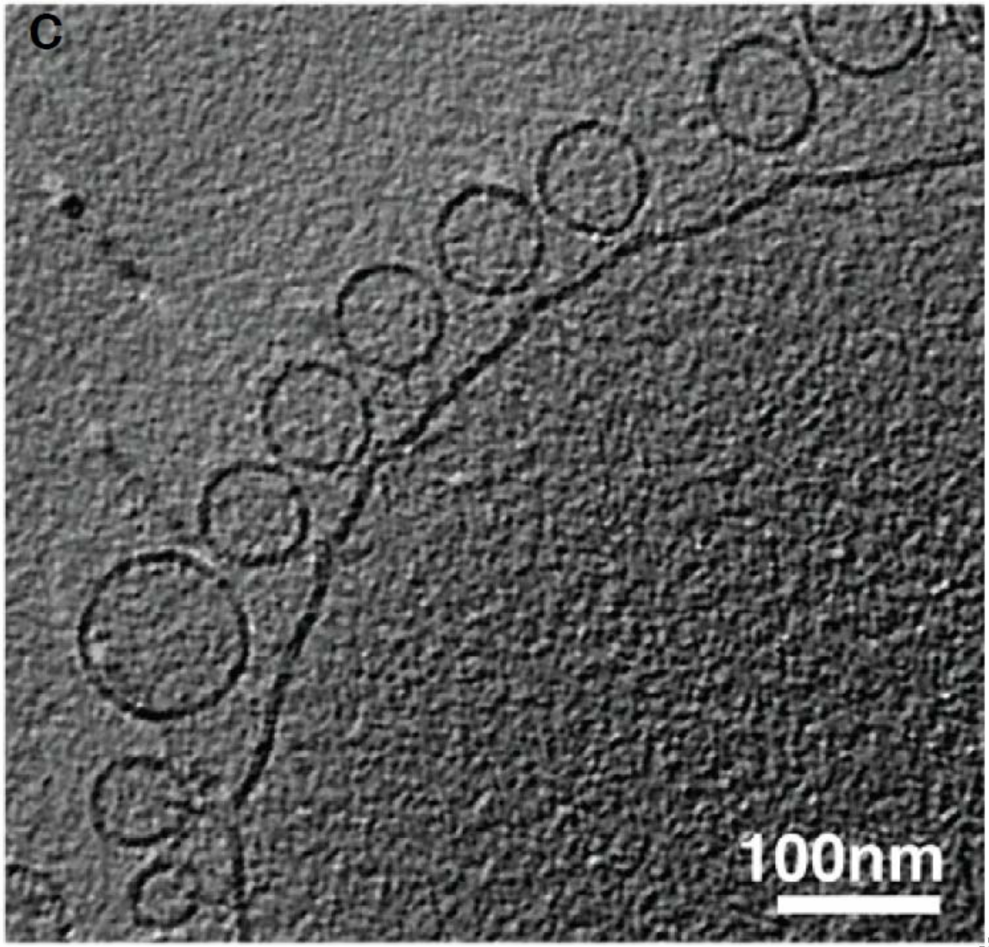
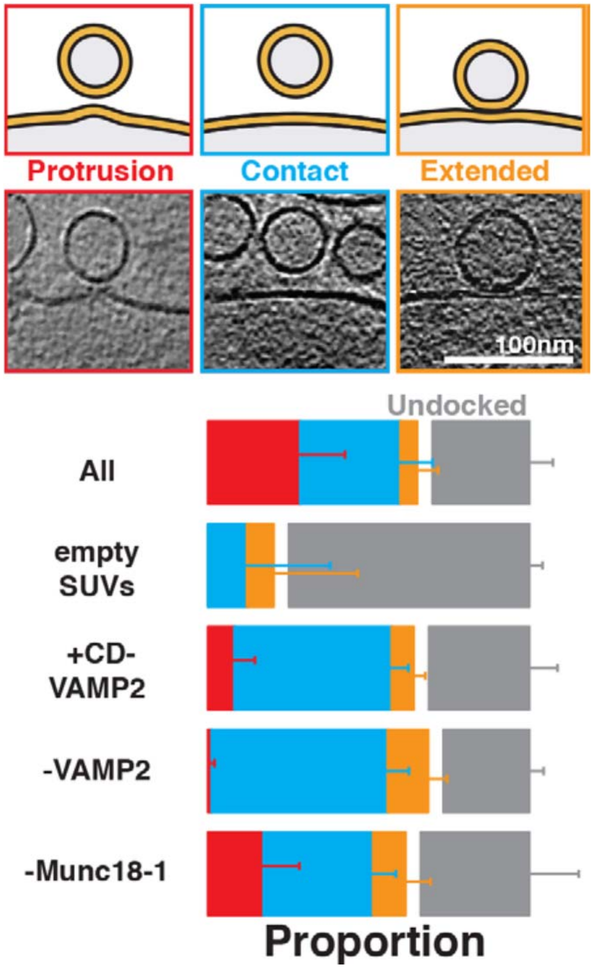
# Lecture 8: Tomography (part 2)

1. Interpretation of EM tomograms
2. Denoising algorithms
3. Segmentation approaches
4. Identification of features of interest
5. Subtomogram averaging techniques
6. Methods of EELS and EF-TEM

