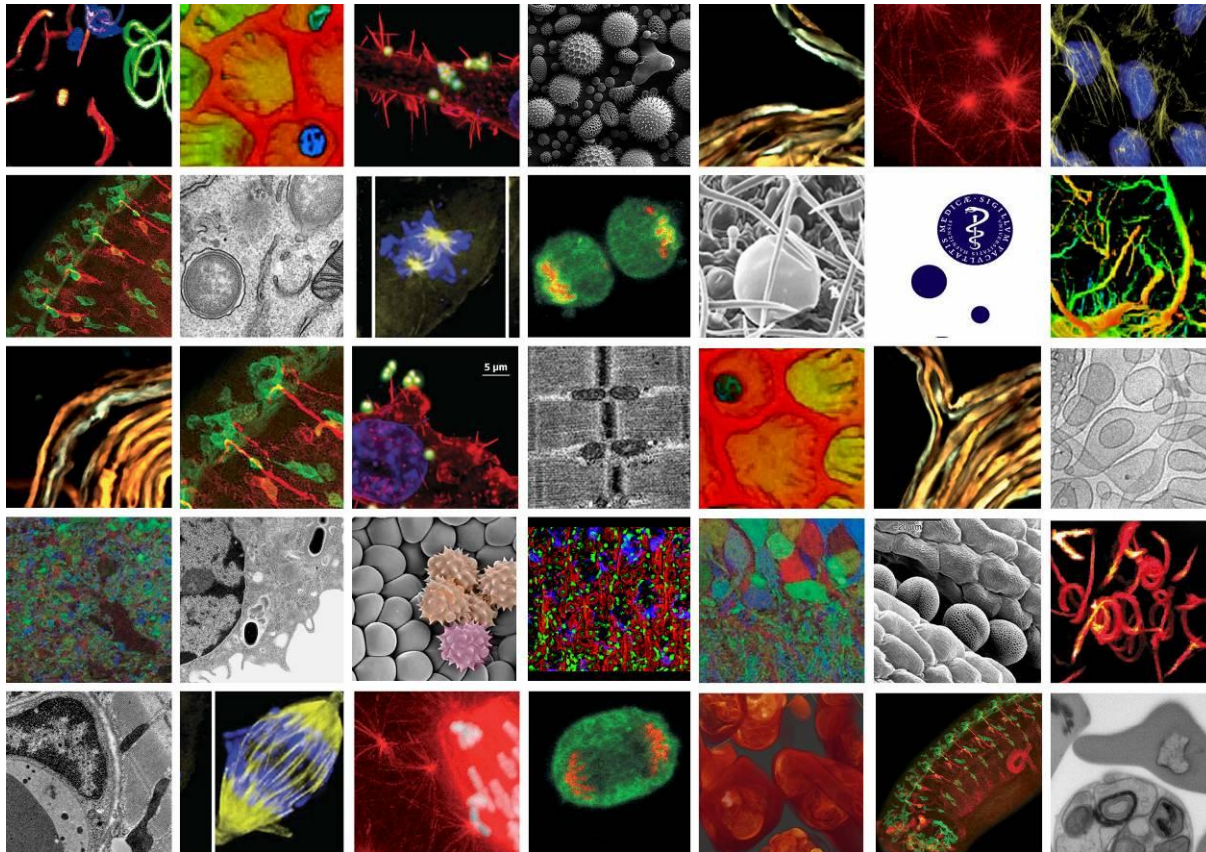
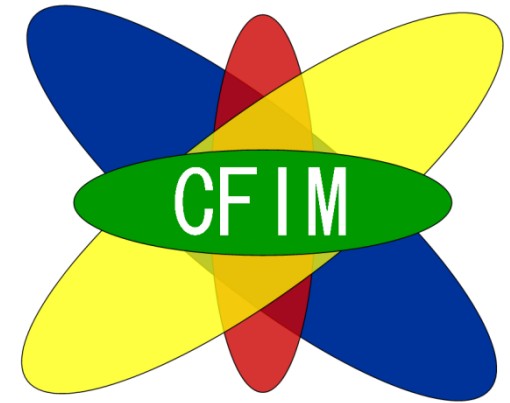


