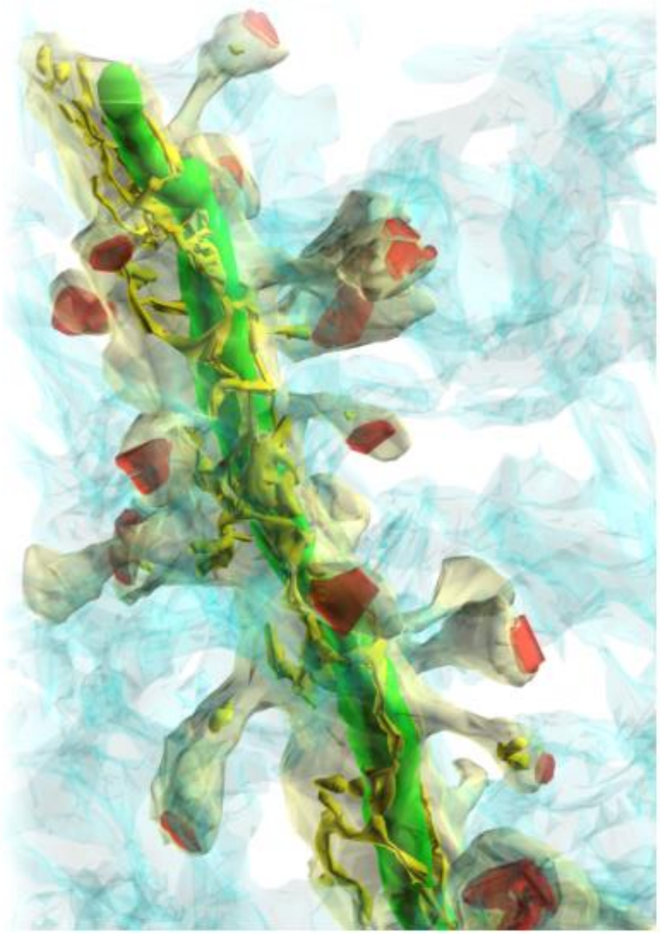
Understanding Synapses in the Brain



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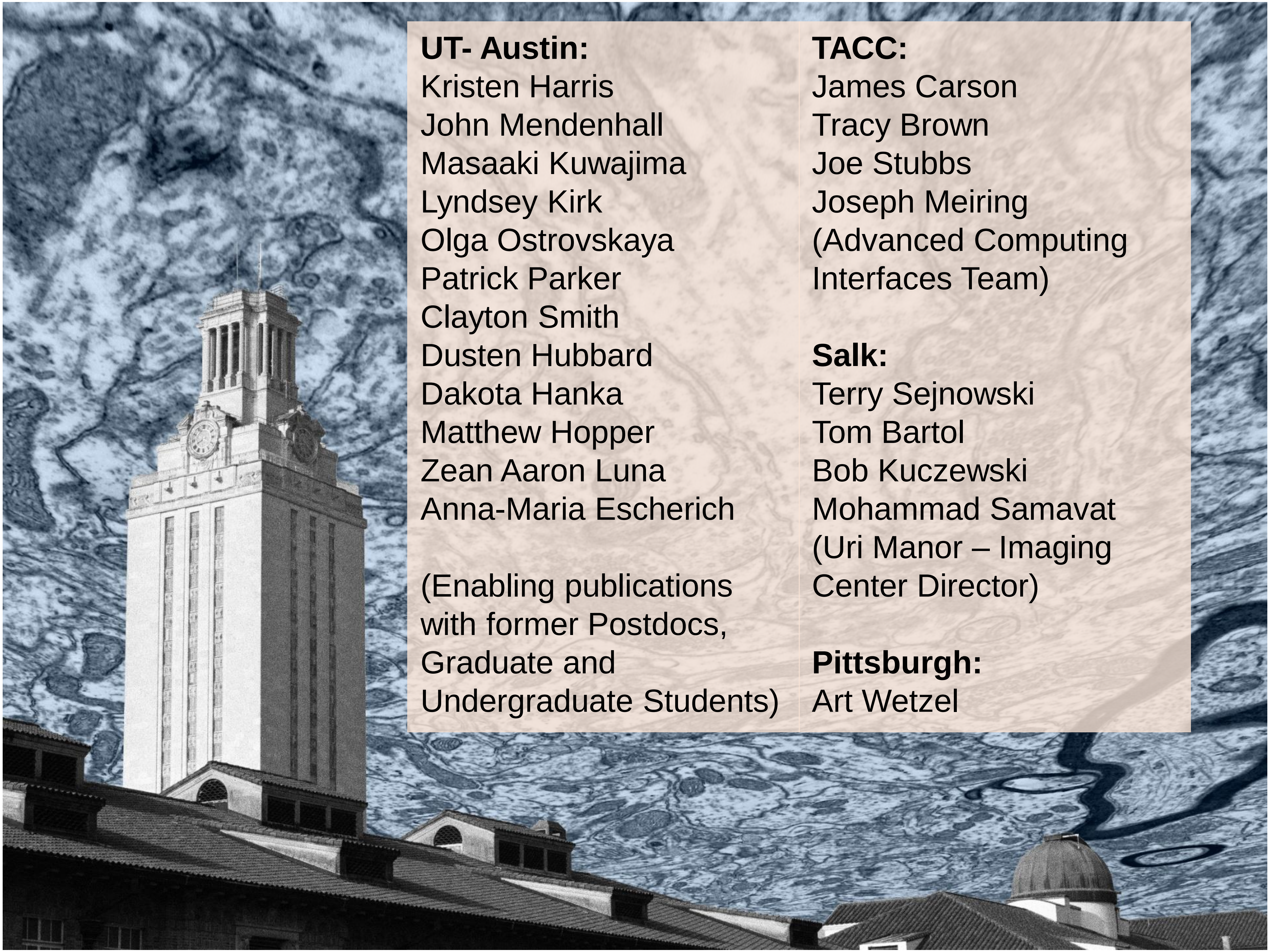
Salk Institute for Biological Studies

5 April 2019 NSF Large Facilities Workshop – Austin
Envisioning the future of facility science and cyberinfrastructure
NeuroNex Award No.1707356



UT- Austin:
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(Enabling publications with former Postdocs, Graduate and Undergraduate Students)

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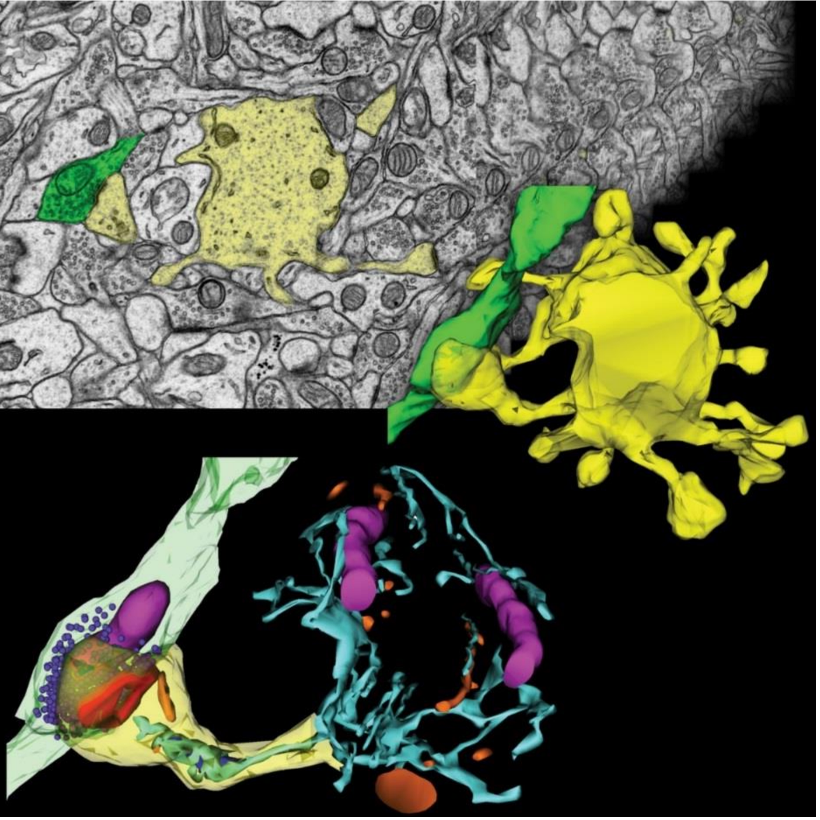
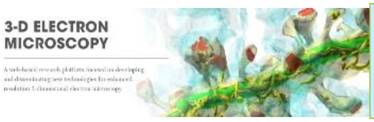


2013 Kuwajima, Spacek, Harris
Neuroscience 251:75

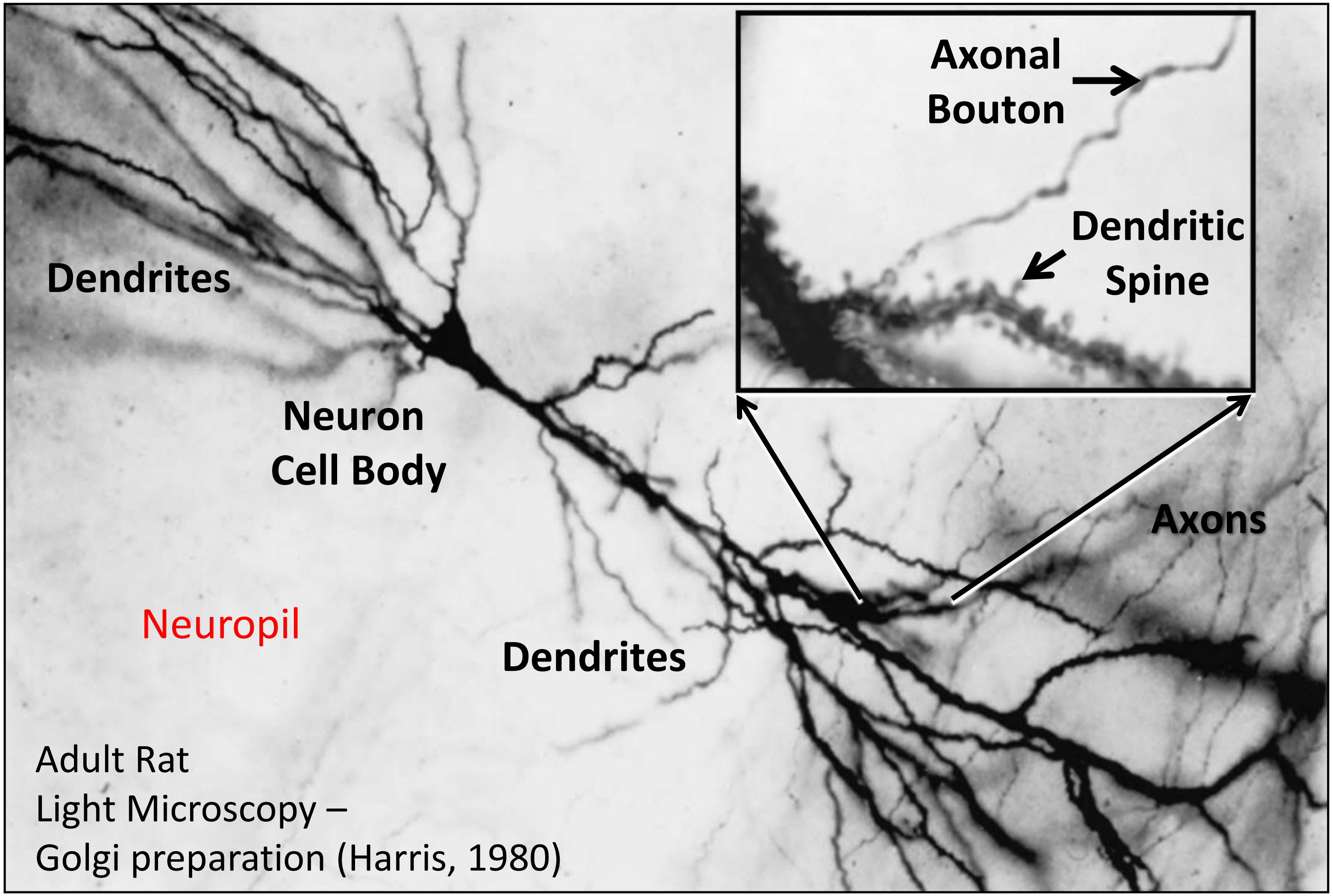
Goals for Today

- What is a Synapse?
- How does coordination with a cyberinfrastructure facility help us to understand synapses.



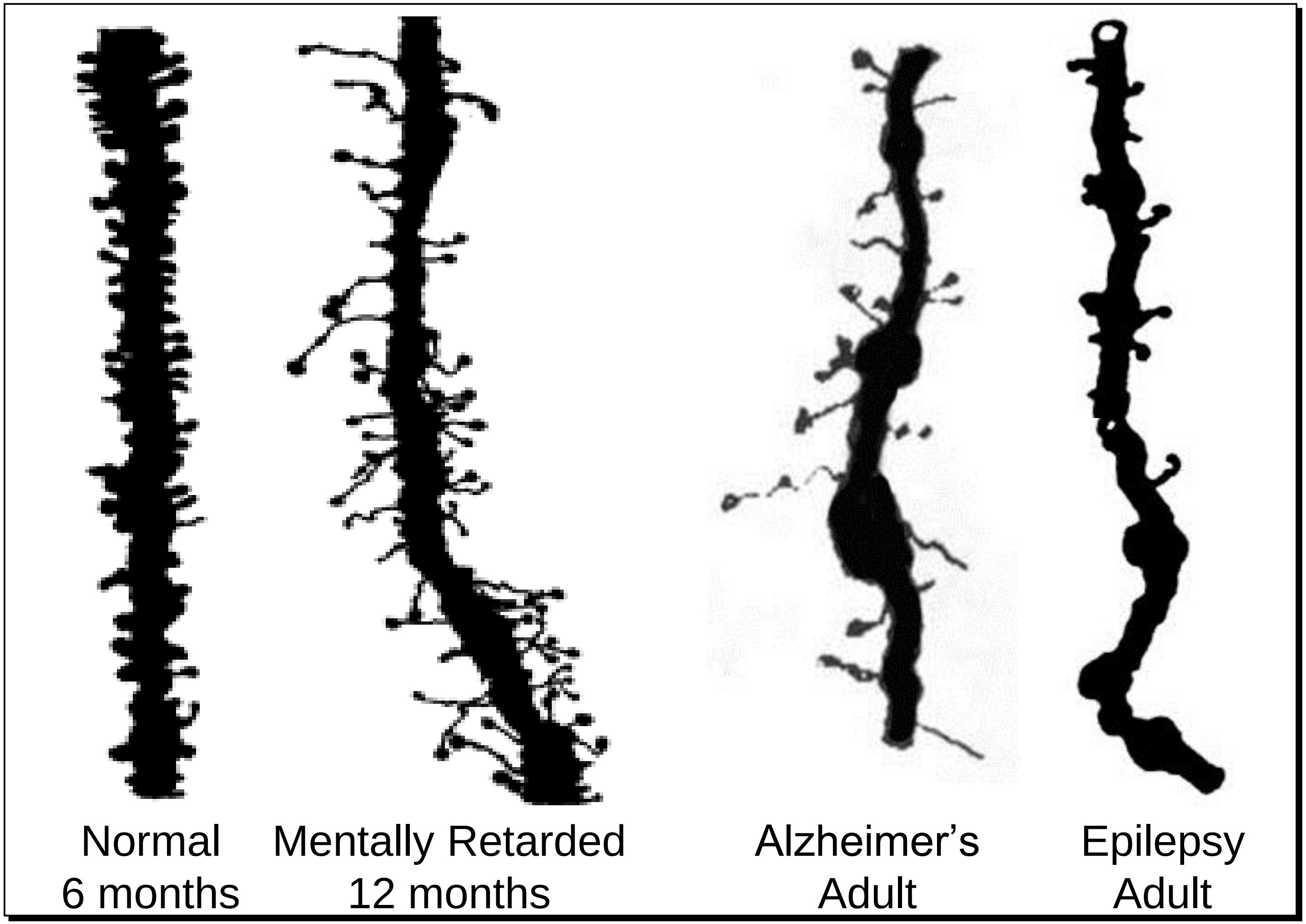
5 April 2019 NSF Workshop
NeuroNex Award No.1707356

Synapses are the sites of communication between neurons.



