

The Brain as a Source of Big Data

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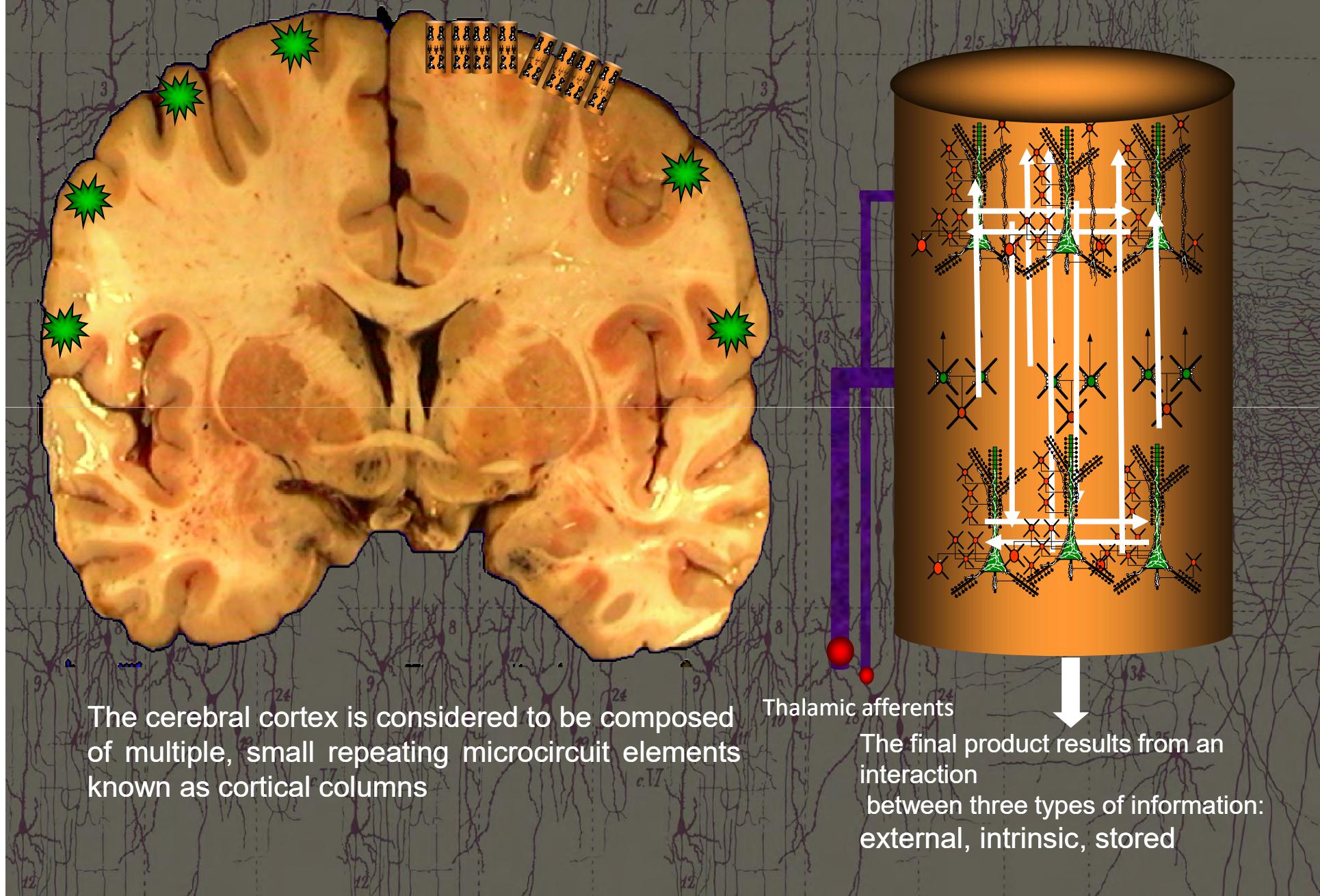
cei
tec
bio



Human
Brain
Project

- Why is the brain a potential source of big data?
- What kind of technologies will help to obtain and analyze brain data?
- What would be the potential uses of these data?

The brain is an extraordinarily complex organ



The brain as a potential source of big data

- The brain is a very complex structure
- The connectivity of the brain spans several scales, from nm to cm
- The physiology of the brain is only incompletely understood
- Brain diseases are poorly known and difficult to treat

The brain can be studied with many different technologies for different purposes

- Structural and functional *in vivo* brain imaging
- Electrophysiology
- Magnetoencephalography
- Light microscopy (conventional, fluorescence, confocal, etc.)
- Electron microscopy
- Molecular biology
- Genetics
- Cognitive neuroscience ...

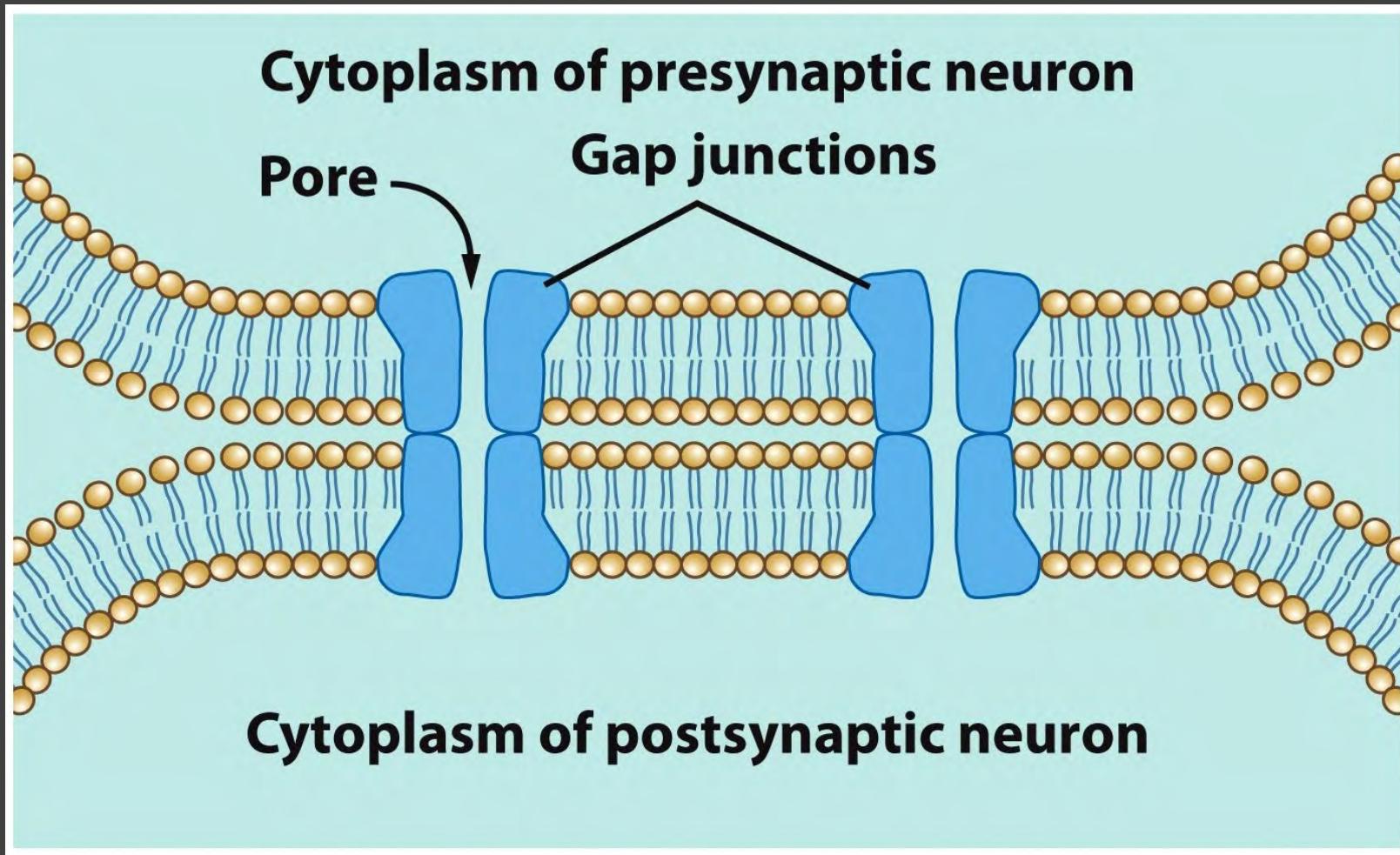
Here we will focus on the study of synapses with the electron microscope

- Synapses establish the connections between neurons and between neurons and other types of cells
- They are key to the regulation of the flow of information within the brain
- They are plastic structures, so they can adapt to different circumstances
- They are involved in learning and memory
- They are visible with the electron microscope

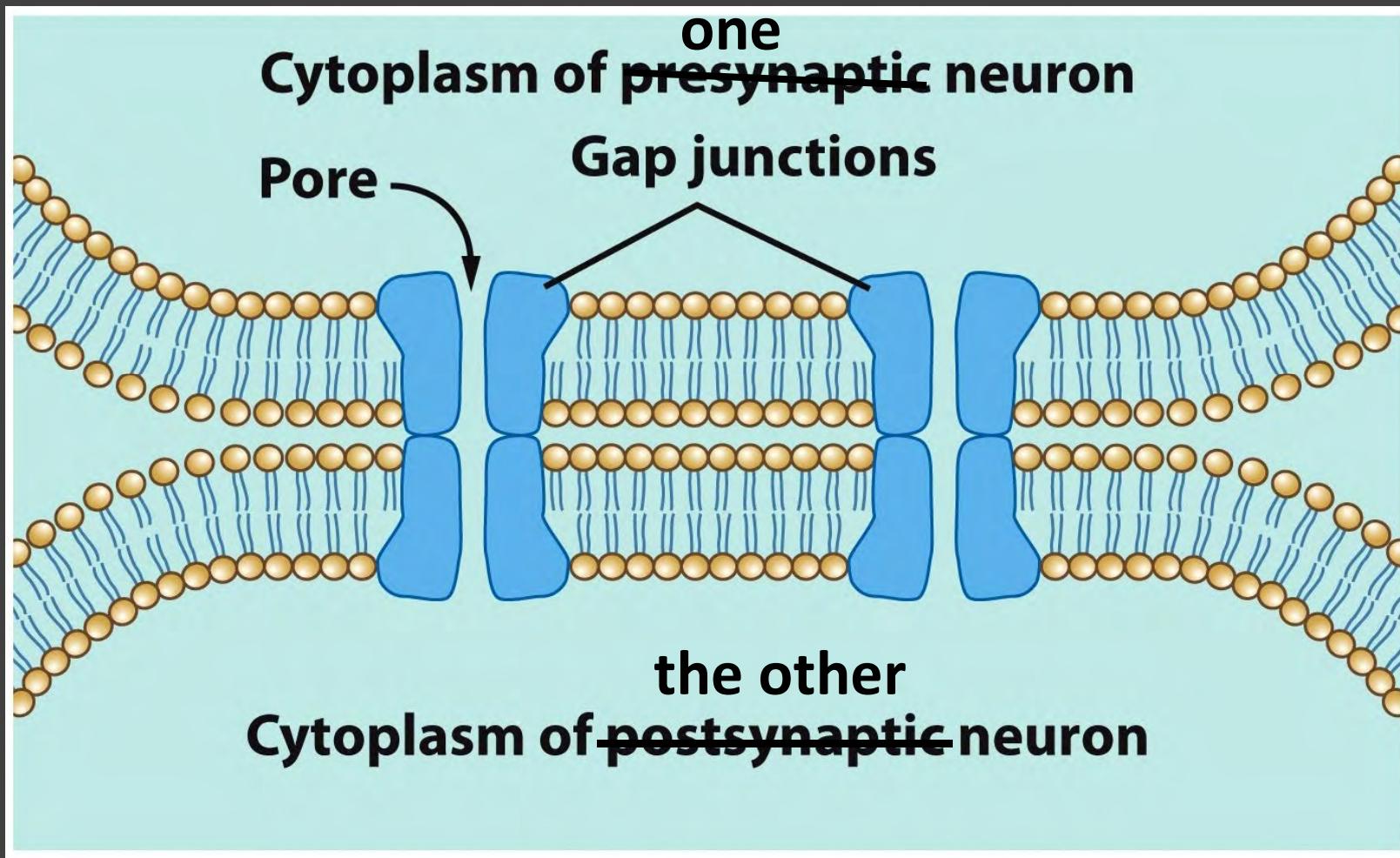
Neurons communicate through synapses

- Electrical
- Chemical

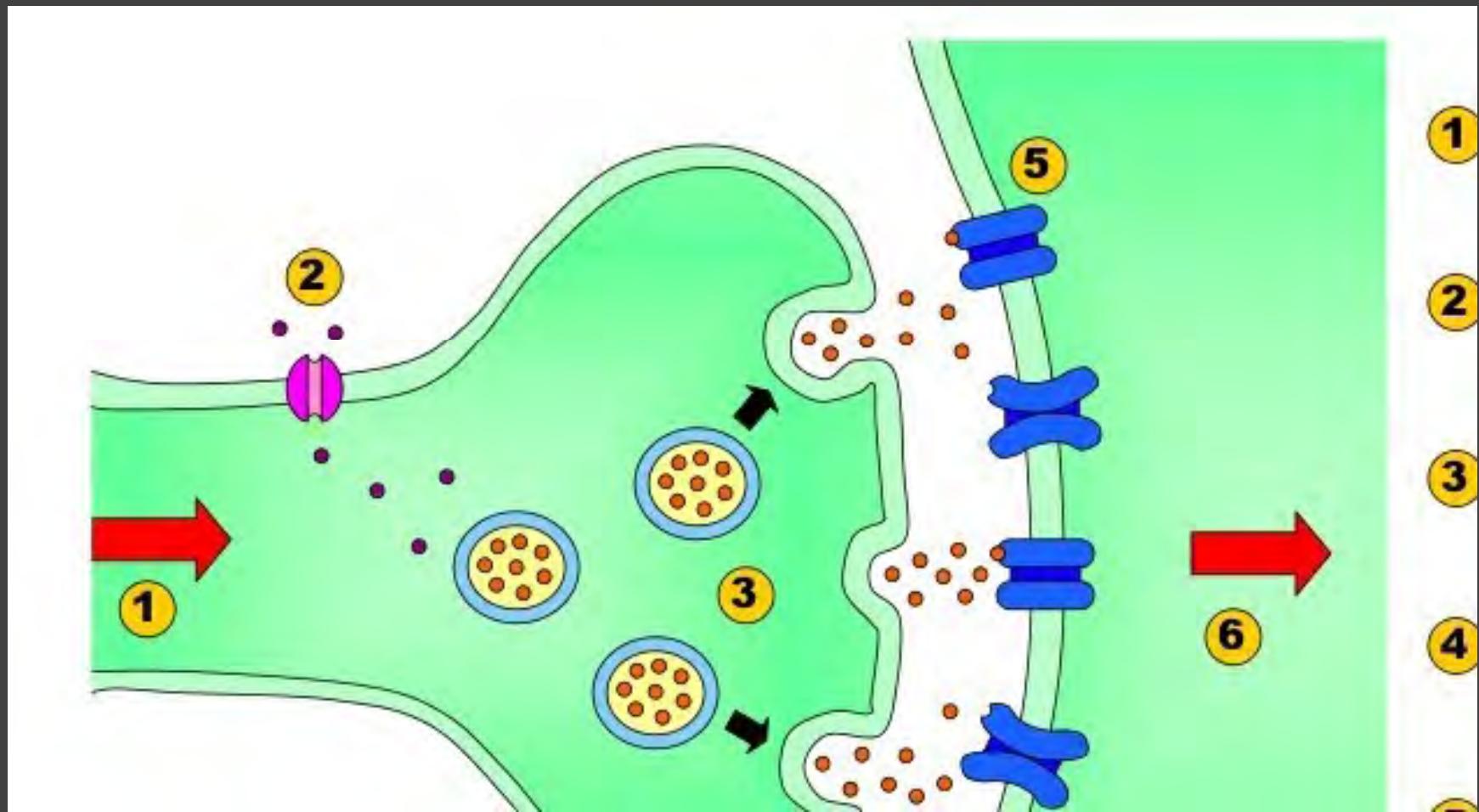
Electrical synapse



Electrical synapse



Chemical synapse

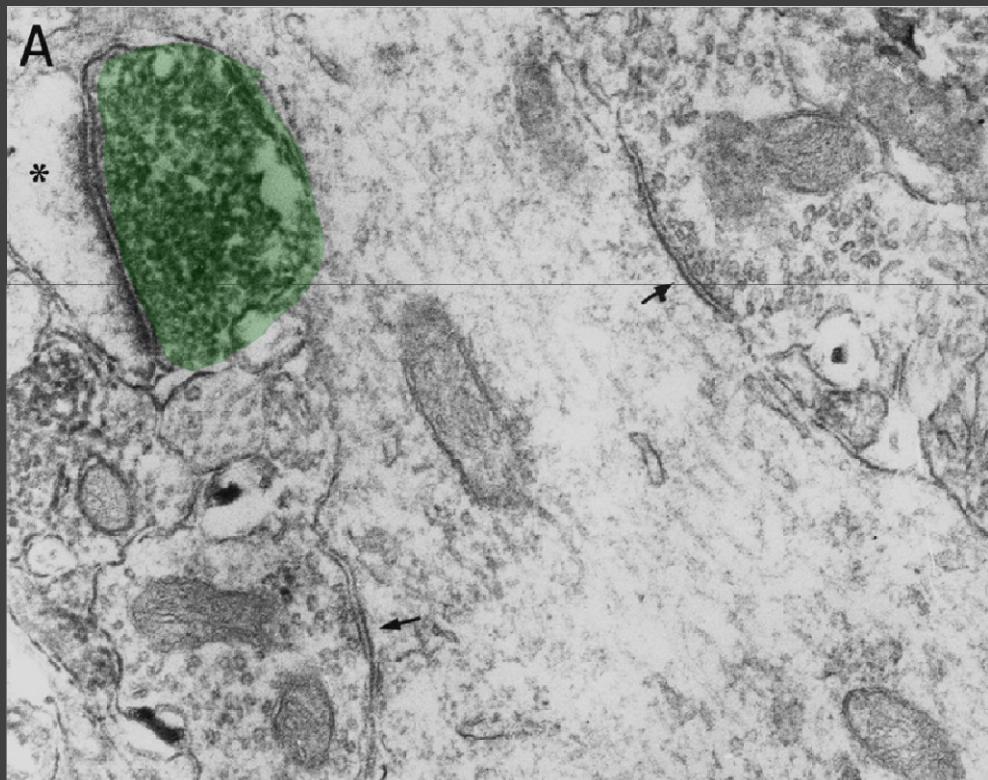


Chemical synapse

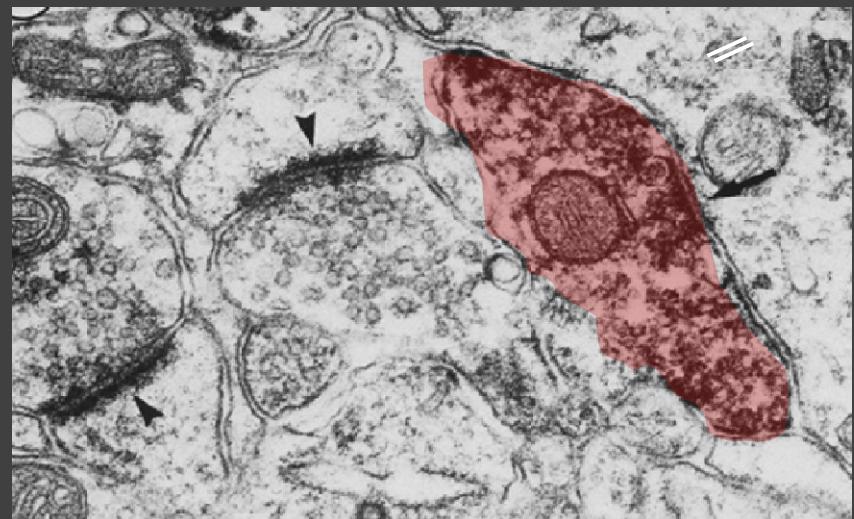


Classification of Cortical Synapses

Asymmetric synapses:
Excitatory (Glutamate)



Symmetric synapses:
Inhibitory synapses
(GABA)



Chemical synapses are:

- Much more complex BUT:
 - Unidirectional
 - Different neurotransmitters
 - Excitatory or inhibitory
 - They can be regulated!!!!

SYNAPSES

Type

Position

Number

Size

Origin-Target

Energy requirements

Pathology ...

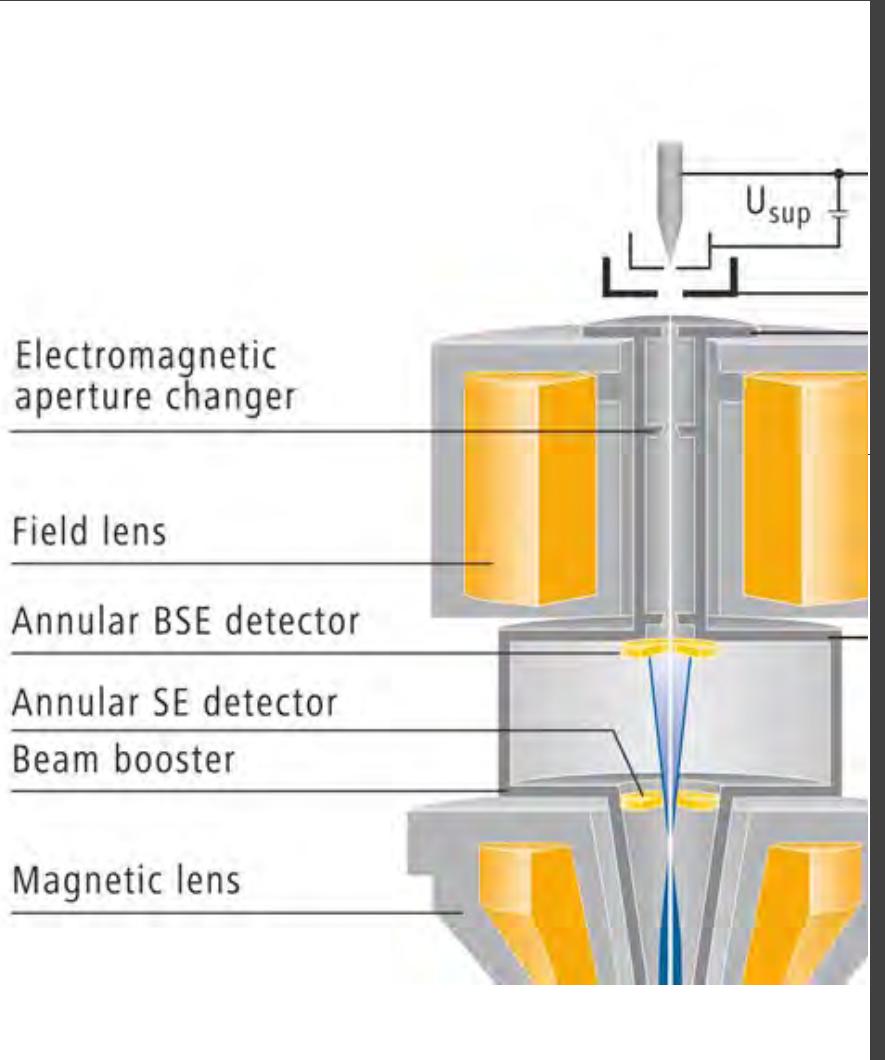


The dual-beam
microscope:
Focused Ion Beam /
Scanning Electron
Microscope (FIB/SEM)

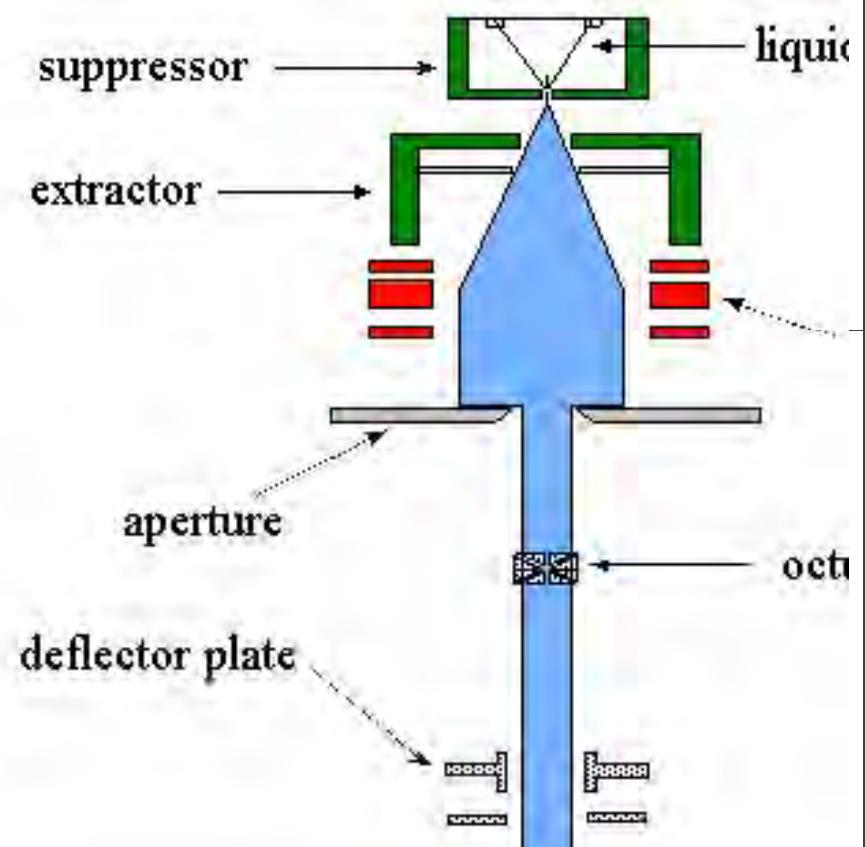
What is a FIB-SEM?

