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Stimulated Raman scattering microscopy with spectral focusing of 2-ps laser pulses for higher spectral resolution and signal-to-background ratio

Kota Koike[a,b], Nicholas Smith[c], and Katsumasa Fujita*[a,b,d]
[a]Department of Applied Physics, Osaka University, 2-1 Yamadaoka, Suita, Osaka, Japan, 565-0871; [b]Advanced Photonics and Biosensing Open Innovation Laboratory, AIST - Osaka University, 2-1 Yamadaoka, Suita, Osaka, Japan, 565-0871; [c]Immunology Frontier Research Center, Osaka University, 2-1 Yamadaoka, Suita, Osaka, Japan, 565-0871; [d]Transdimensional Life Imaging Division, Institute for Open and Transdisciplinary Research Initiatives, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan
*fujita@ap.eng.osaka-u.ac.jp; phone +81-6-6879-7847

ABSTRACT

We propose the use of 14 ps chirped laser pulses in stimulated Raman scattering (SRS) microscopy to improve the spectral resolution and signal-to-background ratio (SBR) in SRS imaging. We developed a single-grating-based pulse chirper and implemented it into an intensity-modulation SRS microscope to stretch the excitation pulse width from 2 to 14 ps. We confirmed that the 14 ps pulses provide a spectral resolution of 2 cm^{-1} by measuring the SRS spectra of diamond crystals. We found that the 14 ps pulses have smaller nonlinear background signals and improve SBR in SRS imaging of various samples due to the instantaneous narrow-band excitation and low peak power. Our technique can broaden the application of the 2 ps intensity modulation SRS microscopy by improving the spectral resolution and sensitivity.

Keywords: stimulated Raman scattering microscopy, pulse shaping, label-free, Raman scattering, spectral focusing

1. INTRODUCTION

Stimulated Raman scattering (SRS) microscopy enables high-speed and label-free chemical imaging by coherent excitation of molecules [1,2]. Although SRS is a nonlinear process and shorter pulses can generate a large signal amount [3], 2 ps pulses are the most commonly used laser source for SRS microscopy. Owing to the narrow spectral band width, picosecond pulses used in SRS microscopy can provide Raman spectral shapes similar to the spontaneous Raman with reduced nonlinear background signals [4]. However, to the best of our knowledge, the regime with pulse widths above 10 ps has not been exploited for SRS imaging, despite its great potential for high spectral resolution and sensitivity. Here, we propose the use of picosecond spectral focusing to obtain 14 ps laser pulses and improve the spectral resolution and signal-to-background ratio (SBR) in SRS microscopy. We developed single-grating-based pulse chirpers, which allowed us to switch the pulse width between 2, 7, or 14 ps without affecting the beam path after the chirper. With this long pulse regime, we show that a 7-fold increase in spectral resolution and 3-fold increase in SBR can be obtained compared to conventional SRS excitation with 2 ps lasers.

2. EXPERIMENTAL

2.1 Experimental setup

Fig.1 shows a schematic of an intensity-modulation SRS microscope equipped with pulse chirpers. We used a 2 ps laser pulse (Emerald Engine, APE) at 1031 nm with a repetition rate of 80 MHz as a Stokes beam for SRS imaging. As a pump beam, we used a wavelength-tunable laser pulse (Levante Emerald, APE) from 660 to 990 nm. The intensity of the Stokes beam was modulated by an EOM (APE) at 20 MHz with a 90% modulation depth. A motorized-optical delay line was placed in the pump beam path to ensure the temporal overlap between the pump and Stokes pulse trains. Both laser beams were introduced into the chirp systems individually, where we can easily switch the pulse widths between 2 (no-

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chirp), 7, and 14 ps (see Figure 2 for detail). After the chirpers, the beams were picked-off by mirrors (PM) and spatially combined by a short-wavelength pass dichroic mirror (DM). We performed laser scanning using a pair of galvanometer mirrors in front of an entrance port of the microscope body. Water immersion objective lenses (Plan Apo IR 60x, 1.27 NA, Nikon) were used to focus and collect the beams. After blocking the Stokes beam by optical bandpass filters (BPF, Semrock), we detected the pump beam intensity using a large-area silicon photodiode (PD, S3590-09, Hamamatsu Photonics). The PD was reverse-biased with 90 V by DC power suppliers to increase the saturation threshold and the response bandwidth. The signal from PD was pre-filtered with a bandpass filter (BBP-21.4+, Mini-Circuits). The signal was then sent to a lock-in amplifier (UHFLI, Zurich Instruments) for SRS signal demodulation. A reference signal at 20 MHz was provided from the EOM. The demodulated signal was sent to PC to reconstruct SRS images.

