Aalto University

otananno

Nanomicroscopy Center

Microscopy of Nanomaterials: SEM Lecture

Ramzy Abdelaziz

Nanomicroscopy Center

ramzy.abdelaziz@aalto.fi

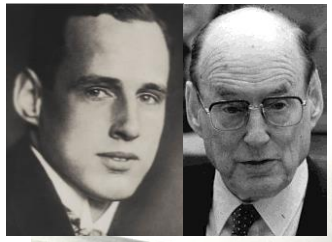
Learning Outcomes

- Basic knowledge about the main SEM component and what happens to the electrons in the SEM; how they are generated, interact with specimens and are then detected

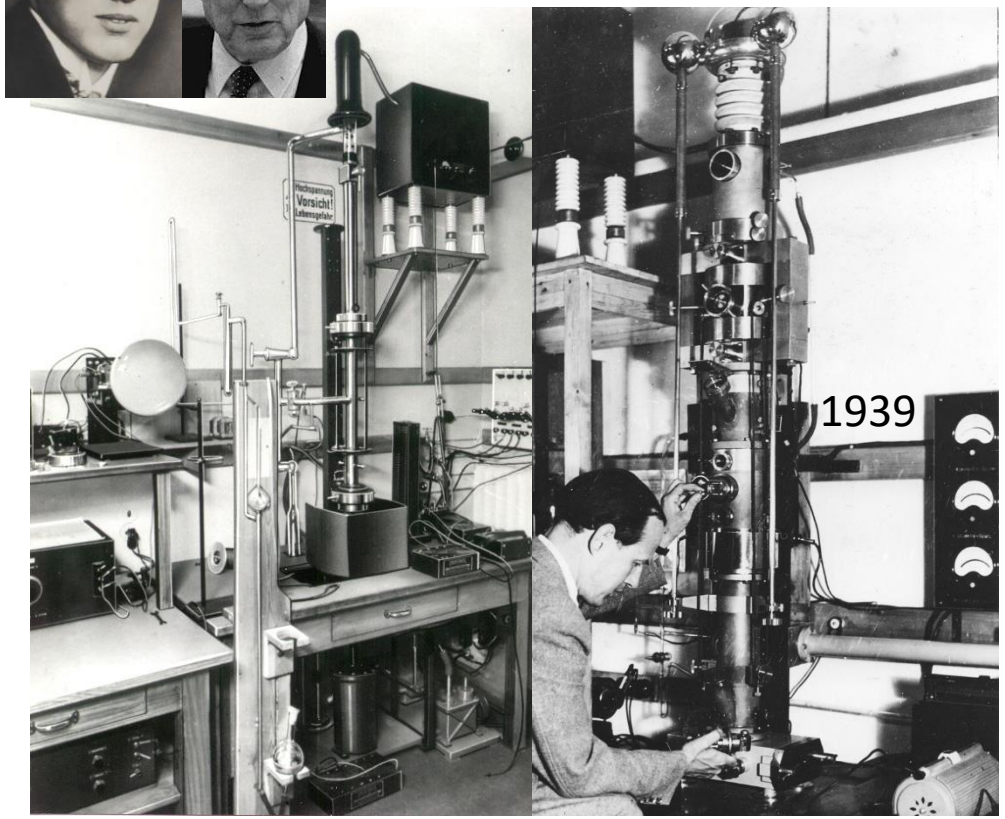
- Learning about SEM beam alignment (astigmatism and wobble), magnification, focus, brightness, contrast, parameters affecting the quality (resolution) of the image

- Understanding SEM observations, sample preparation methods, and EDS

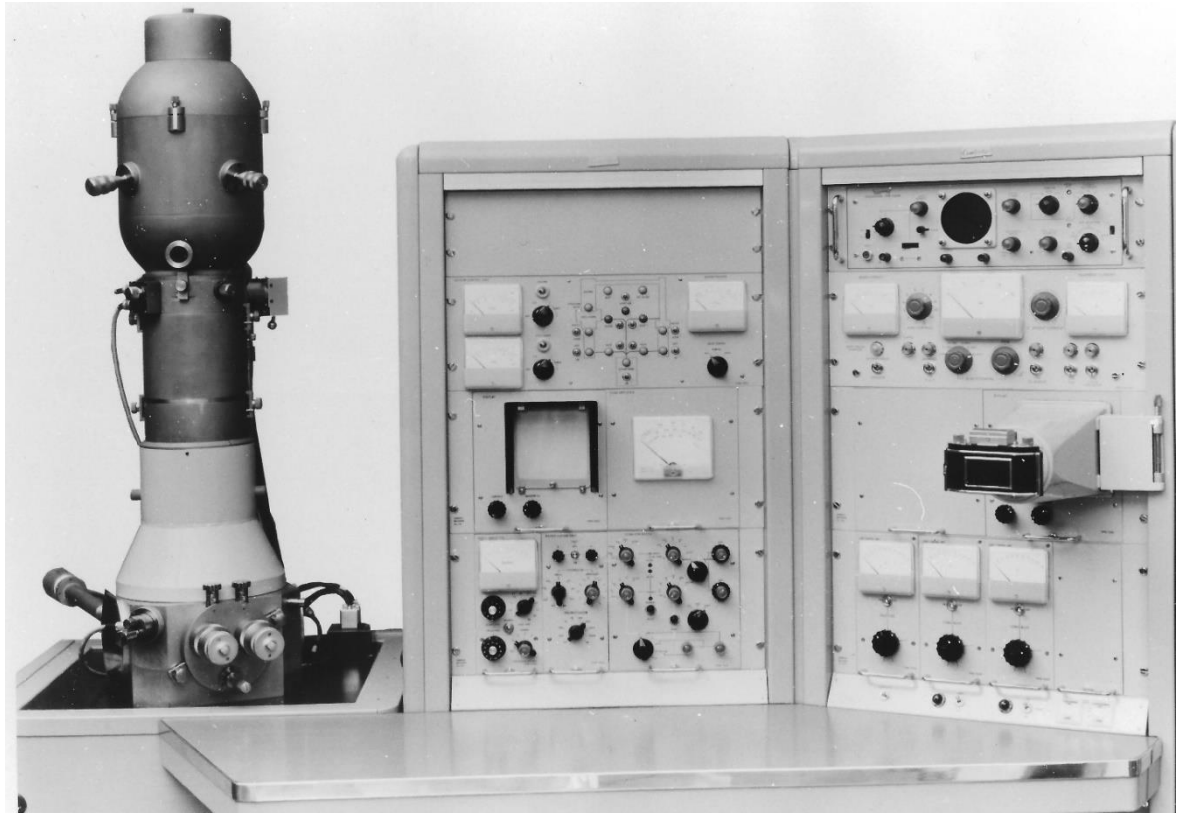
The First SEM



Prof. Manfred von Ardenne
(1907 – 1997)



The first SEM was invented and built by
Manfred von Ardenne in Germany **1937**



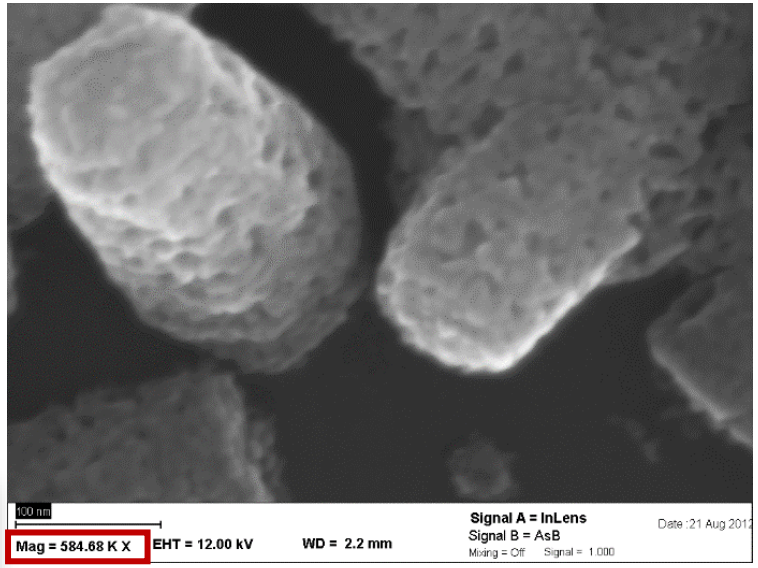
Stereoscan MK1, the first commercial SEM
Cambridge Scientific Instrument Company in **1965**

https://www.vonardenne.biz/ja/company/manfred-von-ardenne/

What is SEM?

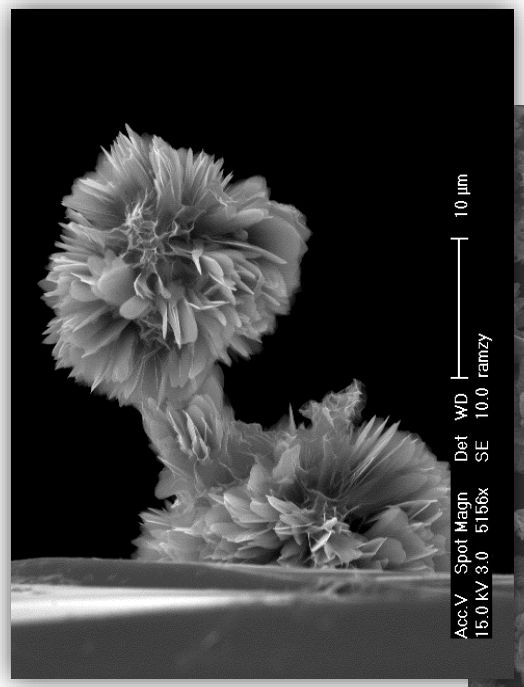
Scanning electron microscope (SEM)

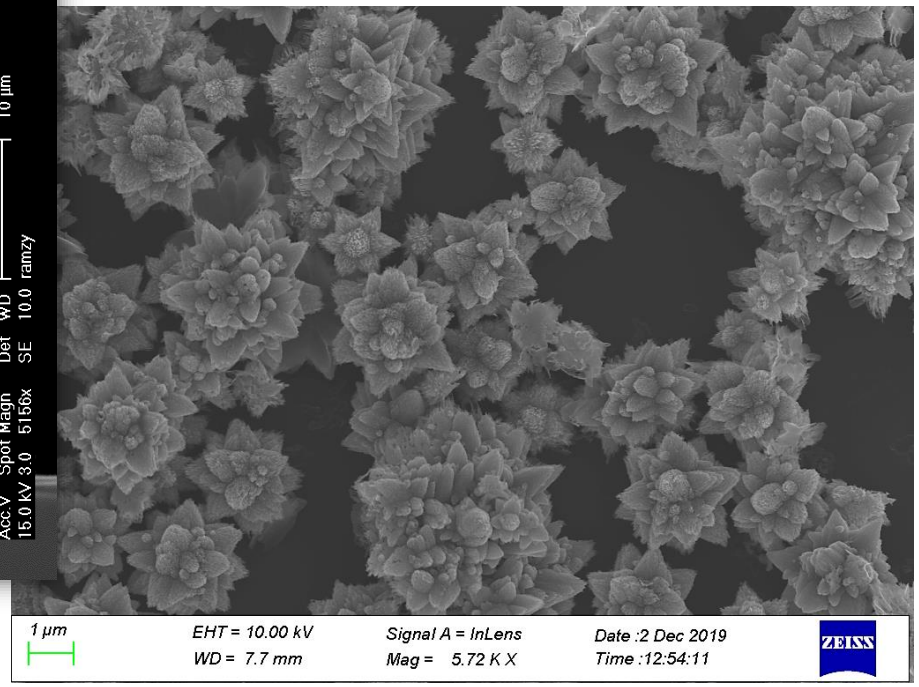
- A type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons
- The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample



Why SEM?

- Magnification range 10 to 1 million times
- Resolution of ~ 0.5 nm
- Excellent depth of field (3D appearance)
- Relatively easy sample prep.

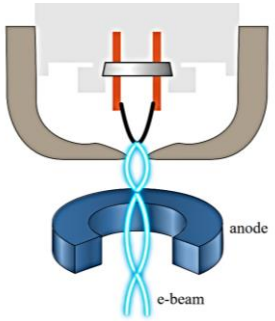


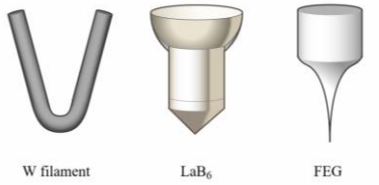


SEM Components

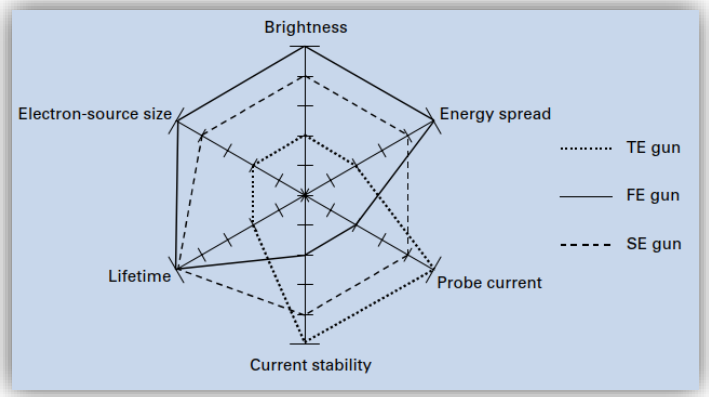
✓ Electron Gun

- Thermionic gun
- Field-Emission Gun (FE)
- Schottky-Emission Gun



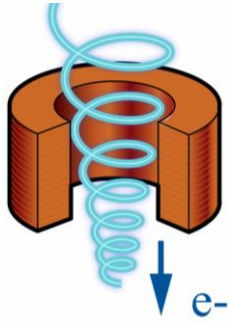


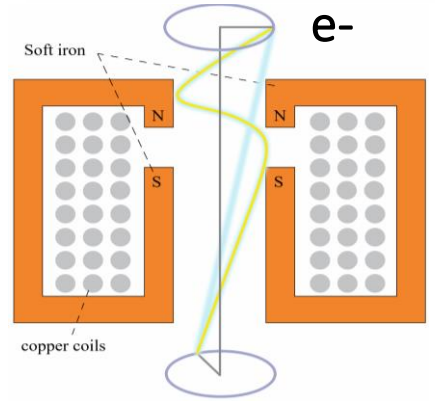
Up to 40 keV



✓ Condenser Lenses

Electromagnetic lenses
focusing the beam
→ fine electron probe





✓ Objective aperture

To control the amount
of probe current

✓ Scan coils

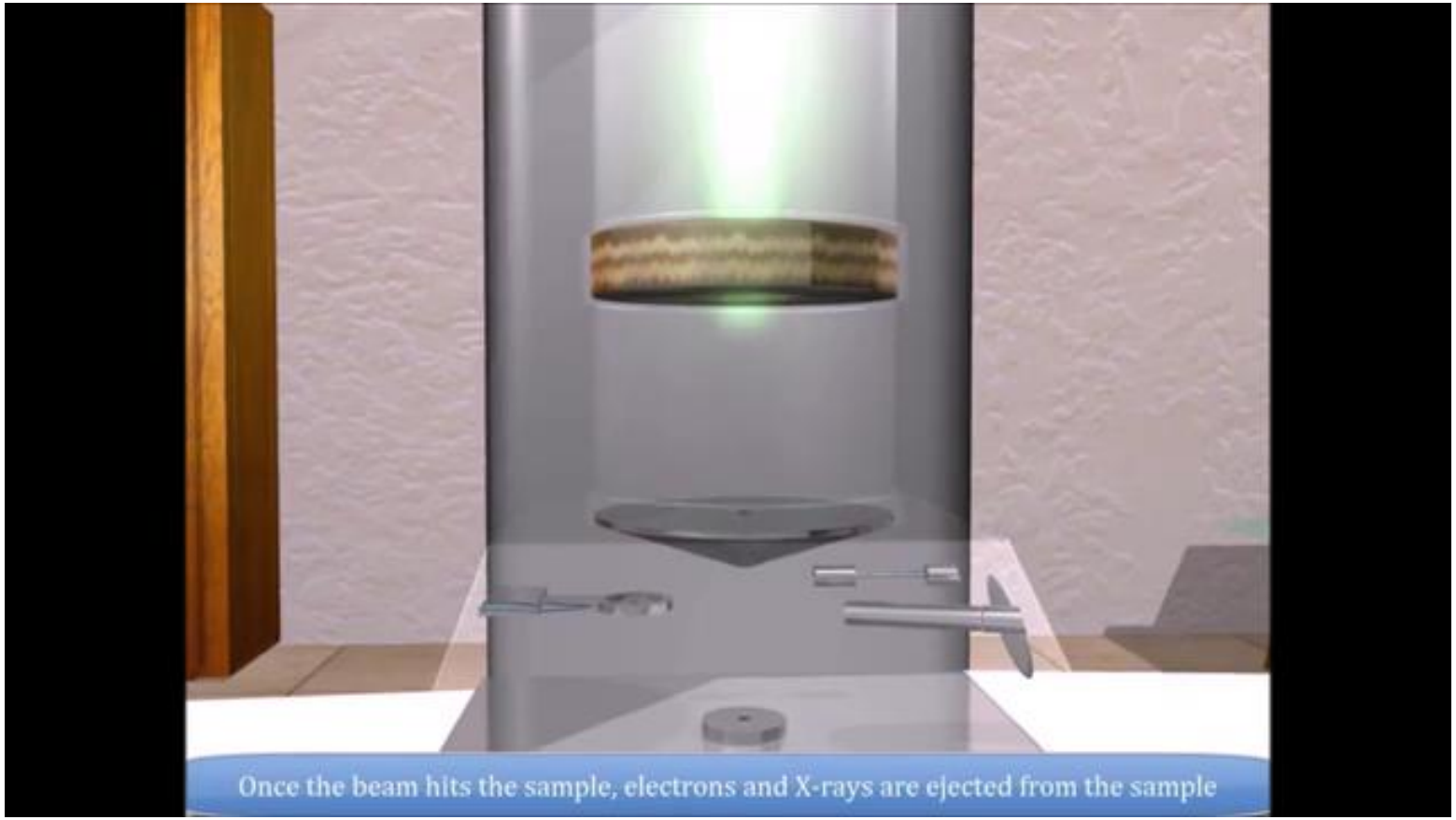
Two pairs of electromagnetic
deflection coils to construct the
image point-by-point and line-by-line

✓ Objective Lens

- Focus the beam onto the sample
- Influence over the diameter of the spot size of the electron beam

- ✓ Astigmatism correction coil
- ✓ Vacuum system
- ✓ Water chilling system
- ✓ Column
- ✓ Specimen chamber
- ✓ Detectors
- ✓ Imaging system

SEM Operation

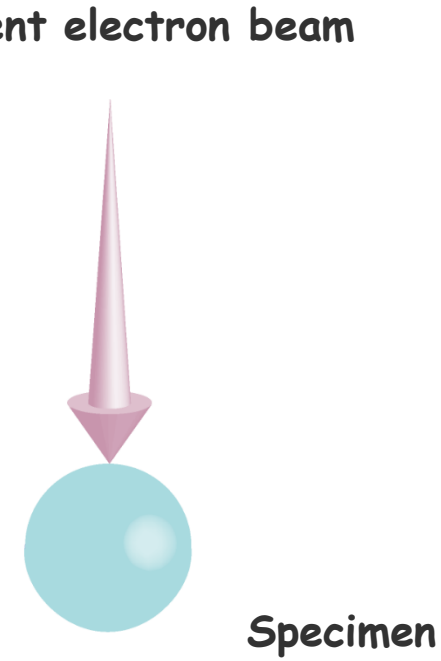


https://www.youtube.com/watch?v=Vs360UarP1U

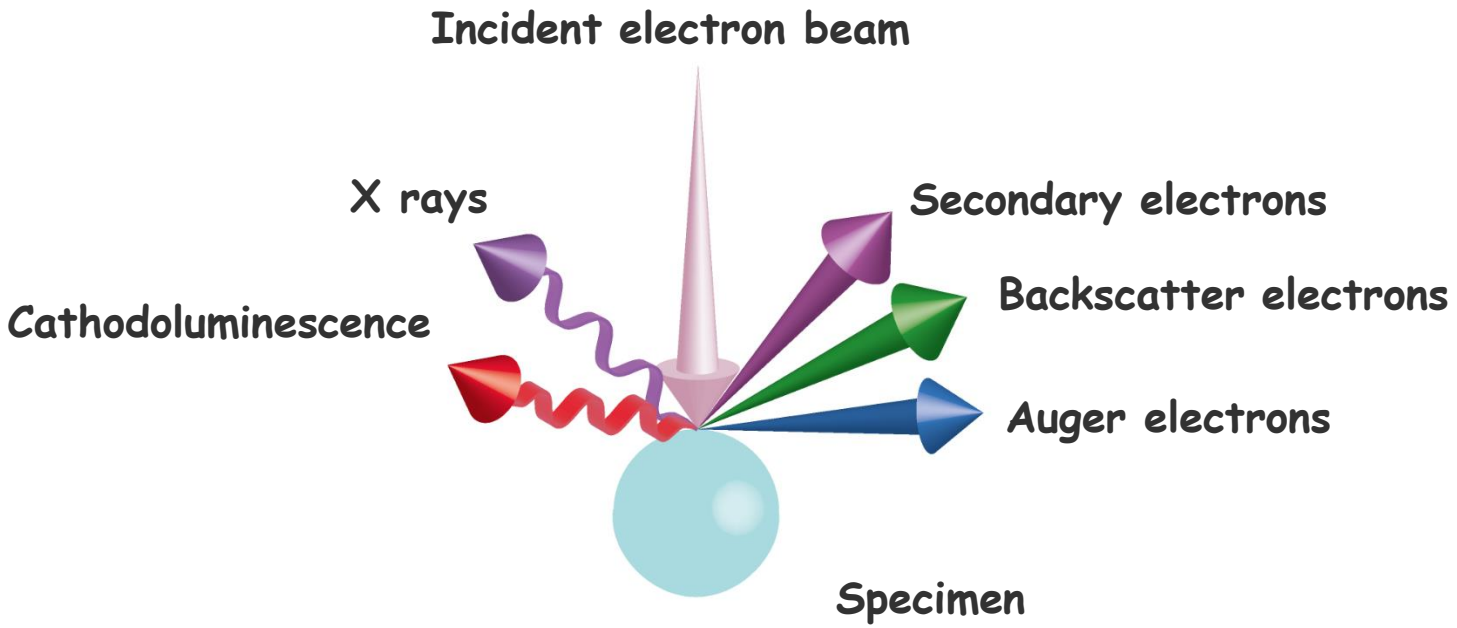
Electron Matter Interaction in SEM

Specimen

Electron Matter Interaction in SEM

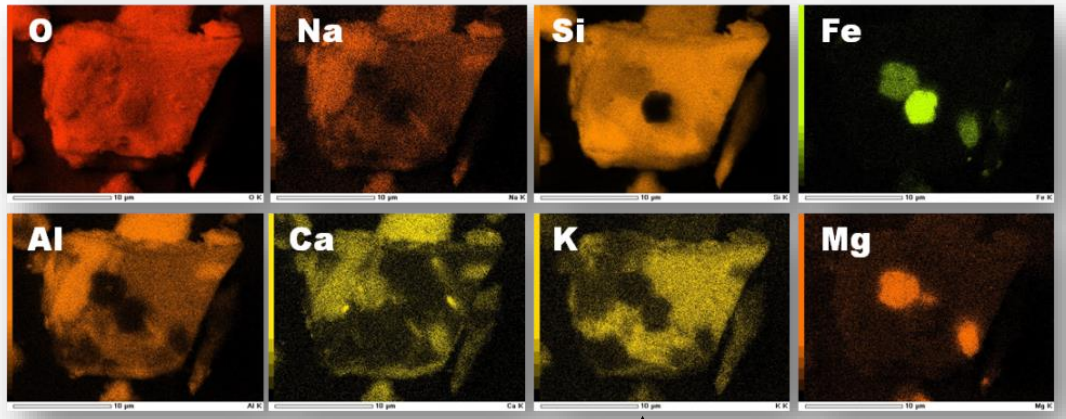


Electron Matter Interaction in SEM

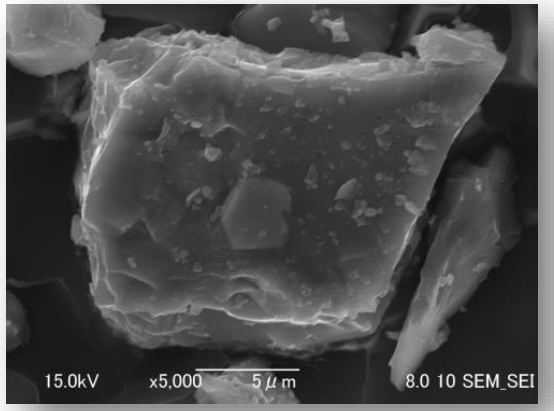


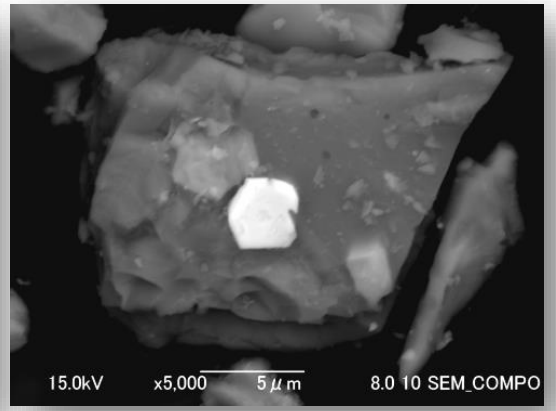
- When irradiating the material with electron beam in vacuum, various signals are emitted
- Electron microscopes can obtain various information of substances by using these signals

