

3.5 Electron microscopy

Cellular Mechanisms of Brain Function

Graham Knott



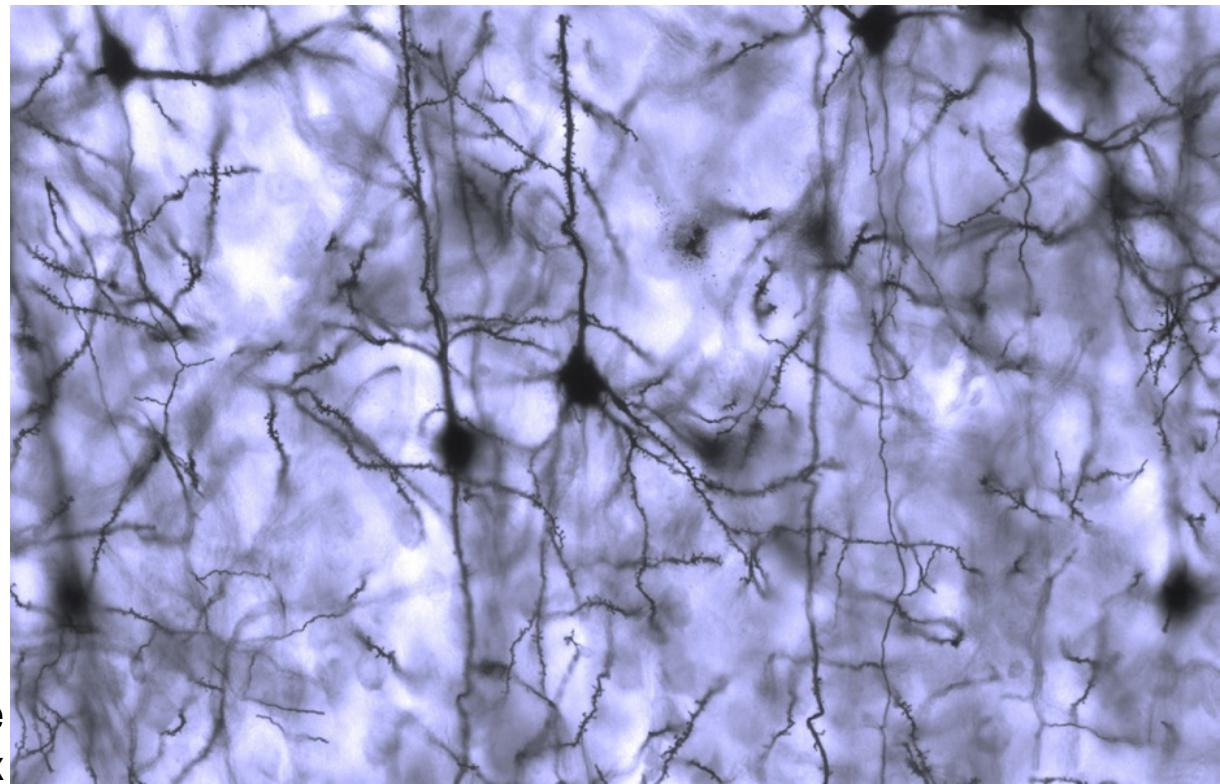
Graham Knott – Head of BioEM at EPFL



Electron microscopy of neuronal structure

- Preparing brain samples for EM
- Principles of transmission and scanning EM
- Imaging volumes using TEM and SEM

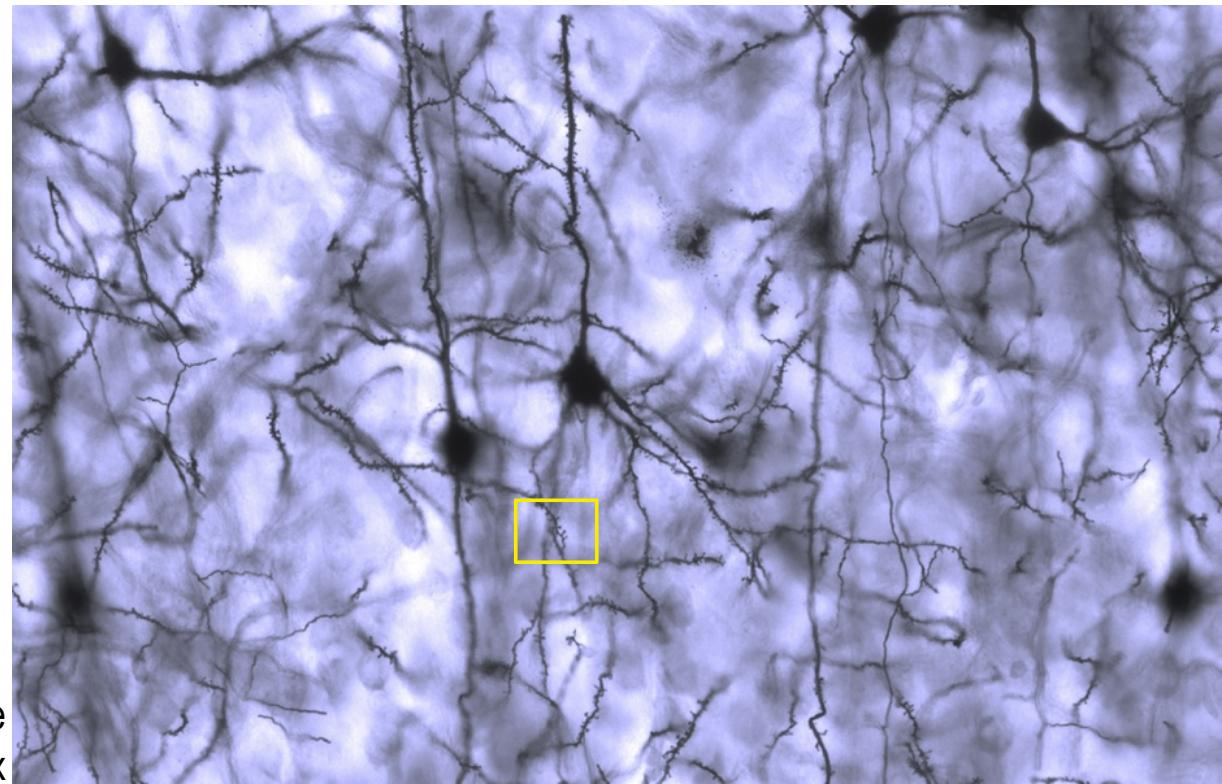
Imaging neuronal structure - light microscopy



