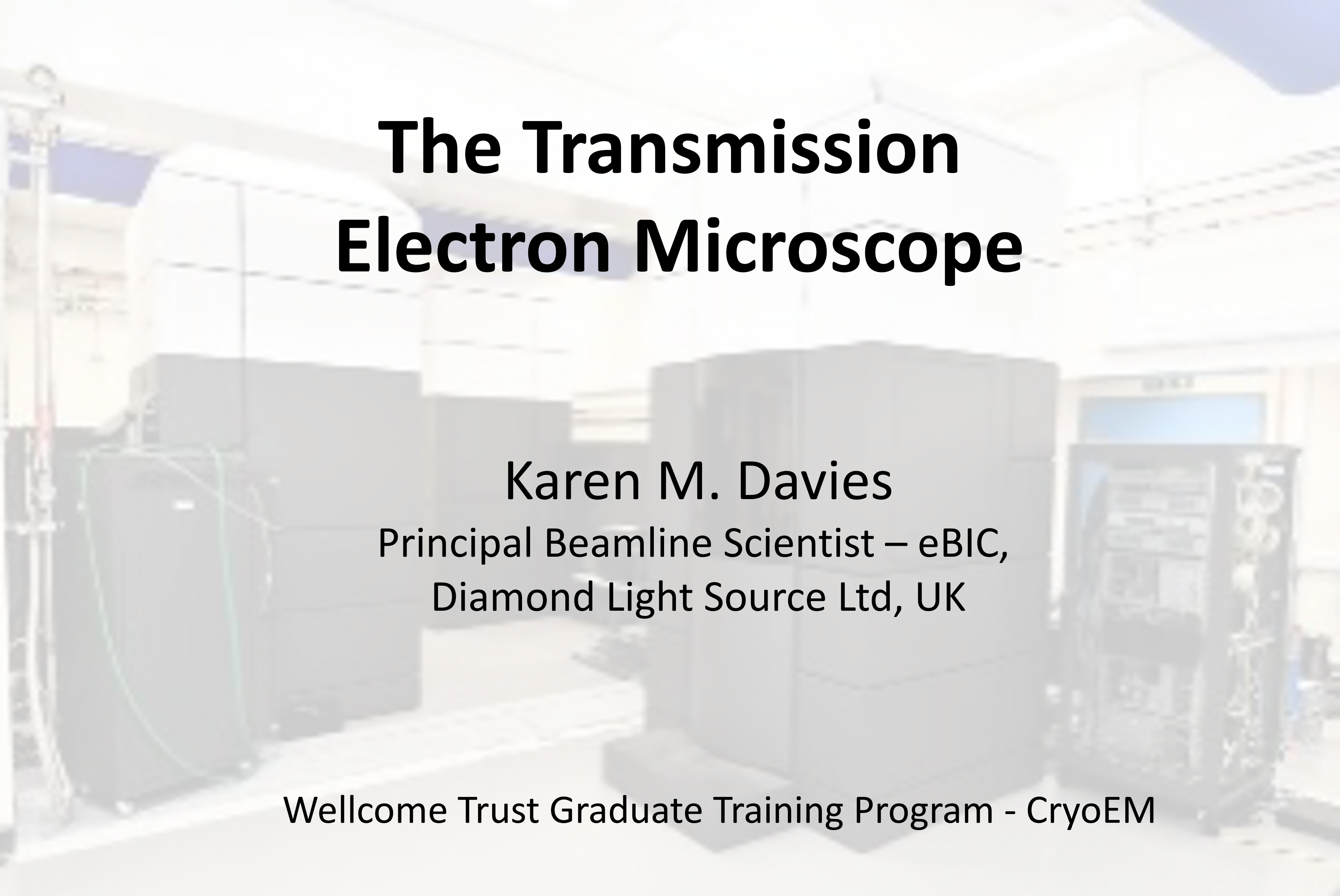


The Transmission Electron Microscope

Karen M. Davies

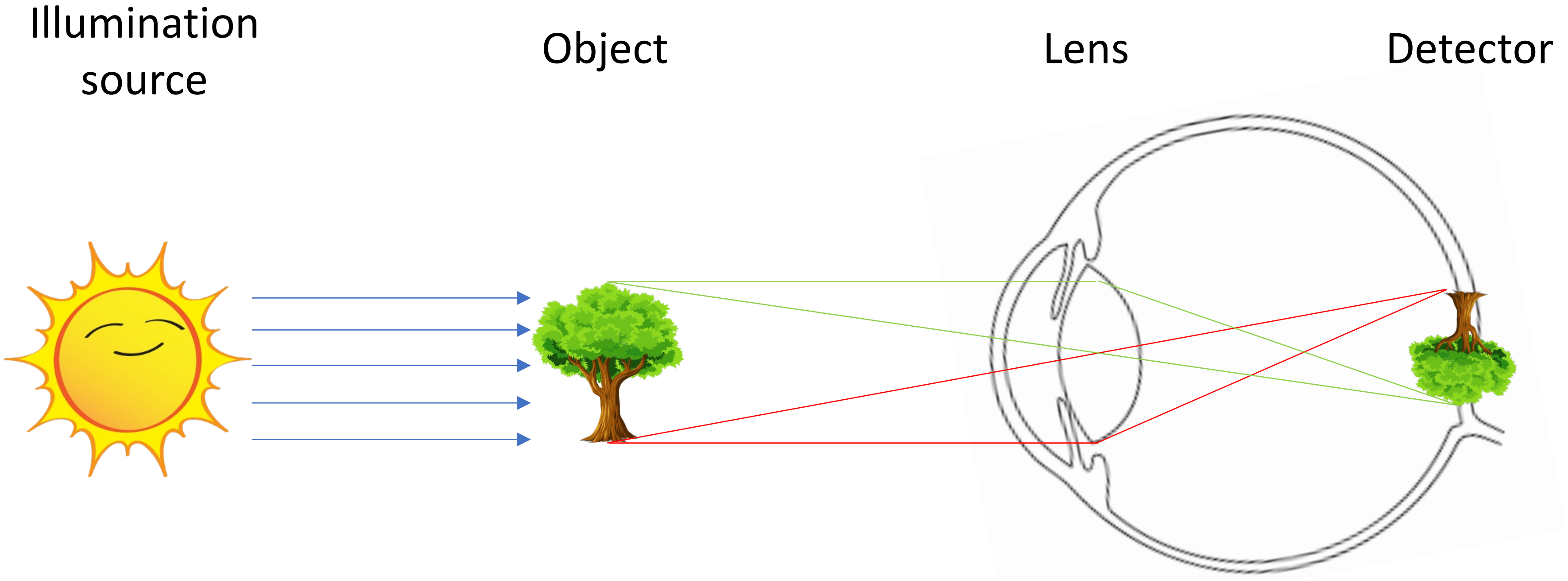
Principal Beamline Scientist – eBIC,
Diamond Light Source Ltd, UK

Wellcome Trust Graduate Training Program - CryoEM

Outline

- Basic image formation – eye vs. microscope
- Hardware of a transmission electron microscope:
  - Vacuum system
  - Electron source
  - Types of lenses
  - Apertures
- How the microscope works:
  - Condenser system
  - Objective lens
  - Projection system
- Aberrations and correctors
- Detection system
- Suggested Reading

Basic Image Formation



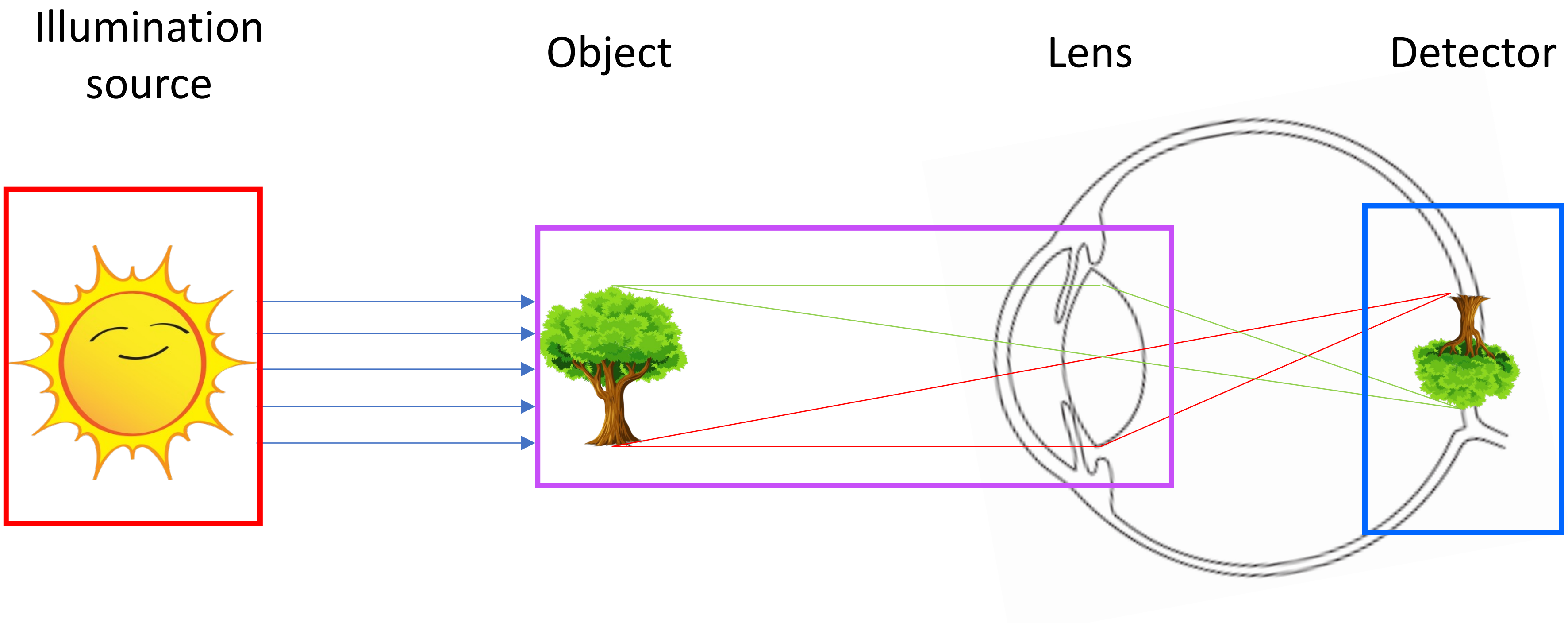
Illumination source

Object

Lens

Detector

Basic Image Formation

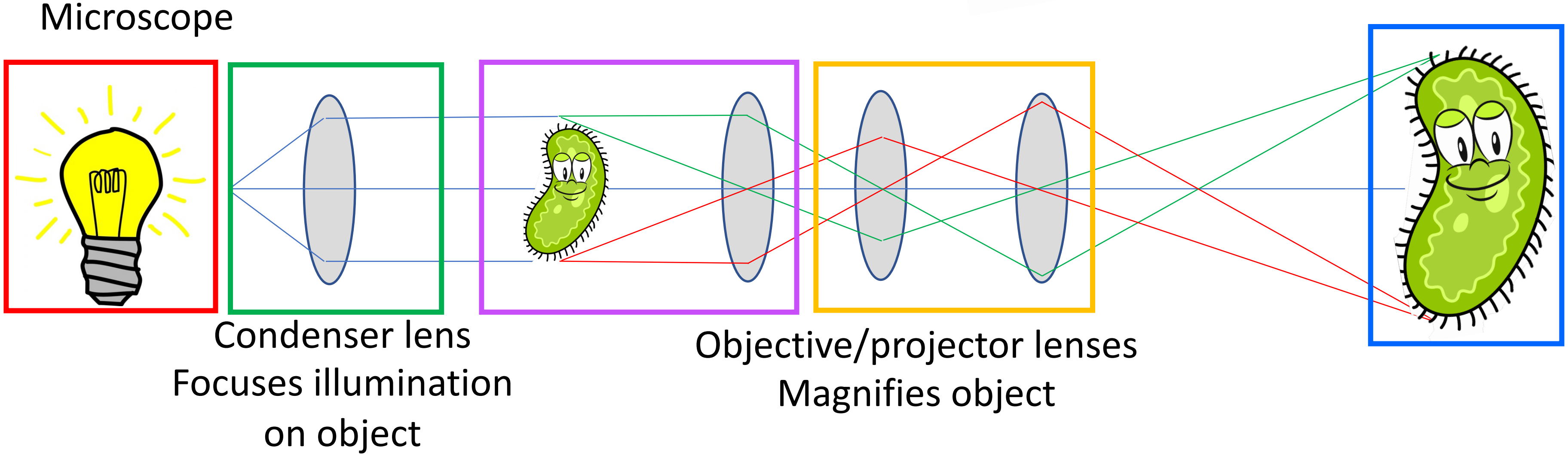


Illumination source

Object

Lens

Detector

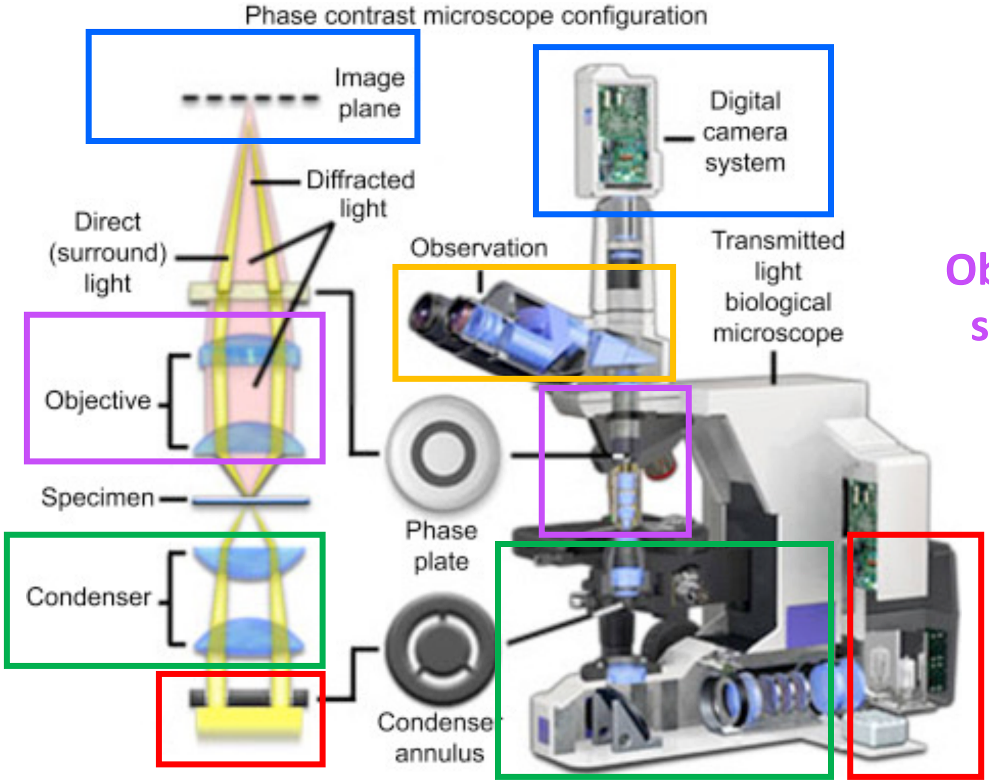


Microscope

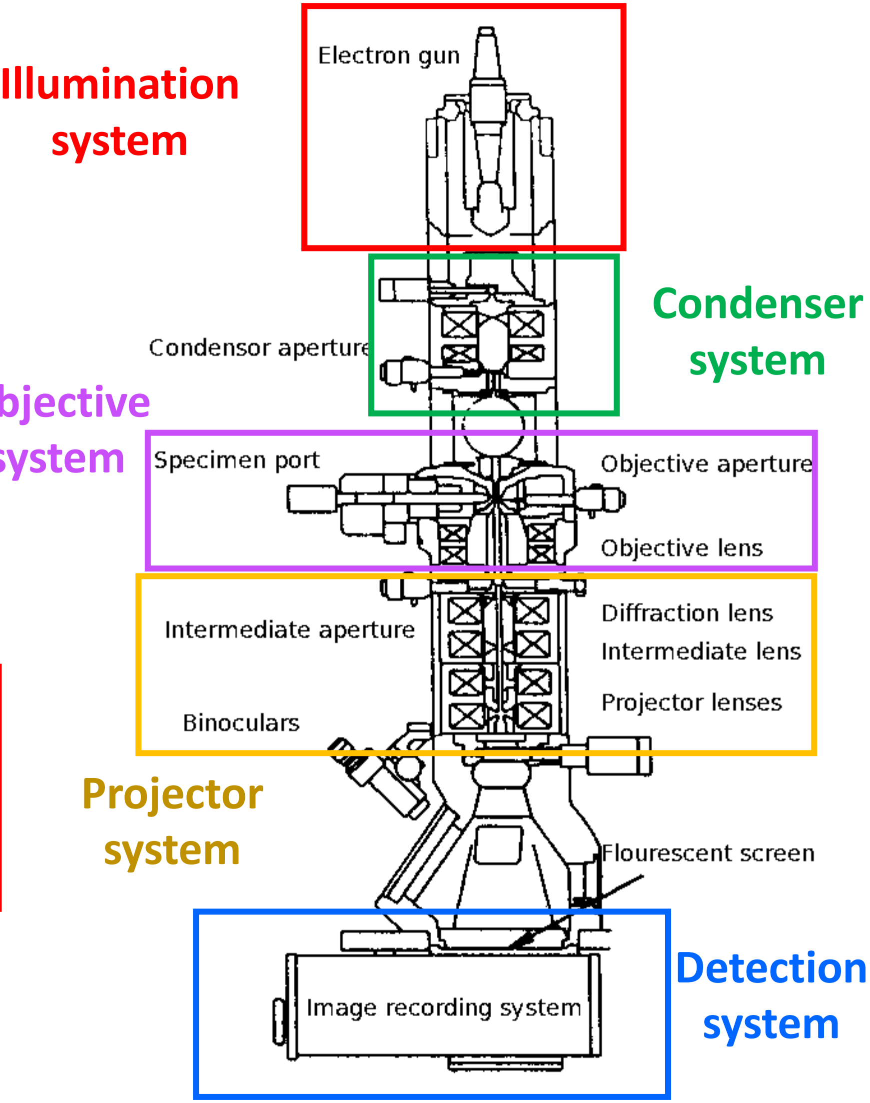
Condenser lens
Focuses illumination on object

