ELSEVIER Nuclear Instruments and Methods in Physics Research A 467–468 (2001) 841–844

NUCLEAR INSTRUMENTS & METHODS IN PHYSICS RESEARCH Section A

www.elsevier.com/locate/nima

Soft X-ray microscopy to 25 nm with applications to biology and magnetic materials

G. Denbeaux[a,*], E. Anderson[a], W. Chao[a,b], T. Eimüller[c], L. Johnson[a], M. Köhler[c], C. Larabell[d], M. Legros[a], P. Fischer[c], A. Pearson[a], G. Schütz[c], D. Yager[d], D. Attwood[a,b]

[a] *Center for X-Ray Optics, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA*
[b] *Electrical Engineering and Computer Sciences Department, University of California, Berkeley, CA 94720, USA*
[c] *Experimental Physics IV, University of Würzburg, D97074 Würzburg, Germany*
[d] *Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA*

Abstract

We report both technical advances in soft X-ray microscopy (XRM) and applications furthered by these advances. With new zone plate lenses we record test pattern features with good modulation to 25 nm and smaller. In combination with fast cryofixation, sub-cellular images show very fine detail previously seen only in electron microscopy, but seen here in thick, hydrated, and unstained samples. The magnetic domain structure is studied at high spatial resolution with X-ray magnetic circular dichroism (X-MCD) as a huge element-specific magnetic contrast mechanism, occurring e.g. at the $L_{2,3}$ edges of transition metals. It can be used to distinguish between in-plane and out-of-plane contributions by tilting the sample. As XRM is a photon based technique, the magnetic images can be obtained in unlimited varying external magnetic fields. The images discussed have been obtained at the XM-1 soft X-ray microscope on beamline 6.1 at the Advanced Light Source in Berkeley.

Keywords: X-ray microscopy; Cryogenic preservation; High resolution; Magnetic imaging

1. Introduction

The XM-1 X-ray microscope is located at the Advanced Light Source and provides high spatial resolution imaging of thick ($<10\,\mu m$) samples. The design allows a high throughput of a variety of samples in a wide variety of applications including biology, environmental science and materials science [1–3].

The illumination is provided by bending magnet radiation from the Advanced Light Source which is projected onto the sample through a condenser zone plate lens. The present condenser zone plate has a diameter of 9 mm, an outer zone width of 55 nm, and 41,000 zones. The illumination energy can be changed by the linear monochromator, which is composed of the condenser zone plate and a pinhole (approximately 10 μm aperture) near the sample plane (typically 100 μm from the sample plane). Due to the chromatic aberrations of zone plates, simply shifting the distance between the

*Corresponding author. Fax: +1-510-486-4550.
E-mail address: gpdenbeaux@lbl.gov (G. Denbeaux).

0168-9002/01/$ - see front matter
PII: S0168-9002(01)00480-6

condenser and the pinhole/sample plane shifts the illumination energy, which can be changed between 250 and 900 eV, and has been measured to have a spectral resolution of $E/\Delta E = 700$ [4].

The radiation passing through the sample is projected through the micro zone plate onto a CCD camera. The present micro zone plate has an outer zone width of 25 nm and a diameter of 63 μm. Both the micro zone plate and the condenser zone plate were fabricated by electron beam lithography by Erik Anderson at the Nanofabrication Laboratory in the Center for X-ray Optics [5]. The CCD camera is a 1024×1024 pixel array which is back-thinned and back-illuminated. It has a quantum efficiency of approximately 60–70% in the range of energies that the microscope operates.

Sample positions and focus can be pre-selected in a custom Zeiss Axioplan visible light microscope which is mutually indexed with the sample stage of XM-1. X–Y position accuracy is typically 2 μm over a 3 mm field with focal accuracy of 1 μm. This helps to allow the high throughput of samples. During a typical day, hundreds of images are collected.