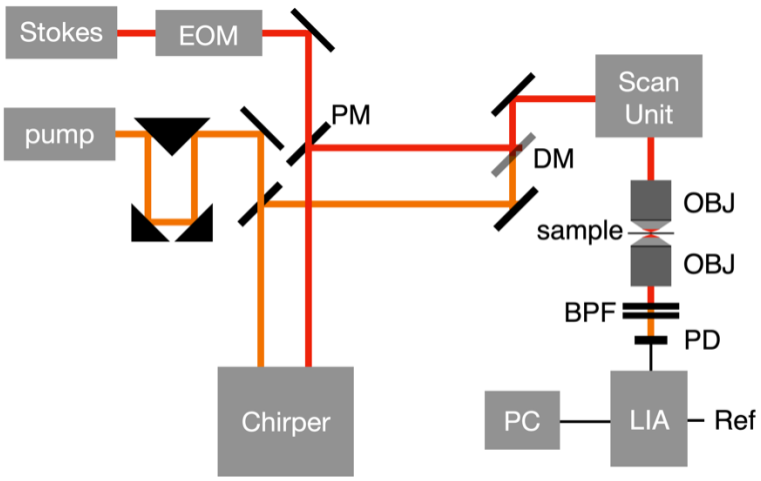

Figure 1. An SRS microscope equipped with pulse chirpers. EOM for electro-optic modulator; PM for pick-off mirror; DM for dichroic mirror; FM for flip mirror; OBJ for objective lens; BPF for optical bandpass filter; PD for photodiode; Ref for reference signal; LIA for lock-in amplifier.

Fig. 2(a) shows a detailed schematic of the chirper we have developed for the picosecond spectral focusing. It consists of a single grating and several sets of retroreflectors (RFs) for the pump and Stokes beam, respectively. Fig. 2(b) shows how to switch the pulse width between 2 ps and other pulse width modes. As for the 2 ps mode, we changed only the beam heights using RF. As for the 7 and 14 ps modes, we used diffraction gratings to chirp the laser pulses. The grating was inserted into the beam path, and each beam was diffracted four times at different grating positions. The output beams were maintained at the same position as the 2 ps mode (Fig. 2(b)). The throughput of average power was around 78% for the pump (847 nm) and 83% for the Stokes beam, and there was no significant power loss other than diffraction gratings.

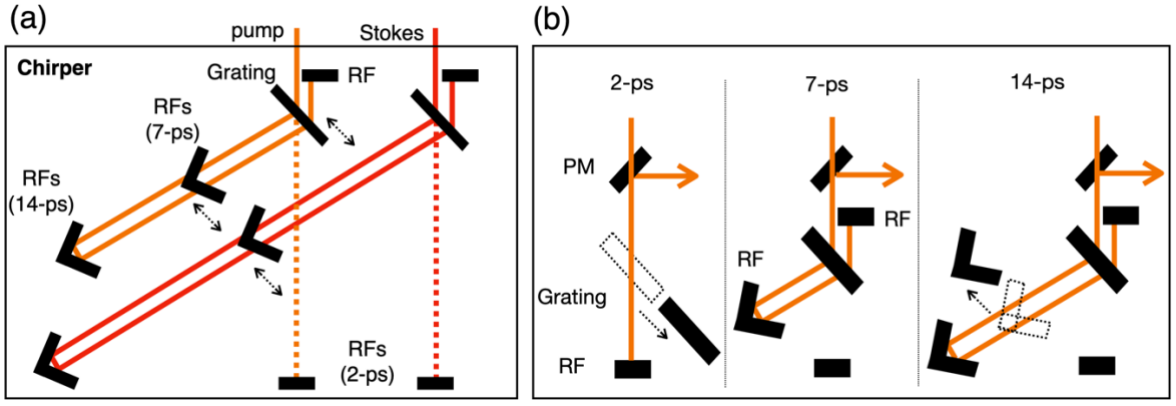

Figure 2. (a) Optical system of a single-grating chirper. RF; retroreflector. (b) Switching the pulse width by moving grating and RFs.

2.2 Spectral resolution

We performed SRS spectroscopy of diamond crystals to determine the spectral resolution of the developed SRS microscope for each pulse widths. The diamond crystals have a Raman peak at 1332.5 cm^{-1} with a spectral width of 1.7 cm^{-1} [5,6]. The crystals (IRM2-4, Tomei Diamond) were immersed in water and sandwiched with two coverslips. The pump beam wavelength was tuned from 904.8 (1354.7 cm^{-1}) to 908 nm (1315.8 cm^{-1}), and the SRS signal of the diamond crystals was obtained at each wavenumber with each pulse width. Table. 1 shows the full-width-at-half-maximum (FWHM) of the diamond peak calculated from the Lorentzian fitting function. The experimental spectral resolution was then determined from the measured FWHMs [7]. As a result, we achieved the spectral resolutions of 3.71 and 1.85 cm^{-1} for the 7 and 14 ps modes, respectively. The theoretical spectral resolution was also estimated from the experimental values of pulse widths [7,8]. The pulse widths were obtained by $sech^2$ fitting to the autocorrelation functions measured using an autocorrelator (pulseCheck SM2000, APE). From these values, we estimated the theoretical spectral resolutions of 11.26, 2.87, and 1.49 cm^{-1} for 2, 7, and 14 ps modes, respectively. There was a 24-30% difference between the experimental and theoretical values, presumably due to the presence of higher-order dispersion in the original 2 ps laser pulse, which became pronounced due to chirping. Nevertheless, we achieved a spectral resolution of 2 cm^{-1} in SRS microscopy, which allows us to resolve most of the Raman bands of biomolecules.