Light micrograph of mouse
cerebral cortex

Cellular Mechanisms of Brain Function

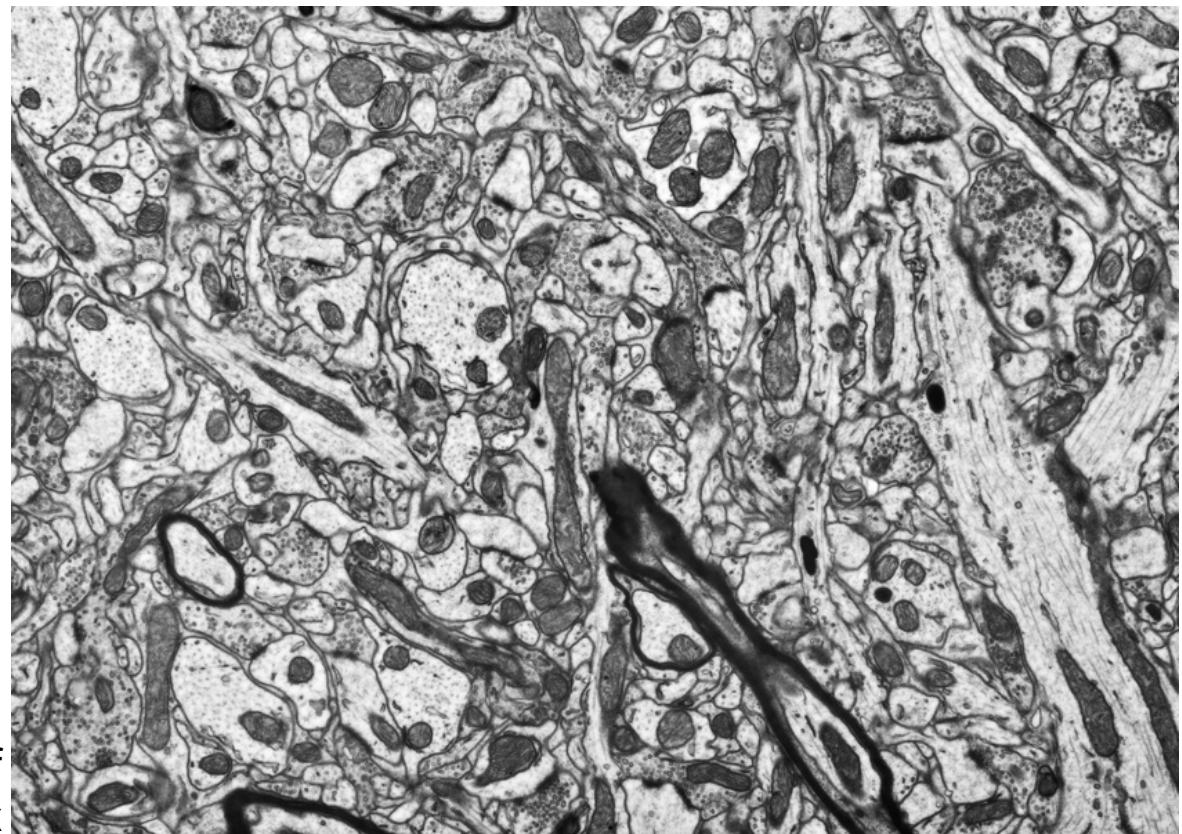
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Light micrograph of mouse
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Cellular Mechanisms of Brain Function

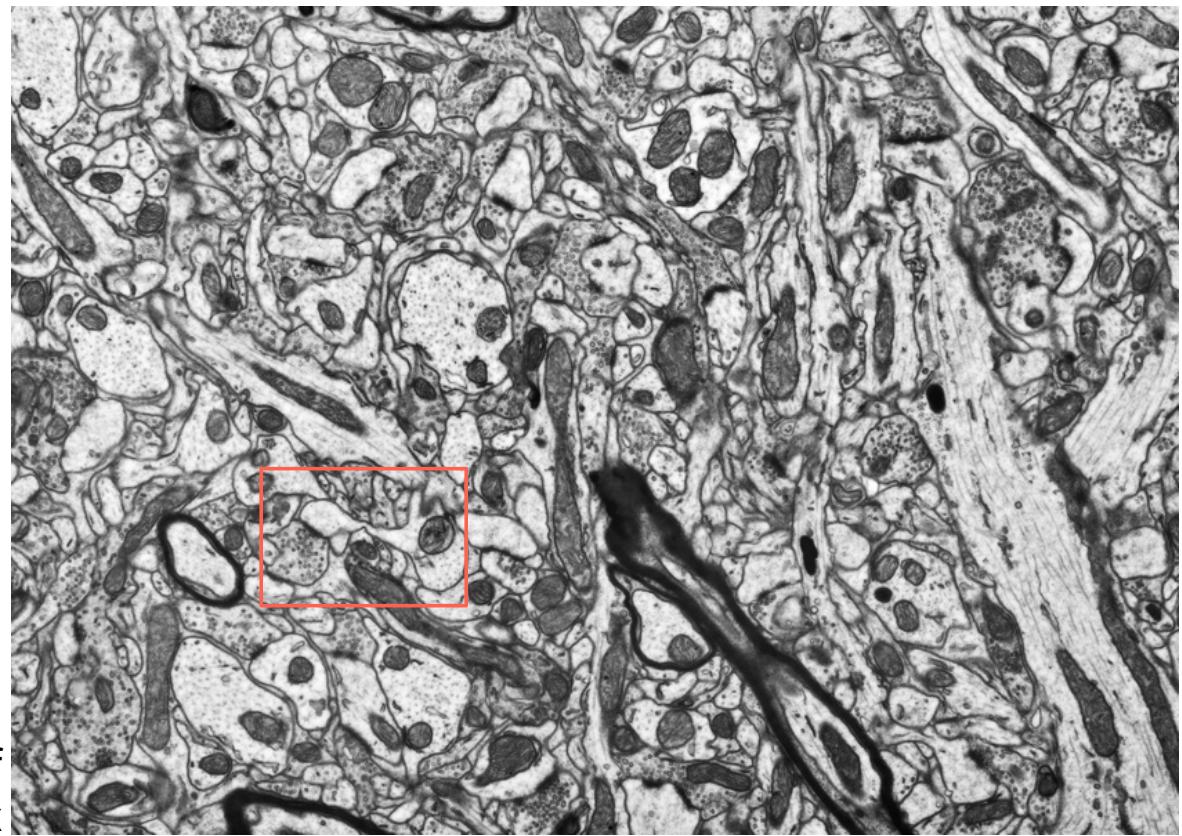
Imaging neuronal structure - electron microscopy