Volcanic Ash



Volcanic Ash





Incident Electron beam

X-rays

Secondary electrons

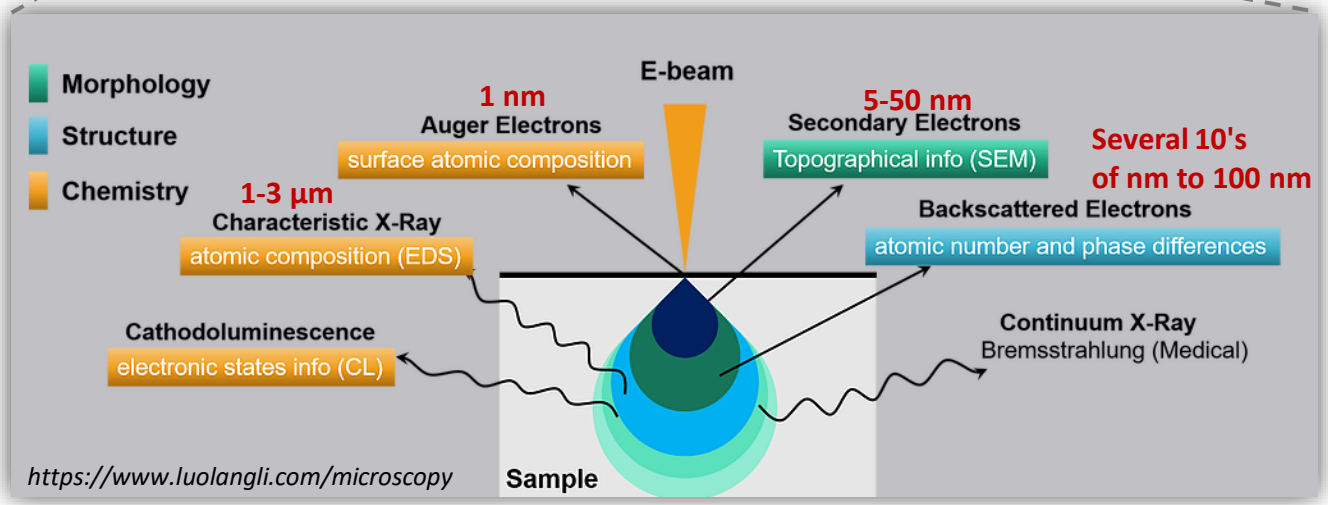
Backscatter electrons

Cathodoluminescence

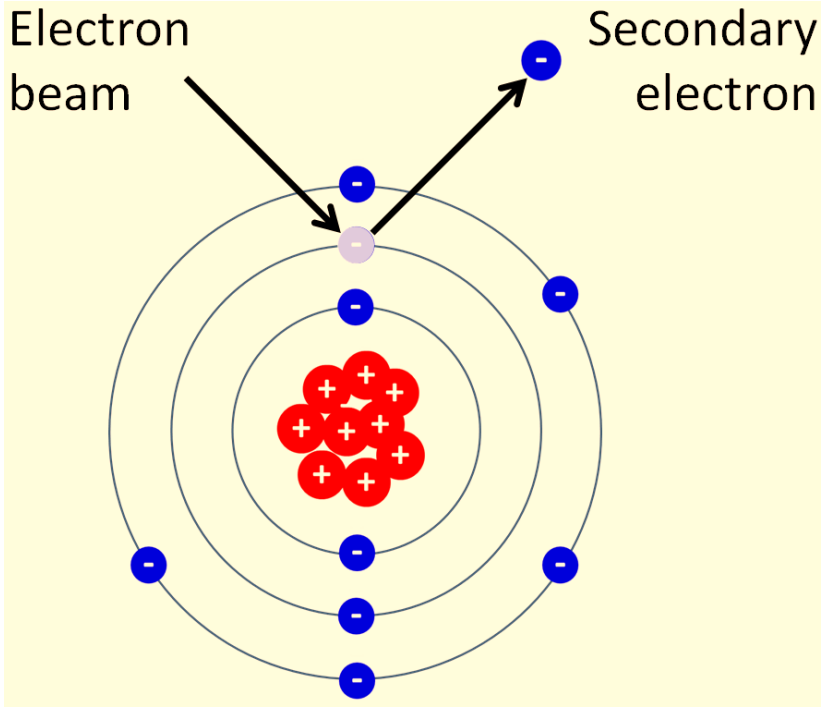
Auger electrons

Specimen

From Jeol

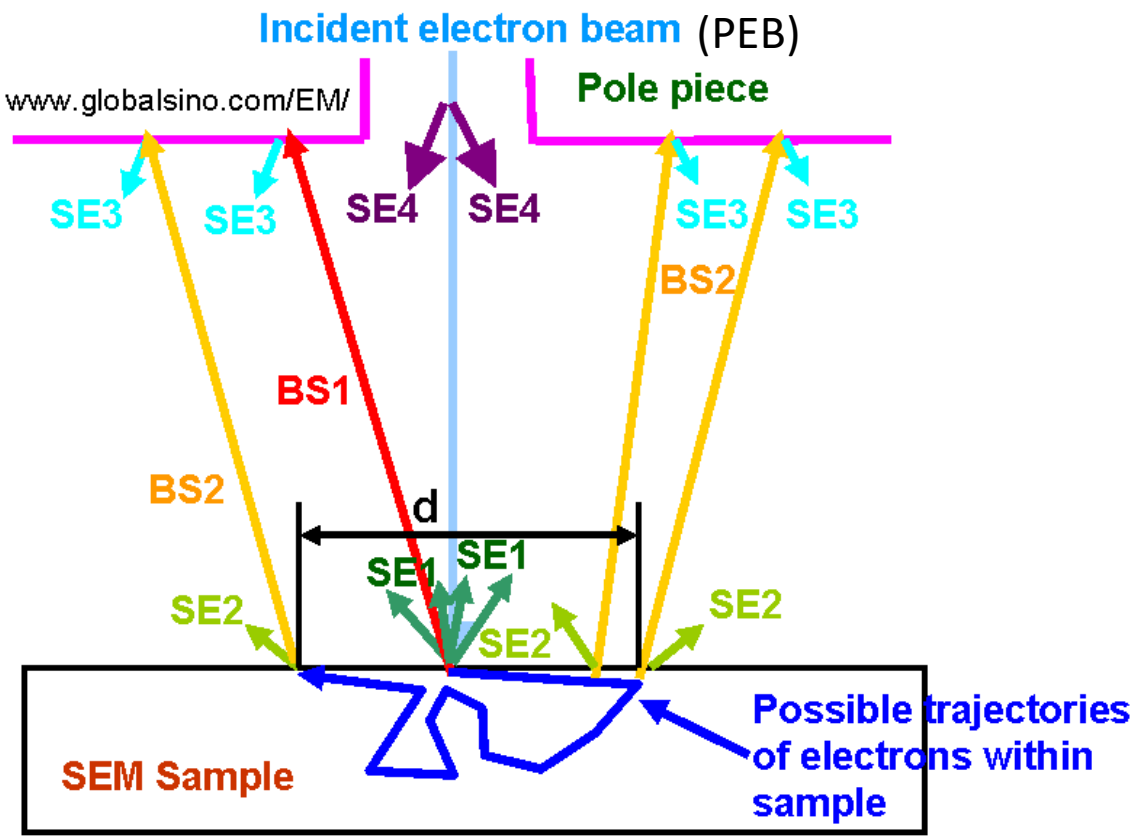


Secondary Electrons (SE)



- Inelastic collision between the primary electrons (PE) and the valence electrons of the constituent atoms in the specimen → ejection of secondary electrons (SE)
- Very low energy electrons ~ 2-50 eV
- Generated close to surface (5-50 nm)
- Topography contrast, provide particularly good edge detail

Types of SE



- Primary incident beam → SE1

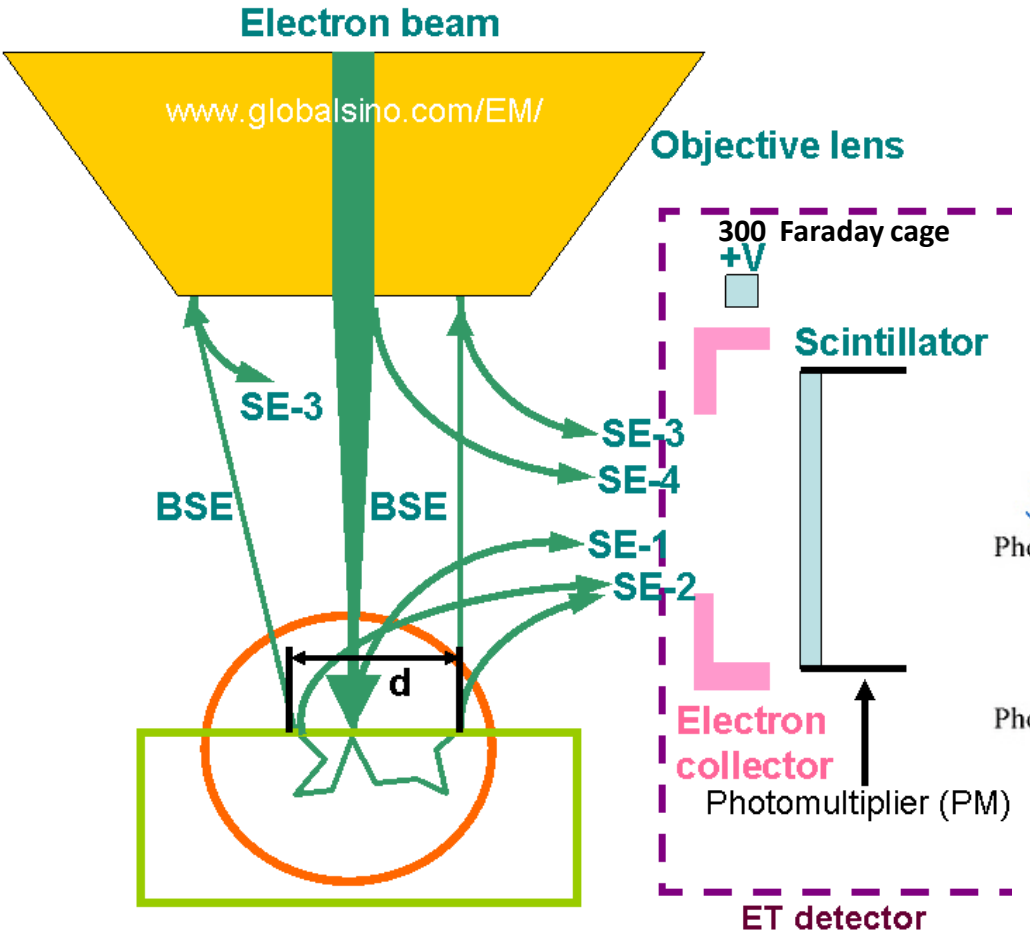
Some Z contrast

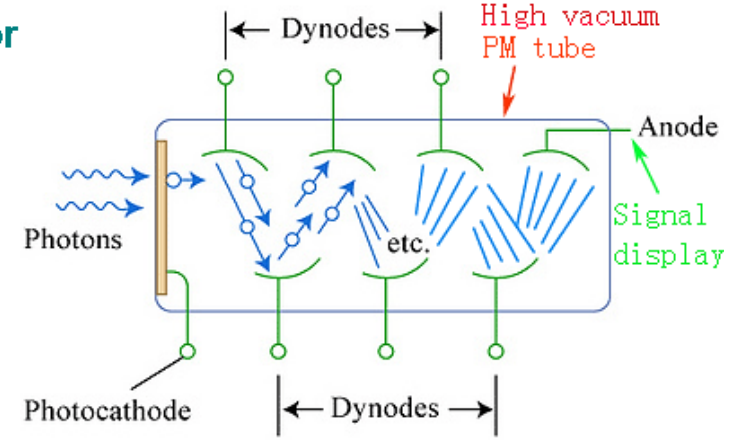
- BSE as they leave the specimen → SE2
- BSE colliding with chamber or lens system → SE3

- PIB strikes an aperture within the electron column → SE4

Everhardt-Thornley Detector (ETSE)

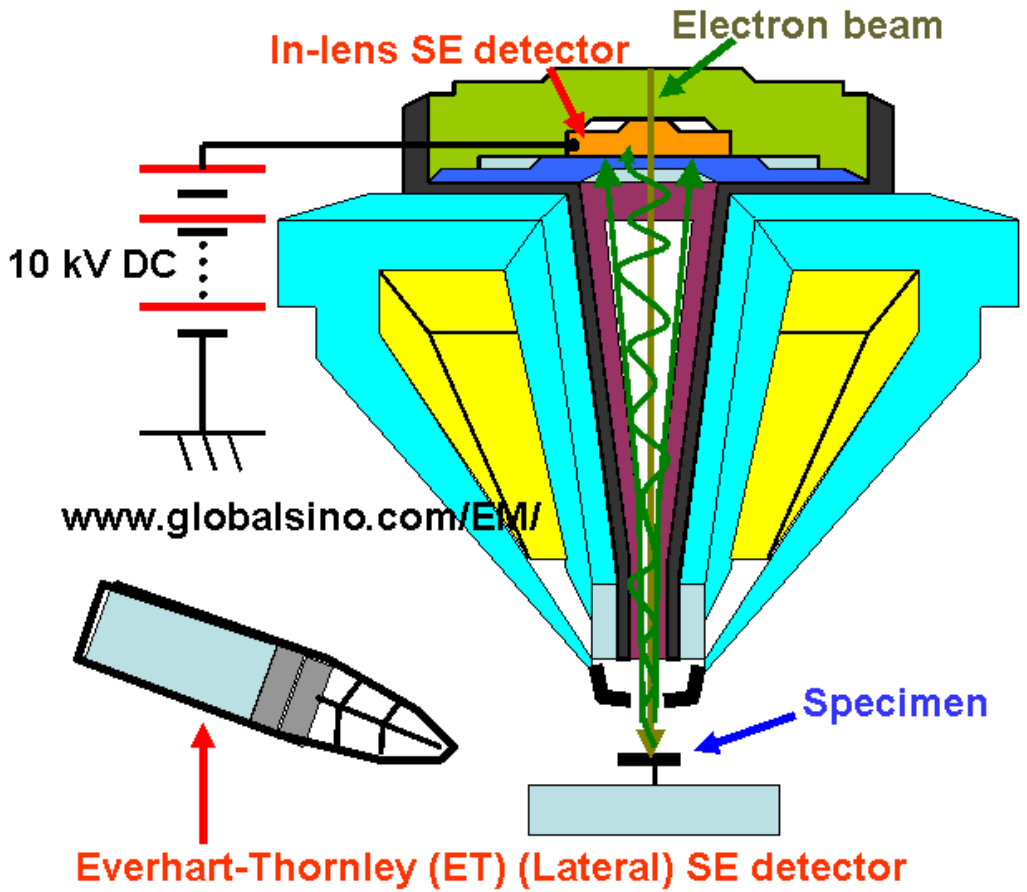
- Main components: a collector grid, a scintillator, and a photomultiplier





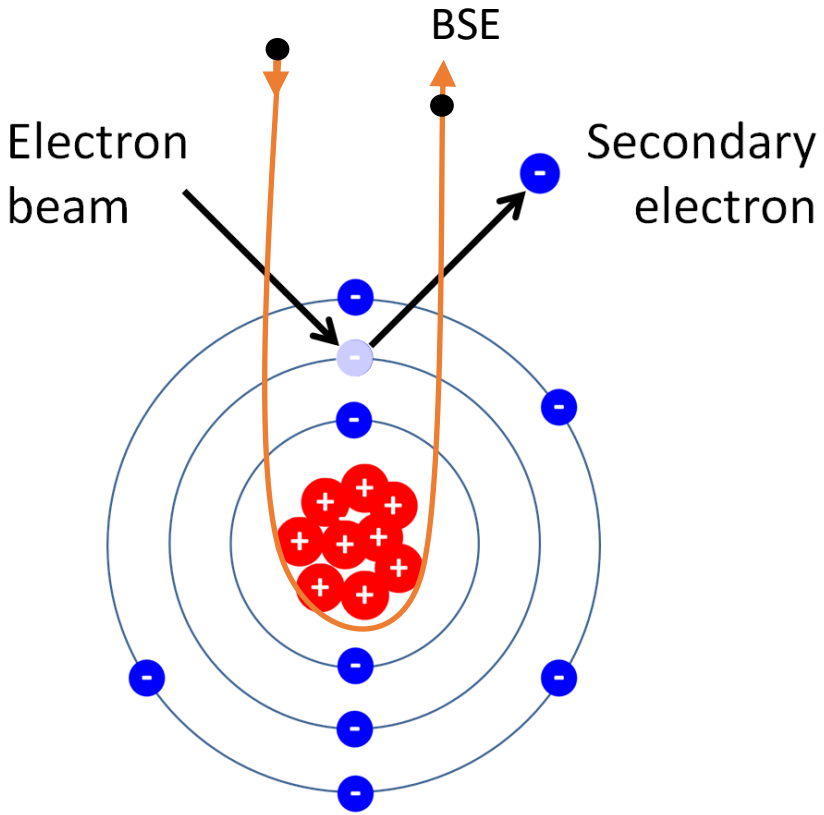
- Mainly SEs (< 50 eV) are pulled toward the scintillator by a high potential (300 V) on the collector grid (Faraday cage)
- A **scintillator** (fluorescent substance) is used to convert electrons to visible light that is amplified by a **photomultiplier** (PM)
- A high positive bias (10 kV) on the scintillator attracts and accelerates SEs enough to be converted to light photons
- The light is conducted through a guiding tube (LG) to a photomultiplier
- The photons converted back to electrons at the **photocathode**, the electrons are accelerated and multiplied by the dynodes

In-lens SE Detector



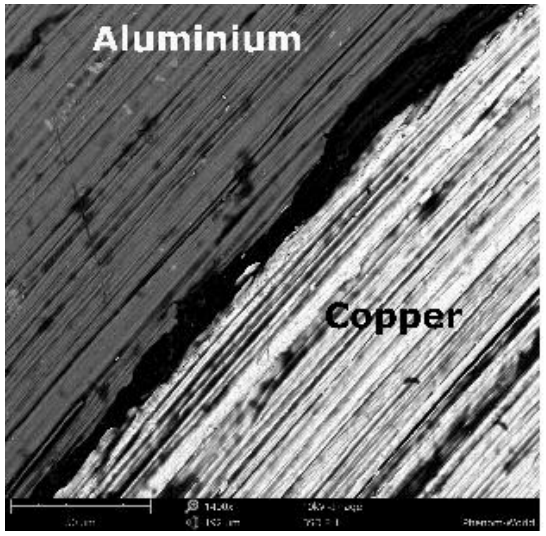
- Mostly collect SE1
- SE1 carry the highest spatial resolution information
- Ideal for very fine structures with short WD

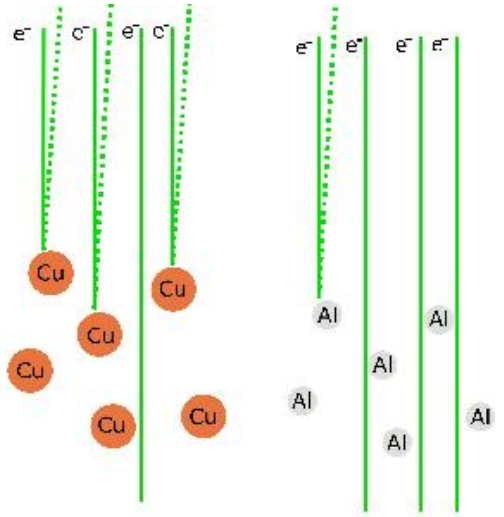
Backscattered electrons (BSE)



- Elastic scattering of the primary electrons (PE) by atom nucleus
- PE is deflected by the electrostatic field of the positive nucleus

- Tiny particles (electrons) collide with larger particles (atoms)
- Larger atoms are a lot stronger scatterers of electrons compared to light atoms
- Hight energy electrons same as the incident electrons
- Generated from deeper layers (several 10's of nm to 100 nm)
- Material contrast, depend on atomic number (Z)



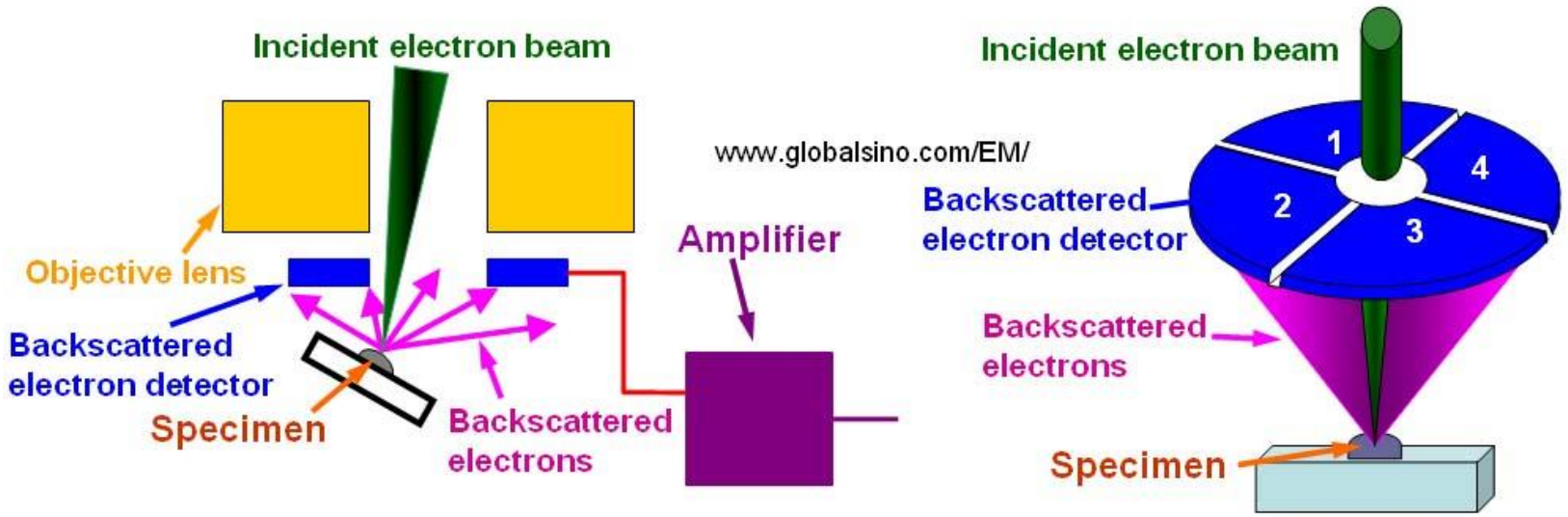


https://www.azom.com/article.aspx?ArticleID=14309

Copper atoms (higher Z) scatter more electrons back towards the detector than the lighter aluminum atoms and therefore appear brighter in the SEM image

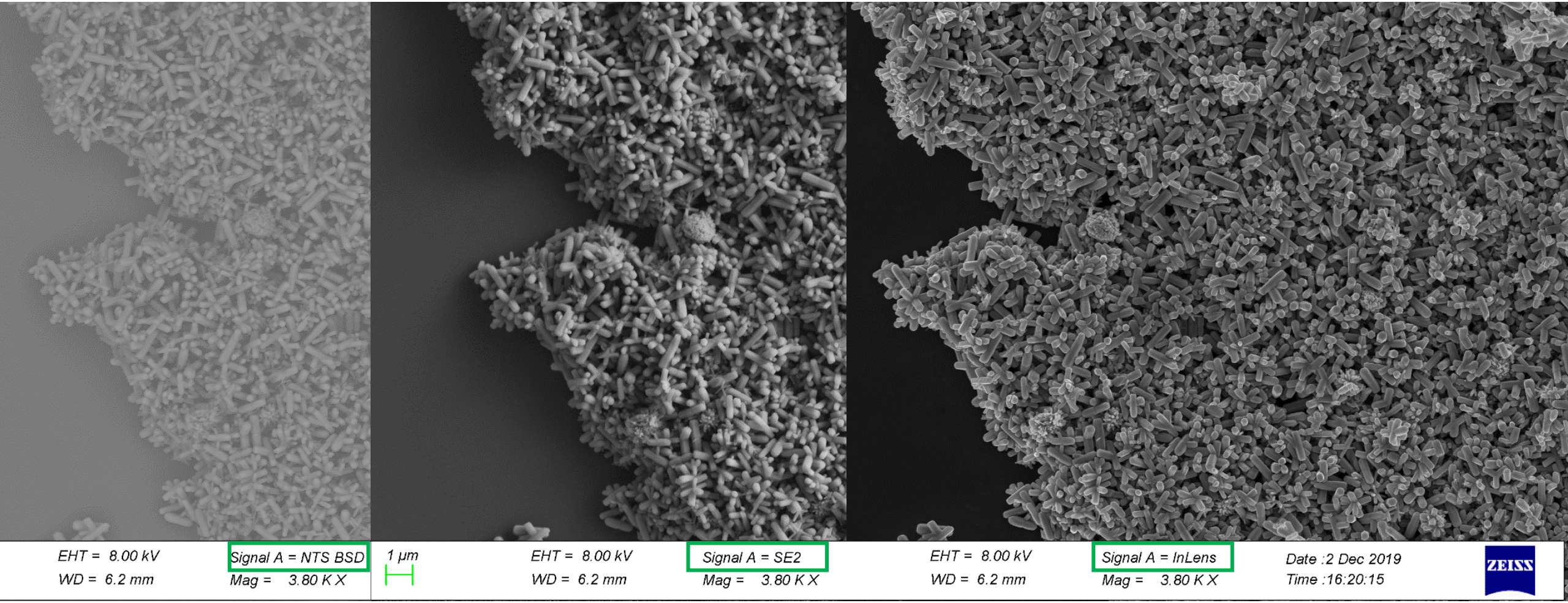
BSE Detector

Solid-state BSE detector

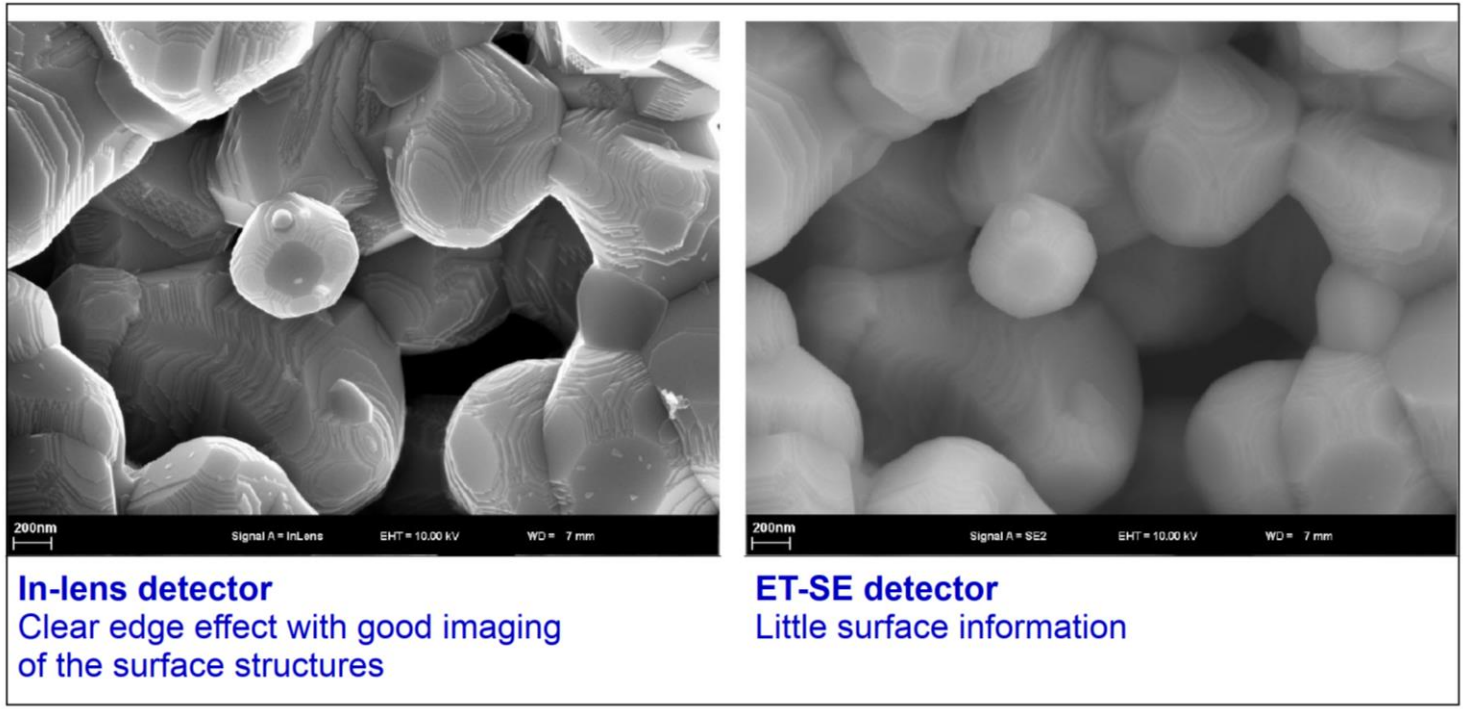


- BSEs detector is usually a four quadrant solid state detector (p-n junction) that is placed directly above the specimen
- BSEs that hit the detectors excite the silicon electrons, generating an electron-hole pair
- The p-n junction is linked to two electrodes, one of which attracts the electrons and the other the holes, thus producing an electrical current, which can be amplified

