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Light-Microscopy-Based Sparse Neural Circuit Reconstruction: Array Tomography and Other Methods

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Efficient neural circuit reconstruction requires sufficient lateral and axial resolution to resolve individual synapses and map a large enough volume of brain tissue to reveal the molecular identity and origin of these synapses. Sparse circuit reconstruction using array tomography meets many of these requirements but also has some limitations. In this minireview, the advantages and disadvantages of applicable imaging techniques will be discussed.

Key Words: Neural circuit reconstruction, Array tomography, Light microscopy

Mapping the neural circuits of the brain is a challenging task. Neurons communicate with each other through specialized structures called synapses, significant fraction of which are close to or smaller than the light diffraction limit. The physiological properties of synapses can vary depending on many factors, such as their size and molecular composition. To make the problem even more difficult, synapses are packed in an extremely high density (approximately 1 per m^3) in most of brain regions (DeFelipe et al., 1999). To understand how circuits are wired, identifying the origins of the synapses, which can be over a millimeter away, is also necessary. Therefore, an ideal technique for efficient circuit reconstruction requires (1) sufficient lateral and axial resolution to resolve individual synapses, (2) the ability to reveal the molecular identity and source of these synapses, and (3) coverage of a large enough volume of brain tissue to map an entire unit of the neural circuit of interest (e.g., entire cortical layers). In addition, the readiness of image segmentation technique is also critical for the acquisition of statistical information. In this minireview, I discuss new toolsets for neural circuit mapping.

For many years, transmission electron microscopy (TEM) has been the gold standard for circuit reconstruction because of its resolution although it lacks throughput capacity. Recently developed automated three-dimensional electron

microscopy techniques such as serial block-face scanning microscopy (Briggman et al., 2011) and TEM camera array (Bock et al., 2011) as well as multi-beam scanning electron microscopy combined with the development of large volume tissue processing (Mikula et al., 2012) and automated section collection (Hayworth et al., 2006), have enabled the acquisition of large volumes (Lee et al., 2016; Morgan et al., 2016). The impact of these efforts is tremendous and reveals more information about neural networks than was previously possible. However, the limited compatibility of these techniques with labeling for molecular identity or input sources, as well as experimental cost leaves room for improvement.

On the other hand, most conventional microscopy techniques, such as confocal microscopy, does not provide sufficient resolving power for reliable synapse detection (Mishchenko, 2010). Per theoretical predictions, much of this unreliability is due to limited axial resolution. Improved axial resolution dramatically improves synapse detection accuracy (Rah, 2013).

Array tomography (AT) is a high-resolution imaging technique based on wide-field fluorescent imaging of arrays of ultrathin serial sections followed by computational reconstruction (Micheva & Smith, 2007; Fig. 1). Because the imaging is performed on ultrathin sections, the Z-axis

resolution of this technique is determined by the thickness of the section instead of by Abbe's rule. Synapses can be resolved with approximately 80% accuracy by AT compared to TEM (Rah, 2013). Multiple axonal origins can be distinctively labeled using different fluorescent proteins, which articulate the origins of the synapses. Since every voxel of the tissue has the same chance of antibody labeling, the molecular identity of the synapses can be examined in a quantitative manner. Moreover, antibody staining on thin sections can be easily removed and the sections can then be restained. Repeated cycles of antibody staining and stripping allows for detailed investigation of the proteomic diversity of synapses of interest (Micheva et al., 2010). Gathering the necessary information about a neural circuit from AT images is relatively simple because of high contrast and isotropic resolution. In terms of imaging area, AT is applicable to a sufficiently large volume of brain tissue to cover entire cortical layers (Rah, 2013) or hippocampal CA1 pyramidal neurons (Bloss et al., 2016).

