Study & Analysis of Acute Lymphoblasic Leukemia Blood Cells Using Image Processing

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ABSTRACT: Microscopic analysis of peripheral blood smear is important step in detection of leukemia. Microscopic analysis of blood smear is time consuming, tedious and is governed by hematopathologists clinical experience. To address this problem, an efficient methodology for analysis of peripheral blood samples is required to be developed. In this paper, we have developed methodology for automatic detection and classification of Acute Lymphoblastic Leukemia (ALL) using image processing techniques and machine learning methods.
Segmentation methods play a crucial role in the automated disease identification process. Segmentation of the normal lymphocyte cell and malignant lymphoblast cell images into constituent morphological regions, novel segmentation scheme have been proposed. It calculates morphological parameters from those cells and finally it classifies the presence of the acute lymphoblastic leukemia. The segmentation process produces enhanced images for each blood cell, containing the cytoplasm and the nucleus regions. To automatically recognize lymphoblast and detect ALL in peripheral blood samples, an efficient methodology is proposed. Morphological, textural features are extracted from the regions of the lymphocyte images.
The sub classification of ALL based on French-American-British (FAB) criteria is essential for prognosis and treatment planning.

KEYWORDS: Leukemia, Morphological Analysis, Leukocyte, Lymphocyte cells, Morphological Indexes, Segmentation

I. INTRODUCTION

Leukemia is a cancer of white blood cells, this disease develops in the bone marrow spongy tissue that fills the inside region of the bones. There are four major different types of leukemia, which progress in cancer patients according to the growth speed and the improper overproduction of leukemic cells. Images of the infected cells, these forms have unique features which can be visually observed by a trained expert using microscopic images of the infected cells. However, the identification task is usually difficult due to the variety of features and the often unclear images which cause missing out on vital indicators to which form of leukemia is being observed. There are four major forms of leukemia; Acute lymphoblastic leukemia (ALL), Acute myelogenous leukemia (AML), Chronic lymphocytic leukemia (CLL) and Chronic myelogenous leukemia (CML) [4]. The early diagnosis of the leukemia type provides the appropriate treatment for that particular type. The currently used diagnostic methods rely on analyzing immunephenotype, cytogenetic abnormality. These diagnostic methods require sophisticated expensive laboratories. Morphological analysis methods, on the other hand, rely mainly on analyzing images of leukemic cells, and can be also used in correlation with cytogenetics Thus, it can be suggested that using morphological analysis methods for identifying the different leukemia types based mainly on images can greatly reduce the cost of performing type identification tests. Acute Lymphocytic Leukemia (ALL), also known as acute lymphoblastic leukemia is a cancer of the white blood cells, characterized by the overproduction and continuous multiplication of malignant and immature white blood cells (referred to as lymphoblast's or blasts) in the bone marrow. ALL produces a lack of healthy blood cells due to an abnormal number of malignant and immature white blood cells Unfortunately, the initial symptoms of ALL are quite aspecific generalized weakness, anemia, frequent fever and infections, weight loss and loss of appetite, excessive bruising or bleeding from wounds, nosebleeds, bone pain, joint pains, breathlessness, enlarged lymph nodes,

liver and spleen. If the described symptoms are present, blood tests such as a full blood count, renal function, electrolytes and liver enzymes and blood count have to be done [2]. Once the blast-cells invasion starts, blast cells can be detected into the peripheral blood. Principal cells present in the blood are the red blood cells, and the white cells (leucocytes). ALL symptoms are associated only to the lymphocytes [3].

Hence, the observation of the peripheral blood film by expert operators is one of the diagnostic procedures available to evaluate the presence of the acute leukemia. This analysis suffers from slowness and it presents a not standardized accuracy since it depends on the operator's capabilities and tiredness. Conversely, the morphological analysis just requires an image not a blood sample and hence is suitable for low-cost, standard-accurate, and remote diagnostic systems. The system firstly individuates in the blood image the leucocytes from the others blood cells, then it extract the lymphocyte cells (the ones interested by acute leukemia), it extracts morphological indexes from those cells and finally it classifies the presence of the leukemia using classifier. The overall system focuses on segmentation of lymphocyte cell and how to extract a suitable set of morphological indexes from the leucocytes in order to identify the blast cells by a classifier.

