




Miniature structured illumination microscope for *in vivo* 3D imaging of brain structures with optical sectioning: supplement

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Miniature Structured Illumination Microscope for *in vivo* 3D Imaging of Brain Structures with Optical Sectioning

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Supplementary Materials

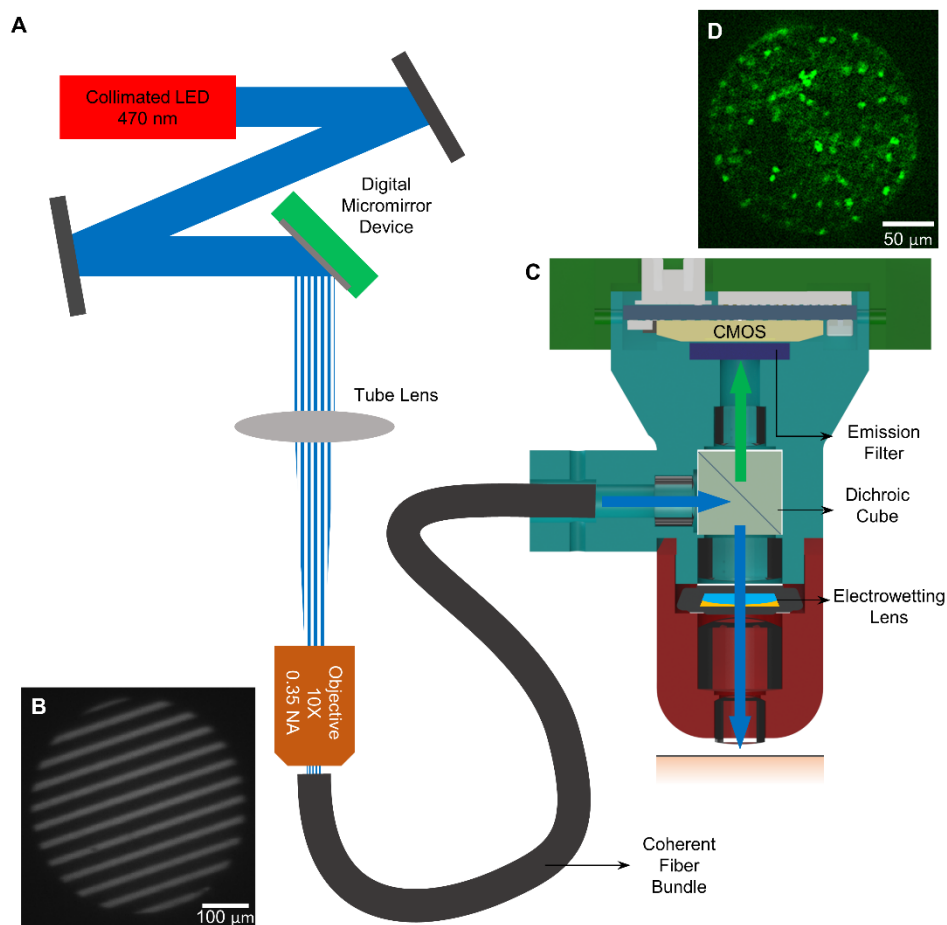


Figure S1: (A) Schematic of the experimental setup; (B) SIM pattern on the distal end of the fiber; (C) Cross-sectional view of the SIMscope3D; (D) SIM reconstructed image of the fixed tissue sample.

