3-Dimensional Observation of a Phytoplankton by Using a Full-Field Imaging Soft X-ray Microscope

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1. Introduction

In observation of planktons, an optical and an electron microscope are typically used. However, the optical one has lower limit of spatial resolution and the electron one requires vacuum condition and some processes such as coating, staining and slicing, on the samples. Then, a full-field imaging soft X-ray microscope (XM) bridges these two microscopes for high spatial resolution and high transmittance. The high transmittance of the X-ray gives the XM advantage to access the sample easily. The XM can handle relatively thick sample without any additional processes and vacuum condition. Especially, using the X-rays in water window region (λ: 2.3~4.4 nm) enables the observation of the sample even in watery condition. Moreover, 3-dimensional observation can be performed by using computed tomography (CT) technique without any destructive processes on the sample. Therefore, the XM is suitable approach to observe the biological specimens such as planktons and cells. We have been developing CT system with the XM at BL12 [1, 2]. In this report, 3-dimensional structure of a phytoplankton, *Skeletonema potamos*, which was identified at Lake Biwa, was observed by using this system.

2. Experimental

Preparation of Sample

The phytoplankton was fixed on a fine tip of a glass capillary tube whose tip was lengthened by a puller, PC-10 (Narishige), with use of crystal bond under an optical

microscope. The sample was dried in the air.

Experimental

For acquisition of whole data set, 75 X-ray projection images were acquired with rotating the sample 2.4 degree each (180 degree rotation). The X-ray wavelength of 1.81 nm was used for its high yield and high transmittance. Then, magnification of the optical system was 672. The exposure times were 2 min. I_0 images (*i.e.* image without the sample) were acquired every 5 projections to compensate decay of storage ring current. The cross sectional images were reconstructed with convolution-backprojection algorithm.

3. Results and Discussion

Three-dimensional image consists of a stack of the reconstructed cross sectional images. From this image stack, cross sectional images on the longitudinal directions (*i.e.* direction of the rotational axis) were extracted in Fig. 1. In these images, contents are seen at the middle of the frustule indicated by dashed circles. They are considered as residual of pigment tissue. At the valve surface in Fig. 1(b), several pores (arrows) penetrate the valve mantle. Diameters of these pores are nearly 120 nm.

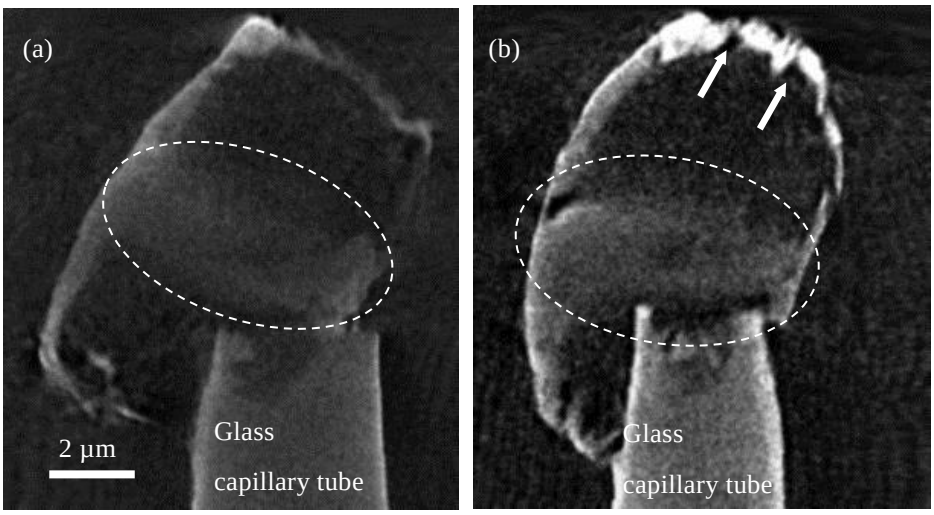

Figure 1 Cross sectional images of the phytoplankton and the glass capillary tube, (a) 0 and (b) 90 degree. Residuals and pores are indicated as dashed circles and arrows, respectively.

The volume rendering image of the phytoplankton is shown in Fig. 2. The volume rendering process was performed by using ImageJ software with VolumeJ plugin [3]. Elevations (indicated by a dashed circle) are seen around the edge of the valve face.

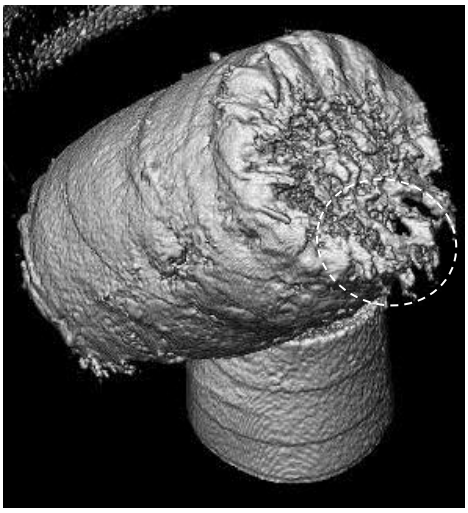

Figure 2 Volume rendering image of the phytoplankton.

4. Conclusions

Non-destructive 3-dimensional observation of the phytoplankton was performed and its morphology, such as the elevations on the valve surface, pores and residuals, was observed. However, it was impossible to find the phytoplankton which remained their fine junctions to connect each cell, intecalary fultoportula processes, on the valve surface. Quality of morphology of the phytoplankton highly depends on their cultivation. Condition and timing of their cultivation are under discussion.

Acknowledgement

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References

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