Current status and future prospects of an automated sample exchange system PAM for protein crystallography

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Current status and future prospects of an automated sample exchange system PAM for protein crystallography

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Abstract. To achieve fully-automated and/or remote data collection in high-throughput X-ray experiments, the Structural Biology Research Centre at the Photon Factory (PF) has installed PF automated mounting system (PAM) for sample exchange robots at PF macromolecular crystallography beamlines BL-1A, BL-5A, BL-17A, AR-NW12A and AR-NE3A. We are upgrading the experimental systems, including the PAM for stable and efficient operation. To prevent human error in automated data collection, we installed a two-dimensional barcode reader for identification of the cassettes and sample pins. Because no liquid nitrogen pipeline in the PF experimental hutch is installed, the users commonly add liquid nitrogen using a small Dewar. To address this issue, an automated liquid nitrogen filling system that links a 100-liter tank to the robot Dewar has been installed on the PF macromolecular beamline. Here we describe this new implementation, as well as future prospects.

1. Introduction

Structure-based drug design (SBDD) and fragment-based drug design (FBDD) both enlarge the scope of macromolecular structure analysis. In addition, studies of membrane proteins and protein complexes also require the investigation of a large number of proteins and their structures. Advances in beamlines have reduced the time required for X-ray experiments; consequently, the rate of sample manipulation in experimental hutches during beamtime is increasing. In prior years, before each diffraction experiment, users usually need to enter the hutch, dismount a previous cryo-pin, mount a new one, and center the new sample on the rotation axis of the diffractometer. Sample-exchange robots can substantially reduce the time required for these procedures. Various automated sample-exchange systems have been developed and are currently in use at many synchrotron facilities [1-7]. At the Structural Biology Research Center of the Photon Factory (PF), we have implemented a PF automated mounting system (PAM) for sample-exchange robots on our macromolecular crystallography beamlines. To achieve stable and efficient operation, we are also upgrading the experimental systems.

2. Current status of the PAM sample exchange robot

2.1. System overview

Figure 1 shows the up-to-date PAM sample-exchange robot installed at the low-energy beamline BL-1A. PAMs were developed based on the Stanford Auto-Mounter (SAM) [1] with some modifications

to fit our beamlines. Notably, the double-tong system [3] has also been implemented on the BL-1A PAM in order to decrease the time required for exchanging the cryo-pins.

We are now operating sample exchange robot PAMs at five PF macromolecular crystallography beamlines, BL-1A, BL-5A, BL-17A [8], AR-NW12A [9] and AR-NE3A [10], respectively. In addition, we have incorporated an automated loop-centering function and management software for fully automated data collection into the GUI beamline control software [10]. About 25% of researchers currently use the PAMs in expectation of fast (10 seconds) sample exchange [3]. The reason for the relatively low proportion can be explained as follows. Some users want to bring crystallization plates and fish crystals to the beamline, and hence cannot use robots. Users bringing few samples also do not need a robot. Finally, not all users have cassettes and tools. Expecting an increase of the PAM users, we are planning to distribute cassettes and tools to many beamline users in a new project entitled "Platform for Drug Discovery, Informatics, and Structural Life Science (PDIS)".

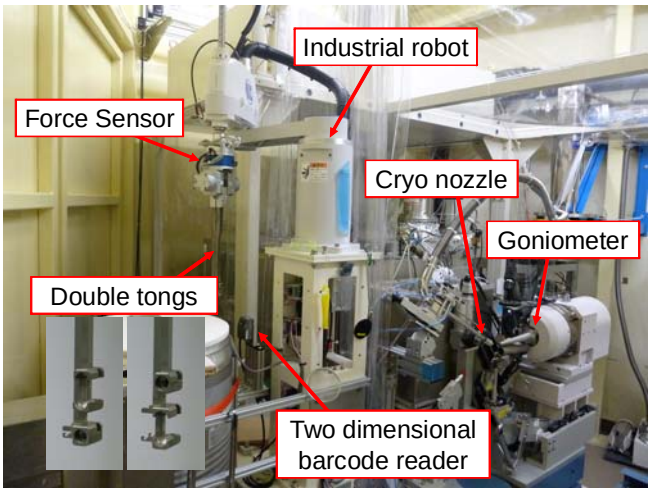

Figure 1. Sample exchange robot PAM installed in low-energy beamline BL-1A. PAM can mount 288 samples (using SSRL cassette) stored in a liquid nitrogen Dewar continuously.

