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# White Blood Cells' Segmentation for the Detection of Acute Lymphoblastic Leukemia

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**Abstract—***Acute lymphoblastic leukemia (ALL) is the most common hematological neoplasia of childhood. The characterization of ALL is figured out by rapid uncontrolled growth of immature leukemic cells (named as blast cells) in bone marrow, lymphoid organs etc. The most important preliminary step in the diagnosis of ALL is the morphological analysis of the blood and bone marrow smear under the microscope. Nowadays, this is performed manually by skilled operators. The process have numerous drawbacks, such as slowness of the analysis, non-standard accuracy, dependency on the operator skills etc. Also the nonspecific nature of the signs and symptoms of ALL often leads to wrong diagnosis. The low cost and efficient process is to use a computerized system for image analysis of stained blood microscopic images for quantitative examination and ALL detection. This paper presents a description of a robust segmentation technique combining a few segmentation subsystems, which makes the further process of feature extraction and classification of blood microscopic images easier for diagnosing the presence of ALL and will help out to make a perfect automated system in future.*

**Keywords:** *Acute lymphoblastic leukemia (ALL), Image segmentation, Automatic cell identification, Cell features extraction, Lymphocyte classification.*

## 1. INTRODUCTION

Leukemia, the name comes from the ancient greek 'leukos' (white) and 'haima' (blood). It is a type of cancer of the blood or bone marrow characterized by an abnormal increase of immature white blood cells called 'blasts'. Large numbers of white blood cells are detected, when a blood sample slide is viewed under a microscope. These extra dysfunctional white blood cells can also interfere with the other cells present in blood, causing a harmful imbalance in the blood count. Due to the rapid spread of the blasts in the blood stream and in other vital organs, it can be fatal for our body if left untreated [1]. It mainly affects young children and adults over 50. Above 90% of leukemias are diagnosed in adults [2]. Early diagnosis of the disease is crucial for the recovery in the case of children. The observation of the peripheral blood slide by expert operators is one of the diagnostic procedures available [1]. The cytogenetics and immunophenotyping diagnostic methods are currently preferred for their great accuracy rather than the method of blood cell observations which depends on the operator capabilities and tiredness. Instead of these processes, the morphological analysis just requires an image of blood slide, not a blood sample, and hence it is suitable for low cost, standard, accurate and remote diagnostic systems.

## 2. CLASSIFICATION OF LEUKEMIA

A blood microscopic image is shown in Fig. 1. The principal cells present in blood are RBCs, WBCs, and Platelets.

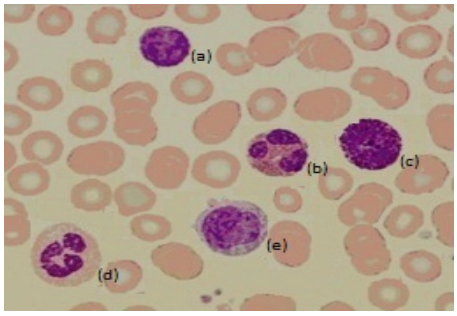
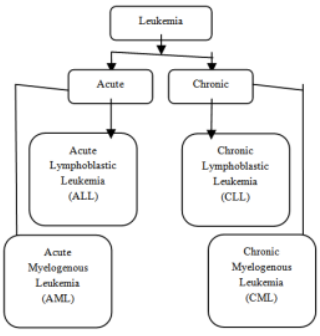



**Fig. 1: White blood cells marked with colorant : (a) lymphocyte, (b) neutrophil, (c) basophil, (d) eosinophil, (e) monocyte. Other elements are red blood cells.**

In the blood slide image, the WBCs are bigger than RBCs. WBCs are subdivided in Granulocytes (Neutrophil, Eosinophil, Besophil) and Agranulocytes (Monocyte, Lymphocyte). Leukemia can be detailed as a spectrum of diseases which affects the blood, bone marrow and lymphoid system. Clinically and physiologically leukemia is subdivided into acute and chronic forms.

