Notes of Advanced Physics Laboratory (2017)

AN INTRODUCTION TO
electron microscopy

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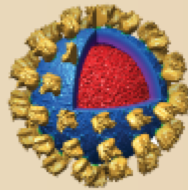
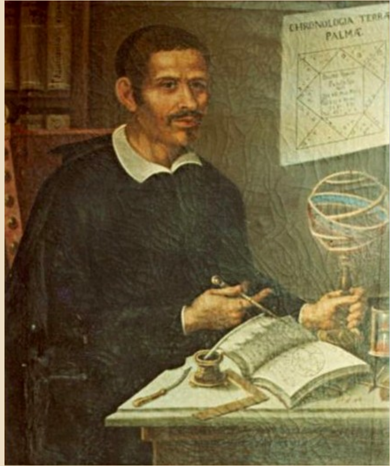
Credits to: FEI introduction to microscopy 2013, J. S Arino (2012), S Bhargava (2012)



Historic Figures in optical microscopy



Hooke's microscope (~1660)

Giovan Battista Odierna (1597-1660) astronomer but using lenses to observe the microscopic world (L'occhio della Mosca, 1654).
Galileo Galilei invented the compound microscope "occhiolino" in 1609 (2 lenses).

Robert Hooke (1635-1703) Micrographia (1665) with observations and figures made with microscopes (and telescopes) coining the term cell

Antony van Leeuwenhoek (1632-1723)
Royal Society published in 1673 observations with a high-quality single-lens optical microscope

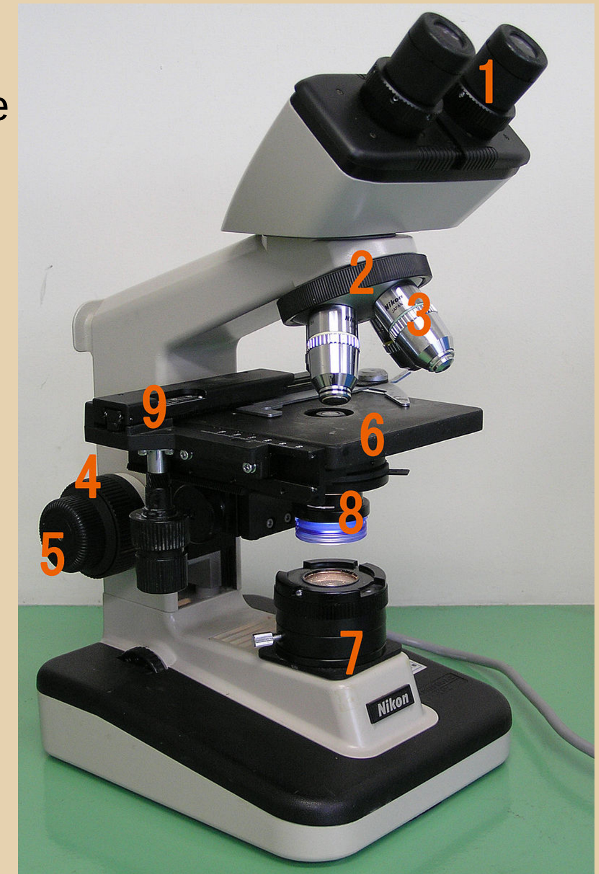
Ernst Abbe (1840-1905)
Dir. At Zeiss
Apochromatic (no distortion) lens, improving optics, resolution limits for optical microscopes

Microscope by Carl Zeiss (1879) with optics by Abbe

Typical microscope components

All modern optical microscopes designed for viewing samples by transmitted light share the same basic components of the light path. In addition, the vast majority of microscopes have the same 'structural' components

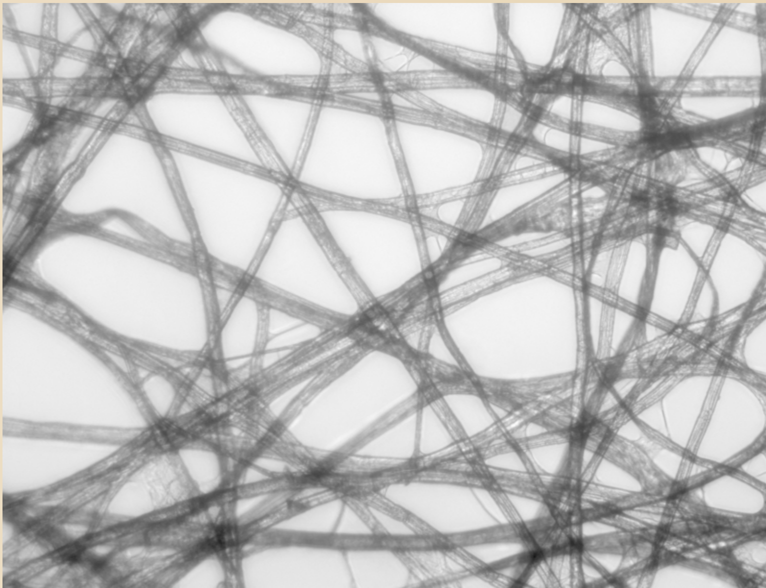
Eyepiece (ocular lens) (1)
Objective turret, revolver, or revolving nose piece (to hold multiple objective lenses) (2)
Objective lenses (3)
Focus knobs (to move the stage)
Coarse adjustment (4), Fine adjustment (5)
Stage (to hold the specimen) (6)
Light source (a light or a mirror) (7)
Diaphragm and condenser (8)
Mechanical stage (9)

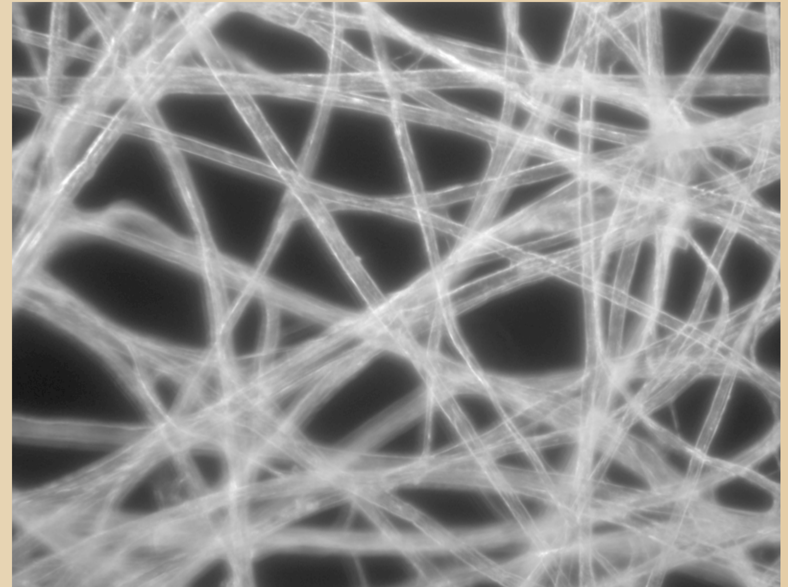


Illumination techniques

Many techniques are available which modify the light path to generate an improved contrast image from a sample. Major techniques for generating increased contrast from the sample include cross-polarized light, dark field, phase contrast and differential interference contrast illumination. Tissue paper

Dark field illumination, sample contrast comes from light scattered by the sample.





Bright field illumination, sample contrast comes from absorbance of light in the sample.

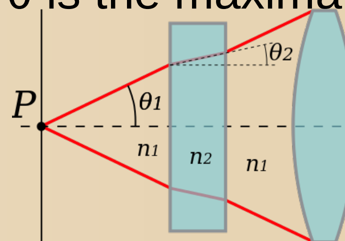
Typical optical (light) resolution

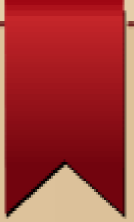
Assuming that optical aberrations in the whole optical set-up are negligible, the resolution d, can be stated as:

$$d = \frac{\lambda}{2NA}$$

Usually a wavelength of 550 nm is assumed, which corresponds to green light. With air as the external medium, the highest practical NA is 0.95, and with oil, up to 1.5. In practice the lowest value of d obtainable with conventional high quality lenses is about 200 nm.

The numerical aperture (NA) of an optical system is a dimensionless number that characterizes the range of angles over which the system can accept or emit light.Numerical aperture is commonly used in microscopy to describe the acceptance cone of an objective. the numerical aperture of an optical system such as an objective lens is defined by NA= n sin θ where n is the index of refraction of the medium in which the lens is working (1.00 for air, 1.33 for pure water, and typically 1.52 for immersion oil) and θ is the maximal half-angle of the cone of light that can enter or exit the lens.

