Modern 3D electron microscopy for mapping neuronal circuit connectivity

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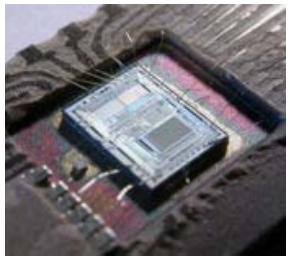
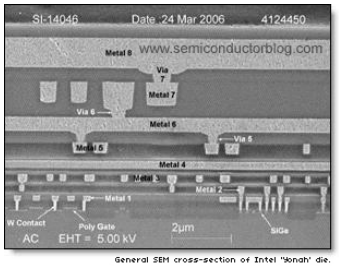
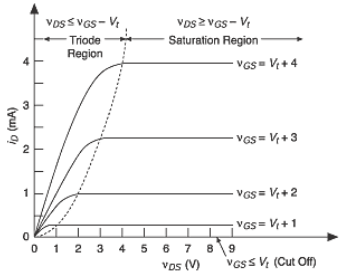
Reverse engineering neuronal circuits

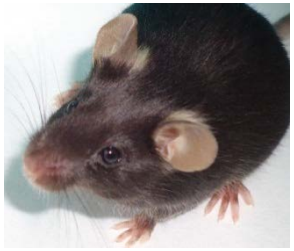


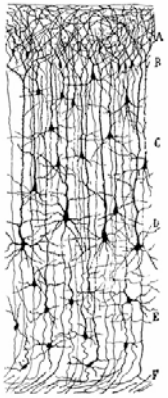
$2 + 2 = 4$



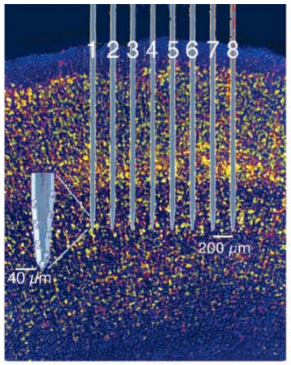
General SEM cross-section of Intel "Yonah" die.



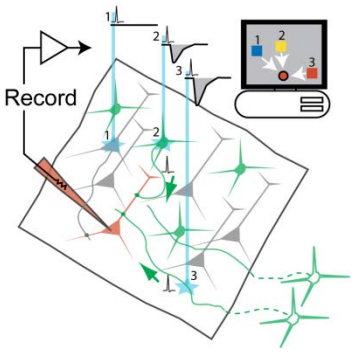




Cajal, 1904



Buszaki, 2004



Luo et al 2009

Behavior

Map structure

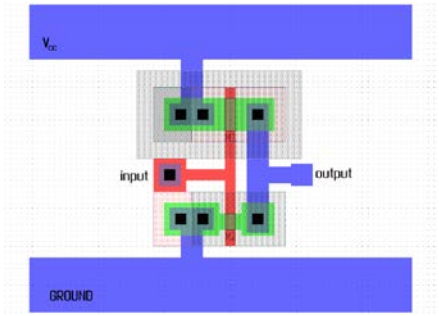
Measure function

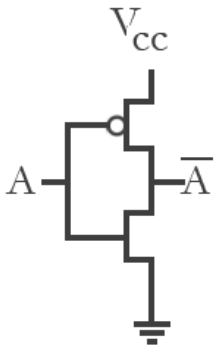
Manipulate circuit

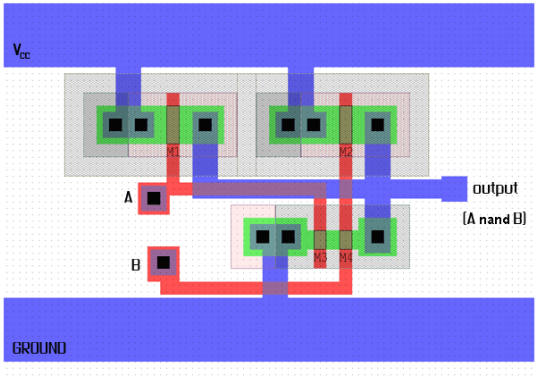
Network-level computational model

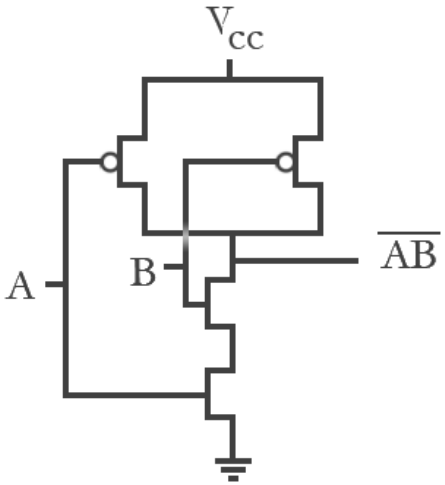
Synaptic connectivity is necessary, but not sufficient, to understand the neural basis of behavior.

But, the hope is that structure tells us something about function:

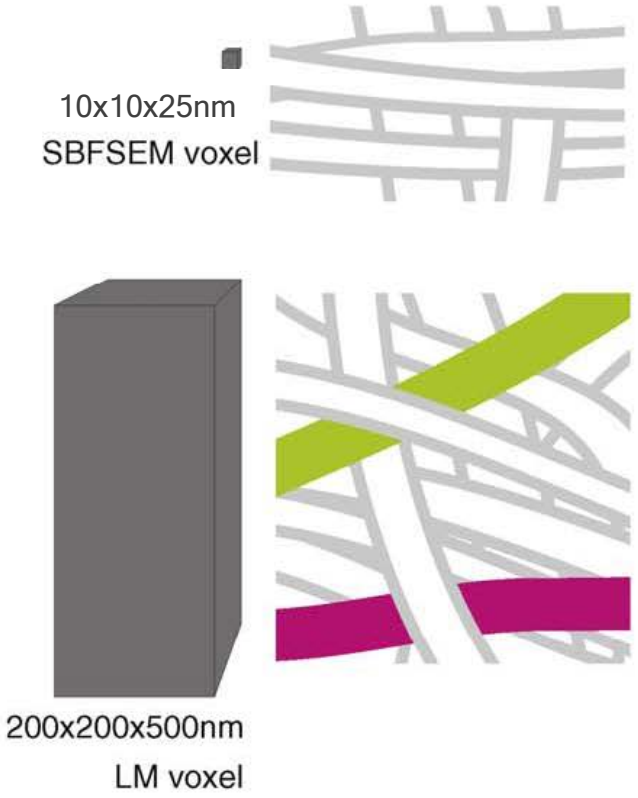


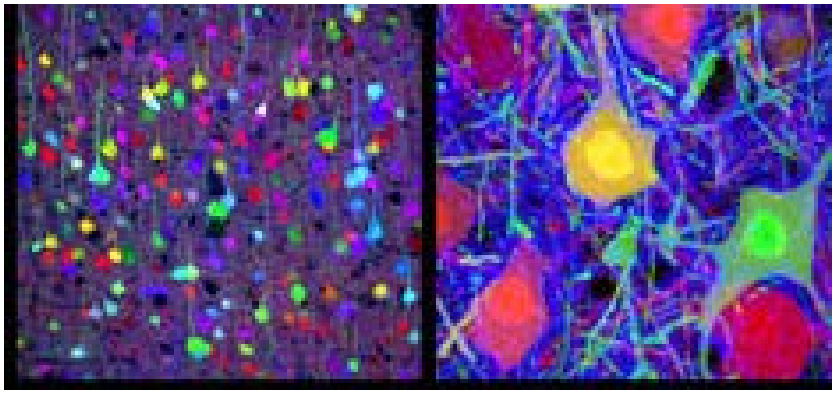






Electron microscopy is needed for dense synaptic reconstruction



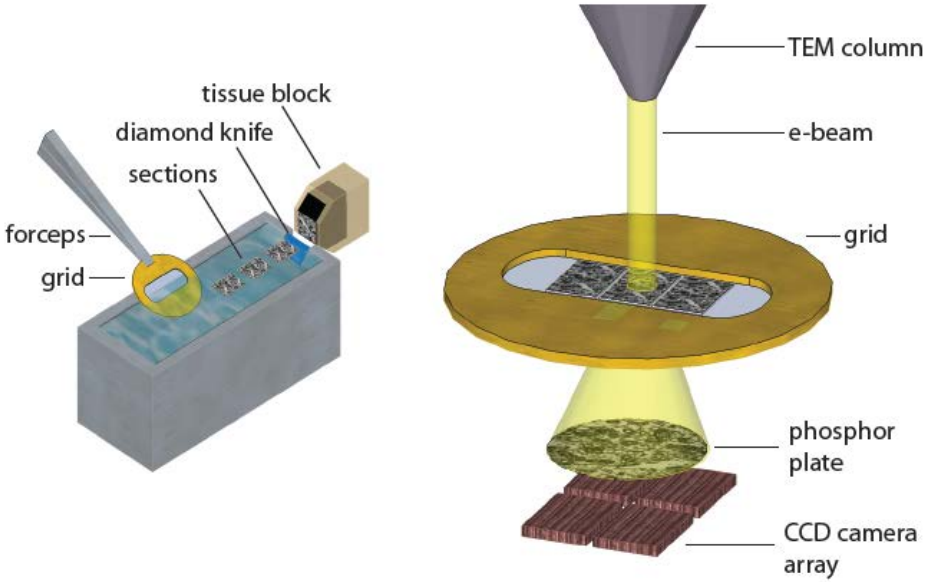


Livet et al 2007

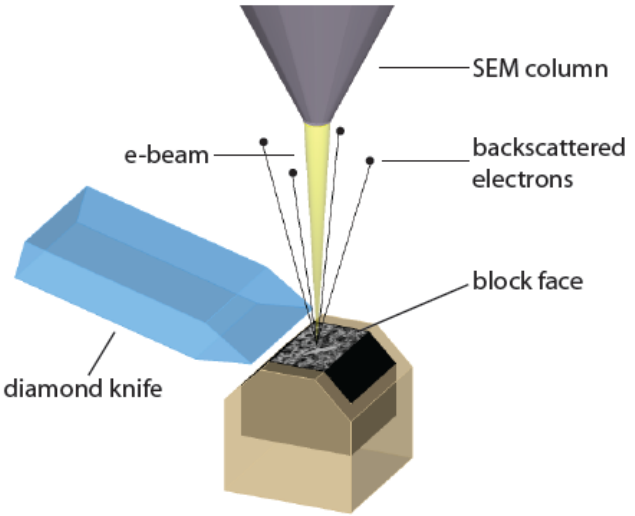
Helmstaedter M, Briggman KL, Denk W (2008) 3D structural imaging of the brain with photons and electrons. Curr Opin Neurobiol

3D electron microscopy techniques

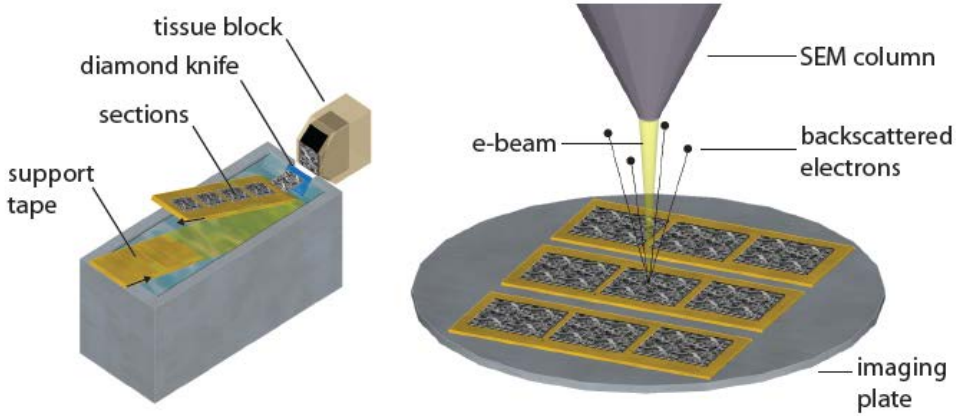
Serial section TEM (ssTEM)



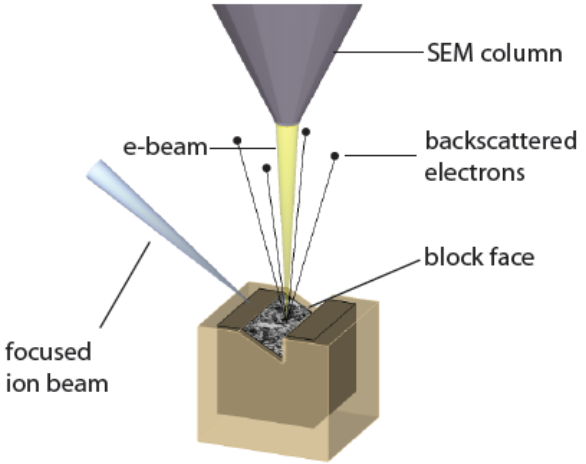
Serial block-face SEM (SBEM)



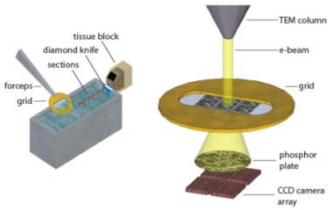
Automated tape collection ultramicrotomy (ATUM)

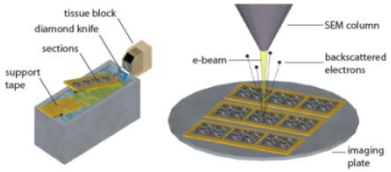


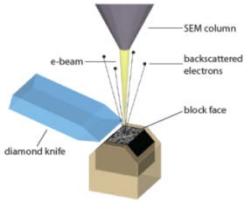
Focused ion beam SEM (FIBSEM)

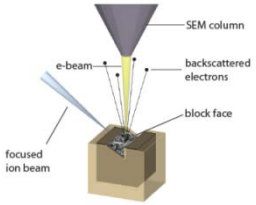


Briggman KL, Bock DD (2012) Volume electron microscopy for neuronal circuit reconstruction. Curr Opin Neurobiol.

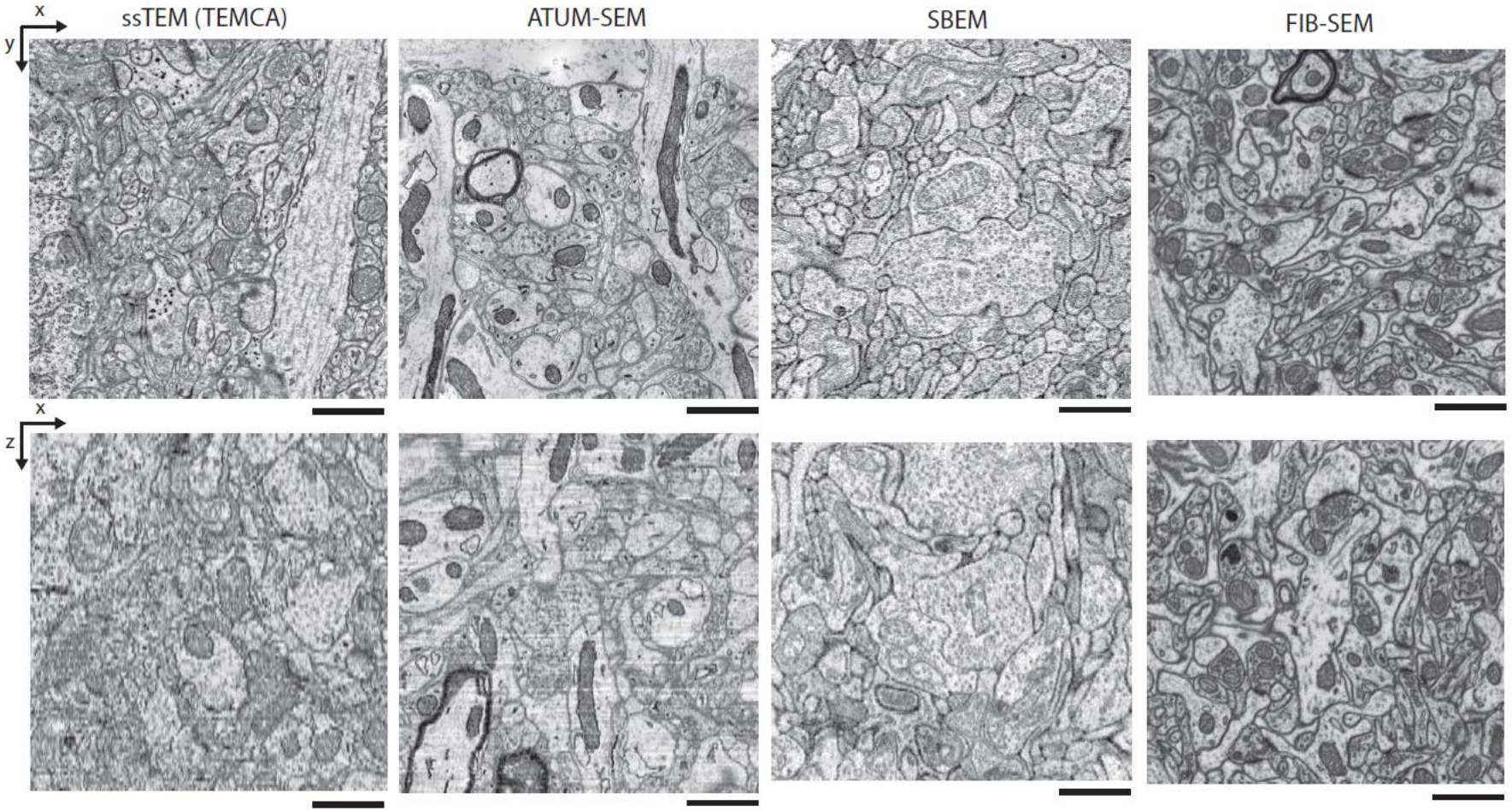






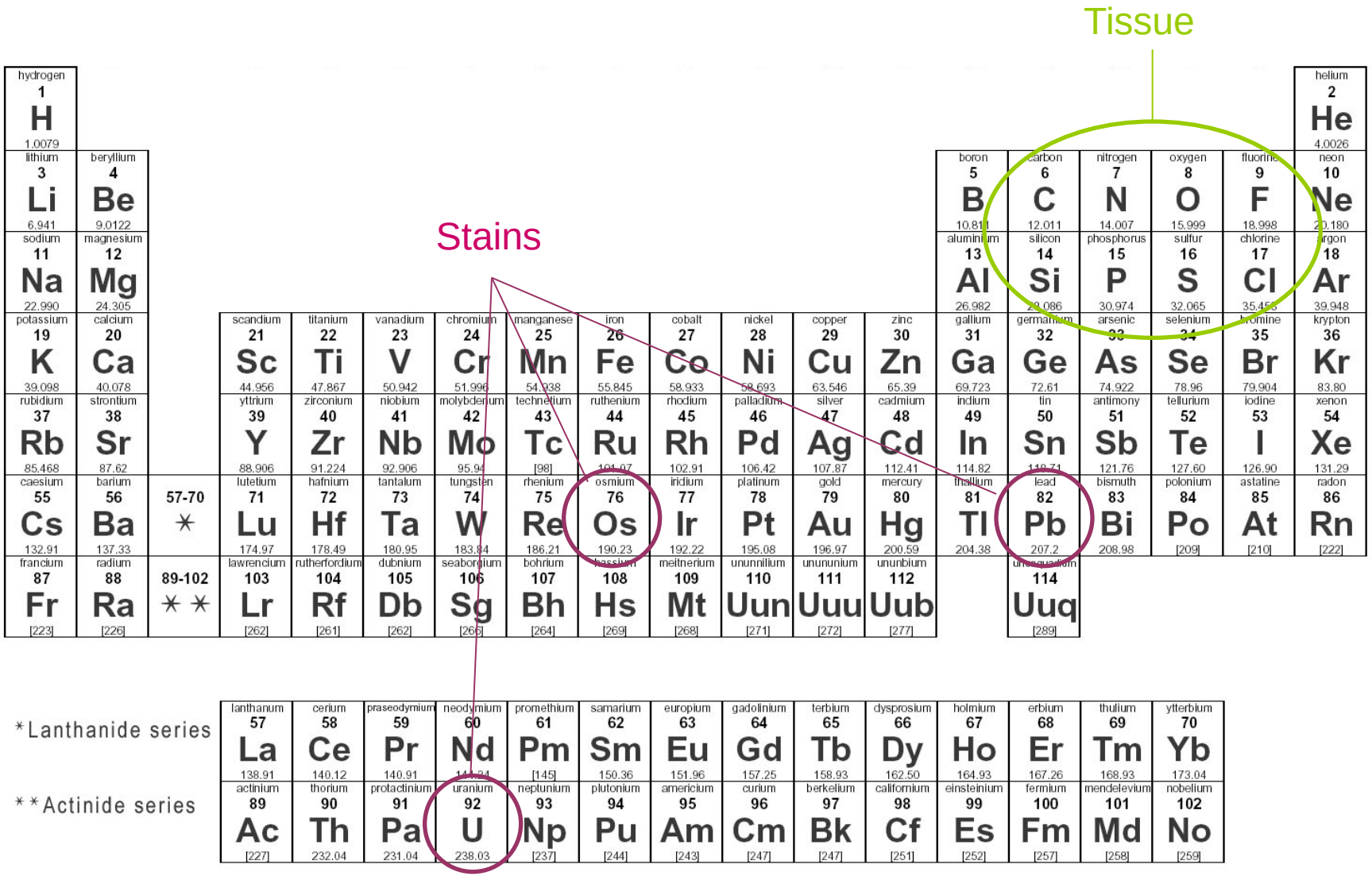


ssTEM (TEMCA) | ATUM-SEM | SBEM | FIB-SEM



ssTEM (TEMCA)	ATUM-SEM	SBEM	FIB-SEM
Poor isotropic resolution	Good isotropic resolution	Good isotropic resolution	Best isotropic resolution
Non-destructive	Non-destructive	Destructive	Destructive
Section distortions	Section distortions	No distortions	No distortions
Large field of view	Large field of view	Large field of view	Limited field of view

Conventional heavy metal stains



Contrast in block face SEM

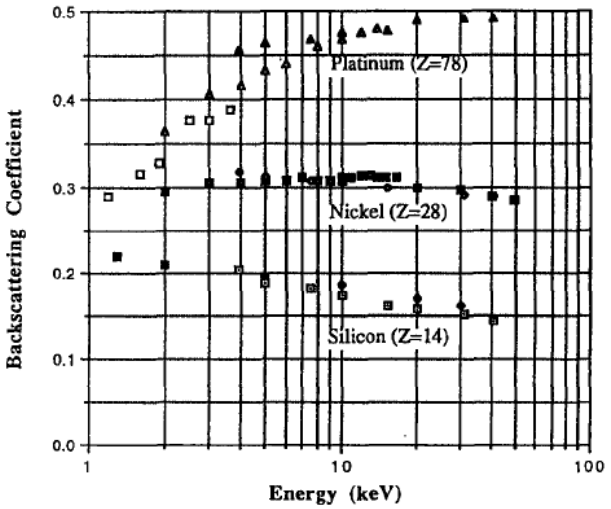
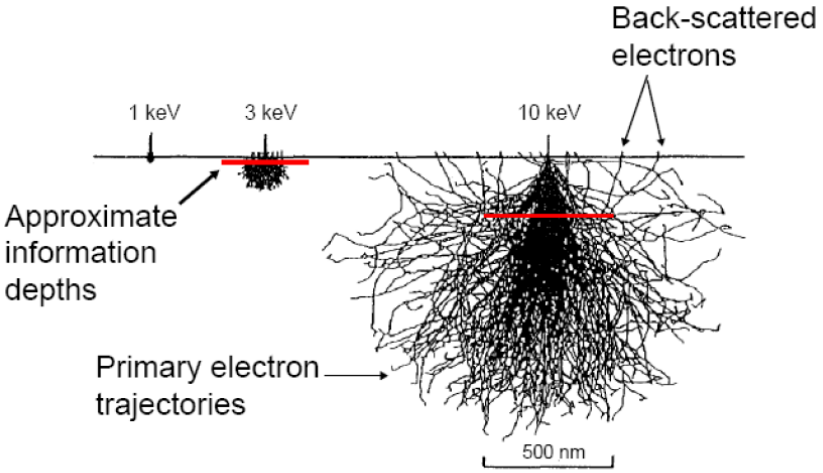
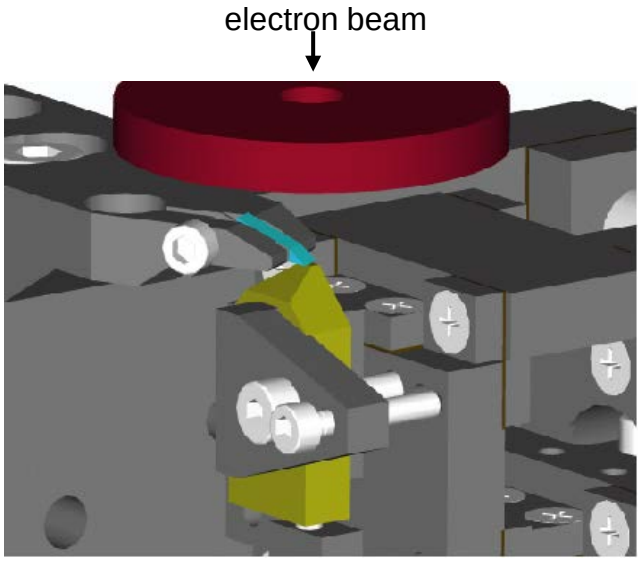
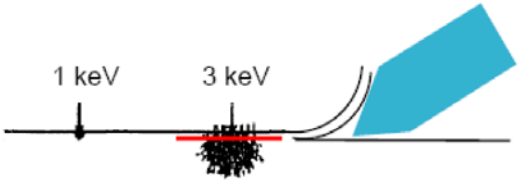


Fig. 6. Variation in the backscattering yield coefficient η for silicon, nickel and platinum as a function of the incident energy. Data taken from references in Joy (1995b).



Modified from Joy & Joy (1996)





Leighton, S. B. (1981). SEM images of block faces, cut by a miniature microtome within the SEM - a technical note. Scan Electron Microsc, (Pt 2): 73-6.
Denk W, Horstmann H (2004) Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLoS Biol 2(11): e329.

