

User's Guide to Macromolecular Crystallography Experiments at SSRL

January 24, 2018

This document contains information about doing experiments at the macromolecular crystallography beamlines at SSRL. An html version of this documentation can be found at http://smb.slac.stanford.edu/users_guide/

Contents

1	How to become an SSRL user	5
1.1	Writing a proposal	5
1.2	Beamtime requests	6
1.3	Other requirements	6
2	Experiment policies	6
2.1	Beamtime shifts schedule	6
2.2	User responsibilities	7
2.3	When to contact SSRL staff	8
2.4	Off-line use of computer resources	9
2.5	Use of the SAM robot for sorting samples between cassettes and/or unipucks	9
2.6	Biohazards	9
2.7	On-site handling of samples	10
2.7.1	Heavy metal use	10
2.7.2	Propane and ethane use	10
2.7.3	Liquid nitrogen use	11
2.7.4	Skin and eye frostbite burns	11
2.7.5	Asphyxiation	11

2.7.6	Instructions for liquid nitrogen disposal	13
2.7.7	Compressed gas experiments	13
3	On-site experiments	14
3.1	On-site safety protocols	15
3.2	Safety in the experimental hutch	15
3.3	Mounting samples	16
3.4	Making room to safely mount samples	16
3.5	Mounting samples in the cryostream.	16
3.6	Mounting crystals at room temperature	19
3.7	Sample illumination	20
3.8	Checking out	20
4	Remote experiments	21
4.1	New remote users	21
4.2	Preparing a remote experiment	21
4.3	Remote data collection	22
4.4	Remote experiment support	23
5	Using the SSRL Automated Mounting (SAM) system	24
5.1	Overview	24
5.2	Sample pin selection and preparation	25
5.2.1	Allowed types of pins	25
5.2.2	Pin preparation	26
5.2.3	Pin testing	28
5.2.4	Re-using pins	29
5.3	Loading and shipping SSRL cassettes	29
5.3.1	SSRL Cassette kit	29
5.3.2	Avoiding ice	31
5.3.3	Loading samples in the SSRL cassette	33
5.3.4	Preparing cassettes for shipment	35
5.4	Loading and shipping Uni-Pucks	37
5.5	Storing sample information: The Excel spreadsheet	38

5.5.1	Uploading an Excel spreadsheet	38
5.5.2	Assigning the Excel spreadsheet to a beamline	40
5.5.3	Using the Default spreadsheet	40
5.5.4	Editing the spreadsheet	41
6	Data collection and processing	41
6.1	Automated crystal screening	41
6.1.1	Selecting samples for data collection	44
6.2	Setting up and starting data collection	45
6.2.1	Notes on strategy for monochromatic and high resolution experiments	47
6.2.2	Notes on strategy for MAD and SAD experiments	48
6.2.3	Multicrystal data collection strategy	49
6.3	Data processing	50
6.3.1	Data processing environment	50
6.3.2	Data processing documentation	50
7	Referencing SSRL	51
7.1	References to supported software/hardware	52
8	Frequently Answered Questions	54
8.1	Data collection	55
8.1.1	How can I convert from wavelength to energy?	55
8.1.2	What is the optimal beamstop to sample distance?	55
8.2	Sample mounting	55
8.2.1	What is the size of the white box on the Blu-Ice sample video?	55
8.2.2	The automated loop centering leaves the camera near the medium zoom level. Is there anything I can do?	55
8.3	Detectors	56
8.3.1	Can I display the image header?	56
8.3.2	How do I determine the direct beam position?	56
8.4	Pilatus and Eiger detectors	56
8.4.1	What is the optimal oscillation angle per image with the Pilatus and Eiger detectors	56
8.4.2	What is shutterless data collection? Can I turn it off?	57
8.4.3	Why can't I pause data collection during shutterless data collection?	57

8.4.4	The data collection takes a long time to start	57
8.4.5	Can the Pilatus 6M detector resolve large unit cells?	58
8.4.6	How do I know if I am overloading the detector?	58
8.5	Determining data collection strategy	58
8.5.1	What is a reasonable exposure time for beamline X?	58
8.5.2	How long do I need to expose a crystal at SSRL for RIP phasing?	58
8.6	Beamlines	59
8.6.1	BL12-2	59
8.6.2	BL9-2	60
8.6.3	BL14-1	60
8.7	SPEAR3 operation	60
8.7.1	How can I monitor the SPEAR3 beam?	60
8.7.2	How often is the beam refilled?	60
8.7.3	Does the frequent fill mode affect the diffraction images?	60
8.7.4	How does the search and reset procedure work?	61
8.8	Problems during data collection	61
8.8.1	Is there beam in the hutch?	61
8.8.2	Why do I get blank diffraction images?	61
8.8.3	Why can't I open Blu-Ice?	62
8.8.4	Why is Blu-Ice not responding?	63
8.8.5	What does the message "Detector error" mean?	63
8.8.6	When should I optimize the beam?	64
8.9	Data processing and graphics software	64
8.9.1	I get an HTTP error when trying to display or refresh a page in Web-Ice	64
8.9.2	Why doesn't program X run?	64
8.9.3	Why is iMosflm/HKL2000/other running slow?	64
8.9.4	Why can't I autoindex my images?	64
8.9.5	How do I display the JPEG snapshots of the crystal?	65
8.9.6	Are the International Tables for Crystallography available?	65
8.9.7	Is it OK to leave files on the /data disk?	66
8.10	Remote access	66
8.10.1	How can I monitor the SPEAR3 beam remotely?	66

8.10.2	HKL2000 displays a screen size error	66
8.10.3	I cannot open an ADXV window from Blu-Ice	66
8.10.4	Why can't I connect to the NX server?	66
8.11	Computers	66
8.11.1	Can I change the default settings for my SSRL account?	66

1 How to become an SSRL user

There are several mechanisms to become an SSRL user, mutually compatible:

- **Submitting a proposal.** Proposals can be submitted for single experiments, for a program (i.e. different research problems or projects scientifically linked or related to each other) or for rapid access ("hot" new projects).
- Setting up **Participating Research Team** (PRT access)
- **Scientific collaborations with SSRL staff**

Additional information about proposal submission including links to the appropriate forms and contact information to become a PRT or set up a collaboration with staff can be found at:

<http://smb.slac.stanford.edu/forms/becominguser>

1.1 Writing a proposal

A successful proposal has three components:

1. Important structural targets, and/or a novel and useful experiment
2. Sound justification of the need for synchrotron time
3. Experience and previous results (can you carry out the experiment)

Make your proposal strong by balancing all three components; make a reasonable estimate of time and determine the appropriate beamlines¹ that could be used for your experiment; contact any support staff member beforehand for advice if you have any questions. Looking at other successful proposals can be helpful.

Examples of justifications for synchrotron time include:

- Data collection at energies (wavelengths) not available in the home lab. Required for MAD/SAD experiments.

¹<http://smb.slac.stanford.edu/>

- Increased intensity to push the resolution limit. What resolution do you have, why do you need to extend it further? What question cannot be answered at the current resolution?
- The ability to resolve longer unit cells. What can the system at home resolve, what can you expect to resolve at the synchrotron? Look into the beamline characteristics, do a quick calculation.
- Fast automated screening and characterizing many samples using the SAM robot² and automated sample analysis. What percentage of crystals provide good diffraction, how many need to be screening to find one suitable for data collection? How long would it take to do screening at home?

1.2 Beamtime requests

Spokespersons with active proposals are notified via email to submit a request. New requests are required three times a year and are due two months in advance each scheduling period.

Beamtime request forms are available from the URAWI database URAWI database³

The forms provide options to request collaboration with staff, use of the sample mounting robot (section 5) and remote data collection (section 4). **Note:** First time users are currently requested to collect data at the beamline once or to attend a training workshop⁴ at the SSRL before they perform the experiment remotely.

If you need an SSRL SMB Unix account (e.g. you have never collected data at the SSRL Macromolecular Crystallography beamlines before or you started a new group) please follow the instructions to request one⁵.

If you have an active proposal and need beamtime urgently, please contact Lisa Dunn⁶; there may be short notice openings because of cancellations or other reasons.

1.3 Other requirements

For information on additional administrative requirements once beam time has been assigned, please consult the SMB "Forms" menu.

2 Experiment policies

2.1 Beamtime shifts schedule

Unless informed otherwise by the beamline support staff, the beamtime starts at **3:00 pm** of the first day allocated to the experiment and end at **11:00 am** of the last day; an exception to this rule are

²<http://smb.slac.stanford.edu/facilities/hardware/SAM/>

³<https://www-ssrl.slac.stanford.edu/URAWI/beamTimeRequestForm.html?proposalNo=8045&btrType=MC&btrId=&method=>

⁴<http://smb.slac.stanford.edu/news/workshops/>

⁵<http://smb.slac.stanford.edu/facilities/computing/account.html>

⁶<http://smb.slac.stanford.edu/staff/>

the first day of Accelerator Physics and Accelerator Maintenance, when the beamtime usually ends at 6 am. Accelerator shifts are usually scheduled on Monday and Tuesday every two weeks, and are indicated in the schedules⁷. Also, shorter shifts are sometimes scheduled on BL12-2, with different starting and finishing times. You will be informed by staff about unusual starting and finishing times.

2.2 User responsibilities

Both **on-site** and **remote users** are responsible for:

- Ensuring that one experimenter who has attended the beamline orientation participates in the experiment at all times.
- Contacting SSRL staff in case of a problem.
- Backing up data before their run has ended (users transferring data should log onto the SMB computer **smbcopy** for this purpose).
- Reporting missing or malfunctioning equipment.
- Protecting the SSRL computers from unauthorized access by logging out or locking the terminal screen whenever not using them.

Users collecting data **on-site** are also responsible for the following:

- If applicable, reading and complying with the policies regarding use of heavy metals (section 2.7.1), propane (section 2.7.2), compressed gases (section 2.7.7) and liquid nitrogen use (section 2.7.3).
- Knowing and following all applicable safety protocols.
- Posting the proper safety protocols and signs required for their experiment.
- Cleaning up the beamline and lab areas before their run has ended.

On site users should not attempt:

- Making repairs or realigning beamline optics.
- Removing covers or unplugging detectors and other electrical equipment.
- Rebooting computers.
- Using SSRL computers other than those provided at the assigned beamline.

⁷<http://smb.slac.stanford.edu/schedule/>

2.3 When to contact SSRL staff

For information on beamtime requests, scheduling or beamtime, e-mail Lisa Dunn⁸.

For inquiries about facilities, experimental capabilities, or related topics, contact the assigned support staff scientist found in the user support schedule⁹. Please use the contact information provided below.

For information on how to ship dewars or equipment to SSRL, consult the shipping procedures¹⁰. For further inquiries send e-mail to dewars@smb-mail.slac.stanford.edu.

To request a computer id and questions about a user unix account, contact Thomas Eriksson¹¹

During a **remote experiment**:

- For any problems, contact support staff by phone during normal working hours 9 am - 9 pm and by e-mail outside of normal working hours. Remote access experiments are not typically supported outside of normal working hours, therefore if time is lost, additional time will be provided the next day or in the immediate future.
- For questions about SPEAR3 status call the Duty Operator: 650-926-4040.

During a **experiment on-site**:

- For general questions, data processing help or to report non-critical problems, contact the assigned support staff scientist on the beamline cell phone **during the hours 9 am-9 pm or by e-mail anytime**.
- For a problem that makes it impossible to continue the experiment, on-site users may contact support staff by phone or e-mail any time. In an emergency, they may try contacting other staff scientists currently assigned to other beamlines (see the user-support schedule).
- For questions about SPEAR3 status or to reset beamline alarms, call the Duty Operator by dialing 4040 from any SSRL phone.

For sample container exchanges and other support that requires staff to be on-site:

- For weekdays, arrangements can be made with the assigned user support staff for cassette exchanges during normal working hours 9 am - 9 pm.
- For weekends and holidays, support staff will set the cassette exchange time.

<p>Important: Note that there is no beamline support staff on site during the weekend or outside normal working hours; issues that require staff being present at SSRL at these times will be dealt with at the staff's earliest convenience.</p>
--

⁸<http://smb.slac.stanford.edu/staff/>

⁹http://smb.slac.stanford.edu/schedule/sch_staff.cgi

¹⁰<http://smb.slac.stanford.edu/forms/shipping/>

¹¹<http://smb.slac.stanford.edu/staff/>

2.4 Off-line use of computer resources

- No restrictions.

2.5 Use of the SAM robot for sorting samples between cassettes and/or unipucks

Blu-Ice provides an interface¹² for moving samples between cassettes and/or uni-pucks. This facility is available to SSRL users during their scheduled beamtime. The following policies apply:

- Sample sorting is considered to be part of the experiment and should be completed by the end of the scheduled beamtime.
- This service is not available during beamline maintenance periods, accelerator physics or SPEAR3 shutdowns.
- All the SSRL cassettes or unipucks used to move samples must be supplied by the user, and mounted on the beamline robot dewar at the start of the experiment or during normal working hours.
- Staff may not be called at off hours or on weekends to load a cassette in the dewar for sample sorting.

2.6 Biohazards

Biohazardous materials include infectious agents and hazardous biological materials as described by the United States Department of Health and Human Services, Centers for Disease Control¹³ for infectious agents and by the National Institutes of Health¹⁴ for recombinant DNA molecules.

All experiments involving biohazardous materials must be clearly identified and categorized according to their biosafety level and carried out according to the guidelines set by Stanford University¹⁵ Currently, the following restrictions apply for biohazardous samples:

- Level 1: No restrictions
- Level 2: Users will be requested to submit a form¹⁶ for review by the Stanford University Administrative Panel on Biosafety, which will communicate any special controls or requirements to the user. **Users must adhere to the approved protocol and notify the safety officer/safety coordinator before making any changes.**
- Levels 3 and 4: Due to the levels of engineering and administrative controls needed for biosafety level 3 and 4 hazards, use of these agents is currently not permitted at SSRL.

¹²http://smb.slac.stanford.edu/facilities/software/blu-ice/sort_tab.html

¹³<http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

¹⁴http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

¹⁵http://www.stanford.edu/dept/EHS/prod/researchlab/bio/Biosafety_Manual/Biosafety_Manual_complete.pdf

¹⁶<http://www.stanford.edu/dept/EHS/prod/researchlab/bio/docs/APBform.doc>

2.7 On-site handling of samples

These policies must be followed by users bringing their samples to SSRL. We recommend that remote users follow the same or similar safety protocols when applicable.

2.7.1 Heavy metal use

The following protocol is required for on-site preparation of heavy atom solutions or crystal soaking. It does not apply to use of frozen pre-soaked crystals.

- Post the "**Caution: Heavy Metal Solutions**" sign in the working area prior to usage.
- Ensure that containers of solutions containing heavy atoms are clearly labeled, identifying contents, owner's name, contact telephone number and date.
- Heavy metal solutions can only be made up in one of the Biotechnology Laboratories, and safety glasses and gloves shall be worn. Containers of volatile heavy metal solutions shall be opened in a working hood in one of these laboratories. The area in the hood must be lined with absorbent material.
- Quantities of less than 1 ml of solution, containing less than 0.1 M of heavy metals, may be removed from the Biotechnology laboratory in labeled and tightly sealed containers and transported to the sample preparation area at the beamline for soaking crystals.
- Soaking crystals at the beamline shall be performed in the Heavy Metal containment tray; **absolutely no exceptions**. The tray is located next to the computer table. Contact support staff if you can not locate the containment tray. The tray should be lined with absorbent material.
- Spills outside the containment tray shall be wiped up immediately with absorbent material (present at the beamline toolboard). The SSRL Safety Office must be notified immediately of any heavy metal spill that escapes the containment tray. Please contact the Beamline Duty Operator for this to occur.
- Any liquid or solid waste, including gloves, absorbent material and other contaminated material should be bagged, clearly labeled and disposed of as hazardous waste. The SSRL Safety Office should be contacted for proper disposal.
- All heavy atom solutions shall be returned to the user's home laboratory at the finish of the experiment.

2.7.2 Propane and ethane use

The following protocol is required for bottles/canisters of propane or ethane gas used at SSRL for the purpose of flash-cooling samples. The use of prefrozen samples containing propane or ethane does not require any special protocol.

- The ”**Caution Flammable Gas**” sign must be posted at the work area prior to usage. Only small bottles/canisters are allowed at SSRL (lecture bottles). The propane/ethane bottle shall be set up for dispensing in a clear open space.
- The bottle must be secured to the table.
- **No electrical appliances or ignition sources, such as a wax melter, microscope, etc. are allowed to be in this area.**
- The amount of liquid propane/ethane dispensed for flash cooling should not exceed 10 mL.
- After dispensing the gas, ensure the bottle valve is firmly closed.
- Empty gas bottles shall be returned to the experimenter’s home institution.

2.7.3 Liquid nitrogen use

This section describes hazards and proper handling procedures for work with liquid nitrogen

2.7.4 Skin and eye frostbite burns

Direct contact with liquid nitrogen, metal or other material that is in contact with liquid nitrogen or cold nitrogen gas can cause freezing of exposed tissue.

- Follow all documented procedures for a given task and any additional instructions that may be posted at the work site.
- Wear the appropriate Personal Protection Equipment (PPE) appropriate for the task.

2.7.5 Asphyxiation

In confined areas, there is a risk of asphyxiation by displacement of oxygen. Do not use liquid nitrogen in a confined space unless the maximum allowable amount of liquid nitrogen has been posted by the SSRL Safety Officer or if the room is equipped with an oxygen deficiency (OD) alarm.

