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Automated leukocyte recognition using fuzzy divergence

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ABSTRACT

This paper aims at introducing an automated approach to leukocyte recognition using fuzzy divergence and modified thresholding techniques. The recognition is done through the segmentation of nuclei where Gamma, Gaussian and Cauchy type of fuzzy membership functions are studied for the image pixels. It is in fact found that Cauchy leads better segmentation as compared to others. In addition, image thresholding is modified for better recognition. Results are studied and discussed.

1. Introduction

Recently, pathological image processing has become very important for better characterization, visualization as well as disease recognition in order to strengthen digital microscopy. Among various pathological studies, characterization of blood parameters viz., erythrocytes, leukocytes and platelets, is essential in case of many vital diseases e.g. anemia, hepatitis, diabetes, allergy, leukemia, cancer, AIDS, psoriasis, etc. Out of these three blood cells, microscopic evaluation of leukocyte is still a challenging task to hematologists, being indispensable in diagnostics with malignance suspicious. In conventional pathological evaluation, the medical experts visually characterize leukocytes as per their clinico-pathological understanding and experience. Such visual analysis is time consuming, difficult to reproduce and subjective, which result in larger inter and intra-observer variations. In order to facilitate such conventional mechanism, a computer aided leukocyte recognizer is developed here using fuzzy divergence method, which is helpful in understanding the morphological or/and textural changes over disease mapping (especially leukemia types).

Few studies have been reported in the literatures. Liao and Deng (2002) have shown segmentation of white blood cell (WBC) based on gray level thresholding. In addition, thresholding combined with morphology operation is also a well known method (Leite and Dorini, 2006; Theera-Umpon and Gader, 2000; Anoraganingrum, 1999; Harlik and Stemberg, 1983; Angulo and Flandrin, 2003; Beucher and Meyer, 1992; Ruberto and Dempster, 2002). Kumar and Sreenivas (2002) used teager energy operator to segment nucleus boundary. Umpon proposed an idea of fuzzy c-means combined with morphology in WBC segmentation (Theera-Umpon and Gader, 1992; Thera-Umpon, 2000, 2003). Umpon also introduced patch based WBC nucleus segmentation using fuzzy clustering ((Umpon, 2005) and extraction of granulometric features of nucleus using pattern spectrum (Lain et al., 2000). Liao and Deng (2002) introduced the concept of shape analysis and Jiang (Jiang et al., 2003) established the idea of the scale space filtering and HSV histogram clustering for segmenting WBC. Anoraganingrum (1999) used median filtering, thresholding and mathematical morphology in cell segmentation. Jun recommended PCNN in noise removal combined with median filter (Mao-jun and Zhao-bin, 2008). A texture based approach in recognition of leukocyte and textural feature extraction method using GLCM (gray level co-occurrence matrix) was suggested by Sabino (2004). An automated detection and analysis of leukemia using K-means clustering followed by EM algorithm were proposed by Sinha and Ramkrishna (2004). Scotti (2005) established automatic morphological analysis for acute leukemia detection and Serbouti et al. (1991) initiated segmentation and classification methods for leukemia detection from peripheral blood images. Mehnert and Jackway (2007) used an improved seeded region growing algorithm for cell segmentation. Hitong applied fuzzy cellular neural network to WBC segmentation (Hitong et al., 2007). Even, automated cell-counter system viz., laser-based citometer does not allow direct morphological subclassification of leucocytes. Flow citometry based blood cell count is suggested by Dumpy (2004).

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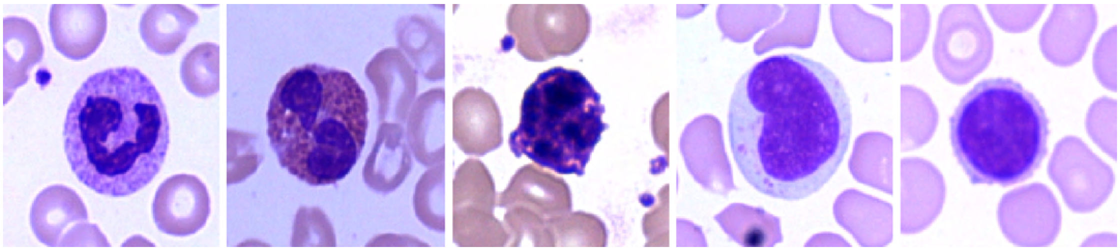

Fig. 1. Light microscopic view of five different types of stained leukocytes.

In the context of routine screening of differential counts, leukocyte segmentation is very challenging task because of the occurrence of too much noise in the images. Generally the noise appears due to dusts in the lens as well as staining intensity variation. In practice, difficulty mainly arises in segmenting the nuclei of basophil and eosinophil from cytoplasm since there is an insignificant difference in the intensity level in the nuclei-boundary. These pixels in the boundary region have partial belongingness to both the regions and hence should be modeled by fuzzy logic. Till date no such fuzzy-induced segmentation approach is reported for leukocyte recognition in the literature.

