



**INSTITUTE**



**OF SCIENTIFIC INSTRUMENTS**

The Czech Academy of Sciences

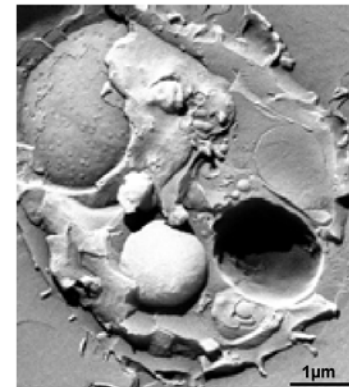
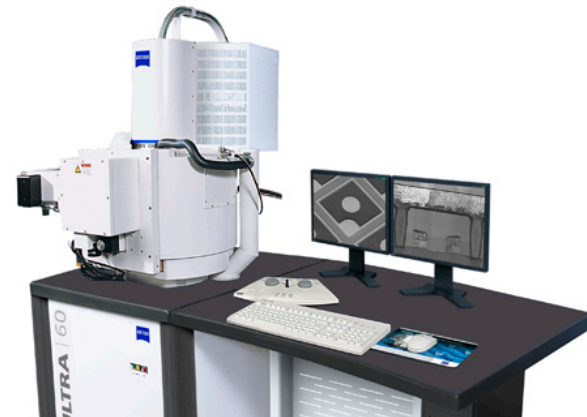
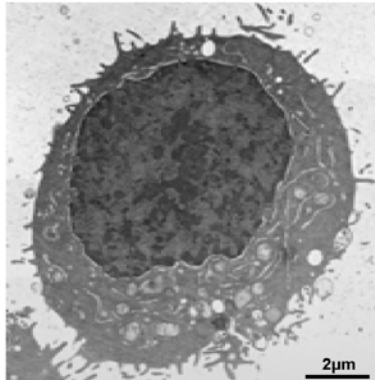
# Principles of scanning electron microscopy

Vladislav Krzyžánek

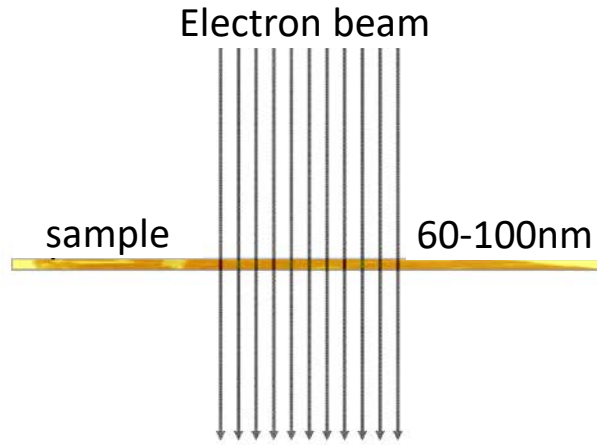
25<sup>th</sup> September 2023, Prague  
course “Advanced Methods of Scanning Electron Microscopy”

Transmission electron microscope (TEM)

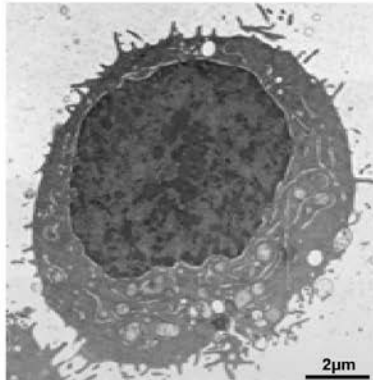
Scanning electron microscope (SEM)



## Transmission electron microscope (TEM)

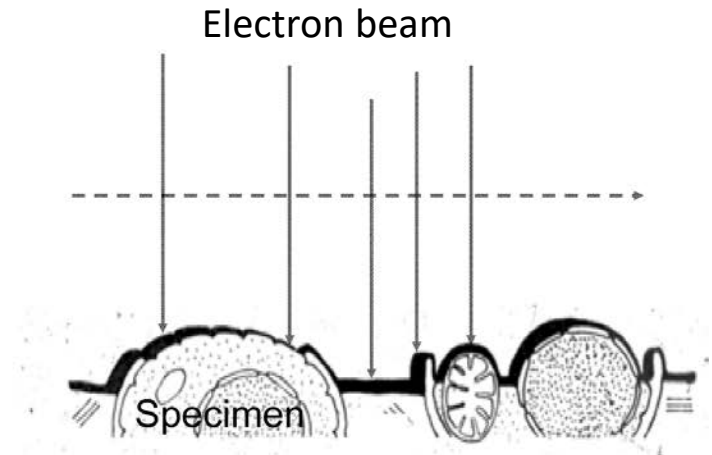


Projection

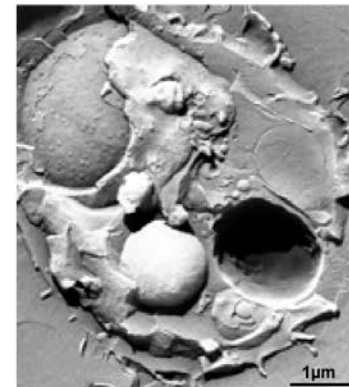


Typical electron energies: 60 – 300 keV

## Scanning electron microscope (SEM)



Surface



≤30 keV

- Principle of SEM
- Electron-matter interaction
- Main parts of SEM
- Types of detectors, their usage
- Other configurations
- Multimodal imaging
- Conclusion

## How SEM works

Electron gun



- Rest mass  $m_0 = 9.1 \times 10^{-31}$  kg
- Charge  $Q = -e = -1.602 \times 10^{-19}$  C
- Rest energy  $E_0 = m_0 c^2 = 511$  keV

- Planck constant  $h = 6.6256 \times 10^{-34}$  Nms

- A non-relativistic approach:

- Energy

$$E = eU = \frac{1}{2} m_0 v^2$$

- Momentum

$$p = m_0 v = \sqrt{2m_0 E}$$

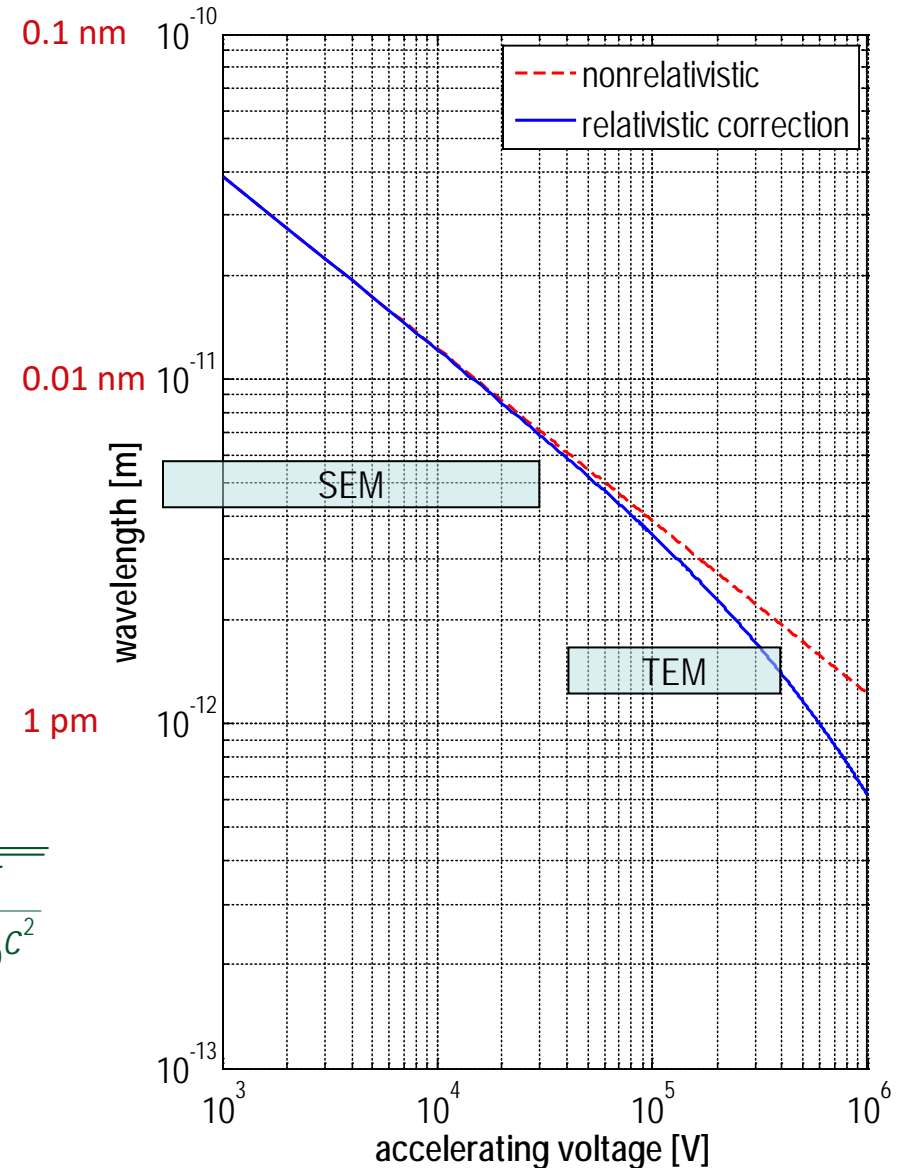
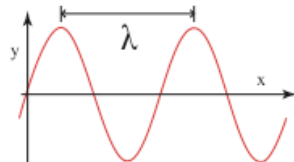
- de Broglie wavelength

$$\lambda = \frac{h}{p} = \frac{h}{m_0 v} = \frac{h}{\sqrt{2m_0 E}}$$

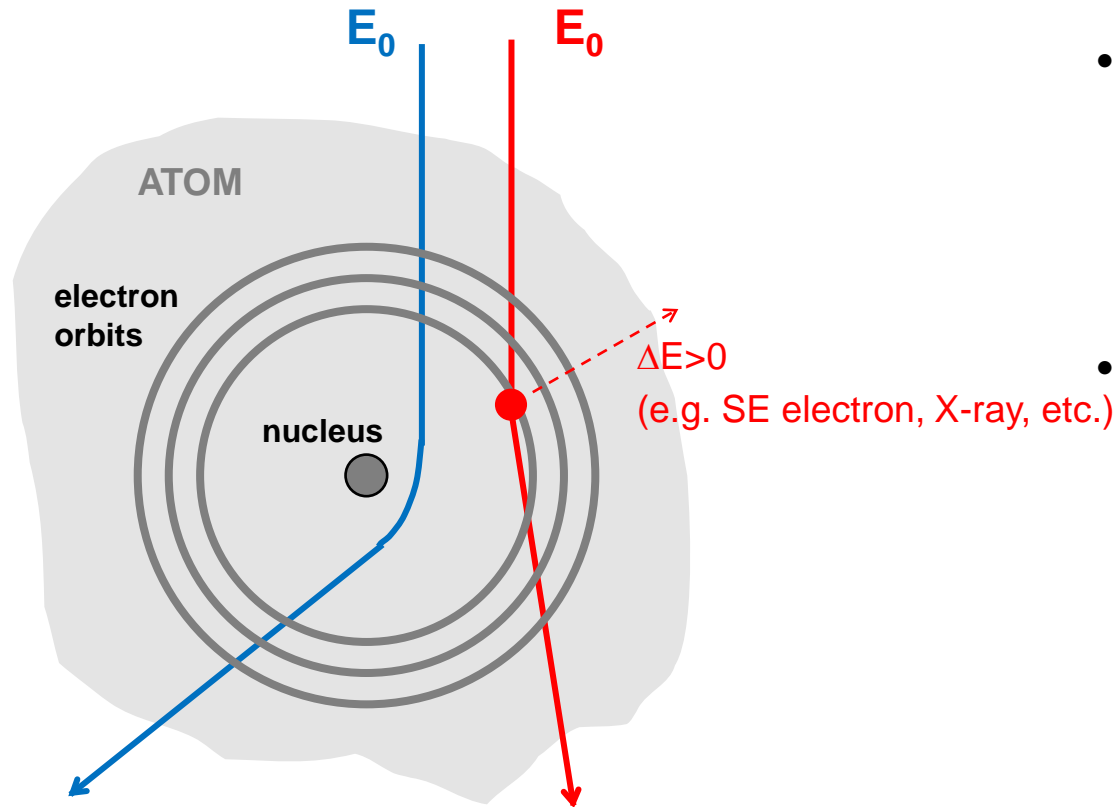
- Wavelength including relativistic correction