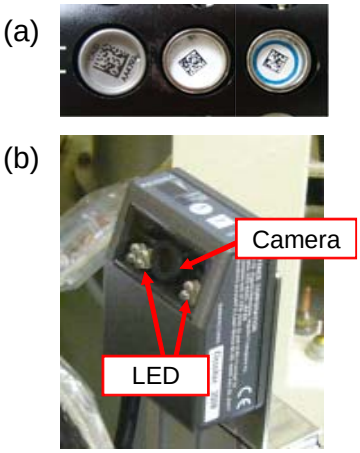

Figure 2. Two dimensional barcode. (a) Cryo-pins with 2D barcode on the market. (b) Implemented barcode reader.

2.2. Two-dimensional barcode

The automated data collection experiments are carried out according to a sample data description file. This file includes sample data such as cassette name, sample position, sample name, X-ray exposure time and rotation angles. Here users give an independent cassette name to each cassette. Prior to the automated experiment, the users had to place the cassettes in the robot Dewar and then carefully input the positions and cassette names into the GUI beamline control software. The GUI software then would execute sample mounting, loop-centering and data collection in the order entered in the file.

A clear drawback of this implementation appears when users make a mistake entering positions and cassette names, resulting in the wrong experiments being performed. Giving each cassette an ID that can be recognized by the robot automatically prevents such errors. To identify the cassette, a commercially available cryo-pin, which contains a two-dimensional barcode (figure 2a), is placed in a predetermined cavity of the cassette. In addition, the users prepare a table file that includes the cassette name and the barcode number. The users have to upload the sample data description file and this table file before the experiment. After checking the cassette type, the PAM reads the barcode on the cryo-pin using a two-dimensional barcode reader (figure 2b) and recognizes the positions of all the cassettes present inside the Dewar. Then the GUI software carries out automated data collection according to the sample description file. Currently, the barcode pins are used only for the identification of cassettes. For the new PDIS project, more samples will come to our beamlines. We are now modifying the

control software and the form of the sample description file in order to read all barcodes mounted by the PAMs and record them in the files.

2.3. The automated liquid nitrogen filling system

The level of liquid nitrogen (LN_2) has to be kept within certain limits during data collection. Simple automated LN_2 filling systems (figure 3a) have been installed at many facilities with LN_2 pipeline in the experimental hall. When the LN_2 level reaches the lower limit, a LN_2 level controller opens a valve and the LN_2 is supplied from the LN_2 pipeline until the upper limit is reached.

However, no LN_2 pipelines are installed at the experimental hall of the PF. At first, we added LN_2 manually using 5 or 10 liter LN_2 containers every 3 or 4 hours. Next, we installed the LN_2 pipeline between the robot Dewar and a self-pressurized LN_2 tank. We have to keep the pressure of the self-pressurized tank below 0.05 MPa in accordance wth the PF safety rules. Since it was difficult to maintain this pressure, we have developed the automated LN_2 filling system shown in figure 3b. In this system, an N_2 gas cylinder supplies pressure-regulated gas to the LN_2 tank.

