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Efficient Enhancement and Segmentation of Leukocytes From Microscopic Images

Syed Hamad Shirazi[1], Arif Iqbal Umar[1], Saeeda Naz[1,4], Nuhman Ul Haq[2], Muhammad Imar Razzak[3]

[1]Department of Information Technology, Hazara University, Mansehra, Pakistan
[2]Department of Computer Science, COMSATS Abbottabad Pakistan
[3]Department of Computer Science King Saud University, KSA
[4]GGPDC No.1 Abbottabad, Higher Education Department, KPK, Pakistan

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ABSTRACT

Differential counting of various types of blood cells provides vital information for the the analysis of wide range of diseases in blood cells. Until now, traditional manaul approaches are practiced for the diagnosis of blood cell's diseases. However, these orthodox methods are subject to sampling error, tedious and too much time consuming. The accuracy of such methods rely on the expertise of an expert, while the accuracy of automated blood cells analyzer depends on the segmentation of objects present in the blood cell images. Despite a lot of efforts still accurate segmentation and blood cell counting is a major challange. Complexity of shapes and overlapping of blood cells objects in microscopic images make the segmenation process more difficult. In this paper we have proposed a novel method for blood cells image segmentation.We have used curvlet transform along with wiener filter for image enhancement and noise removal. For image segmentation and boundary detection we have used Gram-Schmidt orthogonalization and snake algorithm respectively.
KEYWORDS: Segmentation, RBC,WBC, Curvelets, Wiener Filter, SEM.

1. INTRODUCTION

In the field of computer vision, digital image processing and pattern recognition image segmentation is a technique that divide a gray level or color image into homogenious regions. Image segmentation is arguably the most important low level task of computer vision. The main purpose of segmentation process is to identify clusters and extract useful information in image. The the accuracy of blood cell counting rely heavily on the results of image segmentation. For blood testing blood cell counting is used to
count the number of blood cells (WBC, RBC) in blood sample. There are two methods in practice for blood cell counting.

For blood cell counting two approaches are in practice manual blood cell counting and automated blood cells counting. Manual blood cells counting is performed by the well trained physician through visual assesment of blood smears by means of light microscope [1]. This approach require more time and human efforts along with specialized instruments and specialized personnels. The other approach is automated blood cell count which uses automated blood cells image segmentation, pattern recognition and computer vision techniques to analayze blood cells microscopic images. The quantification of these cells is vital for the diagnosis and detection of numerous diseases like cancer and various other fatal diseases [18]. As compared to manual methods automated blood cell counting is more flexible and yeild accurate results.

However; still there is a great potential for furher improvements, especially the segmentation of overlapping objects in microscopic images is challenging task in performing accurate blood cells counting and analysis.

Hematologists or pathologist in cell based diagnosis using cell count, geomatrical features of cells and their distribution for decision making.In literature mumerouse computer vision technique are proposed to extract and retrieval useful information from microscopic and medical images.The complexit of cell shapes and overlapping of cell boundaries makes manual analysis difficult. The same problems are also requiresconcentration during the automation of blood cell analysis. The computer aided systems for blood cell analsysis depends on the accurate segmentation of blood cell images. To increase the accuracy disesease detection and accelerate the diagnosing process the development of state of the art automated systems is vital.

Corresponding Author: Saeeda Naz Department of IT Hazara University, Mansehra Pakistan
{syedhamad, saeedanaz292}@gmail.com,

For feature extraction and classification phase accurate segmentation of blood cell images is vital. The aim of image segmentation is to trace different objects existing in SEM images. The image segmentation not only focus on the partitionning of image into objects and regions but it also focus on the discrpancy among various regions[2]. Region base segmentation and contour based image segmentation are the two main approaches of image segmentation. Morphological operators and watershed segmentation are the technique belonging to ragion based segmentation. The process of automated blood cells analysis consists of four steps viz preprocessing, image segmentation, feature extraction and classification shown in figure 1. In this paper our work is confined to image pre-processing and image segmentation of leukocyte.

