NewSpec inspireSTEM education

Electron Microscopy and Hitachi TM4000Plus Overview

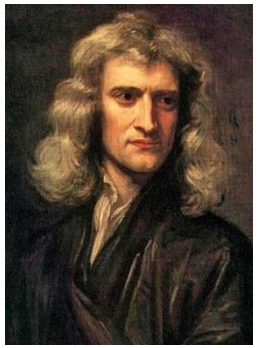
Coordinator: Jessica Jones

Service and Support:

Karloskar Hall, Luke Basso,

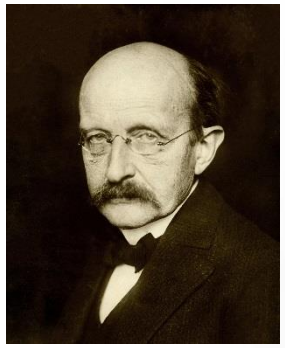
Derrick Choy and Andrew Gibson-White

Some of the giants whose shoulders we stand on

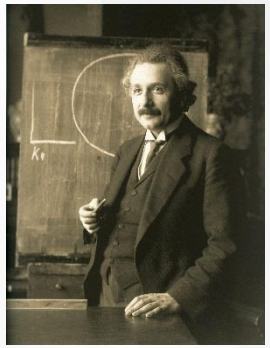


Isaac Newton
(1643-1727)

Thomas Young
(1773-1829)



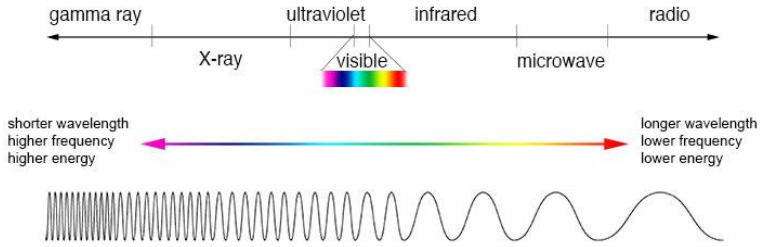
Max Planck
(1858-1947)

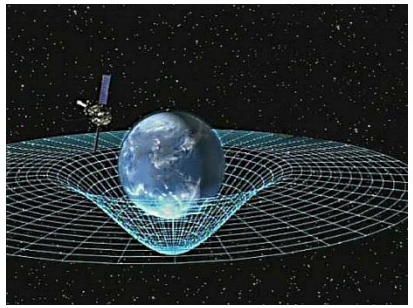


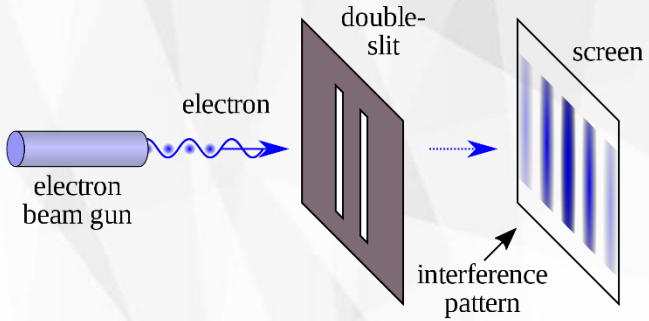
Albert Einstein
(1879-1955)

Louis de Broglie
(1892-1987)

Ernst Ruska
(1906-1988)







Technology changes how humans view the world/Universe

Walking
Speed: 5 km/h
Began: ~3.5 Million years ago

Steam train
Speed: 100 km/h
Began: Early 1800s

Horse/Horse and Carriage
Speed: 8 km/h
Began: ~3500 BC

Modern Car
Speed: 100 km/h
Began: Early 1950s

Bicycle
Speed: 24 km/h
Began: 1500s

Modern Plane
Speed: 800 km/h
Began: 1950s

Contents

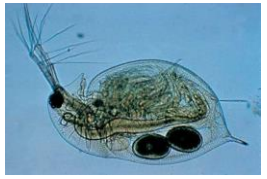
- What is Microscopy
  - Optical Microscopes
  - Optical vs Electron microscopy
- Overview of an electron microscope
- Physics of an electron microscope
- General components of an electron microscope
  - More detailed look at the TM4000/TM4000Plus microscope

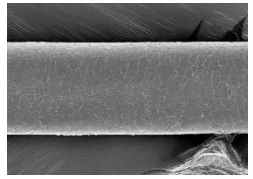
What is microscopy?

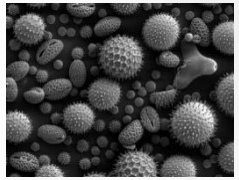
- A technical field making use of microscopes to view things that are not able to be seen with the naked eye.
- Sub-fields within microscopy;
  - Optical microscopy
    - Conventional, fluorescence, confocal, multiphoton etc.
  - Scanning probe microscopy
  - **Electron microscopy**
    - **SEM, TEM, STEM, FIB etc**

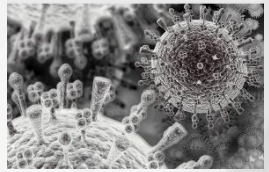
What do microscopes allow us to see?

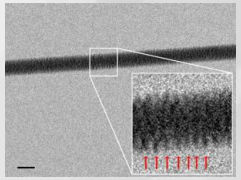

Honeybee

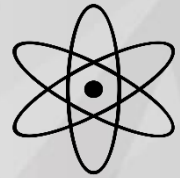

Water flea


Hair


Pollen


Virus


DNA


Atom

100 mm | 10 mm | 1 mm | 100 µm | 10 µm | 1 µm | 100 nm | 10 nm | 1 nm | 1 Å

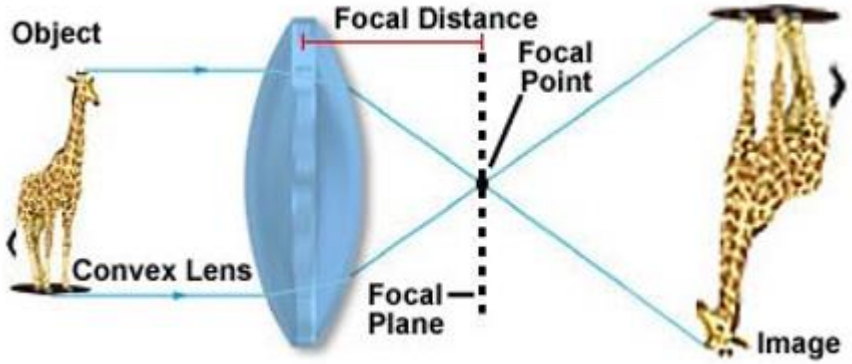
Optical Microscopes

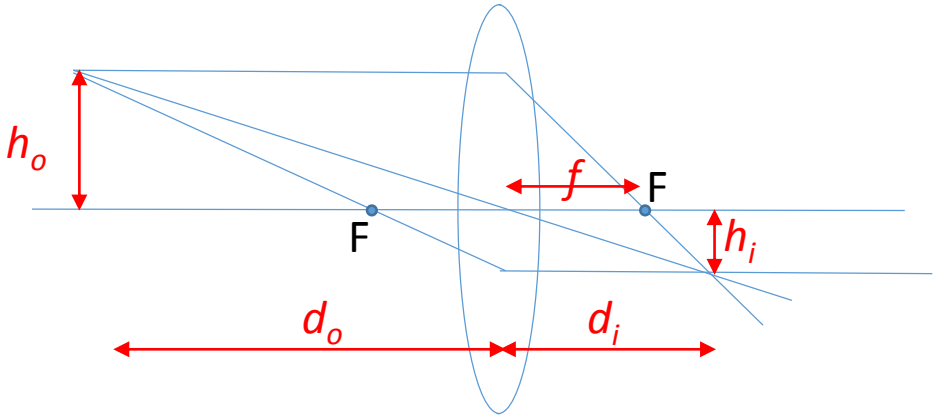
- Designed to produce magnified visual or photographic images of small objects.
- The microscope must accomplish three tasks:

  1) produce a magnified image of the specimen
  2) separate the details in the image,
  3) and render the details visible to the human eye or camera.

This group of instruments includes not only multiple-lens designs with objectives and condensers, but also very simple single lens devices that are often hand-held, such as a magnifying glass.

Optical Microscopes





An optical microscope, using one or more lenses produces an enlarged image of an object that is located at the focal plane of the lens / lens system.

The thin lens equation is

$$\frac{1}{d_o} + \frac{1}{d_i} = \frac{1}{f}$$

The magnification is given by

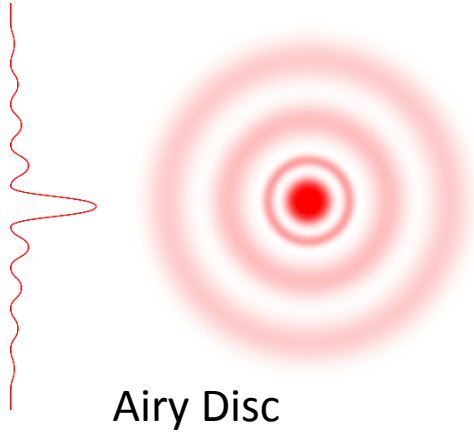
$$\frac{h_i}{h_o} = -\frac{d_i}{d_o} = m$$

Limitations of Optical Microscopes

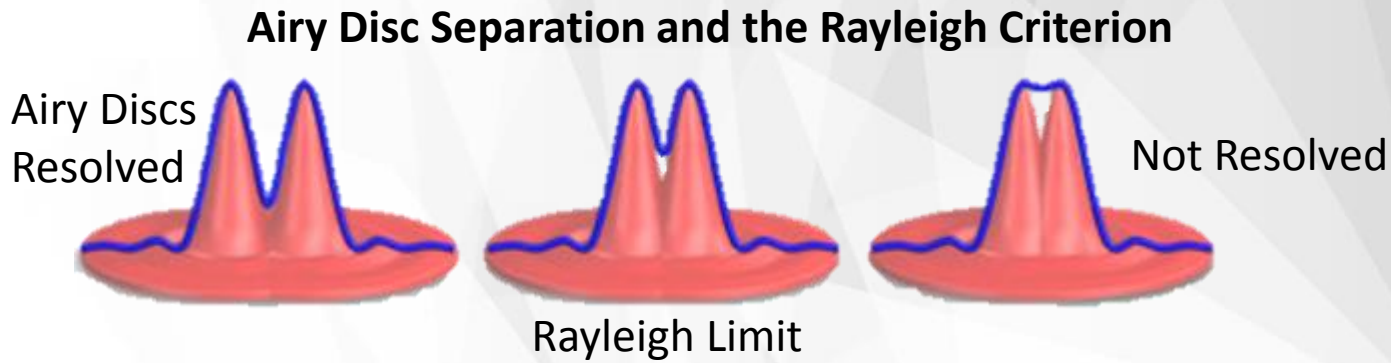
- ‘Low’ resolution (sub micron) due to diffraction limit
  - Standard optical microscopes work with visible light 400-700 nm
- Magnification (Max ~x500 to x1500)
- Depth of field

Diffraction Limit

- At high magnifications an image of point objects may be distorted
  - The point objects can appear as fuzzy discs that are surrounded by diffraction rings (Airy discs).
- When diffraction rings occur it limits ability of the microscope to resolve fine details of the sample.

