A number of initiatives to extend the capabilities for macromolecular diffraction at CHESS are underway; particular emphasis is placed on the development of an excellent facility to deal with microcrystals, i.e. crystals with a diffracting volume less than about $10^{-5} \text{mm}^3$. Several projects related particularly to microcrystallography, but also of use with larger crystals, are in various stages of completion.

The remote crystal-centering system, which has been running stably on all MacCHESS stations for the past several running periods, has received many positive comments by users and staff. Inside the hutch, a high-quality digital video camera is operated by a server which provides the video stream to clients and controls zoom and focus; outside the hutch, on the station computer, a graphical interface is employed to view the crystal, adjust zoom and focus (by passing commands to the server), and center the crystal (by clicking on it, which sends commands to a Compumotor controller, via the data collection software, to move the appropriate motors).

A new version of the system, incorporating a number of enhancements and user-requested features, will be put into service during the summer running period. Built on the Java programming language, the new remote centering system is a single program that can act as either server or client. This will allow a user to make adjustments to a sample using the same interface whether inside or outside the hutch. In addition, projects are underway to incorporate digital image enhancement and fully automatic sample centering in future releases.

A recently-installed variable-intensity “macro-LED” light source, together with a simple piece of thin foam mounted below the beamstop to provide backlighting, aids in visualizing small crystals. Microfibers which fluoresce green when hit by the X-ray beam are used to match the height of the spindle rotation axis with the beam, and to set the cross-hairs displayed on the GUI (Fig 1) - there is no longer a need to run in and out of the hutch adjusting bits of burn paper to locate the beam!

The crystal automounter at F1 is now completed, tested, and ready for use (Fig 2). This device is based on the ALS automounter, with modifications developed at NSLS and here at CHESS. Up to 64 crystals at a time can be stored in a Dewar for automatic mounting. The ADX data collection software has been modified to permit automounting of a crystal before an exposure; the capability of screening a series of crystals will be available by fall 2005. To make it easier for first-time users to prepare samples for automounting, a kit of “pucks” for holding crystals, associated tools, and instructions (a DVD prepared at NSLS) may be borrowed from CHESS (Fig 3).

**Fig 1:** Fluorescent microfiber in use to determine beam position and adjust spindle height. The centering GUI provides the image of the fiber, a “midpoint tool” (the white lines), rotation of the spindle, and translations of the goniometer head (rotation and translation buttons not shown); a separate interface **rspec** is used to adjust the optical table height to place the center of spindle rotation on the X-ray beam. This image has been slightly processed for increased contrast.

**Fig 2:** Automounter and new lighting in place at F1. The gripper is the part of the automounter that actually picks up the crystal (from the Dewar below; the white slide just under the gripper moves out of the way), rotates, and puts it on the goniometer. The holes to the right of the slide are for the liquid nitrogen autofilling apparatus, which was removed for the photo.

**Fig 3:** Sample mounting kit to be loaned to users.
A helium-filled enclosure to reduce background scatter has been tested. Helium is substituted for nitrogen in the cryostream, without changing the 100 °K temperature of data collection. A plexiglass box, about 15 cm. on a side, with openings for spindle, collimator, cryostream, and diffracted rays, surrounds the crystal; it takes about 20 minutes to flush the box with helium. Diffraction patterns, shown in Fig 4, demonstrate a substantial reduction in background by going from nitrogen to helium; signal/noise for weak reflections is enhanced by a factor of 2-5. Improved signal-to-noise ratios not only yield better data statistics, but can also increase the practical limit of resolution of the data. This is particularly true of microcrystals, for which reflections tend to be weak in comparison to background scattering.

The visibility of fluorescent protein crystals is much enhanced over that of plain protein crystals when the sample is illuminated with short-wavelength (e.g. green) light and viewed through a long-wavelength (e.g. red) filter (Fig 5). Samples as small as 500 nm in diameter have been located in preliminary tests of this method.

Most crystallographers are familiar with the "Izit" test for protein crystals, in which a commercial blue dye is introduced into hanging drops containing already-formed crystals. While salt crystals remain colorless, protein crystals become stained and are easy to identify. Experiments conducted at MacCHESS indicate that many common dyes used in fluorescence microscopy are readily taken up in this manner by protein crystals; it is also possible to incorporate a dye into protein crystals during their growth, simply by including the dye in the crystallization solution. Beautiful red crystals, for example, may be produced from the industry standard lysozyme recipe by simply setting up hanging drops containing 1 μL of protein solution + 1 μL of well solution + 1 μL of dilute rhodamine in water.

In the cases studied so far, the dye does not significantly affect crystal quality or growth conditions. Preliminary diffraction data (see images in Fig 4) appear identical to those from undyed crystals.

MiTeGen (http://www.mitegen.com/), the company founded by Cornell physics professor Rob Thorne to make microfabricated crystal mounting scoops, has added some useful new products. In addition to the original MicroMounts™ for freezing small crystals, mounts are available for low-stress mounting and freezing of thin plates (MicroMesh™) and for room temperature mounting (MicroRT™) without the hassle of traditional X-ray capillaries. The MicroMesh tool can also be used to scoop up and freeze multiple microcrystals at one time – very useful for screening a flock of tiny crystals.

Experimental use of narrow-bandpass multilayer optics for crystallography is reported by Ulrich Englich on page 30.

Facility upgrades for improved efficiency include:

- upgraded computing equipment - data collection is now run by new AMD Opteron computers running Linux and equipped with 2 TB RAID systems for storage of user data, and dual-processor Opterons are replacing the aging Alphas for data processing (see article by Ernie Fontes, et al., "Networking and Computing at CHESS" on page 17 for more information).
- coordinated operation of software - scripts are in place to start, stop, or restart various processes needed for data collection, including crystal centering, with a single command. Communication between components of the MAD data collection software has been improved.
- quantum-4 upgrade - the computers that operate the Q-4 CCD detector used at F3 have been replaced, and their software modified, to reduce the dead time between exposures.