Transmission Electron Microscopy I

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What are we talking about?

1. Quick Review
- What is electron microscopy?
- TEM vs SEM

2. Basics of TEM
- Resolution in the TEM
- Inside the TEM
- Electron Gun/Lens Review

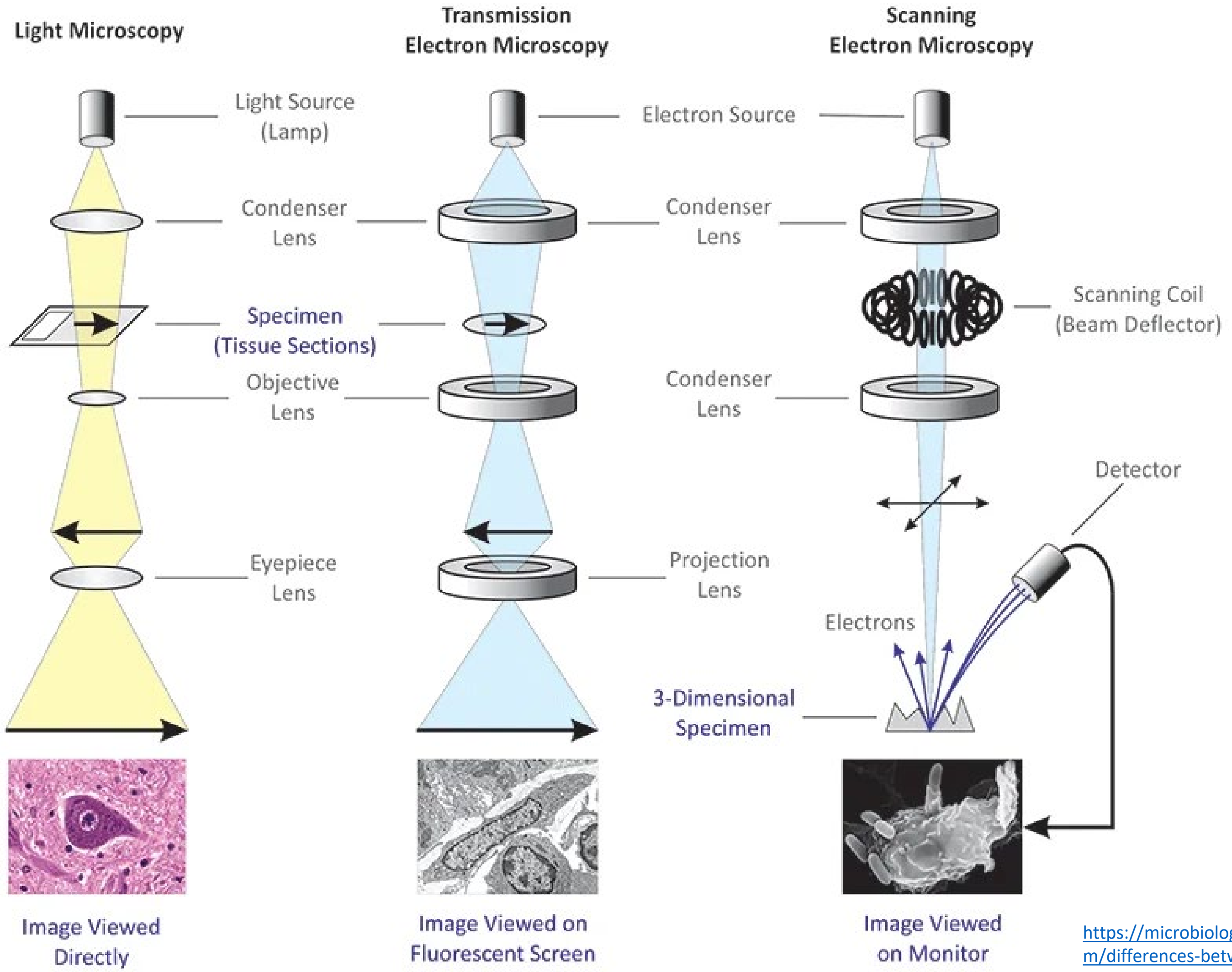
3. TEM Imaging
- Beam Shape
- Electron-Sample Interactions
- Image and Contrast Formation

What is Electron Microscopy (EM)?

Electron Microscopy is an imaging technique that allows us to see things at much higher magnification and resolution than is possible through light microscopy.

- **Light Microscopy**
  - Illumination Source: Photons
  - Samples: No special preparations
  - Lenses: Glass
  - Resolution: ~200 nm
  - Magnification: ~1500X

- **Electron Microscopy**
  - Illumination Source: Electrons
  - Samples: Vacuum Compatible
  - Lenses: Electromagnetic
  - Resolution: ~ 0.1 nm (1 Angstrom)
  - Magnification: ~1,000,000 (+) X