**Table 1: Major kinds of leukemia**



Except these four types, there are also HCL, T-PILL, adult TCL etc.; But Acute Lymphoblastic Leukemia (ALL) detection is the main purpose of this paper. The classification of ALL is done by morphological analysis in the French-American-British (FAB) method [3]. Recently it has been reported with the non-image based immunologic classification [1]. The lymphoblasts of different variability and patterns are analyzed using the FAB method [4] and shown in Fig. 2.

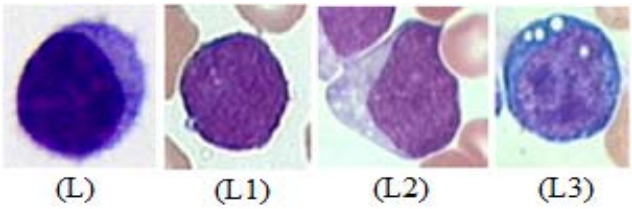



**Fig. 2: Morphological variability of blast cells according to FAB classification: (L) healthy lyphocytic cell, (L1), (L2), (L3) are lymphoblasts.**

- **L1**–Blasts are homogeneous and small with round nuclei and inconspicuous nucleoli. Cytoplasm is scanty and without vacuoles.
- **L2**–Blasts are large and heterogeneous with large and cleft nuclei. Large nucleoli are present. Cytoplasm is often abundant and may contain vacuoles.
- **L3**–Blasts are moderate large and homogeneous with round oval shape nuclei. One or more prominent nucleoli are present. Volume of cytoplasm is moderate and contains vacuoles.

## 3. DIAGNOSIS

The early and fast identification starts with the Complete Blood Count (CBC) [5]. If CBC is abnormal, then the patient is suggested to perform the bone marrow biopsy. The study of morphological bone marrow and peripheral blood film analysis is done by a hematologist [6]. Manual examination of the slides is subjected to bias, resulting in inconsistent and subjective reports.

An automated blood slide image analysis system can be a useful tool. The segmentation step is very crucial; because, the accuracy of the subsequent feature extraction and classification depends on the correct segmentation of WBCs. An automated method can be divided into following steps [5].

i. **Segmentation**–Based on the different characteristics, the cells are separated from the background.
ii. **Identification of WBCs**–By using color information and an accurate algorithm, the WBCs and RBCs are classified.
iii. **Identification of Lymphocytes**–By analyzing the shape of the nucleus, the lymphocytes can be distinguished from the other WBCs.
iv. **Identification of candidate Lymphoblasts**–By the analysis of morphological deformations, candidate lymphoblasts are identified.

Only a few attempts of partial/full automated system for leukemia detection based on image processing exists today. Ceske used automatic thresholding method [7]. The two step segmentation processing using HSV color model along with K-mean clustering method is utilized by Sinha and Ramakrishnan [8]. Watershed transform method has been reported in [9]. Cell segmentation using contour model has been presented in [10]. A system for classification of single white cells has been presented in [11]. Each of these techniques has some drawbacks or limitations. If some techniques are combined for segmentation, they will present a prominent output image, from which accurate feature extraction and classification can be done. The following section describes three best segmentation techniques which are combined to have clear and exact lymphocyte cell segmentation. The first technique provides the features of a lymphocyte from a grayscale image, the second technique is used to have the color features, and the third technique is taken into account for the distance measurement between those lymphocytes.

## 4. SEGMENTATION METHOD 1 (EDGE DETECTION TECHNIQUE)

The proposed system for segmentation is shown in Fig. 3.

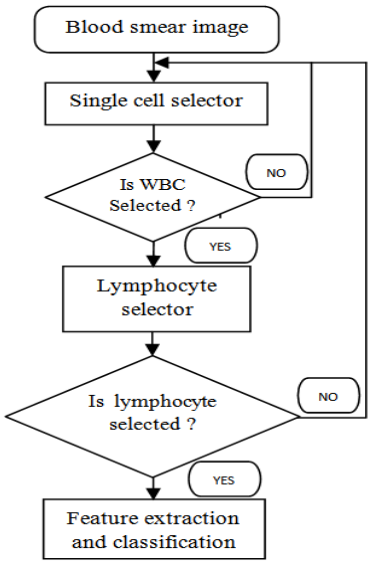



**Fig. 3: System for segmentation.**

It individuates the leukocytes followed by the contrast enhancement of the image; then extraction of the lymphocytes is done and finally classification is done using the lymphocytic identifier.

