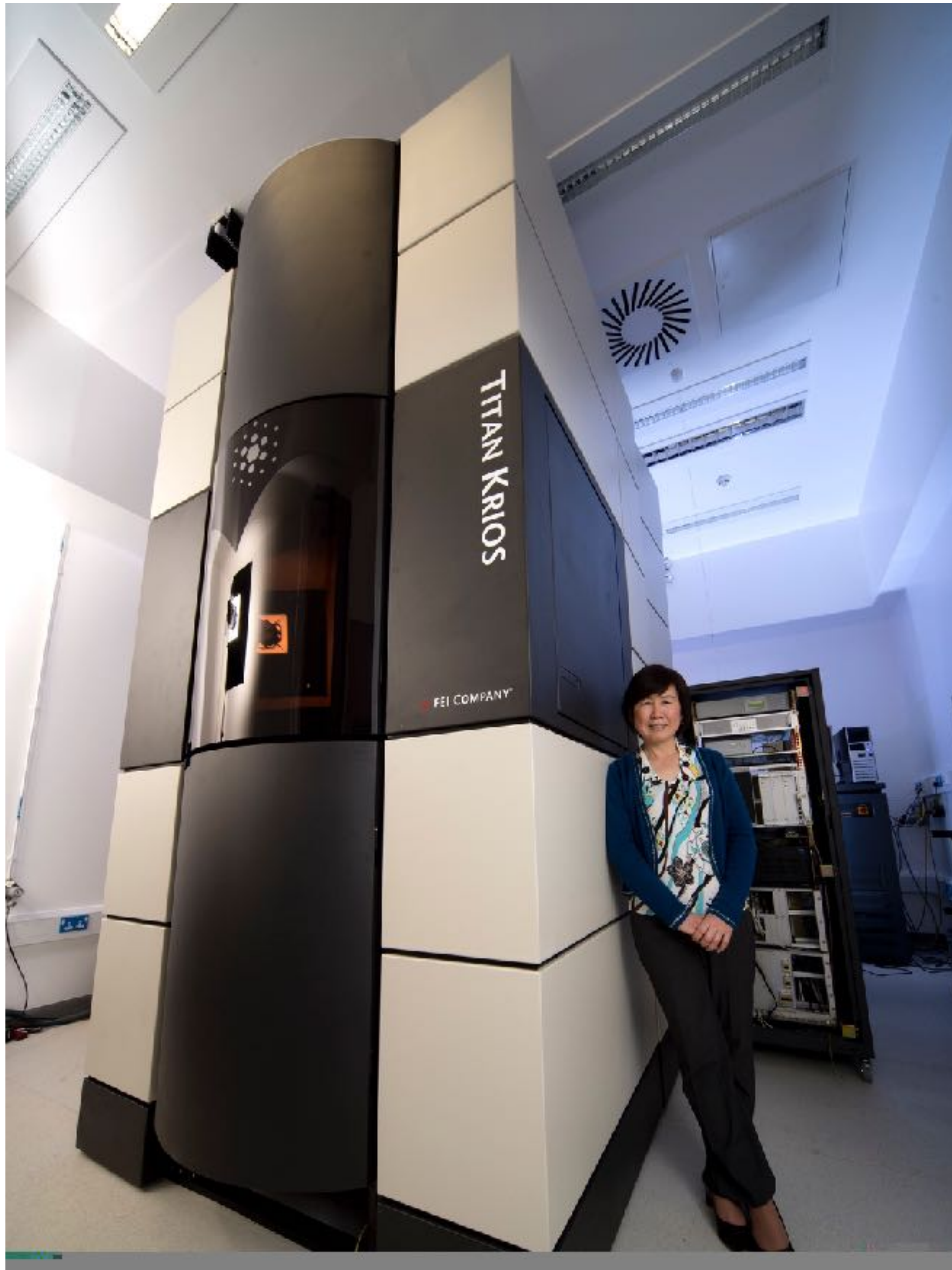
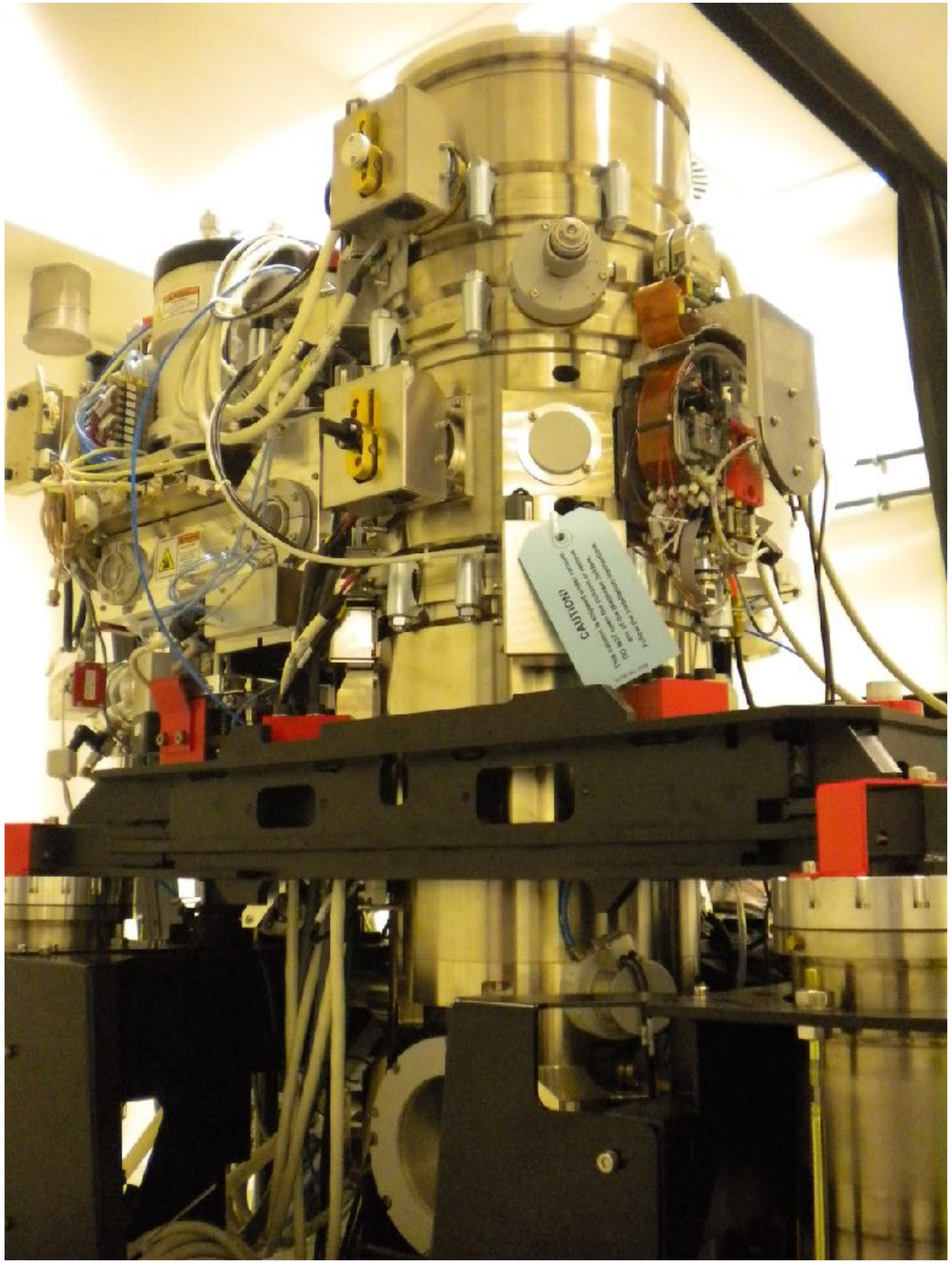
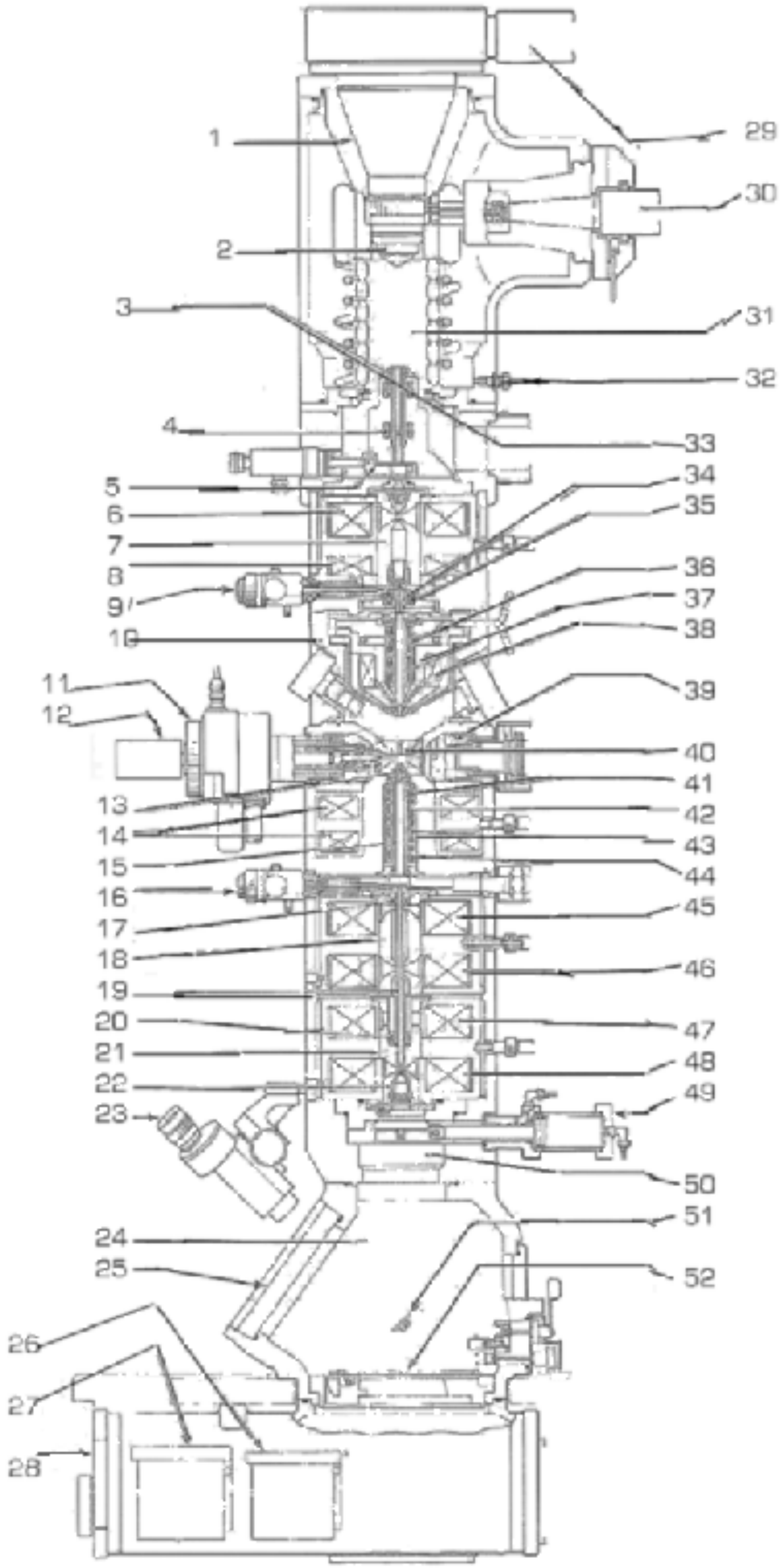
Electron microscope physics & optics

