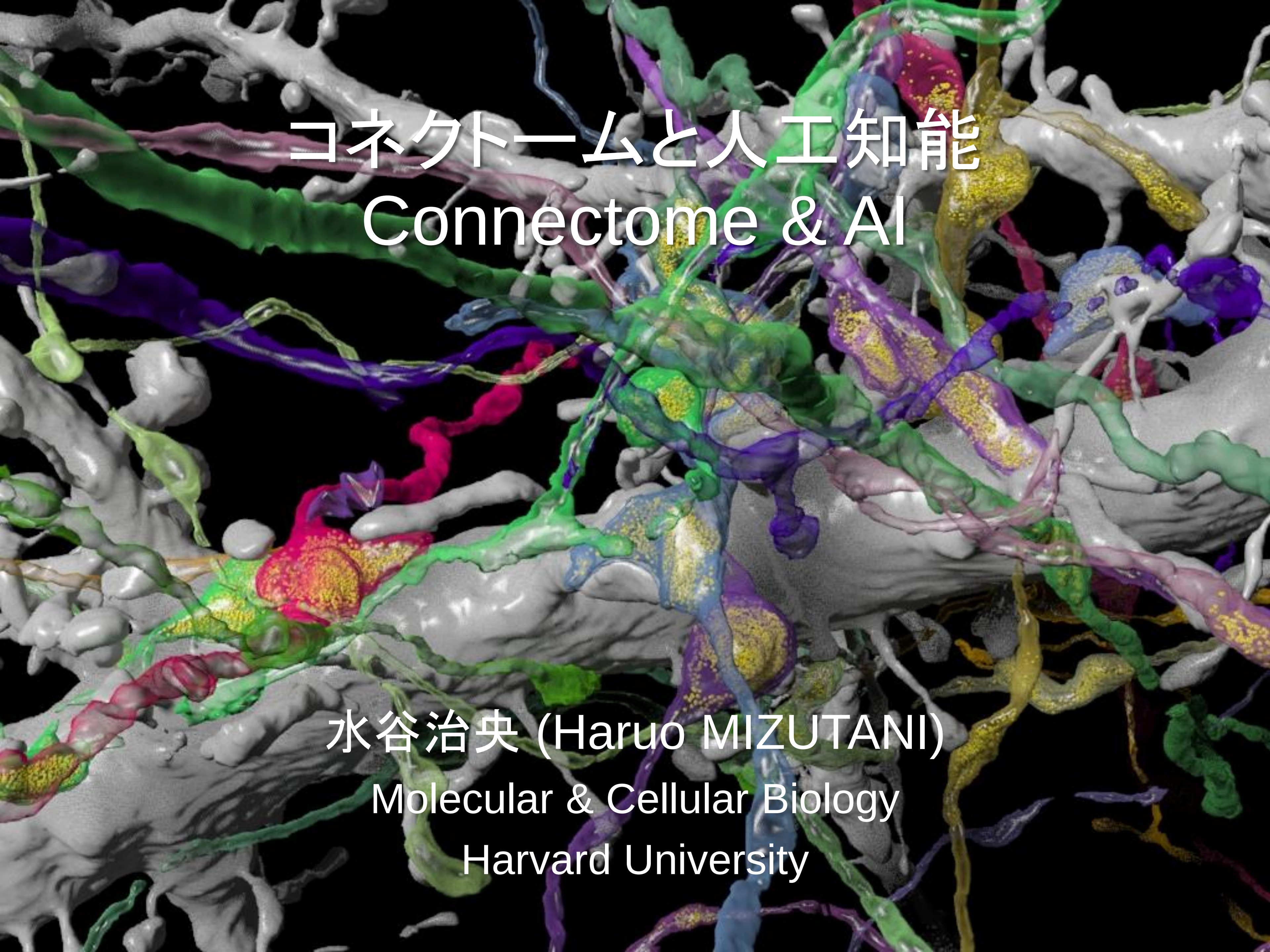


コネクトームと人工知能
Connectome & AI

水谷治央 (Haruo MIZUTANI)

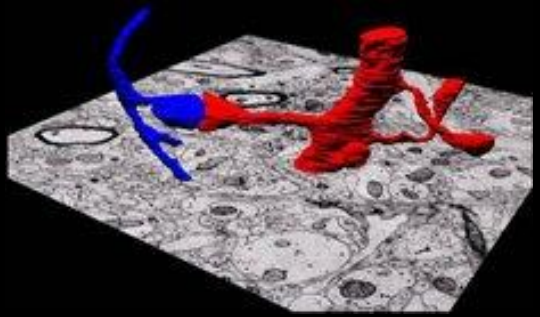
Molecular & Cellular Biology

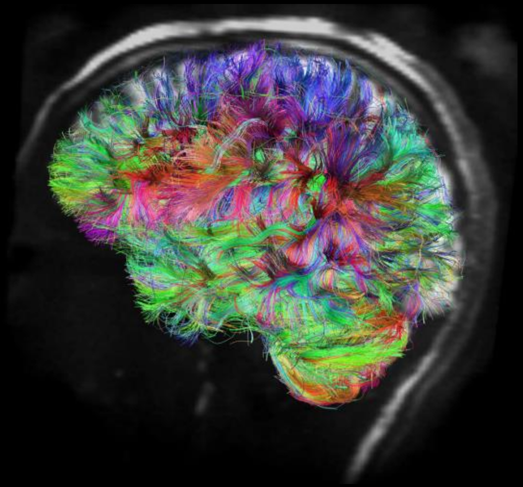
Harvard University

Connectomics

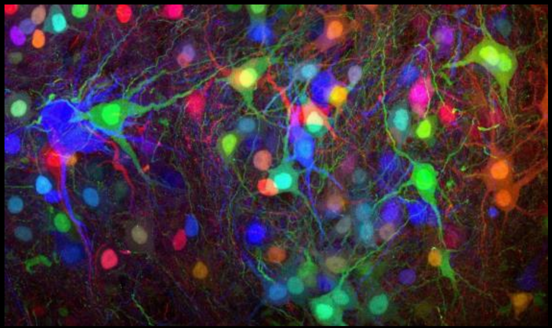
- Connectomics aims at deciphering all (synaptic) connections between all neurons and clarifying wiring diagram of the neuronal networks.

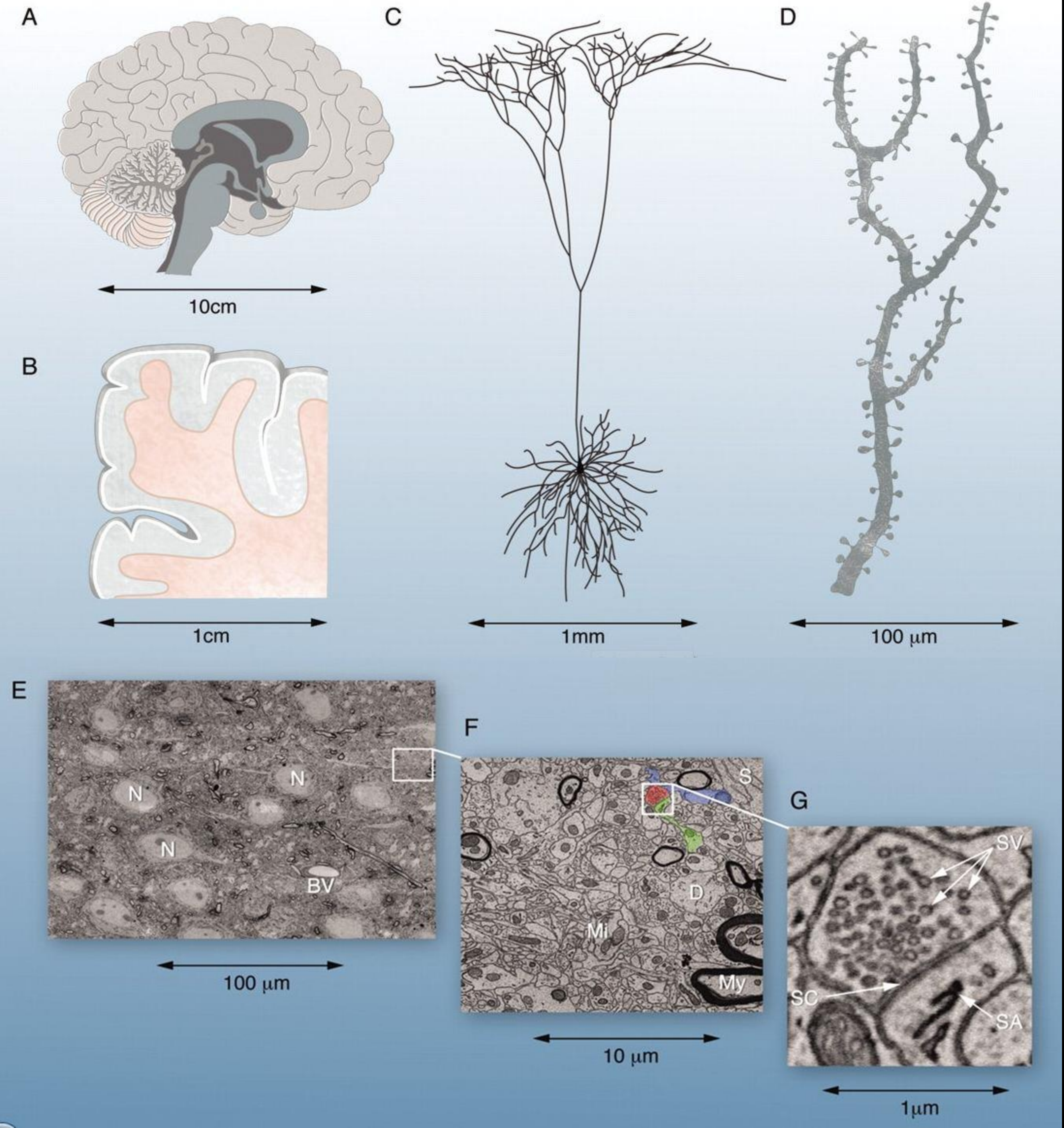
- Scale of Connectomics
  - Microscopic
    - Electron Microscopy
  - Macroscopic
    - MRI (Diffusion Tensor Imaging)





- Light Microscopy



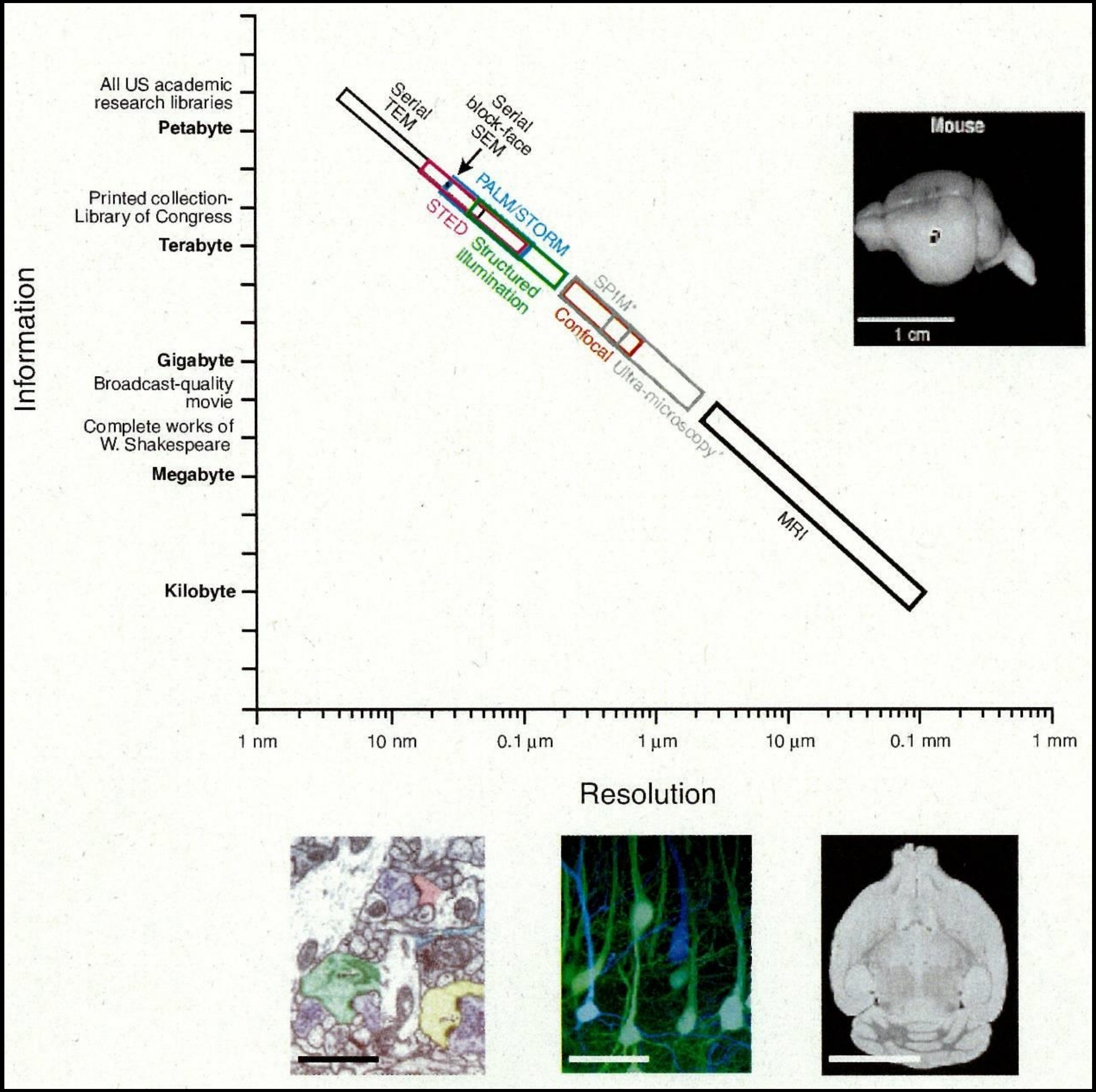


The brain is organized over sizes that span 6 orders of magnitude

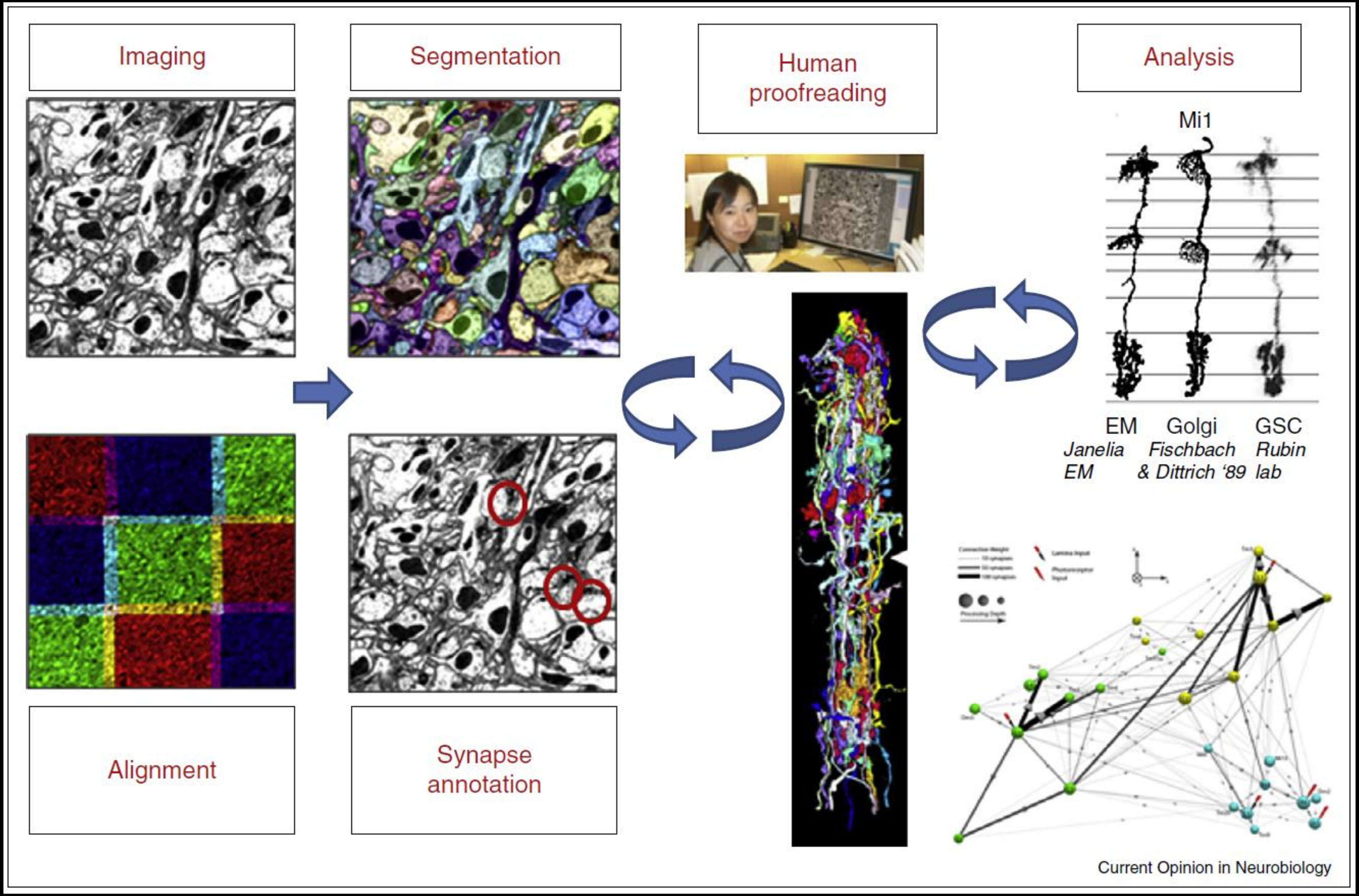
An MRI voxel ($1mm^3$) is 1 trillion times larger than an electron microscopy voxel ($100nm^3$)