Transmission electron micrograph of
mouse cerebral cortex

Cellular Mechanisms of Brain Function

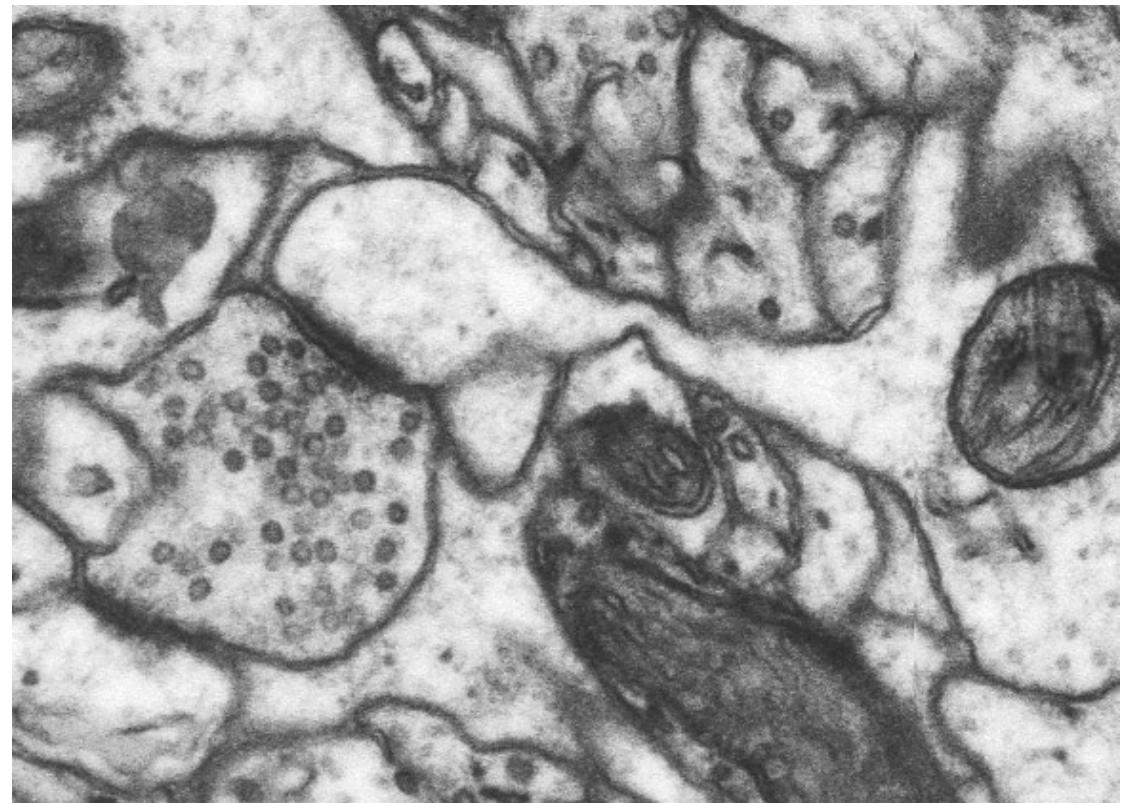
Imaging neuronal structure - electron microscopy



Transmission electron micrograph of
mouse cerebral cortex

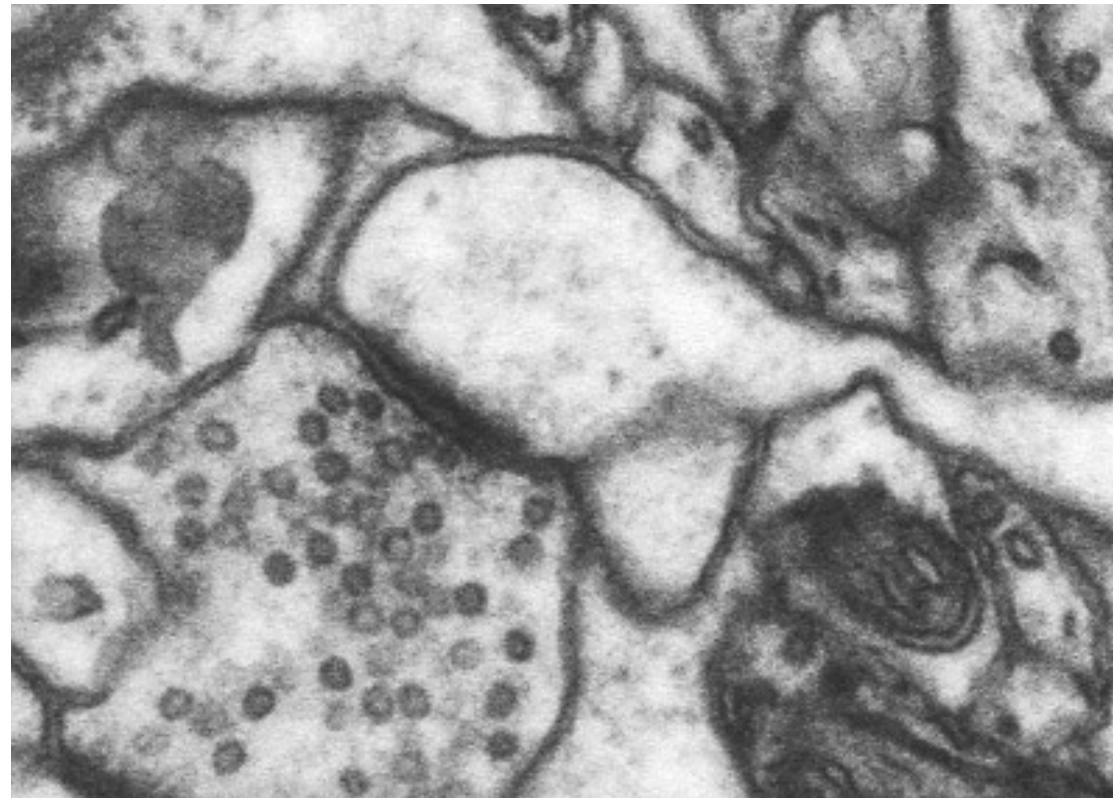
Cellular Mechanisms of Brain Function

Imaging neuronal structure - electron microscopy



TEM of a single synaptic connection

Imaging neuronal structure - electron microscopy



TEM of a single synaptic connection

Electron microscopy



Considerations for EM of biological structures

- Samples contain atoms of low molecular weight
- Most biological samples contain water
- High vacuum
- Intense heat of the e beam
- Size of Specimen

