# BL45XU
# Structural Biology III

## 1. Introduction

BL45XU is a macromolecular crystallography (MX) beamline designed for high-throughput diffraction measurements, utilizing the data collection system ZOO for automated measurements [1]. The development of peripheral technologies associated with automatic measurements, such as an automatic processing pipeline for diffraction data and simplification of information communication with users, is under way. 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 $5.70 \times 10^{12}$–$1.75 \times 10^{13}$ photons/s at 12.4 keV.

## 2. New Detector Eiger 16M

A new detector, EIGER X 16M (Fig. 1), was installed in March 2024. Compared with the conventional PILATUS3 6M detector, the dead time between readings of consecutive frames has been improved from 0.95 ms to 3 μs, enabling continuous readouts with duty cycles exceeding 99%. The new detector enables measurement at 100 Hz instead of 50 Hz for the duty cycle.

With the increased speed of measurements, it became necessary to accelerate the diffraction image analysis during diffraction scans. Therefore, we introduced two high-performance computing machines equipped with 64-core CPUs (Fig. 2), which enabled faster and more stable image analysis. Additionally, we enhanced the data analysis environment by upgrading the memory and updating the analysis software.

Fig. 1. EIGER X 16M.

Fig. 2. High-performance computing machines: (top) the server for downloading diffraction images from the detector server, (2nd & 3rd) the image analysis server for diffraction scan, and (bottom) the detector server.

## 3. Development of Uni-Puck exchange system for continuous automatic measurement

The puck exchanger system consists of a multi-axis robot that exchanges Uni-pucks between a stocker, which holds up to 42 Uni-Pucks, and the sample exchange robot, SPACE II [3]. This system enables automated measurements over a continuous period of 2 to 3 days.

To achieve stable operation, we have continued to conduct trial measurements of non-proprietary user samples. During this process, the issue that the heater used to maintain the temperature of the rotation axis in the storage section of the high-capacity stocker became excessively hot was observed. To address this, thermocouples were installed for temperature monitoring as a safety measure. Additionally, a problem occurred in which sample pucks were not released properly from the multi-axis robot hand responsible for transporting them. To mitigate this issue, we increased the torque of the motor in the robot hand. Moving forward, we will continue trial operations that include user samples, aiming for full-scale operation in the near future.

## 4. Crystallization plate observation system

We are developing a device to observe crystallization plates before measurements on the beamline, enabling the prerecording of crystal positions within the plate. The device is equipped with three cameras: a low-magnification camera for observing the entire crystallization plate and identifying its type, a medium-magnification camera for determining the positions of crystallization drops within the plate, and a high-magnification camera for observing the crystal positions within the drops. By placing the crystallization plate on a designated arm, it can be moved between the three cameras. In the future, we aim to develop a system that, upon placing the plate, will automatically determine the crystal positions and register them in a database.

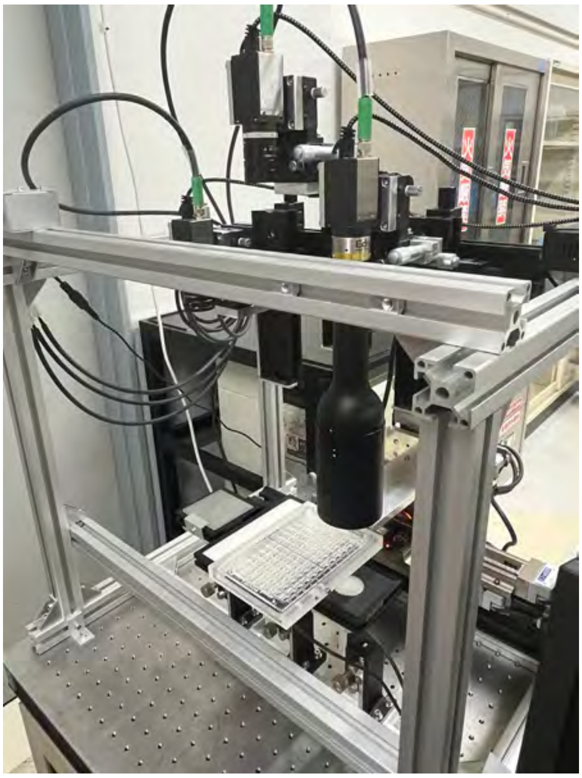

Fig. 3. Crystallization plate observation device.

## 5. Crystal preparation facility

The automation of diffraction data collection from macromolecular crystals at BL45XU has significantly accelerated the acquisition of structural analysis data. It is now routine to collect 200–250 datasets within 24 hours. This highly efficient system is being applied across various areas of structural biology, including structure-based drug development/discovery (SBDD). SBDD leverages the structural information of target proteins in complexes with molecules such as inhibitors, cofactors, substrates, and synthesized compounds. Recently, screening for new molecules that bind to target proteins using a library of ‘fragment compounds’ with a molecular weight of 300 Da or less has become feasible thanks to high-throughput structural analysis. The technique, known as crystallographic fragment screening (CFS), is further supported at SPring-8 by a dedicated crystal preparation facility adjacent to BL45XU, which is used for CFS as well as other crystallization experiments. Here, users can prepare protein crystals using crystallization robots and inspect them on crystallization plates. Moreover, the automated introduction of tiny volumes of solution, such as fragment compound solutions, is possible with the acoustic liquid handler Echo, specifically for CFS. In FY2023, to enhance automation and efficiency, the latest crystal imager, RockImager 360, was installed. This facility is now utilized for CFS by pharmaceutical companies.

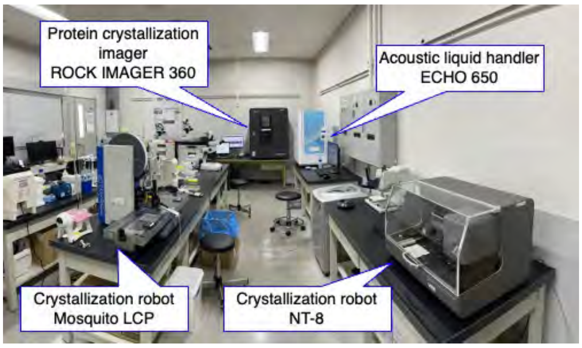

Fig. 4. Crystal preparation facility at the outer room of BL45XU.

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