The Transmission Electron Microscope

Karen M. Davies
PBS – eBIC,
Diamond Ltd, UK

Wellcome Trust Graduate Training Program - CryoEM

Aluminum Σ 11(113) / [ˉ 100] grain boundary, G.H. Campbell, LLNL

Outline

Why Electrons?

Types of interactions

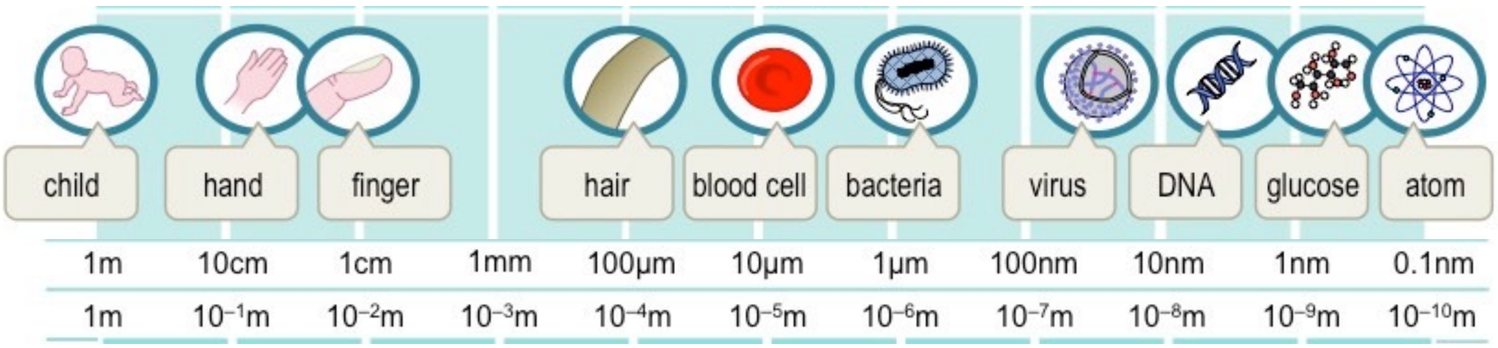
The TEM

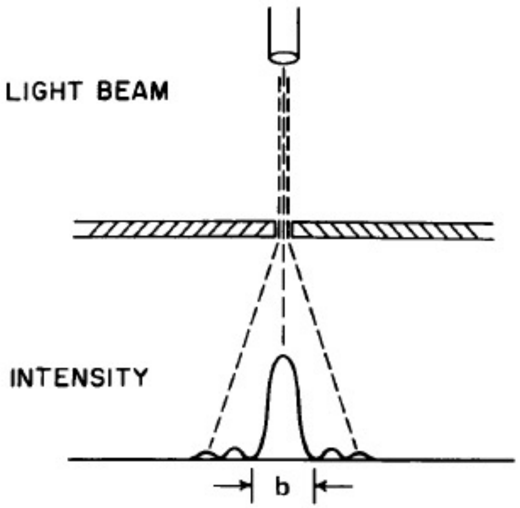
- Vacuum system
- Electron source
- Lenses
- Condensor system
- Objective Lens
- Imaging system

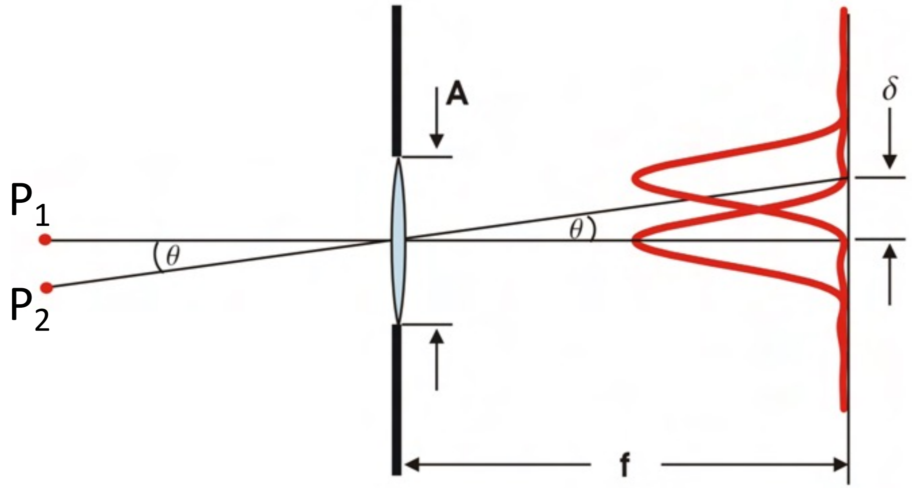
Aberrations and correctors

Suggested Reading

Why Electrons?







$$\delta_{min} = \frac{0.61\lambda}{n \sin\beta}$$

$$(n \sin\beta = NA)$$

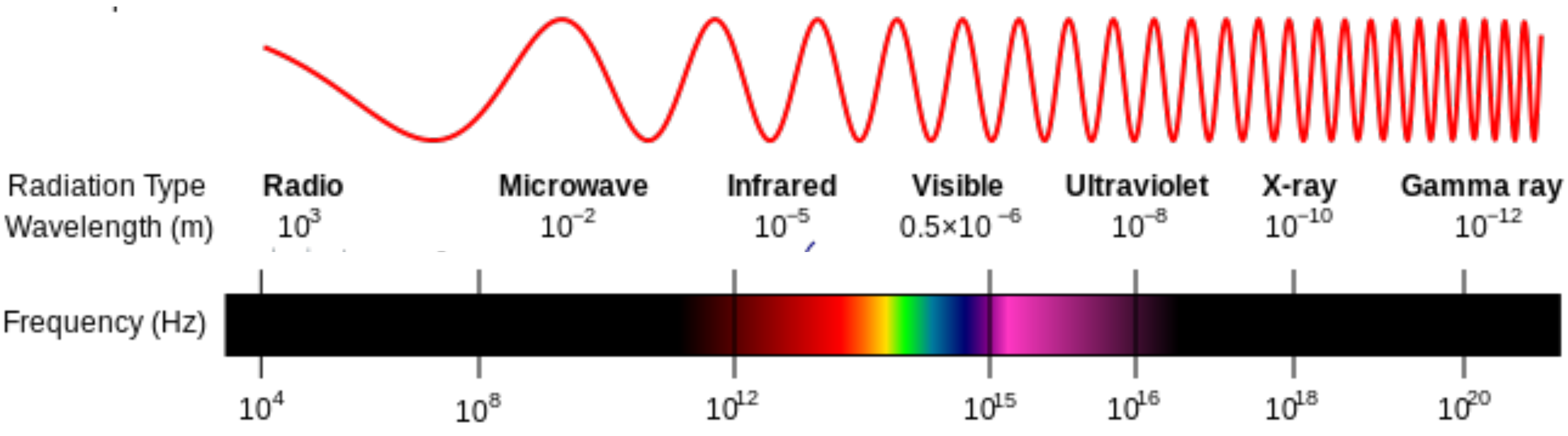
Limiting aperture → diffraction
Point source → airy disk (b)

Rayleigh's criterion: to resolve two point sources, P1 airy disk maxima overlays first minima of P2.

Resolution dependent on wavelength and numerical aperture (NA)

In EM, NA tends to unity → To image 1Å, λ = 0.5Å

Why Electrons?



$$\lambda = \frac{h}{p} = \frac{h}{m_e v}$$

Debroglie equation

Electrons act as both particles and waves

The wavelength of electrons depends on velocity

We can control wavelength of electrons by voltage

To match x-rays we need a voltage between 1-1000kV

Higher voltage = shorter wavelength.

But higher energy = more specimen damage

Biological TEM operate at 300kV max: λ~0.2pm

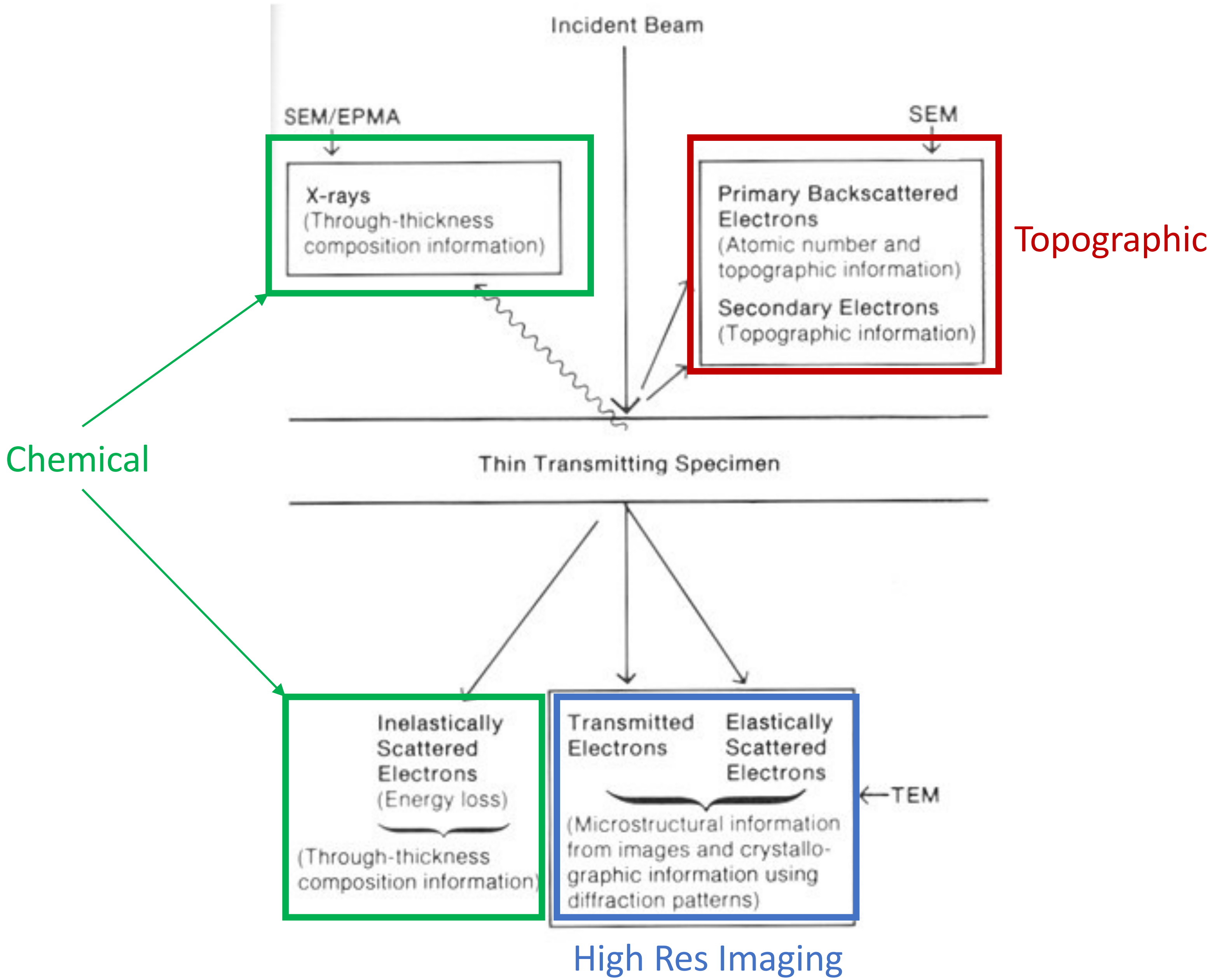
$$\lambda(nm) \approx \sqrt{\frac{1.5}{V}}$$

Current max resolution

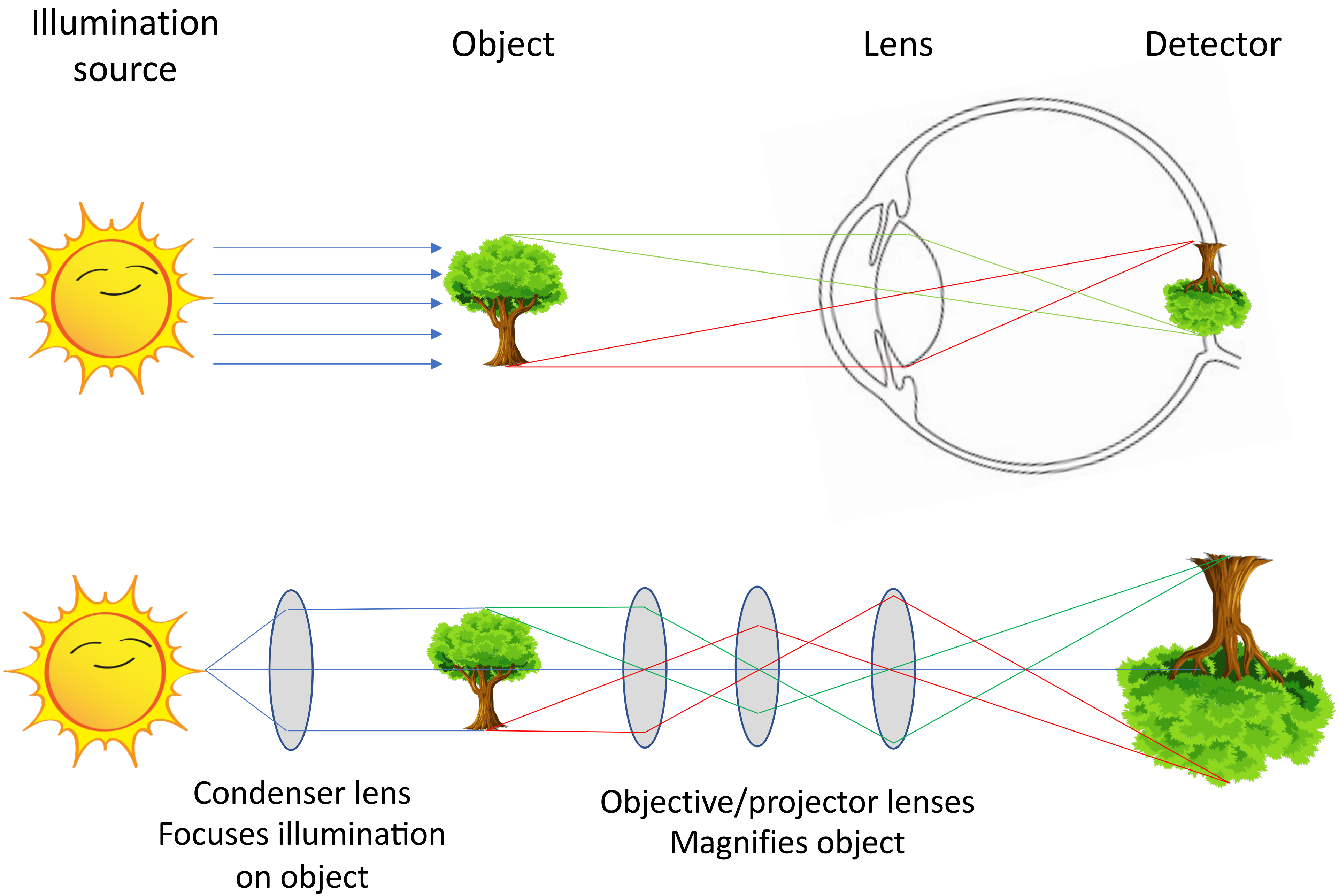
Materials: 0.05 nm

Biology: 0.14 nm

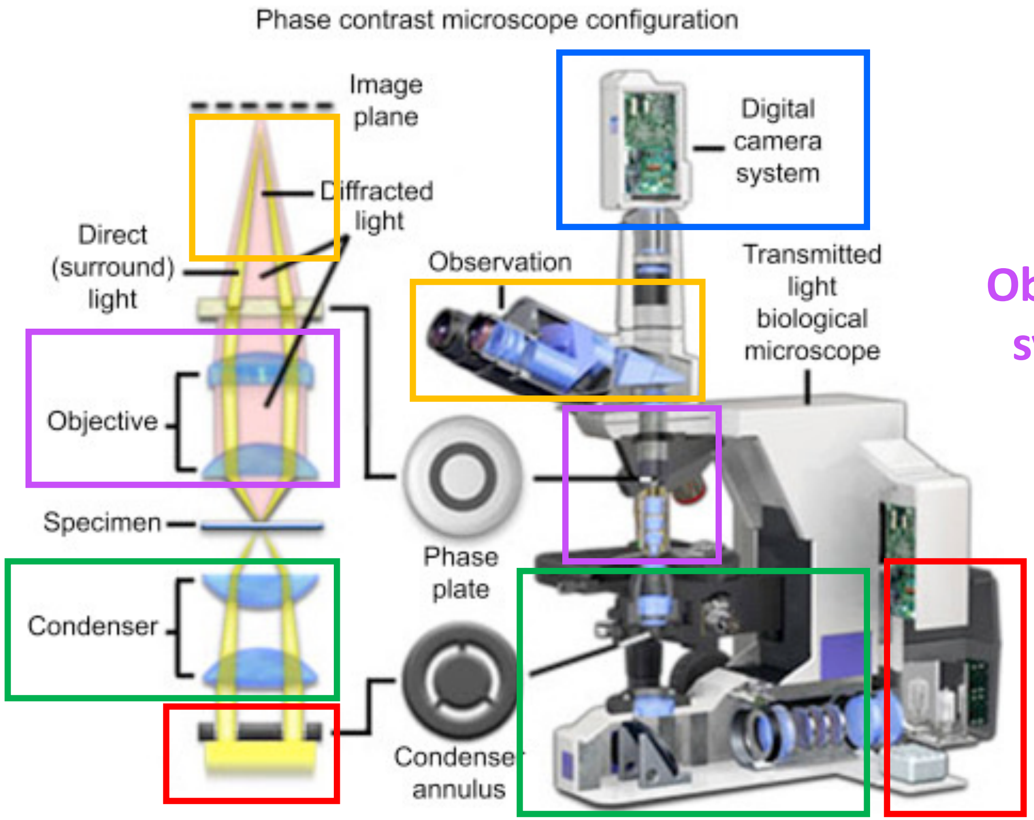
Signals from Electron Interaction with Matter