Preparing biological samples for EM



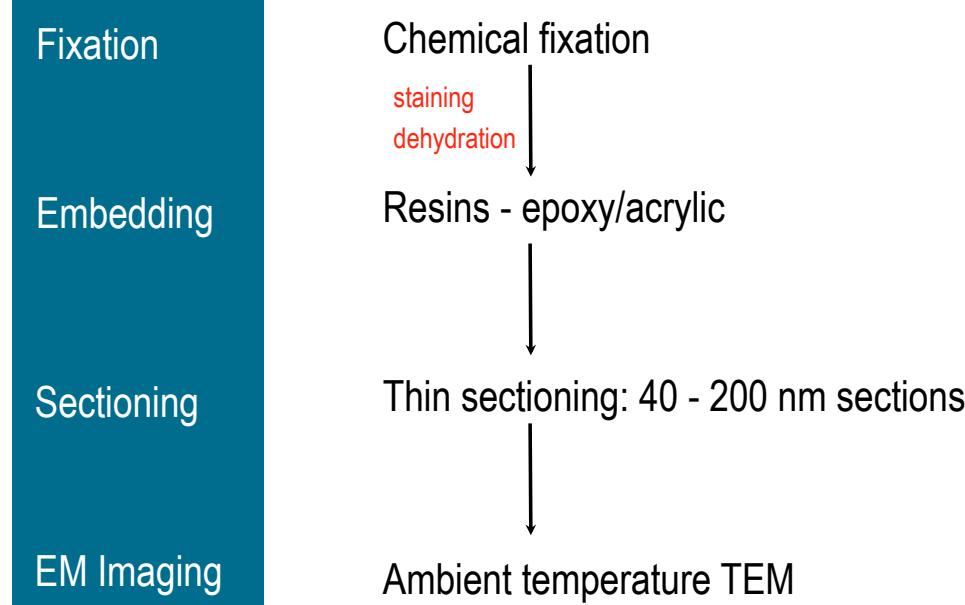
Fixation

Embedding

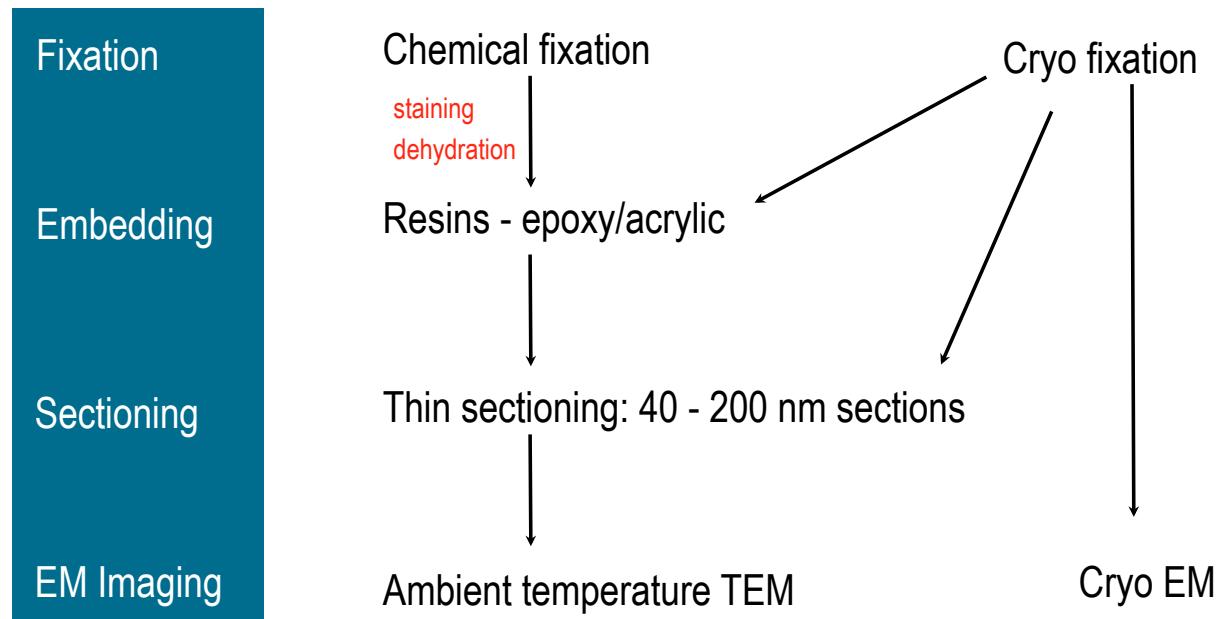
Sectioning

EM Imaging

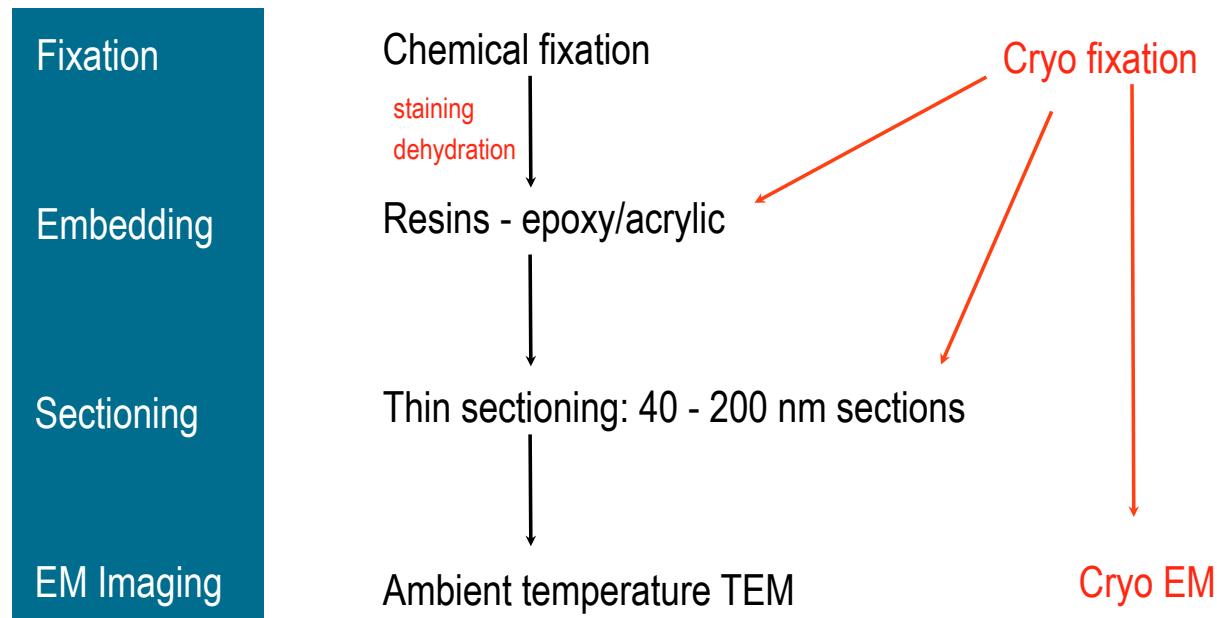
Preparing biological samples for EM



Preparing biological samples for EM



Preparing biological samples for EM



Fixation



Preservation of freshly killed material in a state that most closely resembles the structure when living.

Fixation

Chemical fixation

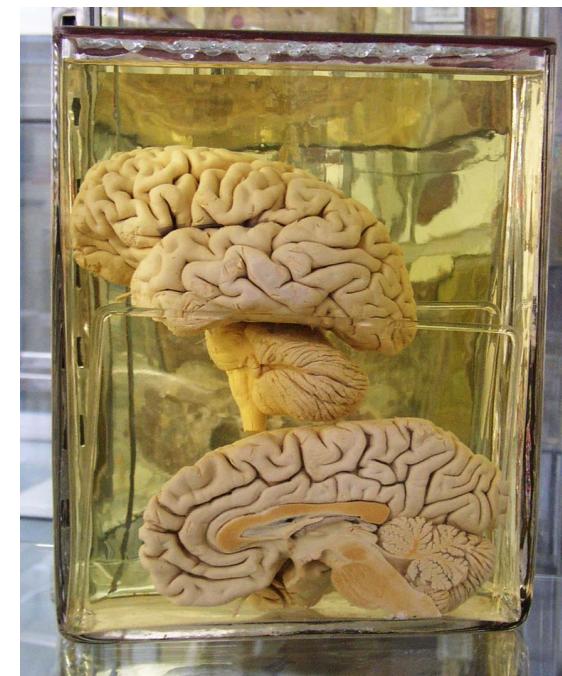
- Coagulative fixation - acids, ethanol



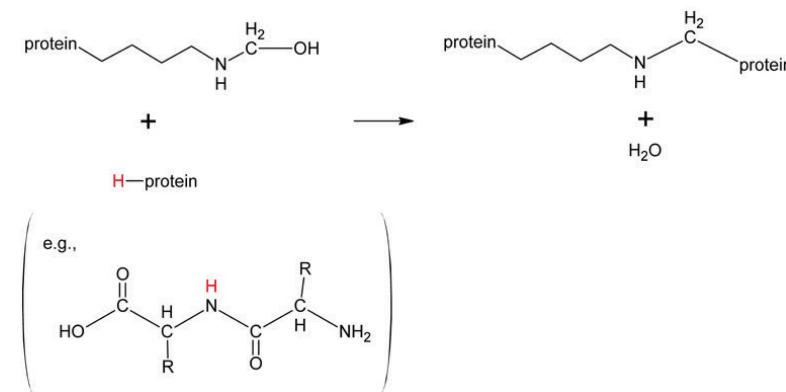
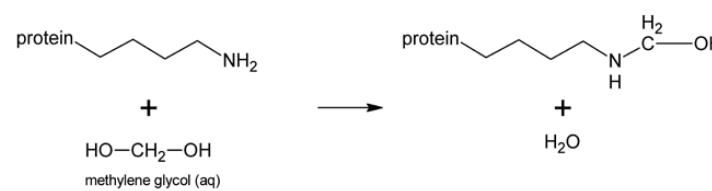
Fixation

