Supplementary to Basic anatomy of the electron microscope

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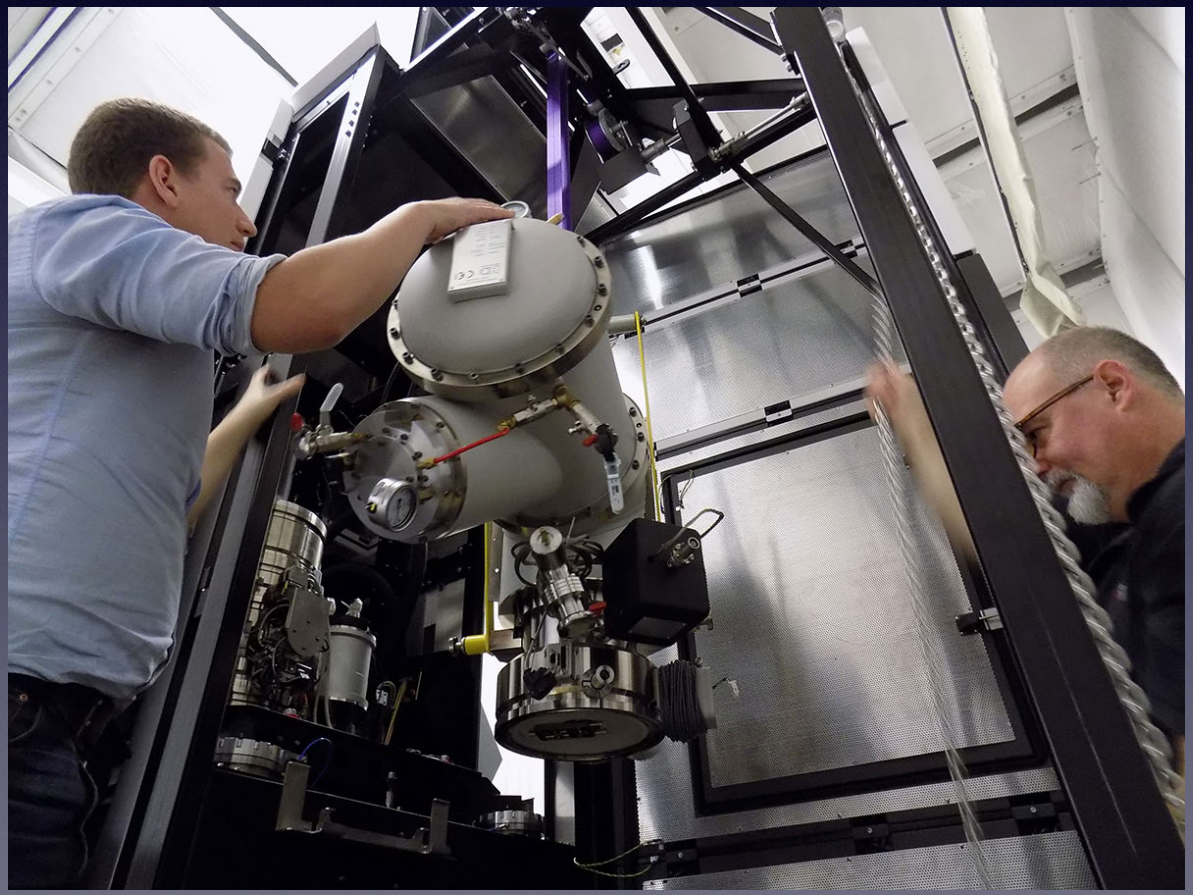
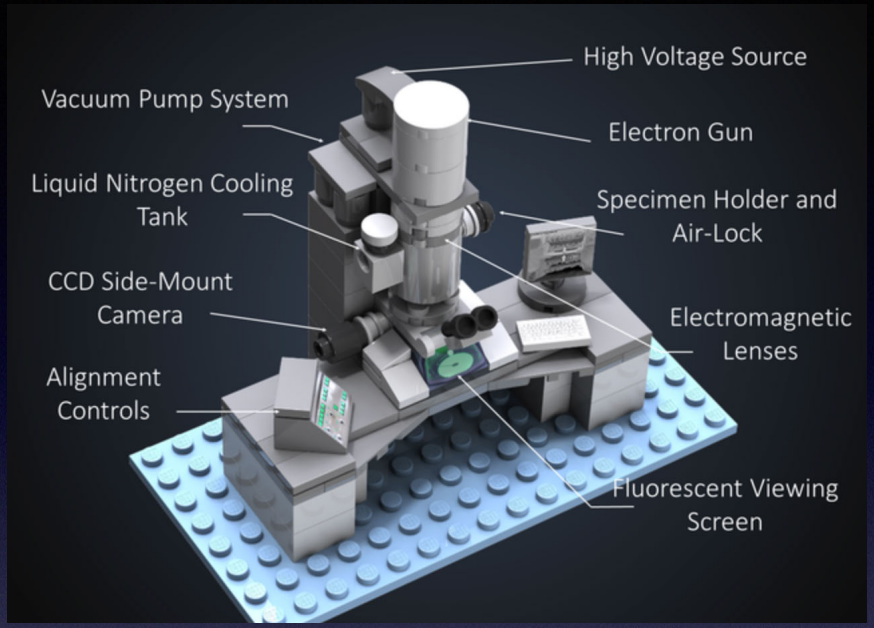
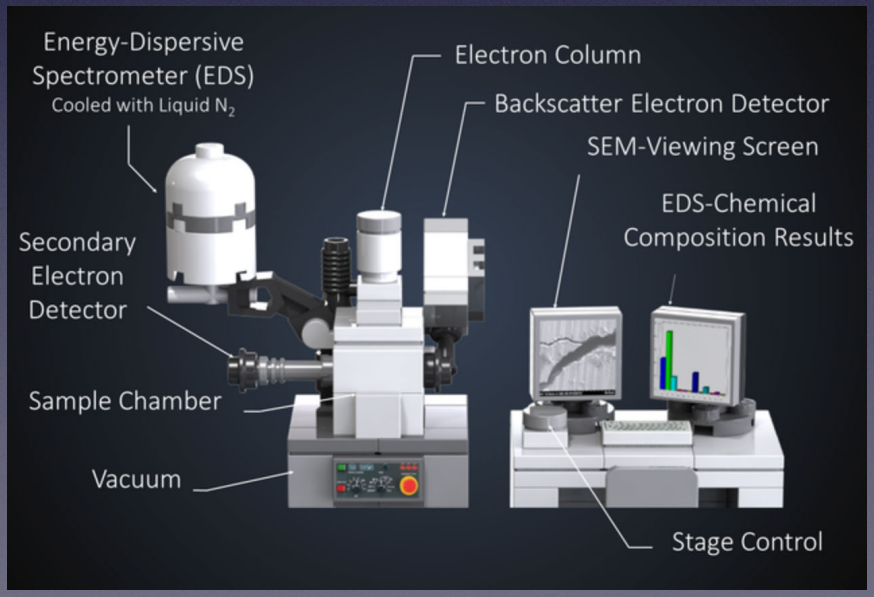