Objective/projector lenses
Magnifies object

Light vs. Electron Microscope

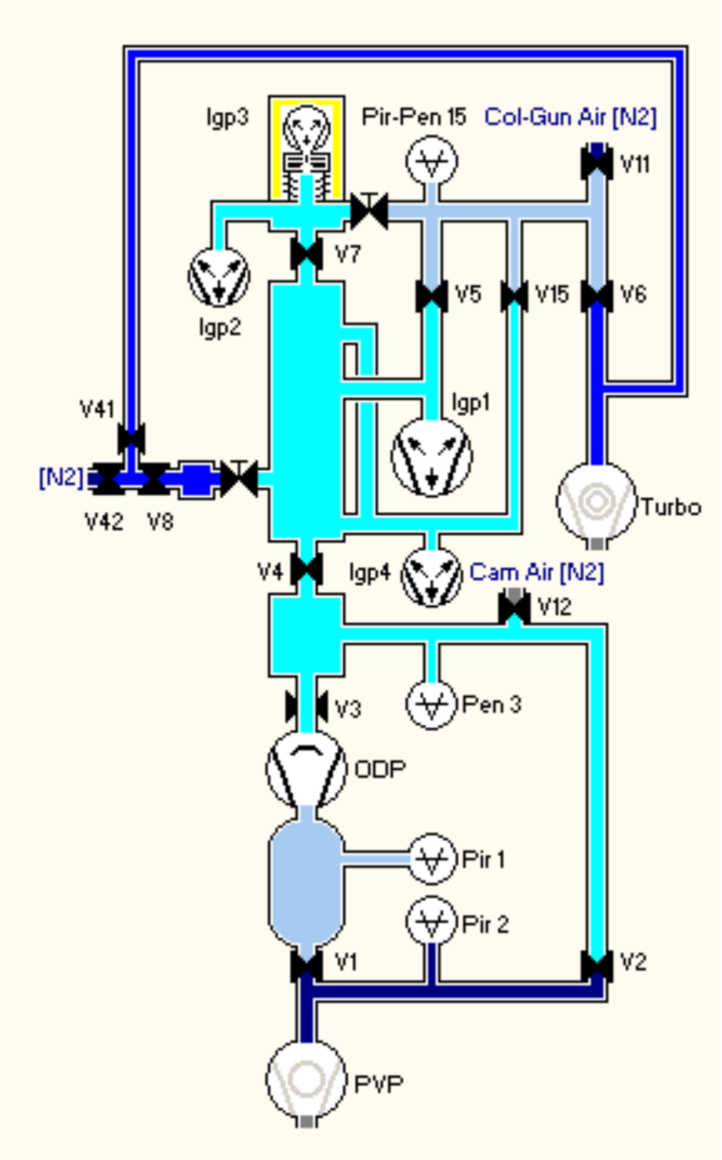


Light

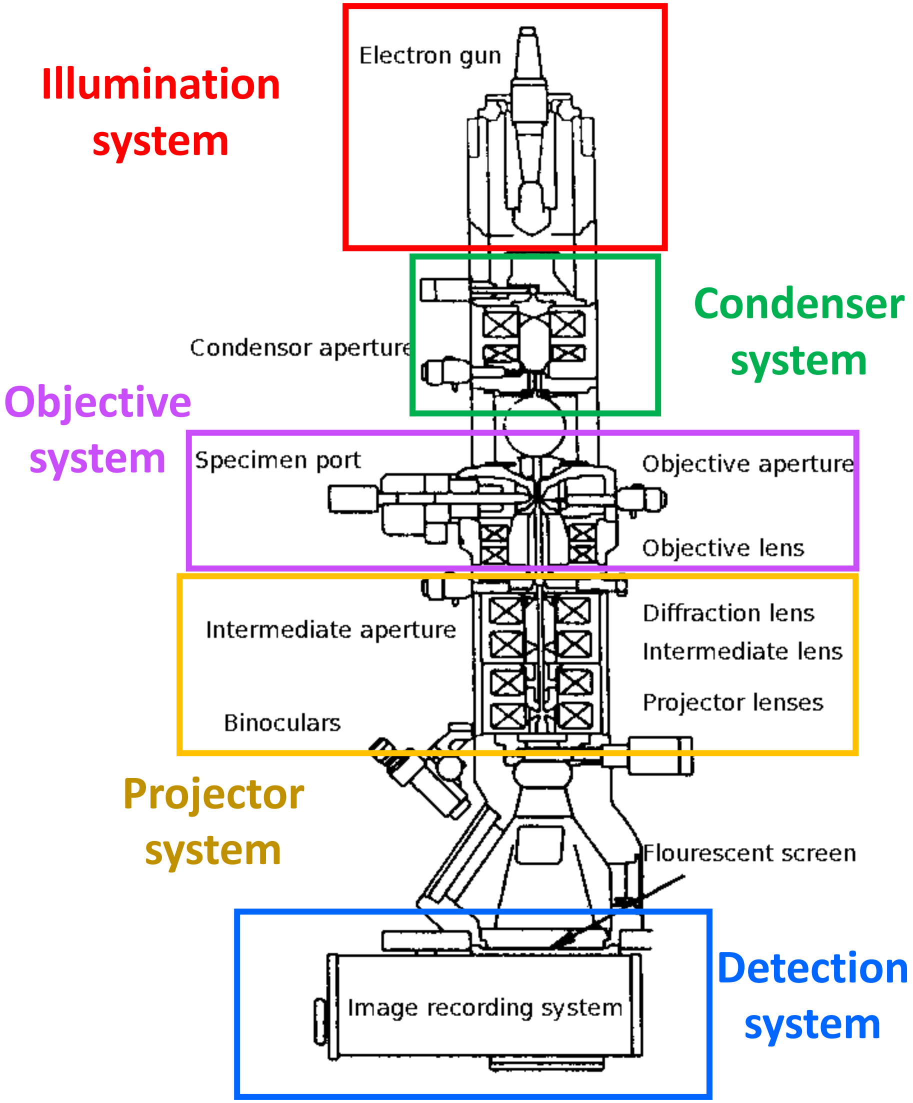


Electron

The Vacuum System



Vacuum system



Illumination system

Condenser system

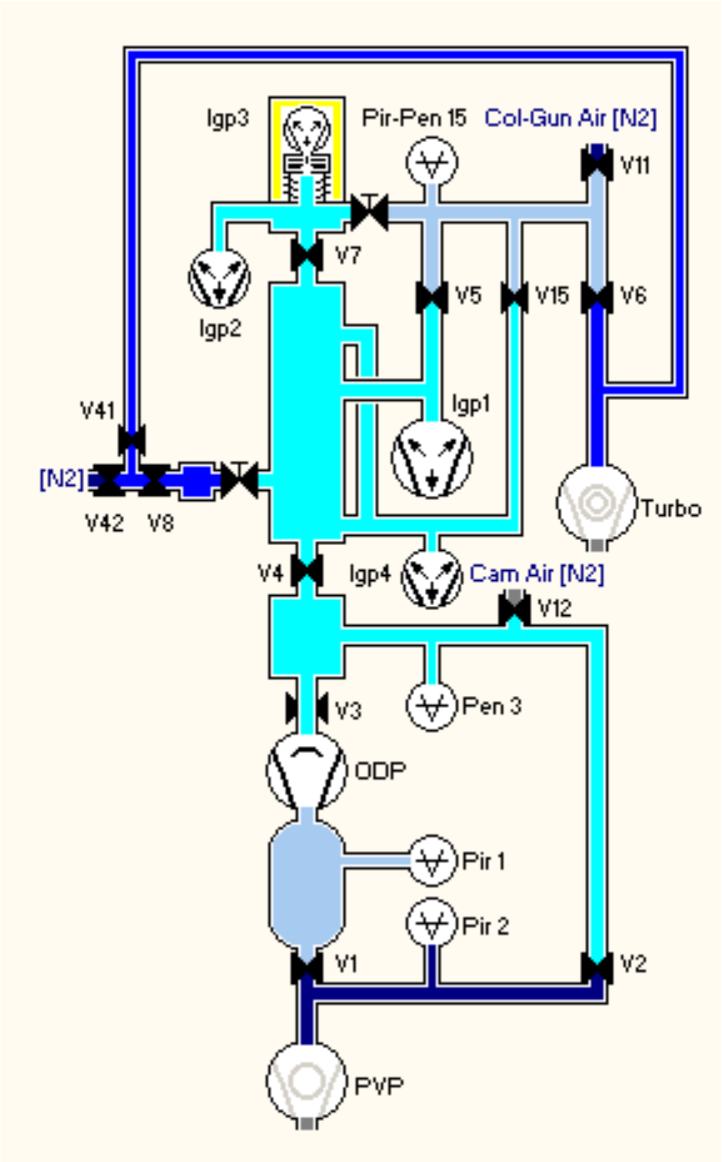
Objective system

Projector system

Detection system

Electron

The 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 (PVP) – 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

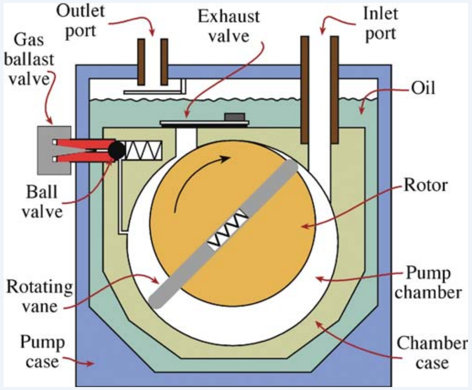
🟦 Room Pressure: 10^5 Pa
🟦 Rough Vacuum: 100-0.1 Pa
🟦 Low Vacuum: 0.1 - 10^{-4}
🟦 High Vacuum: 10^{-4} - 10^{-7} Pa
🟨 Ultra High Vacuum: 10^{-7} Pa and below

Vacuum System

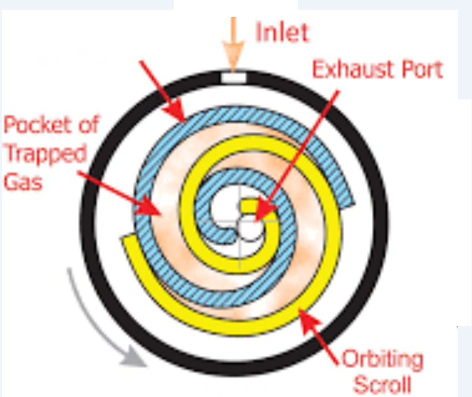
Atmosphere

Low vacuum

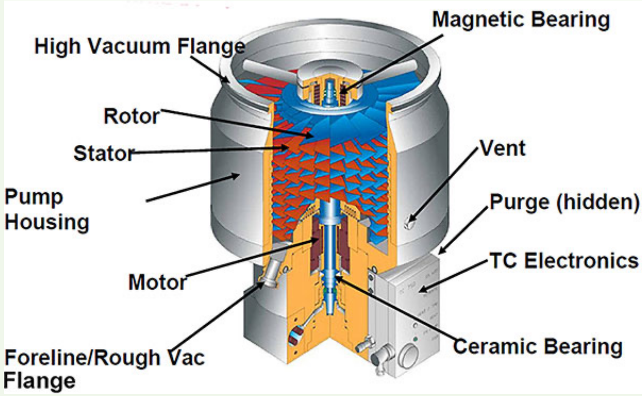
Low vacuum



Rotary pump



Scroll Pump

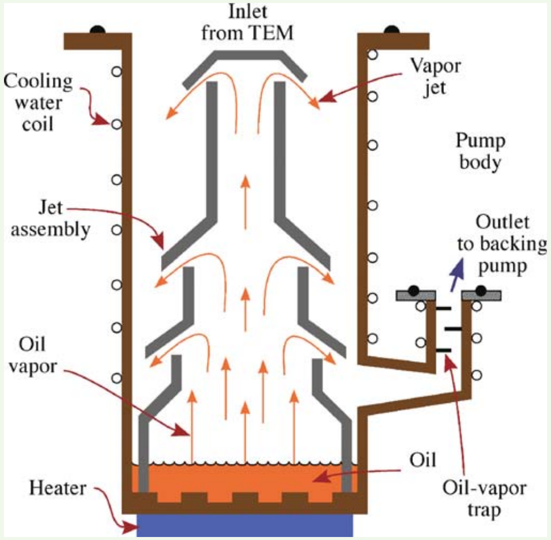


Turbo Pump

Increasing Vacuum

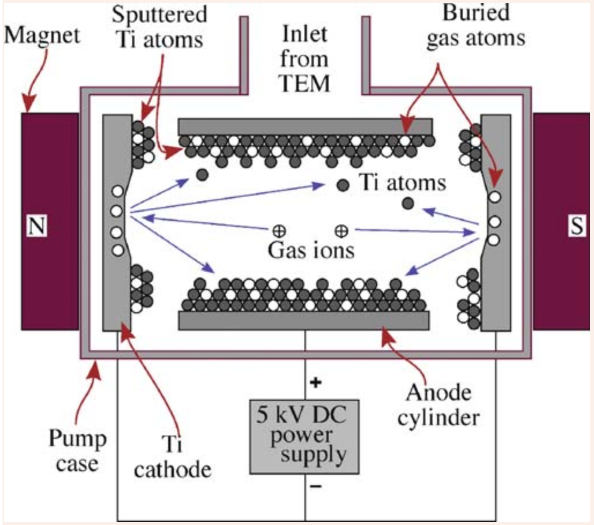
high vacuum

High vacuum

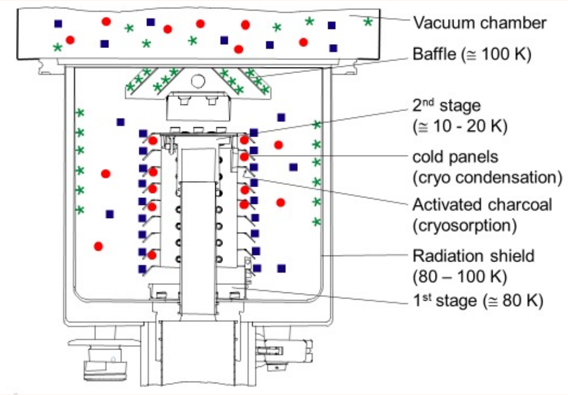


Oil Diffusion Pump

Low vacuum



Ion Getter Pump

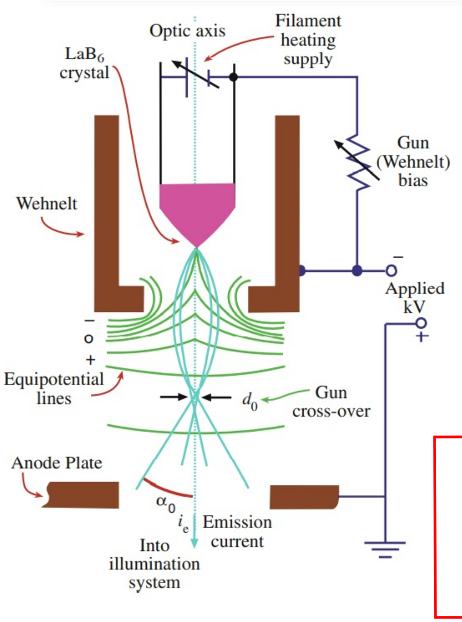


Cryo-pump

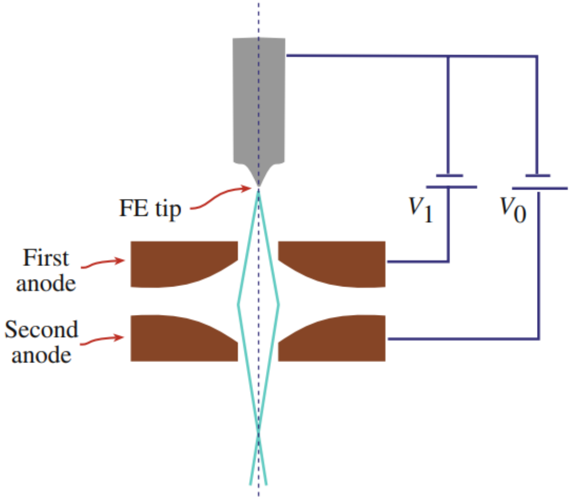
Images from Williams and Carter “Transmission Electron Microscopy” (2016) & internet/youtube

Electron Source

Thermionic



Field Emission



Gun quality:

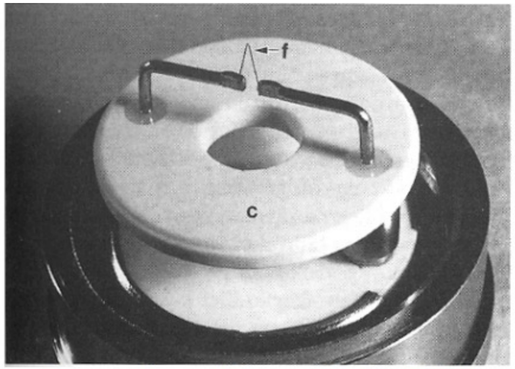
Temporal coherence – Wavelength spread

Spatial coherence – Angular spread

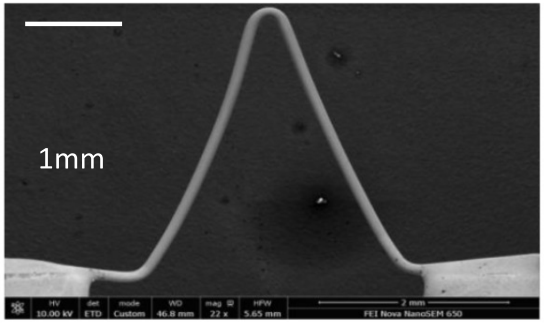
	Units	Tungsten	LaB$_6$	Schottky FEG	X-FEG	Cold FEG
Work function, Φ	eV	4.5	2.4	3.0		4.5
Richardson's constant	A/m^{2}K^2	6×10^9	4×10^9			
Operating temperature	K	2700	1700	1700		300
Current density (at 100 kV)	A/m^2	5	10^2	10^5		10^6
Crossover size	nm	$> 10^5$	10^4	15		3
Brightness (at 100 kV)	A/m^{2}sr	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

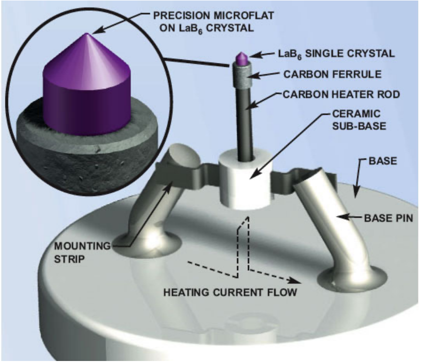
Tungsten (W)