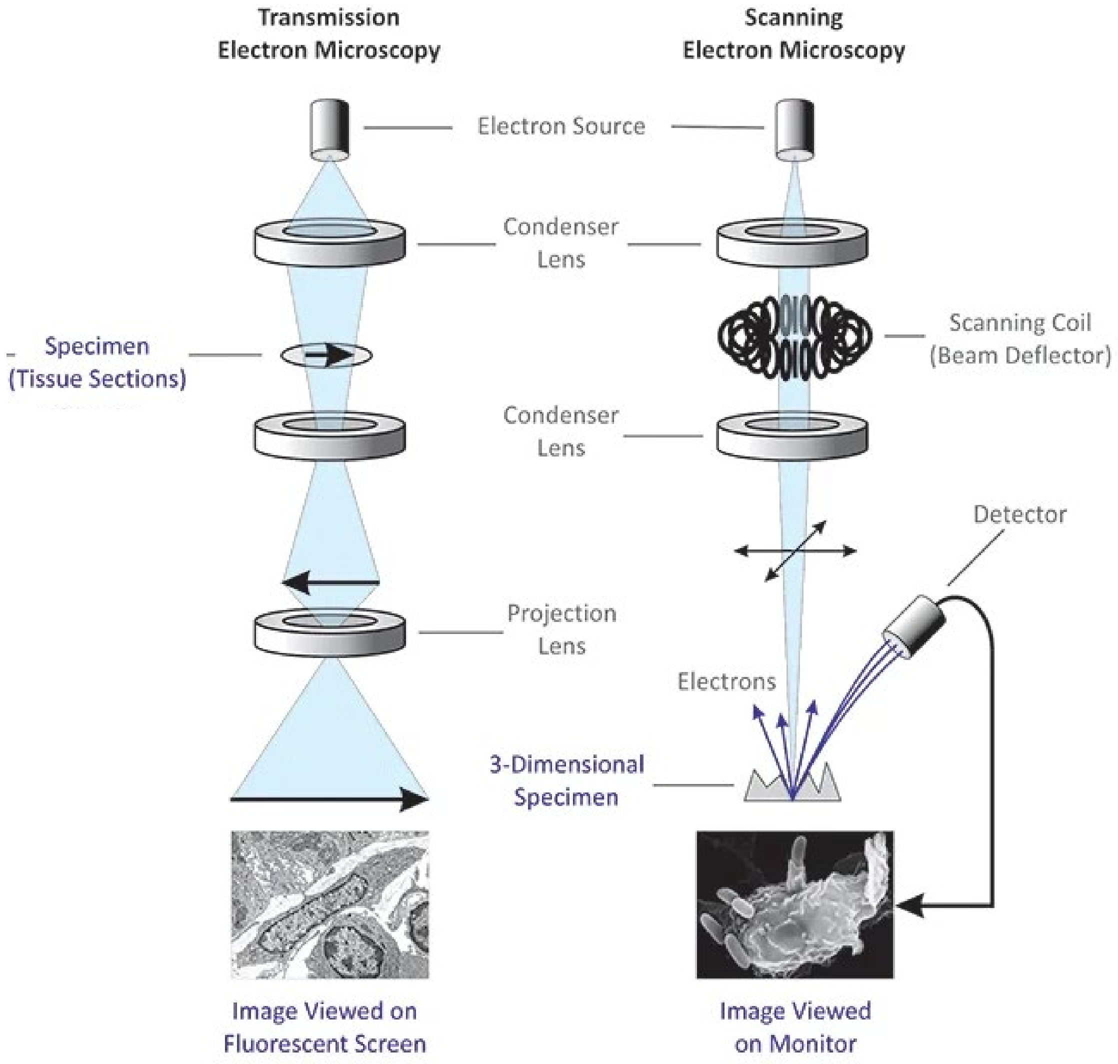


https://microbiologyinfo.com/differences-between-light-microscope-and-electron-microscope/

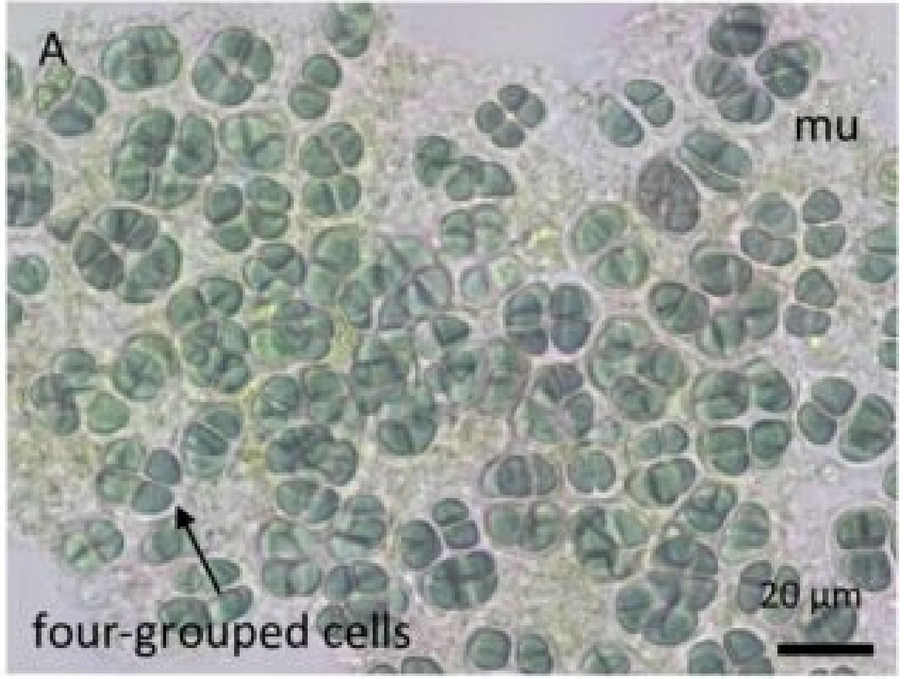
TEM or SEM?

- **Transmission Electron Microscopy (TEM)**
  - Electron beam is SPREAD and TRANSMITTED through the entire sample area
  - Samples are THIN and FLAT
  - Electron Accelerations: 75 – 300 kV
  - Resolution: ~0.2, up to 0.1 nm

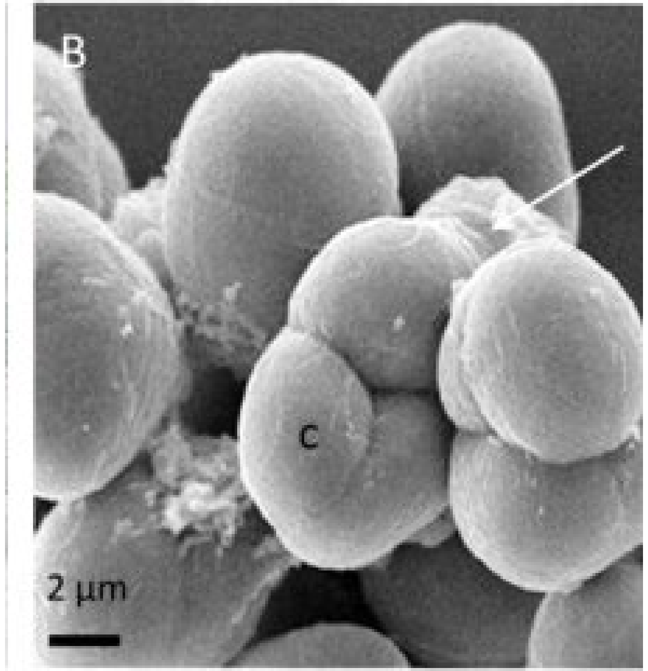
- **Scanning Electron Microscopy (SEM)**
  - Electron beam is FOCUSED and SCANS the surface of the sample
  - Samples are typically 3D
  - Electron Acceleration: 1 – 30kV
  - Resolution: ~ 1 - 20 nm



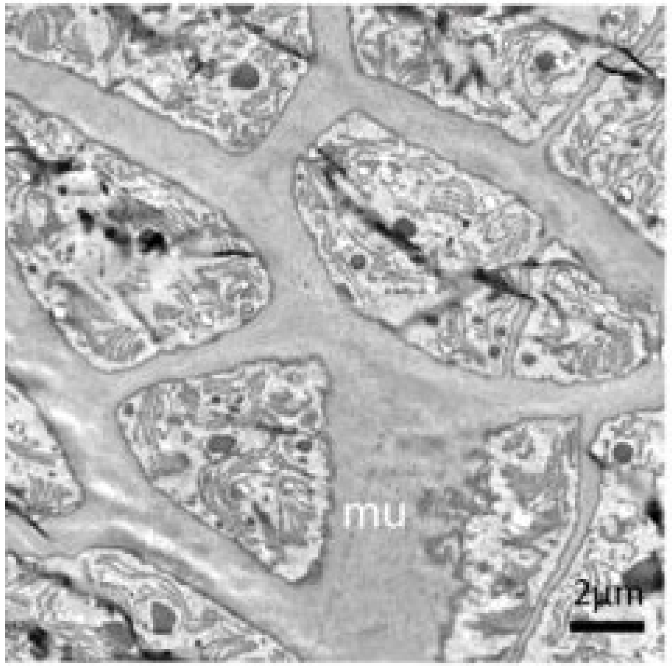
Resolution Limits of Light and Electron Microscopy



