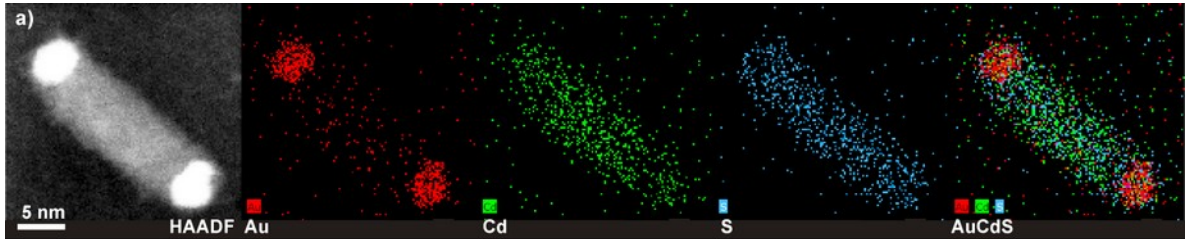
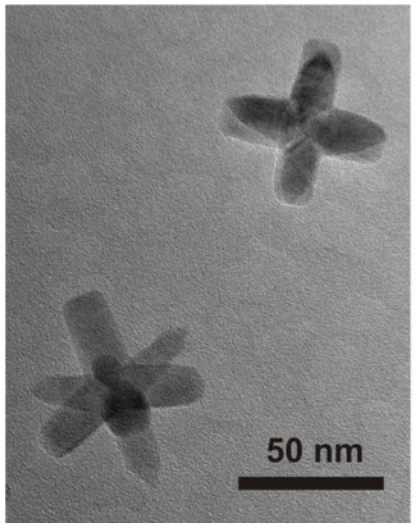
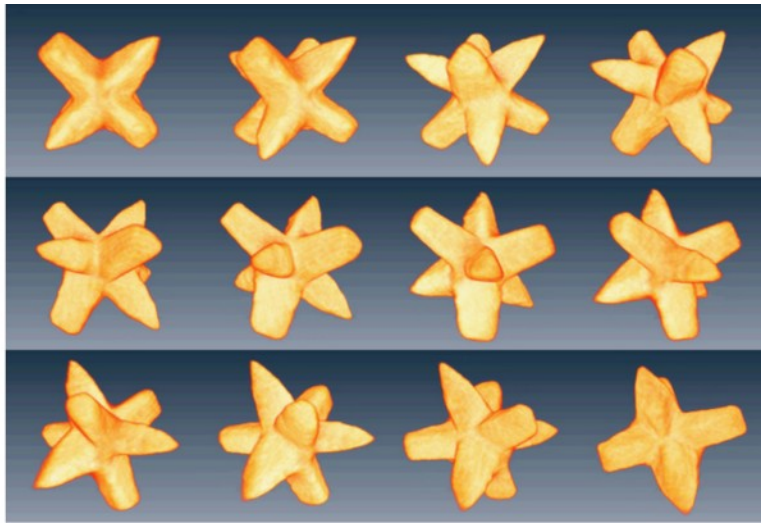
Daring to Try

Electron Microscopy







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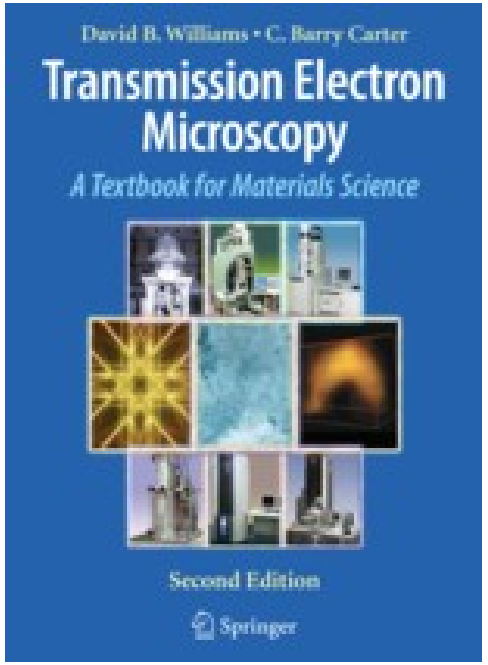
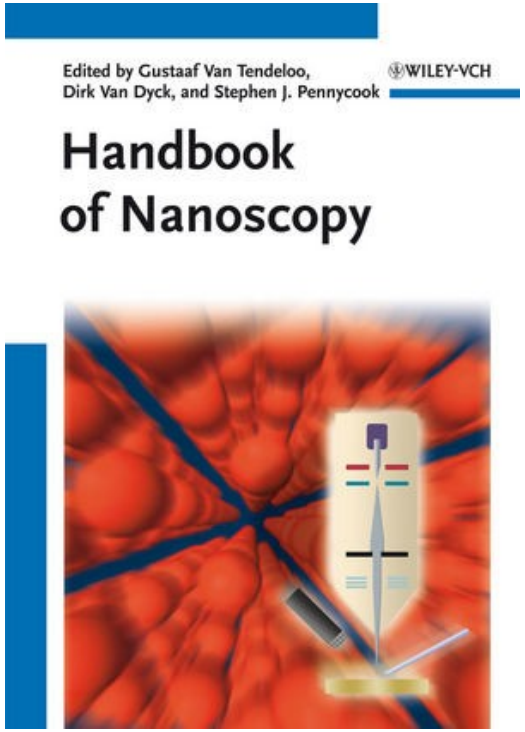
Molecular Biophysics

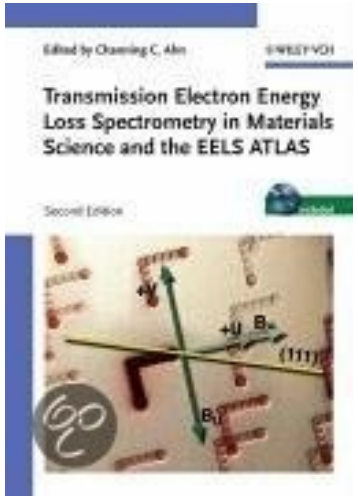
Slides & images by Profs. Gerritsen, van Huis, and the Internet

Shameless Plug for "Advanced Microscopy"

- Transmission Electron Microscopy
  - Williams and Carter
- Handbook of Nanoscopy
  - Van Tendeloo et al. (Eds).
- EELS Atlas





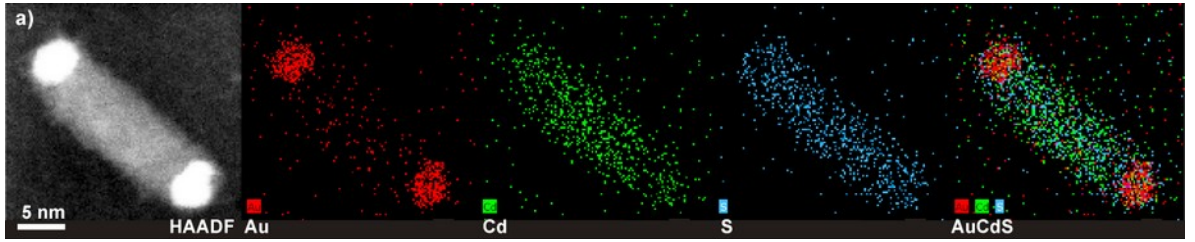


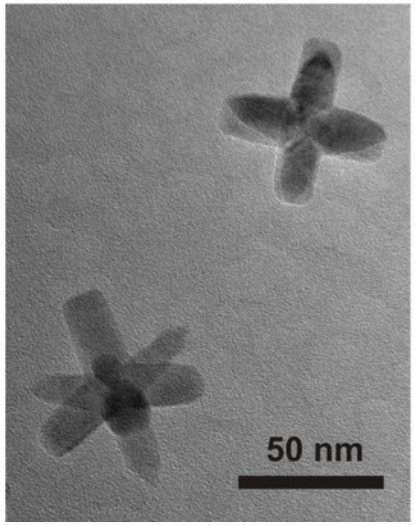
Reading list for NS-EX423M Advanced Microscopy Block 2

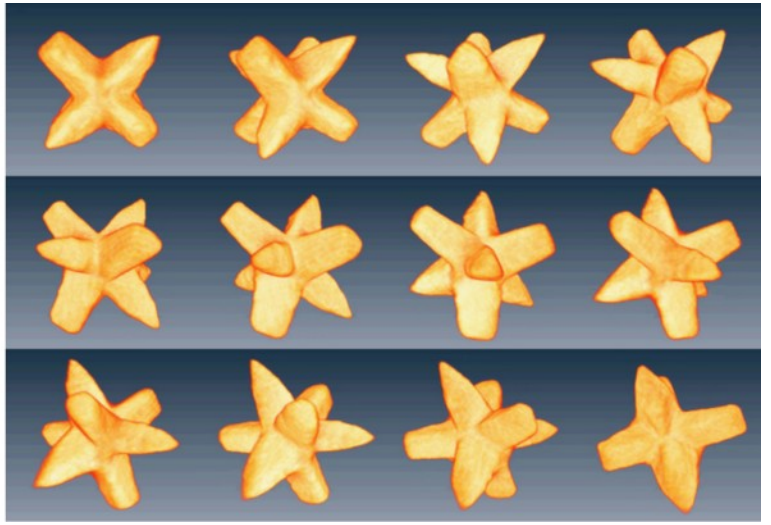
Daring to Try

Electron Microscopy

Part 1: History







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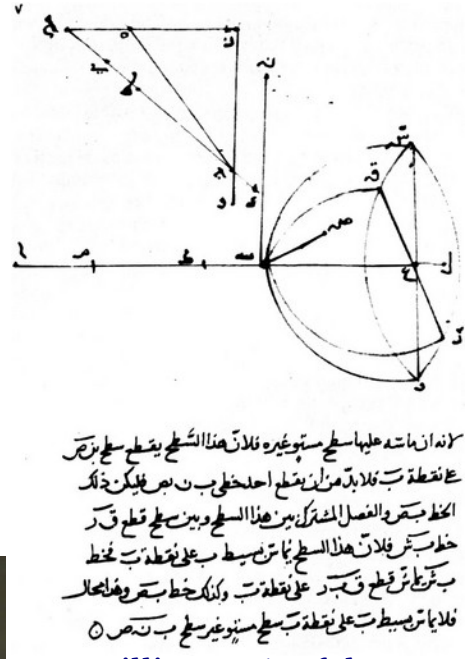
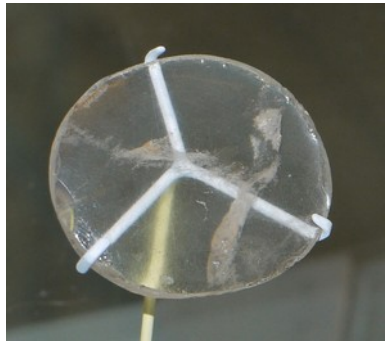
Ridiculously Abridged History of Optics & Microscopy

(Re?)Discovery of the law of refraction ('Snell's Law') by Ibn Sahl

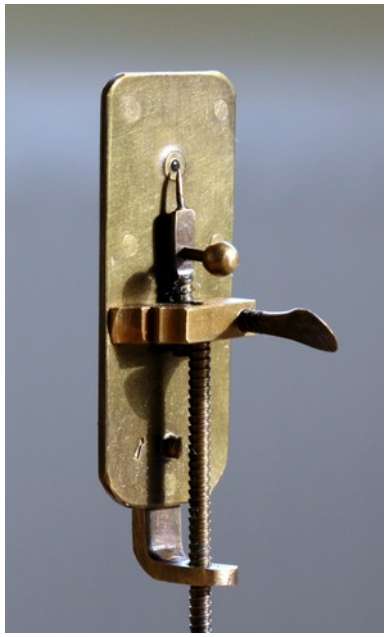


Milli MS 867, fol. 7r, Milli Library, Tehran

Nimrud Lens
~ 730 BCE
(British Museum)



Possibly already known by Ptolemy



Simple micro-scopes in biology
Required reference to Antonie van Leeuwenhoek

Confocal Microscope (1943 Koana, 1957 Minsky)

The Nobel Prize in Chemistry for Cryo Electron Microscopy

Ernst Abbe: resolution is limited

1850 1873 1900 2017

Modern Microscopes
Zeiss Optics founded,
Köhler illumination

1931: First Electron Microscope (TEM)

Compound and Achromatic Lenses

Year 1 730 BCE ca. 150 CE 983 CE 1621 1687 ~ 1750 1850-1900 2017

Utrecht University

Students interested in this subject may also be interested in courses by the programme "History and Philosophy of Science (HPS)"!

