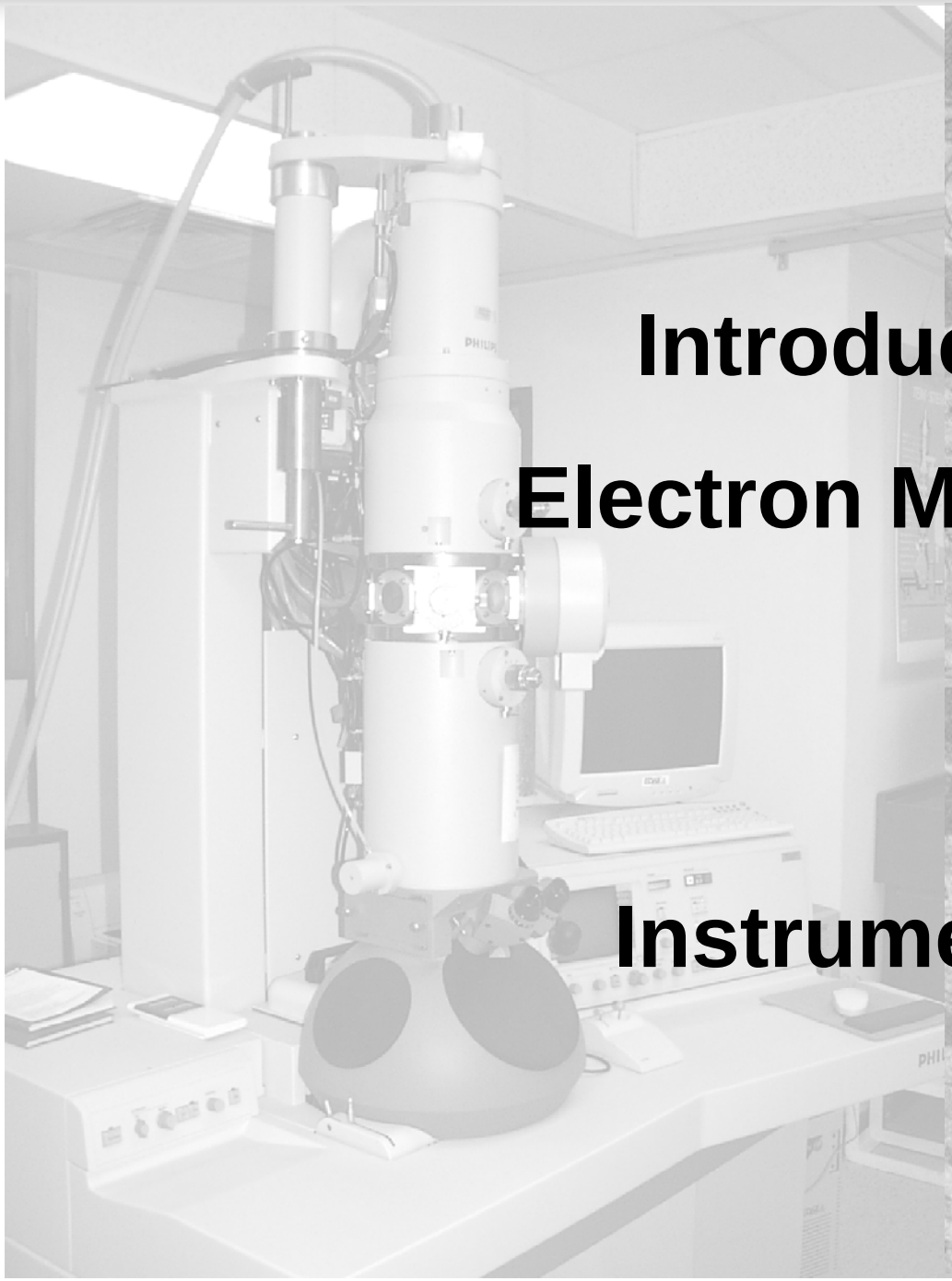
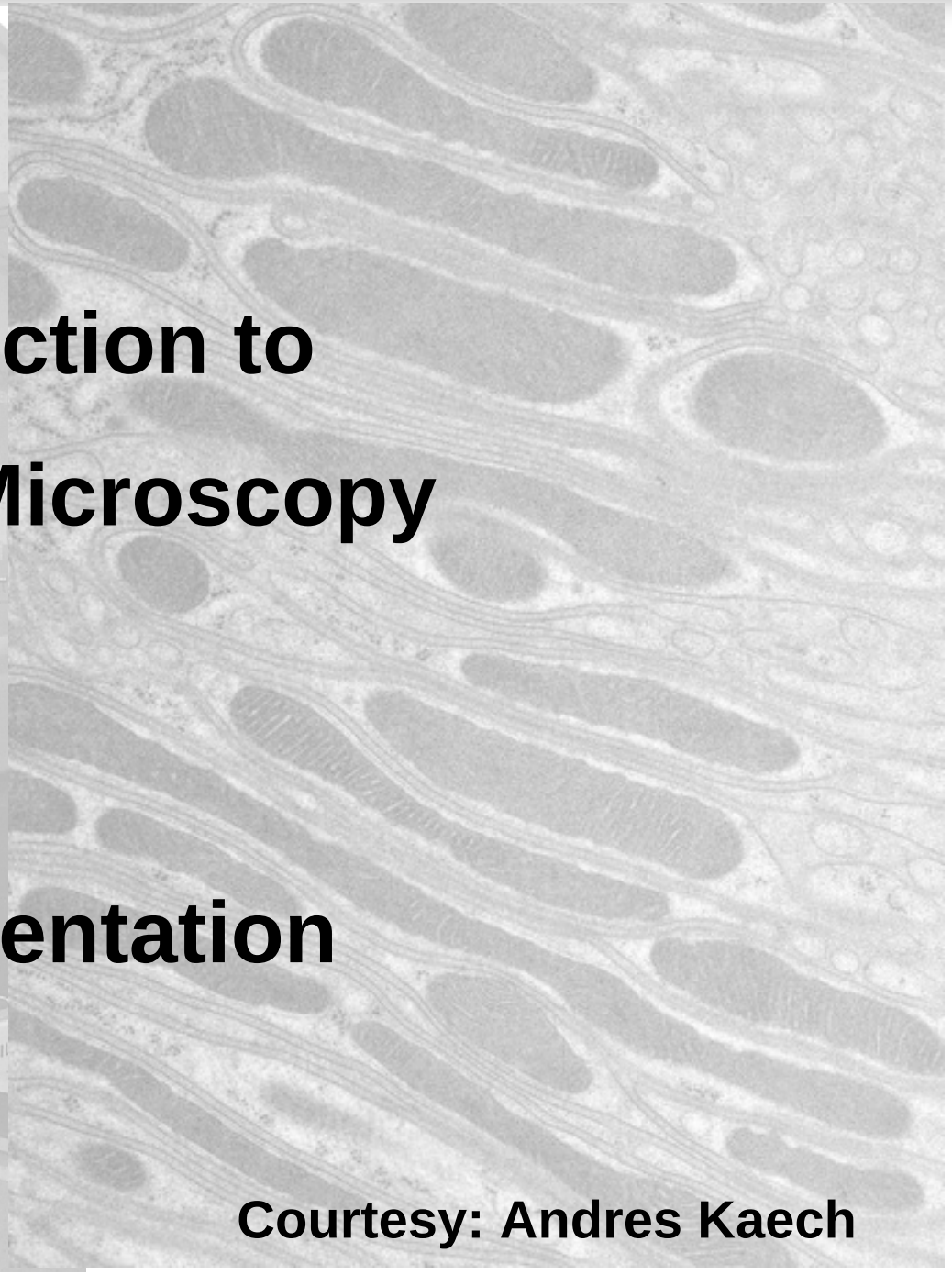


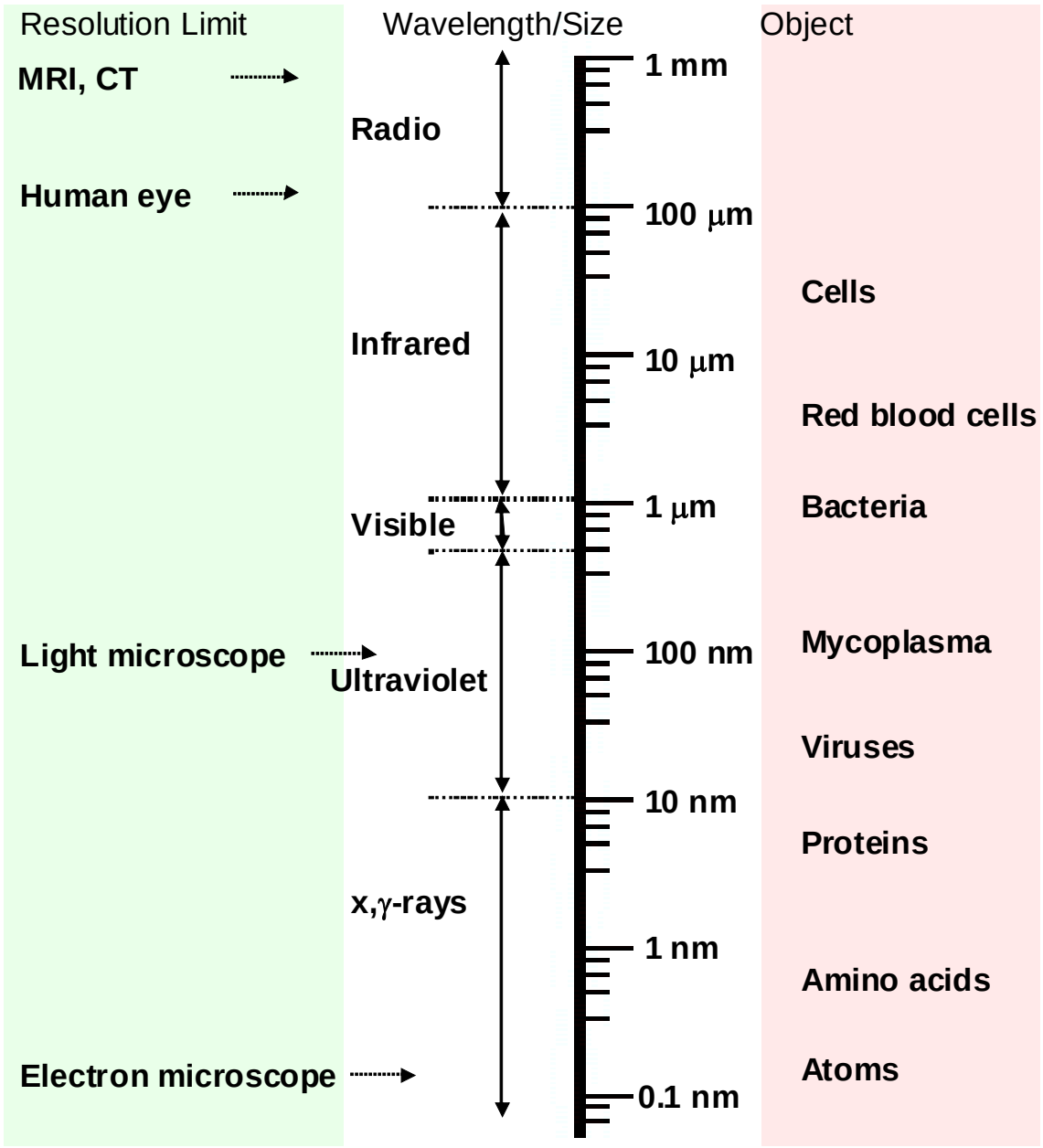
Introduction to Electron Microscopy

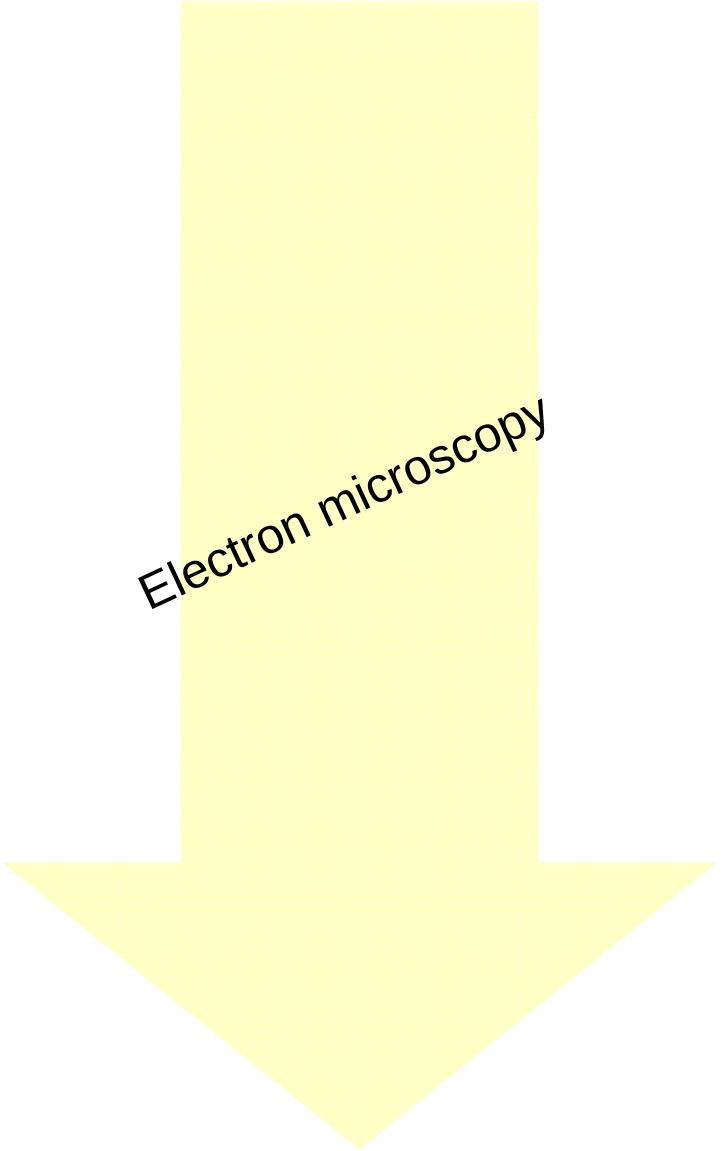
Instrumentation



Courtesy: Andres Kaech

Why electron microscopy?

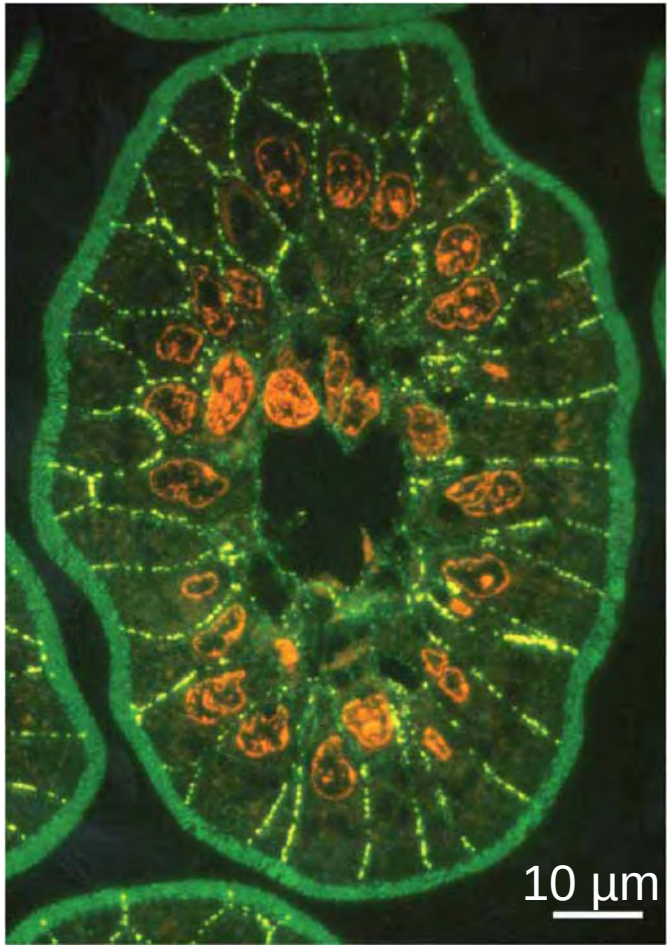




Why electron microscopy?

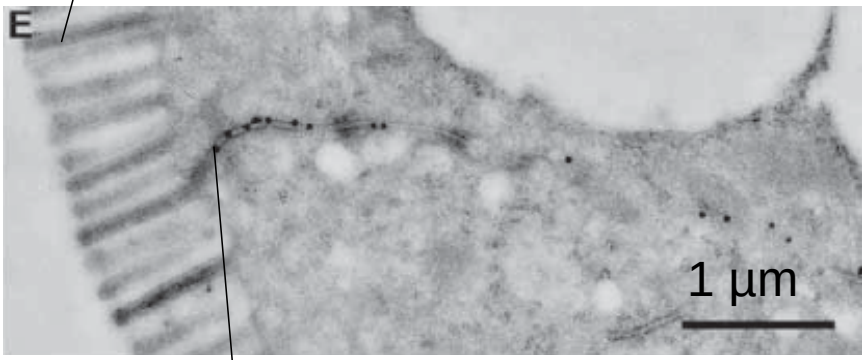
Rat intestine

Light microscopy



Green…F-actin
Yellow…ß-catenin
Orange…Nuclei

Electron microscopy

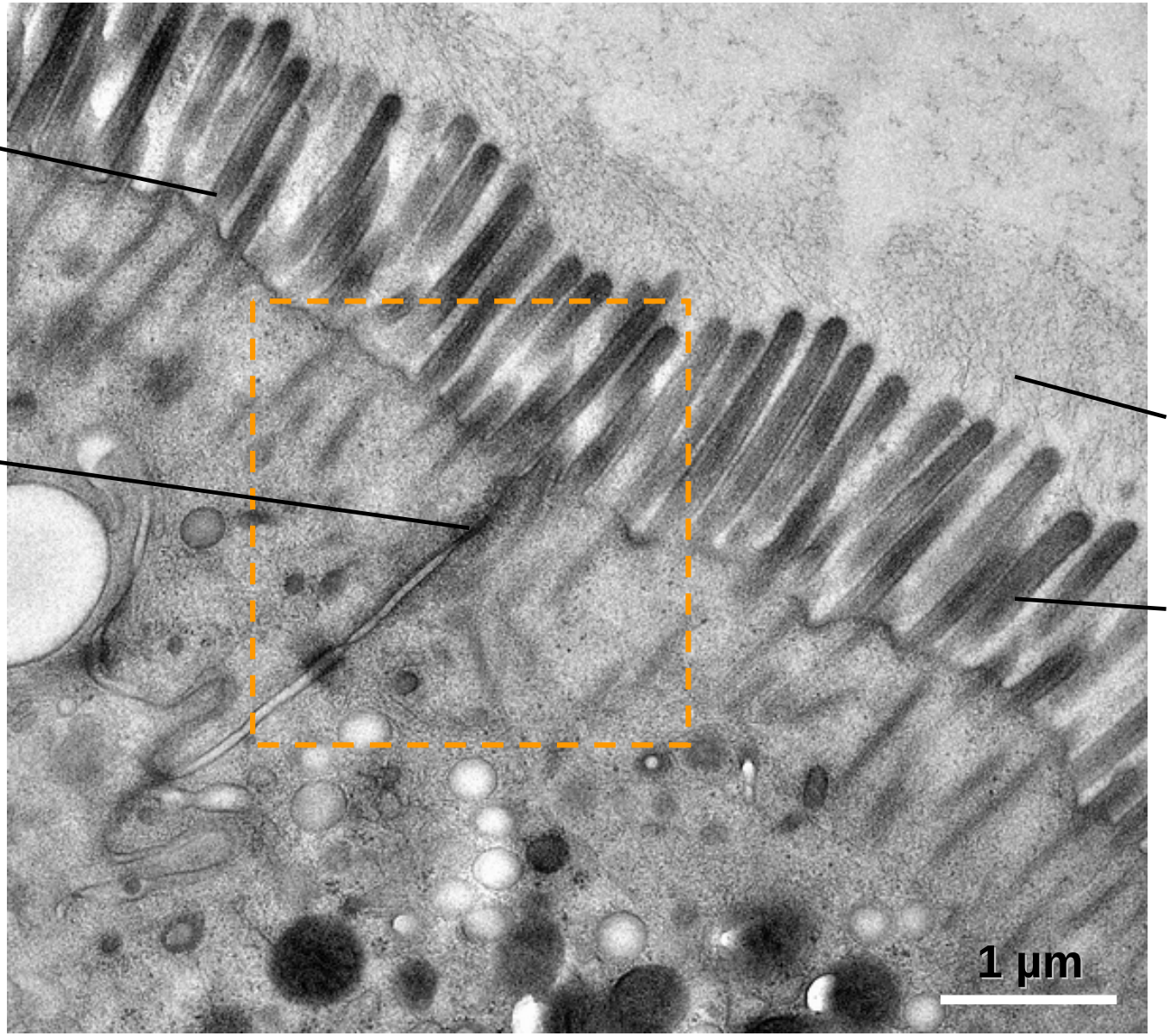


Adherence junction: ß-catenin visualized by immunolabelling using „immunogold“

Schwarz & Humbel 2007: Methods in Molecular Biology, vol. 369, Electron Microscopy: Methods and Protocols, Second Edition
Edited by: J. Kuo © Humana Press Inc., Totowa, NJ

Why electron microscopy?

