Deep-ultraviolet resonance Raman spectroscopy for biomolecular imaging and analysis in cells

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Raman spectroscopy enables chemical analysis and imaging of biomolecules in cells without any molecular labeling. With excitation by deep-ultraviolet (DUV) light, Raman scattering of nucleotide bases and aromatic amino acid residues is resonantly amplified and selectively observed in a cell, while excitation by the visible (or near-infrared) light, which is often used for Raman spectroscopy in biology, results in less signal from these molecules (see Fig. (a)).

We performed DUV resonance Raman imaging [1] and analysis[2] of nucleotide bases and aromatic amino acid residues in cells for the first time. Fig. (b) shows a DUV Raman image of HeLa cells. The image shows intensity distribution of a 1490 cm\(^{-1}\) band, seen in Fig. (a) as the intense and well-isolated band, and assigned to guanine and adenine. In the image, one big and one small spots seen in the middle of the right bottom cell are highlighted. These spots are recognizable as nucleoli where rDNA and rRNA densely exist. Around the nucleoli, mesh-like structure can be recognized. This structure can correspond to distribution of nucleosomes. The Raman intensity is also high at both wings of the cell. These regions correspond to the cytoplasm where rRNA, mRNA, and tRNA spread over.

For nucleotide imaging, Raman scattering was excited by frequency-doubled Ar\(^+\) laser of \(\lambda=257.2\) nm. The wavelength is the best among CW laser lines for resonantly exciting Raman scattering of nucleotide bases. The laser beam was focused by a microscope objective (NA=1.35) onto the bottom of a cell in the inverted microscope configuration. Scattered light from the sample was collected with the objective, and recorded as a spectrum with an cooled CCD camera. For each spectral measurement, the laser intensity at the focus was set at 200 \(\mu\)W/\(\mu\)m\(^2\), and the sample was exposed to the excitation beam only for 0.2 sec. In order to get a 2-D hyperspectral image, we raster-scanned the sample over a fixed excitation focal spot with acquiring single Raman spectrum for each scanning step. The scanning step length was set at 333 nm, the length larger than FWHM of the Airy disk planar profile (~200 nm), so that biomolecules are not cumulatively degraded by the excitation beam during imaging.

For bimolecular analysis, we switched the laser line to \(\lambda=244\)nm. The wavelength is good for resonance excitation of both nucleotide bases and aromatic amino acids. With increasing exposure duration (up to 12.5 sec), Raman bands of these biomolecules diminished, while a photoproduct Raman band grew. By exponential function fitting analyses, intensities of those two biomolecular bands were correlated with sample exposure duration at different intensities of excitation light.

These studies can shed light to a new aspect of Raman spectroscopy for biomolecular imaging and analysis of cells and expand its applications in biology and medicine.