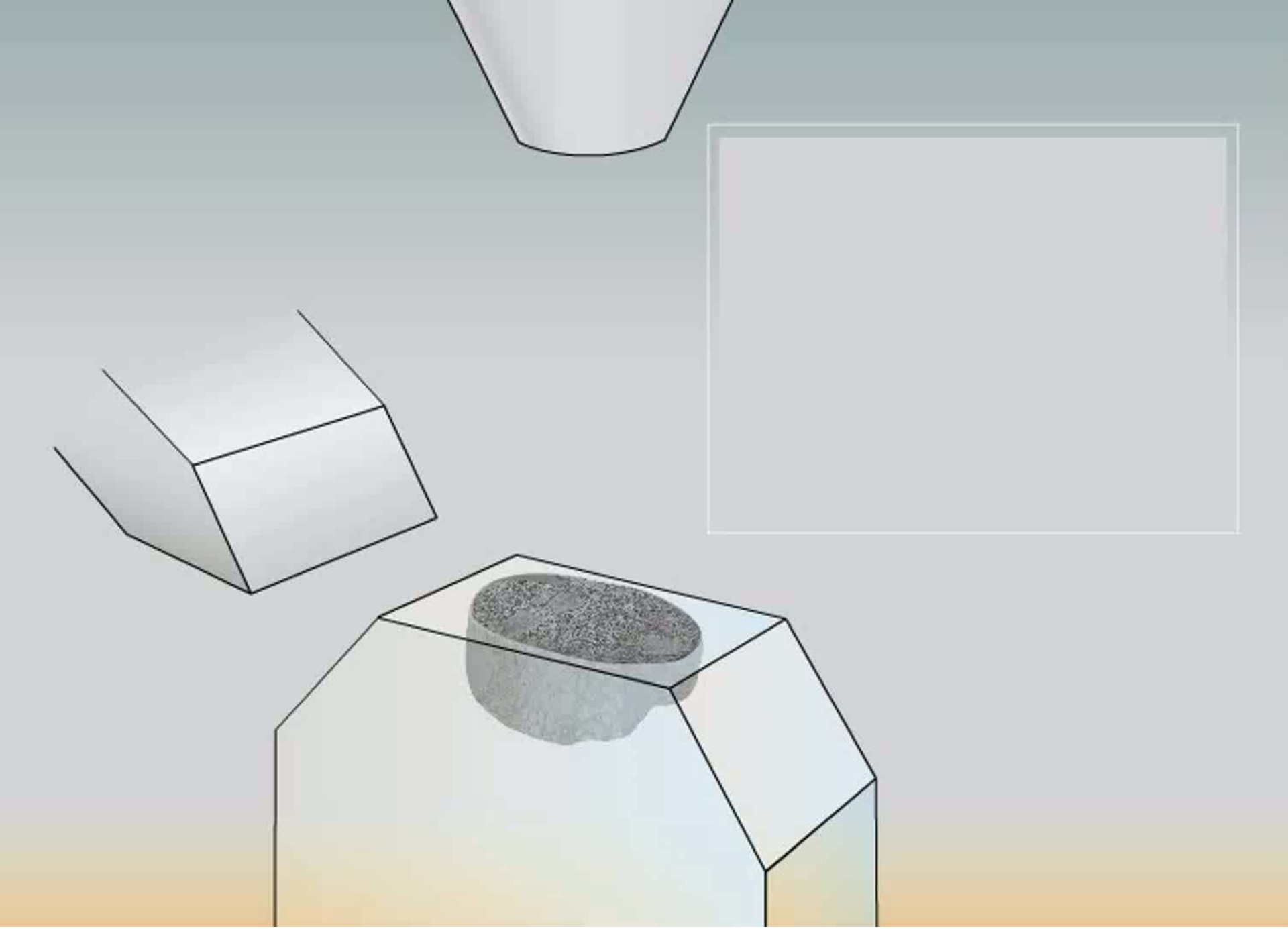


Illustration: Julia Kuhl

What SBEM volume is currently feasible?

C. elegans

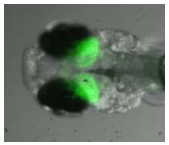
1 mm

10^{-3} mm^3
10^2 neurons

Retina

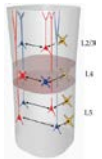
10^{-2} mm^3
10^4 neurons

Fish brain

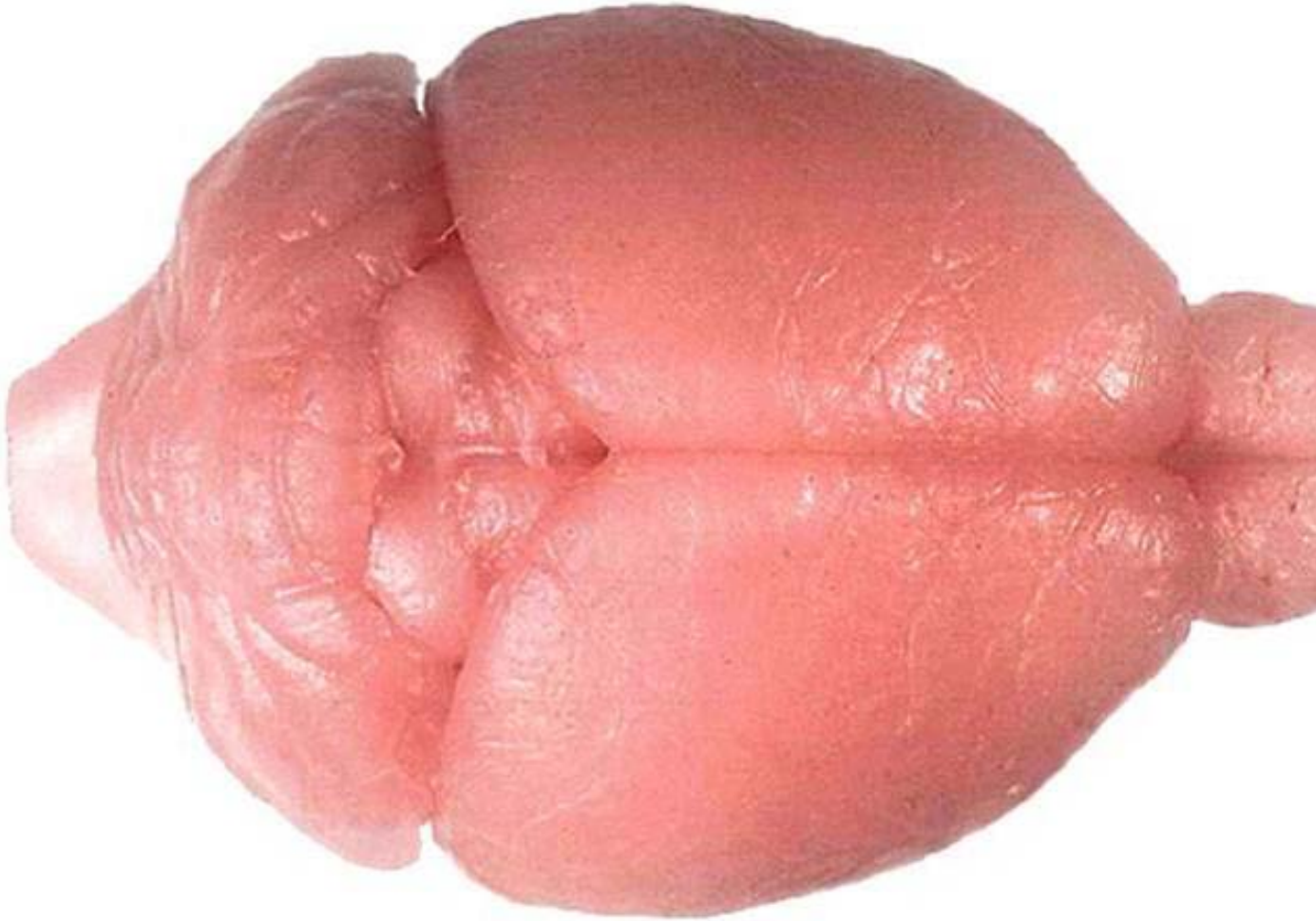


10^{-1} mm^3
10^5 neurons

Mouse barrel column



10^{-2} mm^3
10^4 neurons

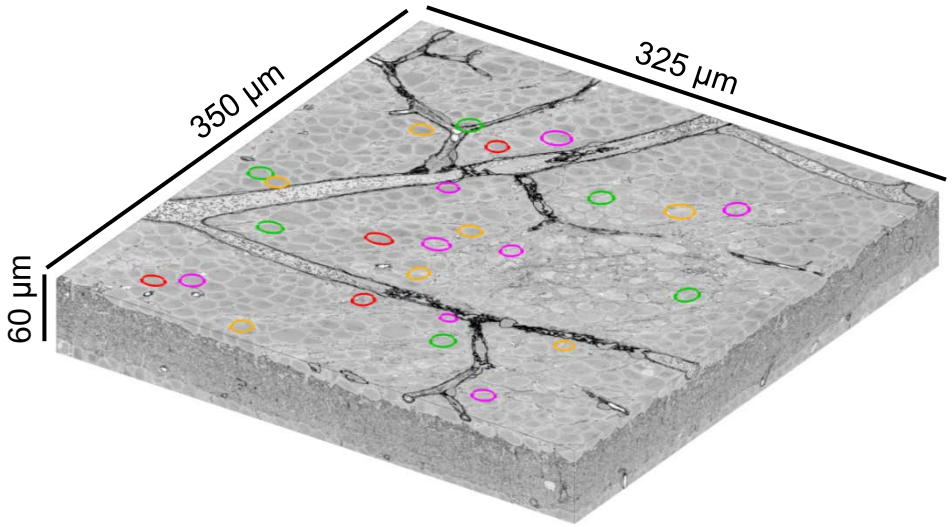


Mouse brain

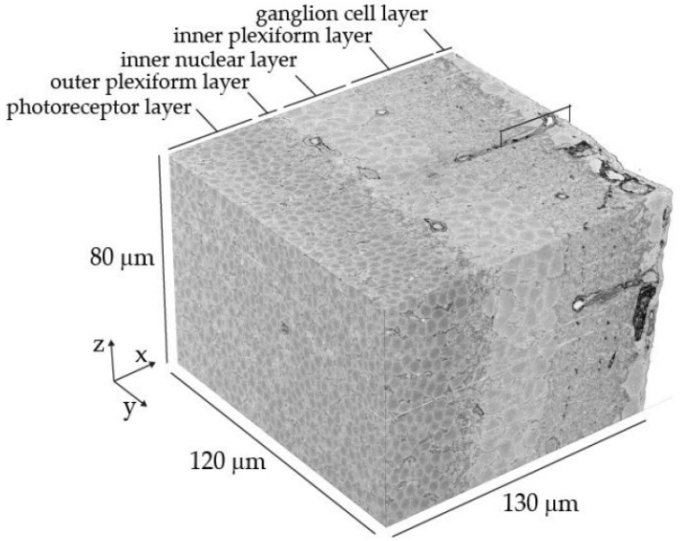
10^3 mm3
10^8 neurons

Neuronal circuit analysis of SBEM mouse retina data / are connectomes useful?

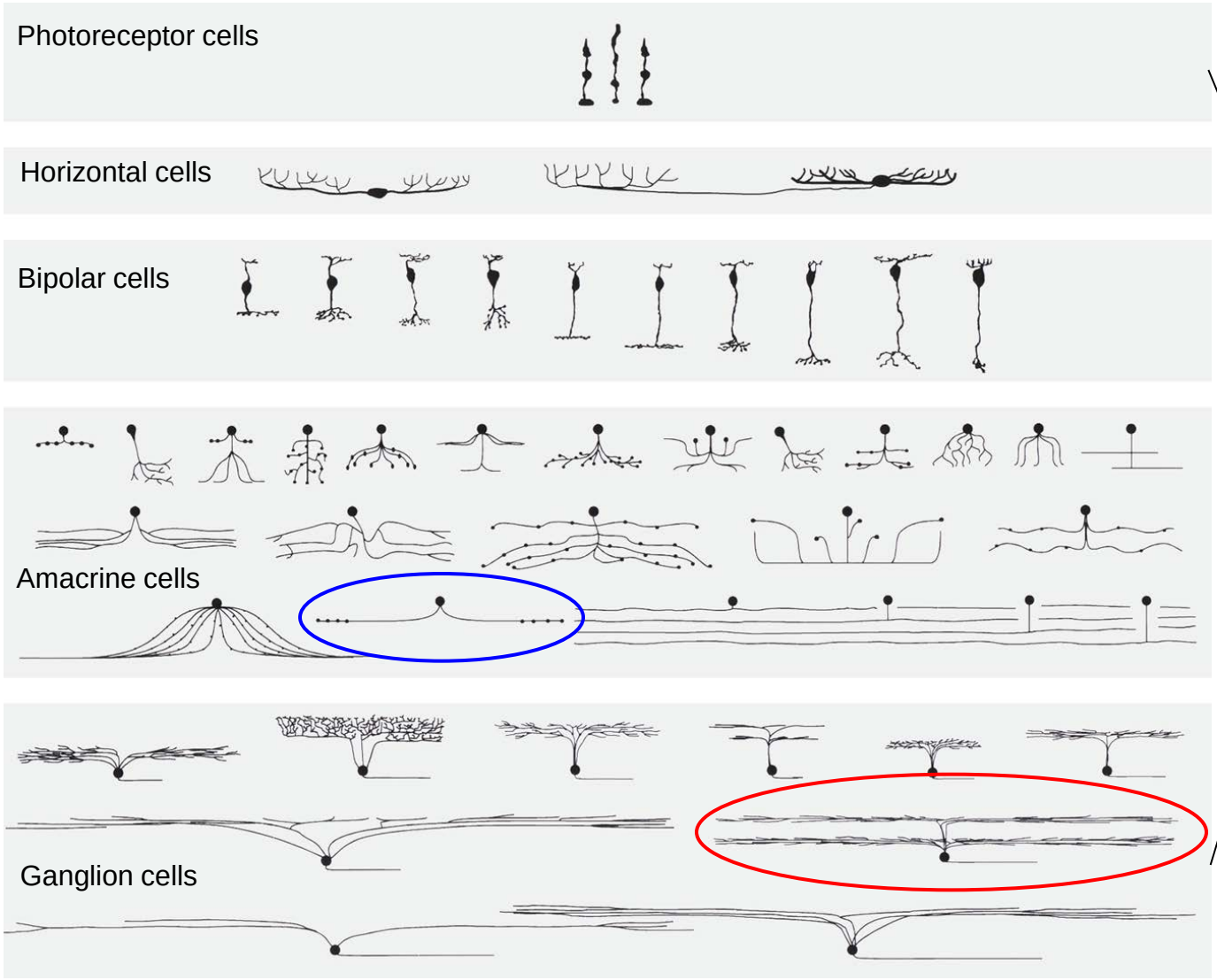
Sparse, targeted reconstruction
following 2-photon calcium imaging

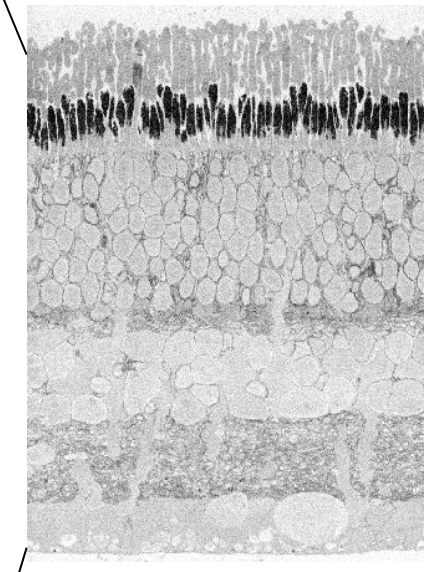


Dense, comprehensive reconstruction



The mammalian retina

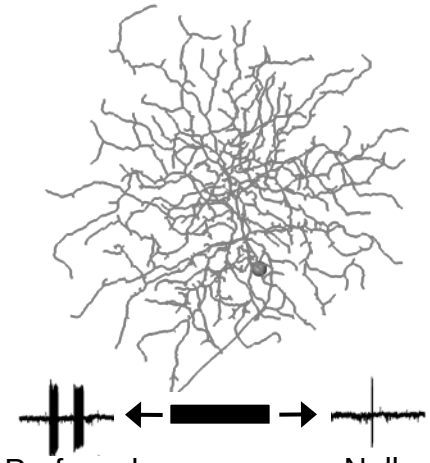




Masland (2001)

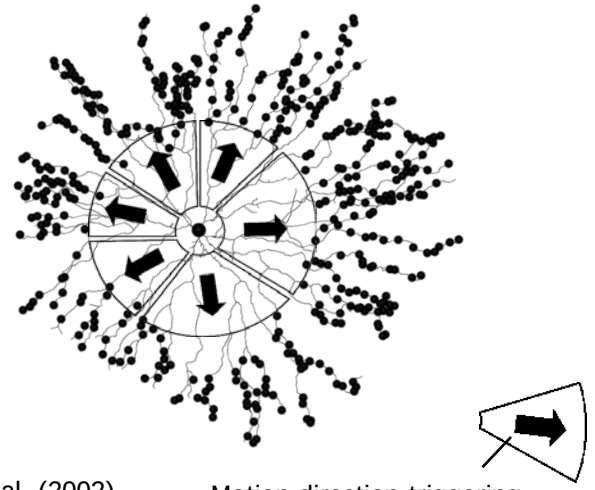
Sparse reconstruction of direction-selectivity circuitry

DS ganglion cell



Linear direction-of-motion detector

Starburst amacrine cell

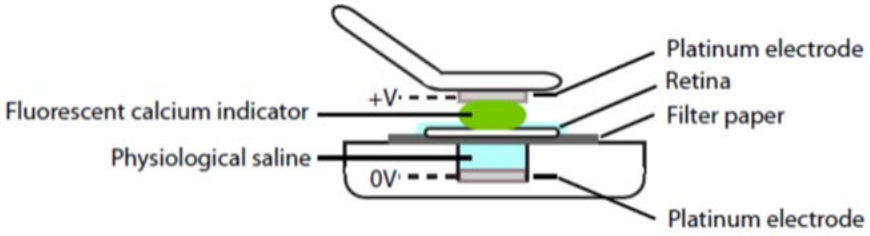


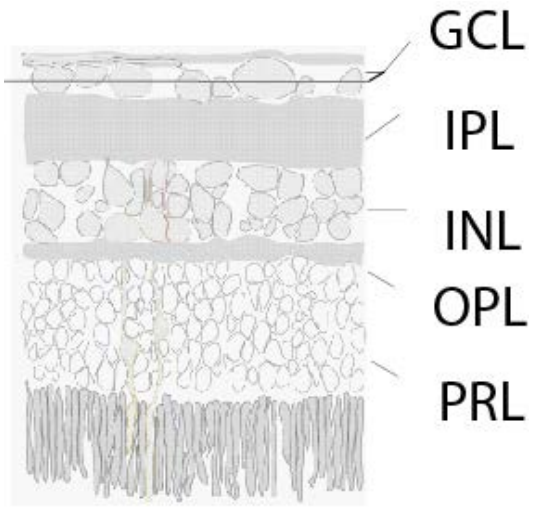
Radial direction-of-motion detector

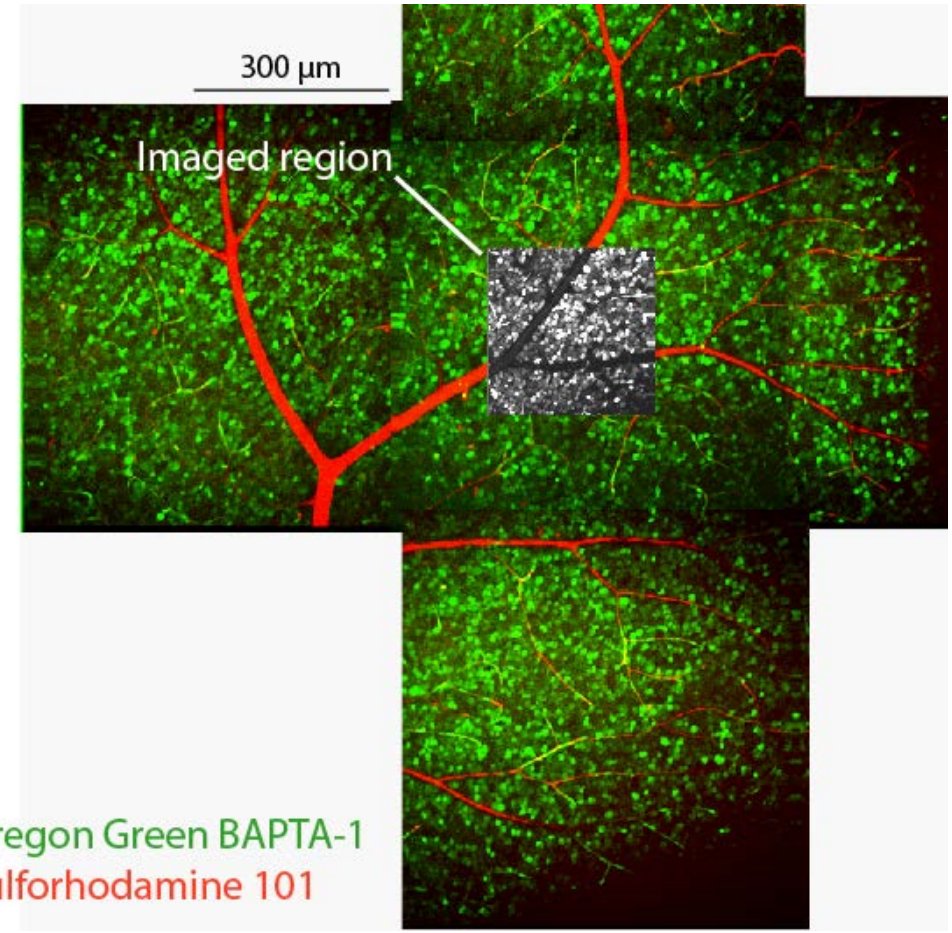
Euler et al. (2002)

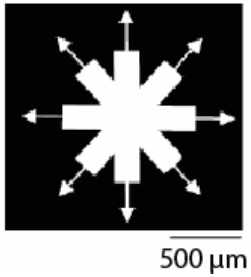
Motion direction triggering
GABA and/or ACh release from SAC

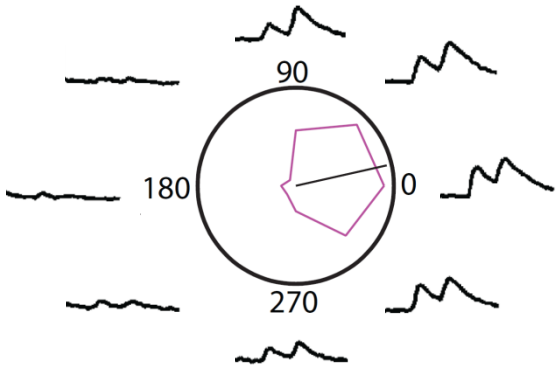
Bulk electroporation and 2P imaging of GCs



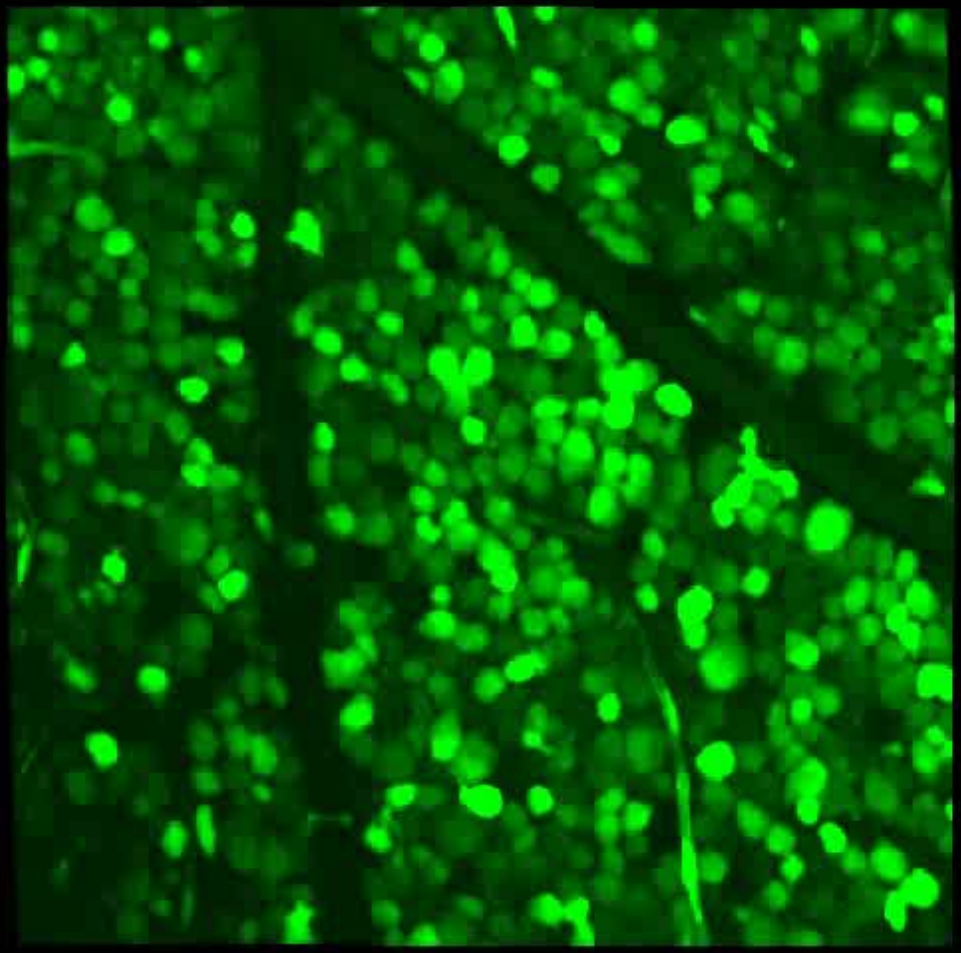




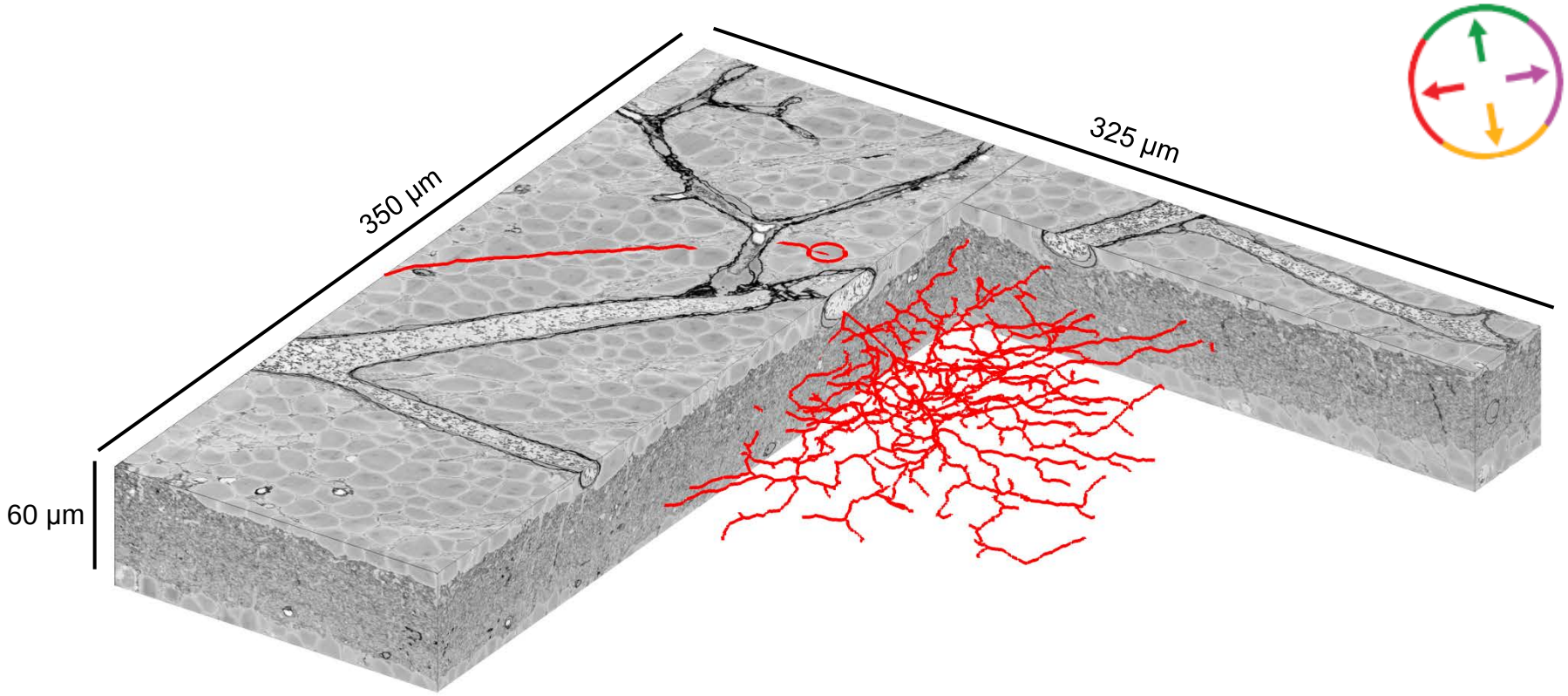


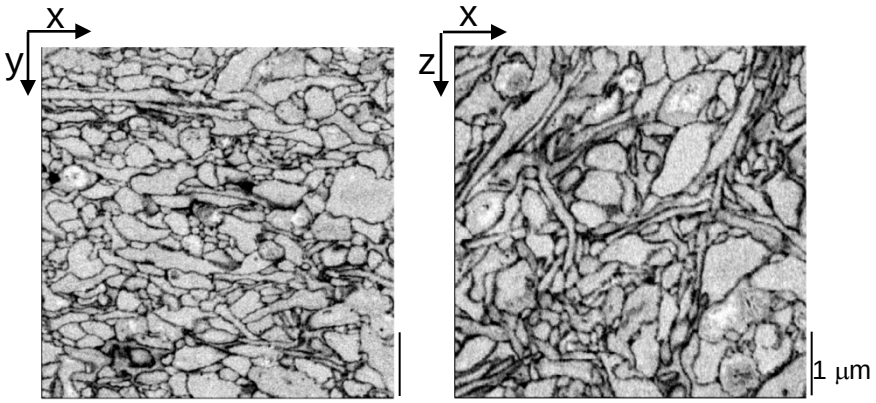


Briggman KL & Euler T. (2011) Bulk electroporation and population calcium imaging of the adult mammalian retina. J Neurophys