- All experiment hutches are equipped with OD alarms. Immediately exit the hutch if the alarm sounds. If the OD alarm sounds when you are outside the hutch, do not enter the hutch. **Do not open the door until the alarm has stopped.**
- The experimental floor is also equipped with oxygen alarms, which can activate if, e.g. a liquid nitrogen line ruptures or leaks. If the experimental floor OD alarm sounds, treat it like an emergency alarm: exit the building through the nearest exit and do not re-enter until the duty operator indicates that it is safe to do so.
- Cold rooms in general are not equipped with OD alarms. Do not exceed the posted amount of liquid nitrogen allowable in the confined space. If there is no posting, do not bring liquid nitrogen into the confined space.

Table 1: Required PPE for handling Liquid Nitrogen

	Safety Glasses	Cryogenic Gloves	Goggles or Face Shield	Long Pants without cuffs or Apron	Closed-toe shoes	Insulated tool handles
Fill dewar with close-loop transfer line	X	X				
Fill dewar with open flow delivery line		X	X	X	X	
Dewar to dewar transfer	X	X		X	X	
Removing/storing items in dewars	X	X		X	X	
Transporting open dewars holding less than 0.5 L	X	X				
Disposing of liquid nitrogen by pouring on ground	X	X		X	X	
Disposing of liquid nitrogen by bubbling warm nitrogen gas	X	X				
Manipulating protein crystals in dewars	X					X
Transporting dewars or tanks with lids or closed valves						
Disposing of liquid nitrogen by evaporation						

2.7.6 Instructions for liquid nitrogen disposal

- Use a warm nitrogen bubbler to evaporate liquid nitrogen when possible.
- Small amounts of liquid nitrogen (less than 0.5 Liters) can be poured carefully in an open area on the floor.
- Larger amounts must be poured in an open area outside the building.

2.7.7 Compressed gas experiments

The following policies apply for work at the SSRL involving gases delivered in compressed gas cylinders (CGC):

- Requests to work with CGC must be indicated the Beam Time Request Form¹⁷ (typically submitted three times per year). To make a request after the form has been submitted, contact the SSRL User Office.
- Standard instructions and safety protocols are to be applied when using the SSRL pressurization cells:
 1. The SSRL pressure cells may only be used as specified in the instructions¹⁸.
 2. The CGC must be secured at all times during the experiment.
 3. Only support staff are allowed to replace an empty CGC.
- The following experiments require specific safety protocols to be developed by SSRL staff; this requires at least a 1 month lead time before the beam time:
 1. Experiments using CGC directly purchased and shipped by the user.
 2. Experiments involving flammable, toxic or corrosive gases (e.g. O₂, CO).
 3. Experiments involving gases conducted in the cold room or other confined space.

For case 2 and 3, complete the Hazards Form available from the User Research Administration Database¹⁹ is required for cases 2 and 3.

- Xenon and Krypton CGC are supplied by the SSRL SMB group. Other gases may be purchased through SSRL by filling the specialty gas purchase form²⁰. Setting up an SSRL account²¹ is required for purchasing items through the SSRL.

¹⁷<http://smb.slac.stanford.edu/forms/beamtime/>

¹⁸<http://smb.slac.stanford.edu/facilities/hardware/pressurecell>

¹⁹<http://www-ssrl.slac.stanford.edu/URAWI/>

²⁰http://www-ssrl.slac.stanford.edu/userresources/forms/form_gas.html

²¹http://www-ssrl.slac.stanford.edu/userResources/documents/user_acct.pdf

3 On-site experiments

- Please make sure that you complete all required training on-line before arriving at SSRL to expedite check-in and badging. If you have a valid user badge, you can proceed straight to SSRL.
- If you shipped frozen samples, the dewar should be at the beamline. If it is not present, check the dewar receiving area downstairs in building 120.
- Incubators with adjustable temperature are available at each beamline to store samples; there are also cold rooms (4 C) in the sample preparation lab. You can store pre-frozen samples in the storage dewar provided at the beamline.

Note: The sample storage facilities at the beamline are meant to be used only for the duration of the experiment; if you require longer term storage facilities please contact the user support staff. Samples left at the beamlines may be accidentally destroyed or disposed of.

- When you arrive at the beamline call your assigned support scientist. The name and contact information will be written on the beamline white board. After you have contacted the support person, you may call the duty operator: Either call the extension number **4040** or dial 161 to access the intercom system and ask the duty operator to call the beamline extension number (written on the beamline phone). Repeat the message twice.
- When you arrive, the hutch door will usually be **open**. Do not enter the hutch if the door is closed without first contacting support staff.
- Log in to one of the terminals at the beamline using your SSRL Unix account and password. If the terminal is locked by another user, you can switch to your account or log out the previous one by simultaneously typing the keys “Ctrl-Shift-Backspace”. You may not be able to start Blu-Ice until staff enables you to use the beamline.

Note: The terminals at the beamline are used primarily to run the beamline control software. Although you have access to the data disks where your images are stored, you will only be able to run data processing software by logging in to the servers dedicated to that purpose from the terminal. Read about data processing (section 6.3).

- Contact your support person if you experience difficulties logging in. For information on the beamline computer environment consult the document at <http://smb.slac.stanford.edu/facilities/computing>.
- Read the beamline policy documents (section 2), paying particular attention to the safety procedures that apply to your experiment.
- You can find additional documentation about the available user facilities at: <http://smb.slac.stanford.edu/facilities>

3.1 On-site safety protocols

As per beamline policy (section 2), it is your responsibility to know and follow all safety protocols applicable to your experiment, samples and equipment you bring to the SSRL. Please note the SSRL has policies concerning the use and disposal of propane and ethane (section 2.7.2), liquid nitrogen (section 2.7.3), heavy metals (section 2.7.1) and compressed gases (section 2.7.7). Please follow all the applicable rules.

3.2 Safety in the experimental hutch

To minimize the possibility of accidents while working inside the hutch, there are restrictions on the motors that can be moved from certain locations.

- When the hutch door is open, you must use the computer monitor in the hutch to move the detector or other motors.
- Once the hutch is searched and locked, motors can be moved from the consoles outside the hutch.
- Motor movements can be immediately stopped by pressing any of the large yellow emergency buttons (fig.1). The motor reset button (fig.2) must be pushed to reactivate the motors.

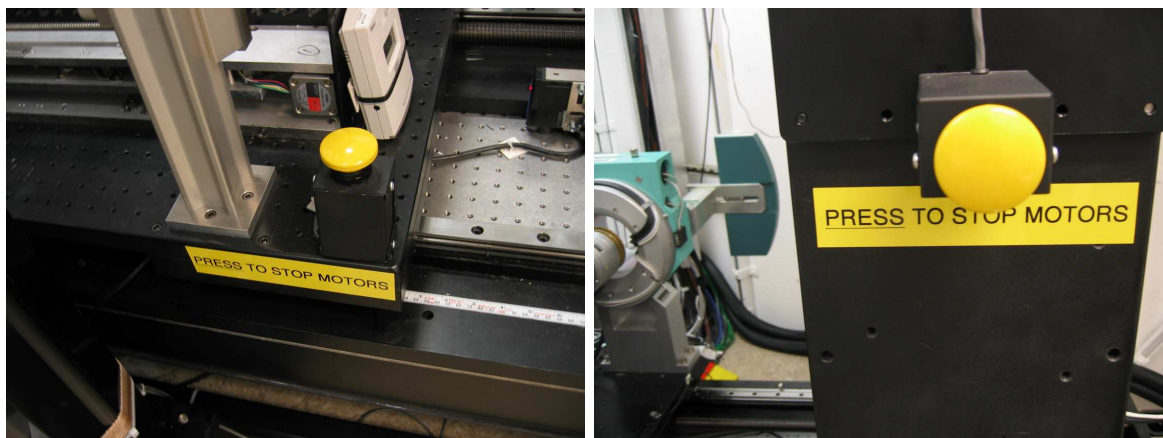


Figure 1: Emergency buttons

Note: If the Blu-Ice status window displays the messages "MOTOR STOP BUTTON LATCHED", an emergency stop has been activated. Press the green reset button to re-enable motor moves.

The hutches are equipped with oxygen deficiency sensors. An alarm will go off if the oxygen content in the hutch falls below 19.5%. This may happen while the nitrogen dewar is being refilled. **Do not enter the hutch or open the door if the oxygen alarm is sounding!** If the duty operator arrives to investigate the oxygen alarm, explain that the alarm will be automatically reset once the dewar has been filled. If the alarm does not subside within a few minutes after the nitrogen filling is finished, call support staff.



Figure 2: Motor reset button, located in the hutch near the door

3.3 Mounting samples

The following instructions describe how to mount samples manually. For automated sample mounting, please refer to the SAM robot use instructions (section 5).

3.4 Making room to safely mount samples

- An X-terminal monitor is available inside the hutch. You can log in at this terminal and start Blu-Ice (see the Blu-Ice documentation²²). To be able to mount samples comfortably, the detector should be moved to about 400-500 mm and the beamstop to 40 mm.
- On BL12-2, make sure that you retract the backlight screen for the On-Axis camera (from the Sample Video interface²³ in Blu-Ice)

3.5 Mounting samples in the cryostream.

- The 4LD filling dewar (fig.3) can be used for dispensing liquid nitrogen. It can be filled up at a filling station. The main filling station is located in building 120 near beamline 9; other filling dewars are available in different locations of the experimental floor.
- Cryo-tools are available on the tool board²⁴.
- A microscope (fig.4) is available for mounting crystals and flash-cooling directly in the cold stream. The microscope can be placed on a small table on top of the dewar inside the hutch. The table (usually located inside the experiment hutch) can be installed as shown below (fig.5). Use the plastic step stored inside the experiment hutch to reach the microscope table.

²²<http://smb.slac.stanford.edu/facilities/software/blu-ice/>

²³http://smb.slac.stanford.edu/facilities/software/blu-ice/sample_tab.html#Sample_Lighting

²⁴<http://smb.slac.stanford.edu/facilities/hardware/other/toolboard.html>



Figure 3: Dewars available at the beamline. The two white Nalgene dewars can be used for short term cassette, pucks or samples storage and transport; the 4LD dewar can be used for dispensing liquid nitrogen.



Figure 4: Beamline microscope

- Roughly align the goniometer for your samples by mounting an empty pin of the same length on the goniometer head and centering it as described in the Blu-Ice documentation²⁵.

²⁵http://smb.slac.stanford.edu/facilities/software/blu-ice/hutch_tab.html

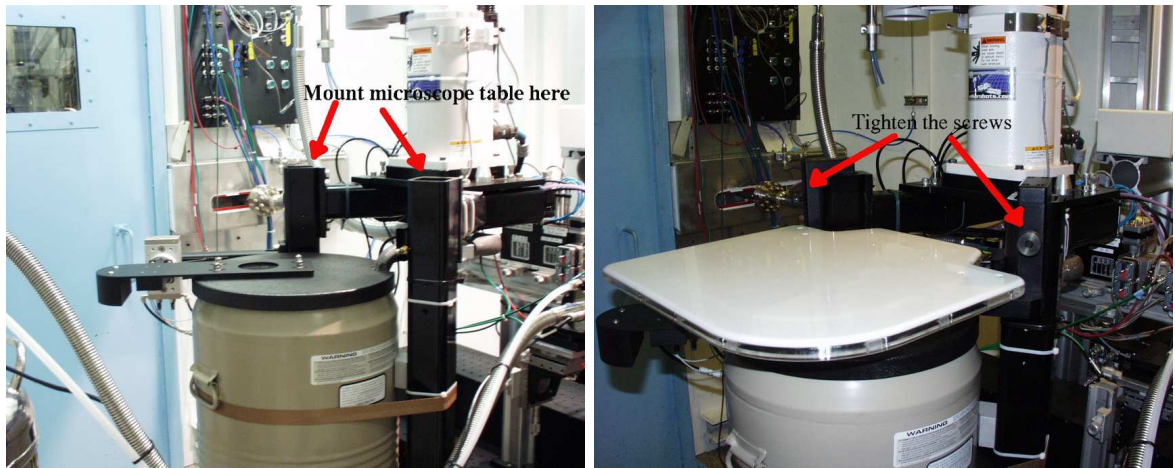


Figure 5: Mounting the microscope table in the experiment hutch

- Verify that you can easily insert the tongs to mount or remove the sample pin.
- Check that the centered loop is in the center of the cryostream. If it is not, contact staff.
- Check that the temperature of the cryo-cooler, displayed on the cryo-controller (fig.6), is about 100 K. Occasionally the temperature may be a few degrees higher. This means that the unit will soon need maintenance, but it should not affect the sample. Do not try to adjust the flow rates as this can cause icing.



Figure 6: Cryo-controller

- If the crystal has snow flakes, it can be cleaned by pouring a small amount of liquid nitrogen (use a cryo-vial) over it. Protect the sample camera (fig.7) by covering it with a clean light

object (for example, the light blue foam covers for the small dewars²⁶).

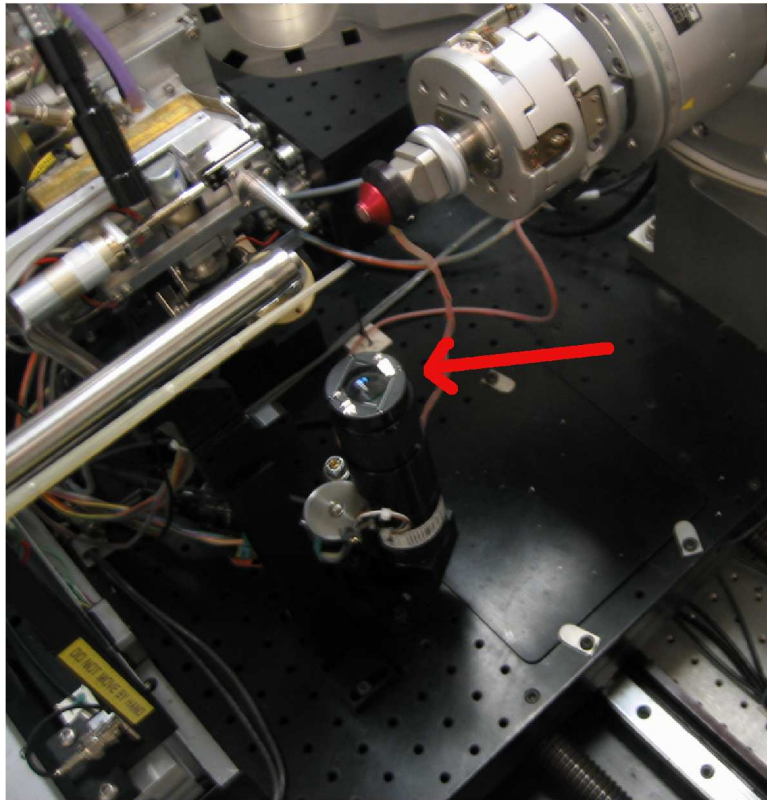


Figure 7: The sample camera

3.6 Mounting crystals at room temperature

The Oxford cryostream can be used to collect data at temperatures other than 100 K. The temperature can be changed at the cryostream controller (fig.6) outside the hutch (**you need to ask the support staff to enable manual control of the cryojet before being able to do this**):

- On the left hand side of the controller, under the temperature display, there is a black button labeled "set". Pressing this button will display the set temperature. Pressing the "raise" red button to the right of the display while pressing the "set" button will increase the set temperature.
- Once the set temperature has reached the desired value, release the buttons. Monitor the temperature display to find out when the system reaches the new set temperature.
- In some cases you might have to adjust the translation of the cold stream nozzle to accommodate the capillary or plastic sleeve. Please contact staff in this case.

²⁶http://smb.slac.stanford.edu/facilities/hardware/other/beamline_toolboard/bin21.htm

3.7 Sample illumination

In the case that the overhead lamp does not provide adequate illumination, the side light source can be used to better view the sample. Use the Blu-Ice interface²⁷ to turn the light on and adjust the intensity of the light until you obtain a clear view of the sample. When using automated centering of the loop, the software will temporarily turn off the side light source. The software will also turn off the light if the software has been idle for some time.

On BL12-2, make sure to insert the backlight screen to be able to see small samples using the on-axis camera.

On some beamlines, a Visex microscope²⁸ is available to detect the crystal. The alternative, is to use low dose X-ray rastering²⁹ to find the sample.

3.8 Checking out

- Make sure that all data are backed up or transferred to your home computer. Read the instructions for using the available backup facilities³⁰.
- Return all the items you have borrowed to the toolboard; if the small open dewars still contain liquid nitrogen you may leave them to empty and dry off on the table (as far away from the edge as possible).
- Throw away all garbage. Recycle white paper and printouts in the blue recycling bins near the beamline printer. Put used sharps and glass in the sharps boxes provided. Disposal of hazardous material should be arranged with the safety officer (Matt Padilla, ext. 3861).
- Log in to the User Research Administration Database³¹ and fill out the end of run summary. Be as specific as possible when reporting problems at the beamline. For problems requiring prompt attention, you can also send an e-mail to your support staff contact person.
- If you finish, or know in advance that you will finish your experiment more than two hours early, call your contact person on the beamline phone or, outside normal working hours, send them an e-mail.
- Users may leave an experiment running at the end of their run, as long as it finishes before 11 am. Leave a note or e-mail support staff and leave a phone number where you can be reached. Experiments running unattended after 11 am may be stopped, unless previously arranged with support staff.

²⁷http://smb.slac.stanford.edu/facilities/software/blu-ice/sample_tab.html#Sample_Lighting

²⁸<http://smb.slac.stanford.edu/news/Visex.pdf>

²⁹http://smb.slac.stanford.edu/facilities/software/blu-ice/raster_tab.html

³⁰<http://smb.slac.stanford.edu/facilities/computing/backup.html>

³¹<http://www-ssrl.slac.stanford.edu/URAWI/Login.html>

4 Remote experiments

Complete protein crystallography experiments can be carried out from remote locations using remote access applications³². Users wishing to collect data remotely should indicate this on their beamtime request form.