BSD SED In-lens SED

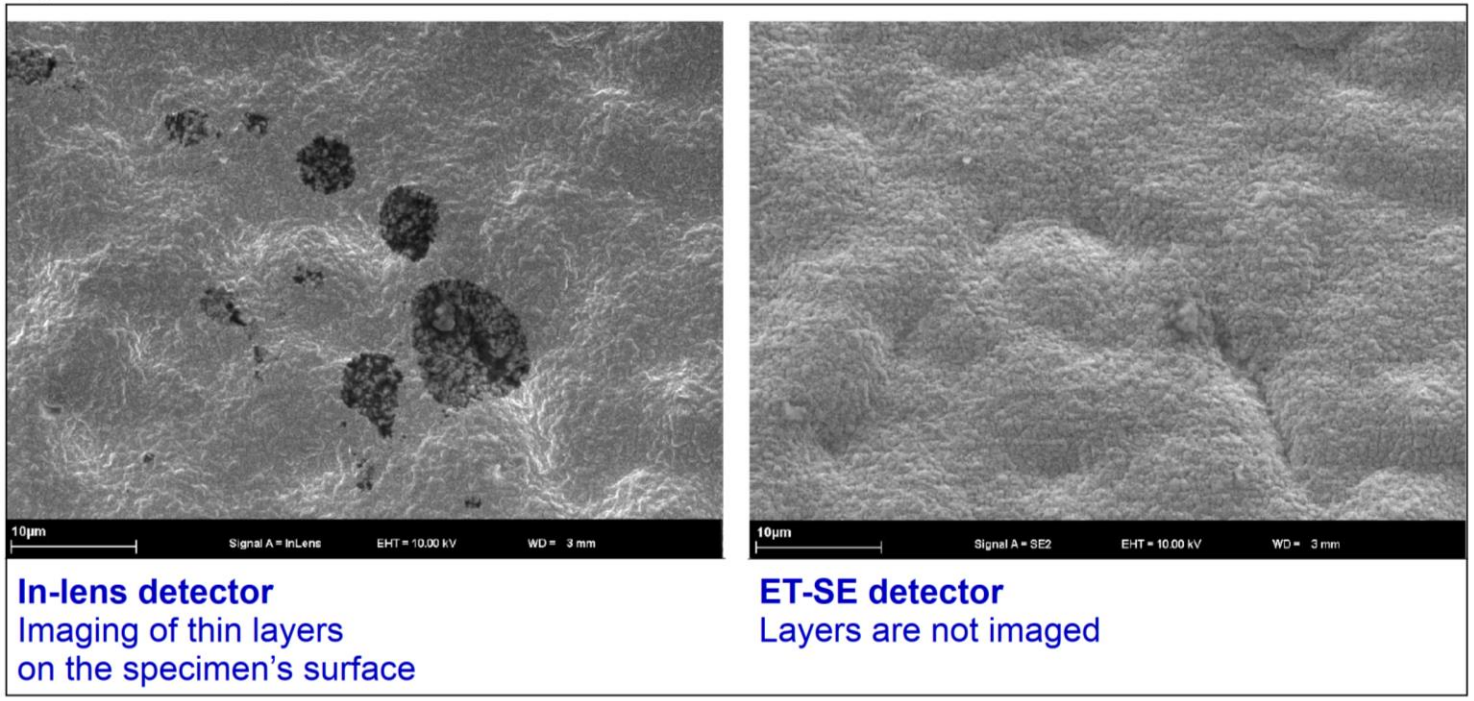


In-lens vs SE2



In-lens detector
Clear edge effect with good imaging of the surface structures

ET-SE detector
Little surface information

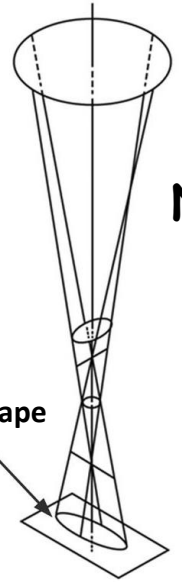


In-lens detector
Imaging of thin layers on the specimen's surface

ET-SE detector
Layers are not imaged

Sigma User Guide

Astigmatism

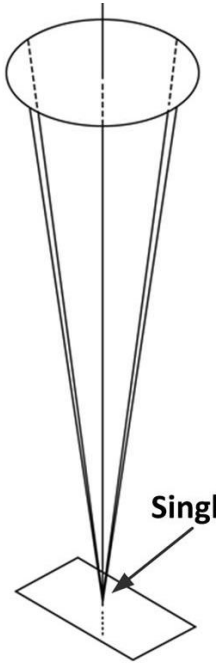


Astigmatism

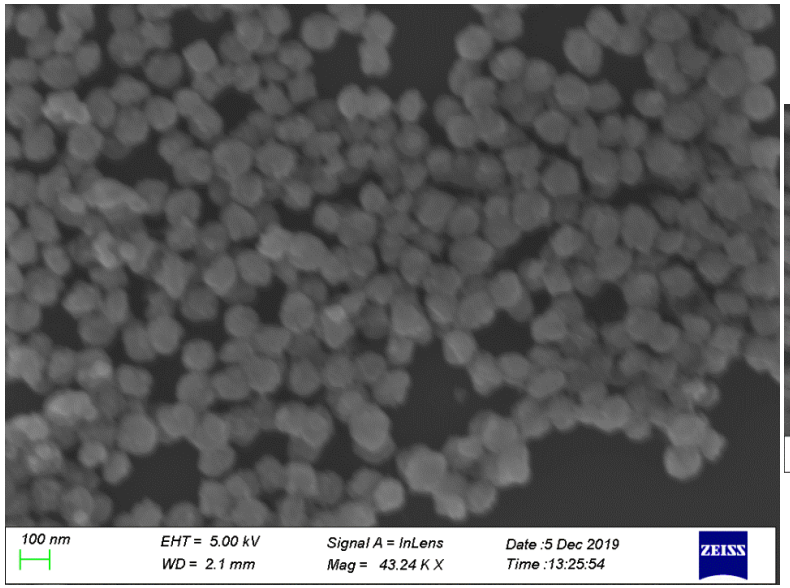
Non-spherical electron beam

How to correct?

- Go to high mag.
- Focus (between elongations)
- Stigmator

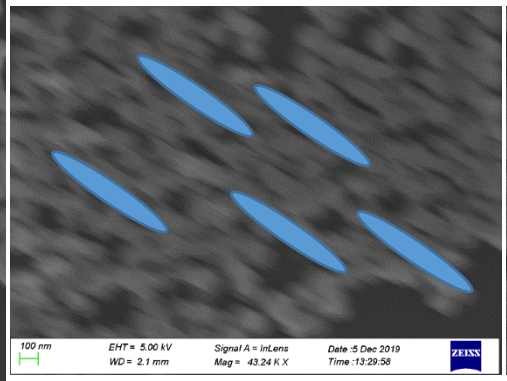


3



Blurred image

1 Underfocus



2 Overfocus

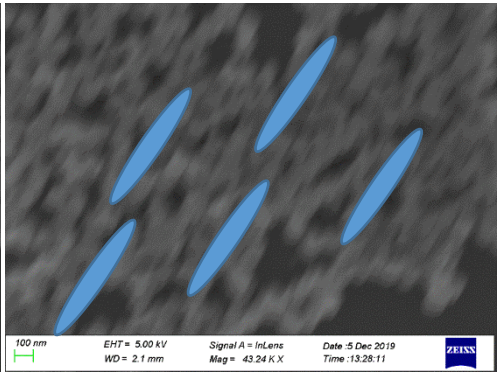


Image of defocus

4

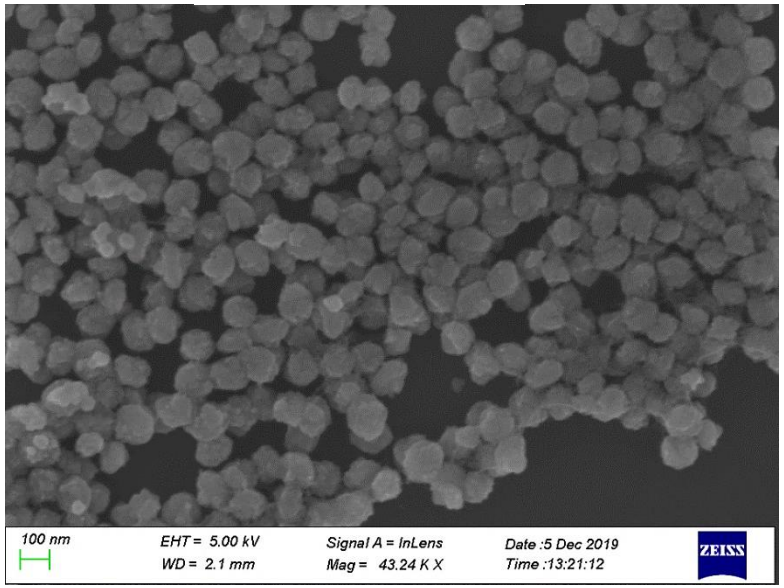
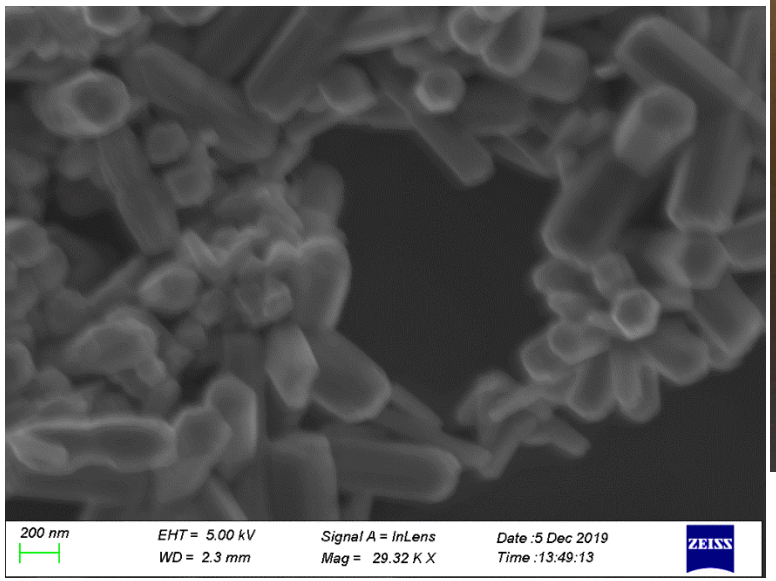


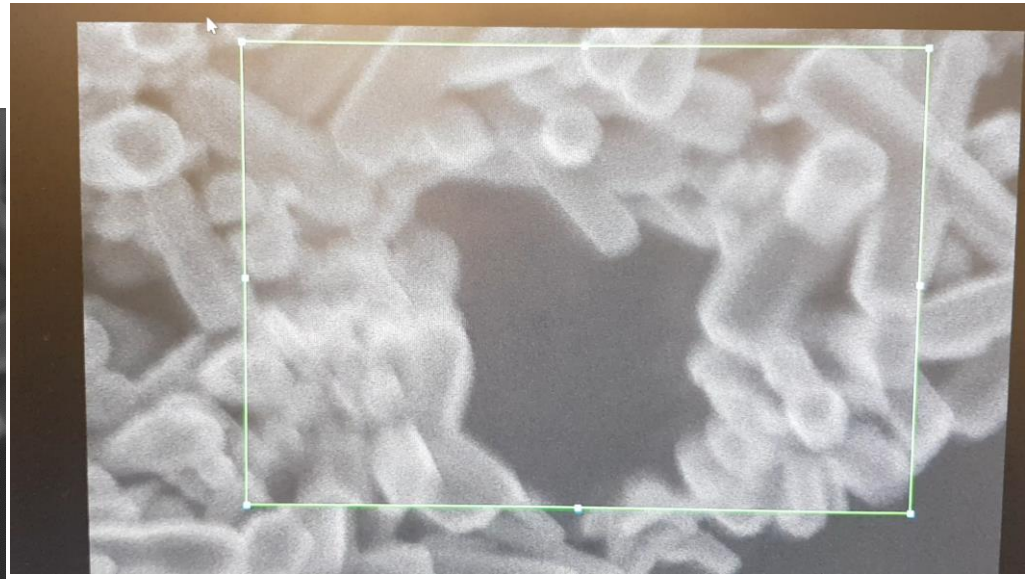
Image of In-focus
After astigmatism correction

Wobble Aperture Alignment

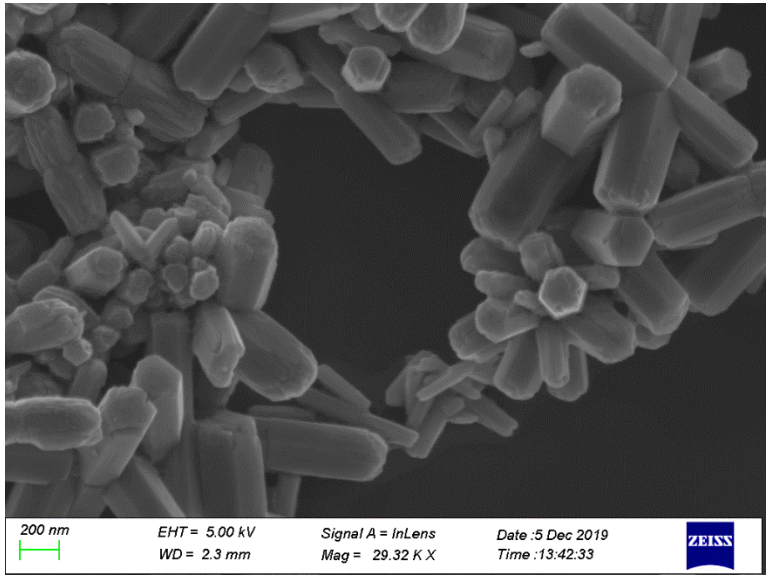
- Ensuring that the apertures are centered with respect to the beam and thus the optical axis of the microscope (perfectly perpendicular to lenses)
- **HT wobbling**: is done by changing the acceleration voltage of the microscope
- If an objective aperture is not centered the image will move when you try to focus it. The way to correct this is to wobble the current to the objective lens and align the aperture to minimize movement in both the X and Y plane.
- This correction is done at successively higher magnifications—course to fine adjustment.
- The screen will "breath", pumping in and out of the screen.

Before wobbling adjustment
Unfocused image (blurred)



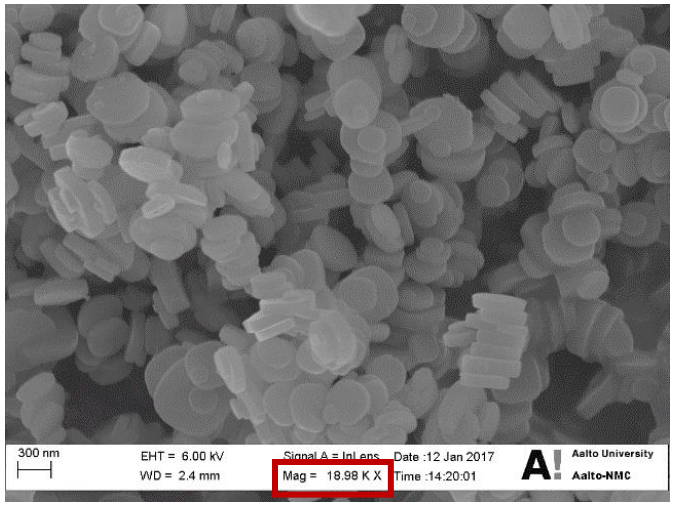


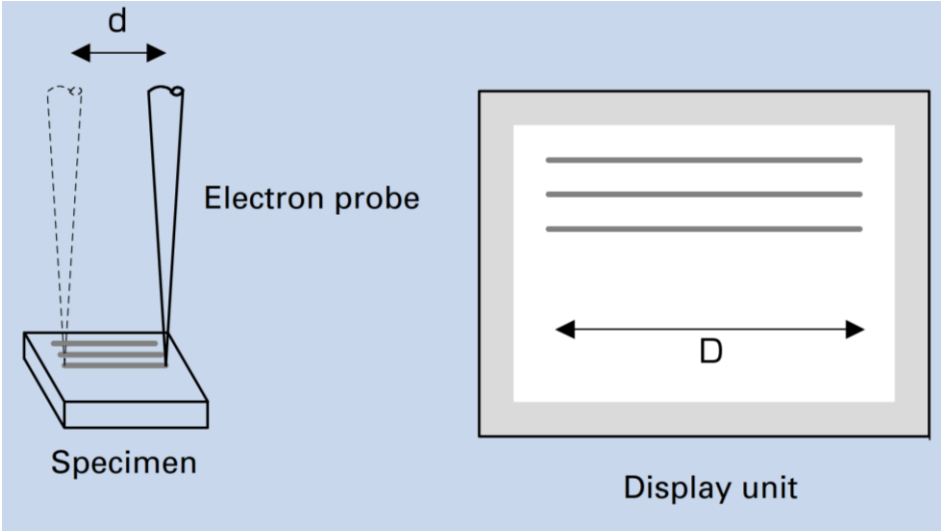
After wobbling adjustment
Focused image (sharp)



Magnification

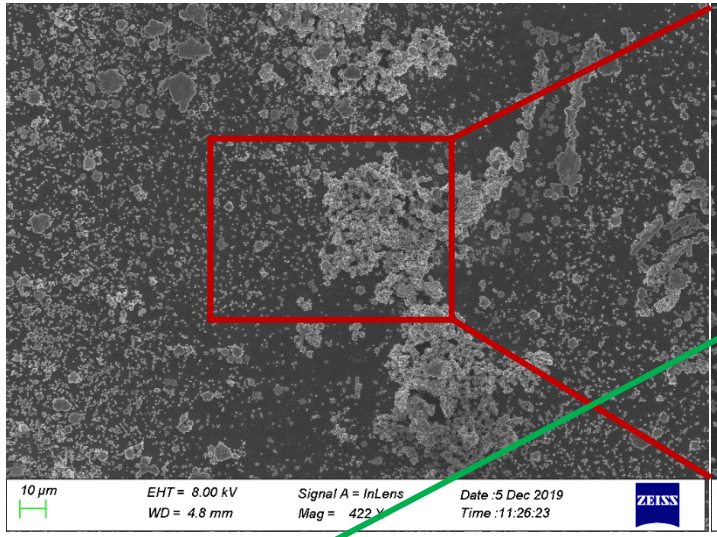
- The specimen surface is two-dimensionally scanned by the electron probe
- SEM image appears on the monitor screen of the display unit

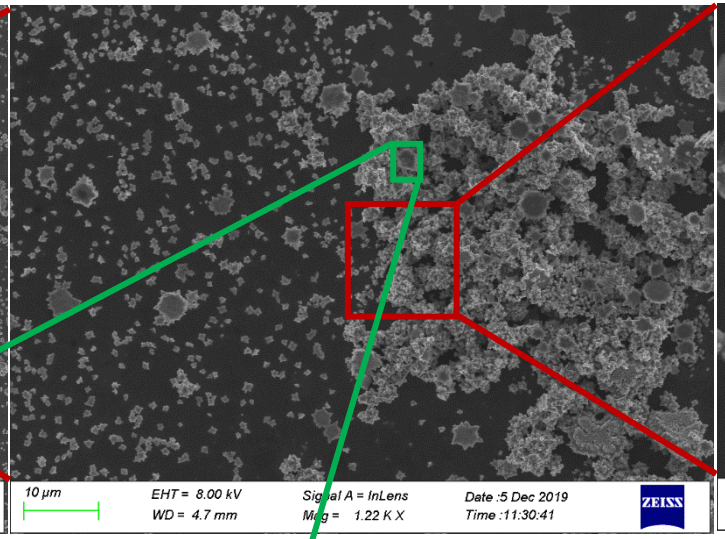


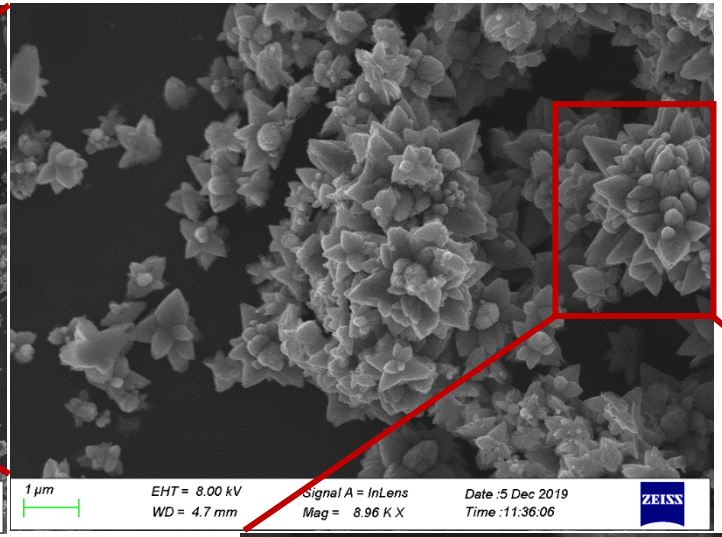


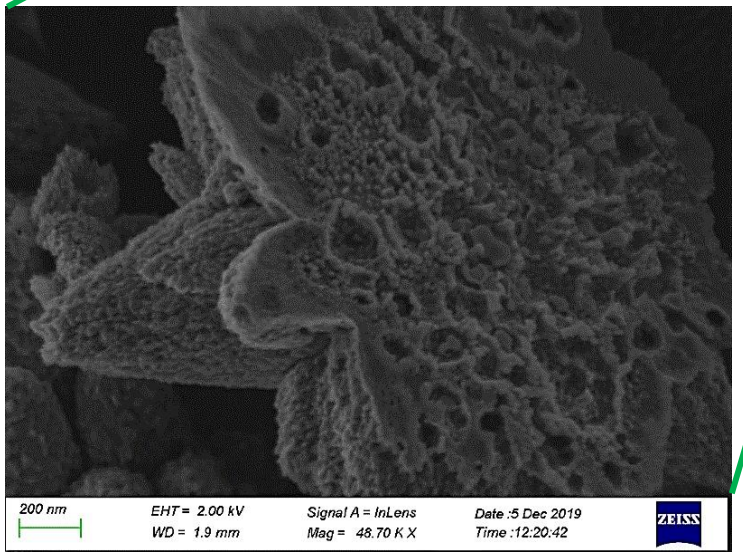
- SEM magnification = $\frac{\text{a length measured from the SEM monitor (D)}}{\text{length measured on the sample (d)}}$

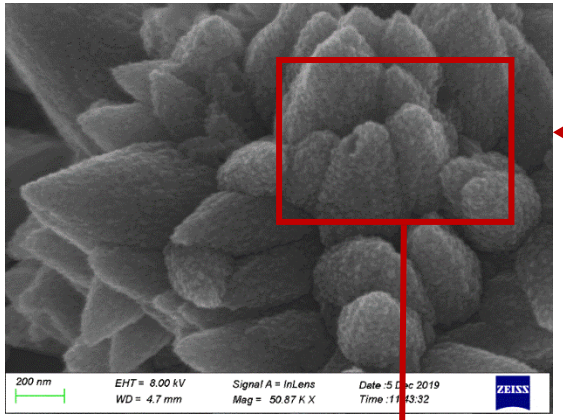
Magnification (M)	Example 1 – HFOV Image width D	Scan length d
10	24 cm	24.00 mm
100	24 cm	2.40 mm
1,000	24 cm	0.24 mm
10,000	24 cm	24.00 um
100,000	24 cm	2.40 um
1,000,000	24 cm	0.24 um

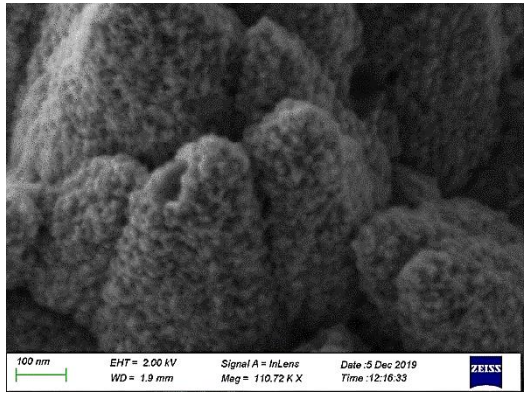

10 µm
EHT = 8.00 kV
WD = 4.8 mm
Signal A = InLens
Mag = 422 X
Date :5 Dec 2019
Time :11:26:23
ZEISS

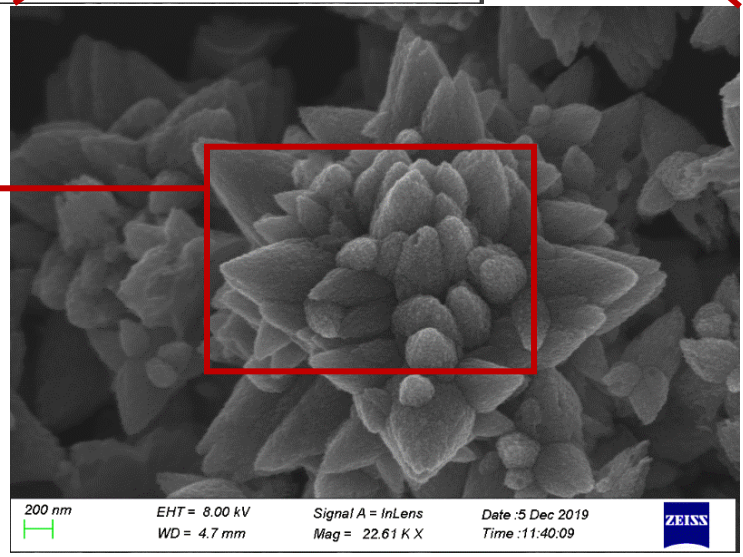

10 µm
EHT = 8.00 kV
WD = 4.7 mm
Signal A = InLens
Mag = 1.22 K X
Date :5 Dec 2019
Time :11:30:41
ZEISS


1 µm
EHT = 8.00 kV
WD = 4.7 mm
Signal A = InLens
Mag = 8.96 K X
Date :5 Dec 2019
Time :11:36:06
ZEISS


200 nm
EHT = 2.00 kV
WD = 1.9 mm
Signal A = InLens
Mag = 48.70 K X
Date :5 Dec 2019
Time :12:20:42
ZEISS

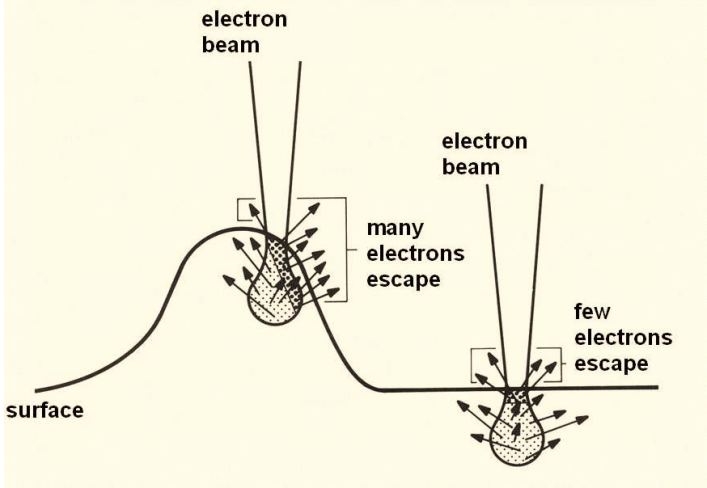

200 nm
EHT = 8.00 kV
WD = 4.7 mm
Signal A = InLens
Mag = 50.87 K X
ZEISS

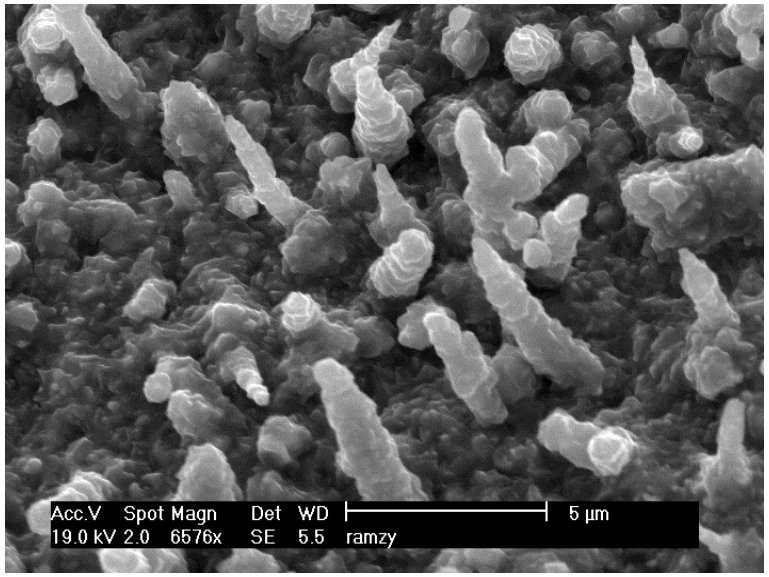

100 nm
EHT = 2.00 kV
WD = 1.9 mm
Signal A = InLens
Mag = 110.72 K X
Date :5 Dec 2019
Time :12:16:33
ZEISS