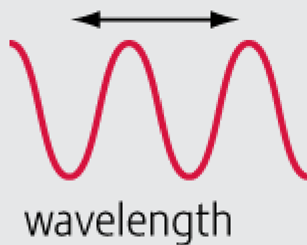


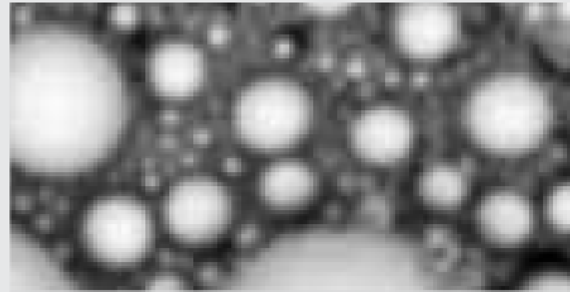


How is Resolution Affected by Wavelength?

poor resolution

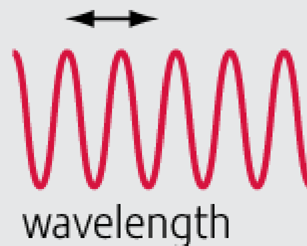
low frequency

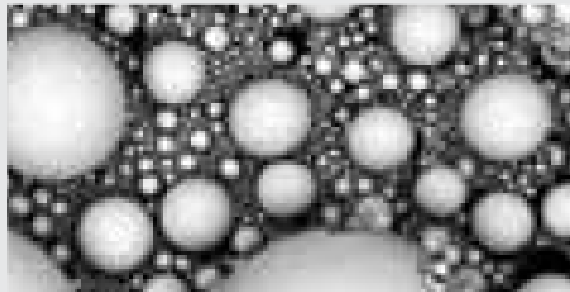




good resolution

high frequency



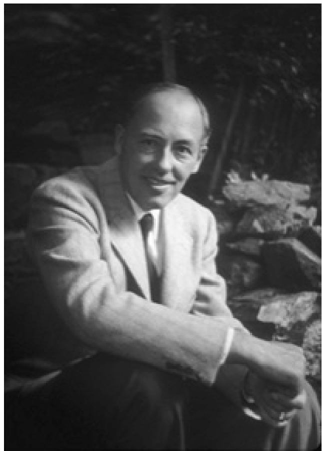


$$\lambda = \frac{h}{p}.$$

The De Broglie's relationships works for massive particles like electrons. So in principle the resolution limit can be pushed down to the atomic size.

Electron Microscopy
the beginners

Ruska-Knoll Microscope

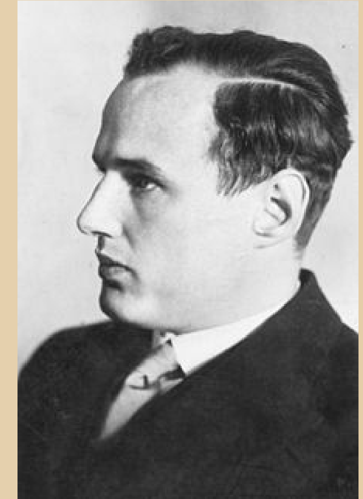


1906–1988

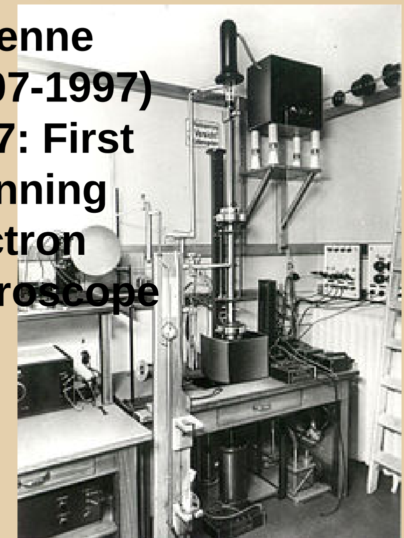
Ernst A. F. Ruska (Nobel prize in Physics 1986)

1897-1961

Max Knoll (1897-1969)
1931: First transmission electron microscope



Manfred Von Ardenne (1907-1997)
1937: First scanning electron microscope



Development of electron microscopy

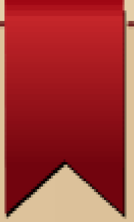
- **Electron microscopes were developed due to the limitations of Light Microscopes which are limited by the physics of light.**
- **In the early 1930's this theoretical limit had been reached and there was a scientific desire to see the fine details of the interior structures of organic cells (nucleus, mitochondria...etc.).**
- **This required 10,000x plus magnification which was not possible using current optical microscopes.**

- The **transmission electron microscope** (**TEM**) was the first type of Electron Microscope to be developed and is patterned exactly on the light transmission microscope except that a focused beam of electrons is used instead of light to "see through" the specimen. It was developed by Max Knoll and Ernst Ruska in Germany in 1931.

- The first **scanning electron microscope** (**SEM**) debuted in 1938 (Manfred Von Ardenne) with the first commercial instruments around 1965. Its late development was due to the electronics involved in "scanning" the beam of electrons across the sample.

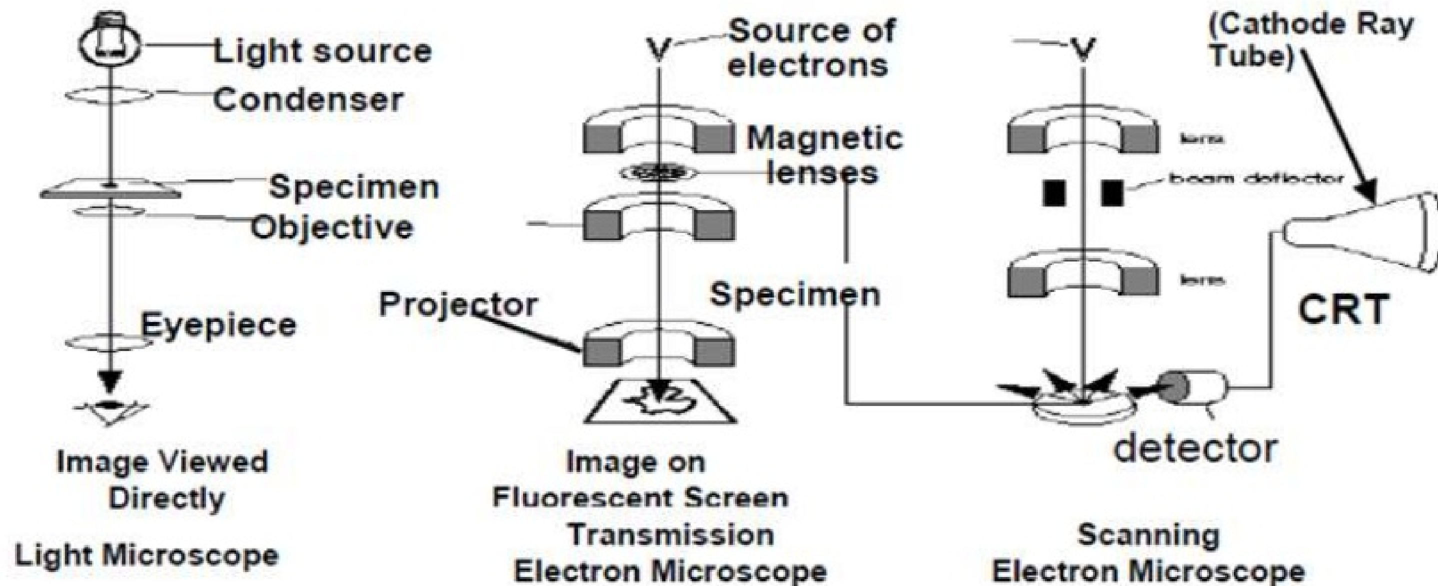
A **scanning electron microscope** (**SEM**) is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition, and other properties.

Comparing Microscopes



	OPTICAL MICROSCOPE	ELECTRON MICROSCOPE
The source of illumination	illuminating the sample	light is replaced by an electron gun built into the column
The lens type	Glass lenses	Electromagnetic lenses
Magnification method	Magnification is changed by moving/changing the lens	Focal length is charged by changing the current through the lens coil
Viewing the sample	Eyepiece (ocular)+ digital camera	Fluorescent screen or digital camera
Use of vacuum	No vacuum needed	Entire electron path from gun to camera must be under vacuum

