraction and condition generation

From the extracted features the minimum maximum value for each feature is found & taken as reference value while deciding type of WBC, as shown in figure 7. In this, first WBCs from sample microscopic blood image are segmented by various segmentation methods. Segmented WBCs are used to obtain features viz.; area, solidity, length and circularity. Next step is to compute minimum and maximum value for each feature for refference. These values are further used for classification. For counting Condition check and for decision making computed features such as length, nucleus area, circularity and solidity these features are used for further classification. First we have calculated minimum and maximum value for every feature for each type of WBC. We have set five conditions based on these features.

Figure 8 shows flow chart for WBC classification and counting. First condition relate with basophil. Condition checking using look up table considering of refference value is done to find that whether the calculated value of length, area, solidity and circularity for sample WBC lie in the range of calculated minimum and maximum value of length, area, solidity and circularity of basophil. If the checking fulfills this condition then sample WBC is of type basophil. Otherwise the sample features are checked for second condition, where the procedure similar to basophile is adopted to neutrophil class. Otherwise sample is subjected to third condition. Third condition includes same parameters for the eosinophil. The sample WBC is stamped eosinophil if it passes the test of minimum maximum range of refference parameters. Otherwise it is subjected to fourth condition.

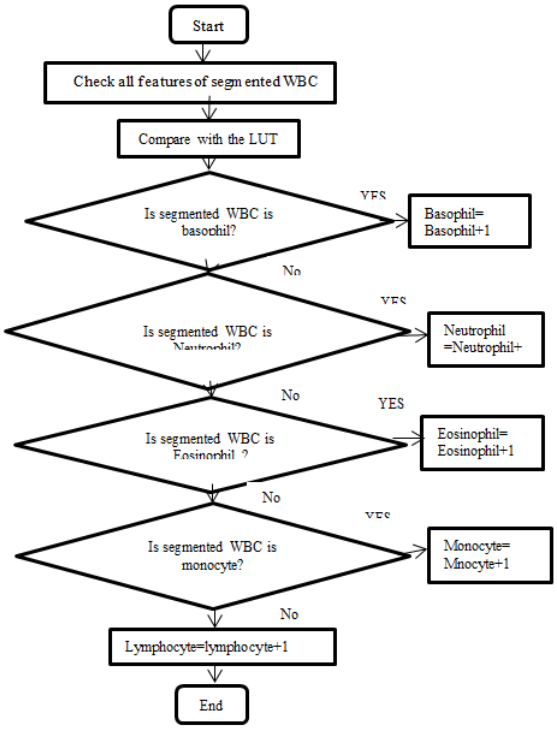

Fig. 8. Flow chart for classification and counting of WBC

Fourth condition includes maximum and minimum value for monocyte. If sample WBC fulfills these conditions then it is monocyte, otherwise sample WBC is declared as lymphocyte. Thus we have found the classified WBCs & further used for WBC counting. While generating blood report we have mentioned total count along with individual count for each type of WBC for respective sample image.

Counting: In this section we have noted counting results. For which, after classification counted the total number of WBC as well as count of each type of WBC using simple count algorithm. For this, initially we took basophil count as to zero, while classifying if sample WBC is passed check test of basophil, then the count of basophil is incremented by one. Likewise we performed the same operation for remaining four types of WBC viz. lymphocyte, eosinophil, monocyte and neutrophil. Once we found the count for each type of WBC, we calculated the total number of WBC present in sample microscopic blood image by adding those count together. We have applied the same classification and counting procedure for all images obtained after application all of implemented segmentation.

V. Results

Results of nucleus segmentation obtained using watershed transform are given in figure 9. In this figure, first column a, corresponds to the original microscopic blood image. Column b, corresponds to SMMT simplified image. Here we can observe that SMMT operated image contains part of WBC clearer than rest of the parts in microscopic blood image. Third column that is column c, shows gradient enhanced image, where we can see the edges of WBC very clearly. Result image in column d, shows segmented nucleus from the microscopic blood image which is the result of segmented nucleus.

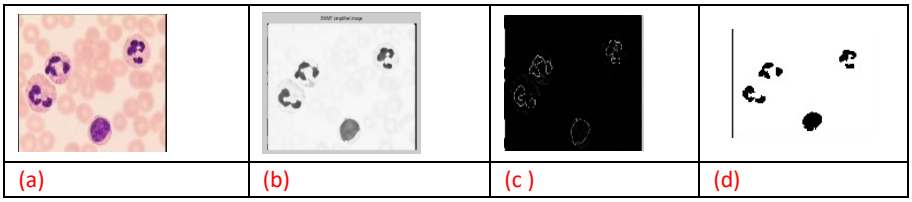

Fig. 9. a)Original WBC image b) Image in Fig a simplified by SMMT c)Gradient of original image d)segmeted nuceus by watershed transform

Results of nucleus segmentation obtained using level set method are given in Figure 10. In this figure first column corresponds to the original microscopic blood image, column b, shows SMMT simplified image. Column c, shows results of thresholding. Column d, shows edges of threshold image detected by canny edge detection method. Column e, shows results of erosion. Column f, shows results for addition of binary image and edges of that image. Column g, shows segmented nucleus which is found by subtracting eroded image from added image (image shown in column f).