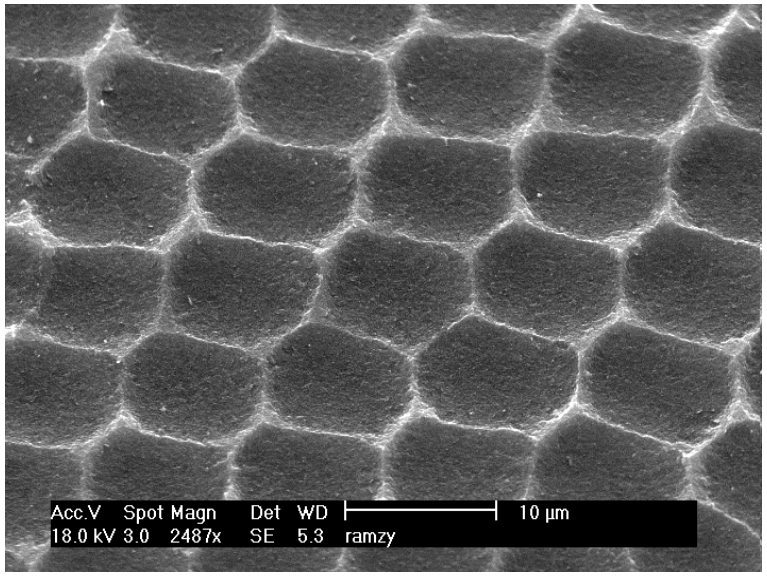

200 nm
EHT = 8.00 kV
WD = 4.7 mm
Signal A = InLens
Mag = 22.61 K X
Date :5 Dec 2019
Time :11:40:09
ZEISS

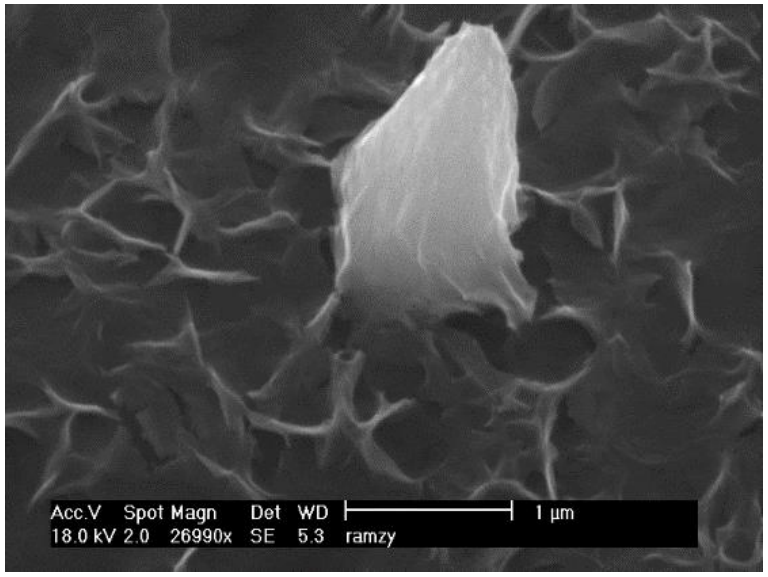
Edge Effect & Topography Contrast with SEs

- SEs → morphology & surface topography
- Contrast is dominated by the so-called edge effect
- SEs ↑ can leave the sample @ edges than in flat areas → brightness↑



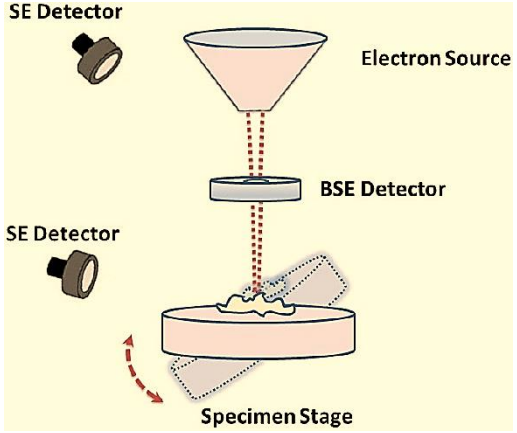




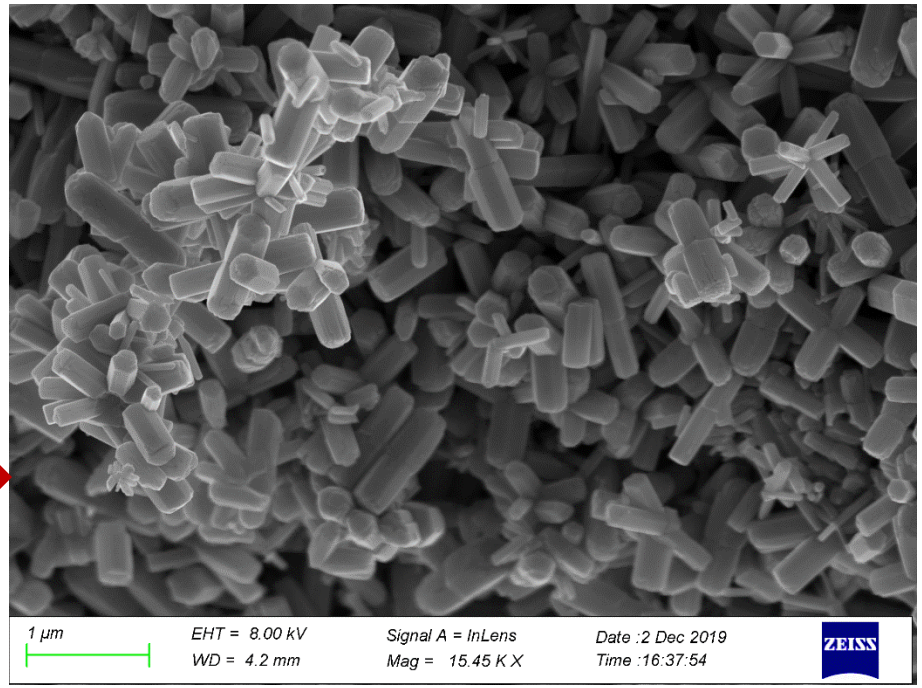


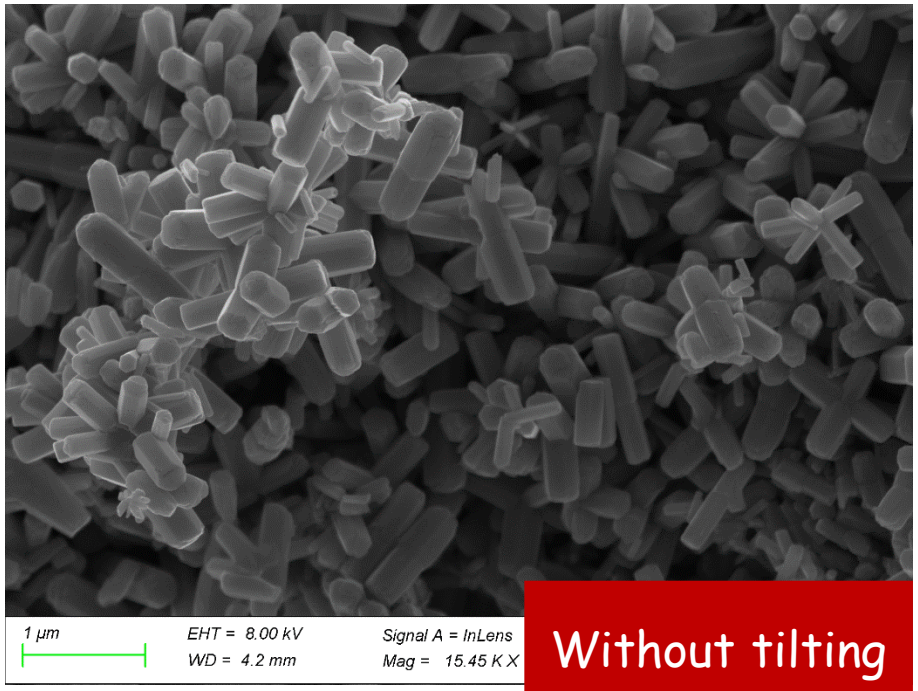
Tilting & 3D Effect

InLens detector:
sample tilted with 10°

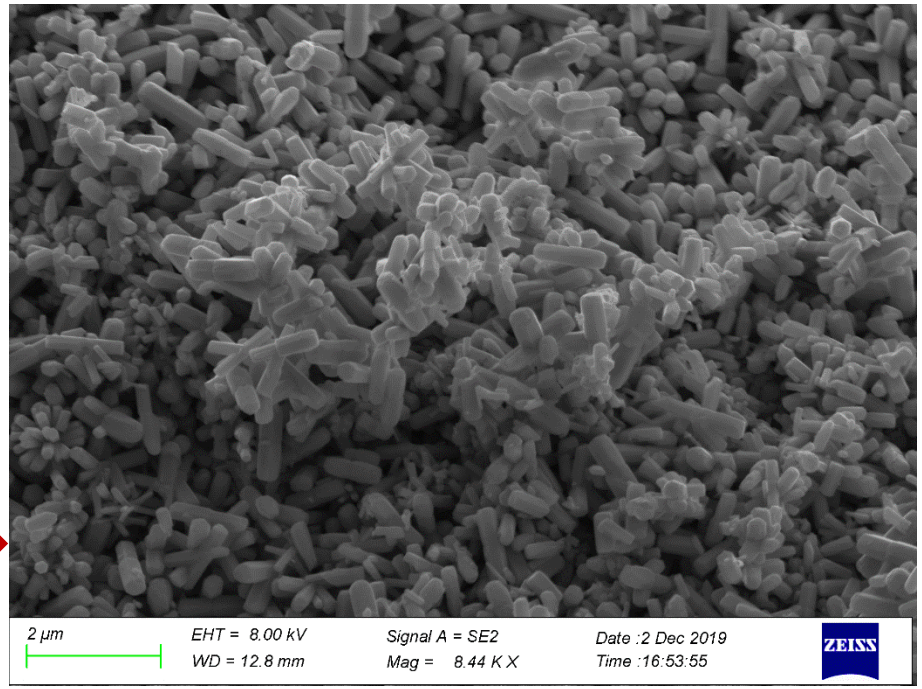


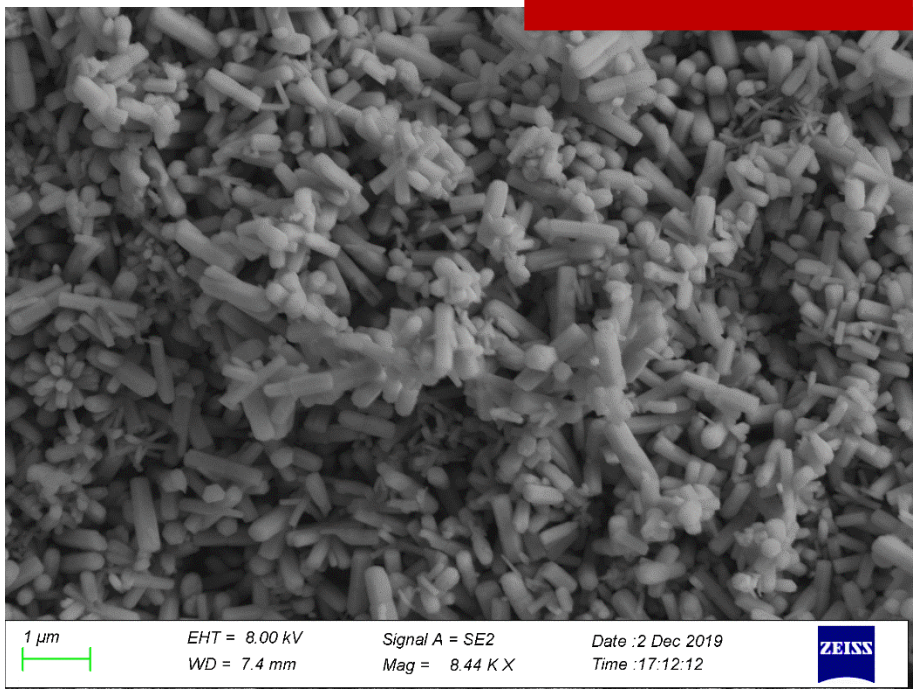
SE2 detector:
sample tilted with 30°





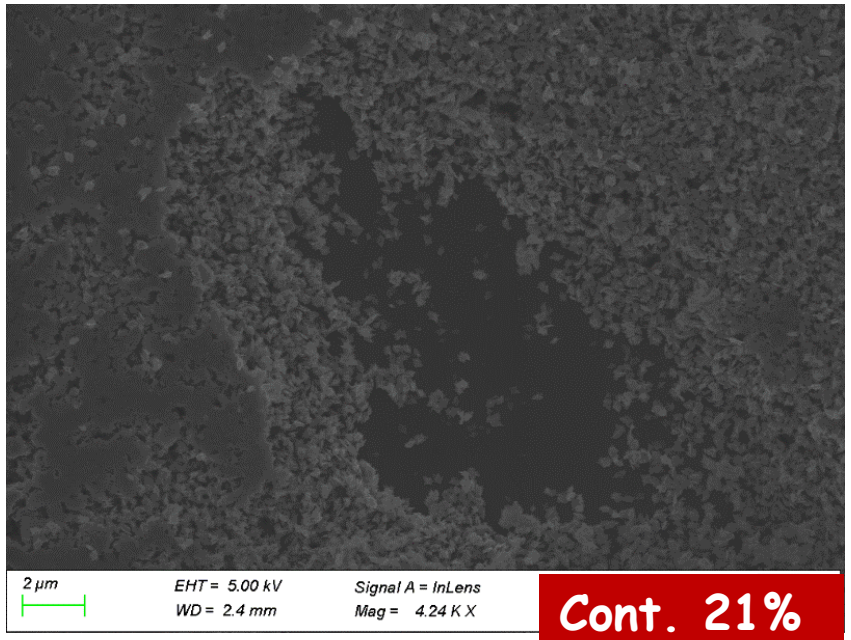
Without tilting

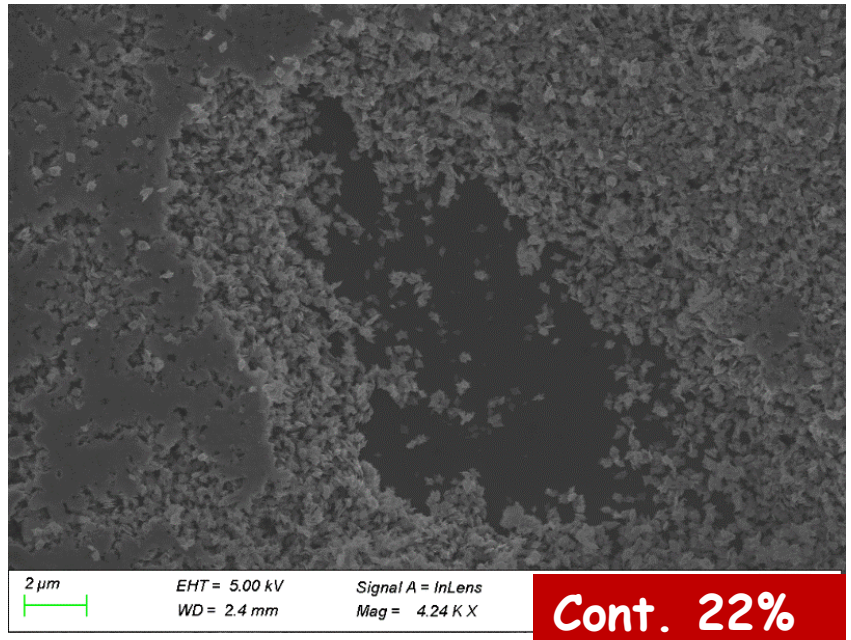




Contrast

- Measure of the difference between the highest and lowest density regions of the image

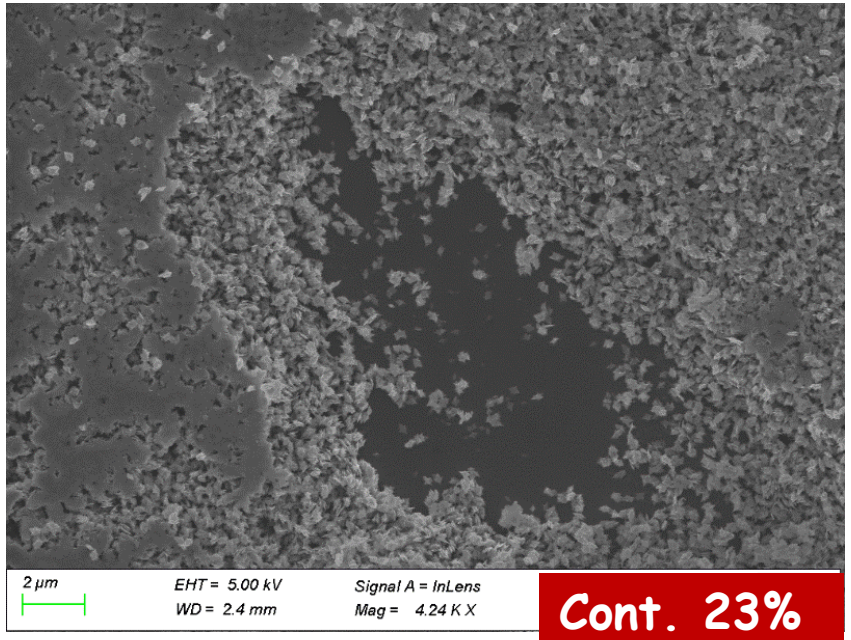


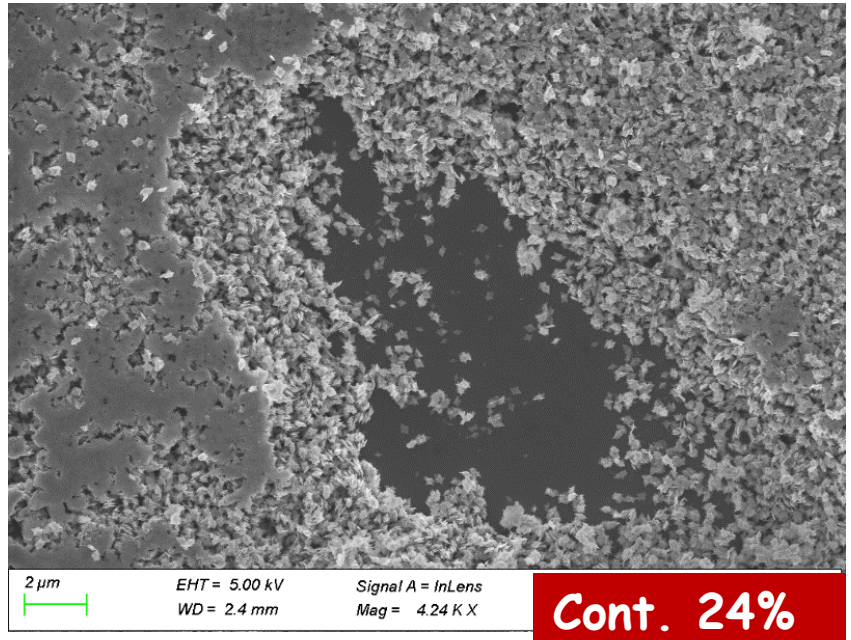


- **Contrast = $(S_2 - S_1)/S_2$**

Where…

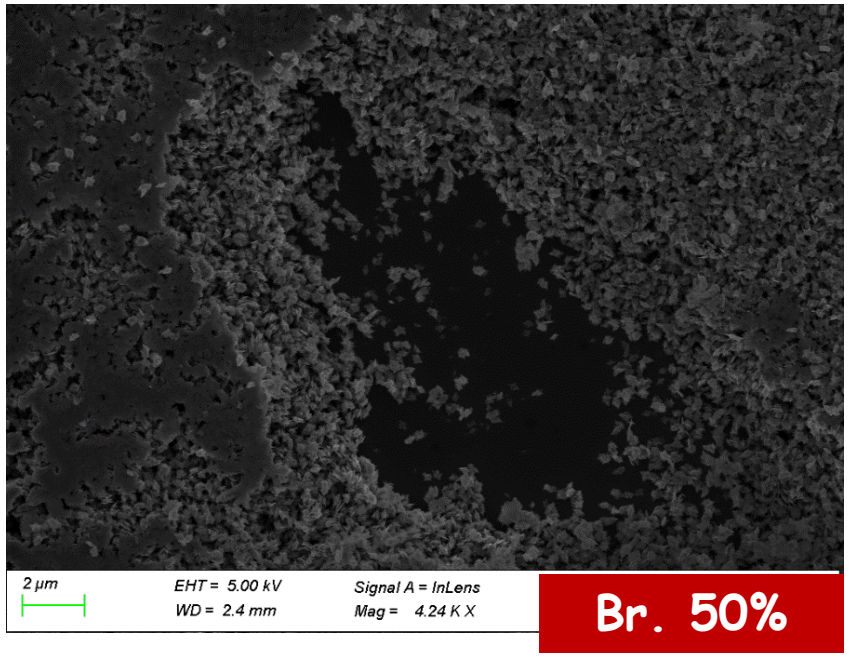
S_2: signal from the **feature of interest**
S_1: is the **background** signal
$S_2 > S_1$

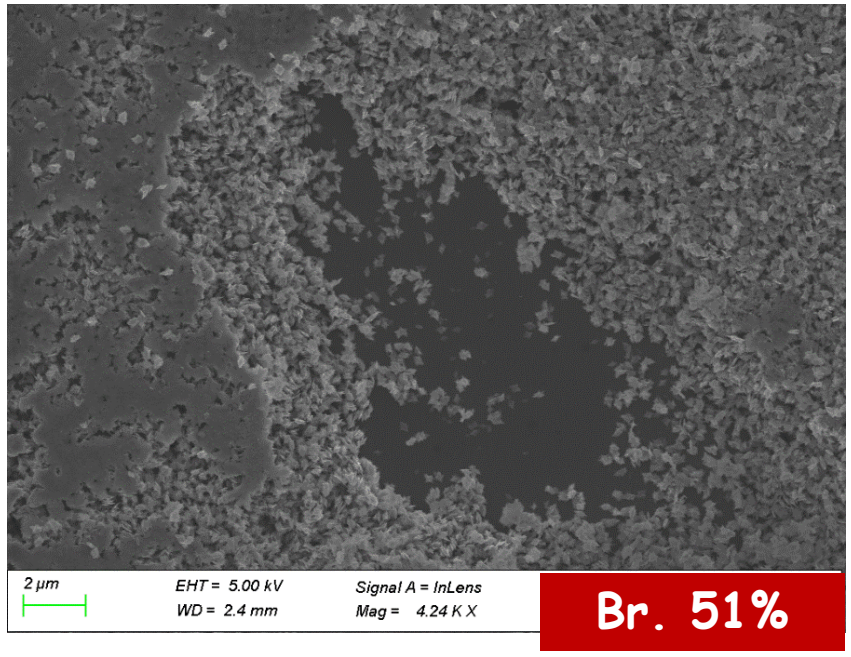


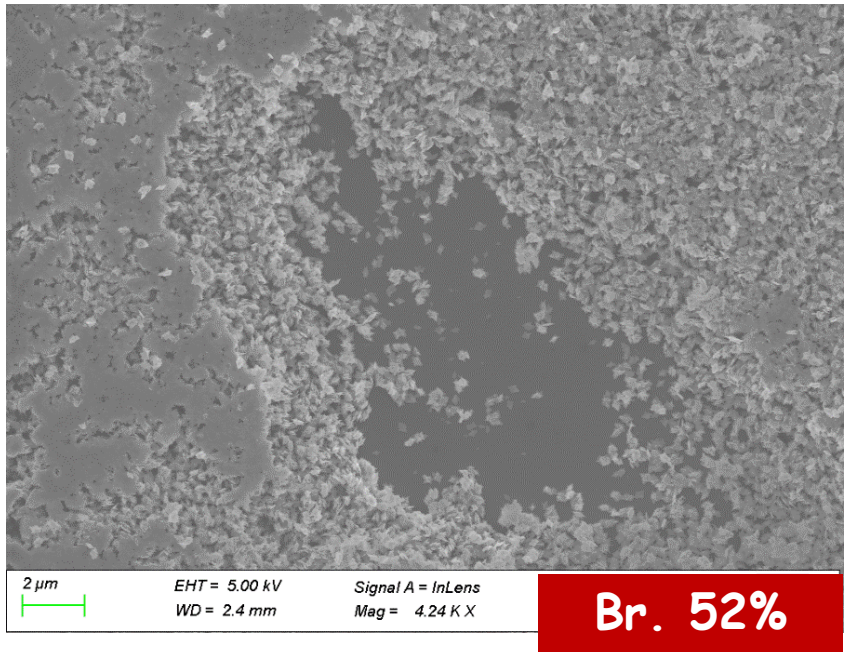


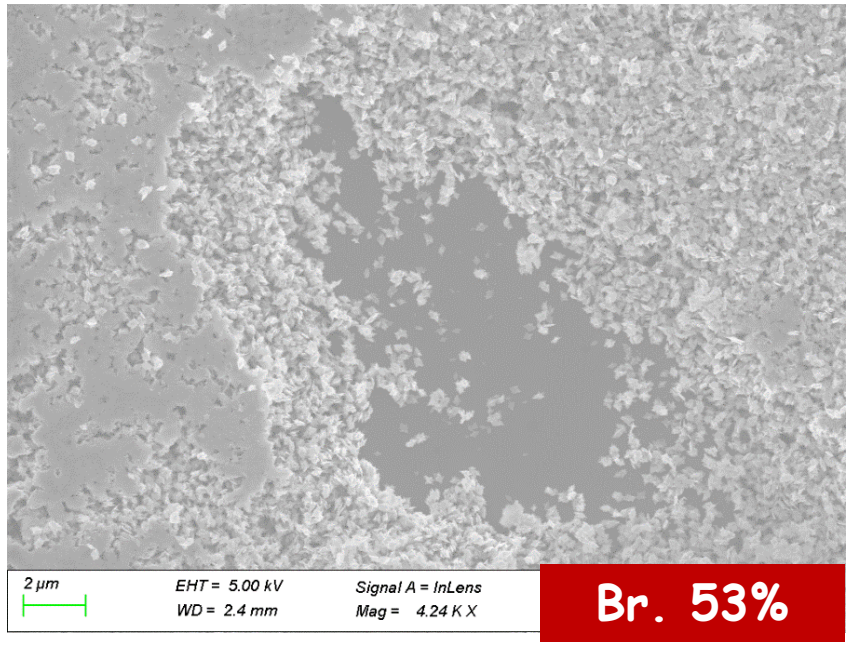
Brightness

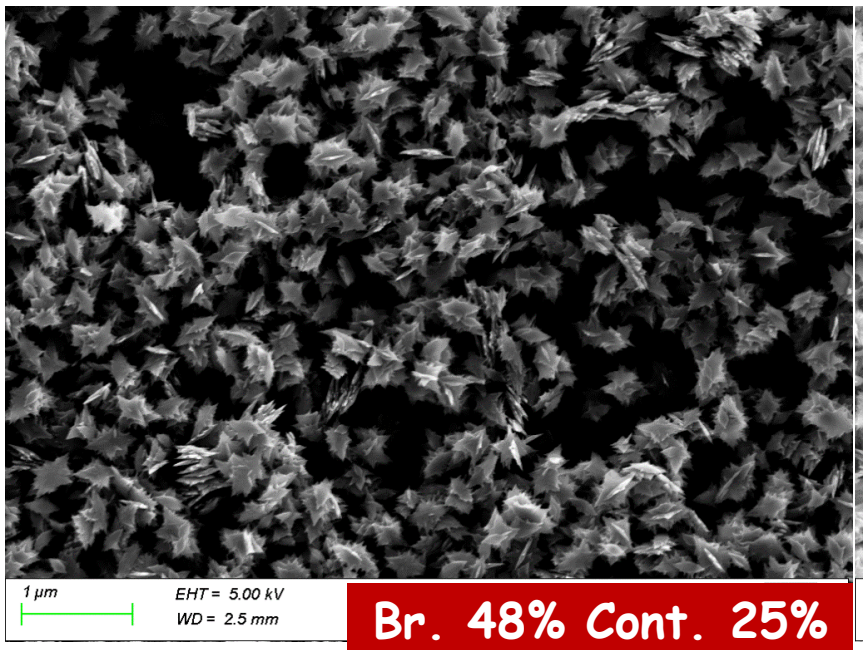
- Measure of the overall density of an image. Brighter images have less density than darker images