# Examples of EM Tomograms

## SNARE-mediated membrane fusion

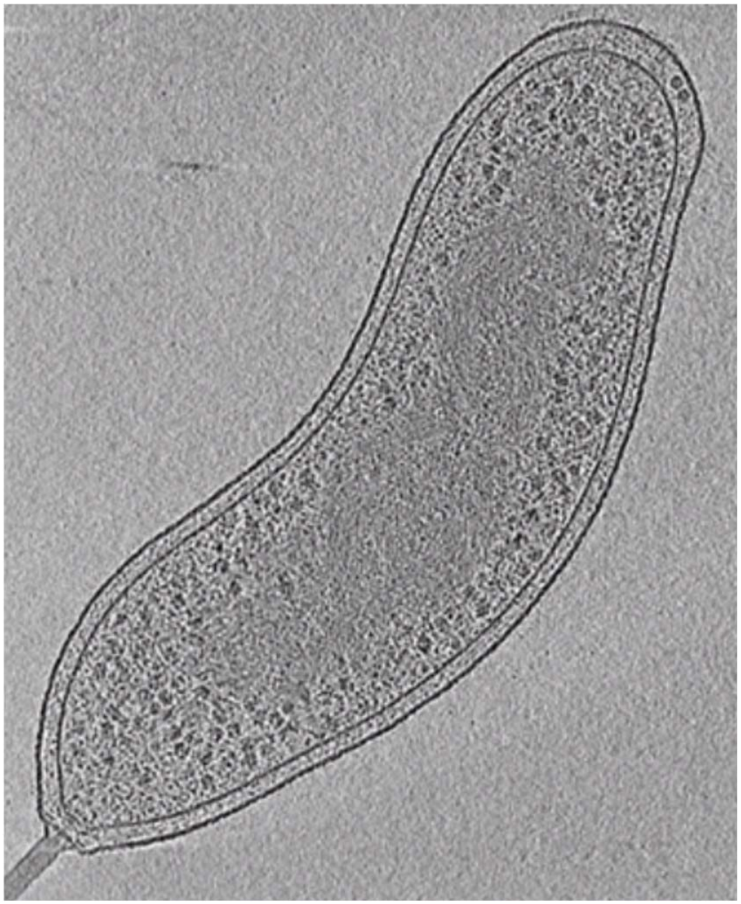


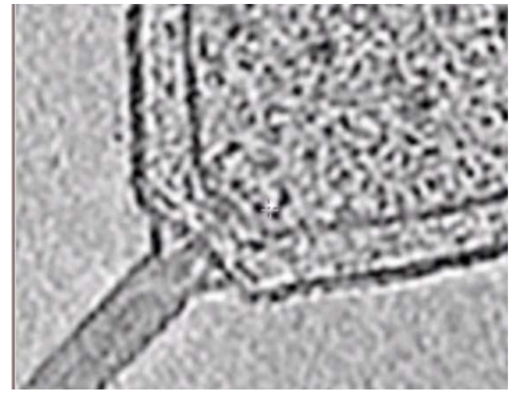



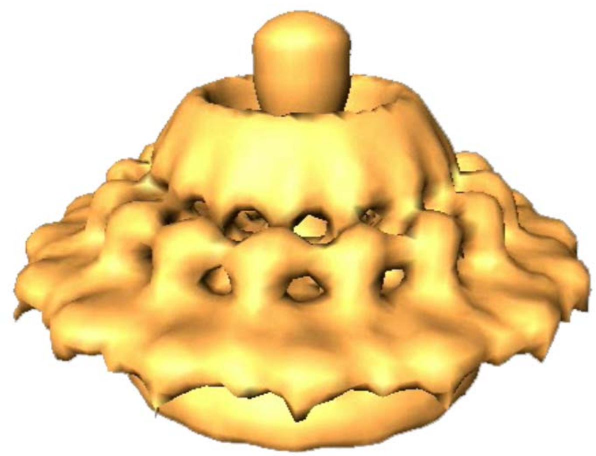

Bharat, TAM et al. (2009) *EMBO Rep*., 15, 2014

# Examples of EM Tomograms

## Bacterial flagellar motor



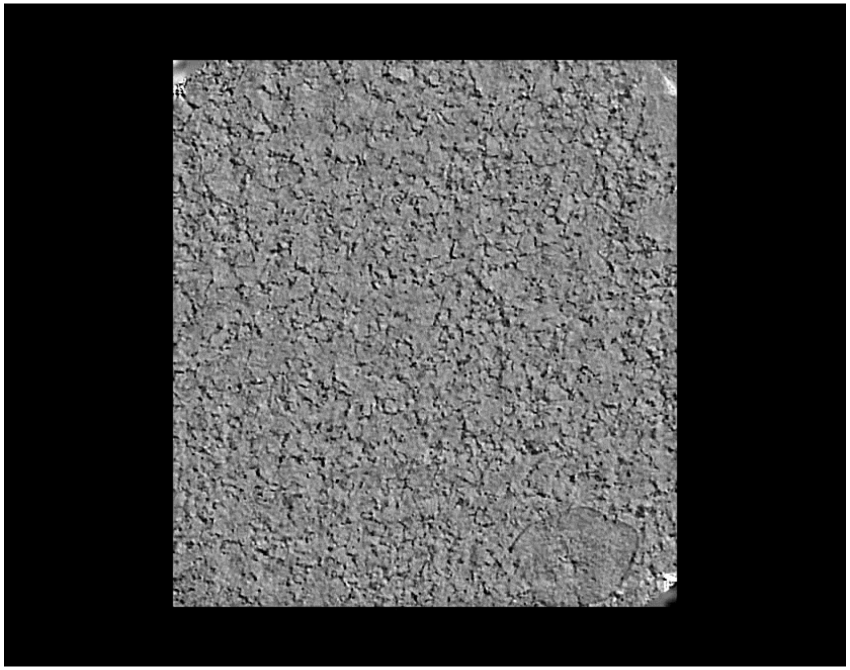


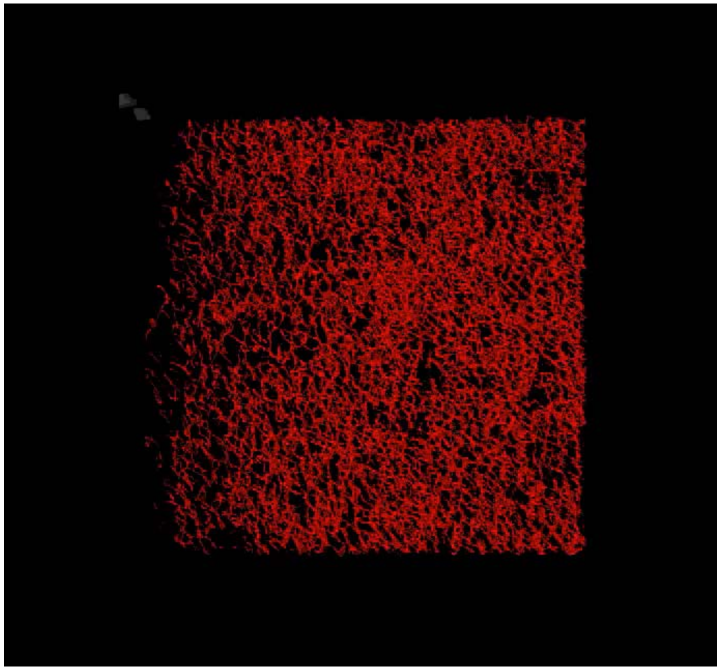


Murphy, GE et al. (2006) *Nature*, 442, 1062

# Examples of EM Tomograms

**Red Cell Cytoskeleton**





**Nans, A et al. (2011) *Biophys. J.*, 101, 2341**

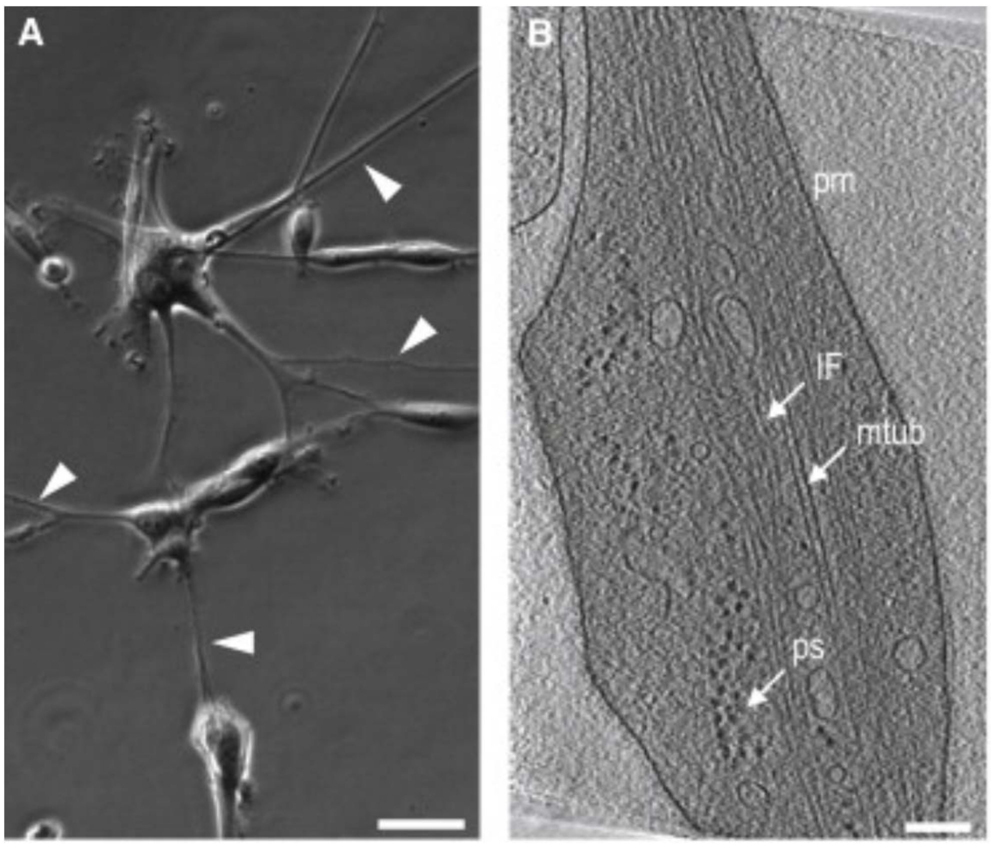