Lichtman & Denk 2011

Scale: Devices & Resolution

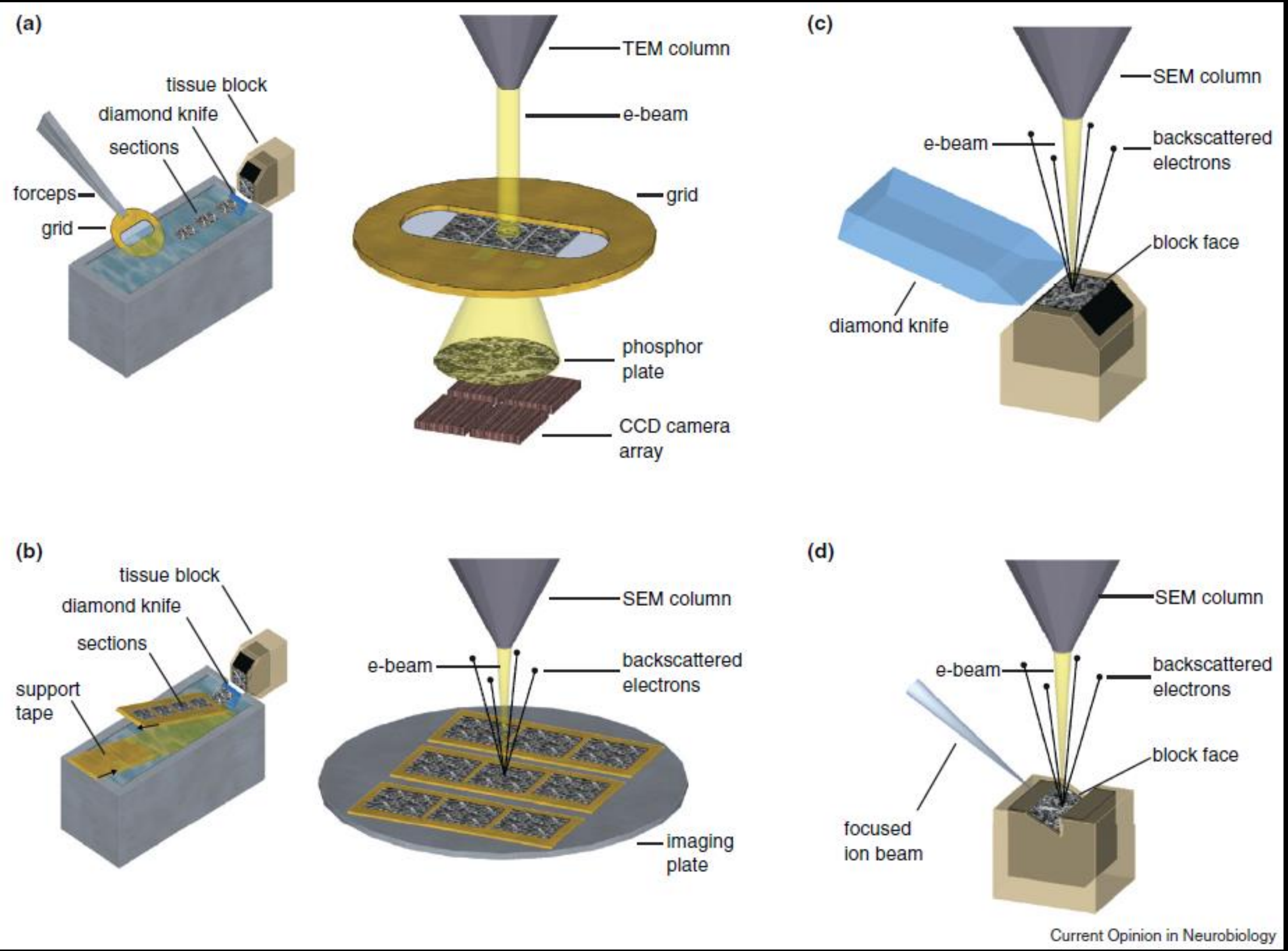


Workflow for large-scale neuron reconstruction

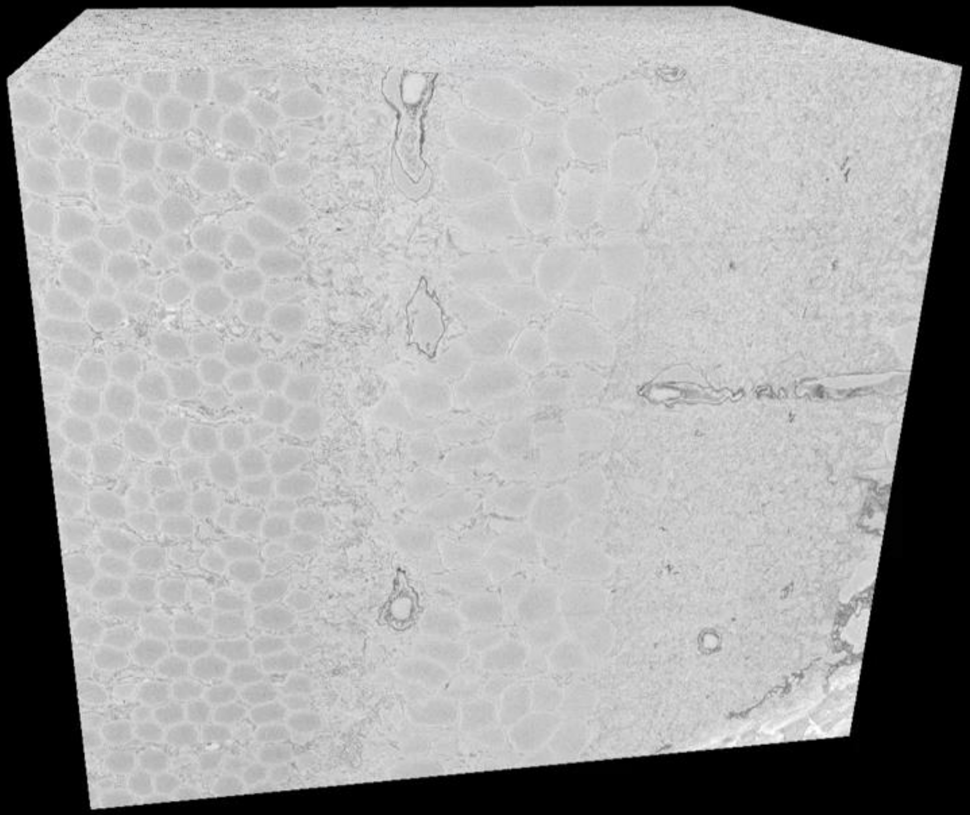


Plaza et al. (2014). Curr Op Neurobiol 25, 201-210.

3D EM techniques

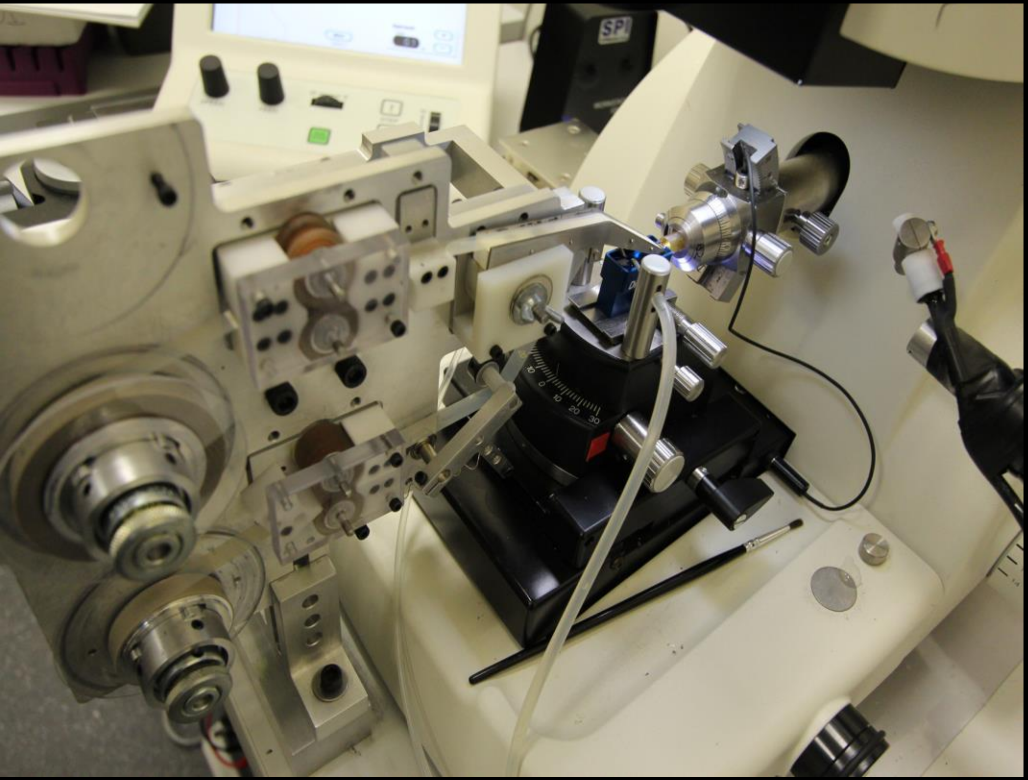


Retina neural circuit reconstruction
SBF SEM



Helmstaedter et al., Nature 500, 168-174 (2013)

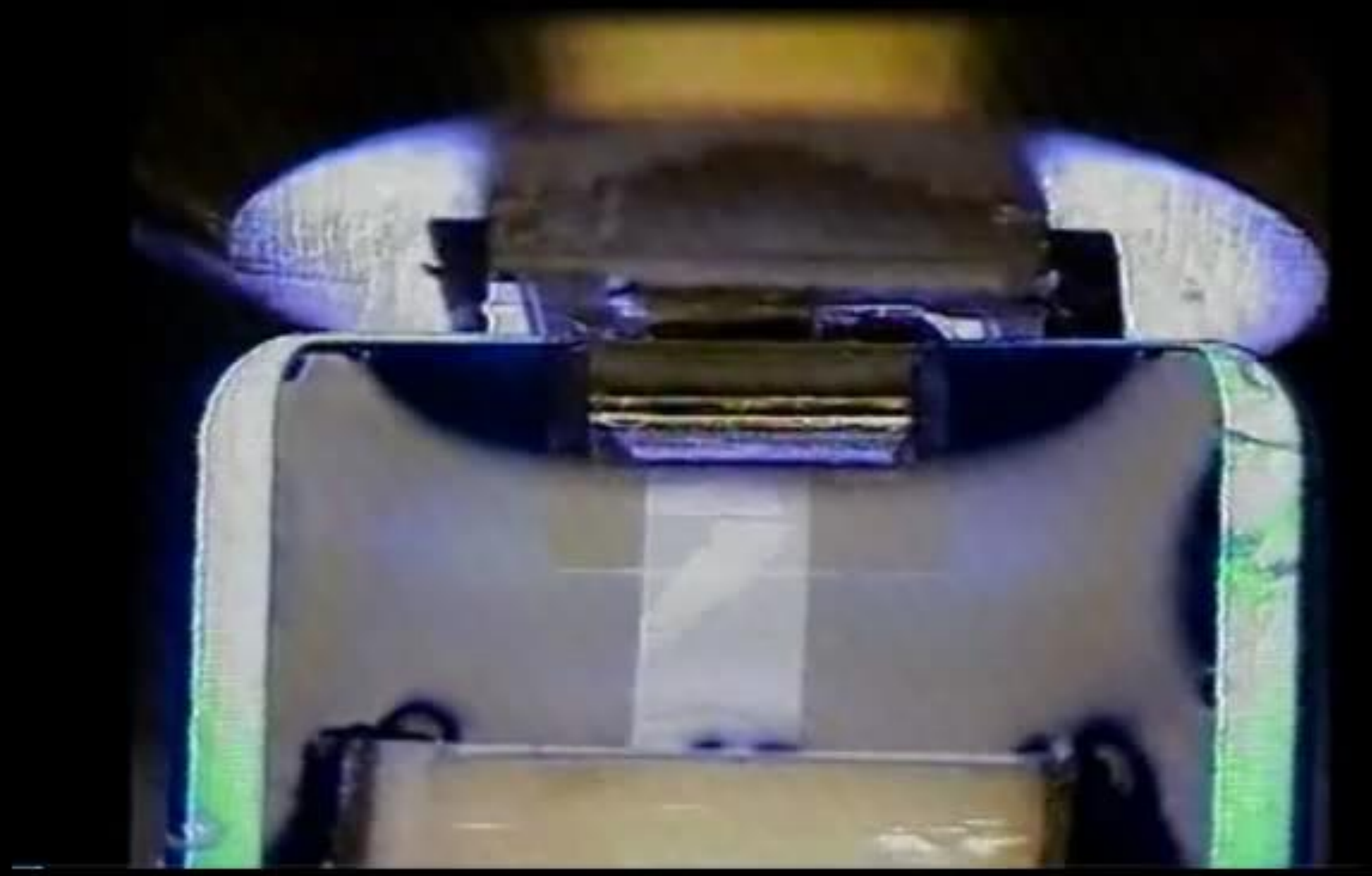
Automatic Tape-collection Mechanism for Ultramicrotomes

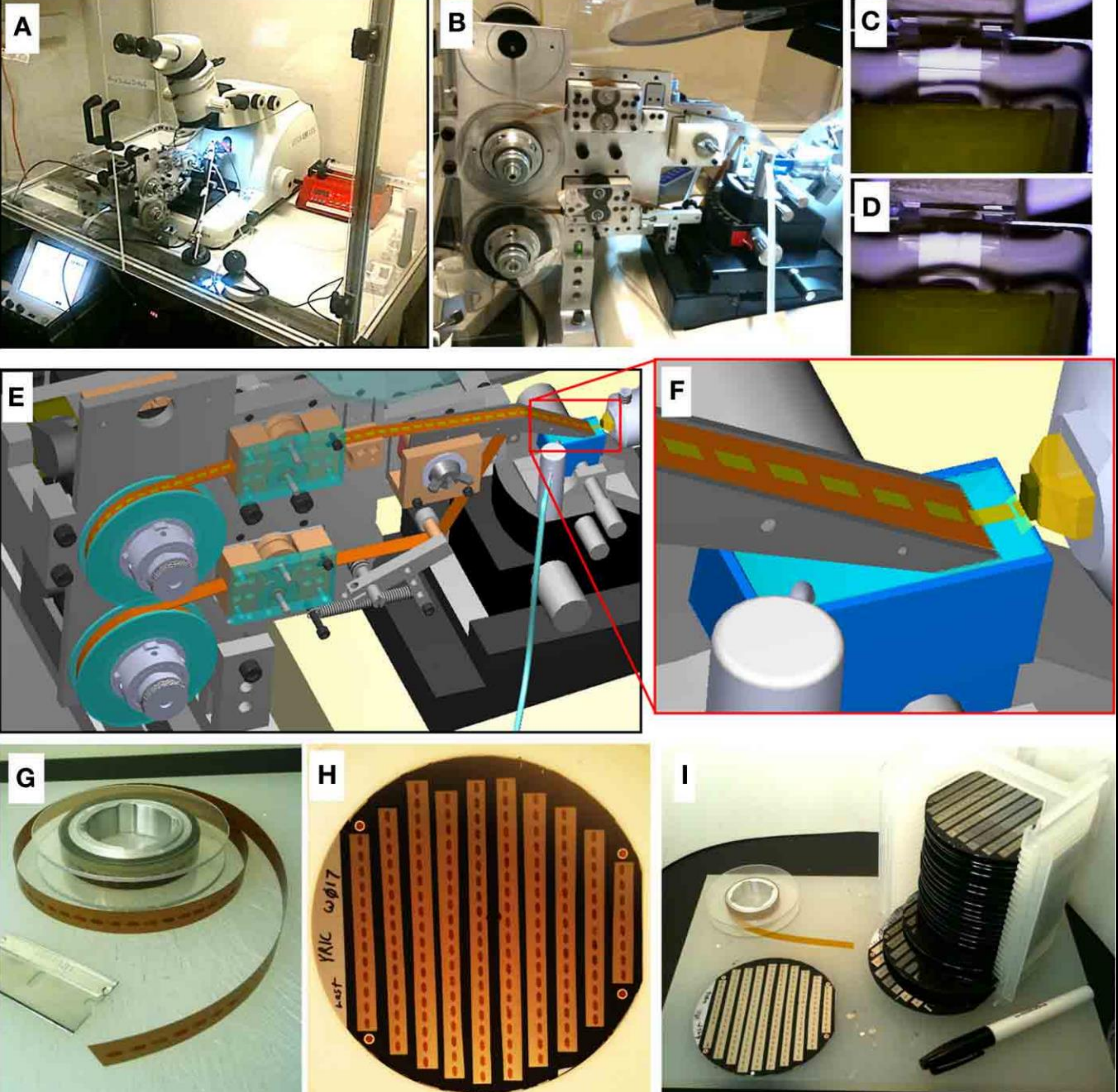


- Lossless & relatively damage free sectioning
- Nondestructive sectioning
- Stable and sturdy after sectioning
- allows for:
  - Immunohistochemistry (the proteome meets the connectome)
  - Multiscale imaging (same section re-imaged many times at different magnifications)
  - Post sectioning intensification methods for added contrast
  - Multi-instrument image acquisition multiplexing or more exotic solutions for speed

