Raman microscopy for label-free observation of living cells

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ABSTRACT: Raman scattering can be used to observe biological molecules without any labeling because of its capability of detecting vibration frequency. Since molecular vibration is strongly related to the structure, condition and environment of molecules, combination of Raman scattering and optical microscopy gives us deep insights about biological molecules in cells. We have developed a slit-scanning Raman microscope, where a line-shaped laser focus illuminates a sample to detect Raman scattering from cellular molecules at multiple points simultaneously, resulting in an image acquisition rate around 100 times higher than conventional confocal Raman microscopy. Figure 1 shows Raman images of living HeLa cells reconstructed by the distribution of Raman peak intensity at 752, 1684, and 2857cm^{-1} that can be assigned to the porphyring breathing mode of cytochrome, amide-I vibration mode in protein beta sheet and CH_2 stretching vibration mode in lipids, respectively. Since we used a CW laser with 532 nm wavelength for the light source, cytochromes were observed with strong contrast in Fig.1 a) through the resonant Raman scattering which is given by light absorption at the 520-560 nm range of heme containing ferric iron and porphyrin ring. We also applied slit-scanning Raman microscopy to observation of cellular molecules with surface enhanced Raman scattering (SERS). Strong Raman signals from a macrophage incubated with gold nanoparitcles of 50nm diameter were successfully observed with high spatial selectivity and temporal resolution [3].

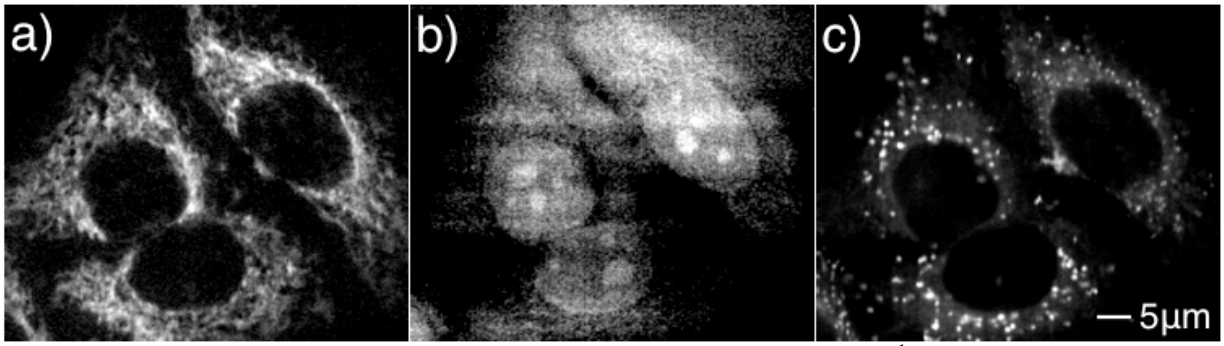

Figure 1. Raman images of living HeLa cells. a) cytochrome (752 cm^{-1}:porphyrin breathing mode), b) protein (1684 cm^{-1}:amide-I, beta sheet), c) lipid (2857 cm^{-1}:CH_2 stretching). The images consist of 291×215 pixels, and image acquisition time was about 25 minutes.

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