IMAGING

Tunable light-sheet microscopy

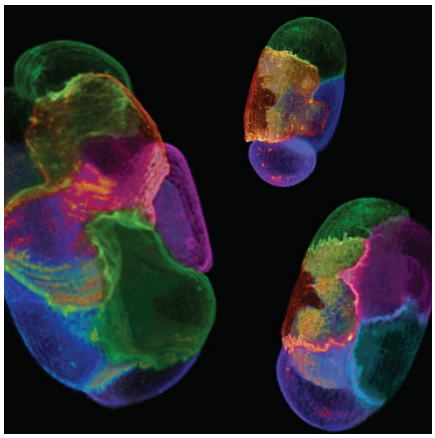
Tiling light-sheet microscopy improves live cell imaging of multicellular organisms.

Light-sheet microscopy offers high spatial resolution and imaging speed over relatively large sample volumes at low doses of light, making it very attractive for live cell imaging. Ideally, light sheets would be large and uniformly thin, which would allow for high image resolution in three dimensions over a large field of view and throughout an entire specimen. In reality, no such light sheet exists, despite many advances toward this goal. Most cutting-edge light-sheet microscopes perform best on relatively small samples, such as single cells.

Liang Gao and his colleagues at Stony Brook University sought to develop a new approach to imaging large, multicellular specimens with subcellular resolution. Gao, who worked at the forefront of light-sheet microscopy development with Eric Betzig, recalls, "After I worked in Eric's lab for four years, I realized that there was very little space left for me to keep improving the imaging ability of light-sheet microscopy on cultured cells." So he developed tiling light-sheet selective-plane illumination microscopy (TLS-SPIM) to overcome some of the barriers with existing microscopes.

In TLS-SPIM a small but thin light sheet is tiled quickly within an image plane until it illuminates the entire field of view. The fluorescence emitted from the center of each tiled light sheet is used to generate the final image. This works as a substitute for an ideal large and thin light sheet, and it allows higher resolution of larger fields of view in three dimensions. The researchers demonstrated the power of this approach by imaging developing worm embryos.

The TLS-SPIM microscope employs two binary spatial light modulators to allow users to select from among Gaussian, Bessel and lattice light sheets depending on the imaging application. Users can switch between the different light sheets in real time on the basis of their imaging results, a feature that caused Gao to reflect on an important message of his work: "The benefit of light-sheet microscopy in 3D imaging doesn't come by default. It requires optimization of the light sheet based on the sample and application." He hopes that making such optimization straightforward expands the utility of light-sheet microscopy.



3D volume renderings of worm embryos at 4-, 7–8- and 8–12-cell stages (clockwise from upper right). Individual cells are shown in different colors.

A drawback of using tiled light sheets is that imaging speed is reduced because of the time it takes to image one field of view with multiple light sheets. To minimize this issue, TLS-SPIM also allows users to choose the size of the tiled light sheets. Smaller light sheets yield higher resolution at the cost of imaging speed, whereas larger tiled light sheets increase imaging speed, but at the cost of resolution. In an attempt to optimize this tradeoff, the team developed a method for switching rapidly between high-speed and high-resolution modes, which allowed them to get good spatiotemporal resolution of the migration of mesodermal cells in the tailbud of a zebrafish embryo.

TLS-SPIM should be a very practical tool for studying multicellular organisms at the subcellular level. Looking back, Gao was surprised by the low photobleaching rate of samples imaged by TLS-SPIM. He attributes this to better light confinement in the tiled sheets versus in a single light sheet used to cover the same field of view. He hopes to use this method to better understand embryogenesis. He notes that improved optical and computational tools are needed to continue driving live cell imaging and the analysis of multicellular organisms forward, and he is also working toward these goals.

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RESEARCH PAPERS

Fu, Q. *et al.* Imaging multicellular specimens with real-time optimized tiling light-sheet selective plane illumination microscopy. *Nat. Commun.* **7**, 11088 (2016).

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