The Advanced Light Source and CSMP

Increasing the structure determination success rate

1 Crystal triage technology

a) Simulate the experiment to discover minimum required data quality

b) Understand radiation damage

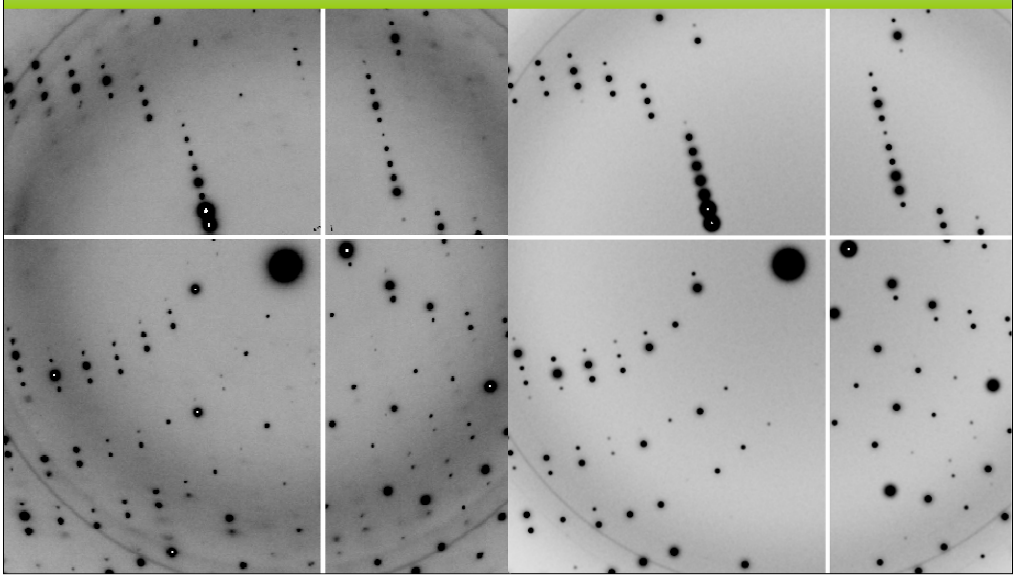
c) Combine these criteria into smarter strategy programs

2 Improve screening infrastructure

a) offline targeting and unattended execution

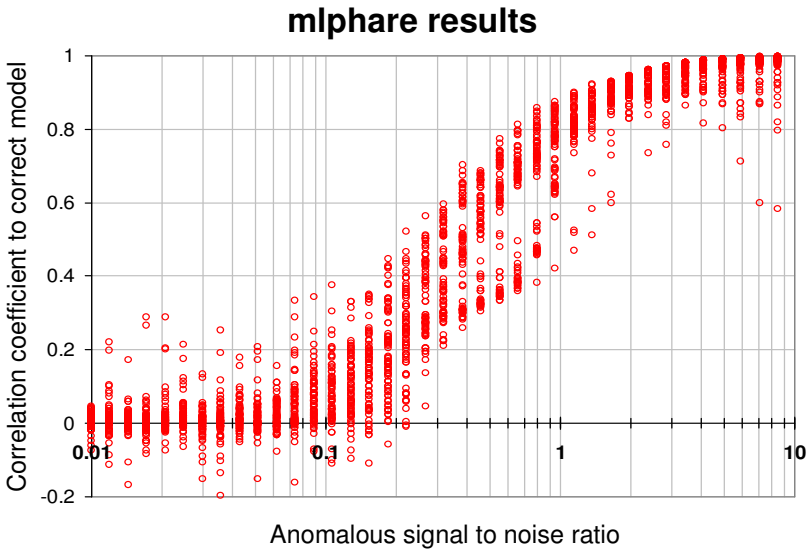
b) Room temperature tray screening

Is it real, or is it MLFSOM?

