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Methyl green and nitrotetrazolium blue chloride co-expression in colon tissue: A hyperspectral microscopic imaging analysis

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ABSTRACT

Histological observation of dual-stained colon sections is usually performed by visual observation under a light microscope, or by viewing on a computer screen with the assistance of image processing software in both research and clinical settings. These traditional methods are usually not sufficient to reliably differentiate spatially overlapping chromogens generated by different dyes. Hyperspectral microscopic imaging technology offers a solution for these constraints as the hyperspectral microscopic images contain information that allows differentiation between spatially co-located chromogens with similar but different spectra. In this paper, a hyperspectral microscopic imaging (HMI) system is used to identify methyl green and nitrotetrazolium blue chloride in dual-stained colon sections. Hyperspectral microscopic images are captured and the normalized score algorithm is proposed to identify the stains and generate the co-expression results. Experimental results show that the proposed normalized score algorithm can generate more accurate co-localization results than the spectral angle mapper algorithm. The hyperspectral microscopic imaging technology can enhance the visualization of dual-stained colon sections, improve the contrast and legibility of each stain using their spectral signatures, which is helpful for pathologist performing histological analyses.

1. Introduction

Colorectal cancer is a major health burden and the second leading cause of cancer-related deaths, with a worldwide cumulative incidence rate of 9.4%, affecting both men and women. Although several colon cancer diagnosis methods exist, for example, frequency domain fluorescence imaging technique [1], 64-section CT colonography [2], and confocal Raman microscopy [3], colonoscopy biopsy remains the standard diagnostic method for colorectal cancer. Traditional colonoscopy biopsy diagnosis is usually performed by experienced physicians by visual inspection of the suspicious lesion or a polyp at the most basic level. Then the biopsied polyp or lesion tissues are inspected by pathologist using light microscopy. Although this step is reasonably accurate in detecting cancer and diagnosing the stage of cancer, it still fails to detect small flat adenomas and cannot reveal information on the origin of the disease in terms of mutations and cancer markers [4]. Therefore, new methods for the early detection of colorectal cancer based on biochemical changes, rather than morphological ones are required. Hyperspectral microscopic imaging technology is a promising method for the definition of biomarkers for colorectal cancer.

Hyperspectral imaging, also known as imaging spectroscopy, is a technology that integrates traditional imaging and spectroscopy methods to obtain both spatial and spectral information of an object on the Earth. Goetz et al. proposed applications in remote sensing using hyperspectral imaging in the late 1980s [5]. Today hyperspectral imaging has been successfully applied in mining, military, geology, agriculture, environmental science, and climate change research [6]. As the spectral signatures of biological tissues are generally closely related with the pathological changes, hyperspectral imaging technology also has been extended to the field of biomedical engineering as a means of estimating the physiological status of biological tissues. In recent years, researchers have developed various hyperspectral imaging systems to capture spectral and spatial images of tissue sections for histological and immunohistochemical analysis. For example, Levenson et al. developed a type of inverse Fourier transform multi-pixel spectroscopy and found that spectral variations in staining behavior correlate with alterations in subcellular macromolecular composition [7]. This is one of the earliest studies on the relationship between pathology and the observed cytology using microscopy spectral imaging technology. Papadakis et al. later developed a spectral microscope system (SMS) for the quantitative assessment

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and mapping of the concentration of stains used to label estrogen receptors in breast cancer tissue samples [8]. Li et al. developed a pushbroom microscopic hyperspectral imaging system to evaluate the protective effect of erythropoietin on diabetic retinal cells dyed with hematoxylin [9]. Larsen et al. presented hyperspectral image analysis combined with histology to detect and characterize advanced atherosclerotic plaques in vitro [10]. These studies show that hyperspectral imaging can obtain both structural and biochemical information of tissue sections—information which makes it possible to be applied to biochemical analysis. In addition, its performance improvement over visual examination and traditional grayscale or RGB color imaging has led to the development of novel, qualitative, as well as quantitative tools for interpreting conventionally stained pathology samples [11]. Colon sections also have been analyzed by hyperspectral imaging systems. Gentry et al. investigated the application of an information-efficient spectral imaging sensor (ISIS) for colon cancer screening [12]. Then, Davis et al. presented a mathematical algorithm to perform spectral/spatial analysis on normal and malignant colon tissue and found differences among cellular constituents of cell nuclei, cytoplasm and lamina propria/lumens [13]. These differences can be expanded to cytologic preparations seeking greater sensitivity and specificity in discriminating the populations under study. Afterwards, a wavelet based segmentation algorithm was proposed by Rajpoot et al. to segment hyperspectral human colon tissue cell images into their constituent parts by exploiting the spatial relationship between these parts [14]. The classification of hyperspectral colon tissue cells using morphological analysis of gland nuclei cells, 3D spectral/spatial analysis (SSA) and 2D spatial analysis (SA) methods also have been proposed by researchers [15,16]. In a more recent study by Nallala et al., an IR spectral imaging system was used for histopathological recognition in colon cancer diagnosis [17]. All these studies demonstrate that hyperspectral imaging could be a valuable complementary tool to be used alongside conventional histopathological tissue examination in colon cancer. On the other hand, co-expression of disease markers in colon tissues is also very important for colon cancer diagnosis and pathology studies. For instance, Peng et al. proposed the co-expression of nuclear and cytoplasmic HMGB1 in colon cancer cells and found that the co-expression of HMGB1 is inversely associated with the infiltration of CD45RO+ T cells and prognosis in patients with stage IIIB colon cancer [18]. More recently, there are some studies addressing the CD133 and CD44 co-localization of named cancer stem cell (CSC) antigens in colon cancer patients [19,20].