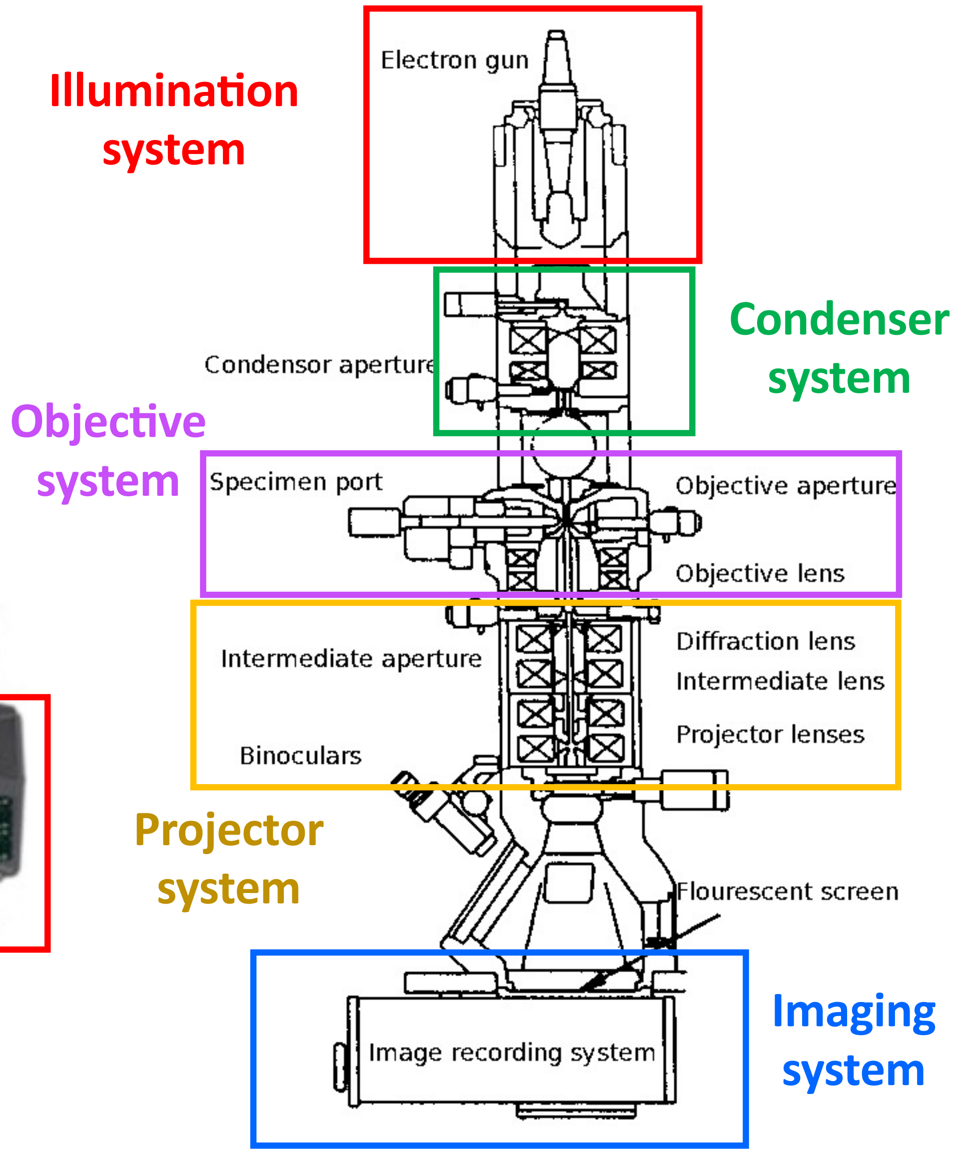
Basic Image Formation



Light vs. Electron Microscope

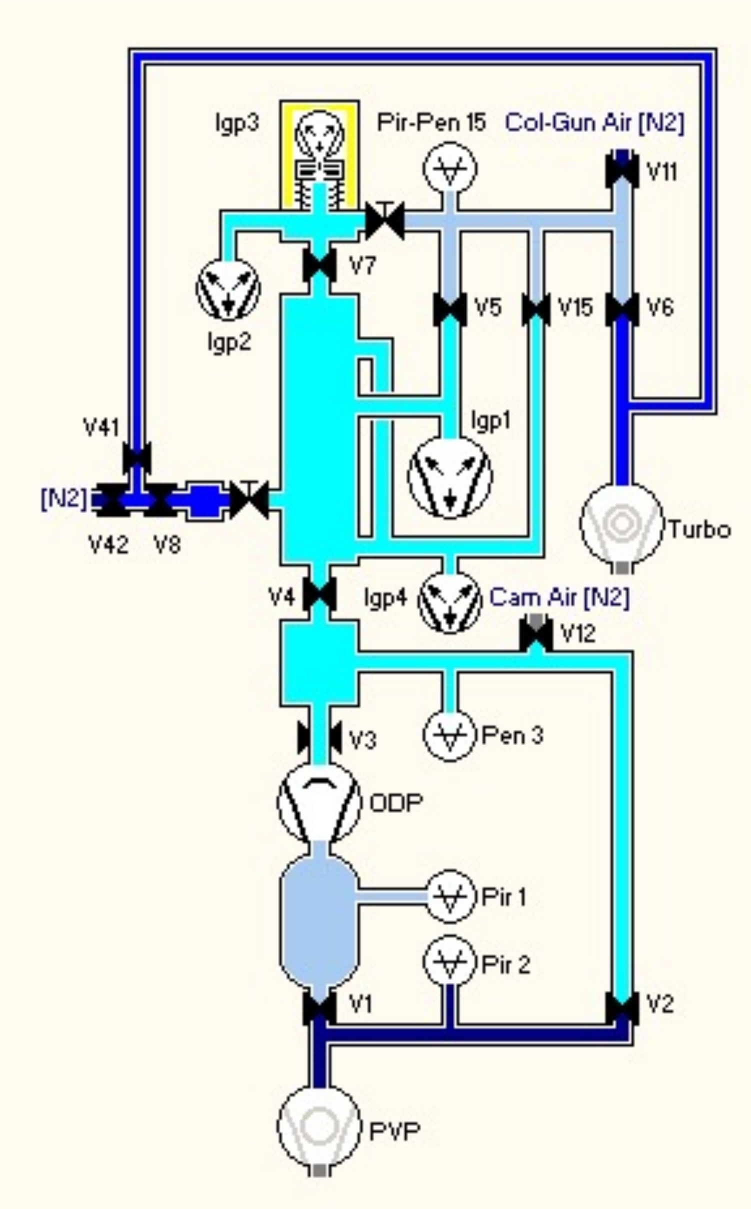


Light

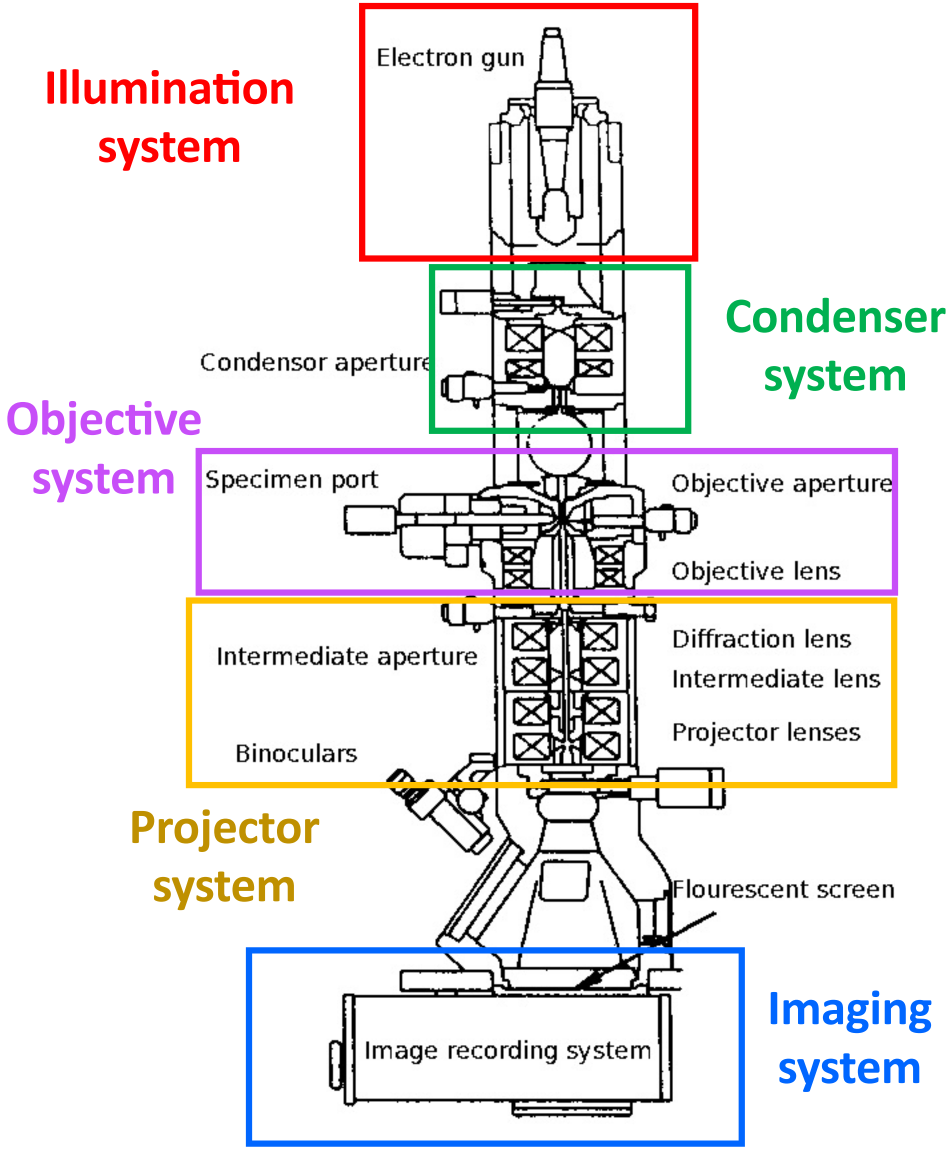


Electron

Light vs. Electron Microscope

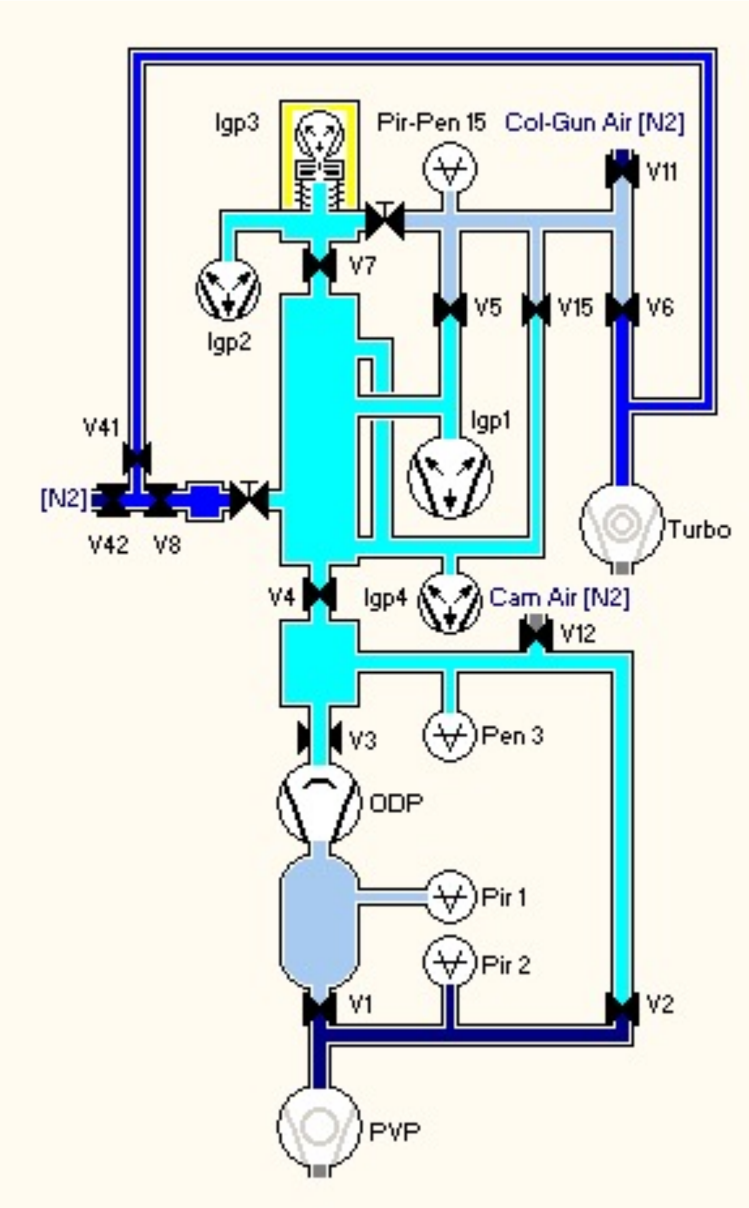


Vacuum system



Electron

Vacuum System



Vacuum system

Why operate in vacuum?

- Electrons interact well with matter
- Mean-free-path length: 20 cm in air
  2 km in vacuum

How is vacuum achieved?

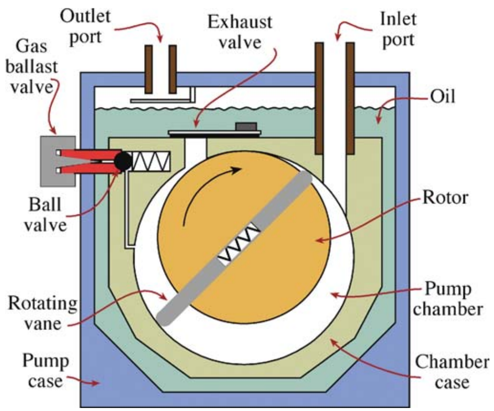
- Rotary pump – ATM to rough vac.
- Oil diffusion pump (ODP) / Scroll Pump - low vac
- Turbo pump – high vacuum
- Ion getter pump (IGP) – ultra high vacuum
- Cryo-pump/trap – high vacuum

How is vacuum monitored?

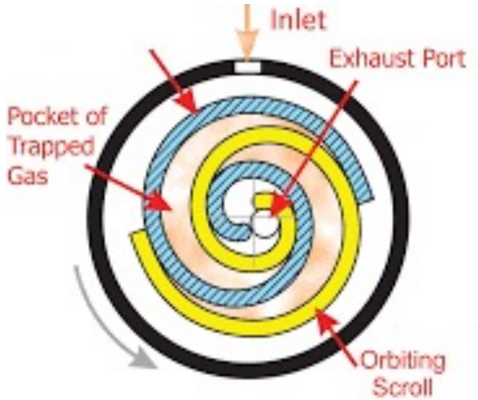
- Pirani gauge (Pir) – ATM to low vac
- Penning gauge (Pen) – high vac
- Current readout (IGP) – ultra high vac

1Pa = 0.01 mbar
Room Pressure: 10^5 Pa
Rough Vacuum: 100-0.1 Pa
Low Vacuum: 0.1 - 10^{-4}
High Vacuum: 10^{-4} - 10^{-7} Pa
Ultra High Vacuum: 10^{-7} Pa and below

