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Advanced beamline automation for biological crystallography experiments

Carl Cork,[a] James O'Neill,[b] John Taylor[a] and Thomas Earnest[a]*

[a]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA, and [b]Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

Correspondence e-mail: tnearnest@lbl.gov

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An automated crystal-mounting/alignment system has been developed at Lawrence Berkeley National Laboratory and has been installed on three of the protein-crystallography experimental stations at the Advanced Light Source (ALS); it is currently being implemented at synchrotron crystallography beamlines at CHESS, NSLS and the APS. The benefits to using an automounter system include (i) optimization of the use of synchrotron beam time, (ii) facilitation of advanced data-collection techniques, (iii) collection of higher quality data, (iv) reduction of the risk to crystals and (v) exploration of systematic studies of experimental protocols. Developments on the next-generation automounter with improvements in robustness, automated alignment and sample tracking are under way, with an end-to-end data-flow process being developed to allow remote data collection and monitoring.

1. Introduction

Automation of the structure-determination process, from target selection to structure interpretation, significantly benefits the biology community by providing tools for improved and more rapid structure determination (Abola *et al.*, 2000; Rupp *et al.*, 2002). These new developments are especially beneficial, and even critical, to efforts in structural genomics and rational drug design, where large numbers of variant structures are to be solved. Automation also significantly benefits the broader structural biology community by increasing the overall speed and accuracy of data collection and by providing the capability for the rapid screening of crystals to select those with the best merit for data collection. An automated crystal-mounting/alignment system (automounter) has been developed at Lawrence Berkeley National Laboratory and installed on three of the protein-crystallography experimental stations at the Advanced Light Source (ALS) synchrotron (Snell *et al.*, 2004). Systems of similar purpose for rotating-anode sources have been developed at Abbott Laboratories (Muchmore *et al.*, 2000), as well as for experimental stations at the Stanford Synchrotron Radiation Laboratory (Cohen *et al.*, 2002). Recently, the LBNL automounter system has been implemented at the National Synchrotron Light Source, the Cornell High Energy Synchrotron Source and the Advanced Photon Source through open collaborations with scientists and engineers at those facilities. European efforts, particularly at the European Synchrotron Radiation Facility (ESRF) and as part of the Structural Proteomics in Europe (SPINE) project, have also produced automated mounting systems that are currently in use. Commercially available systems are also available, particularly through Rigaku, MAR Research and Bruker. A summary of current systems along with references can be found at the RoboSync site maintained by SSRL (http://smb.slac.stanford.edu/robosync).

There are several benefits to using an automounter system.

(i) It facilitates optimum use of synchrotron beam time. Crystallography experiments are installed in radiation-shielded hutches, which are inaccessible during data collection. Changing the crystal manually requires opening and closing the hutch, initiating the interlocks and performing a hutch search. This typically takes several minutes. The automounter significantly reduces both the sample-mounting time and the number of required hutch accesses.

(ii) It facilitates advanced data-collection techniques. An experimental station can be fully automated, including integrated data collection and processing, whereby the structure-solving software can influence the data-collection process (*e.g.* crystal ranking and data-collection strategy determination).

(iii) It facilitates the collection of higher quality data. The rapid crystal-interchange mechanism enables the researcher to evaluate a large pool of samples and to select the best crystals from the set.

(iv) It reduces the risk to crystals. Automated mounting and dismounting of crystals can be performed much more reliably than manual handling.

(v) It facilitates systematic studies of experimental protocols. Alternative protocols can be performed in a manner and amount that would be impractical for humans to perform manually. Furthermore, this can allow for an intelligent system to 'learn' improved methods of data collection and processing.

The LBNL automounter system was designed for optimal simplicity: it is easy to maintain, highly reliable and task-specific, with a small footprint (since space at many experimental stations is limited) and with random access to a large number of samples stored in liquid nitrogen. Furthermore, the simple design facilitates its implementation at sites external to the developers' institution and provides flexibility for a wide range of configurations. Technical problems, such as keeping the sample at liquid-nitrogen (LN_2) temperature during the whole mounting process, avoiding buildup of ice on external components and reducing thermal expansion and conductance at LN_2 interfaces, have been overcome with thorough testing and refinement. The first-generation implementation is based to a large extent on pneumatic actuators, localized temperature controllers and a few motorized stages that require only a relatively simple control system.