Remote users have access to all experimental facilities, except when they require on-site preparation.

4.1 New remote users

New remote users who have never collected data at SSRL before should get training³³ on the beamline control software and other users facility, unless they can get assistance or instructions from other experienced members of the group. Hands on training can be done at SSRL. Training sessions can be arranged on a need to basis by contacting user administration.

New users who are unable to get hands-on training session at SSRL or at their home institution must schedule a remote training session a few weeks or as soon as possible in advance of their beamtime. It is advised to review this guide, and, where relevant, the video tutorials.

4.2 Preparing a remote experiment

- Determine the beamline contact person for your experiment by consulting the support staff schedule³⁴. Staff contact information is available by clicking on the name of the support person in the **Support Staff** list or by using the support staff web page. Contact the assigned support person before the experiment to provide your contact information during the experiment (preferably a cell phone).
- Download and install the free NX client software as explained in the remote Unix desktop documentation³⁵ or the corresponding video tutorial³⁶. Verify that the NX client can be used to successfully access the SMB computers before the experiment. The user passwords at SSRL expire every six months. If the authentication fails, contact staff so that your password can be reset.

Frequent users should set up e-mail notifications³⁷ and change their password³⁸ before it expires.

Important: Please check the NoMachine site³⁹ periodically (e.g., every few months) for updated versions of the NX client.

- Infrequent remote users are encouraged to arrange a practice run with the beamline simulator in advance of their scheduled beam time.

³²http://smb.slac.stanford.edu/facilities/remote_access/

³³<http://smb.slac.stanford.edu/news/workshops>

³⁴http://smb.slac.stanford.edu/schedule/sch_staff.cgi

³⁵http://smb.slac.stanford.edu/facilities/remote_access/remote_desktop/

³⁶http://smb.slac.stanford.edu/users_guide/tutorials/nxclient-v4.swf

- Carefully read the instructions for preparing and shipping samples to SSRL (section 5). Pay special attention to the following:
 - Only use sample pins that are compatible with the SSRL robot (SAM).
 - Know the safety practices at SSRL for handling liquid nitrogen.
 - Replace the liquid nitrogen in the loading dewar as soon as any ice forms.
- Download an Excel spreadsheet template⁴⁰ for the SSRL cassette or the Unipuck. Fill out a separate spreadsheet for each cassette or adapter (four pucks); see the SAM documentation (section 5.5). Enter the cassette or puck pin number (engraved in the top of the container) in the Excel file **ContainerID** column for proper identification by the user support staff.
- Once the spreadsheet is filled in, upload it into the Sample database⁴¹; for help uploading the spreadsheet see the SAM documentation (section 5.5.1), or the video tutorial^{42,43}. When your beam time begins the excel spreadsheet information can be assigned to a particular beamline and cassette location inside the dewar; see the SAM documentation (section 5.5.2).
- Dewars should arrive at SSRL **1 working day in advance** of beam time; *e.g.*, if the beamtime starts on Monday, the dewar should be shipped so that it arrives on Friday the previous week. **Dewars are not delivered to SLAC during the weekend or on holidays!**

Use the "Shipping Dewars to SSRL form"⁴⁴. The tracking number must be included in the form. Also, make sure that you specify that you are doing a remote experiment by clicking on the corresponding check box and fill up the requested information for return of the dewar.
- After submitting this form, a PDF file will be created that includes your shipping labels and return shipping forms. Save this file, print these forms, attach the shipping labels to each dewar container and insert the return shipping forms inside each container.

Important: Staff will not be able to send your dewar back unless you send the return form!

4.3 Remote data collection

- Remote experiments are scheduled to start at the normal time (3 PM). However, support staff may contact you to start earlier in the day (as early as 11 AM). Please review the experiment policies (section 2.1) that apply to remote access beamtime.
- Support staff will mount your cassette(s), search and lock the experimental hutch, probe cassettes for jammed pins and enable access to Blu-Ice.
- Support staff will then contact you and inform you of the following:

⁴⁰<http://smb.slac.stanford.edu/templates/spreadsheets>

⁴¹<http://smb.slac.stanford.edu/crystal-server>

⁴²http://smb.slac.stanford.edu/users_guide/tutorials/upload_spreadsheet_hotspot2.swf

⁴³If you are using an Excel spreadsheet lacking the ContainerID column, enter the correct cassette pin in the input box in the uploading web page.

⁴⁴<http://smb.slac.stanford.edu/forms/shipping/DewarToSSRL.html>

- Cassette locations (left, middle or right position) in the dewar; you can then assign the excel spreadsheet(s) to the correct location in the dewar from the Sample Database or Blu-Ice as described in the SAM system documentation (section 5.5.2).
 - Pin locations that *might* cause a port jam (when a real port jam is detected during the probing, the corresponding cassette port is automatically disabled).
 - When you can start your experiment.
- Data collection and data processing are carried out in exactly the same way as on-site; see the data collection and processing section (section 6).
 - The beamline can be monitored using the standard video feeds in Blu-Ice or Web-Ice.
 - When you finish your experiment, contact the beamline support staff (use e-mail between 9 pm and 9 am); once staff have been notified, they will put your cassette(s) back into the shipping dewar, attach the return form (supplied by you) and arrange for its shipment. Note that your dewar cannot be shipped unless you provide a return shipping form.
 - Log in to the User Research Administration Database⁴⁵ and fill out the end of run summary.
 - **If you wish to store your dewar at SSRL between experiments**, make sure that you inform the user support person. It is important that you also notify the support person for your next experiment, who may not be the same one as for your current experiment, even if using the same beamline. Please check the Support Schedule.

Note: At the moment users' sample containers may only be stored in the dewar they were shipped, with accompanying paperwork. We are unable to return the dewar and provide our own storage for the cassette or Uni-Puck.

4.4 Remote experiment support

For questions or problems that develop during the experiment, please consult the online documentation or the FAQ (section 8) before contacting support staff. Use the beamline cell phones⁴⁶ or weekend phone to reach staff during call hours (see below), e-mail at any other time.

Information about the beam status is available through the SSRL Web site⁴⁷. You can also call the number (650) 926-BEAM (2326).

Support staff will be on call from 9 am to 9 pm. If the system should stop for some reason after 9 pm, please send an e-mail to the assigned user support staff and the problem will be addressed in the morning. If beam time should be lost, additional time may be assigned at the end of the normally scheduled beam time (11 AM - 3 PM).

Currently the following are **not** supported:

⁴⁵<http://www-ssrl.slac.stanford.edu/URAWI/Login.html>

⁴⁶<http://smb.slac.stanford.edu/staff/beamline-phones.html>

⁴⁷http://www-ssrl.slac.stanford.edu/talk_display.html

- Exchanging cassettes or Unipucks once the beamtime has started. However, staff will exchange the sample containers if the users receive more than one day (three shifts) of beamtime. In this case the exchange will usually take place between 11:00 and 15:00 Pacific time or other convenient time for staff.

The robot dewar holds up to three cassettes or Unipuck adaptors (holding 4 pucks each), therefore up to three cassettes or 12 Unipucks per day of beamtime are allowed. Additional containers may be stored at SSRL for use on future beamtime.

- Manual sample mounting; Automated sample mounting with the SAM robot is required for remote access.
- The use of a Kappa offset (currently incompatible with automated sample mounting).
- Derivatizing samples with the SSRL Xe/Kr pressure cell.
- Tape or FireWire backups. See other options available in the computing resources documentation⁴⁸.
- GL dependent graphics programs such as **O** and **COOT** are now supported by the NX client, but the graphics applications may be slow.

5 Using the SSRL Automated Mounting (SAM) system

5.1 Overview

SAM is a completely integrated hardware and software system for mounting and dismounting pre-frozen protein crystals and screening samples for x-ray diffraction quality in a fully automated or semi-automated fashion. SAM is installed on all of the SSRL macromolecular crystallography beam lines and is seamlessly integrated into the beamline control and data analysis software. Since upgrading the robot system in 2014, the screening sequence (comprising crystal mounting, automatic sample loop centering in the x-ray beam, video and diffraction image acquisition at 0 and 90 degrees, and dismounting) may take less than one minute per crystal. The diffraction images are analyzed and autoindexed on the fly.

Samples may be stored in either SSRL cassettes or Uni-Pucks (fig.8) for use with SAM. If you are scheduled beam time with use of the robot sample mounting system, a cassette kit will be lent to you.

The following sections describe how to prepare your crystals for data collection using the SSRL robotic sample mounting system. Follow these instructions to prepare the sample pins, mount them in an SSRL cassette or Uni-Puck and ship them to the SSRL.

For additional information on the SAM system, see also the guide to automated sample screening (section 6.1). There are also video tutorials⁴⁹ illustrating the sample preparation, sample container loading using SSRL cassettes, and Unipucks and use of the SAM system for data collection.

⁴⁸<http://smb.slac.stanford.edu/facilities/computing/backup.html>

⁴⁹http://smb.slac.stanford.edu/users_guide/tutorials

For information about the cassette kit tools, including drawings and vendor information, see the SAM hardware web pages⁵⁰

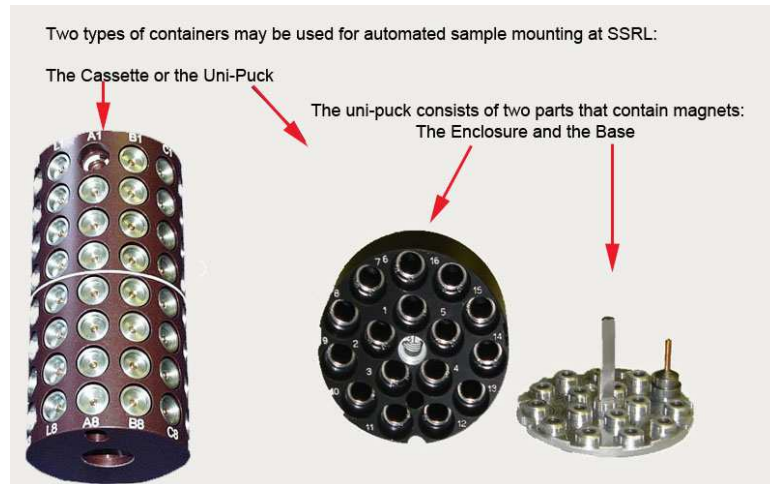


Figure 8: The two types of container for automated sample mounting at SSRL

5.2 Sample pin selection and preparation

To have a successful experiment proper sample pin preparation is essential. The majority of problems we have observed with SAM have been related to use of improper sample pins. To avoid these problems please read the following directions.

5.2.1 Allowed types of pins

The SSRL system supports only Hampton-style CrystalCap Copper Magnetic pins or CrystalCap Magnetic pins. The allowed pins sizes (fig.9) are 16 or 18 mm. The 18 mm size copper pin is preferred.

Compatible Hampton-style sample pins (fig.10) may be purchased from a number of vendors including Hampton Research⁵¹, MiTeGen⁵², Crystal Positioning Systems⁵³ or Molecular Dimensions⁵⁴.

If sample pins are purchased from MiTeGen, use the B1, B1A or B3S with the 18mm MicroMounts, MicroLoops, MicroMeshes or MicroGrippers. The B1, B1A, B3A and B3 bases can also be used with 19 mm mounts. The older B2 base can be used with 11 mm Micromounts and 10 mm nylon loop mounts. Please note that the SSRL loop-centering routine, based on visual analysis, is not yet optimized for use with the MiTeGen MicroMount, these mounts, as well as the loops are well suited for

⁵⁰<http://smb.slac.stanford.edu/facilities/hardware/SAM/>

⁵¹<http://www.hamptonresearch.com>

⁵²<http://www.mitegen.com/>

⁵³<http://www.crystalpositioningsystems.com/>

⁵⁴<http://www.moleculardimensions.com>

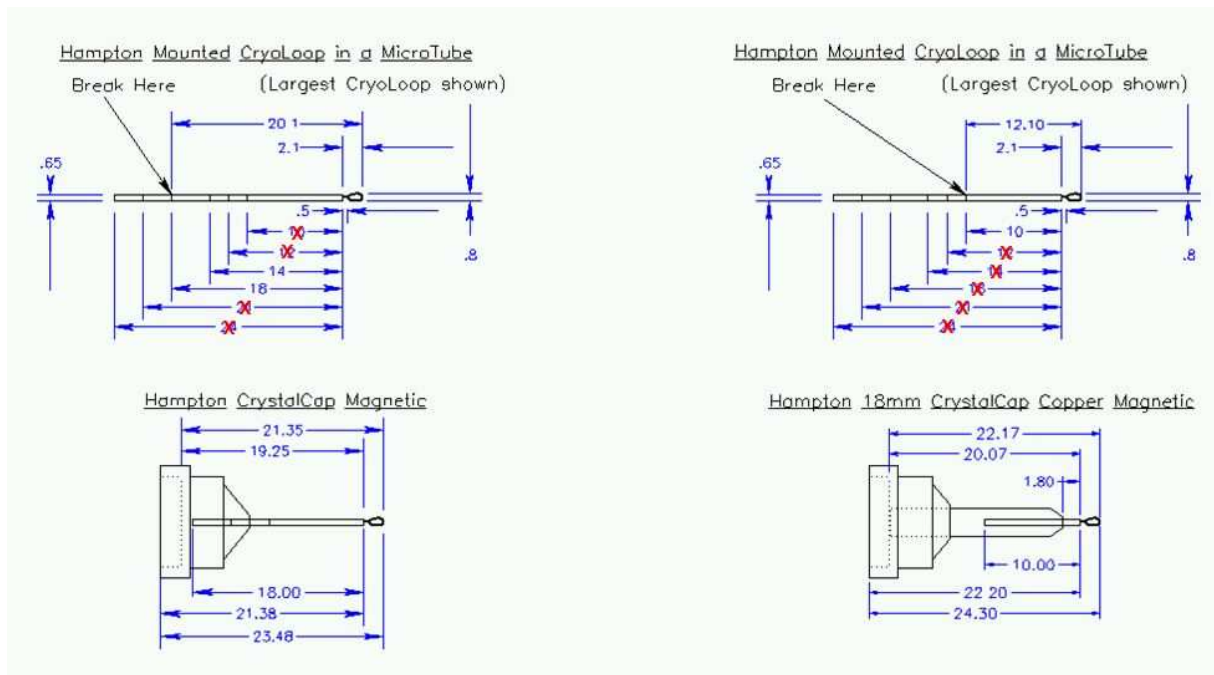


Figure 9: Preferred size of pin. Hampton cryo-loops should be cut at the segment closest to the loop for use with the CrystalCap Copper pin bases, provided with the cassette kits. **Do not exceed the maximum MicroTube length shown in the diagram!** Unacceptable lengths are marked with a red x. The 11 mm MiTeGen Micromounts and meshes should be used with the CrystalCap Copper pin base; longer Micromounts will need to be cut to the proper length before inserting into this pin base.

rastering⁵⁵. Molecular Dimension ActiLoops⁵⁶ and LithoLoops⁵⁷ are also compatible with the robot, if mounted on a solid (non CryoCap) base. **SPINE-standard pins can not be used with SAM.**

We recommend using Hampton-style Copper Magnetic pins because no laser etched lines are exposed. These are the pins supplied with the cassette kit. The microtubes used with Magnetic pins can sometimes break off if bumped (fig.11).

5.2.2 Pin preparation

Microtubes should be affixed inside sample pins using epoxy. Any epoxy with a curing time between 5 minutes and 24 hours should work well for this purpose. We have found that superglue and superglue gel is less reliable than epoxy for affixing microtubes. A number of failures with the SAM system have been attributed to using other types of adhesives on sample pins such as wax, nail polish, and Duco cement. These should not be used (fig.12).

⁵⁵http://smb.slac.stanford.edu/facilities/software/blu-ice/raster_tab.html

⁵⁶<http://www.moleculardimensions.com/products/c455-Mounted-ActiLoops/>

⁵⁷<http://www.moleculardimensions.com/products/c432-Mounted-LithoLoops/>



Figure 10: Commonly available pin types



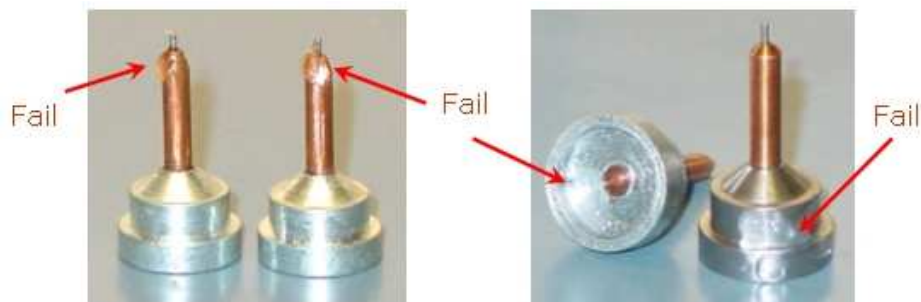
Figure 11: Broken microtubes used with Magnetic pins can make the pin jam inside the cassette port, stopping the robot.

If an adhesive other than Epoxy has been used to affix the microtubes to your sample pins, please let your user-support person know in advance of using them. These pins may cause problems with SAM.

Be careful not to use pins with excess epoxy on the pin base or postpin-excess and do not get grease or excess cryo-protectant on the pin body. At liquid nitrogen temperatures, grease from crystallization trays gets rock hard. If you continually reuse your pins, please also inspect them for corrosion and loose microtubes. Anything that changes the outside form factor of the sample pin could cause the pin not to fit properly in the SAM robot tongs.