SEM



TEM



Images from Duval et al. 2020

NA is 0.95 with air
up to 1.5 with oil

Light Microscopy

$$r = \lambda/(2\mathrm{NA})$$

λ (light) = 400nm

Resolution limit: ~200 nm

Electron Microscopy

$$r = 0.61(C_s\lambda^3)^{1/4}$$

C_s is defined by the objective lens

λe-(200kV) = ~ 0.0025 nm

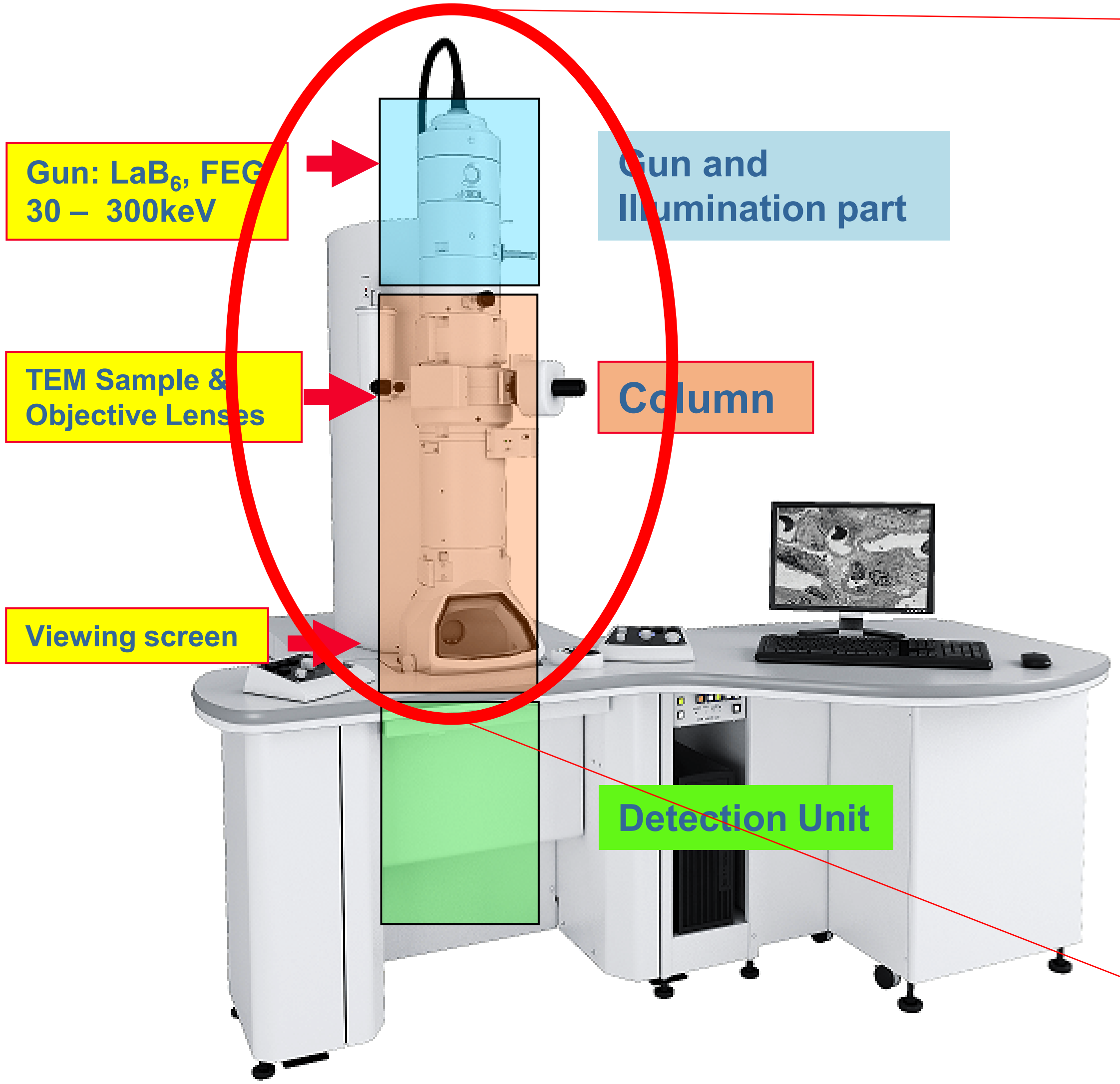
λe-(20kV) = ~ 0.0085 nm

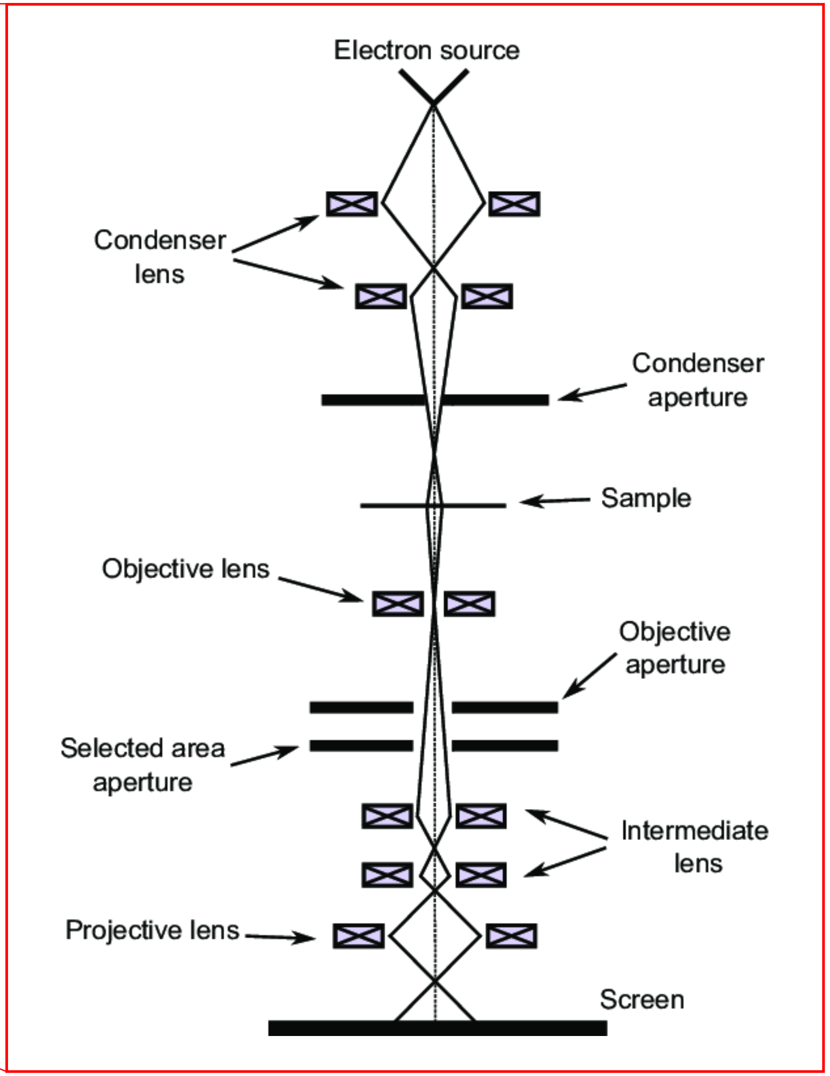
Resolution limit (SEM): ~1 nm

Resolution limit (TEM): ~0.1 nm

* uncorrected systems

Inside the TEM





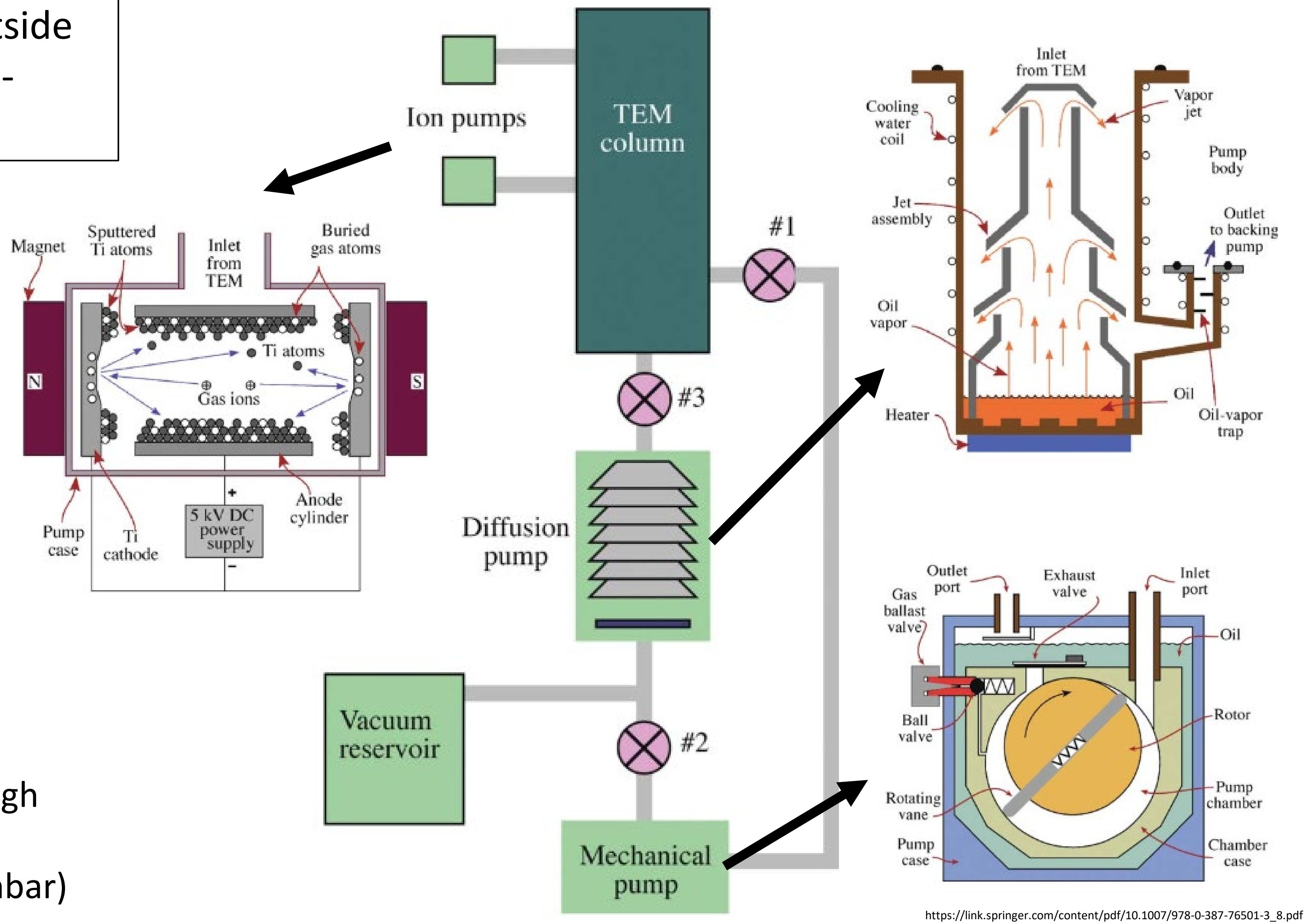
Naresh Marturi. Vision and visual servoing for nanomanipulation and nanocharacterization in scanning electron microscope.. Micro and nanotechnologies/Microelectronics. Universit´e de Franche-Comt´e, 2013.

High Vacuum in the TEM