Electron Microscopy

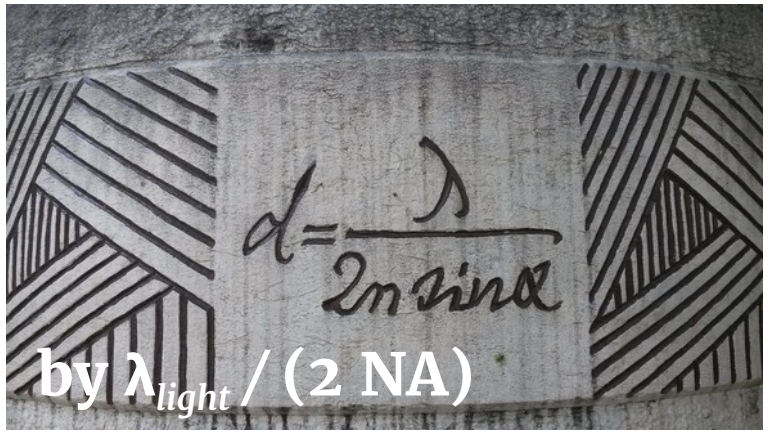
First electron microscope, Ernst Ruska 1931

Electrons:

Particles

Mass $m = 9.109 \times 10^{-31}$ kg

Charge $q = -1.602 \times 10^{-19}$ C $(-e)$



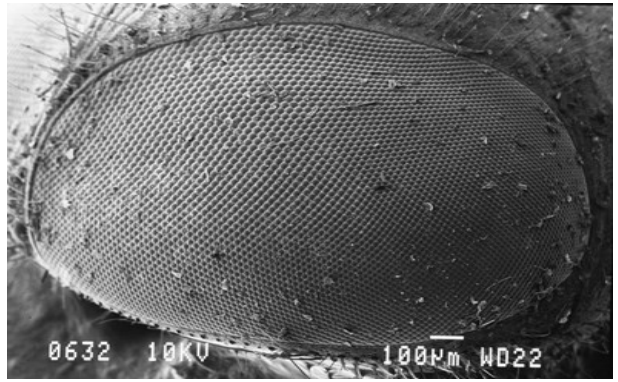
Waves

Ernst Abbe (1873) resolution optical microscope limited by λ_{light} / (2 NA)

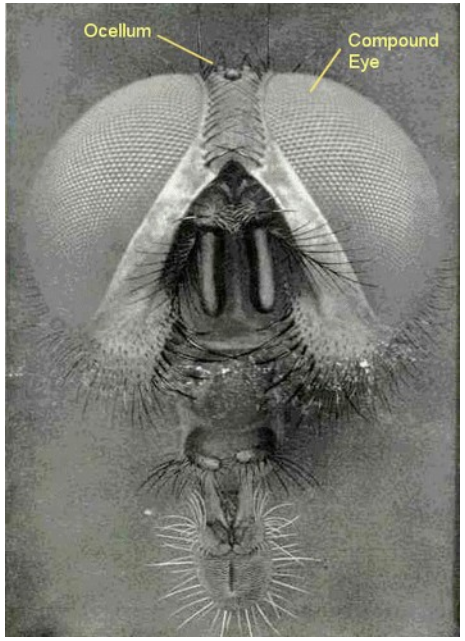
De Broglie (1924) $h = \lambda_e p_e$ (h Plank's constant, p momentum), $h = 6.63\ 10^{-34}$ Js

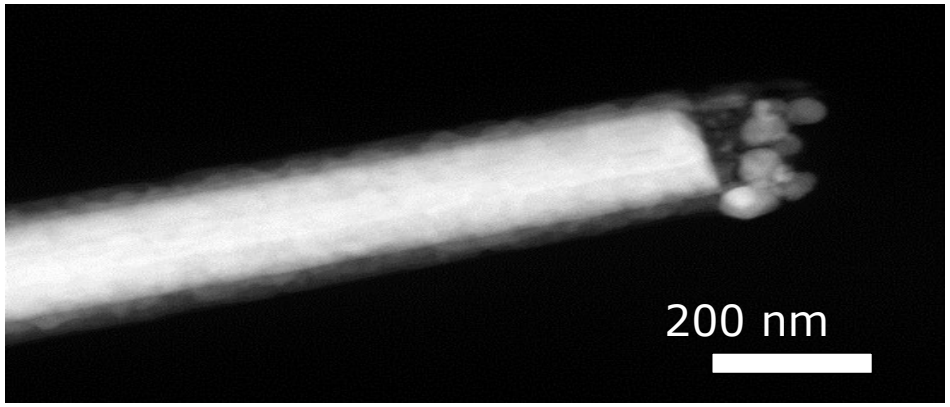
EM application areas:

- Biology: bone, tissue, cells, proteins
- Geology: minerals, ice, meteorites
- Semiconductor industry: devices, interfaces
- Metal industry: aluminum and steel alloys
- Catalysis: particles, porous supports (zeolites)
- Soft materials: polymers, colloids, assemblies
- Nanoscience and nanotechnology

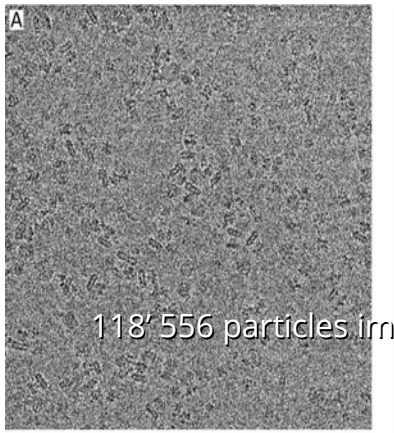


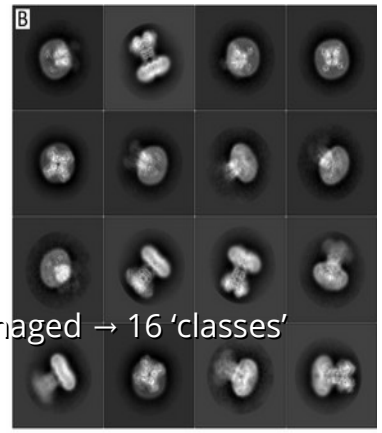
Compound eye of a fly



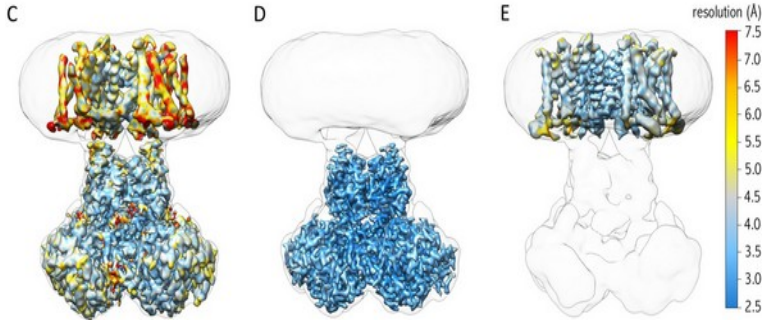


Silver nanowire with Cu_2O shell





118’ 556 particles imaged → 16 ‘classes’

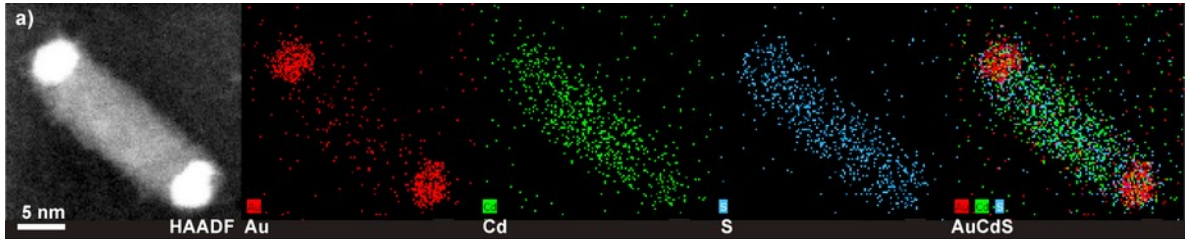


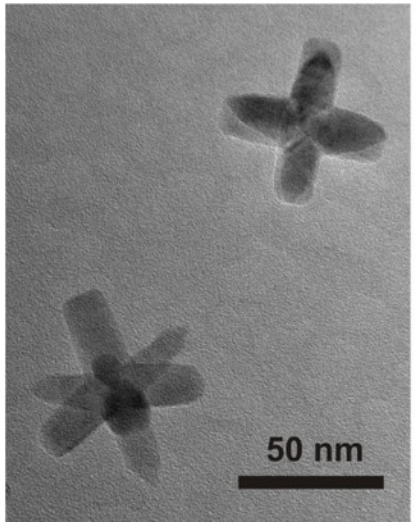
sp-cryo EM of a potassium channel
eLife, 7:e37558, 2018.

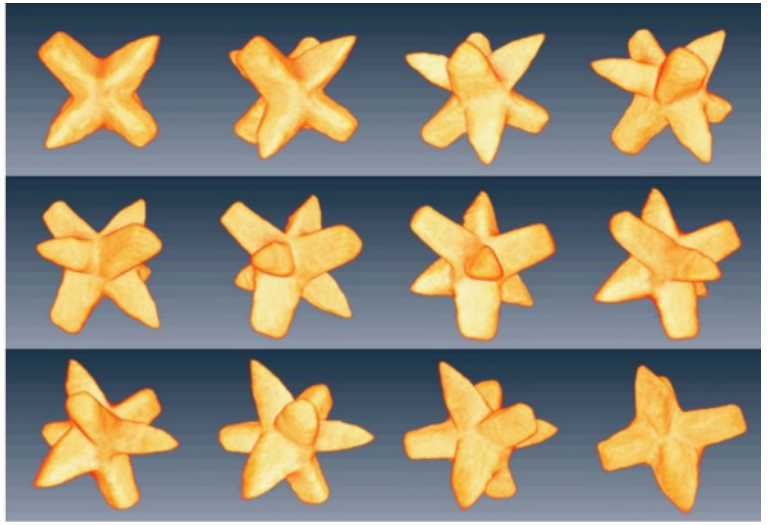
Daring to Try

Electron Microscopy

Part 2: Physics & Engineering







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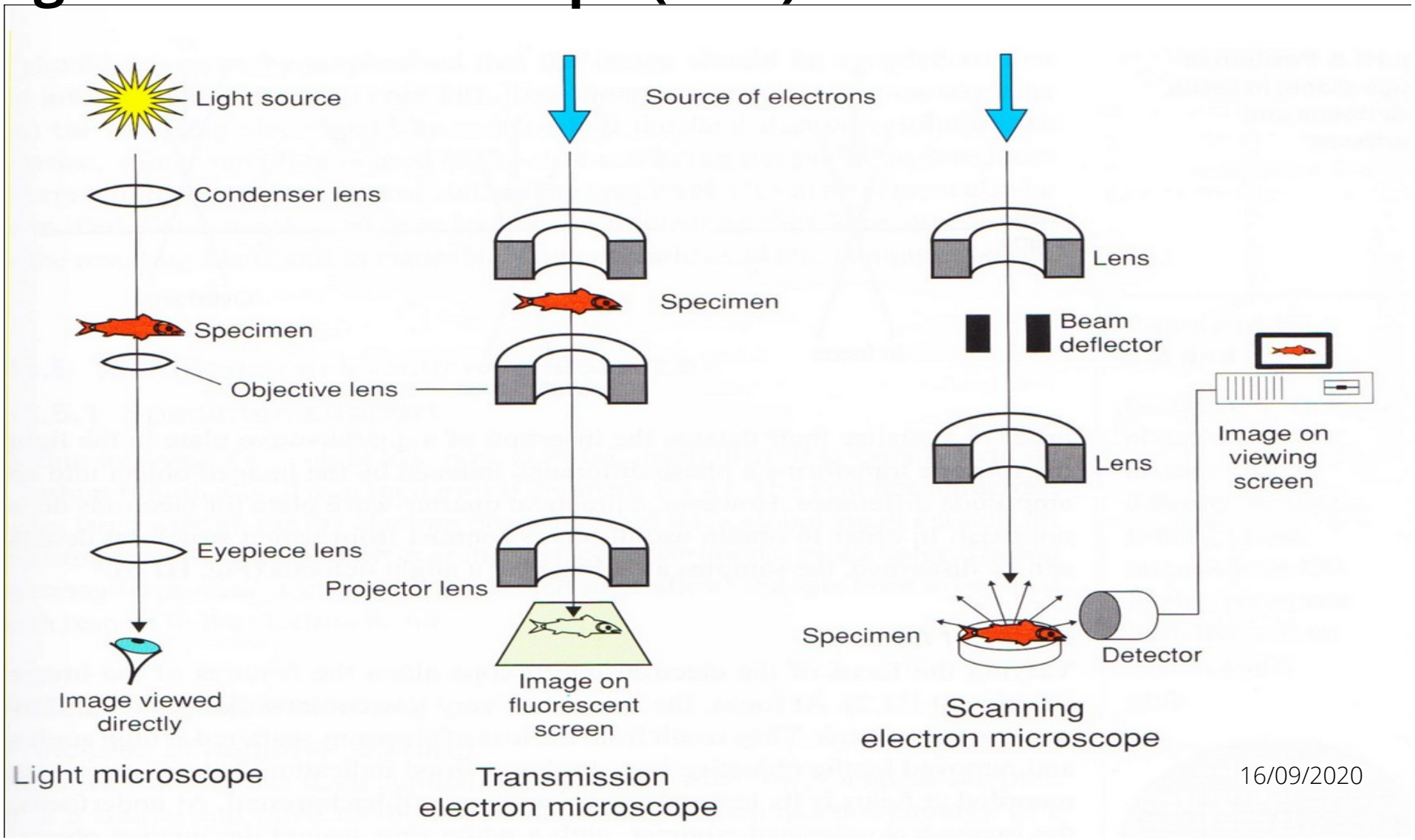
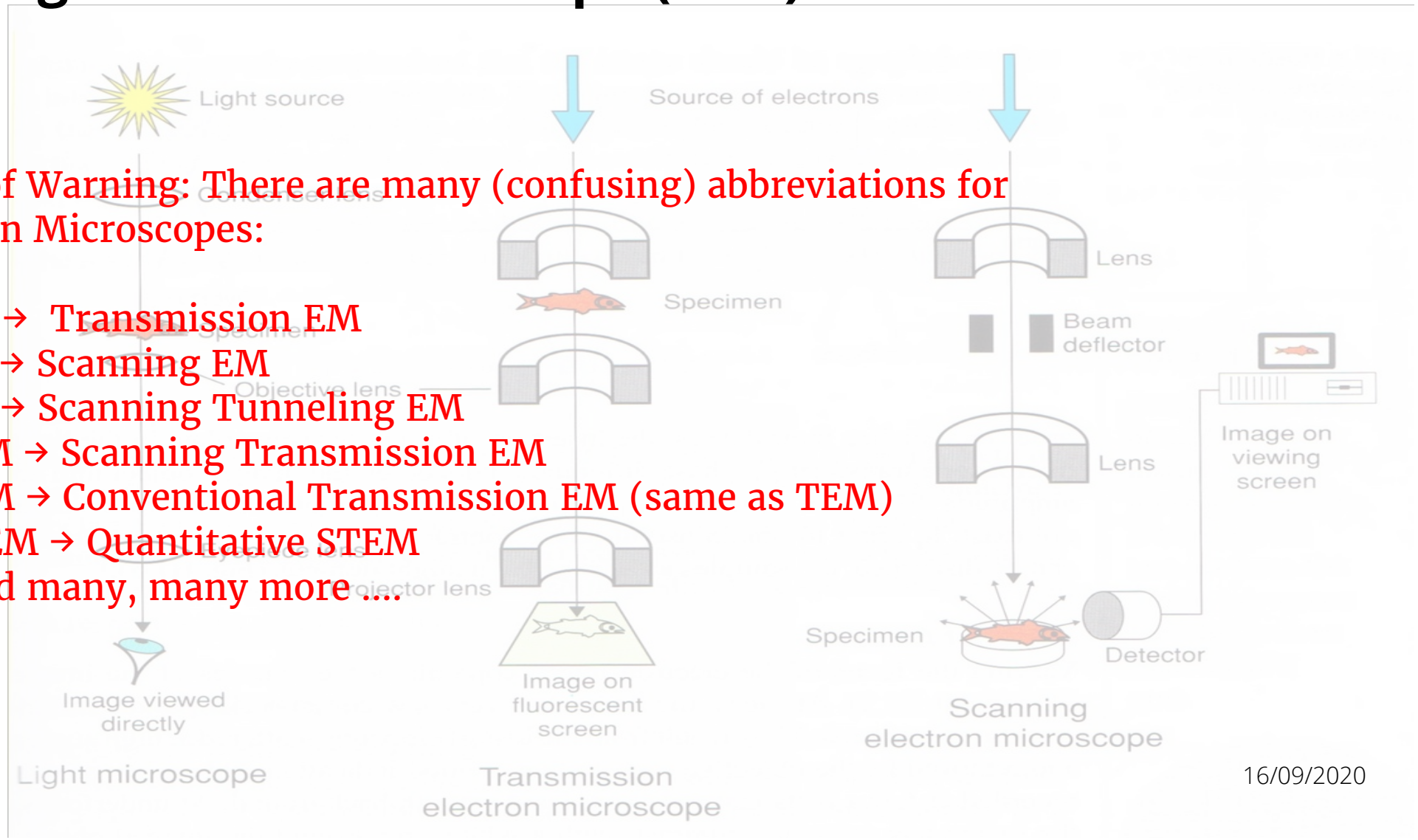
Transmission Electron Microscope (TEM) & Scanning Electron Microscope (SEM)



Transmission Electron Microscope (TEM) & Scanning Electron Microscope (SEM)



Word of Warning: There are many (confusing) abbreviations for Electron Microscopes:

- TEM → Transmission EM
- SEM → Scanning EM
- STM → Scanning Tunneling EM
- STEM → Scanning Transmission EM
- CTEM → Conventional Transmission EM (same as TEM)
- QSTEM → Quantitative STEM
- … and many, many more ….

