Large-scale computational reconstruction of three-dimensional neural connectivity

T. Tasdizen[1], B. W. Jones[1], P. Koshevoy[1], E. Jurrus[1], R. Whitaker[1], M. Hardy[1], C.-B. Chien[1], R. E. Marc[1] and W. Denk[2]

UNIVERSITY OF UTAH[1], Max Planck Institute for Medical Research, Heidelberg, Germany[2] •

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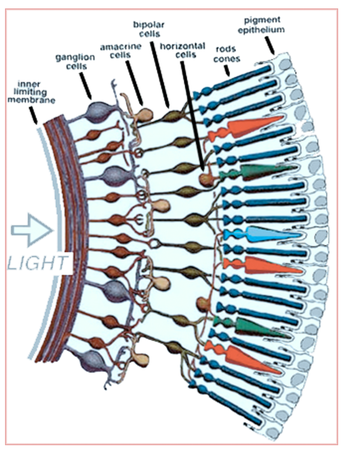
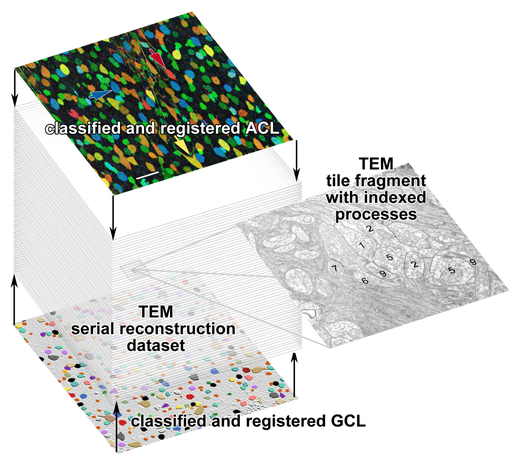
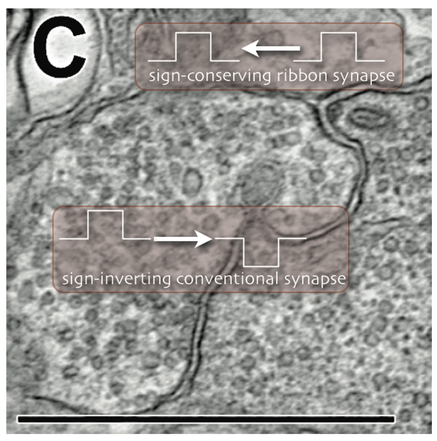
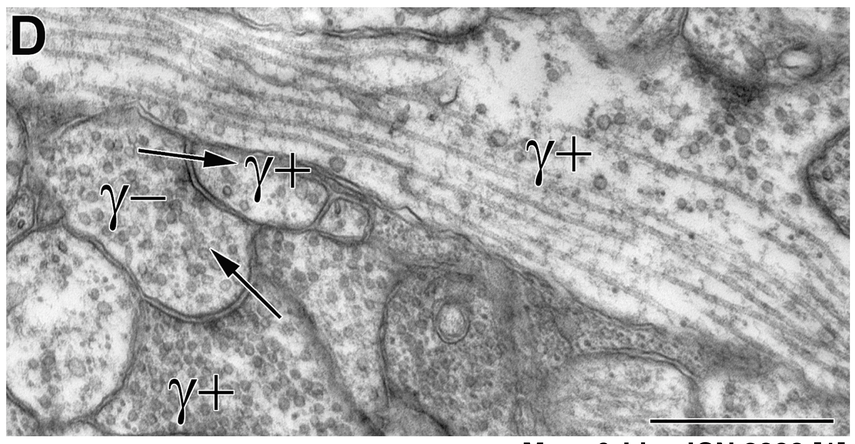
Abstract: We address the problem of building three-dimensional (3D) connectivity maps for neurons from sectional electron microscopy. Sectional data consists of a stack of very high-resolution, two-dimensional images that are oriented to capture cross sections of elongated neuronal processes. High magnification serial microscopy images have the potential to expand the field of neurophysiological modeling by providing ground-truth neuroanatomical data. However, their complexity and vast size make them impractical for human interpretation. This project aims at building tools for automatic and semi-automatic tools to aid researchers analyze such data.









Marc & Liu, JCN 2000 [1]

Retinal signal processing is executed by groups of sign conserving and sign-inverting synapses[1]. The problem is that 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. Our goal is a complete TEM reconstruction of the mammalian retina with all connectivities documented providing for the first time, the ultimate ground truth for retinal circuitry. Before serial-section TEM 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.

