

## A Novel Method For Scanning Electron Microscope Image Segmentation And Its Application To Blood Cell Analysis

Syed Hamad Shirazi<sup>1</sup>, Arif Iqbal Umar<sup>1</sup>, Saeeda Naz<sup>2</sup>, Syed Fahad Shirazi<sup>2</sup>,  
Muhammad Imran Razzak<sup>2</sup>

Department of Information Technology, Hazara University, Mansehra, Pakistan

<sup>2</sup>Department of Telecommunication Engineering ICT, Islamabad Pakistan

<sup>3</sup>Department of Computer Science King Saud University, KSA

<sup>4</sup>GGPDC No.1 Abbottabad, Higher Education Department, KPK, Pakistan

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### ABSTRACT

Automatic Recognition and inspection of human blood cells in microscopic images are of paramount importance, which can assist the hematologist in diagnosing several diseases like Leukemia, AIDS, blood cancer, syndrome, anemia and Malaria etc. Extraction of numerical values from microscopic images represents a tricky challenge. Such type of research efforts include normalization of images, image segmentation followed by feature extraction and its classification. In this paper we have proposed a novel method for blood analysis that automate the diagnosing of several blood disease like RBC, WBC, platelets, leukemia, Aids, syndrome, anemia, malaria, blood disorder, iron deficiency.

**KEYWORDS:** SEM, Segmentation, RBC, WBC, Fuzzy C-Means, GLCM

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### 1. INTRODUCTION

Automated analyses of Microscopic images and pattern recognition approaches have been widely utilized in the area of pathological analysis to help out pathologist in identifying diverse patterns and cells in blood smear. A conventional scanning electron microscopy (SEM) image reveals high contrast and signal to noise ratio (SNR), with capabilities of high magnification ranges from millimeter to nanometer scales. Current advancements in the field of nano-technology reveal that electron microscope image analysis is a powerful tool for analysis of particles and blood cells at minuscule level [1].

The genomic technological advancements have opened a new domain for earlier encounter of diseases that reveals the possibility to minimize the shortcomings of manual detection and recognition technologies. For quantitative blood cell analysis automatic counting machines and flow cytometry are automated instruments for blood cell counting which can examine blood cells but they cannot qualitatively analyze blood cells. Nevertheless 21% of blood samples analyses still need the intervention of microscopic expert review [2]. The microscopy and camera based approaches can perform both the qualitative and quantitative evaluation of blood cells. However, still extensive research is required in this area [3]. Therefore, numerous efforts have already been made to develop camera based automated blood cell analysis using image processing techniques. Blood cell segmentation and morphological analysis and recognition of the entire blood cells are very challenging tasks due to the complexity of the blood slide image, cell shapes and their similarity among themselves.

Blood cell images are very complex due to overlapping of cells. The variance in ration of blood cell in blood samples indicate various diseases i.e. on the basis of the blood cell maturity it can be categorized into 20 different types. For the automatic detection of infection in human blood various works are in progress to analyze the microscopic blood cell images. There are different approaches have been proposed in literature for automatic segmentation and classification blood cells. We propose efficient machine based blood morphological analysis. We target red blood cells, white blood cells and its types, platelets, blood cell disorder, malaria parasites and cancer cell count and their morphological analysis. We propose novel method for cell segmentation and fused different mythology (features, color domain, classifier) in order to get better results. We are expected to detect several diseases such as malaria, blood cancer, thalassemia, Aids, Leukemia, disorder in RBC and WBC etc. efficiently. The objective of this study is to develop an automatic blood sample

analyzer to diagnose the blood disease by performing several automated test. However in this paper our work is confined to the segmentation of blood cell microscopic images.

## 2. RELATED WORK

The microscopic blood cell images contain white blood cells (WBC), red blood cells (RBC) and platelets which are scattered across the background. These blood cells having variations in shape and structure however the images contain complex shapes which makes segmentation and classification tough. The variation in features like shape and size change can be used for the indication of diseases. Similarly white blood cells can be categorized into five different types i.e. lymphocyte, basophil, eosinophil, neutrophil and monocyte [8] which can also be used in detection of diseases.

