Biological Science

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Observation of human HeLa cells with soft X-ray projection microscope

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Introduction

In our continuing attempt to develop projection microscope using focused beam by a Fresnel zone plate, spatial resolution was improved when a pinhole placed in the upstream of a zone plate (pre-pinhole) was applied in order to increase coherent illumination to the zone plate. A test pattern having lines and spaces was resolved to the value better than 0.25 μm with the aid of computer reconstruction of the image with Fresnel fringes [1]. In the present study, we obtained soft X-ray images of human HeLa cells and examined the effect of the pre-pinhole on image formation.

Materials and Methods

Monochromatic soft X-rays of 1.5 nm wavelength were obtained at the beamline 11A and 2C. A typical optical layout of the projection microscope was illustrated in Fig. 1. A pinhole behind the zone plate was installed to remove the higher order diffracted light, and a pre-pinhole before the zone plate, which was placed at the focal point of the focusing toroidal mirror installed at the downstream of the monochromator.

Human HeLa cells were cultured on the SiN window, fixed with glutaraldehyde, and subjected to critical point drying.

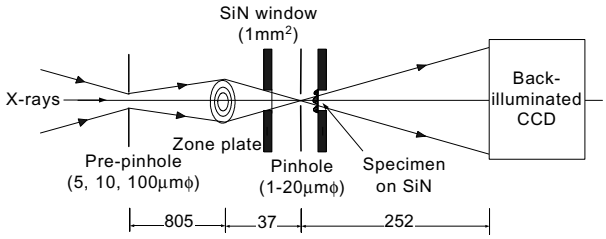

Fig. 1. Optical layout of projection microscope.

Results and Discussion

Figure 2a shows an X-ray image of HeLa cells. The image was taken with 1μmϕ pinhole without pre-pinhole at the magnificaiton of 50. Comapred with an optical microscopic image (Fig. 2b), separate dense parts in the nucleus were clearly contrasted with other cellular region. Identification of the dense structure is a future issue.

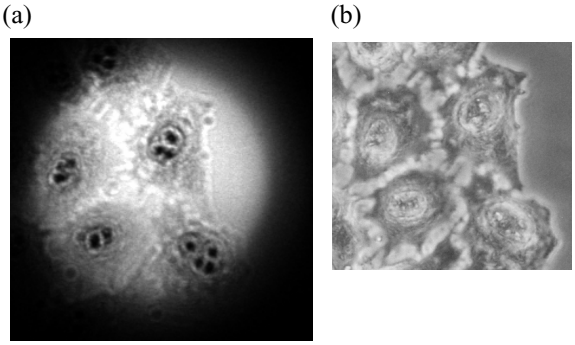

Fig. 2. X-ray image of HeLa cells (a) and the corresponding view by optical microscope (b).

Figure 3 shows images taken with and without pre-pinhole. The image with the pre-pinhole of 100μmϕ (panel b) seems to be slightly improved in its contrast. In accordance with this result, a test pattern with lines and spaces was resolved with decreasing a diameter of the pre-pinhole. Further computer processing of these images with Fresnel fringes would give information about the effect of a pre-pinhole.

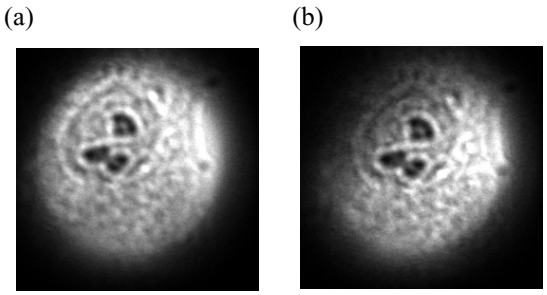

Fig. 3. X-ray images with (a) and without (b) pre-pinhole. Pinhole: 1μmϕ, Magnification: 170

References

[1] A. Ito et al., PF Activity Rep. 2001, 19, 208 (2003).

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