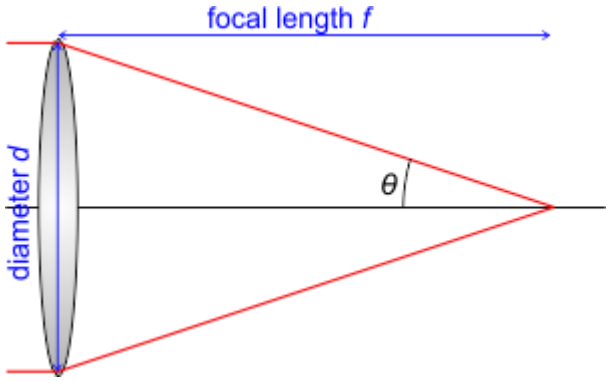


Airy Disc



Diffraction Limit

- The diffraction limit depends on the wavelength (λ) and numerical aperture (NA) of the objective lens.
- Ernst Abbe discovered relationship between wavelength of light, refractive index, NA and final spot diameter;

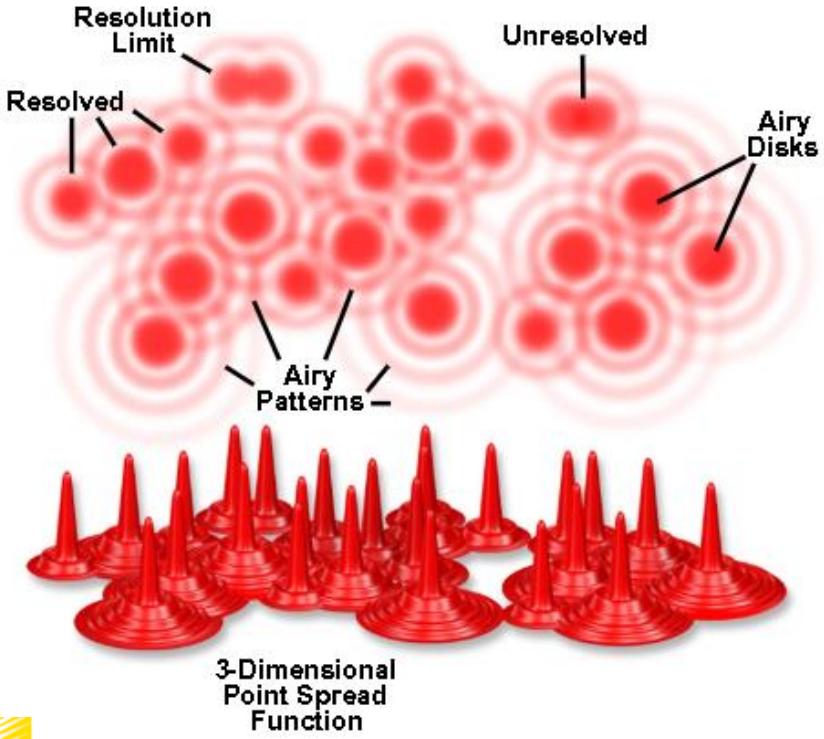


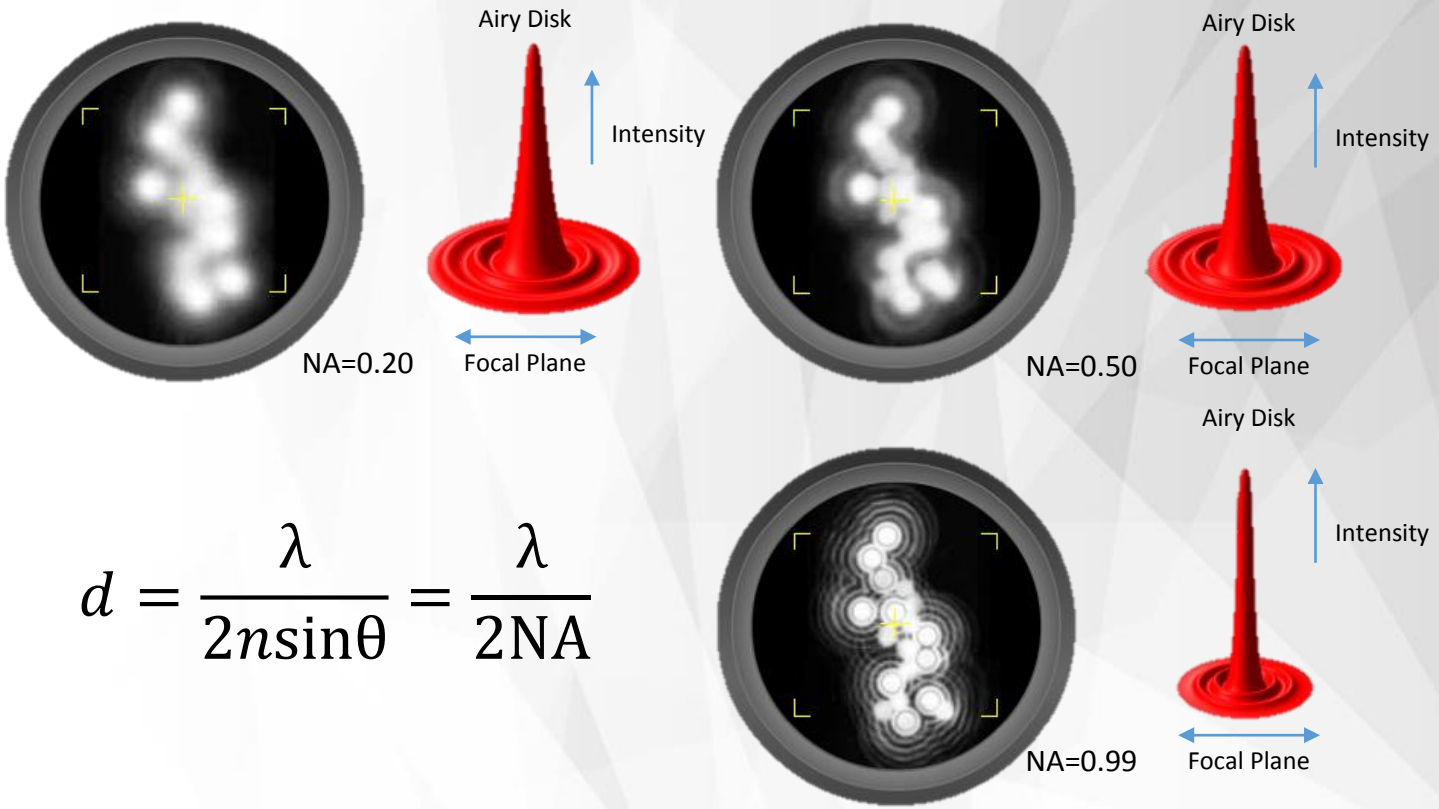
$$d = \frac{\lambda}{2n\sin\theta} = \frac{\lambda}{2\mathrm{NA}}$$

Where
λ = wavelength
n = refractive index
θ = half-angle
d = spot diameter

- Modern optics can reach NA = ~1.4 (immersion oil)
  - Using lower limit of visible wavelength range (400 nm) and a more typical NA of 1.0 → min spot size = 0.2 µm

Diffraction Limit





$$d = \frac{\lambda}{2n\sin\theta} = \frac{\lambda}{2\mathrm{NA}}$$

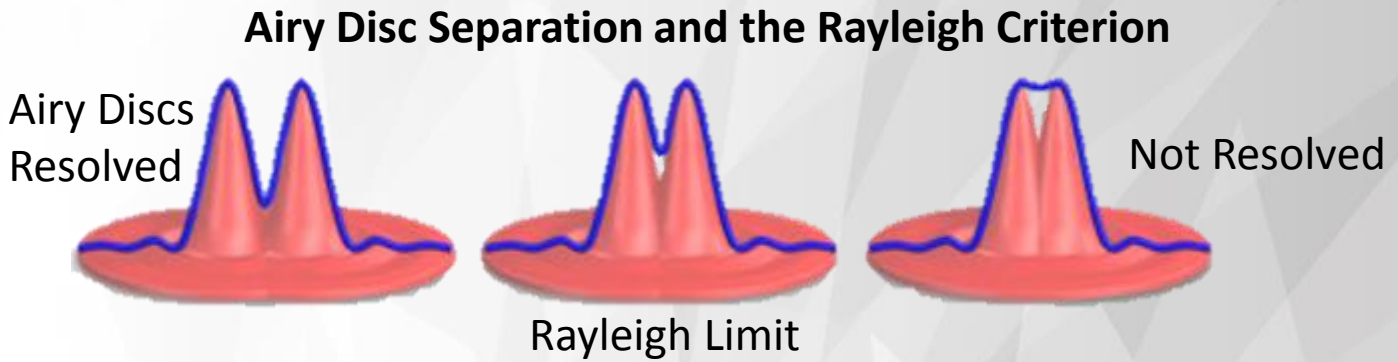
https://www.microscopyu.com/microscopy-basics/resolution

Rayleigh criterion

- Minimum resolvable detail
- For circular aperture;