What we will add

- Different TEMs
- Scope Alignment
- Direct Detection Camera
- SEM anatomy

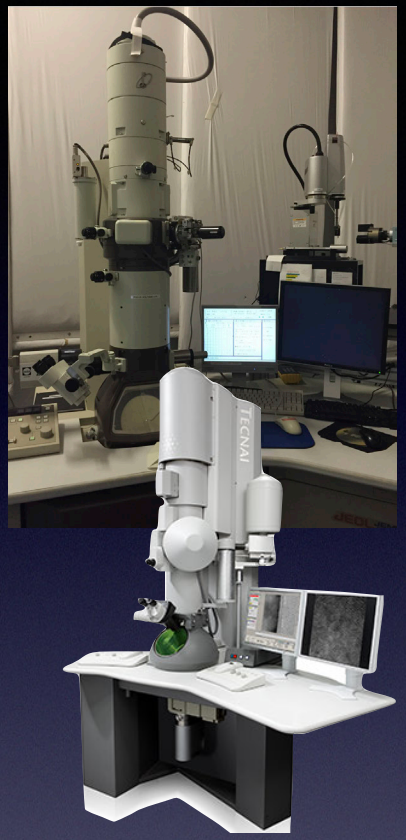


https://ideas.lego.com/projects/102281



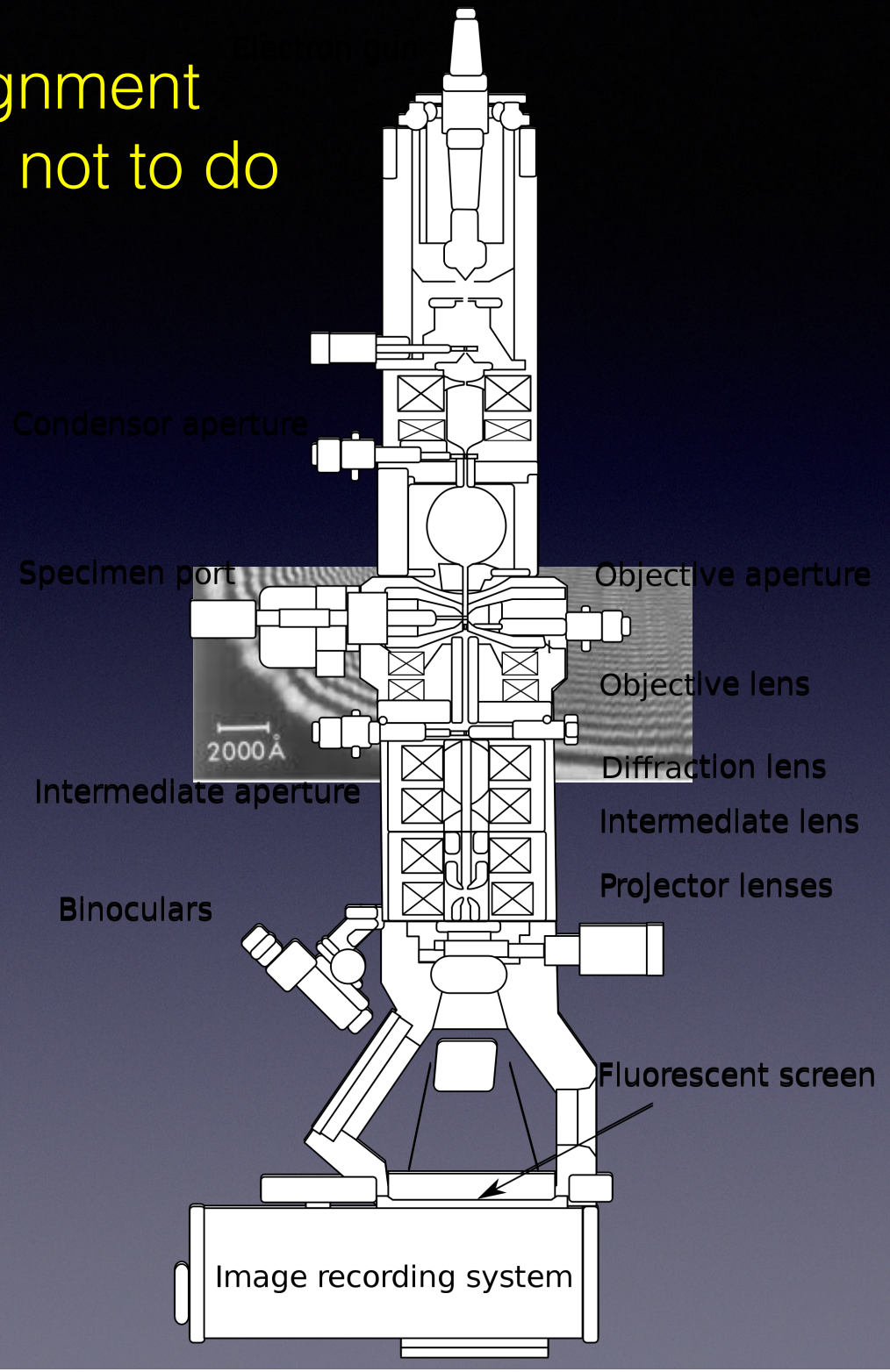
TEMs used in cryo-EM

- 80-120 kV: JEM 1230; Tecnai T12
  - W or LaB6
  - High contrast & robust
- 200 kV: JEM 2100F, Tecnai F20, Talos, Artica
  - FEG
  - 4 Å resolution (3.5 Å with 2d-xtal)
- 300 kV: JEM 3200FSC, Krios, Polara
  - FEG
  - Smaller effect on unwanted lens aberration
  - 2.5-3 Å resolution



