Micro-428 Electron Microscopy

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Nanoscale Metrology Part 2

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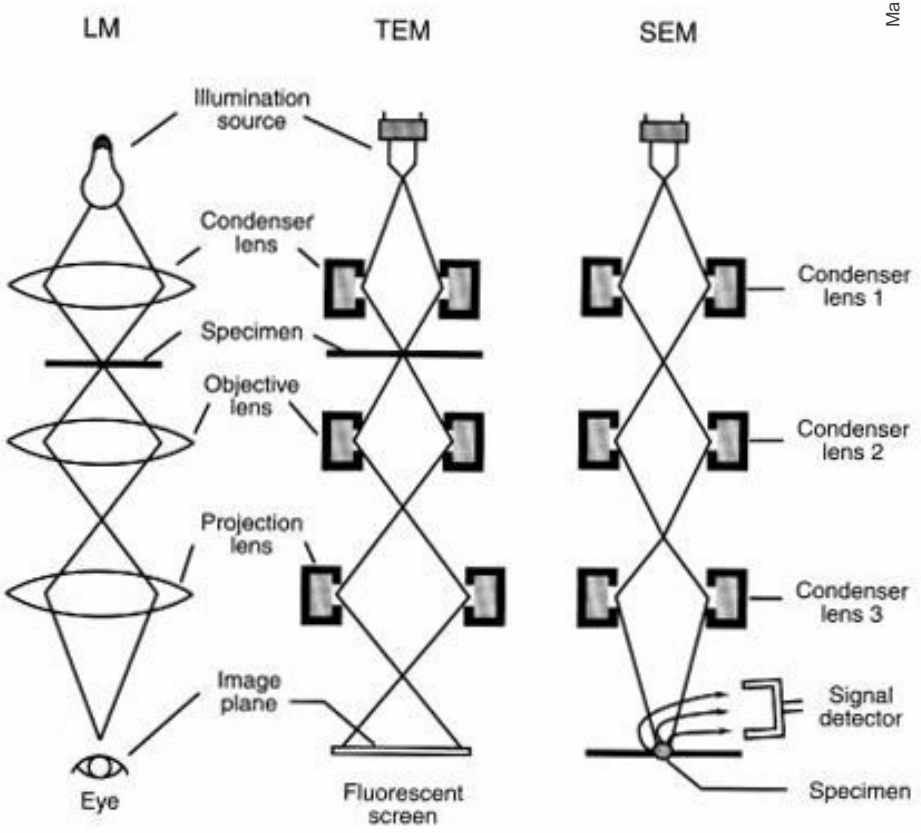
Relationship of optical and electron microscopes

- Electron microscopes use lenses just like optical microscopes.
- Lenses are electromagnetic lenses.
- Most of the optical microscopy methods have an analog in electron microscopy.



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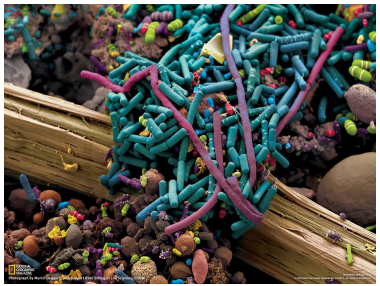
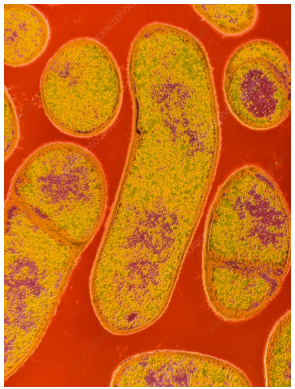
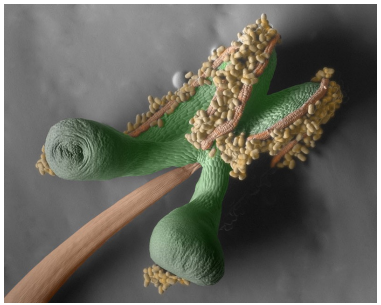
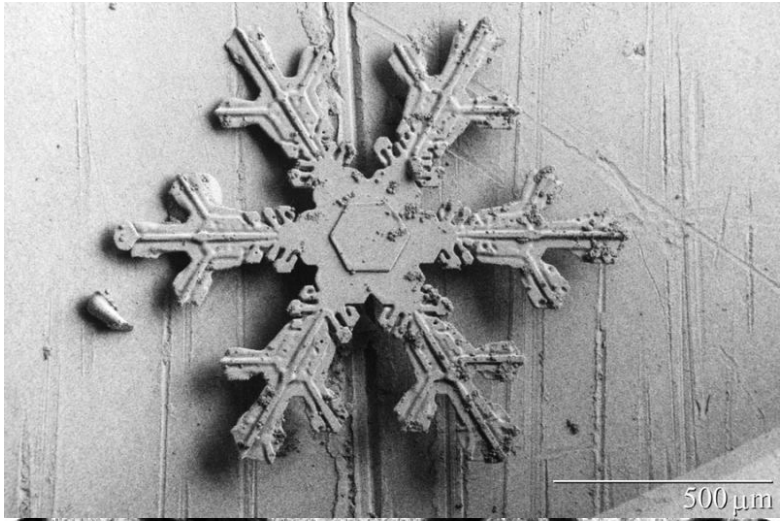
What do EM images look like?

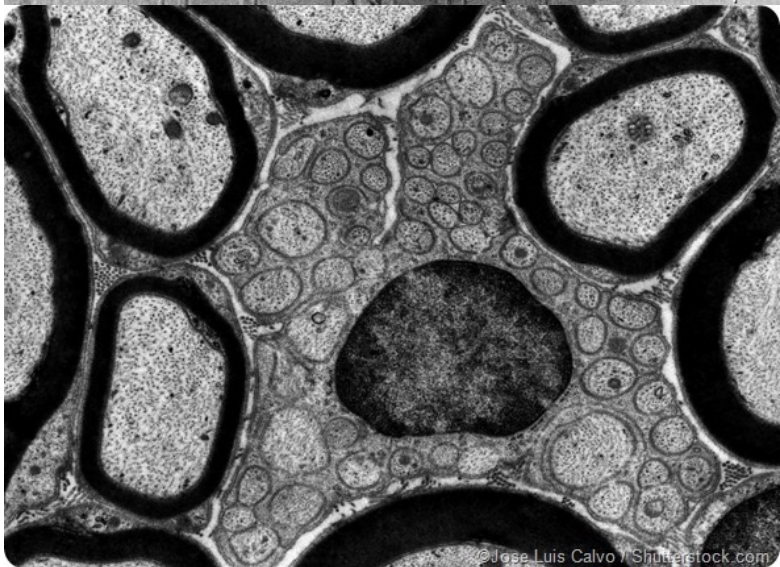
- EM images don't have color! Any color EM image is *false colored.*











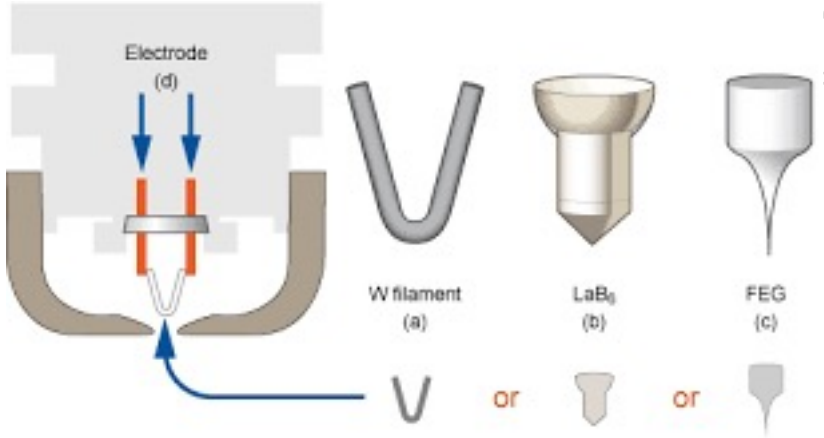
What we need for an electron microscope

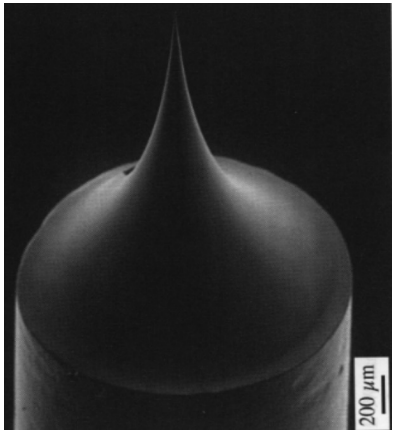
- Source of electrons (“electron lamp”).
- Lenses for electrons.
- Detectors for electrons.

Electron source

We create electrons by pulling them out of a conductor using a strong field and heat.

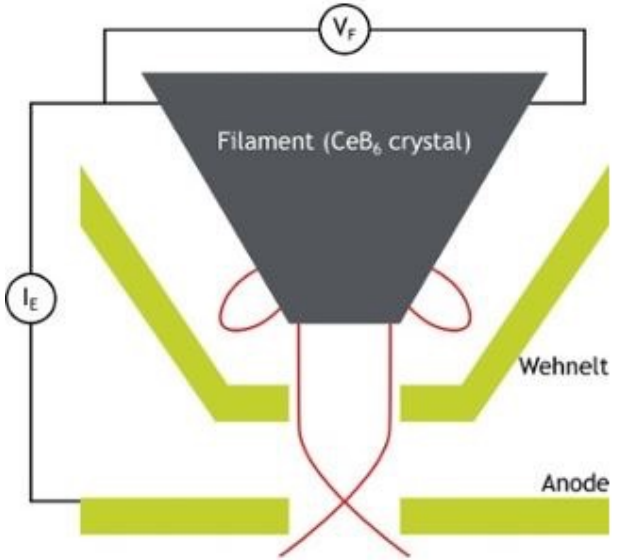
- Thermal emission sources (tungsten filaments): a tungsten filament is heated by passing a current through it. The high temperature makes electrons leave the wire easier.
- Field emission sources: the electron source material is sharpened to a point so that the field lines are there very dense, which makes the electrons leave the material into the vacuum.





Electrons Source

- The electrons then get accelerated by a voltage called the acceleration voltage (in the kV range) towards the anode.
- The Wehnelt cylinder bundles the electrons and ensures that only electrons from a small spot and with a very well-defined energy exit the source.

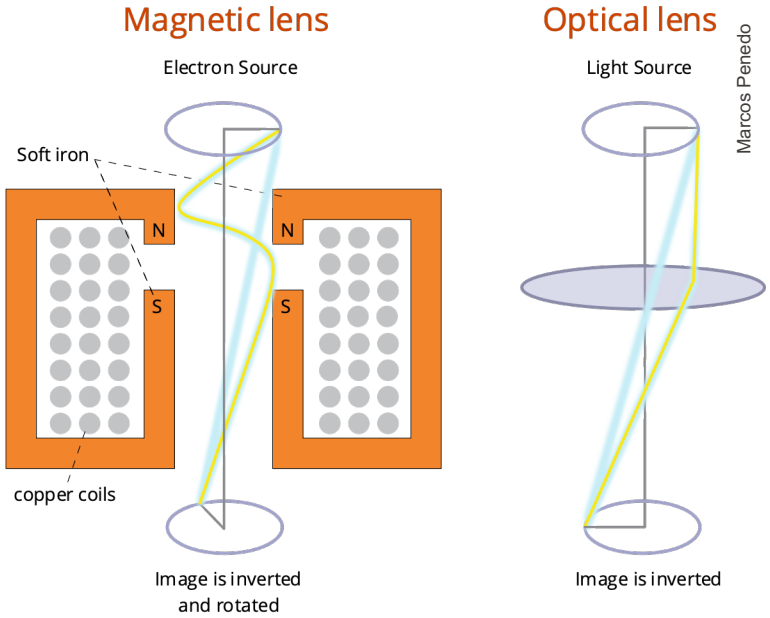


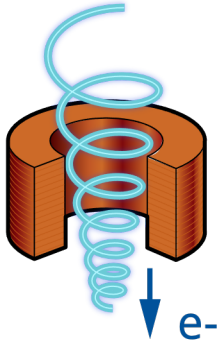
Electrostatic lenses

- When charged particles travel through a magnetic field, they experience the Lorenz force:

$$\mathbf{F} = q\mathbf{E} + q\mathbf{v} \times \mathbf{B}$$

- By sending a current through coils, a magnetic field is generated which deflects the electrons that pass through the lens.
- The focal distance of the length depends on the current through the coils.
- The image of an electromagnetic lens is inverted and rotated.





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Electrons only fly well in vacuum

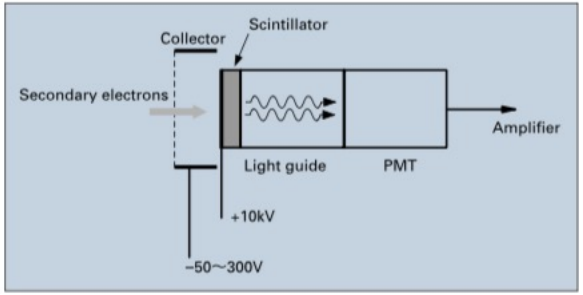
- In air or any other medium, electrons would be stopped by the medium already at very short distances.
- Inside an electron microscope we therefore need a high vacuum or even ultra high vacuum (10^{-5} to 10^{-9} mbar).
- Most electron microscopes use a combination of rotary pumps, turbo pumps, ion getter pumps, or oil diffusion pumps to achieve this vacuum.
- The pressure in the electron gun is lower than the pressure in the sample chamber.

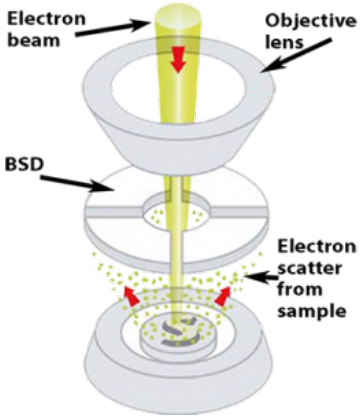
Rotary pump

Turbo pump

Electron detectors

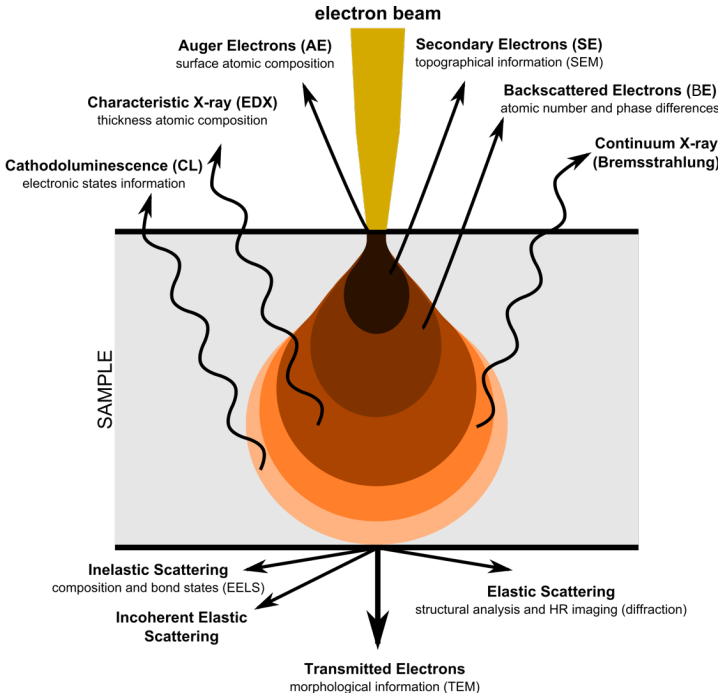
- Transmission electron microscopy (TEM)
  - Film
  - Phosphorous screen
  - CCD cameras
- Scanning electron microscopes (SEM)
  - Secondary electron detectors
  - Backscattered electron detectors





How do electrons interact with the sample

- In EM we have elastic and inelastic interactions of the electrons with the sample.
- In ***inelastic interactions***, the incident electrons (primary electrons) loose energy.
- In ***elastic interactions***, the electrons only change direction, not energy.

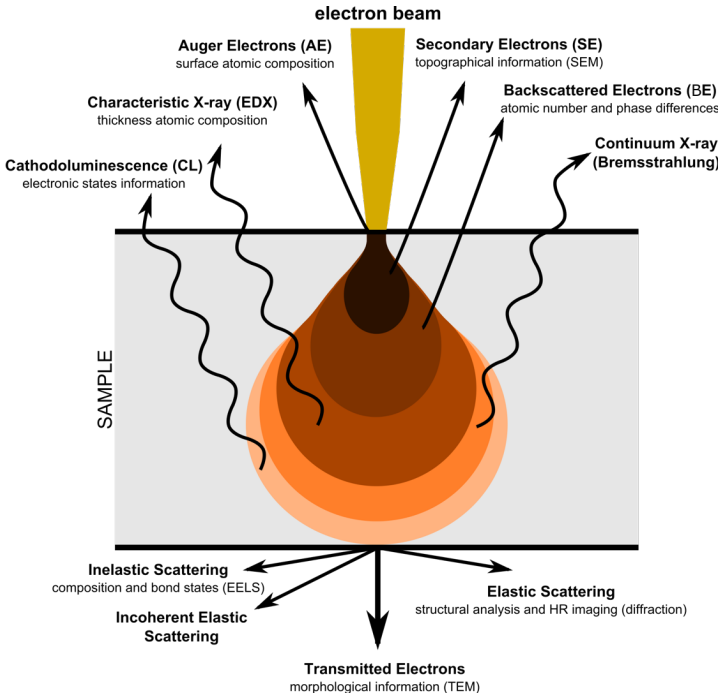


How do electrons interact with the sample

Primary electrons can:

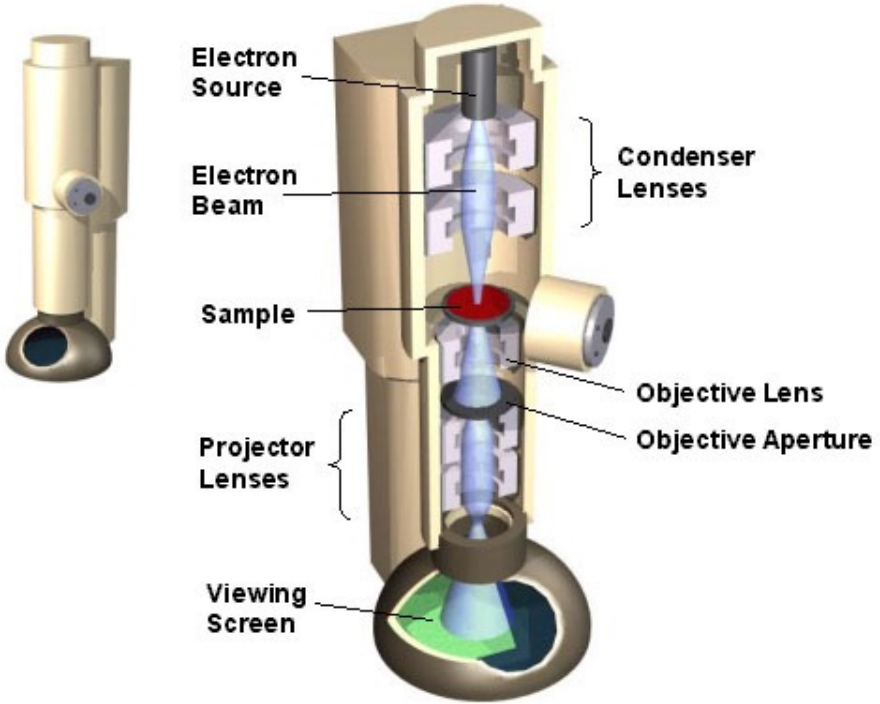
- Induce secondary electrons (SEM)
- Be backscattered
- Create Auger electrons
- Create characteristic X-rays (EDX)
- Create continuous X-rays
- Be transmitted (TEM)
- Be scattered (electron diffraction)

In the different electron microscopy modes, we use different interactions of the electrons with the sample to create an image.

