

Recent Developments In Scanning Microscopy at Stony Brook

C. Jacobsen, S. Abend, T. Beetz, M. Carlucci-Dayton, M. Feser, K. Kaznacheyev, J. Kirz, J. Maser¹, U. Neuhäusler², A. Osanna, A. Stein, C. Vaa, Y. Wang¹, B. Winn, S. Wirick

Department of Physics & Astronomy, State University of New York at Stony Brook, USA

¹*Present address: Advanced Photon Source, Argonne National Lab, USA*

²*Also: Institute for X-ray Physics, Universität Göttingen, Germany*

Abstract. Recent activities in scanning transmission x-ray microscopy at Stony Brook are outlined.

INTRODUCTION

Scanning transmission x-ray microscopes have been developed at Stony Brook since 1980 [1]. We summarize here our recent activities in scanning microscopy using undulator radiation. The Stony Brook STXMs make use of zone plates fabricated in a collaboration with Lucent Technologies Bell Labs [2, 3], and are used by a number of researchers as described in these proceedings.

THE NSLS X-1A MICROSCOPY BEAMLINES

The X-1 undulator at the National Synchrotron Light Source is a high-brightness source of soft x rays [4]. Upgrades to the storage ring, and improvements in its operating mode, have led to a twentyfold increase in source brightness over the past decade. Even so, the horizontal source output is into about 100 spatially coherent “modes,” while a diffraction-limited scanning microscope can only use one spatially coherent mode [5]. As a result, we are able to operate two scanning microscopes at the same time that a spectroscopy beamline (X-1B) is taking beam. The increased horizontal divergence of the source also means that the undulator peaks are spread out to a width of ~20 eV, so that XANES spectra can be acquired without a need to adjust the undulator tuning parameter K (see e.g., [6]).

In 1996, our microscopy beamline underwent a substantial upgrade [7] that included important contributions from H. Ade, C. Buckley, M. Howells, S. Hulbert, I. McNulty, and T. Oversluizen. The upgraded beamline (see Fig. 1) has a separate monochromator for each microscope end station, with a spectral resolving power of between 1000 and 5000, depending on entrance slit width and photon energy. A

stigmatic source is provided with adjustable size, allowing each scanning microscope endstation control over the tradeoff between flux and spatial resolution.

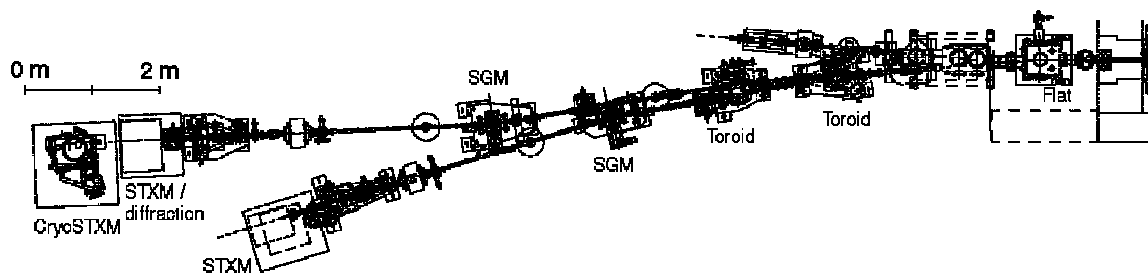


Figure 1. Schematic of the X-1A undulator beamlines at the National Synchrotron Light Source, Brookhaven National Laboratory. The undulator (with 37 periods of 8 cm length) is located about 12 m to the right of the “Flat” mirror at the right end of this diagram.

THE CRYO SCANNING TRANSMISSION X-RAY MICROSCOPE

Radiation damage is a limitation in studies of some specimens, especially wet biological specimens. The use of cryo methods for radiation damage protection in electron microscopy is well established [8-10], and cryo has begun to be employed in x-ray microscopy with great success [11-14].