Mouse intestine

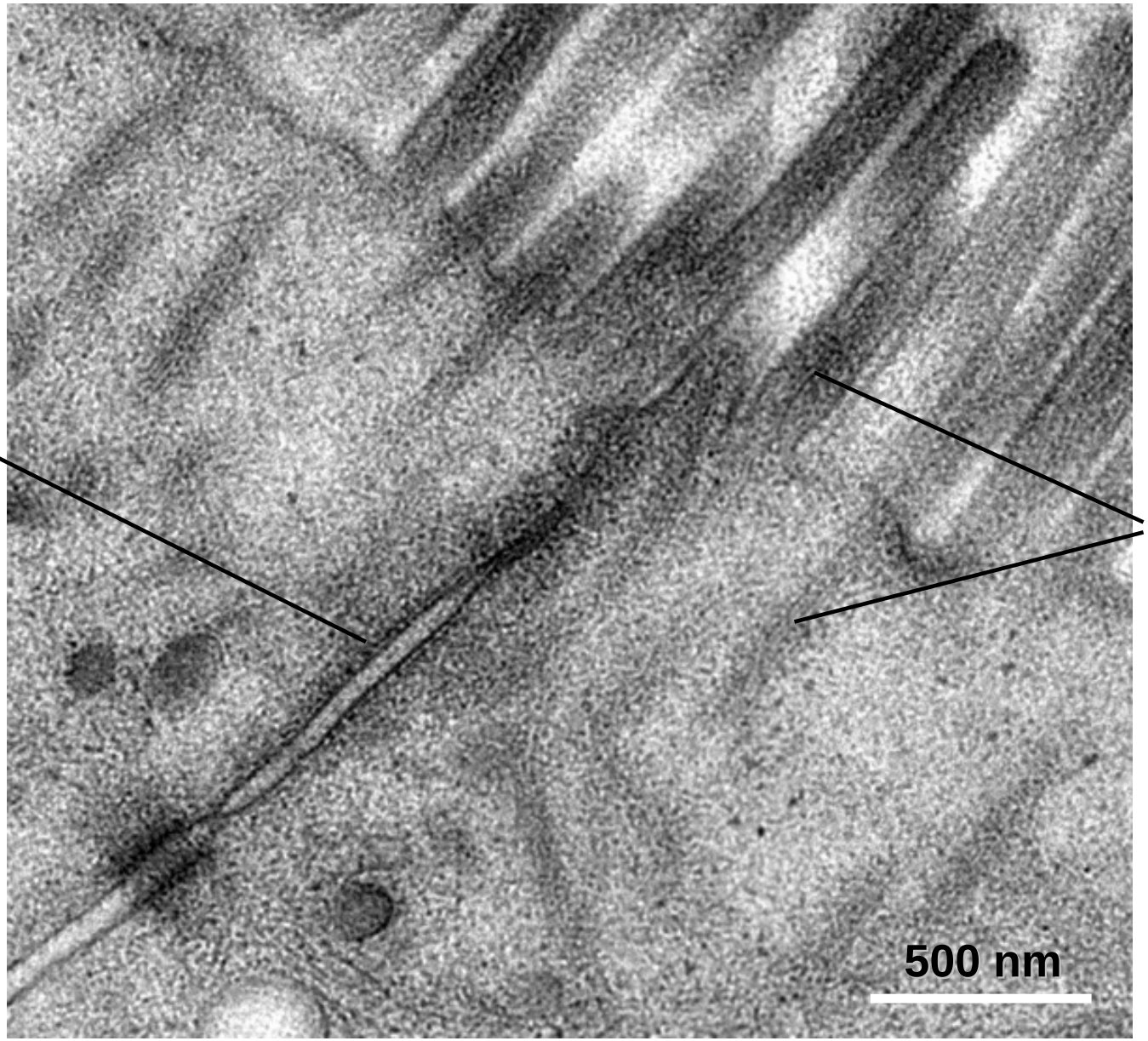


Elektronenmikroskopie ETH Zurich

Specimens courtesy of Bärbel Stecher, Institute of Microbiology, ETH Zurich

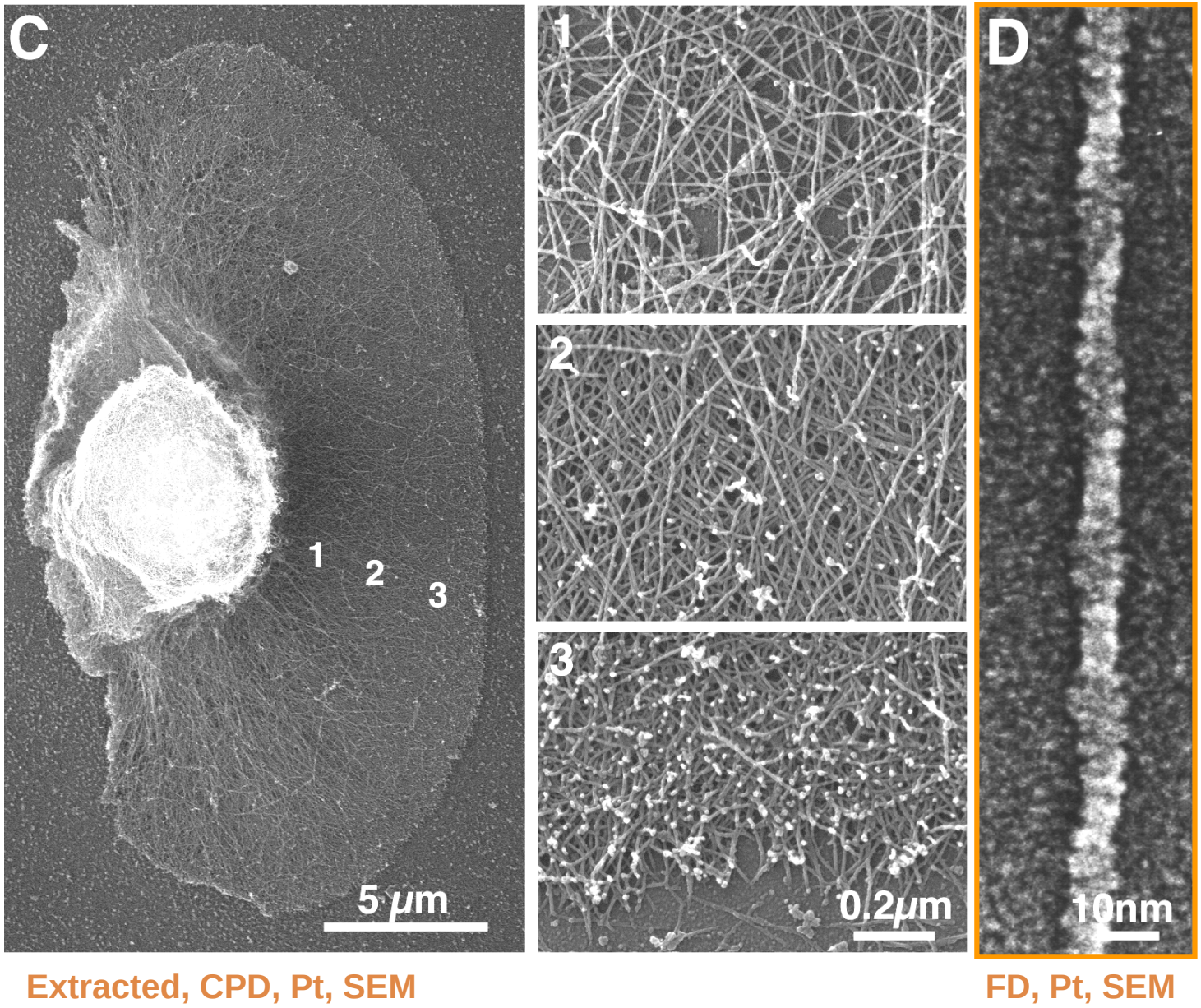
Why electron microscopy?

Mouse intestine



Why electron microscopy?

Up to macromolecular level



Extracted, CPD, Pt, SEM

FD, Pt, SEM

Cytosceleton after extraction

Single filaments and their fine structure are visible (here SEM)

The overall design of an electron microscope

is

similar to that of a light microscope.

From photons to electrons

Light microscopes

Photons

Photons are substituted with electrons

Glass lenses are substituted with electromagnetic and electrostatic lenses

Electron microscope

Electrons

e^-

From photons to electrons

e^- Similarities to photons:

→ Wave-particle duality of electrons

→ Optical properties
(Diffraction, chromatic abberation, spherical abberation, astigmatism etc.)

→ Resolution depends on aperture and wavelength
(Diffraction limited resolution)

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

Resolution of electron microscopes

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

DeBroglie relation: $\lambda = \frac{h}{m \cdot v}$

λ = wavelength
h = Planck's constant (6.6 X 10-27)
m = mass of the particle
v = velocity of the particle

Electron pathes through potential field

$\lambda = \frac{1.23}{\sqrt{V}} nm$

V = accelerating voltage

Abbe's equation $d = \frac{0.61 \cdot \lambda}{n \cdot \sin \alpha}$

$NA = n \cdot \sin \alpha$

For electron microscopes: n ≈ 1 and n*sinα ≈ α

Resolution EM: $d = \frac{0.753}{\alpha \cdot \sqrt{V}} nm$

d = resolution in nm
α = half opening angle of objective (in radians)
V = accelerating voltage

d (100 kV) = 0.24 nm

α ≈ 0.01 radians ≈ 0.6 grad

Resolution of electron microscopes

- Acceleration voltages of electrons:

  Transmission electron microscopes (TEM): 40 – 1200 kV
  Scanning electron microscopes (SEM): 1 – 30 kV

- Effective instrument resolution TEM: ≈ 0.1 nm

- Effective instrument resolution SEM: ≈ 1 nm

However:

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

The types of electron microscopes

Wide field microscopy

Photons

Confocal laser scanning microscope

Photons

Light is substituted with electrons

Glass lenses are substituted with electromagnetic and electrostatic lenses

Transmission electron microscope

Electrons

Scanning electron microscope

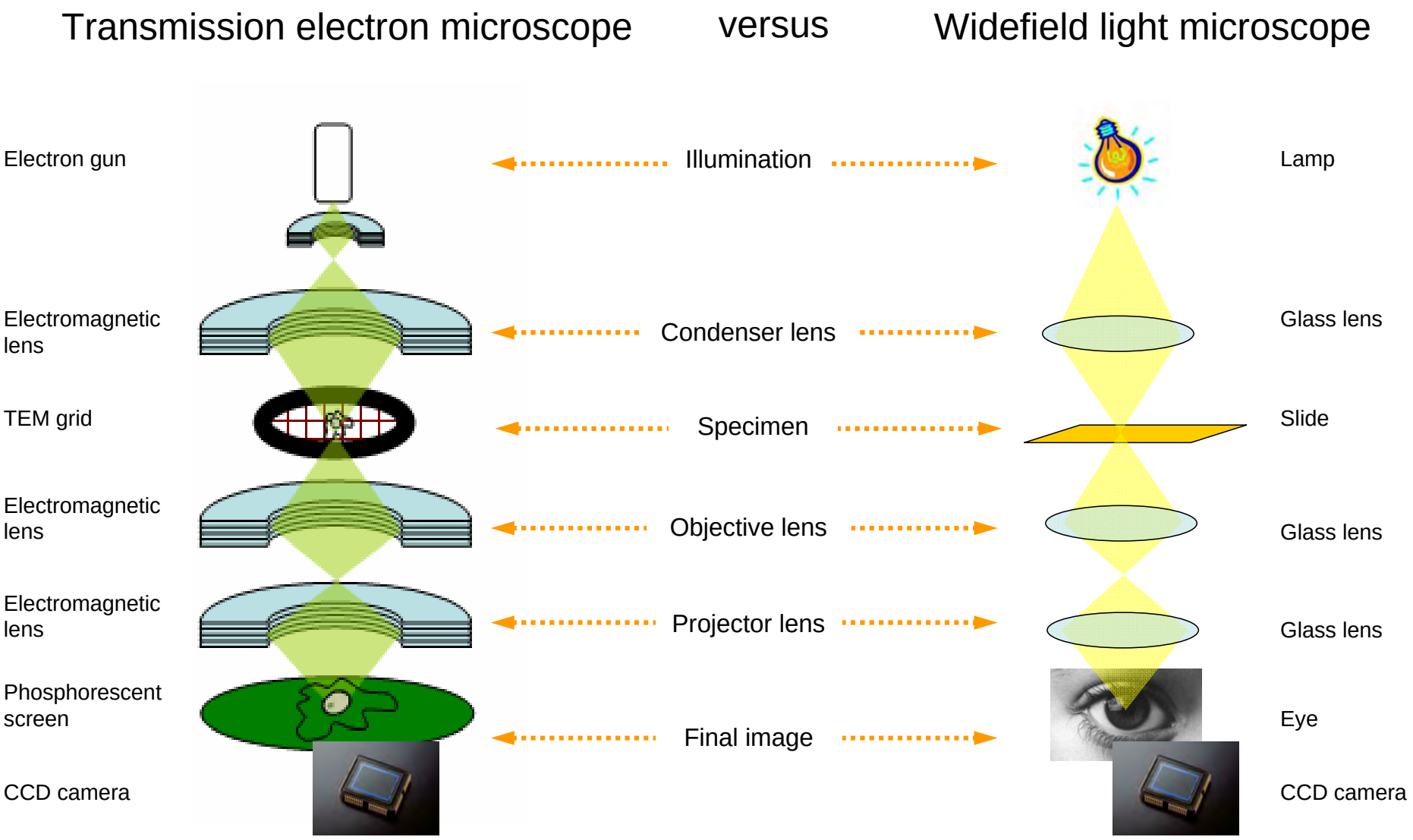
Electrons

The types of electron microscopes

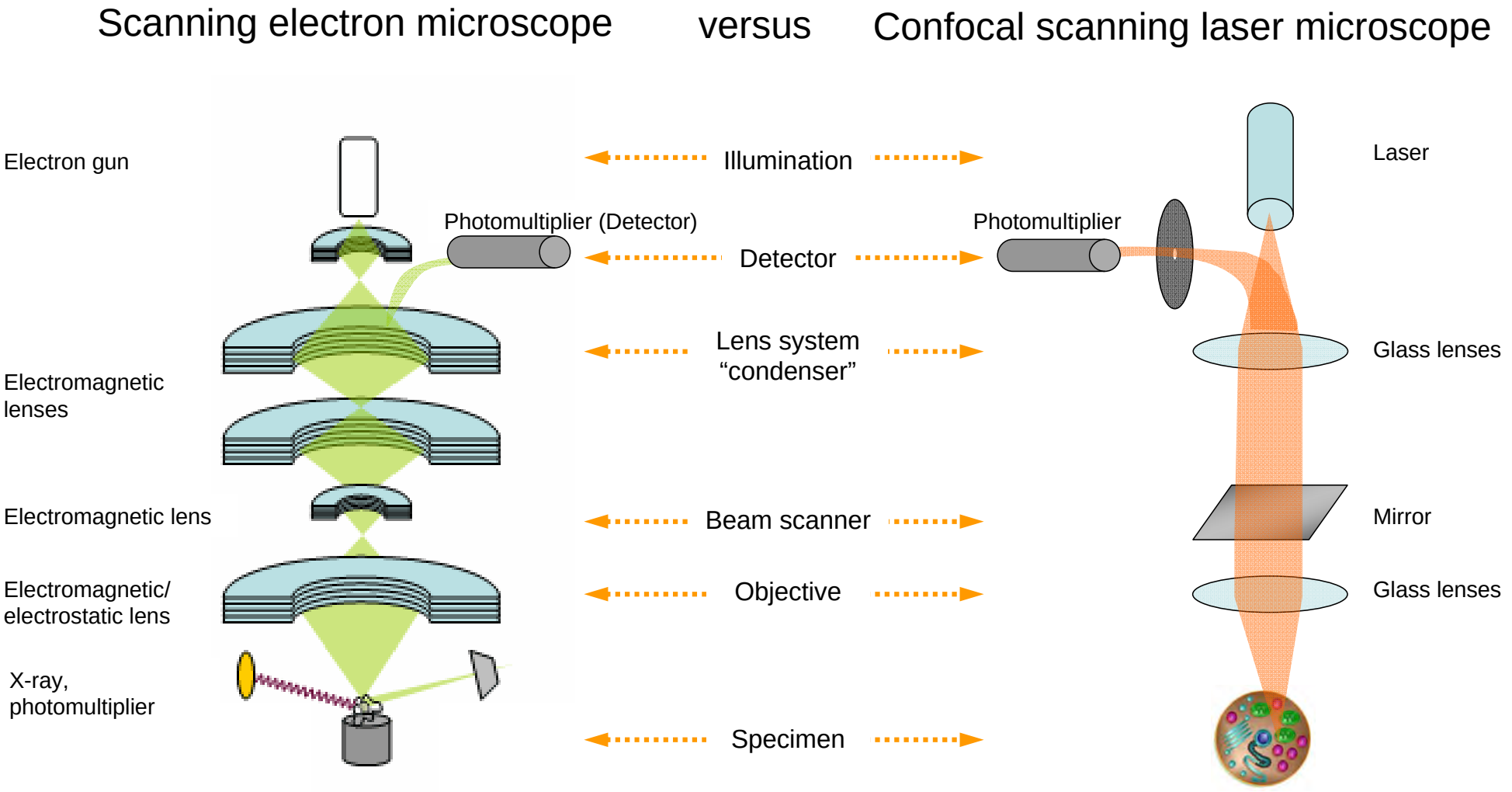
Transmission electron microscope (TEM)

Scanning electron microscope (SEM)

The types of electron microscopes



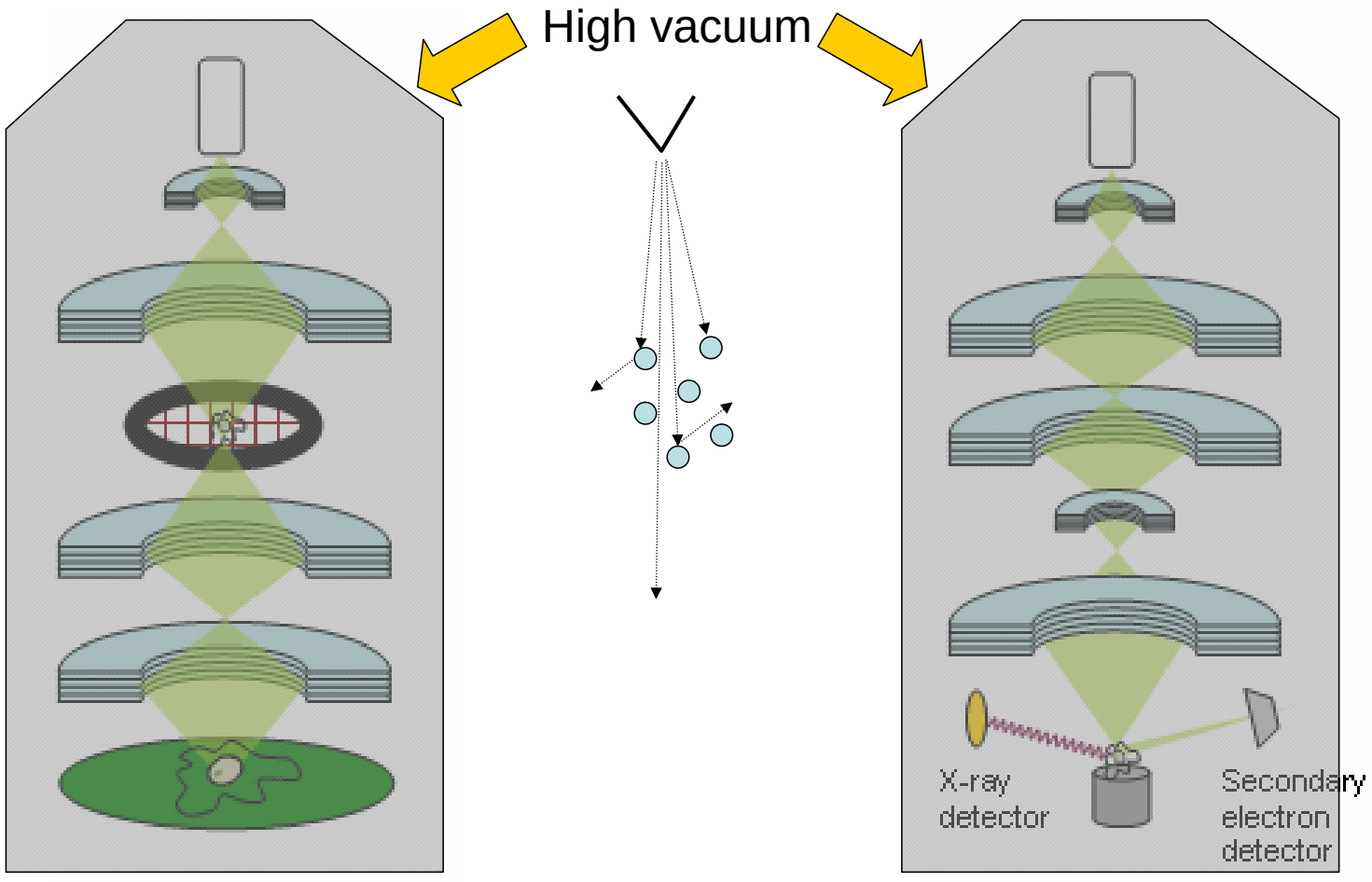
The types of electron microscopes



The types of electron microscopes

Transmission electron microscope (TEM)

Scanning electron microscope (SEM)



Without vacuum:

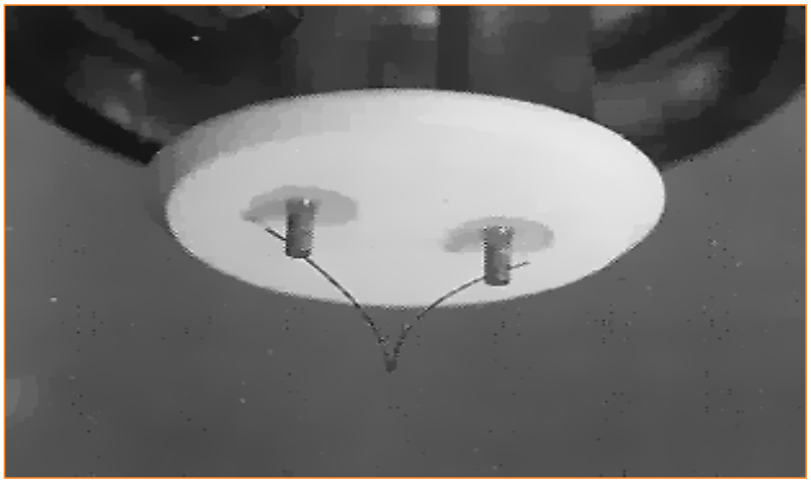
- Electrons would collide with gas molecules
- Electron source (tungsten) would blow

Components of electron microscopes

Electron source (Electron gun)

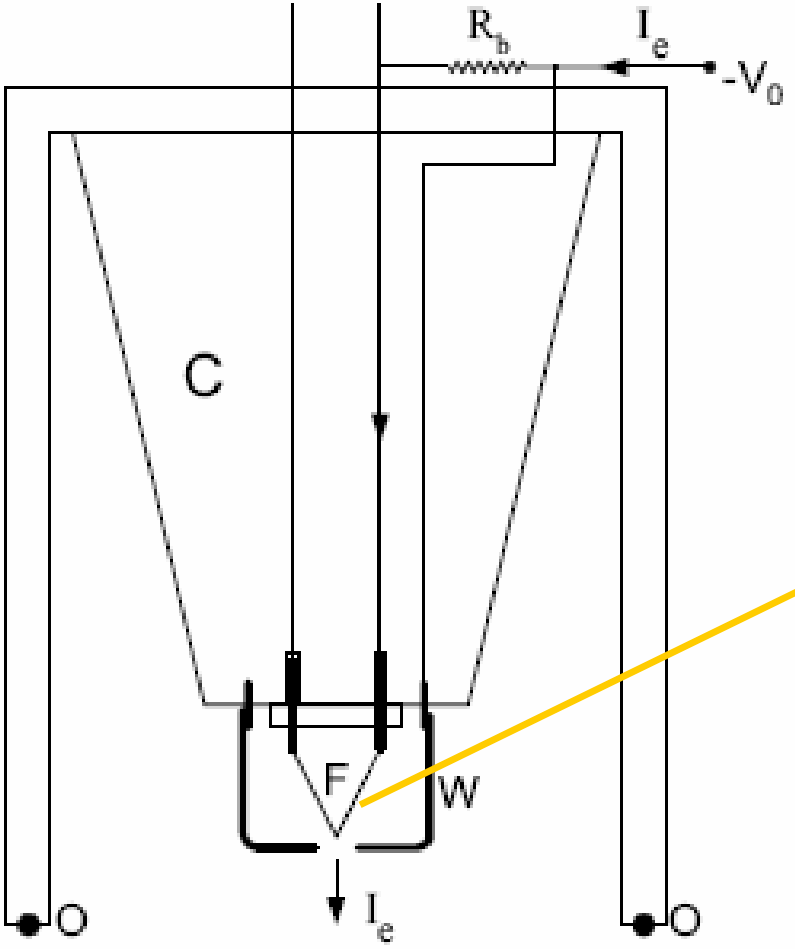
Light microscope: tungsten filament (bright field)

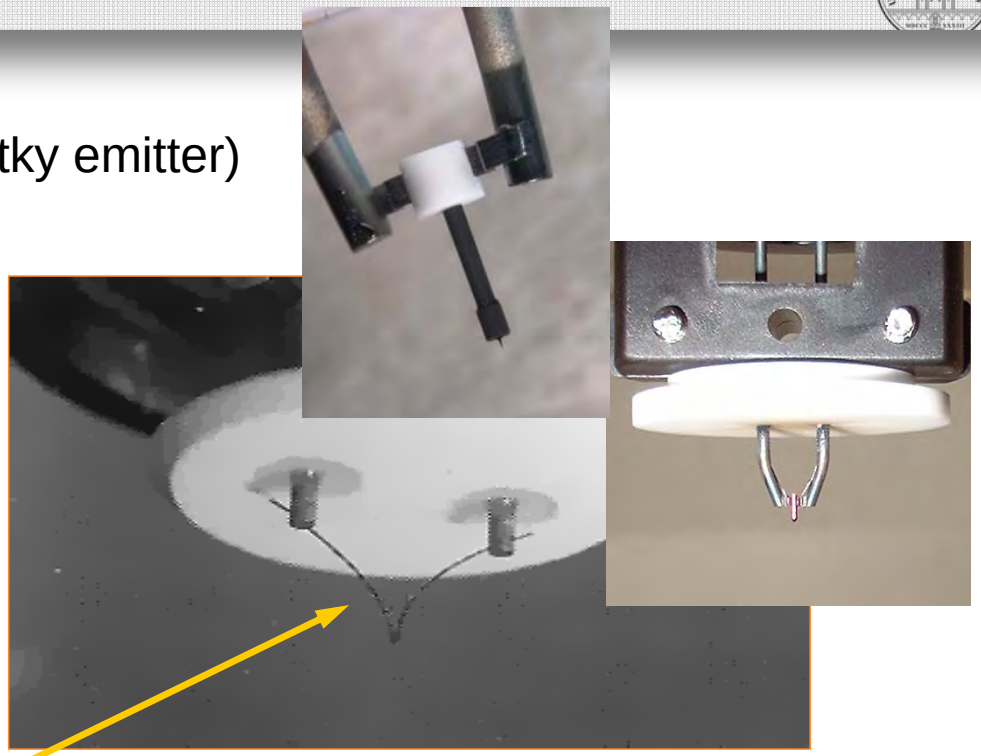
Electron microscope: tungsten filament (common form)



Electron source (Electron gun)

Thermionic emission (tungsten, LaB6, Schottky emitter)





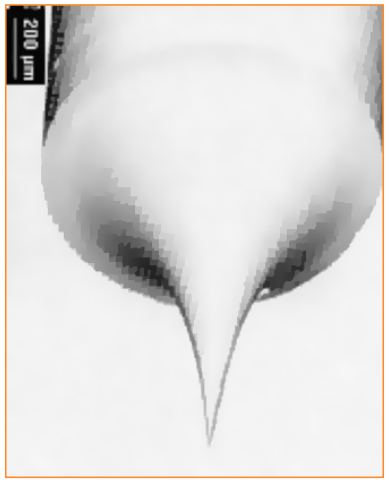
Filament is heated

Electrons are emitted from the tip

F…Filament
W…Wehnelt electrode
C…Ceramic high voltage insulator
Rb…Autobias resistor
Ie…Electron emission current

Electron source (Electron gun)

Cold field emission (quantum-mechanical tunneling)

