



# A new thresholding technique based on fuzzy set as an application to leukocyte nucleus segmentation

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## ABSTRACT

**Background and objectives:** The main aim of this paper is to segment leukocytes in blood smear images using interval-valued intuitionistic fuzzy sets (IVIFSs). Generally, uncertainties occur in terms of vagueness through brightness levels of image. Processing of such uncertain images can be efficiently handled by using fuzzy sets, particularly IVIFSs.

**Methods:** Logarithmic membership function is utilized for computing membership values corresponding to intensities of the pixel. Non-membership function of IVIFS is constructed by using Yager generating function. By varying parameters, 256 IVIFSs are generated. An IVIFS is selected from 256 IVIFSs having maximizing ultrafuzziness along with varying threshold. Threshold is determined by finding an IVIFS with maximum similarity between ideal segmented and segmented results obtained from the proposed method.

**Results:** Quantitatively, the segmented images are evaluated using precision–recall, receiver operator characteristic curves, Jaccard coefficient and measure for structural similarity index along with the time taken for segmenting nucleus, and their results are compared with results of existing methods. Performance measures reveal that the proposed method seems to segment leukocytes better than other comparable methods.

**Conclusions:** Segmentation of leukocytes using the proposed method helps the analyst in differentiating various types of leukocytes and in the determination of leukocyte count, and the counting is essential in finding out diseases related to reduction or surplus quantity of these cells.

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## 1. Introduction

Segmentation is crucial in the field of computer vision. Various algorithms are available in literature for segmentation of micro array images [1], breast images [2,3] and skin images [4] and so on. Immune system protects the body from harmful bacteria, viruses and other pathogens by finding and eliminating them. Leukocytes are the primary constituent in the immune system and play a vital role in identifying and destroying while

there is a pathogenic intrusion into the body. These cells are originated from a multi-potent cell in bone marrow. Leukocytes are mainly divided into two types, namely granulocytes (neutrophils, eosinophils and basophils) and agranulocyte (lymphocytes and monocytes), as shown in Fig. 1.

Naturally, leukocytes occupy certain range in blood stream, namely: lymphocytes, monocytes, eosinophils, basophils and neutrophils represent respectively less than 20–35%, 3–9%, 5%, 1% and 50–70% of all leukocytes. Each class of leukocytes must be in a normal range in a differential leukocyte report. If the

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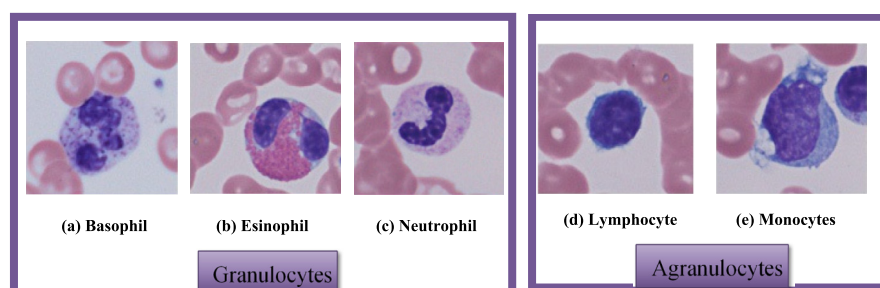


Fig. 1 – Types of leukocytes.

amount of leukocytes exceed or go beneath such range, then health issues may occur. Hence, counting of leukocytes is employed as an identity for finding out disease and it varies with respect to the age of each person. Counting of leukocytes is a tedious and time consuming job for a pathologist. Moreover, analyzing leukocytes by a pathologist depends on the knowledge, eyesight and strength. Hence, it is essential to have an automated system to detect and classify types of leukocytes [5].

Currently available automated cell counters are based on laser light scattering and flow-cytochemical principles, yet 21% of all processed blood samples still require microscopic review by experts [6]. Hence, numerous efforts [6–10] have been developed for automatic cell analysis using image processing. Blood cell images consist of both white and red blood cells scattered across the entire image, however, it is the white blood cells (WBCs) that provide the important information for patient diagnoses, such as leukemia or cancer [7]. In the case of WBC segmentation, an important task is the extraction of WBCs from a complicated background and then segmenting them as the nuclei and cytoplasm.

Due to pale and lucid nature of leukocytes, they cannot be seen clearly through microscope until they are stained. For instance, a mixture of methylene blue and eosin based stains are utilized for staining blood smear images. The effect of staining depends upon time for staining, temperature and concentration of solution. So, one cannot assure that the blood smear images produced in the single laboratory and exploited by the same pathologist are exactly alike. To overcome this problem, image can be analyzed using various rigorous algorithms for accurate, effective identification and classification of normal and abnormal cells. Computer aided techniques are broadly employed in medical diagnosis from the last decades. In recent years, many researchers [5,11–17] concentrated in the formulation of an automated system for identifying and classifying leukocytes. Accurate segmentation of leukocytes is yet an unrevealed problem. There exist some challenges in accurate segmentation of leukocytes.

Several segmentation techniques were introduced by various authors to classify different kinds of images and few of them worked on pathological images [18]. Huang et al. [5] introduced a computerized recognition method for classifying five types of leukocytes. Count of leukocytes in blood cells is essential to detect diseases like leukemia, parasitic fever, and many others. Detection of such diseases have been done by employing various methods, some of them are based on morphological analysis, color analysis [14], clustering [15] and multi-

spectral technique [19]. Yang et al. [16] segmented leukocytes based on components of color spaces. Texture based recognition approach had been introduced in 2004 to recognize types of leukocytes [20]. Neural networks and fuzzy logic approach have been implemented to segment leukocytes in Ref. [21]. Simulating visual attention has been utilized in Ref. [22] to identify leukocytes. Segmentation and counting of lobes of nucleus of leukocytes have been introduced in 2010 by Chan et al. [23]. They found that the lobes increase when there is vitamin B12 deficiency and leukemia.

In this paper, a new automatic segmentation method is introduced based on interval-valued intuitionistic fuzzy similarity measure. Initially, blood smear images in RGB color model is converted into HSI color model since S-channel in HSI space efficiently shows leukocytes in blood smear images [5]. Then, contrast enhanced S-channel is thresholded with  $T \in [0, 255]$ . For each  $T$ , three regions are divided according to mean of the background and object at the threshold  $T$ . Upper and lower membership functions of IVIFS are defined with one free variable  $\sigma \in [0, 255]$  and for each  $\sigma$ , ultrafuzziness of the IVIFS is determined. An appropriate  $\sigma$  for an image is found by maximizing ultrafuzziness. Upper and lower membership degrees of IVIFS are calculated with this  $\sigma$  and their corresponding non-membership values are determined by using Yager generating function. Then, the similarity between ideally thresholded image and the segmented image using each threshold  $T$  are calculated. Finally, an optimal threshold is identified with maximum similarity. Half of the optimal threshold value segments the image into meaningful regions.

The organization of the paper is described as follows. Section 2 briefly discusses some works related to the present study and essentiality of the proposed method. Basic ideas about fuzzy sets and their extended sets are provided in Section 3. Section 4 renders the detailed description of methodology of segmentation based on IVIFS. Quantitative metrics are presented in Section 5, which are useful in evaluating effectiveness of the proposed method over other existing methods. Section 6 renders experimental results and their performance based on evaluation metrics. Conclusion is drawn along with future directions in Section 7.

## 2. Related works and the present study

In this section, some works related to the segmentation of white blood cells are provided based on the category of segmentation.

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