Introduction

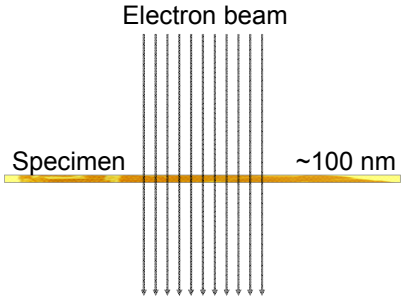
to

Biological Electron Microscopy

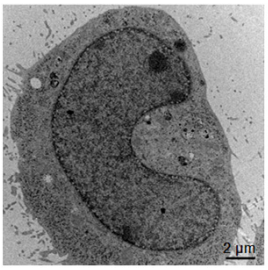
Andres Kaech

The types of electron microscopes

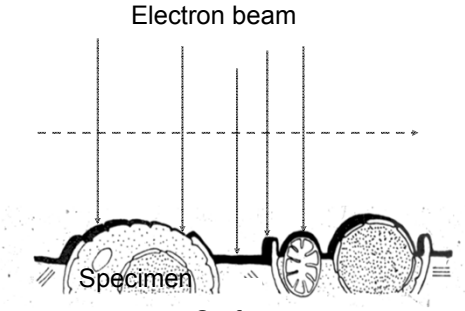
Transmission electron microscope (TEM)



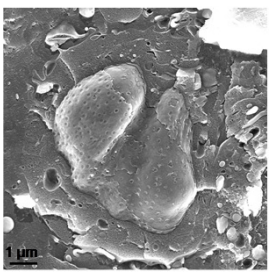
Projection



Scanning electron microscope (SEM)