Ken Hayworth and Richard Schalek

ATUM cutting & collecting sections





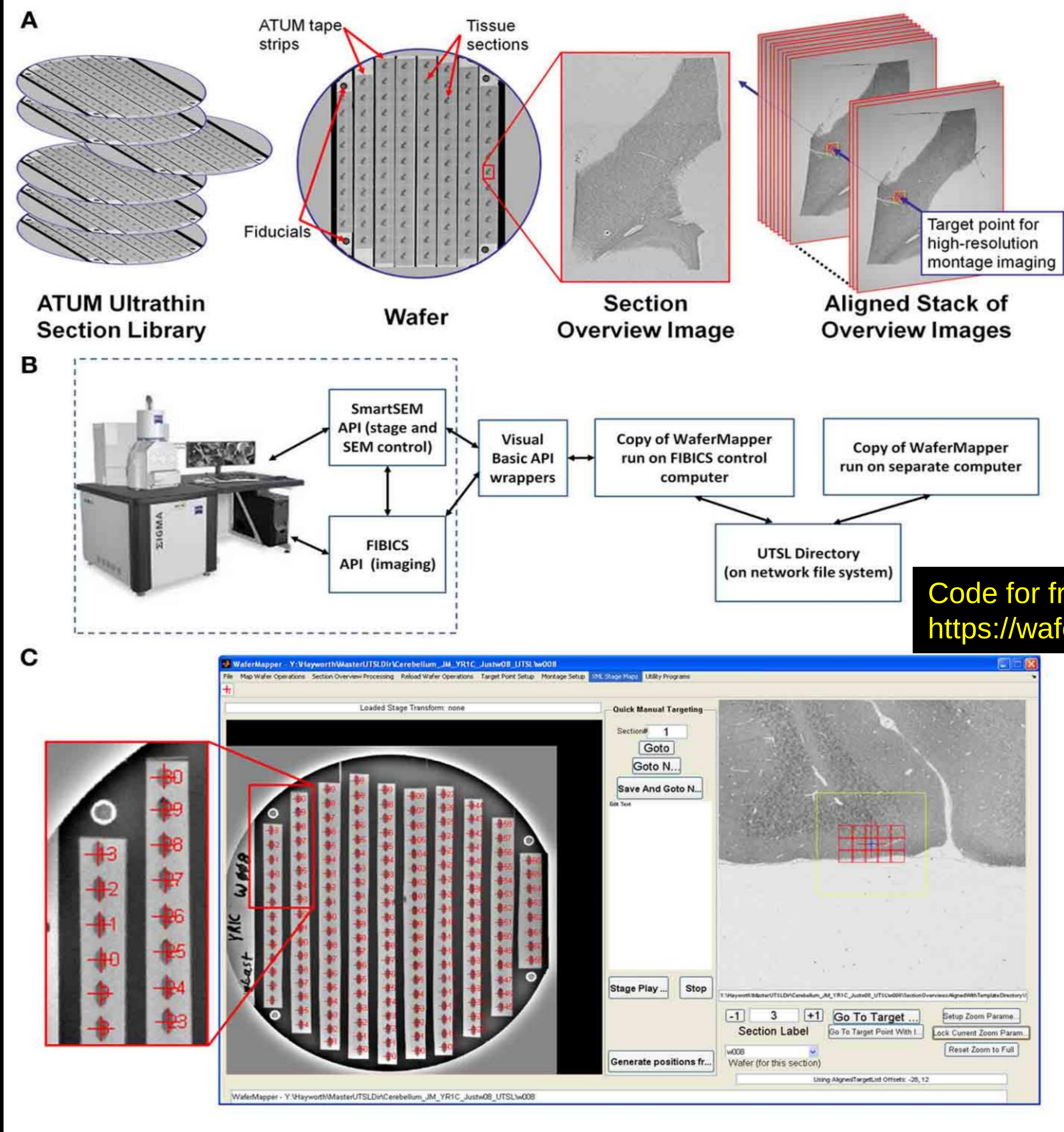
ATUM

(Automatic Tape-collecting Ultra-Microtome)

Commercially Available in Boeckeler Instruments, Inc.