Chemical fixation

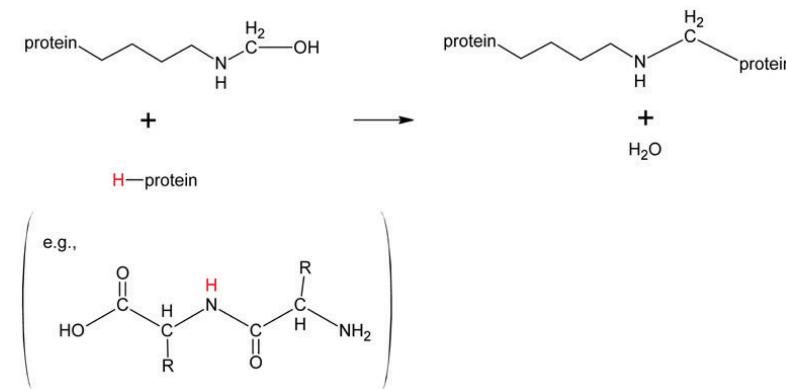
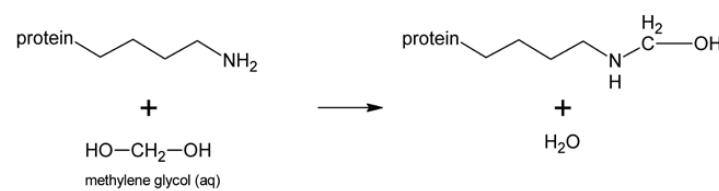
- Coagulative fixation - acids, ethanol
- Non coagulative (polymerizing) - aldehydes



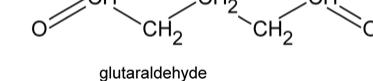
Mechanism of action of aldehydes



Mechanism of action of aldehydes



glutaraldehyde



Staining and embedding brain tissue for EM



3_5 EM_video1

Staining and embedding brain tissue for EM



Heavy metals used to stain molecular complexes

- Osmium tetroxide - membranes and proteins
- Uranyl acetate - basic stain (DNA, RNA)
- Lead citrate - glycoproteins (starch, glycogen)

Staining and embedding brain tissue for EM



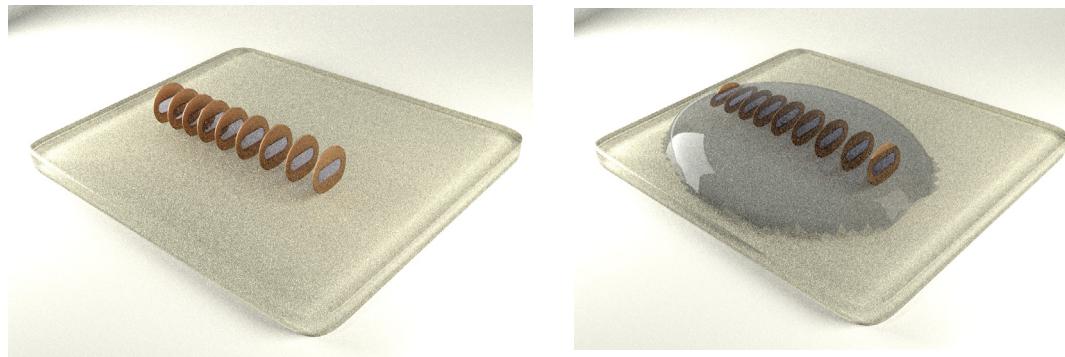
3_5 EM_video2

Cutting thin sections for transmission electron microscopy



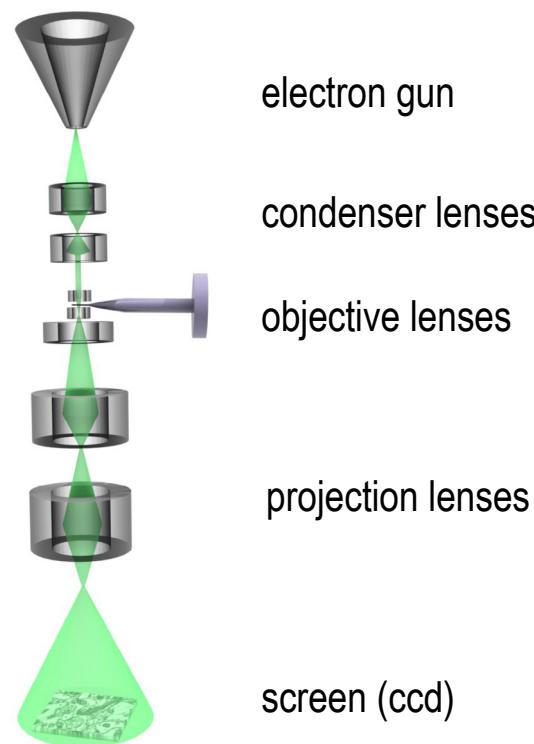
3_5 EM_video3

Post staining of ultrathin sections



Grids held in plastic holders and flooded with stains
(uranyl acetate and lead citrate)

