APPLICATION NOTE

SMZ18 Research Stereomicroscope
AX/AX R Confocal Microscopes

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Multiscale Simultaneous ER and Cytosolic Ca^{2+} Imaging in Plants

Calcium ions (Ca^{2+}) play a central role in cellular signaling, with environmental and developmental stimuli triggering specific increases in cytosolic and endoplasmic reticulum (ER) Ca^{2+} levels. To define the role of the ER in regulating cytosolic Ca^{2+} dynamics, it is essential to analyse Ca^{2+} fluctuations in both compartments in vivo. This is now feasible using genetically encoded fluorescent Ca^{2+} indicators (GECIs) such as ER-GCaMP6-210 and R-GECO1, which form an ideal pair for simultaneous ER and cytosolic imaging. Here, we describe an *Arabidopsis thaliana* transgenic line co-expressing these indicators, enabling visualization of Ca^{2+} responses to stimuli including leaf wounding and extracellular ATP. Our approach allows Ca^{2+} imaging across spatial scales: the Nikon SMZ18 stereomicroscope supports whole-organ observations in adult leaves, while the AX R laser-scanning confocal microscope with silicon objectives enables high-resolution, dual-compartment analyses at the single-cell level in roots. Together, these experiments demonstrate that this dual-sensor line provides a robust platform for investigating ER-related Ca^{2+} signaling across developmental stages.

Keywords: Plant Biology, Calcium imaging, *Arabidopsis thaliana*, confocal microscope, stereo microscope

Rosette-wide Ca^{2+} responses visualized with SMZ18

To demonstrate dual-compartment in vivo Ca^{2+} imaging in intact adult plants, 4-week-old *Arabidopsis thaliana* (hence after Arabidopsis) plants co-expressing ER-GCaMP6-210 and R-GECO1 (Fig. 1) were imaged using a Nikon SMZ18 fluorescence stereomicroscope. Plants were grown in soil and imaged non-invasively using standard GFP and RFP filter sets in combination with a pE300-Ultra LED illuminator. This configuration enabled rapid switching between channels and uniform widefield illumination, making it suitable for whole-plant and whole-organ imaging.
Mechanical stimulation was applied by wounding a rosette leaf with tweezers, following the protocol described in Resentini et al. (2021). Fluorescence signals from both genetically encoded Ca^{2+} indicators were recorded and quantified in the wounded (local, Region of Interest 1:ROI1) leaf and in an unwounded distal (ROI2) leaf (Fig. 2A). Images were acquired at 4 s intervals.

