NEUROSCIENCE

Hybrid volumetric calcium imaging

Hybrid multiplexed sculpted light microscopy enables calcium imaging of volumes as large as half a cubic millimeter.

In neuroscience, the quest is on for imaging approaches that allow for calcium imaging in large volumes with good spatial and temporal resolution, which would facilitate the study of neuronal networks in the brain. However, there are tradeoffs among volume, imaging speed, and resolution, necessitating creative strategies to outsmart this limitation.

Alipasha Vaziri from Rockefeller University in New York and his collaborators have designed and built a microscope based on biological considerations that allows imaging of up to 12,000 neurons in the mouse brain with single-cell resolution and at high speed. To achieve this feat, they combined several previously developed optimizations and built a hybrid multiplexed sculpted light (HyMS) microscope. Key design choices were the use of temporal focusing to shape the excitation light into a larger spot allowing faster scan rates, spatiotemporal multiplexing for near-simultaneous imaging with multiple beamlets, rapid remote scanning, and the combination of two-photon and three-photon illumination for imaging of both shallow and deep subvolumes.

Vaziri and his team demonstrated the capabilities of the two-photon module in a variety of different applications. They imaged layers 1–5 in the posterior parietal cortex at more than 16 Hz by stacking the imaged volumes for each beamlet in the axial direction. Furthermore, they imaged four volumes side-by-side in the primary auditory cortex while providing auditory stimuli. Finally, the team used both the two-photon and three-photon modules to image an entire column in the posterior parietal cortex, as well as part of the underlying hippocampus.

While HyMS microscopy enables calcium imaging in large volumes, it is important to keep in mind that imaging at depth requires higher illumination power than imaging in shallow regions and that the multiplexing approach further increases the illumination power. Vaziri and his team image in a power window that has been considered safe, but potential users of this and related approaches should be mindful of brain heating during imaging.

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Research papers
Weisenburger, S. et al. Volumetric Ca^{2+} imaging in the mouse brain using hybrid multiplexed sculpted light microscopy. *Cell* **177**, 1050–1066 (2019).