

Development of slit-scanning multiplex coherent anti-Stokes Raman scattering microspectroscopy system using elliptical focal spot for low damage and high-speed observation of live cells

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In recent years, researches on dynamic observation of live cells and cytometry using coherent Raman scattering has been actively conducted [1]. However, when observing living cells at high speed, increasing the peak irradiance of the excitation beams to obtain high-intensity coherent Raman scattering signal causes the photo-induced damage to living cells.

In this study, we show the development of a slit scanning multiplex CARS (coherent anti-Stokes Raman scattering) microspectroscopy system using an elliptical spot with parallel excitation/detection to realize low-invasive and high-speed hyperspectral coherent Raman scattering imaging. Results of the numerical calculation show 1.3 times increasing of CARS signal by using an elliptical spot comparing with multiple spots as simple parallel excitation/detection. We also demonstrate multiplex CARS hyperspectral imaging of living 3T3-L1 adipocytes using the newly developed system (Fig. 1). By using the focal spot with an ellipticity of 0.125 and the peak irradiance of 7.1 GW/cm^2 for ω_{pump} beam and 11.4 GW/cm^2 for ω_{Stokes} beam, we succeeded in acquiring a CARS hyperspectral image in the fingerprint region of $(256 \times 256)_{\text{image}} \times 512_{\text{spectrum}}$ pixels in 3.81 seconds. Comparing with the conventional multiplex CARS microspectroscopy [2], we acquired 17,500 spectra per second and realized 14 times faster spectral imaging while suppressing peak irradiance to 1/12.

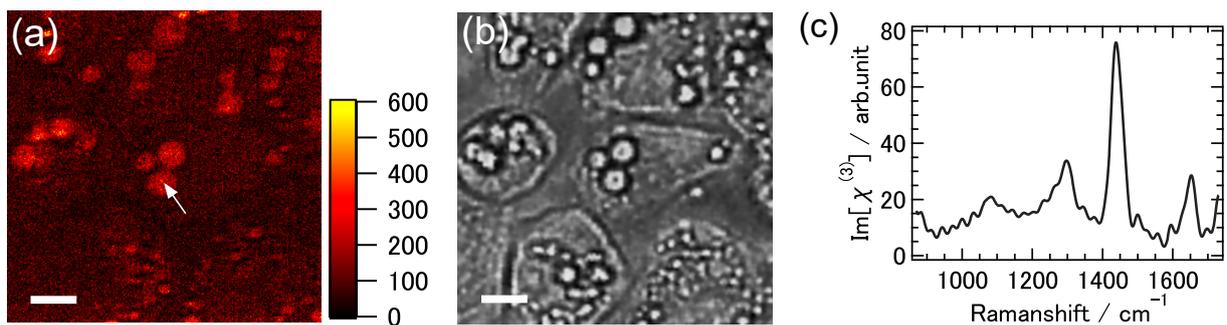


Figure 1: (a) CARS image at 1438 cm^{-1} and (b) bright field image of living 3T3-L1 adipocytes. (c) CARS spectrum at arrows in (a). The incident laser powers of ω_{pump} and ω_{Stokes} were 174 and 88 mW, respectively. The scale bar indicates $20 \mu\text{m}$.

- [1] K. Hitamatsu, *et al.*, “High-throughput label-free molecular fingerprinting flow cytometry,” *Science advances*, **5** (1), eaau0241 (2019).
- [2] H. Kano, *et al.*, “Ultra-multiplex CARS spectroscopic imaging with 1-millisecond pixel dwell time” *OSA Continuum*, **2** (5), 1693-1705 (2018).