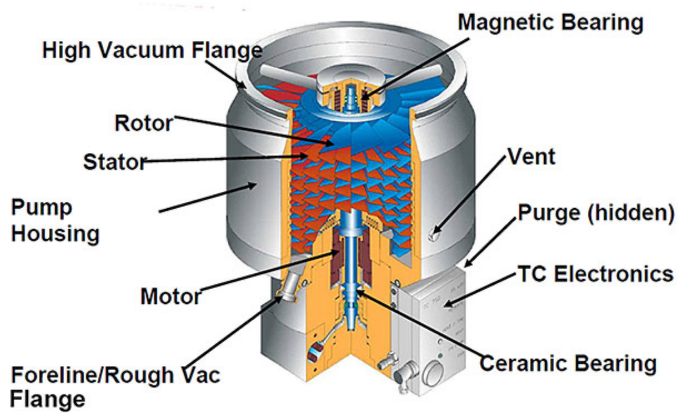
Vacuum System



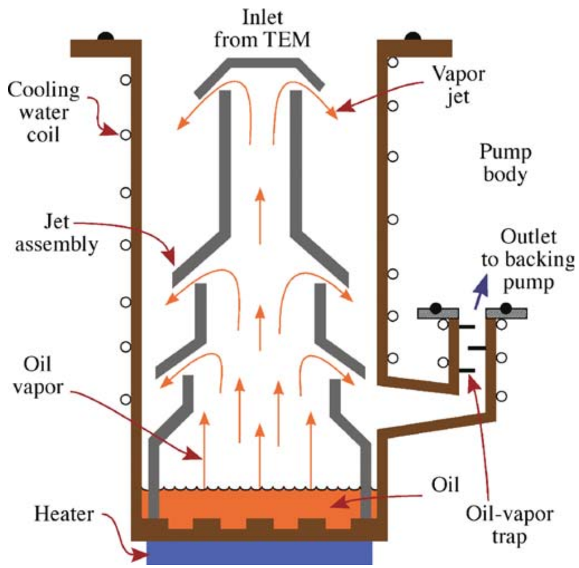
Rotary pump



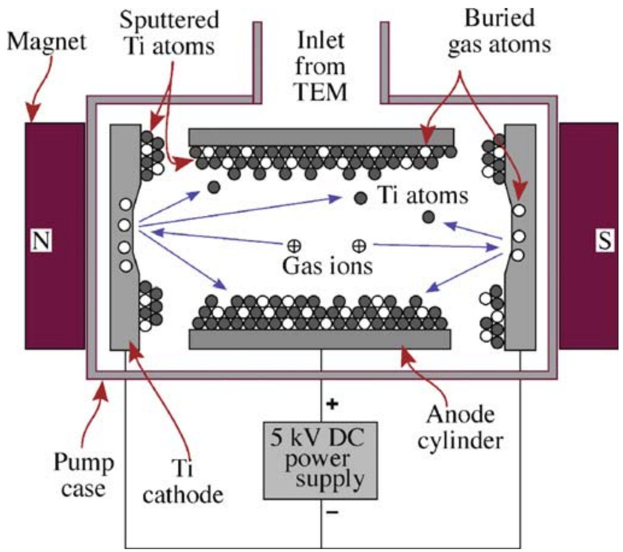
Scroll Pump



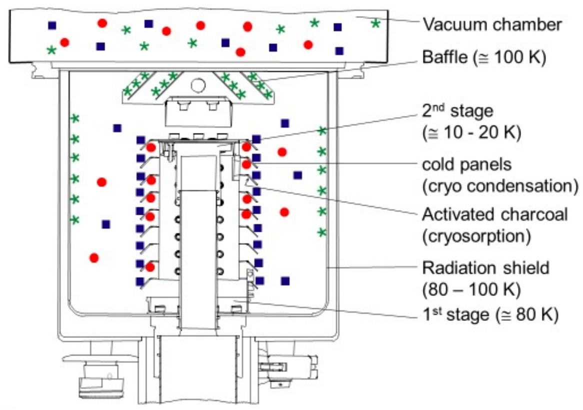
Turbo Pump



Oil Diffusion Pump



Ion Getter Pump

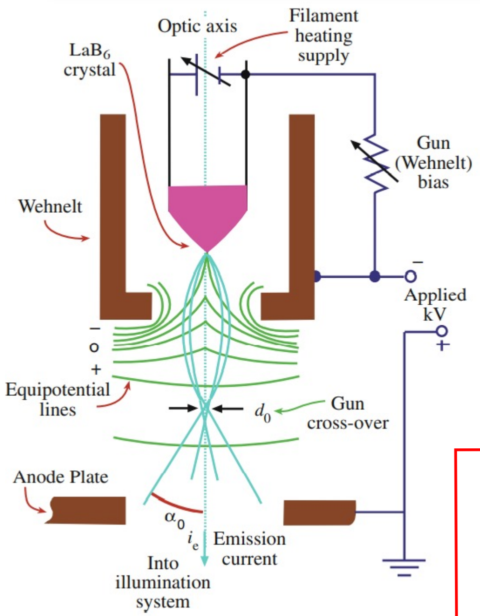


Cryo-pump

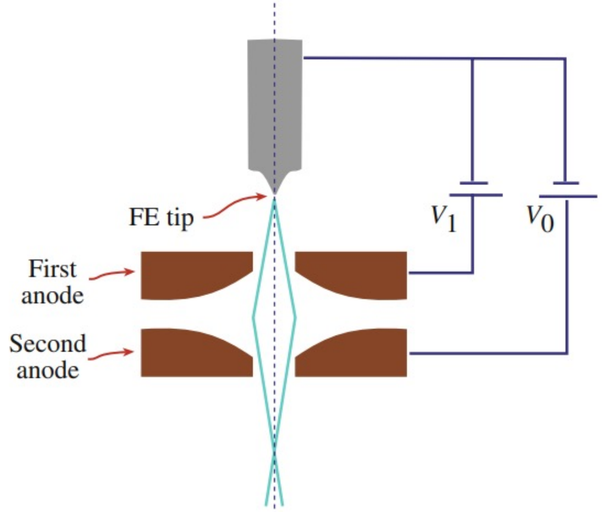
Images from Williams and Carter “Transmission Electron Microscopy” (2016) & internet

Electron Source

Thermionic



Field Emission



Gun quality:

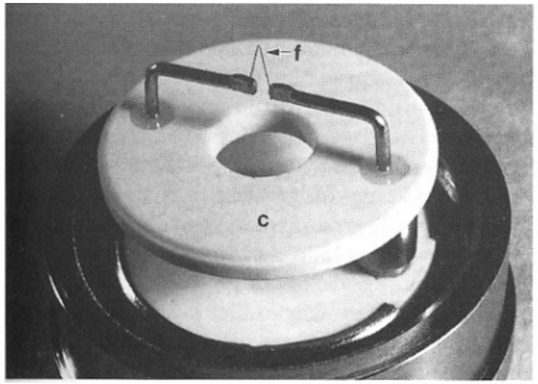
Temporal coherence – Wavelength spread

Spatial coherence – Angular spread

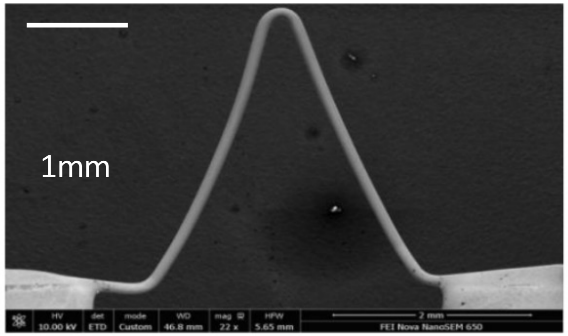
	Units	Tungsten	LaB_6	Schottky FEG	Cold FEG
Work function, Φ	eV	4.5	2.4	3.0	4.5
Richardson's constant	A/m^2K^2	6×10^9	4×10^9		
Operating temperature	K	2700	1700	1700	300
Current density (at 100 kV)	A/m^2	5	10^2	10^5	10^6
Crossover size	nm	$> 10^5$	10^4	15	3
Brightness (at 100 kV)	A/m^2sr	10^{10}	5×10^{11}	5×10^{12}	10^{13}
Energy spread (at 100 kV)	eV	3	1.5	0.7	0.3
Emission current stability	%/hr	<1	<1	<1	5
Vacuum	Pa	10^{-2}	10^{-4}	10^{-6}	10^{-9}
Lifetime	hr	100	1000	>5000	>5000
Cost of tip		£80	£800	£8000	£8000
Time to replace		1-2 days	1-2 days	5-8 days	5-8 days

Filaments

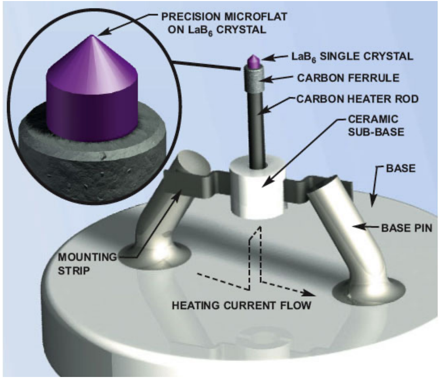
Tungsten (W)

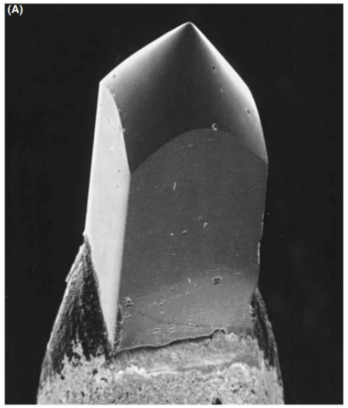


Bozzola and Russell, Fig. 6.22

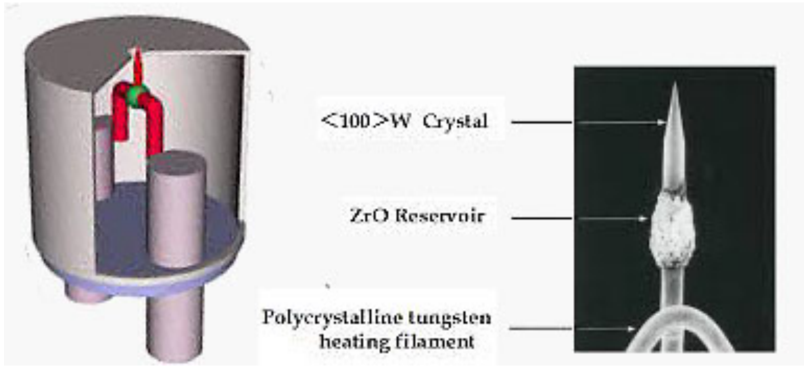


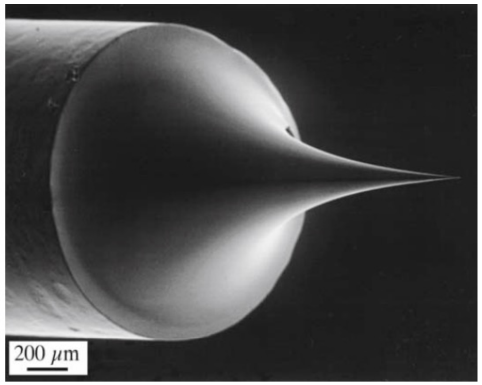
LaB_6



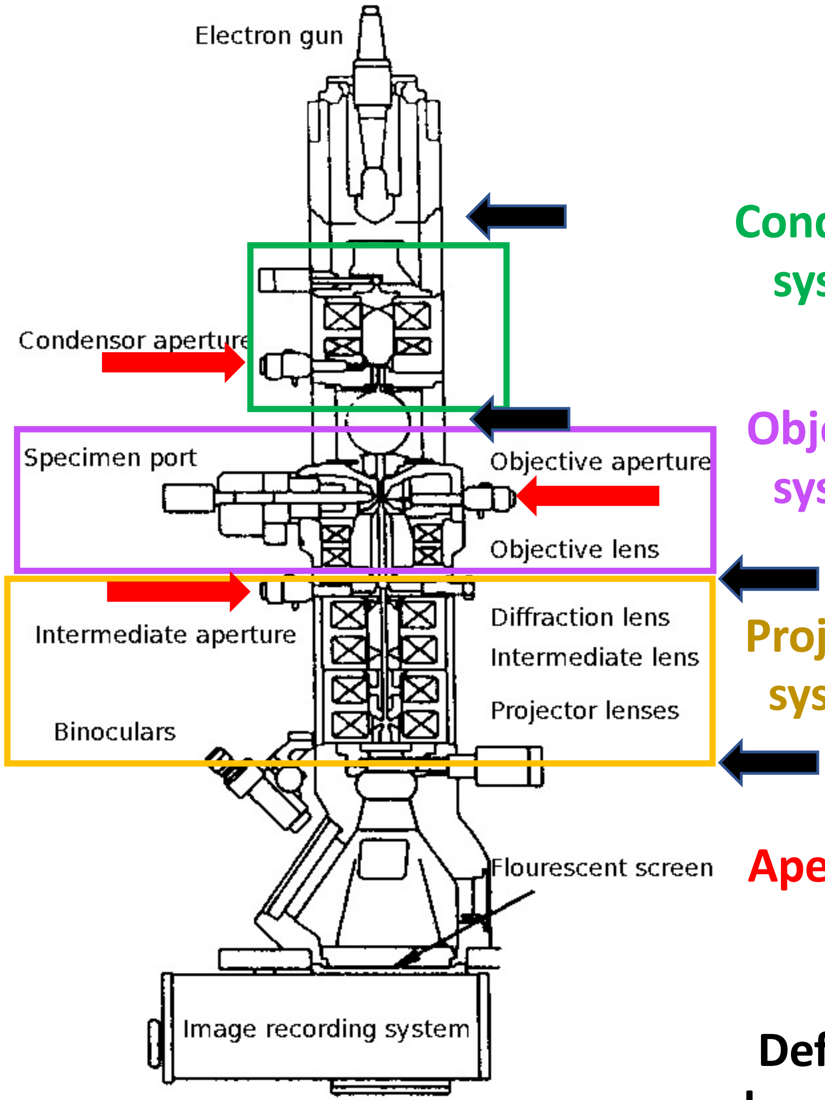


FEG (W)



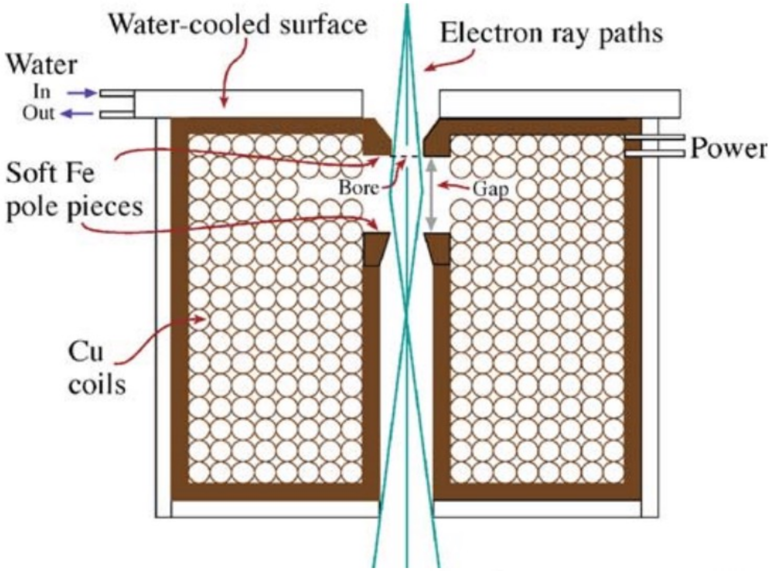


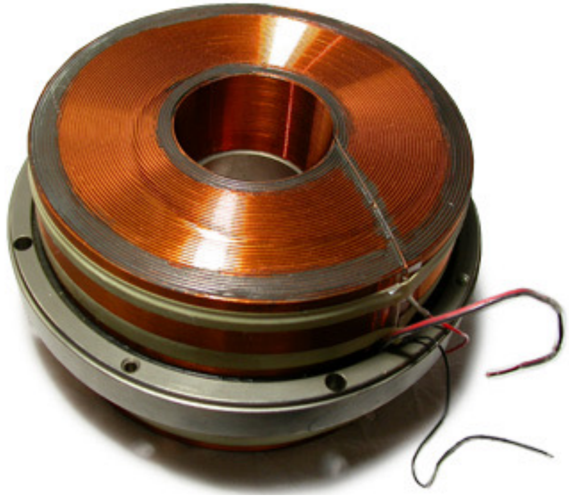
The Lens System

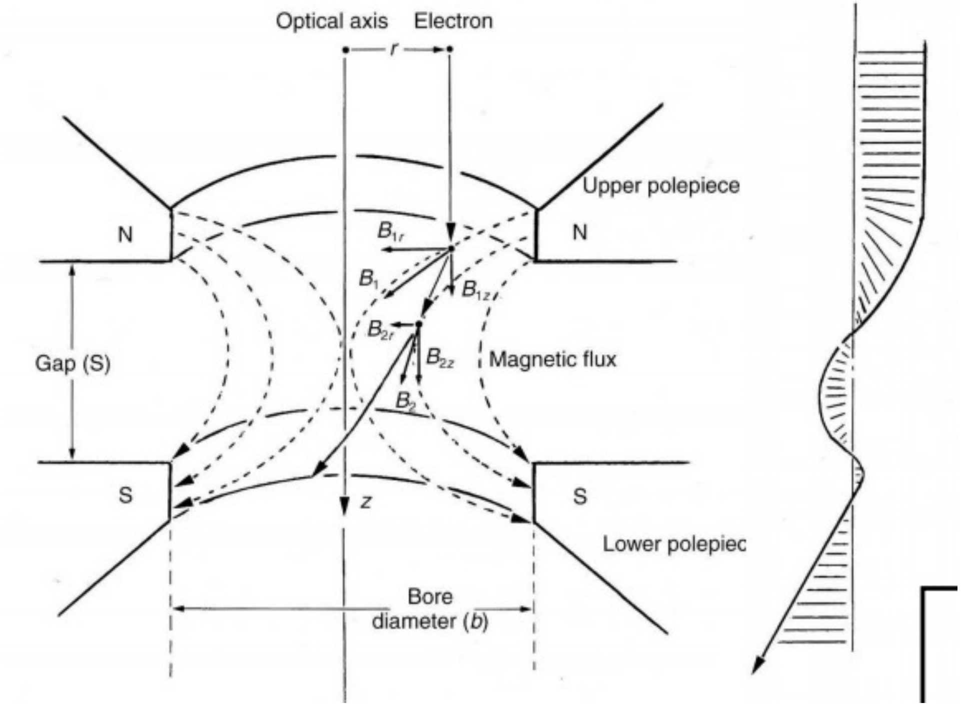


Condenser system	Focuses electrons on specimen (controls illumination cone shape/intensity)
Objective system	Principle Magnification Lens (magnifies sample, 80/100x)
Projector system	Weaker magnification lenses (magnifies sample, 2-10x)
Apertures	Reduces beam intensity Parallelizes beam
Deflector beam coils	Corrects beam shape (stigmator) Corrects beam location/angle (tilt/shift)

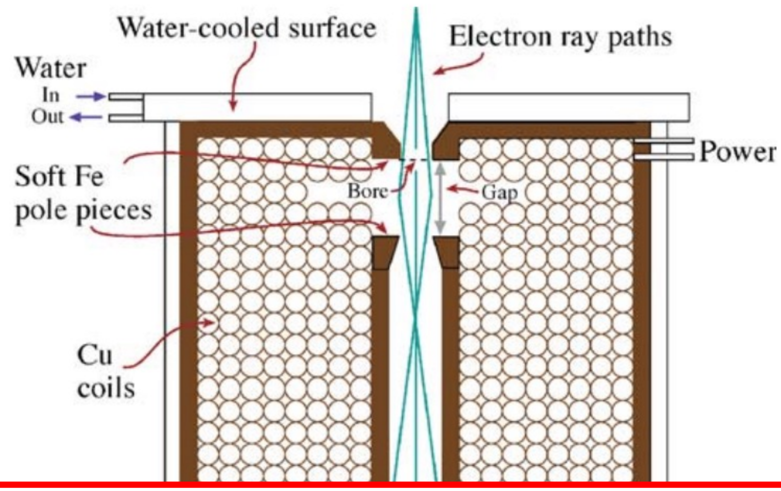
The Condensing Lens

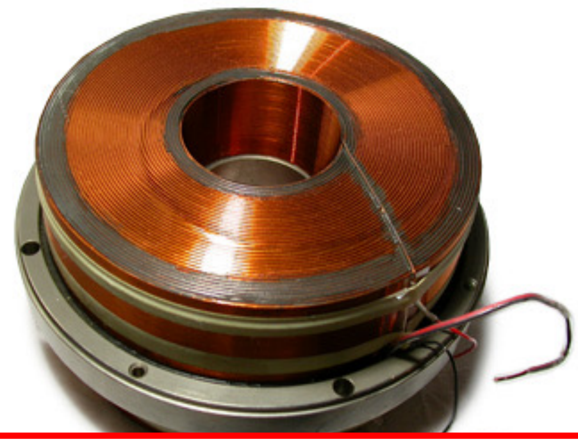






The Condensing Lens



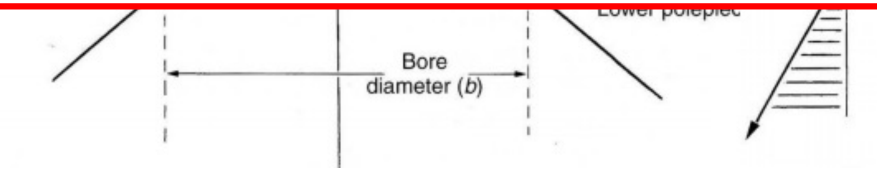


Magnetic lenses are poor quality and have severe aberrations
(aberrations increase with distance from centre)

