## Study of quantitative analytical method for soft X-ray nanotomography at BL-12

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The remarkable features of the soft x-ray microscope (SXM) are the high resolution and high penetrating power. By applying the computed tomography (CT) to the SXM system, the 3-dimensional structure of the sample is obtained as distribution of the physical quantity, linear absorption coefficient (LAC), without any destructive process. This technique is one of the highlights of the SXM and is developed anywhere else [1]. In recent years, our SXM at BL-12 in the SR center has been modified to perform the nano-CT and 3-dimensional structures of the brain cortex of the mouse have been observed [2]. For better performance of the CT, the data acquisition condition, the exposure time and projection number, should be determined by considering the operation time of the SR center, about 5 h [3]. In this study, the condition of data acquirement was investigated from the numerical simulations of the reconstructed images of the phantom. The reconstructed values of the LACs and the spatial resolution of this method were discussed. Additionally, by using this system, 3-dimensional quantitative analysis of the diatom, *Skeletonema potamos*, was performed as an application.

The measurement condition of the CT was determined from the numerical simulation by reconstructing the phantom, which was imitated to the standard sample at the wavelength of 2.4 nm, the glass capillary tube filled with pure water (see below). The simulations were performed by changing the exposure time and the number of the projections with considering the operating time of the SR center as the total measurement time. Then, the exposure times of the projection images of the phantom were simulated approximately by adding the statistical noise caused by the number of the incoming photons to the sinograms. This statistical noise was estimated experimentally from the projection images of various exposure

times. Then the differences of the  $I_0$  image and its smoothened image by using both the median and the Gaussian filter were used. Cross sectional images by the CT were reconstructed by the convolution backprojection method with Shepp-Logan filter. The standard deviations of the reconstructed LACs (for instance. the exposure times of 6, 120 and 720 s) are plotted against the projection number in Fig. 1. The best measurement condition, the



**Fig. 1** Standard deviations of the reconstructed LACs of the phantom with changing the number of the projection and the exposure time.

exposure time of 2 min and 100 projections, was determined from the smallest standard deviation of the water area in the reconstructed image within the total measurement time of 240 min.

The standard deviations of the reconstructed LACs were investigated for the quantitative analysis. Then, the extended glass capillary tube filled with pure water was used as the standard sample. In this experiment, the two measurements were implemented. The 90 projection images of the exposure times of 2 min were acquired with rotating the sample of  $2^{\circ}$  each. In the other measurement, 120 projection images of the exposure times of 80 s were acquired with rotating the sample of  $1.5^{\circ}$  each. Then the wavelength of 2.4 nm in the water window region (*i.e.* between the K absorption edges of carbon and oxygen, 2.3-4.4 nm) was used in common.

The reconstructed cross sectional images are shown in Figs. 2. The reconstructed LACs of the water areas were plotted. In these plots, the LACs of the water were within the standard deviation of the LACs of the water areas in the reconstructed image.

The tungsten needle was used for the evaluation of the spatial resolution and the detection limit of the cross sectional image. The tip of the tungsten needle was fabricated by electro



**Figs. 2** Reconstructed cross sectional images of the standard sample with the measurement conditions of (a) 90 projections and 2 min exposure and (b) 120 projections with 80 sec exposure. The standard deviations of the LACs of the water in the squared areas are plotted in (c).

polishing to obtain the extremely sharp tip smaller than the spatial resolution of the SXM,  $\sim$ 50 nm. The 90 projection images were acquired with rotating angle of the sample of 2° each and the exposure time was 80 s.

In Figs. 3, from the edge response (10-90%) of the projection image of the tungsten needle, the spatial resolution of 143 nm was obtained. On the other hand, that of the cross sectional image was 138 nm (Figs. 4). These resolutions are nearly equivalent.



Fig. 3 (a) The projection image of the tungsten needle and (b) its profile.



Figs. 4 (a) Cross sectional image of the tungsten needle and (b) its profile



**Figs. 5** Cross sectional images of the tungsten needle of the diameter of (a) 156 nm, (b) 142 nm, (c) 76 nm, (d) 57 nm and (e) the relation between their LACs and the detection limit.

Furthermore, the detection limit of the LAC which was defined with three times of the standard deviation of the intensity of the background [4],  $1.01 \times 10^{-4}$  [nm<sup>-1</sup>], was evaluated. Then, the diameter of the tungsten needle was 105 nm.

By using this system, 3-dimensional structure of a diatom, *Skeletonema potamos*, was observed. The sample was attached to the glass capillary tube filled with pure water and was dried. The 3-dimensional rendering image is shown in Fig. 6. The 80 projection images of the exposure times of 150 s were acquired with rotating the sample 2.25 degree. In the cross sectional images of Figs. 7(a) and (b), thicknesses of the outer shell were about 100~300 nm. Their LACs of the outer shell, its material was considered to be like the glass capillary tube made of borosilicate glass.





**Fig. 6** 3-dimensional rendering image of the diatom attached to the glass capillary tube

**Figs. 7** (a), (b) Different cross sectional images of the diatom and the glass capillary tube and (c) their LACs shown in (a) and (b)

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## Reference

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