- It is a “two in one” microscope, also known as “dual-beam” or “cross-beam” microscope
- SEM = Scanning Electron Microscope
- FIB = Focused Ion Beam

Scanning Electron Microscope (SEM)



Focused Ion Beam (FIB)

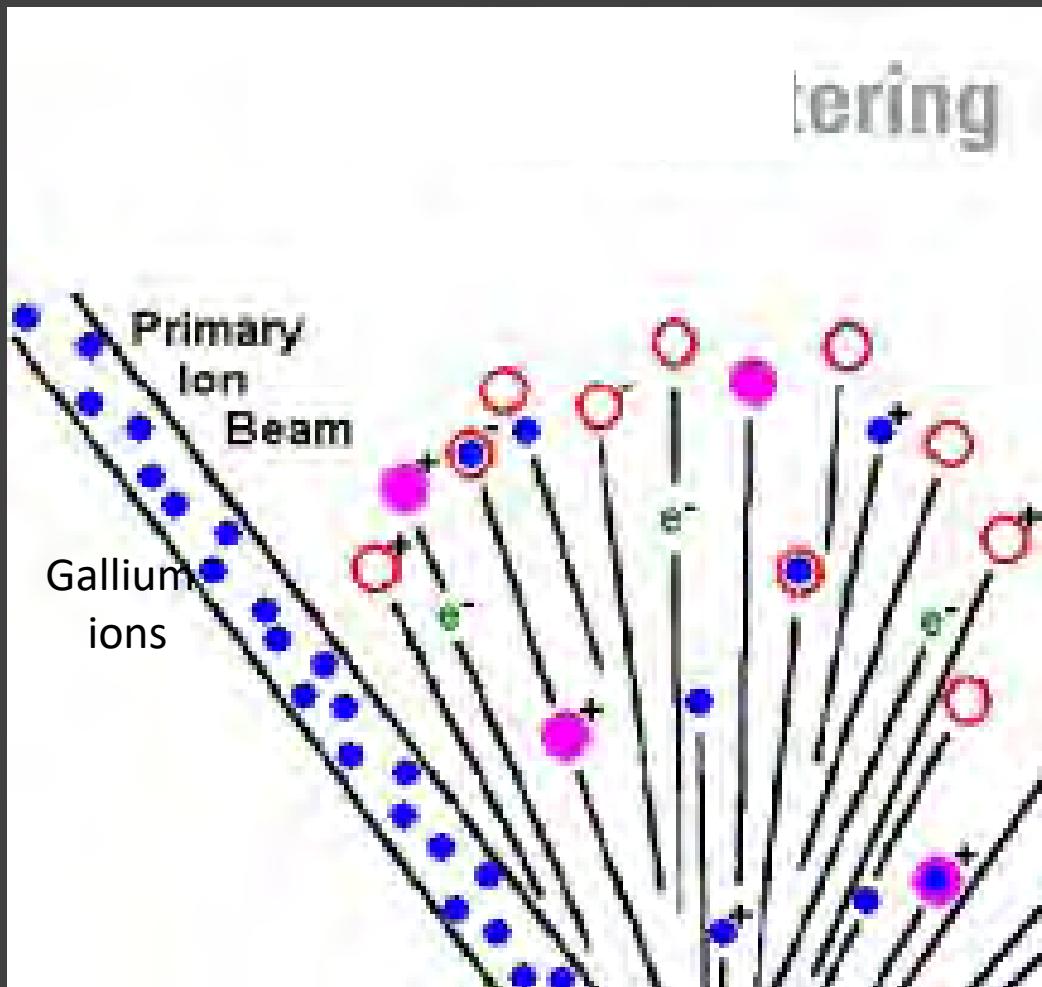




← ION GUN

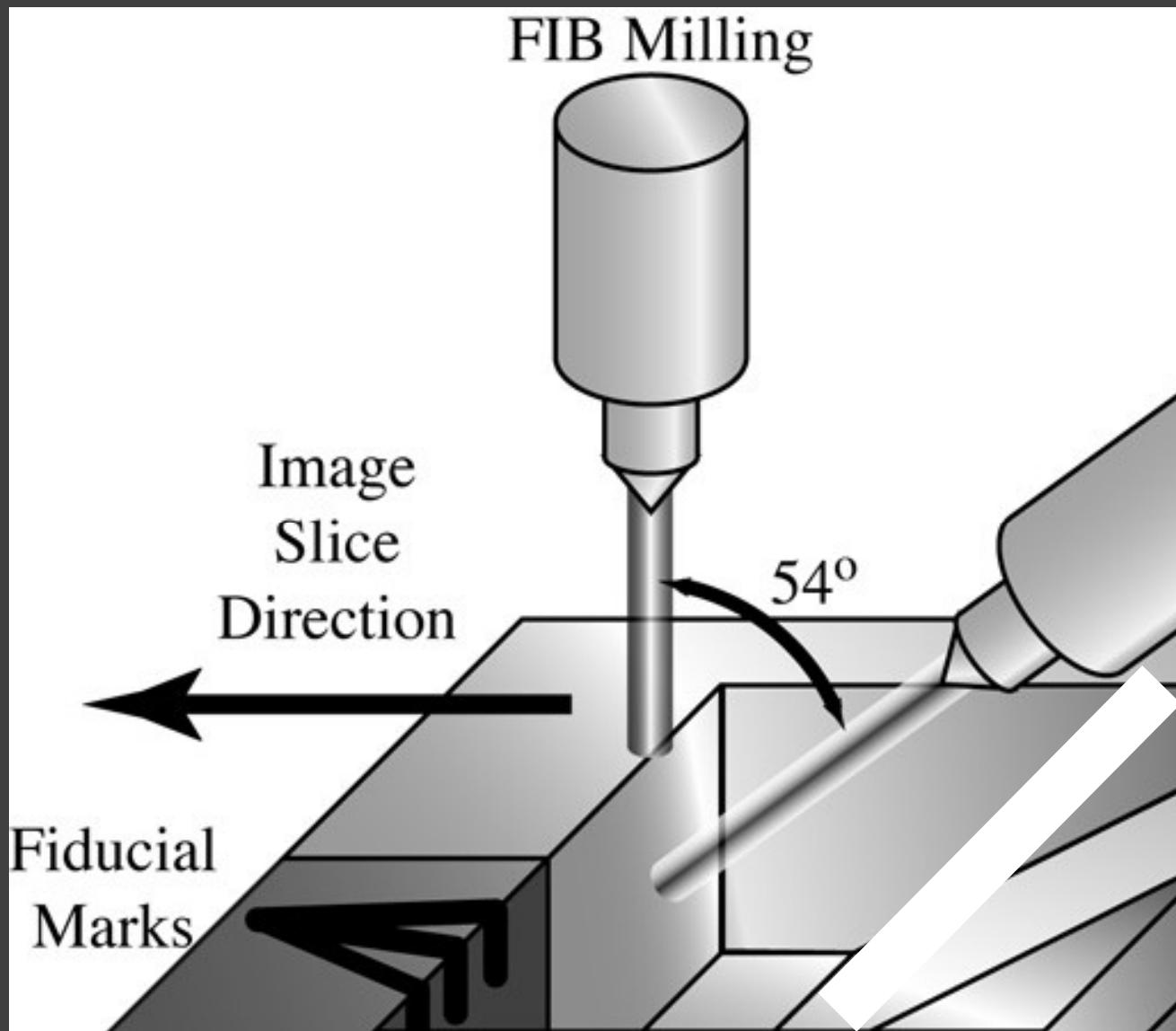
HAN SOLO

Focused Ion Beam (FIB)



The FIB-SEM is very useful to obtain serial images of the brain

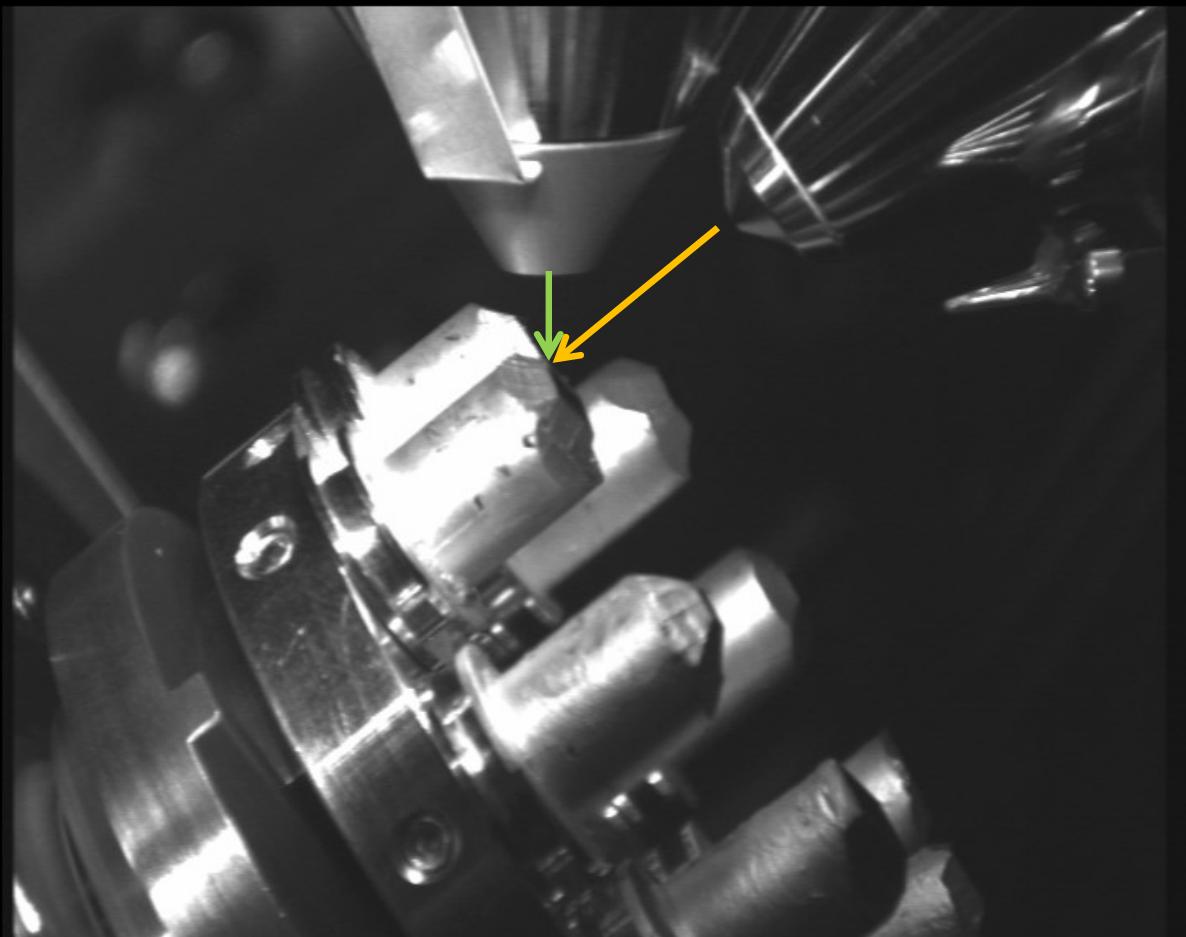
- We use the FIB to mill the sample.
 - We can remove a very thin layer of material from the surface of the sample
- We then use the SEM to acquire an image of the freshly milled surface
 - We use the back-scattered electrons to acquire the image
- We can repeat these processes automatically!!!
 - In this way we can obtain hundreds of serial images separated by a known distance











100 μ m

FIB Lock Mags = No

Mag = 99 X

EHT = 0.00 kV

WD = 1.3 mm

Signal A = TV

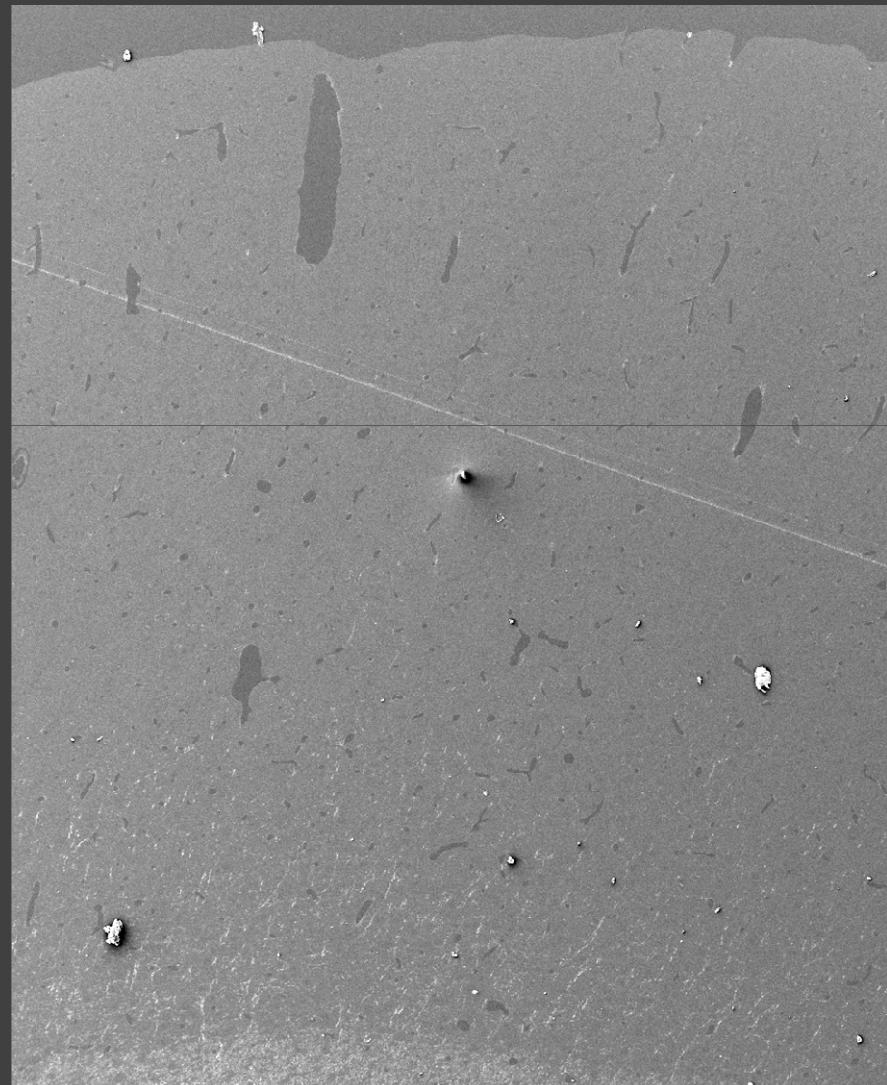
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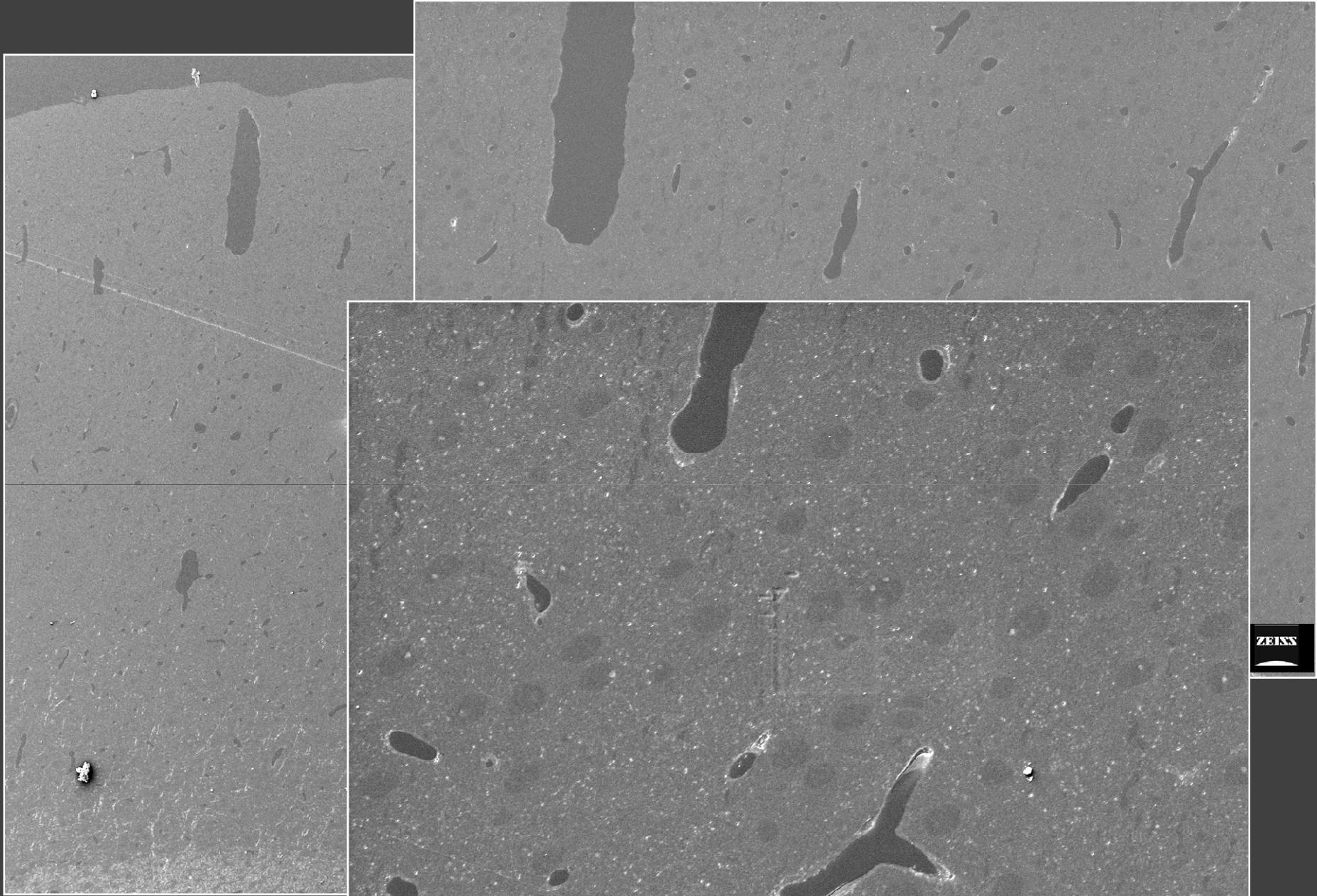
Date :19 Nov 2010

Time :13:02:43



Localization of the region of interest



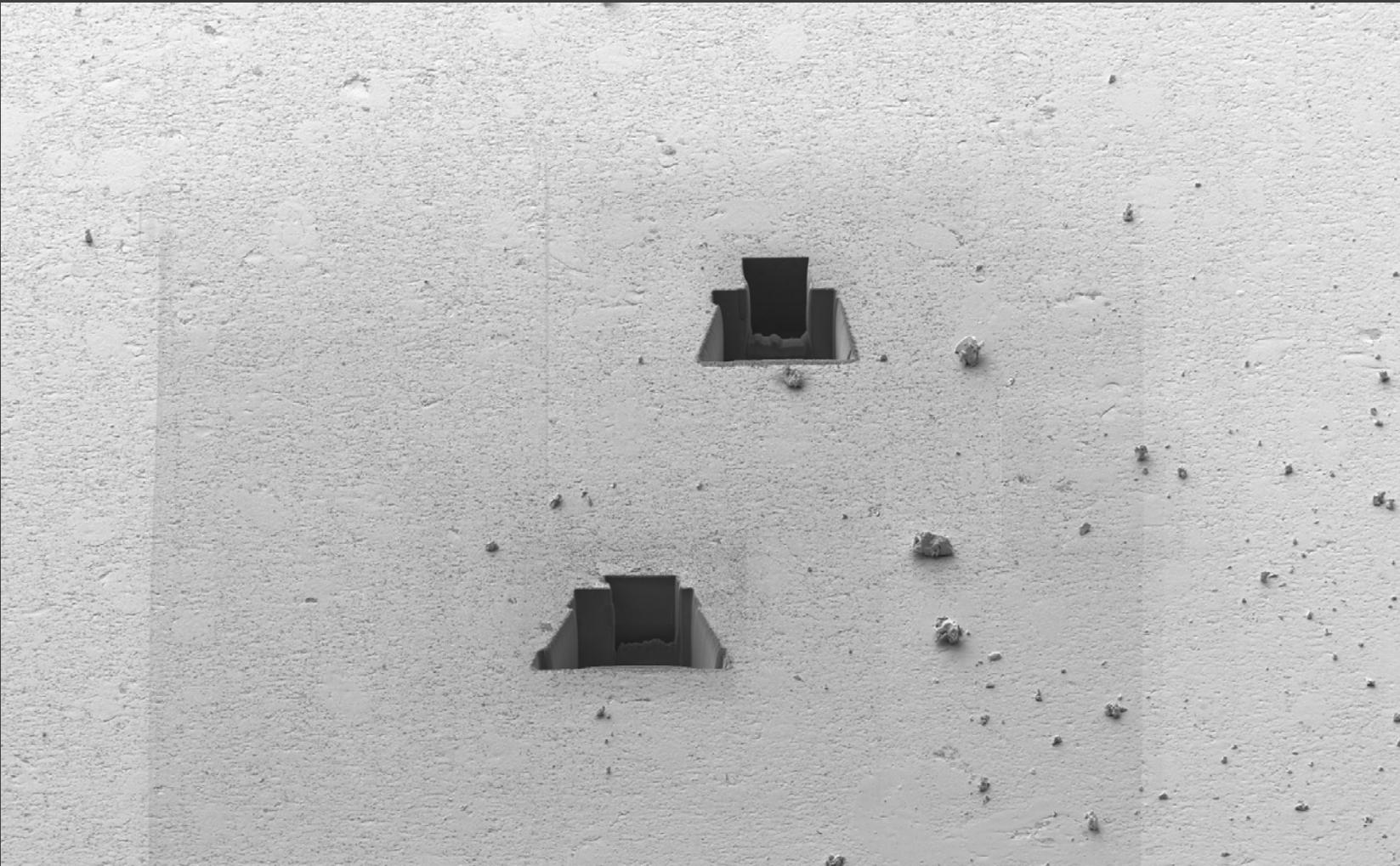
20 μ m

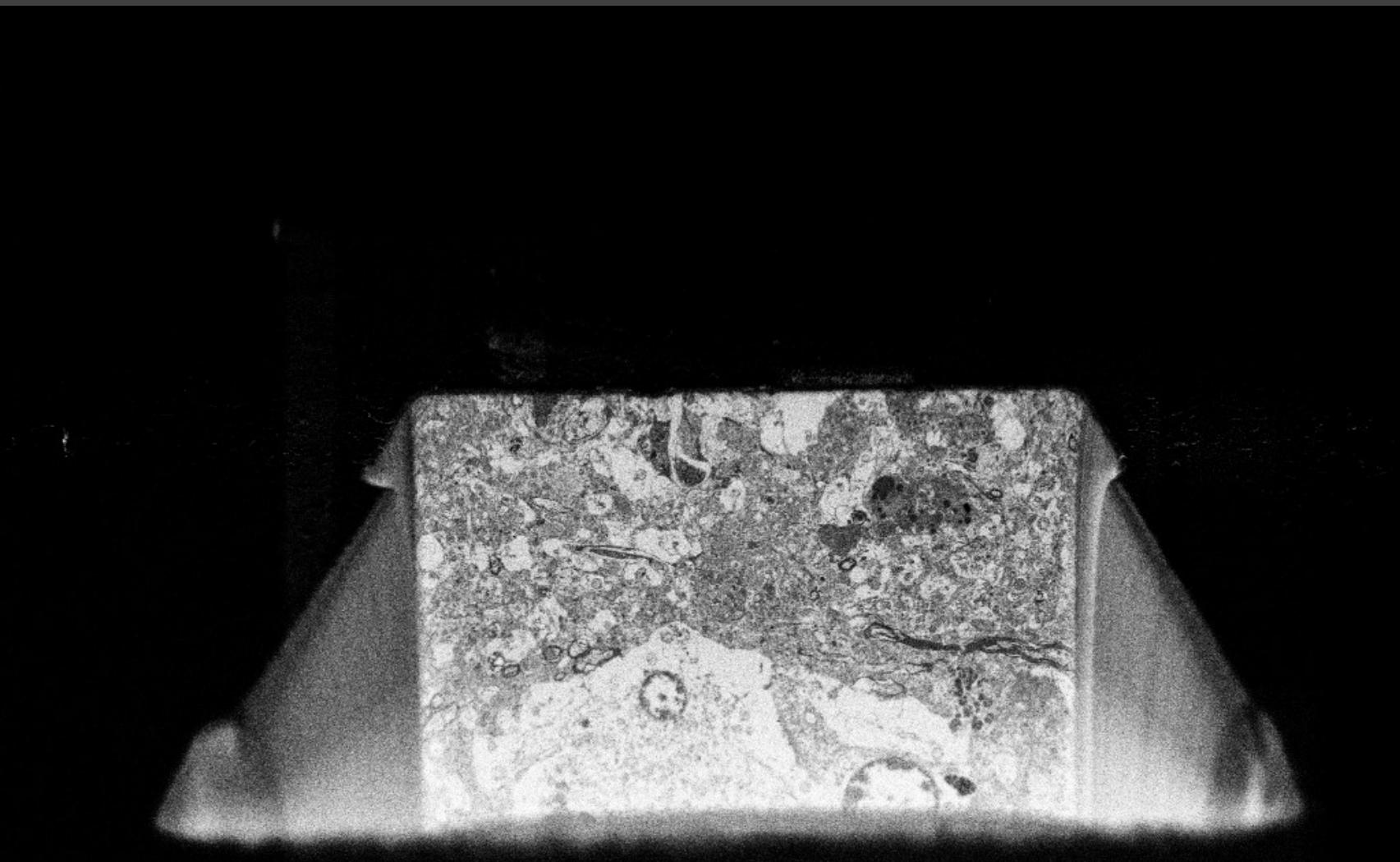
FIB Lock Mags = No

Mag = 1.26 K X EHT = 5.00 kV Signal A = SE2 Date : 17 Feb 2010
WD = 18.6 mm Photo No. = 514 Time : 11:36:51