$$\lambda = \frac{h}{\sqrt{2m_0 E}} \frac{1}{\sqrt{1 + \frac{E}{2m_0 c^2}}}$$

$$\lambda = \frac{h}{mv} \quad f = \frac{E}{h}$$



# How do electrons interact with matter



**Elastic scattering**

$$E_{el} = E_0$$

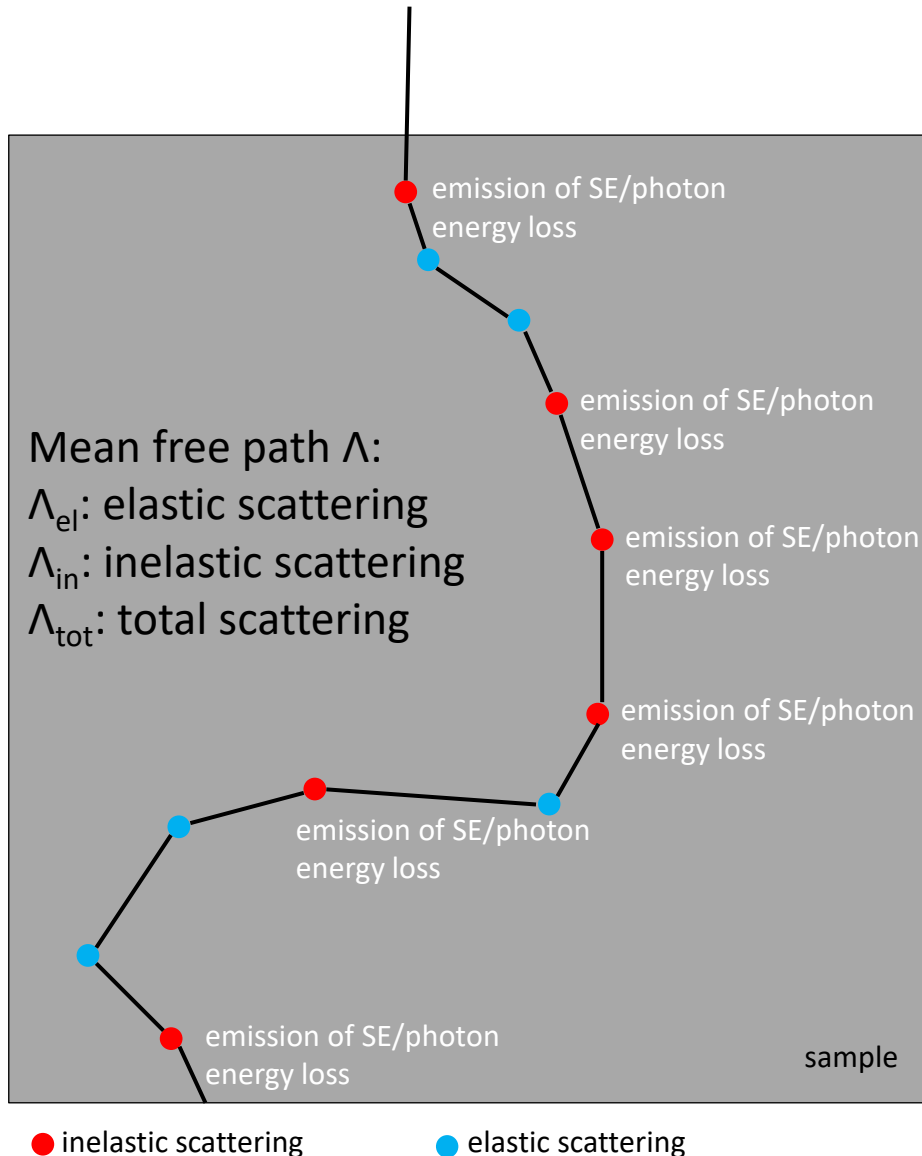
**Inelastic scattering**

$$E_{in} = E_0 - \Delta E$$

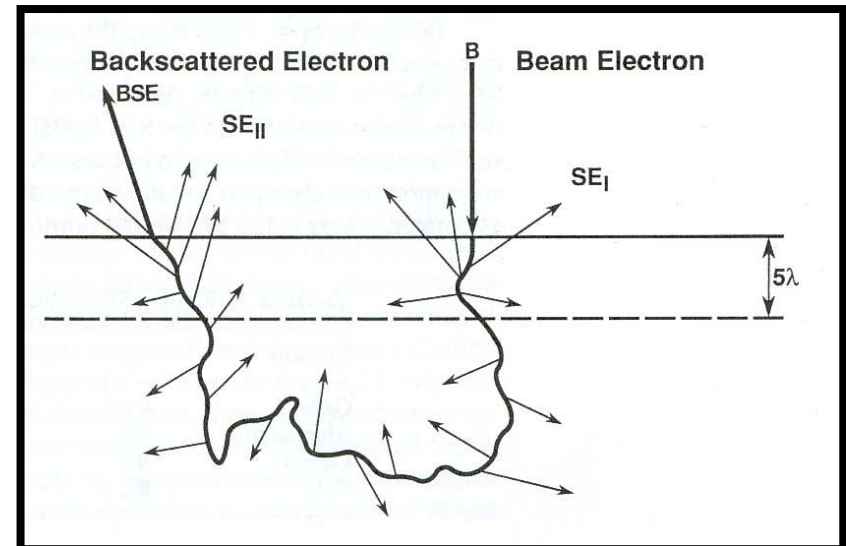
$$\langle \Theta_{el} \rangle \gg \langle \Theta_{in} \rangle$$

- **Unscattered electrons** (no collision)
- **Elastic scattering** on nuclei of atoms of the matter
  - primary electron changes mainly its direction by an angle  $\Theta_{el}$
  - often backscattered electrons
- **Inelastic scattering**
  - primary electron slightly changes its direction by the angle  $\Theta_{in}$  and loses part of its energy  $\Delta E$
  - inner-shell ionization
  - secondary electrons (SE)
  - continuum X-rays (Bremsstrahlung)
  - phonons (lattice vibrations - heat)
  - plasmons (oscillations of loosely bound electrons)
  - cathodeluminescence
  - ...

# Usually multiple scatter occurs



- Scattering occurs depending on the material of the sample and the energy of the electron
- The path of the primary electron can be reversed during the interaction with the sample (backscattered).
- Secondary electrons (SE) have very little energy and therefore only those at the surface get out of the sample
- Due to the loss of energy during inelastic scattering, the primary electron can be absorbed in the sample (electron range)





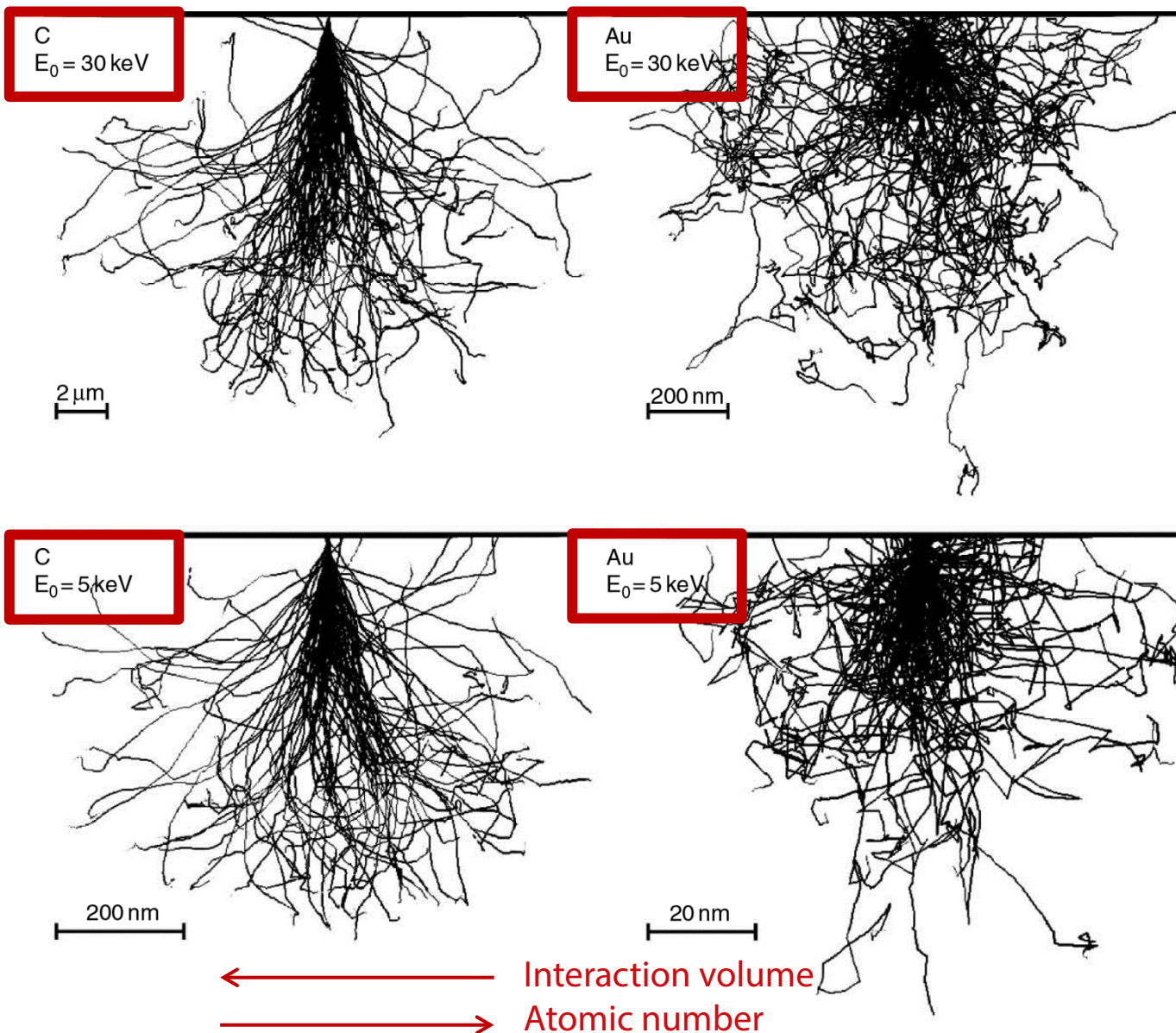
**Table 3.1.** Total elastic cross-sections  $\sigma_{el}$  in units  $10^{-16} \text{ cm}^2$ , elastic mean-free-path length  $\Lambda_{el} = 1/N\sigma_{el}$  in nanometres ( $N = N_A\rho/A$ : Number of atoms per unit volume), total mean-free-path length  $\Lambda_t = \Lambda_{el}/(\nu + 1)$  with  $\nu = \sigma_{inel}/\sigma_{el}$  and the electron range  $R$  in  $\mu\text{m}$  for different elements and electron energies  $E = 1\text{--}50 \text{ keV}$

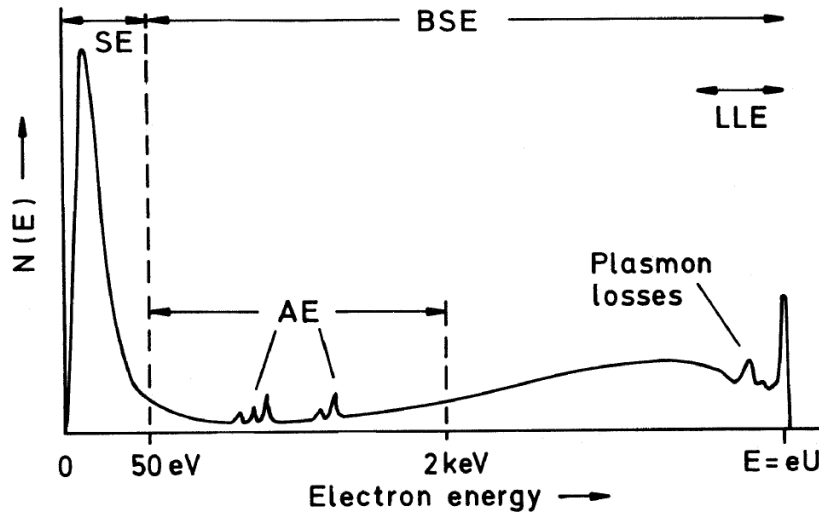
E [keV]		1	5	10	20	30	50	
C Z=6 $\rho = 2 \text{ g/cm}^3$ $\nu \simeq 3$	$\sigma_{el}$	0.65	0.11	0.055	0.027	0.018	0.012	$\times 10^{-16} \text{ cm}^2$
	$\Lambda_{el}$	1.5	9	18	37	55	83	nm
	$\Lambda_t$	0.4	2.3	4.5	9	14	20	nm
	$R$	0.033	0.49	1.55	4.9	9.7	22.6	$\mu\text{m}$
Al Z=13 $\rho = 2.7 \text{ g/cm}^3$ $\nu \simeq 1.5$	$\sigma_{el}$	1.26	0.31	0.16	0.08	0.053	0.034	$\times 10^{-16} \text{ cm}^2$
	$\Lambda_{el}$	1.3	5	10	21	31	49	nm
	$\Lambda_t$	0.5	2	4	8	12	20	nm
	$R$	0.025	0.36	1.14	3.6	7.1	16.7	$\mu\text{m}$
Cu Z=29 $\rho = 8.9 \text{ g/cm}^3$ $\nu \simeq 0.6$	$\sigma_{el}$	1.84	0.64	0.37	0.21	0.15	0.11	$\times 10^{-16} \text{ cm}^2$
	$\Lambda_{el}$	0.64	1.8	3.2	5.6	7.8	10.7	nm
	$\Lambda_t$	0.4	1.1	2.0	3.5	4.9	6.70	nm
	$R$	0.007	0.11	0.35	1.10	2.26	5.1	$\mu\text{m}$
Ag Z=47 $\rho = 10.5 \text{ g/cm}^3$ $\nu \simeq 0.4$	$\sigma_{el}$	3.09	1.15	0.71	0.43	0.32	0.22	$\times 10^{-16} \text{ cm}^2$
	$\Lambda_{el}$	0.5	1.5	2.4	4.0	5.3	7.7	nm
	$\Lambda_t$	0.4	1.0	1.7	2.8	3.8	5.5	nm
	$R$	0.006	0.09	0.29	0.93	1.8	4.3	$\mu\text{m}$
Au Z=79 $\rho = 19.3 \text{ g/cm}^3$ $\nu \simeq 0.2$	$\sigma_{el}$	3.93	1.60	1.05	0.67	0.52	0.37	$\times 10^{-16} \text{ cm}^2$
	$\Lambda_{el}$	0.43	1.0	1.6	2.5	3.3	4.6	nm
	$\Lambda_t$	0.36	0.9	1.3	2.1	2.7	3.8	nm
	$R$	0.003	0.05	0.17	0.51	1.0	2.3	$\mu\text{m}$

$\Lambda$  increases with decreasing Z

$\Lambda$  increases with increasing E

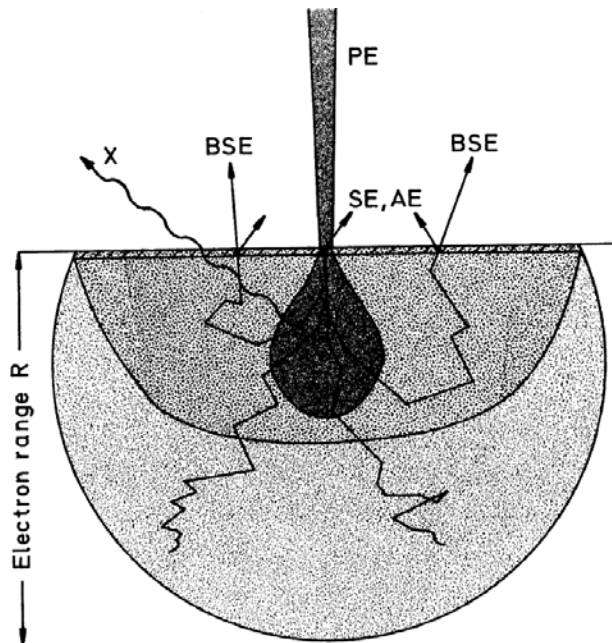
Interaction volume  
Electron energy





Schematic energy spectrum of emitted electrons consisting of

- secondary electrons (SE;  $E_{SE} \leq 50$  eV)
- backscattered electrons (BSE;  $E_{BSE} > 50$  eV)
- and more signals like Auger electrons (AE) or low-loss electrons (LLE) used mainly for material science



