Instrumentation and Technique

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High-resolution phase-contrast CT of biological specimens using zone plate X-ray microscope

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Introduction

A phase contrast X-ray microscope (PC-XM) is a powerful tool for the observation of biological specimens with the minimum radiation dose and adequate image contrast. In our previous report, we have developed a Zernike PC-XM using a Fresnel zone plate and biological specimens were observed with a spatial resolution of 100nm[1]. However, the X-ray images were recorded by the photographic plate due to the low magnification system and the resolution limit of a CCD camera. In this report, we developed a high-magnification PC-XM by using a Fresnel zone plate with a short focal length to obtain a high resolution X-ray image by a CCD camera. Then, a high resolution three-dimensional (3D) observation of a biological specimen was performed using a phase-contrast X-ray computed tomography (PC-CT).

Optical setup

A schematic diagram of the optical setup is shown in Fig. 1. A PC-XM was constructed at BL3C2/3C. The X-ray energy was tuned to be 5keV by Si(111) double-crystal monochromator. X-ray beam was shaped by a Pt pinhole with 50μm in diameter which was set at the upstream part of a sample. A Fresnel zone plate whose outermost zone width was 50nm was used as a microscope objective lens. The focal length was 16mm (E=5keV). A pinhole-type phase plate (Aluminum, 3μm thick, 6μm in diameter) was set at the back focal plane to modulate the phase factor of the diffracted X-rays. The phase modulation was approximately quarter wavelength. A magnified image was detected by a CCD camera (1000×1018pixels, pixel size: 12μm²). Figure 2(a) and 2(b) are the phase contrast X-ray images of a test sample and enlarged image of a white square, respectively. A spatial resolution of 100nm could be obtained in both horizontal and vertical directions using a CCD camera.

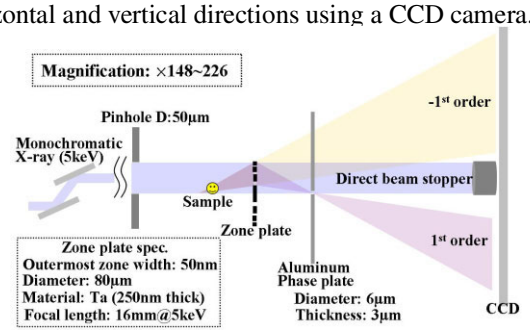

Fig.1 Schematic diagram of Zernike phase-contrast X-ray microscope using a zone plate

PC-CT of a biological specimen

To obtain a tomogram of a phase object, 100 different view angle images through 360° were collected. A conventional convolution back projection method with a Shepp-Logan filter was used for the reconstruction. Pollen (*Erigeron philadelphicus*) was used as a biological specimen. One of the projection images is shown in Fig. 3(a). 2D sectional image at line A is shown in Fig. 3(b). Furthermore, 3D volume images developed using the reconstructed dataset are shown in Fig. 3(c). From the 3D images, an external needle-like structure of pollen can be recognized. A conic object is the tip of a tapered capillary tube which was used to fix the pollen. Although a tomogram did not represent the quantitative phase-shift of a sample, 3D images of a phase object could be obtained with sub-micrometer resolution.

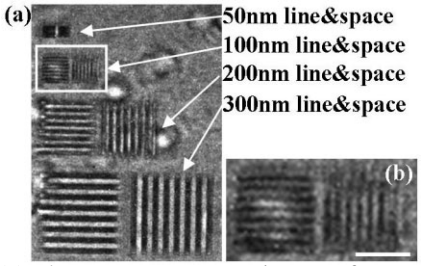

Fig.2(a) Phase contrast X-ray image of a test sample. Exp. 10min. (b) Enlarged image of white square region. Bar: 1μm.

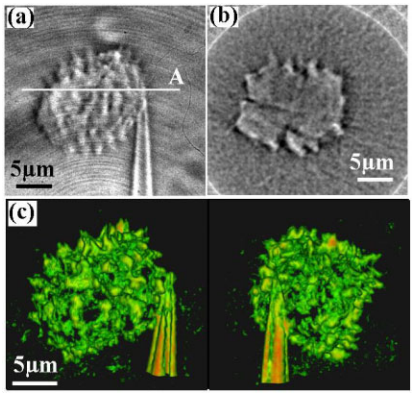

Fig.3 (a) Phase contrast X-ray image of pollen. Exp. 10min. (b) Sectional image at line A. (c) 3D volume images. Voxel size: 160nm³

Reference

[1] N. Watanabe *et al.*, Photon Factory Activity Report Part B, **21**, (2003) 266.

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