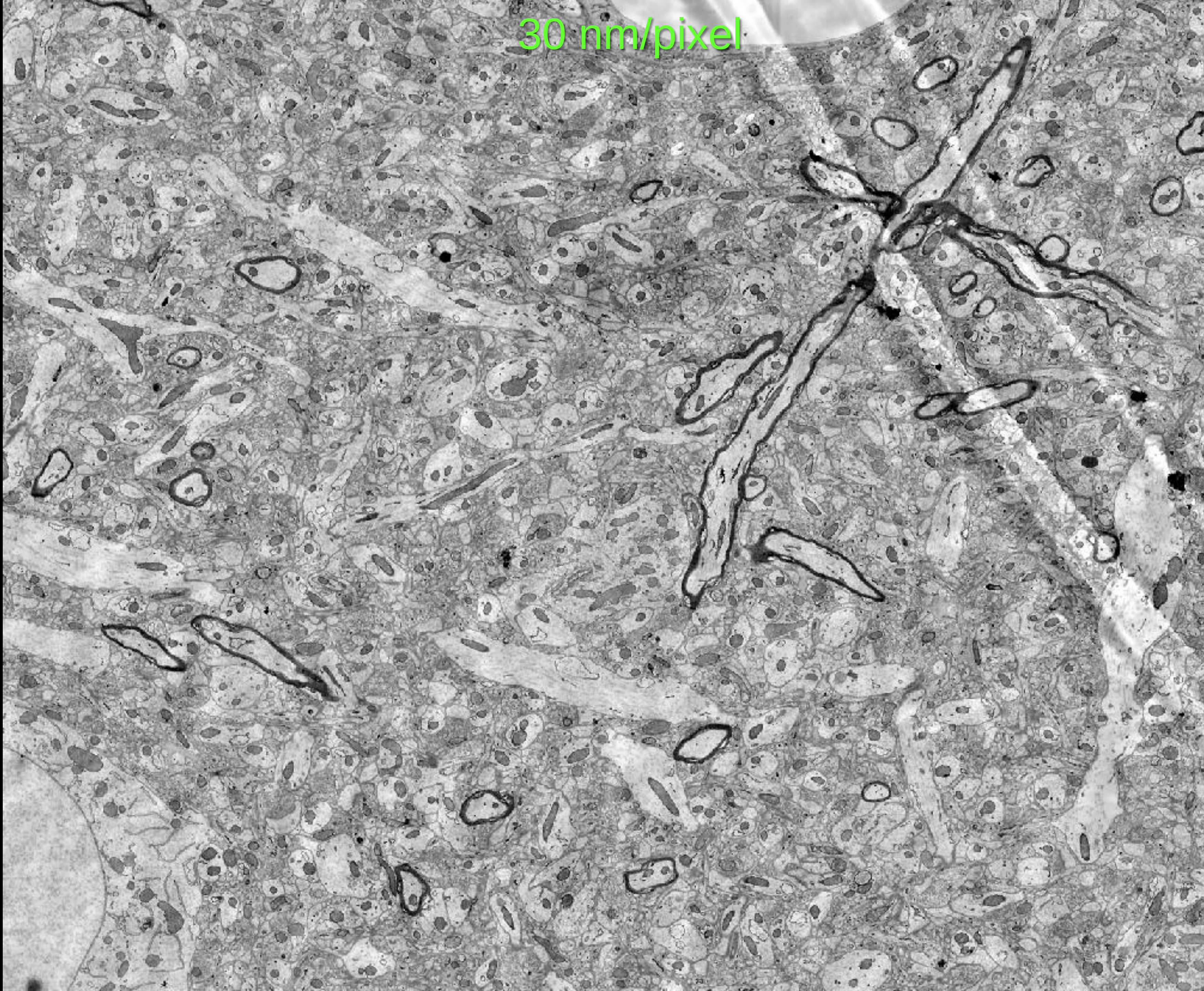
Hayworth et al., 2014

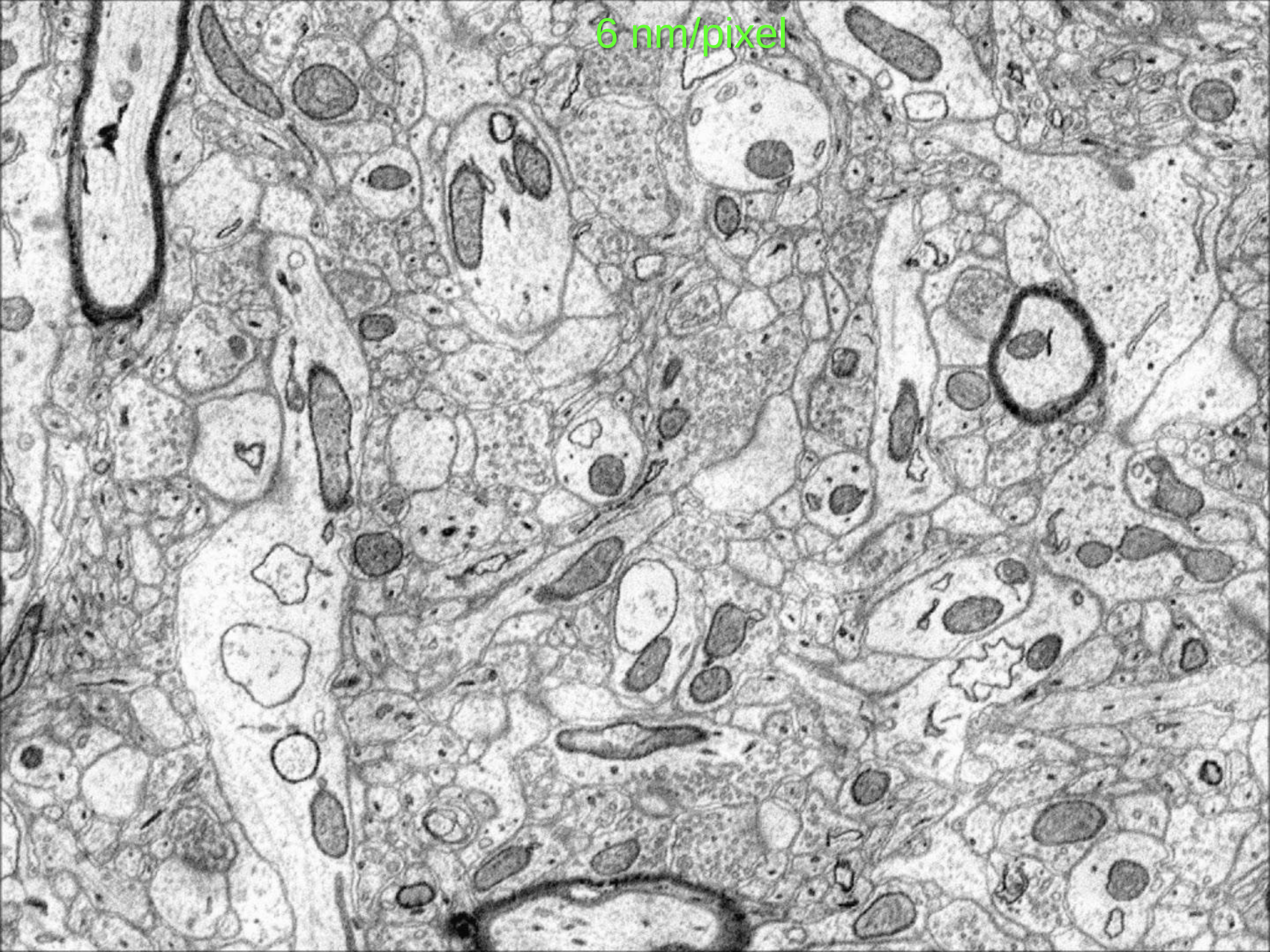
WaferMapper

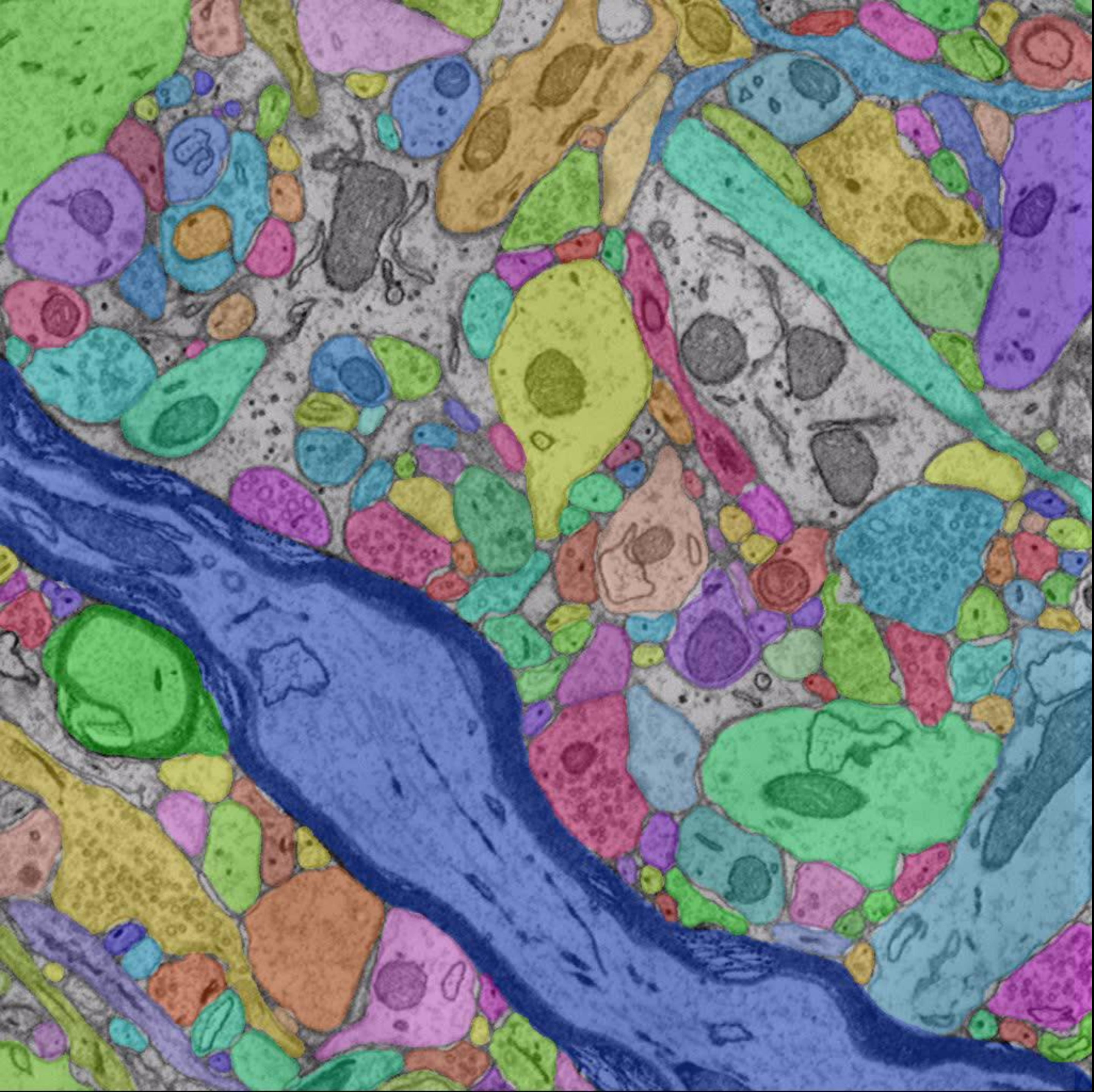


Code for free
https://wafermapper.googlecode.com

Hayworth et al., 2014


30 nm/pixel

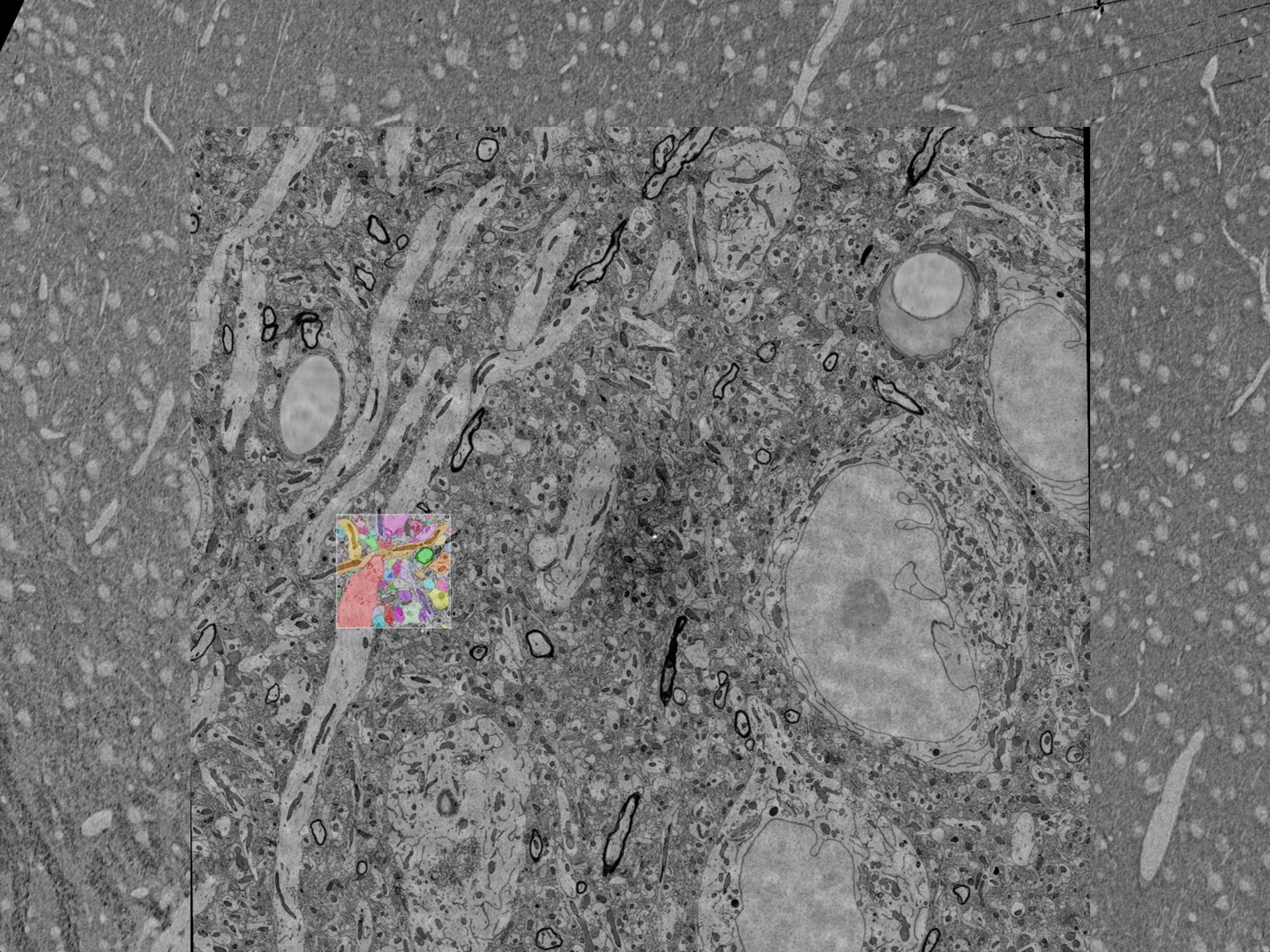

6 nm/pixel

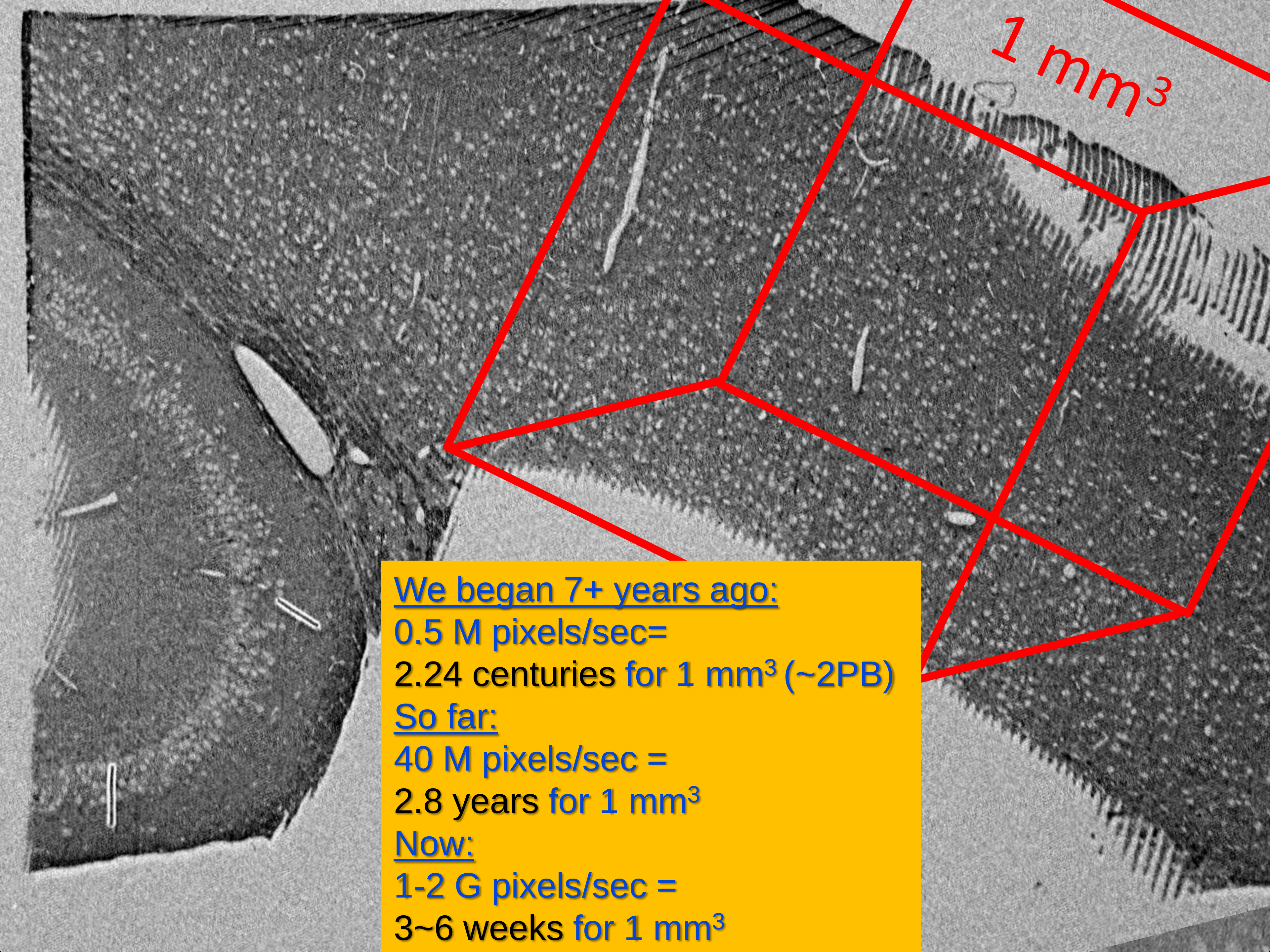


Computer-assisted “VAST” saturated *hand* segmentation of cortex 6 x 6 x 6 µm

216 μm^3 = 2 months

Daniel Berger





We began 7+ years ago:

0.5 M pixels/sec=

2.24 centuries for 1 mm³ (~2PB)

So far:

40 M pixels/sec =

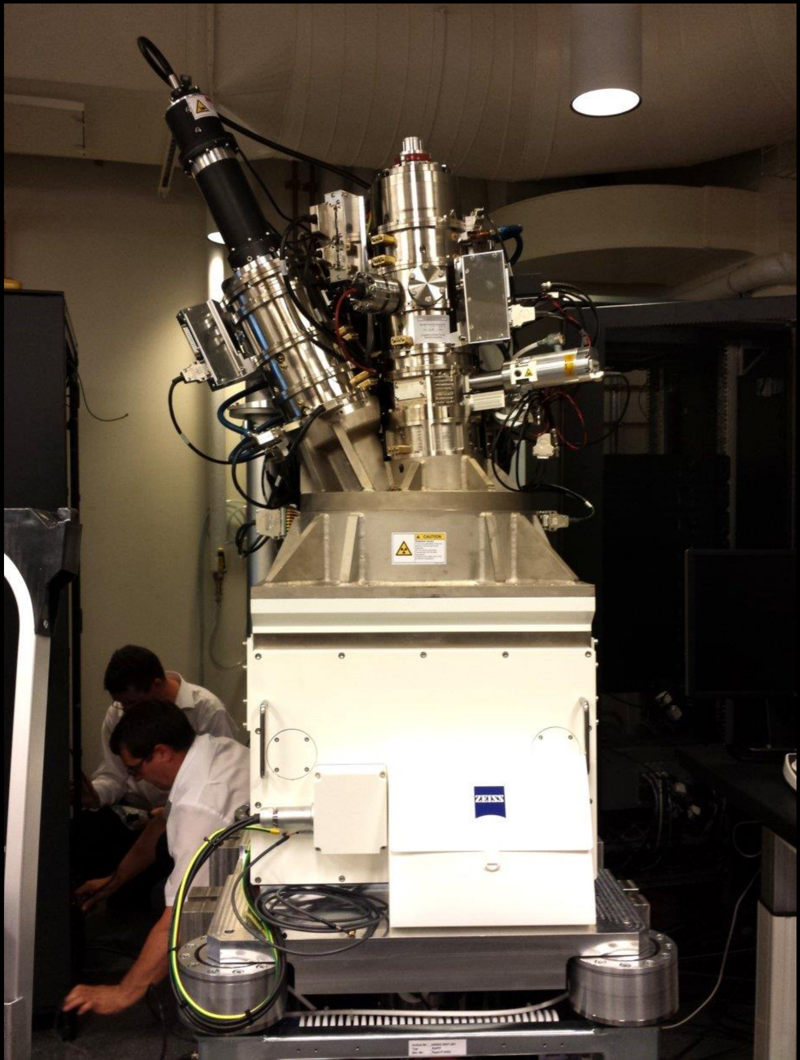
2.8 years for 1 mm³

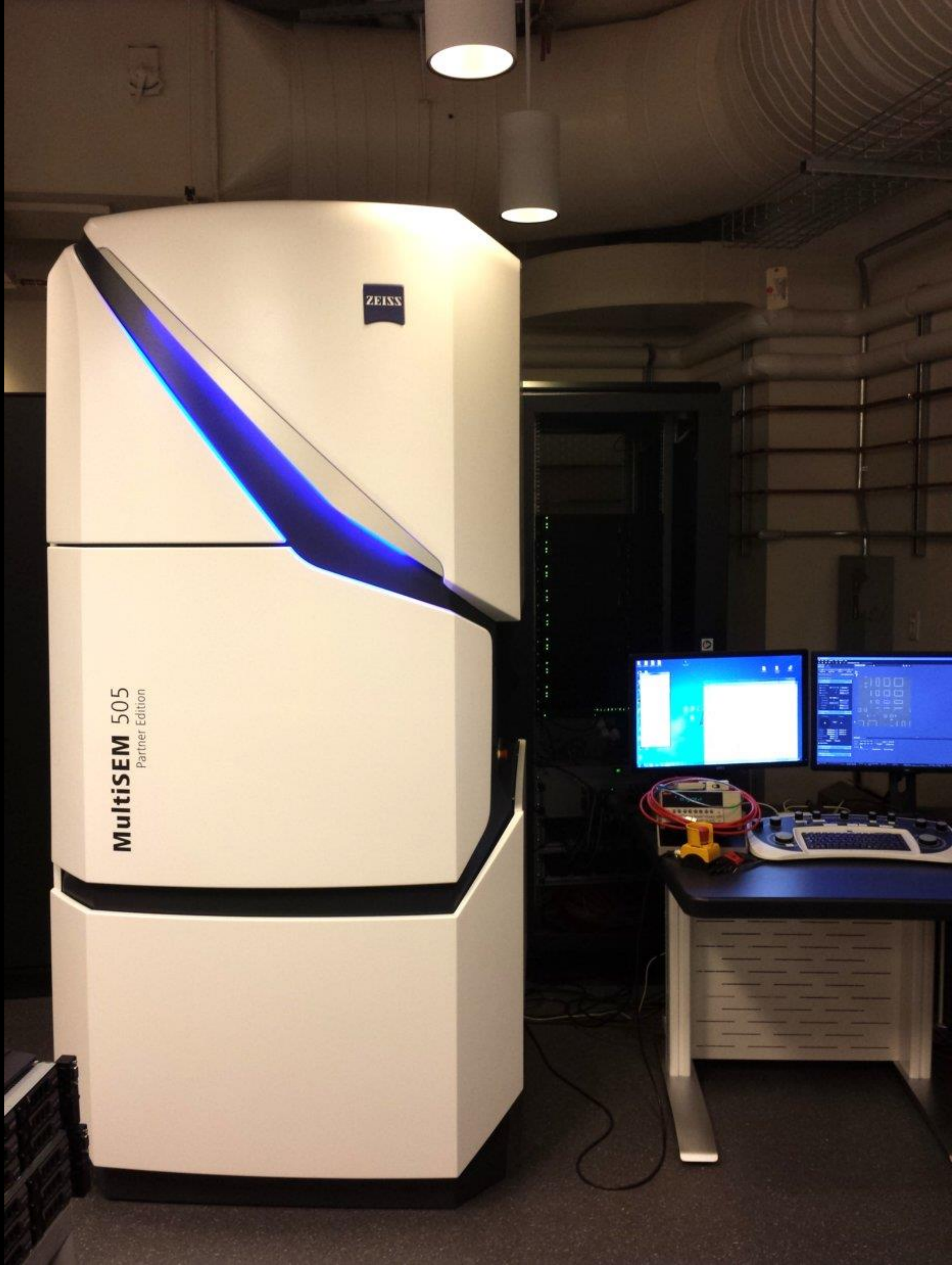
Now:

1-2 G pixels/sec =

3~6 weeks for 1 mm³

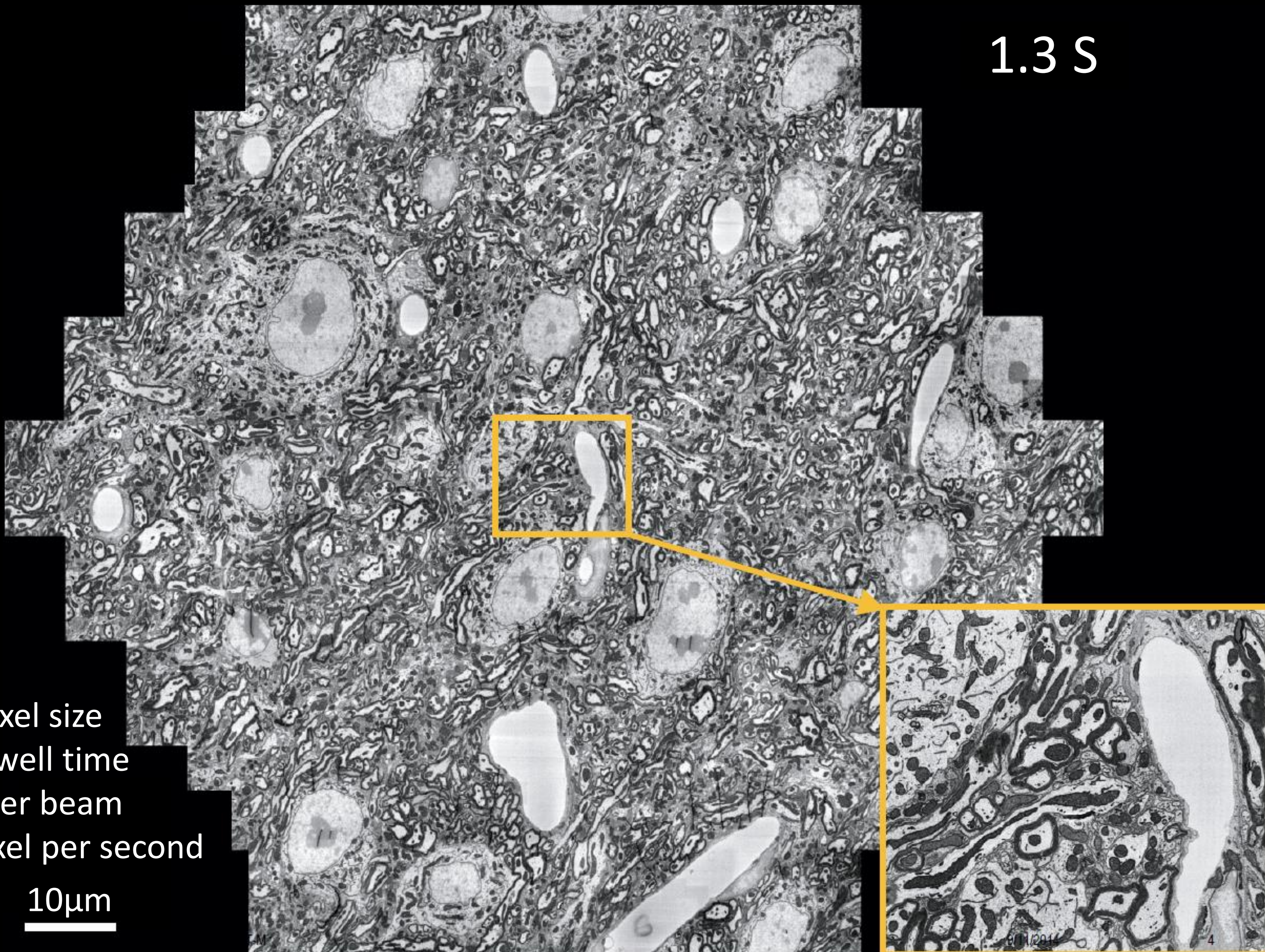
61 multi-beam SEM





Single Hexagon Image

1.3 S



3.8nm pixel size
100 ns dwell time
430 pA per beam
470 Mpixel per second

10μm

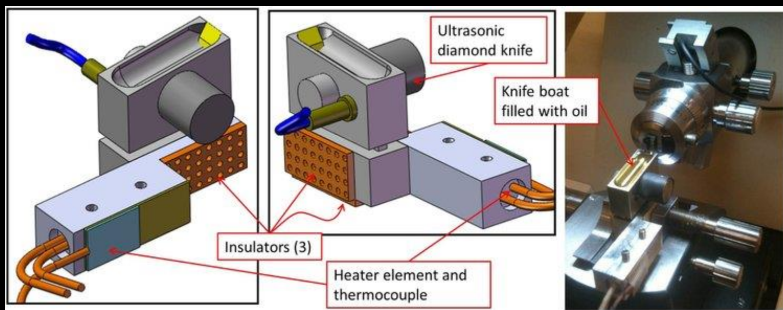
Large-Area Imaging

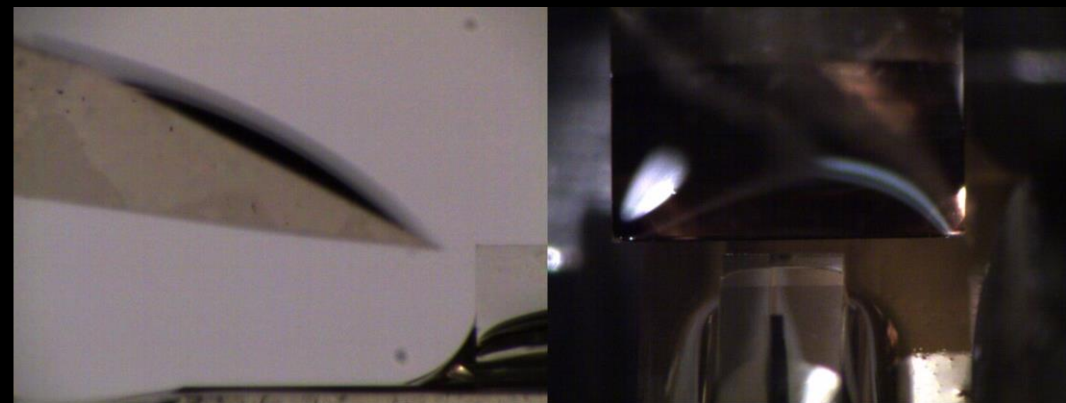
Fully automatic run
492 individual hexagons
2 x 1.6 mm sample size
4 nm pixel size
290 GB file size
100 ns dwell time

