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A combined spatial-spectral method for automated white blood cells segmentation

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ABSTRACT

To overcome the shortcomings in the traditional white blood cells (WBCs) identification methods based on the color or gray images captured by light microscopy, a microscopy hyperspectral imaging system was used to analyze the blood smears. The system was developed by coupling an acousto-optic tunable filter (AOTF) adapter to a microscopy and driven by a SPF Model AOTF controller, which can capture hyperspectral images from 550 nm to 1000 nm with the spectral resolution 2–5 nm. Moreover, a combined spatial-spectral algorithm is proposed to segment the nuclei and cytoplasm of WBCs from the microscopy hyperspectral images. The proposed algorithm is based on the pixel-wise improved spectral angle mapper (ISAM) segmentation, followed by the majority voting within the active contour model regions. Experimental results show that the accuracy of the proposed algorithm is 91.06% (nuclei) and 85.59% (cytoplasm), respectively, which is higher than that of the spectral information divergence (SID) algorithm because the new method can jointly use both the spectral and spatial information of blood cells.

1. Introduction

Microscopic blood cells analysis is a powerful diagnostic tool for many types of diseases. Generally, there are three major cellular constituents of blood: the red cells (or the erythrocytes), the platelets, and the white blood cells (or the leukocytes). Among these kinds of cells, the white blood cells (WBCs) refer to those nucleated cells with diameters ranging from 6 μm to 20 μm which play a very important role in the immune system. These WBCs can eliminate germs such as bacteria and viruses, and fight cancer cells and other toxic substances [1]. Therefore, identification and inspection of WBCs in peripheral blood smear can provide valuable information for disease diagnosis, such as leukemia, blood cancer, and other blood-related diseases, which makes it to be one of the most salient steps in hematological procedures [2]. Traditionally, the manually and semi-automated methods have been used by investigators to recognize and count the leukocyte cells [3,4]. These methods are generally time consuming, tedious, and operator fatigue producing, which can introduce unavoidable subjective bias during the profile selection by the hematologists.

Nowadays, the rapid progress of information technology promotes the automatization of WBCs analysis based on the modern image processing and pattern recognition approaches. This can be called the computer-aided automatic analysis method, which has been used for automatic identification and measurement of WBCs in histological blood cells images. The most important step of the computer-aided automatic analysis method is blood cell segmentation, which can directly influence the feasibility and reliability of analytical results. In the past decades, researchers have proposed different algorithms to segment the WBCs automatically from the microscopy images of blood smears. Harms and his coworker proposed a segmentation strategy that characteristic color difference thresholds for each nucleus and cytoplasm combined with geometric operations, probability functions, and a cell model [5]. This is one of the prior studies on automatic blood cells segmentation. Then, Park and Keller present a water snake (snakes on the watershed) algorithm and applied it to segment the WBCs in bone marrow images [6]. Consequently, different kinds of algorithms have been proposed to segment the WBCs, such as the active contour models [7], the feature space clustering based algorithm [8], the stepwise merging rules and gradient vector flow snake method [9], the multilevel thresholding [10], and the neural network model [11]. These studies have shown that the automated WBCs segmentation methods are faster and more objective than the manually and semi-automatic methods. However, these methods are associated with some other problems. First, most of these methods were based on the RGB color images captured by traditional light microscope which only contain the spatial

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information of blood cells. Second, due to uneven staining, color mixing often occurs which leads to the smooth variations of the average luminance in some regions of the image. In addition, the microscopy imaging conditions, smear thickness, and background illumination may result in biases and changes in shape, color, and scale. All these factors influenced the accuracy of the automatic segmentation results and lead to the algorithms complicated. Therefore, there are still some difficulties and challenging problems to segment WBCs accurately based on the traditional microscopic images.

The microscopy hyperspectral imaging technology may offer a solution for these problems. Hyperspectral imaging, also known as imaging spectroscopy or spectral imaging, is a technology that can provide a digital image with far more spectral (color) information for each pixel than traditional color cameras. The concept of hyperspectral imaging was originally defined by Goetz in the late 1980s and the hyperspectral imaging system was originally developed for the remote sensing of the earth [12]. The advantage of hyperspectral imaging technique is that it can acquire a reflectance spectrum for each pixel in the image, which can be used to classify the surface cover materials that can hardly be identified by traditional gray or color imaging methods. In recent years, this technology has been extended from the remote sensing field to the life science field. Different kinds of microscopy hyperspectral imaging system have been developed and used for biomedical analysis of various biological tissues. For example, Morris et al. have developed imaging spectrometers for fluorescence and Raman microscopic analysis on rat brainstem [13]. Shonat et al. used this technology to generate in vivo hemoglobin saturation (SO_2) and oxygen tension (PO_2) maps in the cerebral cortex of mice [14]. Some more recent studies have developed various microscopy hyperspectral imaging systems based on prism grating prism (PGP), acousto-optic tunable filters (AOTF), liquid crystal tunable filter (LCTF), and variable interference filter (VIF) for different biomedical applications [15,16]. All these studies shown that the microscopy hyperspectral imaging technology can obtain both images (structural information) and spectra (biochemical information) of biological tissues which have the significant advantages in the area of life science. However, only a few detailed investigations on automated WBCs segmentation method based microscopy hyperspectral imaging technology have been reported.

