Improvement of spatial resolution in soft X-ray projection microscope and its application to the observation of biological specimen

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Introduction

We have been developing a projection microscopy system using synchrotron radiation, where an intense X-ray point source is produced by a Fresnel zoneplate in combination with a pinhole (1 μmϕ) located at a focal point of the zoneplate [1]. In the present study, we examined a pinhole of smaller size (0.5 μmϕ) and the use of a prepinhole (10 μmϕ) for the improvement of spatial resolution. We have also tried to image human chromosomes in dry state.

Materials and Methods

The optical layout of the projection microscopy was illustrated in Fig. 1. A series of pinholes with the diameters of 20, 5, 1, 0.5 μm was made on Pt membrane (1.5 μm thickness) using Focused Ion Beam apparatus. Monochromatic X-rays from 1.5 nm to 3.6 nm were obtained at BL-11A. The prepinhole was placed at the position of a focal point of the focusing toroidal mirror installed at the downstream of the monochrometer.

Spatial resolution was evaluated using a test pattern with grooves of defined widths (NTT Advanced Technology Corp. Japan) as described previously [2].

Chromosome specimen from human lymphocytes was prepared by depositing on a SiN membrane and air-dried.

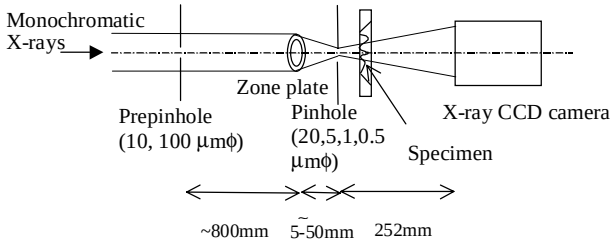

Fig. 1. Layout of projection X-ray microscope.

Results and Discussion

As shown in Fig. 2, the line-spacing of 0.2 μm was clearly resolved using 0.5 μmϕ pinhole in combination with 10 μmϕ prepinhole. This result indicates that the spatial resolution is improved with decreasing a size of the pinhole. Figure 3 shows an image of human chromosomes. An image taken by phase contrast optical microscope is shown for comparison (Fig. 3b). The inner structures seem to be resolved much better than those in the optical microscopic image. Further improvement by image processing with a computer and the imaging of biological specimens such as mammalian cells are in progress.

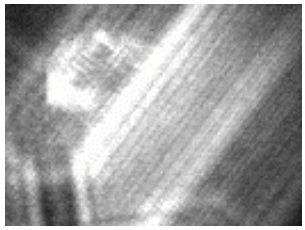

Fig. 2. Image of a test pattern with 0.2 μm spacing at the wavelength of 1.5 nm.

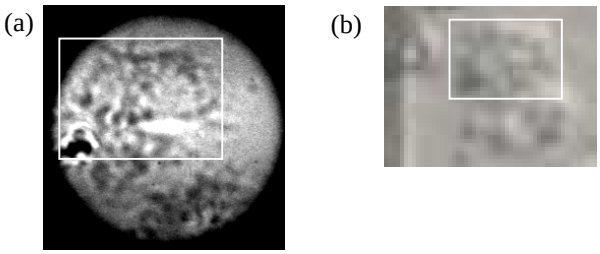

Fig. 3. Images of human chromosomes.
(a) X-ray image at the wavelength of 3.6 nm under the condition of 1 μmϕ pinhole with no prepinhole.
(b) Phase contrast image by optical microscope.
White boxes show the approximate corresponding area.

References

[1] K. Shinohara et al., In "X-Ray Microscopy", (edited by W. Meyer-Ilse et al.), pp. 346-349, New York: American Institute of Physics, (2000).
[2] A. Ito et al., Photon Factory Activity Report 1999, #17, part B, p. 286, High Energy Accelerator Research Organization (2000).

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