NEUROSCIENCE

Monitoring 3D neural activity at large scale

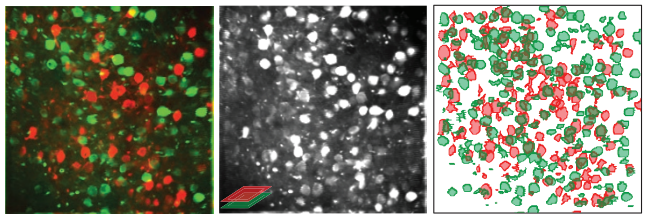
A combination of flexible imaging and computational methods opens new views into the dynamics of activity across large populations of neurons.

One of the goals of the US Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative is to study the dynamics and complexity of neural activity in the brain. This will require the development of methods for imaging many neurons simultaneously and in three dimensions, as well as computational tools that can extract meaningful information from such imaging data sets.

In a collaborative effort, the teams of Liam Paninski and Rafael Yuste at Columbia University addressed this challenge of large-scale activity imaging and analysis. They developed a strategy for simultaneously imaging neural activity in several planes (Yang *et al.*, 2016). The resulting data sets required robust computational algorithms that could disambiguate calcium signals arising from activity in different planes (Pnevmatikakis *et al.*, 2016). Paninski refers to this combination of approaches as computational microscopy. "The algorithms and the imaging are designed together to do something new. This is going to be a big theme over the next several years," Paninski explains.

It is possible to monitor the activity of large populations of cells with a one-photon microscopy approach in transparent brains such as that of the zebrafish. However, imaging in deeper regions of the highly scattering mouse brain requires two-photon illumination and consequently laser-scanning approaches that are inherently slow. "There is no way you can image all the cells with a single laser beam. You have to multiplex the light," says Yuste.

The imaging approach developed by the researchers follows a hybrid strategy in which a spatial light modulator generates multiple laser beams that can scan individual planes and allow simultaneous two-photon calcium imaging in multiple mouse brain layers. Yuste says that they are currently imaging up to seven planes simultaneously. The light emitted from these multiple planes is collected at the same time. "The problem of course is how [to] disambiguate the neurons sitting on top of each other, because the photons are going to be projecting exactly through the same space," Yuste explains.



Sequential and simultaneous imaging followed by demixing of two planes (displayed in green and red, respectively) in the mouse visual cortex. Adapted from Figure 3 in *Neuron*, Vol. **89**, Yang, W. *et al.*, Simultaneous multi-plane imaging of neural circuits, 269–284, copyright (2016), with permission from Elsevier.

The researchers use a three-pronged computational strategy to disambiguate the calcium signals they detect. They simultaneously determine the location of the signals, separate overlapping signals and derive calcium spikes from the data using a non-negative matrix factorization approach. "We can view the whole spatiotemporal activity of the movies that we obtain as a big matrix and then exploit some statistical properties of this matrix … so it can be explained as the product of two much smaller matrices. And this can help us to deduce the noise and extract the information," says Eftychios Pnevmatikakis, now at the Simons Foundation in New York.

This computational approach is generally applicable to calcium imaging data sets obtained with a variety of imaging technologies, which the researchers have demonstrated on data obtained with conventional two-photon microscopes and light-sheet microscopes. But it is particularly beneficial for demixing the signals obtained with the multi-plane approach. Although other methods could potentially disambiguate these data, Yuste says that the non-negative matrix factorization approach was far superior in sorting out which cells the calcium signals originated from.

One prerequisite for data analysis in the context of multi-plane imaging is that there be sufficiently sparse activity and low background. These conditions are met by genetically encoded calcium indicators such as the GCaMP6 family. "With conventional dyes, the background is always bright. When we saw that GCaMP6 started out dark and went bright, we realized that this would give us effective sparsity that we could exploit," says Darcy Peterka, who was then a senior associate in the Yuste group and who led the development of the multiplexing strategy.

With the current two-photon multiplane setup, the researchers can image up to a depth of 600–700 micrometers, according to Weijian Yang. "One way to further improve this is to introduce some adaptive optics," says Yang. "That should dramatically increase the deep tissue imaging." He also thinks that the multi-plane platform could be further expanded via combination with technologies that increase the field of view or imaging speed. "There are various ways of improving things, both on the imaging side and also on the computational side," agrees Paninski. For example, it will be useful to include two-color imaging capabilities and adding options for stimulating neurons using optogenetics.

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RESEARCH PAPERS

Yang, W. *et al.* Simultaneous multi-plane imaging of neural circuits. *Neuron* **89**, 269–284 (2016).

Pnevmatikakis, E.A. *et al.* Simultaneous denoising, deconvolution, and demixing of calcium imaging data. *Neuron* **89**, 285–299 (2016).