Core Facility for Integrated Microscopy



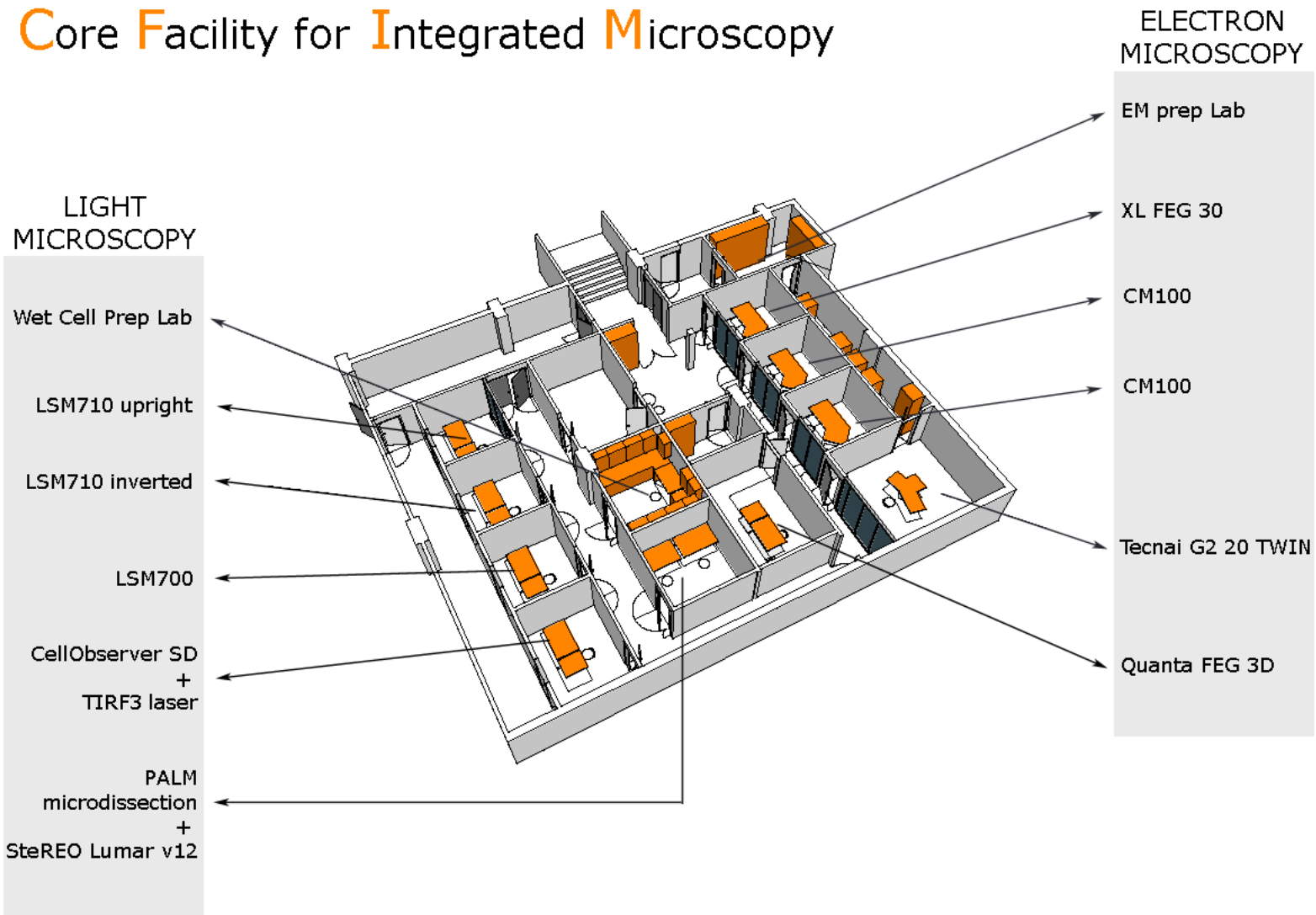
CFIM scope

- Core facility with 'state-of-the-art' electron and light microscopes
- Purpose built facility
- Live cell analysis
- Dedicated support
 - Director
 - Head of Light Microscopy (March 1st, 2010)
 - Head of Electron Microscopy (Ramon Liebrechts)
- User support in 3 levels: DIY, Minimal and Full support
- PhD courses in Light- and Electron Microscopy
- On-line booking on www.cfim.ku.dk
- Wet cell & EM prep lab
- Seminar room
- Image post-processing workstations



Faculty of Health Sciences

Core Facility for Integrated Microscopy



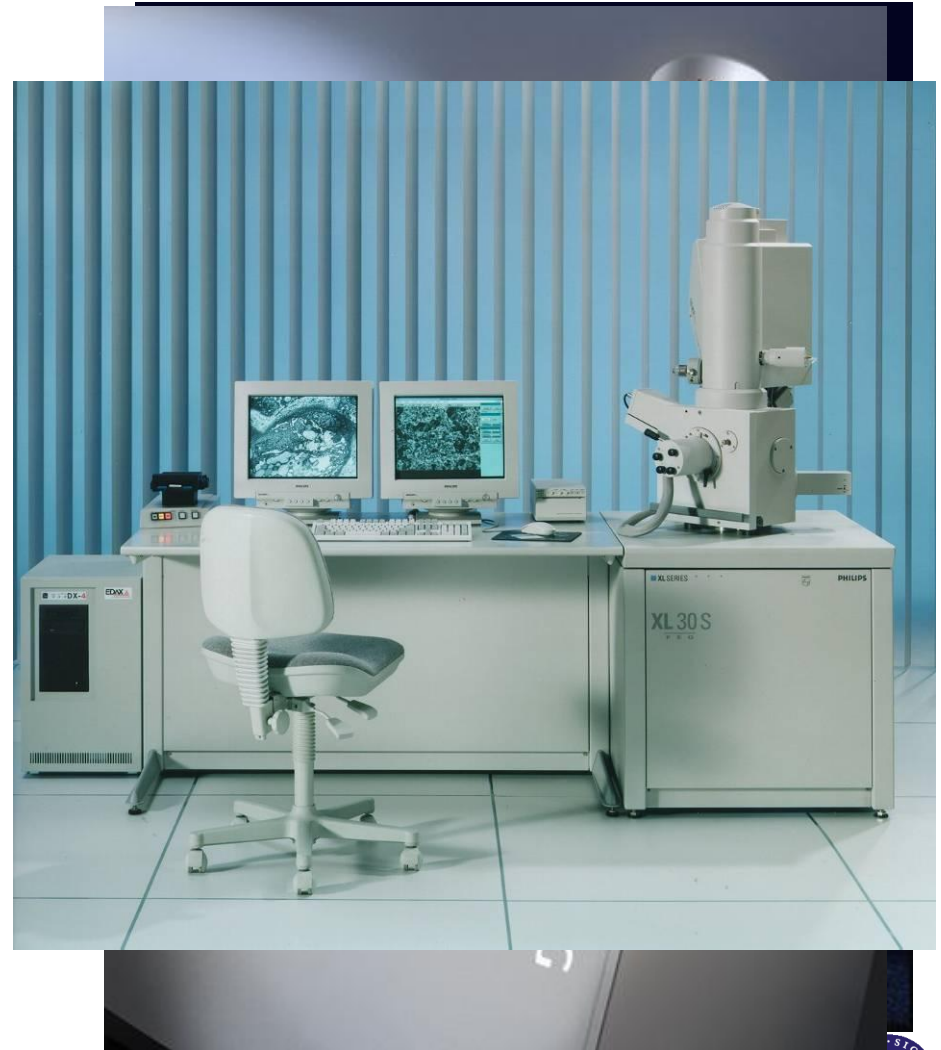
Electron Microscopy

- TEM Tecnai G20 (cryo + tomography)
- *TEM CM 100 (2)
- SEM Quanta 3D (cryo + FIB SEM)
- *SEM FEG30 (SED & BSD)

*Full upgrade of existing microscopes

Support

- Dark room
- Optical bench, diffraction
- Sputter coaters, carbon evaporators etc.



Tomography, what's in a word?

Definition Tomography....

