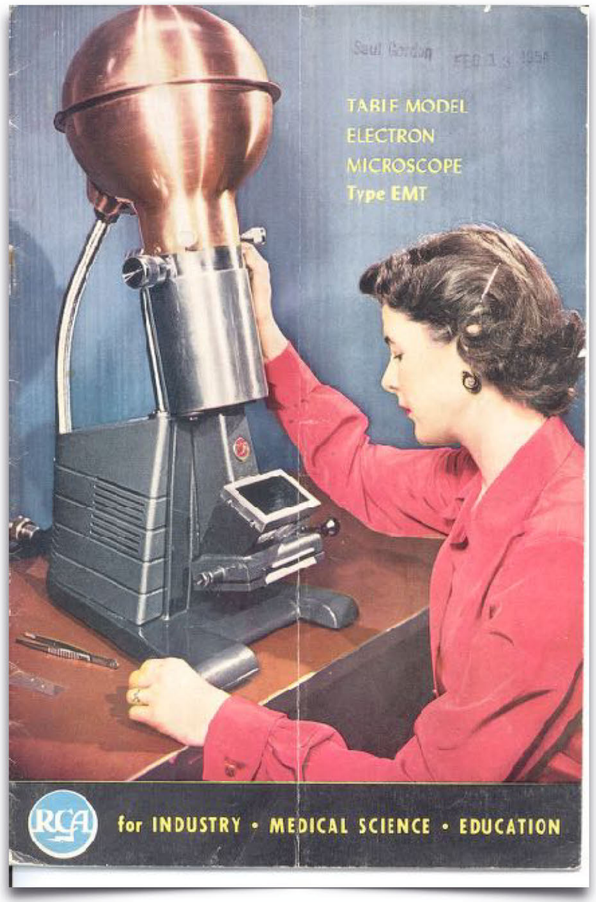
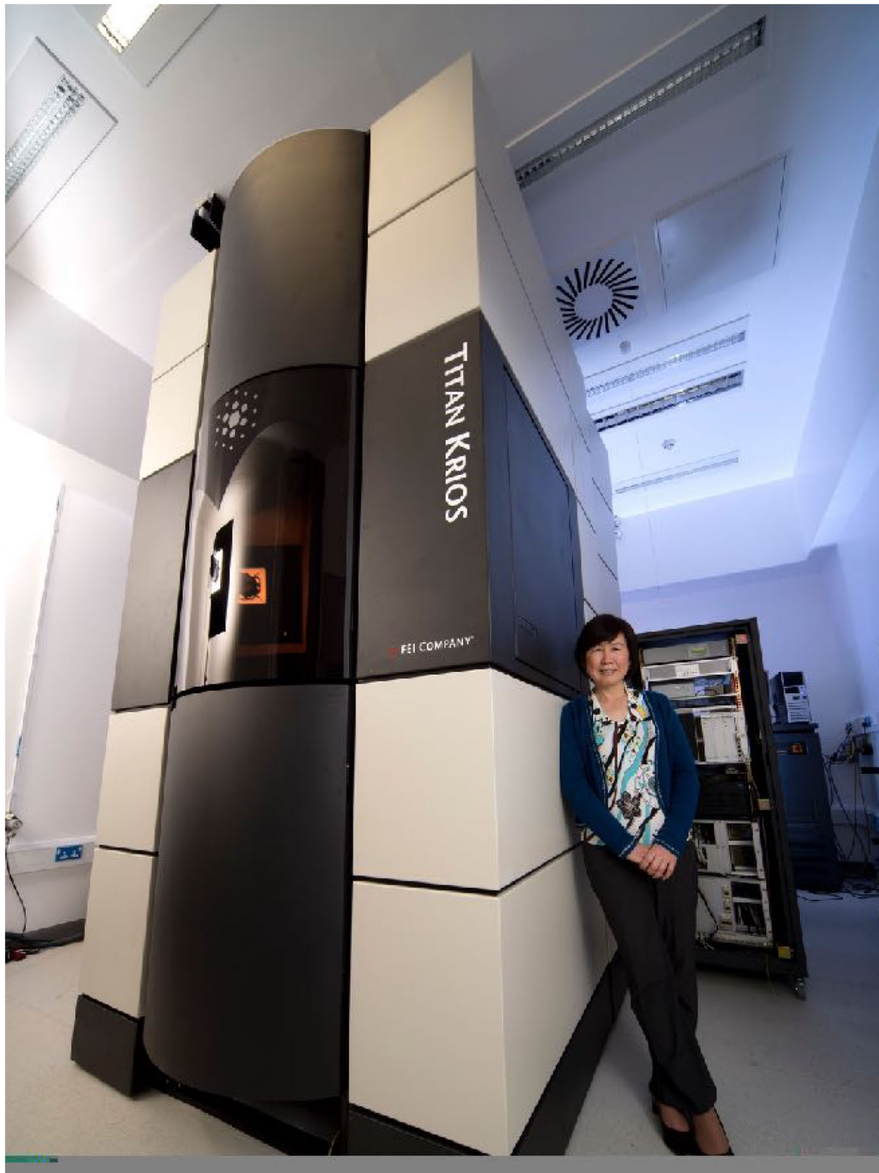
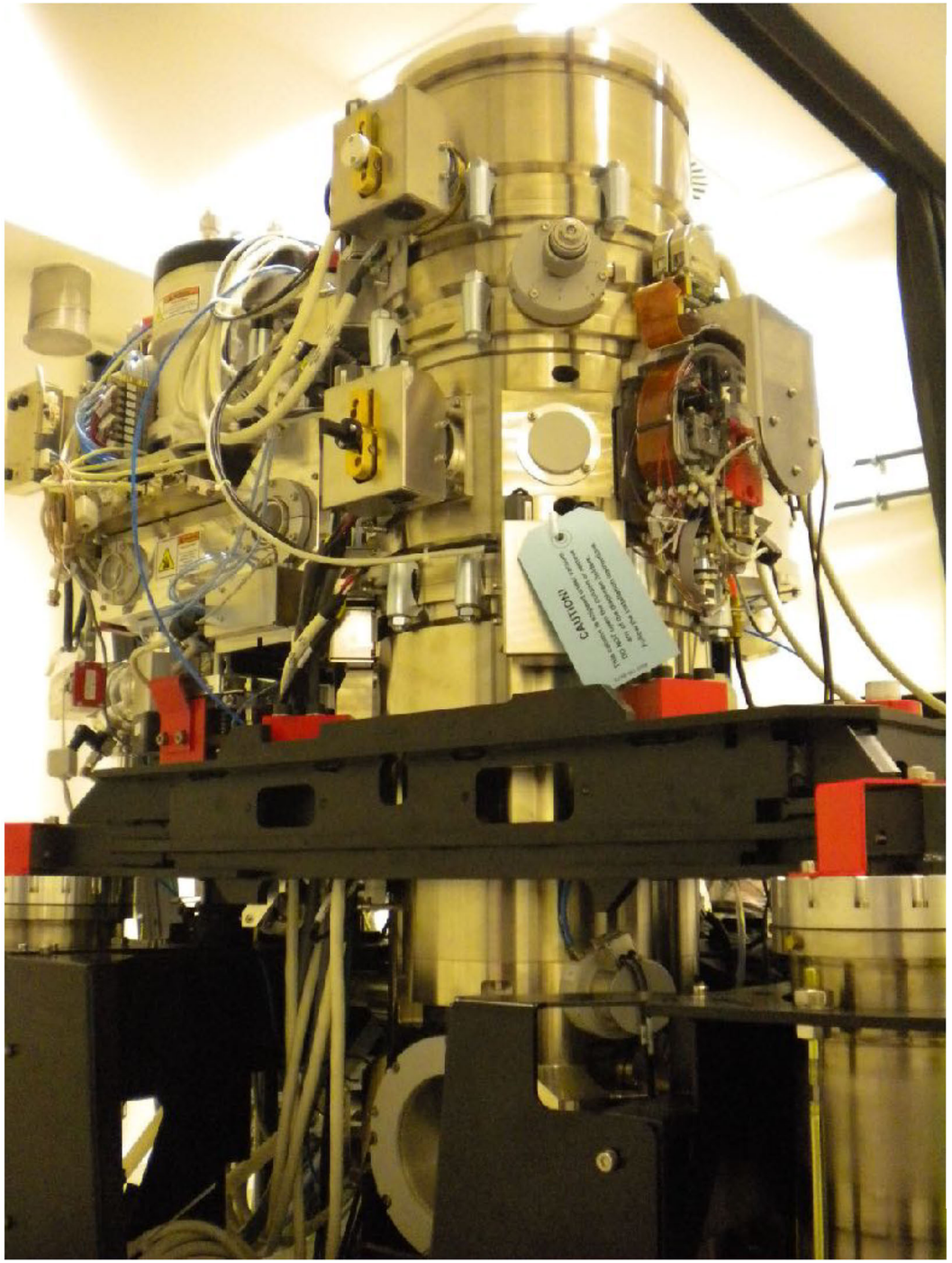
Electron microscope physics & optics

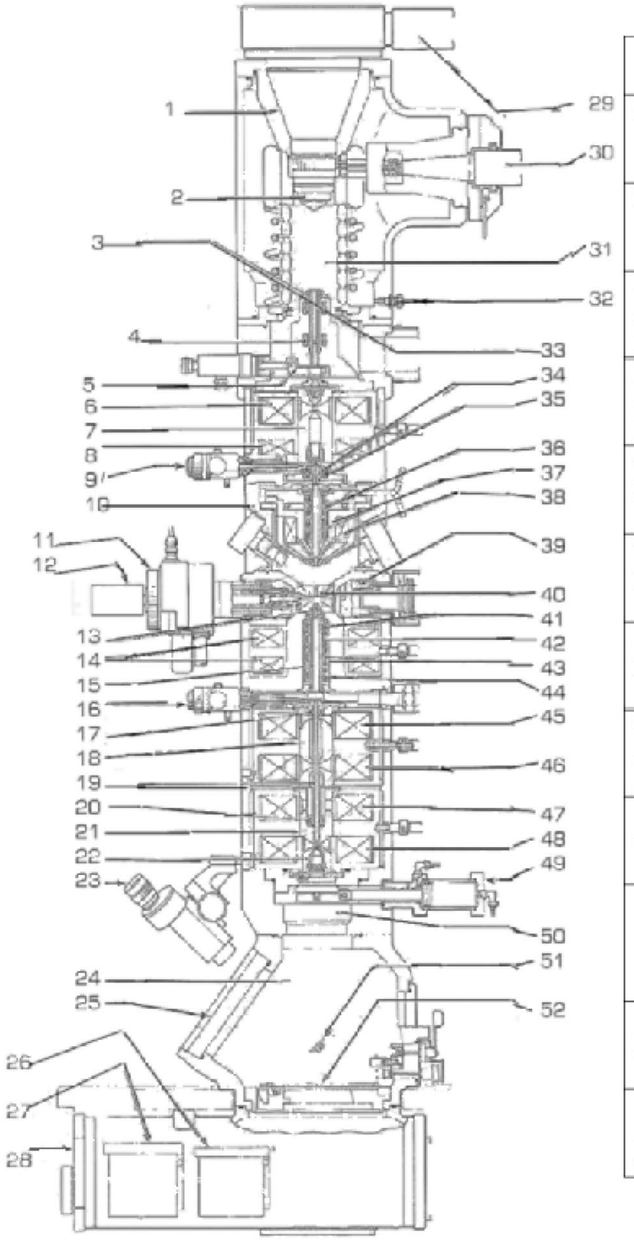
Chris Russo





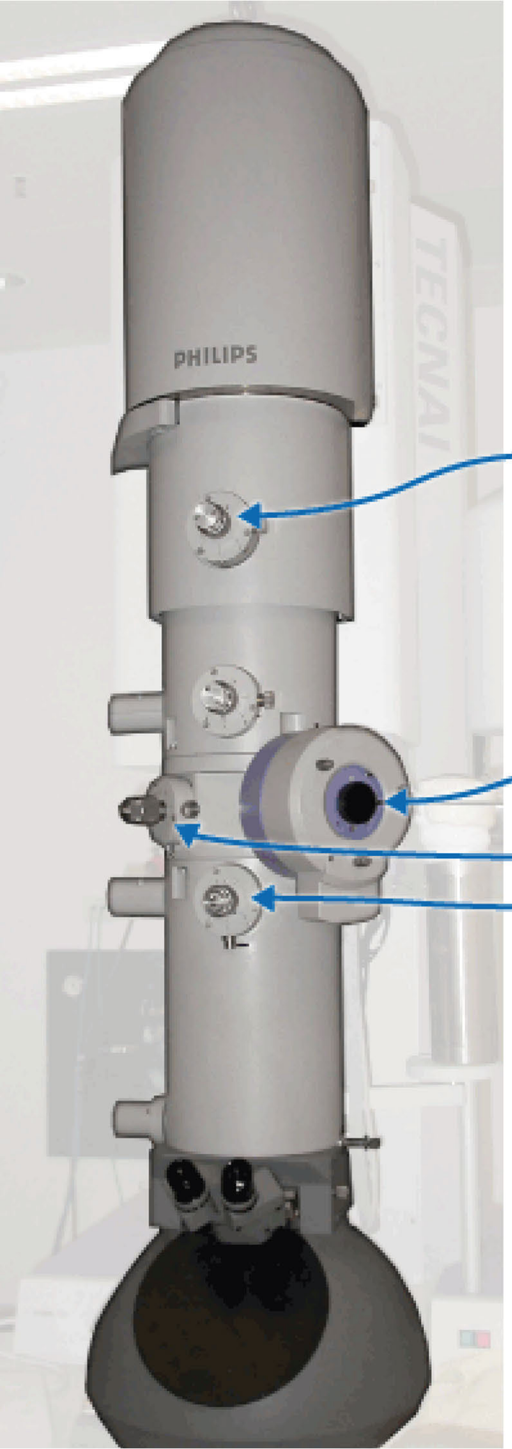
MRC Laboratory of Molecular Biology





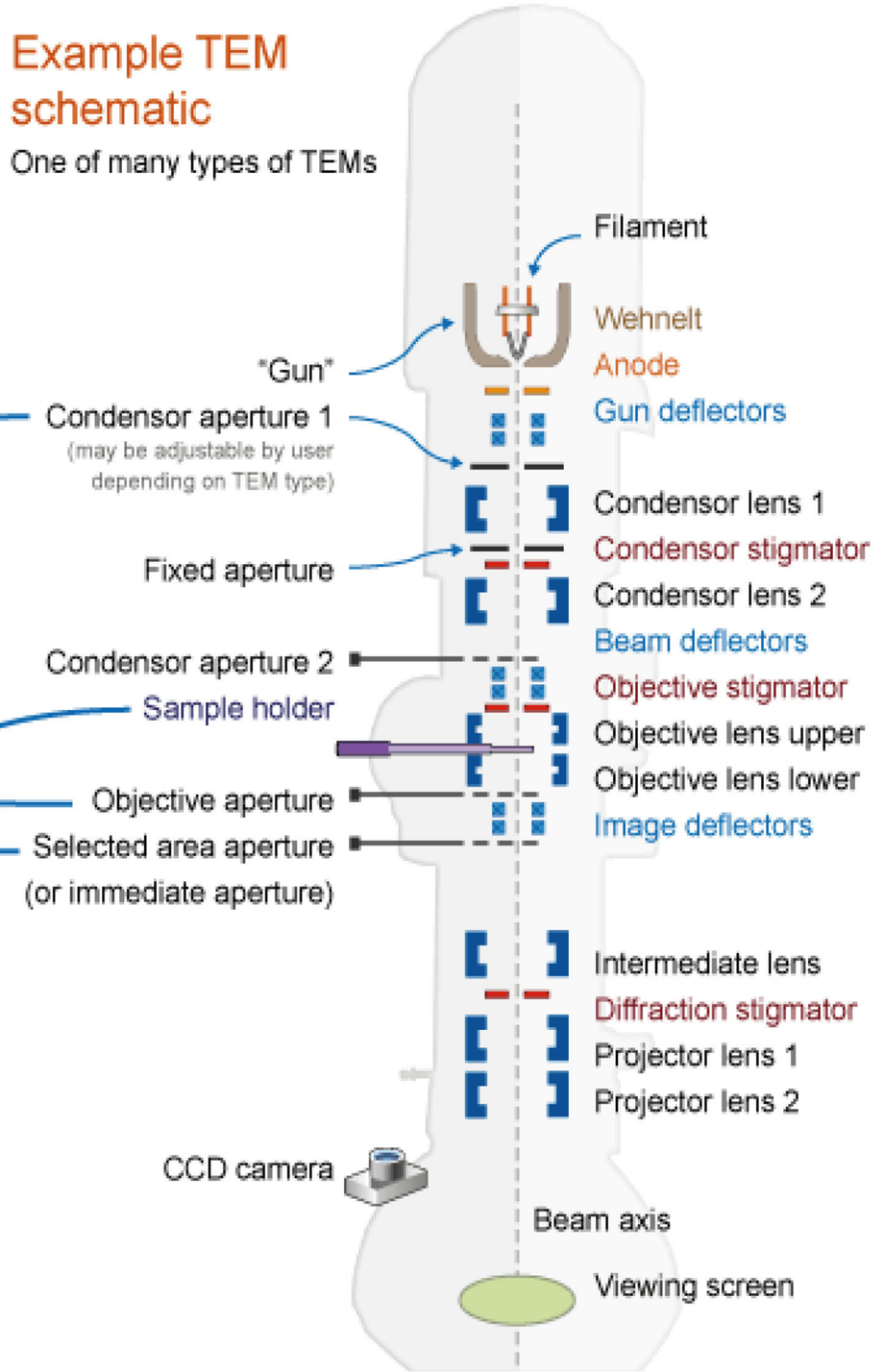
1. Electron Gun	14. Objective Lens Coil	27. Receiving Magazine	40. Objective Polepiece
2. Wehnelt Unit	15. Objective Lens Liner Tube	28. Camera Chamber	41. Objective Lens Stigmator Coil
3. Anode	16. Field Limiting Aperture	29. Lift Arm	42. 1st Image Shift Coil
4. Electron Gun Second Beam Delector Coil	17. Intermediate Lens Stigmator Coil	30. HT Cable	43. Objective Minilens(OM Lens) Coil
5. Anode Chamber Isolation Valve	18. Intermediate Polepiece	31. Anode Chamber	44. 2nd Image Shift Coil
6. 1st Condenser Lens Coil	19. Intermediate Lens Linear Tube	32. Gas Inlet	45. 1st Intermediate Lens Coil
7. Condenser Polepiece	20. Projector Lens Beam Deflector Coil	33. Electron Gun 1st Beam Deflector Coil	46. 2nd Intermediate Lens Coil
8. 3rd Condenser Lens Coil	21. Projector Upper Polepiece	34. Condenser Lens Stigmator Coil	47. 3rd Intermediate Lens Coil
9. Condenser Aperture Assembly	22. Projector Lower Polepiece	35. Spot Alignment Coil	48. Projector Lens Coil
10. Specimen Chamber	23. Binoculars	36. Condenser Lens 1st Beam Deflector Coil	49. Viewing Chamber Isolation Valve
11. Goniometer	24. Viewing Chamber	37. Condenser Lens 2nd Beam Deflector Coil	50. High Resolution Diffraction Chamber
12. Specimen Holder	25. Viewing Window	38. Condenser Minilens(CM Lens) Coil	51. Small Screen
13. Stigmator Screening Cylinder	26. Dispensing Magazine	39. Stage Heater	52. Large Screen

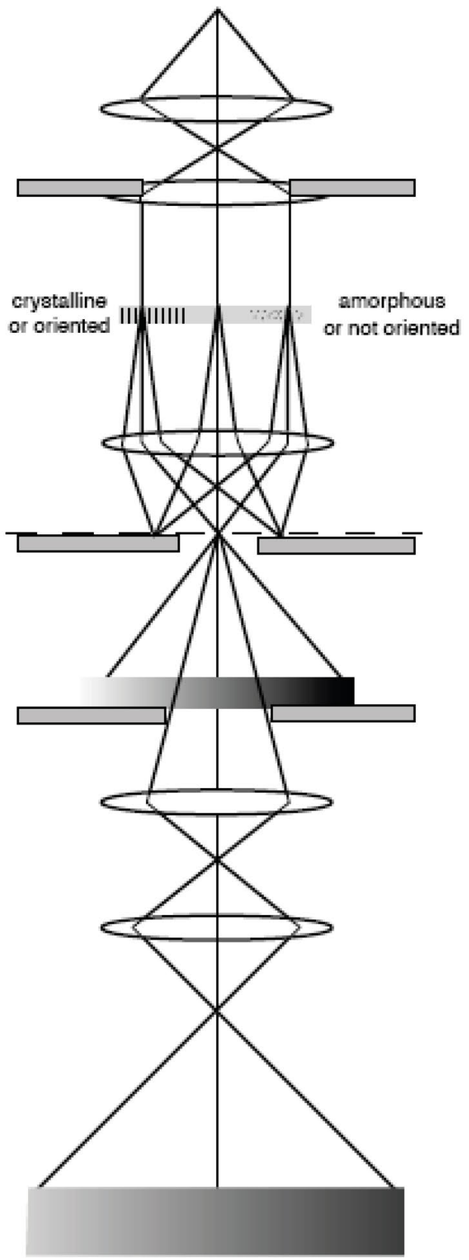
S. Chen



Example TEM schematic

One of many types of TEMs





anode

condenser lens 1

condenser lens 2
condenser diaphragm

sample

objective lens

objective diaphragm
(back focal plane)

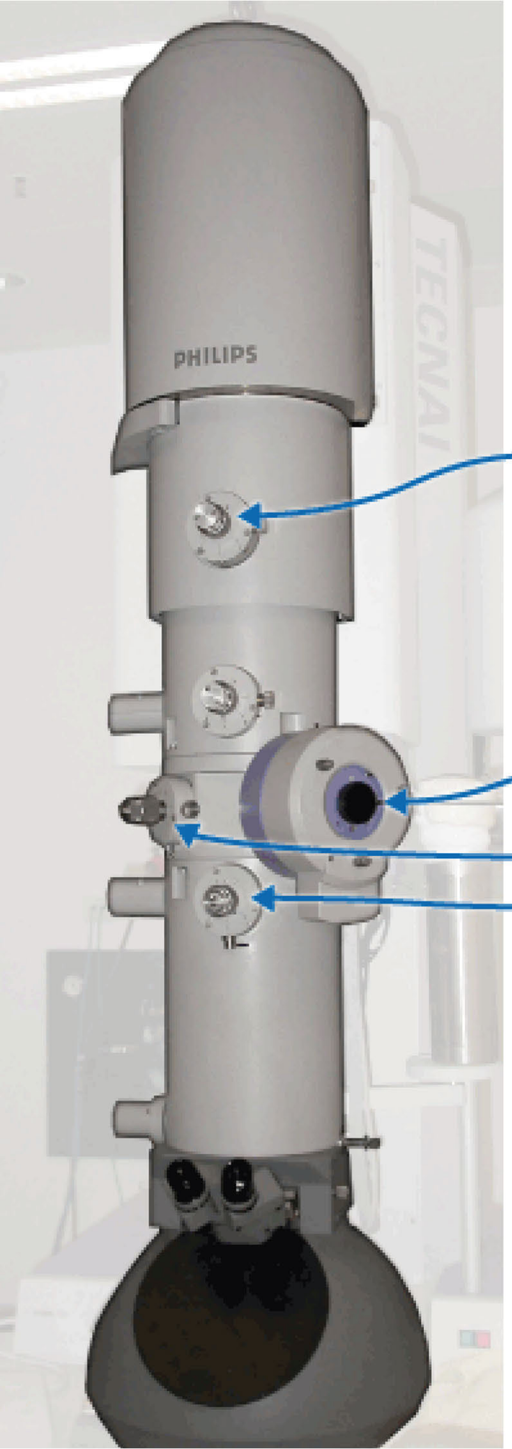
selected area diaphragm

intermediate lens

projective lens

final image

screen



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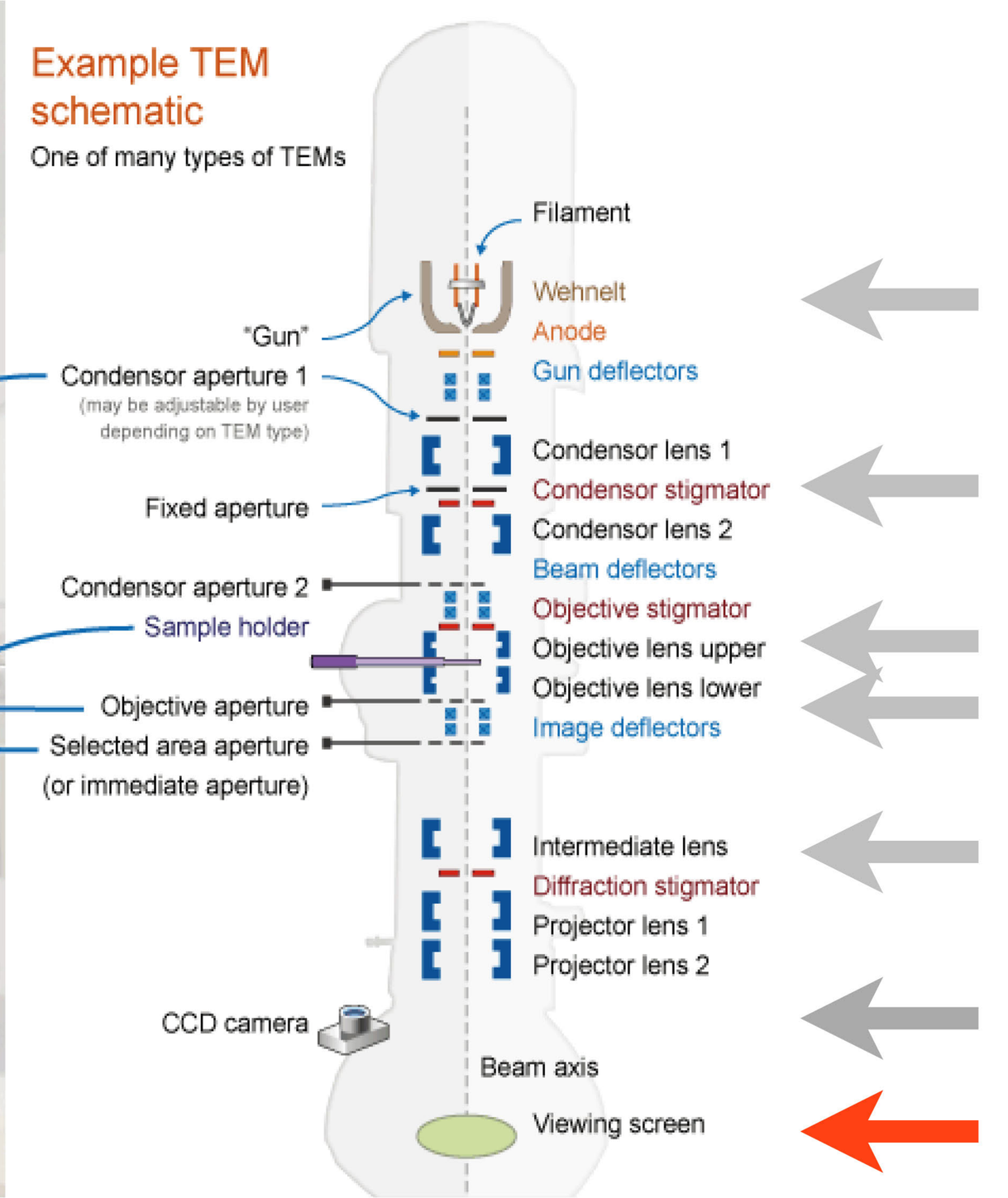

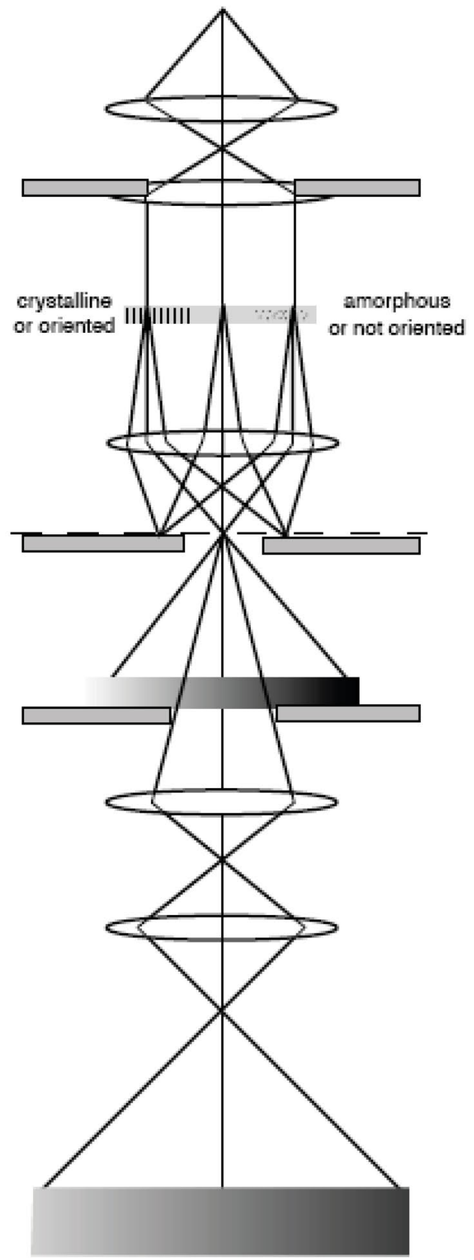

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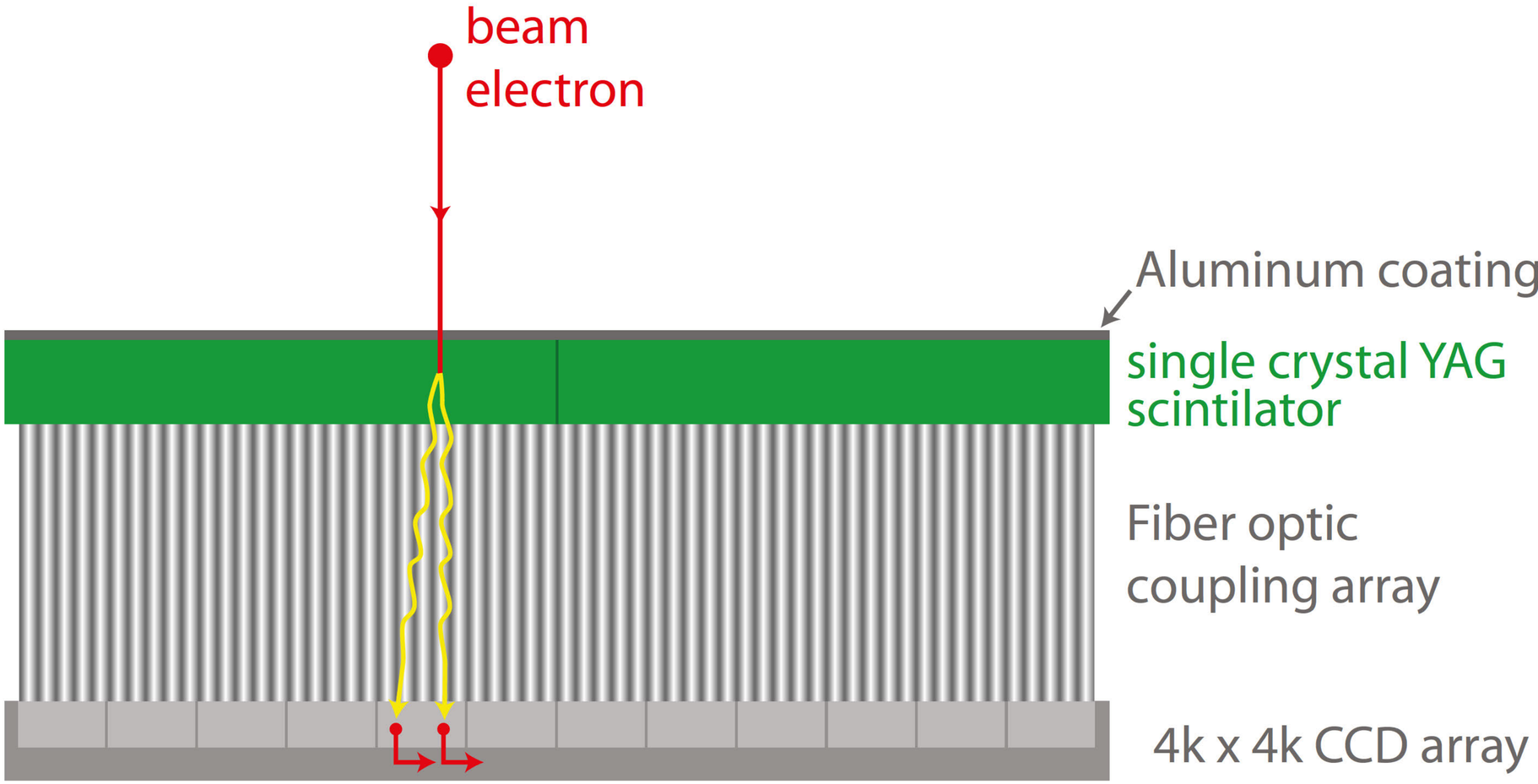
Silver

The original direct electron detector

reduce silver halides exposed to electrons to silver

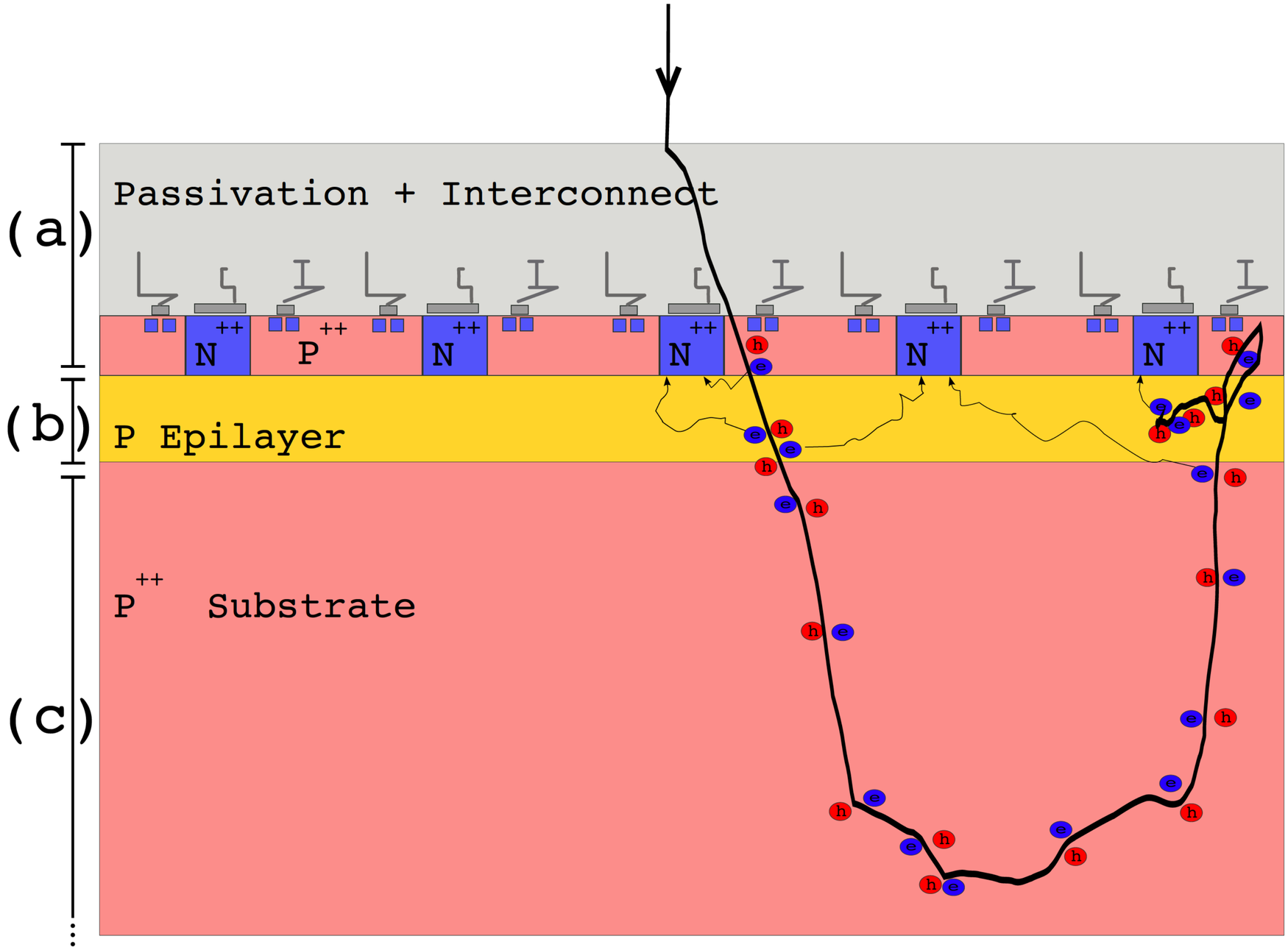
"Conventional" electron microscope camera



convert electrons to photons and back to electrons

Charge
Coupled
Device

Direct electron detector

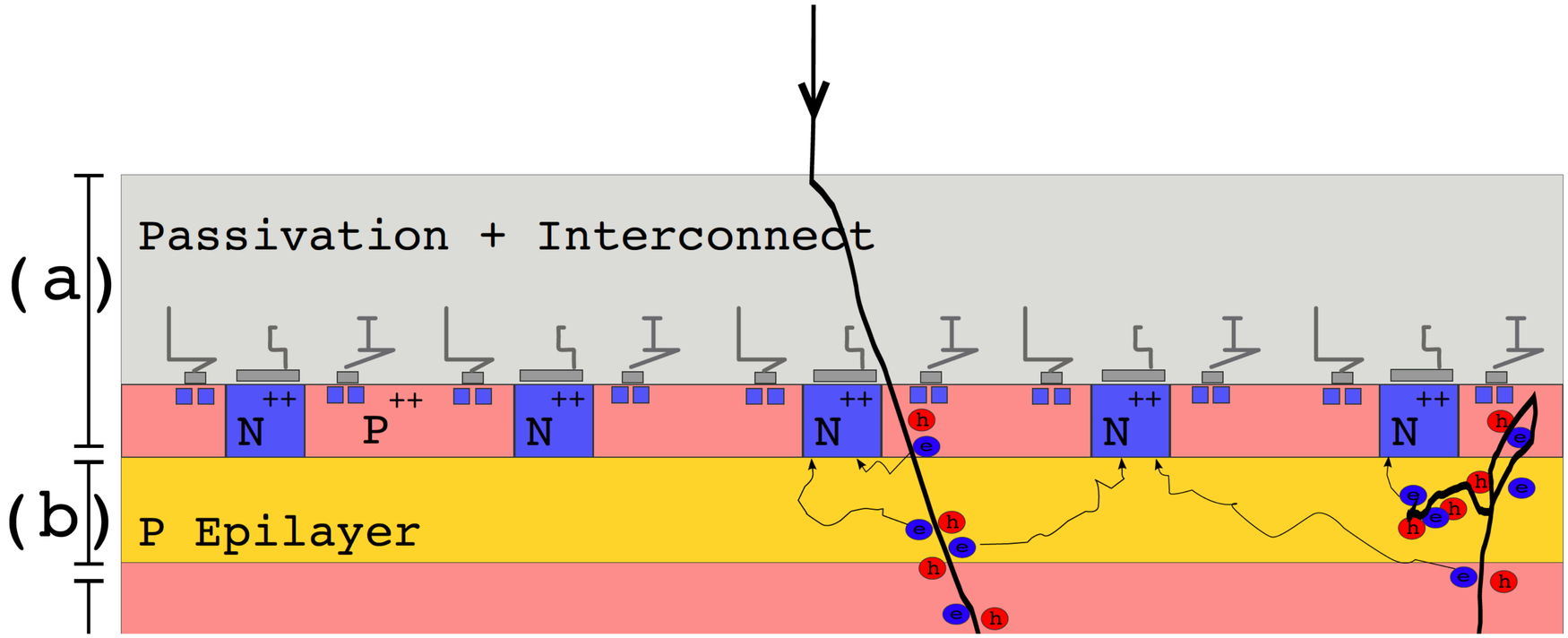


"CMOS detector"