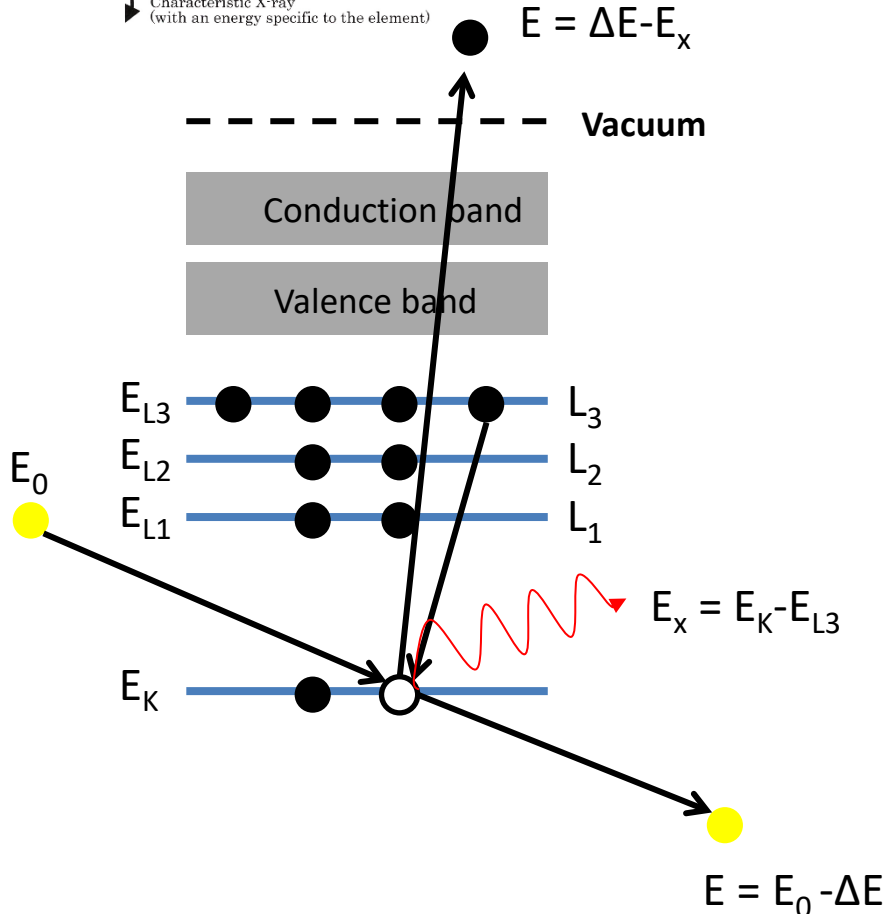
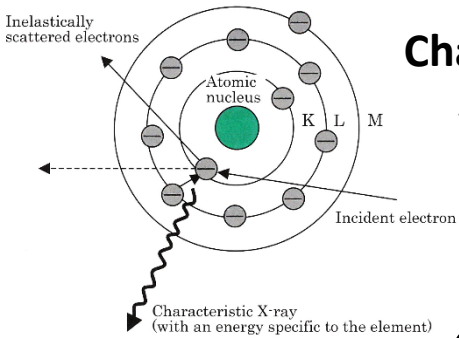
Origin and information depth of

- secondary electrons (SE)
- backscattered electrons (BSE)
- Auger electrons (AE)
- X-ray quanta (X)

in the diffusion cloud of electron range  $R$  for normal incidence of the primary electrons (PE)

# X-rays: Energy dispersive X-ray spectroscopy (EDX)

## Characteristic X-rays



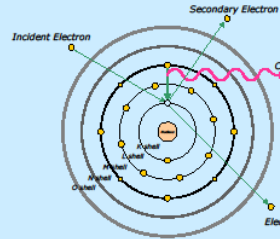
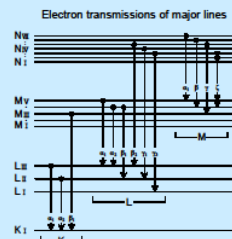
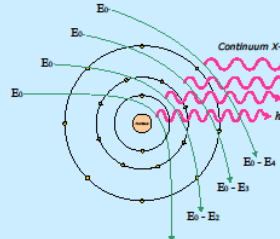
Values for individual elements are well known

Crystal Structures

- Cubic, face centered
- Cubic, body centered
- Cubic
- Hexagonal
- Monoclinic
- Orthorhombic
- Tetragonal
- Rhombohedral

26	55.847	27	58.933	28	58.70
<b>Fe</b>		<b>Co</b>		<b>Ni</b>	
Iron		Cobalt		Nickel	
6.403		6.929		7.477	
0.705		0.776		0.851	

Energy is characteristic of a given element

Characteristic X-rays				Continuum X-rays				Helium									
<b>Hydrogen</b> 1 H 1.01 0.08										<b>Helium</b> 2 He 4.00 0.19							
<b>Lithium</b> 3 Li 6.94 0.53	<b>Beryllium</b> 4 Be 9.01 1.85 K $\alpha$ 0.110	<b>Sodium</b> 11 Na 22.99 0.97 K $\alpha$ 1.041	<b>Magnesium</b> 12 Mg 24.31 1.74 K $\alpha$ 1.253	<b>Boron</b> 5 B 10.81 2.34 K $\alpha$ 0.183	<b>Carbon</b> 6 C 12.01 2.25 K $\alpha$ 0.277	<b>Nitrogen</b> 7 N 14.01 1.14 K $\alpha$ 0.392	<b>Oxygen</b> 8 O 16.00 5.13 K $\alpha$ 0.525	<b>Fluorine</b> 9 F 19.00 1.68 K $\alpha$ 0.677	<b>Neon</b> 10 Ne 20.18 1.20 K $\alpha$ 0.848	<b>Aluminum</b> 13 Al 26.98 2.70 K $\alpha$ 1.486	<b>Silicon</b> 14 Si 28.09 2.42 K $\alpha$ 1.739	<b>Phosphorus</b> 15 P 30.97 3.13 K $\alpha$ 2.013	<b>Sulphur</b> 16 S 32.06 2.07 K $\alpha$ 2.307	<b>Chlorine</b> 17 Cl 35.45 2.2 K $\alpha$ 2.621	<b>Argon</b> 18 Ar 39.95 1.65 K $\alpha$ 2.957		
<b>Potassium</b> 19 K 39.10 0.87 K $\alpha$ 3.312	<b>Calcium</b> 20 Ca 40.08 4.5 K $\alpha$ 3.690	<b>Scandium</b> 21 Sc 44.96 4.5 K $\alpha$ 4.088	<b>Titanium</b> 22 Ti 47.88 5.49 K $\alpha$ 4.508	<b>Vanadium</b> 23 V 50.94 7.14 K $\alpha$ 4.949	<b>Chromium</b> 24 Cr 52.00 7.1 K $\alpha$ 5.411	<b>Manganese</b> 25 Mn 54.94 7.3 K $\alpha$ 5.894	<b>Iron</b> 26 Fe 55.85 7.46 K $\alpha$ 6.398	<b>Cobalt</b> 27 Co 58.93 7.4 K $\alpha$ 6.924	<b>Nickel</b> 28 Ni 58.70 6.49 K $\alpha$ 7.471	<b>Copper</b> 29 Cu 63.55 8.93 K $\alpha$ 8.040	<b>Zinc</b> 30 Zn 65.38 7.4 K $\alpha$ 8.630	<b>Gallium</b> 31 Ga 69.72 7.4 K $\alpha$ 9.241	<b>Germanium</b> 32 Ge 72.59 7.3 K $\alpha$ 9.874	<b>Arsenic</b> 33 As 74.92 7.4 K $\alpha$ 10.530	<b>Selenium</b> 34 Se 78.96 7.4 K $\alpha$ 11.207	<b>Bromine</b> 35 Br 79.90 7.4 K $\alpha$ 11.907	<b>Krypton</b> 36 Kr 83.80 7.4 K $\alpha$ 12.631
<b>Rubidium</b> 37 Rb 85.47 1.53 K $\alpha$ 13.373	<b>Strontium</b> 38 Sr 87.62 2.60 K $\alpha$ 14.140	<b>Yttrium</b> 39 Y 88.91 4.48 K $\alpha$ 14.931	<b>Zirconium</b> 40 Zr 91.22 6.44 K $\alpha$ 15.744	<b>Niobium</b> 41 Nb 92.91 5.4 K $\alpha$ 16.581	<b>Molybdenum</b> 42 Mo 95.94 9.01 K $\alpha$ 17.441	<b>Technetium</b> 43 Tc (97) 98.00 7.4 K $\alpha$ 18.325	<b>Ruthenium</b> 44 Ru 101.07 12.1 K $\alpha$ 19.233	<b>Rhodium</b> 45 Rh 102.91 12.44 K $\alpha$ 2.696	<b>Palladium</b> 46 Pd 106.4 12.16 K $\alpha$ 2.838	<b>Silver</b> 47 Ag 107.87 10.49 K $\alpha$ 2.984	<b>Cadmium</b> 48 Cd 112.40 8.65 K $\alpha$ 3.133	<b>Indium</b> 49 In 114.82 7.28 K $\alpha$ 3.286	<b>Tin</b> 50 Sn 118.69 7.30 K $\alpha$ 3.443	<b>Antimony</b> 51 Sb 121.75 6.62 K $\alpha$ 3.604	<b>Tellurium</b> 52 Te 127.60 6.25 K $\alpha$ 3.769	<b>Iodine</b> 53 I 126.90 4.54 K $\alpha$ 3.937	<b>Xenon</b> 54 Xe 131.30 5.4 K $\alpha$ 4.109
<b>Cesium</b> 55 Cs 132.91 1.87 K $\alpha$ 4.286	<b>Barium</b> 56 Ba 137.34 3.5 K $\alpha$ 4.465	<b>Lanthanoid</b> 57-71	<b>Hafnium</b> 72 Hf 178.49 13.3 K $\alpha$ 7.898	<b>Tantalum</b> 73 Ta 180.95 16.6 K $\alpha$ 8.145	<b>Tungsten</b> 74 W 183.85 19.3 K $\alpha$ 8.396	<b>Rhenium</b> 75 Re 186.21 20.53 K $\alpha$ 8.651	<b>Osmium</b> 76 Os 190.2 22.5 K $\alpha$ 8.910	<b>Iridium</b> 77 Ir 192.22 22.42 K $\alpha$ 9.174	<b>Platinum</b> 78 Pt 195.09 21.37 K $\alpha$ 9.441	<b>Gold</b> 79 Au 196.97 19.88 K $\alpha$ 9.712	<b>Mercury</b> 80 Hg 200.59 14.19 K $\alpha$ 9.987	<b>Thallium</b> 81 Tl 204.37 11.86 K $\alpha$ 10.267	<b>Lead</b> 82 Pb 207.19 11.34 K $\alpha$ 10.550	<b>Bismuth</b> 83 Bi 208.98 9.78 K $\alpha$ 10.837	<b>Polonium</b> 84 Po (209) 209 9.78 K $\alpha$ 11.129	<b>Astatine</b> 85 At (210) 210 9.78 K $\alpha$ 11.425	<b>Radon</b> 86 Rn (222) 222 9.78 K $\alpha$ 11.725
<b>Francium</b> 87 Fr (223) 223 9.78 K $\alpha$ 12.029	<b>Radium</b> 88 Ra 226.03 5 K $\alpha$ 12.340	<b>Actinoid</b> 89-103	<b>Lanthanum</b> 57 La 138.91 6.17 K $\alpha$ 4.650	<b>Cerium</b> 58 Ce 140.12 6.66 K $\alpha$ 4.839	<b>Praseodymium</b> 59 Pr 140.91 6.77 K $\alpha$ 5.033	<b>Neodymium</b> 60 Nd 144.24 7.02 K $\alpha$ 5.229	<b>Promethium</b> 61 Pm (144) 144.91 7.54 K $\alpha$ 5.432	<b>Samarium</b> 62 Sm 150.35 7.54 K $\alpha$ 5.635	<b>Europium</b> 63 Eu 151.96 5.25 K $\alpha$ 5.845	<b>Gadolinium</b> 64 Gd 157.25 7.90 K $\alpha$ 6.056	<b>Terbium</b> 65 Tb 158.93 8.25 K $\alpha$ 6.272	<b>Dysprosium</b> 66 Dy 162.50 8.56 K $\alpha$ 6.494	<b>Holmium</b> 67 Ho 164.93 8.80 K $\alpha$ 6.719	<b>Erbium</b> 68 Er 167.26 9.06 K $\alpha$ 6.947	<b>Thulium</b> 69 Tm 168.93 9.32 K $\alpha$ 7.179	<b>Ytterbium</b> 70 Yb 173.04 6.96 K $\alpha$ 7.414	<b>Lutetium</b> 71 Lu 174.97 9.84 K $\alpha$ 7.654
<b>Actinium</b> 89 Ac 227.03 10.07 K $\alpha$ 12.650	<b>Thorium</b> 90 Th 232.04 11.00 K $\alpha$ 12.967	<b>Protactinium</b> 91 Pa 231.04 10.07 K $\alpha$ 13.288	<b>Uranium</b> 92 U 238.03 18.7 K $\alpha$ 13.612	<b>Neptunium</b> 93 Np 237.05 18.7 K $\alpha$ 13.942	<b>Plutonium</b> 94 Pu (244) 244 18.7 K $\alpha$ 14.276	<b>Americium</b> 95 Am (243) 243 18.7 K $\alpha$ 14.615	<b>Curium</b> 96 Cm (247) 247 18.7 K $\alpha$ 14.953	<b>Berkelium</b> 97 Bk (247) 247 18.7 K $\alpha$ 15.304	<b>Californium</b> 98 Cf (251) 251 18.7 K $\alpha$ 15.652	<b>Einsteinium</b> 99 Es (254) 254 18.7 K $\alpha$ 16.000	<b>Fermium</b> 100 Fm (257) 257 18.7 K $\alpha$ 16.348	<b>Mendelevium</b> 101 Md (258) 258 18.7 K $\alpha$ 16.696	<b>Nobelium</b> 102 No (259) 259 18.7 K $\alpha$ 17.044	<b>Lawrencium</b> 103 Lr (262) 262 18.7 K $\alpha$ 17.392			

**Gold**  
Number: 79  
Atomic mass: 196.97  
Density (kg/m<sup>3</sup>): 19.3  
Characteristic X-ray (keV): 2.120

**Name**: Gold  
**Symbol**: Au

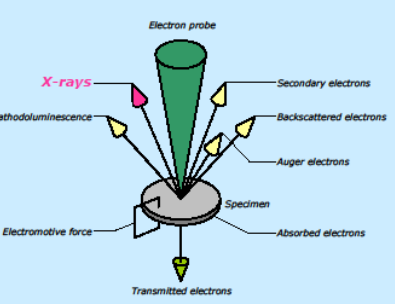
Note: Density  
\* 'C' as (graphite), 'P' as (white),  
'S' as (alpha), 'Sn' as (white)

**Minimum accelerating voltage**

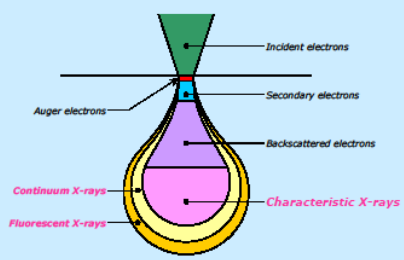
Unable to detect	5kV or higher
10kV or higher	15kV or higher

The colors mean to detect the characteristic X-ray of the lowest energy for each element.

