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Automatic Classification of Leukocytes with Neural Network and Fuzzy Interference Systems

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ABSTRACT: Designing a single automatic and accurate segmentation approach for different classes of white blood cells is a challenging task. This paper presents a fully automated segmentation framework to segment both nuclei and cytoplasm of five major classes of white blood cells in the peripheral blood smears based on color and texture enhancement. Particularly, a new gray-scale transform is generated based on three representative color channels to separate the nuclei from the cytoplasm and background by Poisson distribution based minimum error thresholding. For cytoplasm segmentation, discrete wavelet transform (DWT) and morphological filtering based enhancement procedure is utilized to highlight the cytoplasm and eliminate the small details inside the cells. Finally level set-based refinement and false candidates filtering are applied to obtain the accurate cell segmentation. The proposed approach is evaluated on two sets of peripheral blood smears, and it demonstrates improved segmentation performance when compared with existing methods.

KEYWORDS: White blood cells, cell segmentation, peripheral blood smears, image enhancement.

I. INTRODUCTION

Diagnosis of various blood related diseases, such as the Leukaemia, adopts differential count of white blood cells (WBCs), also called leukocytes, to determine the patient health. These cells protect the body from infections caused by viruses and other foreign invaders like bacteria or fungi, which make WBCs an important part of the immune system. Leukocytes are produced and derived from the bone marrow and circulate through the bloodstream. A change of the number of different WBC subtypes in the blood is utilized as marker for various diseases. Therefore a blood cell count is often utilized for a routine health examination.There are five major subtypes of WBCs :

- neutrophils (50-70%);
- lymphocytes (25-30%);
- monocytes (3-9%);
- eosinophils (0-5%);
- basophils (0-1%).

The ranges within the brackets display the percentage of the corresponding cell subtypes in the blood, which are typical ratios for a healthy person. There are various classification approaches, which can be roughly divided into manual and automated methods of cell classification.

The manual classification is performed by a pathologist through the subjective recognition of cell subtypes on microscopic images of stained cells. This type of analysis does not require complex equipment or highly specialized chemical reagents. To simplify the identification, cells are usually stained with the Kimura stain, which colors cell nuclei in blue. Manual differentiation between varying subtypes is accomplished based on characteristics of the cell

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morphology, like cell size, transparency, granularity, and the shape of the cell nucleus, which are the major differences between the subtypes. Manual classification is widely used in some specific cases of diagnosis and as a “gold standard” for scientific purposes. However, variation of cell morphology within the same cell subtype is very high, and manual classification efficiency is dependent on the pathologist’s qualification and experience.

On the other side, there are various automated classification methods, based on different physical and chemical characteristics of the cells. The main advantage of the automated devices is that they efficiently analyze large number of cells in a short time. Unfortunately, their analyzing workflows include very specific combinations of chemical and physical processes. Hence, manual counting of cells is tedious, time consuming, and susceptible to error. Computer-based imaging systems for peripheral blood analysis are thus highly desirable. These imaging systems generally start with segmentation of WBCs into the nucleus and cytoplasm, and an accurate segmentation is important to achieve accurate analysis in subsequent stages.The majority of existing approaches are semi-automatic, and need to be substantially extended to work with new datasets.

Several methods for color features selection and contrast enhancement have been used to segment the WBC nuclei and/or cytoplasm in one class of leukocytes, such as lymphoblast , or from the five leukocyte classes .

In this work, we propose a fully automatic approach for nucleus and cytoplasm segmentation with color- and texturebased image enhancement. In particular, various color channels are incorporated to generate an enhanced grayscale image to distinguish the cell nucleus, whereas DWT with morphological filtering based enhancement is used to smooth the texture of the cytoplasm to enable easy segmentation. We successfully applied our approach on two different sets of peripheral blood smears, and obtained good nuclear and cytoplasmic segmentation results.

Fuzzy logic models, called fuzzy inference systems, consist of a number of conditional "if-then" rules. For the designer, these rules are easy to write, and as many rules as necessary can be supplied to describe the system adequately.We will analyze the differences between each pixel using a local operator.The fuzzy logic approach can be implemented through a Fuzzy Inference System (FIS) that formulates the mapping from multiple inputs to a single output.It further improves target detection and localization accurately. The experimental results will show that the proposed fuzzy logic approach to pixel-wise change detection provides better outcome than the previous approach.

II. METHODOLOGY

Our proposed approach consists of three stages: nucleus segmentation, cytoplasm segmentation, and cell refinement and filtering. Pre-processing is applied as well to generate a high contrast images by applying automatic contrast stretching.