Structural identification of DSGCs





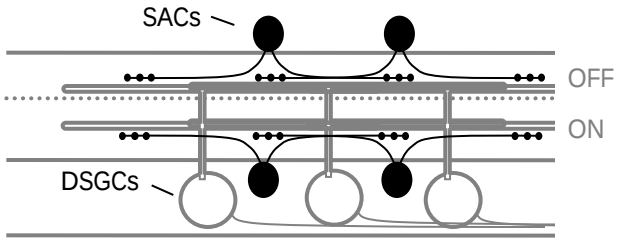
16.5 x 16.5 x 25 nm voxels
~ 1 TByte

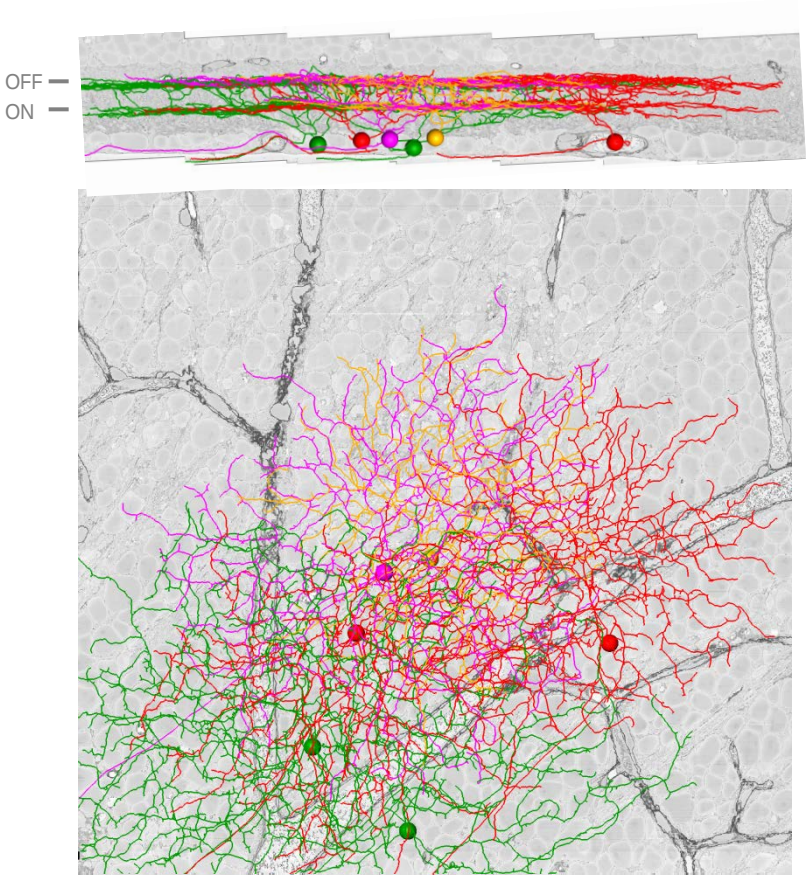
~13,000 25nm sections

0.5 MHz scan rate
~ 7 week acquisition

Briggman KL, Helmstaedter M, Denk W. (2011) Wiring specificity in the direction-selectivity circuit of the retina. Nature

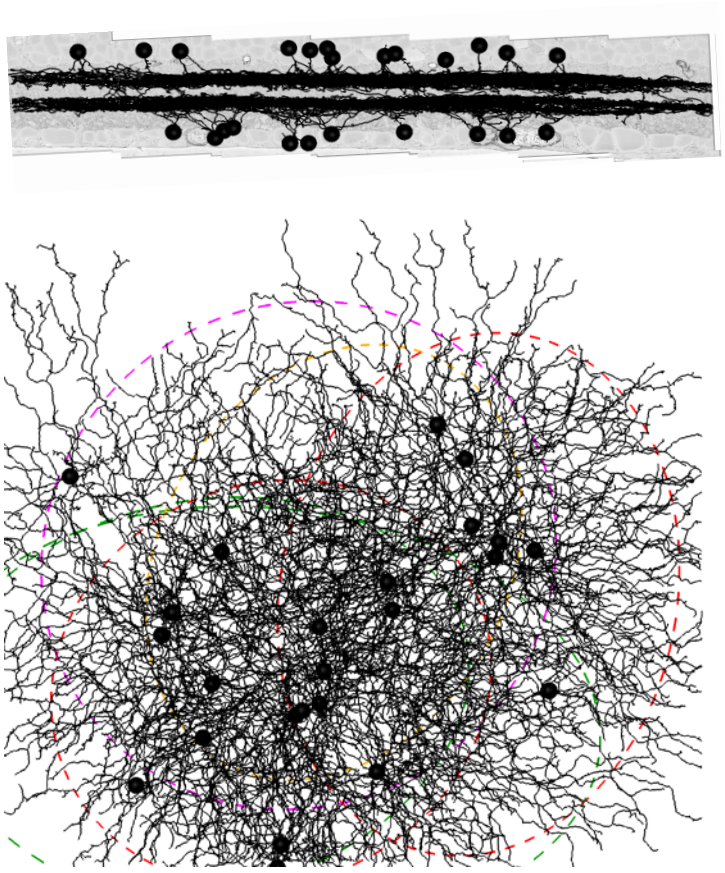
EM reconstruction of DSGCs and SACs



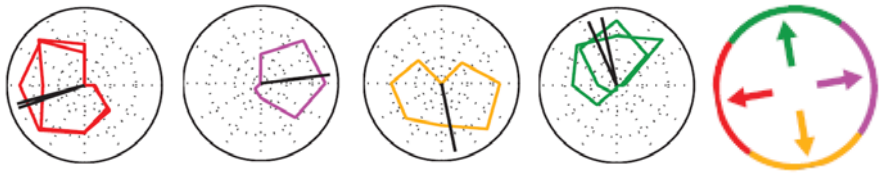


n = 6 DSGCs

100 µm

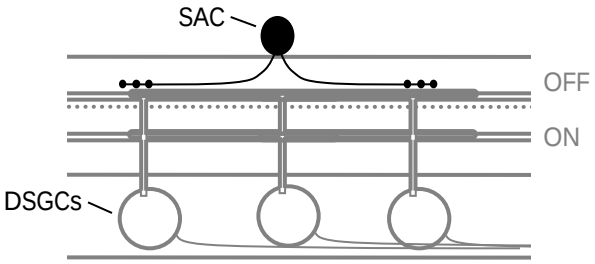


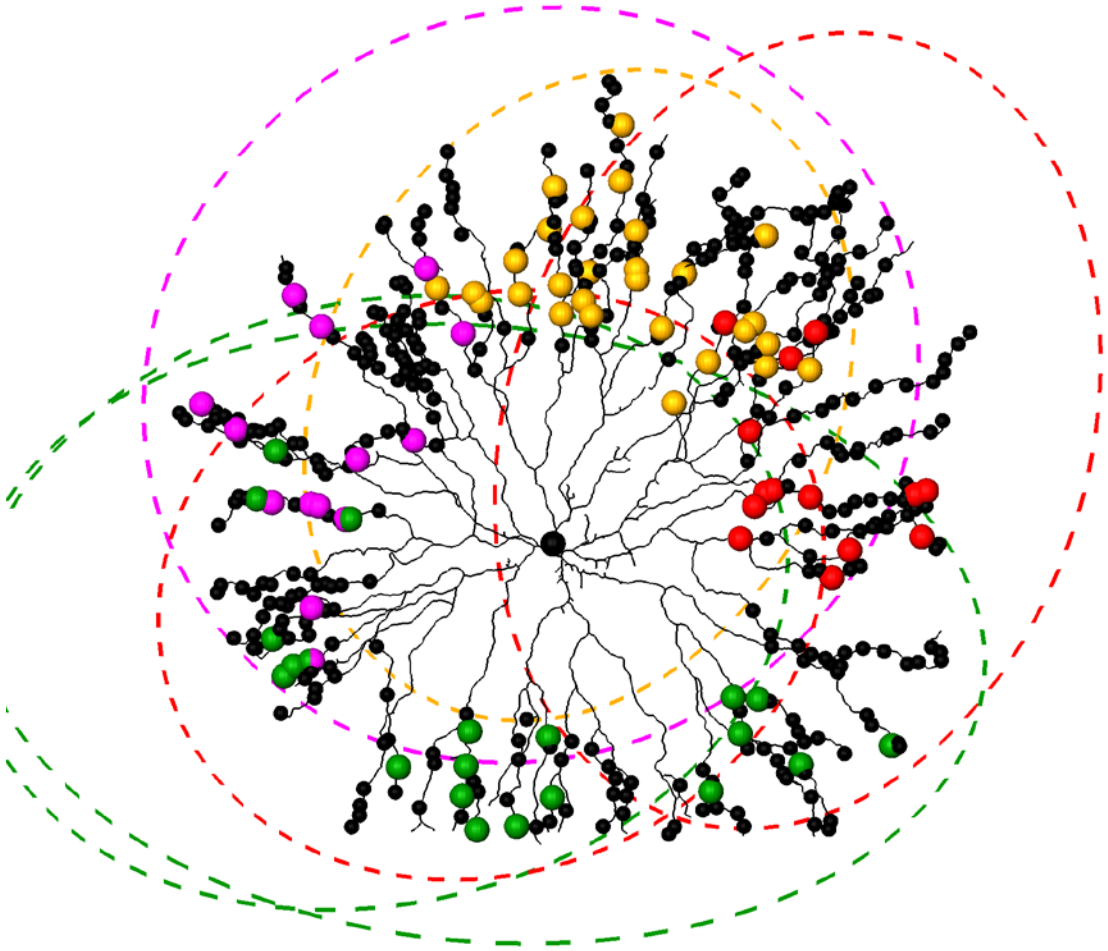
n = 24 SACs (10%)

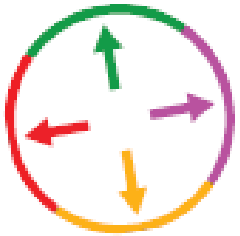


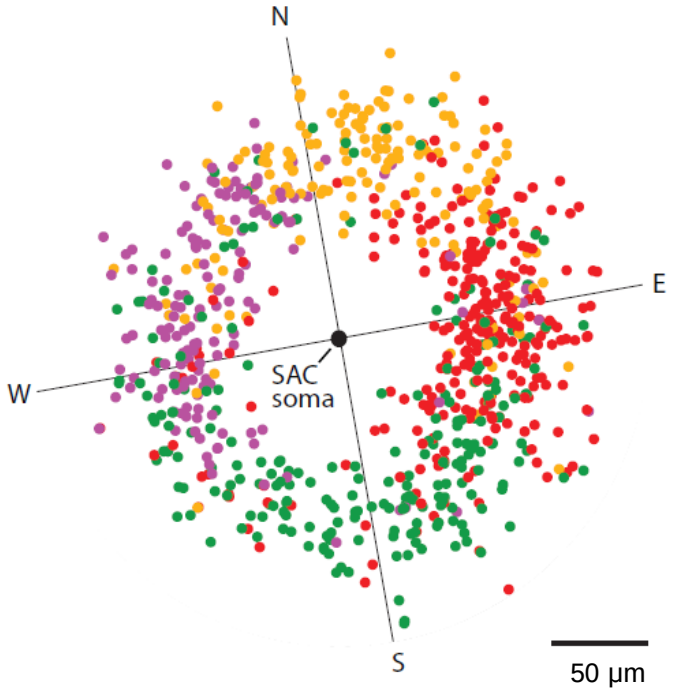
Briggman KL, Helmstaedter M, Denk W. (2011) Wiring specificity in the direction-selectivity circuit of the retina. Nature

Specificity of SAC output synapses





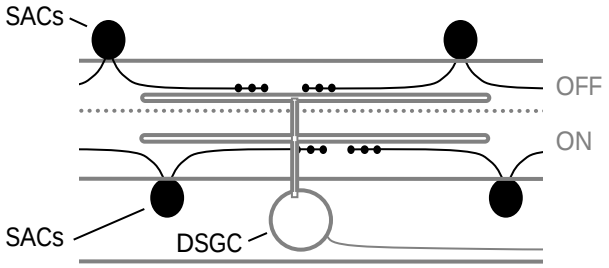




n = 831 synapses, 24 SACs

Briggman KL, Helmstaedter M, Denk W. (2011) Wiring specificity in the direction-selectivity circuit of the retina. Nature

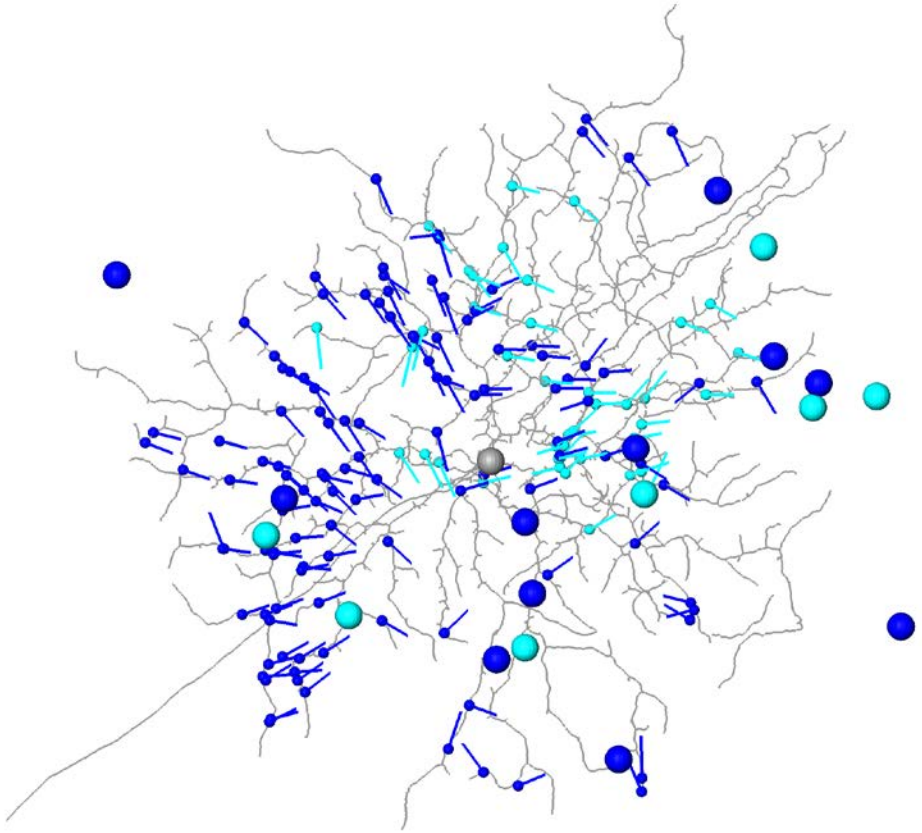
Directional input to DSGCs

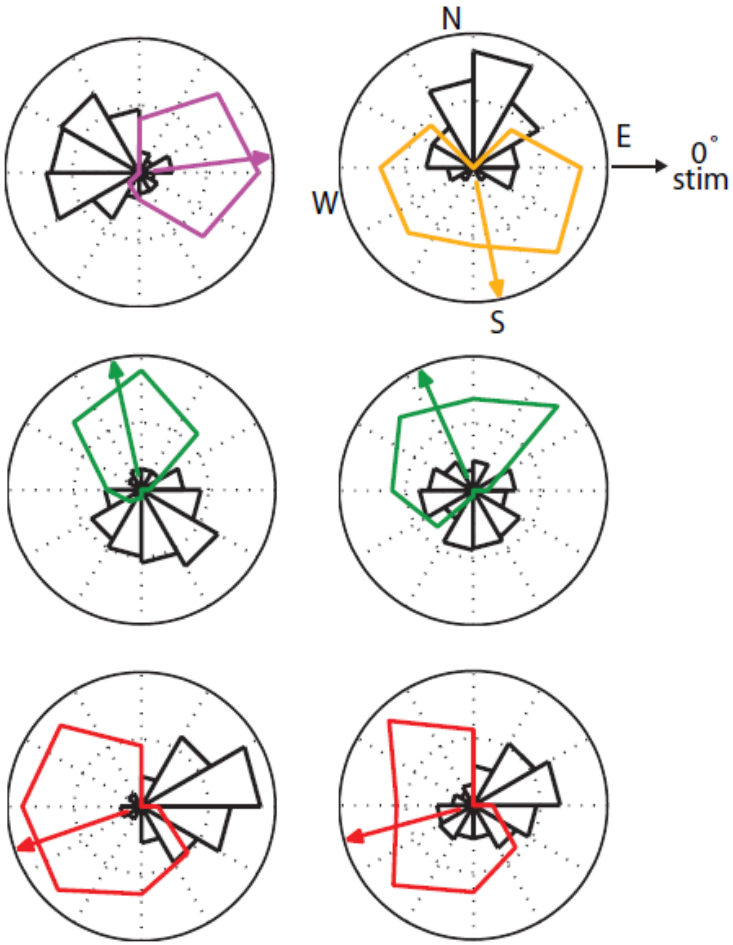


DSGC

On, Off SAC soma

On, Off SAC synapse

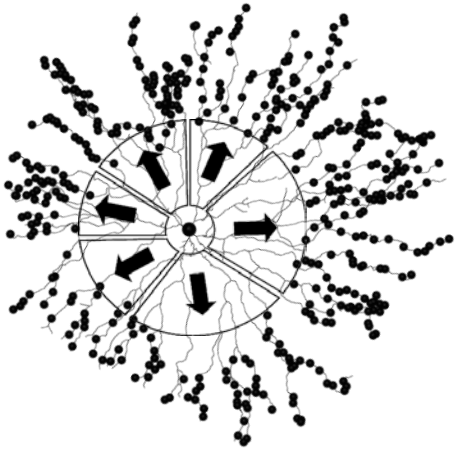




Briggman KL, Helmstaedter M, Denk W. (2011) Wiring specificity in the direction-selectivity circuit of the retina. Nature

Sparse reconstruction of direction-selectivity circuitry

Starburst amacrine cell



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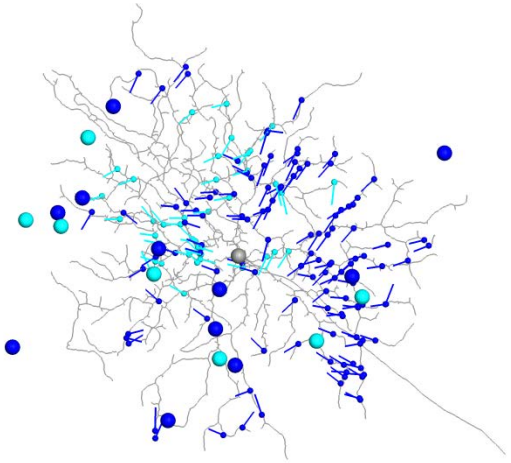

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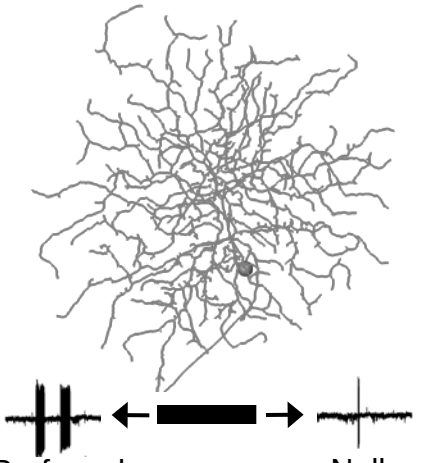
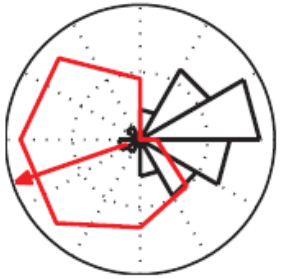
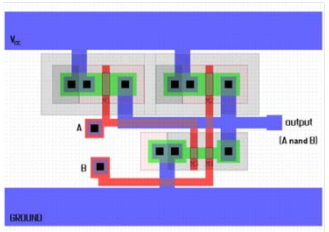
DS ganglion cell

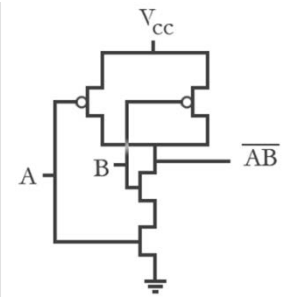


Preferred

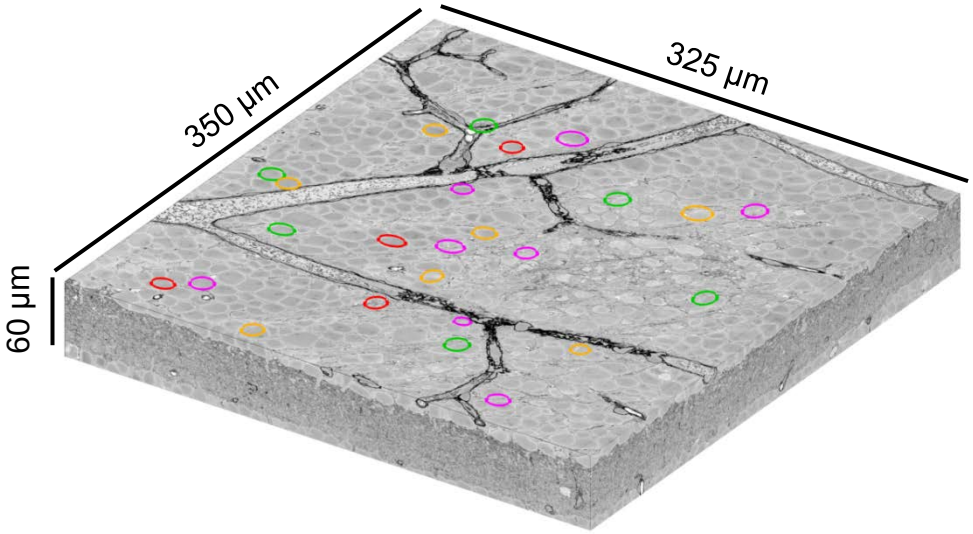
Null

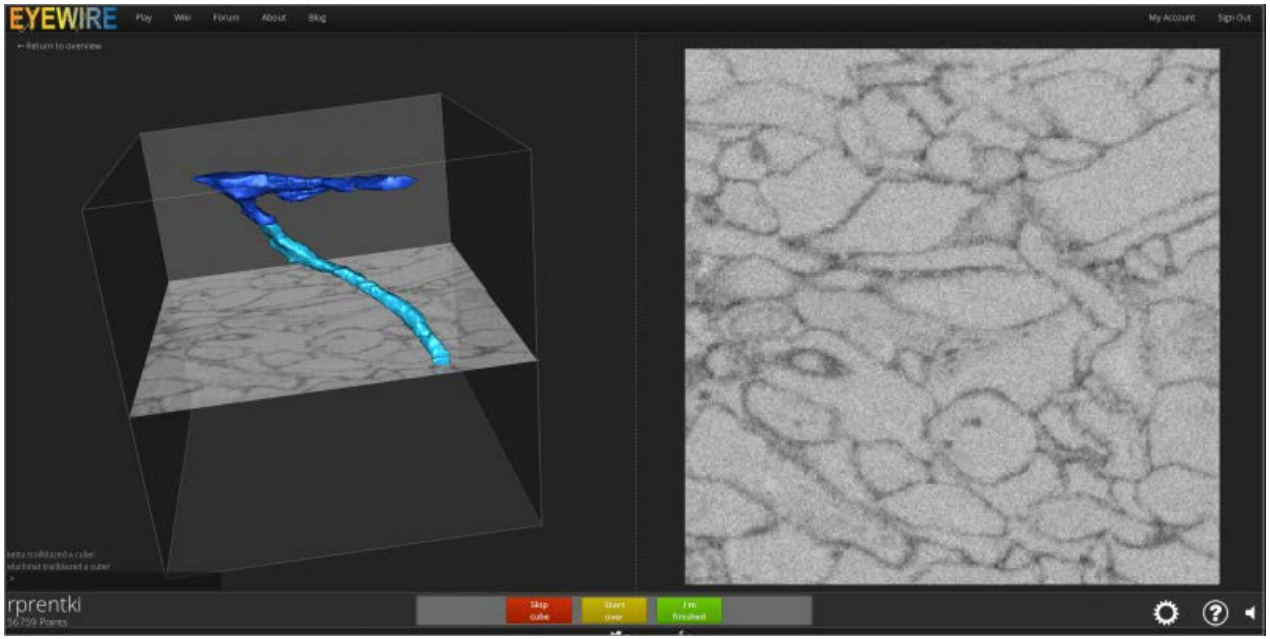






Data is difficult to collect, but ultimately yields rich datasets

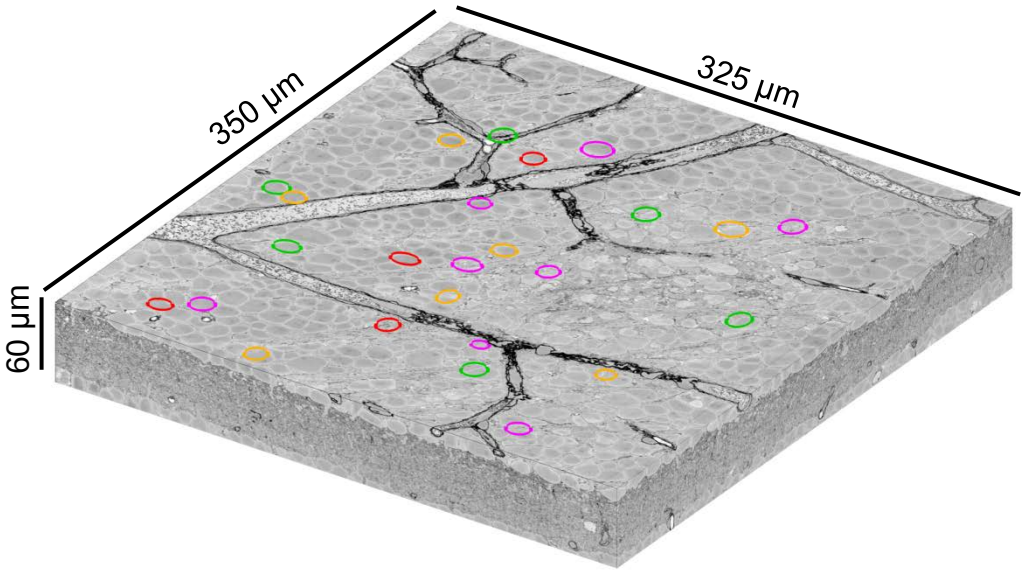




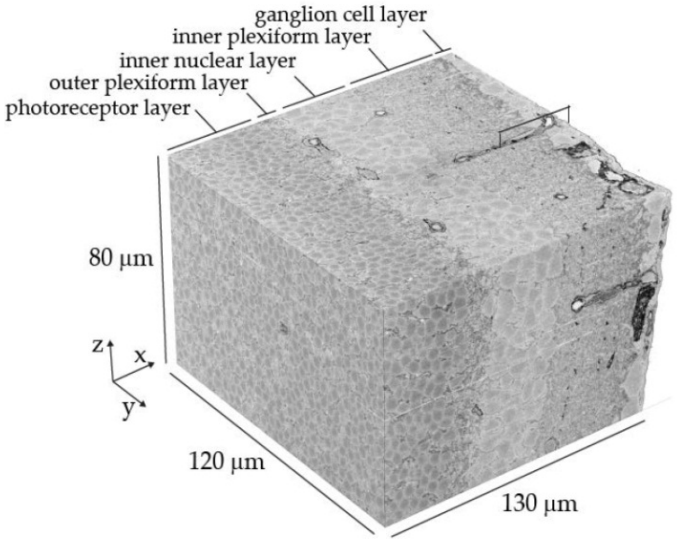
eyewire.org

Neuronal circuit analysis of SBEM mouse retina data / are connectomes useful?