Wave character of the electron

Wavelength electron: $\lambda_e = h/p_e$, *with* $p_e = m_e v_e$, *and h Planck's constant*

Electron in an electric field with potential difference U

Accelerated up to $eU = \frac{1}{2} m_e v^2 = \frac{1}{2} p_e^2/m_e$ $(E_{pot} = E_{kin})$

- $p_e = \sqrt{2 m_e e U}$
- $\lambda_e = \frac{h}{\sqrt{2 m_e e U}} \approx \frac{12}{\sqrt{U}}$ $[Å]$ (U in Volt)

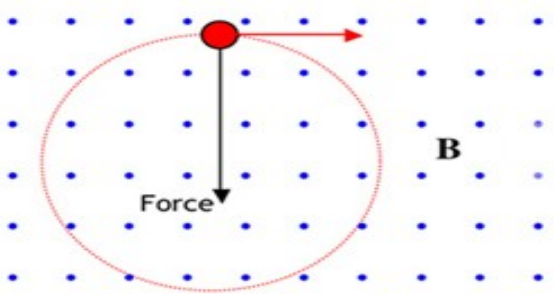
Relativistic effects cannot be neglected:
100 keV → 0.55 c

Typical EM U ~ 40 – 400 keV λ_e = *0.06 – 0.019* Å, with typical resolution ~ λ

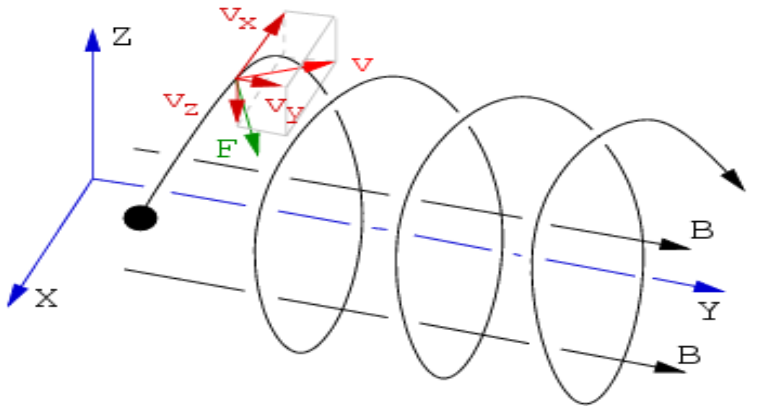
however achievable resolution is 100× worse than this …

Focusing of electrons

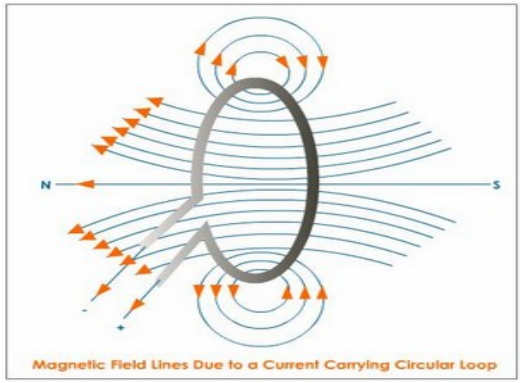
Lorentz force: $F = q\,(v \times B)$ $\quad (F \perp B, v)$



Electron spirals around field lines



Electrons can be focused by magnetic field



Busch (1926), 5 years before EM

Resolution EM

For diffraction limited resolution: $d \sim \lambda_e / \sin \theta$

In EM, θ is very small

EMs *not* diffraction limited

Astigmatism & spherical aberration

In practice (spherical) aberrations dominate the resolution

$$d = (C_s \lambda_e^3)^{1/4}$$

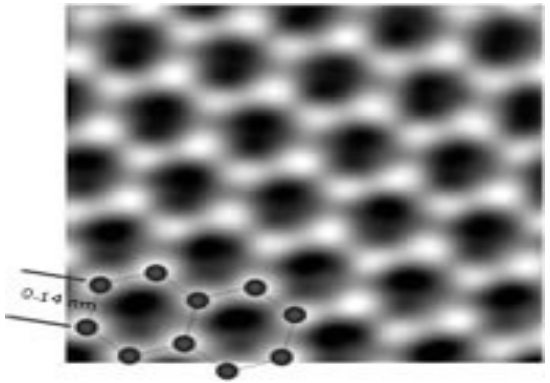
C_s spherical aberration coefficient common value of ~ *1 mm* = 10^7 Å

d = 5 Å (100 keV)

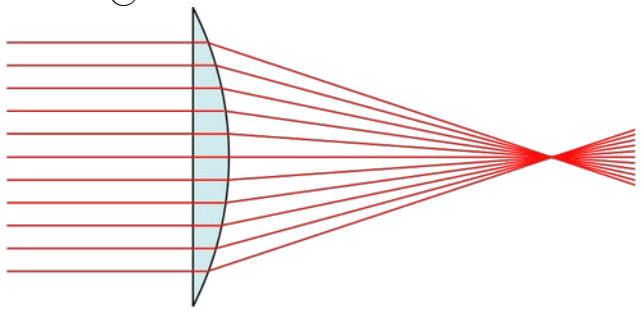
Practical resolution:

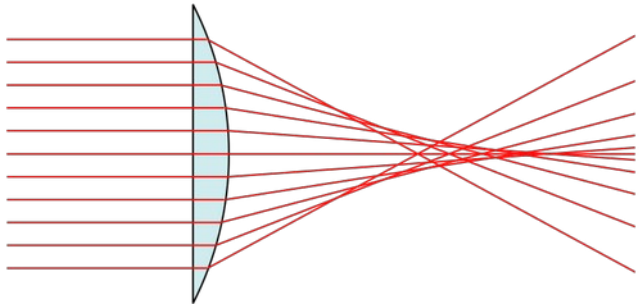
biological specimens: 2–5 nm but possibly down to 3 Å.

material science: d <0.1 nm (atomic resolution)

