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Supplemental information

Instant three-color multiplane fluorescence microscopy

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Supporting material

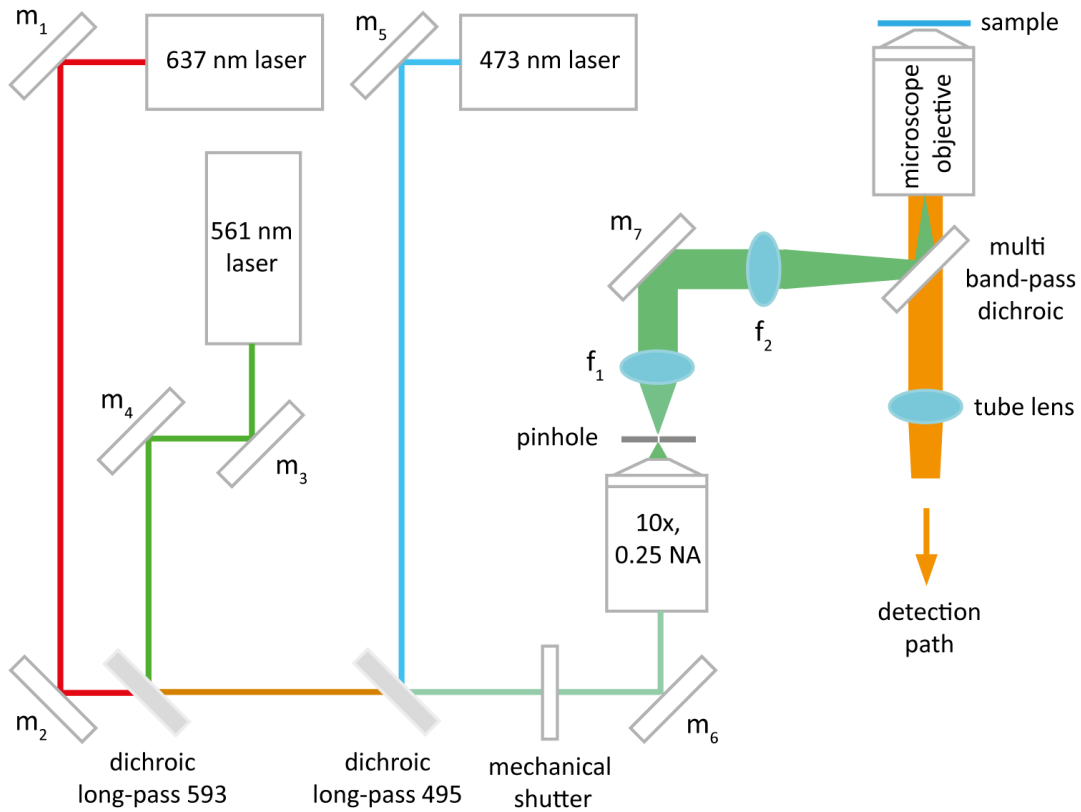


Fig. S1. Illumination path of the three-color multi-plane setup. Three laser beams at 473 nm, 561 nm and 637 nm are co-aligned using a set of dielectric mirrors, m_1 to m_5 , and dichroic long pass filters at $\lambda = 495$ nm and $\lambda = 593$ nm. The beam-expander is comprised of a 10x, 0.25 NA objective ($f = 18$ mm) and lens $f_1 = 200$ mm. A 20 μm pinhole is placed in the common focus position of both lenses to clean up the transverse modes of the beams. Lens $f_2 = 300$ mm focuses the collimated beam at the back focal plane of the objective. A mechanical shutter controls the illumination timing.