Chris Russo



MRC Laboratory of Molecular Biology



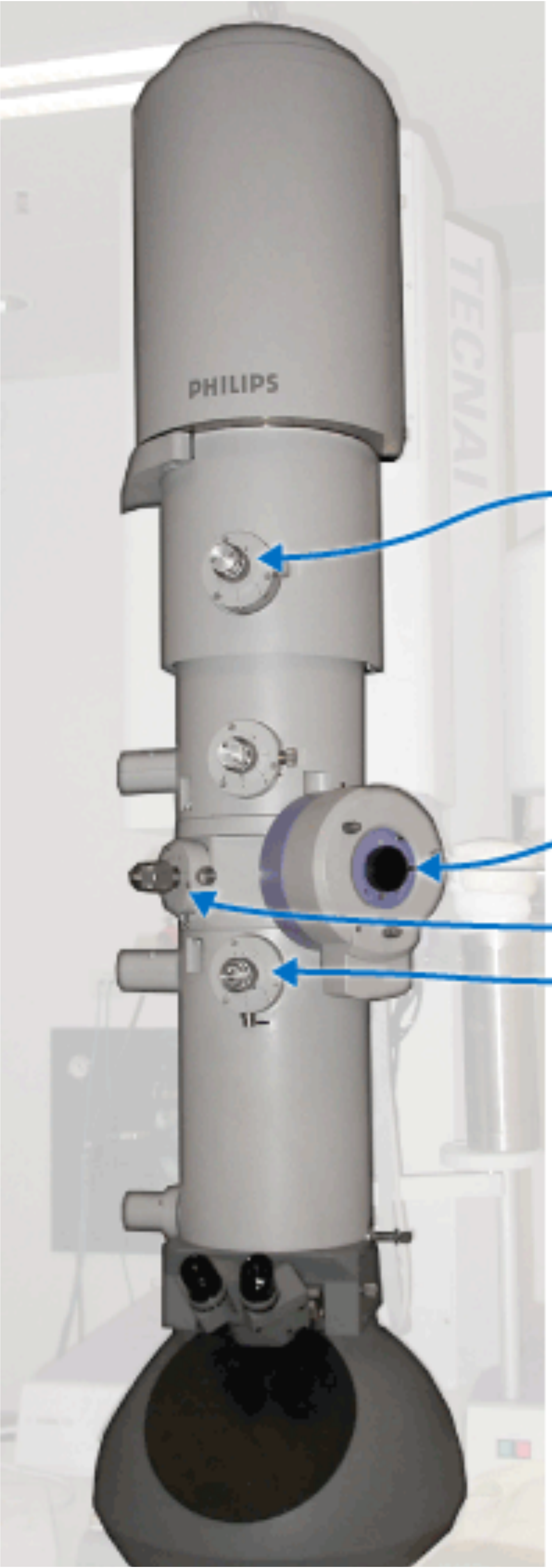


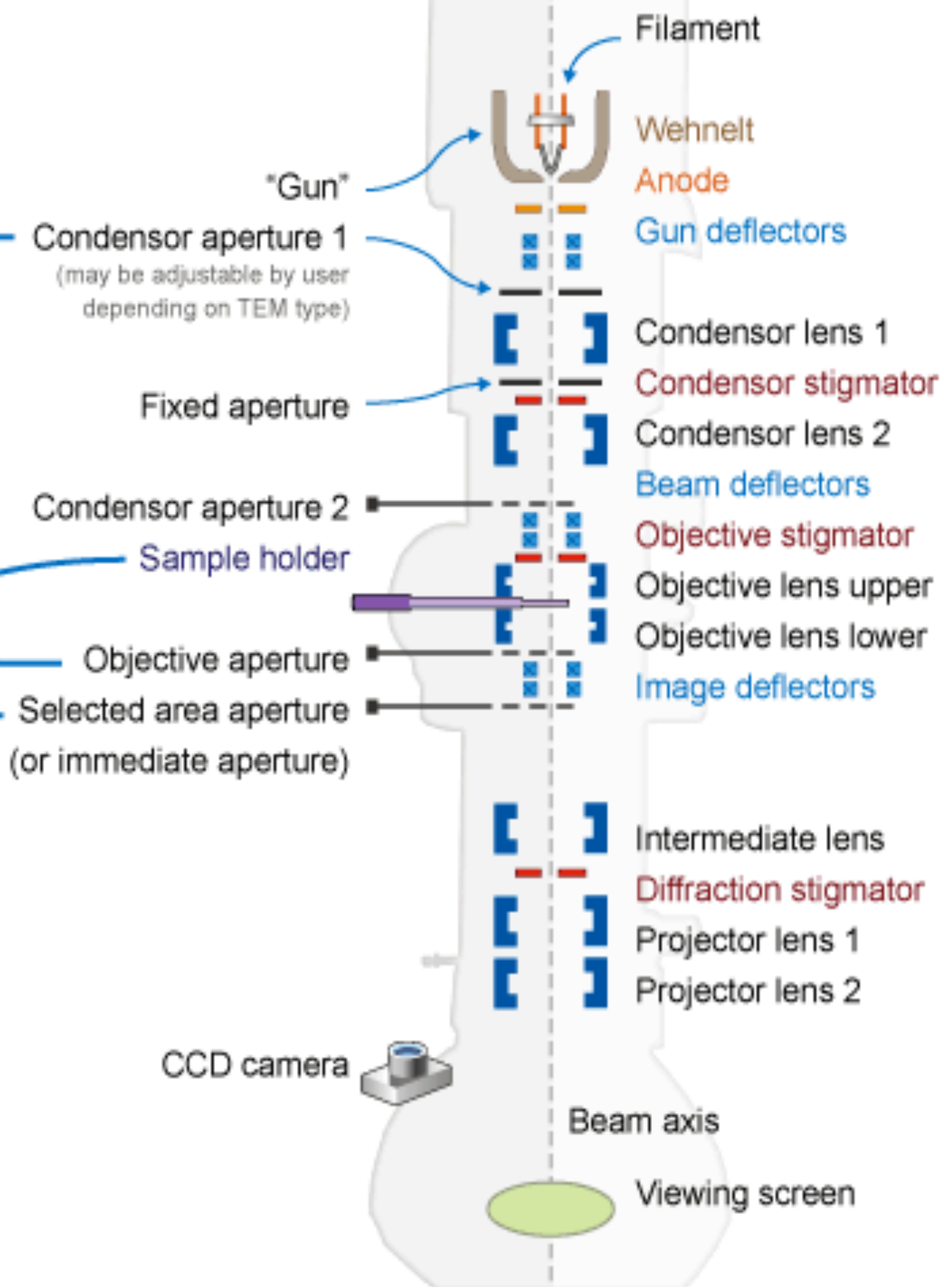
S. Chen

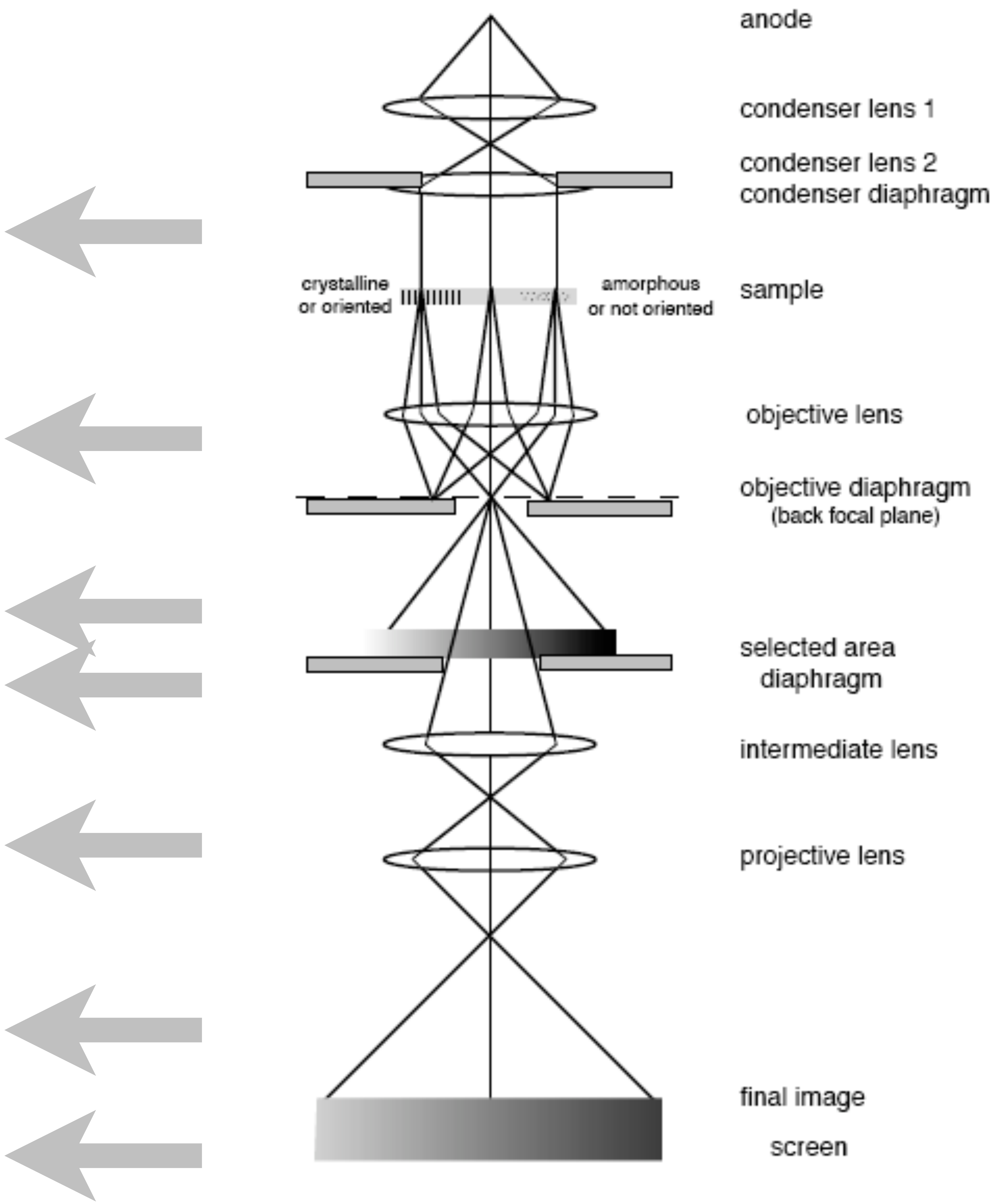
1. Electron Gun	14. Objective Lens Coil	27. Receiving Magazine	40. Objective Polepiece
2. Wehnelt Unit	15. Objective Lens Liner Tube	28. Camera Chamber	41. Objective Lens Stigmator Coil
3. Anode	16. Field Limiting Aperture	29. Lift Arm	42. 1st Image Shift Coil
4. Electron Gun Second Beam Delector Coil	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

