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NAPPN Annual Conference Abstract: Multiplex Immunofluorescence Imaging and Quantification in Plant Cells and Tissues

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Multiplex Immunofluorescence technologies allow simultaneous detection of multiple biomolecules on a single tissue section which permits comprehensive studies of the abundance, spatial distribution, and interactions of different molecules. Plants and their microbial associations pose unique challenges (i.e. chemically resistant cell walls, large air/vacuolar spaces) in such imaging, with very limited adoption. In this project, we aim to develop a versatile and compatible protocol for multiscale plant tissue arrays using multiplex microscopy on the same sample, while preserving cell ultrastructure. We tested different combinations of fixatives and resin types for multiplex cell wall immunofluorescence on confocal microscopy system and correlative ultrastructure on field emission scanning electron microscopy, and applied it on multiple sample types including seedling leaves and roots, different stages of abscission zones and anthers of *Setaria viridis*. We developed a multiplex image processing and analysis pipeline to handle, integrate, visualize, and analyze the data. This pipeline started from multi-modal image registration to overlay images from different cell wall labeling for the same tissue. Then segmentation methods with manual correction were applied to the Calcofluor White stained cell wall images to identify individual cells. An interfaced tool was implemented enabling semi-automatic removal of the space between cells. We extracted multi-dimensional measurements for cell size, shape as well as the fluorescence signal of every cell wall labeling. This quantitative protocol permits studies of localization patterns and interactions of different cell wall components, and can be applied to a wide range of biomolecules and corresponding tissue ultrastructure of the same tissue sections.