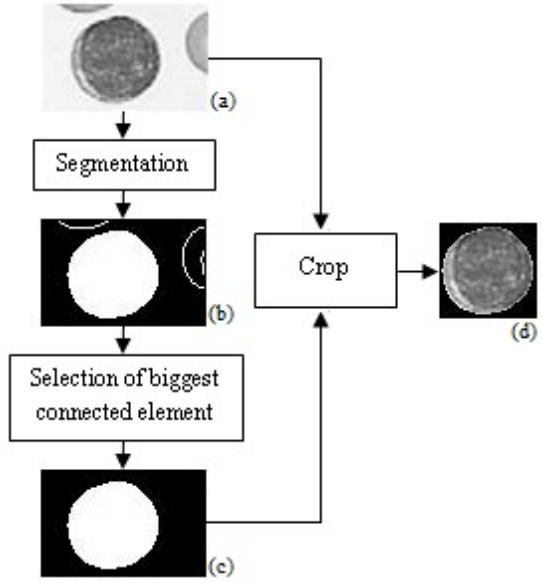



**Fig. 4: Selection of the lymphocytic cell.**
**(a) lymphocyte from image, (b) hole filled image,**
**(c) selected the biggest element, (d) crop lymphocyte image**

The individual lymphocytic image output from the first system is the input of the subsystem shown in Fig. 4. The processing of membrane, cytoplasm and nucleus is done and all the indexes directly go to the classifier [12, 13].

### 4.1 Lymphocytic membrane detection

1) For better performance, sobel filter is used to enhance the border of the membrane.
2) Canny based filters are used for edge detection which reconstructs the border of the membrane.
3) To better connect the separated points of the membrane, the morphological operator 'dilation' is used.
4) After dilation, the internal hole of the connected element with biggest area in the processed image is filled.
5) Using that hole filled image, the next subsystem selects the connected element with biggest area and it corps the selected lymphocyte from the original image.

## 4.2 Lymphocytic Nucleus and Cytoplasm detection

The nucleus and the cytoplasm will have to be selected from the image generated at the previous step.

Otsu's thresholding method shows a good performance in separating the cytoplasm and the nucleus. It simply chooses the gray level threshold to minimize the intraclass variance of the threshold black and white pixels. These processed subimages will be used further for feature extraction.

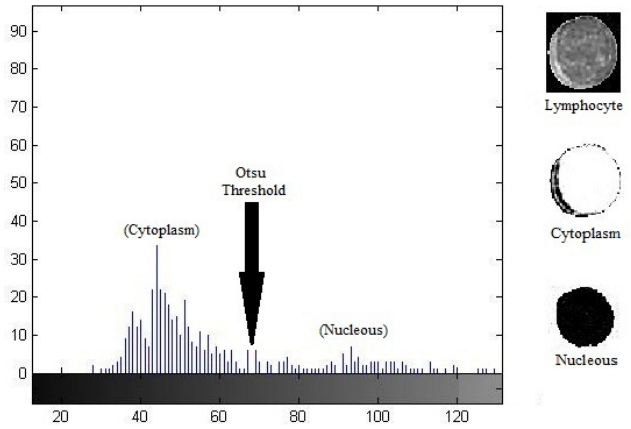



**Fig. 5: Lymhocytic histogram analysis for nucleus and cytoplasm selection.**

## 5. SEGMENTATION METHOD 2 (FUZZY CLUSTERING TECHNIQUE)

The scheme for segmentation using fuzzy clustering technique is shown in the Fig. 6.