At Stony Brook, we have developed a cryo scanning transmission x-ray microscope that makes use of a transmission electron microscope-type specimen holder (see Fig. 2) [13, 15]. With this system, we are able to take specimens that have been prepared on standard 3 mm electron microscope grids and load them into the microscope at temperatures below -150°C , for imaging at temperatures of about -165°C (see Fig. 3).

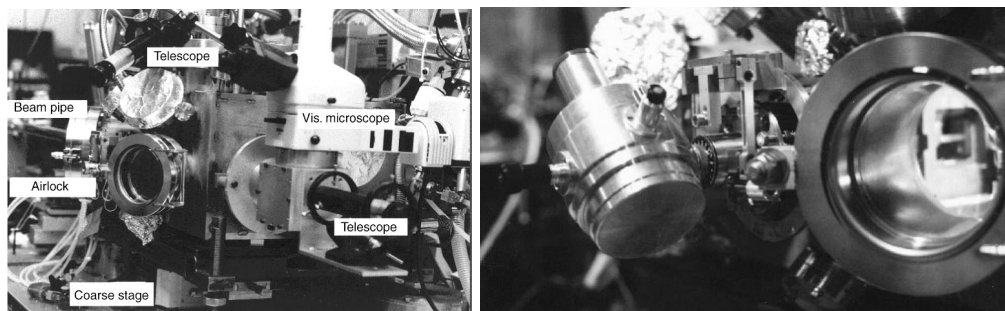


Figure 2. The cryo STXM. The x-ray beam enters through the beam pipe at left. At right is shown a closeup of a cryo holder rotated for tomography experiments; the holder tip can be seen extending across a square cutout in the piezo scanning stage inside the vacuum chamber.

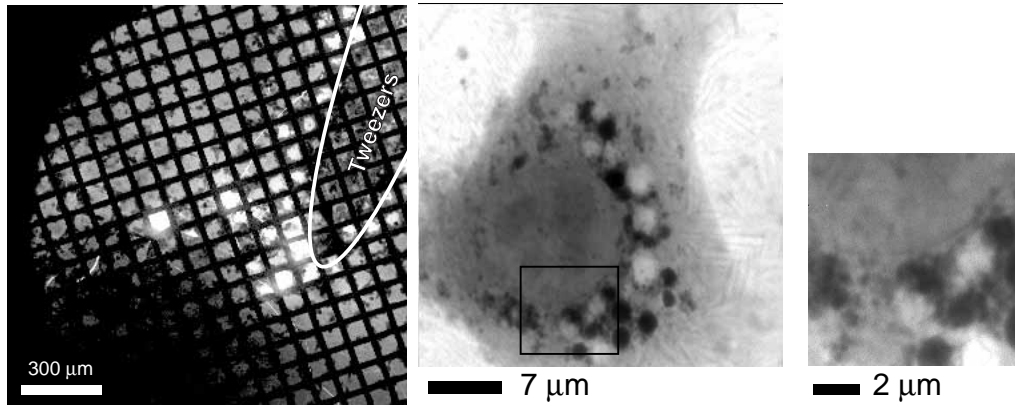


Figure 3. Grids of frozen hydrated cells can be loaded into the cryo STXM, and large area scans can be taken to identify regions with appropriate ice thickness (left). Higher resolution images can then be taken of smaller areas (center, right). Some ice crystal formation can be seen in the medium surrounding the specimen. From Maser *et al.* [15].