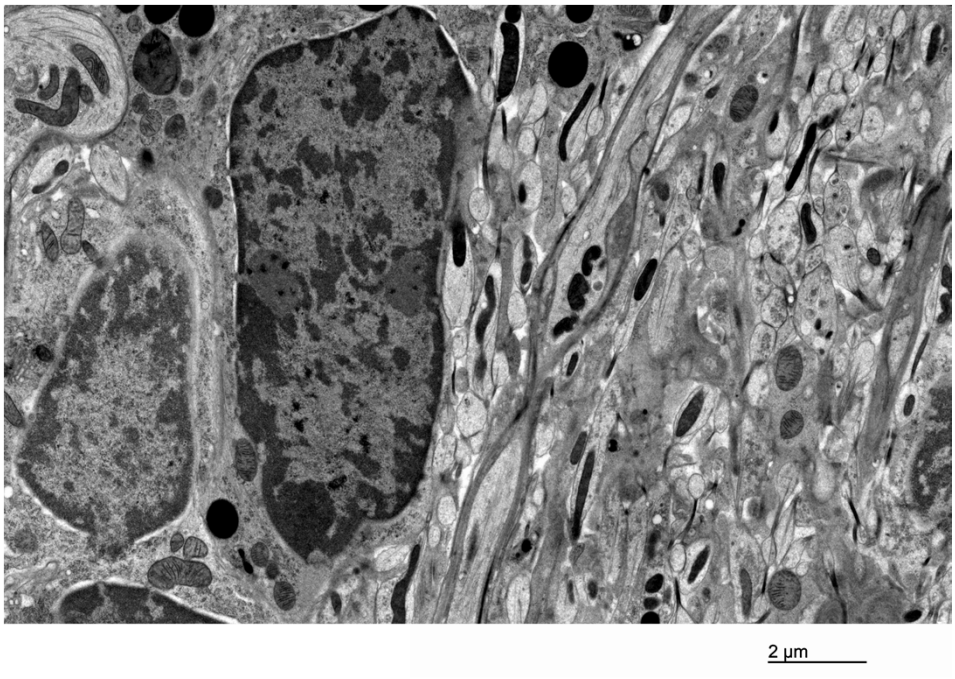
Surface



Hela Cells

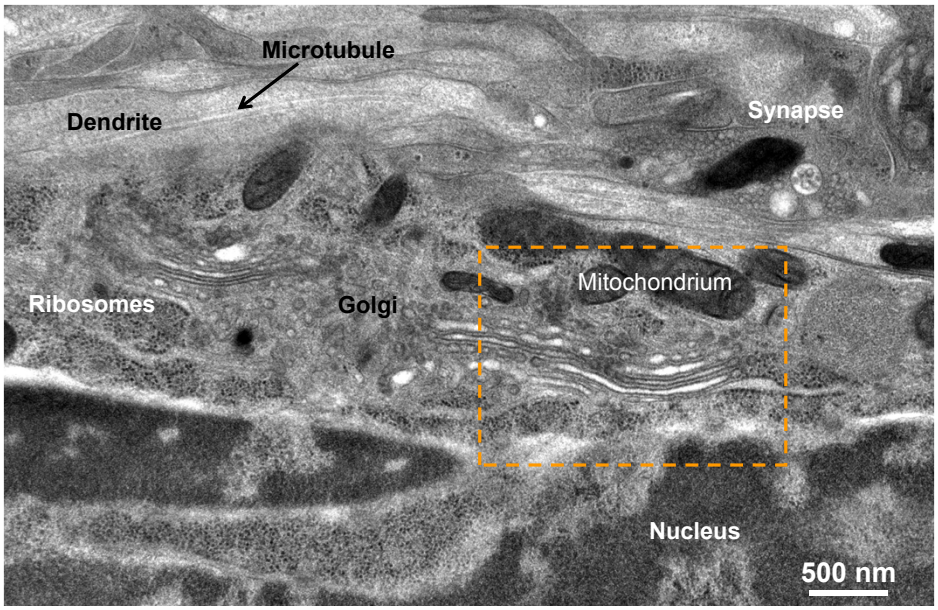
Examples TEM

Mouse cerebellum



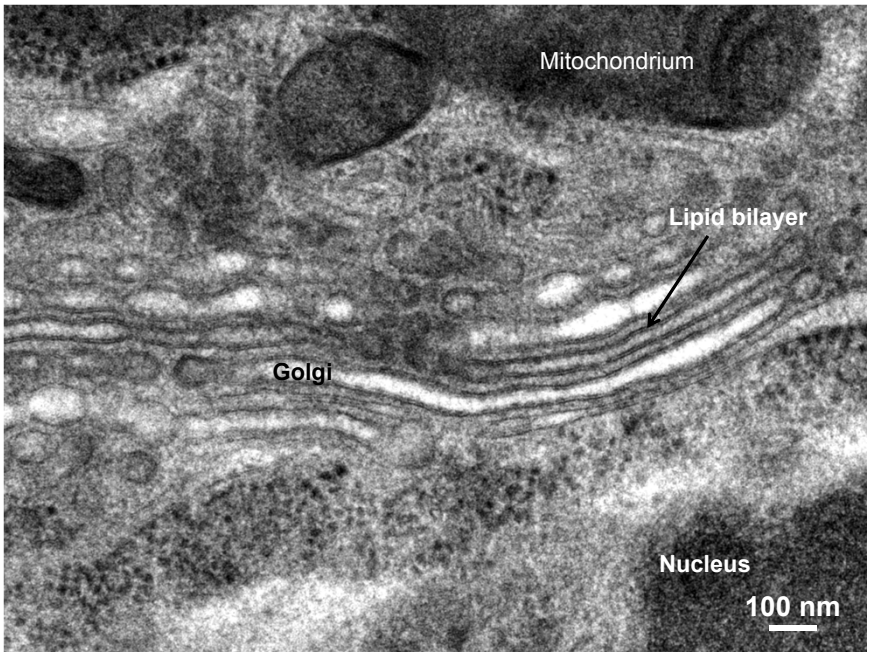
Examples TEM

Mouse cerebellum



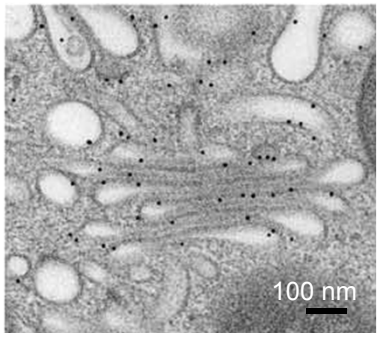
Examples TEM

Mouse cerebellum



Examples TEM

Immunolabelling: Localization of proteins

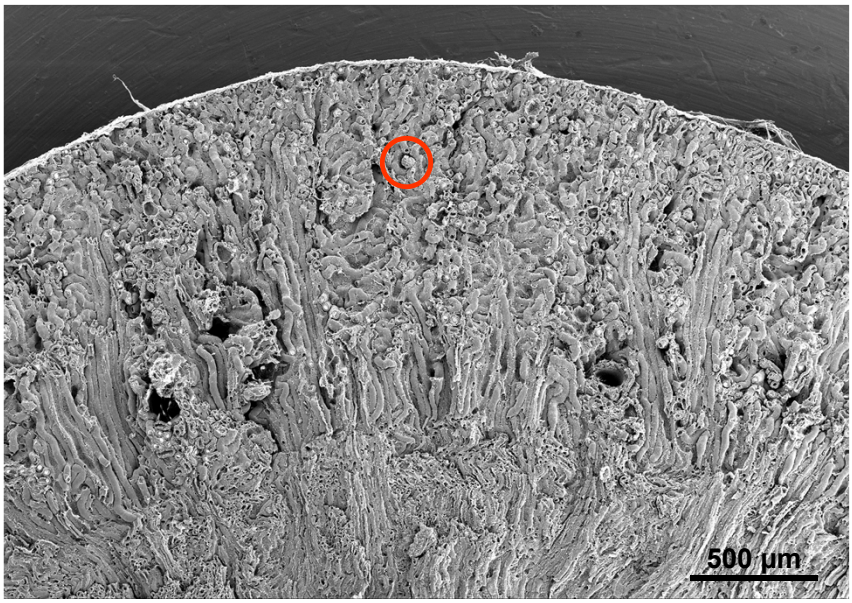


H/K -ATPase in cimetidine-treated resting **gastric parietal cells (rabbit).**

Sawaguchi et al. 2004, Journal of Histochemistry & Cytochemistry

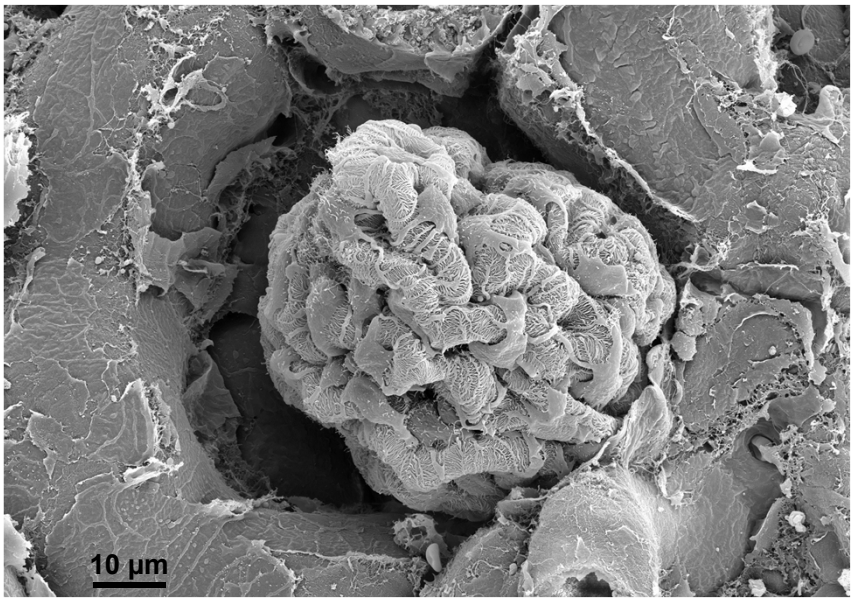
Examples SEM

Mouse kidney



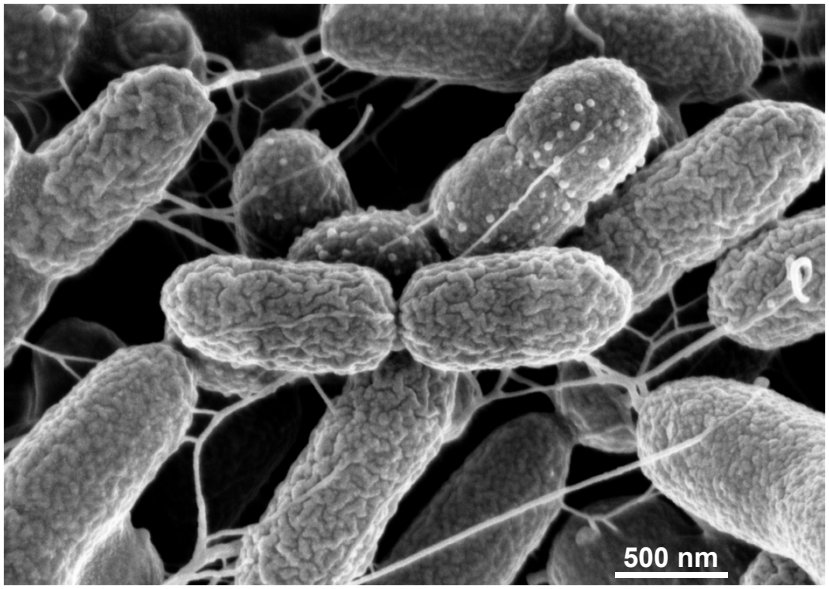
Examples SEM

Mouse kidney (glomerulus)



Examples SEM

Pseudomonas aeruginosa



Properties of electrons

- Very similar to photons:
- Wave-particle duality
- Optical properties
  (Diffraction, chromatic abberation, spherical abberation, astigmatism etc.)
- Resolution depends on aperture and wavelength
  (Diffraction limited resolution)

Abbe's equation d = 0.61 λ/NA $NA = n \cdot \sin\alpha$

Resolution of electron microscopes

The higher the energy of the electrons, the lower the wavelength, the higher the resolution

TEM: 40 – 300 kV

Effective instrument resolution TEM: ≈ 0.5 nm (120 kV)

SEM: 0.5 – 30 kV

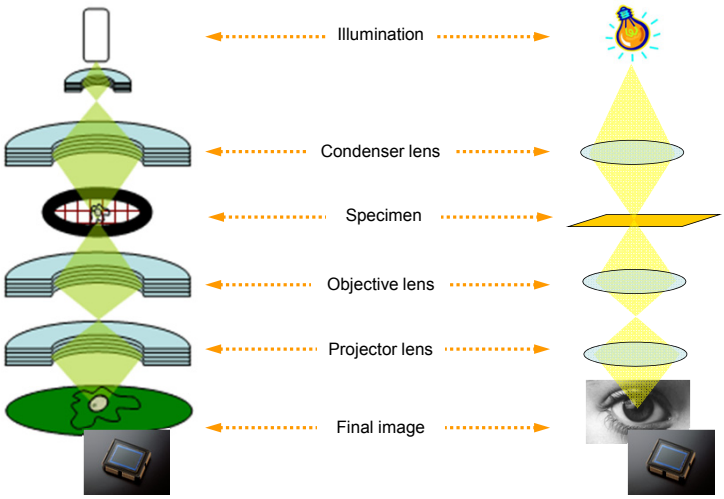
Effective instrument resolution SEM: ≈ 1 nm