### Information from specimen



### Generation depth and space resolution

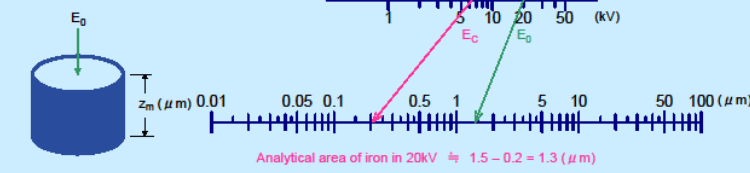


### Analytical area

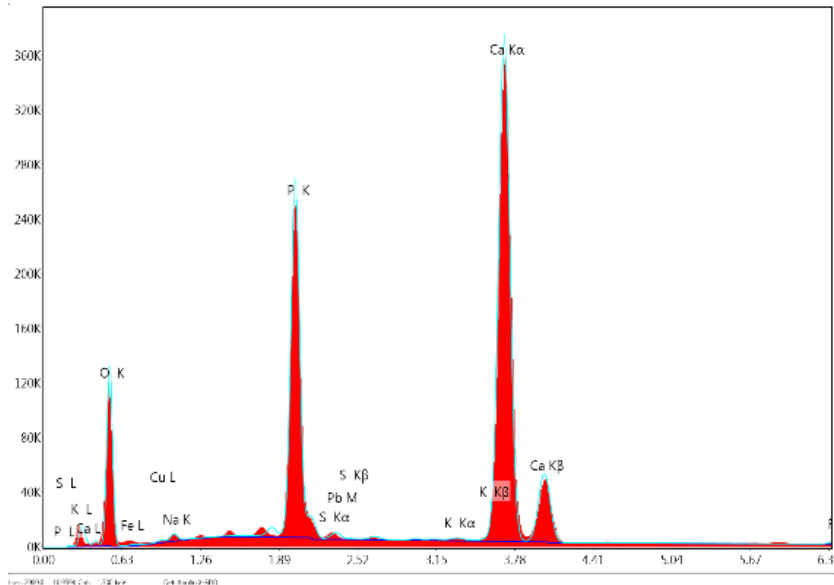
#### Castaing's formula

$$z_m = 0.033 (E_0^{1.7} - E_c^{1.7}) \frac{A}{\rho Z}$$

$E_0$ : Accelerating voltage (kV)  
 $E_c$ : Minimum emission voltage (keV)  
 $A$ : Atomic mass  
 $\rho$ : Density (kg/m<sup>3</sup>)  
 $Z$ : Atomic number

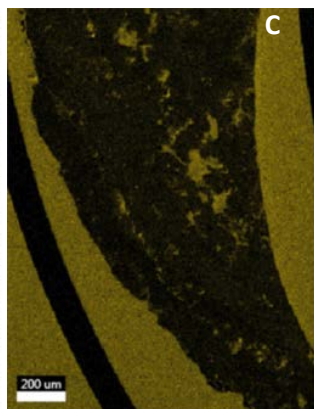
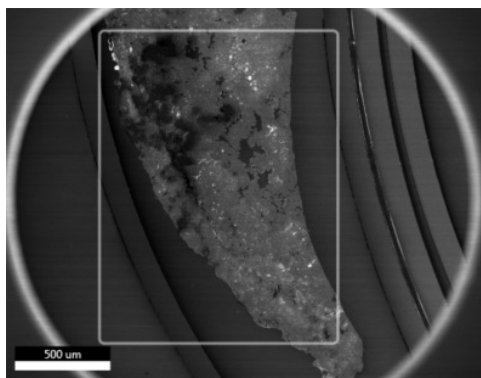


EDX spectrum recorded from an area or point = all elements in one place



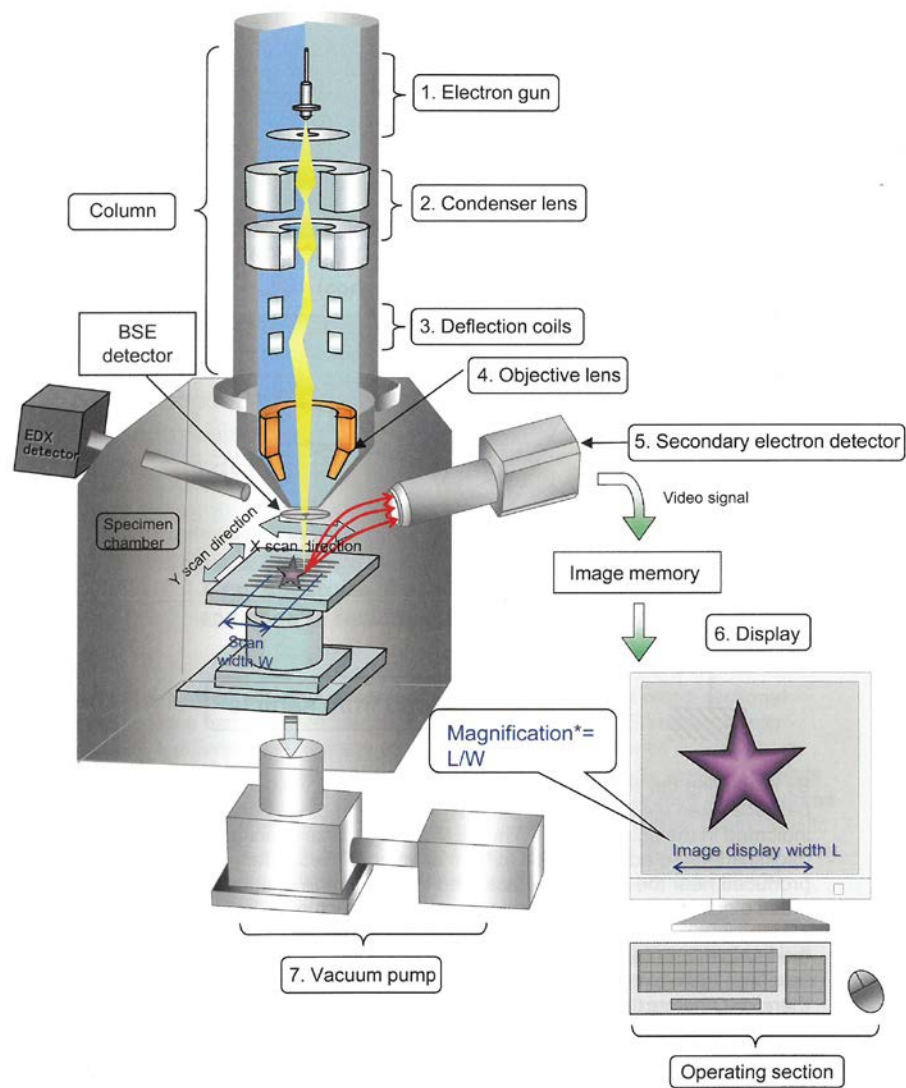
Element	Wt %	Atomic %	Error %	Net Int.	Net Error %
O K	42.04	63.00	9.71	368.04	0.13
Na K	0.60	0.63	9.20	15.80	1.28
P K	15.20	11.76	2.79	1119.15	0.08
S K	0.06	0.04	10.32	3.98	7.24
Pb M	0.57	0.07	5.27	17.70	2.23
K K	0.13	0.08	7.62	8.86	5.97
Ca K	39.54	23.65	1.53	2026.68	0.05
Fe K	1.44	0.62	2.70	33.46	1.04
Cu K	0.42	0.16	6.01	6.04	4.37

Color representation of selected elements in the sample = one element in all pixels



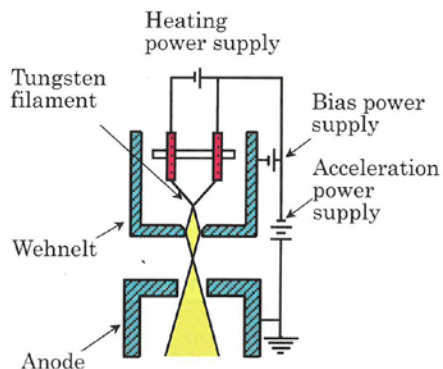
WDX/WDS: wavelength dispersive X-ray spectroscopy (much better energy resolution, but slow)

# Scanning electron microscope (main parts)

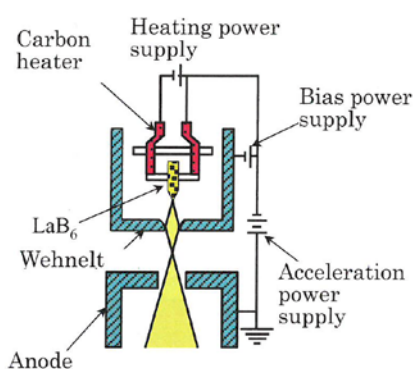


- Electron gun is source of electrons with required parameters
- Condenser lens converge the electron beam emitted from electron gun into finer beam
- Deflection coils are used to scan the electron beam in X and Y directions and change size of the area to be scanned (magnification)
- Objective lens converges electron beam into a fine beam and focus it on the sample surface.
- Detectors for capturing signal for each pixel
- Synchronization of data acquisition and scanning system to receive an 2D image

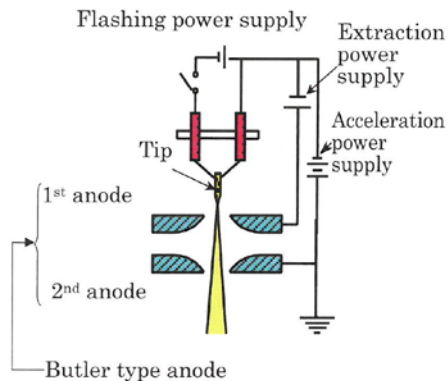
## Tungsten



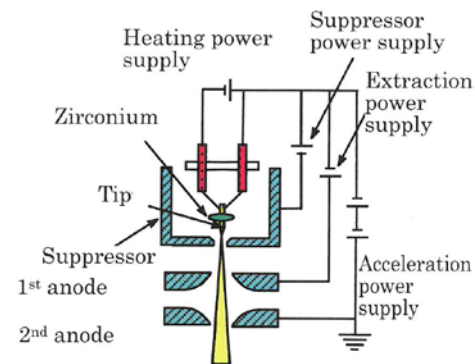
## LaB<sub>6</sub>



## Cold field-emission



## Schottky

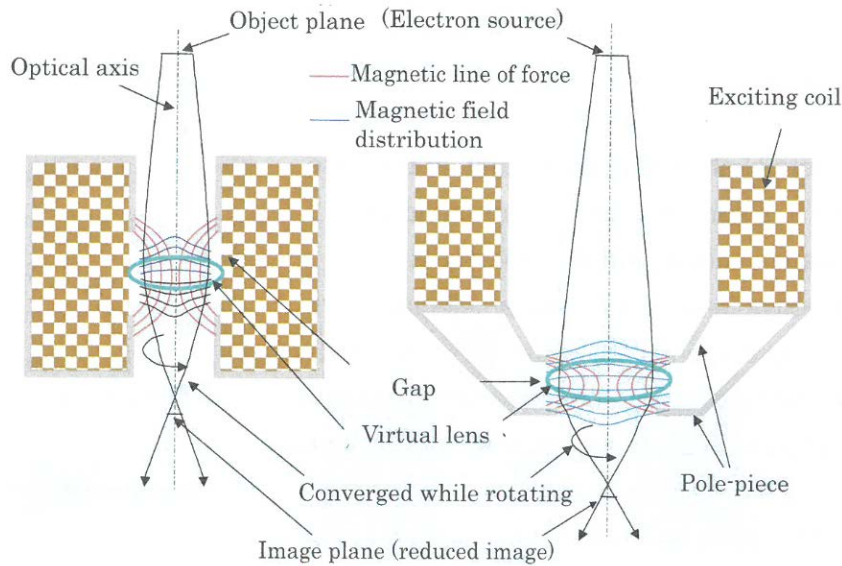


	Tungsten	LaB <sub>6</sub>	CFE	Schottky
Material	Tungsten hairpin	Lanthanum hexaboride single crystal	Tungsten single crystal	Tungsten single crystal/ zirc. oxide
Cathode temp	~2300 °C	~1500 °C	RT °C	~1500 °C
Brightness	10 <sup>6</sup> A/cm <sup>2</sup> ·sr	10 <sup>7</sup> A/cm <sup>2</sup> ·sr	10 <sup>9</sup> A/cm <sup>2</sup> ·sr	10 <sup>8</sup> A/cm <sup>2</sup> ·sr
Energy width	~2 eV	~1.5 eV	0.2-0.3 eV	0.3-1 eV
Max. probe current	~100 nA	~100 nA	~10 μA	~100 nA
Working pressure	10 <sup>-4</sup> Pa	10 <sup>-5</sup> Pa	10 <sup>-8</sup> Pa	10 <sup>-7</sup> Pa
Lifetime	50-100 h	200-1000 h	2-4 y	2-3 y



## Condenser lens

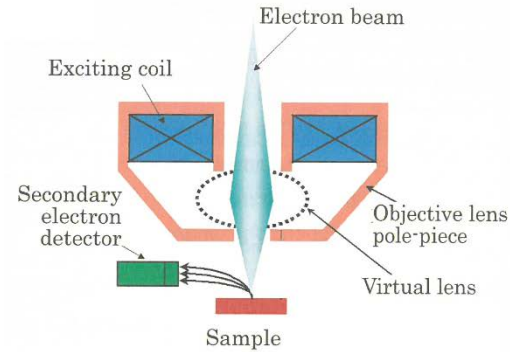
## Objective lens



**Electron lenses in SEM:** magnetic/electrostatic lenses to focus the accelerated virtual electron source from electron gun into a fine probe onto the sample surface.

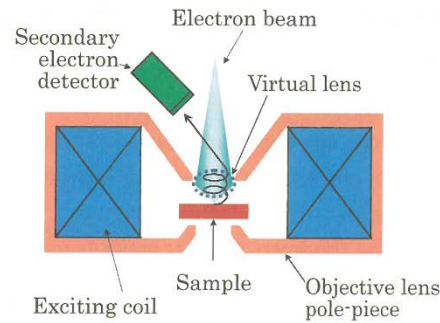
**Condenser lens:** “shaping” electron beam (responsible for its intensity)

**Objective lens:** focusing the electron beam into a fine probe



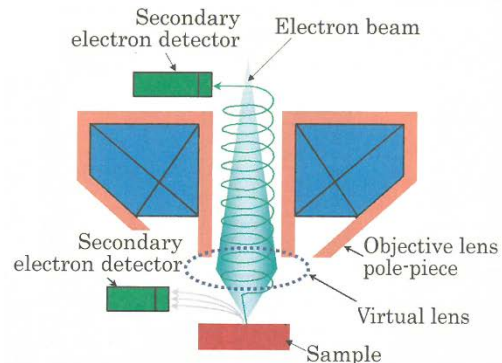
### Standard type

- generally used in low-end SEM
- WD can be high (longer depth of focus)
- optionally can be set also in snorkel type



