IN VIVO QUANTIFICATION OF PLANT CELL COUPLING WITH 4D PHOTOACTIVATION MICROSCOPY

Johannes Liesche, Michael Hansen and Alexander Schulz
Department of Plant Biology and Biotechnology, University of Copenhagen, Denmark
Center for Advanced Bioimaging at University of Copenhagen, Denmark
E-mail: joli@life.ku.dk

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Plant cells are directly connected by plasmodesmata (PD) that form channels through the cell wall and enable the intercellular movement of cytosolic solutes, membrane lipids and signaling molecules. Transport through PD is a key step for the regulation of plant growth, development and adaptation to stresses, such as pathogen attack. The capacity for a specific molecule to pass a specific cell wall interface is therefore an essential parameter for our understanding of these processes.

So far, the degree of cell coupling was derived from frequency and diameter of PD in relevant tissues as assessed by electron microscopy of fixed material. However, PD functionality and capacity can only be determined in live material, not from electron microscopy, which is static and prone to fixation artifacts. Fluorescence recovery after photobleaching (FRAP) has been used to study cell coupling in living tissue [1], but the analysis was limited to a single image plane of homogeneous tissue.

Here we present 4D photoactivation microscopy to quantify PD-mediated cell wall permeability between living pumpkin (Cucurbita maxima) leaf cells with the tracer caged fluorescein [2]. Caged fluorescein can be activated by UV illumination in a target cell and the activated tracer has similar diffusion properties to relevant cytosolic solutes. The experimental procedure resembles that of FRAP. The better signal-to-noise ratio of the photoactivation approach combined with high speed acquisition with a confocal microscope equipped with resonant scanner enabled us to gather all necessary functional and anatomical data for each individual cell from 3D time series. Thereby, we were able to accurately quantify cell coupling between living cells in complex tissue.