2. Experimental station with automounter

The system can be adapted for use with a variety of experimental station configurations. The only significant constraint in the use of this design is that the nitrogen cold-stream subsystem must be oriented off-axis from the primary goniometer axis. The first automounter was installed on experimental station BL5.0.3 at the Advanced Light Source, a fixed-wavelength station with significant access constraints. This system has been in operation since March 2001. Shortly thereafter, systems were installed on a second monochromatic experimental station, BL5.0.1, and also on a multiwavelength experimental station, BL5.0.2 (Earnest *et al.*, 1996; Snell *et al.*, 2004). The automounter configuration is essentially identical for all three experimental stations and increased standardization is under way. We are currently designing a slightly modified configuration which will work within even more spatially constrained experimental enclosures, such as the ALS mini-hutches.

Fig. 1 shows an overview of ALS experimental station BL5.0.2 with an automounter installed. The main components of the automounter setup are a rapid sample-rotation and alignment subsystem, a retractable collimator/beamstop assembly, a retractable cryostream, a long-range video microscope and a sample-mounting subsystem. This station has multiwavelength capability and contains an additional fluorescence monitor for the determination of anomalous absorption edges. The collimator/beamstop assembly and cryostream are retractable to provide access to the rotation and alignment subsystem during sample mounting/dismounting.

2.1. Sample rotation and alignment

A high-speed sample-rotation and alignment subsystem has been developed for the automounter. The crystal-centering process involves the collection of a series of microscope images while rotating the sample over a wide angular range, followed by a sequence of adjustments to center the crystal in alignment with the incident X-ray beam. Another procedure, crystal screening, involves taking a rapid series of diffraction images at 90° angular separation.

The sample-rotation and alignment subsystem designed to meet the above requirements is based on a direct-drive rotary air-bearing stage (Precision Motion Distributors; Fox Instruments) coupled with a three-degrees-of-freedom (3DOF)

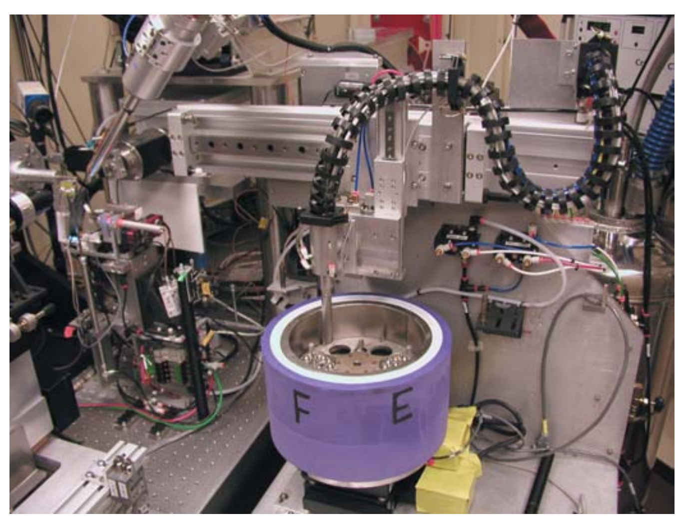

Figure 1
Automounter at the Advanced Light Source beamline 5.0.2. This diagram shows the automounter without the heated lid and liquid nitrogen. The internal baseplate allows for up to six sample-cassette assemblies (each containing up to 16 crystals).

sample manipulator. This system provides a highly variable rotation speed (0.01–360° s^{-1}), high angular resolution (0.00005°) and a small circle of confusion (<1 μm at sample). The 3DOF stage is mounted on the air bearing, allowing remote-controlled alignment of the sample. Two types of 3DOF stages have been developed: a Cartesian *XYZ* stage and a kinematic tripod stage. The measured sphere of confusion of the air-bearing rotation stage and 3DOF system is under 2 μm. The stability and compactness of the tripod stage has made this approach our preferred design. The sample is currently viewed by a long-distance microscope (Questar SZM100; Company Seven), which has variable zoom settings and can be controlled remotely. The sample can be both front- and rear-illuminated to achieve optimum image contrast.

