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Stimulated Raman Scattering Microscopy with High-Speed Wavelength Scanning Laser

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Stimulated Raman scattering (SRS) microscopy becomes an attractive tool in biological applications recently, since it offers a non-invasive label-free imaging with molecular sensitivity. SRS microscopy employs two pulse laser sources with slightly different frequencies which are tightly focused on the sample. If the frequency difference between both lasers coincides with the specific molecular vibration, the SRS process generates the energy transfer from high frequency pulse to the low frequency pulse. Using the lock-in detection method and the modulation of one excitation beam, the small change of pulse intensity due to the energy transfer can be identified [1-3].

Further application in biological imaging requires the simultaneous information of multiple molecules and the fast detection of molecular change [2-5]. Conventional SRS microscopy can achieve a fast detection by employing a high-sensitive optical detector; however, it suffers from the simultaneous detection ability [3]. We developed an SRS microscopy system equipped with a picosecond laser that enables high speed and wide range wavelength scanning. A high-speed wavelength scanning laser is a modified commercial picosecond-pulse mode-locked laser with an acousto optic tunable filter (AOTF), a piezoelectric device from birefringent crystal, installed inside the cavity. AOTF can alter the diffracted wavelength of incident light due to the controlled radio frequency applied to the crystal. However, the synchronization with another laser to generate the SRS process becomes difficult to be achieved because of the repetition frequency shift during the wavelength scanning due to the cavity length change. Thus, one of the cavity mirrors attached to a piezo-actuator and a parallel plate pair mounted on the galvano scanners are introduced into the cavity to compensate the cavity length change and to maintain the mode-locked state of the laser [5]. The AOTF laser can be tuned from 820 nm to 920 nm with the resolution of 0.1 nm. It enables the observation of Raman spectrum of around 1300 cm^{-1} with resolution of 1.2 cm^{-1}.

One important subject in biological study is lipid and SRS microscopy is suitable for lipid observation because lipid abundantly consists of CH structure which has strong Raman signal in specific region around 2845 cm^{-1} [6, 7]. Additionally, CH has an isomorphic structure, CD, which downshifts the Raman signal to the silent region. This CD bond can be used a Raman tag for the biological application in lipid treatments [6]. For observation, we demonstrate the imaging of polystyrene beads and 3T3-L1 adipocytes (fat storing cells) around Raman shift of 2845 cm^{-1} (Figure 1). In the future, we would like to extend our observation range using treated cells with deuterated lipid (lipid with CD structure) at Raman shift of 2100 cm^{-1} and CH 2845 cm^{-1} that belong CD and CH structures, respectively. For this purpose, we should set the pump beam at 709.06 nm; whereas, the Stokes beam from AOTF laser should be altered between 833.60 nm and 888.00 nm.

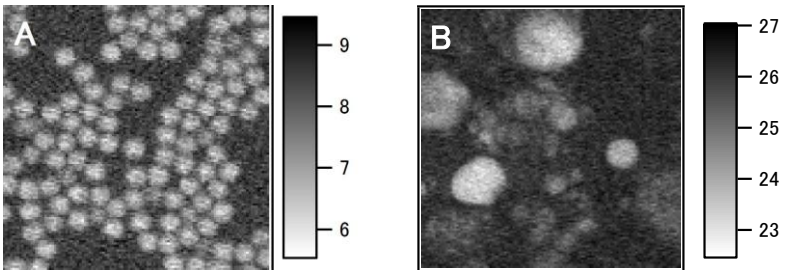

Figure 1. SRS images of (A)polystyerene beads and (B)3T3-L1 adipocytes at 2845 cm^{-1}, 125 x 125 pixels

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