Microscope Alignment
What to do & what not to do

- Do:
  - Start at eucentric height and focus
  - Check if it is already good before attempt
  - Align from top to bottom
- Not to do:
  - ~~Align without a way to undo~~
  - ~~Align when TEM is not stable (i.e., temperature)~~

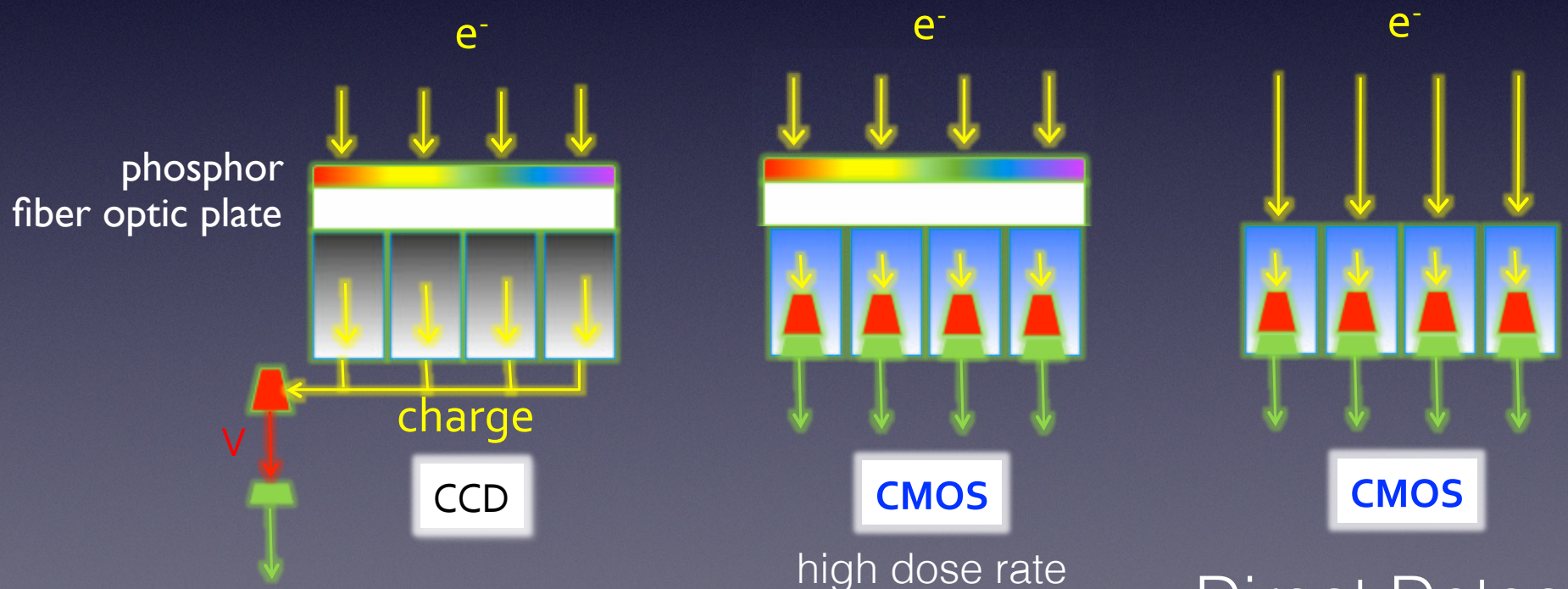


Digital Cameras for TEM

- Photon converted
- Direct sensing

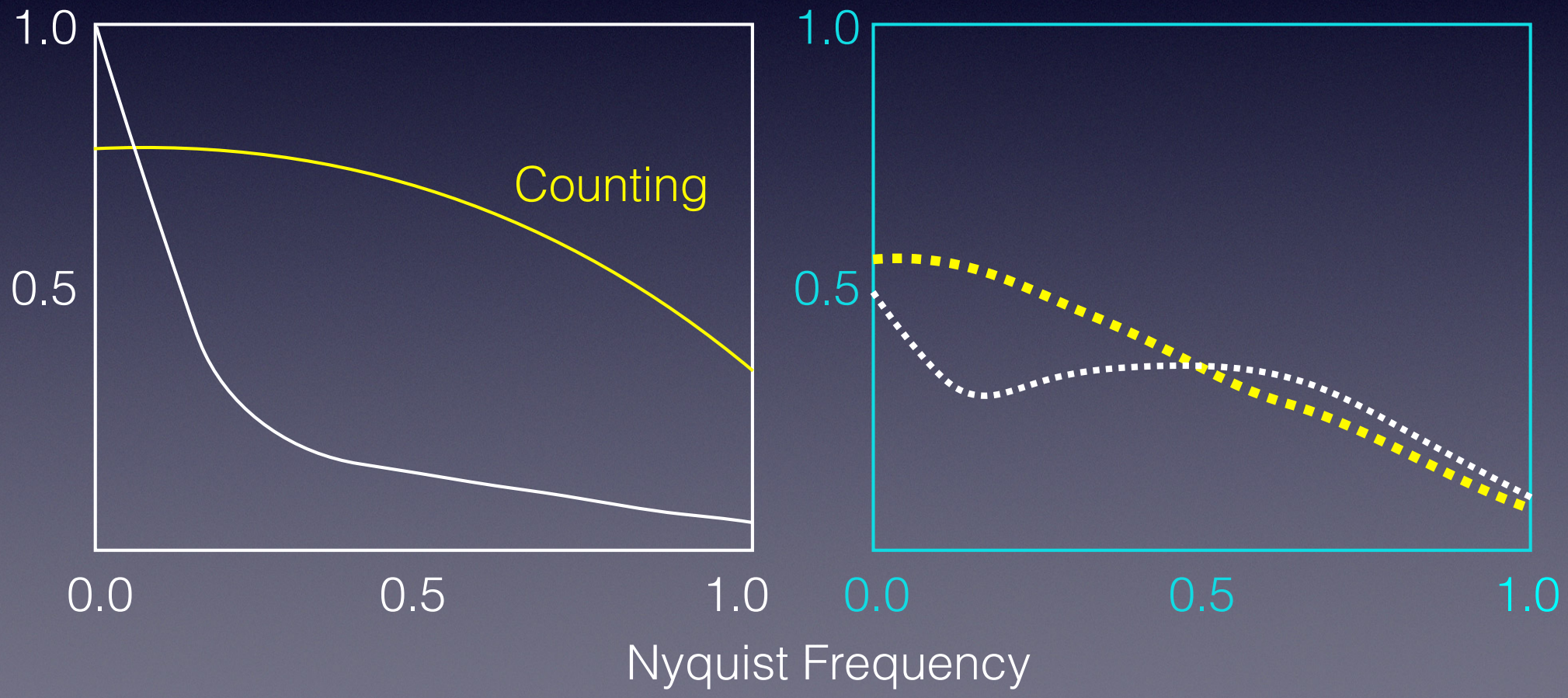
?