In the past decades, researchers have proposed different segmentation algorithms to identify targets from the hyperspectral images. Kruse et al. proposed a spectral angle mapper (SAM) algorithm to detect spectrally active targets in Airborne Visible Infrared Imaging Spectrometer (AVIRIS) data [17]. This is one of the pixel based methods which have been commonly used in hyperspectral images segmentation. Then, some algorithms were proposed to segment and classify objects from the multispectral and hyperspectral images, such as the modified phase correlation (MPC) [18], the active contours and graph cuts [19], the Markov random field (MRF) model-based algorithm [20], and the support vector machines (SVM) [21]. Moreover, some spatial-spectral methodologies which consider spatial information along with spectral information to improve the segmentation accuracy of a hyperspectral image also have been investigated recently [22,23]. These algorithms have been used to analyze the remote sensing images acquired in fields under the vegetation scene, the urban environment, and the AVIRIS Indian Pines data set. However, little studies have been reported on the automated white blood cells segmentation using both the spatial and spectral information extracted from the microscopy hyperspectral images.

In this study, an AOTF based microscopy hyperspectral imaging (MHSI) system was developed and used to capture hyperspectral images of blood smears. A preprocessing method was used to remove the influence of noises and artifacts computationally. Then, an automatic spatial-spectral segmentation algorithm based on the microscopy hyperspectral images of blood smears is presented and used for WBCs segmentation. Unlike those traditional light microscopy based segmentation methods, the hyperspectral based algorithm can segment the WBCs using both the spatial and spectral information of blood smears. The experiment results show that the proposed method is effective for WBCs segmentation.

2. Materials and methods

2.1. Blood smear preparation

The samples used in this study are peripheral blood smears (or peripheral blood films) which are glass microscope slides coated on one side with thin layer of fresh venous blood. After collecting the blood specimen, discard the first few drops of blood and then make the smears as follow: place a drop of blood, about 2 mm in diameter approximately an inch from the frosted area of the slide; lightly balance another slide (spreader slide) on the fingertips and place the spreader slide at a 30° angle in front of the drop of blood; pull the spreader slide back toward the blood droplet and blood spreads along the edge of the spreader slide; quickly push the spreader across the slide with smooth action; allow the blood smears to air-dry completely and finally dyed with Giemsa [24]. Those well-made peripheral smears which are thick at the frosted end and become progressively thinner toward the opposite end are selected and stored for future use.

2.2. Microscopy hyperspectral imaging system and image acquisition

The microscopy hyperspectral imaging system used in this paper consists of a microscope (Nikon 80i, Nikon Co., Ltd., Japan), an AOTF adapter (CVA200-0.55-1.0-L, Brimrose, USA), a SPF Model AOTF controller (VFI-138.5-93-SPS-A-C2, Brimrose, USA), a 1/1.8 in. high-density cooled charge coupled device detector (CCD, DS-2MBWc, Nikon, Japan), a data collection and control module (Camera Control Unit DS-U2, Nikon, Japan), and a personal computer (A8800t, Lenovo, China). The front interface of the AOTF adapter was coupled to the microscope with an F-mount adapter and the back end was coupled to the CCD with C-mount. The light source used in the microscope is a 120 W halogen lamp (Epi-illumination, Eclipse 80i, Nikon). The AOTF adapter driven by the SPF Model AOTF controller provides narrow bandwidth, rapid wavelength selection, and intensity control. The minimum wavelength selection sweep interval of the AOTF adapter is 20ns, which makes it possible for the system to capture the microscopy hyperspectral images quickly. The designed wavelength range of the system is from 550 nm to 1000 nm and the spectral resolution is 2–5 nm (2 nm at 543 nm; 5 nm at 792 nm).

The software running on the personal computer can control the AOTF adapter to filter the light passed through the microscope and record the image data on the CCD detector. In this way, the microscopy hyperspectral imaging system can capture hundreds of spectral bands covering the narrow spectral features of the captured blood smears with high accuracy. As shown in Fig. 1, the microscopy hyperspectral data can be visualized as a three dimensional cube because of its intrinsic structure, where the cube face is a function of the spatial coordinates and the depth is a function of wavelength [25]. From the figure it can be seen that the microscopy hyperspectral images of blood smears contain both spectral and spatial information of blood cells, which makes it possible to improve the accuracy of the automated WBCs segmentation with some new hyperspectral based algorithms.