Ultrastructural Mapping of Neural Circuitry, A Computational Framework

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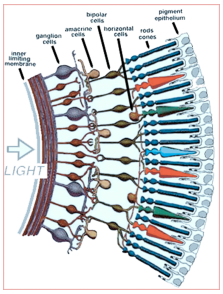
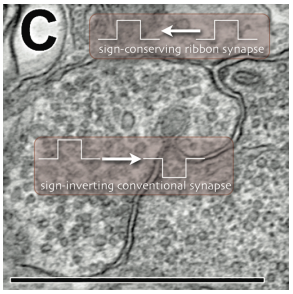
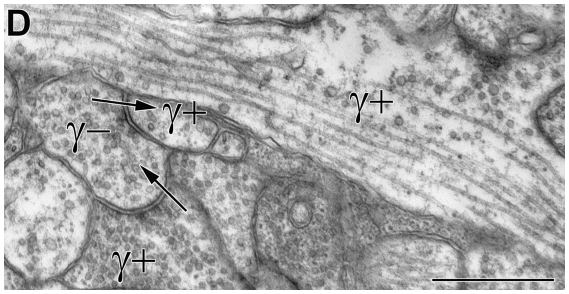
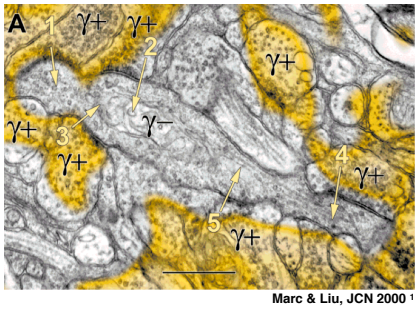
Abstract: Complete mapping of neuronal networks, the creation of connectomes, requires data acquisition at resolutions sufficient to identify synaptic connectivity, robust neuronal classification and a minimum sample size inclusive of all participating neurons in a network. Transmission Electron Microscopy (TEM) remains the optimal tool for connectome mapping, however, the process of reconstructing large, serial section TEM (ssTEM) image volumes is rendered difficult by the need to precisely mosaic distorted image tiles and subsequently register distorted mosaics. Moreover, most molecular neuronal class markers used for class segmentation are poorly compatible with optimal TEM imaging.

We present a complete framework for neuronal reconstruction at ultrastructural resolution allowing the elucidation of complete neuronal circuits. This workflow combines TEM-compliant small molecule profiling with automated image tile mosaicking, automated slice-to-slice image registration and terabyte-scale image browsing for volume annotation. Networks that previously would require decades of assembly can now be imaged, registered and annotated in months. Our framework enables large-scale connectivity analyses of both new and legacy data.









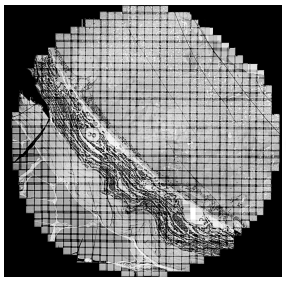
Marc & Liu, JCN 2000 [1]

The mammalian retina is a complex tissue comprised of over 70 types of cells representing complex network and spatial topologies. Retinal signal processing is executed by groups of sign conserving and sign-inverting synapses[1]. However, ultrastructural/molecular mapping demonstrates more micronetwork topologies than are currently used by any model. More problematic is that the most common topologies include concatenated sign-inverting chains of connectivities that are used by no models and are invisible to any methodology other than ultrastructural analysis. Our goal is the first complete connectome: a complete TEM reconstruction of the mammalian retina with all connectivities documented, providing for the first time, the ultimate ground truth for retinal circuitry.

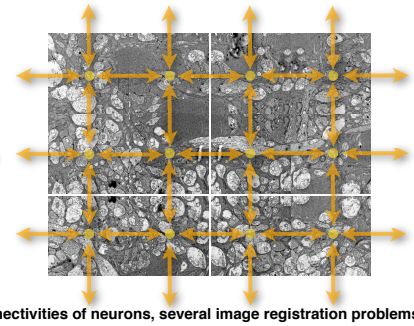
Approach:

1) Calculate the required volume to represent a canonical volume that includes at least 3 copies of the rarest cell types expected.
2) Capture the data at high speed through mosaicked image acquisition of TEM (Serial-EM on JEOL JEM-1400 with Gatan 16k camera).
3) Image layout, mosaic and refinement of captured EM data
4) Feature enhancement (ir-blob) followed by slice to slice registration.
5) Identify all space in retina as belonging to neuronal cell classes as well as glial cell classes through Computational Molecular Phenotyping (CMP).
6) Identify and track processes, synaptic contacts and gap junctional contacts of *all* cell classes in canonical volume.

