1456
doi:10.1017/S1431927622005918
Microsc. Microanal. 28 (Suppl 1), 2022

Tips and Tricks for Volume Electron Microscopy Workflows in Plants

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Volume electron microscopy (vEM) approaches have increasingly provided critical insights into complex and multi-dimensional biological structures, including plants [1]. However, plant specimen face several challenges when processing for microscopy due to their hydrophobic cuticle, chemically diverse cell walls and intercellular air spaces that can collectively impede sufficient chemical fixation and staining. Optimal results for one vEM technique, serial block-face scanning electron microscopy (SBF-SEM), depend on high atomic number contrast agents for improved throughput, resolution, image contrast and sample conductivity.

For conventional chemical fixation, plant tissues were fixed in paraformadehyde and glutaraldehyde followed by osmium tetroxide and then stained with potassium ferrocyanide, osmium-thiocarbohydrazide-osmium (OTO), hot uranium and lead salts and embedded in Quetol resin [2] modified from a large volume brain tissue protocol [3]. For high-pressure freezing and freeze-substitution, samples were fixed in 2% osmium tetroxide with 3% water in acetone, warmed to room temperature rehydrated and stained and embedded as described for conventional chemical fixation. Sample prepared in this way were readily compatible with x-ray microscopy imaged and selected area could be targeted for SBF-SEM images. Deep learning was applied for improved and efficient segmentation of SBF-SEM dataset to visualize and create quantitative 3D volumes. With this approach, chloroplasts, starch, nuclei, Golgi, vacuoles and cell walls could be readily discerned and segmented. These protocols demonstrated the utility of enhanced metalization for high-resolution 3D volume imaging of key plant structures and multiscale correlative workflows of plants.

References:

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