The field of view of the microscope is 10 μm. In order to image larger samples, there is an automated montage assembly process which builds a larger image based on a series of sub-fields [6]. Using cross-correlation techniques, the smaller images are placed at the proper locations creating a nearly seamless montage.

2. Spatial resolution

In order to test the spatial resolution of the microscope, we used test patterns of grating patterns of various linewidths and duty cycles. They were fabricated with the same electron beam lithography tool that is used for the zone plates. An image of one test pattern is shown in Fig. 1(a). This test pattern has lines (light regions) with a width of 15 nm, separated by 45 nm spaces. The intensity across the lines is shown in Fig. 1(b). This shows a contrast of approximately 43%. This pattern of lines and spaces with a half-period of 30 nm can clearly be resolved. Another pattern

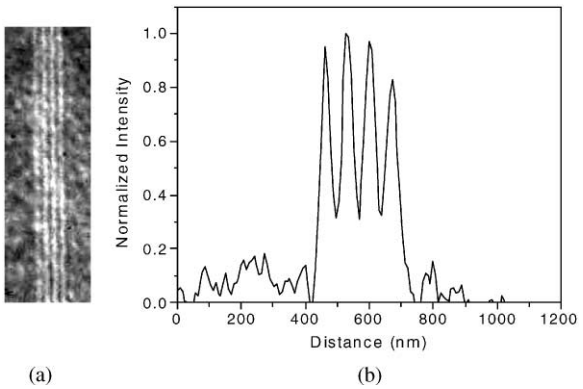

Fig. 1. (a) Image of test pattern with 15 nm lines (light) and 45 nm spaces (dark). The lines and spaces can clearly be resolved. (b) Intensity across the pattern averaged over adjacent line scans. This shows a contrast across the test pattern of approximately 43%.

with a half-period of 25 nm could also clearly be resolved and had a contrast of approximately 24%. We have not yet had a pattern with a half-period smaller than 25 nm, but if we project the modulation to smaller patterns, we expect to cross the Rayleigh Resolution Criterion of 15.3% contrast with a half-period pattern of 23 nm [7].

3. Magnetic materials

X-ray magnetic circular dichroism (X-MCD), i.e. the dependence of the absorption of circularly polarized X-rays onto the projection of the magnetization in a ferromagnetic absorber, yields magnetic contributions to the absorption cross-section as high as 25% for element-specific core level absorption edges, as e.g. the $L_{2,3}$ edges of transition metals. It has been shown recently that in combination with microscopies like photoemission electron microscopy [8] or soft X-ray microscopy [9], this can serve as a huge contrast mechanism to image the magnetic domain structure with high lateral resolution. Working in transmission, the magnetic absorption is conveniently measured by recording the transmitted photon intensities. Elliptically polarized light is obtained at the XM-1 from off-axis bend magnet radiation. A typical example is shown in Fig. 2