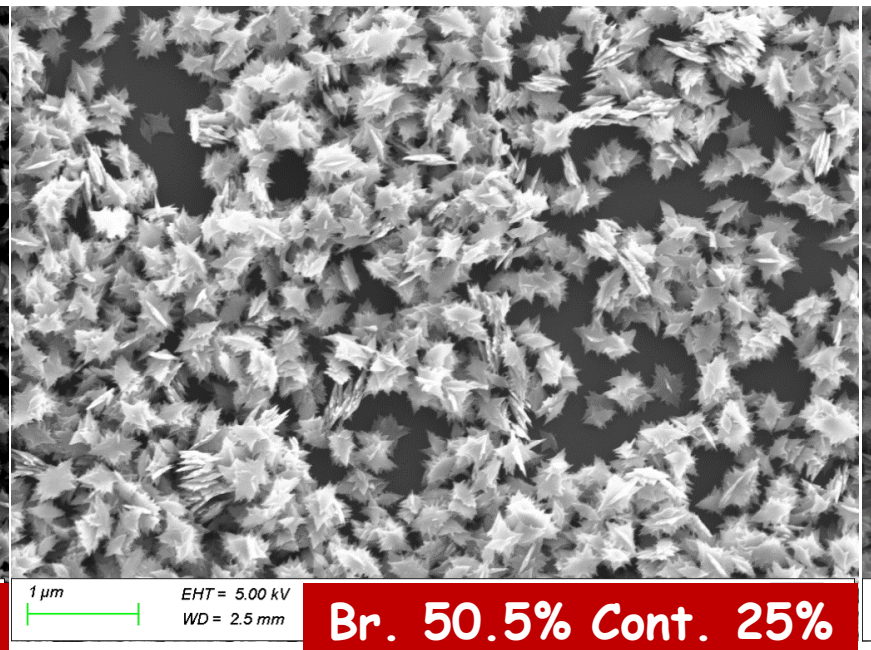


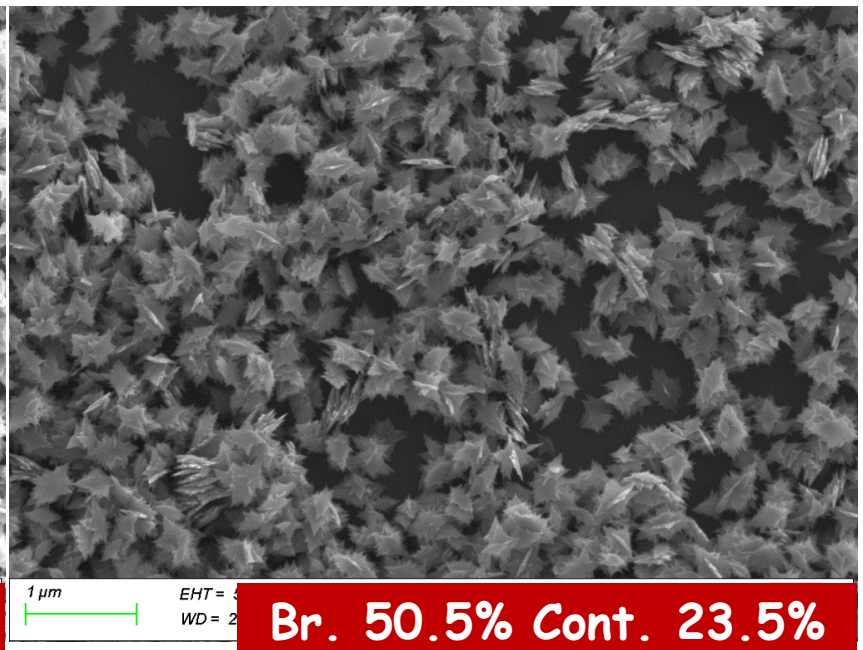


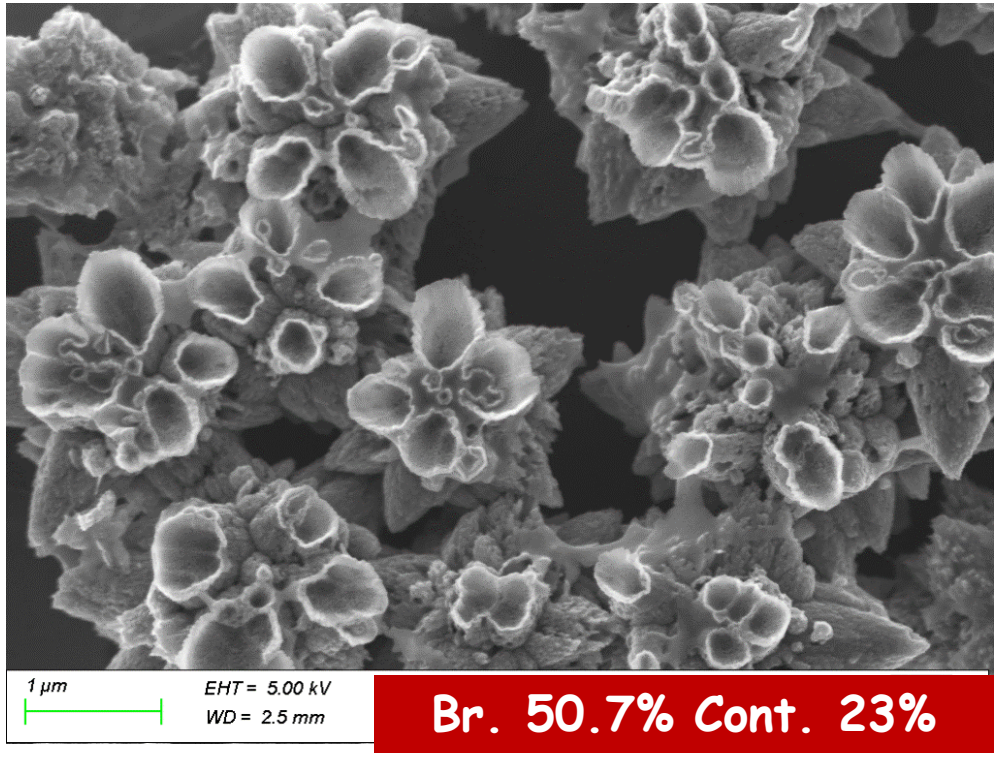


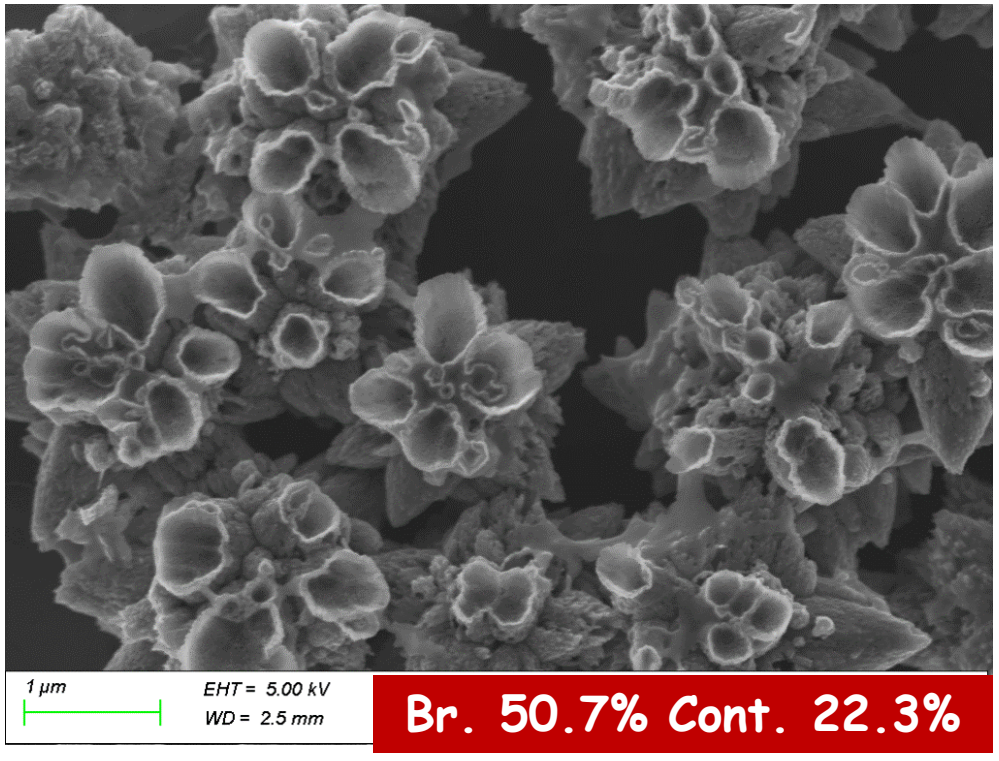



1 µm
EHT = 5.00 kV
WD = 2.5 mm
Br. 48% Cont. 25%

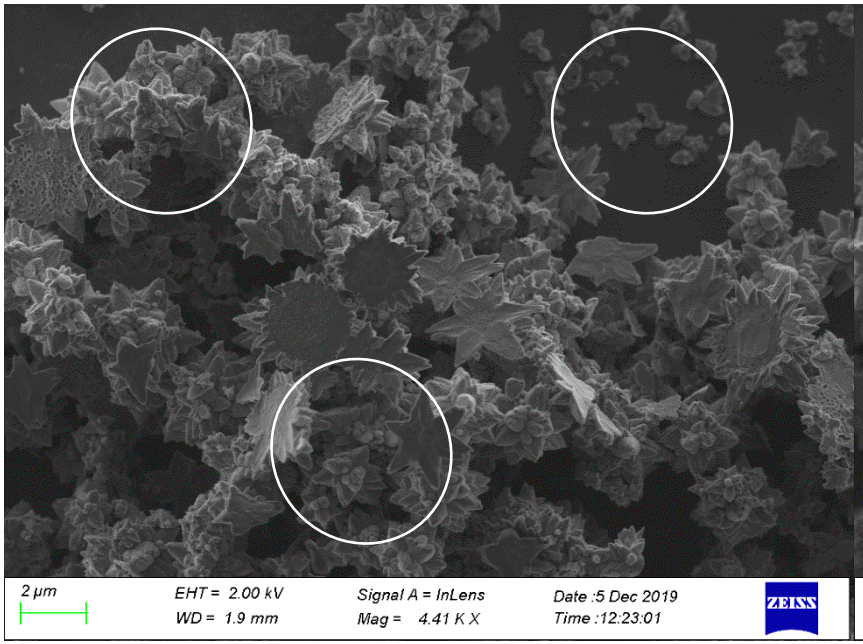

1 µm
EHT = 5.00 kV
WD = 2.5 mm
Br. 50.5% Cont. 25%

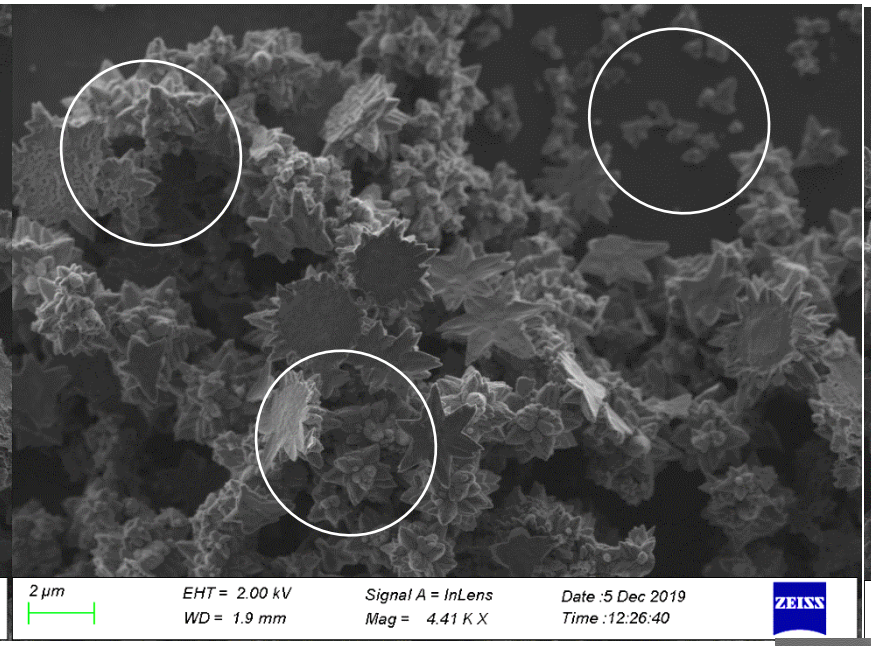

1 µm
EHT =
WD = 2
Br. 50.5% Cont. 23.5%

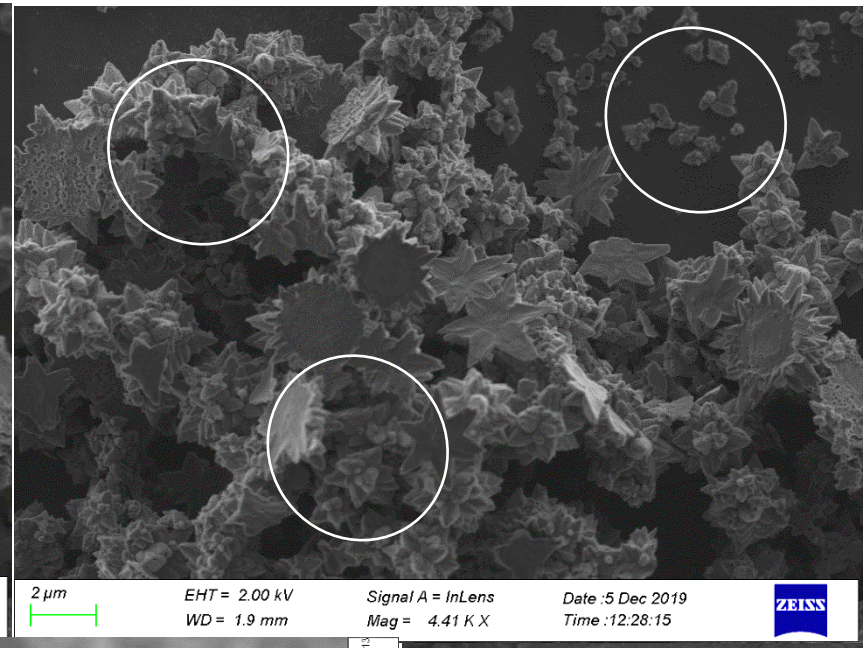

1 µm
EHT = 5.00 kV
WD = 2.5 mm
Br. 50.7% Cont. 23%

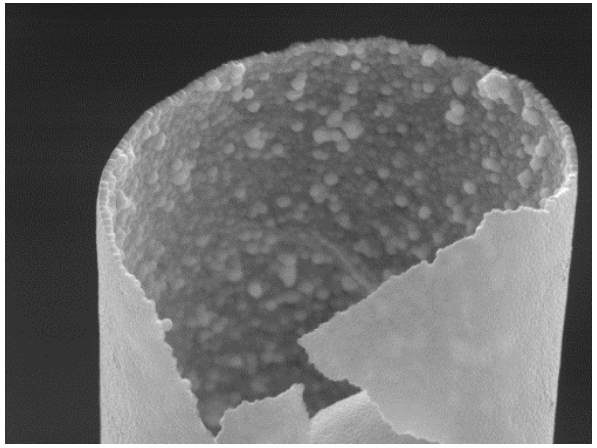

1 µm
EHT = 5.00 kV
WD = 2.5 mm
Br. 50.7% Cont. 22.3%

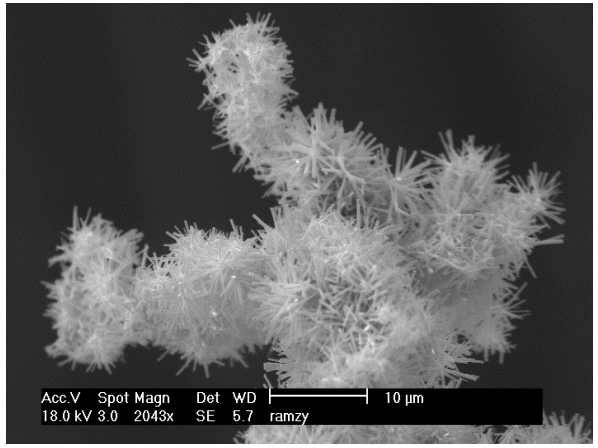
Focus

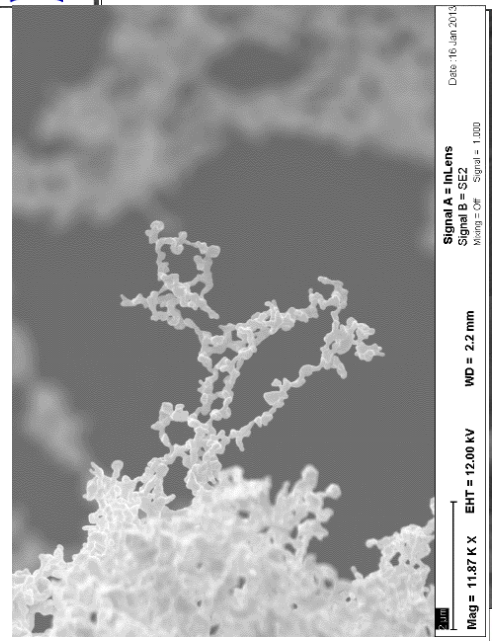

2 µm
EHT = 2.00 kV
WD = 1.9 mm
Signal A = InLens
Mag = 4.41 K X
Date :5 Dec 2019
Time :12:23:01
ZEISS

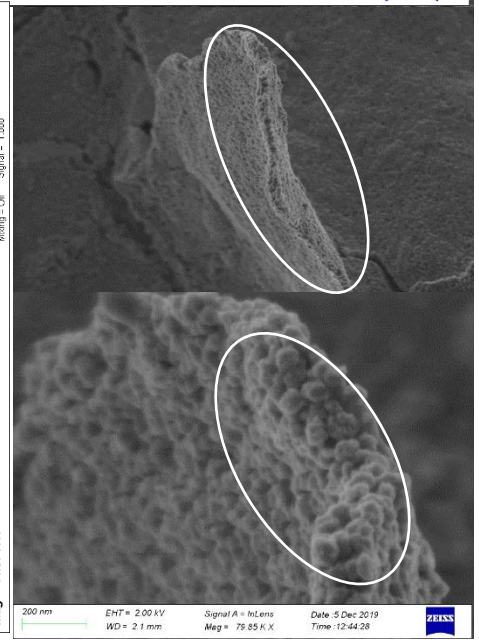

2 µm
EHT = 2.00 kV
WD = 1.9 mm
Signal A = InLens
Mag = 4.41 K X
Date :5 Dec 2019
Time :12:26:40
ZEISS


2 µm
EHT = 2.00 kV
WD = 1.9 mm
Signal A = InLens
Mag = 4.41 K X
Date :5 Dec 2019
Time :12:28:15
ZEISS



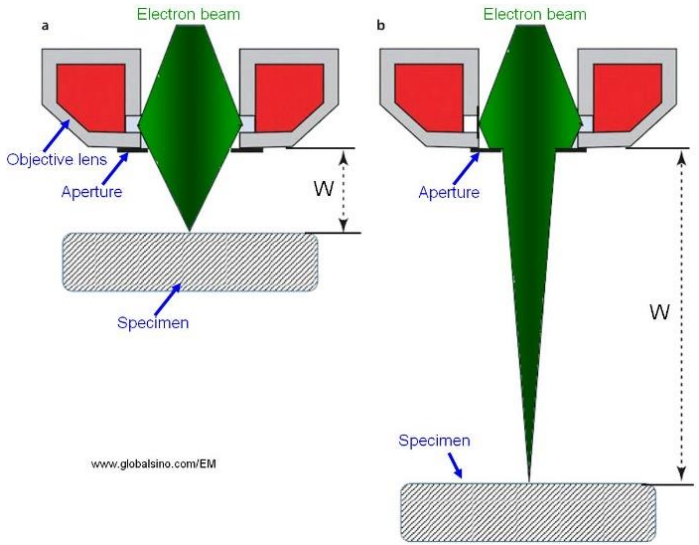

Acc.V Spot Magn Det WD 10 µm
18.0 kV 3.0 2043x SE 5.7 ramzy


Signal A = InLens
Signal B = SE2
Mag = 11.87 K X
EHT = 12.00 kV
WD = 2.2 mm


200 nm
EHT = 2.00 kV
WD = 2.1 mm
Signal A = InLens
Mag = 79.85 K X
Date :5 Dec 2019
Time :12:44:28
ZEISS

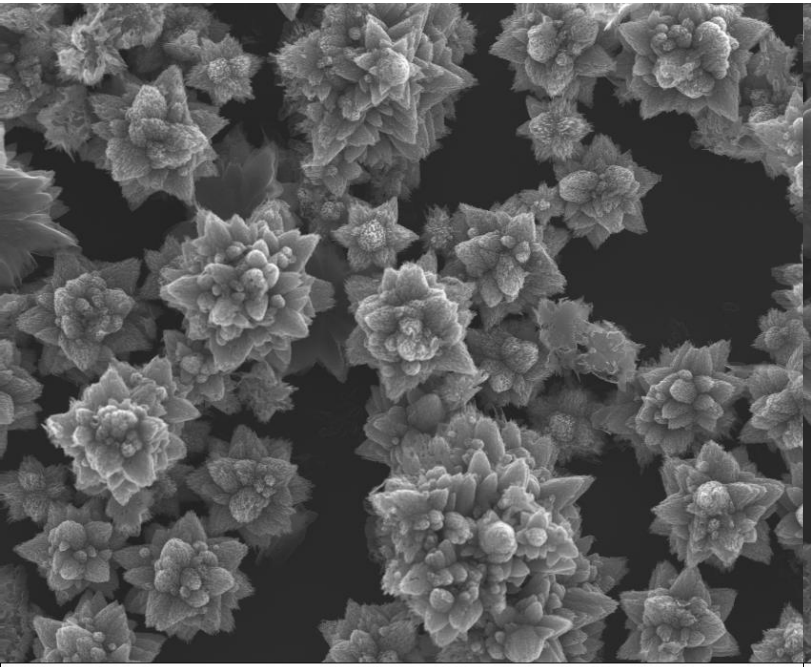
Working Distance (WD) effect

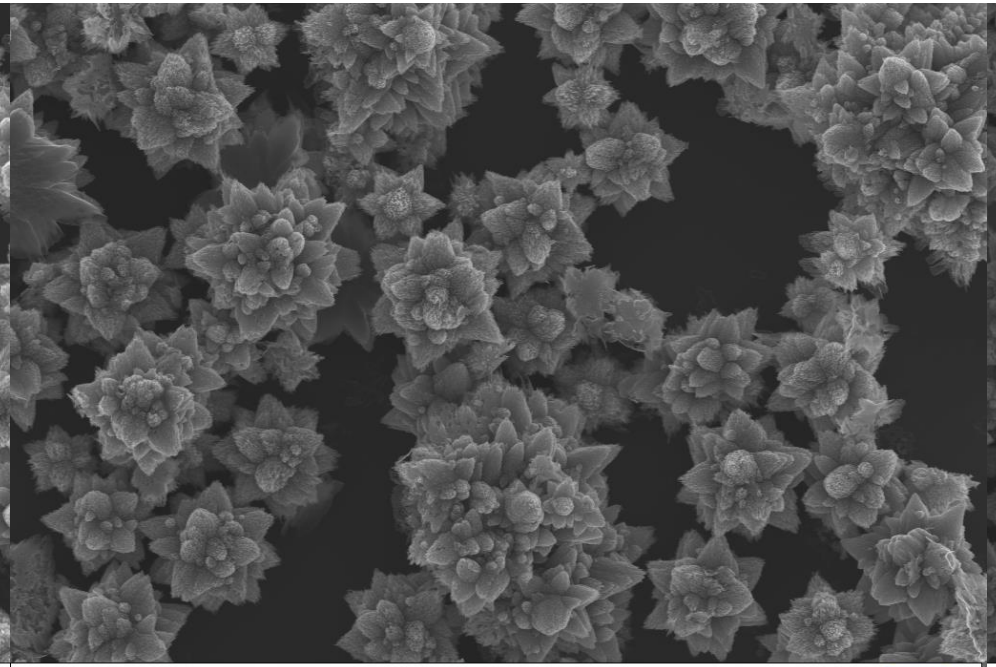
WD: a distance between the specimen and the lower pole piece in SEM system

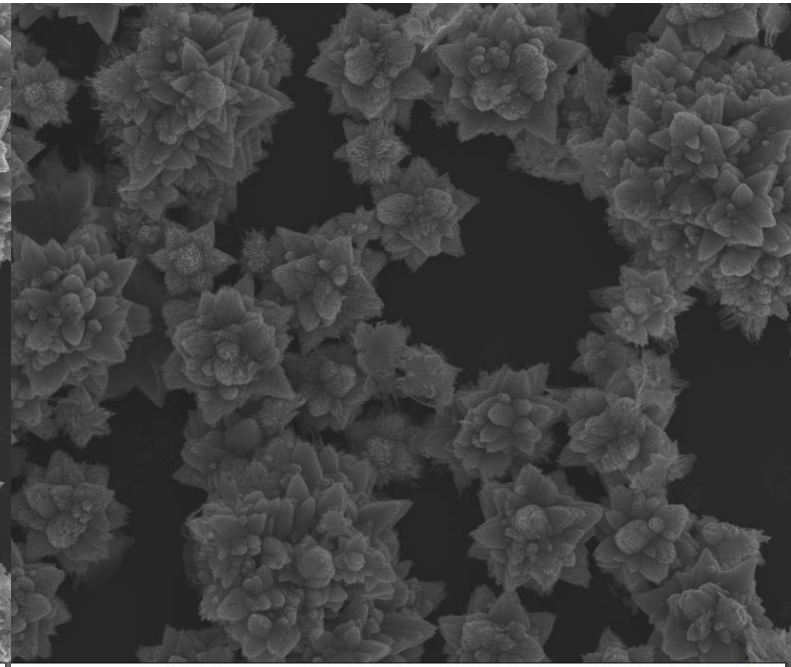


- Short WD
- Small depth of field
- High resolution
- More edge effect
- More charge-up

