New highly efficient and fully automatic MX at SPring-8 BL45XU beamline

Recently, high flux and microfocus X-rays have enabled structural analysis from tiny (less than 20 μm) protein crystals, such as LCP crystals of membrane proteins, at macromolecular crystallography (MX) beamlines. However, there still remains demand for highly efficient data collection from relatively large crystals (crystal size: 50 μm or more) obtained by soaking chemical compounds aimed at, for example, drug discovery. Thus, SPring-8 **BL45XU**, operated as the SAXS beamline, was redesigned and reconstructed as a high-throughput automatic MX beamline with high flux and microfocus X-rays.

The new BL45XU was designed on the basis the undulator beamlines BL41XU and BL32XU [1], at each of which an in-vacuum undulator and double crystal monochromator with Si (111) were installed. Available wavelengths range from 0.775 Å to 1.9 Å (Fig. 1(a)). The size of the focused X-rays at the sample position can be changed from 5 (H)×5 (V) to 50 (H)×50 (V) μm^2 by adjusting the aperture of the virtual source slit and the glancing angles of vertical and horizontal focusing mirrors with a photon flux of 5.2×10^{12} – 1.8×10^{13} photons/s at 1.0 Å (Fig. 1(b)). The diffractometer in the experimental hutch was designed to enable high-speed and fully automatic measurement. The pixel array detector Pilatus 3 6M was installed to enable high-frame-rate measurement. The automatic sample changer with twin arms, SPACE-II [2], enables high-speed sample exchange (Fig. 1(c)). All the motorized axes are controlled by the beamline control software BSS [3]. The automated data collection system *ZOO* [4] has achieved automation for all goniometer-based data collection schemes in protein crystallography via communication with BSS. *ZOO* can estimate the absorbed dose in implemented schemes to mitigate severe radiation damage that may interfere with precise structure analyses (Table 1). The system has enabled 'unattended' automatic measurements. Users can obtain good datasets simply by sending their crystals without the need to visit the SPring-8 site. The automatic and unattended measurements, the so-called "mail-in" service, started at the end of May 2019.

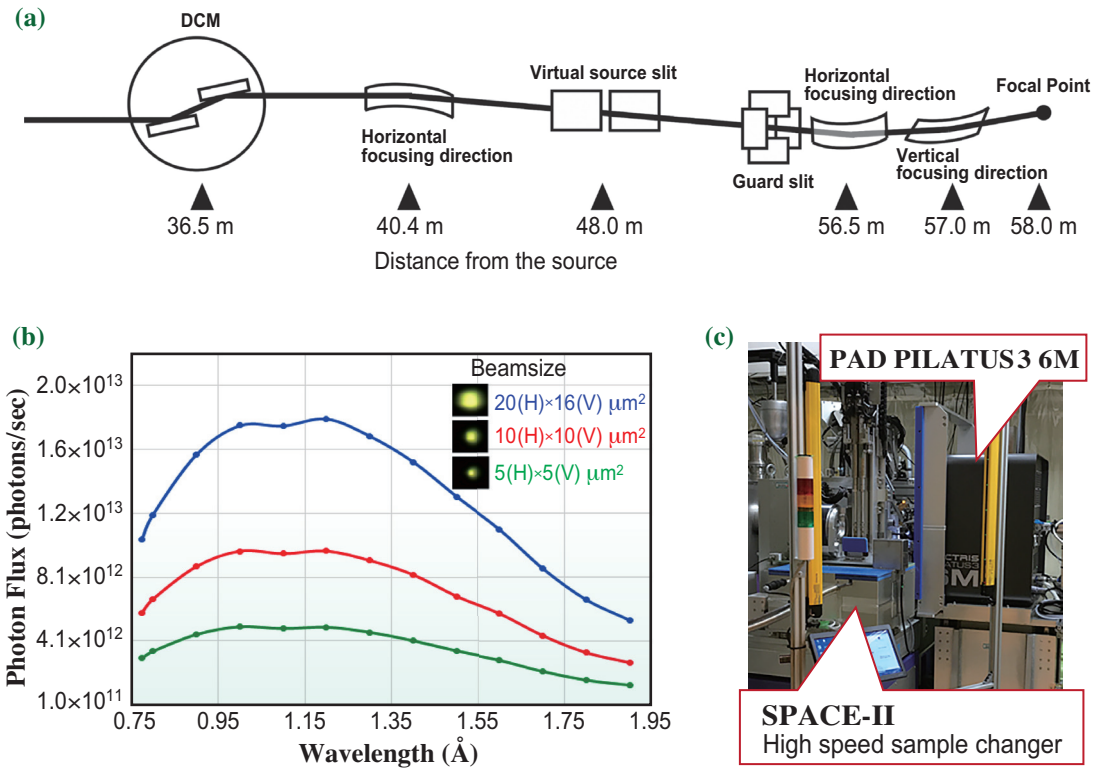

Fig. 1. Overview of BL45XU. **(a)** Layout of the optics. **(b)** Photon flux and beam profiles at the sample position. **(c)** Photograph of BL45XU experimental hutch showing diffractometer (sample changer and pixel array detector (PAD)).

Table 1. Experimental results of automatic measurements using *ZOO* system in BL45XU

Type	Single crystal		Multiple crystals	
Number of Sample	128 pins/8 pucks		73 pins/6 pucks	
Experimental time	8 h 13 min		11 h 19 min	
Ave. exp. Time	3 min 51 s		8 min 23 s	
Beam size	20 (H) × 16 (V) μm^2		20 (H) × 16 (V) μm^2	
(Photon flux)	(1.73×10^{13} photons/sec)		(9.75×10^{12} photons/sec)	
Crystal size	50 – 150 μm		5 - 20 μm (Membrane protein - LCP)	
Data collection by *ZOO*				
Data Collect	121 pins: Single point (102 pins) Helical (9 pins) Total Φ = 360°, (Dose = 10 MGy)		64 pins: Multiple small wedge Total Φ = 10° (Dose = 10 MGy)	
Data processing by KAMO (XDS)				
Processed	121 data (Indexing failed: 5 set)		64 pins, 940 data	Resolution, Completeness, I_0/σ [outer shell]
	Completeness > 94% : 116 data		**Sample A:** (30 pins, 287 data)	1.92 Å , 99.6%, 1.56
	Beyond 2.0 Å	33 data	**Sample B:** (23 pins, 446 data)	2.04 Å , 99.8%, 1.42
	3.0 – 2.0 Å	25 data	**Sample C** (9 pins 190 data)	4.78 Å , 99.8%, 1.94
	Below 3.0 Å	58 data (9.17 – 3.03 Å)	**Sample D** (2 pins, 17 data)	Failed to a small number of data

S. Baba[a,*], K. Hirata[b], N. Mizuno[a] and T. Kumasaka[a]

[a] Japan Synchrotron Radiation Research Institute (JASRI)
[b] RIKEN SPring-8 Center

*Email: baba@spring8.or.jp

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