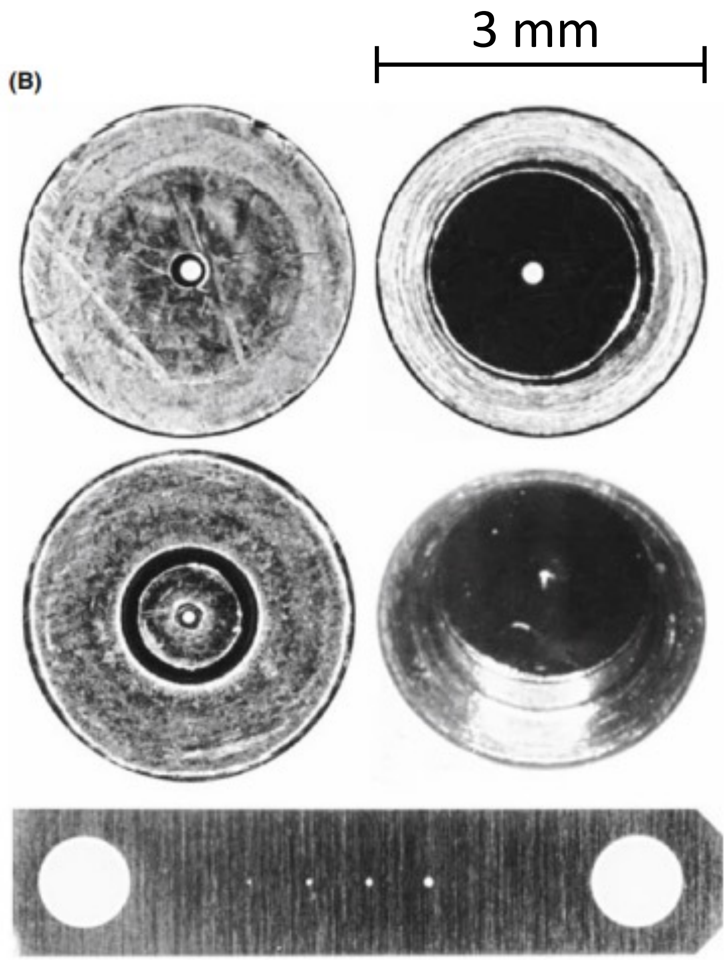
“a magnetic lens is like using the bottom of a soda bottle as a magnifying glass”

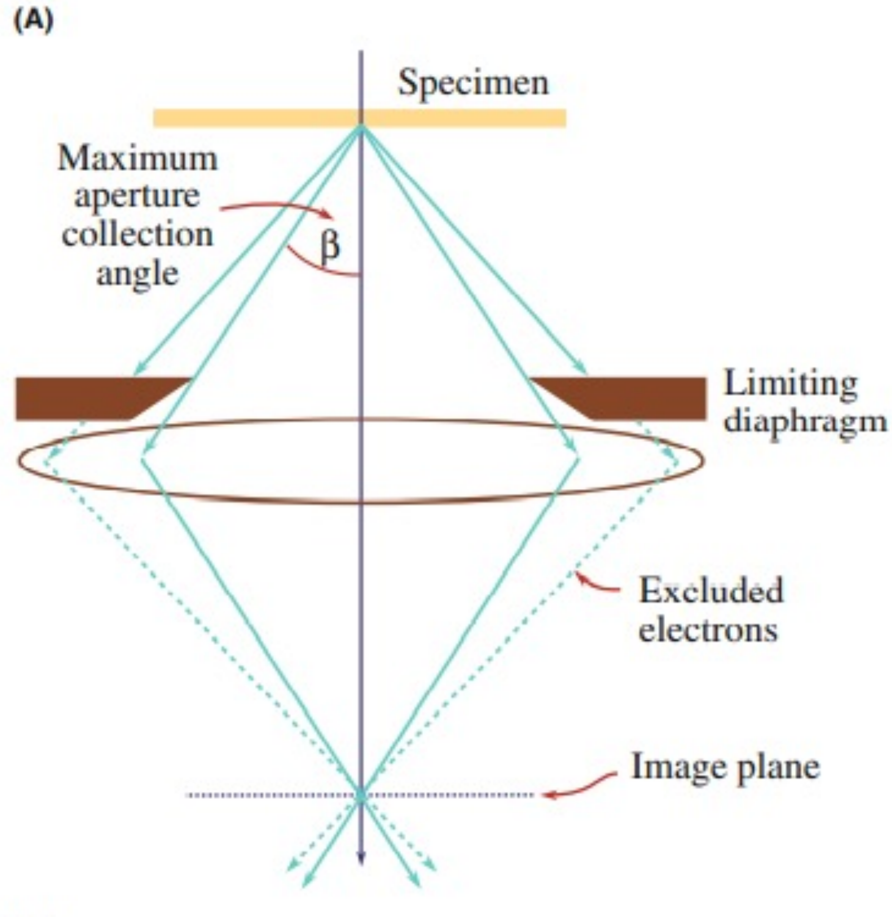
“if our eye lens worked as well as a magnetic lens we would be ***legally blind***”

(quotes in William and Carter)



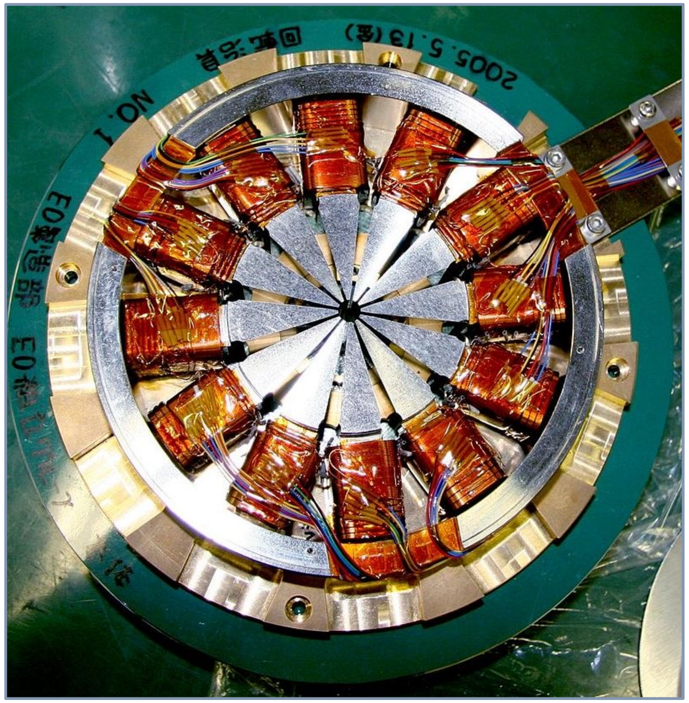
Apertures

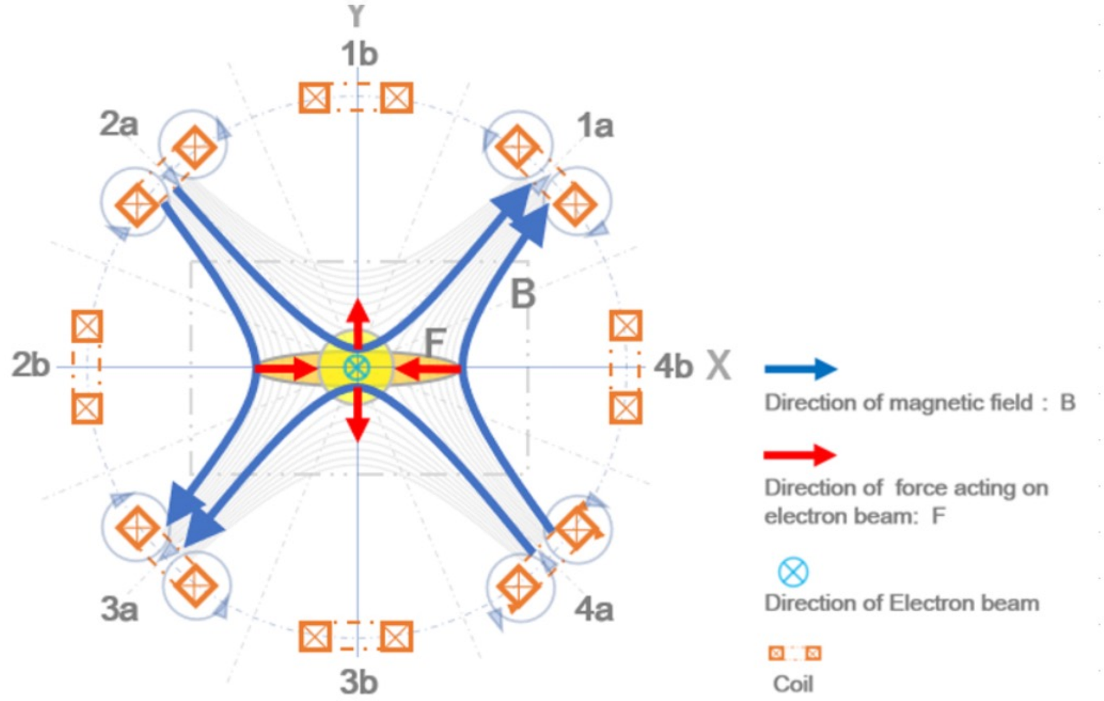




Condenser aperture	Controls beam intensity, parallelity
Objective aperture	Amplitude contrast
Selective area aperture	Diffraction imaging/dark field

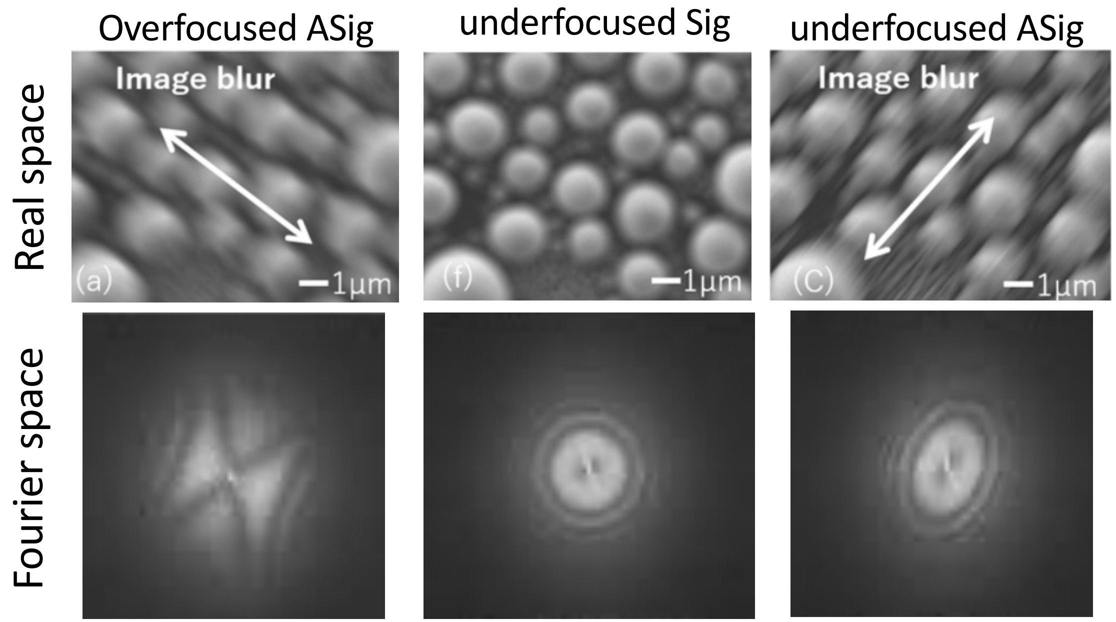
Deflector Coils



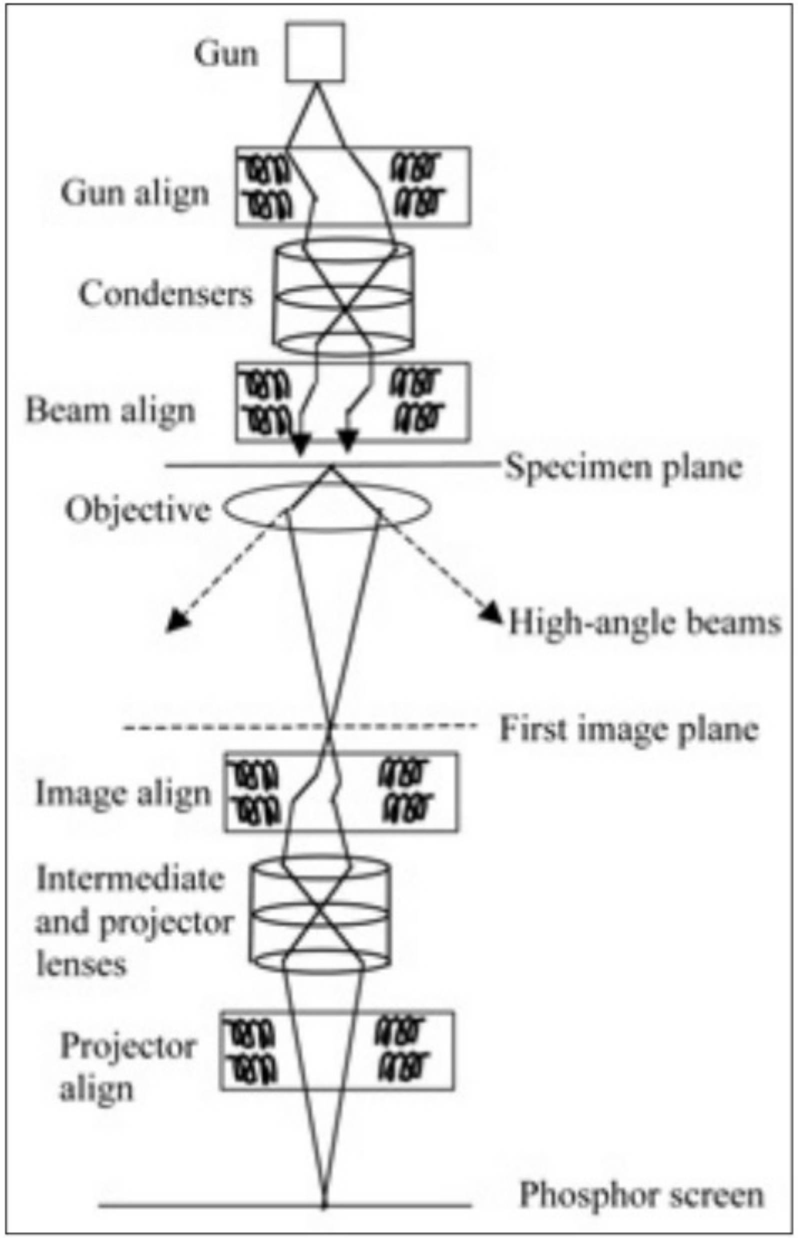


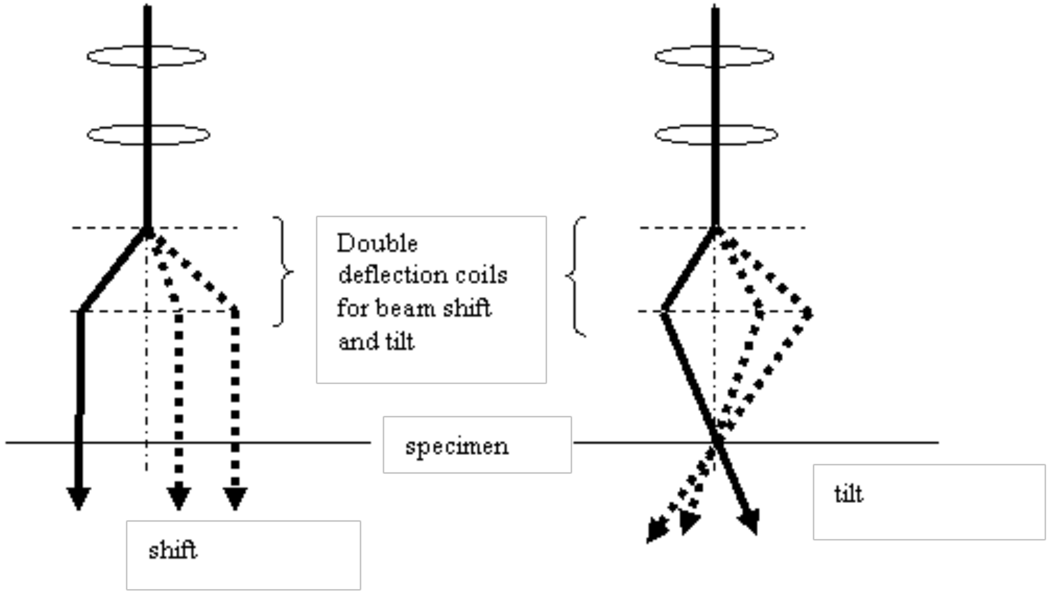
Condenser stigmators
correct beam shape
(circle vs. oval)

