BL45XU
Macromolecular Crystallography II

1. Introduction

BL45XU is a macromolecular crystallography (MX) beamline designed for high-throughput diffraction measurements and utilizing the automated data collection system ZOO[1]. The development of peripheral technologies associated with automated data collection, such as the development of an automatic processing pipeline for diffraction data and the simplification of information communication with users, is underway. The optics layout consists of a double-crystal monochromator of SPring-8 standards, a horizontal focusing mirror, a virtual source slit, and a Kirkpatrick–Baez mirror[2]. The available energy range is 6.5 to 16 keV. The beam size at the sample position can be changed from 5 µm (H) × 5 µm (V) to 50 µm (H) × 50 µm (V) with a photon flux of 5.70×10^{12}–1.75×10^{13} photons/s at 12.4 keV.

2. Development of UniPuck exchange system for continuous automated data collection

We are developing a sample container exchange system that enables the measurement of a larger number of samples in succession. To achieve the stable operation of the device, we have been conducting trial operations using samples from non-proprietary users. To enhance stability, the bent parts of the support pillars of the puck stocker's hand, which moves pucks within the stocker, were replaced with machined parts that offer higher rigidity, ensuring they do not bend even at liquid nitrogen temperatures. Additionally, we attached sensors to the multi-axis robot hand that transports the sample pucks in order to detect malfunctions and protect the samples by moving them in liquid nitrogen and bringing the system to a stop if necessary. Moving forward, we will continue trial operations that include user samples, aiming for full-scale operation in the near future.

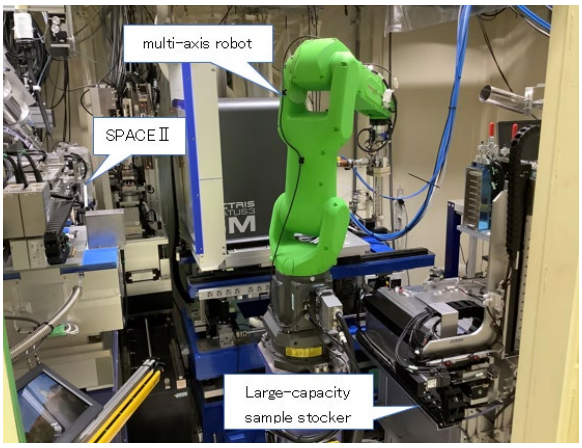

Fig. 1. Sample container exchange system.

3. Crystallization plate observation system

We are developing a crystallization plate observation device that records the positions of crystals within a plate before measurement on a beamline. Three cameras are arranged side by side to observe the entire plate, the crystallization drop positions, and the crystal positions within the drops. By moving the plate, observations can be performed for various applications.

This year, using the In Situ-1 crystallization plate, we developed two systems: **plate_view**, which automatically observes the 96 wells of a single plate, records drop positions, and captures images, and **drop_view**, which observes the recorded drop images and records crystal positions. The **plate_view** system consists of a server that controls plate movement and camera operations, a

WebGUI for sending commands to the server, and a database for recording images and drop positions. Users can initiate observation with a single button on the WebGUI, allowing a single plate to be observed in approximately 30 min. The **drop_view** system enables users to observe drop images recorded by **plate_view** via the WebGUI and manually record crystal positions. The registered crystal positions are stored in a database and, by entering measurement conditions for each recorded crystal, can be used for automated measurements at the beamline.

Currently, **plate_view** is being optimized for faster observation. Simultaneously, **drop_view** is being improved to include AI (deep learning) for crystal identification, in addition to the existing manual crystal position recording.

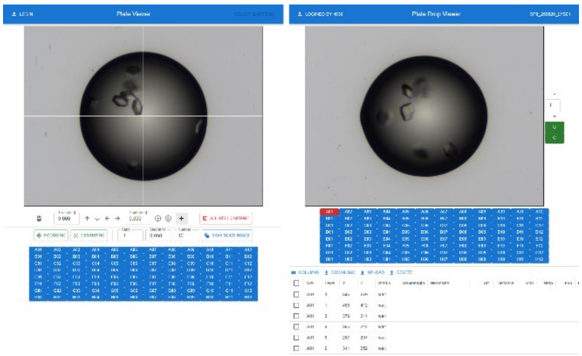

Fig. 2. WebGUI for **plate_view** mode (left) and **drop_view** mode (right).

4. Initiatives for stable operations of automated data collection

Automated data collection continues day and night, even when staff is absent. For this reason, to ensure the stable operation of the beamline, we have implemented a system that constantly monitors various parameters, including the supply status of liquid nitrogen.

This fiscal year, we have added the remaining amount of liquid nitrogen in the liquid-nitrogen drizzling device as a new parameter to be monitored. The system is also equipped with a feature that automatically sends a warning email to staff to notify them of an anomaly when any parameter exceeds its set threshold. Furthermore, we have added a function to attach images from inside the experimental hatch and a snapshot of all parameters to the warning mail, enabling rapid situation assessment when an anomaly occurs.

In addition, during the automated data collection, data processing is performed concurrently with the measurement. This fiscal year, we have added one more computer to facilitate smoother data processing.

5. Crystal preparation facility

Our automated data collection system enables the acquisition of 200–250 datasets from crystals within a single 24-hour period. To leverage this high-throughput capacity for structure-based drug discovery (SBDD), we have developed a comprehensive crystallographic fragment screening (CFS) pipeline. The pipeline features a high-throughput crystallization system with automated liquid handlers: RockImager360 for automated plate observation and the Echo650T acoustic dispenser for direct compound transfer. We provide full support for user experiments at our sample preparation facility, conveniently located adjacent to beamline BL45XU.

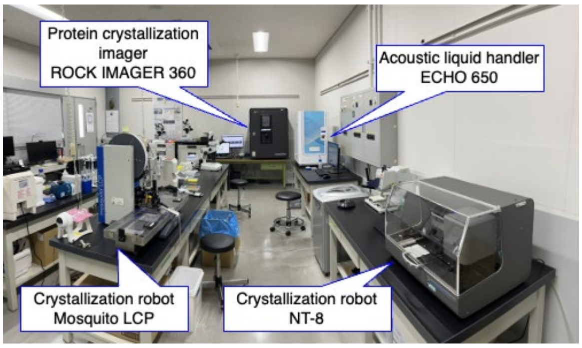

Fig. 3. Crystal preparation facility at the outer room of BL45XU.

6. Public use of CryoTEM

Four CryoTEMs are available for PX-BL users to investigate their sample properties such as monodispersity or conformational heterogeneity. By using these CryoTEMs, users can screen their samples and prepare good grids for high-resolution structure determination. As a sample is not necessarily crystallized, structures with small conformational variations are analyzed, classified and refined to a resolution of up to 2 Å. This technique has been found to be beneficial for those who have had difficulty in crystallizing their proteins of interest.

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References:

[1] Hirata, K. et al. (2019). *Acta Cryst.* **D75**, 138–150.

[2] Goto, S. et al. (2019). *SPIE Advances in X-ray/EUV Optics and Components XIV*, 111080I.