

WHY TO TOPPLE THE CONFOCAL MICROSCOPE: MICROSCOPE WITH VERTICAL SAMPLE MOUNTING FOR PLANT RESEARCH

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The plant root is a widely used model for developmental, physiological and cell biological studies. Until now most of microscopy data studying plant root were acquired with the mean of classical upright/inverted confocal microscope with the horizontal orientation of sample. However this mounting is not natural for seedling growth. As the root tip permanently attempts to bend downwards that is not possible in this setup, the plant is permanently gravistimulated during all measurements. This might influence the data obtained by possible unwanted artifacts and moreover completely enables some type of experiments on growing roots. Therefore new attempts are taken to optimize the microscope setup for the needs of plant growth. The pioneer work [1] describes the confocal microscope Zeiss LSM700 with vertical sample mounting. Here, we present newer version of Zeiss microscope - LSM880 with Airyscan detector in horizontal setup with the vertical sample mounting preserving natural position of root growth. Moreover, our system is equipped with the stage rotation insert that enables further non-invasive application of gravistimulus on the seedlings, growing for longer time under the microscope. In addition to the previously established setup, we have installed high magnification objectives (63x and 100x) with high numerical aperture and optimized immersion fluids and oils. This allows imaging in much higher details in contrast to published setup [1]. To demonstrate benefits of the system we concentrated on auxin signaling and transport during root growth. We compare results obtained on classical LSM880 with horizontal sample mounting and data obtained on vertical sample mounting on our new LSM880 with vertically grown roots. These comparisons show that using new setup, it is possible to detect previously not seen re-distributions of auxin carriers as well as auxin-regulated expression of marker genes.

[1] D. von Wangenheim, R. Hauschild, M. Fendrych, V. Barone, E. Benková, J. Friml “Live tracking of moving samples in confocal microscopy for vertically grown roots.” *Elife*, **6**, e26792 (2017).