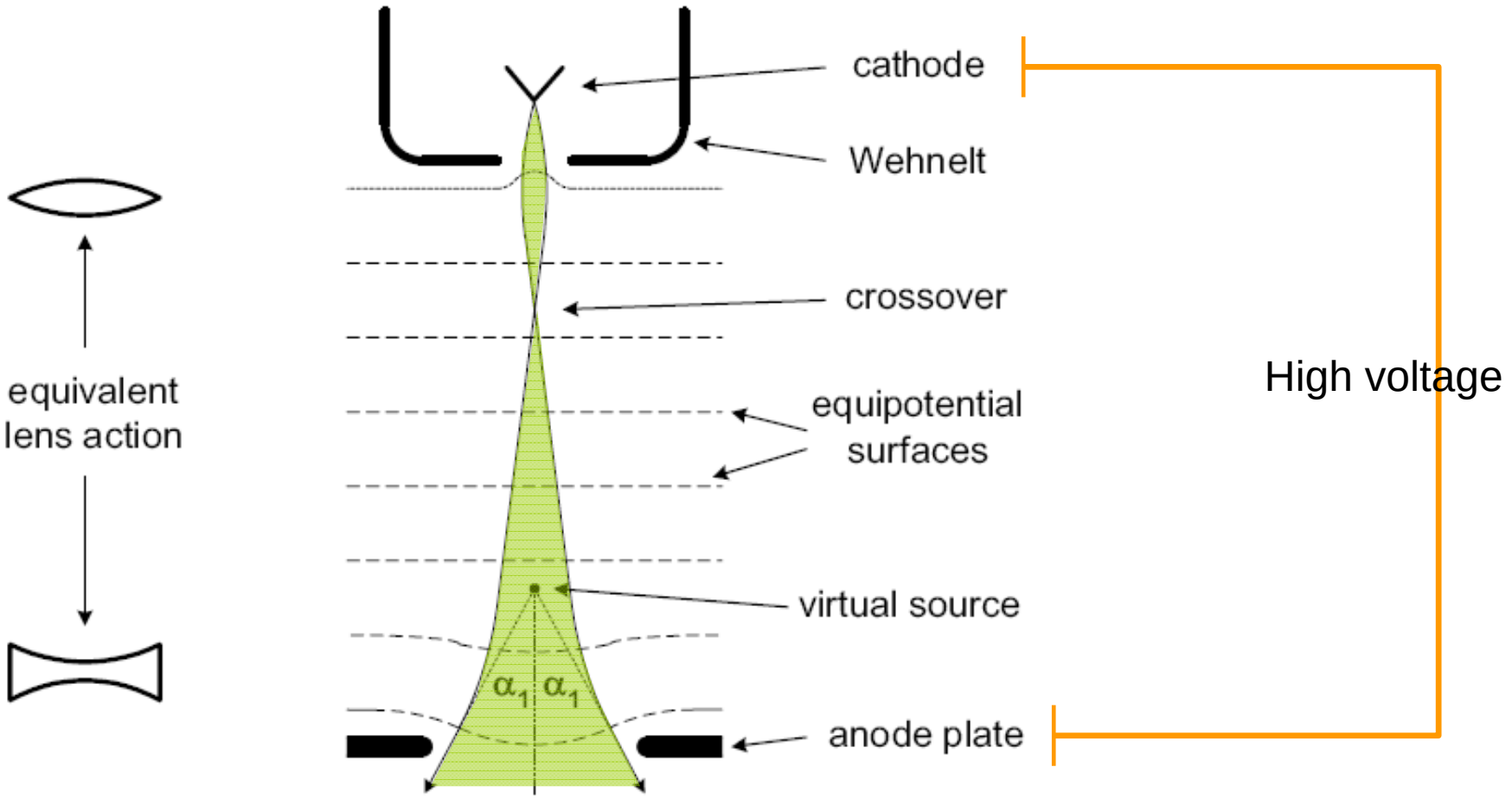


Very fine tungsten tip

No heating required (room temperature)

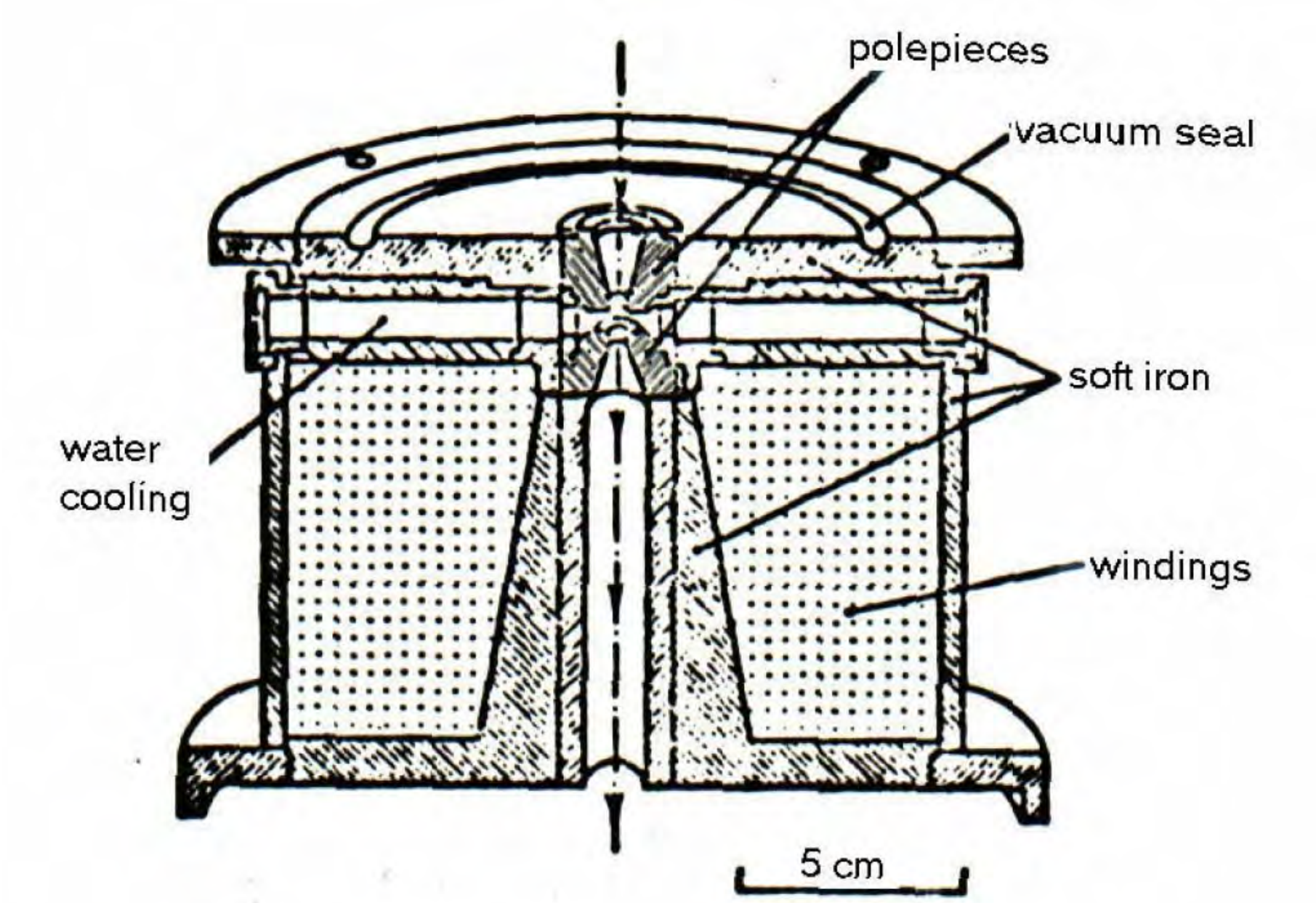
	Thermionic			Cold field emission
	Tungsten	LaB6	Schottky	
Material	W	LaB6	ZrO/W	W
Heating temp. (K)	2700	1800	1800	300
Normalized brightness				
Required vacuum (Pa)	high			Ultra high
ΔE (eV)	Chromatic aberration!			

Electron source (Electron gun)



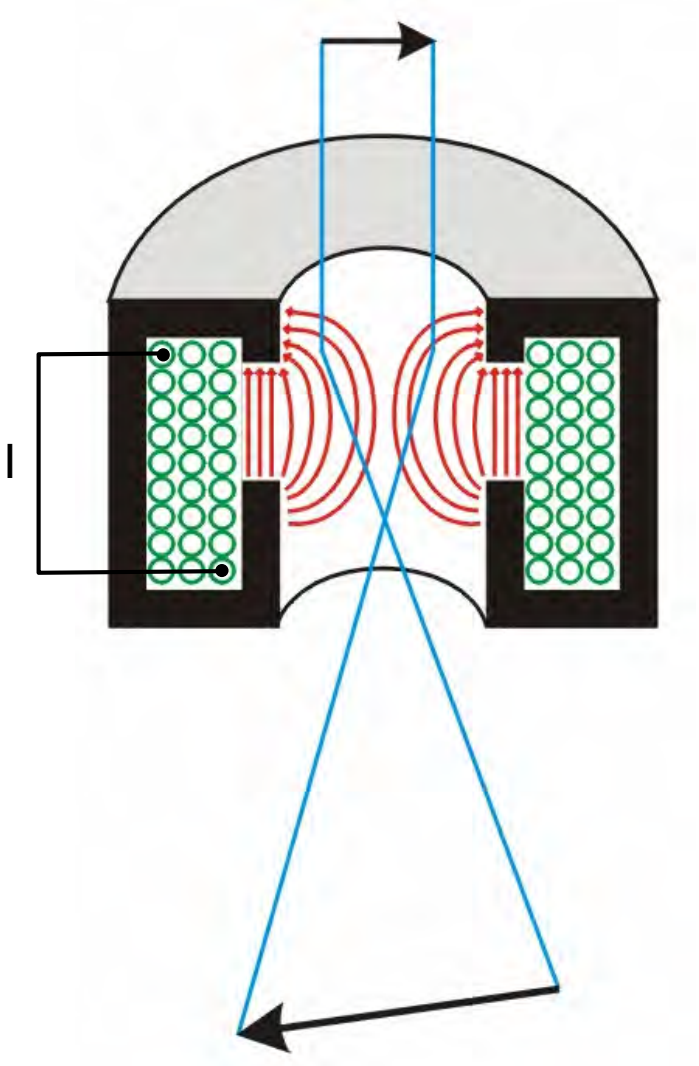
Electromagnetic lenses

Electromagnetic lens of a transmission electron microscope

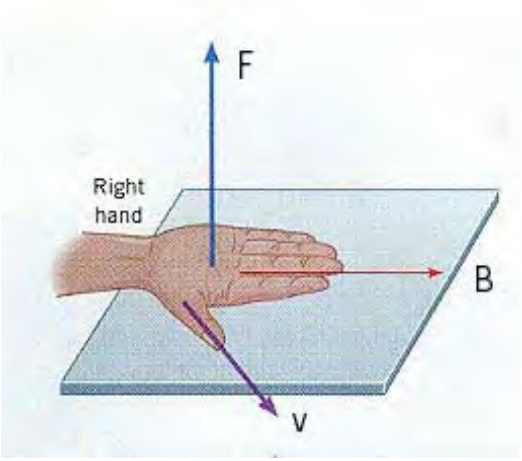


Electromagnetic lenses

Magnetic field depends on current and number of windings



Electrons are deviated in a magnetic field



v…speed of electron
B…magnetic field
F…resulting force

Note: Force is perpendicular to the plain defined by B and v

Electromagnetic lenses

Image rotation:

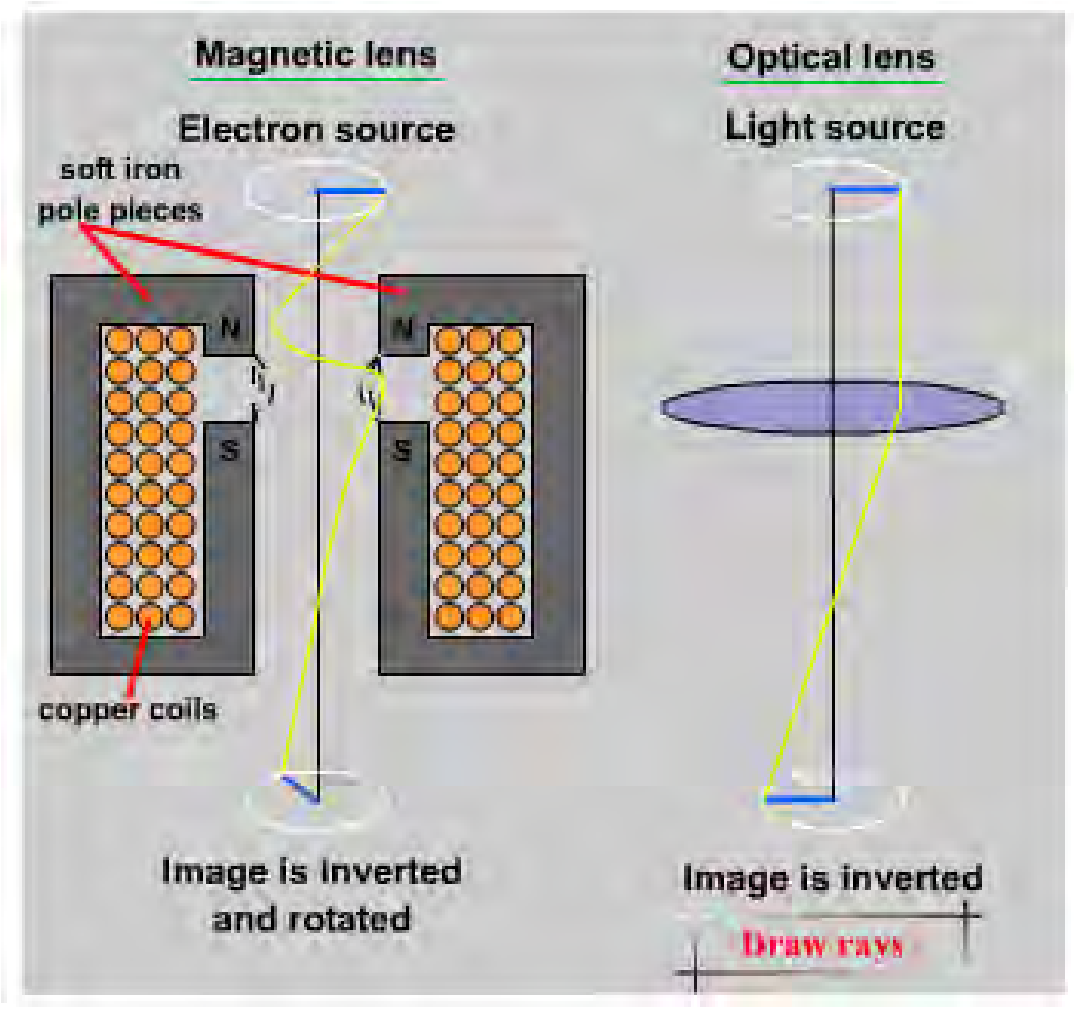
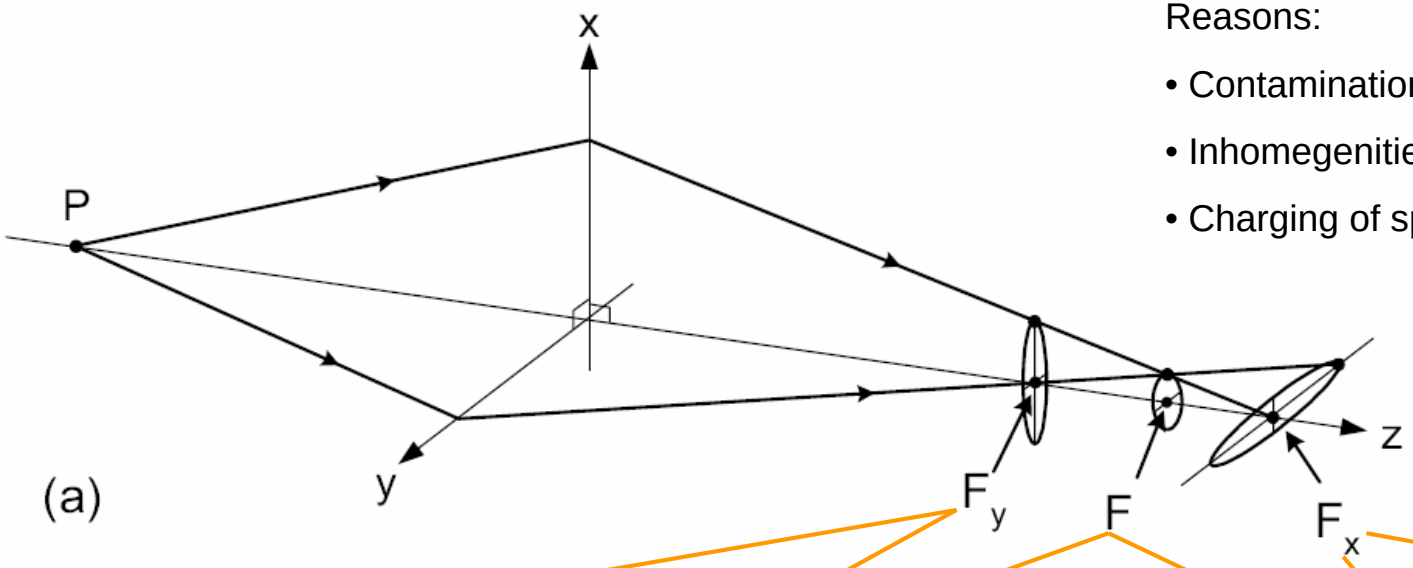


Image rotation is corrected in modern microscopes

Electromagnetic lenses

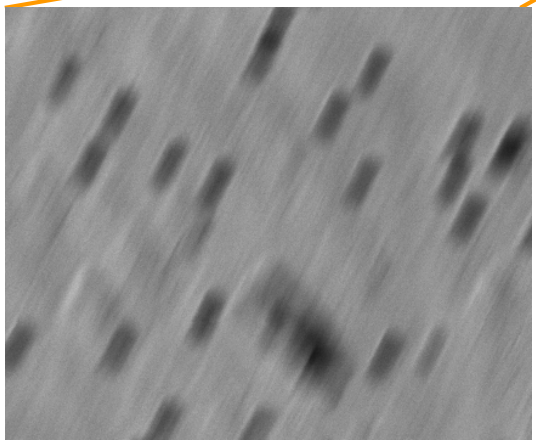
Axial astigmatism of electromagnetic lenses … confusion of the image

Most relevant aberration in biological electron microscopy (in particular SEM)

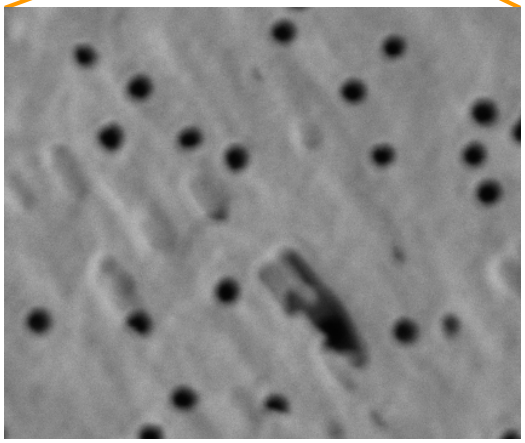


Reasons:

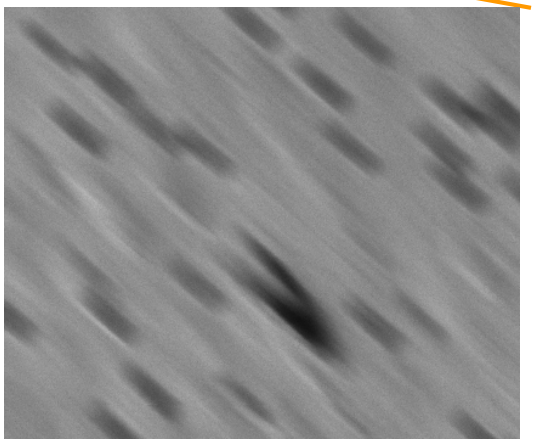
- Contamination of lenses and apertures
- Inhomegenities of the lens
- Charging of specimen



Under focussed image
elliptic deformation



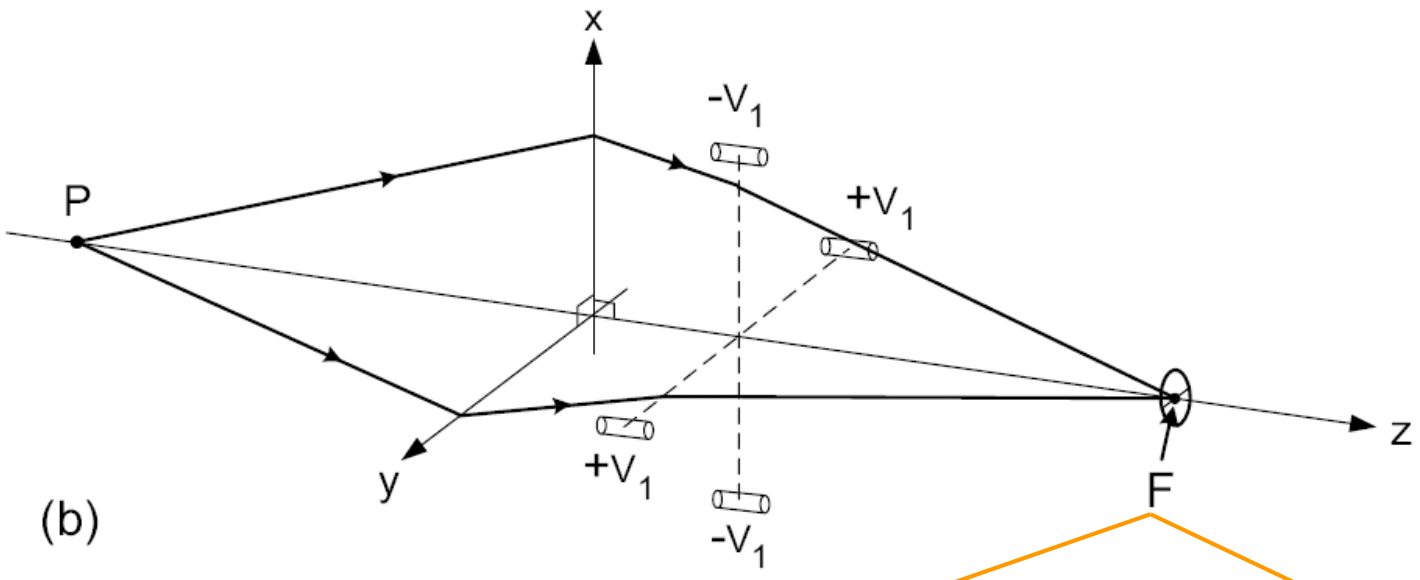
Focus
circle of least confusion

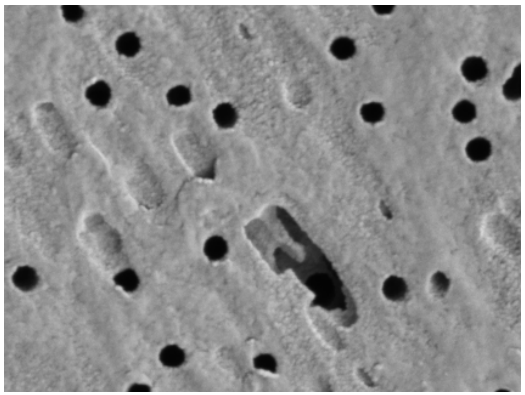


Over focussed image
elliptic deformation

Electromagnetic lenses

Correction of astigmatism with corrector coils



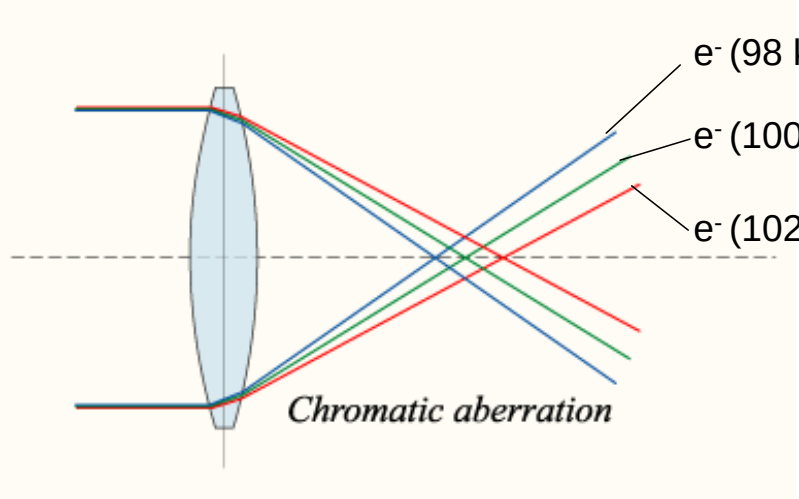


Focus, corrected astigmatism
circle of confusion minimized

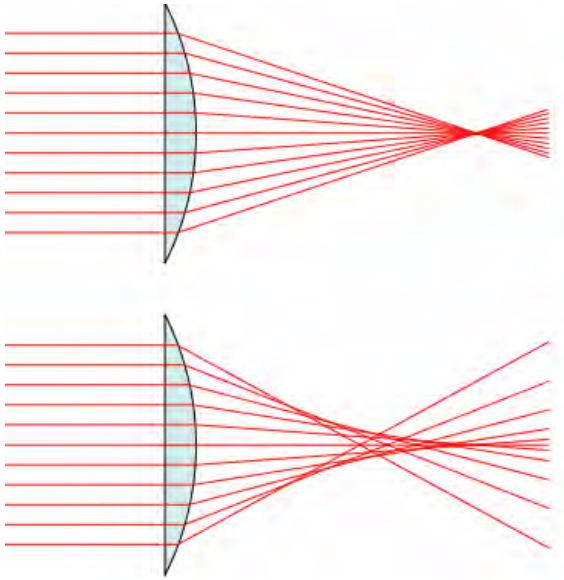
Electromagnetic lenses

Chromatic aberration

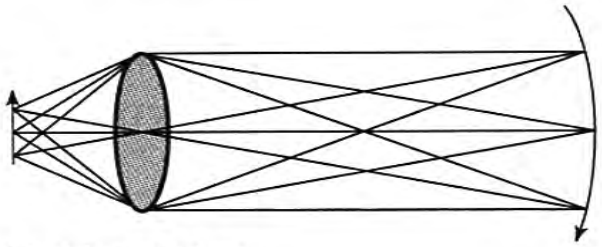
Due to energy difference of electrons (wavelength)