# Examples of EM Tomograms

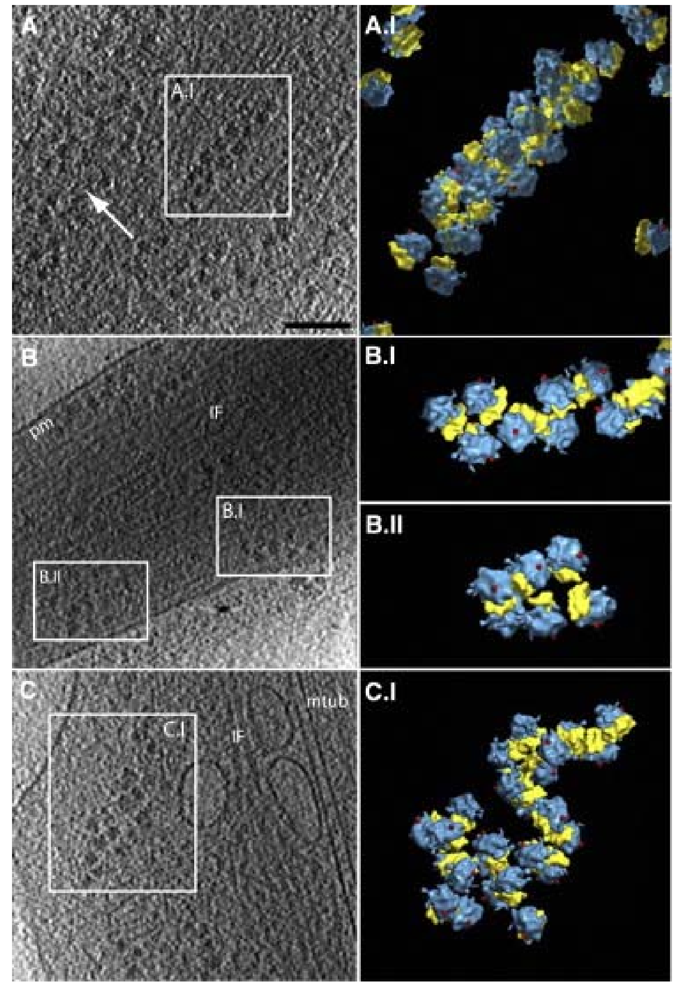
**Golgi apparatus**

**March, BJ (2005) *Biochim. Biophys. Acta*, 1744, 273**

# Examples of EM Tomograms

**Polyribosomes in human glia cells**

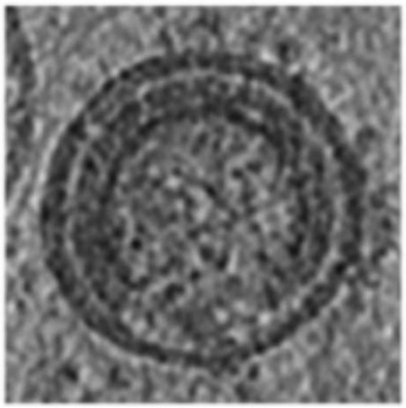


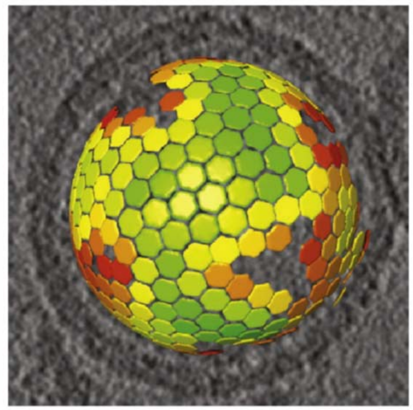



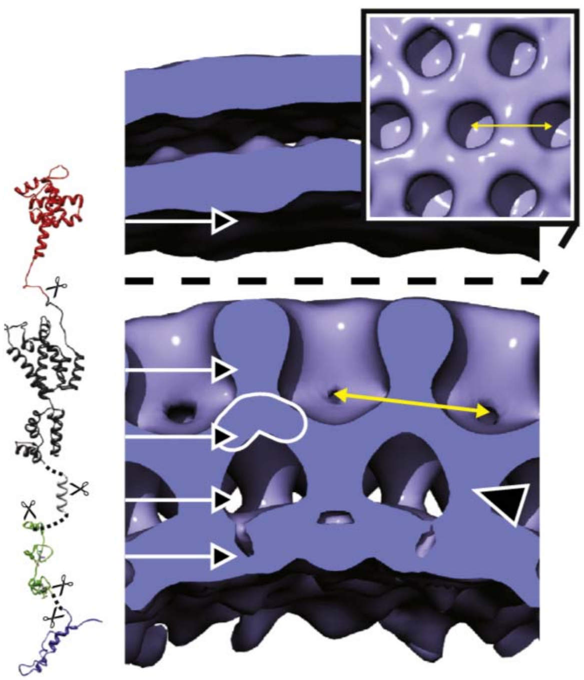

**Brandt, F et al. (2010) *Mol. Cell*, 39, 560**

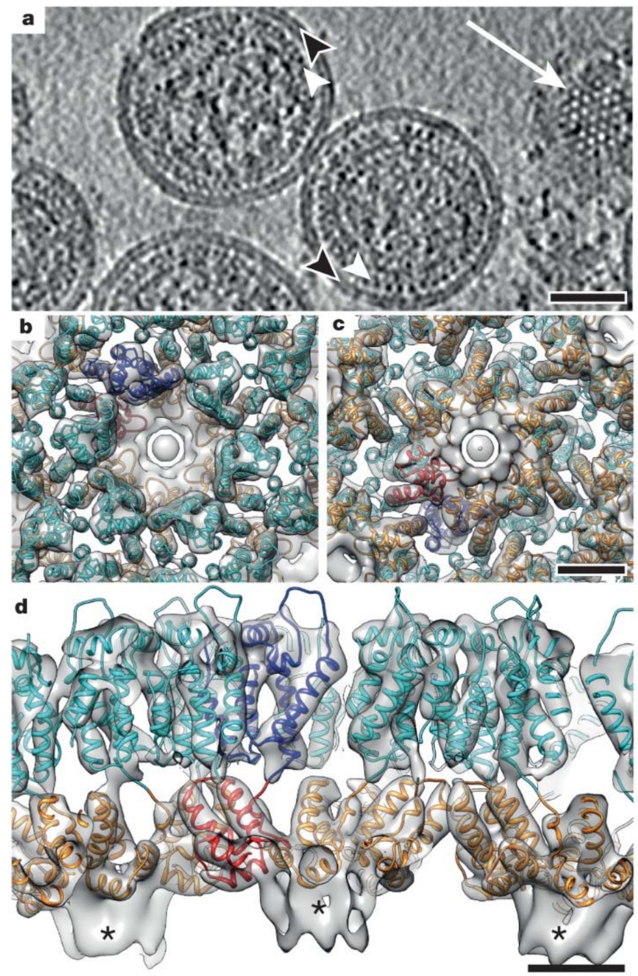
# Examples of EM Tomograms

**Gag lattice of the immature HIV virion**









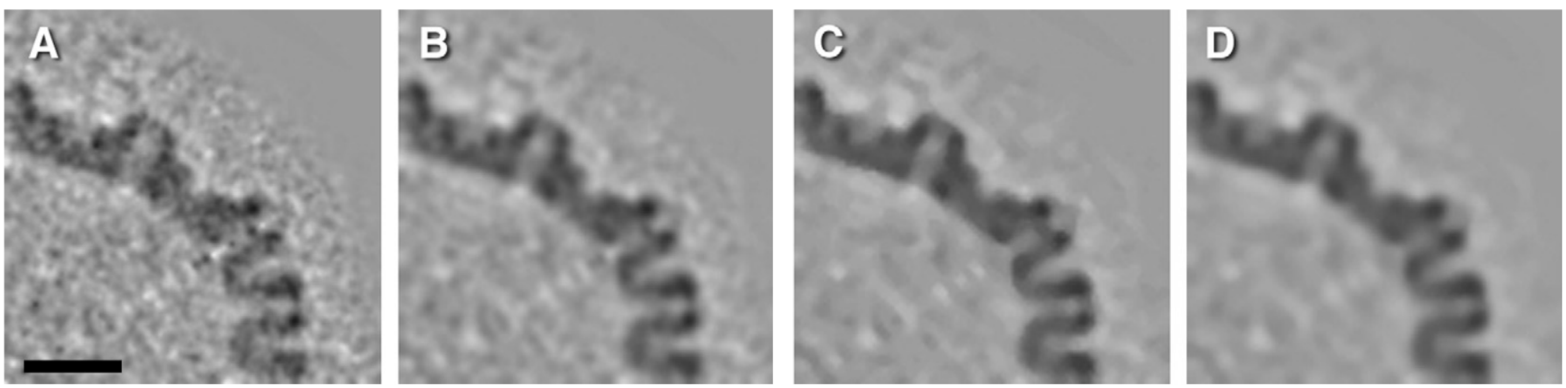

