3-dimensional Observation of Cerebral Cortex of Mouse by Using the Full-field Imaging Soft X-ray Microscope

Takuji Ohigashi[1], Haruo Mizutani[2], Hidetoshi Namba[3], Kuniko Takemoto[4] and Hiroshi Kihara[4]

[1]*Research Organization of Science & Engineering, Ritsumeikan University, 1-1-1 Noji Higashi, Kusatsu, Shiga 525-8577, Japan.*

[2]*Science Integration Program, Organization for Interdisciplinary Research Projects, University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba, Japan*

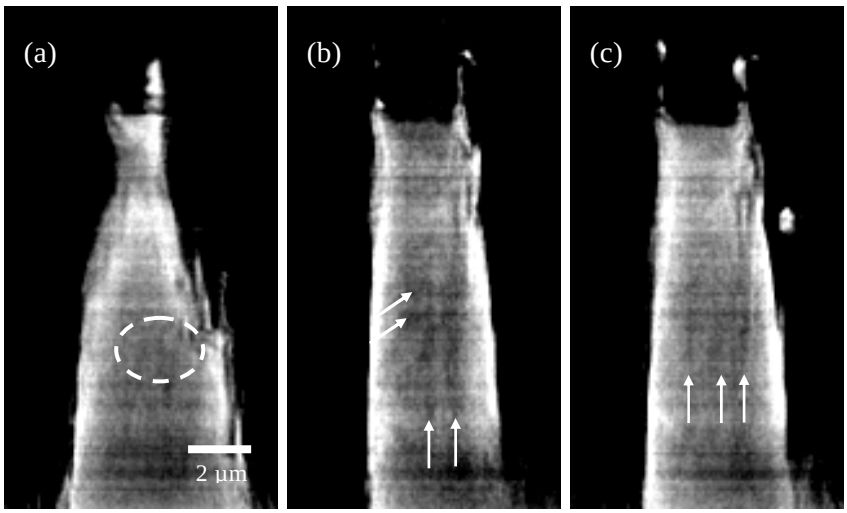
[3]*Department of Physics, Ritsumeikan University, 1-1-1 Noji Higashi, Kusatsu, Shiga 525-8577, Japan.*

[4]*Department of Physics, Kansai Medical University, 18-89 Uyamahigashi, Hirakata, Osaka 573-1136, Japan.*

Investigations for 3-dimensional structures of neuronal network are considered as the key to understand the functions of the brain. Then, depending on the size of the objective organs, high spatial resolution is required for the microscopies. As the major strategy, the electron microscopy has been used for its highest spatial solutions. For obtaining the 3-dimensional image, the images are acquired continuously with cutting the serial thin sections from the sample block and are stacked as 3-dimensional volume [1]. However, this method is not so convenient to observe a number of samples because it requires so much time and processes. Therefore, we noticed using the X-ray microscopy as the best strategy. High penetrating power of the X-ray enables to perform computed tomography, which shows 3-dimensional structures of the sample without any destructive processes. In our previous report, 3-dimensional structures of mitochondria were observed but any other structures of the neuronal network, such as synapse, nerve cord, neuronal cells and so on, were hardly observed [2]. Therefore, we have been improving the optics of the full-field imaging soft X-ray microscope (SXRM) for 3-dimensional observations [3-5] and have considered the condition of the measurement.

Cerebral cortex of a male mouse (C57BL/6J) was observed by the SXRM. The protocol for the sample preparation was described in somewhere [2]. 50 projection images were acquired with rotating the sample 3.6° every. Then, each projection image was obtained by summing two images of exposure time of 4 min at the same tilt angle. Totally, it took over 7 hours. The images were acquired with operating the charge-coupled device camera as 2×2

binning mode. Though this operation mode degrades the spatial resolution of the detector, the narrower width of the projection image reduces requirement of the number of the projection for reconstruction. The wavelength of 1.77 nm was used because of the high throughput of our SXRM and high transmittance for the sample as carbon based material. After normalizing the intensities of the projection images by its and realigning the rotational axis, cross sectional images were reconstructed by home-made software of convolution-backprojection algorithm. The 3-dimemsional image data was constructed by stacking the reconstructed cross sectional images. To observe the 3-dimensional structure, the cross sectional images re-sliced from the 3-dimentional stack along the rotational axis are shown in Figs. 1. In (a), bright spots indicated by dotted circle are attributed to mitochondria. In (b) and (c), streaky structures and dark spots indicated by arrows are attributed to nerve cord. Because of low contrast, volume rendering were not performed. Being compared to the previous results [2], salt-and-pepper noise is obviously decreased but the statistics was still insufficiency to observe the structures clearly.



Figs. 1: Re-sliced cross sectional images along the longitudinal direction of the cerebral cortex of the mouse. (a), (b) and (c) are the cross sectional images along the rotational axis direction re-sliced at different positions, respectively.

References

[1] J. W. Lichtman and J. R. Sanes, *Curr. Opin. Neurobiol.*, **18,** 2008, 346.

[2] T. Ohigashi, H. Fujii, K. Usui, H. Namba, H. Mizutani, K. Takemoto and H. Kihara, *AIP Conf. Proc.*, **1365**, 2011, 124-127.

[3] H. Fujii, T. Ohigashi, H. Namba, K. Takemoto and H. Kihara, *Memoir of the SR Center*, **13**, 2011, 197-200.

[4] T. Ohigashi, H. Namba, K. Takemoto and H. Kihara, *Memoir of the SR Center*, **13**, 2011, 201-203.

[5] T. Ohigashi, H. Fujii, K. Usui, H. Namba, K. Takemoto and H. Kihara, *Memoir of the SR Center*, **12**, 2010, 67-73.