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A Comparative Study on Image Isolation and Classification Techniques in Microscopic Blood Smear Images

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ABSTRACT*:*In relevance to the scope of Biomedical Engineering, Blood Cell Analysis plays a significant role in assisting Medical Practitioner to diagnose the presence or level of severity of diseases. Blood tests usually carried out by pathologists to determine number of blood cell components is very tiring, monotonous, time consuming and requires highly experienced personnel's. The switching of manual methods to completely automized requires image processing and computer vision techniques. This paper reveals most of the segmentation and classification methods of White blood cells (Leukocytes) in microscopic blood smear images. The selection of suitable segmentation technique is a challenging task. The classification technique also depends on success of segmentation. We mainly focus on methods used in segmenting White blood cells and its major types from its RBC's, Platelets and background.

KEYWORDS*:* Leukocytes, segmentation, classification, Machine learning, blood smear images, Medical tests

I. INTRODUCTION

When a doctor suspects any disease, firstly suggests a complete blood test or differential blood test based on severity of symptoms. Complete blood test results in complete count of red blood cells platelets, leukocytes (white blood cells), and erythrocytes (red blood cells). Differential blood test classifies and counts the five major types of leukocytes. Two broad classifications of WBC's are Granulocytes and Agranulocytes. Granulocytes are Eosinophil's, Basophils and Neutrophils. Agranulocytes are Lymphocytes and Monocytes. The WBC count range for a normal adult is 4,500 to 10,000. If the count is above or below this range, it may detect the presence of some diseases. Leukopenia is the low WBC count and may detect the presence of HIV, autoimmune disorders, liver & spleen diseases, and radio therapy and so on. Leucocytosis is the high WBC count that may predict the presence of anaemia, allergies, pregnancy, tissue damages, asthma, blood cancer and many more. Even during the medical treatment continuous monitoring of WBC count has to be done through blood tests to analyse the effect of treatment on the patients. Hence blood tests are very crucial and helpful in correctly diagnosing and treating the diseases.

Automatic Haematology instruments currently available identify and count types of WBC's using laser technology, radio frequency, direct current, impedance method, optimum temperatures and volumes and also with different blood smear slide staining techniques. These instruments provide high level of accuracy and precision in quantifying and identifying normal white blood cells. How-ever these methods are not sensitive in identifying immature, abnormal and blast cells. To overcome this problem, most of the time analysers flag samples with abnormal WBC population indicating the need for peripheral smear examinations to be examined by trained personnel to identify the abnormal cells [1]. Therefore Image Processing and computer vision methods can be thought of to develop and implement highly efficient, robust and cost effective system to identify and analyse blood cells. Intelligent identification and classification of leukocytes is a vital step in analysing many diseases. In recent years many researchers have developed algorithms to segment and classify Leukocytes from microscopic blood images. This paper

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presents a review of some existing techniques available in segmentation and classification of leukocytes (WBC) in microscopic blood smear images.

A powerful alternate to automatic haematology analysers is systems based on image processing and machine learning techniques. Here images of microscopic blood smear are acquired using microscope and camera. Later, these images are analysed using different robust segmentation and classification algorithms which can give accurate count results.

A successful Segmentation highly depends on type of application and algorithm selected which in turn leads to efficient recognition and classification. Over the decades, many research scholars are continuously working on different traditional and hybrid techniques to find optimized solution. Unfortunately failed to prove the best one since no single technique is applicable to all applications. The following paragraphs discuss various segmentation and classification techniques analysed by many researchers in segmenting White blood cells and its types from background and other components of blood.

Rong Chu, et al. proposed a method to segment WBC using contour sub image Cosegmentation method. All types of white blood cells: Eosinophil, Basophil, Neutrophil, Monocyte, Lymphocyte can be segmented using this hybrid method [7]. Huey Nee Lim, et al. proposed a combination of k-means clustering for color based clustering and watershed transform based morphological segmentation for segmenting only WBC's [8]. Khaled A. Abuhasel, et al. [9] suggested a technique to isolate WBC nucleus using modified gram Schmidt method and region growing method to segment cytoplasm of WBC. Jyoti Rawat et.al [6] reviewed on different techniques like global thresholding, color space transformation, morphological methods for segmenting and classifying all types of White blood cells: Eosinophil, Basophil, Neutrophil, Monocyte, Lymphocyte and concludes that blob analysis method proves better.