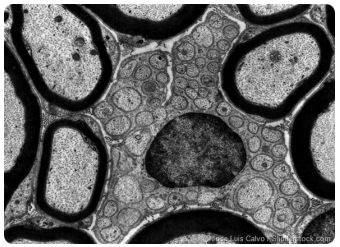


Transmission electron microscopy TEM

- TEM uses the primary electrons that are transmitted through the sample to form an image.
- Samples must be thin!!!
- Depending on the density and atomic number of the material in the sample, electrons will be more or less transmitted through the sample.
- The transmitted electrons are refocused to form a magnified image on a phosphorous detector



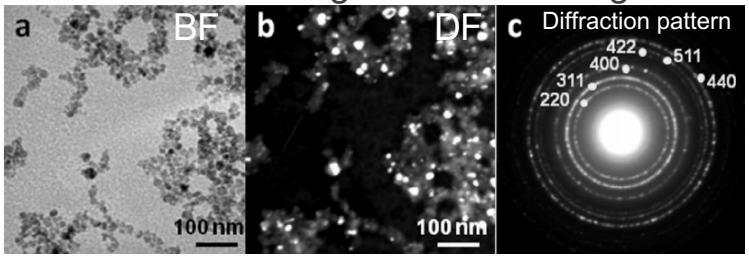
Transmission electron microscopy



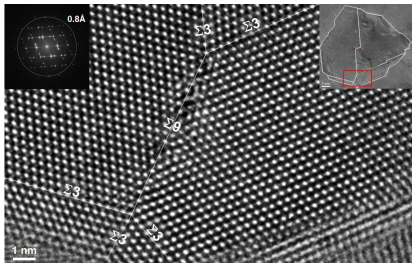
Bright field TEM image

There exist a large number of TEM techniques, some are very similar to their optical counterparts

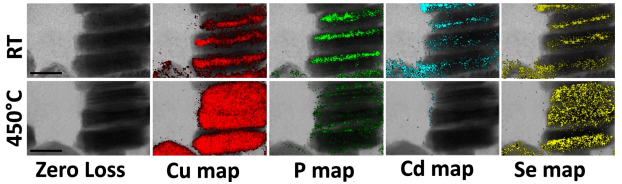
- Bright-field
- Dark-field
- High-resolution TEM (HR-TEM)/phase contrast
- Energy filtered TEM (EFTEM)
- Electron Diffraction



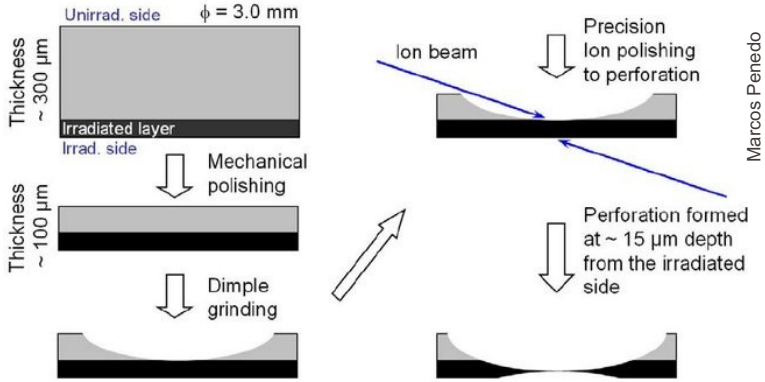
HR-TEM



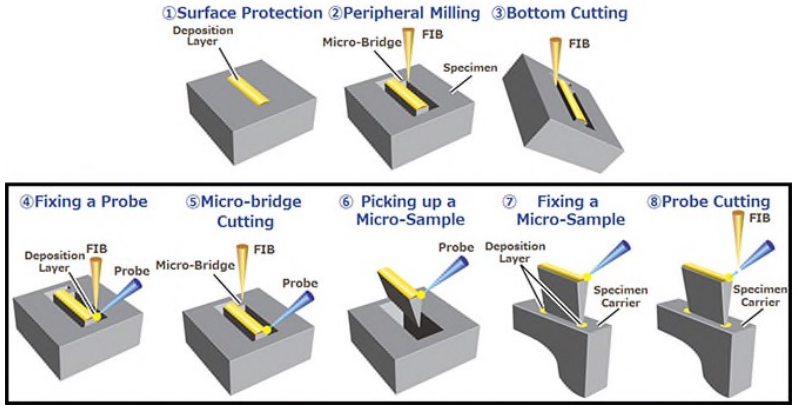
EFTEM

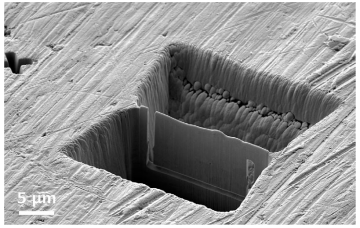


Sample preparation for TEM



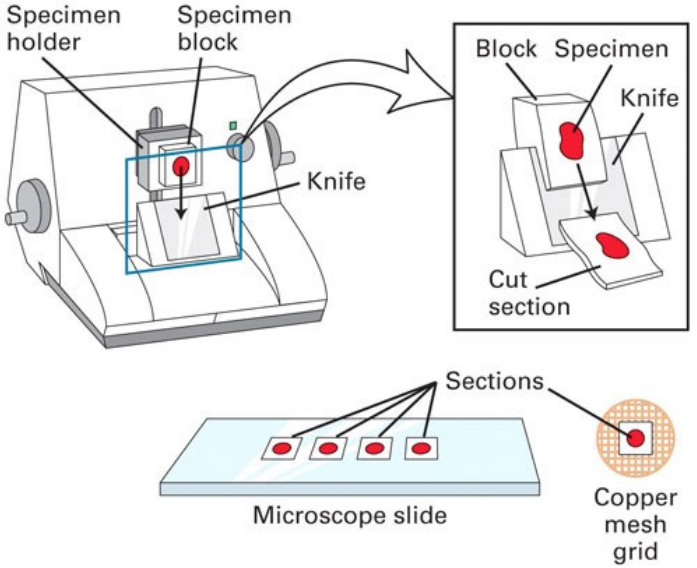
- Because electrons have to traverse the sample, the sample needs to be thin in order to observed by TEM (100-200nm).
- Samples generally have to be thinned down:
  - Mechanical cutting, polishing and dimple grinding (down to ca 10um)
  - Chemical thinning
  - Electrolytic thinning
  - Ion thinning
  - Ultramicrotome
  - FIB milling





Ultramicrotome/Cryo microtome

- Thin sections for TEM can be cut with a ultramicrotome
- Very sharp diamond knives are used to cut thin sections from an embedded sample
- Cryomicrotomes are used for biological samples that were vitrified

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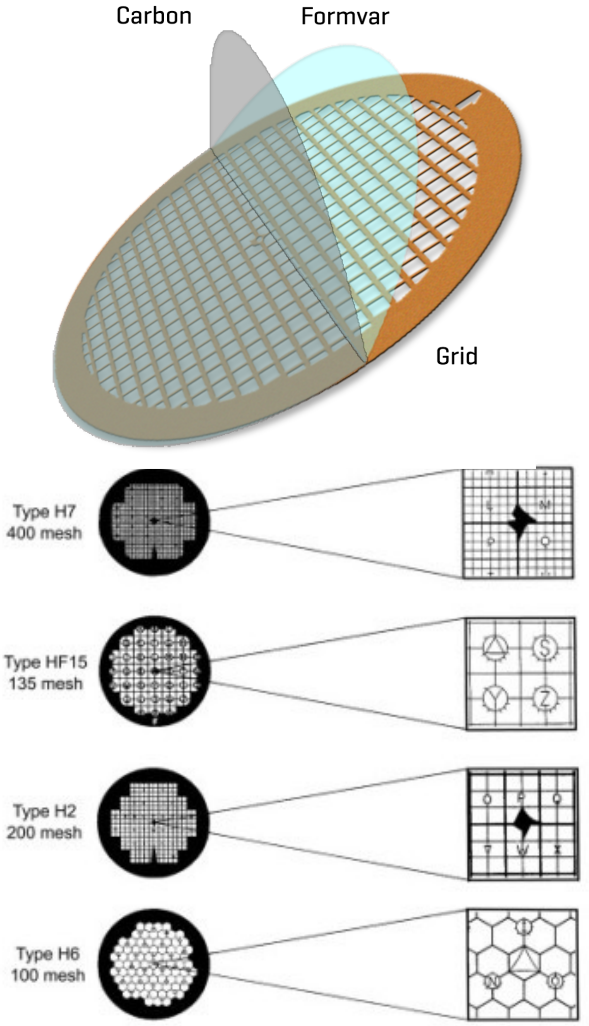
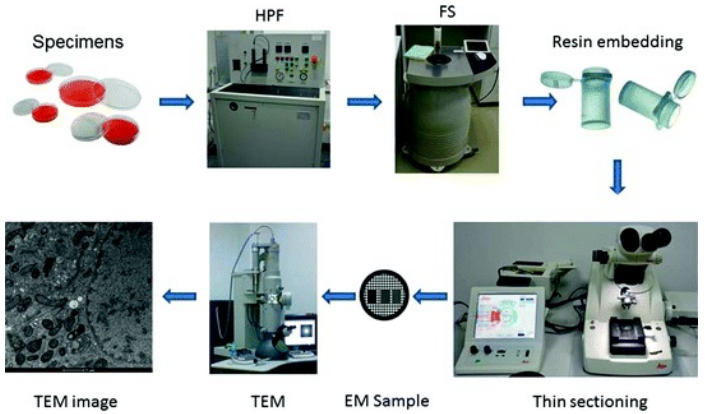
Sample support

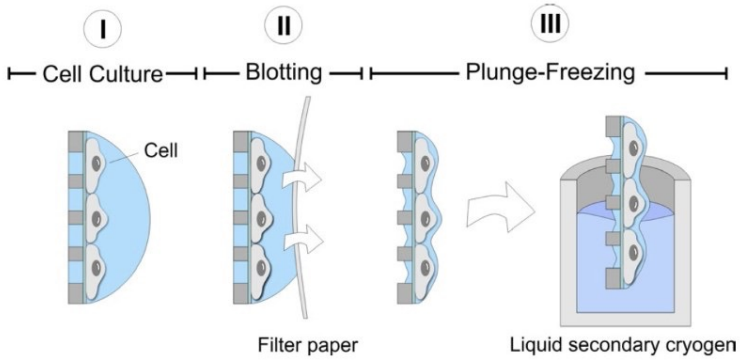
- The sample is deposited on a very thin disk: TEM grid
- The disk typically consists of a grid made of copper, nickel or gold and are coated with a thin layer of amorphous carbon or polymer (formvar)
- Often the grid has a specific shape wit fiducial markers to facilitate finding areas on the grid



Fixation and embedding



- Samples that are not solid (enough) by themselves to be thinned down or cut need to be fixed and embedded
- This is often the case for polymers or biological samples
- For biological samples vitrification (shock freezing) is used with subsequent freeze-fracture or cryo-microtoming. The advantage of shock freezing is that the water forms amorphous ice rather than crystals.



Vitrification in amorphous ice

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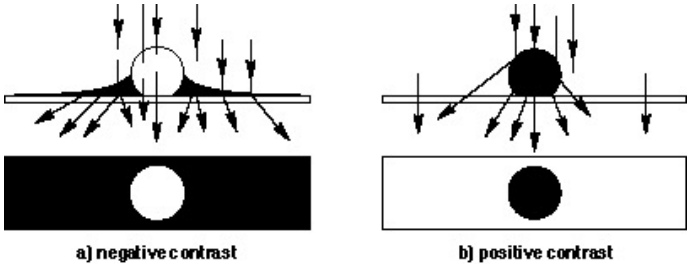
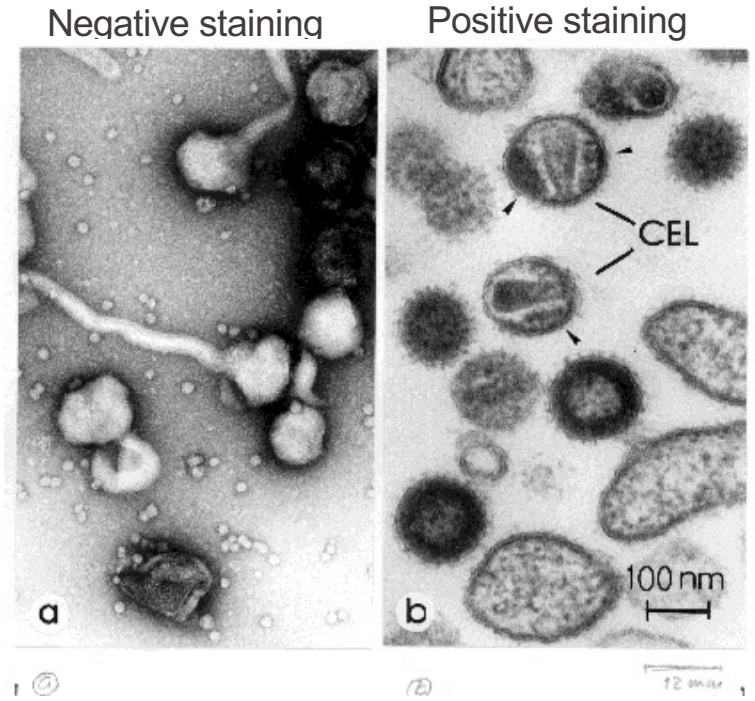
Staining for TEM



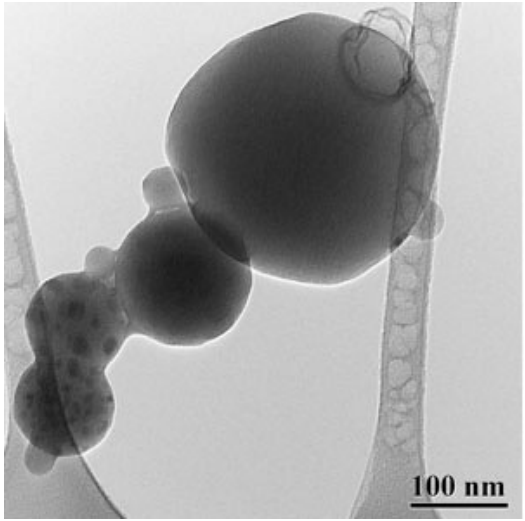
If a sample does not have sufficient electron contrast by itself, the sample needs to be stained or labeled.

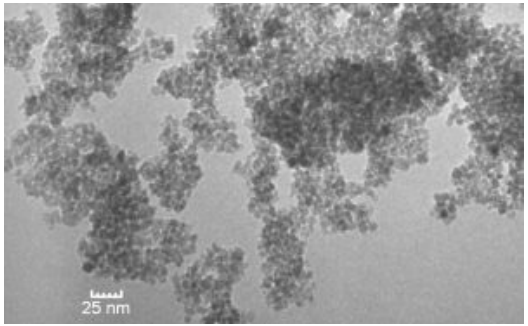
- Positive staining (the sample is infused with electron dense material/salts)
- Negative staining (the sample surrounding is filled with electron dense material)
- (Immuno-)labeling



Bright field TEM

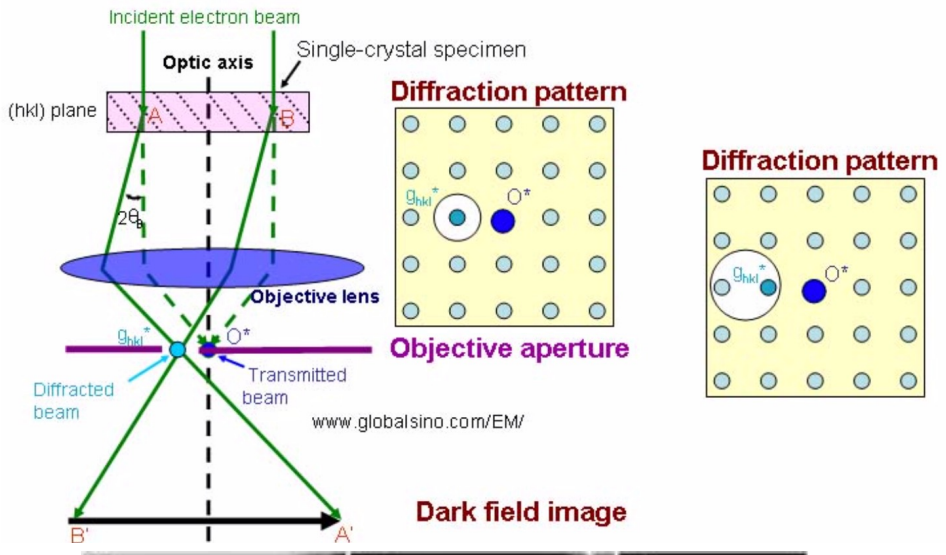
- BF-TEM is the most used form of TEM
- It makes specimens visible based on their ability to scatter electrons. Electron dense materials (higher atomic number) will be darker than materials that have a low interaction with electrons.
- The thickness of the sample also plays a role. Thicker materials will look darker.
- For many biological materials, the contrast is insufficient. Therefore, these samples are stained with heavy metal salts (Ur, Pb, Os)