2.2. First-generation automounter

The sample-mounting robot consists of three main components: the gripper which holds the sample during transport from the Dewar to the goniometer while keeping it at low temperature (Fig. 2), the $XY\Theta$ stage which performs the actual transport (shown in Fig. 1) and the Dewar stage which supports the samples in a regular array submerged in liquid nitrogen and positions the selected sample for access by the transporter (Fig. 1).

The gripper contains a conically shaped brass collet that can be opened and closed using a small pneumatic actuator (Fig. 2). The collet is precooled to ~100 K before engaging the sample holder. A sensor inside the collet is used to monitor its temperature. The inner tube is surrounded by a low thermal capacity outer shroud (very thin stainless-steel tube) in the first implementation of the gripper, which provides a sheath-flow of warm dry gas to reduce icing and frost formation during exposure to ambient moisture-laden air. During continuous use, ice is periodically removed from the gripper by means of an extendable air-jet heater (see Fig. 1). Additionally, the gripper has a small low-force linear stage used for the final positioning of the gripper on the sample during the mounting and dismounting. The sample gripper was originally designed around the standard Hampton metal cap, which is held on the goniometer by a permanent magnet. A modified cap design, which has tighter tolerances and a conical shape rather than a ledge, has been designed to further increase automounter reliability (also available from Hampton Research as Product No. HR4-779). The gripper is mounted on a pneumatic $XY\Theta$ stage, which is used to transport the samples between the goniometer and the storage Dewar. A vertical *Y* stage moves the gripper in and out of the Dewar, a 90° rotational Θ stage orients the gripper either horizontally or vertically and a long horizontal *X* stage moves the gripper between the Dewar and the goniometer mount points. When they are not mounted on the goniometer for data collection, the samples are maintained in a small cylindrical Dewar (see Fig. 1). A custom cassette, which can hold up to 16 crystals, facilitates automated handling and bulk transport. Up to six cassettes (96 samples) can be loaded into the sample Dewar. The Dewar is mounted on an $R\Theta$ motorized stage, which is used to position the selected sample for access by the gripper. The Dewar can also be positioned such that the gripper will be inserted into unoccupied space during a precooling action when LN_2 boiling might endanger samples. The Dewar is automatically filled directly from the facility's LN_2-supply system. During normal operations, an insulated and heated cover reduces icing and LN_2 evaporation. The gripper reaches into the Dewar through a small heated hole in the cover. The sample cassettes are moved in or out of the Dewar through a small access port in the cover. An automated loading mechanism is being developed to facilitate this operation.

The performance of this system was tested for the ability to repeatedly mount crystals without deleterious effects (Snell *et al.*, 2004), since the automounter provides the capability to rapidly screen a large number of crystals and choose the one with the most promising parameters. Screening requires this ability and the automounter must provide a robust and reliable approach to selecting the optimal crystal.

Several improvements to the gripper system have been made since the original design and are shown in Fig. 3. These includes a more rigid central collet subassembly to make picking and placing more reliable, reduction of the outer shroud diameter to fit MSC-style pucks as well as ALS-style, a change of the collet design to use a more elastic material (aluminium bronze), use of standard tubing sizes for ease of manufacturability and use of welded parts rather than hard solder for improved durability.