~60 min imaging time

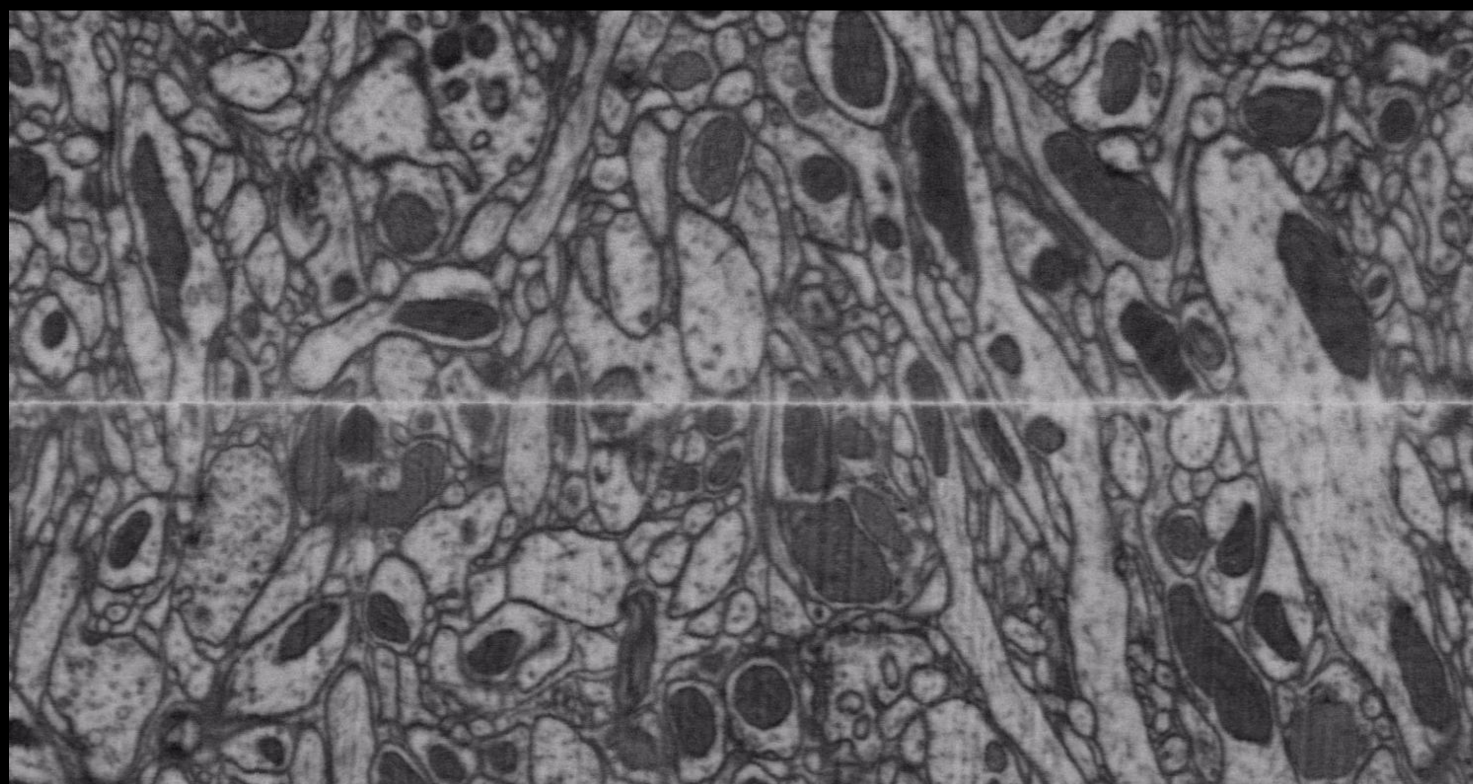
1 mm

Ultrathick sectioning (20 µm)



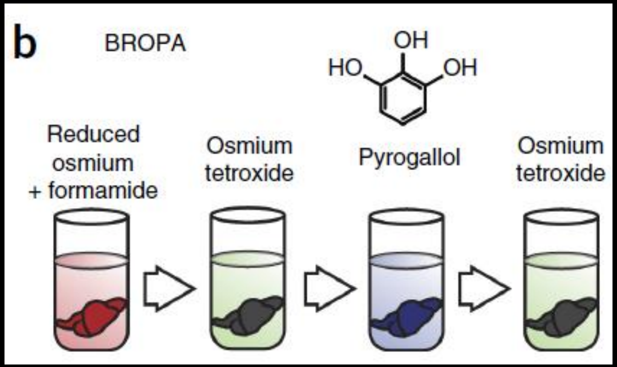


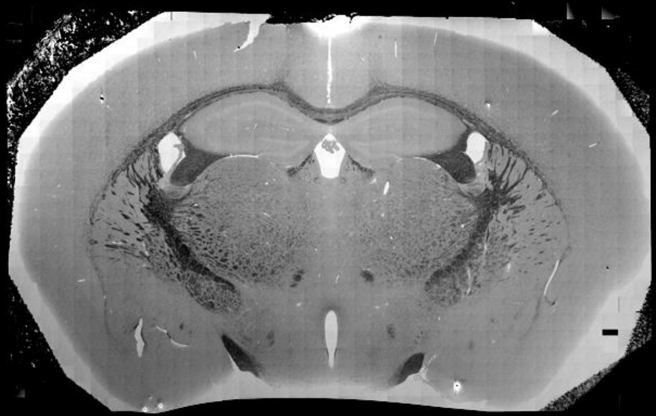
30 nm lost →



Hayworth et al., Nat Methods, 12:319-22 (2015)

Whole mouse brain EM imaging





Mikula & Denk @ Max Plank
Nat Methods.12:541-6 (2015)

80nm/pixel resolution

Whole mouse brain

- Mouse-brain volume: 0.5ml

- Number of voxels at 80 nm isotropic resolution: 10^{15}
- (500 hard disks, $100 each -> $50k)
- Imaging time at 40 MHz: 280 days

- Number of voxels at 20 nm isotropic resolution: $6x10^{16}$
- (30,000 hard disks, $100 each -> $3M)
- Imaging time at 40 MHz: 50 years

- 5nm isotropic resolution: $3.6x10^{18}$
- (1,800,000 hard disks, $100 each -> $180M)
- Imaging time at 40 MHz: 3000 years

Whole mouse brain

- Mouse-brain volume: 0.5ml

- Number of voxels at 80 nm isotropic resolution: 10^{15}
- (500 hard disks, $100 each -> $50k)
- Imaging time at **2 GHz: 6~10 days**

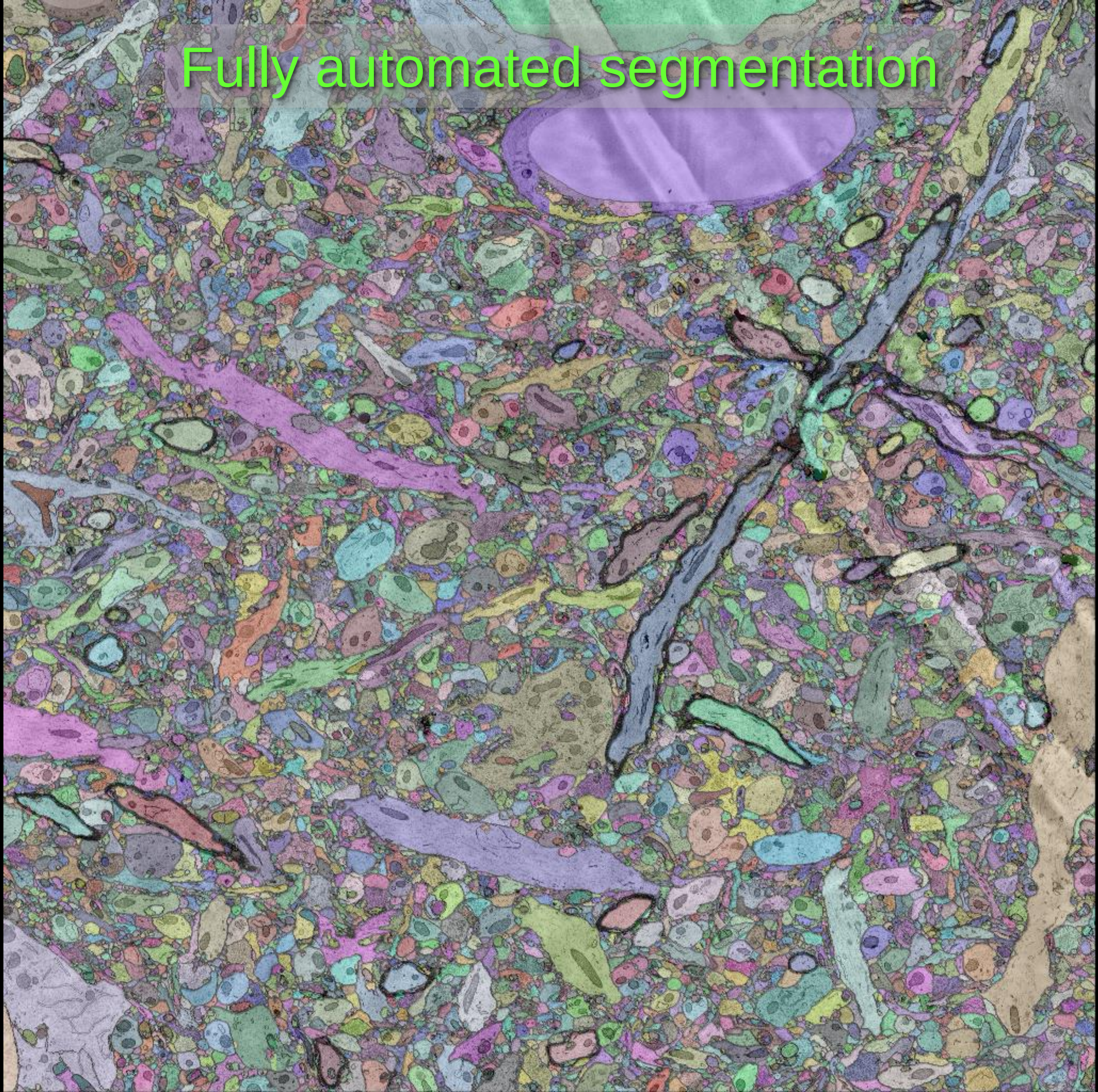
- Number of voxels at 20 nm isotropic resolution: $6x10^{16}$
- (30,000 hard disks, $100 each -> $3M)
- Imaging time at **2 GHz: 1~2 years**

- 5nm isotropic resolution: $3.6x10^{18}$
- (1,800,000 hard disks, $100 each -> $180M)
- Imaging time at **2GHz: 60~100 years**

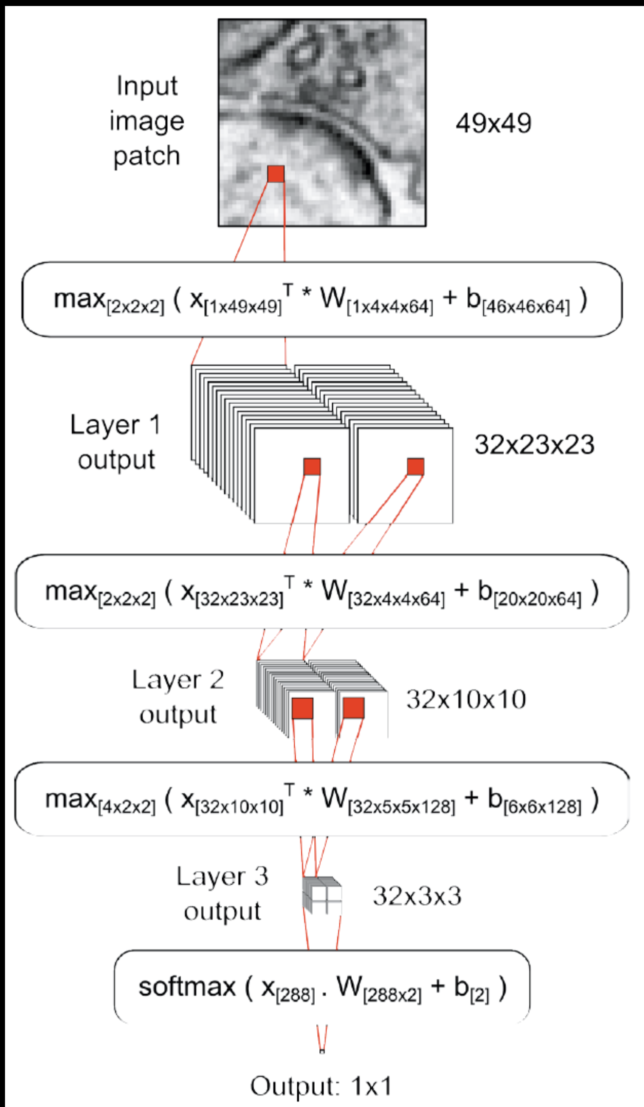
Challenges for Data Acquisition

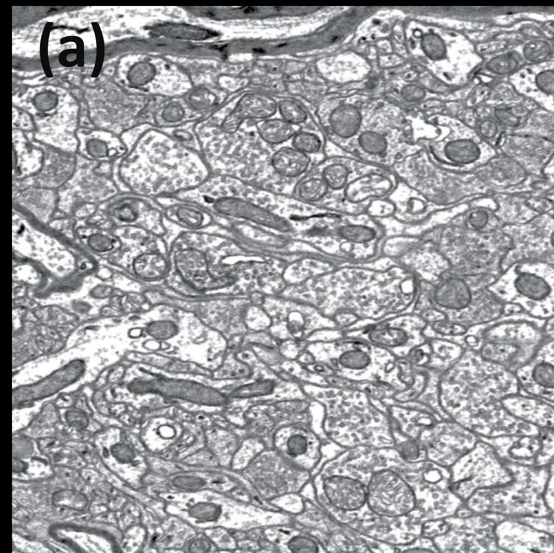
- Speed
  - 1-2G pixel/sec, 6 weeks for $1mm^3$ @4x4x30nm
  - Whole mouse brain: 1 year with 400 beams, 7 mSEM
- Resolution
  - 6.25 x speed at 10nm/pixel, 1 week for $1mm^3$
  - Whole mouse brain: 2 months with 7 mSEM
- Cutting with big diamond knifes
  - Hot diamond knife
  - Larger than 1 cm diamond knife
  - Replacing knife
- Deep staining