Figure 12: Use Epoxy to affix the microtubes to the sample pins. Glues other than Epoxy have been observed to cause sample mounting errors.



Warning: Do not use pins with excess epoxy on the copper post.

If you would like to mark your sample pins different colors, use permanent marker for this purpose. Paint or nail polish should not be used as this can change the form-factor of the pin or be sticky. Hampton Research now sells a new pre-assembled Copper Magnetic sample pin (catalog number HR5-112) which is already color coded according to the size of the nylon loop attached. It also has an alpha numeric code and bar code.

5.2.3 Pin testing

All pins must be tested to ensure they fall within the allowed tolerances. We have found some irregular pins that if used would damage the SSRL sample mounting system. To test your pins, place them on the end of the Pin Tester magnetic tool (fig.13) on the red line. Only use pins that completely cover the red mark without forcing. Pins that fail this test should not be used!



Figure 13: Use this tool (included with the SAM kit) to test all your pins before use with the SAM robot

5.2.4 Re-using pins

Broken micro-tubes (fig.11) and torn nylon loops are usually a result of mishandling sample pins in preparation for reuse. In particular pins should not be piled together in a container when washing and drying them. To wash pins, place them individually on a magnetic tray. Hang the tray upside down to dry the pins. It is important to store your sample pins in a safe place when they are not in use. The microtube storage rack (fig.14), available through Fisher, is a useful storage location for extra pins.

5.3 Loading and shipping SSRL cassettes

5.3.1 SSRL Cassette kit

If you are scheduled beamtime with use of the robot sample mounting system, a cassette kit (fig.15) will be lent to you.

For additional information about the kit tools, including drawings and vendor information, please see the SAM hardware web pages⁵⁸.

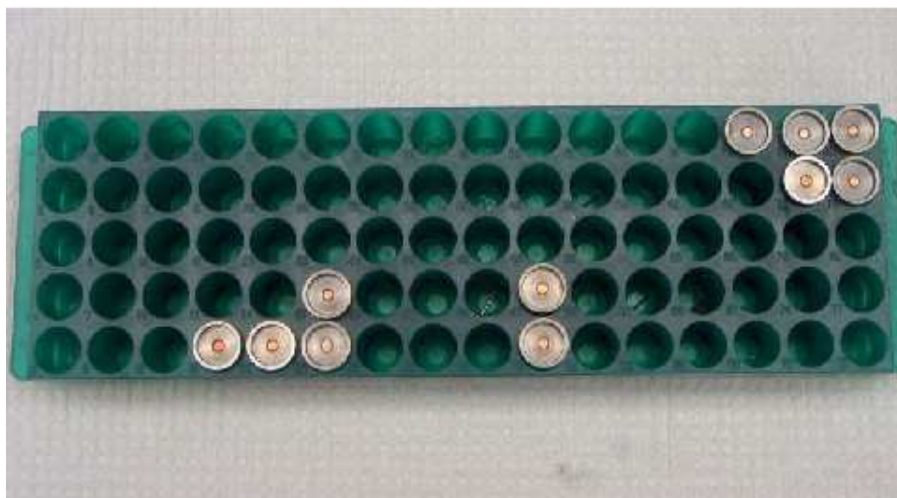


Figure 14: Storing pins for re-use.

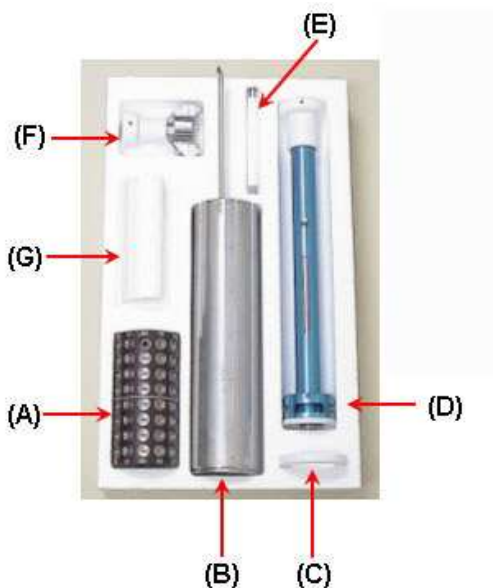


Figure 15: Cassette Kit: (A) Sample Cassette and 96 Hampton pins (microtubes and loops not included) (B) Dewar Canister - replaces stock canister in dry shipping dewars (C) Teflon Ring - to support the canister in the shipping dewar (D) Transfer Handle - for transferring cold cassettes (E) Magnet Tool - to mount pins in cassette and to the test size of pins (F) Guide Tool - to aid in mounting pins into cassettes with the magnetic tool (G) Styrofoam Spacer - to keep the cassette in place when shipping one cassette; not shown: A dewar for mounting crystals into the cassette.

The cassette contains 96 sample ports each port contains a ring magnet which holds in the sample pin. A cutaway view of the cassette is shown below (fig.16). The ring magnets are shown in green. The magnets are held in place by a polycarbonate washer shown in white. The washers are removable

so any broken ring magnets may be replaced.

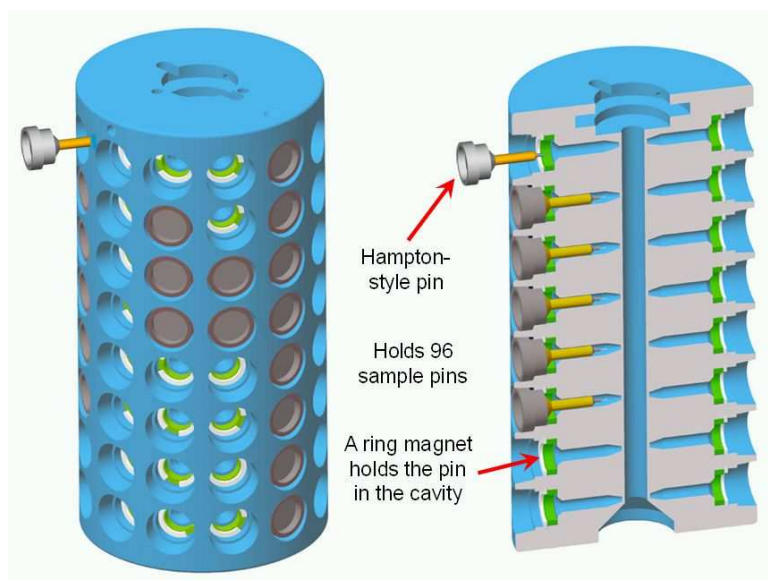


Figure 16: Cassette diagram. The ring magnets are shown in green and the washers holding the magnet are shown in white

Cassettes should not be stored where magnetic debris can get inside the ports. Before using your cassette, it should be inspected to ensure the ports are all empty.

The transfer handle is used to safely transport cold cassettes. To attach the transfer handle (D) to a sample cassette (A) (fig.17) first place the locking pins into the slots at the top of the cassette. Then push down the handle, and rotate clockwise until the handle locks in place.

The slotted guide tool (fig.18) consists of a long magnetic wand with two slots in the middle and a loading guide with a track on the handle that accommodates the slots on the wand tool. To flash freeze samples, the wand may be inserted into the top of the loading guide. To transfer pre-frozen samples it is important to keep the samples at liquid nitrogen temperatures during transport into the cassette. The wand should be inserted from the side of the slotted guide tool to keep the sample under liquid nitrogen during transfer. Use the slot closest to the red (or maroon) side of wand (weak magnet) for loading samples.

When unloading samples the wand is turned around and the opposite slot and stronger magnet is used.

The circular cutout in the loading dewar may be used to hold samples in cryo-vials or pucks for transfer.

5.3.2 Avoiding ice

The most common problems we see observe with cassette loading is the accumulation of ice in the liquid nitrogen bath. Ice will stick to your sample as it is transferred through the liquid nitrogen on

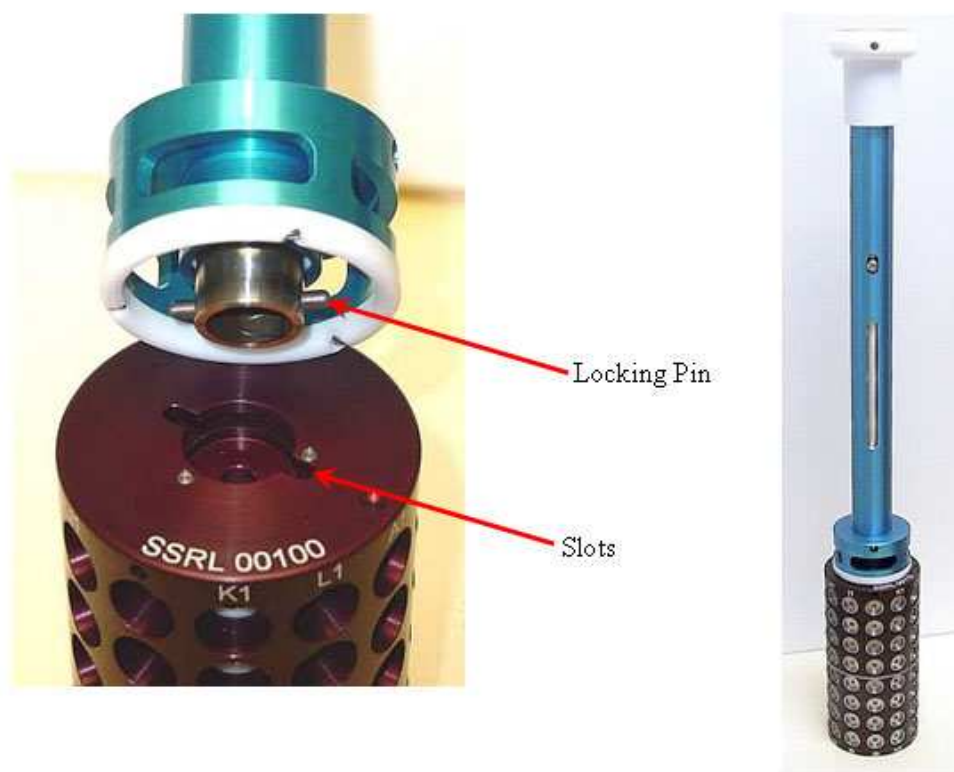


Figure 17: Attaching transfer handle to cassette

the way to the cassette port. It can also fall inside the cassette ports. If excess ice is observed in the bath, the cassette should be stored in a cold dry-shipping dewar and the loading dewar emptied, dried and refilled with liquid nitrogen before proceeding.

Important: To prevent ice from accumulating in the liquid nitrogen, the cassette should not be stored in the loading dewar for more than **20 minutes** without exchanging the liquid nitrogen.

To avoid ice accumulation, it may be useful to load cassettes under a fume hood or a dry box (as an example, see a description of Nham Nguyen's setup at http://smb.slac.stanford.edu/facilities/hardware/cassette_kit/Ice_free_Nham.pdf). If not using a fume hood or a dry box, cover the dewar with the lid when you are not mounting crystals. To prevent ice from falling into empty cassette ports, some users advise filling empty ports with blank pins. Each blank pin is removed just before inserting a sample pin into the port.

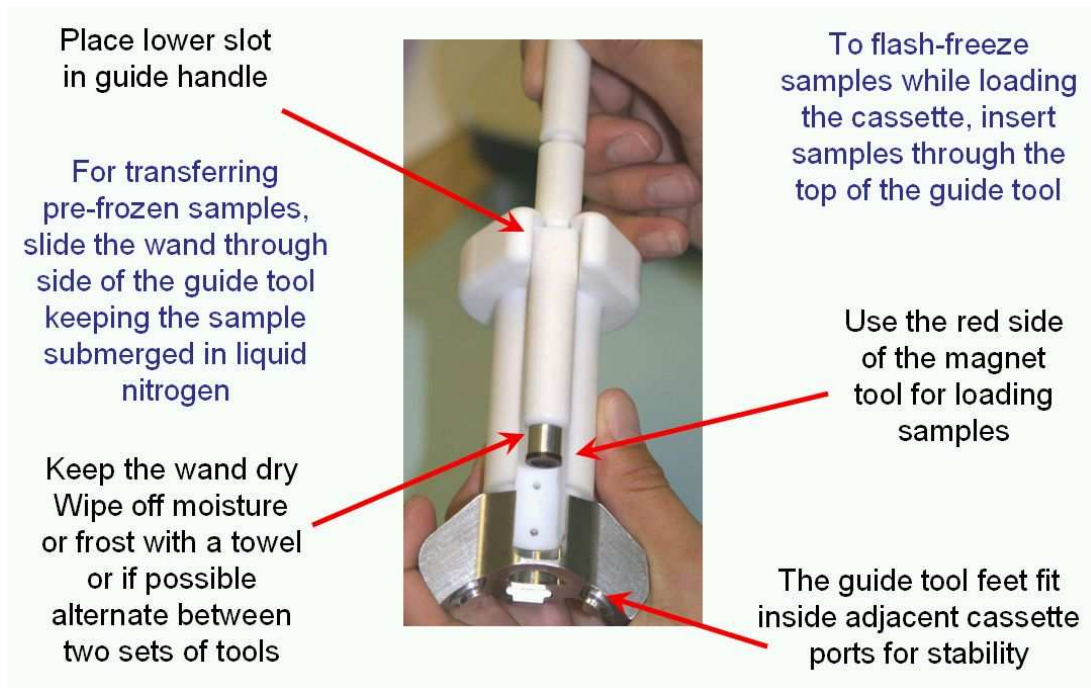


Figure 18: Guide tool

5.3.3 Loading samples in the SSRL cassette

Important: Please read the safety notes (section 2.7.3) before working with liquid nitrogen and make sure you are familiar with the liquid nitrogen safety procedures at your institution.

1. The custom foam dewar should be filled up to the internal indicator ledge (fig.19.) This takes about 4 Liters of liquid nitrogen; it will be necessary to top off the liquid nitrogen level after inserting a warm cassette.
2. Once the dewar is filled and equilibrated, place the cassette in the dewar and tilt the transfer handle until it rests inside the side notch. Then push the cassette forward (fig.20a) until the bottom of the cassette is touching the edge of the dewar.
3. The guide tool may be pre-cooled on the side dewar shelf (fig.20b). To prevent condensation on the metal portions of the guide, it may be stored on the shelf between loading samples.
4. Rotate the sample cassette with the transfer handle to access the desired cassette port.
5. Place the guide tool (F) on the cassette (fig.21a) centered on the port.
6. To flash freeze a sample:
 - (a) Put a pin onto the red side of the magnet tool (E) and pick up a crystal (fig.21b,c).



Figure 19: Filling the custom foam dewar

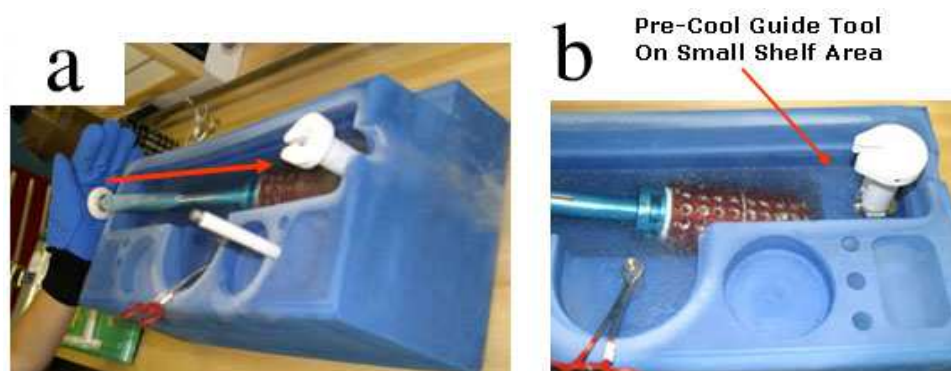


Figure 20: **a.** Placing the cassette in the foam dewar. **b.** Storing the guide tool.

- (b) Flash-freeze the crystal (fig.22) by placing the pin through the handle of guide tool and into the cassette port, minimizing the time the crystal is in the air.

7. To transfer a pre-frozen sample from a vial (or puck):

- (a) Tilt the vial and use the red side of the magnetic wand tool to remove the sample pin (fig.23b,c). Be careful to keep the sample under liquid nitrogen at all times. (Vials or pucks may be placed in the round cutout inside the dewar.)
- (b) Slide the lower slot of the magnetic wand tool into the guide tool (fig.24) while keeping the sample under liquid nitrogen.
- (c) Once the magnetic wand tool is in the center of the guide tool, the wand tool may be pressed against the back of the guide and pushed downward placing the pin into the port. (It is sometimes helpful to rotate the wand while it is pushed down to break any ice that may have formed between the magnetic wand tool and sample pin.) The magnetic tool may then be pulled up, removed and the next sample transferred.