- Long WD
- Large depth of field
- Low resolution
- Less edge effect
- Less charge-up

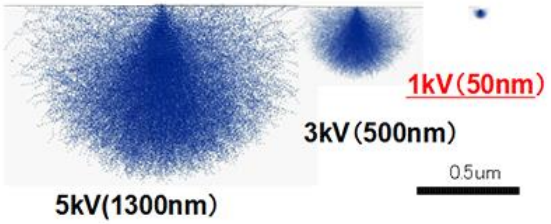






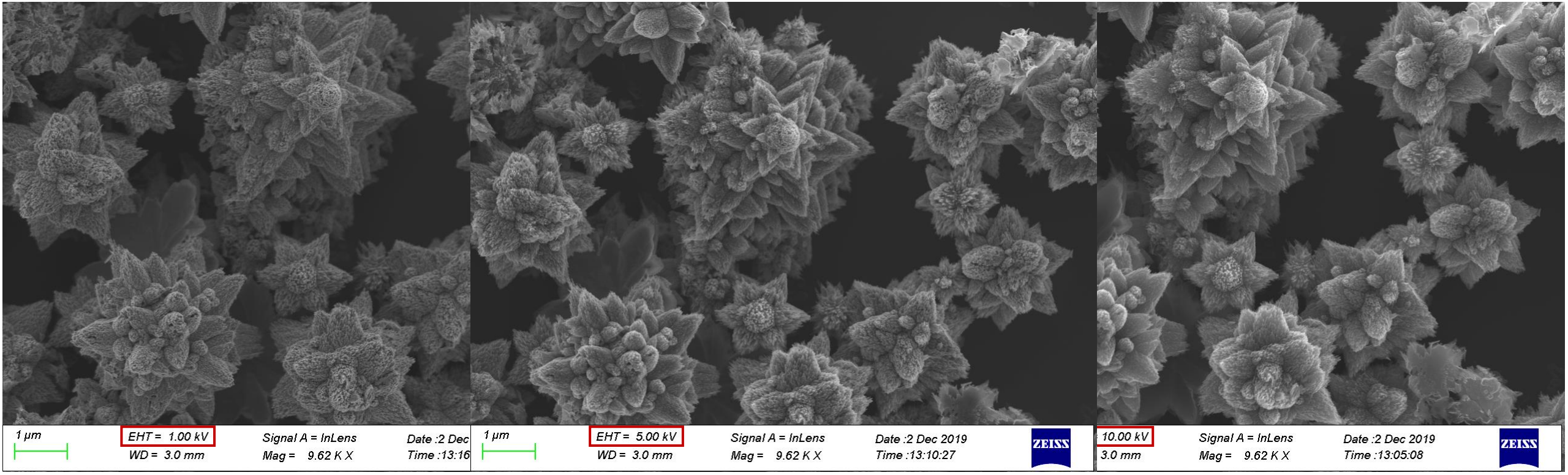
Accelerating Voltage effect

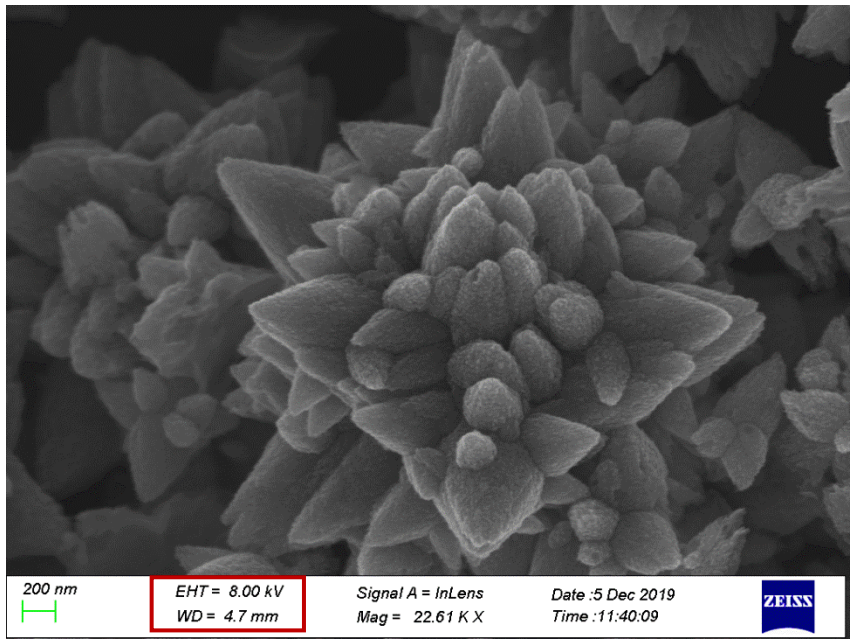
- Low AV
- Clear surface structures
- Low resolution
- Less edge effect
- Less charge-up
- Less damage

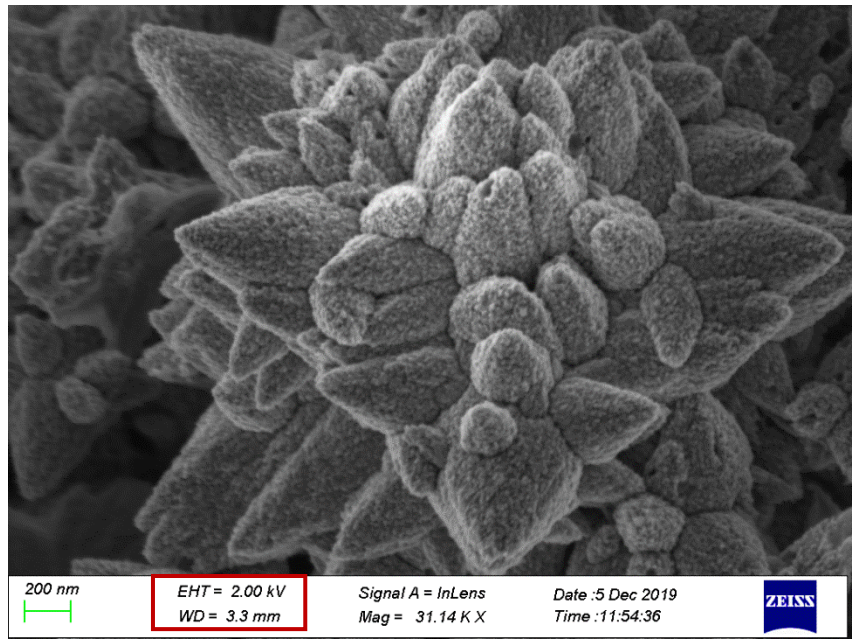


$$R = \frac{0.0276\ A\ E^{1.67}}{(Z^{0.89}\ \rho)}\ \mu m$$

- High AV
- Unclear surface structures
- High resolution
- More edge effect
- More charge-up
- More damage

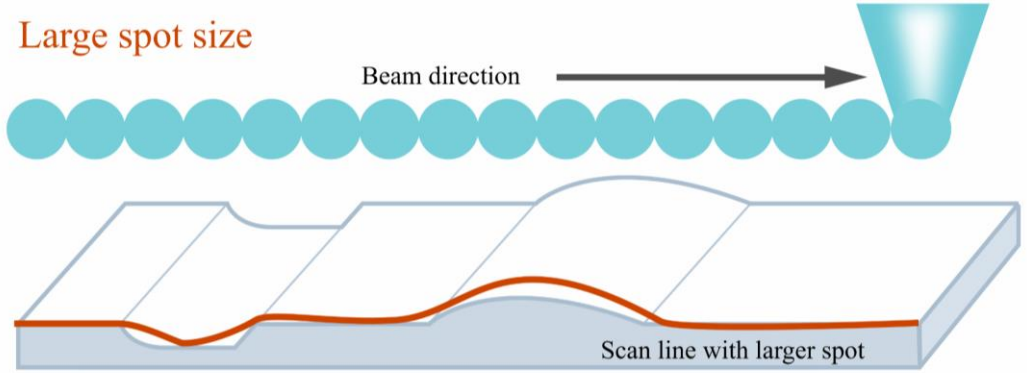



200 nm
EHT = 8.00 kV
WD = 4.7 mm
Signal A = InLens
Mag = 22.61 K X
Date :5 Dec 2019
Time :11:40:09
ZEISS

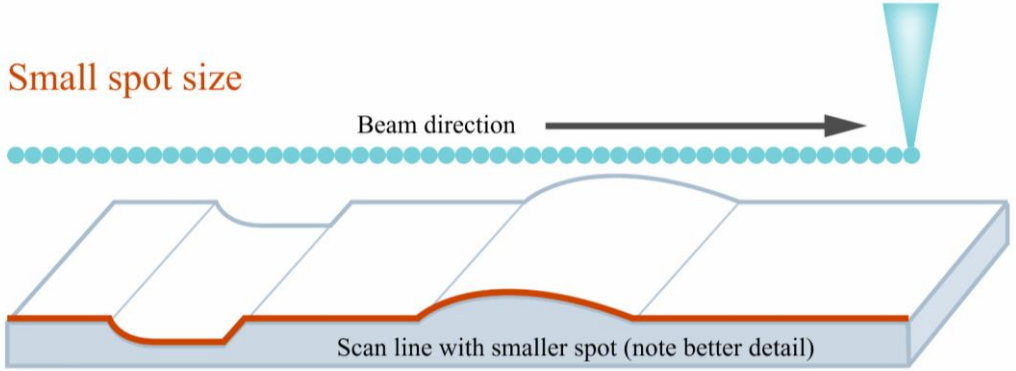

200 nm
EHT = 2.00 kV
WD = 3.3 mm
Signal A = InLens
Mag = 31.14 K X
Date :5 Dec 2019
Time :11:54:36
ZEISS

Spot Size Effect

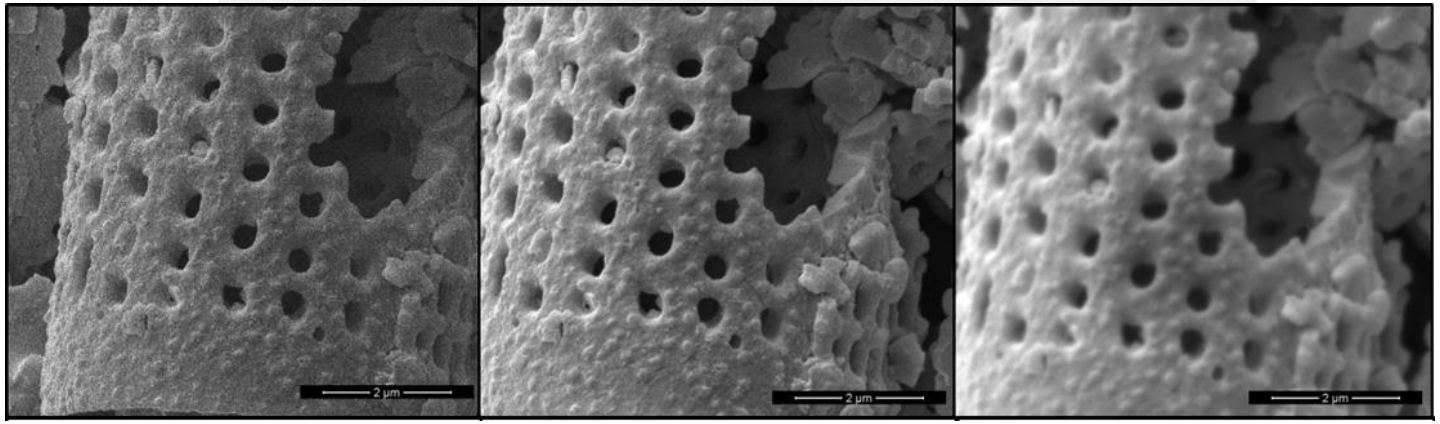
The diameter of the final beam spot onto the sample



- Large current
- Low resolution
- Small depth of field



- Less current
- High resolution
- Greater depth of field
- Clear surface structure



Small spot size

Large spot size

Sample preparation

- **Solid or powder samples (perfect dry)**

Put C tape tape on sample stub

↓

Mount the sample on the tape

↓

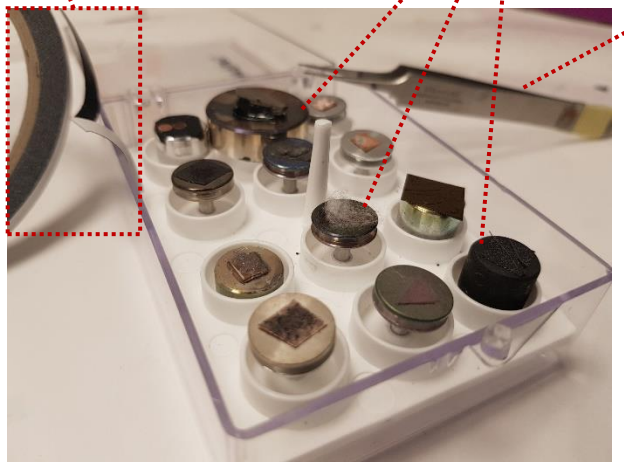
Non-conductive samples
Metal sputter coating

Conductive samples

Double-sided carbon or Cu adhesive tape

SEM stubs

Tweezer



- **Biological or food samples (wet)**

Cutting

↓

Pre-fixation

(e.g., with Glutaraldehyde)

↓

Post-fixation

(e.g., with Osmium tetroxide)

↓

Dehydration

(e.g., with ehtanol ascending series)

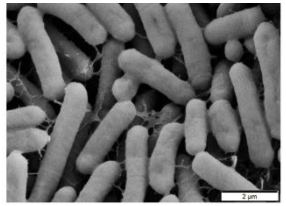
↓

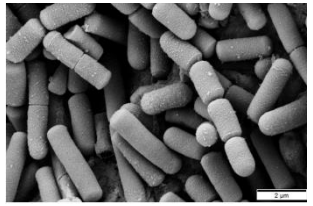
Drying

(e.g., critical point drying, freez drying or air drying)

↓

Metal sputter coating





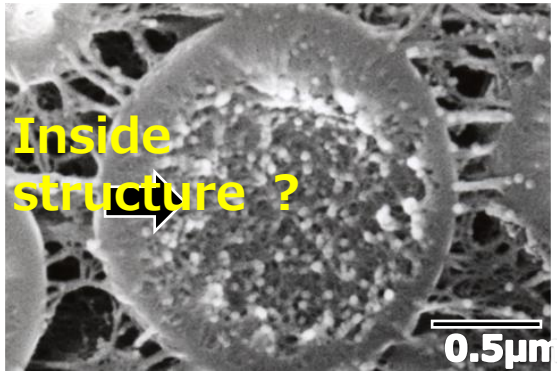
Bacterial cells, Flattened, shrunken
Unclear surface structures

Well rounded and fulsome while the dividing cells are well exposed

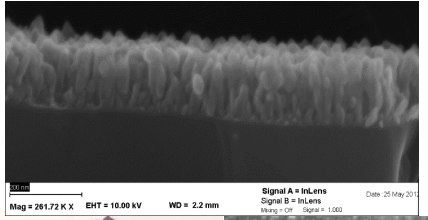
Murtey, M. D., & Ramasamy, P. (2016). Sample preparations for scanning electron microscopy–life sciences. In Modern electron microscopy in physical and life sciences. IntechOpen.

➢ Making a cross section sample

Cryo-SEM (freeze fracturing) method



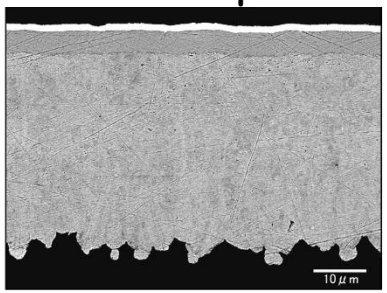
Specimen： Polyvinyl acetate

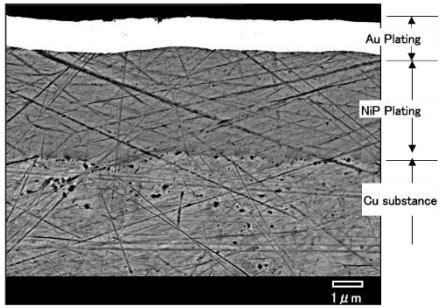


Cross section method	Applicable
Cleavage	Polymer
Freeze fracturing method	Polymer Bio
Mechanical polishing	Metal Semiconductor Sintered body
Microtome	Polymer Bio
FIB	Semiconductor Metal

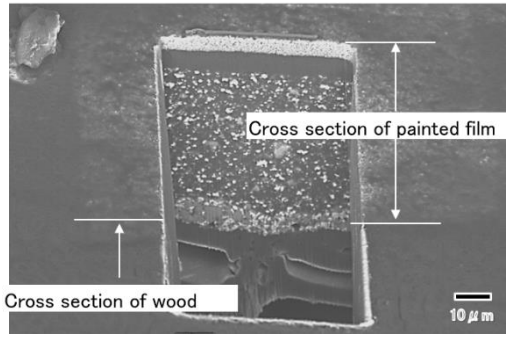
Specimen

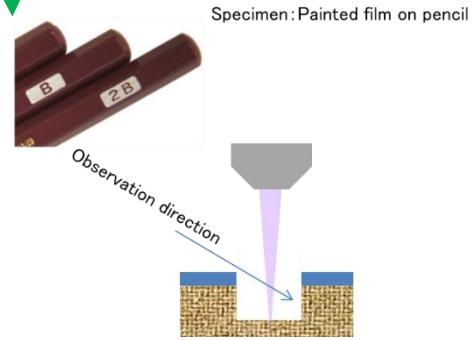
Mechanical polishing



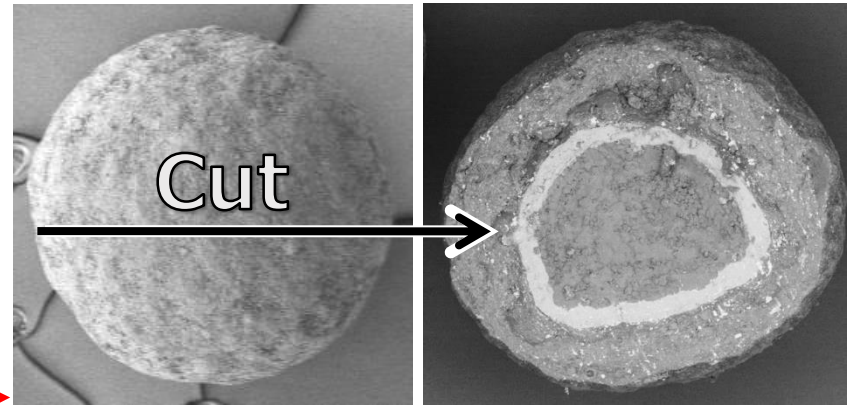


By FIB

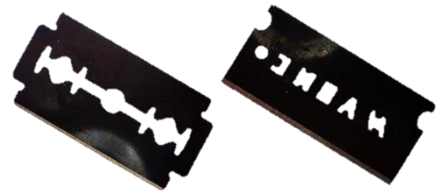




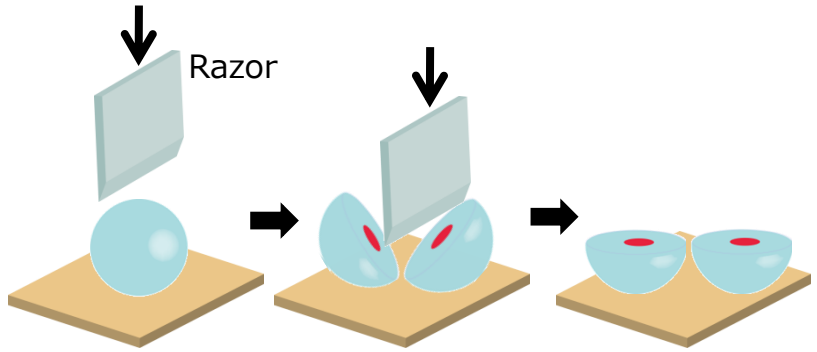
Cleaved by double edge razor



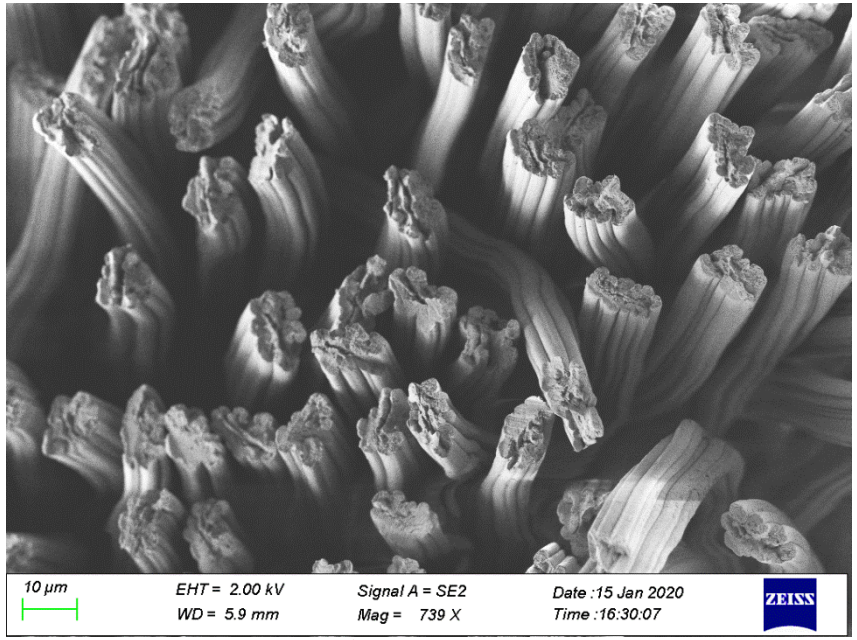
Specimen： Granule
(Flue Medicine)

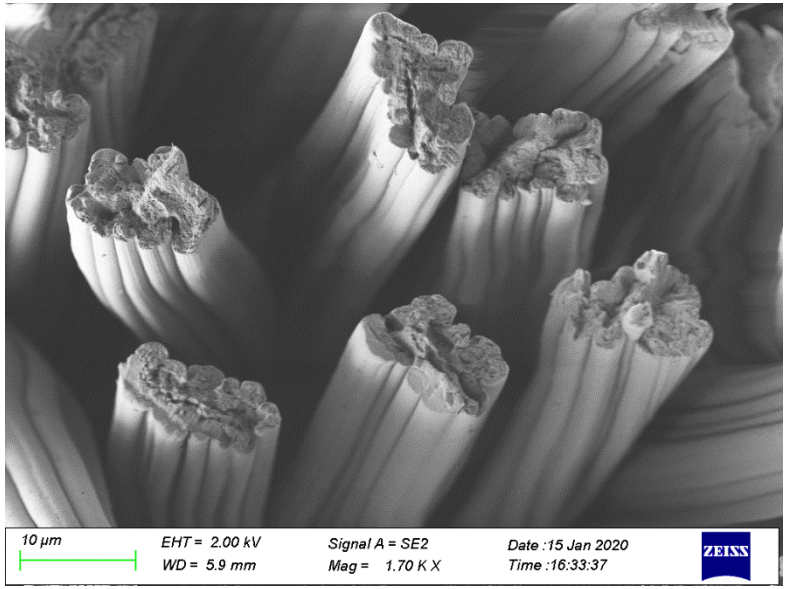


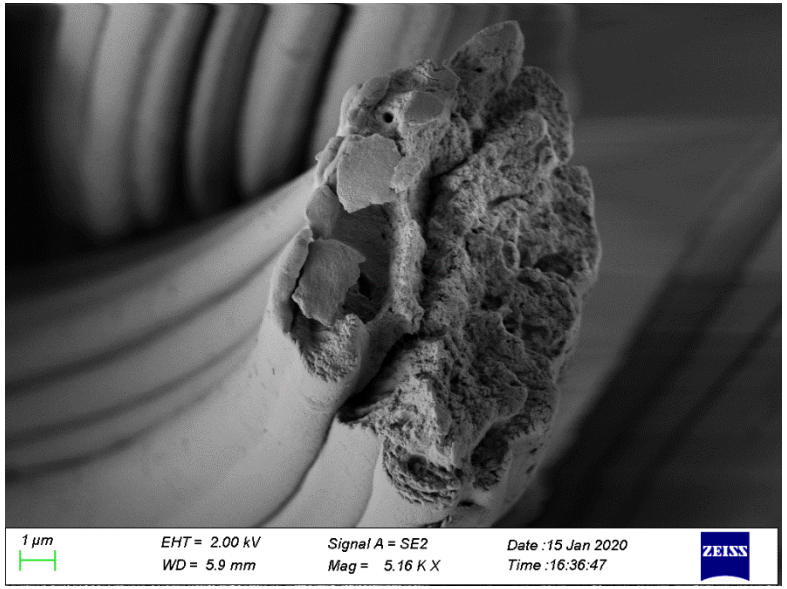
Double or single edge razor



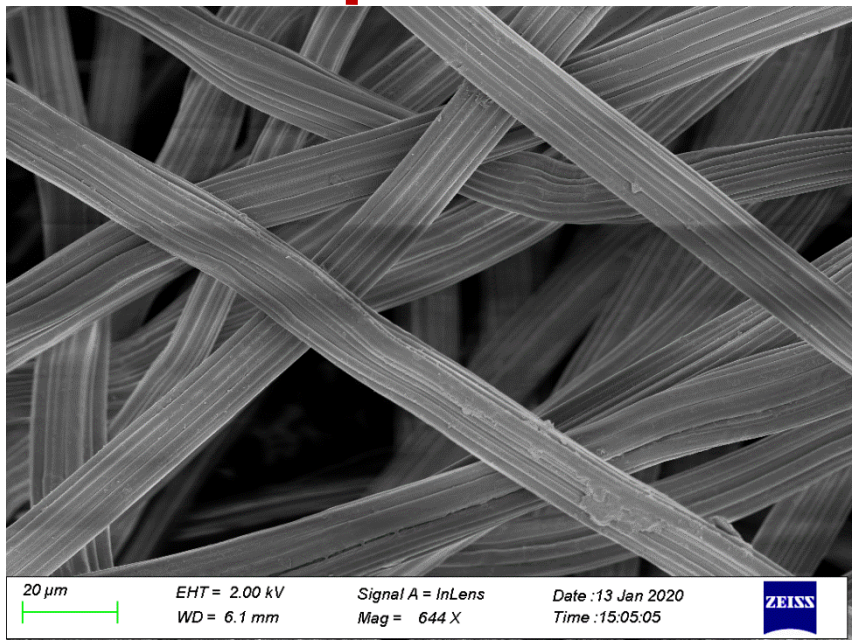
Double-sided tape





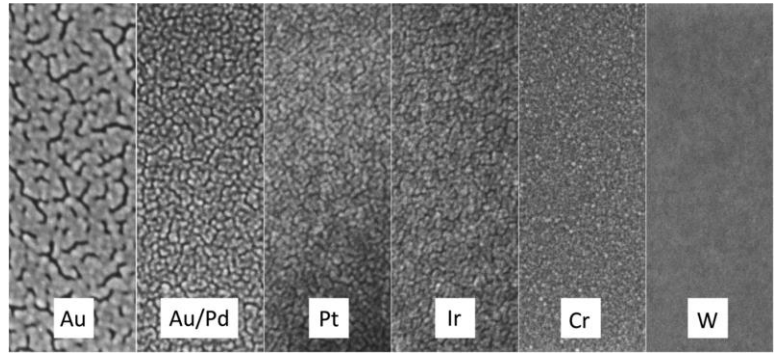


1. Liquid nitrogen
2. Single edge razor
3. Sputter coating



Cellulose fiber

Sputter Coating

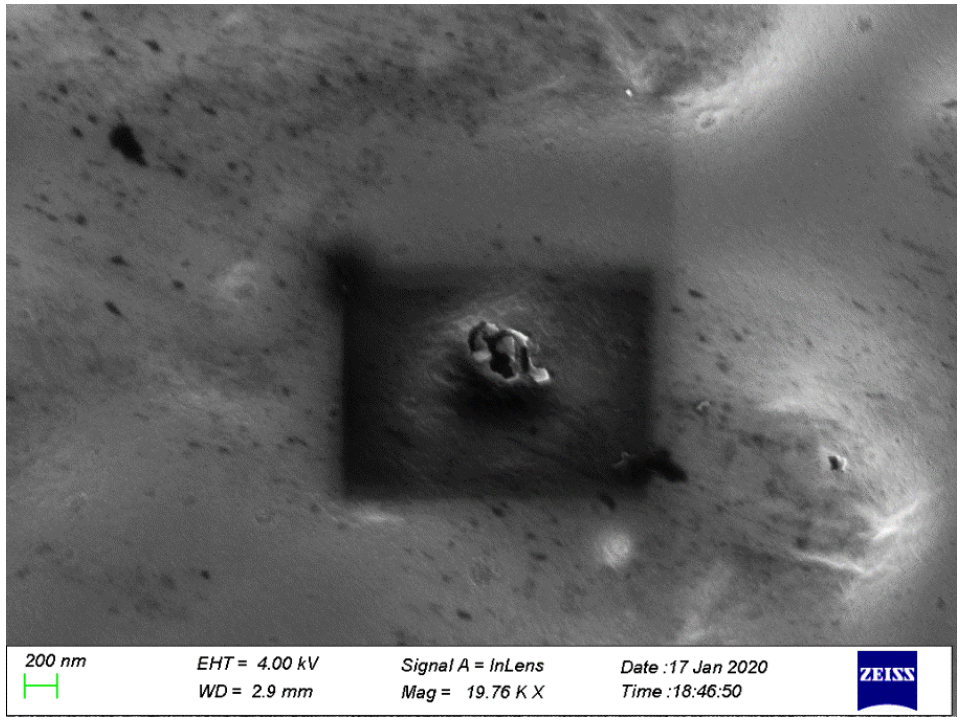


Sputter Material	Grain Size[a]	Typical Maximum Magnification[b]	Relative SE yield[c]	Relative Sputter Rate[d]	Vacuum Requirements
Au	10–12 nm	10,000×	High	10	Modest
Au/Pd	4–8 nm	25,000×	High	9	Modest
Pt	2–3 nm	50,000×	High	6	Stringent
Ir	1–2 nm	100,000×	High	4	Stringent
Cr	1–2 nm	100,000×	Moderate	5	Stringent
W	< 1 nm	200,000×	High	2	Stringent

https://www.cambridge.org/core/journals/microscopy-today/article/target-material-selection-for-sputter-coating-of-sem-samples/089A8657A8345CFFCF963BED868578D4/core-reader