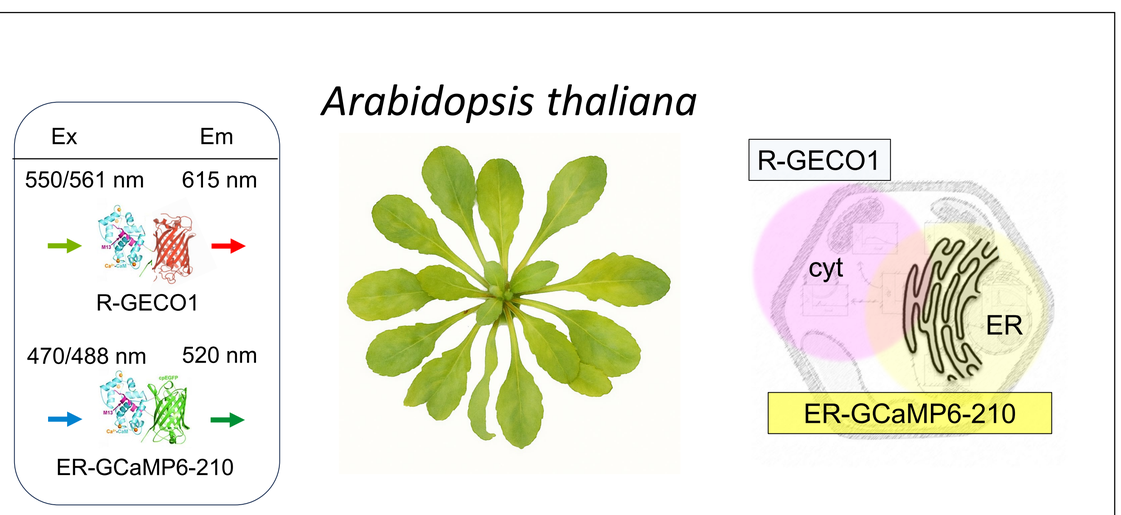

Figure 1: Graphical summary

The SMZ18 stereomicroscope allowed visualization of large-scale Ca^{2+} signaling events across the rosette, revealing stimulus-induced Ca^{2+} increases in both the cytosol and ER (Fig. 2B). Signals originated primarily in vascular tissues and propagated through the leaf lamina and petiole, eventually reaching distal leaves (Fig. 2B-E). These experiments highlight the suitability of the SMZ18 platform for monitoring long-distance Ca^{2+} signaling dynamics at the whole-plant level. Quantitative analysis of fluorescence changes within defined ROIs showed that ER Ca^{2+} accumulation consistently followed the cytosolic Ca^{2+} increase and exhibited more sustained kinetics (Fig. 2B, C).

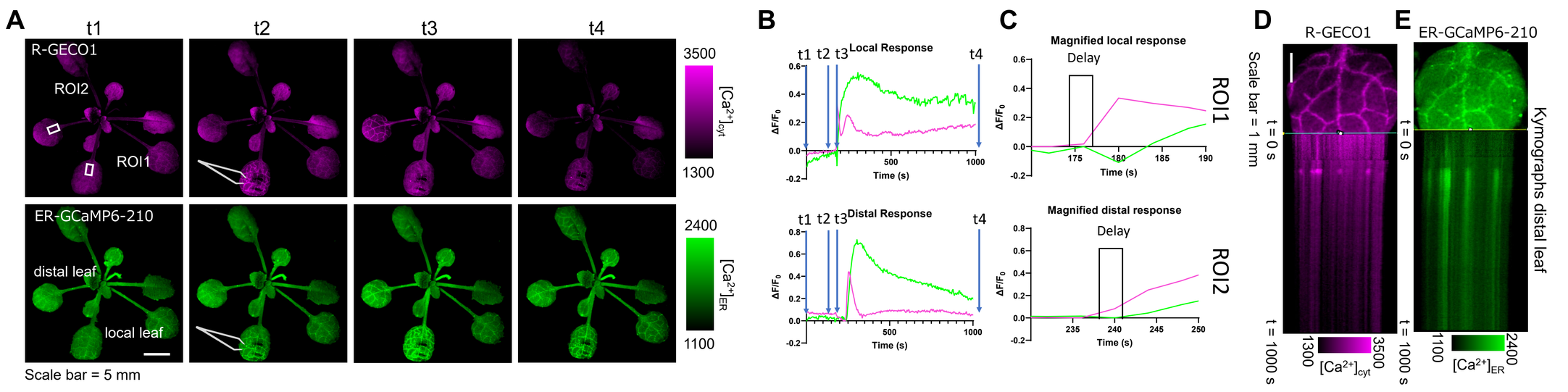

Figure 2: Simultaneous cytosolic and ER Ca^{2+} analyses in Arabidopsis leaves of adult plants challenged by leaf wounding.
(A) Representative images of a mature R-GECO1 × ER-GCaMP6-210 plant following local leaf wounding. Cytosolic Ca^{2+} is reported by R-GECO1 (magenta), and ER Ca^{2+} by ER-GCaMP6-210 (green). Wounding was applied to a local leaf. Forceps indicate wounding site. **(B)** Normalized fluorescence changes of R-GECO1 and ER-GCaMP6-210 in the wounded local leaf (ROI1, top) and a distal systemic leaf (ROI2, bottom) over time after wounding. Time points t1, t2, t3, and t4 correspond to the images shown in (A). **(C)** Delay of the ER-GCaMP6-210 fluorescence increase relative to the R-GECO1 fluorescence increase. **(D)** Kymograph from a 1,000 s time-lapse acquisition of R-GECO1 fluorescence in the distal leaf. **(E)** Kymograph from a 1,000 s time-lapse acquisition of ER-GCaMP6-210 fluorescence in the distal leaf.

High-resolution dual-compartment Ca^{2+} imaging in root cells

The stereomicroscope enabled visualization of spatially extended Ca^{2+} waves across whole organs, providing valuable insight into large-scale signaling patterns. To complement these observations with high-resolution, single-cell dual-compartment Ca^{2+} imaging, and to precisely resolve short delays between cytosolic and ER Ca^{2+} dynamics, Arabidopsis seedling root tips were subsequently imaged using a Nikon Ti2-E equipped with AX R laser-scanning confocal microscope. The AX R system was configured with dual GaAsP spectral detectors, allowing simultaneous acquisition of ER-GCaMP6-210 and R-GECO1 signals and eliminating temporal offsets associated with sequential imaging. Images were acquired at 3 s intervals, providing sufficient temporal resolution to resolve Ca^{2+} dynamics. Cytosolic Ca^{2+} transients were induced by perfusion with 0.1 mM ATP for 3 min using a previously described microfluidic delivery system (Resentini et al., 2021).