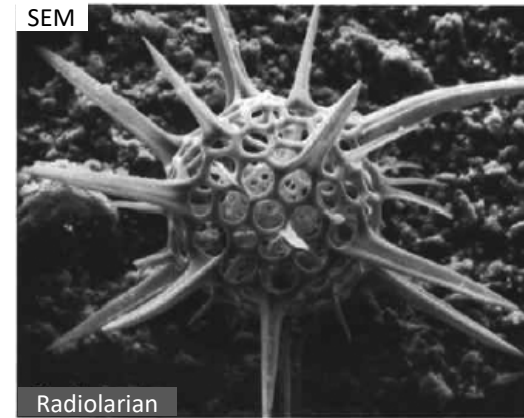
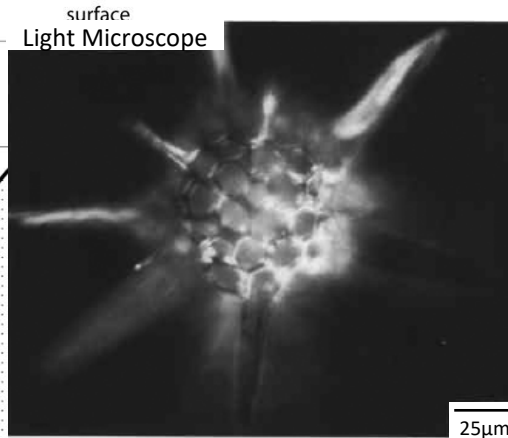
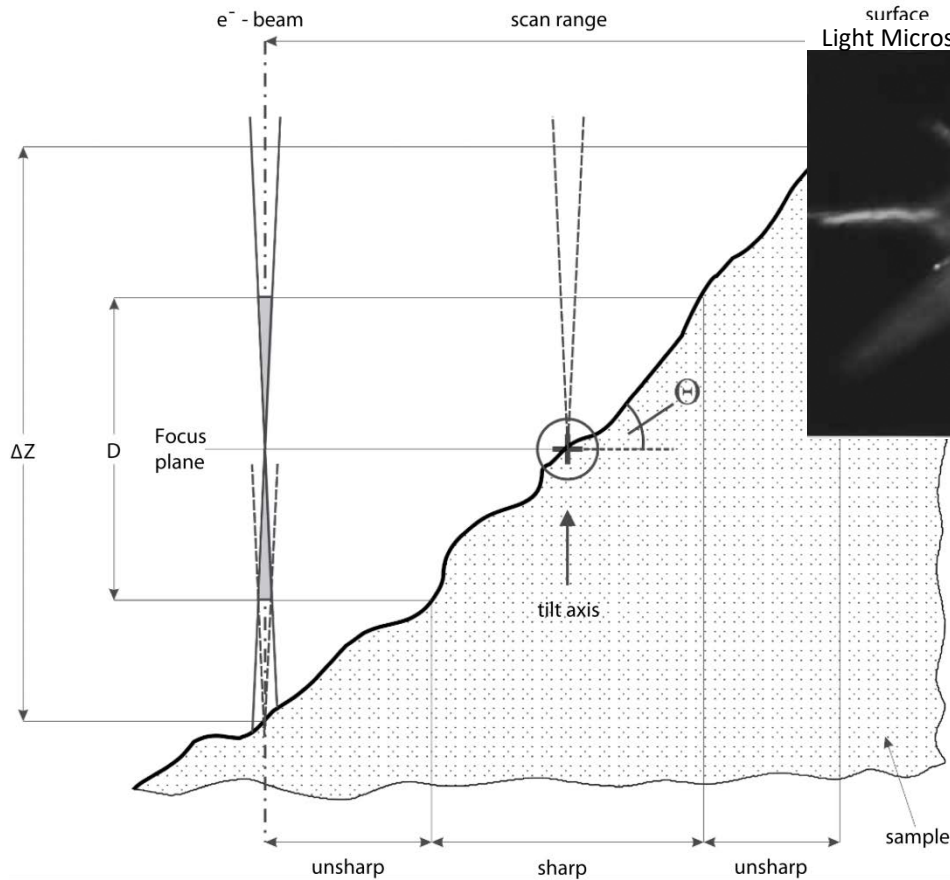
### In-lens type

- best resolution SEM
- only short WD
- limited sample size



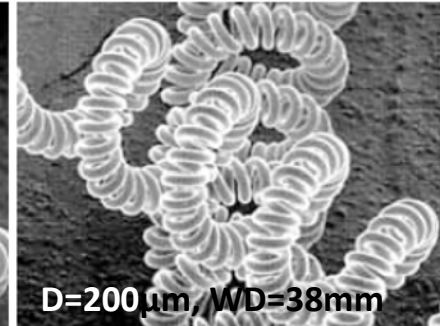
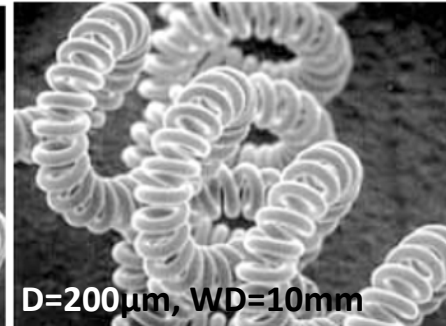
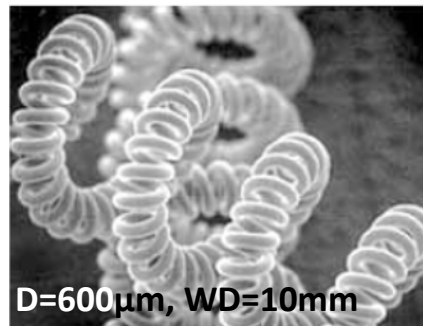
### Semi in-lens (snorkel) type

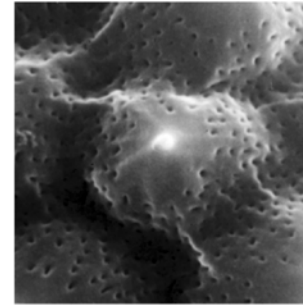
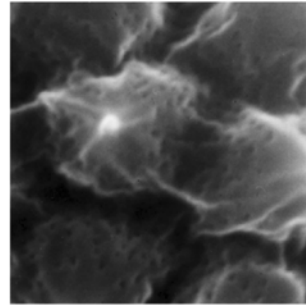
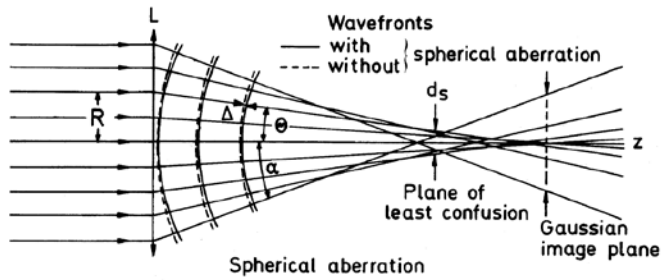
- high-end SEM
- virtual lens close to sample (sample in EM field)



Magnification	Depth of focus (example)	
	Light microscope	SEM
10	60 $\mu$ m	1000 $\mu$ m
100	8 $\mu$ m	100 $\mu$ m
1,000	0.2 $\mu$ m	10 $\mu$ m
10,000	---	1 $\mu$ m

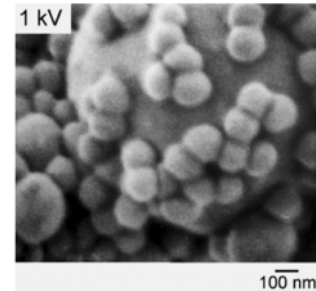
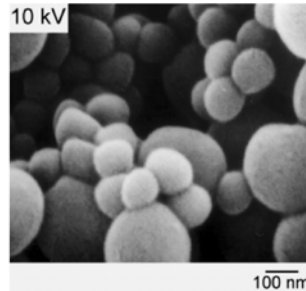
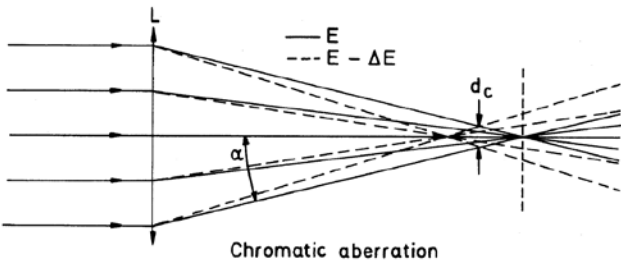
Effect of aperture diameter ( $D$ ) and working distance ( $WD$ ) of depth of focus  
(Light bulb coil)





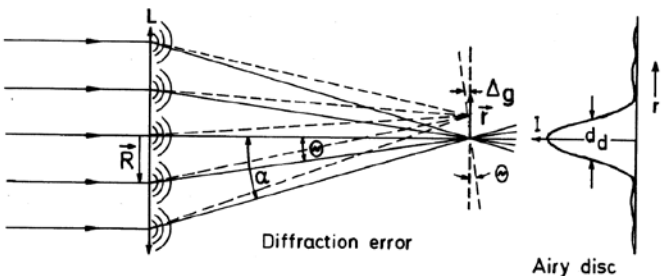
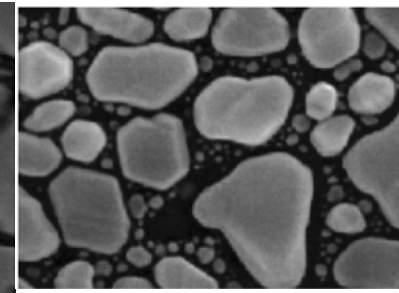
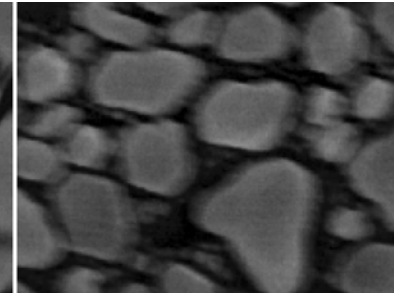
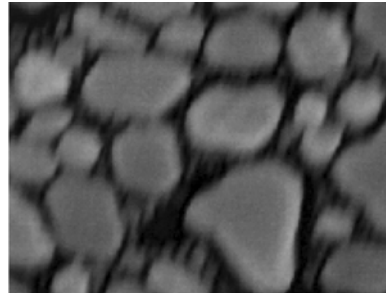
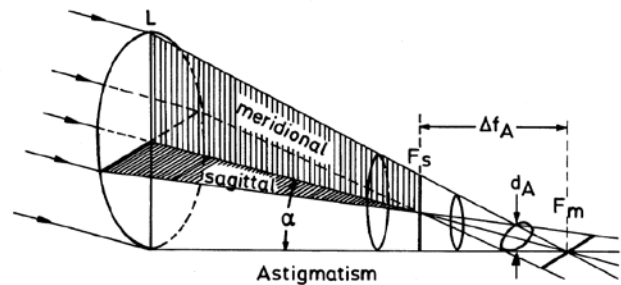
$$d_s \sim \frac{1}{2} C_s \alpha^3$$

$C_s$  ... spherical aber. coef.



$$d_c \sim C_c (\Delta E/E_0) \alpha$$

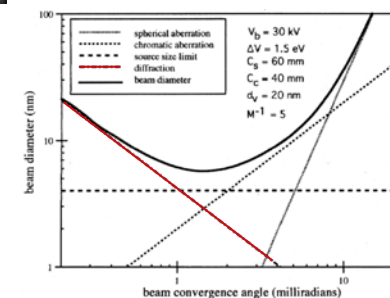
$C_c$  ... chromatic aber. coef.



$$d_d = 1.22 \lambda / \alpha$$

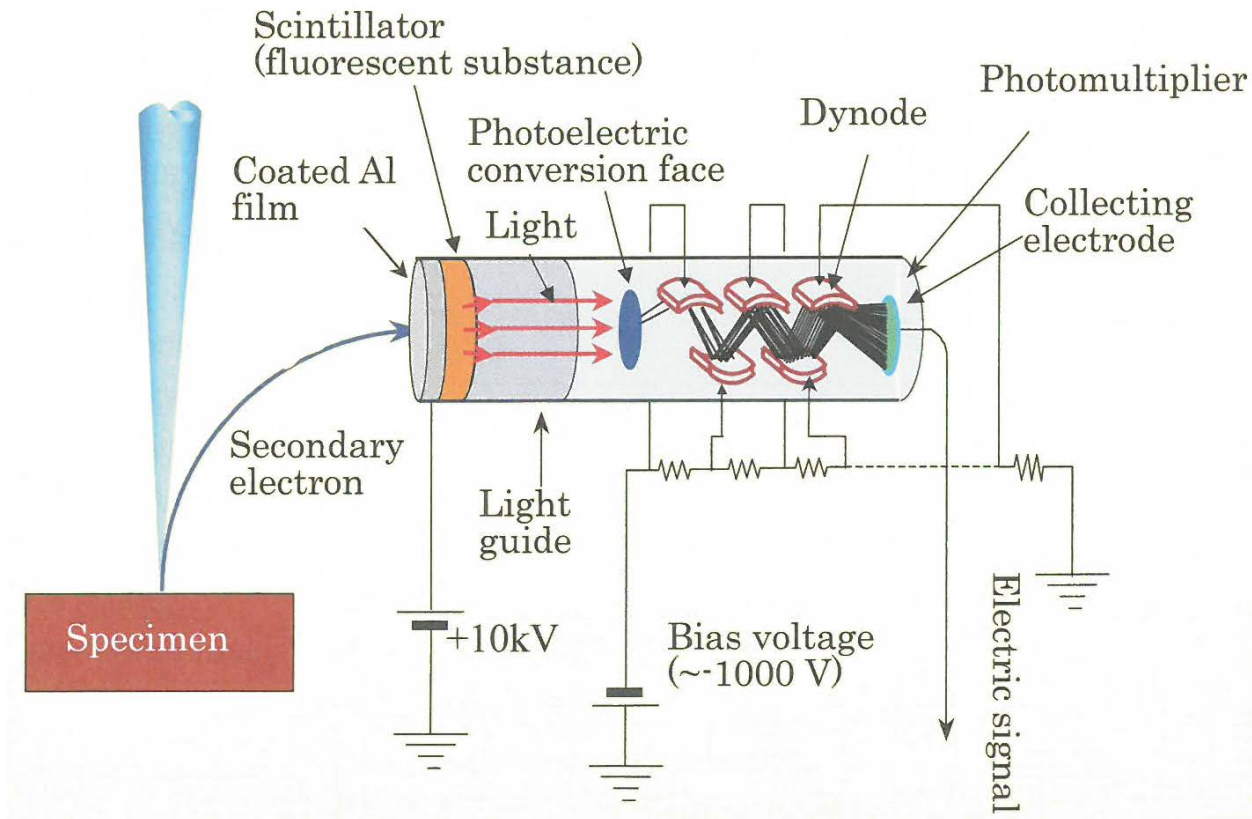
$\lambda$  ... wavelength of electrons

Increasing  $\alpha$  effects spherical and chromatic aberrations being higher



**Everhart-Thornley (ET) detector**

- SE electrons (very low energy  $<50$  eV) are accelerated towards 10 kV electric field and hit against the scintillator for conversion into the light signal which leads to the photomultiplier tube (PMT) through a light pipe



# Signals in SEM and their detection

## X-ray detector detector:

EDX, WDX detectors

## CL detector

Cathodoluminescence (CL)  
electronic states information

(~1-5 μm)

Characteristic X-ray (EDX)  
thickness atomic composition

Auger Electrons (AE)  
surface atomic composition

(~1 nm)

Secondary Electrons (SE)  
topological information (SEM)