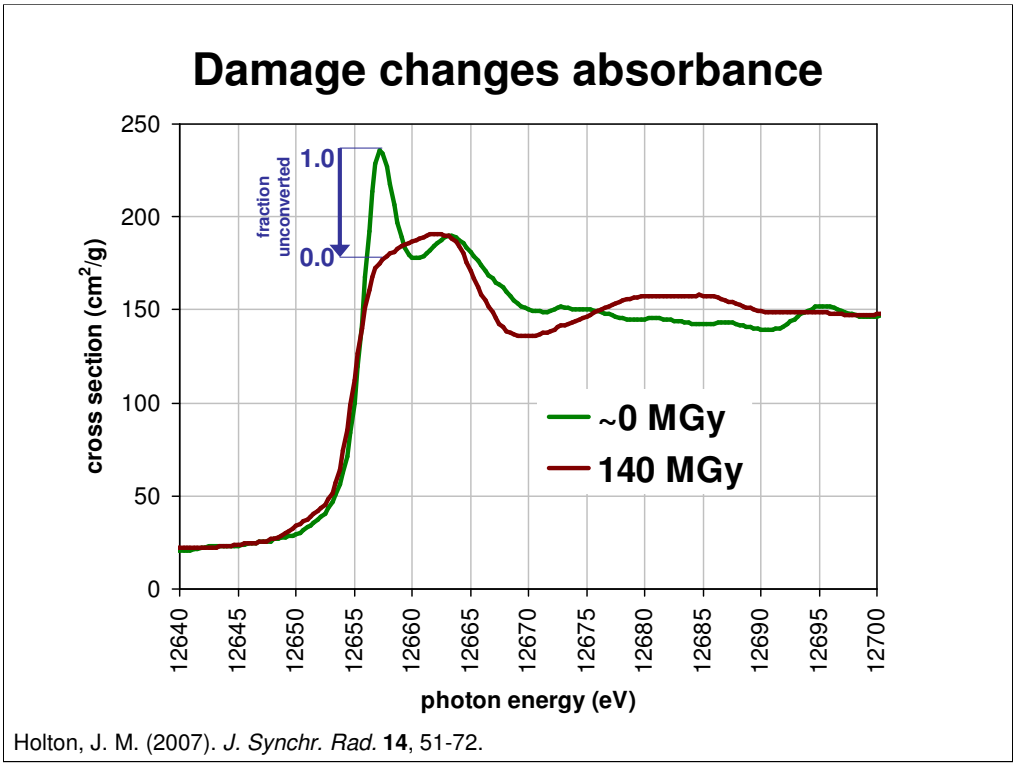


A diffraction-image simulator called MLFSOM is being developed. The image on the left is a real diffraction pattern from a lysozyme crystal. The image on the right is a simulation of the same experiment. The simulator uses real-world physical parameters such as beamline flux, crystal size, detector sensitivity and other camera parameters to predict the observed diffraction pattern. These two images are on the same intensity scale.

