Quantitative analysis of morphological feature of cell nuclei in Cerebellum cortex using Array Tomography and Deep Learning

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Array tomography (AT) provides three-dimensional (3D) information by observing serial sections using scanning electron microscopy (SEM). Compared with serial block-face SEM and focused ion beam (FIB)/SEM, AT has several advantages: (a) High lateral resolution, (b) Standard staining with uranium and lead, allowing easy comparison of images captured by SEM and transmission electron microscopy (TEM), (c) Repetitive observations of samples, enabling hierarchical analysis from low magnification to high magnification, and (d) Low installation cost. In many cases, however, images of serial sections have been taken manually with labor and time. In addition, manual segmentation has been usually required. To solve these issues, we developed automatic image capturing software for AT with SEM. We also tried to use deep neural network to assist segmentation of the images.

Fig. 1 shows flow diagram of observation and analysis. Mouse cerebellum cortex was fixed with glutaraldehyde and osmium tetra oxide, and was embedded in epoxy resin. Then serial sections were prepared using ultra microtome and scooped on silicon substrate, and then stained with uranyl acetate and lead citrate. In our automatic observation software, a corner edge of each section was used as an origin of the section to capture the related positions through the serial data set. The software well controlled SEMs (JSM-7800F and JSM-7900F; JEOL Ltd.) during the automated observation. For the object segmentation, convolutional neural network (CNN) algorithm was used to extract cell nuclei from each image. After the automatic recognition, we manually verified and corrected the result. The 3D reconstructed data set was then quantitatively analyzed.

The SEM system provided comparable images to those by TEM (Fig. 2), and allowed us to identify cellular types by morphological characteristics of the nuclei. The CNN-based segmentation process dramatically reduced the labor to extract objective structure from the 3D dataset than manual segmentation. Image of extracted nuclei was binarized and ratios of dark and bright areas were calculated (Fig. 3). Features of chromatin pattern among cell types will be discussed in the congress.

The present SEM system with AT would be valuable not only for basic biology, but also for pathological analysis. The automated processes will further facilitate these availabilities.

References

[1] Micheva and Smith, Neuron, **Vol. 55.**, page 25-36. (2007).