Table 1. Measurement of spectral resolution and pulse width.

	SRS peak FWHM	Experimental spectral resolution	Theoretical spectral resolution
2-ps	13.24 cm^{-1}	13.13 cm^{-1}	11.26 cm^{-1}
7-ps	4.08 cm^{-1}	3.71 cm^{-1}	2.87 cm^{-1}
14-ps	2.51 cm^{-1}	1.85 cm^{-1}	1.49 cm^{-1}

2.3 Signal-to-background ratio

We investigated the background suppression capability of longer pulses. We used polystyrene (PS) beads with a diameter of 4.5 μm as a sample. PS has two Raman bands at 1583 and 1602 cm^{-1}, corresponding to C=C stretching and ring-skeletal stretching modes, respectively [9]. The PS beads were attached to a MAS-coated coverslip immersed in water and then sandwiched by another coverslip. We performed SRS spectroscopy/imaging of the beads with different pulse widths and compared the SBRs between the SRS images. The imaging parameters used for this measurement were as follows. The scanning area was 38x38 μm^2 with pixel numbers of 64x64. The pixel dwell time was 100 μs with a time constant of 10 μs and 4th low-pass filter order. The average powers of the pump and Stokes beams were 15 and 25 mW for the 2 ps mode, and 40 and 70 mW for the 14 ps mode. Fig. 3(a) shows SRS spectra of PS beads obtained from the mean value of PS beads SRS signals at each wavenumber. In the 14 ps spectrum, the two Raman bands at 1583 and 1603 cm^{-1} were resolved due to the higher spectral resolution. The two peaks were not resolved with the 2 ps excitation due to the relatively large excitation bandwidth of more than 10 cm^{-1}. Fig. 3(b) shows the SRS images at the on-resonance and off-resonance wavenumbers obtained by the 2 ps and 14 ps modes. We used 2.7 times higher average powers in the 14 ps to obtain the same signal level as the 2 ps mode at the SRS peak. A significant difference was observed in the off-resonance image. The background signal level of 14 ps was smaller than 2 ps. This may be due to the narrower excitation bandwidth and lower peak power of 14 ps laser pulses, resulting in decreases in other nonlinear background effects (e.g., XPM) and the excitation of Raman peak shoulder. We calculated the signal-to-background ratio from the SRS images. The SRS signal and background values were defined as the mean value of PS beads signals at the on and off-resonance images. The 2 ps SBR was 13. In contrast, the 14 ps SBR was 36. The SBR improvement was around 2.8. We experimentally confirmed the SBR improvement using longer pulses due to higher spectral resolution and lower peak power excitation.

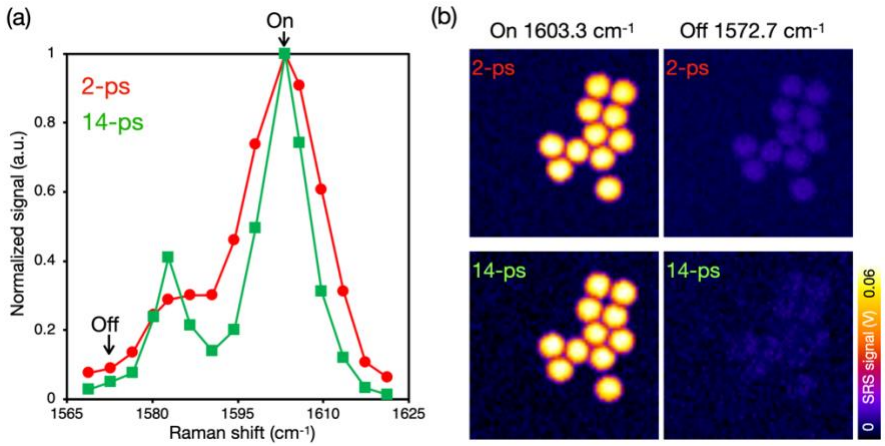

Figure 3 (a) SRS spectra of PS beads obtained at different pulse widths. (b) SRS images at the SRS on-resonance (1603 cm^{-1}) and SRS off-resonance (1573 cm^{-1}) obtained with the 2 (red) and 14 ps mode (green). The same color scale was used for all images.

3. CONCLUSION

In conclusion, we developed an easy-to-build single-grating chirper that allowed us to obtain the chirped 14 ps laser pulses in both pump and Stokes beams. We have shown that the 14 ps pulses provided spectral resolution of 2 cm^{-1} based on the SRS measurements of diamond crystals. Due to the narrow instantaneous spectral width and low peak powers of the 14 ps laser pulses, SRS imaging of samples with high spectral resolution and SBR was realized. The proposed methods can be easily incorporated into any SRS microscope based on a 2 ps laser system to improve the imaging sensitivity.

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