Example TEM schematic

One of many types of TEMs

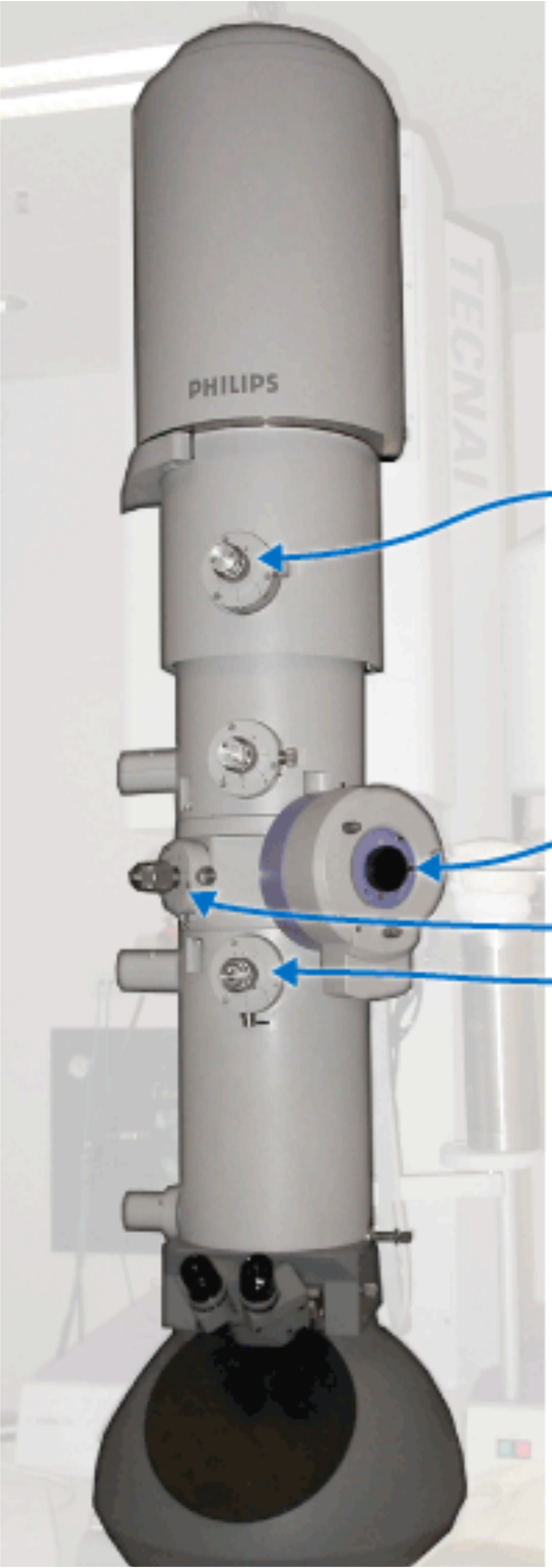


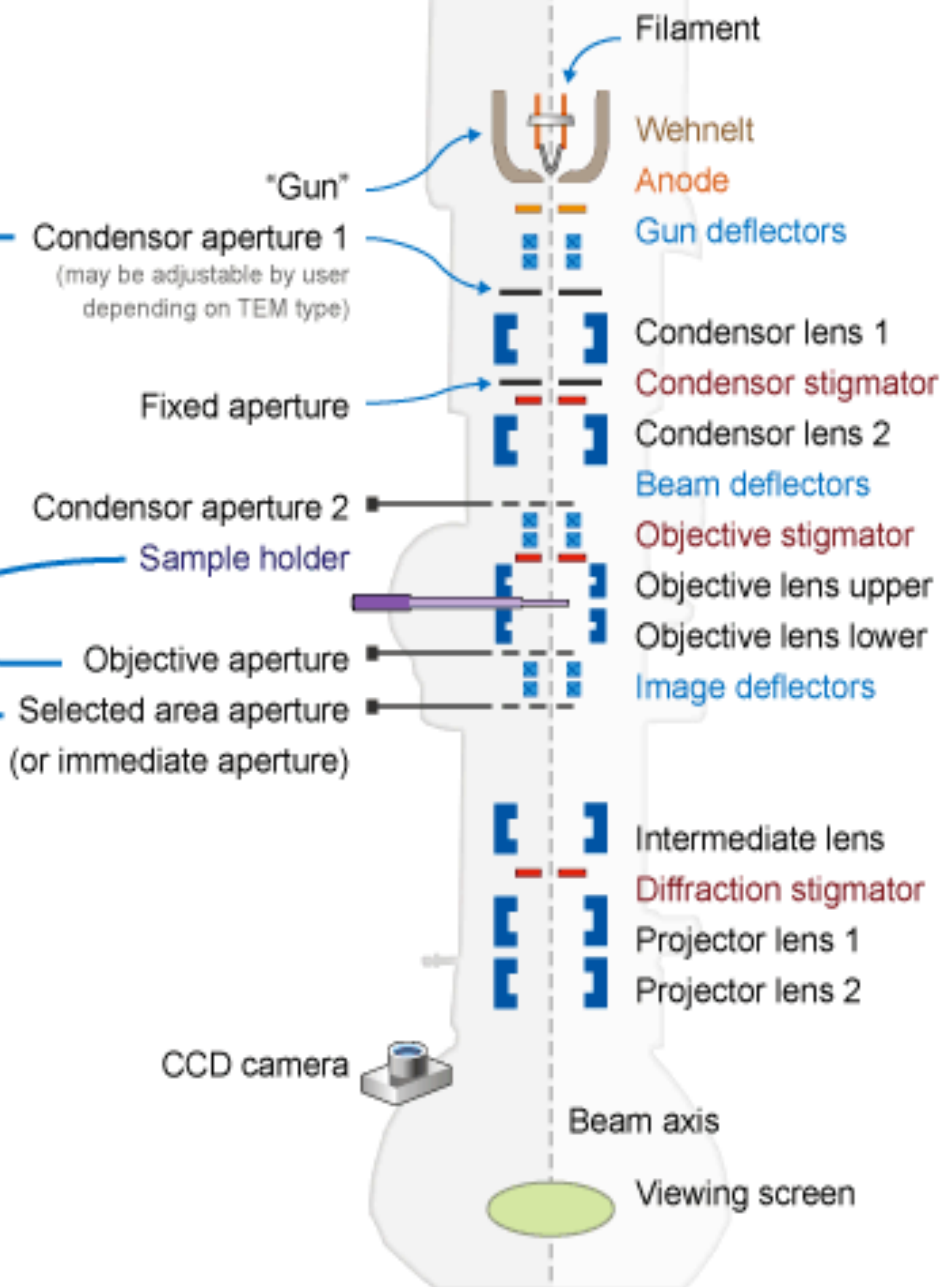


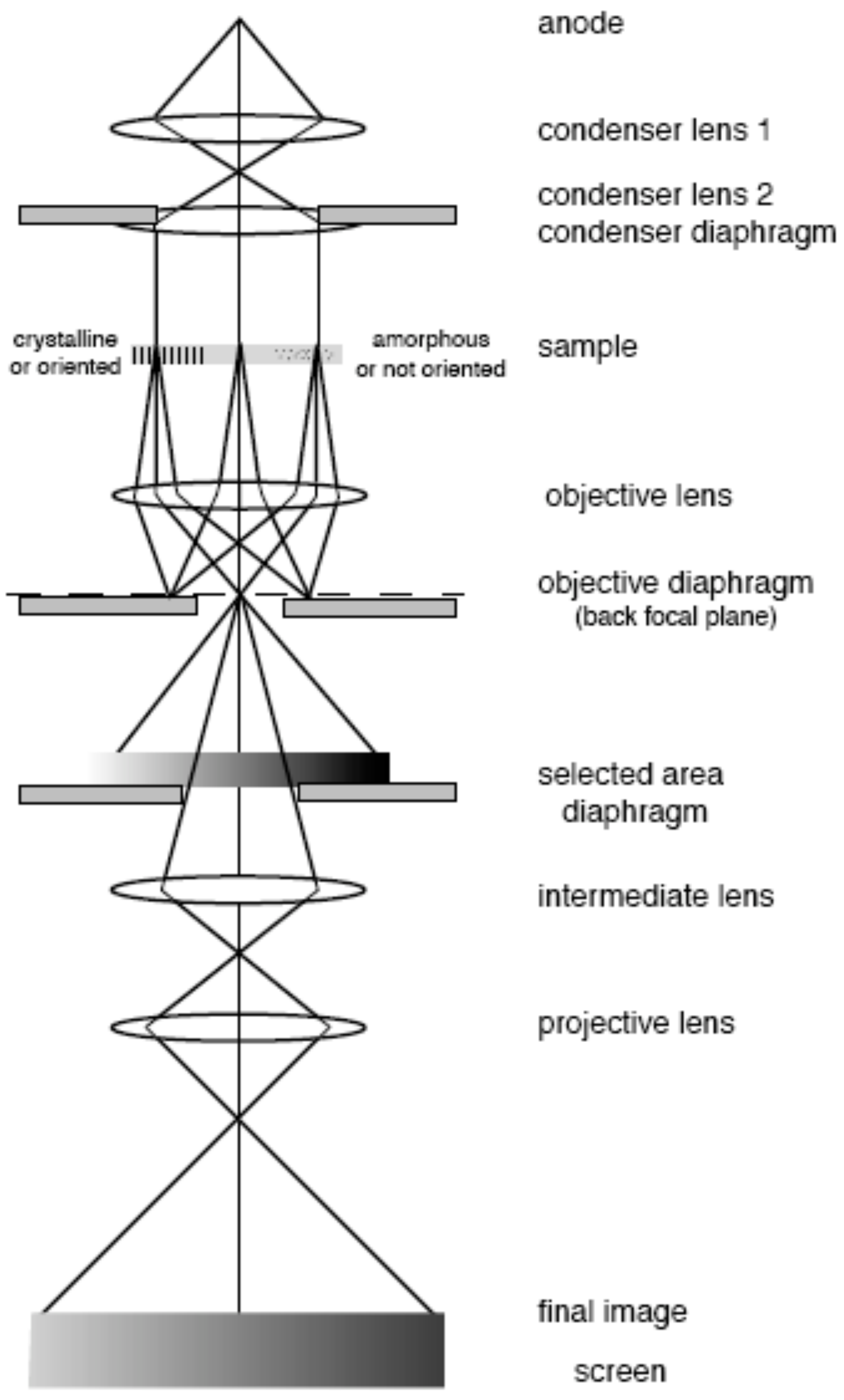