Parameters for 5300x, 1200 dpi/tile, 13.5 Mb/tile @ 8 bits

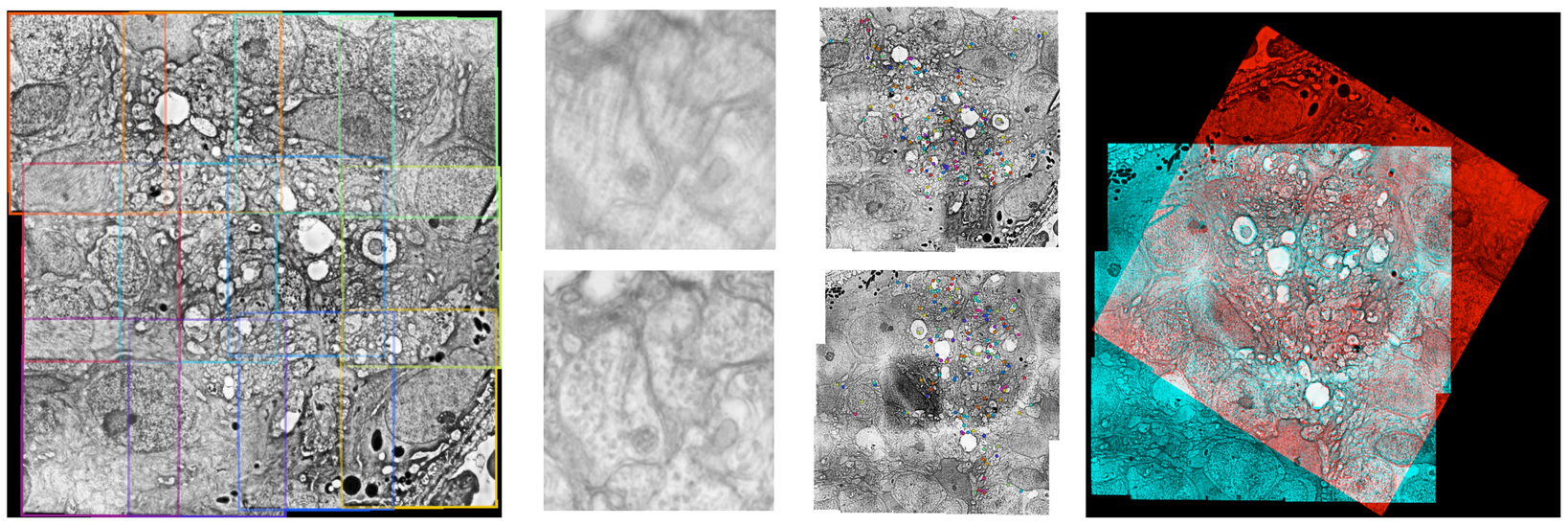
Images/plane at approx 10% overlap	230
Gb/plane	3.03
Gb/stack at approx 20% overlap	842
Total Images	63,887
Acquisition workdays @ 60s/image	133
Images/workday	480
Section size	= 0.2 mm x · 0.2 mm y · 90 nm
Stack thickness	= 0.025 mm = 278 sections (ipl)
Film size	= 73 mm · 87 mm, 6351 mm2
Film dimensions @ 1200dpi	= 3449 x 4110 = 13.5 Mb @ 8 bits
Film resolution @ 1200dpi	= 3.99 nm/pixel

Population sampling parameters

Sampling Area (mm2)	0.04
BCs sampled	1600
ACs sampled	800
GCs sampled	200
dACs sampled	100

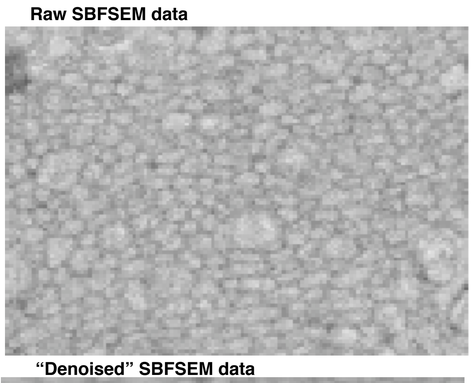
Synaptic sampling parameters

Sampling Area (mm2)	0.04
BC density	40000/(mm2)
Ribbons/BC	15
Ribbon synapses	24000
Conventional synapses/ribbon	2
Total synapses	72000
Synapses scored/hr	10
Total workdays	900
Total work years	3