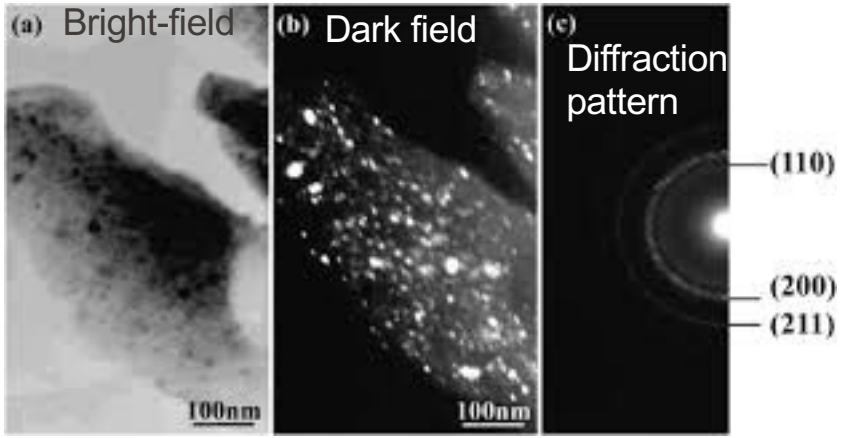




Dark field TEM

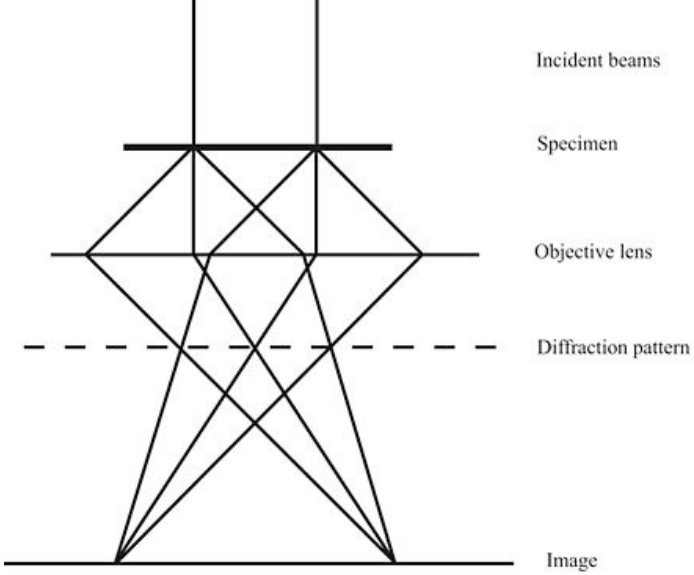
- In contrast to bright-field imaging, in dark field imaging one does not look at the un-diffracted primary electron beam but forms an image using only selected diffraction order.
- Dark field TEM shows where there are areas of crystallinity and gives contrast to their orientation

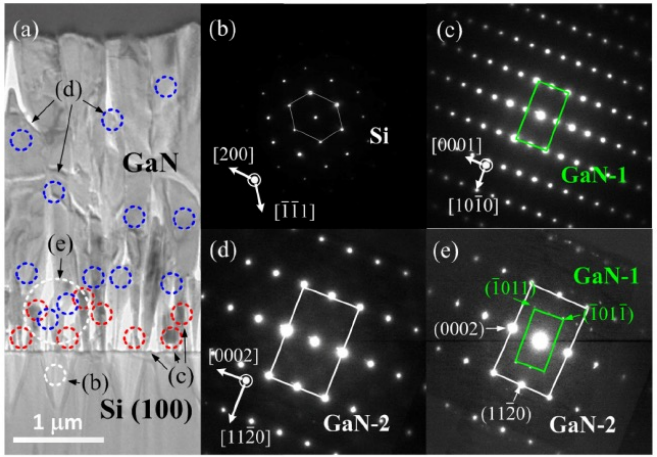




Electron Diffraction

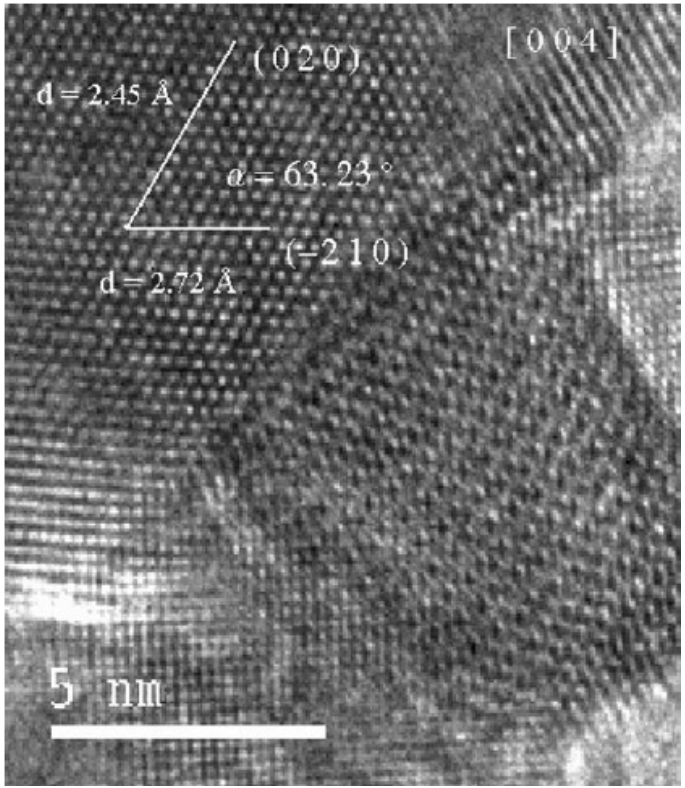
- Because electrons have a wavelength, they get diffracted by the electrostatic potential of the sample they pass
- When all these electrons are collected by the objective lens, they can be focused to create an image on the image plane.
- If the phosphorous screen is put in the back focal plane of the objective lens however, it reveals the diffraction pattern. In the back focal plane, all electrons that are diffracted into the same direction are collected in one point.





HR-TEM (=phase contrast TEM)

- HR-TEM can be used to resolve atomic lattices, defects and interfaces
- It requires very thin samples
- In HR-TEM, the phase information of multiple electron beam paths is combined to obtain a high-resolution representation of the periodic sample potential
- The diffraction pattern is the **Fourier transform** of the periodic potential for the electrons in two dimensions. In the objective lens all diffracted beams and the primary beam are brought together again; their interference provides a back-transformation and leads to an enlarged picture of the periodic potential.



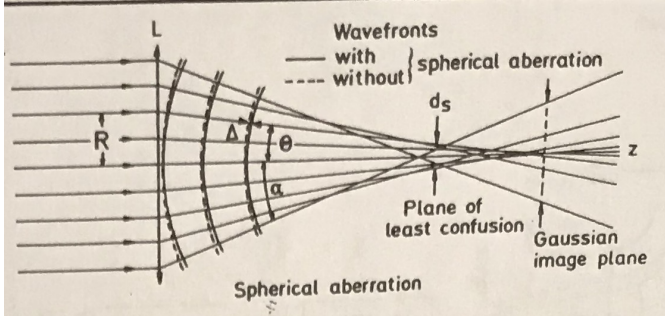
Limitations of TEM

- Requires very thin samples and extensive sample preparation
- The preparation can alter the sample structure
- Low throughput
- Small field of view can make the interpretation difficult
- 3D information is compressed into to 2D

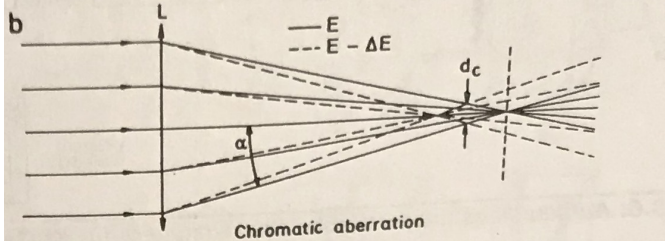
Resolution limit in TEM

Lens errors:

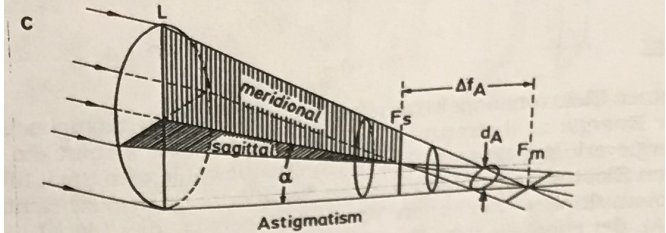
- Spherical aberrations
- Chromatic aberrations
- Astigmatism
- Diffraction error



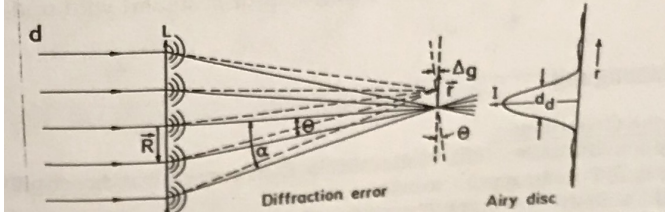
Spherical aberration



Chromatic aberration



Astigmatism

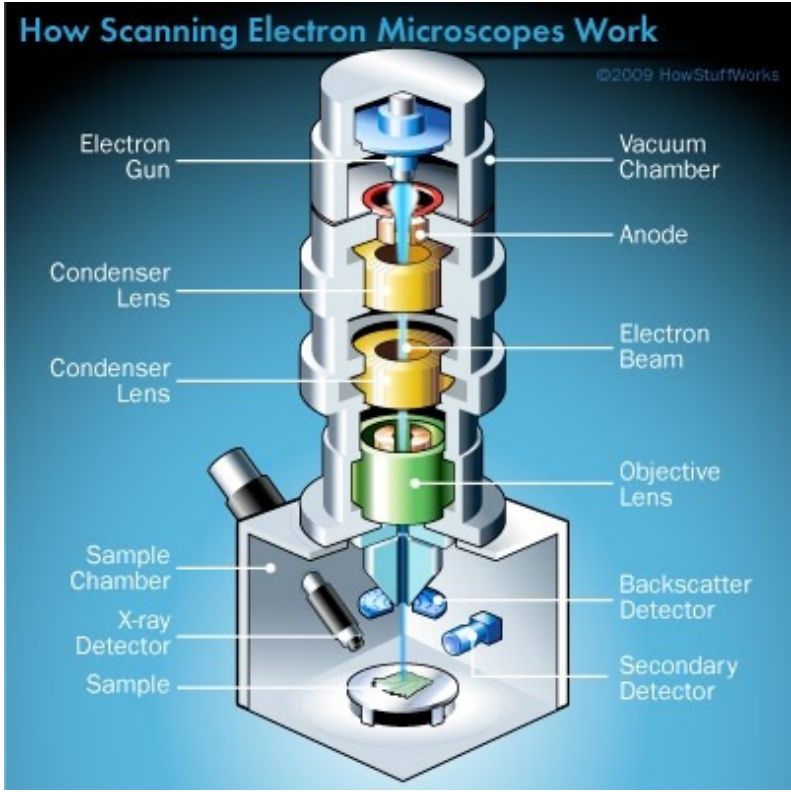


Diffraction error

Airy disc

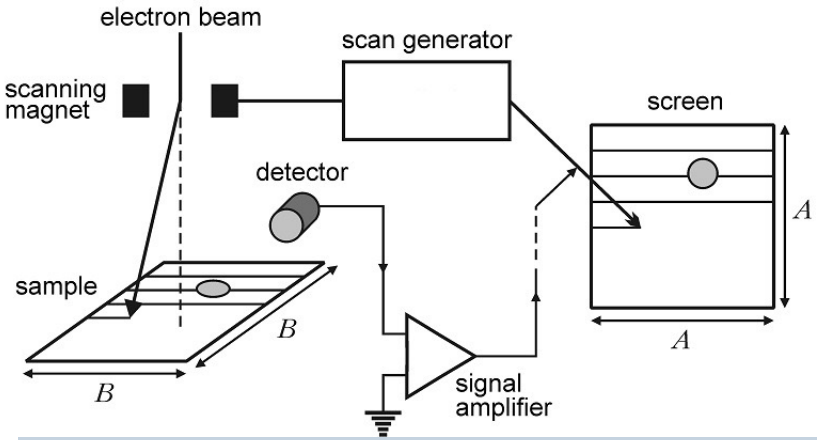
Scanning electron microscopy SEM

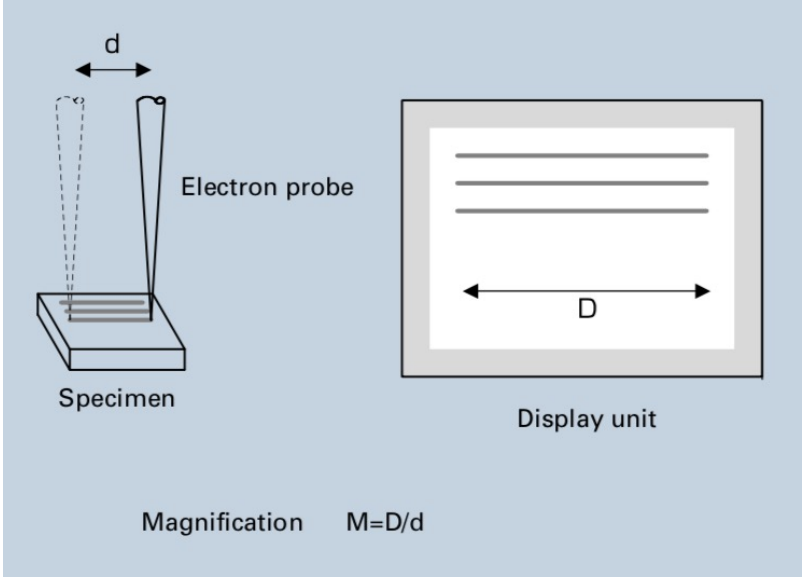
- The SEM is a scanning technique: no actual image is formed!
- Instead of projecting the whole image at a time, the focused electron beam is raster-scanned over the surface to measure the interaction of the electrons with the sample one pixel at a time.
- The image is then generated in the computer from time sequence data
- The primary electrons used for SEM are secondary electrons (SE) or back-scattered electrons (BE).
- Sample can be thick but should be conductive.



Magnification of an SEM

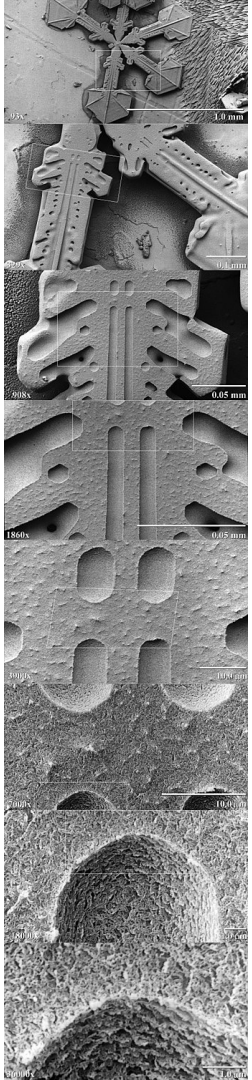
- In SEM we focus the electron beam spot on the surface
- The spot is then scanned over the surface in a raster pattern
- For each point we record the beam/sample interaction with dedicated detectors
- The magnification of an SEM is therefore given by the ratio of the scan-size to the size of the display unit





Advantages of SEM

- Large field of view combined with high magnification possibility
- Easy sample preparation
- Large depth of field



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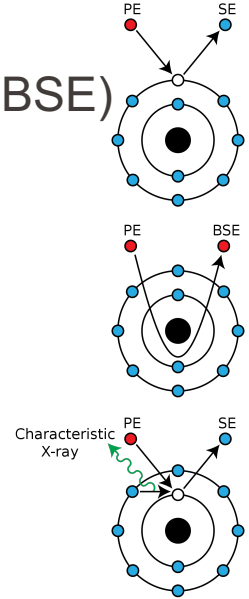
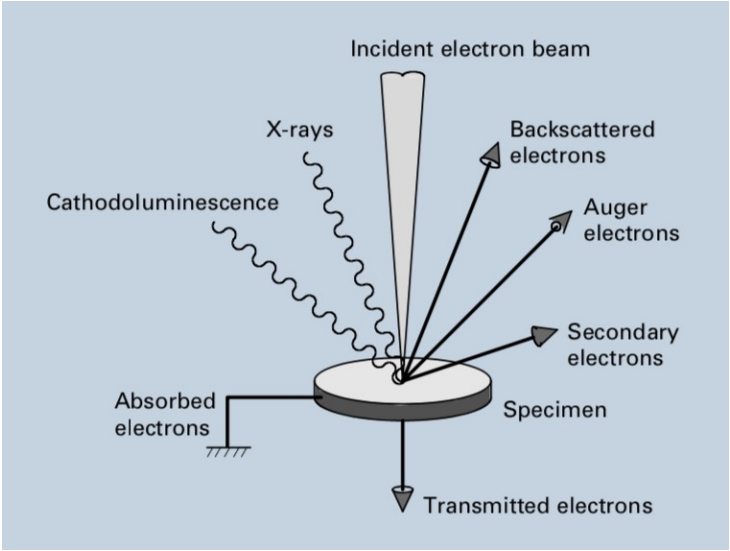
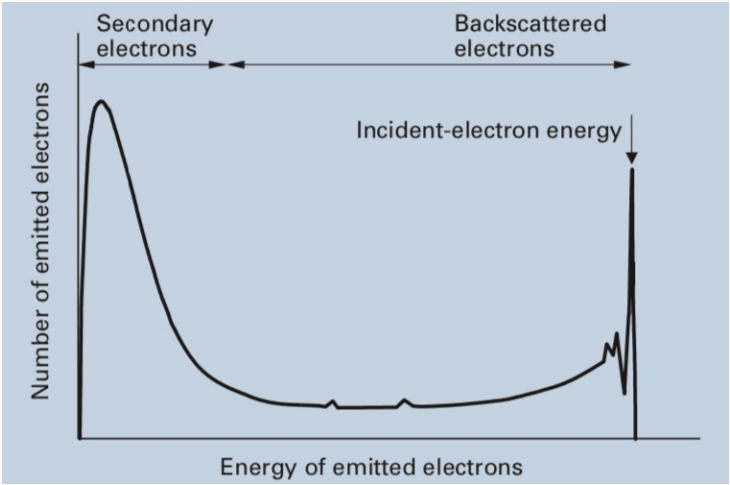
What we see in SEM

- The SEM records for each position on the sample the result of the primary beam hitting the sample. The main signals recorded are:
  - Secondary electrons (SE)
  - Back-scattered electrons (BSE)
  - Characteristic X-rays