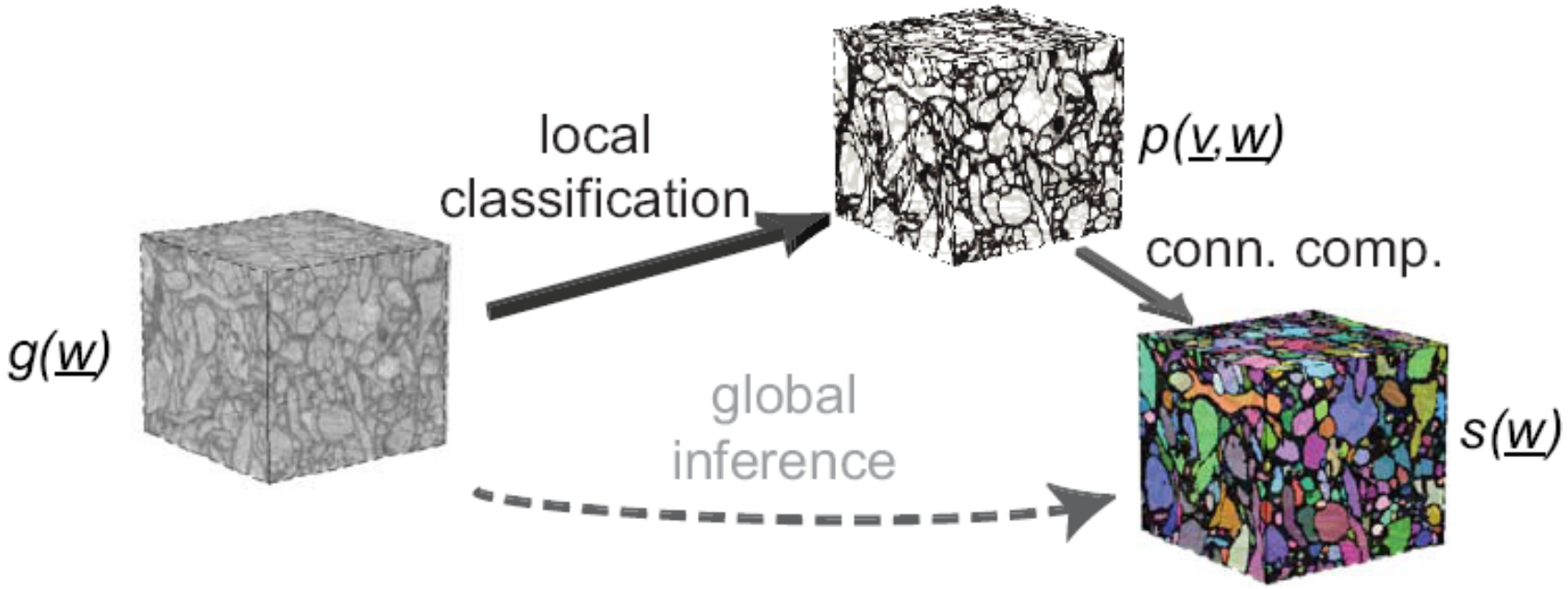
Sparse, targeted reconstruction
following 2-photon calcium imaging



Dense, comprehensive reconstruction

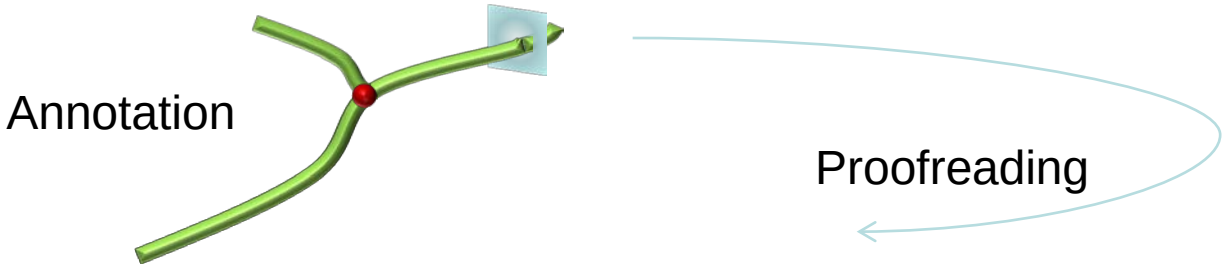


Automated image analysis?

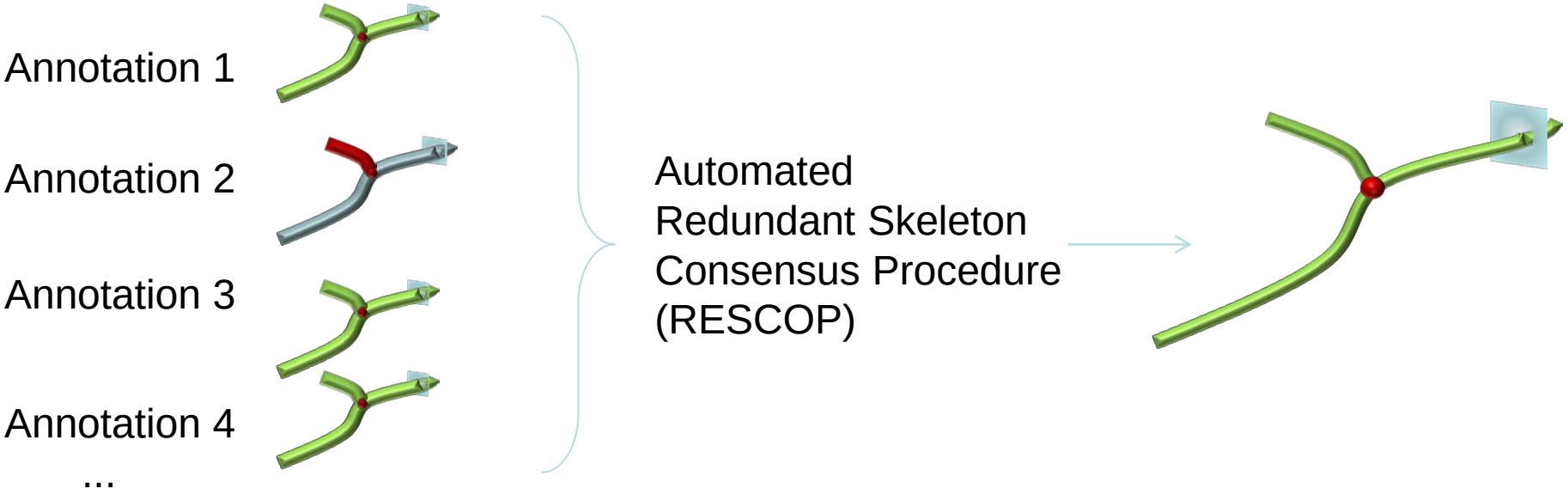


Forward-only annotation: efficiently scalable

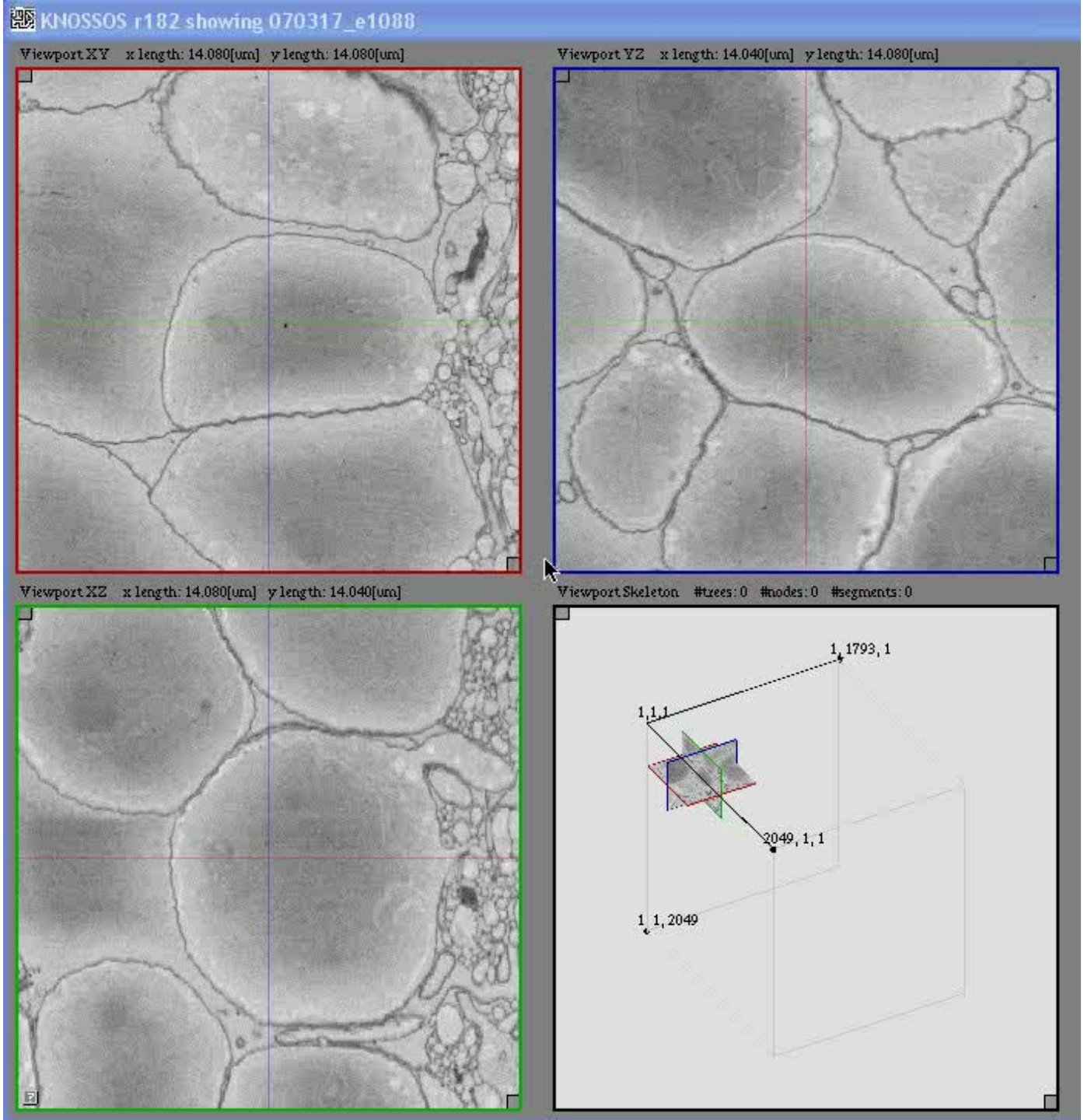
(Iterated) proofreading



Forward-only annotation + consensus



Helmstaedter M, Briggman KL, Denk W. (2011) High accuracy neurite tracing for high-throughput neuroanatomy. Nature Neurosci

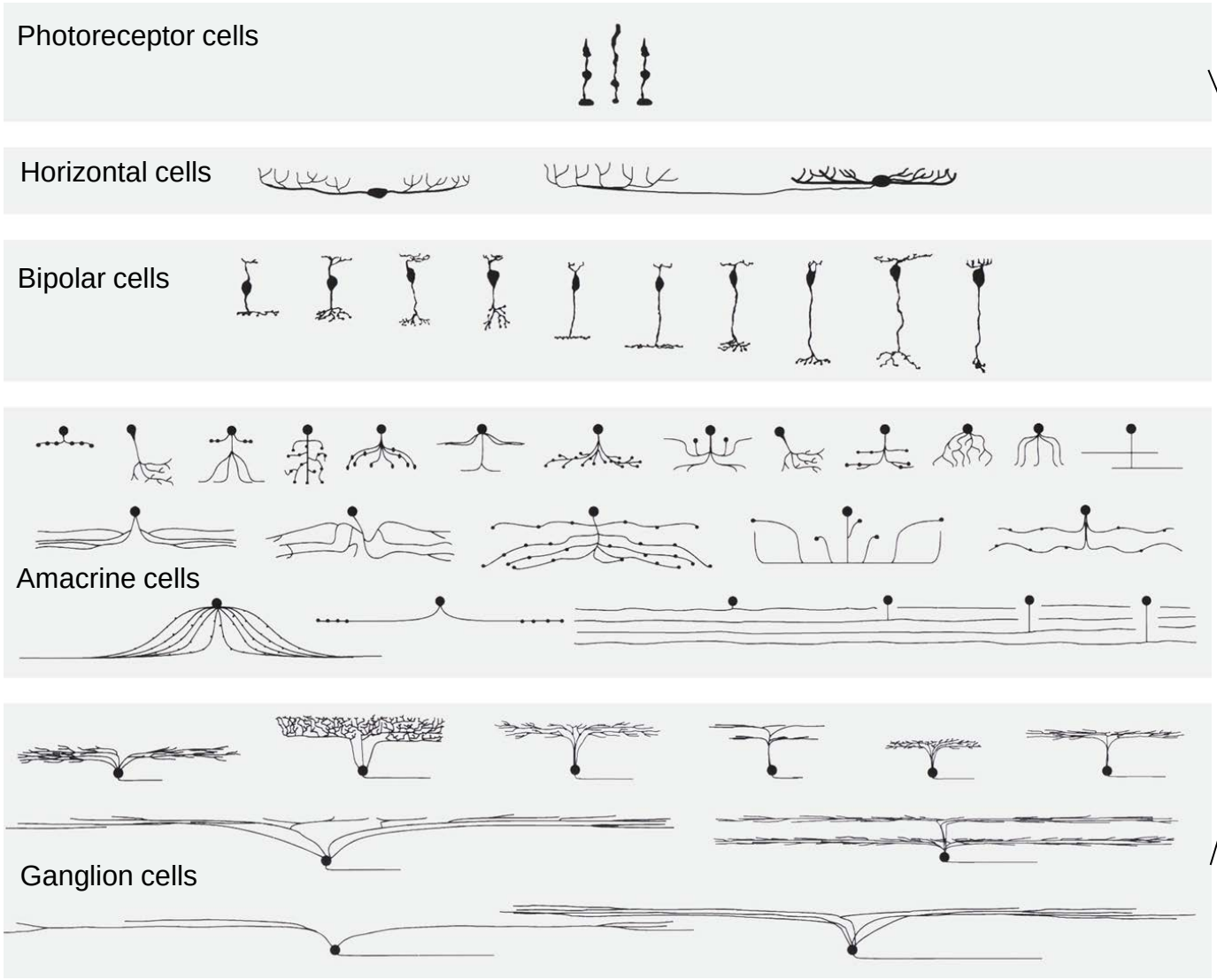


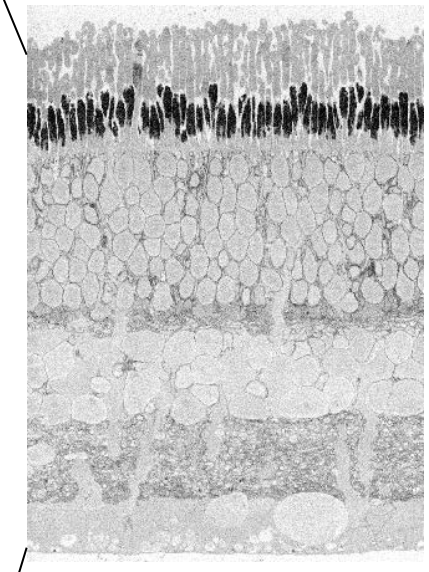
Helmstaedter
Briggman
Denk (2011)
Nature
Neuroscience

KNOSSOS
programmed by
Jörgen Kornfeld,
Fabian Svara

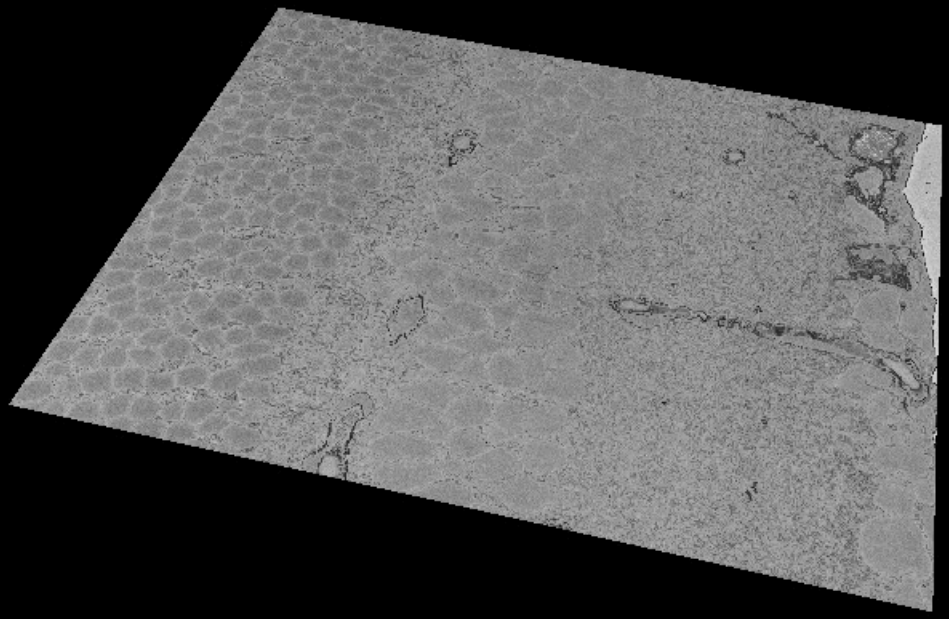
knossostool.org

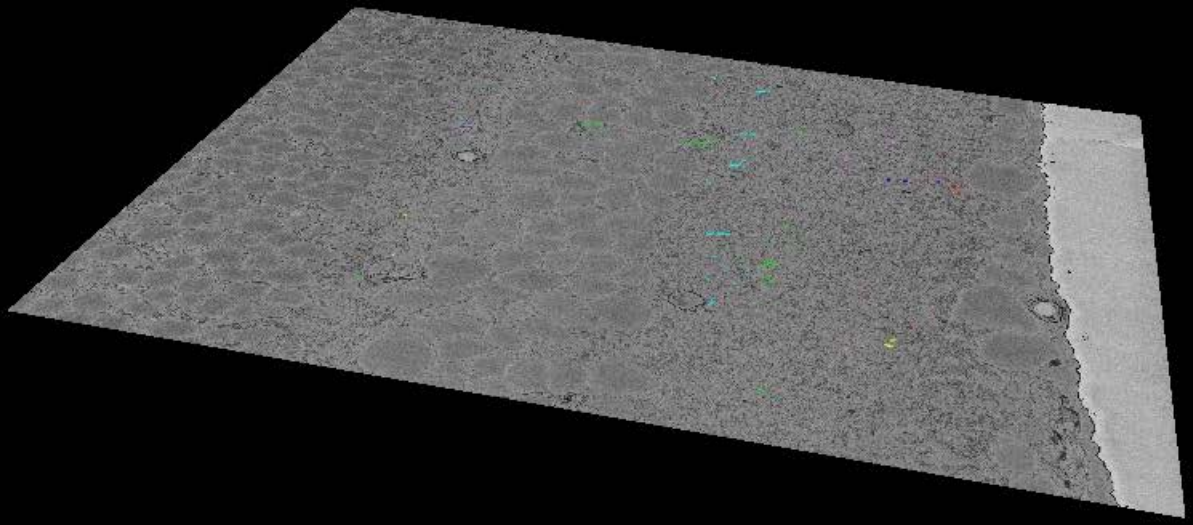
The mammalian retina



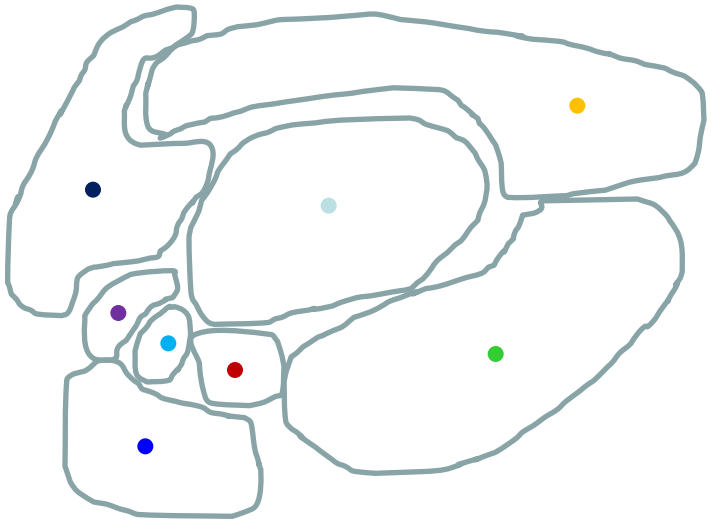


Masland (2001)

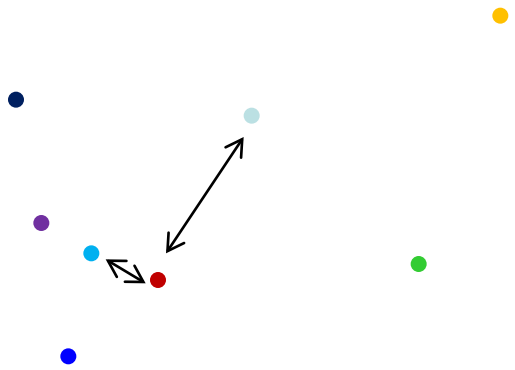




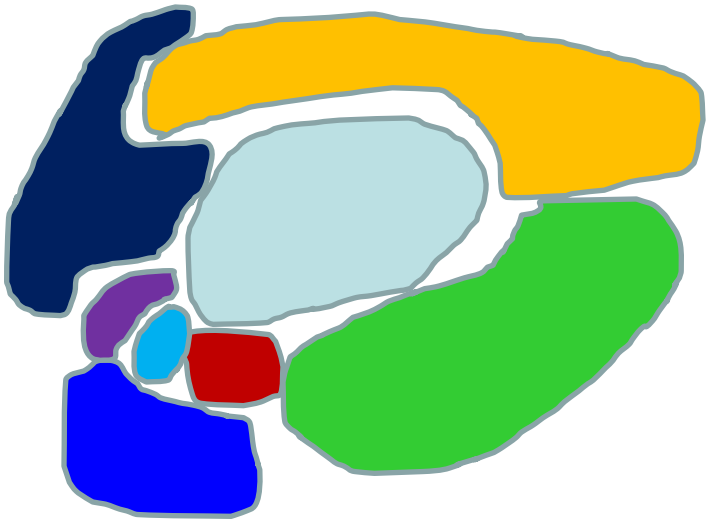
Skeleton annotation is insufficient for contact detection

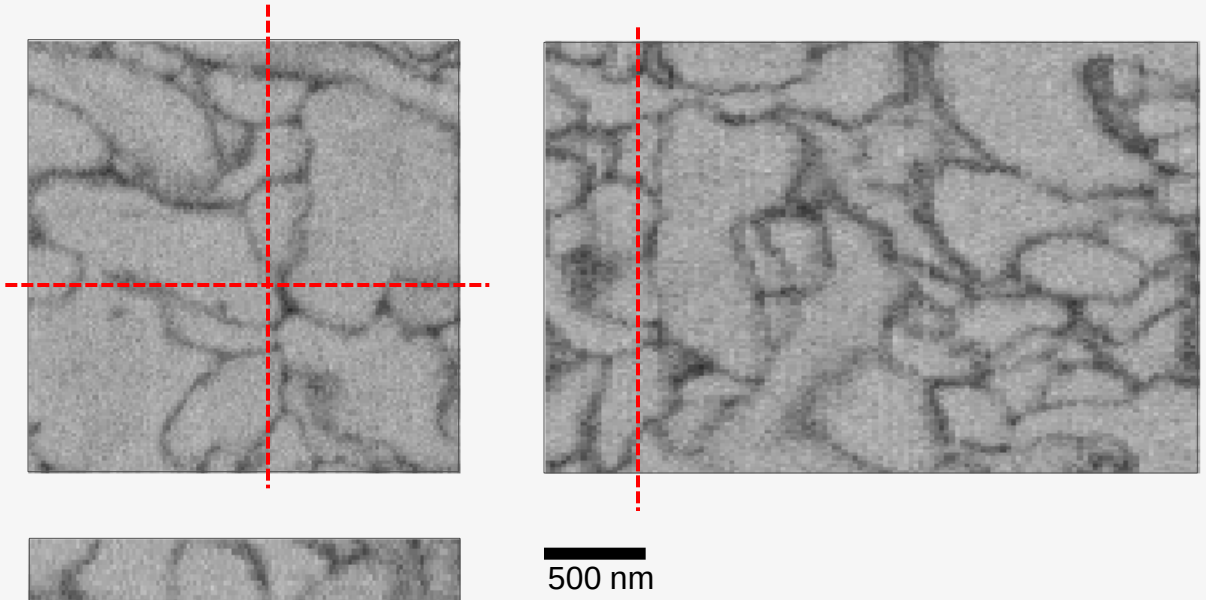


Skeleton annotation is insufficient for contact detection

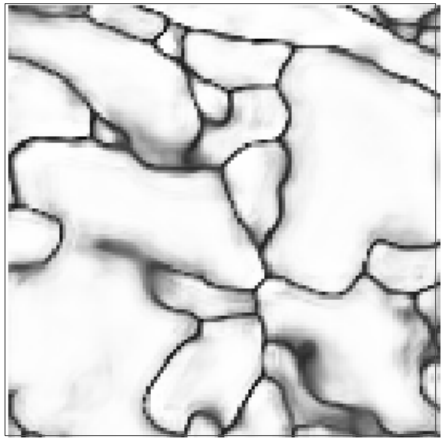


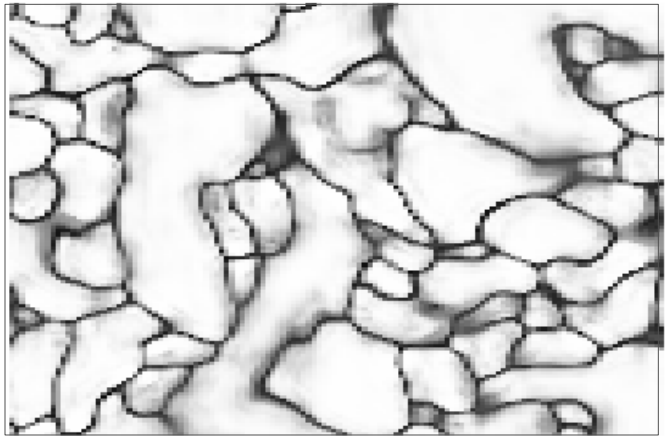
Skeleton annotation insufficient for contact detection:
“growing out“ volumes required