In this paper, we investigate the potential of using hyperspectral microscopic imaging technology to enhance co-localization analysis in colon tissue sections stained by both methyl green and nitrotetrazolium blue chloride. A homemade hyperspectral microscopic imaging system based on the acousto-optic tunable filter (AOTF) was used to capture the hyperspectral microscopic images of dual-stained colon tissue sections. Then, the normalized score algorithm is presented and used for co-expression of the two stains. This algorithm can generate the co-expression results using spectral information of colon sections, which can be used to help pathologists diagnose colon diseases. The following sections describe the hyperspectral microscopic imaging (HMI) system, the normalized score co-expression analysis method, and experimental results.

2. Materials and methods

2.1. System configuration and data format

In our previous study, we developed an acousto-optic tunable filter (AOTF) based HMI system composed of six parts: a microscope (80i, Nikon, Japan) for signal collection, an AOTF adapter (CVA200-0.55-1.0-L, Brimrose, USA) for wavelength selection, a SPF Model controller (VFI-138.5-93-SPS-A-C2, Brimrose, USA) for AOTF control, a high-density cooled charge coupled device (CCD, DS-2MBWc, Nikon, Japan) for transmitted light detection, a data collection and control module (DS-U2, Nikon, Japan), and a personal computer (A8800t, Lenovo, China) for image processing and display [21]. The AOTF adapter used in the system is a rapid wavelength scanning solid-state device that operates as a tunable optical bandpass filter based on light-sound interactions in a TeO_2 crystal. The spectral filtering interval is unequal at different wavelengths due to the physical characteristics of the AOTF (usually narrower at shorter wavelengths and wider at longer wavelengths). In this system, the available wavelength range is from 550 nm to 1000 nm, with a spectral resolution from 2 nm to 5 nm (2 nm at 543 nm and 5 nm at 792 nm).

In this paper, we use the HMI system to observe dual-stained colon sections and perform co-expression analysis. Hyperspectral microscopic images were acquired using a 1/1.8 in. high-density gray CCD camera. The camera can capture each band image with a resolution of 1024×1024 pixel (12 bit per pixel). The pixel size of the CCD is 6.45×6.45 μm and the sensitivity is equivalent to ISO350. A scene of hyperspectral microscopic data with 80 single band images usually consists of approximately 160 MB data. Data was 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 data is a data cube, where the (x, y) face of the cube is a function of the spatial coordinates and the z dimension is a function of the wavelength. The hyperspectral microscopic data contain both spatial and spectral information of colon tissues, which makes it possible to perform the co-expression analysis of dual-stained tissues.

2.2. Calibration

An important step of the hyperspectral imaging approach is ensuring that the HMI system can provide an accurate measurement of the transmittance spectra [22]. Therefore, all acquired hyperspectral microscopic images must be calibrated before further processing to remove the influence of the varying spectral response originating from illumination system, transmittance of optics, and detector. This step is also important for ensuring reproducible results and for comparing spectra across multiple systems. To acquire the calibration data, a coverslipped slide containing no sample with approximately 95% transmittance across the 550 nm to 1000 nm spectral range is selected as the calibration specimen. The calibration data was collected at the beginning of a capture process and was used throughout the process as long as the imaging and illumination conditions did not

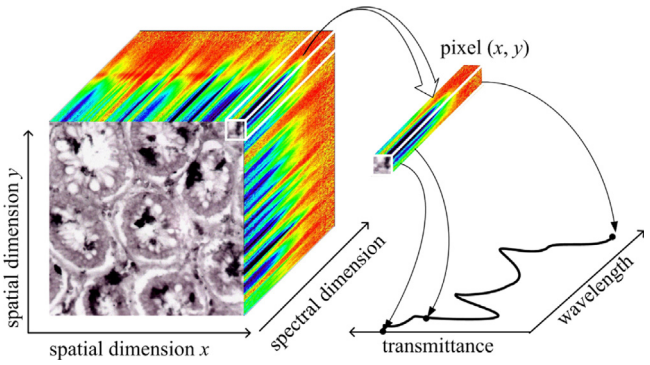

Fig. 1. The BSQ hyperspectral microscopic data cube. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)