6) Browse, identify and track neurons, processes and synapses

This ultrastructural mapping framework removes four major barriers to large scale ssTEM reconstruction: mosaicking, registration, viewing, and annotation. While mathematically robust tools have long existed for analyst-guided non-linear mosaicking and registration and many solid efforts have been made to provide small-volume tools, the scale of ssTEM canonical volume reconstruction precludes a user-guided software solution, instead demanding computational automation. The ability and availability of our software tools, ir-fft / ir-refine-translate / ir-refine-grid to automatically mosaic individual tiles and ir-stos-brute / ir-stos-grid to automatically register mosaics means that we have enabled any laboratory to build high-performance ssTEM volumes. The Viking viewer enables resolution independent image browsing, identification and tracking of complex data in large terabyte scale connectome volumes. Since scanned film imagery can be readily managed, we have also enabled volume construction and exploration of many extremely high quality ssTEM datasets produced over the past three decades.

For complete identification of neural connectome volumes, computational molecular phenotyping is fundamental. Without these methods of cell identification, ssTEM volumes have limited value as post-hoc determination of neuronal connectivity multiplies the difficulties of determining network properties and parameters making determinations statistical rather than absolute.

Viking is a web based multi-user collaborative volume viewer written in C#. Viking functions as the interface to the terabyte scale volume. Volume images/transforms and annotations are exposed by an HTTP web server and web service respectively. Viking adds each slice to slice image transform together to create a transformation for each section into the volume. Sections are warped to volume space as they are displayed using XNA. The user can navigate to sections within the volume, zoom in or out in a resolution independent fashion and create/browse/modify metadata associated with structures, locations and features. Annotations are stored on a remote Microsoft SQL server and exposed via a Windows Communication Foundation based web service. The flexible schema allows the tracking of any biological structures users choose to define. This scalable approach permits massively parallel viewing and annotations of datasets. (Viking! © James Anderson).

Gallery:

Screen capture of ON cone bipolar cell axon tracking in Viking. These profiles are approximately 400-500 nm across, at the practical edge of reconstruction limits in light microscopy applications.

A rendering of three All amacrine cells (All), a rod bipolar cell (Rod BC) and OFF (OFF cBC) and ON (ON cBC) cone bipolar cells. Synapses are visualized with small red and blue spheres, gap junctions are visualized with small yellow spheres demonstrating laminations and connectivities of the All amacrine cell circuit.

This image is captured four slices into the volume of a bipolar cell, moving from the inside of a bipolar cell to the inside of a GABA+ amacrine cell. The synaptic ribbon has been imaged enface.

Nanoribbons from a cone bipolar cell axon onto two amacrine cells, one with a feedback synapse. Nanoribbons are common structures in retina that are below the limits of optical resolution (~100-200 nm) and are also undefined in the literature. This is important as they break commonly accepted lamination rules in retinal circuitry. These rules hold that ON axons do not make outputs into the OFF layer.