**Briggs, JA et al. (2011) *J.Mol.Biol.*, 410, 491**

**Schur, FK et al. (2015) *Nature*, 517, 505**

# Denoising algorithms

**Linear filters:**
averaging neighborhood voxels
Gaussian filter or other function

**Median filters:**
local filters that estimate the voxel value based on the neighbors
wavelet filtering
non-linear anisotropic diffusion
bilateral filtering



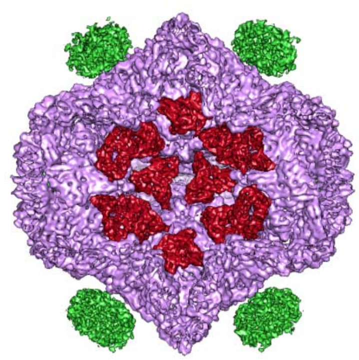

no filter bilateral median NAD

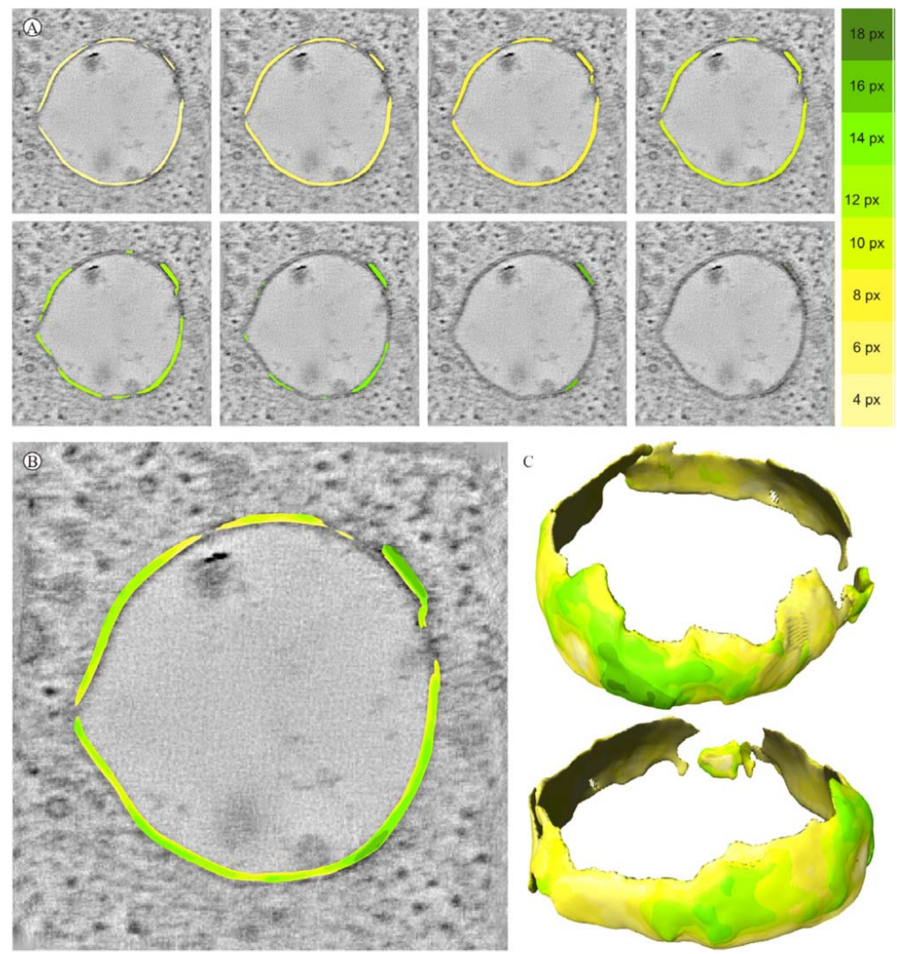
# Segmentation Techniques

- thresholding and masking
- manual segmentation
- watershed segmentation
- segmentation with eigenvectors
- segmentation using prior knowledge