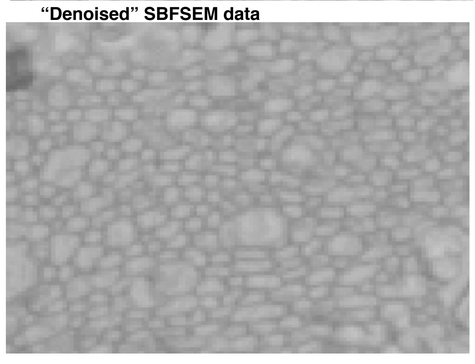


Results of mosaicking 12 images into a single slice are shown above (1st column). By using nonlinear distortion models, very good matching accuracy was obtained in the tile overlap areas (2nd column). The actual size of this image is approximately 11K x 10K pixels. Processing time was approximately 3 hours. The slice-to-slice registration application requires using fiducial points because direct image matching is not computationally feasible. Fiducial points detected at the extrema of a difference-of-Gaussian pyramid (3rd column)[2]. Results of slice-to-slice matching with this approach are shown in the 4th column.

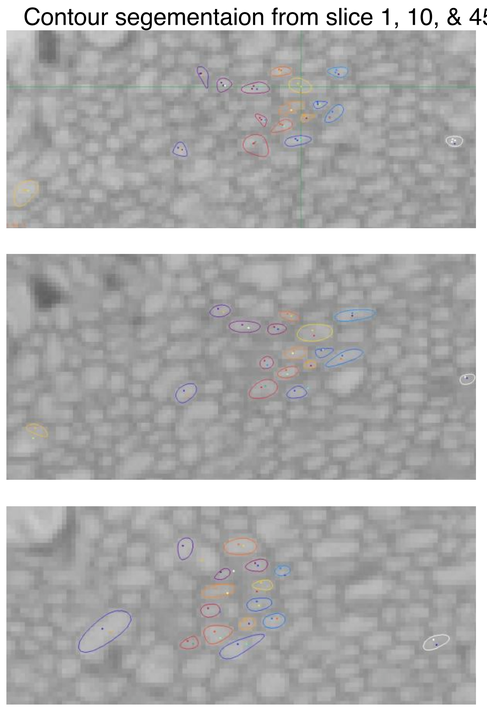
Raw SBFSEM data



"Denoised" SBFSEM data

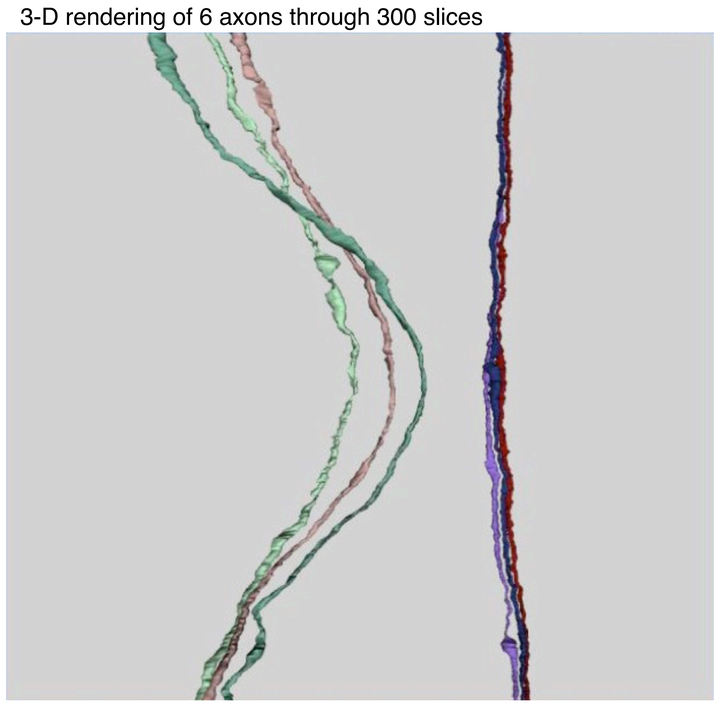


Contour segementaion from slice 1, 10, & 45



Rendering of axon contours

3-D rendering of 6 axons through 300 slices



The second driving application is the study of the organization of axons in the optic tract of the wildtype and mutant zebrafish. 3D cell segmentations can divulge precisely how retinal axons maintain and rearrange their neighbor relationships in the optic tracts. Optic tract axons are mapped in whole embryonic zebrafish brains using a new sectioning/imaging technique called serial block-face scanning electron microscopy (SBFSEM)[3]. SBFSEM images have worse signal-to-noise ratio than TEM images and require a denoising preprocessing step (1st column)[4]. We have developed an active contour segmentation + Kalman filter tracking[5] approach to track axons in the optic tract from SBFSEM images (2nd and 3rd columns). This method has yielded very promising results; it has been demonstrated to successfully track individual axons up to 600 slices without user intervention (fourth column). The results were validated by comparison to manual tracking by an expert.

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