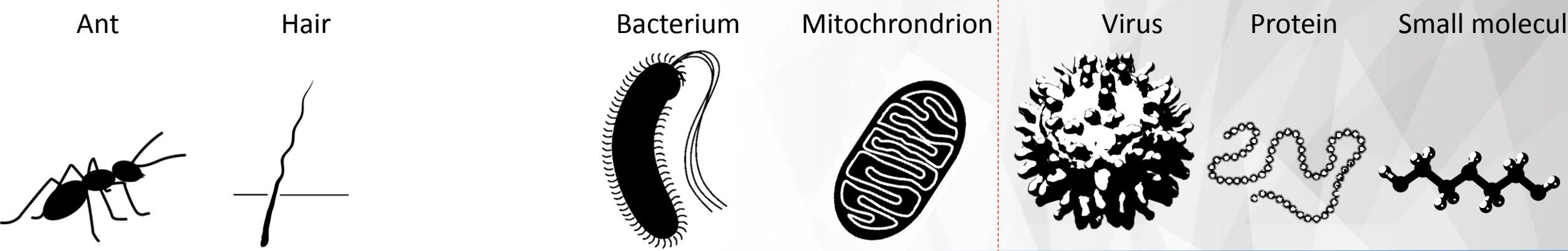
$$d_0 = 0.61\frac{\lambda}{n\sin\alpha}$$

- For electron microscope n≈1
- For TEM nsinα ≈ α



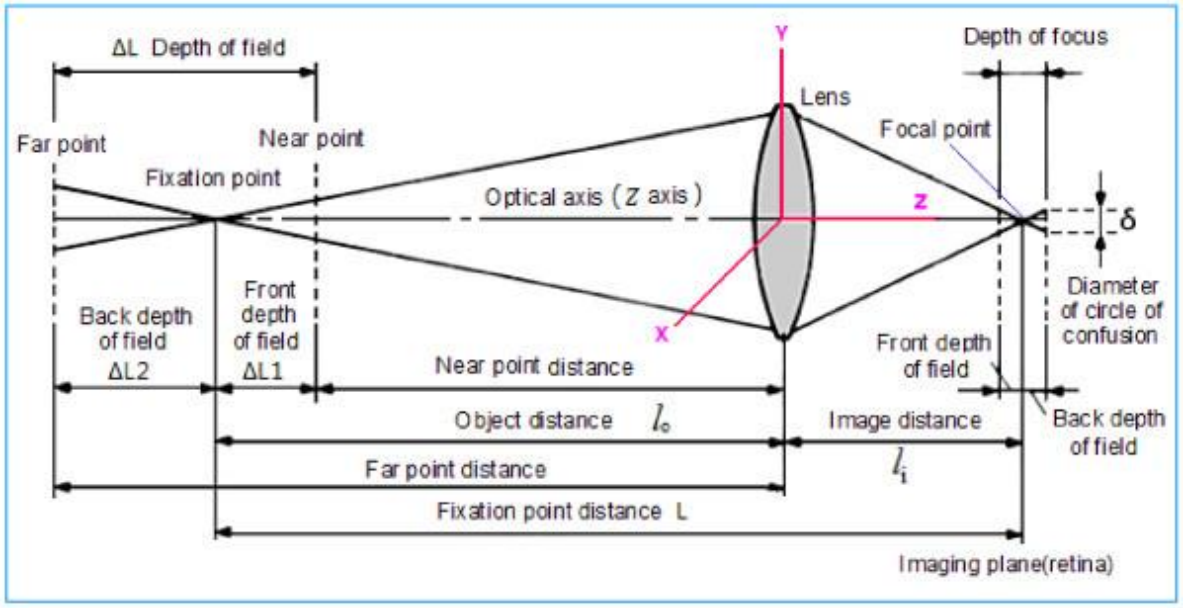
Limitations of Optical Microscopes
Diffraction Limit

Abbe's Diffraction Limit (0.2 µm)



Depth of field

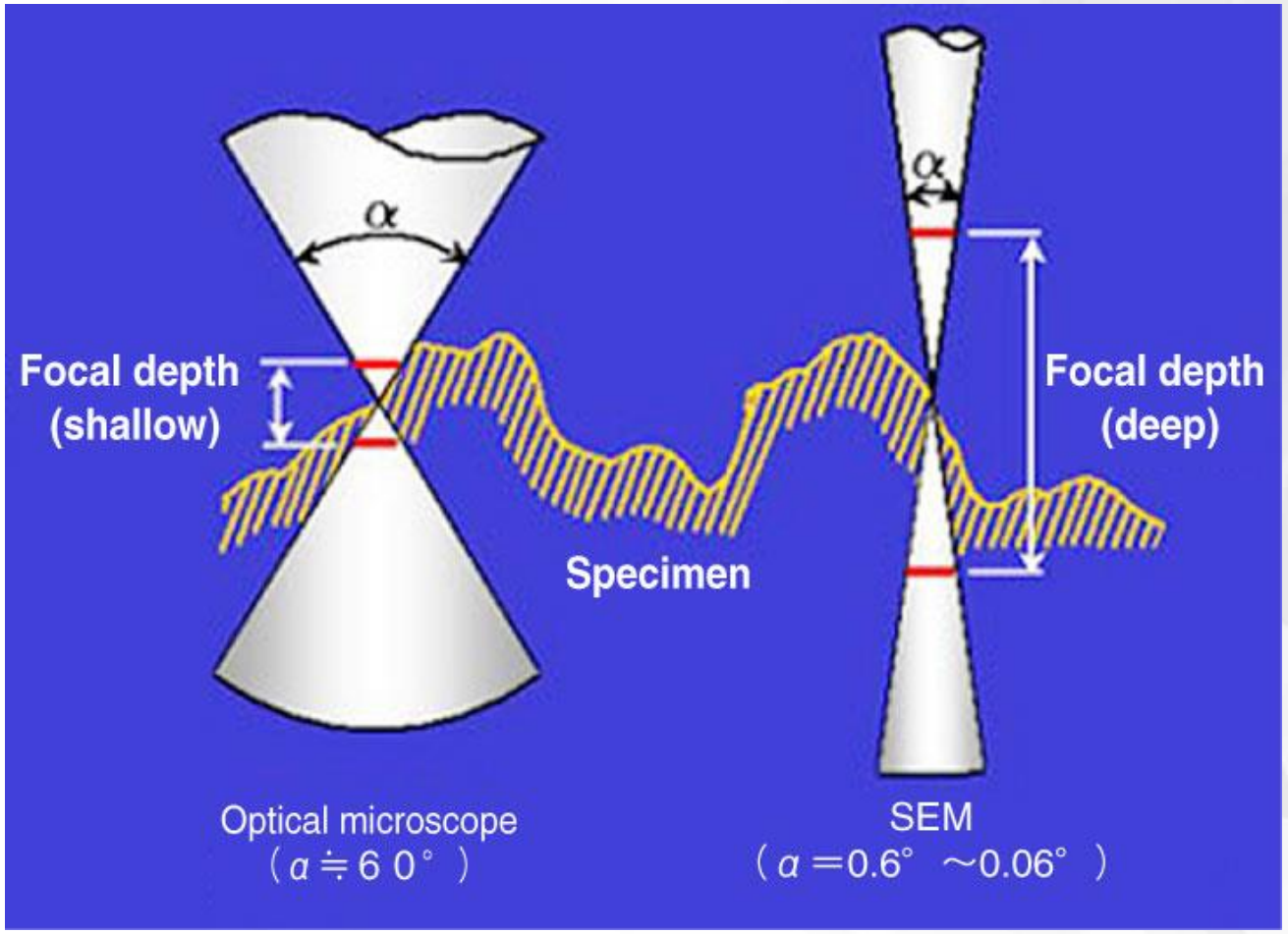
- The range of distances at the image plane where the object will remain at an acceptably sharp focus.



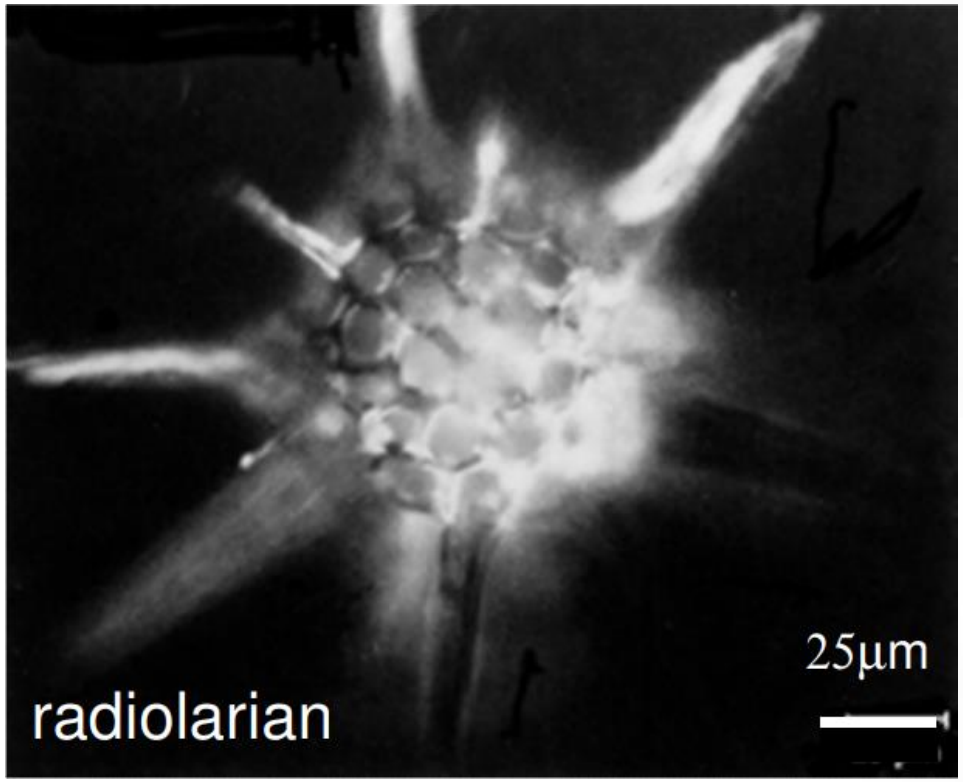
$$d = \frac{n\lambda}{NA^2} + \left(\frac{n}{M.NA}\right)e$$

λ = wavelength of illumination
n = refractive index of the imaging medium
NA = Objective numerical aperture
M = Objective lateral magnification
e = smallest distance that can be resolved by a detector that is placed in the image plane of the objective.