ZEISS

Excavation of a "trench" on the surface of the sample





10 μ m

FIB Lock Mags = Yes

Mag = 3.63 K X

EHT = 2.00 kV

WD = 5.1 mm

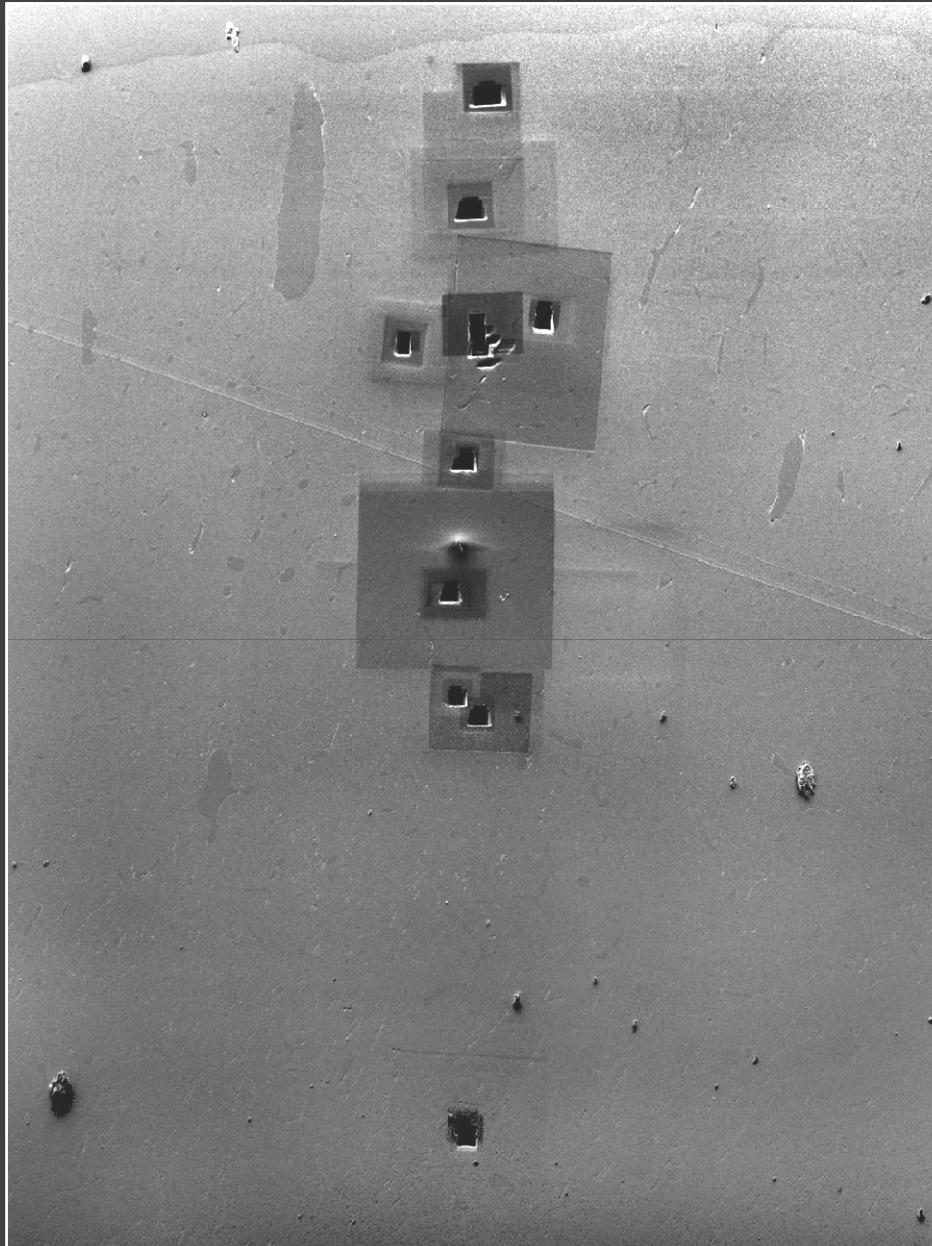
Signal A = ESB

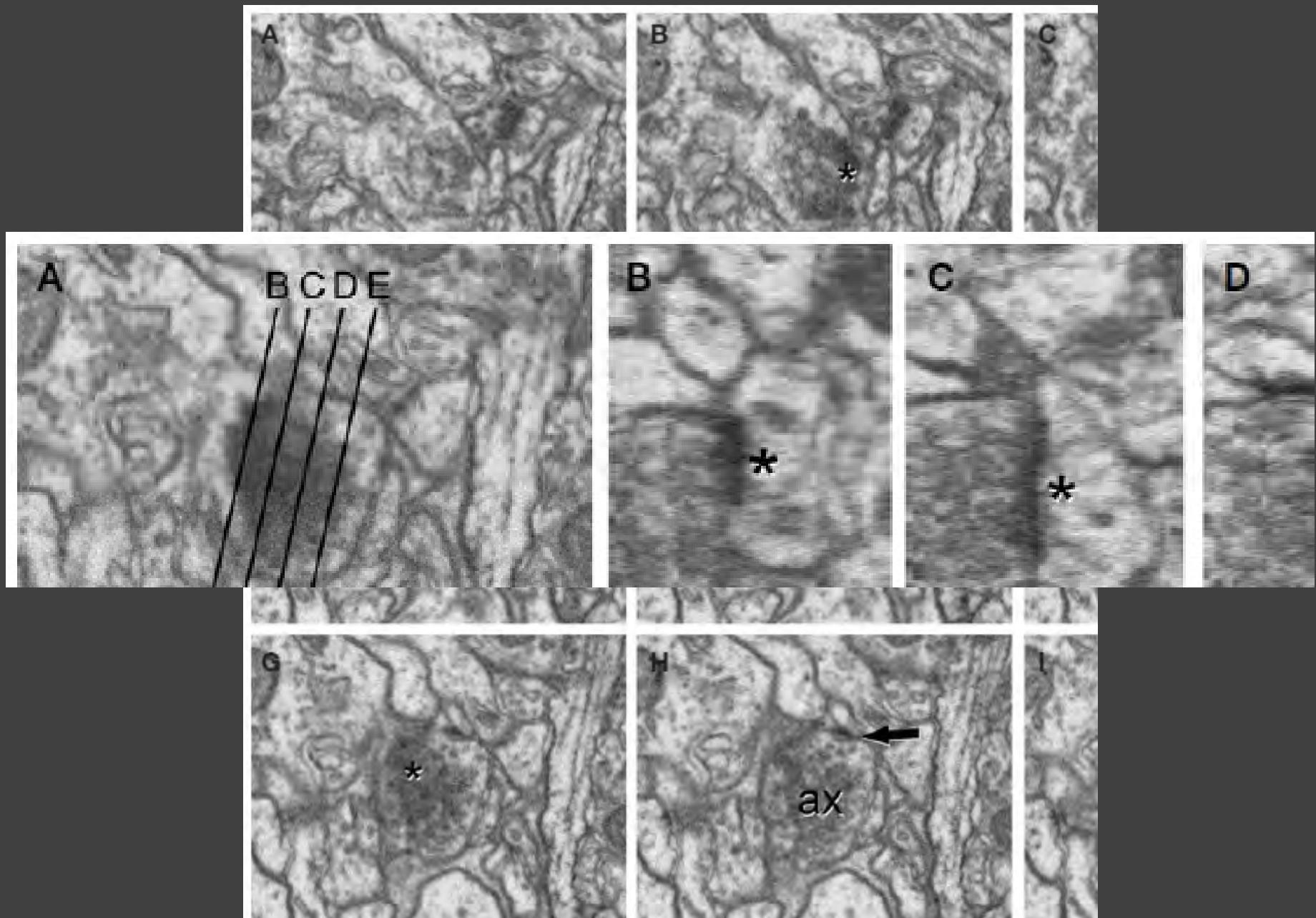
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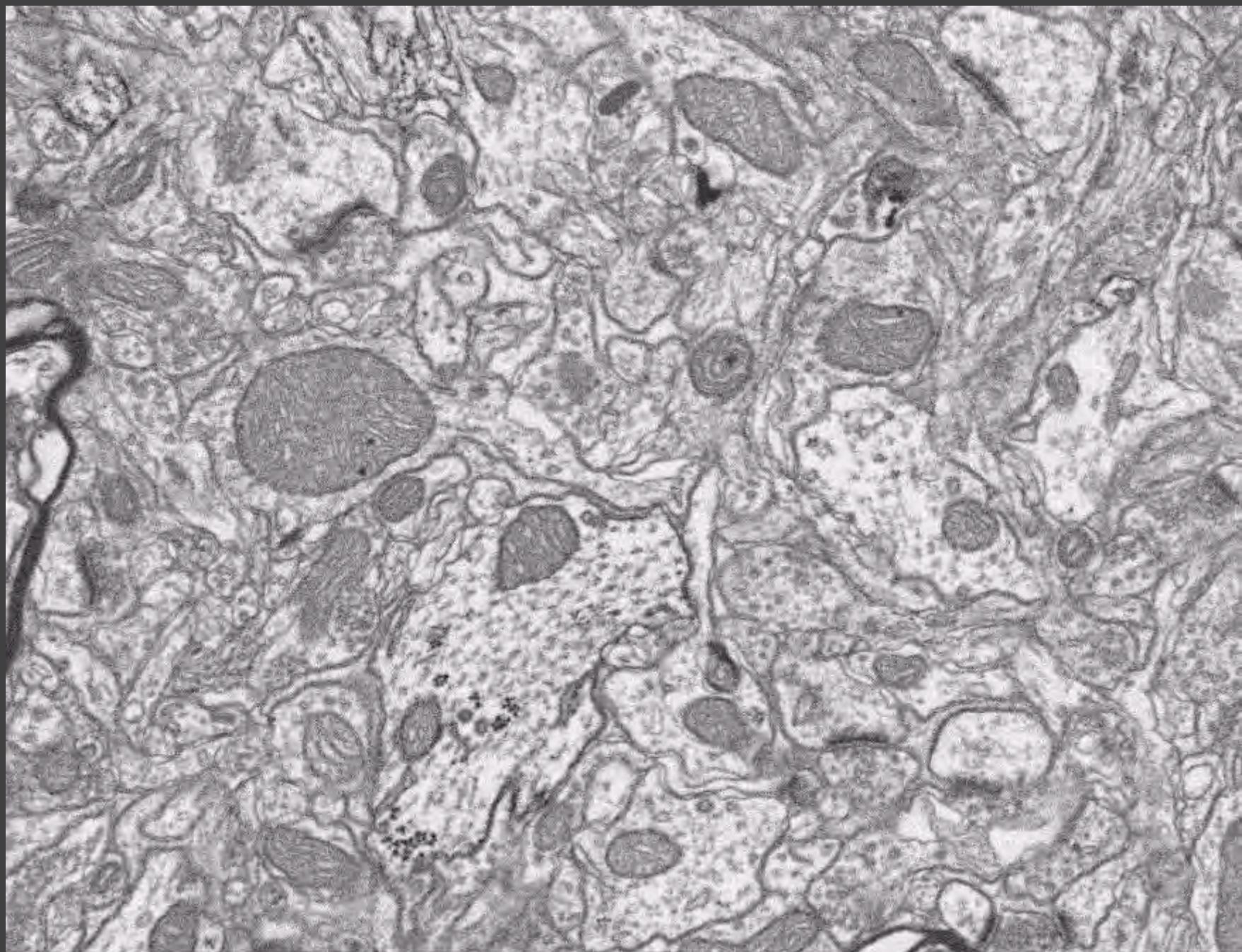
Date : 30 Nov 2009

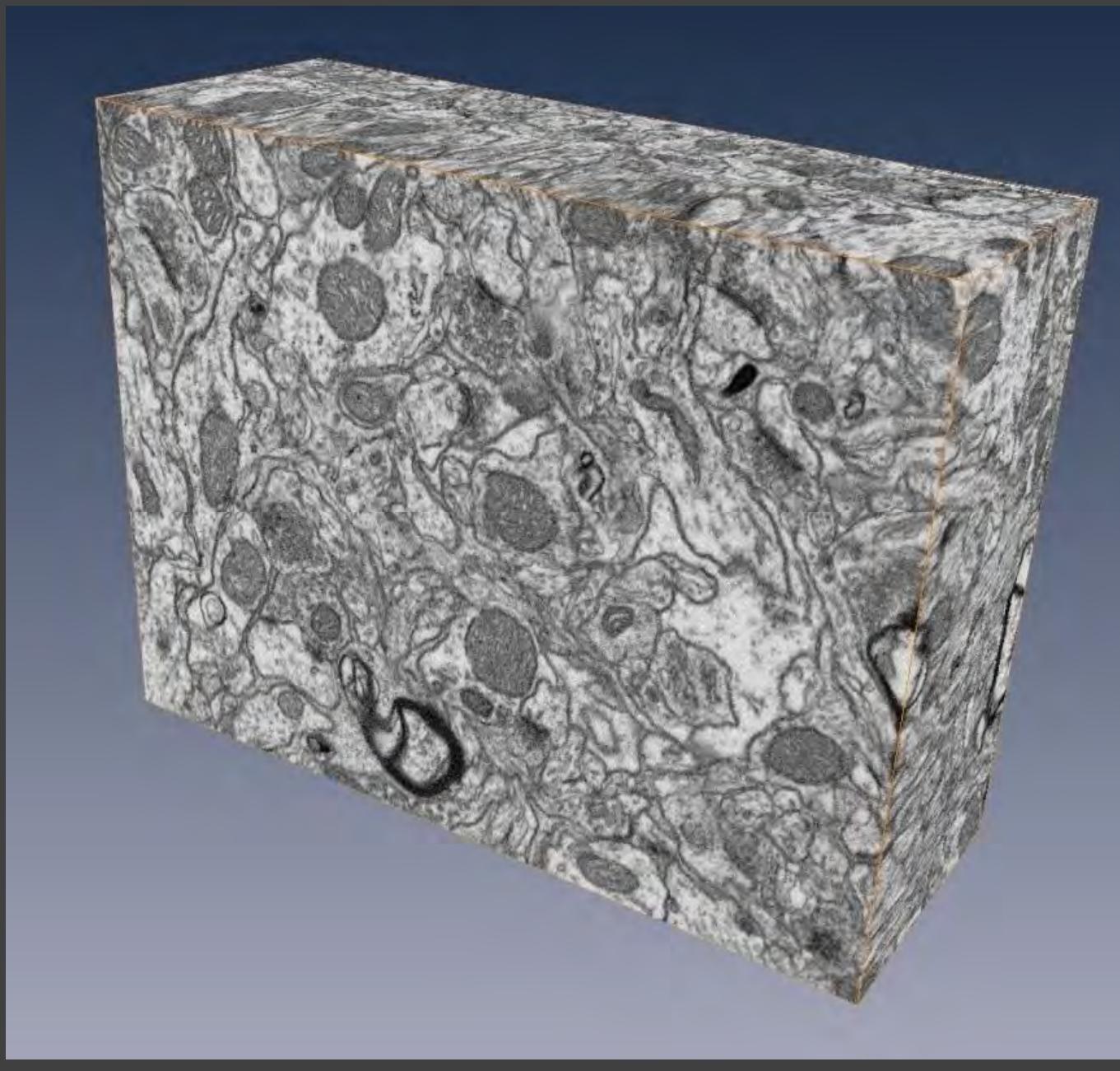
Time : 17:38:27





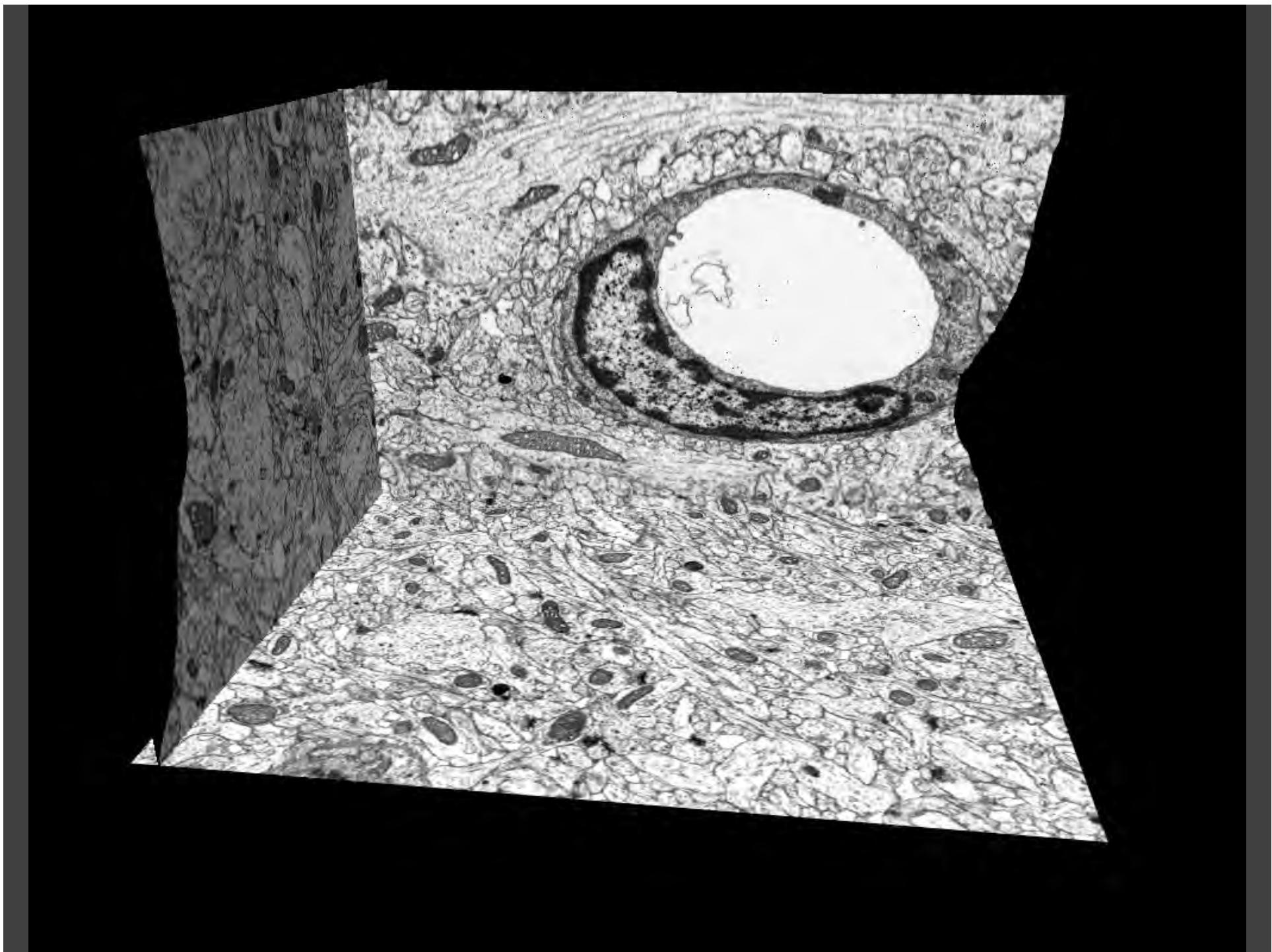






What are serial sections used for?

- We can pile up-serial images to form a brick or stack of serial sections
- The stack of serial sections represent a piece of tissue in 3D
- Anything that is visible inside the stack can be reconstructed in 3D
- Visualization and analysis in 3D is specially useful for the study of the very complex structure of the brain



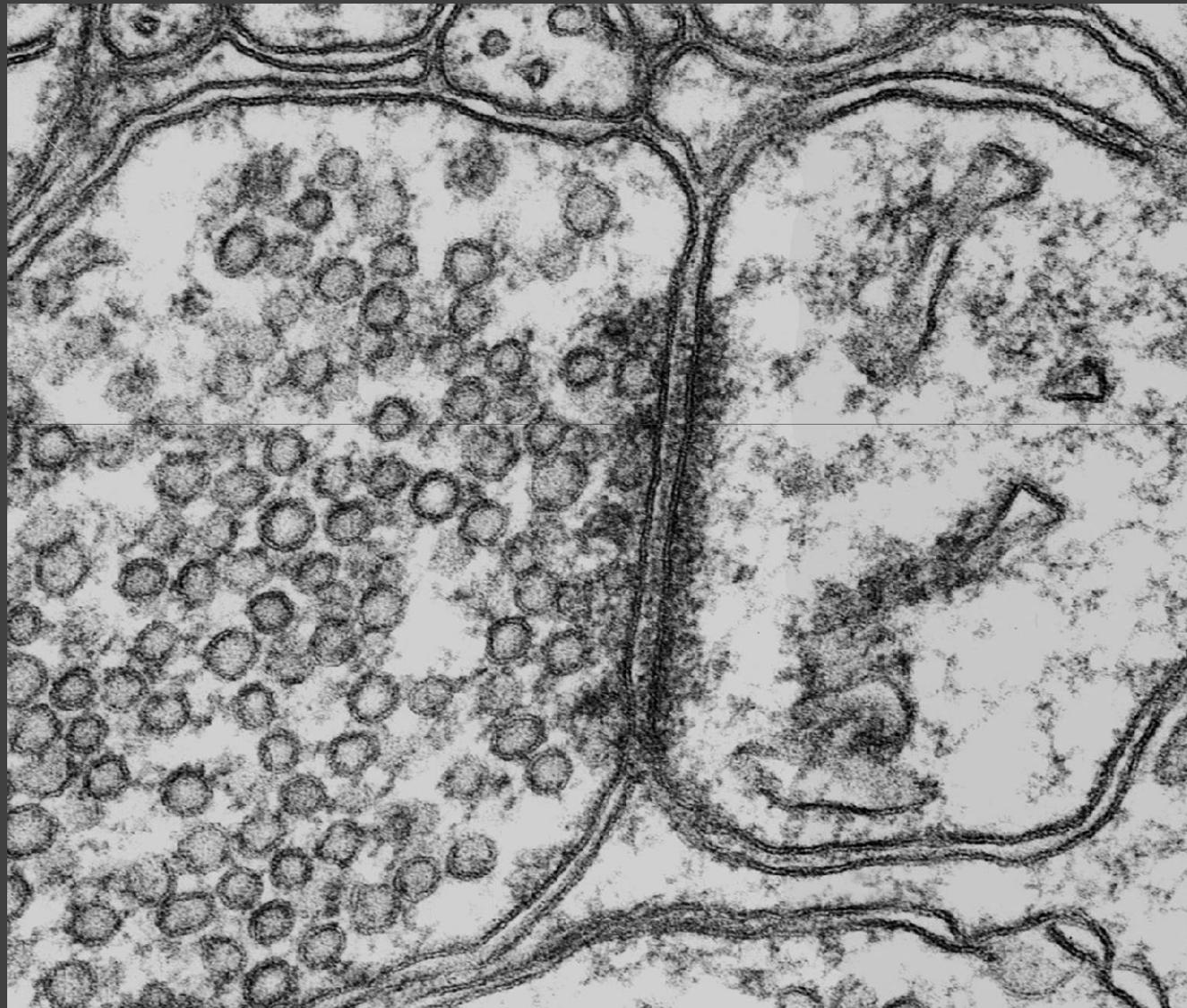
But we need dedicated software

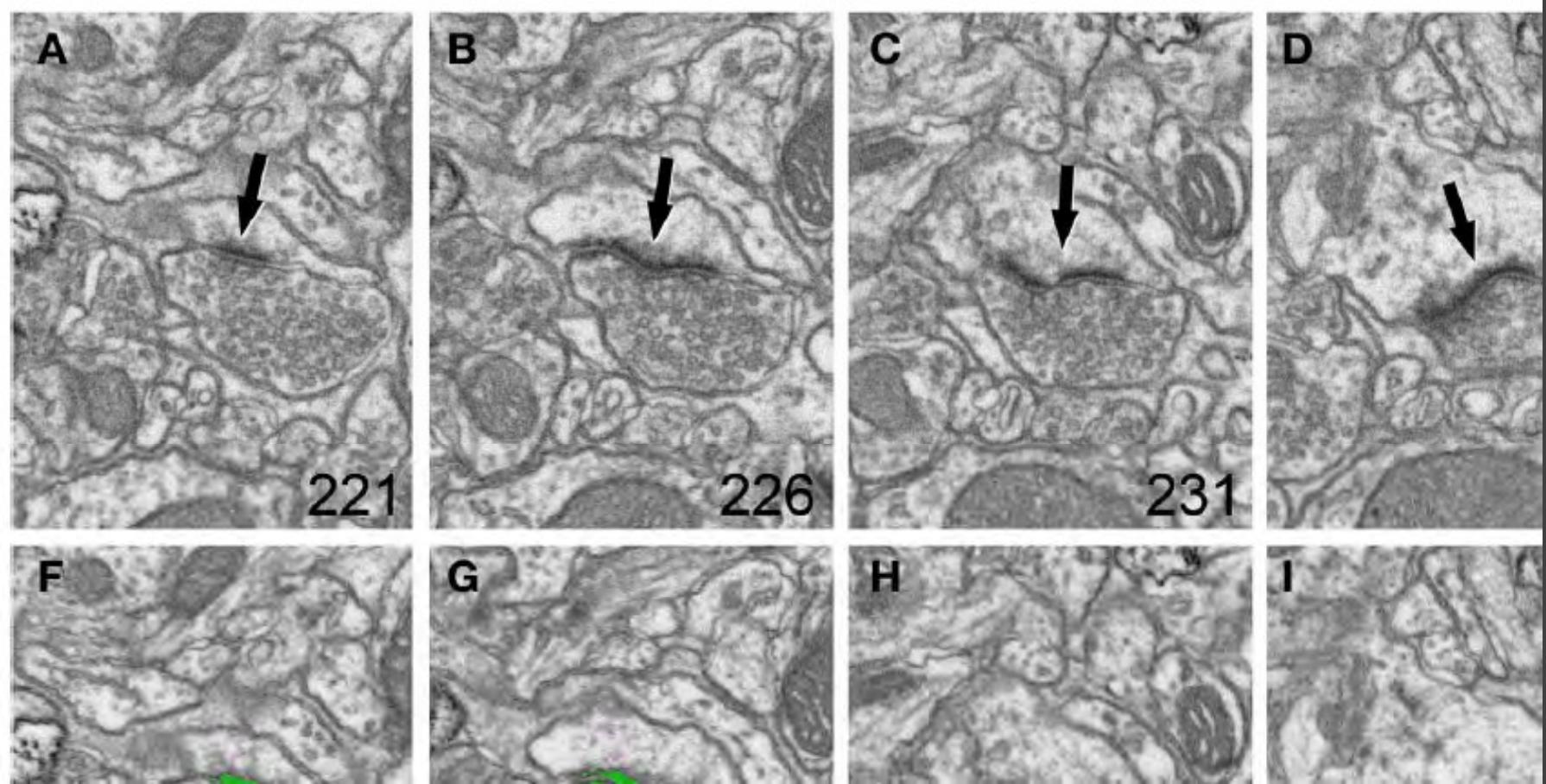
- Now that large amounts of serial sections can be obtained automatically the main problem is how to analyze the stacks of sections
- We have developed “Espina”, a software tool that allows us to visualize, navigate and analyze 3D stacks of the brain
- It can provide quantitative information about virtually any structure that is visible inside our 3D stacks

