BL45XU
Structural Biology III

1. Introduction

One of the important roles of macromolecular crystallography (MX) beamlines is to provide the experimental environment for high-throughput diffraction data collection using high-flux X-rays from a synchrotron. In 2018, the beamline BL45XU was renewed to be dedicated to high-throughput diffraction experiments of protein crystals through automatic measurements. 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 [1] (Fig. 1). The available energy range is from 6.5 to 16 keV. The beam size at the sample position can be changed from 5 (H) × 5 (V) to 50 (H) × 50 (V) μm^2 with a photon flux of 5.70×10^{12}–1.75×10^{13} photons/s at 12.4 keV.

2. More accurate and efficient automatic measurement

The beamline is well optimized for automatic data collection. The automatic measurement system, the ZOO system [2], and automatic data processing using the KAMO system [3] were originally developed at BL32XU. To launch the systems at this beamline, which have different instruments, we optimized the settings and parameters for the systems. In particular, the algorithm for finding diffraction spots was optimized to improve the accuracy of the crystal positions determined from the images collected by two-dimensional (2D) X-ray raster scan. In addition, we optimized the measurement and analysis conditions of the 2D X-ray raster scan for application in the ZOO system for data collection from low-molecular-weight organic crystals, which yield fewer diffraction spots in each image than protein crystals.

The sample exchange robot SPACE-II [4] accommodates 128 sample pins with eight UniPucks. At present, the automatic measurement of samples takes less than one day under the fastest measurement condition. For highly efficient measurement, it is necessary to handle more UniPucks continuously. Therefore, we developed and introduced an original puck-stocker to exchange more UniPucks automatically (Fig. 2). The puck-stocker can store 42 UniPucks and transfer these UniPucks to SPACE-II automatically to achieve nonstop measurement over 3 days. Its setup and tuning are in progress.

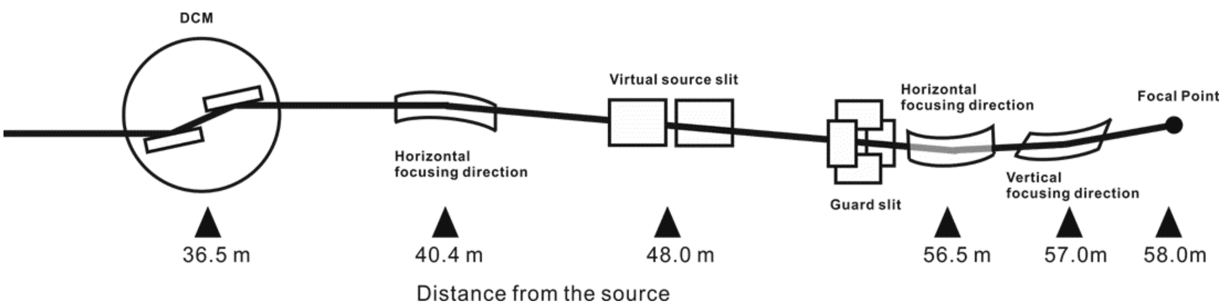

Fig. 1. Optics layout at BL45XU.

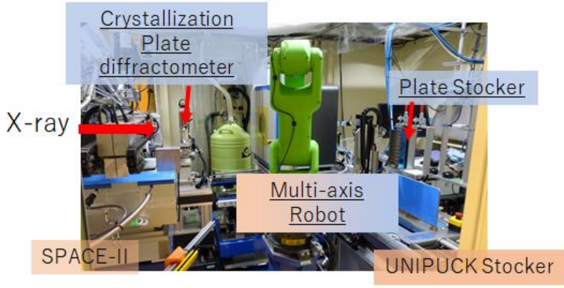

Fig. 2. Diffractometer components in Exp. Hutch.

These implementations improve the data collection efficiency and then the total amount of data reaches up to 4 TB in a day. With our old local computing system shared with PX-BLs, it was difficult to process the data without delay. To overcome this bottleneck and establish on-the-fly processing, we replaced the old system with a new one consisting of 4 servers with 64 CPUs or more (Fig. 3).

Fig. 3. On-the-fly automatic data processing system.

3. Crystallization plate in situ diffraction measurement

The collection of diffraction data from the crystals in the crystallization plate at room temperature is suitable for screening for crystallization conditions because it eliminates troublesome work of cryoprotection and picking up crystals with a cryoloop. To meet these needs, an *in situ* diffraction measurement system for crystallization plates is developed at BL45XU. The diffractometer for *in situ* diffraction is installed between the experimental stage for diffraction measurement at cryogenic temperatures and the detector stage. In the automatic data collection at cryogenic temperature, plate grippers attached to 3-dimensional stages, an on-axis microscope, and a beam stopper are withdrawn away from the beam axis. When the beamline is operated in the plate mode, they are inserted on the beam axis (Fig. 4). These two modes can be switched automatically in about 5 min. The gripper of the plate holder is compatible with SBS standard plates. The plate can be rotated around the vertical axis by ~30°, because the gripper is attached to the goniometer. At present, crystal centering operation and registration in measurements are performed manually with the beamline control software BSS. As with ZOO automatic measurement, the obtained data were processed with the clustering of small-wedge data and the acquisition of datasets by data merging using the KAMO system.

Fig. 4. Diffractometer for crystallization plate in situ measurement.

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