However, circuit reconstruction with AT is a rather fragile and laborious process that involves handling 100-nm thick serial sections. On top of that, a synapse detection accuracy of 80% may be enough for some biological questions, but certainly not for all. In the following sections, I review the pros and cons of other imaging techniques for studying neural circuits. Selective-plane illumination microscopy (SPIM) is now widely used for efficient optical sectioning because of its high-contrast with reduced photo damage. The volume of reconstruction can be limited by the working distance of the lens. For that reason, conventional SPIM is more commonly used with brain clearing methods for area-to-area connectivity reconstruction where the observation of cell bodies and bundles is sufficient (Tomer et al., 2015, but see Cella Zanacchi et al., 2011).

Many super-resolution microscopy techniques now also provide improved axial resolution. For instance, with interferometric photoactivated localization microscopy (iPALM), three-dimensional stochastic optical reconstruction microscopy (3D-STORM), one can acquire better axial and lateral resolution than one can possibly slice without the hassle of laborious serial sectioning (Huang et al., 2008; Shtengel et al., 2009). A potential drawback of these techniques is the severe limitations in depth and field-of-view. The use of selective fluorophores and slow imaging speeds also require improvement before these techniques can be used for large-volume circuit reconstruction. It is important to remember that imaging thick specimens with high axial resolution is accompanied by antibody penetration issues. Clever sample preparation followed by conventional microscopy provides a good alternative for this issue. GRASP or green fluorescent protein (GFP) Reconstitution Across Synaptic Partners utilizes overexpressed GFP-fusions of interacting presynapse- and postsynapse-specific proteins (Feinberg et al., 2008; Kim et al., 2011). Although there are concerns about the synaptogenic feature of overexpressed synaptic proteins (Graf et al., 2004; Scheiffele et al., 2000), the number of synapses as well as the subcellular localization of synapses were in agreement with previous studies at least in gross level (Kim et al., 2011). Expansion microscopy uses the physical expansion of a polymer network that is covalently anchored to a specific location within the specimen (Chen et al., 2015, 2016). Considering the expansion factor of the specimen, an effective resolution of approximately 70 nm lateral and 200 nm axial could be achieved even with conventional confocal microscopy. There is no obvious evidence that this technique affects the overall structure of neurons, which would suggest uneven expansion or distortion of the neuronal structure.

The methods described above and other techniques may be perfectly adequate for testing some hypotheses but none of them is a magic bullet. The combination of these technologies would lead to a powerful tool for large-volume neural circuit
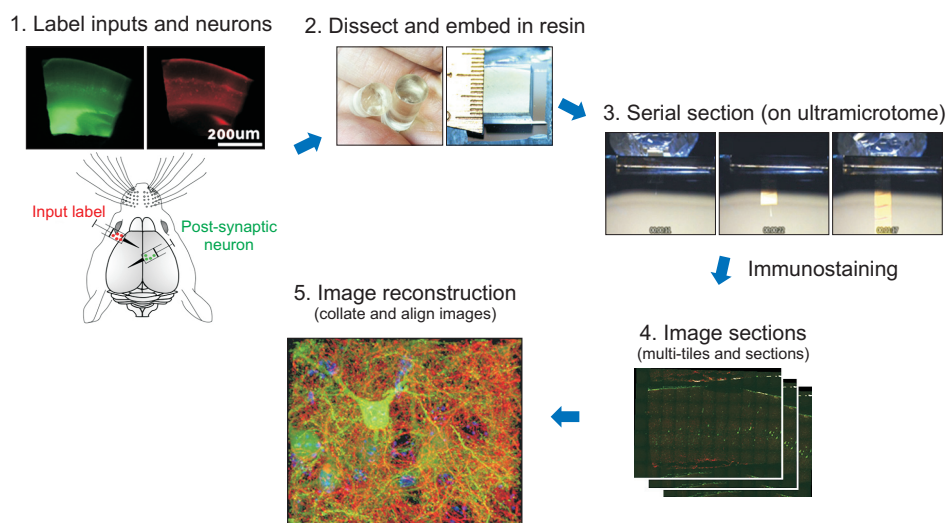

Fig. 1. Schematic diagram of the array tomography.

reconstruction (Nanguneri et al., 2012; Punge et al., 2008; Sigal et al., 2015).

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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