Complementary
Metal
Oxide
Semiconductor

G. McMullan

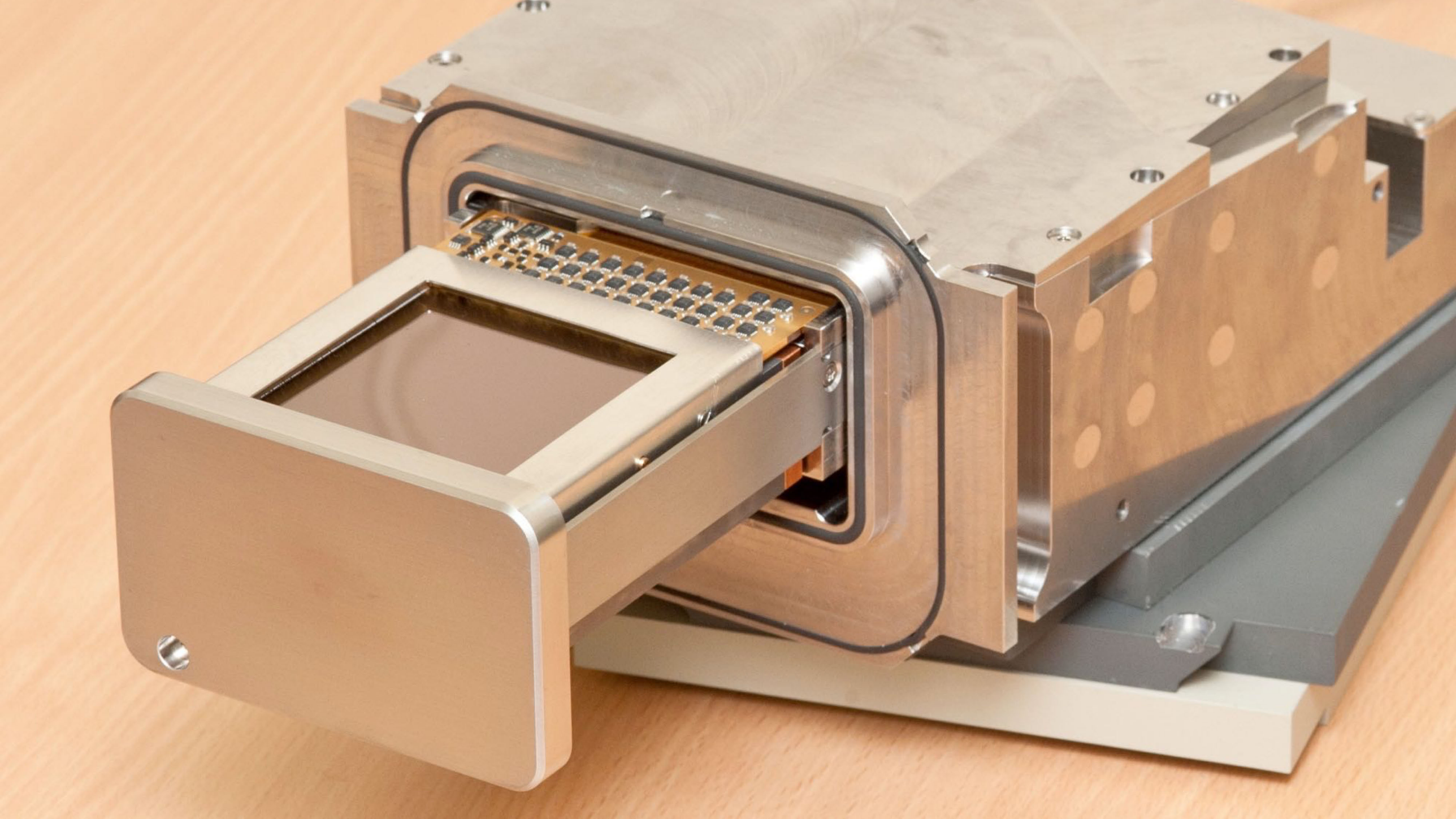
Direct electron detector



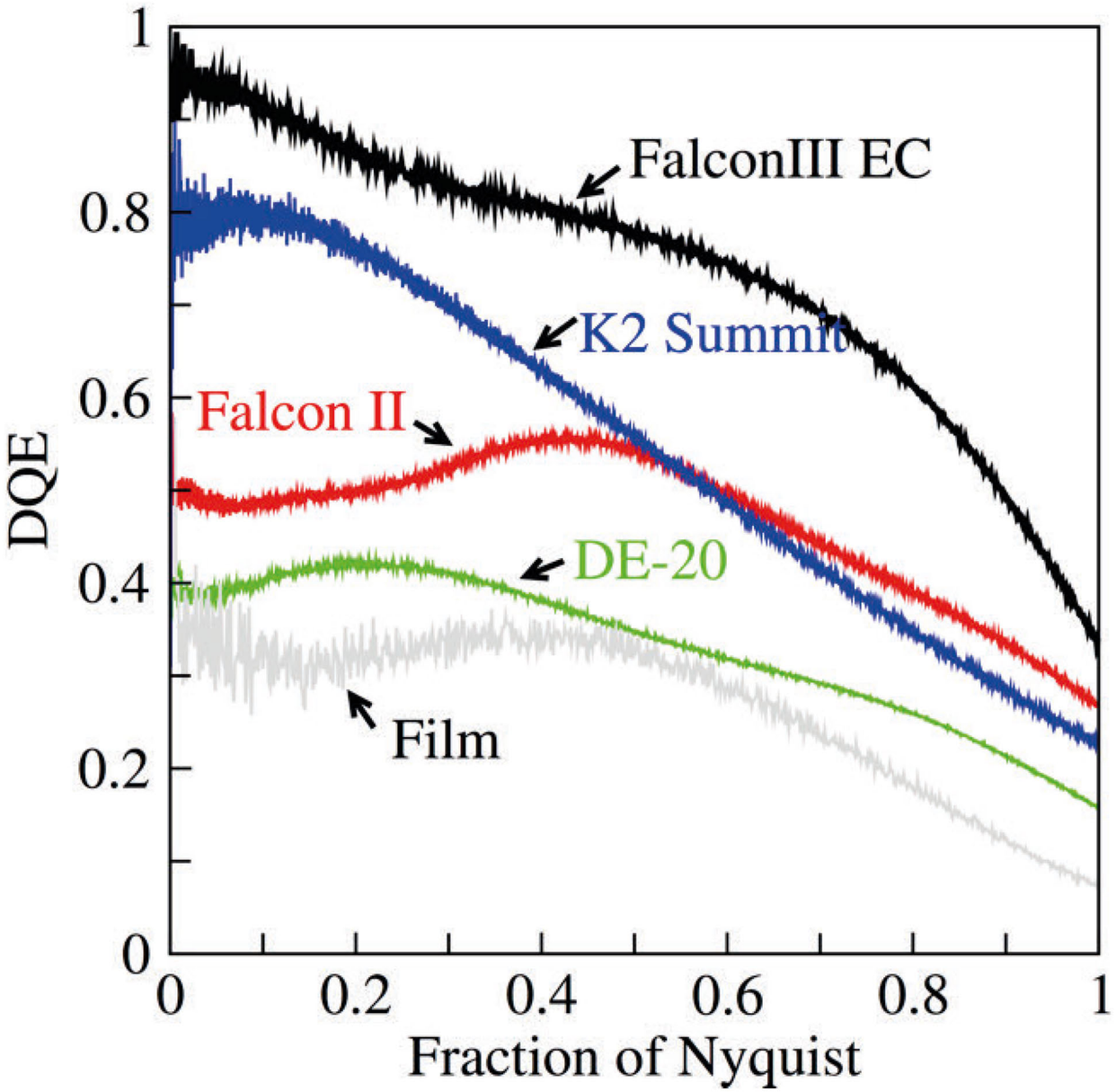
"Back-thinned"

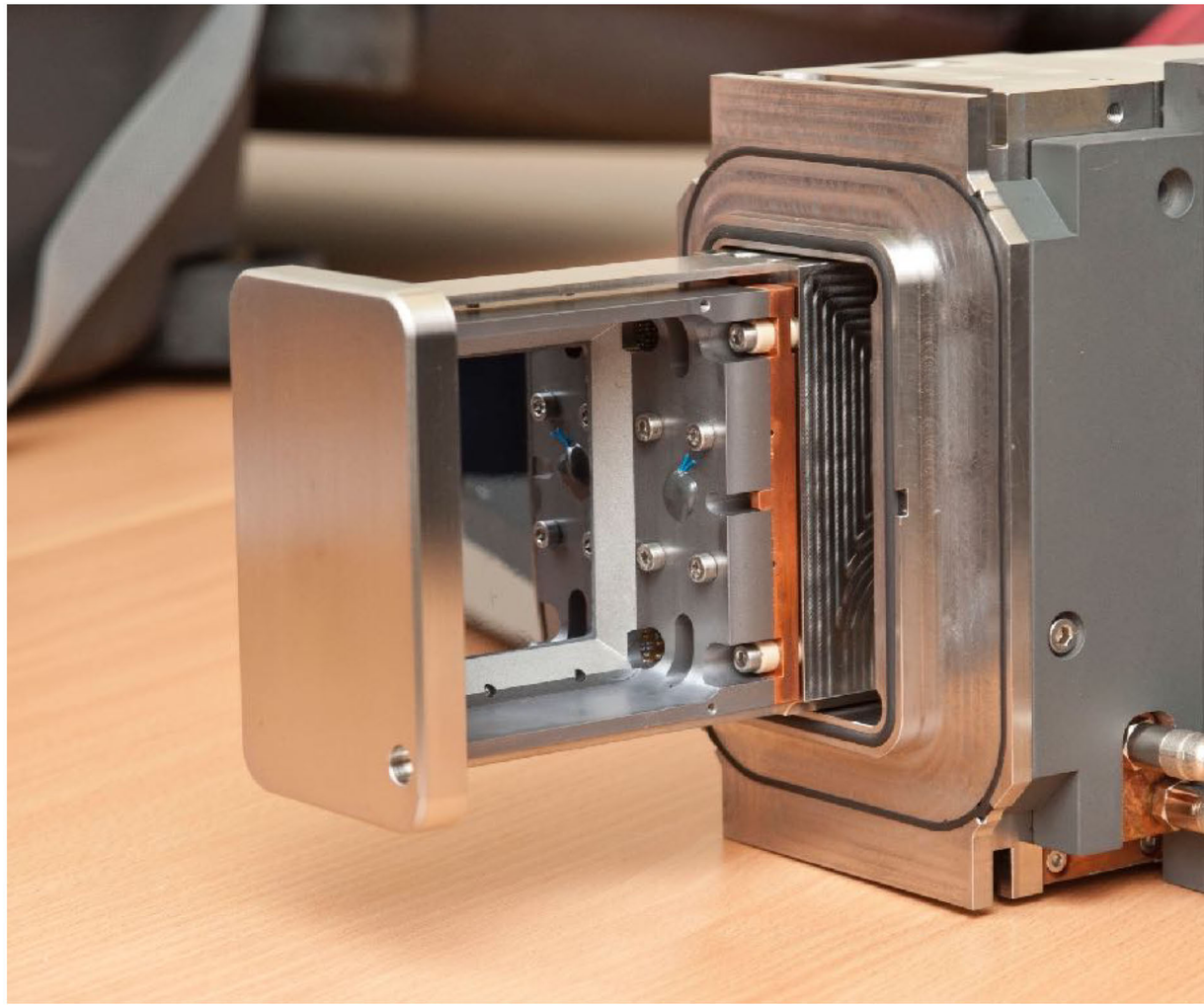
~500 → 50 → <30 μm

G. McMullan

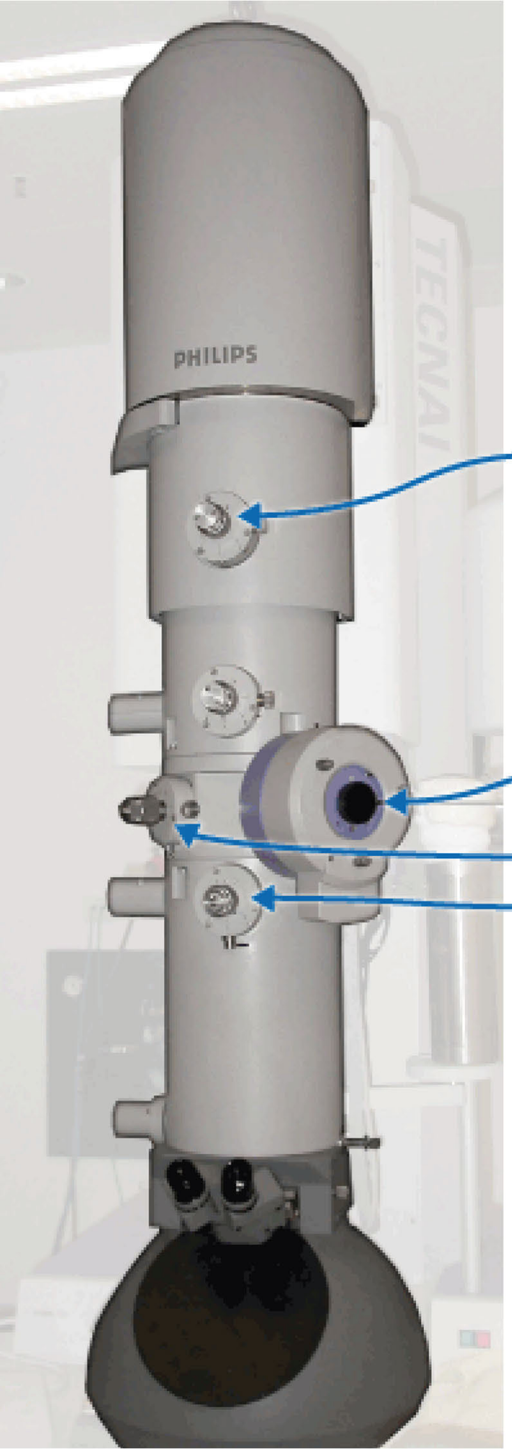


Detector quantum efficiency





G. McMullan



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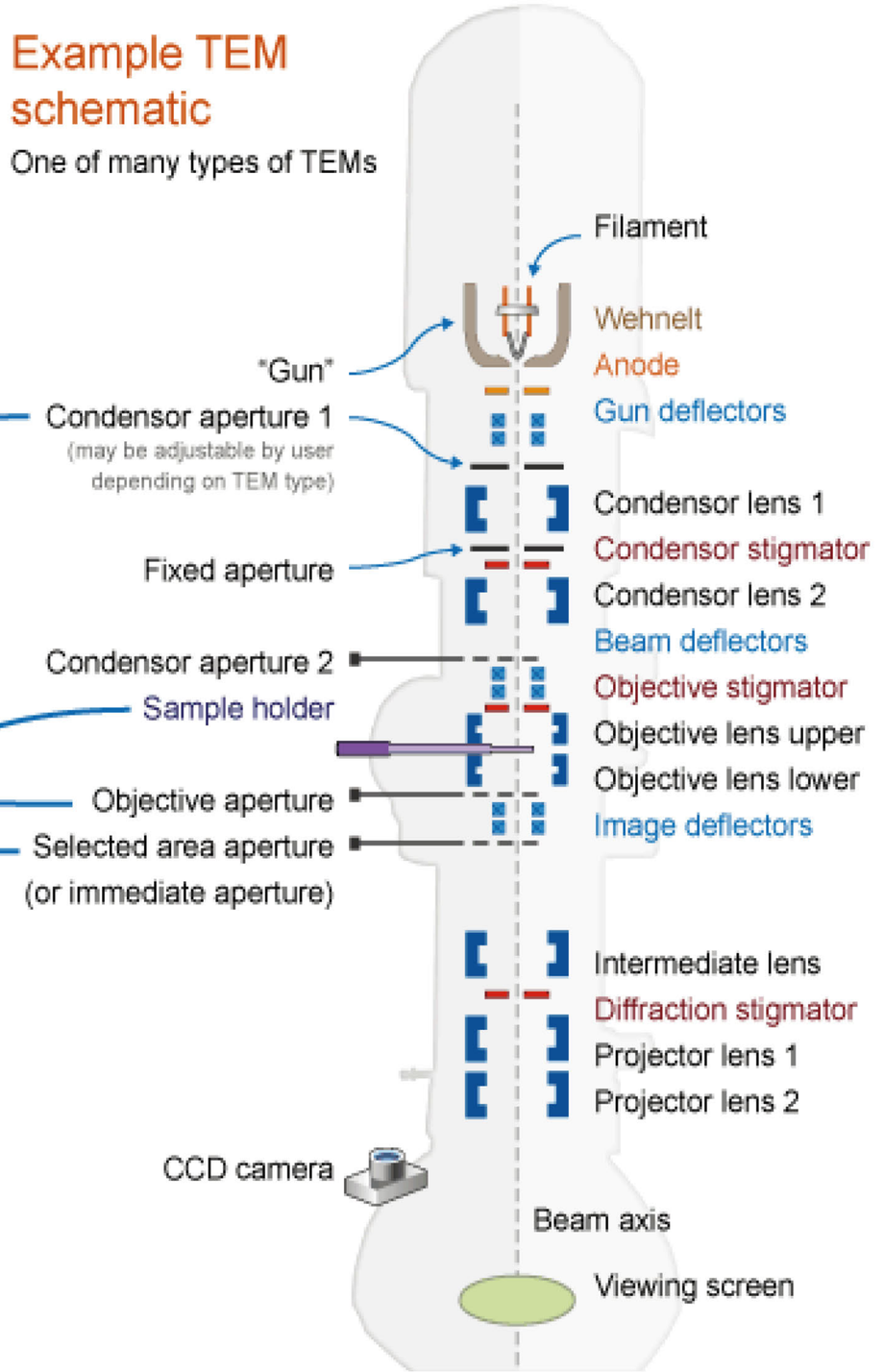
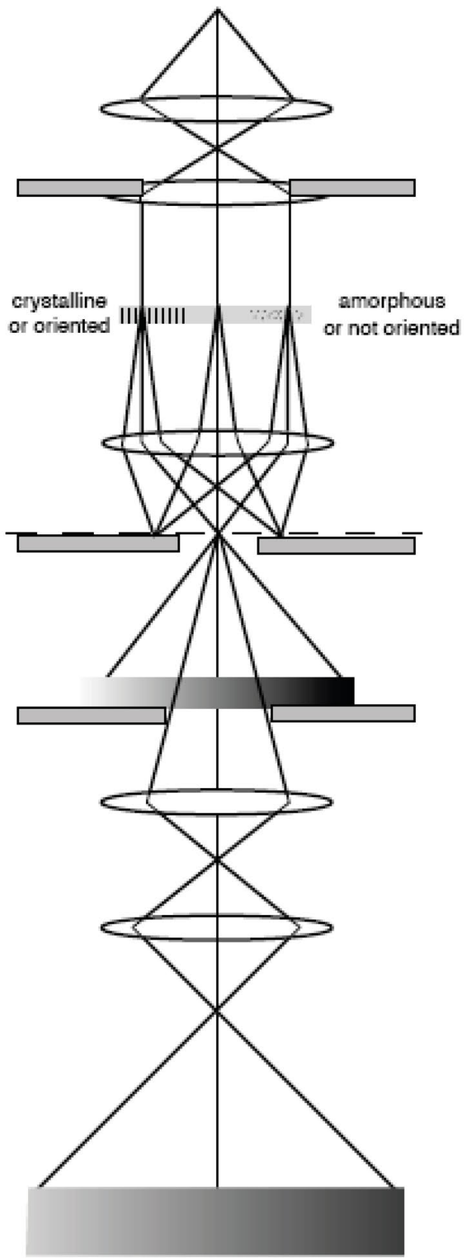
One of many types of TEMs





anode

condenser lens 1

condenser lens 2
condenser diaphragm

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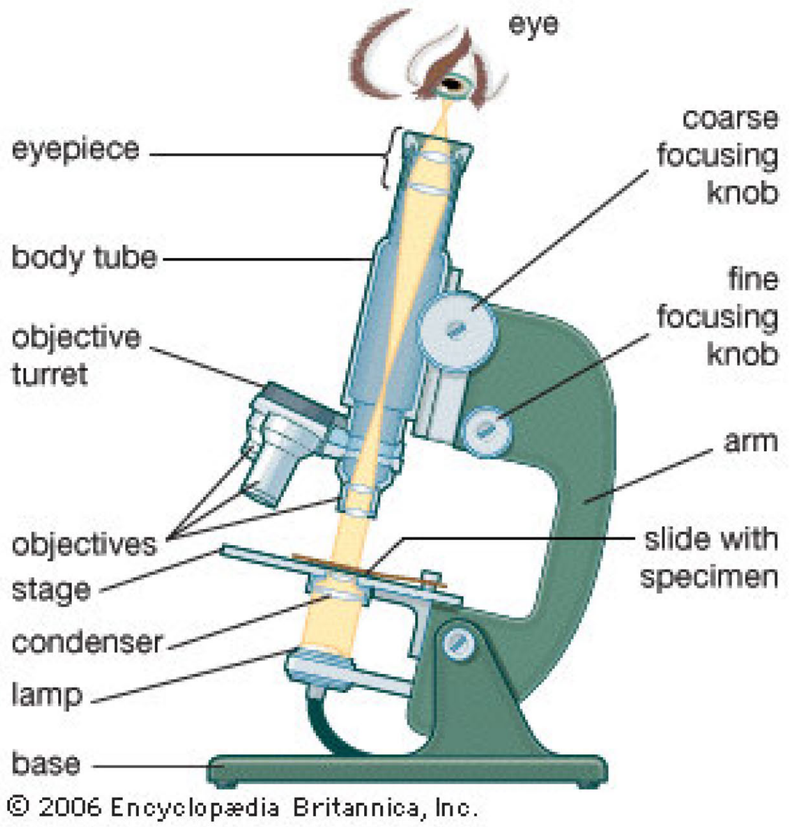
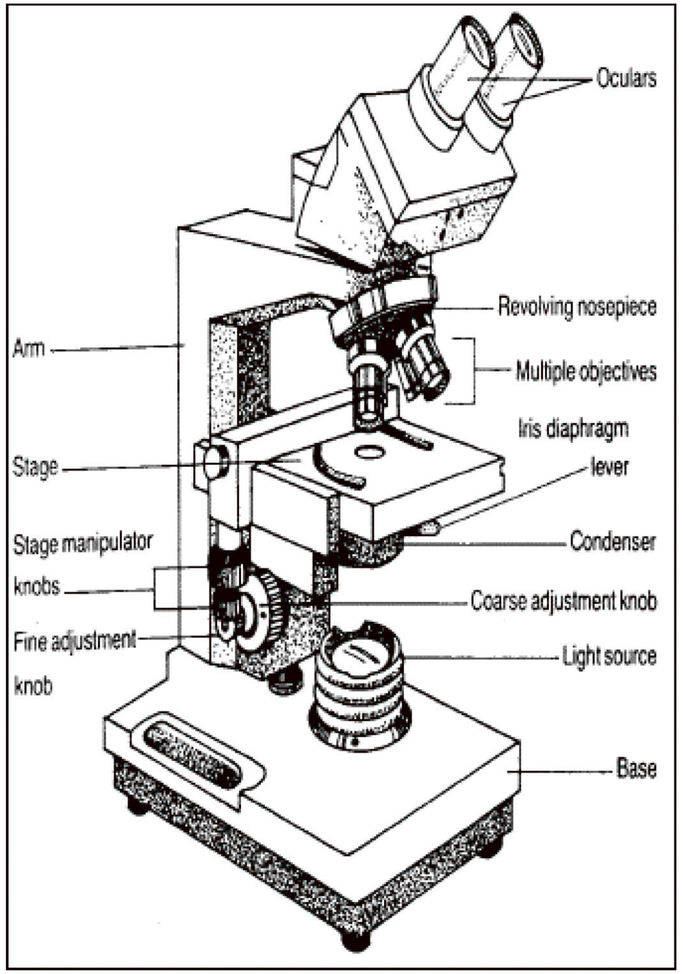
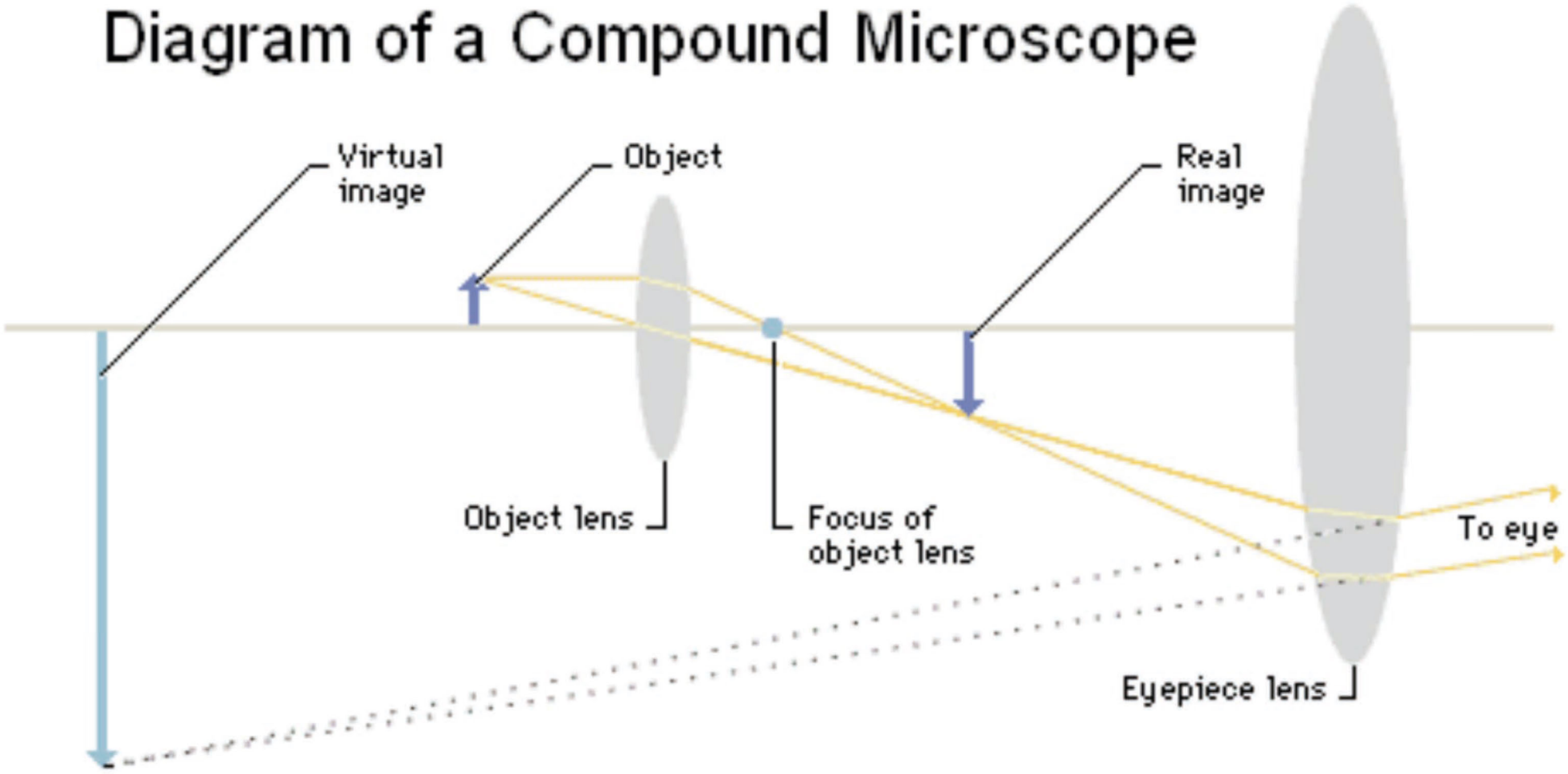
The Optical Microscope

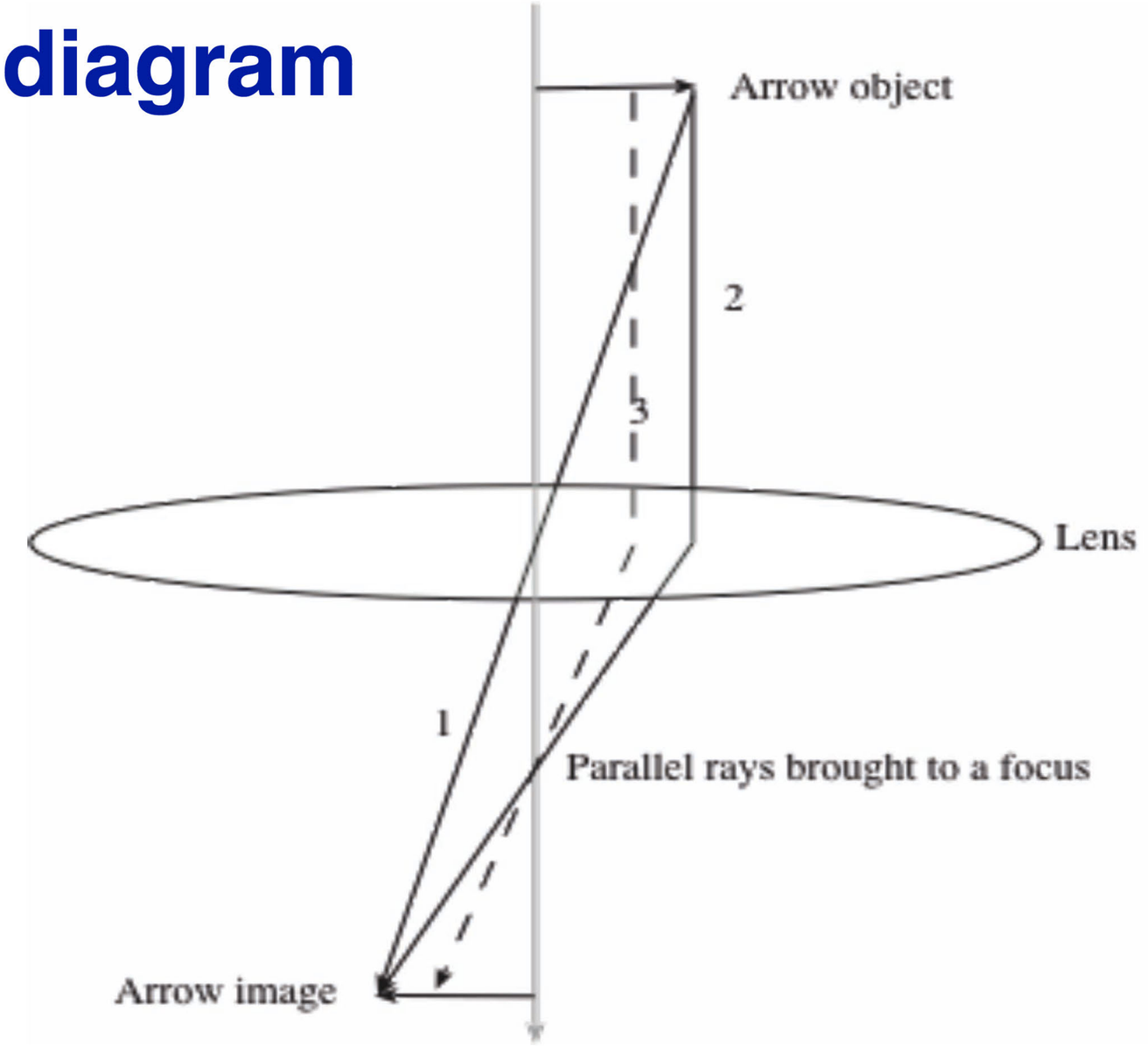




Optical Microscope Lens Diagram



Convex lens ray diagram



Important diagram
(Draw in ~10 seconds)

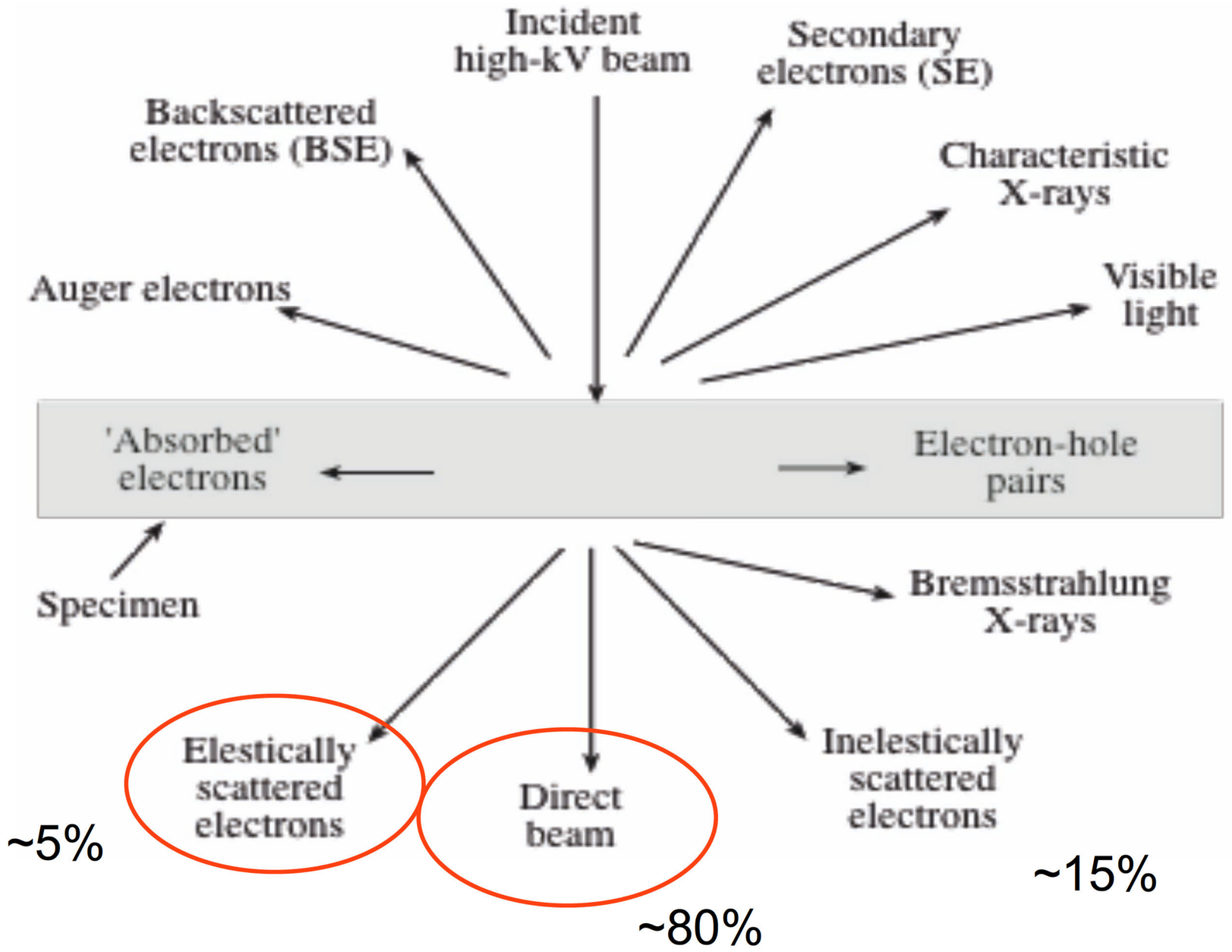
Steps are 1, 2, 3

Very basic electron image formation

- Part of the beam electrons hit the nuclei or electrons of the atoms in specimen, and they are “scattered”
- Scattered electrons can be removed using apertures
- Dense sections in the specimen (i.e. stained parts) cause more scattering and are dark in the image plane
- The most important factor in image formation in TEM is **scattering**

- (NOTE! In light microscopy: it’s absorption, in phase contrast microscopy, it's photon scattering)

Large Number of Signals



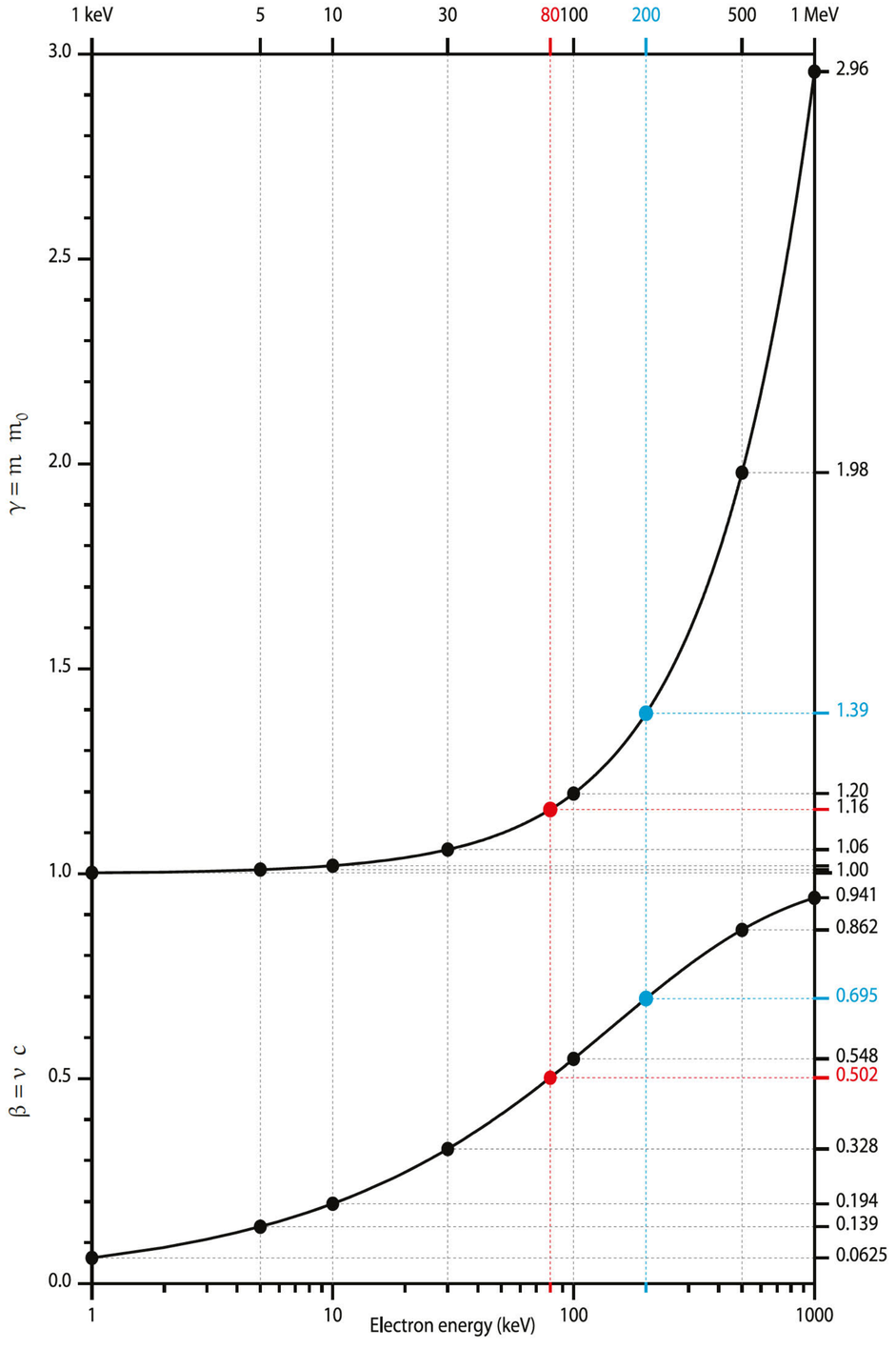
Properties of electrons are used for simple calculations

Table 2.1. Properties of the electron.

