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COMMENTARY

Towards Semi-Automatic Reconstruction of Neural Circuits

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The spatial layout of neuronal arbors and their synapses in the brain, also known as the wiring diagram or the connectome,[1] may provide the basis for formulating experimentally testable hypotheses of neural circuit function. In the brain, the small dimensions of terminal dendrites and synapses, and the anatomical flexibility of neuronal arbors implementing the same function in the brains of two different individuals, conspire against the standard scientific approach of examining a small piece of tissue and generalizing to the rest. Furthermore, the identification of the synaptic contacts between neurons is not sufficient to capture important aspects of circuitry function such as neuromodulation,[2] which operates via volume release and difusion through the extracellular space. To obtain a connectome complete with spatial information, scientists must image the brain at nanometer resolution and reconstruct neuronal arbors, synapses, and glial cells. So far, only electron microscopy (EM) has proven effective,[3] if limited to relatively small volumes of brains[4,5,6] or to complete nervous systems of small organisms such as *C. elegans*.[7,8]

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Imaging a complete brain at nanometer resolution has proven troublesome. FIBSEM[9] has so far demonstrated that excellent, continuous and isotropic volumes of small brain volumes can be obtained if the

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sample can be sufficiently stained prior to imaging. For large volumes, all suitable EM-based approaches require physically sectioning the tissue,[6,10,11] and deliver anisotropic image volumes not free of artifacts. Loss of tissue may occur at multiple stages, including sectioning, counterstaining for contrast enhancement, or in handling events. But if preserving the integrity of serial sections is hard, reconstructing the neurons contained in said sections and identifying all synaptic contacts correctly remains an open and very hard problem.

The first notable EM-based reconstruction of a nervous system was that of *C. elegans*[7] from serial section transmission EM (ssTEM). The reconstruction was performed by tracking the location of sectioned neuronal processes and synapses on transparencies overlaid on printed photographs, and recording the adjacency matrix in a spread sheet. Since then, monumental increases in computerized data storage and processing capacity have enabled automatic image acquisition (e.g. Leginon[12]) and automatic image volume composition from serial sections robust to non-linear deformations, missing sections and noise.[13,14] The problem of algorithmically reconstructing neurons and synapses from anisotropic volumes has attracted considerable attention from the computer vision community, and significant progress has been made in developing algorithms,[15,16] particularly for recognizing cytoplasmic membranes.[17,18]

The state of the art methods for reconstructing neuronal circuits include computer-assisted image browsing and bookkeeping, with humans annotating by hand every section of a neuron and every synapse.[4,5,6] Despite the task being tedious and error-prone, humans currently outperform all automatic methods in correctness, and also in speed when reconstructing arbors with skeletons rather than volumes.

To automate the reconstruction of arbors, a researcher starts by labeling all cytoplasmic membranes in a volume; this is the gold standard–the best human effort. With these labels, a classifier (e.g. a neural network, a random forest) is trained to recognize membranes by observing many instances of the area or volume around pixels or voxels containing membrane. Upon applying the trained classifier to the original image data, the gold standard labels serve as ground truth for the classifier output, providing the means to quantify its accuracy. The trained classifiers can only, in general, be as good as the human annotator. But if the gold standard contains a small number of errors, the classifier may have learned to ignore them given the much larger amount of correct labels.[18] Measuring the discrepancy between human annotations and the result of the trained classifier requires great care. In anisotropic EM volumes some membranes are ambiguous, and prior knowledge is required. Such information can be obtained from the sample prior to EM imaging by labeling and imaging with light microscopy multiple neurons with multiple colors with the brainbow genetic construct,[19] which would serve as ground truth for the resolvable parts of the arbor of

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such neurons. In practice this approach has yet to succeed, presumably because of sample degradation prior to fixation for EM. In sufficiently stereotyped nervous systems such as *C. elegans* or *Drosophila* larva, statistical information about neuronal arbor configuration can be gathered from systematic imaging of individual neurons in numerous individuals. Ambiguity can also be resolved by considering multiple interpretations simultaneously and resolving for the entire serial section set.[16]

All existing automated methods for reconstructing circuits from anisotropic EM volumes perform reasonably well with perfect data, and so far no approaches have been published capable of handling the messy reality of serial section EM. While all in the neural circuit reconstruction field await eagerly the development of FIBSEM technology for large-scale imaging with its promise of artifact-free isotropic EM volumes, we must handle imperfect anisotropic EM image volumes today and for the foreseeable future.

In this issue, Jurrus et al. present their approach to neuronal arbor reconstruction from EM volumes. Their work walks the extra mile and not only demonstrates the applicability of their approach for two widely different EM volumes, one from ssTEM of *C. elegans* and another from SBFSEM[10] of mouse brain, but also deliver their novel algorithms in an end-user software application, NeRV. Their approach is based on three key steps. First, an iterative membrane recognition algorithm based on artificial neural networks operating directly on Gaussian-processed and CLAHE-enhanced[20] images that is capable of utilizing information from adjacent sections, cleverly bypassing the anisotropic cliff. The performance of the approach is impressive and, remarkably, avoids altogether the expensive computation of the filter banks traditionally used to extract pixel-wise features that are then fed to machine learning classifiers. Second, their algorithm for linking 2d profiles into a 3d neuronal process includes information from more than the adjacent sections, which is key for handling noise on the tissue sections that may occlude some profiles. Third, they have built in NeRV the tools necessary for manually correcting the output of the automated approaches, rerunning these as desired, joining neuronal processes into neuronal arbors, and then visualizing and correcting the results. This comprehensive approach is modular and open to further improvements by replacing specific components.

The approach by Jurrus et al. could be positively described as semi-automatic, implying that human intervention will be necessary and that it is then best to facilitate it. Magnificient software applications like ilastik[21] provide means to interactively and iteratively train a machine learning classifier with examples, and delivers its best results in isotropic FIBSEM volumes. With their approach, Jurrus et al. provide a semi-automated approach for neural arbor reconstruction on anisotropic volumes, ready for use today. This novel approach, well grounded in the realities of EM-based reconstructions, is a step forward from computer-assisted manual neuronal arbor reconstruction methods such as provided by KNOSSOS[5] or TrakEM2,[22] and pushes the field towards the mid-term goal of conceiving a computerized automatic reconstruction approach that requires human interaction only for bits too tough for algorithms to chew.

But much remains to be done. A noise-aware version of the algorithm by Funke et al.,[16] capable of handling branching and termination events when reconstructing neuronal arbors, would reduce the need for manually editing all branch points in NeRV–an operation that in NeRV, being a desktop application, can only be performed by one human at a time. A web-based large image volume visualization system such as CATMAID,[23] enhanced for neural circuit reconstructions and powered by the algorithms of Jurrus et al. and others, could allow hundreds of humans to cooperatively and semi-automatically reconstruct neural circuits. And a whole new class of algorithms which are but just barely starting to appear,[24] aiming at analyzing reconstructed neuronal arbors and indicating potential points of error, would finally make fast reconstructions also accurate.

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