Biplab Kanti Das, et al. [11] proposed a technique to detect cytoplasm and nucleus of blood cells using thresholding, morphological analysis and contouring on 20 images of color sample blood image and reports 85% of accuracy. Chaitali Raje, et al. [10] proposed a method of segmenting nucleus in WBC using Otsu's thresholding and statistical parameters like Standard Deviation and Mean. Mostafa Mohamed et al. [13] tested on 365 blood images using Gray scale contrast enhancement and filtering method for automatic WBC nuclei segmentation and reports 79.7% of accuracy but did not overcome the problem of non-homogeneous lighting and over staining [15].

R. Adollah et al. [5] Segmented leukocyte from background using multilevel thresholding technique but this method may not be applicable for dark or bright images. N H Abd Halim et al. [17] worked on image quality and Extraction of nucleus from WBC using a global contrast stretching and HSI color models. Jun Duan et al. [18] proposed a color image segmentation technique Integrating color histogram and region growing and merging-based for segmenting nucleus and cytoplasm from WBC and states that results vary if there is cell adhesion. Subrajeet Mohapatra et al. [2][4] also worked on Leukocyte color based segmentation using a rough set based clustering approach for Nucleus and cytoplasm of WBC extraction. P Sukumar et al. [19] proposed a technique to identify abnormal WBC nucleus in cervical cancer cells using Fast particle swarm optimization with ELM method (extreme learning machine, standard particle swarm optimization) also calculates Execution time of the techniques. F Boray Tek et al. [20] worked on segmentation of various components of blood by computing Cell area using area granulometry. Minimum area watershed transform, circle radon transform are used for segmentation. Transform used is not completely capable of detecting under or over segmentation. Pramit Ghosh, et.al. Makes use of HSI color model, shape and color characteristics, Fuzzy logic functions to identify the 5 types of leukocytes. It finds that nucleus of monocyte is oval and that of lymphocyte is circular. Considering height and width of cell nucleus, identification of monocyte and lymphocytes can be done. Average color and saturation helps in detecting neutrophils. Fuzzy logic assigns two colors red and blue which is used to detect eosinophil and basophil [3].

Vanika Singhal, et.al. Proposes a method to detect abnormal lymphocyte from a given blood image. Local Binary Pattern, a classifying texture feature is used to identify blast cell. It encounters various features of shape like perimeter, area, convex area, solidity, eccentricity, major axis length, compactness and orientation to segment nucleus from cytoplasm. Data set are trained using Support vector machine. System reports 89.72% of accuracy [12].

Sheng-Fuu Lin, et.al. Focusses on un-uniform stain caused due to methods involved in preparation of blood smear and dying agents. It presents a feature of nuclei lobes to differentiate nucleus granulocyte from agranulocytes. To segment the nucleus, Region growth algorithm is used. Redescription algorithm is used to identify bi or multilobed nucleus. Shape features used to classify cells are relative area, variance of boundary curvature and distance of center to boundary, roundness, solidity and number of lobes. Texture features used are energy, contract, correlation, entropy and homogeneity. Multiple SVM is used to train the images. Approximately 85% of accuracy in detecting all types of WBC's is reported [14].

S. H. Rezatofighi S. H, et.al. Proposed a method to isolate WBC nuclei using Gram Schmidt Orthogonalization. This technique is used to strengthen desired vectors of colors and weaken the undesired vectors of colors. Three vectors are selected due to large variation in colors. Reports 93% of accuracy. It imposes restrictions in application on new data in which it requires calculation of new vectors. Suggests that methods not based on color features are complex and require more time [21]. A.S.Abdul Nasir, et.al. Presents a combined methods of linear contrast technique used to enhance the quality of image and unsupervised k-means clustering to obtain fully segmented abnormal leukaemia images. By application of median filtering and region growing segmented nucleus is obtained. This work reveals that most of the WBC information is hidden in H component and structure of nucleus can be analysed better using S component of HSI Model [22].