Rest mass		m_0	=	9.1091×10^{-31} kg
Charge		e	=	-1.602×10^{-19} C
Kinetic energy		E	=	eU
				1 eV $= 1.602 \times 10^{-19}$ J
Velocity of light		c	=	2.9979×10^{8} m s^{-1}
Rest energy		E_0	=	$m_0c^2 = 511$ keV
Spin		s	=	$h/4\pi$
Planck's constant		h	=	6.6256×10^{-34} J s $= 4.136 \times 10^{-15}$ eV·s

Nonrelativistic	$(E \ll E_0)$			Relativistic $(E \sim E_0)$	
Newton's law	$\boldsymbol{F} = \frac{\mathrm{d}\boldsymbol{p}}{\mathrm{d}\tau}$	F	=	$\frac{\mathrm{d}}{\mathrm{d}\tau}(mv)$	(2.7)
Mass	$m = m_0$	m	=	$m_0/\sqrt{1 - v^2/c^2}$	(2.8a)
Energy	$E = eU = \frac{1}{2}m_0v^2$	mc^2	=	$m_0c^2 + eU = E_0 + E$	(2.9)
		m	=	$m_0(1 + E/E_0)$	(2.8b)
Velocity	$v = \sqrt{2E/m_0}$	v	=	$c\sqrt{1 - \frac{1}{(1 + E/E_0)^2}}$	(2.10)
Momentum	$p = m_0v = \sqrt{2m_0E}$	p	=	$\sqrt{2m_0E(1 + E/2E_0)}$	(2.11)
			=	$\frac{1}{c}\sqrt{2EE_0 + E^2}$	
Wavelength	$\lambda = \frac{h}{p} = h/\sqrt{2m_0E}$	λ	=	$h/\sqrt{2m_0E(1 + E/2E_0)}$	(2.12)
			=	$hc/\sqrt{2EE_0 + E^2}$	

Reimer 2008



Example: how many electrons are in the column at a time?

Some words: Volts vs. electron Volts

dose, fluence, flux

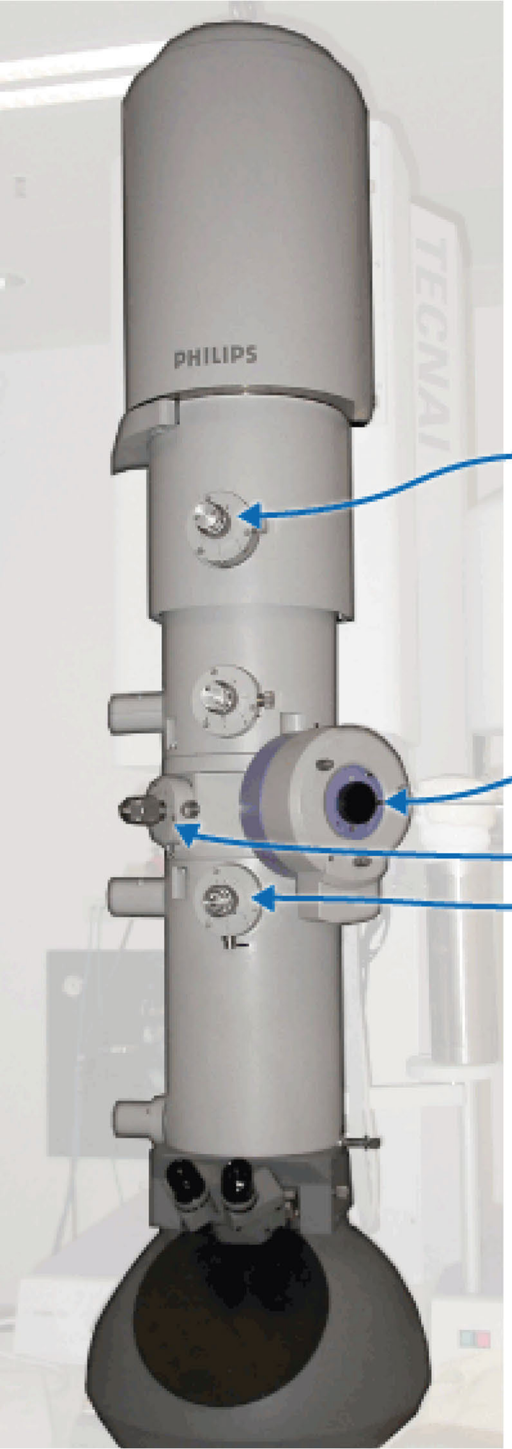
~~electron density~~

Limitations of electron beam instruments

- Vacuum
- Damage Damage Damage Damage Damage Damage Damage Damage

Electron microscopes are used to simulate damage in the core of a nuclear reactor!

- Electron lenses terrible (relative to photon lenses) and hard to make
- Have to record many many noisy images, *lots* of data (just ask Jake & Toby!)
- Charging: non-conductive samples charge up and act like lenses
- Samples must be very thin and are quite fragile, move around in the beam and are often difficult to make
- Expensive (From £300k to £10M) Krios is £3000/day



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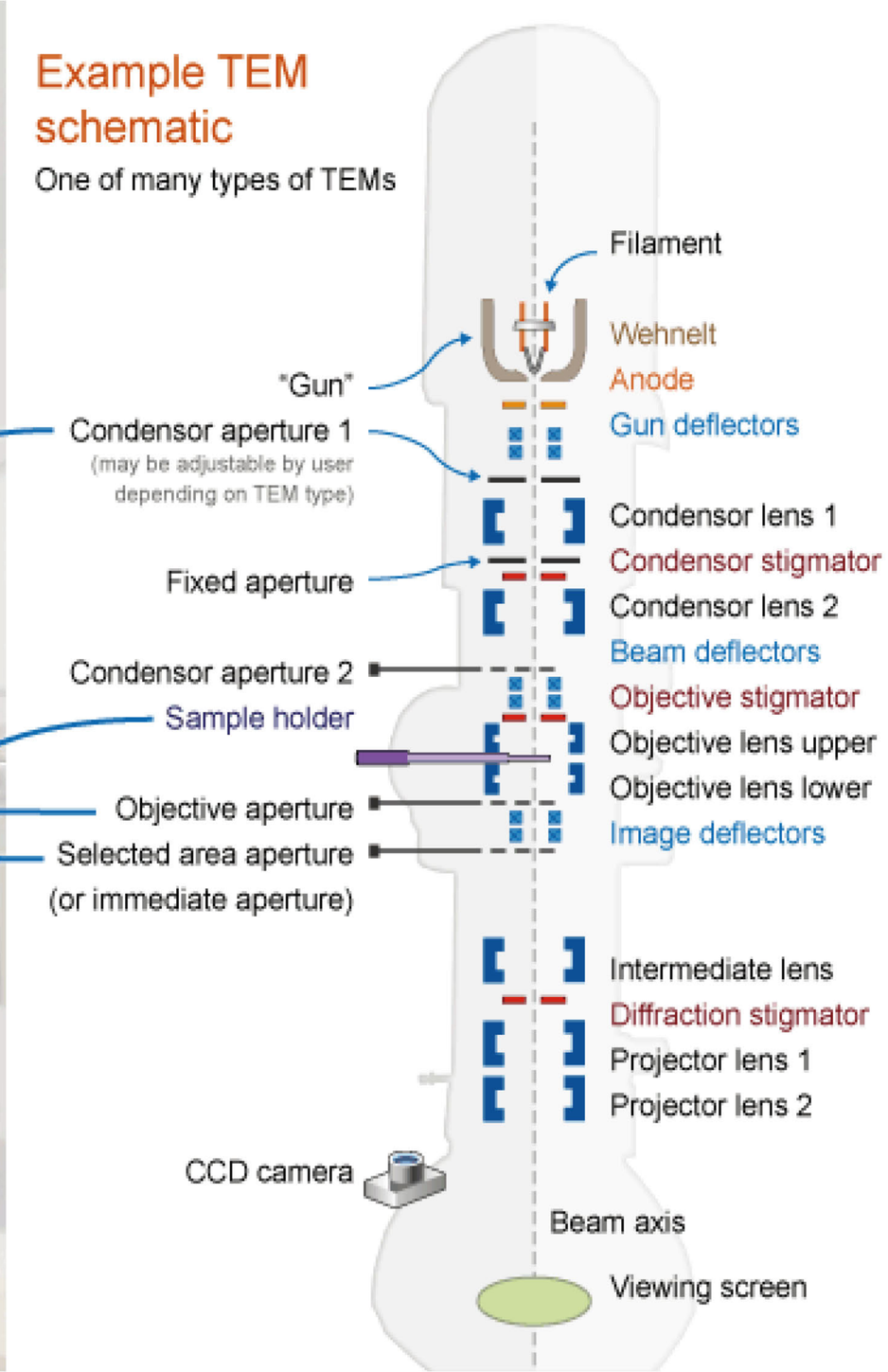
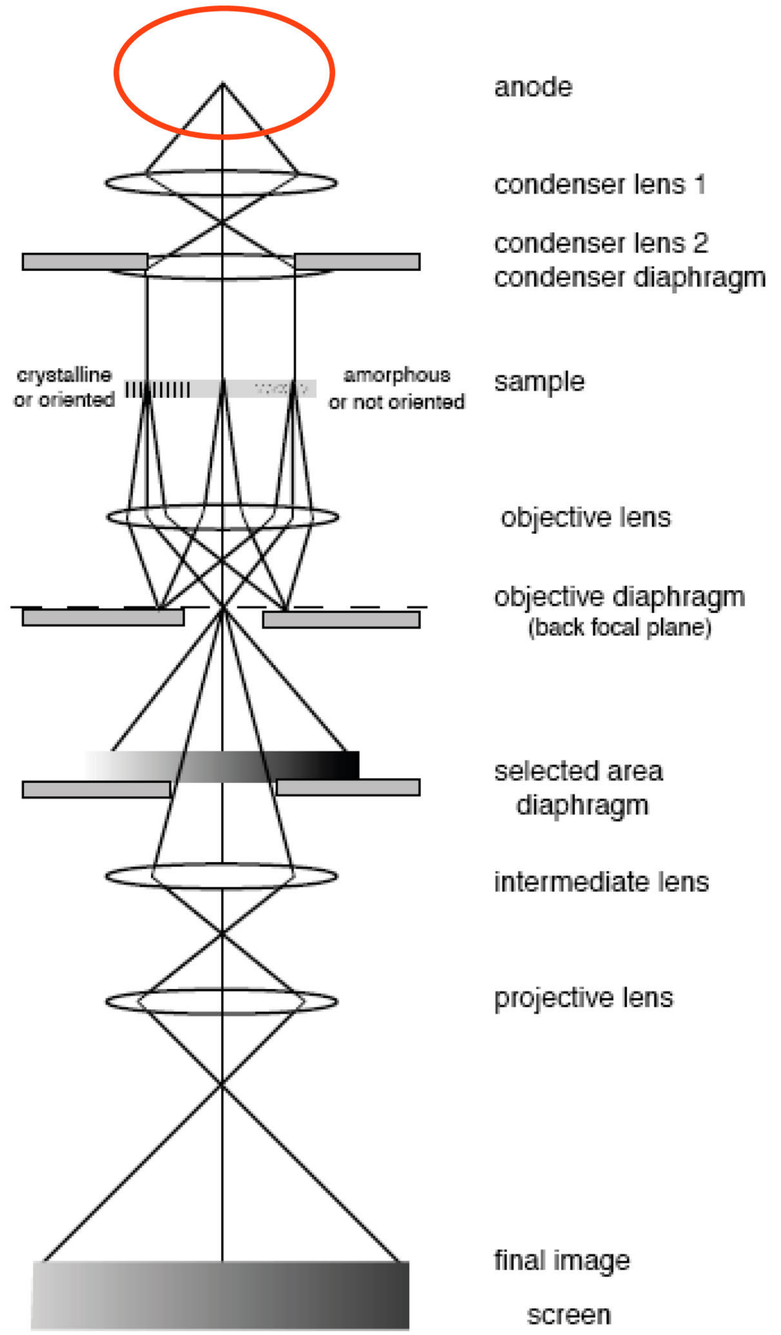
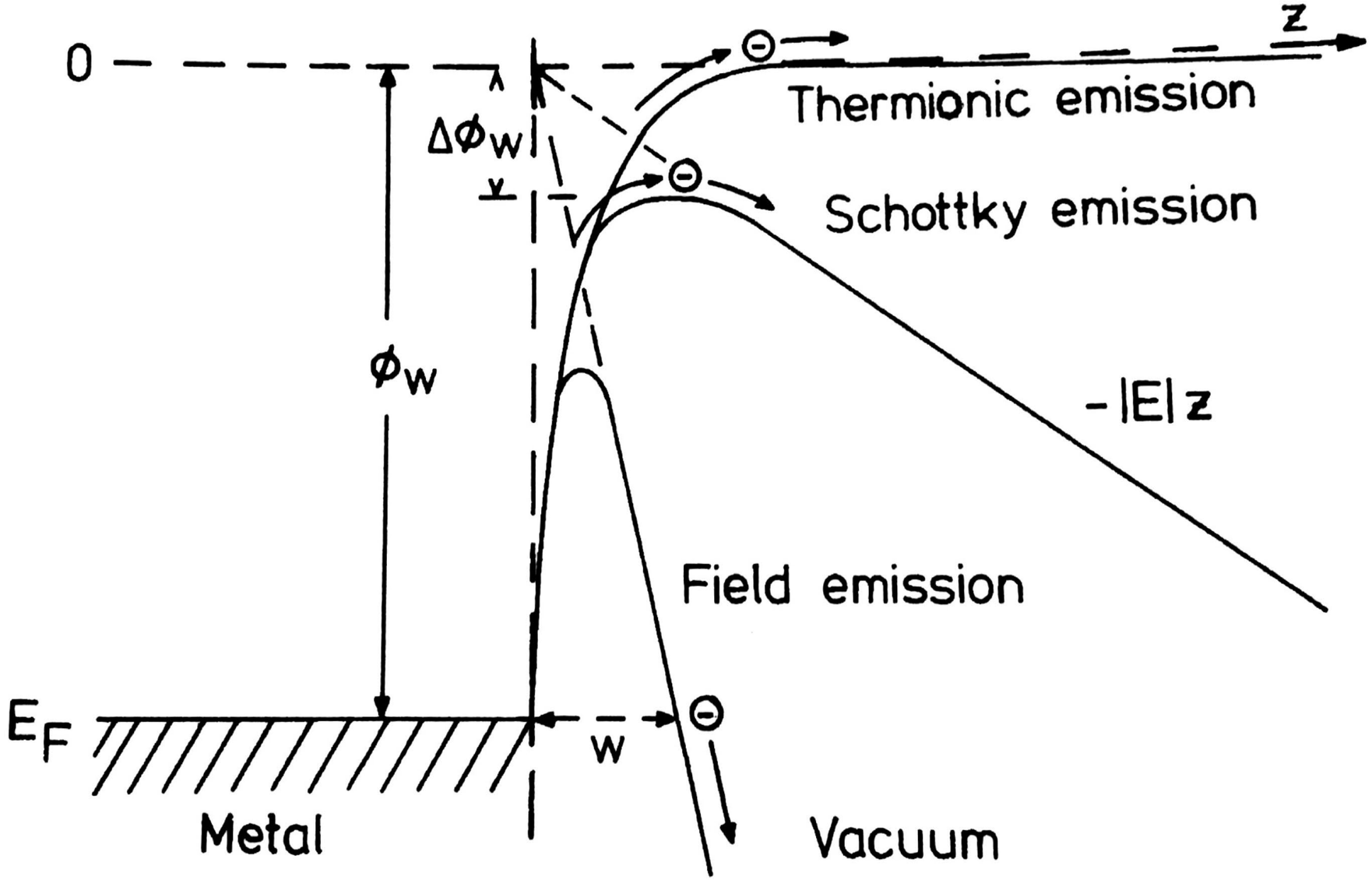




How to get electrons



Reimer 2008

Thermal Emission Source

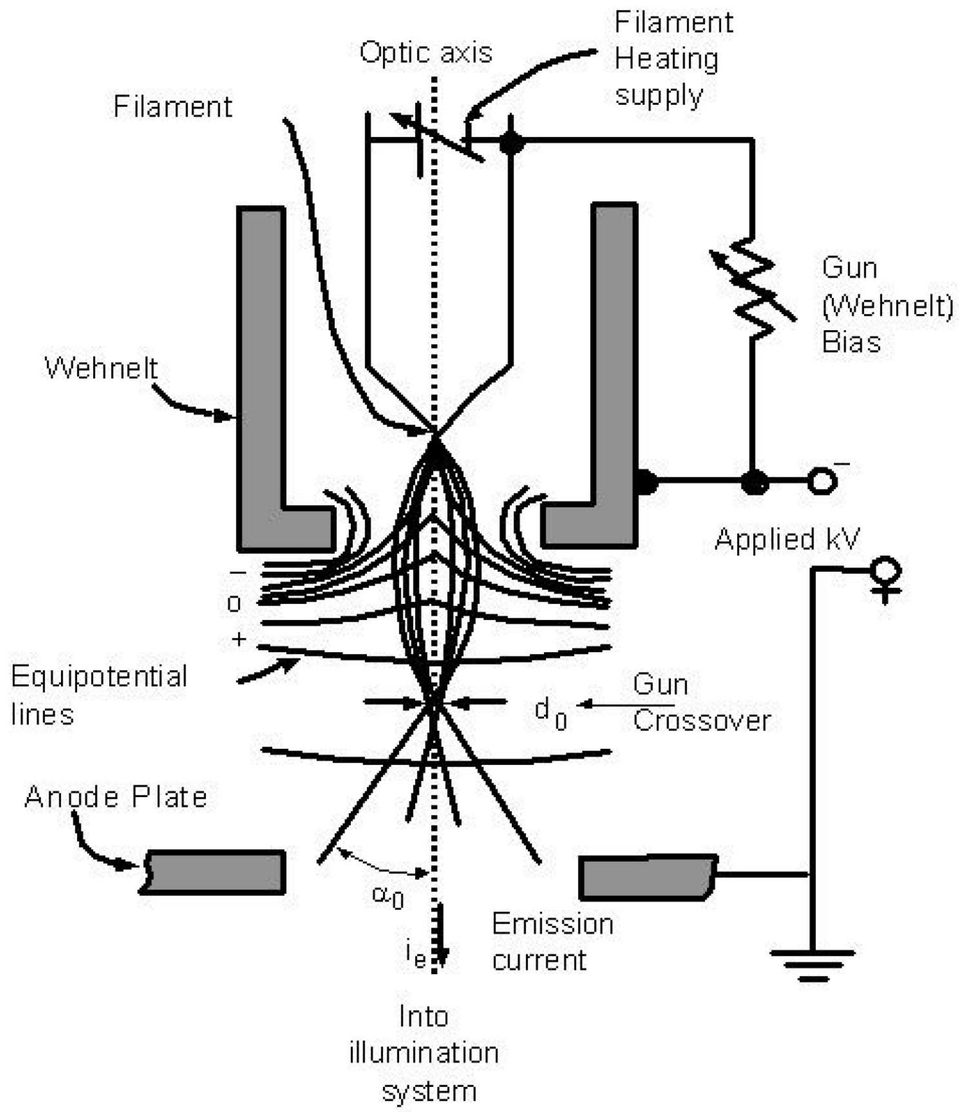
Important ideas

Wehnelt is the first lens

Anode plate

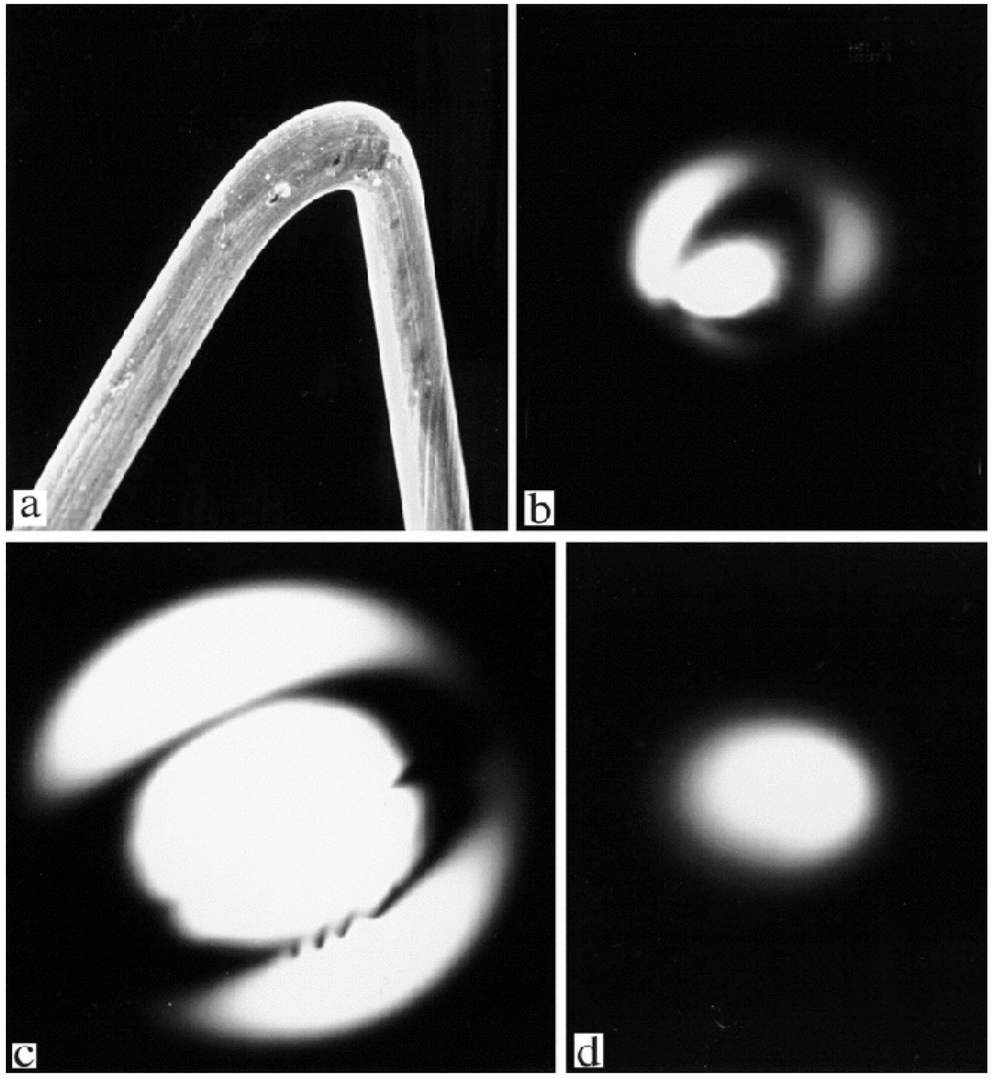
Dimensions

Cross-over



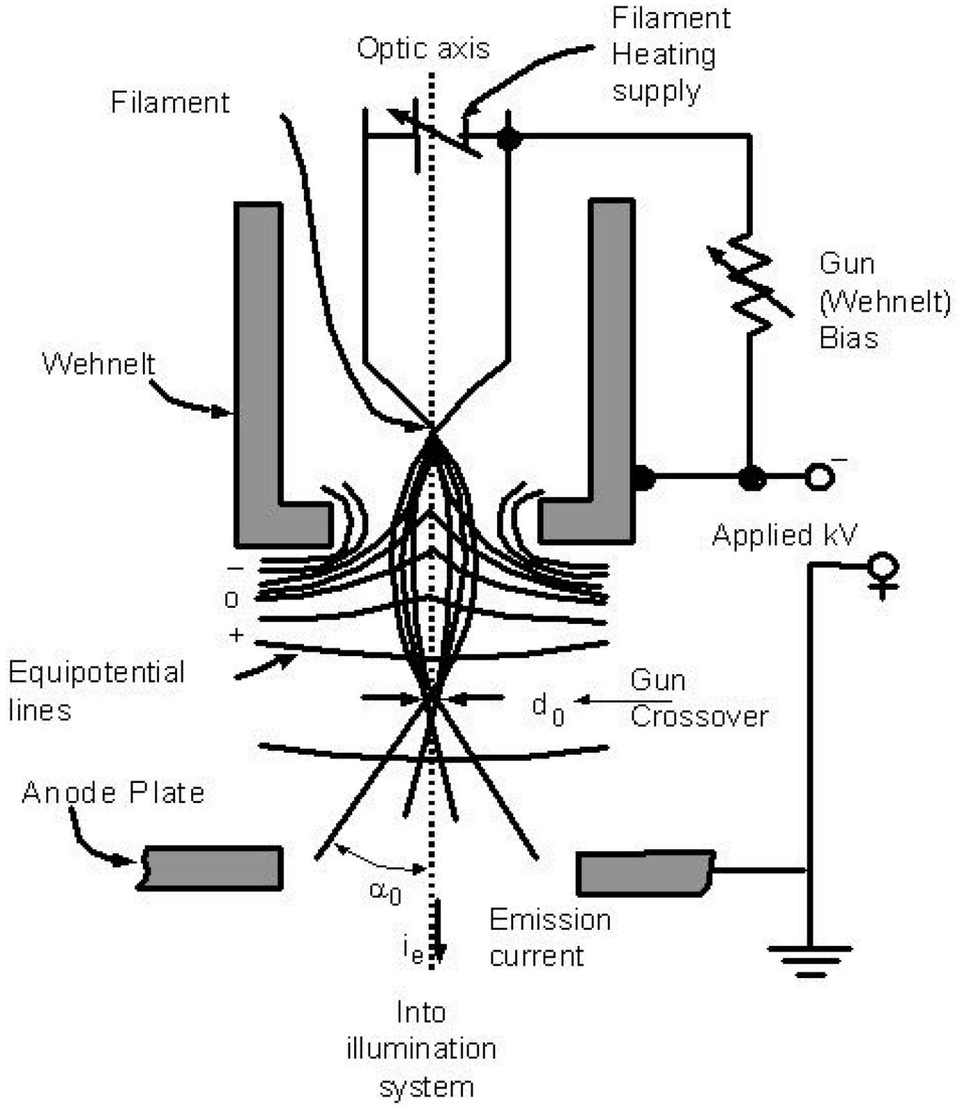
Hairpin filament

Underfocused
& off center



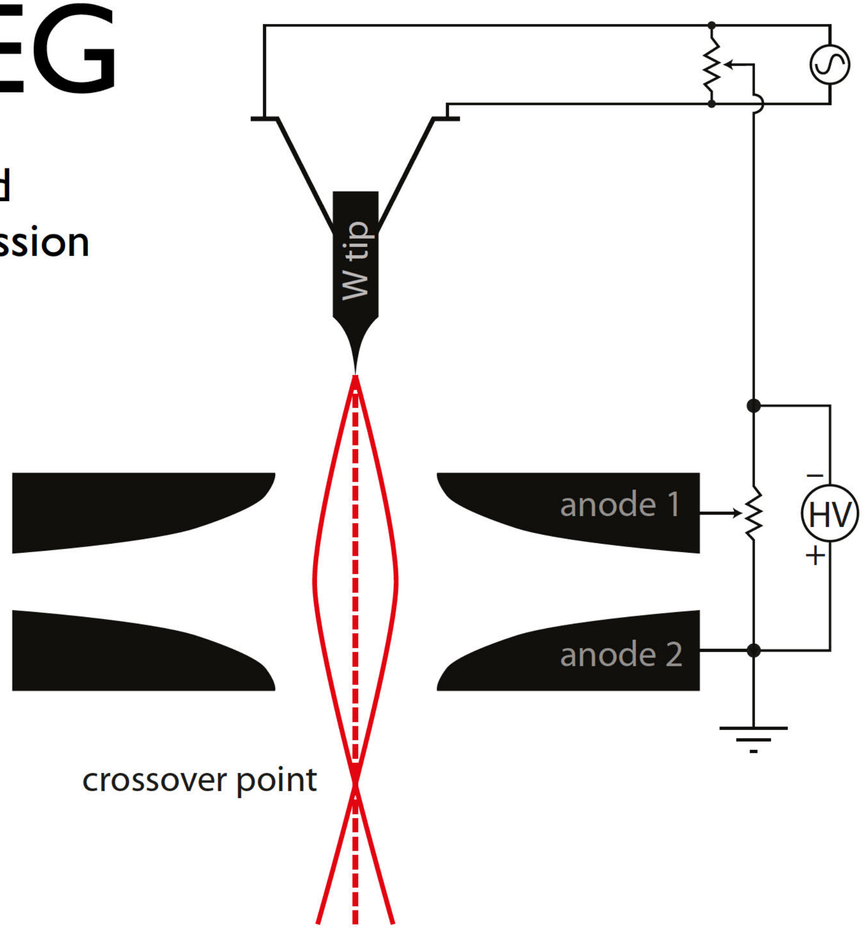
Underfocused but
centered

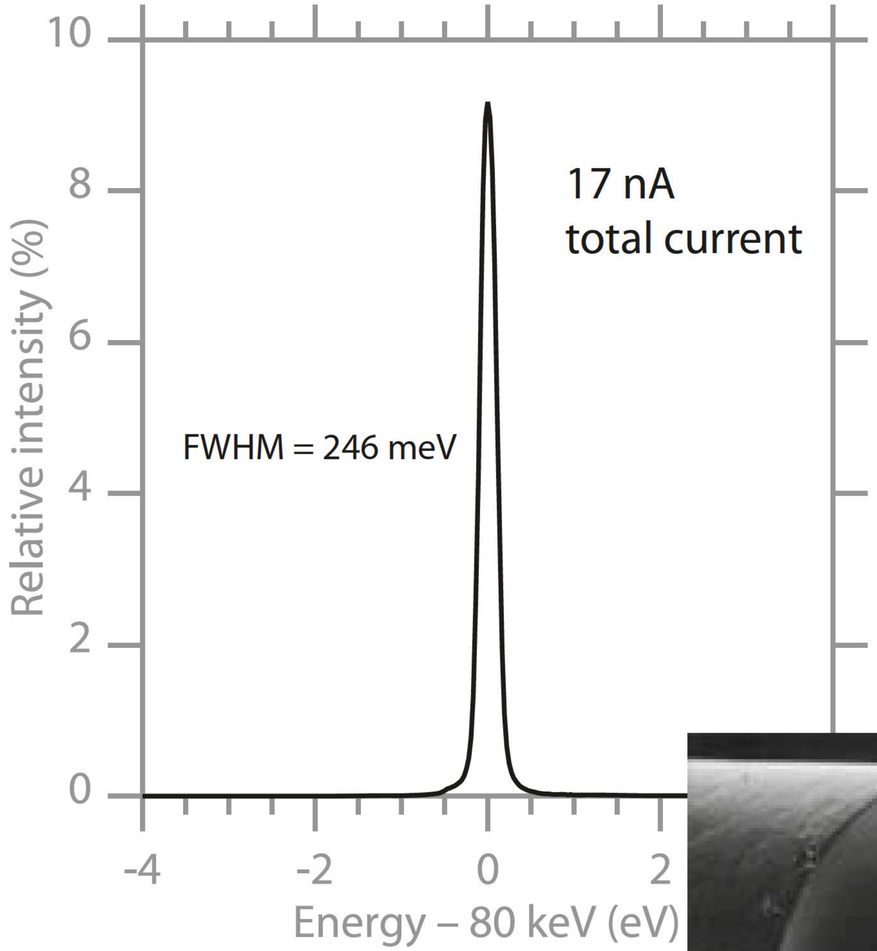
Centered &
focused



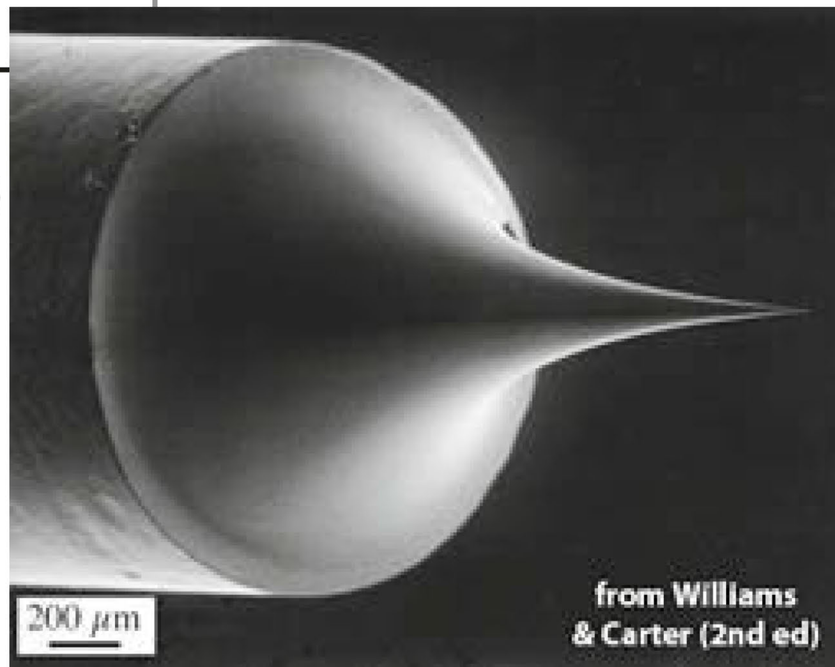
FEG

Field
Emission
Gun



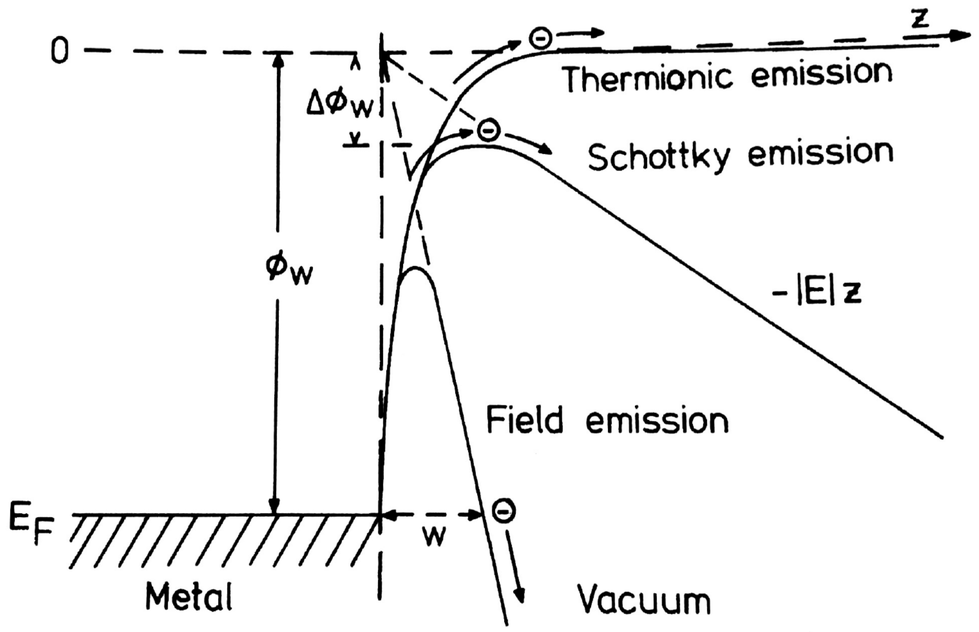


Brighter,
More coherent
More monochromatic source
crudely, think ‘laser vs. light bulb’



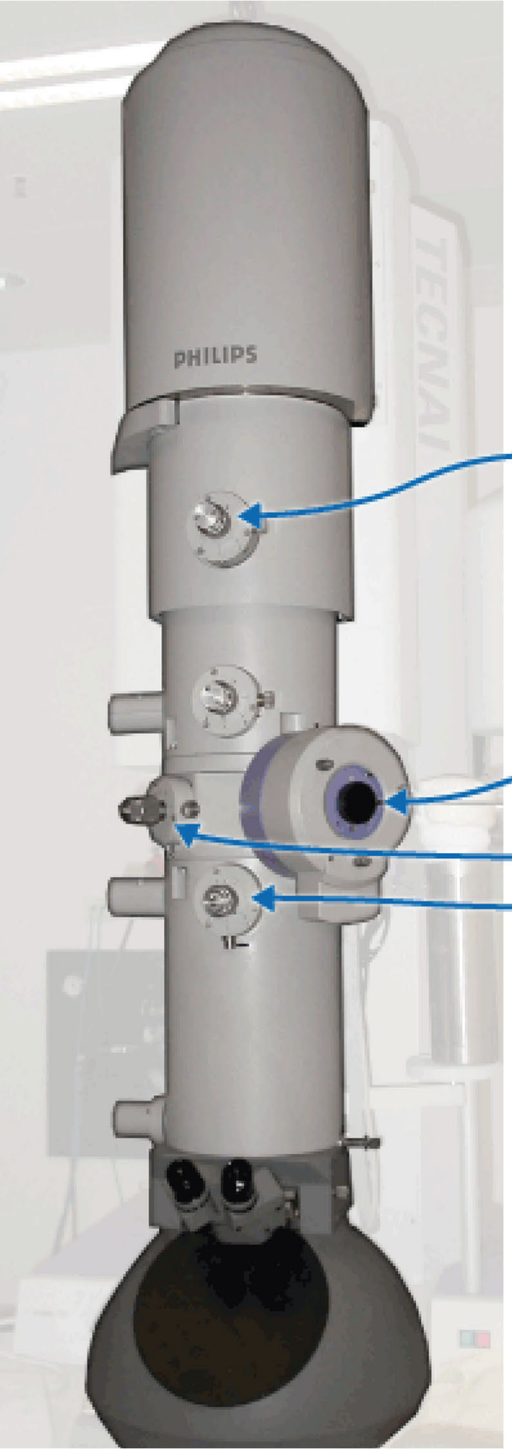
Russo 2010

Characteristics of Electron Sources



	Units	Tungsten	LaB_6	FEG
Operating Temperature	K	2700	1700	300
Current Density	A/m^2	$5x10^4$	10^6	10^{10}
Crossover size	μm	50	10	<0.01
Energy spread	eV	3	1.5	0.3
Stability	% / hr	<1	<1	5
Vacuum	Pa	10^{-2}	10^{-4}	10^{-8}
Lifetime	hr	100	500	>1000

