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Imaging X-Ray Microscope with Zone Plates and Its Application to Biological Specimens at UVSOR

N. Watanabe[1], S. Aoki[1], Y. Shimanuki[2], K. Kawasaki[2], M. Taniguchi[3], E. Anderson[4], D. Attwood[4], D. Kern[5], S. Shimizu[6], H. Nagata[6], Y. Horikawa[7], S. Mochimaru[7] and H. Kihara[8*]

[1]*Institute of Applied Physics, Tsukuba University, Tsukuba 305, Japan*
[2]*Department of Oral Anatomy, Tsurumi University, Yokohama 230, Japan*
[3]*Department of Physics, Nagoya University, Nagoya 464, Japan*
[4]*Lawrence Berkeley Laboratory, Berkeley, CA, USA*
[5]*IBM Research Center Yorktown Heights, NY, USA*
[6]*Nikon Corp., Nishi-ooi, Shinagawa-ku, Tokyo 140, Japan*
[7]*Olympus Optical Co. Ltd., Hachioji-shi, Tokyo 192, Japan*
[8]*Physics Laboratory, Kansai Medical University, Hirakata 573, Japan*

INTRODUCTION

Soft x-ray microscopes offer observation methods for wet biological specimen with higher resolution than that of optical microscopes (Sayre et al. 1977, Rudolph et al. 1992). We have been assembling imaging microscopes with zone plates at UVSOR BL8A [synchrotron radiation facility (750 MeV, 200mA) at Institute for Molecular Science, Okazaki, Japan] (Watanabe et al. 1992).

In our previous report, a 63nm line and space pattern could be resolved, and dry biological specimen could be observed (Watanabe et al. in press). As a next step, an environmental chamber (wet cell) was made and wet biological specimens were observed at 2.4nm. In the present microscope, the numerical aperture of a condenser zone plate (CZP) was much smaller than that of an objective zone plate (OZP). To adjust both the numerical apertures, an ellipsoidal condenser mirror system was made and imaging test was performed at 3.2nm.

OPTICAL SYSTEM

The optical arrangement is shown in Fig.1. The characteristics of the zone plates are CZP (diameter: 4.3mm, outermost zone width: 0.25μm, material: Au 0.2μm, support: SiN 0.1μm) and OZP (diameter: 50μm, outermost zone width: 45nm, zone material: Ni 0.13μm, support: SiN 0.1μm) (Anderson et al. 1992), respectively. Characteristics of the other optical elements are the filter (SiN 0.1μm and Ti 55nm), the mask (a 90% transparent Ni mesh of which the ϕ 2.4mm area is covered with Al foil), and the pinhole (ϕ 20μm).

The CZP monochromatized the soft X-ray and condensed it at the properly placed pinhole. A wavelength of 3.2nm was selected for imaging test. The third order radiation of the CZP was used, because the size of the source image at 3.2nm by the first order radiation was large compared with that of the pinhole. The calculated monochromaticity was 108 from the relationship $\lambda/\Delta\lambda = D/2d$, where λ: wavelength, D: a diameter of CZP, d: a larger diameter between that of the pinhole and the image of the source (Niemann et al. 1974).

The specimen was imaged by OZP. The magnification ratio was 740. The image was focussed at the outside of the zero-th order radiation of OZP. A film (Kodak T-max 400) and a microchannel plate (MCP) were set at an image plane. When the MCP was used, images were converted to visible ones by a fluorescent plate (FP), and monitored by a SIT camera(C2400, Hamamatsu Photonics K.K.). The images from the SIT camera were digitized, accumulated, subtracted the background data (accumulated records of the same number of frames without x-ray illumination) and then stored

*To whom correspondence should be addressed.

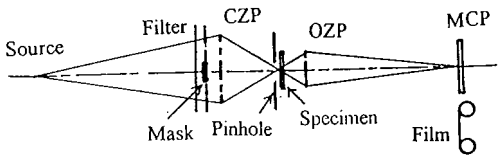

Fig. 1. Schematic of the microscope
CPZ: condenser zone plate, OZP: objective zone plate.

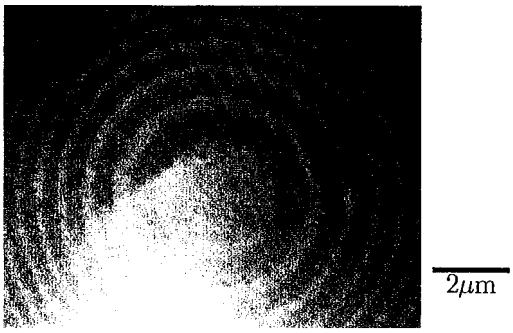

Fig. 2. Zone plate image at 3.2nm.
MCP and SIT camera, accumulation: 8s.

on floppy disks using an image processor (ARGUS-100, Hamamatsu Photonics K.K.). The images of these data were re-displayed on a monitor and were photographed. Sliding the MCP perpendicularly to the optical axis, the film could be used as a detector.

