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Three-dimensional tomography using a soft X-ray holographic microscope and CCD camera

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The depth resolution of a soft X-ray hologram is much worse than its transverse resolution because a single soft X-ray hologram has a small numerical aperture. To obtain a three-dimensional image, in-line holograms of a specimen were recorded from various directions and reconstructed to obtain two-dimensional projection data. Then, a three-dimensional reconstruction was performed by back-projection of these reconstructed holograms. Three-dimensional images of a tungsten wire of diameter 10 μm and a fossil of a diatom were obtained.

Keywords: holography; tomography; soft X-rays.

1. Introduction

Soft X-ray holography has the capability of imaging biological microstructures with high spatial resolution. The resolution of soft X-ray in-line holography with X-ray resists is especially high, up to 56 nm (Jacobsen *et al.*, 1990). Direct recording with an electronic detector, such as a CCD camera, is convenient because no developing process is necessary and it is amenable to digital image processing. However, the typical resolution of a CCD camera is several tens of micrometres, which is much worse compared with that of X-ray resists. Using in-line holography with a point source and enlarging the hologram at the CCD plane by projection, the resolution of the hologram can be improved.

We assembled a soft X-ray in-line holographic microscope with a CCD camera at the Photon Factory at the National Laboratory for High Energy Physics, Japan. A zone plate and a pinhole were used to make a partially coherent point source, and holograms of specimens were enlarged by projection and recorded by a CCD camera. Hologram data were digitized directly, and reconstructed numerically. From the edge profile of a reconstructed image of tungsten wires, the transverse resolution was estimated to be 1.5 μm at a wavelength of 2.34 nm, which was in good agreement with a theoretical value (Watanabe *et al.*, 1997*a*). Several biological specimens were also imaged by this method.

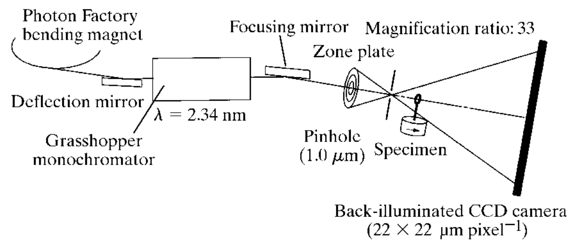


Figure 1
Schematic diagram of the optical system.

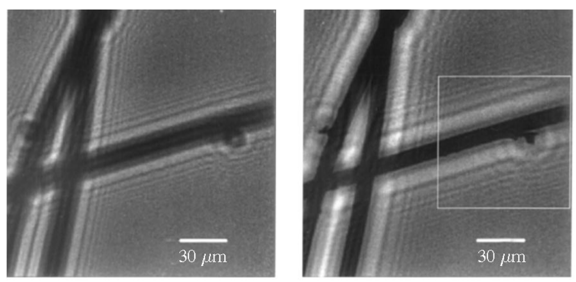
Soft X-ray holograms have a large depth of field because their numerical aperture is very small, thus it is difficult to obtain a three-dimensional image from a single hologram of a specimen. This limitation can be overcome by recording several holograms of the specimen from various directions then reconstructing them according to tomographic principles (Wolf, 1969; McNulty, 1994). McNulty *et al.* (1992) obtained three-dimensional images of a test object of 130 nm width by a combination of soft X-ray Fourier transform holography and diffraction tomography.

In this experiment, soft X-ray holograms of a specimen were recorded from various directions. Then, the holograms were reconstructed to obtain two-dimensional projections of the specimen. A three-dimensional reconstruction was performed by back-projection of the reconstructed holograms.

2. Experiment

The optical system of the holographic microscope is shown in Fig. 1. Synchrotron radiation from the bending-magnet source BL11A at the Photon Factory (2.5 GeV, 300 mA) at the Institute for High Energy Physics, Japan, was used. Monochromatic radiation of 2.34 nm wavelength from the Grasshopper monochromator was focused on a pinhole of diameter 1.0 μm by a zone plate (diameter 1.0 mm, outermost zone width 0.25 μm). X-rays transmitted by the pinhole made a shadowgraph of a specimen on a back-illuminated CCD camera cooled with liquid nitrogen (Astromed Ltd, EEV CCD 02–06, 22 μm × 22 μm pixel^{-1}, 578 × 385 pixels). The pinhole could be regarded as a partially coherent point source, and approximately five diffraction fringes could be observed on the CCD camera. Thus, the shadowgraph could be regarded as the in-line hologram. The specimen was placed on a rotatable stage to obtain holograms from various directions. The distance between the zone plate and the CCD camera was 900 mm, and the magnification ratio was 33. The typical exposure time was 15 min. The numerical reconstruction process has been described elsewhere (Watanabe *et al.*, 1997*a*,*b*).

A hologram of tungsten wires of diameter 10 μm and its reconstructed two-dimensional image are shown in Fig. 2. The transverse resolution, δ_t, was estimated to be 1.5 μm from the edge profile. The depth resolution, δ_l, was estimated to be 0.96 mm, which was calculated from $\delta_l = \delta_t^2/\lambda$, where λ is the wavelength (Jacobsen *et al.*, 1990). The depth resolution was large compared with the thickness of the specimen, and hence the hologram contained no depth information on the specimen. Reconstructed two-dimensional images of the specimen from various directions were measured to use as projection data for tomographic reconstruction. To adjust the rotation axis of the projection data to the



(*a*) (*b*)

Figure 2
(*a*) Hologram and (*b*) reconstructed images of a tungsten wire of diameter 10 μm. The white rectangle in (*b*) indicates the area used for the tomographic reconstruction shown in Fig. 3.