Lim Huey Nee, et.al. Presents a method to segment cell using morphological operations, thresholding, gradient magnitude and

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watershed transform. The work is carried out in three steps like identifying WBC's and blast cells as objects of interest and deleting the background, applying Otsu's global thresholding and finally applying watershed algorithm to segment objects of interest. Author reports drawback of technique as localization of nucleus and cytoplasm are incomplete [23].

Hemant Tulsani, et.al. Presents a method to count all blood cell components. Watershed transform and regional maxima computations are used to segment the cells that solves the problem of overlapping cells. Work begins with smoothening of obtained image and converting to YCbCr color model and binary images. Later different masks are applied on WBC and platelets using morphological and thresholding to compute regional maximal points. Finally performs watershed algorithm on masks to count number of cells. Author suggests that marker based techniques solves the problem of over segmentation associated with watershed transform [24].

Table 1 shows comparison of various segmentation and classification techniques with respect to classifiers used, accuracy and no. of images used.

II. PROPOSED BLOCK DIAGRAM

The figure 1 below shows basic block diagram of Machine learning based classification of WBC's system which consists of a high magnification microscope to view cells on stained glass slides of blood droplets. Later a digital camera if mounted to microscope can be capable of capturing digital images. Using various image processing segmentation techniques, individual cells can be extracted. Classification of these cells into WBC types is carried out by applying machine learning models.

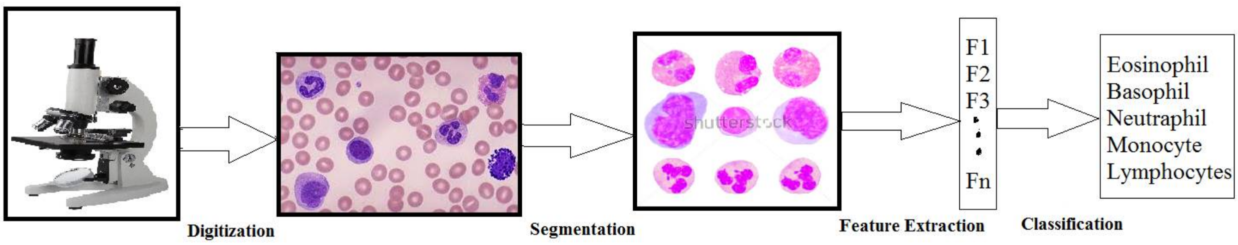

Figure 1: Block Diagram [25]

III. PERFORMANCE PARAMETERS

Medical treatment prescribed by doctors mainly rely on medical test reports. The accuracy of these tests help doctors in predicting presence or absence of diseases. Fortunately tests parameters can be measured using sensitivity, specificity and accuracy. These parameters evaluate how reliably better a test is. Sensitivity measures performance of the test in detecting the presence of a sickness. Specificity decides how best the test is in detecting the absence of a disease. Accuracy determines overall performance of system in correctly detecting presence or absence of a disease.

There are other parameters also that assist in evaluating specificity, sensitivity and accuracy. They are True Positive (TP), True Negative (TN), False Positive (FP), and False Negative (FN). If there is a presence of disease, and test also proves the presence of sickness, then test result is said to be TP. Similarly if there is absence of disease, and test also proves the absence of disease, then test result is said to be TN. If medical test indicates presence of disease, but actually patient is healthy, then result of test is said to be FP. If medical test indicates absence of disease, but actually patient suffers from such disease then test result is said to be FN. Sensitivity measures true positives that are identified correctly by a medical test. Specificity measures true negatives that are detected correctly by medical tests. Accuracy is a measure of both true positives and true negatives.

1. True positive (TP) is no of WBC's (or components of WBC) correctly classified as WBC's.
2. True negative (TN) is the number of other cells correctly classified as other cells.
3. False positive (FP) is no of other cells classified as WBC's.
4. False negative (FN) is no of WBC's classified as others.

