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Leukocyte cells identification and quantitative morphometry based on molecular hyperspectral imaging technology

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ABSTRACT

Leukocyte cells identification is one of the most frequently performed blood tests and plays an important role in the diagnosis of diseases. The quantitative observation of leukocyte cells is often complemented by morphological analysis in both research and clinical condition. Different from the traditional leukocyte cells morphometry methods, a molecular hyperspectral imaging system based on acousto-optic tunable filter (AOTF) was developed and used to observe the blood smears. A combined spatial and spectral algorithm is proposed to identify the cytoplasm and the nucleus of leukocyte cells by integrating the fuzzy C-means (FCM) with the spatial K-means algorithm. Then the morphological parameters such as the cytoplasm area, the nuclear area, the perimeter, the nuclear ratio, the form factor, and the solidity were calculated and evaluated. Experimental results show that the proposed algorithm has better performance than the spectral based algorithm as the new algorithm can jointly use the spatial and spectral information of leukocyte cells.

1. Introduction

The morphology examination of blood cells (such as cell size, cell shape, cell hemoglobin concentration, and cell inclusions) in microscope images is a very important aid for the clinical diagnosis in both hematological and in nonhematological diseases. One of the major tasks of blood cell analysis is the identification of leukocyte cells, especially for the acute promyelocytic leukemia, in which a blood smear is very helpful in the rapid diagnosis [1]. Leukocyte cells refer to a family of cells that do not contain hemoglobin with diameters ranging from 6 μm to 20 μm. The presence of unhealthy leukocyte cells often indicates a host of complications such as deficiency of the immune system, coagulation problems, swollen lymph nodes, and other disorders [2]. Therefore, a microscopy examination of an appropriately prepared and well-stained blood smear is necessary and clinically useful in a number of circumstances [3]. Traditionally, the manually and semi-automated methods have been used by an experienced and skilled hematologist or pathologist to identify and analyze the morphology of leukocyte cells [4,5]. However, these examination methods are usually time consuming and susceptible to error due to the variety of blood cell morphology.

With the progress of information science and technology, a number of automated hematology analysis methods and systems have been proposed to handle heavy laboratory workload and to reduce labor cost, such as the Unicel DxH 800, ADVIA 2120i, and XE 2100 [6]. Most of these hematologic analyzers were used for complete blood cell count (CBC), which often cannot classify and confirm cell morphology or cell numbers when the algorithms detect abnormalities. Therefore, different methods have been investigated to identify blood cells and obtain useful information about their morphology from microscope images to help pathologists diagnose diseases. For example, Gelsema et al. have presented an image segmentation method based on the principle of multiple gray level thresholding to identify white blood cells and classified them into different clinically important types [7]. This is one of the earliest studies on blood cell identification and classification based on 2D microscopy images. Then the support vector machines (SVM) were applied to recognize six types of white blood cell from manually captured 24-bit color pictures of 720 per 480 size [8]. Theera-Umpon and Dhompongsa analyzed a set of white blood cell nucleus based features using mathematical morphology method [9]. In recent studies, different automatic recognition methods to identify blood cells from images captured by light microscopy have been proposed, such as the principal

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component analysis (PCA) and learning vector quantization (LVQ) neural network algorithm [10], the nonlinear functions applied at threshold intervals and distance transform method [11], and the marker-controlled watershed algorithm [12]. These studies have shown that the automated leukocyte cell identification and morphometry analysis methods are faster and more objective than the manually and semi-automatic methods. However, there are still some limitations associated with these 2D microscopy image based methods. For example, the RGB color images captured by traditional light microscope only contain the spatial information of leukocyte cells, which influenced the accuracy of the morphometry analysis and lead to the algorithms complicated. In addition, there are smooth variations of the average luminance in some regions of the blood smear image due to uneven staining and color mixing, which makes it difficult to identify the nucleolus and cytoplasm accurately. Therefore, there are still some challenging problems in automated leukocyte cells identification and quantitative morphometry analysis based on the 2D light microscopy images.

In recent years, the hyperspectral imaging technology has been used to analyze the blood cells. Hyperspectral imaging was originally developed for the remote sensing of the earth, which can acquire a reflectance spectrum for each pixel in the image to classify the surface cover materials that can hardly be identified by traditional gray or color imaging methods. When this technology was extended from the remote sensing field to the hematology field, it can be used to obtain both spatial and spectral information of blood cells for automated identification and classification. Zahniser et al. used two interference filters to obtain four digital images of every cell for leukocyte classification [13]. Although this method just got several images at 490 nm and 600 nm and cannot extract spectra from them, it has verified the importance of spectral information in the automated classification of leukocyte. Then Monici et al. demonstrated that lymphocytes, monocytes, neutrophils and eosinophils can be distinguished according to the intensity and spectral shape of the autofluorescence emission in the visible range from 440 to 580 nm [14]. Afterwards, Levenson et al. discussed the spectral imaging perspective on cytomics by classifying the major blood elements [15]. Guo et al. presented the multispectral imaging technique for white blood cell segmentation by applying the support vector machine (SVM) directly to the spectrum of each pixel [16]. Neugebauer et al. presented the Raman spectroscopic characterization of different cells that can be found in peripheral blood such as leukocytes, leukemic cells and solid tumor cells [17]. More recent studies by Berezhnyy and Berezhna show that the multi-spectral imaging technique also can be used to detect circulating endothelial cells (CECs) in human blood samples [18]. All these studies show that the use of spectral information in automated blood cells analysis could simplify algorithm design and aid in classification performance. However, little detailed investigations on automated leukocyte cells identification and morphological parameters calculation using both spatial and spectral information contained in molecular hyperspectral images have been reported.