Example TEM schematic

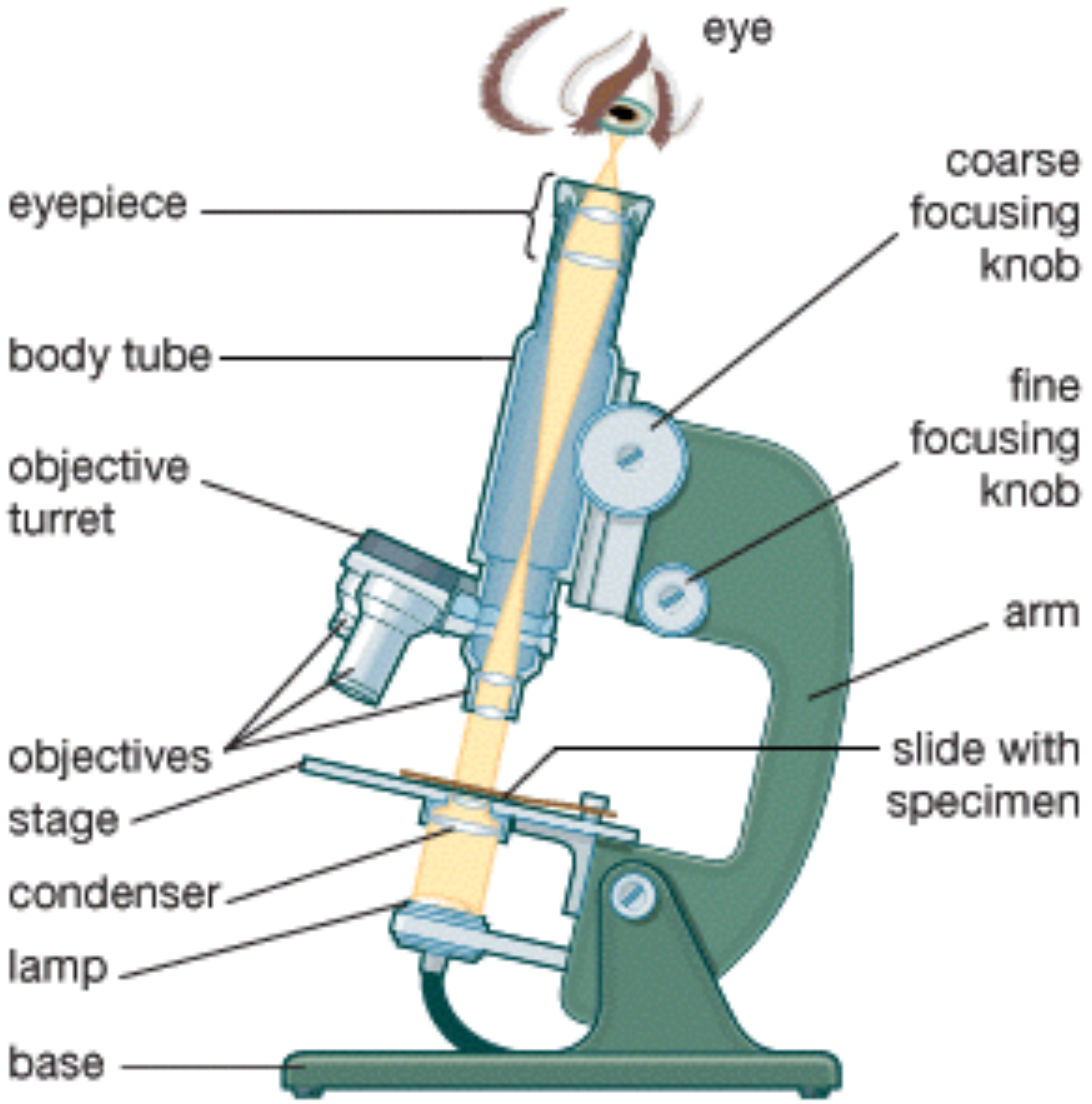
One of many types of TEMs

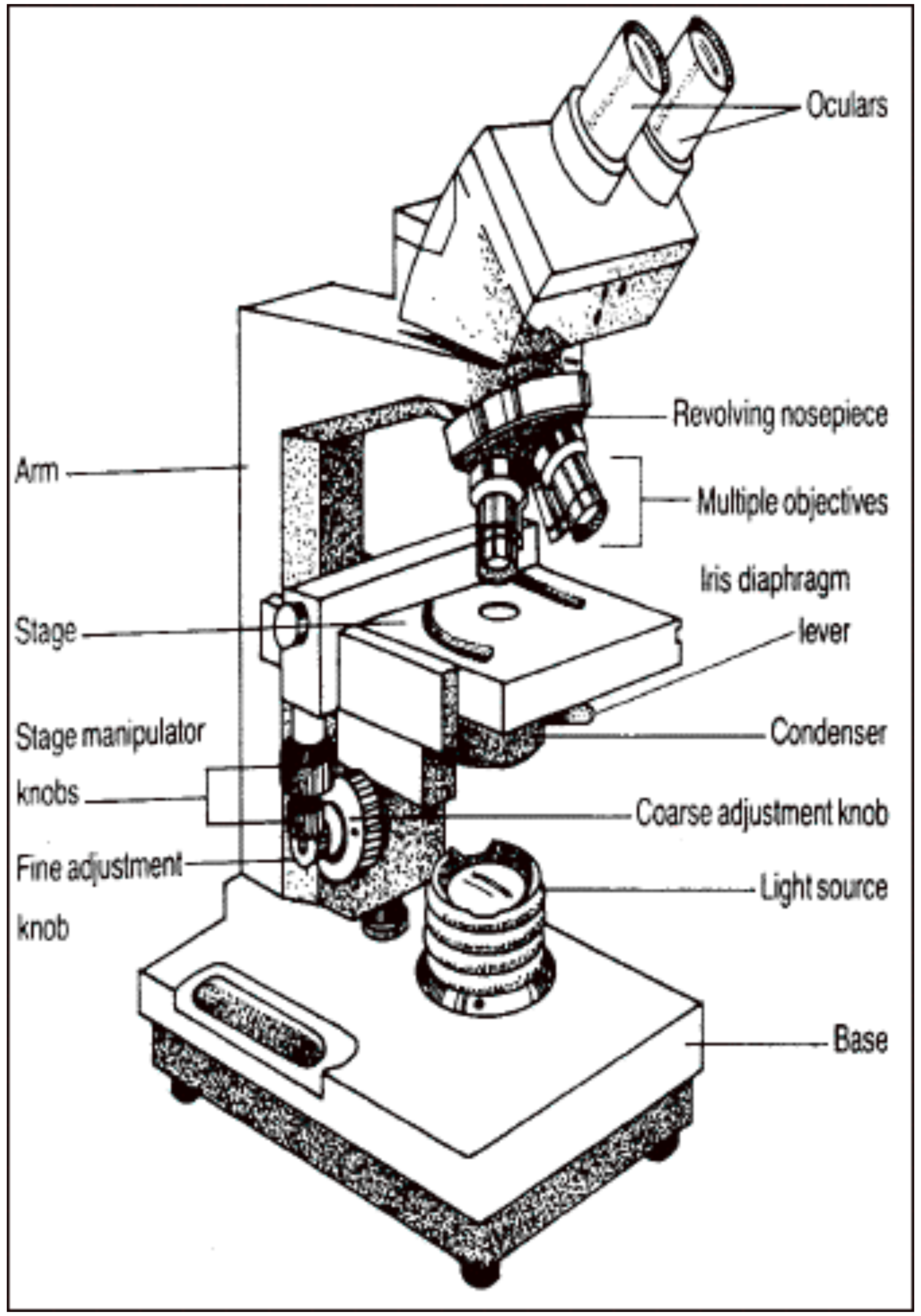




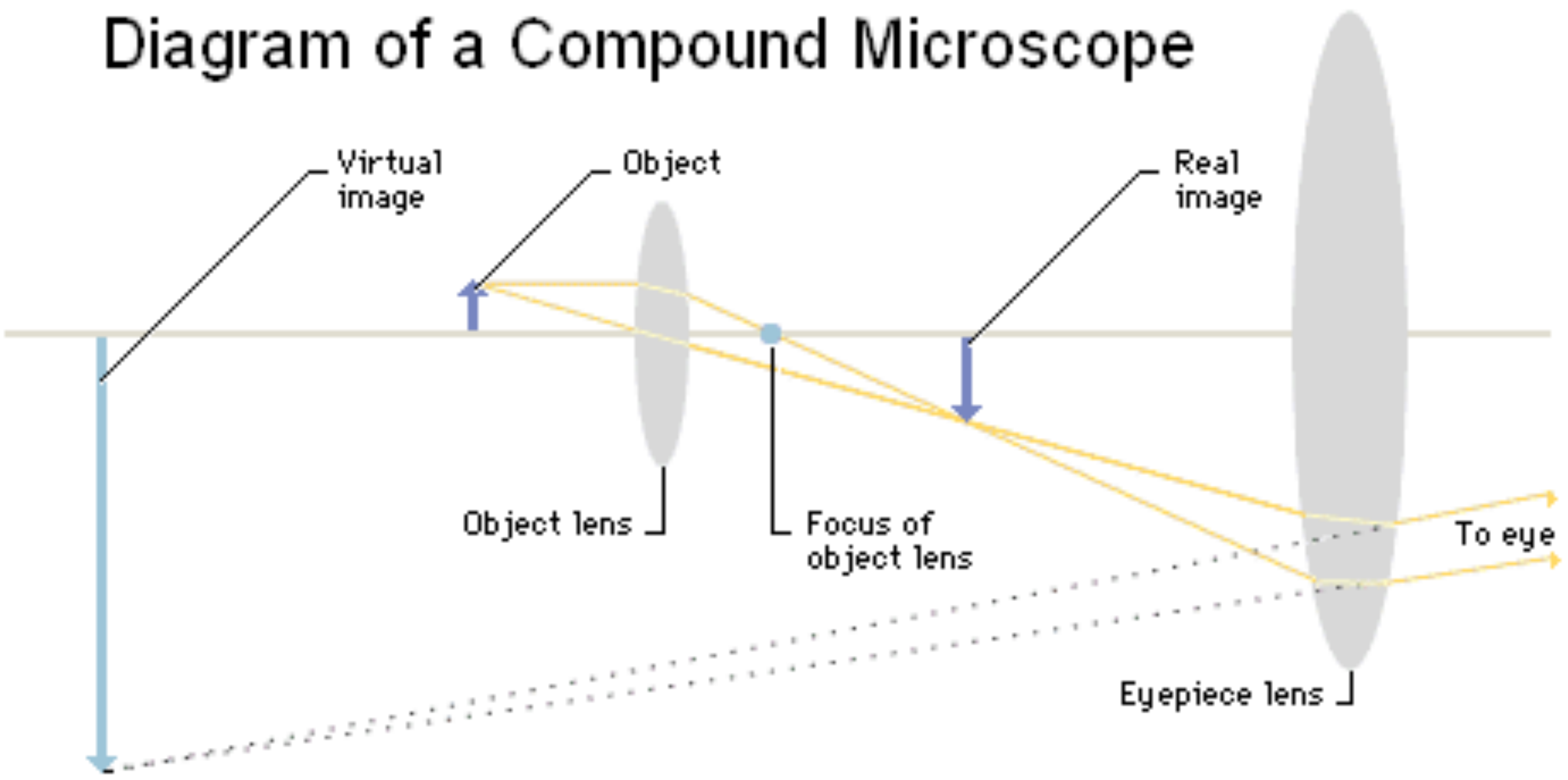