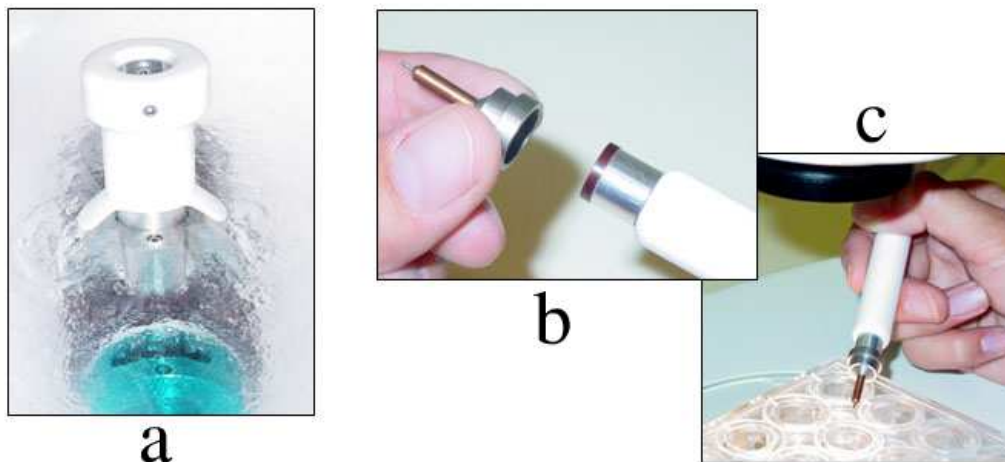


Figure 21: **a.** Placing the guide tool into the cassette. **b.** Putting the pin on magnet tool. **c.** Picking the crystal.



Figure 22: Flash freezing the crystal

As you fill the cassette we recommend you record the location of each crystal as described in the sample Excel spreadsheet documentation (section 5.5). A separate file should be created for each cassette.

5.3.4 Preparing cassettes for shipment

Important: It is strongly recommended to test the shipping dewar⁵⁹ prior to shipping samples to make sure that the samples will be kept cold before arrival.

- Prepare the dewar for shipping⁶⁰; place the Teflon support ring (C) inside the shipping dewar

⁶⁰http://smb.slac.stanford.edu/facilities/hardware/cassette_kit/New_Dewar_Preparation.pdf

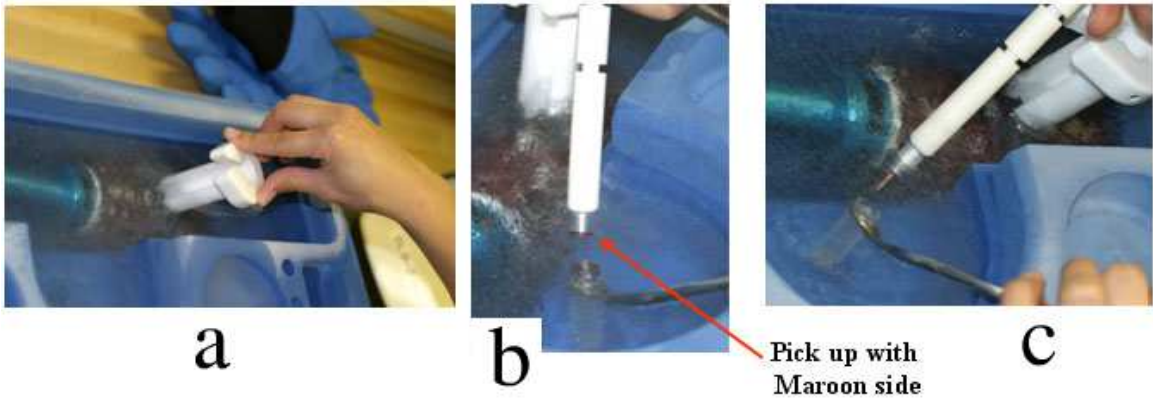


Figure 23: **a.** Placing the guide on the cassette. **b.** Picking the pin. **c.** Transferring the pin

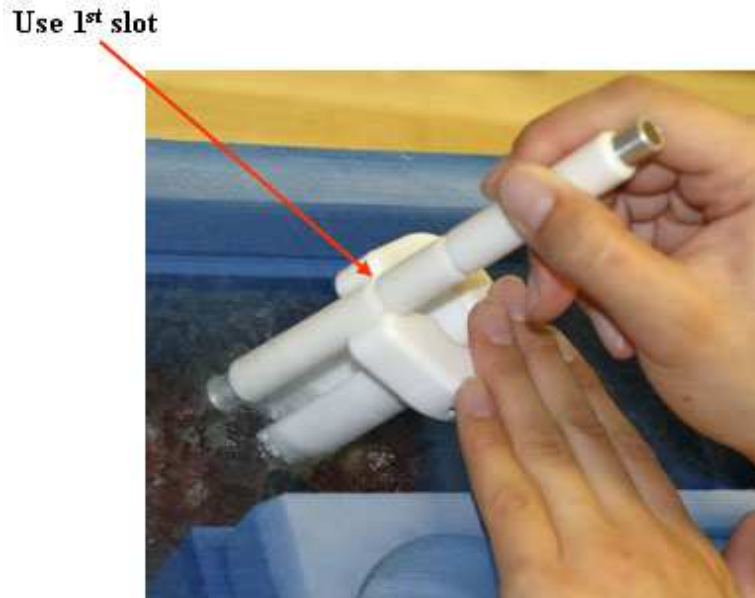


Figure 24: Using the guide slot under liquid nitrogen.

(fig.25) before inserting the canister (G) and fill the dewar with liquid nitrogen in the usual manner.

- Transfer the cassette into the canister in the dewar, minimizing the time the cassette is in the air. Remove the transfer handle from the cassette by pushing down on handle and turning counter-clockwise to release it.
- Two cassettes may be shipped inside one canister. When shipping just one cassette, place the Styrofoam spacer (F) on top of the cassette to keep cassette in place during shipment.
- The cassettes are compatible with most dry shipping dewars. We recommend using the combination of a MVE model SC4/2V, Taylor Wharton CX100 or CXR100R cryogenic shipping dewar



Figure 25: Placing the Teflon support ring (C) inside the shipping dewar

with a Taylor Wharton TAY CX10-8C00 dewar container.



Styrofoam spacer

For information on how to ship dewars to and from SSRL see the User Shipments web page⁶¹.

5.4 Loading and shipping Uni-Pucks

For information about loading and shipping samples in the Uni-Puck see:

http://smb.slac.stanford.edu/facilities/hardware/cryotools/Uni-puck/Uni-puck_Directions.pdf

Important: It is strongly recommended to test the shipping dewar prior to shipping samples to make sure that the samples will be kept cold before arrival.

⁶¹<http://smb.slac.stanford.edu/forms/shipping/DewarToSSRL.html>

5.5 Storing sample information: The Excel spreadsheet

Sample information is entered into a Excel spreadsheet file. The spreadsheet has a specific format for use with the SSRL Sample Database (fig.26) and data collection applications.

The following instructions explain how to download, fill up and upload a spreadsheet. An on-line video tutorial is also available.

- You can download a spreadsheet template⁶² for the SSRL cassette or the Uni-puck adapter (holding up to four pucks).
If your browser cannot display Excel spreadsheets, click the **Save to file** button in the pop-up menu; navigate in the dialog box to the directory of choice and click **Save**.
- Type in the information for the samples in the cassette. **Important:** Make sure that "text" format is used.
- If you are sending more than one cassettes, use one spreadsheet per cassette. The Uni-puck spreadsheet holds information for four pucks (labeled A, B, C and D).
 - * The **ContainerID** field is used to uniquely identify the cassette. You should enter the number engraved on your cassette or puck in this column; this is useful to verify that the spreadsheet position specified in the software matches the actual position of the cassette in the robot dewar. An ever safer method to prevent against container misidentification is the use of a barcoded pin⁶³ in the A1 position of the SSRL cassette or any Uni-puck.
 - * The **Port** field indicates the position of the sample in the SSRL cassette. **ID** and **Directory** are used by the screening software to identify and store files. The other fields are not used by the system, but they are intended to help track and identify each sample.

Important: Use only alphanumeric characters with no blank spaces for the Crystal ID. Special characters, brackets and spaces are not valid in image file names and will cause the screening to stop.

- * After downloading or editing the spreadsheet, verify that it is saved as a 'Microsoft Excel Worksheet' or *.xls.

See also information on editing the spreadsheet (section 5.5.4).

- You can also download spreadsheet templates from the Sample Database interface:
<http://smb.slac.stanford.edu/crystal-server>
See log-in instructions in the following section (section 5.5.1).

5.5.1 Uploading an Excel spreadsheet

Uploading the spreadsheet from a Web browser

Once the Excel spreadsheet has been filled out it can be transferred to the Sample Database at the URL:

<http://smb.slac.stanford.edu/crystal-server/>

⁶²<http://smb.slac.stanford.edu/templates/spreadsheets>

⁶³http://smb.slac.stanford.edu/facilities/hardware/SAM/barcode_pin/

	A	B	C	D	E	F	G
1	ContainerID	Port	CrystalID	Protein	Comment	Metal	Priority
2	00196	D6	T3605	MB2347A	SAD data set	Se	1
3	00196	D5	T3604	MB2347A	MAD data set	Se	1
4	00196	D3	T3602	MB2347A			2
5	00196	D4	T3603	MB2347A			2
6	00196	C6	T3597	MB2347A			3
7	00196	C7	T3598	MB2347A			3
8	00196	C5	T3596	MB2347A			3
9	00196	C8	T3599	MB2347A			3
10	00196	C4	T3595	MB2347A			3

Figure 26: Formatted Screening System Excel Template.

- Log in to the database interface using your account name and password. You also need cookies and javascript enabled in your browser to log in and upload the spreadsheet
- Once you are logged into the system you should see the page shown below (fig.27).

The screenshot shows the 'Sample Database' interface. At the top right, it says 'You are bluser' with a 'Logout' link. Below this is a navigation bar with 'Sample Database' and 'Logout'. A menu bar contains 'Upload Spreadsheet', 'Use SSRL template', 'Use PUCK template', and 'Template: SSRL Cassette Template' with a 'Download' button. Below the menu bar is a search bar with 'Search Field: SIL ID', 'Filter 4925', and a 'Search' button. To the right of the search bar, it says 'Cassettes 1 - 1 of 1 [first | prev | next | last]'. Below the search bar is a table with columns: 'SIL ID', 'Uploaded Spreadsheet', 'Upload Time', 'Commands', and 'Beamline'. At the bottom left, there is a link 'Click here to view User's Guide.'

Figure 27: The Sample Database interface.

- Click on **Upload Spreadsheet**. You will be directed to the **Upload Excel File** page.
- Enter the name of the spreadsheet (you will need the full directory path, for example: /home/yourid/filename.xls) or click on the **Browse** button to search for the file. If you have trouble locating the file, verify that the filter for types of files is *.* or *.xls in the **Choose File** dialog box. Once the file is located in the dialog box, select **Open**.
- Enter the Cassette number in the Cassette Pin box. If the Excel file does not contain a **ContainerID**, it will be generated and assigned the value you enter. On the other hand, if the spreadsheet already contains a ContainerID, the value you enter here will be ignored. Do not modify the default **Spreadsheet name** unless you changed it in your Excel file.
- To finish uploading the file to the Database, click **Upload**. If your spreadsheet is not in a standard format (e.g., empty or duplicated **CrystalID**, no **ContainerID**) the interface will apply and display the necessary corrections in a separate page. Scroll to the bottom of this page and click on **Display Cassettes**. This will take you back to the home page, displaying the new spreadsheet entry (fig.28).
- To screen more than one cassette, click on "Create New Entry" again and upload the corresponding spreadsheet, *etc.*



Please [logout](#) if you are not bluser. [Upload Spreadsheet](#) [Use SSRL template](#) [Use PUCK template](#) Template:

Search Field: Filter: for example, 100*, 1*0 Cassettes 1 - 1 of 1 [first | prev | next | last]

SIL ID	Uploaded Spreadsheet	Upload Time	Commands			Beamline	
4925	cassette_myo_gdlyso_new.xls	2006-10-23 17:01:59.0	view/edit	Download Results	Download Original Excel	Delete	None <input type="text"/>

[Click here to view User's Guide.](#)

Figure 28: Uploaded spreadsheet information.

- Entries can be removed from the database by clicking **Delete entry**.

Important: If you do not wish to fill up the spreadsheet, it is possible to use a Default Spreadsheet (section 5.5.3).

Uploading the spreadsheet from Blu-Ice

Once you beamtime has started and you have been enabled to start the experiment, it is possible to access the Sample Database directly from the screening tab in Blu-Ice: Clicking the **Web** button will launch a web browser and you can upload the file as described in the previous section.

5.5.2 Assigning the Excel spreadsheet to a beamline

The information in the database may be assigned to a beamline once beamline access has been permitted. On the Sample Database page (fig.28), the last entry (**Beamline** column) is used to assign the Excel spreadsheet to a particular beamline or to a particular cassette location. A total of 3 cassettes can be placed in the cassette storage dewar and are labeled 'left', 'middle' and 'right'. Select the appropriate position (staff will let you know which one to use) and beamline by using the drop down menu. If the assignment fails, ask the support staff to verify that your account has beamline access.

The spreadsheet assignment can also be done directly from Blu-Ice using the **Cassette** drop-down menu.

5.5.3 Using the Default spreadsheet

Sample screening without a spreadsheet is possible, but the results of the screening will not be saved. To avoid this problem, log in to the Sample Database interface as described above and click **Use Default Spreadsheet**. Select the cassette type (SSRL or Puck adapter), type in the cassette number if known and click **Submit**. A cassette named **cassette_template.xls** will appear in your spreadsheet list. The spreadsheet can be assigned to a beamline and dewar position as usual.

5.5.4 Editing the spreadsheet

The easiest way to make extensive changes to an uploaded Excel spreadsheet is to download the file to the local computer, then edit it and upload the modified file as explained in the previous section (section 5.5.1). The link **Download Original Excel file** can be used to retrieve the original uploaded file, without any edits or results. If you wish to modify the file after screening results are available, use the **Download Results** link instead.

You can edit the Excel file on the Unix beamline computer with the OpenOffice software⁶⁴. To run OpenOffice from the beamline computers type:

```
% ooffice filename.xls
```

It is also possible to edit selected fields for a sample by clicking on **View/Edit**. This displays an HTML version of the spreadsheet (fig.29)

- Check the Port containing the sample you wish to edit
- Click on the **Edit Crystal** button on top of the spreadsheet.
- Edit the information in the field(s) of your choice
- Click on **Save changes** to save your edits or **Cancel** to reset the old values.
- Download the modified spreadsheet to the local computer by clicking on the **Download Results** link.

During the experiment, Blu-Ice and Web-Ice can also be used to edit selected fields. Consult the Blu-Ice⁶⁵ and Web-Ice⁶⁶ documentation to find out how to edit the spreadsheet on these applications.

6 Data collection and processing

The following sections describe how to carry out an experiment at from sample screening to full data collection making use of the integrated data collection and analysis environment at SSRL. For additional help setting up data collection, please consult the Blu-Ice documentation⁶⁷.

6.1 Automated crystal screening

The high throughput screening system implemented at SSRL makes it possible to automatically collect and analyze test images and fully characterize the sample in a semi- or fully automated fashion.

To set up automated crystal screening follow the steps below or watch the video tutorials "Uploading the Sample Spreadsheet"⁶⁸ and "Automated screening from Blu-Ice"⁶⁹.

⁶⁴<http://www.openoffice.org/>

⁶⁵http://smb.slac.stanford.edu/facilities/software/blu-ice/screen_tab.html

⁶⁶http://smb.slac.stanford.edu/facilities/remote_access/webice

⁶⁷<http://smb.slac.stanford.edu/facilities/software/blu-ice/>

⁶⁸http://smb.slac.stanford.edu/users_guide/tutorials/upload_spreadsheet_hotspot2.swf

⁶⁹http://smb.slac.stanford.edu/users_guide/tutorials/screening.swf

Sample Database

Cassette List → Show Sil 4925

Display Type: Image Display Type:

Row	Port	CrystalID	Protein	Images	Comment	Score	UnitCell	Mosaicity	Rmsr	BravaisLattice	Resolution	SystemWarning	Move
0	A1	CassetteID				0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
1	A2	A2	myo	A2_003.mccd A2_004.mccd	myoglobin	0.822	76.92 76.92 38.36 90.0 90.0 90.0	0.150	0.083	P4,P422	1.180		
2	A3	A3	myo	A3_001.mccd A3_002.mccd	myoglobin	0.822	76.92 76.92 38.36 90.0 90.0 90.0	0.150	0.083	P4,P422	1.180		
3	A4	A4	myo		myoglobin	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
4	A5	A5	myo		myoglobin	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
5	A6	A6	myo		myoglobin	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
6	A7	A7	myo		myoglobin	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
7	A8	A8	myo		myoglobin	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
8	B1	B1	myo		myoglobin	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
9	B2	B2	myo		myoglobin	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
10	B3	B3	myo		myoglobin	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
11	B4	B4	myo		myoglobin	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
12	B5	B5	myo		myoglobin	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
13	B6	B6	myo		myoglobin; used for 9-1	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
14	B7	B7	myo		myoglobin; used for 9-2	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		
15	B8	B8	myo		myoglobin; not good	0.000	0.0 0.0 0.0 0.0 0.0 0.0	0.000	0.000		0.000		

Figure 29: Editable HTML version of the spreadsheet.

1. Upload a spreadsheet containing sample information to <http://smb.slac.stanford.edu/crystal-server> as described in the SAM guide (section 5.5.1) . You can do this any time before or during beamtime.

Note: If no spreadsheet is available, sample characterization must be carried out for each sample manually or with the Web-Ice Autoindexing interface (the latter will keep a permanent record of the results which can be accessed by the user).

2. Once you are enabled to use the beamline, assign the spreadsheet (section 5.5.2) to the cassette or Unipuck position in the beamline dewar (if you are a remote user, the beamline support staff will tell you the correct position or assign it for you).

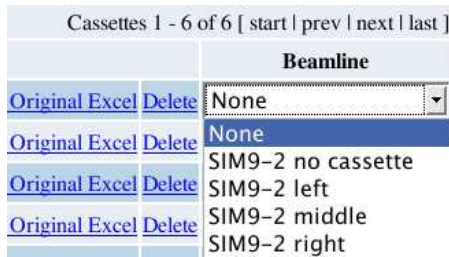


Figure 30: Selecting a cassette position.