II. RELATED WORK

Liao and Deng *et al.* [7] introduced a novel WBC image segmentation scheme which is based on simple thresholding followed by contour identification. This algorithm works with an assumption that the cells are circular in shape, hence is not at all suitable for irregularly shaped lymphoblast's (malignant lymphocytes). Angulo *et al.* [8] proposed a two stage blood image segmentation algorithm based on automatic thresholding and binary filtering. This scheme exhibits good segmentation performance in terms of cytoplasm, nucleus and nucleolus extraction in lymphocyte images. All these come at the cost of higher computational time due to the two stage segmentation process. Umpon *et al.* [9] introduced patch based WBC nucleus segmentation using fuzzy clustering. Even if the nucleus segmentation is accurate, there is no provision for cytoplasm extraction which is equally important for leukemia detection. Dorini *et al.* [10] used watershed transform based on image forest transform to extract the nucleus. Concurrently, size distribution information is used to extract the cytoplasm from the background including RBC. While effective for nucleus segmentation this method fails when the cytoplasm is not round. Meurie *et al.* [11] given an automatic segmentation scheme based on combination of pixel classification. However, despite hybridization of classifiers the average segmentation performance is not so high. Further the use of multiple classifiers increases the average running time. Ko *et al.* [12] proposed a hybrid leukocyte segmentation method which gives stepwise merging rules based on mean shift clustering and boundary removal rules with a GVF snake model. Two different schemes are employed independently to extract the cytoplasm and nucleus of the leukocyte. However, the segmentation accuracy for cytoplasm needs further improvement and computation time has to be reduced. Scotty *et Al.* [13] proposed a method for automated classification of ALL in gray level peripheral blood smear images. As per the experiments conducted by them on 150 images it has been concluded that lymphoblast recognition is feasible from blood images using morphological features. However, use of Otsu thresholding in image segmentation and feed forward neural network for feature classification is the cause of low recognition rate.

III. PROPOSED WORK

Initial screening of Acute Lymphoblastic Leukemia (ALL) begins with microscopic analysis of peripheral blood smear samples to detect the presence of immature lymphocytes or blast cells (lymphoblasts). However, in an alternate approach presence of ALL can be diagnosed through lymphocyte image analysis based blast counting method. In such an automated blast counting approach it is required to differentiate lymphoblasts from mature lymphocytes, and is performed using image processing and machine learning based methods. To analyze the differences in lymphocytes it is important to segment such cell images into individual morphological regions as depicted in Figure.1.
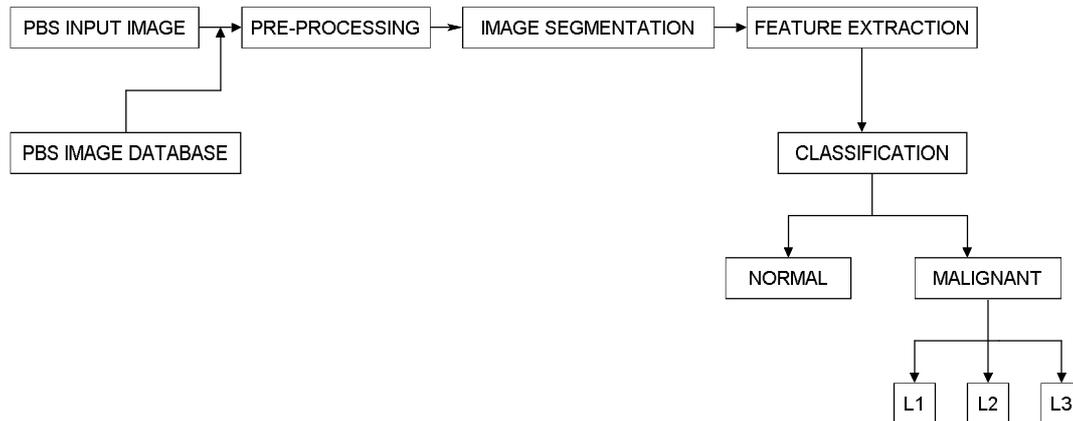
Fig.1. Structure of modules composing the acute lymphoblastic leukemia (ALL) classification system