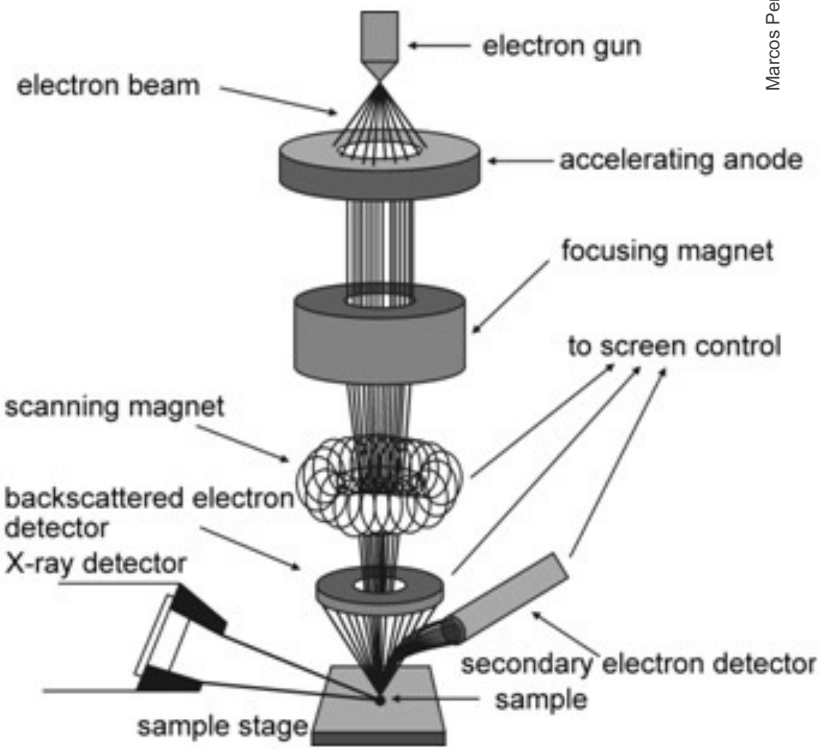




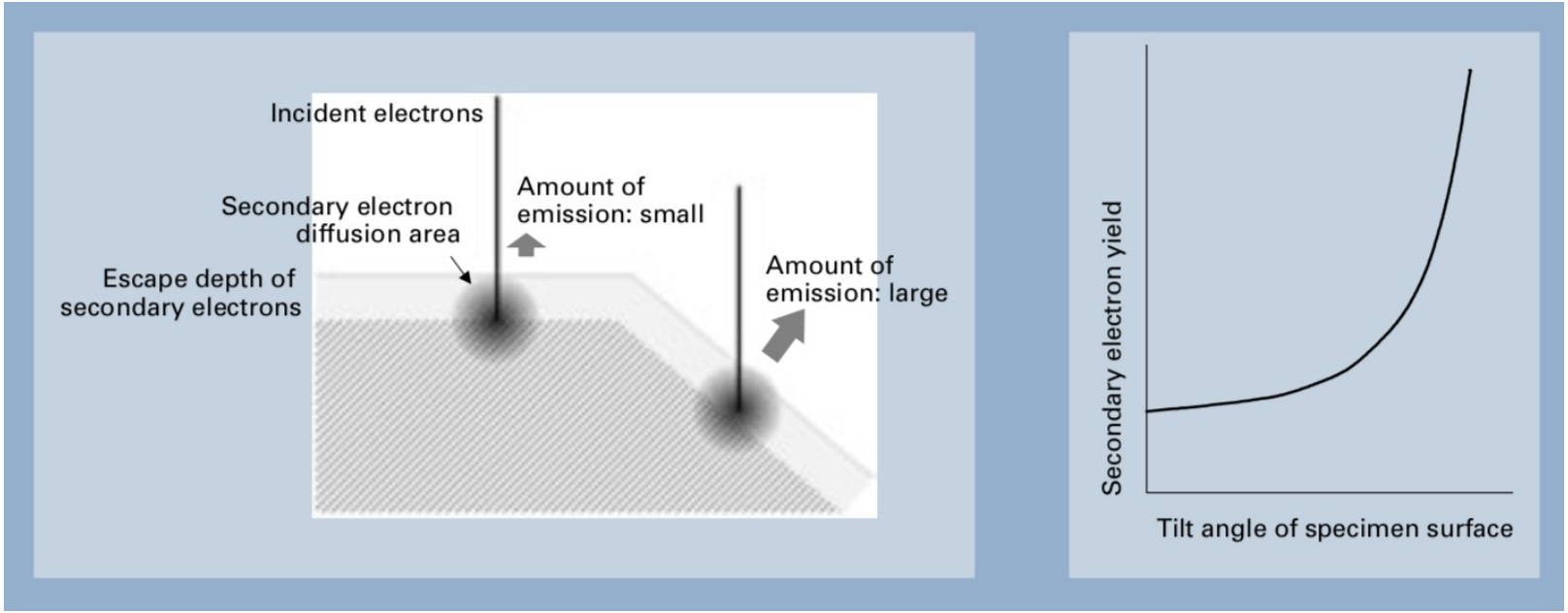


Secondary electron image

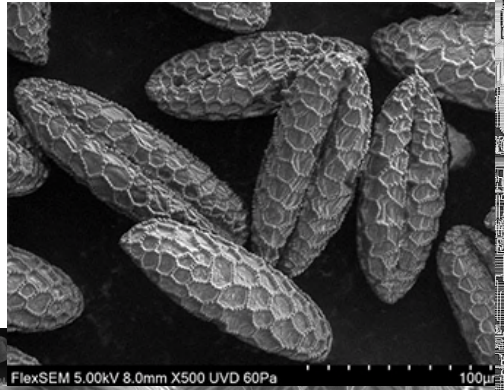
- Secondary electrons are collected via a low collection voltage into the SE detector (Everhart-Thornley detector)
- The number of SE is dependent on the angle between the surface and the position of the SE detector. The number of SE determines the brightness of the image at that position
- SE image provides information about topography
- SE are strongly affected by surface charge

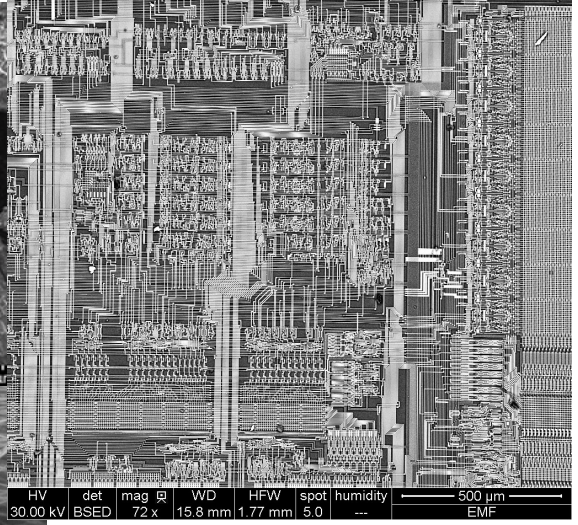


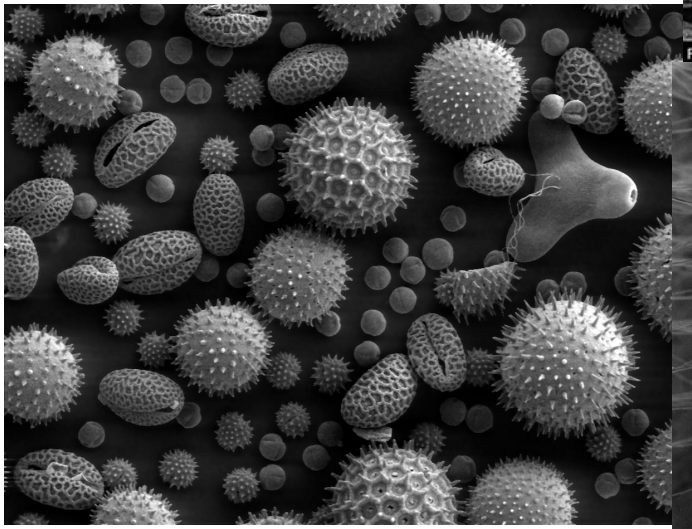
Contrast from Secondary electrons

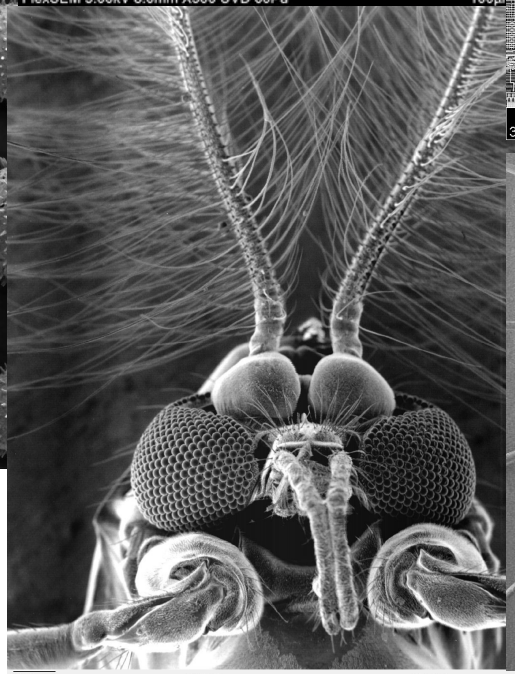


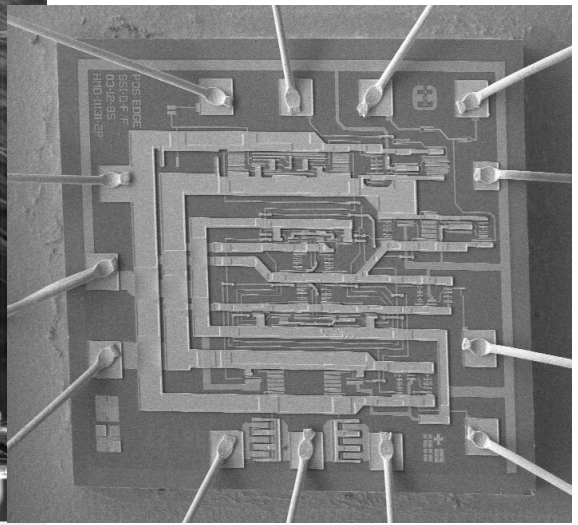
- The number of secondary electrons that leave the sample and reach the detector depends on the angle and ”line of sight” to the detector.
- This makes the SE image look as if it is a shadowed image with illumination from one side. It makes the images appear “3D”.


FlexSEM 5.00kV 8.0mm X500 UVD 60Pa


HV
30.00 kV
det
BSED
mag
72 x
WD
15.8 mm
HFW
1.77 mm
spot
5.0
humidity
---
500 µm
EMF

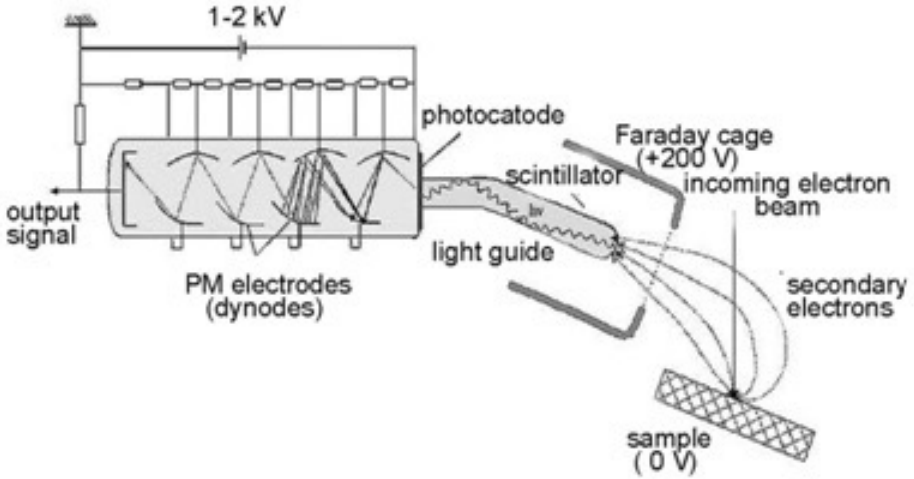






SE detector
Everhart-Thornley detector

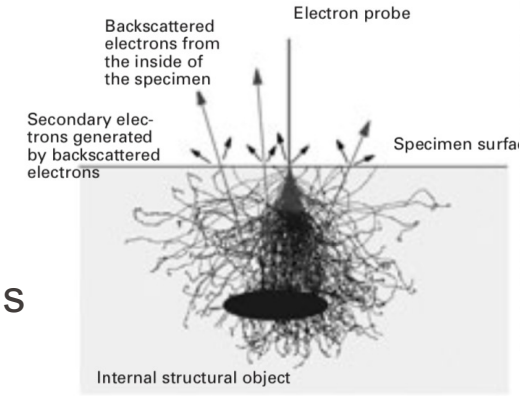
- SE get attracted to the Faraday cage by a low voltage
- Electrons hit the scintillator and generate photons
- The photons are then guided to a photocathode of an electron multiplier tube which amplifies the electrons until they can be detected as a signal current

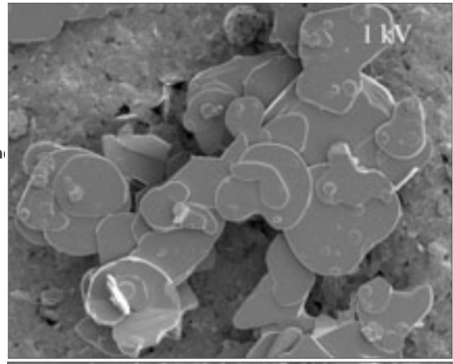


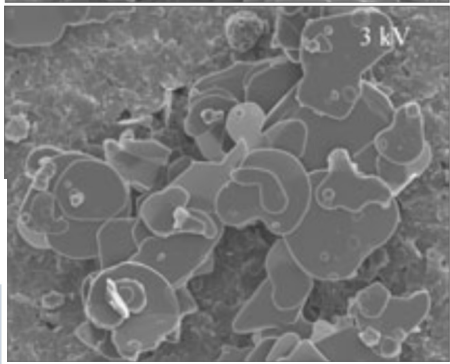
Acceleration voltage

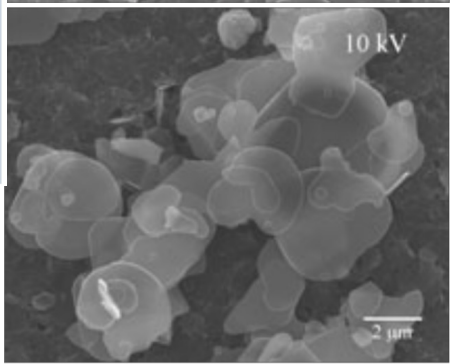
- How fast the electrons are, has an influence on the contrast and the resolution.
- The higher the energy of the electrons is, the deeper into the sample they penetrate.
- If there is structure underneath the surface, that structure will be superimposed on the surface structure, thereby degrading the resolution.
- A higher acceleration voltage decreases the magnitude of lens aberrations and therefore results in a smaller spot size.

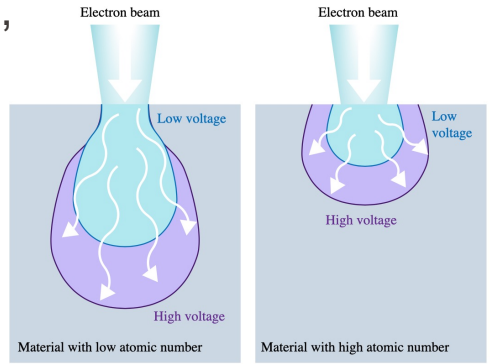
The best acceleration voltage depends on the sample being imaged!





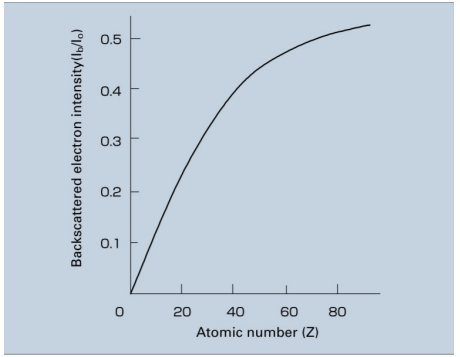


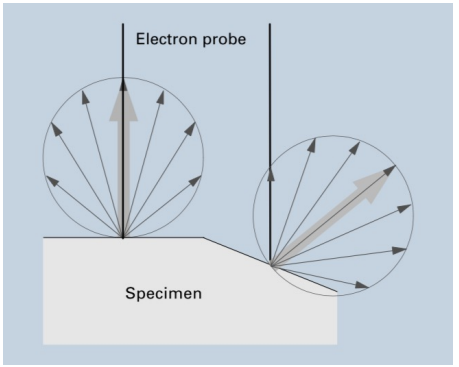


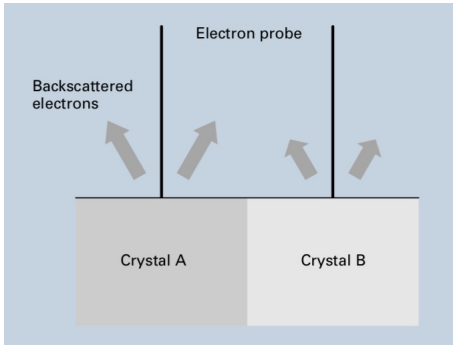


Back-scattered electrons

- The probability of primary electrons to be scattered back increases with higher atomic number (Z) of the material. This gives the BE image material contrast
- The angle of the surface also affects the direction in which the BE are reflected. The BE image therefore also has topography contrast.
- The back-scattering efficiency also depends on the crystal orientation for crystalline materials.

