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Imaging soft X-ray microscope at Rits Synchrotron Radiation Center

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An imaging soft X-ray microscope with zone plates has been installed at Rits SR Center (Ritsumeikan University, Kusatsu, Japan). With this microscope, specimens were set in air, which made it possible to investigate the specimens without breaking the vacuum of the microscope. The specimens can be prefocused with an optical microscope. Dry and wet biospecimens in air were observed. A new optical system was designed to improve the resolution.

Keywords: X-ray microscope; zone plate; Rits SR Center.

1. Introduction

Soft X-ray microscopy offers observational methods for the biological and materials sciences with much higher resolution than optical microscopes and lower radiation doses compared with electron microscopes (Sayre *et al.*, 1977).

An imaging soft X-ray microscope using synchrotron radiation and zone plates has been developed for the first time by the Göttingen University group, and is now applied to observe wet biological specimens (Rudolph *et al.*, 1992; Schmahl *et al.*, 1996).

Since 1987, we have been developing an imaging soft X-ray microscope with zone plates at UVSOR BL8A [synchrotron radiation facility (750 MeV, 200 mA) at the Institute for Molecular Science, Okazaki, Japan] (Watanabe *et al.*, 1996, 1997). In April 1996, this microscope was transferred to Rits SR Center BL-12 (Hirai *et al.*, 1997). Rits SR is usually operated at an energy of 575 MeV with an initial beam current of 300 mA (Iwasaki *et al.*, 1997). The critical wavelength of the radiation is 1.47 nm. Its beam size is 0.28 × 2.6 mm (2σ). This change of synchrotron radiation facility provided at least two advantages: firstly, we can have an exclusively used X-ray microscope station and, secondly, we can use a brighter source.

This report describes the present status of the imaging soft X-ray microscope station, some results, and a new optical system designed to improve the resolution.

Table 1
Characteristics of the zone plates.

	CZP1	CZP7	OZP
Diameter (μm)	4300	9000	50
Number of zones	4300	41890	277
Outermost zone width (nm)	250	53.7	45
Zone material	0.2 μm Au	0.3 μm Ge	0.13 μm Ni
Support material	0.1 μm SiN	0.1 μm Si	0.1 μm SiN

2. Soft X-ray microscope station

The optical system of the imaging X-ray microscope is basically the same as that of the Göttingen X-ray microscope (Niemann *et al.*, 1994) (Fig. 1). It consists of a filter (4 μm Al + 0.1 μm SiN + 55 nm Ti), a central stop (2.4 mm diameter), a condenser zone plate (CZP1 in Table 1), a pinhole (20 μm diameter), a specimen, an objective zone plate (OZP; Table 1) (Anderson & Kern, 1992) and a back-illuminated CCD (SITe SI502A: 512 × 512 pixels, each 24 μm × 24 μm).

X-rays from the source are monochromatized and condensed at the pinhole by the CZP. Transmitted X-rays through a specimen are magnified by the OZP and are imaged on the CCD. A camera with film (Fuji Minicopy HRII) is also used as a detector.

This optical system was mounted on a bench, as shown in Fig. 2. The vacuum pipe was cut into two pieces: a condenser part and an imaging part. A mirror chamber and an acoustic delay line were set upstream of the bench. The specimen cell was placed in air. SiN was used for windows to separate the vacuum from the atmospheric pressure. An optical microscope was set on the same stage with the vacuum chamber of the CZP to observe specimens at the same position as in the observation with X-rays. The CZP chamber can be moved back and forth along the optical axis with a pneumatic cylinder. The CZP chamber and an optical microscope are placed on the same moving stage. They are switched with a pneumatic cylinder.

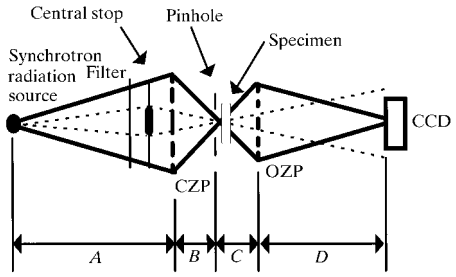

Figure 1
Schematic diagram of the optical arrangement of the X-ray microscope system. CZP, condenser zone plate; OZP, objective zone plate; CCD, back-illuminated CCD (*A*, 6900 mm; *B*, 300 mm; *C*, 2.5 mm; *D*, 670 mm).

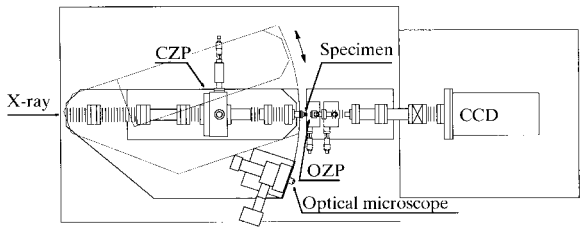

Figure 2
Schematic diagram of the X-ray microscope system. A vacuum pipe with the CZP and an optical microscope were set on the same moving stage.