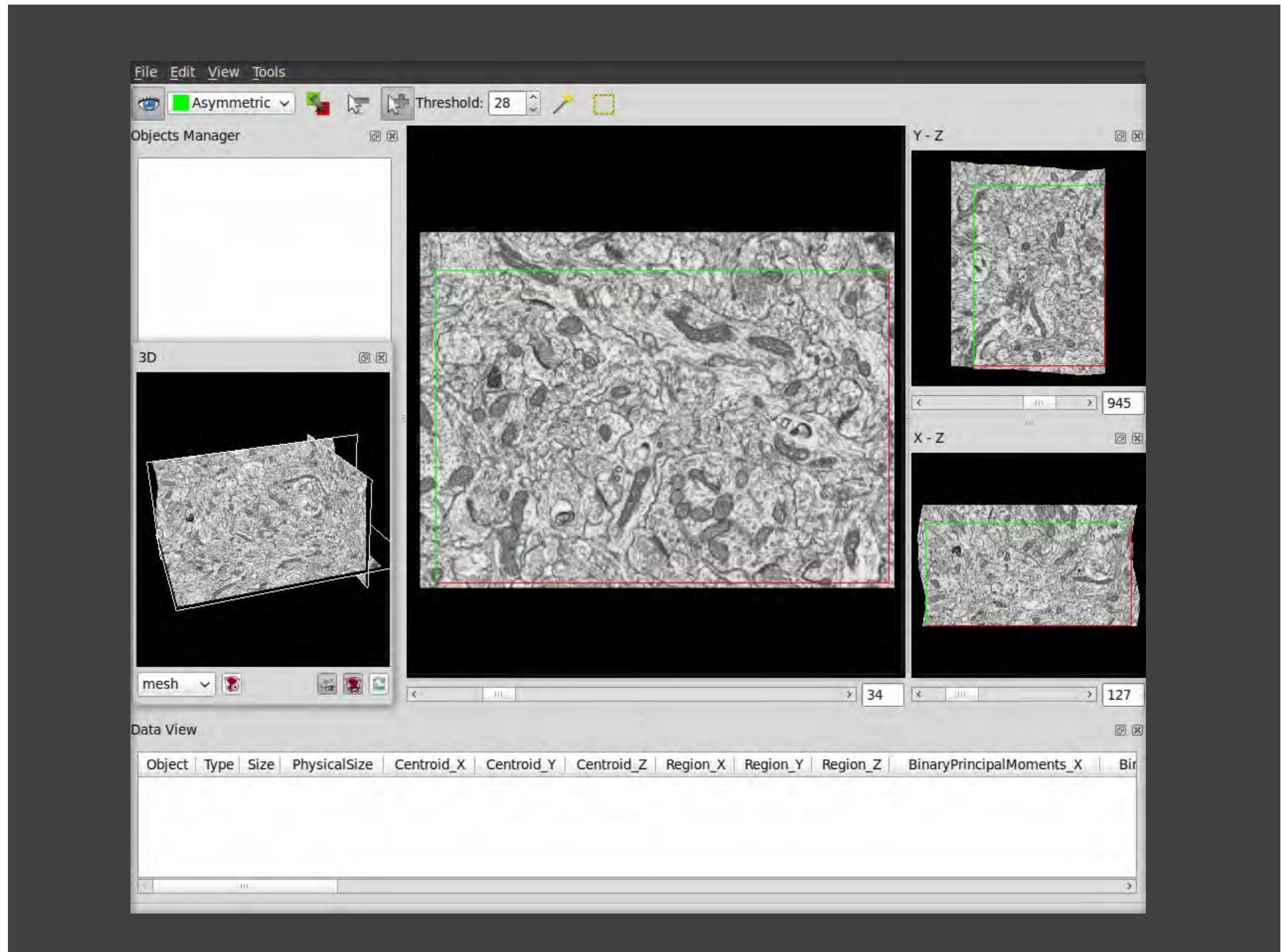
Espina

A new tool for segmentation and
image analysis

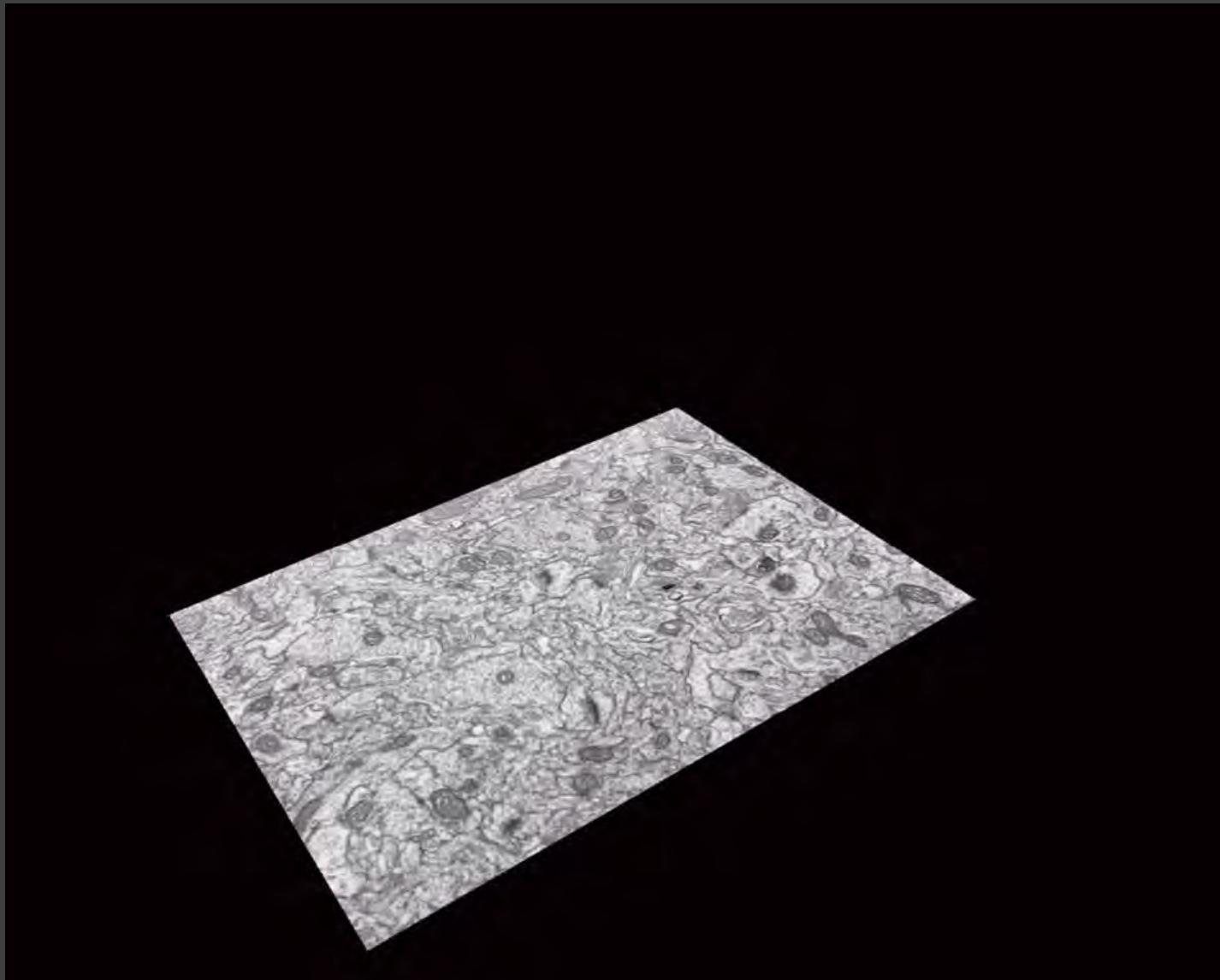
How to identify synapses







Spatial distribution of synapses



Merchan-Perez et al., Cerebral Cortex 2014

Tree basic patterns of spatial distribution:

Regular
Clustered
Random (CSR)

Spatial statistics tools

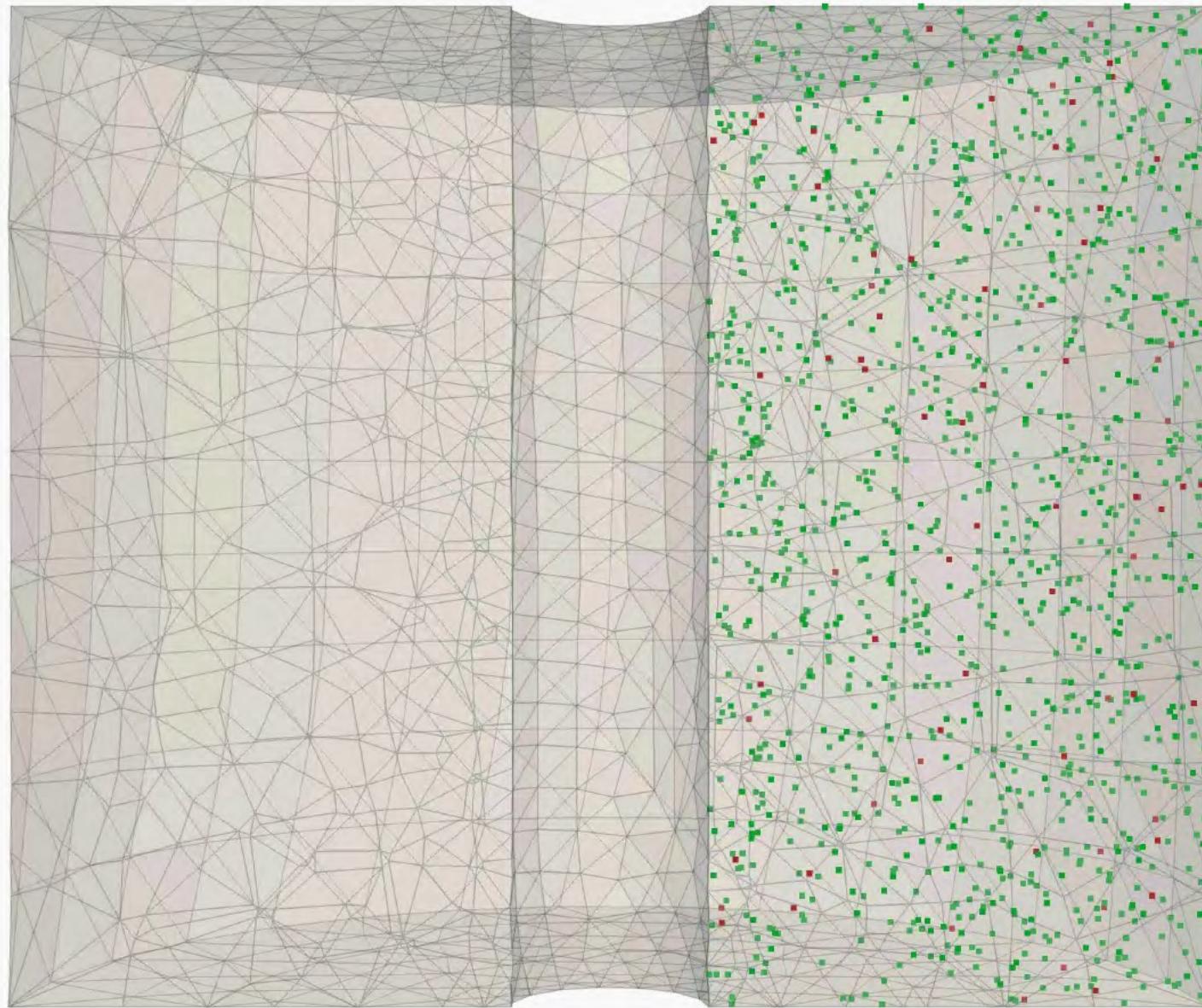
- G function (nearest neighbor)
- F function (empty space)
- K and L functions

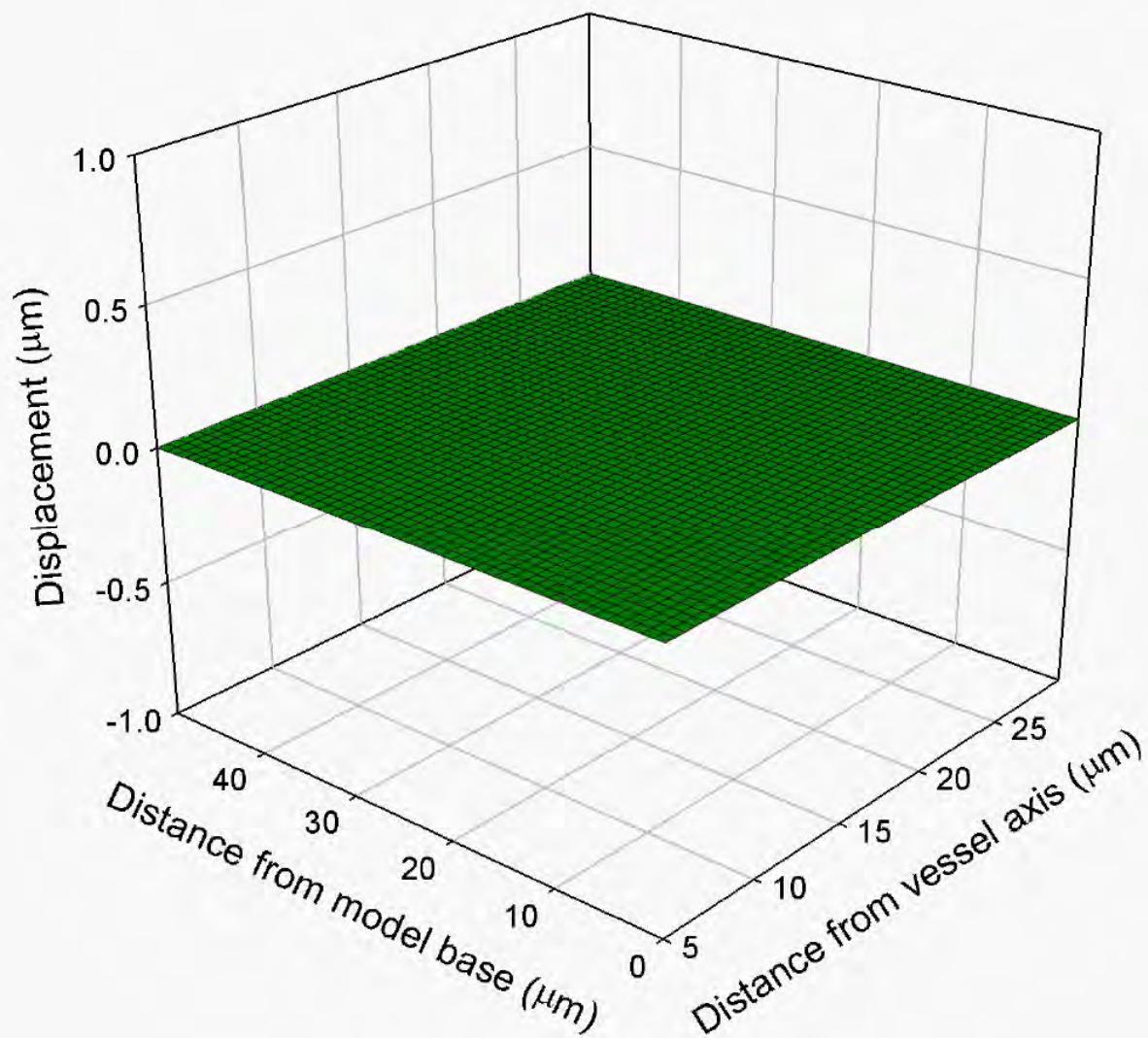
The RSA model

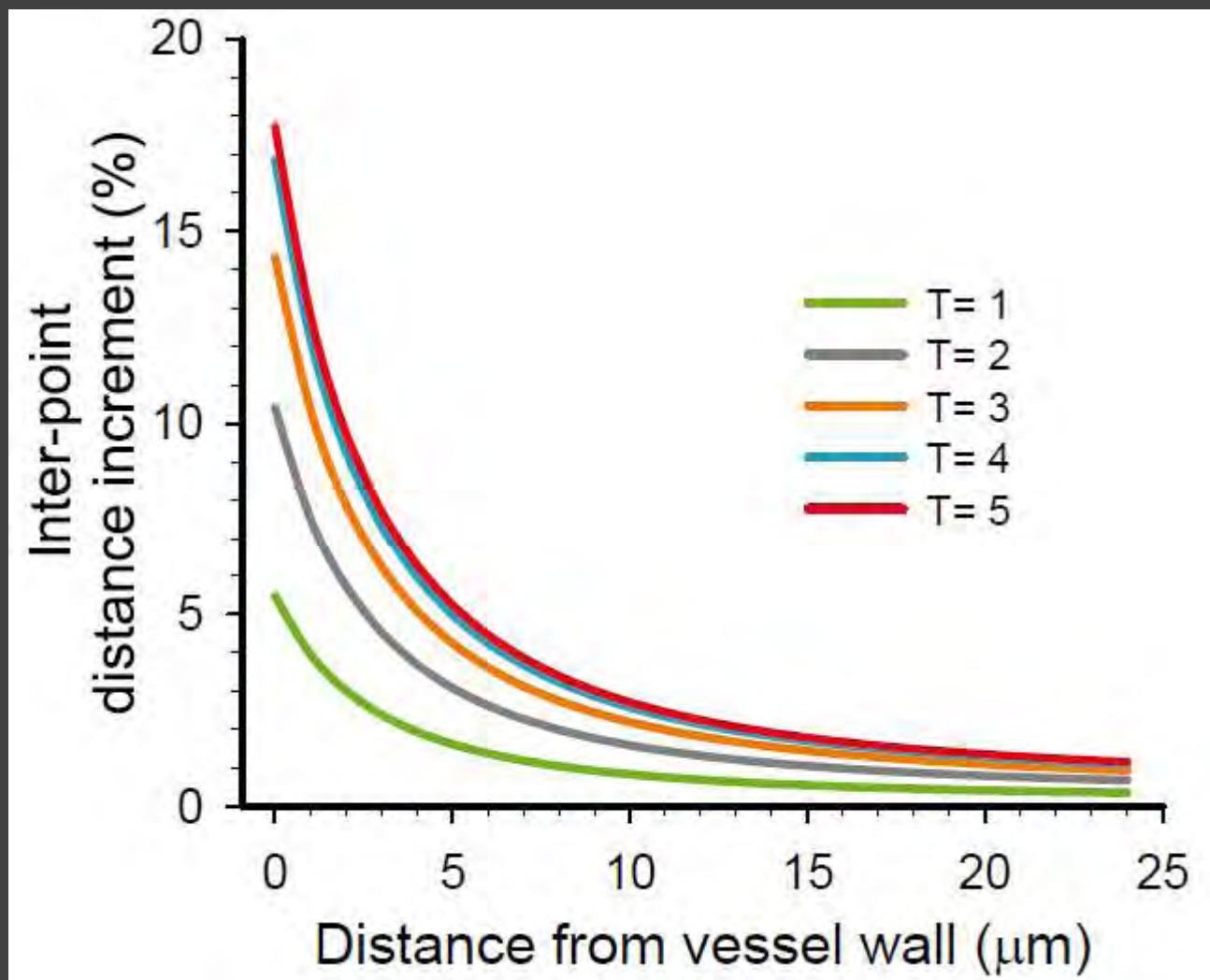
Merchan-Perez et al., Cerebral Cortex 2014

Does position of synapses matter?

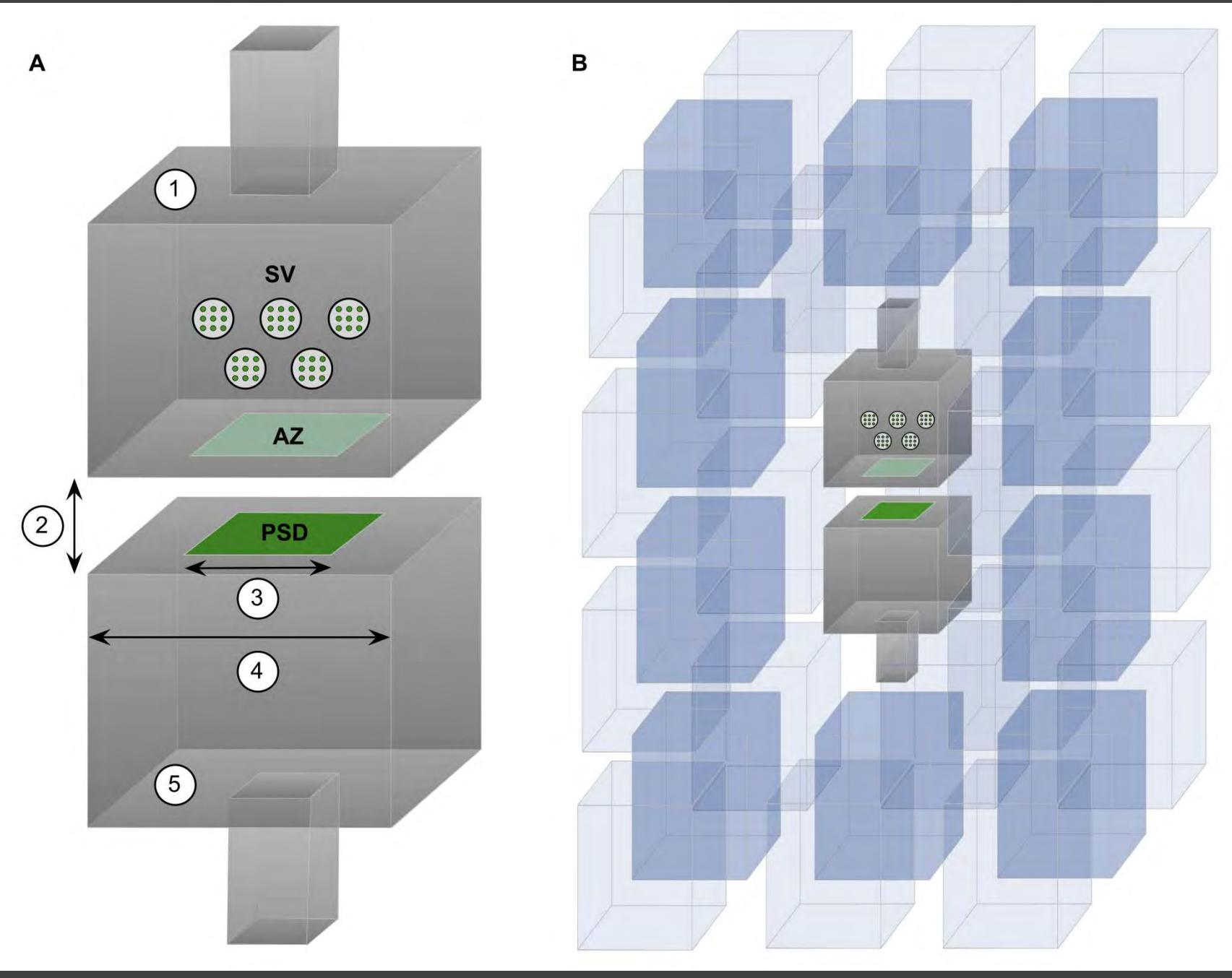
Modeling the positions and
movements of perivascular synapses

