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Editorial: Electron-Microscopy-Based Tools for Imaging Cellular Circuits and Organisms

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Editorial on the Research Topic

Electron-Microscopy-Based Tools for Imaging Cellular Circuits and Organisms

Electron microscopy (EM)-based reconstruction of neuronal circuits from serial ultrathin sections was introduced more than three decades ago (White and Keller, 1987). Initially, all the steps were conducted manually, including cutting serial ultrathin sections using the ultramicrotome, image capturing with transmission electron microscope (TEM), and reconstruction using cardboard pieces of selected profiles of neural structures to provide the impression of depth. In the 1990s, computer software assisted reconstruction methods to make it more efficient were introduced (Harris et al., 1992; White et al., 1994). This reconstruction analysis software was used in a limited number of laboratories where good skills for obtaining serial ultrathin sections had been established and, thus, significant and valuable results were obtained. In general, however, this reconstruction technology was not popular because of a high demand on skills to obtain high quality serial ultrathin sections. In the early 2000s, a number of groups, with many represented in this special issue, started to conduct neural network analyses with reconstruction of serial sections by adapting new EM technologies such as focused ion beam-scanning electron microscopy (FIB-SEM; Knott et al., 2008), serial block-face electron microscopy (SBEM; Denk and Horstmann, 2004; Ohno et al., 2014), automated tape-collecting ultramicrotomy (ATUM) with SEM (Terasaki et al., 2013), transmission electron microscope camera array (TEMCA; Bock et al., 2011), and transmission-mode SEM (Kuwajima et al., 2013). These approaches have been modified and improved vigorously (Kubota et al., 2018a,b), and a large amount of noteworthy results were published in the last decade (Tomassy et al., 2014; Kasthuri et al., 2015; Lee et al., 2016; Villa et al., 2016; Schmidt et al., 2017; Takemura et al., 2017; Bae et al., 2018). The size of EM volume data sets has grown year by year, and it could be huge especially when data are obtained with high-throughput EM systems of either TEMCA (Bock et al., 2011; Lee et al., 2016), multi-beam SEM (Eberle and Zeidler; Shibata et al.) or parallel processing with multiple single beam SEM systems (Plaza and Funke; Scheffer.) For instance, a 100 cubic μm EM data set with 5 nm/pixel and 30 nm z-step of mouse cortex block, which amounts to 1.3 TB, was obtained that provides a sufficient resolution to detect synaptic contacts. This data set should contain about 1,000,000 synapses (Merchan-Perez et al., 2009) and be sufficient in volume to include many different kinds of connections among a wide variety of cortical neuron subtypes and afferent axonal fibers from other brain regions. Such large volume EM data sets could not be acquired with the conventional manually operated EM using the ultramicrotomes and TEM (White and Keller, 1987; Kubota and Kawaguchi, 2000; Kubota et al., 2015; Marc et al.).

The success of large volume EM acquisition using these new EM systems has created an issue, i.e., how to process large image data sets thus obtained. Soon it became obvious that it was difficult to handle large EM volume data sets using conventional 3D reconstruction image processing computer applications that had been developed for the conventional EM data sets obtained with the ultramicrotome and TEM. Therefore, new image processing tools that can handle large volume data sets have been developed. For example, NIH imageJ plugins provide useful tools to stitch tiles for montage and to align serial section images (Cardona et al., 2012). The current bottle neck is the segmentation process. Currently, the majority of researchers working on EM volume data pursue segmentation of their data obtained from brains of a wide variety of animal species: *Caenorhabditis elegans* (Mulcahy et al.), leech (Pipkin et al.), Drosophila (Takemura et al., 2017), Zebrafish (Wanner and Vishwanathan), mouse (Maclachlan et al.), rat, marmoset, and human, and use manual image processing applications including: VAST, Reconstruct, Knossos, and others (Fiala, 2005; Dorkenwald et al., 2017; Berger et al.). To achieve segmentation easily and efficiently, automated segmentation computer applications have been developed (Januszewski et al., 2018; Lee et al., 2019) and used for many EM volume data sets. Segmentation performance has increasingly been improved and achieved coverage of ~90% of the volume, but it is not yet perfect (Plaza and Funke). Annotators are used to fix segmentation errors to create correct wiring of brain networks. This can be done manually using image software with a proof-reading function (Zhao et al.; Katz et al.). Hopefully, segmentation performance will improve further in the near future to reach an almost 100% success rate while reducing the time required for the proof-reading process. Toward this goal, the histological process should be improved (Hua et al., 2015; Mikula and Denk, 2015; Mikula, 2016; Genoud et al.; Maclachlan et al.; Nguyen et al.) and image processing tools with better performance (Berger et al.; Jorstad et al.; Titze et al.) should be developed. Finally, despite these technological advances, analyzing fully segmented EM volume data sets can be done only manually by researchers so far, who must have a good knowledge and understanding of brain networks. In addition, automated cell type identification tool (Schubert et al., 2019), automated synapse detection tool (Staffler et al., 2017), and correlated light and electron microscopy methods (Kubota et al., 2015; Wanner and Vishwanathan) are useful for neural network analyses. This special issue covers most of the cutting-edge 3D-EM methods currently available.

On September 8th, 2017, I contacted Shawn Mikula at the Max Planck Institute in Martinsried, to ask him if he would be willing to work as co-editor with me on a special issue of Frontiers in Neural Circuits Research Topic "Volume electron microscopy for neuroscience." I wanted Shawn to be my partner to edit the special issue because I knew that he had a deep knowledge not only for the EM volume data set analysis but also histology, chemistry, mathematics and other areas. He immediately accepted the invitation and we chose the title of the Research Topic "Electron-Microscopy-Based Tools for Imaging Cellular Circuits and Organisms." We started to invite contributors to the special topic issue at the end of October, 2017. Shawn invited many excellent researchers who have been developing image analysis applications or systems and/or working on large volume EM data sets. His selections indeed led to the success of this special topic issue. Subsequently, Shawn joined my laboratory at the National Institute for Physiological Sciences in Okazaki, Japan, briefly from January 2nd to March 30th, 2018. After visiting his family in the USA in April and May of 2018, he moved to the Keio University School of Medicine in Tokyo. With great sadness and most unfortunately, on July 8th, 2018 we lost Shawn tragically, when we just started reviewing a few manuscripts submitted.

This special issue reflects Shawn Mikula's great interest in the brain network architecture and his commitment to introduce the best technology available to all researchers conducting neuroscience research with EM volume data set analyses. I took over all the editorial work after Shawn was lost and always kept these convictions with me during the editorial work. I hope he would appreciate the results. Finally, I express my sincerest condolences and special thanks to Shawn Mikula's family.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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