Tomos = section, slice, cutting

Electron Tomography

Computer Tomography

X-ray Tomography

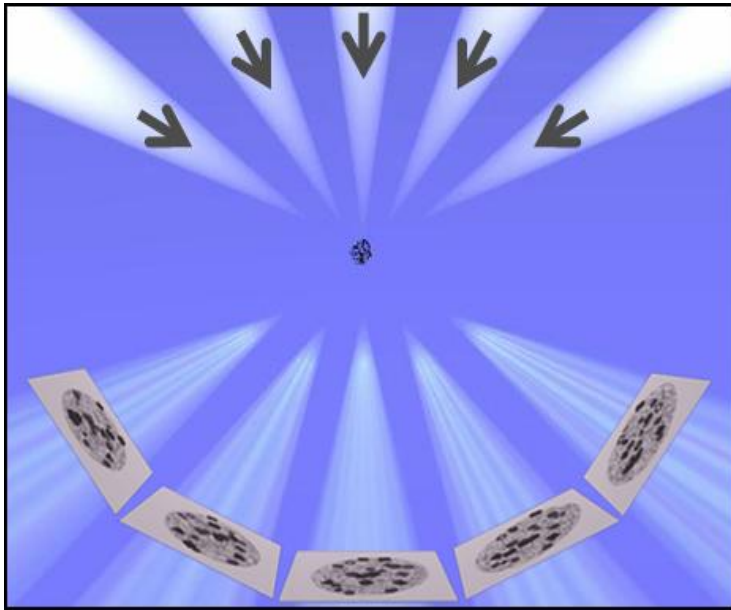
Polytomography



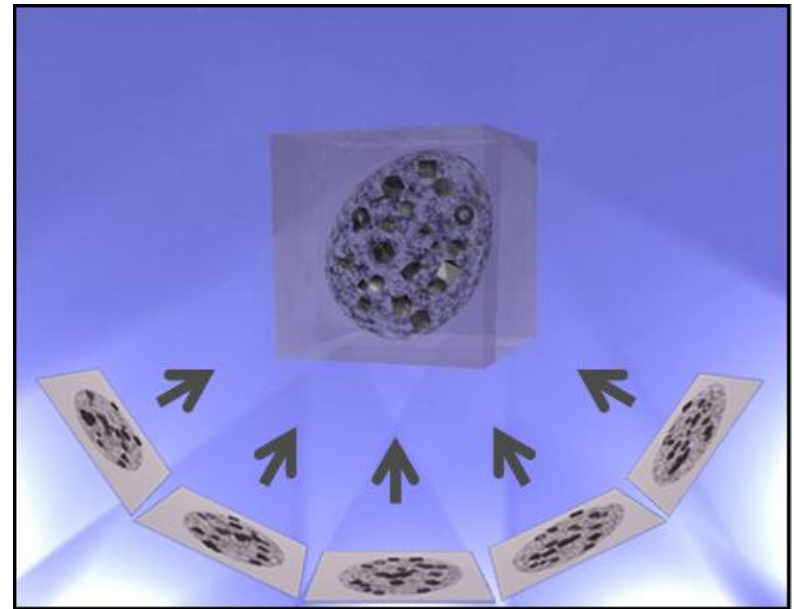
Electron Tomography

Principle

3D-object => set of 2D-projections



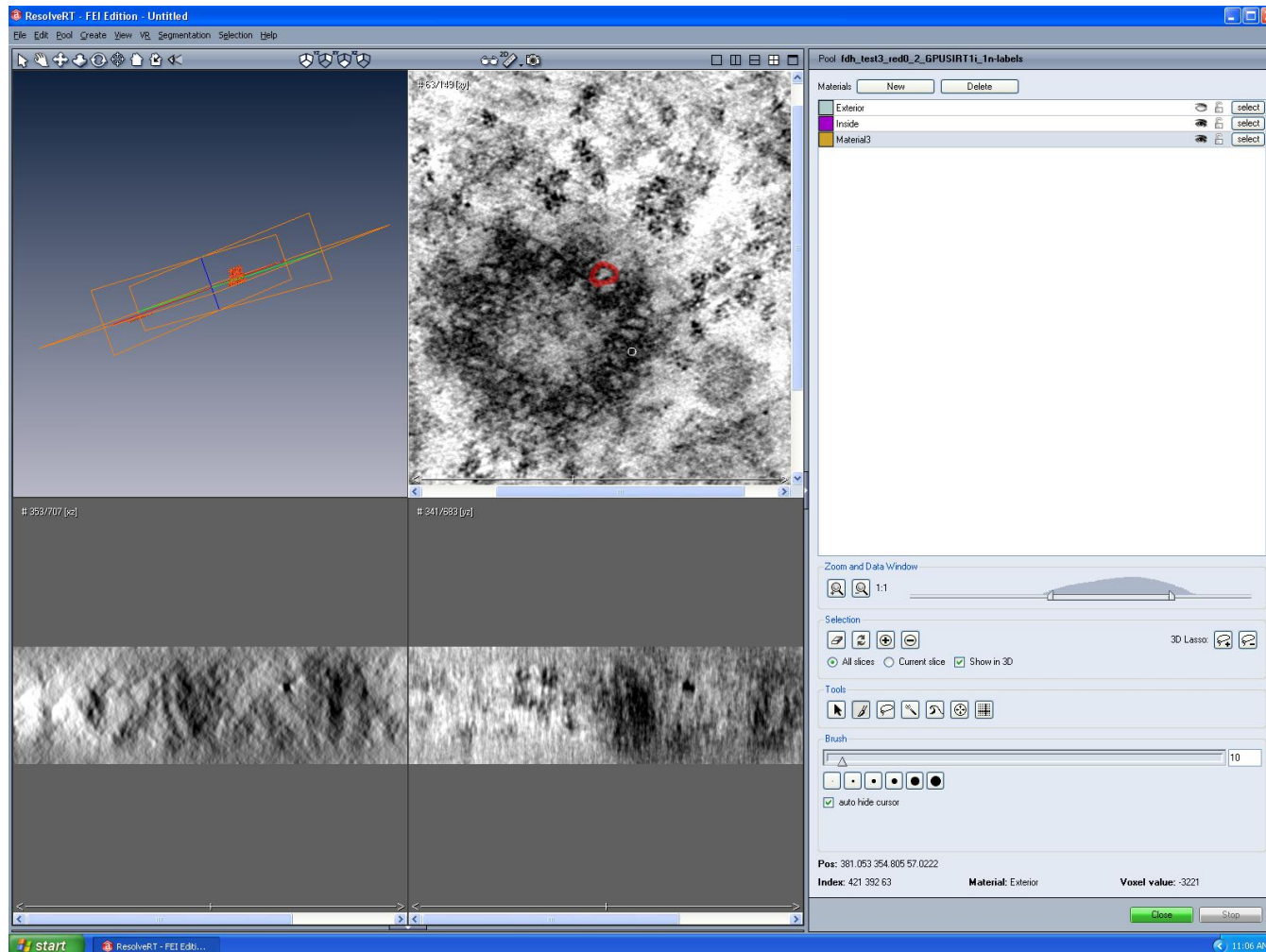
2D-projections => 3D-reconstruction



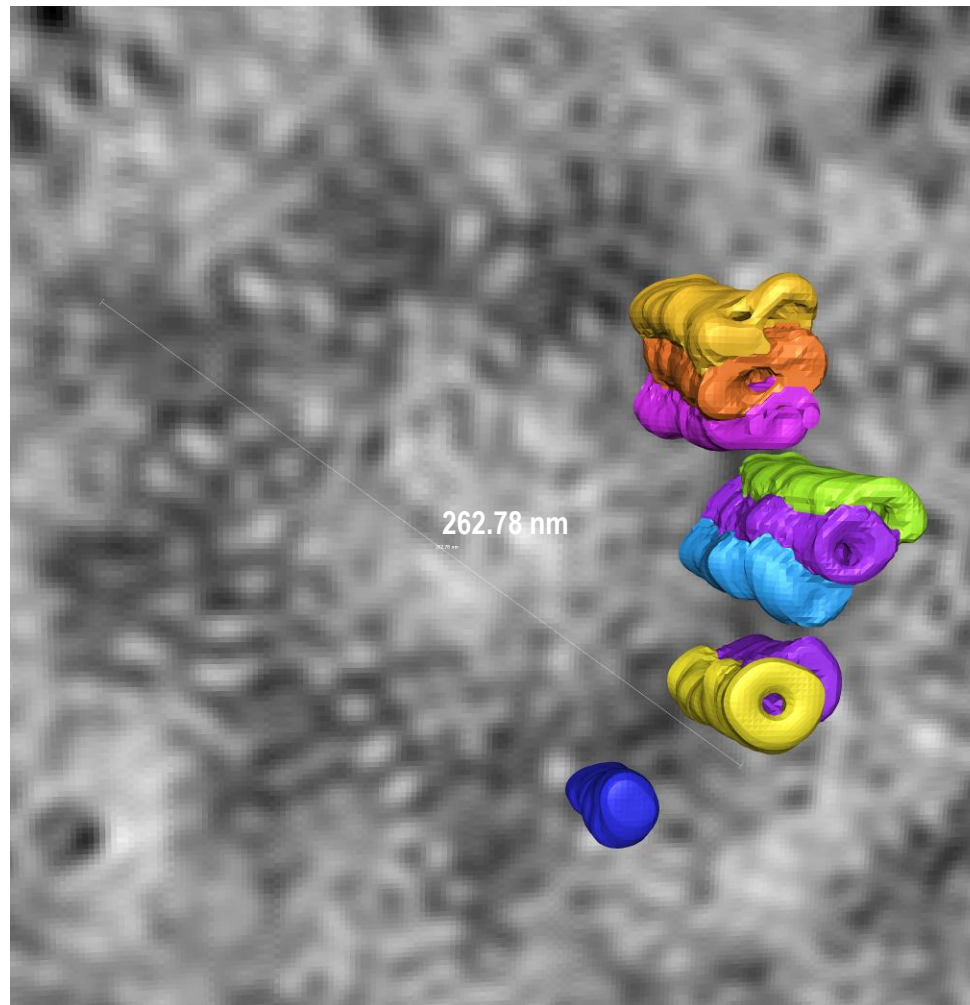
S. Nickell, C. Kofler, A. Leis, W. Baumeister: Nature Reviews Molecular Cell Biology