2.3. Sample transport and storage

A sample-cassette system was developed, as described in Snell *et al.* (2004), to facilitate the rapid loading of multiple frozen samples into the automounter system. The cassette system was also designed to enable efficient and safe transport and storage of frozen samples using conventional shipping Dewars. The main considerations for the design were high sample density and compatibility with the Taylor–Wharton CP100 dry-shipping Dewar. One assembly can hold 16 samples and consists of a cassette plus a magnetic base. For transportation and storage, the cassette and base are locked together by two springs on the side of the assembly. A central post in the base is used to protect the samples and to guide the cassette during assembly. The noncircular shape of the post provides the correct orientation between the two parts. A magnetic sheet on the bottom of the base holds the samples within the base and also holds the base within the automounter. The cassettes are labeled with cryo-compatible barcodes for tracking. Seven cassette assemblies can be stored in a CP100 Dewar. The pucks are placed between the shelves of a cylindrical carrier and springs hold them in place. The samples are stored upside down so some liquid nitrogen can be preserved within the cassettes during transport. To secure the pucks during transportation, a metal rod is inserted into the carrier, passing through all the cassette assemblies. The sample-cassette system and associated tools are commercially available (Boyd Technologies). Fig. 4 shows future improvements in the cassettes, with bases on pedestals for better gripping and the use of magnetic material for the pedestals to minimize the failures that occassionally occur with the currently used cut magnetic sheets.

2.4. Control system

The different motions of the automounter can be actuated from a software interface either individually or through scripts which run a sequence of motions (protocols). Several standard protocols, such as 'MOUNT' and 'UNMOUNT', are built into the integrated control system. The image of the crystal is shown on a video monitor. In the case of semi-automated crystal centering, the user clicks on the crystal and it is moved to the center position by the motorized 3DOF stage on the goniometer (*i.e.* its final position will coincide with the green cross on the image, which marks the projection of the intersection of the X-ray beam and the goniometer center of rotation on the microscope axis). Afterwards the crystal is rotated 90° and centered again. For 'autocentering', a protocol is run which consecutively centers the loop at both 0 and 90°. The current autocentering protocol only centers the loop and can fail under adverse illumination conditions. Further development is required before this protocol is ready for routine use. Under remote control, the goniometer can be moved to any angle, the zoom level on the microscope can be changed, the intensity of the front and rear illuminators can be adjusted and automounter mount/dismount operations can be activated. A MySQL relational database system is used to track precise sample progress at the beamline. Information about the crystals, such as the puck ID and position index, the organization it belongs to *etc.*, is stored in the database. The sample cassettes are labeled with barcodes and can be scanned before they are put into the automounter storage Dewar. This system also assigns information on experimental parameters to the samples.

The beamline-control system and graphical interface, Distributed Control System (DCS), is written in Java, C++ and C. It currently includes capabilities for crystal mounting, alignment and data collection. Protocols can be scripted into the software that allow for automated mounting, alignment and data collection of one or more images on all 96 crystals in the sample-storage Dewar.

3. Development of next-generation automounter

The first-generation automounter features a transport subsystem based on a set of very compact pneumatic stages. This has the advantage of being very low cost, has excellent