Backscattered Electrons (BSE)  
atomic number and phase differences

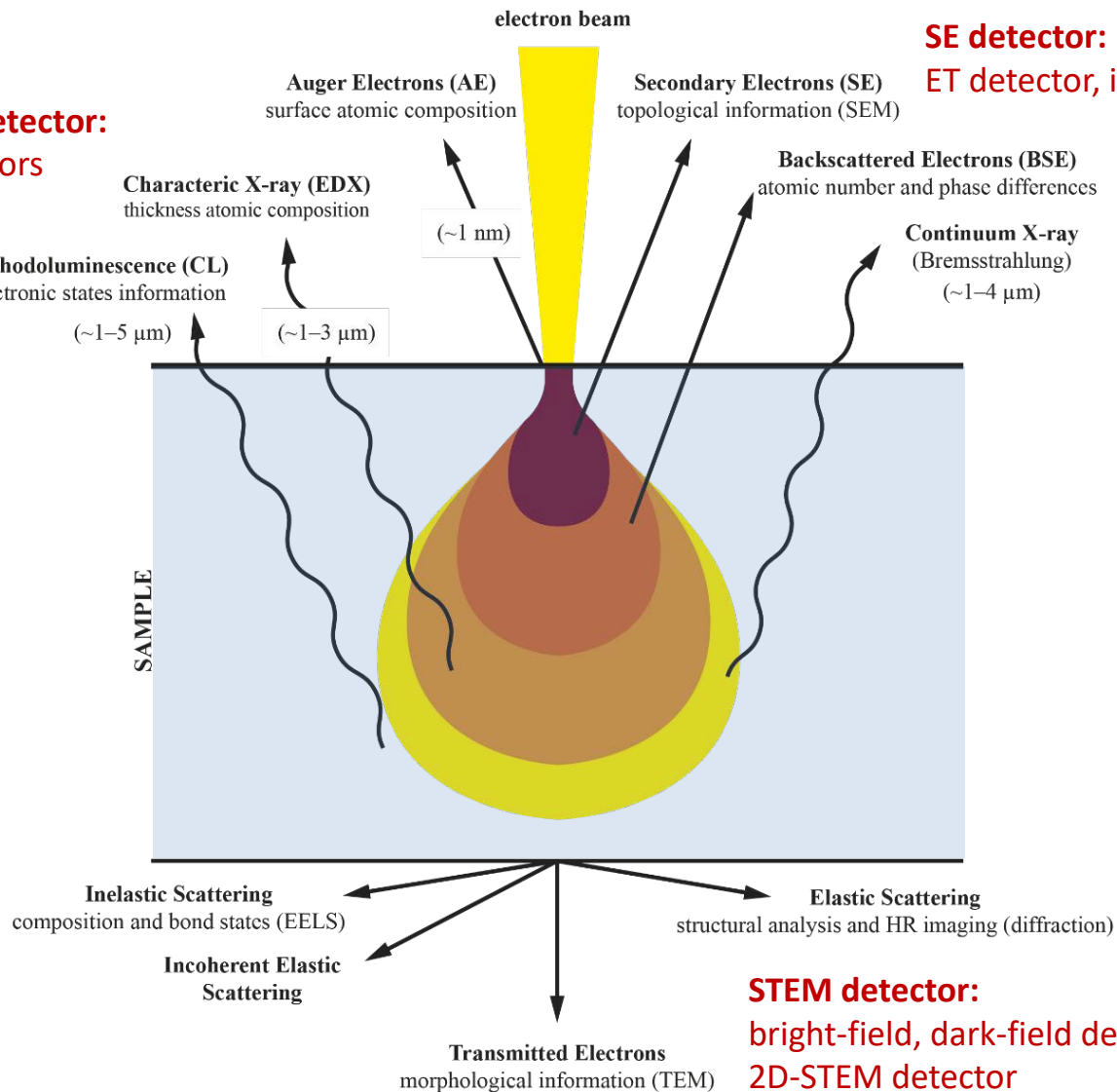
Continuum X-ray  
(Bremsstrahlung)  
(~1-4 μm)

## SE detector:

ET detector, in-lens (TLD) detector

## BSE detector:

retractable BSE detector,  
in-lens detektor

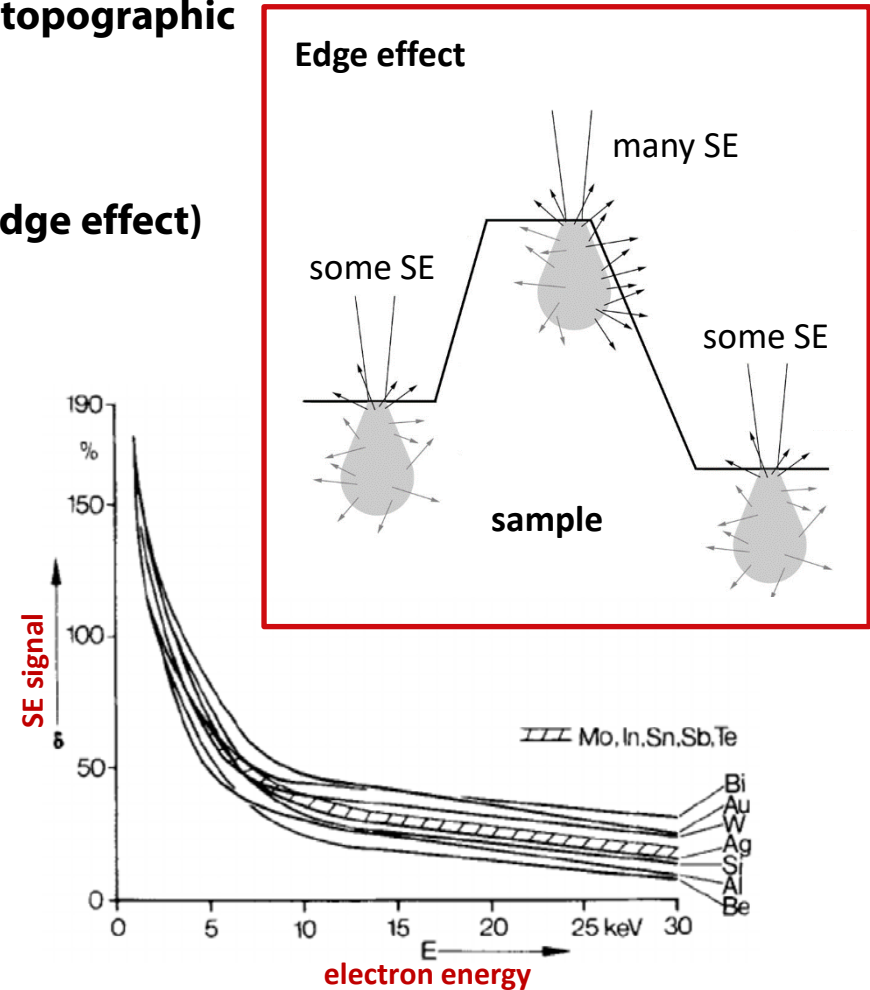
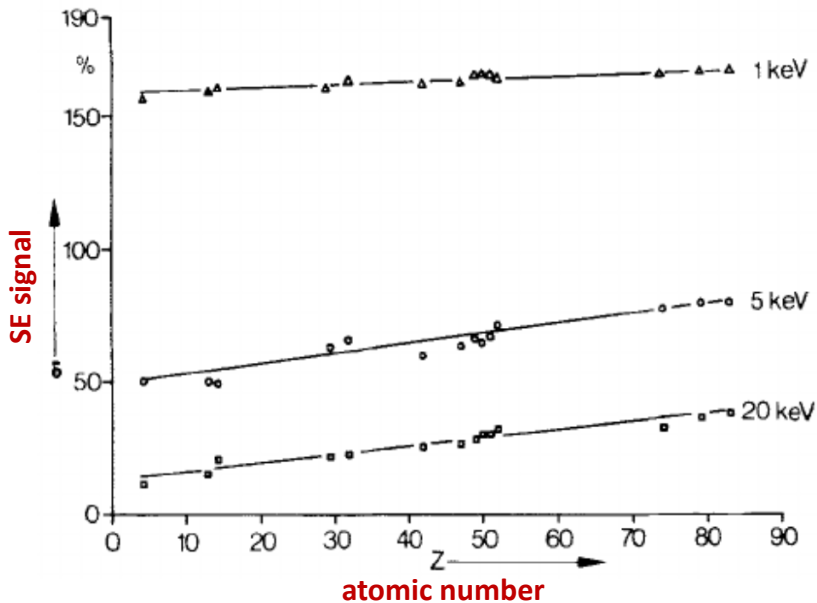


## STEM detector:

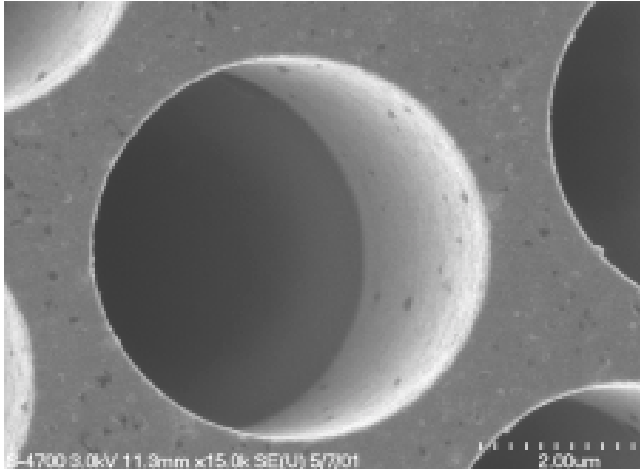
bright-field, dark-field detectors,  
2D-STEM detector

## SE signal:

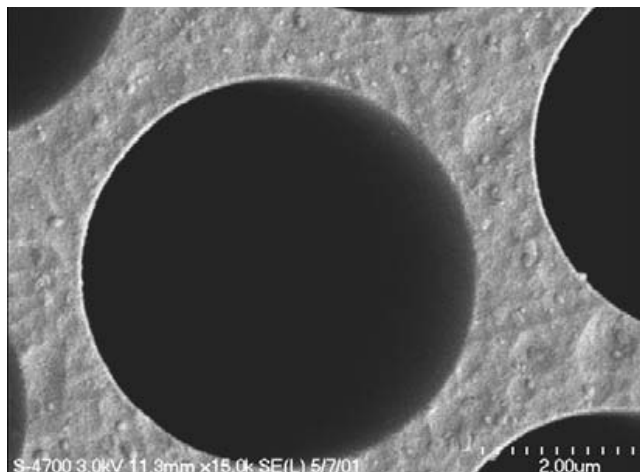
- is formed by very low energy of secondary electrons ( $\leq 50$  eV) generated from the collision between the primary electrons and loosely bound outer electrons
- gives **mainly information about topography (topographic contrast)**, less about composition
- **increases with decreasing electron energy**
- **weaker dependence on atomic number Z**
- is **strongly dependent on tilt of the sample (edge effect)**



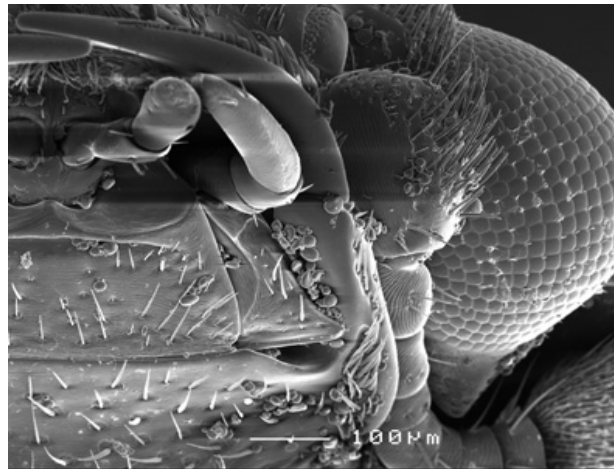
- SE detector type
- upper “in lens” detector



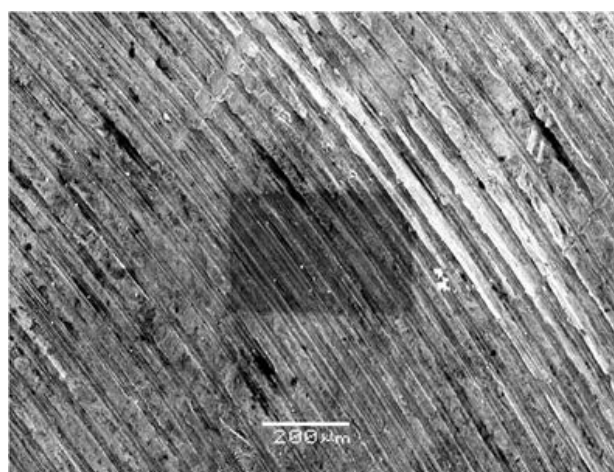
- lower “ET” detector



- Artefacts
- charging

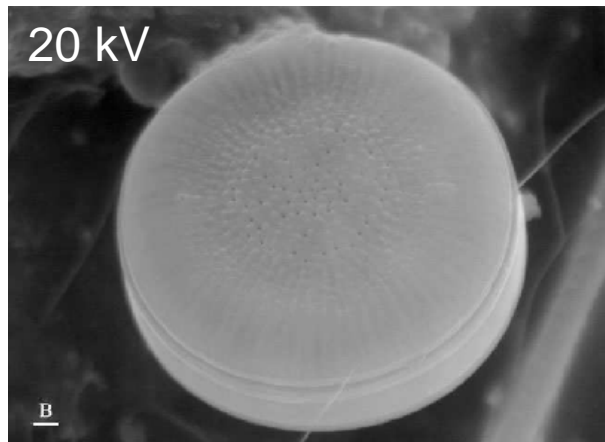
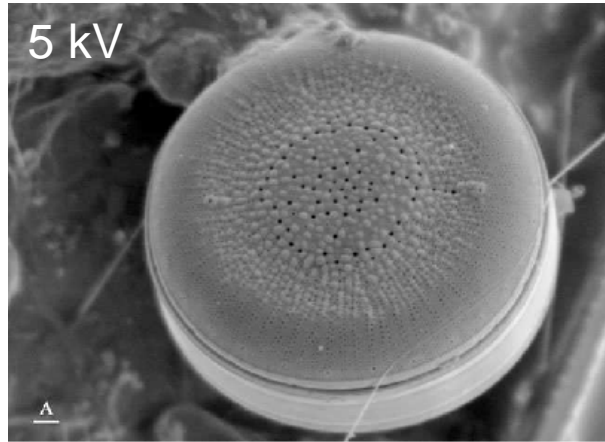


- contamination



- In non-conducting samples the electron probe current remains in sample → accumulation of charge → artefacts mainly for SE (*reducing probe current, lowering  $E_0$ , imaging with BSE, metal coating,...*)
- Result of interaction of electron beam with residual gasses and hydrocarbons on the sample surface (*ensure cleanliness, decrease probe current, use plasma cleaner,...*)