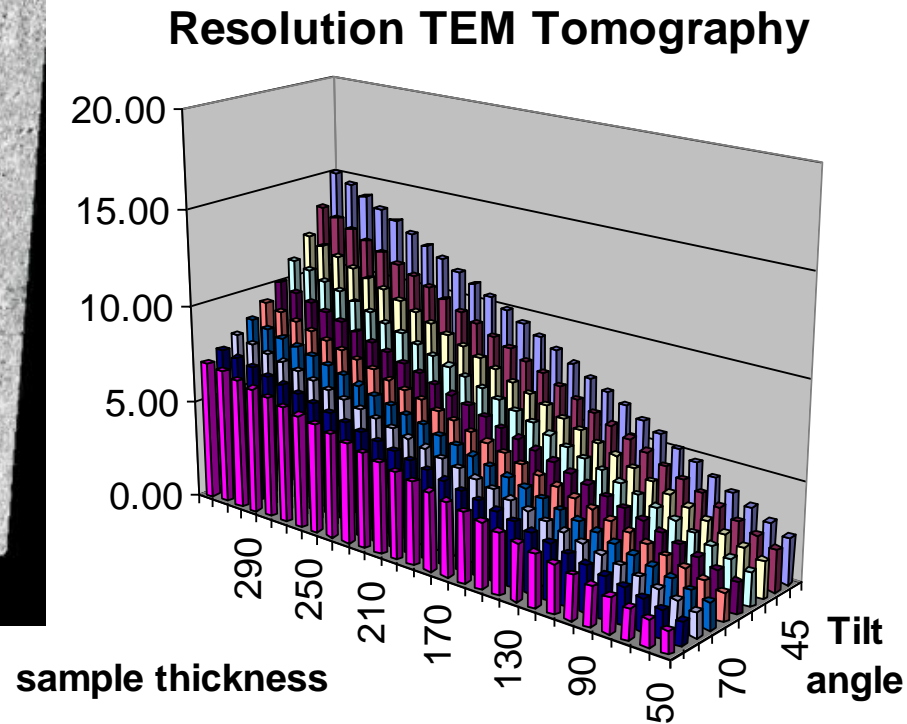
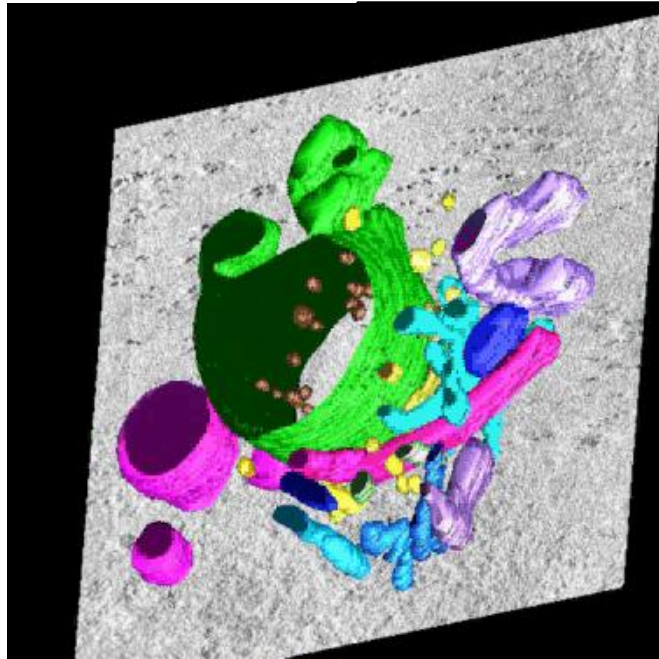
Visualized 3D reconstruction in ResolveRT using Label fielding allows the user to accentuate particular structures



Visualized 3D reconstruction in
ResolveRT using Label fielding allows the
user to accentuate particular structures



Resolution TEM Tomography



$$d = \frac{\pi \cdot D}{N} \sqrt{\frac{(\alpha + \sin \alpha \cos \alpha)}{(\alpha - \sin \alpha \cos \alpha)}}$$

D = sample thickness

α = Max Tilt angle

N = 140 images

d = resolution

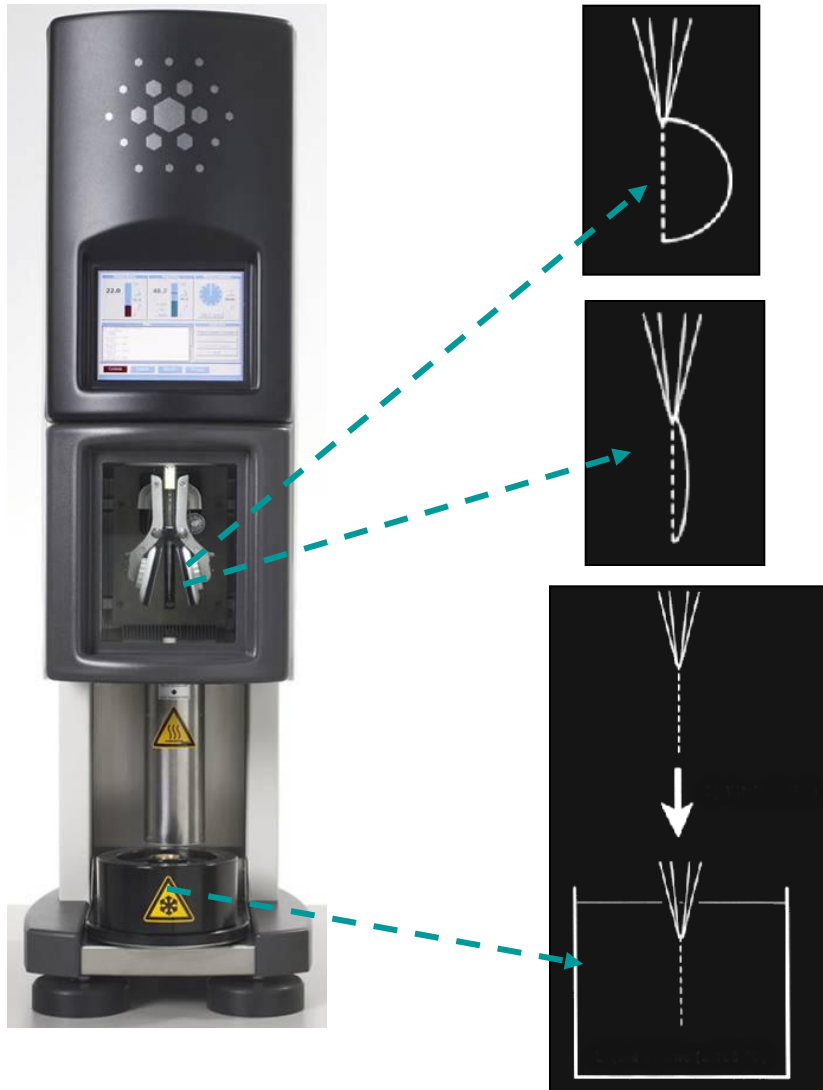


Vitrification of suspensions using the Vitrobot Mark IV



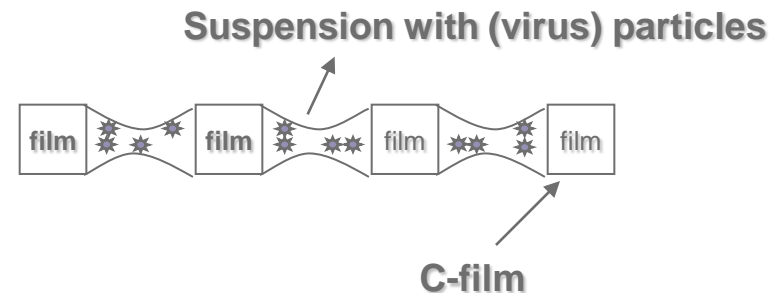
Computer controlled environmental conditions (as defined by the user) allows reproducible vitrification of suspensions.