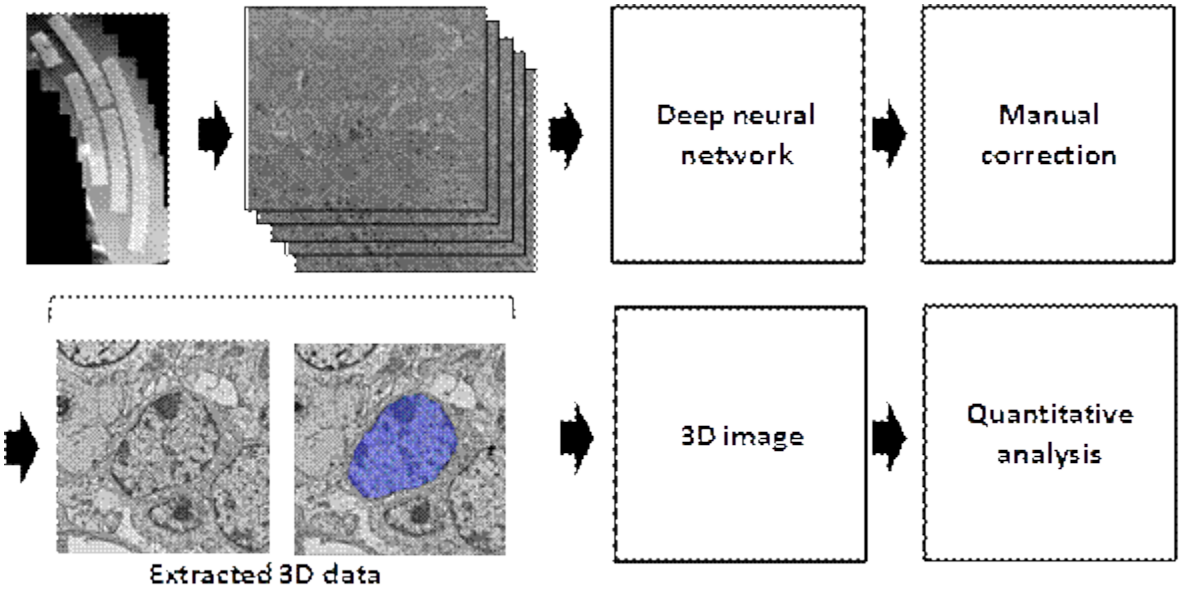

Fig. 1 Flow diagram of Array Tomography and data analysis. Deep nerual netowrk was used to assist extraction of data and segmentation.

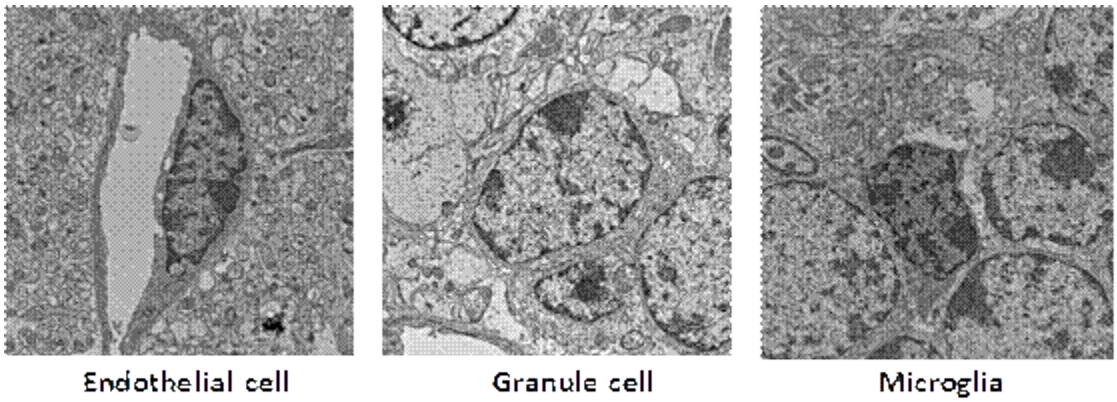

Fig. 2 Nuclei of various cells captured using the SEM system. Since the contrast of these images was comparable to that obtained by TEM, cell types could be easily identified by structural information of the cells.

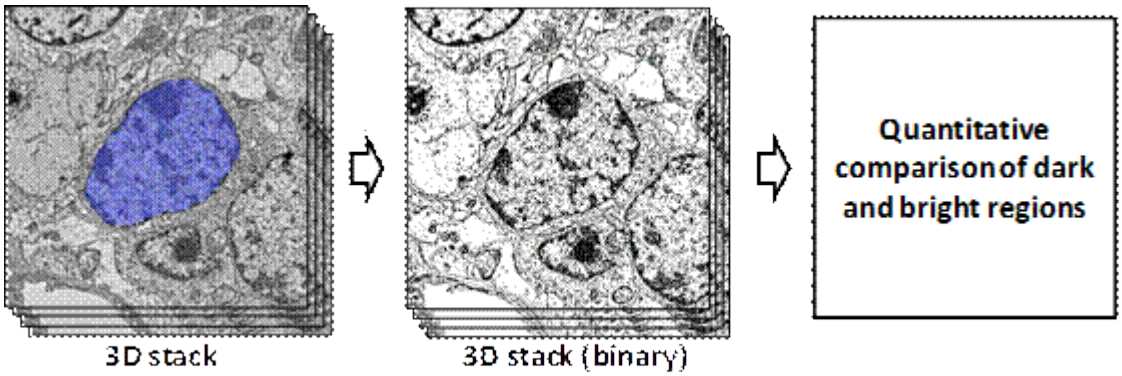

Fig. 3 Extraction of nuclear structure and quantitative measuring of dark and bright regions in nuclei.