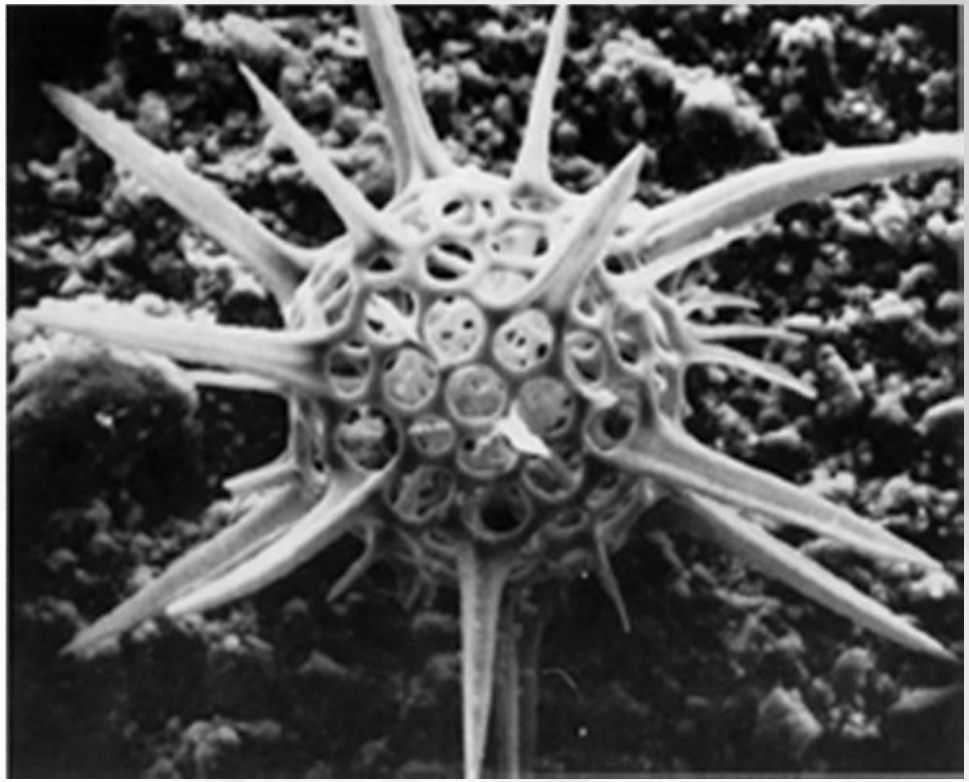
Depth of field



Depth of field (OM vs SEM)



Optical microscope



SEM

J.I. Goldstein et al., eds., Scanning Electron Microscopy and X-Ray Microanalysis, (Plenum Press,NY,1980)

How we get past the limitations of visible light?

- Advanced optical techniques
  - Localisation techniques
    - STORM, dSTORM, PALM, fPALM, PALMIRA, SPDM, GSDIM, BaLM, and PAINT
  - Structured illumination microscopy (SIM)
  - Stimulated emission depletion (STED)
- Shorter wavelengths?
  - Conventional optical microscopes more or less limited to visible light
  - Need something to produce shorter wavelengths and a way to detect them

Electron Microscopy was born

- 1924: Louis DeBroglie (Pronounced DeBroy) $\lambda_e = \frac{h}{p}$
- For things travelling near the speed of light the relationship to momentum is actually $E^2 = m_0^2c^2 + p^2c^2$ (Einstein)
- 1931 Max Knoll and Ernst Ruska built first **T**ransmission **E**lectron **M**icroscope (**TEM**)
- Late 1930's electron microscopes with resolutions of 10 nm
- 1944 electron microscopes with resolutions of 2 nm

NOTE: Theoretical resolution of an optical light microscope is 200 nm

The wavelength of an electron

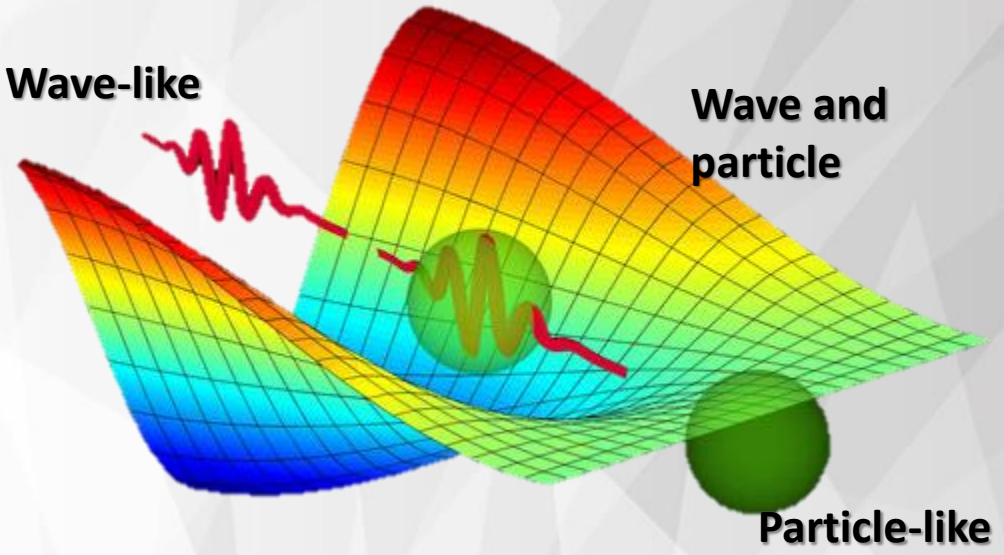
De Broglie relationship $\lambda = \frac{h}{p}$

Momentum is product of mass and velocity of a particle $\lambda = \frac{h}{m_0 v}$

Velocity of electrons determined by accelerating voltage $eV = \frac{1}{2} m_0 v^2$

∴ Velocity of electron calculated by $v = \sqrt{\frac{2eV_{acc}}{m_0}}$

∴ Wavelength of electron calculated by $\lambda = \frac{h}{\sqrt{2m_0 eV}}$



Where;

h = Planck's constant (6.626×10^{-34}Js)

m = electron rest mass (9.109×10^{-31}kg)

e = electronic charge (1.60×10^{-19}C)

V_{acc} = accelerating voltage (typ. 0.1-30 kV for SEM)

v = velocity

c = speed of light

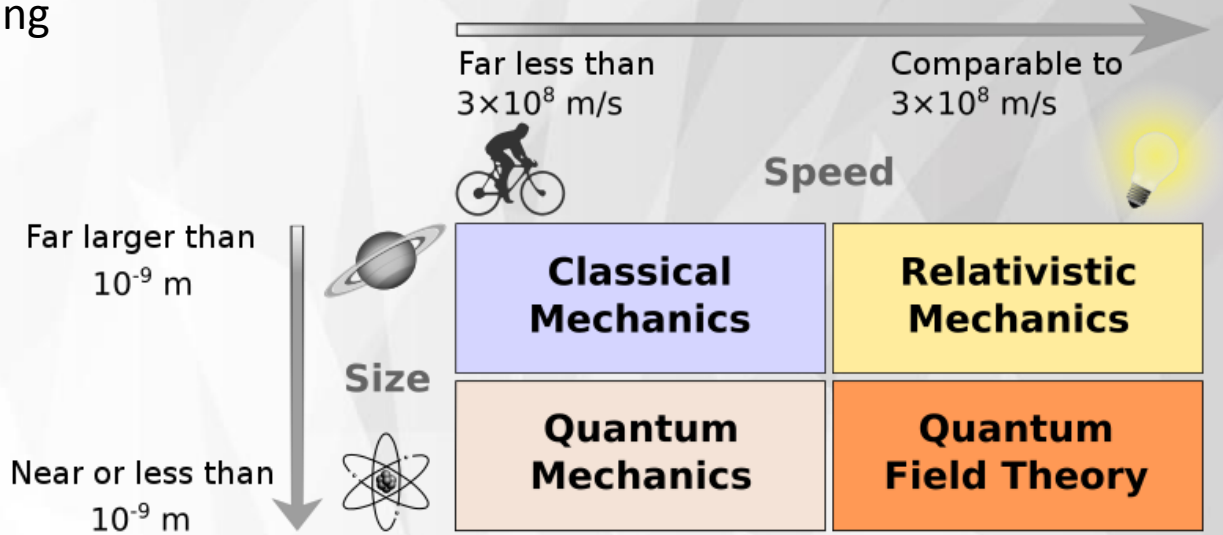
The wavelength of an electron

However high energy electrons are accelerated to velocities approaching the speed of light, so relativistic effects such as an increase in mass must be taken into account when calculating the wavelengths of the electrons.

$$V_{acc}^{*} = V_{acc}\left(1 + \frac{e}{2m_0c^2}V_{acc}\right)$$

$$= V_{acc}(1 + 9.78 \times 10^{-7}V_{acc})$$

$$\therefore \lambda = \frac{h}{\sqrt{2m_0eV_{acc}^{*}}} \qquad v = c\sqrt{1 - \left(1 + \frac{eV_{acc}}{m_0c^2}\right)^{-2}}$$



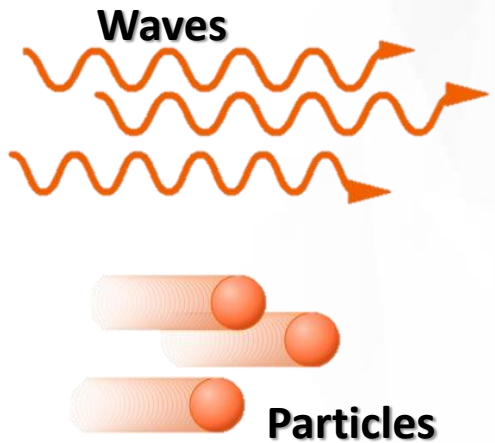
Where;