Because electrons are sensitive to outside forces, TEMs must be kept under high-vacuum conditions (10^{-8} - 10^{-10} mbar)

There are generally 3 types of vacuum pumps used in the TEM:

1. **Roughing/Mechanical Pump**
   Belt-driven rotary pump with or without oil
   Low vacuum pump (0.01 mbar)
2. **Diffusion Pump**
   Oil-based pump that pulls air out of the column
   High vacuum pump (0.01 - 10^{-10} mbar)
3. **Ion Pump**
   Remove gas and air via electrical and chemical attraction
   Placed next to gun & sample, where high vacuum is most important
   Only efficient at high vacuum (< 10^{-4} mbar)



https://link.springer.com/content/pdf/10.1007/978-0-387-76501-3_8.pdf

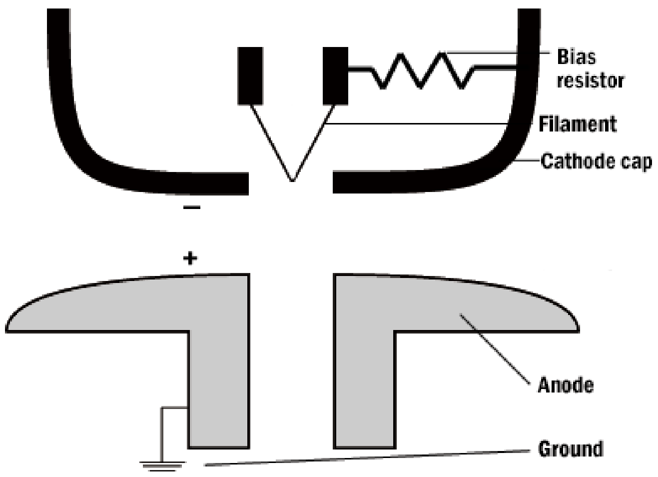
How are electrons generated – Thermionic Electron Guns

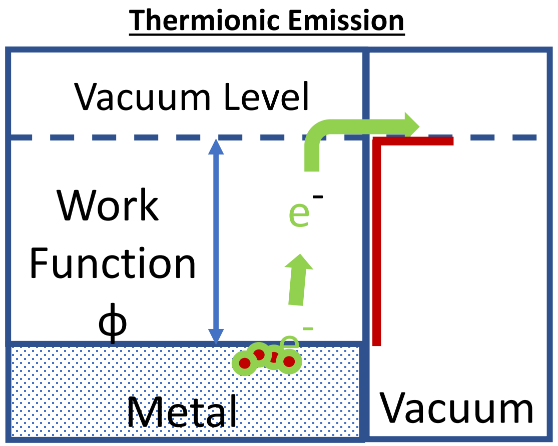
Thermoionic Emission electron guns work similarly to a light bulb.