In this work, an automated segmentation mechanism for nuclei-based leukocyte recognition is designed using fuzzy divergence (Chaira and Ray, 2003a,b, 2004) measure followed by preprocessing. Particularly, Gamma, Gaussian and Cauchy type of membership functions are considered in order to develop the measure. Here the thresholds are selected in the gray scale on the basis of obtained divergence values. In fact, it is found that the threshold i.e., half of the gray value corresponding to minimum divergence provides better segmentation in comparison with gray value of the same while Cauchy membership function governs the measure.

2. Materials and method

2.1. Human blood smear preparation

Study subjects are considered in the Dept. of Hematology, Midnapur medical College & Hospital and Medipath Laboratory, Midnapur, West Bengal through randomization to collect blood samples. Afterwards, blood smear is prepared on a clean and disinfected slide and stained with Leishman for visualizing different cellular counterparts. In the laboratory, firstly 5–7 drops Leishman is applied to the slide with the specimen. After 5 min, 10–12 drops of a buffer solution (pH 6.8) is added and mixed with the stain, then the specimen is left staying for 20–30 min, then washed off with the distilled water and dry the washed slide.

2.2. Microscopic imaging and acquisition

The images are optically grabbed by Leica Observer (Leica DM750, Leica Microsystems (Switzerland) Limited) under 100× oil objective (NA 1.515) having 0.064 μm resolution (Fig. 1).

2.3. Preprocessing

The input images are the microphotographs of Leishman's stained slides from normal blood smears. For study 150 image frames of size (2048 × 1536) are taken. As per the requirement, region of interest (ROI) of size (420 × 400) enveloping leukocyte is selected from the acquired original images. The raw ROI very often needs to be preprocessed for noise reduction, which is mainly due to staining procedure. Here, in fact, we use Wiener filter (Gonzalez and Woods, 2002; Jain, 1989) to reduce the background noise of ROI. It is a type of linear filter that tailors itself to the local image variance. Wiener performs little smoothing with large variance. Next, the noise reduced ROI is considered for contrast enhancement with the help of Laplacian filter (Gonzalez and Woods, 2002; Jain, 1989). This filter is basically a derivative operator which sharpened the image but drives constant areas to zero as well as restores the gray level tonality of ROI image.

2.4. Fuzzy divergence approach to leukocyte segmentation

In pathological evaluation, leukocytes are usually recognized by the experts because of significant morphometric variation of their nuclei. Towards this, segmentation of nuclei from background is necessary. However, the selection of the threshold is very difficult in segmenting the nuclei. Blood smear image consists of erythrocytes, leukocytes and platelets. In this paper, our focus is to segment the leukocyte nuclei from the background using fuzzy divergence minimization approach. Our approach is one such approach that proves as a sound scheme to segment nucleus distinctly and provides a comparison among three different fuzzy membership functions: Gamma, Gaussian and Cauchy.

There are various ways of choosing the threshold of an image. Basically, thresholding processes are based on the image histogram (Gonzalez and Woods, 2002). The region between two successive peaks is the region for searching the minimum value which will be set as the threshold point for thresholding the object from background. If there are more valleys (multilevel histogram) succeeding and preceding peaks of each valley are noted and accordingly the search regions are selected. And hence for unimodal case linear search is employed. Thresholding of image can be done in two ways viz., fuzzy threshold selection (Chaira and Ray, 2009) and non-fuzzy threshold selection (Abutaleb, 1989; Brink and Pendock, 1996). In case of crisp image the pixels are precise and regions are well defined. But while threshold is selected from the histogram, deep valleys of histogram cannot be located properly. Thus object may not be separated appropriately. But in fuzzy image this problem can be overcome during thresholding. So in this case we attempt to fuzzy threshold selection for segmenting the nuclei from blood image background.

2.4.1. Fuzzy image and membership functions

A gray image is a combination of gray values. Let an image A of size $M \times N$ and having L gray levels, be defined as a combination of gray levels and its membership value $\mu(f_{ij})$ of $(i,j)^{\text{th}}$ pixel:

$$A = \{f_{ij}, \mu(f_{ij})\}, \quad \forall f_{ij} \in A \tag{1}$$

where $0 \le \mu(f_{ij}) \le 1$. The grade of membership $\mu(f_{ij})$ maps the pixel gray level f_{ij} to positive real numbers in the interval [0,1]. In an image, the count of a particular gray level 'f' denotes the number of occurrences in the image. For a particular given threshold value t, the object and background is separated distinctly. The average gray level of background and object region are given by the following