11A,12A/2003G326

Improvement of soft X-ray projection CT microscope

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Introduction

Since soft X-ray is in the spectral region of "Water-window", it is dominant in the observation of a living cell. In other word, soft X-ray gives a good contrast for wet specimens. In a projection microscopy, the specimen should be brought close to the light source that is located at the position of a pinhole with a diameter of 1-5μmϕ when the microscope raises its magnification. Therefore, the authors have exercised the ingenuity to hold and rotate a specimen close to the pinhole.

In this study, the magnification and specimen holder were improved on the precedent soft X-ray projection microscope[1]. The lateral magnification of the microscope was fixed at x107 or x134. As an X-ray CCD camera was a back-illuminated type of 512x512 pixels (24.8μm/pixel), its field of view became 118μm or 95μm square at the above magnifications. The experimental setup is shown in Fig. 1. The experiment was conducted at the beam line 11A and 12A. Monochromatic soft X-ray was used at the wavelengths of 15 - 25 angstrom (0.83 - 0.50 keV in energy). The specimen, the rotation stage, and the imaging area of the CCD camera were in vacuum. The specimen was bonded on the tip of the rotating shaft and observed under the low vacuum of 10^{-4} Torr. An X-Y stage can be controlled from the outside of the vacuum.

Reconstructed CT images

Each projection image was integrated in its intensity for 2 – 5 minutes to improve the signal-to-noise ratio. It took 3-4 hours to obtain a set of projection images. The projection image blurs because of fringes caused by Fresnel's diffraction. This study eliminates such fringes on the projection image with the iteration process, which repeats the calculation between Fresnel and inverse Fresnel transformation. Figure2 shows the CT image of a glass capillary reconstructed by the filtered back projection from the projection images at an interval of 3 degrees all round the specimen. The outer diameter of the capillary was estimated as about 6 μm, while inner diameter was about 4.2 μm. We confirmed that the pipe structure was clearly reconstructed with enough contrast by using the iteration process. CT reconstructed image of HeLa cells were also shown in Fig. 3. The HeLa cells were cultivated on a mylar film on a single-hole mesh for 12 hours. Cells were fixed with glutaraldehyde and then dried; (a) Air-drying and (b) Critical point drying. Because the single-hole mesh was slightly listed, the specimen was away from the rotation axis. As the mesh's edge shadowed the X-ray light, the projection images could not be obtained in the angular range of about 100 degrees in total. The lacked images were interpolated with the dummy data at the cell's position in their angles. In the CT reconstructed image, the internal structure could not be clearly visualized because of the Fresnel's diffraction, while the nucleus and other intracellular structures were recognized.

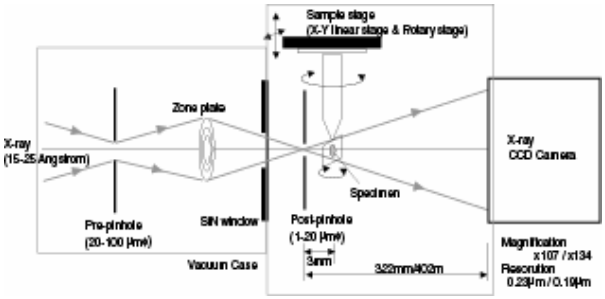

Fig. 1 Soft X-ray projection CT microscope

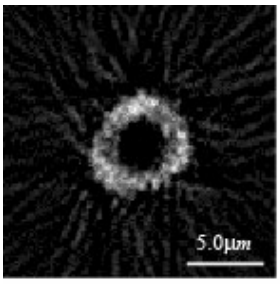

Fig. 2 CT image of glass capillary with iteration process.

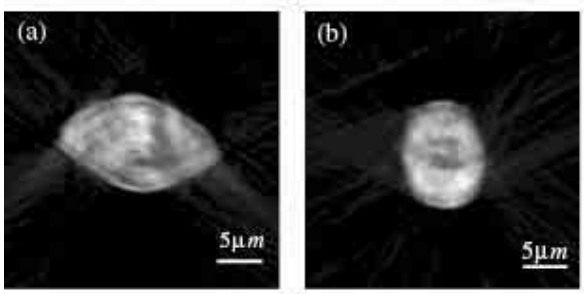

Fig. 3 CT image of HeLa cells without iteration process. (a) Air-drying (b) Critical point drying.

References

[1]T. Shiina et al., PF Activity Rep. #22 Part B, 231 (2004)
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