Transmission electron microscope



Transmission electron microscope



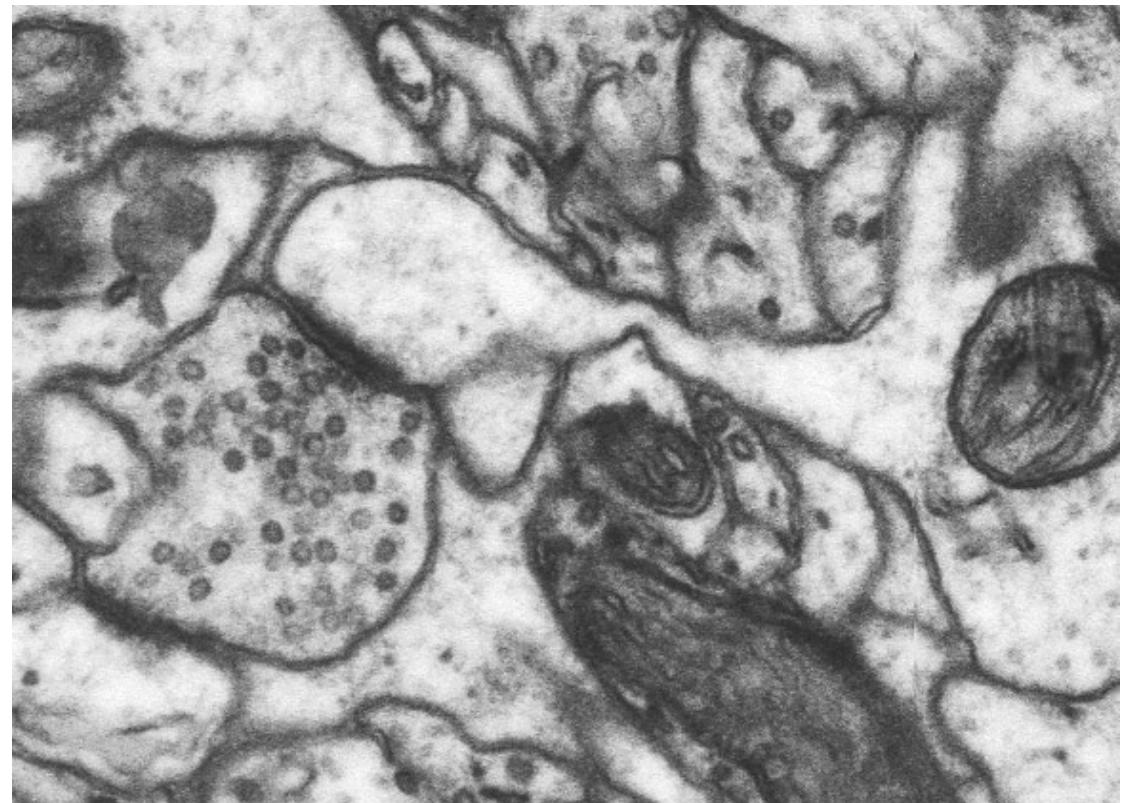
3_5 EM_video4

Transmission electron microscope



3_5 EM_video5

Imaging neuronal structure - electron microscopy



TEM of a single synaptic connection

Serial TEM imaging

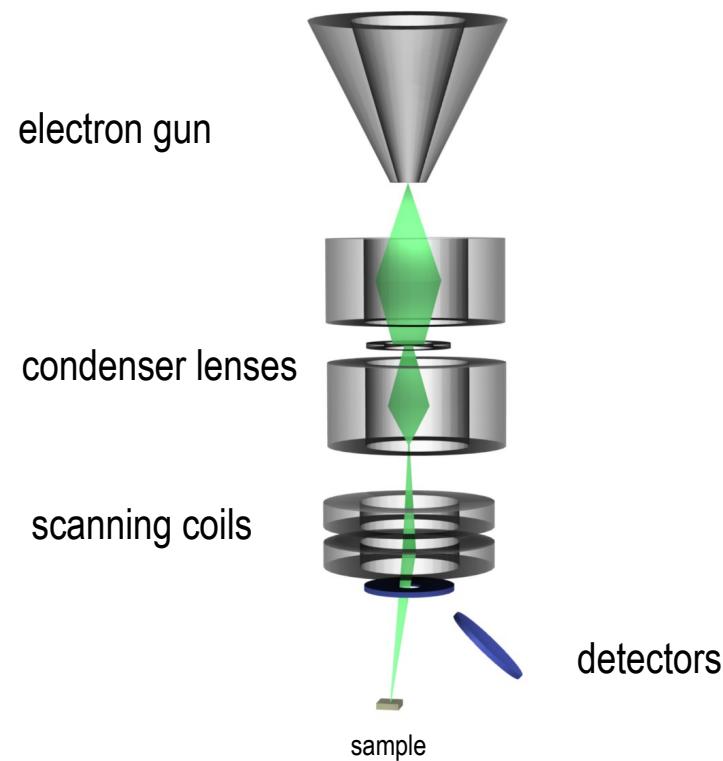


3_5 EM_video6

Serial transmission electron micrograph of
mouse cerebral cortex

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Scanning electron microscope