Objective stigmators
correct image



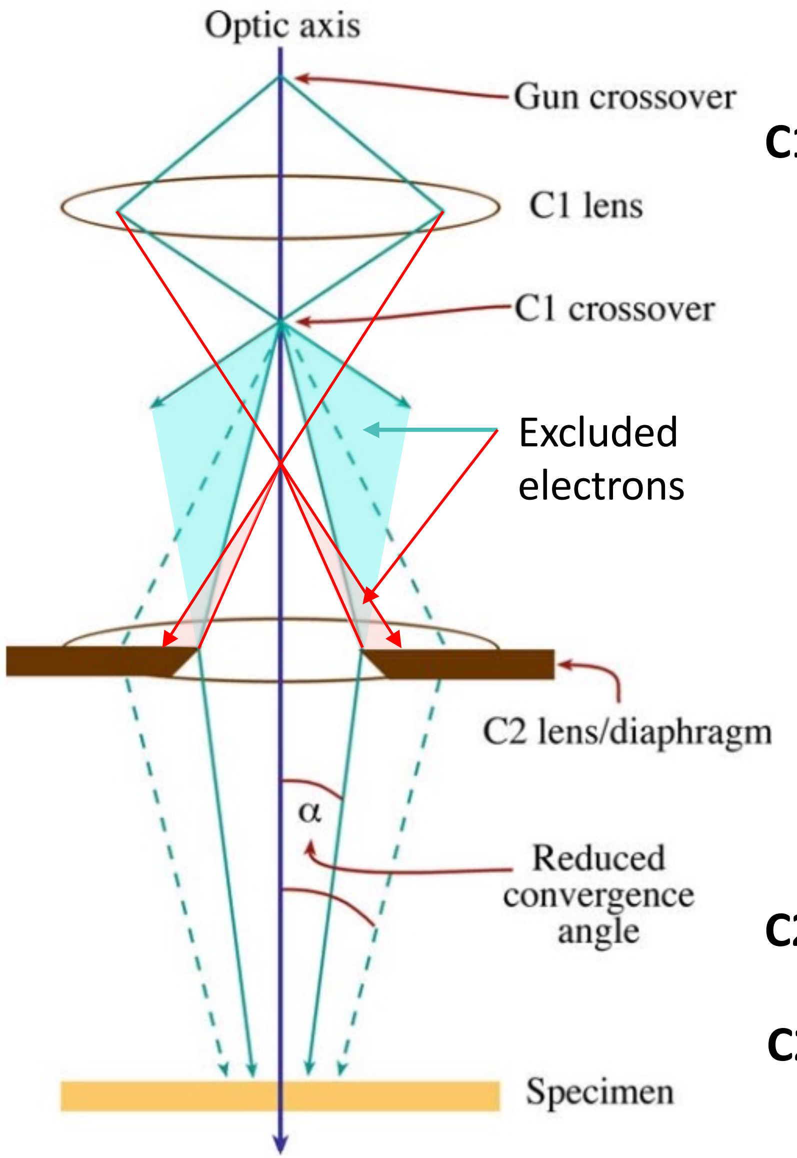
Shift/Tilt Coils





1. Get the beam down the scope
2. Make sure beam is parallel/aligned to optical axis (bright field imaging)
3. Low dose imaging
4. Automatic procedures (e.g. eucentric height/focus)

Condensor System



Gun tilt/shift: sets up beam to enter condenser system on optical axis

C1 = Spot size, controls beam size and quality

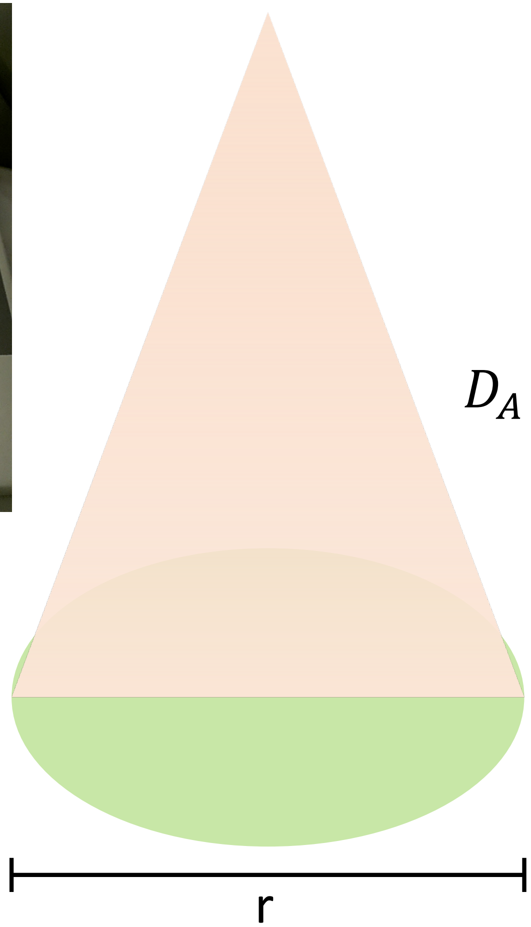
Spot 9: Strongest lens setting
Highest crossover
Dimmest beam and smallest focused beam
Most coherent little spatial divergence
Only most parallel electrons reach specimen

Spot 1: Weakest lens setting
Lowest crossover
Brightest & largest focus beam diameter
Least coherent
Greatest spatial divergence
Majority of electrons reach specimen

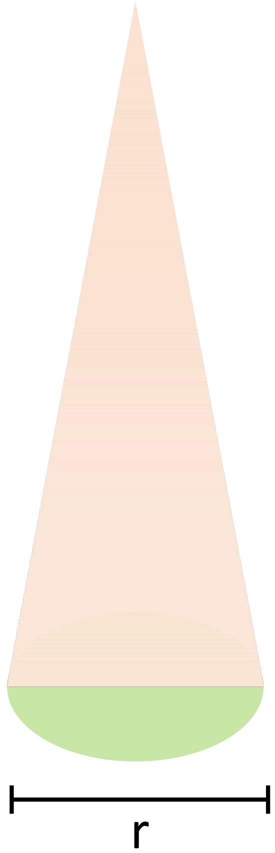
C2 aperture = limits amount of electrons reaching sample

C2 lens = Intensity knob. Controls diameter of beam reaching sample ➔ (Dose/beam intensity)

Note on C2 Lens and Dose



$$D_A = \frac{D_T}{A} = \frac{D_T}{\pi r^2}$$



Dose in an area increase 4x when you half the radius.

USE THIS INTENSITY CONTROL KNOB WISELY!

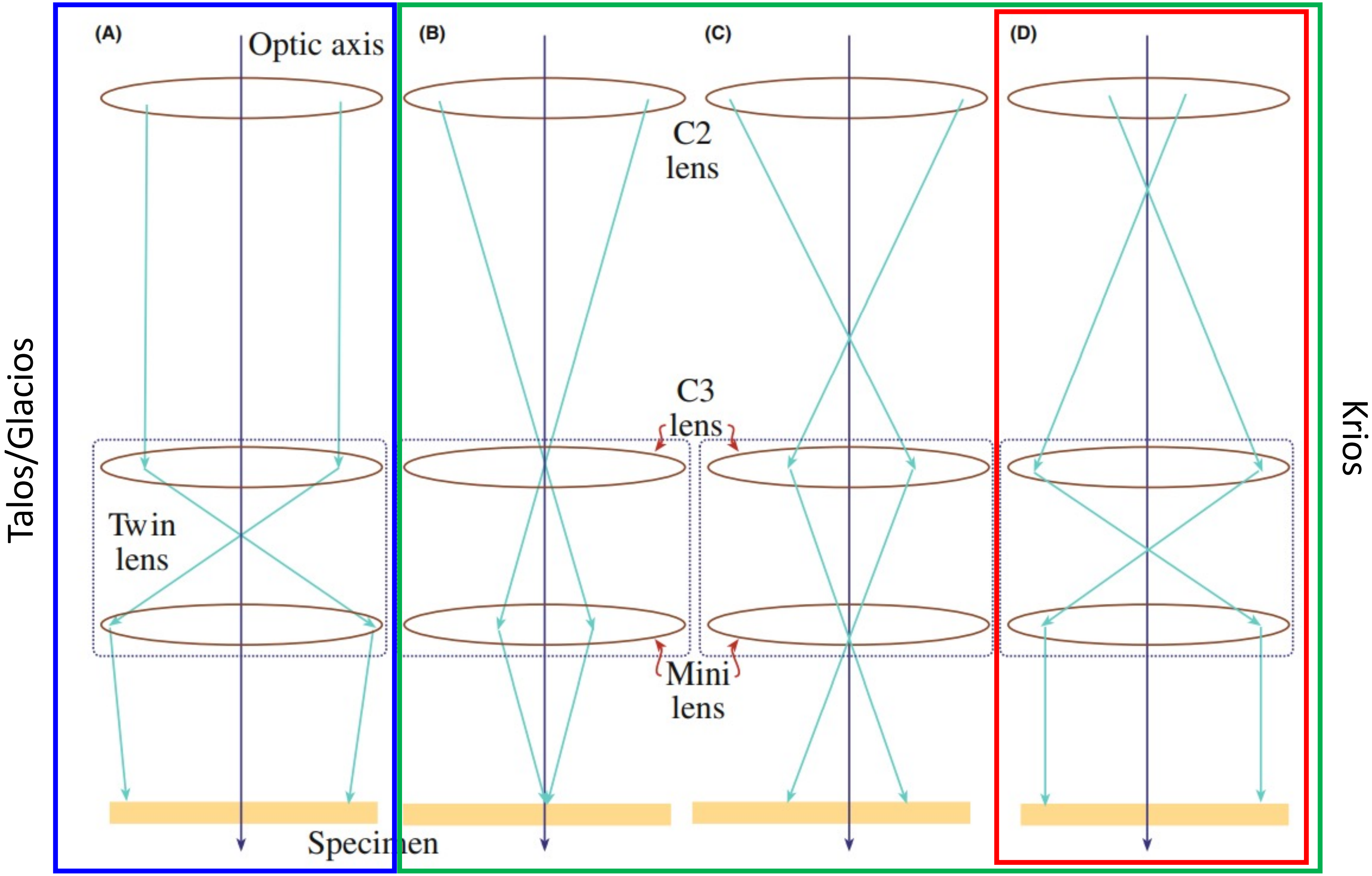
DA = Dose per unit area

DT = Total dose In beam

A = Area

r = Beam Radius

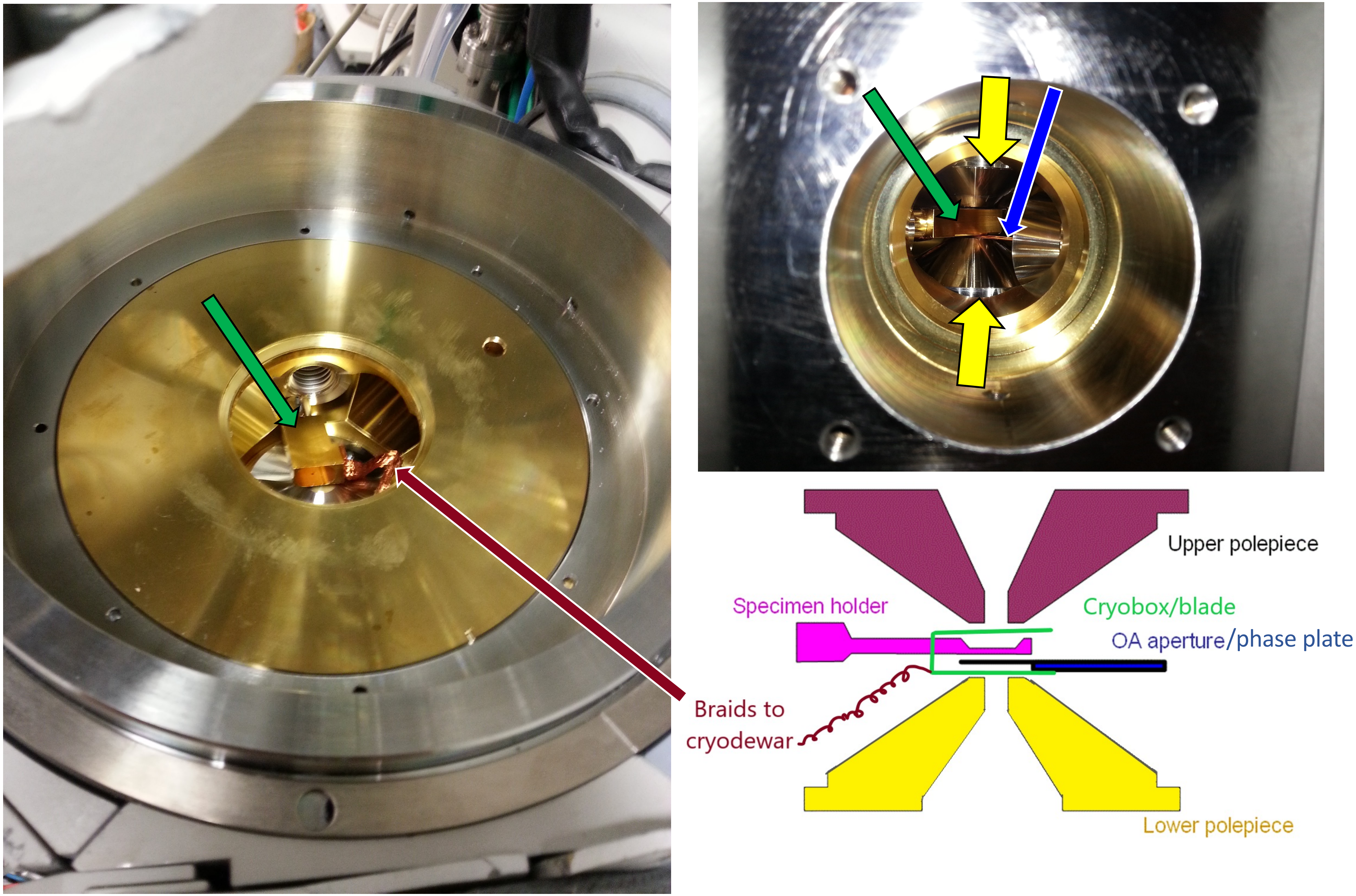
C3 Lens (Krios)



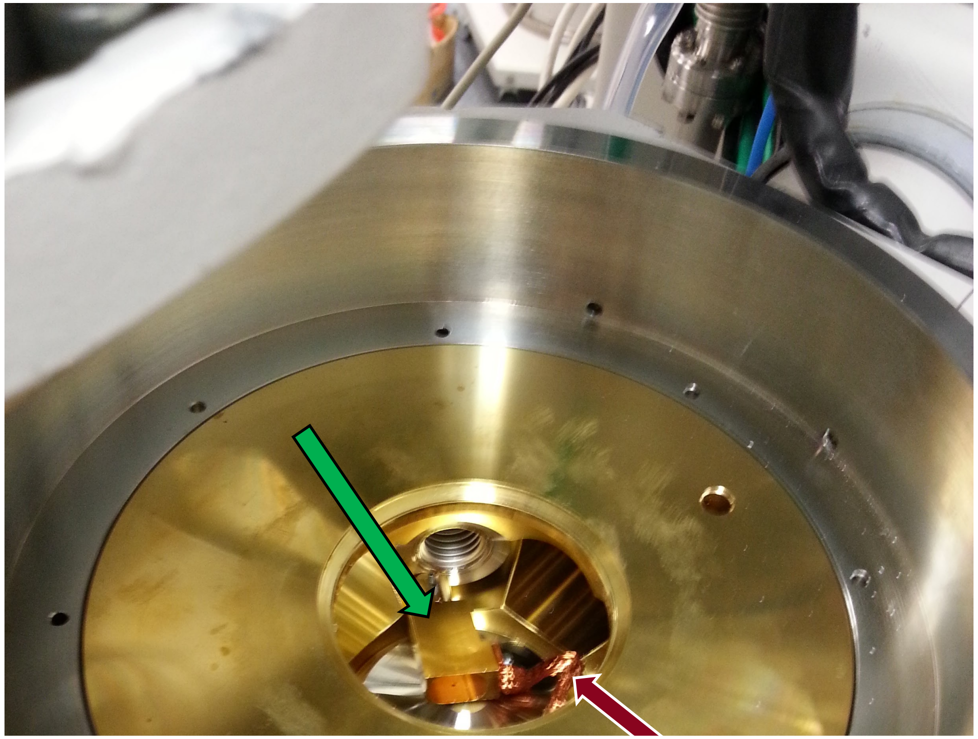
Parallel illumination of specimen reduces aberrations