MAD phasing simulation

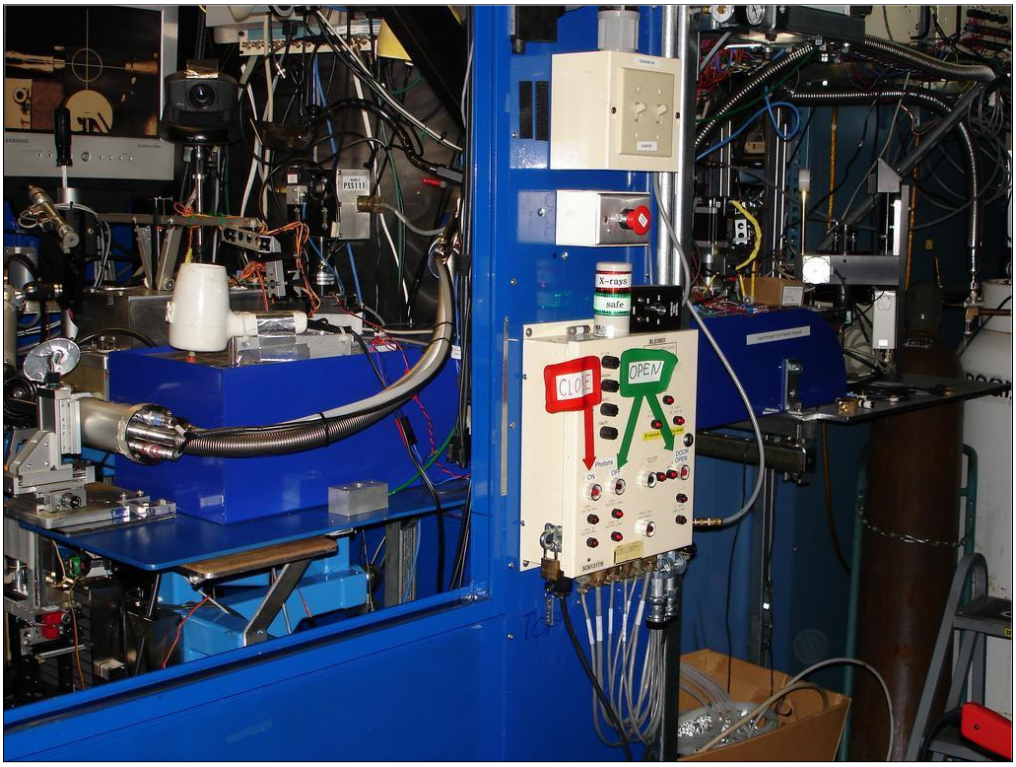


A simplistic simulation reveals the data quality required to solve a MAD structure. Structure factors were calculated from three PDB files (ovalbumin, lysozyme, and GCN4-p1-N16A) with a random number of randomly distributed “heavy-atom sites” with random occupancy to generate ~1000 test “data sets”. Random noise with a constant Gaussian distribution was added to the calculated intensities to simulate a background-dominated experiment. Maximum-likelihood phases were then extracted from the resulting anomalous differences using the “correct” heavy-atom constellation. The y-axis of this graph is the correlation coefficient between the electron density map obtained with these phases with the electron density map calculated from the original PDB file. The “threshold of interpretability” for an electron density map by eye appears to be around 0.5. The x-axis of this graph is the ratio of the known anomalous difference magnitudes to the average noise in the anomalous difference “measurements”. It is remarkable that there is virtually no difference in final map quality between an anomalous signal to noise ratio of 1 or 10, and there is a sharp drop-off in map quality below an anomalous signal-to-noise ratio of 1.