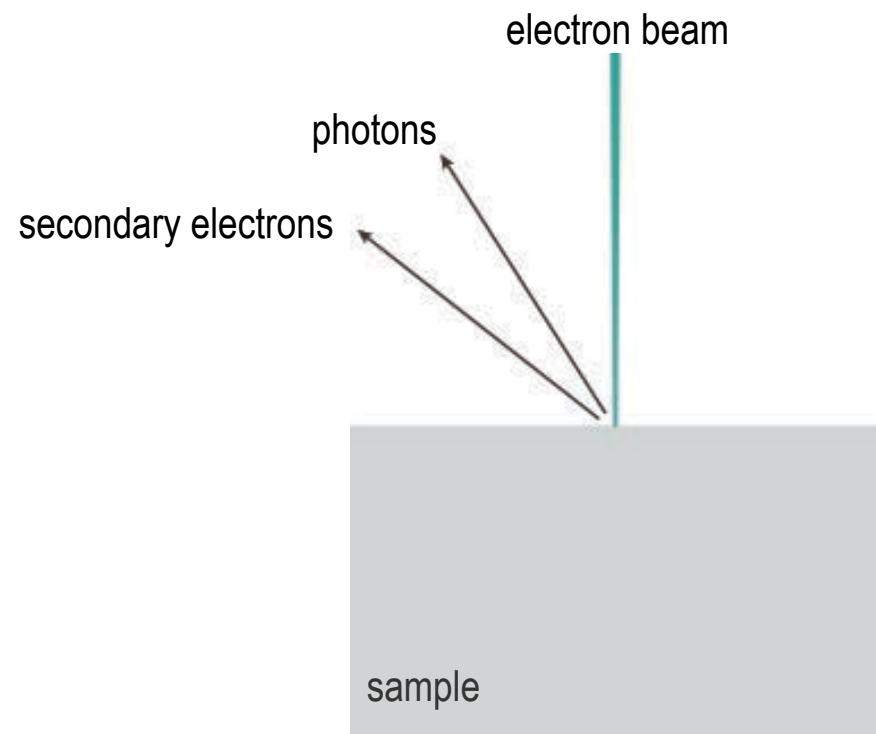


Scanning electron microscope

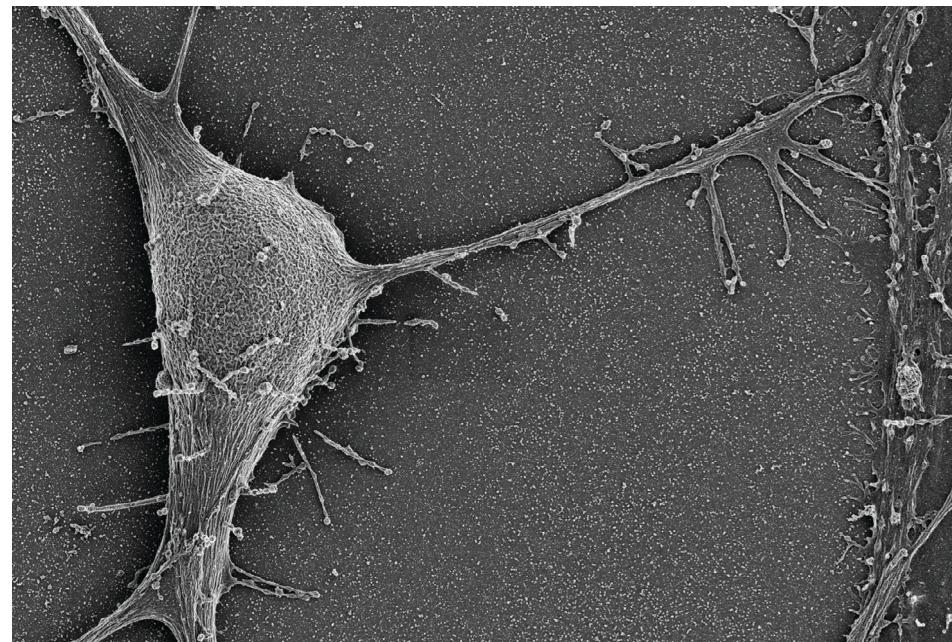
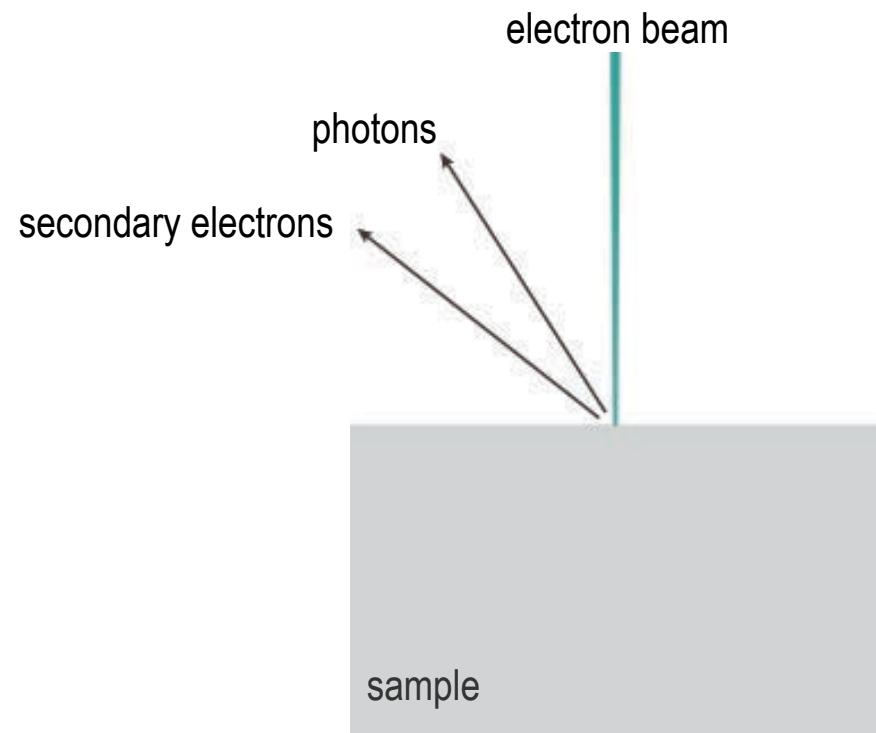


3_5 EM_video7

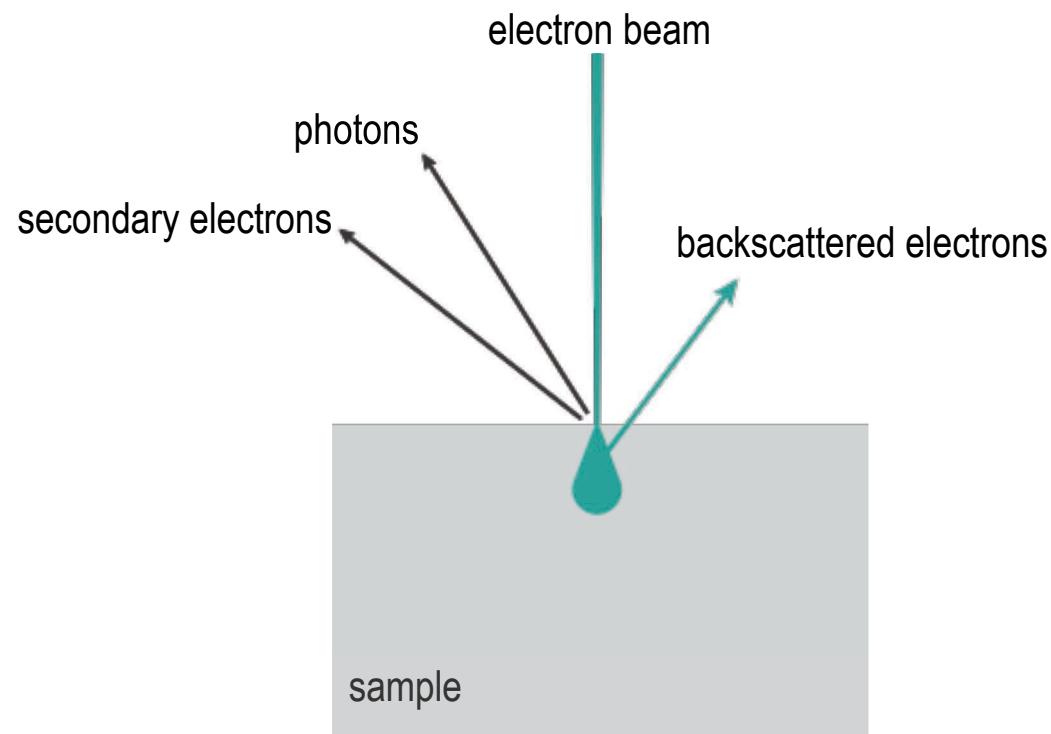
Scanning electron microscope



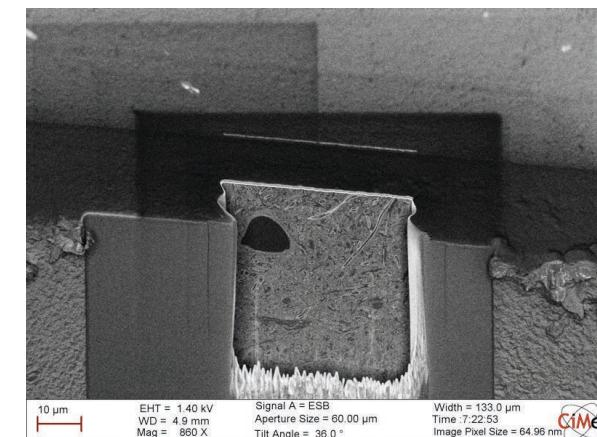
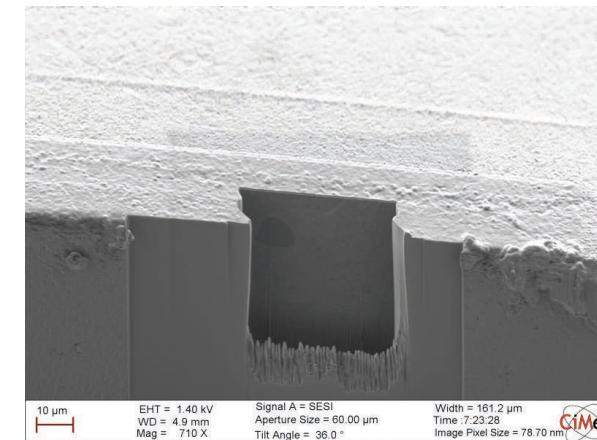
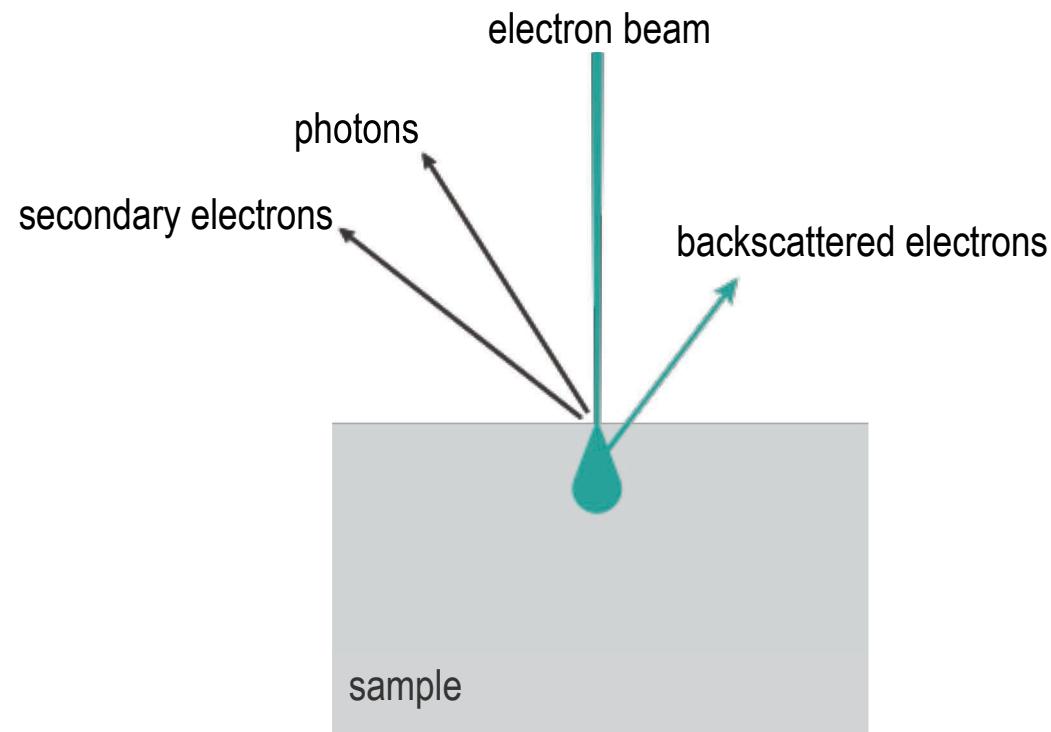
Scanning electron microscope