2. RELATED WORK

In the recent decade, the interest of researchers is increasing in the development of algorithms for automated analysis of medical images and especially blood cell image. Researchers have made a lot of improvements by the integration of artificial intelligence, image processing, and pattern recognition and computer vision techniques to the blood cell analysis. There are several automated systems developed for medical diagnosis which are helpful for doctors to detect diseases particularly in red blood cells(RBC) and white blood cells(WBC) which is vital for curing the disease[5].

The leukocytes provides protection to the body against infections and viral attacks. The quantification of leukocytes plays an essential role in the diagnosis of several disease. WBC consists of two types of cells granular (polymorph-nuclear cells) white blood cells and non-granular (mononuclear cells) white blood cells.The granulocytes are further divided into three categories of granules i.e. basophiles, nutrophils and eosinophiles, while the non-granular cells consists of only tow types of cells lymphocytes and monocytes [6]. WBC cells are colorless which requires the process of staining with different chemicals to make leukocyte colorful and visible under microscope. The staining process causes variation in color intensity. During the image acquisition and formation of blood smear microscope images the quality of image may be distorted by various types of illuminations. These images are also affected by camera lenses and exposure of time as well.

Image enhancement is essential to resolve the illumination issues. In literature several image enhancement techniques are applied to remove noise. Histogram equalization is used for the contrast enhancement of RBC in [7][21]. In [8] the authors have used adaptive histogram equalization for the image enhancement [9][10]. Illumination correction is performed in [11] by utilizing pre-defined illumination factor. The authors in [12] have applied paraboloid modal for illumination correction to resolve the issues relevant to feaatures visibility under white light. Now a days polarized filters are used in light sources, however; issues related to color illumination are still existing and require image enhancement.

Image segmentation is the most imprtant phase in automated blood cell analysis. This is also very challenging phase because of complex cell nature and overlapping of cells. The image segmentaion of microscopic blood cell images yeilds different single cell images. These images can be further divided into three distinct regions i.e. cytoplasm, cell nucleus and the background. A lot of work in literature is focused on the segmentation of white blood cells and its differential counting. In [13] the authors have proposed edge detection technique based on HIS (Hue Saturation Intensity) model is used for segmentation [13]. In [14] color features based selection and histogram thresholding is utilized for cytoplasm and nucleus segementation. Non supervised technique for nucleus detection is performed in [15] they have used the G channel of RGB coordinates for nucleus color segmentation.

The blood cell image segementation algorithms can be classified into three broad categories: Graph cut based segmentation, traditional segmentation and active countour models[15]. The traditional method for segmentation is based on state of the art watershed segmentation, edge detection and thresholding methods for segmentation.The WBC segmentation is a challenging task because of overlapping of cells and complexity of shapes of different types of leukocytes. Moreover, the instability of staining process causes variation in color and intensity of image frequentyly. Meanwhile, there are other factors which also contribute in making the segmentation process more tedious like variation in morphology of all classes of leukocytes,

light and noise variations. A novel approach for segmentation of WBC is presented in [16]. The have used color and shape features of the nucleus. For classification they have used SVM[17]. In automated blood cell analysis blood cells image segmentation is most challenging task, becuase it has great influence the performance of the classification. Despite numerous efforts on image segmentation a lot of improvements are required. Still the segmentatiion of overlapping blood cells, complex objecs and shape variations present in the microscopic image. In this article our work is focussed on blood cell image enhancement and image segmentation.

3. METHODOLOGY

Automated blood cell analysis comprising of image acquisition, image preprocessing, image segmentation, feature extraction and classification. Our work in this paper is confined to image enhancement and segmentation. The block diagram in figure1 shows our proposed methodology for microscopic image segmentation and enhancement.