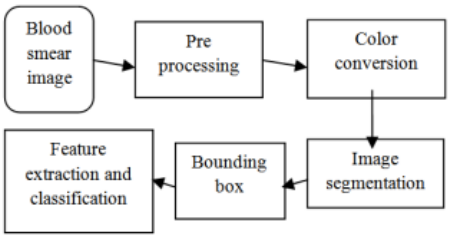



**Fig. 6: System for segmentation.**

The proposed system takes the blood slide image as input. The objective is to separate the WBCs from the background using color based clustering in L*a*b* color space and finally separate the nucleus and cytoplasm [14].

### 4.3 Input image and pre processing

Image of blood slides are captured and taken as input of the module. During image acquisition, noise can be accumulated. Selective median filtering and unsharp masking is done before the processing.

### 4.4 Conversion of color space

L*a*b*color space is mostly applied in color based clustering. In this color representation technique, the 3D RGB color space is reduced to 2D L*a*b* color space. It consist of luminosity layer L* and color representative chromaticity layers a* and b*. As images in RGB color space are difficult to segment, images are converted to L*a*b* before clustering.

### 4.5 Segmentation procedure

The initial segmentation is performed using Gustafson Kessel clustering, an improved version of fuzzy clustering technique, where an attempt of partitioning is done such that the objects within each cluster are closer to each other and far from the objects of other clusters. Each pixel of an object is classified into four clusters which represent the regions of RBCs, WBC nucleus, cytoplasm, background stain.

Another segmentation using nearest neighbor classification is done as WBC cytoplasm and RBCs are classified in same cluster. The mean color of each region is calculated in a*b* space and each pixels in L*a*b* space is labeled to a particular color. Next the nucleus segmented image is reconstructed from the labeled image.

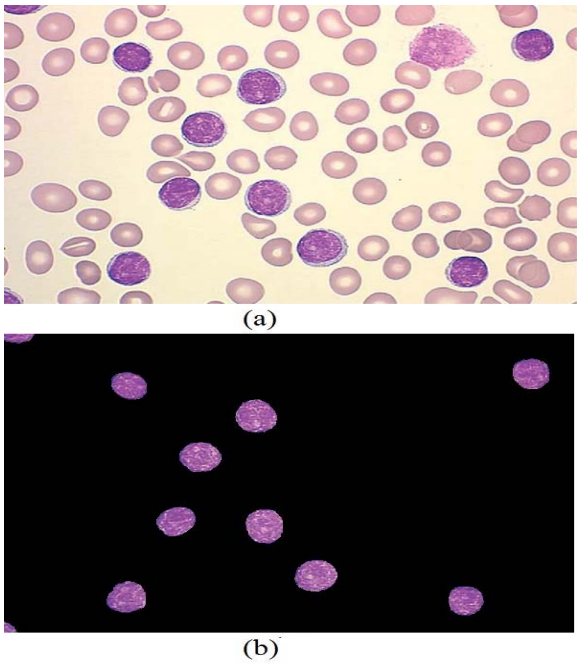



**Fig. 7. Two stage segmentation results. (a) Input image, (b) Segmented image**

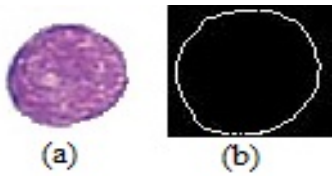



**Fig. 8: Nucleus boundary detection. (a) Nucleus image, (b) Edge image.**

### 4.6 Bounding box

From the nucleus segmented image, the WBCs are classified according to their morphology and lymphocytes are selected. As the cluster image is too large for further feature extraction, bounding boxes are used to contain single nucleus per image.

## 6. SEGMENTATION METHOD 3 (MATHEMATICAL CALCULATION TECHNIQUE)

The figure shows the simplest method to diagnose ALL having a very simple image segmentation method using arithmetical operations. The blood slide image is the input of the system, from which lymphocytes and lymphoblasts are differentiated via a proper segmentation method [15].