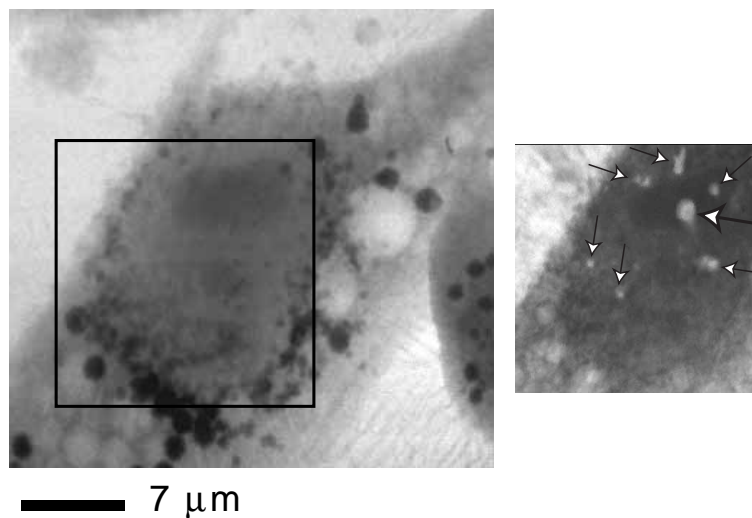


Figure 4. Several submicron regions of a frozen hydrated fibroblast were exposed to a dose of $\sim 10^{10}$ Gray. The image at left was taken *afterwards*, and shows no obvious sign of radiation damage. Upon slow warming of the sample, the free radicals created by exposure were able to diffuse and react, creating holes in the specimen (at right). From Maser *et al.* [15].

We have carried out studies of mass loss in the cryo STXM. In these studies, we find that radiation doses of up to about 10^{10} Gray can be tolerated by frozen hydrated fibroblasts before noticeable changes are observed (see Fig. 4) [15]. We have also used the cryo STXM to obtain the first 3D reconstruction of a frozen hydrated eucaryotic cell (see Fig. 5) [16].

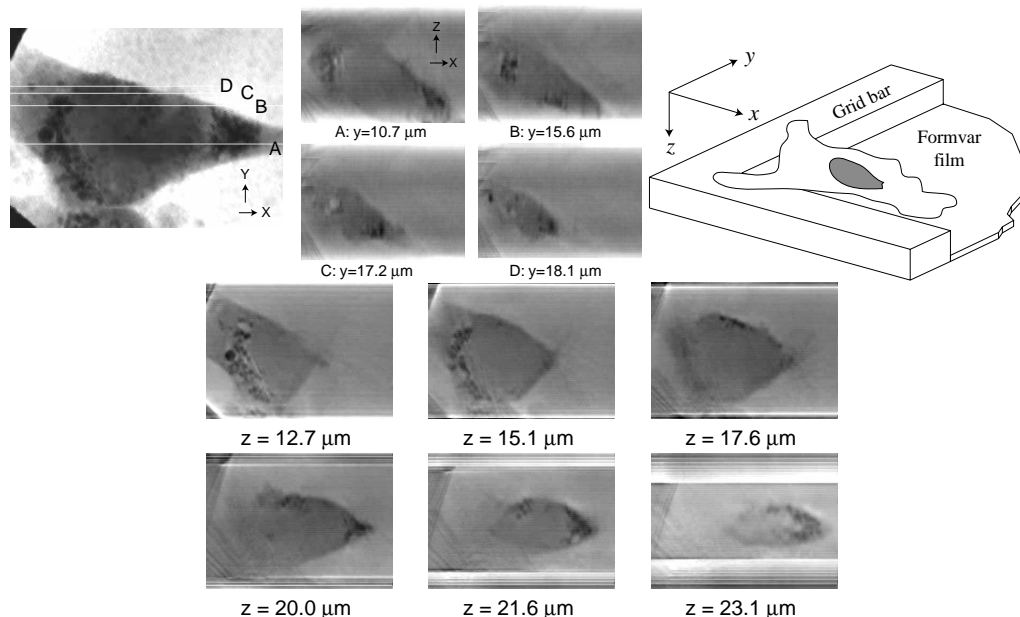


Figure 5. Tomography of a frozen hydrated fibroblast using cryo STXM. At top left is the 0° tilt image of a frozen hydrated fibroblast. The tomographic dataset, consisting of images from -55° to $+60^\circ$ in 5° tilt increments acquired over a 36 hour period, was reconstructed using ART. Y slices from the indicated planes into the paper are shown at top middle, and sections from different depth planes along the beam direction are shown at bottom left. The orientation of these axes, and of the cell, is shown at top right. In several cases, lipid-rich vesicles can be resolved in the reconstruction which lie on top of each other in the 0° tilt image. From Wang et al., [16].