3. Start Blu-Ice and select the Screening Tab. Select the samples you wish to screen, the input directory and image collection parameters as described in the Blu-Ice manual. Some tips:
 - Collect two images at different phi orientations in order to trigger automated autoindexing (check the first two "Collect Image" entries⁷⁰ in the Action Sequence list of the Blu-Ice screening tab). If you collect only one image, the spots will be analyzed and assigned a score, but autoindex will not be carried out.

Important: The images must be collected to your area in the /data disk. Please do **not** try to use a specific data disk (eg /data1 , /data2 , etc.) in the directory path. Use /data/"your-id", or else the software may fail to write the image to the disk.

- You may pause the screening after the loop has been centered. This allows you to adjust the sample centering using the click to center option. This is only recommended if you use loops much larger than the crystals (which is not a good idea). It is faster to select a large beam size⁷¹ in the Blu-Ice hutch tab and screen all the samples without pauses.
- It is possible to pause the data collection after the test images have been collected - this will trigger automated strategy calculation for this sample following autoindex. This option is **not recommended**, unless you are very sure that you want to collect data from this particular sample (*e.g.*, if the samples have already been screened). It is more time efficient to screen all samples automatically and then remount the best ones for data collection.

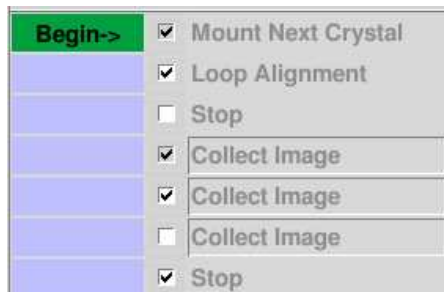


Figure 31: Screening sequence.

⁷⁰http://smb.slac.stanford.edu/facilities/software/blu-ice/screen_tab.html#Action_Sequence

⁷¹http://smb.slac.stanford.edu/software/blu-ice/hutch_tab.html#Adjusting_Beam_Size_and_Energy

Tip: It is possible to use automated sample characterization with the Blu-Ice screening tab without using the robot (in manual mode). The software will prompt you to change the sample in this case

6.1.1 Selecting samples for data collection

A summary of the autoindexing results (symmetry, resolution and mosaicity estimates and a score) will be written to the sample list information displayed in Blu-Ice and Web-Ice shortly after the images have been collected. This information can be used to help select the best samples for collection of a complete data set. Follow the instructions below or consult the Video Tutorial⁷²

To see the screening results in the Blu-Ice screening tab, make sure that you use the "Results" view⁷³ to see these columns.

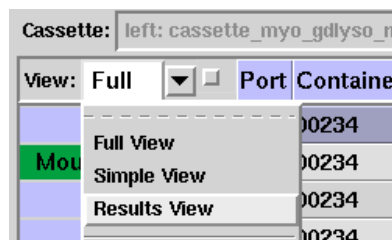


Figure 32: Displaying the Result columns in Blu-Ice.

You can inspect the results in detail with **Web-Ice**:

1. Use the **Web** button⁷⁴ above the sample information list: Click on the arrow to open the drop down menu, and select Web-Ice. Blu-Ice will open a new browser window (if you do not see the browser window, look for minimized or hidden browser windows). You will be directed to the Web-Ice Screening tab⁷⁵.



Figure 33: Launching a web browser window running Web-Ice from the Blu-Ice screening tab.

2. In the Web-Ice Cassette Summary page sort the samples by score, mosaicity, resolution, or rmsd by clicking on the title of the corresponding columns.
3. Select the sample you want to inspect and click on **Cassette Details** (in the gray navigation toolbar). This will let you see the analyzed image, spot statistics, crystal JPEG and autoindexing results for that particular sample.

⁷²http://smb.slac.stanford.edu/users_guide/tutorials/select_crystal2.swf

⁷³http://smb.slac.stanford.edu/facilities/software/blu-ice/screen_tab.html#Customizing

⁷⁴http://smb.slac.stanford.edu/facilities/software/blu-ice/screen_tab.html#Uploading_Spreadsheet

⁷⁵http://smb.slac.stanford.edu/facilities/remote_access/webice/Screening_Crystals.html

Port	CrystallID	Protein	Images	IceRings	Comment	Score	UnitCell	Mosaicity	Rmsd	BravaisLattice	Resolution
A1	CassetteID		CassetteID_001.mccd CassetteID_002.mccd	3 3		0.822	76.92 76.92 38.36 90.00 90.00 90.00	0.15°	0.083 mm	P4,P422	1.18 Å
A2	mvo		A2_003.mccd	3	mvooglobin						

Figure 34: Web-Ice Screening: cassette summary page.

Important: Do not rely blindly on the score or other image statistics as a means of selecting the best crystal - always inspect the results displayed in the **Cassette Details** page.

Header | Spot Statistics | Crystal Image | **Autoindex** | Details

Figure 35: Cassette details navigation in Web-Ice Screening tab: Header displays the image header; Spot Statistics displays the results of image analysis before autoindexing; Crystal image shows a camera shot of the crystal; Autoindex shows the autoindex results and image score; Details is a directory browser and image display tool to inspect log and output files.

6.2 Setting up and starting data collection

Once the optimal sample for data collection has been selected, monochromatic (non-anomalous), and simple MAD and SAD experiments from a single crystal can be set up in a very easy way using Web-Ice. Follow the steps below or look at the Video Tutorial⁷⁶

1. Mount the sample (either manually (section 3.3) or using the robot. If you use the robot, select the sample in the sample information list in the Blu-Ice Screening tab and select the **Stop** immediately following **Loop Alignment** in the Action sequence widget (fig.31); then click the Start button.
2. Move the sample camera zoom to High, and adjust the sample centering if necessary. If the box defining the beam is much larger than the crystal, adjust the beam size
3. Go back to the Blu-Ice screening tab and start Web-Ice (fig.33) - or go to the URL <http://smb.slac.stanford.edu/webice/> and log in using you Unix user ID and password.
4. Once in Web-Ice select the **Autoindex Tab**.

⁷⁶http://smb.slac.stanford.edu/users_guide/tutorials/strategy3.swf

5. Select **New run** from the gray toolbar menu in the Autoindex tab. Enter a unique run name (the software will ask you to try again if the run name has already been used under your user name)

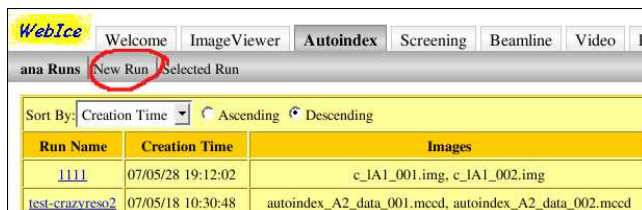


Figure 36: How to generate a new run in Web-Ice.

6. Select the option to collect 2 images and autoindex. If required, by the software, select the beamline from the drop down menu on the right of the gray navigation toolbar.

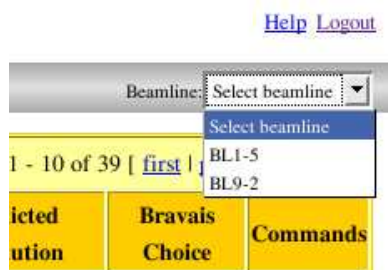


Figure 37: Selecting a beamline in Web-Ice.

7. The program will take you through all the steps to set up the test image collection and (for MAD and SAD) fluorescence scan. Some tips:
 - Use the screening results to set up the optimal parameters. If you want to increase the resolution, remember to increase the exposure time too; however, it is important you do not overload spots, this can cause a problem with the exposure time estimate.
 - If you know that your crystals consistently index in a higher symmetry than the correct one (e.g., a monoclinic crystal with the β angle close to 90 degrees), supply the correct Laue group and cell.
8. Once you have started the autoindex/strategy run, you can monitor progress by looking at the **Beamline log** and **Autoindex Log** in the **Log** page in Web-Ice. The **Setup page** also prints messages as the results become available. Inspect the autoindexing results in the **Autoindex Summary page**, image integration results in **Solutions page**, and (very important!) look at the images in the **Predictions page**. For MAD and optimized SAD experiments, examine the **Scan**. If everything looks fine, look at the **Strategy page**.

Important: Never collect data without inspecting the test diffraction images and the predicted pattern

9. If the crystal space group is well known, make sure that the strategy for the correct Laue symmetry is selected (remember that if the Laue symmetry is not declared when setting up the run, the strategy selected by default corresponds to the lowest symmetry for the best autoindexing solution).
10. If the predicted resolution is lower than the target resolution, try recollecting the test images with a longer exposure time. Do this by clicking on the **Recollect** button; the program will display a page where you can edit the data collection parameters. **Note:** Increasing the exposure time per image will increase the radiation dose by the same amount. E.g., if the estimated dose is 1.5e7 Gy, you will reach the limit by doubling the exposure time. Always verify that the dose limit is not exceeded unless you have additional good quality crystals.
11. If the predicted resolution is higher than the target, recollect the test images by clicking on the **Recollect** button. This will not only test if the diffraction limit estimated by the software is correct, but will also recalculate the correct oscillation angle per image and optimal exposure time, both of which are dependent on the resolution. Use the initial exposure time (used to collect the first test images) and the new sample to detector distance recommended by the software.

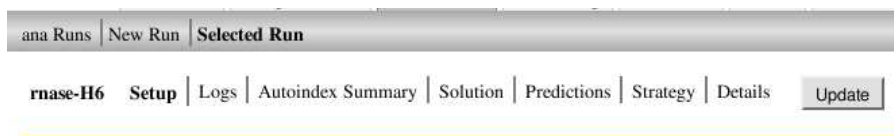


Figure 38: Results menu for a Selected Run in the Web-Ice autoindex tab.

12. Once you have obtained a satisfactory strategy (see the notes below), start data collection from by clicking on the **Collect** button. This will simultaneously export the data collection to Blu-Ice AND start the collection. If you wish to make additional adjustments (e.g. enabling dose mode⁷⁷), you can **Export** the strategy to Blu-Ice. Like the **Collect** button, this will create a run in Blu-Ice, but will not initiate collection. This will provide you the chance to edit the experimental parameters in Blu-Ice; once this is done, start the data collection as described in the Blu-Ice documentation
13. Pausing and interrupting the data collection can be done at any time from Blu-Ice.

6.2.1 Notes on strategy for monochromatic and high resolution experiments

For monochromatic experiments the priority is to maximize **unique completeness**. If the estimated dose for the experiment is low enough, consider collecting additional data beyond the starting or ending phi to increase data redundancy.

Completeness for the low resolution shells is important, so decrease the exposure time or use additional attenuation if the strategy page displays a warning about overloaded spots in one or both of the images.

⁷⁷http://smb.slac.stanford.edu/facilities/software/blu-ice/collect_tab.html#Dose_mode

If ultra-high resolution data are required and you are on a beamline equipped with a CCD detector (e.g. BL14-1) it may not be possible to collect to the resolution limit without overloading the low resolution reflections. In this case, collect an additional low-resolution pass:

- Run Web-Ice to calculate the strategy for the high resolution pass. Once this is done, **export the strategy to Blu-Ice**. This will create a data collection run in Blu-Ice but will not initiate data collection.
- Examine the high resolution test images to determine the resolution d at which **no overloaded reflections are present**.
- Run Web-Ice again to determine the strategy for the low resolution pass. The target resolution for this pass should be $1/2 \text{ \AA}$ above the resolution d mentioned in the above step. This will allow the data sets to be scaled together. Export the low pass strategy to Blu-Ice.
- Start data collection from Blu-Ice. To ensure proper measurement of the critical low resolution reflections, it is recommended to collect the low resolution pass first (if the high resolution data are collected first and the crystal degrades, proper scaling of the two passes may be difficult).

A low pass may not be necessary when collecting thin phi sliced data on the high dynamic range Pilatus detector, but check the reflections at low resolution.

6.2.2 Notes on strategy for MAD and SAD experiments

For MAD and SAD experiments it is very important to limit the dose received by the crystal during the experiment. The Web-Ice strategy already incorporates some mitigation procedures (e.g., data collection in wedges, use of two wavelengths for MAD experiments). If the Web-Ice strategy still results in a dose exceeding or at the limit given by the software, consider **decreasing the exposure time**. This can be done manually either after exporting the data collection parameters to Blu-Ice, or in the edit window Web-Ice displays prior to initiating data collection. The dose is proportional to exposure time, so reducing the exposure time by half will reduce the dose by half at the only expense of a slightly lower data resolution.

For certain space groups and crystal orientations, it is also possible to reduce the absorbed dose by selecting the phi range to maximize unique data set completeness (maximizing Bijvoet pair completeness is the default strategy for MAD and SAD). This usually works for MAD data with a medium to strong anomalous signal.

In unfavorable cases (very small, weakly diffracting crystals) it may be impossible to collect a data set without inflicting serious radiation damage to the crystal. In this case, several crystals will most likely be required for successful structure solution.

Low signal experiments

If the expected anomalous signal is very low (less than 1 % of the average reflection intensity), collection of additional redundancy may be required for structure solution. This can be done by manually adjusting the ending phi before proceeding with data collection in Blu-Ice or Web-Ice. As mentioned above, the exposure time may also require adjusting to avoid excessive irradiation of the crystal.

6.2.3 Multicrystal data collection strategy

On the most intense beamlines, particularly BL12-2, it is possible to collect data from small crystals of dimensions less than 20 microns. However, because diffracted intensity decreases with the crystal volume faster than the deposited dose, use of such small crystals often prevents being able to collect a full data set from a single crystal before radiation damage severely affects the data. This is also often the case for data collection at above cryo and room temperatures, where often crystals last about a factor of 100 less than at cryo. In these cases, it is necessary to stitch a data set from data collected from different crystals. For high symmetry space groups it is often possible to obtain good completeness by starting data collection on a random orientation for each crystal. For low symmetry or scarce samples, it is useful to determine a data collection strategy for each crystal that maximizes the total completeness. This can be achieved with the collection strategy tool available in Web-ice⁷⁸.

Some tips for multicrystal experiments:

- It is a good idea to estimate the dose before starting data collection so that you have an idea of how long the crystal will last. Collecting a low dose low resolution data set from a single crystal may also give you some idea of the total lifetime of the crystals in the beam and help you plan the experiment.
- Do not overexpose the crystal: When aiming for high resolution, it is tempting to use very long exposure times to measure very faint spots at 2 /AA/ in test shots...only to find that not only have those spots vanished after collecting the first image, but you cannot even merge those single shots together. In general, slightly lower resolution data are better than no data.
- To maximize the lifetime of the crystal, always expose as much volume as possible: Fully bathing large crystals in the beam at room temperature will result in a longer lifetime (or give higher resolution data for the same total exposure time) than making the beam small and shooting several data sets on different parts of the crystal.
- It is important to process the data on the fly. If there is a large variation of the unit cell or other lack of isomorphism between different crystals, you may need to collect data from more samples than anticipated to achieve a quality data set. A program such as “pointless”⁷⁹ or “sortmtz”⁸⁰ can be used to combine the reflections in a single file previous to scaling. When using autoxds⁸¹, use the name_xds.mtz files as input to obtain a single file. Note that pointless can also be used to ensure that the indexing for the different crystals is consistent in the space groups where this can be an issue.
- Autoindexing only a few degrees of data may not always be reliable. It is useful to provide the indexing software with the symmetry and unit cell if known. When using the autoxds script this can be done from the command line.
- Similarly, pointless can misidentify the space group in an incomplete data set, as there may not be enough reflections to fully characterize all symmetry elements presents in the

⁷⁸http://smb.slac.stanford.edu/facilities/remote_access/webice/Autoindex_strategy_calculat.html

⁷⁹<http://www.ccp4.ac.uk/ccp4bin/viewcvs/pointless/pointless.html?rev=1.1.1.2pointless>

⁸⁰<http://www.ccp4.ac.uk/html/sortmtz.html>

⁸¹[/baseurl/facilities/software/XDS/](#)

crystal. If this becomes a problem, the option “-c” will force the program to use the input space group.

6.3 Data processing

6.3.1 Data processing environment

The following directories are automatically created the first time you log in to a SSRL px computer (these directories are accessible from *all* computers):

- **/data/username** This directory is used for storing images. We recommend that you also use this directory for data processing.
We also recommend creating subdirectories in your data directory for each data set you collect.
- **/data/username/templates** This directory is a symbolic link to the directory containing the SSRL-specific data processing input and shell files.

Use the remote data processing servers (pxproc01 - pxproc16) to process the data; from the beamline workstations, use the SSRL menu option of the Xfce panel (fig.39) in the Linux beamline computers and the remote Unix desktop⁸². You can also right-click on the Linux Xfce desktop and select *Data Processing* from the desktop menu. Clicking on **Select Least Loaded** displays the load (fig.40). Avoid using a computer is the load is close to the total number of CPUs (displayed next to the computer name).

Note that, since 2017, Web-Ice attempts automated data processing after a data set has been collected. The purpose of this tool is to provide quick feedback of the data quality. Please consult the Web-Ice data processing help.

