Poster

Current status of microspectroscopic systems for the macromolecular crystallography beamline AR-NW12A at the Photon Factory

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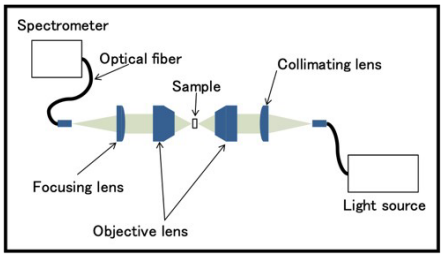
X-ray crystallography using high-brilliance synchrotron radiation sources is a powerful tool for elucidating the three-dimensional structure of proteins at the atomic level. Conversely, vibrational spectroscopy can precisely analyze the chemical bonds that determine the chemical properties of the atoms that consist of proteins. Therefore, X-ray crystallography and vibrational spectroscopy, such as Raman and UV-visible absorption, are complementary methods used to analyze the functional mechanisms of proteins.

In the macromolecular crystallography beamline AR-NW12A at the Photon Factory, we have been developing instruments for UV-visible absorption spectroscopy and Raman spectroscopy and improving their utilization environment to establish the beamline where X-ray crystallography and spectroscopic methods can be used seamlessly. Microspectroscopic systems have been developed in the laser booth beside the beamline equipped with laser interlocks. The UV-visible absorption and Raman microspectroscopic systems in an off-line (no X-ray use) environment are currently available.

Fig. 1 shows the schematic view of a UV-visible absorption microspectroscopic system. The available beam size is 50 – 200 mm at the sample point, and the detector can be measured between 250 and 800 nm. The cryostream device is also installed, enabling measurements at 95K. We have already obtained some results by utilizing this system [1].



	Collimated beam : 12 mm		
Diameter of optical fiber (μm)	100	300	600
Beam size at focal point (μm)	56 x 55	105 x 107	199 x 204
Beam profile			

Figure 1. Schematic view of UV-visible absorption microspectroscopic system (Left) and available beam sizes (Right).

However, it is essential to consider the thickness of the crystals when crystals are used for microspectroscopic experiments. It should be noted that the microspectroscopic experiments with a thick crystal is difficult to measure due to the significant light absorption by the crystal. In many cases, spectroscopic measurements are performed on the same crystals as X-ray diffraction experiments, and the thickness of crystals used for X-ray diffraction experiments is unsuitable for spectroscopic experiments.

Furthermore, solvent and frost around the crystal cause serious background noise, resulting in a poor signal-to-noise ratio for the microspectroscopic absorption spectra. Therefore, a protein crystal shaping machine using a 193 nm deep-UV laser [2] was installed to adjust crystal thickness and remove the solvent, enabling microspectroscopic measurements with a high signal-to-noise ratio.

[1] Nakamura, R., Hikita, M., Ogawa, S., Takahashi, Y. & Fujishiro, T. (2020). *FEBS J.* **287**, 1138-1154.

[2] Kawano, Y., Hikita, M., Matsugaki, N., Yamamoto, M. & Senda, T. (2022). *Acta Cryst.*, F**78**, 88-95.

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