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Multi-type hyper-spectral microscopic imaging system

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ABSTRACT

We present a hyper-spectral microscopic imaging system capable of obtaining both transmission- and fluorescence-spectral images for biological measurements. For obtaining high quality images of samples with low self-fluorescence, large aperture optics and low vibration elements were implemented in the system. This study focused on obtaining different types of spectral information, i.e., transmission and fluorescence, to study the structure of the objects and observe the similarities and differences in composition at different positions. By employing this system to investigate bee pollen, we observed a special thin-film structure, which cannot be detected using conventional microscopic systems.

1. Introduction

An imaging system is one of the most important tools required to study an object. However, conventional imaging systems only offer morphological information, which is an obvious disadvantage for scientific analysis. To overcome this limitation, spectral images, especially hyper-spectral images (HSI) that contain continuous and relatively complete records of spectral response to materials in a certain wavelength range, were developed. Objects with different chemical compositions have different spectral responses and can be distinguished from each other by their spectra. Accordingly, HSI systems are widely applied in various areas to identify the composition, status, or features of objects precisely. For example, Brigitte Mayinger et al. [1] designed a system for the endoscopic detection of esophageal cancer in 2001 by using imaging spectroscopy, and Deborah Lau et al. [2] reported a method for the elemental analysis of paintings using Raman spectral imaging. Other applications such as 3D image acquisition [3] and automatic target detection using spectral Images [4] have also been reported. Moreover, the development of laser technology and 2D filter devices has enabled hyper-spectral imaging to be applied for microscopic analysis [5,6]. For example, with a hyper-spectral microscopic imaging system, it is possible to obtain information about living cells [7] or to distinguish normal and cancerous cells [8].

There are several hyper-spectral technologies that can be used to achieve different spectral images, such as absorption spectroscopy [9], Fourier-transform infrared spectroscopy [10], and Raman spectroscopy [11]. However, in general, some materials that are almost transparent or opaque are not suitable for absorption or transmission spectroscopy, and other materials do not generate sufficient self-fluorescence. Therefore, none of these technologies can be used to analyze all types of materials. Moreover, in some cases, the difference in the spectral response for two different materials is too subtle. Multi-type hyper-spectral detection can provide more comprehensive information, but most hyper-spectral imaging studies have focused only on a single type of spectral information. Only some researches about multi-mode hyper-spectral imaging system

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with fluorescence and reflectance detection were reported [12–15], and most of these are not the microscopic system. For improving the detection and obtaining more information from samples, we carry out the research of design and application of a multi-type hyper-spectral microscopic imaging system.

The simplest way to set up a multi-type hyper-spectral microscopic imaging system is to combine a fluorescence spectral system with a transmission spectral system because the structure and spectral detection range of the two systems are quite similar. In this study, we designed such a hyper-spectral microscopic imaging system, and used the bee pollen as test sample to investigate the detection ability of it due to its relative large size and strong self-fluorescence. Several studies on pollen have been reported [16–20], such as studies on obtaining morphologic information of bee pollen by using a scanning electron microscope (SEM). The SEM can obtain more morphologic details than the conventional microscope because of its high spatial resolution. For example, with a conventional bright-field microscope, the bee pollen of the jasmine flower has been observed as a pellet with a hole at the center, while morphologic information obtained using an SEM indicated that it is not a hole but rather a thin film in a fluted structure [21]. However, SEMs are too expensive and can only obtain morphologic information. Though the spatial resolution of our proposed system is similar to that of a conventional bright-field microscope, we were able to determine that the center of the bee pollen is a thin film, rather than a hole, by using the proposed system alone. Based on the fluorescence spectral images, we could confirm that the composition of the thin film is the same as the rest of the pollen. Hence, we were able to obtain the transmission spectrum at the thin film in the bee pollen; it is difficult to obtain the transmission spectrum at other locations. These experimental results were based on multi-type spectral detection. Neither conventional microscopy nor a single-type hyper-spectral imaging system is capable of obtaining such data.

2. Design of the multi-type hyper-spectral imaging system

In our multi-type hyper-spectral microscopic system, the light passing through or being emitted from the sample will pass through the filter, focal lens, objects, and microscopic system to be imaged by the CCD. In this process, the light intensity will be decreased by certain factors. For studying light attenuation, a model was set up as follows:

(i) The relationship between the light intensity on the sample $\left(I_{sample}\right)$ and the CCD (I_{CCD}) can be expressed as

$$I_{CCD} = \frac{(1-\partial_\lambda)(1-\partial_o)I_{sample}}{\beta}. \tag{1}$$

Here, $\beta$is the magnification factor of the microscopic system. Only light with a particular wavelength can pass through the filter, while the other light will be absorbed or reflected; the loss coefficient of this process is represented as ∂_λ. The factor ∂_o is used to describe the loss of the light escaping from the optical system.

(ii) The responsiveness R of the CCD is related to the light intensityI_{CCD}, exposure timet_e, and the quality factor K associated with the characteristics of the CCD. Thus, the factor R can be described as

$$R = K \times I_{CCD} \times t_e = \frac{K \times t_e \times (1-\partial_\lambda)(1-\partial_o)I_{sample}}{\beta}. \tag{2}$$

The higher the value of R is, the higher the signal-to-noise ratio that can be obtained. As our microscopic system has a tunable filter, it has the significant disadvantage of a low optical transmission rate, especially when measuring samples with low self-fluorescence. Thus, in order to obtain high-quality images, the large-aperture elements, such as an objective with a large numerical aperture (low ∂_o) or a 2D filter with a wide field of view (low ∂_λ), can be used to improve the optical signal collection. The most commonly used 2D filters are the acousto-optic tunable filter (AOTF) and liquid-crystal tunable filter (LCTF), both of which have already been widely used in microscopic studies [22,23]. In addition, an increase in the exposure time (long t_e) also has a beneficial impact on the intensity of the optical signal. However, if vibrations are present in the system, a longer exposure time leads to a loss in sharpness in the resulting images. Thus, mechanical vibrations should be avoided during spectral scanning. In this study, we chose an LCTF for our experimental system because of its high rejection rate of out-of-band transmission and the absence of mechanical vibrations.

The structure of the experimental setup is shown in Fig. 1. It consisted of a hardware and software part.

The hardware of the system comprises a 2/3-in CCD (SONY.INCEX view HAD ICX285) with an image size of 1360 × 1024 pixels, zooming lenses with magnification ranging from 1 × to 7 ×, an LCTF (CRI.INC, VariSpec VIS) covering the spectral range of 420–720 nm, a dichroic mirror (Thorlabs, Inc., DMLP425R) to separate the laser and optical signal, a 10 × infinity-corrected imaging microscope objective (Olympus, Inc., RMS10X) with a large numerical aperture (N.A. = 0.25), and two different light sources for spectroscopy. A 20 mW laser diode (LD) emitting at λ = 410 nm was used to excite the fluorescence, and a catadioptric white LED light source was used for transmission spectroscopy. The optical characteristics of the laser, LED, and dichroic mirror are shown in Fig. 2.

Software is used to control the gain factor, exposure time, sweep range, and step length. The minimum step length of spectral scanning and the maximum acquisition speed of the system are 5 nm and 50 ms, respectively, as determined by the LCTF characteristics. The exposure time of the system is adjustable from 1/44000–115 s. The time series of the CCD and LCTF are shown in Fig. 3. We apply median filtering program to reduce the salt and pepper noise, and extract the spectral data by a MATLAB program in the computer.