3. Results

3.1. *Performance test*

The optical performance was estimated using a zone plate (OZP) as a specimen. Fig. 3 shows an image of the zone plate at a wavelength of 3.2 nm. The observable finest zone width was 63 nm. In contrast, the observable finest zone width was 0.1 μm at a wavelength of 0.90 nm. This is probably due to low monochromaticity of illumination at wavelengths of 0.90 nm.

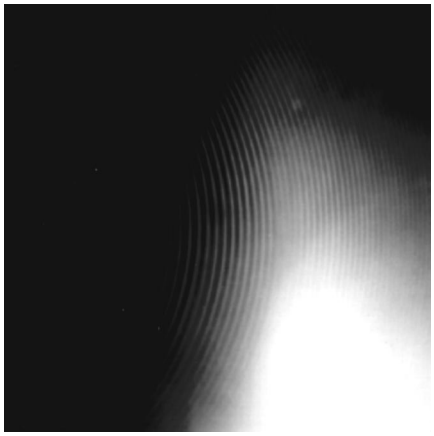

Figure 3
An X-ray micrograph of a zone plate at a wavelength of 3.2 nm, taken using a camera with Minicopy film (exposure time 10 min).

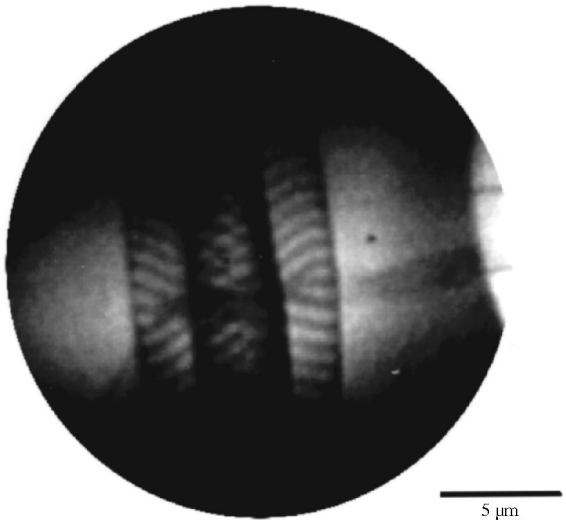

Figure 4
An X-ray micrograph of a diatom at a wavelength of 3.2 nm, taken using a camera with Minicopy film (exposure time 10 min).

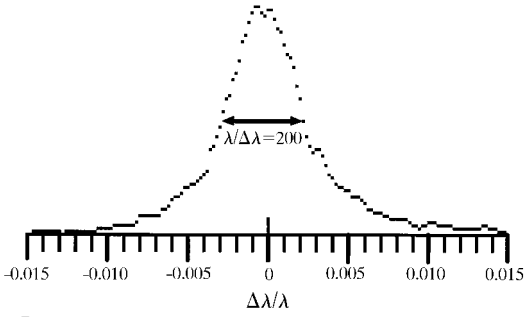

Figure 5
Monochromaticity (λ/Δλ) of the new optical system at a wavelength of 3.3 nm.

3.2. *Observation of biospecimens*

Several biospecimens, such as diatoms, crab muscular tissues, granule cell layers in rat cerebellum, and blood cells of ascidian, were observed (Hirai *et al.*, 1997; Watanabe *et al.*, 1996, 1997). Fig. 4 shows an image of a diatom at a wavelength of 3.2 nm. The diatom was boiled in sulfuric acid for about 30 min to remove organic matter. The skeletons thus prepared were washed with water and air dried. A 0.5 μm width of skeleton was clearly observed.

Wet biospecimens were observed by using a cell covered with thin polyimide foils (0.35 μm thick) supported by thick polyimide foils (Kapton tape) (Takemoto *et al.*, 1997).

4. New optical system

To improve the present system, we have replaced the CZP with a grazing new one (CZP7 in Table 1) and inserted a plane mirror (Si, grazing incident angle 40 mrad) upstream of the CZP as a low-pass filter to cut off high energy, which protects the CZP against heat load. The CZP was fabricated at Göttingen (Schmahl *et al.*, 1993), where the groove efficiency was measured as 7.5% at a wavelength of 2.5 nm.

In this system, the monochromaticity (λ/Δλ) was calculated to be 200 at a wavelength of 3.3 nm with the source of 0.28 × 2.6 mm (2σ) in the central region of the source image of 6 × 30 μm at a distance of 7.05 m (Fig. 5) (B. Niemann, private communication). This will improve the spacial resolution of the X-ray microscope drastically.

5. Conclusions

We have been constructing an imaging soft X-ray microscopy system with zone plates. A 63 nm line and space pattern was resolved at a wavelength of 3.2 nm, whereas a 0.1 μm line and space pattern was resolved at a wavelength of 0.90 nm. Dry and wet biospecimens, such as diatoms, crab muscular tissues, granule cell layers in rat cerebellum, and blood cells of ascidian, in atmospheric pressure, were observed.

A new optical system has also been designed and set up to improve the resolution.

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