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doi:10.1017/S1431927621010552
Microsc. Microanal. 27 (Suppl 1), 2021
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Tools and Approaches for Assembly, Review, and Analysis of Large-Scale Electron Microscopy

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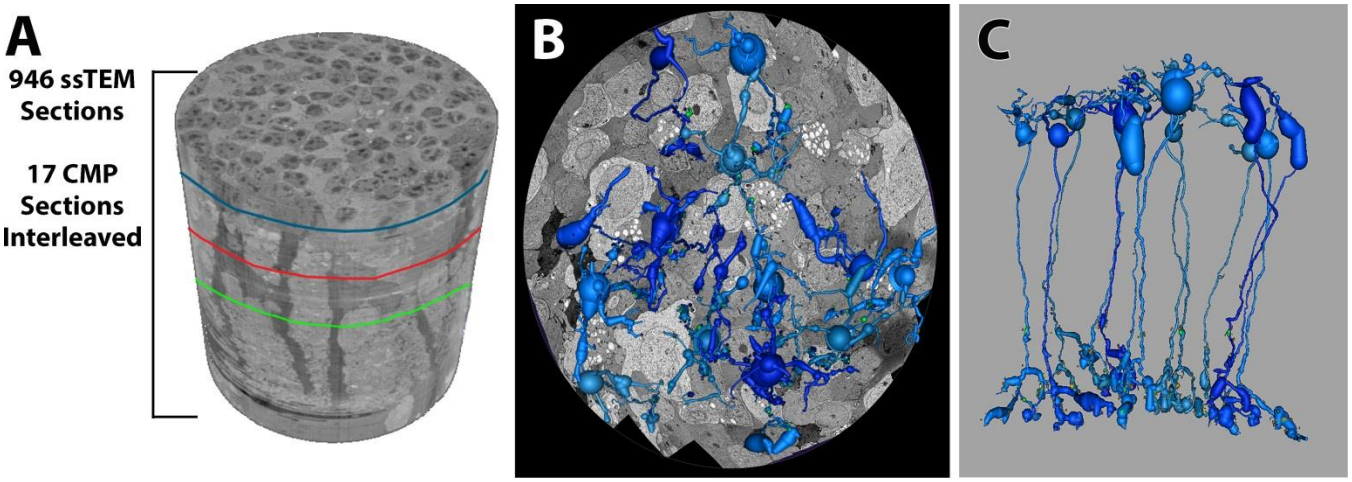
Even though electron microscopy has been around as a technology since the late 1930s and early 1940s, the approaches for dealing with data coming out of electron microscopes have not substantially changed for decades. For a long time, advances in the field were limited to higher power instruments and newer, or different modalities, but the amount of data that was coming off these instruments had not changed significantly until recently.

New approaches to electron microscopy that leverage hybridization of light and ultrastructural imaging, new CryoEM approaches, large-scale initiatives in connectomics, and 3-dimensional reconstruction of both biological and materials samples have driven the expansion of data intensive ultrastructural microscopy. These efforts in combination with a dramatic drop in storage costs beginning about a decade ago, and automation capabilities in the instruments themselves that lead to extended periods of unsupervised operation have enabled tremendous increases in the amount of data that laboratories have been able to generate. However, the ability to generate large amounts of data creates new problems. Now labs have to store the data, structure the data, view and browse the data, and then annotate and analyze the data to properly appreciate the value of our new found abilities to generate large amounts of ultrastructural data.

Because of the complexity and cost of these tasks, most of this truly large-scale work is being accomplished by large microscopy groups at research institutions with large endowments, and these approaches are not typically available to the small lab, or the traditional core facility.

This talk will address all of these issues through the "lens" of our decade long efforts in retinal connectomics initiatives from a single laboratory, but the principles are generally applicable to other areas outside of neuroscience, including the larger fields of bioscience and materials science. Additionally, we'll discuss the importance of large-scale ultrastructural data, along with operational advantages to core facilities of introducing tools for large-scale ultrastructural data capture, and include a discussion of software tools to automate image capture, assembly, visualization, and annotation.



Retinal Pathoconnectome 1 (RPC1) (A) Description of the RPC1 volume. (B) Overlay of a top down view of the 3D renderings of all confirmed 16 rod bipolar cells (RodBCs) and cone-contacting rod bipolar cells (XRodBCs) in the RPC1 volume on a representative TEM section from RPC1. (C) Vertical view of the 3D reconstruction of RodBC and XRodBCs in RPC1.

https://doi.org/10.1017/S1431927621010552 Published online by Cambridge University Press

Figure 1. Retinal Pathoconnectome 1 (RPC1) (A) Description of the RPC1 volume. (B) Overlay of a top down view of the 3D renderings of all confirmed 16 rod bipolar cells (RodBCs) and cone-contacting rod bipolar cells (XRodBCs) in the RPC1 volume on a representative TEM section from RPC1. (C) Vertical view of the 3D reconstruction of RodBC and XRodBCs in RPC1.