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Author, Year	Work	Classifiers	Accuracy	Remarks	No of sample Images
Dipti Patra, et al. 2010 [2]	A fuzzy clustering based two stage color segmentation strategy is employed	Discriminative features: nucleus shape, texture are used for detection. Shape features: Hausdorph Dimension & contour signature. Support vector machine (SVM) for classification	95%	Advantage over existing schemes is images considered are smear images with many lymphocytes	108 blood smear images
Pramit Ghosh, et al. 2011 [3]	Work is to detect different WBC's	Image enhancement is done using Laplacian filter. Thresholding is used to segment, HIS color model and fuzzy logic functions are used	97.33%	Specifically not mentioned techniques only algorithms are explained.	150samples of blood image are taken.
Subrajeet Mohapatra, et al. 2011 [4]	Work on Leukocyte color based segmentation	A rough set based clustering approach	Not mentioned	Improved rough k-means clustering is highlighted	100 images
R.Adollah, M Y Mashor, 2011. [5]	Work on segmentation of microscopic bone marrow images	Multilevel Thresholding	Not mentioned	Works well on normal images but not on too dark or bright images	2 images Normal bone marrow and ALL
Jyoti Rawat, et al. 2015 [6]	Review on different techniques by different researchers	Global Thresholding, color space transformation, Morphological methods.	Approximately 80%	States that blob analysis method proves better	Not mentioned
Rong Chu, et al. 2015 [7]	A new method to obtain entire WBC contour	Sub image Cosegmentation method based on region-based active contour model.	92.3%	Successfully solves Adhesion problem	103 blood cell images
Huey Nee Lim, et al. 2015 [8]	A combination of Color and Morphological based techniques	k-means clustering for Color based clustering and Watershed transform based morphological segmentation	Claims to be 100% accurate but no record of it.	Overcomes the problem of over segmentation	Not mentioned
Khaled A. Abuhasel, et al. 2015 [9]	Method to segment Nucleus and Cytoplasm of White Blood Cells.	Modified Gram-Schmidt method for segmenting nucleus of WBC and Region growing method to segment cytoplasm of WBC.	95.21% for Lymphocyte cell type. 92.31% for Neutrophil cell type: with comparison to manual segmenting.	Not mentioned how feature extraction and classification is carried out.	100 samples of microscopic WBC images
Chaitali Raje, et al. 2014 [10]	Method of Segmenting nucleus in WBC	Statistical parameters like Mean and Standard Deviation and Otsu's thresholding based segmentation.	Good performance	Fully segmented nucleus is not obtained	128 microscopic blood slide images
Biplab Kanti Das, et al	To detect nucleus and	Thresholding, morphological analysis and	85% of accuracy	Not clearly mentioned	20 images

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2014 [11]	cytoplasm of blood cells	contouring		about techniques used	
Vanika Singhal, et al. 2014 [12]	Methodology for automatic detection of Acute Lymphoblastic Leukemia	Segmentation is done using HIS color model, thresholding and morphological operation like dilation is used.	89.72% of accuracy	Methods for segmentation, feature extraction and classification are clearly mentioned	75 blast cell images and 65 normal cell images.
Mostafa Mohamed, et al. 2012 [13]	Method for automatic WBC nuclei segmentation	Gray scale contrast enhancement and filtering	79.7%	Major problem is non-homogeneous lighting and over staining.	365 blood images
Sheng-Fuu Lin, et al. 2011 [14]	A method to ease the influence of non-uniform stain and extract the nucleus of WBC	Region growing algorithm for segmentation, shape feature is extracted using distance transformation and mean-shift to analyze no of lobes of nucleus, classification is done using support vector machine	Min of 76.47%	An attempt to solve problem of non-uniform stain is considered by modification of RGB channels.	400 images
Behrouz Far, et al. 2012 [15]	Technique for automatic blood cell nuclei segmentation	Gram-Schmidt Orthogonalization technique for segmentation and morphological operations used to enhance the segmentation.	85.4%	Non-homogeneous lighting and over staining is the main reason of segmentation inaccuracy	367 blood images
Cecilia Di Ruberto, et.al. 2016 [16]	Technique to segment and count leukocytes using learning by sampling	Nearest Neighbor and SVM for Segmentation. Counting by Hough Transform	99.2%	Considered only normal cells. Future work may segment blast cells.	367 blood smear images of size 640X 480
N H Abd Halim, et al. 2011 [17]	To improve image quality	A global contrast stretching and segmentation WBC based on HIS color space	Not mentioned	Details about image acquisition is missing	Not mentioned
Jun Duan, et al. 2011 [18]	Color image segmentation technique	Integrate color histogram based method and region growing and merging-based segmentation method	Not mentioned	Segmentation results vary if there is cell adhesion.	Not mentioned
P Sukumar, et al. 2015 [19]	To detect abnormal WBC nucleus in cervical cancer cells	Fast particle swarm optimization with ELM method (extreme learning machine, standard particle swarm optimization)	90%	Execution time of the techniques are mentioned.	50 images
F Boray Tek, et al. 2005 (Springer) [20]	To segment various components of blood.	Cell area is computed using area granulometry. Minimum area watershed transform, circle radon transform.	95.40%	Transform used is not completely capable of detecting under or over	20 microscopic blood cell images