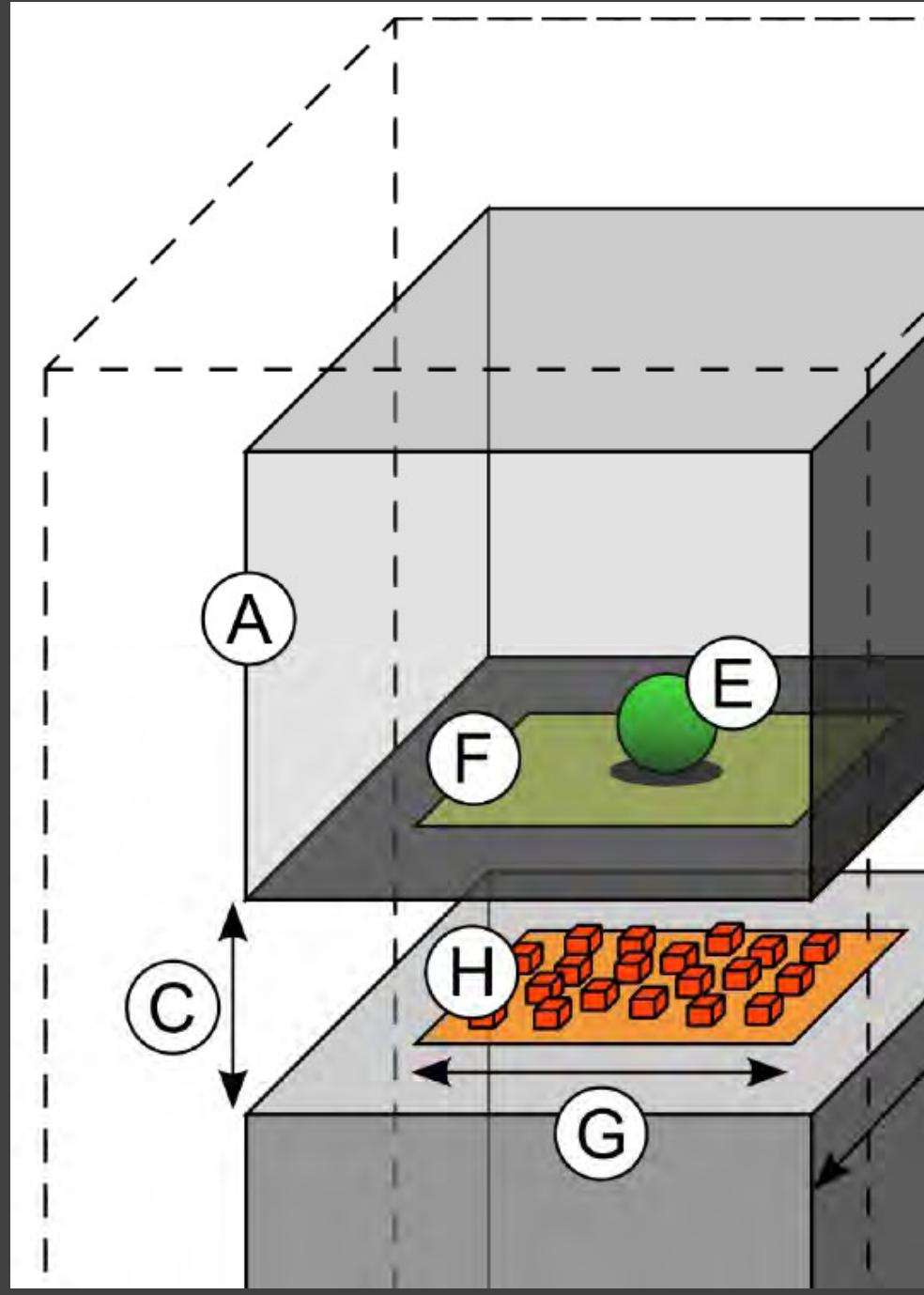




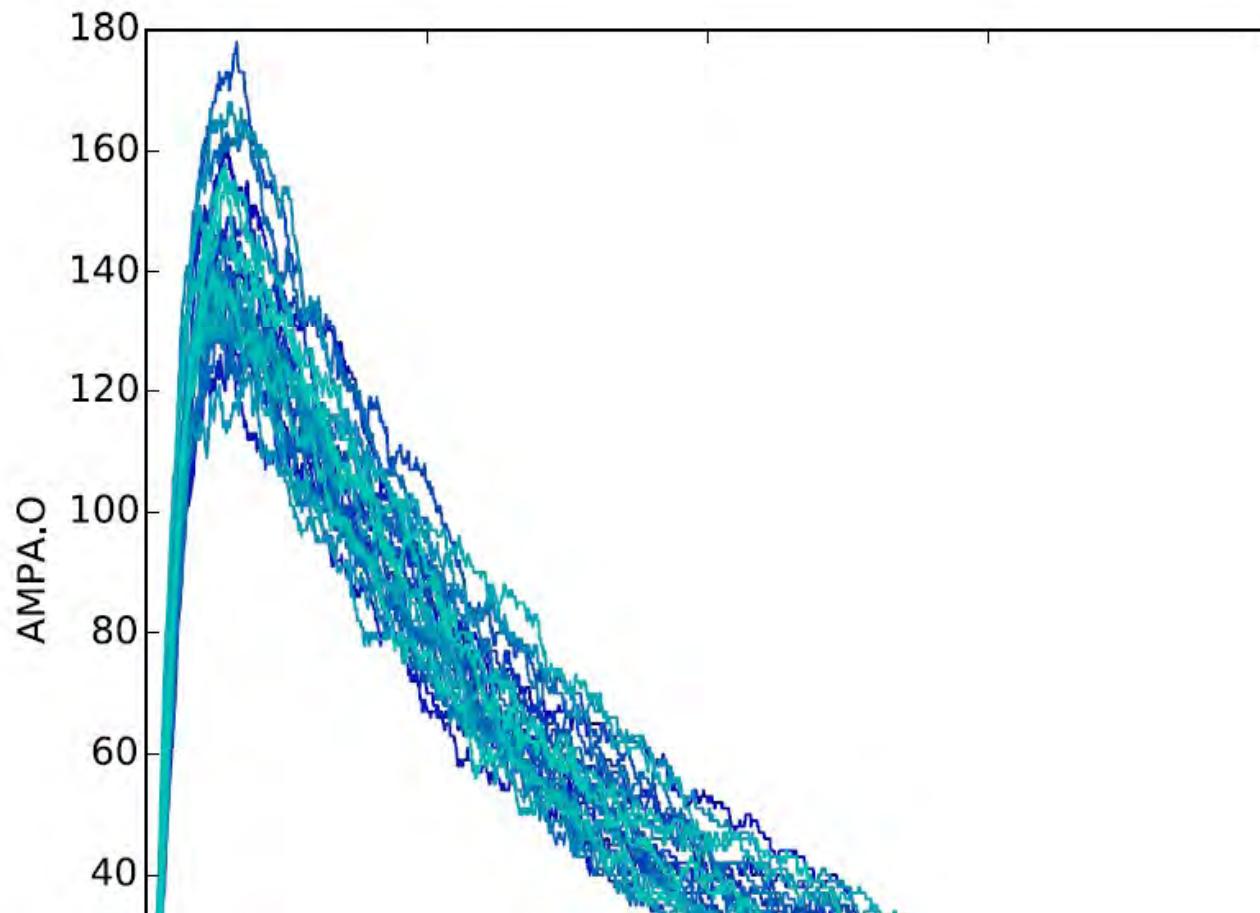


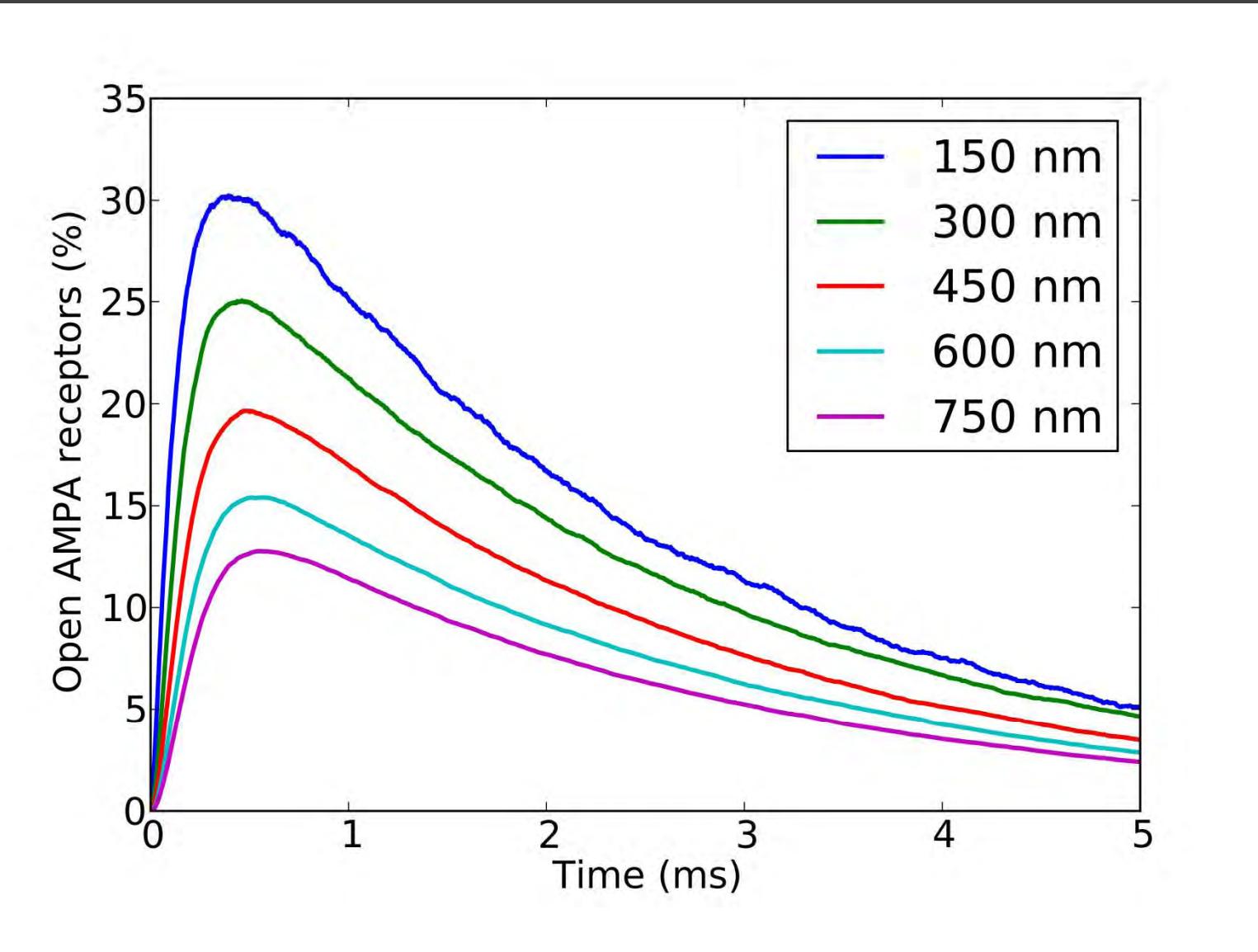
Does size affect the behavior of
synapses?

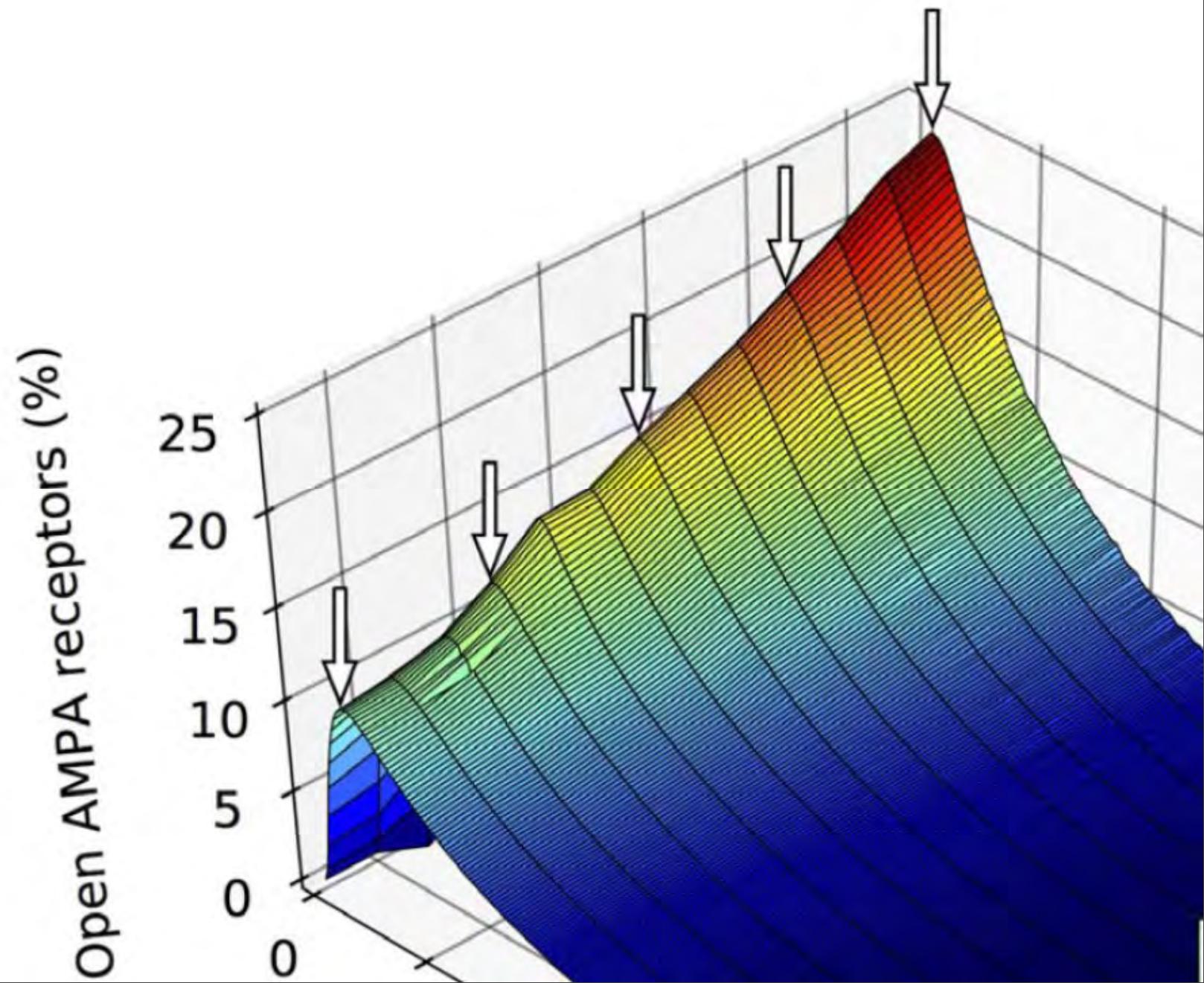




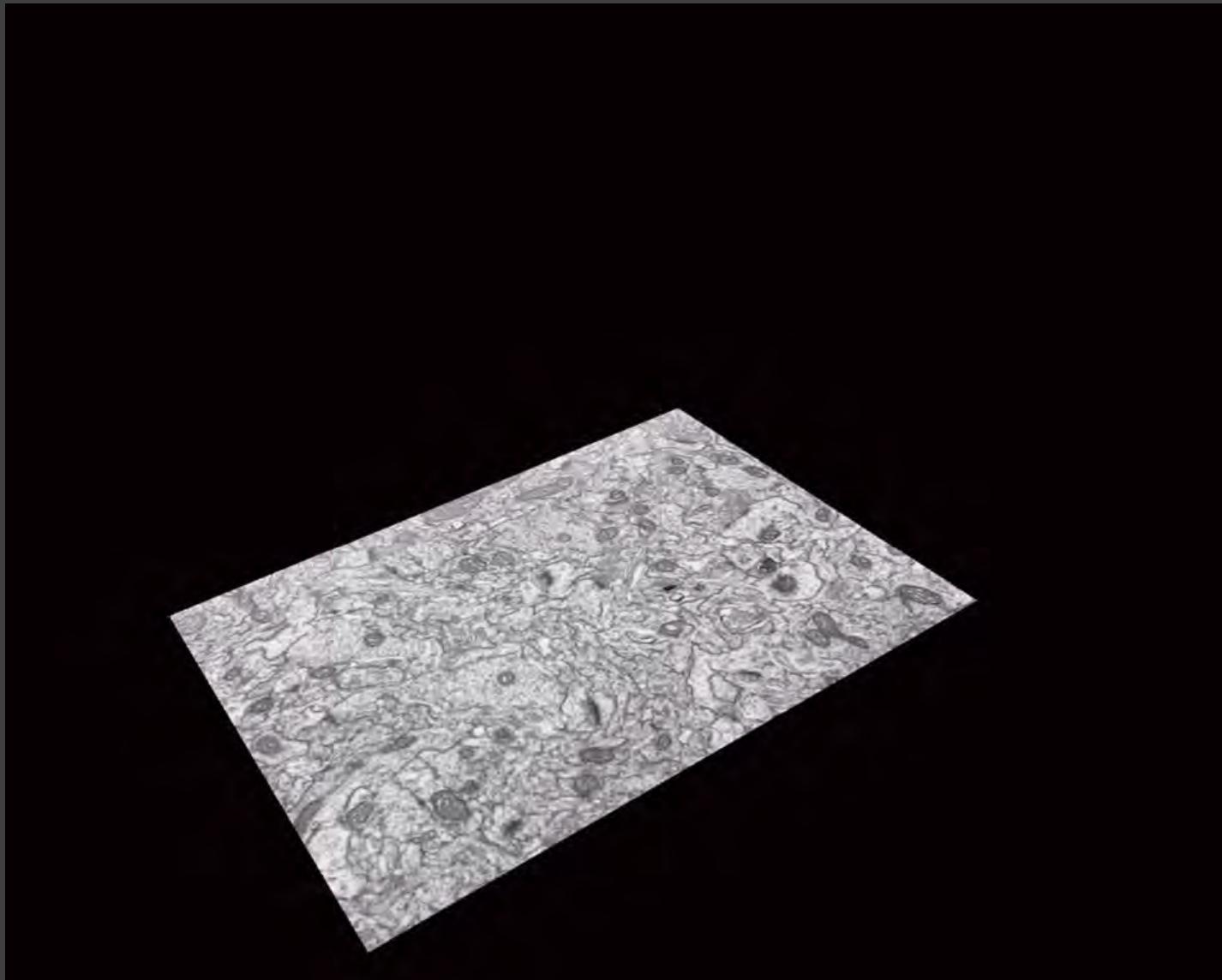
$cAMPA = 3000, cT = 7500, Ls = 750, Hc$



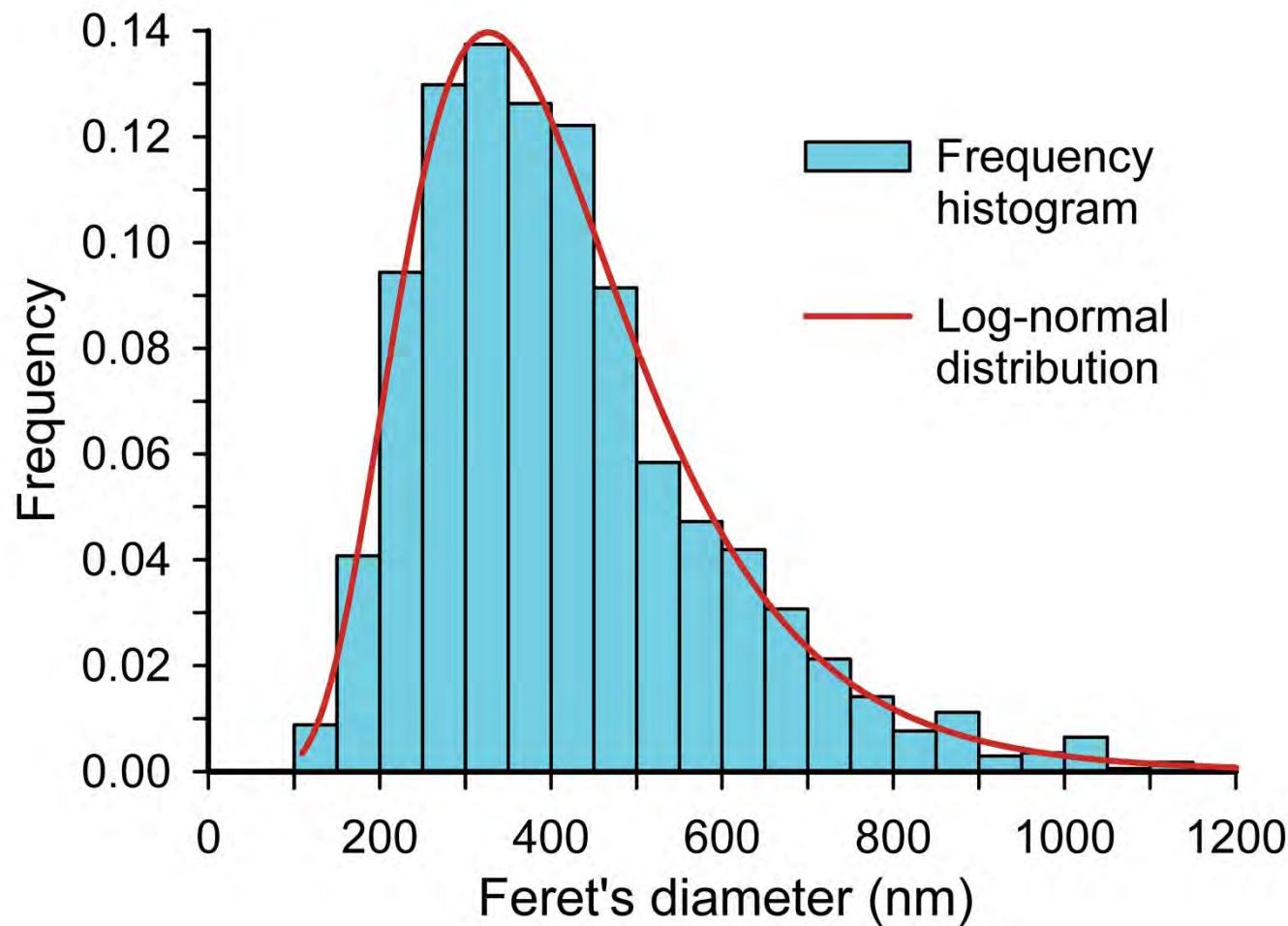


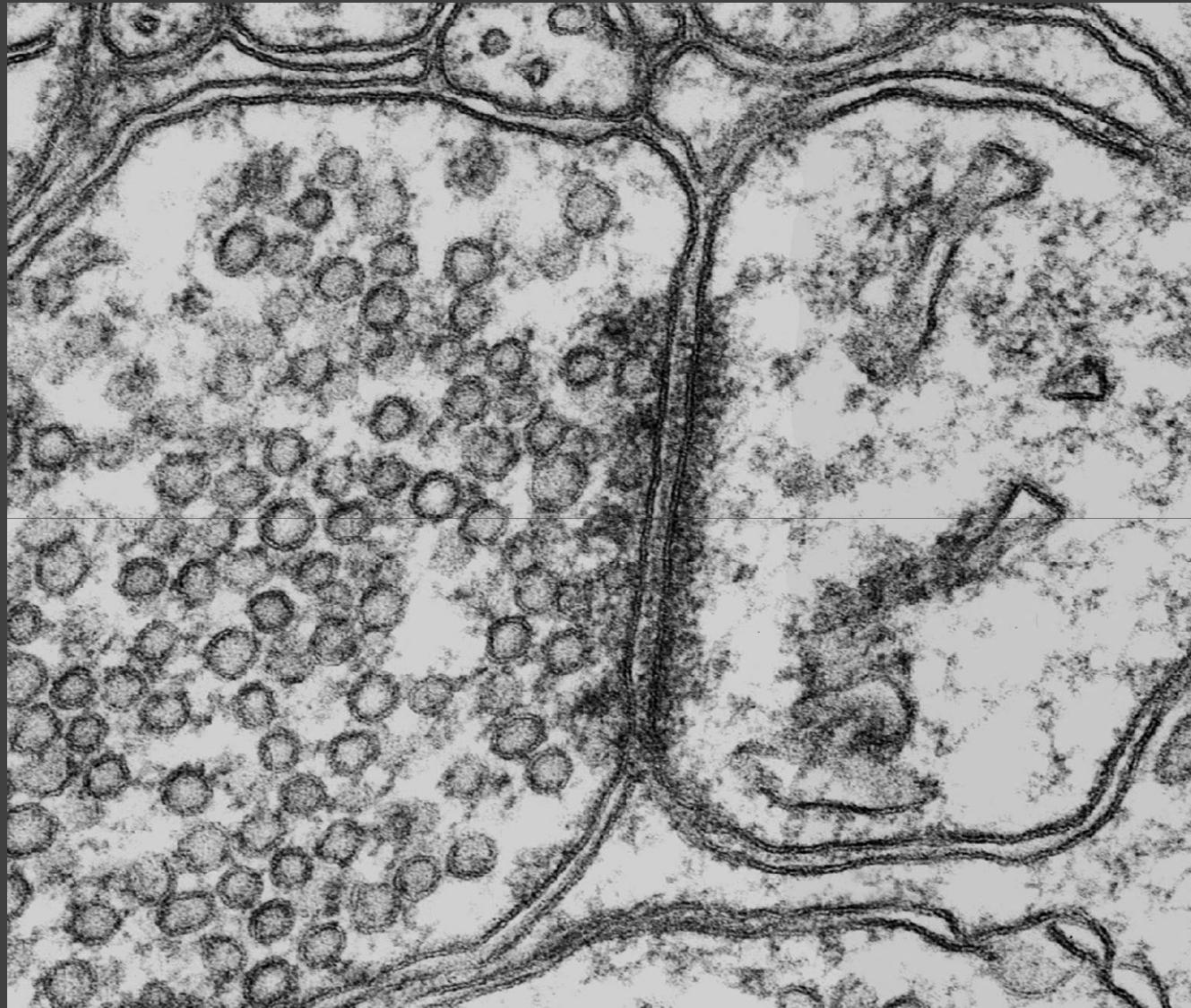


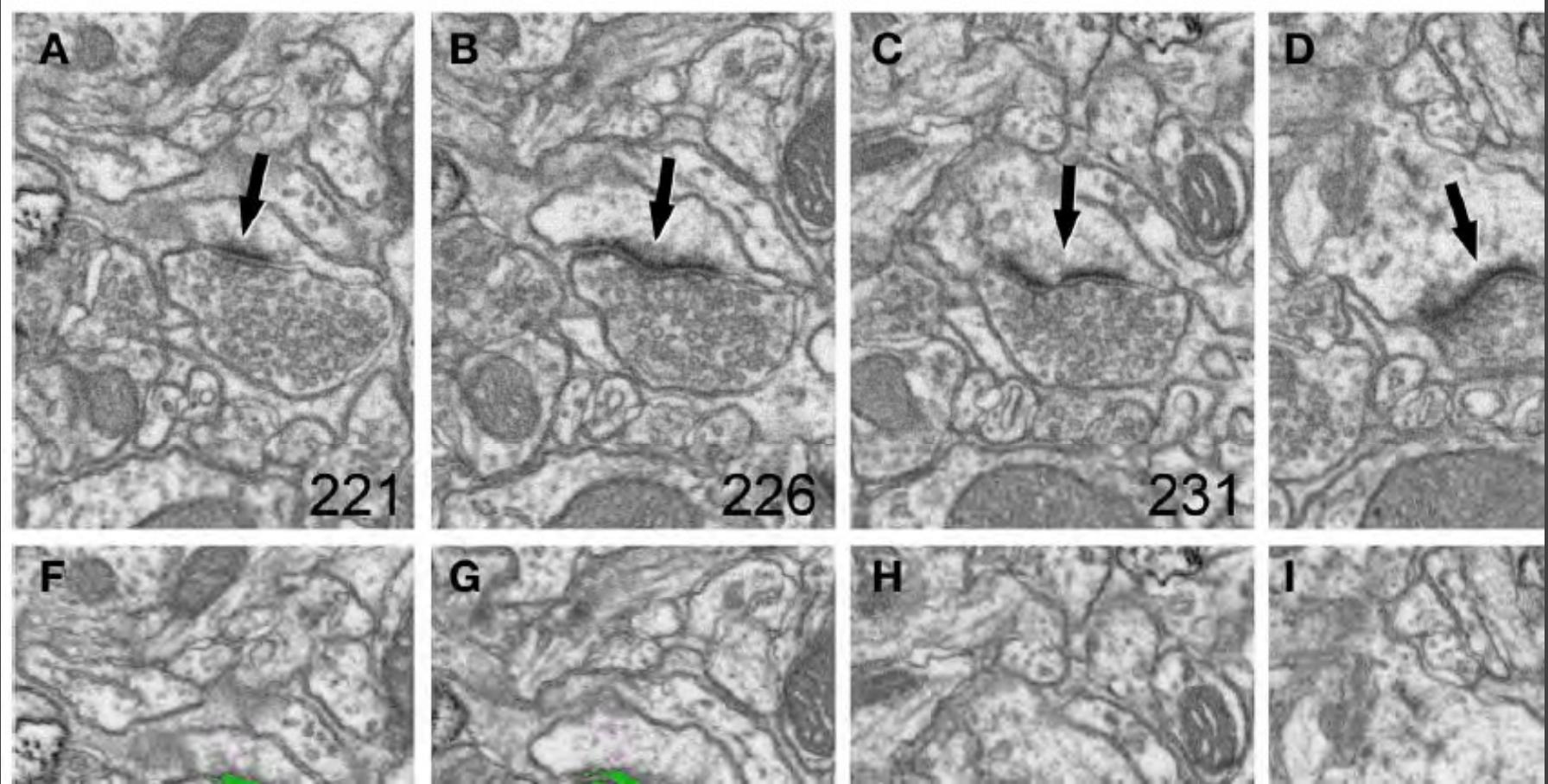
What is the best way to measure the size of synapses?