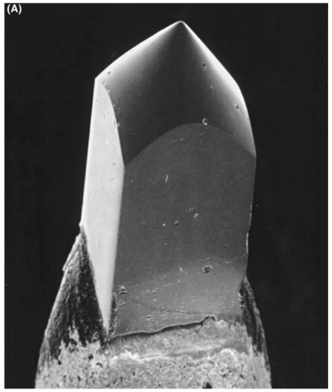


Bozzola and Russell, Fig. 6.22

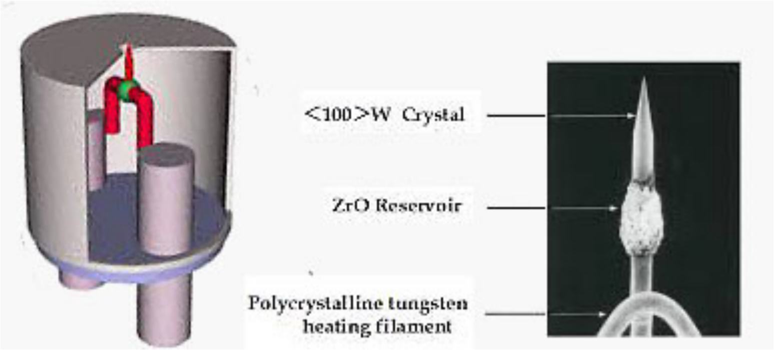


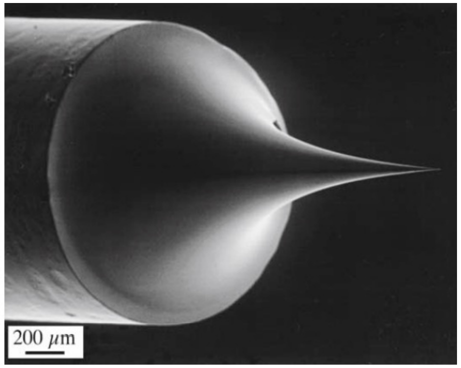
LaB$_6$





FEG (W)

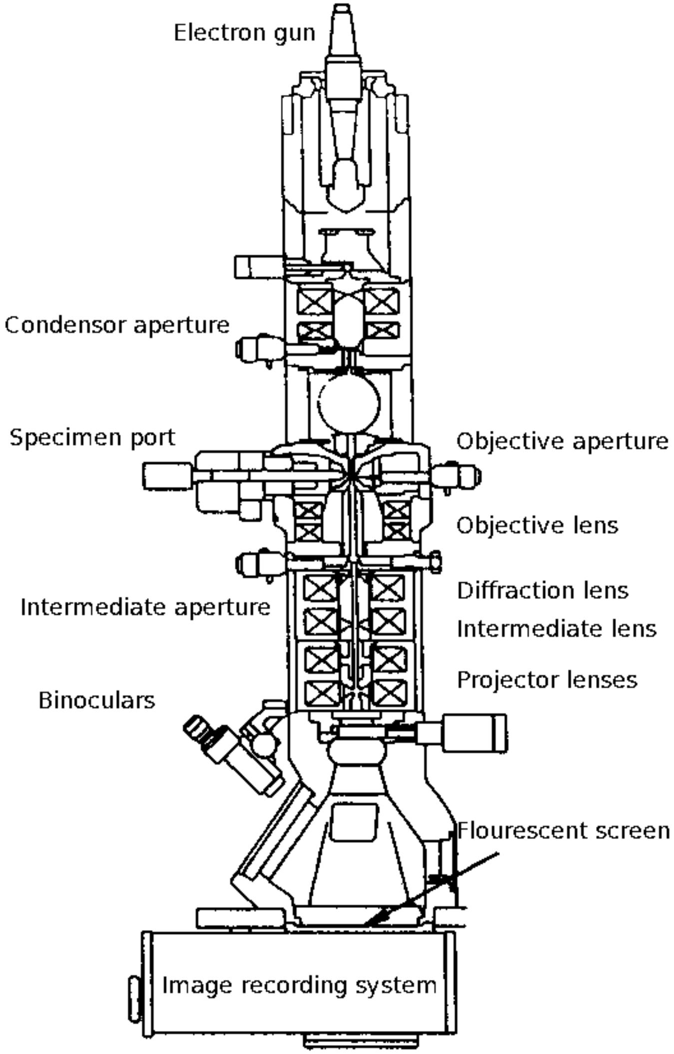




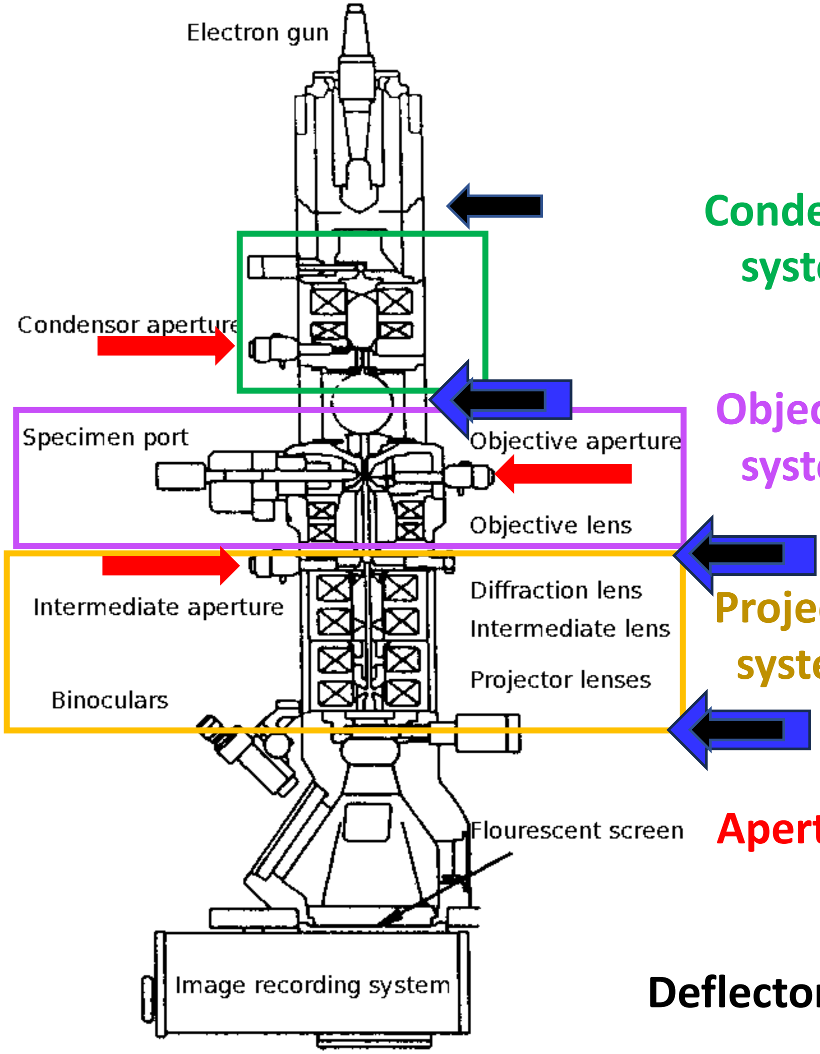
Thermionic

Field Emission

The Lens System – Types of Lenses



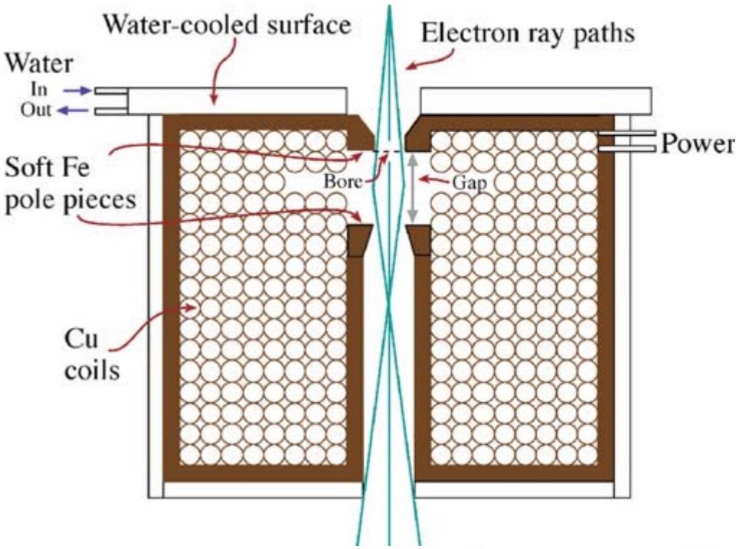
The Lens System – Types of Lenses

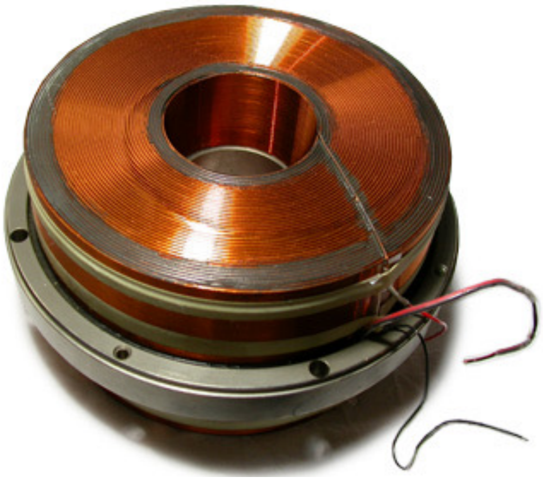


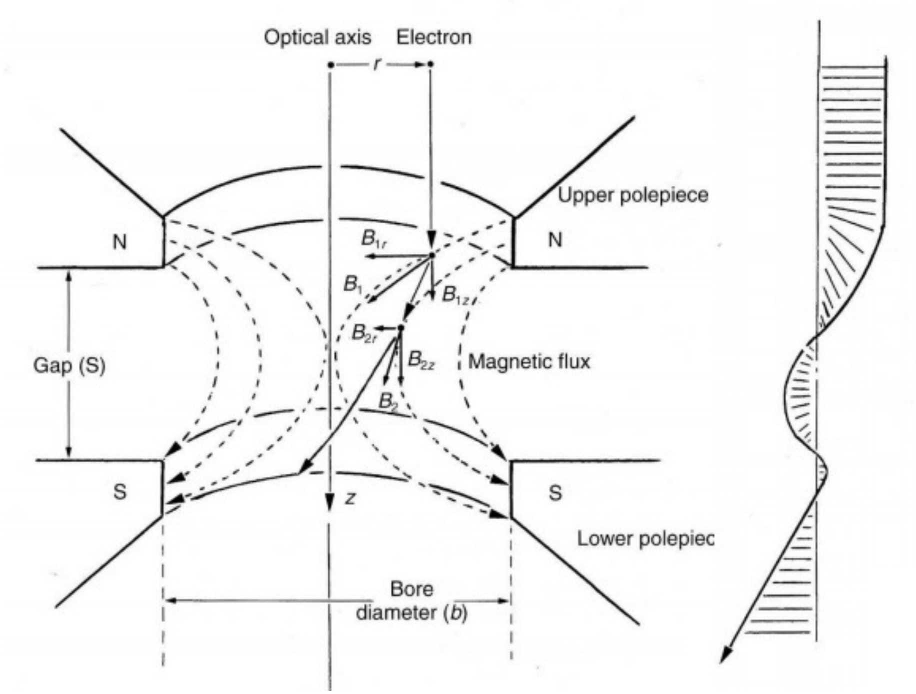
Condensing lenses

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

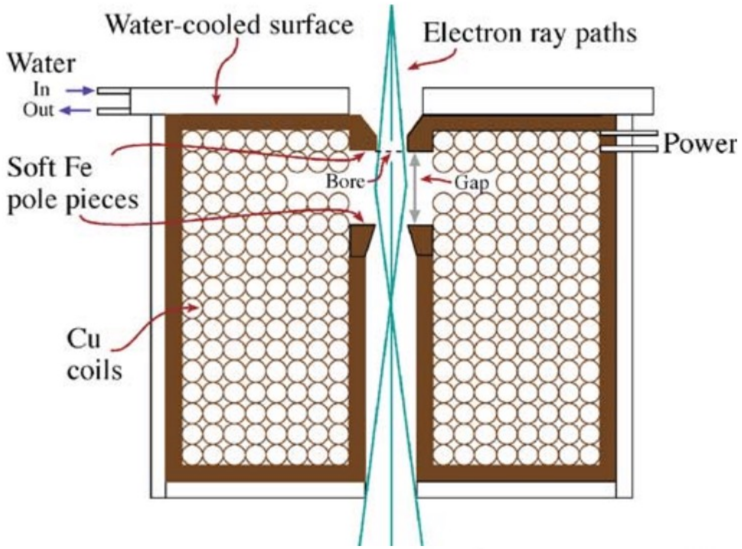
The Imaging (Condensing) Lens

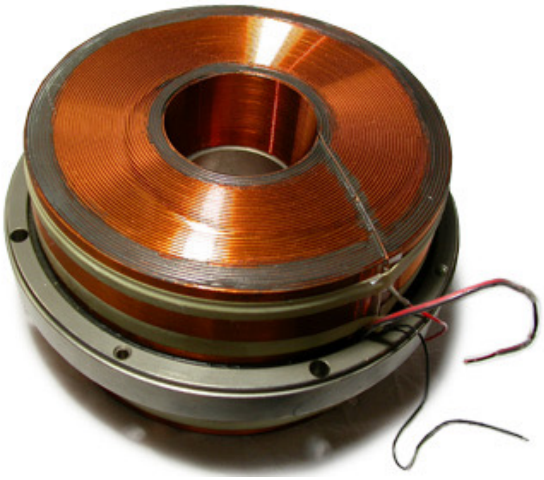






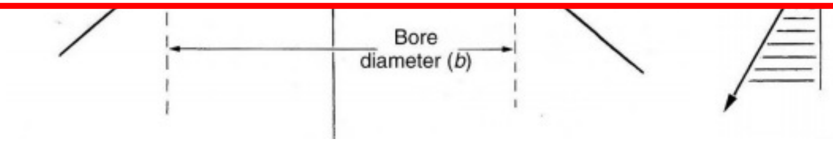
The Imaging (Condensing) Lens





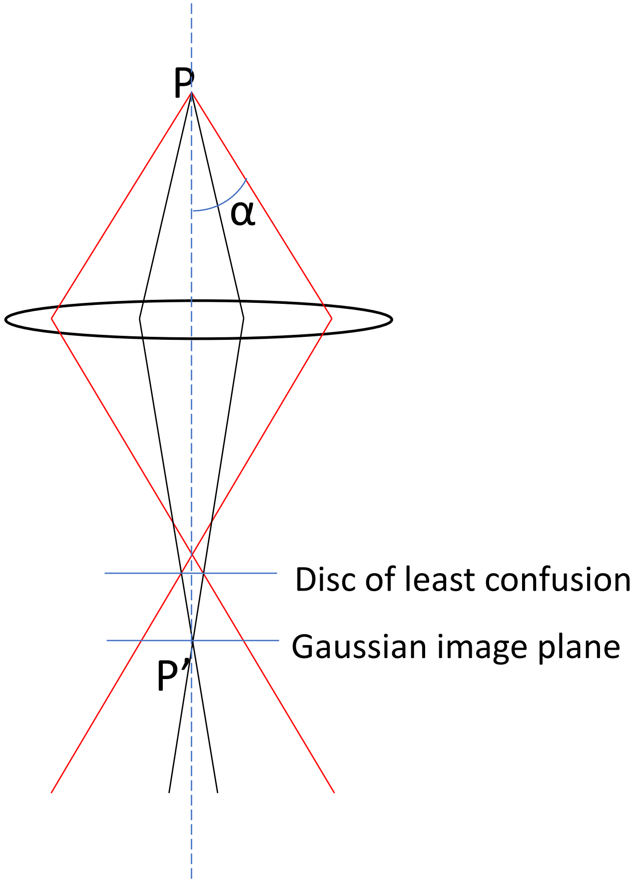
Magnetic lenses are poor quality and have severe aberrations:

Spherical aberration (position)
Chromatic aberration (wavelength)
Coma (angle of entry)
Stigmation – defects in magnetic field symmetry



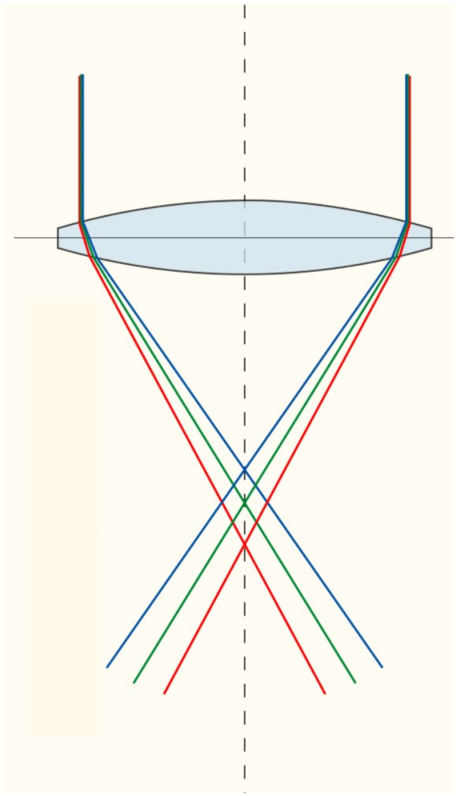
Aberrations from Condensing Lenses

Spherical C_s



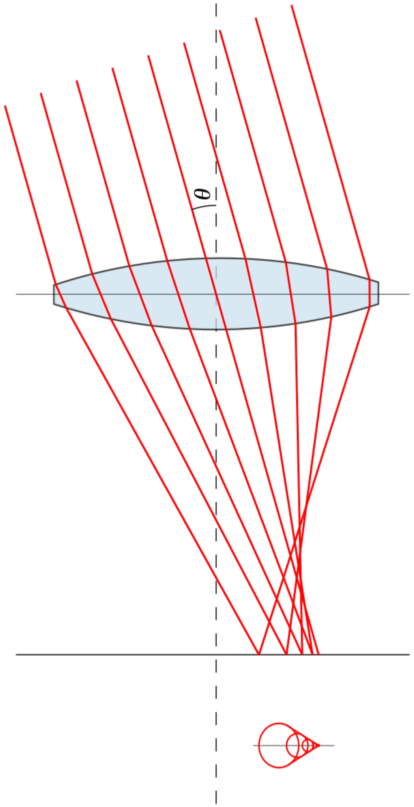
- Lenses are strongest at edge
- Thus off-axis electrons bent more than on-axis
- Different focus points

Chromatic C_c



- Electrons of different wavelength focused by different amount
- Focus plane different for different wavelengths