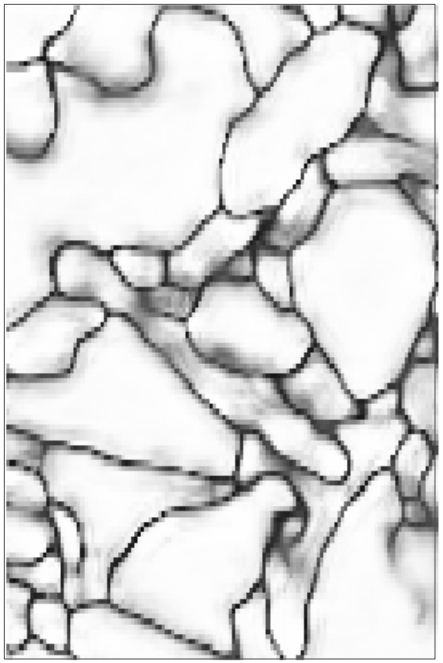


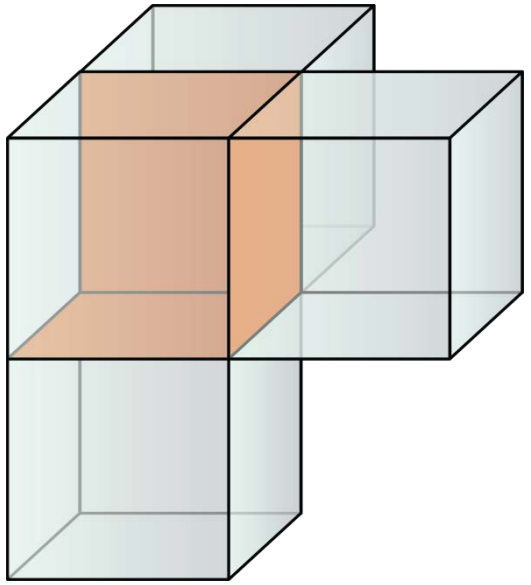

500 nm

Convolutional-Neural-Network Voxel-Connectivity Classifier



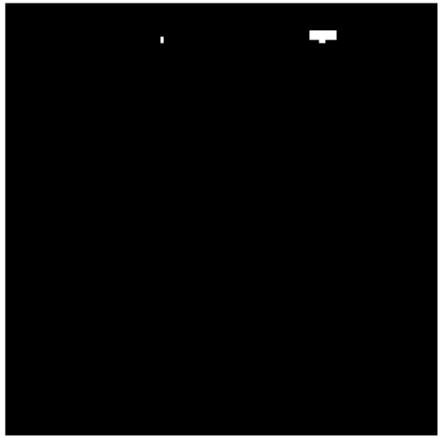


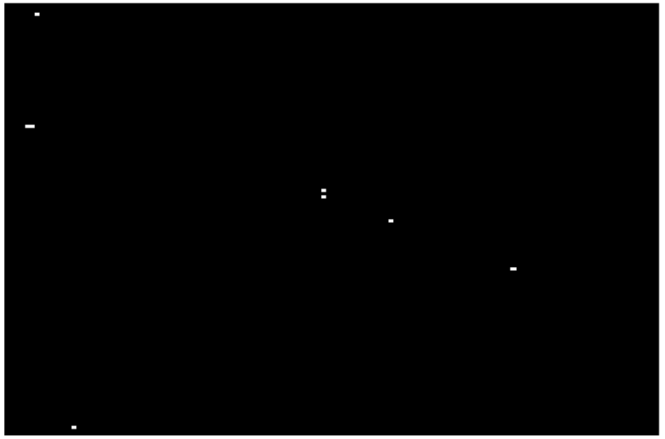


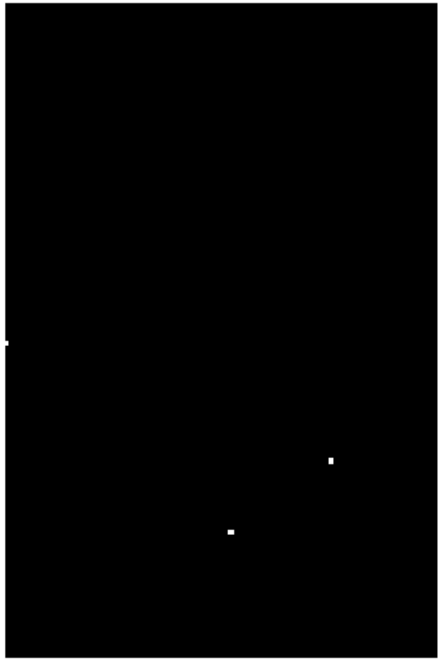


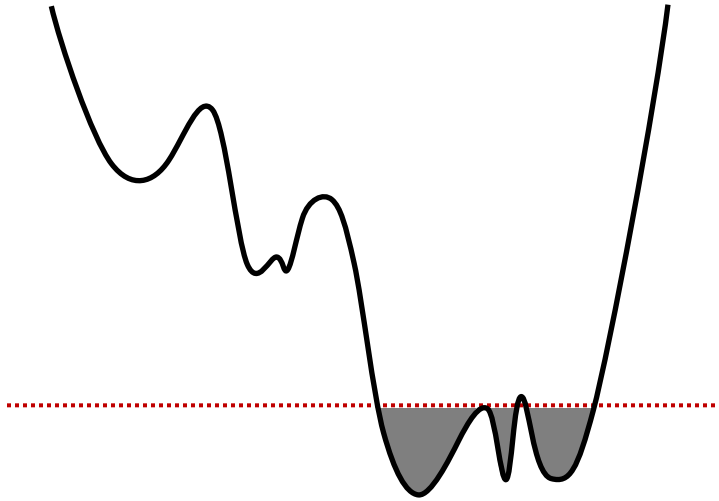
with Srini Turaga, Viren Jain, Sebastian Seung (MIT)

