

# Microcrystallography at MacCHESS

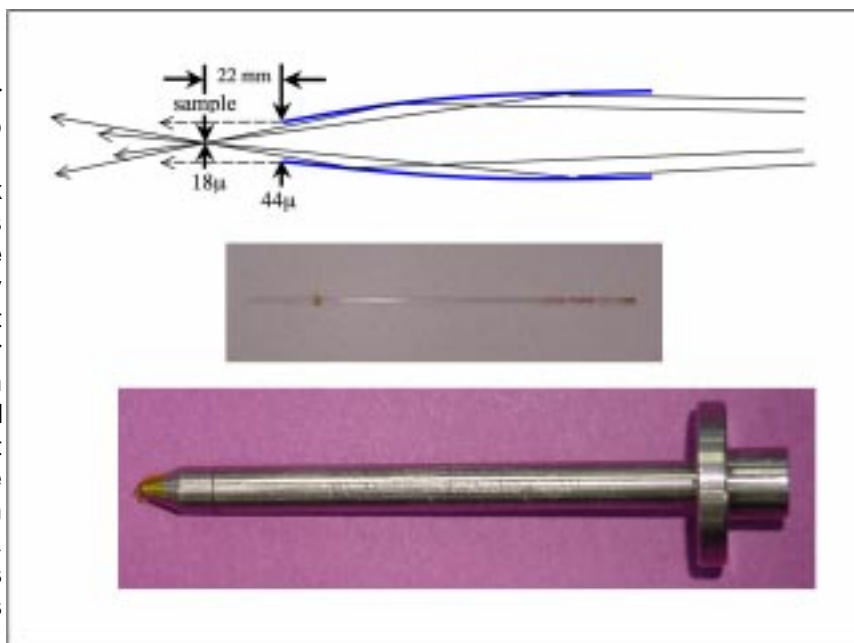
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It is not uncommon for users to bring crystals smaller than 30  $\mu\text{m}$  in size to MacCHESS hoping to obtain data. Indeed, many important proteins only produce crystals in this size range. With a beam diameter of 100  $\mu\text{m}$ , scattering from excess cryoprotectant and mounting loop material can degrade signal. Such crystals are often difficult to see, and present special problems such as increased radiation damage. The MacCHESS microcrystallography initiative is bringing a number of major improvements to our beamlines over the course of the next year that will dramatically enhance data collection on tiny crystals.

## Optics

Smaller x-ray beam diameters allow for reduced background scatter but also produce fewer photons per second overall unless a corresponding increase in flux is engineered. Custom capillary optics designed by Bilderback and Huang have achieved an 11-fold gain over the already doubly focused monochromatic beam at the F1 station [1-3]. With a beam diameter (FWHM) of 18  $\mu\text{m}$  and a typical beam divergence of 1.7 mrad, the optic should provide adequate spot separation for most common unit cell sizes (Figure 1). The capillary optic enclosure has been designed for easy interchange with a standard 100 $\mu\text{m}$  collimator for cases where lower beam divergence is necessary.



**Fig 1:** Single-bounce capillary optics for microcrystallography. The doubly-focused monochromatic beam at F1 station is focused to 18  $\mu\text{m}$  by a glass capillary (middle) resulting in an 11-fold gain in intensity. The capillary is housed in a standard collimator assembly for ease of interchange (bottom).

## Camera design

The introduction of air bearings for the oscillation stage, scheduled for installation on all stations during the Spring of 2004, will enable rapid rotation ( $180^\circ$  in 0.5 sec) with very high angular resolution ( $0.001^\circ$  steps with  $\pm 0.0002^\circ$  accuracy). This development should dramatically speed up both remote centering operations and MAD data collection. Based on recent tests with realistic load conditions, the phi axis is also expected to exhibit minimal translational wobble ( $0.025 \mu\text{m}$ ). With 18  $\mu\text{m}$  beam diameters and 10  $\mu\text{m}$  crystals, rotational stability is essential. Precise position control is also essential, and our improved oscillation camera design is capable of submicron sample translations.

## Remote centering and sample visualization

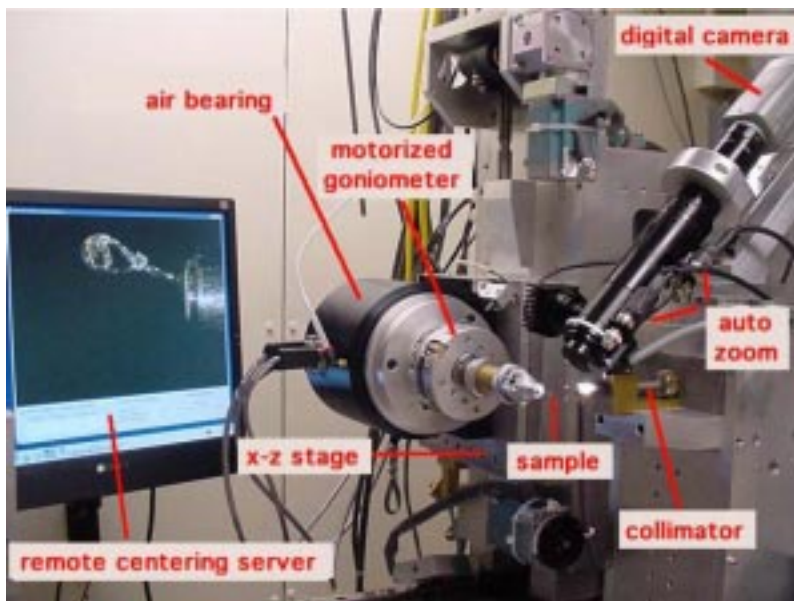
Remote sample centering and manipulation is an important component of future crystallography, not just in connection with automated sample mounting, but as a means of achieving greater accuracy in beam placement on the sample. Our graphical user interface (Figure 2) is a client-server system in which the in-hutch computer server broadcasts MPEG4-encoded images to connected client computers outside the hutch. The unique high-resolution digital video stream (1024x768 pixel images at 10-15 frames/sec) is not only capable of resolving greater detail than conventional