Adult Rat
Light Microscopy –
Golgi preparation (Harris, 1980)

Diseases of Dendrites and Spines



Normal
6 months

Mentally Retarded
12 months

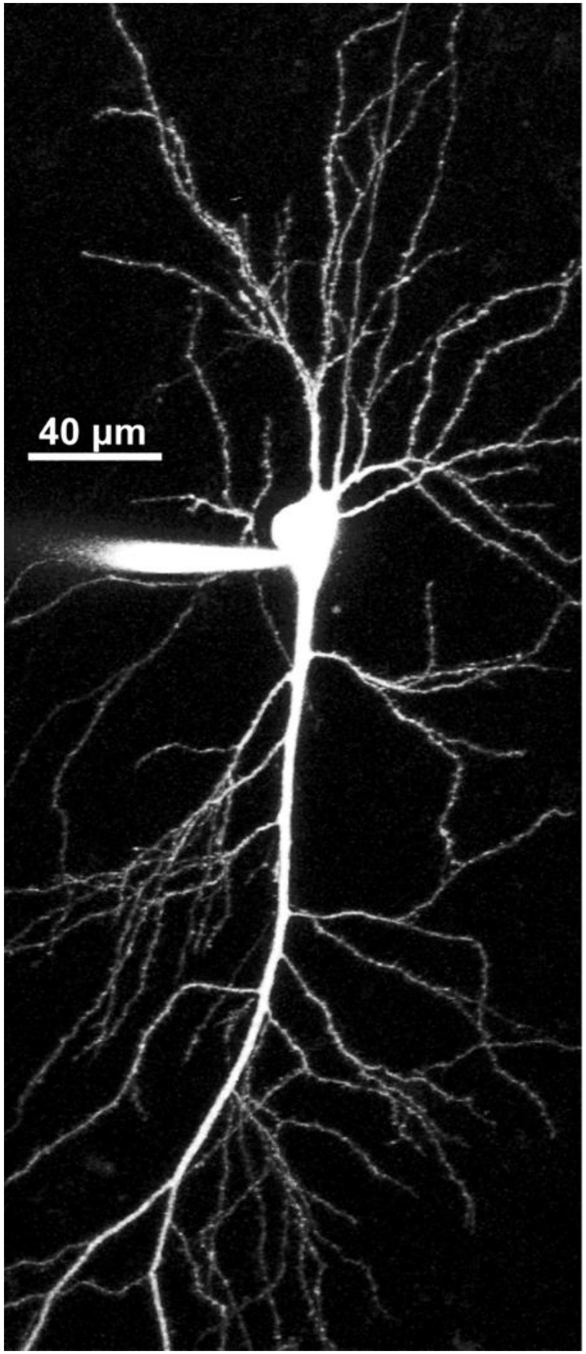
Alzheimer's
Adult

Epilepsy
Adult

Purpura 1974

Schiebel 1983, 1986

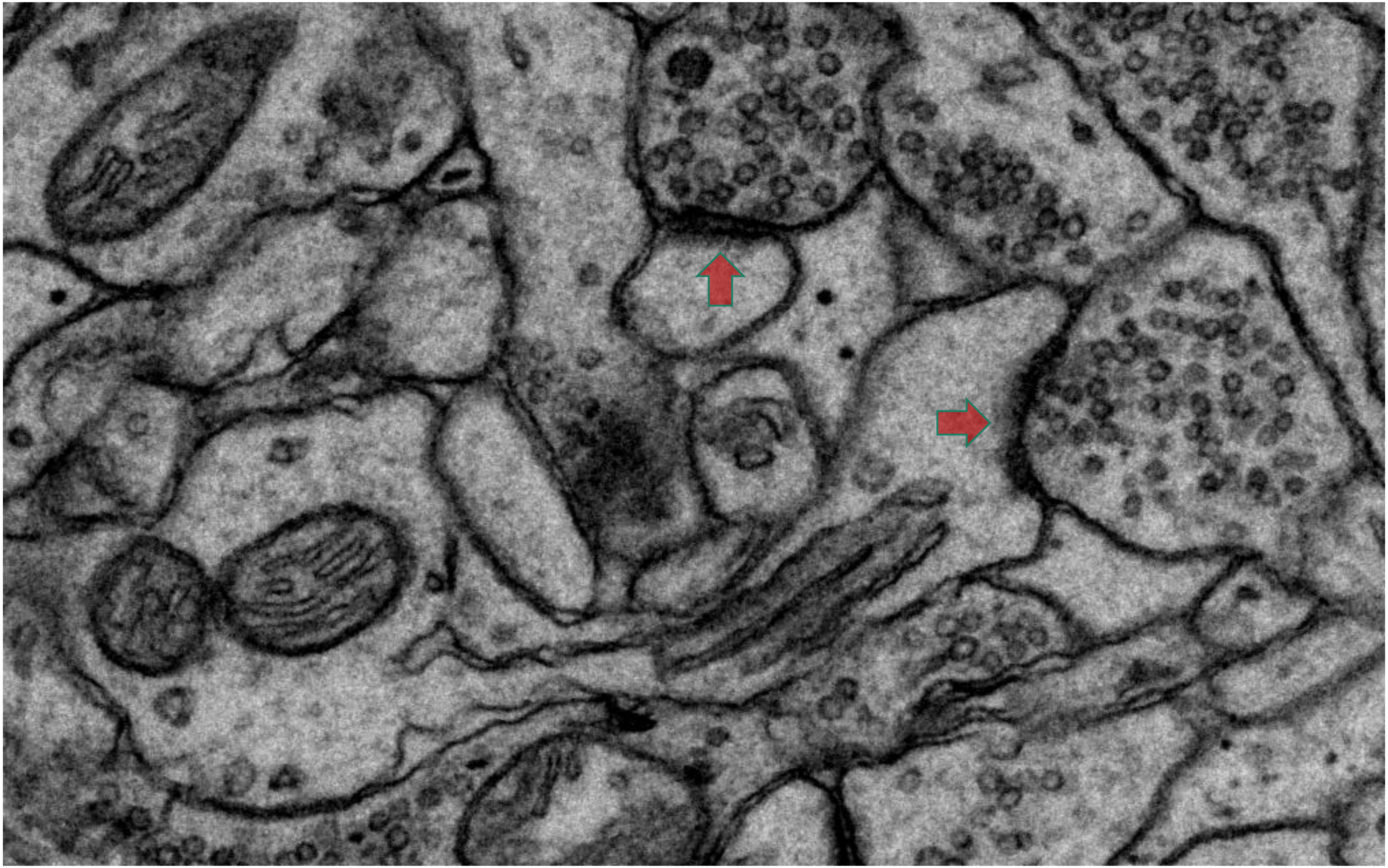
Modified from Fiala, Spacek, Harris 2002, Brain Res. Rev. 39:29-54.



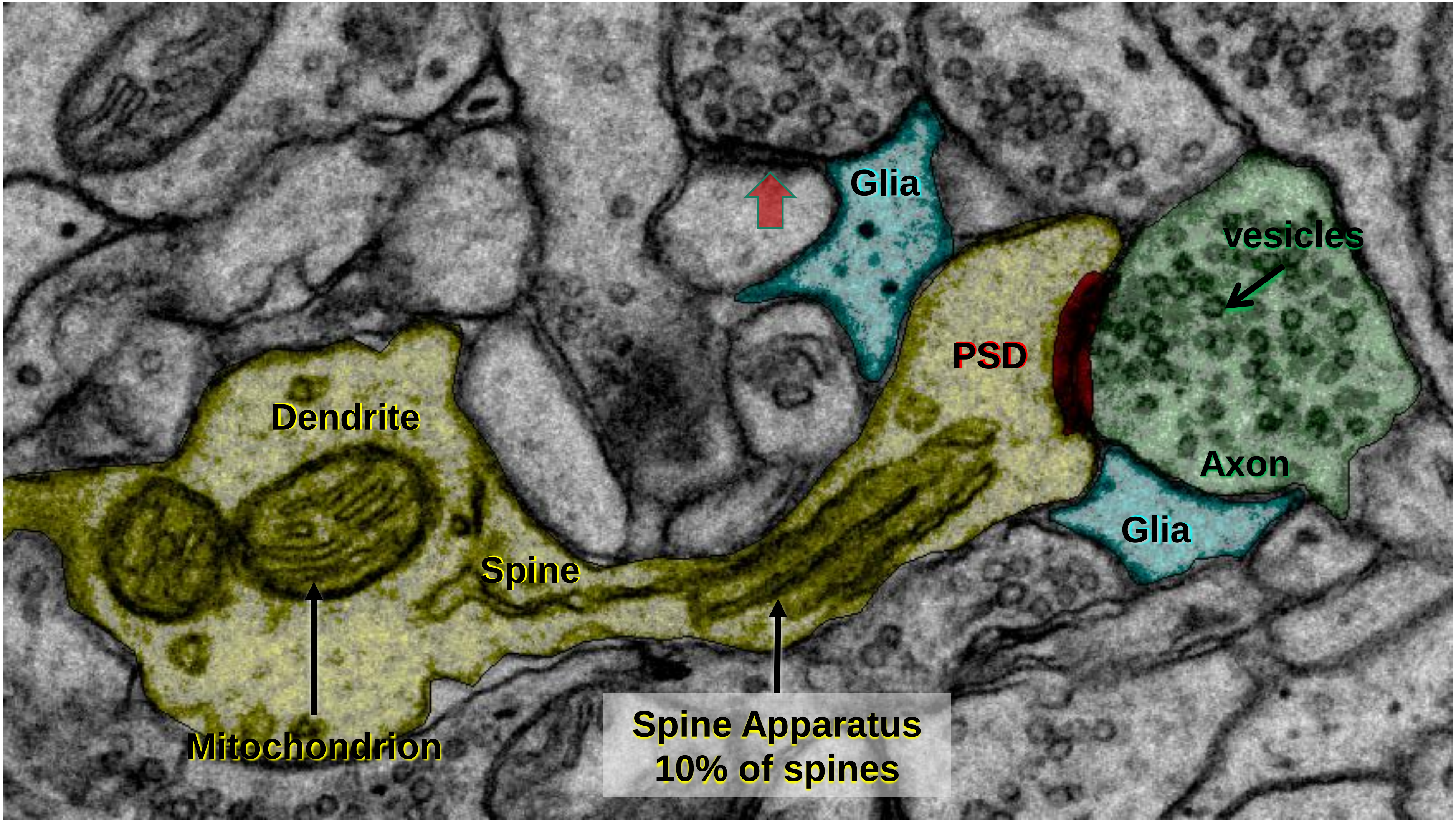
Light Microscopy Provides

- Large fields
- Visualization of living cells over time
- But, not enough resolution to study synapses.
- Need Electron Microscopy (EM)

Synapses on Dendritic Spines in EM

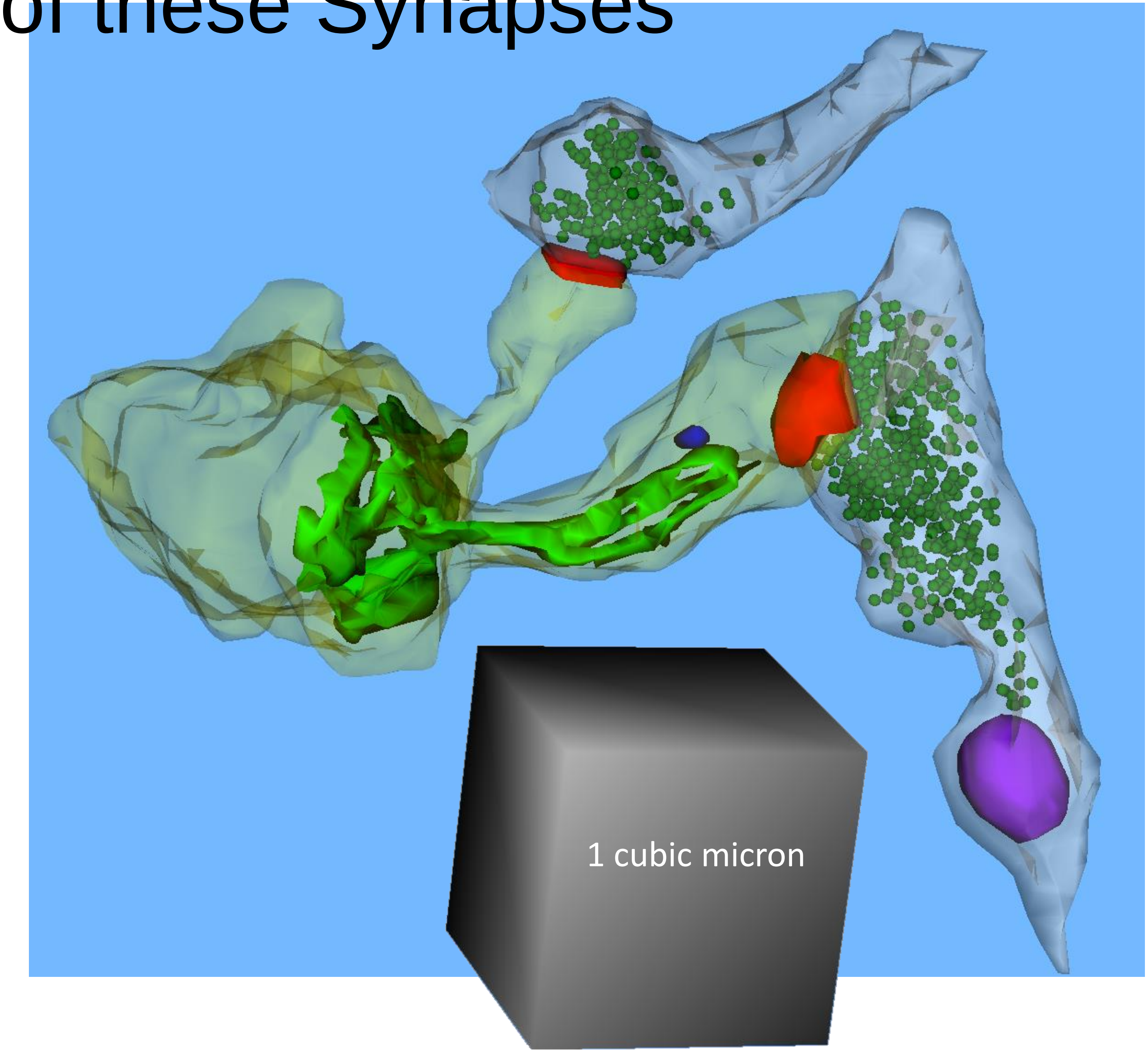


Synapses on Dendritic Spines in EM



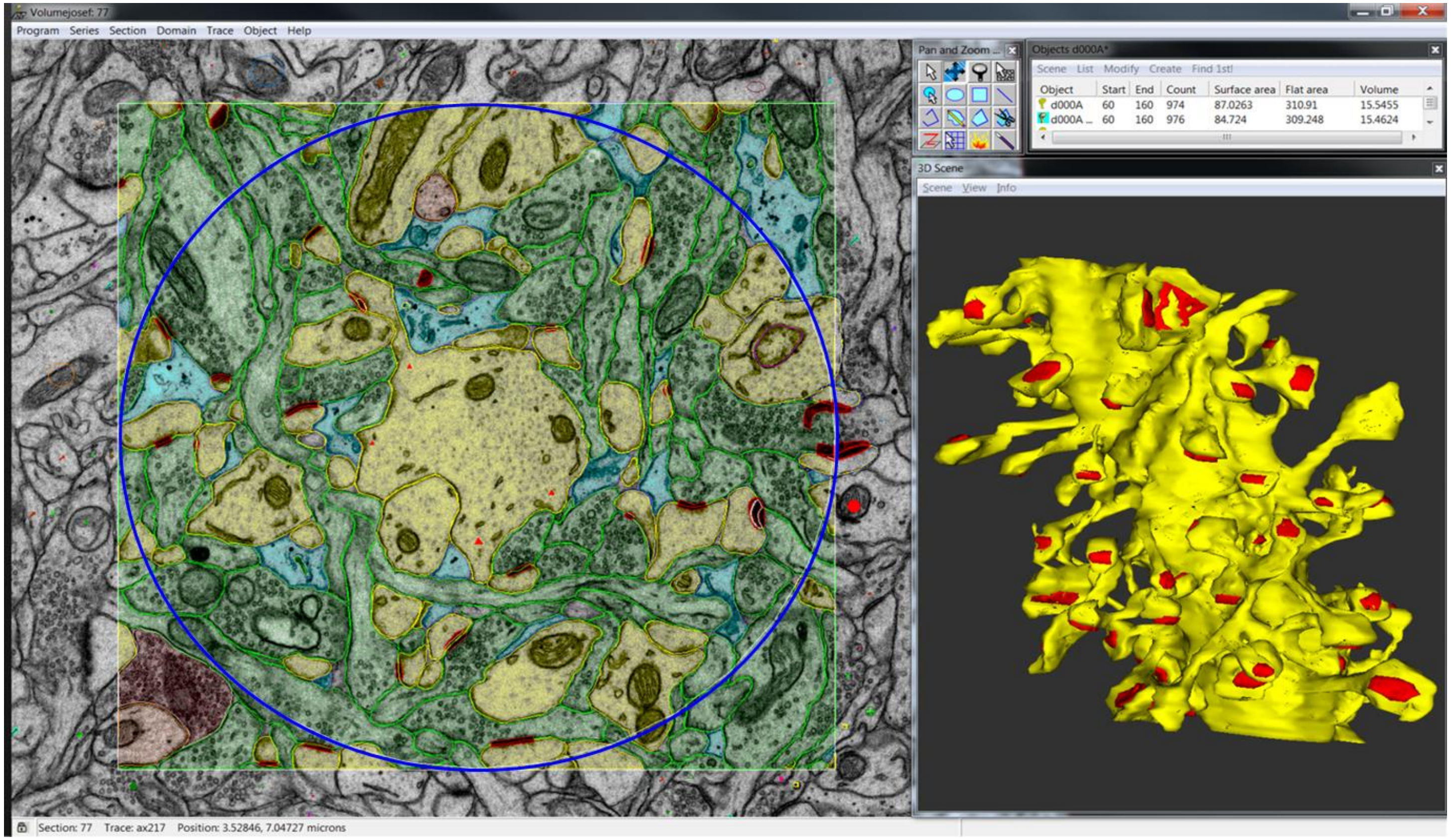
Spine Apparatus
10% of spines

3D of these Synapses



3DEM reveals subcellular resources in context of synapses

John Fiala
Harris Lab
1999…



Object	Start	End	Count	Surface area	Flat area	Volume
d000A	60	160	974	87.0263	310.91	15.5455
d000A ...	60	160	976	84.724	309.248	15.4624