Coma



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

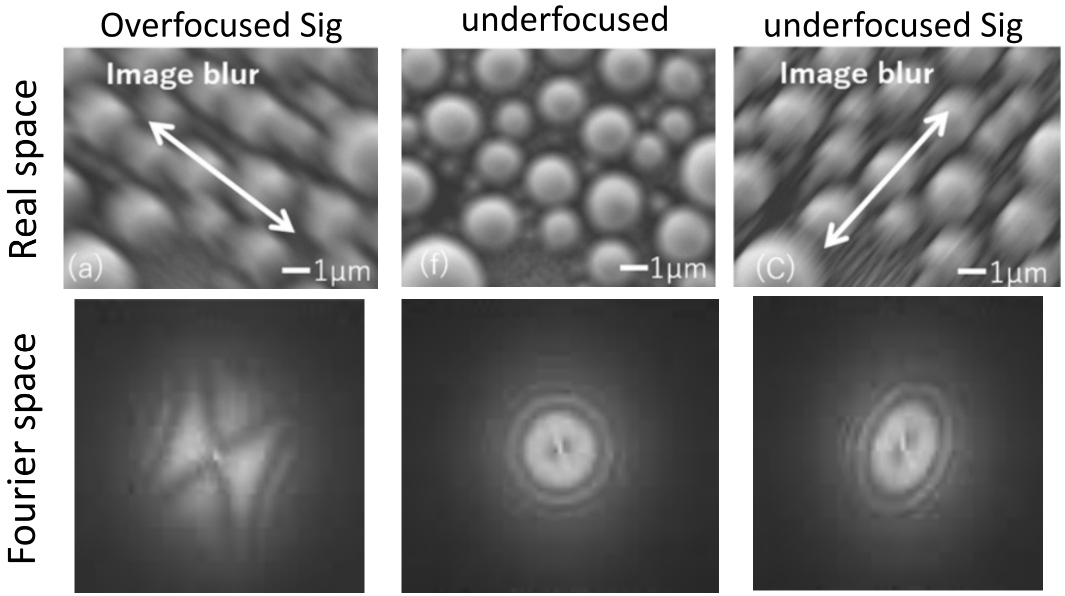
Quadrupoles = Stigmators

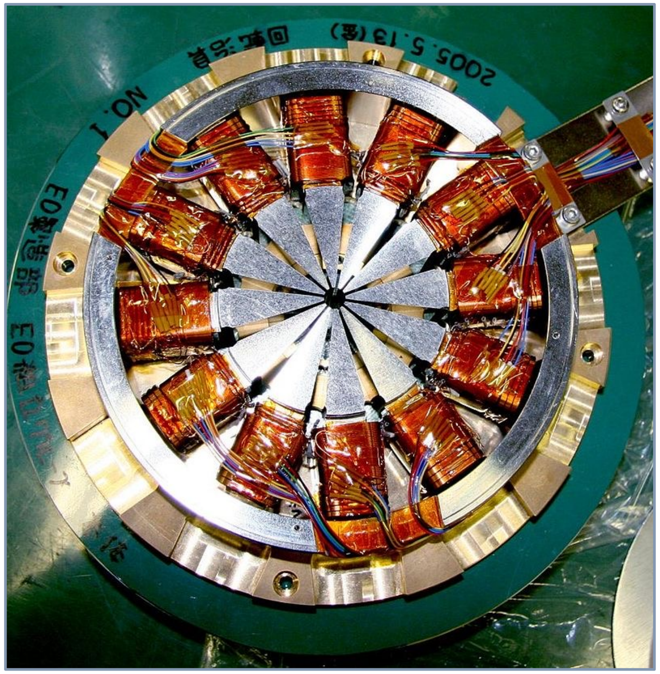
Stigmation is caused by:

1) Magnetic lens defects
2) Contamination in pole gap

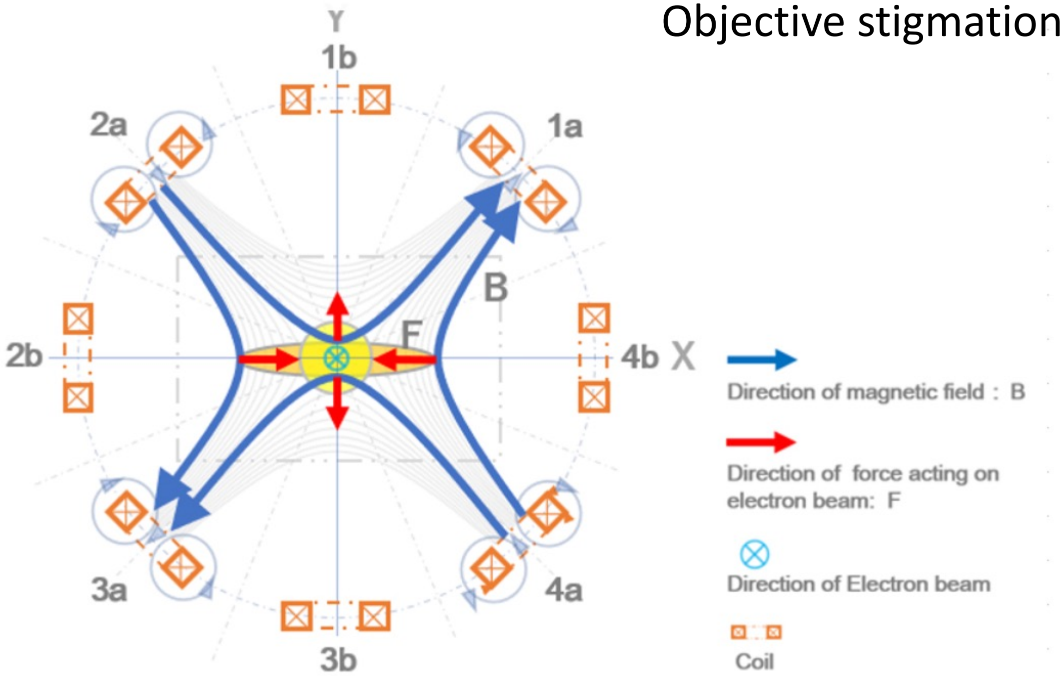
Condenser stigmators
correct beam shape
(circle vs. oval)

Objective stigmators
correct image





Objective stigmation



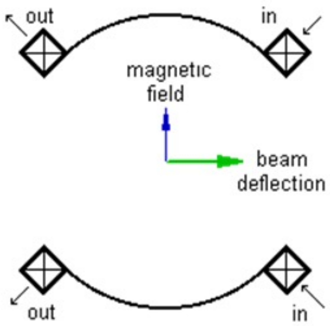
→ Direction of magnetic field : B

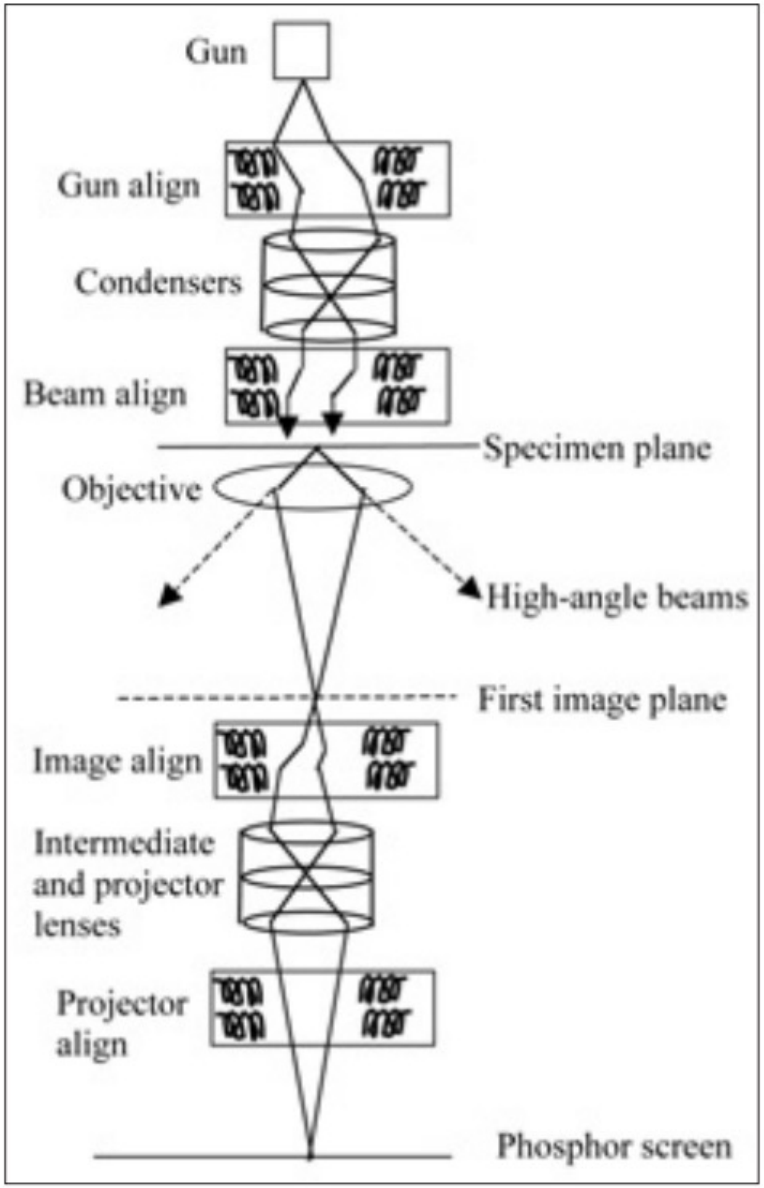
→ Direction of force acting on electron beam: F

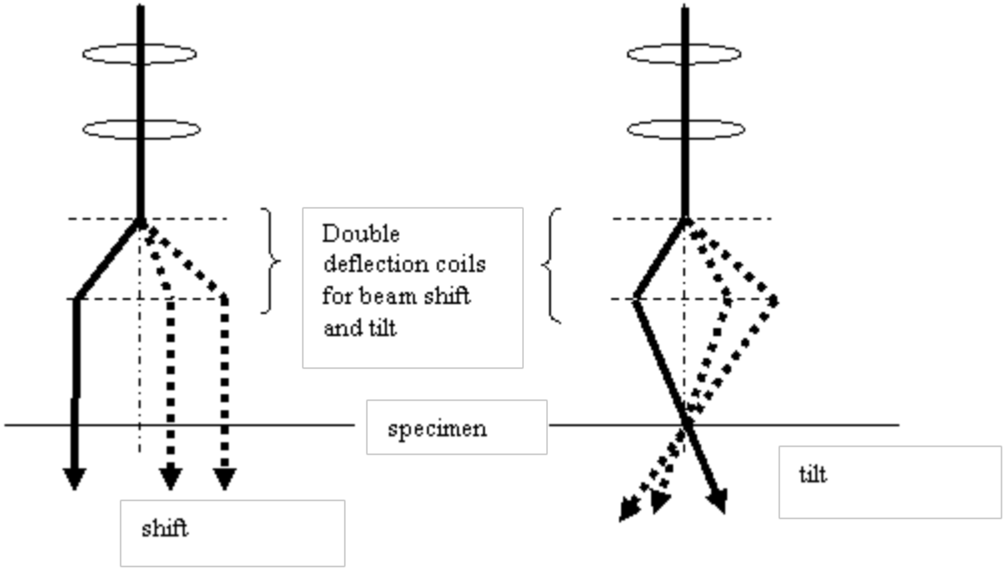
⊗ Direction of Electron beam

⊠ ⊠ Coil

Shift/Tilt Coils

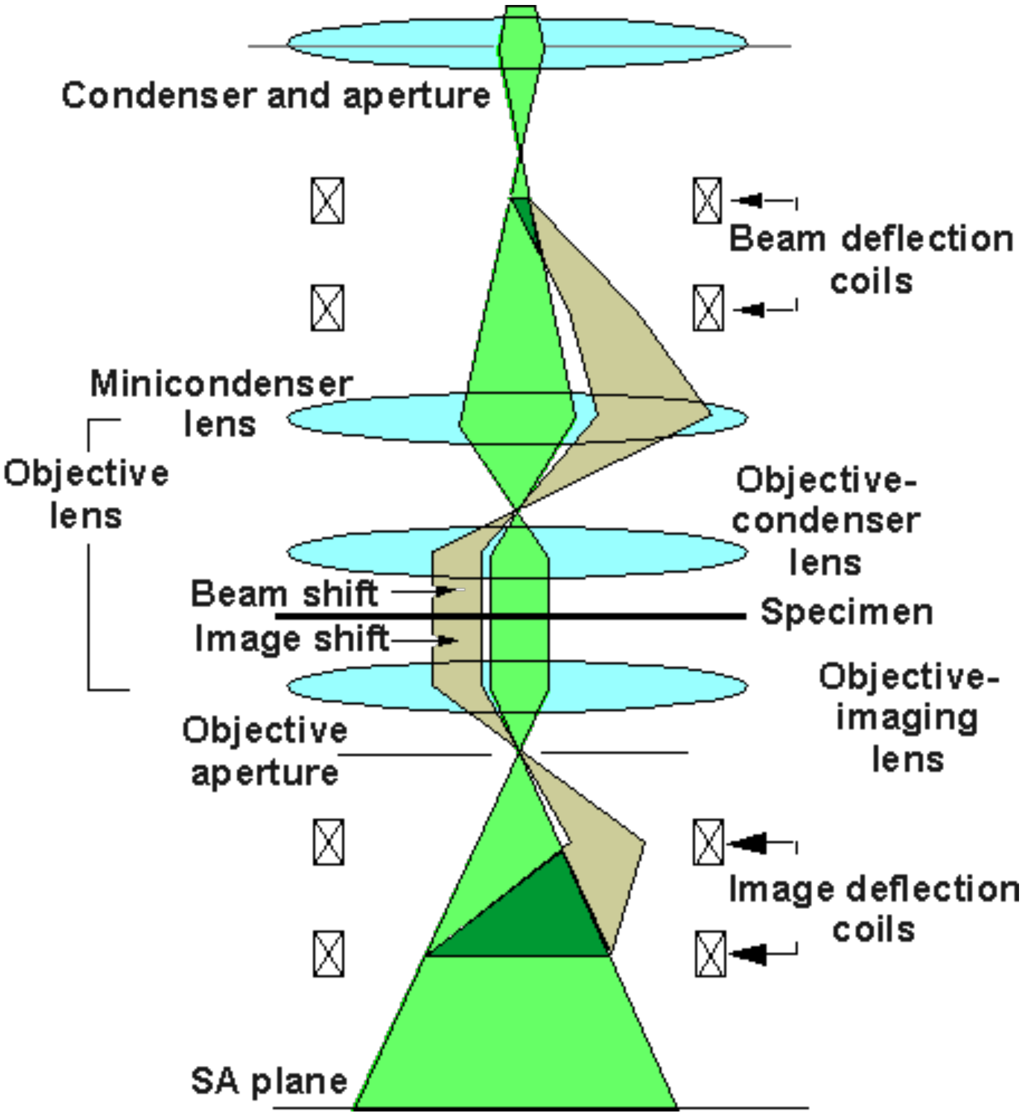






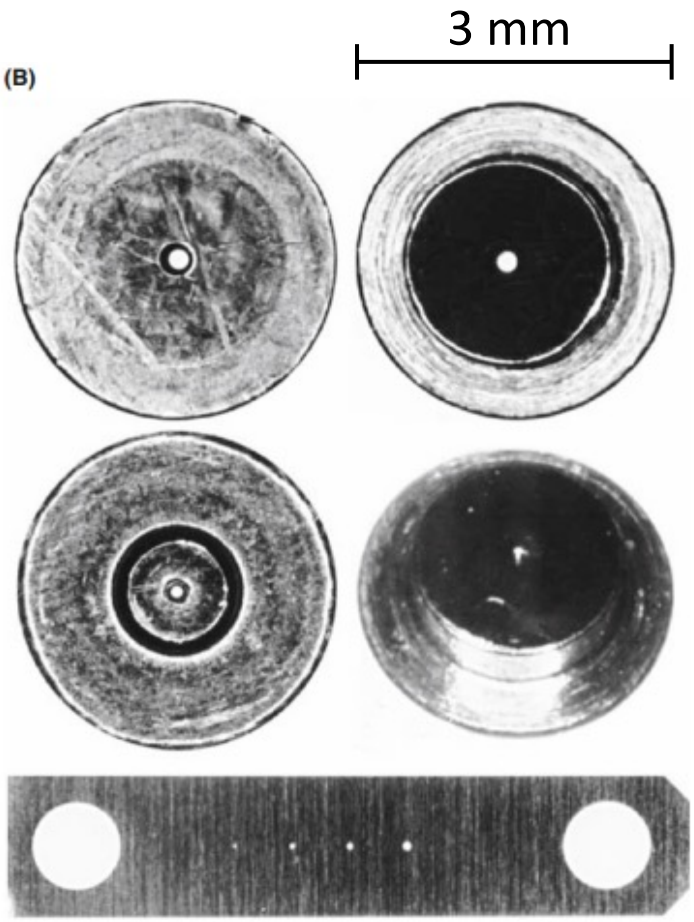
1. Get the beam down the scope
2. Make sure beam is parallel/aligned to optical axis (bright field imaging)
3. Low dose imaging
4. Automatic procedures (e.g. eucentric height/focus)

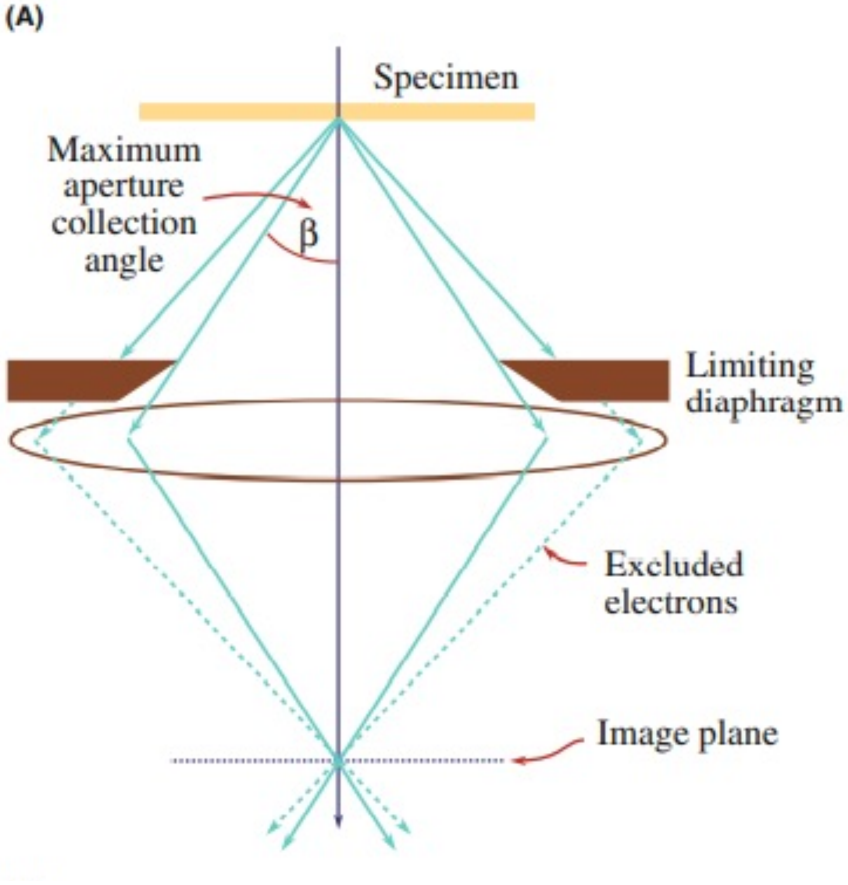
Use of Deflection Coils in Low Dose Imaging



https://forum.scilifelab.se/t/creating-optics-groups-from-epu-afis-data-and-more/122

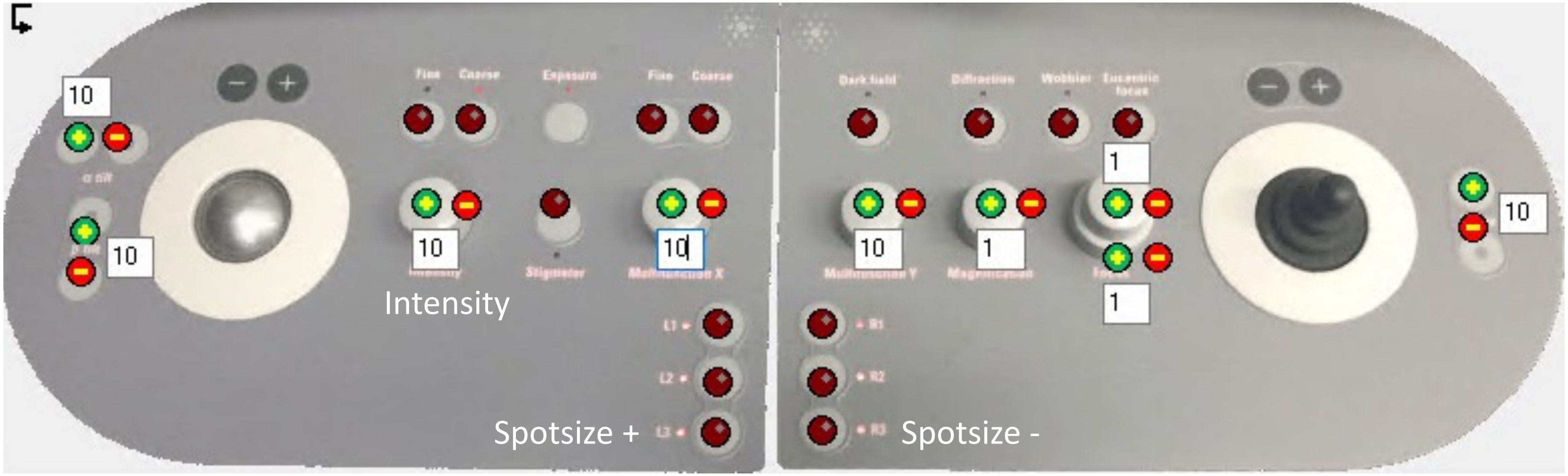
Apertures





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

What is happening when I operate the microscope?