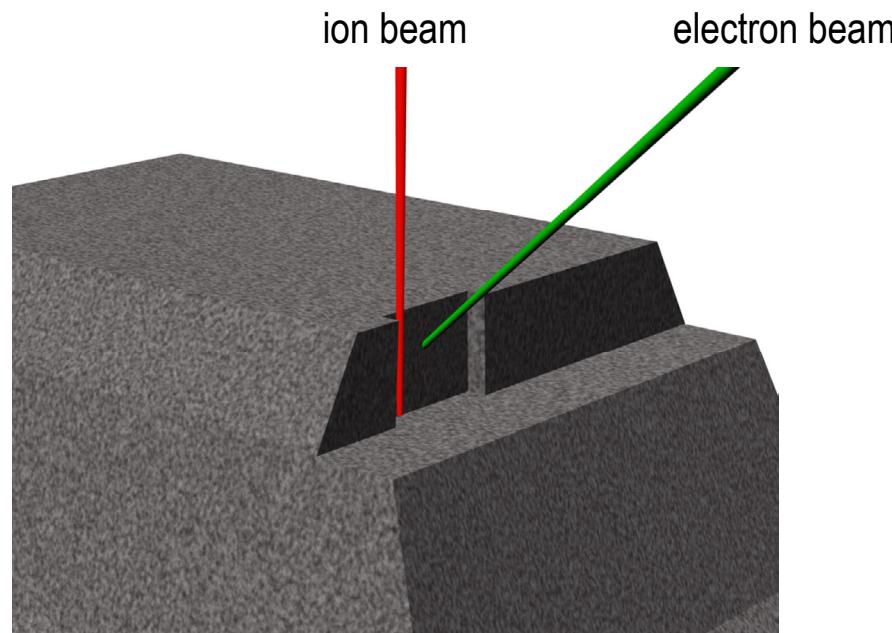
Scanning electron microscope



Scanning electron microscope



Block face scanning electron microscope



Focussed ion beam scanning electron microscope

Cellular Mechanisms of Brain Function

Block face scanning electron microscope



3_5 EM_video8

Block face scanning electron microscope



3_5 EM_video9

Focussed ion beam scanning electron microscopy of brain tissue

Cellular Mechanisms of Brain Function

Block face scanning electron microscope

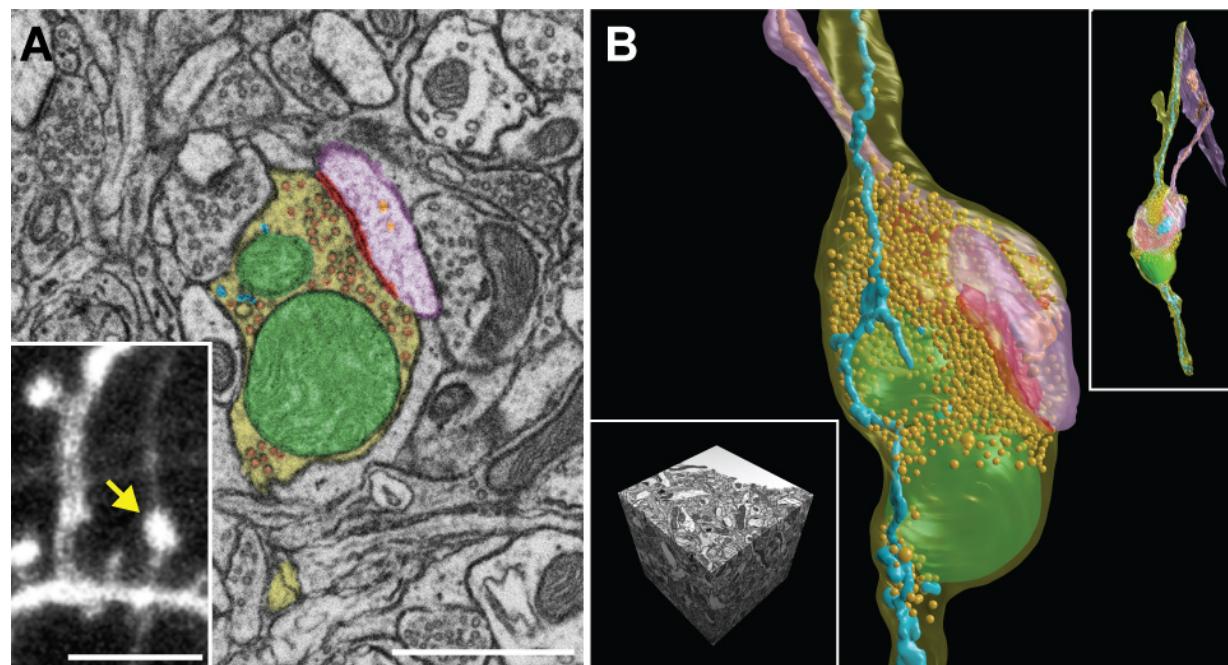


3_5 EM_video10

arbitrary slice planes through bouton of interest

Cellular Mechanisms of Brain Function

Synaptic ultrastructure



Maco et al, PLoS ONE, 2013

Cellular Mechanisms of Brain Function