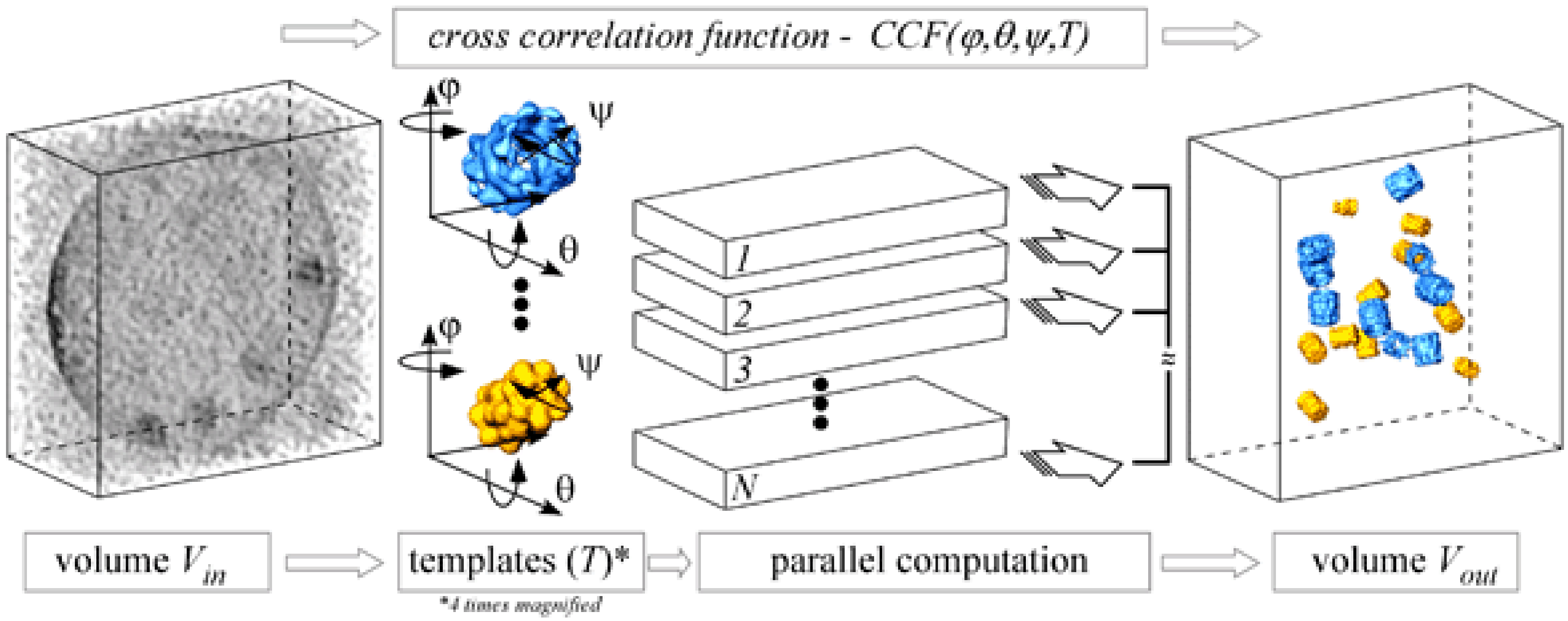
    - tubular structures

    - membranes





# 3D Template Matching

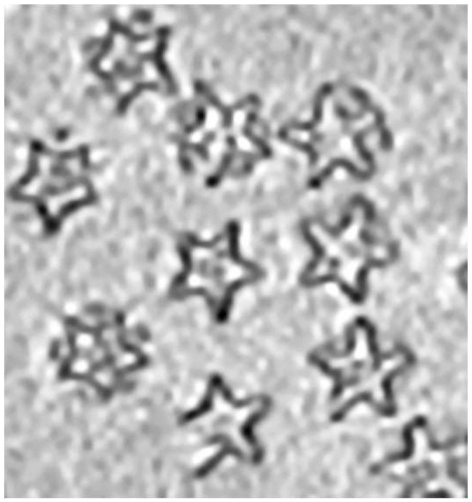


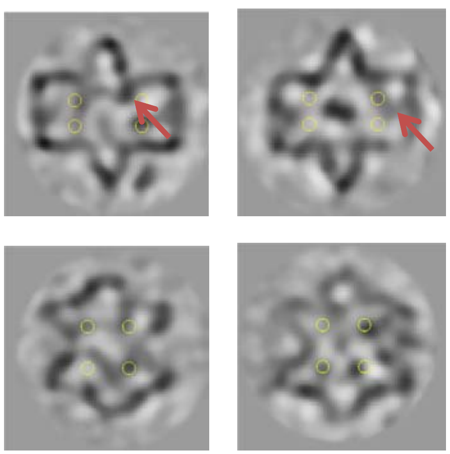

**Further considerations:**

Missing wedge
Local variance at each angle
Peak detection
Validation

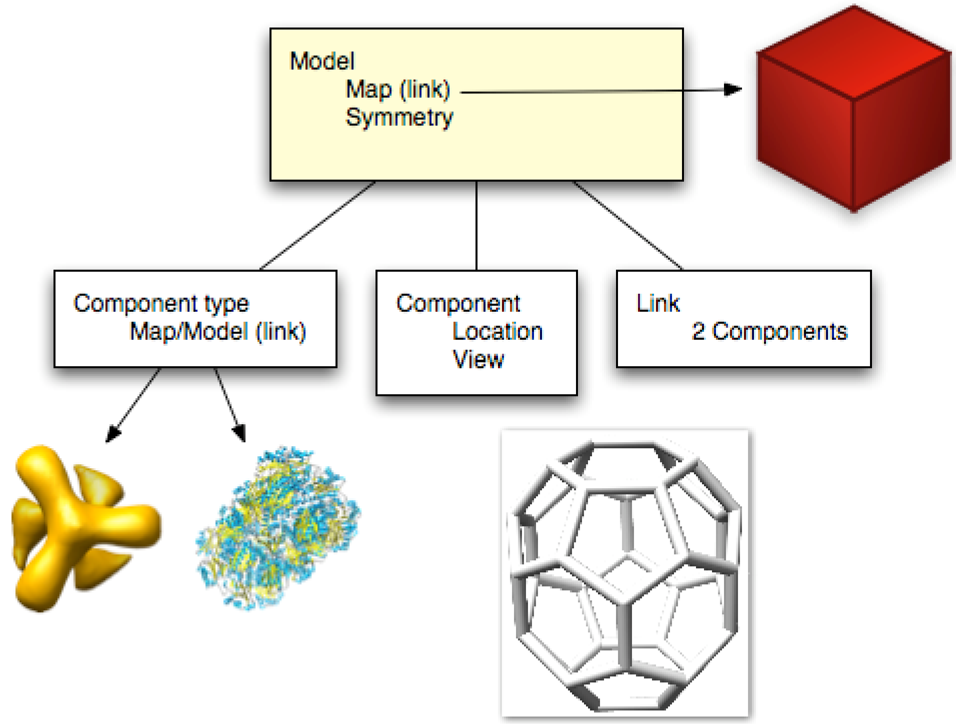
# Subtomogram Averaging

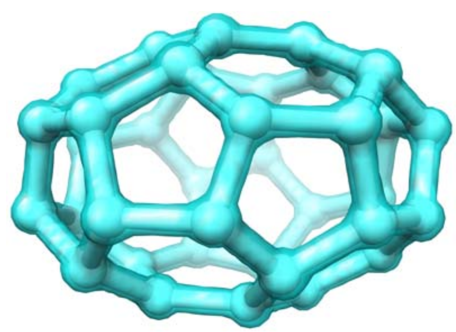
**Subtomograms**

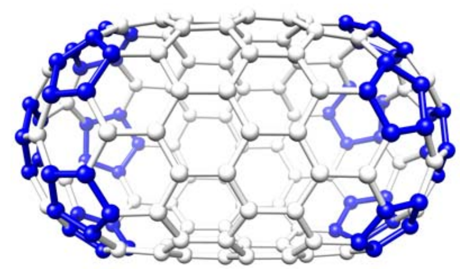




**Modelling of subtomograms**

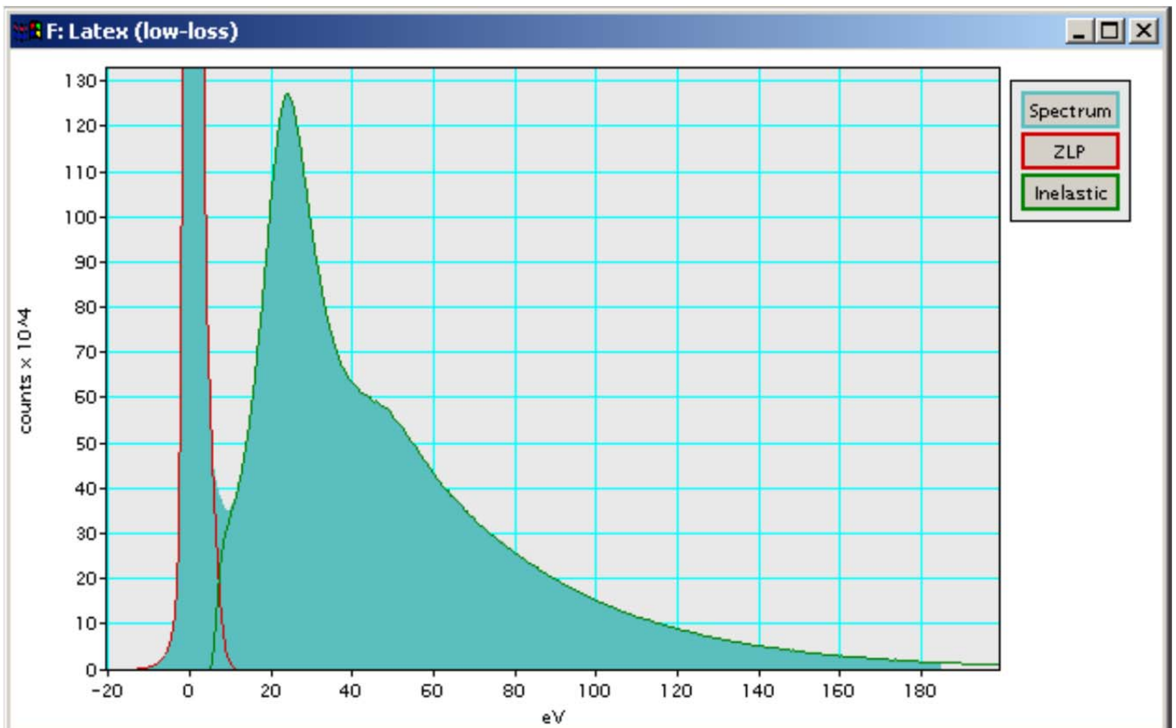


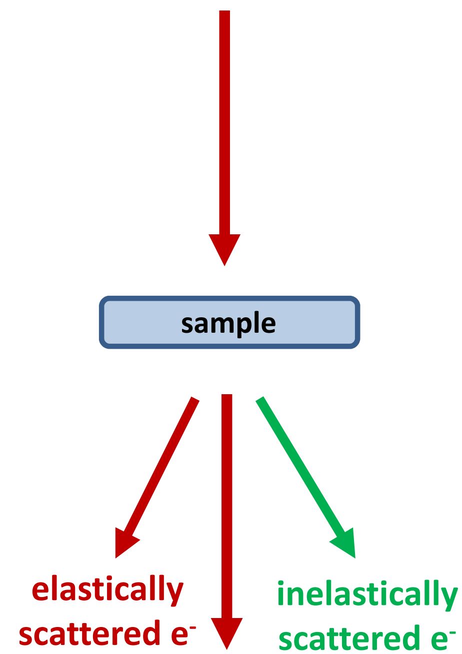





# EELS & EF-TEM Techniques

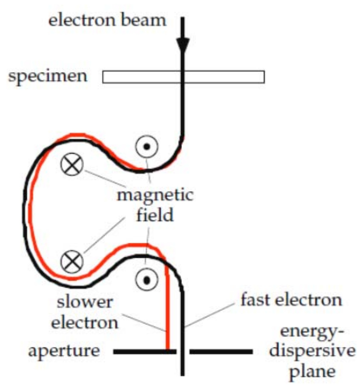
1) Expose specimen to mono-energetic electron radiation
2) *Inelastic* scattering in the specimen => poly-energetic electron beam