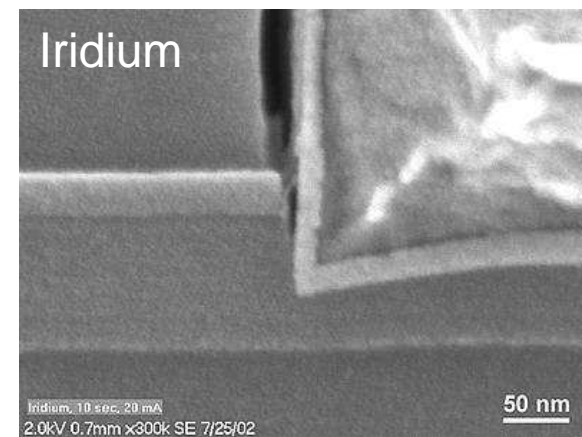
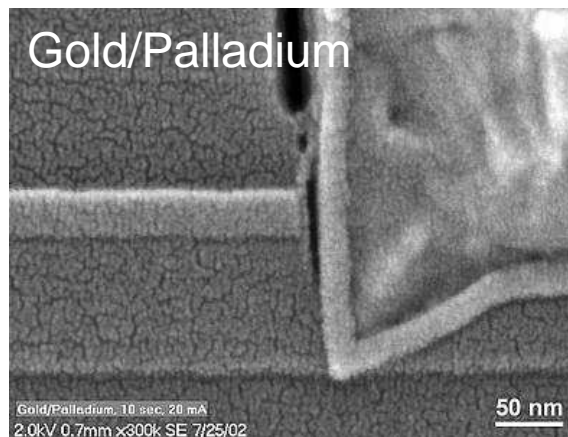
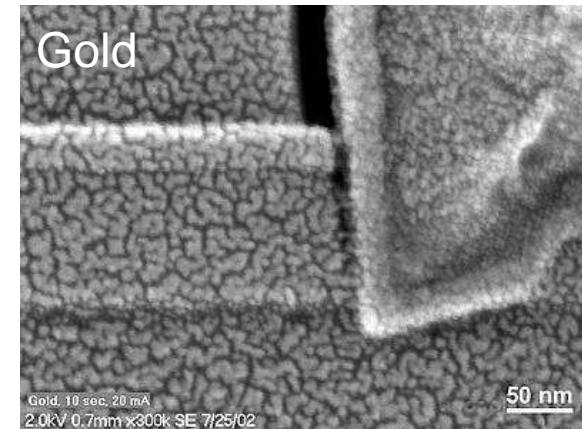
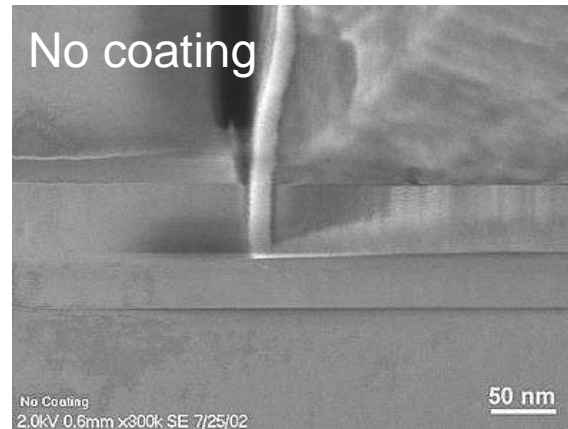
- Effect of penetration depth



Bar: 1  $\mu\text{m}$  (Sample: Diatom)

- Enhancement of contrast by metal coating to reduce

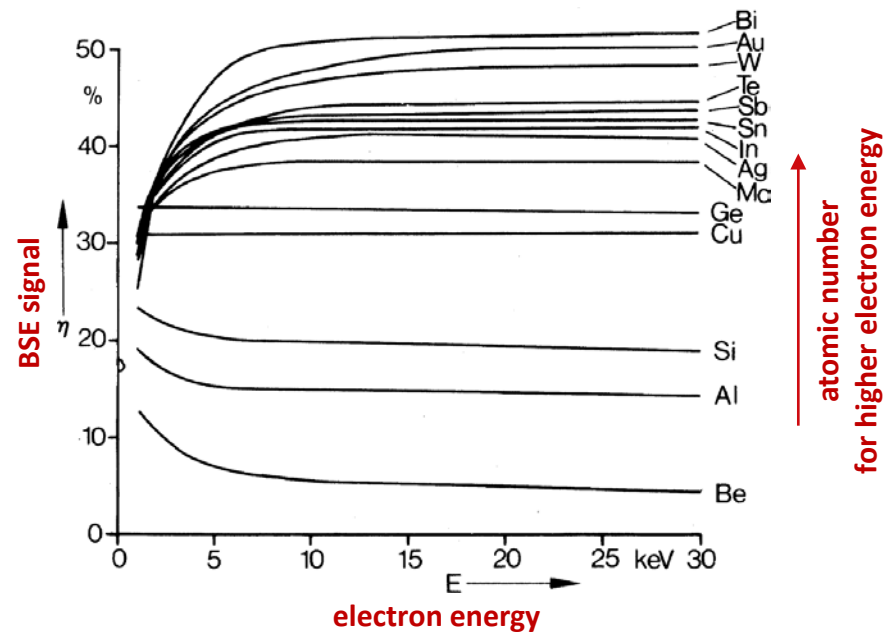
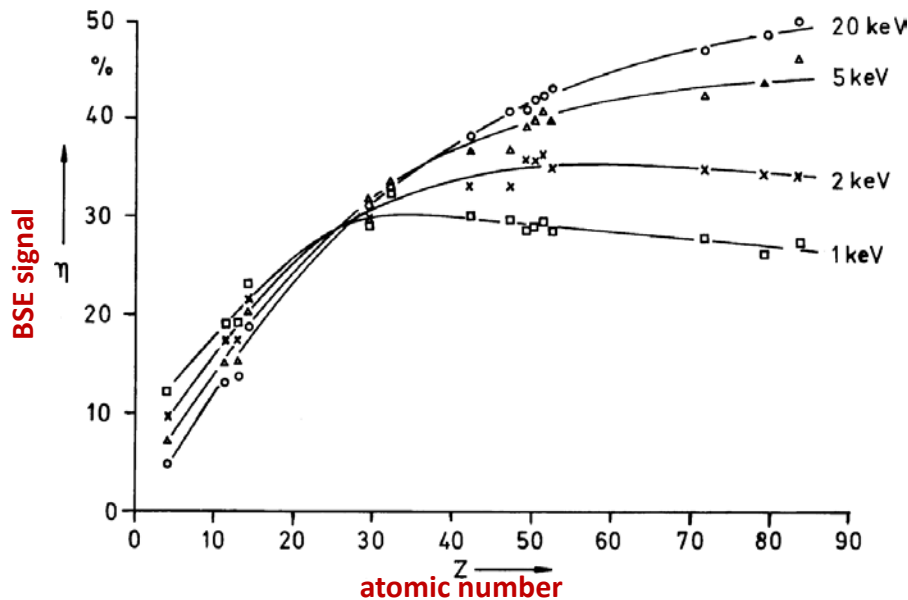
- Interaction volume
- Charging effect
- Beam damage



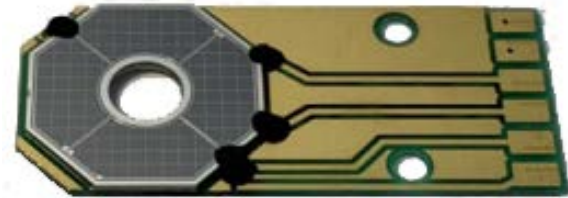


## BSE signal:

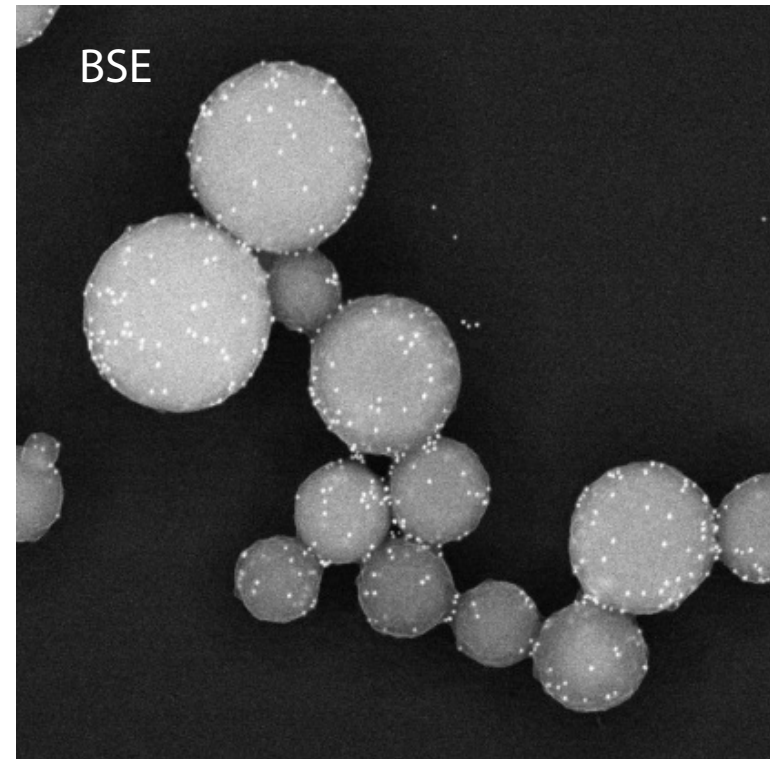
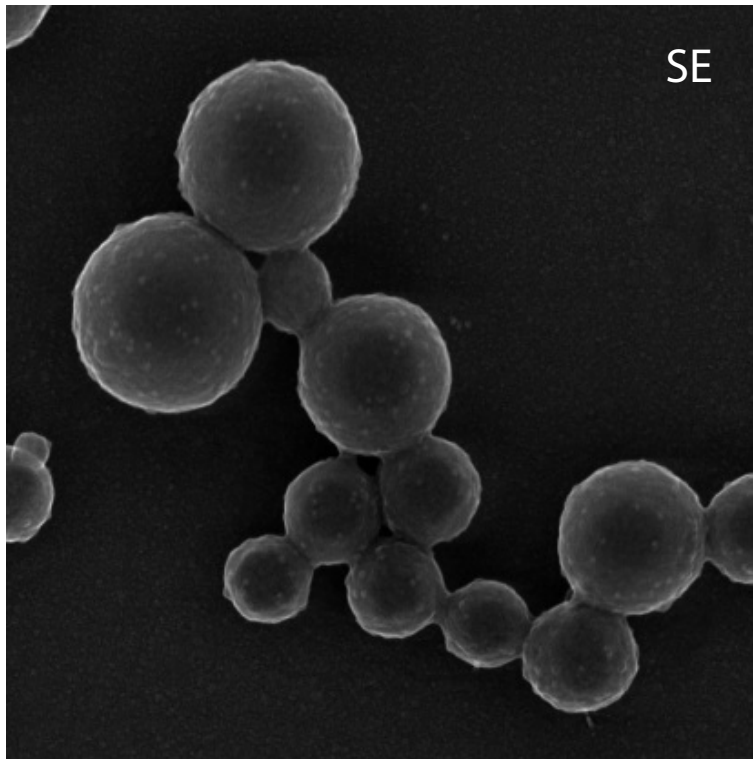
- is formed by primary electrons which are backscattered (all electrons in the energy range from 50 eV to  $E_0$ )
- in general number of BSEs is much lower than SEs
- gives **mainly information about material (material contrast)**, less about topography
- **increases with increasing atomic number  $Z$**  up to  $E_0 \sim 30$  keV
- increases with increasing tilt of the sample
- is **almost independent on energy for  $E_0 > 10$  keV**



- **BSE detector type**
- **retractable BSE detector** (standard BSE like ET for SE)
- annular active area (well defined)
- very effective
- basic types
  - scintillator based (usually YAG detector)
    - usually 1 segment
  - semiconductor detector
    - usually multi-segment
  
- **in-lens detector** (only for snorkel type objective)
  - various possibilities depending on SEM producer and SEM type

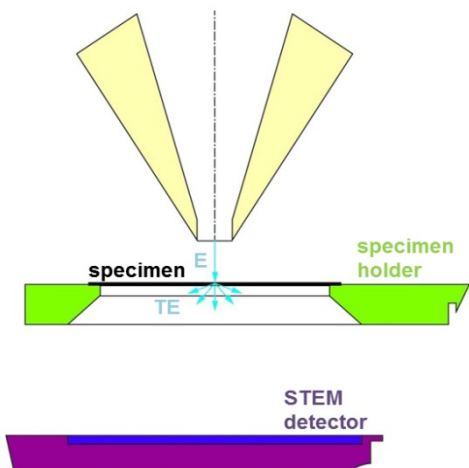


- Immunogold labeling of surface proteins (rubber nanoparticles isolated from *Taraxacum*)

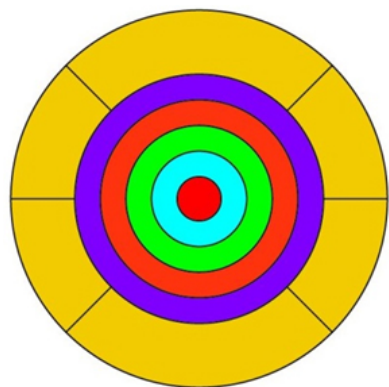


SE: sample surface information (topography)

BSE: due to targeted immunolabeling (gold nanoparticles bound to immunoglobulin) the possibility to identify specific parts on the sample (bright Au nanoparticles)

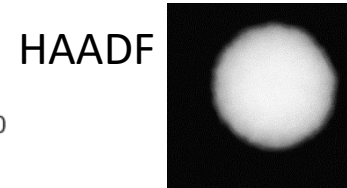
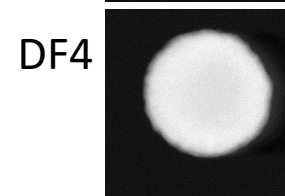
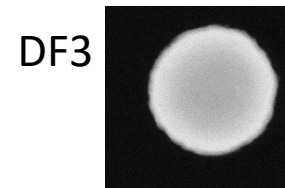
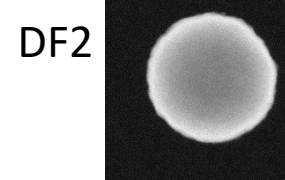
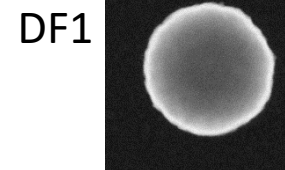
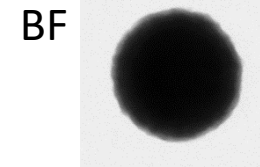
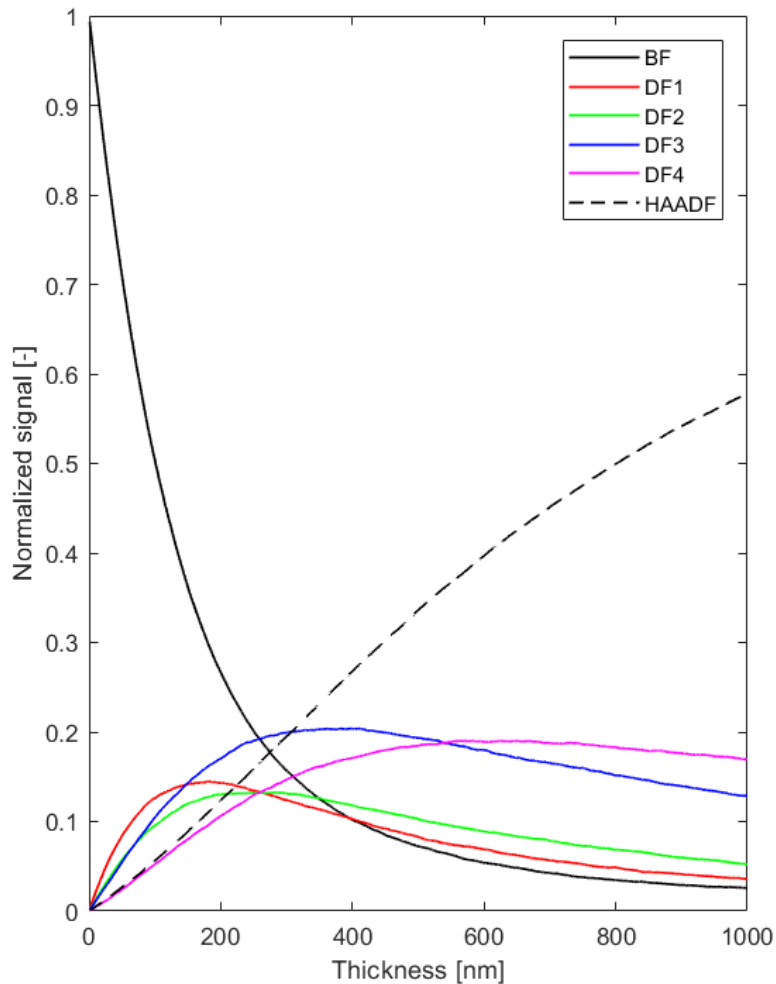