The Optical Microscope

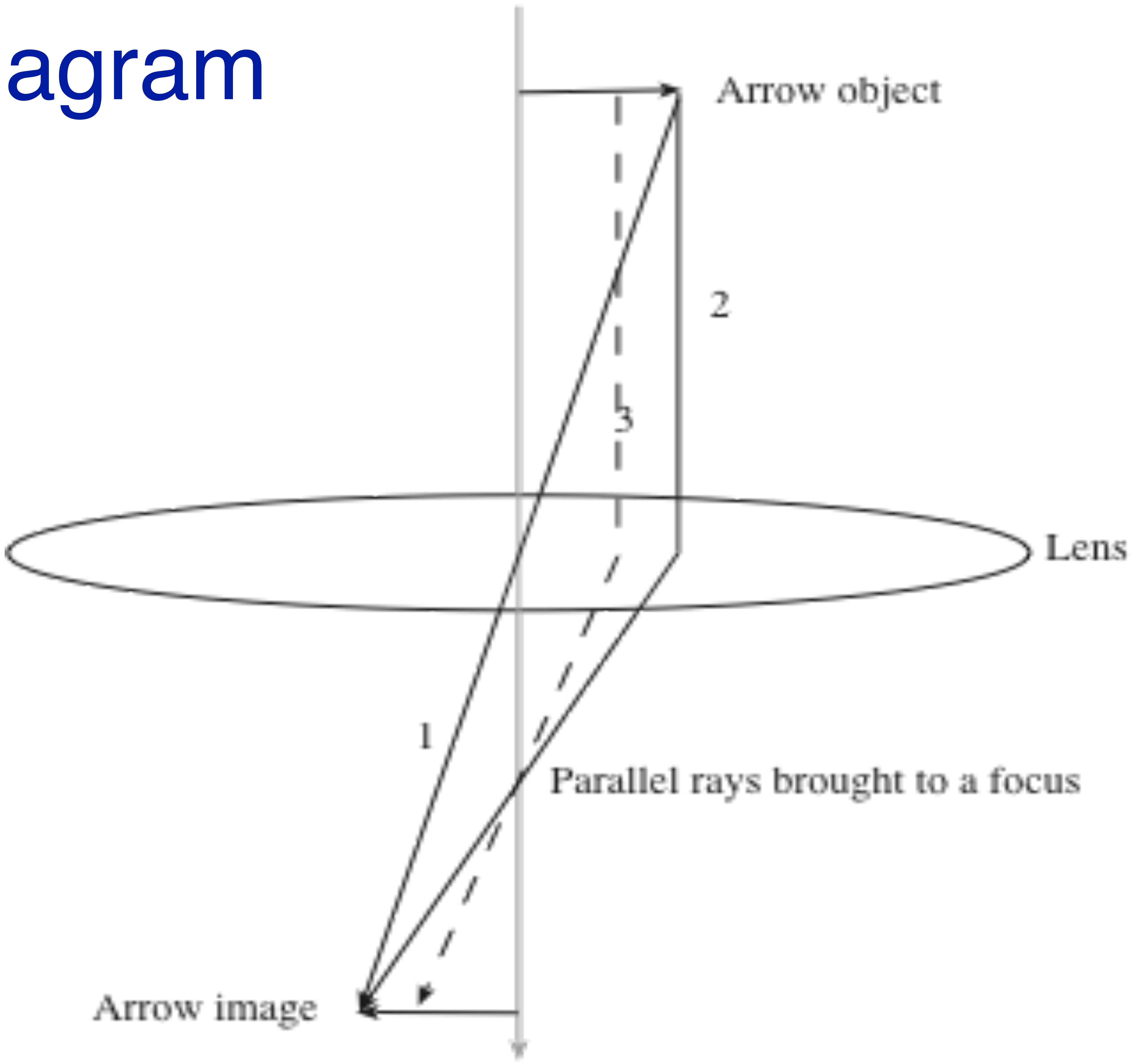




Optical Microscope Lens Diagram



Convex lens ray diagram



Important diagram
(Draw in ~10 seconds)