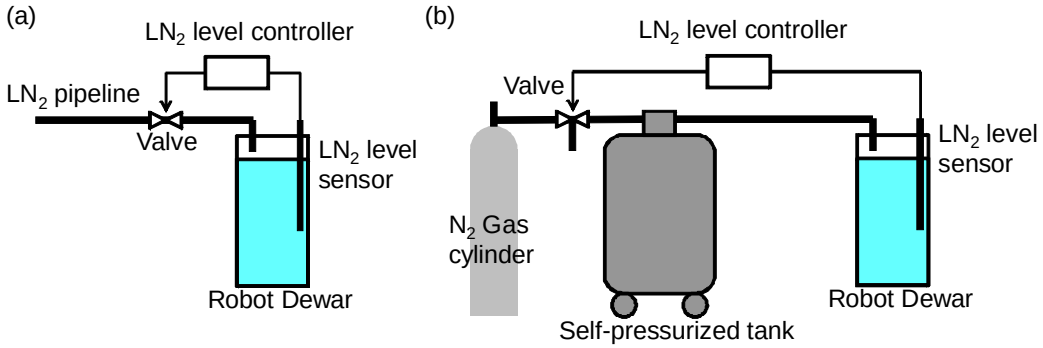

Figure 3. Automated liquid nitrogen filling system. (a) Simple LN_2 auto-filling system. The valve is opened / closed by the controller according to the LN_2 level. (b) Proposed LN_2 filling system using N_2 gas cylinder and LN_2 tank.

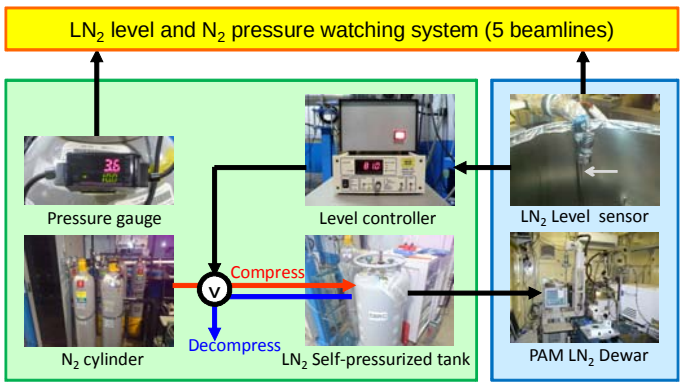

Figure 4. Installed auto-LN_2 filling system and status watching system.

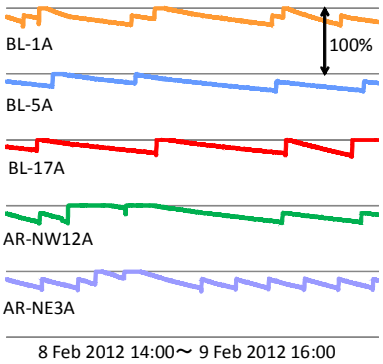

Figure 5. Examples of transition of LN_2 level of all beamlines

The developed automated LN_2 filling system links a 100-liter, self-pressurized tank placed beside the experimental hutch to the PAM LN_2 Dewar inside the experimental hutch. This system has been installed on the PF macromolecular beamlines (figure 4). When the LN_2 level reaches a pre-determined lower limit, the controller opens the valve and N_2 gas is supplied (red "Compress" arrow in figure 4). Then the LN_2 flows to the PAM LN_2 Dewar. When the LN_2 level reaches a pre-fixed upper limit, N_2 gas in the LN_2 tank is discharged through the three-way valve (blue "Decompress" arrow in

figure 4). A level and pressure-monitoring system collects data from the LN_2 level sensor and N_2 pressure gauge of all the beamlines and sends e-mail to the beamline staff when the cylinder and/or the tank need to be exchanged.

Figure 5 shows examples of transitions of LN_2 levels at our five beamlines. The LN_2 levels were controlled stably between lower (70%) and upper (90%) levels. When the controller opens the valve, N_2 gas passes in the pipeline from the LN_2 tank until the pipeline becomes cold. The N_2 gas causes turbulence on the LN_2 surface and the LN_2 level decreases slightly.

3. Conclusions and future prospects

The PAMs are operating at the PF macromolecular crystallography beamlines, BL-1A, BL-5A, BL-17A, AR-NW12A and AR-NE3A. To date, nearly 50,000 samples have been mounted by the PAMs. We have upgraded the experimental systems to achieve stable and efficient operation. In order to distinguish between many cassettes and prevent human error, we have also implemented a two-dimensional barcode system into the PAM. To maintain the LN_2 level in the Dewar, an automated LN_2 filling system was developed and installed at our beamlines. Interestingly, this system does not require installation of a LN_2 pipeline.

In the future, the size of the LN_2 Dewar will be increased, for higher throughput and fully automated data collection at AR-NE3A. With this new implementation, the capacity of the LN_2 Dewar will double. The automated LN2 filling system will be used without any modification.

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