Katsumasa Fujita

Department of Applied Physics, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871 Japan

fujita@ap.eng.osaka-u.ac.jp

High-sensitivity Raman imaging of cryofixed biological samples

Raman imaging is used in various applications to provide spatial distribution while analyzing molecular components in a sample. In recent years, the improvement in spatial resolution has enabled the acquisition of spectral distributions in complex samples such as cells and advanced studies for new applications of Raman spectroscopy in biological research. However, there have been challenges, such as the weak Raman scattering that makes it difficult to detect low-concentration molecules and to visualize moving samples. In this research, we developed a Raman microscope that can extend the exposure time while maintaining a cellular environment by freezing the cells [1]. On the microscope stage, cell samples were rapidly frozen [2] and measured while keeping the sample at a low temperature. This approach significantly improved the signal-to-noise ratio from prolonged observation time, confirming practical enhancements in wavenumber and spatial resolution. Additionally, due to the high sample stability at low temperatures, the bleaching effect of resonant Raman scattering is reduced, and Raman peaks, which are not observed at room temperature, were observed. Furthermore, this technique can be easily combined with other observation methods, such as fluorescence microscopy, and high-sensitivity and motion-artefact-free observation can be achieved in multi-modal imaging, allowing us to visualize intracellular molecules and chemical environments in biological samples more accurately.

References

[1] M. Yamanaka, K. Tsuji, et al., bioRxiv 2023.08.01.551103.
[2] K. Mizushima, Y. Kumamoto, et al., submitted.