Reimer 2008



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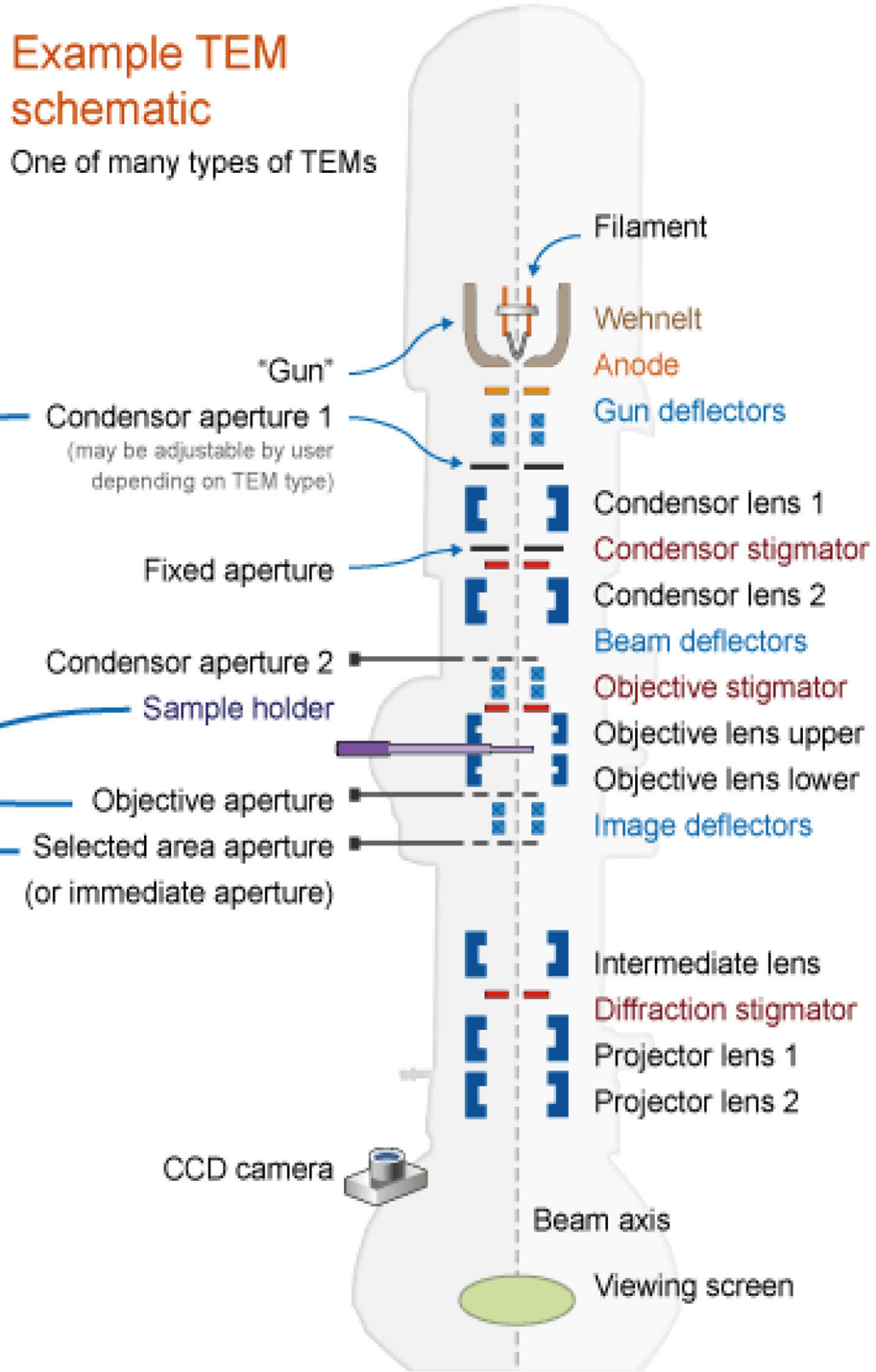

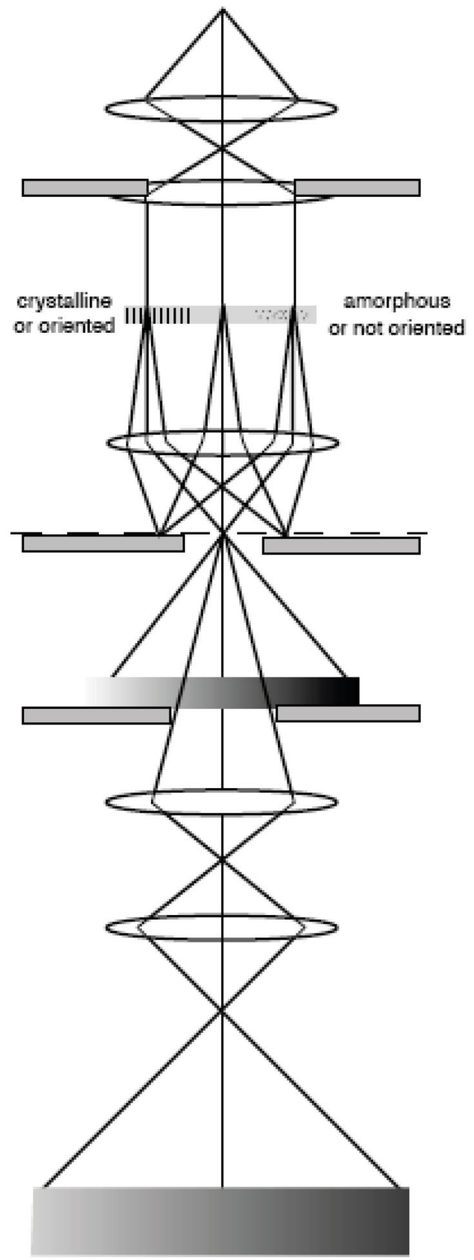

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objective diaphragm
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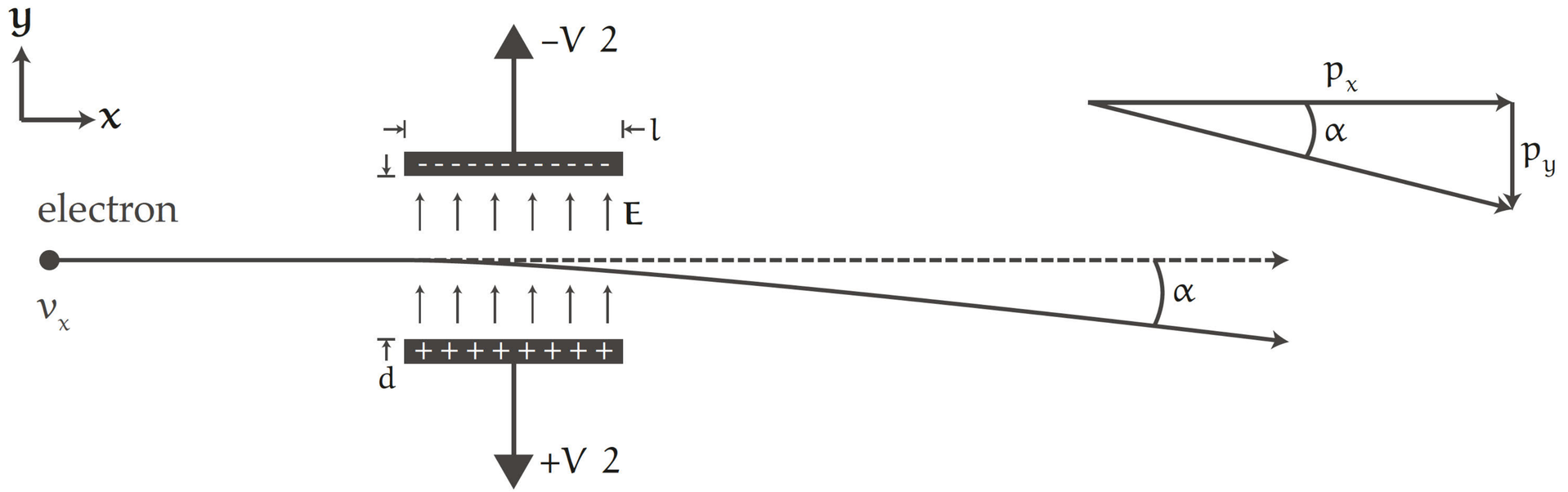
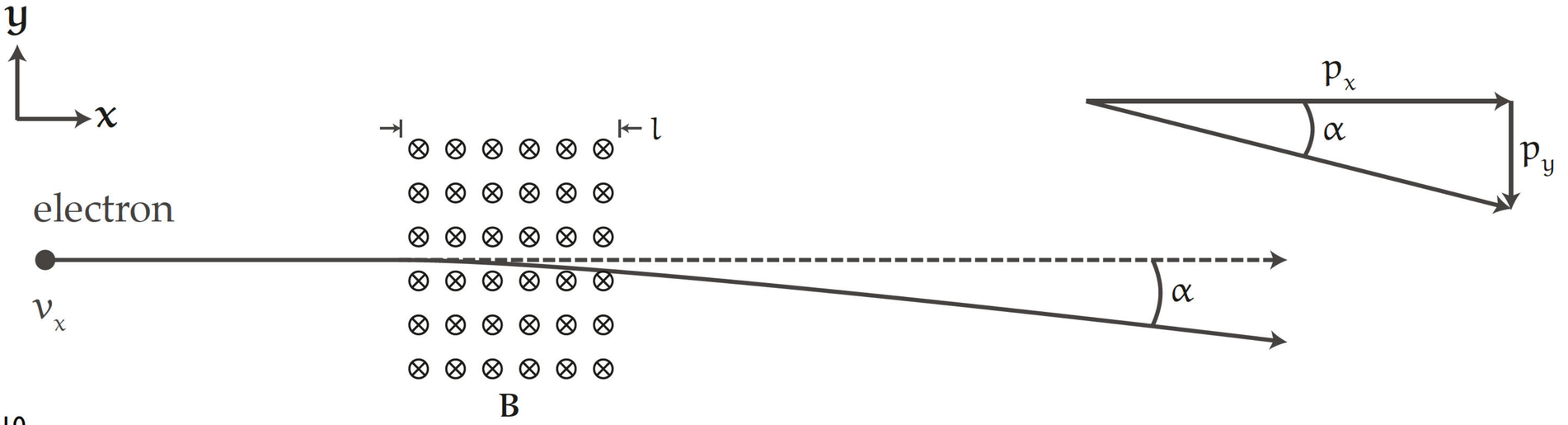
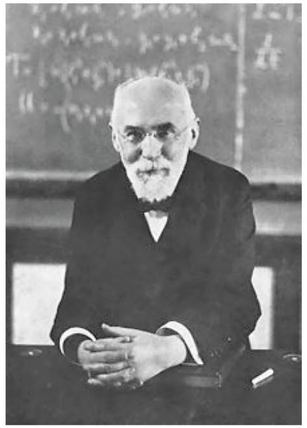
The Lorentz force

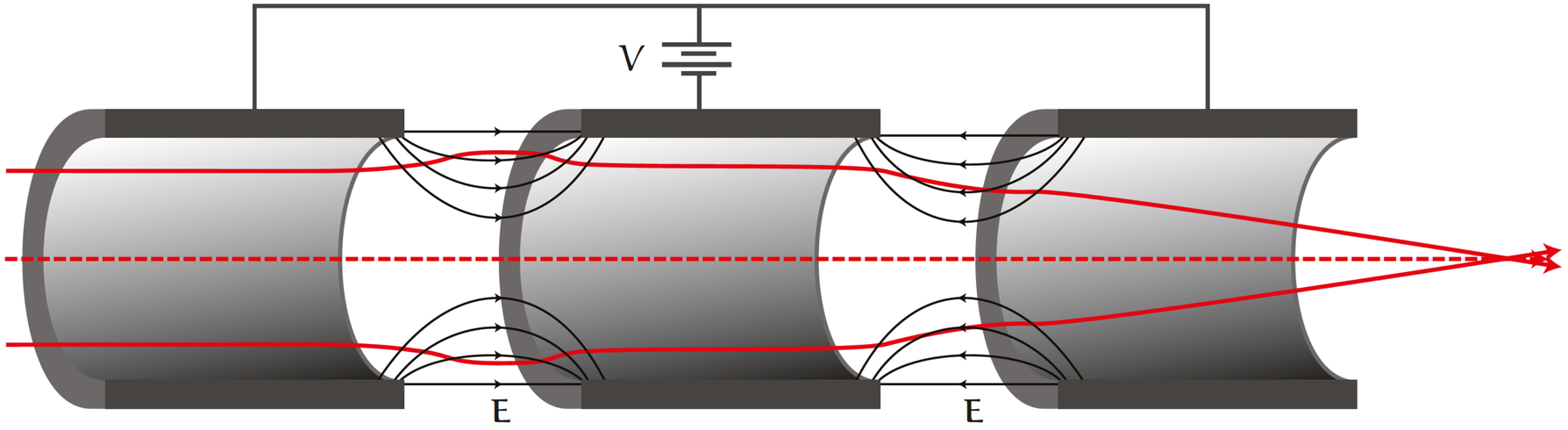
$$\mathbf{F} = -q_e(\mathbf{E} + \mathbf{v} \times \mathbf{B})$$

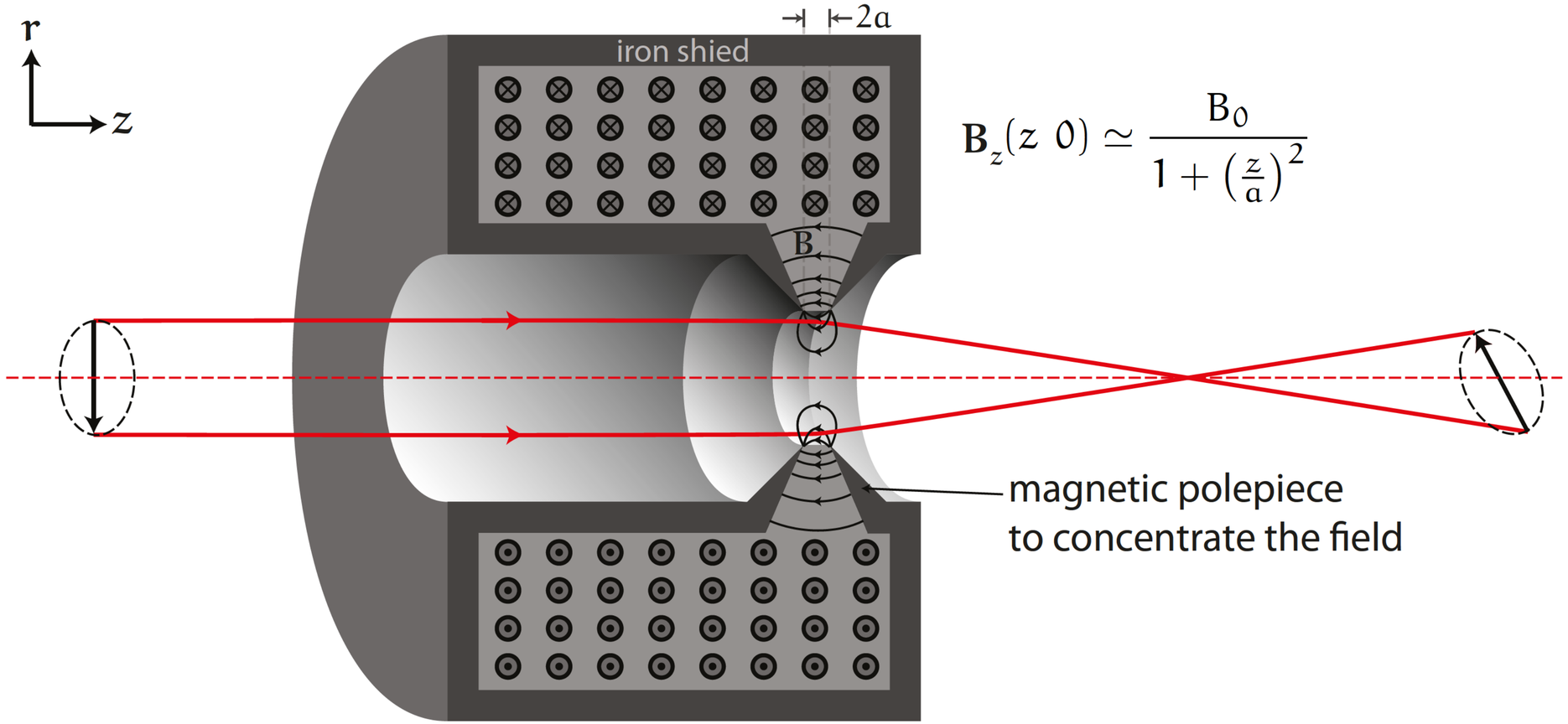






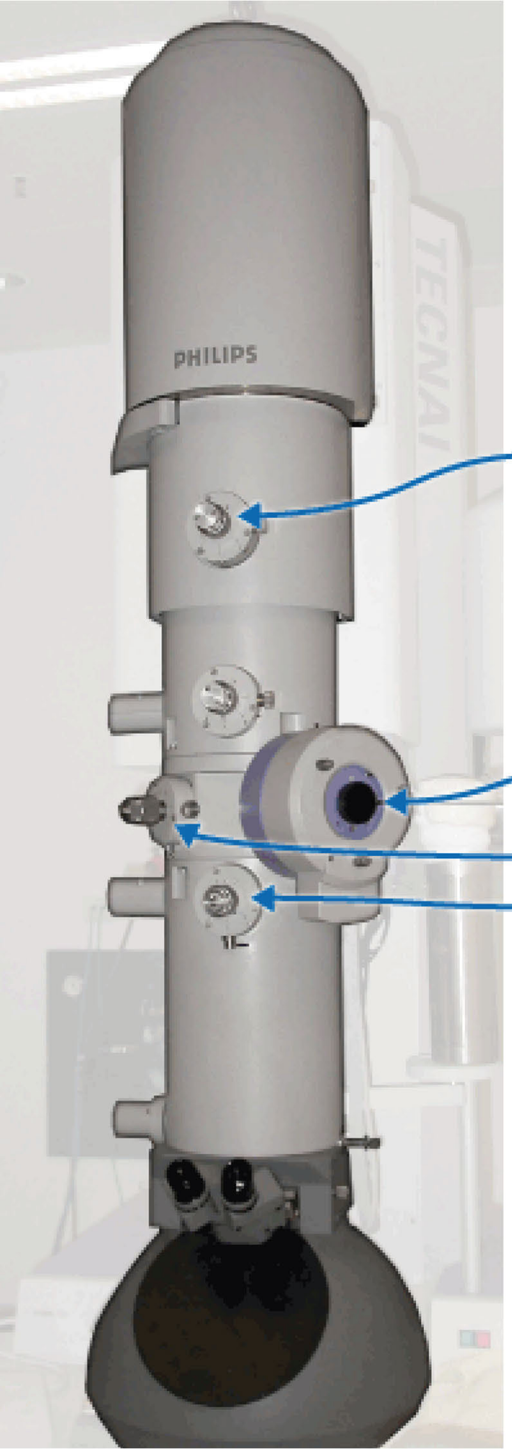
Russo 2010





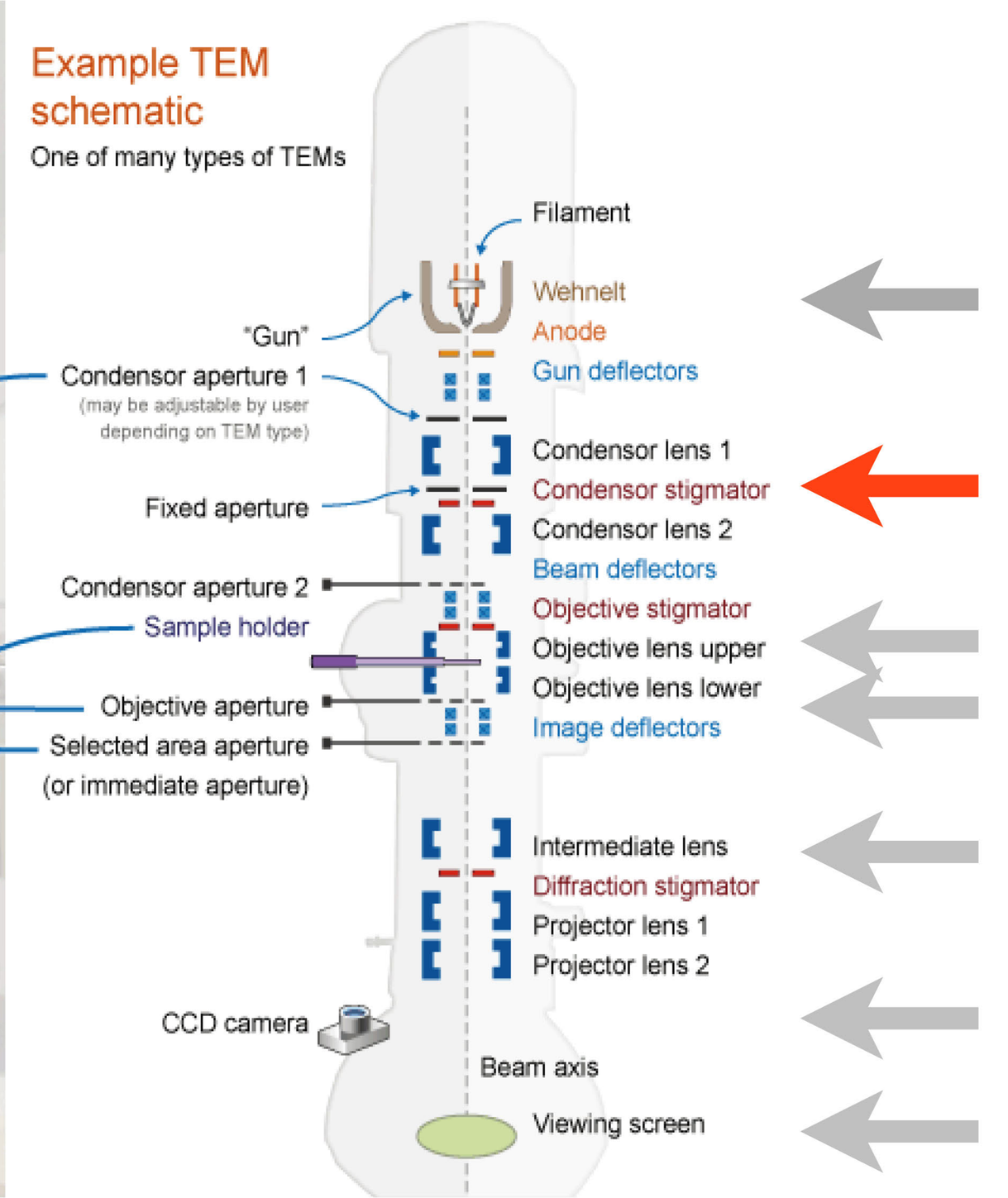
$$B_z(z\ 0) \simeq \frac{B_0}{1+\left(\frac{z}{a}\right)^2}$$

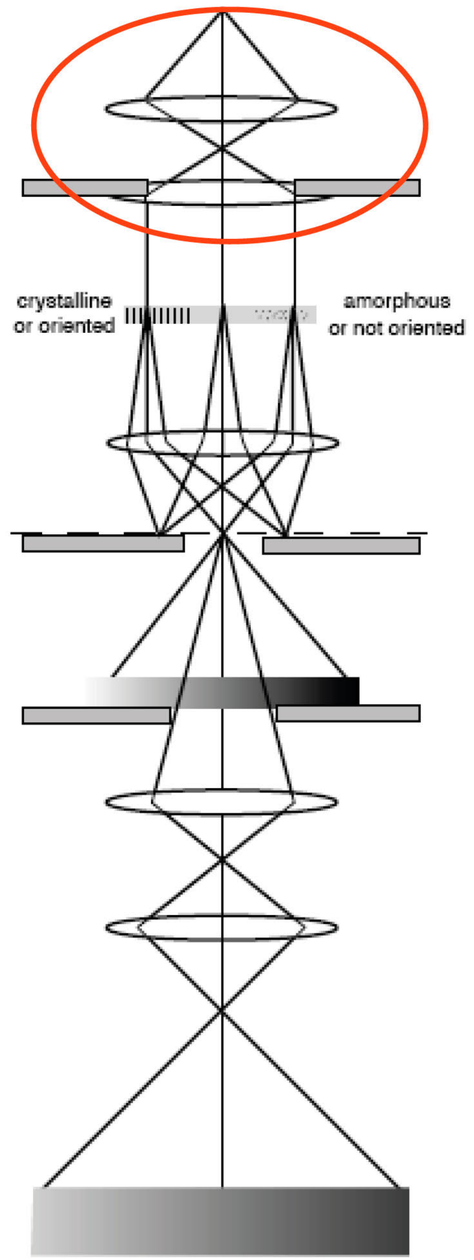
Russo 2010



Example TEM schematic

One of many types of TEMs





anode

condenser lens 1

condenser lens 2
condenser diaphragm

sample

objective lens

objective diaphragm
(back focal plane)

selected area diaphragm

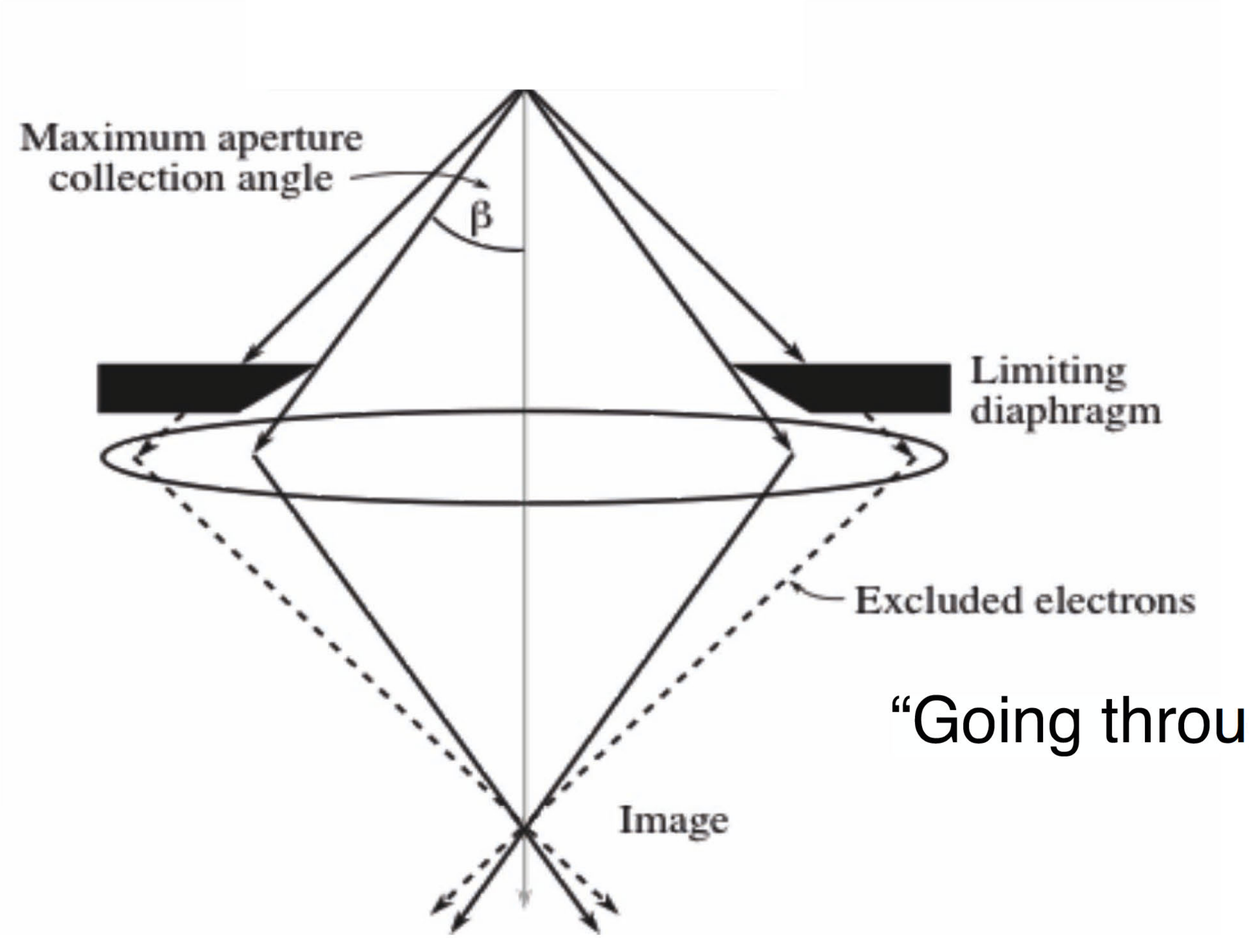
intermediate lens

projective lens

final image

screen

Diaphragms & Apertures

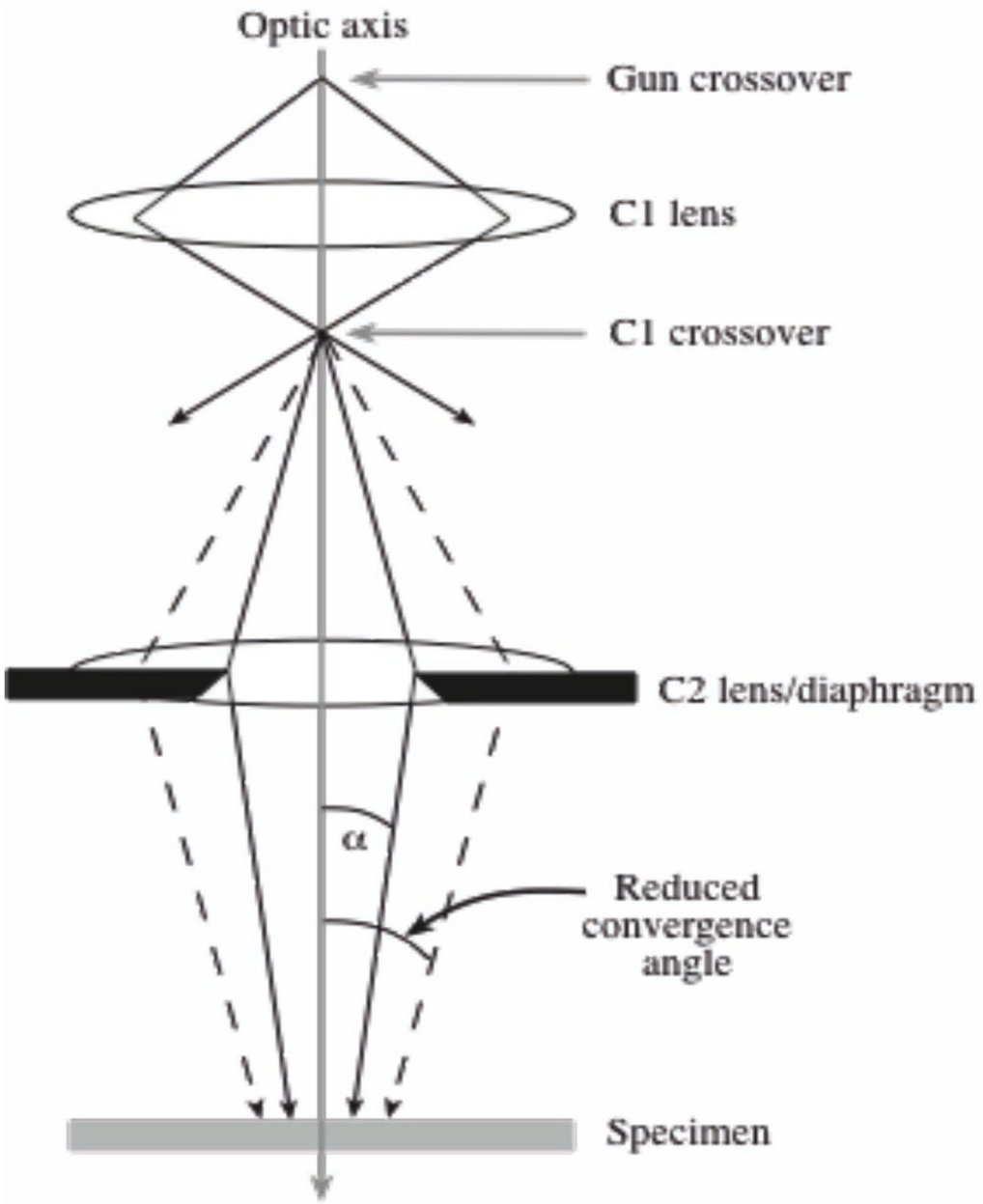


“Going through the door”

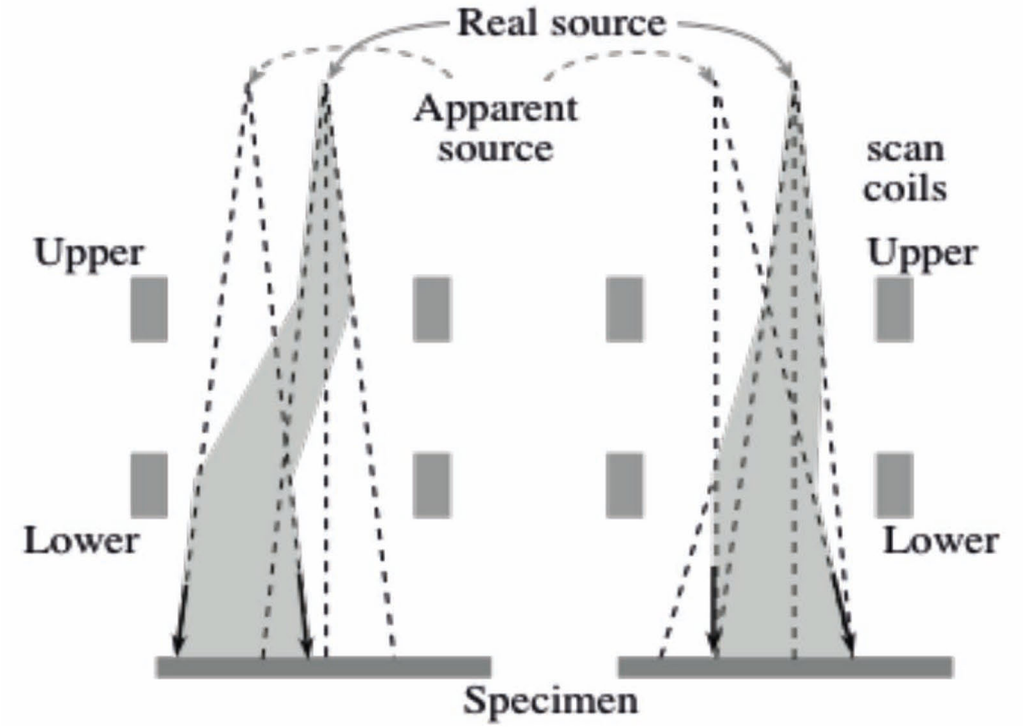
The Basic Electron Condenser System

Most TEMs 2 lenses + 1 aperture

Krios: 3 lenses + 1 aperture



Shift and tilt through a lens

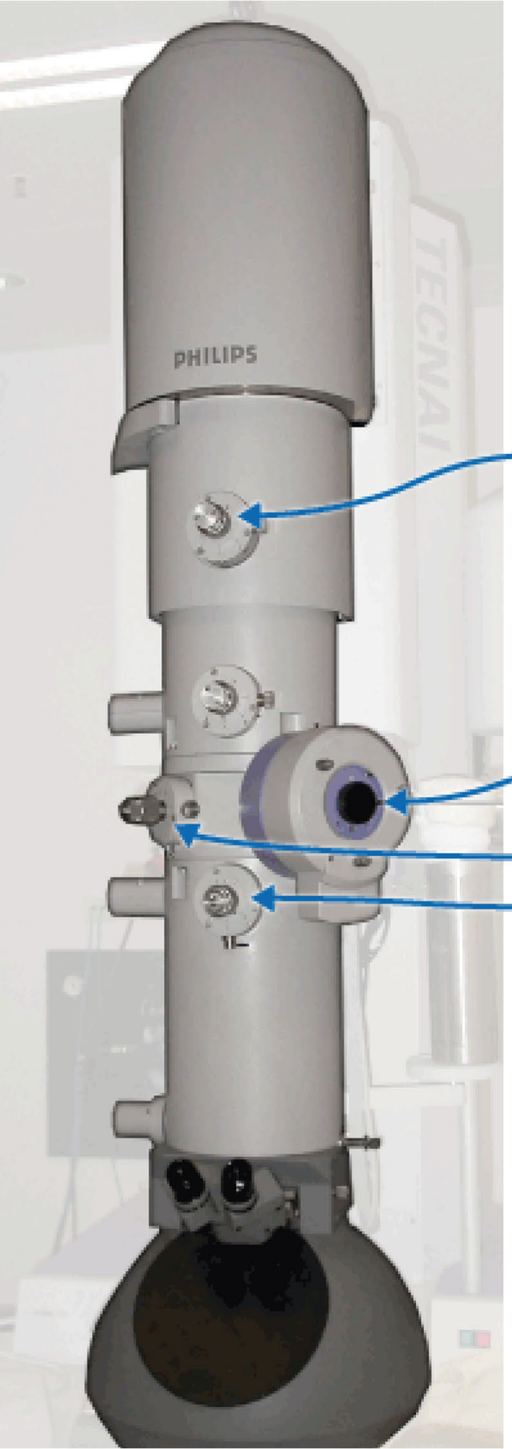


Shift

Tilt

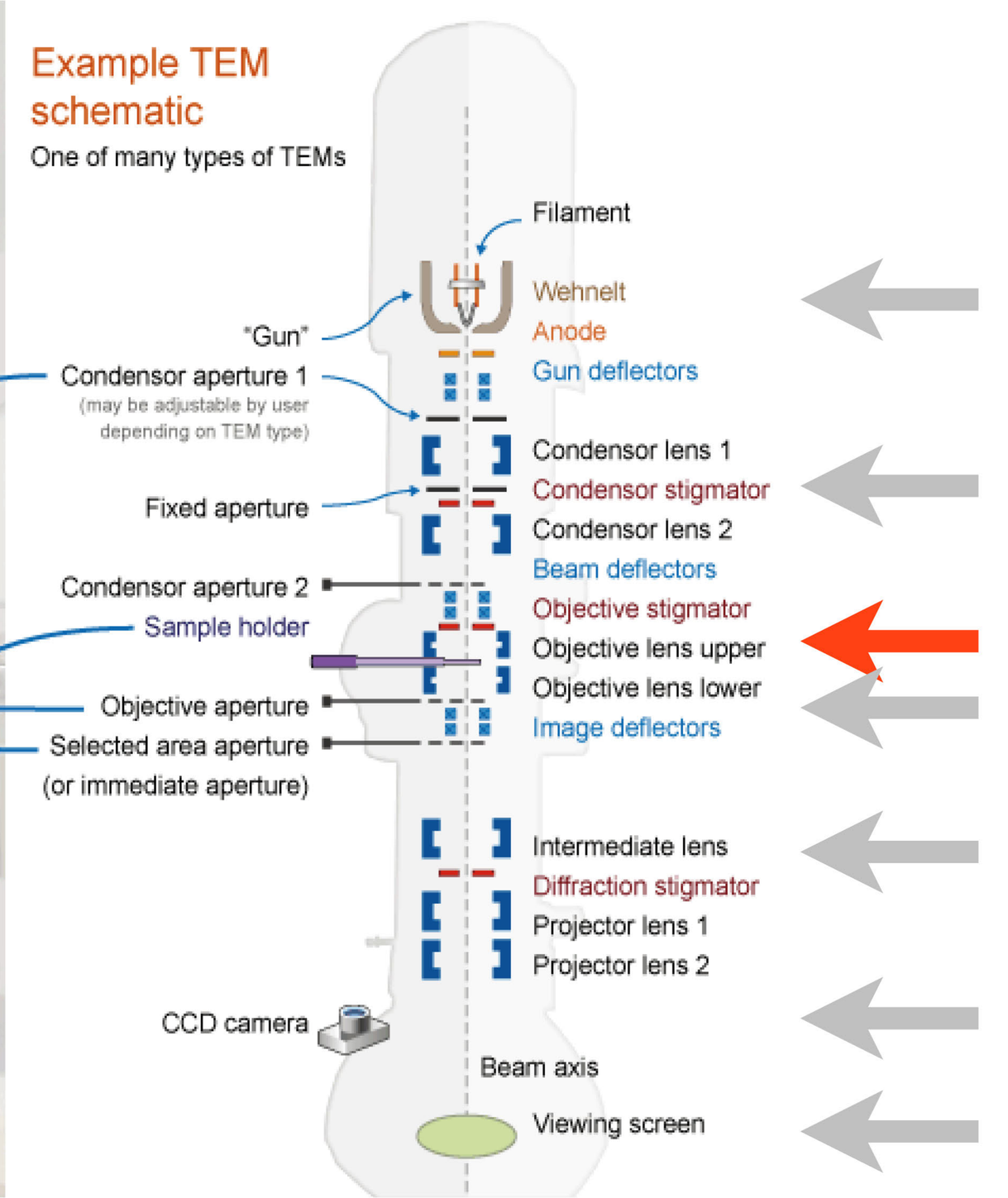
Same direction -> Different area

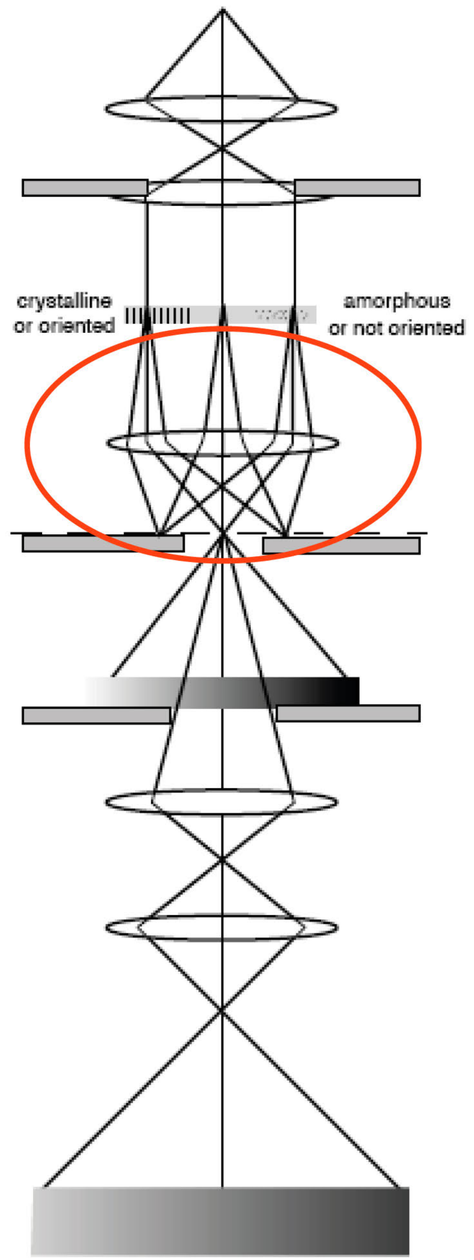
Same area -> Different direction



Example TEM schematic

One of many types of TEMs





anode

condenser lens 1

condenser lens 2
condenser diaphragm

sample

objective lens

objective diaphragm
(back focal plane)