Comparison of OM,TEM and SEM



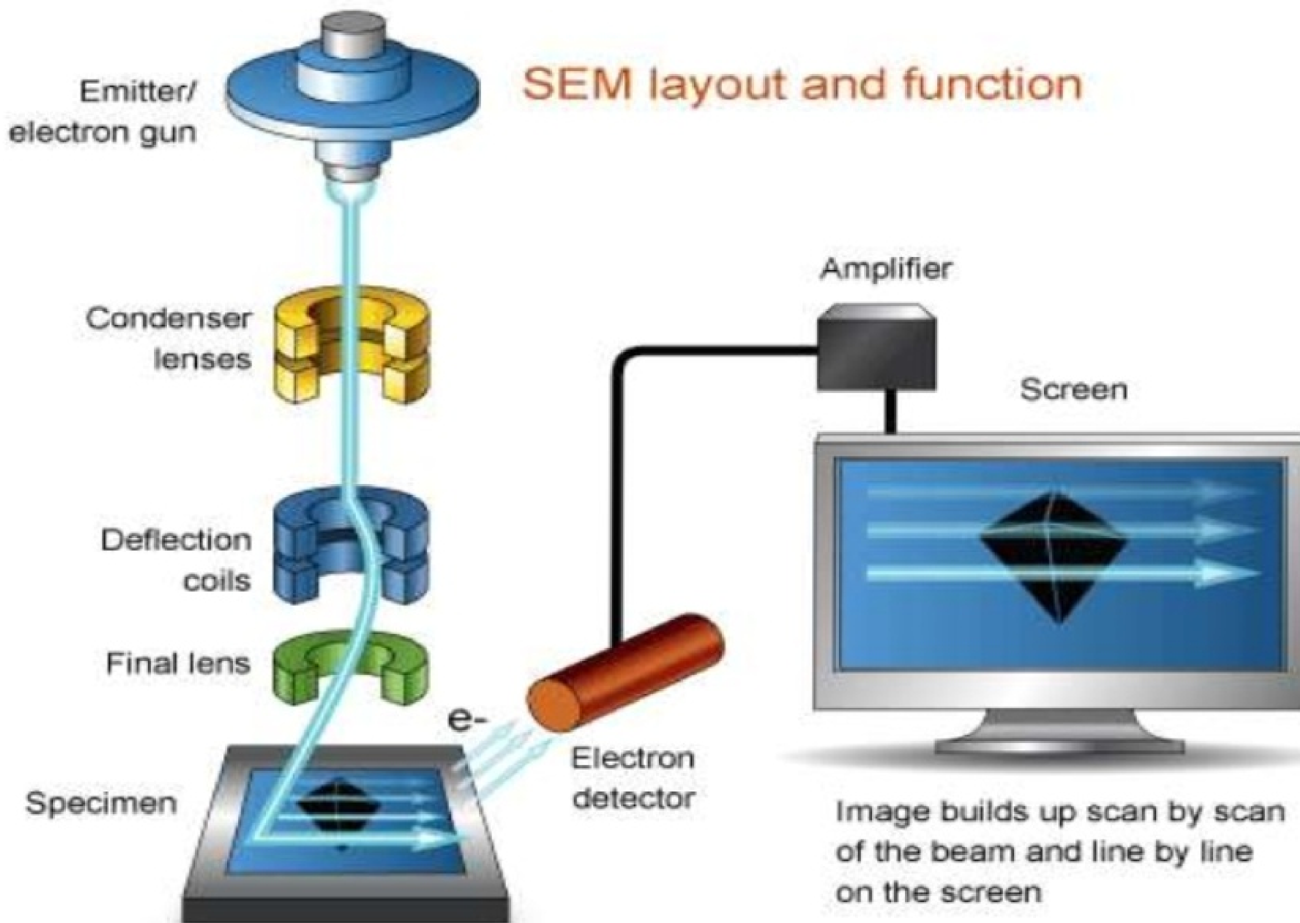
Principal features of an optical microscope, a transmission electron microscope and a scanning electron microscope, drawn to emphasize the similarities of overall design.

Advantages of Using SEM over OM

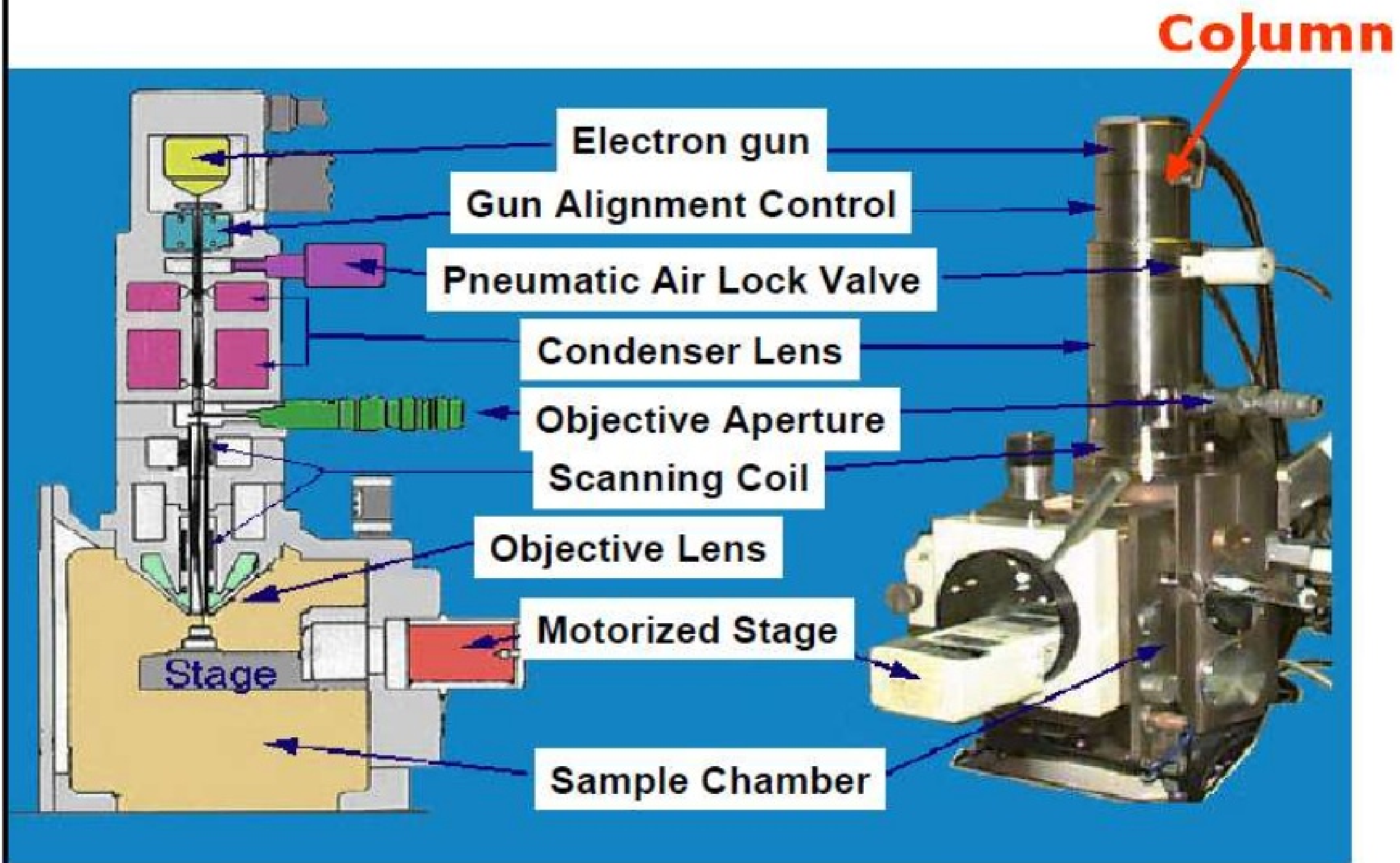
	Magnification	Depth of Field	Resolution
OM:	4x – 1400x	0.5mm	~ 0.2 μm
SEM:	10x – 500Kx	30mm	1.5nm

- The SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time and produces an image that is a good representation of the three-dimensional sample.
- The combination of higher magnification, larger depth of field, greater resolution, compositional and crystallographic information makes the SEM one of the most heavily used instruments in academic/national lab research areas and industry.

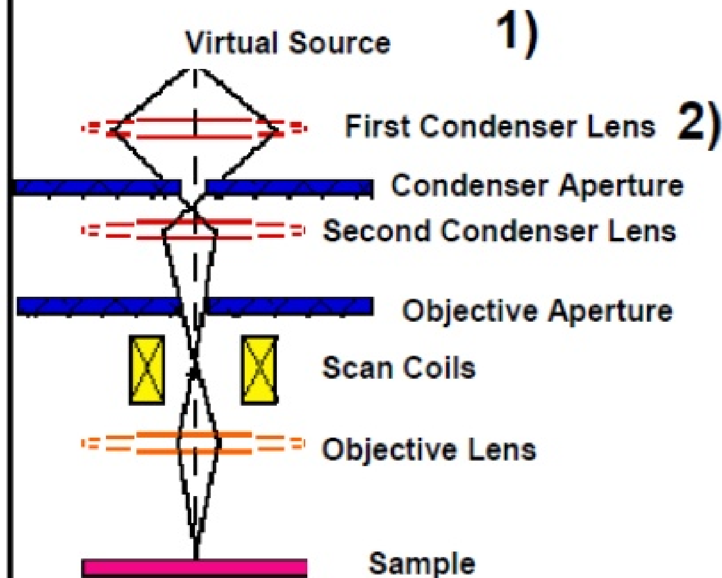
How does SEM works? Image formation



How does SEM works?



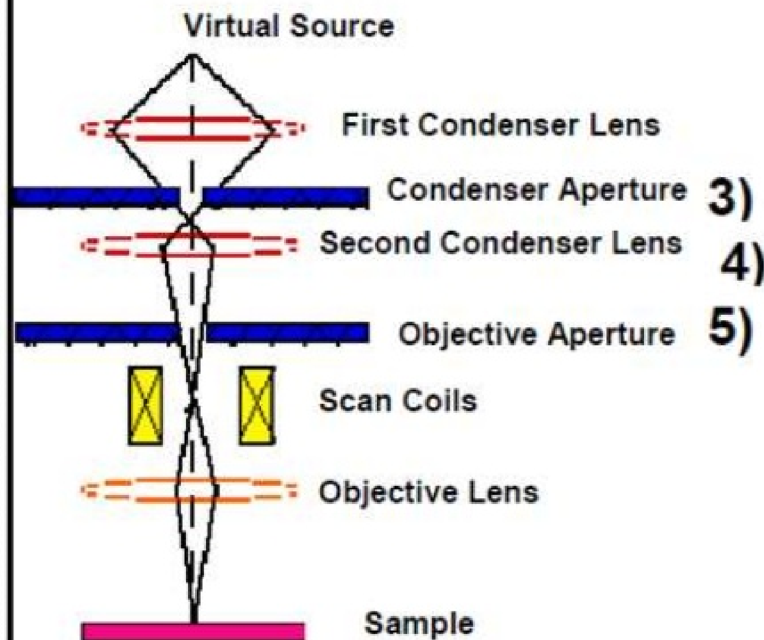
Scanning Electron Microscope



1) The "Virtual Source" at the top represents the electron gun, producing a stream of monochromatic electrons.