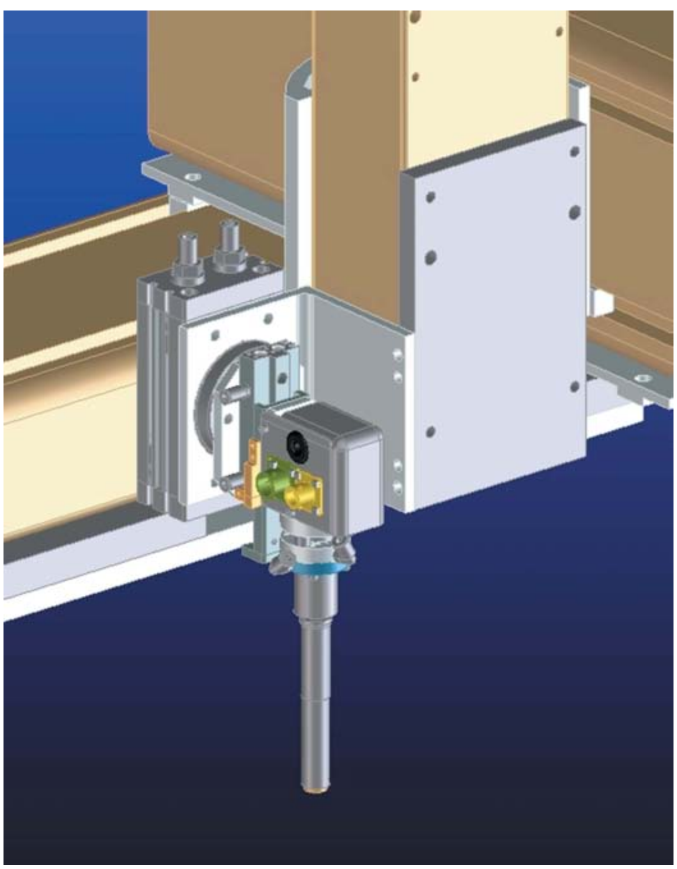

Figure 2
Gripper assembly, version 2 (V2), mounted on the motorized stages of a next-generation robot. Improvements in robustness and servicability have been incorporated as described in the text. The first-generation automounters can also be retrofitted to incorporate these improvements.

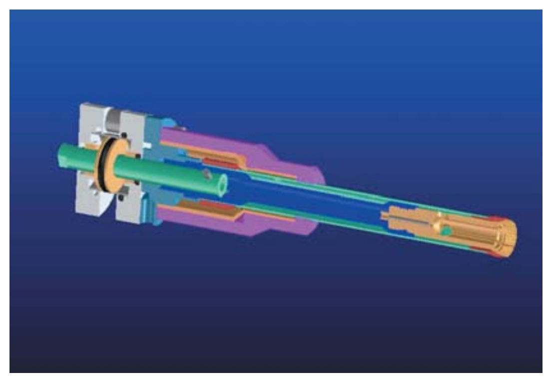

Figure 3
Inner view of V2 gripper assembly. Improvements over the version 1 gripper design include ease of assembly, serviceability and improved precision. The V2 design incorporates a more rigid collet subassembly, more elastic aluminium bronze collet and improved manufacturing processes.

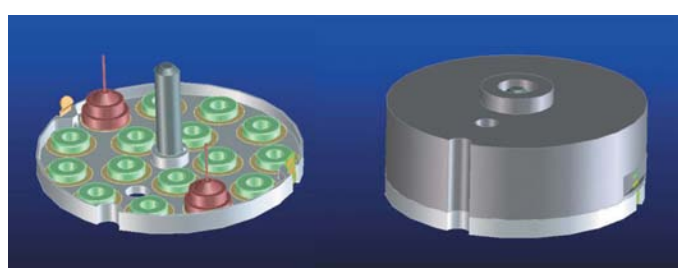

Figure 4
Future sample-cassette assembly modifications, including the use of high-field-strength magnetic inserts in place of the current low-field-strength magnetic sheet, shown without top (left) and with top (right). This system allows for preservation of a dry and cold environment during shipping and storage and is dimensioned to fit into a standard shipping Dewar.

speed performance and is relatively safe to operate (low-force actuators). However, the pneumatic stages are somewhat difficult to align relative to the sample positioner and the storage Dewar. The design also requires a motorized $R\theta$ stage under the storage Dewar in order to position the sample beneath the gripper. In the development of the next-generation system, the pneumatic and the $R\theta$ stages will be replaced with a single motorized *XYZ* Cartesian stage (see Figs. 5 and 6). The stage is equipped with absolute encoders for error-free initialization. Realignment is accomplished by adjustment of software parameters, not mechanical shims and clamps. Additional research and development will be required to develop appropriate collision sensors and personnel-protection equipment. Software development will also be required for interfacing to the robot controller and for optimizing the gripper transport protocols. We will also add new capabilities to the system beyond our current design (*e.g.* new docking procedures and a special storage crib for alignment tools).