Results of cytoplasm segmentation obtained using mathematical morphology are given in Figure 11. In this figure column a, corresponds to the microscopic blood image, second column corresponds to SMMT simplified image. Column c, shows results for bottom hat transform. Here bottom-hat transform is applied to emphasize brighter regions. Column d corresponds to results

of flood fill operation which fills all small holes. Column e corresponds to results of addition of previously segmented nucleus into the resultant image after flood fill operation. Finally seventh column, column f, gives us segmented cytoplasm from the microscopic blood image, by applying MM operator .

Results of cytoplasm segmentation obtained using granulometric analysis are given in Figure 12. In this figure column a, corresponds to the microscopic blood image, second column corresponds to binary image which is result of thresholding. Column c corresponds to results of opening, where size of structuring element for this opening is same as size of RBC. Finally column d, shows segmented cytoplasm by comparing opening results with previously segmented nucleus.

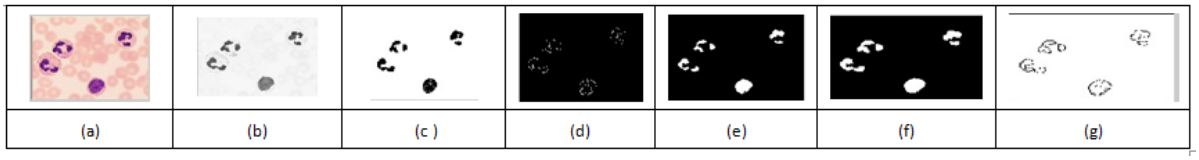

Figure 10 a) Original WBC image b) Image in Fig a simplified by SMMT c)thresholded image d)Edges obtained by canny edge detection method. e)result of erosion. f) result of addition of edges and thresholded image. g) Segmented Nucleus

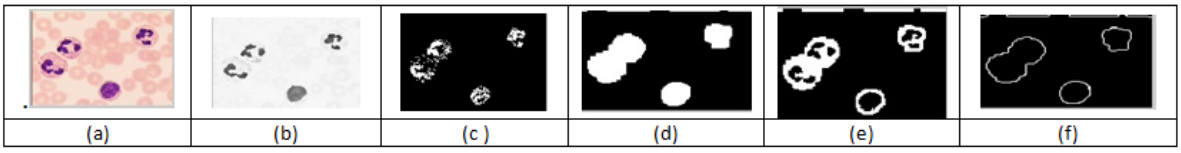

Fig. 11. a)Original WBC image b) Image in Fig a simplified by SMMT c)result of bottom hat transform d) result of flood fill operation. e)result of erosion. f) result of addition ofprevious;y segmented nucleus. g) Segmented cytoplasm.

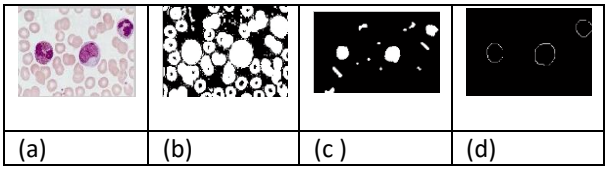

Fig. 12 a)Original WBC image b) Image in Fig a simplified by SMMT c)thresholded image d)Edges obtained by canny edge detection method. e)result of erosion. f) result of addition of edges and thresholded image. g) Segmented Nucleus

Tables I and Table II show result analysis, of WBC count obtained by four segmentation methods viz., nucleus segmentation by watershed transform and Level set method and cytoplasm segmentation by MM operator and granulometric analysis applied on 46/10 WBC image samples of non-overlapping WBC images/ overlapping WBC images respectively. These tables also include comparison of practically obtained individual WBC count for five types of WBC viz.; basophil, eosinophil, lymphocyte neutrophil and monocyte obtained after segmentation by four methods and actual total WBC count (i.e. pathology lab results of WBC count).

Table III shows the accuracy of WBC segmentation results for WBC images with no overlapping and for WBC images with overlapping. It is seen from this table that, accuracy of watershed method is good for overlapping as well as non-overlapping WBC images. It is 86.95% for non-overlapping WBC images and 50% for overlapping images. Accuracy of level set method for non-overlapping WBC image is 76% . Cytoplasm segmentation result by MM operator for non-overlapping images is 78.56% which is 58.7% more accurate than that obtained by granulometric analysis and results of granulometric analysis for images with overlapping WBCs is 40% which is ~10% more than that by level set segmentation. So for non-overlapping & overlapping blood cell images Granulometric analysis method is found to perform poorly. Performance of Level set method & MM operator are similar.

Table IV shows the accuracy of WBC count with reference to lab results. WBC count results after watershed transform is ~70% which is ~15% greater than level set method. MM operator gives 50% accuracy for WBC count which is 25% greater than granulometric analysis.