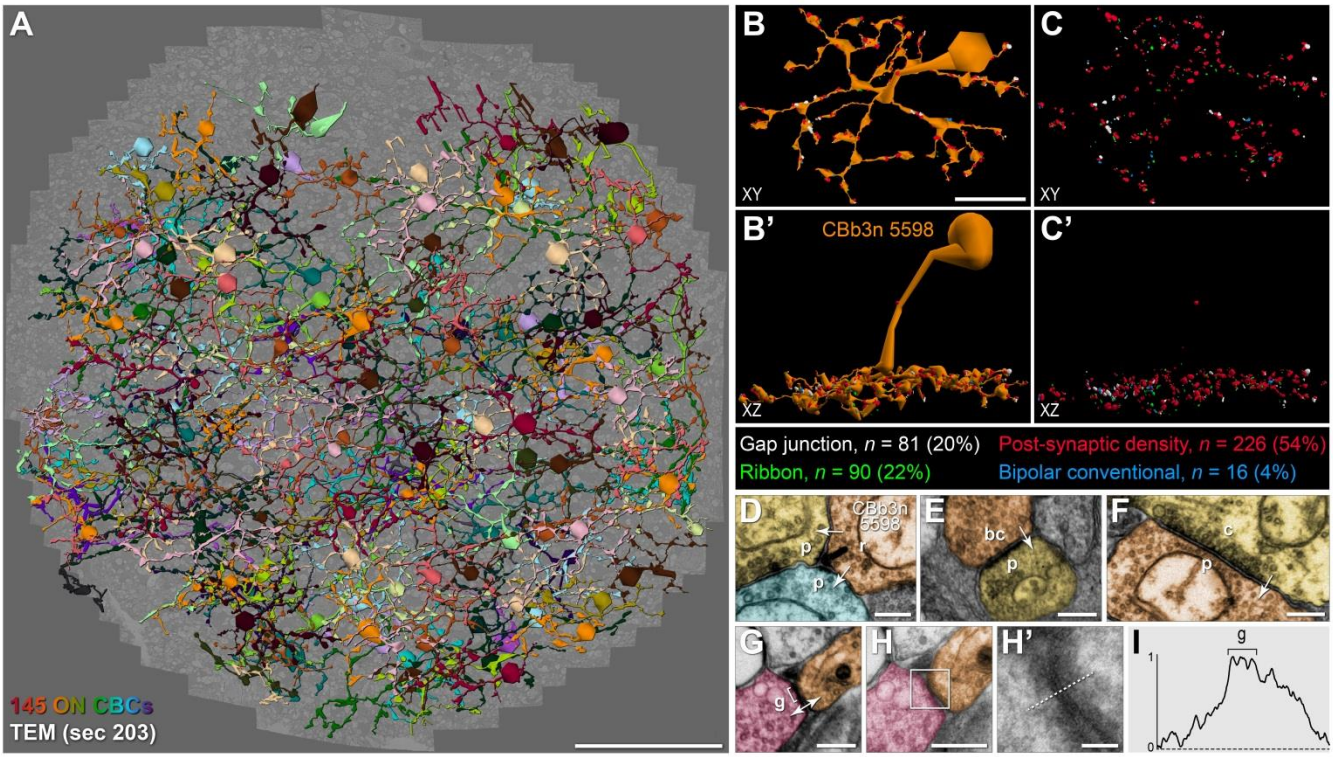


Figure 2. Ultrastructural reconstruction of ON CBCs and their synaptology. RC1 is a 0.25 mm diameter volume of a light-adapted 13-month old female Dutch Belted rabbit retina, built by automated transmission electron microscopy and computational assembly at 2.18 nm/px resolution. ON CBCs and their coupling partners were annotated using the Viking Viewer for Connectomics and their connectivity explored with 3D rendering and network graph visualization leveraging a spatial database and established computational geometry analysis methods. (A) 3D computer reconstruction of the 145 ON CBCs contained in RC1 superimposed on a TEM section from RC1. (B,C) 3D rendering of CBb3n 5598 and its 413 identified synapses. (D-G) Representative examples of synaptic contacts as viewed in the Viking Viewer (native 2.18 nm resolution). (H-J) Gap junctions were validated by 0.27 nm resolution recapture with goniometric tilt as necessary. (I) Normalized plot of the image density profile taken along dotted line in H'. Scale bars: (A) 50 µm; (B) 10 µm; (D-H) 250 nm; (H') 50 nm. Abbreviations: bc, bipolar conventional pre-synapse; c, conventional pre-synapse; g, gap junction; p, post-synaptic density; r, ribbon.

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https://doi.org/10.1017/S1431927621010552 Published online by Cambridge University Press

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https://doi.org/10.1017/S1431927621010552 Published online by Cambridge University Press