Generally, there are five major steps of analyzing hematological images [3] , [ 4] ,[ 8].

- a. Image Acquisition
- b. Preprocessing
- c. Segmentation
- d. Feature Extraction
- e. Classification

Various techniques for contrast enhancement and illumination are proposed in literature. In [9] authors proposed an approach for contrast enhancement of parasite and RBC. The Technique is based on histogram equalization. The authors in [10] have used histogram equalization for image enhancement. In [11] [12] illumination correction is used for image enhancement. The authors in [13] used diagonal model for illumination modeling. Morphological operation has been widely applied in literature [4, 14]. Sabino et al. performed G channel histogram calculation, by identifying a threshold of the value ranges between (100, 200) for generation of a binary image [15]. In order to remove illumination and reduce noise, several research methods have been presented in literature as discussed above. The preprocessing phase can be enhanced by using the multispectral images along with each separate channel form both HSV and RGB domain.

In the analysis of automatic blood imaging procedures, the most significant and challenging part is segmentation because the blood cells are often overlaid with each other and is the basis of quantitative analysis [16]. Moreover, the color and intensity of blood slide image often vary due to instability of staining. Cell morphology, light variation and noise are the other factors that makes segmentation difficult task. Blood cell manual segmentation by human for visual assessment requires a large amount of tedious work and prone to error due to subjective expert view [2]. Recent study has suggested several segmentation methods for blood cell segmentation but the reported segmentation results still need enhancement [8][31]. Liao and Deng introduced shape analysis for white blood segmentation first [17]. Approaches for blood cell segmentation are classified into three categories i.e. graph cut based approaches, active contour model and traditional approach for segmentation [4]. The traditional approaches for image segmentation are based on water-shed methods, thresholding and edge detection. Leukocyte segmentation is an uphill task, because of the presence of overlapping cells which are often overlaid with each other. Due to instability in staining variation in color and intensity of an image occurs which makes segmentation more difficult.

In the meantime, other factors also contribute to the difficulty in segmentation, like: five types of leukocyte (WBC) and variation in their morphologies and noise. In literature different segmentation methods are used for the segmentation of blood cells microscopic images like region growing it is a fast and reliable method for uniform images but this method is inconsistent as for as the variability in images is concerned. Similarly watershed segmentation also produces poor results when the images contain overlapping complex objects [1]. Similarly active contours based method [30] also suffers from overlapping objects and the presence of overlapping objects makes the segmentation process more tedious.

Textural [18-20] and color features [20-22] are very imperative to segregate cells from each other and its background. They are also extensively used for blood cell recognition, texture features. Color features plays important role in order to differentiate similar shapes and overlapped cells. The blood cell images are composed of three color components: red, green and blue, for each of the pixels in the images. The color characteristics and other features are need to be calculated in each of the color components i.e. each of the malaria parasites features a particular color tone (blue ring).

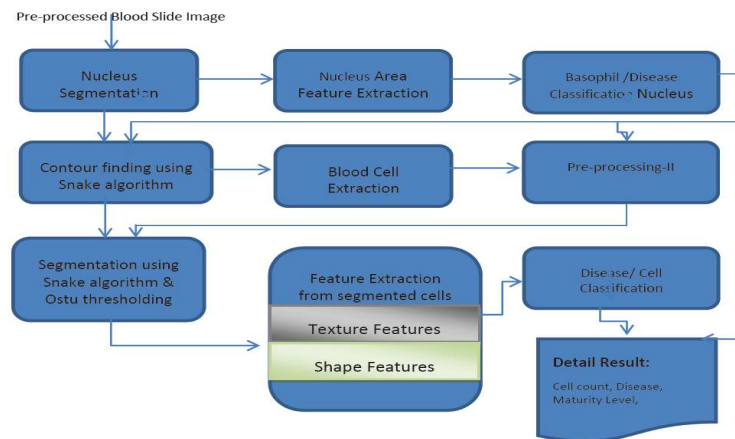
Rezatofighi and Zadeh, Sabino et al. and Li et al. extracted morphological features i.e. area, circumference, shape factor, rectangle factor etc. [3] , [14] , [23] as well as chromatic features that are used

after morphological identification in order to increase accuracy [23].Huang et al. extracted 85 features, including 5 shapes and 20 texture features, to identify the type of leukocyte [24].They have used PCA a statistical method to transfer 85 features into 7 features.