3) Image-forming electrons are selected by scattering angle
4) Diffraction contrast (interference of scattered and unscattered electrons)

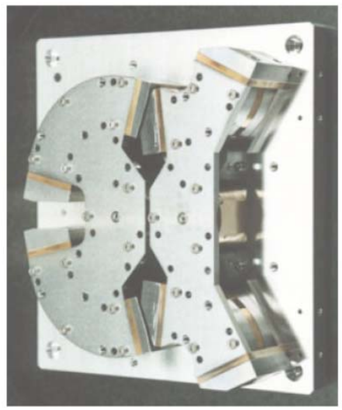


# Energy Filters

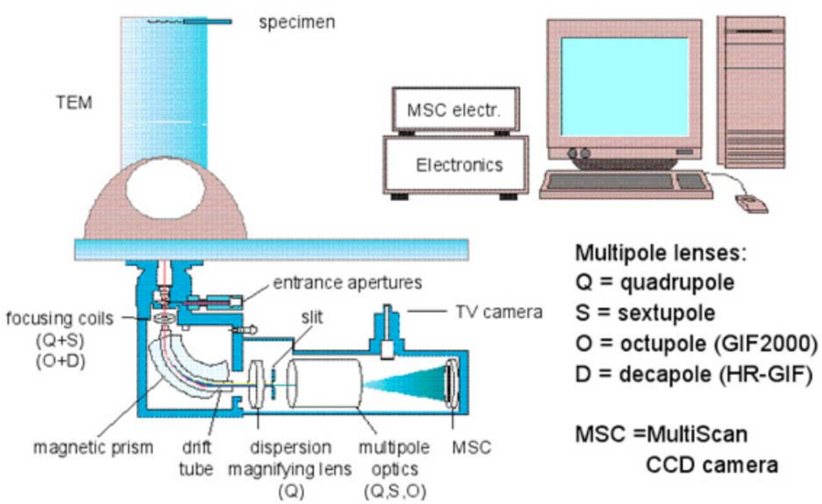
### In-column energy filter (JEOL)



**Advantages:**
- Less aberrations
- Larger scattering angles
- Larger fields of view

### Post-column energy filter (Gatan)

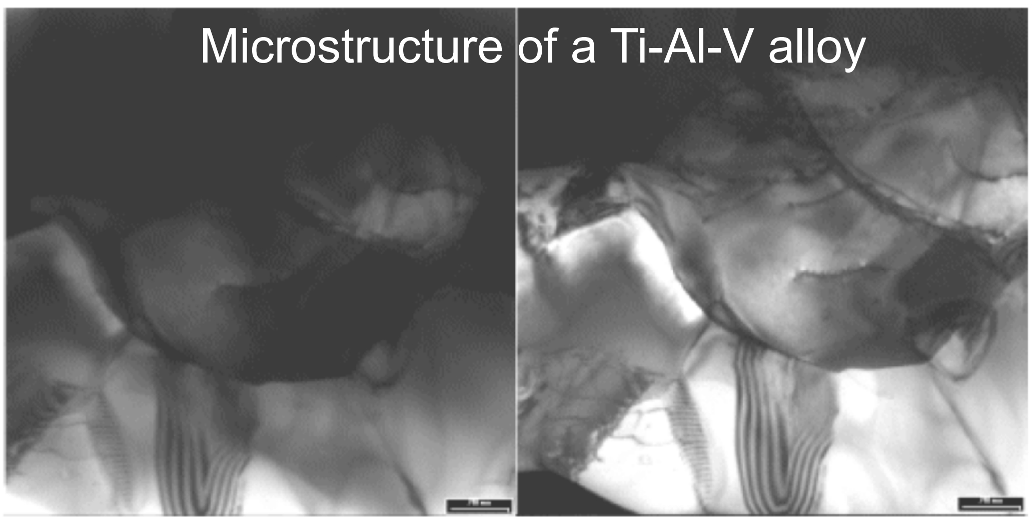



**Advantages:**
- Can fit any microscope
- Recording of filtered and unfiltered data is possible

# Zero-Loss Imaging (EF-TEM)

Slit aperture is centered on the zero-loss peak of the EELS spectrum
–> typical slit width = 10-20 eV
–> only electrons that suffered no energy loss in the specimen can pass
–> only elastically scattered electrons arrive at the electron detector