The proposed systems has in input a color image and it produces in output a list of sub-images containing one-by-one the white cells present in the input image and a estimation of the mean cell diameter. Subsequent modules of the final system will exploits this outputs to detect the presence of acute leukemia. The system is designed to be capable to process images grabbed by a commercial digital camera during normal microscope observation of a blood film. In the image are present the principal components of blood (basophil, eosinophil), lymphocyte, monocyte, neutrophil. If acute Leukemia is present it shows heterogeneous morphological variations in lymphocytes (area, circularity, compactness, nucleus non-uniformities, etc.). Those features can suitably be measured in the following modules of the final system only if the selection of white cells is accurate and successful.

The main modules which compose the overall system are plotted in Fig.1. The PBS input image module firstly selects the peripheral blood smear image. After selection of image, pre-processing is performed which filter out the noise and adjust the contrast of the image. It has been composed by pre-filtering. Secondly, the morphological operations performed on the input image. White-cells Identifier module selects the white cells present into the image by separating them from others blood's components (red cells and platelets). Typically, the main source of error is related to the strong morphological similarities between the components of the leucocytes family (lymphocytes, monocytes, etc.), conversely is much less probable to classify the other blood components (such as red cells) as lymphocyte than vice versa [17]. In general, the first three modules of the system in Figure 3.1 can select sub-images of lymphocytes from the blood film image with high accuracy. The sub-system which has to recognize if a lymphocyte is blast or normal, the features of input image are extracted. From the features extracted the input image is classified as normal Lymphocyte or Lymphoblast. Further Classification of Acute Lymphoblastic Leukemia uses KNN classifier for the classification of ALL subtypes.

The propose system has follows main processing steps:
- To preprocess the image in order to reduce acquisition noise and background non uniformities.
- To perform segmentation for achieving a robust identification of white cells.
- Extraction of heterogeneous morphological variations in lymphocytes features from segmented images.
- Classification of Acute Lymphoblastic Leukemia.

IV. MATERIALS AND METHODS

A. *PERIPHERAL BLOOD SMEAR DATA BASE*

The database is provided by the M. Tettamanti Research Center for Childhood Leukemias and Hematological Diseases, Monza, Italy. The dataset consists of 80 images and it globally contains about 8400 blood cells, 150 of them are lymphocytes labeled by expert oncologists as normal or blast. The Image data base is provided by Fabio Scotti, Department of Information Technologies, University of Milan, Crema, Italy. For each image in the dataset, the classification/position of ALL lymphoblasts is provided by expert oncologists. The Images of the dataset has been

captured with an optical laboratory microscope coupled with a Canon Power Shot G5 camera. All images are in JPG format with 24 bit color depth, resolution 2592 x 1944.

B. PREPROCESSING.

Noise may be accumulated during image acquisition. All the test images are subjected to selective median filtering. Minute edge details of the microscopic images are perfectly preserved even after median filtering. Unsharp masking is performed to sharpen the image details making the segmentation process easier.

C. IMAGE SEGMENTATION.

Recognition of leukemia in blood samples is based on morphological variation of WBC. Such alterations can only be measured with segmented nuclei and cytoplasm. Accuracy of leukemia detection solely depends on leukocyte segmentation; thus, a suitable method has to be employed for morphological region extraction.

