Multi-color, stain-free medical imaging by stimulated Raman scattering microscopy

Yasuyuki OZEKI †

† Department of Electrical Engineering and Information Systems, The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8656, Japan

E-mail: † ozeki@ee.t.u-tokyo.ac.jp

Abstract This paper introduces stimulated Raman scattering (SRS) microscopy for stain-free biomedical imaging. We show that our high-speed spectral microscopy is capable of multicolor imaging of various types of tissues.

Key words **Raman microscopy, stimulated Raman scattering**

1. Introduction

Raman microscopy is attracting much attention because Raman scattering gives vibrational spectroscopic information of biomolecules, allowing us to observe unstained (i.e. transparent) biological samples. Previously, Raman imaging suffered from slow imaging speed and low image resolution, and therefore applications were limited. To overcome this, various technical developments have been made including (a) multi-focus/line-scanning geometries [1], and (b) the use of coherent Raman effects [2]. Indeed, stimulated Raman scattering (SRS) microscopy [3-5], which is categorized in (b), allows not only fast imaging but also quantitative analysis because SRS signal is proportional to the density of molecules of interest. In order to fully utilize these advantages, we recently developed high-speed SRS spectral microscopy [6], which allows stain-free, multi-color imaging of tissues within a short period of time.

In this paper, I introduce our SRS spectral microscopy system and show imaging results of unstained tissues.

2. SRS spectral microscopy

Figure 1 shows the schematic of SRS microscopy. Two-color laser pulses, which are called pump and Stokes, are used, and either the pump or the Stokes beam is intensity-modulated beforehand. Then these pulses are focused on a sample. When the optical frequency difference matches the vibrational resonance frequency of sample molecules, SRS occurs. As a result, the pump pulses are attenuated, while the Stokes pulses are amplified. Therefore the intensity modulation is transferred to the other pulse train. Then the SRS-induced modulation transfer is measured by the photodetection followed by the lock-in detection. Images are taken by scanning the laser beam with a beam scanner (not shown.) In order to discriminate different constituents in SRS microscopy, we conduct SRS spectral imaging, where the laser wavelength is scanned to acquire a set of SRS images at various vibrational frequencies.

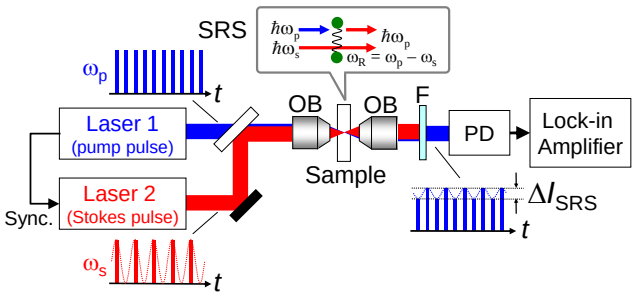

Fig. 1. Schematic of SRS microscopy. OB: objective lens, F: filter, PD: photodiode.

We have developed a high-speed SRS spectral microscopy system [6]. As a light sources, we employed a 76-MHz picosecond Ti:sapphire laser for pump pulses and a 38-MHz wavelength-tunable Yb fiber laser system for Stokes pulses. The tuning mechanism of the latter relies on the bandpass filtering of broadband pulses produced by an Yb fiber oscillator and post amplification with Yb fiber amplifiers. The transmission wavelength of the filter can be changed by controlling the galvanomirror scanner (GS) in the filter. The tunability of the Stokes pulses is ~300 cm^{-1} and the spectral width is ~3 cm^{-1}. The pump and Stokes lasers are synchronized by a feedback loop. Note that the repetition rate of Yb oscillator is half that of Ti:sapphire laser. This situation is equivalent to the intensity modulation of Yb laser pulses at the maximum modulation frequency. Then these two-color pulses are combined and led to a video-rate laser-scanning SRS microscope. By using this system, we were able to acquire SRS images with 500 × 480 pixels at a frame rate of 30.8 frames/s, while the filter was controlled in a frame-by-frame manner. We also developed a method for analyzing spectral images based on a modified version of independent component analysis (ICA), which allows blind separation of several constituents. The images given by ICA can be used for producing a multi-color image, while the vibrational spectra given by ICA can be used to assign the images.

3. Tissue imaging

Figure 2(a) shows the imaging results of a rat liver tissue. In order to have good signal-to-noise ratio, we repeated 10 times the acquisition of 91 spectral images at wavenumbers from 2800 to 3100 cm^{-1}. Nevertheless, the total acquisition time is less than 30 s. By combining IC images, we could obtain a multicolor image shown in Fig. 2(a). Various structures in the liver tissue such as lipid droplets (A), cytoplasm (B), fibrous texture (C), nucleus (D), and water-rich region (E) can be seen with different pseudo-colors, and their morphological shapes and locations are clearly visualized, which would be useful for medical diagnosis. Figure 2(b) shows a 3D image of fibrous textures around a blood vessel. This image was taken by two-wavenumber imaging at 2850 and 2950 cm^{-1}, followed by ICA. The acquisition time is less than 6 s. Figure 2(c) shows SRS images of mouse intestine taken at different depths separated by 5.6 μm. The acquisition time was 24 s. We could successfully visualize the 3D structure of nuclei and cytoplasm of intestinal villi. In this way, SRS spectral microscopy allows rapid label-free imaging, and will be useful for medical imaging.

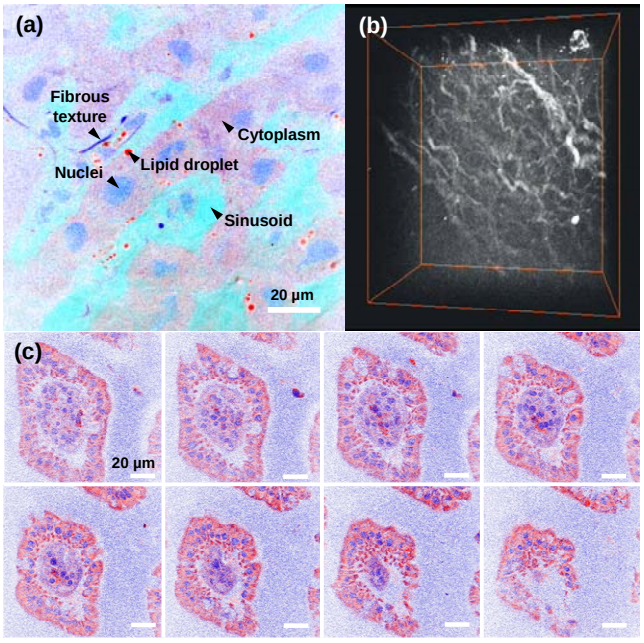

Fig. 2. Experimental results of stain-free imaging with SRS spectral microscopy [6]. (a) Multi-color image of rat liver. (b) 3D image of fibrous texture in blood vessel in rat liver. (c) Sectioned images of mouse intestine.

References

[1] A. F. Palonpon *et al.*, Nature Protocol **8**, 677 (2013).

[2] W. Min *et al.*, Annu. Rev. Phys. Chem. **62**, 507 (2011).

[3] C. W. Freudiger *et al.*, Science **322**, 1857 (2008).

[4] P. Nandakumar *et al.*, N. J. Phys. **11**, 033026 (2009).

[5] Y. Ozeki *et al.*, Opt. Express **17**, 3651 (2009).

[6] Y. Ozeki *et al.*, Nature Photon. **6**, 845 (2012).