TABLE 1. Result analysis for wbc count of non-overlapping WBC images by various methods

Sample image	No. of Basophil					No. of Eosinophil					No. of Lymphocyte					No. of Neutrophil					No. of Monocyte				
	A	PRW	PRL	PRM	PRG	A	PRW	PRL	PRM	PRG	A	PRW	PRL	PRM	PRG	A	PRW	PRL	PRM	PRG	A	PRW	PRL	PRM	PRG
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	1*	1*	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
11	0	0	0	0	0	1	0	1	1	1	0	0	0*	-2	-2	1	-2	-5	-5	-5	0	0	0	1*	1*
12	0	0	0	0	0	1	1	0	0	0	1	0	0*	0*	0*	2	-5	-3	-3	-3	2	0	-1	-1	-1
13	0	0	0*	0*	0*	0	0	0*	0*	0*	1	0	0	0	0	0	-1	1	1	0*	0	0	0*	0*	0*
14	1	0*	**0**	**0**	**0**	2	0*	**0**	**0**	**0**	0	0	**0***	**0***	**0***	1	1	**0***	**0***	**0***	1	0*	**0**	**0**	**0**
15	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0***	**0***	**0***	**1**	**0***	**0**	**0**	**0**	**3**	**0***	**0***	**0***	**0***	**0**	**0**	**0**	**0**	**0**
16	**0**	**0**	0	0	0	**1**	**0***	0	0	0	**0**	**0**	-1	0	0	**1**	**0***	-1	0	0	**0**	**0**	-6	1	0
17	0	0	1	0*	0*	0	0	0	0	0	0	0	0	0	0	0	0	-1	-1	0	1	1	0	0	0
18	1	0*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0	1	1	0*
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0*	1	1	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0*	0*	0*	1	1	-2	0*	0*	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	1	0*	0	0	-1	1	-2	1	1	1	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0

TABLE II. RESULT ANALYSIS FOR WBC COUNT OF OVERLAPPING WBC IMAGES BY VARIOUS METHODS

Sample image	No. of Basophil					No. of Eosinophil					No. of Lymphocyte					No. of Neutrophil					No. of Monocyte				
	A	PRW	PRL	PRM	PRG	A	PRW	PRL	PRM	PRG	A	PRW	PRL	PRM	PRG	A	PRW	PRL	PRM	PRG	A	PRW	PRL	PRM	PRG
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
3	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-2	-2	-2	0*	0	0	0	0	0
6	0	0	0	0	0	0	-1	1	-1	0	0	-2	-1	-2	0	1	1	1	1	0	0	-3	-4	-4	0
7	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	-2	0	0	0	0	-1	2	2	2	2	1*
8	1	0	0*	0*	0*	0	0	0	-1	-1	0	0	0	0	-1	0	-1	-1	-4	-1	0	0	0	0	-3
9	0	0	0	0	0	0	-1	0	0	0	0	0	0	-1	-1	1	-2	-3	-2	-2	0	0	0	0	0
11	0	0	0	0	0	1	0*	1	1	1	0	0	-7	-8	-8	1	-2	-10	-10	-10	0	0	0	0	0
25	1	0*	0*	0*	0*	0	0	0	0	0	0	0	0	0	0	0	-1	-1	-1	-1	0	0	0	0	0
48	0	0	0	0	0	0	-1	0	0	0	5	1*	2*	2*	2*	0	-1	-3	-3	-3	0	0	0	0	0

TABLE III. RESULT ANALYSIS FOR SEGMENTATION RESULT FOR OVERLAPPING AND NONOVERLAPPING OF WBC IMAGES.

	Accuracy for overlapping images		**Accuracy for non-overlapping images**	
Total number of blood images	**10**		**46**	
Segmentation method used	**Total number of blood images with accurately segmented WBC**	**% Accuracy**	**Total number of blood images with accurately segmented WBC**	**% Accuracy**
Watershed Transform	5	**50**	40	**86.95**
Level-set Method	3	30	35	76
MM Operator	3	30	36	78.26

Granulometric analysis	4	40	9	19.56

TABLE IV. RESULT ANALYSIS FOR WBC COUNT.

	Accuracy for count
Watershed Transform	69.64
Level-set Method	55.35
MM Operator	50
Granulometric analysis	25

VI. Conclusion

The main goal of this research was for detection and classification of white blood cells from microscopic images using four different segmentation methods of image processing. Results of four segmentation methods applied on microscopic blood image for WBC segmentation are discussed followed by, feature extraction, classification and counting of WBC. This goal was largely met, with the exception of a manual step required during report generation. As a component of this, we attempted to determine which of a number of investigated classification techniques provides the best automated segmentation results for the classification of white blood cells into their five major types (Neutrophils, Lymphocytes, Monocytes, Eosinophil and Basophils), based on a limited data set of visual images from classification results, we found that our classification is good to achieve the accuracy, with an error rate of 2%. We have found that classification result after segmentation is 70% which is our unique contribution.

Future scope

It has been found that this project helps to obtain 70% accuracy for classification and counting for microscopic blood images. These accuracy leads to be improved by using training and testing algorithm like ANN. As training testing algorithm requires more number of sample images we have not gone to use such algorithm. Some improvement in processing of overlapped images is needed which will drive for further improvement in segmentation.

Our method requires manual involvement for report generation. Further advancement in report generation can make this whole process automatic.

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