2) The stream is condensed by the first condenser lens (usually controlled by the "coarse probe current knob"). This lens is used to both form the beam and limit the amount of current in the beam. It works in conjunction with the condenser aperture to eliminate the high-angle electrons from the beam.

Scanning Electron Microscope

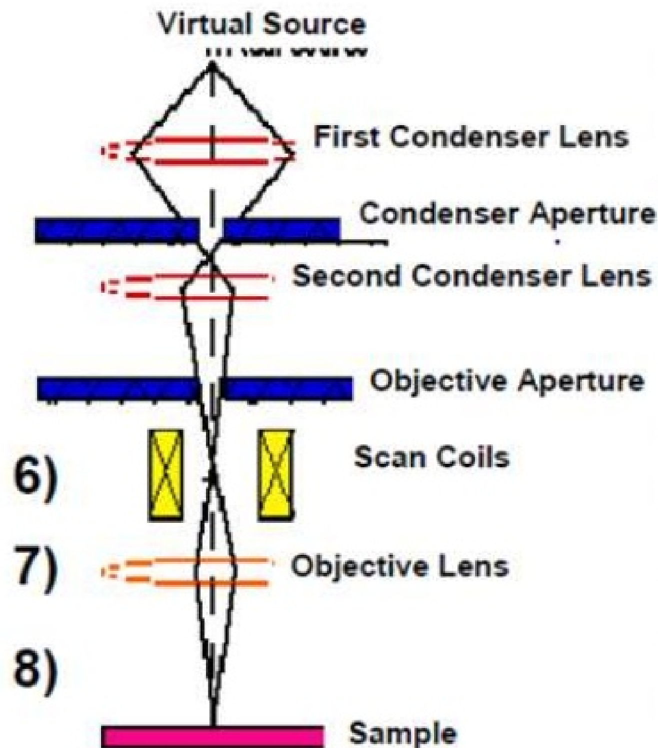


3) The beam is then constricted by the condenser aperture (usually not user selectable), eliminating some high-angle electrons.

4) The second condenser lens forms the electrons into a thin, tight, coherent beam and is usually controlled by the "fine probe current knob".

5) A user selectable objective aperture further eliminates high-angle electrons from the beam.

Scanning Electron Microscope

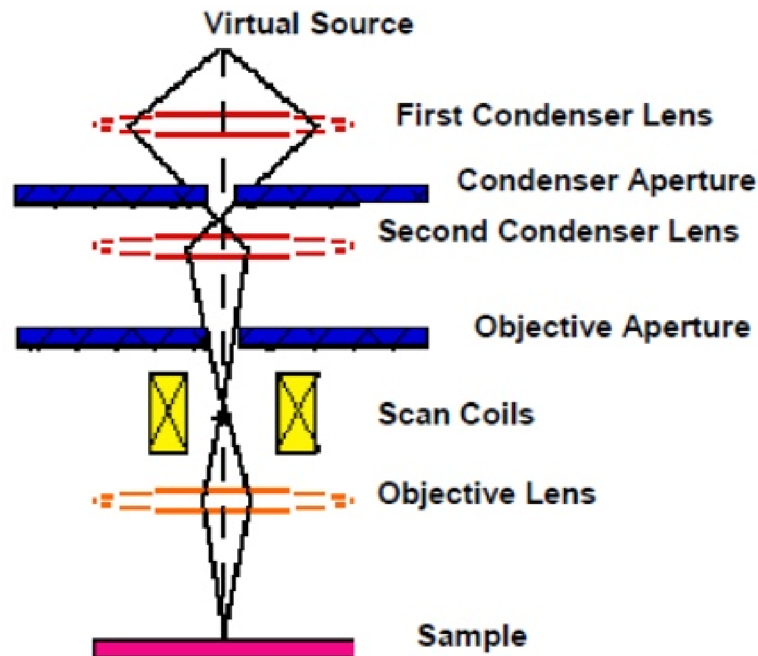


6) A set of coils then "scan" or "sweep" the beam in a grid fashion (like a television), dwelling on points for a period of time determined by the scan speed (usually in the microsecond range).

7) The final lens, the objective, focuses the scanning beam onto the part of the specimen desired.

8) When the beam strikes the sample (and dwells for a few microseconds) interactions occur inside the sample and are detected with various instruments.

Scanning Electron Microscope

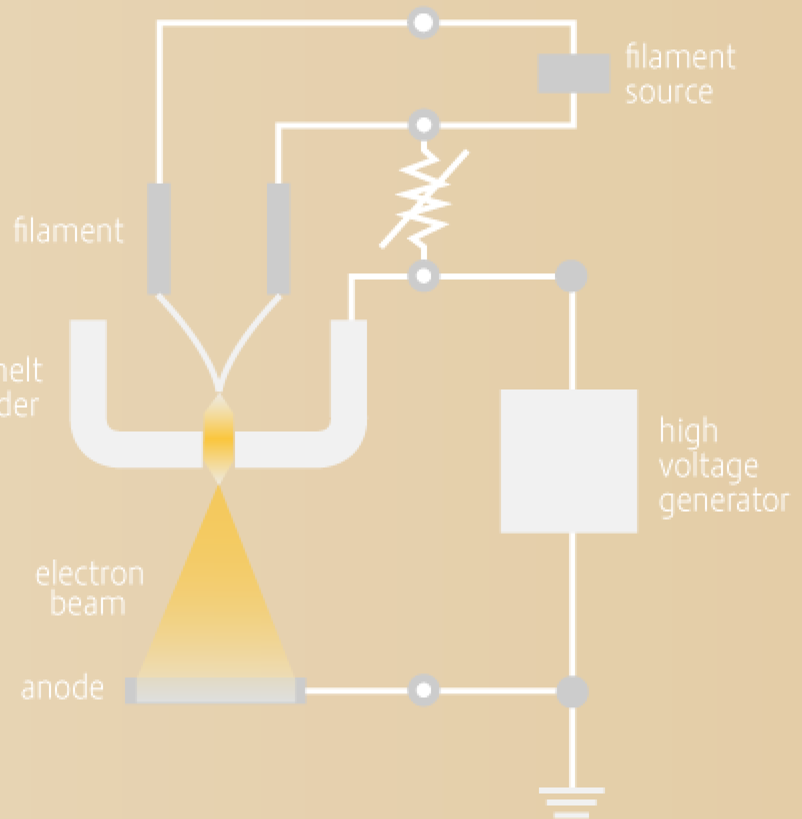


9) Before the beam moves to its next dwell point these instruments count the number of e^- interactions and display a pixel on a CRT whose intensity is determined by this number (the more reactions the brighter the pixel).

10) This process is repeated until the grid scan is finished and then repeated, the entire pattern can be scanned 30 times/sec.

CORE TECHNOLOGY: The Electron Gun

Three main sources
- of electrons:
  - Tungsten
  - LaB6 (lanthanum hexaboride)
  - Field Emission Gun (FEG)
  - Different costs and
- benefits of each



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Electron guns

Types of electron guns

Heated tungsten

A heated filament made from the metal tungsten. Much in the way that an incandescent lightbulb works, the high voltage that is fed through the filament causes electrons to be kicked off the filament. The amount of energy required is known as the work function.

Lanthanum hexaboride (LaB_6)

The LaB_6 filament is also a thermal filament. However, its work function is lower than for a tungsten filament, so it is more efficient.

Tungsten field emission gun (FEG)

The FEG gun is not a thermal filament. Instead, electrons are expelled by applying a very powerful electric field very close to the filament tip. The size and proximity of the electric field to the electron reservoir in the filament causes the electrons to tunnel out of the reservoir.