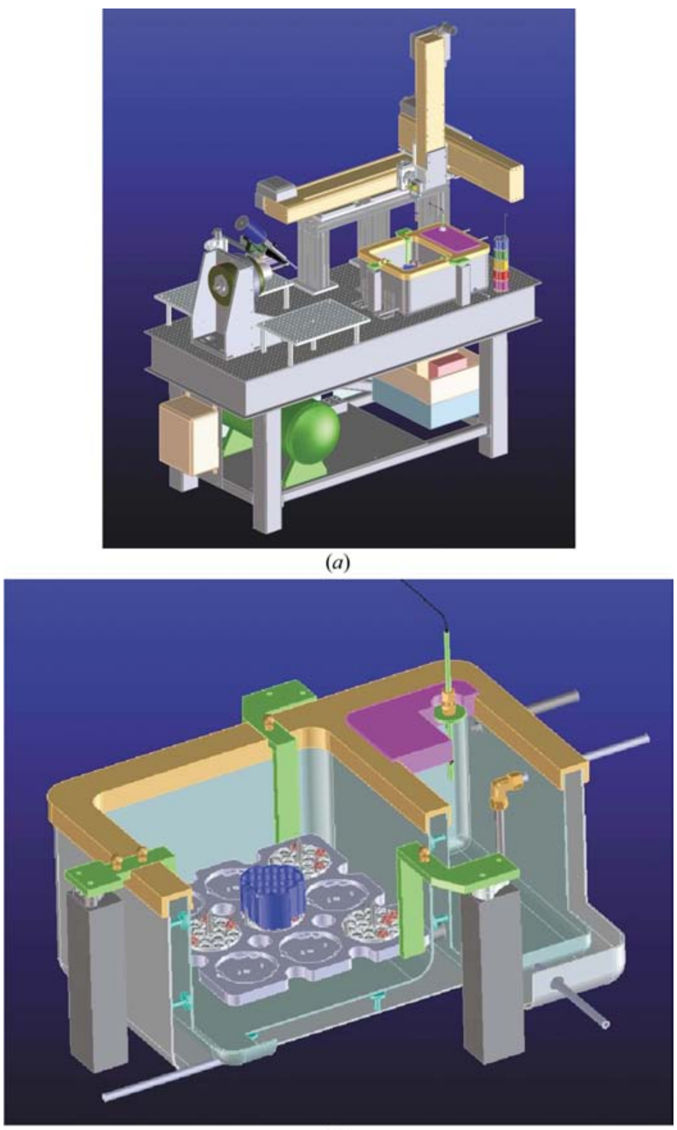
(*a*)
(*b*)

Figure 5
Computer-assisted design drawing of the next-generation development system showing full system (*a*) and cut-away view of the Dewar (*b*). The cassette-mounting plate is suspended independently of the Dewar and features externally viewable alignment balls.

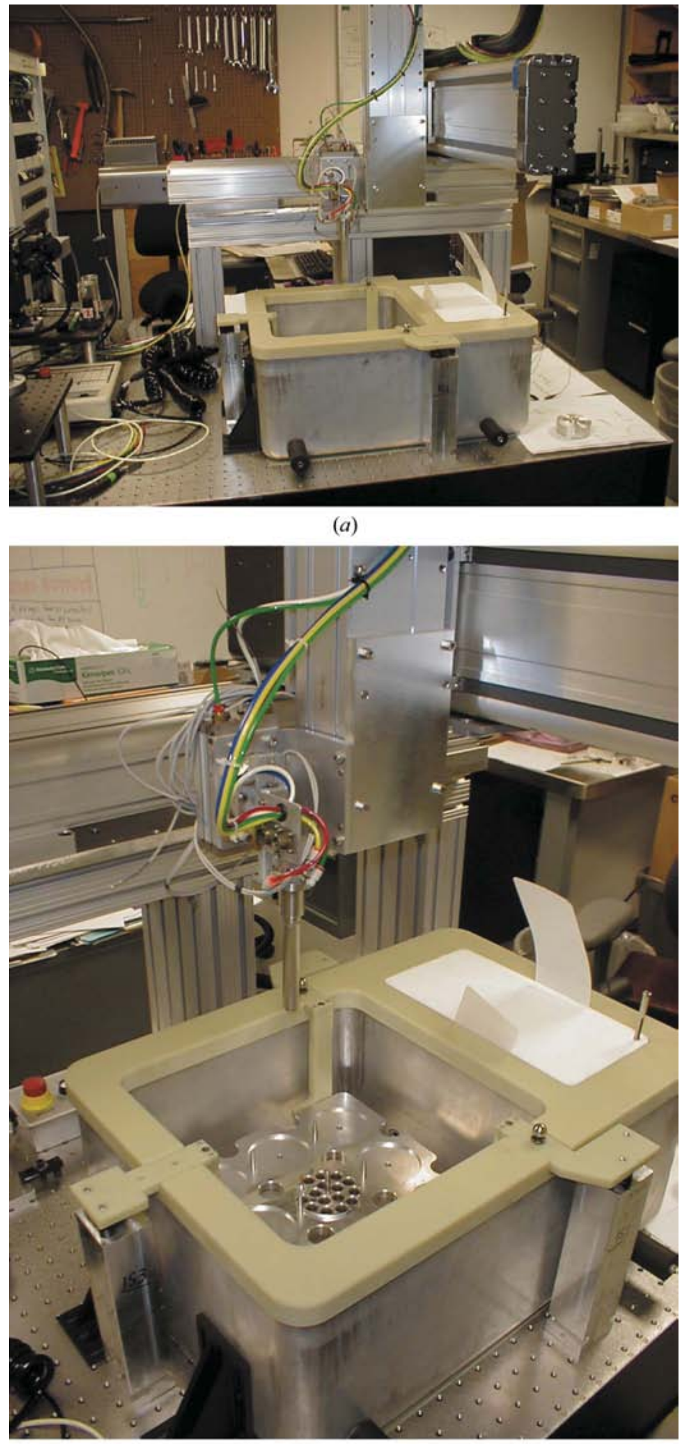
(*a*)
(*b*)