Vitrification process described in steps.



Incubation of suspension on grid (holey carbon, lacey film or Quantifoils) at constant temperature and humidity

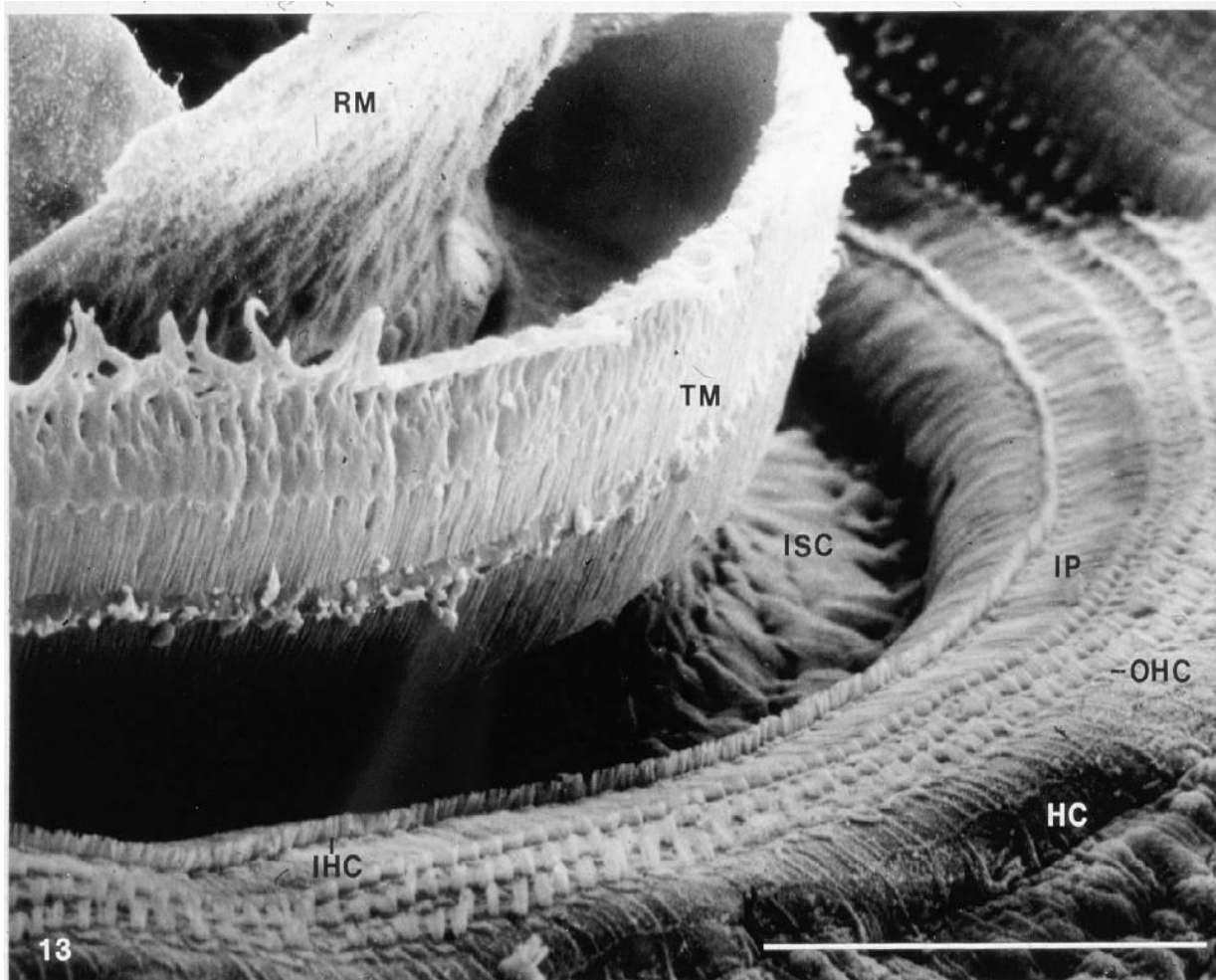
Automated Blotting



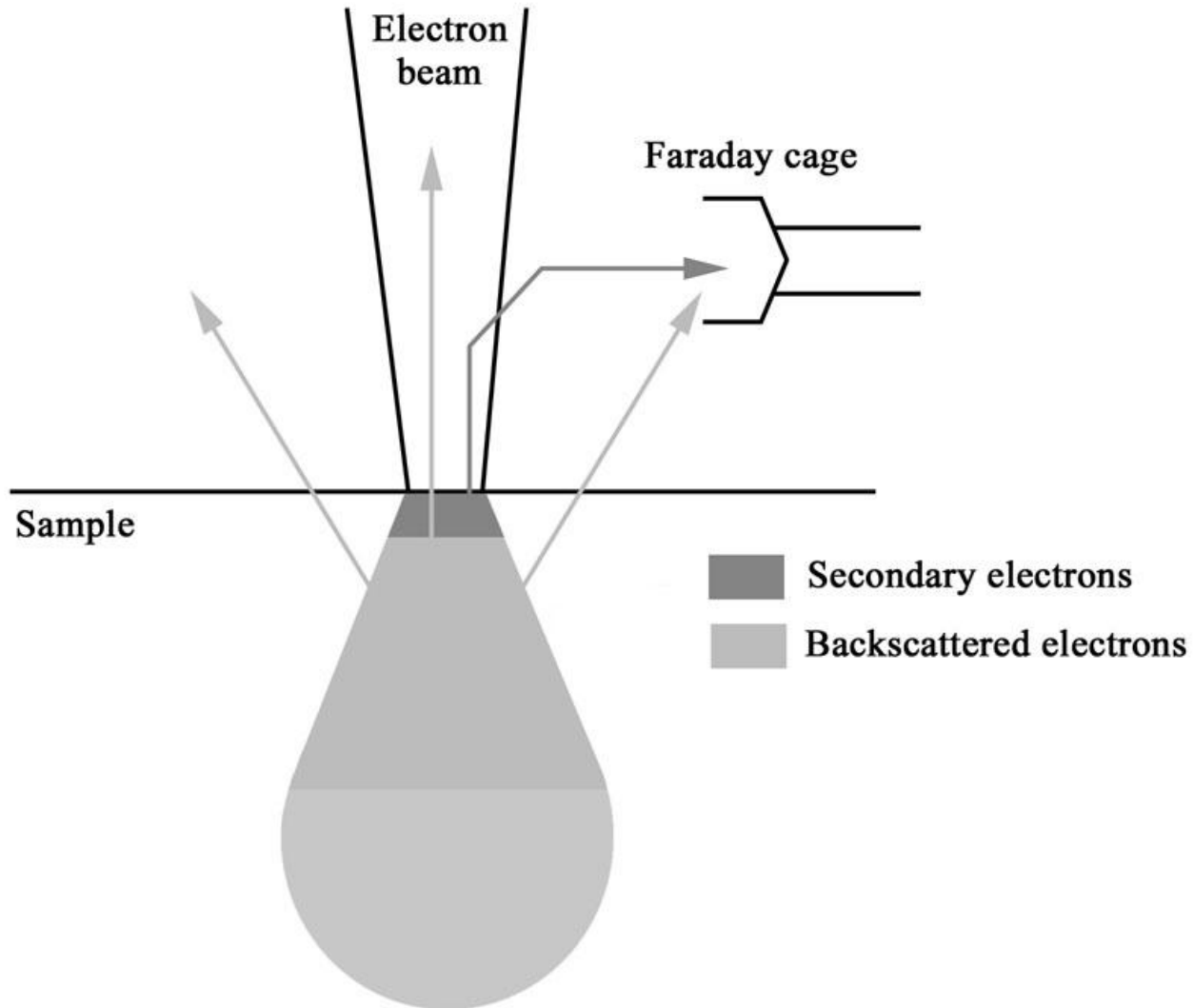
Plunge freezing in liquid propane or ethane