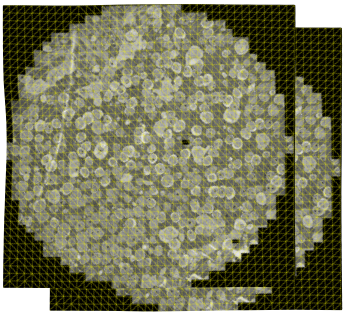
2) Automated Capture



3) Automated Layout and refinement of aberrations



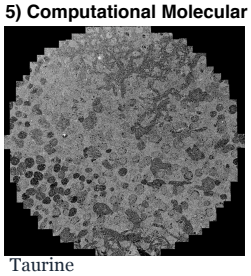
4) Automated slice to slice registration

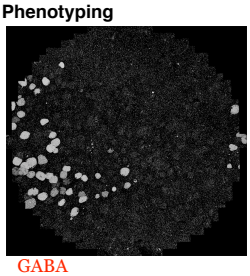


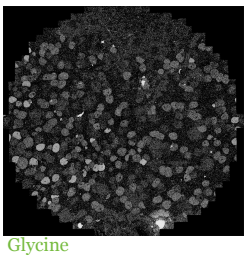
341 Sections
90nm thick sections
~32GB/Section
~1000 tiles/section
4096x4096 pixels/tile
2.18 nm/Pixel
16.5 TB after processing

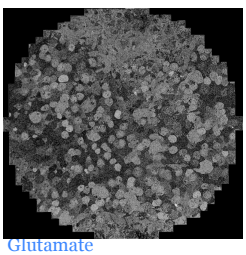
Before ssTEM images can be used to reconstruct connectivities of neurons, several image registration problems must be addressed. The first problem arises due to the large sample size and limited field of view of the microscope: each section must be assembled from many overlapping tiles, a process also referred to as mosaicking. The second problem is the co-registration of slice mosaics into a single three-dimensional volume. In both problems, non-linear distortions of individual images must be corrected within the plane and each image plane needs to be corrected to the adjacent image plane.

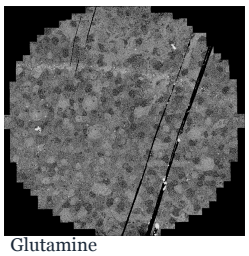
5) Computational Molecular Phenotyping

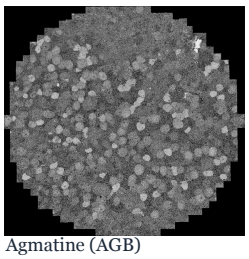

Taurine

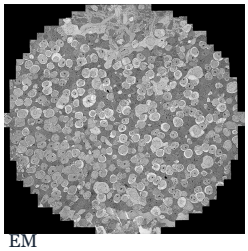

GABA

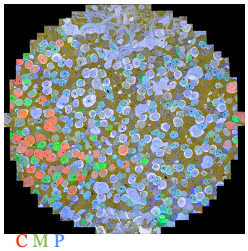

Glycine

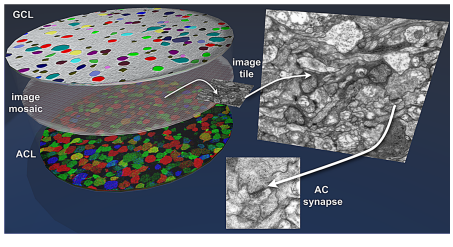

Glutamate


Glutamine


Agmatine (AGB)


EM


C M P



Of the neurons, there are at least 26 different types of amacrine cells, 13 types of bipolar cells and 14 types of ganglion cells in the retina. These cell classes assemble into distinct network motifs that form retinal signaling pathways into potentially thousands of network motifs. The only way to truly derive the identity of network motifs is to map the identity of cells participating in the network and determine the type, location and connectivities of the synaptic contacts.