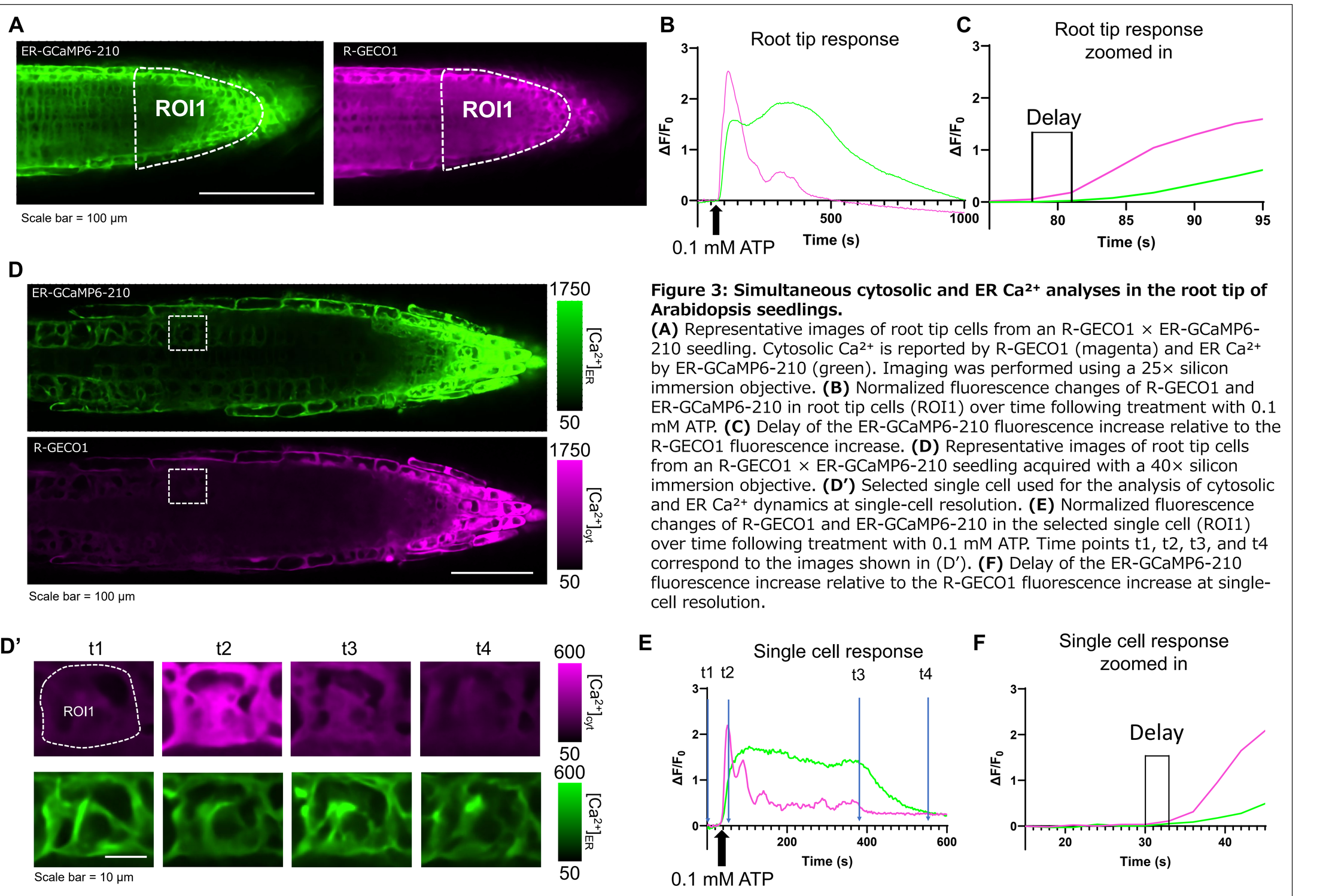

Figure 3: Simultaneous cytosolic and ER Ca^{2+} analyses in the root tip of Arabidopsis seedlings.
(A) Representative images of root tip cells from an R-GECO1 × ER-GCaMP6-210 seedling. Cytosolic Ca^{2+} is reported by R-GECO1 (magenta) and ER Ca^{2+} by ER-GCaMP6-210 (green). Imaging was performed using a 25× silicon immersion objective. **(B)** Normalized fluorescence changes of R-GECO1 and ER-GCaMP6-210 in root tip cells (ROI1) over time following treatment with 0.1 mM ATP. **(C)** Delay of the ER-GCaMP6-210 fluorescence increase relative to the R-GECO1 fluorescence increase. **(D)** Representative images of root tip cells from an R-GECO1 × ER-GCaMP6-210 seedling acquired with a 40× silicon immersion objective. **(D')** Selected single cell used for the analysis of cytosolic and ER Ca^{2+} dynamics at single-cell resolution. **(E)** Normalized fluorescence changes of R-GECO1 and ER-GCaMP6-210 in the selected single cell (ROI1) over time following treatment with 0.1 mM ATP. Time points t1, t2, t3, and t4 correspond to the images shown in (D'). **(F)** Delay of the ER-GCaMP6-210 fluorescence increase relative to the R-GECO1 fluorescence increase at single-cell resolution.

Initial imaging was performed using a 25× silicon immersion objective, enabling visualization of the entire root tip with high signal-to-noise ratio and minimal phototoxicity (Fig. 3A). Analysis of the meristematic root zone revealed that, as observed in leaves, ER Ca^{2+} accumulation followed cytosolic Ca^{2+} elevations, displaying delayed peak responses and slower recovery kinetics (Fig. 3B, C). For detailed single-cell analysis, imaging was further refined using a 40× silicon immersion objective combined with digital zoom (Fig. 3D, D'). This configuration provided enhanced spatial resolution while maintaining high sensitivity, enabling precise temporal measurements of Ca^{2+} fluxes between cellular compartments. Under these conditions, simultaneous dual-channel imaging can allow a precise determination of the delay between the onset of cytosolic Ca^{2+} increase and subsequent ER Ca^{2+} uptake (Fig. 3E, F).
