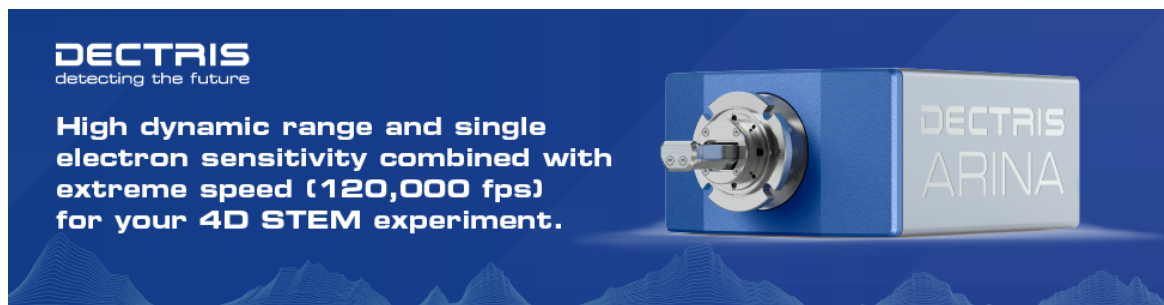


An Optimized Optical Clearing Pipeline for Intact 3D Plant Imaging and Visualization of Fluorescent Probes

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Meeting-report

An Optimized Optical Clearing Pipeline for Intact 3D Plant Imaging and Visualization of Fluorescent Probes

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Here we report an adapted version of the ClearSee¹ tissue clearing technique optimized for plant tissues, which enables whole plant imaging to visualize stains and expressed fluorescent proteins. This approach facilitates protein and vascular system localization in intact tissue in 3D, while retaining cellular-level structure, permitting the exploration of plant connectome interactions.

Fixation and embedding of plant tissue for molecular interrogation has historically relied on techniques such as histological staining and immunohistochemistry as the foundation of plant cell biology studies, which required sectioning the tissue, labeling the section, imaging each section and then reassemble the images into a 3D representation of the structures of interest. Recent advances in fixation and tissue clearing techniques such as PEGASOS, SeeDB1, ScaleA22, iDISCO, and X-CLARITY have enabled intact imaging of animal organoids, embryos, brains and other organs. However, less attention has been given to the 3D imaging of intact plant tissues and organs. Here we present a fundamental shift from section reconstruction to tissue clearing and autofluorescence reduction, allowing for intact plant 3D visualization while retaining endogenous molecular structures of interest.

Plants transport sugars and other organic compounds from source tissues, like leaves, via the phloem (sieve elements), whereas water and inorganic ions move through the xylem². How the vasculature connects to sink tissues, like roots, flowers and seeds, is intimately linked to the mechanism through which solutes are loaded and unloaded². In this study we utilized intact young plants and tissues from *Arabidopsis thaliana* expressing phloem-localized fluorescent proteins, including leaves, crowns (combination of stems and flowers), flowers (individual open flowers), siliques (green seed pods at varied developmental stages) and roots. Our goal was to optimize the clearing protocol while retaining fluorescence of endogenous GFP and RFP proteins expressed in genetically modified plants. Additionally, we aimed to identify the interactions of these expressed phloem proteins with the xylem for comparative imaging in vivo and in situ. For this purpose, plants expressing fluorescent proteins were grown in soils doped with Rhodamine WT. At the appropriate stages, the plants were harvested, fixed, cleared, mounted in refractive index matching media, and imaged using a laser scanning confocal microscope with appropriate immersion optics to validate the various treatment steps.

We will present the details of the techniques used, describing the optimal chemical fixation, tissue clearing reagents and times, optimal refractive index matching for the imaging systems at hand, imaging conditions, and data management pipelines used in our shared research facility. This has allowed us to visualize expressed fluorescent proteins, including GFP and RFP, simultaneously with Rhodamine WT labeling in whole intact tissues, while minimizing loss of structural and molecular integrity.

As studies continue to strengthen clearing, labeling, imaging and visualization methods for intact biological tissues, so too will imaging, analysis, and computational technologies. With concerted and collaborative efforts, molecular and optical profiling of plant biological systems will find many more applications in the near future.



Fig. 1. Macro image of crown region of *Arabidopsis thaliana* used in this study.

A major hindrance to applying clearing techniques to plant material is the cell wall, comprised mainly of



Fig. 2. 2D maximum projection of confocal z-series of *Arabidopsis thaliana* GFP (*SUC2p::SUC2-GFP* – membrane-bound GFP (fused to SUC2), green) and Rhodamine WT (xylem-mobile fluorescent dye applied via soil, red) localization in plant crown region.

References

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