Section: 77 Trace: ax217 Position: 3.52846, 7.04727 microns

http://synapseweb.clm.utexas.edu > Tools > Software

Cruise Through Hippocampal Neuropil

Credits

University of Texas at Austin

Josef Spacek, Kristen Harris, Larry Lindsey, Patrick Parker
Chandra Bajaj, Jarred Bowden

The Salk Institute

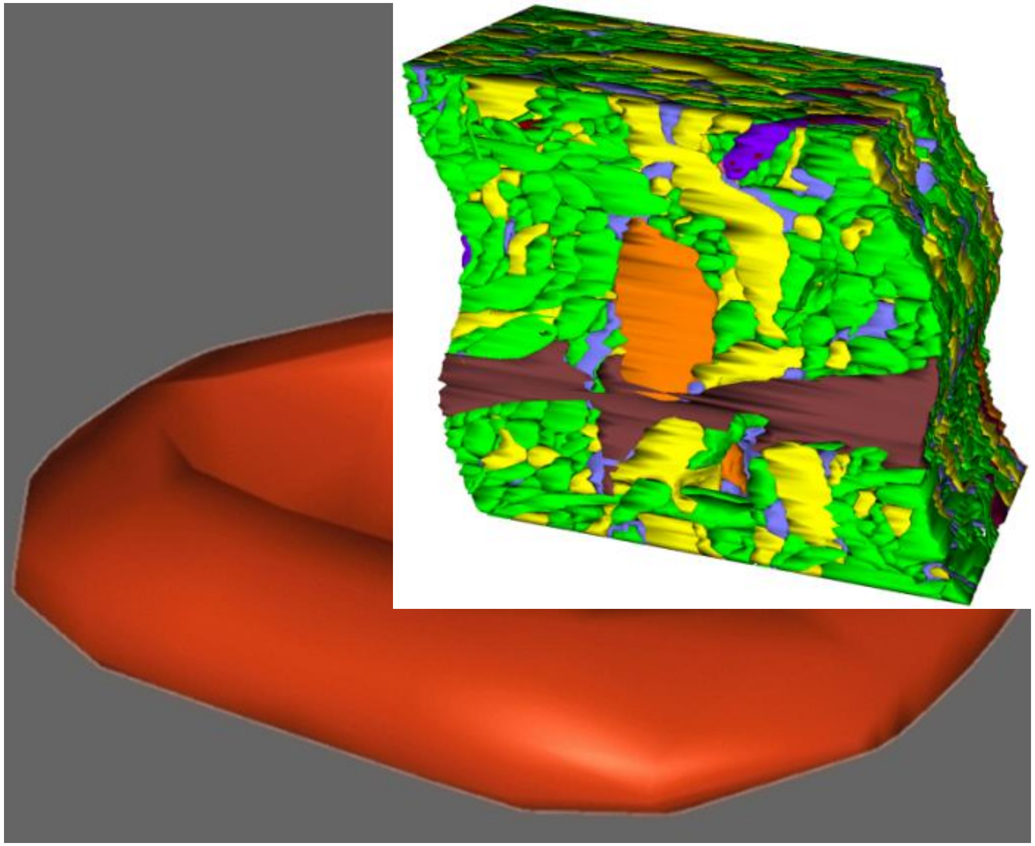
Justin Kinney*, Tom Bartol, Terry Sejnowski
Dan Keller, Varum Chaturvedi

3D – Blender
Music by: Camille Saint Saëns, Carnival of the Animals, Aquarium

Available: YouTube – 2014 New York World Science Festival with narration
https://www.youtube.com/watch?v=hpxHSISSUes&feature=youtu.be
Or on SynapseWeb with other tutorials:
https://synapseweb.clm.utexas.edu/tutorials - bottom of the page.

First fully reconstructed volume.

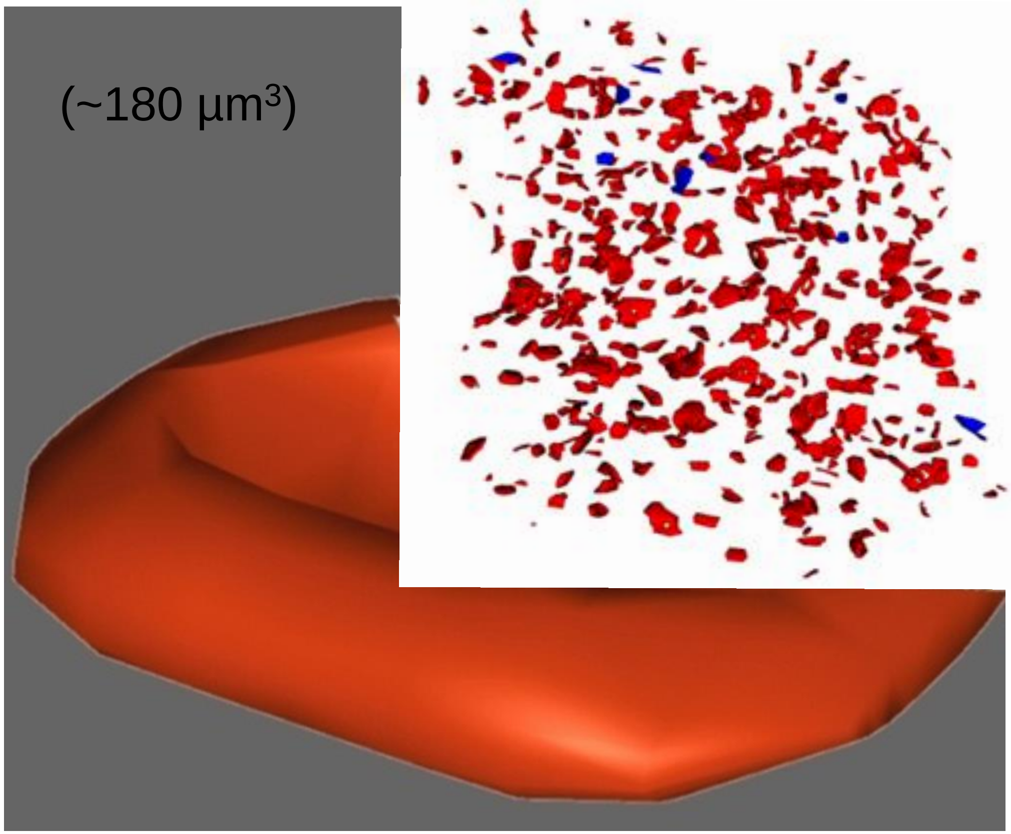
Josef Spacek
Charles University
Hradec Kralovè
Czech Republic



(~180 µm^3)

Harris et al. (2015) (Nature) Sci. Data

~500 (498) synapses in volume = 1 RBC!!



Repeat ~8 trillion times for 1 human brain (1500 cm^3)
Current methods, > 8 trillion years of human labor!

Many papers have resulted from sharing this dataset.

Harris et al. (2015) (Nature) Sci. Data

NSF NeuroNex Hub Motivation:

- Variance in synapses is not known.
    - dimensions, connectivity, and content across cell types, brain regions, species….
- Knowledge needed to assess if model systems represent human brain synaptic functions.
- Current approaches are limited by
    - resolution
    - inefficient and insufficient data collection
    - analysis bottlenecks
    - use and dissemination of data and knowledge

NSF NeuroNex Hub
Aims:

1) Collect, compare, and share nanoscale volumes of synaptic neuropil across brain regions and species.
2) Improve axial resolution with tilt tomography on the scanning electron microscope.
3) Integrate and test software tools to enhance analysis of synaptic neuropil.
4) Integrate and disseminate enhanced imaging output and tools with high performance computing.

NSF NeuroNex Hub
Progress so far

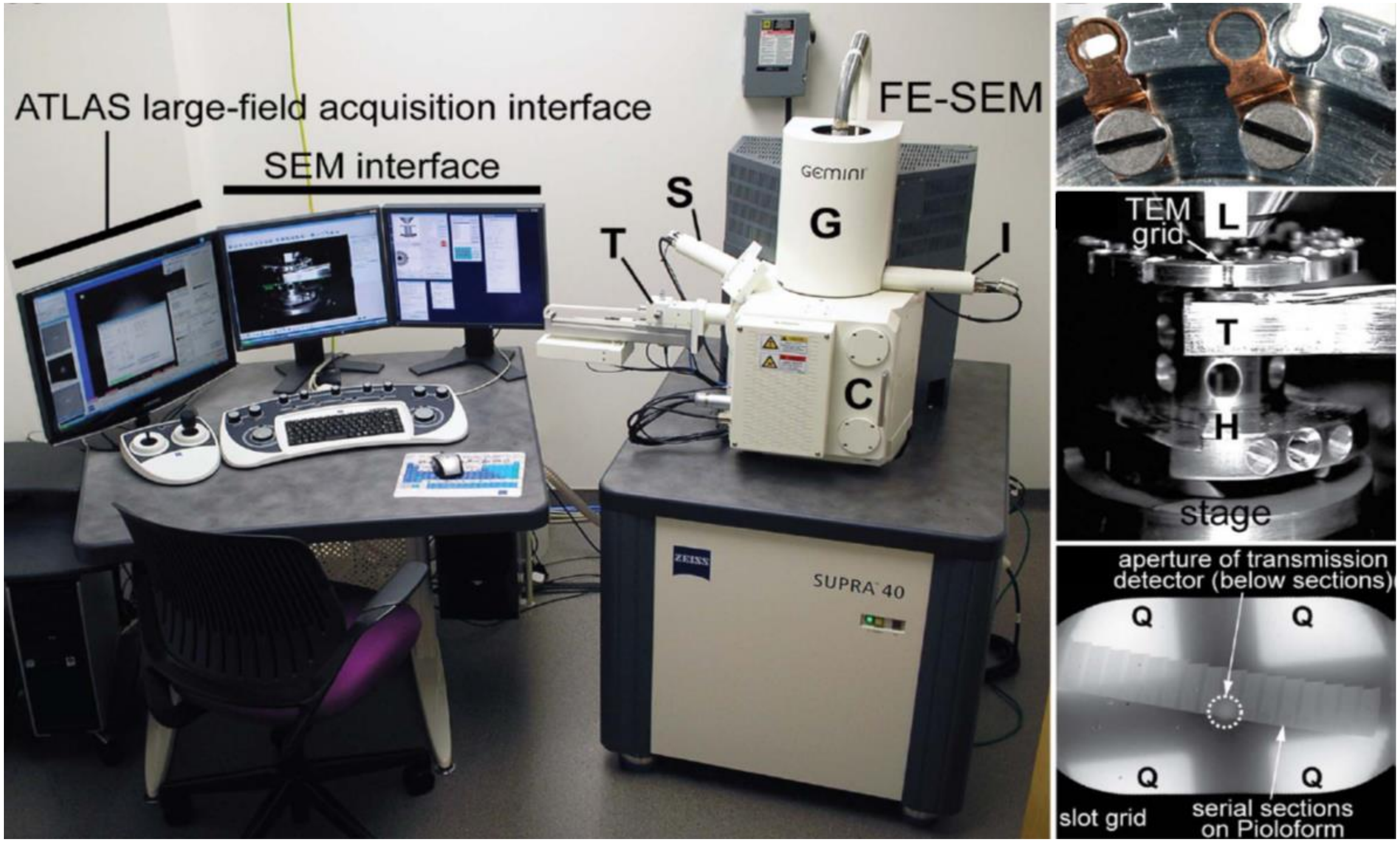
- **First Human tSEM series posted**
- **Designed and implemented “tomoSEM”**
- **Collected 1st conical tilt images**
- **Launched Portal at TACC**
- Procured new tSEM and ultramicrotome
- Refined *en bloc* staining
- Refined Neuropil Tools and Virtual Ultramicrotome
- Initiated ReconstructJAVA with SWiFT-IR alignment
- Created Github sites for software development
- Created Github testbeds for data sharing
- Held first community workshop at UT-Austin
- Initiated multiple collaborations
- Attended Brain Initiative meeting
- Publishing papers, talks, and conferences

(Details on pages 2-3/17 annual report and 2-4/18 interim report.)

Scanning Electron Microscopy in the Transmission Mode (tSEM)

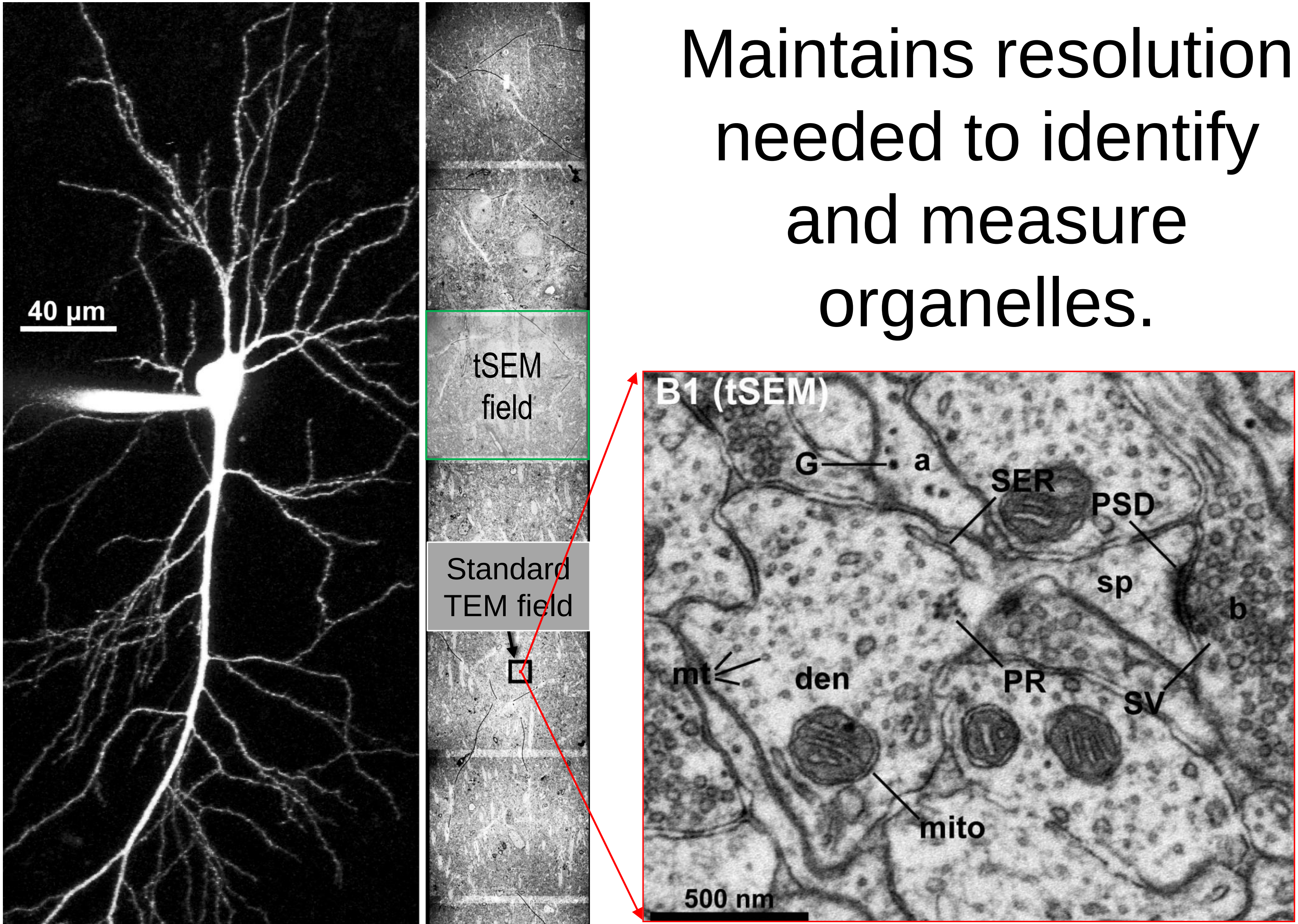
Masa Kuwajima

John Mendenhall



Kuwajima, Mendenhall, Lindsey, Harris (2013) PLOS 1

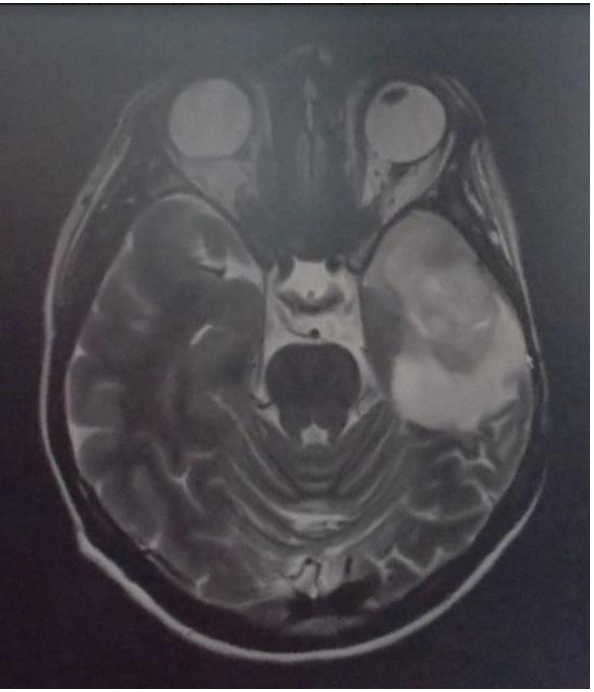
Maintains resolution needed to identify and measure organelles.