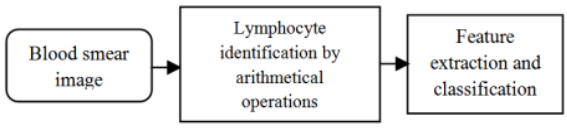



**Fig. 9: System for segmentation.**

### 6.1 Procedure of segmentation

1) The RGB input image is fed to the system and converted into grayscale.
2) The grayscale image is processed in two ways. In the first part, normal contrast stretching is done. In the second part, contrast enhancement is done to the input grayscale image using histogram equalization.
3) Now, a new image is created by adding both of the images produced in step 2.
4) Again a new image is created by subtracting the histogram equalized image from the image obtained in step 3.
5) The final image is created by adding the images obtained in step 3 and 4. In this image all other components of the blood is removed with a minimum effect of distortion over nucleus. A 3-by-3 minimum filter can be used to remove the noise and preserve the edges.
6) Then a global threshold Otsu's method is applied to convert the image into binary. Morphological opening can be used to remove the small pixel groups. Also neighboring pixels are connected to form the objects.

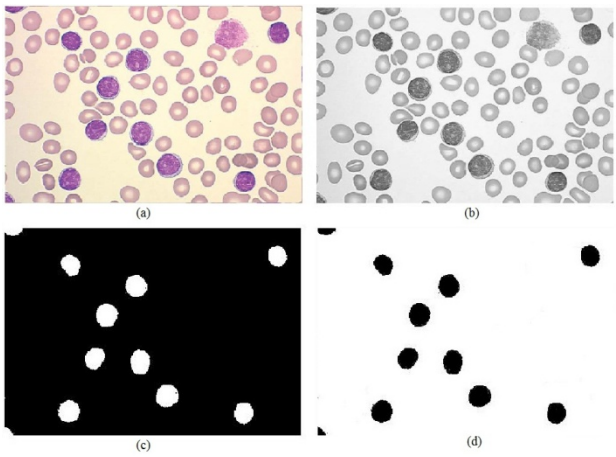



**Fig. 10. (a): Original image, (b) Grayscale image, (c) Image after segmentation, (d) Image after thresholding,**

By following this process the lymphocytes are extracted from the blood slide image. Then they are taken individually for feature extraction and classification. Also the image after segmentation is taken for counting the number of lymphocytes (normal or diseased) in a unit area.

## 7. FEATURE EXTRACTION

All the segmentation methods stated above produces a set of lymphocyte images, along with the subimages of its cytoplasm and nucleus. The processed subimages can be used to extract the gray level intensity and pattern features as described below.

1) Shape features are most important to classify a blast cell. The features that can be extracted, are, area, perimeter, compactness, solidity, circularity, major axis length etc. Perimeter roughness of the nucleus can be measured using fractral geometry.
2) Nucleus texture measurement like granularity, uniformity, homogeneity, can be done using the co-occurrence matrix for each boxed segmented nucleus image.
3) Color features can also be extracted from RGB color space as well as HSV color space.

## 8. CLASSIFICATION

For classification, the features extracted in the previous section are compared with previously stored features of normal lymphocytes and as a result the classifier detects whether the input lymphocytic cells are blast or not. The capability of the selected features in separating the normal lymphocyte from blasts can be qualitatively evaluated by plotting the classes with respect to three most relevant features: cell area, nucleus area and gray intensity of the cytoplasm. The k Nearest Neighbor (kNN) is considered as the best classifier family. A feed forward neural network or Bayes Normal classifier can also be used. When the number of samples increases, it is suitable to use FF-NN. A support vector machine (SVM) may be used when classification lies in HSV color space. This hyper plane classifier separates the surface in the input space of the data set.

## 9. CONCLUSION

The prominent segmentation of WBCs for feature extraction and thus the detection of ALL from blood microscopic images is the main theme of the paper. The various techniques of WBC segmentation and classification are stated in this paper. The variability of the procedures of segmentation is asserted briefly. The details of the procedures are given in [12, 13, 14, 15] respectively. All these three methodologies offer remarkable classification accuracy. Though there are some basic problems regarding segmentation like, if foreground and background are not clear in image, or bounding box problem to a particular cell or selection fluctuation between lymphocyte and monocyte for almost same morphologies. The future goal is to create a robust automated system according to the modules or may be accumulating a few segmentation modules that will fetch the image, process it correctly and efficiently and produce an accurate datasheet as an output.

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