C3 lens provides parallel illumination but only at certain C2 values

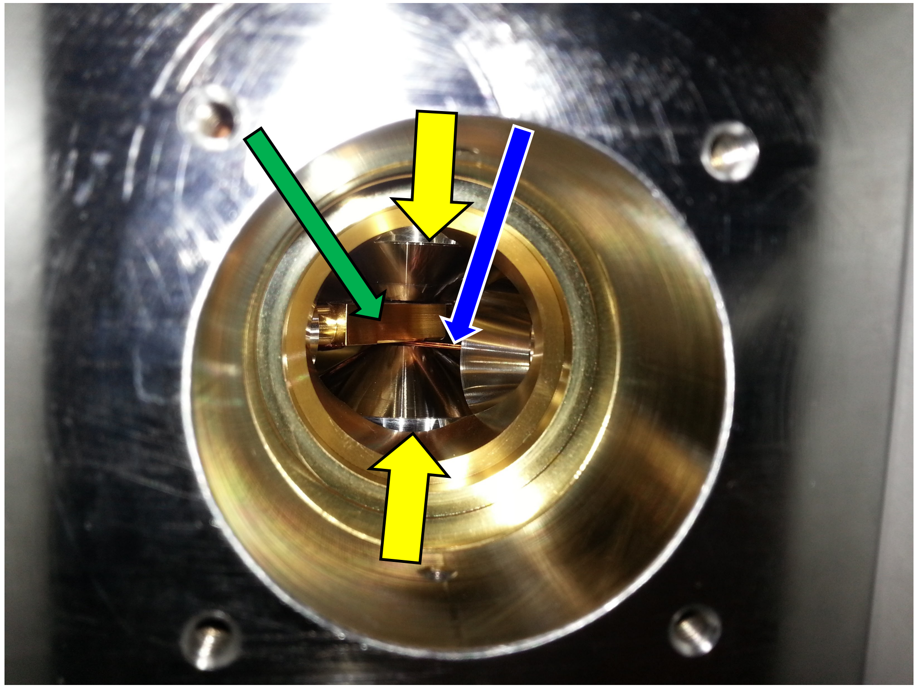
Objective System

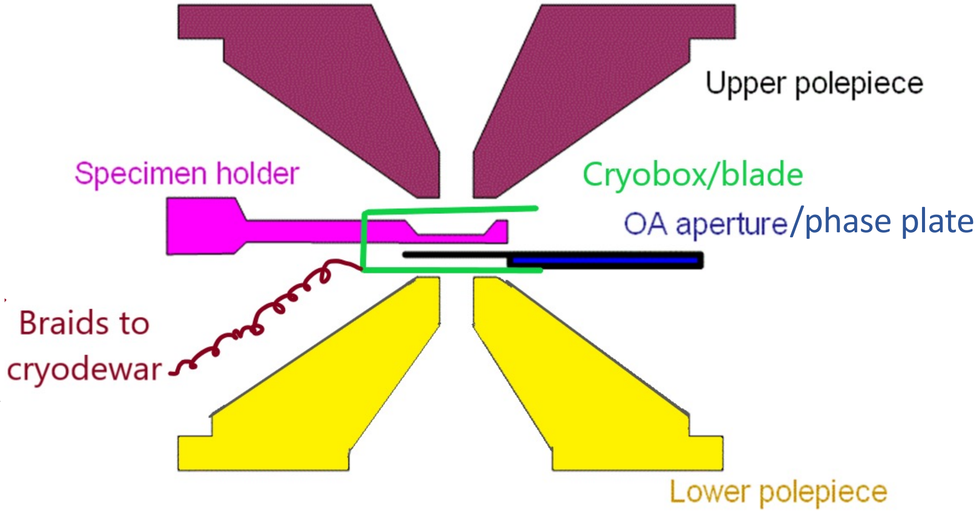


Objective System



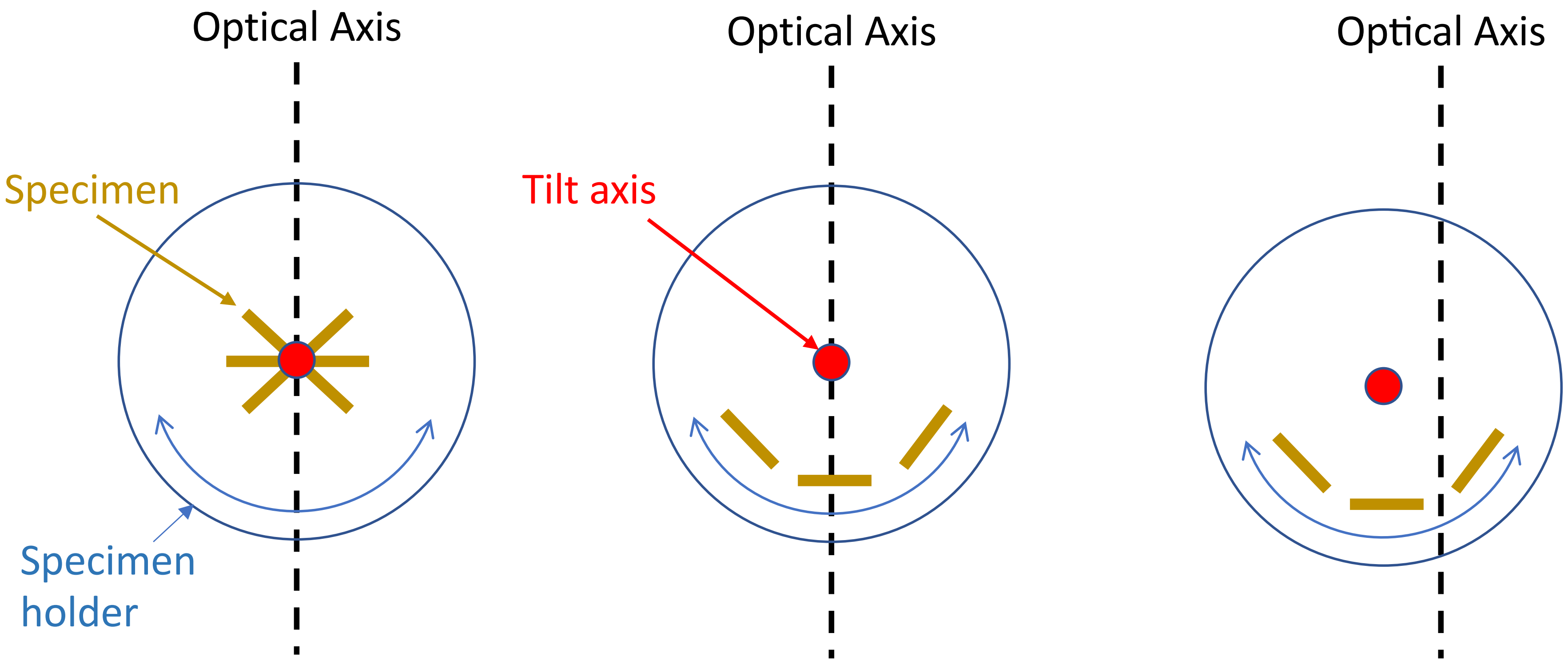
- Cryoblades cooled to LN_2 temp by braids
- Cryoblades colder than sample → cryotrap
- Keeps sample clean and cold by absorbing contaminant from sample/column
- Cryoblades needs to be warmed up regularly to remove contaminants → cryocycle
- Cryocycle: turns off IGP, pumps specimen chamber with Turbo





Gap between pole pieces is spherical aberration (Cs). Smaller gap = better.

Eucentric Height



Excellent!

Tilt axis aligns on optical axis and eucentric focus

Specimen does not move when tilted

Bad

Specimen moves when tilted

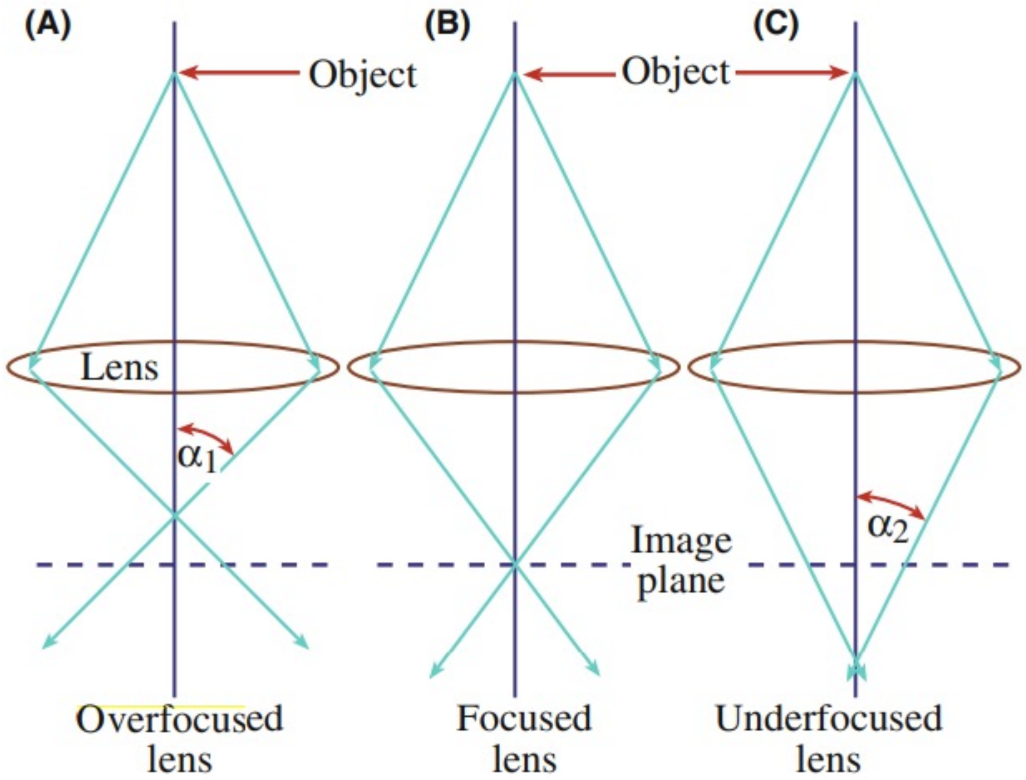
Change specimen Z-height

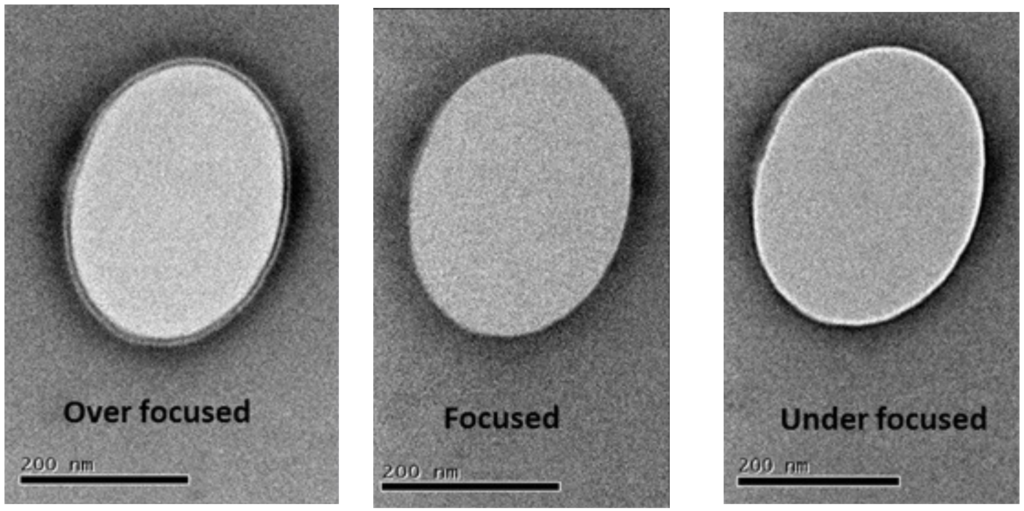
Very Bad

Specimen moves when tilted

Change specimen Z-height
Software or engineer required to align tilt axis with optical axis

Focus





Focus knob modulates objective lens current changes clarity of image (focus)

If crossover above image plane - overfocused

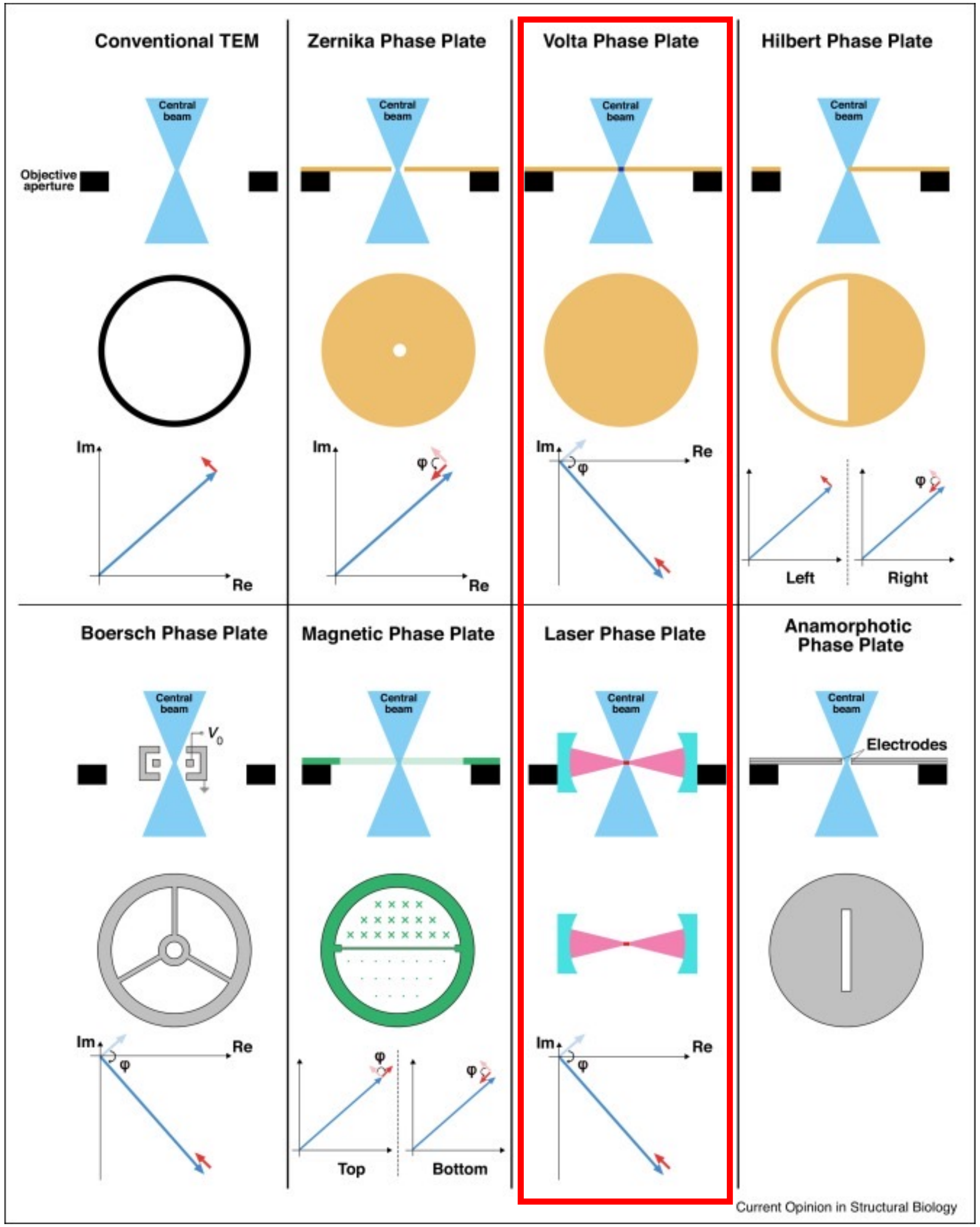
If crossover below image plane - underfocused

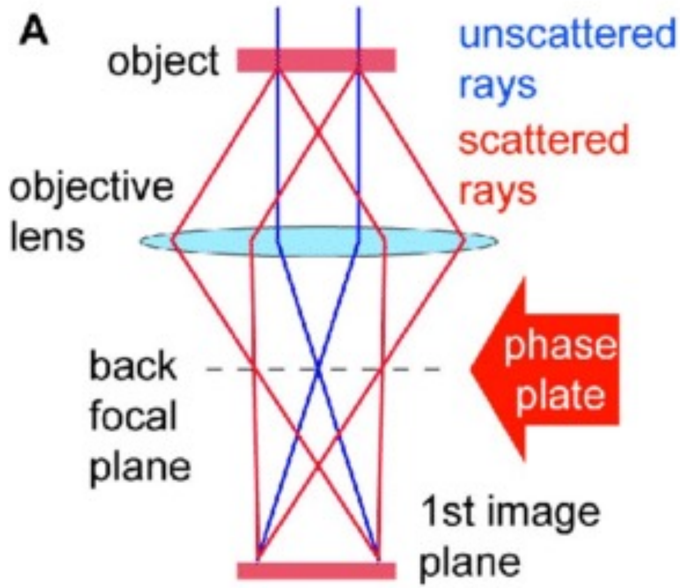
Biological samples contain light atoms so minimal phase shifts occurs between scattered and unscattered rays

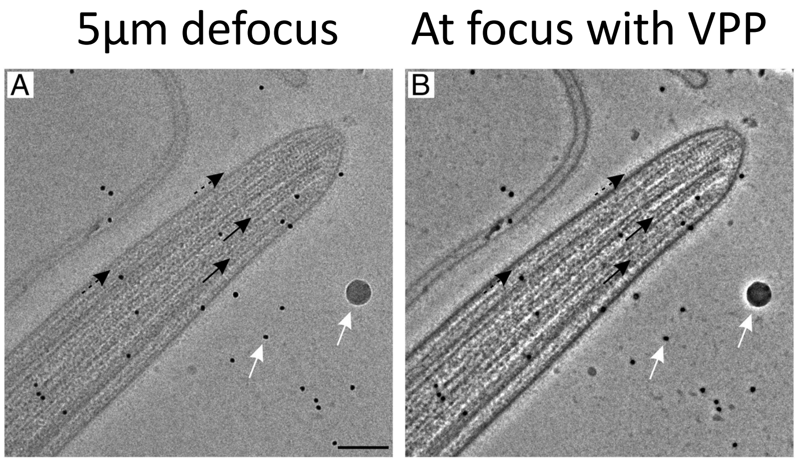
→ At focus, very little contrast

To see sample, either image underfocus or use phase plate

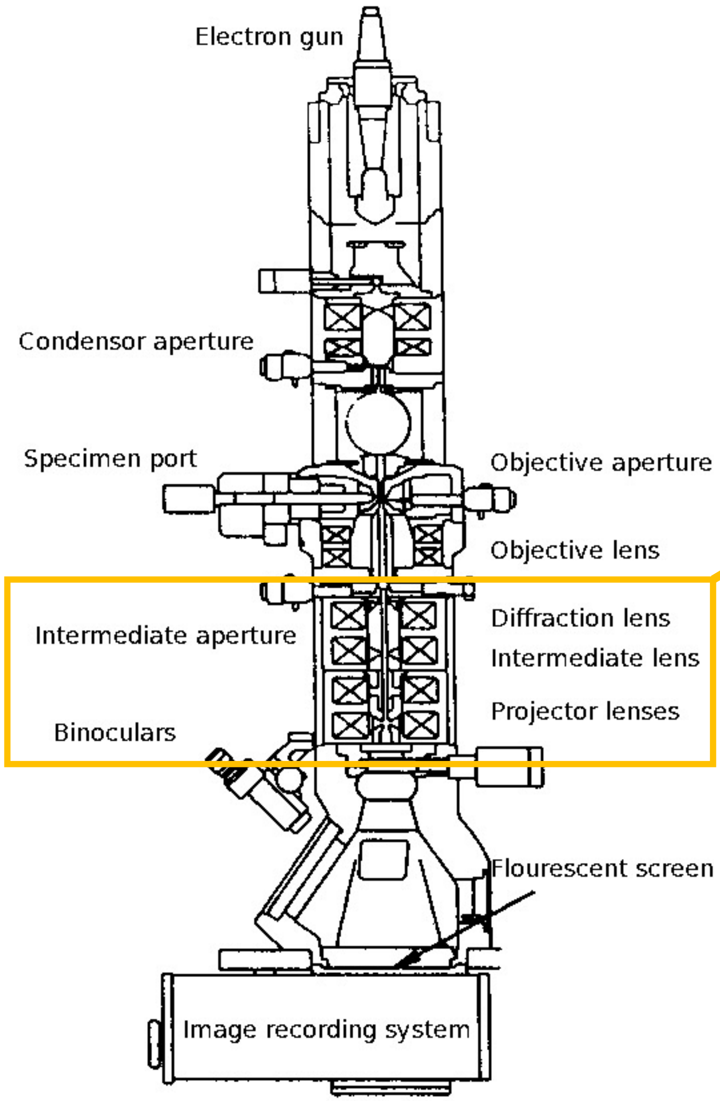
Phase Plates







Projector System



Projector system

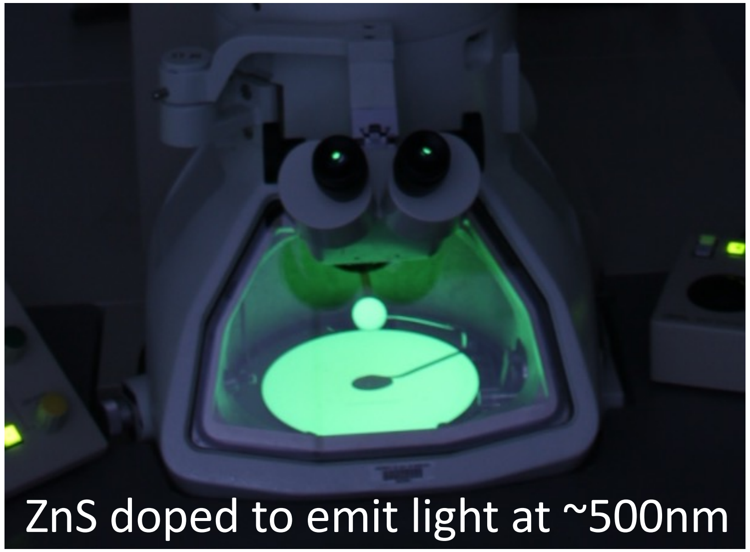
Responsible for magnification in SA mode (record mode)

