Deep UV resonant Raman microscopy of nucleotide distribution in a cell

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Raman microscopy for cellular imaging is useful for biomolecular distribution analysis without any labeling. Resonant Raman effect highlights the distribution of specific molecules where excitation light energy is resonant with electronic transition. We employed deep UV (DUV) to resonantly observe nucleotide bases in a cell with high sensitivity. This is the first report presenting DUV Raman microscopy images of a cell.

An obtained Raman spectrum of HeLa cell is shown in the left panel of Figure. The spectrum shows a prominent band at 1489 cm\(^{-1}\), which is assigned to nucleotide bases, guanine and adenine. Intensity distribution of this band is shown in the right panel of Figure. The cell seen at center has four bright areas, indicated by arrows. Two of them situated close together near the center correspond to DNA localization at nucleoli in the nucleus, while the others correspond to RNA in cytoplasm.

Images were acquired with a purpose-made microscope. 257 nm cw line was used for excitation. Sample was irradiated at 0.1mW/µm\(^2\) via an objective having NA=1.35. Scattering light was dispersed by a grating with 1800 G/mm and measured by a CCD. For the imaging, sample stage was raster-scanned with a step of 0.5 µm every 0.75 sec spectral acquisition.

The achievement of this research relies on handling molecular photodegradation that is caused by DUV. To avoid molecular photodegradation by DUV as much as possible, we optimized the intensity, exposure duration, and focal volume of excitation, the system detection throughput, and the scanning step, based on our previous study [1].

The results presented in this research extend the potential of Raman microscopy as a selective probe of DNA and RNA in a cell with high sensitivity. This achievement is important for future works on understanding a cellular activity such as apoptosis and mitosis.


Fig. A spectrum derived of cytoplasm region in a HeLa cell (left), intensity distribution of 1489 cm\(^{-1}\), which is assigned to guanine and adenine vibrational modes (right).