Initial seed generation at a high threshold

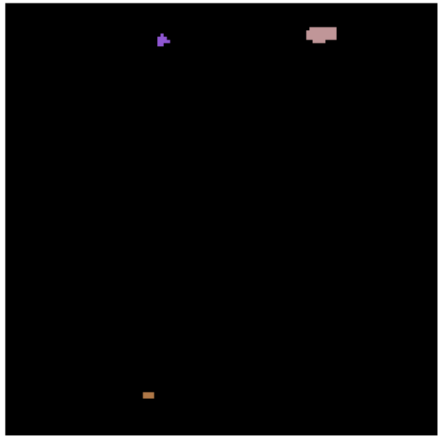


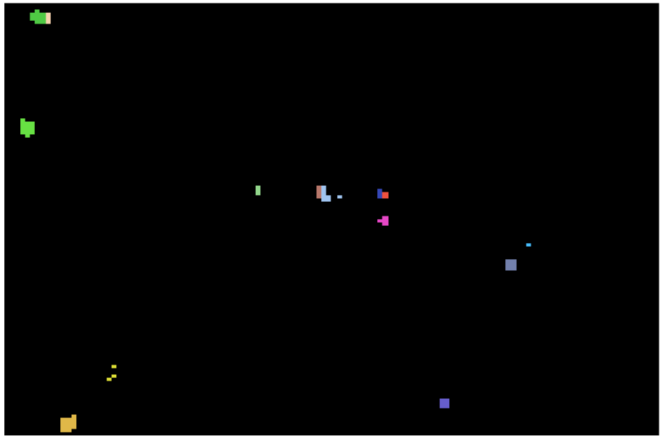


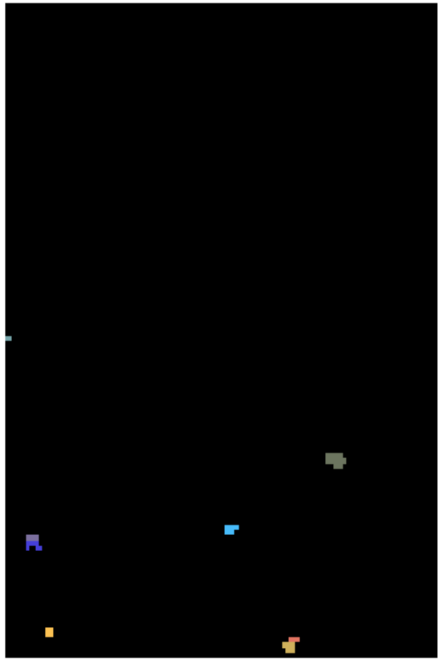


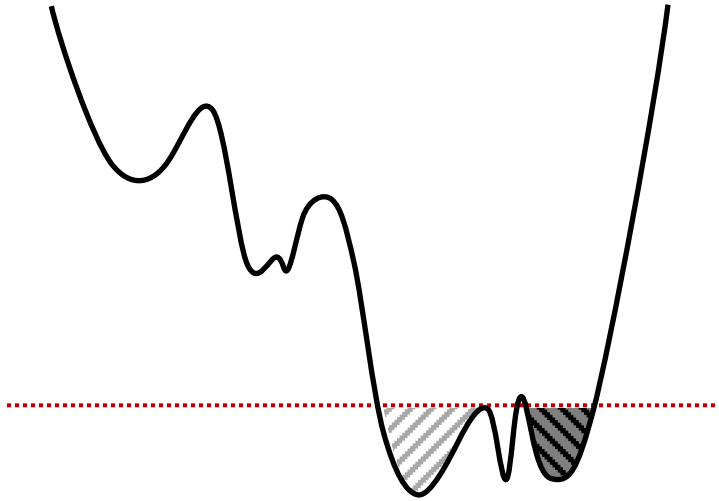


Initial seed generation at a high threshold

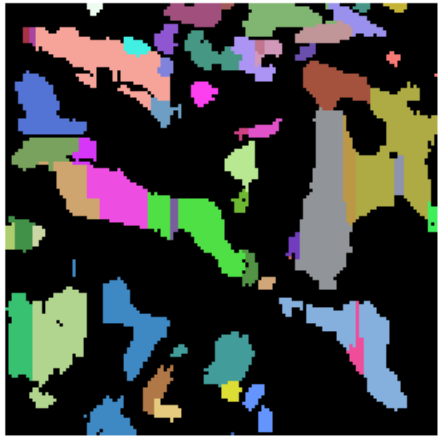


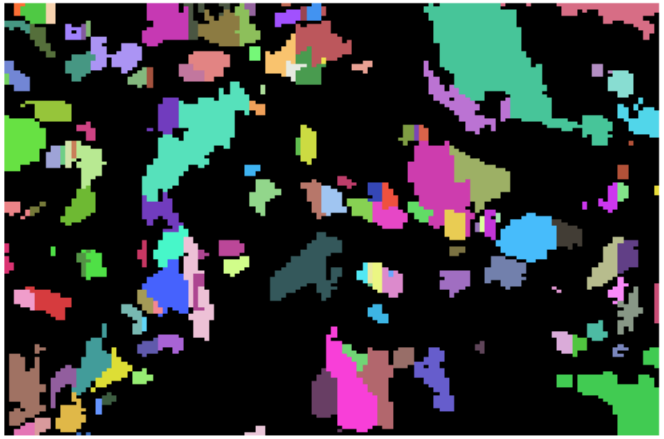


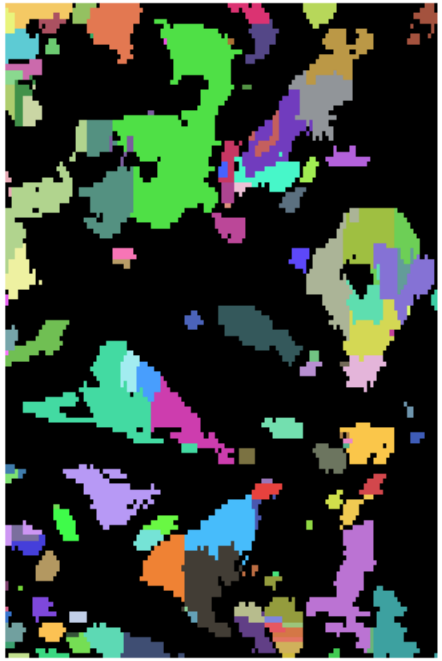


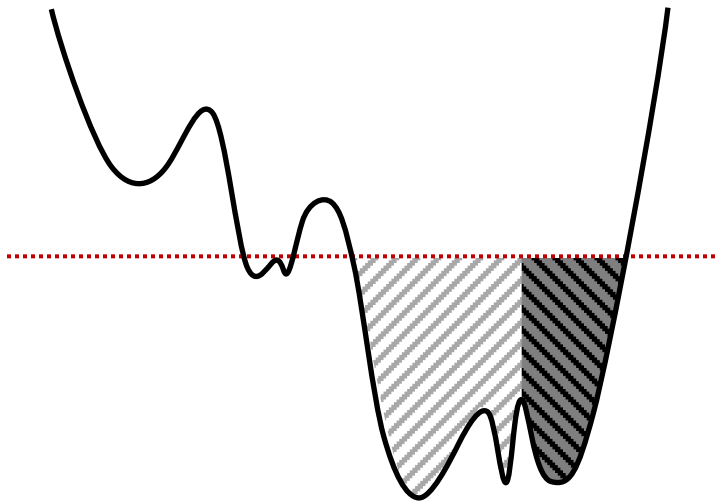


Growing objects

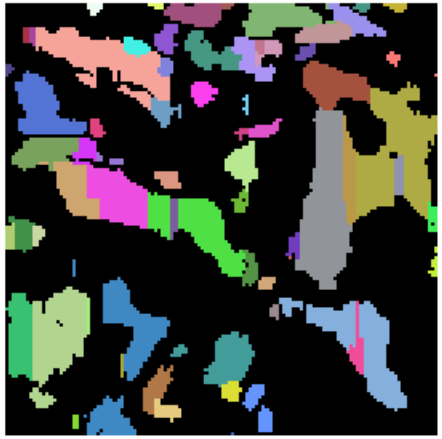


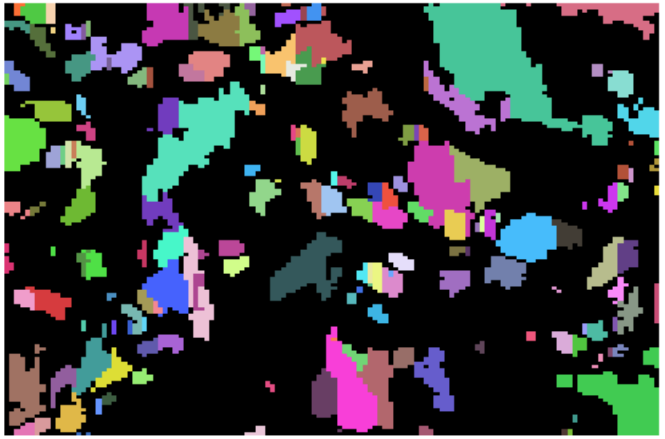


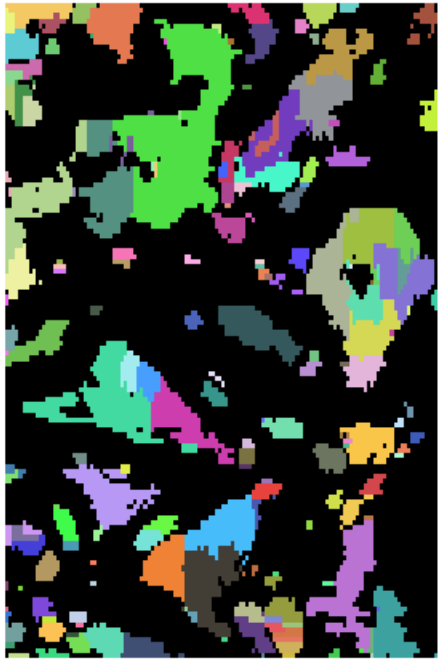


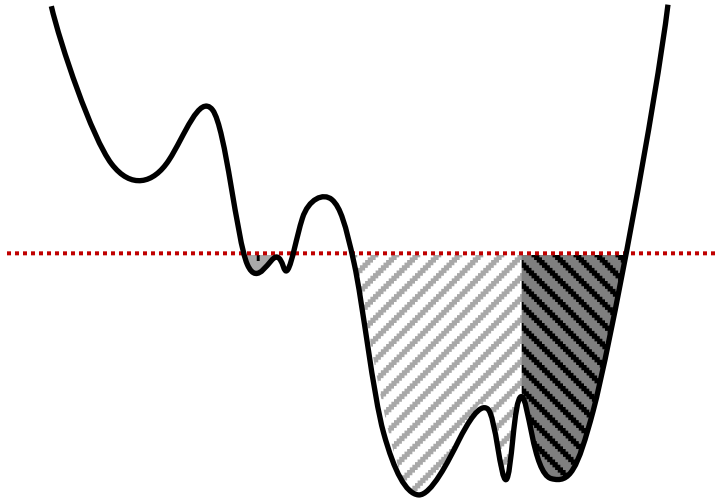


Re-seeding

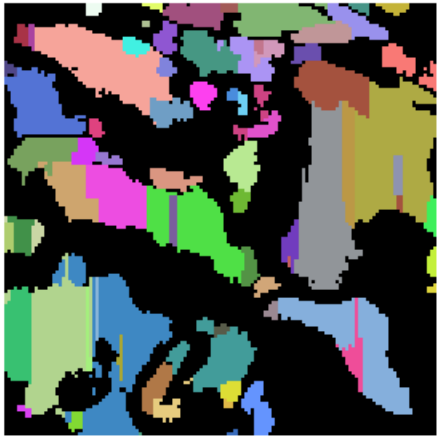


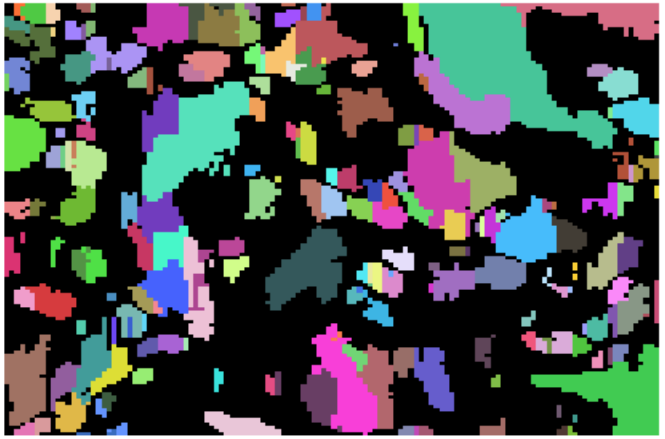


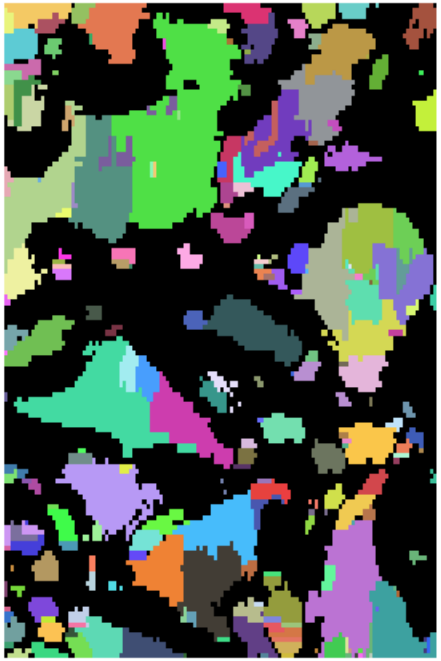


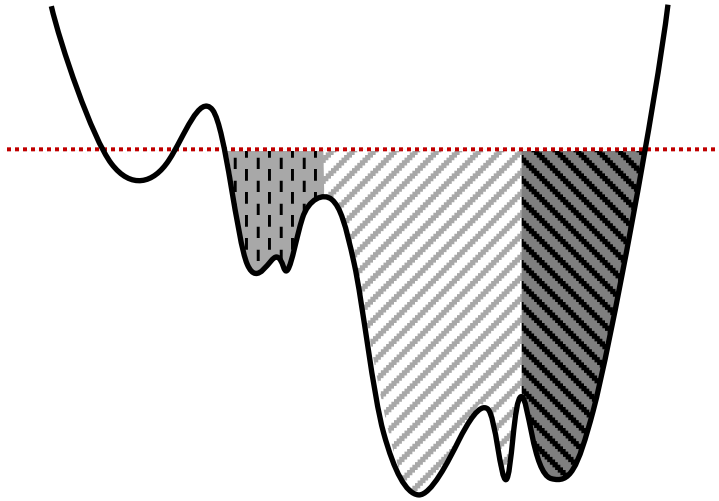


…Growing…

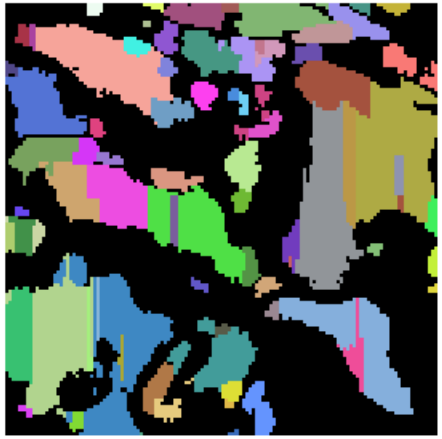


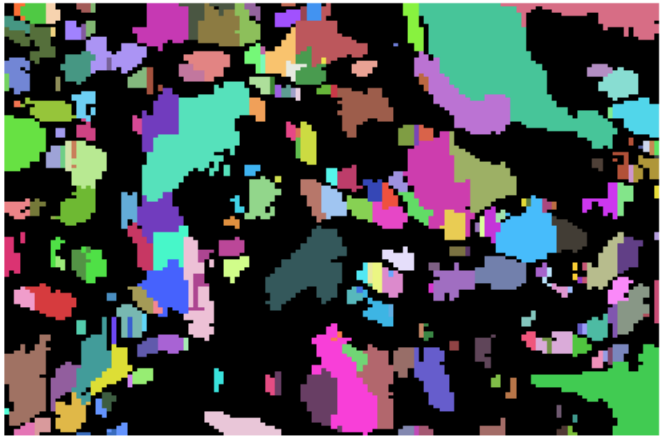


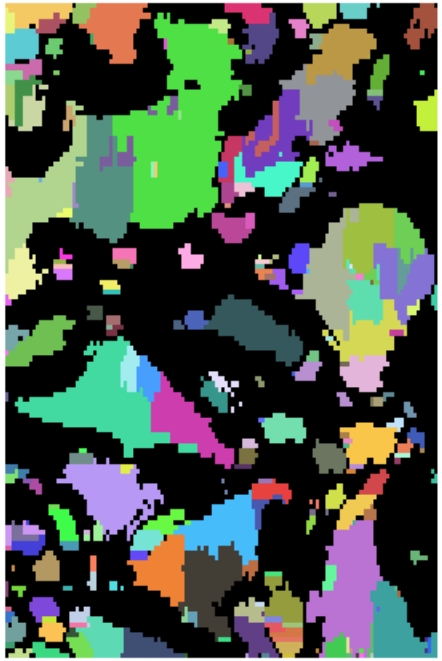


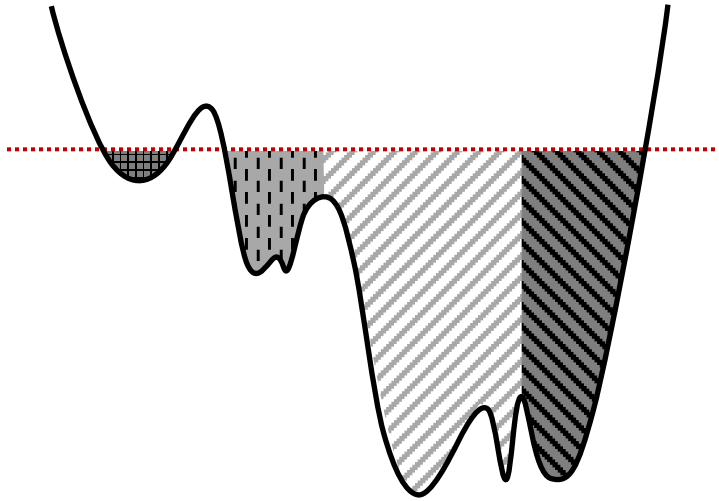


Re-seeding

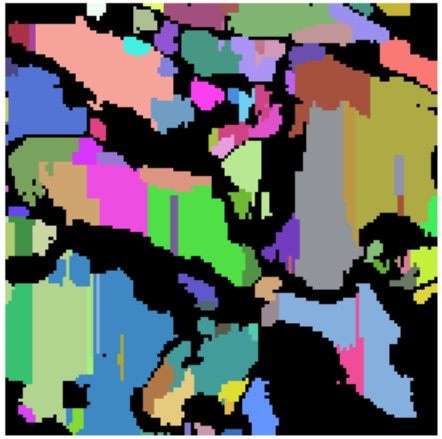


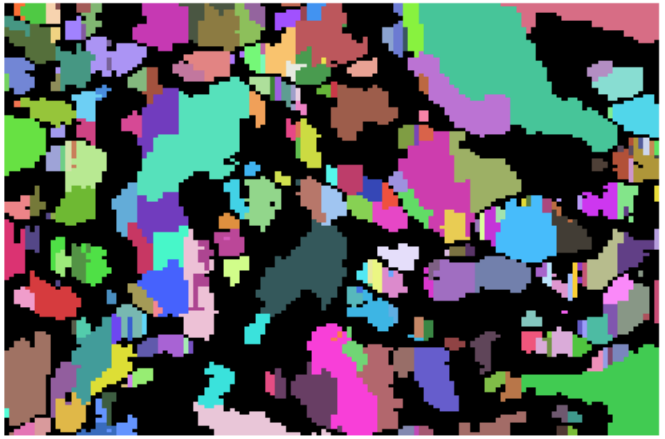


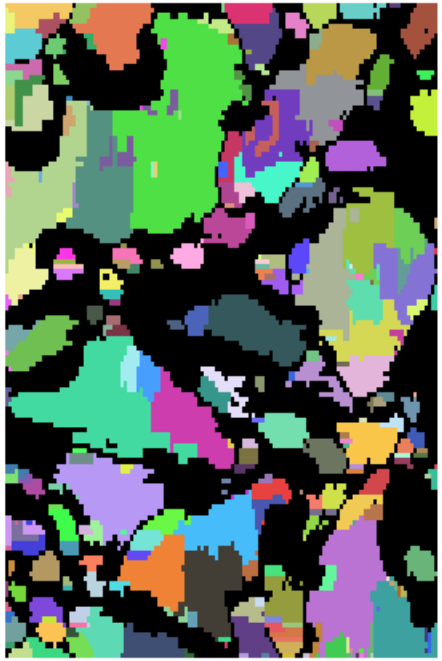


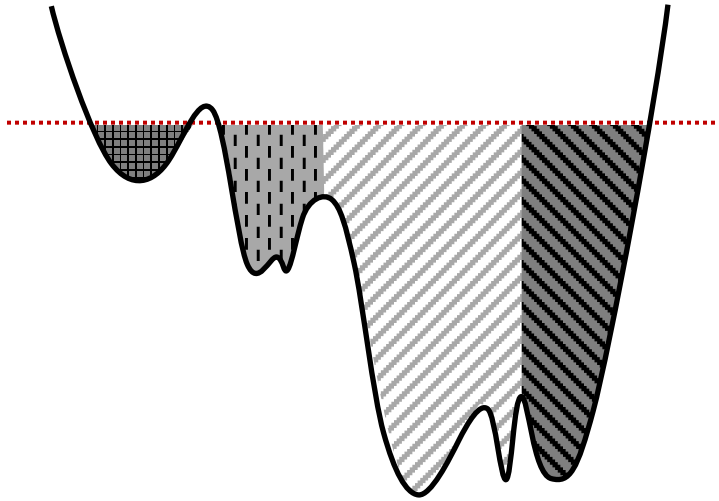


…Growing…

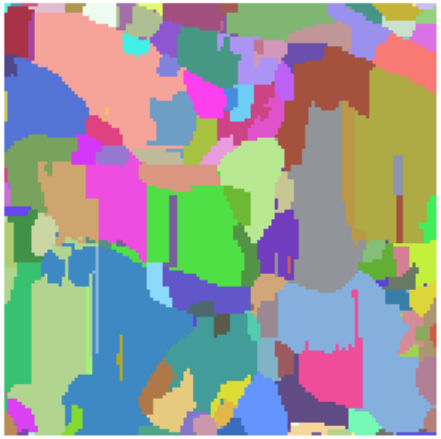


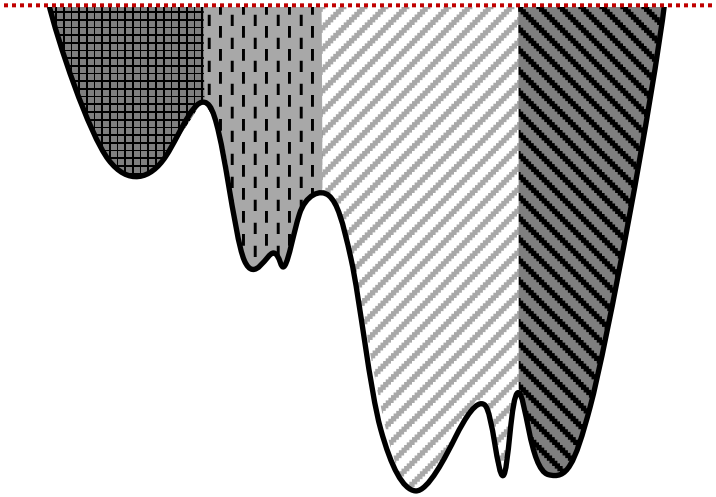


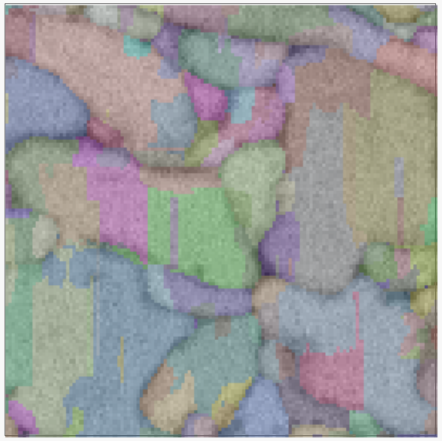


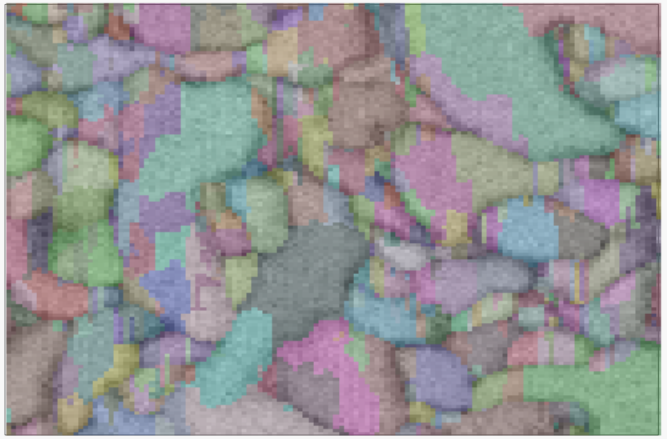


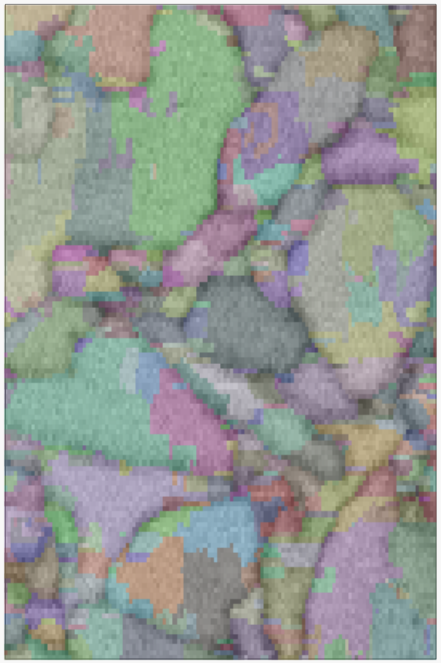
Grown to confluence



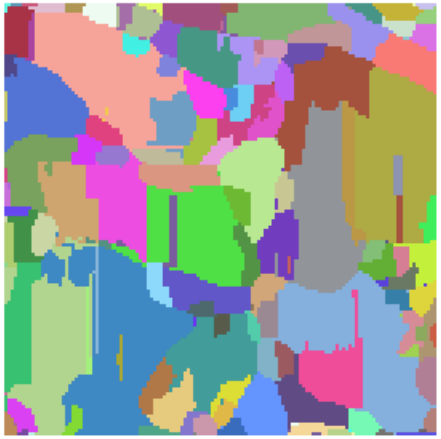


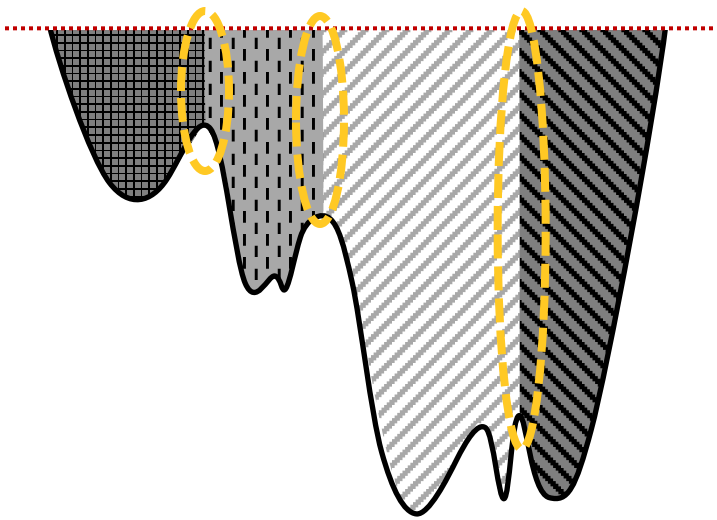




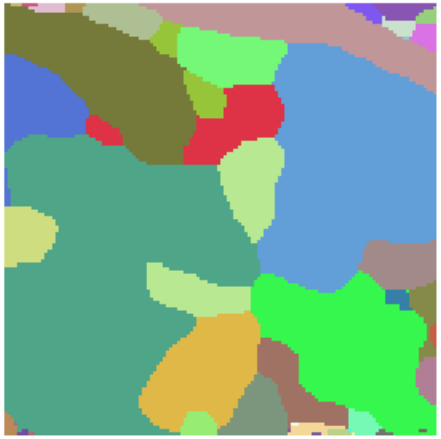


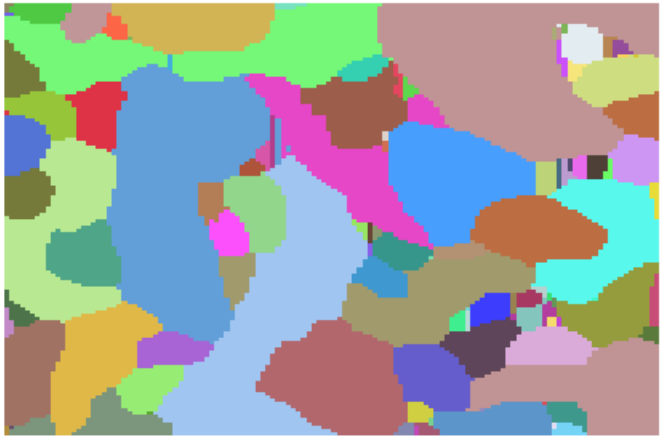
Examine contact areas

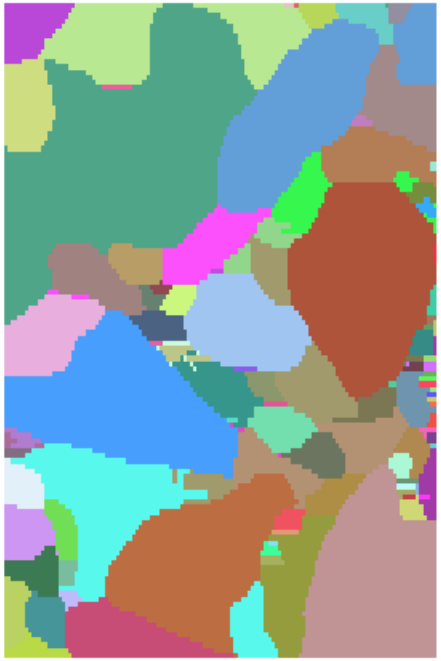


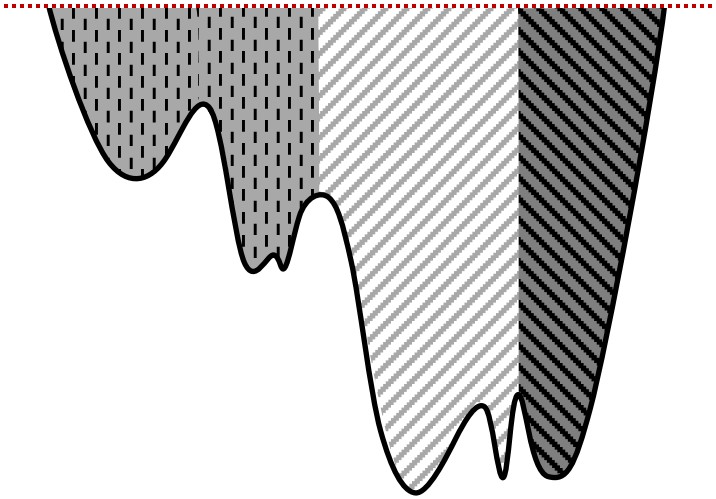


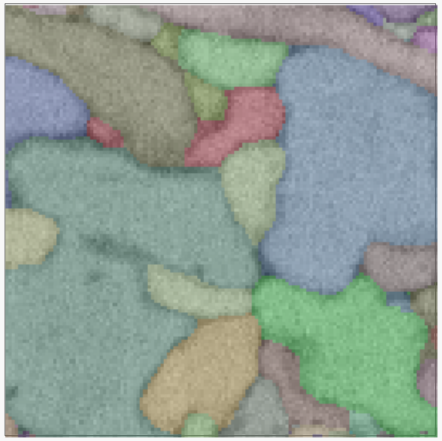
Merging (several iterations with different rules)