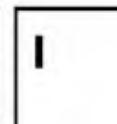
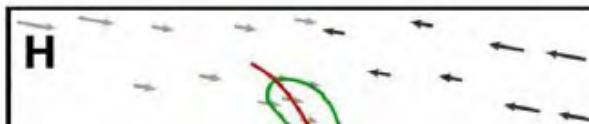
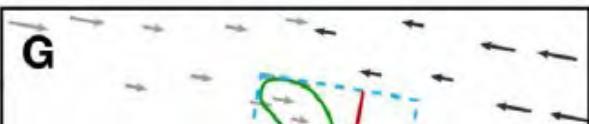
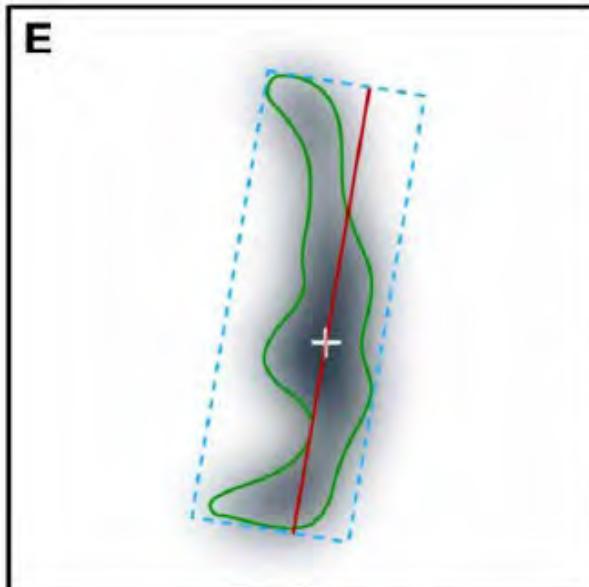
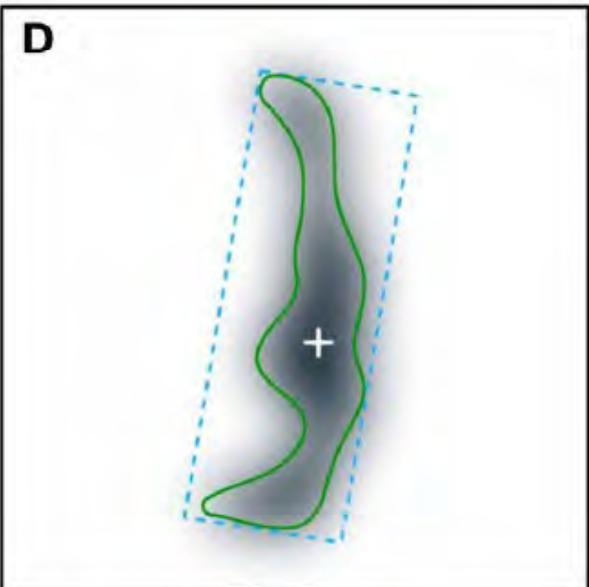
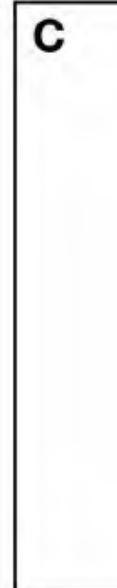
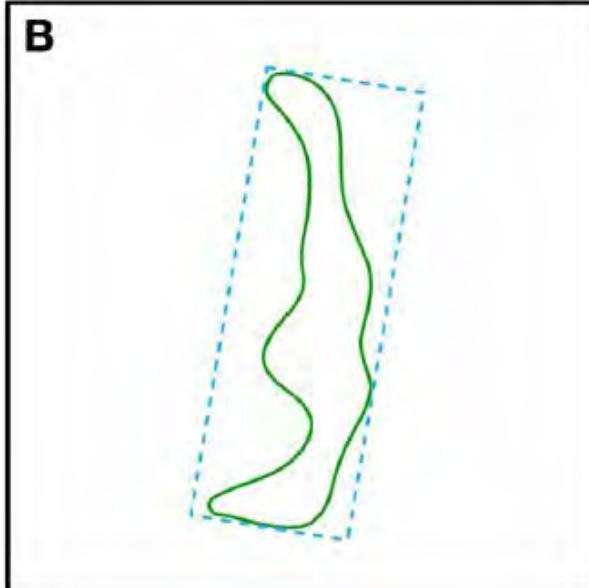
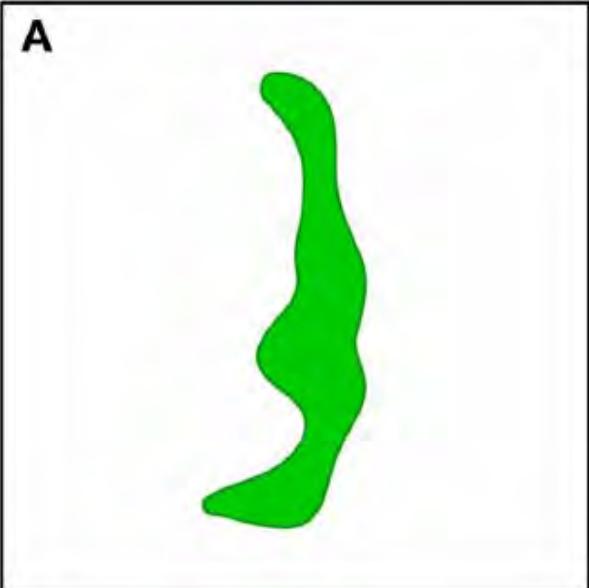


Merchan-Perez et al., Cerebral Cortex 2014









K



L



M

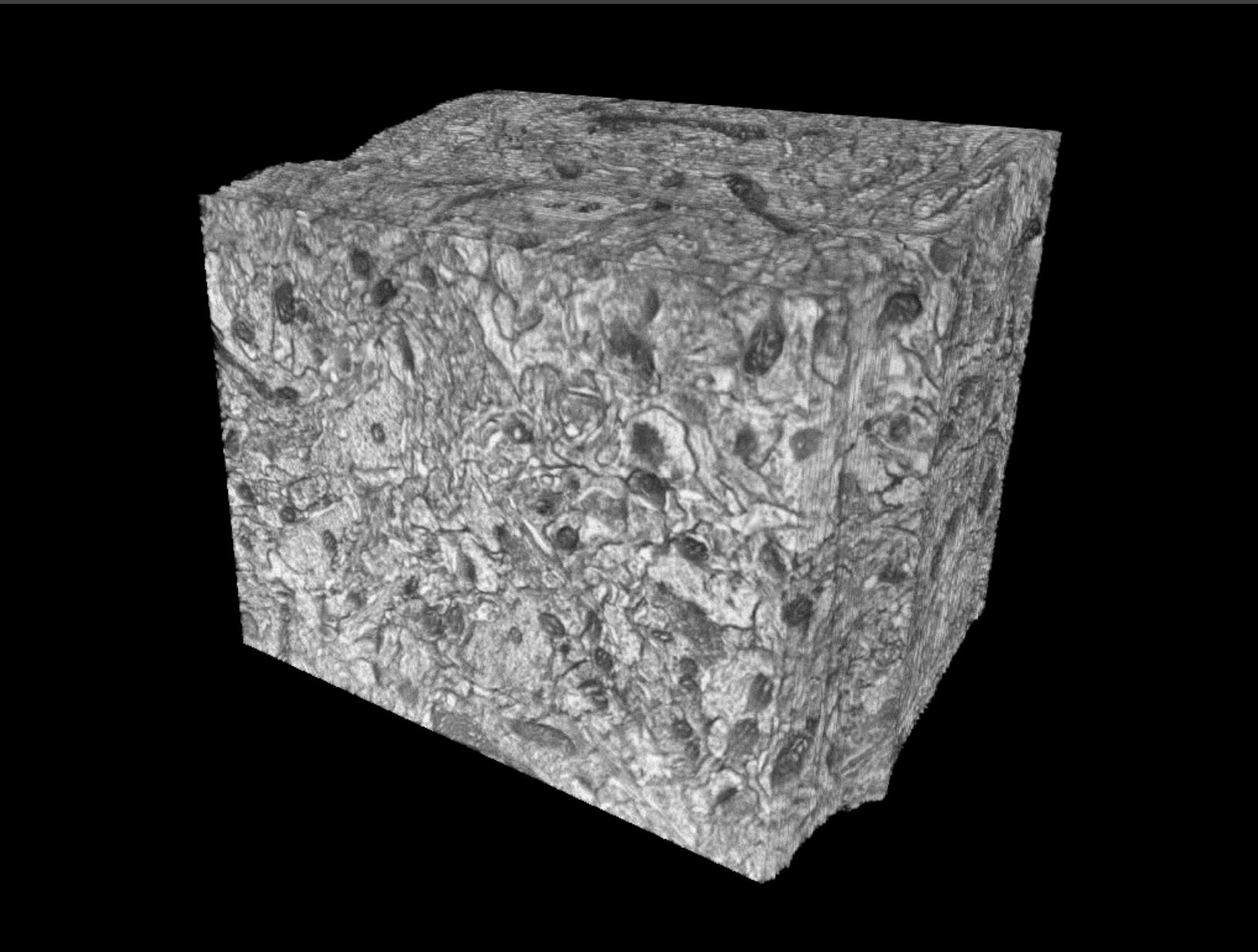


N

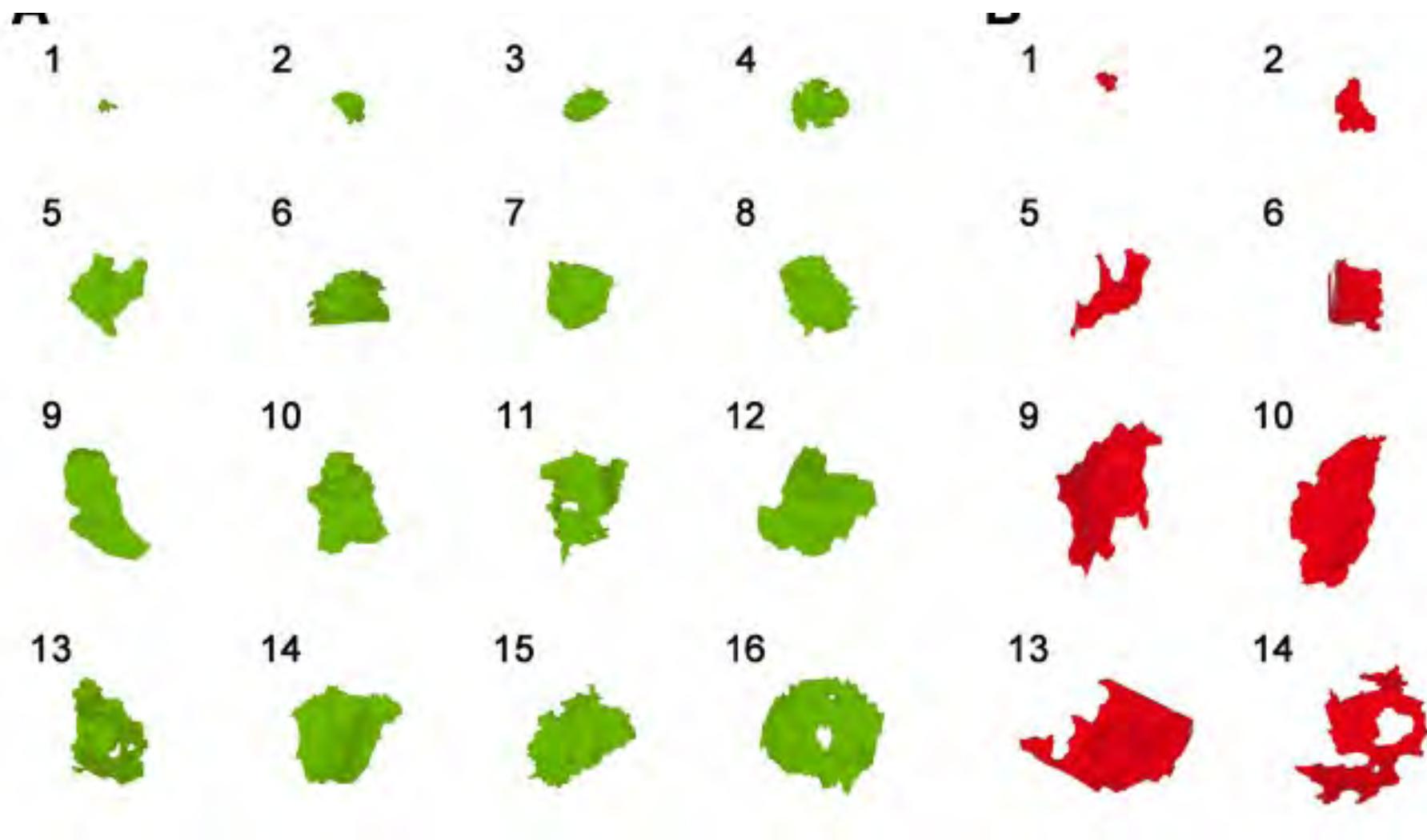




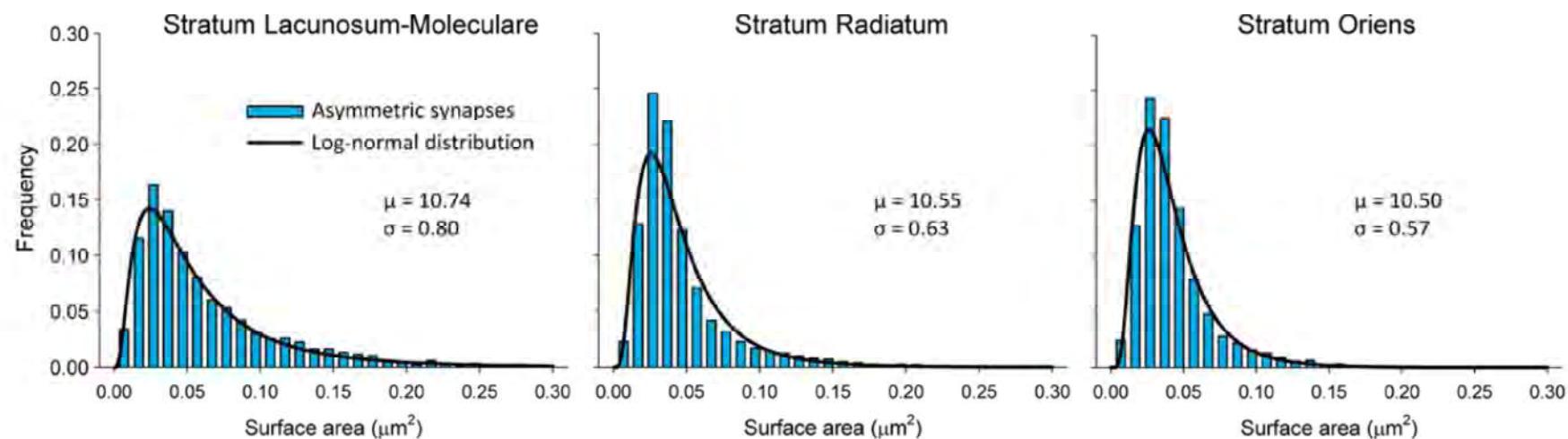
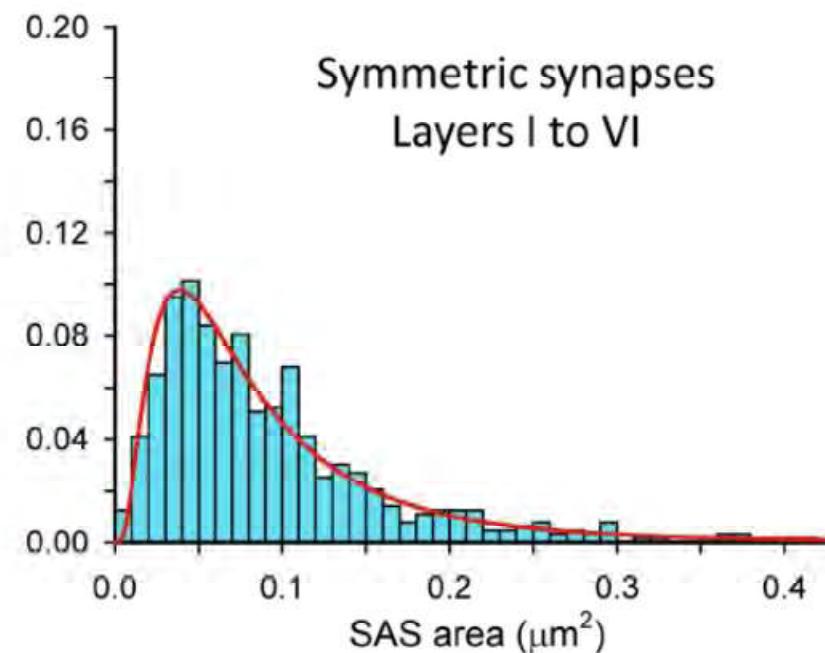
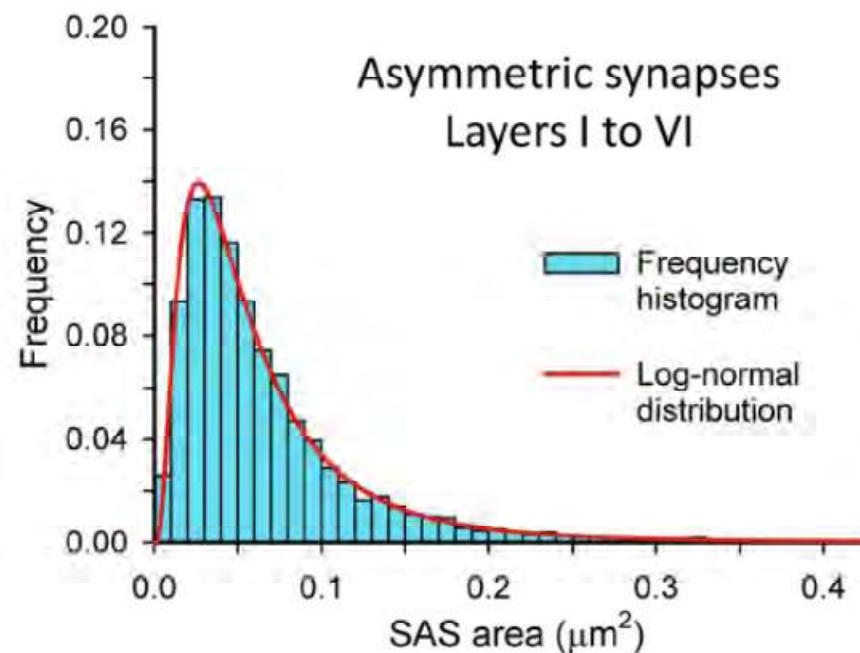
Segmented Synaptic Junctions
Synaptic Apposition Surfaces