The aim of feature selection is dimensionality reduction of feature vector attributes smaller than the original and generates a new feature vector with a higher discrimination power in order to improve the classifier performance. Several classifiers have been reported for the computerized recognition of malarial parasites form blood cells images [32]. Bayes classifier and different types of Artificial Neural Networks (ANNs) [3, 18, 25, 26] local linear map [16], SVM [3][27] K-mean [26][28] and fuzzy system[29] has been extensively used as classifier in the literature for blood cell recognition. To overcome the effects of disease it is important to detect and diagnose it in the earliest stage.

### 3. METHODOLOGY

We have proposed a novel blood sample analysis and recognition approach that automate the diagnosing of several blood disease like RBC, WBC, platelets, leukemia, syndrome, anemia, malaria, blood disorder, iron deficiency etc. Blood cell segmentation and morphological analysis is one of the most important and challenging phase in machine base blood analysis due to the complex cell nature uncertainty in microscopic videos. We propose a novel method for blood cell segmentation and morphological analysis by fusing the color domain and using color features along with textural and geometrical features. The research not only focus on the detection of specific disease or cell count but also perform several test in order to find the maturity level by performing the cell morphological analysis. We will use both spectral imaging and HSV color space in order to differentiate similar cells and overcome the illumination issue. We will focus on text features, GLCM as well as shape defining features and for classification, SVM and neural network will be use along with fuzzy divergence. The fuzzy divergence will help us to define the maturity level of attacked virus or specific disease.



**Figure 1**Proposed solution

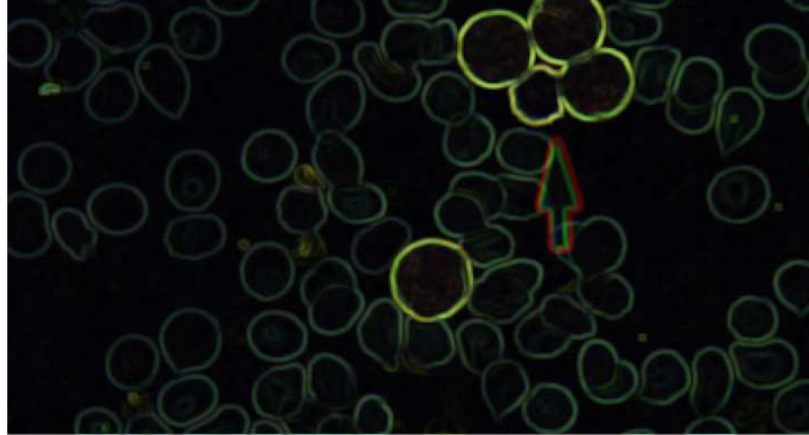
#### 3.1. Illustration of proposed Methodology

With respect to implementation point of view, Intelligent Blood sample is divided into five steps.

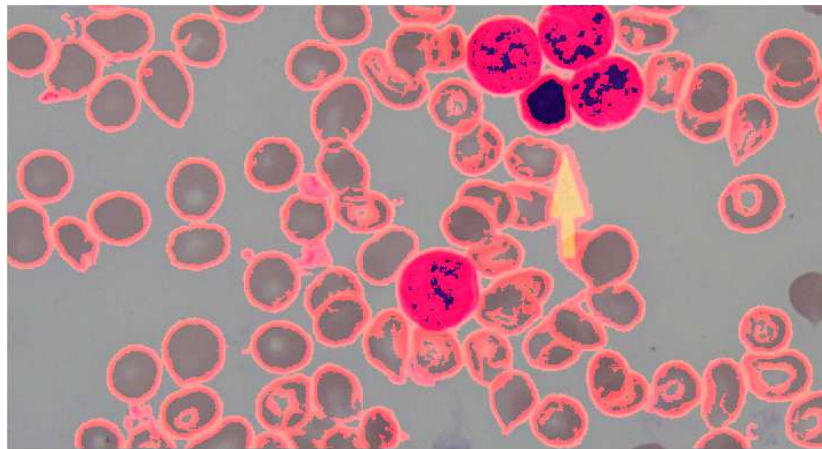
1. In the first phase, we have build a database of blood cell images that are captured through multispectral microscope. Based on the target operations, several blood cell images were taken at different scaling factor and different camera resolution in order to increase the visibility factor.
2. We have used several filters both in spatial domain and frequency domain i.e. Wiener and Laplace filters in order to remove the noise and segment the required images form the background.
3. We have used the Gram-Schmidt orthogonalization and snake algorithm segment the blood cell, their nucleus and cytoplasm. The Gram-Schmidt methodproduces a set of vectors. Each feature vector will be reflected as vector and elements of this feature vector will be pixel intesities in RGB and LUV space. Moreover, we have used snake algorithm in a case when edges are not clear and well defined
4. GLCM is used for the computation of summing all the texture information in segmented blood cell