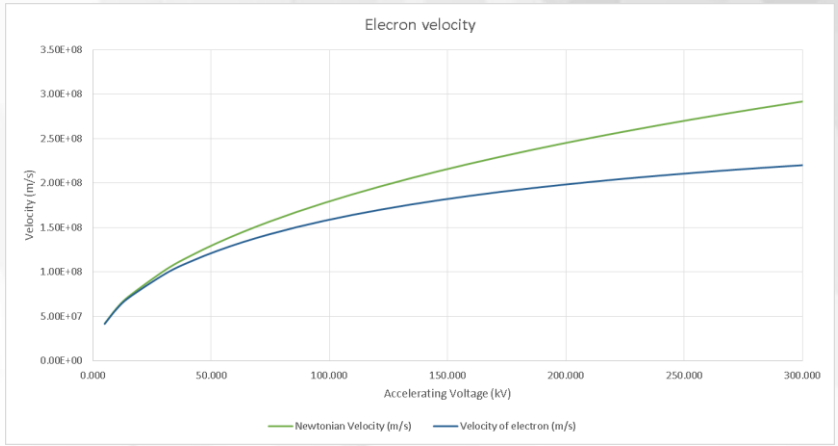
V_{acc}^{*} = Corrected accelerating voltage

The wavelength of an electron

Accelerating voltage, V_{acc} (kV)	Relativistically corrected V_{acc} (kV)	Corrected λ (nm)	Corrected λ (Å)	Corrected λ (pm)	Newtonian Velocity (m/s)	Velocity of electron (m/s)	Ratio to speed of light (v/c)
5	5.024	0.017314	0.173140116	17.314	4.19E+07	4.16E+07	0.1388
10	10.098	0.012213	0.122131996	12.213	5.93E+07	5.84E+07	0.1949
15	15.220	0.009948	0.099479973	9.948	7.26E+07	7.10E+07	0.2370
30	30.879	0.006984	0.069840314	6.984	1.03E+08	9.84E+07	0.3282
300	387.947	0.001970	0.019704026	1.970	3.25E+08	2.33E+08	0.7763

Name	Symbol	Base 10
centi	c	10^{-2}
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}

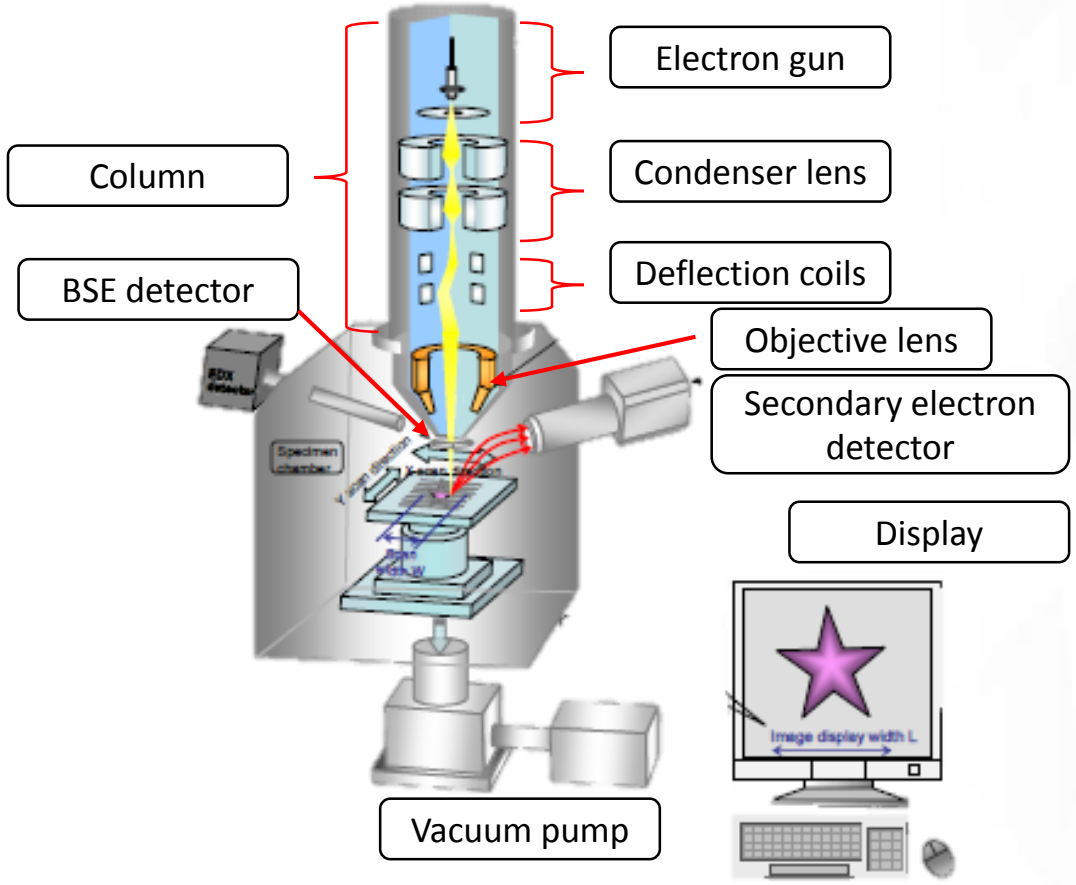




Optical Microscope Vs Electron microscope

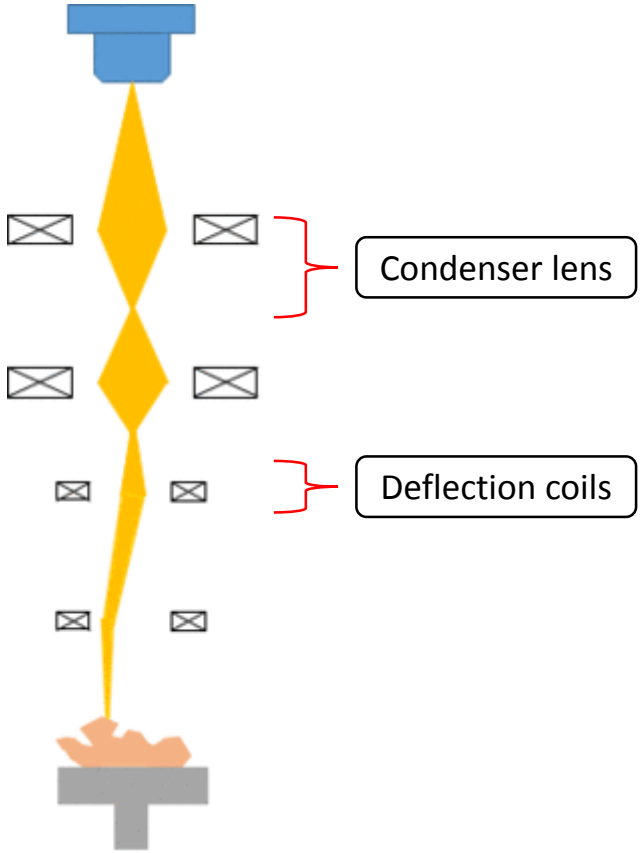
	Optical Microscope	Electron microscope
Wavelength	400-700 nm	~0.01 nm @ V_{acc}= 15 kV
Typical resolution	200 nm	0.5 nm
Magnification	x1-x1500	x10-x300,000 (SEM)
Depth of Field	Up to ~3 µm	mm
Image	Colour	Grayscale
Vacuum	Not Required ∴ **Can** observe lice cells/processes	Required ∴ **Can't** observe live cells/processes
Compositional information		Can provide elemental information

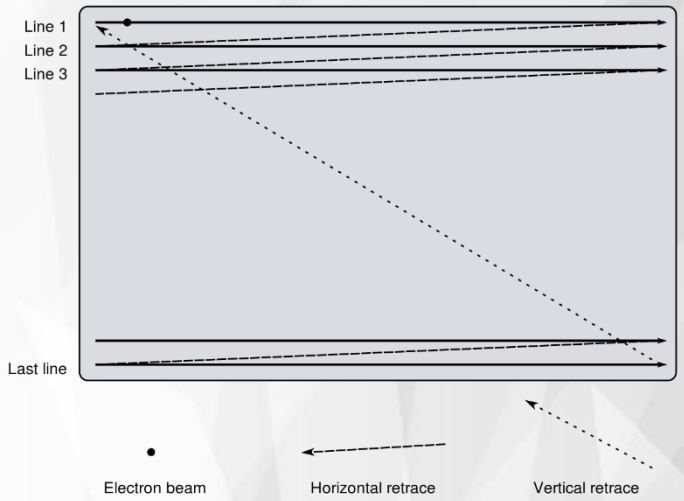
How an SEM works – a look Inside a SEM

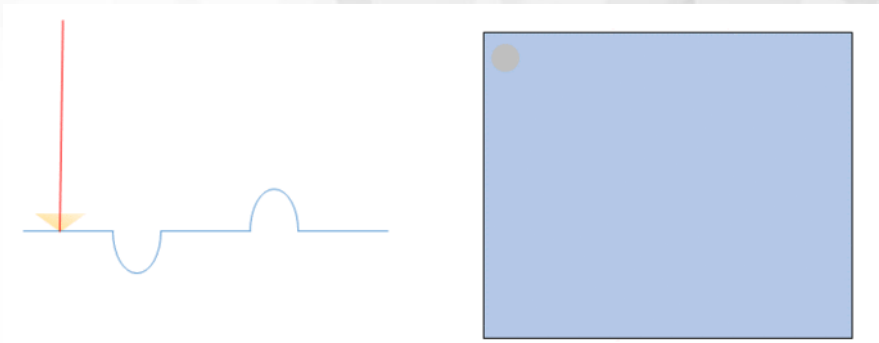


- Source / electron gun
- Column
  - Condenser lenses
  - Deflection coils
  - Objective lens
- Chamber
- Detectors
- Vacuum system
- Display