ROOM TEMPERATURE SCANNING MICROSCOPY

While the cryo STXM offers important new capabilities for imaging, tomography, and spectroscopy of biological specimens, the majority of the studies carried out at X-1A make use of our older STXM [17, 18] to examine room temperature specimens. First tests of a new replacement for this microscope, plus improved x-ray detectors, are described in these proceedings [19].

Both the room temperature STXM and the cryo STXM have available a new mode of operation where a sequence of images is acquired at various photon energies [20]. These image “stacks” are then aligned to remove any position shifts from image to image, and absorption spectra can then be obtained from single image pixels or from user-defined regions of pixels (see Fig. 6).

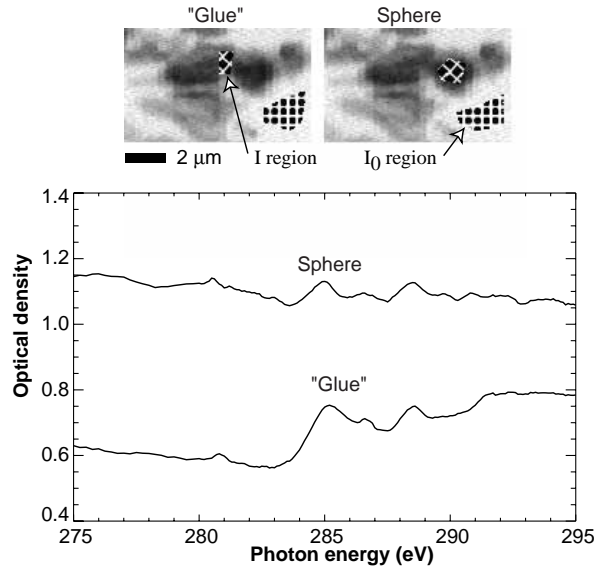


Figure 6. Images (top) and absorption spectra (bottom) of a region of the interplanetary dust particle L2009J4. A spectromicroscopy “stack” of images was taken over an energy range of 270 to 310 eV; the particular image shown twice above was taken at 296.25 eV., with a pixel size of 24 nm. From such an image sequence, one can define incident flux I_0 regions, and calculate the absorption spectrum for all other pixels or for groups of pixels as a transmitted flux I region. The larger grains of material (including the spherical grain at right) in this interplanetary dust particle thin section show relatively little carbon content, while the material surrounding the spheres (labeled “glue” in the image) shows more pronounced carbon content, as indicated by the increase in absorption near the carbon edge in the “glue” spectrum. Specimen prepared by L. Keller and imaged by G. Flynn *et al.*; for details see [20].

APPLICATIONS OF THE X-1A MICROSCOPES

Besides those applications briefly outlined here, the scanning transmission x-ray microscopes at X-1A are used for studies of polymers (see *e.g.*, [21] and papers in these proceedings), colloid chemistry and environmental science (see *e.g.*, [22] and papers in these proceedings), and other research areas described in these proceedings.

ACKNOWLEDGEMENTS

We gratefully acknowledge support from the Office of Biological and Environmental Research, U.S. DoE under contract DE-FG02-89ER60858, the National Science Foundation under grants DBI-9605045 and ECS-9510499, and the Alexander von Humboldt Foundation (Feodor-Lynen Fellowship, JM). This work was carried out at the National Synchrotron Light Source at Brookhaven National Laboratory, which is supported by the U.S. Department of Energy.