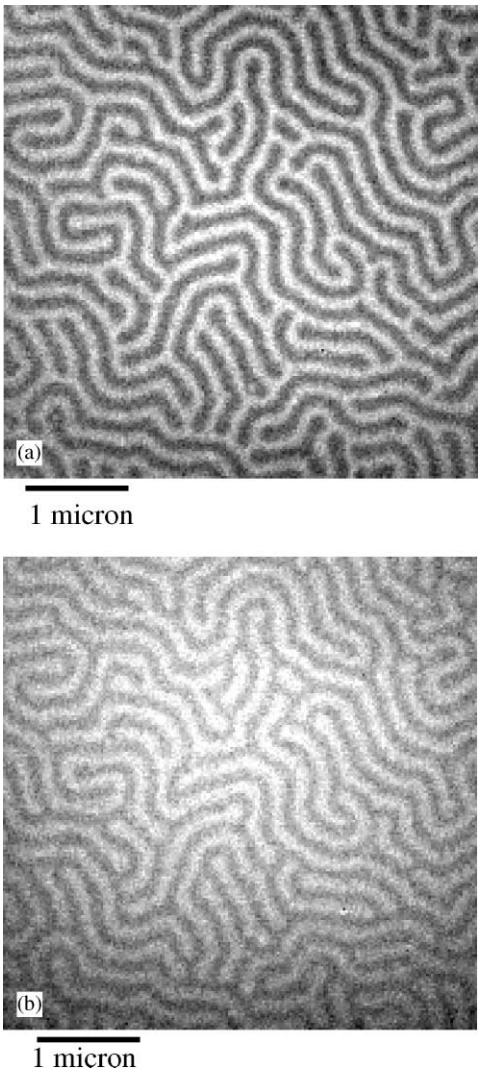

Fig. 2. Images of magnetization within an iron gadolinium multilayer, imaged at the iron L_{III} (a) and L_{II} (b) edges (707.5 and 720.5 eV, respectively). The expected contrast reversal of X-MCD between the edges can be seen.

where the self-organized magnetic domain pattern is shown. It has been obtained in a (0.4 nm Fe/0.4 nm Gd) $\times$ 75 multilayer system with a pronounced out-of-plane anisotropy. To account for the limited penetration of soft X-rays, it has been prepared on a 35 nm Si_3N_4 membrane. The dark/light regions correspond to the Fe magnetization with its direction pointing in/out of the paper plane. As expected from the different spin–orbit coupling, the magnetic contrast has a reversal between the L_3 and the L_2 edges which is a direct proof of the magnetic character of the pattern observed.

This is a photon based magnetic microscopy; thus, in principle, the domain structure can be recorded in unlimited external magnetic fields, which is of outstanding importance e.g. to proof the functionality of current devices, like magnetic sensors, MRAM, etc. The XM-1 is currently limited to apply magnetic fields up to $\pm$ 1000 Oe fields; however, this will be extended by a factor of three in the near future.

Since it is the projection of the local magnetization onto the photon propagation direction providing the contrast, in-plane magnetization, which is the most favorable configuration for magnetic systems of low dimensionality can be addressed by tilting the sample relative to the photon propagation direction. The first results have been recently obtained at the XM-1 [10]. Furthermore, the M-TXM allows to distinguish between in-plane and out-of-plane contributions to the magnetic domain structure. Together with the high sensitivity down to a few nanometers thickness due to the large magnetic contrast, this technique allows the study of magnetic microsctructures and the reaction to external fields in current technologically relevant magnetic systems, like magnetic sensors (GMR, TMR), nanostructures, patterned media (MRAM) and high density storage media (magneto-optics).

4. Cryogenic sample preservation

In order to preserve the structural integrity of biological samples, a cryogenic sample holder has been built. The radiation dose for a typical exposure is approximately 10^7 Gy, which in some circumstances is enough to change the morphology of hydrated, room temperature biological samples [11,12]. The samples are frozen at a rate of about 3000°C/s to a temperature of about -130°C where they are maintained. The quick rate of freezing and the low final temperature are necessary to prevent the formation of ice crystals on a scale large enough to damage the sample.

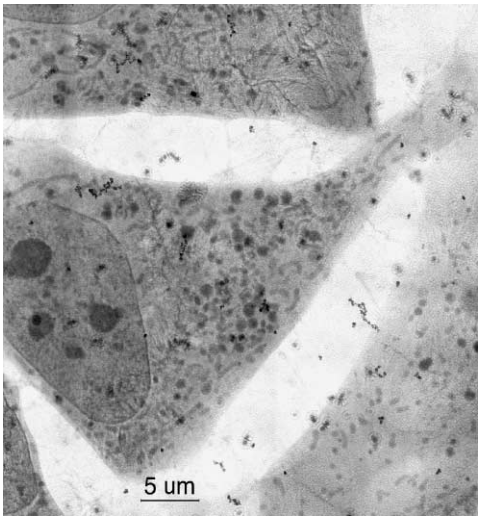

Fig. 3. Image of cryogenically preserved 3T3 fibroblast cells.

