Water in a living cell probed by a Raman microscopy

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We have used Raman microscopy to investigate the structures and functions of biomolecules and cells, especially in relation to water [1–4]. In this presentation, we propose that "intracellular water" can be a new parameter for understanding the intracellular environment.

[Water distribution in a cell] Plotting the Raman intensity of a single HeLa cell in the C-H and O-H stretching Raman band regions (Figure 1) showed that the O-H Raman intensity of water was higher in the nucleus than in the cytoplasm [1]. Calculation of the difference spectrum subtracting the cytoplasm from the nucleus confirmed a positive peak in the O-H stretching region and a negative peak in the C-H stretching region. These results indicate that the water density in the nucleus is higher than in the cytoplasm. The water density in the cytoplasm was estimated to be about 3% smaller than that in the nucleus. The intracellular environment is highly crowded with biomolecules such as proteins and lipids, called macromolecular crowding. In this study, the magnitude of molecular crowding in the nucleus is found to be smaller than that in the cytoplasm. We proposed to evaluate macromolecular crowding from the Raman image of water in each organelle.

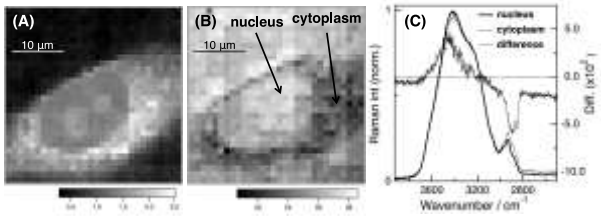

Figure 1: Raman images of a HeLa cell in the C–H (A) and O–H (B) stretching bands. (C) Difference Raman spectrum (solid) of a HeLa cell obtained by subtracting the spectrum of cytoplasm ((b) dotted) from the spectrum of nucleus ((a) thin-solid) [1].

[Label-free evaluation of intracellular temperature] We have applied Raman imaging of intracellular water to evaluate the temperature in a single living cell in a label-free manner [1]. This method utilizes the change in the shape of the O-H stretching Raman band of water with temperature. First, the temperature dependence of the O-H stretching band of the medium, nucleus, and cytoplasm was measured to obtain a calibration curve between temperature and the Raman band in each region. Next, using this calibration curve, we evaluated the temperature at each region and its change after adding FCCP (uncoupling reagent) into the cell. We succeeded in measuring the temperature increase in the cytoplasm after adding FCCP using the O-H stretching band (Figure 2). We were also able to obtain the temperature gradient between the cell and the medium. This label-free imaging using water is a promising new method for intracellular temperature studies.

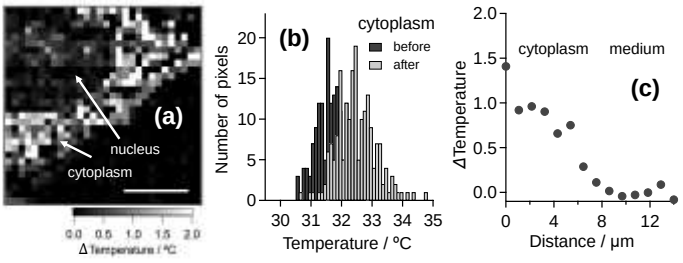

Figure 2: (a) The image of temperature change in a HeLa cell by subtracting the temperature image before the FCCP treatment from that after the FCCP treatment. (b) Histograms of cytoplasm temperature before and after the FCCP treatment. (c) Cross section of the temperature change from cytoplasm to medium [2].

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