Together, these results demonstrate how complementary Nikon imaging platforms can be used to investigate Ca^{2+} dynamics across spatial scales, from whole-plant responses using fluorescence stereomicroscopy to single-cell, dual-compartment analyses using high-speed confocal imaging. This integrated approach provides a robust in vivo framework for probing ER Ca^{2+} handling and the activity of ER-localized Ca^{2+} transporters under physiologically relevant conditions. Indeed, we recently demonstrated in Kuang et al. (2025) that the same approach and technology can be applied to perform similar analyses in other compartments, such as the chloroplast and cytosol.

Conclusions

This application note builds on our recent publication (Resentini et al., 2021), which described the development of the Arabidopsis double-sensor line and similar experiments to those presented here. In the present study, we apply this dual-sensor approach using two complementary Nikon imaging platforms: the fully automated SMZ18 stereomicroscope for widefield, whole-organ observations, and the AX R laser-scanning confocal microscope for high-resolution, fast dual-channel imaging. The AX R system, equipped with dual spectral detectors, enables precise definition of emission windows to minimize fluorescence crosstalk between ER-GCaMP6-210 and R-GECO1 and allows true simultaneous acquisition of both channels. Moreover, the new 25× and 40× Silicon Immersion Objectives are particularly well suited for high-resolution imaging of in vivo samples, such as seedling root cells, by better matching the refractive index of aqueous environments, thereby reducing optical mismatch and improving image quality.

References

1. Resentini, F., Grenzi, M., Ancora, D., Cademartori, M., Luoni, L., Franco, M., Bassi, A., Bonza, M. C., & Costa, A. (2021). Simultaneous imaging of ER and cytosolic Ca^{2+} dynamics reveals long-distance ER Ca^{2+} waves in plants. PLANT PHYSIOLOGY, 187(2), 603–617. Kuang
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Product information

SMZ18 Research Stereomicroscope

A high-performance research stereomicroscope that provides both brightness and high-resolution.

- 18:1 zoom ratio for macro-to-micro imaging
- High-resolution SHR Plan Apo optics with excellent color fidelity
- Improved fluorescence performance with high S/N ratio

Product information is here

AX/AX R Confocal Microscope

Supports high-speed, high-resolution, large field-of-view confocal imaging, with reduced phototoxicity to living cells and photobleaching.

- High speed: Up to 720 fps (resonant at 2048 x 16 pixels)
- High resolution: Up to 8K (galvano)/ 2K (resonant)
- High throughput: Ultra-wide field of view of 25 mm

Product information is here