Figure 6
Implementation of next-generation system in the robotics development laboratory. The Dewar contains two interconnected chambers, one for liquid-nitrogen filling and the other for sample assemblies. (*a*) shows a partially assembled system consisting of a three-axis Cartesian robot, V2 gripper and stationary sample Dewar. (*b*) is a close-up view of the mounting plate.

The current storage Dewar design incorporates a cylindrical vacuum-insulated vessel with an internal platform for supporting the sample cassettes. The subassembly is mounted on an $R\theta$ stage that positions the sample directly beneath the fixed transport stage pickup location. The samples are kept a few centimetres below the LN_2 surface and the liquid level is controlled within 4–5 mm. The storage Dewar subsystem is the most problematic component of our current design. Condensation from the Dewar tends to accumulate on the $R\theta$ stage and leads to maintenance problems with the stage and motors. The LN_2-level controls are also difficult to make reliable with a moving Dewar design. Finally, system alignment is much more complicated with the somewhat sloppy tolerances of the vessel design. The $R\theta$ stage has been replaced with the overhead-mounted motorized Cartesian XYZ stage (see Figs. 5 and 6). This will remove all moving parts from the area susceptible to condensation buildup. The cylindrical Dewar will be replaced with a rectangular design for better space utilization and sample-handling capacity. A stationary Dewar design will also permit us to use a secondary LN_2-supply Dewar for better level control and buffer capacity (Fig. 6). A gravity-levelling design is used in which the storage and supply Dewars are coupled directly by a flexible transfer line and the supply Dewar is equipped with a reliable float-valve level control. For support and alignment of the samples within the Dewar, we plan to use interchangeable cassette-mounting plates for incorporation of either LBNL or Rigaku-style cassettes.

This system is being coupled to smart cameras that feature image-recognition capabilities to facilitate a fully automated alignment utilizing alignment balls located external to the Dewar. These balls will be precisely aligned to the base plate such that locating them allows the precise calculation of the positions of the crystal under liquid nitrogen in the Dewar. In addition to the automated alignment capabilites that are being designed into this system, the elimination of the moving stages under the Dewar along with encoded stages offer a route to more robust and servicable systems for the future.