In this study, an AOTF based molecular hyperspectral imaging (MHSI) system was used to observe blood smears. An automated algorithm based on the fuzzy C-means (FCM) and the spatial K-means was proposed to identify the cytoplasm and the nucleus from leukocyte cells. Then the morphological parameters of leukocyte cells were defined and calculated based on the identification results. Unlike those existing identification algorithms, the proposed method can recognize leukocyte cells using both spectral signatures and structural features of blood cells, which makes it possible to improve the accuracy of morphometry analysis. The combined spatial-spectral morphometry algorithm, the implementation of this algorithm, the experimental process, and the performance of the proposed method are illustrated and discussed in the following sections.

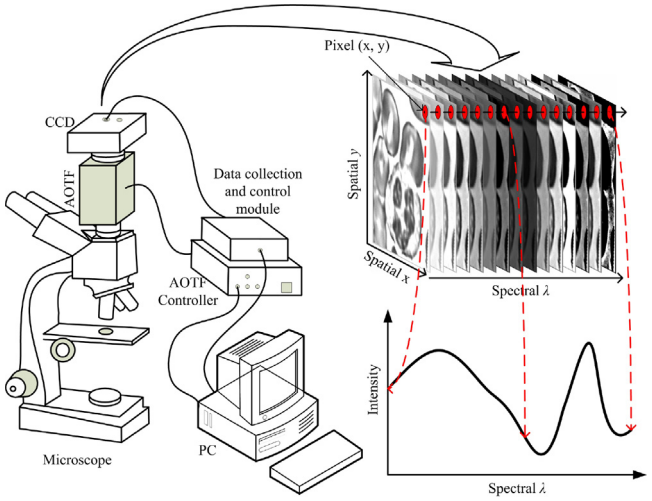

Fig. 1. Configuration of MHSI system and molecular hyperspectral data cube.

2. Materials and methods

2.1. MHSI system

In our previous study, we have developed a pushbroom microscopic hyperspectral imaging system by coupling an ImSpector spectrograph (Specim Ltd., Finland) to a microscope (E400, Nikon, Japan) [19]. This system needs to scan in one spatial dimension of sample which leads to system structure complex and time consuming for data collection. Therefore, we developed a staring imaging mode MHSI system to capture molecular hyperspectral images of blood smears. As shown in Fig. 1, the MHSI system is made up of six parts: a microscope, an AOTF adapter, a SPF Model AOTF controller, a 1/1.8 in. high-density cooled charge coupled device (CCD) detector, a data collection and control module, and a personal computer. The AOTF adapter used in this system is a rapid wavelength-scanning solid-state device that operates as a tunable optical band pass filter [20]. The transmittance light collected by the objective lens can be filtered by the AOTF adapter and imaged on the CCD detector. With wavelength switching at the narrow bandwidth by the AOTF adapter, different single band images can be captured by the CCD detector and the molecular hyperspectral data cube can be obtained by switching wavelength continuously. Unlike the pushbroom imaging system, no spatial scan is required to obtain a complete scene of molecular hyperspectral images, which makes the structure of the new system based on AOTF more simple and compact, and more suitable for blood smears detection.

The designed wavelength range of the MHSI system is from 550 nm to 1000 nm and the spectral resolution is 2–5 nm (2 nm at 543 nm and 5 nm at 792 nm). As each single band image captured at a certain wavelength contains 1024 × 1024 pixels (12 bit/pixel), a scene of molecular hyperspectral data with 80 single band images consists of approximately 160 Mb data. It will take at least 6.67 s to capture the 80 band data cube as the maximum capture speed of the CCD camera is 12 frames per second (fps) at 1024 × 1024 pixels resolution. The images were stored in band sequential (BSQ) file format, which is optimal for spatial (x, y) access of any part of a single spectral band with each line of the data followed immediately by the next line in the same spectral band. As shown in Fig. 1, the BSQ hyperspectral data can be visualized as a 3 dimension cube (x–y–λ dimensions) where the cube face is a function of the spatial coordinates and the depth is a function of the wavelength. Therefore,