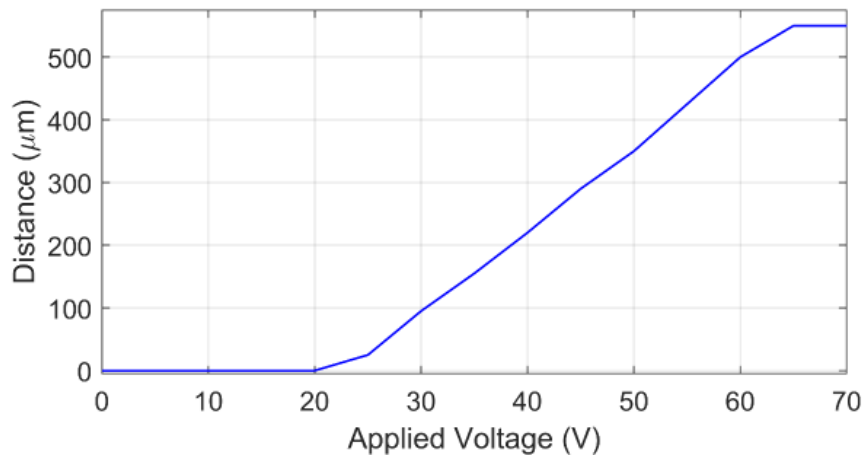


Figure S2: Axial depth calibration of the SIMscope3D as a function of the electrowetting lens applied voltage.

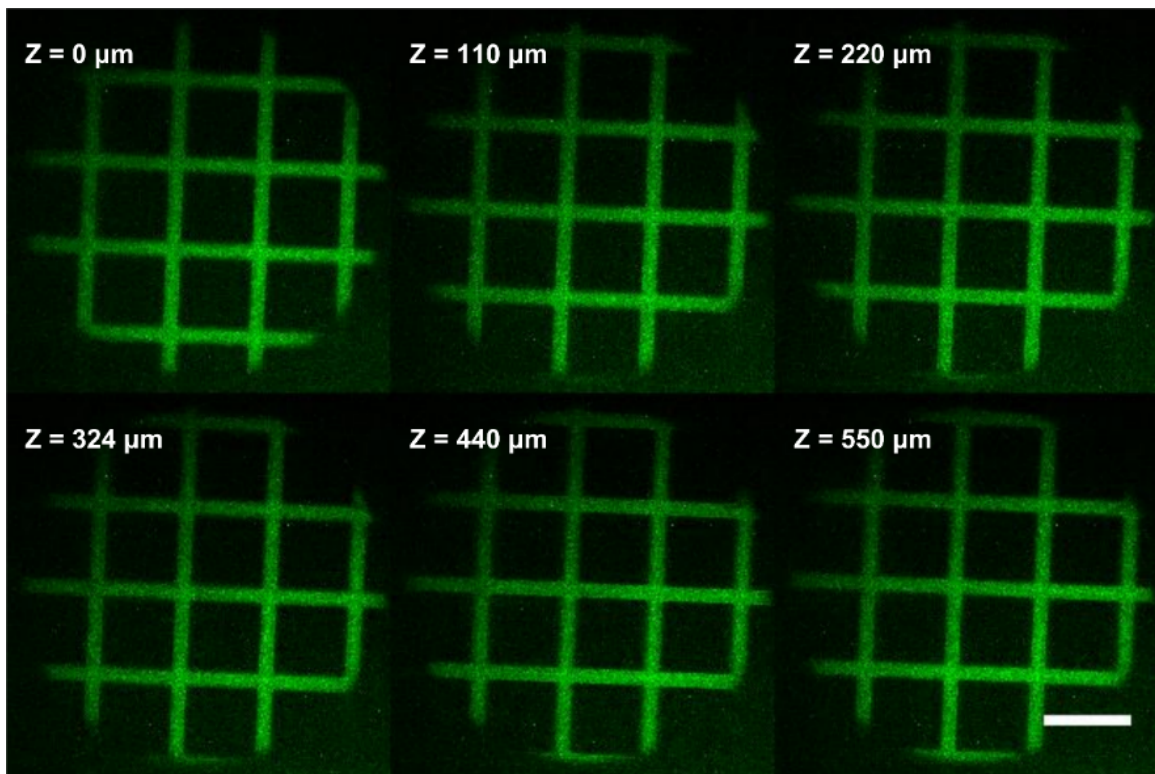


Figure S3: FOV calibration of the SIMscope3D at various axial depths using a 50 μm square grid pattern based on axial calibration from Figure S1. Scale bar is 50 μm.

