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FINAL REPORT

APPROACH: We have developed a soft x-ray microscopy beamline (X-1A) at the National Synchrotron Light Source at Brookhaven National Laboratory. This beamline has been upgraded recently to provide two endstations dedicated to microscopy experiments. One endstation hosts a brand new copy of the redesigned room temperature scanning x-ray microscope (STXM), and the other end station hosts a cryo STXM and the original redesigned room temperature microscope, which has been commissioned and has started operation. Cryo STXM and the new microscope use the same new software package, running under the LINUX operating system. The new microscope is showing improved image resolution and extends spectromicroscopy to the nitrogen, oxygen and iron edges. These microscopes are used by us, and by users of the facility, to image hydrated specimens at 50 nm or better spatial resolution and with 0.1-0.5 eV energy resolution. This allows us to carry out chemical state mapping in biological, materials science, and environmental and colloidal science specimens. In the cryo microscope, we are able to do chemical state mapping and tomography of frozen hydrated specimens, and this is of special importance for radiation-sensitive biological specimens.

We have also developed new techniques such as an automated method for acquiring "stacks" of images for spectromicroscopic analysis, and methods for obtaining real-space images from the soft x-ray diffraction patterns of non-crystalline specimens. The user program provides opportunities for collaborators and other groups to exploit the techniques available and to develop them further.

RESULTS TO DATE: The cryo STXM effectively removes radiation damage as a limitation to soft x-ray microscopy studies in biology. With the cryo STXM, former graduate student Y. Wang has obtained a 100x100x250 nm 3D reconstruction of a frozen hydrated 3T3 fibroblast. More recently Ph. D. recipient Barry Winn has obtained an even higher resolution 3D image of a yeast cell. This is the highest resolution 3D image of the entirety of structures within a frozen-vitrified, non-chemically-fixed eucaryotic cell (rather than just of labeled structures). This instrument received a 1999 R&D 100 award.

PhD recipient A. Osanna has also demonstrated the ability to exploit x-ray absorption near-edge structure (XANES) resonances for chemical state mapping in the cryo STXM. With co-PI C. Jacobsen, and an improved version of his automated data acquisition program STACKS, she applied principal component analysis (PCA) to isolate major components milk. In addition, in collaboration with Dr. Rod Balhorn of LLNL, they used PCA and XANES spectromicroscopy to map protamine 1, protamine 2, protamine 2 precursor, and histone content in human sperm to better understand the relationship between DNA packing and male infertility. With the room temperature STXM, former graduate student U. Neuhaeusler used XANES to study how layered double hydroxides and clays cage oil in a new surfactant-free emulsion developed by S. Abend and G. Lagaly of U. Kiel, Germany; these new emulsions offer improved environmental and toxicological properties for cosmetics and oral drug delivery systems. Postdoc Thorsten Schaefer has used XANES to study the effects of organic coatings on clays on the transport of radionuclides in ground water.

Former graduate student J. Miao obtained images of microfabricated test patterns at high resolution by reconstructing their soft x-ray diffraction patterns; this offers an approach to excede the 30 nm resolution limit of today's zone plates. This work was published in the July 22, 1999 issue of Nature, and has attracted much attention, including a news story in Science (July 23). In a major step toward extending this

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technique to biological applications, graduate student David Shapiro recently obtained diffraction patterns from frozen hydrated yeast cells. The hardware was modified to allow higher resolution diffraction patterns to be collected, and to obtain in-situ low-resolution x-ray micrographs. These micrographs can be used to fill the hole in the diffraction pattern caused by the beam stop, and to create the "finite support", needed for the reconstruction of the high-resolution image.

These and other results are described further in an Adobe Acrobat file available at http://xray1.physics.sunysb.edu/downloads/brochure.pdf

DELIVERABLES: In 2000, we provided 134 days of experimental time on the room temperature STXM to collaborators and outside users for their studies of bone tissue, polymers, geochemical specimens, and soil and environmental specimens. We had the following publications:

1. M. Feser, T. Beetz, M. Carlucci-Dayton, and C. Jacobsen, ``Instrumentation advances and detector development with the Stony Brook scanning transmission x-ray microscope," in W. Meyer-Ilse, T. Warwick, and D. Attwood, eds., X-ray Microscopy: Proceedings of the Sixth International Conference, (Melville, NY), American Institute of Physics, 2000, pp. 367-372.

2. C. Jacobsen, S. Abend, T. Beetz, M. Carlucci-Dayton, M. Feser, K. Kaznacheyev, J. Kirz, J. Maser, U. Neuhaeusler, A. Osanna, A. Stein, C.Vaa, Y. Wang, B. Winn, and S. Wirick, ``Recent developments in scanning microscopy at Stony Brook," in W. Meyer-Ilse, et al. (ref.1), pp. 12-18.