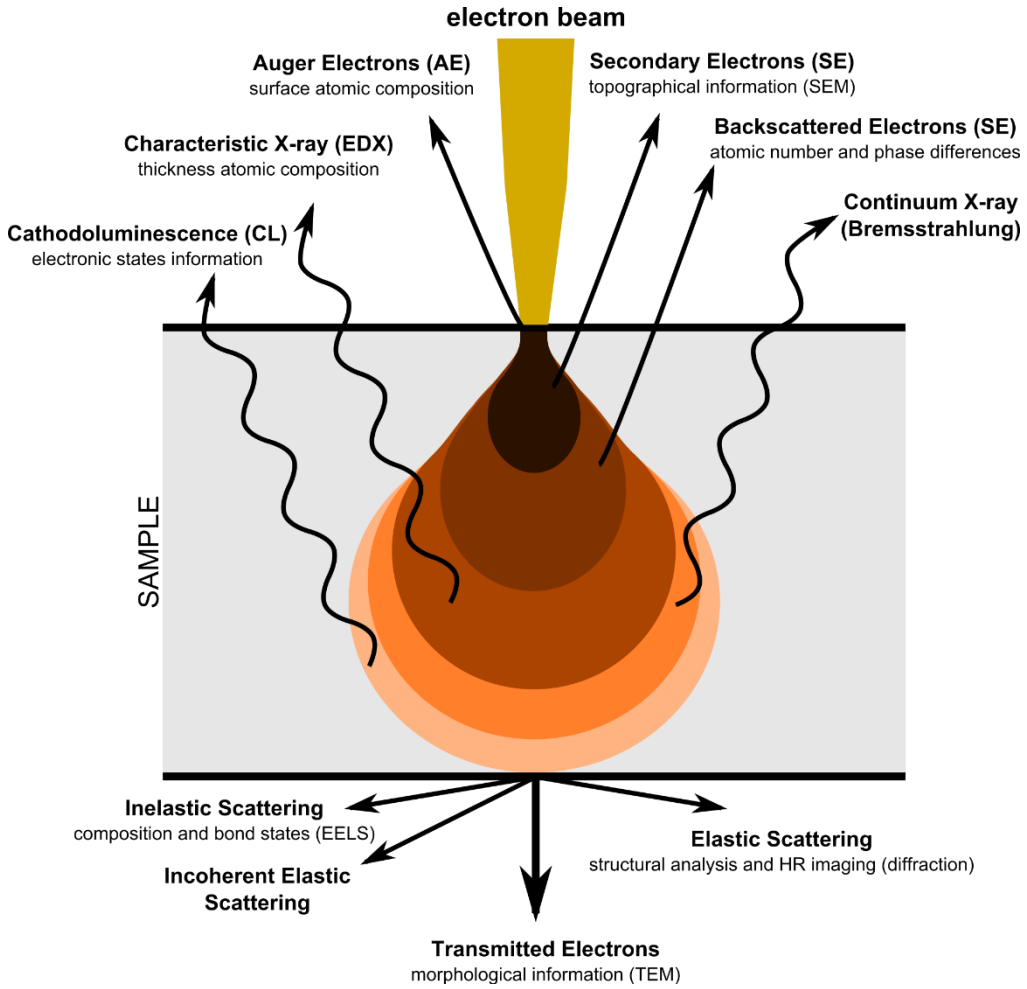
How the SEM ‘builds’ an image







Signals generated



- **Back-scattered electrons (BSE)**

  Electrons that a reflected back from the sample by elastic scattering.

  Intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen.

- **Secondary electrons (SE)**

  Electrons generated as ionisation products.

  Called secondary as they are generated by the primary radiation source.

  Gives surface information.

Signals generated

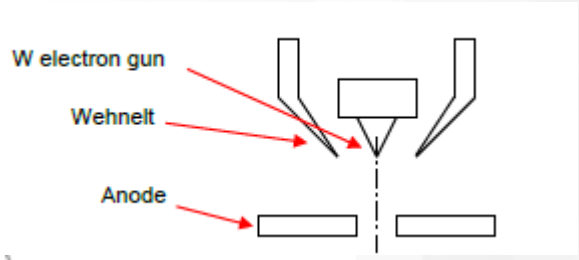
- Characteristic X-rays

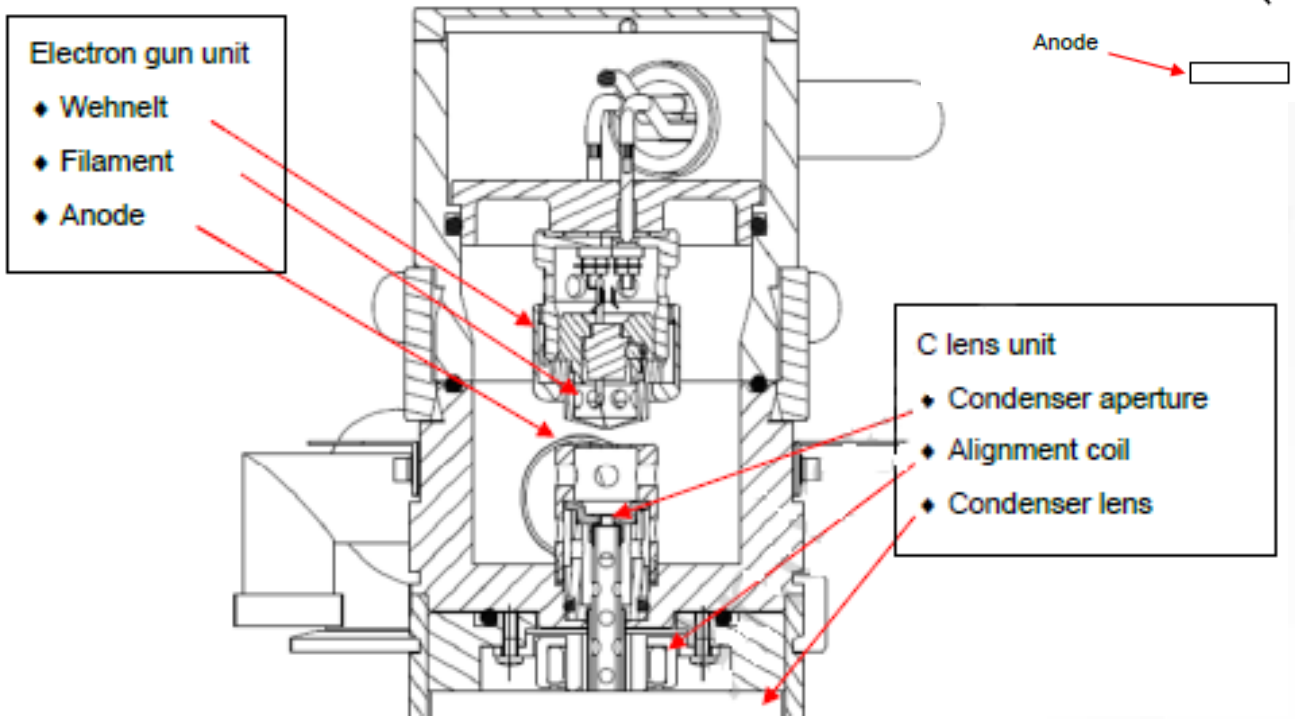
  X-rays emitted when electron beam removes an inner shell electron from the sample causing a higher energy electron to fill the shell and release energy.

  Used to identify the composition and measure the abundance of elements in the sample.

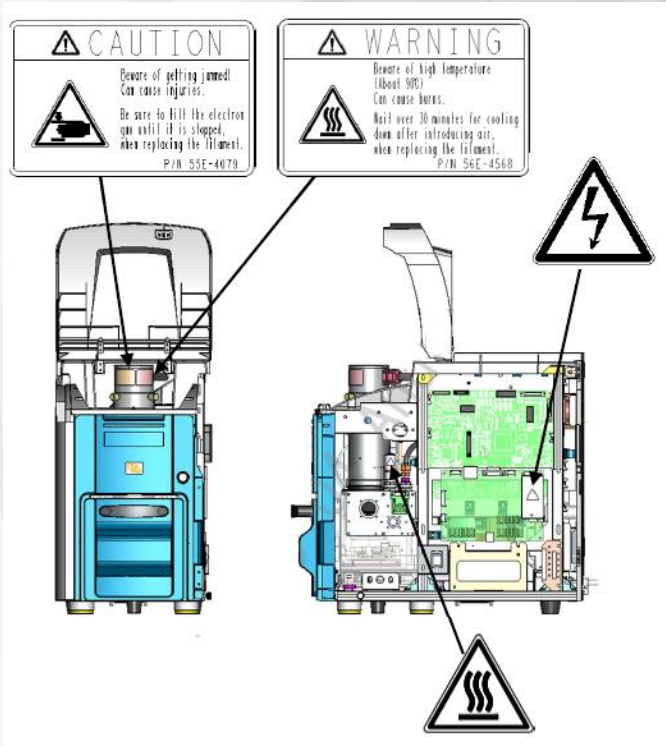
  NOTE: The SEM is shielded and no hazard from exposure to x-rays

Electron Gun





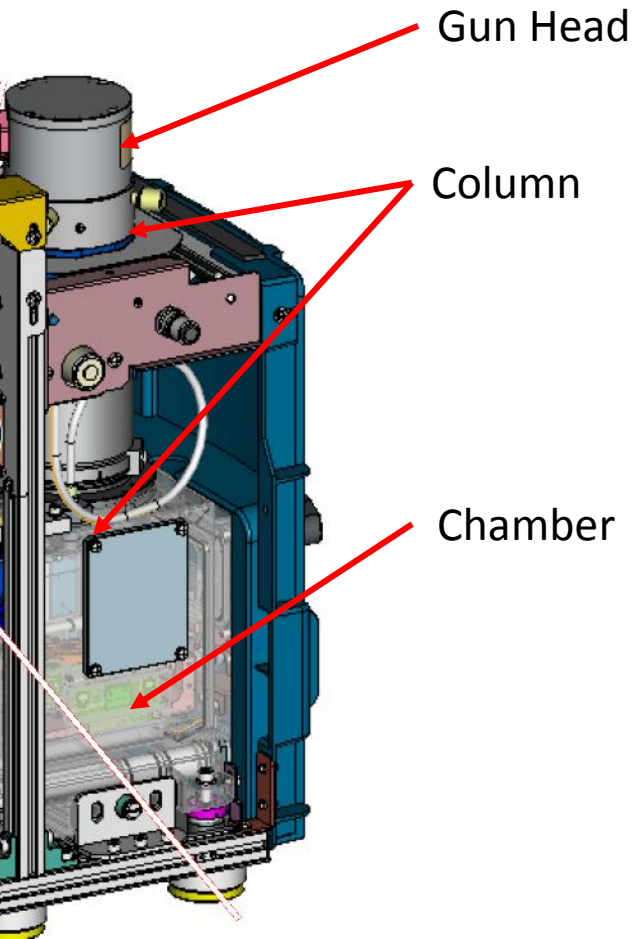
Metal	Melting temp. (K)	Heating temp of filament in TM4000 (K)
W, tungsten	3650	~2700