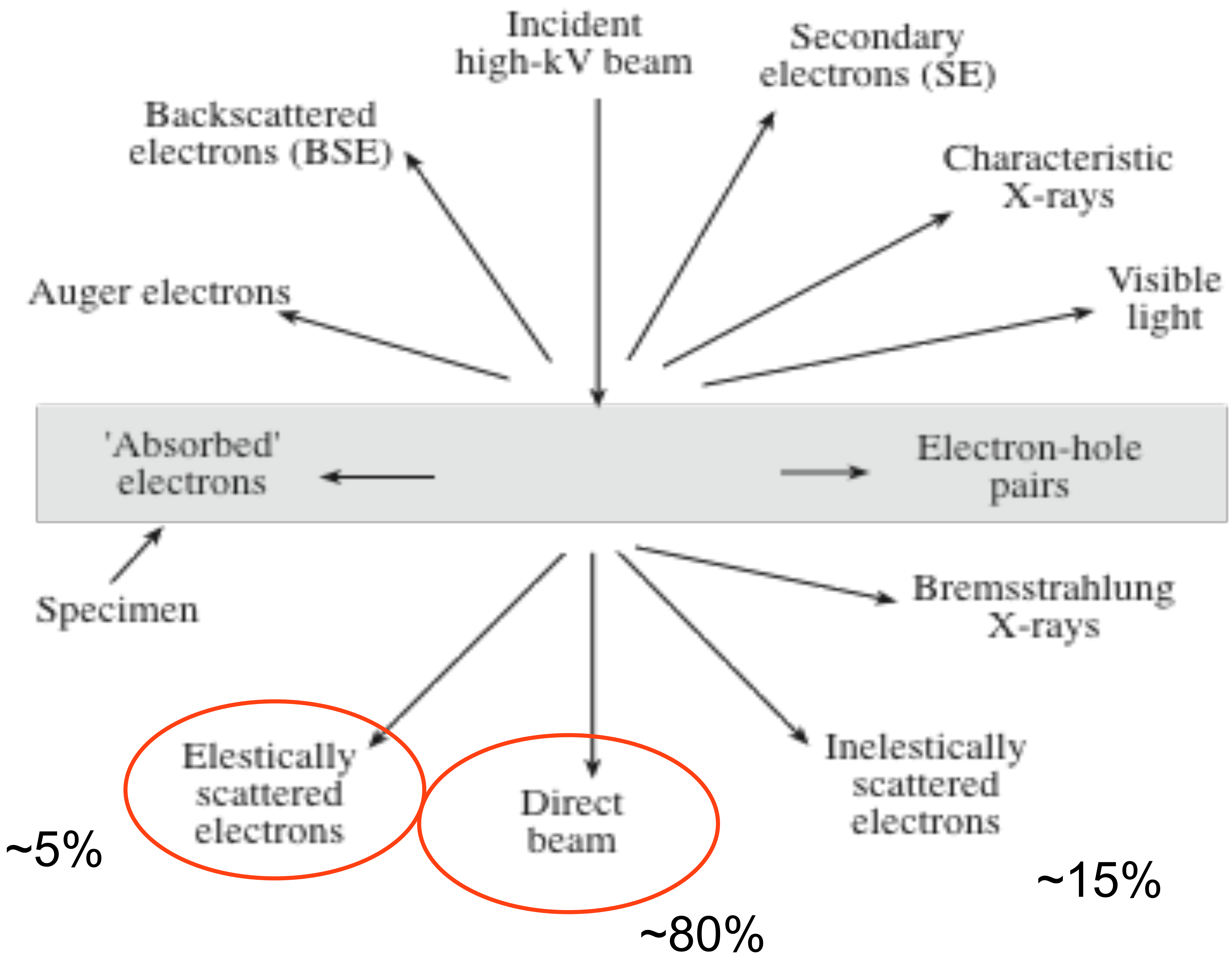
Steps are 1, 2, 3

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



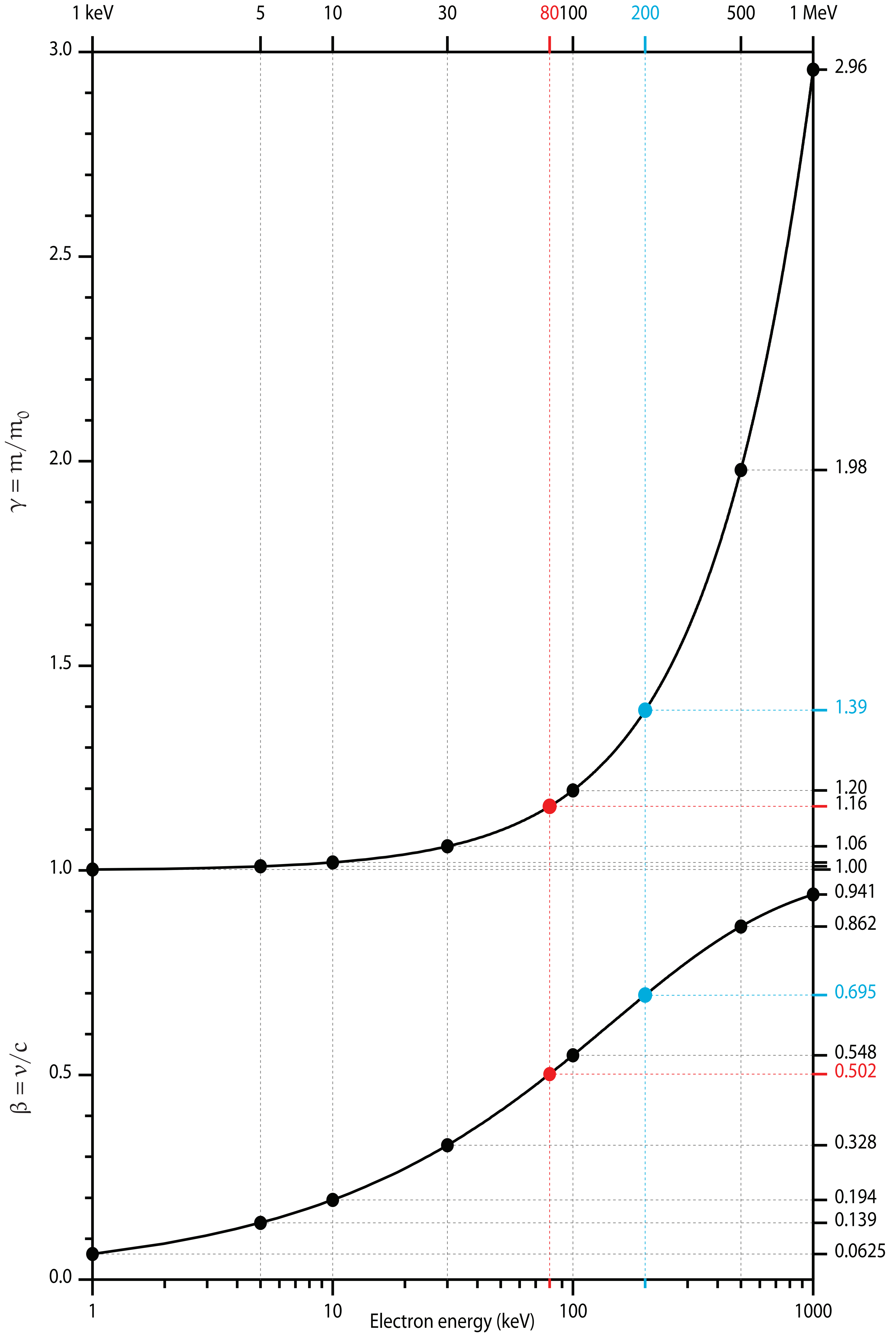
Properties of electrons are used for simple calculations

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
			$1\ \text{eV} = 1.602 \times 10^{-19}$ J
Velocity of light	c	$=$	$2.9979 \times 10^{8}\ \text{m s}^{-1}$
Rest energy	E_0	$=$	$m_0c^2 = 511$ keV
Spin	s	$=$	$h/4\pi$
Planck's constant	h	$=$	6.6256×10^{-34} J s $= 4.136 \times 10^{-15}\ \text{eV}\cdot\text{s}$