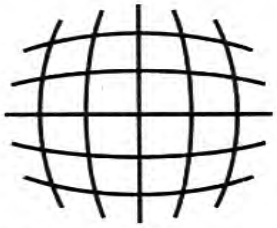


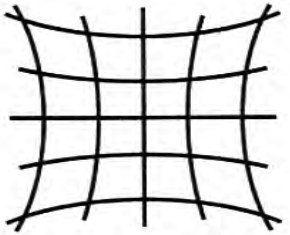
Spherical aberrations



Curvature and distortion of field

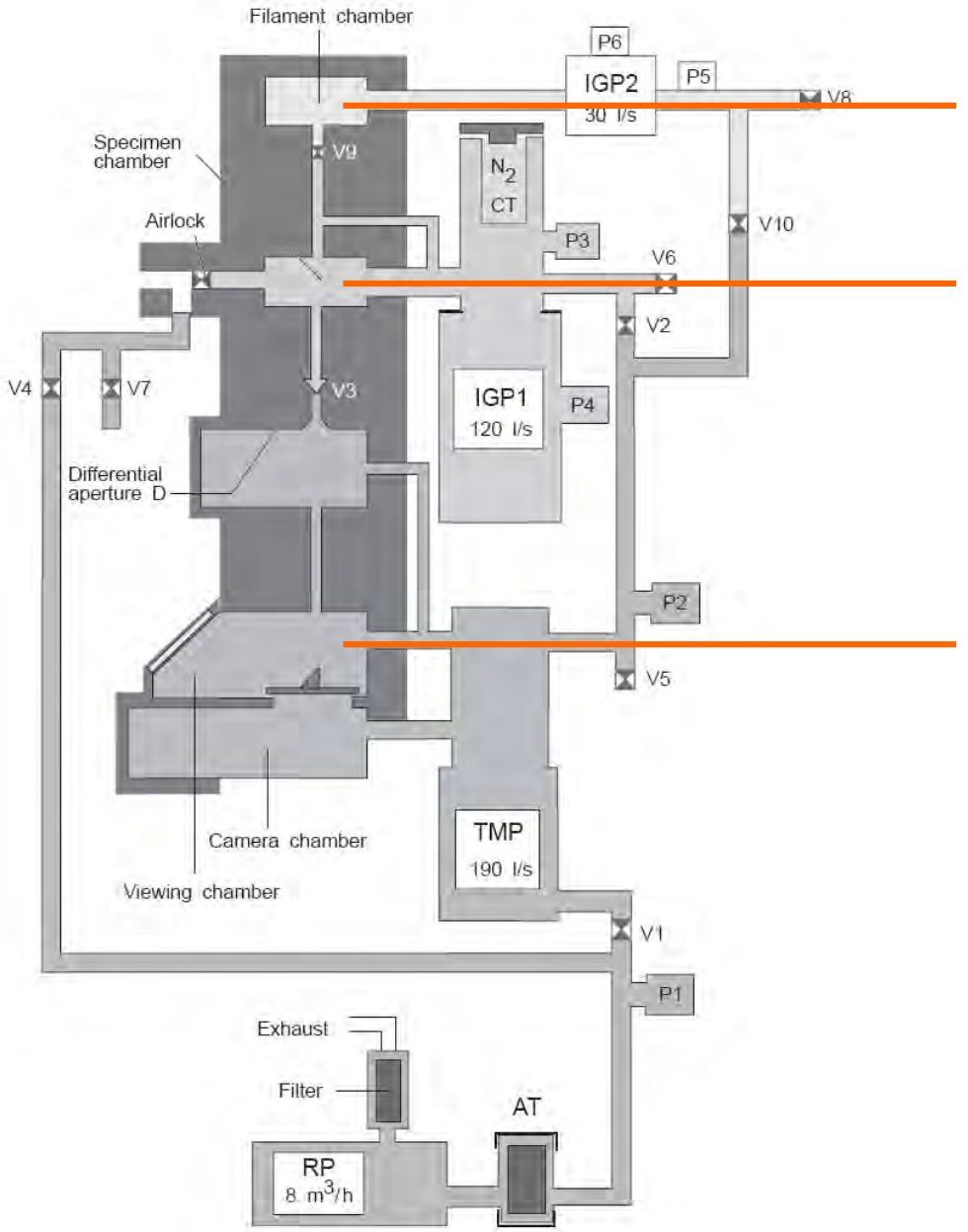

Field curvature


Barrel distortion


Pincushion distortion

Vacuum systems

Transmission electron microscope



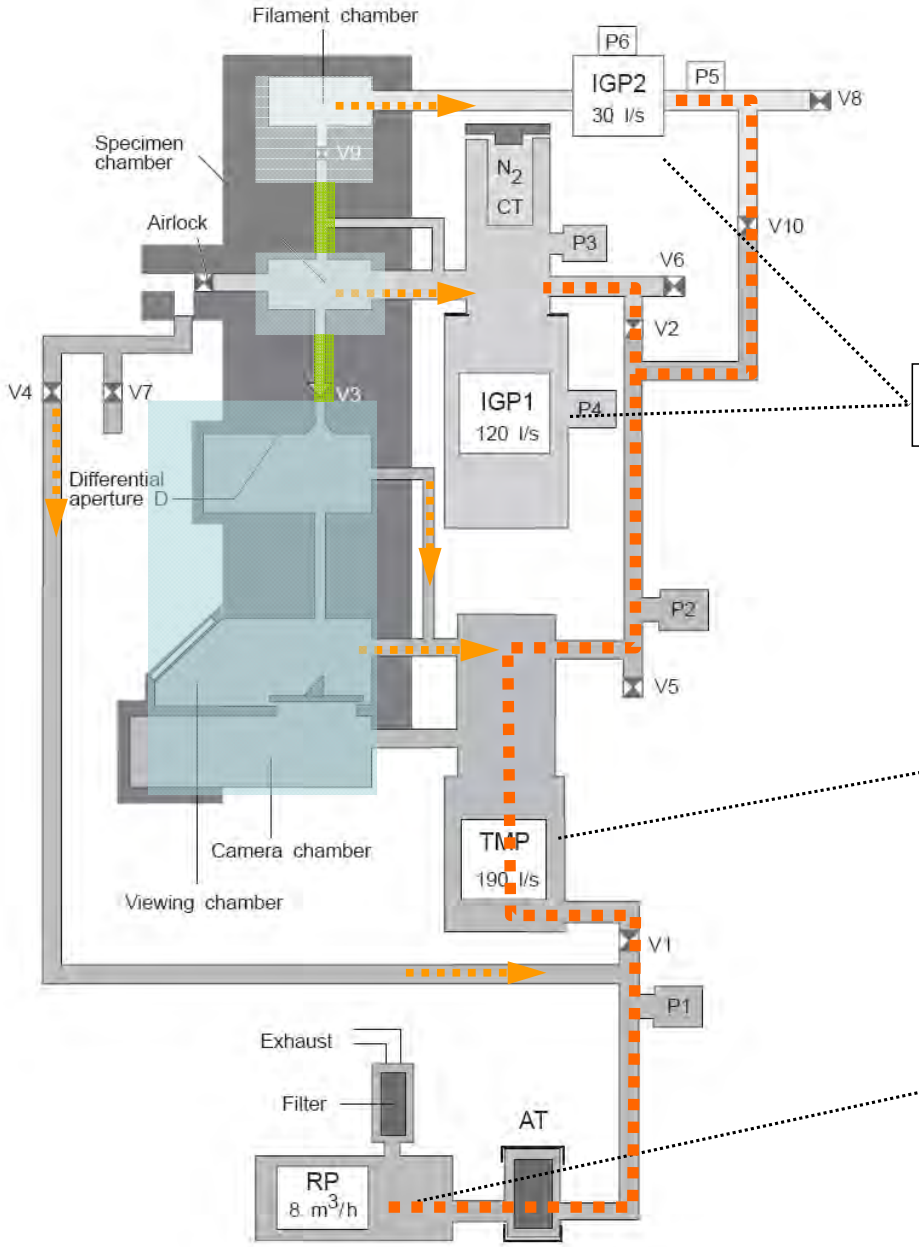
Filament chamber
Ultra high vacuum: < 10^{-9} mbar

Specimen chamber
High vacuum: ~ 10^{-7} mbar

Viewing chamber
High vacuum: ~ 10^{-5} mbar

Vacuum systems

Transmission electron microscope



10^{-7} - 10^{-10} mbar

Ion getter pump / Oil diffusion pump

10^{-5} - 10^{-7} mbar

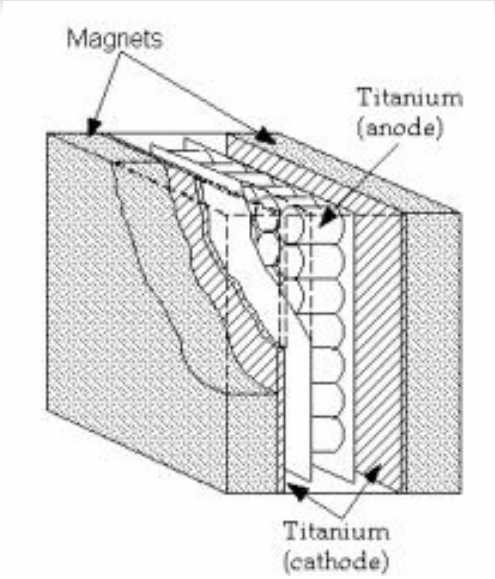
Turbo molecular pump

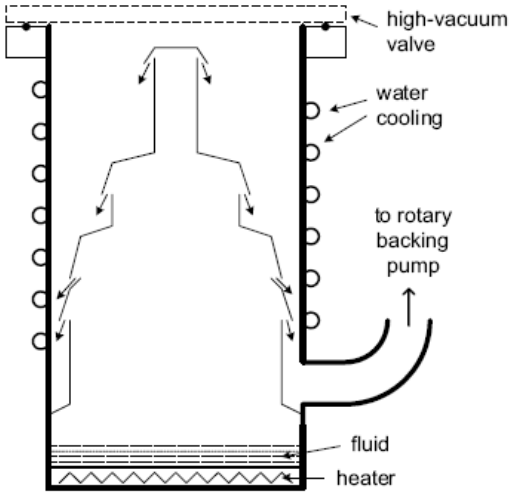
10^{-0} - 10^{-2} mbar

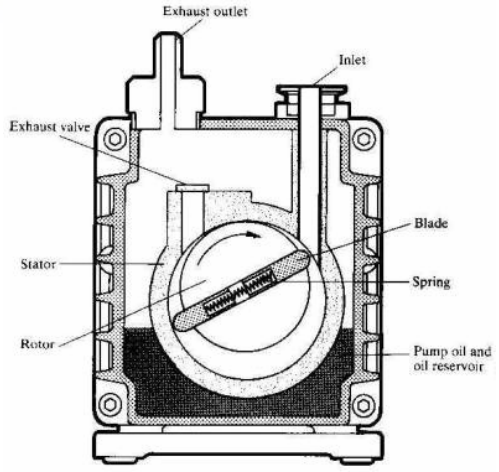
Rotary pump

Atmosphere: 1000 mbar

Vacuum systems







10^{-7} - 10^{-10} mbar

Ion getter pump / Oil diffusion pump

10^{-5} - 10^{-7} mbar

Turbo molecular pump

10^{-0} - 10^{-2} mbar

Rotary pump

Atmosphere: 1000 mbar

Vacuum systems

Properties of vacuum systems

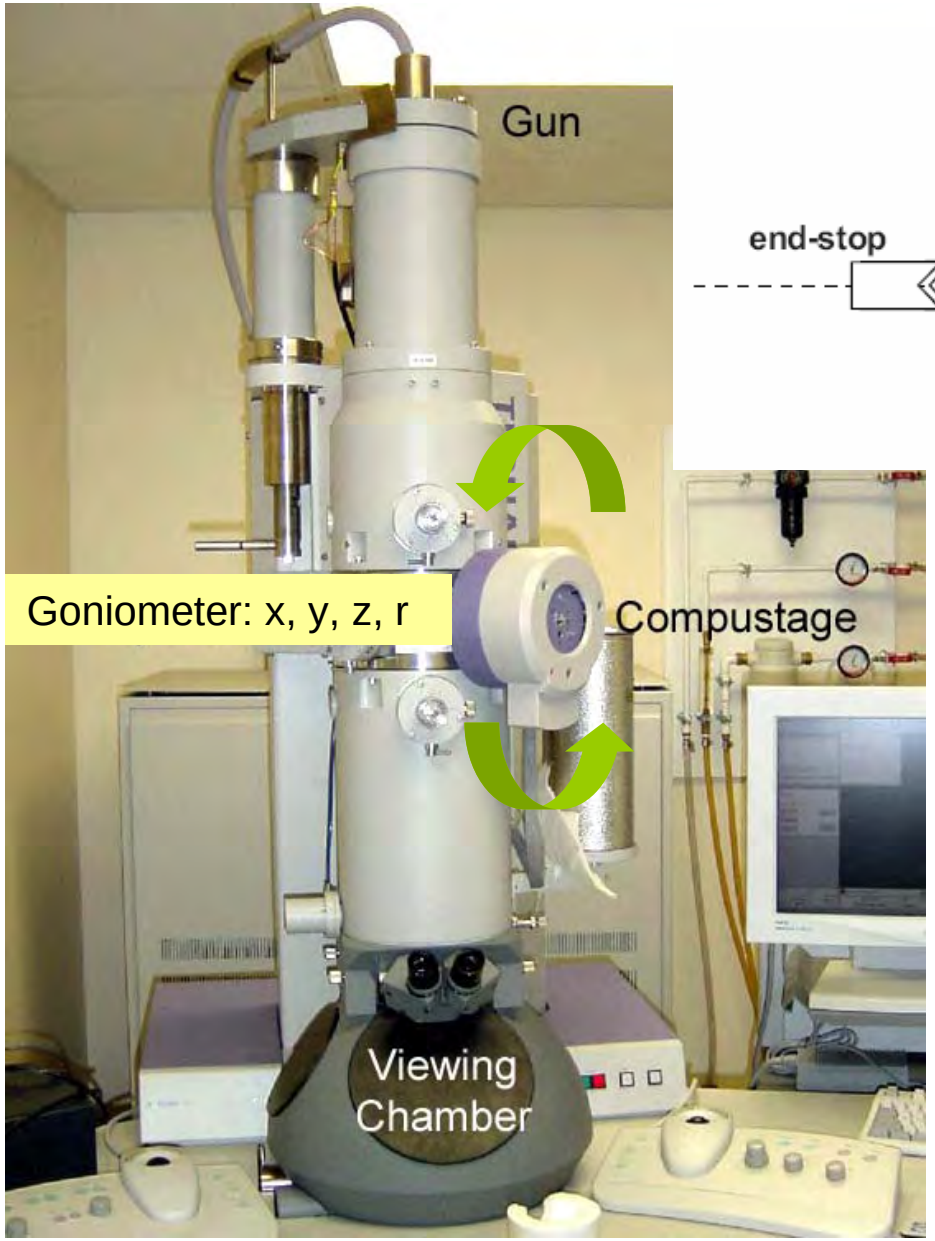
- High vacuum systems always require a sequence of different vacuum pumps
- Differential vacuum is maintained by small openings between “chambers” and location of the pumps
- Pumping efficiency depends on the gas

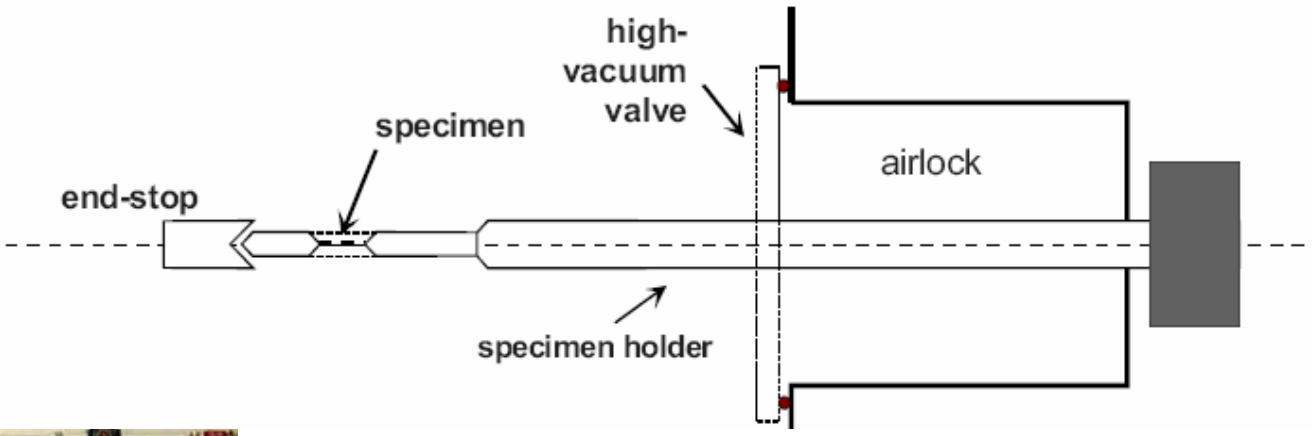
Vacuum systems have to be kept clean:

- No volatile components (fatt, oil, water)
- Air-lock for transfer of specimen into vacuum
- Vent with dry nitrogen gas

Specimen holders and stages

Transmission electron microscope

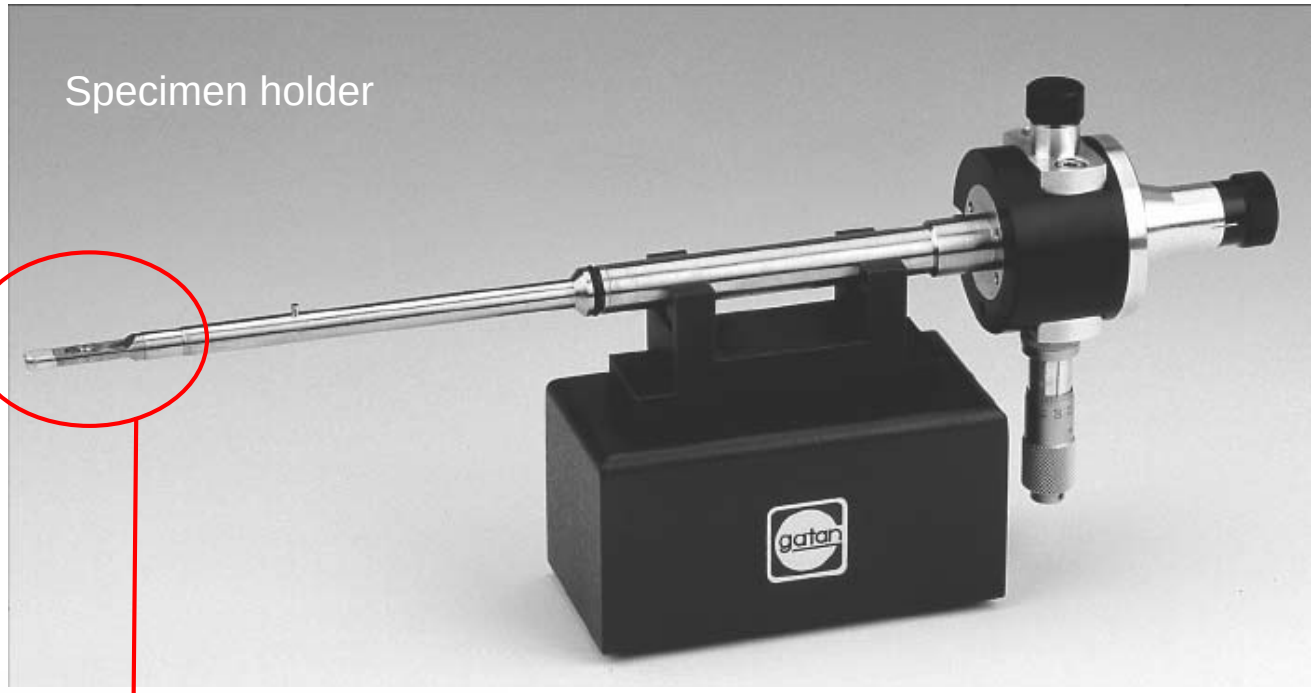


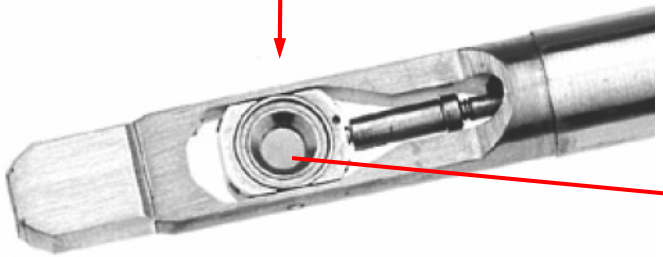


Specimen size:

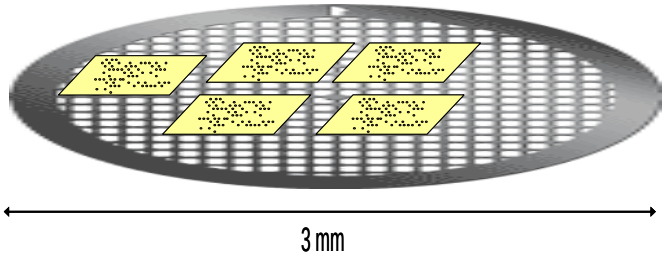
- 3 mm in diameter!
- Ca. 100 nm in thickness (electron transparent)

Specimen holders and stages



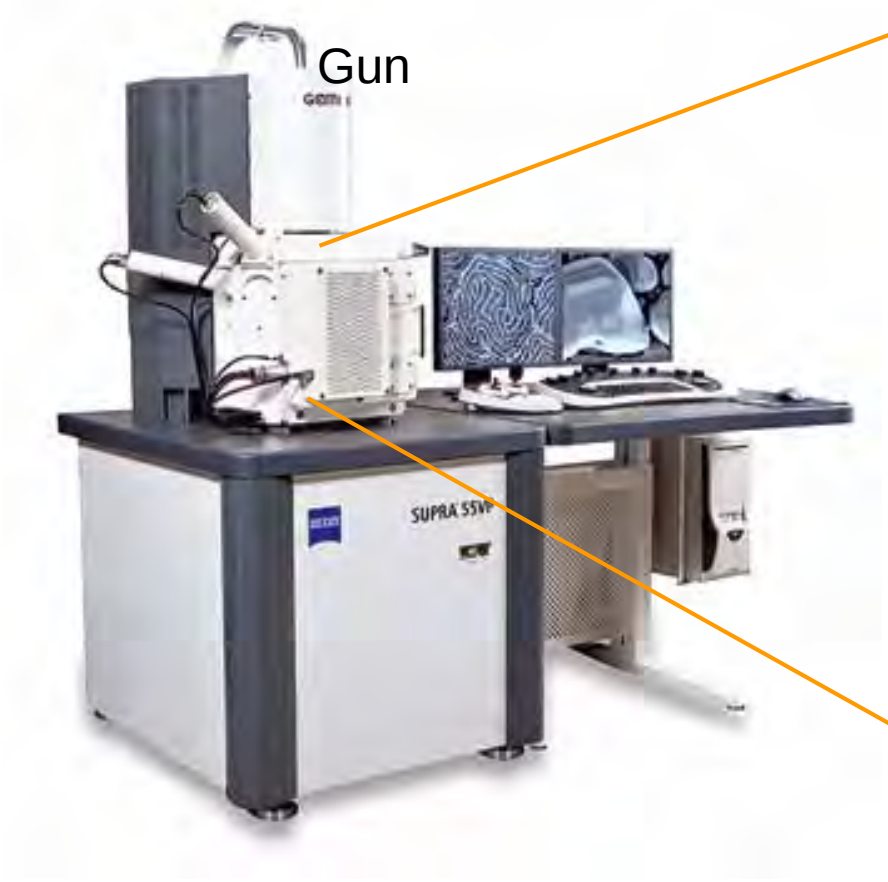


Specimen on a TEM grid

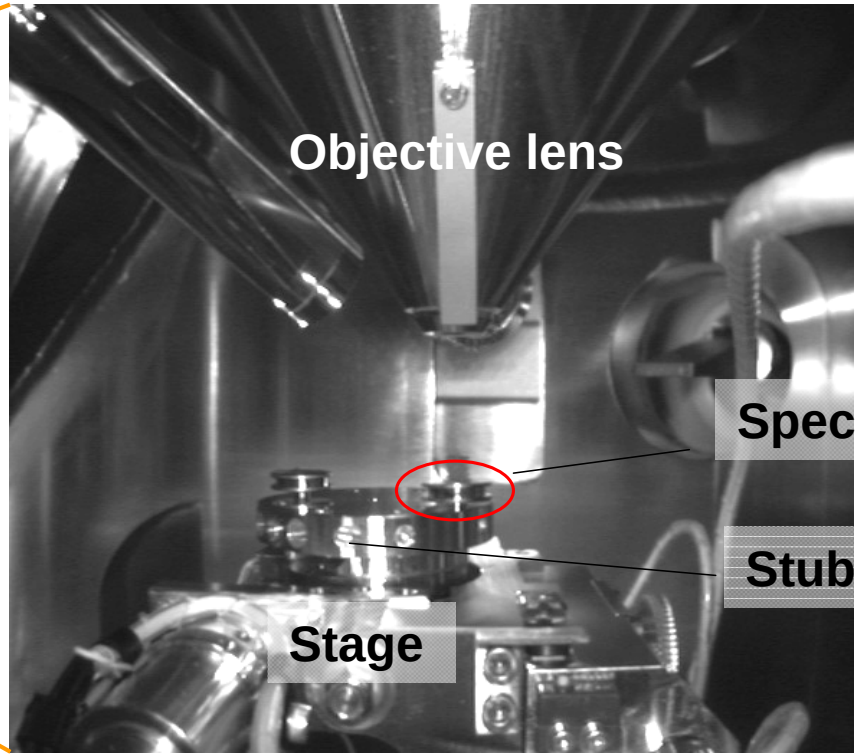


Specimen holders and stages

Scanning electron microscope



Viewing chamber = Specimen chamber



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

Specimen size:

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

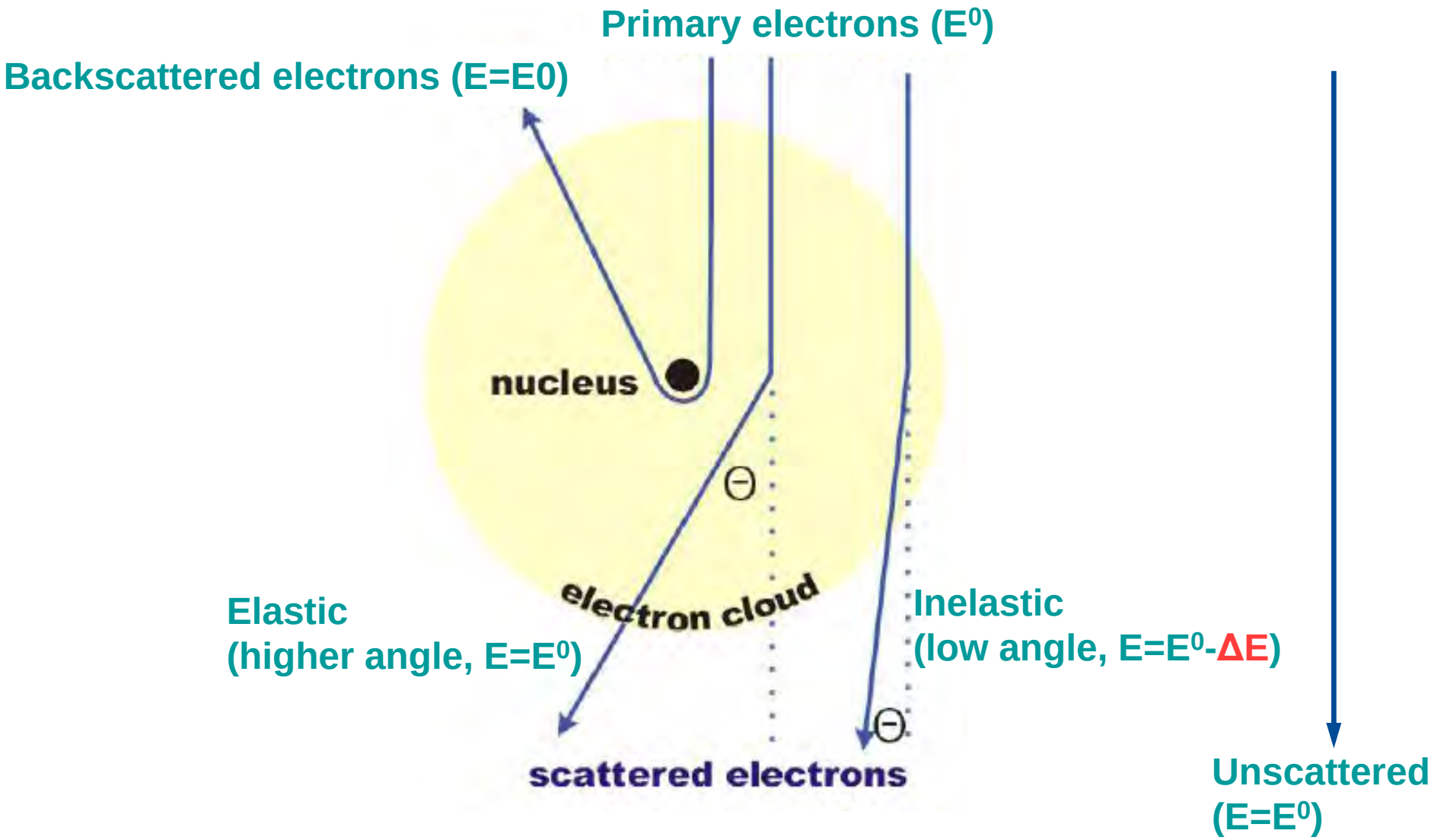
Specimen holders and stages

NOTE:

- Stages and goniometer must be extremely stable and precise!
- Any drift will cause unsharp images, in particular at high magnifications

Electron - specimen interactions

Electron – specimen interactions



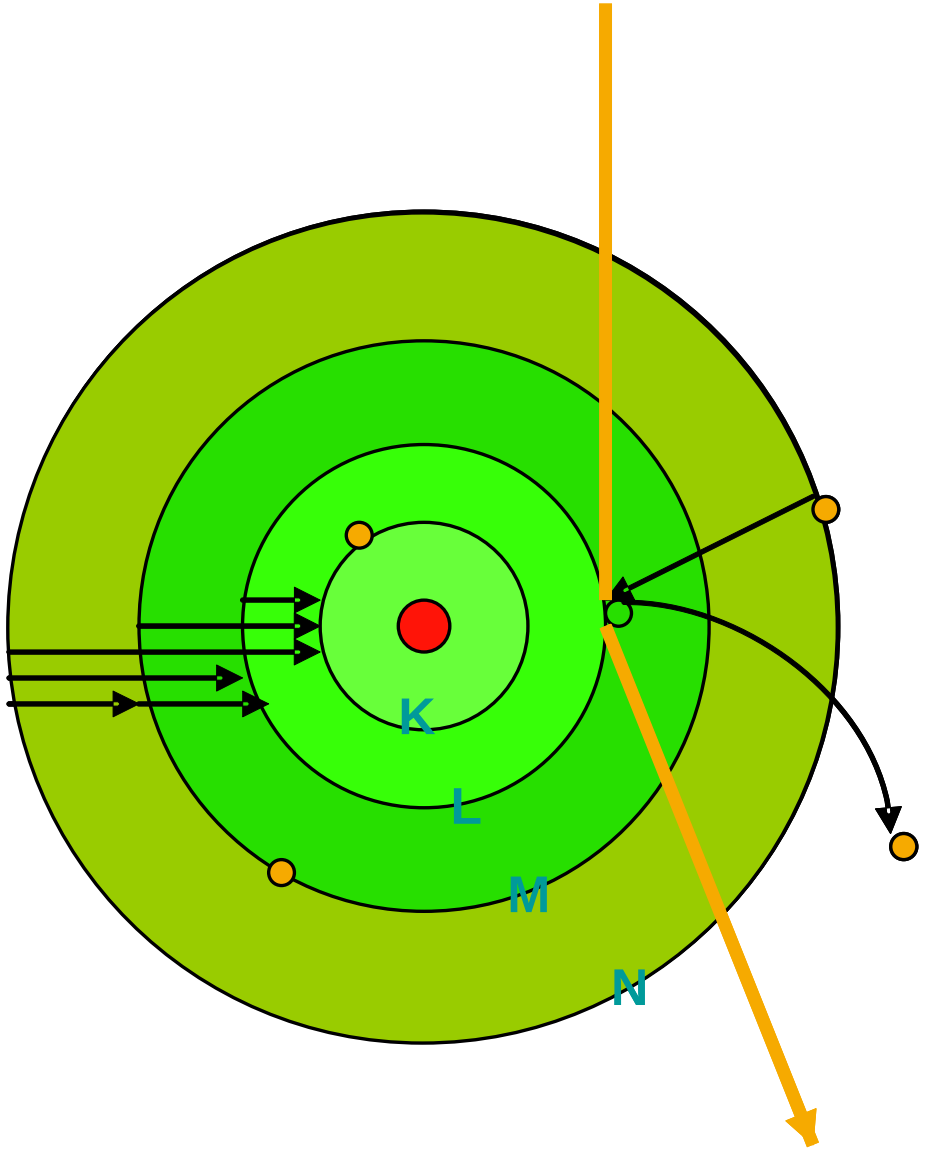
Electron – specimen interactions

Inelastic scattering:

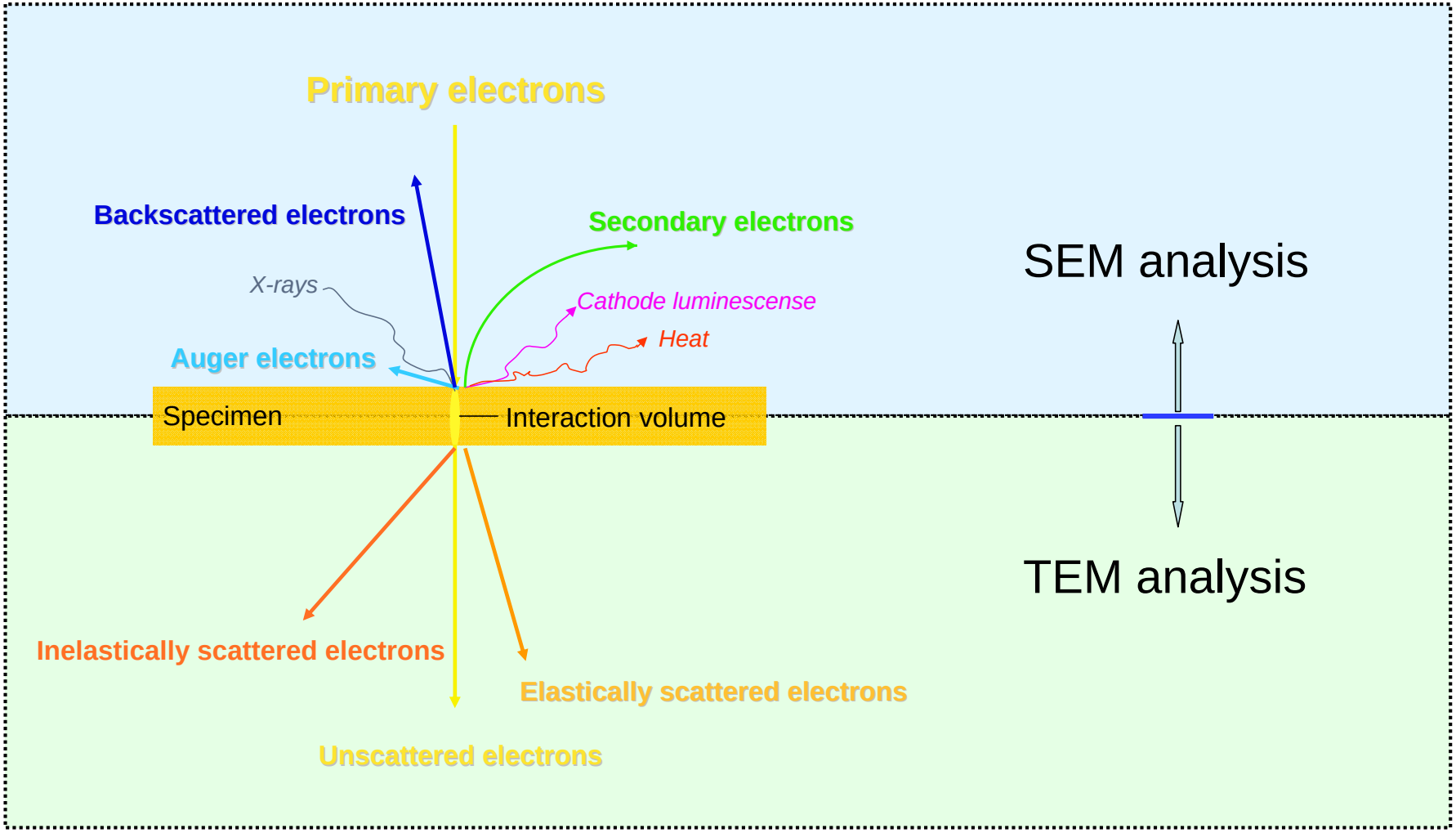
Primary electrons hit electrons of the specimen atom

Energy is transferred from the primary electron to the specimen

Emission of electrons and radiation

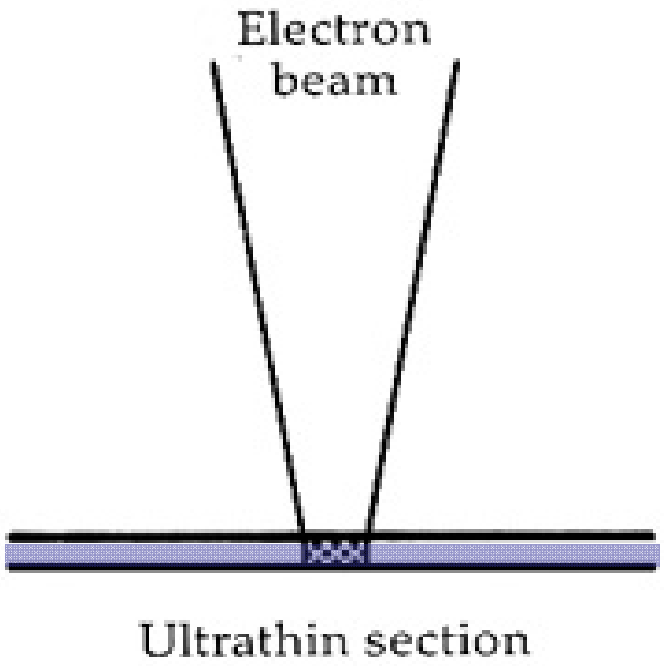


Electron – specimen interactions

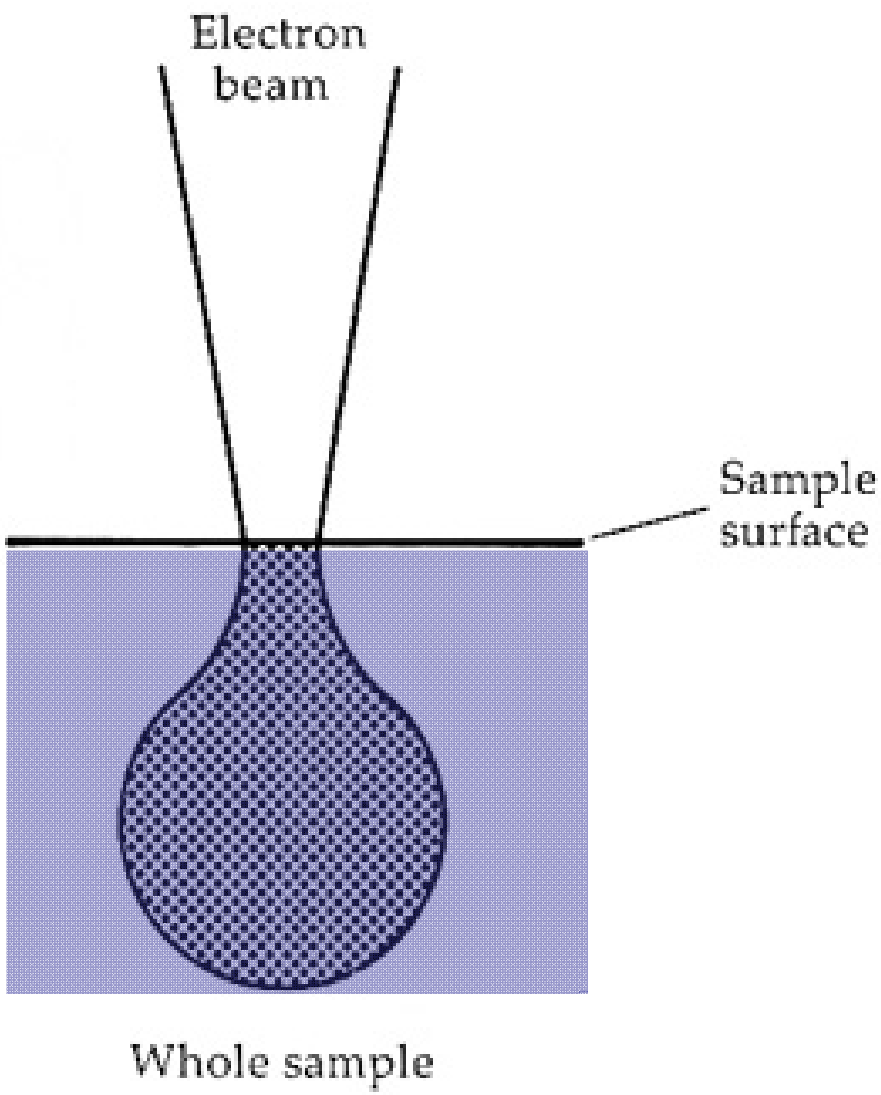


Electron – specimen interactions

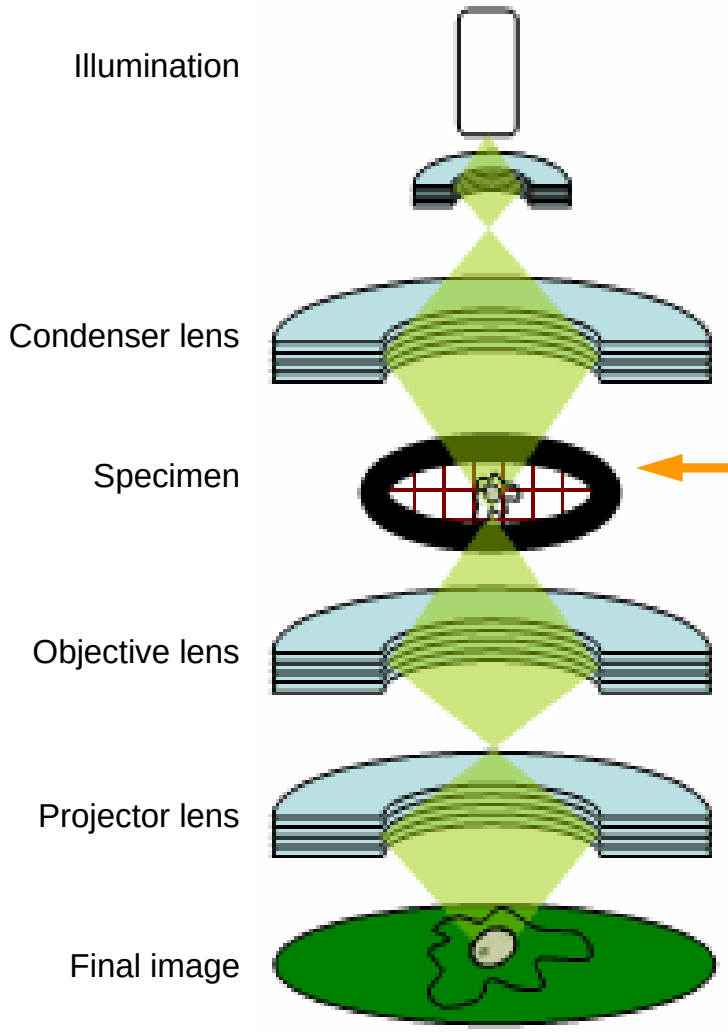
TEM



REM



Imaging in the transmission electron microscope



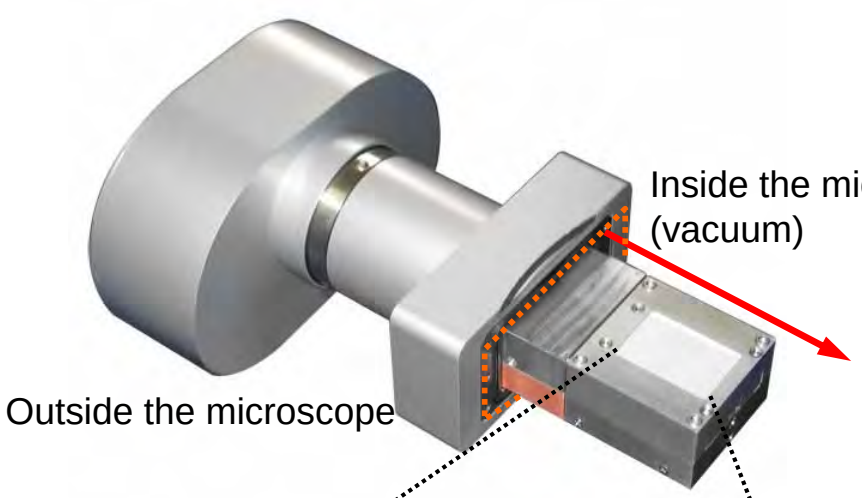
Specimen: Electron transparent
(very thin: 100 nm)

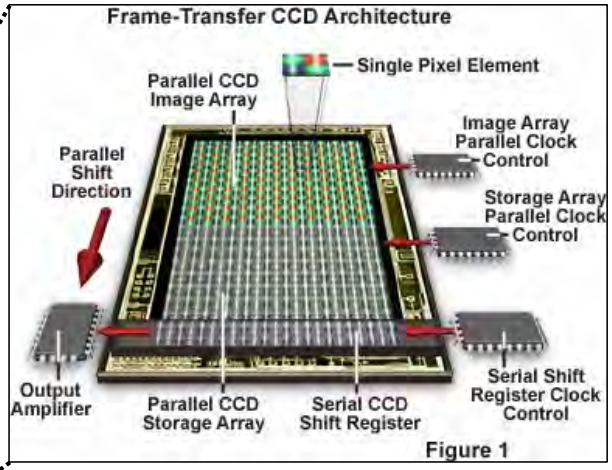
Image: 2D projection of a volume

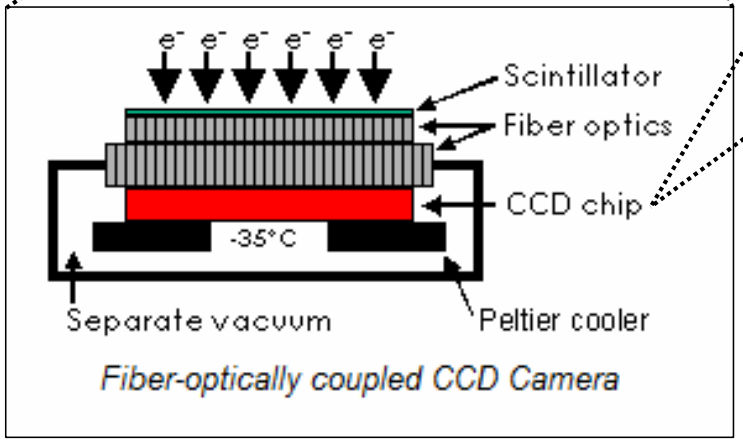
- CCD camera
- Phosphorescent screen
- Conventional photosensitive film

Imaging in the transmission electron microscope

The CCD camera for electron microscopy







- Electrons need to be converted to photons (scintillator)
- CCD has to be protected from electron bombardment

Imaging in the transmission electron microscope

Contrast formation in TEM

- Absorption of electrons
- Scattering of electrons
- Diffraction and phase contrast

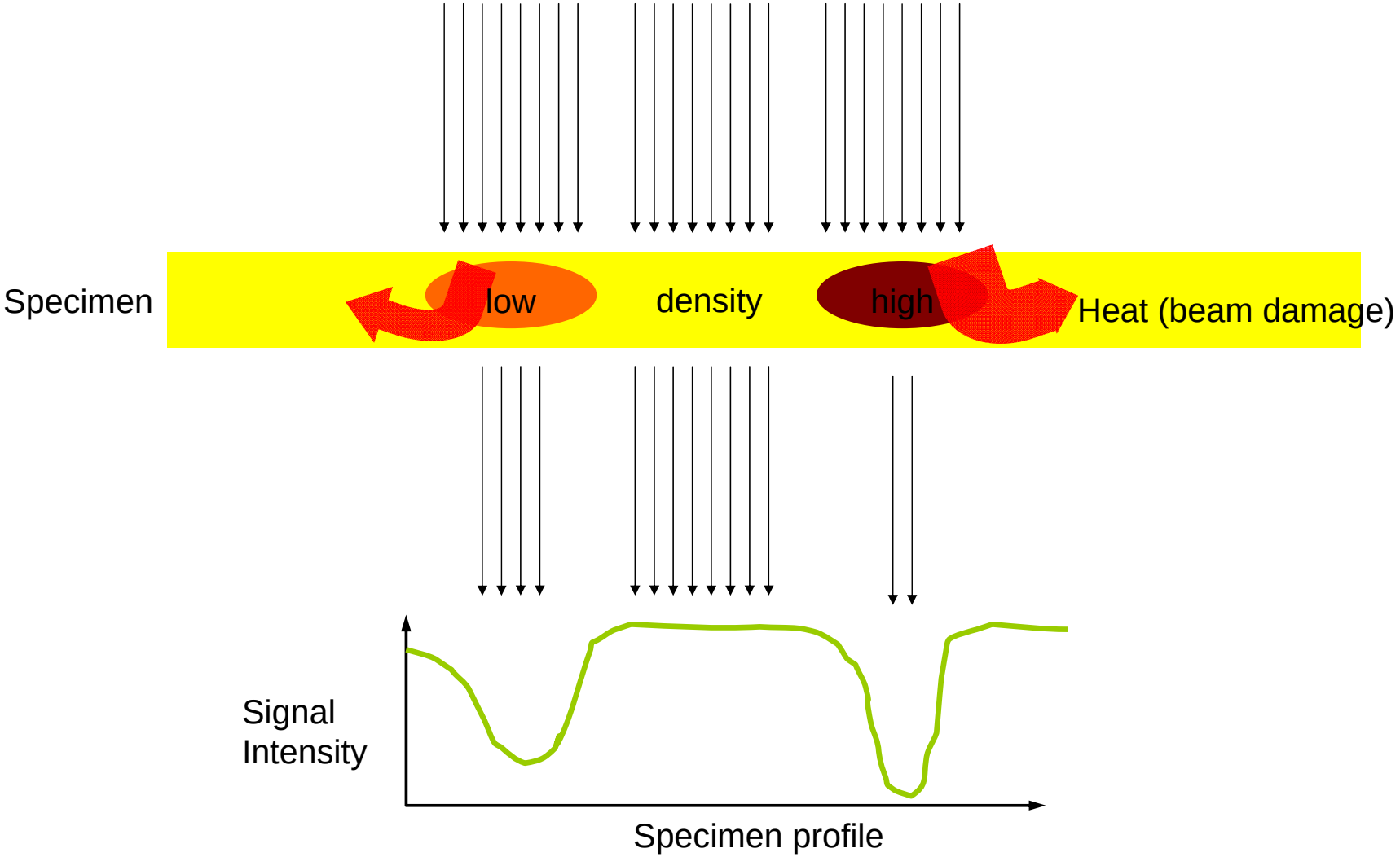
NOTE: All mechanisms occur at the same time (superposition)

Question: Which mechanism is most relevant for biological specimens?