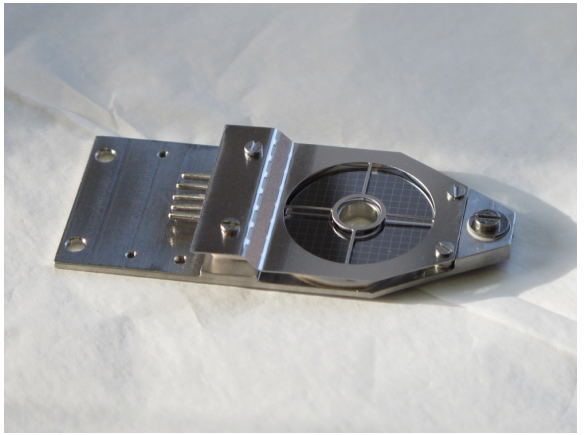


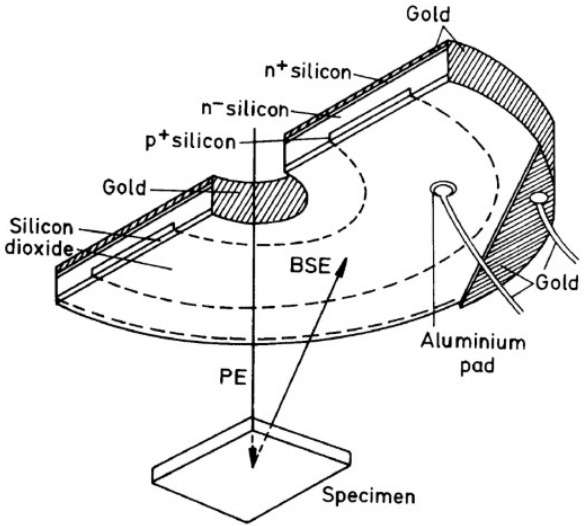




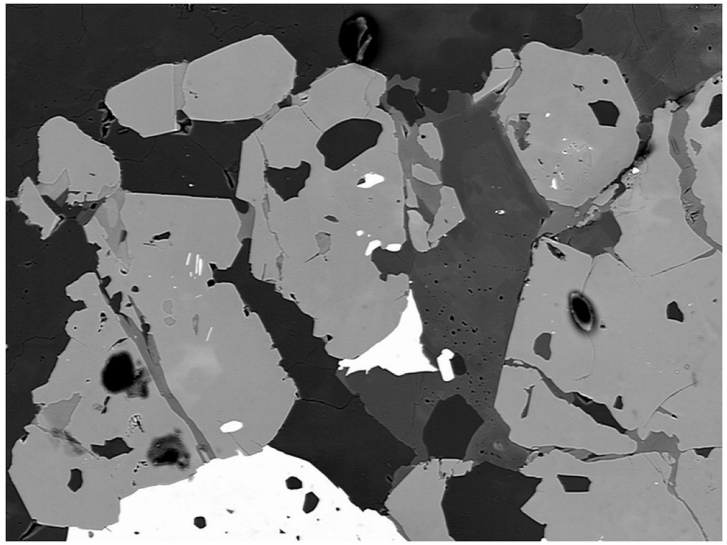
BE detector

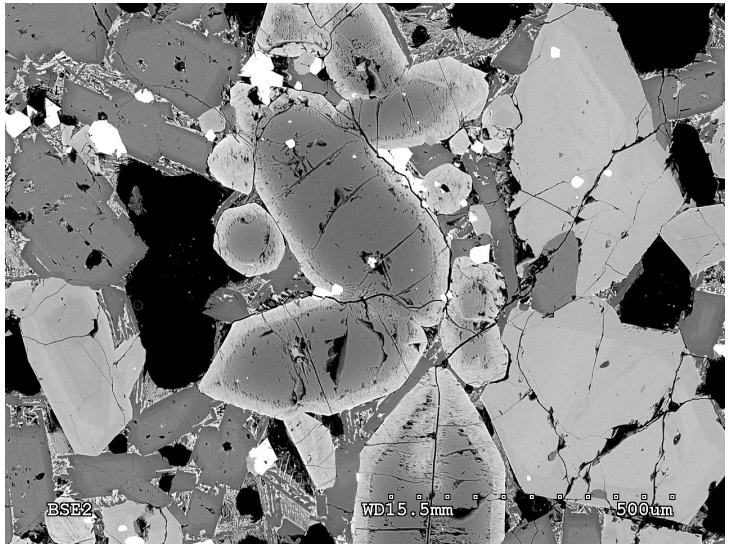
- The BE detector is located around the the pole-piece of the electron gun
- Since back-scattered electrons have a relatively large energy, no additional acceleration is required to cause ionization. They can directly generate electron hole-pairs in a semiconductor device.





BE-images

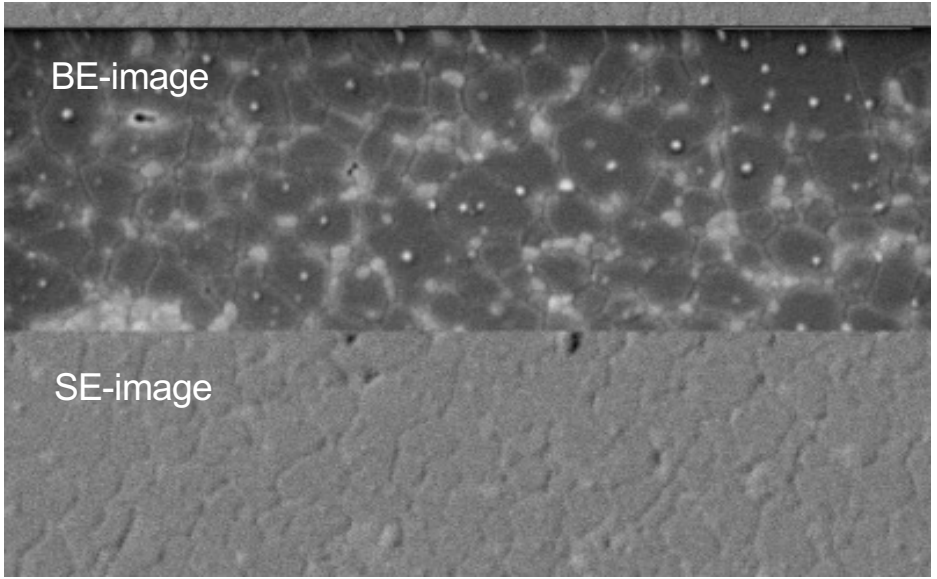


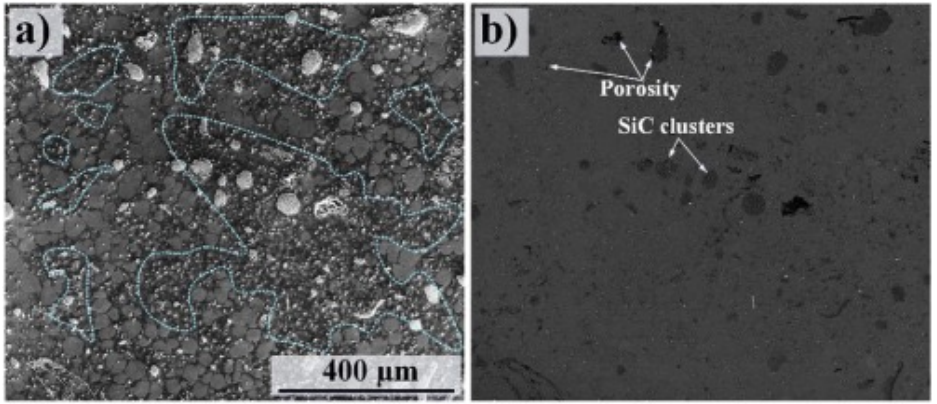


- BE electrons can come from deeper inside the sample. This let them look “underneath” the surface, but it also reduces the resolution
- BE images are good for obtaining materials contrast or crystallographic contrast

BE image and SE image provide complementary information

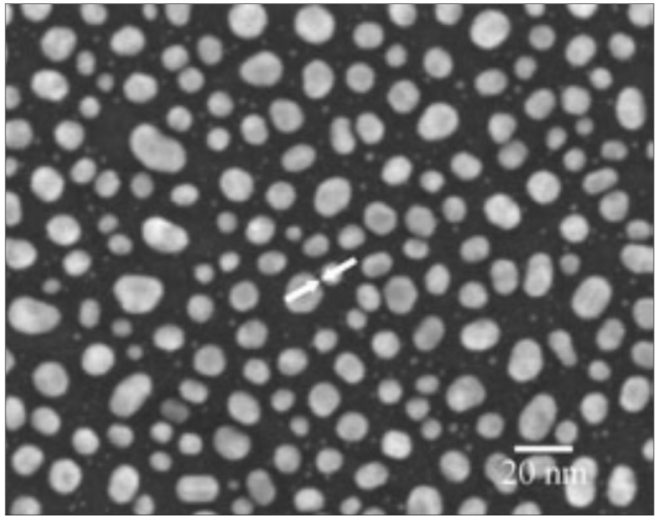
- BE and SE image can be recorded at the same time

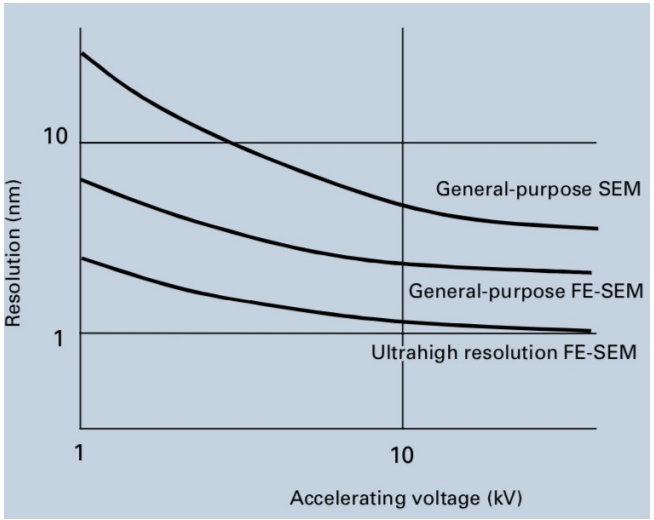




Resolution vs resolving power

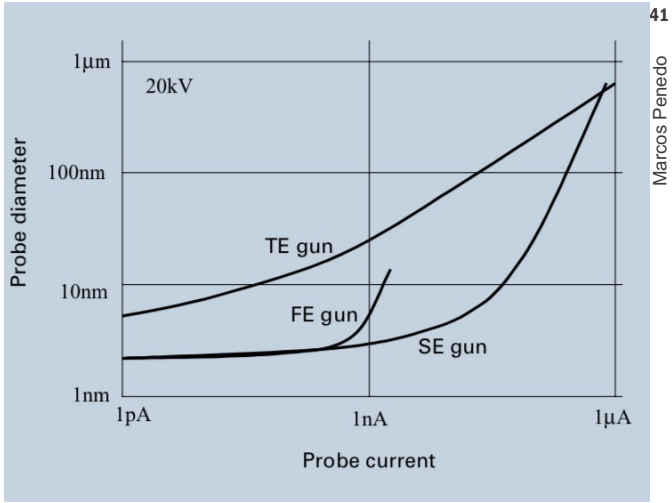
- The ***resolving power*** is the minimum distance that can be separated as two distinguishable points using the instrument
- Resolving power is measured under ideal conditions with easily measured samples (for example gold evaporated on carbon)
- The ***resolution*** is the minimum distance that can be separated as two distinguishable points in the (SEM) image. The resolution is determined also by the sample, imaging parameters, the status of the instrument

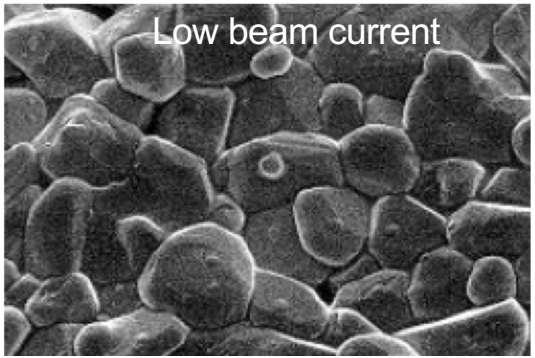


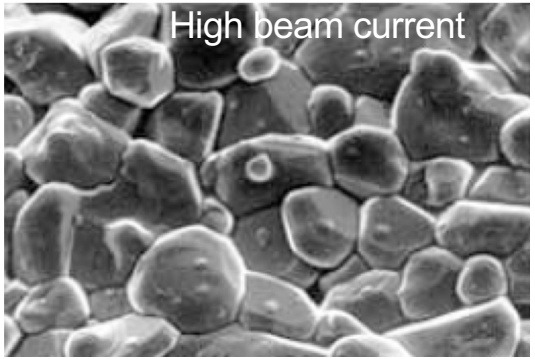


Effect of probe/beam current on resolution

- The probe current can be set in the SEM by changing the voltage on the anode of the electron gun or by changing the aperture.
- The higher the probe current, the better the signal to noise ratio, but the larger the beam size.
- The higher the probe current, the more issues there are with charging.

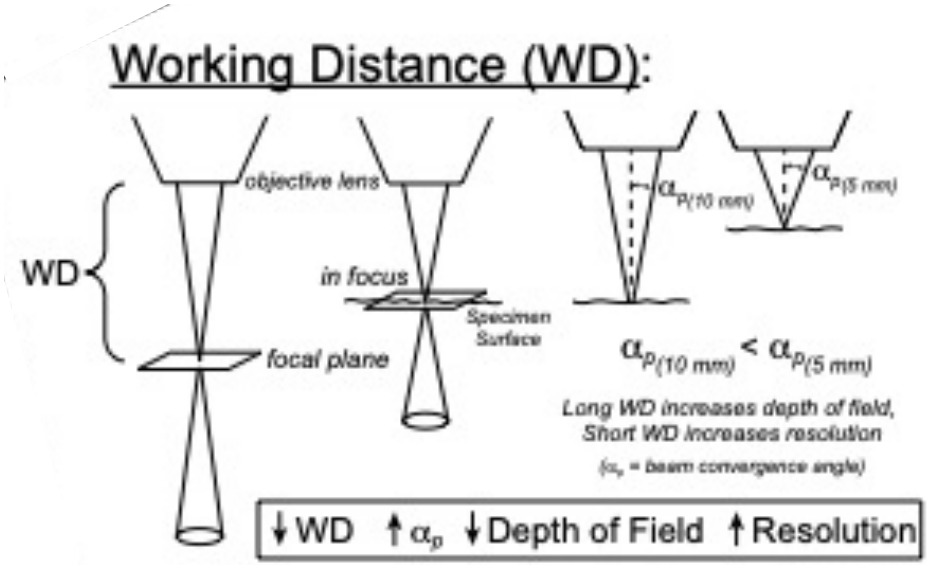






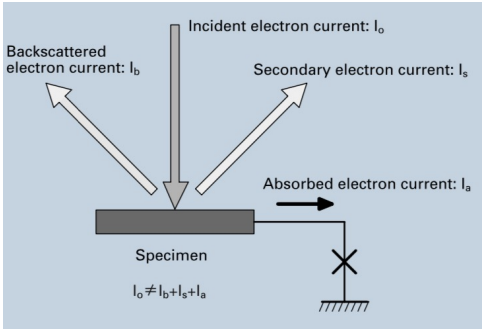
Working distance

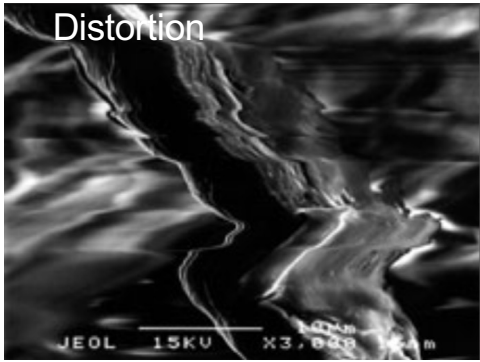
- Working distance has an influence on the depth of field, the larger the working distance the larger is the depth of field.
- Decreasing the working distance will increase the spatial resolution. However, at reduced working distances focusing becomes more and more difficult.

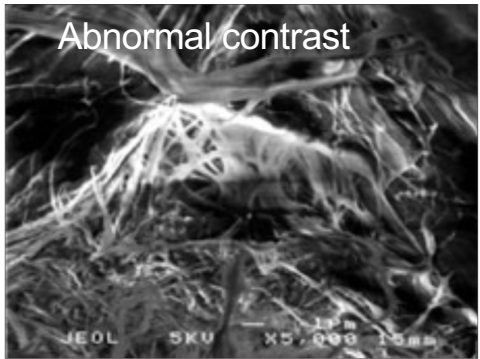


Charging

- Because we are bombarding the sample with electrons, the charges accumulate in the sample if they can not flow away through a conductive path.
- Charging occurs when the number of incident electrons is larger than the number of the electrons that exit from the specimen.
- A negative charge is accumulated in the irradiated point, resulting in a large negative potential in this point.
- This negative potential affects the incoming electron beam and leads to image distortion an abnormal contrast
- Charging is a problem for non-conducting samples







SEM Sample preparation

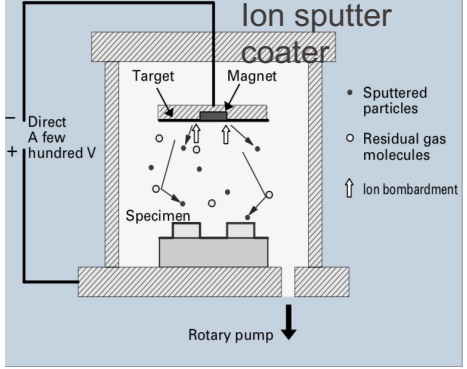
Requirements for SEM sample

- The surface to observe is exposed.
- The specimen is firmly fixed to the specimen mount.
- The specimen has conductivity in principle.
- Sample is vacuum compatible.

Samples can have many forms: fracture surfaces, polished surfaces, powders, natural surfaces, microfabricated surfaces.

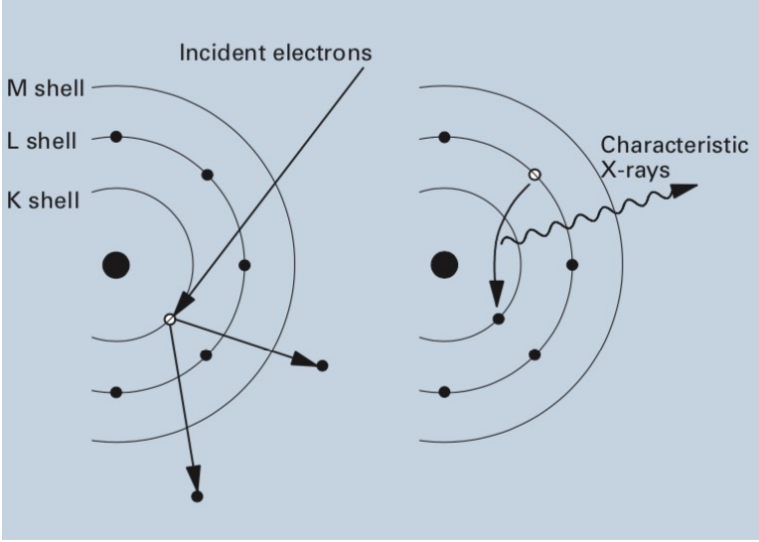
Sample coating and contacting

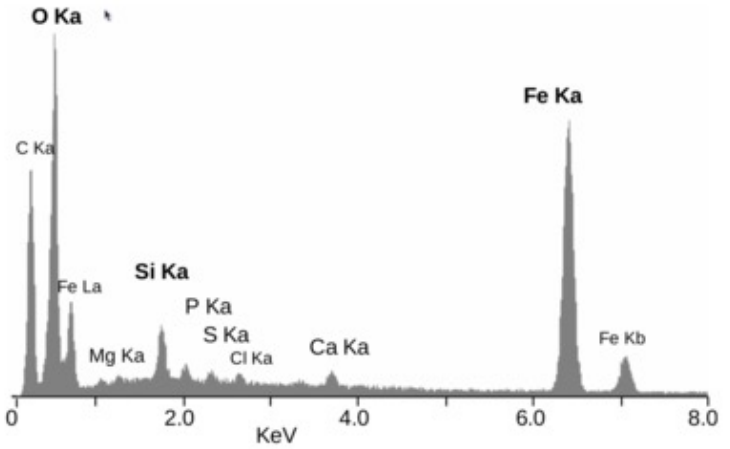
- SEM samples need to be conductive
- If they are not conductive, they need to be coated with a thin coat of conductive material (metal or carbon)
- Typical coating methods:
  - Sputtering
  - Evaporation
- Typical coating thickness: 10's of nm
- Samples need to be conductively connected to the specimen holder using either clamping, conducting glue, silver paste or carbon tape



EDX
X-ray analysis

- When electrons interact with atoms, they can generate characteristic X-rays.
- When a primary electron kicks out an electron from an inner orbital of the atom, it creates a vacancy. This vacancy is filled by an electron from an outer orbital. The difference in energy between the electron in the inner orbital and the outer orbital is emitted as an X-ray photon.
- The energy spectrum of that X-ray photon is characteristic for the atom.