Nonrelativistic	$(E \ll E_0)$			Relativistic $(E \sim E_0)$	
Newton's law	$\boldsymbol{F} = \frac{d\boldsymbol{p}}{d\tau}$	$\boldsymbol{F}$	$=$	$\frac{d}{d\tau}(m\boldsymbol{v})$	(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

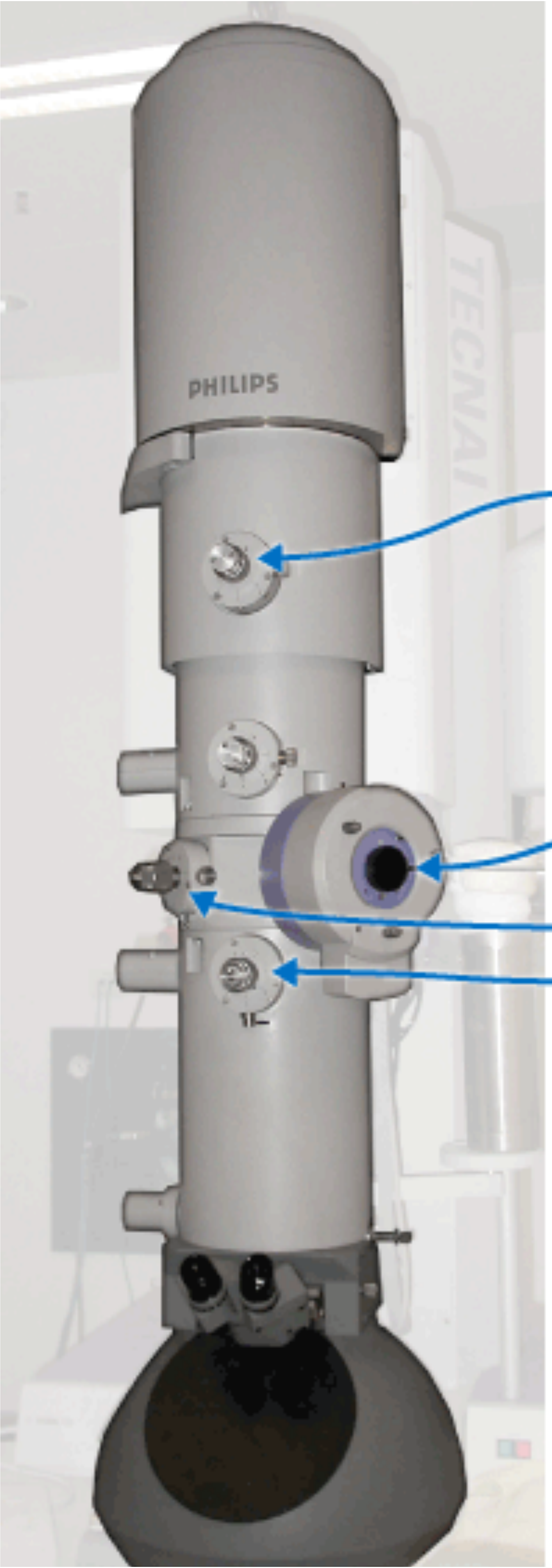
- Vacuum
- Damage Damage Damage Damage Damage Damage Damage Damage

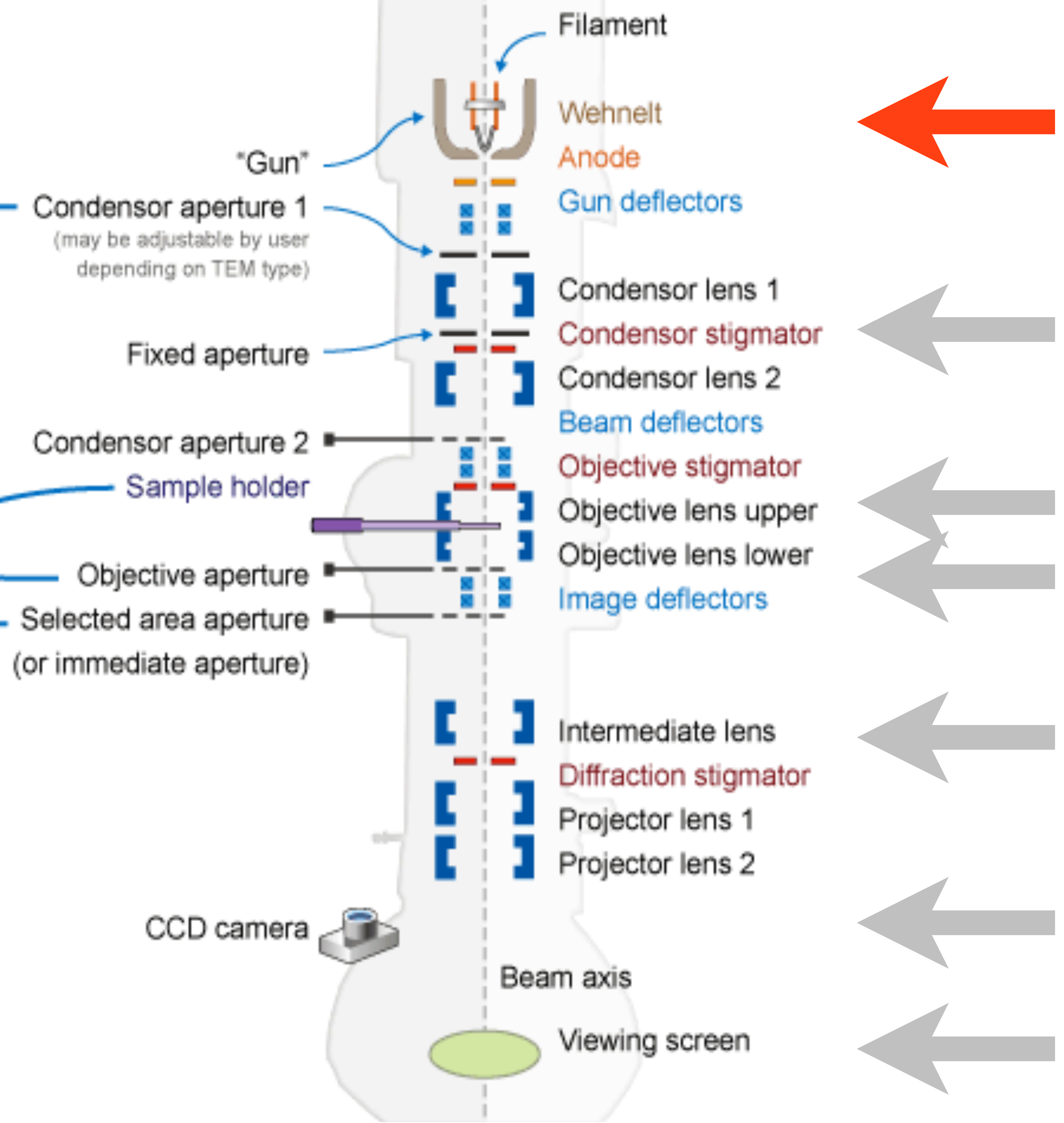
  Electron microscopes are used to simulate damage in the core of a nuclear reactor!

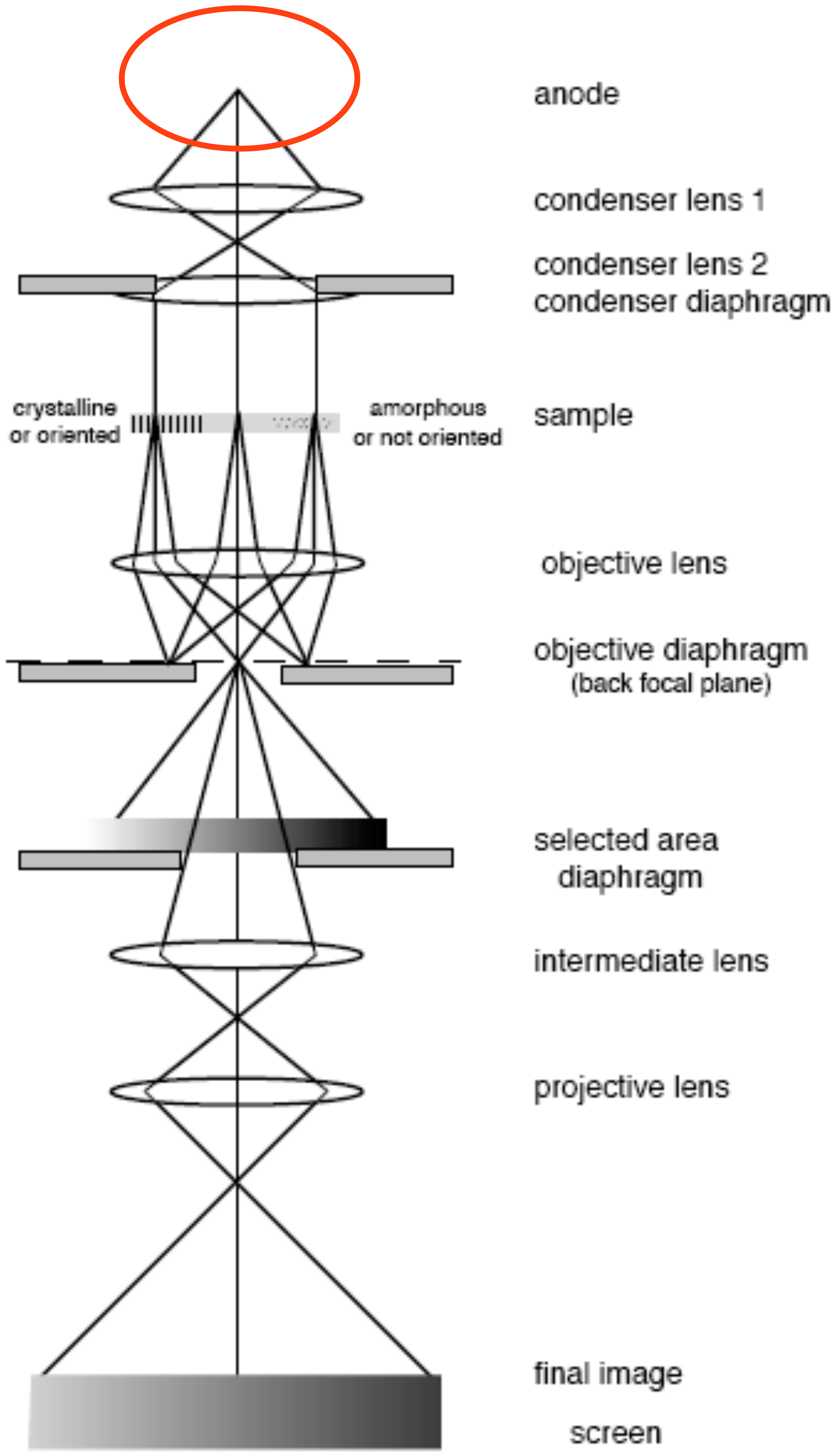
- Electron lenses terrible (relative to photon lenses) and hard to make
- Have to record many many noisy images, lots of data (just ask Jake & Toby!)
- Charging: non-conductive samples charge up and act like lenses
- Samples must be very thin and are quite fragile, move around in the beam and are often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