The freezing is accomplished by blowing helium gas, cooled to liquid nitrogen temperature, across the sample. Once the sample is properly frozen, it is able to withstand many exposures without any apparent changes in the morphology. During one test, the edge of the nuclear membrane of a fibroblast cell was imaged 40 times with no noticeable changes due to the accumulated radiation dose. Fig. 3 shows a cryogenically preserved 3T3 fibroblast cell. The cryogenic preservation allows imaging of the cell with remarkable detail.

5. Conclusion

Recent qualitative improvements including improved spatial resolution, cryogenic sample preservation, and the ability to image magnetic domains within samples provide new opportunities for X-ray microscopy as an important scientific tool. In particular, soft X-ray microscopy in combination with X-MCD for imaging of magnetic microstructure is a powerful tool for technologically relevant current nanopatterned magnetic media. With these capabilities, the XM-1 is in use for high resolution imaging of a wide variety of scientific studies including biology and magnetic materials.

References

[1] W. Meyer-Ilse, G. Denbeaux, L.E. Johnson, W. Bates, A. Lucero, E. Anderson, The high resolution X-ray microscope, XM-1, in: W. Meyer-Ilse, T. Warwick, D. Attwood (Eds.), X-Ray Microscopy, Vol. 507, American Institute of Physics, Melville, 2000, pp. 129–134.
[2] W. Meyer-Ilse, H. Medecki, L. Jochum, E.H. Anderson, D. Attwood, C. Magowan, R. Balhorn, M. Moronne, D. Rudolph, G. Schmahl, Synchrotron Radiat. News 8 (1995) 23–33.
[3] P. Fischer, T. Eimüller, G. Schütz, G. Bayreuther, S. Tsunashima, N. Takagi, G. Denbeaux, D. Attwood, magnetic domains in nanostructured media studied with M-TXM, J. Synchrotron Rad. 8 (2001) 325.
[4] G. Denbeaux, L.E. Johnson, W. Meyer-Ilse, Spectromicroscopy at the XM-1, in: W. Meyer-Ilse, T. Warwick, D. Attwood (Eds.), X-Ray Microscopy, Vol. 507, American Institute of Physics, Melville, 2000, pp. 478–483.
[5] E.H. Anderson, D.L. Olynick, B. Harteneck, E. Veklerov, G. Denbeaux, W. Chao, A. Lucero, L. Johnson, D. Attwood Jr., Nanofabrication and Diffractive Optics for High-Resolution X-ray Applications, J. Vac. Sci. Technol. 18 (6) (2000) 2970.
[6] B. W. Loo Jr., W. Meyer-Ilse, S.S. Rothman, J. Microsc. Part 2, 197 (2000) 185.
[7] M. Born, E. Wolf, Principles of Optics, 6th Edition, Cambridge University Press, Cambridge, 1980.
[8] J. Stohr, Y. Wu, B.D. Hermsmeier, M.G. Samant, G.R. Harp, S. Koranda, D. Dunham, B.P. Tonner, Science 259 (1993) 658.
[9] P. Fischer, G. Schütz, G. Schmahl, P. Guttmann, D. Raasch, Z. Phys. B 101 (1996) 313.
[10] P. Fischer, T. Eimüller, G. Schütz, M. Köhler, G. Bayreuther, G. Denbeaux, D. Attwood, Study of in-plane magnetic domains with Magnetic Transmission X-ray Microscopy, J. Appl. Phys. (2001), submitted.
[11] G. Schneider, Ultramicroscopy 75 (1998) 85.
[12] J. Maser, C. Jacobsen, J. Kirz, A. Osanna, S. Spectro, S. Wang, J. Warnking, Development of a cryo scanning transmission X-ray microscope at the NSLS, in: J. Thieme, G. Schmahl, D. Rudolph, E. Umbach (Eds.), X-ray Microscopy and Spectromicroscopy, Springer, Berlin, 1992, pp. I–35.