3. C. Jacobsen, G. Flynn, S. Wirick, and C. Zimba, ``Soft x-ray spectroscopy from image sequences with sub-100 nm spatial resolution," Journal of Microscopy, vol. 197, no. 2, pp. 173-184, 2000.

4. J. Maser, A. Osanna, Y. Wang, C. Jacobsen, J. Kirz, S. Spector, B. Winn, and D. Tennant, ``Soft x-ray microscopy with a cryo STXM: I. Instrumentation, imaging, and spectroscopy," Journal of Microscopy, vol. 197, no. 1, pp. 68-79, 2000.

5. J. Miao, J. Kirz, and D. Sayre, ``The oversampling phasing method," Acta Crystallographica D: Biological Crystallography, vol. 56, pp. 1312-1315, 2000.

6. U. Neuhaeusler, C. Jacobsen, D. Schulze, D. Stott, and S. Abend, ``A specimen chamber for soft x-ray spectromicroscopy on aqueous and liquid samples," Journal of Synchrotron Radiation, vol. 7, pp. 110-112, 2000.

7. U. Neuhaeusler, S. Abend, S. Ziesmer, D. Schulze, D. Stott, K. Jones, H. Feng, C. Jacobsen, and G. Lagaly, ``Soft x-ray spectromicroscopy on hydrated colloidal and environmental science samples," in W. Meyer-Ilse, et al. (Ref 1) pp. 323-328.

8. A. Osanna and C. Jacobsen, ``Principle component analysis for soft x-ray spectromicroscopy," in W. Meyer-Ilse, et al. (Ref 1) pp. 350-357.

9. D. Tennant, S. Spector, A. Stein, and C. Jacobsen, ``Electron beam lithography of Fresnel zone plates using a rectilinear machine and trilayer resists," in W. Meyer-Ilse, et al. (Ref 1) pp. 601-606.

10. S. Vogt, H. N. Chapman, C. Jacobsen, and R. Medenwaldt, ``Dark field x-ray microscopy: the effects of condenser/detector aperture," Ultramicroscopy (accepted), vol. 0, p. 0, 2000.

11. Y. Wang, C. Jacobsen, J. Maser, and A. Osanna, ``Soft x-ray microscopy with a cryo STXM: II. Tomography," Journal of Microscopy, vol. 197, no. 1, pp. 80-93, 2000.

12. B. Winn, H. Ade, C. Buckley, M. Feser, M. Howells, S. Hulbert, C. Jacobsen, K. Kaznacheyev, J. Kirz, A. Osanna, J. Maser, I. McNulty, J. Miao, T. Oversluizen, S. Spector, B. Sullivan, S. Wang, S. Wirick, and H. Zhang, ``Illumination for coherent soft x-ray applications: the new X1A beamline at the NSLS," Journal of Synchrotron Radiation 7, 395-404 (2000)

13. F. Polack, D. Joyeux, M. Feser, D. Phalippou, M. Carlucci-Dayton, K. Kaznacheyev, and C. Jacobsen, ``Demonstration of phase contrast in scanning transmission x-ray microscopy: comparison of images obtained at NSLS X1-A with numerical simulations," in W. Meyer-Ilse, et al. (Ref.1) pp 573-580.

14. C. Schmidt, J. Thieme, U. Neuhaeusler, U. Schulte-Ebbert, G. Abbt-Braun, C. Specht, and C. Jacobsen, ``Association of particles and structures in the presence of organic matter," in W. Meyer-Ilse, et al. (Ref. 1) pp 313-318

15. J. Miao and D. Sayre, " On possible extensions of X-ray crystallography through diffraction-pattern oversampling", Acta Cryst. A56, 596-605 (2000).

16. J. Miao, P. Charalambous, J. Kirz and D. Sayre, "Extending the Methodology of X-ray Crystallography to Non-Crystalline Specimens", in: Synchrotron Radiation Instrumentation: Eleventh National Conference, P. Pianetta et al. eds. (American Institute of Physics, Melville, NY. 2000) pp. 3-6.

17. M. C. Dayton, ``Nomarski differential phase contrast using a scanning transmission x-ray microscope," 2000. MSI thesis, Department of Physics and Astronomy, SUNY at Stony Brook.

18. B. L. Winn, "Tomography with a cryogenic scanning transmission x-ray microscope." PhD thesis, Department of Physics and Astronomy, SUNY at Stony Brook, Nov. 2000.

19. A. Osanna, "Soft x-ray spectromicroscopy in a cryo scanning transmission x-ray microscope." Ph.D. thesis, Department of Physics and Astronomy, SUNY at Stony Brook, 2000.

Co-PI Chris Jacobsen has been named recipient of the 2001 Presidential Award of the Microprobe Analysis Society as the "Outstanding Young MAS Scientist related to his pioneering work in x-ray microscopy".

Reprints were removed for separate processing - 5/28/02