Resolution of biological objects limited by **specimen preparation**:
Practical resolution: **>** 1 nm

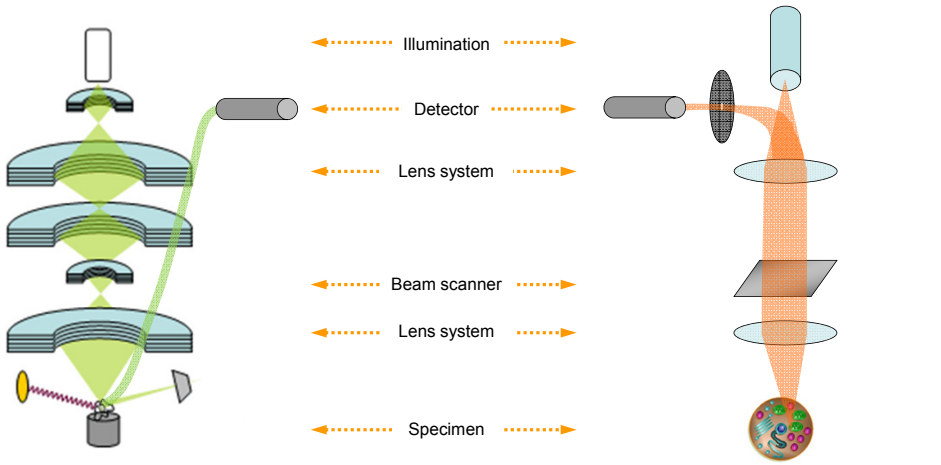
Transmission electron microscope vs. Widefield light microscope

Transmission electron microscope

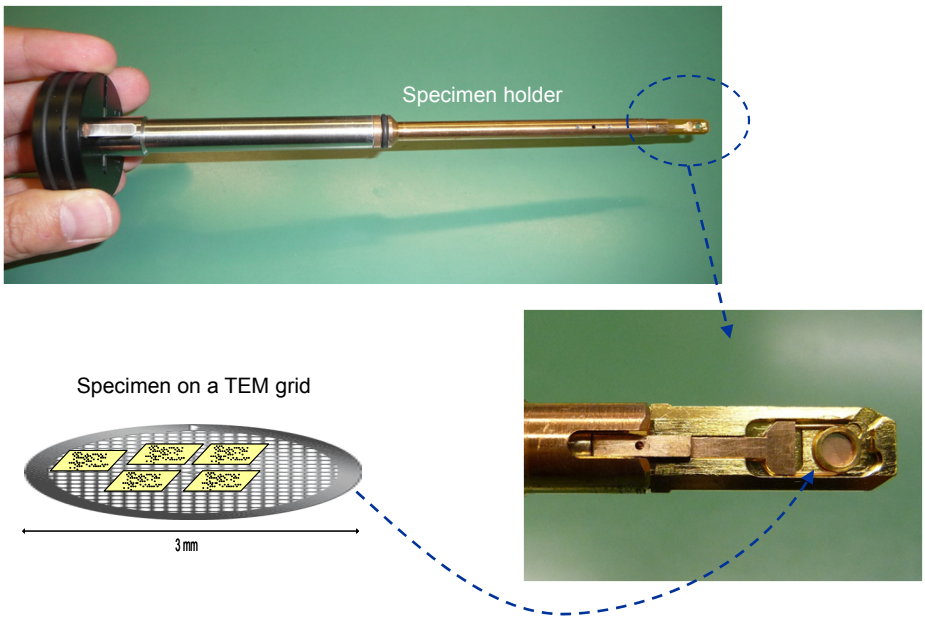
Widefield light microscope



Scanning electron microscope vs. Confocal laser scanning microscope

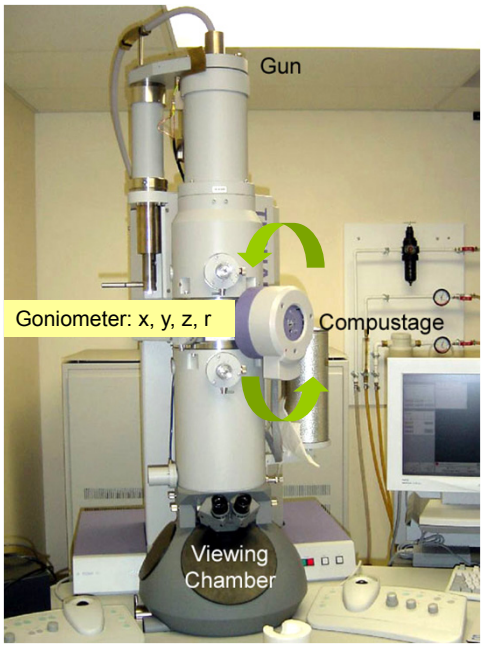


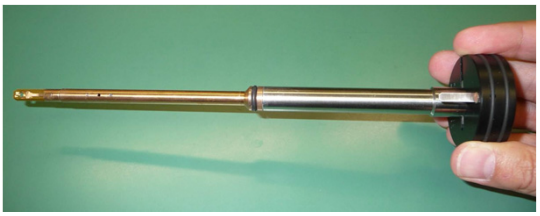
Specimen holders and stages - TEM



Specimen holders and stages - TEM

Transmission electron microscope



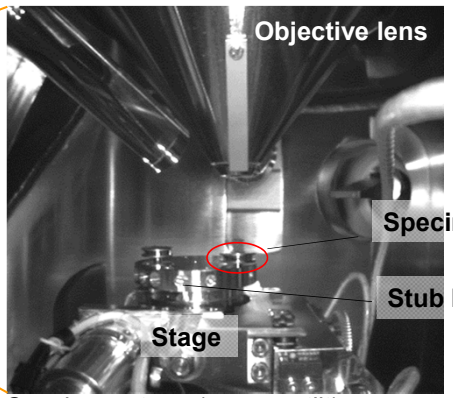


Specimen size:

- 3 mm in diameter!
- Ca. 100 nm in thickness (electron transparent)

Specimen holders and stages - SEM

Scanning electron microscope



Specimen stage (x, y, z, r, tilt)

Specimen size:

- 100 mm in diameter
- 2 cm in z-direction (not electron transparent)

Biological sample preparation for electron microscopy

Biology

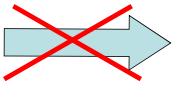
Aqueous/hydrated

Soft

Light elements
(C, O, H, N, S, P etc.)

"Large"

Not suitable for EM



Electron microscope

High vacuum

Electron beam

Sensitive to vibration/motion
(High magnifications)

Biological sample preparation for electron microscopy

Biology

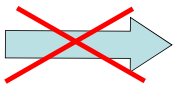
Aqueous/hydrated

Soft

Light elements
(C, O, H, N, S, P etc.)

"Large"

Not suitable for EM



Biological samples need to be transferred into a solid state...