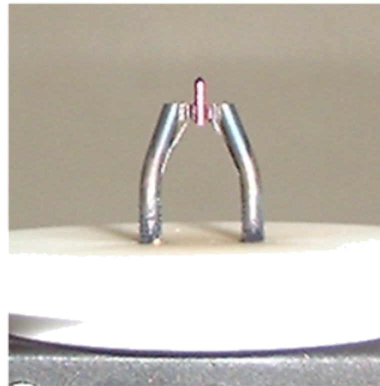
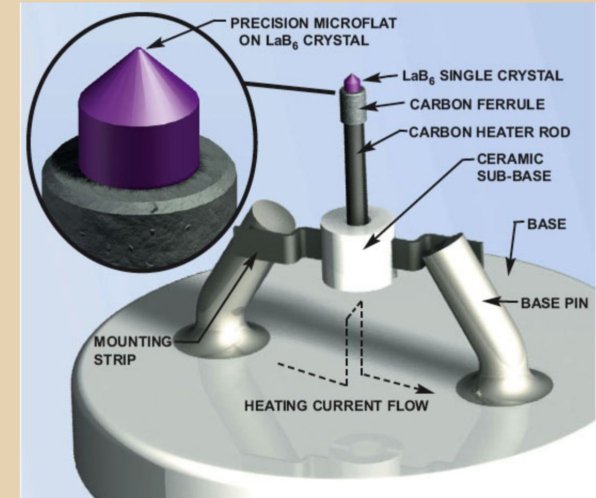
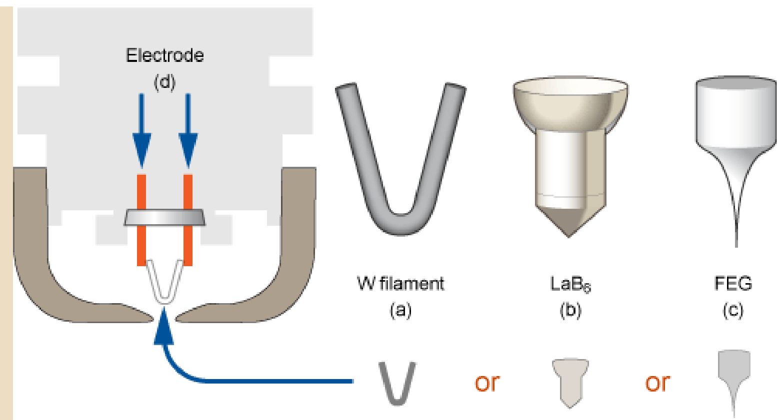


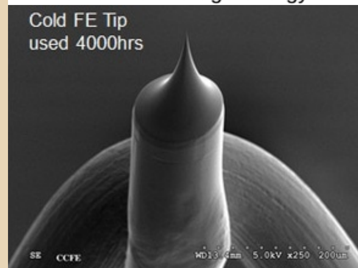
Figure 1. A LaB_6 filament.





Temporal coherence

Because the emitted electrons in the various types of guns are heated, their energy distribution is not a sharp peak. Instead, they have a Boltzmann distribution that can vary widely depending on the type of filament. For a good microscope, you want $\Delta E/E$ to be as small as possible, that is, the energy distribution to be a small fraction of the average energy of the electrons. Following is a list of the average energy distributions:

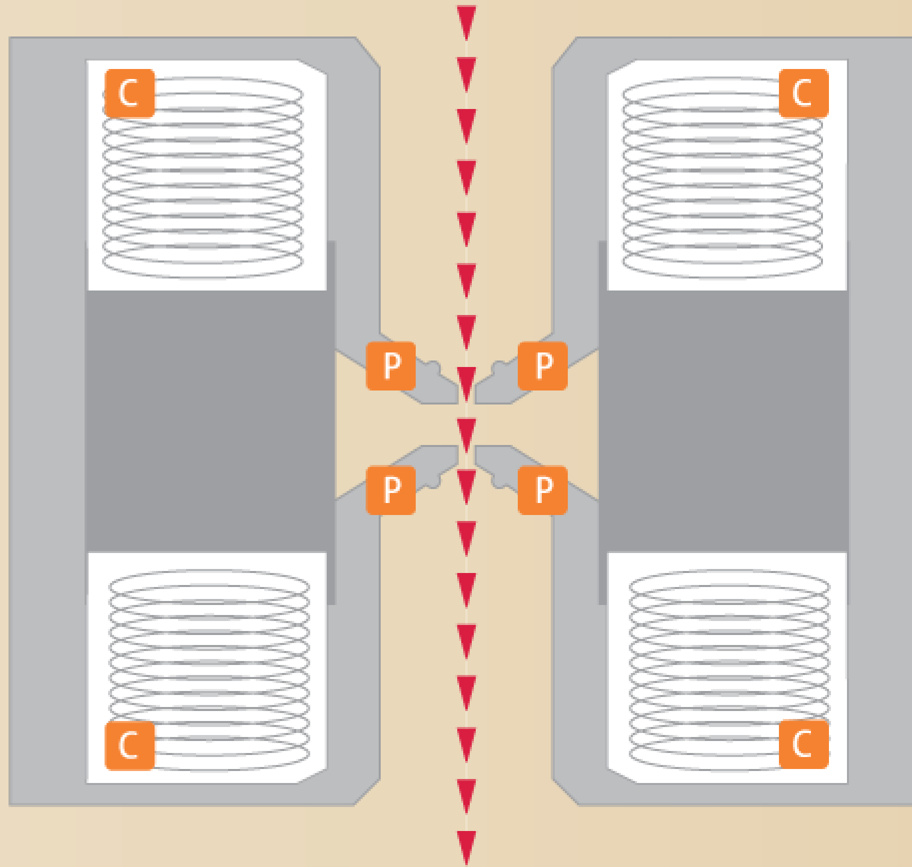


Filament	Energy Distribution (ΔE)
Heated tungsten	2.5 eV
LaB_6	1.5 eV
Warm FEG	1.0 eV
Cold FEG	0.25 eV

It is clear that the FEG based microscopes have an advantage over the other types. For this reason, they are most often used for high resolution imaging. Ideally, cold FEGs are the best. However, they suffer from rapid degradation and are not economical.

CORE TECHNOLOGY: Electromagnetic Lenses

electron beam



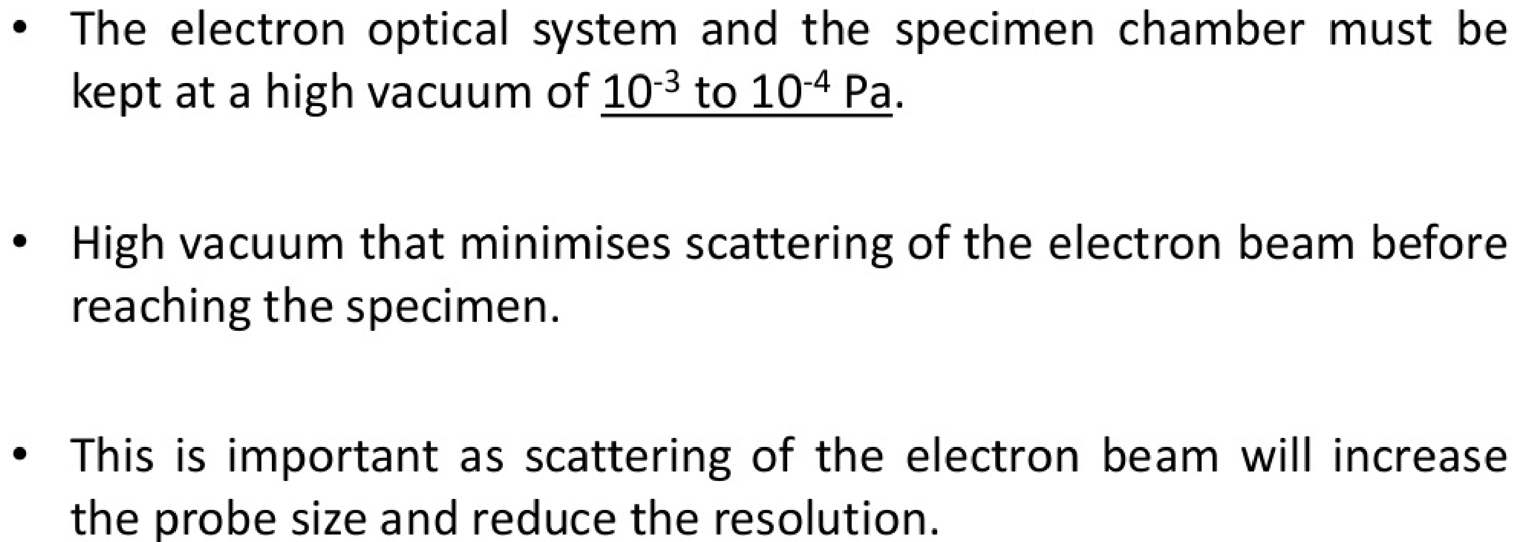
C electrical coil

P soft iron pole piece

The electron beam size and direction can be finely tuned through EM lenses. Need advanced electronics.

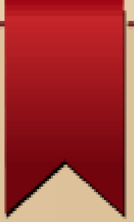
CORE TECHNOLOGY: Vacuum

Electrons can freely propagate only in high-vacuum conditions. So an efficient pumping system is necessary for the column.



- The electron optical system and the specimen chamber must be kept at a high vacuum of 10^{-3} to 10^{-4} Pa.
- High vacuum that minimises scattering of the electron beam before reaching the specimen.
- This is important as scattering of the electron beam will increase the probe size and reduce the resolution.

SEM sample preparation



- **Cleaning the surface of the specimen**

- The proper cleaning of the surface of the sample is important because the surface can contain a variety of unwanted deposits, such as dust, silt, and detritus, media components, or other contaminants, depending on the source of the biological material and the experiment that may have been conducted prior to SEM specimen preparation.

