Development of microspectrophotometer for the macromolecular crystallography beamline at the Photon Factory, Japan

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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. On the other hand, vibrational spectroscopy is the effective technique for detecting the electronic and chemical changes of proteins. Therefore, X-ray crystallography and vibrational spectroscopy, such as Raman and UVVisible absorption, are complementary methods that have been used to analyze the functional mechanisms of proteins.

In order to perform the spectroscopic study at macromolecular crystallography beamline at the Photon Factory, the development of spectroscopic systems for both off-line and on-line has been started at beamline AR-NW12A. For development of offline spectroscopic system, the laser booth has been built beside of control cabin of AR-NW12A.

After the end of the construction of the laser booth, we installed the laser, goniometer and so on. And we started to develop the microspectroscopic system for off-line spectroscopy, and so far, the UV-Visible absorption system and Raman spectroscopy system have now been in general user operation.

The UV-visible absorption system has a beam size of 100-300 μm at the sample point and the detector can be measured between 250 nm to 800 nm. We prepare the two different systems for the UV light region (250 – 400 nm) and visible light region (400 – 800 nm), and are used in different ways depending on the measurement region. The optimal crystal thickness is ~50 μm for UV system and ~150 μm for visible system. In this system, we have already obtained some results and published the paper [1].

The off-line spectroscopic system for Raman spectroscopy is utilizing backscattering method, the beam size at sample point is 50 to 200 μm, it depends on the diameter of the optical fiber. And the available wavelength is 488, 514.5 and 647.1 nm.

Herein, we describe the outline of the offline UV-Visible and Raman spectroscopic instrumentations. The continuing development of the online spectroscopic instrumentation is also outlined. In the future, macromolecular crystallographic beamline users will be able to not only determine the atomic structure of their samples but also to explore the electronic and vibrational characteristics of their sample, before, during and after data collection.

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

Keywords: X-ray crystallography; Spectroscopy; Raman; UV-Visible absorption