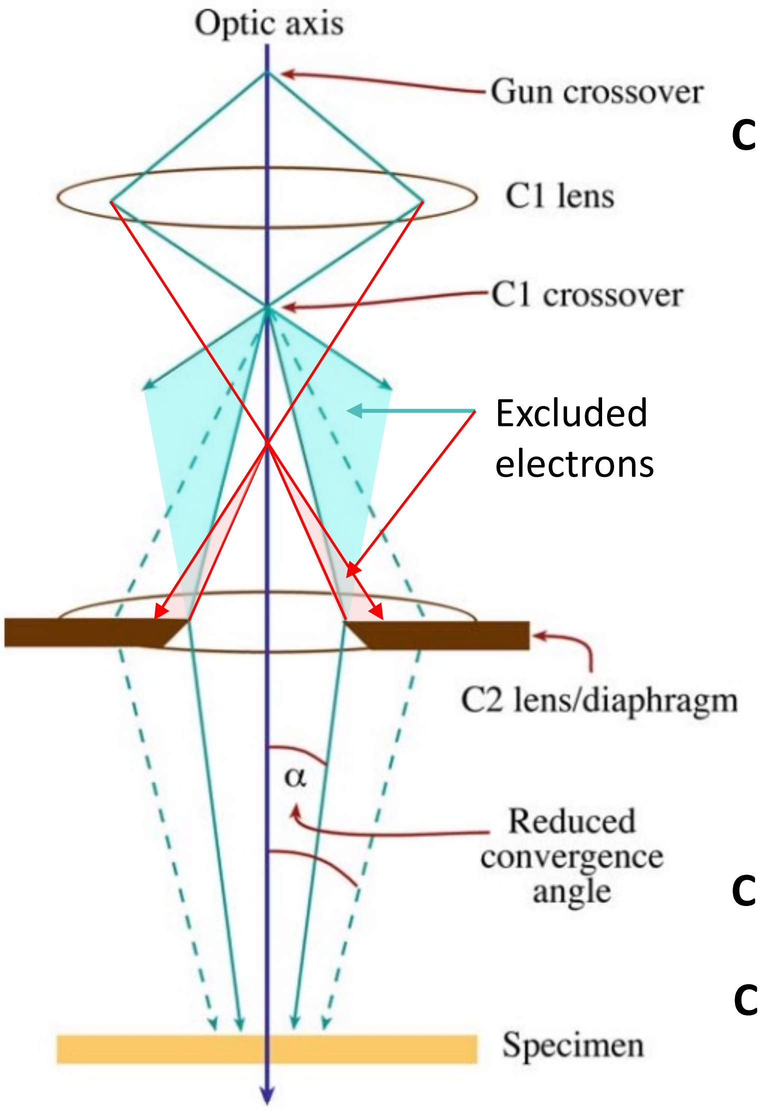


Intensity

Spotsize +

Spotsize -

Condenser System



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

C1 = Spot size, controls beam size and quality

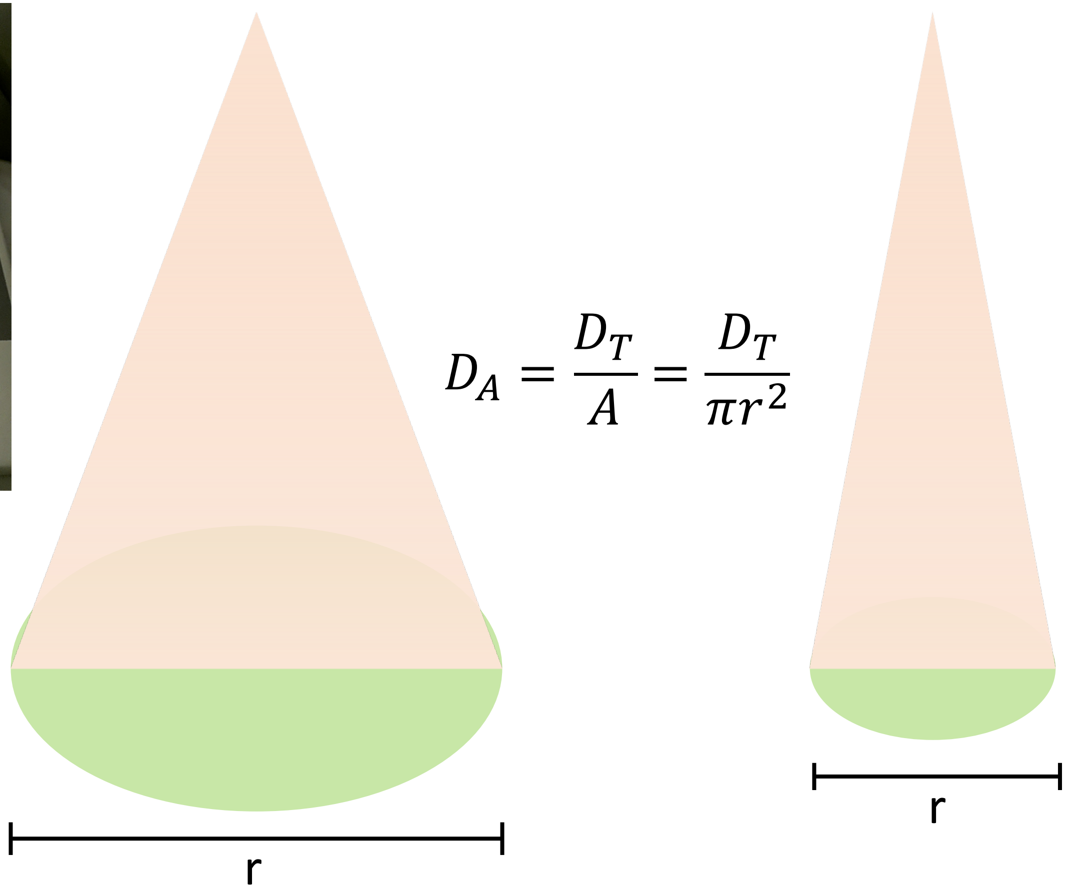
Spot 9: Strongest lens setting
Highest crossover
Dimmest beam and smallest focused beam
Most coherent little spatial divergence
Only most parallel electrons reach specimen

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



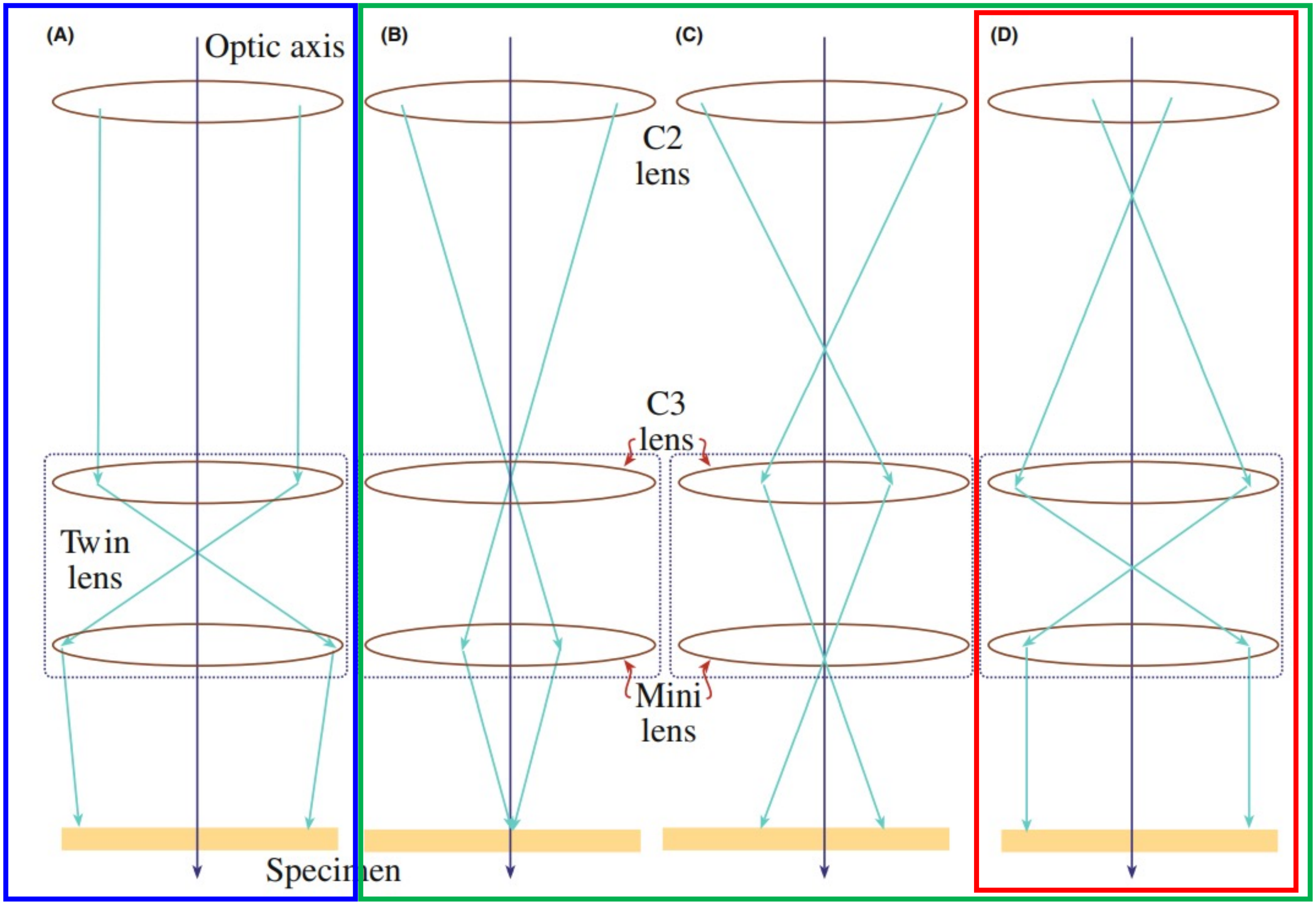
$$D_A = \frac{D_T}{A} = \frac{D_T}{\pi r^2}$$

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)



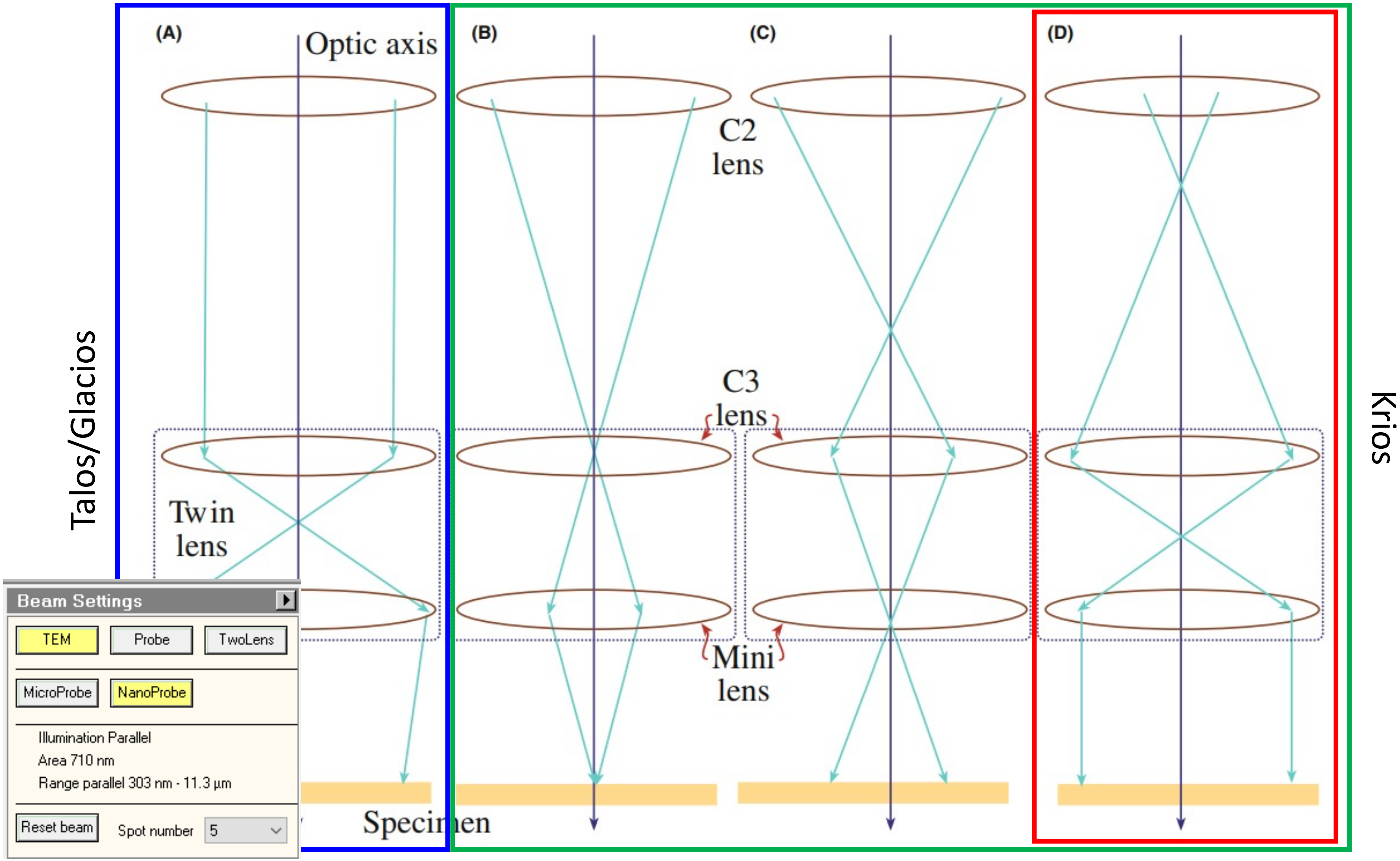
Talos/Glacios

Krios

Parallel illumination of specimen reduces aberrations

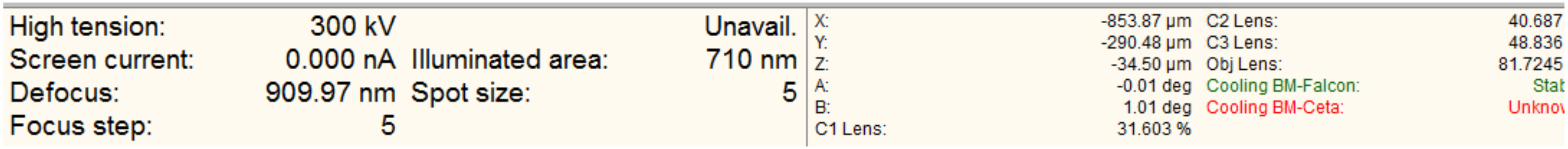
C3 lens provides parallel illumination but only at certain C2 values

C3 Lens (Krios)