- **Stabilizing the specimen**

- Stabilization is typically done with fixatives. Fixation can be achieved, for example, by perfusion and microinjection, immersions, or with vapours using various fixatives including aldehydes, osmium tetroxide, tannic acid, or thiocarbohydrazide

- **Rinsing the specimen**

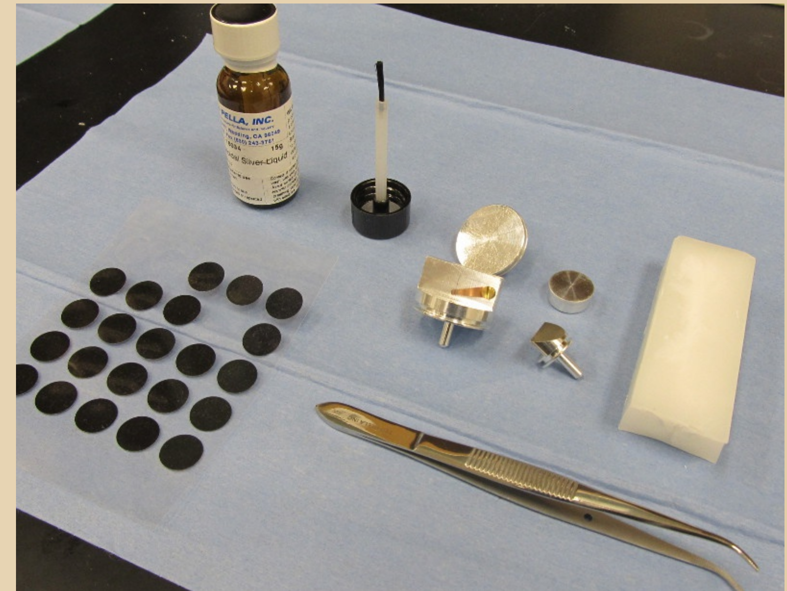
- After the fixation step, samples must be rinsed in order to remove the excess fixative.

- **Dehydrating the specimen**

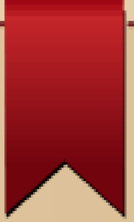
- The dehydration process of a biological sample needs to be done very carefully. It is typically performed with either a graded series of acetone or ethanol.

- **Drying the specimen**

- The scanning electron microscope (like the transmission electron microscope) operates with a vacuum. Thus, the specimens must be dry or the sample will be destroyed in the electron microscope chamber. Many electron microscopists consider a procedure called the Critical Point Drying (CPD) as the gold standard for SEM specimen drying. Carbon dioxide is removed after its transition from the liquid to the gas phase at the critical point, and the specimen is dried without structural damage.



SEM sample preparation (cont.)

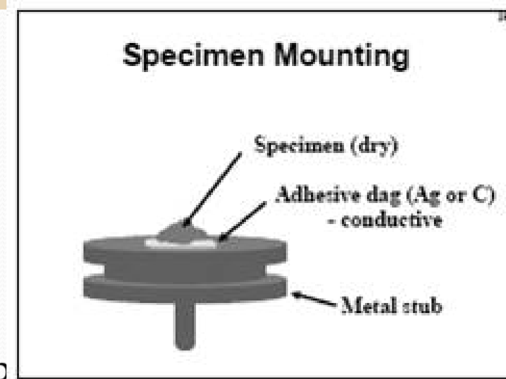


- **Mounting the specimen**

  - After the sample have been cleaned, fixed, rinsed, dehydrated, and dried using an appropriate protocol, specimens must be mounted on a holder that can be inserted into the scanning electron microscope. Samples are typically mounted on metallic (aluminum) stubs using a double-sticky tape. It is important that the investigator first decides on the best orientation of the specimen on the mounting stub before attaching it. A re-orientation proves difficult and can result in significant damage to the sample.

- **Coating the specimen**

  - The idea of coating the specimen is to increase its conductivity in the scanning electron microscope and to prevent the build-up of high voltage charges on the specimen by conducting the charge to ground. Typically, specimens are coated with a thin layer of approximately 20 nm to 30 nm of a conductive metal (e.g., gold, gold-palladium, or platinum).

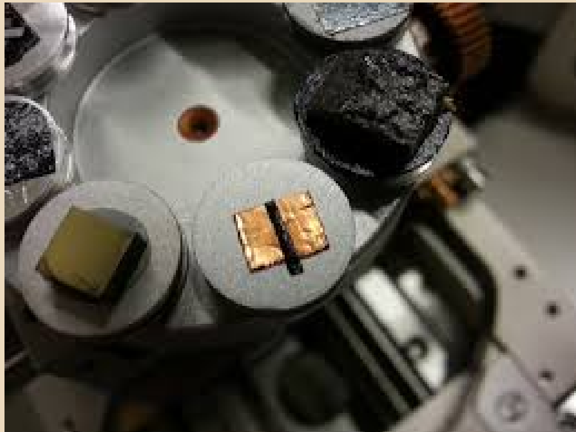


Sputtering system (Cr, Au, C)

Stubs and samples for SEM

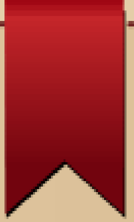
- Stubs with samples (of various shapes) are placed in a revolver and inserted in the SEM chamber

Coffee beans



A spider coated in gold, having been prepared for viewing with a scanning electron microscope

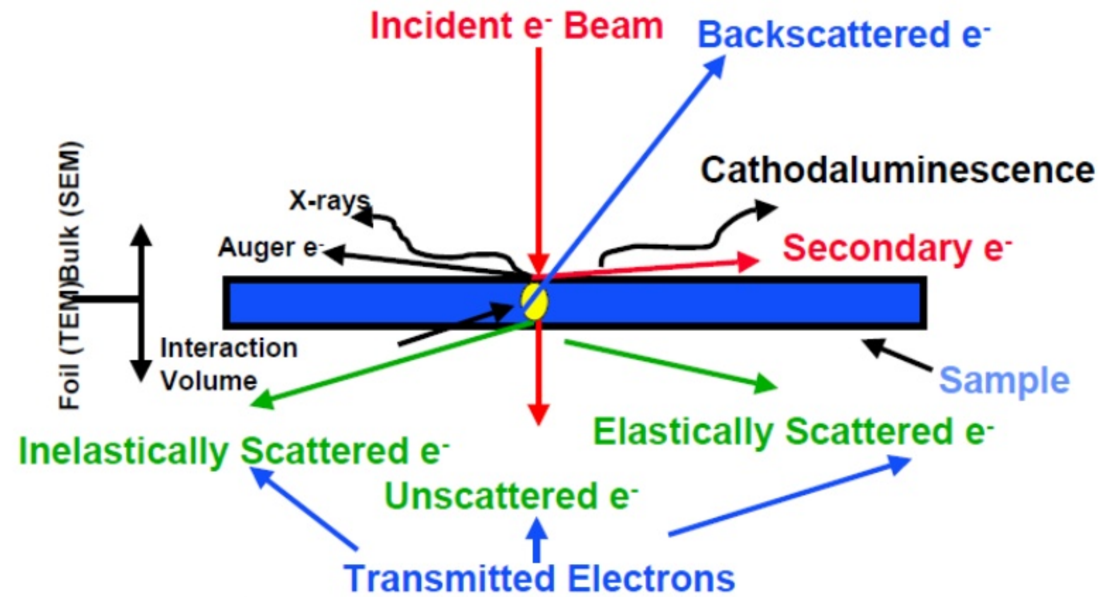
Physics of SEM

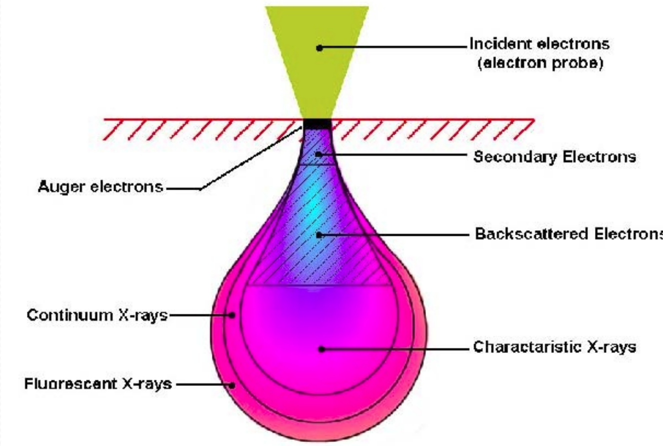


1. Electron optical column consists of:
– electron source to produce electrons
– magnetic lenses to de-magnify the beam
– magnetic coils to control and modify the beam
– apertures to define the beam, prevent electron spray, etc.