A **filament,** typically made of tungsten or lanthanum hexaboride (LaB6), is the **electron source**

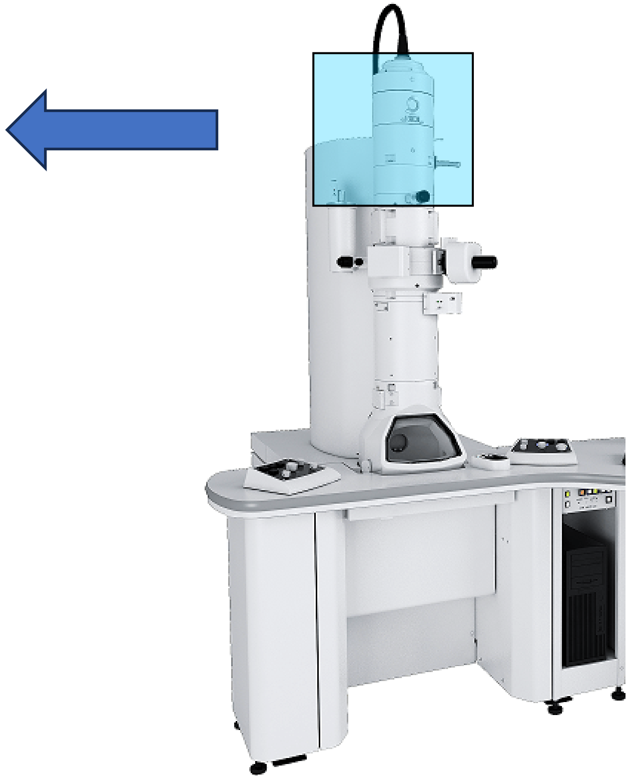
The **filament is heated** to release electrons, and the **Cathode cap** (Wehnelt cylinder) creates **negative bias** to focus the released electrons to a beam shape

The **anode** below the cap creates **positive attraction** of the negative electrons to accelerate down the column





Gun and Illumination part



https://cmrf.research.uiowa.edu/transmission-electron-microscopy

How are electrons generated – Field Emission Electron Guns

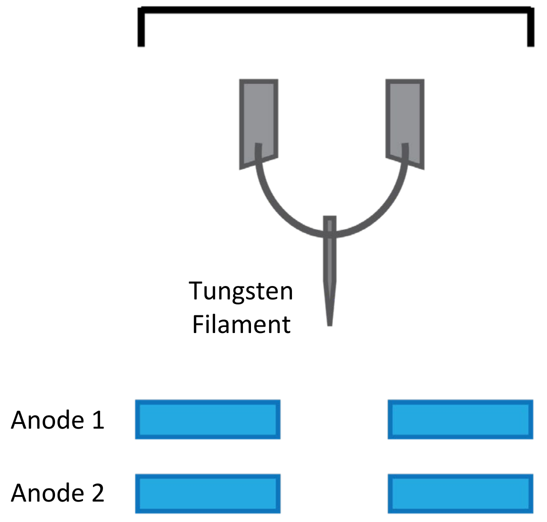
Field Emission electron guns use electrostatic forces with or without heat.

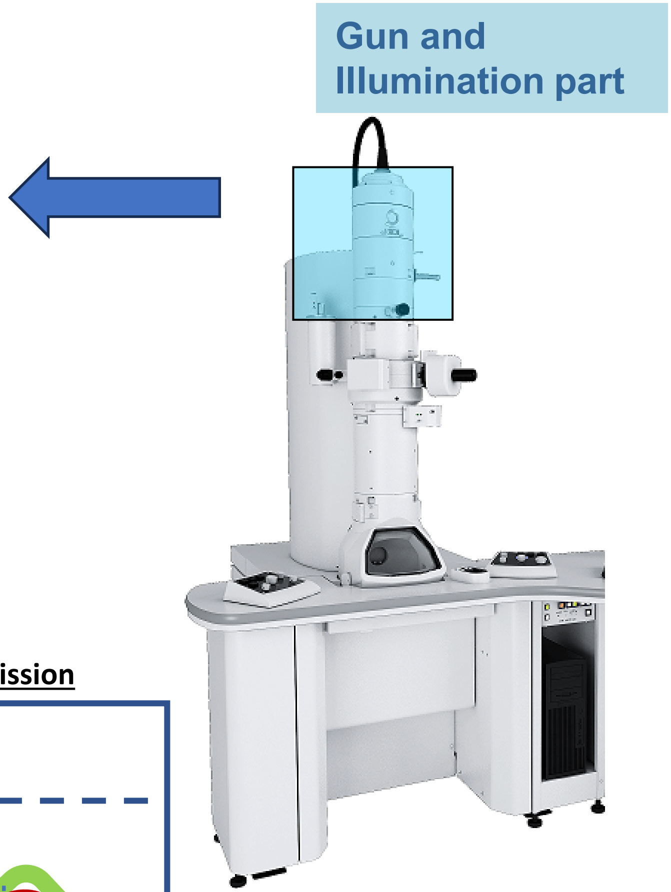
Cold Field Emission uses electrostatic forces alone to accelerate electrons down the column

Schottky Field Emission uses both electrostatic forces and thermionic emission through the addition of a zirconium oxide coating

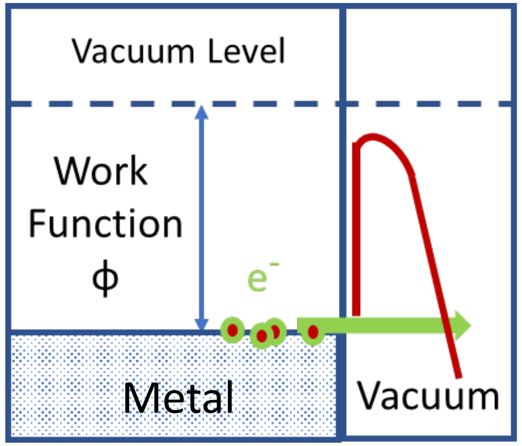
Tungsten is used as the electron source in both Schottkey and Cold FEGs

Field Emission Source

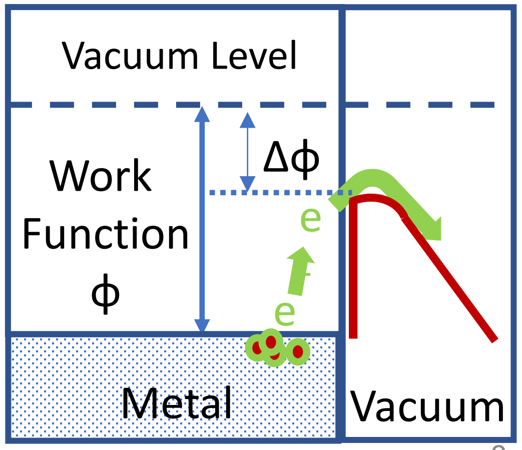




Cold Field Emission



Schottky Field Emission



Which Electron Gun is Best for Your Application?