- CCD Charge Coupled Device
- CMOS Complementary Metal Oxide Semiconductor



Detector Performance Characterization

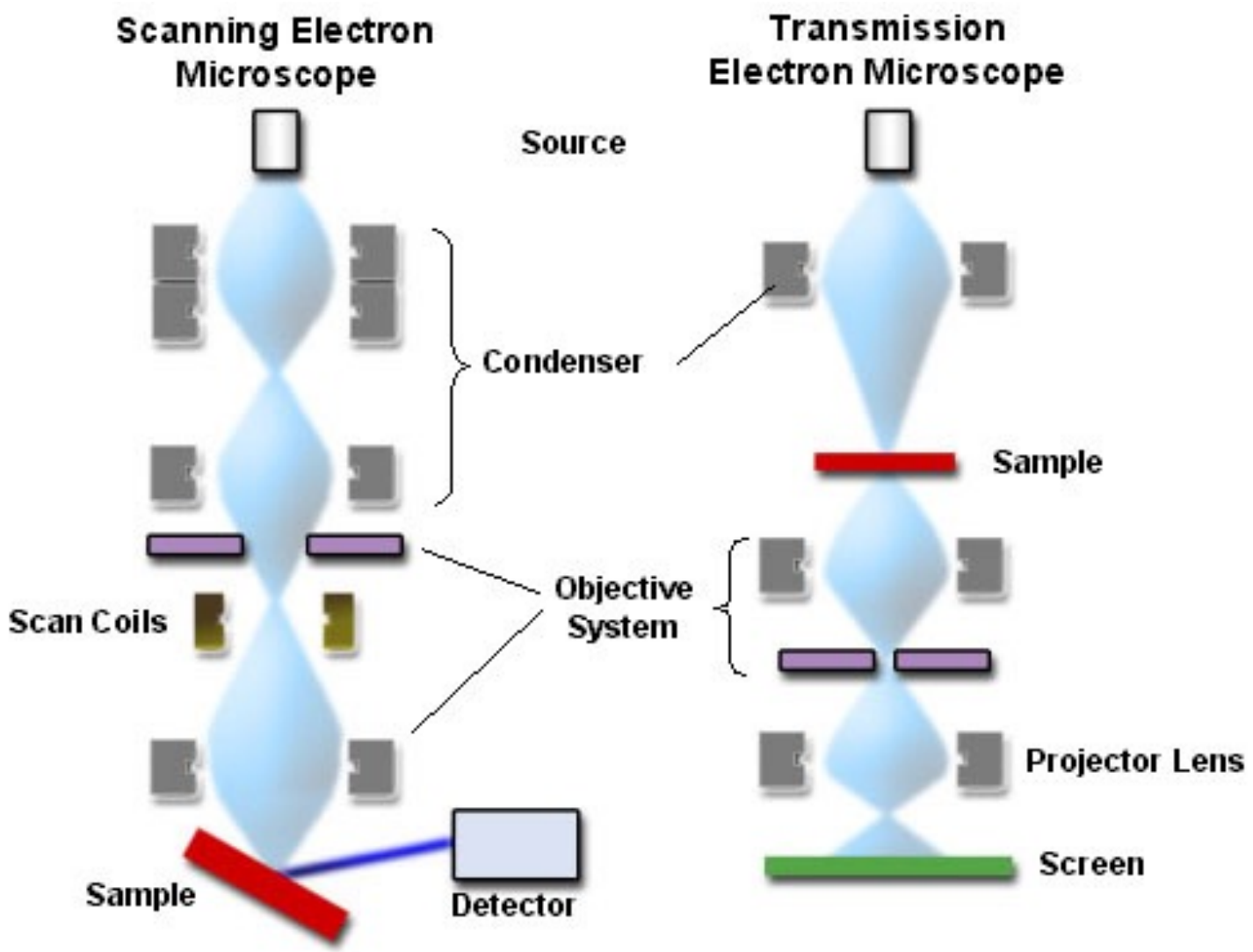
- MTF (Modulation Transfer Transform)
  - contribute to signal envelope
- DQE (Detector Quantum Efficiency)
  - S/N over spatial frequency range