...keeping the sample close to the native state

Electron microscope

High vacuum

Electron beam

Sensitive to vibration/motion
(High magnifications)

Resistant to high vacuum

Resistant in electron beam

Thin – permeable for electrons
(for TEM)

Contrast

Biological sample preparation for electron microscopy

Biology

Aqueous/hydrated

Soft

Light elements
(C, O, H, N, S, P etc.)

"Large"

Not suitable for EM

Any treatment changes the specimen!

Electron microscope

High vacuum

Electron beam

Sensitive to vibration/motion
(High magnifications)

Resistant to high vacuum

Resistant in electron beam

Thin – permeable for electrons
(for TEM)

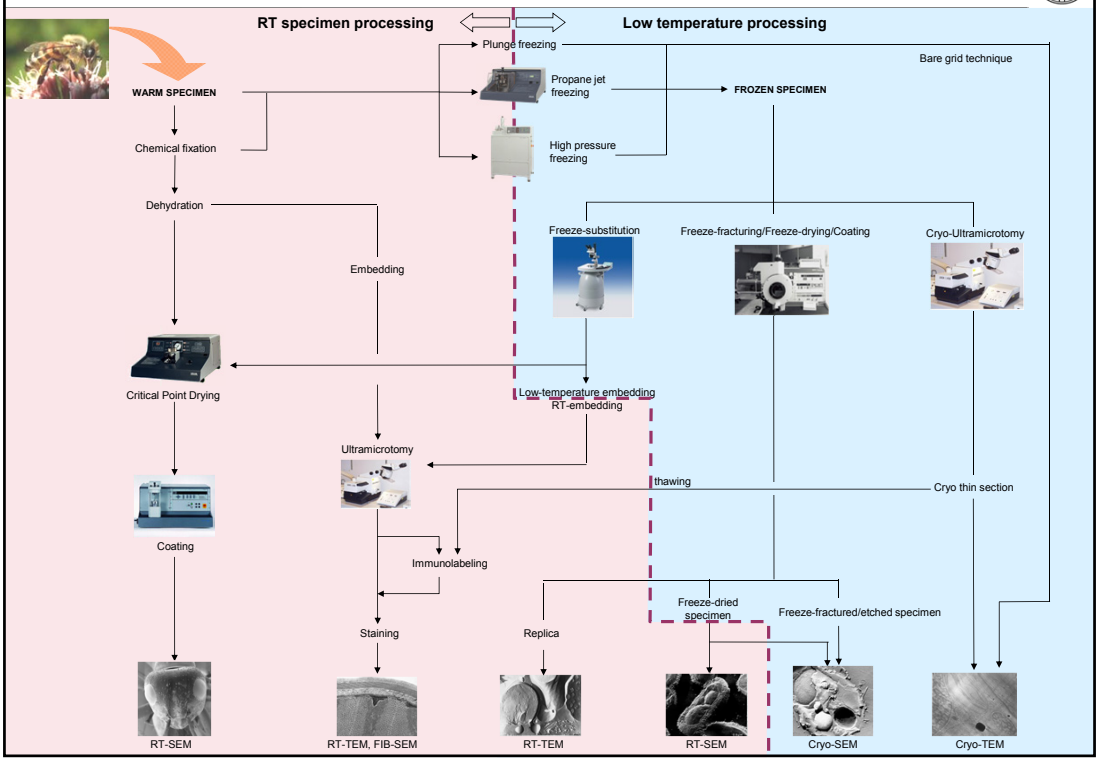
Contrast

Biological sample preparation for electron microscopy

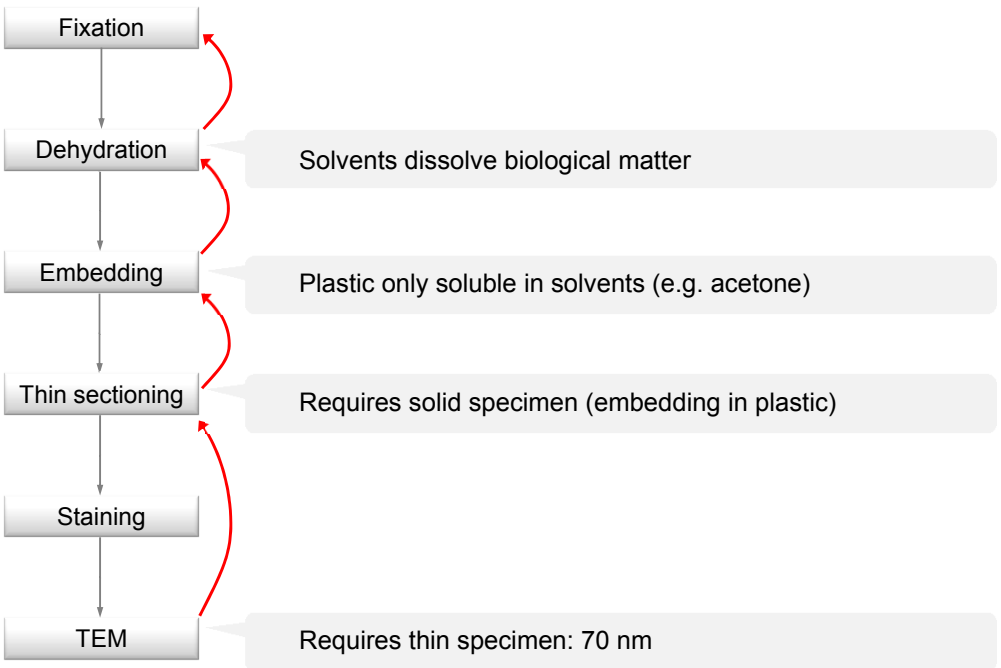
What is (was) this?

2 cm

Preparation pathways overview



Sample preparation steps for TEM



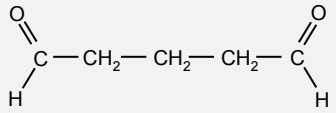
Room temperature processing for TEM

Fixation
Dehydration
Embedding
Thin sectioning
Staining
TEM

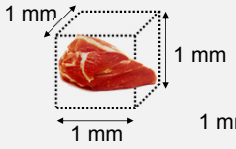
Stabilization of biological material

Chemical fixation (cross-linking) with Aldehydes, Ur^{2+}…

Glutaraldehyde



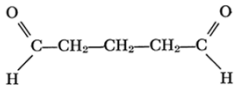
Maximum size for good preservation



1 mm^3: penetration within 30-60 min at 20-37°C

Room temperature processing for TEM

Glutaraldehyde versus Formaldehyde



- Mainly used for electron microscopy
- Irreversible fixation and polymerisation
- Destroys antigens considerably: Not suitable for subsequent immunolabelling
- Needs to be prepared freshly (prevent polymerisation prior to fixation)
- Needs to be buffered (reaction causes drop of pH)

- Mainly used for LM
- Reversible fixation and polymerisation
- Destroys much less antigens than Glutaraldehyde
- Used for EM only in combination with Glutaraldehyde
- Needs to be prepared freshly (prevent polymerisation prior to fixation)
- Needs to be buffered (reaction causes drop of pH)

Room temperature processing for TEM

Fixation
Dehydration
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Staining
TEM

Post-fixation with OsO_4

Osmiumtetroxide 

- Cross linker of unsaturated lipids, proteins & phenolic compounds
- Provides contrast
- Can solubilise some proteins

Room temperature processing for TEM

Fixation
Dehydration
Embedding
Thin sectioning
Staining
TEM

…often additional step after fixation:

Contrast enhancement – block staining with uranyl-acetate (Ur^{2+})

Room temperature processing for TEM

Fixation

Dehydration

Embedding

Thin sectioning

Staining

TEM

Substitution of water with solvent (ethanol, acetone)
Usually performed with gradient of different concentrations.