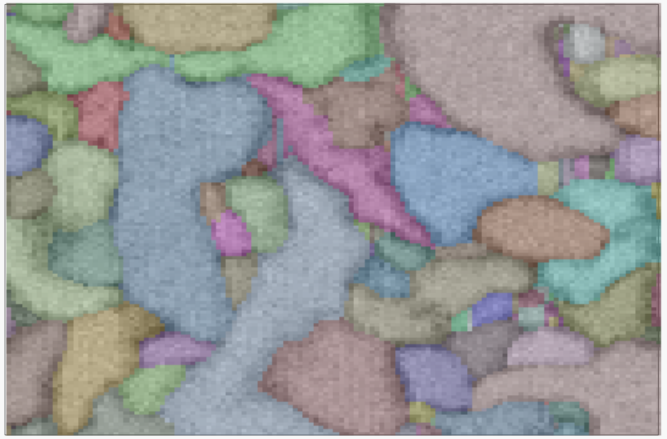


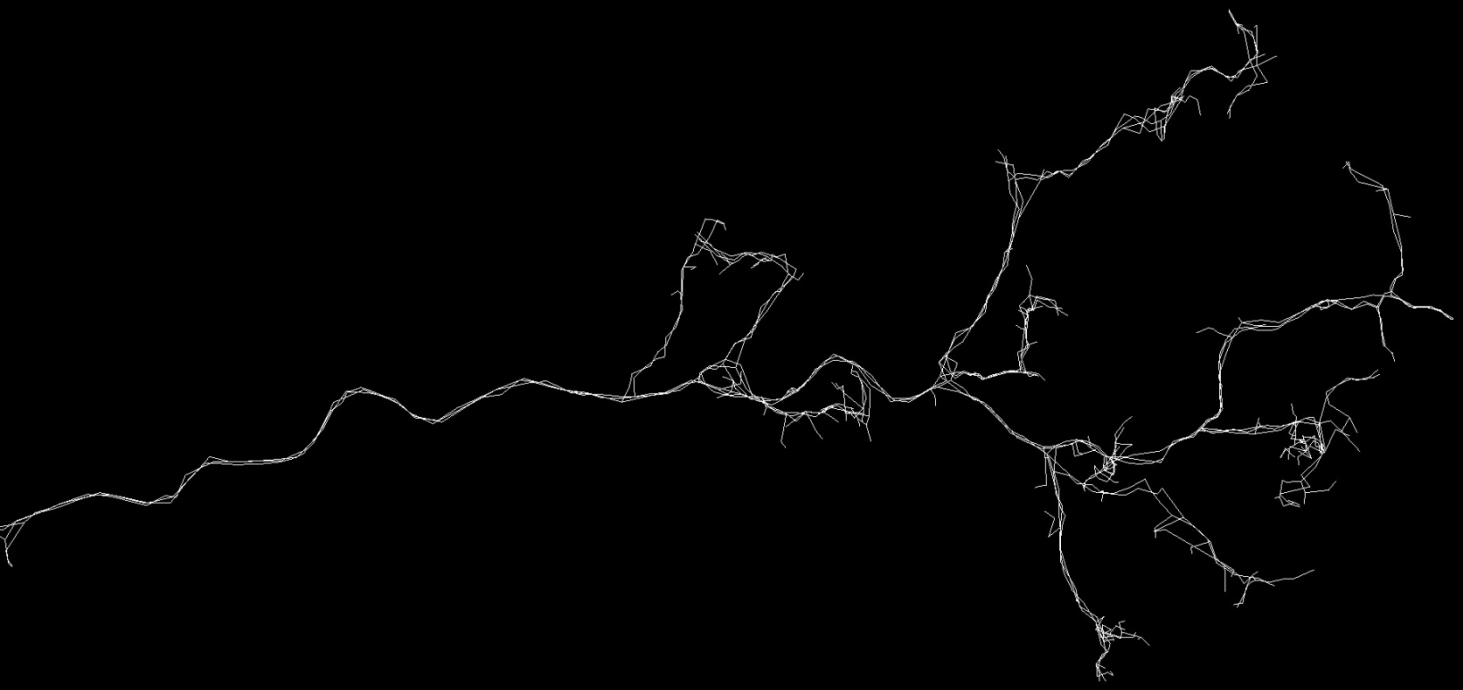


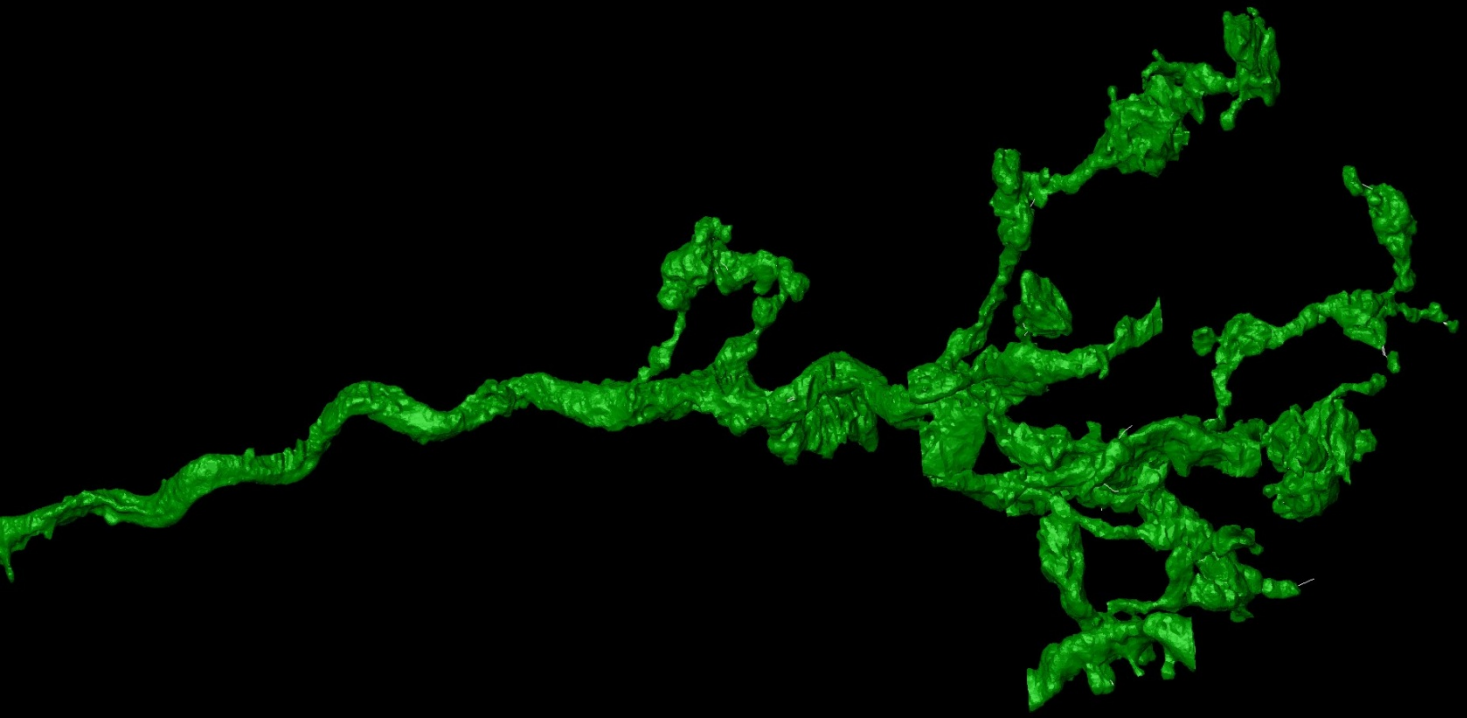


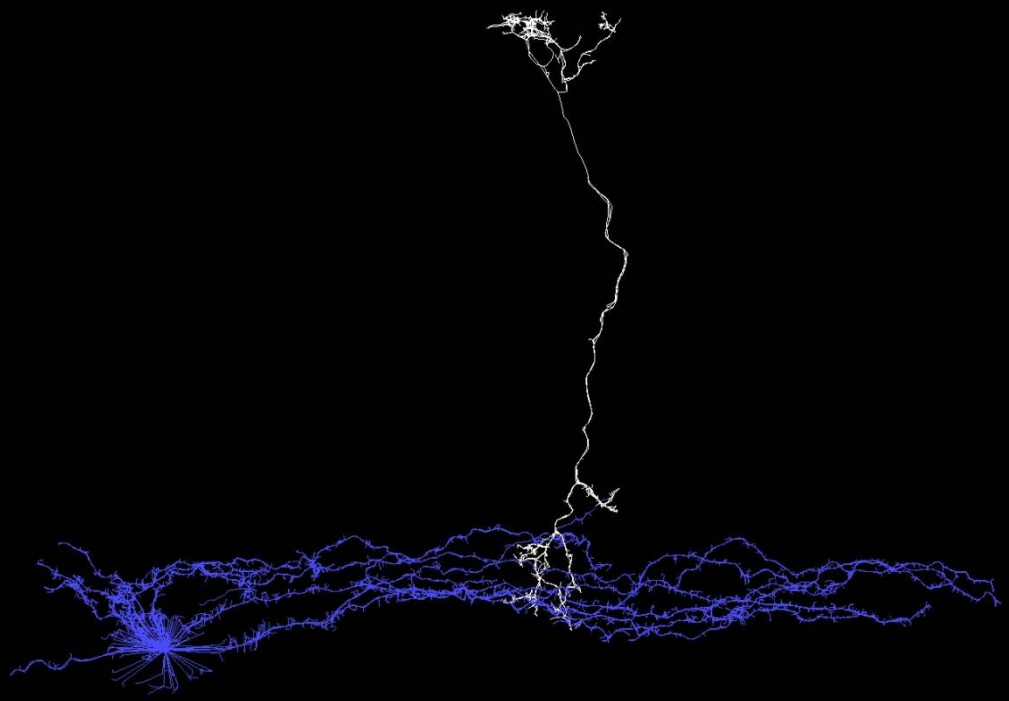


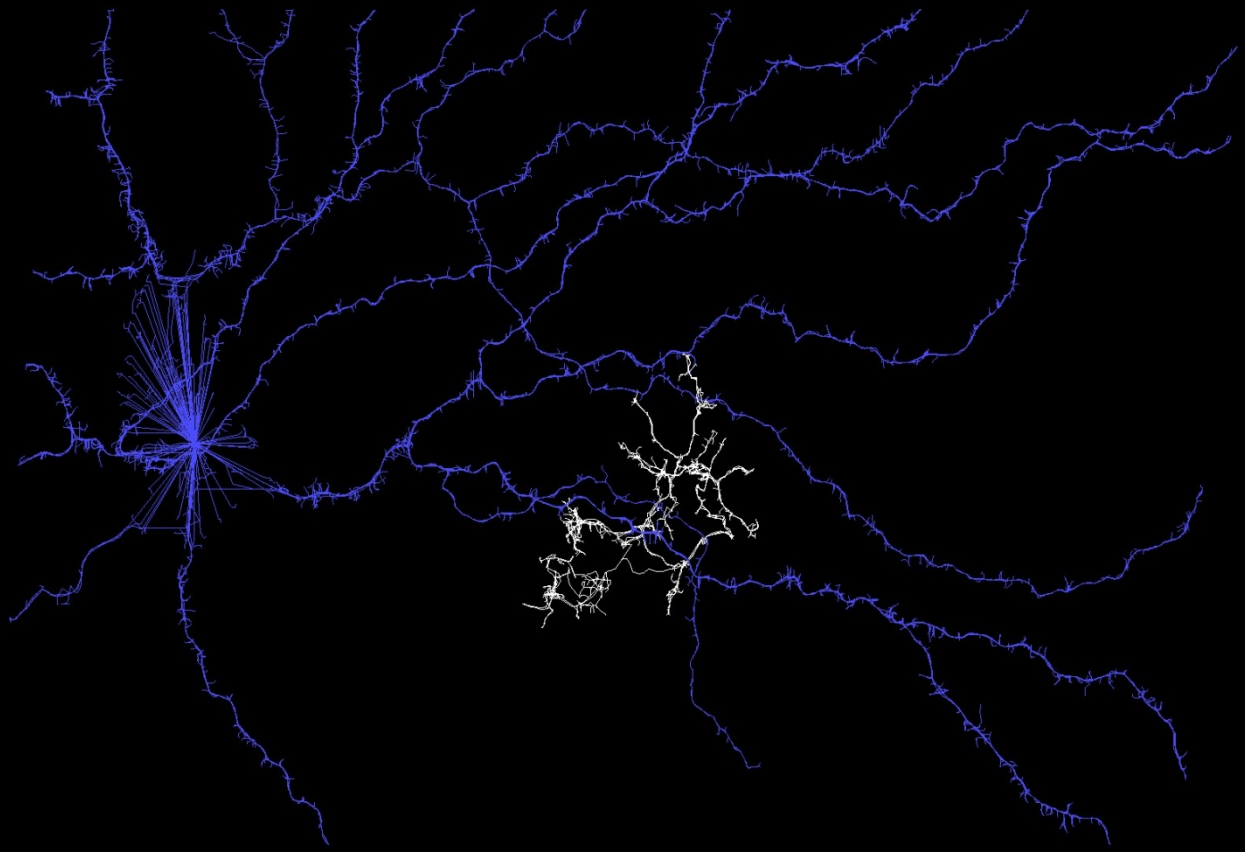


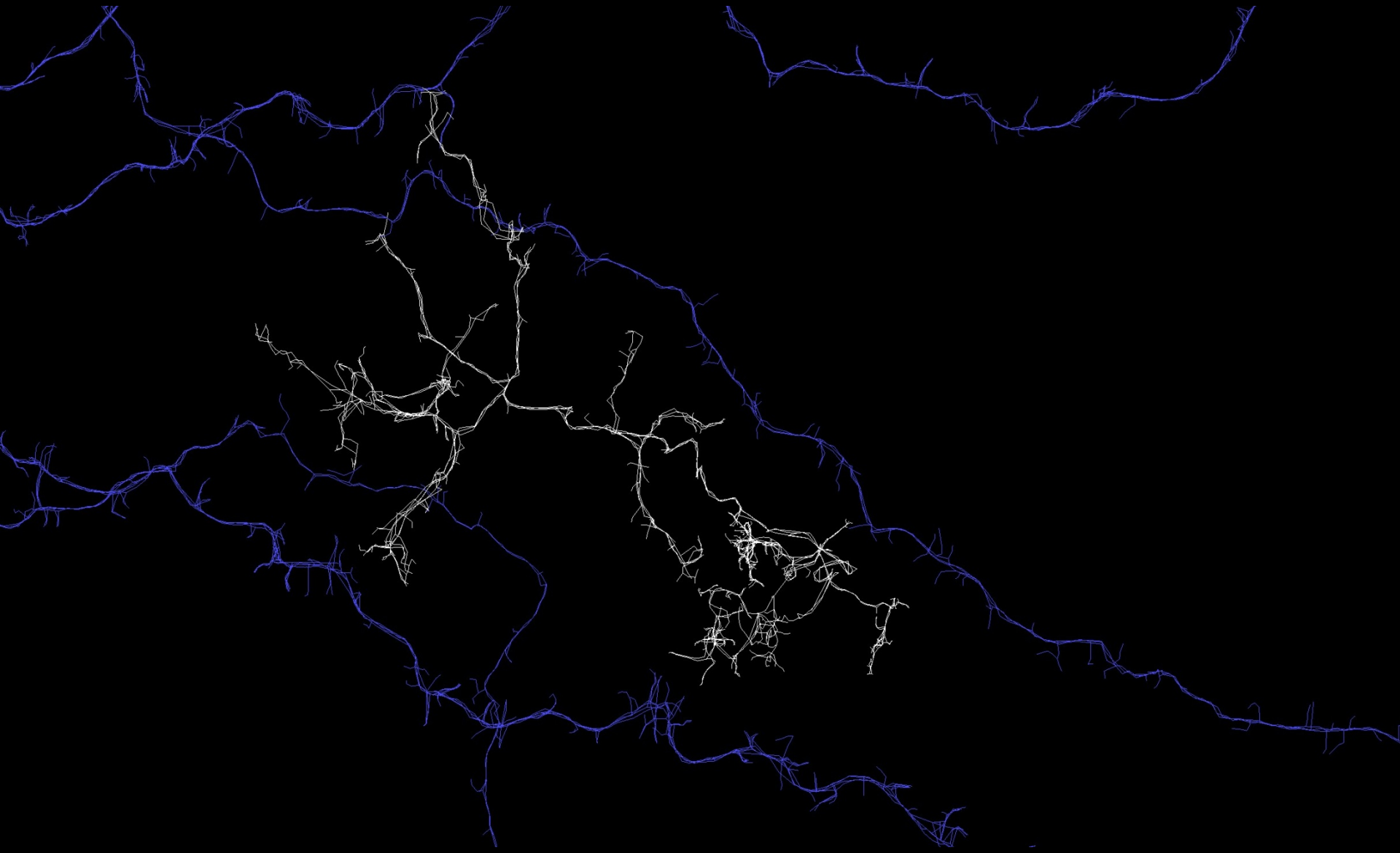


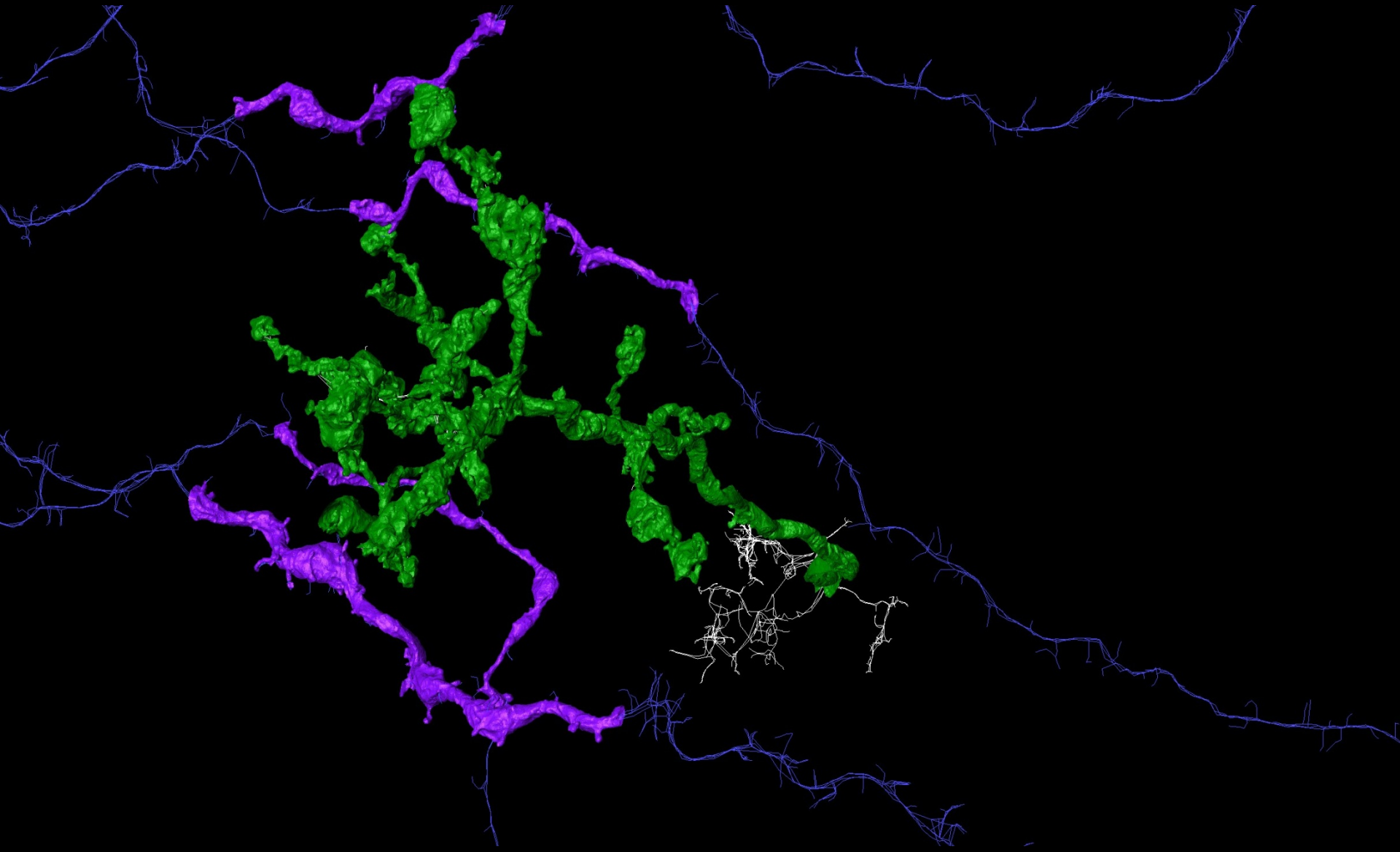


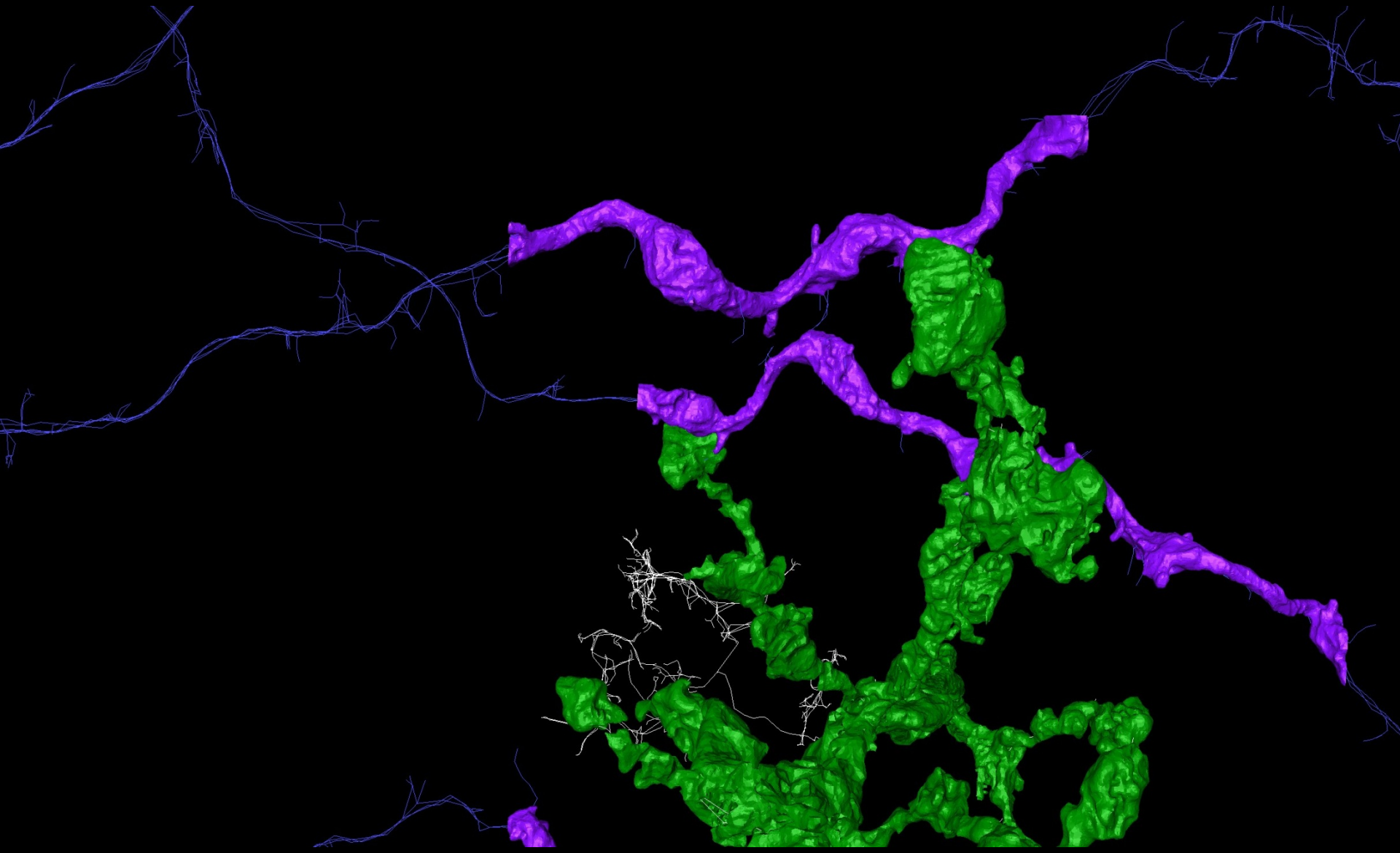


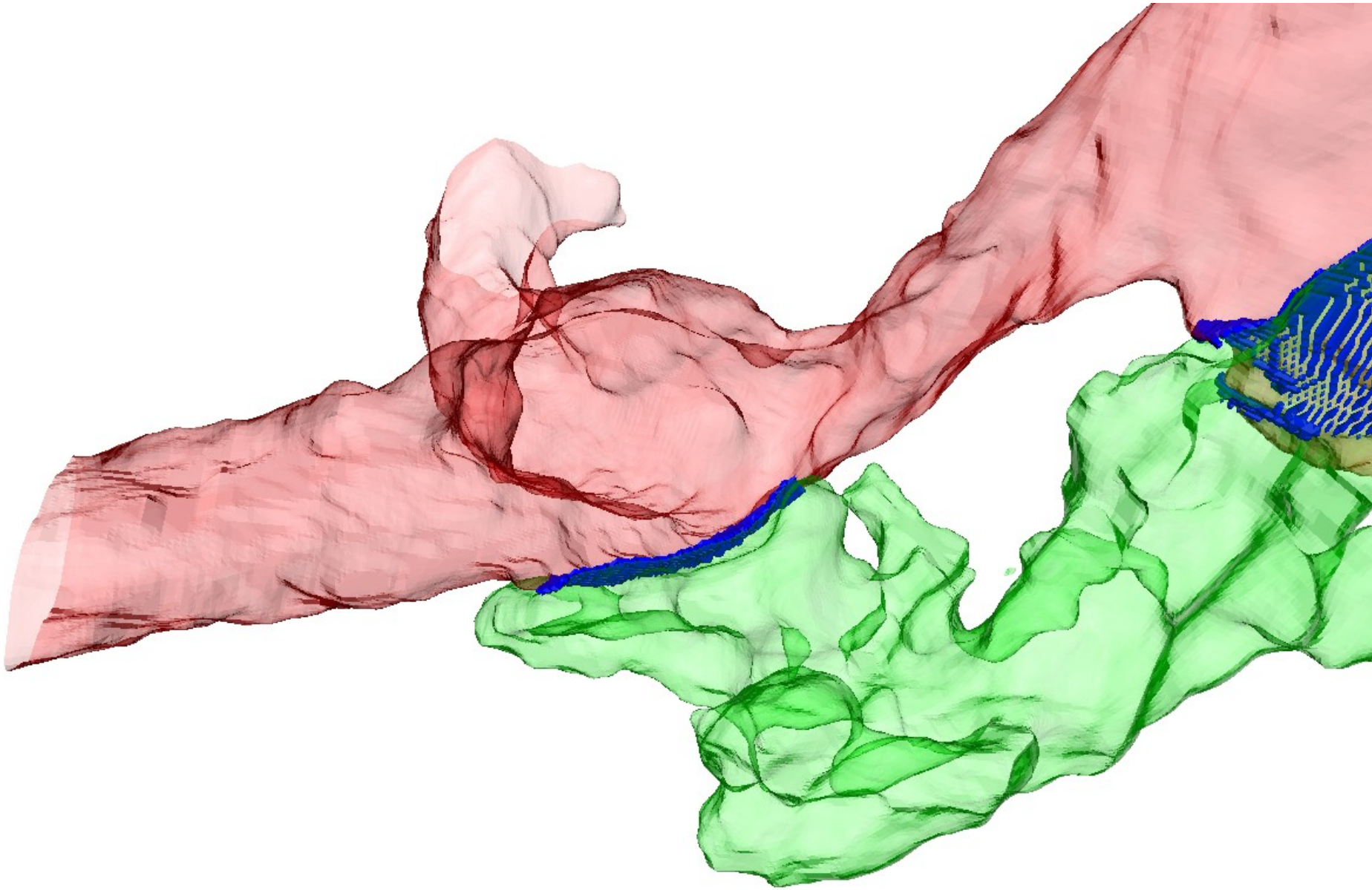


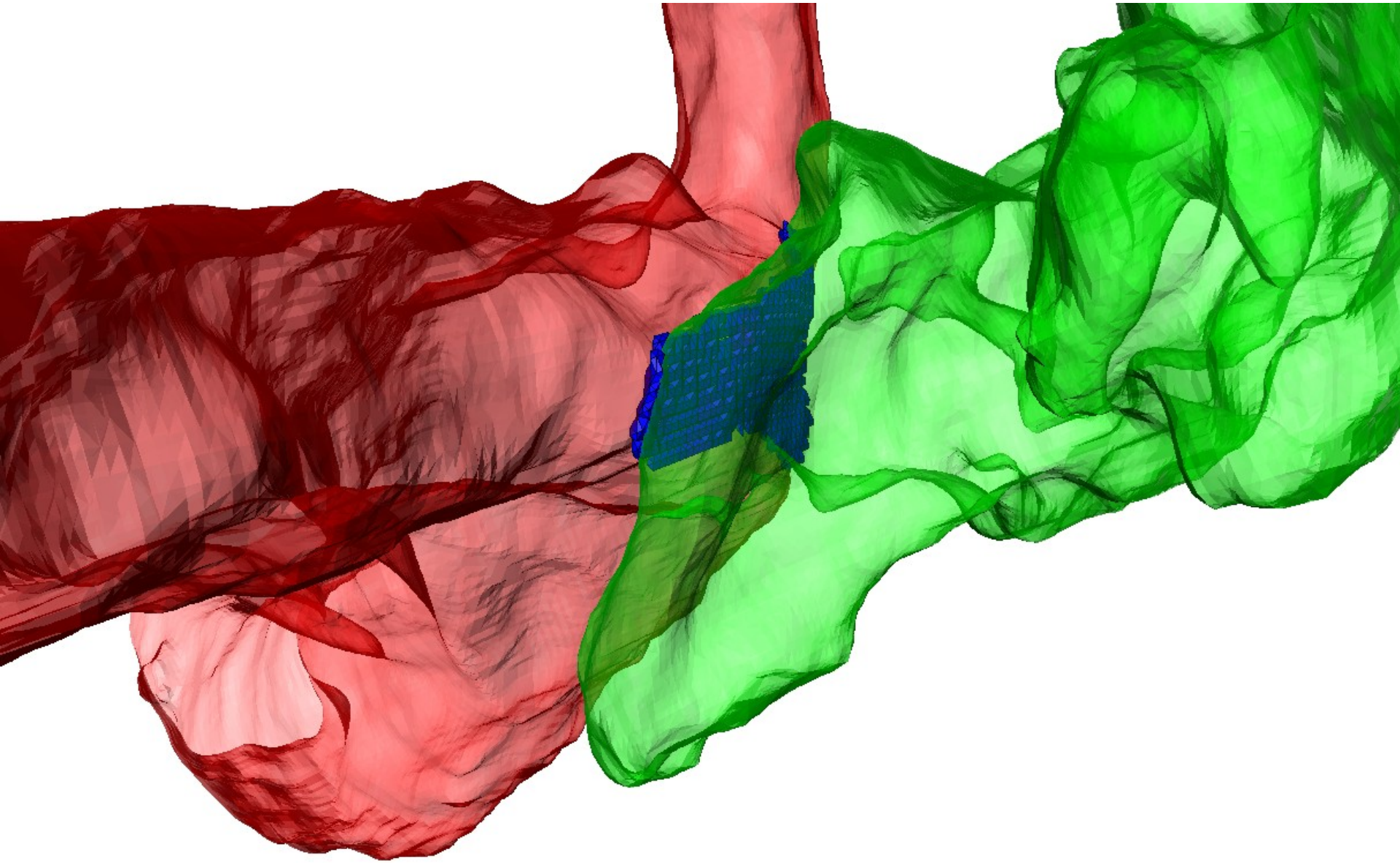




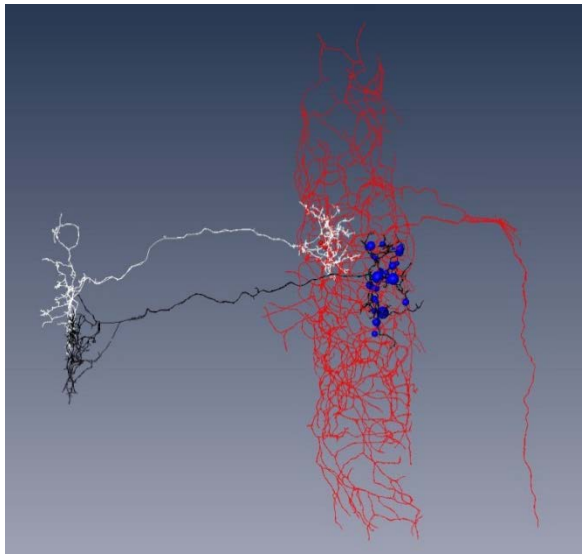








Dense reconstruction of mouse retina

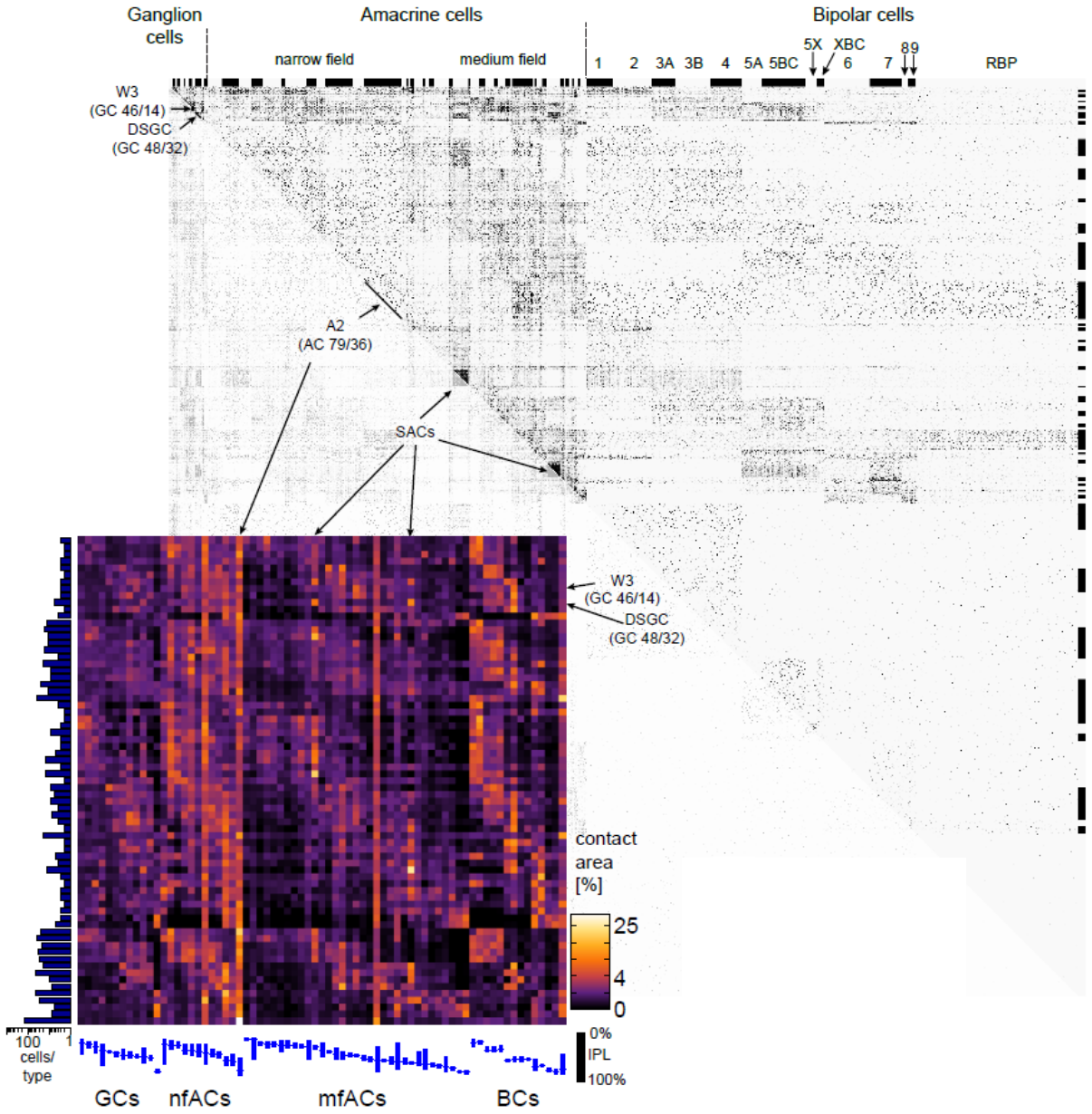


Zeroes rule out possible circuits

Defined cell-types by connectivity

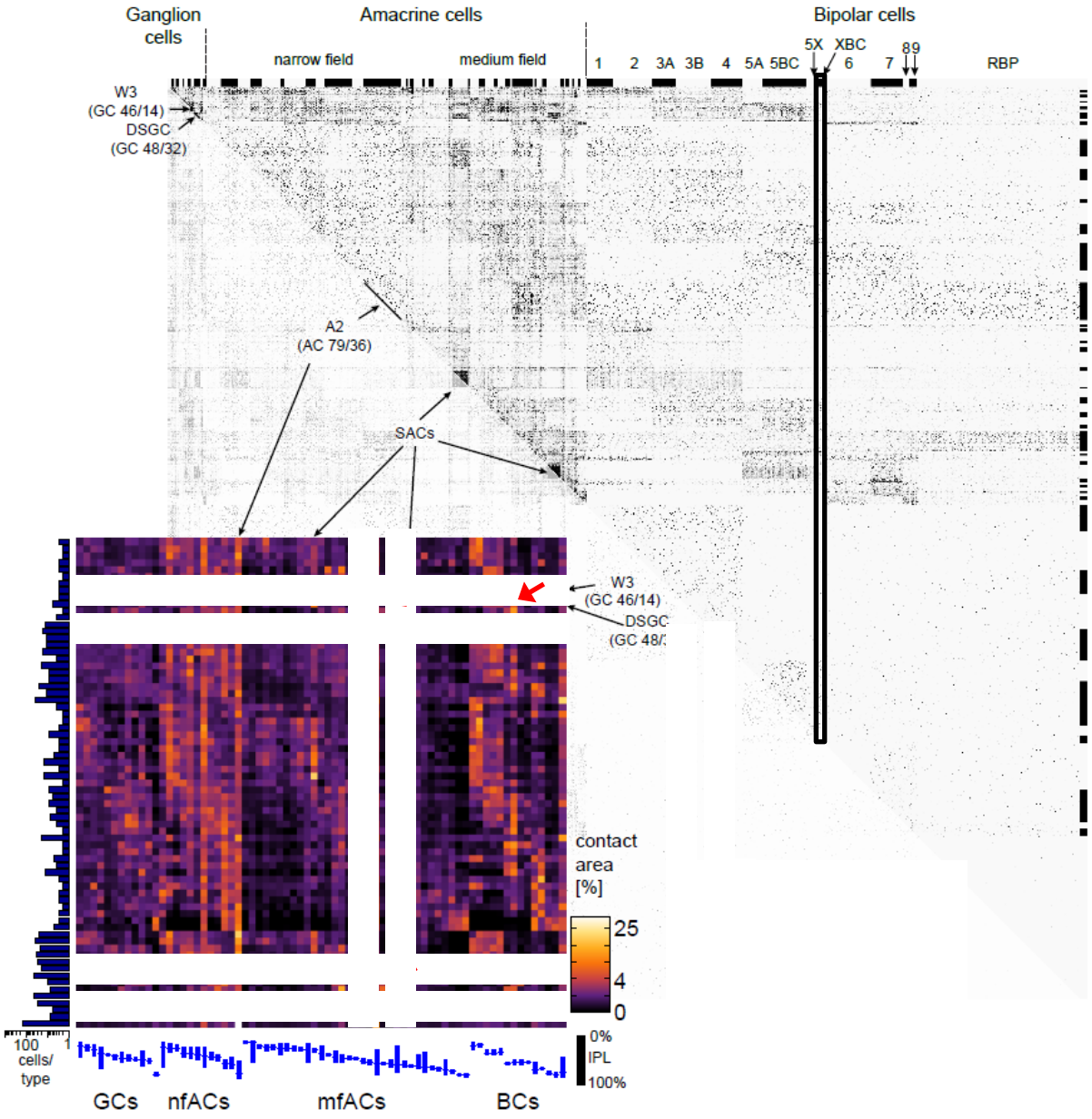
Identified novel cell types

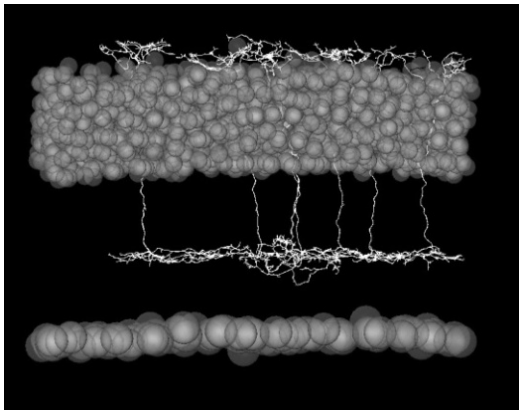
Proposed novel circuits

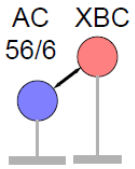


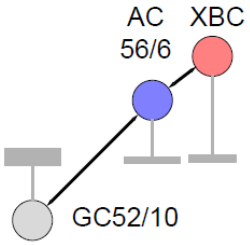
Helmstaedter M, Briggman KL, Turaga SC, Jain V, Seung HS, Denk W. Connectomic reconstruction of the inner plexiform layer in the mouse retina. Submitted