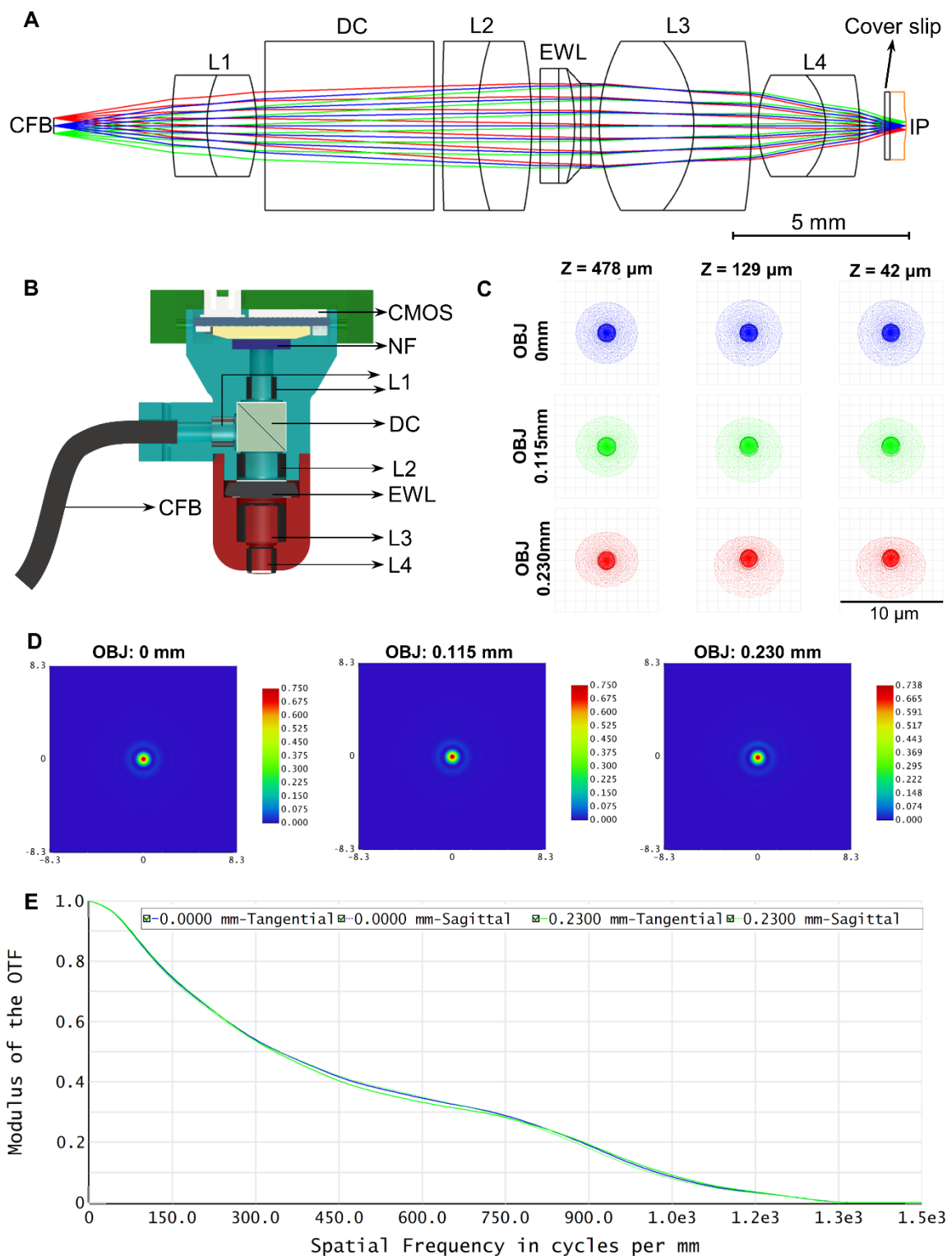


Figure S4: (A) Zemax OpticStudio design of the excitation path at 470 nm for the SIMscope3D. The dichroic cube (DC) is modeled as a 5 mm path length BK-7 window. The fiber bundle (FB) provides the excitation light for the SIMscope3D. Lenses (L1 to L4) are achromatic doublets, and the electrowetting lens (EWL) provides depth scanning of the imaging plane (IP) of up to 550 μm below the coverslip. The imaging plane has a field curvature of 2.71 mm; (B) Cross-sectional view of the SIMscope3D CAD design with all the components listed. CFB: Coherent Fiber Bundle,