Contamination in SEM

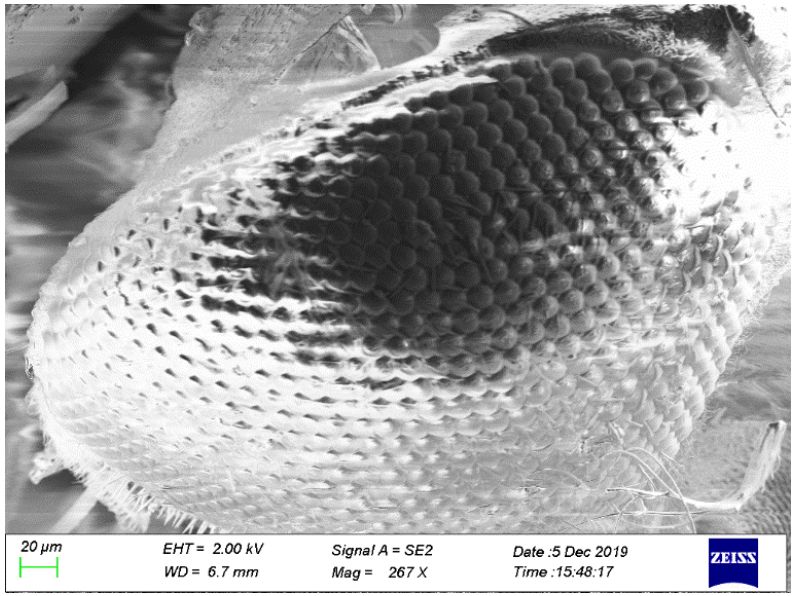
Interaction of the electron beam with residual gases and hydrocarbons on the specimen surface

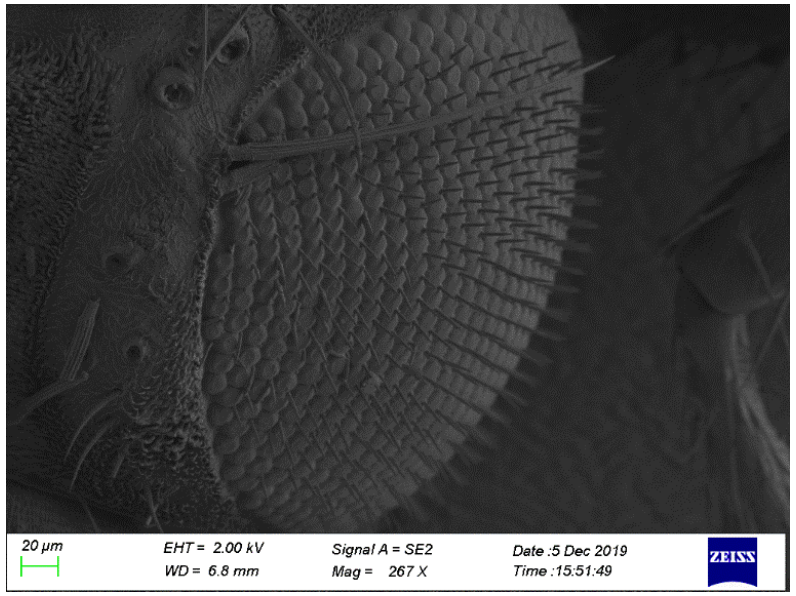


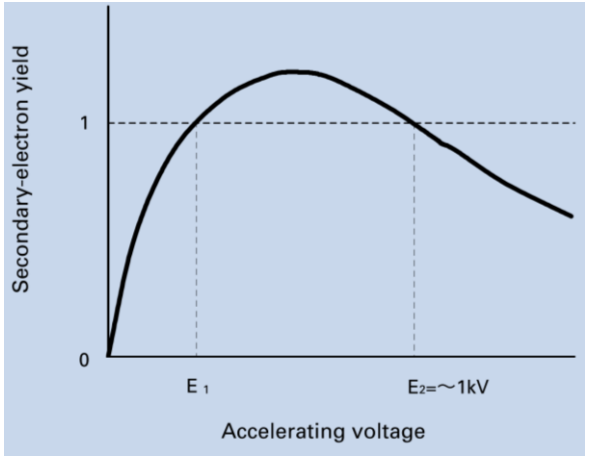
- Ensuring the cleanliness of specimens (heat, UV or plasma cleaning)
- Decreasing the probe current
- Only from low magnification imaging to higher
- Aligning the microscope on areas of the specimen not used for imaging

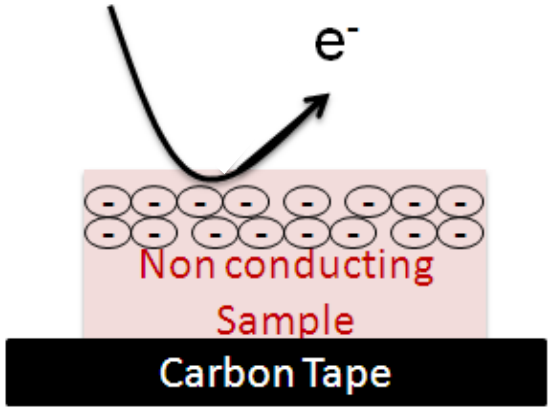
Charging in SEM

- Charging is a result of electrons becoming trapped within the sample→ sample to "glow"
- No conducting path for electrons to flow from the sample surface to ground
- Drifting, blurring, low contrast and false Image

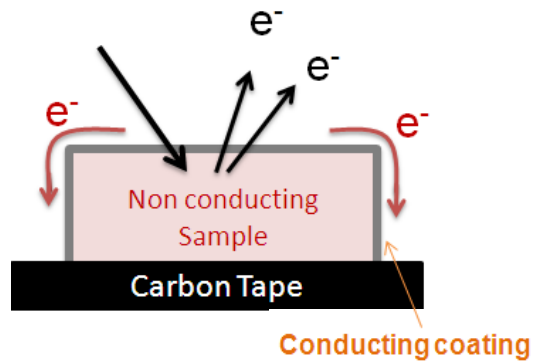








Very less e⁻ escaping from specimen



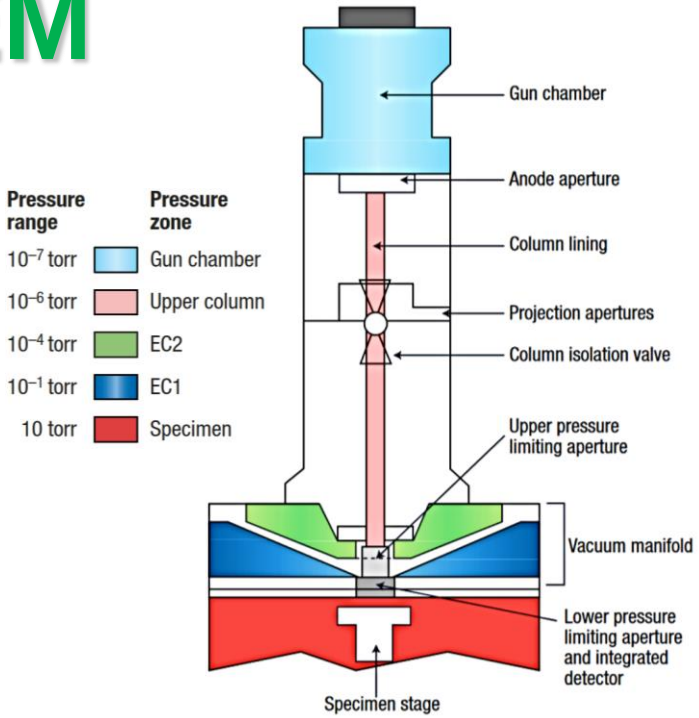
The developed charges are passed to the ground via the conducting layer

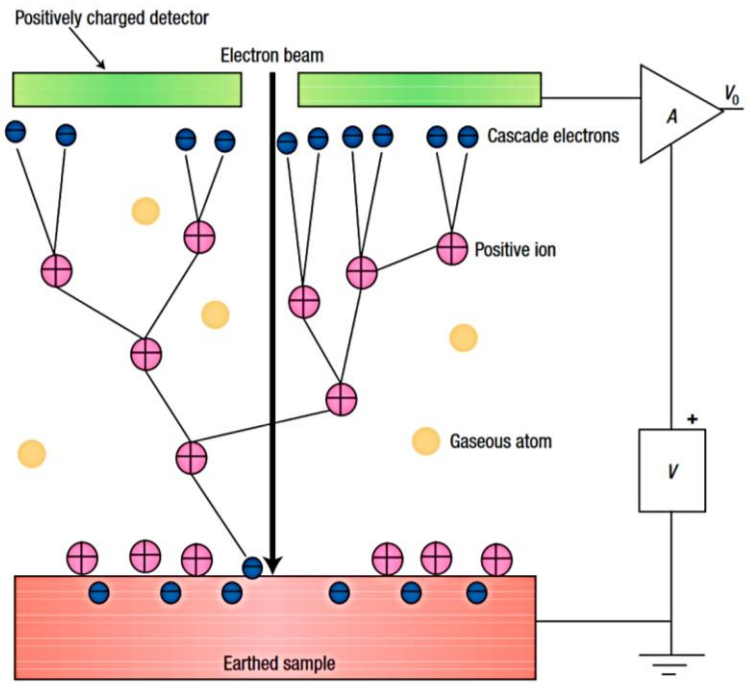
http://vlab.amrita.edu/?sub=3&brch=263&sim=1596&cnt=3340

Environmental SEM

Three operating modes:

- High vacuum (HV),
- Variable pressure (VP)
  (10 - 133 Pa, N2, air, H2O)
- Environmental mode (EP)
  (10 - 3000 Pa, N2, air, H2O)



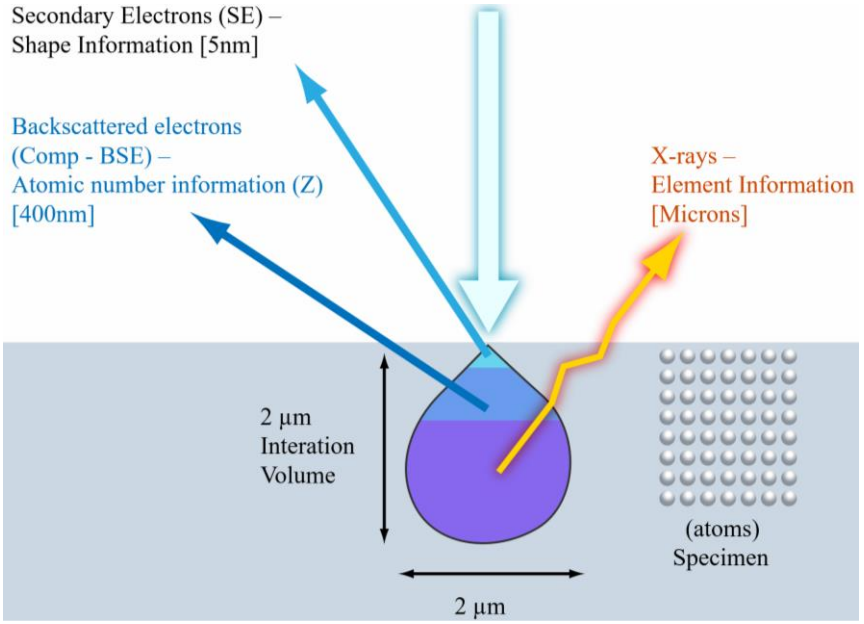


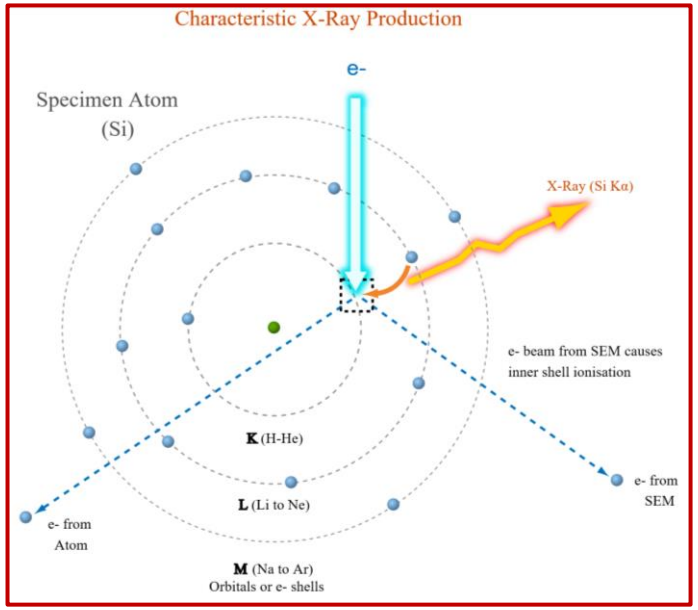
- Wet (or dry) & nonconductive (uncoated) sample can be imaged
- Gaseous environment (oxygen, nitrogen, argon, and water vapor)
- Series of different pressure zones
- The gas molecules are ionized by the electrons emitted from the sample
- **Daughter electrons** produced in the ionizing collisions
- All the electrons produced are drawn towards the **positively biased detector**
- The **positive ions** drift back and hence serve to **compensate charge** build-up at the surface of insulators

Donald, Athene M. "The use of environmental scanning electron microscopy for imaging wet and insulating materials." Nature materials 2.8 (2003): 511-516.

Energy Dispersive Spectroscopy

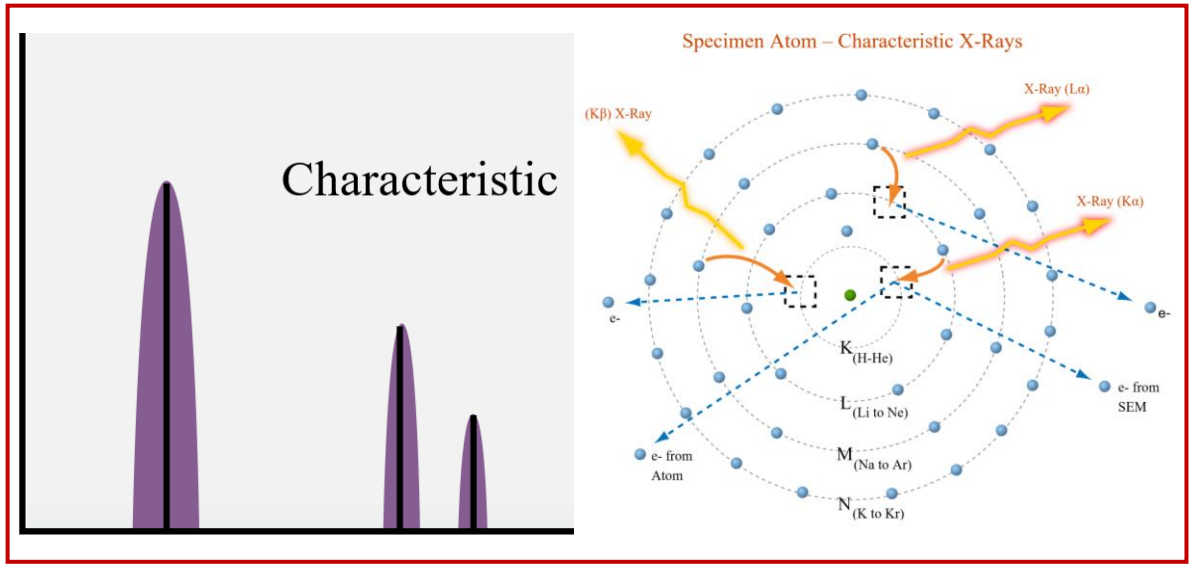
- EDS, (EDX) or (XEDS)
- A qualitative and quantitative X-ray microanalytical technique → chemical composition: Z >3 (Z >2 is now achievable under some circumstances)
- Primary electrons interact with the atoms. Bremsstrahlung X-rays (braking radiation, Continuum or background X-rays) + Characteristic X-rays are produced
- The production of Characteristic X-rays is a two-stage process
  - Ionisation: an electron is removed from one of the inner shells of the atom by an electron from the primary (ionized unstable atom)
  - Relaxation: the atom regains stability when an electron from an outer shell fills the inner shell vacancy and an X-ray photon is emitted.
  - The energy of the emitted X-ray is equal to the difference between the ionisation energies of the electrons involved in the transition.
- The X-rays are detected by an Energy Dispersive detector
- The typical spatial resolution for X-ray microanalysis is a few microns
- The detection limit in the range 0.1-0.5 wt%

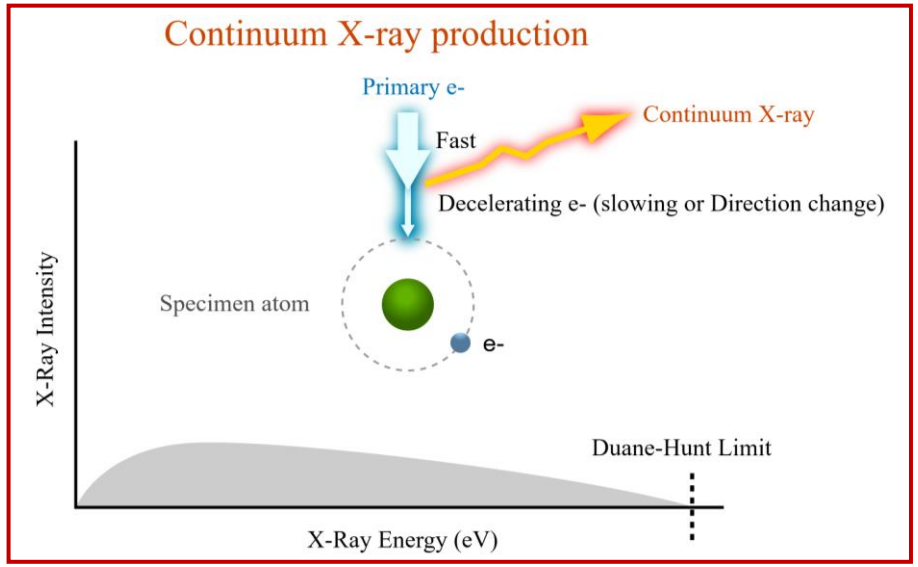




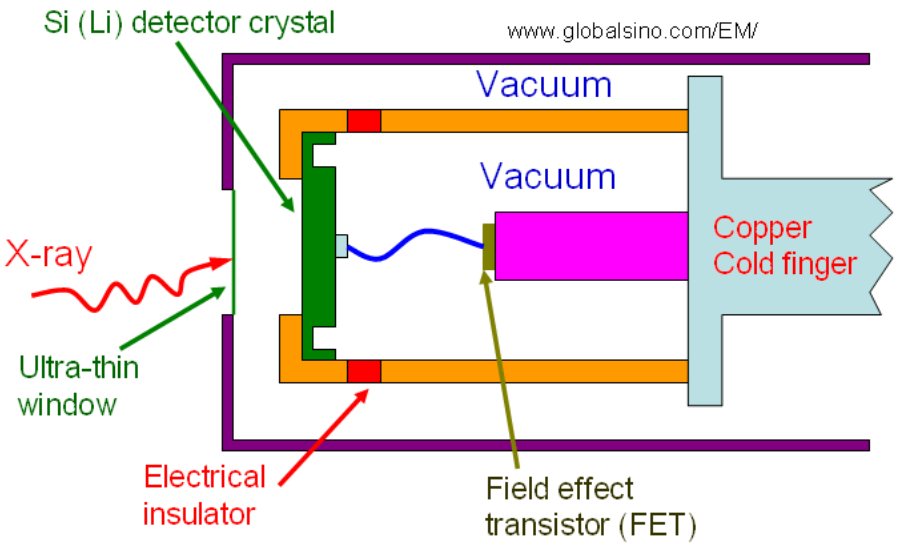
https://myscope.training/

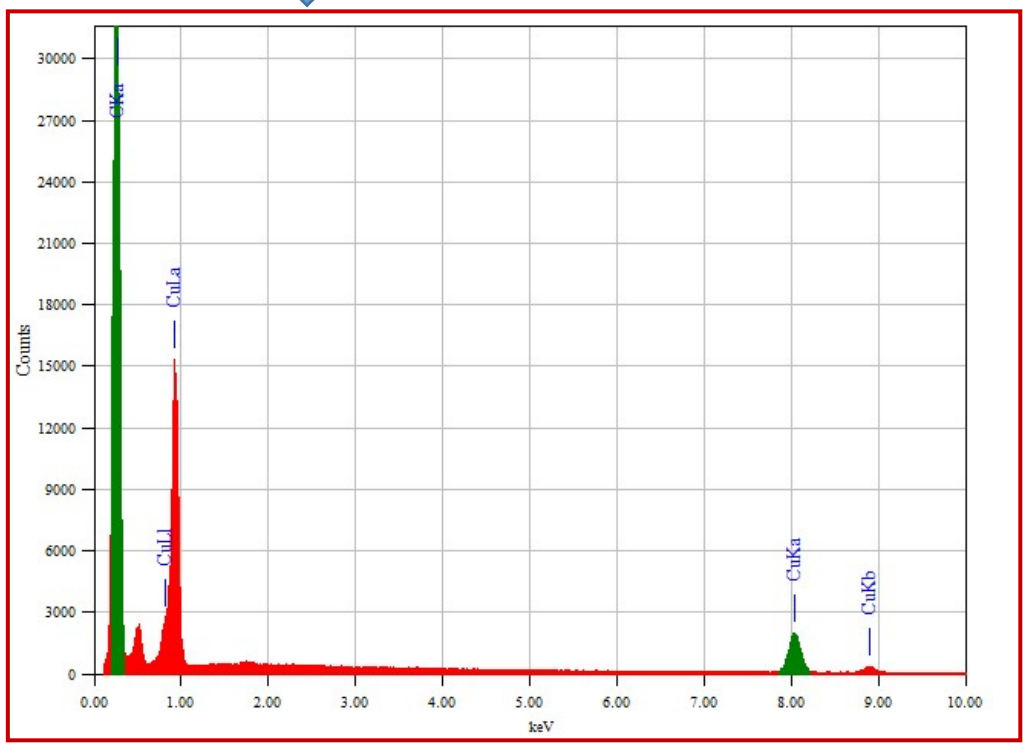
For Si, the ionisation energy of the K shell is 1.84 keV, the ionisation energy of the L shell is ~0.10 keV and the ionisation energy of the M shell is ~0.01 keV.





EDS detector with a solid state crystal





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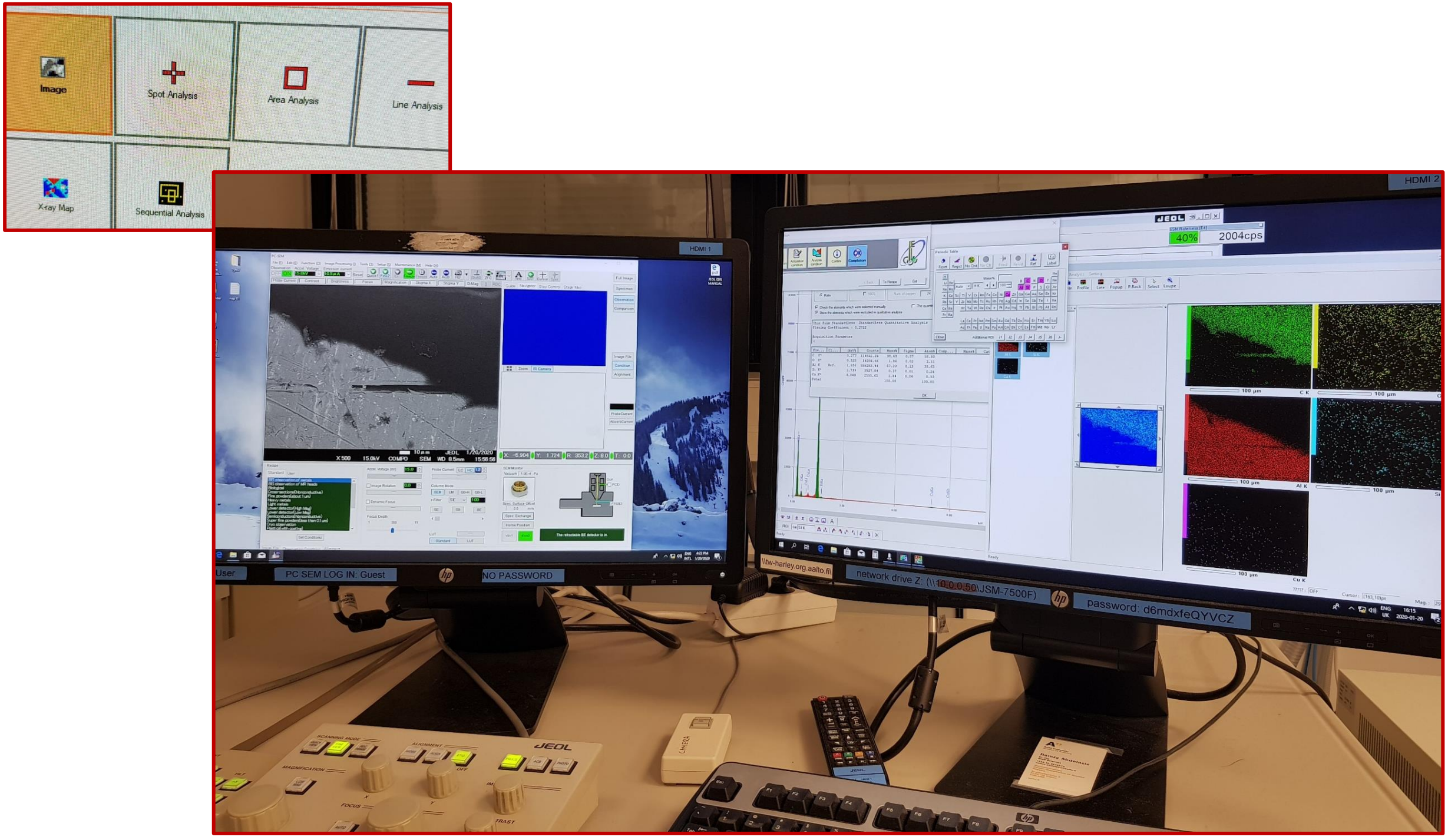
Image
Spot Analysis
Area Analysis
Line Analysis
X-ray Map
Sequential Analysis
HDMI 1
HDMI 2
PC SEM LOG IN: Guest
NO PASSWORD
User
JEOL
40%
2004cps
network drive Z: (\\10.0.0.50\JSM-7500F)
password: d6mdxfeQYVCZ

nature COMMUNICATIONS

How to describe the SEM image?