Fully automated segmentation



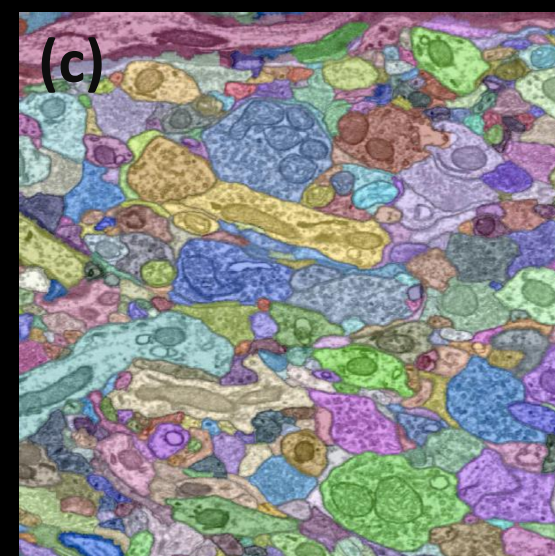
Deep Learning for the Connectome





4-layer deep network

(a) Original EM image
(b) Deep network output
(c) Segmentation pipeline output

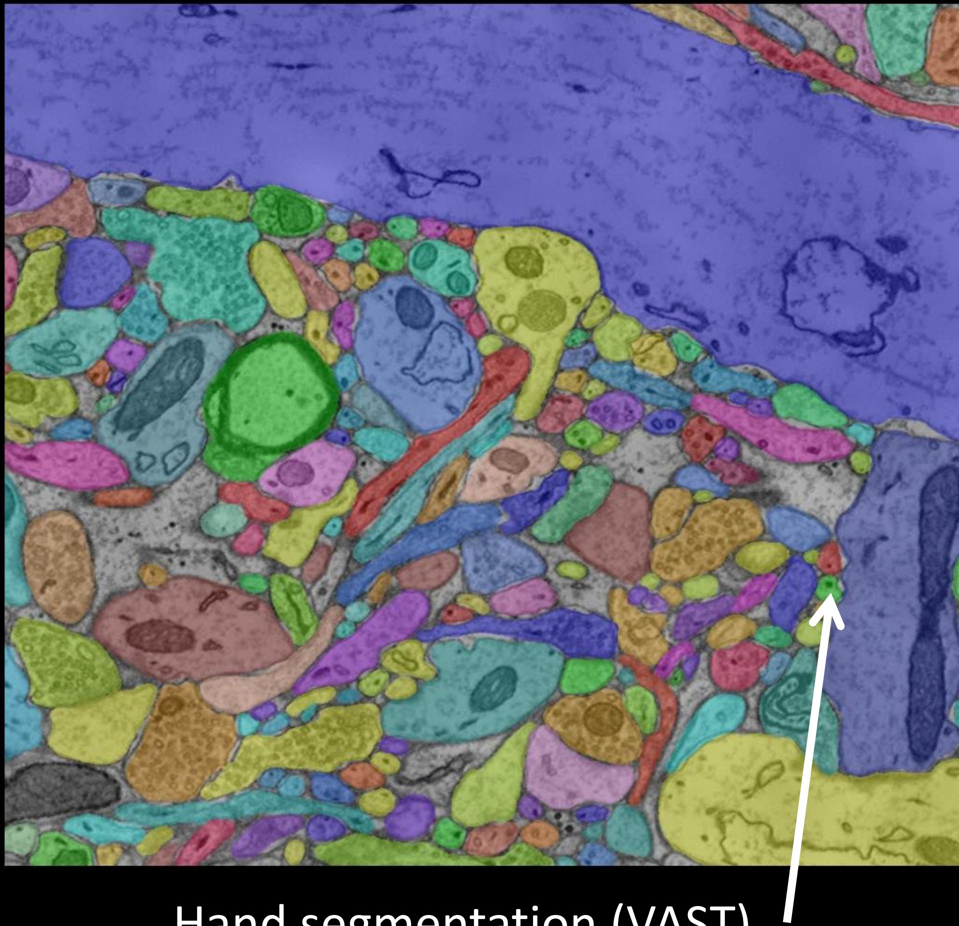


http://www.rhoana.org/

Full Automatic vs Hand Segmentation

Peril: still not quite good enough without substantial human editing

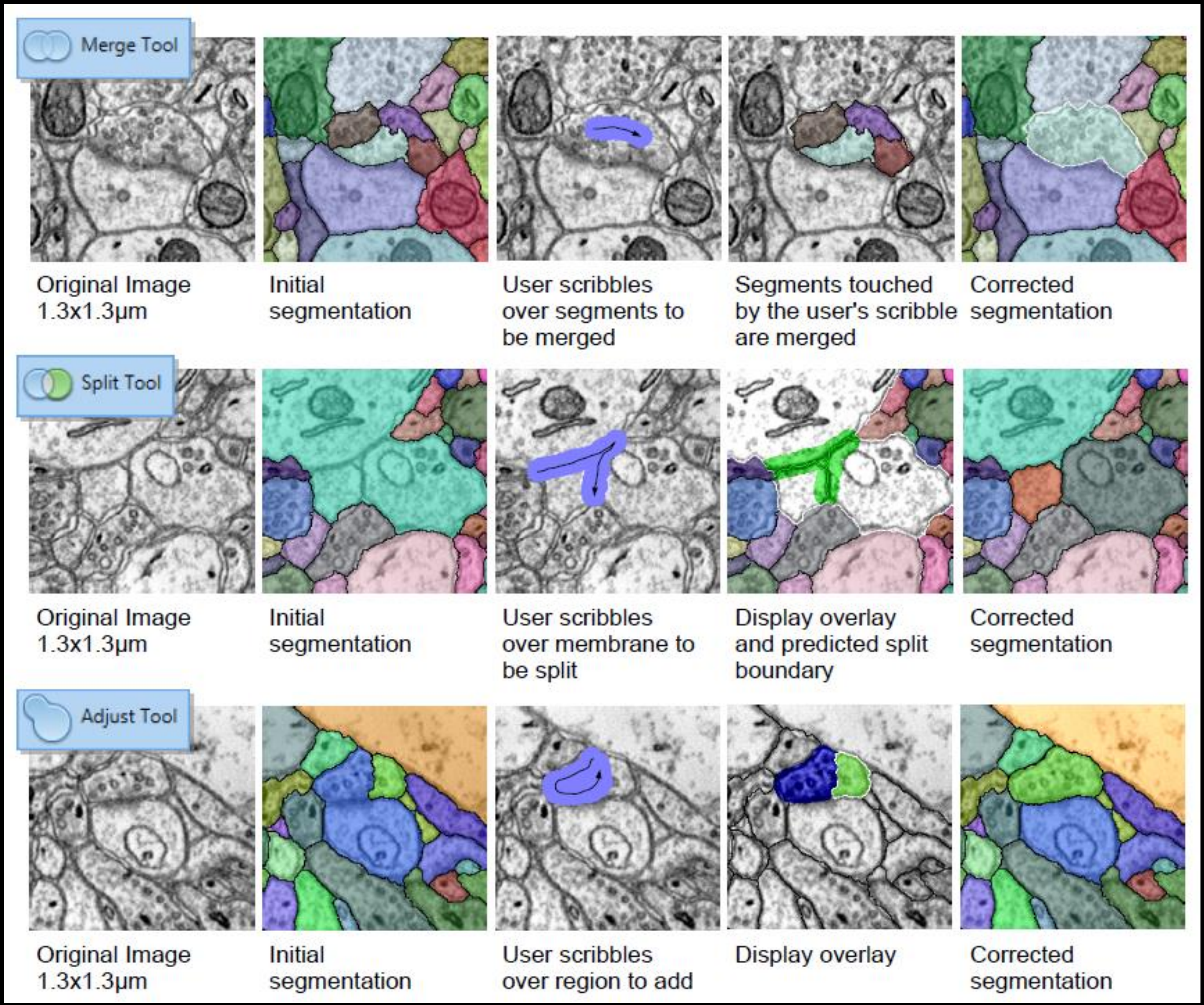
Fully automatic (FUSION)



Hand segmentation (VAST)

Kasthuri et al., Cell 2015
Seymour Knowles-Barley with Hanspeter Pfister et al

Mojo: Connectome Annotation Tool



http://www.rhoana.org/

Challenges for Data Analysis

- Very computationally intensive
  - 38 million flops per output required to process image data at 0.85TB/day
    - 250 nVidia Tesla K40 GPU ($2M: $8,000/K40)
- Learning algorithm errors
  - 0.9/um^3 split human operation required to correct for merger errors
  - 5.8/um^3 merge human operation required to correct for split error
- Proof Reading
  - Intensive proof time

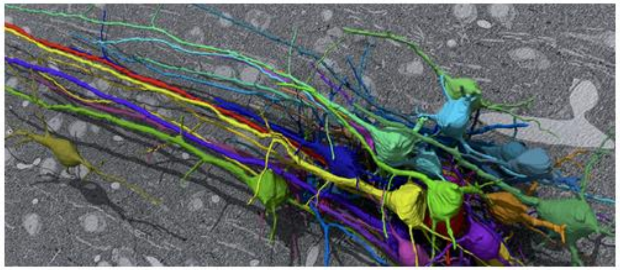
Challenge on 3D segmentation

SNEMI3D: 3D Segmentation of neurites in EM images

Home | Leaders Board | Forum | Evaluation | Organizers | My account | Register

ISBI 2013 challenge: 3D segmentation of neurites in EM images

Welcome to the server of the **first challenge on 3D segmentation of neurites in EM images!**



Group of pyramidal neurons of mouse cortex reconstructed from SEM images (Daniel Berger).

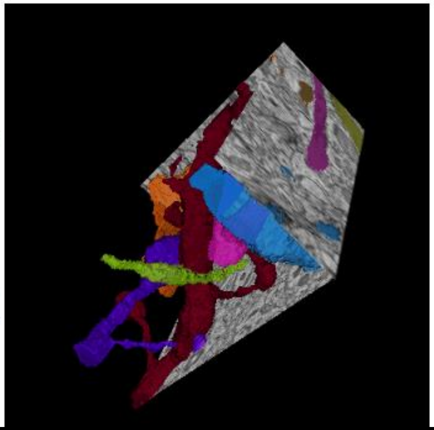
The challenge is organized in the context of the IEEE International Symposium on Biomedical Imaging (San Francisco, CA, April 7-11th 2013). If you wish to participate, please register now to be able to download the training and test data sets and upload your own results.

Background and set-up

In this challenge, a full stack of electron microscopy (EM) slices will be used to train **machine-learning algorithms** for the purpose of **automatic segmentation of neurites in 3D**. This imaging technique visualizes the resulting volumes in a highly anisotropic way, i.e., the x- and y-directions have a high resolution, whereas the z-direction has a low resolution, primarily dependent on the precision of serial cutting. EM produces the images as a projection of the whole section, so some of the neural membranes that are not orthogonal to a cutting plane can appear very blurred. None of these problems led to major difficulties in the manual labeling of each neurite in the image stack by an expert human neuro-anatomist.

In order to gauge the current state-of-the-art in automated neurite segmentation on EM and compare between different methods, we are organizing a 3D Segmentation of neurites in EM images (SNEMI3D) challenge in conjunction with the ISBI 2013 conference. For this purpose, we are making available a large training dataset of mouse cortex in which the neurites have been manually delineated. In addition, we also provide a test dataset where the 3D labels are not available. The aim of the challenge is to compare and rank the different competing methods based on their **object classification accuracy** in three dimensions.

Challenge format



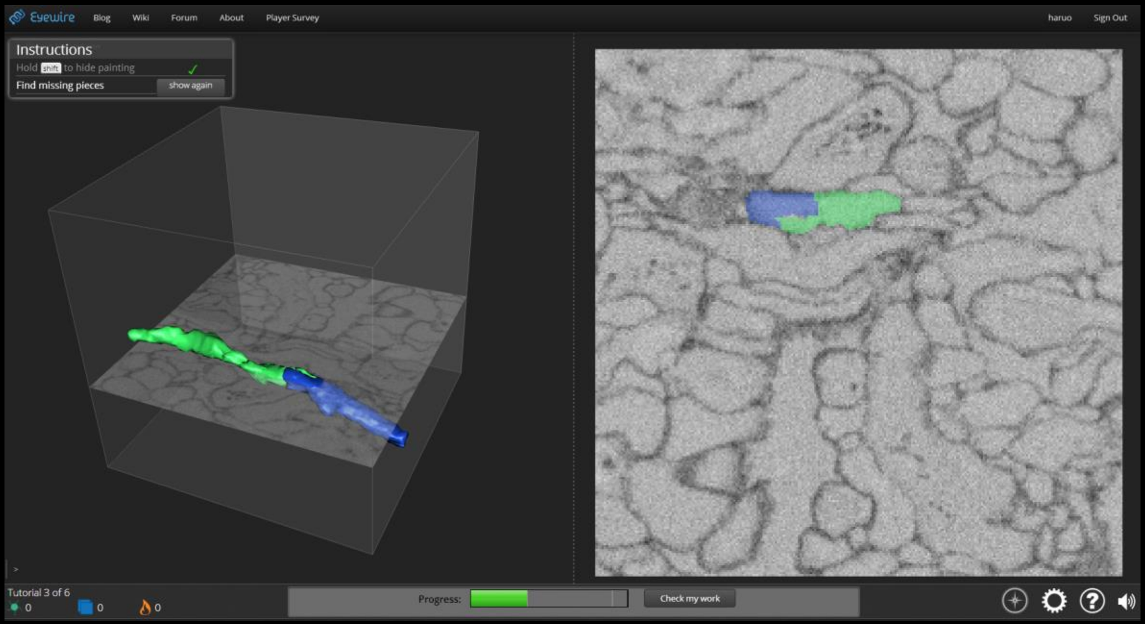
Teams wishing to participate in the challenge should register and download the training data for algorithmic tweaking and tuning. The teams should then evaluate their segmentation performance on **two test sets:** one already released with the training set that will decide the progress winners, and a second set that will be released five days before the final deadline. Finally, the participants are expected to submit an abstract describing the method that was used together with the final results by the established deadline (see important dates below). On the day of the ISBI challenge workshop itself, the best methods at the time will be expected to be explained in an oral presentation. The challenge day will conclude with a round-table discussion of the obtained results.

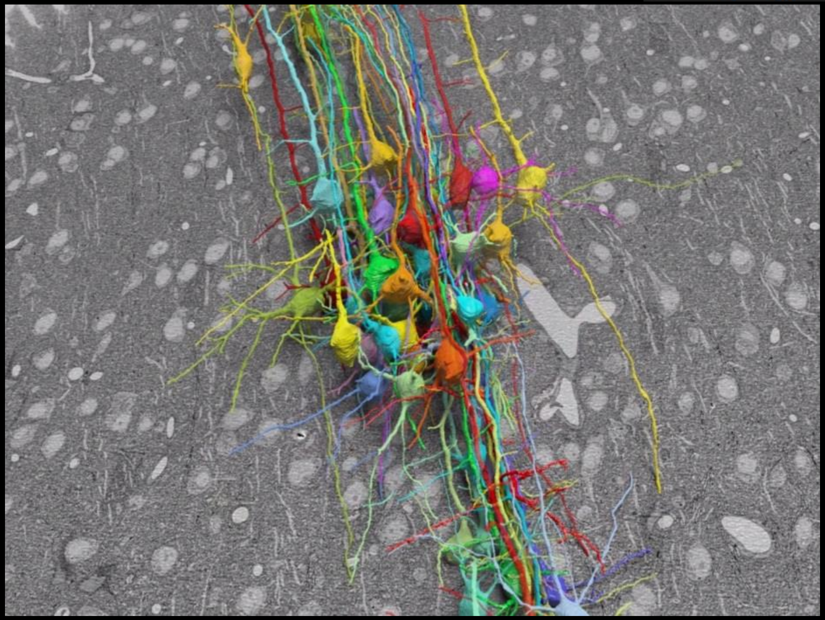
Challenge final winners: the dates of the challenge conclusion along with the release of the second test set are to be announced. The final ranking with the best methods will be made public at a conference yet to be decided. In the weeks following the challenge, participating teams will be invited to contribute to a joint paper describing and summarizing the challenge outcome, which we will then submit to a high-impact journal in the field.