Type of Source	Thermionic	Cold Field Emission	Schottky Field Emission
Source Material	W, LaB_6	W (310)	ZrO/W(100)
Coherence	Lowest, Low	Highest	High
Work Function ϕ (eV)	4.5, 2.7	4.5	2.8
Emission Current Stability (%/hr)	±0.05, ±0.1	±3	±<0.3
Flashing	n/a	Every 6-8 hrs	n/a
Required Vacuum (mbar)	10^{-5} - 10^{-7}	10^{-9}	10^{-8}
Lifetime (hours)	100, 1000	10^4	10^4
Cost	Low	High	High

Depends on your application!

Electromagnetic Lenses - Review

Condenser Lens:

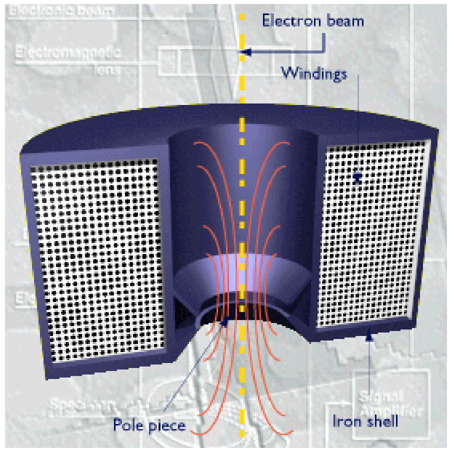
TEMs can have 2 or 3 condenser lenses. In a 2-condenser system, **C1** controls the **spot size** and **C2** controls the **convergence** of the beam on the sample

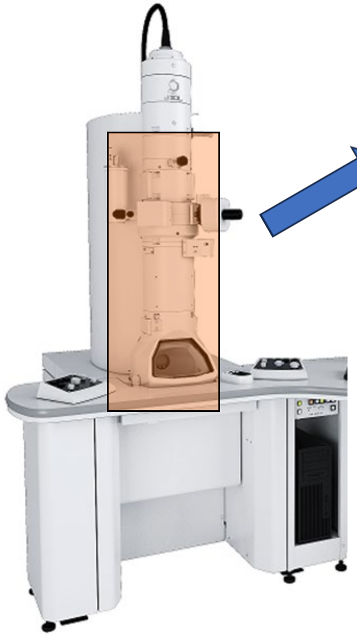
Objective Lens:

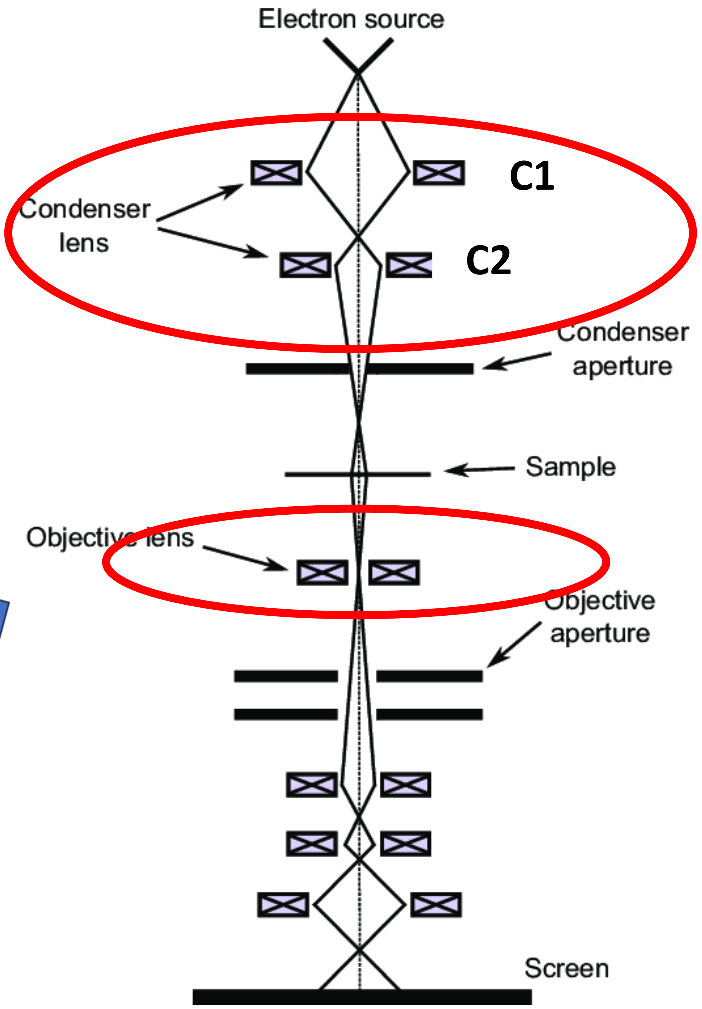
focuses the image of the sample

Cross section of electromagnetic lens

A Guide to X-Ray Microanalysis, Oxford Microanalytical Instruments







Types of Lens Aberrations

- **Spherical Aberrations:**
  - Magnetic fields within the lenses are not uniform and focused to different points
- **Chromatic Aberrations:**
  - Electrons with different energies are focused to different points
- **Astigmatism:**
  - Caused by uneven lens strength
  - **Condenser lens astigmatism** affects beam shape
  - **Objective lens astigmatism** affects image focusing