Imaging in the transmission electron microscope

Contrast formation in TEM

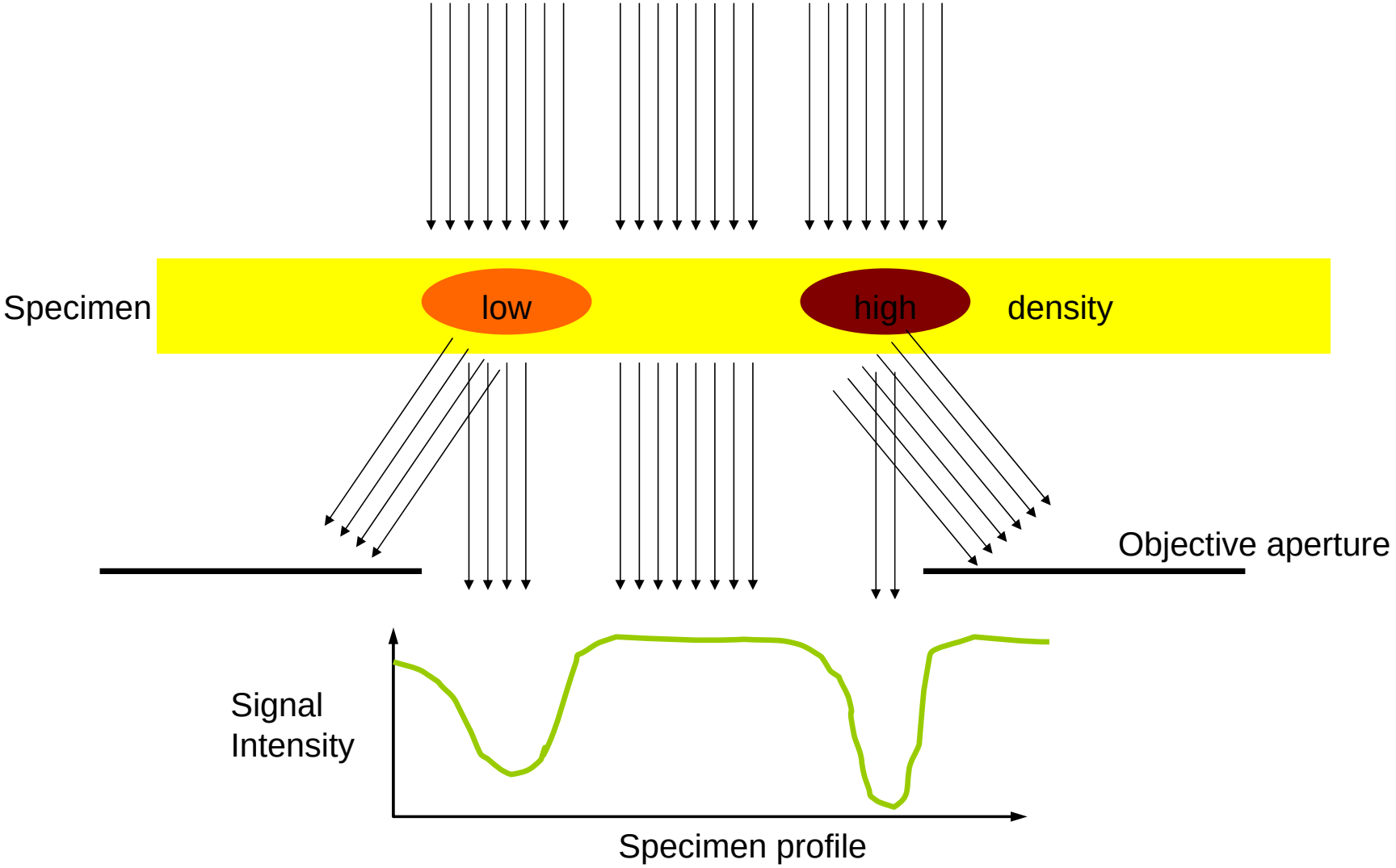
- Absorption of electrons
- Scattering of electrons
- Diffraction and phase contrast



Imaging in the transmission electron microscope

Contrast formation in TEM

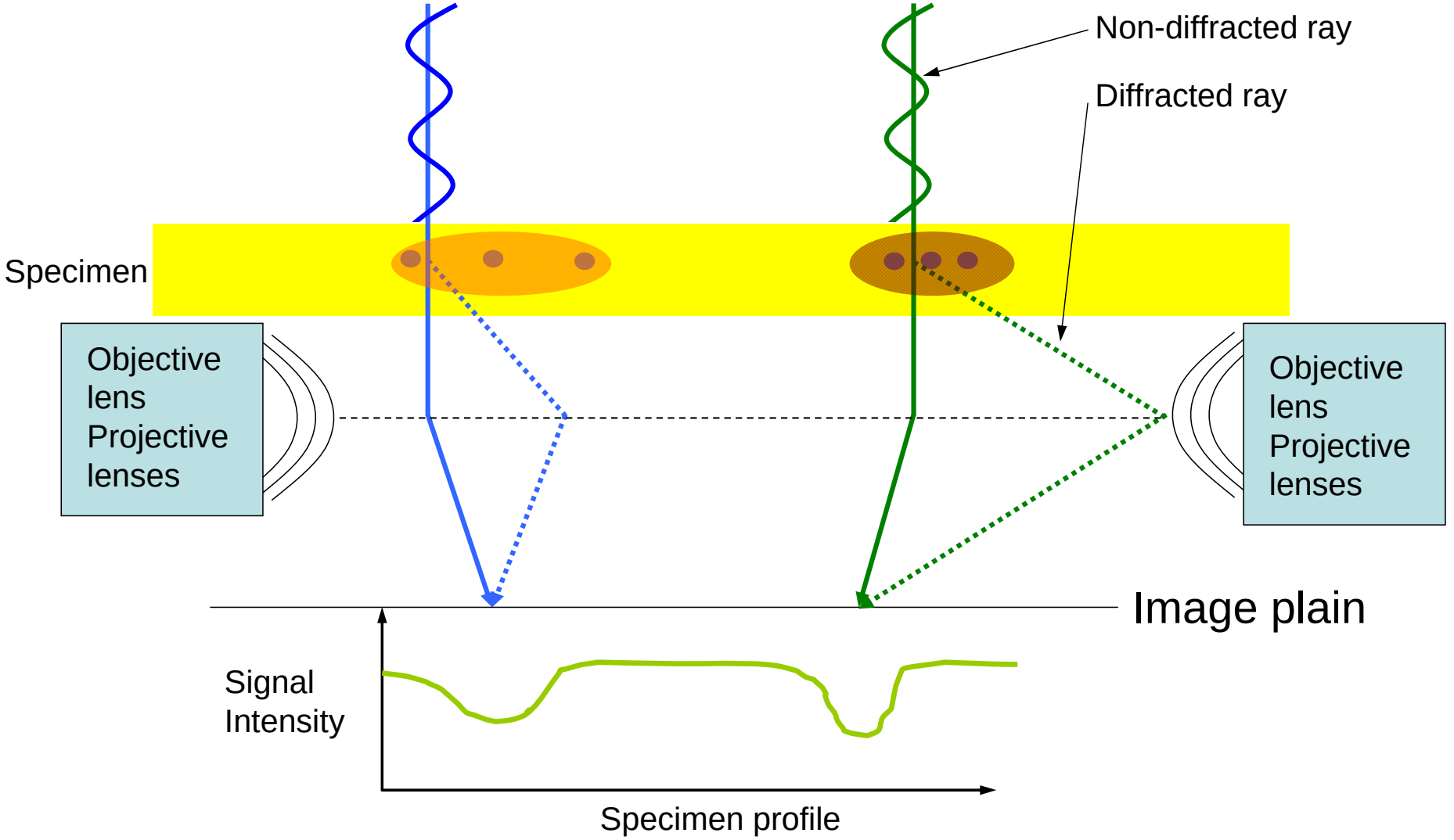
- Absorption of electrons
- **Scattering of electrons**
- Diffraction and phase contrast



Imaging in the transmission electron microscope

Contrast formation in TEM

- Absorption of electrons
- Scattering of electrons
- Diffraction and phase contrast



Imaging in the transmission electron microscope

Contrast formation in TEM

Biological specimen consist of light elements:

- Absorption weak
- Scattering weak
- Diffraction and phase weak

} “NO CONTRAST”

Contrast enhancement required:

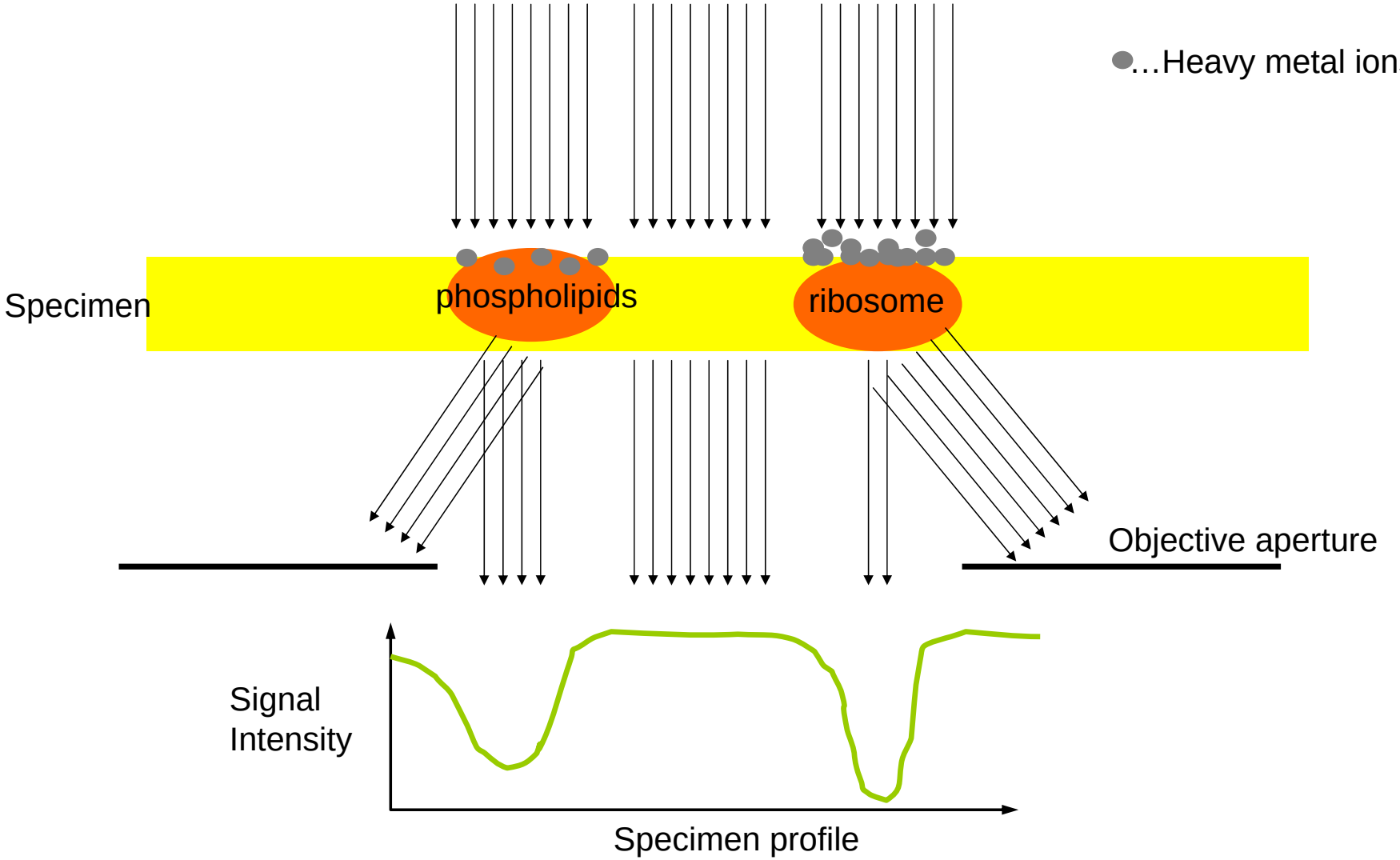
➡ Treatment with heavy metals (Ur, Pb, Os)!

➡ Heavy metals attach differently to different components

Imaging in the transmission electron microscope

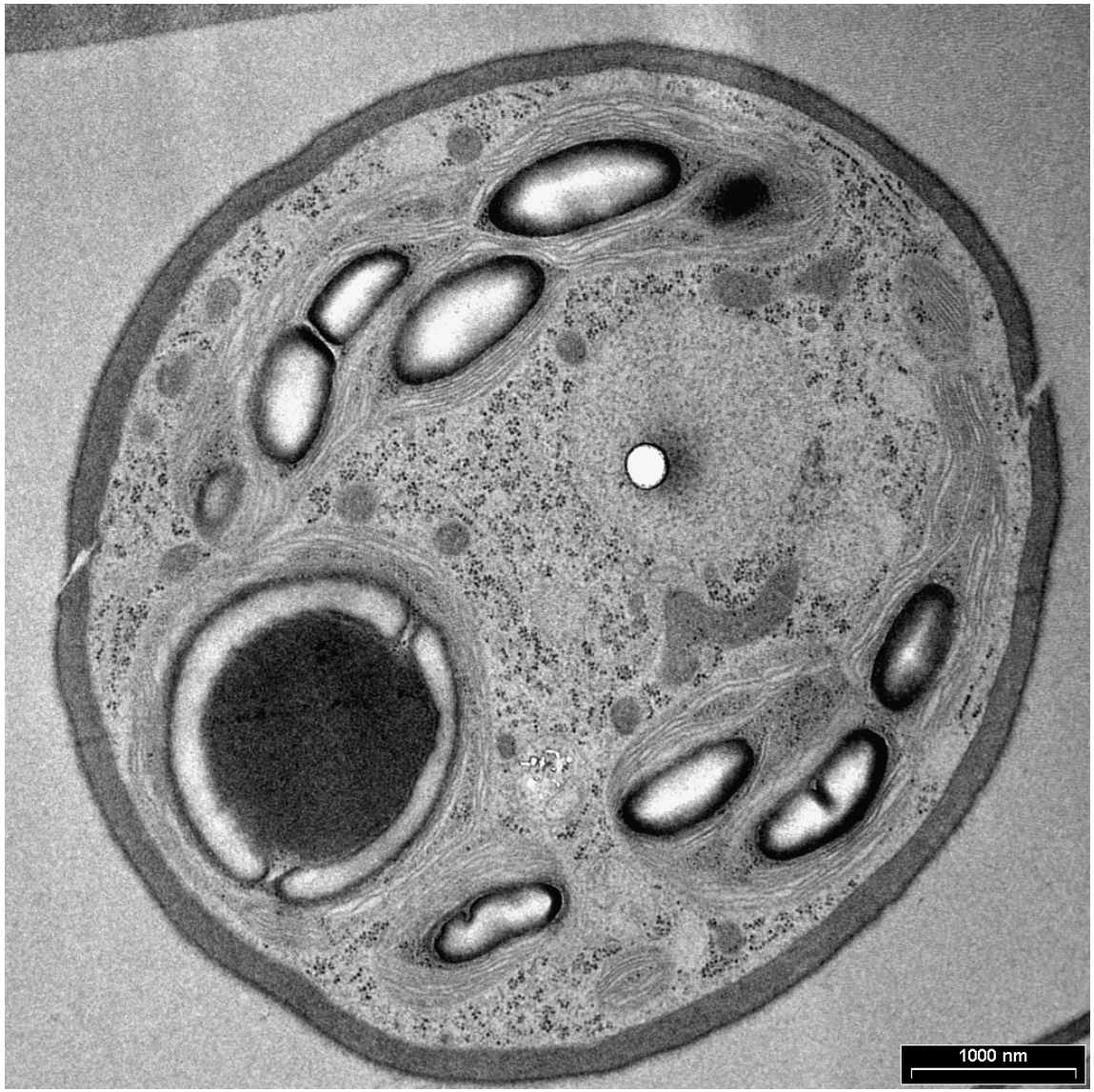
Main contrast formation in plastic embedded specimens

- Absorption of electrons
- **Scattering of electrons through heavy metals**
- Diffraction and phase contrast



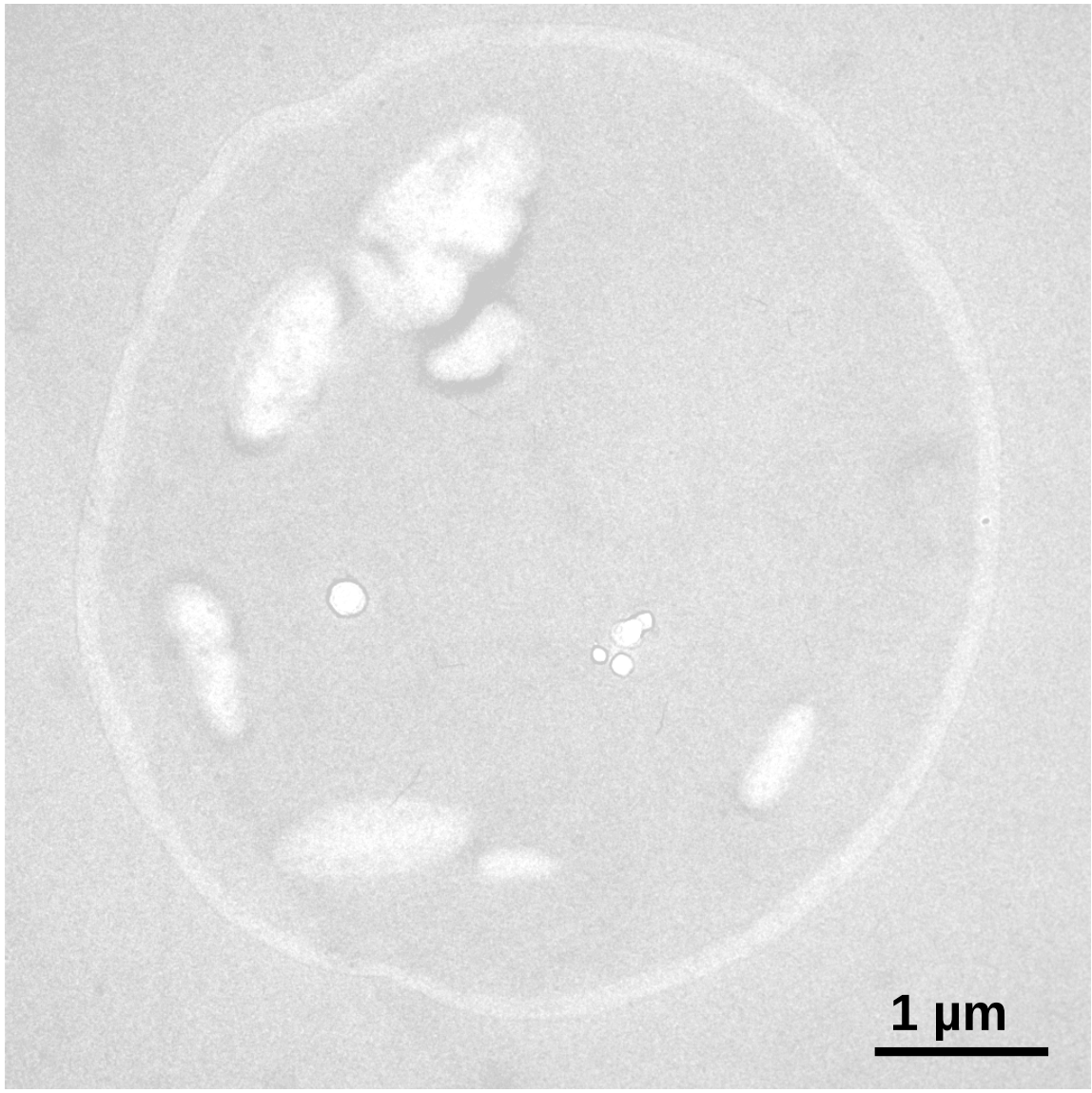
Imaging in the transmission electron microscope

Thin section of alga stained with heavy metals (Ur, Pb)



Imaging in the transmission electron microscope

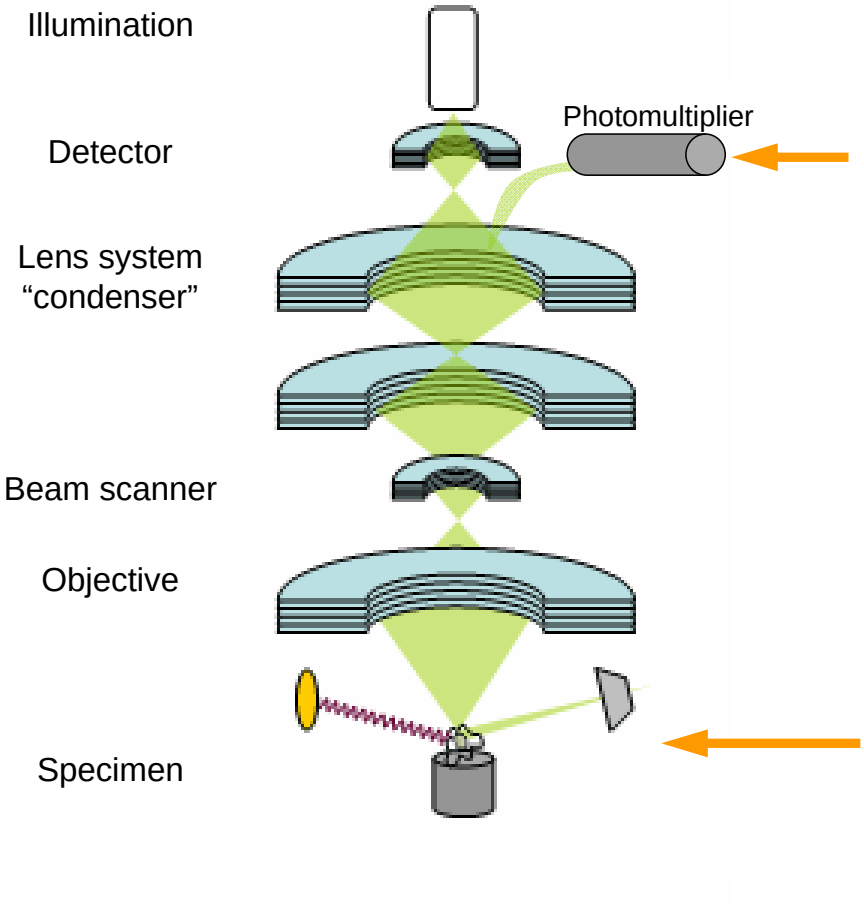
Thin section of alga without heavy metal staining