REFERENCES

1. Rarback, H., et al., Scanning X-ray Microscopy -- First Tests with Synchrotron Radiation, in Scanned Image Microscopy, E.A. Ash, Editor. 1980: London. p. 449-456.
2. Spector, S., C. Jacobsen, and D. Tennant, Process optimization for production of sub-20 nm soft x-ray zone plates. *Journal of Vacuum Science and Technology*, 1997. B 15(6): p. 2872-2876.
3. Tennant, D., et al. Electron beam lithography of Fresnel zone plates using a rectilinear machine and trilayer resists. These proceedings.
4. Rarback, H., et al., Coherent radiation for x-ray imaging---the soft X-ray undulator and the X1A beamline at the NSLS. *Journal of X-ray Science and Technology*, 1990. 2: p. 274-296.
5. Jacobsen, C., J. Kirz, and S. Williams, Resolution in soft x-ray microscopes. *Ultramicroscopy*, 1992. 47: p. 55-79.
6. Jacobsen, C., et al., The X-1A Scanning Transmission X-ray Microscope: Optics and Instrumentation, in *X-ray Microscopy IV*, V.V. Aristov and A.I. Erko, Editors. 1994, Bogorodskii Pechatnik: Chernogolovka, Russia. p. 304-321.
7. Winn, B., et al., X1A: second generation undulator beamlines serving soft x-ray spectromicroscopy experiments at the NSLS. *Reviews of Scientific Instruments*, 1996. 67(9): p. 1-4.
8. Taylor, K. and R. Glaeser, Electron diffraction of frozen, hydrated protein crystals. *Science*, 1974. 106: p. 1036-1037.
9. Taylor, K.A. and R.M. Glaeser, Electron microscopy of frozen hydrated biological specimens. *Journal of Ultrastructure Research*, 1976. 55: p. 448-456.
10. Echlin, P., *Low-Temperature Microscopy and Analysis*. 1992, New York: Plenum Publishing.
11. Schneider, G., et al., Cryo x-ray microscopy. *Synchrotron Radiation News*, 1995. 8(3): p. 19-28.
12. Schneider, G. and B. Niemann, Cryo x-ray microscopy experiments with the x-ray microscope at BESSY, in *X-ray Microscopy and Spectromicroscopy*, J. Thieme, et al., Editors. 1998, Springer-Verlag: Berlin. p. 1-25-34.
13. Maser, J., et al., Development of a cryo scanning x-ray microscope at the NSLS, in *X-ray Microscopy and Spectromicroscopy*, J. Thieme, et al., Editors. 1998, Springer-Verlag: Berlin. p. 1-35-44.
14. Schneider, G., Cryo x-ray microscopy with high spatial resolution in amplitude and phase contrast. *Ultramicroscopy*, 1998. 75: p. 85-104.
15. Maser, J., et al., Soft x-ray microscopy with a cryo STXM: I. Instrumentation, imaging, and spectroscopy. *Journal of Microscopy* (in press)
16. Wang, Y., et al., Soft x-ray microscopy with a cryo STXM: II. Tomography. *Journal of Microscopy* (in press).
17. Jacobsen, C., et al., Diffraction-limited imaging in a scanning transmission x-ray microscope. *Optics Communications*, 1991. 86: p. 351-364.
18. Zhang, X., et al., Micro-XANES: chemical contrast in the scanning transmission x-ray microscope. *Nuclear Instruments and Methods in Physics Research*, 1994. A 347: p. 431-435.

19. Feser, M., et al. Instrumentation advances and detector development with the Stony Brook scanning transmission x-ray microscope. These proceedings.
20. Jacobsen, C., Wirick, S., Flynn, G., Zimba, C. Soft x-ray spectroscopy from sub-100 nm regions. *Journal of Microscopy* (in press).
21. Ade, H., et al., X-ray spectroscopy of polymers and tribological surfaces at beamline X-1A at the NSLS. *Journal of Electron Spectroscopy and Related Phenomena*, 1997. 84: p. 53-72.
22. Neuhäusler, U., et al., Soft x-ray spectromicroscopy on solid stabilized emulsions. *Colloid & Polymer Science*, 1999. 277: p. 719-726.