Illumination Parallel
Area 710 nm
Range parallel 303 nm - 11.3 µm

Spot number 5



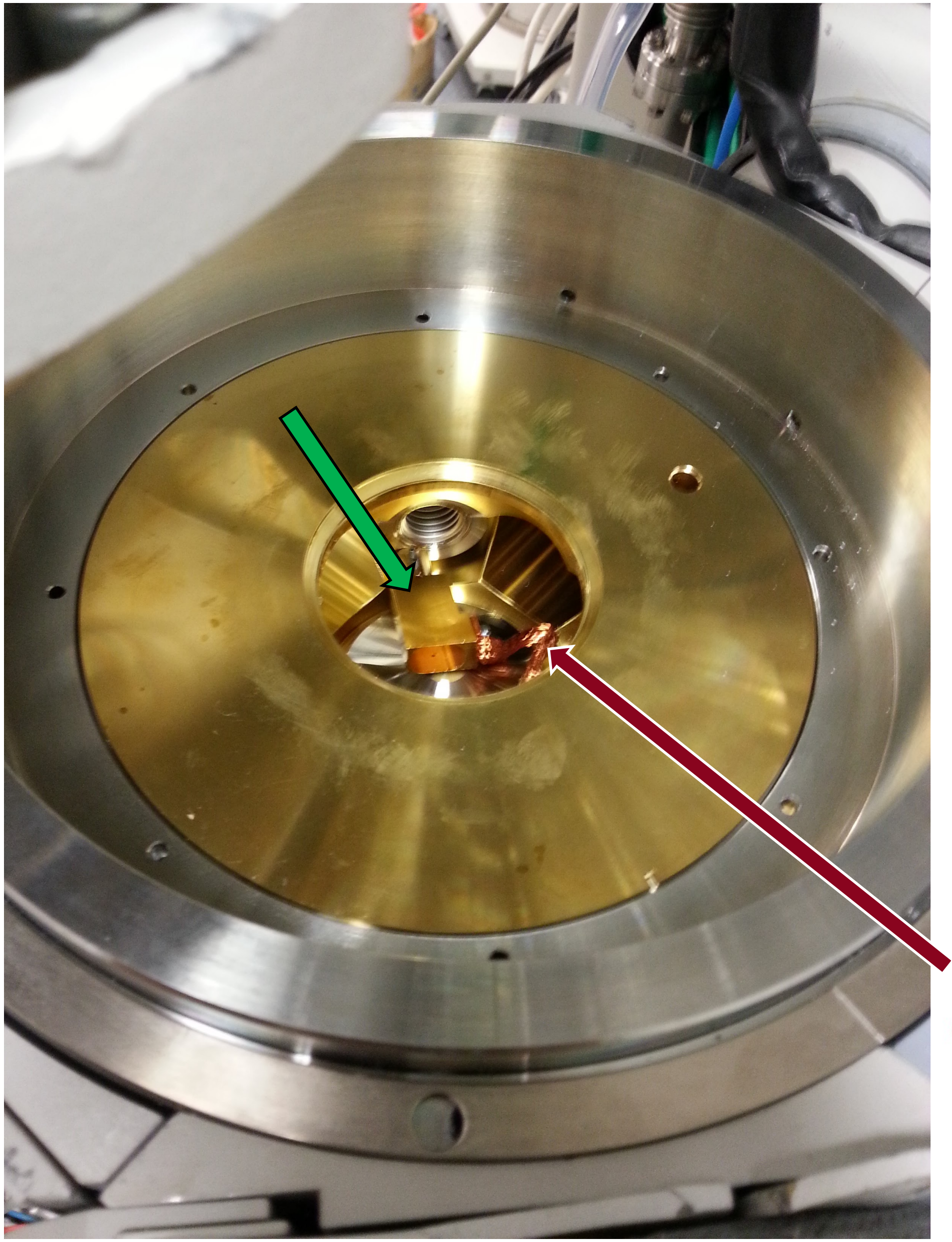
High tension: 300 kV
Screen current: 0.000 nA
Defocus: 909.97 nm
Focus step: 5

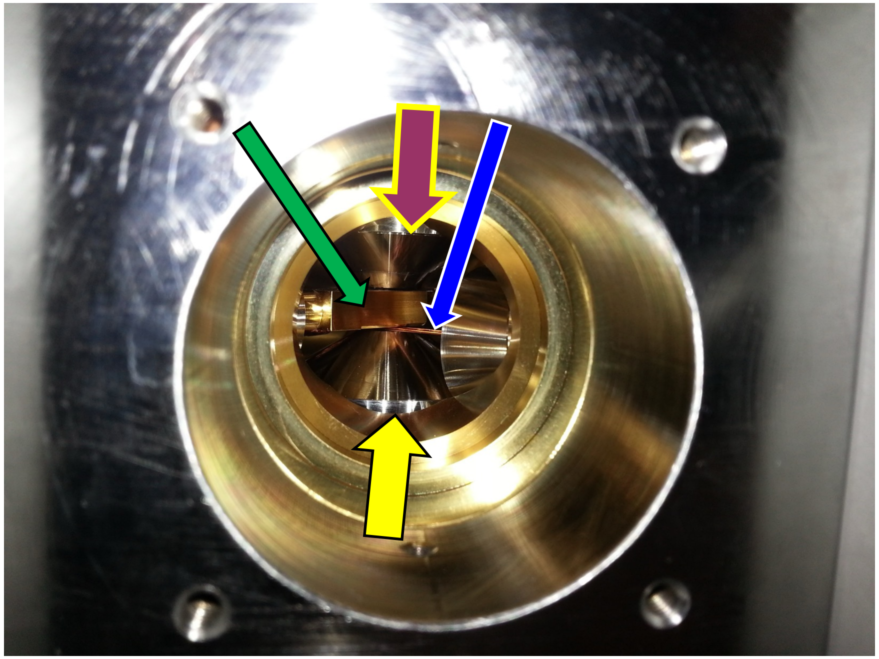
Unavail.
Illuminated area: 710 nm
Spot size: 5

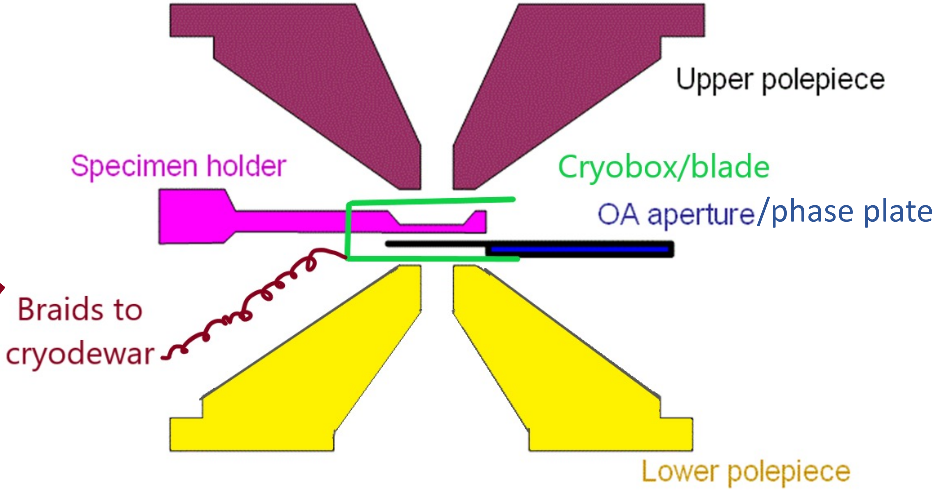
X: -853.87 µm
Y: -290.48 µm
Z: -34.50 µm
A: -0.01 deg
B: 1.01 deg
C1 Lens: 31.603 %

C2 Lens: 40.687
C3 Lens: 48.836
Obj Lens: 81.7245
Cooling BM-Falcon: Stat
Cooling BM-Ceta: Unknov

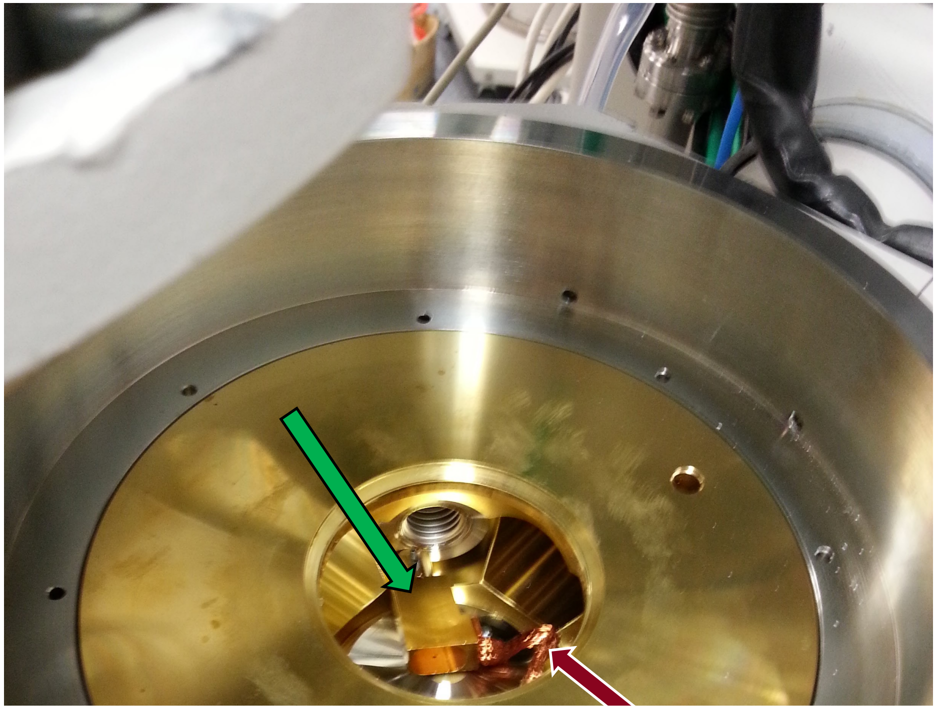
Objective System – Mag and Specimen



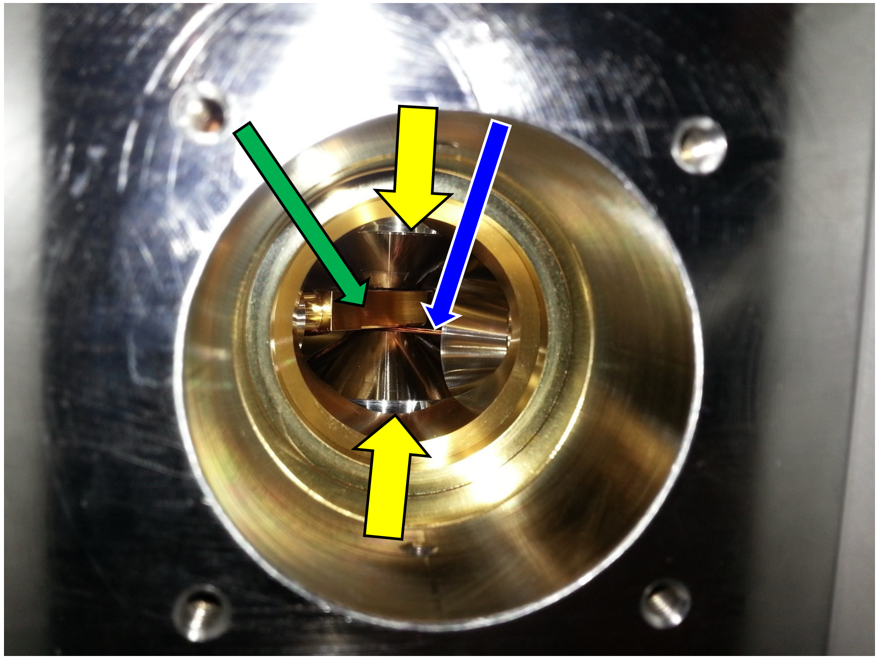


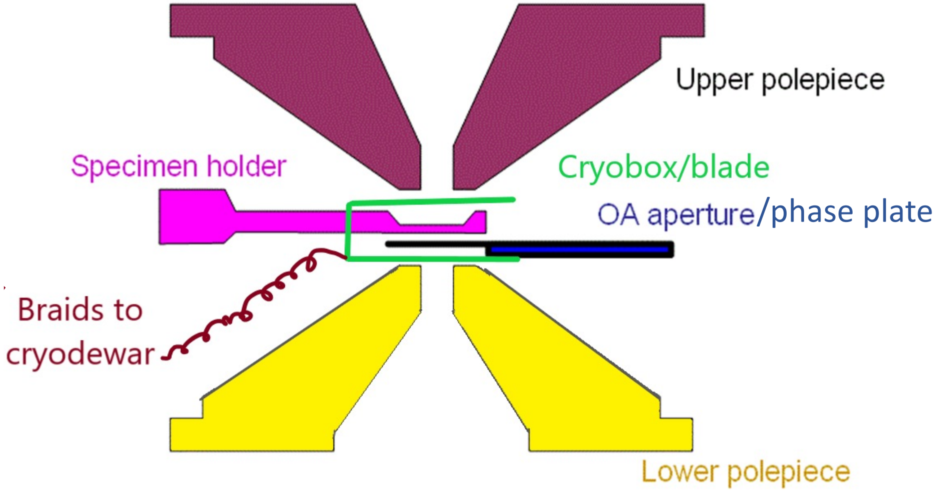


Objective System – Mag and Specimen



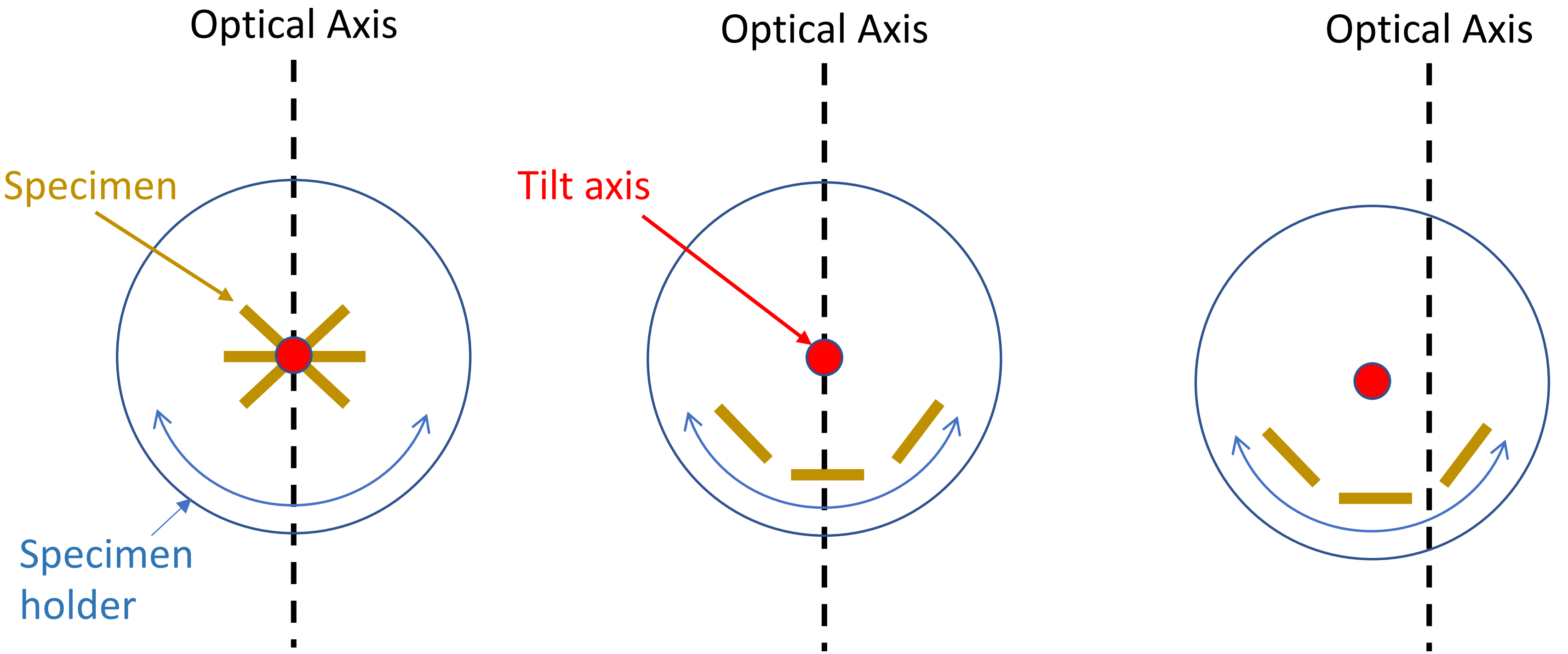
- Cryoblades cooled to LN_2 temp by braids
- Cryoblades colder than sample → cryotrap
- Keeps sample clean and cold by absorbing contaminant from sample/column
- Cryoblades needs to be warmed up regularly to remove contaminants → cryocycle
- Cryocycle: turns off IGP, pumps specimen chamber with Turbo





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

Objective System - Eucentric Height



Excellent!

Tilt axis aligns on optical axis and eucentric focus

Specimen does not move when tilted

Bad

Specimen moves when tilted

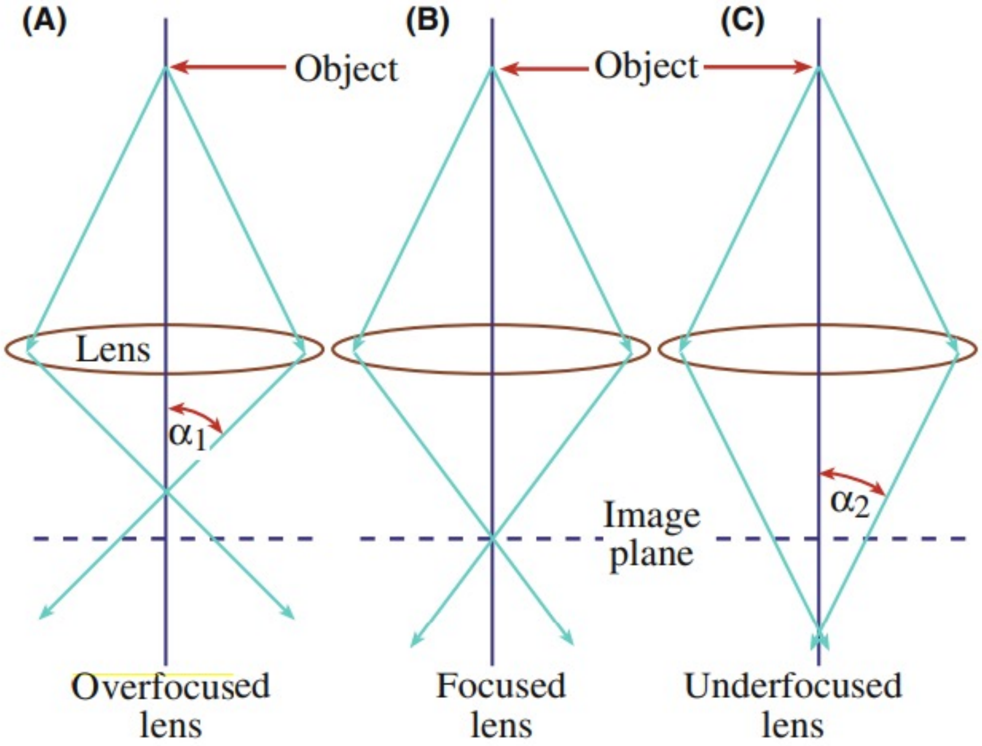
Change specimen Z-height

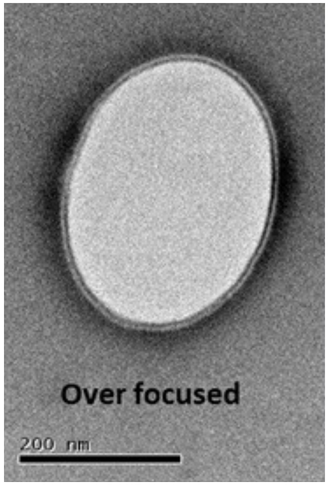
Very Bad

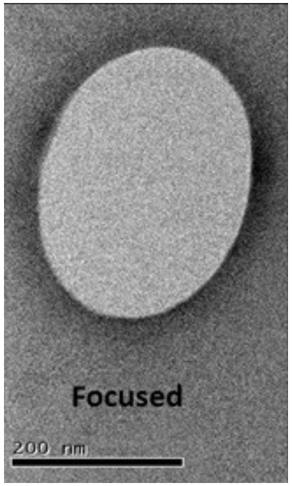
Specimen moves when tilted

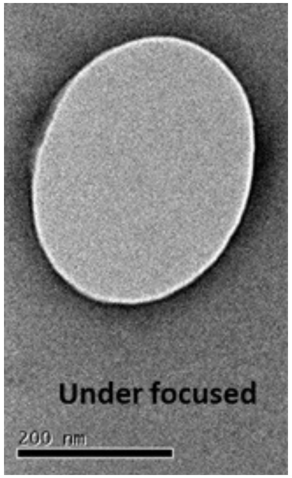
Change specimen Z-height
Software or engineer required to align tilt axis with optical axis