4. Sample tracking and remote data collection

Sample tracking, currently performed ‘by hand’ or using barcoding, remains an issue for automated data collection. New miniature RFID (radio-frequency identification) technologies are very promising and could be used in conjunction with the two-dimensional barcodes. RFID systems function by attaching small programmable ‘transponders’ to the sample and querying them with RF-transmitting ‘readers’. The RFID systems that we are considering are of the ‘passive’ low-frequency type because of their ability to read in the presence of LN_2 and metal surfaces. The main limitation with this design is that the receiver antennae need to be located within 5–10 cm of the transponder. One vendor (Research Instruments Ltd) offers an RFID system with cryogenic transponders. Very small transponders (2 × 10 mm) can be attached to sample cryomounts. Significant research and development will still need to be performed to determine the optimum antennae configurations and to determine a design that can be used for both sample and cassette tracking within the storage Dewar.

Ultimately, the software system is envisioned as an end-to-end data-flow system which handles the information and operations required to conduct all tasks from the time a researcher creates an experiment plan until the resulting data are returned. We are basing our overall design for remote data collection on the architecture being developed for the Atacama Large Millimeter Array (ALMA) Project (Schwarz *et al.*, 2002), although recognizing that there are differences in scale and features needed by this system and the case that we are addressing. A slightly modified version of their end-to-end data flow is shown in Fig. 7. One may view the system either from the perspective of the researcher or the facility. The researcher is interested in how the observing process flows through the different parts of the system, as illustrated by the outer set of lines in Fig. 7. The researcher (shown as the actor symbol on the leftmost side) initiates the cycle by creating and submitting a measurement plan to the Plan Compiler. The Compiler converts the plan into a set of Scheduling Blocks (SBs). Once each SB has been defined it is stored in the Archive and considered for scheduling whenever available instrument conditions and resources make its execution feasible. If all other factors are equal, the ready-to-run SB with the highest priority will be chosen and executed by the Sequencer. The execution of an SB generates a set of raw data, which is both saved in the Archive and forwarded to the Screening Pipeline. The Screening Pipeline performs a rapid assessment of sample quality and orientation and feeds this information back to the Scheduler to influence subsequent processing. Parameter data derived from the Screening Pipeline is passed on to the Reduction Pipeline for final processing of the archived raw data. These components are already in place within our control system. New modules can be easily inserted into the data flow. As can be seen from the above discussion, the measurement system is primarily a service-based architecture. The researcher submits the measurement plan and then monitors the system while it proceeds, mostly

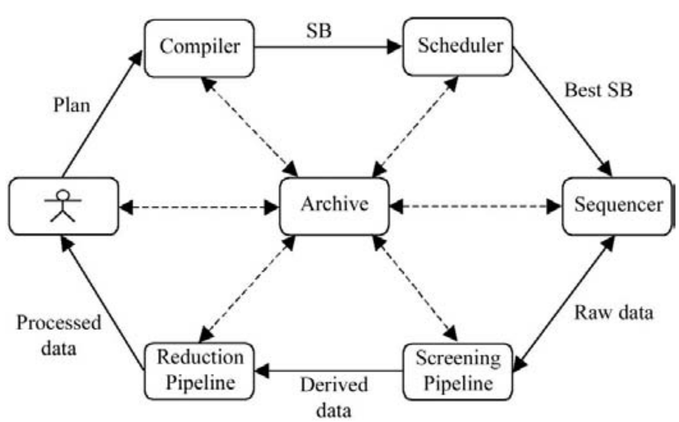

Figure 7
End-to-end data-flow model for remote operation, based on ALMA model for radio astronomy. A similar approach can be utilized for multi-user remote operation of protein crystallography beamlines coupled with high data throughput.

independently, to process the samples. However, the Scheduling Blocks will be designed to provide checkpoints where the processing is stopped until guidance is received from the researcher. Currently, the Researcher and Operator/Scheduler are usually the same person. In this case, the plan–measure–approve–measure process is all performed locally. However, we see a strong preference from several researchers to separate these roles and to permit remote interaction for the Researcher. We plan to implement a prototype system that will provide secure web services for remote interaction with the measurement system. At the moment, we believe that this can be implemented using Java servlet technology, such as Apache Tomcat. However, during the design phase, it might be decided to use an enterprise Java Beans (EJB) application server, such as JBoss, in order to take advantage of the large number of additional built-in services.

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