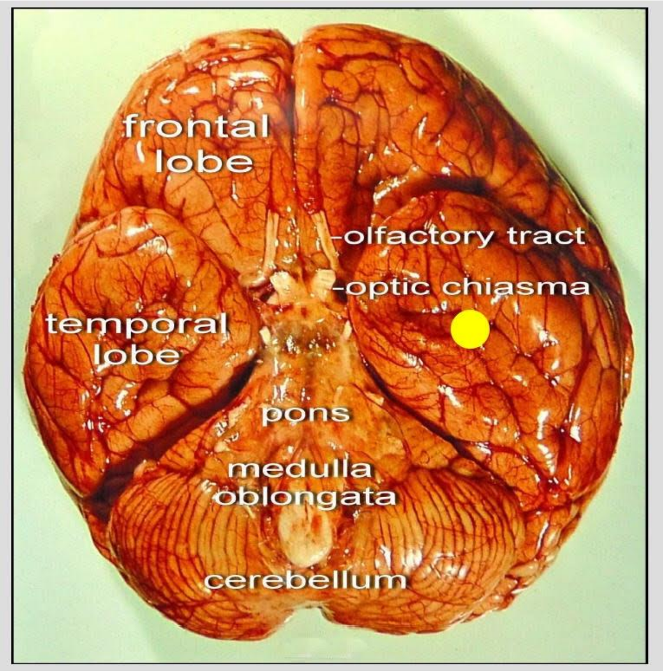
Randy Chitwood, UT Austin

Kuwajima M., Mendenhall JM, Lindsey LF, Harris KM (2013)

Human Neocortex Location: Epitumorous Temporal Lobe

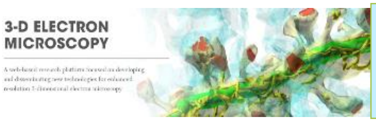


Josef Špaček – MRI of tumor

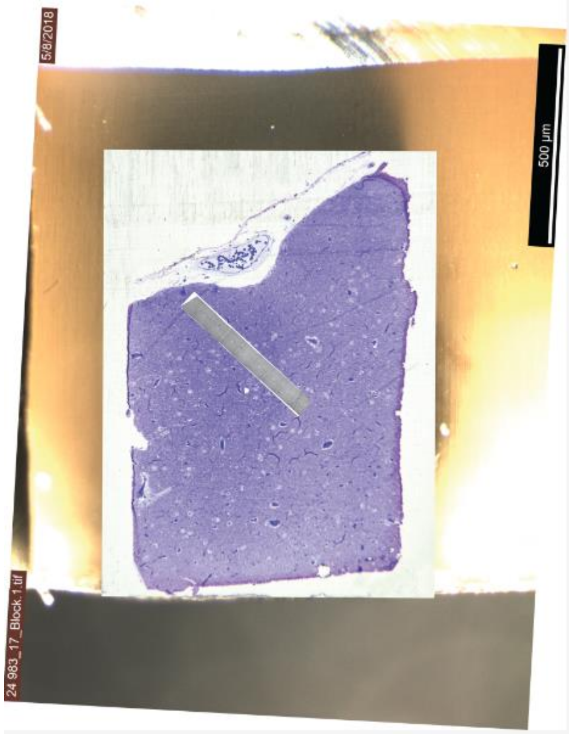


Yellow dot, location

Woman 56, anaplastic astrocytoma



Human Neocortex
Series Location: Layer II



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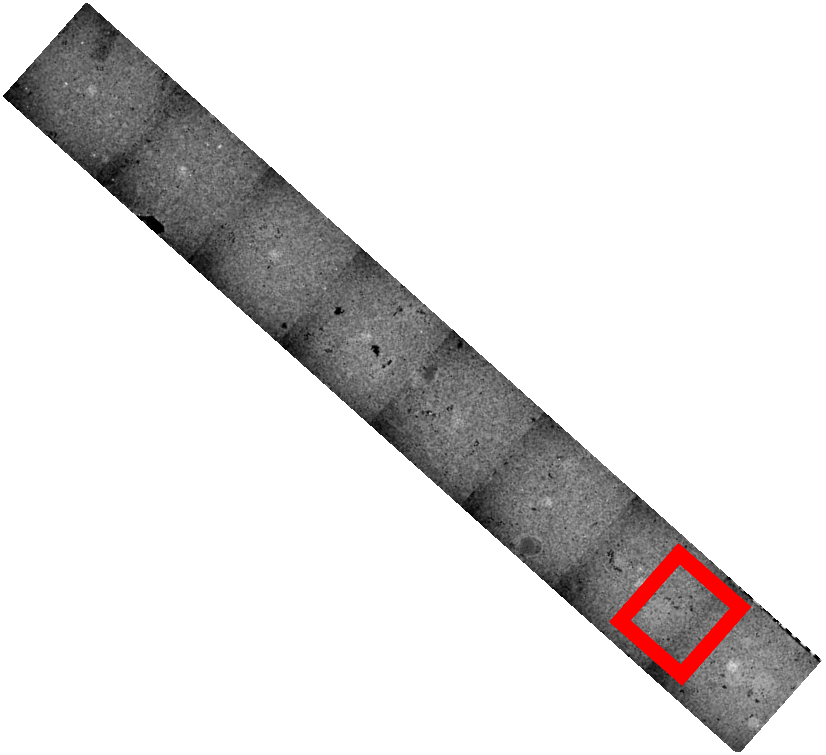
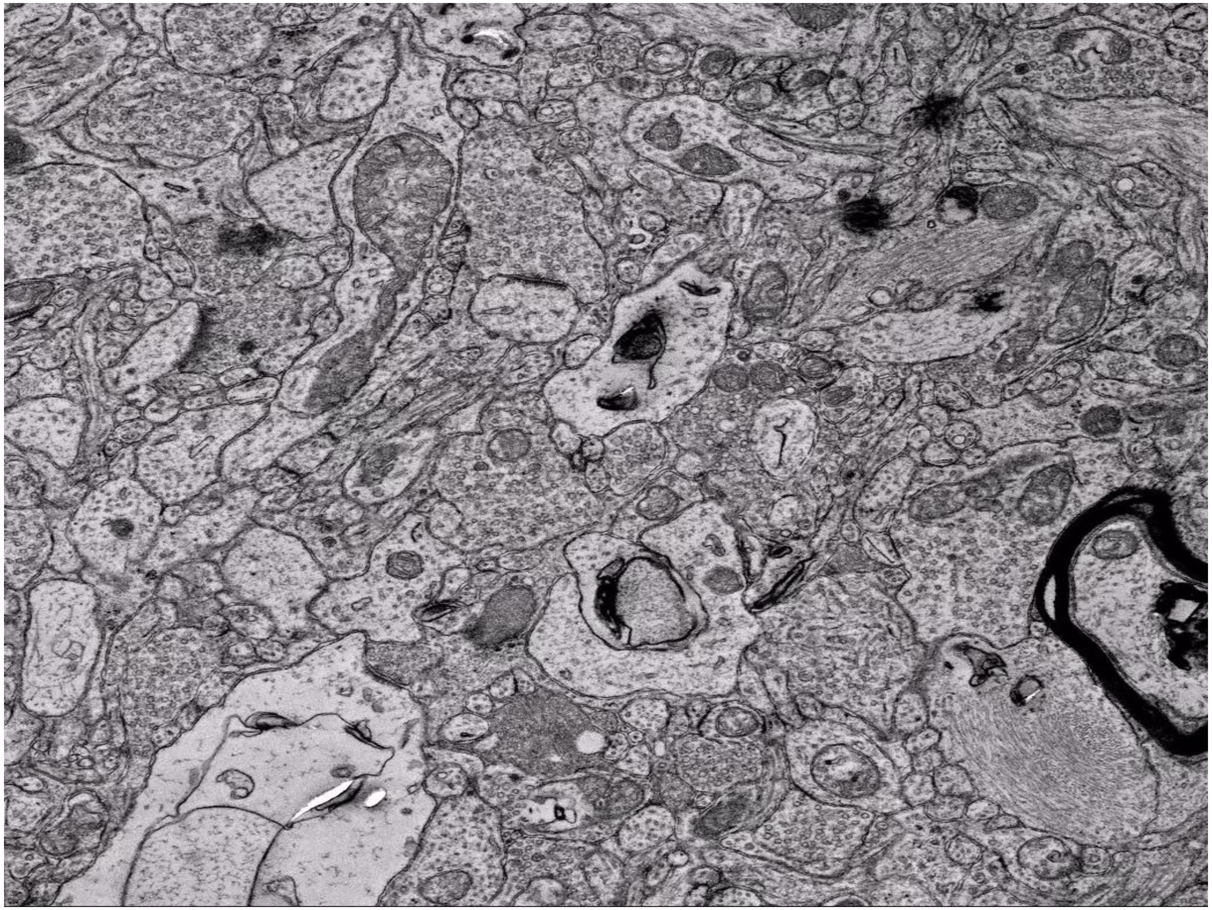
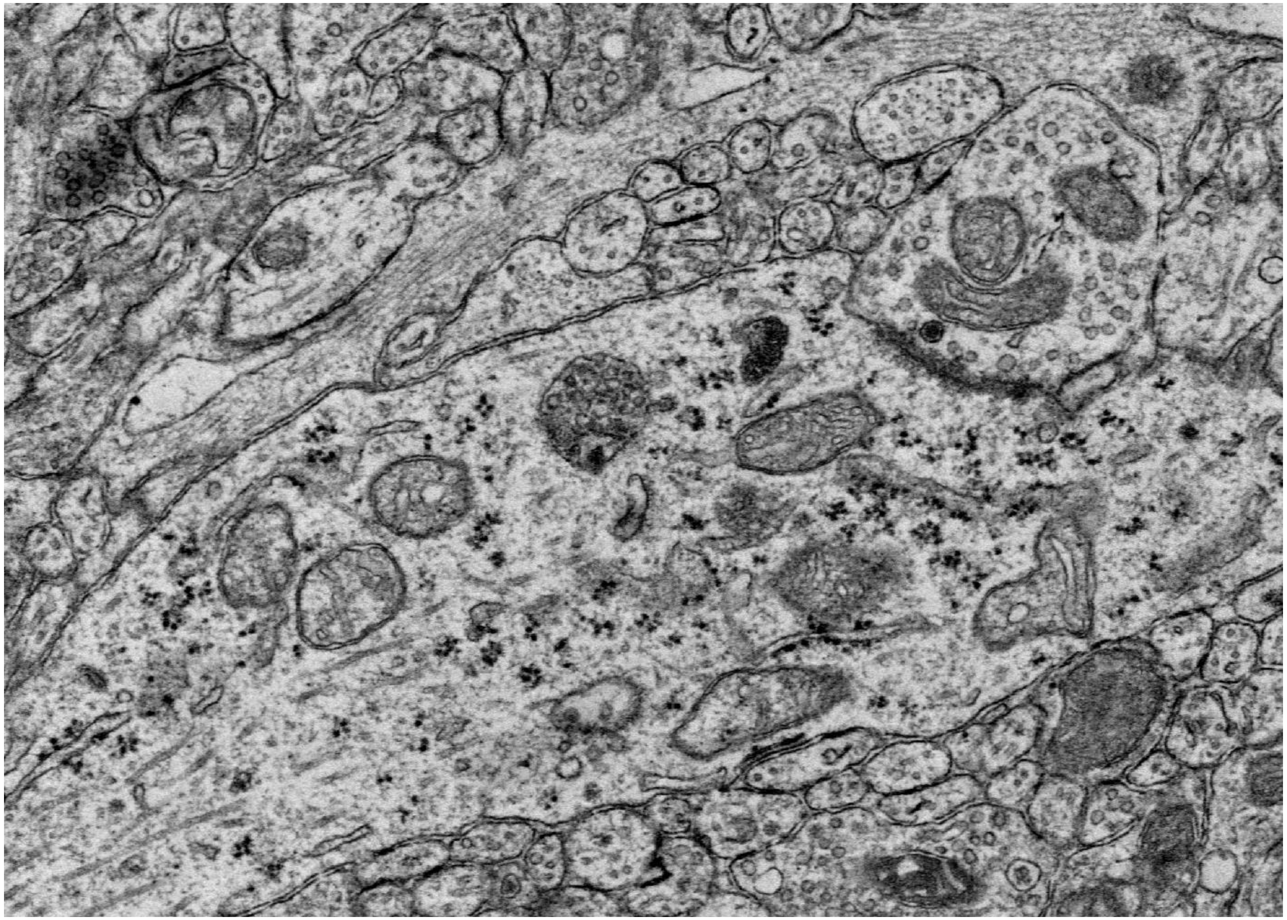


Aligned and cropped using TrakEM2

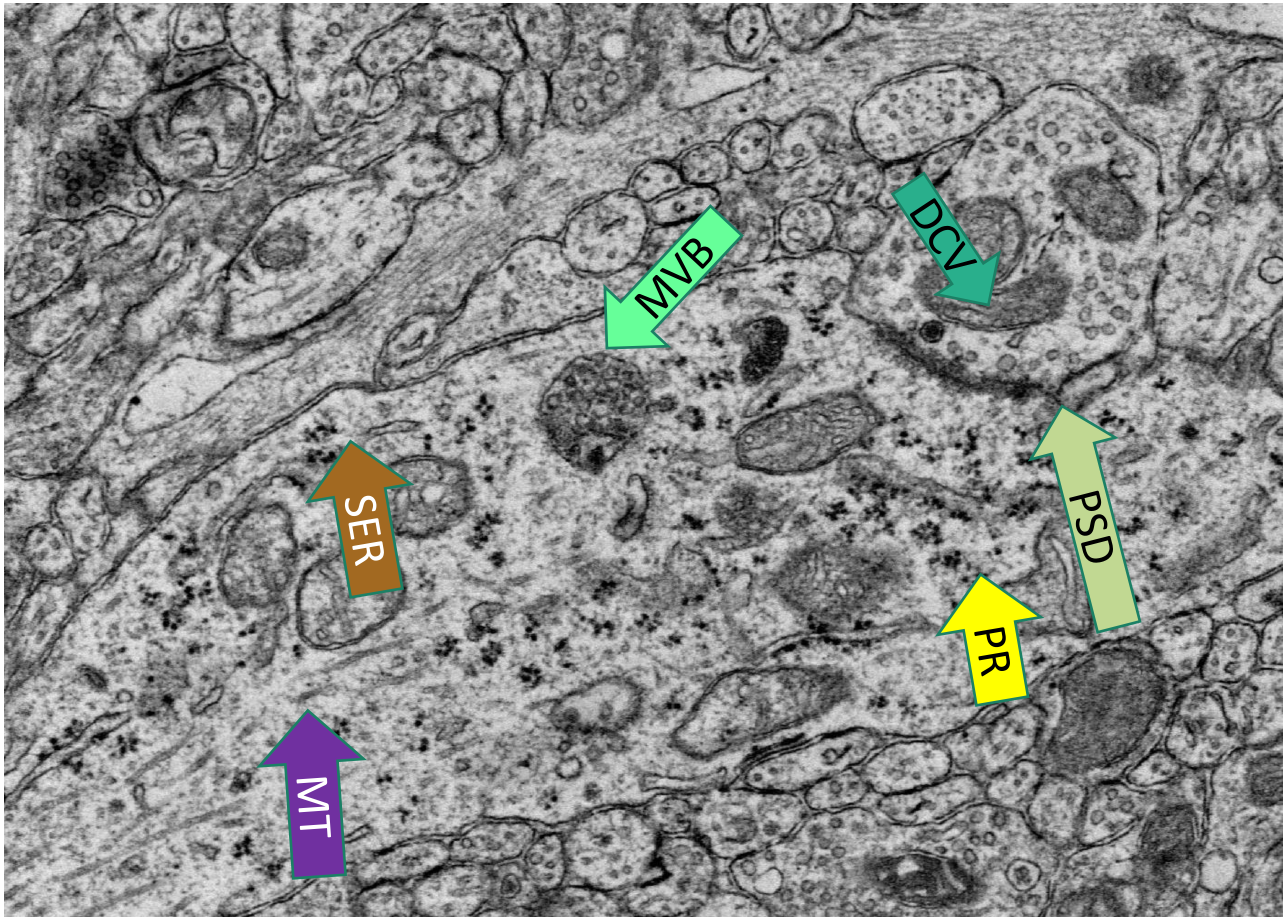


Human Neocortex
Layer II

Human Neocortex – Layer II
Great tissue preservation

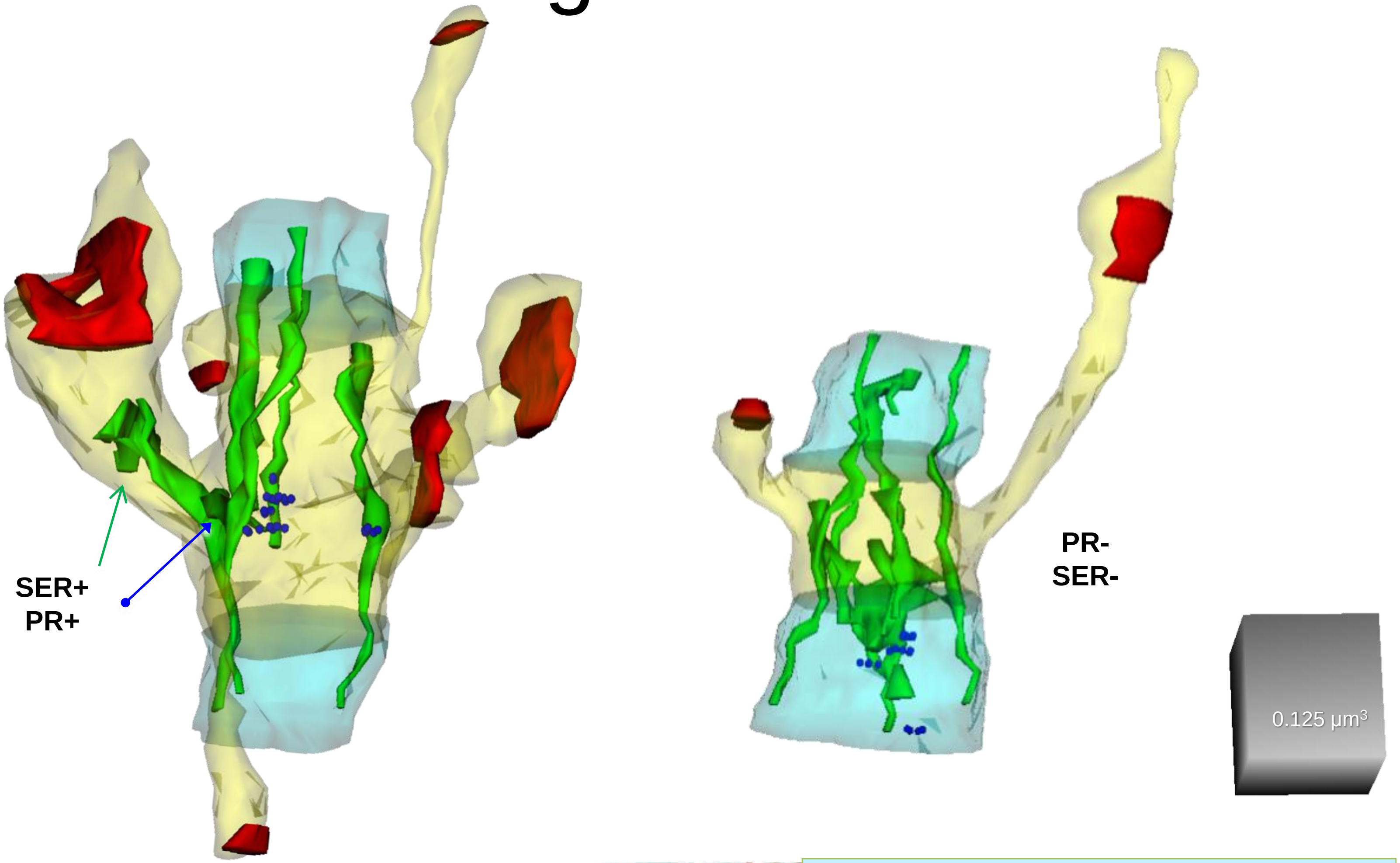


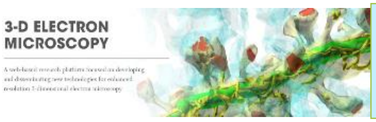
Human Neocortex – Layer II
Great tissue preservation