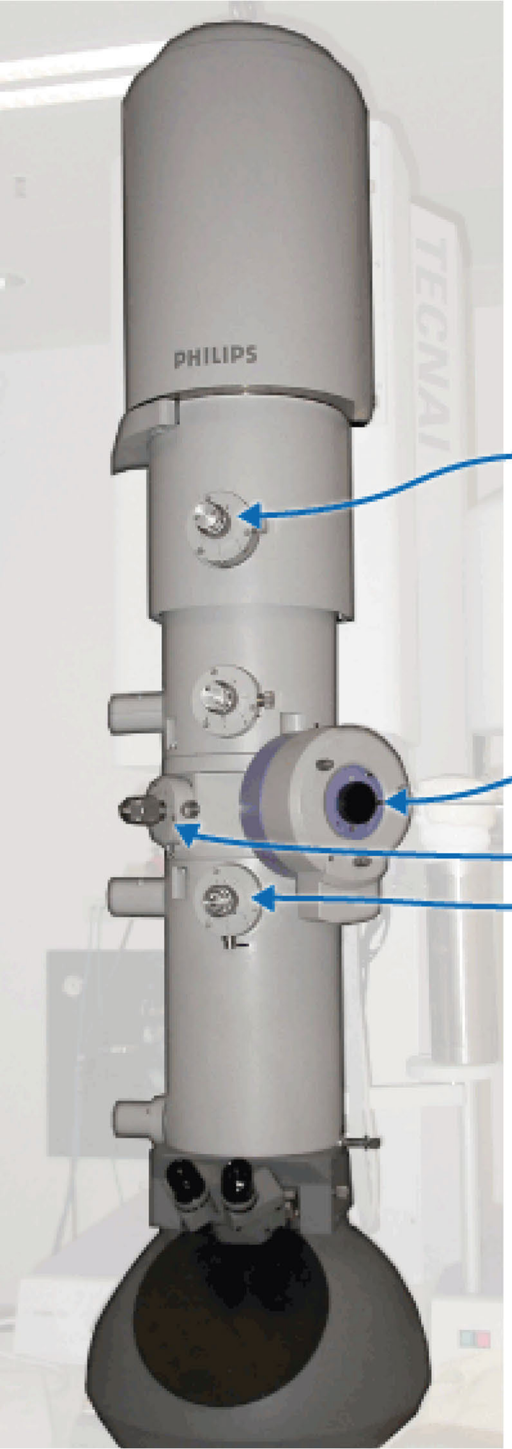
selected area diaphragm

intermediate lens

projective lens

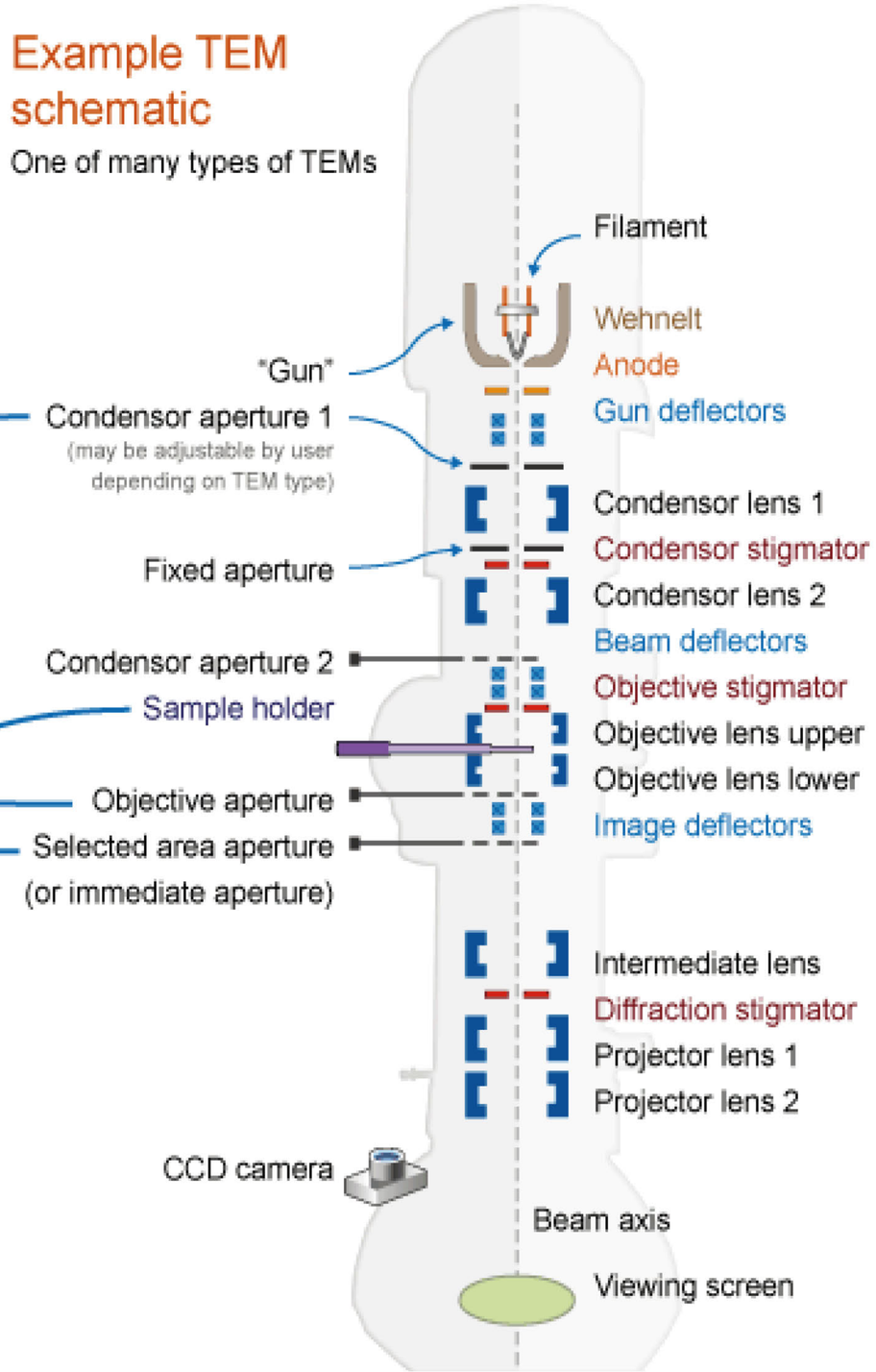
final image

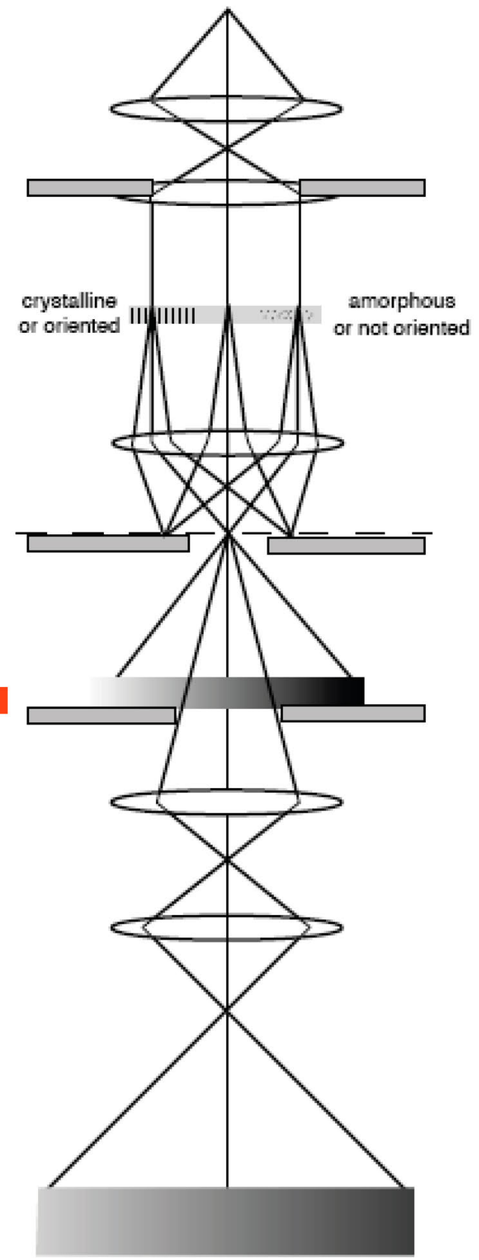
screen



Example TEM schematic

One of many types of TEMs





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condenser lens 2
condenser diaphragm

sample

objective lens

objective diaphragm
(back focal plane)

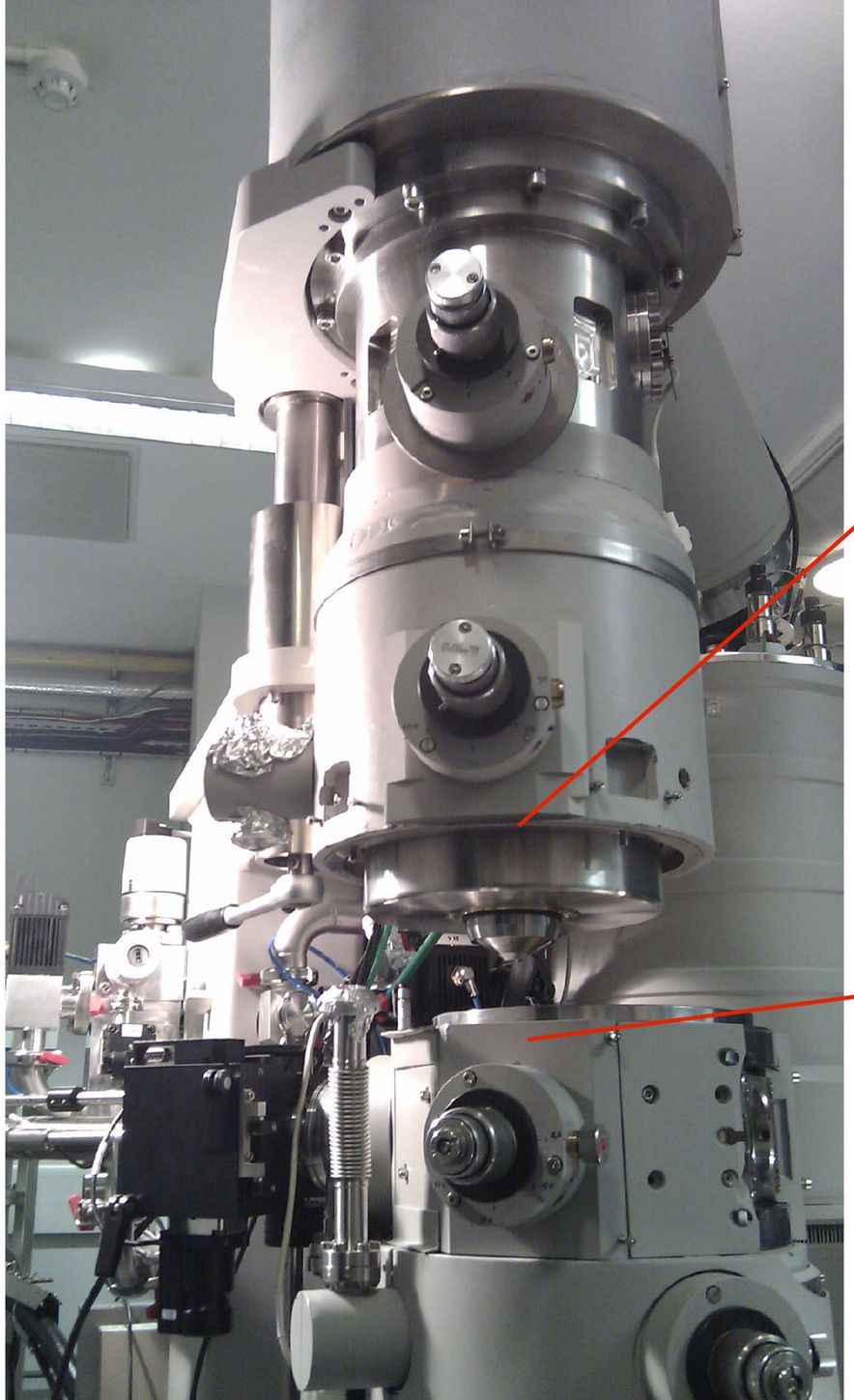
selected area diaphragm

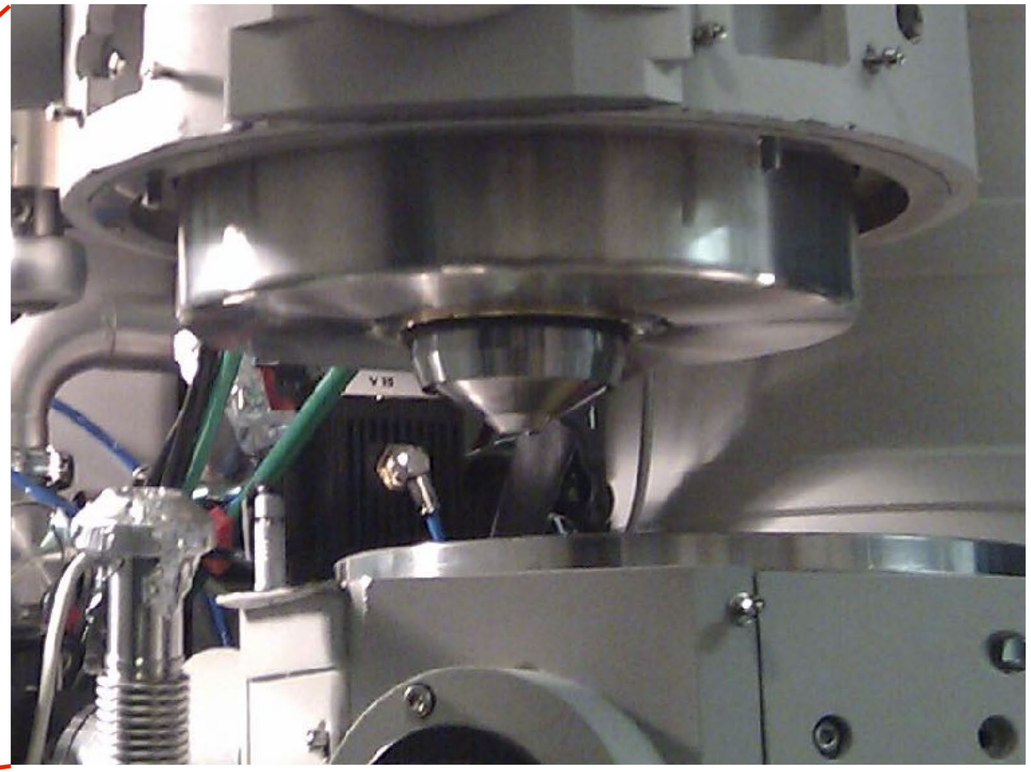
intermediate lens

projective lens

final image

screen



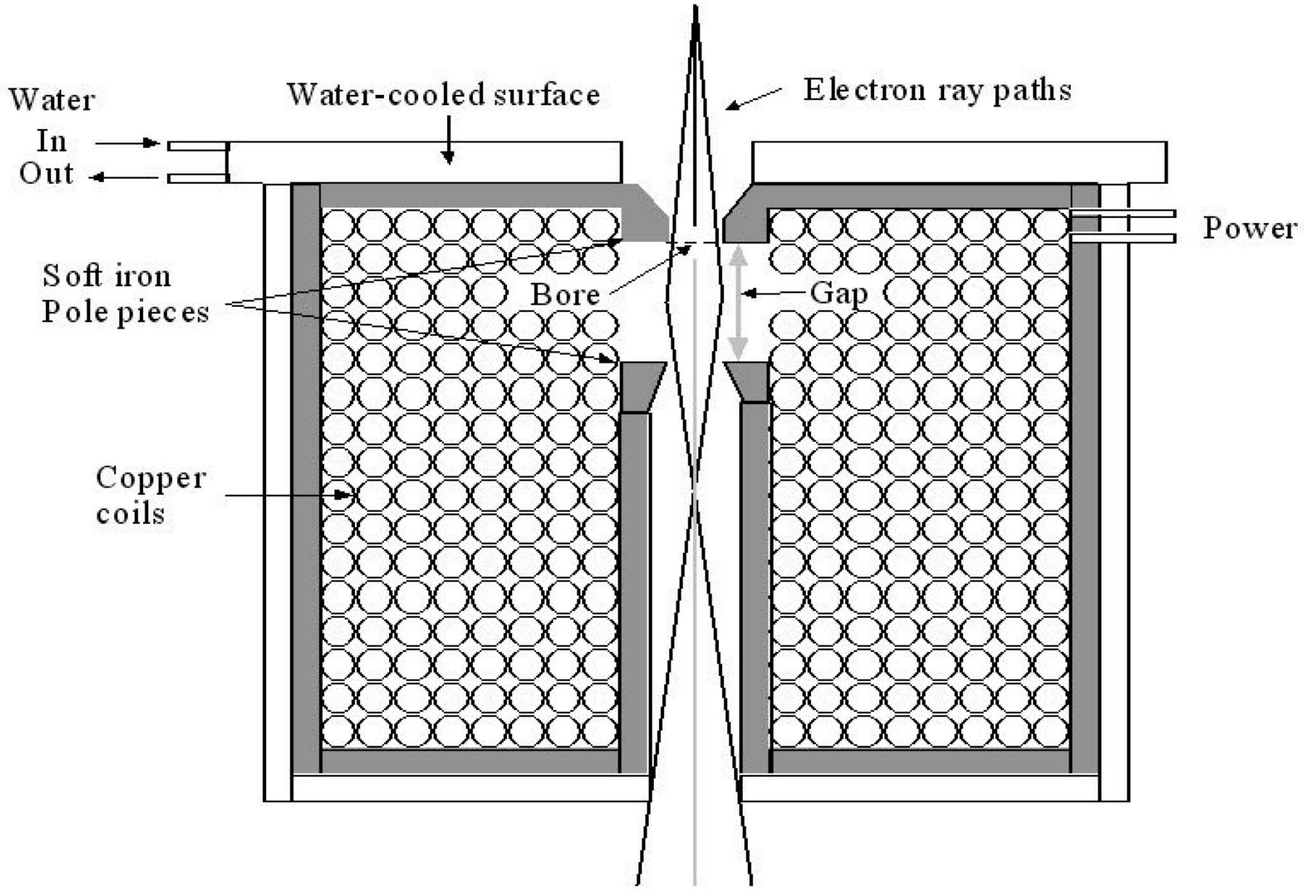


Magnetic Lens

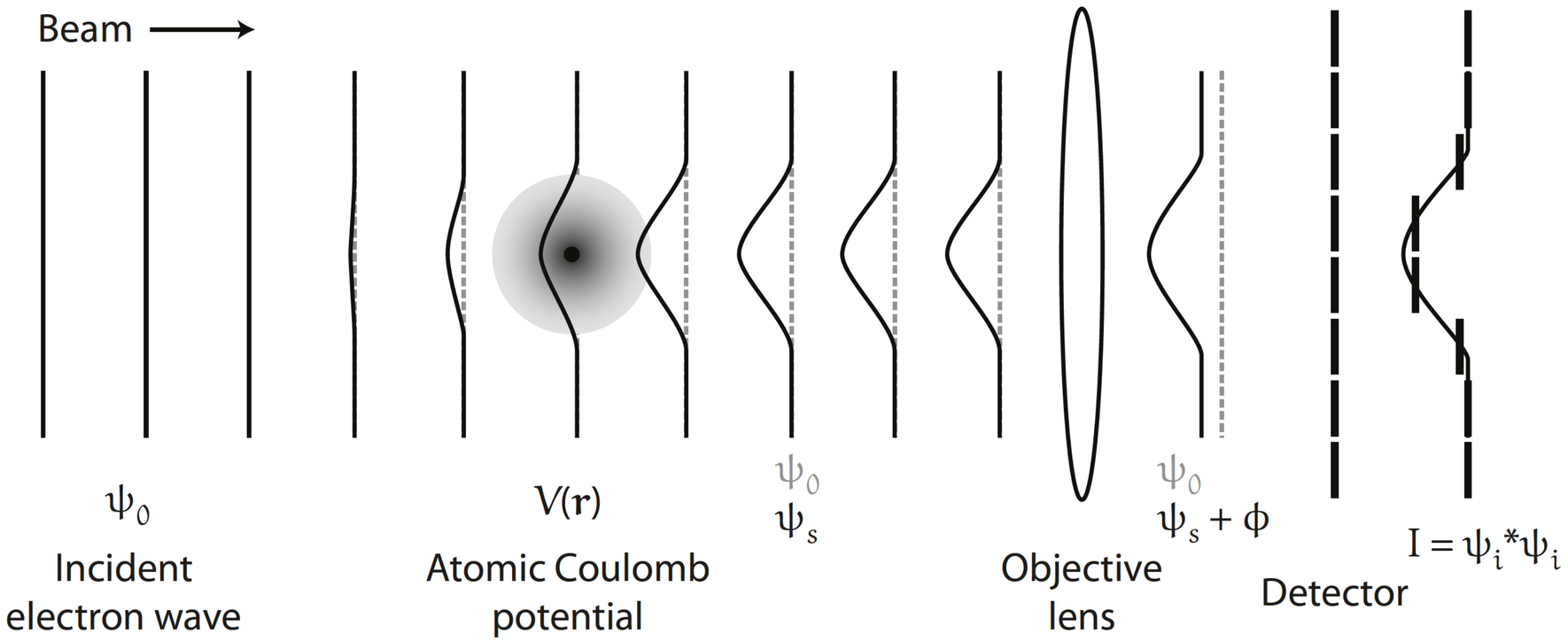
Concentrate flux

Field varies

Zero force on axial electrons



Phase contrast and the perfect objective lens



The objective lens is *far* from perfect

How bad is the objective lens?

Really bad

Electron lens aberrations

$$B(\mathbf{k}) = \exp\left[i\frac{2\pi}{\lambda}W(\mathbf{k})\right]$$

2.2: Description of aberration constants to 6th order

A_0	Lateral image shift
A_1	Two-fold astigmatism
C_1	Defocus
A_2	Three-fold astigmatism
B_2	Axial coma
A_3	Four-fold astigmatism
S_3	Axial star aberration
$C_3 = C_s$	Spherical aberration
A_4	Five-fold astigmatism
D_4	Three-lobe aberration
B_4	Fourth-order axial coma
A_5	Six-fold astigmatism
S_5	Fifth-order star aberration
C_5	Fifth-order spherical aberrat
R_5	Fifth-order rosette aberratior

$$\begin{aligned}W(\mathbf{k}) =& \Re\{A_0\lambda\mathbf{k}^* \\ &+ \frac{1}{2}A_1\lambda^2\mathbf{k}^{*2} + \frac{1}{2}C_1\lambda^2\mathbf{k}^*\mathbf{k} \\ &+ \frac{1}{3}A_2\lambda^3\mathbf{k}^{*3} + \frac{1}{3}B_2\lambda^3\mathbf{k}^{*2}\mathbf{k} \\ &+ \frac{1}{4}A_3\lambda^4\mathbf{k}^{*4} + \frac{1}{4}S_3\lambda^4\mathbf{k}^{*3}\mathbf{k} + \frac{1}{4}C_3\lambda^4\mathbf{k}^{*2}\mathbf{k}^2 \\ &+ \frac{1}{5}A_4\lambda^5\mathbf{k}^{*5} + \frac{1}{5}D_4\lambda^5\mathbf{k}^{*4}\mathbf{k} + \frac{1}{5}B_4\lambda^5\mathbf{k}^{*3}\mathbf{k}^2 \\ &+ \frac{1}{6}A_5\lambda^6\mathbf{k}^{*6} + \frac{1}{6}S_5\lambda^6\mathbf{k}^{*4}\mathbf{k}^2 + \frac{1}{6}C_5\lambda^6\mathbf{k}^{*3}\mathbf{k}^3 + \frac{1}{6}R_5\lambda^6\mathbf{k}^{*5}\mathbf{k} + \cdots\end{aligned}$$

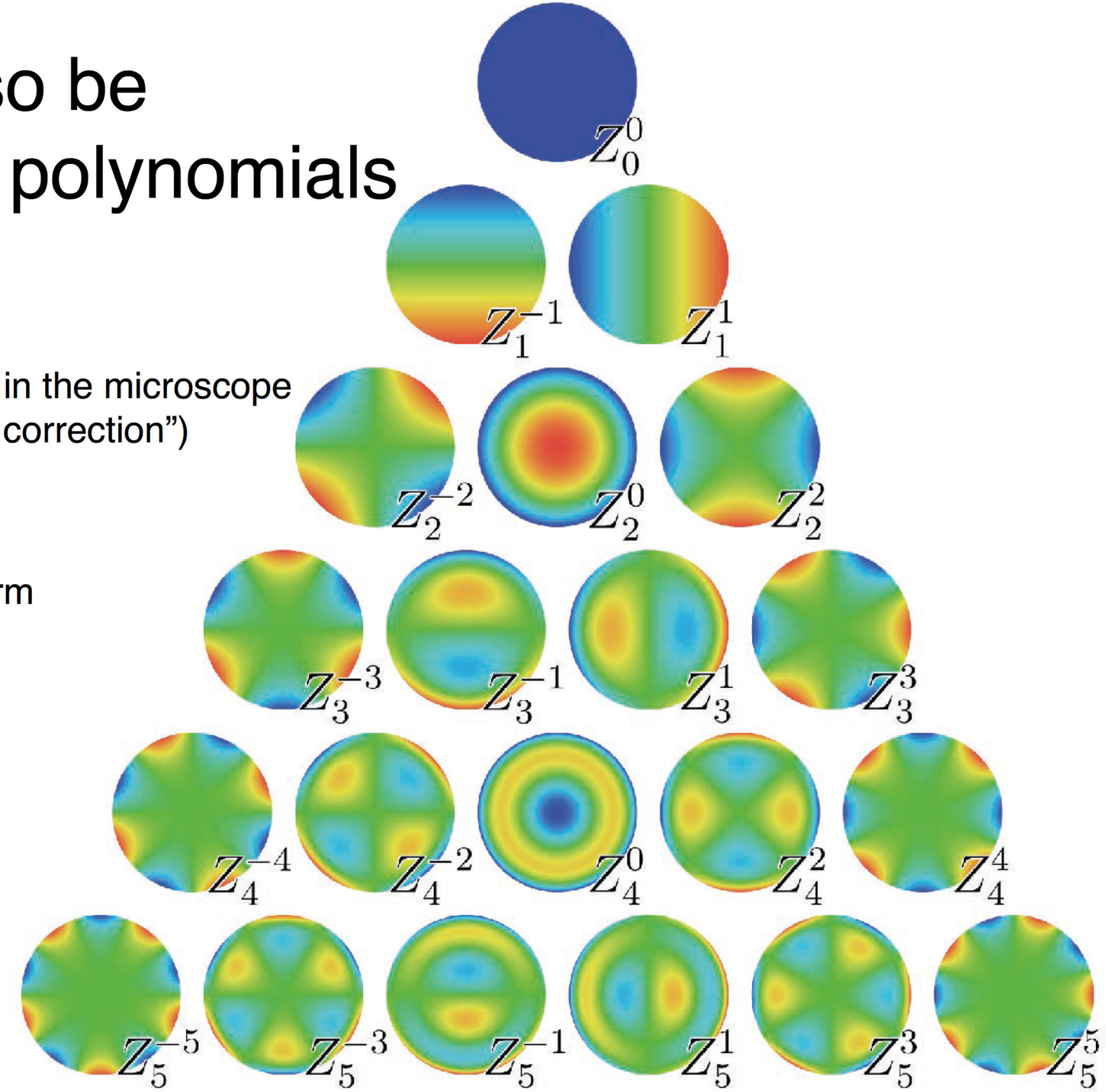
Lens aberrations can also be visualized using Zernike polynomials

Aberrations are corrected with additional lenses in the microscope or in software after the image is collected (“CTF correction”)

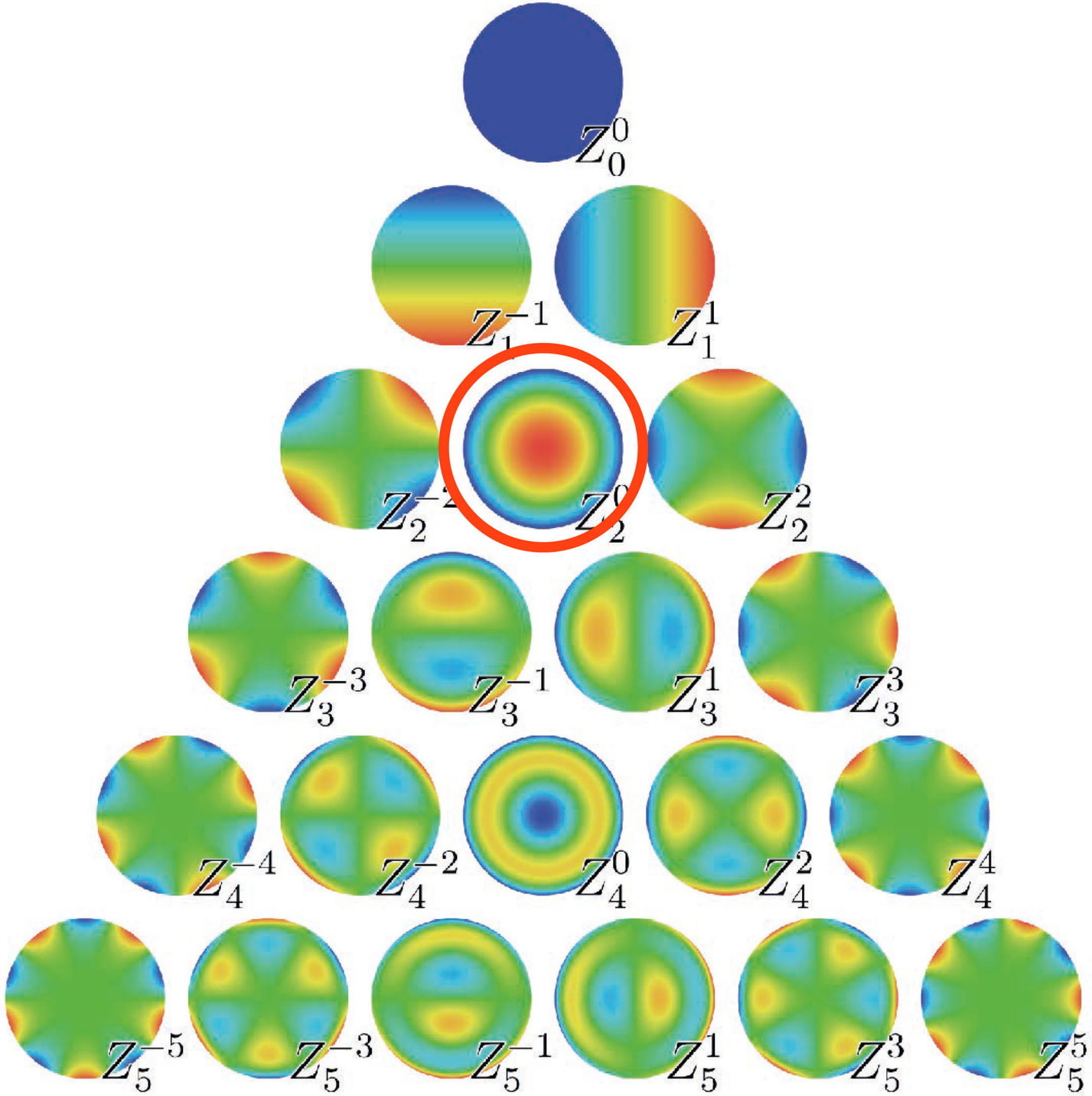
Complete set of orthogonal functions

Zernike transform analogous to Fourier transform

Frits Zernike,
1953 Nobel Prize in Physics
inventor of phase contrast microscopy



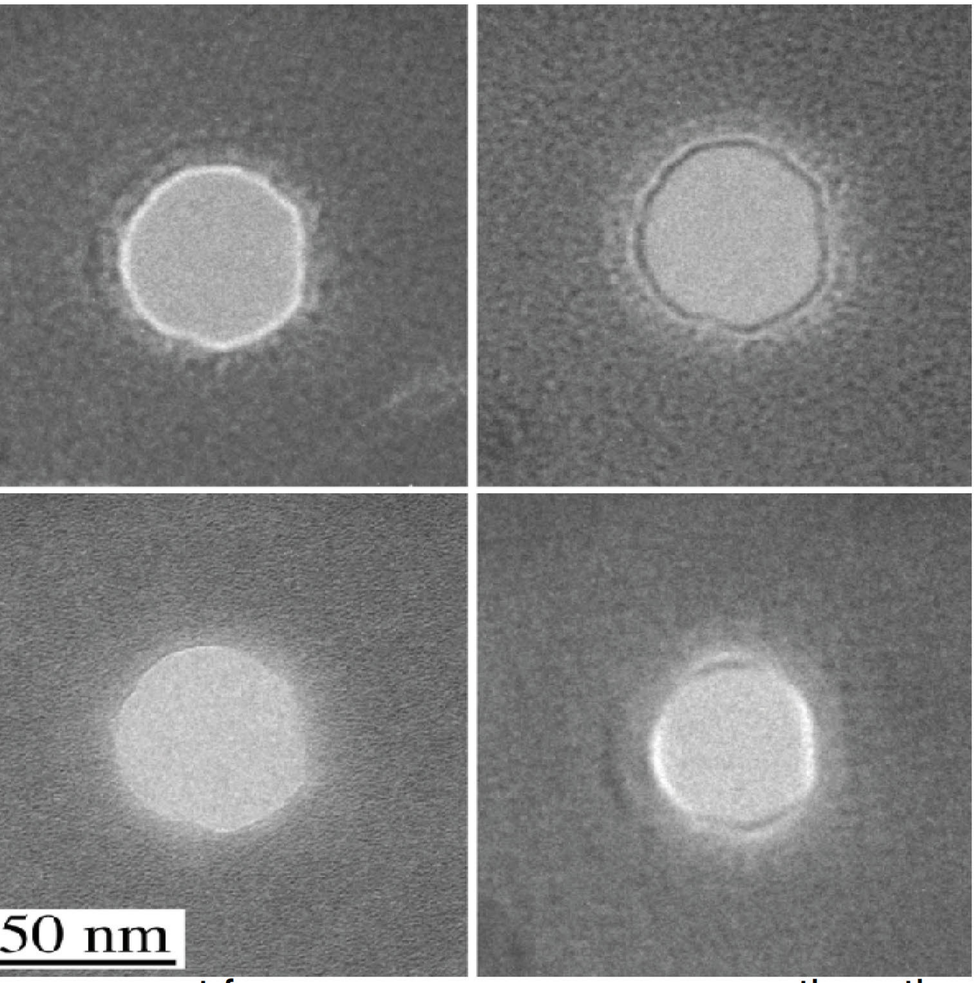
Defocus


Z_0^0
Z_1^{-1}
Z_1^1
Z_2^{-2}
Z_2^0
Z_2^2
Z_3^{-3}
Z_3^{-1}
Z_3^1
Z_3^3
Z_4^{-4}
Z_4^{-2}
Z_4^0
Z_4^2
Z_4^4
Z_5^{-5}
Z_5^{-3}
Z_5^{-1}
Z_5^1
Z_5^3
Z_5^5