Morphological operations are performed on the input image. Whereas erosion and dilation are considered the primary morphological operations and the operations of opening and closing are secondary operations and are implemented using erosion and dilation operations. Mathematical Morphology refers to a branch of nonlinear image processing and analysis that concentrates on the geometric structure within an image, it is mathematical in the sense that the analysis is based on set theory, topology, lattice, random functions, etc.

For the selection of membrane of lymphocyte sobel edge enhancing technique is used. This step enhances the borders of the membranes [26] in order to better perform the subsequent edge detection steps. An edge-enhanced gray level image is hence produced. This processing step is recommended since it helps to better segment grouped cells.

D. FEATURE EXTRACTION MODULE

Processes image containing a lymphocyte cells and it produces in output a set of morphological indexes. The classification module processes those indexes in order to classify the cell as lymphoblastvblast or normal. The two modules perform the automatic morphological analysis of lymphocyte images. The following morphological, textural features are measured from the binary gray image version of the nucleus and cytoplasm image regions respectively of each lymphocyte image. Area, Total White Cells, Total Black Pixels, Perimeter, Eccentricity, Solidity, Form Factor.

E. CLASSIFICATION

In pattern recognition, classifiers are used to divide the feature space into different classes based on feature similarity. Depending on the number of classes each feature vector is assigned a class label which is a predefined integer value and is based on the classifier output. Each classifier has to be configured such that the application of a set of inputs produces a desired set of outputs. The entire measured data is divided into training and testing data sets. The training data is used for updating the weights and the process of training the network is called learning paradigms. The remaining test data are used for validating the classifier performance. In this study, we propose the use of KNN classifiers for labeling each lymphocyte sub image as normal or malignant sample based upon a set of measured features.

V. SIMULATION RESULTS

As per previous discussion it is well understood that ALL is detected on the basis of the presence or absence of immature lymphocytes or lymphoblasts in PBS samples. Therefore, lymphocytes in PBS samples must be characterized as malignant or normal based on certain fixed pathological criteria defined for the screening of ALL. In this regard, an automated system has been developed, and experiments are conducted using the above configuration and the results are presented in this section.

Following cases are tested for the analysis of Acute Lymphoblastic Leukemia (ALL).
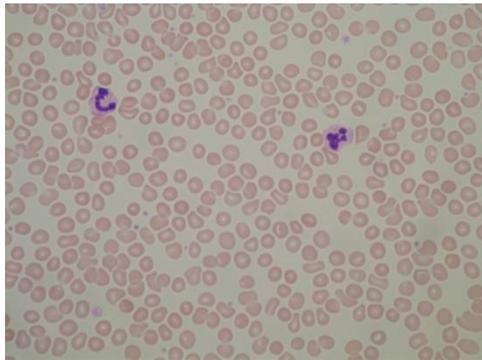
A. NORMAL CASE:
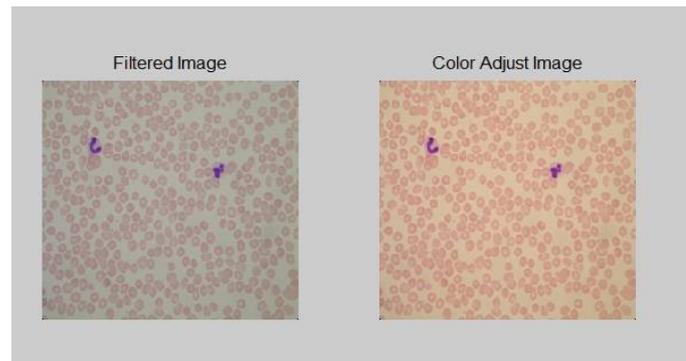

Fig.2. PBS image of normal Patient.

Fig.3. Enhanced PBS image of normal Patient.