Ready for investigation in amorphous ice

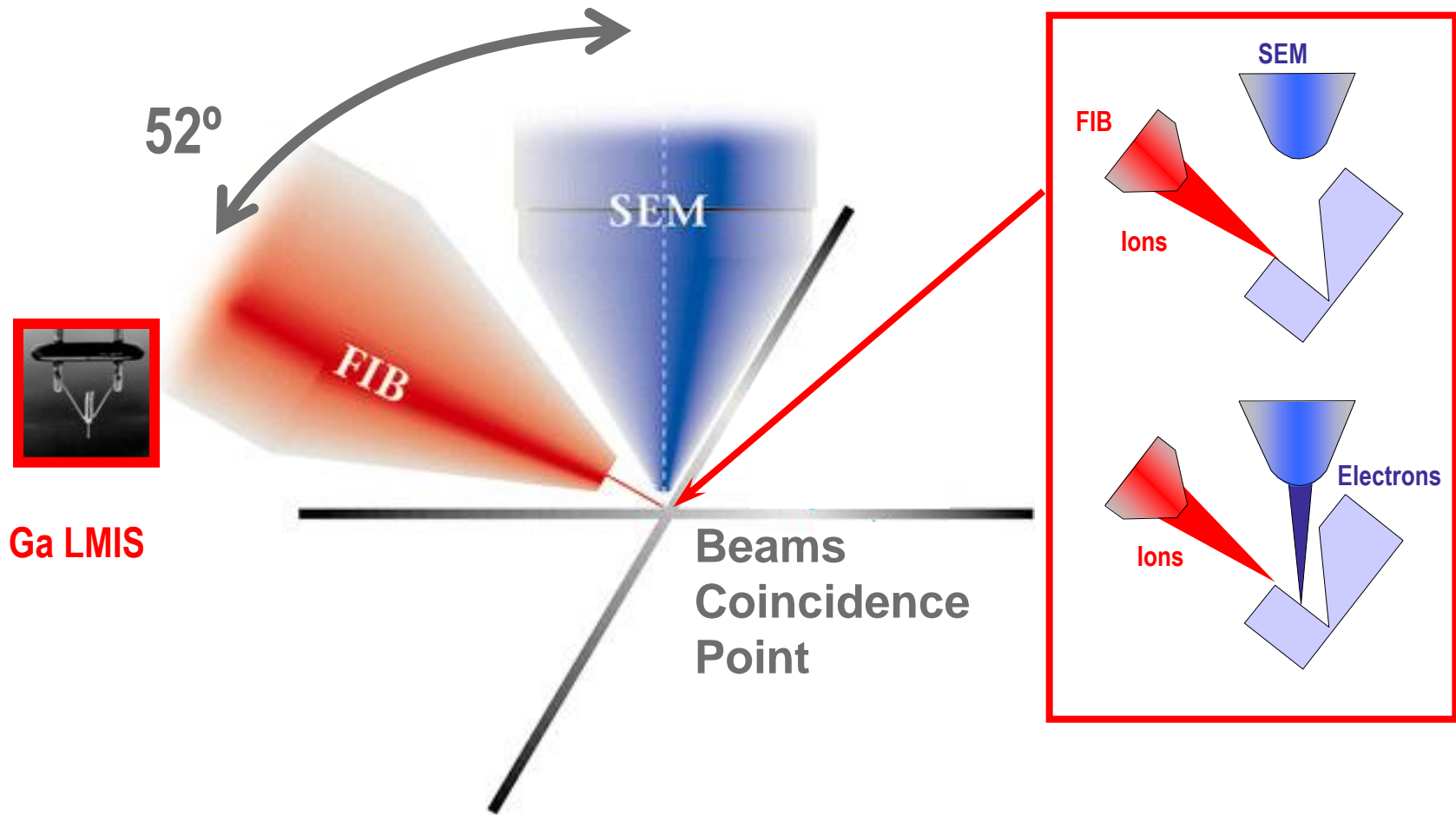
Scanning Electron Microscopy



SEM



Principle of a DualBeam

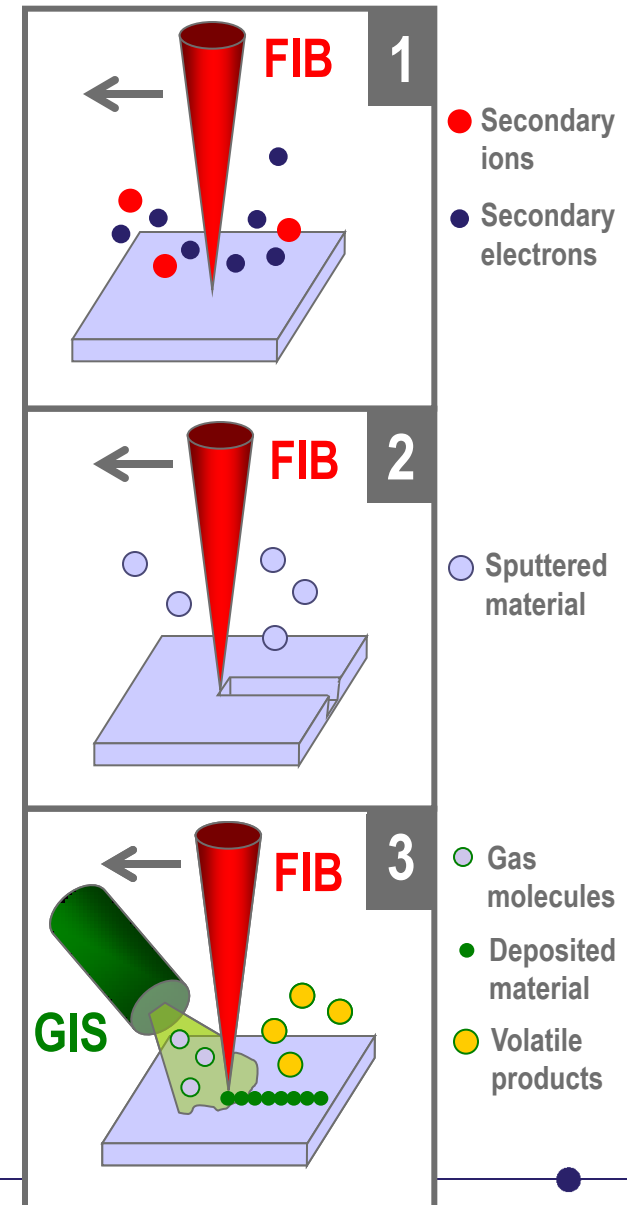


FIB : Three Basic Operating Modes

1. Emission of secondary electrons and ions
 - **FIB Imaging**
2. Sputtering of substrate atoms
 - **FIB milling**
3. Chemical interactions
 - **FIB deposition / enhanced etch**

