Soft X-Ray Microscope with Zone Plate at UVSOR


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A soft X-ray microscope with zone plates was set up at UVSOR [synchrotron radiation facility (750MeV, 200mA) at Institute for Molecular Science, Okazaki, Japan]. A 50nm line & space pattern could be resolved at \( \lambda = 3.2 \text{ nm} \). With an environmental chamber (wet cell) using SiN windows, wet biological specimens, such as lettuce protoplasts, rabbit myofibrils, tubulin of COS cell and Deinococcus radiodurans strains, could be observed at \( \lambda = 2.5 \text{ nm} \). In the present microscope, the numerical aperture of the condenser was much smaller than that of the objective. To adjust both the numerical apertures, an ellipsoidal condenser mirror system was tested, and preliminary result (an image of Cu mesh, 12.7\,\mu m pitch) was obtained.

1. INTRODUCTION

Soft X-ray microscopy has a potential to observe wet biological specimen with higher resolution than that of optical microscopy and with lower radiation damage than that of electron microscopy [1]. A soft X-ray microscope using synchrotron radiation and zone plates was constructed for the first time by Gottingen university group, and it is now applied to observe wet biological specimens [2].

We have been assembling soft X-ray imaging microscopes with zone plates at UVSOR BL8A [synchrotron radiation facility (750MeV, 200mA) at Institute for Molecular Science, Okazaki, Japan] to observe hydrated biological specimen since 1987 [3-5]. This report shows the present status of the soft X-ray microscope at UVSOR.

2. OPTICAL SYSTEM

A zone plate is a circular diffraction grating with radially increasing line density. When \( dr_n \) is the outermost zone width of a zone plate, the theoretical resolution is given by \( 1.22 \times dr_n \) according to the Rayleigh criterion [6]. As the focal length is proportional to \( 1/\lambda \), where \( \lambda \) is the wavelength, a zone plate combined with a pinhole acts as a linear monochromator. For imaging with a zone plate, a monochromaticity of approximately \( \lambda/\Delta \lambda = n \) is necessary to avoid the image degradation by the chromatic aberration, where \( n \) is the zone number of the zone plate.

The optical arrangement is shown in Fig.1. The optical system consists of two zone plates. One is a condenser zone plate (CZP), which is used as a linear monochromator, and the other is an objective zone plate (OZP). The characteristics of the zone plates are shown in Table 1. The theoretical resolution of OZP with monochromatic radiation is 55nm.

Characteristics of the other optical elements are a filter (SiN 0.1\,\mu m and Ti 55nm), a mask (a 90% transparent Ni mesh of which the \( \phi 2.4 \text{ mm} \) area is covered with Al foil), and a pinhole (\( \phi 20 \text{ \mu m} \)).
The synchrotron radiation from the bending magnet of UVSOR BL8A was used. The filter prevented the heat load damage of CZP. CZP was located 8m downstream from the source. CZP monochromatized the soft X-ray and condensed it at the pinhole. A wavelength of 3.2nm was selected for imaging tests. The calculated monochromaticity was 108 from the relationship $\lambda/\Delta\lambda=D/2d$, where $D$: a diameter of CZP, $d$: a larger diameter between the pinhole and the image of the source [7]. The third order radiation of CZP was used, because the size of the source image of the first order radiation at 3.2nm was large compared with that of the pinhole. Using the third order radiation, the size of the source image was reduced about one-third, and the monochromaticity was increased. The mask prevented the zeroth order radiation of CZP from reaching the pinhole.

The specimen was imaged by OZP with a magnification ratio of 740. The image was focused at the outside of the zero-th order radiation of OZP to prevent it from reaching the image field. A film (Kodak T-max 400) and a microchannel plate (MCP) were set at the image plane. When the MCP was used, the 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, the background data subtracted (accumulated records of the same number of frames without x-ray illumination) and then stored 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.

3. PERFORMANCE TEST

Figure 2 (a) and (b) shows the image of a zone plate at 3.2nm as a specimen, which has the same specification as OZP in Table 1. The outermost zone of 45 nm width is almost resolved in Fig. 2 (b). Figure 3 shows a diatom image at 3.2nm.

Figure 2 (b) shows that the experimental resolution is worse compared with the theoretical...
one (55nm). This is probably due to low monochromaticity of illumination. A monochromaticity up to \( \lambda/\Delta\lambda = 278 \) was necessary to avoid the chromatic aberration, but the present microscope had the theoretical monochromaticity of \( \lambda/\Delta\lambda = 108 \).

4. OBSERVATIONS OF WET BIOLOGICAL SPECIMENS

For Observations of hydrated biological specimens, an environmental chamber (wet cell) was made. The wet cell has SiN foils of 0.1 \( \mu \)m thickness and 200 \( \mu \)m square to maintain atmospheric pressure against a high vacuum environment. SiN foils were made at the center of Si wafers of 10mm square. A schematic of the wet cell is shown in Figure 4.

![Figure 4. Schematic of the wet cell.](image)

The right side Si wafer was sealed by adhesive and the left side one was sealed by an O-ring. A wet specimen was placed between two SiN foils, which was separated by spacers of 3 \( \mu \)m thickness. A Cu #2000 mesh of 3 \( \mu \)m thickness was put between SiN foils to prevent a specimen from moving out.

A wavelength of 2.5nm was selected for imaging, as the transmittance of a water layer is better than that for 3.2nm soft X-rays. The magnification ratio was 570. The other optical parameters was the same with the above ones at 3.2nm.

Figure 5 is a Letus protoplast image. Figure 6 is an image of COS cell fixed by folmaldehyde, tubulin of which was stained by immunogold labeling. This specimen was observed in collaboration with Dr. A. Yamamoto, Kansai Medical University. Figure 7 is an image of rabbit myofibrils, which was taken in collaboration with Prof. S. Ishiwata, Waseda University. Figure 8 shows an image of Deinococcus radiodurans strain MR1. This specimen was observed in collaboration.
with Dr. I. Narumi, Japan Atomic Energy Research Institute. In the observation, this strain moved to the outside of the imaging field easily during soft x-ray illumination. It is necessary to stop its motion for observations of such a mobile biological specimen.

5. ELLIPSOIDAL CONDENSER MIRROR SYSTEM

In the present microscope, the numerical aperture of illumination was small compared with that of OZP, and the zeroth order radiation of OZP was concentrated about the optical axis. To avoid overlapping it with a focused image, the image were focused at the outside of the zeroth order radiation. An uniformity of an image was considered to be degraded in such off-axis imaging.

To make an imaging area on the optical axis, an ellipsoidal condenser mirror system with the same numerical aperture as that of OZP was designed (Table 2). The optical system is shown in Fig. 9. An imaging test was performed at 3.2nm and a magnification ratio of 320. The soft X-ray beam size at the specimen plane was approximately φ20μm in diameter. Fig. 10 shows an image of Cu mesh (#2000, pitch:12.7μm).

6. NEW X-RAY MICROSCOPE CHAMBER.

Using the wet cell, wet biological specimen can be observed. However, it is better access to specimens to place an environmental chamber in air under investigation. A microscope of this type was developed by Gottingen university group [8].

A soft X-ray microscope chamber to place an environmental chamber in air has been developing now at UVSOR. The experiment using this chamber is currently under way.

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REFERENCES