Protocol for rapid clearing and staining of fixed Arabidopsis ovules for improved imaging by confocal laser scanning microscopy

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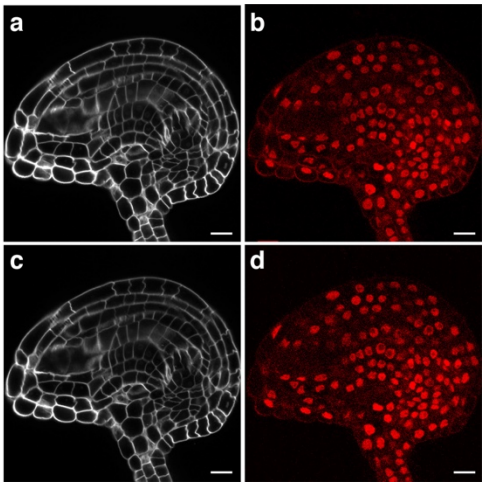
Comparison between HyD and PMT detectors



Confocal micrographs show mid-optical sections through fixed mature ovules from Col-0 plants carrying a pP16::H2BV:tdTomato reporter and stained with SR2200. Images were obtained using an upright Leica TCS SP8 X WLL2 HyVolution 2 (Leica Microsystems) equipped with GaAsP (HyD) detectors, PMTs, and a 63x glycerol objective (HC PL APO CS2 63x/1.30 GLYC, CORR CS2).

(**a,b**) Image acquisition with HyD detectors.

(**a**) SR2200: 405 diode laser 0.10%, HyD 420 nm – 480 nm, detector gain 10.

(**b**) tdTomato: 554 White laser 4%, HyD 570 nm – 630 nm, detector gain 80.

(**c,d**) Image acquisition with PMTs.

(**c**) SR2200: 405 diode laser 0.10%, PMT 420 nm – 480 nm, detector gain 600.

(**d**) tdTomato: 554 White laser 12%, PMT 570 nm – 630 nm, detector gain 800.