

SSRL beamline Standard Operating Procedures

If in doubt, ask!

These SOPs are updated as necessary, so please check for updates before data collection. The newest version can always be found at:

<http://molobs.caltech.edu/SSRL.htm>

Failure to comply with the directions in these SOPs may result in revocation of your privilege to use this facility.

Beamline website

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

Getting Started

People that intend to collect data at BL12-2 must have (or create) an individual Google **Gmail account** (no lab accounts or group accounts – everyone must have an individual account for tracking purposes). If this account is not regularly checked, you should forward mail from this Gmail account to an account you monitor, as most communication about the beamtime will be done using this Gmail account.

Email the Molecular Observatory (MO) at molecularobservatory@gmail.com using your Gmail address. Ask to have the **SSRL beamtime calendar** shared with you. You will then be able to see the calendar of beamtime availability and shipping dates by logging into Google Calendar. The calendar is called “SSRL beamtime.”

Using your Gmail account, **create a calendar** that will be used to request SSRL beamtime. From your Gmail account, click “Calendar” in the top left corner. On the Calendar page, under “My Calendars” on the left, click “Add.” Give this calendar the name “myname_xtals” and share it with molecularobservatory@gmail.com with Permission Settings “See all event details.” Click “Add Person” and “Create Calendar.” Signing up in this round about way ensures the privacy of each individual signing up for beamtime. See below for instructions on how to sign up for beamtime using this calendar.

If your lab is using Google calendar for other signups, and you wish to have the beamtime calendar visible in your lab’s calendar, feel free to send a separate email to molecularobservatory@gmail.com to request that the beamtime calendar be shared with your lab account. Remember, however, that beamtime requests will not be accepted from lab accounts.

MO will collect **contact information** from you including cell phone number, Gmail address, Caltech email address, account numbers, lab affiliation and position in that lab. Some of this information will be shared with the SSRL staff and other beamtime users for the purpose of communication during beamtime.

You will be assigned a MO SSRL beamline **user name** that will consist of three letters that are your initials and two numbers. This user name will identify you in the spreadsheets used to keep track of crystals sent to the SSRL.

Signup for beamtime

Scheduled SSRL beamtime can be found in the calendar as soon as this information is available. It is your responsibility to **monitor the calendar** for available beamtime.

The day before the start of an upcoming beamtime, there will be an event in the beamtime calendar called “shipping”. In order to sign up to send crystals to the SSRL, you should **create an event on the “shipping” day**.

Please create this event in the **box at the top** of the day in the calendar (ie, as an “all day” event) rather than at a specific time during the day, so that your request is not overlooked.

To create an event, click on the calendar to open a pop-up window. Under “What:” type your name and number of crystals you wish to send. Using the “Calendar” drop-down box, choose the calendar **“myname_xtals”** that you have previously shared with MO (see instructions above for creating this calendar). Click “Create event” and check to make sure your event was created correctly.

Please create this event as soon as you know you would like to send crystals so preparations can be made based on the total number of crystals to be shipped. Do not wait until shipping day to post notification of your intent to ship crystals.

If there are not enough participants two working days (excluding Fridays) before the scheduled start, we might forfeit that beamtime.

Note also, that you will not be able to see any other people's entries on the shipping day, only yours.

Shipping samples to SSRL

You should “freeze” your samples before shipping day and store them in liquid nitrogen; **please do not start looping and freezing crystals the day of the shipping**, as this time is needed to coordinate sample loading for shipping.

Mount your crystals in loops that are of approximately the same size as the crystal. This makes it less likely that you will lose crystals and makes auto centering less problematic since samples are centered based on the loop, not the crystal. Below are the **recommended loops**.

Note that SPINE pins CANNOT be used.

http://hamptonresearch.com/product_detail.aspx?cid=24&sid=152&pid=570

Familiarize yourself with the **cassette loading procedure** ahead of time and make sure you use approved pins!

http://smb.slac.stanford.edu/users_guide/manual/Using_SSRL_Automated_Mounti.html#automated-mounting

Keep track of your samples. A data sheet named “Crystals” is included among the Standard Forms.

You will be contacted by email and/or phone to schedule the transfer of your samples from a storage dewar to the SSRL cassettes. This happens on the day marked “shipping” in the beamtime calendar.

Please retain a flexible schedule. **All sample loading must be finished by 15:30 (3:30pm)** to ship from Caltech in time.

MO will share spreadsheet documents with you via Google Documents cataloguing all the crystals shipped to the beamline.

Once your samples are in the cassette, **update the correct spreadsheet(s)** (based on cassette number) with your crystal information. Please do this immediately; once the spreadsheets are uploaded, it is hard to correct them, especially once data collection has begun.

Do NOT change the entries for PORT.

Do NOT delete lines.

CrystalID entries have to be unique. It's safest to leave them alone.

You will be assigned a MO username, please use this in the name field.

Only use characters (A-Z,a-z), numbers (0-9) and the dash (-) in your protein name. Positively NO (I \ " ? * _ or space) characters!

Blu-Ice and Data Collection at SSRL BL 12-2

You will need to install **NoMachine NX client** on a computer in order to access the SSRL beamline computers remotely.

http://smb.slac.stanford.edu/facilities/remote_access/remote_desktop/nx.shtml

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

Make sure you are comfortable with **Blu-Ice** data collection software and data collection strategies in general (see separate SOP below for some useful tips) beforehand. Synchrotron time is valuable and we want to make the best use of it. Training sessions are available, or you can learn with an experienced user while he/she collects for you. **Please plan training in advance of beamtime usage.**

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

Connect remotely and open Blu-Ice. Make sure the directory in the top right corner of the Screening tab reads /data/rees. If this is not the case, press the “default” button right next to it.

Do not screen using the microbeam, **use a beam size as large as your largest loop for screening.**

Line your samples up for **auto-screening**, do not manually mount and dismount each crystal. Also, please disable the two optional stops. This speeds up the process by about 100%!

Use **Webice** to evaluate your screening.

<https://smb.slac.stanford.edu/webice/ShowLoginForm.do>

Line your “best guys” up for another screening, this time with both stops enabled. This time you may use the microbeam, manual recentering, rastering, and all the bells and whistles.

Wait until the automatic processing is done; start Webice strategy and import the run. If you repeated screening of one crystal several times, you might need to edit the setup and choose the most recent pictures.

Make sure your contact information is up to date and **read the emails** that are sent by the SSRL staff. Quite often, new developments at the beamline are described and Dos and Don'ts are updated. **Answer your phone and be on call** when waiting for beamtime use.

Directory structures are coded in the sample spreadsheet, currently:

MO/YMMDD/group/user/project/cassette/port.

Do NOT change the names of directories and do NOT create any subdirectories for image storage/collection.

You should create subdirectories for **data processing**. When you create processing directories, and you wish to link to images for more convenient access, DO NOT create links like “ln -s ../.". This will create

infinite directory trees that will cause problems for our evaluation software. There are many powerful and convenient ways to link your images. I (Jens) will be glad to help you.

If you wish to have a different directory structure, you can create that during your backup of the data. (See below.)

Data collection is around the clock. We try to accommodate special circumstances, but we try to distribute the grave yard and weekend shifts fairly; so expect to collect over night or on Saturday evenings.

MO transfers the data to a central server at Caltech, which you can access to copy data to an external hard drive. You may copy your data independently; but remember, no images should be stored in your home directory on the MO server.