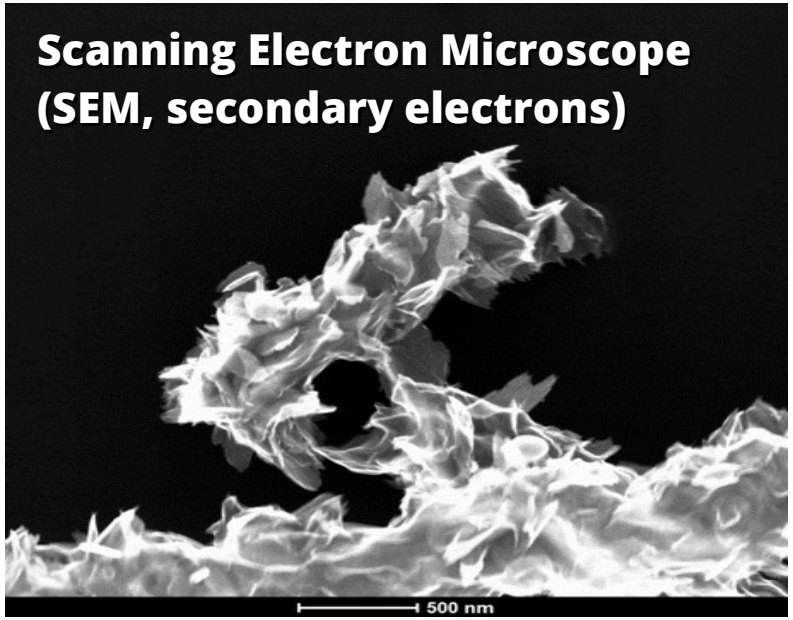


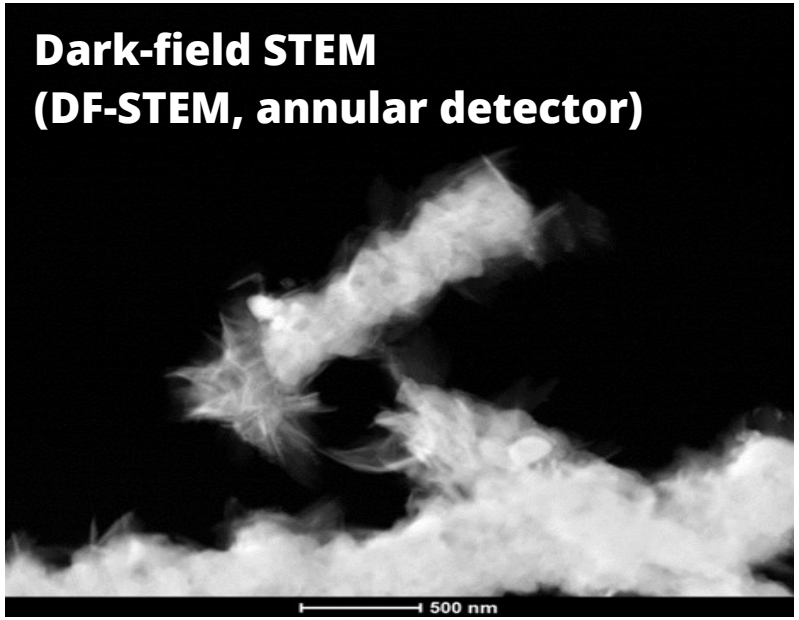
Single graphene sheet
@ 200 keV

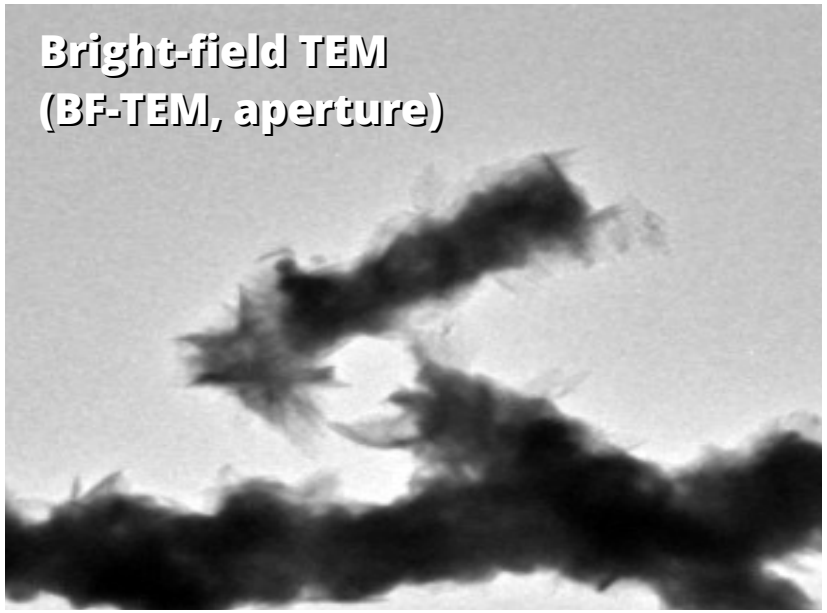


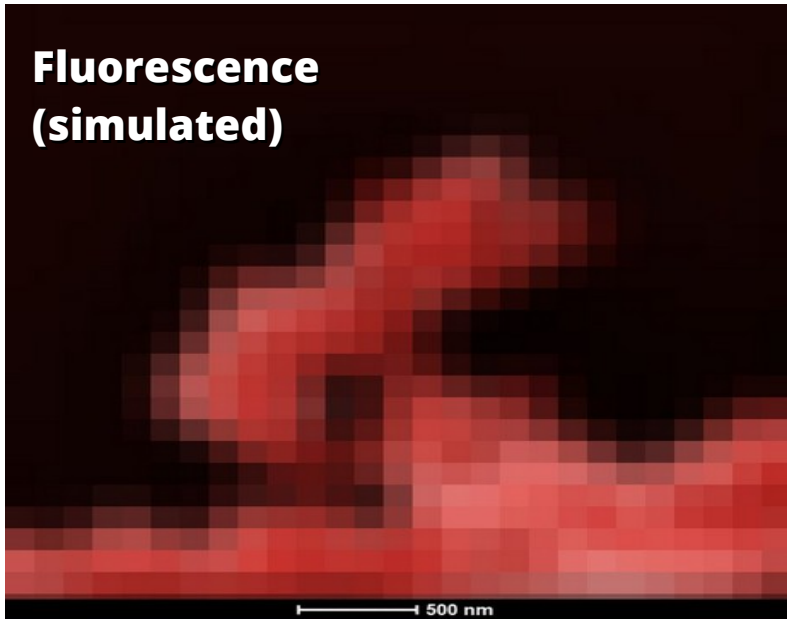


Imaging Modes



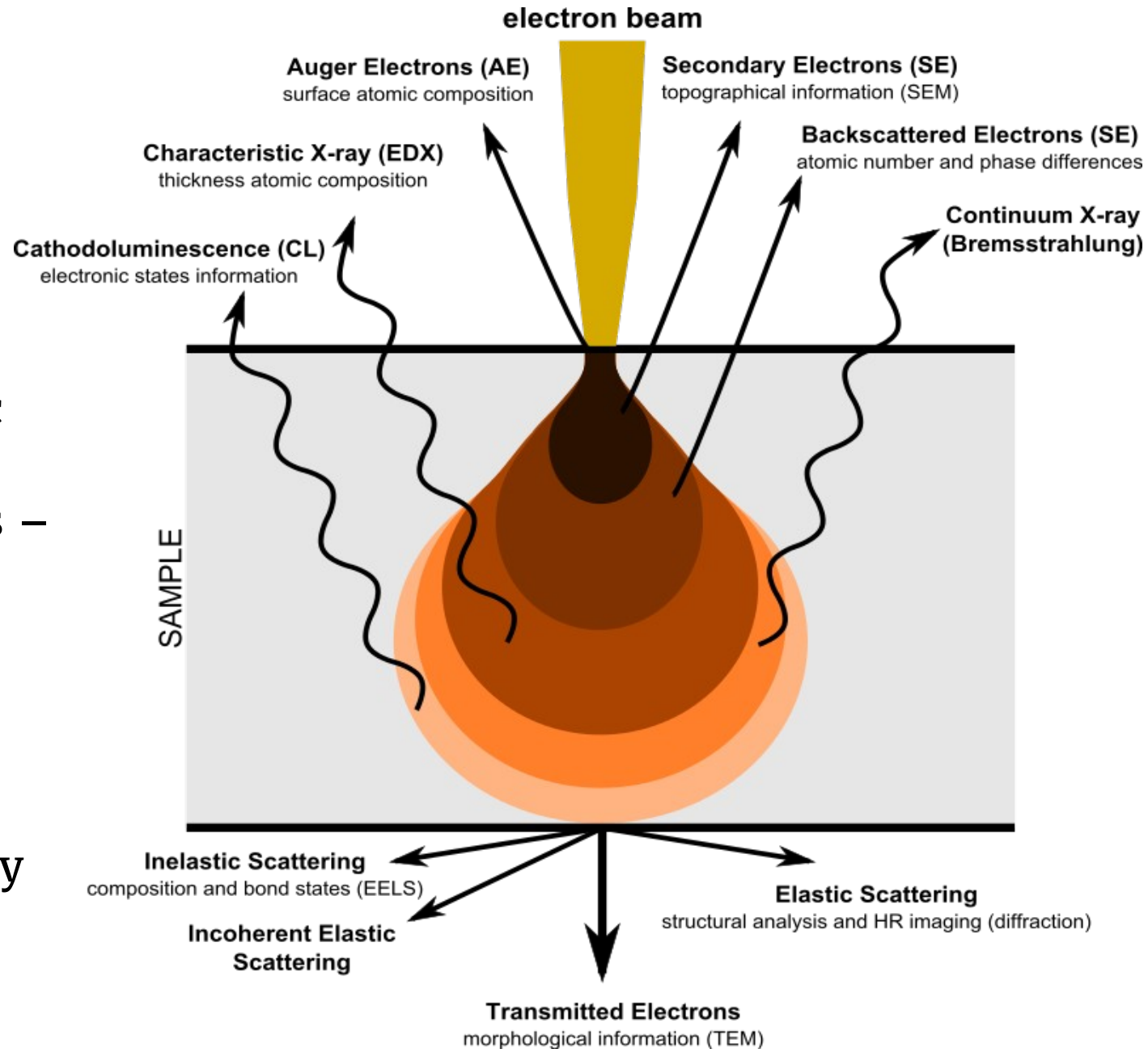






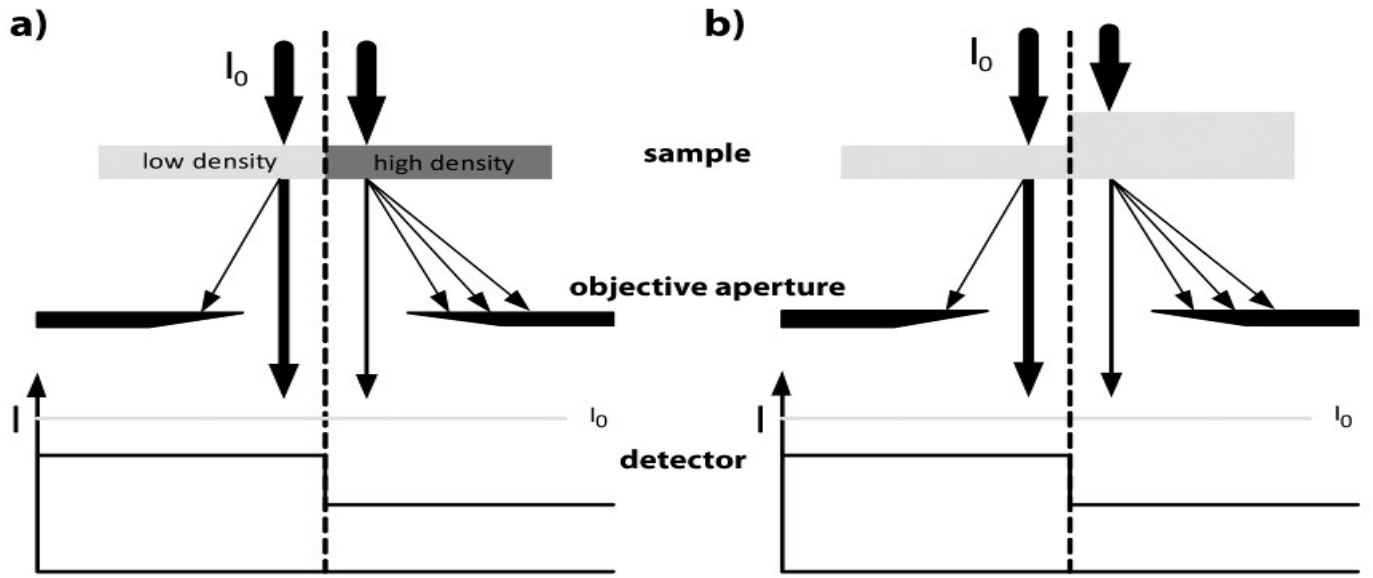
EM: Electrons Interact With the Sample

- Different qualities of interaction:
  - Topographic (surface)
  - Atomic Number
  - Luminescence (electronic states)
  - X-Ray or Auger Electrons – atomic composition
- Elastic and Inelastic Processes
- Sample will be damaged by the imaging process!



TEM image formation – contrast modes

- Mass-thickness contrast



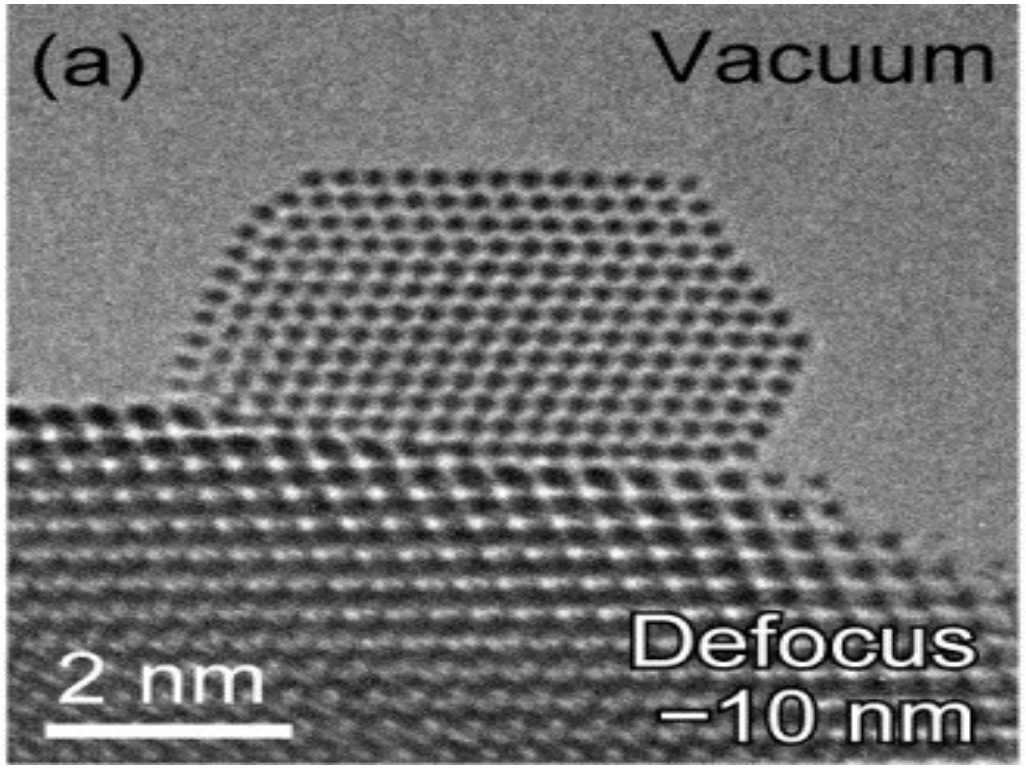
$I = I_0 \exp(\sigma \cdot t)$

t is sample thickness

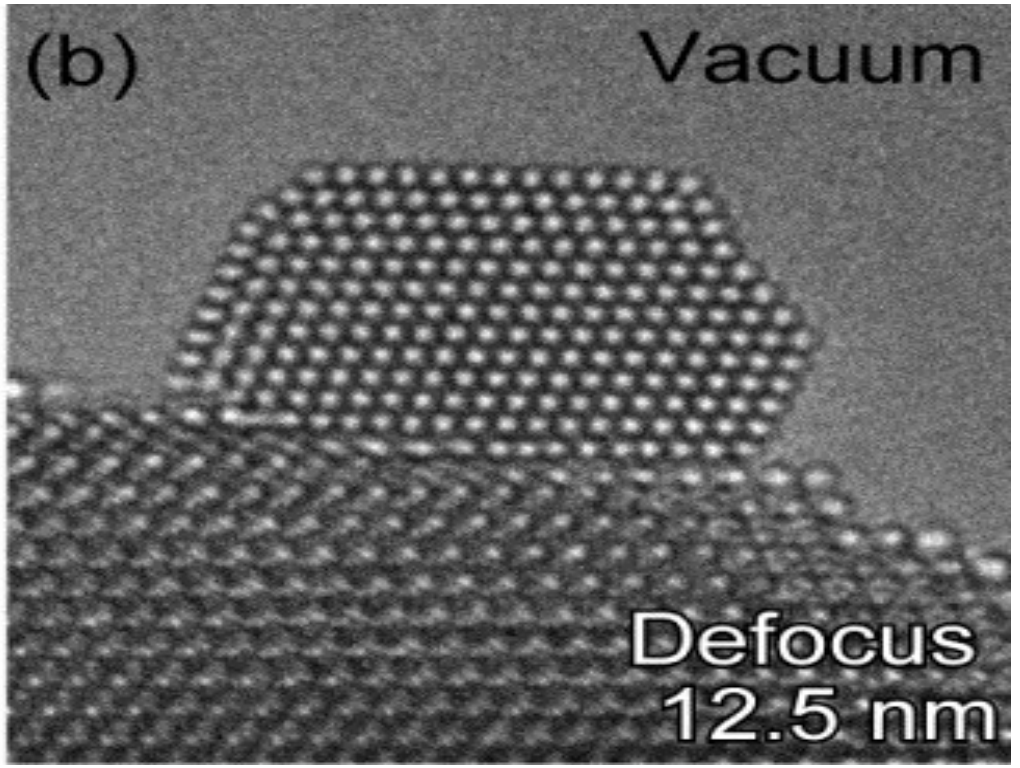
σ is material-dependent scattering cross-section

BF-TEM imaging issues – Phase Information

The focus manipulates the phase information, and can make the dots (columns of atoms) in HR images look either black or white.



underfocus



overfocus

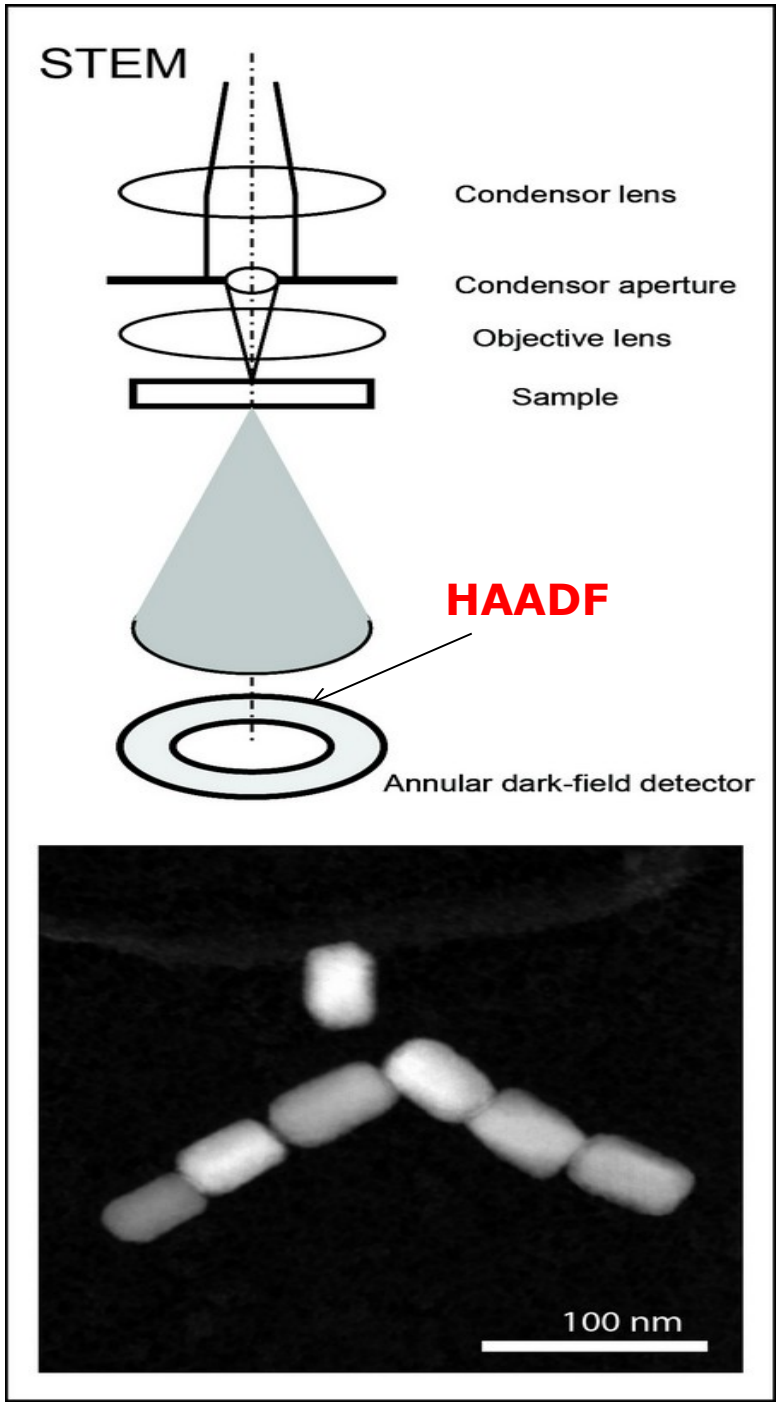
S. Takeda et al, Ultramicroscopy (in press)