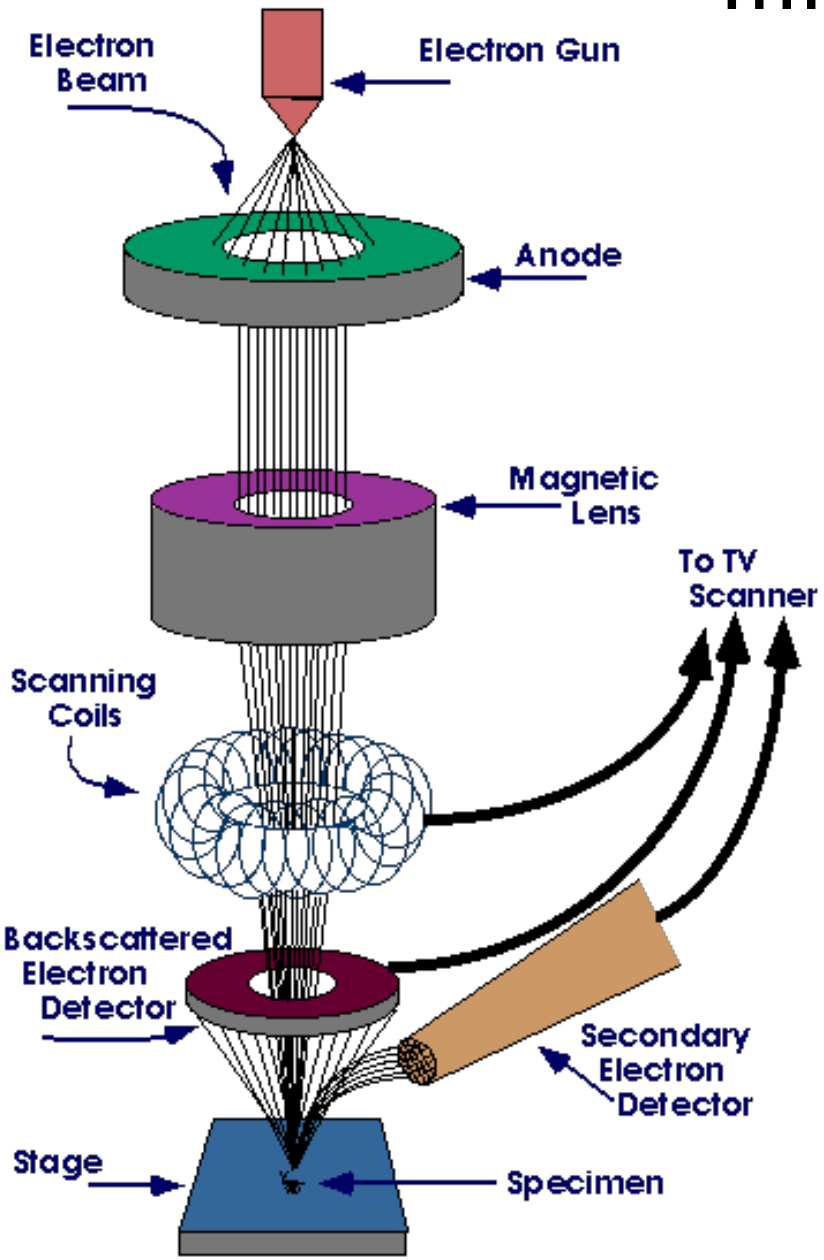
Recommended Reading

- Instrument Manual
- Light and Electron Microscopy
  - E. M. Slayter and H.S. Slayter (**ISBN-13:** 978-0521339483)
- Internet

Basic anatomy of a scanning electron microscope



Basic anatomy of a scanning electron microscope



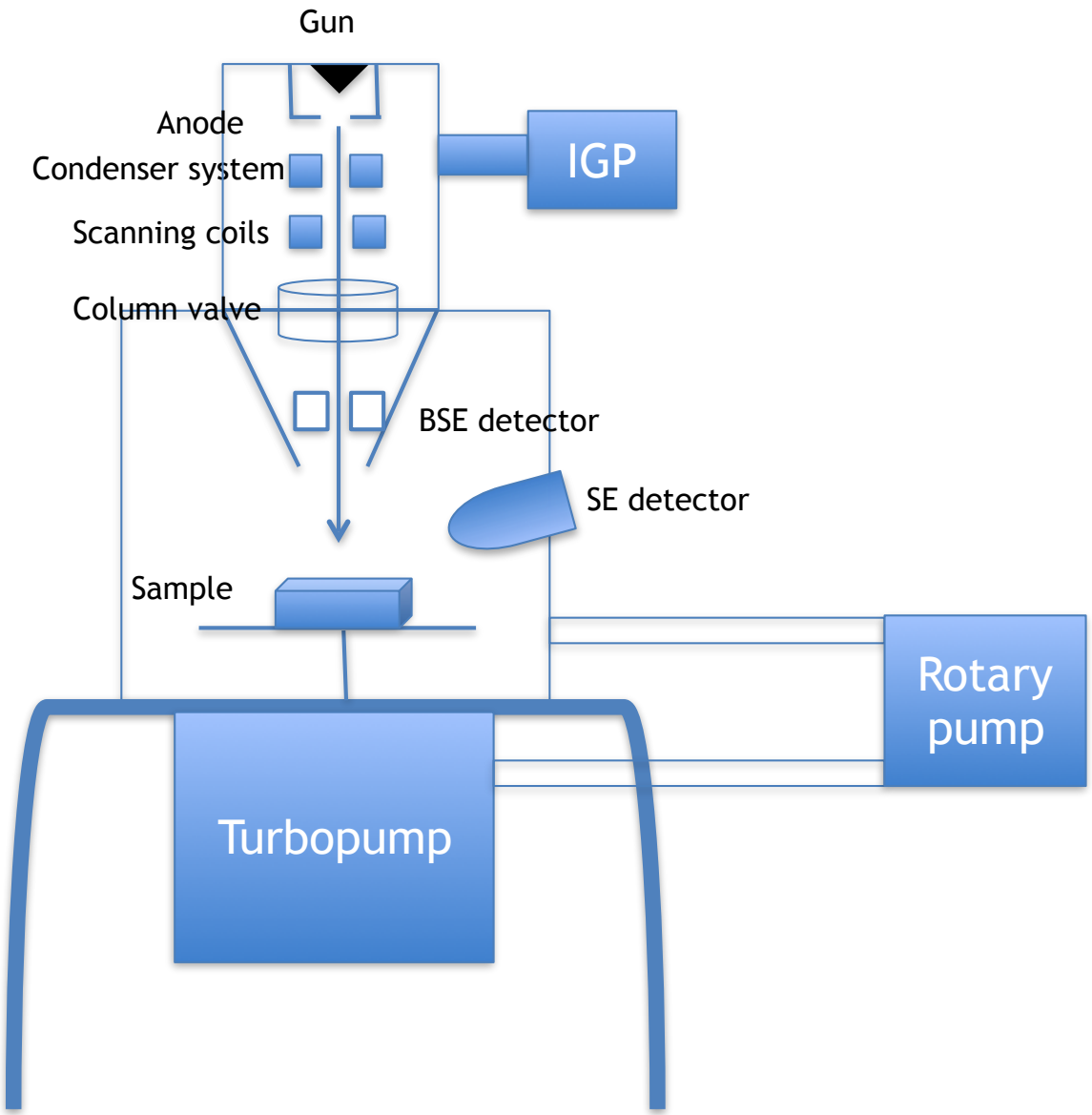
Electron gun: range from tungsten filaments in lower vacuum SEMs to FEGs which need modern high vacuum SEMs

