Deep UV confocal Raman microscopy: label-free bio-molecular analytical imaging

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Raman scattering microscopy is a promising method for analytical imaging of bio-molecules in a cell without labeling with fluorescing dyes or proteins [1]. Since the cross-section of Raman scattering at molecules is extremely small compared to fluorescence or infrared absorption, coherent nonlinear process such as CARS and SRS and plasmonic enhancement at metallic particles as TERS have been applied for the scattering enhancement [2]. The combination of above two mechanisms has also been proposed and demonstrated [3]. Line illumination with a polychromator and a 2D-CCD is effective and practical for low-power short-exposure excitation of spontaneous Raman scattering imaging [4].

In this presentation, we discuss another enhancement mechanism, resonant Raman scattering. Scattering efficiency of deep ultraviolet excitation is much higher, typically $x10^8$, than that of visible/near-infrared excitation, due to the resonance Raman scattering process for bio-molecules, especially nucleotides and protein molecules in a cell. We have developed a confocal deep UV microscope, in which a frequency-doubled multi-line Argon ion laser (244 and 257nm) was used with silica and calcium fluoride optical components and lenses, and a scintillated EM-CCD [5]. A liquid-immersion objective was developed based on Schwarzschild design with NA of 0.9, field of view of 100 μm, covering the range between deep UV and near infrared [6]. For suppressing the photo-degradation of molecules, we have proposed to dope trivalent lanthanide ions in solution for energy transferring from bio-molecules to lanthanide ions [7]. Experimental results of deep-UV imaging and spectroscopy of Adenine/Guanine distribution in a cell have been shown. The mechanism of suppression of photo-degradation is discussed. For super-resolution imaging, we use TERS tips coated either with aluminum or indium grains on silicon dioxide cantilever [8, 9].

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