ARTICLE

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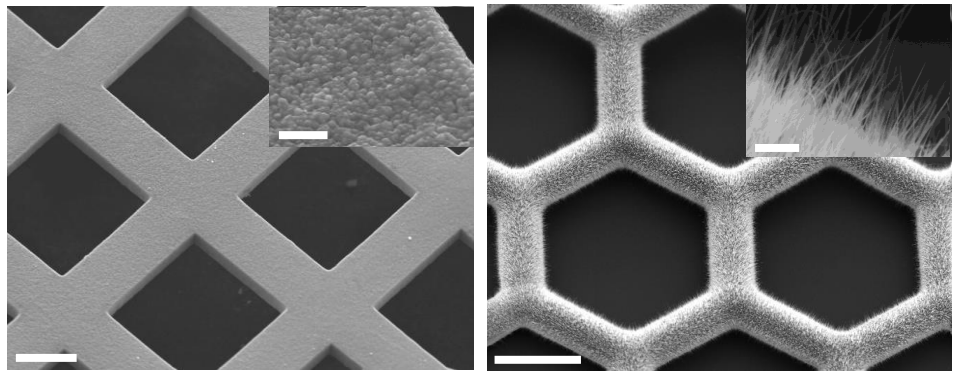
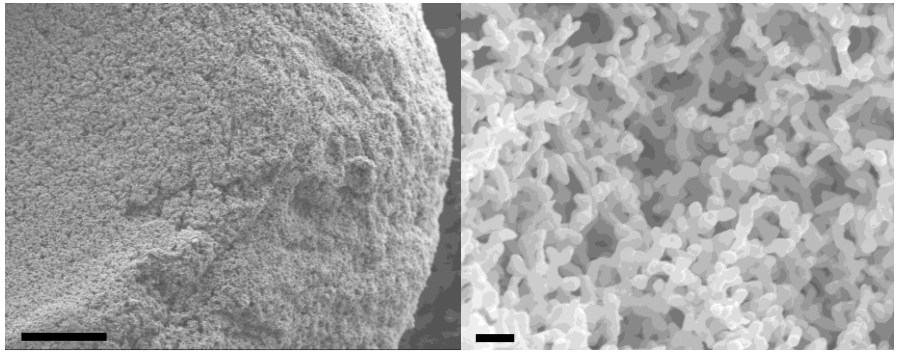
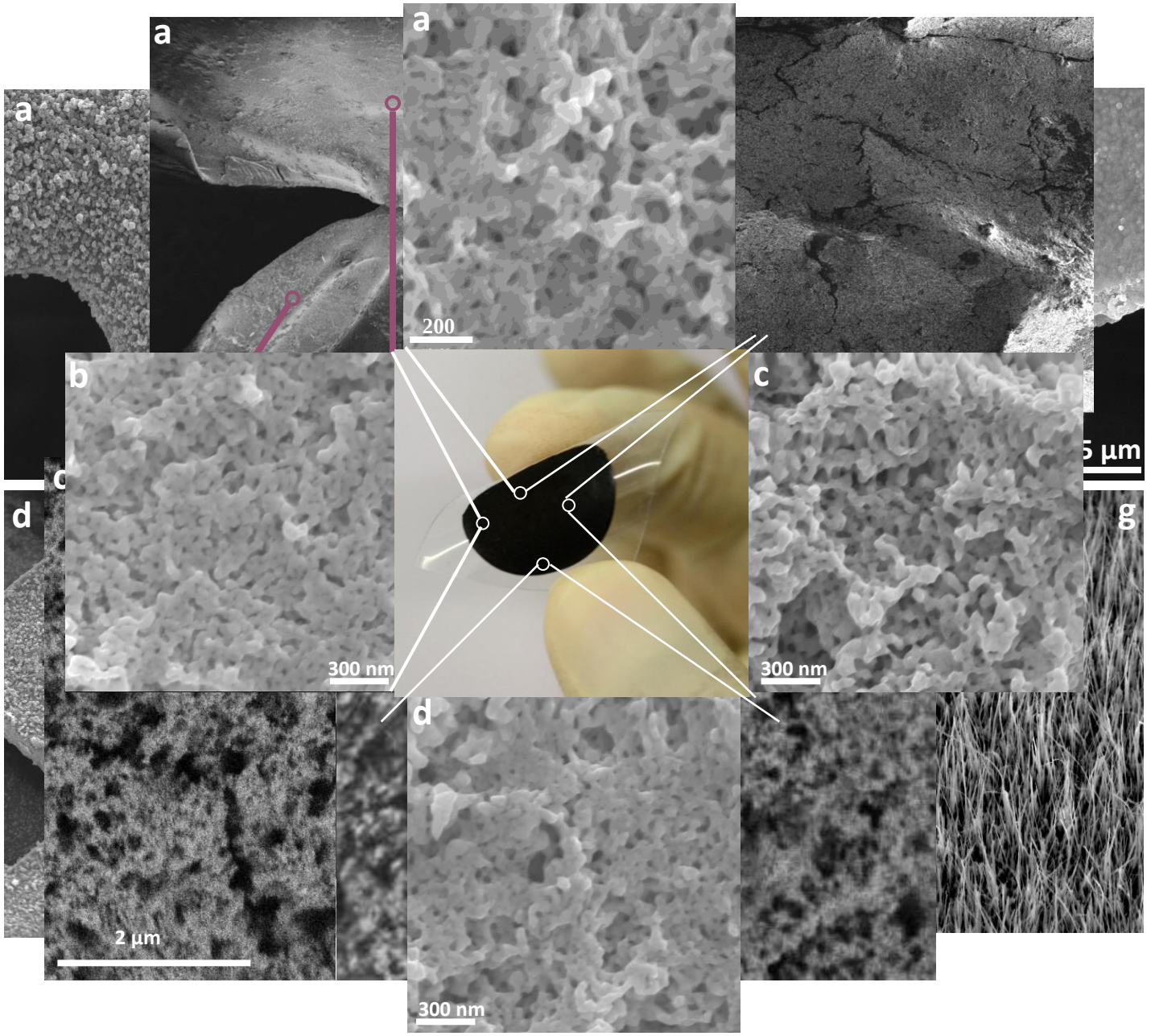
Green chemistry and nanofabrication in a levitated Leidenfrost drop

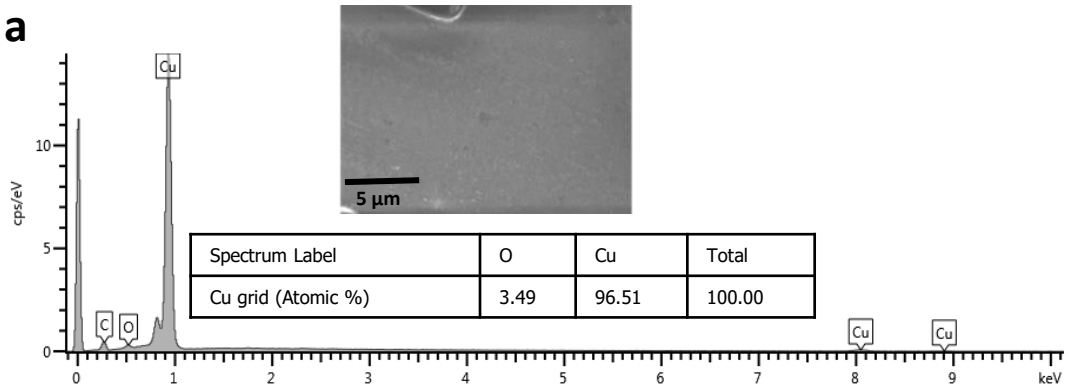
Ramzy Abdelaziz[1], Duygu Disci-Zayed[1], Mehdi Keshavarz Hedayati[1], Jan-Hendrik Pöhls[1], Ahnaf Usman Zillohu[2], Burak Erkartal[3], Venkata Sai Kiran Chakravadhanula[3,†], Viola Duppel[4], Lorenz Kienle[3] & Mady Elbahri[1,2]





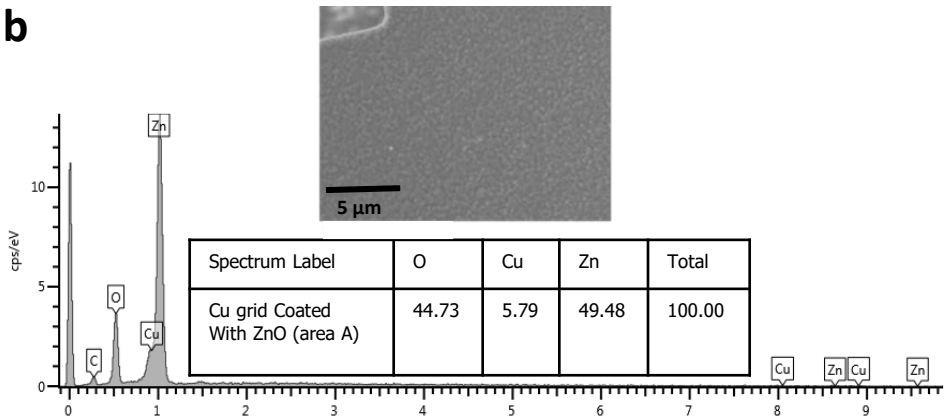


a



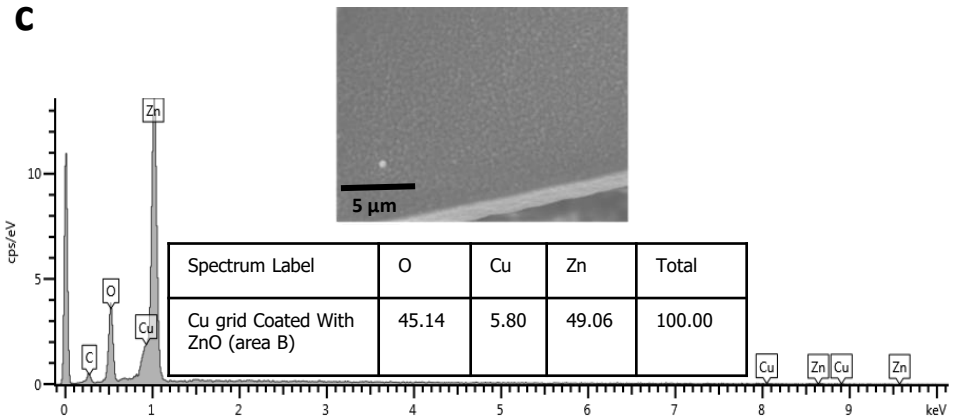
Spectrum Label	O	Cu	Total
Cu grid (Atomic %)	3.49	96.51	100.00

b



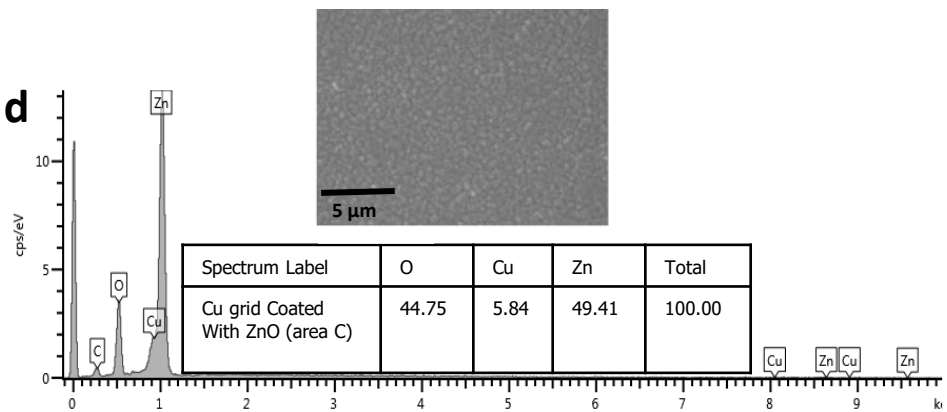
Spectrum Label	O	Cu	Zn	Total
Cu grid Coated With ZnO (area A)	44.73	5.79	49.48	100.00

c



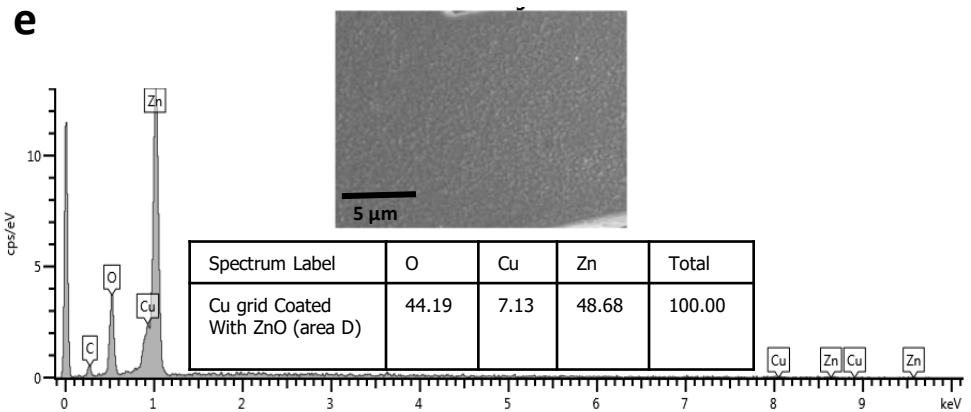
Spectrum Label	O	Cu	Zn	Total
Cu grid Coated With ZnO (area B)	45.14	5.80	49.06	100.00

d

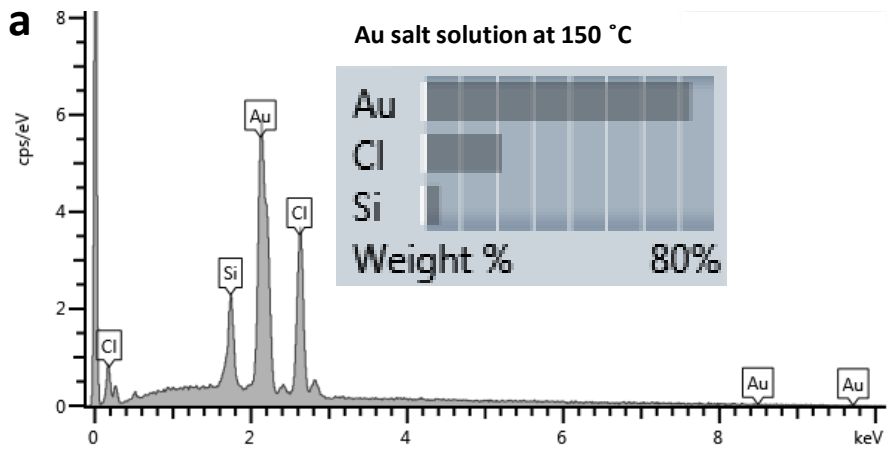


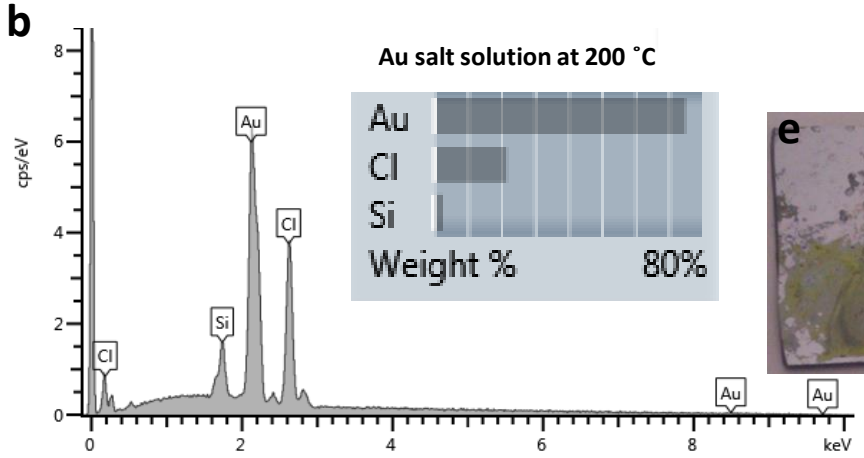
Spectrum Label	O	Cu	Zn	Total
Cu grid Coated With ZnO (area C)	44.75	5.84	49.41	100.00

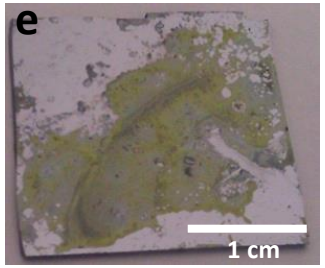
e

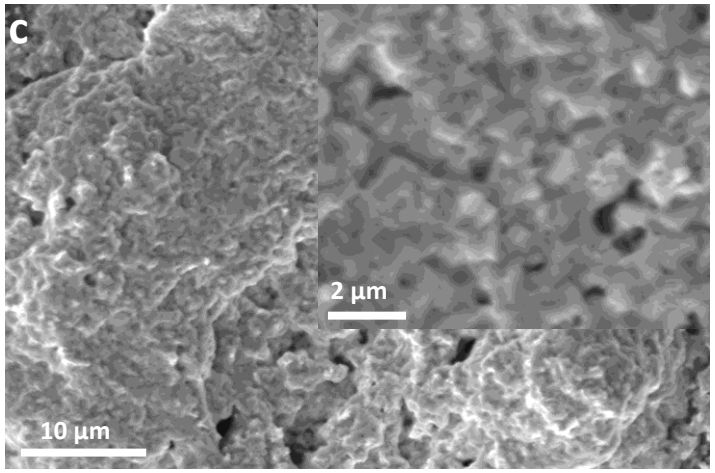


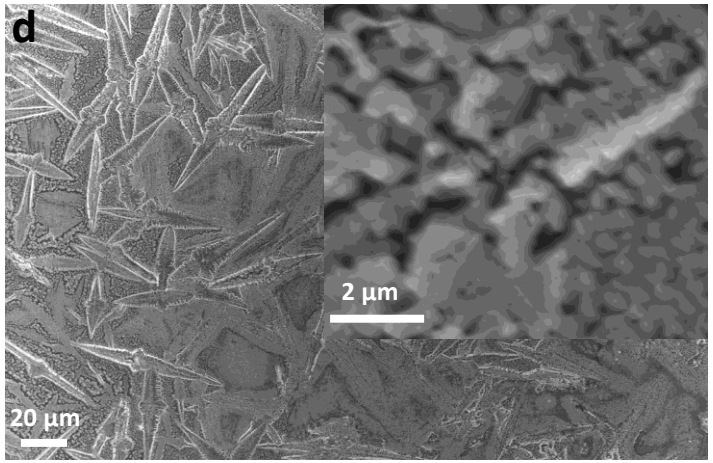
Spectrum Label	O	Cu	Zn	Total
Cu grid Coated With ZnO (area D)	44.19	7.13	48.68	100.00


a
Au salt solution at 150 ˚C
Au
Cl
Si
Weight %
80%
cps/eV
keV
Au
Cl
Si
Cl
Au
Au


b
Au salt solution at 200 ˚C
Au
Cl
Si
Weight %
80%
cps/eV
keV
Au
Cl
Si
Cl
Au
Au


e
1 cm


c
2 µm
10 µm


d
2 µm
20 µm

nature COMMUNICATIONS

ARTICLE

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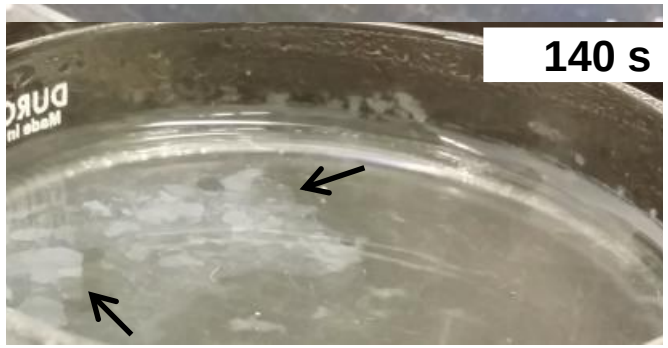
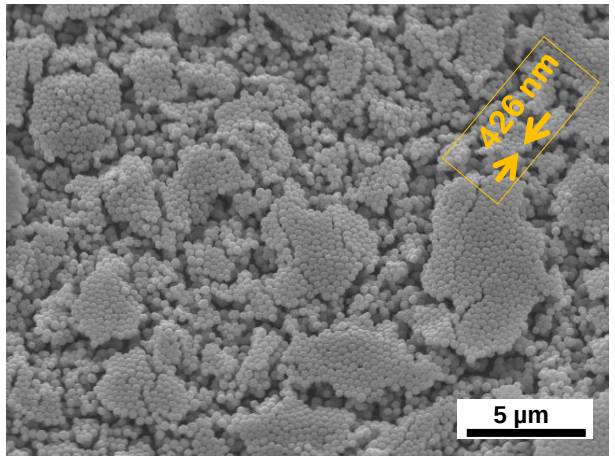
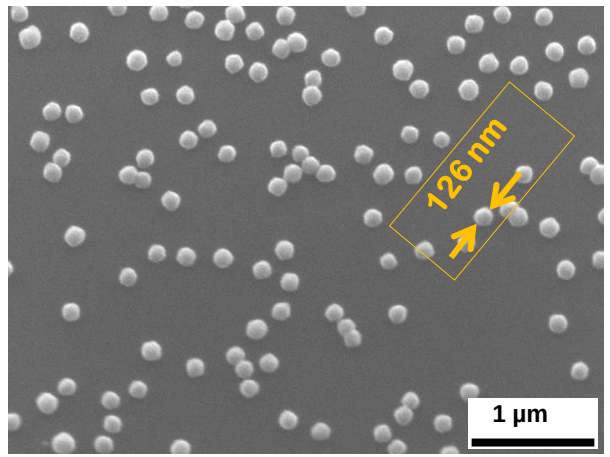
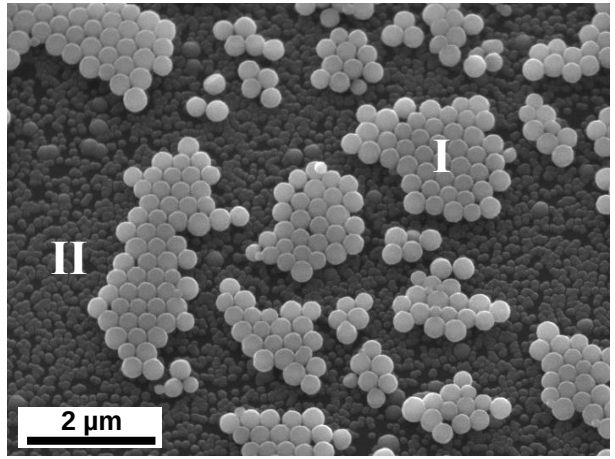
Underwater Leidenfrost nanochemistry for creation of size-tailored zinc peroxide cancer nanotherapeutics

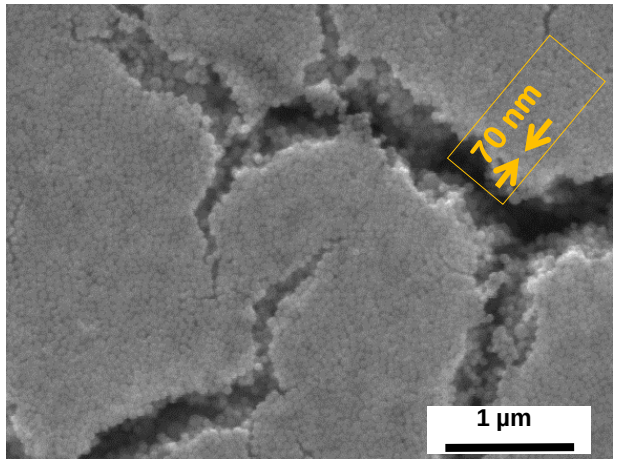
Mady Elbahri[1,2,3], Ramzy Abdelaziz[1], Duygu Disci-Zayed[2,4], Shahin Homaeigohar[1], Justyna Sosna[5,6], Dieter Adam[5], Lorenz Kienle[7], Torben Dankwort[7] & Moheb Abdelaziz[1,2]

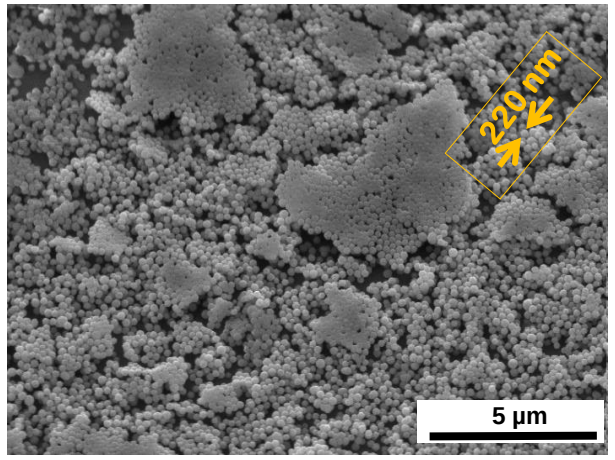


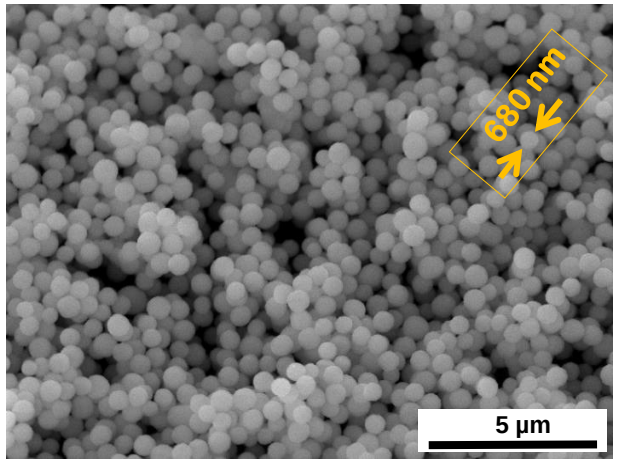










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ara research