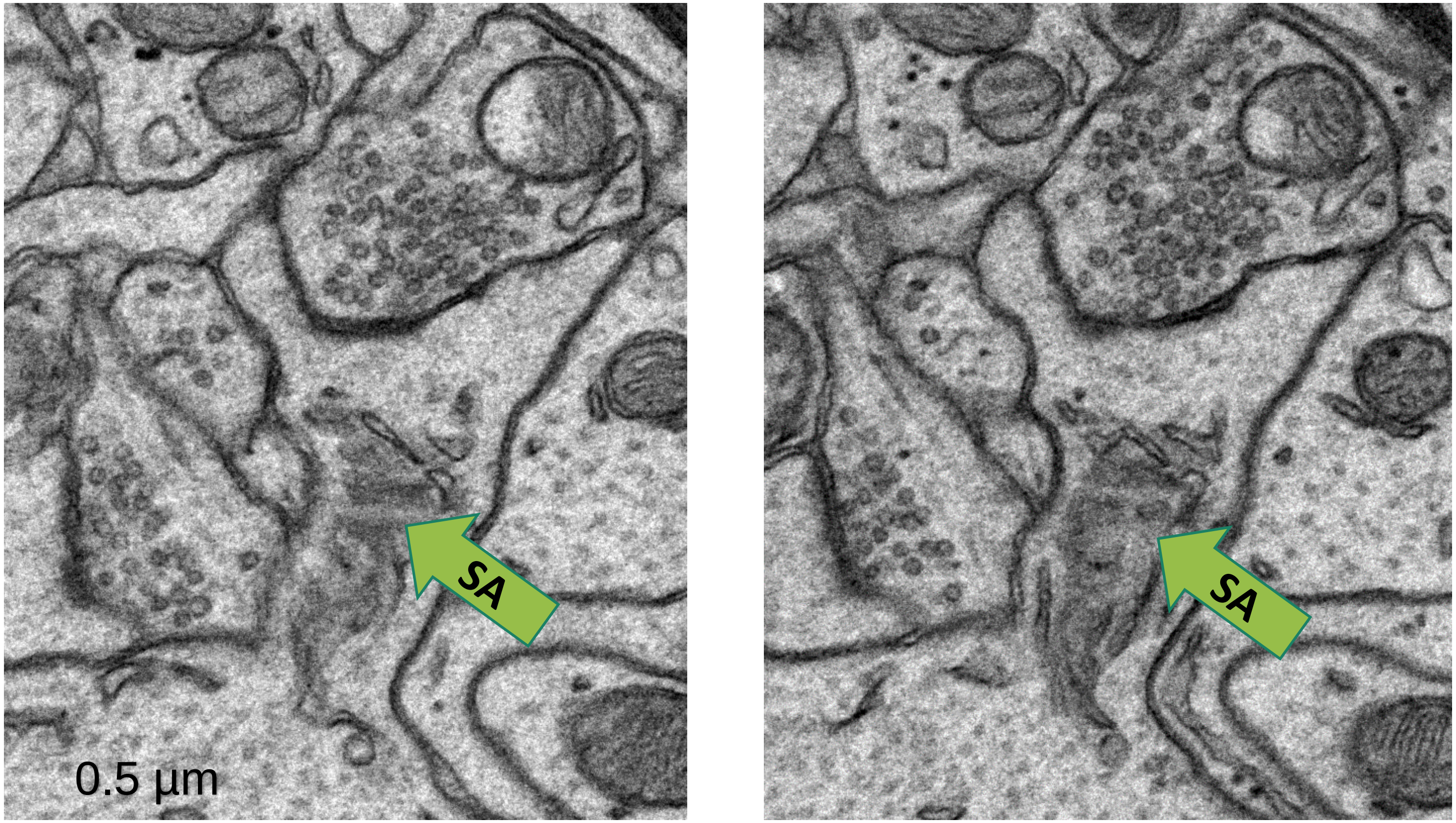
Human Neocortex – Layer II
Great tissue preservation

Integrate Cell Biology in Understanding Neural Circuits

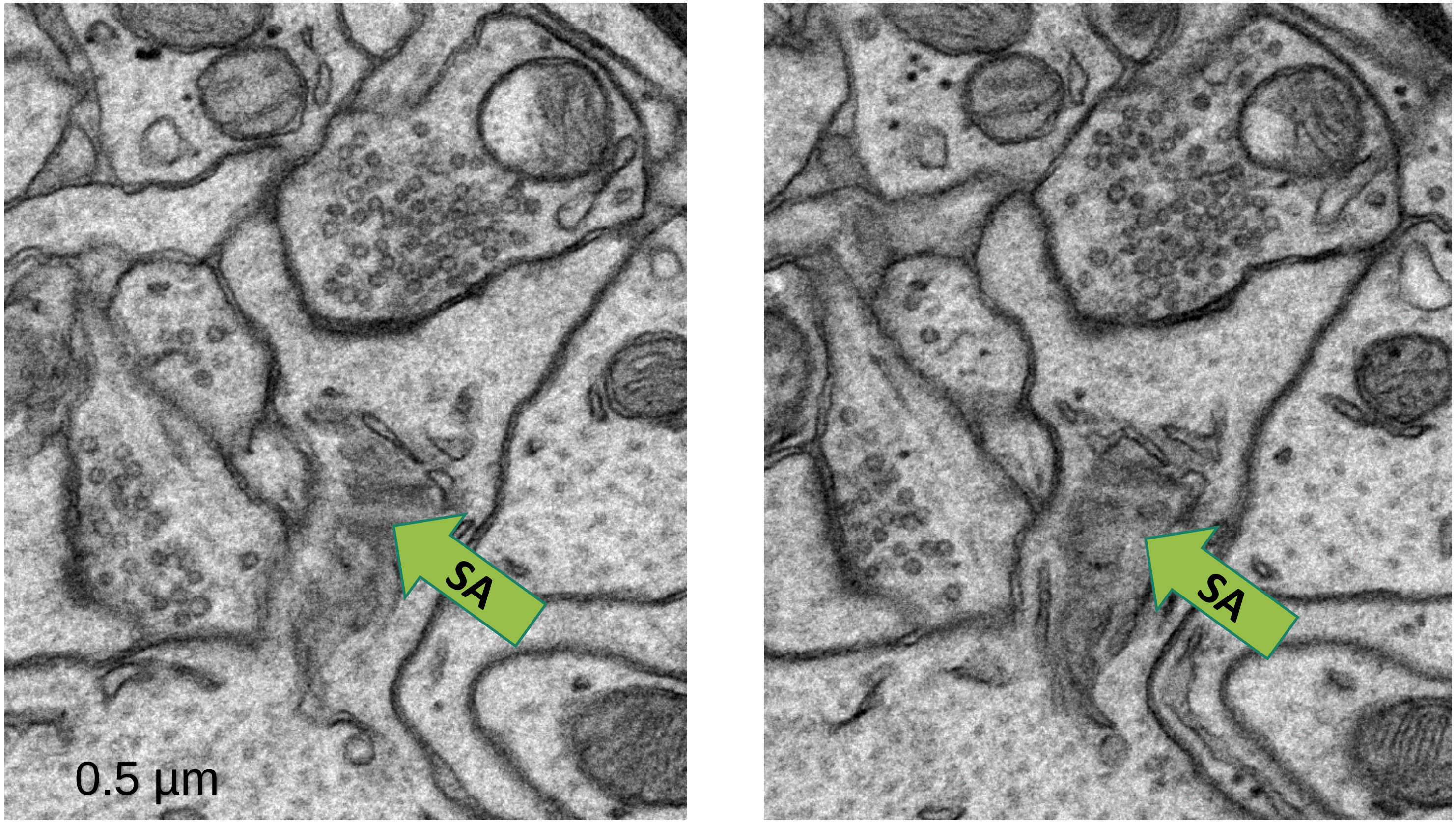




Need EM Tomography
E.g. to know the spine apparatus



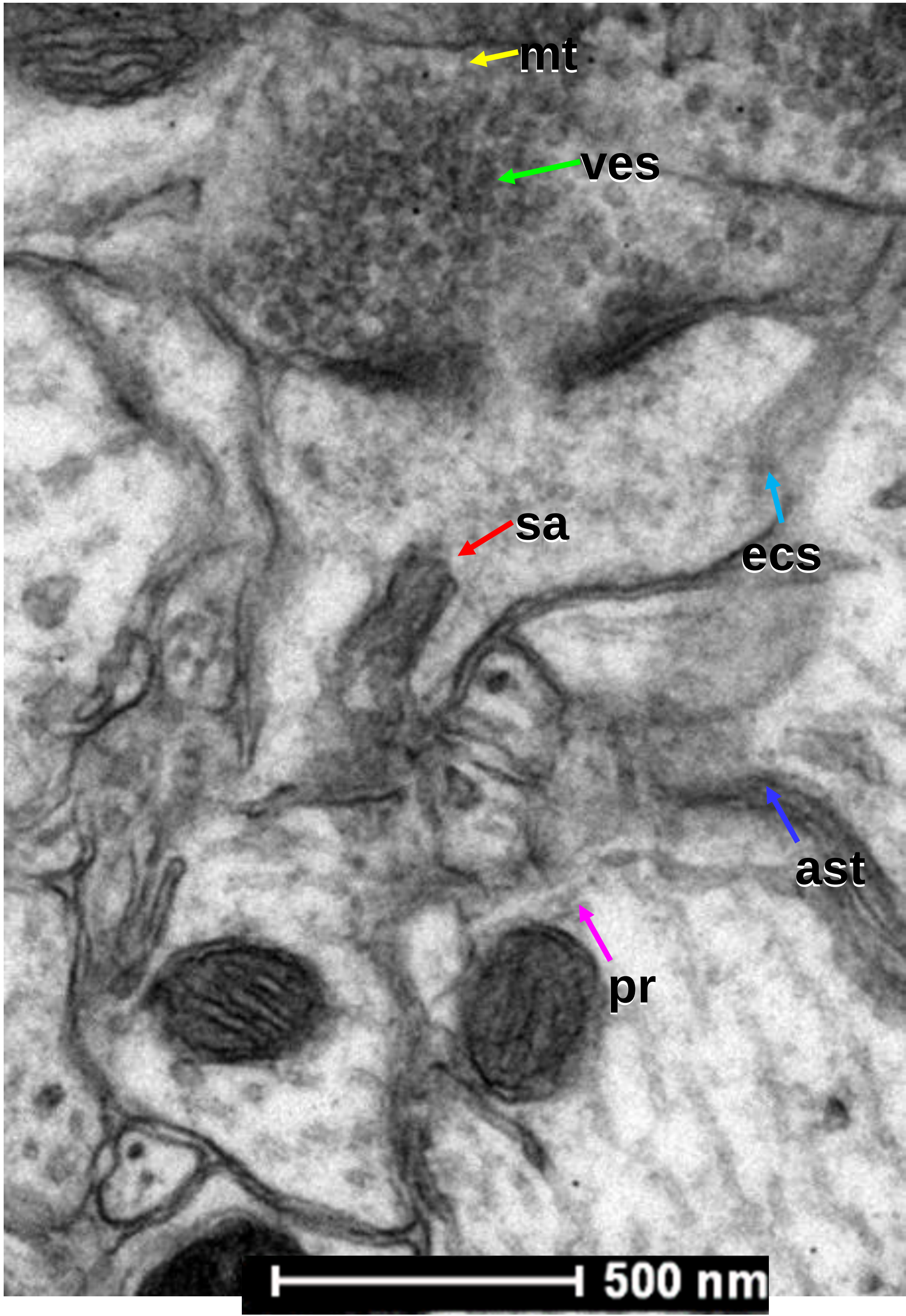
EM Tomography needed even for thin sections – 50 nm:



Thin cut sections ~50 nm: Fragile and still obscure

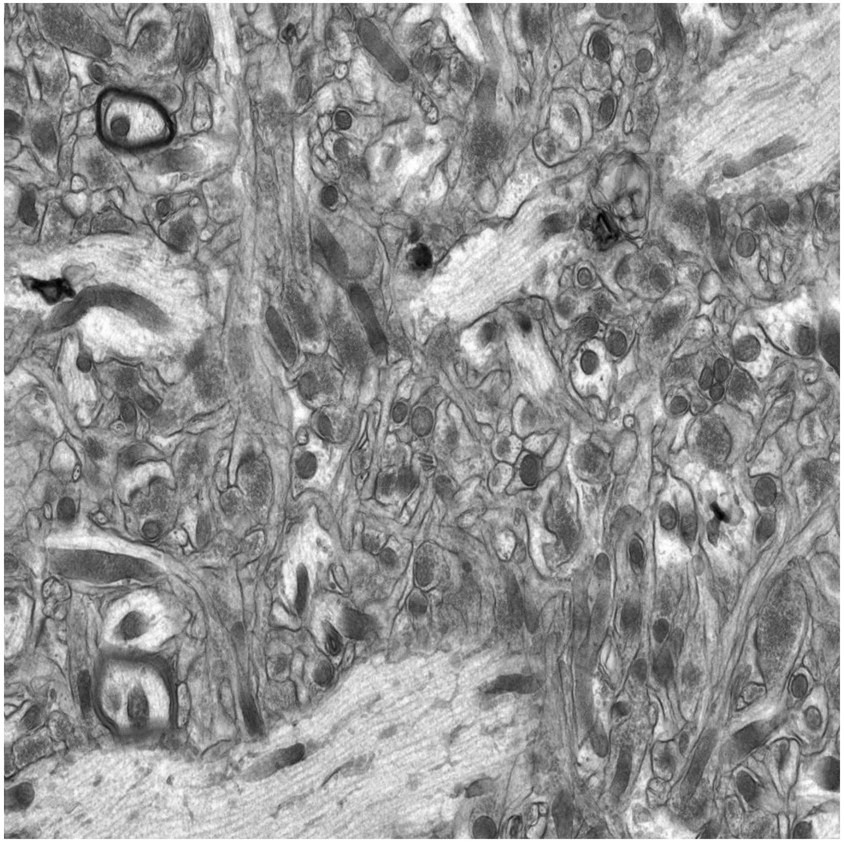
TEM Tomography Reveals obscured ultrastructure.