analog video webcams, but offers the possibility of real-time digital image enhancement. For the first time, users can zoom to arbitrary magnification without needing to readjust display crosshairs. Accurately calibrated distance measures and a beam spot circle scale automatically with zoom. Phi rotation and pin translation are coordinated with the data collection software so that future data collection protocols may involve sample translation during exposure. Rapid image-based point-and-click crystal centering is expected to come online at F1 during the Summer 2004 running period.

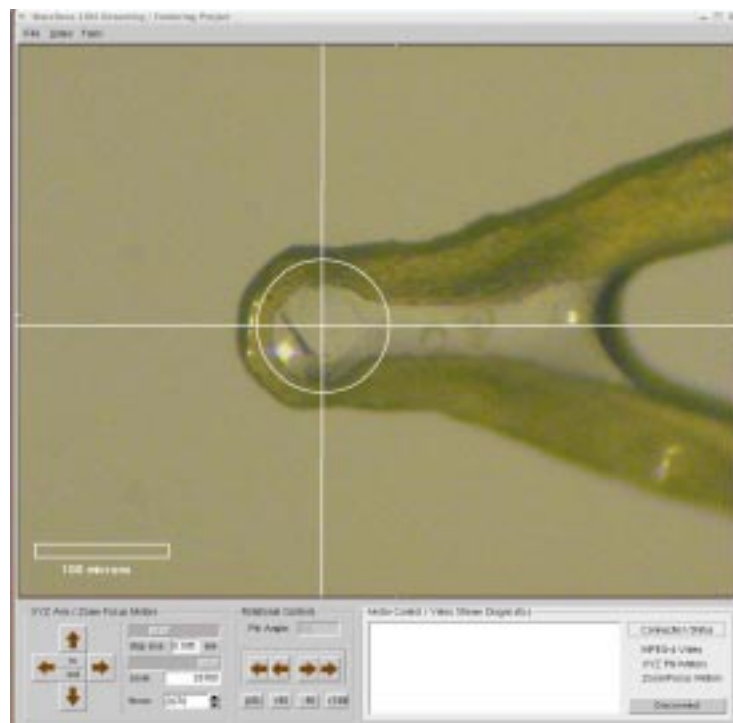
## Sample micromounts

Harvesting and mounting crystals smaller than 20  $\mu\text{m}$  presents a special challenge. Background scattering from loop material and cryoprotectant fluid are significant factors in the degradation of signal. Robert Thorne's group (Cornell, Physics) has produced special microfabricated crystal mounting loops that produce less scatter than conventional nylon-based loops and are designed to allow excess cryoprotectant to be more easily removed (Figure 3) [4]. Early experience at MacCHESS indicates that these new mounts will be an essential component of the microcrystallography effort.

**Fig 3:** Graphical user interface for remote centering. A high-resolution digital video stream produced by the server within the experimental hutch is decompressed and displayed by a client program running anywhere on the network. A microfabricated crystal mount designed by the Thorne group is shown.



**Fig 2:** New high-precision x-ray camera design (shown in F2 station). An air-bearing produces stable and rapid  $\phi$ -axis rotation. A motorized goniometer head is combined with high-resolution streaming digital video to enable remote operation.



## References:

- [1] D.H. Bilderback & R. Huang, "X-ray Test of Microfocusing Mono-capillary Optic for Protein Crystallography", Nucl. Instrum. & Meth. **A467** 970-973 (2001).
- [2] R. Huang & D.H. Bilderback, "Simulation of Microfocused Image Size from a One-bounce Glass Capillary", Nucl. Instrum. & Meth. **A467** 978-981 (2001).
- [3] R. Huang, and D. H. Bilderback, "Secondary Focusing for Micro-Diffraction Using One-Bounce Capillaries", submitted to proceedings of Synchrotron Radiation Instrumentation 2003 meeting held August 25-29, 2003 in San Francisco. Preprint available on-line at... [http://erl.chess.cornell.edu/Pub\\_Bilderback\\_group/Huang\\_Bilderback\\_Secondary\\_Focusing\\_SRI2003.pdf](http://erl.chess.cornell.edu/Pub_Bilderback_group/Huang_Bilderback_Secondary_Focusing_SRI2003.pdf)
- [4] R.E. Thorne, Z. Stum, J. Kmetko, K. O'Neill and R. Gillilan, "Microfabricated Mounts for High Throughput Macromolecular Cryocrystallography", J. Appl. Cryst. **36**, 1455-1460 (2003).