Beam energy: 0.2 – 40 keV is focused by a condenser lens system into a spot of 0.4 – 5 nm

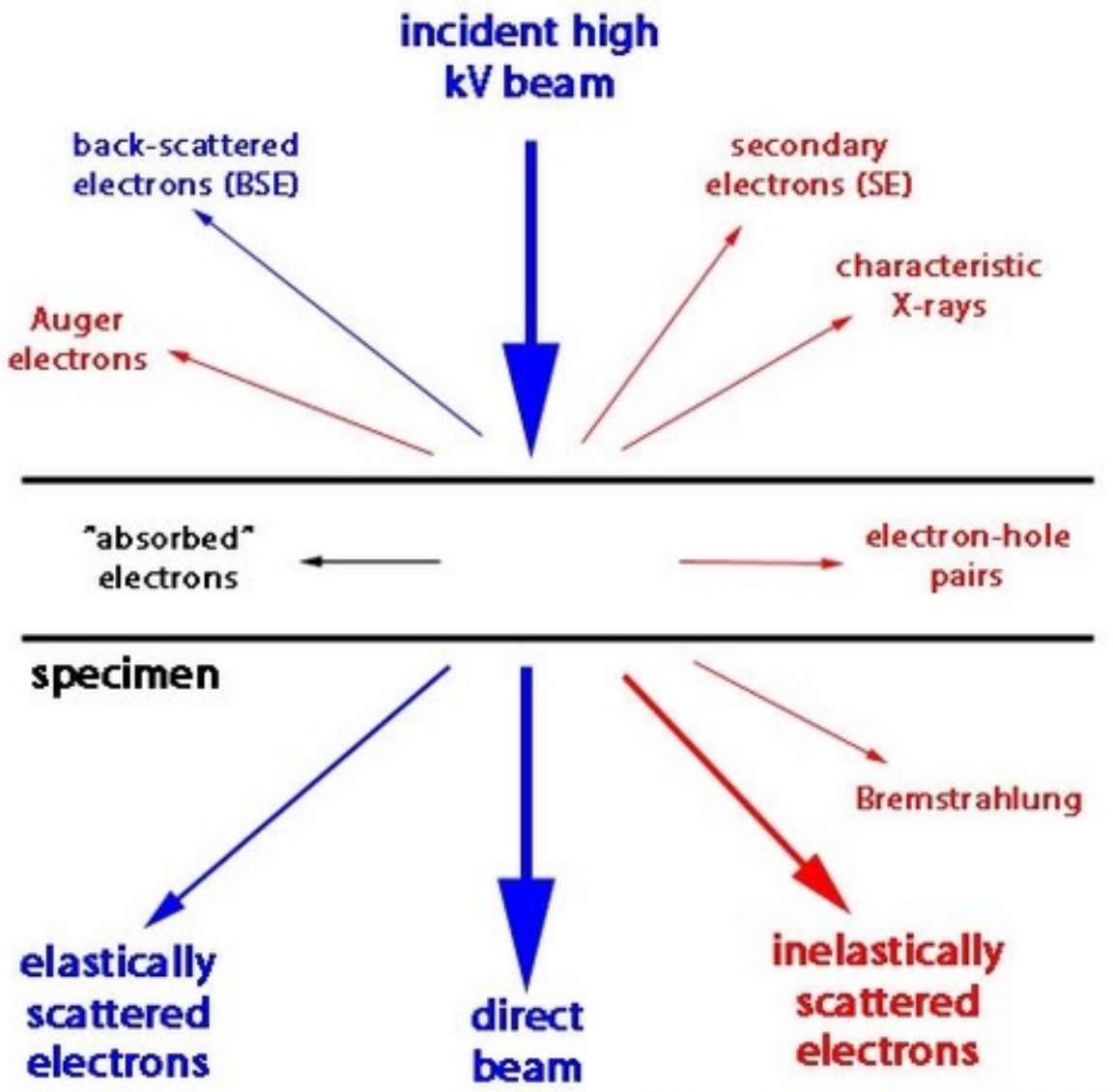
Beam is deflected by very fast scanning coils and rasters the sample surface