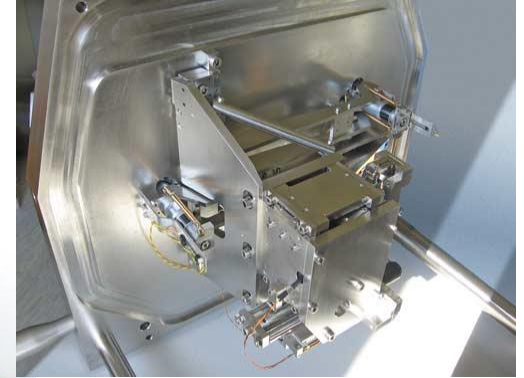
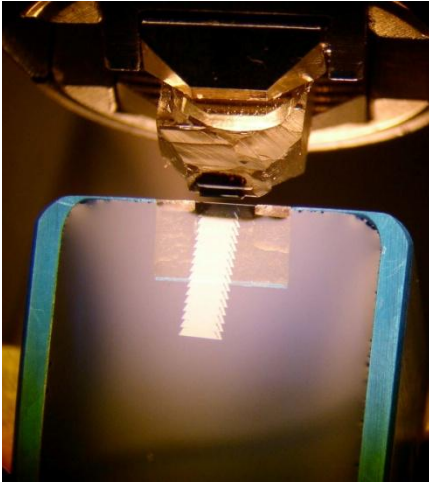
Other effects :

- Ion implantation
- Displacements of atoms in the solid (induced damages)
- Heating

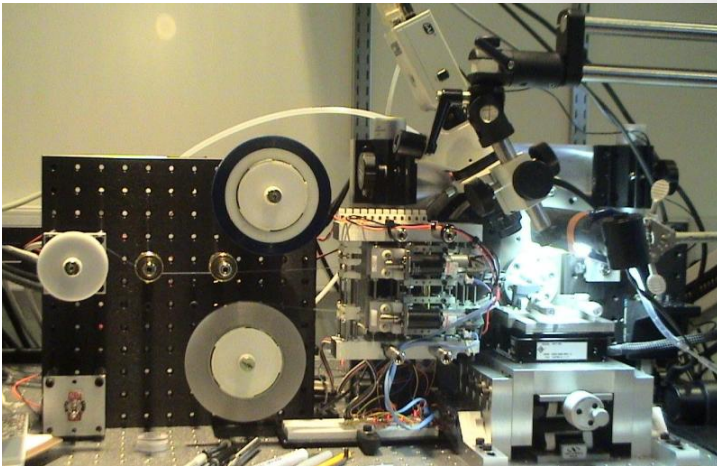


Tomography

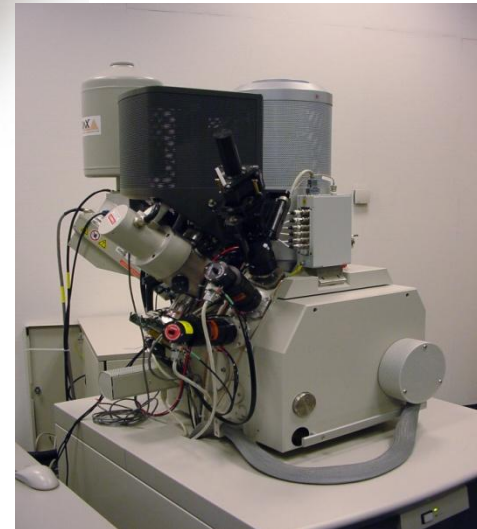
Principle



Source : Gatan 3View information



Source : Atlum website (Harvard University, J Lichtmann)



