IMAGING

Volumetric functional imaging two ways

Bessel focus scanning and stereoscopy speed up volumetric brain imaging.

Methods for imaging neurons firing within the living brain have helped researchers begin to understand neuronal networks with high spatiotemporal resolution. However, many of these methods involve two-photon (2P) laser-scanning fluorescence excitation. Although this has advantages for optical sectioning, it is limited in terms of imaging volume for monitoring rapid events in conventional point-scanning modes.

Two papers describe methods for relatively large-volume neuronal imaging at speeds fast enough for activity mapping. Na Ji and colleagues at Janelia Research Campus describe an approach that they developed called Bessel focus scanning. This method uses point-scanning 2P microscopy but bypasses imaging of individual stacks in the axial direction by scanning an axially elongated Bessel beam in 2D. This allows the rapid acquisition of projected views of 3D volumes at video rate.

To set their work apart from related extended-depth-of-field methods, the authors developed an optical setup optimized for *in vivo* brain imaging; their system allows fast volumetric imaging with resolution good enough to image small structures such as dendritic spines and axial boutons. The team demonstrated their approach by carrying out functional imaging in mouse and ferret visual cortexes, the hindbrains of zebrafish larvae, and fruit fly brains in response to visual stimuli. The authors note that despite many advantages, the method works best on sparsely labeled samples, the axial resolution is compromised relative to that achieved with conventional approaches, and signals from different neurons may need to be separated computationally.

David Tank and colleagues at Princeton University describe the development of volumetric 2P imaging of neurons using stereoscopy (vTwINS), another method for fast volumetric functional brain imaging. The method is conceptually similar to Ji's in that it uses an elongated point-spread function (PSF) to gain volume information. In vTwINs, the elongated PSF is split, separated and angled inward to create a "V" shape. When scanned, this PSF yields a 2D projection image that can be used to extract depth information. An advantage of this approach is that it is useful for imaging densely labeled samples. The team demonstrated the power of their approach by carrying out large-scale recording of calcium transients in the visual cortex and hippocampus of awake mice.

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RESEARCH PAPERS

Lu, R. *et al*. Video-rate volumetric functional imaging of the brain at synaptic resolution. *Nat. Neurosci*. http://dx.doi.org/10.1038/nn.4516 (2017).

Song, A. *et al*. Volumetric two-photon imaging of neurons using stereoscopy (vTwINS). *Nat. Methods* **7**, 420–426 (2017).