NEUROSCIENCE

Automating brain mapping

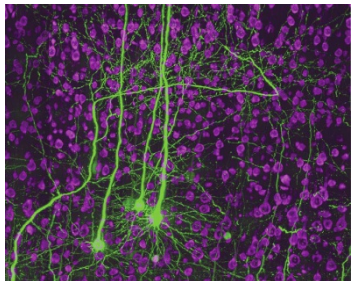
Two new platforms allow single neurons to be imaged throughout the mouse brain.

Large-scale neuroscience projects such as the BRAIN Initiative seek, among other goals, to map the projections of individual neurons throughout the whole brain. Current techniques for this rely on either bulk labeling of bundles of axons or sparse labeling in combination with serial tissue sectioning and manual tracing of axons between sections. The former approach is relatively easy to carry out but lacks sufficient resolution to identify the projections of individual neurons, whereas the latter approach allows for single-cell resolution but is exceedingly time-consuming. Two new studies describe methods for automated whole-brain single-cell tracing that overcome many of these limitations.

In one paper, Qingming Luo of Huazhong University of Science and Technology and colleagues describe a high-throughput method for whole-brain fluorescent imaging (Gong *et al.*, 2016). The method builds upon previously developed platforms in which a resin-embedded brain goes through automated cycles of imaging with a microscope and sectioning with a vibratome to expose a new, un-imaged tissue surface. The authors improved upon previous iterations of the method by using structured illumination microscopy (SIM), which allows for much faster data acquisition than other types of microscopy. In the past, the trade-off for this speed has been lower resolution, but simple adjustments made by the researchers allowed them to increase the resolution to around that of confocal microscopy.

The new method also implements real-time staining of cell nuclei just prior to each imaging cycle. The nuclear staining reveals known cytoarchitectonic landmarks in the brain, such that fluorescently labeled neurons can be localized to particular regions, making the process of axon tracing much easier and more accurate. The authors demonstrated the utility of their technique in tracing individual neurons in multiple regions of the mouse brain.

The work of labeling neurons and of sectioning and imaging tissue is only one barrier to mapping axon projections. Manually tracking a labeled axon between serial sections can be even more difficult and time-consuming. Jayaram Chandrashekar and Eugene Myers of the Janelia Research Campus, along with their colleagues, recently described a platform for automated imaging and reconstruction of individual neurons throughout the brain (Economo *et al.*, 2016).



Fluorescently labeled neurons and their projections (green) and surrounding nuclei (purple) in the mouse cortex. Adapted with permission from Gong *et al.* (2016).

Similar to the work of Luo and colleagues, this automated platform alternately images and sections tissue; however, the system uses two-photon laser scanning microscopy rather than SIM. In addition, the researchers incorporated a step in which the whole brain is optically cleared, facilitating tracing of fluorescently labeled axons.

The authors also developed a computational platform for automatic image registration and axon tracing, which should save researchers a lot of time and tedium. The authors provide extensive validation of this automated process, showing that it is able to follow individual axons far from their neuronal cell bodies, with low error rates even when labeled axons cross over each other.

These new methods promise to facilitate the mapping of neuronal projections throughout the mouse brain, and the improvements in speed move researchers closer to being able to map projections in the brains of larger animals. Maps generated using these techniques will undoubtedly be a valuable resource for the neuroscience community as it seeks to better understand the way the brain is wired, and how this wiring leads to function.

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RESEARCH PAPERS

Gong, H. *et al.* High-throughput dual-color precision imaging for brain-wide connectome with cytoarchitectonic landmarks at the cellular level. *Nat. Commun.* **7**, 12142 (2016).

Economo, M.E. *et al.* A platform for brain-wide imaging and reconstruction of individual neurons. *Elife* **5**, e10566 (2016).