

Selective Observation of Water near Biological Membranes using Attenuated Total Reflection Raman Microscopy

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Recently, Raman microscopy has attracted much attention as a label-free technique for observing living cells. We have focused on intracellular water, an intracellular molecule that cannot be labeled, as a label-free detection by Raman microscopy. This study investigated water molecules around biomembrane interfaces using Raman microscopy. The states of water molecules around biomembranes are proposed to be important for membrane functions; however, the detailed nature of water around membranes is still unknown because of insufficient measurement techniques. In this study, we constructed a total internal reflection (TIR) Raman microscope that can selectively observe molecules around membrane interfaces. Only molecules near the interface can be monitored using evanescent light generated under a TIR condition. The microscope was constructed with an objective lens-type configuration using an inverted confocal microscope and a high NA objective lens, which enables the measurement of a living cell incubated in a glass-bottomed dish. The absence of spectral distortion associated with the TIR condition was confirmed by the fluorescence of the nanoparticle. The constructed microscope was applied to the observation of water on a self-assembled monolayer fabricated on a cover glass, and the C-H stretching bands of the monolayer were observed to be enhanced under the TIR condition. We then measured TIR Raman spectra of a cultured HeLa cell and the obtained results were discussed based on the previous report on the TIR spectra of water using a prism-type configuration.