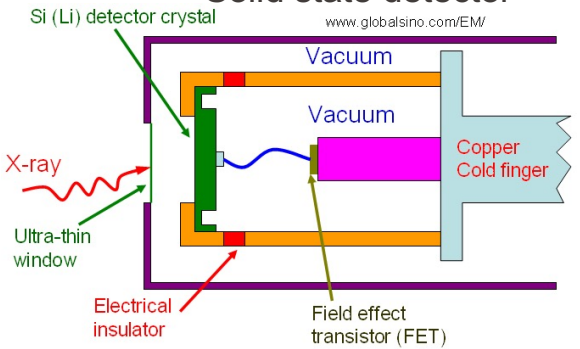




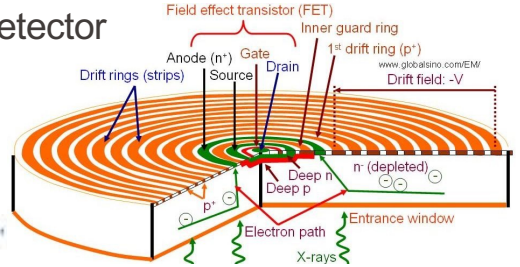
EDX detectors

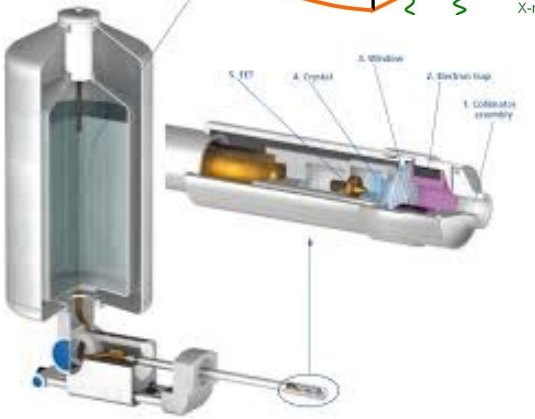
- The X-rays need to be translated into signal that is proportional to the energy of the X-ray photon.
- EDS detector contains a crystal that absorbs the energy of incoming X-rays by ionization, yielding free electrons in the crystal that become conductive and produce an electrical charge bias.
- The X-ray absorption thus converts the energy of individual X-rays into electrical voltages of proportional size; the electrical pulses correspond to the characteristic X-rays of the element.
- EDX detectors need to be cooled either through liquid nitrogen or Peltier element.

Solid state detector



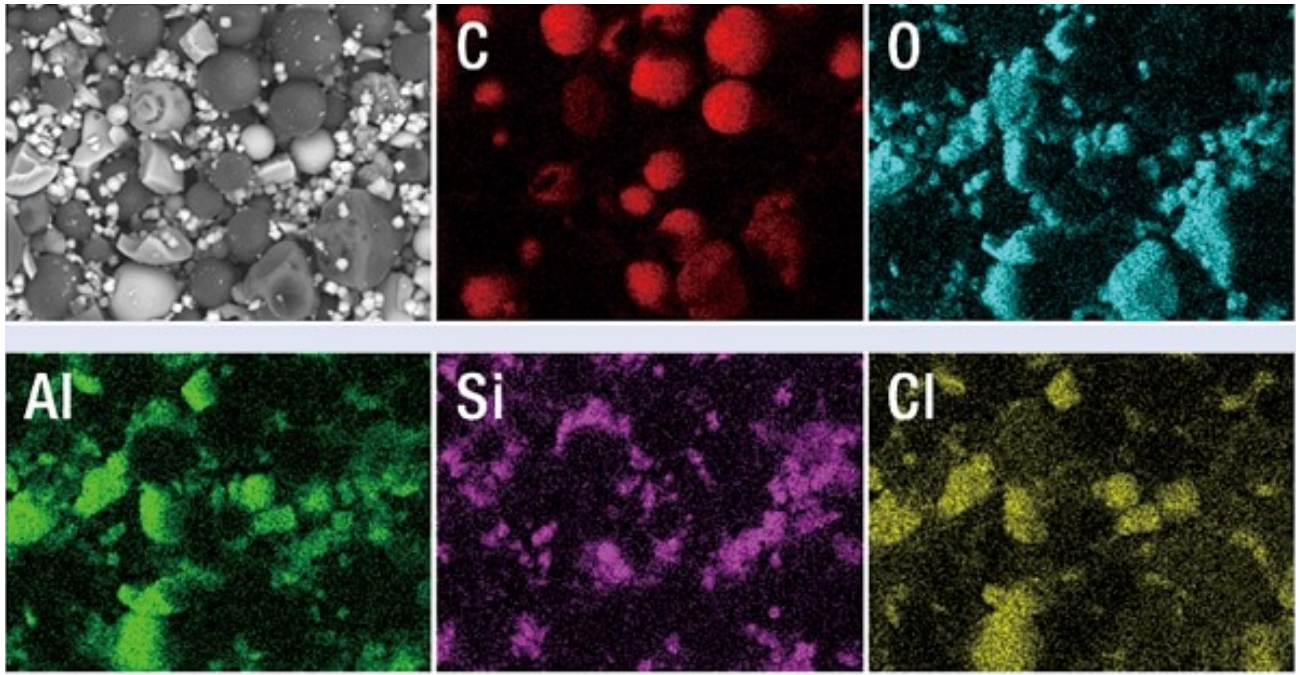
Silicon drift detector





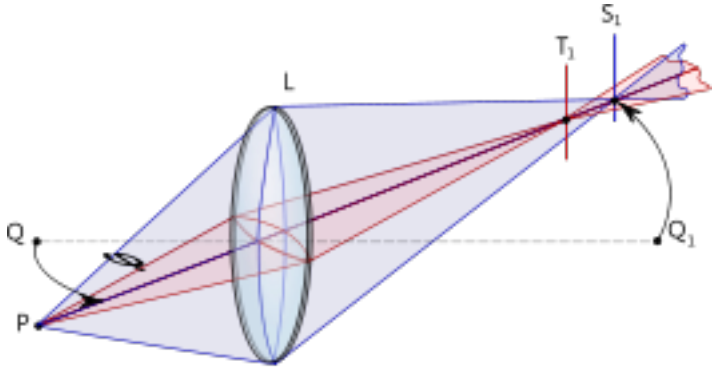
Elemental mapping with X-rays

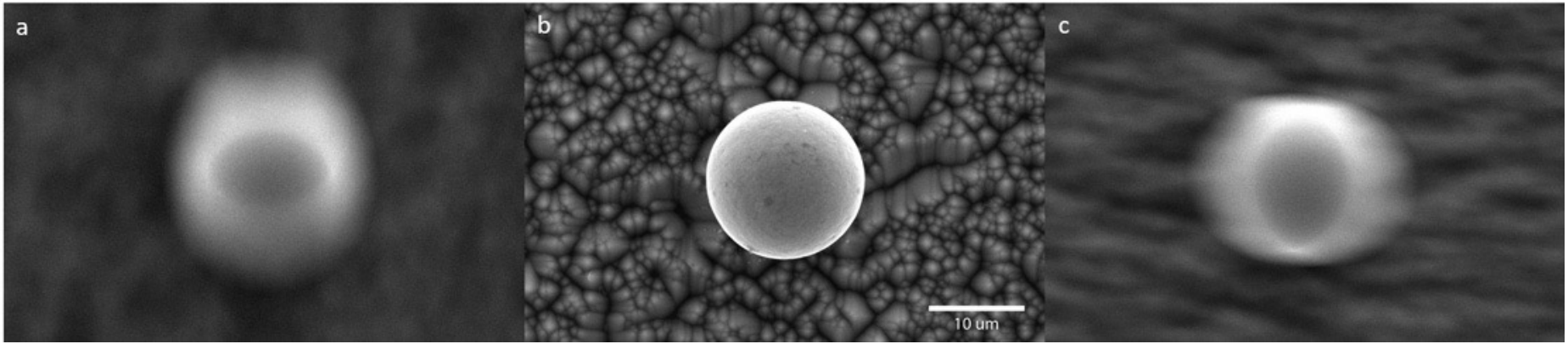
- From the energy spectrum we can determine what atoms are in our sample.



SEM image problems: Astigmatism

- Due to imperfections in the SEM electron optics, the beam is not perfectly round but rather spherical.
- This results in distortions or streakiness in the image.





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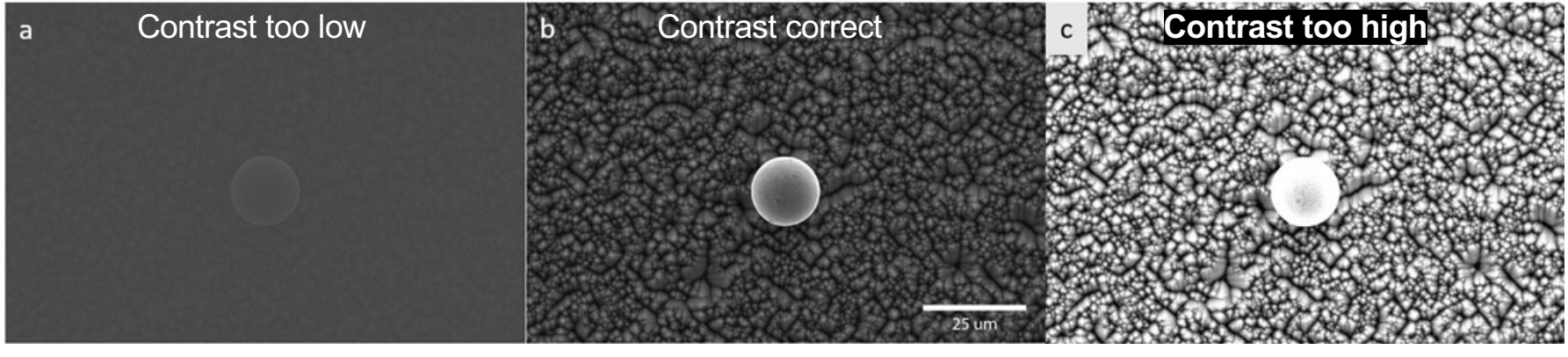
SEM image problems

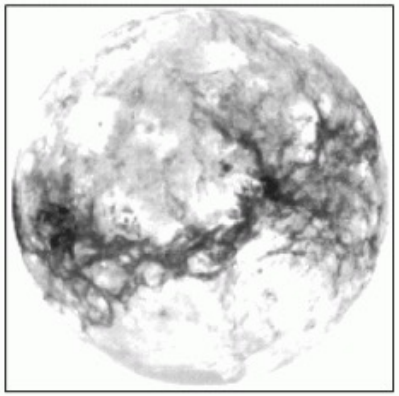
Contrast

- The SEM image is a virtual reconstruction of the sample and does therefore not correspond to a real image.
- The data processing needed to create the sample can affect the clarity of the image. Primarily contrast and brightness are important.
- Contrast is the number of grayscale intensities that the imaging data is displayed.
- In the intensity histogram the contrast adjusts the width of the distribution.

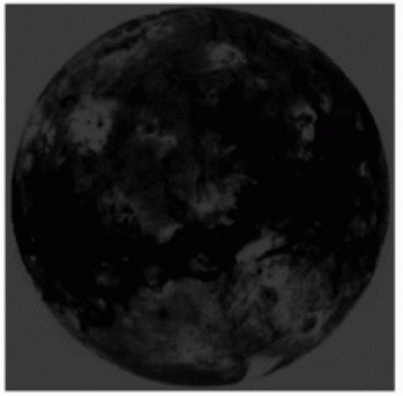


SEM image problems
Brightness

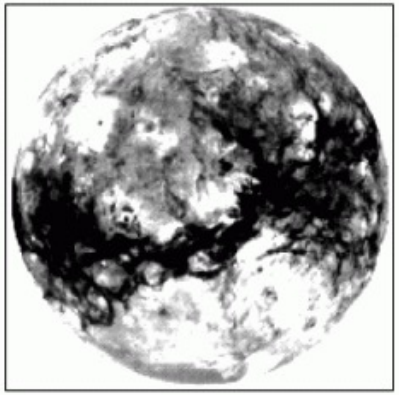
- Brightness is the intensity at which a pixel with median intensity is displayed.
- In the intensity histogram, the brightness shifts the peak left or right



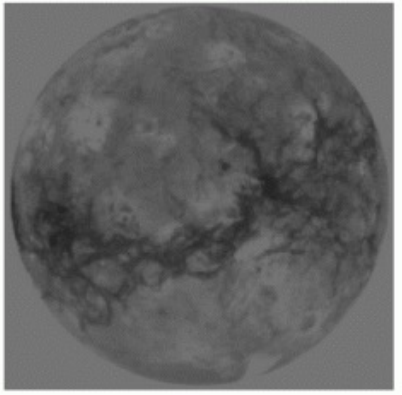
a. Brightness too high



b. Brightness too low



c. Contrast too high

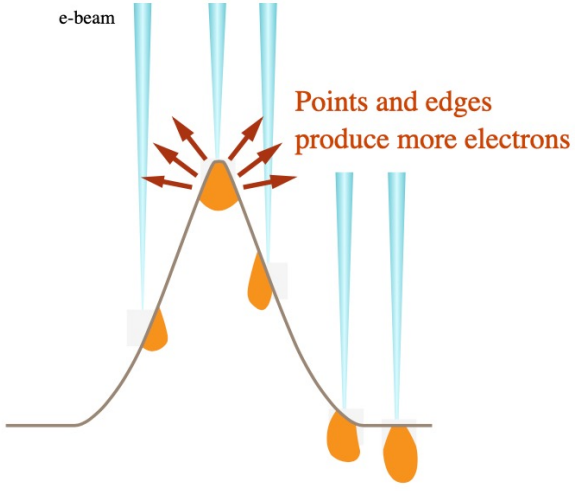


d. Contrast too low

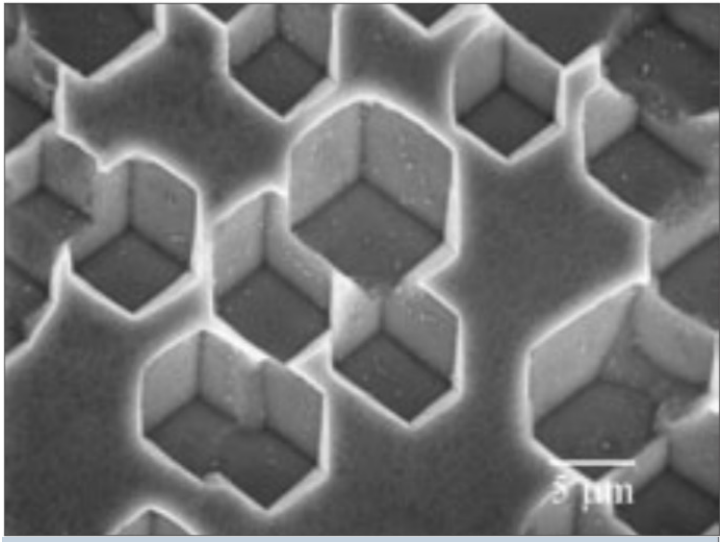
FIGURE 23-10
Brightness and contrast adjustments. Increasing the *brightness* makes every pixel in the image becomes lighter. In comparison, increasing the *contrast* makes the light areas become lighter, and the dark areas become darker. These images show the effect of misadjusting the brightness and contrast.

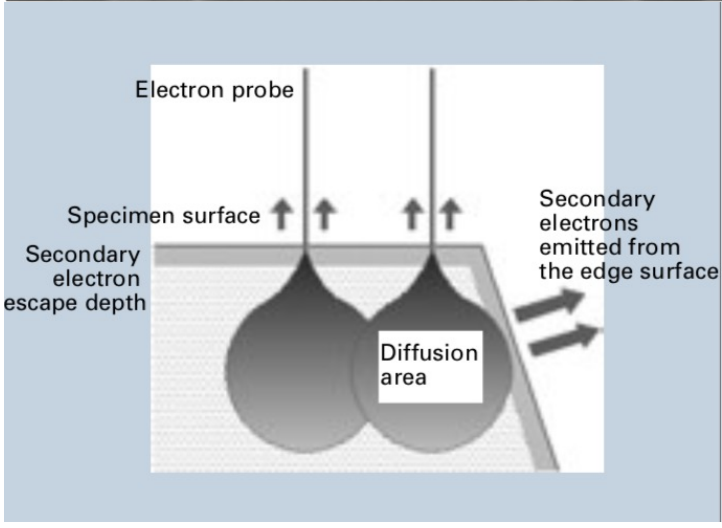
Edge effects

- Sharp edges appear brighter because SE electrons can be collected from the whole interaction volume.



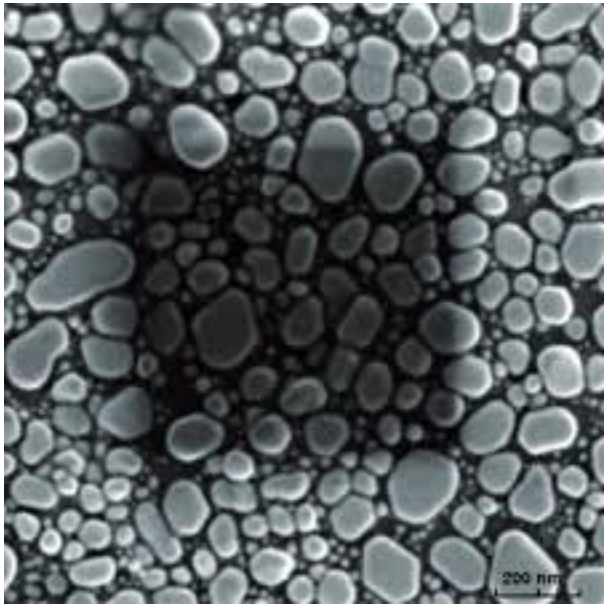
Changes in the interaction volume with topography





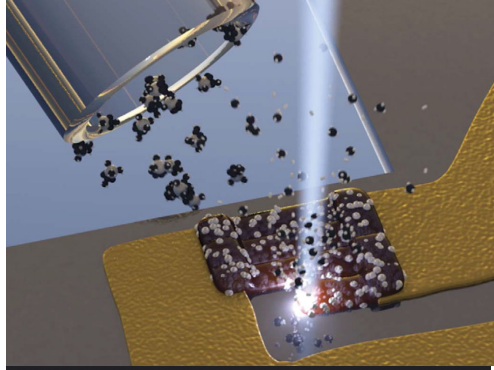
Beam induced contamination

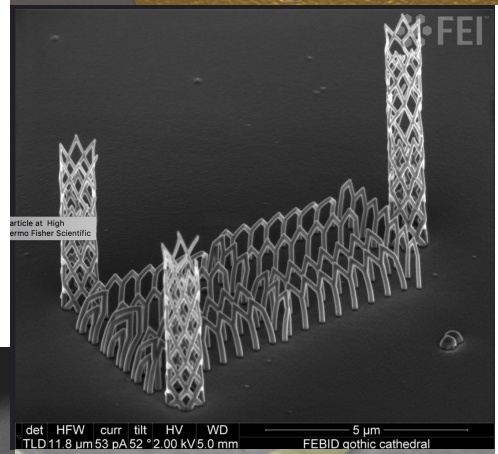
- Although the SEM is under vacuum, there are always some contaminants in the chamber, for example hydrocarbons.
- When the electron beam hits these contaminations, the molecules dissociate and form radicals which in turn find other radicals to react with.
- The dissociated hydrocarbons are re-deposited as C carbon contamination in the are where the SEM beam was rastered.