image. Blood cells classification accuracy may also be affected by the shape features i.e. size, shape, area etc. We have used shape feature along with textural features and morphological features.

5. Multilayer perceptron neural network is used for the blood cell recognition. The objective of this stage is to recognize the segmented image.



**Figure 2: Boundary Detection**



**Figure 3: Texture image**

### 3.1. Validation of Results

Statistical measures sensitivity (also known as true positive rate or recall rate) is used to find the proportion of actual positives which are identified correctly. Specificity (also known as true negative rate) is used to identify the proportion of those negatives which are correctly recognized.

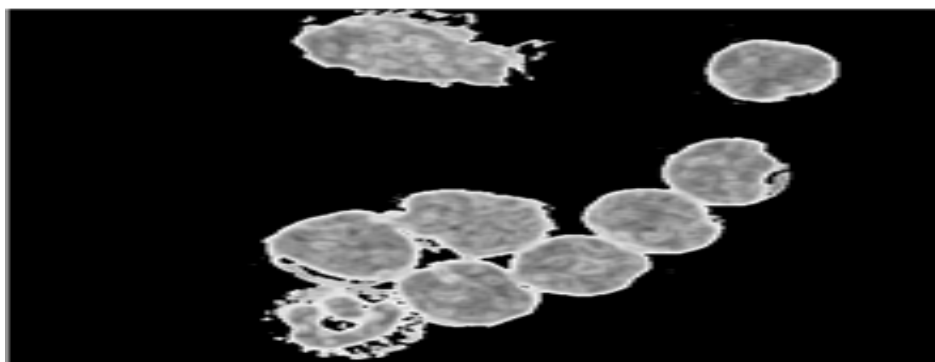
**Table 1: Specificity and sensitivity**

	Blood smear with disease	Blood smear without disease	Sum
Positive	A	b	a+b
Negative	C	d	c+d
	a+c	b+d	

From the above table 1 (a+c) means that all the blood smears with disease and (b+d) means all the blood smear without disease.

$$\text{Sensitivity} = a / (a+c)$$

$$\text{Specificity} = d / (b+d)$$



**Figure 4: Segmented Image of WBC**

#### 4. CONCLUSION AND FUTURE WORK

In this paper we have discussed the problems pertaining to automatic identification of cell in blood smear microscopic images. We have proposed an automated SEM image analysis method for the identification and classification of blood cells. We have identified several problems related to image preprocessing, segmentation feature extraction and classification. In image preprocessing the problems arises due to color variations, illumination and noise presence in microscopic images. In literature different methods are presented but it is difficult to say that there is no generalized method of image normalization which can increase the overall accuracy of classifier. Another main hurdle in automated blood cell analysis is segmentation which has direct impact on the performance of classification. That is why accurate segmentation is vital which the main focus of our work and our method has accurately segmented the overlaid and complex structures present in microscopic images of blood cells.

Another major challenge of this area is the lack of large dataset for the testing of this automated system which makes the trustworthiness of prevailing systems uncertain. Further it also required the manual analysis of each image by the expert so that the results can be compared with each other and manual data. In our proposed method we have addressed these major challenges and hopefully we will get fruitful results in feature extraction and classification phase.

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