Scattering in STEM mode: BF, ADF and HAADF

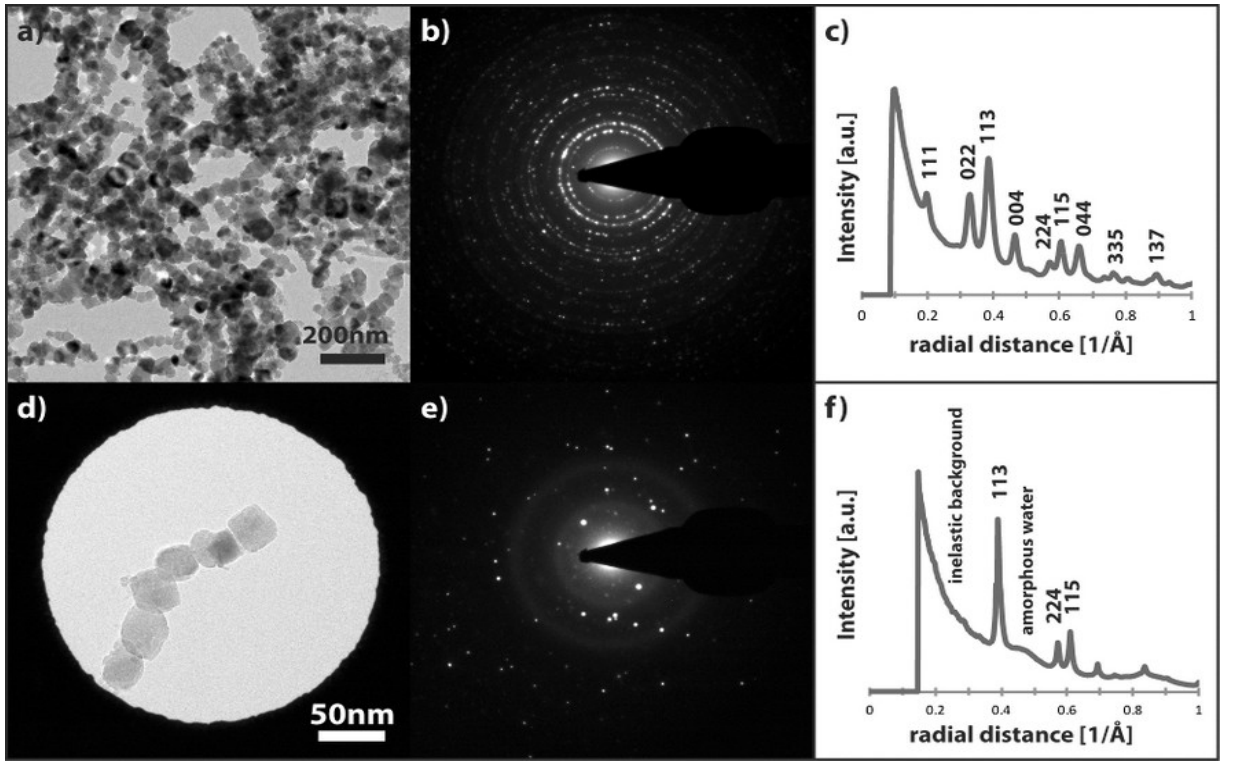
- Which electrons are detected with HAADF-STEM?
- Only strongly scattered electrons, mostly elastic Rayleigh scattering
- Quantitatively sensitive to the atomic element, so-called **Z contrast**:

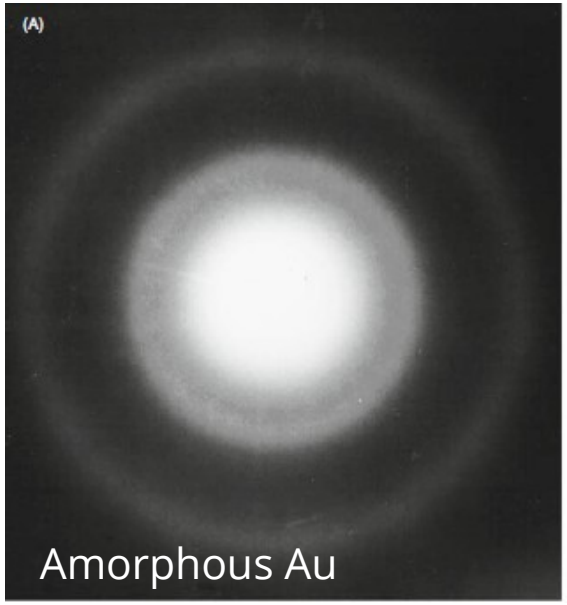
  $I \sim Z^2$

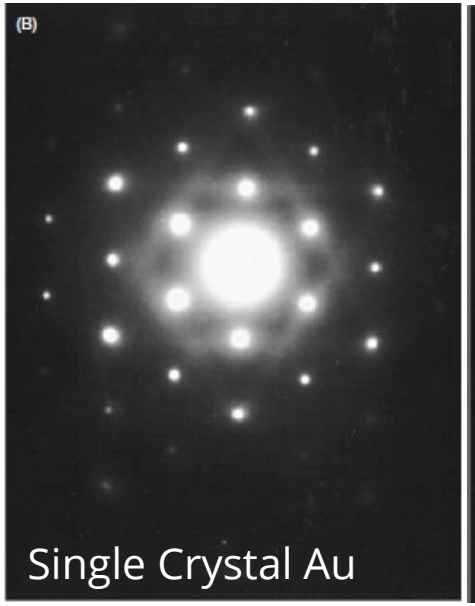
- **Not** sensitive to light elements Z<10 (cutoff)

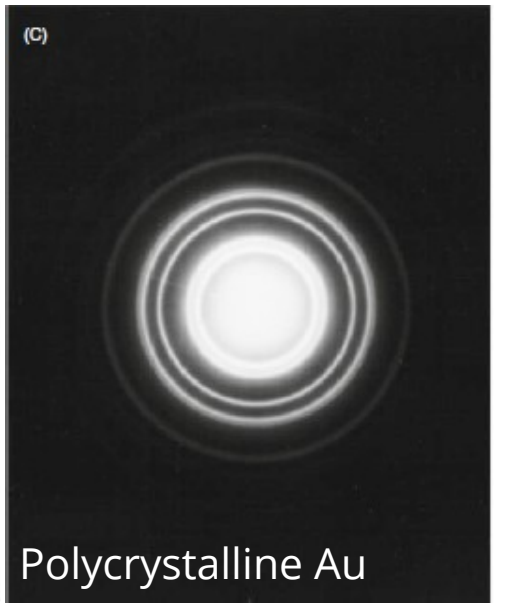


Electron Diffraction







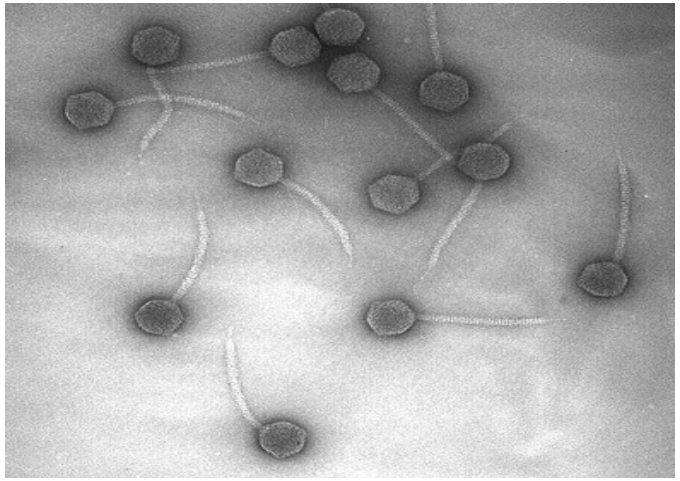


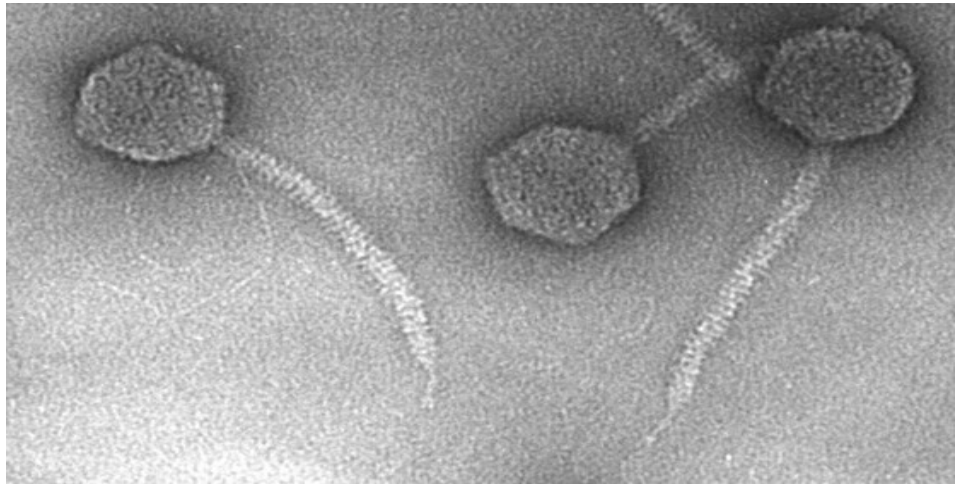
Specimen preparation

Specimen preparation critical

Biological molecules are *not* electron dense (CNO – Z = 6, 7, 8!)
Staining: Treatment with heavy metals (Uranyl acetate, Osmium tetraoxide)
Label proteins of interest using antibodies + nanogold particles

Negative staining: Embed specimen in electron dense medium

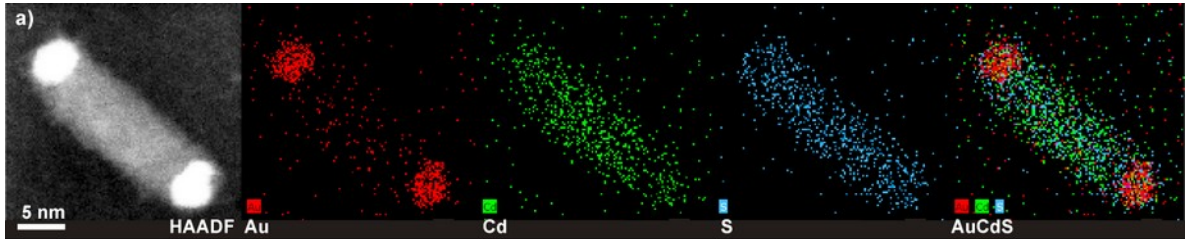


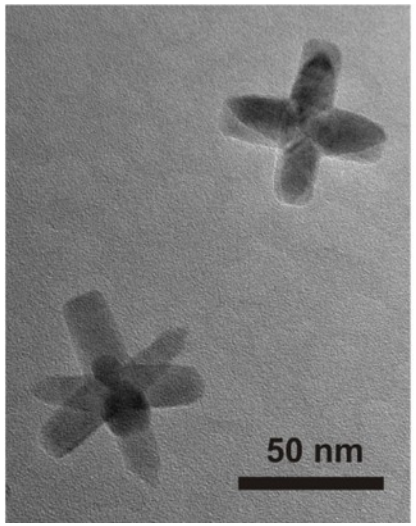


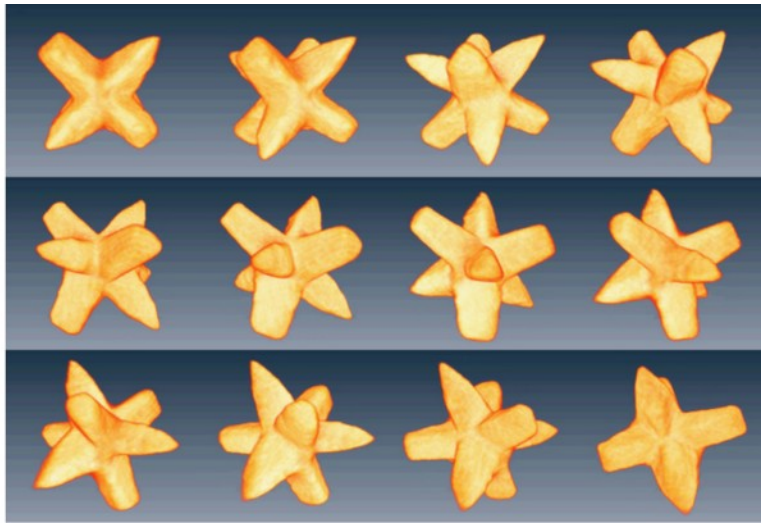
Daring to Try

Electron Microscopy

Part 3: The Nobel Prize







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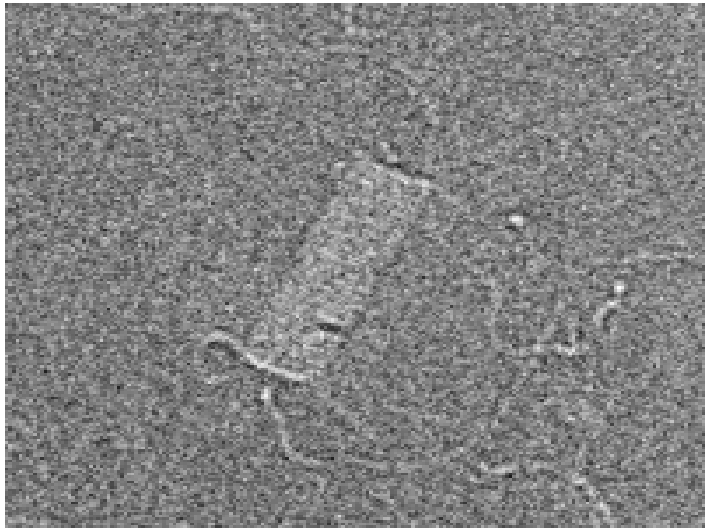
Slides & images by Profs. Gerritsen, van Huis, and the Internet

Cryo-EM (TEM)

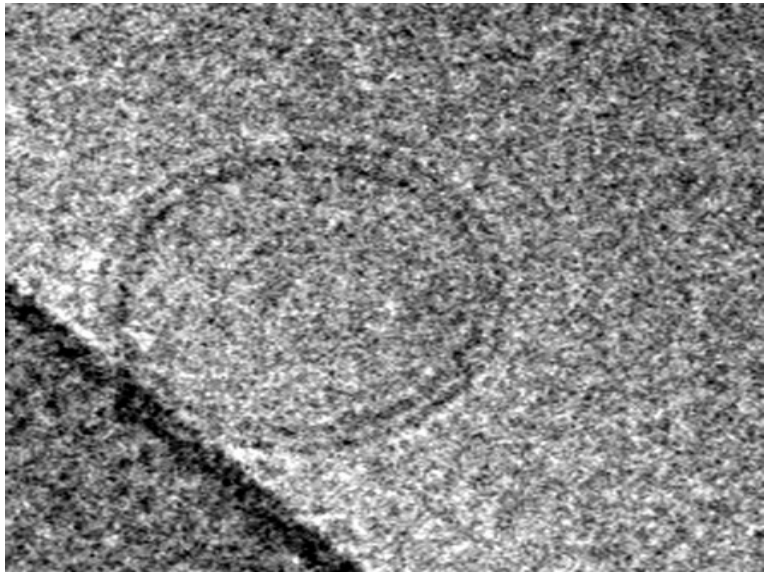
Cryo-EM:

- *Rapid* cooling in e.g. liquid nitrogen
- Preserves native structures, reduces radiation damage
- No staining required (but noisier images)

- *Flash cooling* in liquid ethane or propane (<< 77 K) avoids ice crystals (vitreous ice)
- Some measurements done at 4 K.



