Improvement of Automated Crystallization and Monitoring System in PF

We developed a fully automated protein crystallization and monitoring system (PXS) in 2003 and have been operating and improving the system. Here, we summarize the improvements made to the PXS, now named PXS2. The minimum sample volume is reduced to 0.1 μl, the resolution of captured images is increased to 5 M pixels, and a low-temperature incubator is installed. In addition to the vapor diffusion method, the PXS2 can handle bicelle and LCP methods for membrane protein crystallization. These improvements expand the applicability of the PXS2 and could reduce the bottleneck of X-ray protein crystallography.

Today, once a protein crystal is obtained, X-ray diffraction data are easily collected and the structures are quickly determined. However, obtaining good-quality crystals remains a bottleneck in the process. In the actual experimental procedure, hundreds of crystallization conditions must be tested for one purified sample. Since this simple repetitive work is a dull and time-consuming step, researchers want to automate it. To meet this demand, we developed a large-scale crystallization system at KEK-PF in 2003 [1]. Screening experiments of crystallization conditions involve not only making crystallization drops but also continuously observing the drops because nobody knows when crystals will appear in the drops. Therefore, we developed a system to integrate the part that makes the crystallization drops and another part that observes the drops. The system consists of the following modules: dispensers to make crystallization drops, storage for crystallization plates, an observation device to capture images of crystallization drops, a device for transporting plates between them, and an observation interface to check the images of drops. All of these are integrated, and are controlled by unified software. Each unit is modularized and exchangeable for future improvements.

The original system, named PXS, was developed in 2003, and many improvements have been made to date [2]. The performance of the current system, named PXS2, is described below (**Table 1** and **Fig. 1**). The original PXS can handle a minimum 0.5 μL sample to make crystallization drops. To reduce the total sample volume, we installed in the PXS2 a tip-exchange syringe-type dispenser (Mosquito LCP, SPT Labtech, UK), which can dispense 0.1–0.2 μL of sample solution. Since sample preparation, i.e. protein purification, is the most complicated and time-consuming step in a series of experiments, the dispensing of smaller volumes has been widely welcomed by users. The imager used to observe crystallization drops is also improved. The 0.3 M pixel color CCD camera in the PXS has been upgraded to a 5 M pixel CCD device that reconstructs an image with less out-of-focus from multiple images with different focal positions (RockImager2, Formulatrix, USA). In addition, a new device capable of observing second harmonics generation (SHG) and UV-fluorescence (UV-TPEF) was introduced (SONICC, Formulatrix, USA). This helps to judge whether crystals found in the drops are protein crystals or salt crystals. Since crystallization is affected by temperature, a 4°C incubator with a built-in observation device was installed in addition to 20°C incubators. The storage for crystallization plates at 20°C has been increased from 400 to 1,530 in the PXS2.

Table 1: Specifications of PXS2

Crystallization methods	Vapor diffusion Bicelle, LCP
Sample dispensing volume	0.1–0.2 μL
Plate-making speed	3 min 30 sec
Plate-sealing method	Crimping
Plate incubator (20°C)	1,530 plates (5 units)
Plate incubator (4°C)	400 plates (1 unit)
Observation resolution	5 M pixels
Observation speed	4 min 50 sec
SONICC observation system	SHG, UV-TPEF

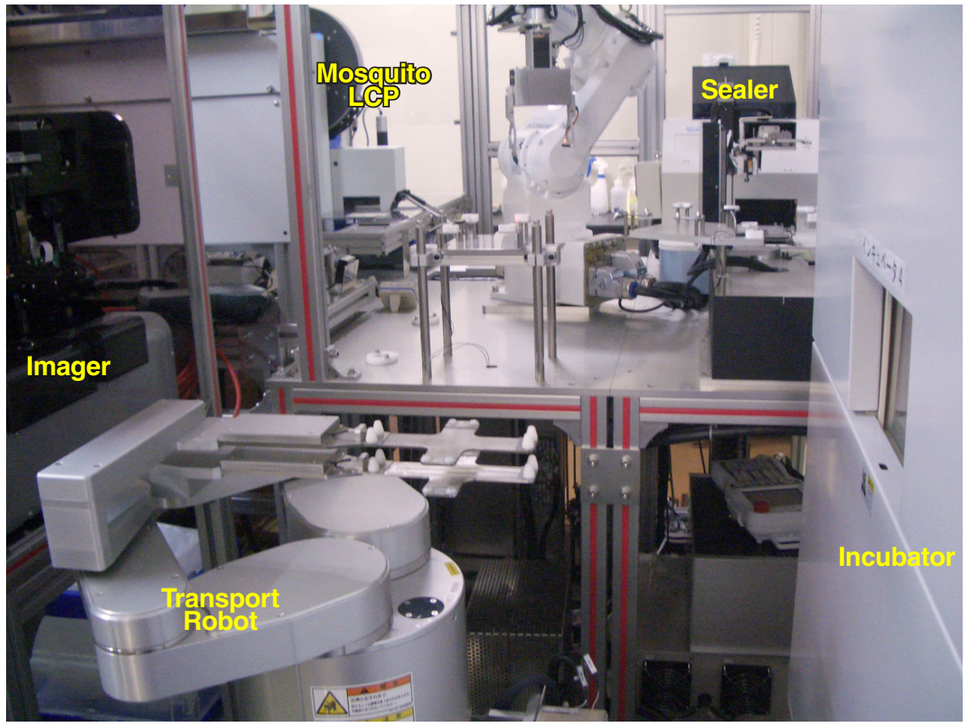

Figure 1: Overview of PXS2.

We also installed new functions in the system. The original PXS supports the vapor diffusion crystallization method, which is the most widely used method for soluble proteins but is often not suitable for membrane proteins. For crystallization of membrane proteins, we made it possible to support the bicelle method by examining and optimizing the dispensing parameters in the PXS2. For the LCP method, we developed an SBS format folder to make the LCP plate compatible with our system. Now, the PXS2 can handle both soluble and membrane proteins for crystallization screening. Images of the crystallization drops are captured automatically, however, judging when and where crystals appear in the drops has been done manually and is laborious work. For example, to screen 500 kinds of crystallization conditions, we would need to observe a total of 12,000 drops during 3 months if each crystallization drop is checked twice a week. Therefore, we developed a system that automatically judges the presence or absence of crystals in drops using machine learning. We connected crystallization screening and diffraction experiments at PF seamlessly by developing an in-situ X-ray diffraction measurement technique and linking two databases of the PXS2 and protein crystallography beamlines, thus enabling the results of crystallization conditions and in-situ X-ray diffraction data to be merged.

Currently, many protein structures are determined by single-particle analysis using cryo-electron microscopy, in addition to X-ray crystallography. Since each method has strengths and weaknesses, these two methods will continue to be used in a complementary manner. Our automated crystallization system will support X-ray crystallography in the future.

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