Objective System - Focus









Modulating objective lens current changes clarity of image (focus)

If crossover above image plane - overfocused

If crossover below image plane - underfocused

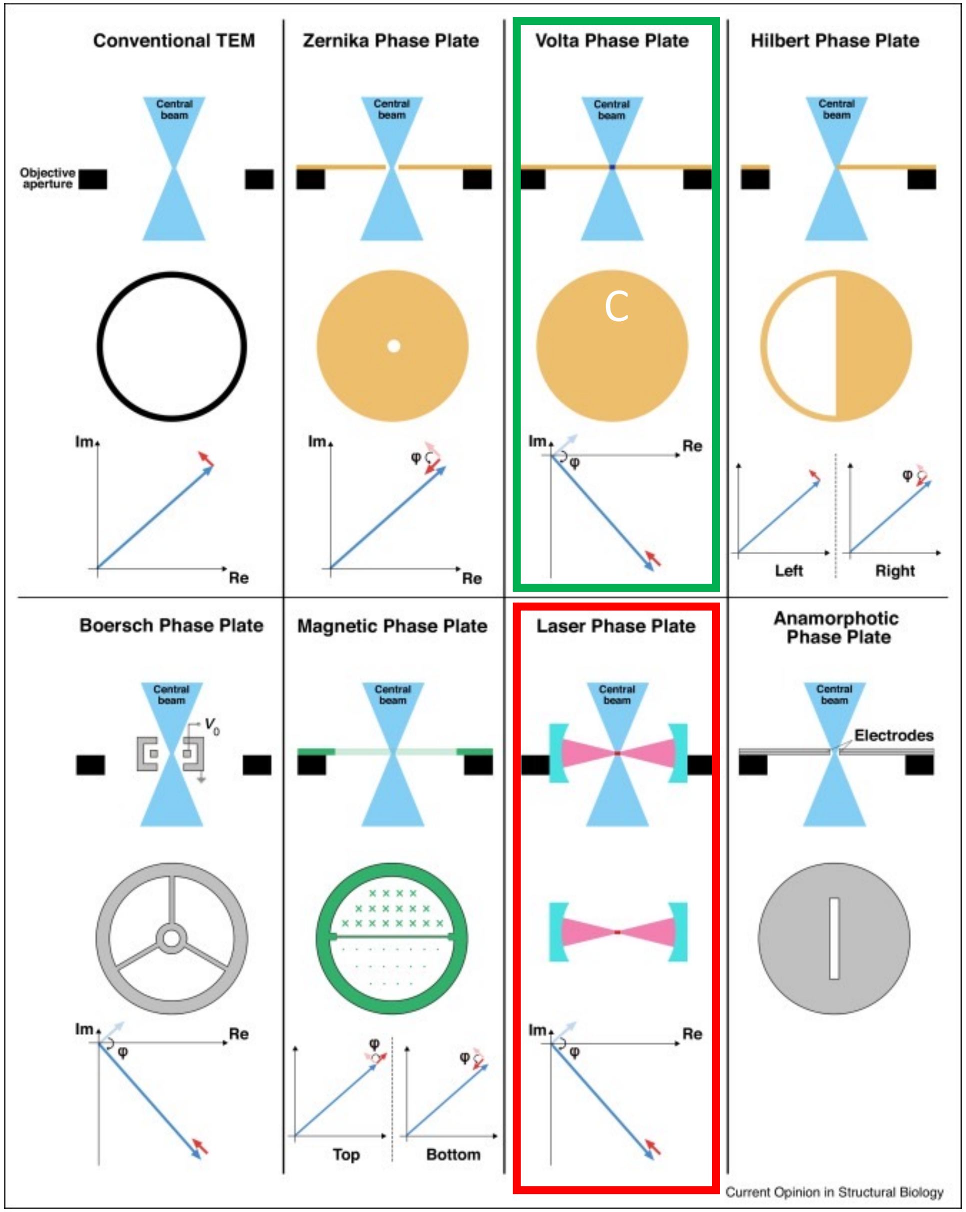
Biological samples contain light atoms so minimal phase shifts occurs between scattered and unscattered rays

→ At focus, very little contrast

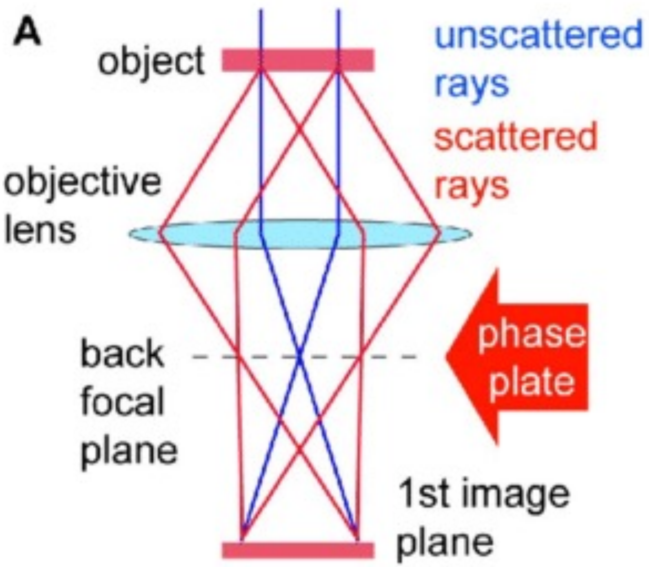
To see sample, either image underfocus or use phase plate

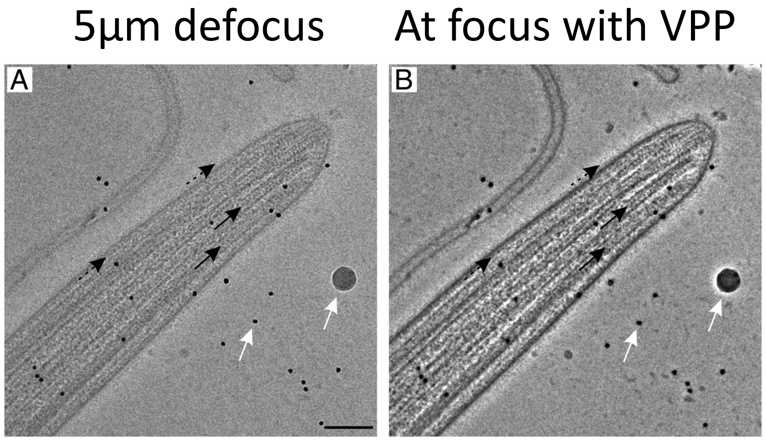
Imaging underfocus = CTF issues

Objective System - Phase Plates



Current Opinion in Structural Biology

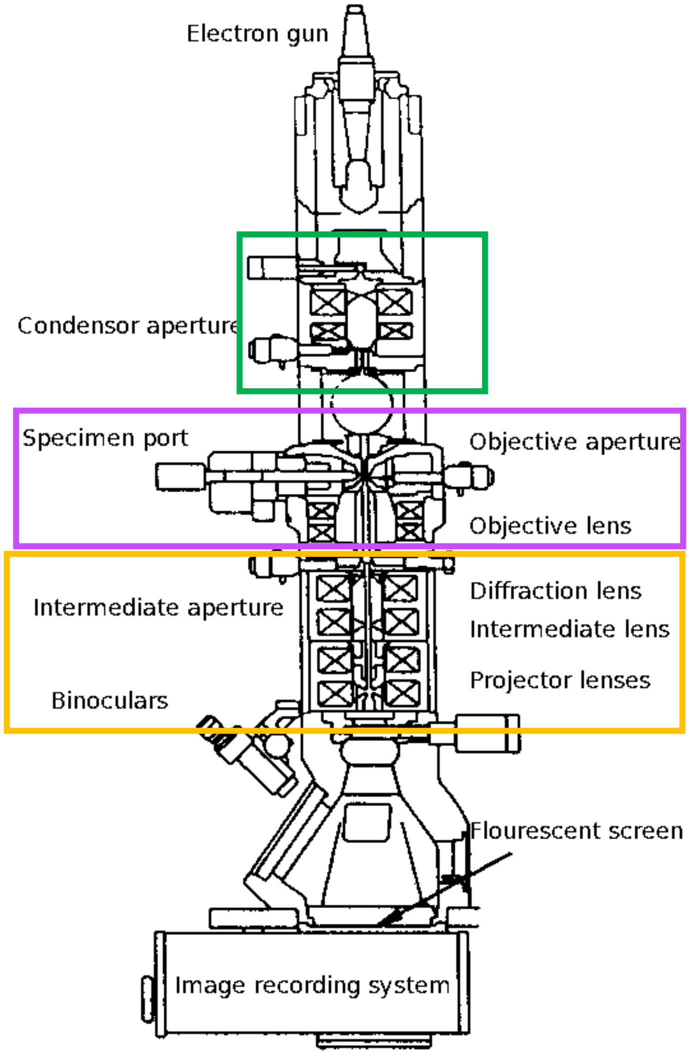




5µm defocus

At focus with VPP

Projection System



Condenser system

Objective system

Projector system

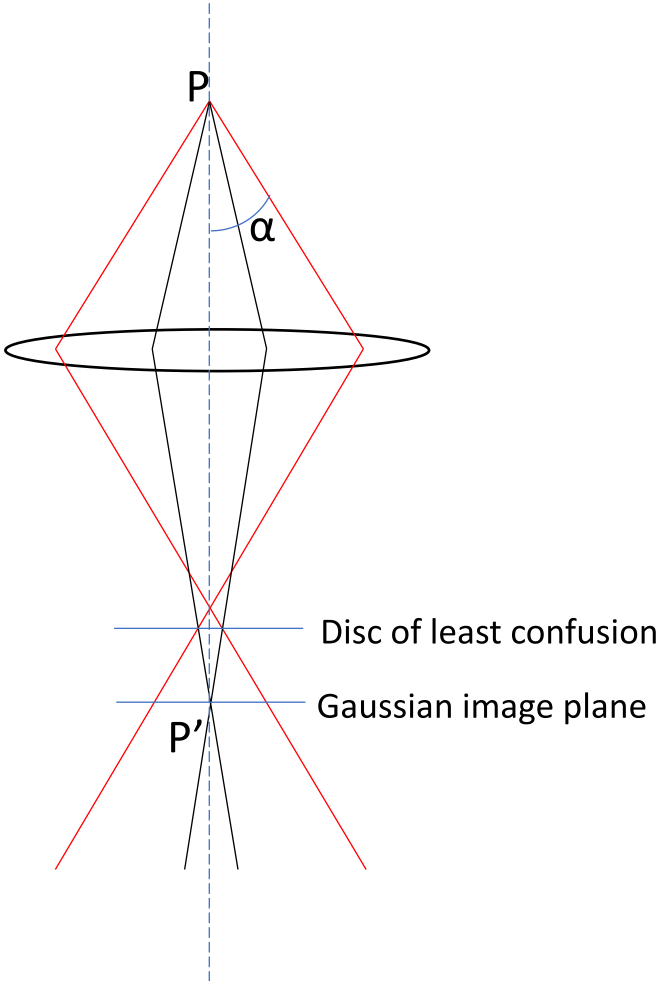
- Bunch of low strength lenses to increase magnification
- Tend to be ignored as aberrations outweighed by objective lens

Aberration Correctors

Magnetic lenses are poor quality and have severe aberrations:

Spherical aberration (position)
Chromatic aberration (wavelength)
Coma (angle of entry)
Stigmation – defects in magnetic field symmetry

Spherical Aberration (Cs) – Cs Corrector



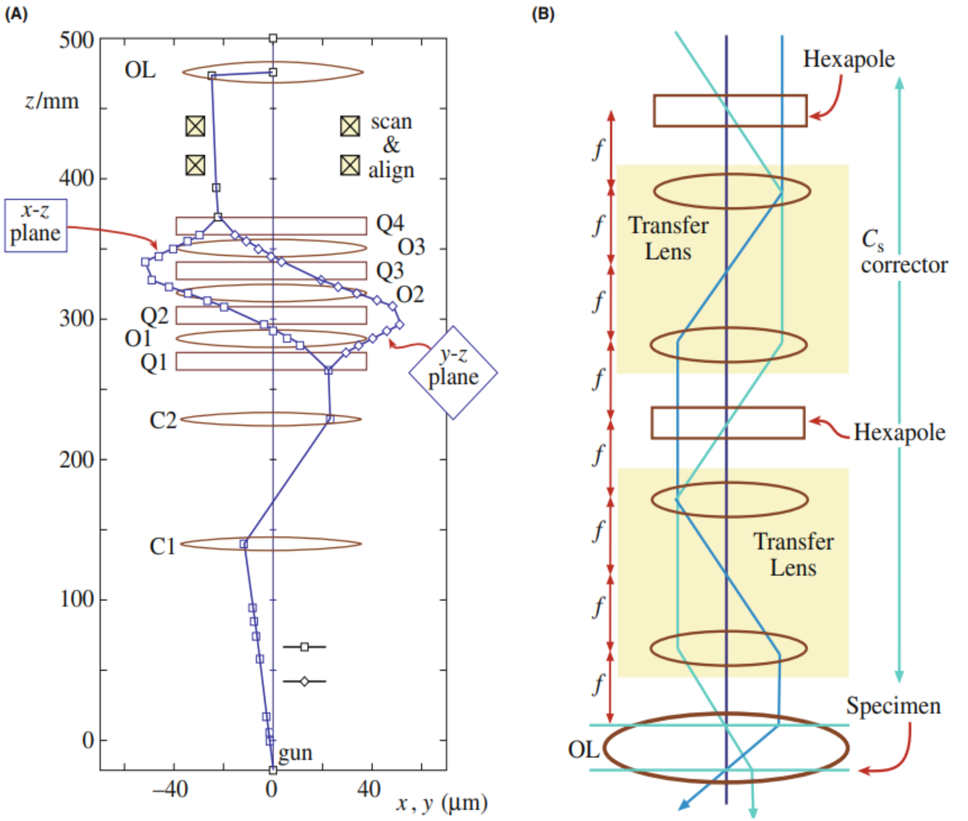


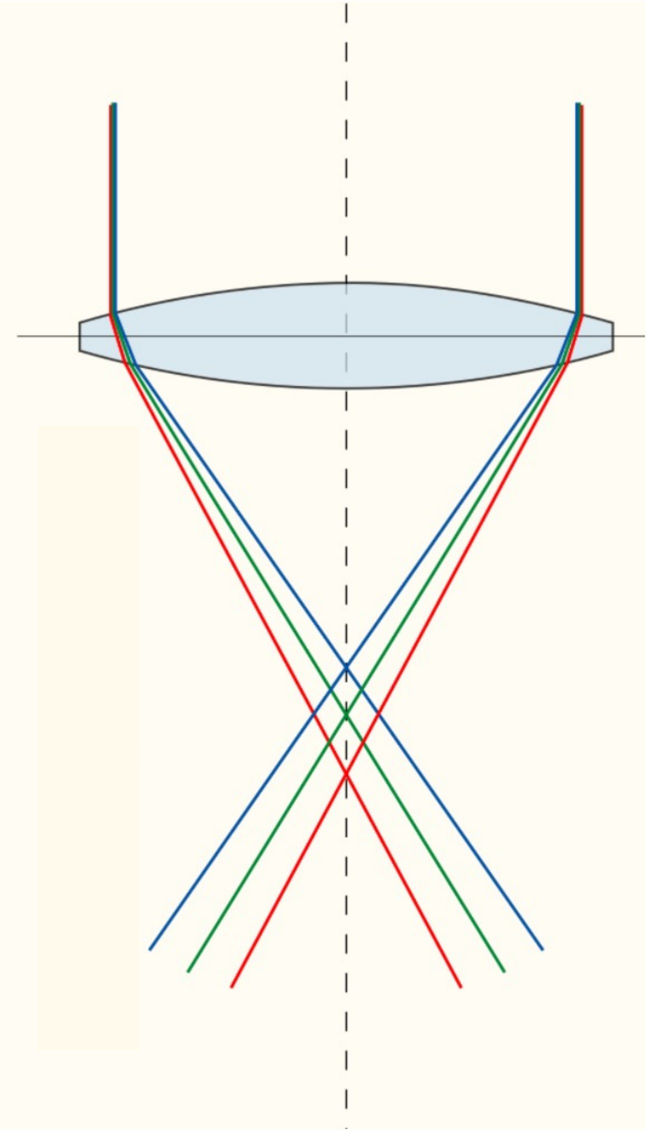
FIGURE 6.12. Ray diagrams showing how the two different commercial systems use (A) multiple quadrupole (Q) and octupole (O) lenses (Nion) or (B) hexapole and other transfer lenses (CEOS) to correct for C_s.