- 150 nm thick section
- 15 nm virtual sections
- Limitation:
  - small field size (~1 μm^2)



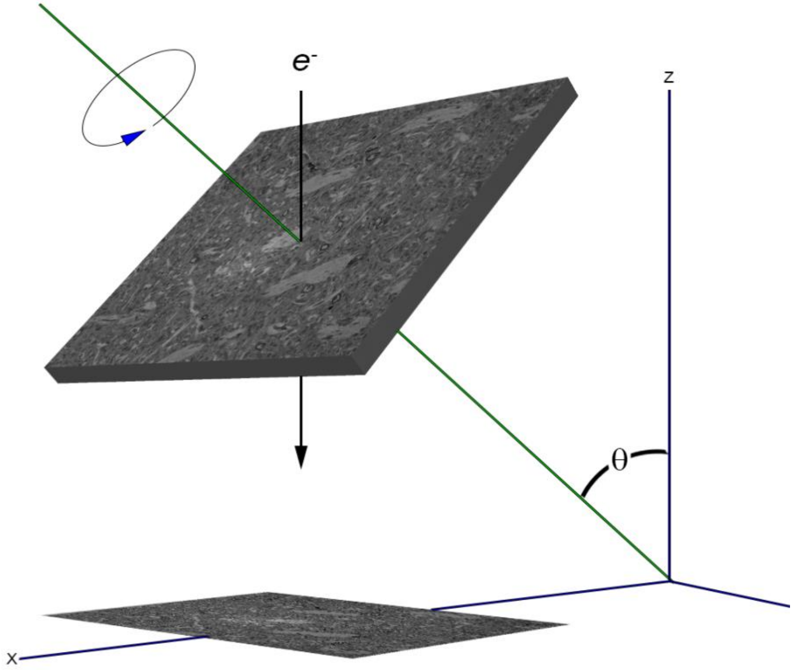
tomoSEM (Tomography in tSEM)

- Large field
  - 2,500 -10,000 μm^2
- Fewer, thicker sections (250 nm)
  - 40 instead of 200 serial sections
  - Less human cut time
  - Stronger sections
  - En bloc stain homogeneity
  - Few or no flaws
- 10-15 nm virtual z
  - Reveals buried structures
  - Better auto-segmentation
- Total Images (no big deal):
  - 40 sections * 25 image / section = 1000
  - Automatable for multi-image,
  - Multi-section, multi-grid
  - Human time minimal – setup

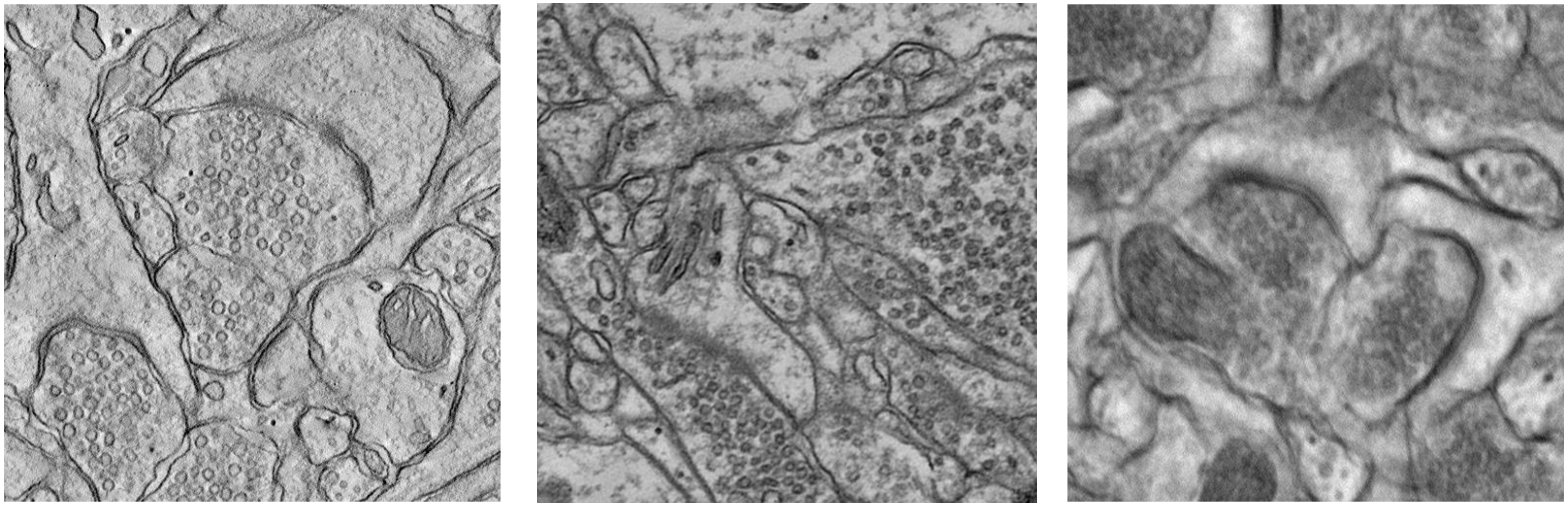


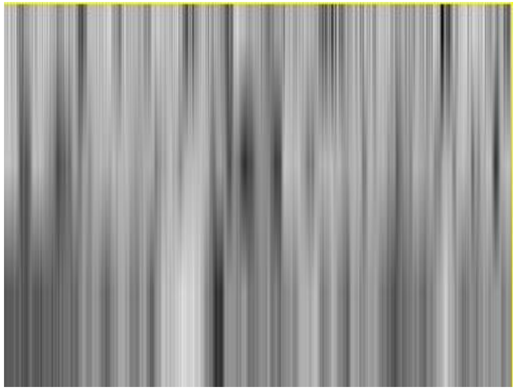
tomoSEM
Conical Tomography:

- Single tilt Angle
- One dynamic focus transform
- Conventional stage and detector
- Rotation to 220 degrees.
- Rotation 360 degrees if flip the grid.



Equal time and advantages

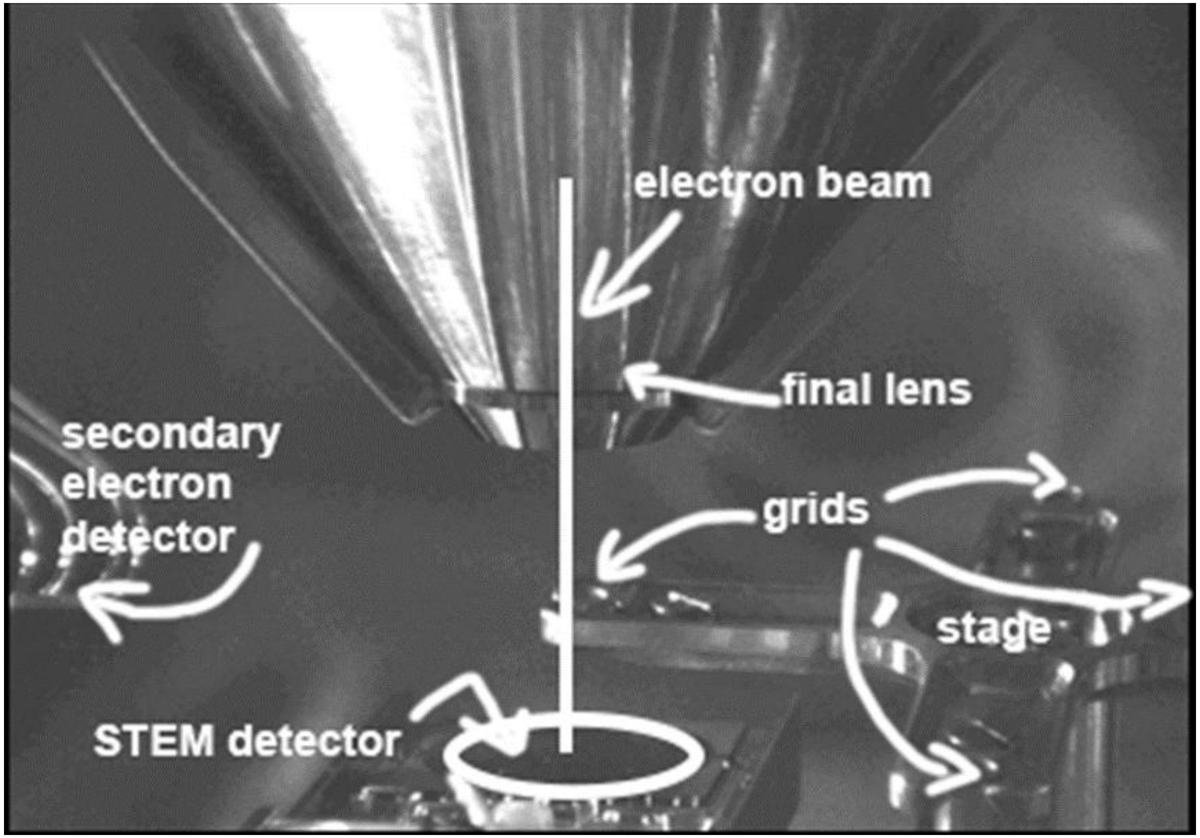




- Tilt tomography proposes 5x fewer sections cut for same total tissue volume
- Requires 5x more images for total volume than conventional ssTEM
- Results in thinner virtual sections
    - More accurate identifications and measurements
    - Improved auto-segmentation

Conical tomography acquisition:

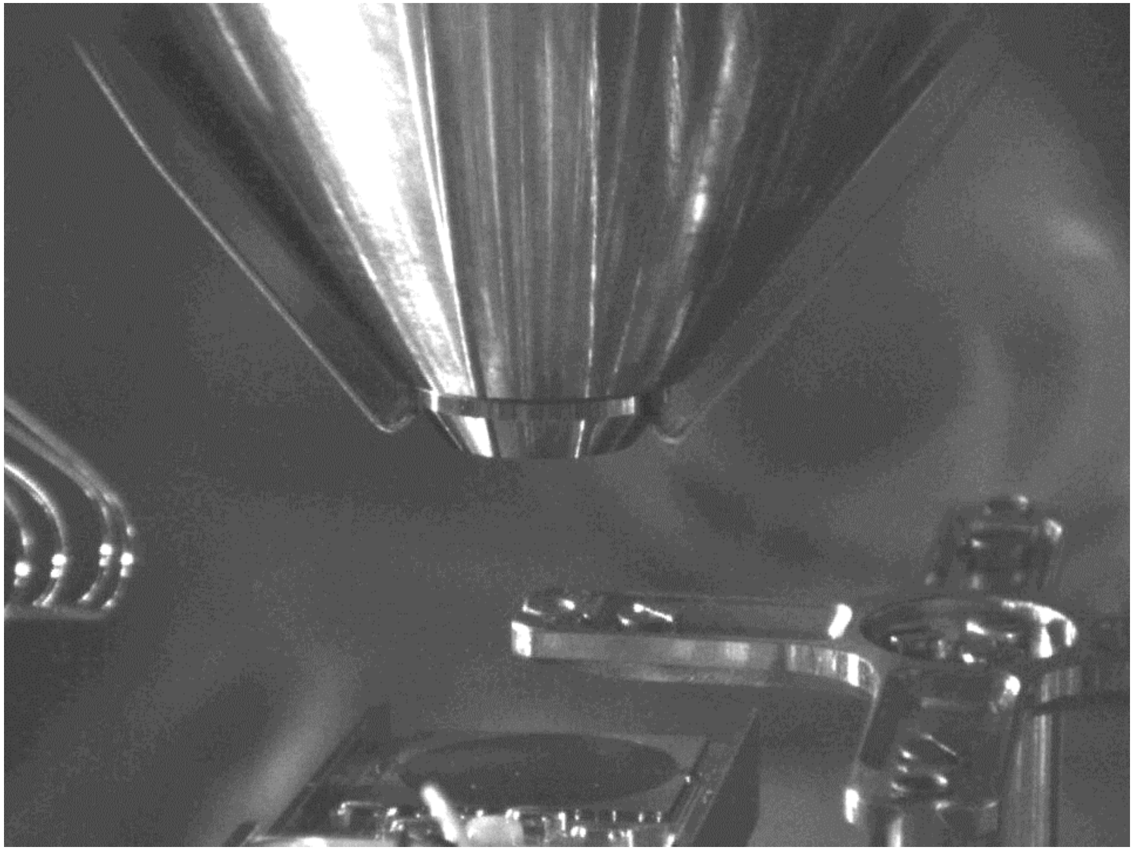
Zeiss
Merlin field emission SEM
A-STEM detector
Custom Grid holder
5 axis eucentric stage
John Yorston
Kirk Cyzmmek



Sequential tSEM images acquired at single tilted plane by rotation around ROI.

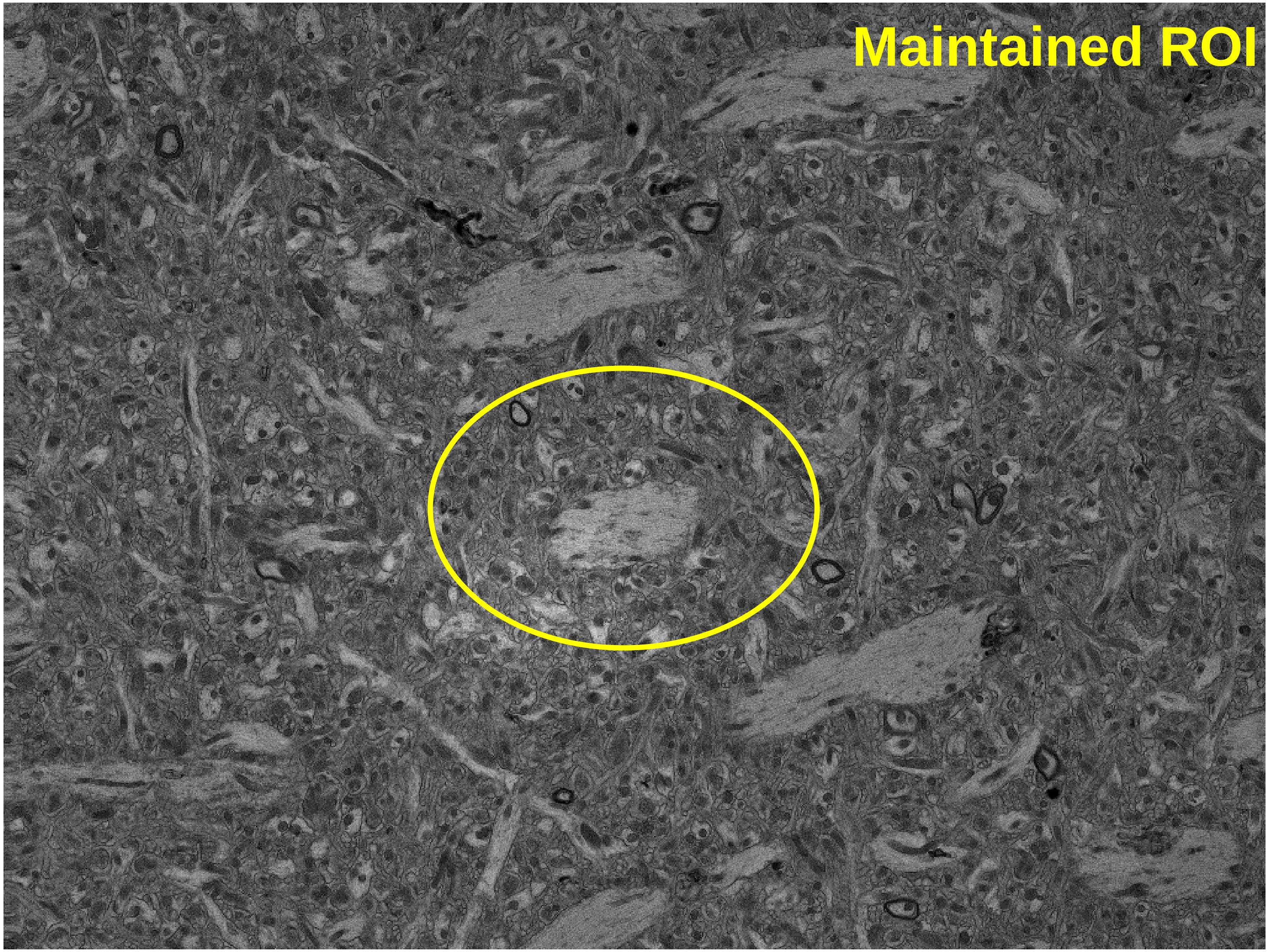
Conical tomography acquisition:

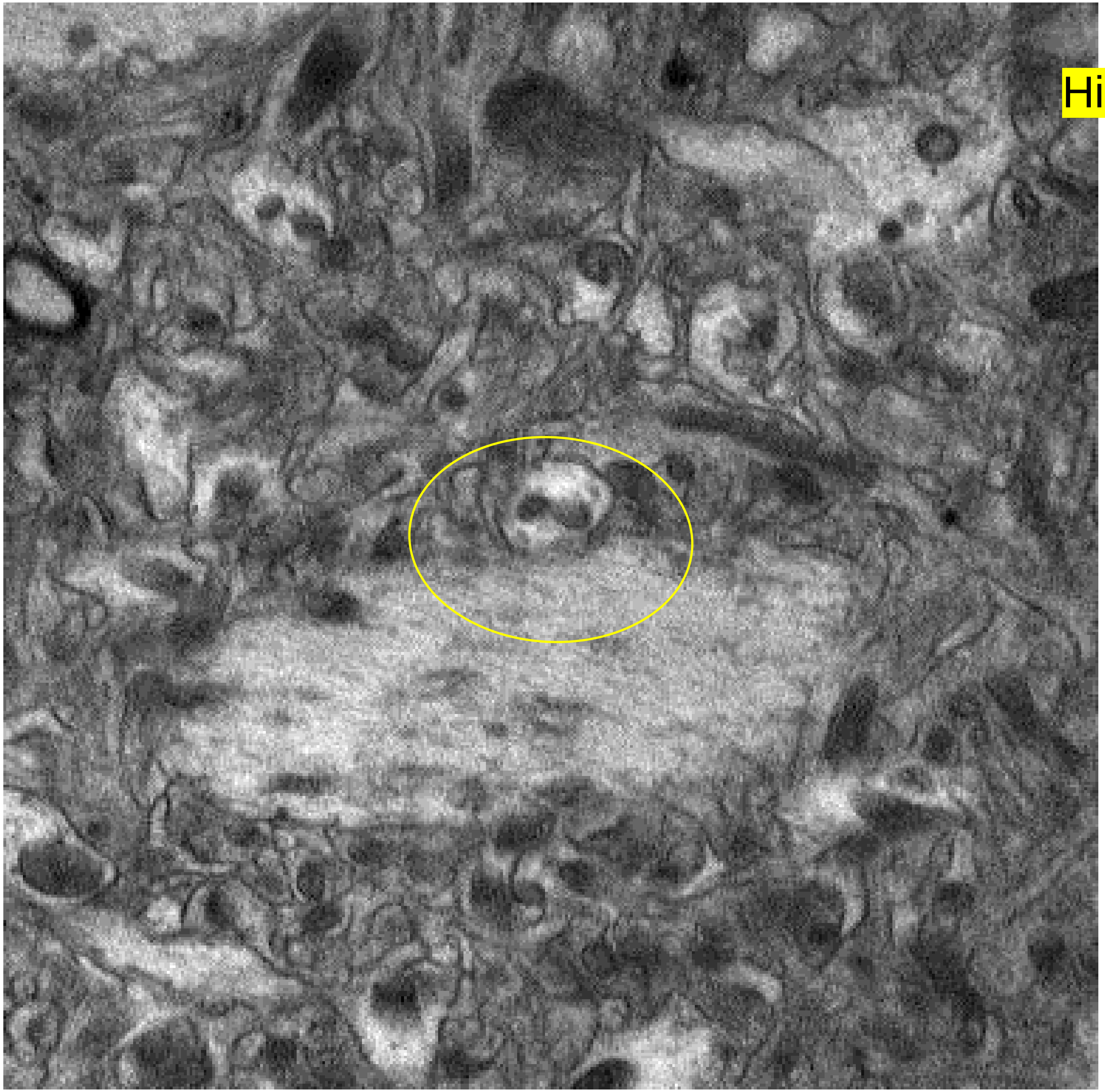
Zeiss
Merlin field emission SEM
A-STEM detector
Custom Grid holder
5 axis eucentric stage
John Yorston
Kirk Cyzmmek



Sequential tSEM images acquired at single tilted plane by rotation around ROI.