Focus terminology

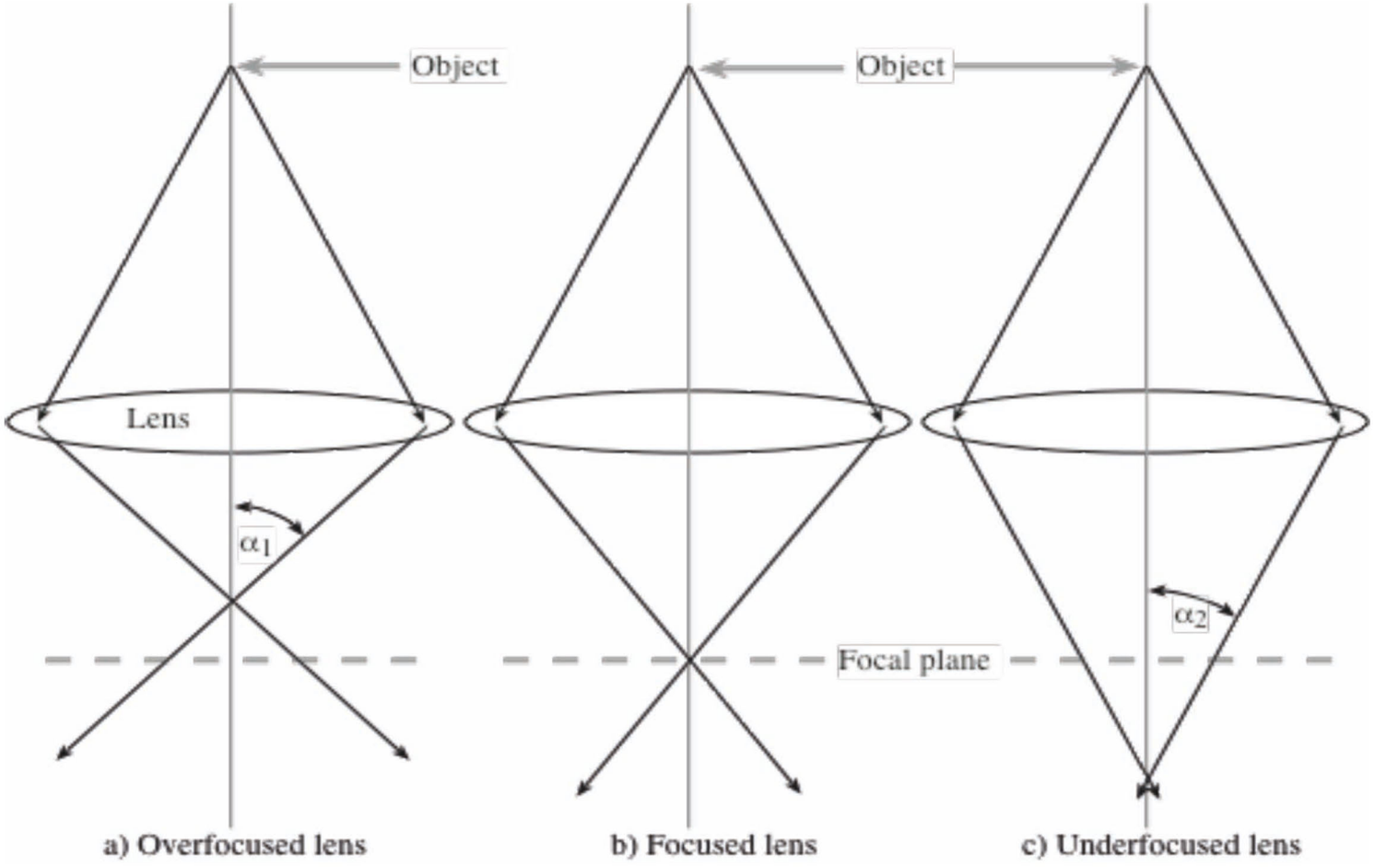
underfocus

overfocus



exact focus

astigmatism

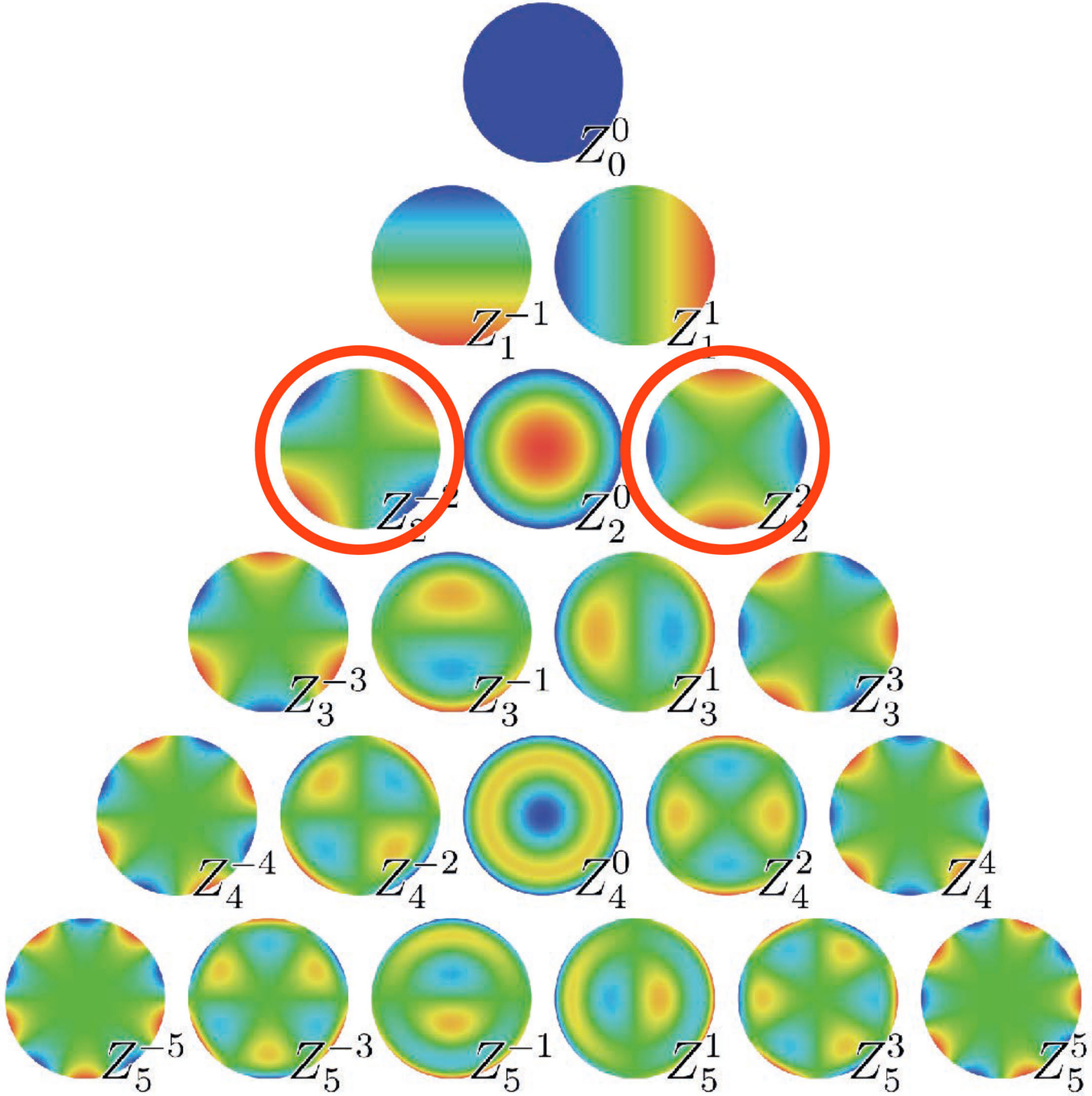


Too strong

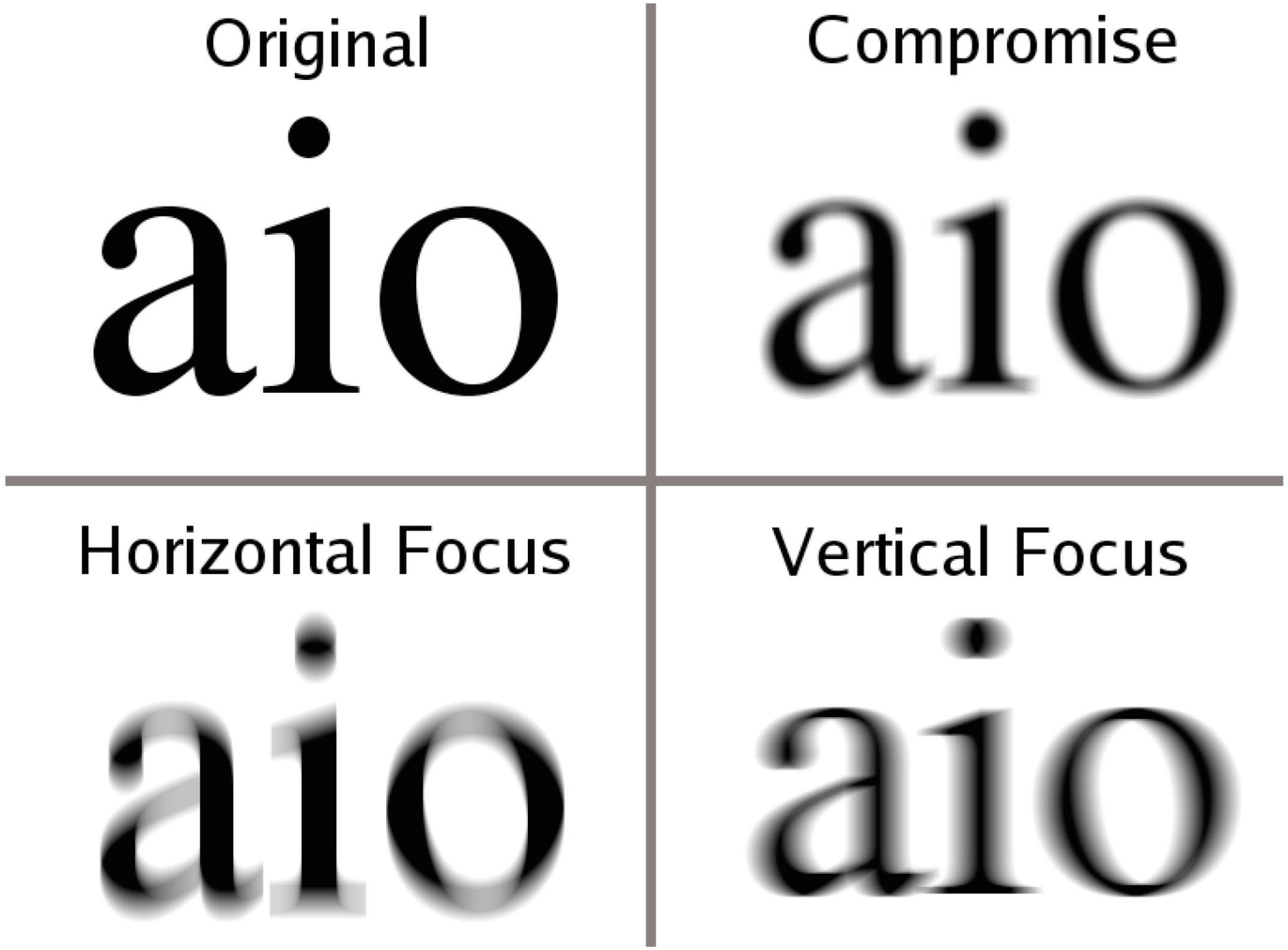
Just right

Too weak

Astigmatism



Astigmatism (example)



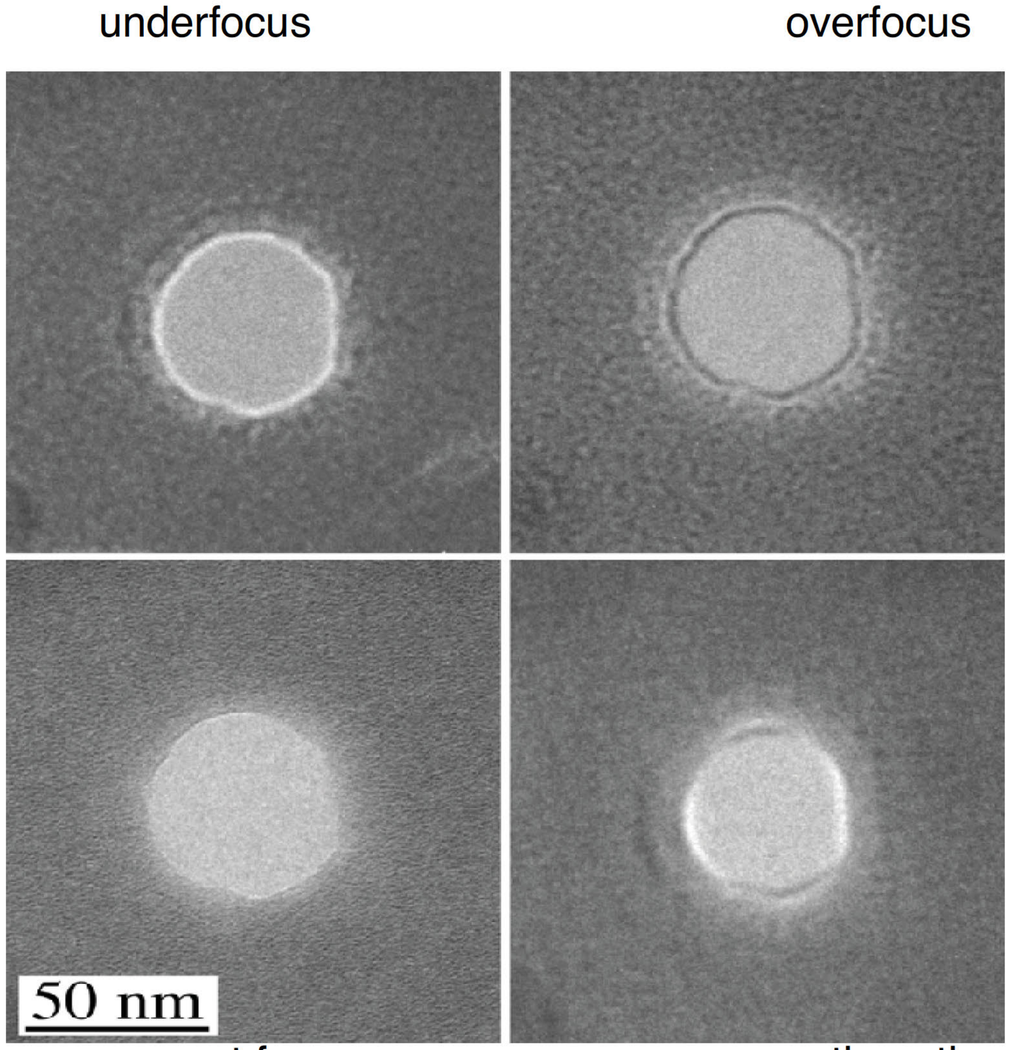
Astigmatism Correction

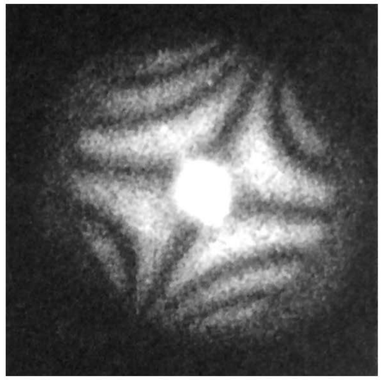
Correcting the
astigmatism
on the objective lens

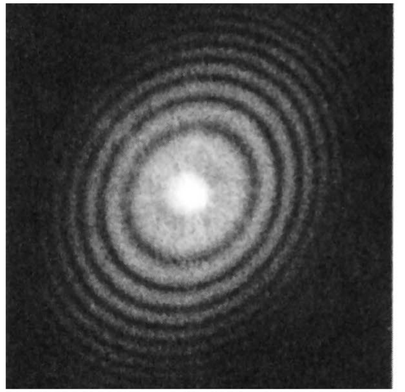
Routine alignment using
Fresnel fringe

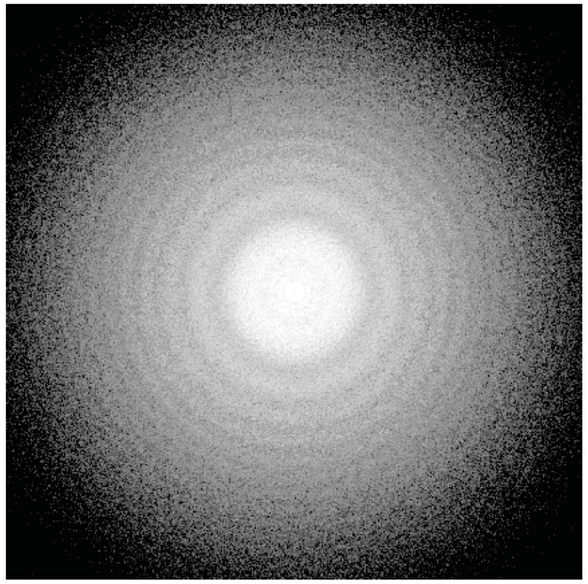
More accurate with FFT

Remember to
correct the
condenser lens too





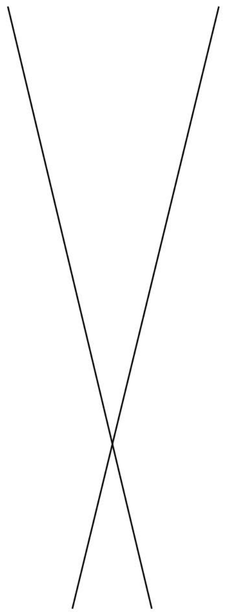




Beam Astigmatism Correction

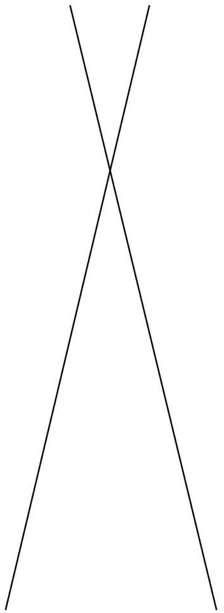
Just change focus

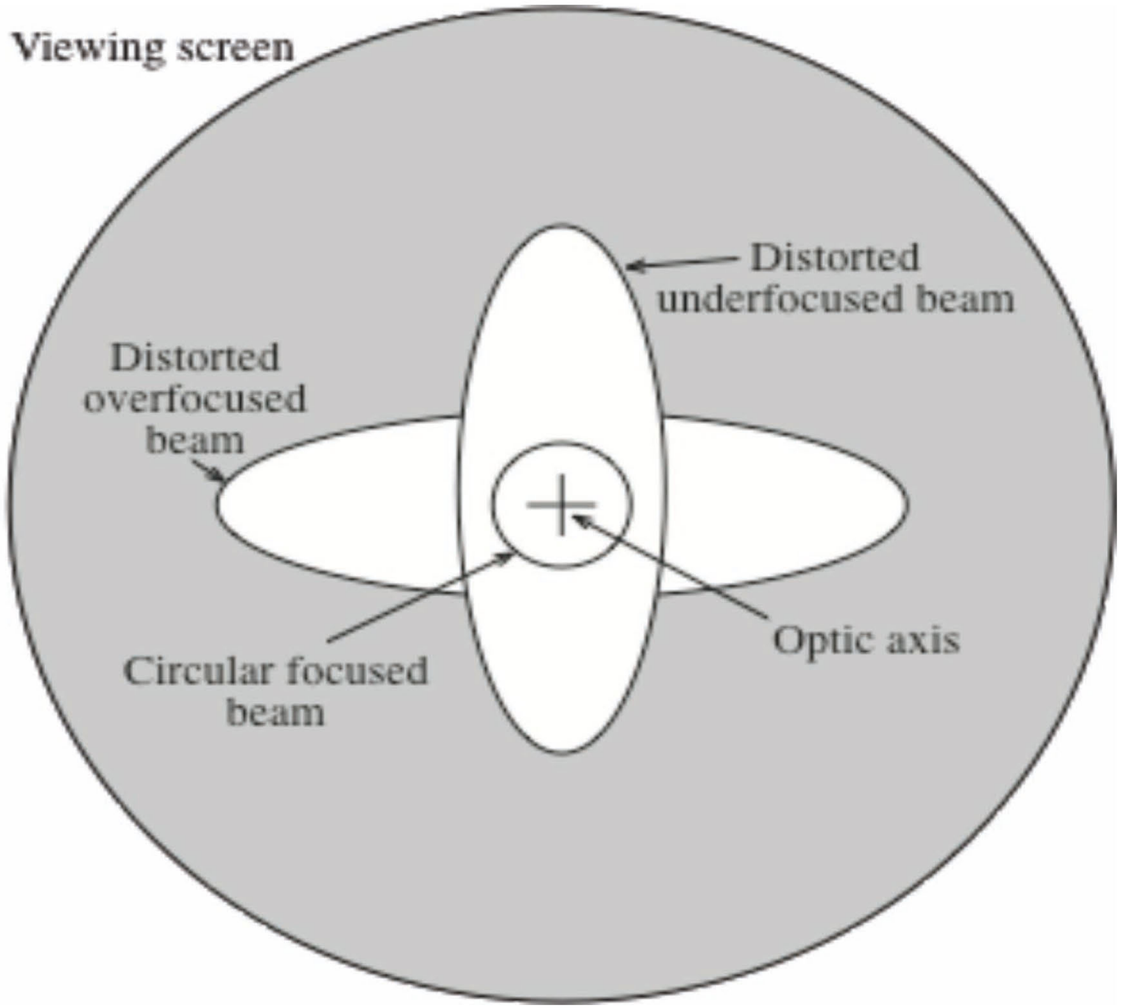
Underfocus



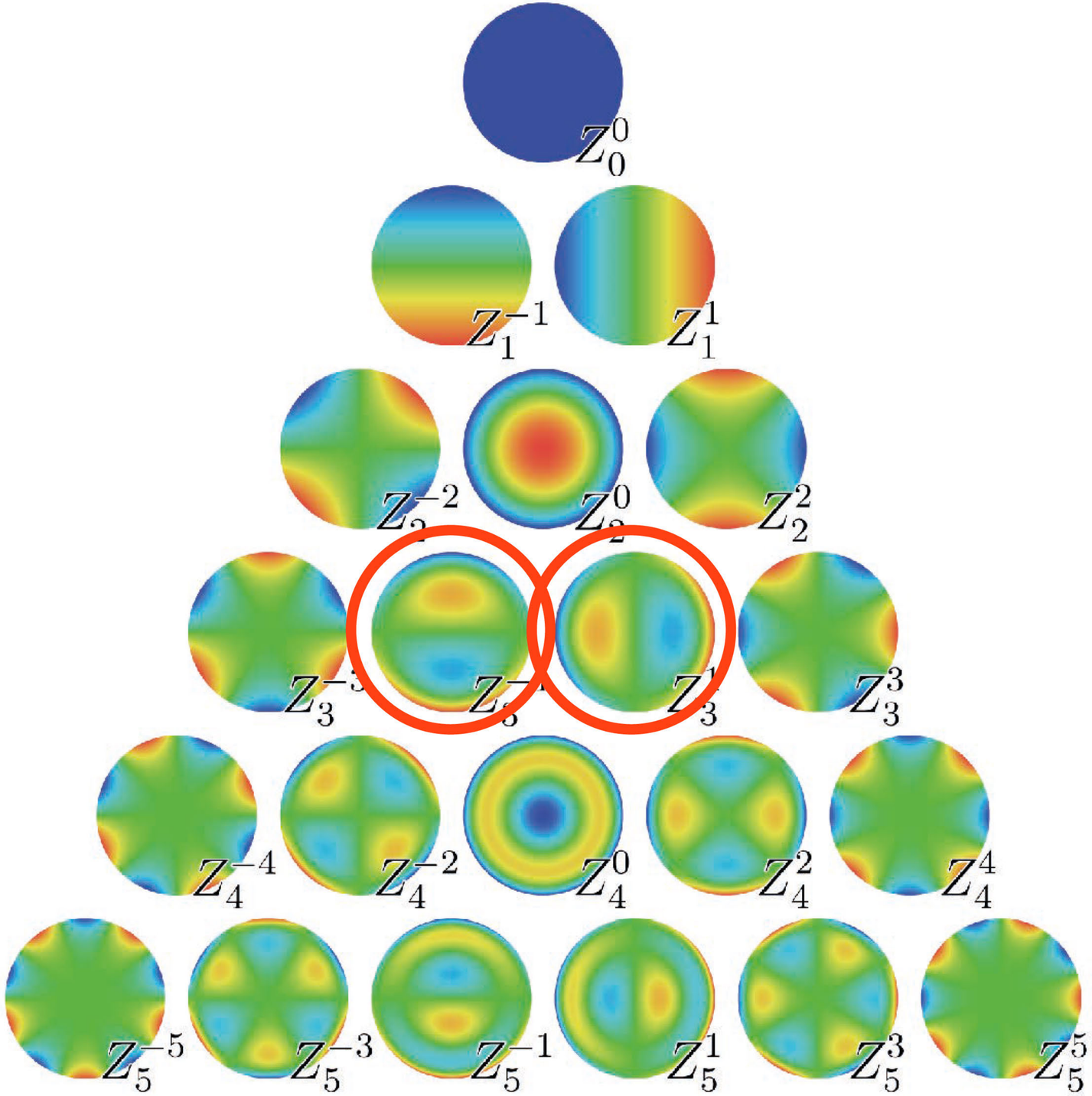
Overfocus

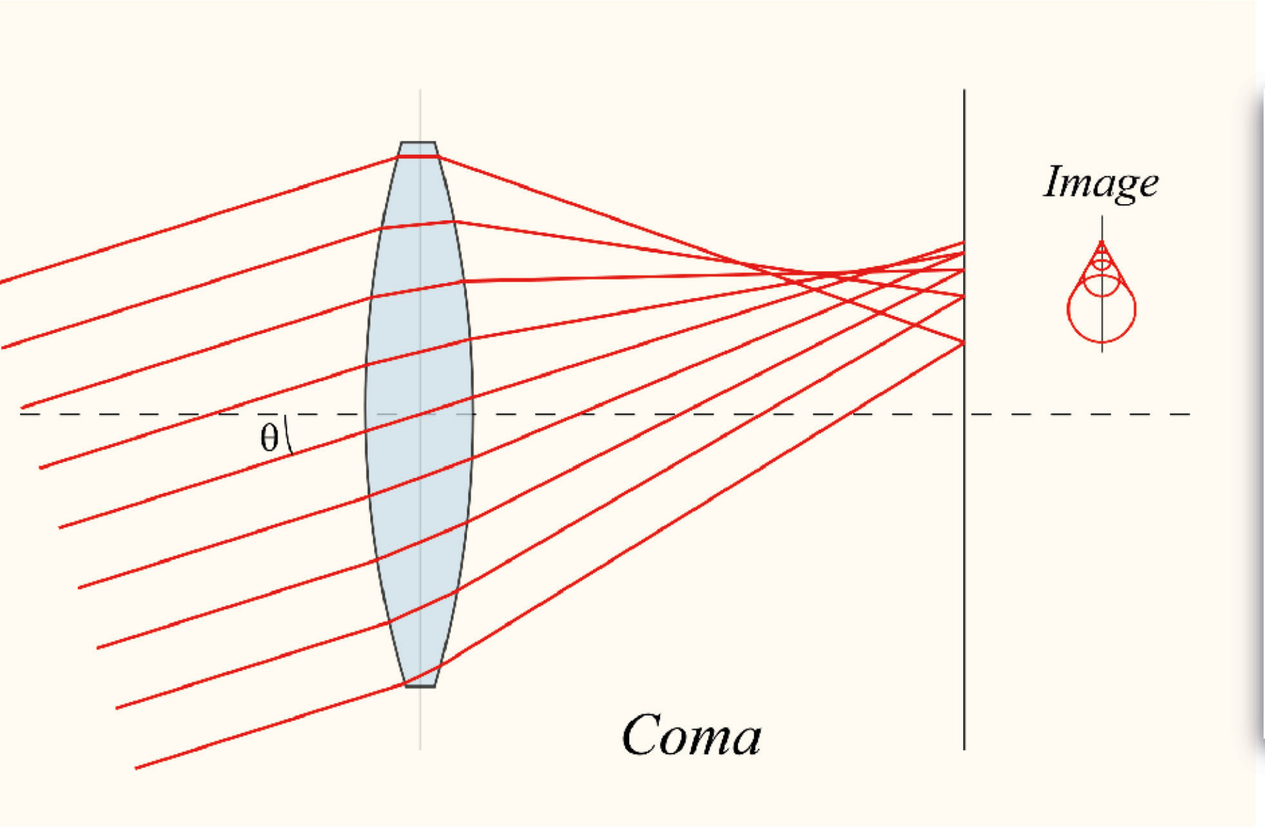
90°->

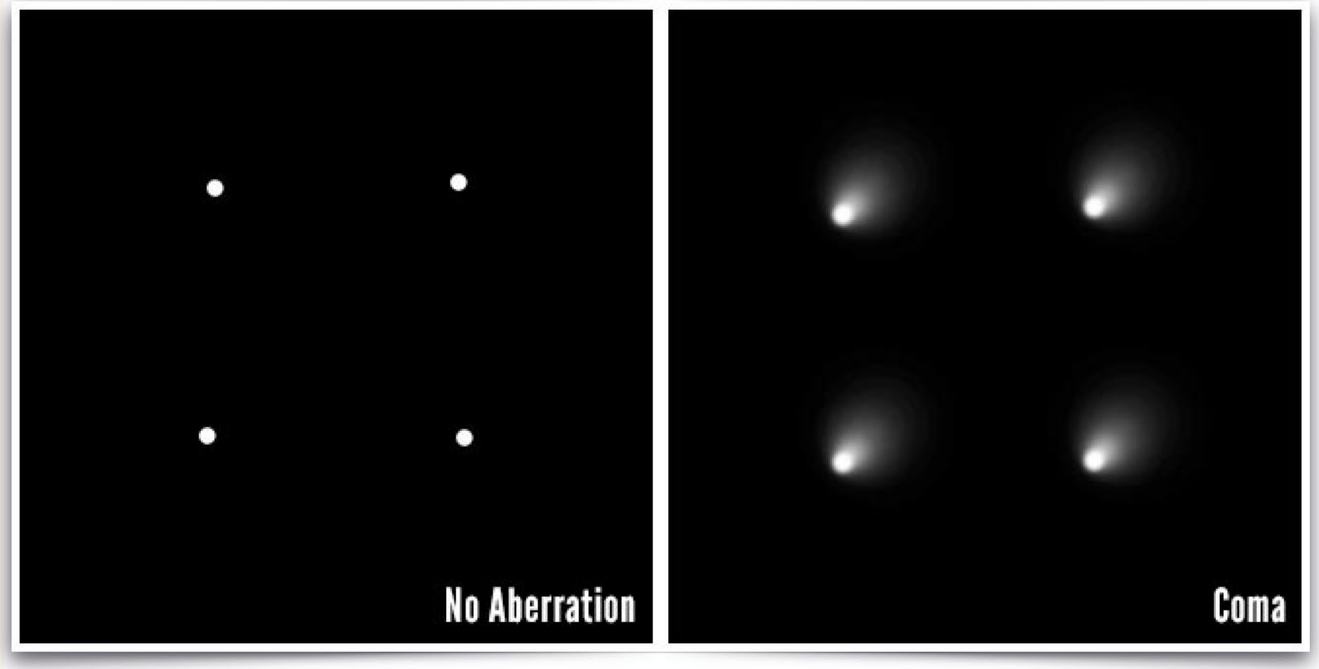




Coma


Z_0^0
Z_1^{-1}
Z_1^1
Z_2^{-2}
Z_2^0
Z_2^2
Z_3^{-3}
Z_3^{-1}
Z_3^1
Z_3^3
Z_4^{-4}
Z_4^{-2}
Z_4^0
Z_4^2
Z_4^4
Z_5^{-5}
Z_5^{-3}
Z_5^{-1}
Z_5^1
Z_5^3
Z_5^5

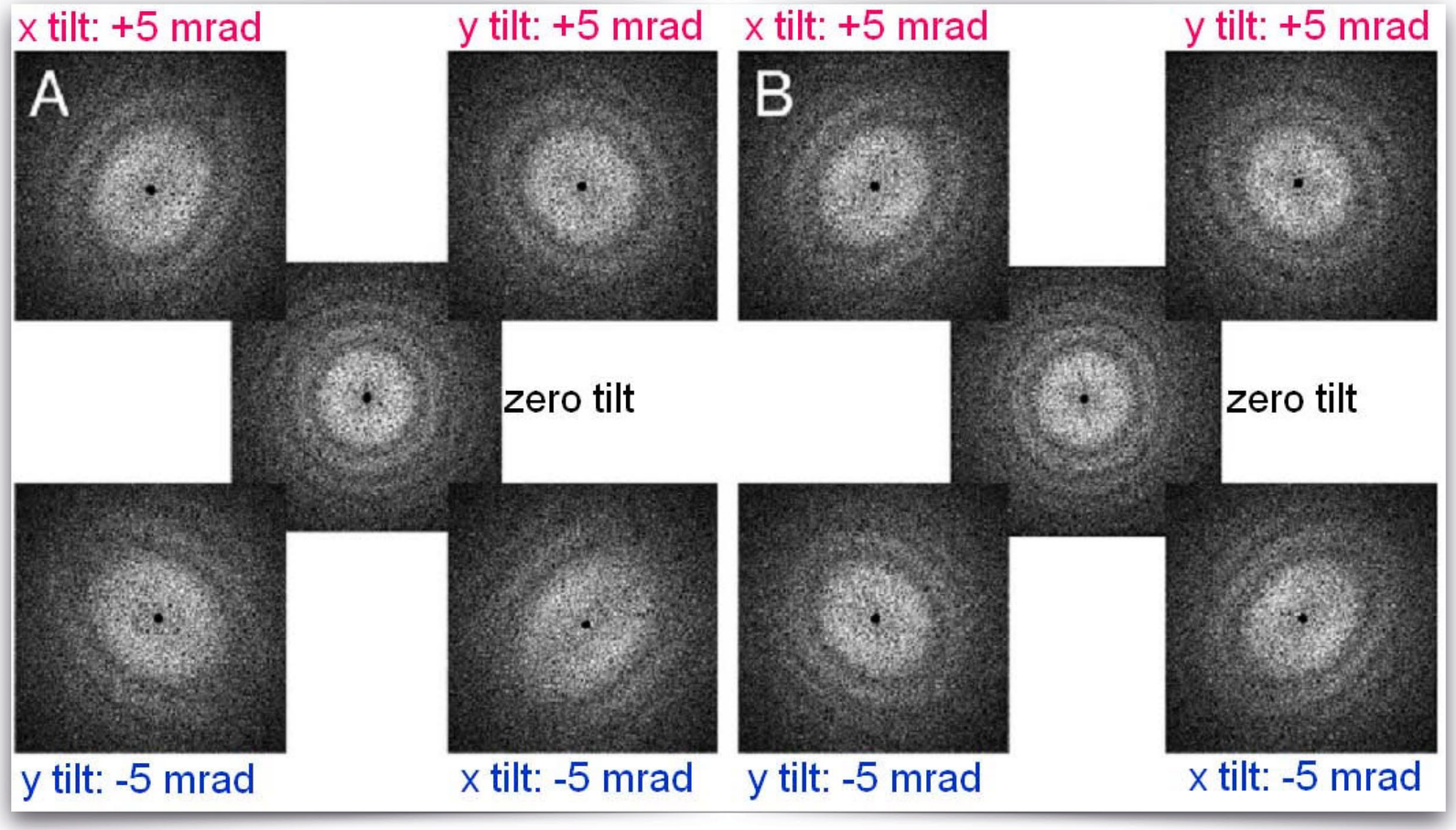




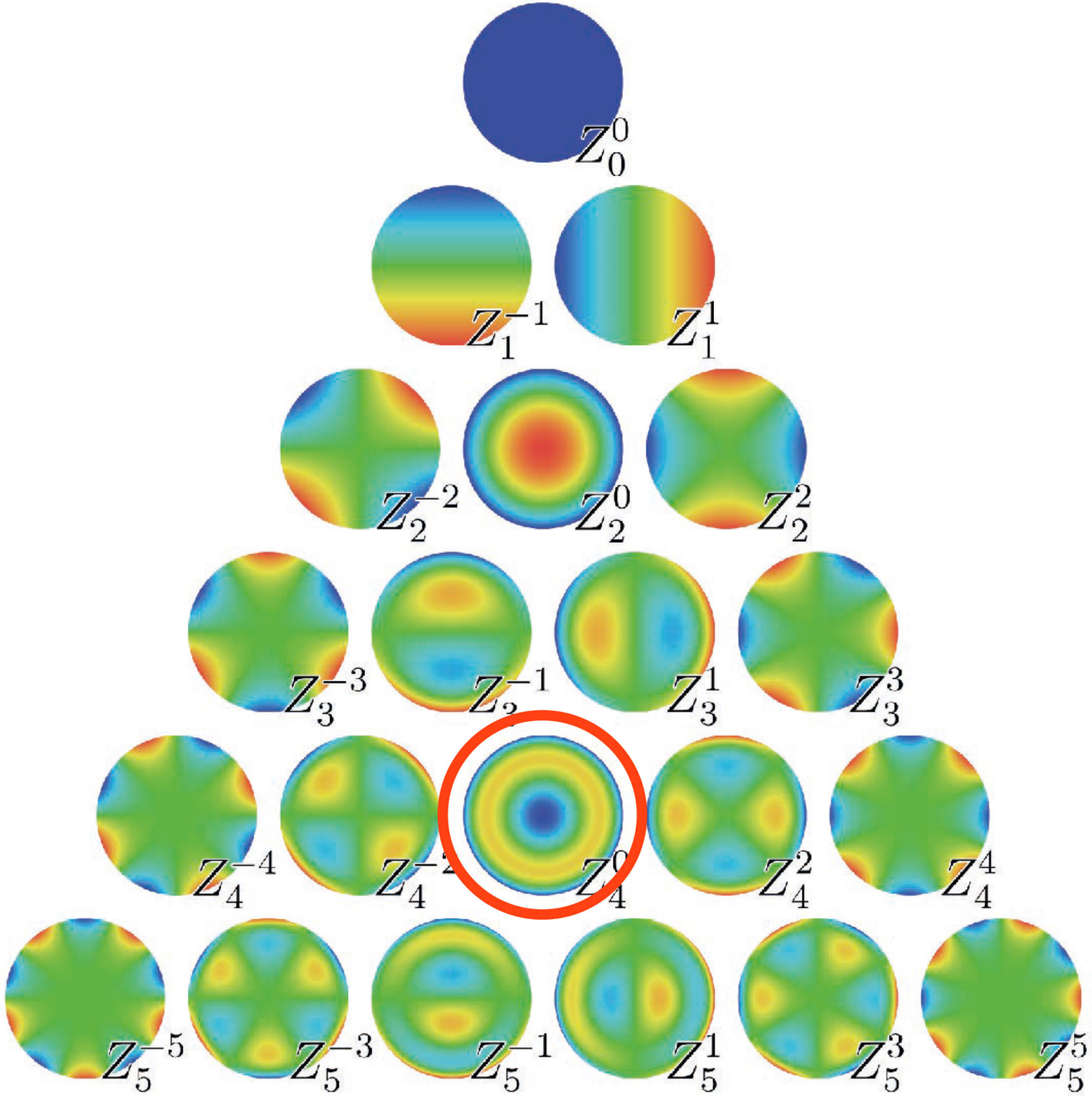
Example image from I. Norman

Reducing coma by minimising beam tilt

1. Voltage centring
2. Current centring
3. Zemlin Tableaux



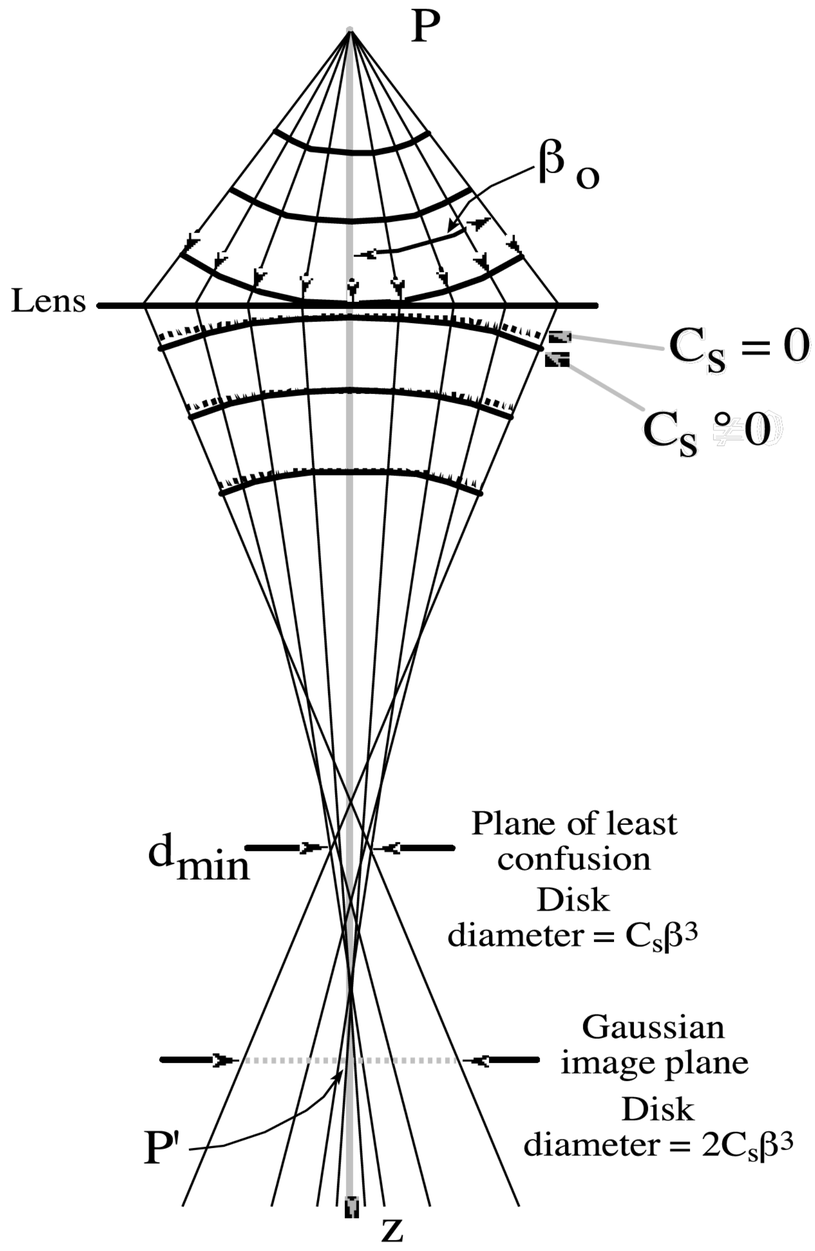
Spherical aberration



Spherical Aberration

Lens is stronger off axis

Plane of least confusion



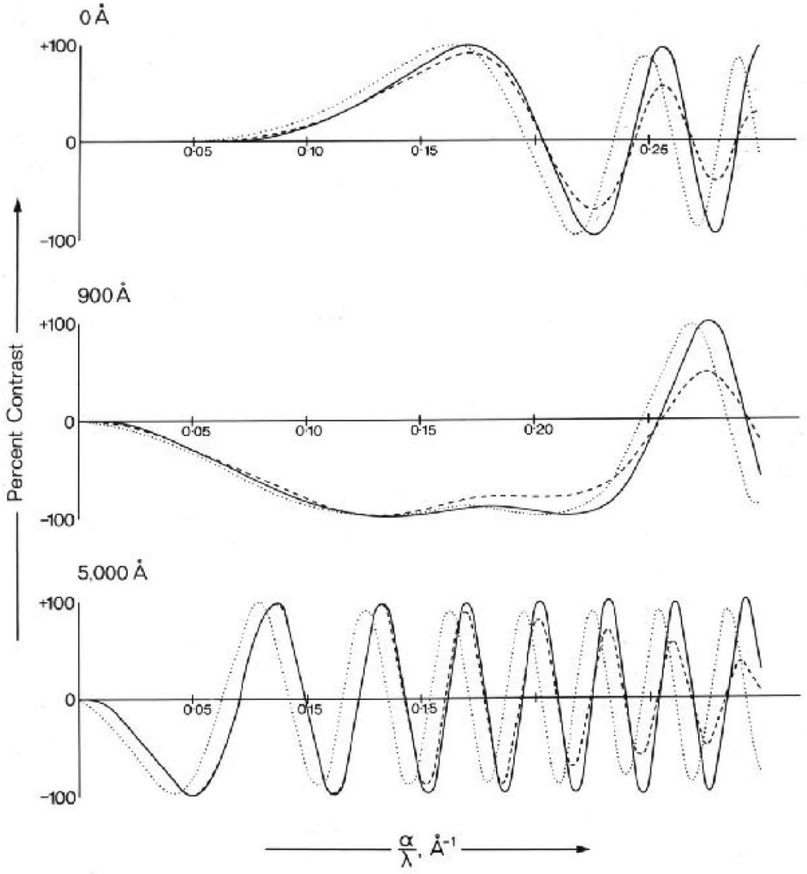
CTF

Contrast
Transfer
Function

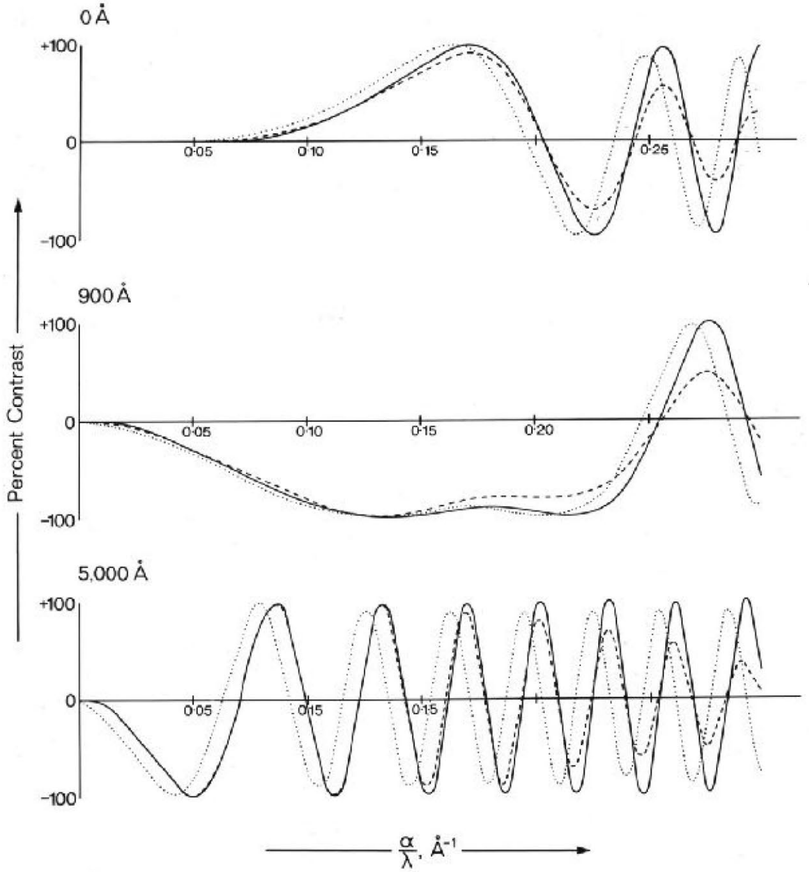
Phil. Trans. Roy. Soc. Lond. B. **261**, 105–118 (1971) [105]
Printed in Great Britain