Important dates

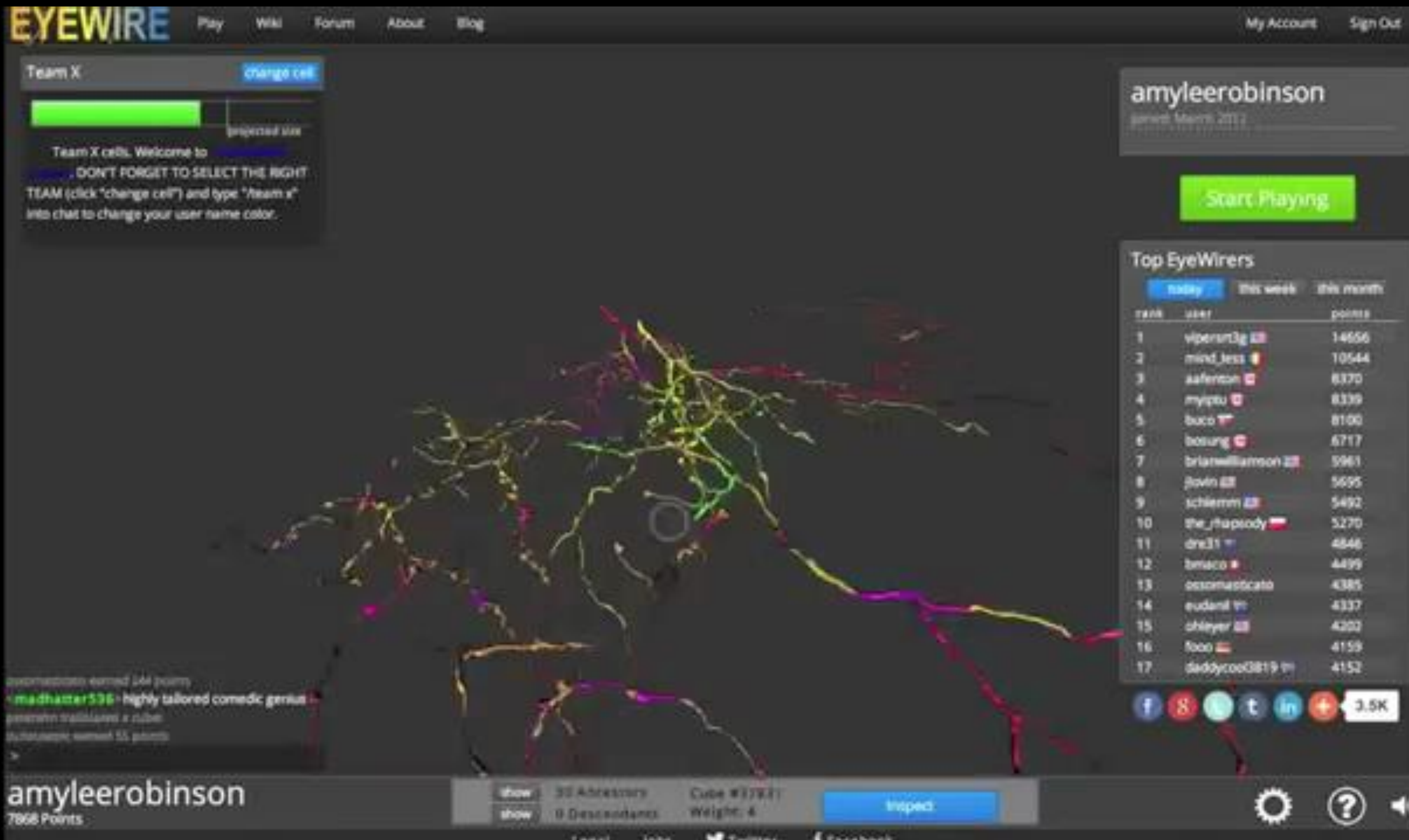
http://brainiac2.mit.edu/SNEMI3D/

EyeWire: neural annotation game





EyeWire Domo



Global gamer density

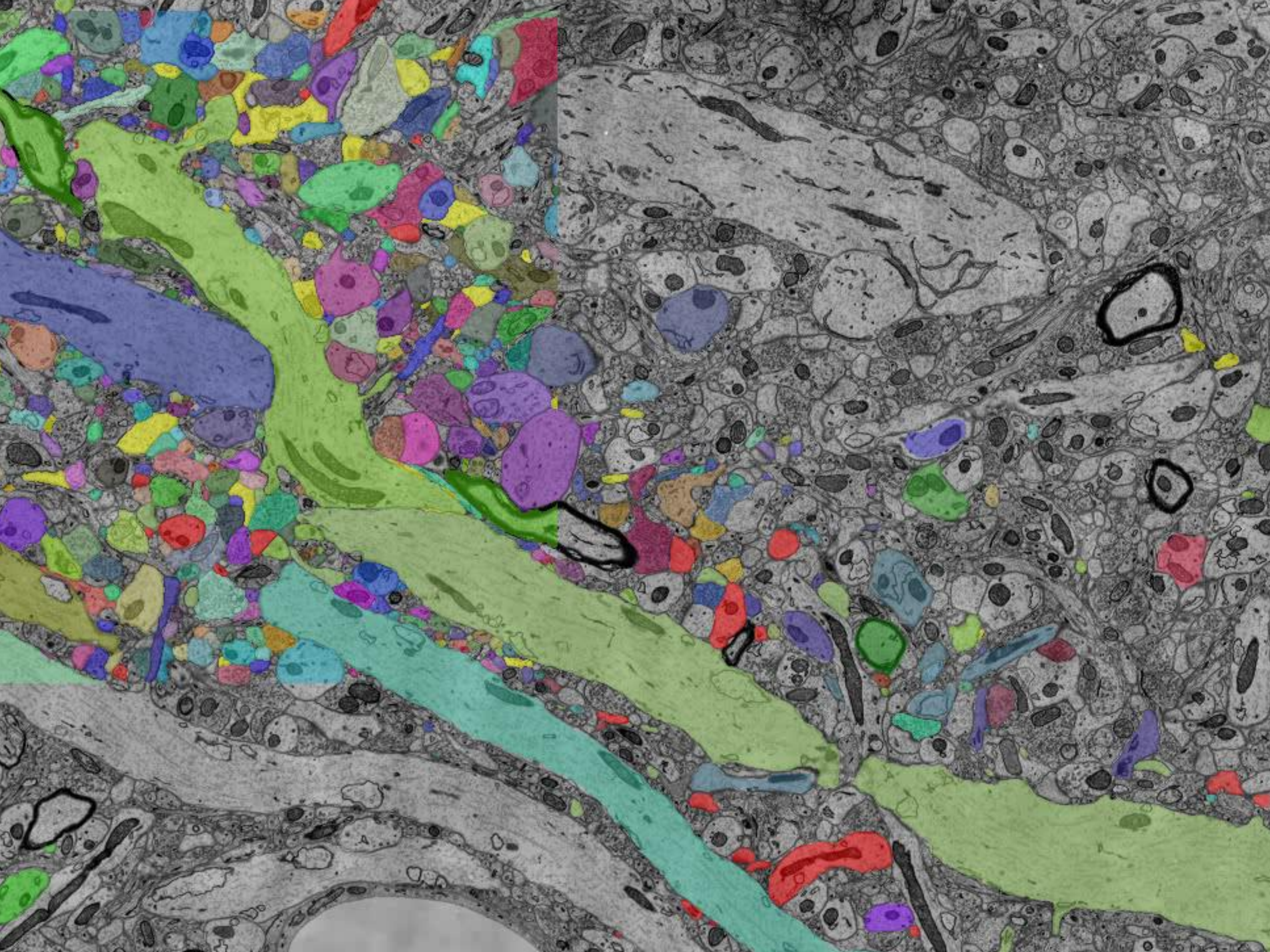
- Japan: 63/100 gamers
- USA: 55/100 gamers
- Europe: 13/100 gamers

0.14 yeas per day

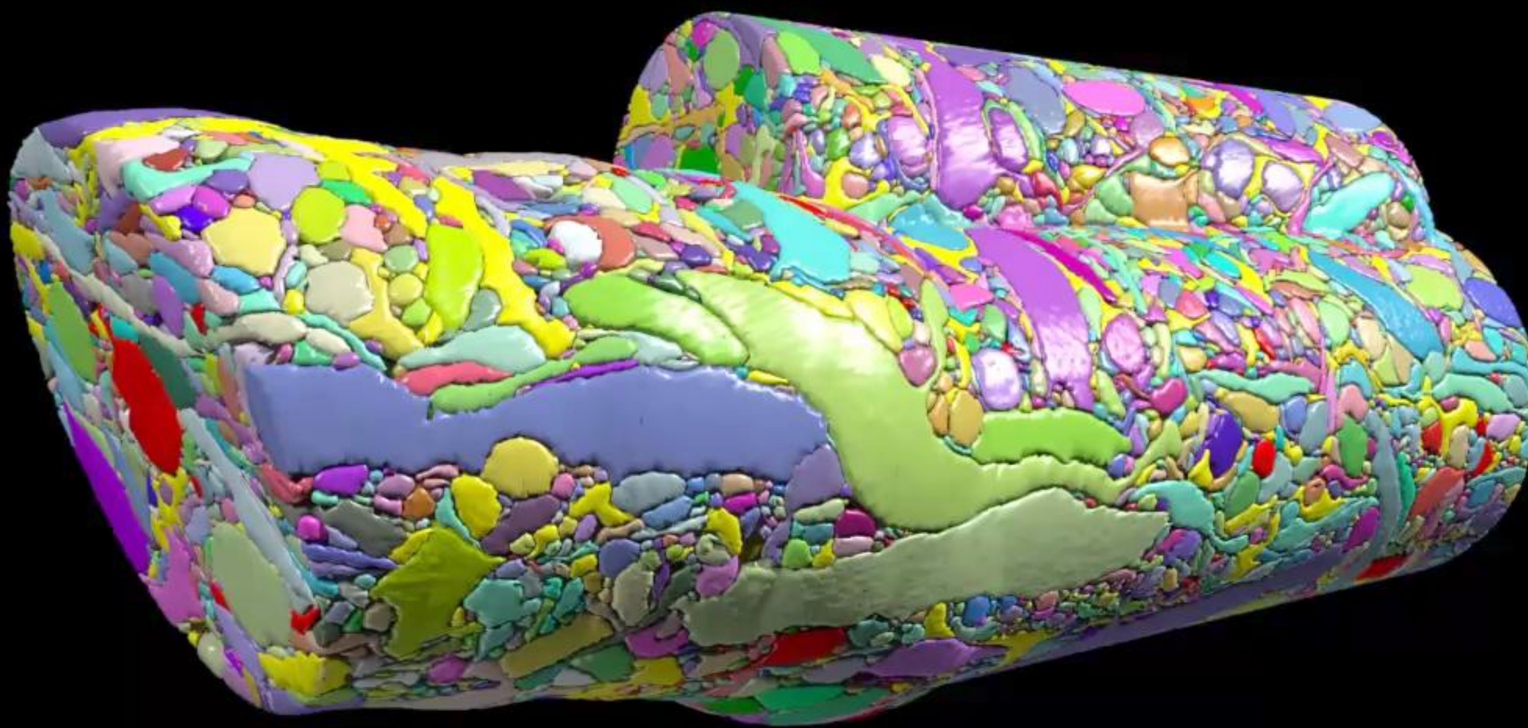
Scaling up

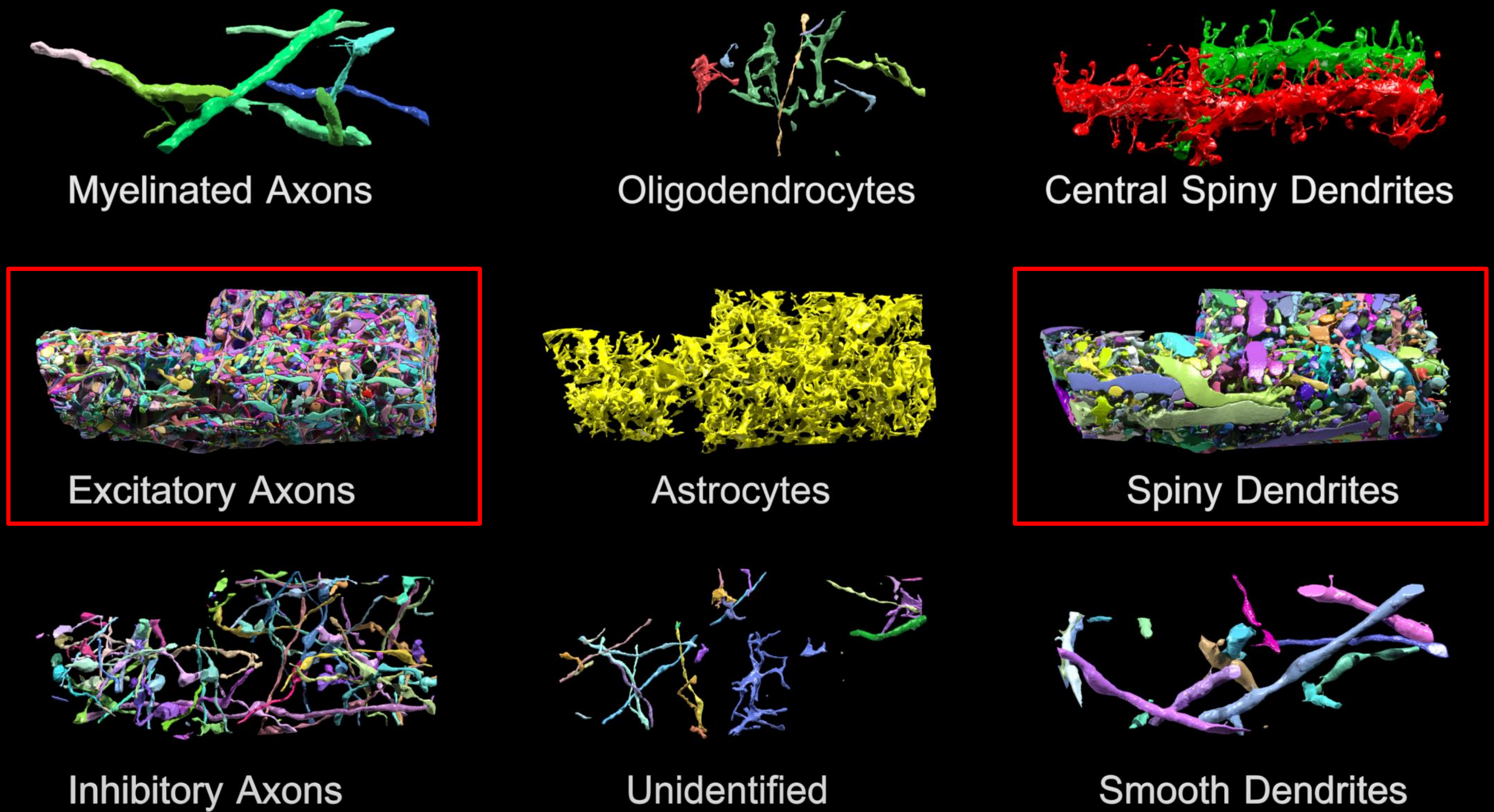
terabyte	$(0.1\ mm)^3$	retinal column
petabyte	$1mm^3$	cortical column
exabyte	$1cm^3$	mouse brain
zettabyte	$1000cm^3$	human brain

- Angry Bird
  - 600 years per day (5 million hours per day)
- EyeWire
  - Only 2 days required for $1mm^3$ annotation
    - If gamers would play as much as Angry Bird



Three-cylinder decomposition by type



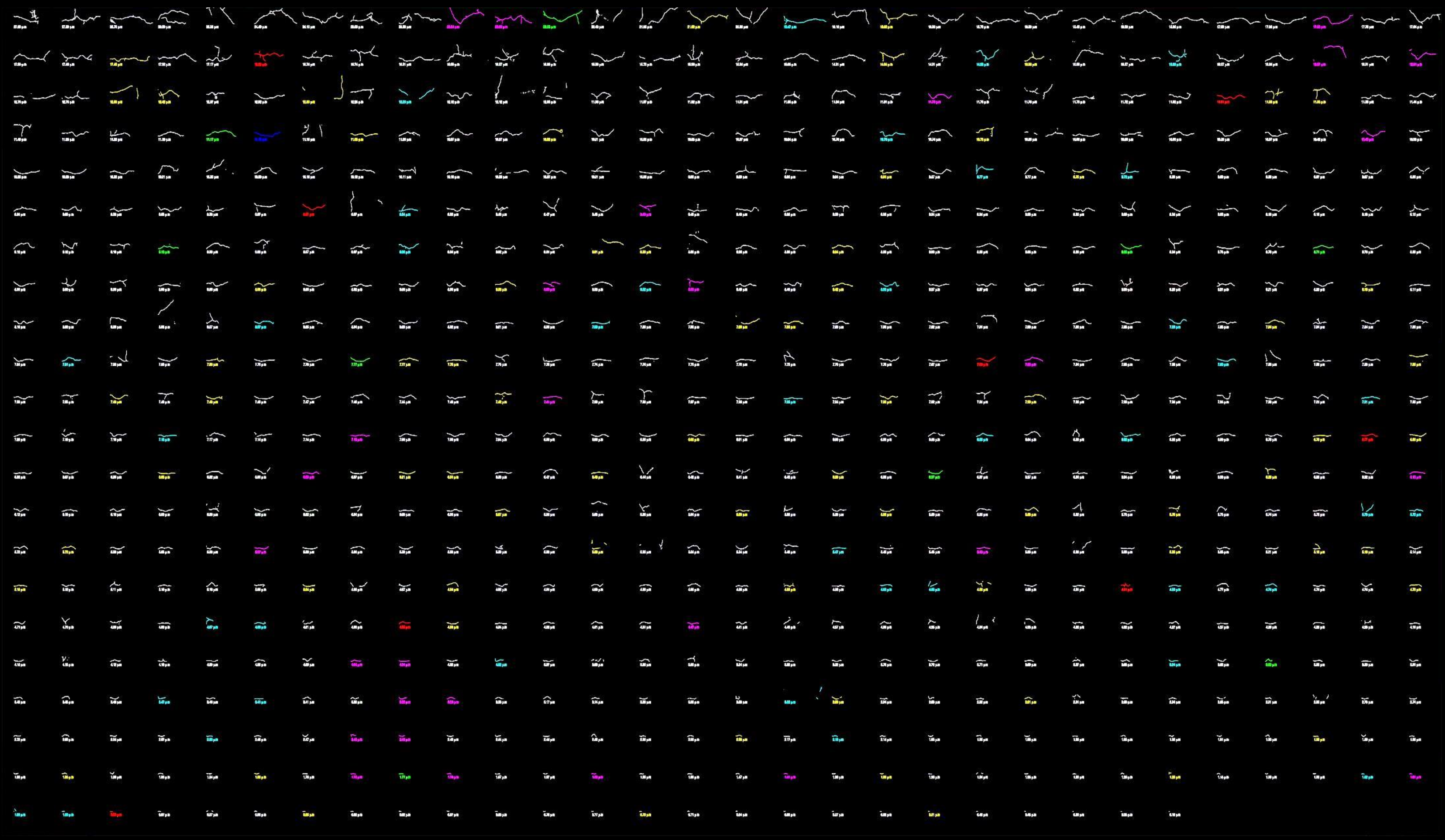

Myelinated Axons
Oligodendrocytes
Central Spiny Dendrites
Excitatory Axons
Astrocytes
Spiny Dendrites
Inhibitory Axons
Unidentified
Smooth Dendrites

What's in the cylinders?