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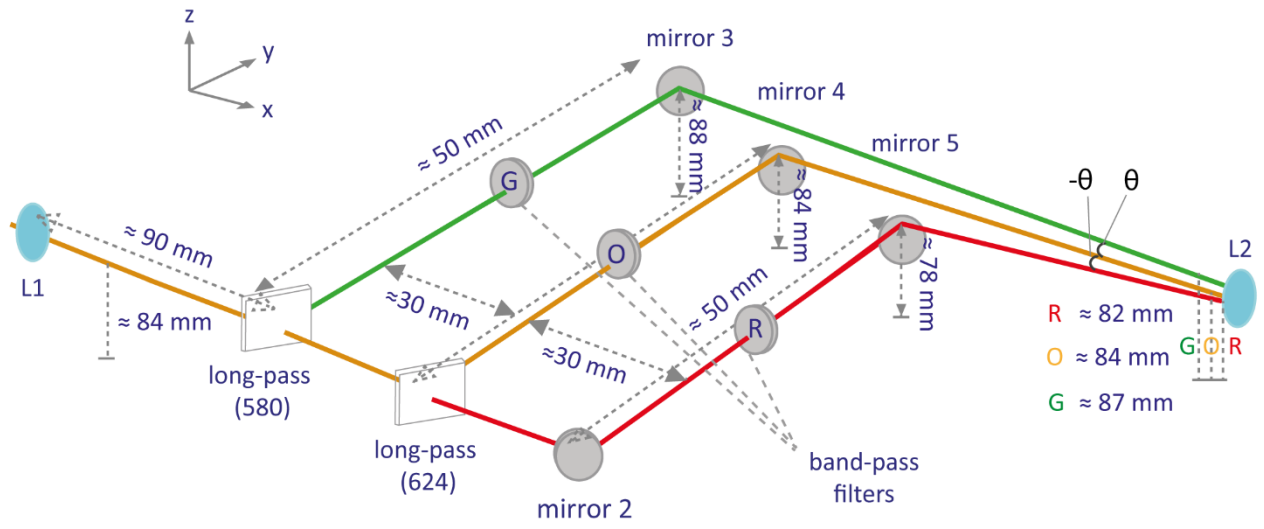


Fig. S2. Detailed design of the three-color beam splitter (spectral unmixing) unit.

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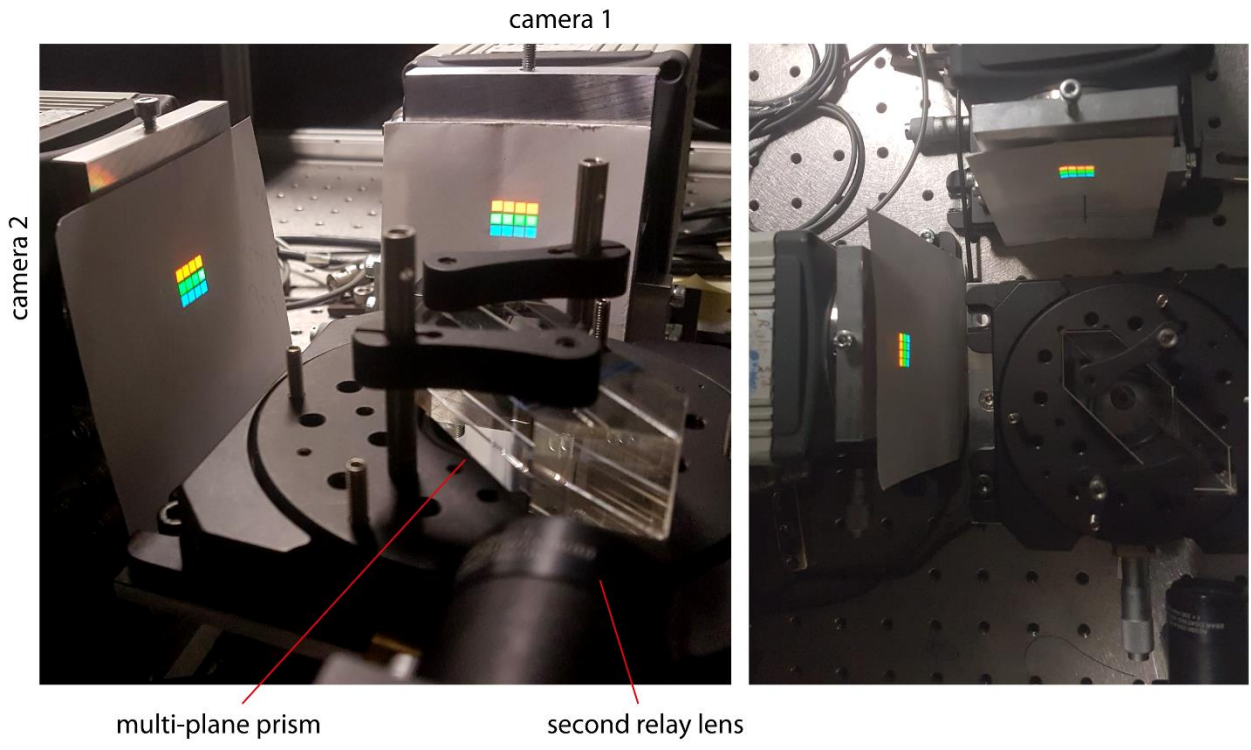