2. Vacuum systems consists of:
– chamber which "holds" vacuum, pumps to produce vacuum
– valves to control vacuum, gauges to monitor vacuum

3. Signal Detection & Display consists of:
– detectors which collect the signal
– electronics which produce an image from the signal

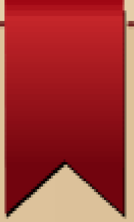


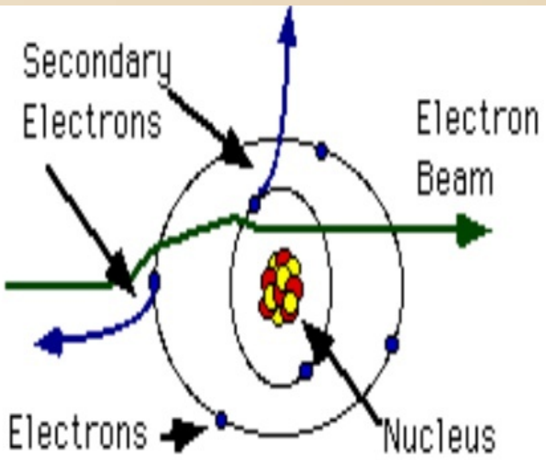


Electron Beam Interaction Diagram

Probing depth and ultimate resolution depend on the detected signal

Secondary and backscattered electrons



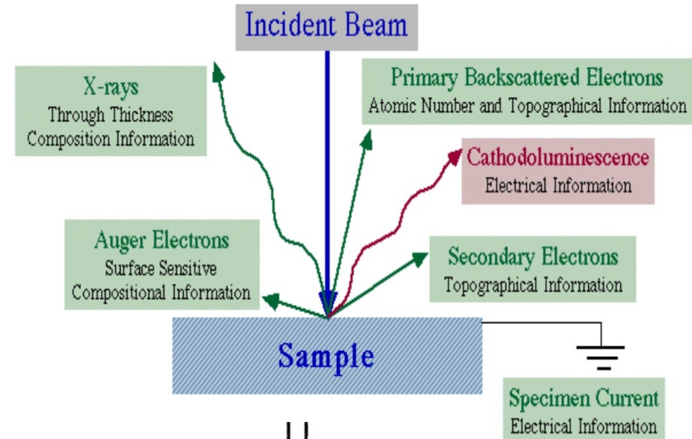


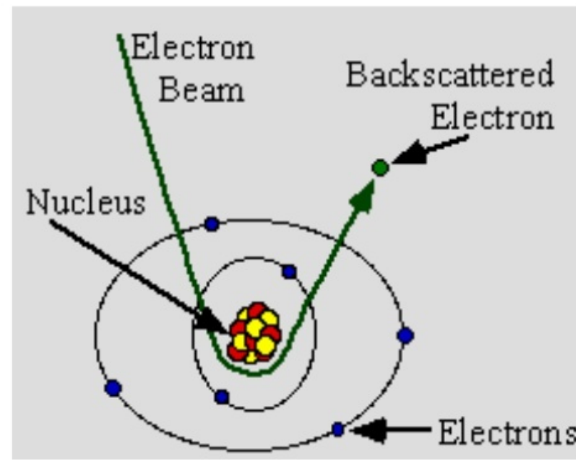
Secondary Electron Generation

-*sample* electrons ejected by the primary beam [green line]

-low energy

-surface detail & topography





Backscattered Electron Generation

-SEM-BSE

-*primary* beam electrons

-high energy

-composition and topography [specimen atomic number]

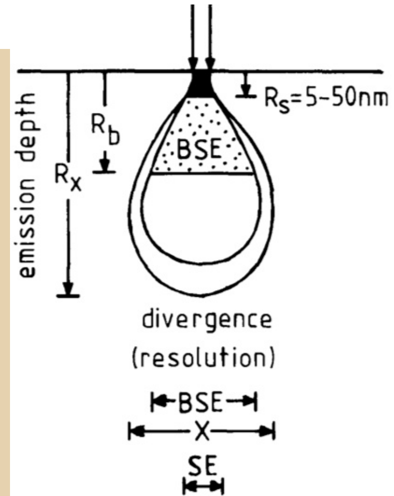
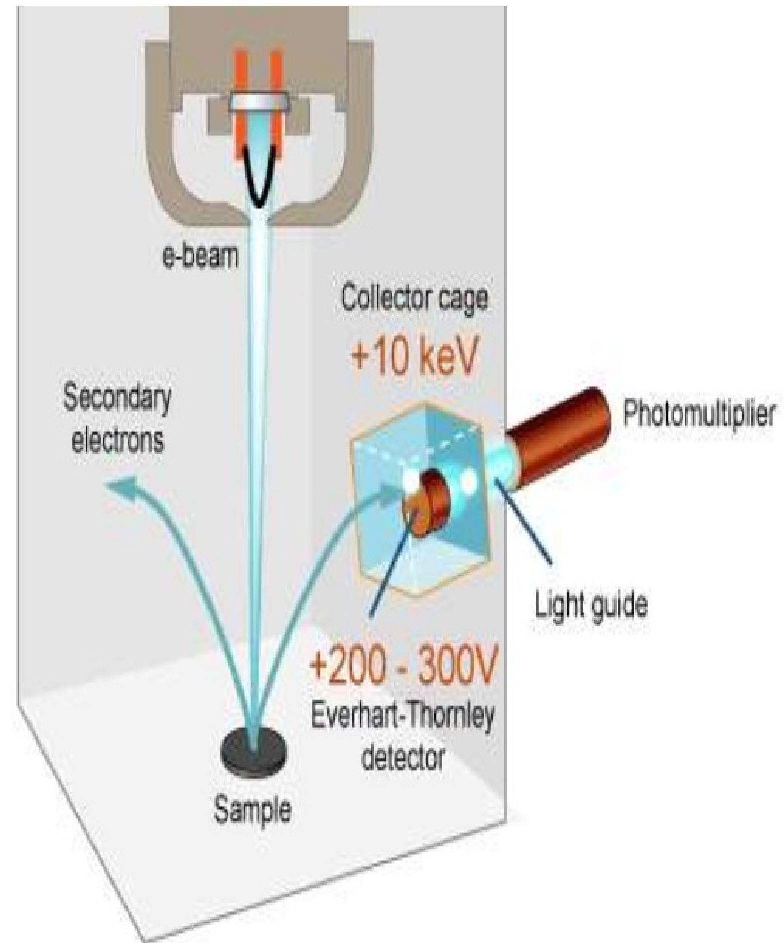


FIG. 2. Comparison of penetration ranges (R_s, R_b, R_x) and spatial resolutions (SE, BSE, X) for secondary electron, backscattered electron and X-ray emission signals from elements with low to medium atomic numbers. After Goldstein and Yakowitz (1975).

Secondary electron detection

- **Secondary electron detector (SED) –**
  - Everhart-Thornley Detector
  - Due to the low energies of secondary electrons (SE) (~2 to 50 eV) they are ejected only from near-surface layers.
  - Therefore, secondary electron imaging (SEI) is ideal for recording topographical information.
  - To attract (collect) these low-energy electrons, usually around +200 to 300V is applied to the cage at the front end of the detector.
  - A higher kV (7 to 12kV) is applied inside the cage i.e. to the scintillator, to accelerate the electrons into the scintillator screen.



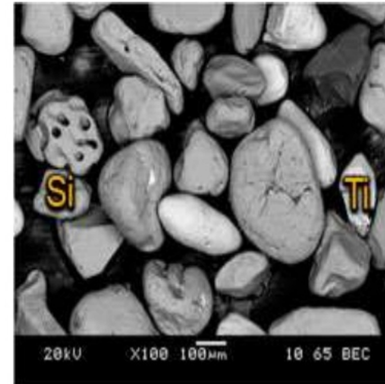
Backscattered electrons

- High atomic number (Z) -> greater elastic scattering & shorter penetration depth
- Greater elastic scattering → better spatial resolution
- Materials with low Z have greater inelastic scattering
- High Z materials appear brighter

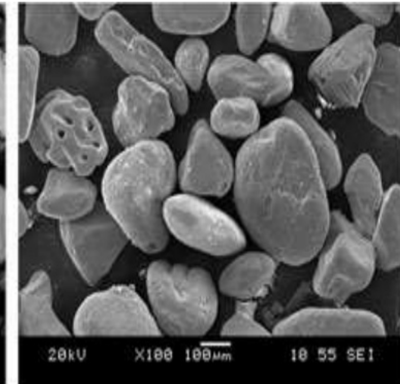
-Detect composition differences
-Show topography
-Show crystal orientation
-Show grain boundaries, phase boundaries, and other crystal features

Backscattered electron (BSE) images

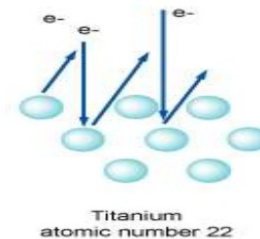
- Backscattered (BS) electrons are high-energy electrons (>50 eV) ejected back out from the sample. These BSE are used to produce a different kind of image.
- Such an image uses contrast to tell us about the average atomic number of the sample.
- The higher the average atomic number, the more primary electrons are scattered (bounced) back out of the sample. This leads to a brighter image for such materials.
- For example, a grain of sand (in a mixture of mineral sand) that is made up of a titanium mineral looks whiter than a grain made of a silicon material (Ti versus Si). In the image (next slide), the left picture is taken using backscattered electrons. Here there is a difference in contrast between the grains labelled Si and Ti whereas in the right image, taken using secondary electrons, there is no difference in contrast between these grains.