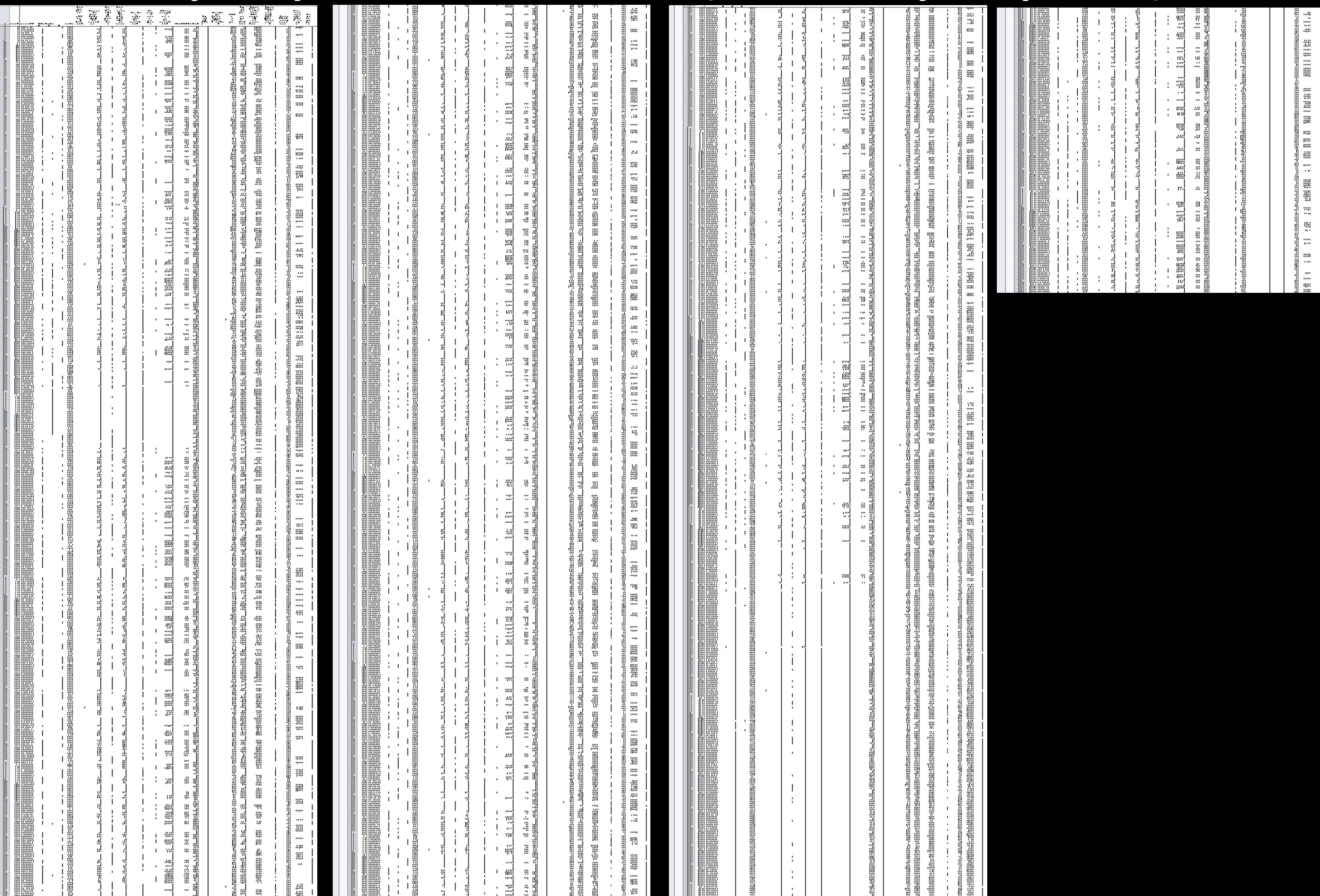
- 1407 axons
  - 94% excitatory
  - 5% inhibitory
  - 1% ?
- 193 dendrites (~7-fold fewer than axons)
  - 91% excitatory "spiny"
  - 9% inhibitory "smooth"
- 1700 synapses in 1500 mm^3 (1.13 synapses/mm^3)
  - 92% excitatory
    - 92% on spiny dendrites
    - 8% on smooth dendrites
  - 8% inhibitory
    - 93% on spiny dendrites
    - 7% on smooth dendrites

N Kasthuri, D Berger, et al

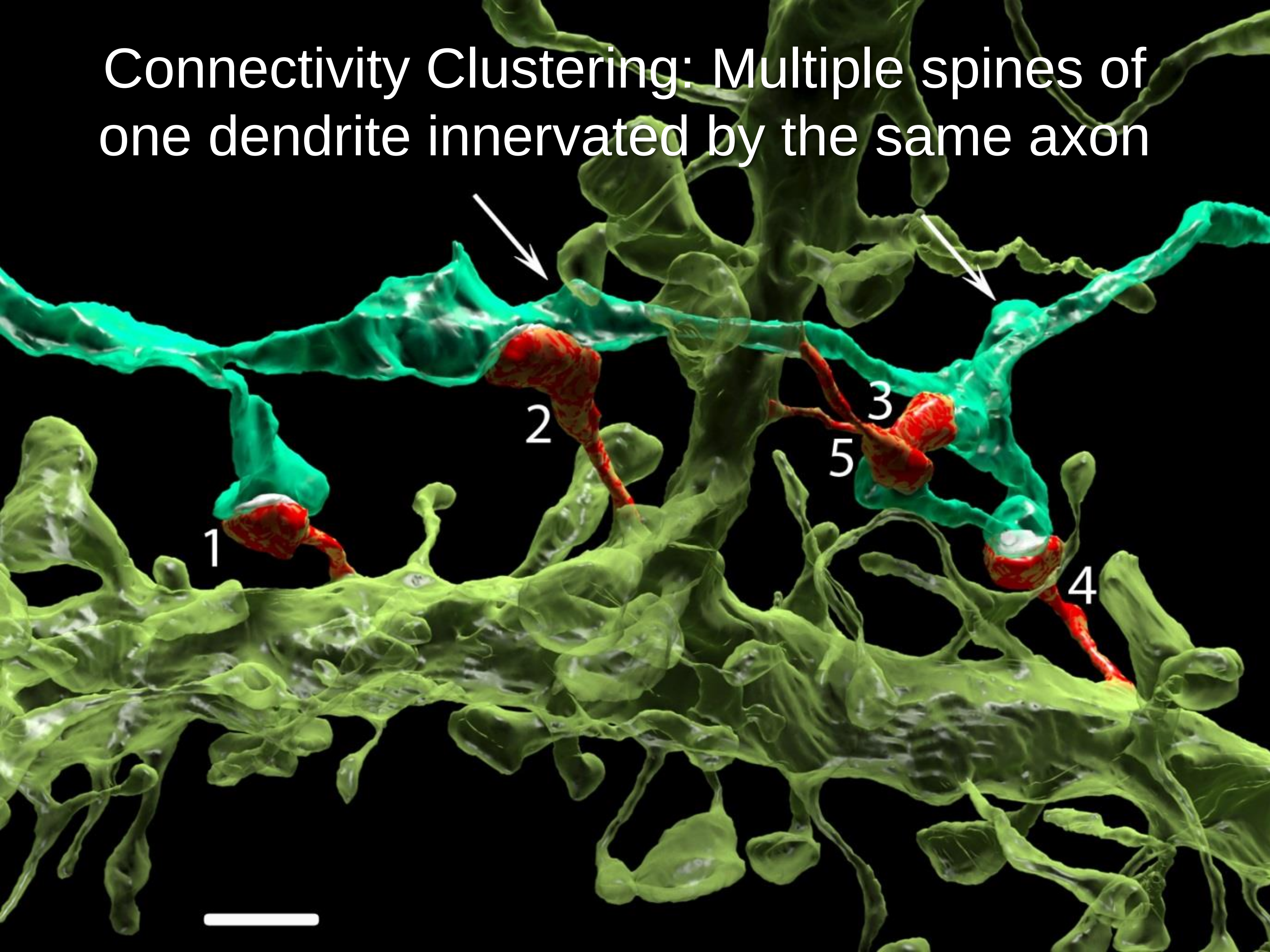
Database of all the axons in red cylinder

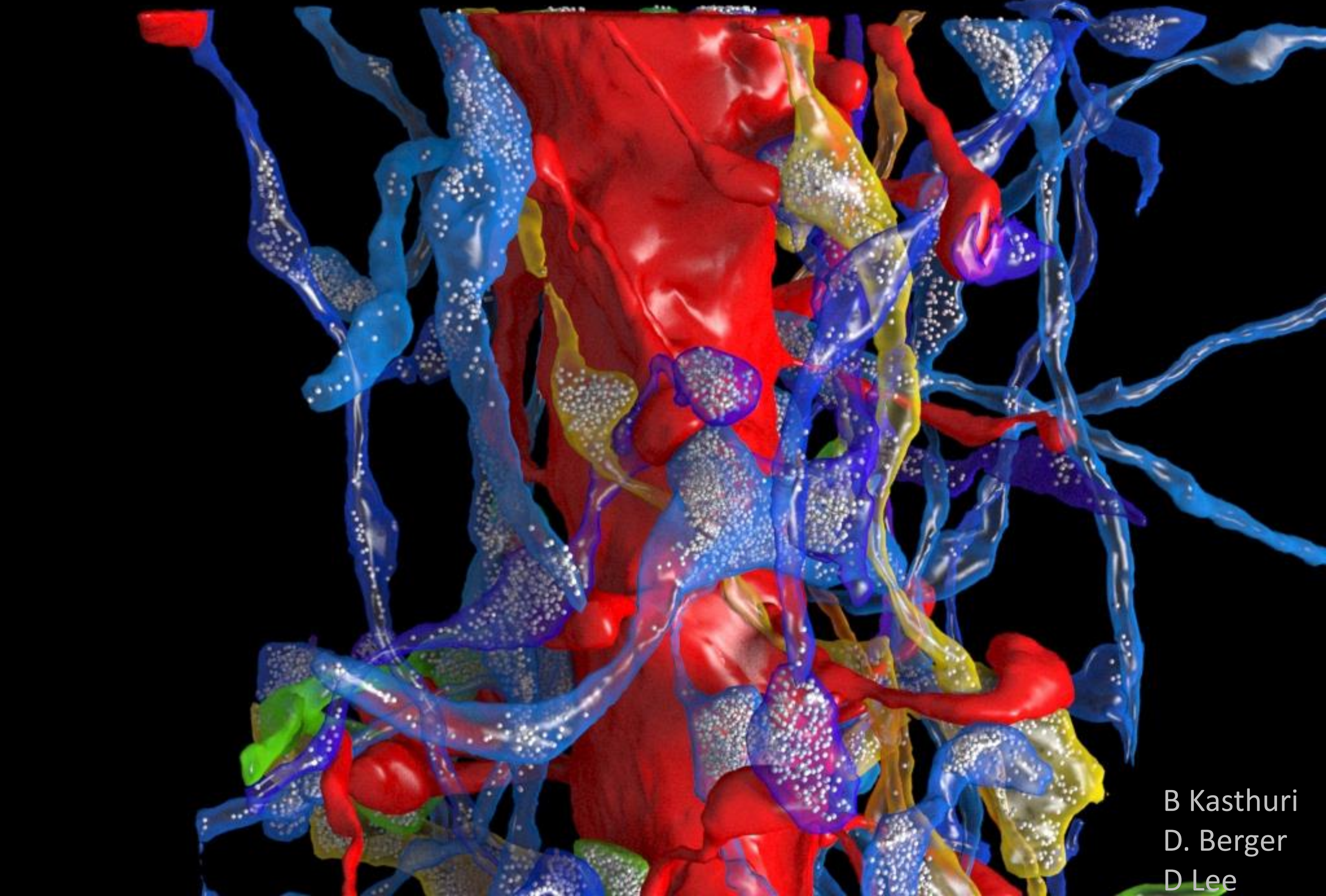


Synapse database (1700 synapses)

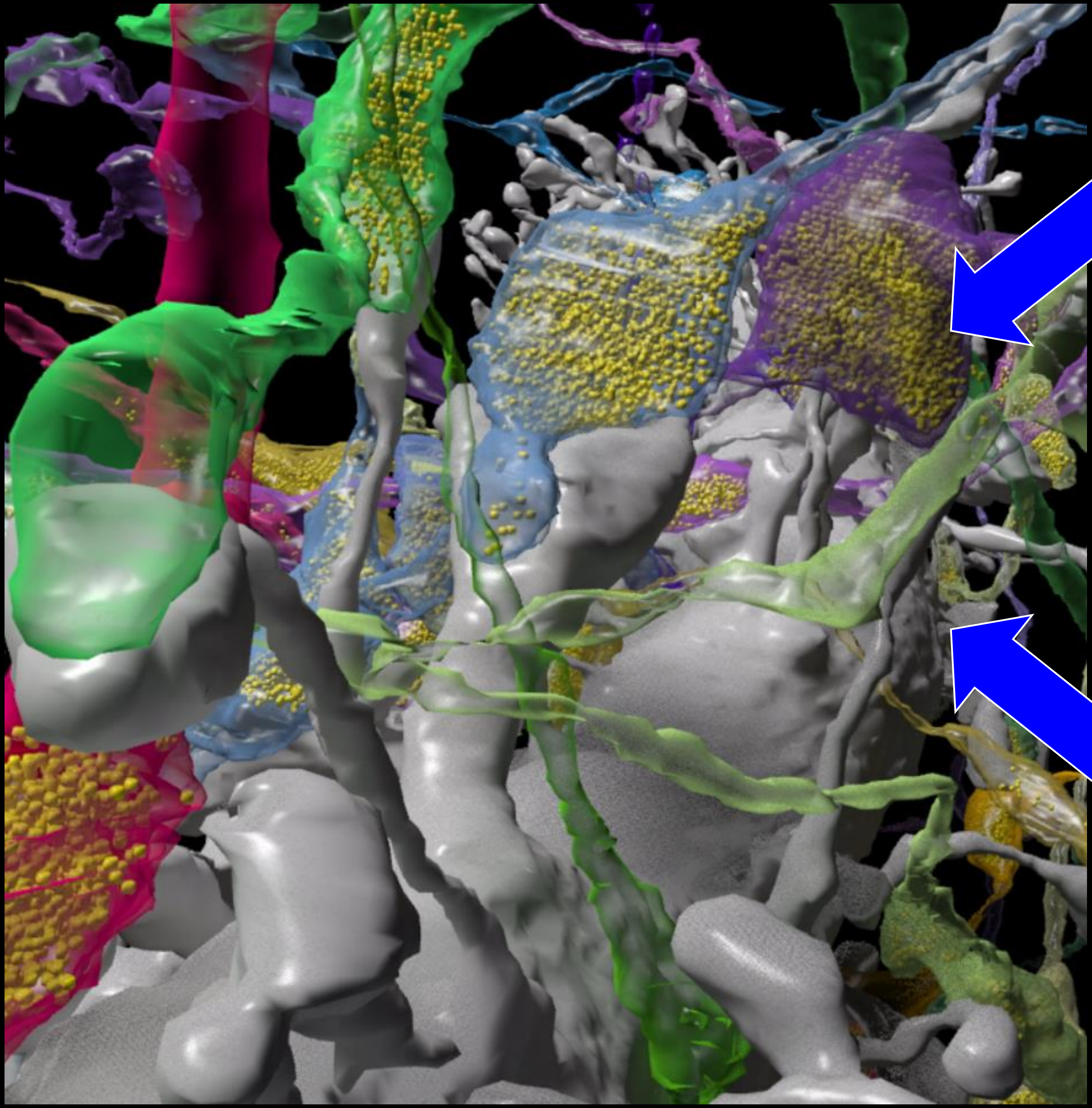


Connectivity Clustering: Multiple spines of one dendrite innervated by the same axon




B Kasthuri
D. Berger
D Lee

Real Brains in the Computer



Synapse Catalogs

Anatomy
Form of neuron
Size of neuron
Number of neuron
Length of axons & dendrites
Projection of axons

Function
Electrical properties
Spike timing
Transduction speed
Transmission efficacy
Neurotransmitters
Receptors
Expressed genes
Physiological significance
Disease (dysfunction)

コネクトームの応用先（?）

- 特定の技術の運用が上手い人と下手な人
  - 自動車の運転
  - 作曲、絵画
  - スポーツ選手
- 病理が難解な神経疾患の機構解明
  - 統合失調症、うつ病、自閉症
- アインシュタインの脳解析
  - 天才性の追究

Jeff Lichtman Lab

- Bobby Kasthuri (Argonne/UChicago)
- Daniel Berger
- Richard Schalek
- Seymour Knowles-Barley
- Josh Morgan

- Ken Hayworth (Janelia)
- Dong-il Lee
- Allysa Wilson
- JC Tapia (Columbia)
- David Hildebrand (Engert)
- Verena Kaynig (Pfister)

- Hanspeter Pfister
- Nir Shavit (MIT)
- Art Wetzel (CMU)
- Aravi Samuel
- Florian Engert
- Takao Hensch
- David Clapham
- Michael E Greenberg

NIH
NIMH (Conte Center)
Dept Army (MURI)
HFSP
IARPA