Imaging in the scanning electron microscope

Imaging in the scanning electron microscope

Scanning electron microscope

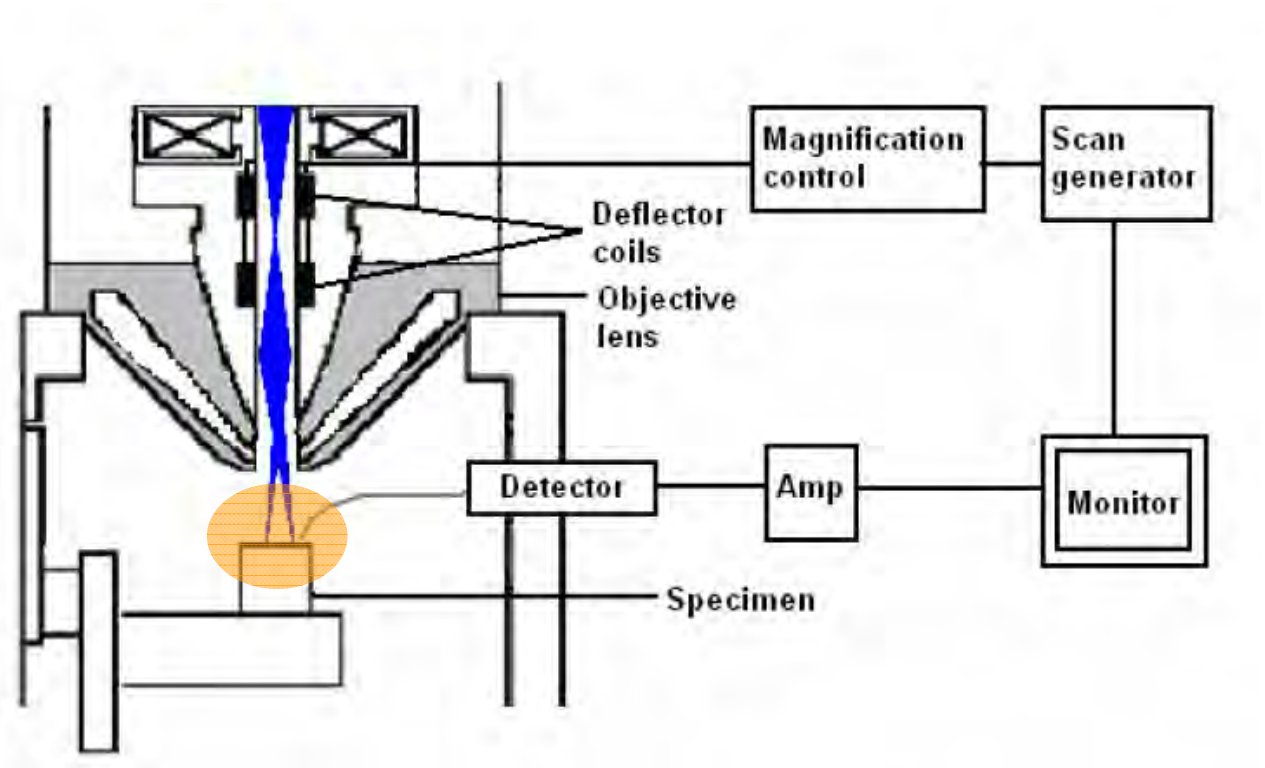


- Photomultiplier
- No CCD camera

Specimen: Bulk specimen

Imaging in the scanning electron microscope

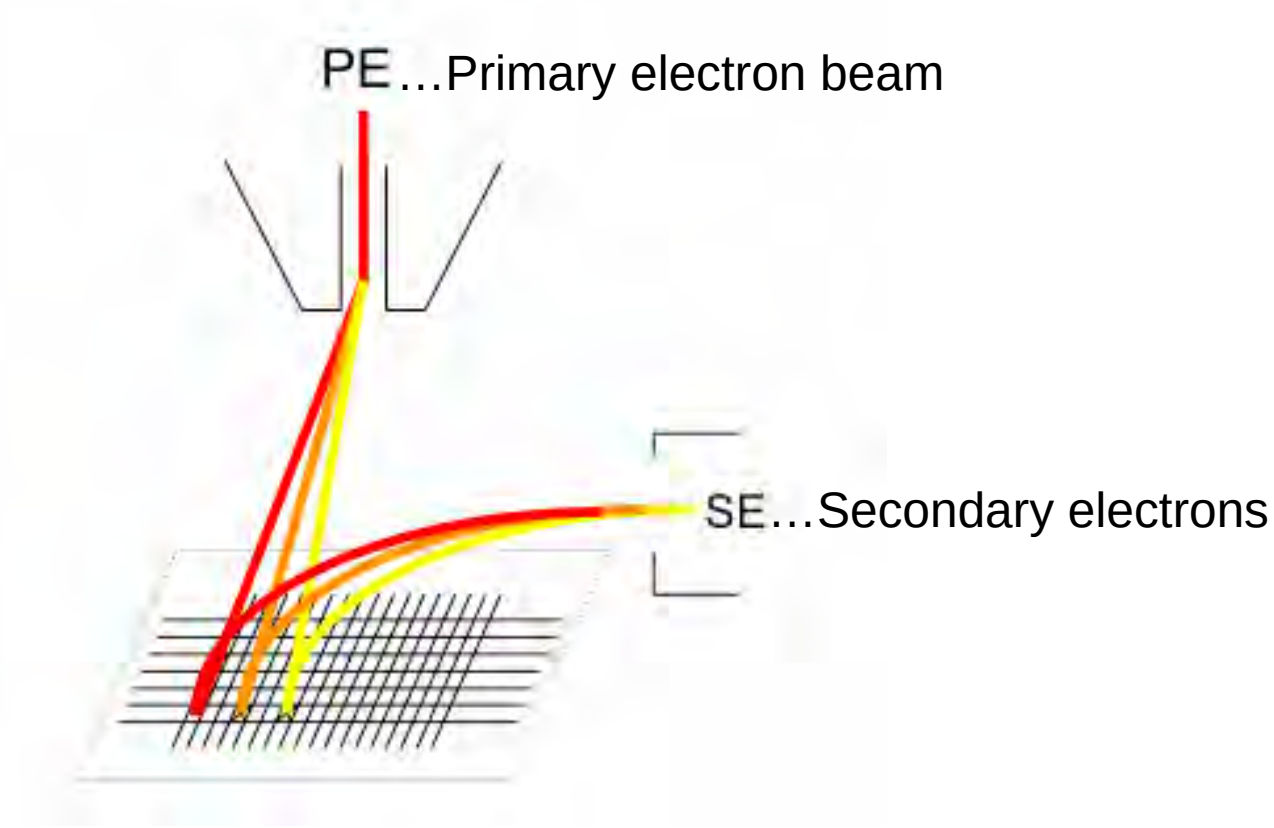
Scanning and signal detection



Scanning of the specimen

Imaging in the scanning electron microscope

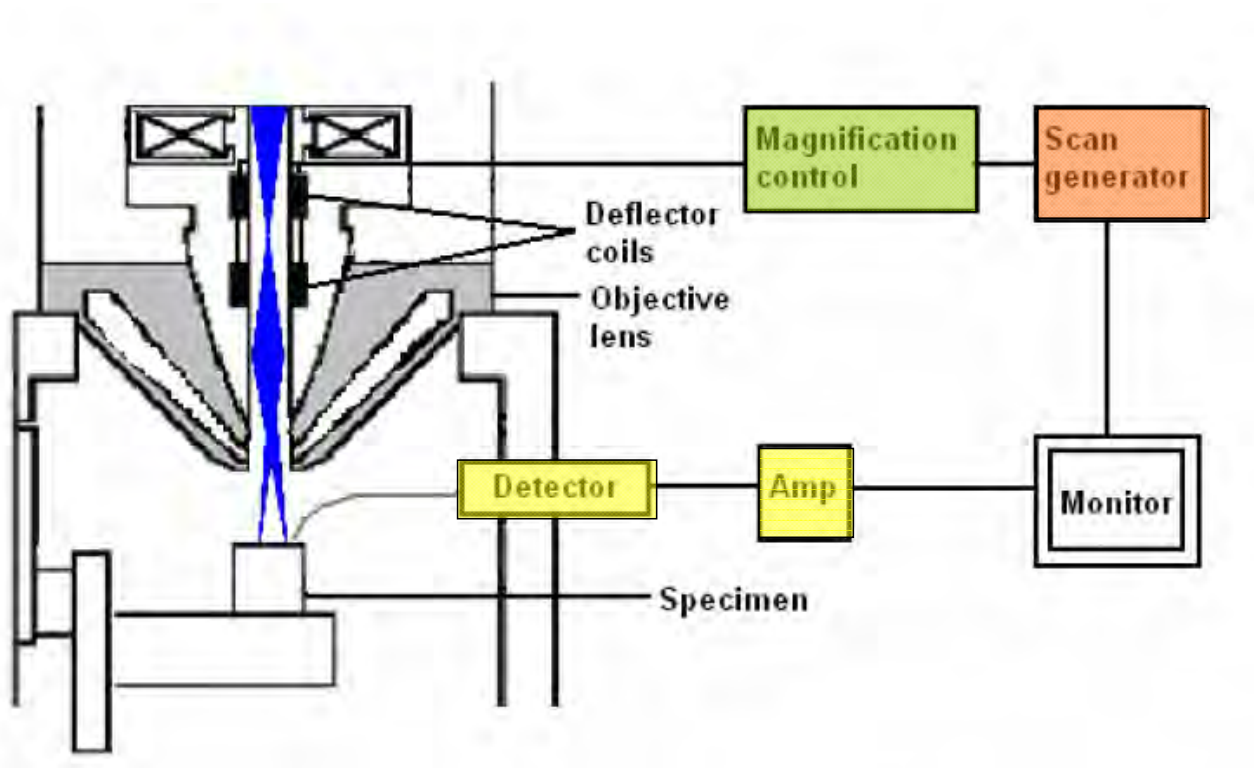
Scanning and signal detection



The focused electron beam is moved from one pixel to another. At every pixel, the beam stays for a defined time and generates a signal (e.g. secondary electrons) which are detected, amplified and displayed on a computer screen.

Imaging in the scanning electron microscope

Scanning and signal detection



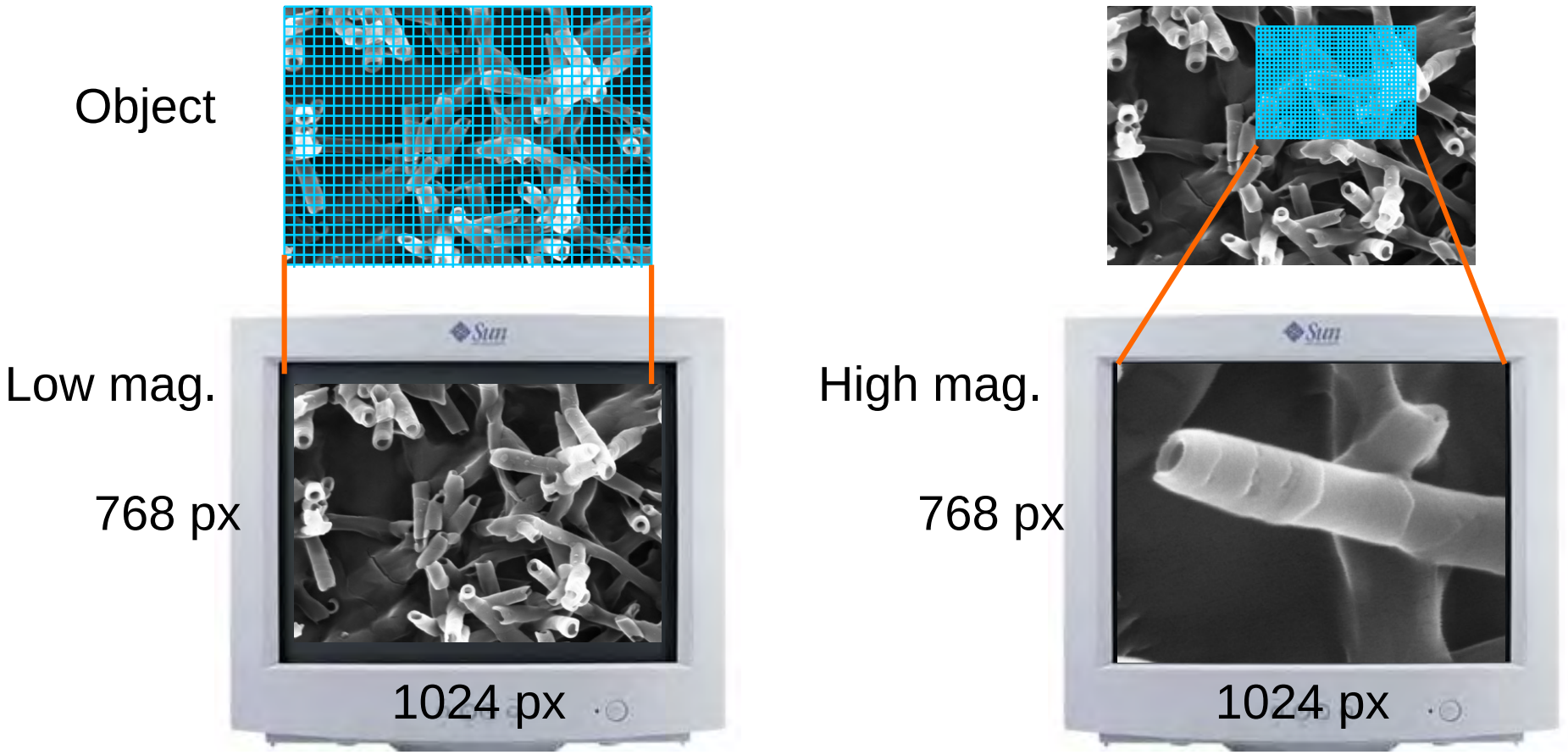
The scan generator synchronizes the scanning of the specimen with the display of the detected, amplified signal.

Imaging in the scanning electron microscope

Magnifying in scanning electron microscopes

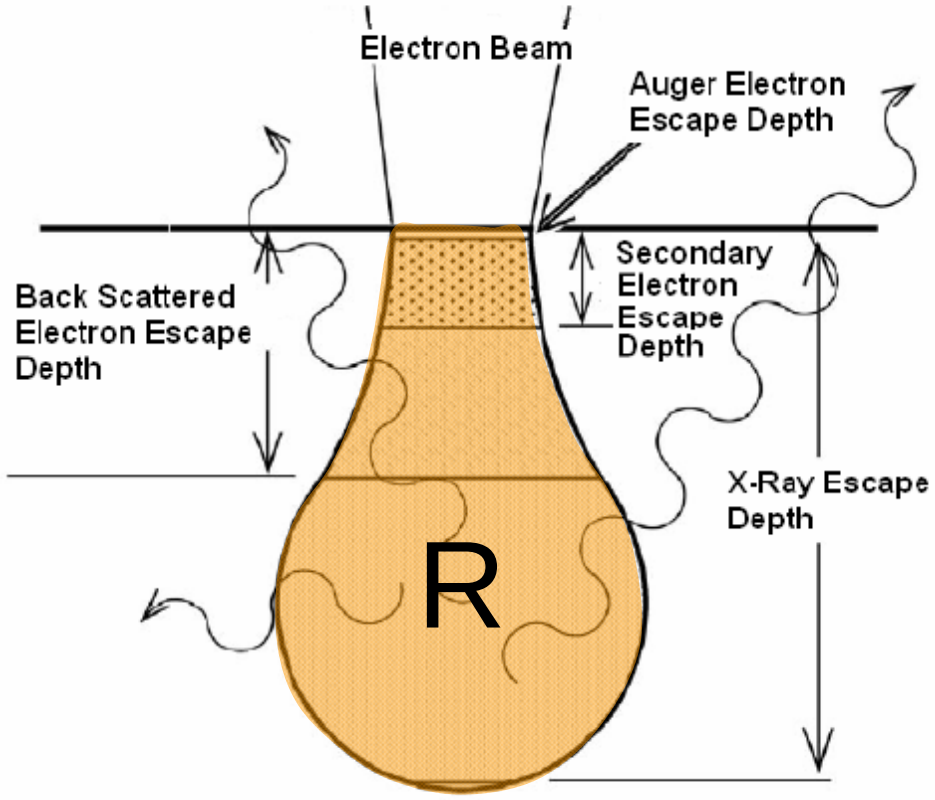
Achieving higher magnifications:

- A smaller area is scanned with the same number of pixels.
- The scanned pixels are smaller
- The signal is displayed on the computer screen at constant pixel size



Imaging in the scanning electron microscope

Signal and detection



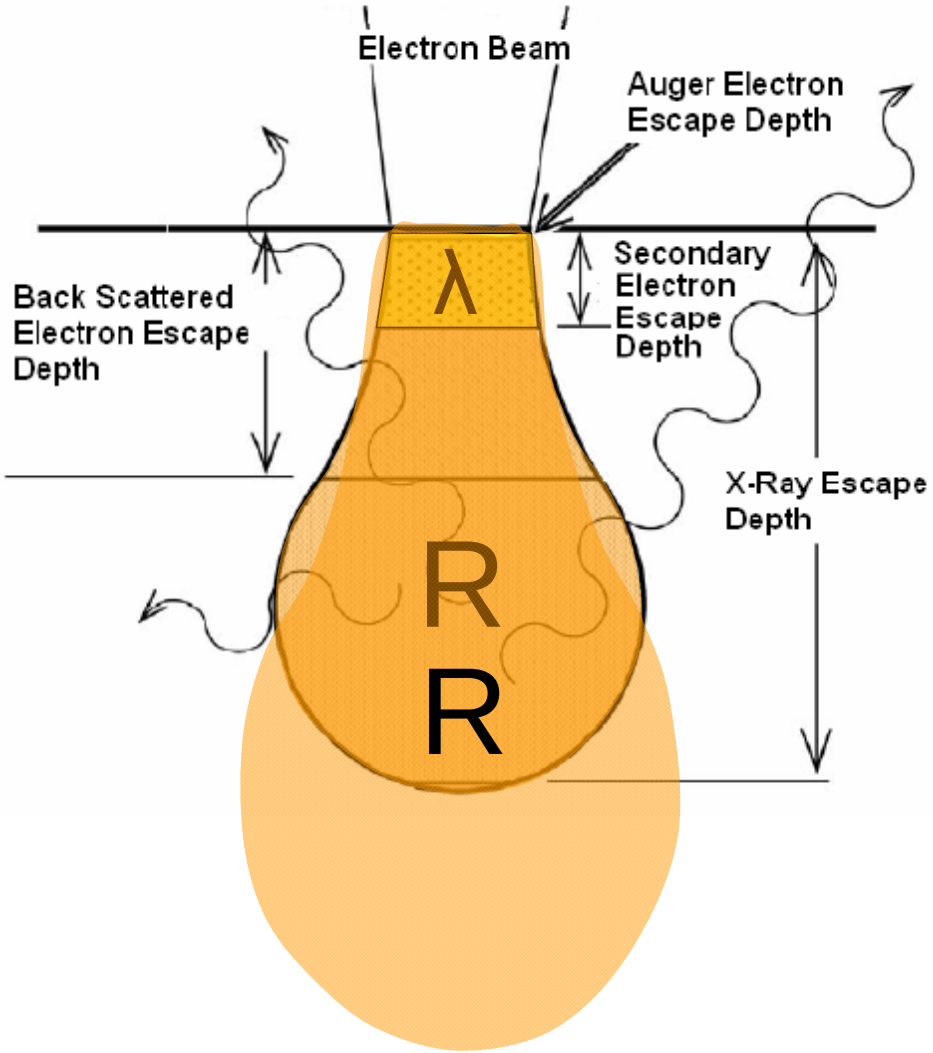
R…interaction volume

Imaging in the scanning electron microscope

Contrast based on SE

R dependent on density of material (Z) and acceleration voltage of PE (0.1 - 30 kV)

Energy of SE independent of acceleration voltage of PE



- R decreases with increasing Z
- R increases with increasing acceleration voltage

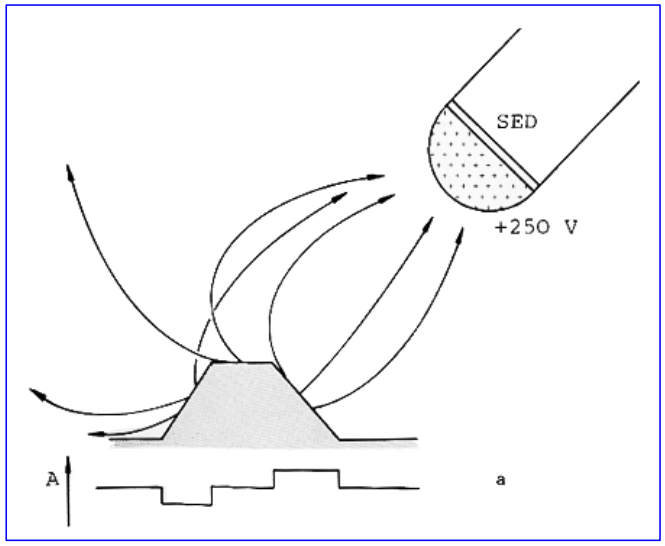
- λ independent on acceleration voltage (but not the number of emitted electrons!)
- λ decreases with increasing Z (density)

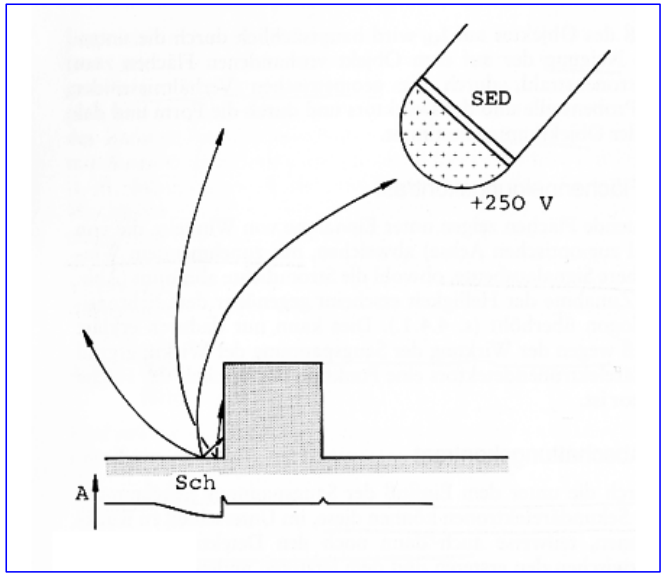
- λ C: 10 – 100 nm
- λ Cr: 2 – 3 nm
- λ Pt: 1 – 2 nm

Imaging in the scanning electron microscope

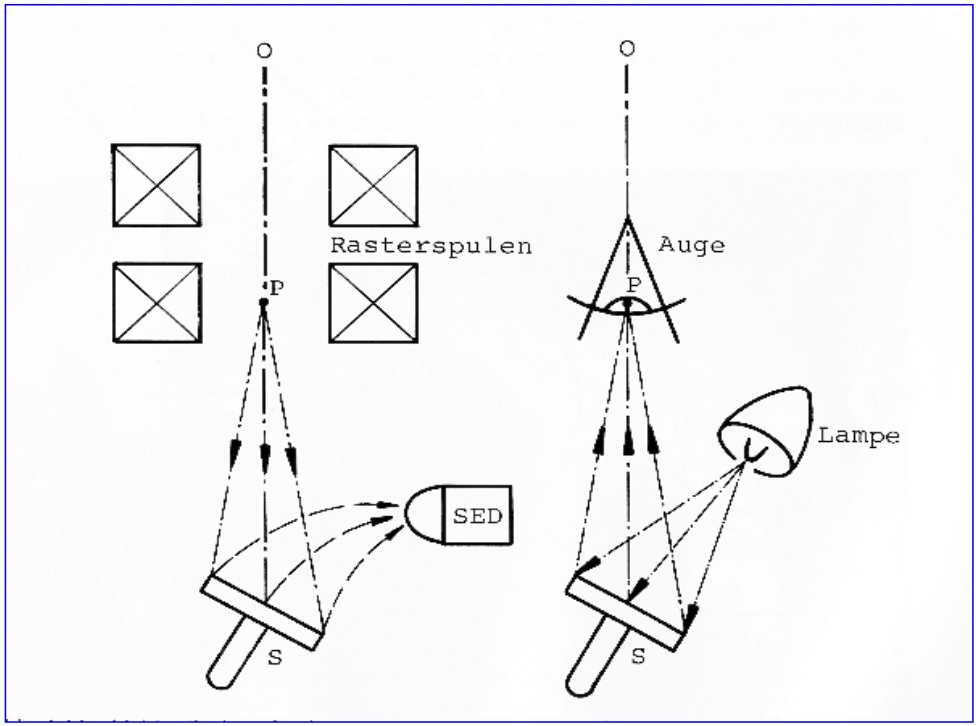
Contrast based on SE

R ≤ λ: Little SE contrast = f (Detectorgeometry)



