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A portable confocal hyperspectral microscope without any scan or tube lens and its application in fluorescence and Raman spectral imaging

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ABSTRACT

In this study, a portable confocal hyperspectral microscope is developed. In traditional confocal laser scanning microscopes, scan lens and tube lens are utilized to achieve a conjugate relationship between the galvanometer and the back focal plane of the objective, in order to achieve a better resolution. However, these lenses make it difficult to scale down the volume of the system. In our portable confocal hyperspectral microscope (PCHM), the objective is placed directly next to the galvomirror. Thus, scan lens and tube lens are not included in our system and the size of this system is greatly reduced. Furthermore, the resolution is also acceptable in many biomedical and food-safety applications. Through reducing the optical length of the system, the signal detection efficiency is enhanced. This is conducive to realizing both the fluorescence and Raman hyperspectral imaging. With a multimode fiber as a pinhole, an improved image contrast is also achieved. Fluorescent spectral images for HeLa cells/fingers and Raman spectral images of kumquat pericarp are present. The spectral resolution and spatial resolutions are about 0.4 nm and 2.19 μm, respectively. These results demonstrate that this portable hyperspectral microscope can be used in in-vivo fluorescence imaging and in situ Raman spectral imaging.

1. Introduction

Fluorescence/Raman imaging is one of the most important imaging technologies in biological imaging. Fluorescent signal can help to provide a high contrast image in biological study. Owing to its fingerprint characteristic to molecule structure, Raman imaging has been well-established as a useful tool in biology [1–3], pharmacology [4–8], and food-safety investigations [9,10]. Furthermore, fluorescence/Raman spectral imaging technology provide a 4D information (spatial and spectral) of a target, which has a great significance in pharmacology and biomolecular study [11]. Recently, by using imaging spectrometer and scanning method, hyperspectral imaging technology, with sub-nanometer spectral resolution and micrometer spatial resolution, has been developed [12–14]. Meanwhile, various portable fiber Raman probes has been used in biomedical applications, such as endoscopes and colonoscopies. Schwab and McCreery developed a fiber probe, consisting of a multitude of collections fibers surrounding a single excitation fiber, to improved collection efficiency [15]. And this arrangement is prevalently used and improved for Raman spectroscopy [16–21]. Dual fiber probe with optical filters was also investigated in order to prevent the reentry of specularly reflected laser light into collection fiber [22–24].

On the other hand, capable of optical sectioning and able to provide greater resolution than conventional imaging, confocal laser scanning microscope (CLSM) have been intensively investigated and applied in biological and medical imaging area. In a traditional CLSM, a rotated grating is utilized to perform spectral imaging. However, the spectral resolution is relatively low.

Since high spatial resolution hyperspectral imaging and fiber Raman probe have immense applications in biomedical and food-safety area, a portable confocal hyperspectral microscope (PCHM) is developed in this study. The system is aimed at utilizing the fiber spectrometer to achieve spectral image with a considerable collection efficiency. As fiber spectrometer usually has a low optical sensitivity compared with a high-end spectrometer which was widely used in previous investigations, it is necessary to improve the detection efficiency. In our PCHM, the galvomirror is placed directly next to the objective, though adopting the confocal detection mode. In this

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way, the detection efficiency for signal light can be enhanced compared with the traditional confocal microscope setup with scan lens and tube lens. Meanwhile, the removal of the scan lens and tube lens further reduce the size of the system. We experimentally demonstrate the diverse hyperspectral image capability of this system by obtaining the fluorescence spectral image of cells and Raman spectral image of kumquat pericarp.

2. Methods and materials

2.1. Optical configuration of the portable confocal hyperspectral microscope

In a traditional CLSM, scan lens and tube lens are usually utilized to build up a conjugate relationship between the galvanometer and the back focal plane of the objective, to implement imaging with higher resolution and intensity homogeneity at the imaging sample. However, the employment of both scan lens and tube lens makes it difficult to scale down the system. Besides, scan lens and tube lens are used to expand the excited laser beam, but it would also scale down the diameter of signal light beam. Signal light beam will have a lager focal spot after passing though the focal lens before pinhole, thus would be partially blocked by the pinhole. Therefore, the existence of scan lens and tube lens reduce the detection efficiency. In addition, absorption and reflection of lens also deteriorate the detection of signal light. To improve the signal light detection efficiency and reduce the volume of this system, a PCHM is developed without using scan and tube lens.