Room temperature processing for TEM

Fixation

Dehydration

Embedding

Thin sectioning

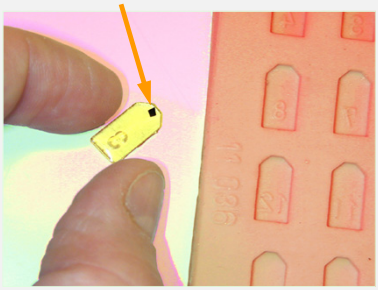
Staining

TEM

Infusion with “plastic” formulation followed by polymerisation

- Plastic formulations consist of monomers, hardener, accelerator
- Polymerization by heat or UV light
- Epoxy resins, acrylic resins
- Note: Resins are toxic and allergenic

Specimen embedded in Epon



Room temperature processing for TEM

Fixation

Dehydration

Embedding

Thin sectioning

Staining

TEM

Cutting sections of ca. 70 nm -> electron transparent

Ultramicrotomy

Room temperature processing for TEM

Fixation

Dehydration

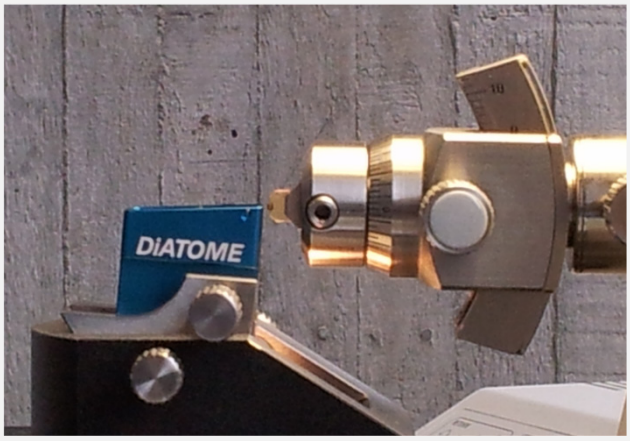
Embedding

Thin sectioning

Staining

TEM

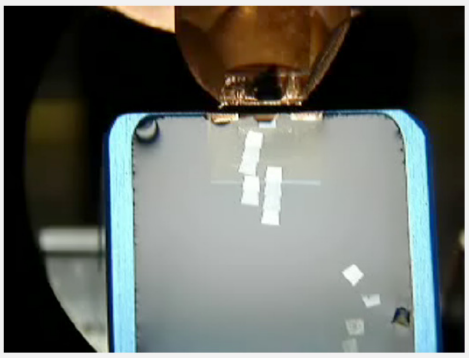
Cutting sections of ca. 70 nm -> electron transparent

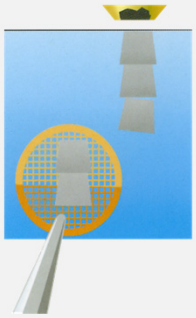


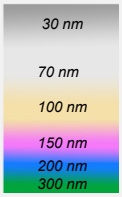
Room temperature processing for TEM

Fixation
Dehydration
Embedding
Thin sectioning
Staining
TEM

Cutting sections of ca. 70 nm -> electron transparent



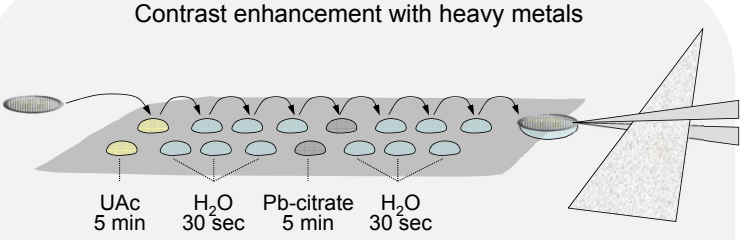


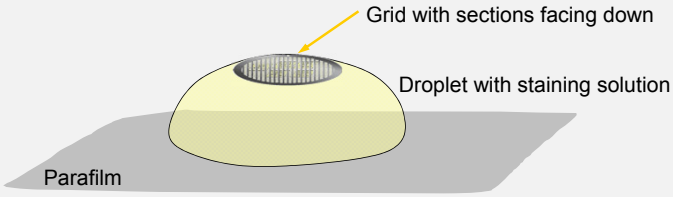


Room temperature processing for TEM

Fixation
Dehydration
Embedding
Thin sectioning
Staining
TEM

Contrast enhancement with heavy metals



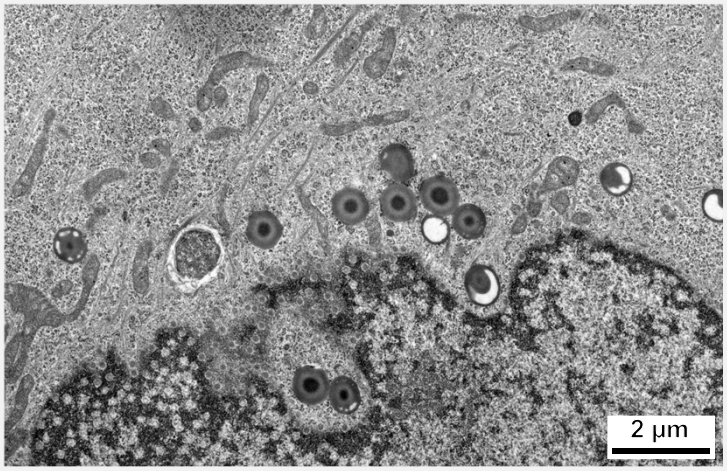


- Uranium ions: phosphate groups of lipids (membrane contrast)
- Lead ions preferably bind to proteins

Room temperature processing for TEM

Fixation
Dehydration
Embedding
Thin sectioning
Staining
TEM

Interpretation/orientation



HEP2 cells infected with *Chlamydia pneumoniae*

Room temperature processing for TEM

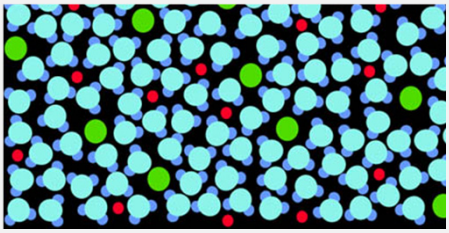
Fixation	Aldehydes: Slow (seconds to minutes), lots of artefacts like shrinkage… OsO_4: Depolimerisation of proteins
Dehydration	Shrinkage Conformational changes of proteins Loss of lipids
Embedding	Mechanical effects Loss of Lipids Shrinkage during polymerisation
Thin sectioning	Compression, knife marks
Staining	Staining artefacts (precipitation of heavy metals)
TEM	Interpretation mistakes

Cryo preparation for TEM

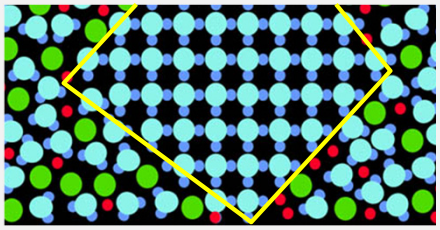
Fixation
Dehydration
Embedding
Thin sectioning
Staining
TEM