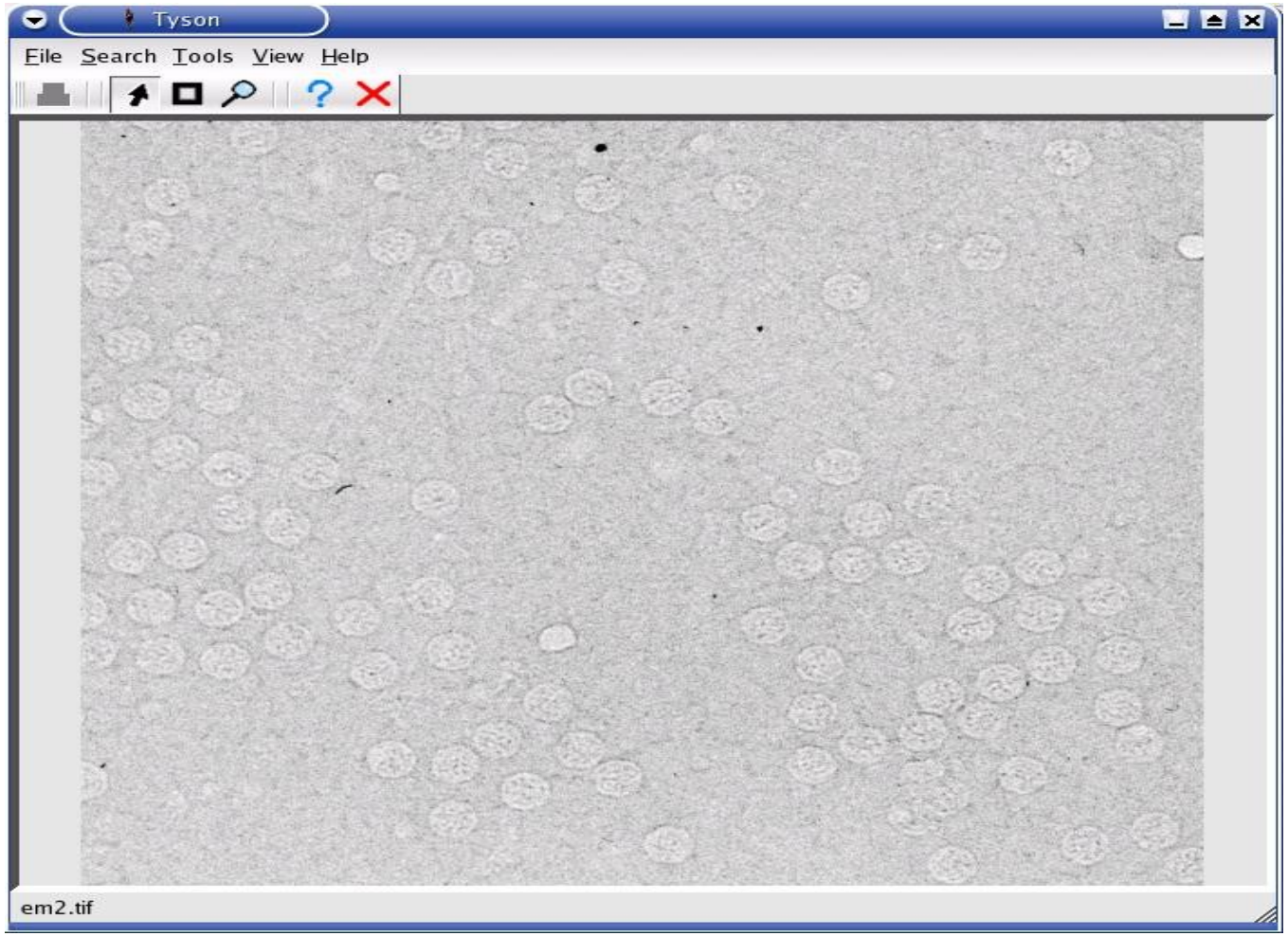
RNA-DNA hybrids vitreous ice by cryo-EM.
Inner diameter ~33 Å and outer diameter ~ 166 Å.



Cryo-EM image SUV.

Single particle cryo imaging

- Many particles imaged at ‘random’ orientations



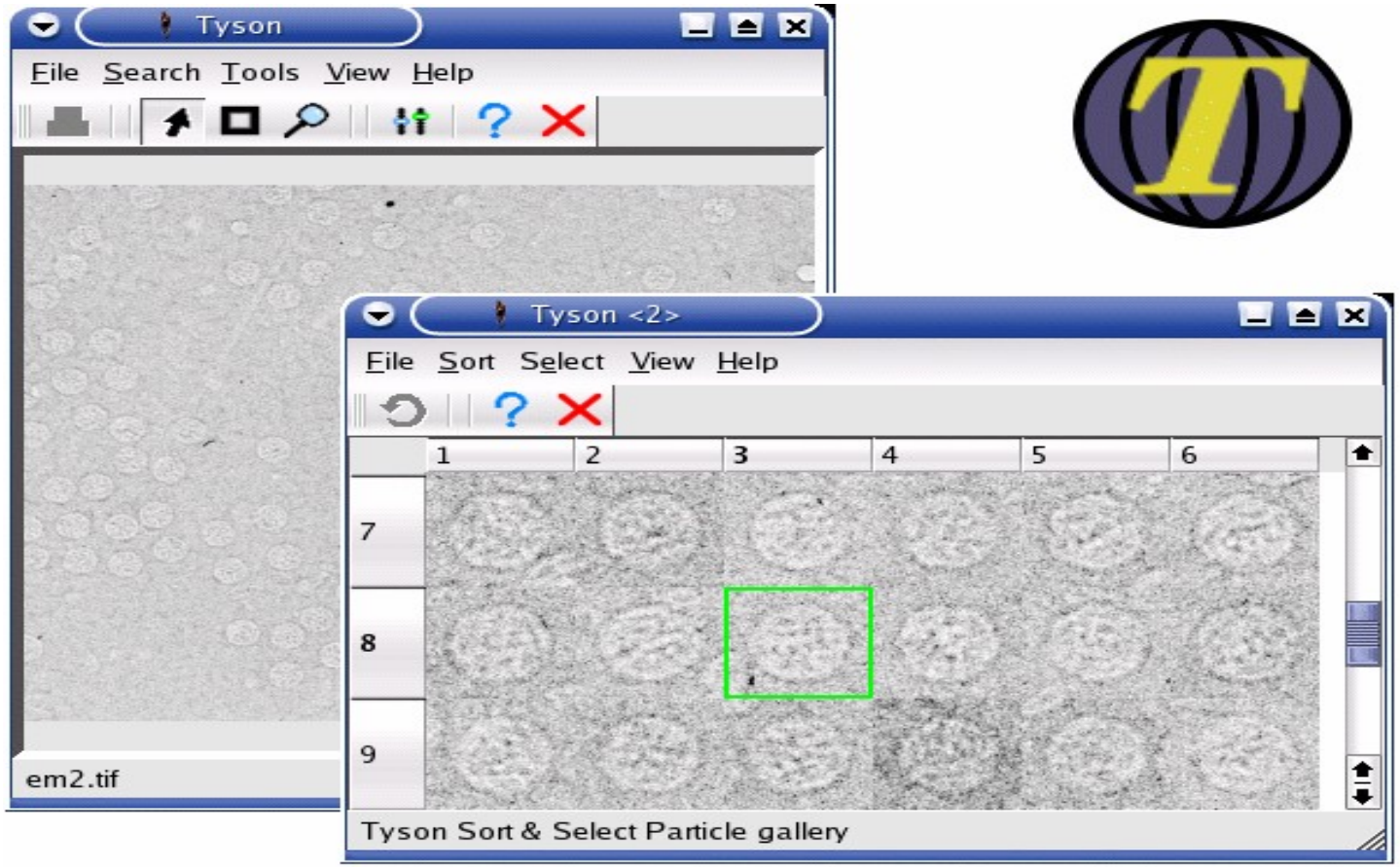
bacteriophage MS2 (cryo-EM)

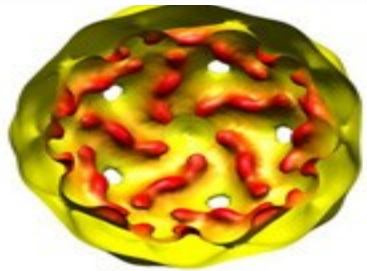
(virus that infects bacteria)

Single particle cryo imaging

- Many particles imaged at ‘random’ orientations
- Smart averaging of particles with same orientation (use symmetry), pattern recognition

➔ Determine 3-D structure from projections of different orientations





bacteriophage MS2

Source: Serious Science Channel
"Single-Particle Electron Microscopy – Richard Henderson"
Link: Youtube

2017 Nobel Prize
Cryo EM

"for the developing **cryo-electron microscopy** for the high resolution structure determination of biomolecules in solution."

- Jacques Dubochet
- Joachim Frank
- Richard Henderson

link:
The Nobel Prize in Chemistry 2017

Video Discussion

Technological Improvement vs.
Methodological Improvement

Gradual Improvement of
Original Idea

Discovery vs. Invention

Gradual Grow vs Hype
People start to use method / it
can become useful to them

Many (small) ideas have to
come together

Everything has been studied,
end of Structural Biology?

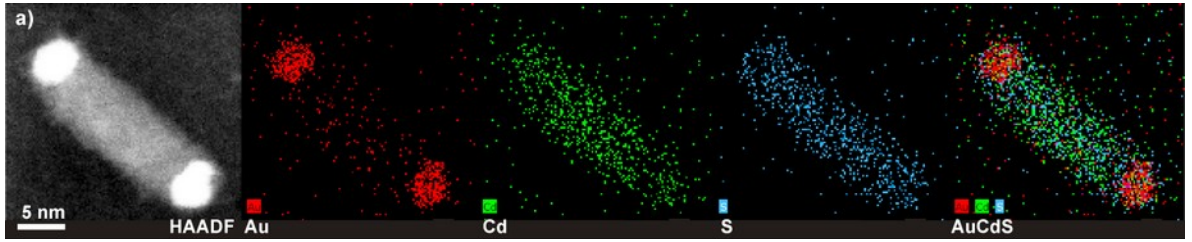
Direct Electron Detectors

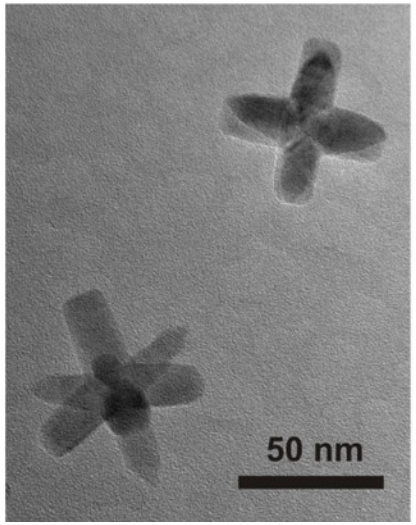
DOI 10.1016/bs.mie.2016.05.056 McMullan, Farqui, and H
enderson; Methods in Enzymology

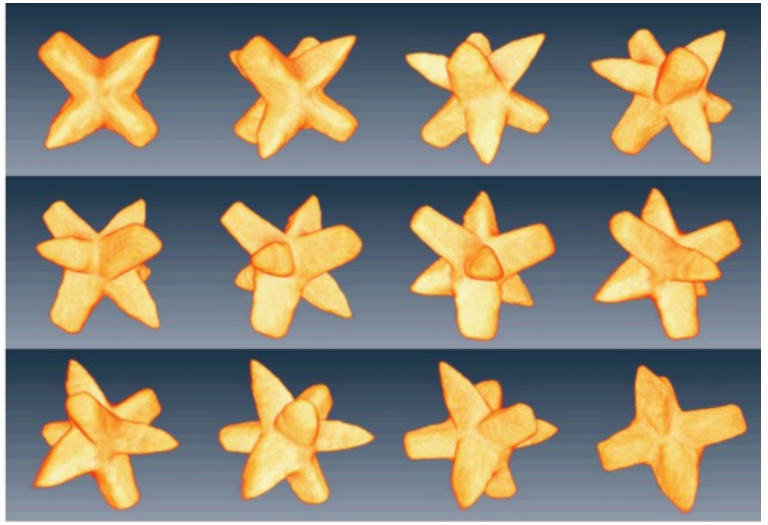
Daring to Try

Electron Microscopy

Part 4: The Future





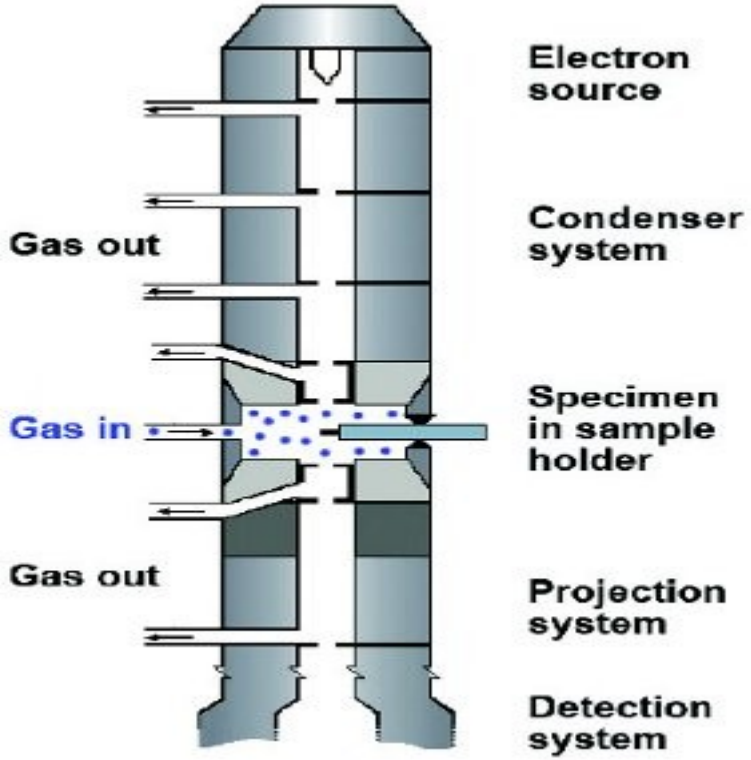


Dr. Gerhard A. Blab
Molecular Biophysics

Slides & images by Profs. Gerritsen, van Huis, and the Internet

Environmental TEM (E-TEM)

