Super-resolution vibrational imaging by using saturation of stimulated-Raman scattering

Kota Koike[1], Giulia Zanini[2,3], Hiroyuki Kawagoe[1], Ryosuke Oketani[1], Nicholas Isaac Smith[4], and Katsumasa Fujita[1,*]
[1] Eng., Osaka Univ., [2] Nanoscopy, IIT, [3]DIFI, Univ. of Genoa, [4]iFReC, Osaka Univ.
E-mail: fujita@ap.eng.osaka-u.ac.jp

Raman scattering microscopy is a powerful technique for label-free imaging based on molecular vibration in biological samples [1]. However, the efficiency of spontaneous Raman scattering is low, and the improvement of both sensitivity and spatial resolution has been difficult. With the exploitation of coherent Raman scattering, which produces a stronger vibrational signal, the enhancements of spatial resolution and contrast has been achieved in coherent anti-Stokes Raman scattering (CARS) microscopy by combining with saturated excitation (SAX) microscopy [2]. SAX microscopy exploits the nonlinear response caused by saturated excitation in order to shrink the point-spread function (PSF) [3].

Stimulated Raman scattering (SRS) microscopy is also a coherent Raman imaging technique. SRS arises from the imaginary part of the third-order susceptibility $\chi^{(3)}$ of the sample. SRS microscopy has a powerful potential for bio-imaging due to its high sensitivity, high contrast and the reduction of non-resonant background [4,5]. The saturation of SRS signal is reported at higher excitation intensity [6] and opens up the possibility to apply the SAX concept for obtaining a resolution improvement also in SRS microscopy, as previously demonstrated in CARS microscopy.

Here, we theoretically show the spatial and spectral resolution improvement in SRS microscopy using the concept of SAX microscopy [7]. We performed the calculation of the SRS signal intensity and PSFs when a diamond sample is observed at various pump and Stokes beam powers. By subtracting the unsaturated PSF from the saturated PSF, we observed a reduction in the effective size of the PSF (see Figure 1). We confirmed the improvement of the spatial resolution in the subtracted case with an improvement factor of about 1.3 in both lateral and axial directions compared with the conventional saturated condition.

We also performed some experimental measurements using PMMA beads and diamond crystals to demonstrate the applicability of the technique.

We theoretically and experimentally demonstrated that saturation of stimulated-Raman scattering can be effectively used for label-free imaging with higher spatial resolution.

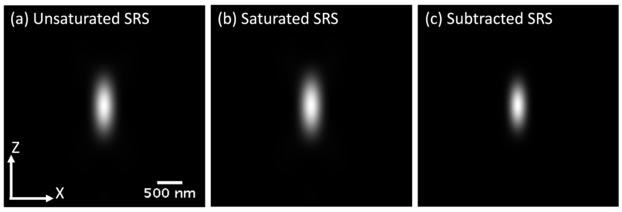

Figure 1. Calculated PSFs in SRS microscopy when a diamond crystal was observed with fixed Stokes intensity of 1×10^{7} mW/cm^{2} and pump intensity of (a) 1×10^{6} mW/cm^{2} (unsaturated condition) and (b) 1×10^{7} mW/cm^{2} (saturated condition). In (c), the subtracted PSF calculated from (a) and (b) is shown. The wavelengths of pump and Stokes beam are 931.88nm and 1064nm in order to match with the vibrational mode of diamond crystals at 1332.5cm^{-1}. The illumination objective lens is water immersion (n=1.333) with NA 1.2.

References

[1] K. Hamada, et al., *Journal of Biomedical Optics*, **13** 4, 044027 (2008)
[2] K. Fujita, et al., *Physical Review Applied*, **4**, 0140101-0140107 (2015)
[3] K. Fujita, et al., *Physical Review Letters*, **99**, 228105 (2007)
[4] X. Sunny Xie, et al., *Science*, **322**, 1857-1861 (2008)
[5] Y. Ozeki, et al., *Optics Express*, **17**, 3651-3658 (2009)
[6] A. A. Betin, et al., *Soviet Journal of Quantum Electronics*, **5**, 11, 1309-1313 (1975)
[7] R. Oketani et al., *Optics Letters*, **42** (3), 571-574 (2017)