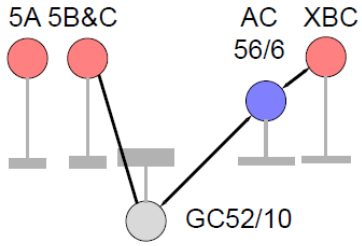
Proposed XBC circuits



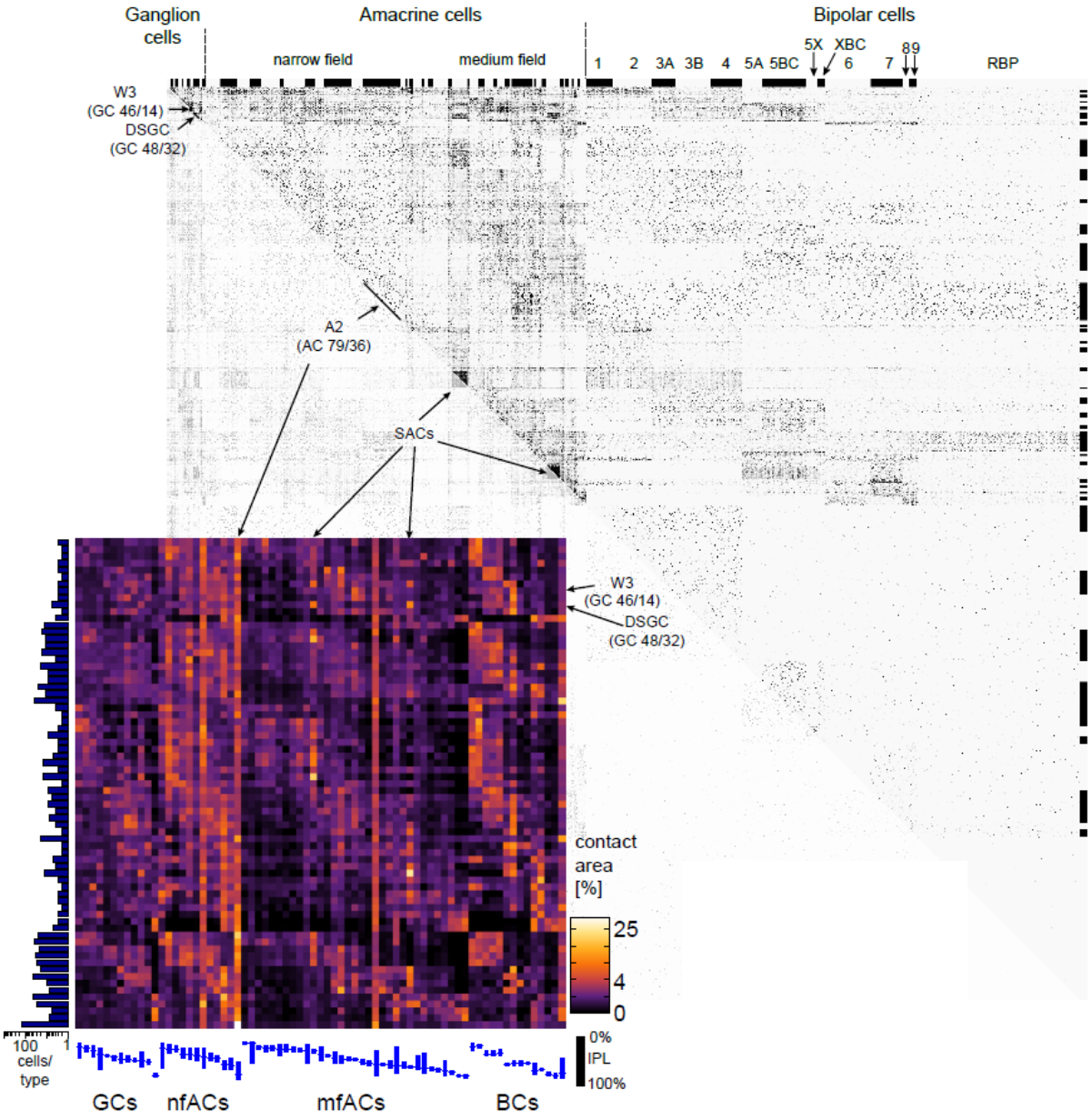


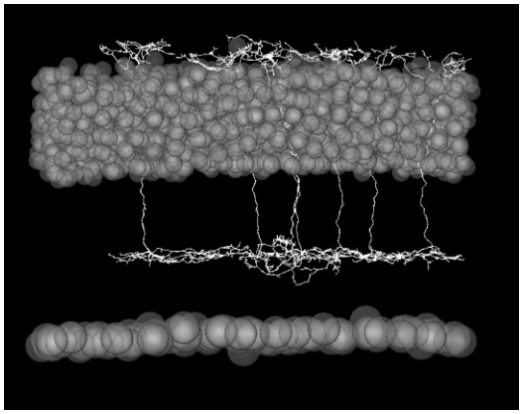


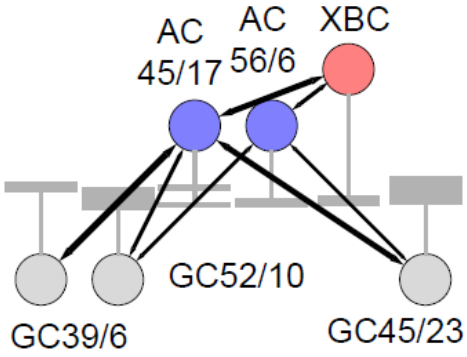




Proposed XBC circuits

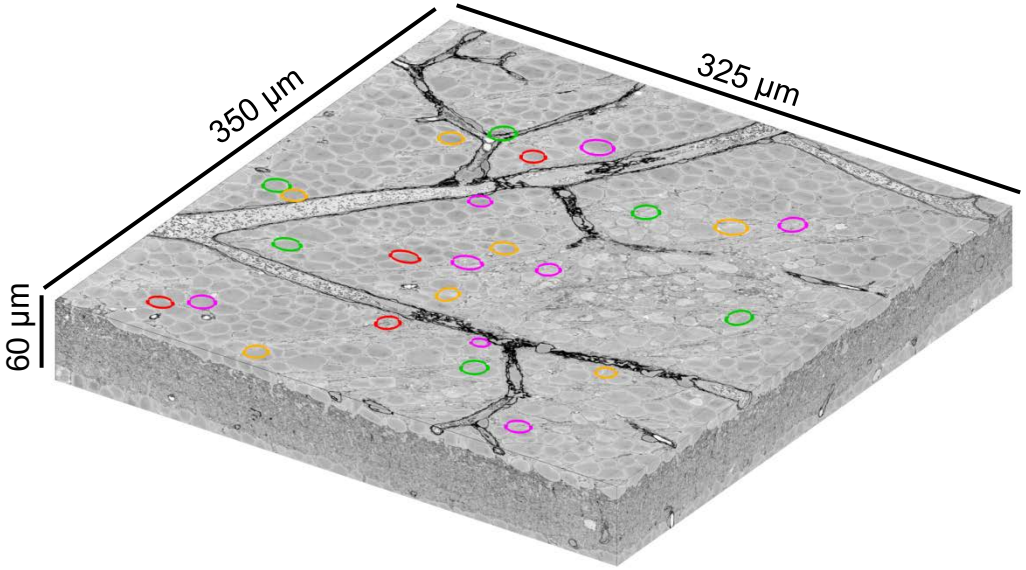




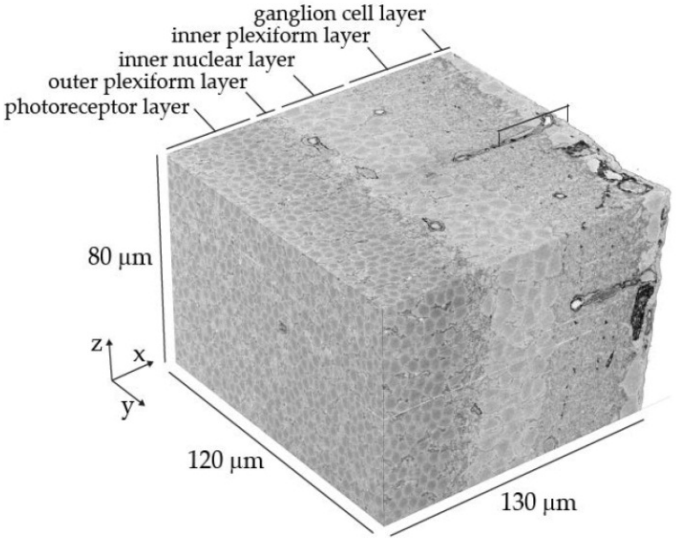


Neuronal circuit analysis of SBEM mouse retina data / are connectomes useful?

Sparse, targeted reconstruction
following 2-photon calcium imaging

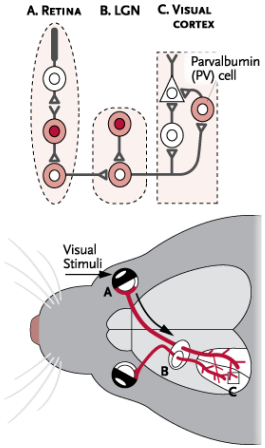


Dense, comprehensive reconstruction

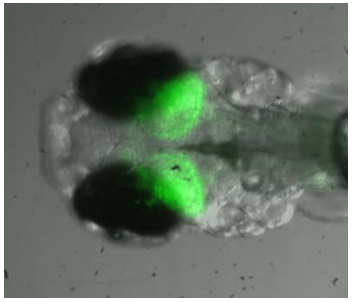


Dense, comprehensive reconstruction
following volume 2-photon calcium imaging

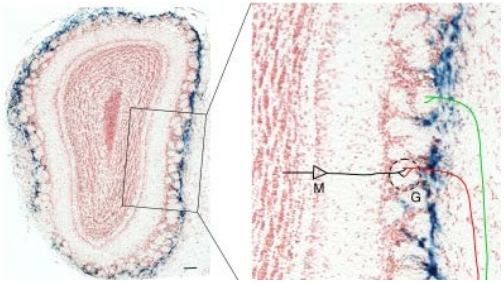
What we are working on now



1. Connectivity of retinal ganglion cell axons in the LGN and SC
   - combine long range axon tracing with locally dense EM reconstruction



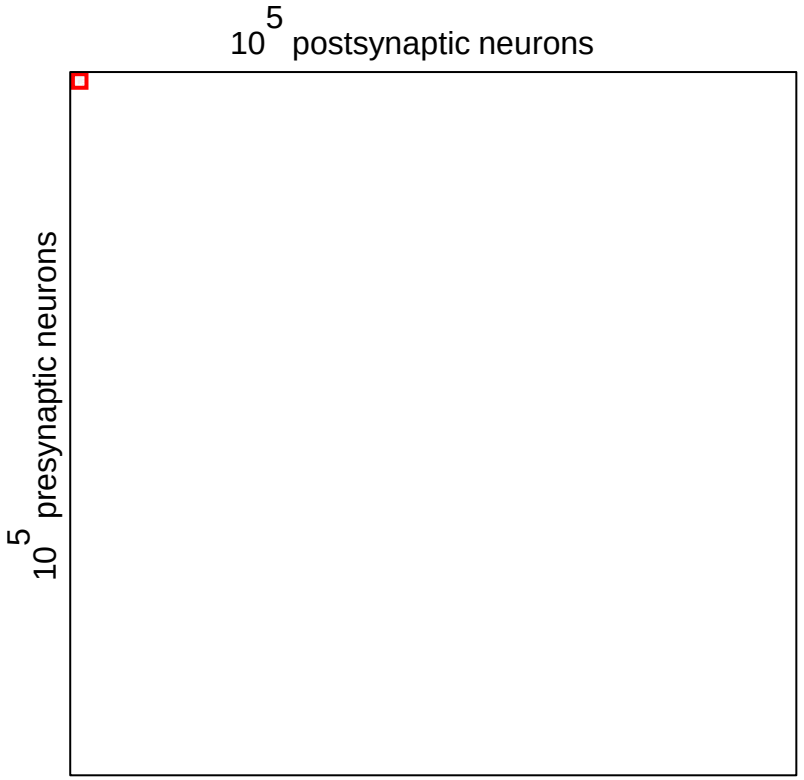
2. Functional imaging in the optic tectum with subsequent dense EM reconstruction
   - possible to acquire entire visual pathway

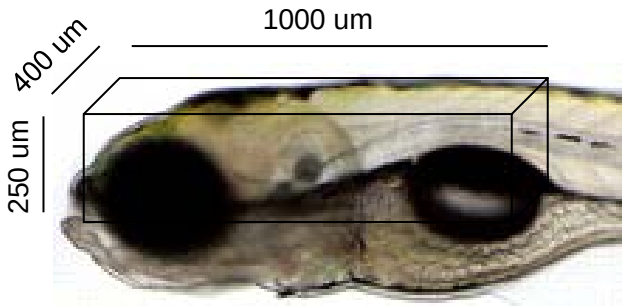


3. Olfactory bulb glomerulus dense EM reconstruction
   - possible to acquire olfactory ‘column’

Is a connectome of the larval zebrafish feasible?

Yes, but how long will it take?





Acquisition: 4-6 months, 60 x 10^6 µm^3

Analysis: 2 hrs / µm^3 (manual annotation)

= 57700 work years

Let's assume a 5 year goal:

11540 human annotators for 5 work years

115 human annotators for 5 work years

with a 100 speed-up using ML algorithms

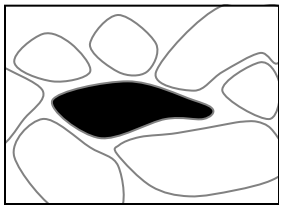
11540 times speed-up (~2.6 s / µm^3) using machine learning algorithms

How can we speed up analysis?

1. Make the data easier to analyze

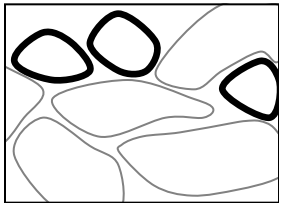
Staining strategies

Intracellular injection



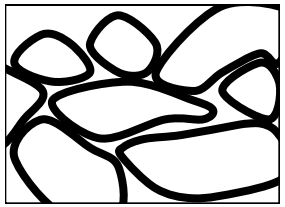
individual cells

Genetic targeting



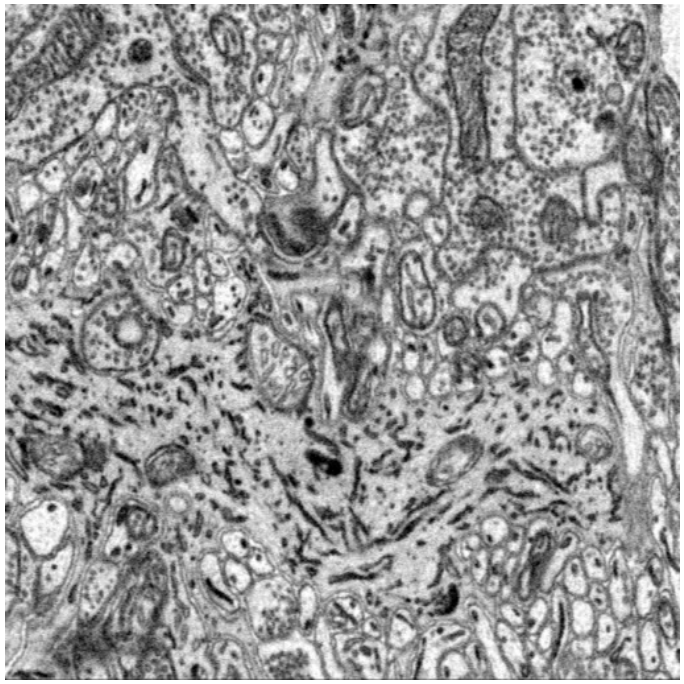
subpopulations

Surface labeling



full volumes

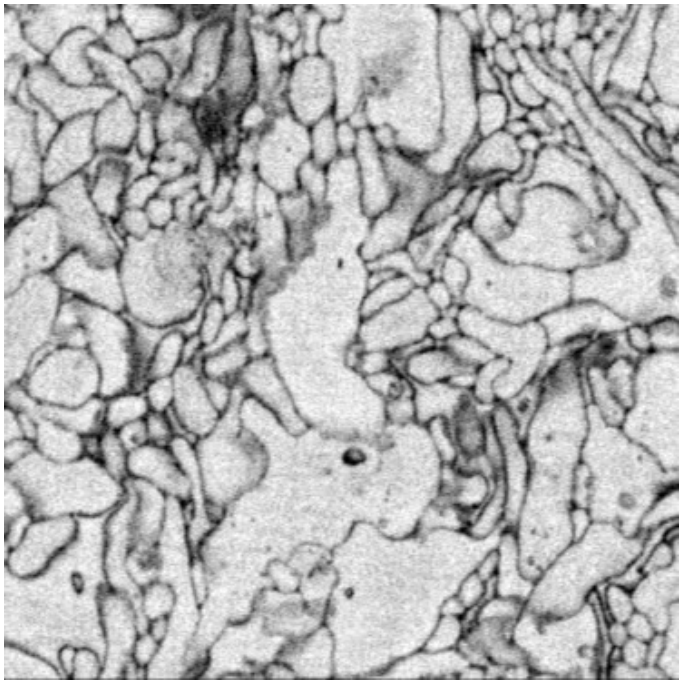
Conventional *en bloc* stains
OsO4, uranyl acetate, lead citrate



12 x 12 x 25 nm^3

1μm

HRP surface labeling

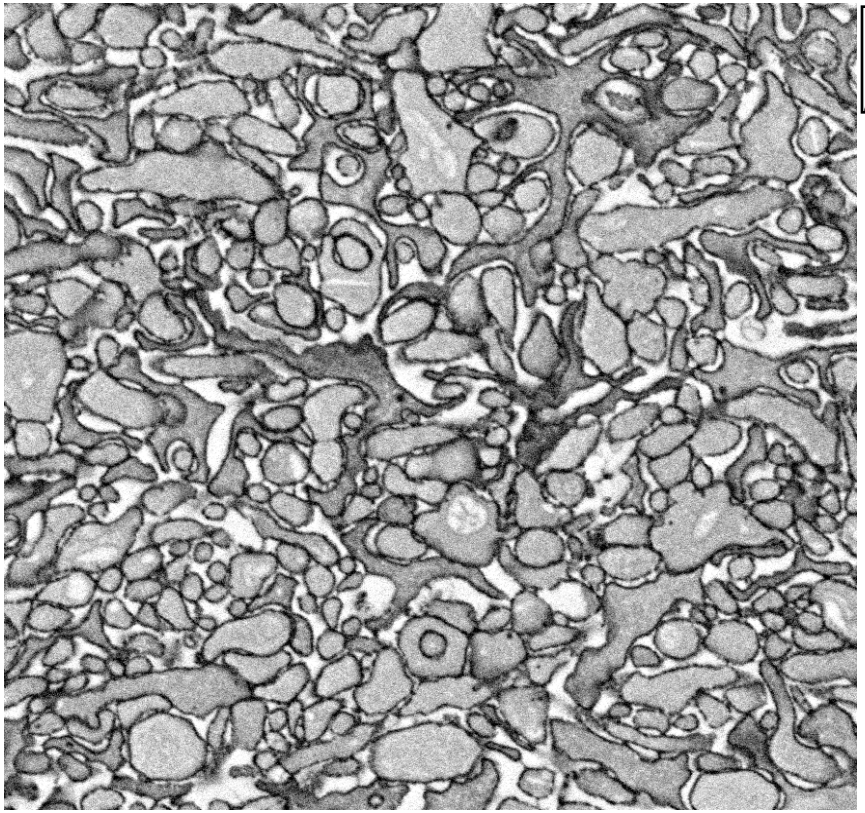


16.5 x 16.5 x 25 nm^3

1μm

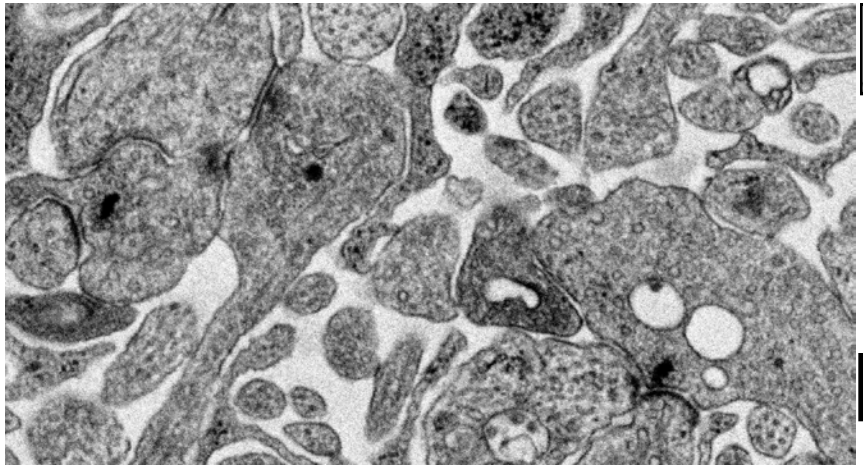
Mouse retina, inner plexiform layer

Extracellular space preservation



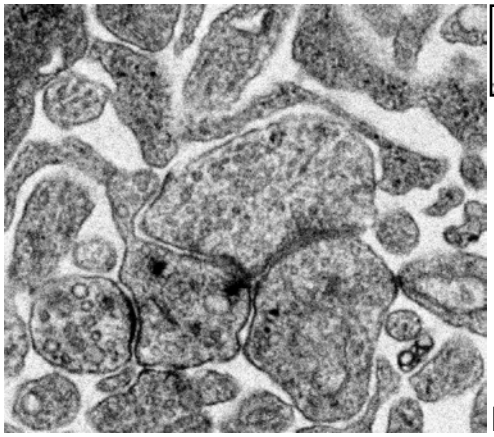
+ DAB

1µm



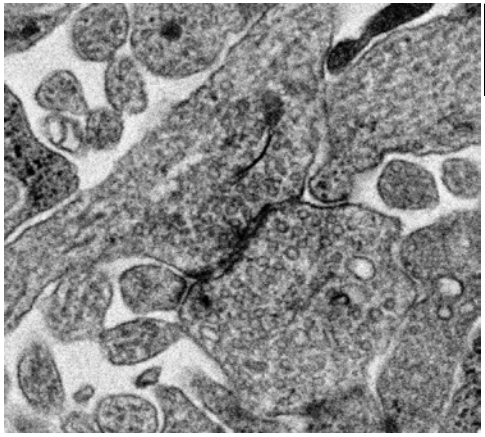
- DAB

100nm



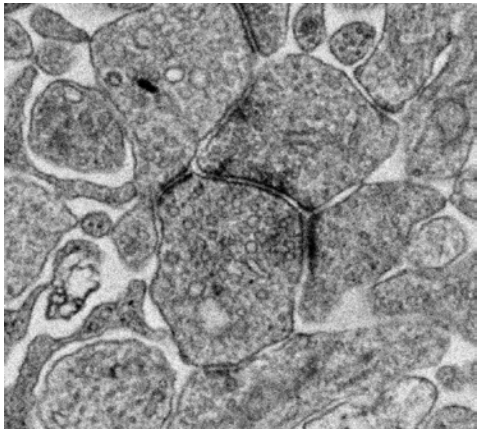
- DAB

100nm



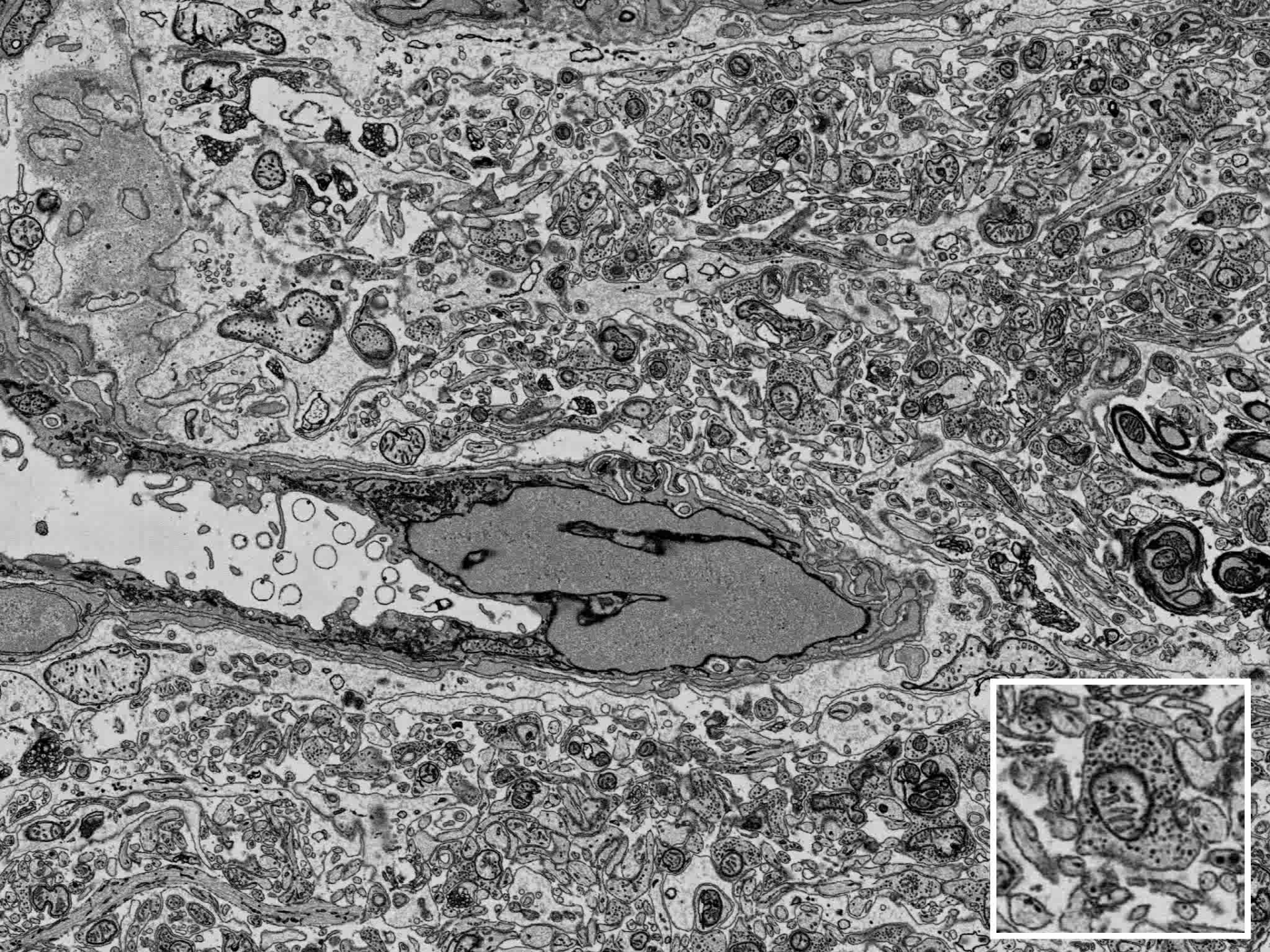
- DAB

100nm



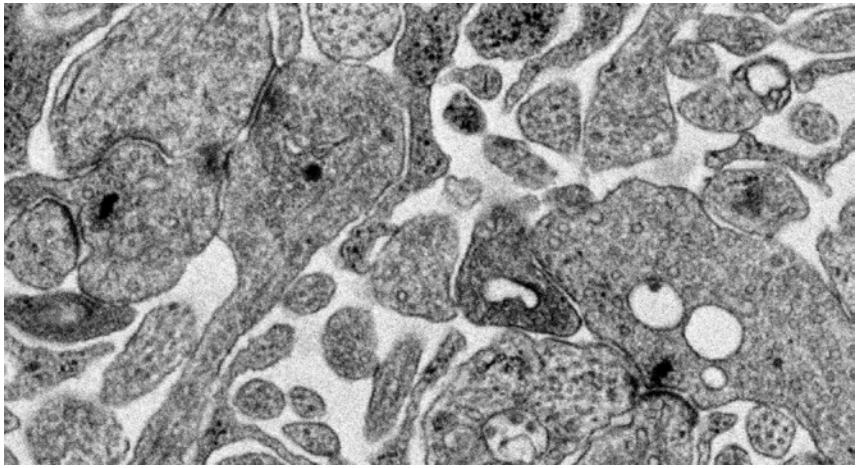
- DAB

100nm



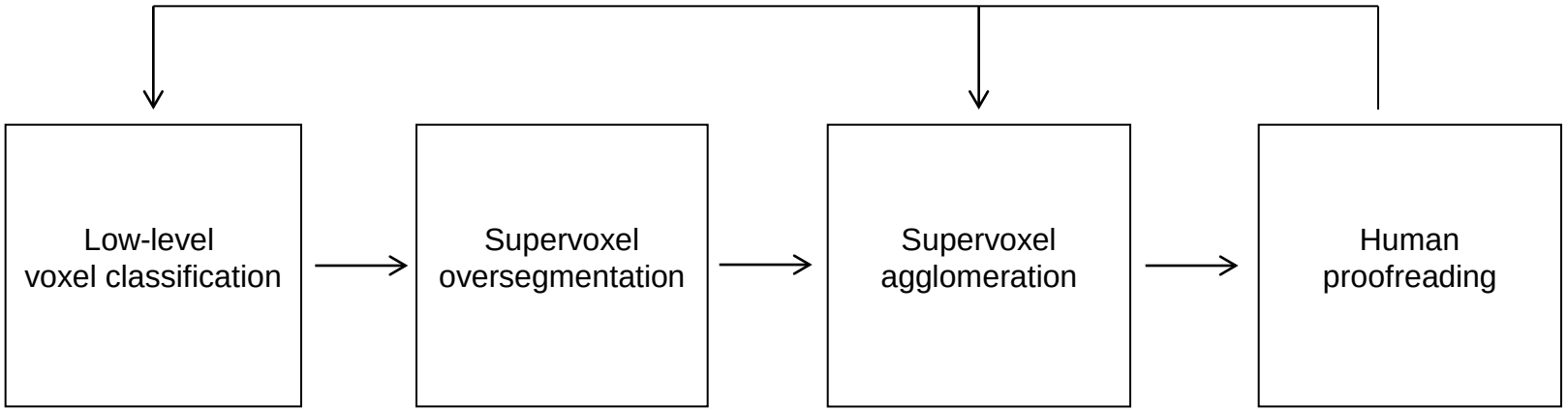
How can we speed up analysis?

1. Make the data easier to analyze



2. Position ourselves to take advantage of latest ML algorithms

NIH analysis pipeline (in progress)



e.g. Alex Krizhevsky's deep conv. NN code

e.g. conn. comp

e.g. feature-based learning

similar to eyewire.org but fun, and mobile

Acknowledgments

SBEM / Retina

Winfried Denk (MPI, Heidelberg)
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John Kaufhold
Marta Pallotto

Machine Learning

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Srini Turaga (UCL)
Sebastian Seung (MIT)

Zebrafish Collaborators

Harry Burgess (NICHD)