For more information about the beamline machines, consult the web document <http://smb.slac.stanford.edu/facilities/computing/>

6.3.2 Data processing documentation

Commonly used software packages for data processing are available at the macromolecular crystallography beamlines. If you are unfamiliar with a particular application, consult the relevant documentation:

- Web-Ice manual:
http://smb.slac.stanford.edu/facilities/remote_access/webice/Data_processing.html
- iMosflm tutorial:
<http://smb.slac.stanford.edu/facilities/software/mosflm/>
- HKL2000 tutorial:
<http://smb.slac.stanford.edu/facilities/software/hkl2000/>
- XDS tutorial:
<http://smb.slac.stanford.edu/facilities/software/xds/>

⁸²http://smb.slac.stanford.edu/facilities/remote_access/remote_desktop/

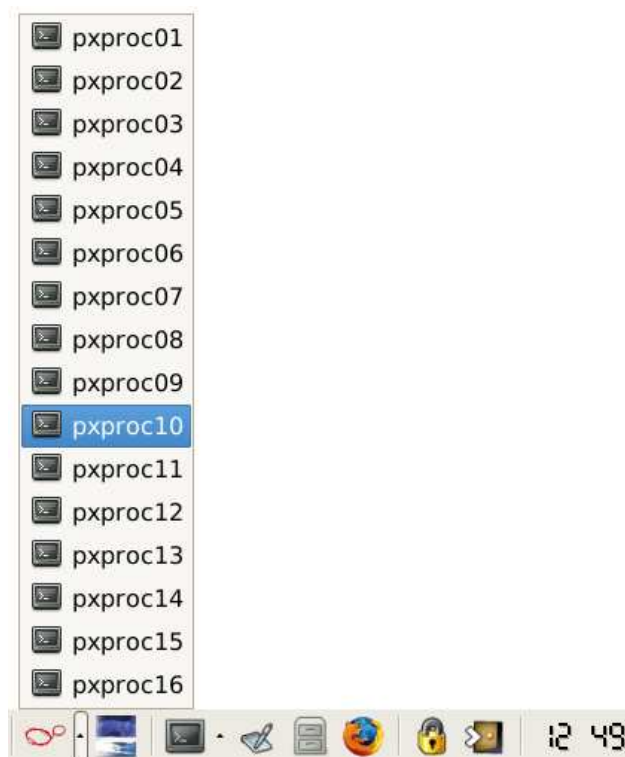


Figure 39: Logging to the data processing servers from the Linux Xfce panel.

- MAD/SAD scripts documentation:
http://smb.slac.stanford.edu/facilities/software/MAD_scripts/

For a complete list of supported and unsupported software installed in the SSRL computers, see http://smb.slac.stanford.edu/facilities/software/xtal_software

7 Referencing SSRL

The acknowledgement section of publications that are based in part or fully on data collected at the macromolecular crystallography beamlines should contain the text cited in the SSRL publication report page⁸³.

You are also encouraged to acknowledge your support staff contact person if they provide help during your beamtime. If the support staff make particularly valuable suggestions or contribute in a non negligible way to the success of the experiment, you may consider making them coauthors in resulting publications.

It is very important that you tell us about publications relevant to work conducted at SSRL. Please fill out the publication form⁸⁴.

⁸³<http://www-ssrl.slac.stanford.edu/content/publications/ssrl-publications-reports>

⁸⁴http://smb.slac.stanford.edu/forms/reporting/form_publication.shtml

Host	arch/CPUs	1m	5m	15m
blcpu6	x86_64/8	0.00	0.00	0.00
blcpu7	x86_64/8	0.00	0.00	0.00
blcpu8	x86_64/8	0.93	0.98	0.94
blcpu9	x86_64/8	0.00	0.00	0.00
blcpu10	x86_64/8	1.01	1.04	1.01
pxproc01	x86_64/16	0.24	0.14	0.07
pxproc02	x86_64/16	0.20	0.25	0.25
pxproc03	x86_64/16	0.00	0.04	0.01
pxproc04	x86_64/16	0.13	0.09	0.07
pxproc05	x86_64/16	0.10	0.08	0.02
pxproc06	x86_64/16	0.24	0.10	0.02
pxproc07	x86_64/16	0.39	0.14	0.07
pxproc08	x86_64/16	0.03	0.10	0.06
pxproc09	x86_64/16	0.19	0.09	0.02
pxproc10	x86_64/16	0.17	0.10	0.08
pxproc11	x86_64/16	0.00	0.03	0.05
pxproc12	x86_64/16	0.03	0.07	0.08
pxproc13	x86_64/16	0.00	0.03	0.35
pxproc14	x86_64/16	0.00	0.03	0.03
pxproc15	x86_64/16	0.01	0.02	0.00
pxproc16	x86_64/16	0.02	0.06	0.02

Figure 40: System load window

7.1 References to supported software/hardware

The following are references of supported software/hardware at the SSRL. Please use the appropriate references in your publications.

Data collection

Remote access Soltis, S. M., Cohen, A. E., Deacon, A., Eriksson, T., Gonzalez, A., McPhillips, S., Chui, H. J., Dunten, P., Hollenbeck, M., Mathews, I. I., Miller, M., Moorhead, P., Phizackerley, R. P., Smith, C., Song, J., van dem Bedem, H., Ellis, P., Kuhn, P., McPhillips, T., Sauter, N., Sharp, K., Tsyba I. and Wolf G. New paradigm for macromolecular crystallography experiments at SSRL: automated crystal screening and remote data collection. (2008) *Acta Cryst.* **D64**, 1210-1221.

Blu-Ice: McPhillips, T. M., McPhillips, S. E., Chiu, H. J., Cohen, A. E., Deacon, A. M., Ellis, P. J., Garman, E., Gonzalez, A., Sauter, N. K., Phizackerley, R. P., Soltis, S. M., Kuhn, P. Blu-Ice and the Distributed Control System: software for data acquisition and instrument control at macromolecular crystallography beamlines. (2002) *J. Synchrotron Rad.* **9**, 401-406.

Automated sample mounting system: Cohen, A. E., Ellis, P. J., Miller, M. D., Deacon, A. M. and Phizackerley, R. P. An automated system to mount cryo-cooled protein crystals on a synchrotron beamline, using compact sample cassettes and a small-scale robot. (2002) *J. Appl. Cryst.* **35**, 720-726.

Russi, S., Song, J., McPhillips, S.E., and Cohen, A.E. The Stanford Automated Mounter: pushing the limits of sample exchange at the SSRL macromolecular crystallography beamlines. (2016) *J. Appl. Cryst.* **49**, 622.

Fluorescence scan analysis: Evans G., Pettifer R. F. CHOOCH: a program for deriving anomalous- scattering factors from X-ray fluorescence spectra. (2001) *J. Appl. Cryst.* **34**, 82-86.
<http://www.gwyndafevans.co.uk/chooch.html>

Derivative preparation with SSRL Xe-Kr cell: Cohen, A., Ellis, P., Kresge, N. & Soltis, S. M. MAD phasing with krypton. (2001) *Acta Cryst.* **D57**, 233-238.

Web-Ice

Web-Ice: Gonzalez, A., Moorhead, P., McPhillips, S. E., Song, J., Sharp, K., Taylor, J. R., Adams, P. D., Sauter, N. K. & Soltis, S. M. *Web-Ice*: Integrated Data Collection and Analysis for Macromolecular Crystallography. (2008) *J. Appl. Cryst.* **41**, 176-184.

Mosflm: Leslie, A. G. Integration of macromolecular diffraction data. (1999) *Acta Cryst.* **D55**, 1696-1702.

Labelit: Sauter, N. K., Grosse-Kunstleve, R. W. & Adams. Robust indexing for automatic data collection. (2004) *J. Appl. Cryst.* **37**:399-409.

Spotfinder/DISTIL: Zhang, Z., van den Bedem, H., Sauter, N. K., Snell, G. & Deacon, A. Automated diffraction image analysis and spot searching for high-throughput crystal screening. (2006) *J. Appl. Cryst.* **39**, 112-119.

Best: A. N. Popov and G. P. Bourenkov. Choice of data-collection parameters based on statistic modeling. (2003) *Acta Cryst.* **D59**, 1145-1153.

Raddose: Paithankar, K. S., Owen, R. L. and Garman, E. F. Absorbed dose calculations for macromolecular crystals: improvements to RADDPOSE (2009) *J. Synchrotron Rad.* **16**, 152-162; Murray, J. W., Garman E. F. and Ravelli. R. B. G. X-ray absorption by macromolecular crystals: the effects of wavelength and crystal composition on absorbed dose. (2004) *J. Appl. Cryst.* **37**, 513-522.

Data processing with Web-Ice

Autodrug: Tsai, Y., McPhillips, S.E., Gonzalez, A., McPhillips, T.M., Zinn, D., Cohen, A.E., Feese, M.D., Bushnell, D., Tiefenbrunn, T., Stout, C.D., Ludaescher, B., Hedman, B., Hodgson, K.O., and Soltis, S.M. AutoDrug : fully automated macromolecular crystallography workflows for fragment-based drug discovery. (2013) *Acta Cryst.*, **D69**, 796-803.

Data processing with the MAD scripts

Pointless, Aimless, Truncate, Scaleit, fft: Winn, M. D. *et al* Overview of the CCP4 suite and current developments. (2011) *Acta Cryst.* **D67**, 235-242.

Solve: Terwilliger, T. C., Berendzen J. Automated MAD and MIR structure solution. (1999) *Acta Cryst.* **D55**, 849-861.

Resolve (density modification): Terwilliger, T. C. Maximum likelihood density modification (2000) *Acta Cryst.* **D56**, 965-972.

Resolve (model-building): Terwilliger, T. C. Automated main-chain model-building by template-matching and iterative fragment extension. (2003) *Acta Cryst.* **D59**, 38-44.

<http://www.solve.lanl.gov/>

Shelxd: Schneider, T. R. & Sheldrick, G. M. Substructure solution with SHELXD. (2002) *Acta Cryst.* **D58**, 1772-1779.

Shelxe: Sheldrick, G. M. Macromolecular phasing with SHELXE. (2002) *Z. Kristallogr.* **217**, 644-650.

Anything that starts with shelx...: Sheldrick, G. M. A short history of SHELX (2008). *Acta Cryst.* **A64**, 112-122.

<http://shelx.uni-ac.gwdg.de/SHELX/>

Data processing with HKL2000

Otwinowski, Z., Minor, W. Processing of X-ray diffraction data collected in oscillation mode. (1997) *Methods Enzymol.* **276**, 307-326.

<http://www.hkl-xray.com/>

Data processing with XDS and autoxds

XDS: Kabsch, W. XDS. (2010) *Acta Cryst.* **D66**, 125-132.

XDS and XSCALE: Kabsch, W. Integration, scaling, space-group assignment and post refinement (2010). *Acta Cryst.* **D66**, 133-144.

autoxds: Gonzalez, A and Tsai, Y. (2010)

http://smb.slac.stanford.edu/facilities/software/xds/#autoxds_script

Graphics software

Coot: Emsley, P. and Cowtan, K. Coot: Model-Building Tools for Molecular Graphics. (2004) *Acta Cryst.* **D60**, 2126-2132.

8 Frequently Answered Questions

If you have questions that are not covered below, please consult the on-line documentation or use our local Google search.

Contact support staff⁸⁵ for additional questions. During your beamtime, use the beamline cell phones⁸⁶.

8.1 Data collection

8.1.1 How can I convert from wavelength to energy?

In Blu-Ice, click on the units next to the energy input box to toggle between wavelength (Å) or energy (eV or keV).

8.1.2 What is the optimal beamstop to sample distance?

The backstop should be placed at a position where it allows collection of reflections in the 30-40 Å resolution range. At most wavelengths, it will be possible to collect even lower resolution, however, this will be at the expense of additional air scatter that may obscure weak reflections and reduce the diffraction signal over noise.

Using Web-Ice⁸⁷ to calculate the data collection strategy will automatically calculate a reasonable beamstop to sample distance. In addition, the Blu-Ice resolution predictor shows the low resolution limit at the given beamstop and energy values.

8.2 Sample mounting

8.2.1 What is the size of the white box on the Blu-Ice sample video?

The white box in the sample camera video displayed on Blu-Ice represents the approximate FWHM beam size at the sample position at all the zoom levels (unlike the box displayed in the monitors at the beamline, which does not change size with the camera zoom. With the **on-axis camera**, the box shows what parts of the sample to beam hits. With the **orthogonal sample camera**, the vertical dimension of the box shows the size of the beam at 90 degrees.

8.2.2 The automated loop centering leaves the camera near the medium zoom level. Is there anything wrong?

No, this is normal operation. The system uses the medium zoom level to do the alignment, in case large loops are used.

Also, during the crystal screening mode, maximum zoom is used for recording JPEG images of the crystal.

⁸⁵<http://smb.slac.stanford.edu/staff/staffpage.php>

⁸⁶<http://smb.slac.stanford.edu/staff/beamline-phones.html>

⁸⁷http://smb.slac.stanford.edu/facilities/remote_access/webice

8.3 Detectors

8.3.1 Can I display the image header?

You can look at the image header with the Web-Ice Image Viewer⁸⁸; The program ADXV also displays the header.

8.3.2 How do I determine the direct beam position?

The default beam position, should usually be at the nominal centers of the detectors (within 1 pixel) listed below (in mm):

- MAR325 CCD: 162.5, 162.5
- Q315 CCD: 157.5, 157.5
- Pilatus 6M: 211.8, 217.3
- Eiger 16M: 155.6 163.9

When a detector offset is selected in the Blu-Ice hutch tab, the data collection software writes the true beam center coordinates C_x, C_y to the image header. **Note:** For CCD detectors and the Pilatus 6M, The convention used at SSRL may not be the same as on other sites. If the detector is offset and you cannot process the data, try flipping the C_y coordinate as described below (section 8.9.4).

8.4 Pilatus and Eiger detectors

8.4.1 What is the optimal oscillation angle per image with the Pilatus and Eiger detectors

To get the best data, we recommend 0.1 or 0.2 degrees oscillations and short exposures per image. However, a larger oscillation (0.5 -1 degree) should be used for crystal screening or test shots. If in doubt, use the default parameters in Blu-Ice; we also advice to use Web-Ice to calculate the data collection strategy.

Important: Thin sliced images with the correct exposure time look very weak - this is to be expected, as all the reflections are partials, and you should refrain from increasing the exposure time until the pattern looks strong, since this may result in rapid radiation damage. Even if you do not exceed the maximum allowed dose with an increased exposure time, consider increasing the data multiplicity instead.

Small oscillation data can be processed at SSRL in a very straightforward manner either with XDS (see also the SSRL script autoxds⁸⁹) HKL2000 (from July 2010) and the most recent version of MOSFLM can be also used, although we have found that XDS tend to give the best results for oscillations much smaller than the crystal mosaicity.

⁸⁸http://smb.slac.stanford.edu/facilities/remote_access/webice/Image_Viewer.html

⁸⁹http://smb.slac.stanford.edu/facilities/software/xds/#autoxds_script

8.4.2 What is shutterless data collection? Can I turn it off?

Because both the Pilatus and Eiger detectors have a very short readout time compared with the typical exposure time, it is not necessary to stop the phi rotation or close the shutter while the detector reads out the image; this results in faster data collection and, for very short exposures, may reduce systematic errors. The default setting for data collection mode is to collect the entire data set without closing the shutter or stopping phi.

For MAD and SAD data collections the shutter will close and phi will stop at the end of each wedge to change the energy or the crystal orientation. For native data sets, entering a wedge value different than the default of 180 will also cause the shutter to close, dividing the data collection run into several shutterless intervals. This can be advisable to be able to pause the data collection at some point - for instance, in order to check the preliminary results of data processing (see following section)

Choosing a wedge equal to the oscillation range effectively turns off shutterless data collection. This is **not** recommended, with the possible exception of MAD or SAD experiments on crystals that suffer significant radiation damage after a single shot.

8.4.3 Why can't I pause data collection during shutterless data collection?

The "**Pause**" button in the Blu-Ice data collection tab is designed to wait until the shutter closes (so that the last image is not bad). This means that after pressing this button, data collection will continue until the end of the current wedge.

Similarly, if there is a SPEAR3 current dump in the middle of the data collection, the collection will not pause, but proceed to the end of the wedge. Multiple images will be blank. For this reason, it is important to always monitor data collection with the Pilatus or Eiger detector during shutterless data collection- or use wedges small enough that can be recollected without increasing dramatically the total dose absorbed by the crystal.

The **Abort button** can be used to stop the data collection, although a few images will be collected before the command takes effect. If you wish to resume the data collection after the pause, remember to recollect the last image written to disk.

8.4.4 The data collection takes a long time to start

The Pilatus detector is programmed to change the gain at an energy of 9000 eV. Setting the new gain for all the pixels can take about two minutes and this will happen every time you start a data collection run at an energy that crosses that threshold in either direction: *eg.* if you collect at 12000 eV on one run and 8000 eV in the next run or *vice-versa*. If you want to do a MAD experiment on an absorption edge below 9000eV and the remote energy is above 9000eV consider manually selecting a different remote energy. Ask the support person for advice.

While the Eiger detector takes a shorter time to reset the gain (about 20 seconds), it does this over a shorter energy change, and it will always take this extra time to change between the edge and remote energies during MAD experiments.

8.4.5 Can the Pilatus 6M detector resolve large unit cells?

Despite the Pilatus 6M relatively large pixel size compared to CCD detectors or the Eiger, in our experience it is possible to resolve closely spaced diffraction spots (see for example, the PDB structure 3M8C); note that, unlike the CCD, this type of detector has a zero point spread function, which contributes to limit the spot size.

8.4.6 How do I know if I am overloading the detector?

The Pilatus 6M and Eiger 16M have a large dynamic range: 1,048,576 counts. When the dynamic range is exceeded, the counter starts from zero again. The only indication for this is that holes may be observed in peaks. Blu-Ice marks in yellow pixels above 64,000 counts. These are not overloaded, but the coloring makes it easier to spot true overloads.