Magnification knob changes strength of projector lens

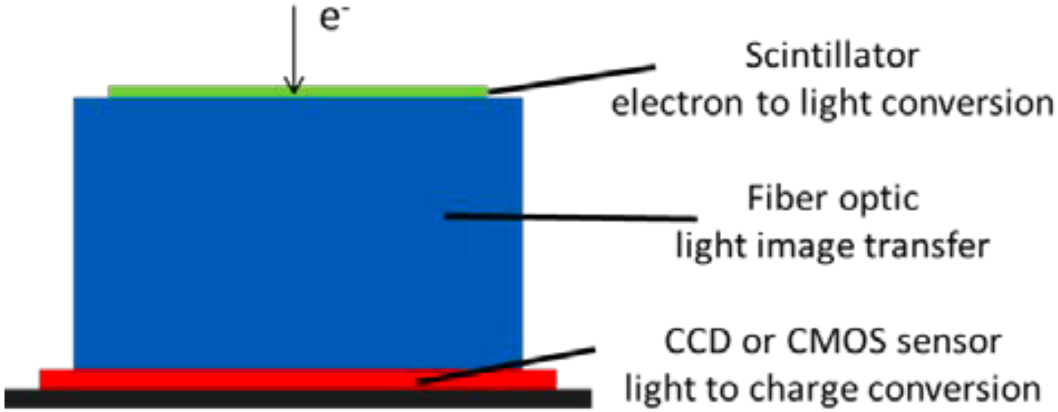
Objective stays constant (prevents hysteresis)

Imaging System

Fluorescent Screen



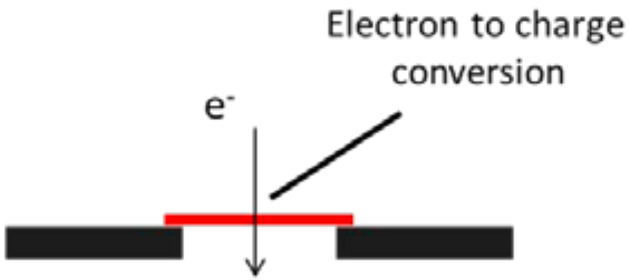
Charge coupled device (CCD)



Film + Scanner

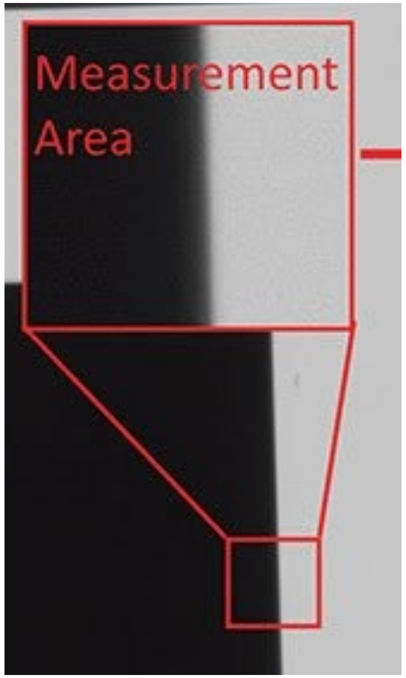
Direct Electron Detectors (DEDs)

(e.g. K3/Falcon)



Camera Quality Measurement

Modular transfer function (MTF)



How fast does intensity change at sharp edge?

Fast change, great camera, able to capture high resolution data

Slow change, poor camera, resolution limiting

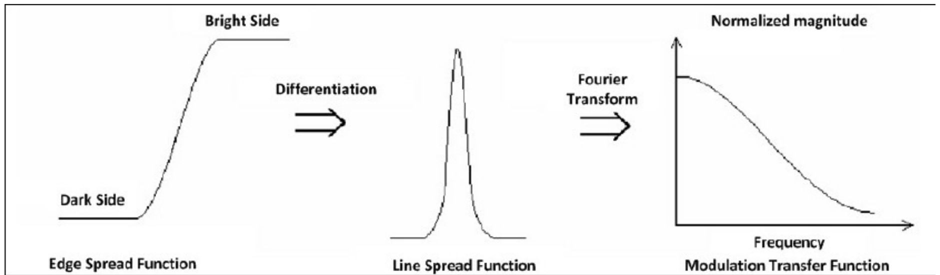
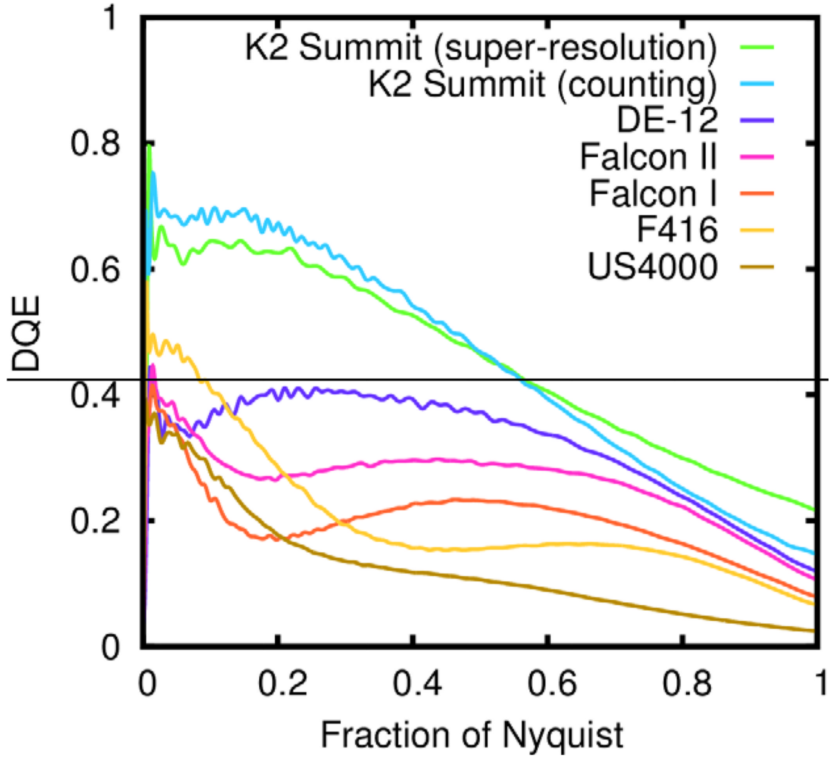


Figure 3. Computation of the Modulation Transfer Function using the knife-edge target.

Detection Quantum Efficiency (DQE)

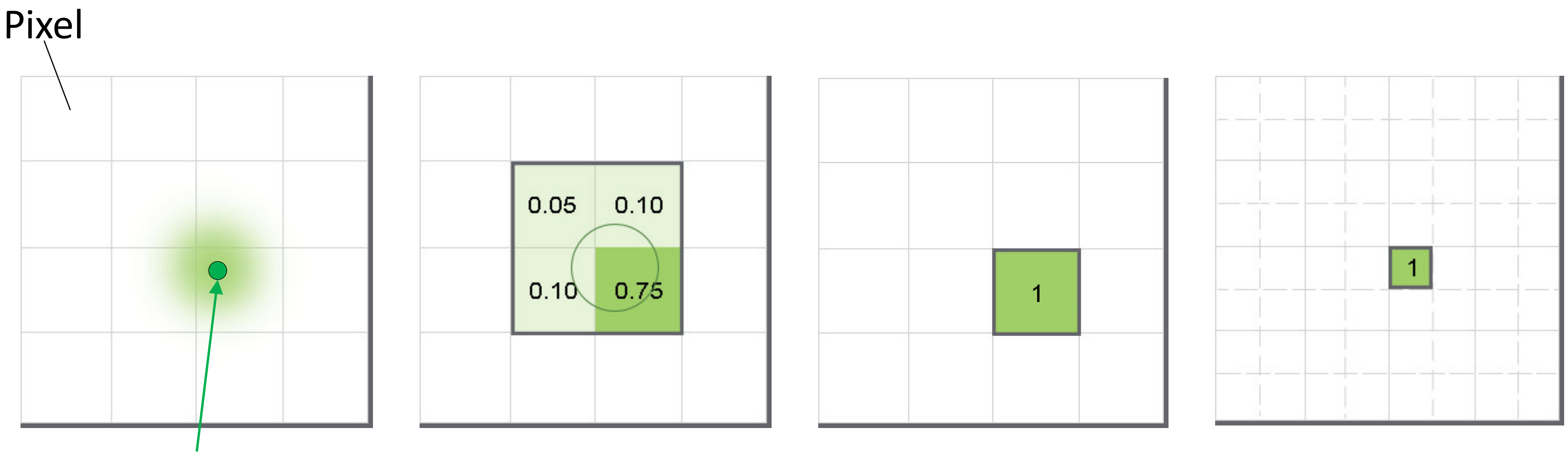


$$DQE = \frac{SN_{out}^2}{SN_{in}^2}$$

DQE = 1, excellent camera, no loss of signal

Greater the Fraction of Nyquist, > resolution

Integrated vs Counted (super-resolution) Mode



Incoming Electron | Integration mode | Counting mode | Super-resolution

Electron hits pixel array and causes charge spread

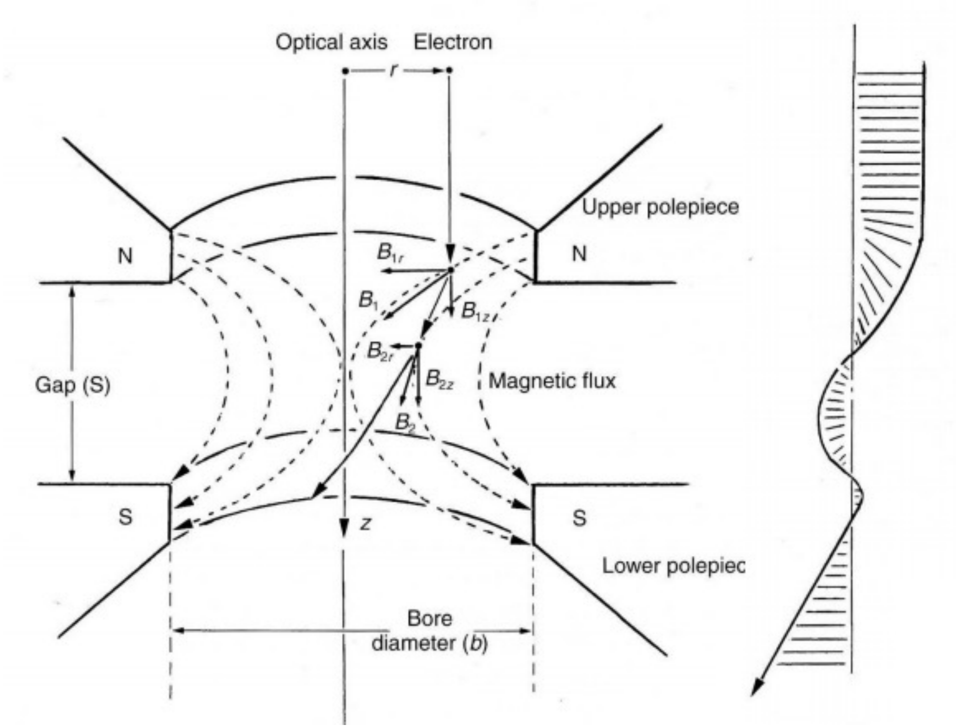
Integrating mode reads out accumulated charge in each pixel

Assumes $1e^-$ in, $1e^-$ out. Calculates most probably location of electron hit.

Subdivides pixel to give more accurate location of electron hit.

Nyqyist: maximum resolution of detector (2*px size)
represented by sine wave where 1px white, next black (smallest sampling frequency)

Lens Aberrations



Lenses imperfect

Deflect beam differently depending on:

- Entrance location
- Angle of entry
- Wavelength

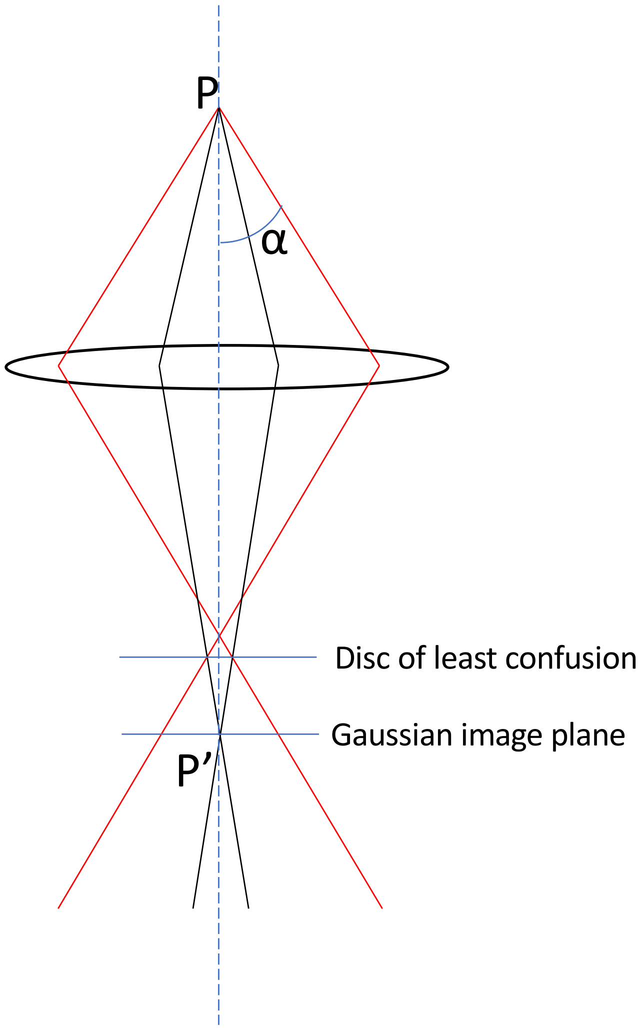
Aberration greatest furthest from optical axis.