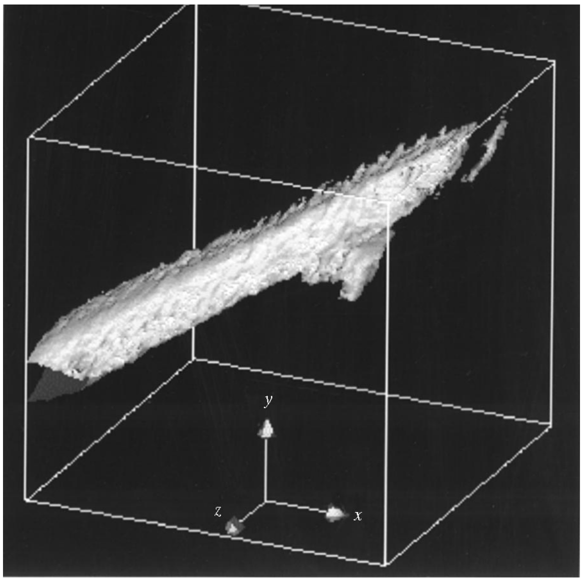


Figure 3
A reconstructed three-dimensional image of the tungsten wire. A 85 μm^3 volume is shown in the white cubic frame.

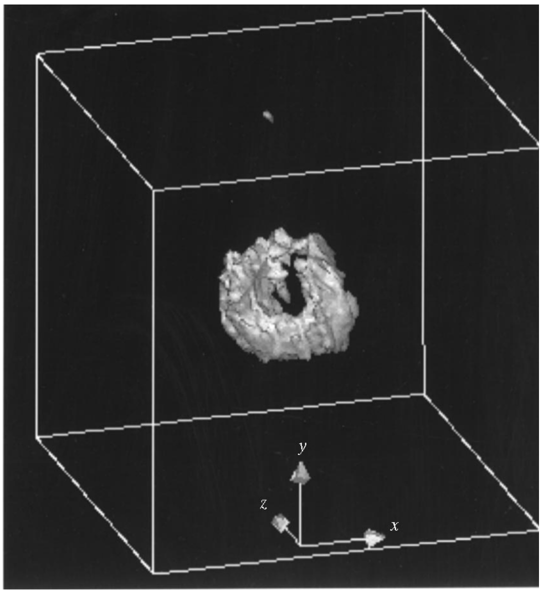


Figure 4
A reconstructed three-dimensional image of a fossil of a diatom. A 85 μm^3 volume is shown in the white cubic frame.

centre of these images, appropriate corresponding points in these images were selected; these images were cut into 128 × 128 pixel images, locating the corresponding points at the centres of the images. One pixel width corresponded to 0.67 μm at the specimen stage. To make these data proportional to the attenuation coefficient of the specimen, the pixel data were replaced by $\log(I_{\max}/I)$, where $I_{\max}$ was the maximum pixel value of the image and I was the pixel data value.

A three-dimensional image of 128 × 128 × 128 pixel volume was calculated by back-projection of the above projection data of 128 × 128 pixels per projection. The projection data were essentially cone-beam projections. However, a parallel-beam reconstruction algorithm of tomography was used because the difference of the ray trajectories between cone beams and parallel beams was smaller than the resolution of 1.5 μm in the reconstructed volume in this experiment. The projection data were simply projected to the reconstructed volume without filters.

3. Results and discussion

A three-dimensional view of a reconstructed image of the tungsten wire is shown in Fig. 3. The surfaces in Fig. 3 have an equal attenuation coefficient of the reconstructed image. The image was obtained from seven holographic views recorded between normal incidence (0°) and 42° at an interval of 7°. A piece of debris attached to the wire was clearly observed. The depth resolution estimated from Fig. 3 was less than 10 μm, which was much smaller than that of the single hologram. However, the image depth resolution was worse along the optical axis (z direction in Fig. 3). This was due to the lack of projection data at large incident angles. Using algebraic reconstruction algorithms (Kak & Slaney, 1988), this distortion seems to be partially improved. This is currently under way. Fig. 4 shows a three-dimensional view of a reconstructed image of a fossil of a diatom. The image was obtained from seven holographic views recorded between −42° and 42° at an interval of 14°.

Using the filtered back-projection algorithm (Kak & Slaney, 1988), the reconstructed three-dimensional image was degraded. This was considered to be due to twin-image noises of the reconstructed holograms, which were emphasized by the filter. Thus, the two-dimensional projection data were simply projected to the reconstructed volume without the filter. The back-projection process seems to suppress the twin-image noises during the superposition of the projection data of various directions.

The transverse resolution of this experiment was limited by the secondary source size of 1.0 μm. However, the combination of a soft X-ray undulator and a high-resolution zone plate has been used to make a soft X-ray microprobe with a resolution of 55 nm (Jacobsen *et al.*, 1994). The current resolution limit seems to be easily overcome by using this combination.

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