2.1. Nucleus Segmentation

The color of image contains rich information that is usually lost during the conventional conversion of color images to the gray-scale domain. This loss of information could result in poor segmentation due to the similar intensities between the nuclei, cytoplasm, and background. To this end, we propose a new way to generate the gray-scale image, which helps to highlight the nuclei by arithmetic processing of three color channels comprising more information about the nuclei. Two color spaces (RGB and CIE LAB) are incorporated to generate the gray-scale map.

CIE LAB separates the lightness/contrast from colors in three channels (the luminance channel L, and the coloropponent channels A and B). The L channel shows similar contrast to the original color image, and it is useful to capture the contrast between the nuclei and cytoplasm. The A channel exhibits the change between red and other colors, and this information helps to differentiate between nucleus and cytoplasm. Based on these characteristics, we propose to use these two channels along with the red channel in RGB color space to convert the color image to gray-scale image, in which the cell nuclei would become more salient. In this way, we can obtain a good threshold accurately separating the nuclei.

Let I_R denote the red channel in the RGB color space equalized by , and I_L denote the L band in the LAB color space. The R, L, and A channels are incorporated as follows:

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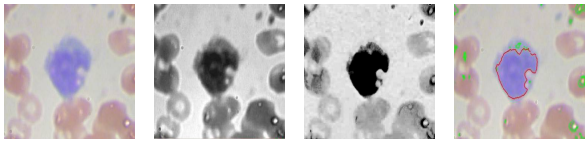
$$I_b(x,y) = (I_R(x,y) + I_L(x,y)) - I_A(x,y) \quad (1)$$

wherex and y represent the coordinates of a pixel. This process removes most of the non-WBC structures while sharpen-



(a) (b) (c) (d)

Fig. 2: (a) The original color blood image, (b) the gray-scale image obtained by conventional conversion of the pre-processed color image, (c) our enhanced gray-scale image, and (d) the nuclear segmentation, where the green line shows the thresholding results, and the red line shows the final nucleus segmentation after filtering step (see Section 2.3).

ing the nuclei. Subsequently, the Poisson distribution based minimum error thresholding algorithm is applied on the obtained gray-scale image to get the nuclei candidates. Fig. 2 illustrates the nucleus segmentation approach.

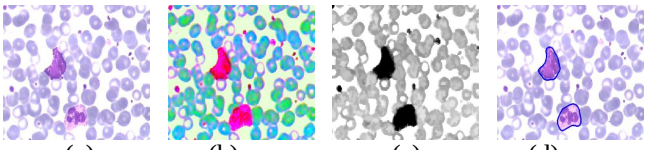
2.2. Cytoplasm Segmentation

The segmentation of WBC cytoplasm is very challenging since it is almost transparent and exhibits fuzzy textures. The existence of nucleus structure, small holes, and noise distributed around and inside the cell boundaries also introduce more difficulties. In addition, the high accumulation of red blood cells and their interference with the WBC boundaries further complicate the segmentation of WBC boundaries. To tackle these difficulties, we propose an image enhancement technique based on discrete wavelet transform (DWT) and morphological filtering to eliminate the noise and fine details, and increase the contrast between the cytoplasm and the other structures.

Firstly, the decorrelation stretch enhancement method is applied to the pre-processed color image to enhance the cytoplasm contrast. DWT is then used to eliminate the nuclear details inside the cell and the background noise. In particular, the image is decomposed into four sub-band by 'Haar' DWT of scale 1; the low sub-band, and three high sub-bands. All the details in the three high sub-bands are ignored by assigning the coefficients in these sub-bands to zeros. Then, the original low sub-band and the three modified high sub-bands are combined to get an enhanced image with good discrimination from the background and reduced fine details (see Fig. 3(b)).

In this reconstructed image, the cytoplasm has darker intensities in the green channel compared to the red/blue channels. The green channel is thus extracted and smoothed using morphological filtering by reconstruction (opening and closing) to further enhance the discriminative characteristics of cytoplasm. Using this enhanced green channel, the cytoplasm candidates are then extracted by Otsu's thresholding .

To perform the morphological filtering, the bright and dark regions smaller than a disk structural element are first eliminated from the foreground and background, respectively.



(a) (b) (c) (d)

Fig. 3: (a) The original color blood smear image, (b) the result of our DWT-based enhancement procedure, (c) the smoothed image by morphological filters-by-reconstruction, and (d) the final cytoplasmic segmentation.