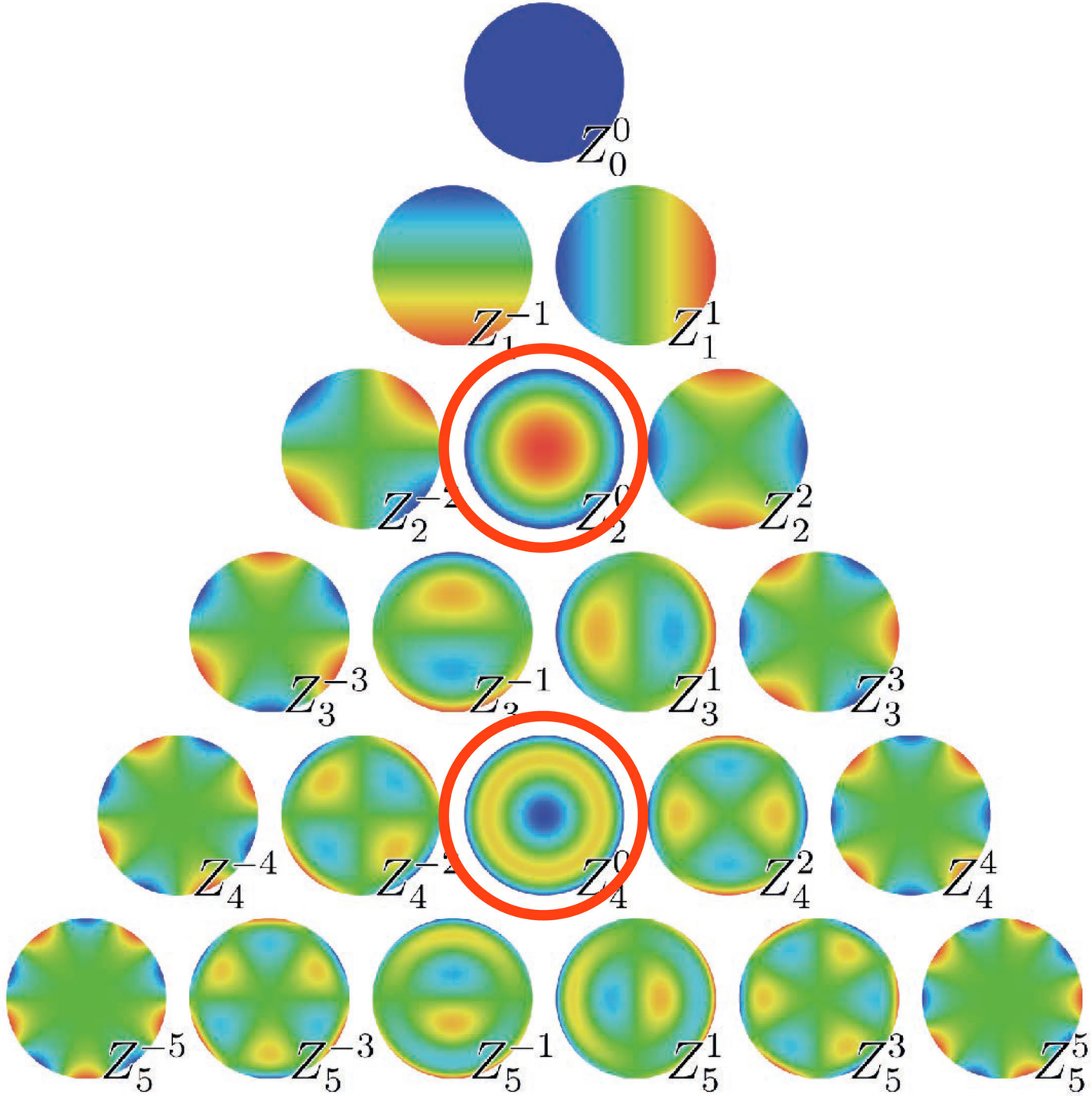
Measurement and compensation of defocusing and aberrations by Fourier processing of electron micrographs

By H. P. Erickson and A. Klug, F.R.S.
Medical Research Council Laboratory of Molecular Biology, Cambridge



Correct with software instead: CTFFIND, GCTF or similar

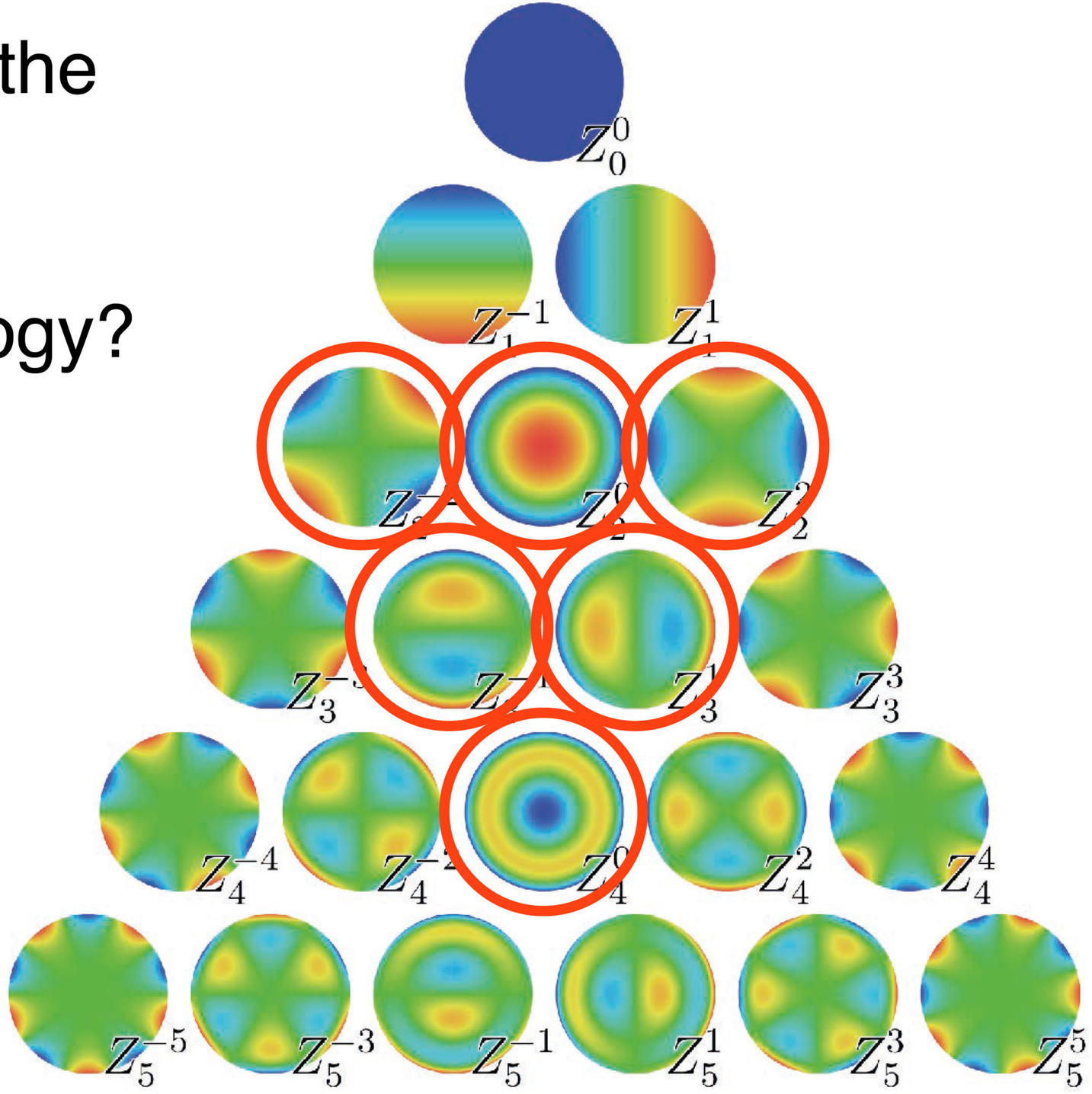




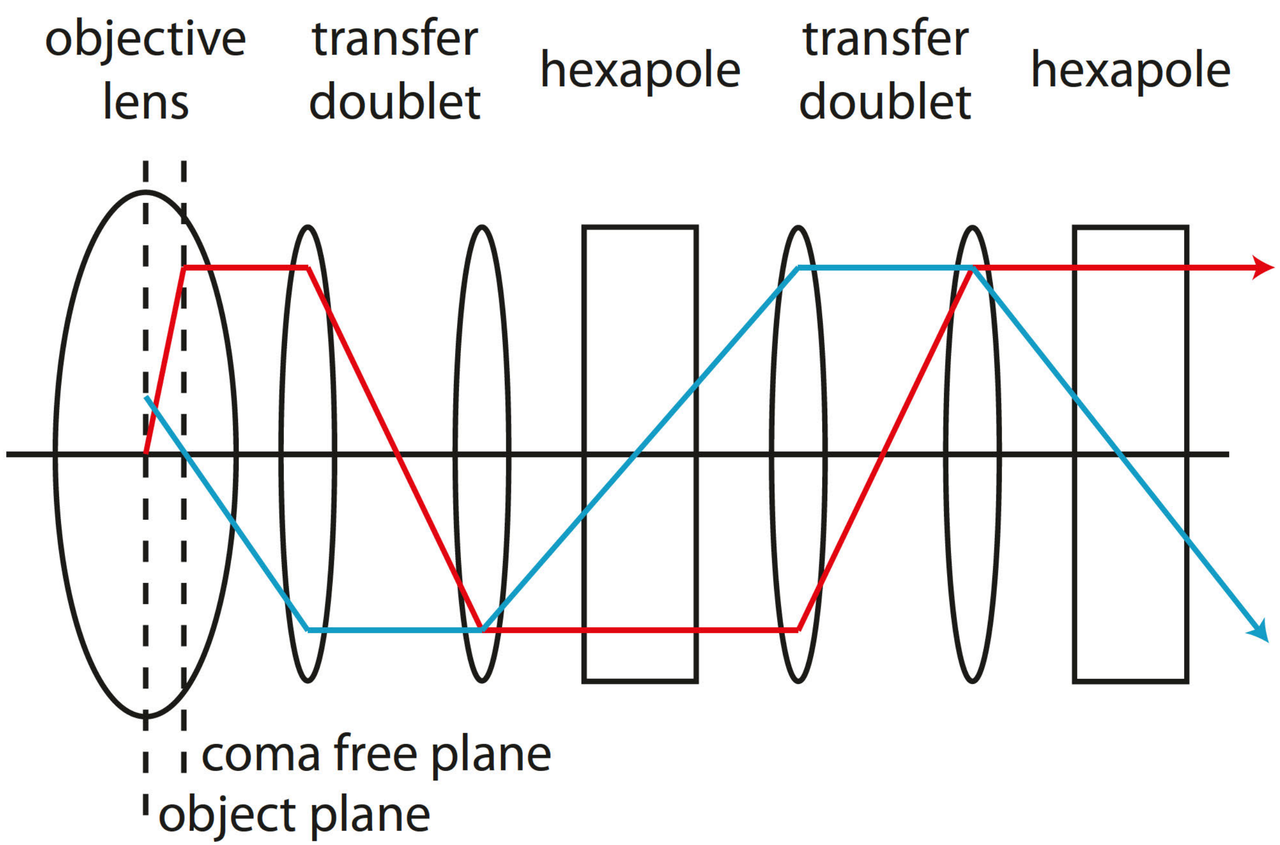
What about the rest of the Lens aberrations?

Do they matter for biology?

not till < 2 Å



Aberration corrector



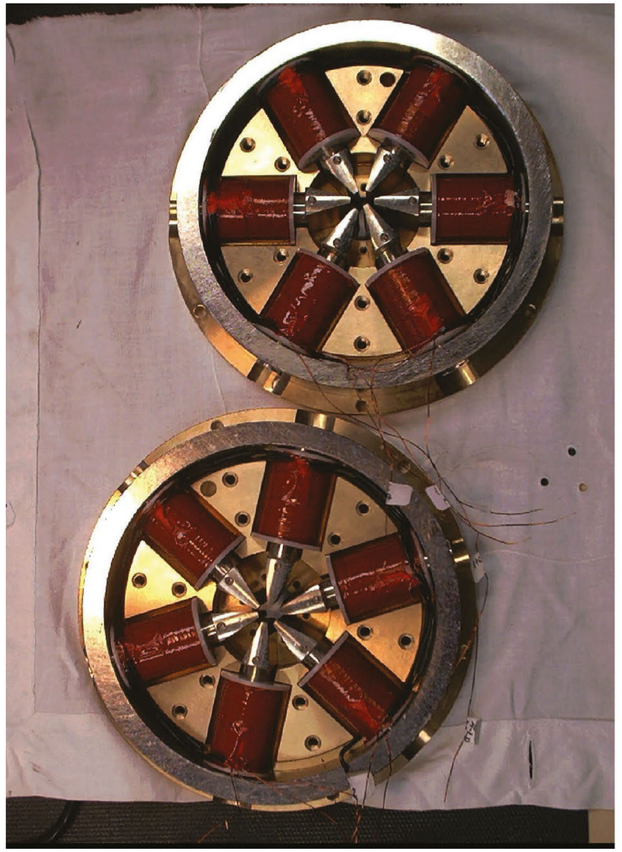
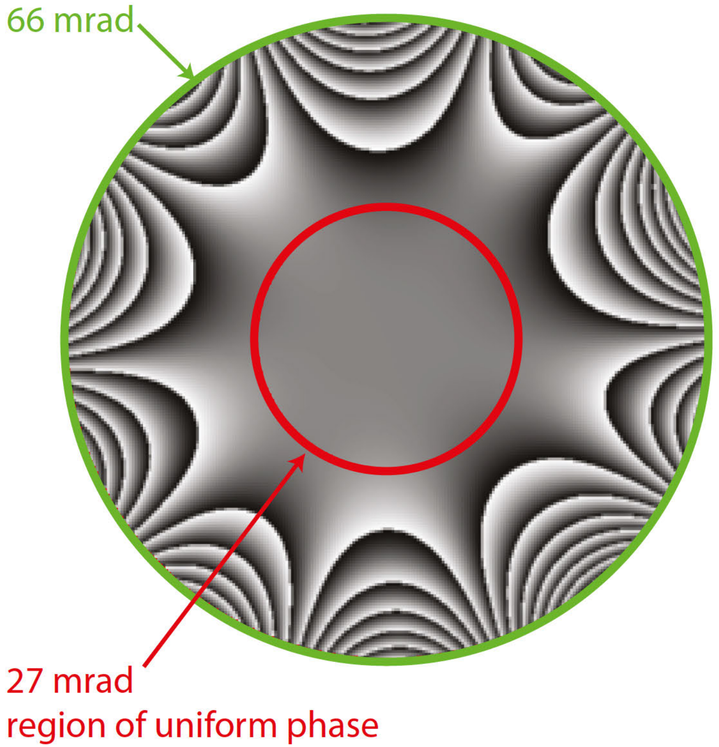


Image courtesy of M. Haider

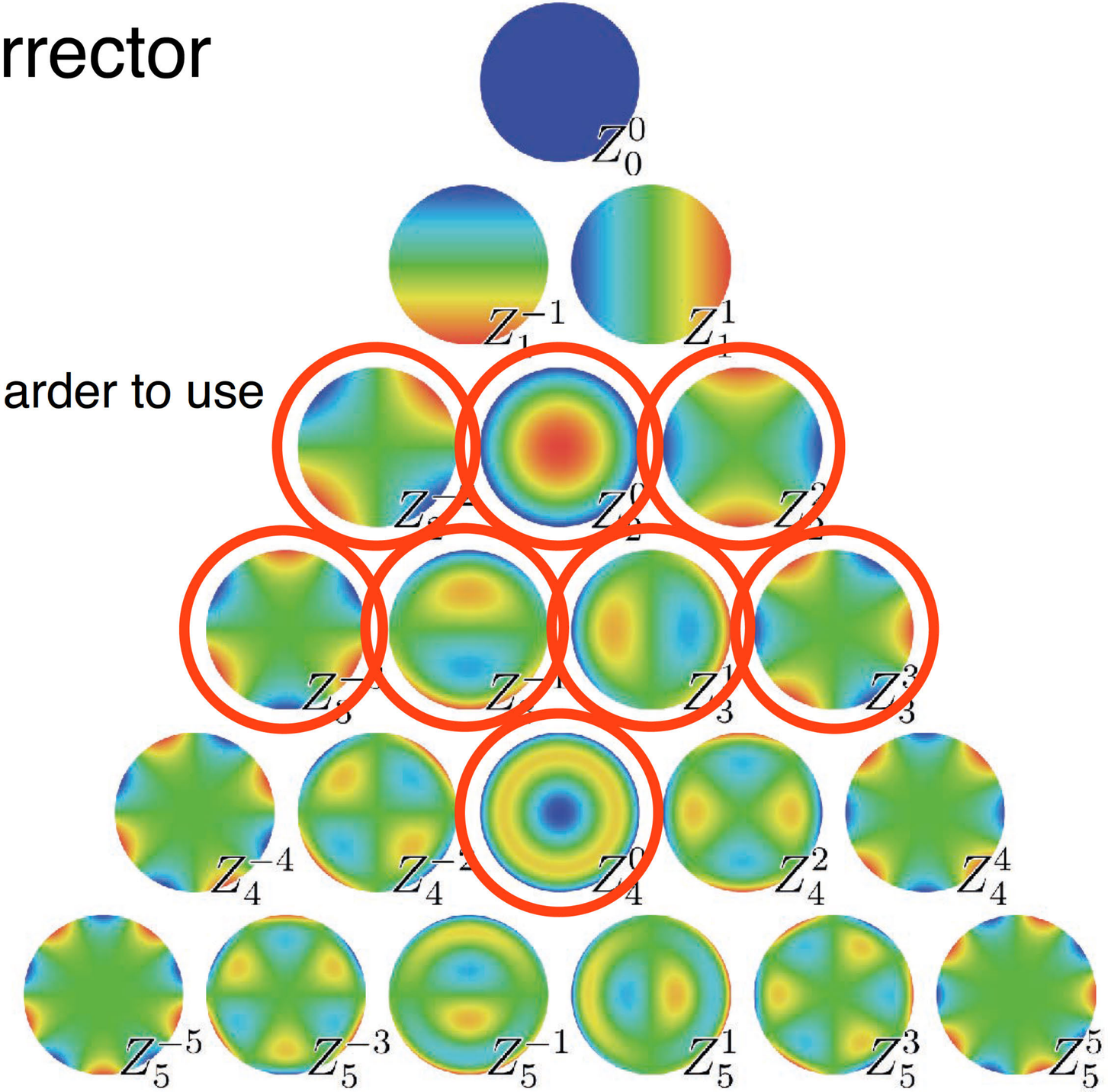
3rd order aberration corrector

for going from 2 Å to 0.5 Å
or low energies (< 100 keV)

expensive, slows data collection, harder to use
no advantage for most projects

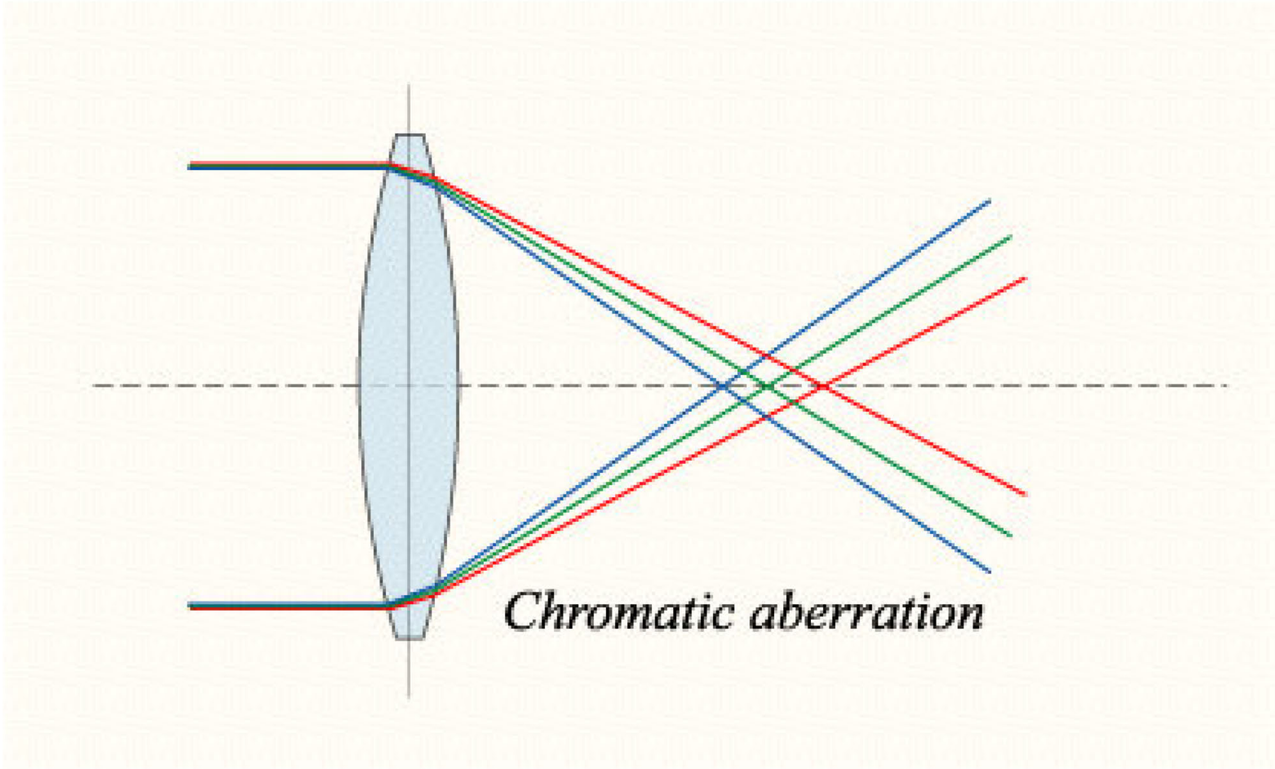


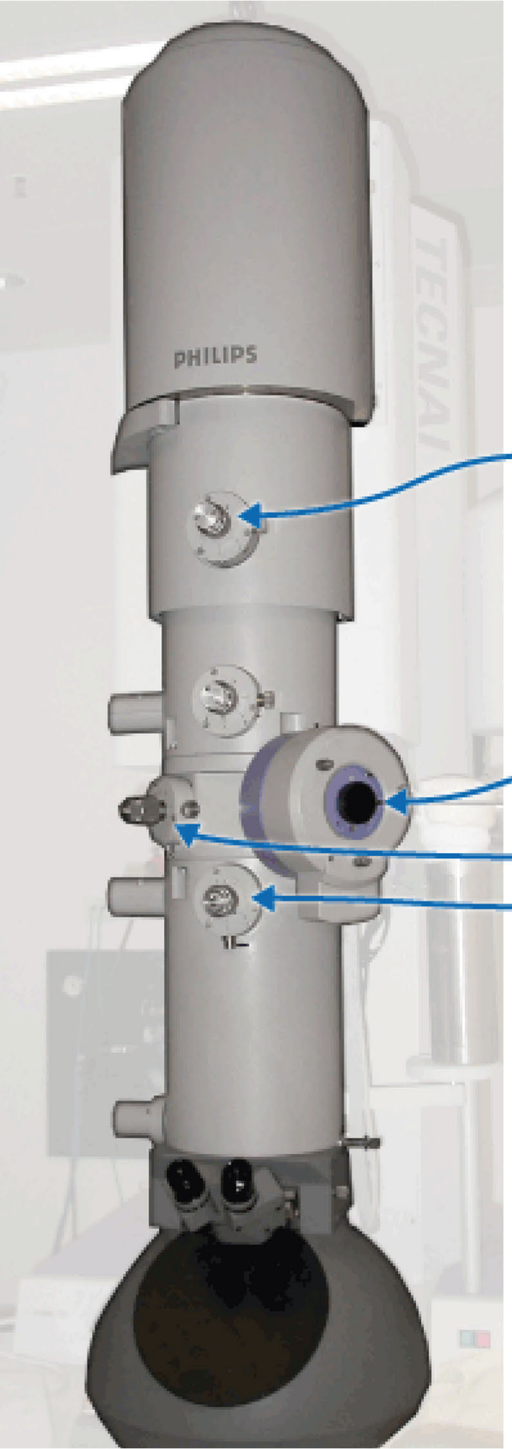
Russo 2010



Chromatic Aberration

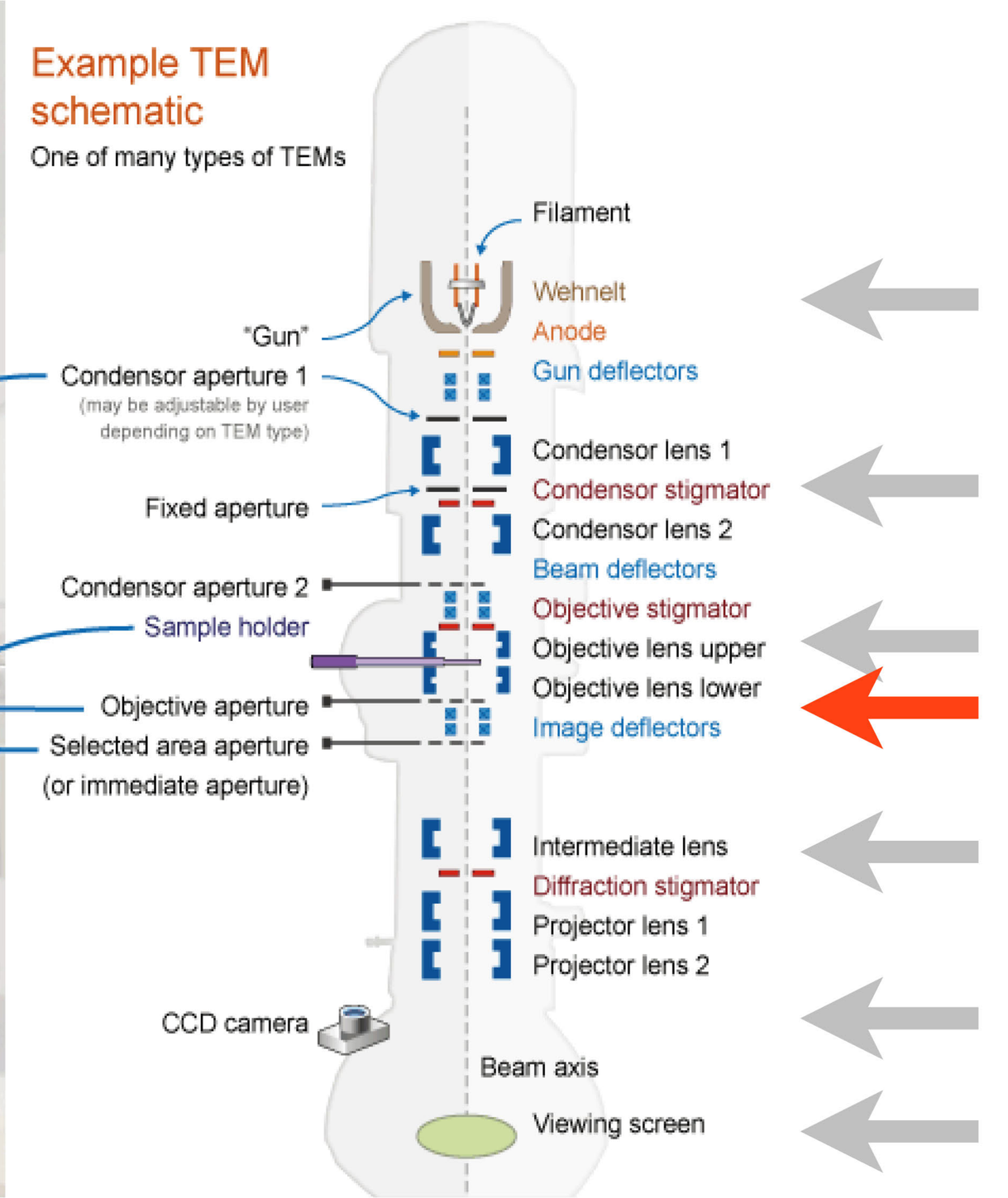
- Different wavelengths focus at different planes

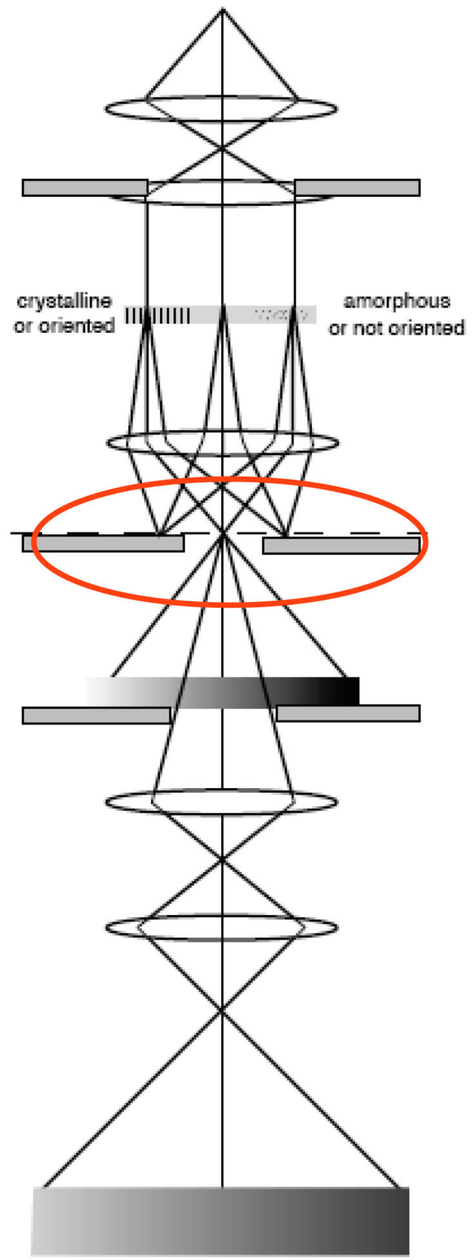




Example TEM schematic

One of many types of TEMs





anode

condenser lens 1

condenser lens 2
condenser diaphragm

sample

objective lens

objective diaphragm
(back focal plane)

selected area diaphragm

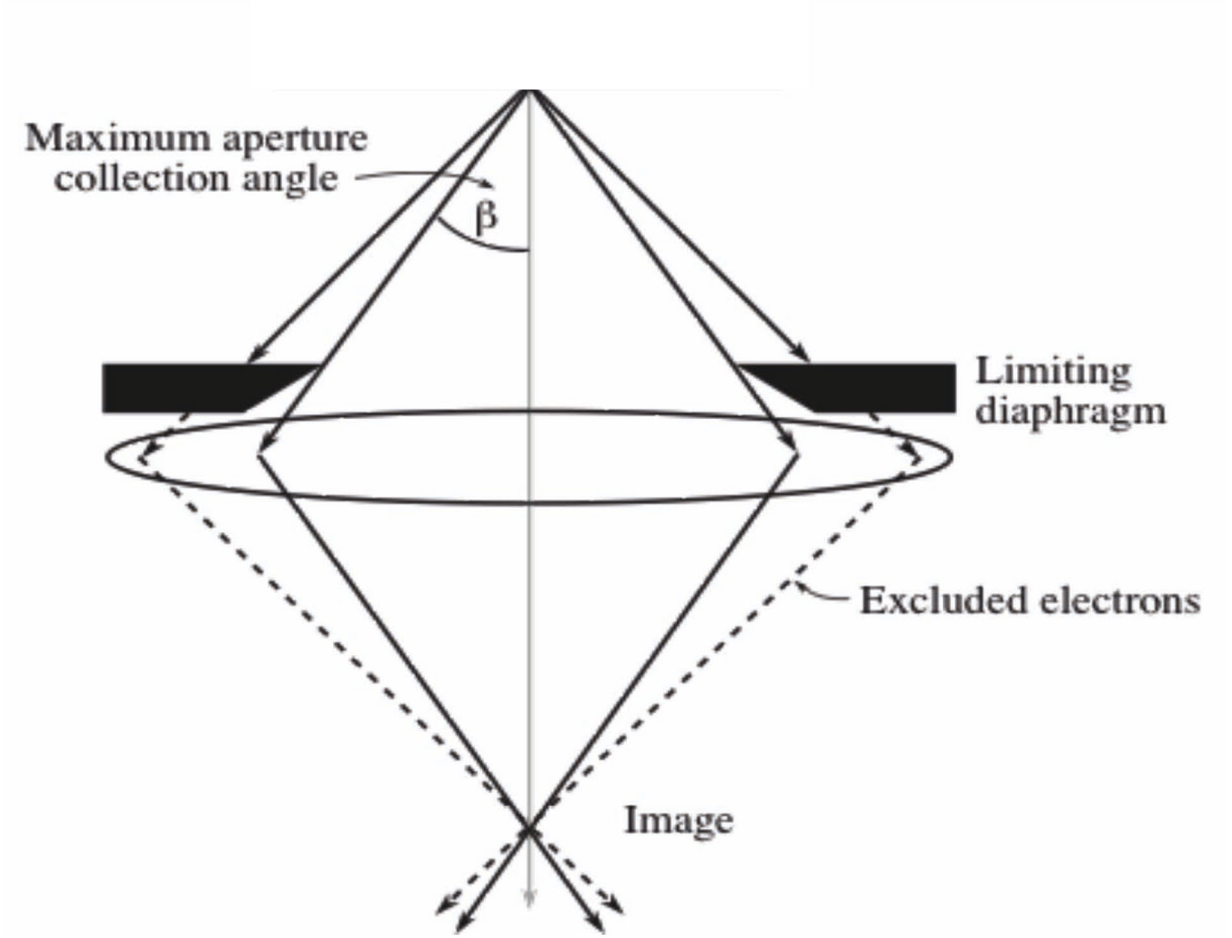
intermediate lens

projective lens

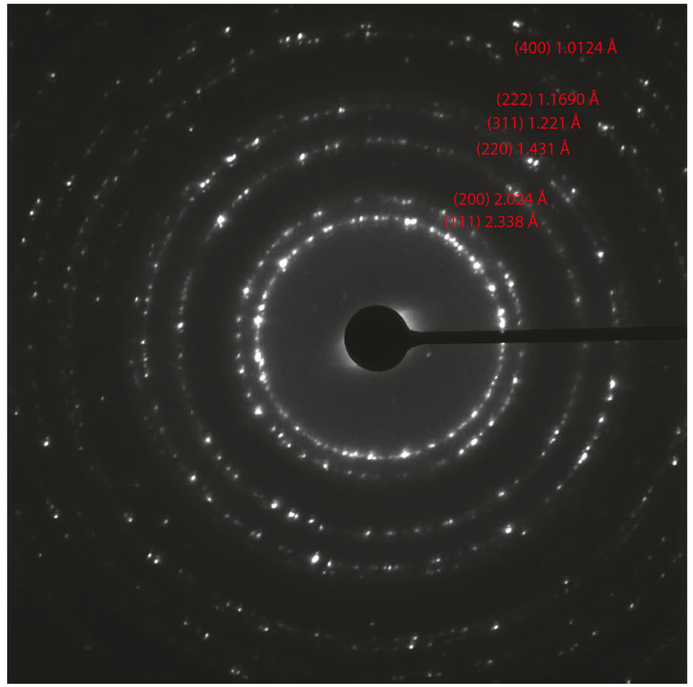
final image

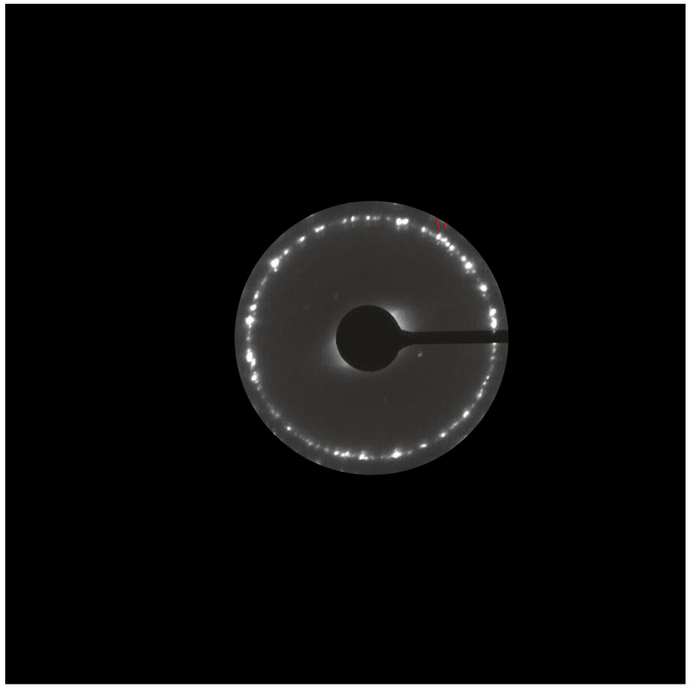
screen

Objective aperture

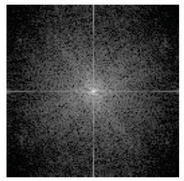


Objective aperture



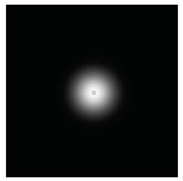


Beware...

