NEUROSCIENCE

# Coloring electron microscopy connectomes

FluoEM aligns axon reconstructions from fluorescence and 3D electron microscopy on the same tissue to allow multicolor labeling of distant inputs.

Brain-connectivity mapping is a major goal in neuroscience. Large-scale 3D electron microscopy (EM) allows high-resolution reconstructions of limited tissue volumes that can contain axons from diverse and distant origins. Electron contrast-based labeling of different axons, however, can disrupt tissue and is challenging. Newer methods permit multicolor labeling by correlating fluorescent microscopy (FM) and EM data with precision adequate for neuronal bodies, but not thinner axons.

Moritz Helmstaedter and colleagues from the Max Planck Institute for Brain Research in Germany have developed FluoEM, a method for registering FM and EM axon reconstructions from the very same tissue. The key, they found, was realizing that most axons follow a unique trajectory, even within distances found in typical EM volumes. "This turns coregistration into a problem of finding the axon that best explains what we see in light—a much better constrained problem," says Helmstaedter.

With FluoEM, the researchers first align EM and FM datasets using blood vessels in the tissue volume. This has an error of about 5 µm—wider than an axon. They select a box with dimensions of this error, through which passes an FM-reconstructed axon. They then trace all EM-reconstructed axons that traverse the box, until they find a match that always stays within an error's radius of the FM-reconstructed axon. This process allows them to refine the registration and assign additional axons.

FluoEM on one-tenth of a 1-mm$^3$ sample would require an estimated 5,300 hours, which is reasonably fast. More important, FluoEM opens up a potentially unlimited fluorescent color space for use with EM. "In large animals, the hope to get entire brains is far on the horizon," says Helmstaedter. "Until then, we now have a way to label all the many interesting input sources for a given set of circuits. FluoEM is primarily an extension of connectomics to the longest scale, without having to image entire brains." ❐

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Research papers
Drawitsch, F. et al. FluoEM, virtual labeling of axons in 3-dimensional electron microscopy data for long-range connectomics. *eLife* **7**, e38976 (2018).