- Create a gas environment very close to the specimen (pressure ~ 1 mbar)
- Use a differential pumping system to prevent that the electrons have to travel through a long gas column (degrading the resolution)

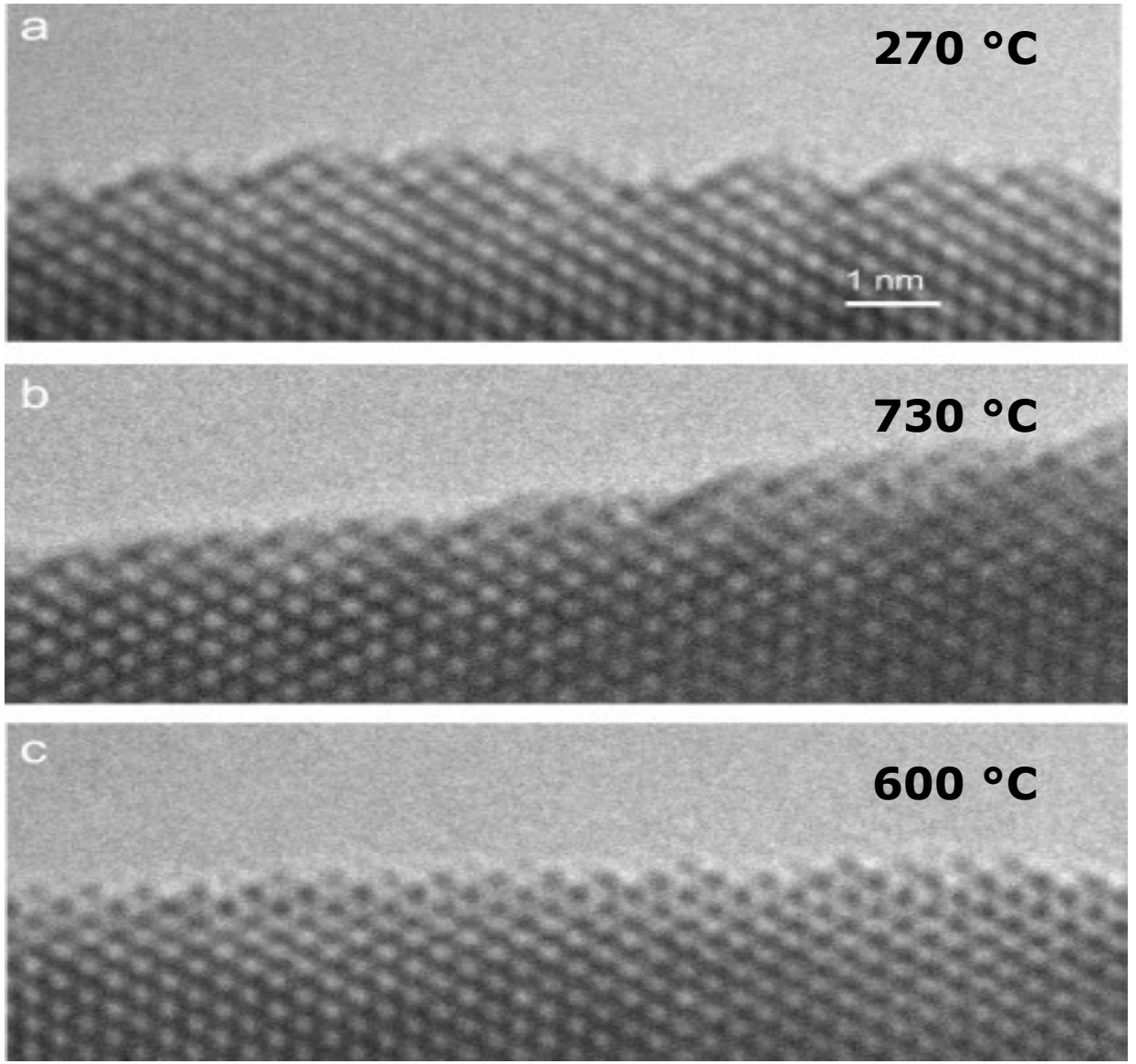


Environmental TEM

- Example:

  Dynamic changes at the surface of cerium oxide nanoparticles during redox reactions

- Gas environment of 0.5 Torr (0.7 mbar) of H_2



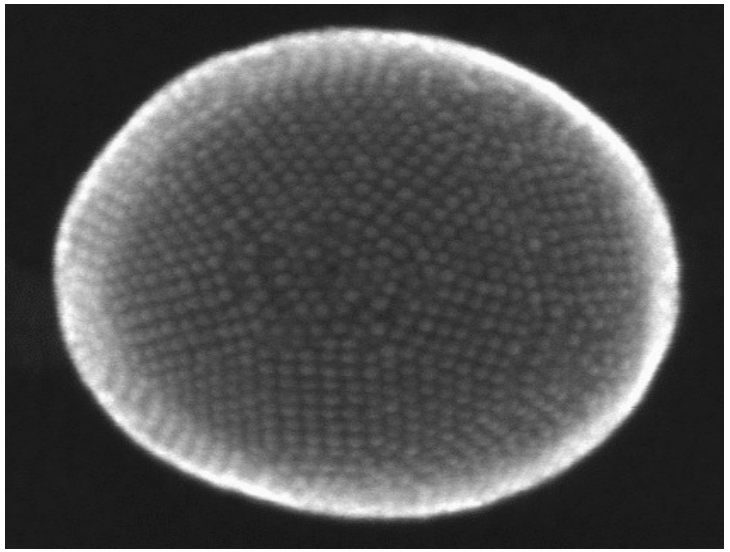
P.A. Crozier et al, *Ultramicroscopy* **108** (2008) 1432

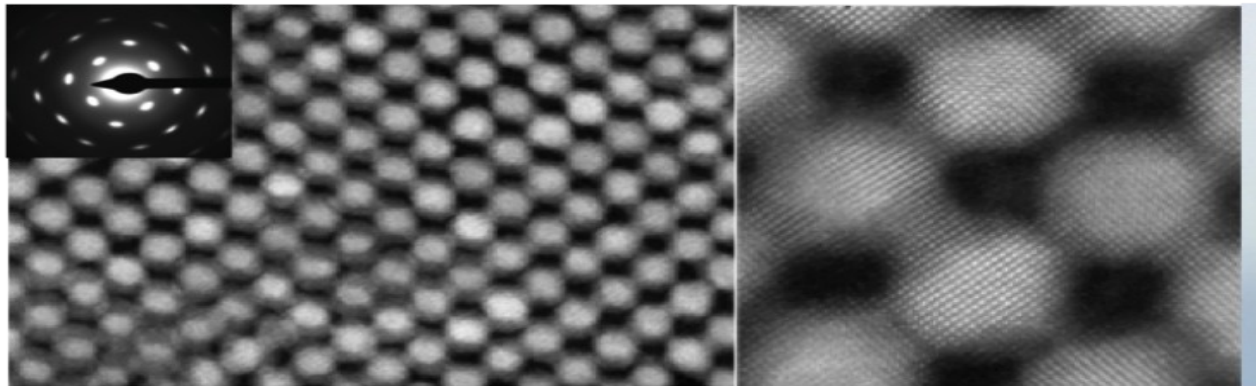
Liquid cell TEM

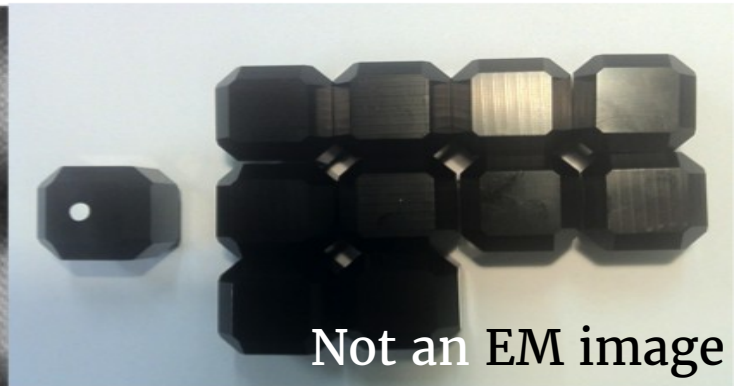
- Study *self-assembly* (SA) in confined geometries

- Utrecht University:
  - Group Marijn van Huis (3D)
  - Group Daniel Vanmaekelbergh (2D)

- 3D and 2D assembly: TEM studies
  - Group Sara Bals, Staf van Tendeloo (Antwerp)







Self-assembly in Liquid Cell TEM

- Self-ordering of octapods into strings

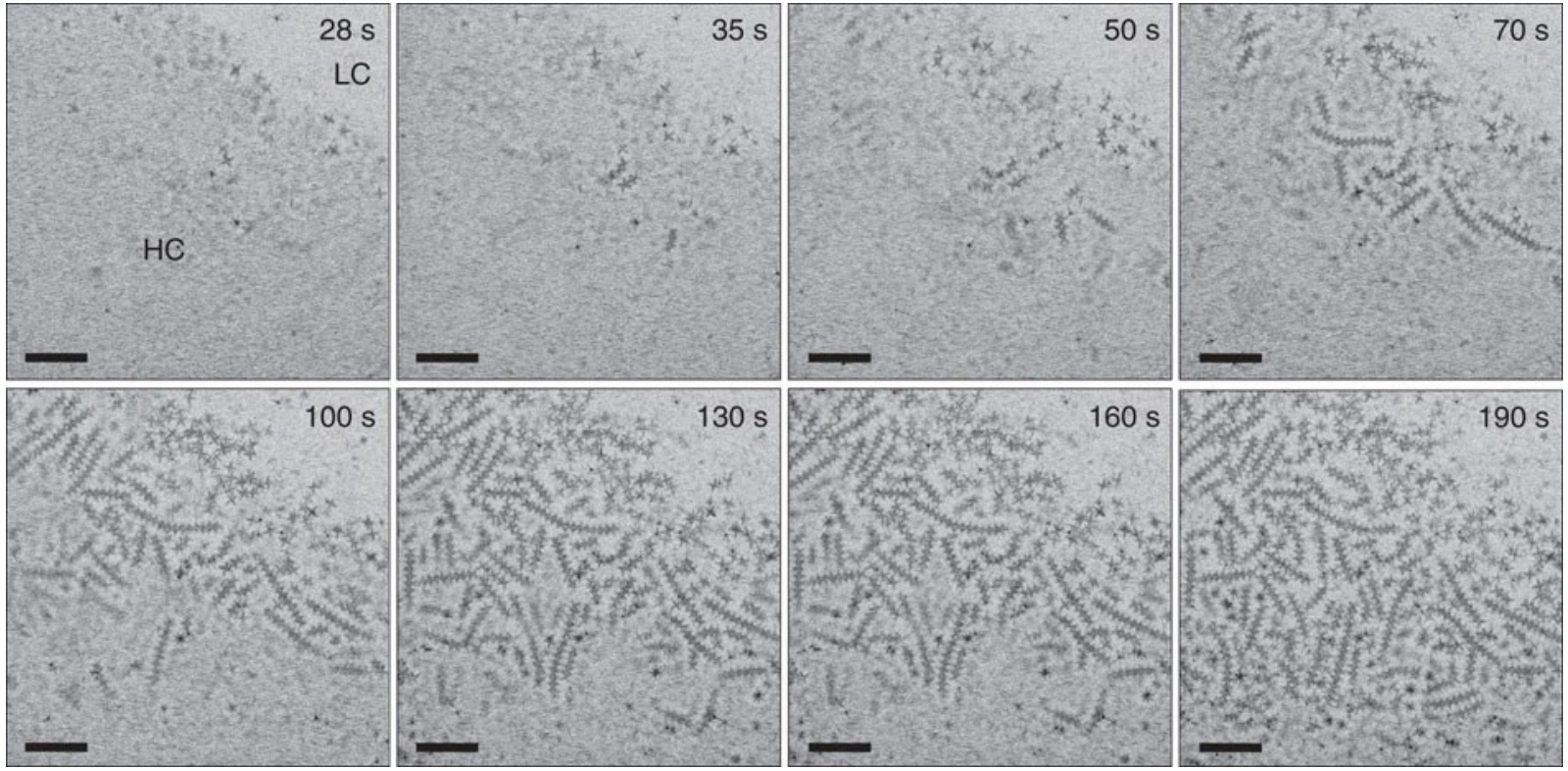


Figure 2 from:

E. Sutter, L. Manna *et al.*,

In situ microscopy of the self-assembly of branched nanocrystals in solution

Nature Commun. **7** (2016) 11213

DOI: 10.1038/ncomms11213

Scale bar: 500 nm

STEM in solution

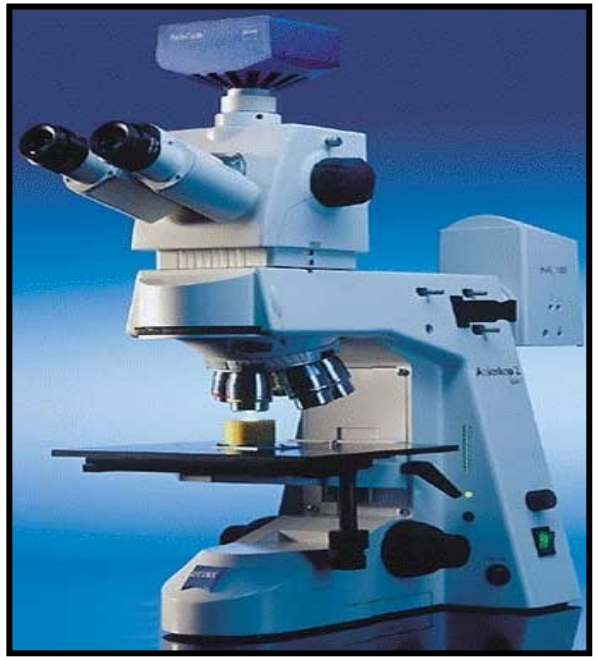
movie: https://www.nature.com/articles/ncomms11213#Sec11