Multisegment STEM detector



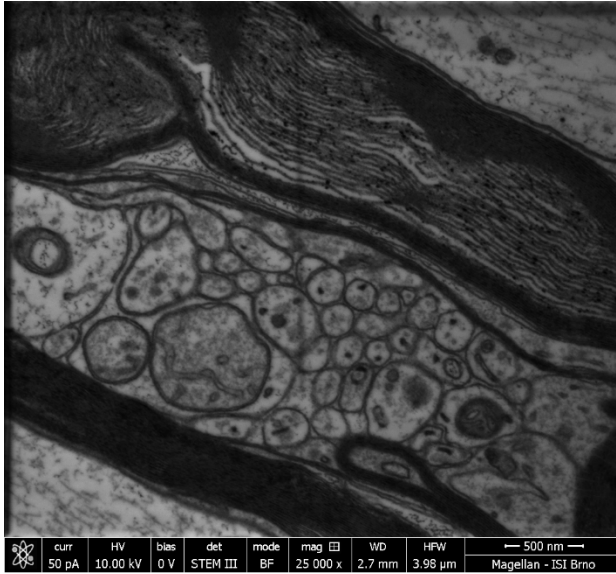
- BF**
- DF1**
- DF2**
- DF3**
- DF4**
- HAADF**

Monte Carlo simulations for STEM signals



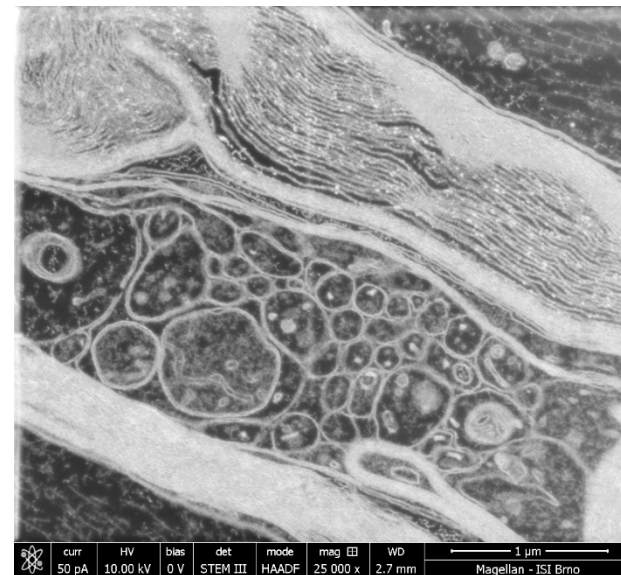
10 kV, 50 pA

STEM BF



BSE

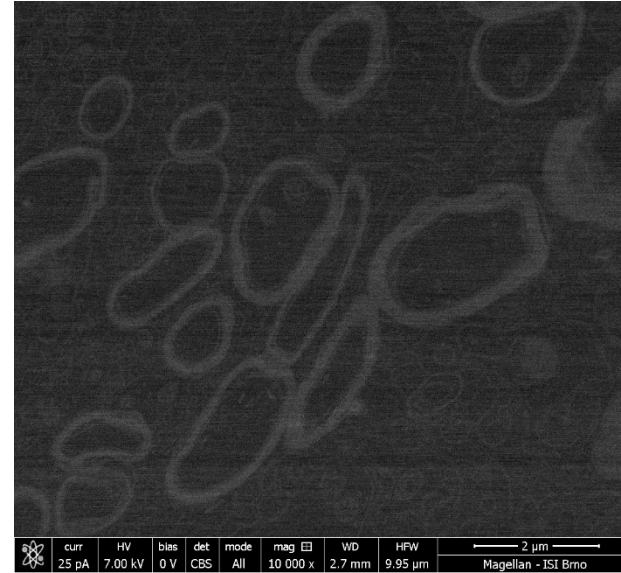
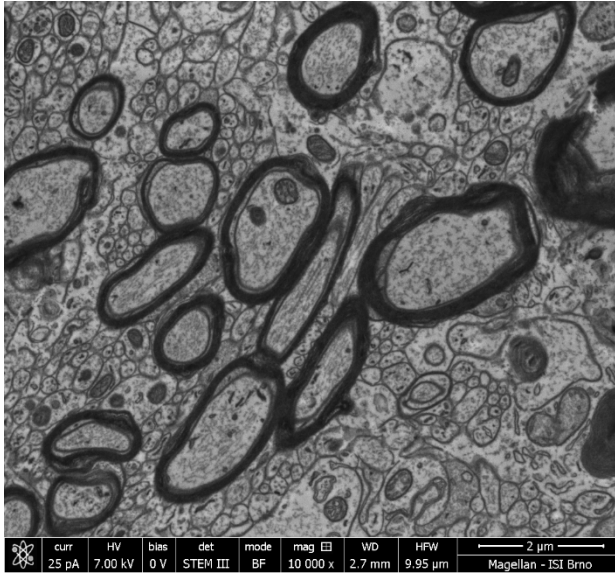
STEM DF1



STEM HAADF

7 kV, 25 pA

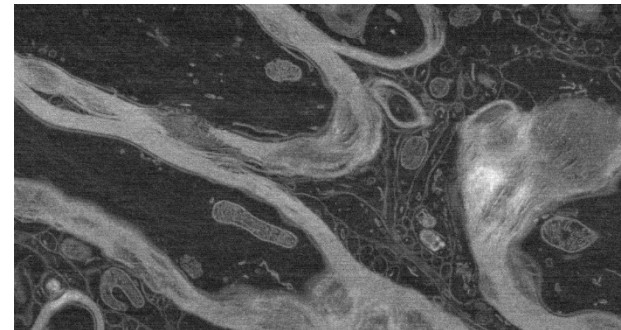
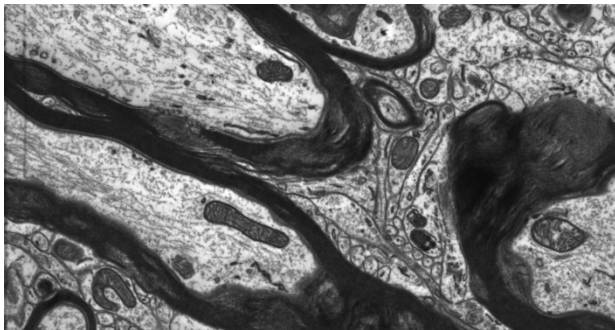
STEM BF



BSE

7 kV, 100 pA

STEM BF



BSE

## STEM-in-SEM

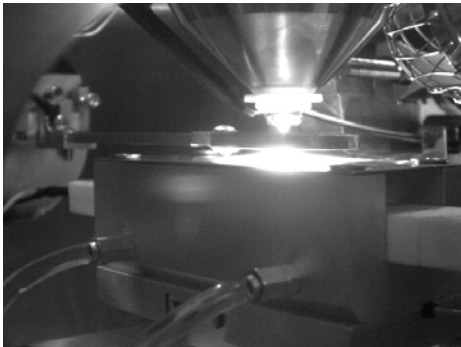
- can be used for imaging of ultrathin sections like TEM (less comfortable)
- can be used as imaging without need of staining, see *Microorganisms* **11** (2023), 888

## ESEM (Environmental SEM)

- specimen chamber pressure up to 3000 Pa (various gasses possible)
- different detectors required
- convenient for insulating samples, vacuum sensitive samples (biological),...

## in-situ SEM, cryo-SEM

- hot stage / mainly for material sci.
- cryo-stage / mainly for life science

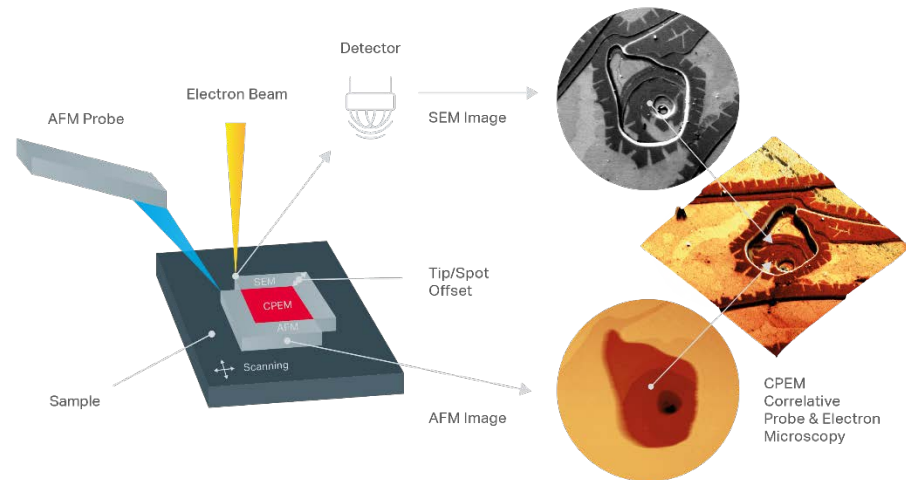


## FIB-SEM, SBF-SEM

- focused ion-beam implemented into SEM
- ultramicrotome implemented into SEM
- → see another lecture

## Combination with AFM

- integration of AFM into SEM



# Multimodal imaging (correlative imaging)

Cooling of the sample to cryo temperatures - reduction of surface contamination together with higher resistance to the electron beam

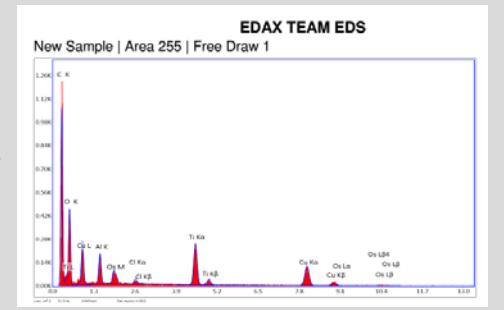
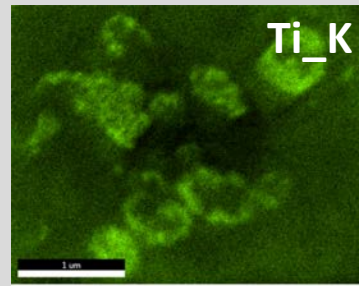
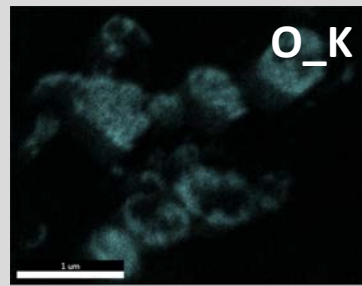
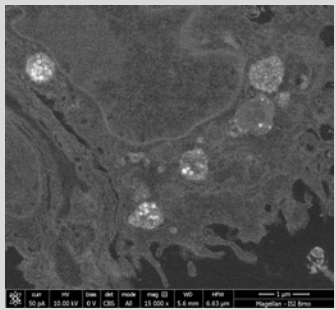
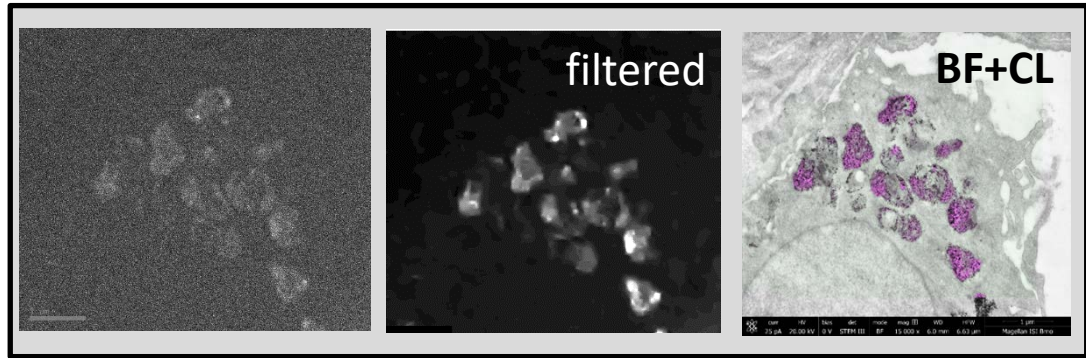
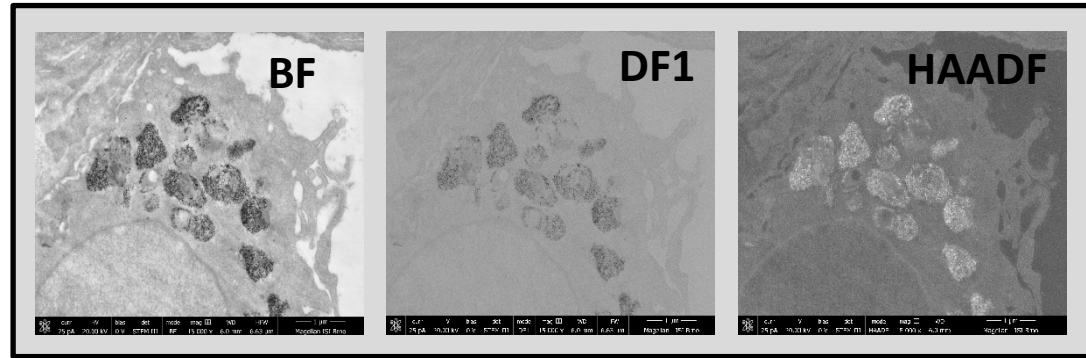
**SAMPLE**  
Titanium particles  
in lung cells

BSE

STEM

CL

EDX





- SEM is a versatile instrument that can be used for many purposes and can be equipped with various accessories
- Secondary electrons (SE): mainly topography
- Backscattered electrons (BSE): mainly material contrast
- Transmitted electrons (STEM): mass-density contrast
- X-ray: chemistry (composition)
- **Use the full power of your SEM - play around with the settings (modern SEMs offer many options)**
- EM manufactures:





Vladislav Krzyžánek  
[krzyzanek@isibrno.cz](mailto:krzyzanek@isibrno.cz)

- There are more free programs, e.g.
- CASINO "monte CARlo Simulation of electroN trajectory in sOlids"  
<https://www.gegi.usherbrooke.ca/casino/>
  - Version 2 is simple for users (only films) ... *recommended for beginners*
  - Version 3 works in 3D (user-defined shapes) ... *more complicated*
- Movie in Czech: Kukátko do nanosvěta z cyklu NEZkreslená věda  
<https://youtu.be/2vYquoVWLqQ>