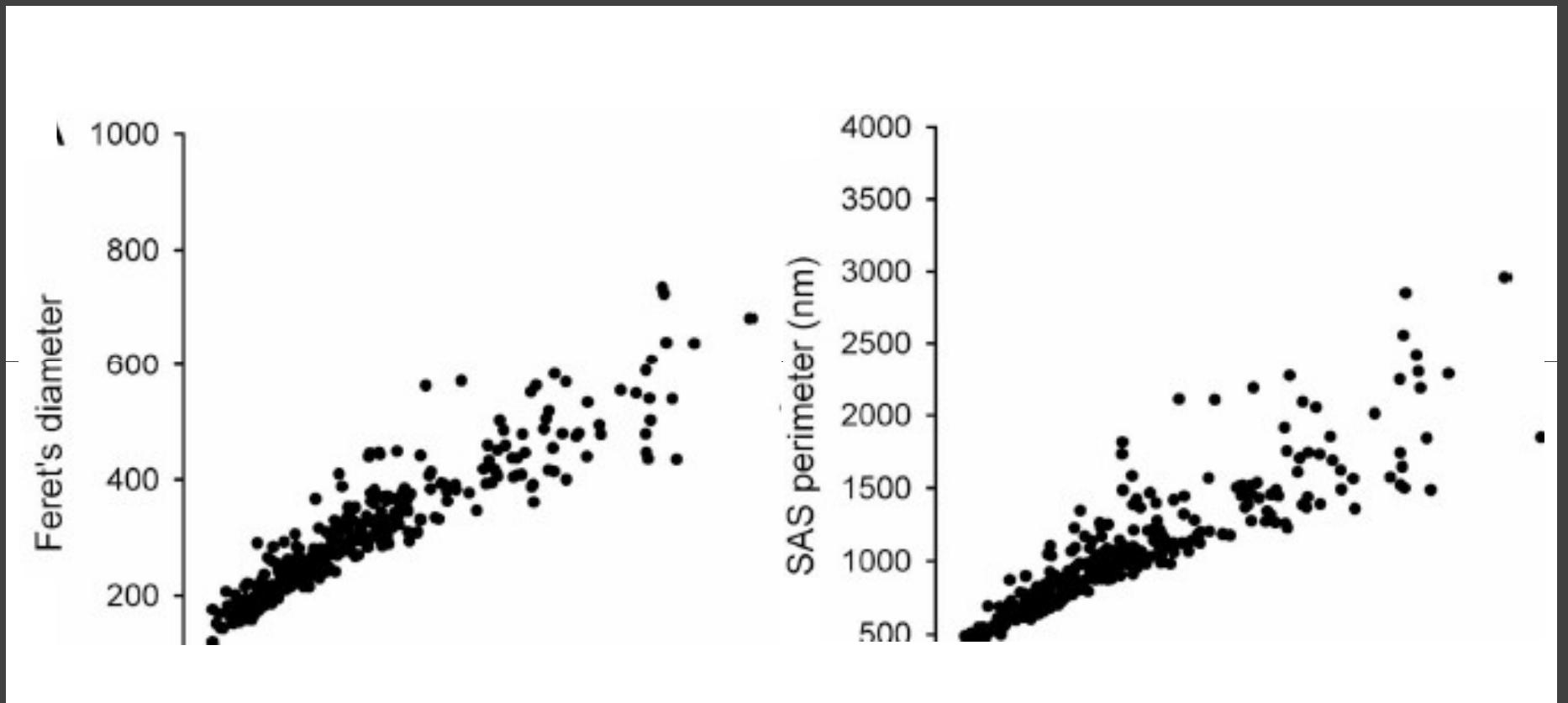


Shape of synapses



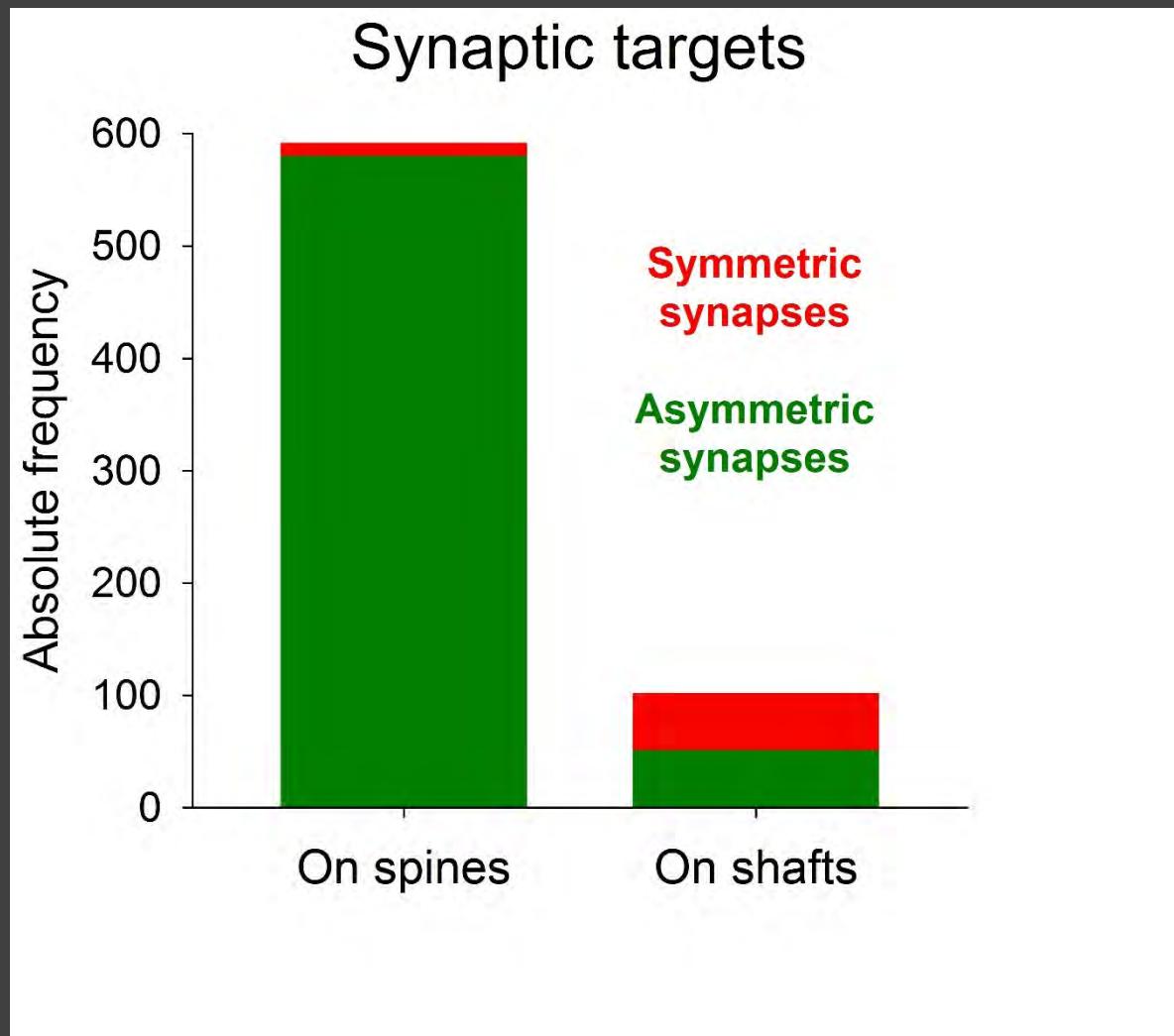
Size of synapses



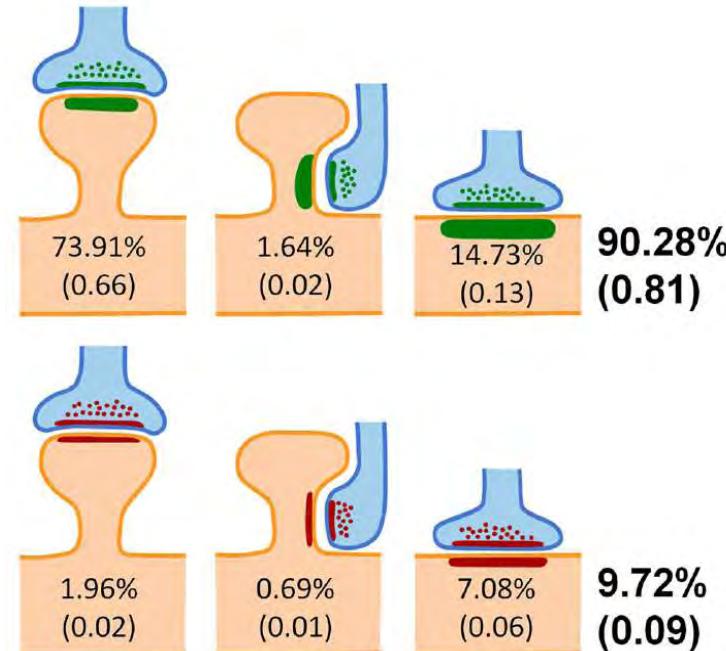


Morales et al., 2013. *Front. Neuroanat* 7, 20

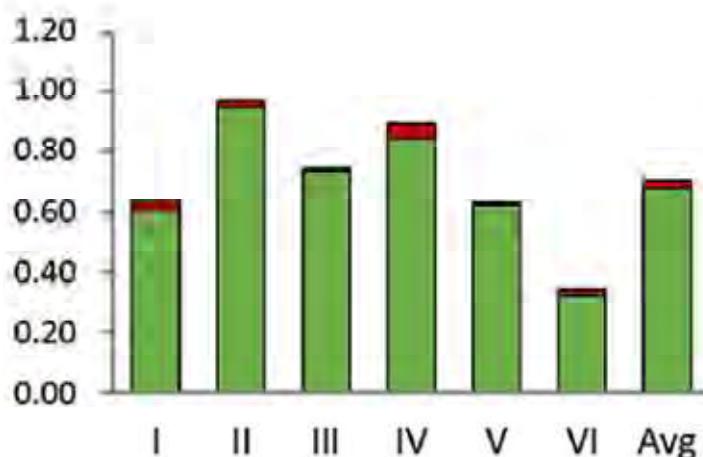
Synaptic targets



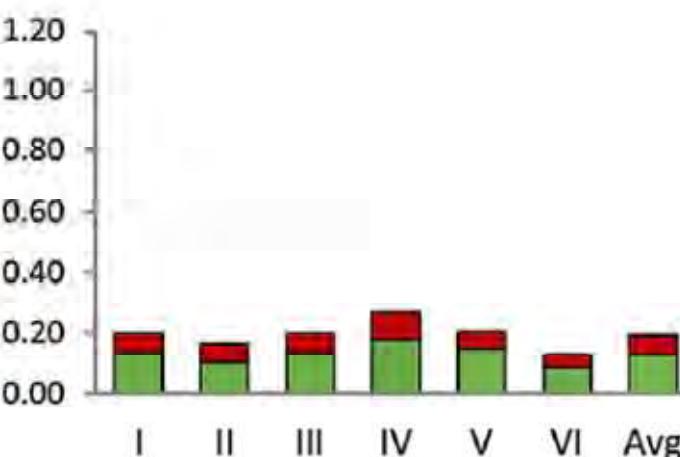
Distribution of excitatory and inhibitory synapses on dendritic spines and dendritic shafts



Spines



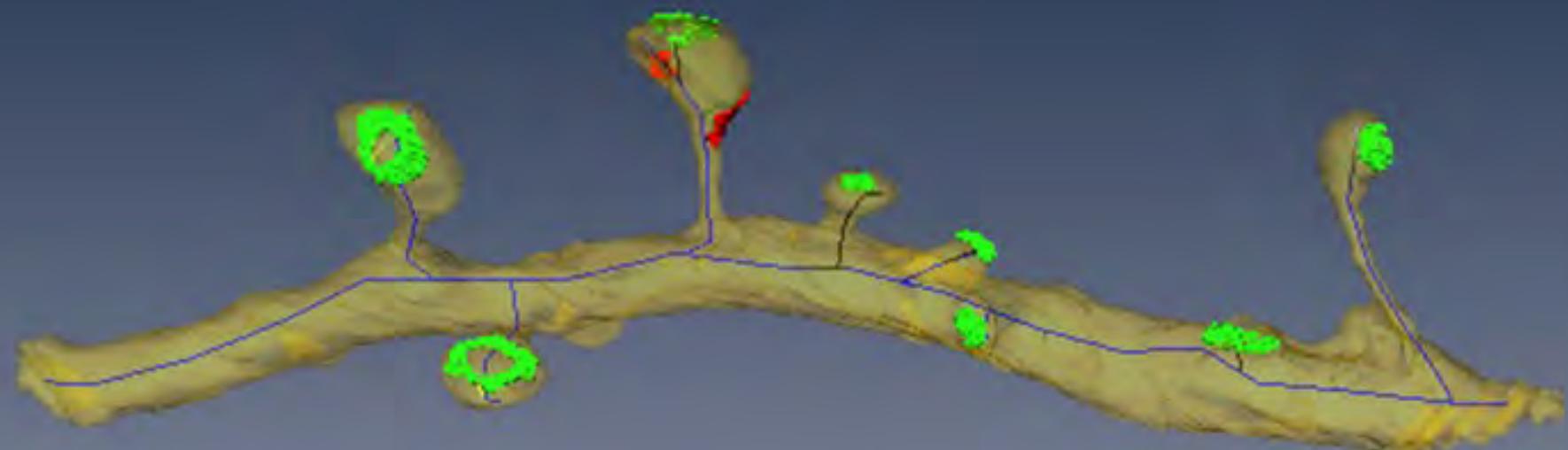
Dendritic shafts



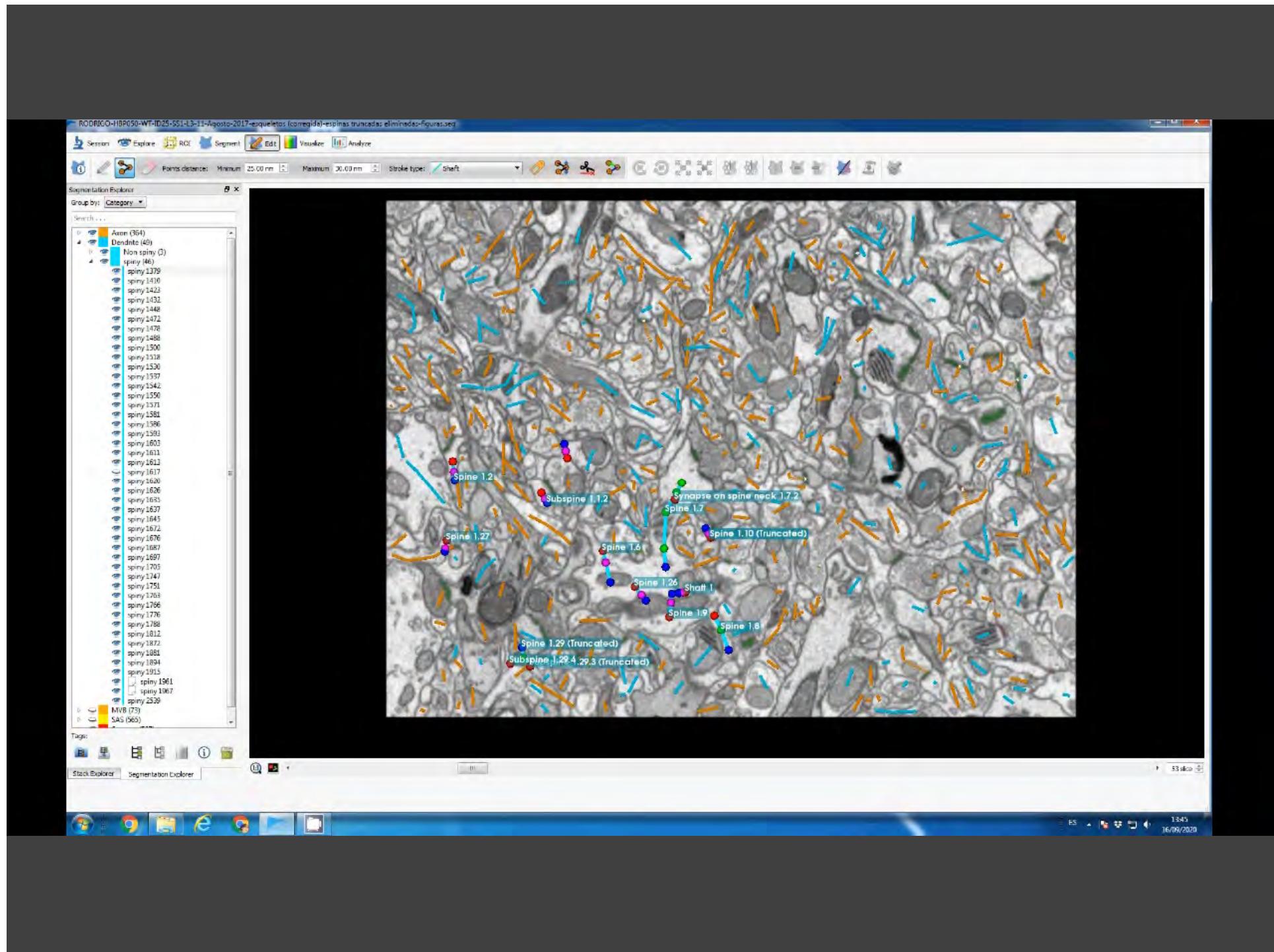
Tracing brain connectivity at the synaptic level

- Development of Espina software is still progressing
- We have developed a new tool within Espina that allows us to trace axons and dendrites, and to connect them through synapses
- These traces represent a simplified version of axons and dendrites (skeletons) and they can be analyzed and measured individually or collectively

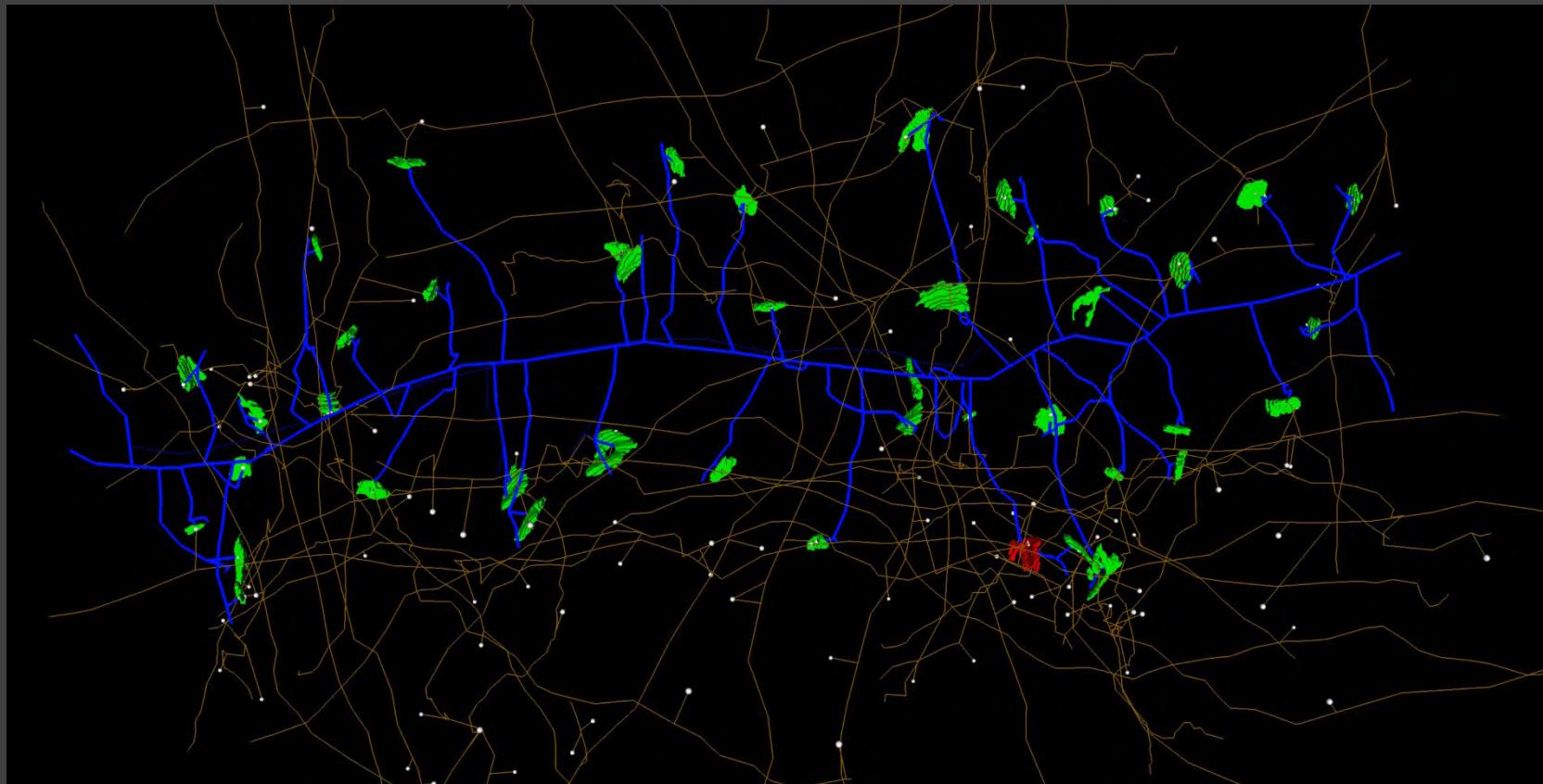
Tracing the “skeletons” of axons and dendrites



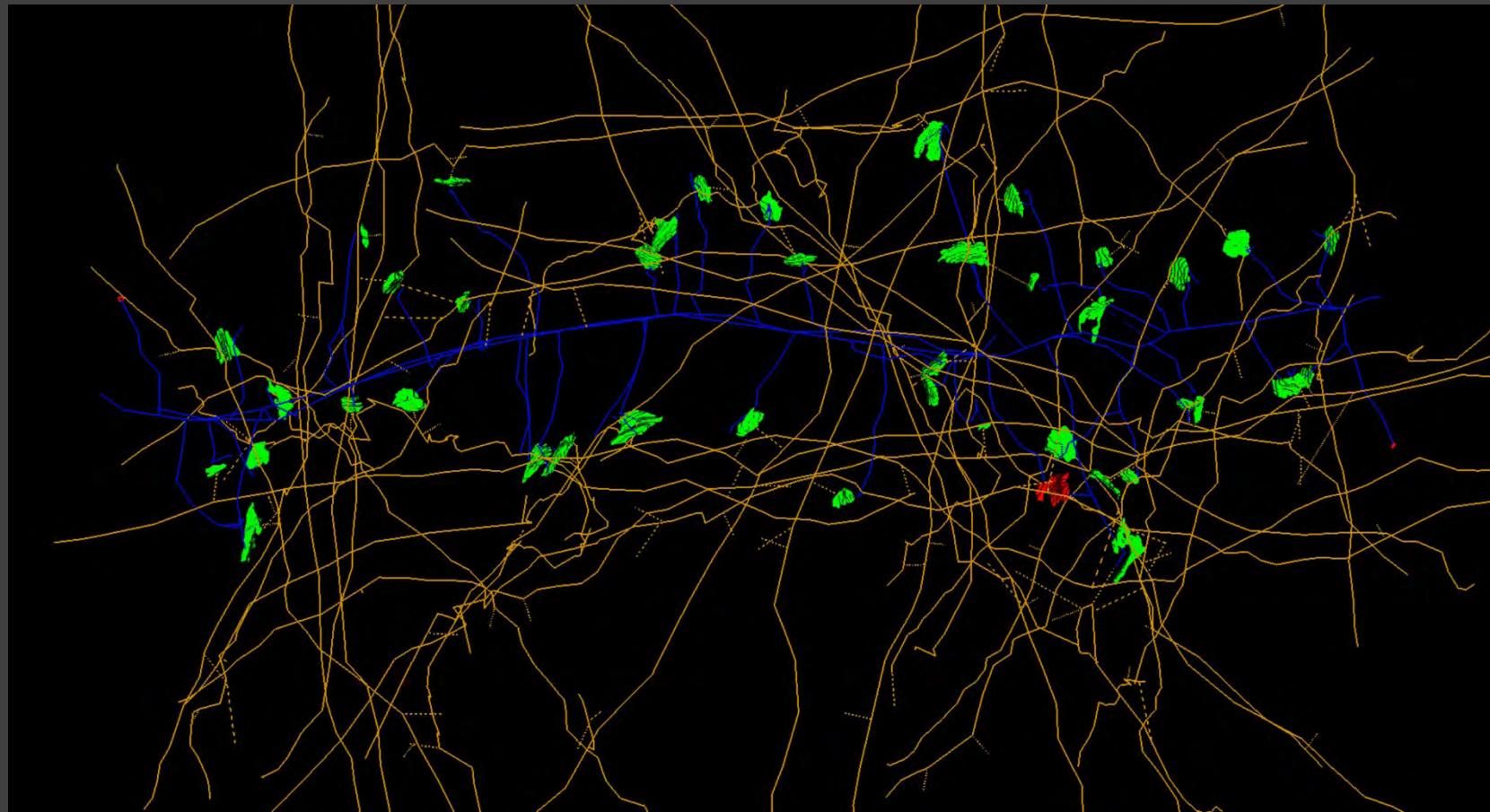
A dendritic segment with its corresponding “skeleton” and synapses



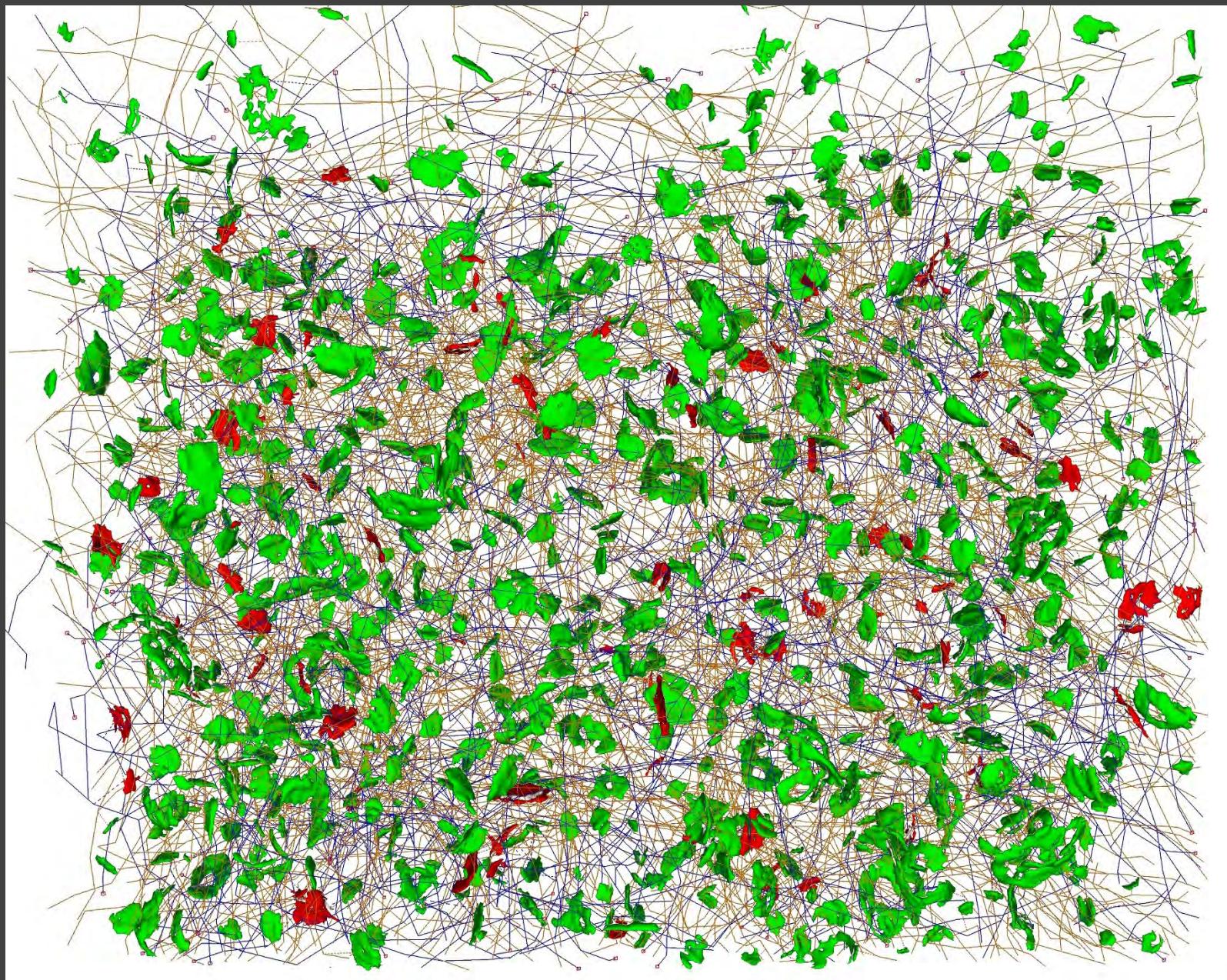
Dendrite with its synapses



Dendrite with its synapses and axons



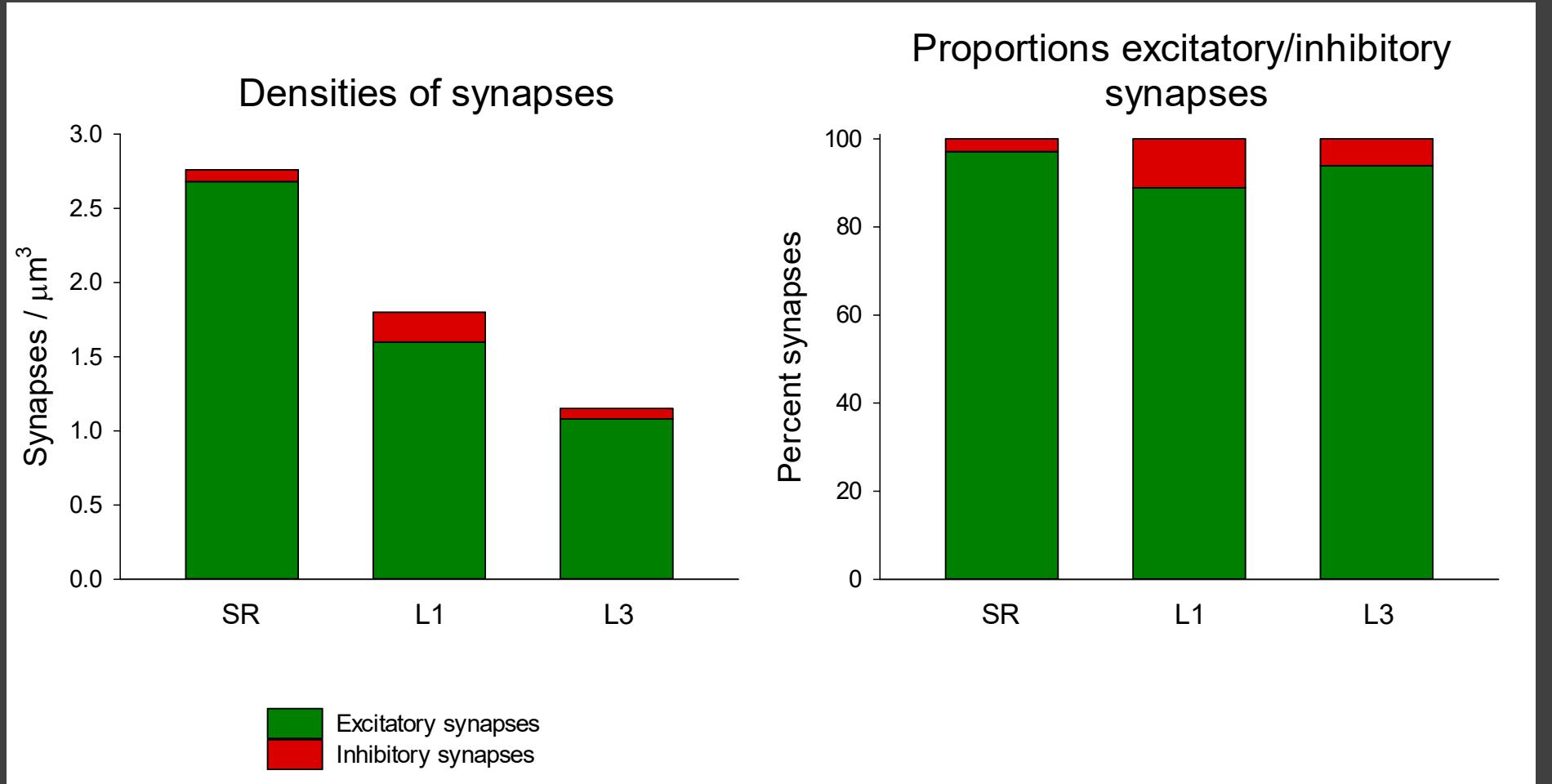
All dendrites, axons and synapses within a brain sample