Fig. S3. Two views of the output multi-planes in blue, green, and red spectral channels.

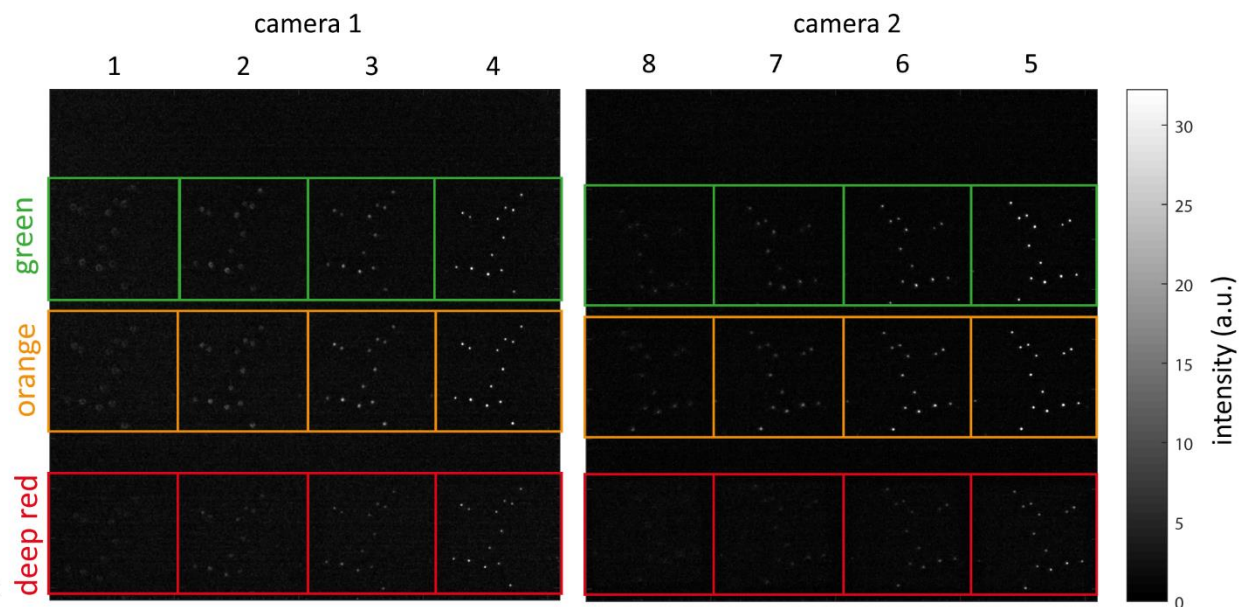


Fig. S4. Raw data of three-color eight-planes from tetra spectral beads in one scanning step of the calibration measurement. Similar intensity magnitude of tetra spectral beads in different color channels at corresponding axial planes (columns) demonstrate the minimal axial color aberration of the imaging system. In this particular scan position, beads are in focus at nominal plane #5. The numbers above images represent the corresponding planes as depicted in Fig. 1. The height of planes from surface coverslide increases from the lowermost plane (#1) to the topmost plane (#8).