3D- Reconstruction in the past

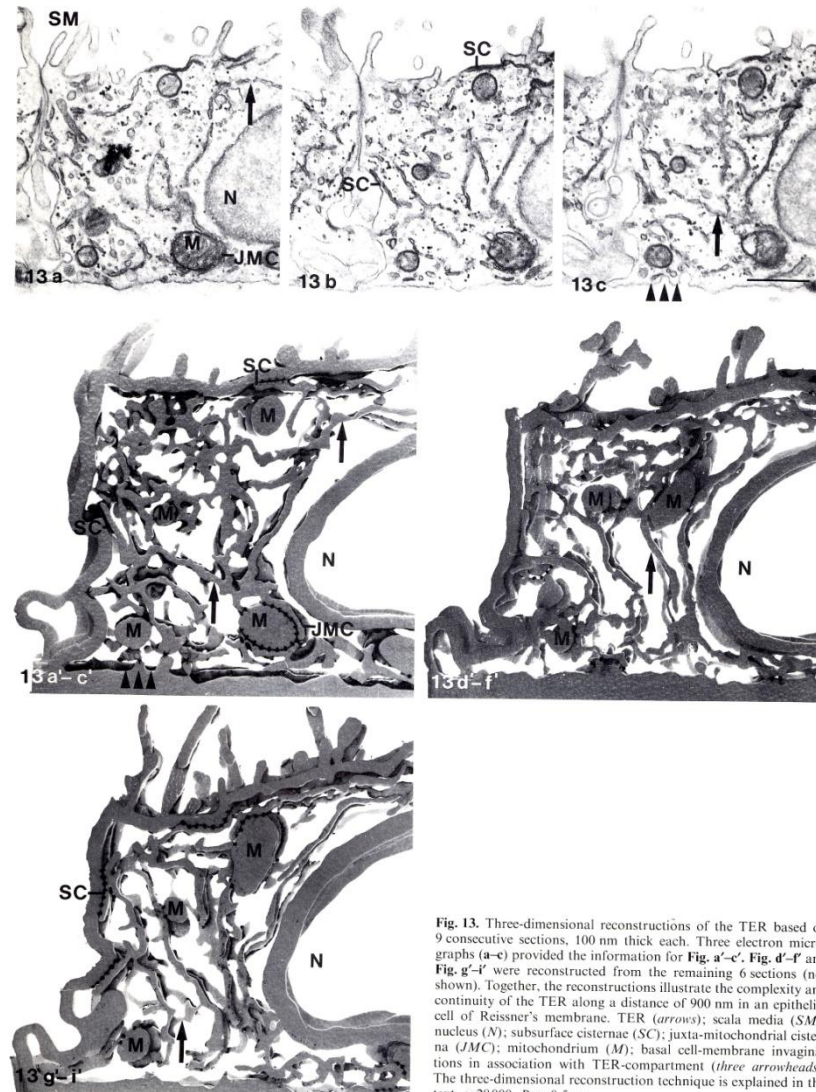
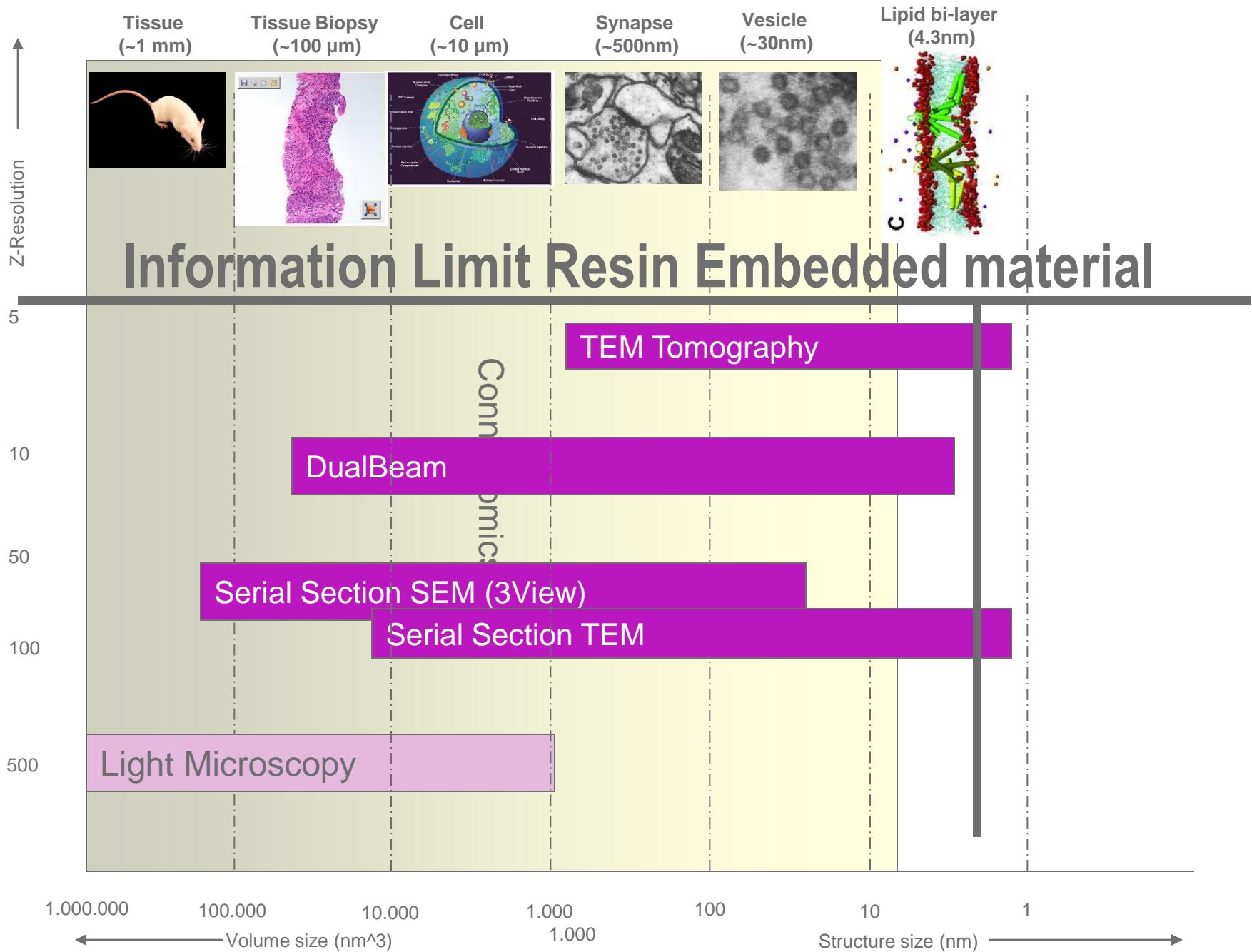
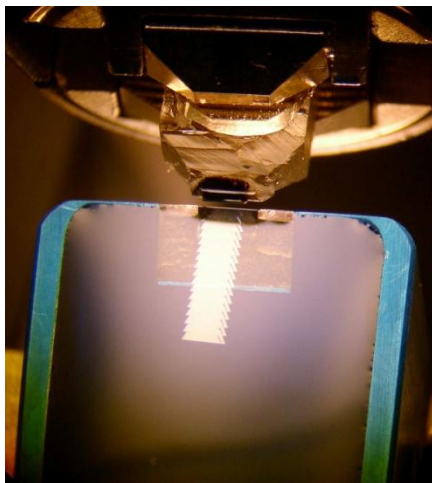


Fig. 13. Three-dimensional reconstructions of the TER based on 9 consecutive sections, 100 nm thick each. Three electron micrographs (a-c) provided the information for Fig. a'-c'. Fig. d'-f' and Fig. g'-i' were reconstructed from the remaining 6 sections (not shown). Together, the reconstructions illustrate the complexity and continuity of the TER along a distance of 900 nm in an epithelial cell of Reissner's membrane. TER (arrows); scala media (SM); nucleus (N); subsurface cisternae (SC); juxta-mitochondrial cisterna (JMC); mitochondrion (M); basal cell-membrane invaginations in association with TER-compartment (three arrowheads). The three-dimensional reconstruction technique is explained in the text. $\times 28000$. Bar: 0.5 μ m

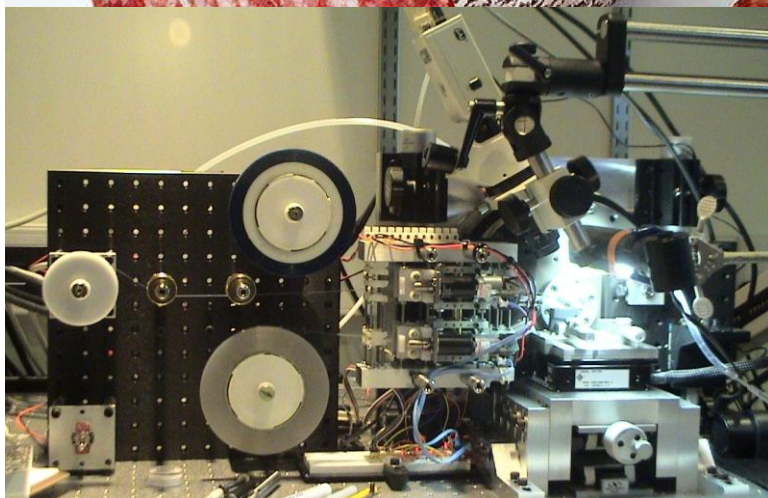
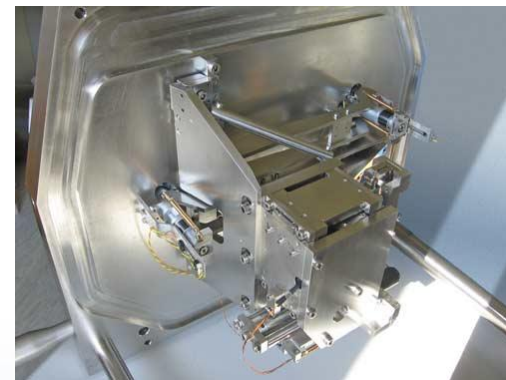


Resolution *Poly*Tomography (X, Y, Z)



(2, 2, 80) nm

(40, 40, 70) nm



(4, 4, 10) nm

(4, 4, 80) nm