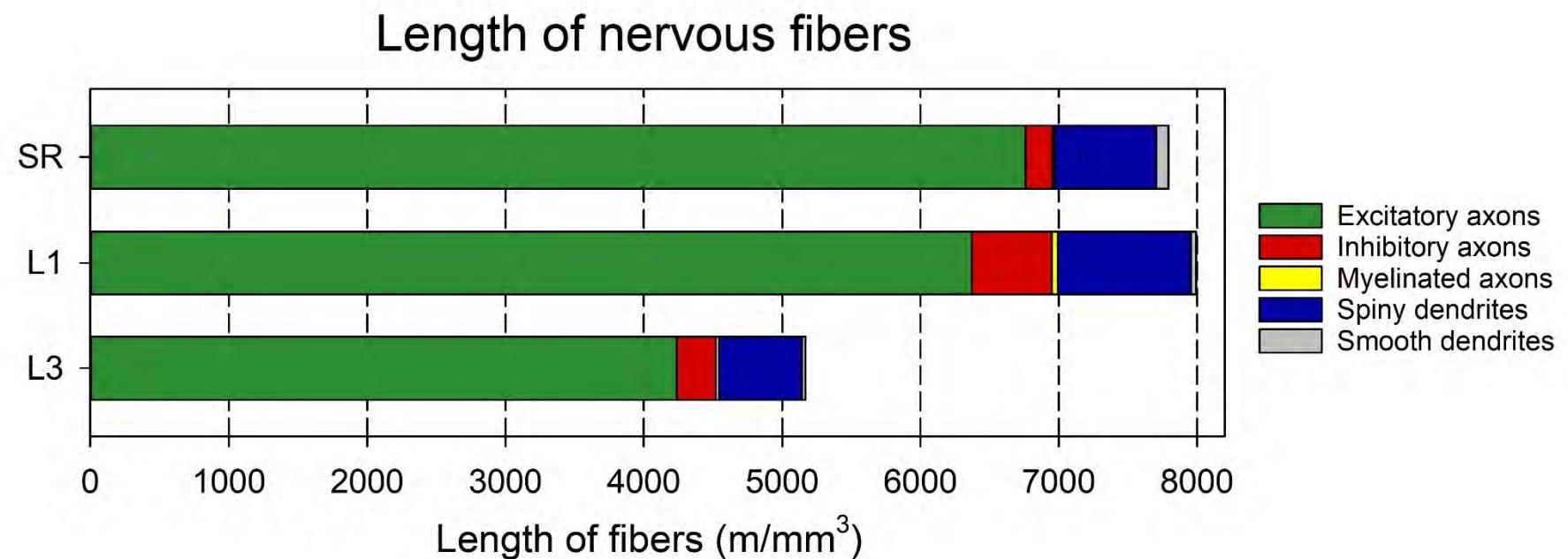
The digital “FIBERprint”

- Number and distribution of synapses
- Numbers and proportions of dendrites and axons
- Lengths of dendrites and axons
- Linear densities of synapses in dendrites (spines and shafts)
- Linear densities of synapses established by axons (excitatory and inhibitory) on dendritic spines and dendritic shafts

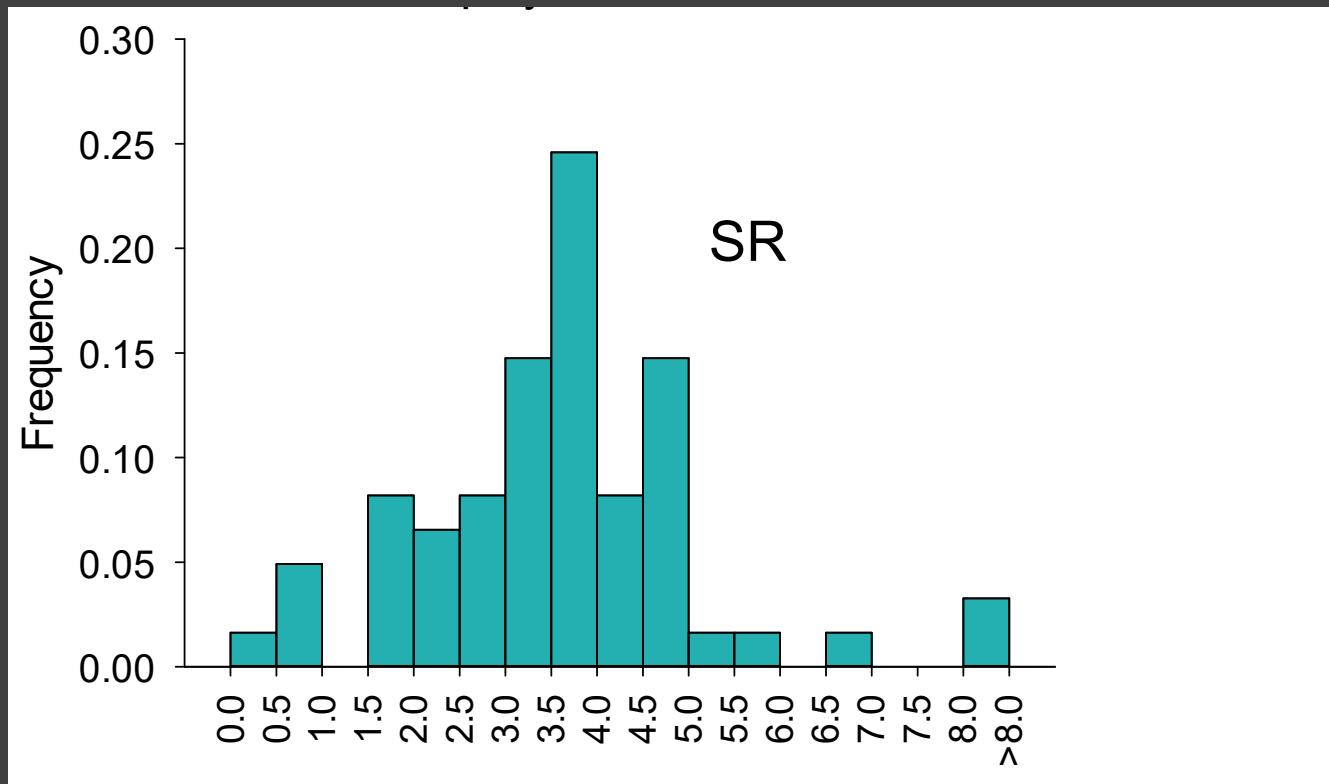
Densities and proportions of inhibitory and excitatory synapses



Lengths of axons and dendrites in three brain regions (m/mm^3)

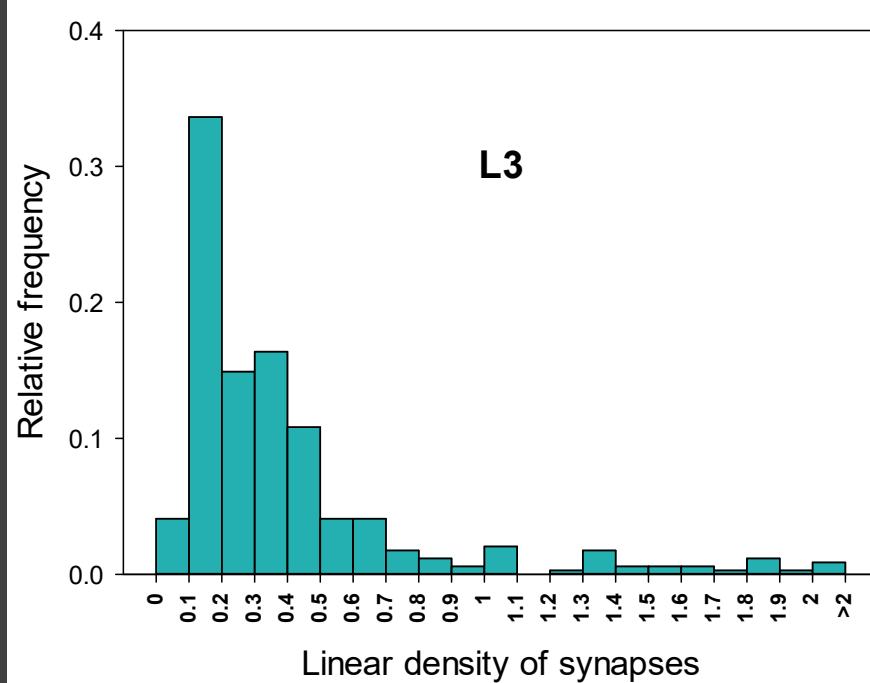


Linear density of synapses on dendrites (synapses/ μm)

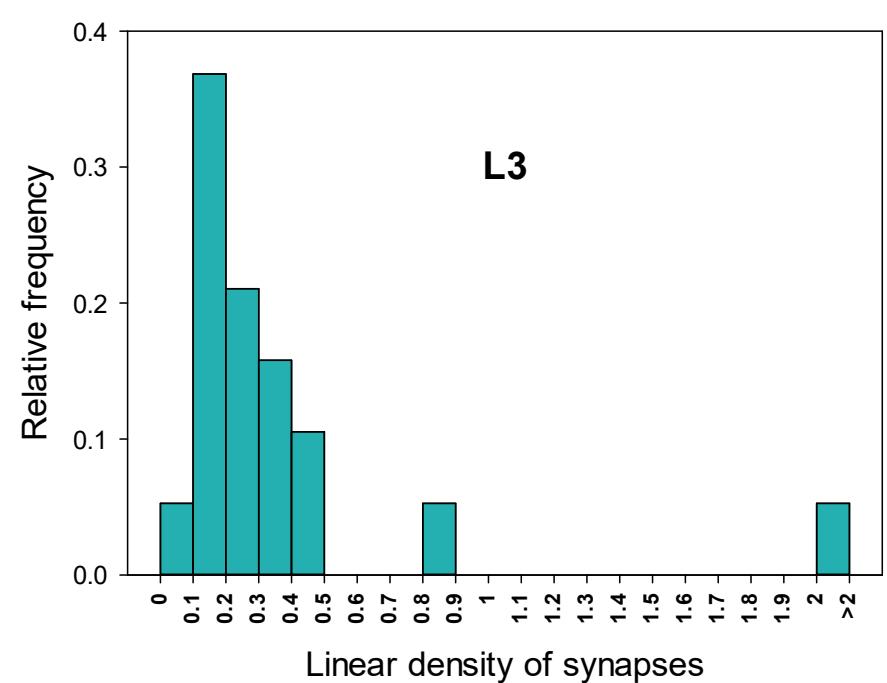


Linear density of synapses established by axons (synapses/ μm)

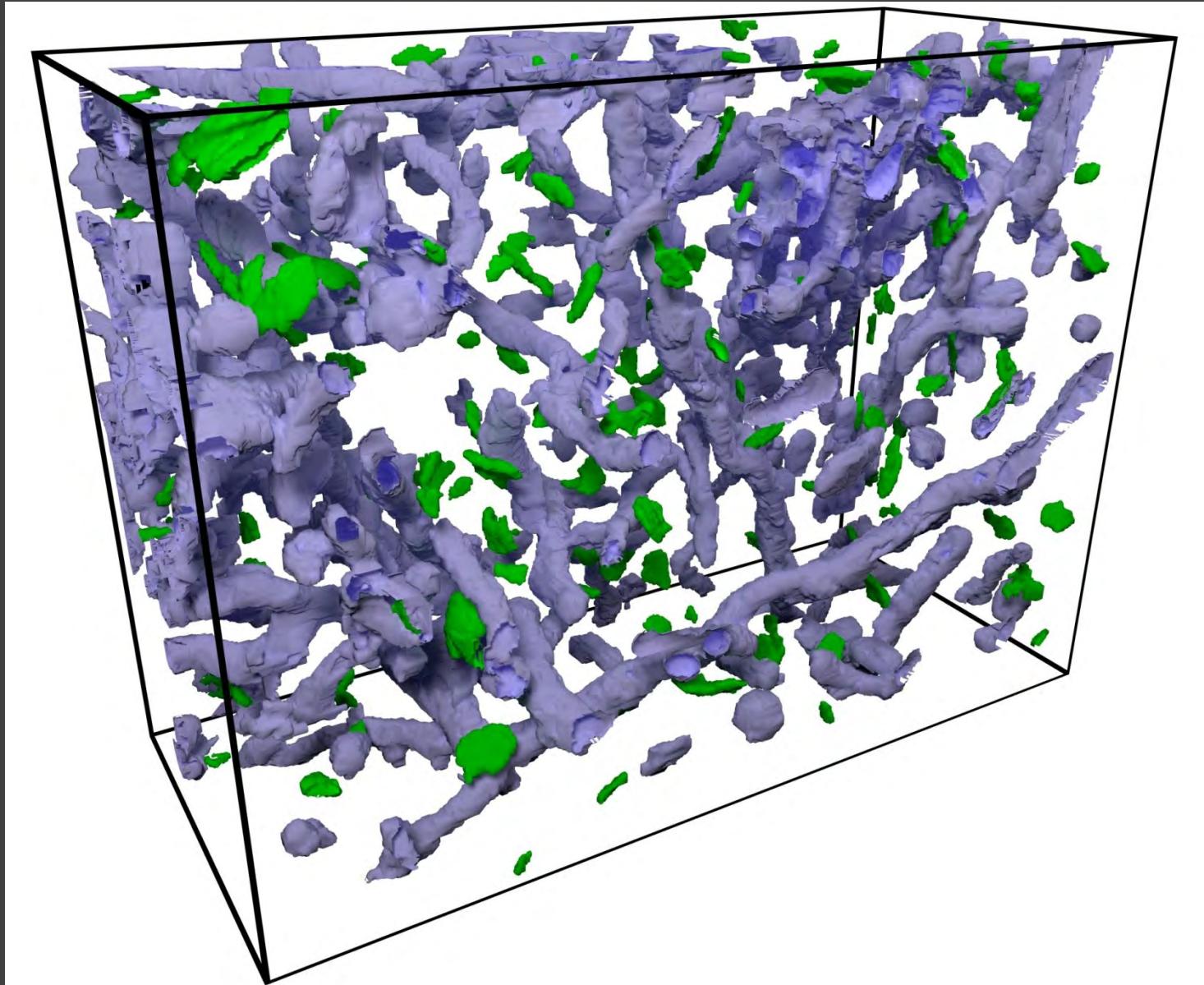
Excitatory axons

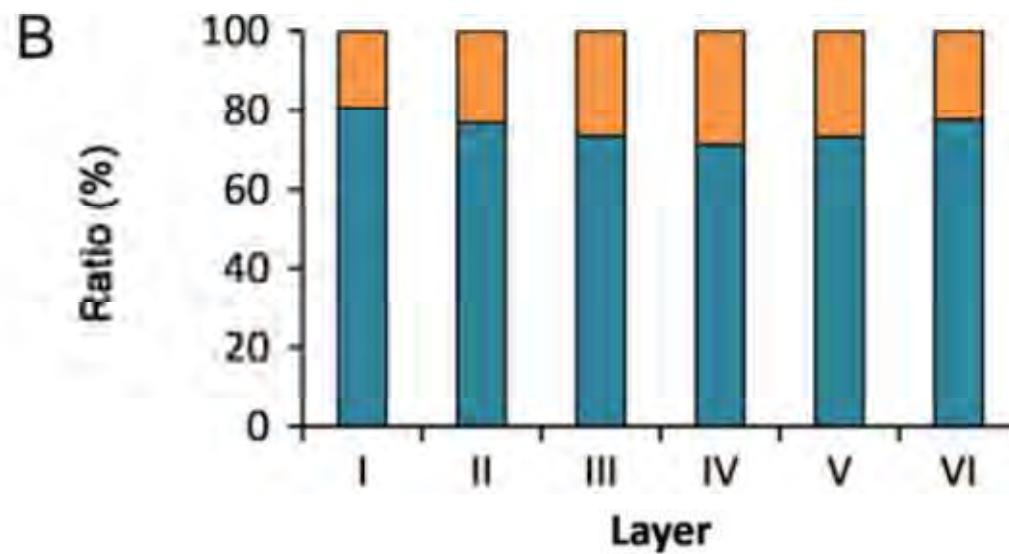
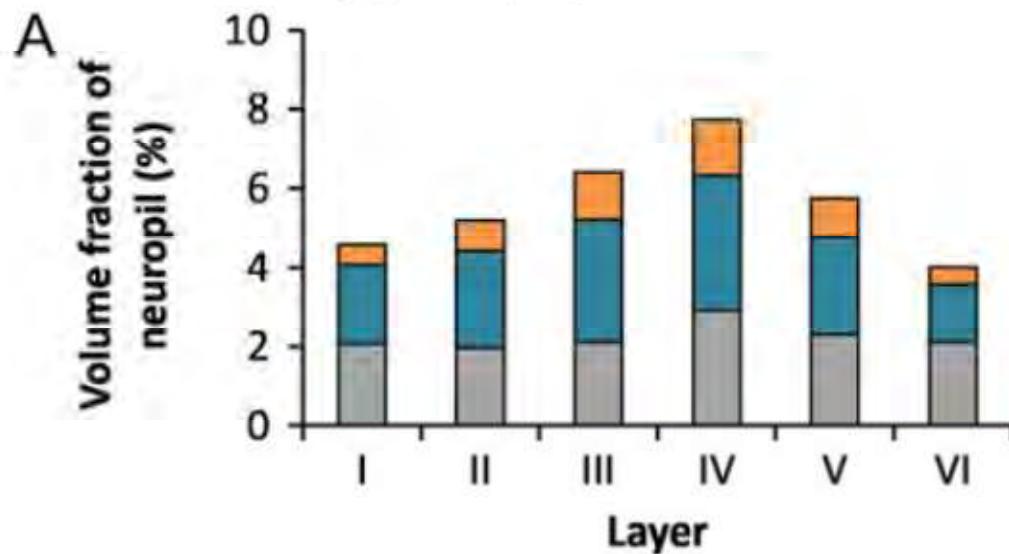


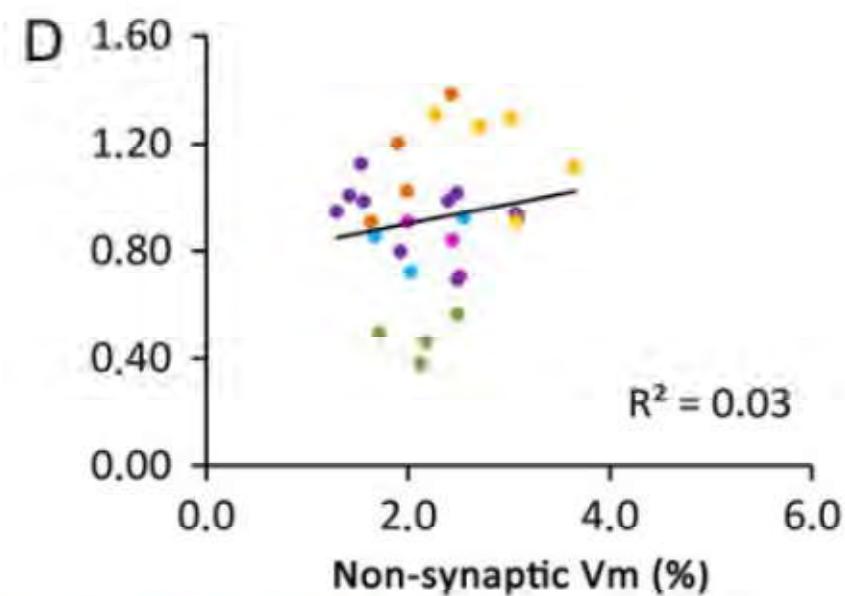
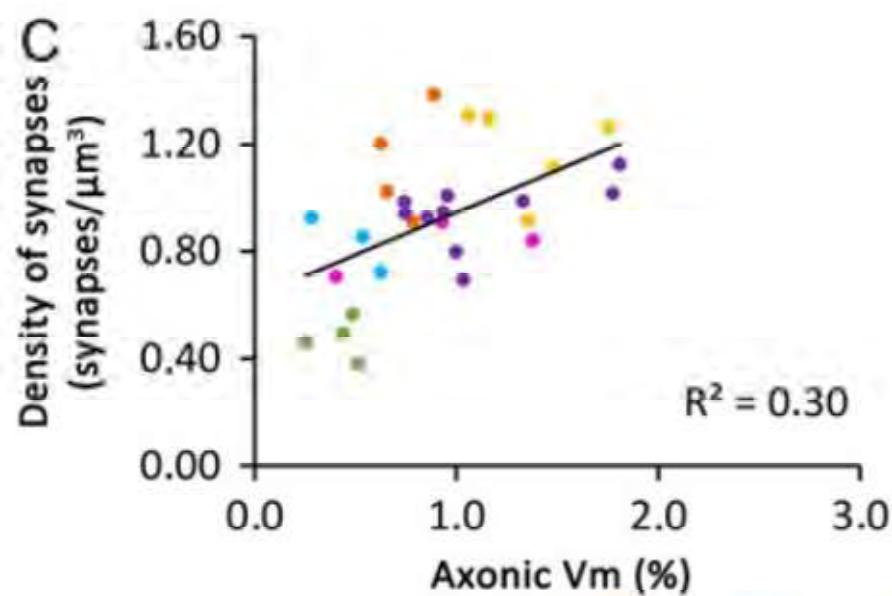
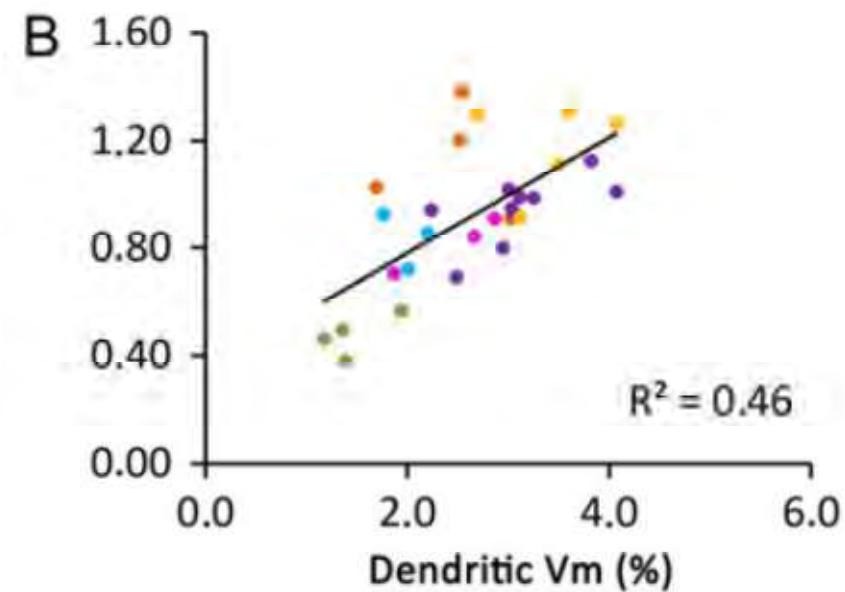
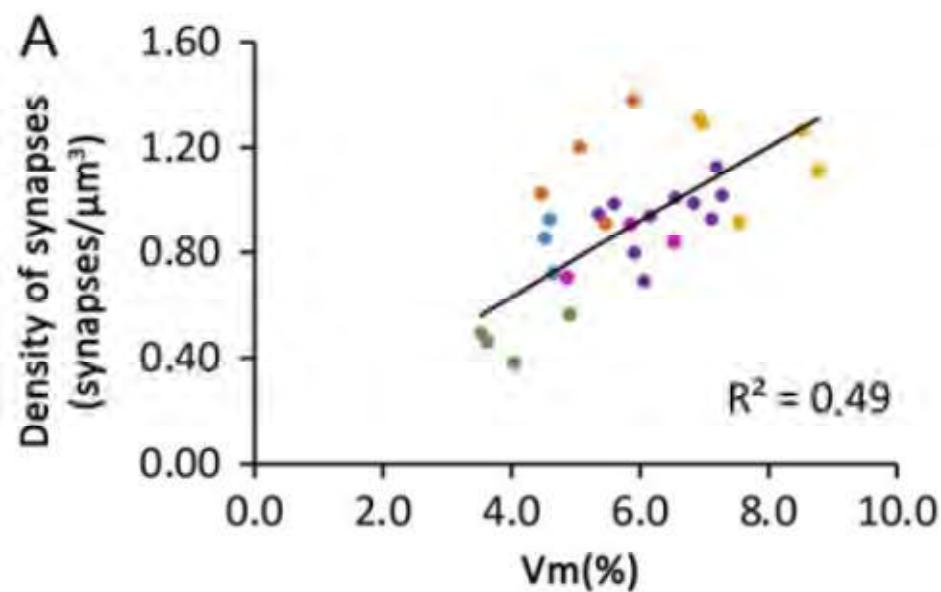
Inhibitory axons



Synapses and Mitochondria







Layer I II III IV V VI

Alzheimer's disease