Unlike an integrating detector the pixel array detectors have a count-rate limit (the counts per unit of time rather than the total number of counts over the entire exposure). Pixels that exceed the maximum count rate (recorded in the image header) are flagged in red.

8.5 Determining data collection strategy

8.5.1 What is a reasonable exposure time for beamline X?

If in doubt, use the default values: in the Blu-Ice Collect tab, click the **Default** button; in the Screening Tab, click **Reset defaults**. If your crystals diffract very poorly or you are collecting the images at extra long or short wavelengths you may have to increase the time.

We recommend to use Web-Ice⁹⁰ to determine the optimal exposure time from the initial test shots of the crystal.

8.5.2 How long do I need to expose a crystal at SSRL for RIP phasing?

For radiation damage sensitive samples, the best strategy is to do a two-wavelength MAD or SAD experiment without exceeding the maximum recommended dose (use Web-Ice to obtain an estimate of the absorbed dose). Overdosing the crystal results in a unit cell expansion which most often prevents accurate measurement of any kind of phasing signal in the data. Radiation induced intensity difference are no easier to measure than anomalous or dispersive differences in this case.

For some derivatives (e.g., brominated DNA), the heavy atom may become cleaved at very low doses. The program SHARP has been reported to deal well with this particular case, using the loss of occupancy of the anomalous scatterer to enhance MAD or SAD phases, as long as the total dose is kept to a reasonable value (i.e., you should not exceed the dose limit in Web-Ice).

⁹⁰http://smb.slac.stanford.edu/facilities/remote_access/webice/Autoindex_strategy_calculat.html

8.6 Beamlines

8.6.1 BL12-2

BL12-2 is an undulator beamline with microfocus capabilities and high flux. Here are the major characteristics of BL12-2:

- **Variable beam size** : While other SSRL beamlines operate at a fixed focus, and the beam size is changed by opening or closing collimating slits upstream of the sample. On BL12-2, however, the beam can be focused down to 50x20 (h/v) microns continuously.

Important: Because the full beamline flux can be concentrated on a very small spot, it is important to avoid overexposing the samples. We strongly advise to use Web-Ice to calculate the data collection strategy, and to inspect the estimated dose before deciding on the exposure time and beam attenuation.

In addition, it is possible to use smaller beam sizes (eg, 10x10) by inserting a microcollimator in the beam. To use this feature, check the **”microbeam”** button of the required size in Blu-Ice.

As a rule, the microbeam should only be used to study very small crystals; for crystals larger than 10-20 microns it will be usually more advantageous to use the ”normal” beam focused to match the crystal size.

- **High zoom on-axis camera:** Besides the sample camera viewing the crystals from a below, a high zoom camera can be used to see the sample from the beam direction. The on-axis camera video feed can be accessed from the Blu-Ice video widget, by clicking the **On-Axis** button. The **Back Light** can be inserted in conjunction with the **Light intensity** bar to obtain the optimal sample visualization.
- **Automated beam optimizations using a special sample:** The beamline software performs an automated sample optimization periodically, using a special metal sample mounted by the robot. This will take place between sample mounting and dismounting cycles - ie, the software will wait until the sample currently in used has been screened or collected from and dismantled before mounting the special sample. The optimization takes about 10 minutes. After it is completed, the new crystal sample will be mounted automatically. Since 2016, it is possible to stop the beam optimization at any time. Doing this is **not** recommended if you are using or planning to use the microcollimators, since they require a very precise degree of component alignment. **If using the microcollimators**, after skipping one or more optimizations, please check the “Optimize beam” button in the hutch tab. If the color has changed to green, use it to optimize the beam. Never use the “Abort” button to stop the optimization.
- **Fast energy changes.** Beam optimizations are not required after a change of energy, so this only takes a few seconds. The only exception is when changing the energy below or above 9keV, because the gain of the Pixel Array Detector is energy-dependent and it needs to be changed at that value; this procedure is automatic, and it takes about 2 minutes.

8.6.2 BL9-2

BL9-2 is a wiggler beamline. The beam size can be continuously changed by collimating the beam by a pair of slits. The slit size is controlled by specifying the beam size in the Blu-Ice interface - note that making the beam size smaller or larger than the limits displayed in Blu-Ice will not change the beam size.

BL9-2 is equipped with a microspectrophotometer, useful to monitor photoreduction when working with samples with a metal center. See the Blu-Ice documentation⁹¹ for more information about this instrument.

Because beam optimizations are not required following a change of energy, this is a very fast procedure. The only exception is when changing the energy below or above 9keV, because the gain of the Pixel Array Detector is energy-dependent and it needs to be changed at that value; this procedure is automatic, and it takes about 2 minutes.

8.6.3 BL14-1

BL14-1 is a bending magnet beamline. Although the total flux is lower than on other beamlines, this is compensated somewhat by a relatively small beam size (less than 0.1 mm). The beam size can be changed by collimating the beam by a pair of slits. The slit size is controlled by specifying the beam size in the Blu-Ice interface.

8.7 SPEAR3 operation

8.7.1 How can I monitor the SPEAR3 beam?

The beamline status and current are displayed in the Blu-Ice status bar⁹².

Additional information about SPEAR3 (including status of all beamlines and the 24-hour fill history) is displayed on a monitor at the beamline (top left corner of the console) and on the SSRL SPEAR3 Web page⁹³. See also "Monitoring the SPEAR3 status remotely" (section 8.10.1)

8.7.2 How often is the beam refilled?

SPEAR3 operates in frequent fill mode. The beam is topped up every 5 minutes. Data collection can continue normally during the injection in frequent fill mode. The normal variation of the beam intensity between fills is less than 1%. In this mode, the displayed current will always be close to the maximum injected current.

8.7.3 Does the frequent fill mode affect the diffraction images?

Under normal data collection conditions, we cannot detect any substantial differences between the quality of the data collected in frequent fill mode and with constant stored beam. It is also

⁹¹http://smb.slac.stanford.edu/facilities/software/blu-ice/microspec_tab.html

⁹²http://smb.slac.stanford.edu/facilities/software/blu-ice/getting_started.html#Status_Bar

⁹³http://www-ssrl.slac.stanford.edu/talk_display.html

impossible to determine whether an injection took place during the collection of a diffraction image by examining the reflections in that image.

If a manual injection takes place, the data collection software will stop and restart when the temperature of the beamline optics has stabilized. If an image was being collected during manual injection, it will be recollected.

A message is displayed in the Blu-Ice status box while the beam is stabilizing following a manual injection.

8.7.4 How does the search and reset procedure work?

Completely search the hutch for persons before activating the search reset. The hutch door must be closed and locked before the search alarm stops ringing, otherwise the procedure must be repeated.

8.8 Problems during data collection

8.8.1 Is there beam in the hutch?

If the beamline is open and Blu-Ice repeatedly displays the message "waiting for beam", check that the beamline stoppers switch is open on the key panel in the control rack (all green LEDs should be lit). Remote users can see the stoppers LEDs by selecting the appropriate panel video preset⁹⁴ in Blu-Ice or Web-Ice. If this is the problem, on-site users should repeat the hutch search. Remote users must call staff or, outside normal working hours, the duty operator (650-926-4040).

If the stoppers are open, try reoptimizing the beam. If the beam optimization does not solve the problem, call support staff.

8.8.2 Why do I get blank diffraction images?

To determine the cause of blank diffraction images, follow these steps:

1. If there is no image displayed, try opening the image with a different program (e.g, ADXV)
If the image looks only blank on Blu-Ice or Web-Ice, the image server may have crashed. Contact support staff (please, send an e-mail during non-working hours, as this problem does not affect data collection).
2. Check the contrast in the image display: Images with no diffraction spots displayed at a high contrast level can hide diffraction features for thin-sliced images.
3. Verify that there is beam in the hutch (see the previous question). Try reoptimizing the beam.
4. Check the beam attenuation: The attenuation level depends on the beam energy, so a filter combination appropriate for data collection at a high energy can fully block the beam if you have changed to a lower energy.

⁹⁴http://smb.slac.stanford.edu/facilities/software/blu-ice/video.html#Panel_View

5. Check that the detector cover is not on. Remote users can use the “Overview” preset in the hutch video⁹⁵ in Blu-Ice or Web-Ice.
6. If nothing is blocking the detector look at the shutter controller to determine if the shutter is opening. The switch on the controller should be on ”auto” and a red LED light should light up when the shutter is open, as shown in shutter-controller (fig.41). Remote users can use the Panel camera to view the shutter controller. In addition, when the shutter opens you should be able to see an increased reading for the I_beamstop beam monitor. The I_beamstop reading will be low at long wavelengths (low energies). Verify that there is beam on the beamstop by going to a shorter wavelength (higher energy).



Figure 41: Shutter controller showing open shutter status

7. If there is diffuse diffraction but no spots, check the centering of the crystal. Open up the slits to increase the beam size and collect images at different crystal orientations. If you get diffraction with a large beam size or at some phi positions but not others, the phi axis may be misaligned. Contact support staff.
8. If there is no difference with large slits or at different phi positions, the crystal may not diffract (even if it was diffracting previously). Check the cryojet temperature (Blu-Ice will display an error in the status display if the temperature raises above 120 K); dismount the sample and try another sample. If you do not observe any diffraction or scatter from any sample, contact support staff.

8.8.3 Why can't I open Blu-Ice?

The first time you log in to Blu-Ice it will request your password. Make sure that it is typed correctly. If you cannot log in, contact the user support staff and let them know what Unix account you are using.

If you cannot start Blu-Ice from the icon in the XFCE menu (fig.39):

1. Open a terminal on the local workstation or NX client.

⁹⁵http://smb.slac.stanford.edu/facilities/software/blu-ice/video.html#Hutch_View

2. Log in to a different beamline workstation. For example, if you are trying to open Blu-Ice from bl92a, log in to bl92b or bl92c. Use the command:

```
>ssh bl92a
```

```
>go
```

Note: The NX client tries to open Blu-Ice on the "c" workstation; if Blu-Ice fails to open, make sure that you use the "a" or "b" computer.

8.8.4 Why is Blu-Ice not responding?

- Verify that the Blu-Ice window is **active**.
- If you lose permission to connect to Blu-Ice (the DCSS server is off), contact support staff.
- If some buttons are inactive (grayed out) move the mouse over the button. Blu-Ice will display a message explaining why the button is inactive. If the message tells that the control software for a hardware component is off-line ("DHS off-line") contact support staff.
- If the Blu-Ice status window displays the messages "MOTOR STOP BUTTON LATCHED", an emergency stop button may have been depressed accidentally. To reset the motors, press the green motor reset button (fig.2). If collecting data remotely call support staff, or, after working hours, try contacting the duty operator at 650 926 4040.
- If the entire Blu-Ice interface fails to respond, there might be a pop-up window showing an error hiding behind another window. If you cannot find it or cannot clear the error, try exiting the Blu-Ice client and starting a new one. If the fault persists, call support staff.
- Sometimes, a computer crash or a network problem can hang the system. In this case, other programs and processes will also be affected. If the window manager program is hanging, log on to the host computer from another terminal and kill the processes. To list processes:
>ps -u "your_id"
and kill them with
>kill -9 "process_id"
- If all the computers at the beamline are hanging or data collection will not proceed, the file system may have crashed. Contact support staff.

8.8.5 What does the message "Detector error" mean?

A detector error message in Blu-Ice can have many different causes; although often staff intervention is required in order to continue data collection, it is a good idea to retry the image collection before calling staff, as some errors (e.g., a transient network glitch) do not disable the detector permanently.

Important: Note that the "Detector Error" message will still be displayed after a problem with the detector has been fixed; the message will only disappear once an image has been collected without errors.

8.8.6 When should I optimize the beam?

It is advised to optimize:

- After changing the wavelength *from the Hutch tab*. Automatic optimization is performed after wavelength changes during MAD data collection.
- After changing the beam size, specially when going from the large to a smaller beam size, or on BL12-2, when using a microcollimator several hours after the last optimization.

Automatic optimization is also performed at regular intervals during data collection and therefore, manual optimization should not be necessary.

8.9 Data processing and graphics software

8.9.1 I get an HTTP error when trying to display or refresh a page in Web-Ice

Often this will be a transient server glitch. Try refreshing the page again. If the problem persists, contact support staff (use e-mail outside working hours)

8.9.2 Why doesn't program X run?

Most crystallographic software packages are only installed on the data processing servers (section 6.3) and not on the local beamline computers. Graphics programs are installed only on the local beamline workstations.

8.9.3 Why is iMosflm/HKL2000/other running slow?

Check the relative load of the data processing server as described in "data processing environment" (section 6.3).

8.9.4 Why can't I autoindex my images?

The detector may have been offset from Blu-Ice: Check the detector positioner vertical and horizontal values in the Blu-Ice hutch tab. If they are not 0, look at the center coordinates in the image header (the Web based software Web-Ice can be used to display the image header).

Web-Ice, the program LABELIT, and the mosflm and autoxds scripts provided at the SSRL can use the image header coordinates directly for autoindexing. However, if you use HKL2000, iMosflm or have your own scripts to run XDS, you must specifically provide the offset center as follows:

- For HKL2000 or iMosflm:
 - y = Detector height (mm) - Cy (CCD detectors and Pilatus)
 - y = Cy (Eiger)
 - x = Cx (all detectors)

- For XDS:
 - x = Cx/pixel size (all detectors)
 - y = Detector height (pixels) - Cy/pixel size (CCD detectors and Pilatus)
 - y = Cy/pixel size (Eiger)

Indexing may also fail if the diffraction is weak, if there are many ice rings or if there is a double lattice. Editing the spots manually often circumvents these problems. Web-Ice uses a different spot-finding algorithm and it is worth trying if other software fails.

If the diffraction pattern is misindexed (this should be very rare, but is a possibility if the spots are very close and the r-merges after scaling are above 20 or 30%) try using Web-Ice (Web-Ice does not assume that the input center is correct, but searches for the optimal coordinates over a small area).

Local users can mounting samples manually determine the accurate center position by following these steps:

1. Move the detector to the distance used for data collection.
2. If the resolution at the edge of the detector is 3Å or higher, use the Si sample, otherwise use the polyethylene sample. Both samples are located in the same compartment on the beamline tool board.
3. Collect a diffraction image. For the Si sample, use a delta phi of 15 degrees and 5 s exposure time. For the polyethylene sample, use a delta phi of 0.02 degrees and a 1 s exposure time. If the detector saturates, attenuate the beam.
4. Run the program **center** on one of the blcpu servers to calculate the direct beam position from the image.

Important: The beam center position in the image header should be accurate to within 0.1mm. In the extremely rare event that it is off by a larger amount, make sure to tell user support staff.

8.9.5 How do I display the JPEG snapshots of the crystal?

You can use the screening tab or the image tab in Web-Ice to display the crystal snapshots. You may also use the program **display** (from a Linux or Unix shell).

8.9.6 Are the International Tables for Crystallography available?

On-line Space Group diagrams are available at <http://smb.slac.stanford.edu/facilities/software/spacegroups/>. This site is for local access only; the NX Client can be used to view the tables remotely.

The full International Tables are available at <http://it.iucr.org/>. The IUCr site is fully accessible from SSRL or the NX client (or off-site if your local institution has a license).

8.9.7 Is it OK to leave files on the /data disk?

Users are responsible for backing up by their data by the end of their beam time. Images stored on the /data disk can be deleted at any time. Special requests to keep files on /data should be made to support staff. The /home area can be used to store small files indefinitely.

8.10 Remote access

8.10.1 How can I monitor the SPEAR3 beam remotely?

The beamline status and current SPEAR3 intensity are displayed in Blu-Ice⁹⁶. The video tools in Blu-Ice and Web-Ice can also be used to look at the beamline SPEAR monitor⁹⁷; in addition, the SPEAR3 status and fill history can also be accessed via the web⁹⁸.

For updates or inquiries about SPEAR3 you can call the duty operator 24/7 at 650 926-4040, or the beam information line at 650 926-BEAM (2326).

8.10.2 HKL2000 displays a screen size error

In order to run HKL2000, the NX client window must be at least 1100 x 900 pixels. If HKL2000 gives the error "HKL2000 requires screen width larger then 1100 and screen height larger then 900 (*sic*)", enlarge the NX client window as described in the remote desktop configuration⁹⁹ (see section "Tuning the configuration" near the end of the page).

8.10.3 I cannot open an ADXV window from Blu-Ice

There is a limit on the number of ADXV processes allowed to run on the NX server in order to stop it from running out of memory (currently the limit is 5). To inspect new image, close some old ADXV windows. You can also use Web-Ice¹⁰⁰ to inspect the images.

8.10.4 Why can't I connect to the NX server?

Please consult the remote desktop documentation¹⁰¹.

8.11 Computers

8.11.1 Can I change the default settings for my SSRL account?

Click on the **Settings** icon in the Xfce panel (fig.39) to access the Settings Manager GUI. **Exception:** Use only the Blank Screen as a screen saver (animated screen savers use a lot of CPU, which can affect remote access through the NX client).

⁹⁶http://smb.slac.stanford.edu/facilities/software/blu-ice/getting_started.html#Status_Bar

⁹⁷<http://smb.slac.stanford.edu/facilities/software/blu-ice/video.html#SPEAR>

⁹⁸http://www-ssrl.slac.stanford.edu/talk_display.html

⁹⁹http://smb.slac.stanford.edu/facilities/remote_access/remote_desktop/nx-4-NX.shtml

¹⁰⁰http://smb.slac.stanford.edu/facilities/remote_access/webice/Image_Viewer.html

¹⁰¹http://smb.slac.stanford.edu/facilities/remote_access/remote_desktop/