Cryo-Immobilization
Stabilization of biological material by freezing

Liquid water and vitrified water



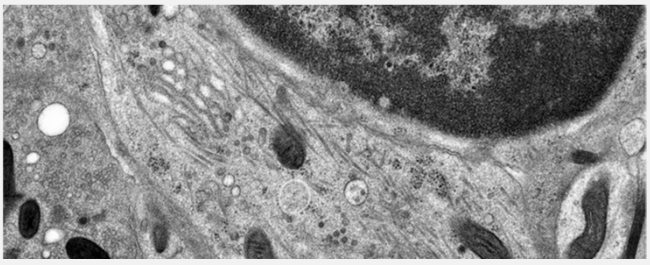
Frozen water with ice crystals



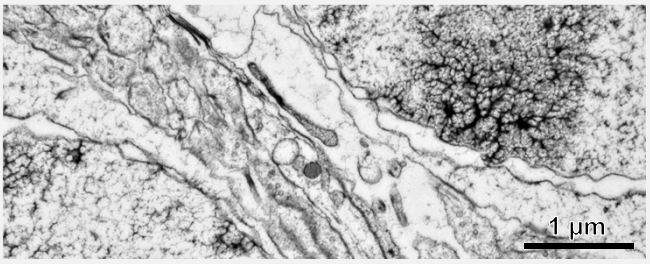
Cryo preparation for TEM

Fixation
Dehydration
Embedding
Thin sectioning
Staining
TEM

Well frozen mouse cerebellum



Not well frozen mouse cerebellum



Cryo preparation for TEM

Fixation

Dehydration

Embedding

Thin sectioning

Staining

TEM

Plunge freezing in liquid ethane/propane:

- Only suspensions (< 1 µm) or thin tissues containing anti-freeze

Slam freezing:

- Suspensions and thin tissues (few µm, only front well frozen ca. 1 µm)

Propane jet freezing (JFD):

- Adequate freezing of suspensions not thicker than 15 µm
- Thicker specimen require anti-freeze

High pressure freezing (HPM)

- Freezing under high pressure (2100 bar)
- Adequate freezing of samples up to 200 µm thickness without anti-freeze

Cryo preparation for TEM

Fixation

Dehydration

Embedding

Thin sectioning

Staining

TEM

Plunge/slam freezer

Propane jet freezer

High-pressure freezer

Relative sizes

Cryo preparation for TEM

Fixation

Dehydration

Embedding

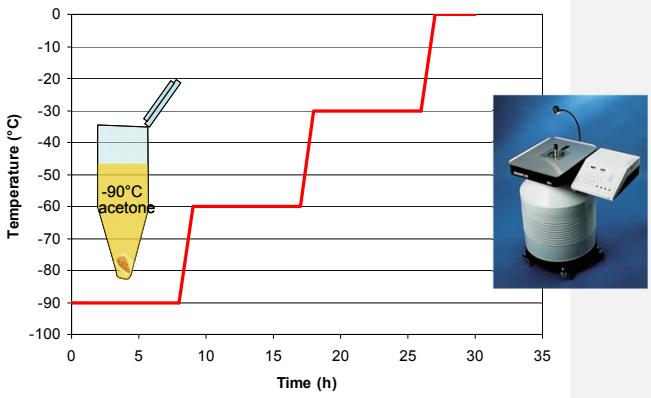
Thin sectioning

Staining

TEM

Freeze-substitution

Substitution of water/ice with solvent (ethanol, acetone)
Usually combined with simultaneous fixation with chemicals (OsO_4, Uranyl-acetate…)