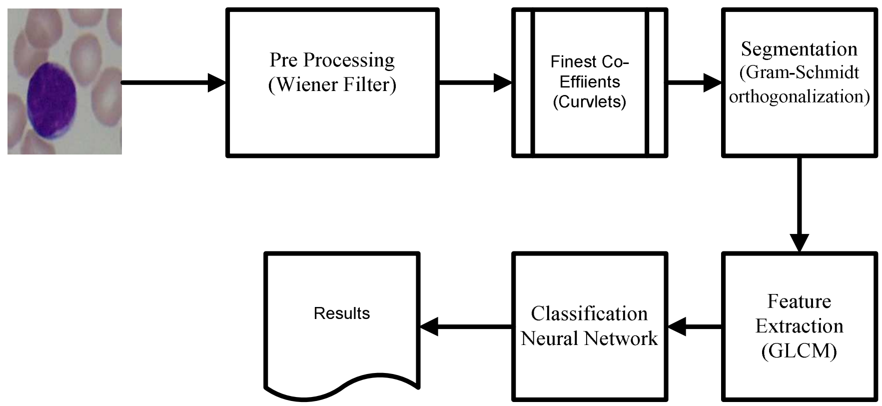

Figure 1: Proposed solution

3.1. Image smoothening Blood cells microscopic images are acquired by using bright field microscopy. Different illuminators like HBO, XBO and LED compromise the quality of image. The variations in illumination causes degrade the efficiency of both the automated and manual blood analysis. This may also lead to biased analysis of blood cells. Therefore, image enhancement is required to minimize the cuases of variations. This preprocessing will improve the image segmentation which will improve the accuracy of blood cell classification and counting.

For image preprocessing we have used Wiener filter to remove the noise and effects of illuminators. The Wiener filter not only remove noise it also conserves the edge details. Wiener filter is statistical in nature due to which it adopts least square (LS) approach for image restoration or signal recovery insipte of noise presence. Wiener filter can not be directly applied to 3D images. Therefore, separation of RGB channel is vital. We have applied Wiener filter to each RGB channel separately. It calculates the mean and variance of each pixel around. In order to get the finest detailed co-efficients of noise free image we have applied Forward Discrete Curvelet Transform (FDCT). As blood cell microscopic images contain complex and overlapping objects therefore, curvelet transform is capable of sensing the contours and curvy edges. Curvelets has high capability of capturing singularities along with multi directional sensitivity.

To extract feature points edge and singularity details are used. After gaining the highest detailed coefficients Inverse Discrete Curvelet Transform is applied to high frequency band to acquire the detailed image. This detailed image now contains the stronger and better edges than the input image and would achieve better in lending edge details to the segmentation step.

3.2. Image Segmentation

The image segmentation is the utmost vital phase in automated hematological image analysis. The ultimate results of subsequent stages depend on the accuracy of segmentation. Our proposed image segmentation technique is based on Gram-Schmidt orthogonalization and snakes algorithm.

For segmentation, we have used Gram-Schmidt process[19][20]. This method is used in mathematics and above all in numerical analysis for orthogonizing a set of vectors in the inner product space. The Gram-Schmidt process each feature is considered as vector. The pixel intensities in RGB space are the elements of each vector.

The Gram-Schmidt method accepts linearly independent set S={v1,v2,v3...Vn} and generate orthogonal set S={u1,u2,u3...un}. Where u and v denotes the vectors having inner product <u ,v>.

$$u1 = v1 \qquad e1 = \frac{u1}{|u1|}$$
$$u2 = v2 - Proj_{u1}^{v2} \qquad e2 = \frac{u2}{|u2|}$$
$$u3 = v3 - Proj_{u1}^{v2} - Proj_{u2}^{v3} \qquad e3 = \frac{u3}{|u3|}$$

$$W_k = v_{vk} - \sum_{j=1}^{k-1} Proj_{uj}^{vk} \qquad (1)$$

Calculating the Wk, in equation 1 by using Gram-Schmidt method, by applying it to a color image in order to exaggerate the required color vk. By applying this process we get a composite image the region of interest with the required color have maximum intensity while the remaining have minimum color intensity. It requires proper thresholding which produce the desired segmentation. This process amplifies the desired color vector while reduce or weakening the undesired vectors.

The snake algorithm performs better segmentation where the edges are not well defined. The snake algorithm is useful in shape detection. It can be parameterized contour defined within image domain. Forces within the image domain control the bending features of the line. Forces like image gradient pushes the snake towards the image features. As the snakes coordinate vector can be defined as C(s)=(x(s),y(s)) so the total energy can be represented as.

$$E(s) = \int_0^1 \left(E_{int}(Cs) + E_{ext}(f,(Cs))\right) ds \qquad (2)$$

Where in equation 2 E_{ext} is the image forces, while f is the image intensity and E_{int} is represent the internal energy of the snake produced due to the discontinuity. The E_{int} inflict the snake to be small and smooth. It eliminated the wrong solution. The E_{ext} represent the external energy which is useful for detection of the objects and boundaries in the image.

