Biology, Medicine, Medical diagnosis, Drug discovery

Raman microscopy for label-free imaging and analysis of frozen cells

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Abstract

We present a newly developed cryo-Raman microscopy technique that enables high-sensitivity, label-free molecular imaging of biological samples maintained at cryogenic temperatures. By rapidly freezing specimens at around -185 ℃ with liquid cryogens, our method preserves the chemical and structural state of cells and tissues while effectively minimizing laser-induced damage. As a result, we obtained Raman signals approximately eight times brighter than those achieved by conventional methods, allowing for long-term, stable observation and high-resolution mapping of molecular distributions and chemical states. This breakthrough opens new avenues for studying low-concentration biomolecules and transient biochemical events that were previously difficult to capture. We anticipate that cryo-Raman microscopy will contribute significantly to a wide range of fields, including cell biology, medicine, and pharmaceutical sciences.

Background & Results

Raman microscopy has long held promise as a noninvasive imaging tool for biological samples, as it can visualize the spatial distribution of diverse molecular species without the need for fluorescent labeling. However, the intrinsic weakness of Raman scattering often necessitates intense laser irradiation, which can damage sensitive biological specimens, alter their chemical states, or induce photobleaching. Additionally, living samples experience ongoing changes in molecular composition and organization, complicating the accurate acquisition of high signal-to-noise ratio Raman data.

Our solution is to cryogenically freeze the samples, immobilizing their molecular structures in a state close to that of native conditions. By maintaining low temperatures throughout the observation, we minimize structural rearrangements, slow down degradation processes, and reduce photodamage under laser illumination. Using our cryo-Raman microscope, we achieved significantly enhanced signal intensity—roughly eightfold compared to conventional room-temperature measurements—and extended observation times exceeding ten hours. This improvement enabled the detection of subtle molecular vibrations, identification of additional molecular species, and spatial mapping of redox states within biological tissues. For instance, we successfully visualized the distribution and oxidation-reduction states of cytochromes in ischemic heart tissues, providing the first spatially resolved spectroscopic images of biochemical changes that could not be observed in unfixed, living samples.

Significance of the research and Future perspective

The cryo-Raman microscope technology addresses longstanding challenges in sensitivity, stability, and molecular specificity in live-cell imaging. By enabling long-term, high-resolution, label-free analyses of biomolecules at cryogenic temperatures, it paves the way for more accurate studies of dynamic biological events and low-abundance molecules. The technique is poised to advance fundamental understanding of cellular processes, support the evaluation of frozen cells, and inform drug discovery and development. Ultimately, cryo-Raman imaging may accelerate progress across life sciences, contribute to sustainable healthcare solutions, and support global health and well-being initiatives.

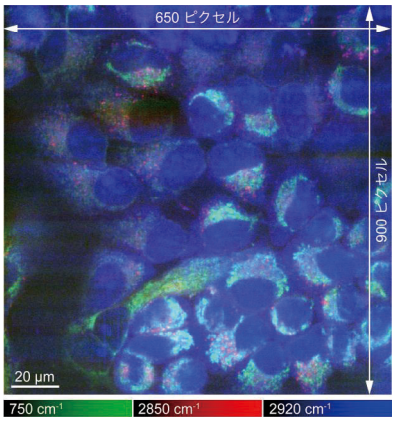

Figure 1. Raman image of rapidly frozen HeLa cells with high signal-to-noise ratio and large field-of-view. The image acquisition time was 10 hours. The distribution of Raman signals from cytochromes (750 cm^{-1}), lipids (2850 cm^{-1}), proteins (2920 cm^{-1}), are indicated in green, red, and blue, respectively.

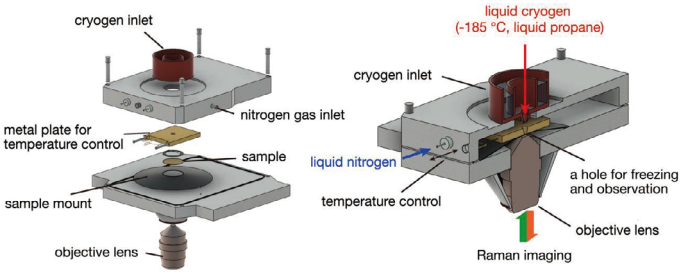

Figure 2. Schematic of the sample area in the developed cryo-Raman microscope. A cover glass with cultured cells is placed on the sample mount, and a metal plate is brought into contact (left). The cryogen enters through the inlet and a hole in the metal plate, coming into direct contact with the sample for rapid freezing. The sample temperature is controlled by the metal plate with liquid nitrogen circulation and a heater inside (right).

Patent	2 PCT applications filed.
Treatise	Mizushima, Kenta; Kumamoto, Yasuaki; Tamura, Shoko et al. Raman microscopy of cryofixed biological specimens for high-resolution and high-sensitivity chemical imaging. Science Advances. 2024, 10, eadn0110. doi: 10.1126/sciadv.adn0110 Dodo, Kosuke; Fujita, Katsumasa; Sodeoka, Mikiko. Raman spectroscopy for chemical biology research. J. Am. Chem. Soc. 2022, 144,(43), 19651−19667. doi: 10.1021/jacs.2c05359
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