Cryo preparation for TEM

Fixation

Dehydration

Embedding

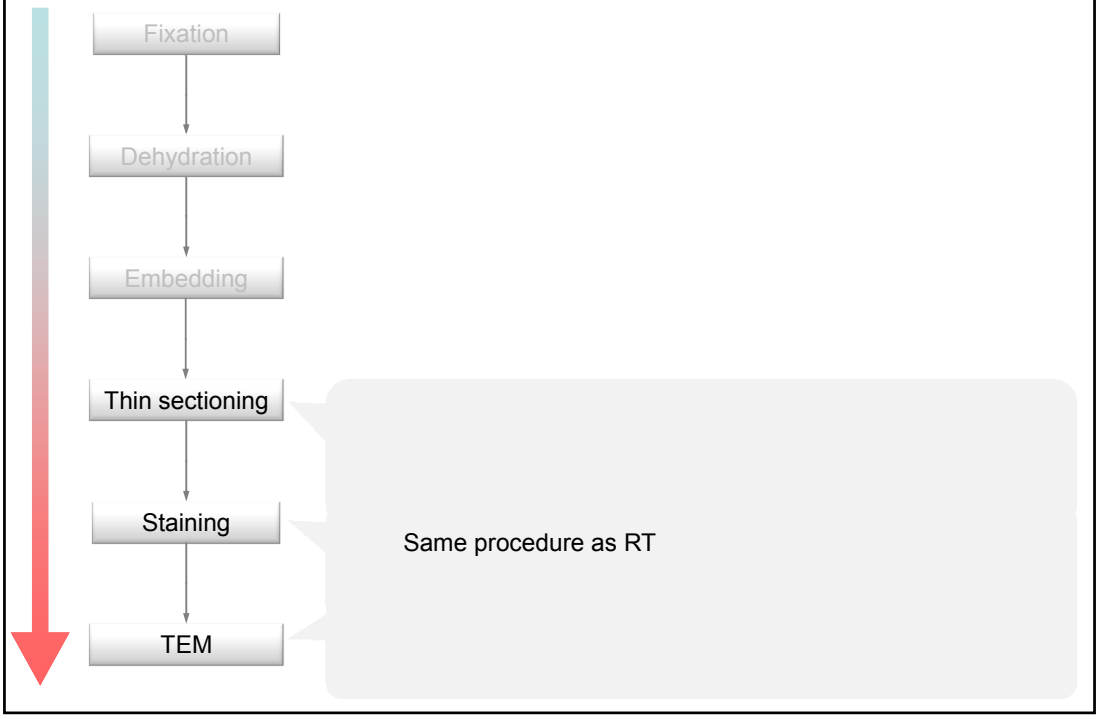
Thin sectioning

Staining

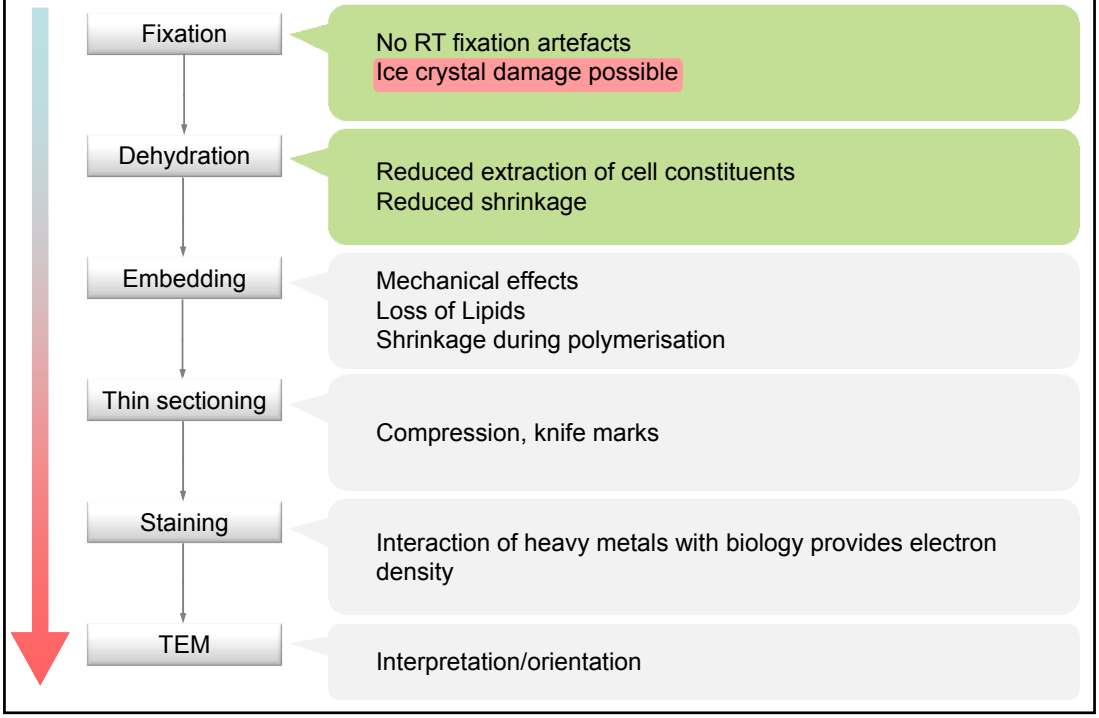
TEM

Infusion with “plastic” formulation followed by polymerisation at low or room temperature

Cryo preparation for TEM



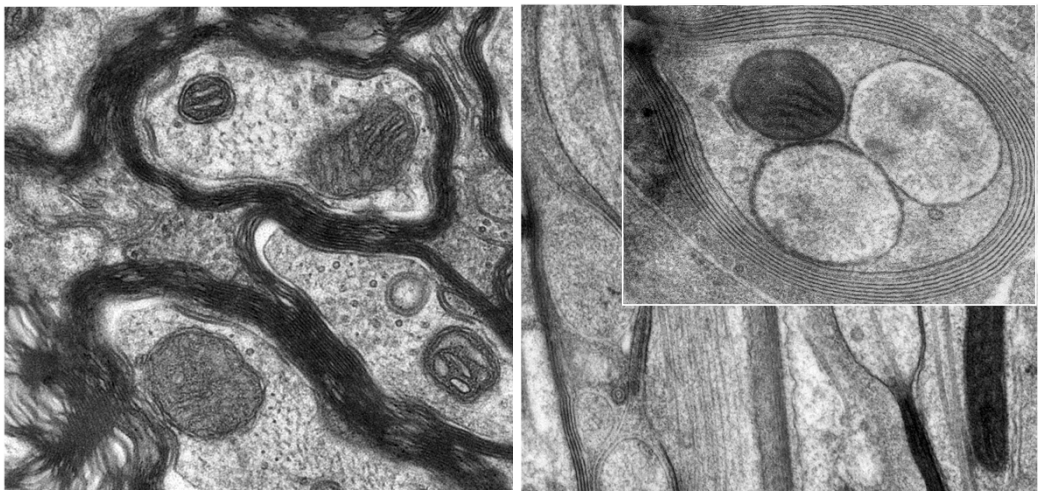
Cryo preparation for TEM



Room temperature vs. cryo preparation

Thin sections of plastic embedded mouse cerebellum

Conventionally fixed (glutaraldehyde) High pressure frozen



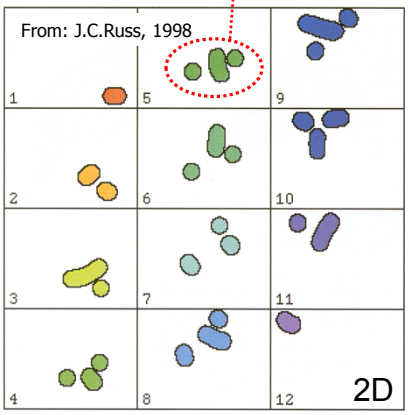
Specimen courtesy of Bettina Sobottka, Neurologische Klinik, University of Zurich

500 nm

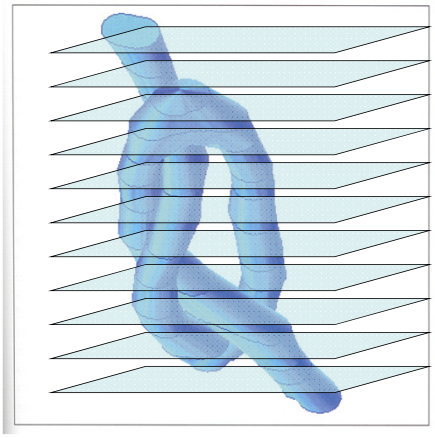
How do we get to 3D

Ultrastructural details, interaction of organelles

Are these objects related to each other?

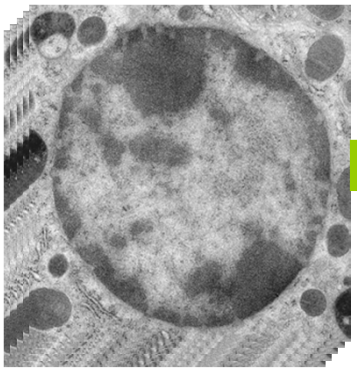


3D



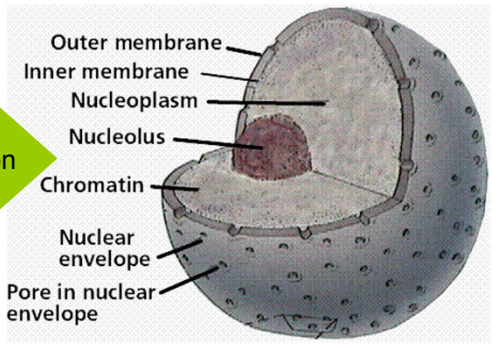
3D reconstruction of 2D projections
of a 3-dimensional object

2D projections



Reconstruction

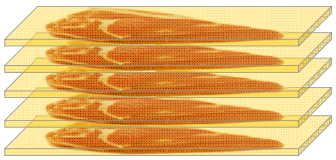
3D rendered object



Electron Tomography

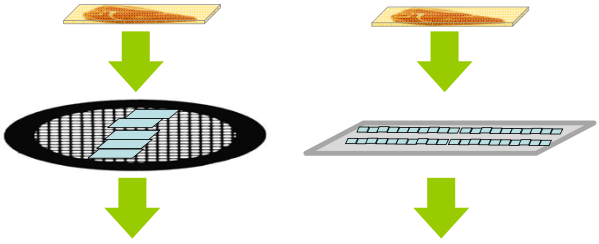
3D reconstruction based on:

I) Imaging of serial sections



Thickness of section for EM: 50 - 300 nm

→ Z resolution limited to section thickness

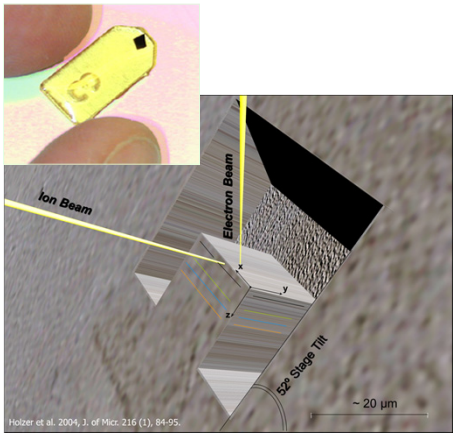


Electron Tomography

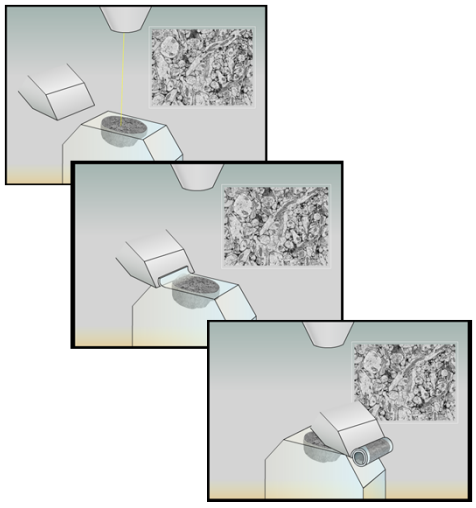
3D reconstruction based on:

I) Imaging of serial sections

Focused ion beam SEM
Ablation of material with Ga-ions

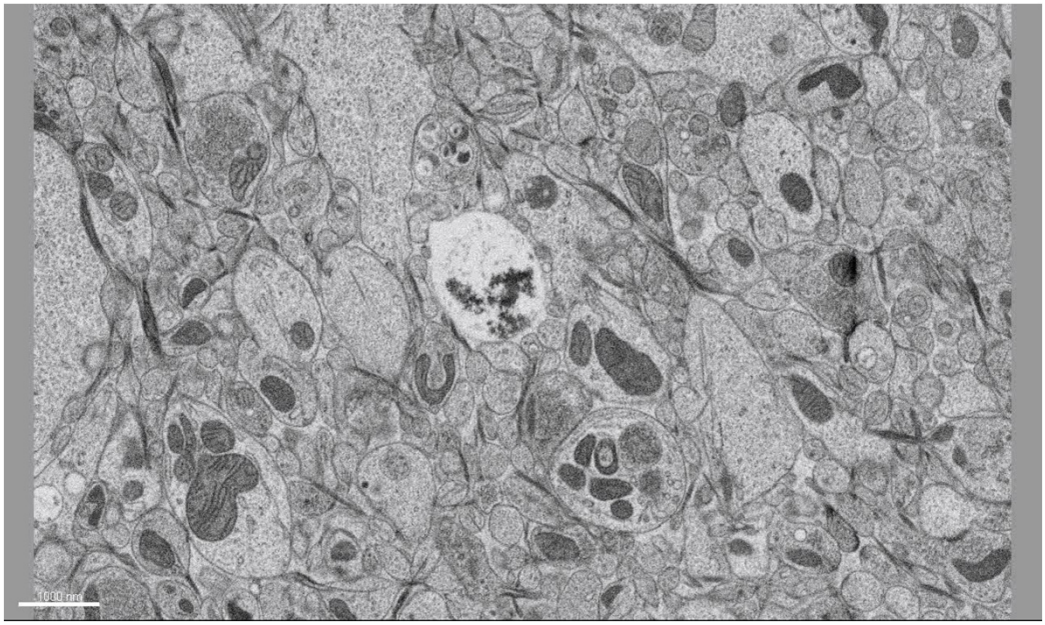


Ultra-microtome in SEM



Electron Tomography

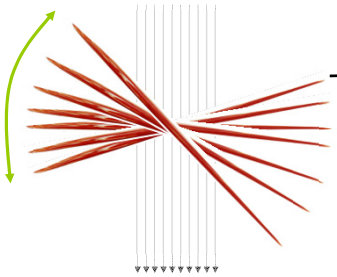
High-pressure frozen and embedded mouse brain (hippocampal slice culture)



Electron Tomography

3D reconstruction based on:

II) Projection of a tilt series



Thickness of sections for tilt series: ~70 - 300 nm

→ Z resolution depending on number of different projections: 5 - 20 nm

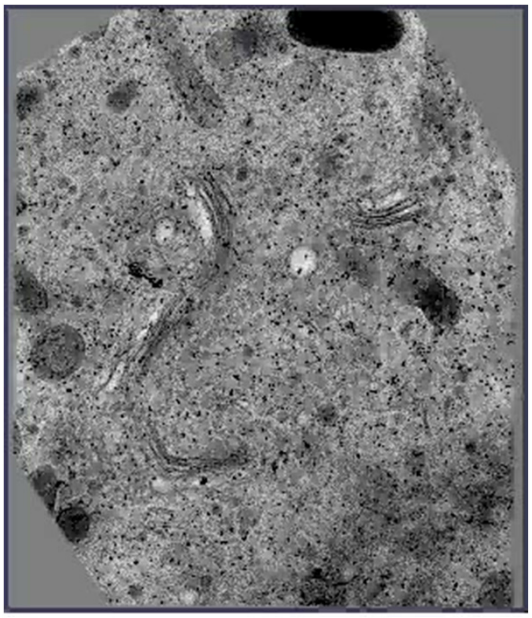
NOTE: A combination of both methods is possible

3D high resolution of several successive sections

Imaging in TEM (RT or CRYO)

Electron Tomography

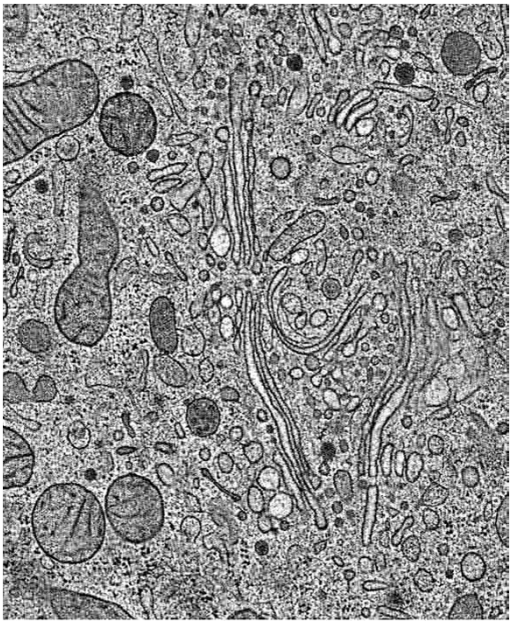
Aligned projections of a
Pancreatic beta cell line: Double-tilt series, ±60°, 1.5° increment, section 400 nm thick



Marsh 2005

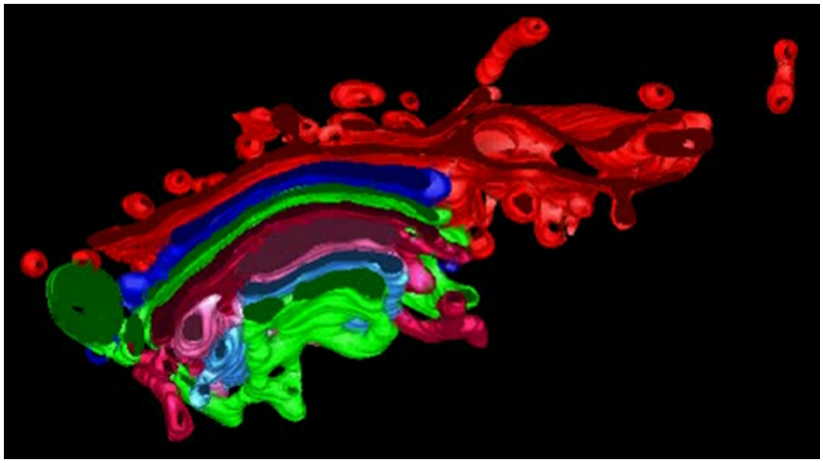
Electron Tomography

Calculation of tomograms based on projections – virtual sections



Marsh 2005

Rendering of segmented structures



Marsh 2005