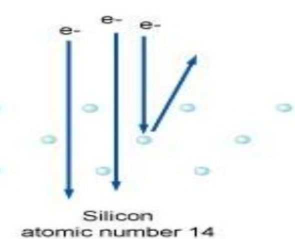


Backscattered electon image (BSE)



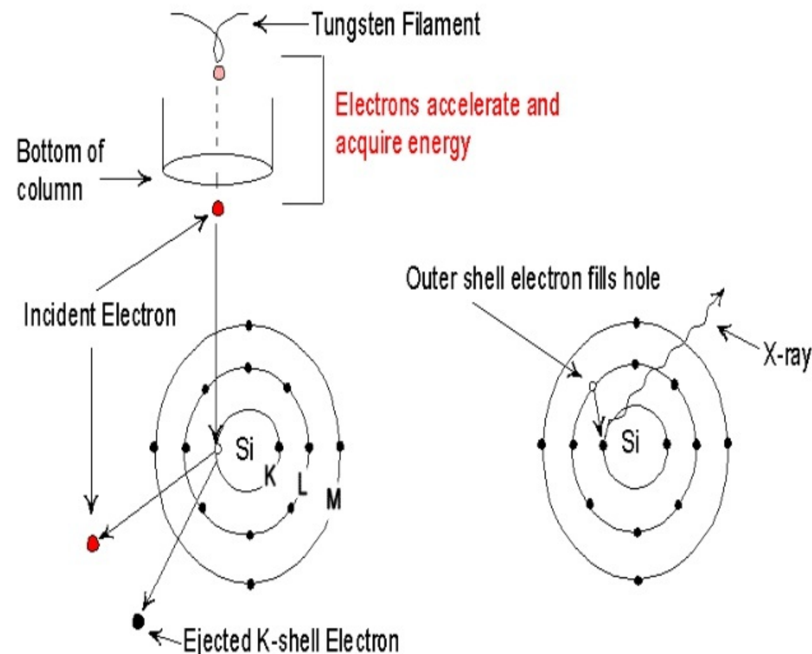
Secondary electron image (SE)



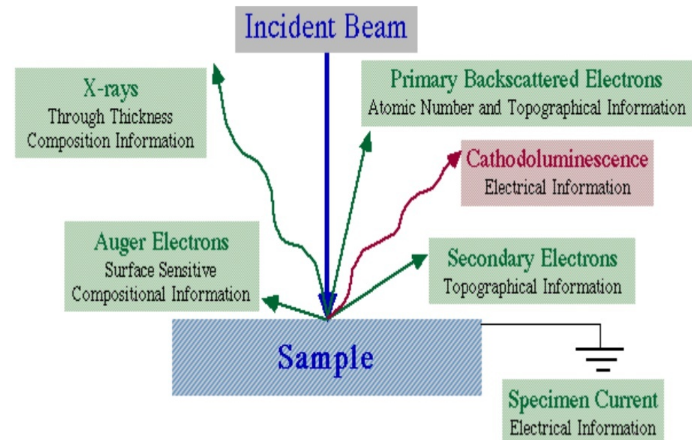


x-ray generation

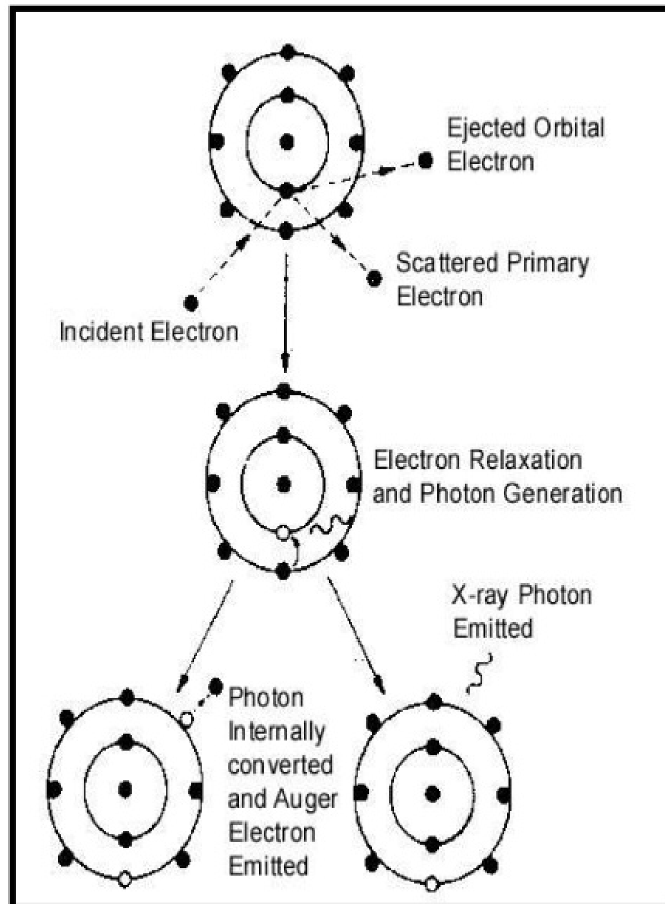
X-Rays Generation



- X-Rays are produced to balance the difference of energy when an electron from outer shell replaces the one from inner shell.



x-ray generation



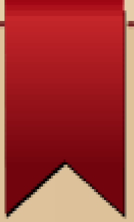
- An incoming high-energy electron dislodges an inner-shell electron in the target, leaving a vacancy in the shell
- An outer shell electron then "jumps" to fill the vacancy
- A characteristic x-ray (equivalent to the energy change in the "jump") is generated

or

- The energy released from an electron replacement event produces a photon with an energy exactly equal to the drop in energy.

1. Elemental identification
2. Chemical characterization
3. Quantitative analysis

SEM @Unicam (2016)



SEM Optics and detectors @Unicam

Electron Emitter	Thermal field emission type, stability better than 0.2 %/h
Acceleration Voltage	0.02 – 30 kV

Detectors included in Base System

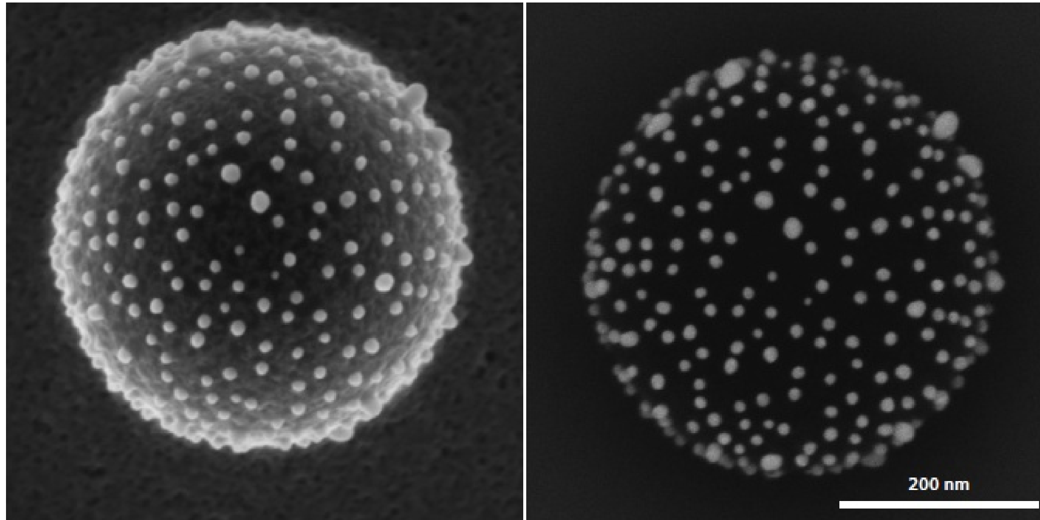
- Inlens Secondary Electron detector
- Everhart Thornley Secondary Electron detector
- High efficiency VPSE detector (included in variable pressure option)

Specimen Stage

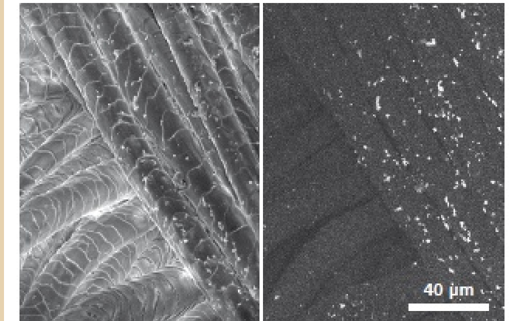
5-axes motorized eucentric specimen stage

Gemini technology. Schematic cross-section of Gemini optical column with beam booster, Inlens detectors and Gemini objective.

BSE and secondary electrons images



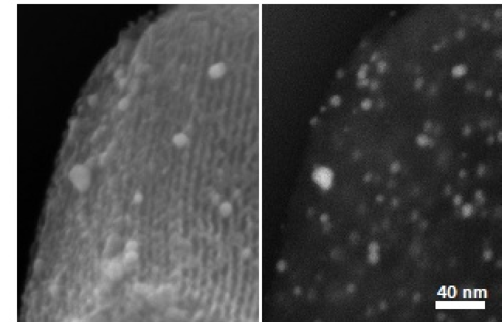
Precursor material for functional surface, gold nanoparticles on polystyrol sphere, imaged with GeminiSEM 500, at 3 kV. Left: Inlens SE image, surface topography. Right: EsB image, material contrast. Sample: courtesy of N. Vogel, University Erlangen-Nuremberg, Germany.



Silver nanoparticle coated natural fibers, imaged with NanoVP at 80 Pa, at 10 kV. Left: Inlens SE, surface detail. Right: Inlens EsB, silver particles. Both images acquired in parallel. Sample: courtesy of F. Simon, Leibniz-Institute for Polymer Research Dresden e.V., Germany.

Magnification	12 – 2,000,000 ×

Resolution*	ZEISS GeminiSEM 300
	0.8 nm @ 15 kV
	1.4 nm @ 1 kV



Catalysts, Zeolite with Ag nanoparticles, imaged at 5 kV using dual channel Inlens SE detector (left) and EsB detector (right). Sample: courtesy of G. Weinberg, Fritz-Haber-Institute of the Max-Planck society, Germany.