The optical performance was estimated using a zone plate as a specimen. The characteristics of the zone plate was the same with the OZP. Figs.2 and 3 show images of the zone plate at 3.2nm. Almost the outermost zone of 45 nm width can be resolved in Fig. 3.

OBSERVATIONS OF WET BIOLOGICAL SPECIMENS

For observation of wet biological specimens, an environmental chamber (wet cell) with SiN (0.1μm thickness) windows was made as shown in Fig.4. Imaging tests were performed at 2.4 nm with the magnification ratio of 570. Fig. 5 shows an image of a wet lettus protoplast. Fig.6 shows an image of Deinococcus radiodurans strain.

ELLIPSOIDAL CONDENSER MIRROR SYSTEM

In the present microscope, the numerical aper-

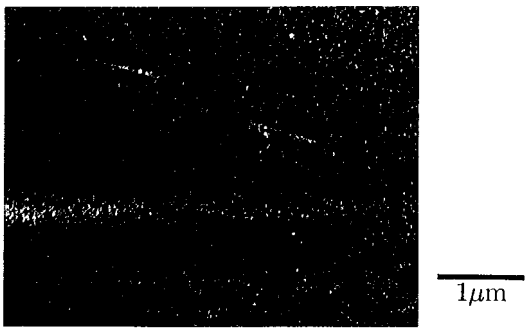

Fig. 3. Zone plate image at 3.2nm.
Film(T-max 400) used as a detector, exposure: 2min.

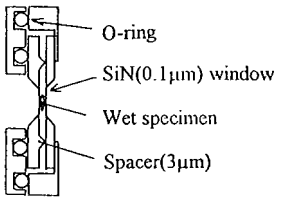

Fig. 4. Schematic of the wet cell.

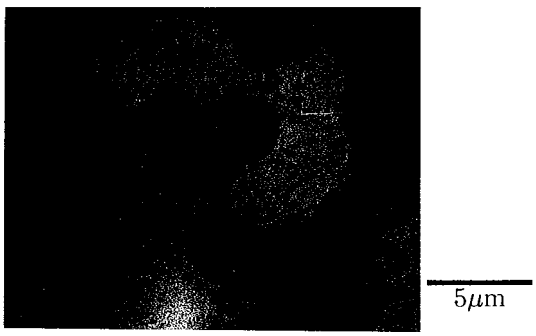

Fig. 5. Lettus protoplast image at 2.5 nm.
Film (T-max 400) used as a detector, exposure: 5min.

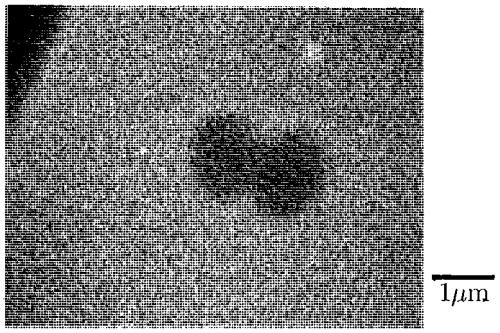

Fig. 6. Deinococcus radiodurans strain image at 2.5 min.
Film (T-max 400) used as a detector, exposure: 2min.

ture of CZP was much smaller than that of OZP. To adjust both the numerical apertures, CZP with the same outermost zone width of OZP has to be made. It is difficult to manufacture such a zone plate with a large diameter. Then an ellipsoidal condenser mirror system was designed (Fig.7), and imaging test was performed at 3.2nm. A magnification ratio of soft x-ray was 320. Fig. 8 shows an image of Cu mesh (#2000).

DISCUSSION

The theoretical resolution of OZP is 55nm (1.22 × the outermost zone width). The experimental resolution is worse compared with the theoretical one. This is probably due to low monochromaticity of illumination and the second order radiation of CZP.

In the observation of Deinococcus radiodurans strain (Fig.6), specimens moved to the outside of the imaging field during soft x-ray illumination, and specimens that maybe died could be imaged.

It is necessary to stop its motion for observations of such a motile biological specimen. It is possible to substitute the ellipsoidal mirror condenser system for a condenser zone plate to adjust both the numerical apertures of a condenser and OZP.

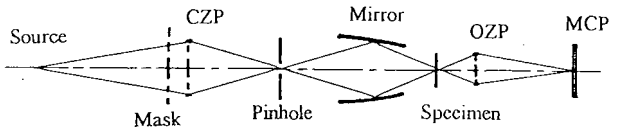

Fig. 7. Ellipsoidal condenser mirror system.
CZP: condenser zone plate, OZP: objective zone plate.

Fig. 8. Cu #2000 mesh image.
MCP and SIT camera, exposure: 16s.

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