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Multilayer beam splitter advances light sheet fluorescence microscopy **FREE**

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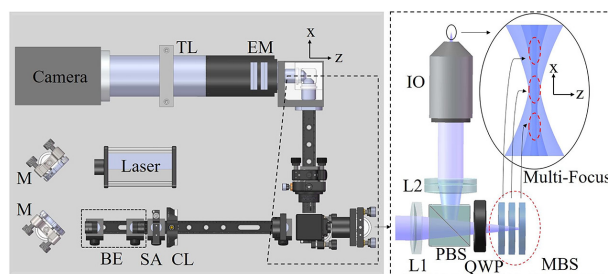


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Multilayer beam splitter advances light sheet fluorescence microscopy

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Technique extends field of view and improves uniformity while maintaining resolution.



With high resolution and low-phototoxicity, light sheet fluorescence microscopy (LSFM) has become an indispensable bioimaging tool that enables researchers to create 3D images of biological samples, including tissue, organelles, and live animal embryos.

To produce even finer details of biological samples, LSFM must generate thinner light sheets, which would also further reduce photobleaching for longer term imaging. However, this is at the cost of a smaller field of view.

To address this challenge, researchers have proposed various solutions, including using phase filters, multiphoton excitation, and multiple beams. But these approaches require precise synchronization, which significantly increases imaging system complexity.

In a more simplified approach, Li et al developed a LSFM device that includes a multilayer beam splitter (MBS) to generate multiple focal planes at different focal distances. The resulting overlapped light sheets extend the field of view by 1.7 times for double-focus and by 2.6 times for triple focus, leading to high-resolution imaging at homogeneous illumination and large-scale sharp optical sectioning capability.

The multi-focus module is integrated into OpenSPIM, a widely used, open-source light sheet system platform. The device comprises a polarizing beam splitter, quarter-wave plate, and the MBS. The researchers tested the device on HeLa cancer cells and 3-day-old zebrafish.

The MBS consists of several beam splitters and a mirror, which produces consecutive beams with different divergence, resulting in the simultaneous imaging of different focal planes within a specimen. Spacers with variable thickness can adjust the focus shift between neighboring beams.

“Easy implementation of the multilayer beam splitter makes our LSFM system highly efficient, low cost, compact, and largely compatible with other LSFM systems,” author Karl Zhanghao said.

Source: “Axially overlapped multi-focus light sheet with enlarged field of view,” by Hongjin Li, Zihan Wu, Zhichao Yang, Karl Zhanghao, Peng Xi, and Dayong Jin, *Applied Physics Letters* (2021). The article can be accessed at <https://doi.org/10.1063/5.0049013>.

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