The imaging system consists of a fiber-coupled laser source, an optical scanning units, a set of filters, an imaging objective and a detection module. Fig. 1(a) provides a schematic of the optical setup of the PCHM. Fig. 1(b) shows the corresponding photograph of the PCHM. A single-mode fiber-coupled 532 nm laser is collimated and injected into the system. Then it is deflected by a mirror mounted on a right-angle kinematic mirror mount (KCB1/M; THORLABS), and transmitted into a galvanometer mirror (GM) by a dichroic beam splitter (LPD02-532RU-25; Semrock), which is mounted on a kinematic dichroic filter mount (DFM/M; THORLABS). The galvanometric mirror is used to perform scanning imaging. The signal light emitted from the sample is collected by the same objective, and transmitted through the GM and dichroic beam splitter. A long pass edge filter (BLP01-532R-25; Semrock), which is assembled in DFM/M, is used to eliminate the excitation light source. Then the signal light is reflected by a mirror mounted on KCB1/M and focused into a multimode optical fiber by an aspheric achromatic lens (49–622; Edmund), which is mounted on a z-axis translation mount (SM1Z; THORLABS). Here, the fiber is utilized as a pinhole to reject the noise out of focus and is fixed on an SMA fiber adapter plate (SM1SMA; THORLABS), which is assembled on a thread cage plates (CP02/M, THORLABS). Finally, the signal is collected through the fiber by a detection module, which can be a photodiode (PD), a photomultiplier tube (PMT), or a spectrometer. The image processes are controlled by a home-made LABVIEW software running on a personal computer equipped with multifunctional data acquisition cards (NI USB-6356; National Instruments). As the GM raster scans the sample, the signal from each pixel is collected simultaneously and displayed on the computer screen in real time. Imaging can be performed in reflectance mode, fluorescence mode and Raman mode. A beam splitter is assembled in DFM/M when working in a reflectance mode, instead of a dichroic beam splitter when working in fluorescence mode or Raman mode. Commercially-available fiber spectrometer, USB2000+ and QE65000 (Ocean Optics) are utilized in this system in spectral mode, the spectral resolution of which are 1 nm and 0.4 nm, respectively.

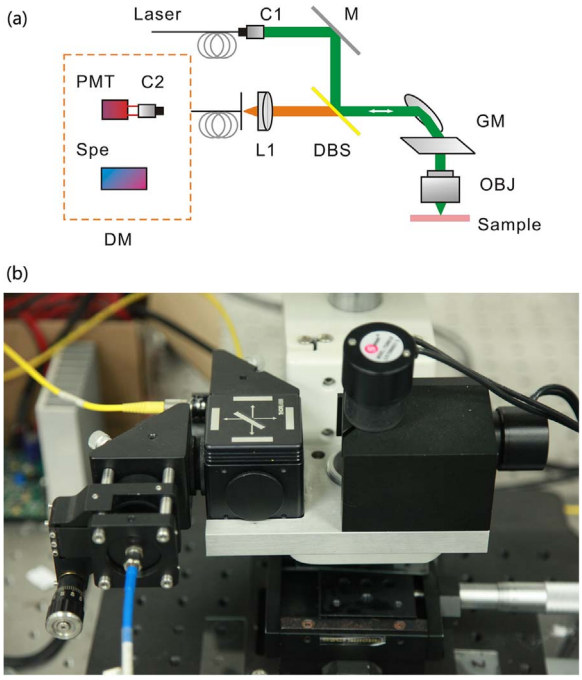

Fig. 1. (a) Schematic of the portable confocal spectral microscope. M: mirror. DBS: dichroic beam splitter. GM: galvanometer mirror. OBJ: objective. C1 and C2: fiber collimator. DM: detection module. L1: aspheric achromatic lens. (b) Photograph of the portable confocal spectral microscope tabletop implementation.

2.2. Materials

Polymer microspheres, red fluorescing, 1% solids, and Rhodamine 6G were purchased from Duke Scientific Corp and Sigma-Aldrich, respectively. HeLa cells were labeled with labeled with 2,3-Bis(4-(phenyl(4-(1,2,2-triphenylvinyl)phenyl)amino)phenyl) fumaronitrile(TTF)encapsulated organically modified silica (ORMOSIL) [25]. They were cultivated in Dulbecco minimum essential media (DMEM) with 10% fetal bovine serum (FBS), 1% penicillin, and 1% amphotericin B, at 37 °C and 5%, and were grown overnight on 35 mm petri dishes with cover slips. After 24 h, the diluted solution of about 25 μg/mL TTF-ORMOSIL nanoparticles was utilized to treat the HeLa cells for 30 min. Then the HeLa cells were washed thrice by phosphate buffered saline (PBS, pH=7.4, 10 mM) and treated with paraformaldehyde in for 15 min. After that, the cells were washed thrice by PBS again and the cover slips with cells were sealed with glycerin onto the glass slides.

3. Results and discussion

3.1. Characterization of lateral spatial resolution

The lateral spatial resolution of PCHM was characterized in reflectance mode by imaging a US Air Force (USAF) target (R1DS1P; THORLABS). The sample was captured by a 20×1.0 NA (Numerical Aperture) water objective (N20X-PFH; Olympus), with entrance pupil about 20 mm. The diameter of the laser beam was adjusted to be 5 mm in this work, in order to make sure the whole excitation laser beam is able to transmit through the objective and focus on the sample during the laser scanning process. However, this results in a relatively lower resolution according to Rayleigh formula. Fig. 2(a) shows that the microscope can distinguish the group 7, element 6 of R1DS1P, in which the line width is 2.19 μm. As this is the minimum spacing of the test target, we believe the actual lateral resolution of this system is superior to 2.19 μm. According to the NA of objective and input laser, the theoretical spatial resolution is 1.3 μm (0.61×λ/NA×4), where the factor 4 stands for the ratio between entrance pupil and laser beam. Fig. 2(b) was obtained by using photodiode detector (PDA100A-EC; THORLABS) as the detection module, while Fig. 2(c) was obtained by