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Blur elimination of cell image with soft X-ray projection CT microscope

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Introduction

A projection microscope has a characteristic point to zoom in a specimen easily. It is also preferable to TEM to observe a soft specimen. 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. Because of the projection microscopy, the specimen should be placed 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. To observe living cells, a soft X-ray imaging microscope has been developed with the resolution of 20 nm[1]. The imaging microscope is so tunnel-visioned (~10 μm) that it needs high positional accuracy for a specimen hold. The projection microscope has the advantage of the wide viewing area, while the diffraction effect cannot be negligible, resulting in a decrease of the image resolution. To obtain images with high-resolution, image processing is essential to eliminate the effect.

In this study, we observed cell samples in a glass capillary and on some sorts of thin films with the developed projection CT microscope.

Observation and blur elimination

The experimental setup is shown in Fig.1[2]. Monochromatic soft X-rays were used at the wavelengths of 15 - 25 angstrom (0.83 - 0.50 keV in energy). The lateral magnification of the microscope was fixed at x107. An X-ray CCD camera was a back-illuminated type of 512x512 pixels (24.8μm/pixel). Its field of view became 114μm square at the magnification. The specimen, the rotation stage, and the imaging area of the CCD camera were in vacuum. The sample holder, which is attached on a center shaft of a rotating stage, was refined in its feature. The glass capillary with the diameter of 5-10 μm could be fixed and rotated in the view area of CCD.

Figure 2 shows the observed images of cultured human cells. In Fig.2(a), MOLT-4 cells were fixed and dried in a glass capillary. To enhance the outlines of the cells, image contrast was adjusted. We also tried to use new transparent thin films to observe cells through them with good transparent efficiency. Figure 2(b) is the example; a HeLa cell on a carbon film.

The blur elimination was studied with the aid of Fourier transformation-based iteration process, as shown in Fig. 3[3]. It was confirmed that the features and intracellular details of a HeLa cell were well represented in the blur-eliminated image. We have also reconstructed CT image from the all-round blur-eliminated image by rotating the sample.

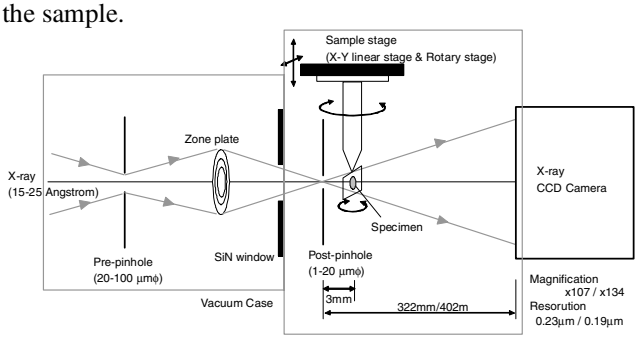
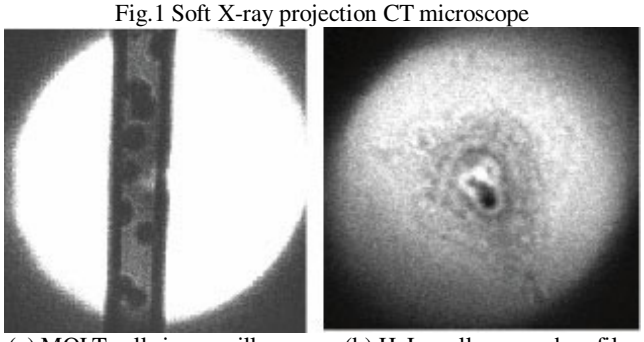
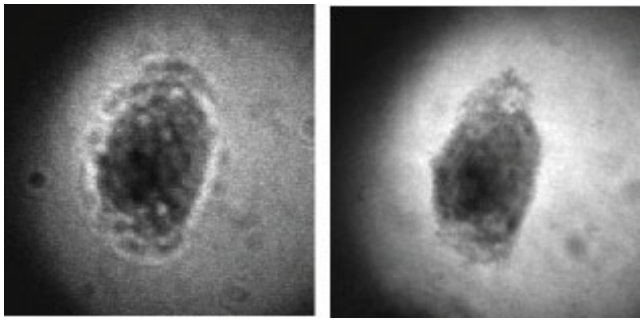


Fig.1 Soft X-ray projection CT microscope



(a) MOLT cells in a capillary (b) HeLa cell on a carbon film

Fig.2 Cell observation with a projection CT microscope.



(a) Observation Image (b) Blur-eliminated image

Fig.3 Image processing with Fourier transformed iteration process (image of a HeLa cell on mylar film).

References

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