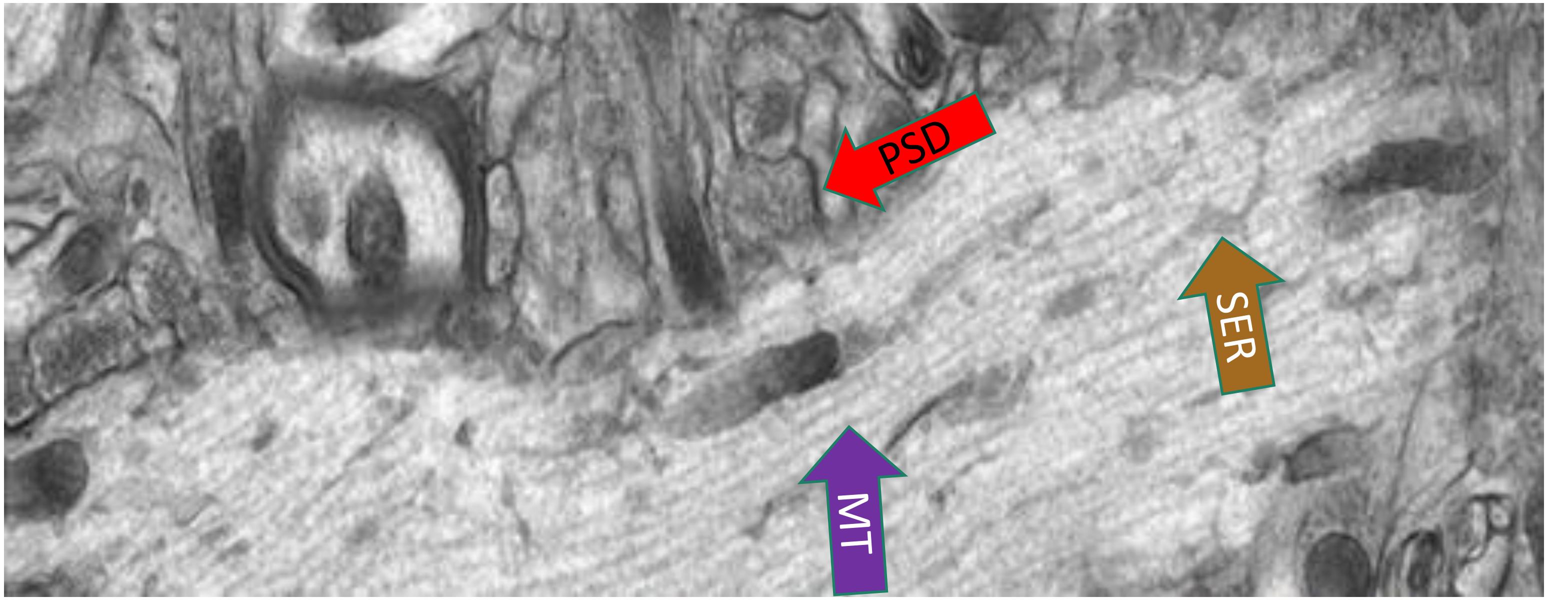
First Conical Tilt images:





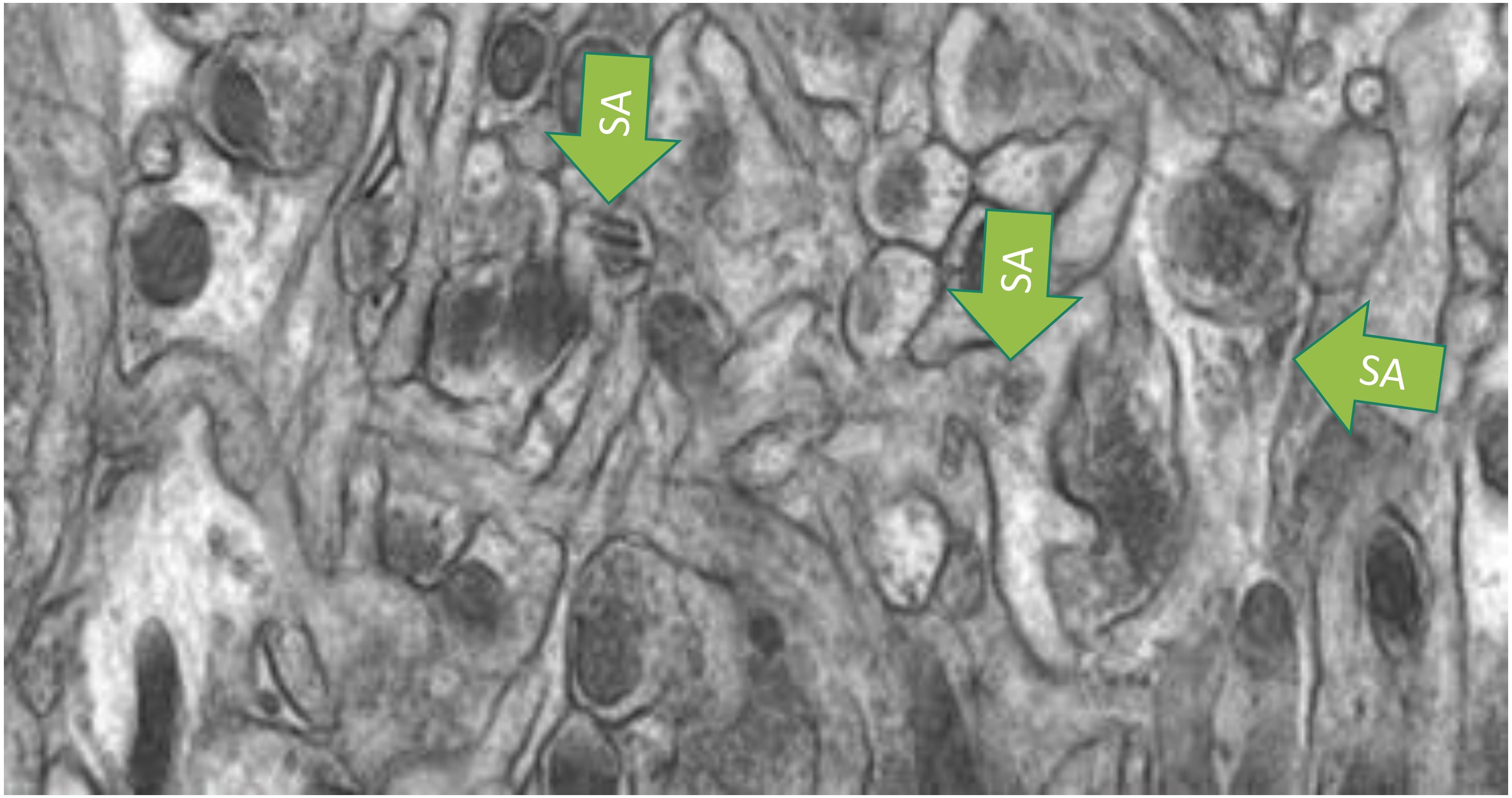
High Mag

tSEM of 250 nm thick sections



- 15 kV – good contrast, signal throughout section depth.
- Can recognize many structures in regions of interest.

tSEM of 250 nm thick section



- Can even recognize spine apparatuses (SA)

Next Goal for tomoSEM: (Techniques in hand)

Resolution (pixel size):

X-Y = 2 nm, Z = 10-15 nm

X-Y field:

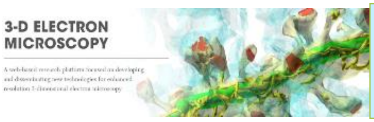
2,500 -10,000 µm^2

Total extent in Z:

200 @250nm = 50 µm

Total tomoSEM Volume:

500,000 –1,000,000 µm^3

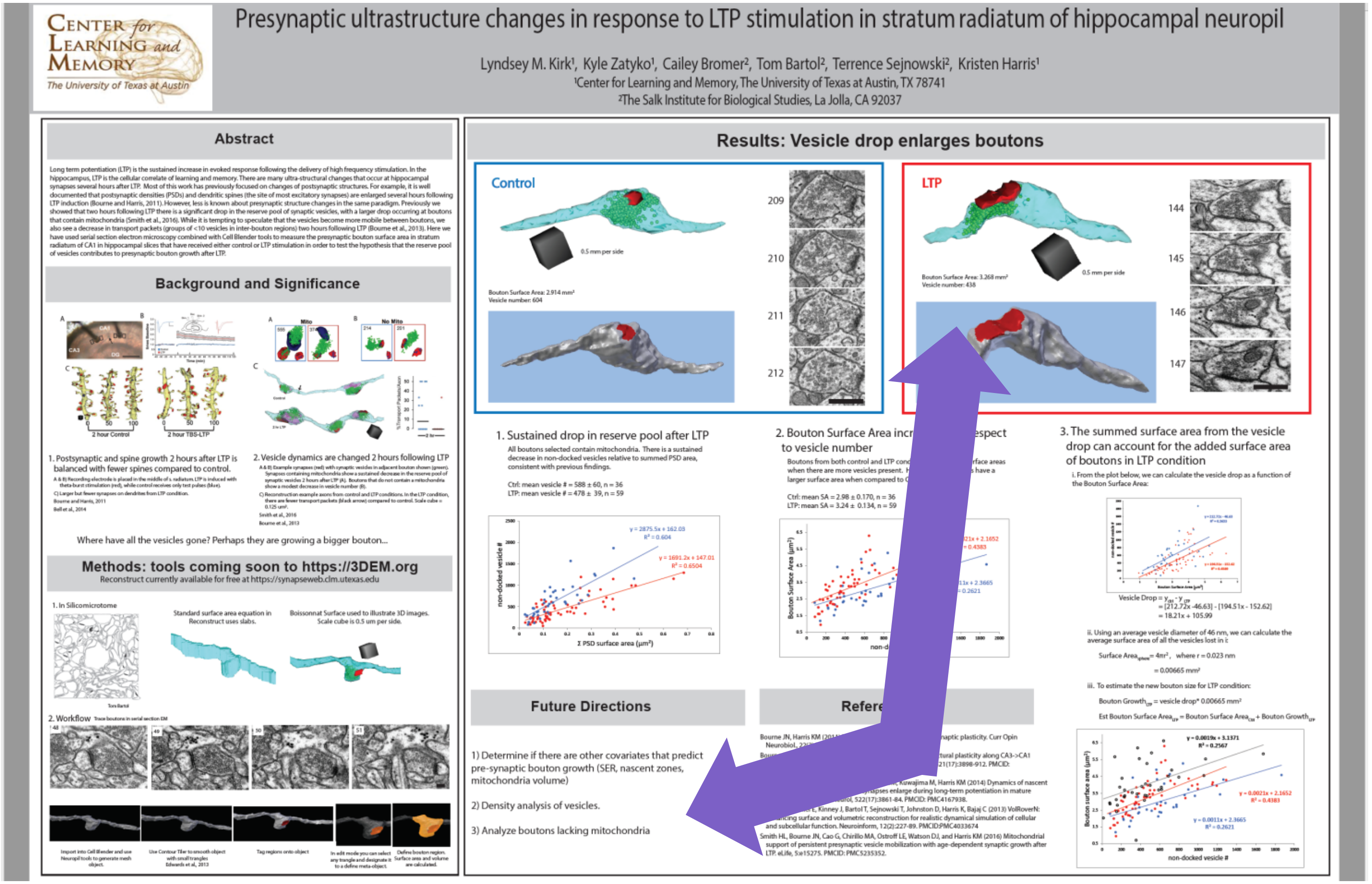


Aim 3:

- Integrate and test software tools to enhance analysis of synaptic neuropil.

- Developed in Sejnowski Lab
- Tested in Harris Lab
- To be Shared on TACC - Carson

Neuropil Tools for Accurate Surface Areas – Axon example:



Presynaptic ultrastructure changes in response to LTP stimulation in stratum radiatum of hippocampal neuropil

CENTER for LEARNING and MEMORY
The University of Texas at Austin

Lyndsey M. Kirk[1], Kyle Zatyko[1], Cailey Bromer[2], Tom Bartol[2], Terrence Sejnowski[2], Kristen Harris[1]
[1]Center for Learning and Memory, The University of Texas at Austin, TX 78741
[2]The Salk Institute for Biological Studies, La Jolla, CA 92037

Abstract

Long term potentiation (LTP) is the sustained increase in evoked response following the delivery of high frequency stimulation. In the hippocampus, LTP is the cellular correlate of learning and memory. There are many ultra-structural changes that occur at hippocampal synapses several hours after LTP. Most of this work has previously focused on changes of postsynaptic structures. For example, it is well documented that postsynaptic densities (PSDs) and dendritic spines (the site of most excitatory synapses) are enlarged several hours following LTP induction (Bourne and Harris, 2011). However, less is known about presynaptic structure changes in the same paradigm. Previously we showed that two hours following LTP there is a significant drop in the reserve pool of synaptic vesicles, with a larger drop occurring at boutons that contain mitochondria (Smith et al., 2016). While it is tempting to speculate that the vesicles become more mobile between boutons, we also see a decrease in transport packets (groups of <10 vesicles in inter-bouton regions) two hours following LTP (Bourne et al., 2013). Here we have used serial section electron microscopy combined with Cell Blender tools to measure the presynaptic bouton surface area in stratum radiatum of CA1 in hippocampal slices that have received either control or LTP stimulation in order to test the hypothesis that the reserve pool of vesicles contributes to presynaptic bouton growth after LTP.

Background and Significance

1. Postsynaptic and spine growth 2 hours after LTP is balanced with fewer spines compared to control.

A & B) Recording electrode is placed in the middle of s. radiatum. LTP is induced with theta-burst stimulation (red), while control receives only test pulses (blue).

C) Larger but fewer synapses on dendrites from LTP condition.

Bourne and Harris, 2011

Bell et al., 2014

2. Vesicle dynamics are changed 2 hours following LTP

A & B) Example synapses (red) with synaptic vesicles in adjacent bouton shown (green). Synapses containing mitochondria show a sustained decrease in the reserve pool of synaptic vesicles 2 hours after LTP (A). Boutons that do not contain a mitochondria show a modest decrease in vesicle number (B).

C) Reconstruction example axons from control and LTP conditions. In the LTP condition, there are fewer transport packets (black arrow) compared to control. Scale cube = 0.125 um³.

Smith et al., 2016

Bourne et al., 2013

Where have all the vesicles gone? Perhaps they are growing a bigger bouton…

Methods: tools coming soon to https://3DEM.org

Reconstruct currently available for free at https://synapseweb.clm.utexas.edu

1. In Silicomicrotome

Tom Bartol

Standard surface area equation in Reconstruct uses slabs.

Boissonnat Surface used to illustrate 3D images. Scale cube is 0.5 um per side.

2. Workflow Trace boutons in serial section EM

Import into Cell Blender and use Neuropil tools to generate mesh object.

Use Contour Tiler to smooth object with small trangles
Edwards et al, 2013

Tag regions onto object

In edit mode you can select any trangle and designate it to a define meta-object.

Define bouton region. Surface area and volume are calculated.

Results: Vesicle drop enlarges boutons

Control

0.5 mm per side

Bouton Surface Area: 2.914 mm²
Vesicle number: 604

LTP

0.5 mm per side

Bouton Surface Area: 3.268 mm²
Vesicle number: 438

1. Sustained drop in reserve pool after LTP

All boutons selected contain mitochondria. There is a sustained decrease in non-docked vesicles relative to summed PSD area, consistent with previous findings.