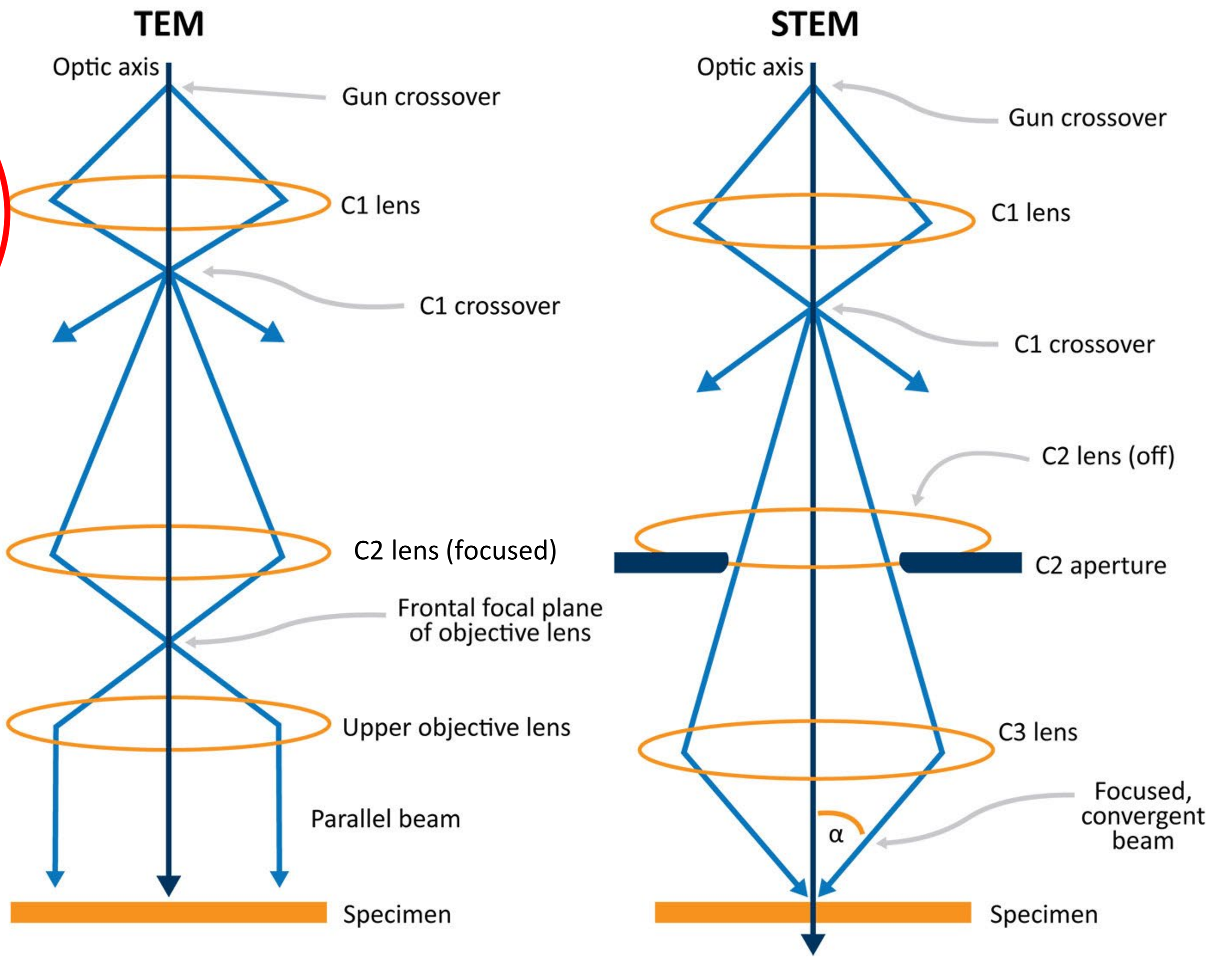
Parallel vs. Convergent Beams

Standard TEM uses the C1 and C2 lenses to form a parallel beam at the specimen plane

- **Single-Particle Analysis (SPA)**
- **Electron Tomography (ET)**
- **Micro-electron Diffraction (MED)**

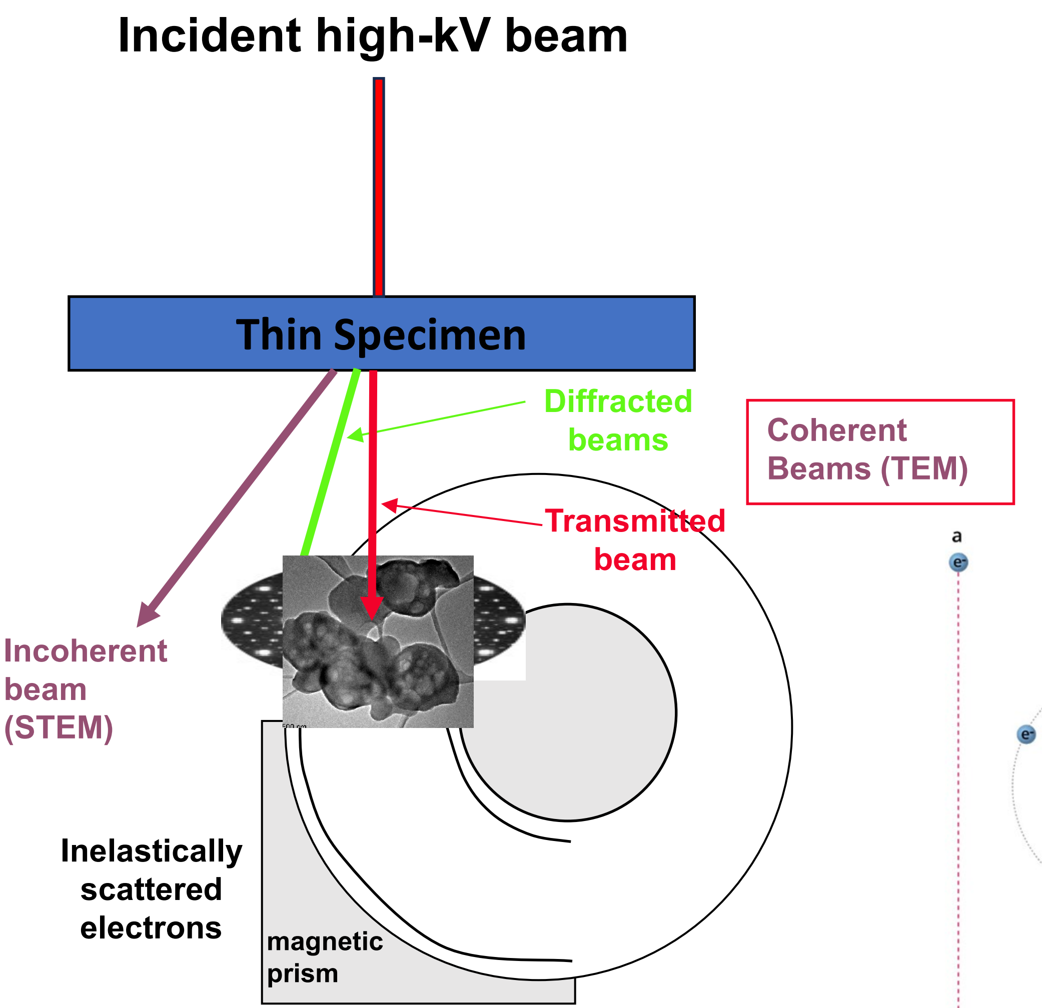
Scanning-TEM (STEM) turns off the C2 lens, allowing a focused, convergent beam at the specimen plane

- **Brightfield STEM**
- **Annular Dark Field STEM (ADF)**
- **High-angle annular darkfield (HAADF)**



https://www.nanoscience.com/techniques/scanning-transmission-electron-microscopy/