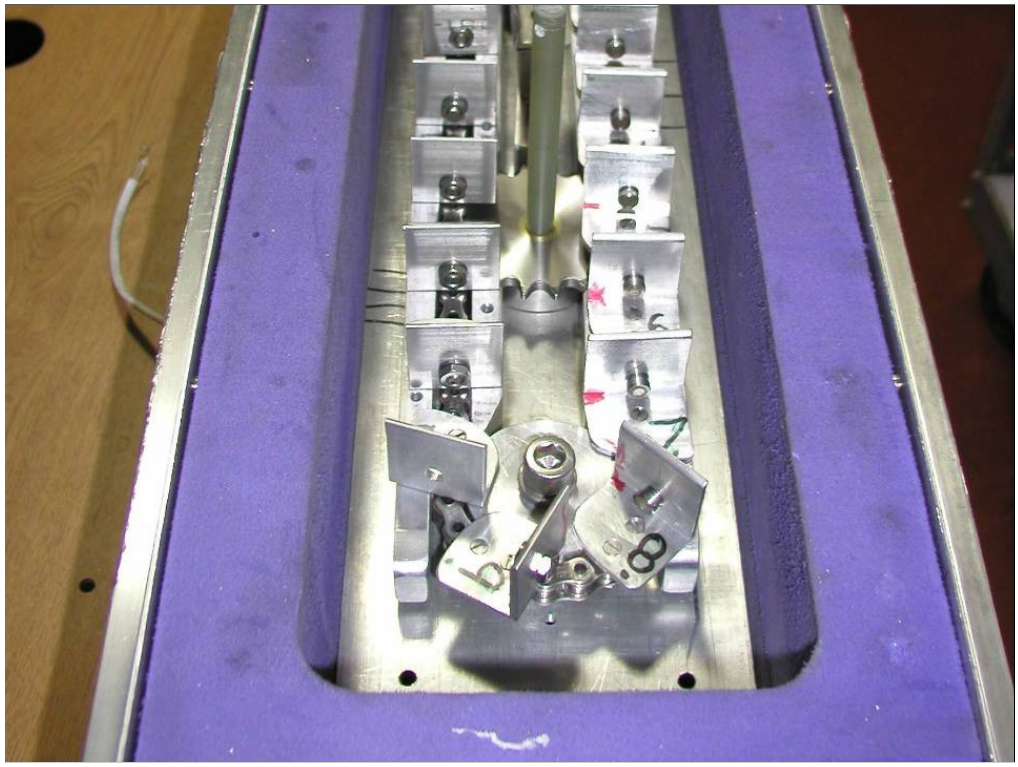


Holton, J. M. (2007). *J. Synchr. Rad.* **14**, 51-72.

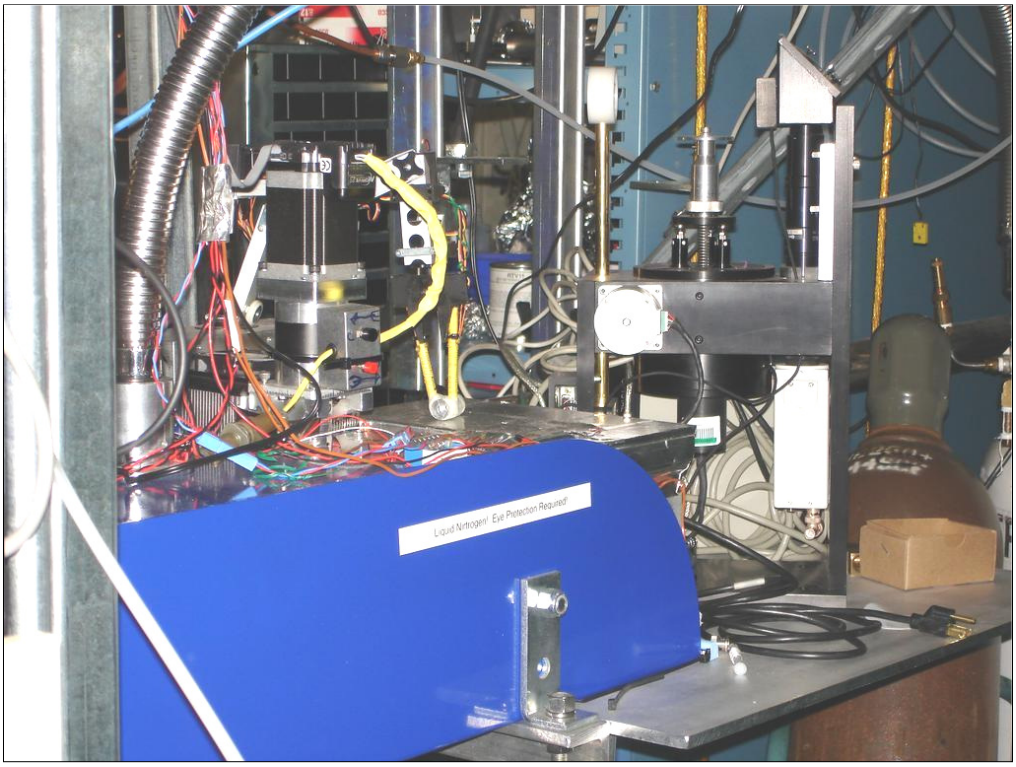
Radiation damage to Selenomethionine can be monitored by x-ray absorbance spectroscopy. The rate of damage varies widely. Sometimes it is slower than the visible loss of diffraction quality, and sometimes it is up to 5x faster. The damage rate to Se-C bonds is site-specific and depends on the microscopic structure of the sample and cannot be simply predicted. However, the condition of the Se-C bonds can be probed before and after each data collection. This type of damage assay is extensible to Br and Pt derivatives, and perhaps to others. A user-friendly utility for monitoring damage in real time at 8.3.1 is under development.