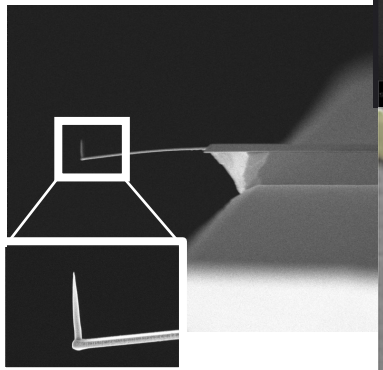


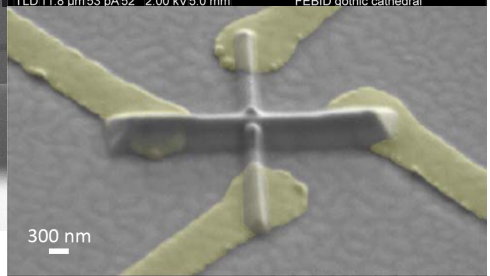
Electron beam induced deposition - FEBID

- The effect of electron beam induced dissociation of molecules can also be used for nanoscale 3D printing.
- Special pre-cursor gasses are added to the vacuum chamber very close to the sample. The beam dissociates the pre-cursor and the material redeposits where the electron beam hits the surface.
- By steering the beam in the desired pattern one can print quasi arbitrary shapes.



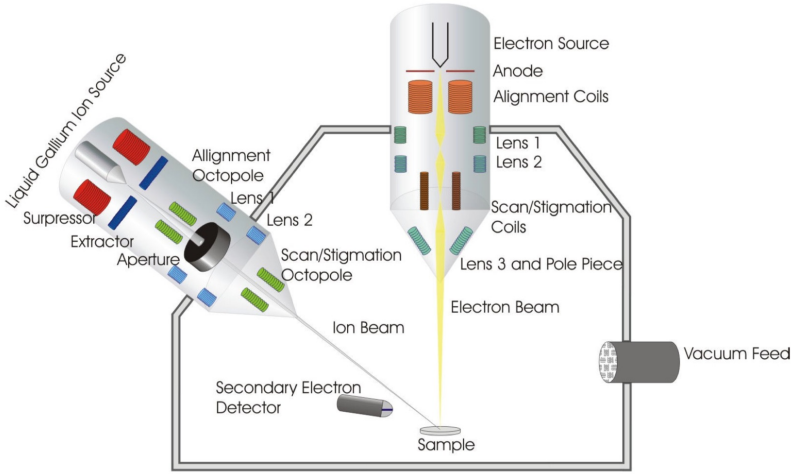


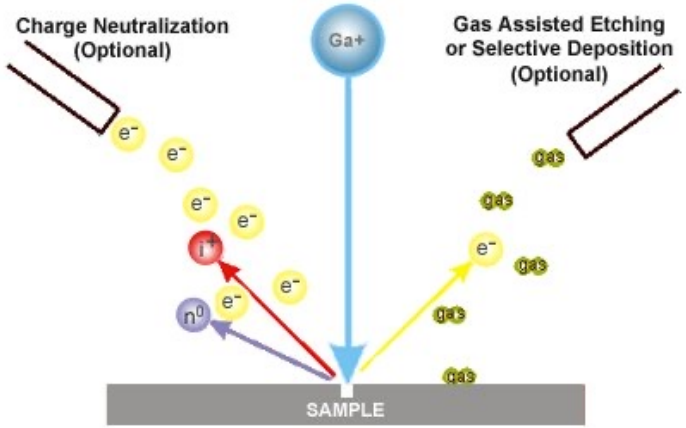




Focused Ion Beam systems

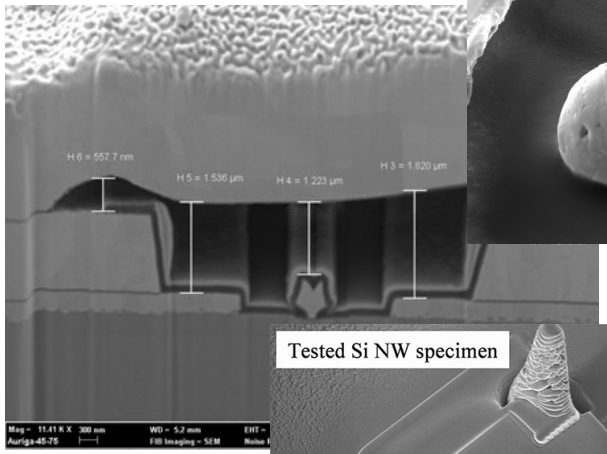
- FIB systems use ions (Ga, He, O,…) instead of electrons to generate a beam.
- Because ions have much higher mass per charge, they can be used to bombard a surface and etch it.
- FIB systems are primarily used for nanoscale etching of material.
- FIB and SEM are often combined in a dual beam microscope DBM.

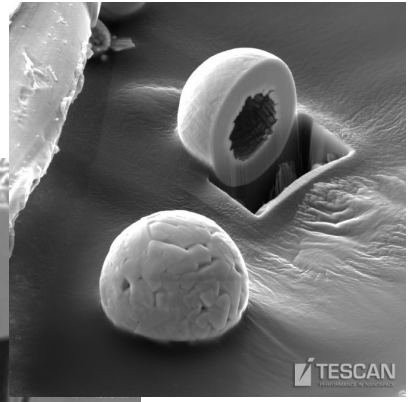


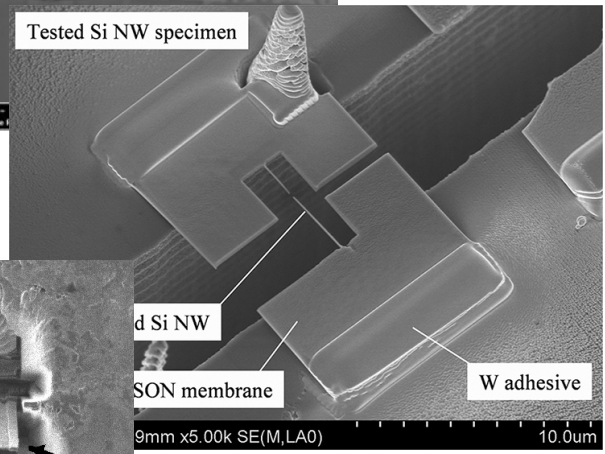


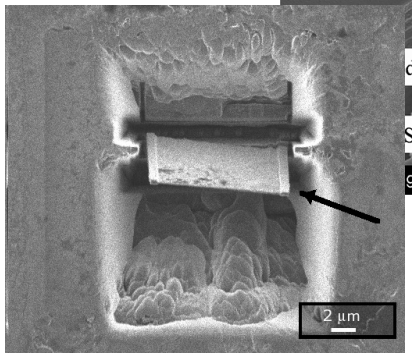
FIB uses

- FIBs are often used when very small, very clean cuts need to be made, for example for defect analysis or microfabrication device analysis.
- It can however also be used for nanofabrication (for example nanowires or nanopores).
- It is often also used for TEM sample preparation.

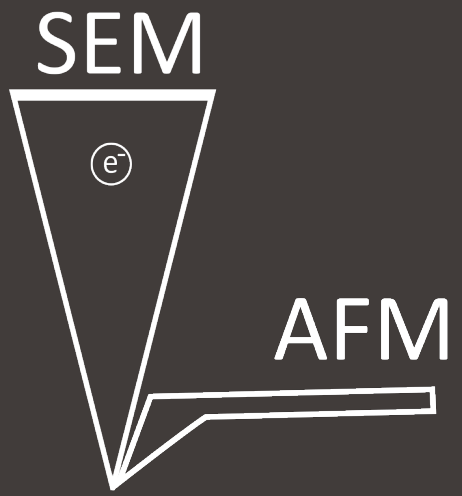




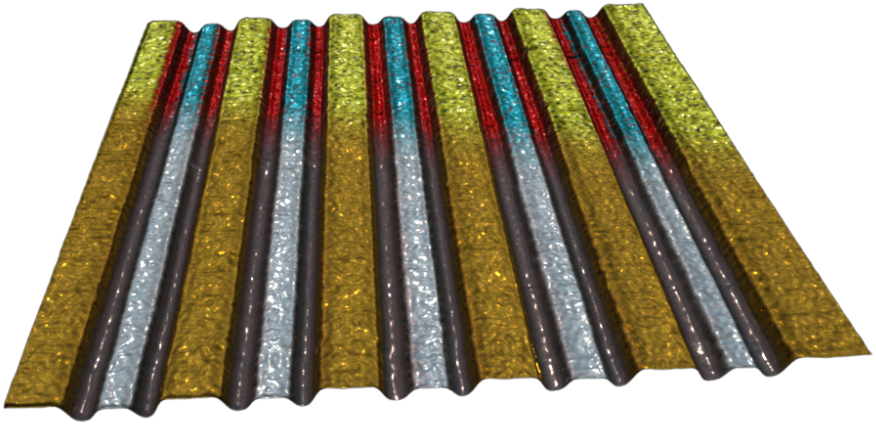




Combined AFM with SEM



Combined SEM & AFM



Gold and Aluminum on Silicon wafer imaged with AFM and SEM/EDX

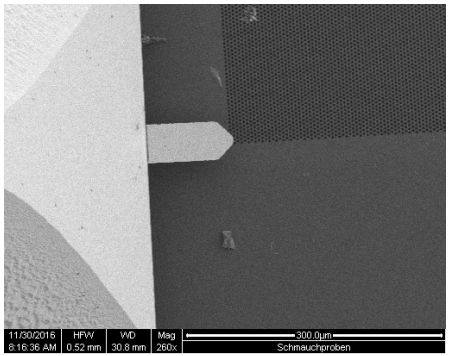
With AFM you get:

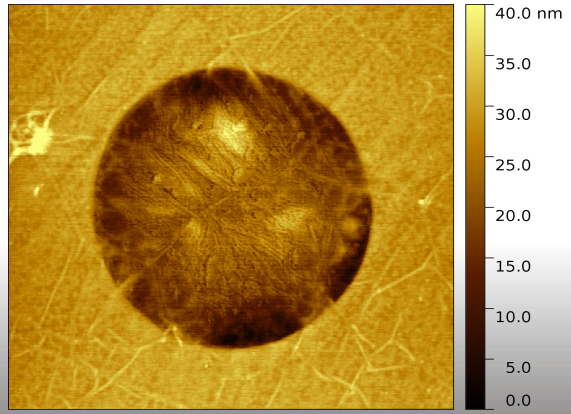
- Topography
- Conductance
- Mechanical properties
- Magnetic properties
- …

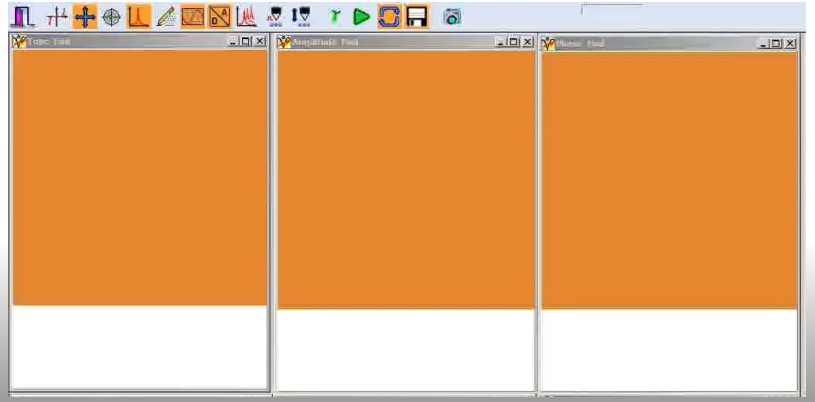
With SEM you get:

- Elemental contrast
- Large area view
- Nanoscale 3D printing
- ...