Fig.2 shows the normal PBS image of patient. Which shows only two white blood cell for normal condition. This input image is pre processed for further image processing operations performed to obtain clean clear region of interest (ROI). Next stage of operation is Segmentation, for segmentation of lymphocyte cell firstly image is converted to Gray scale and the same image is then converted to binary image for performing the morphological operations on the image. Erosion and Dilation these two mathematical morphological operations are performed on the input image. Canny edge detection operation is applied on the morphologically processed image as result lymphocyte areas in the image are segmented. Fig.4 shows gray scale image binary image, eroded and dilated image and last is segmented image.
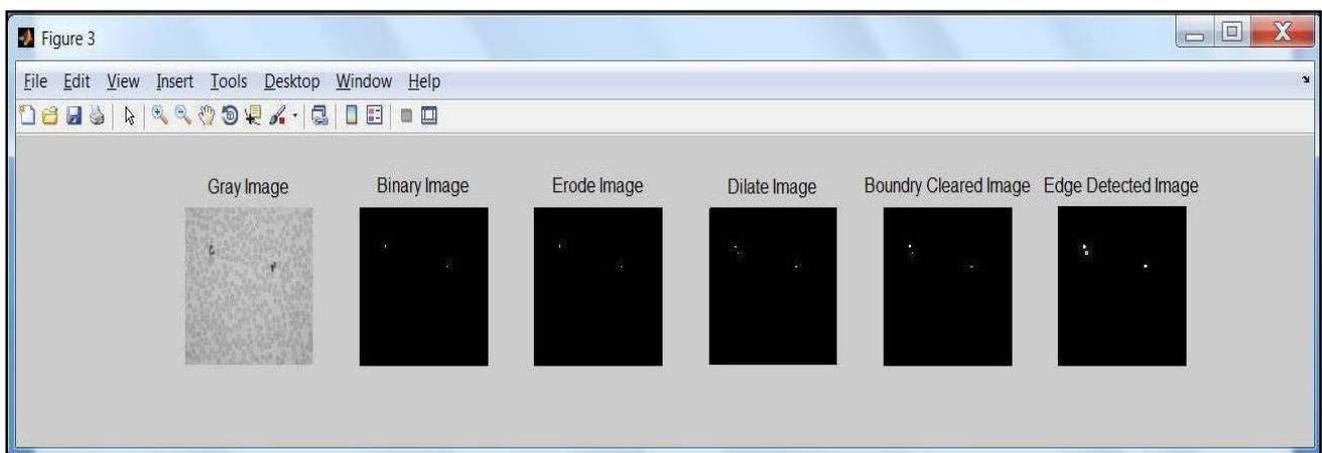

Fig.4 Morphological Operated and Segmented image of Normal Patient

Fig.5 shows the PBS image of ALL patient. Which shows only seven white blood cell for abnormal condition. This input image is pre processed for further image processing operations performed to obtain clean clear region of interest (ROI). Next stage of operation is Segmentation, for segmentation of lymphocyte cell firstly image is converted to Gray scale and the same image is then converted to binary image for performing the morphological operations on the image. Erosion and Dilation these two mathematical morphological operations are performed on the input image. Canny edge detection operation is applied on the morphologically processed image as result lymphocyte areas in the image are segmented. Fig.7 shows gray scale image binary image, eroded and dilated image and last is segmented image.
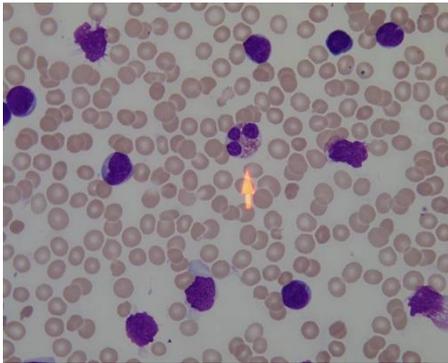
B. ABNORMAL CASE:
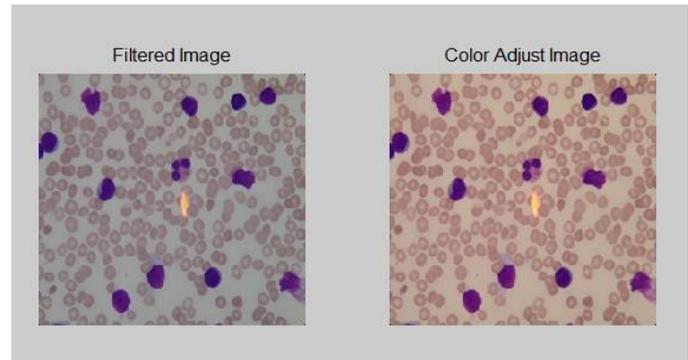