Cryogenic sample conveyor labyrinth installed in beamline 8.3.1. Samples can be inserted into the external port of the conveyor (right) and moved into the robot-accessible internal port (left) without ever turning off the x-rays and disrupting ongoing data collection.

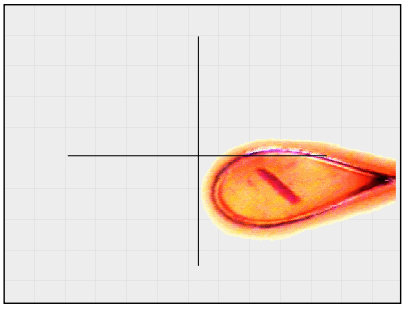


Sample conveyor internals with roller chain. Each magnet on the chain is the same as the one on the goniometer so any sample that can be mounted in the beamline can be mounted in the conveyor. When in operation, all of these “cogs” are under liquid nitrogen, which acts as a lubricant for the chain.

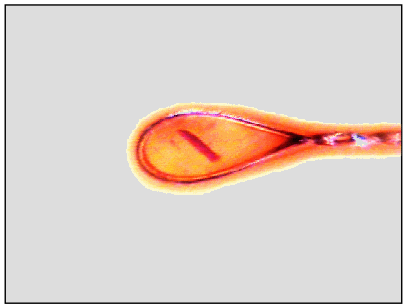


The Offline Target Indication System is now being installed on the external port of the sample conveyor. This station will be used to center and photograph samples. The images of the centered sample can be used to re-center it at a later date.