Imaging of thick specimen:




When inelastically scattered electrons also reach the image plane:
- image is affected by chromatic aberration => unfocused image
- image is blurred and background is diffused => low contrast

**=> Energy filtering allows imaging of thick specimen (both materials and biological samples)**

# Electron Energy Loss Spectroscopy (EELS)

Imaging the energy-dispersive plane of the energy filter onto the image plane

1) Identification of elements (EELS elements tables)

2) EELS Quantification (thickness measurements)

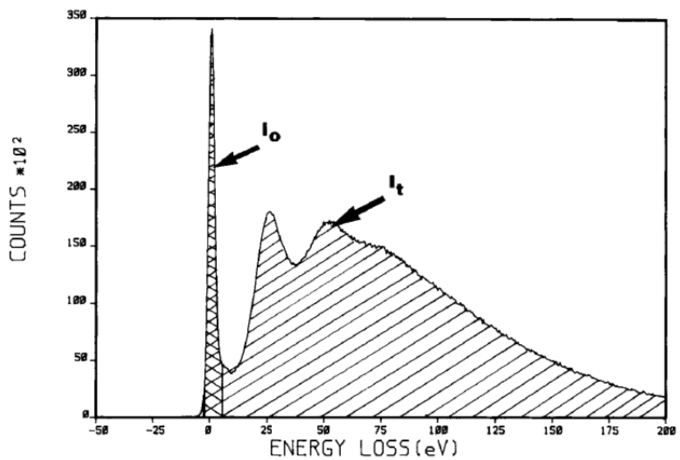
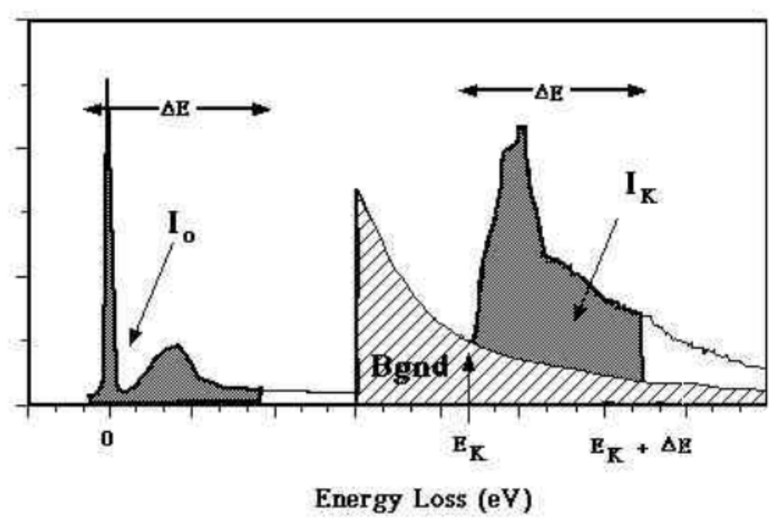



Fig. 1. Typical energy-loss spectrum from a 400-nm carbon film showing $I_t$ and $I_0$ in equation (1).



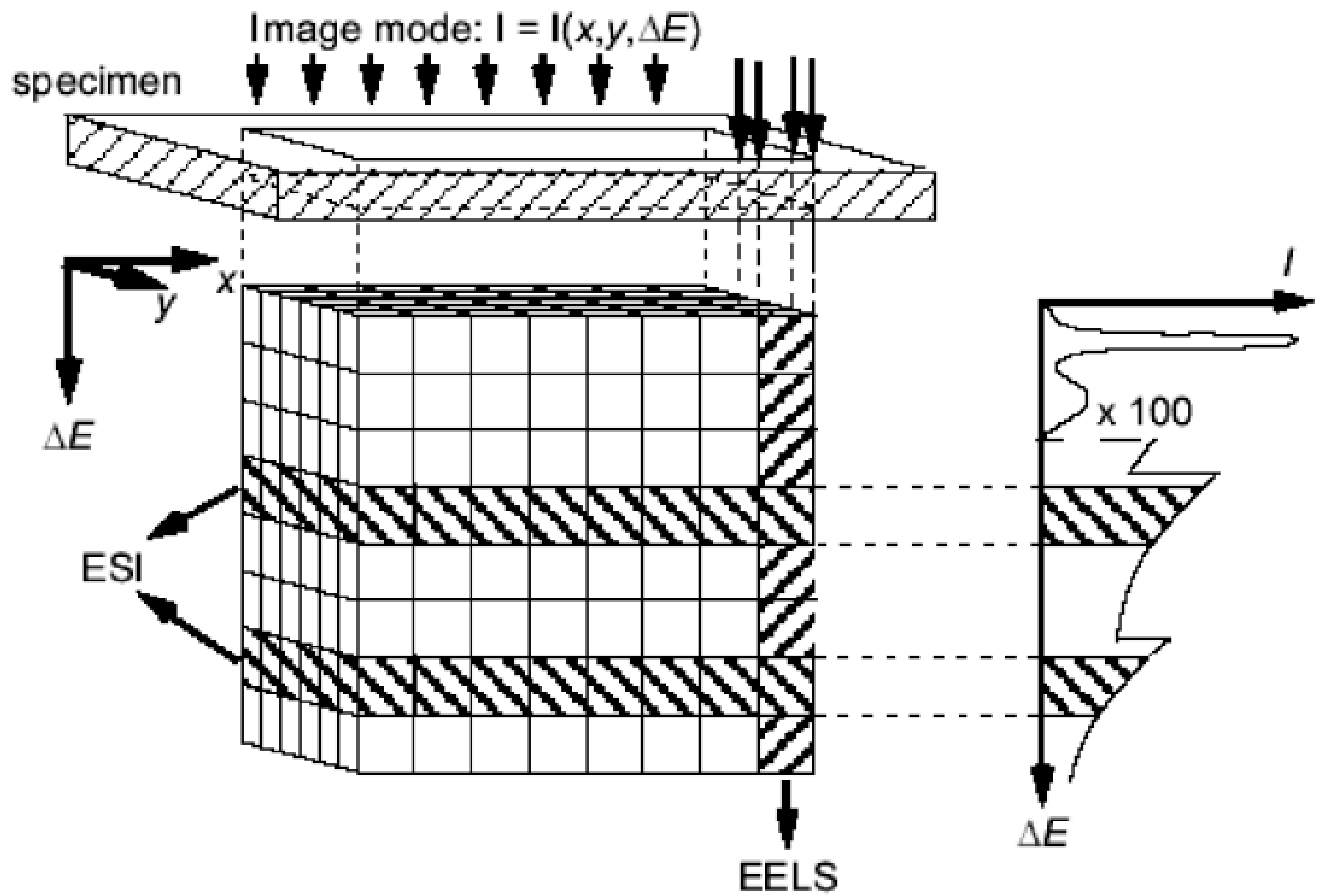

**EELS LOG-RATIO TECHNIQUE**

$I_0$ … electrons in the zero-loss peak
$I_t$ … electrons in the EEL spectrum
$\lambda$ … mean free path for inelastic scattering
t … specimen thickness