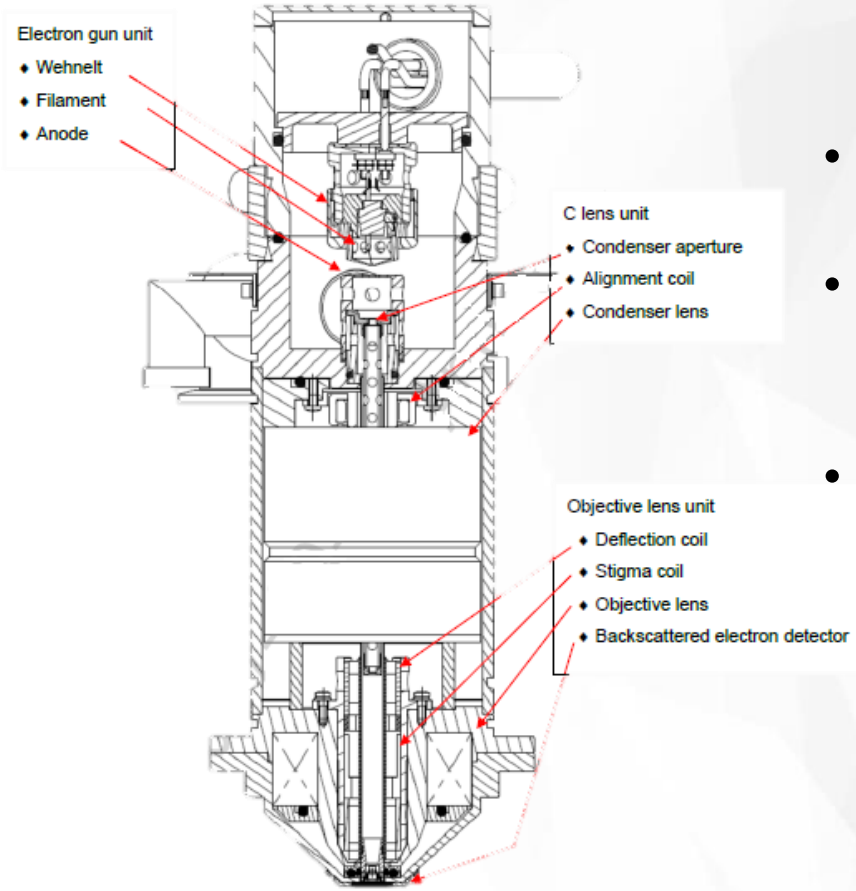


Accelerating Voltage

- The accelerating voltage determines the velocity of the electrons
- Higher kV will give you higher resolution, but potentially more charge-up on non-conductive samples.
- Lower kV will give clearer surface structures, but lower resolution.
  - Also less charge-up and less potential damage to samples.

Column

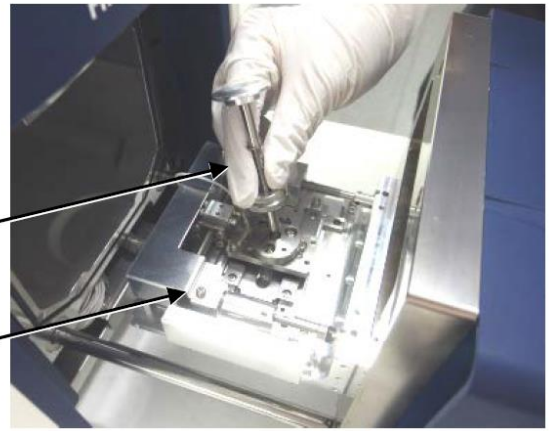


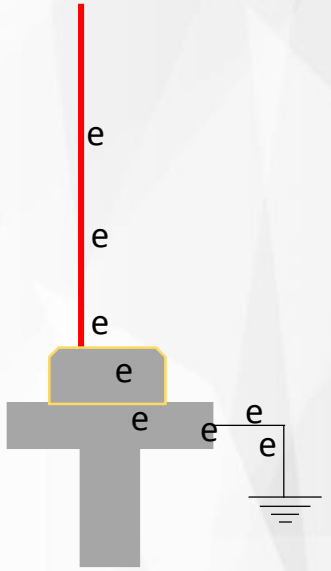


- From gun head to chamber
- Electromagnetic ‘optics’ to shape and deliver a small beam diameter to sample
- Deflection coils to “scan” beam across sample

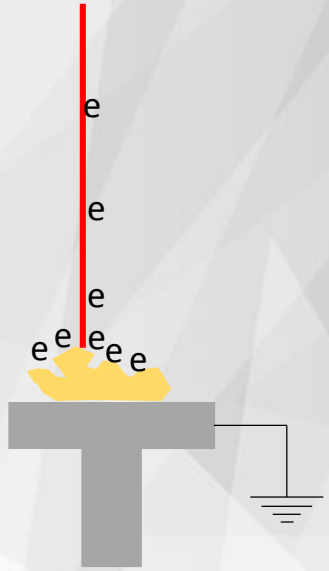
Sample / Stage

- Stage grounded
- Charging



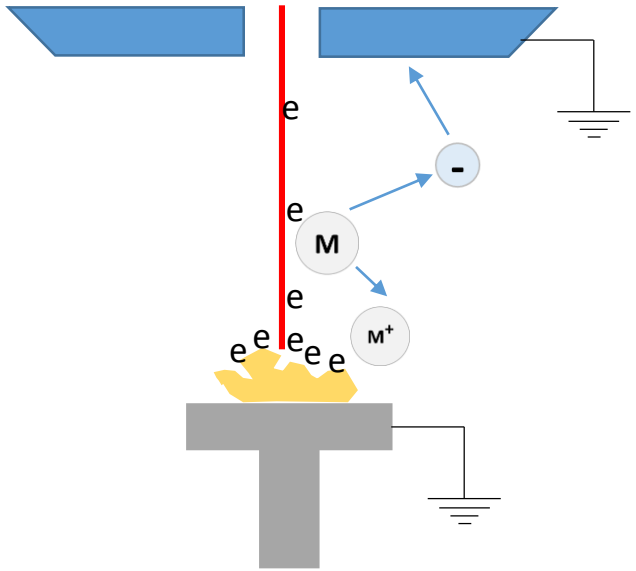


Conductive sample



Non-Conductive sample

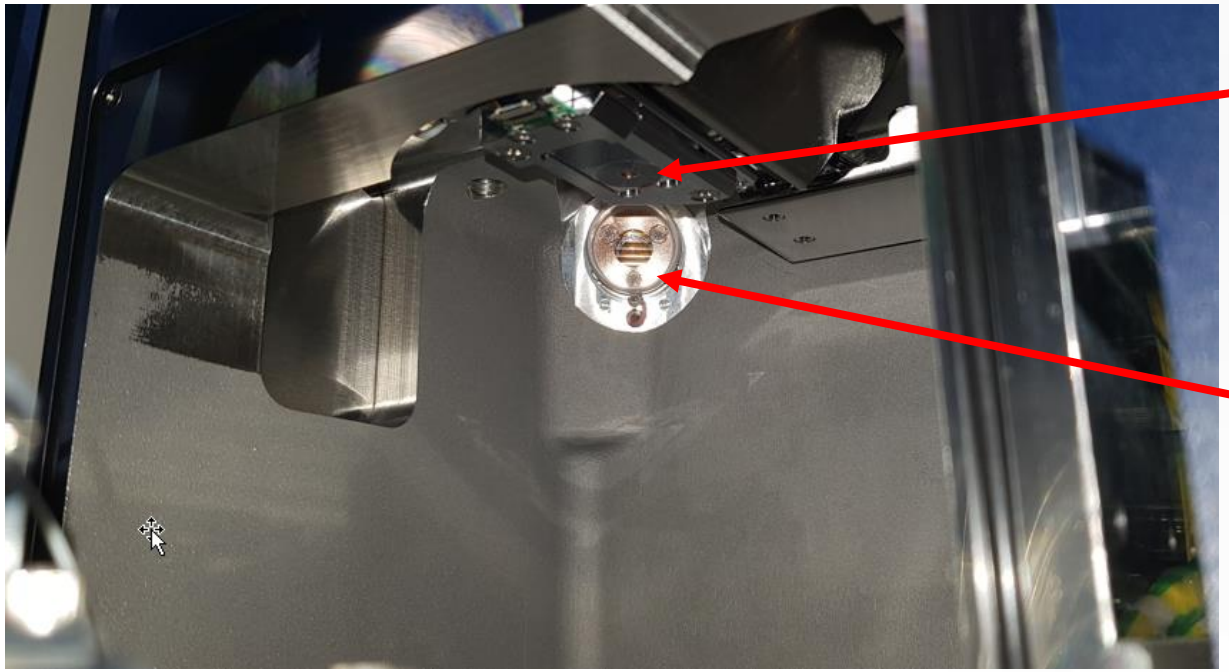
Charge up reduction modes

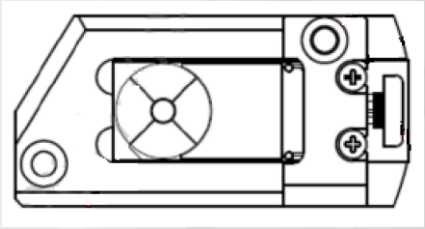


Non-Conductive sample

- In lower vacuum levels (more residual gas in chamber, mainly Nitrogen)
- Primary electrons collide with residual gas
- Positive ions generated that help neutralise negative charge on the surface of specimen.