Fujikura FIGH-10-500N, L1: Edmund Optics 3mm Dia. x 9mm FL MgF2 coated achromatic doublet lens, L2: Edmund Optics 5mm Dia. x 20mm FL MgF2 coated achromatic doublet lens, L3: Thorlabs 5mm Dia. x 7.5mm FL A coated achromatic doublet, L4: Edmund Optics 3mm Dia. x 4.5mm FL MgF2 coated achromatic doublet lens, DC: Dichroic Cube, Shanghai Optics cemented cube with dichroic coating reflecting 440-480 nm and transmitting 510-550 nm, EWL: Electrowetting Lens, Varioptic Corning A25H, NF: Notch Filter or the Emission Filter, CMOS: CMOS image sensor, Ximea MU9PM-MBRD, IP: Imaging Plane; (C) Full field spot diagram corresponding to object field positions 0mm, 0.115mm and 0.23mm at depths 42 μ m, 120 μ m and 478 μ m, here object field position corresponds to the location of the fiber in the CFB; (D) Huygens point spread function at the imaging plane (130 μ m in the tissue) for various object positions; (E) Huygens modulus of the optical transfer function for various object field positions in the fiber bundle.

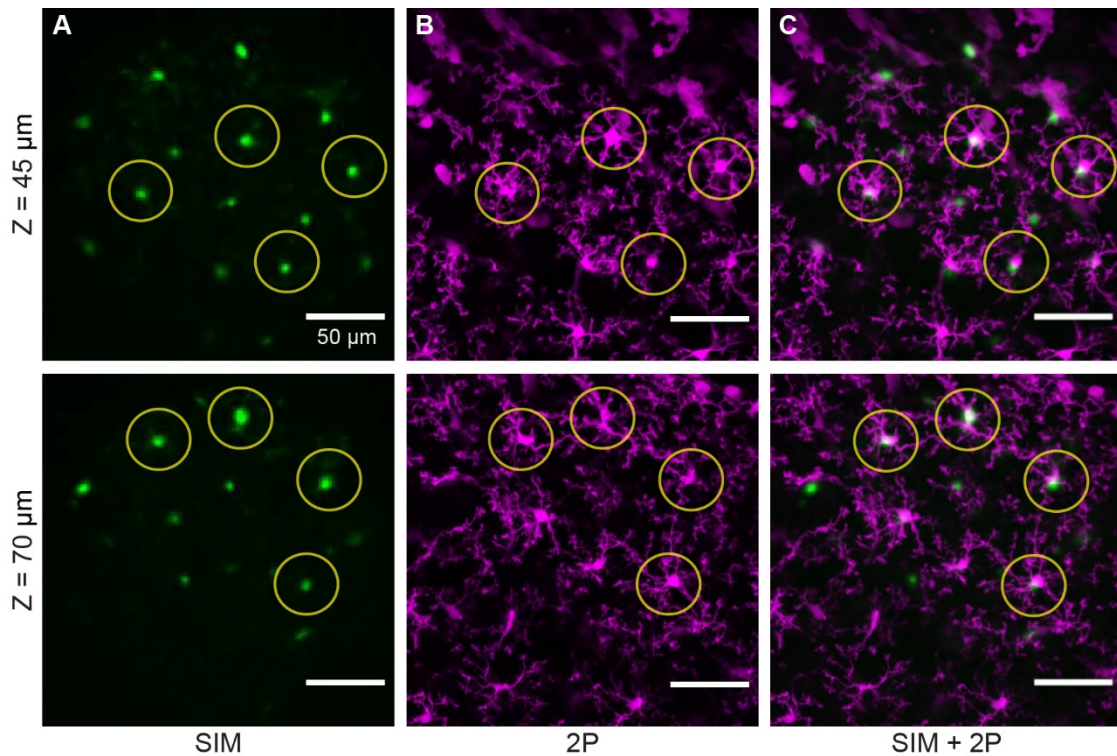


Figure S5: (A) Average Z projection (over 15 μ m) of SIM image stack at two depths (top = 45 μ m; bottom = 70 μ m). The same FOV is used for all panels and ROIs indicate the same cells in each panel at a given depth. (B) Average Z projection of 2P image stack at same depths as A, with same Z depth range. (C) Panels A and B overlaid with areas of direct overlap shown in white. Imperfect overlap may be due to cell migration between imaging. Coverslip was slightly angled for 2P imaging which caused the FOV to be obscured at depths above \sim 45 μ m.

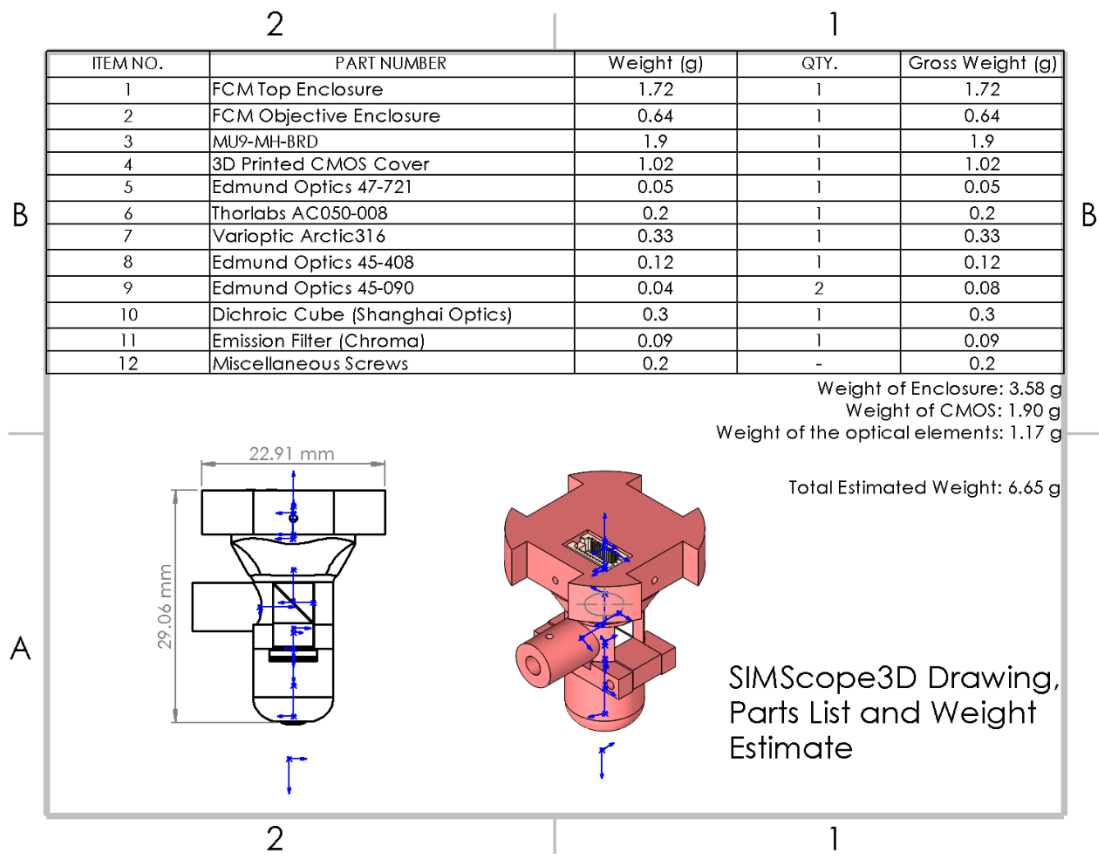


Figure S6: List of components and estimated weights for SIMscope3D. The optical elements weigh approximately 1.2 g, with the CMOS and the enclosure contributing approximately 1.9 g and 3.6 g. The total weight of SIMscope3D is 6.7 g.