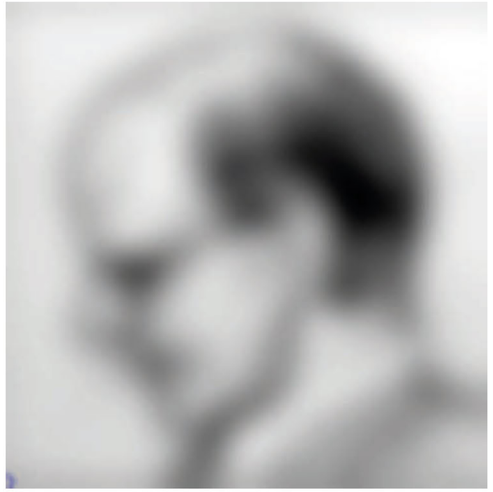


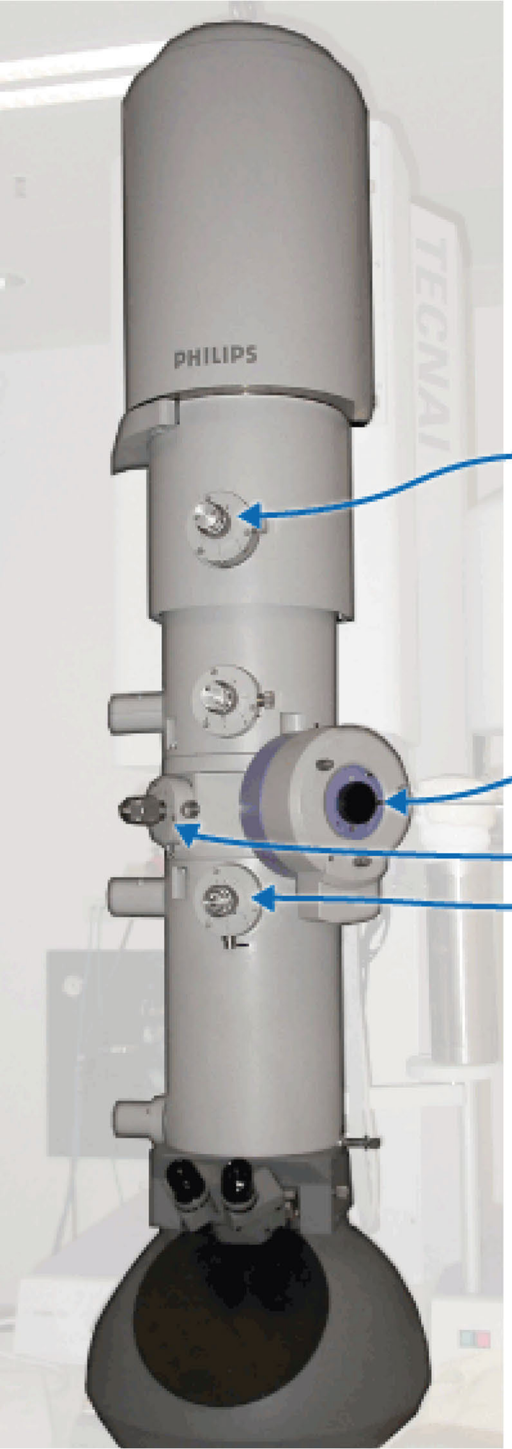
FT

x



Low-pass filter





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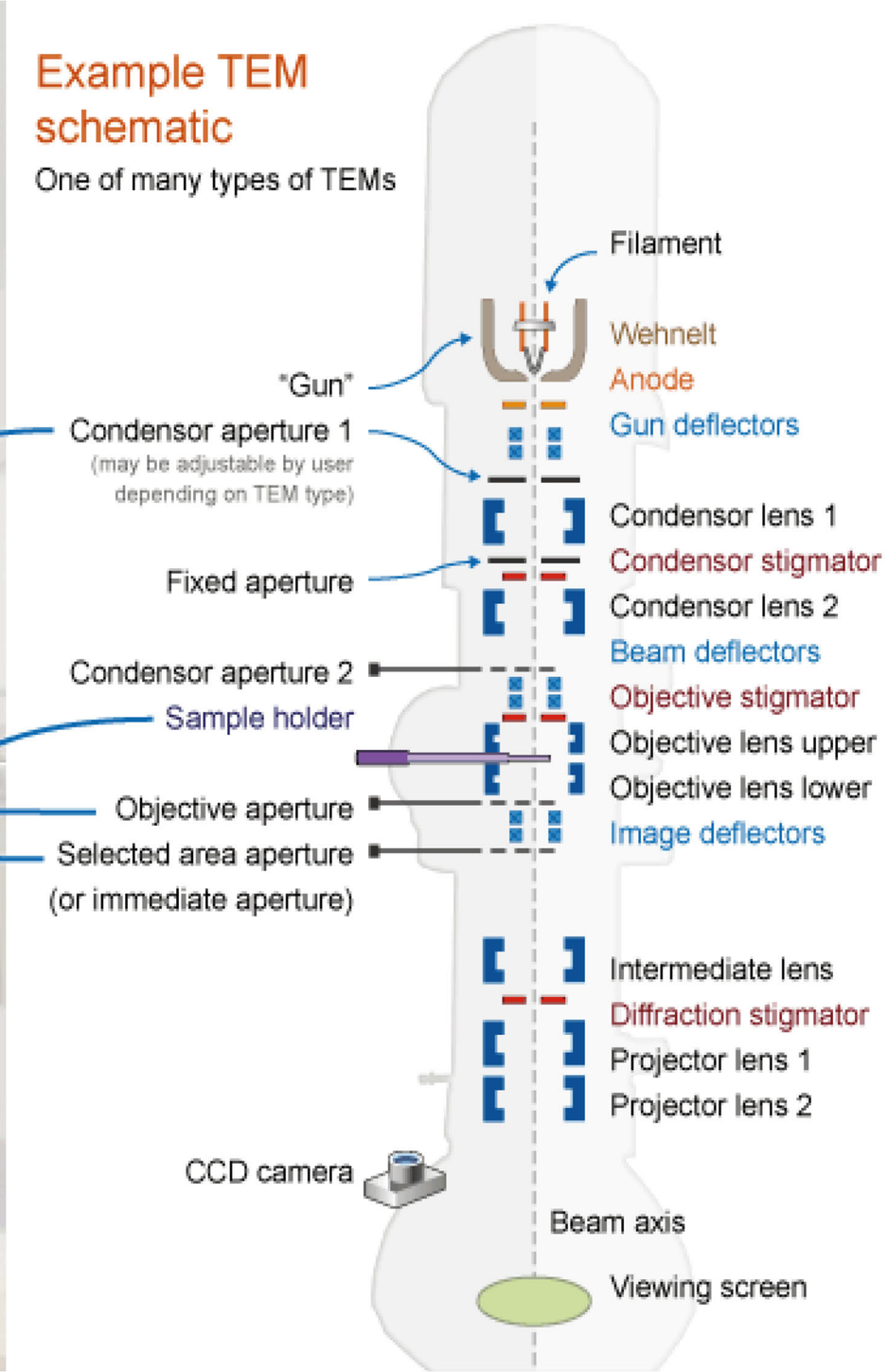
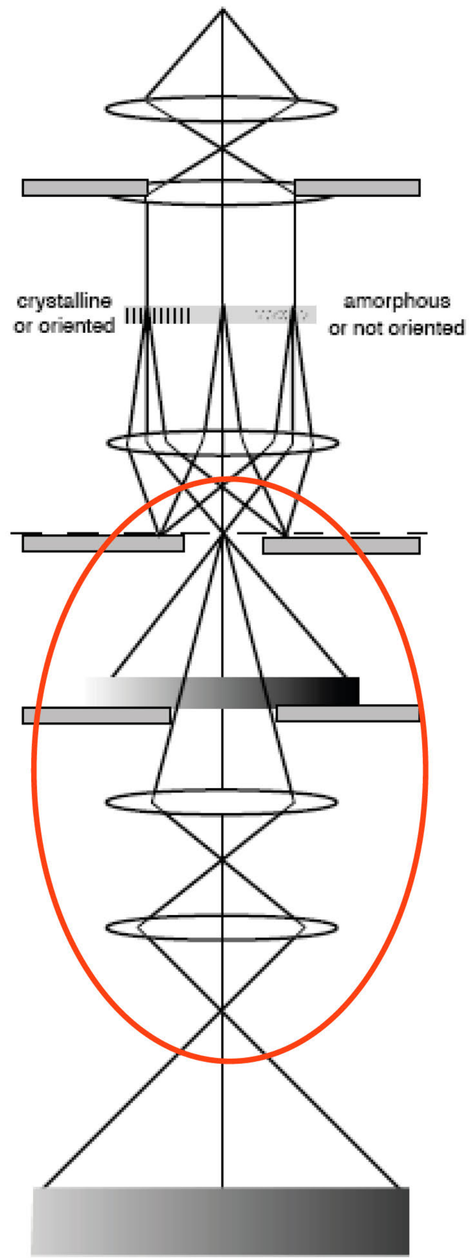
One of many types of TEMs





anode

condenser lens 1

condenser lens 2
condenser diaphragm

sample

objective lens

objective diaphragm
(back focal plane)

selected area diaphragm

intermediate lens

projective lens

final image

screen

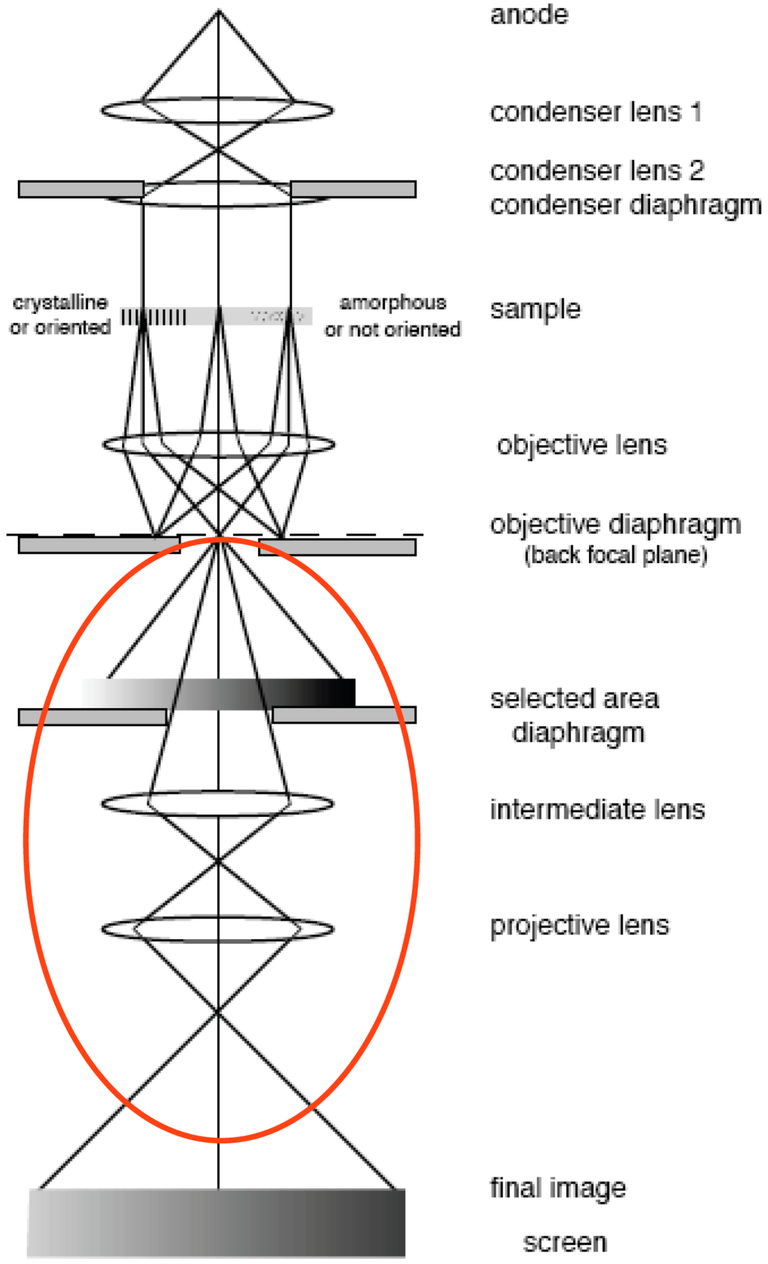
Magnify it!

Intermediate & projector lenses magnify the image created by the objective lens

Goal: take image created by the objective lens and match it to the detector with as little distortion as possible (don't forget Niquist…)

Nearly perfect lenses b/c very small angles used

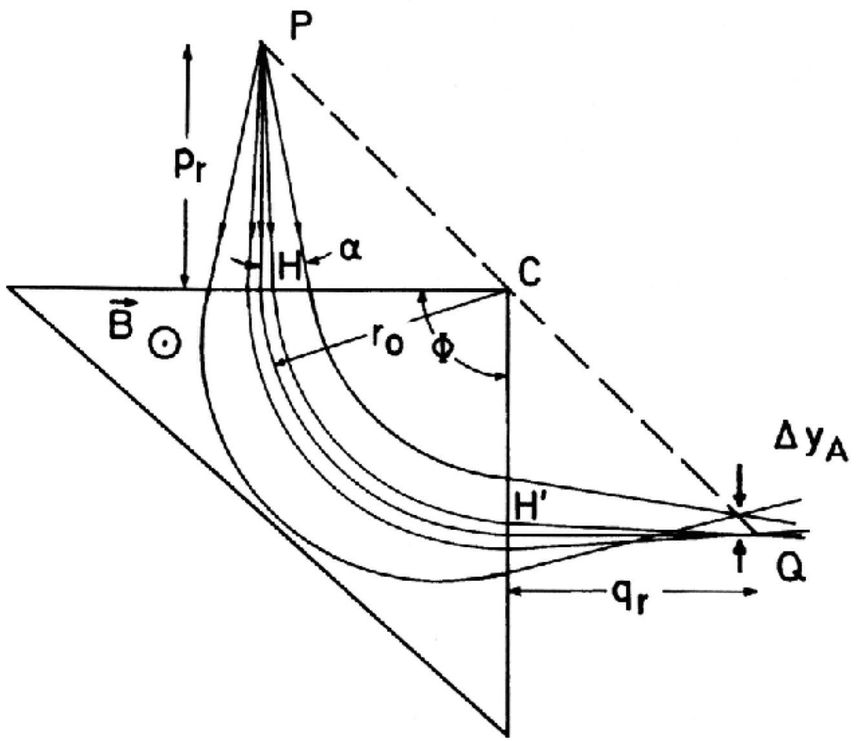
Beware: If not aligned properly, projector lenses can distort image causing differential magnification and other weird effects (barrel, etc.)

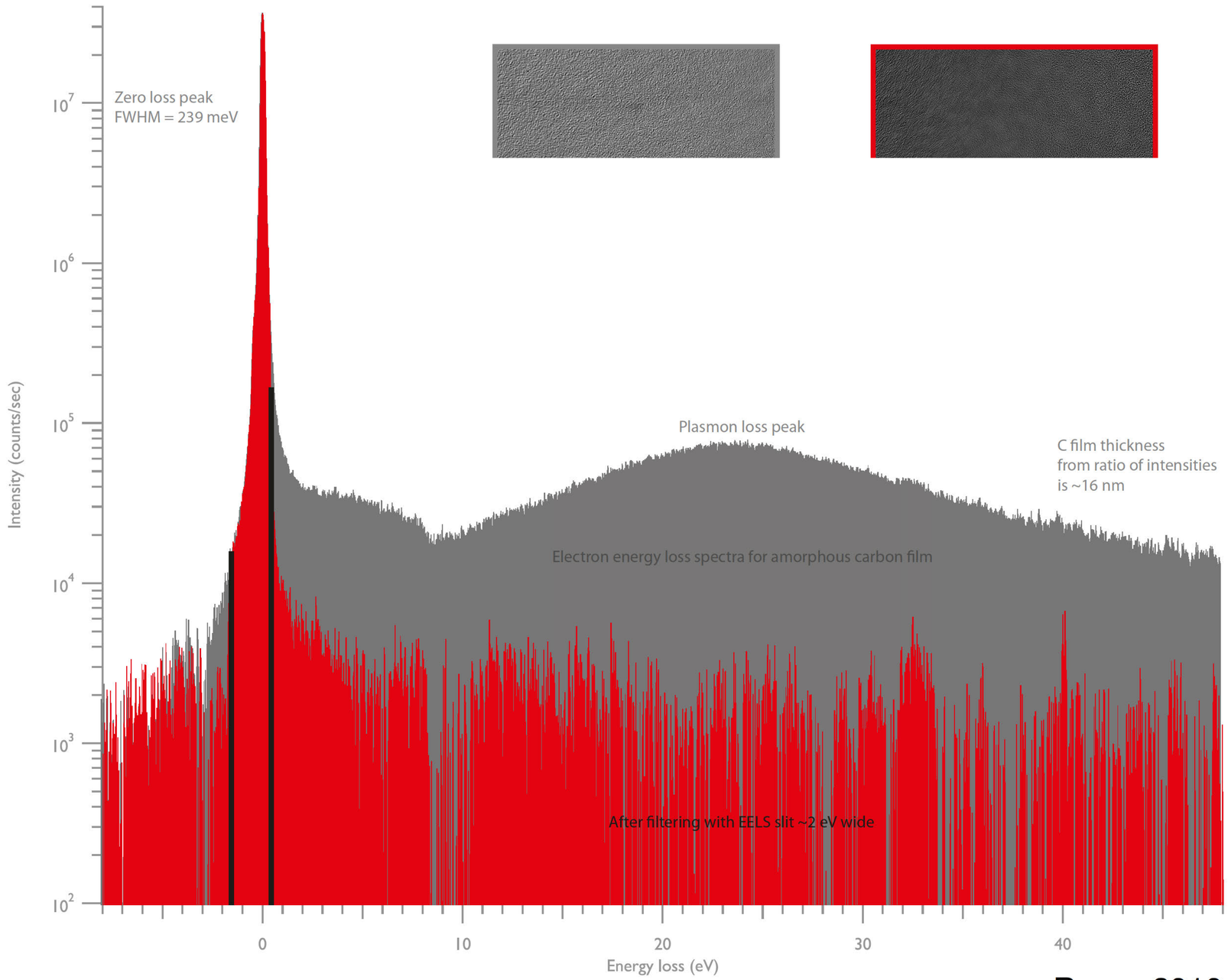


Energy filter

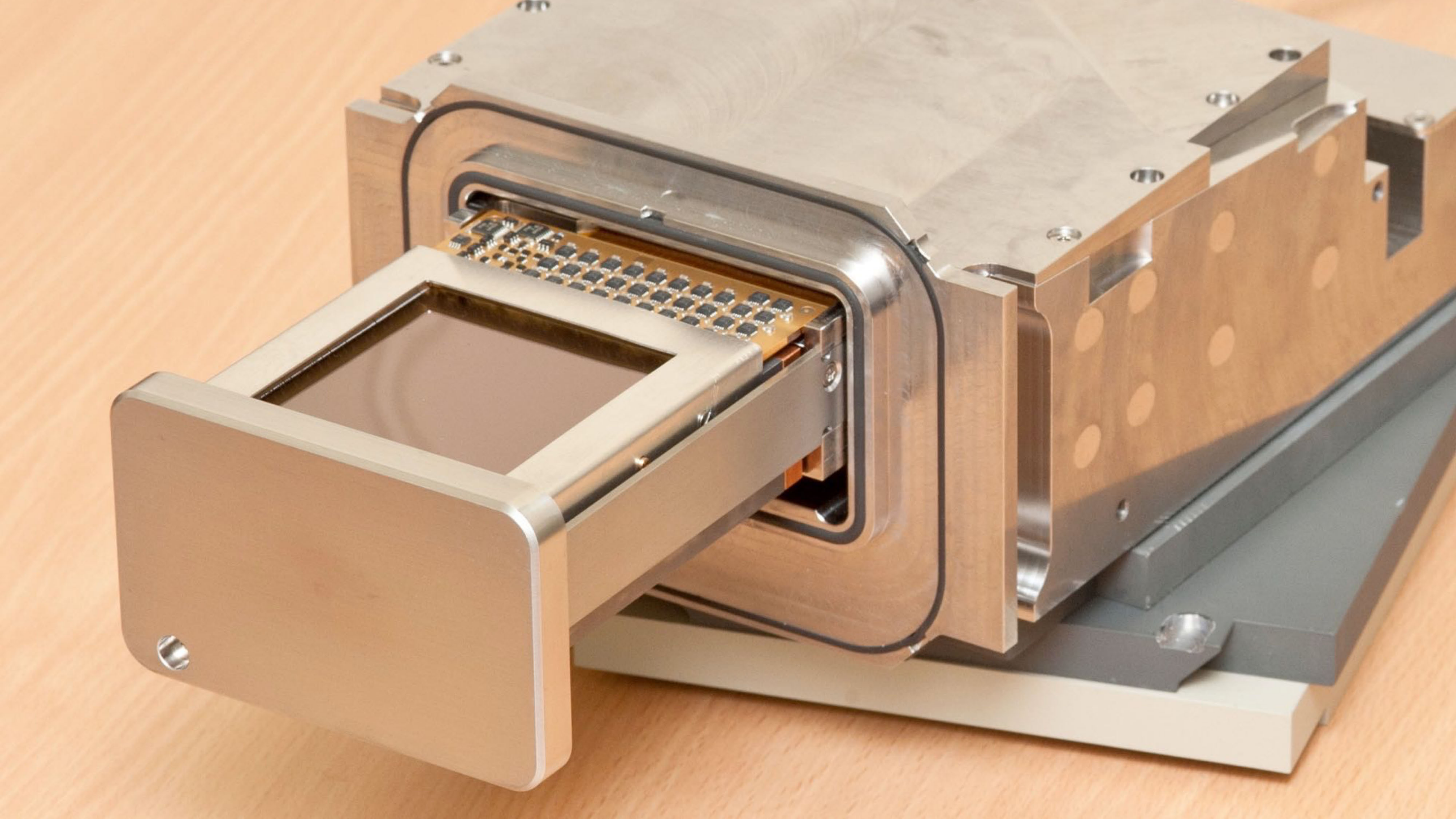
Think: "prism"

$$\mathbf{F} = -q_e(\mathbf{E} + \mathbf{v} \times \mathbf{B})$$





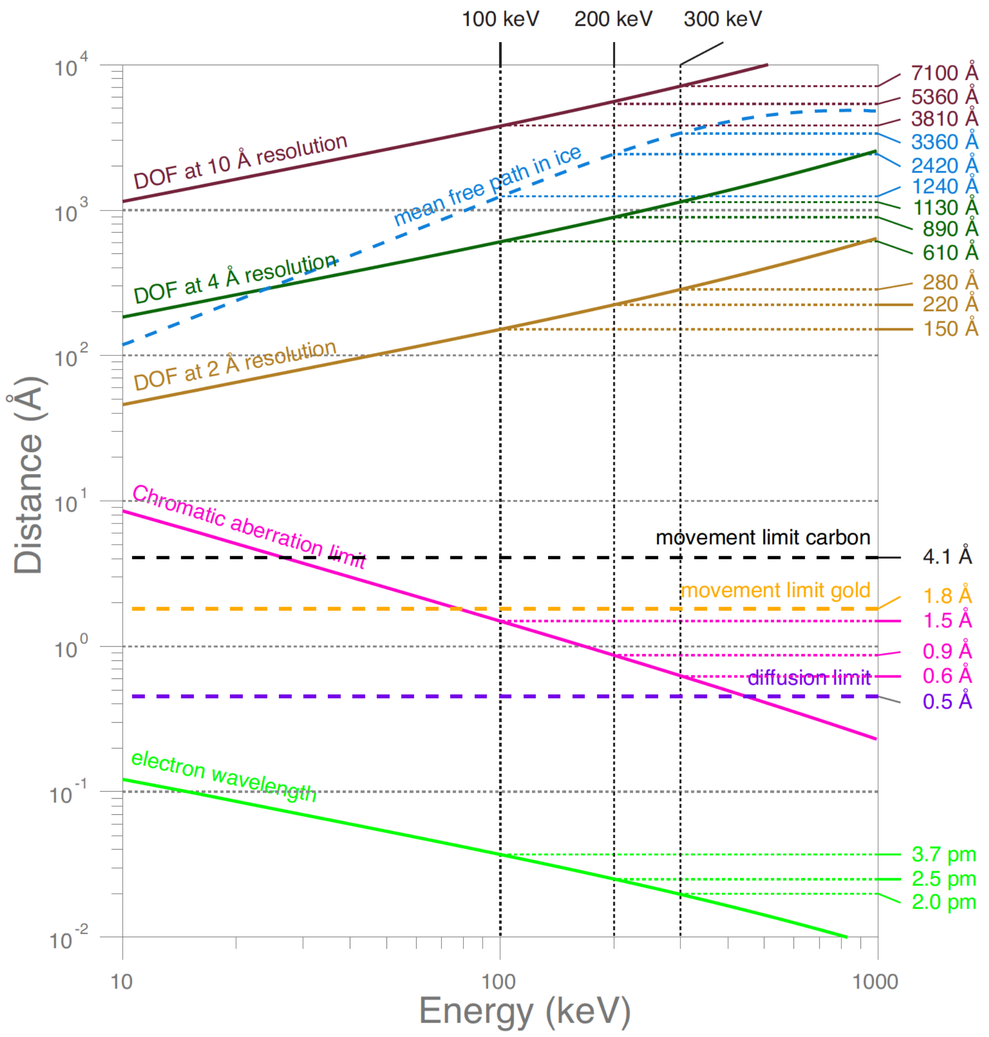
Russo 2010



Physical vs. practical limits in cryo-EM

- Optics
- Damage
- Detection
- Movement
- Specimen thickness
- “Charging”
- Surface interactions

- Specimen preparation and evaluation
- Mass
- Compositional heterogeneity
- Microscope time / cost
- Data processing time / difficulty



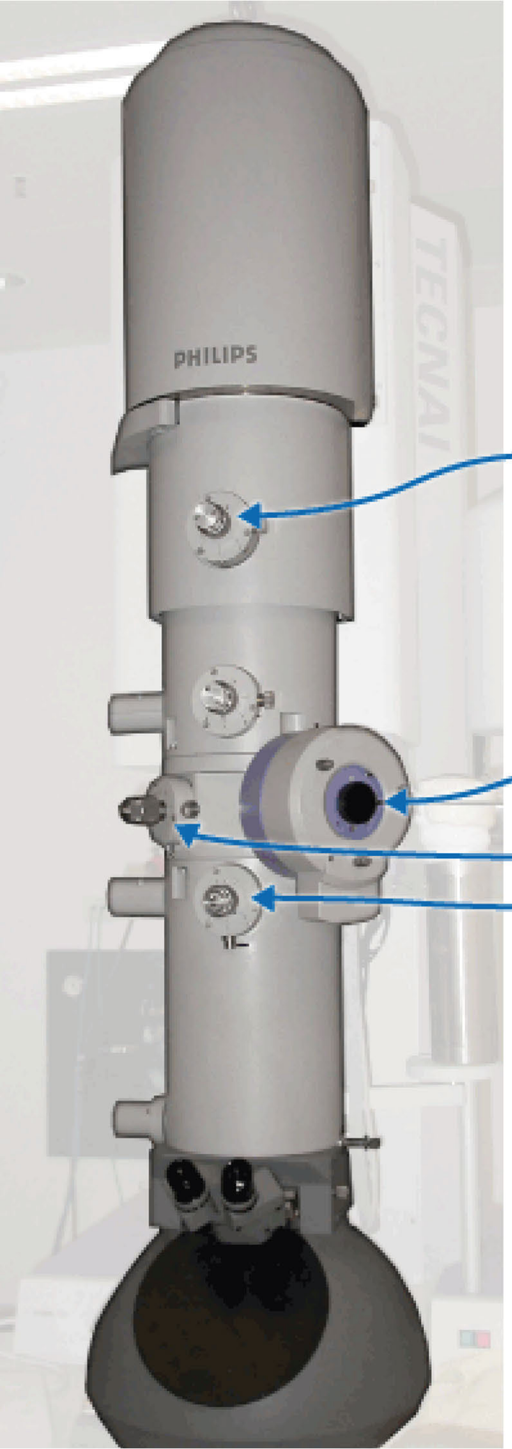
Electron energy scaling limits relevant to cryo-EM

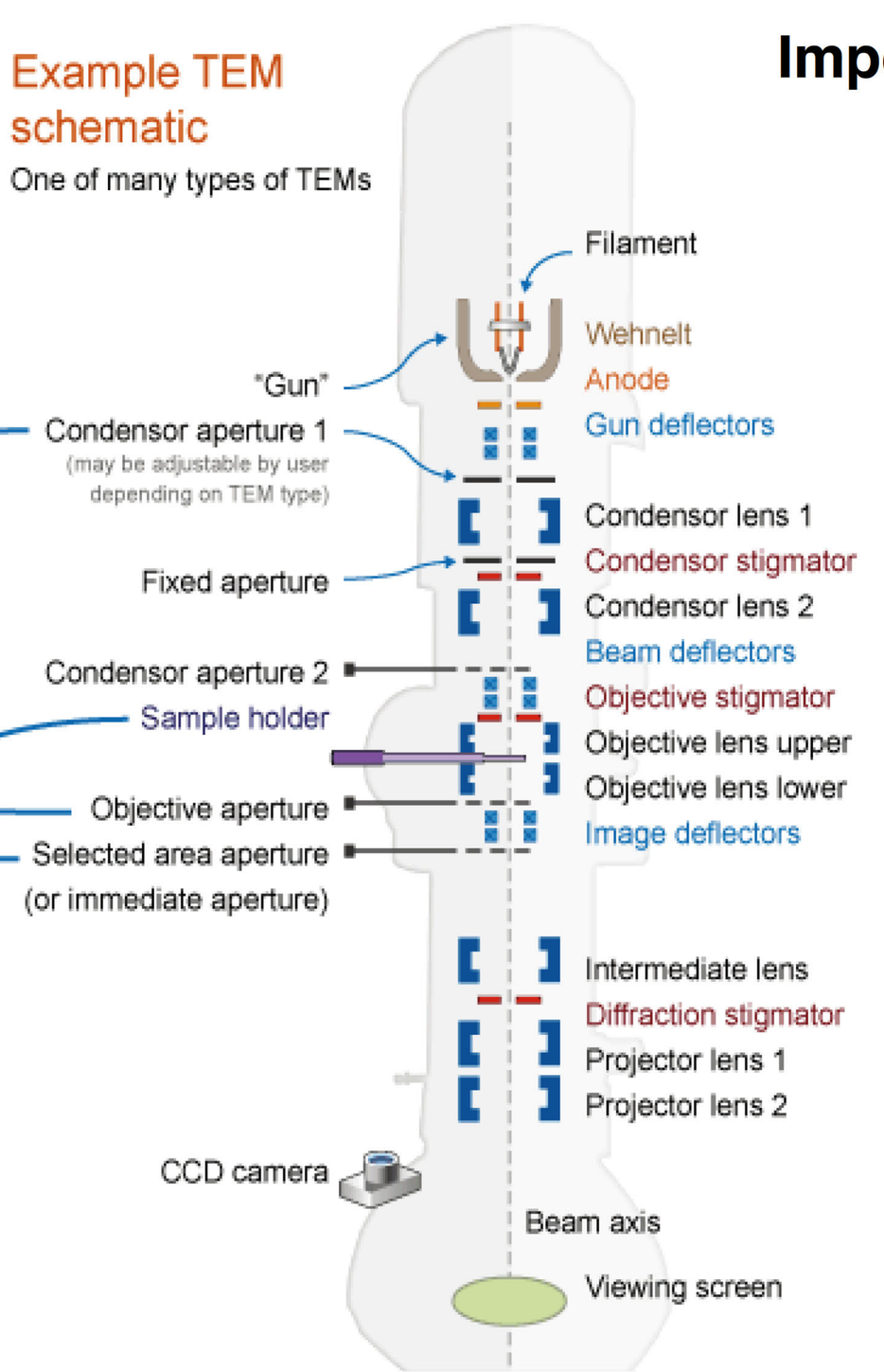
- Wavelength
- Optics
- Depth of field (DOF)
- Mean free path (inelastic)
- Particle movement

Russo & Passmore 2016b

“Progress in science depends on new techniques, new discoveries and new ideas, probably in that order.”

–Sydney Brenner





Important hardware advances in CryoEM

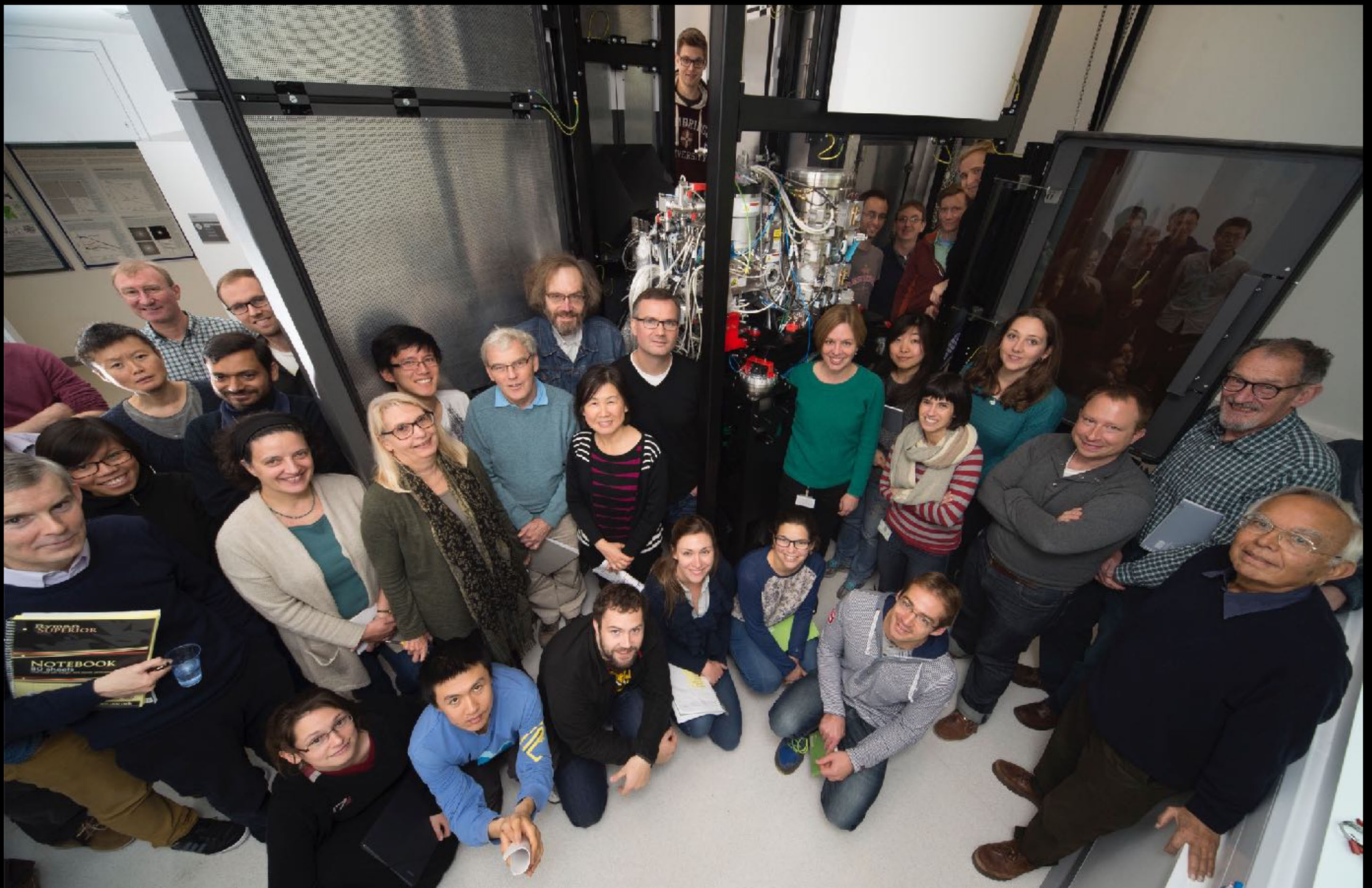
- Electron sources
- Stable lenses and power supplies
- Improved high vacuum systems w/ anti-contamination systems
- High-resolution objective lenses
- Low drift, low vibration, sample stages and cryo-specimen holders
- Stable specimen supports
- Computer control and automation of microscope lenses, stages and controls
- Methods for measuring and correcting lens aberrations
- Improved detectors

“Progress in science depends on new
techniques, new discoveries and new ideas,
~~probably~~ in that order.”

sometimes, *but not always*

–Sydney Brenner

Thanks!



Greg McMullan
Wasi Faruqi
Shaoxia Chen
Christos Savva
Giuseppe Cannone
Tony Crowther
Lori Passmore
Nigel Unwin
Richard Henderson

LMB workshops

LMB Scientific Computing

LMB IT

LMB Visual Aids

Suggested Reference Books

Hecht, *Optics* (any edition)

D. B. Williams and C. B. Carter, *Transmission Electron Microscopy* (any edition)

Glaser, et al., *Electron Crystalography of Biological Macromolecules*

J. Frank, *Three-Dimensional Electron Microscopy of Macromolecular Assemblies*

J. C. H. Spence, *High Resolution Electron Microscopy*, (Third edition)

J. Cowley, *Diffraction Physics* (Second Edition)

Kohl & Reimer, *Transmisison Electron Microscopy* (Fifth Edition)

Methods In Enzymology Vol. 481-483 G. Jensen, editor

Methods In Enzymology Vol. 579 R.A. Crowther, editor