Electron-Sample Interactions



Transmitted electrons: form micrographs

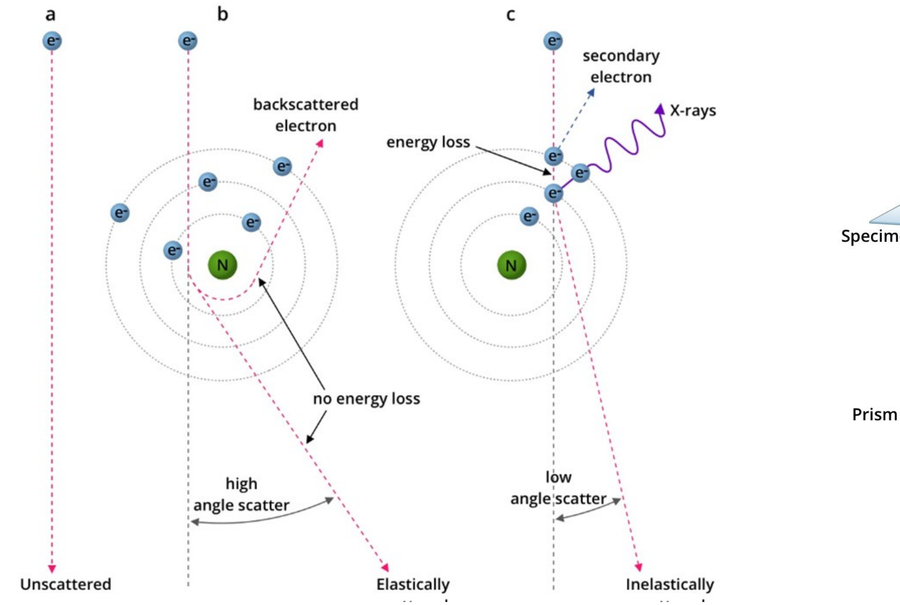
Diffracted electrons: form diffraction patterns

Coherent beams: wavelength stays in phase, used in TEM imaging and diffraction

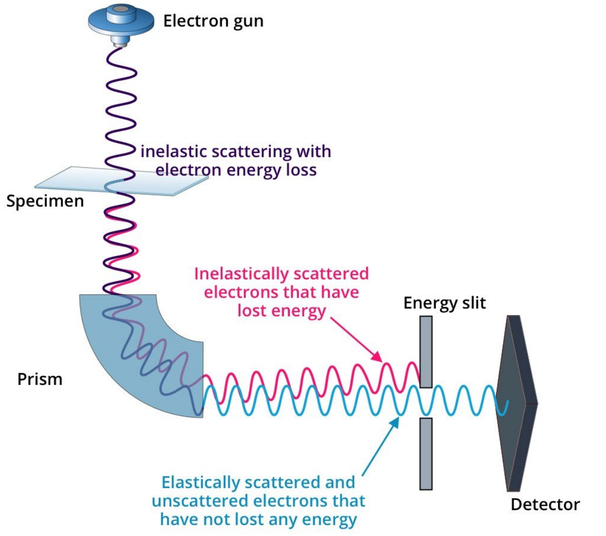
Incoherent beams: wavelengths out of phase, used in STEM imaging

Elastically scattered electrons: no energy loss

Inelastically scattered electrons: energy loss



myscope.training/CRYO_How_images_are_formed



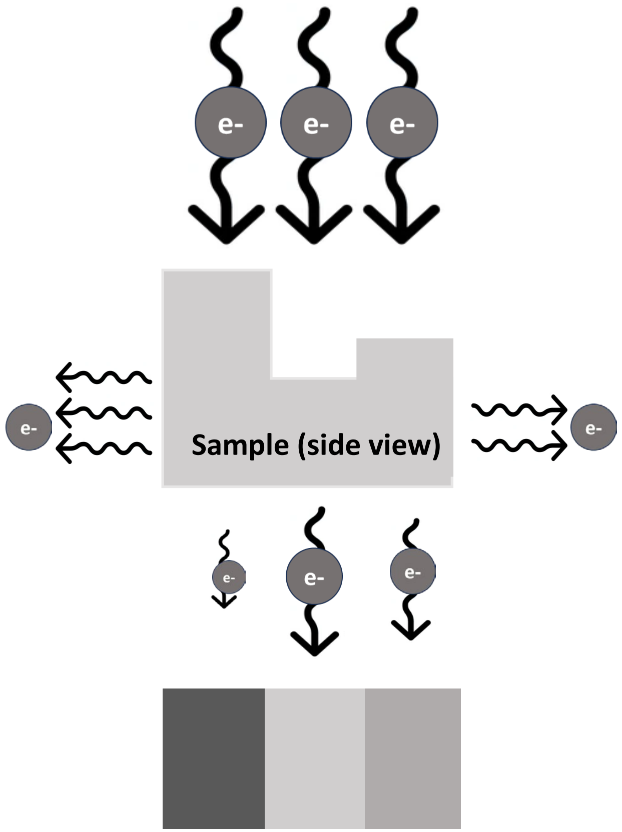
https://myscope.training/CRYO_Electron_energy_filters

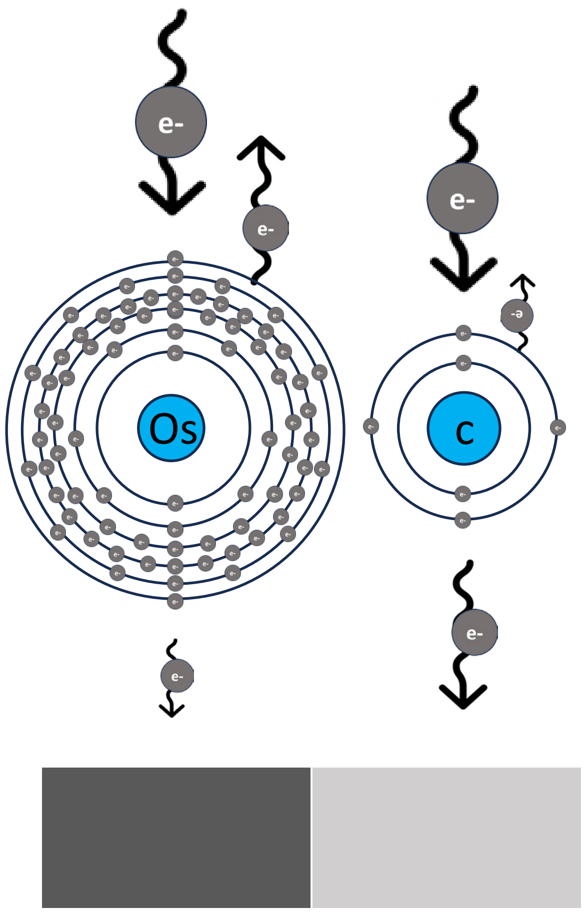
Basic Concepts: Contrast Formation in Transmitted Beam Electrons

Contrast in a TEM sample is affected by the sample thickness and atomic number of the materials being imaged

Thicker samples and materials with higher atomic numbers interact more strongly with the incident electron beam, resulting in less signal reaching the detector and a darker area appearing in the final image.

Bad news for biological samples!!





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