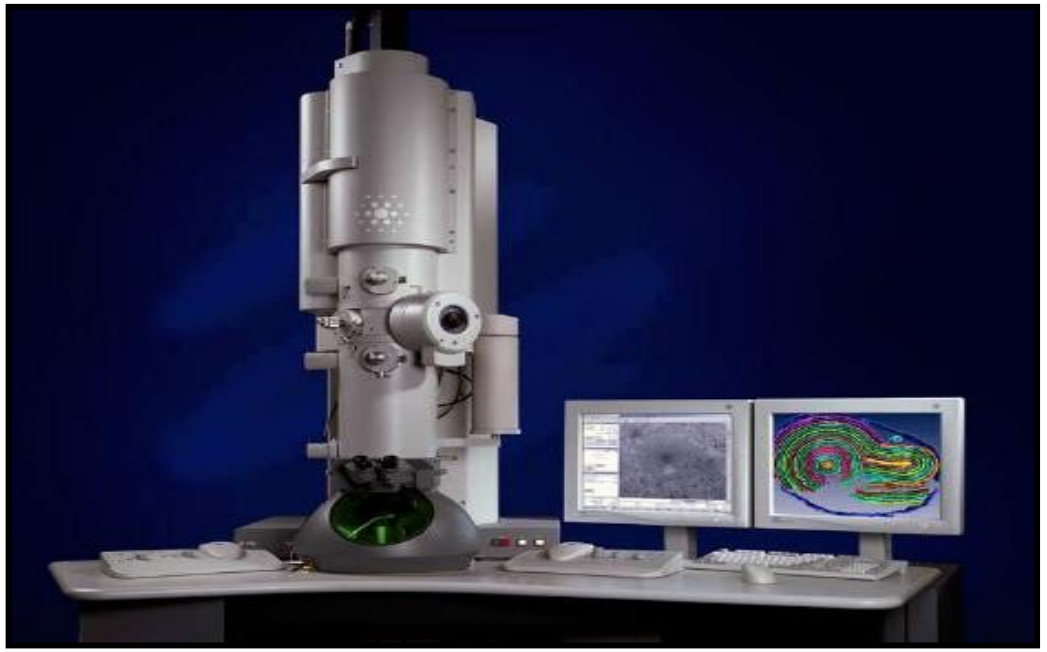
Correlative Microscopy

Standard correlative microscopy in 2 setups

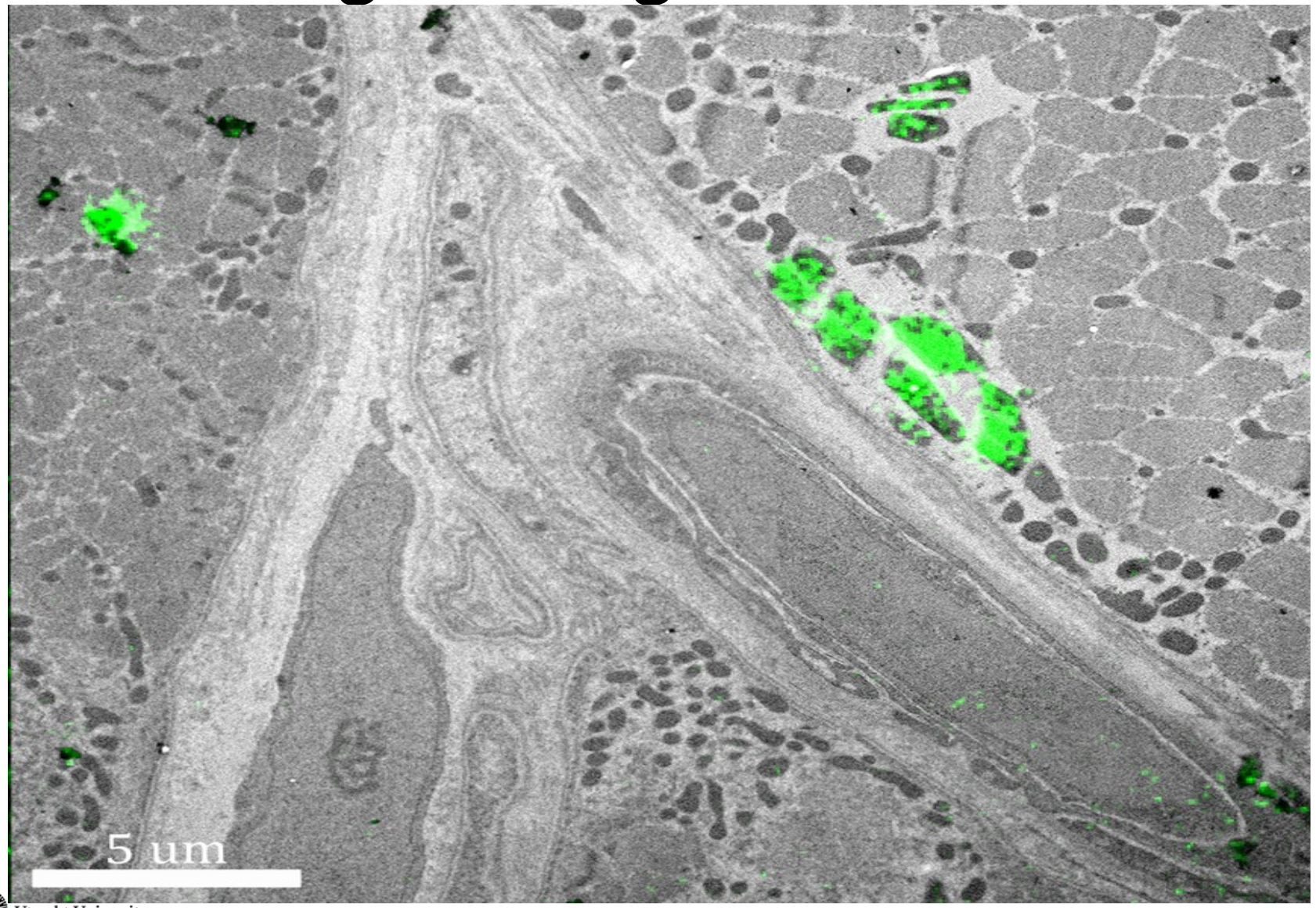
Fluorescence microscope: identify regions of interest

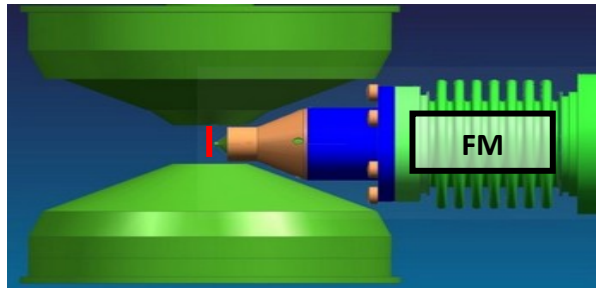
EM: image ultrastructure

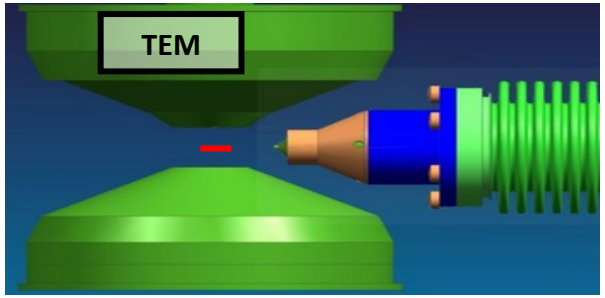


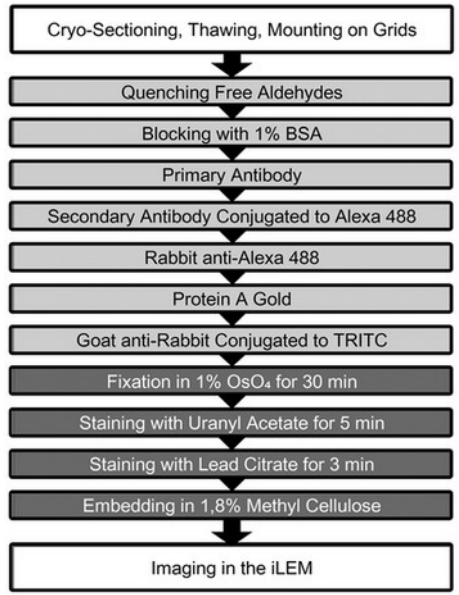


Integrated Light and Electron Microscopy





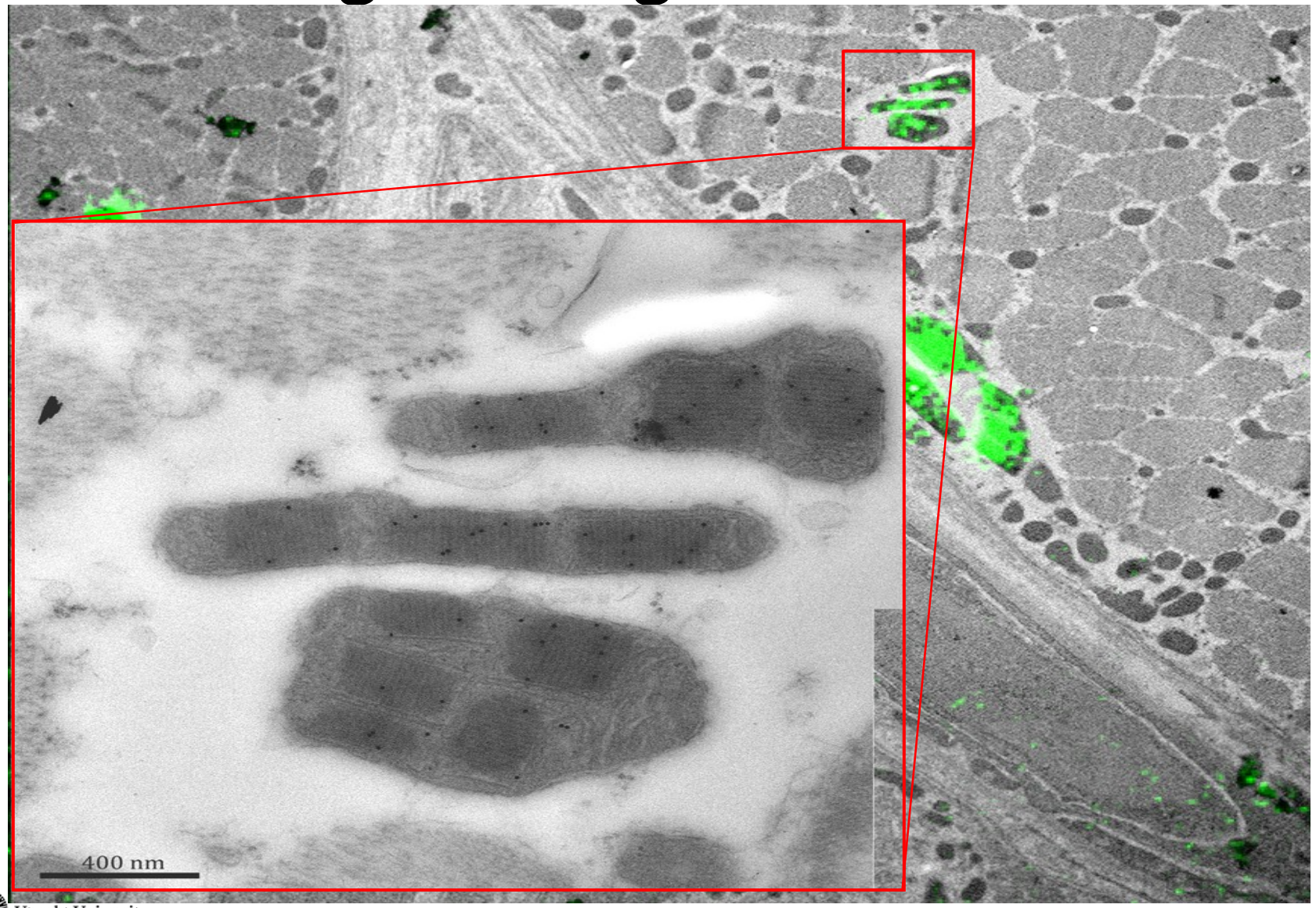


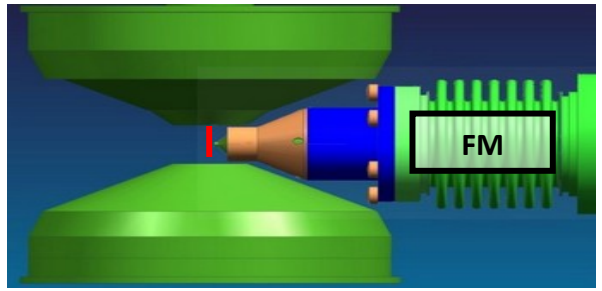


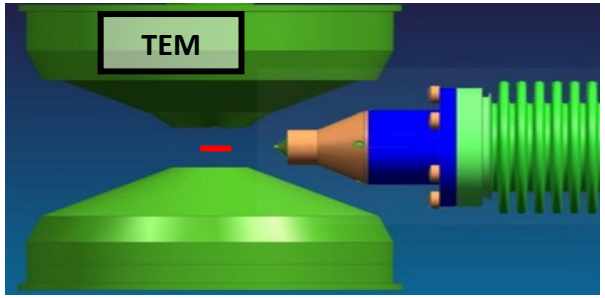
Utrecht University

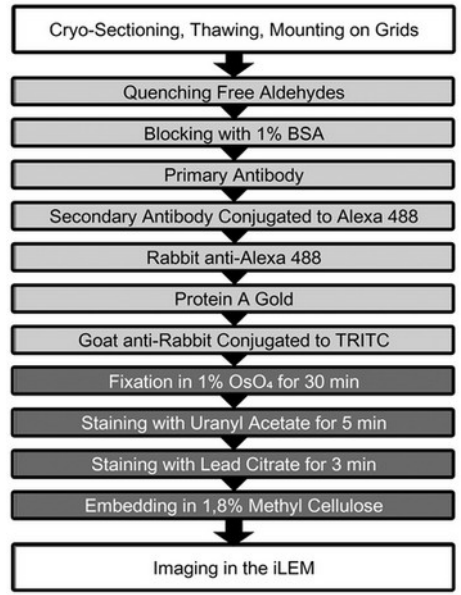
Karreman et al., DOI: 10.1369/0022155412473756 and unpublished data
Muscle tissue

Integrated Light and Electron Microscopy









Karreman et al., DOI: 10.1369/0022155412473756 and unpublished data

Utrecht University

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