Fig.5. Input PBS Image of ALL Patient.

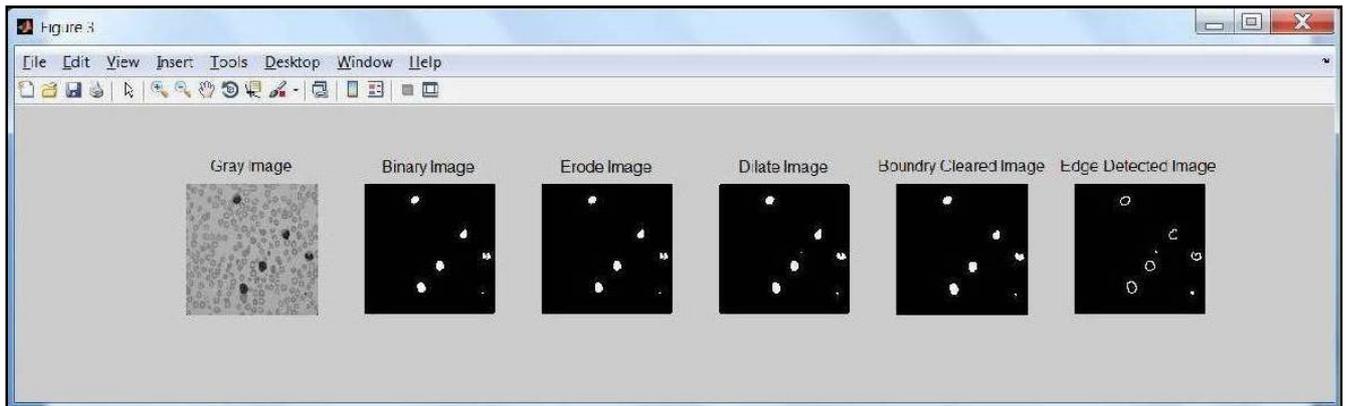Fig.6. Enhanced PBS Image of ALL Patient.

Fig.7. Morphological Operated and segmented Image of ALL Patient

Morphological variations in Parameters obtained from the analysis of normal and abnormal cases are shown in following table.

TABLE I
MORPHOLOGICAL FEATURES OF Normal and malignant lymphocyte

SR.NO.	FEATURES	NORMAL CASE	ABNORMAL CASE
1	TOTAL WHITE CELLS	3.000	00007
2	TOTAL BLACK PIXELS	65501	64343
3	AREA	35.000	01193
4	PERIMETER	12.2400	56.480
5	BOUNDING BOX MIN.	00036	24.500
6	BOUNDING BOX MAX.	00127	00194
7	ECCENTRICITY	0.9979	0.5910
8	SOLIDITY	0.0338	0.0571
9	FORM FACTOR	2.9344	4.7322

VI. CONCLUSION AND FUTURE WORK

A paper presents the analysis and detection of acute lymphoblastic leukaemia from microscope blood film images. In particular the paper proposes method to enhance the microscopic images of blood smear by removing undesired

background noise, a morphological segmentation method for normal and malignant lymphocyte detection and segmentation. Morphological features extracted from the PBS and used for classification of normal lymphocyte and immature lymphocyte also KNN classifier is used for the sub classification of ALL.

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