Clearing of plant tissue without fixation for intra-vital life imaging of virus infection in N. benthamiana leaves

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Kurihara[1] et al. (2015) developed the ClearSee solution to gain an optimal spatial resolution of fluorescence-tagged proteins in plant tissues. ClearSee rapidly diminishes chlorophyll autofluorescence while maintaining fluorescent protein stability. By adjusting the refractive index mismatch, whole-organ and whole-plant imaging can be performed by confocal microscopy in ClearSee-treated samples. Major drawback of this procedure is the fixation of the tissue prior to the ClearSee application, which abolishes the possibility to monitor dynamic processes, e.g. plant virus cell-to-cell movement. So far it is not possible to overcome this crucial limitation. Enabling deep tissue imaging in living and functional plant parts like leaves and stem would dramatically widen the prospective of plant biology based research.
To achieve this goal we are combining a custom build multi-photon intra-vital microscope (Zeiss 880 NLO, Spectra Physics InSight DeepSee Laser) suitable for deep tissue imaging with a modified ClearSee-based protocol. Thus we are able to follow virus infection and movement in living plants specimen such as Nicotiana benthamiana.
For this reason we developed a procedure to observe temporal and spatial plant virus cell-to-cell movement. For a general proof of principle we used Turnip Mosaic Virus tagged with RFP. This modified RFP-Virus is not phloem limited but will allow to estimate the real penetration deepth of the multi-photon set-up using our modified ClearSee protocol in living plant leaves. In 2010 Krenz[2] et al. already showed as proof of concept the movement of Geminivirus Abutilon Mosaic Virus tagged with GFP. The coding region for the coat protein of AbMV was replaced with GFP in a manner that allows GFP expression from the viral genome, thus infected cells express GFP (Krenz et al., 2010). AbMV is phloem-limited, which makes it difficult to monitor by microscopy, since the vascular system is deep in the tissue. By applying our modified ClearSee-based protocol on N. benthamiana leaves infected with AbMV and subsequent deep tissue intra-vital imaging cutting edge microscopy we are able to monitor AbMV movement within the vascular tissue from the onset of infection up to four days of post infection.
This improvement and combination of technologies is very likely to have great potential as it will futurewise allow plant based research to monitor mechanisms of virus proliferation and movement inside living plant tissue of various origin.

1 *ClearSee: a rapid optical clearing reagent for whole-plant fluorescence imaging.*
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2 *Cell-free construction of disarmed Abutilon mosaic virus-based gene silencing vectors.*
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