Spherical Aberration - Entry of electron to lens

Chromatic Aberration - Wavelength of Electron

Coma - Angle of entry

Spherical Aberration (Cs)



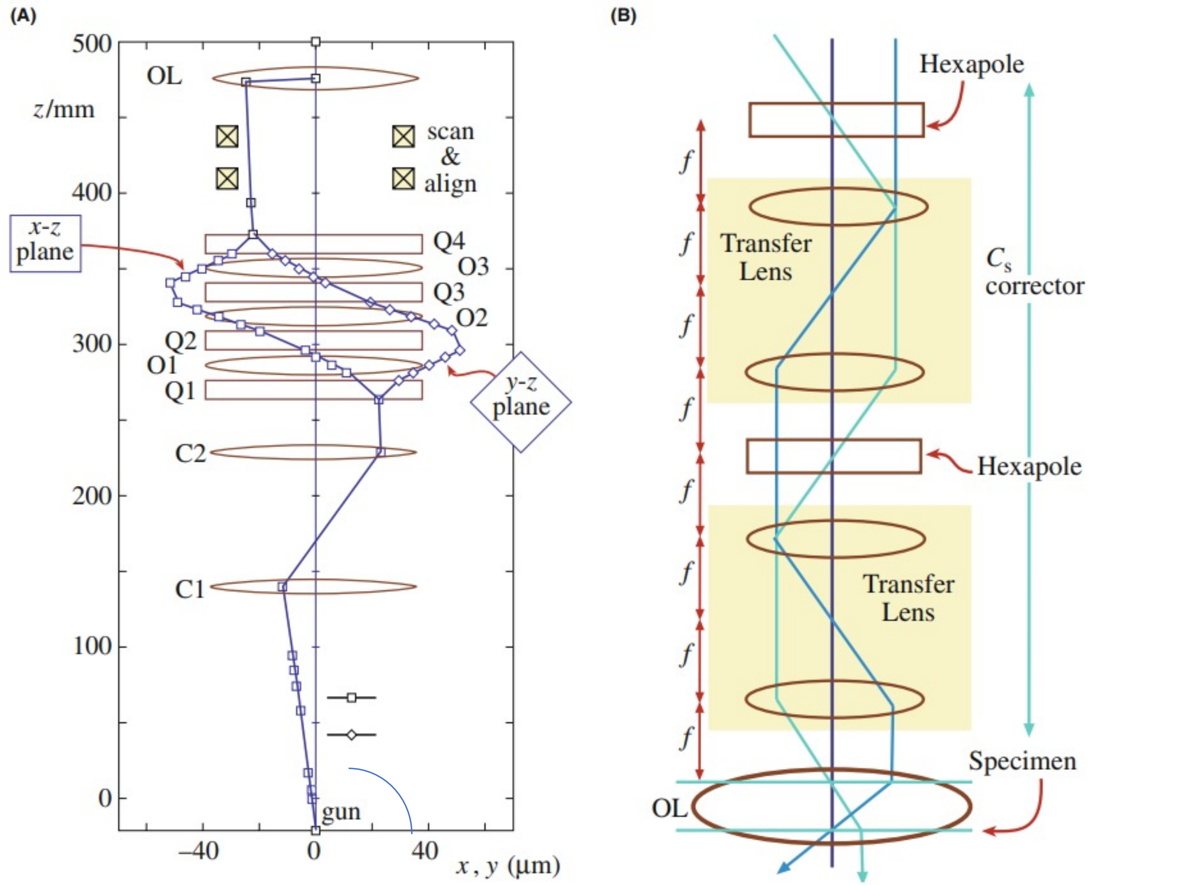
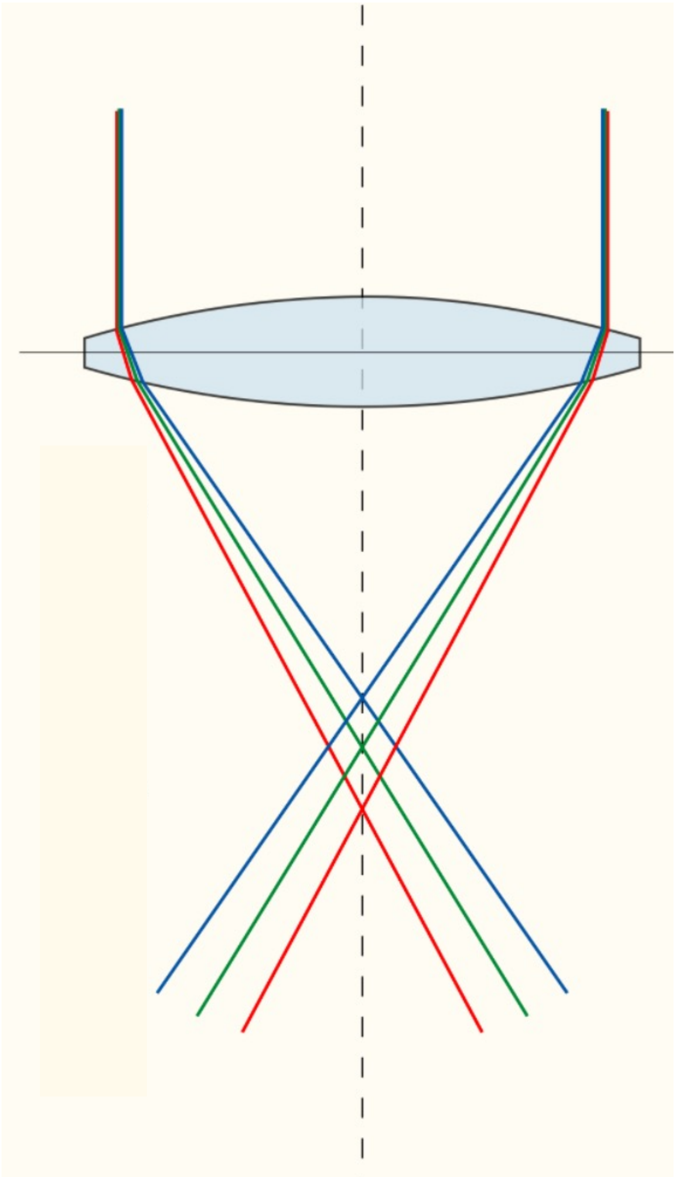


FIGURE 6.12. Ray diagrams showing how the two different commercial systems use (A) multiple quadrupole (Q) and octupole (O) lenses (Nion) or (B) hexapole and other transfer lenses (CEOS) to correct for C_s.

- Lenses are strongest at edge
- Thus off-axis electrons bent more than on-axis
- Different focus points

Cs correctors available on some scopes
Important if imaging at 0.5Å resolution

Chromatic Aberration (Cc)



Electrons of different wavelengths are focused at different point

Cause of wavelength variation:

Electron source

Interaction with sample

(especially thick samples)

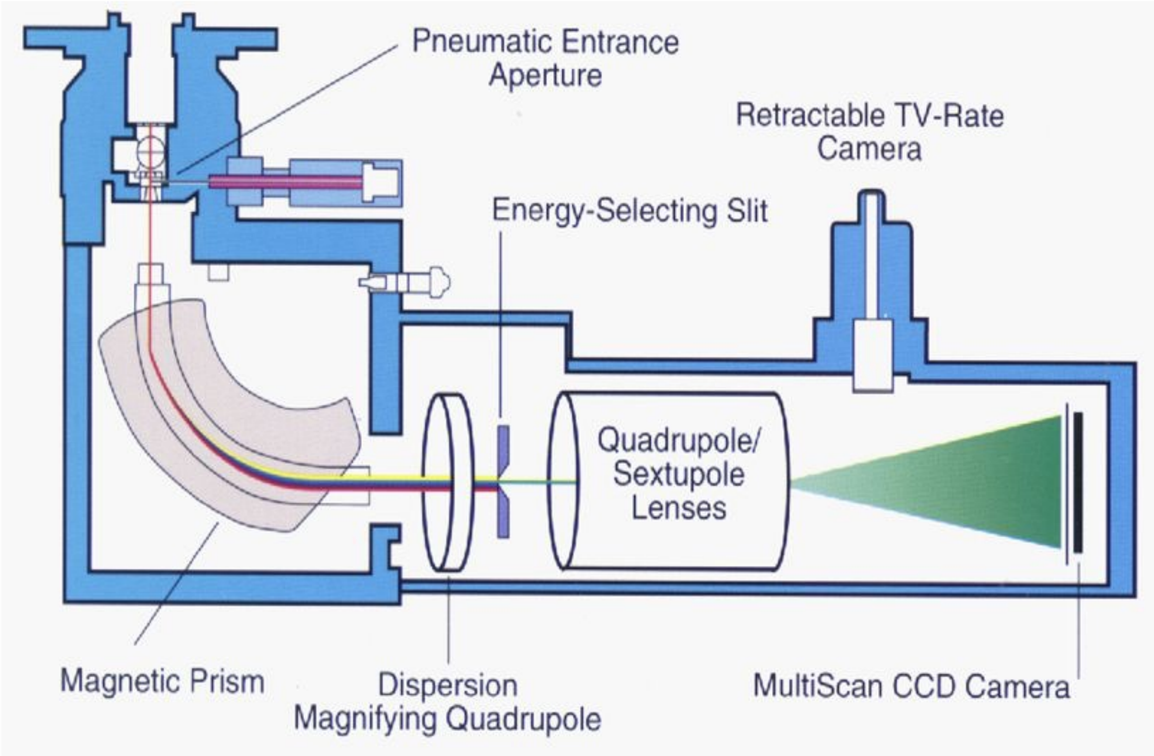
Correct using:

CFEG instead of Tungsten

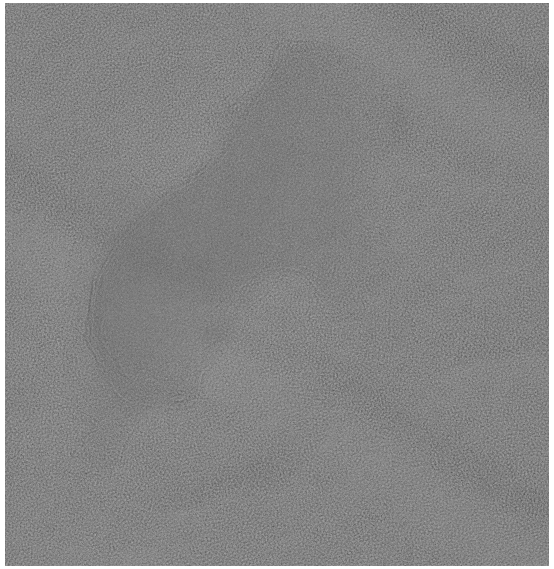
Monochromator after gun

Energy filter after sample imaging

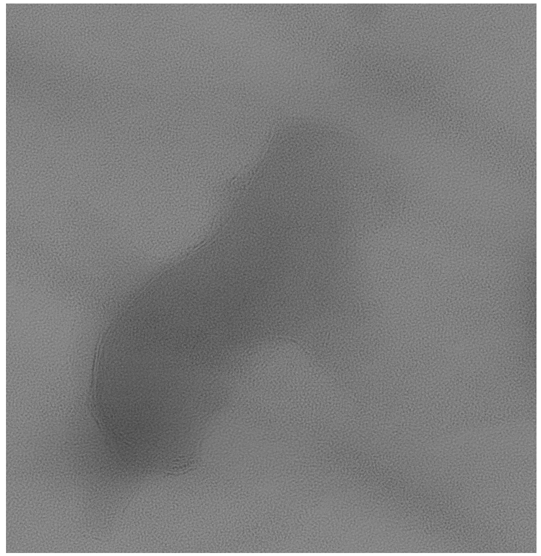
Energy Filter



Unfiltered



20eV Energy Filter



- Elaborate mass spec
- Select for specific wavelength
- Zero-loss imaging (elastically scattered waves)
- EELS – Selects wavelength of inelastically scattered rays – chemical composition

Coma

- Beam enters lens at angle
- Cs causes rays to bend depending on location
- Point source becomes comet shaped

- To correct: Apply +/- beam tilt
- FFT of opposite beam tilts should be identical

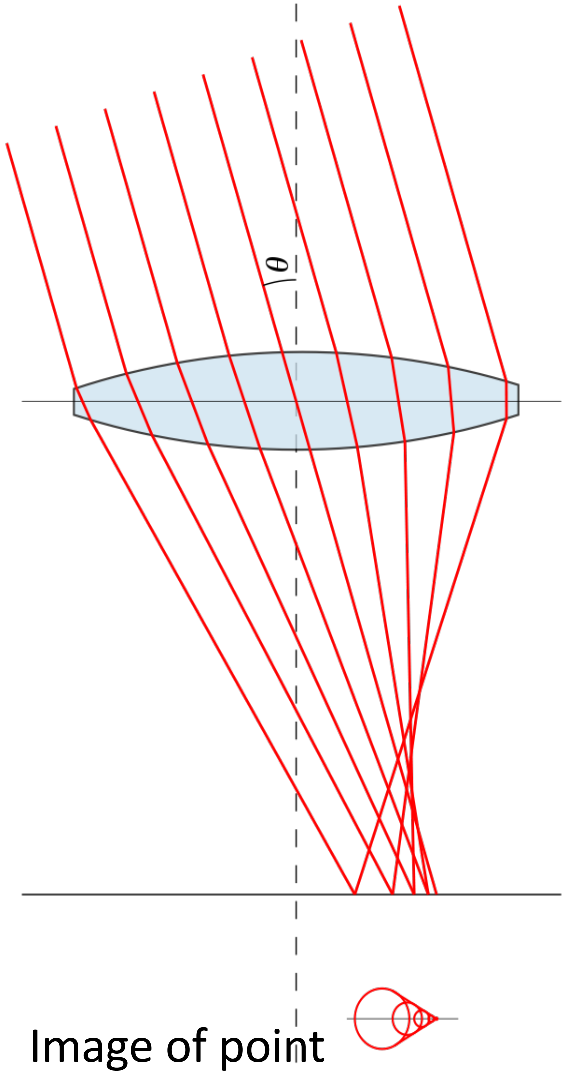
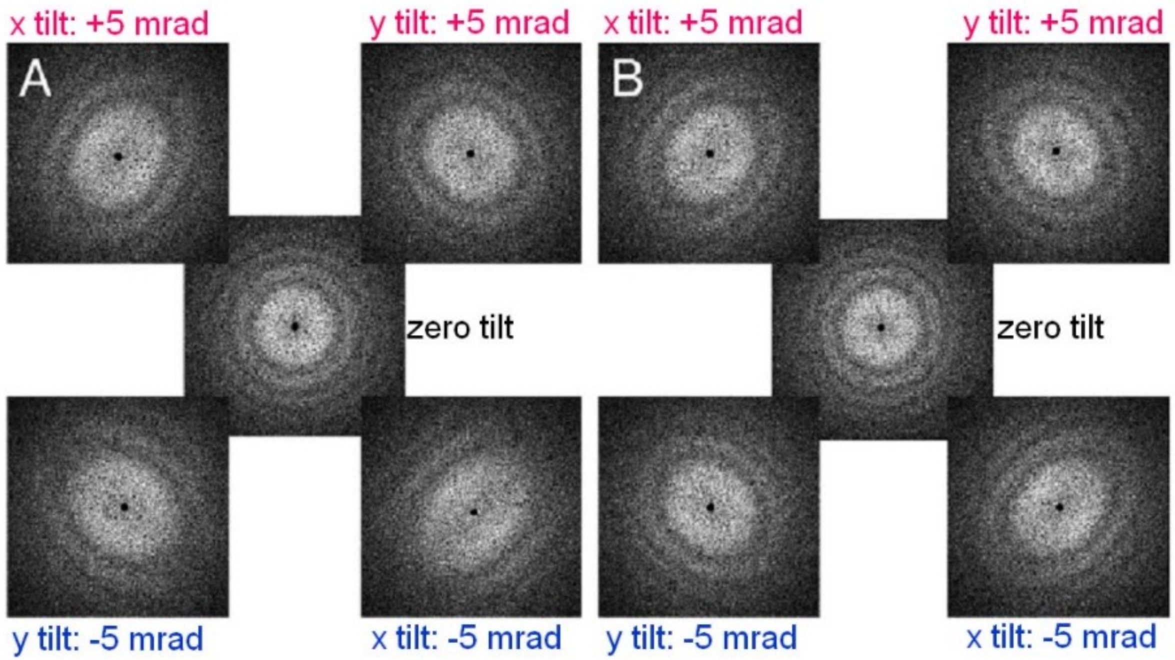
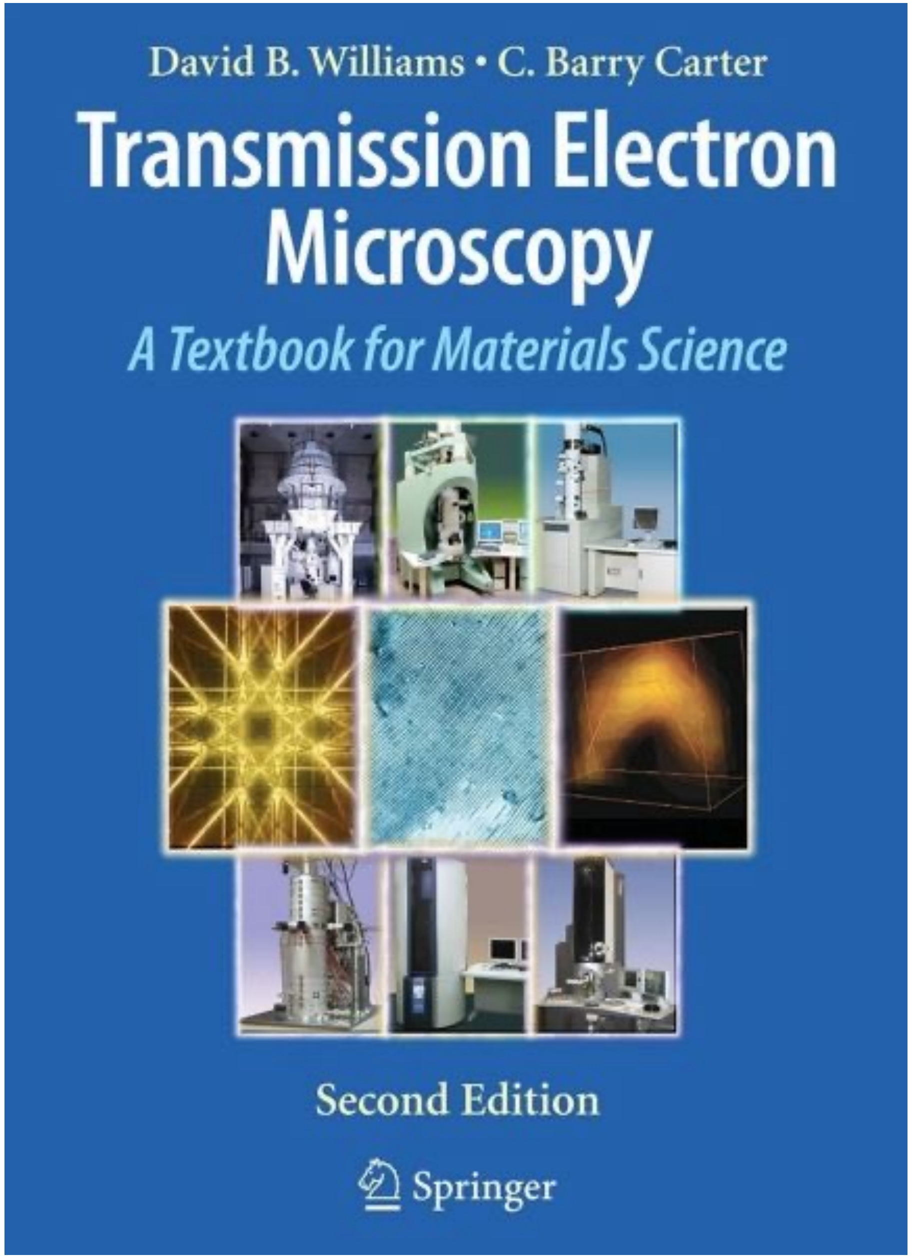


Image of point
looks like a comet



Suggested Reading



http://myscope.training/#/TEMlevel_2_4

Flash of TEM:
http://www.doitpoms.ac.uk/tlplib/tem/illumination.php

http://cryo-em-course.caltech.edu/overview

Youtube