Simple and efficient three-dimensional reconstruction method of plant cells using transmission electron microscope

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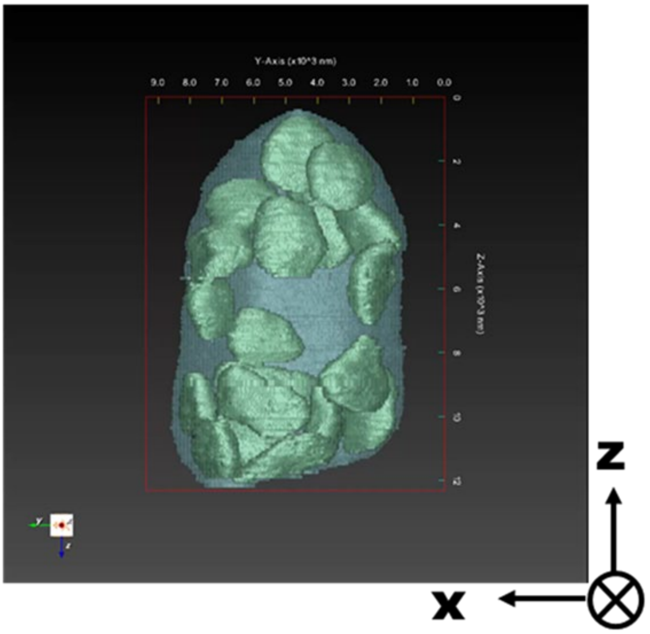
Serial section transmission electron microscopy (ssTEM) is a vital technique in Volume Electron Microscopy (vEM) for unveiling fine structures of cells and tissues in three dimensions (3D). While the ssTEM provides superior X and Y resolution compared to other vEM, it is a challenge to acquire wide field of view images. Conventional mesh grids, for instance, often obscure the desired field of view with mesh bars. Even single-hole grids without bars, the support membrane remains susceptible to tearing and wrinkling, risking the loss of information. To address these challenges, we employed SiN Window Chip [1] for ssTEM. SiN Window Chip has no bars, ensuring uninterrupted observation without any missing information. In addition, its support membrane, made of high-strength silicon nitride film, minimizes the risk of tearing, distinguishing it from single-hole grids. We collected approximately 50 sections, each 70 nm in thickness, onto each of the five SiN Window Chips, resulting in a total of about 250 serial sections. The serial section images of a carrot leaf cell were obtained with a sample holder capable of simultaneously mounting multiple SiN Window Chips. For data acquisition, we used a new 120-kV transmission electron microscope (JEM-120i) equipped with a pole piece that provides high contrast. We attempted to undertake a segmentation analysis of organelles from the 177 TEM images.

Segmentation for annotating regions of interest continues to rely largely on manual methods. This task requires extensive time and effort, a challenge commonly encountered in other vEM as well. To streamline this task, we used convolutional neural networks (CNN) [2], a subtype of deep learning. Only a small number of images were required as training data; chloroplasts were manually segmented from just five images of 512x512 pixels (18.4 nm/pixel). Subsequently, a model was trained using deep learning based on these training data, and chloroplasts were inferred from all TEM images. The entire process, from model training to inference, took only an hour. In contrast, if performed manually, this process would take several weeks. As a result of 3D reconstruction of a cell body and chloroplasts, we gained insights into their shape, distribution, and volume.

Performing 3D reconstruction based on the inference results obtained through deep learning enables streamlined analysis of the shape and distribution of the target tissue. Additionally, the trained model can be applied to different serial section data. We successfully employed the model to other fields of view obtained from the same specimen. Therefore, we anticipate that this method can also reduce the time required for training data creation.

Graphic:



Keywords:

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Reference:

[1] Y. Konyuba et al., Microscopy, 67, 367-370 (2018)
[2] K. Konishi et al., Microscopy, 70, 526-535 (2021)