Typical resolution of SEM is between 1 and 20 nm where the record is 0.4 nm

Basic anatomy of a scanning electron microscope – vacuum system

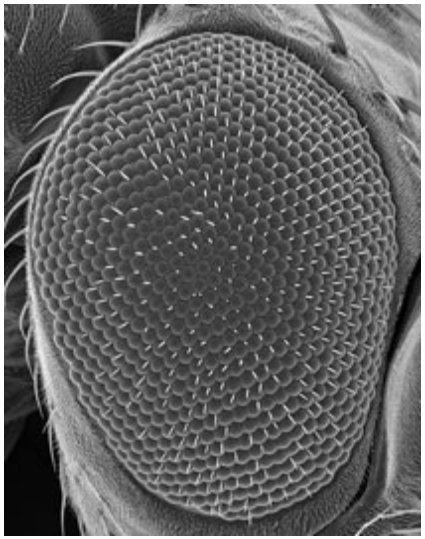


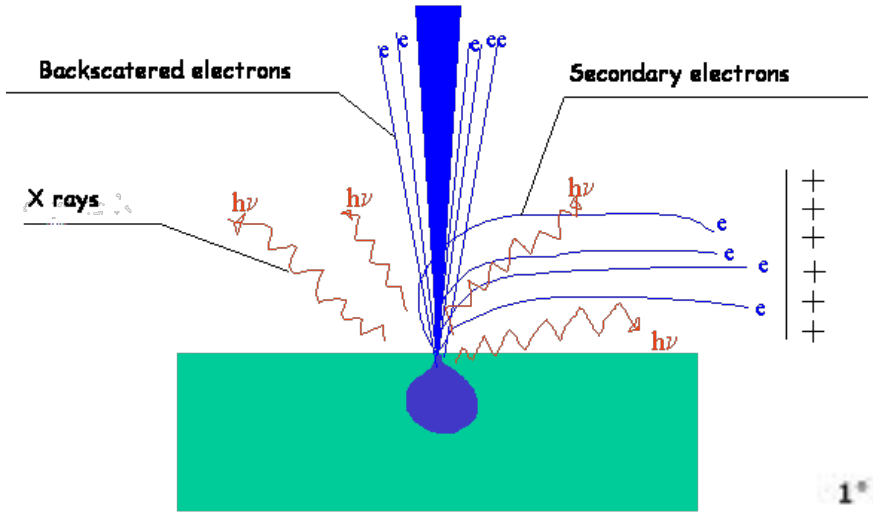
Basic anatomy of a scanning electron microscope –beam sample interactions

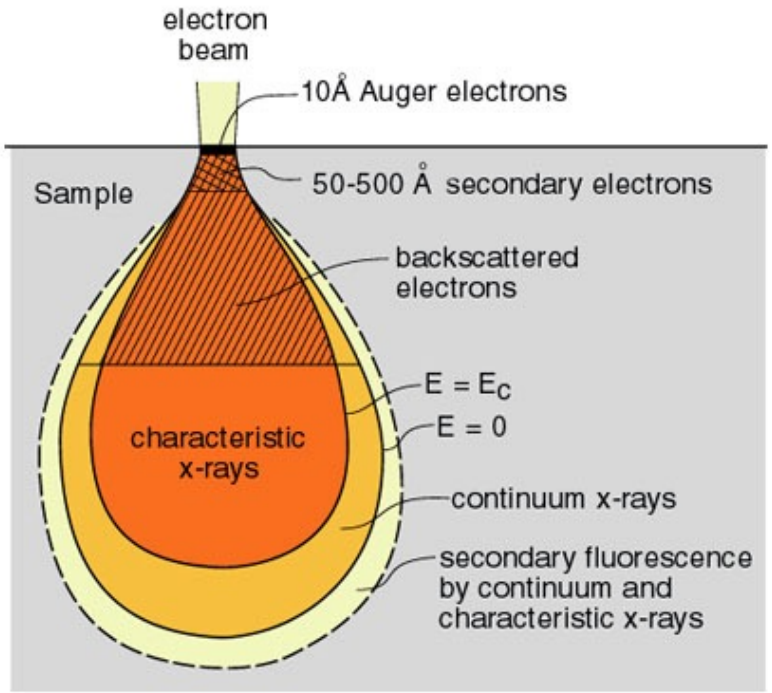


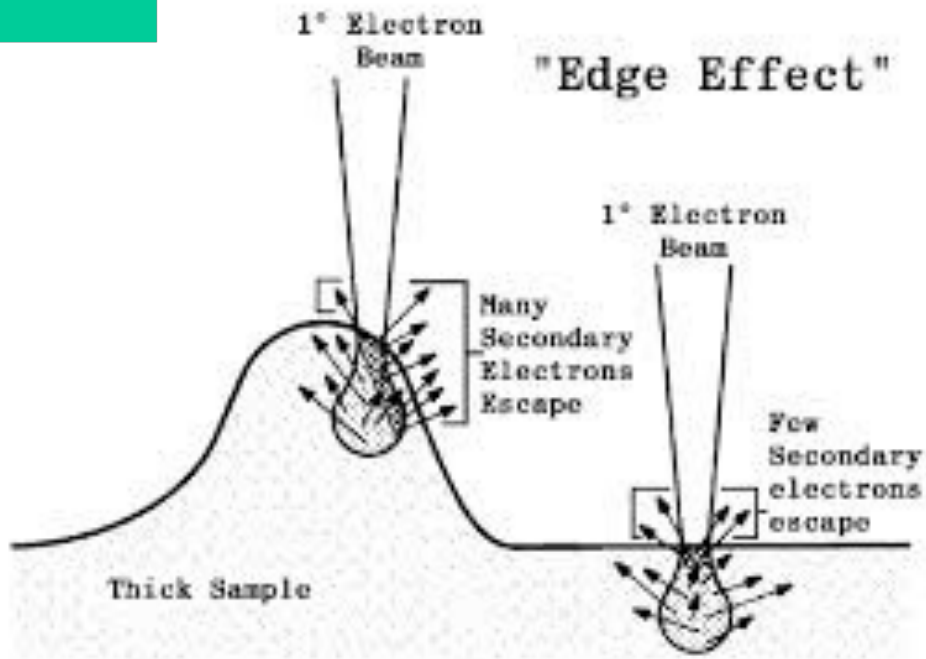
modified from Williams & Carter (1996) Fig. 1.3

Basic anatomy of a scanning electron microscope –beam sample interactions and image formation

