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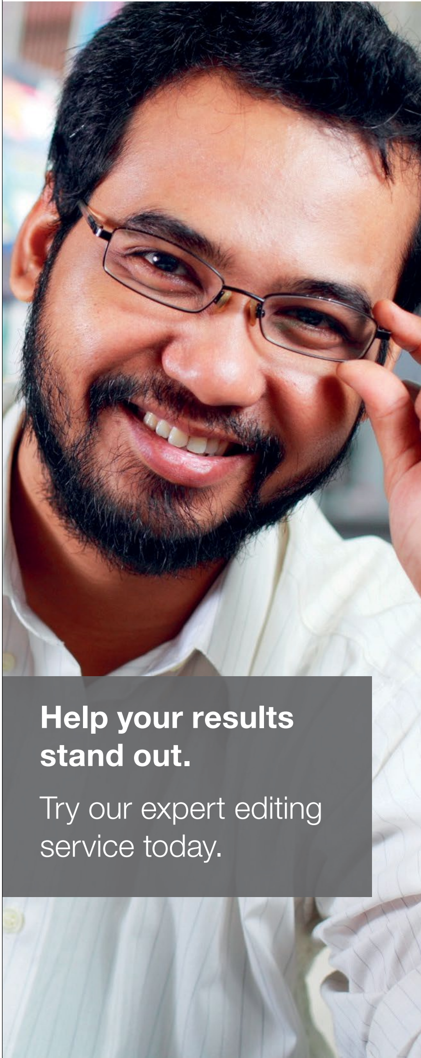

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SPRINGER NATURE

IMAGING

Corrected in vivo imaging

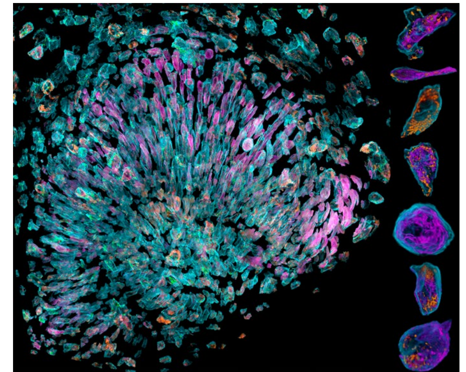
Aberration correction adds depth to light-sheet microscopy.

Light microscopy offers the potential to image in vivo biological dynamics; however, it is challenging to simultaneously achieve both high temporal and high spatial resolution deep inside of living organisms. Light-sheet microscopy (LSM) has risen to this challenge, greatly speeding up volumetric imaging by enabling scientists to image whole planes at a time, instead of points. Despite LSM's promise, optical path distortions, at depths of only tens of micrometers, degrade its image quality. These distortions, or aberrations, can be measured and corrected by adaptive optics (AO). Commonly used in astronomy, where aberrations are weak but rapidly varying, AO offers a great opportunity to image within biological tissue, where aberrations can be extreme but vary slowly in both space and time. Researchers, led by Eric Betzig from the Howard Hughes Medical Institute's Janelia Research Campus, have now merged AO with lattice light-sheet microscopy (LLSM) to enable diffraction-limited high-speed in vivo volumetric optical imaging.

Previously developed by Betzig's lab, LLSM provides a thinner light sheet with less background and higher axial resolution than that achieved with more traditional Gaussian beam light-sheet methods. The LLSM uses a spatial light modulator (SLM) to project an axially confined square or hexagonal optical lattice across the focal plane of the excitation objective; the light sheet is then formed, and imaged, as the focused light is rapidly scanned across the sample. Here, an AO system is integrated into both the excitation and detection pathways to correct for the independent sample- and system-induced aberrations in both pathways.

The AO systems measure distortions with an optical point source: two-photon fluorescence excited by a focused laser beam. This so-called guide star is scanned within the sample while the detected light is descanned, enabling an average wave-front error to be measured across the field of view (up to 60 micrometers in a zebrafish embryo). Surprisingly, application of an average correction for a field of view often yields better results than the use of point corrections.

For the detection path, Betzig's team implemented the AO correction in a traditional way, using a deformable mirror in a plane conjugate to the rear pupil of the objective. However, that was not possible for the excitation path, because only the periphery of the objective is illuminated in LLSM. "We asked, is there a way we can use the same sample-conjugate SLM to also correct for the aberrations?" Betzig recalled. "We weren't sure if it would work, but empirically it worked pretty well." By adding autofocus, the team produced diffraction-limited images that could then be further improved by deconvolution.



AO-corrected image of a developing zebrafish embryo eye (24 hours post fertilization). Reproduced with permission from Liu et al. (2018), AAAS.

By stitching together adjacent aberration-corrected volumes, the authors studied processes ranging from endocytosis in human stem cells to spinal cord development in zebrafish. Open challenges, beyond single-molecule sensitivity and the development of a build-it-yourself next-generation system, include finding a way to extract quantitative insights from increasingly large and complex datasets—what Betzig called "the frontier of microscopy." ❐

Zachary J. Lapin

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Research papers

Liu, T.-L. et al. Observing the cell in its native state: imaging subcellular dynamics in multicellular organisms. *Science* **360**, eaaq1392 (2018).