Ctrl: mean vesicle # = 588 ± 60, n = 36
LTP: mean vesicle # = 478 ± 39, n = 59

2. Bouton Surface Area incr espect to vesicle number

Boutons from both control and LTP cond rface areas when there are more vesicles present. H s have a larger surface area when compared to C

Ctrl: mean SA = 2.98 ± 0.170, n = 36
LTP: mean SA = 3.24 ± 0.134, n = 59

3. The summed surface area from the vesicle drop can account for the added surface area of boutons in LTP condition

i. From the plot below, we can calculate the vesicle drop as a function of the Bouton Surface Area:

Vesicle Drop = $y_{ctrl} - y_{LTP}$
= [212.72x -46.63] - [194.51x - 152.62]
= 18.21x + 105.99

ii. Using an average vesicle diameter of 46 nm, we can calculate the average surface area of all the vesicles lost in i:

$\text{Surface Area}_{sphere} = 4\pi r^2$, where r = 0.023 nm
= 0.00665 mm²

iii. To estimate the new bouton size for LTP condition:

$\text{Bouton Growth}_{LTP}$ = vesicle drop* 0.00665 mm²

$\text{Est Bouton Surface Area}_{LTP} = \text{Bouton Surface Area}_{ctrl} + \text{Bouton Growth}_{LTP}$

Future Directions

1) Determine if there are other covariates that predict pre-synaptic bouton growth (SER, nascent zones, mitochondria volume)

2) Density analysis of vesicles.

3) Analyze boutons lacking mitochondria

Refere

Bourne JN, Harris KM (201 naptic plasticity. Curr Opin Neurobiol., 22(
Bour ctural plasticity along CA3->CA1 21(17):3898-912. PMCID:
Kuwajima M, Harris KM (2014) Dynamics of nascent napses enlarge during long-term potentiation in mature eurol, 522(17):3861-84. PMCID: PMC4167938.
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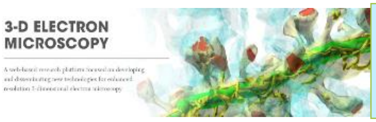
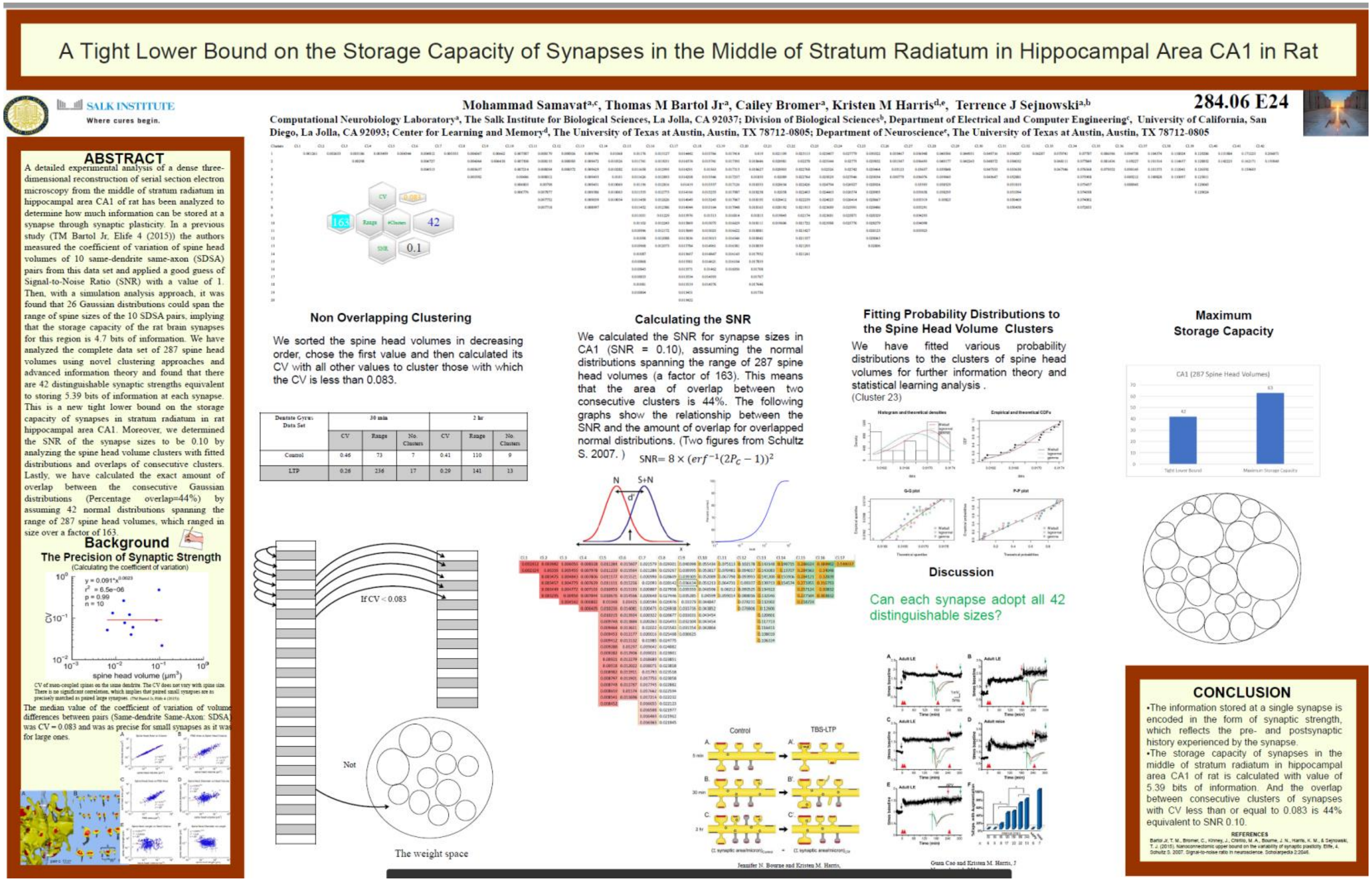


3-D ELECTRON MICROSCOPY

Expanded Analyses of Storage Capacity of Hippocampal Synapses:



A Tight Lower Bound on the Storage Capacity of Synapses in the Middle of Stratum Radiatum in Hippocampal Area CA1 in Rat

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ABSTRACT

A detailed experimental analysis of a dense three-dimensional reconstruction of serial section electron microscopy from the middle of stratum radiatum in hippocampal area CA1 of rat has been analyzed to determine how much information can be stored at a synapse through synaptic plasticity. In a previous study (TM Bartol Jr, Elife 4 (2015)) the authors measured the coefficient of variation of spine head volumes of 10 same-dendrite same-axon (SDSA) pairs from this data set and applied a good guess of Signal-to-Noise Ratio (SNR) with a value of 1. Then, with a simulation analysis approach, it was found that 26 Gaussian distributions could span the range of spine sizes of the 10 SDSA pairs, implying that the storage capacity of the rat brain synapses for this region is 4.7 bits of information. We have analyzed the complete data set of 287 spine head volumes using novel clustering approaches and advanced information theory and found that there are 42 distinguishable synaptic strengths equivalent to storing 5.39 bits of information at each synapse. This is a new tight lower bound on the storage capacity of synapses in stratum radiatum in rat hippocampal area CA1. Moreover, we determined the SNR of the synapse sizes to be 0.10 by analyzing the spine head volume clusters with fitted distributions and overlaps of consecutive clusters. Lastly, we have calculated the exact amount of overlap between the consecutive Gaussian distributions (Percentage overlap=44%) by assuming 42 normal distributions spanning the range of 287 spine head volumes, which ranged in size over a factor of 163.

Background

The Precision of Synaptic Strength

(Calculating the coefficient of variation)

CV of axon-coupled spines on the same dendrite. The CV does not vary with spine size. There is no significant correlation, which implies that paired small synapses are as precisely matched as paired large synapses. (TM Bartol Jr, Elife 4 (2015))

The median value of the coefficient of variation of volume differences between pairs (Same-dendrite Same-Axon: SDSA) was CV = 0.083 and was as precise for small synapses as it was for large ones.

Non Overlapping Clustering

We sorted the spine head volumes in decreasing order, chose the first value and then calculated its CV with all other values to cluster those with which the CV is less than 0.083.

Dentate Gyrus Data Set	30 min			2 hr		
	CV	Range	No. Clusters	CV	Range	No. Clusters
Control	0.46	73	7	0.41	110	9
LTP	0.26	236	17	0.29	141	13

Calculating the SNR

We calculated the SNR for synapse sizes in CA1 (SNR = 0.10), assuming the normal distributions spanning the range of 287 spine head volumes (a factor of 163). This means that the area of overlap between two consecutive clusters is 44%. The following graphs show the relationship between the SNR and the amount of overlap for overlapped normal distributions. (Two figures from Schultz S. 2007.)

$$\text{SNR} = 8 \times (erf^{-1}(2P_C - 1))^2$$

Jennifer N. Bourne and Kristen M. Harris,

Fitting Probability Distributions to the Spine Head Volume Clusters

We have fitted various probability distributions to the clusters of spine head volumes for further information theory and statistical learning analysis .
(Cluster 23)

Discussion

Can each synapse adopt all 42 distinguishable sizes?

Guan Cao and Kristen M. Harris, J

Maximum Storage Capacity

CONCLUSION

- The information stored at a single synapse is encoded in the form of synaptic strength, which reflects the pre- and postsynaptic history experienced by the synapse.
- The storage capacity of synapses in the middle of stratum radiatum in hippocampal area CA1 of rat is calculated with value of 5.39 bits of information. And the overlap between consecutive clusters of synapses with CV less than or equal to 0.083 is 44% equivalent to SNR 0.10.

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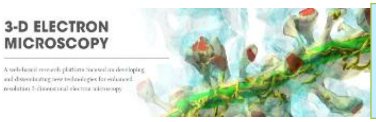
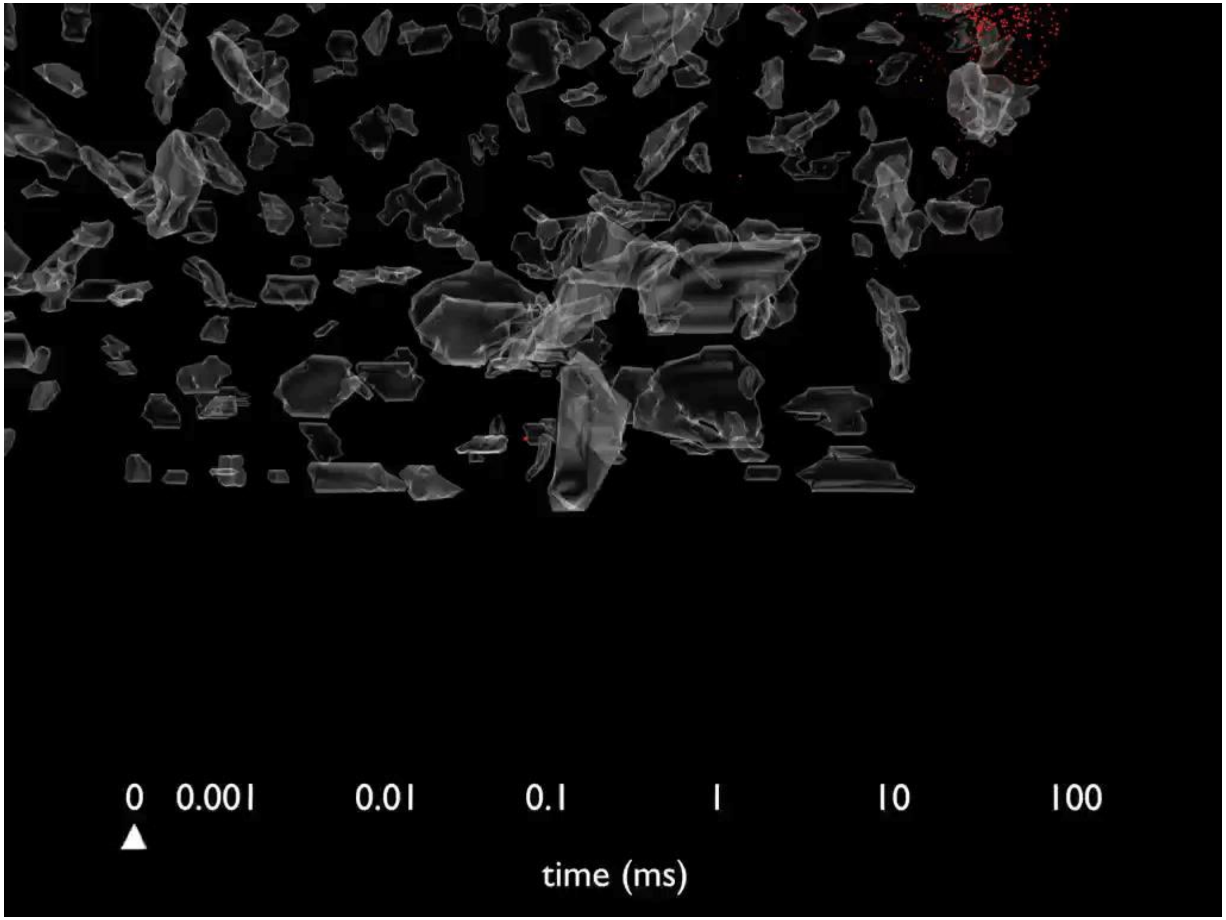
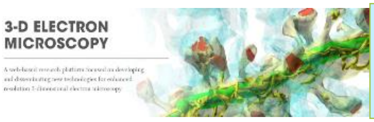


5 April 2019 NSF Workshop
NeuroNex Award No.1707356

Modeling Synaptic Activity in Sleep sharp wave (REM-memory)

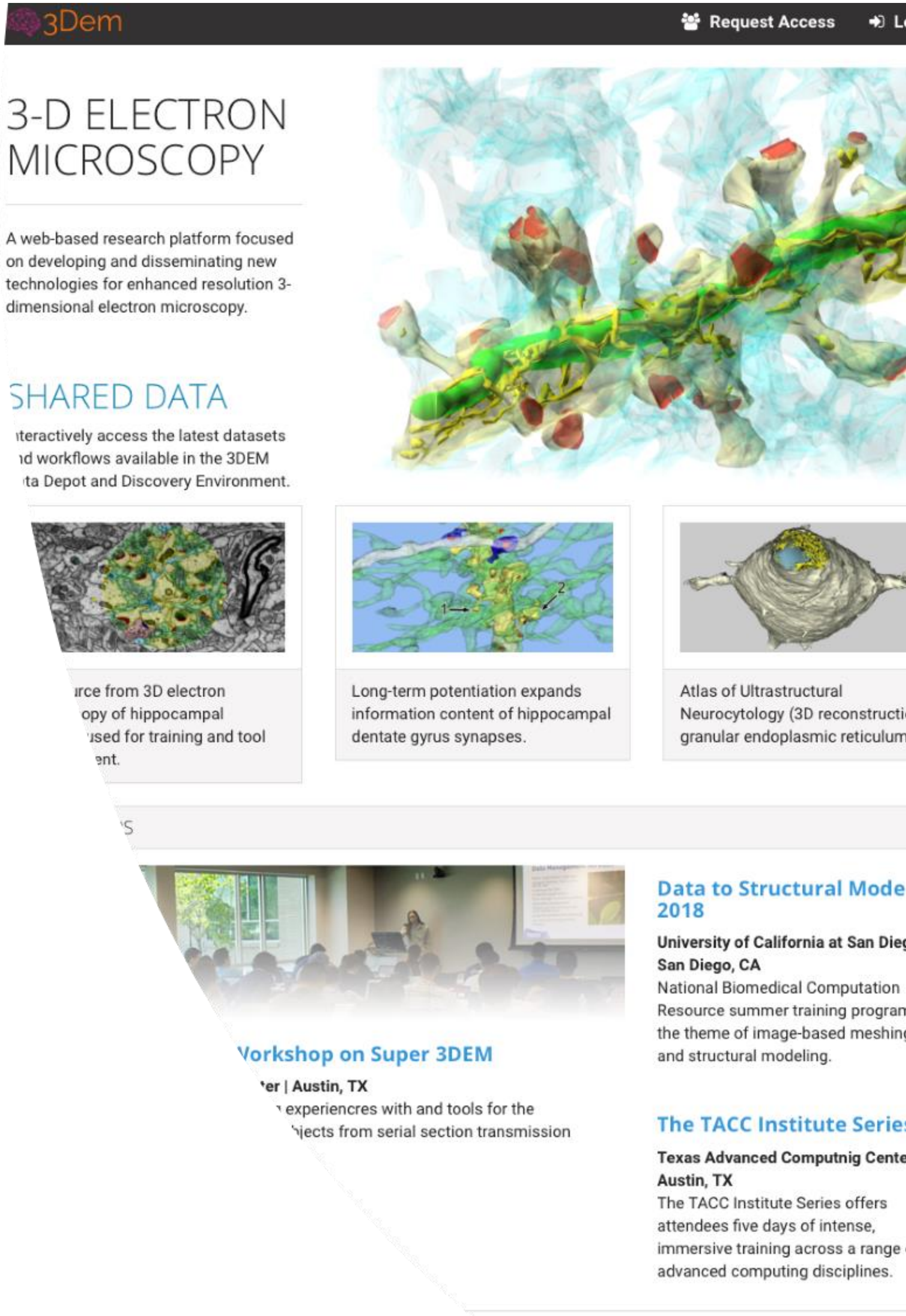


Bartol and Sejnowski, Salk
Blender and MCell modeling



Aim 4:

- Interactive Portal (3DEM.org)
- Live link to EM images
- Incorporate 3DEM tools
- Add community tools
- Workshops and Hackathons
- Disseminate content
- Online tutorials
- Broaden access to 3DEM.org
- Now for James Carson and the portal.



3-D ELECTRON MICROSCOPY

A web-based research platform focused on developing and disseminating new technologies for enhanced resolution 3-dimensional electron microscopy.

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