Images on freely suspended graphene layers

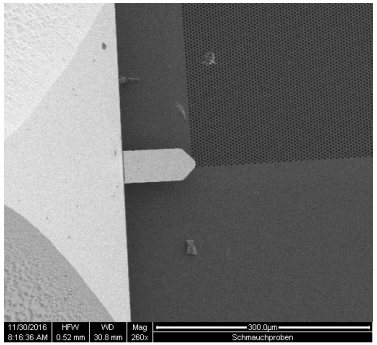


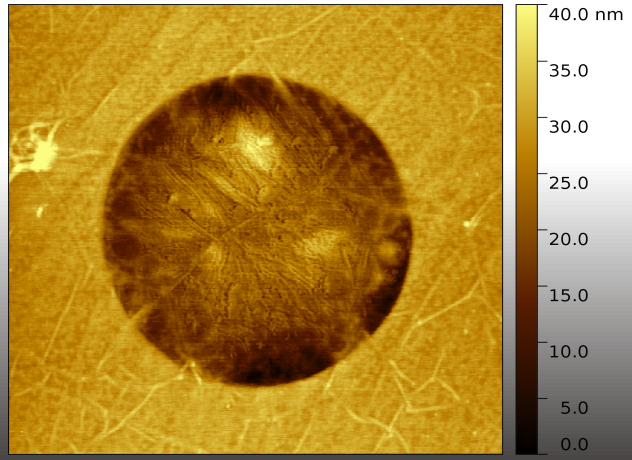




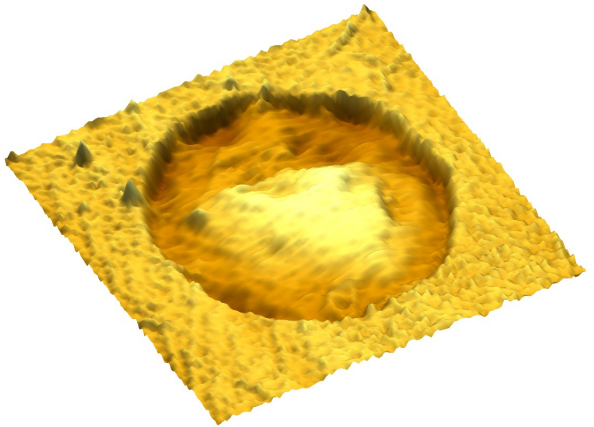
In collaboration with GETec

Images on freely suspended graphene layers



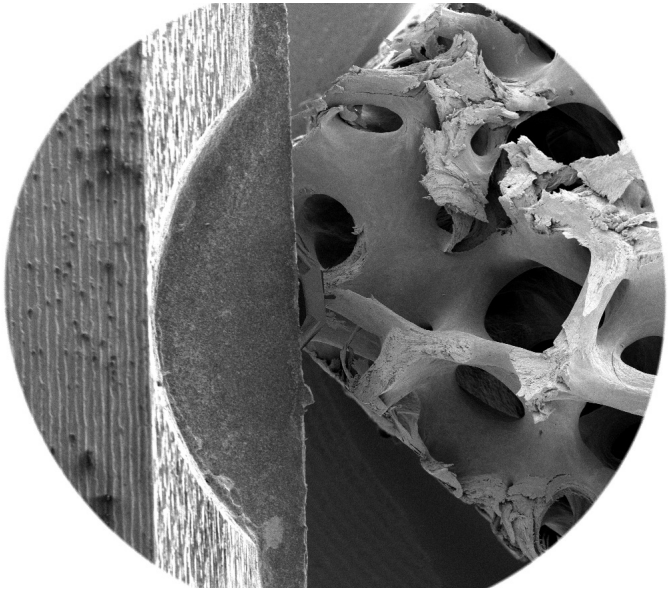


Set-point: 86 %

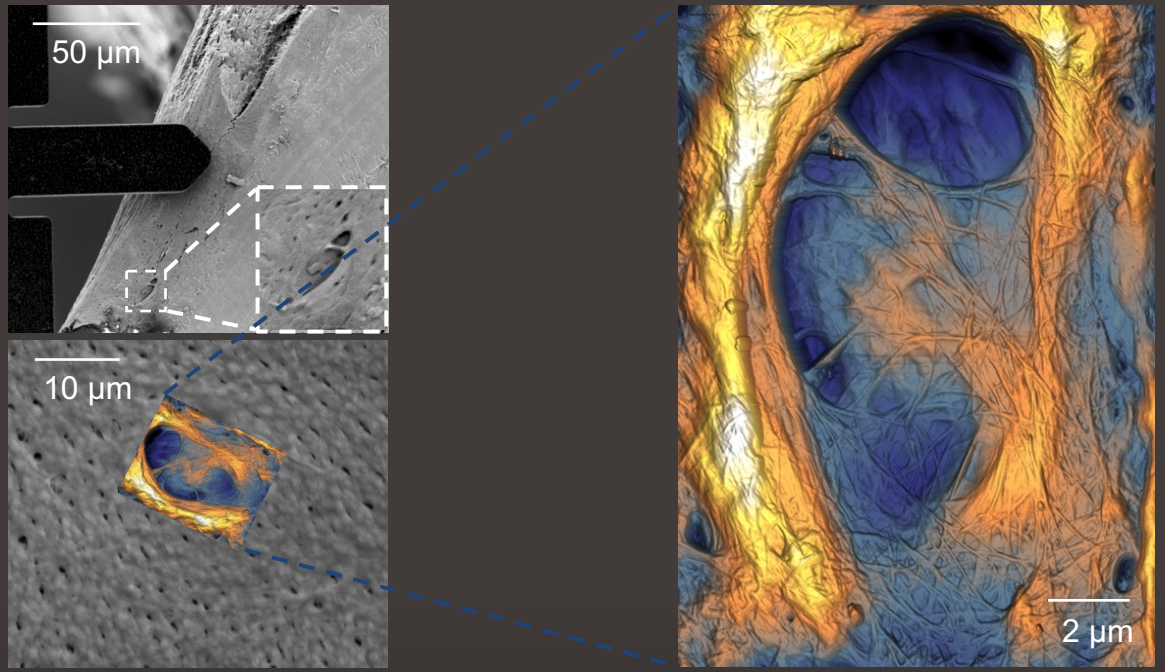


In collaboration with GETec

Correlated AFM- SEM microscopy

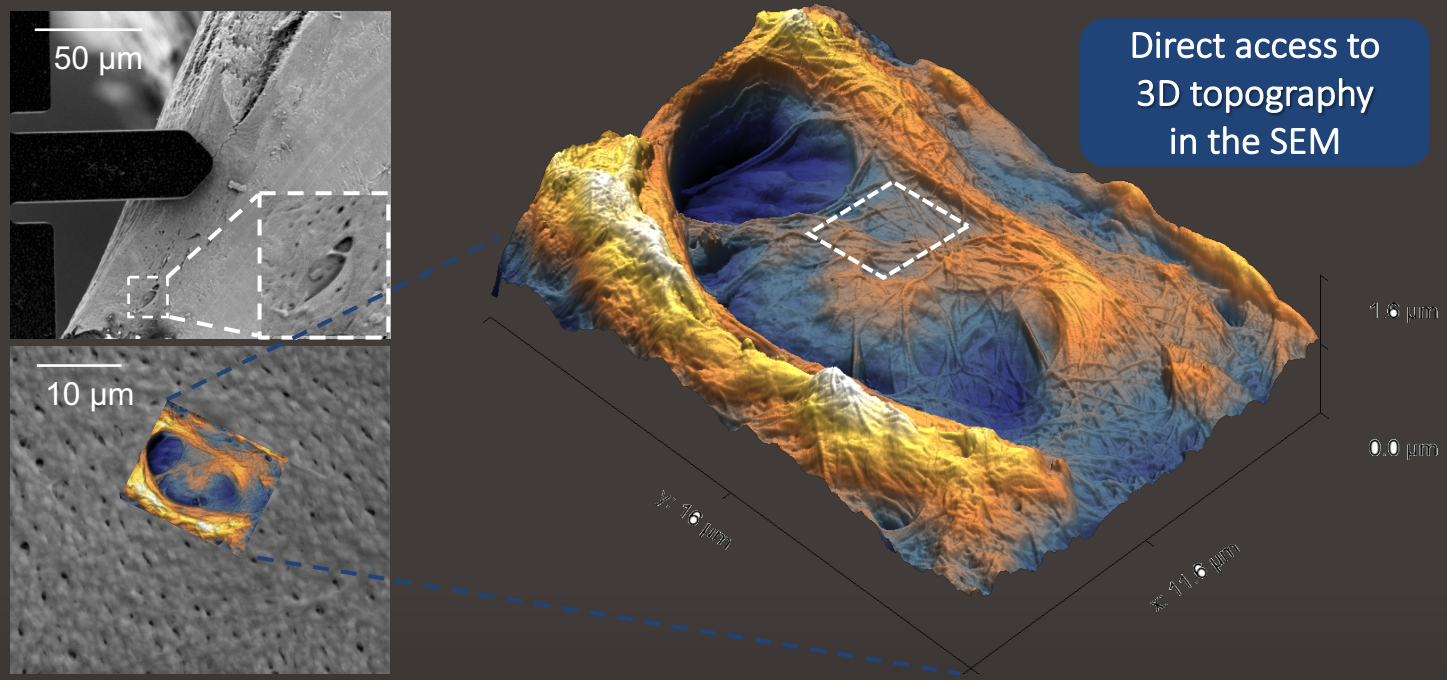


Correlative SEM/AFM analysis of bone tissue

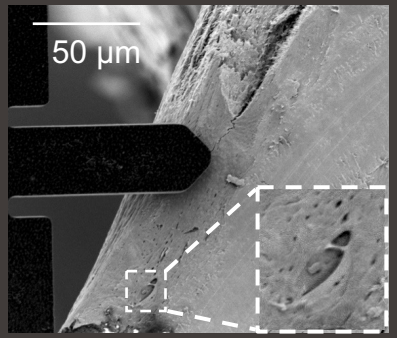


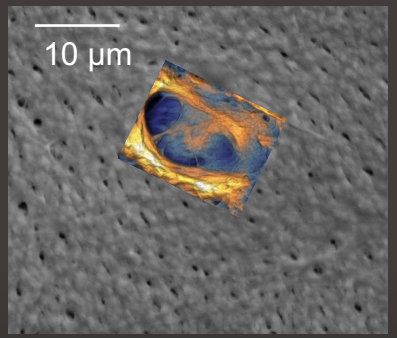
Images taken with AFSEM 1.0 GETec microscopy Ltd.

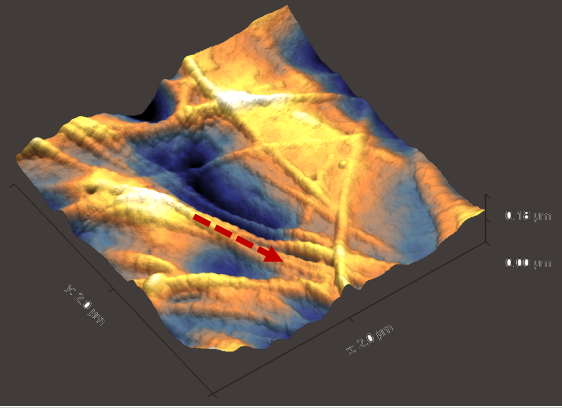
Correlative SEM/AFM analysis of bone tissue



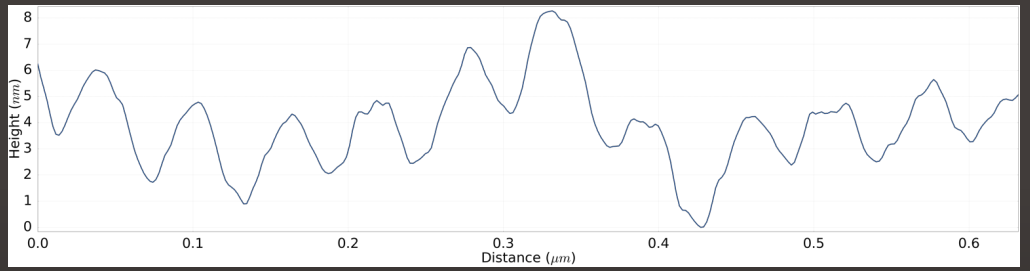
Correlative SEM/AFM analysis of bone tissue



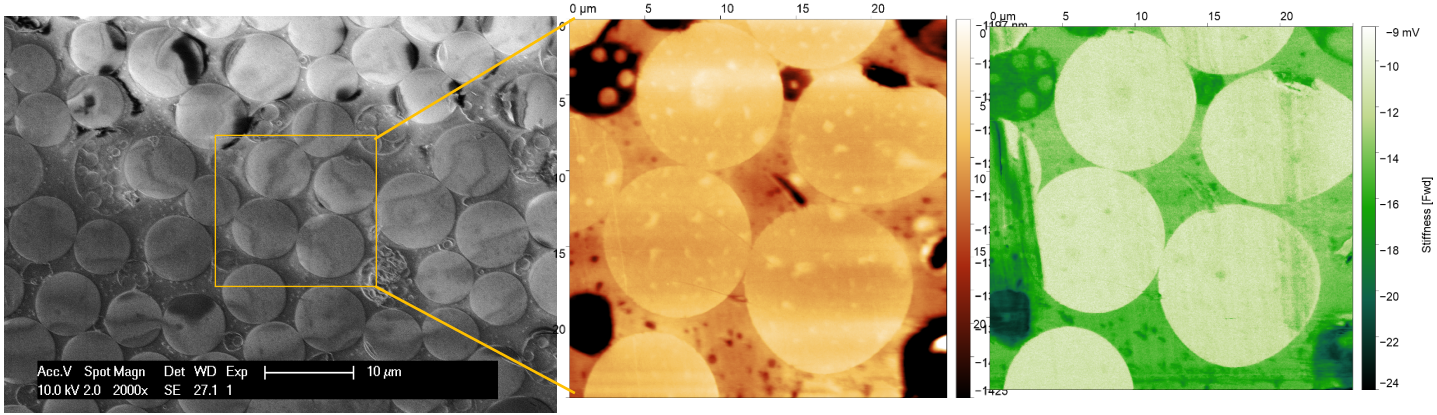




Direct access to 3D topography in the SEM

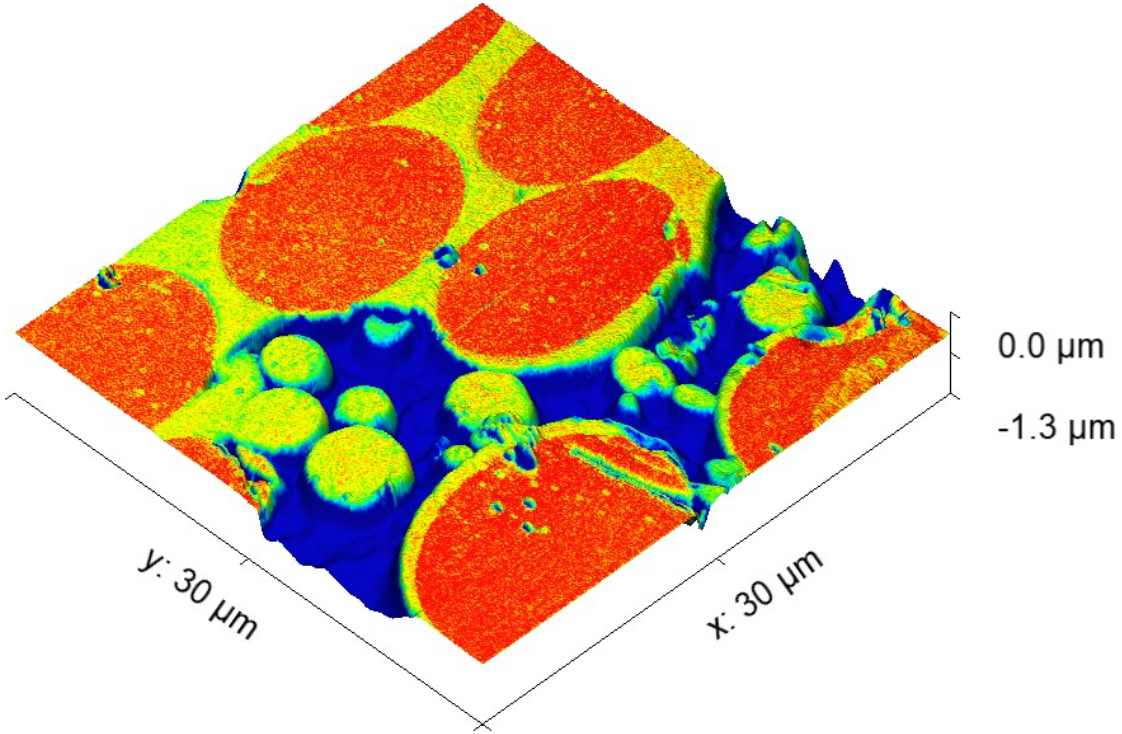


Correlated AFM stiffness measurement with SEM on Polymer composites



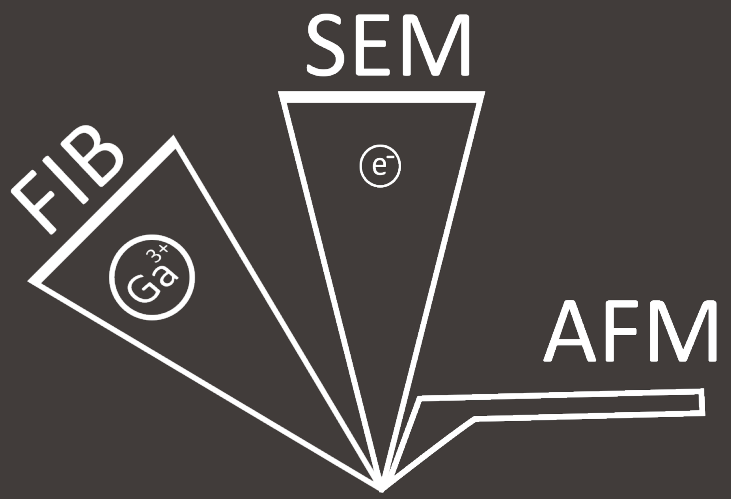
Self healing polymer: Polycaprolactone (25% vol) -glass fiber-epoxy blend. Left SEM image (10kV, SE), middle AFM topography, right: AFM stiffness channel. The stiffness of the 3 phases can be clearly distinguished. The changes in topography of the softer material (PCL) is a result of the polishing process.

Sample provided Michoud lab, EPFL



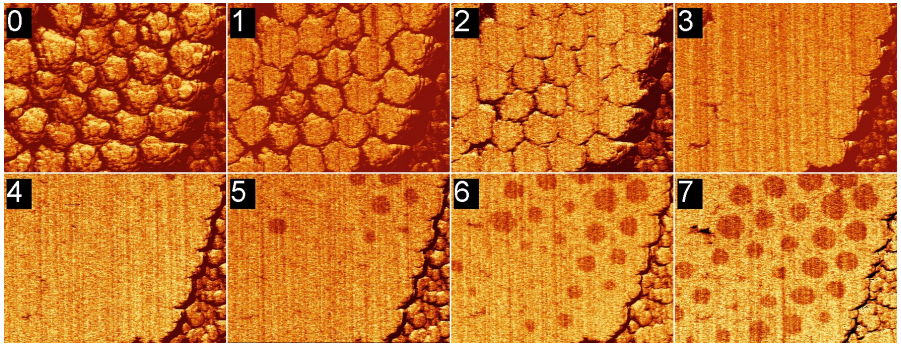
Self healing polymer, sample 3 Polycaprolactone (40% vol)-glass fiber-epoxy blend. Topography (4x scale in Z, with stiffness overlay and light shadows)

Triple-Scanning Microscope

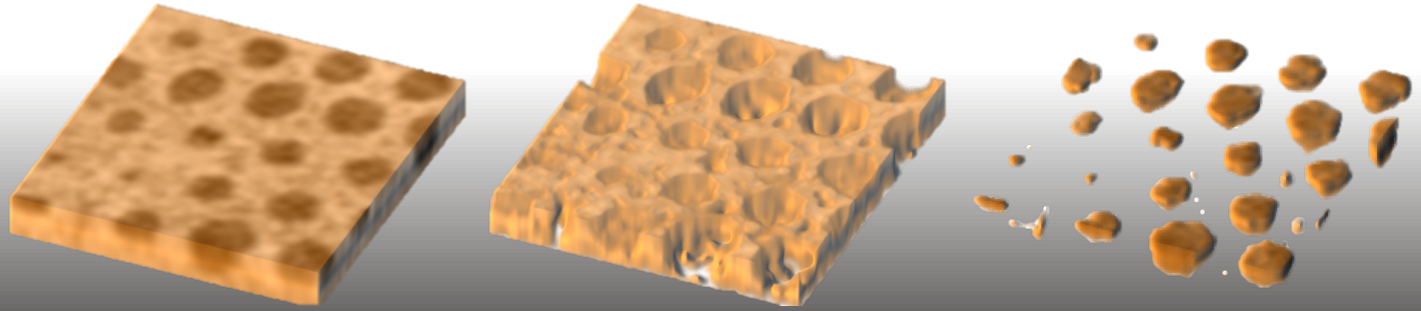


Full 3D AFM System:
Using the AFM + FIB for nanoscale tomography

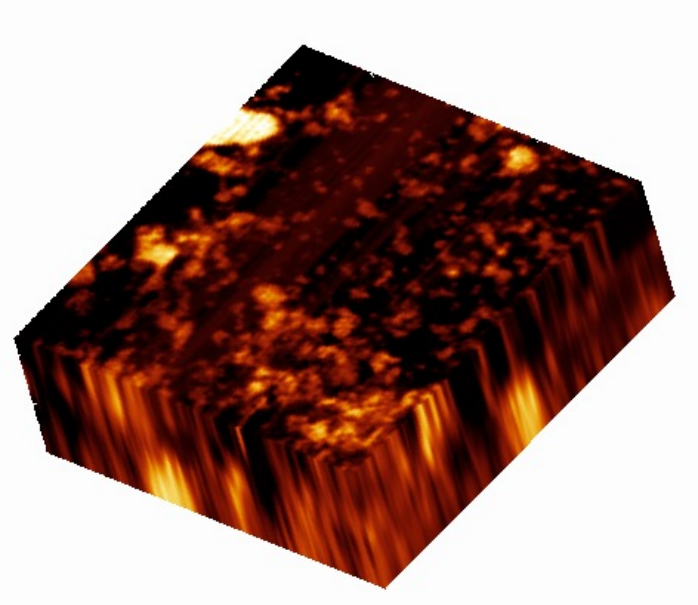
Beads stiffness tomography (raw data)
PS beads 2.5 µm diameter
Aluminum sputtered on top

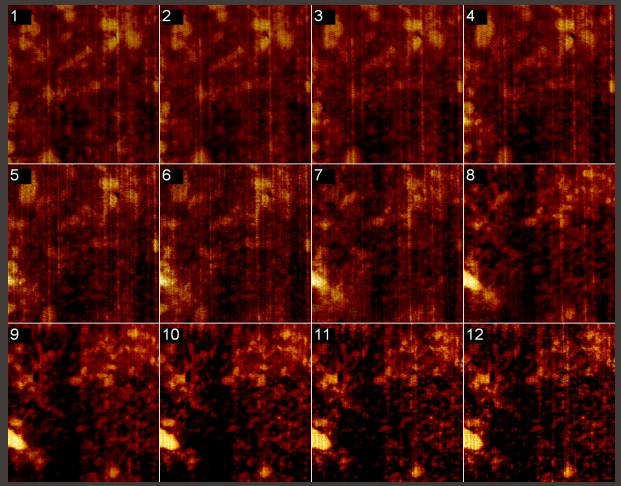


Segmented from raw data



Industrial Black Rubber in 3D





Black rubber stiffness tomography
Stack of 12 images
X - Y size : approx. 10um ; Z size : 2.5 um
Z scale is different from XY scale

Learning outcomes:

- Know basic components of electron microscopes (SEM&TEM: sources, lenses, detectors, pumps, etc).
- Know the different interactions that electrons can have with samples.
- Explain the working principles of SEM, TEM and FIB:
  - How is the magnification achieved?
  - Contrast mechanisms, imaging modes, sample preparation
  - What limits the resolution of the different EMs?
  - What are the advantages of the different EMs?
  - …
- Explain the basic sample requirements and preparations for SEM and TEM.
- Explain the SEM imaging modes SE, BS, EDX.
- Explain what nanofabrication methods are available with SEM/FIB and how they work.