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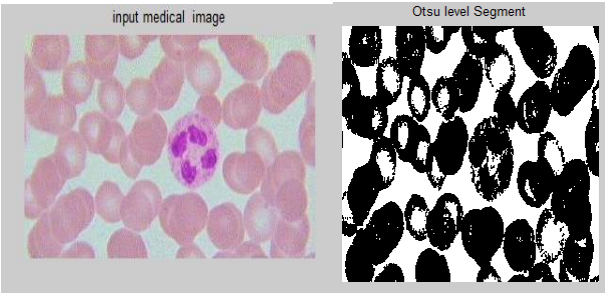
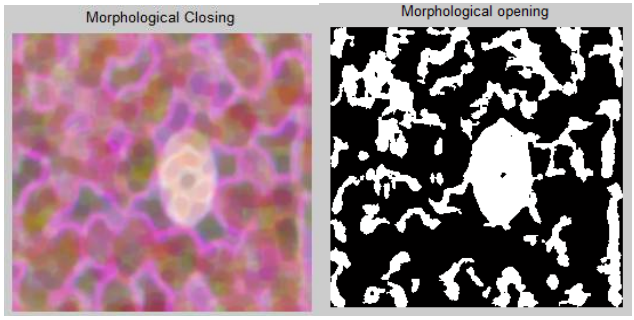


The remaining regions are then iteratively dilated to restore the missing contours using the original image as a reference. The cytoplasm candidates are then extracted by Otsu's thresholding.

2.3. Cell Refinement and Filtering

Lastly, cell refinement and filtering are performed. First, the regularized level set is applied to refine the cytoplasm candidate contour. Opening operation is applied to remove any excessive pixels resulted from small dark structures attached to the cell boundary, followed by dilation to retrieve the boundary pixels missed by the previous step. Second, the false nuclei and cytoplasm candidates are filtered. The false candidates are defined as those objects smaller than being nuclei or cytoplasm, as well as the nuclei regions that are not surrounded by cytoplasm, or cytoplasm regions that do not encompass the nuclei.



III. EXPERIMENTAL RESULTS

The performance of our approach was quantitatively and qualitatively assessed on two blood smears datasets. The images were classified into the five leukocyte classes: basophil, eosinophil, lymphocyte, monocyte, and neutrophil. The ground truth segmentation was manually done by expert haematologist.

The performance of the proposed approach was further compared with the results of two color-based methods for nucleus segmentation on the first dataset. Ideally, we would like to compare our cytoplasm segmentation with other WBC cytoplasm segmentation approaches in the literature. However, we found it is difficult to re-implement the existing approaches reliably based on the provided descriptions and they need to be substantially modified to work with the new datasets.

The results show that the nucleus segmentation of eosinophils is more challenging than the other types of Furthermore, our proposed approach achieved large improvement over the published approaches on the same dataset. With the use of conventional grayscale transform from color images, the contrast enhancement in undesirably increased the intensities of the non-WBC structures and affected the segmentation performance.On the other hand, we designed a new gray-scale transform using two different color spaces. Our method effectively enhanced the contrast between the nuclei of WBCs and the cytoplasm/background, and resulted in the improved segmentation accuracy.

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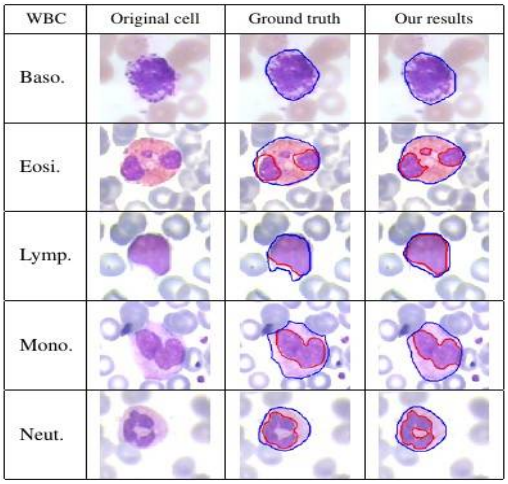

Fig.: Qualitative nuclei and cytoplasm segmentation results on the second dataset, where the red line shows the nucleus segmentation, and the blue line shows the cytoplasm segmentation.

IV. CONCLUSIONS

Segmentation is a crucial step in computer-based haematological images analysis due to the complex nature of the WBCs and the difference between them in shape, texture, color, and density. This paper introduces a fully automatic segmentation framework for five types of white blood cells in the peripheral blood smears. The proposed approach uses color- and DWT-based enhancement procedures to differentiate the nuclei and cytoplasm of WBC and enable easy separation by gray-level thresholding. Finally, the refinement and filtering step is utilized to refine the cell boundaries. Our proposed approach achieved more accurate nucleus segmentation results compared to the other color-based methods, and succeeded in providing good cytoplasm segmentation.

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