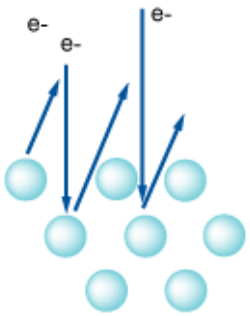




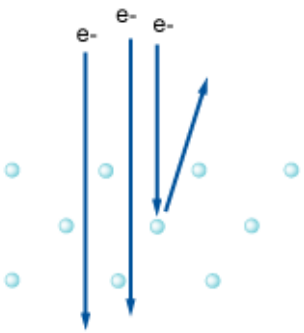




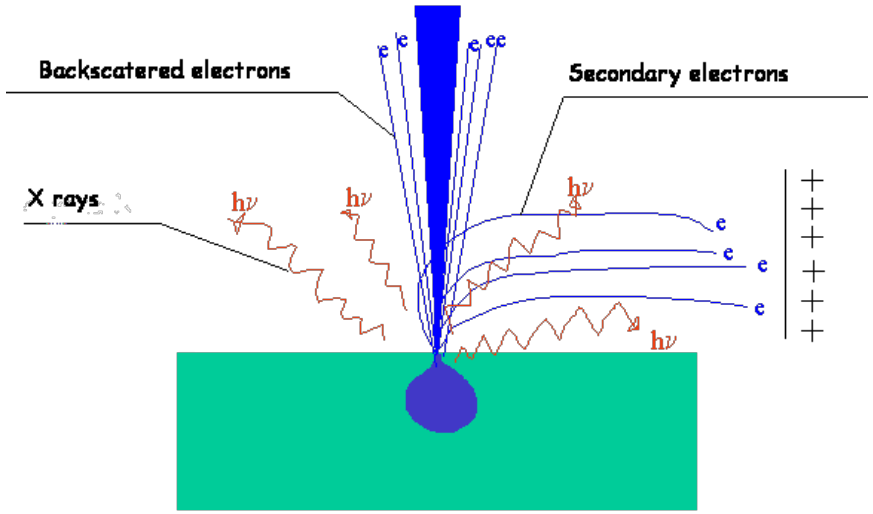
Basic anatomy of a scanning electron microscope –beam sample interactions and image formation

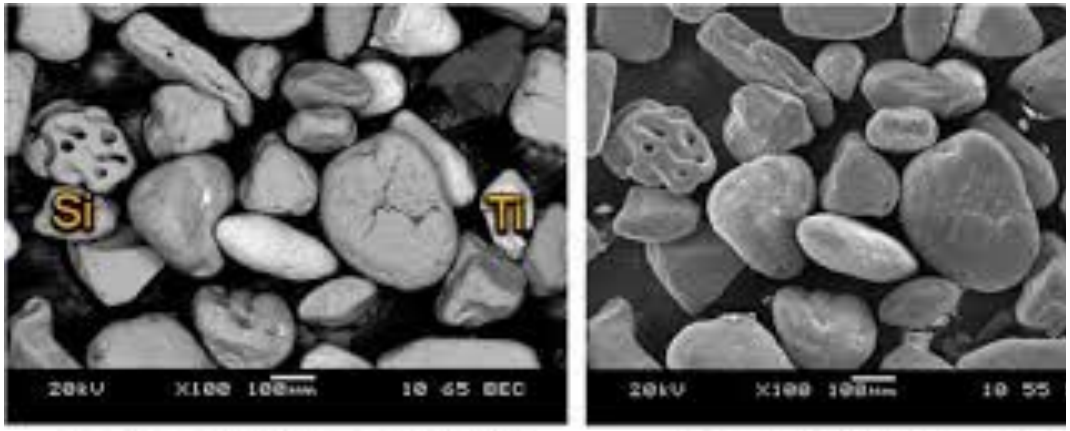


Titanium
atomic number 22



Silicon
atomic number 14





Introduction - Why electrons?
Concept check questions:

- What are the advantages and disadvantages of electrons compared to photons for microscopy? Neutrons?
- What structural biological technologies give higher resolution information than cryo-EM, and what kinds of samples and questions can they address?
- What structural biological technologies complement cryo-EM at lower resolutions, and what kinds of questions and samples do they address?

Electron guns
Concept check questions:

- Where do the imaging electrons in an electron microscope come from?
- What part of the gun is called the "cathode"? What should be called the "anode"?
- What is the accelerator stack a "stack" of?
- What voltages are typically used in transmission electron microscopes? What kinds of electron wavelengths does this correspond to?
- What does it mean to "condition" the gun?
- What is the difference between spatial and temporal coherence?
- What are the three main types of electron guns? What are the advantages and disadvantages of each?

Electron lenses
Concept check questions:

- What is the defining property of a "lens"?
- Why/how do optical lenses focus light?
- Draw a diagram that shows how a lens can be used to form a magnified image. What parameters determine the magnification?
- How do electron lenses focus electrons?
- Why do electron images rotate in an electron lens?
- What are the four main components of an electron lens system? What does each do?

The column
Concept check questions:

- What are the three main lens systems in an electron microscope called?
- What is meant by a "conjugate plane"?
- What are the special names given to the three independent sets of deflectors?
- What current is controlled by the "filament" knob? "emission"? "spot size"? "intensity"? "focus"? "magnification"?
- What is controlled by the "high tension" knob?
- What is a "crossover"?
- Which knob controls whether the microscope is in "LM," "M," or "SA" mode? What currents change?
- What are "pivot points"?
- What does it mean to "align" the microscope?
- What is "hysteresis"?
- What does the "normalize" button do?

The sample chamber
Concept check questions:

- In what directions/ways can the sample be moved while in the microscope?
- What is an "air-lock", and how it is relevant to the sample chamber?
- Where does the sample rest with respect to the objective lens?
- What is the "pole piece gap"?
- What is a "cryo-box"?
- What is "eucentric height"? Is it different for every grid?

Energy filters
Concept check questions:

- Why are EM energy filters used?
- How are "post-column" filters different from "in-column" filters?
- What is a typical slit width for cryo-EM?
- What is a "zero-loss" peak?
- How could an energy filter allow you to image where a particular element was in the sample?

Electron detectors
Concept check questions:

- Name five different types of electron detectors.
- What is a "CCD"? How do they work?
- What are the advantages and disadvantages of film versus CCDs?
- What is meant by "direct" detector?
- What new capabilities do direct detectors provide?

Vacuum systems
Concept check questions:

- Name four different types of vacuum pumps. How does each work?
- Why are so many different types of pumps needed?
- What is a “backing” pump?

Summary, safety
Concept check questions:

- What is the purpose of the heavy lead shielding covering electron microscopes?
- Why do electron microscopes need chilled water?
- Name three lethal and at least one more non-lethal hazards associated with electron cryo-microscopes.