$$\frac{t}{\lambda} = \ln\left(\frac{I_t}{I_0}\right)$$

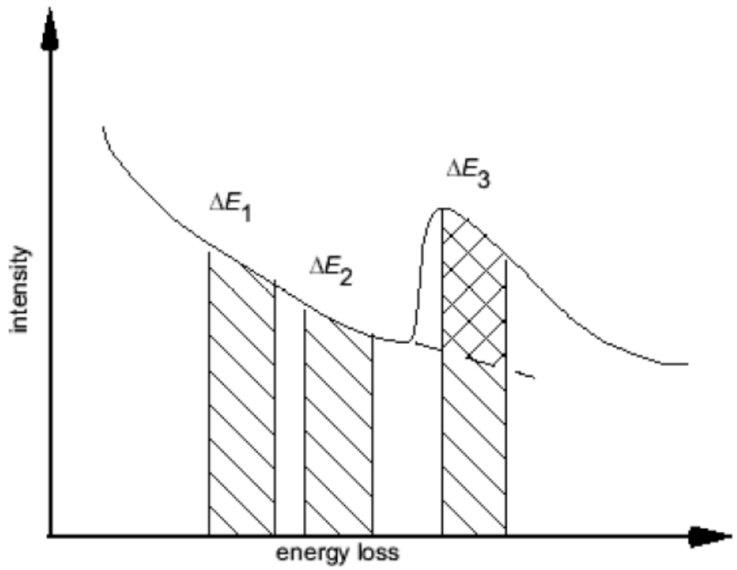
# Electron Spectroscopic Imaging (ESI)

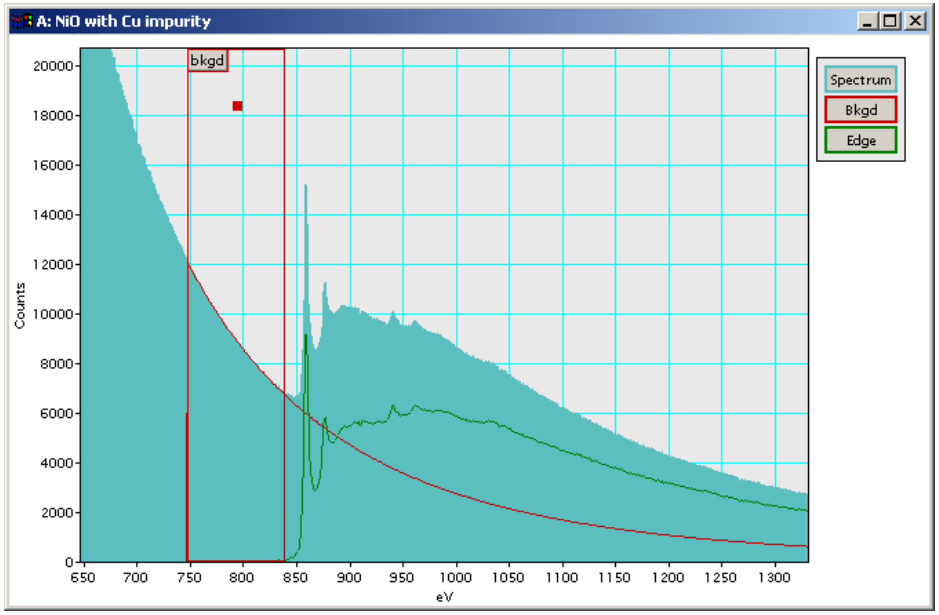
chemical analysis by imaging with element-specific energy-loss windows



# Elemental Mapping Images

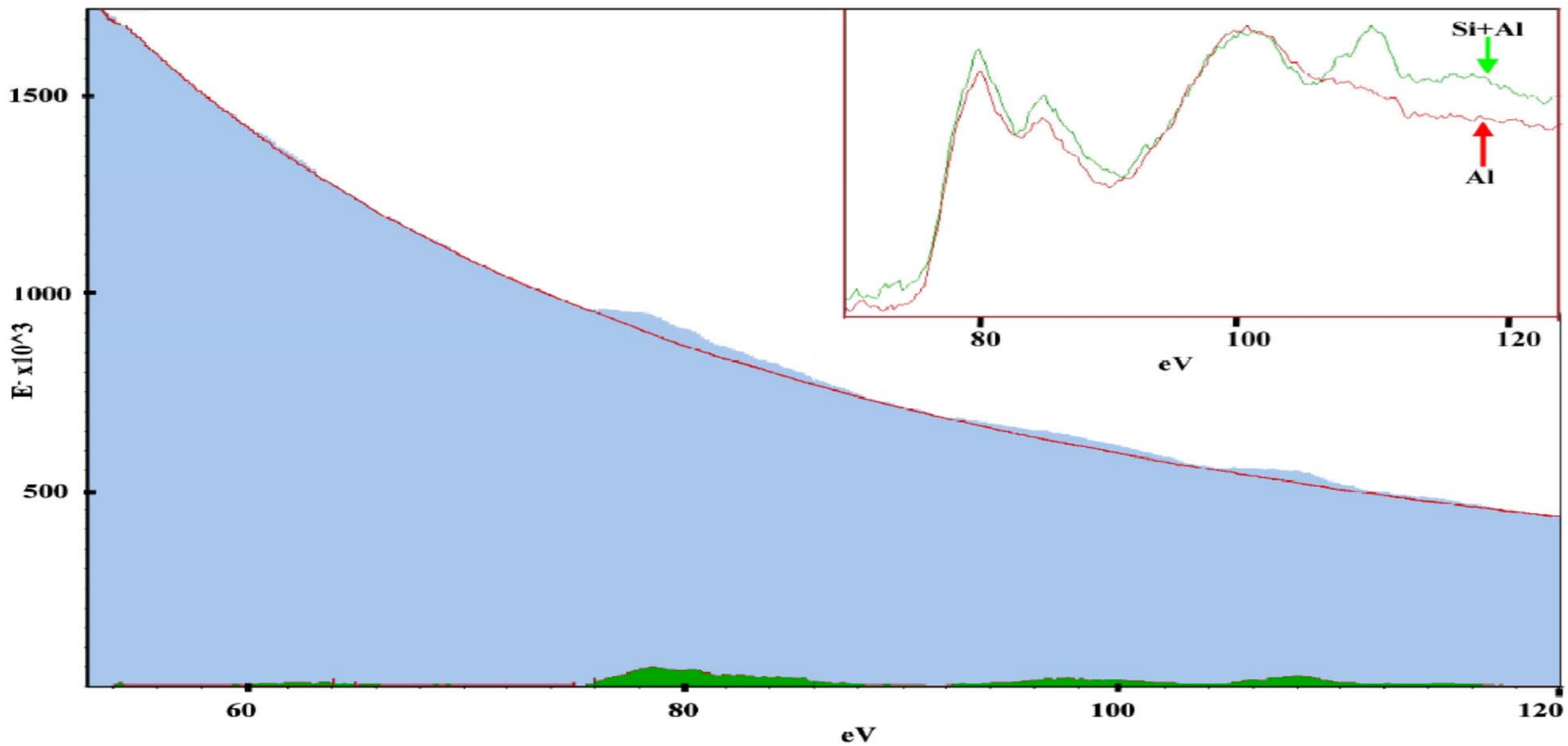
- characteristic edges in the energy-loss spectrum
- onset energy characteristic of atomic species
- concentration of an element can be determined from EELS spectrum
- subtract background for each pixel: *three-window* technique



# EF-TEM Tomography

**Silica–alumina porous composite: 3D elemental mapping**




6 images collected per each tilt image: zero-loss image
L23 edge of Al (59, 70, 81 eV)
L23 edge of Si (99 and 110 eV)

**Ersen & Hirlimann, Microscopy, 1572-79**

# EF-TEM Tomography