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

To reduce Cs:

- Increase lens strength
- Decrease pole gap
- Install Cs corrector (important for imaging < 0.5 Å)

Chromatic Aberration (Cc)



Electrons of different wavelengths are focused at different point

Cause of wavelength variation:

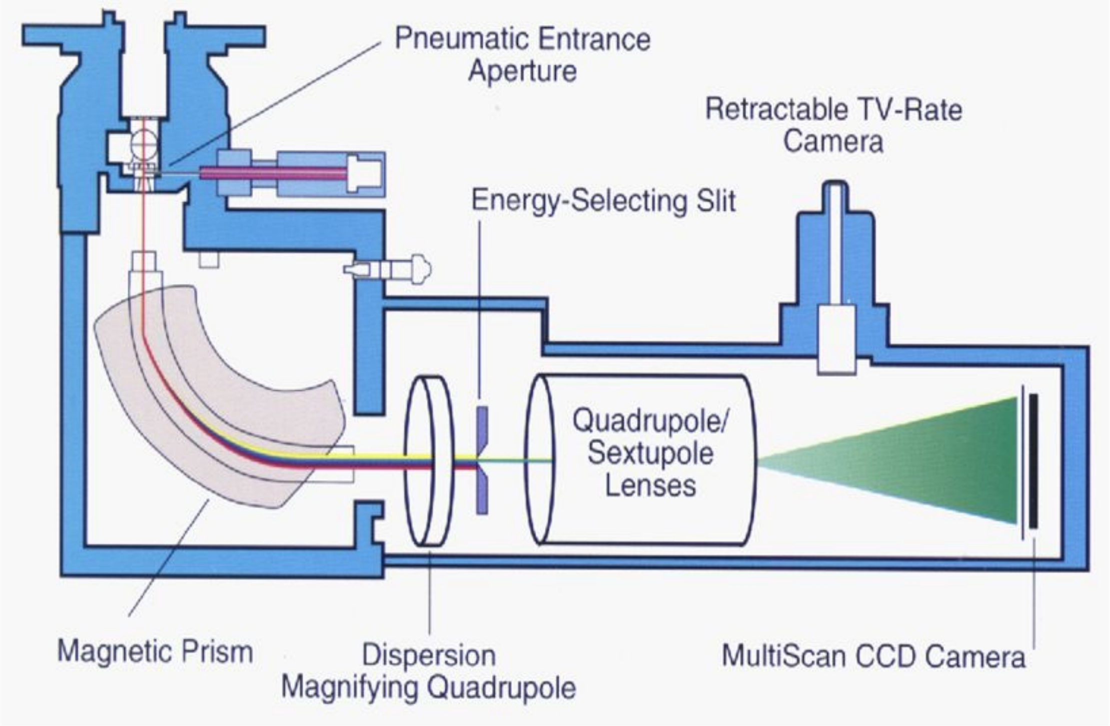
- Electron source
- Interaction with sample
  - (especially thick samples)

Correct using:

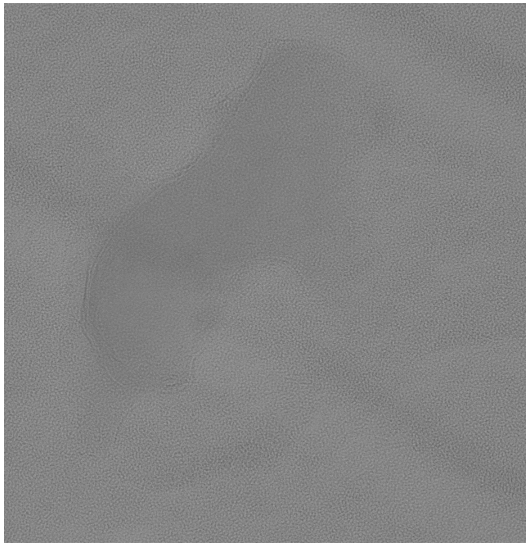
- FEG instead of Tungsten
- Monochromator after gun
- Energy filter after sample imaging

(Important for thick samples)

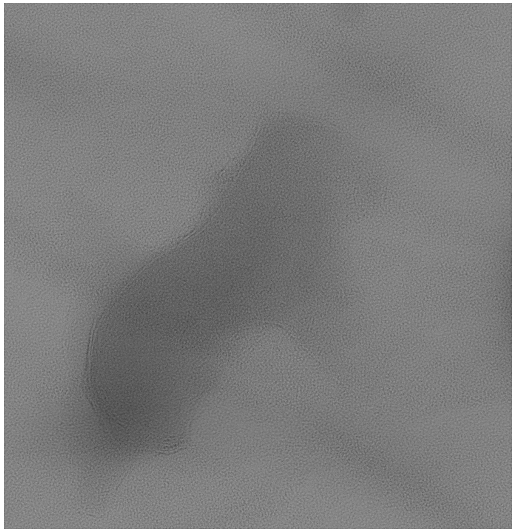
Energy Filter



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

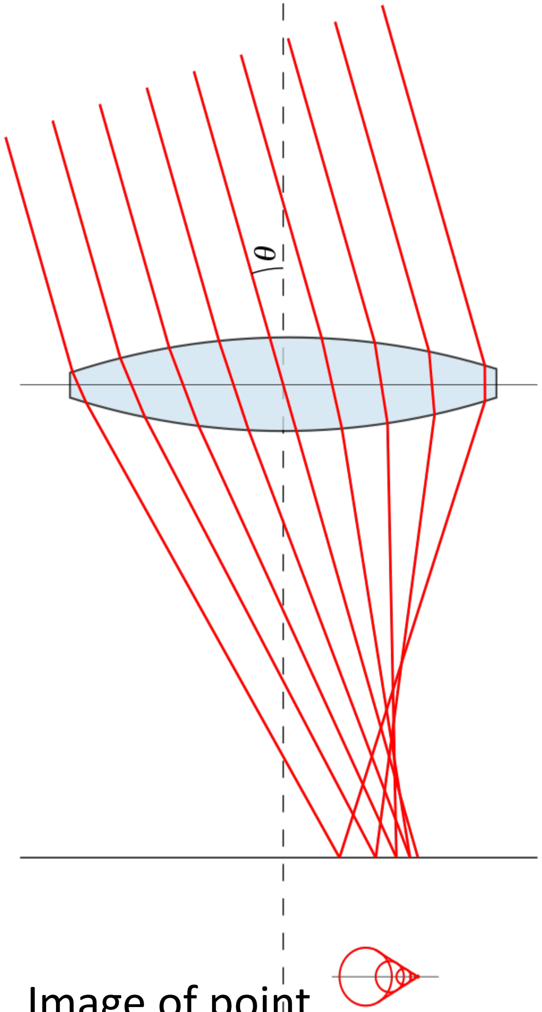
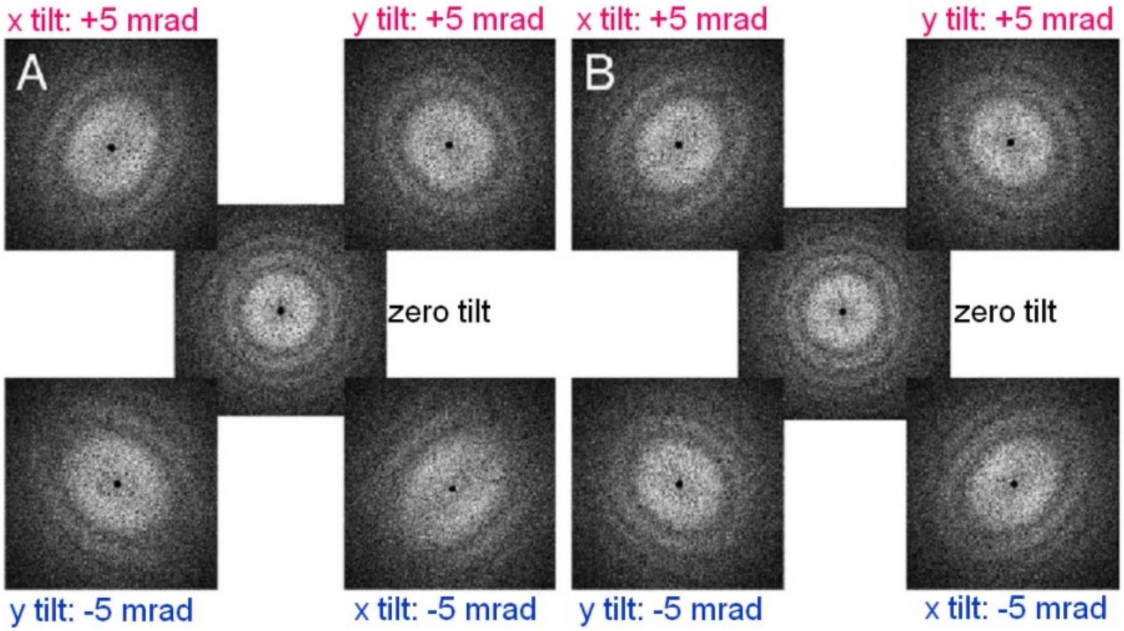


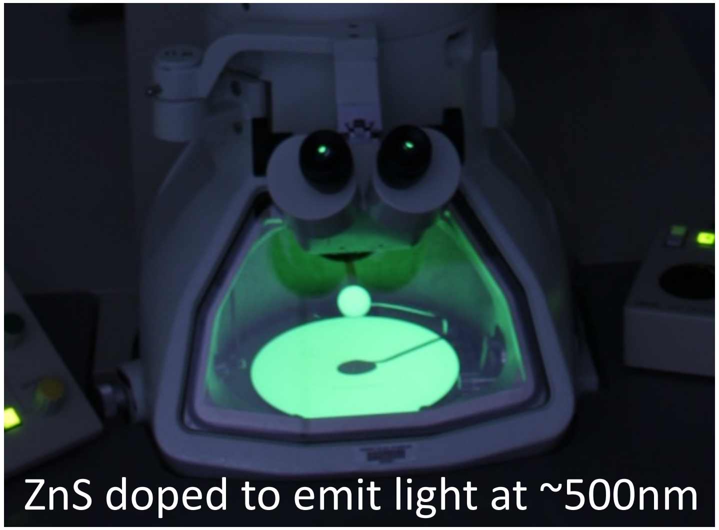
Image of point looks like a comet



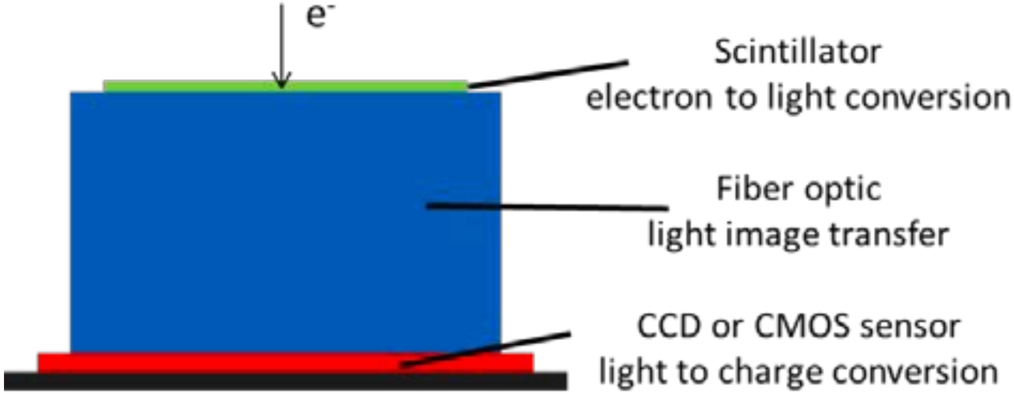
(Important to obtain resolutions beyond 5 Å)

Detection System

Fluorescent Screen


ZnS doped to emit light at ~500nm

Charge coupled device (CCD)



Scintillator
electron to light conversion

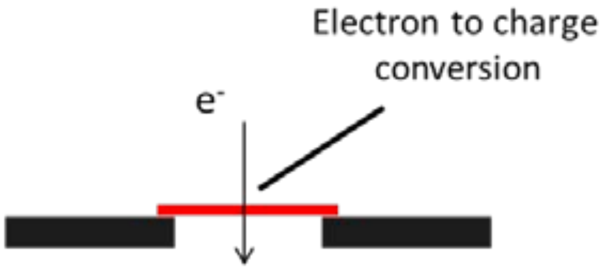
Fiber optic
light image transfer

CCD or CMOS sensor
light to charge conversion

Film + Scanner

Direct Electron Detectors (DEDs)

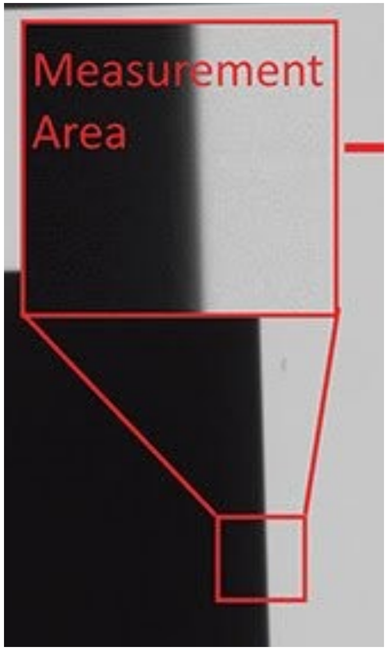
(e.g. K3/Falcon)



Electron to charge
conversion

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

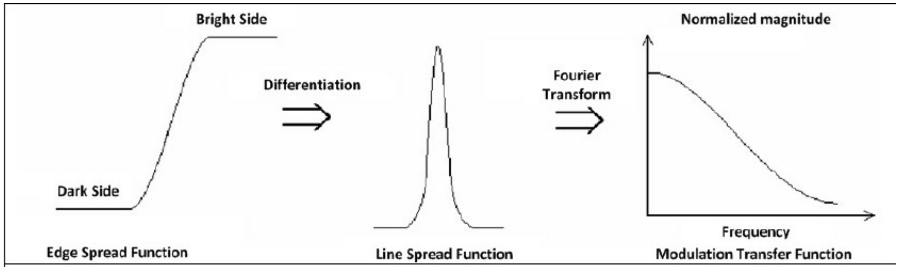
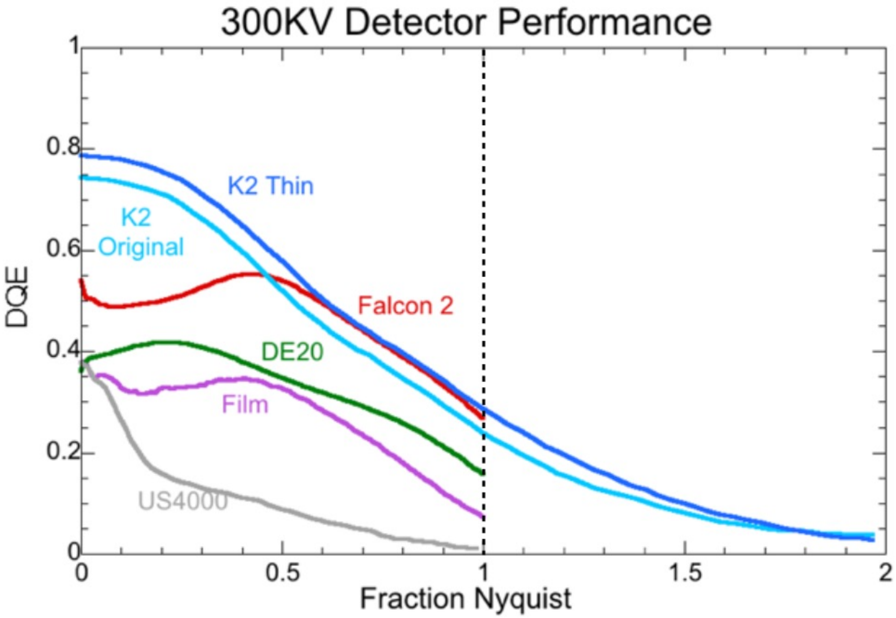


Figure 3. Computation of the Modulation Transfer Function using the knife-edge target.

Detection Quantum Efficiency (DQE)

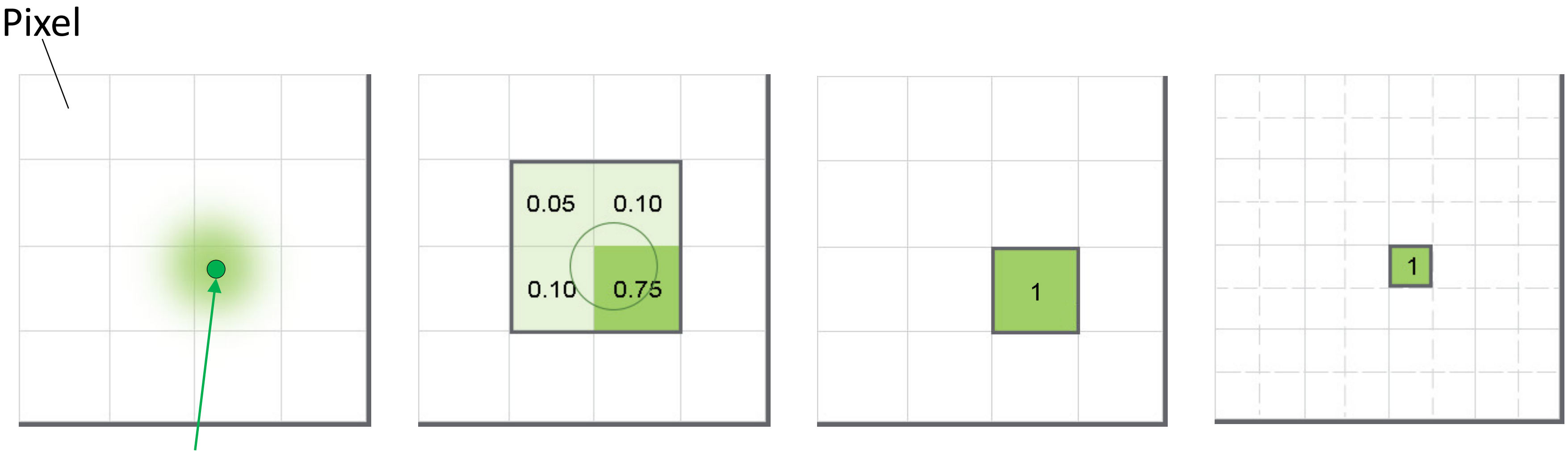


$$DQE = \frac{SN_{out}^2}{SN_{in}^2}$$

DQE = 1, excellent camera, no loss of signal

Nyquist = 1/2px

Integrated vs Counted (super-resolution) Mode



Incoming Electron

Integration mode

Counting mode

Super-resolution

Electron hits pixel array and causes charge spread

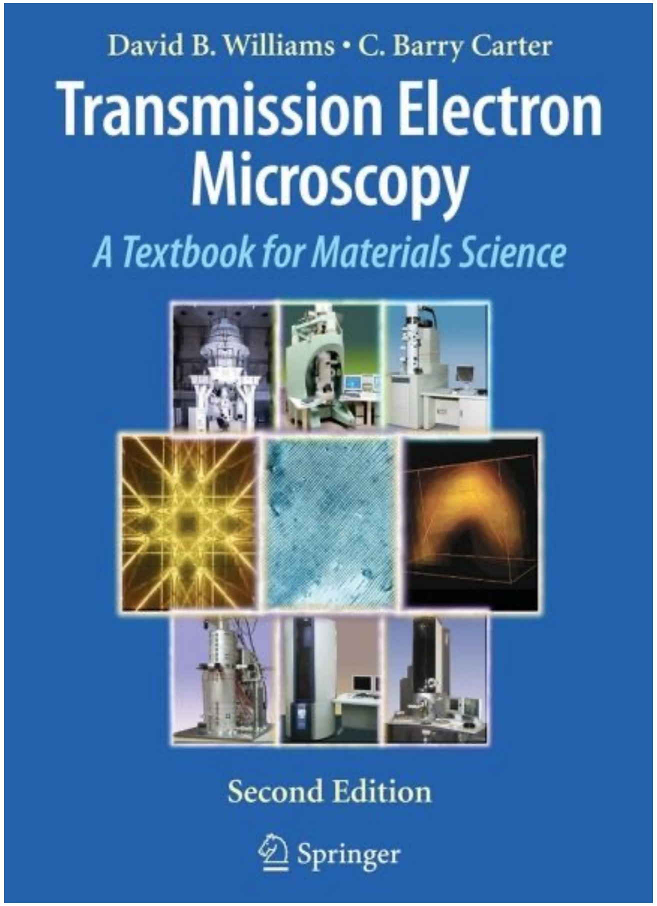
Integrating mode reads out accumulated charge in each pixel

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

Subdivides pixel to give more accurate location of electron hit.

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

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

1.7. Electron Optics — Introduction to Transmisison Electron Microscopy (gduscher.github.io)