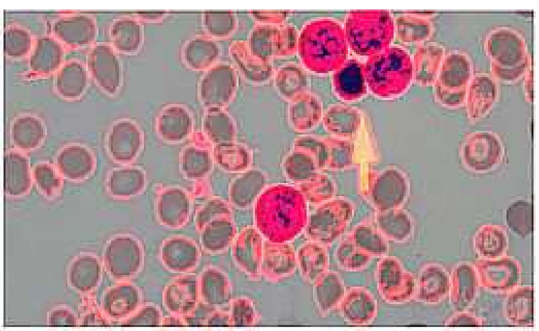
Figure 2: Segmented Image of WBC

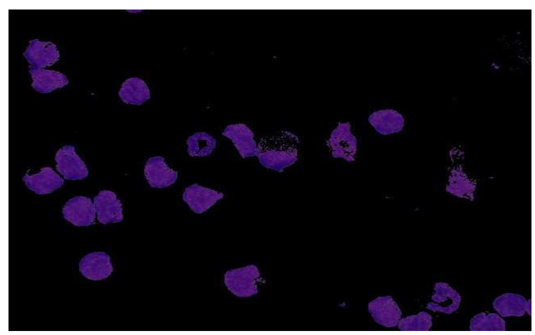
Figure 3: Segmented Image of WBC

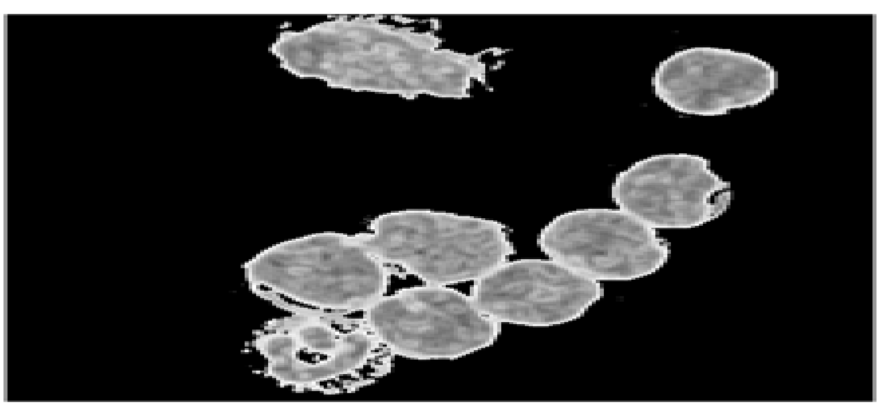
Figure 4: Segmented Image of WBC

Various approaches of white blood cell image segmentation and classification are existing in literature but these methods having limitations. The most popular method are thresholding approaches which is based on otsu thresholding, region growing and watershed segmentation. All these approaches suffer from inconsistencies when the images containing variations. Many authors have used genetic algorithm for WBC segmentation in literature, the main hurdle in accurate blood cell segmentation is the presence overlapping and irrelevant objects. Approaches like Support Vector Machine (SVM), Artificial Neural Networks (ANN), K means clustering and Fuzzy C-means yeilds poor results for those images having complex background and shapes. Despite these problems our proposed method has produced promising results for microscopic images having complex shapes, background and overlapping objects.

4. CONCLUSION

Image enhancement and segmentation are the crucial phases for feature extraction and counting of blood cells. The accurate blood cells segmentation is highly challenging in presence of complex shapes, intensity variations and overlapping objects in blood cells microscopic images. In our proposed method we have used sanke algorithm along with Gram-Schmidt orthogonalization method to tackle inaccurate white blood cell segmentation.We have combined the capablities of snake algorithm and Gram-Schmidt orthogonization method which has produced promising results. For image enhancement we have also combined Discrete Curvelet Transforms (FDCT) and Wiener filter which ensures the accuracy of image segmentation. In our future work we will utilize these results for feature extraction and classification of Leukocytes.

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