Detectors

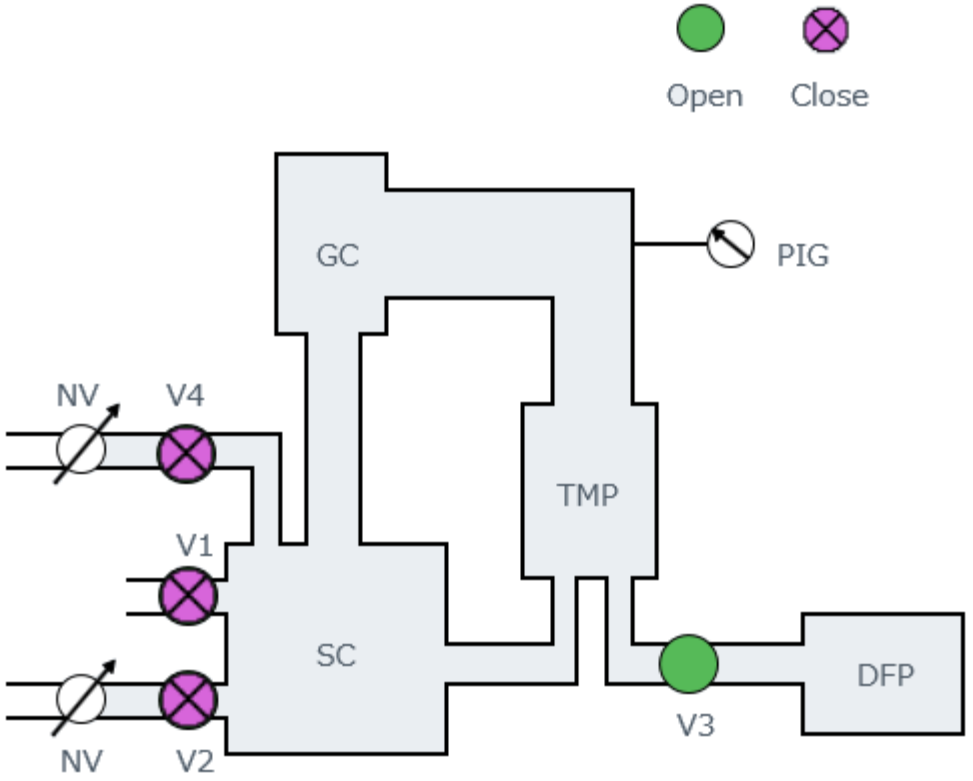




BSE

UVD (SE Detector)

Vacuum system



TMP

DFP

Particle	Mass	
	kg	Relative
Proton (m_p)	1.673×10^{-27}	1836.15
Neutron (m_n)	1.675×10^{-27}	1838.68
Electron (m_e)	9.109×10^{-31}	1

Using the TM4000

Front of TM4000

1. Starting The Instrument

(1) Turn on the earth leakage breaker on the back of the main unit.

(2) Turn on the power switch on the right side of the main unit to start the device.

(3) When the unit is turned on the Evacuation process will start automatically and the EVAC LED will blink BLUE.

If you want to load a sample, press the EVAC/AIR button. The AIR LED will then blink WHITE. Once the specimen chamber is at air, the AIR LED will be stop blinking and remain WHITE.

(4) Turn on the PC. Start the TM4000/TM4000Plus application by double-clicking the TM4000 programme shortcut. The TM4000 start screen will be displayed until the Graphical User Interface (GUI) is loaded.

2. Preparing Specimen

Bulk specimen

(Conductive/Non-conductive)

(5) Put the conductive tape on the specimen stub and attach a specimen on it.

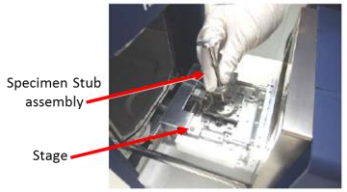
Water / Oil containing specimen

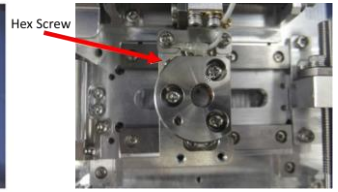
(5) When observing water containing specimens such as biological and botanical specimens, food items and so on, use a paste to stick a specimen on the stub.

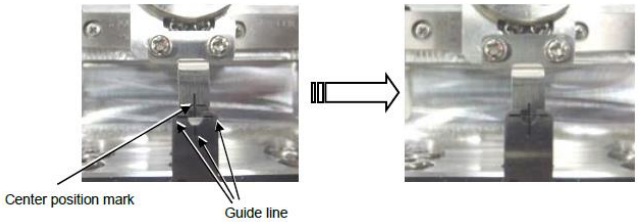
(6) Attach the specimen stub to the specimen holder, and adjust its height using a height gauge so that the gap width becomes about 1 mm.

Using the TM4000

3. Loading the Specimen







(7) Ensure the chamber is at air, ie AIR LED is not blinking and is WHITE.

If the EVAC LED is Blue, press the EVAC/AIR switch. The AIR LED (WHITE) should start blinking. When the AIR LED stops blinking and remains illuminated the chamber is at AIR.

(8) After the AIR LED stops blinking wait approximately five seconds or more before slowly drawing out the specimen stage.

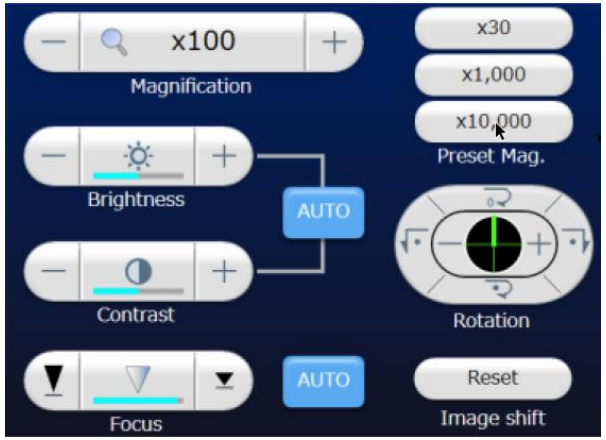
(9) Insert specimen stub assembly into the stage base.

(10) **OPTIONAL** Lightly tighten the hex screw to secure the specimen stub assembly to the stage base.

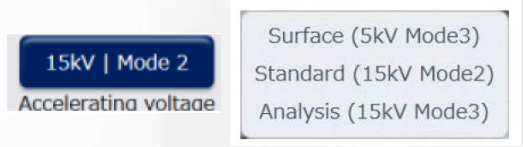
(11) Use the X, Y knobs to centre the specimen stage by lining up the centre position mark with the guides.

(12) Press the EVAC/AIR Switch to evacuate the chamber. The EVAC LED will blink BLUE while the chamber is being evacuated. Once evacuation is complete (~2.5 min) the EVAC LED will stop blinking and remain illuminated (BLUE)

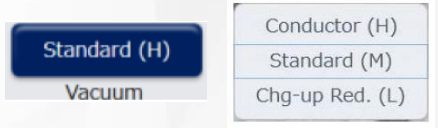
4. Starting Observation



(13) Select Accelerating Voltage / Observation condition.



(14) Select Vacuum level



(15) Select Detector. Can change selection later so not critical to select at this point.

(16) Click the 

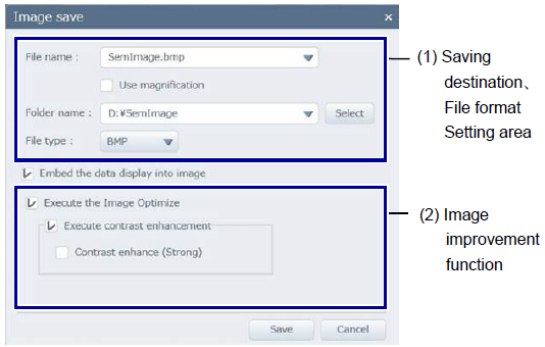
button to turn on the high voltage.

(17) Use the auto functions

a) Set desired field of view using the X, Y knobs on the front of the TM4000.
b) Adjust the Brightness and Contrast (click the Auto B/C button.
c) Perform focusing (click the Auto Focus button)
    a) Perform manual focus adjustment if required.

5. Saving Captured Images

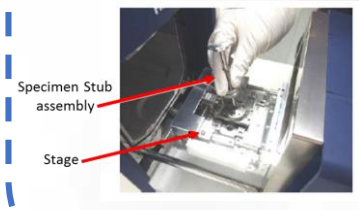
(18) Click the “Save” button to capture the image



NOTE: Quick Save will save the image on the screen, ie lower resolution than if you use the Save button.

(19) After the image capturing process is completed the Save Image dialog will be displayed. Input a file name and save the image.

6. Stopping Observation



(20) Click the Stop button to turn off the high voltage.

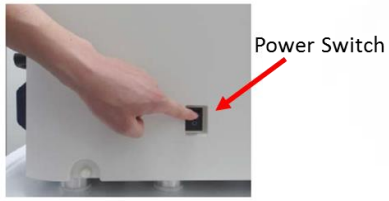
(21) To remove the sample press the EVAC/AIR switch to introduce air into the specimen chamber. Wait until the AIR LED (WHITE) stops blinking and remains illuminated.

(22) Slowly draw out the specimen stage, undo the hex mounting screw and remove the specimen stub assembly from the stage.

(23) Close the specimen stage door.

(24) If you are not looking at any other specimens, press the EVAC/AIR switch to evacuate the chamber. The EVAC LED (BLUE) will blink while the chamber is evacuated and will stop blinking and remain illuminated once the chamber is under vacuum (~2.5 min)

7. Shutting Down The Instrument



(25) Make sure the chamber is under vacuum, ie EVAC LED is not blinking and remains illuminated (BLUE)

(26) Close the TM4000 Graphical User Interface (GUI)

(27) Turn off the Power Switch on the right side of the TM4000.

NOTE: Always leave the chamber under vacuum when system not in use. This helps keep dust and moisture out of chamber to ensure the microscope remains in good condition.

Key Points to remember

- Electron microscopes;
  - Allow **higher magnification**, **better resolution** and **larger depth of field** compared to optical microscopes
  - Can provide information about composition of a sample
    - BSE (compositional information)
    - EDS (x-ray fingerprint of elements)
  - Produce grayscale images while optical microscopes use visible light so provide colour images
  - Require a vacuum system to operate so can’t observe living cells like you can with an optical microscope

Key Points to remember

- In the case of systems like the TM4000/TM4000Plus

Very easy to use with minimal sample preparation

Don’t be intimidated by the microscope and HAVE FUN!