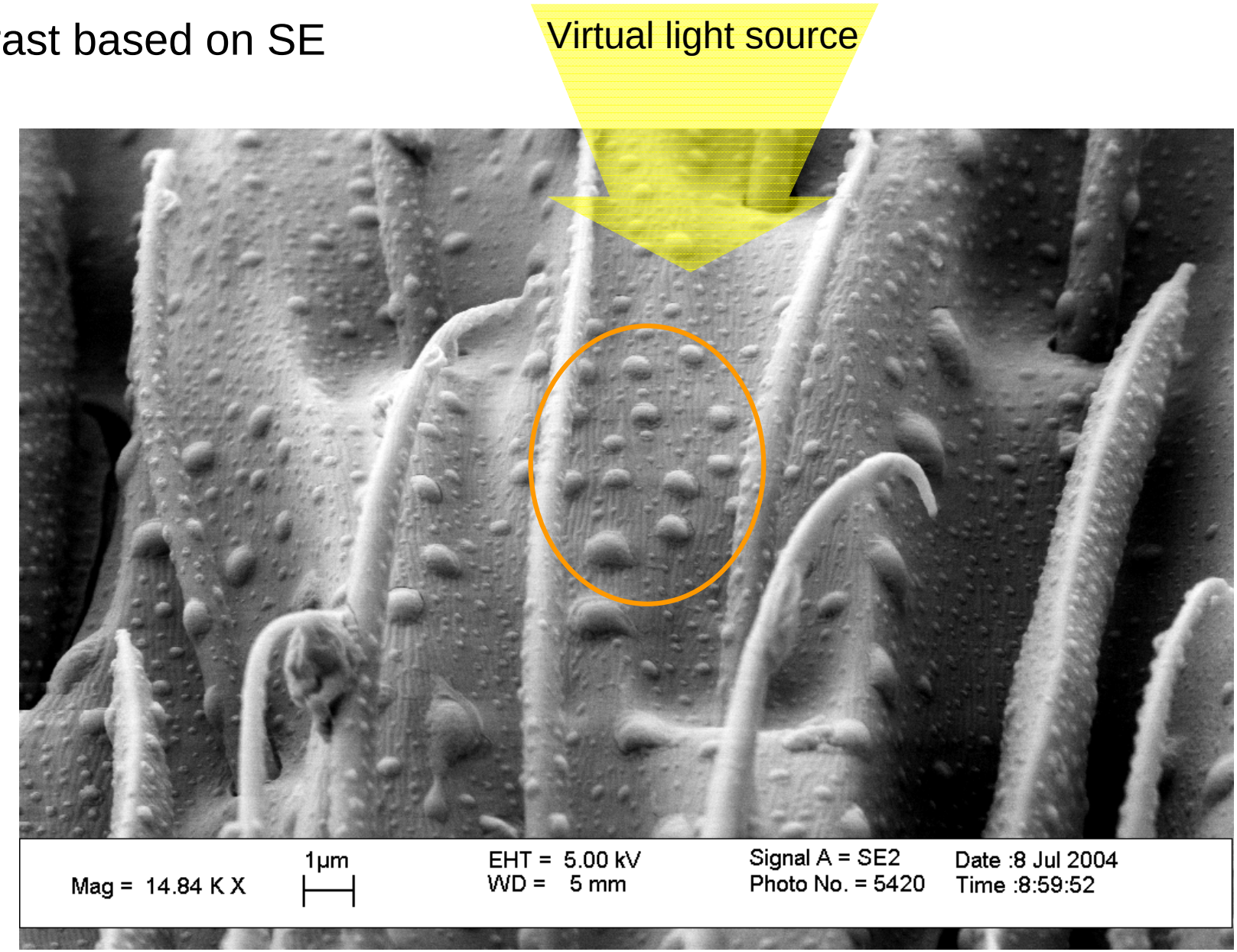


Pseudo 3-dimensional image based on position of SE detector



Imaging in the scanning electron microscope

Contrast based on SE



Leg of an ant, coated with ca. 10 nm Platinum

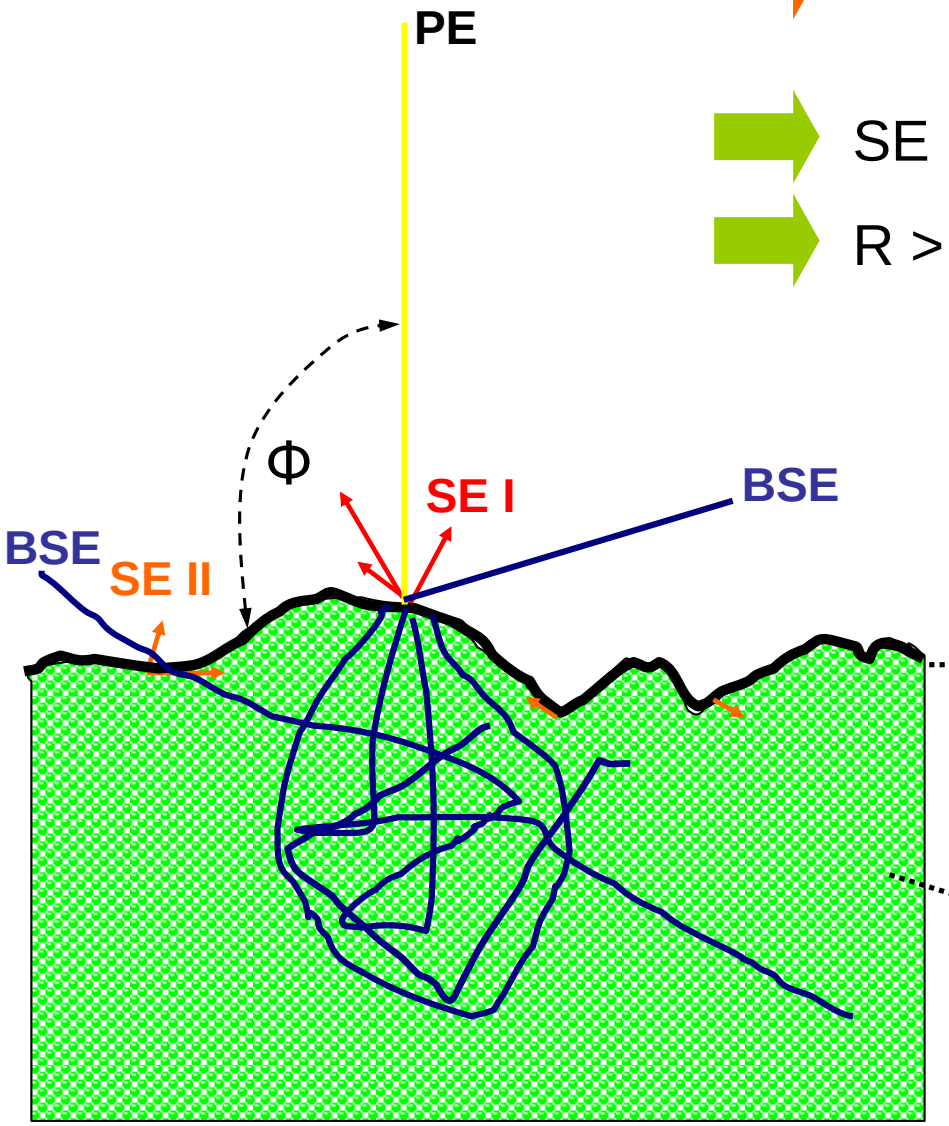
Imaging in the scanning electron microscope

Contrast based on SE: Coating for high resolution SE imaging

THIN (1-4 nm) metal layer, e.g. Pt

SE mainly from metal coat!

R > λ: SE contrast = f(Φ)



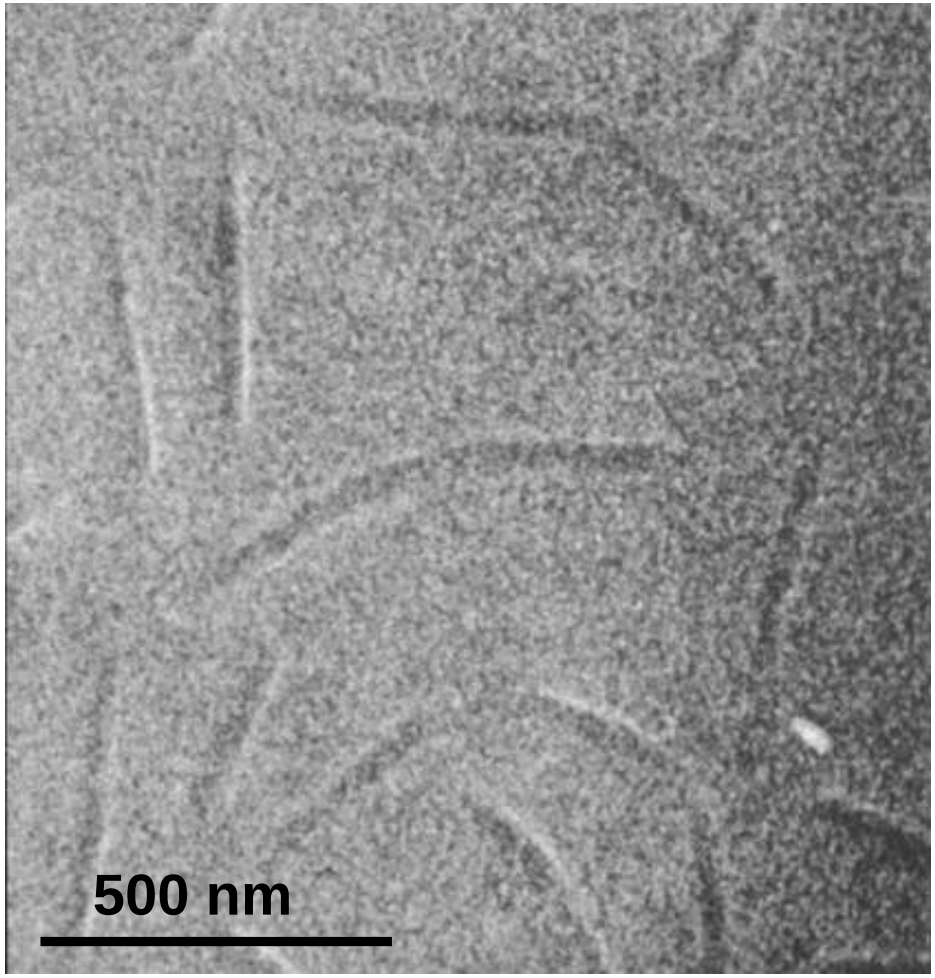
Small excitation volume
High BSE coefficient
SE Escape depth 1-3 nm

Large excitation volume
Low BSE coefficient
SE Escape depth 10-100 nm

Imaging in the scanning electron microscope

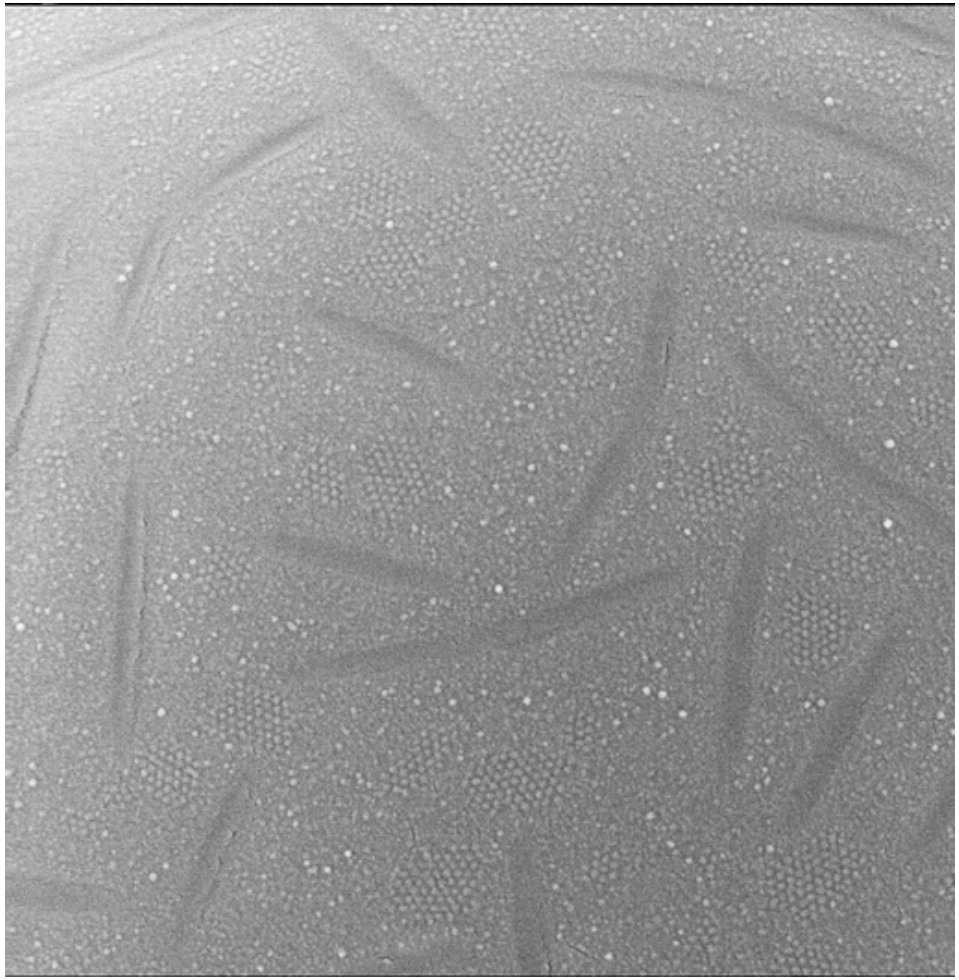
Contrast based on SE: Non-coating vs. coating with heavy metals

Uncoated



Freeze-fractured yeast

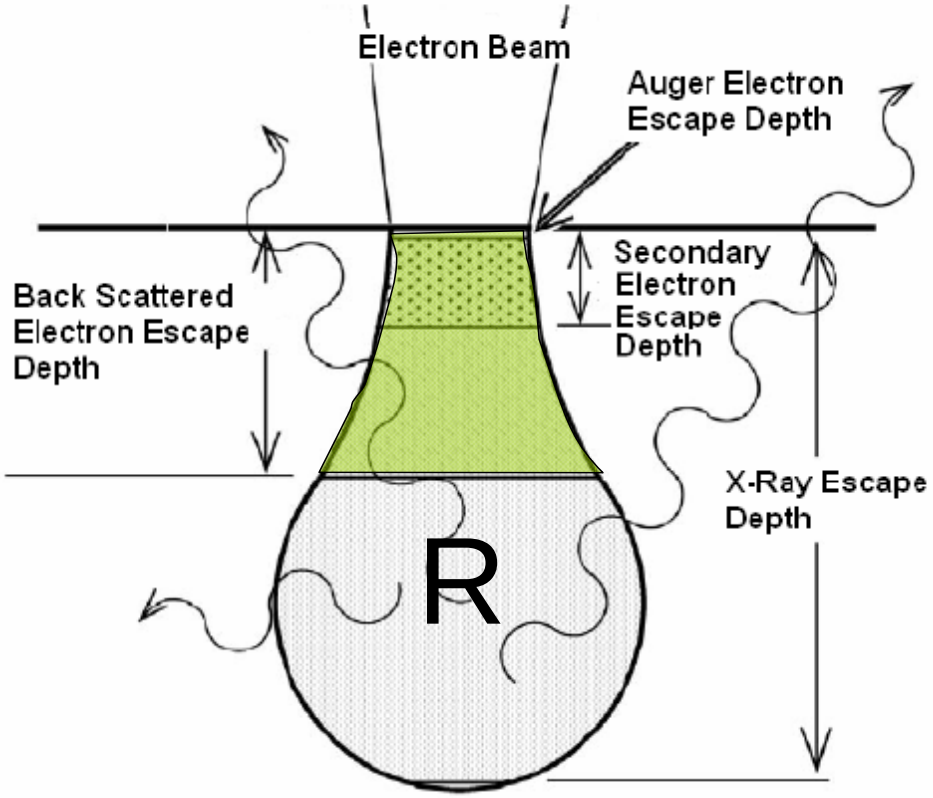
Coated with 4 nm platinum



Imaging in the scanning electron microscope

Contrast based on BSE

R dependent on density of material (Z) and acceleration voltage of PE (0.1 - 30 kV)



Biological material: “No” contrast

BUT:

- Useful if specimen is coated with heavy metals

BSE vs. SE

- Less sensitive to charging (higher energy)
- Less topographic contrast
- More material contrast

Imaging in the scanning electron microscope

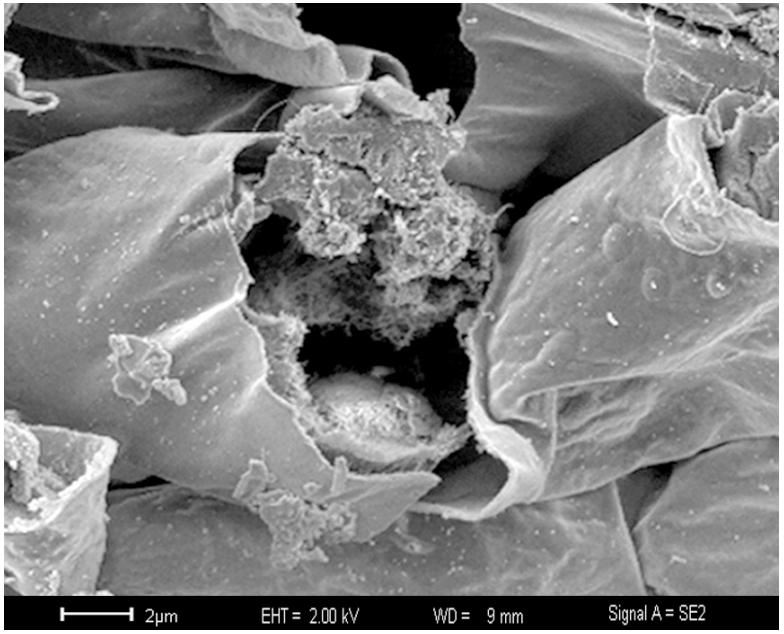
Contrast SE vs. BSE

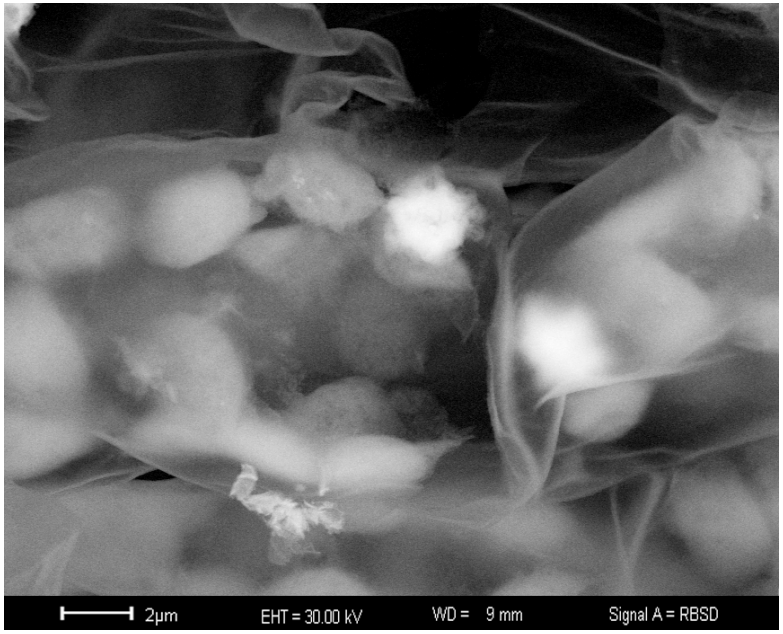
SE signal at 2 kV

➡ Topography

BSE signal at 30 kV

➡ Material





Fractured plant cell containing metal inclusions in chloroplasts

Imaging in the scanning electron microscope

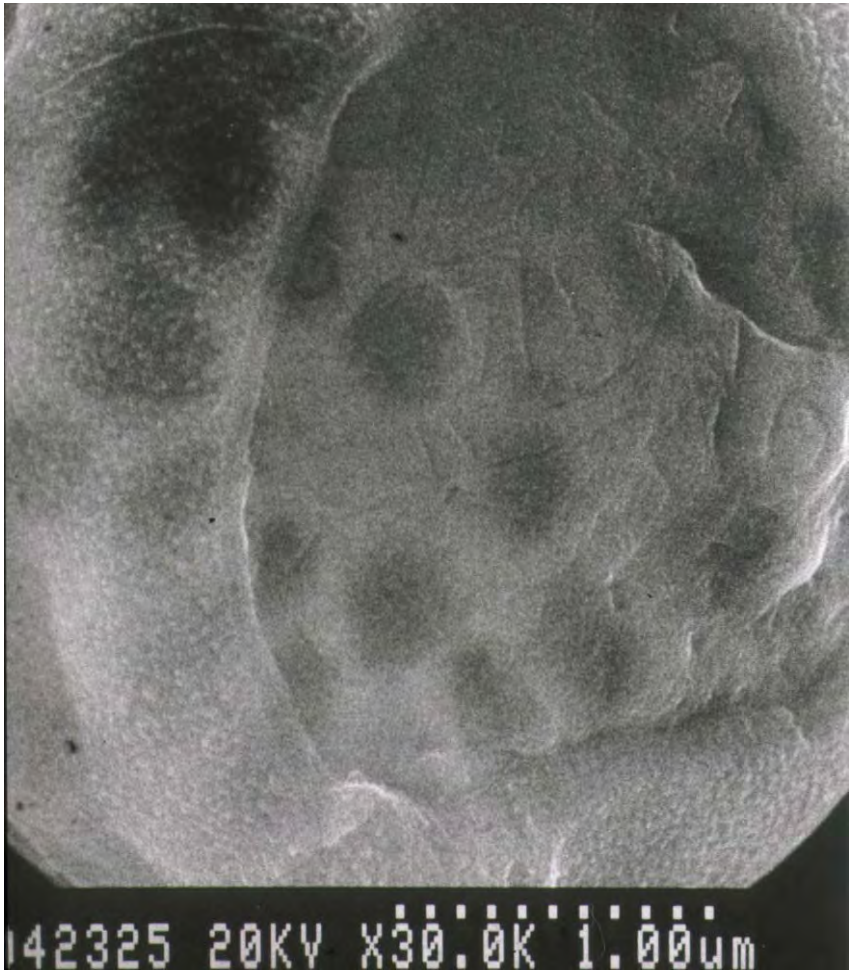
Contrast SE

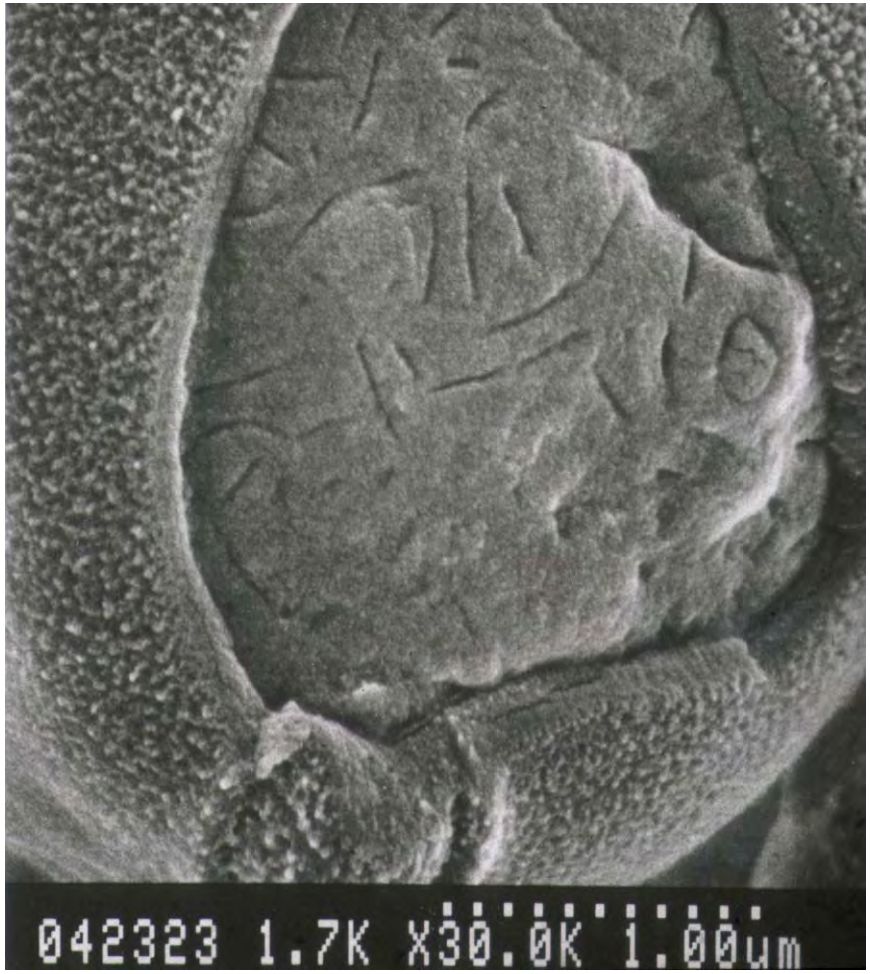
SE signal at 20 kV

➡ Little topography
(Signal based on SE II induced by BSE!)

SE signal at 1.7 kV

➡ Good topography
(Signal based on SE I from surface layer)





Yeast freeze-dried, coated with chromium