Large-volume cryoEM sample preparation for the investigation of the plant-microbiome interaction

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Background incl. aims

In recent years, cryo-electron tomography (cryoET) has proven to be a powerful tool for studying the organization and complexity of cellular ultrastructures in their near-natural state. Nevertheless, its applicability was primarily restricted to single cells and monolayers due to the limited capabilities of volume vitrification techniques. Advances in cryo-electron microscopy (cryoEM) aim to overcome these limitations, which has led to the development of methods that can vitrify larger volumes. Despite these advances, further thinning of samples remained a challenge until focused plasma ion beam (PFIB) microscopy and the lift-out technique were introduced. By combining these improved technologies, we have succeeded in pushing the boundaries of sample preparation for cryoET. In particular, we set out to develop and optimize methods for the preparation of large-volume samples, focusing on samples from fungi and plants to facilitate the study of plant-microbiome interactions at the cellular, and ultrastructural level, and we present our results in this context.

Methods

Samples were collected from an arbuscular mycorrhizal (AMF) fiber network, known for its symbiotic relationship with approximately 80% of plant species. Additionally, samples of plant roots were obtained from both Arabidopsis thaliana seedlings and Ri T-DNA-transformed plant root organs cultivated on a standard MSR medium. Cryogenic preservation by high-pressure freezing (HPF) was performed in 3 mm planchettes and the waffle method on grids. Using hexadecane and phosphate buffer solution as filler to prevent the formation of gas bubbles. Prior to vitrification, all samples were fluorescence stained with Nile Red and Calcofluor to enhance visualization of lipid-rich structures and cell walls, respectively. Following, grids were evaluated and processed in Arctis cryo-PFIB, while planchettes were processed in Helios Hydra V a PFIB dual beam scanning electron microscope. Trenches were prepared, enabling both 3D large-volume imaging and preparation for cryolift-out on planchettes. Lamellae were prepared on grids and from lift-out samples and subsequently transferred to Titan Krios for data collection.

Results
This study demonstrates the successful vitrification of AMF, enabling the visualization of the cellular organization in a near-native state. Fluorescence staining with Nile Red and Calcofluor white facilitated the differentiation of cellular structures, including the distinction between filled and empty AMF hypha. In Addition, 3D volume images of both, the AMF network and plant root tissue, were generated, providing insights into their respective structures. In particular, plant organs provided better results compared to other plant tissue used in this study. Furthermore, the used methodology enabled the imaging of plant tissue in a near-native state without additional fixation steps after HPF, which is a remarkable advance over previous approaches. The combination of methods offers a promising approach for studying plant-microbe interactions on a structural level and provides valuable insights into complex biological systems.

Conclusion
The combination of methods used for large-volume sample preparation in cryoET represents a significant advance in the study of plant-microbiome interactions at the structural level. The inclusion of fluorescent staining in the sample preparation facilitated the identification of areas of interest on the PFIB and enabled the precise alignment for lamella milling of different cellular structures. The acquisition of 3D volume images of both AMF networks and plant organs provides comprehensive insights into their structures and interactions. In particular, our approach demonstrates the enhanced vitrification quality of plant organs over other plant tissues. Furthermore, the ability to image plant tissue in a near-native state without additional fixation steps preserves tissue integrity and ensures accurate visualization of cellular ultrastructure. Overall, our methodology offers a promising opportunity to improve our understanding of plant-microbiome interactions. By elucidating complex dynamics at the cellular level, our work contributes to the broader knowledge of complex biological systems. Further refinement and application of our methodology have the potential to unravel the mechanisms underlying the interactions between plants and microbiomes.

Keywords:

large-volume, plant tissue, host-microbe, mycorrhiza

Reference:

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