Re-centering

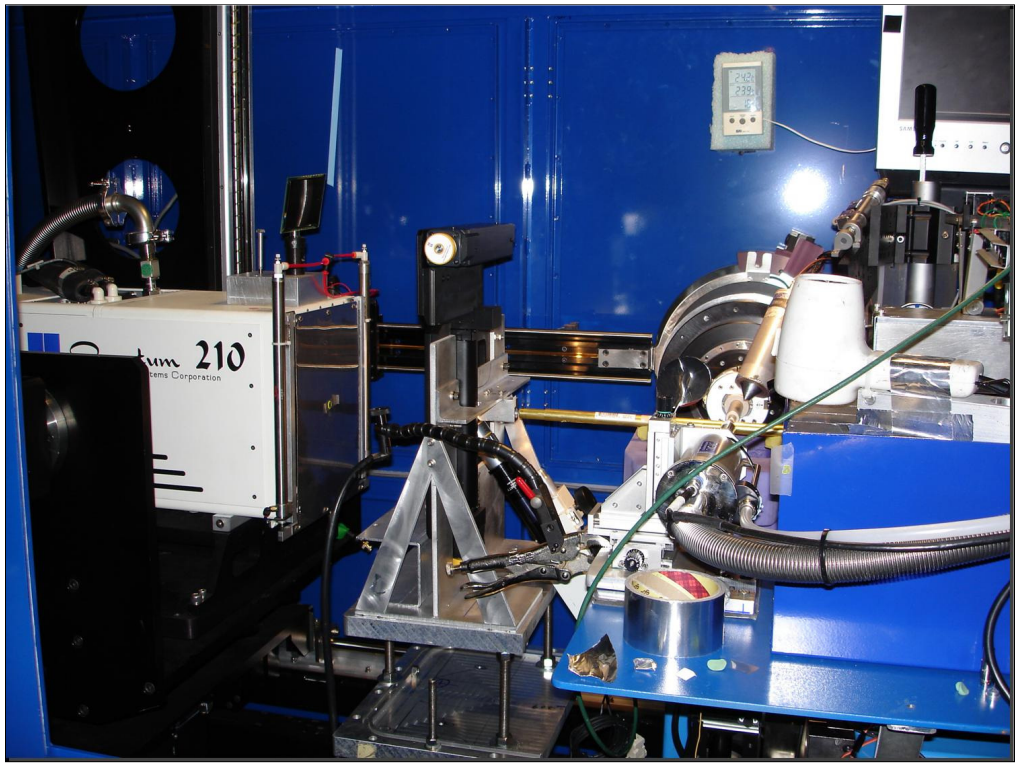


beamline
microscope

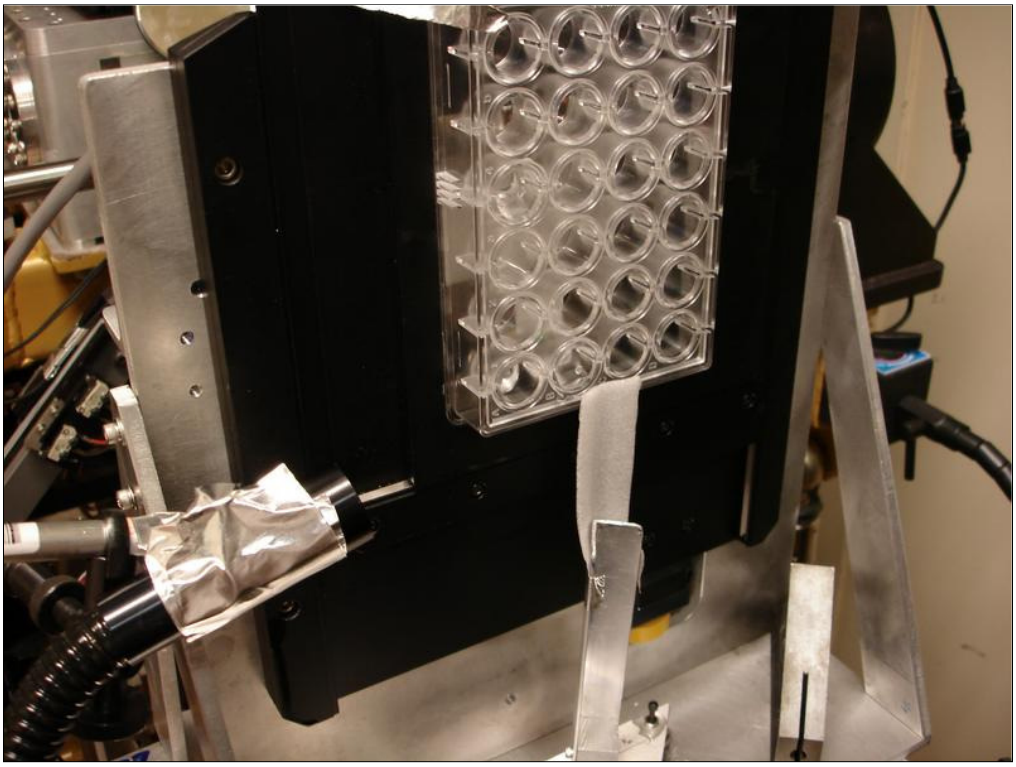


reference
image

Images of a previously centered sample can be used to re-center the sample automatically. A digital image of the sample when it was last centered is scanned over the digital image of the current sample position. The pixel offset that corresponds to the best match between the images is used to determine the physical translation required to place the crystal into the x-ray beam. Precisions of 4-5 microns are routinely achieved. The “phi” rotation of the sample is recovered by rotating the sample in 10 degree increments and finding the rotation that produces an image that best matches the reference image.



The new “tray goniometer” option shown installed in the 8.3.1 hutch. This device takes ~30 minutes to install ~300 mm downbeam from the “normal” protein crystallography sample mounting point. It will accept most any crystallization tray that can be rotated 90 degrees and probe arbitrary points in each crystallization drop with a 50 um x-ray beam.



Downbeam side of the “tray goniometer”. Shown here with a Limbro tray installed. The beamstop is mounted in the foam sheet.