3D label-free imaging of intracellular molecules by using slit-scanning confocal Raman microscopy

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Raman microscopy is a powerful technique that combines the advantages of Raman spectroscopy and optical microscopy. Without labeling, distributions of various biological molecules can be obtained in cells with the benefit of Raman spectroscopy that provides molecular vibrational frequencies indicative of chemical species and structures of biological molecules and their conformational changes caused from their surrounding conditions associated with cellular functions.

A slit-scanning confocal technique has been widely used to analyze biomolecules in living cells because of its higher image acquisition rate compared to that of a conventional point scanning confocal technique. With the slit-scanning technique, specimens are illuminated with a line-shaped focus. Resultant Raman scattering from different positions along the line-shaped focus are imaged at the entrance slit of a spectrometer and multiple Raman spectra are detected simultaneously[1-3]. Since the entrance slit eliminates the scattering light from out-of-focus regions, biological samples can be imaged with the three-dimensional resolution and higher image contrast.

We built a slit-scanning confocal Raman microscope and demonstrated three-dimensional observation of living mammalian cells. Figure 1 shows the three-dimensional Raman scattering image of living HeLa cells acquired with 532 nm excitation and a water immersion objective with an NA of 1.25. Our microscope clearly shows the distributions of mitochondrial structures, proteins, and lipid droplets, with detecting Raman scattering originating from the pyrrole breathing mode in cytochromes, Amide-I vibrational mode of peptide bonds, and CH_2 stretching mode, respectively. We will investigate on other Raman shifts contributed by other cellular components for analysis of cellular functions.

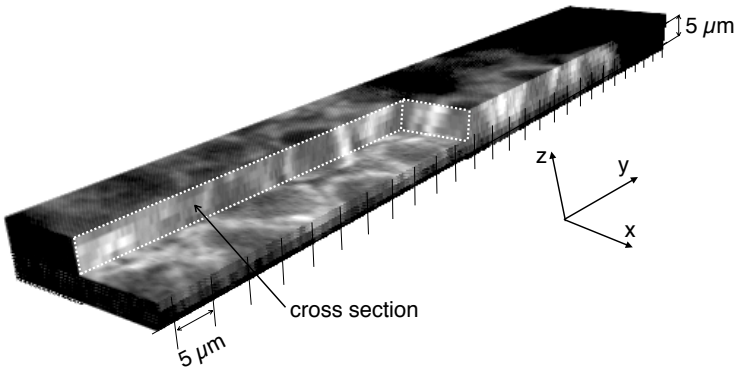

Figure 1: Three-dimensional Raman image of living HeLa cells. Raman images of cytochrome c, protein, and lipid are merged.

References:
[1] D. K. Veirs *et. al.*, *Appl. Opt.* **29**, 4969–4980 (1990).
[2] K. Hamada *et. al.*, *Proc. SPIE* **6443**, 64430Z (2007).
[3] K. Hamada *et. al.*, *Journal of Biomedical Optics* **13**, 044027 (2008).