

High-Resolution, Large Field-of-View, and Multi-View Single Objective Light-Sheet Microscopy

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Light-sheet microscopy is well suited for long-term imaging of living samples due to good optical sectioning and low photo-invasiveness. Among various light sheet methods, single objective light sheet microscopy based on Oblique Plane Microscopy (OPM)¹ is well suited for various sample mounting modalities and high-throughput imaging. Initial design of OPM had low optical resolution due to loss of numerical aperture during remote focusing^{1,2}. This limitation was overcome recently, and sub-cellular resolution was achieved using index mismatched objective lens for remote focusing³⁻⁵.

Here we describe such a single-objective light-sheet microscope that has increased field of view (700 μm * 300 μm * a few mm) and an effective numerical aperture close to 1.0. The high-resolution and large field-of-view are achieved via a custom remote focusing objective and light sheet stabilized stage scanning, a novel scanning modality that extends the imaging volume without compromising imaging speed nor quality.

Moreover, refraction and scattering often pose obstacles to light-sheet propagation and limit imaging depth, especially when imaging large non-transparent samples, such as zebrafish embryo. This is typically addressed by imaging multiple complementary views to obtain high and uniform image quality throughout the sample⁶. In this paper, we also combine the multi-view concept and OPM to extend the illumination coverage of the sample by means of dual orthogonal light-sheet illumination.

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