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				segmentation.	
S. H. Rezatofighi, H. Soltanian-Zadeh ,et.al.2009 [21]	Work on segmentation of WBC's	Method is based on orthogonality theory and Gram-Schmidt Orthogonalization	93.02%	Simple to implement, quick & efficient. But new vectors needed for new images	251 blood smear images
A S Abdul Nasir, et al. 2011 [22]	To obtain a fully segmented abnormal WBC and nucleus	Unsupervised segmentation technique namely k-means clustering	99.02%: WBC, 99.05%: nucleus	Combination between linear contrast technique and color segmentation based on HSI color space	100 images
Lim Huey Nee, et al. 2011 [23]	A work on WBC segmentation	Gradient magnitude, thresholding, morphological operations and watershed transform.	94.5%	Drawback of the system is incomplete localization of the cells of interest.	50 images
Hemant Tulsani, et al. 2013 [24]	An Approach for counting blood cells.	Segmentation using morphological watershed transformation and regional maxima computation	94.44 % counting efficiency	Eliminates problem of overlapping cells. Component labeling algorithm is mentioned used as counting algorithm.	20 blood cell images

Table 1: Comparison of various segmentation and classification techniques

Range of sensitivity lies between 0 and 1. As the value approaches 1, system is highly sensitive in identifying correct WBC.

1 Sensitivity is the probability of correctly identifying a Leukocyte (WBC) cell.

$$Sensitivity = \frac{TP}{(TP + FN)}$$

2 Specificity determines the probability of correctly classifying other cell.

$$Specificity = \frac{TN}{(TN + FP)}$$

Range of Specificity lies between 0 and 1. As the value approaches 1, system is highly specific in detecting other cells.

3 Misclassification is the total number of cells classified incorrectly and is given by

Misclassification= FP+FN

The range of misclassification lies between 0 and 1. 0 indicates absence of wrong classification i.e. all WBC's and other cells are classified respectively. 1 indicates systems incapability in identifying correctly WBC's and others.

4 Accuracy $=\frac{(TP+TN)}{(TP+TN+FP+FN)}$

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Accuracy is systems performance measurement parameter. Its range lies between 0 and 1. 1 indicates 100% accurate system.

Execution Time is the time taken by the system from segmenting, correctly classifying and counting a normal or abnormal WBC's. Execution time should be as minimum as possible. Usually in terms of milli seconds to micro seconds or even less.

The system sensitivity and specificity should approach 100%. Misclassification should approach 0% claiming highly accurate system with value approaching 100%. Execution time should be in terms of micro seconds.

IV. CONCLUSION

This work reveals most of segmentation and classification methods used in isolating and counting WBC's in microscopic blood smear images. Main causes of inaccurate or failure in segmentation step are contract of nucleus, overexposed images, clustering of leukocytes, similar leukocyte erythrocyte color, Some neutrophils not resembling typical multilobed nucleus and some image articrafts.. Before selecting any method thorough study of structure, area, development stages, staining effects, illumination conditions, and contextual information of cells is utmost importance. Most important is to select moderately illuminated and uniformly stained smear slide images. For improved classification accuracy, typical images should be considered in the training set of samples. Contextual information, marker based, color features based segmentation techniques blend completely into such application.

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