Example TEM schematic

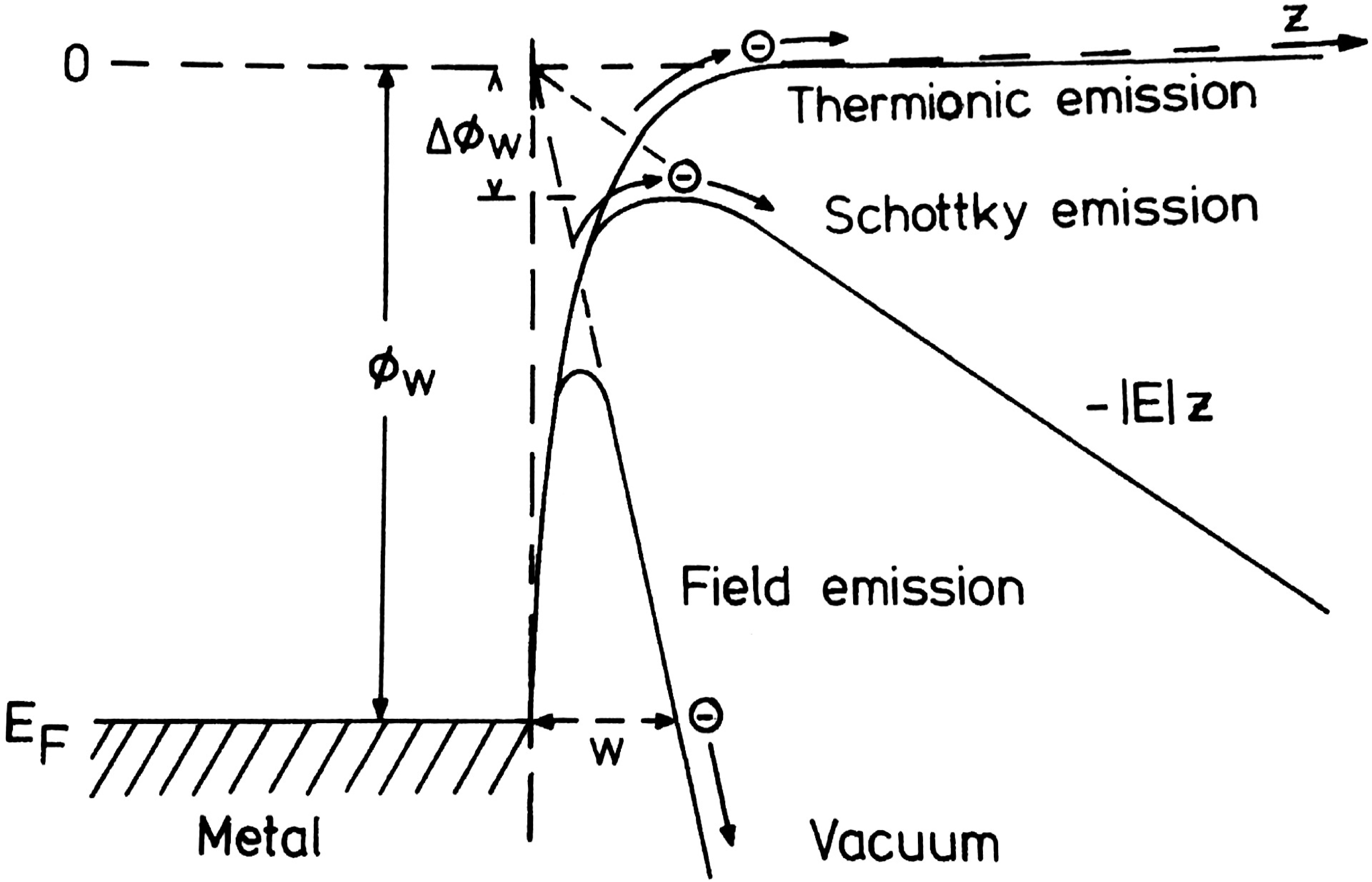
One of many types of TEMs







How to get electrons



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Thermal Emission Source

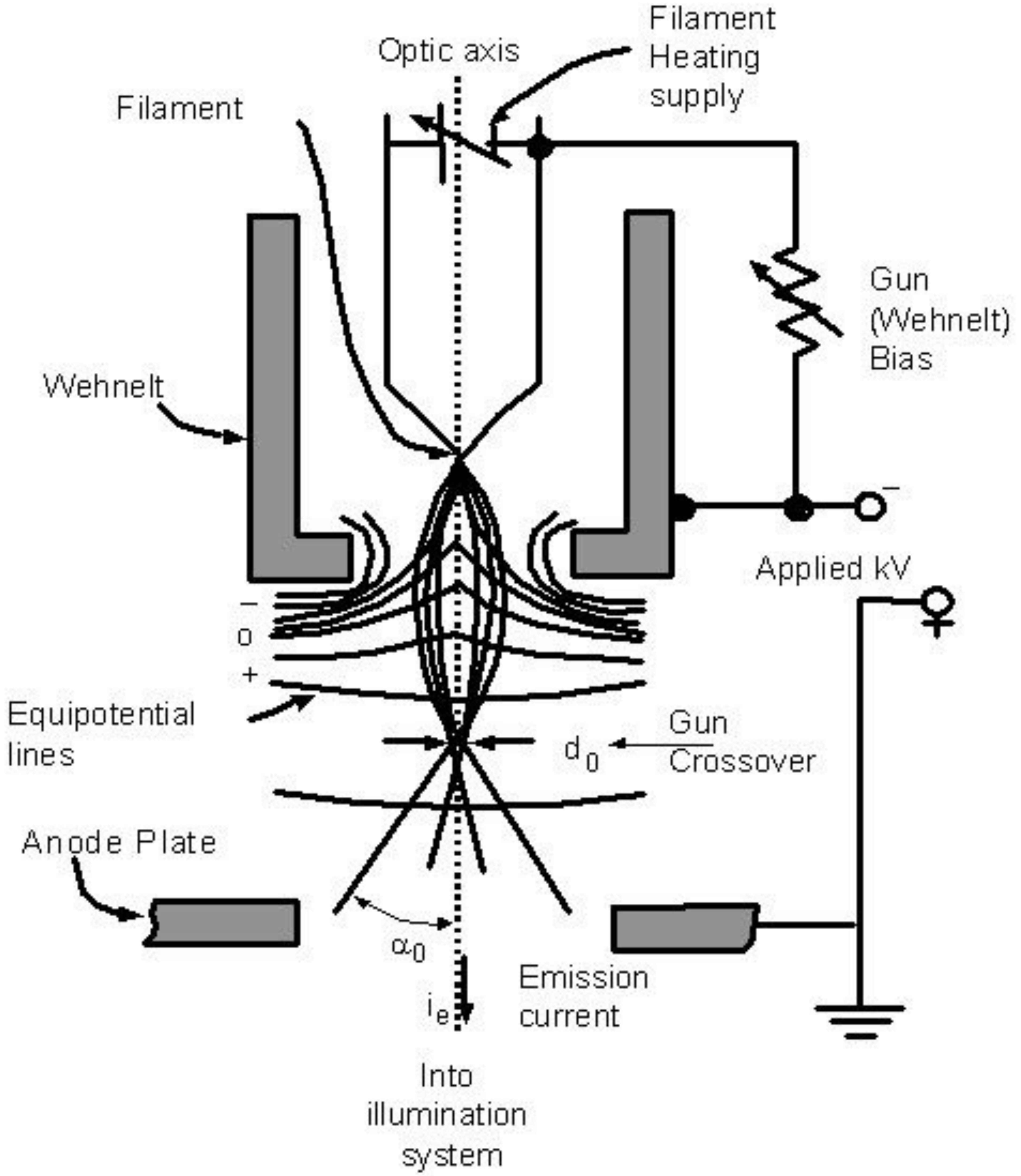
Important ideas

Wehnelt is the first lens

Anode plate

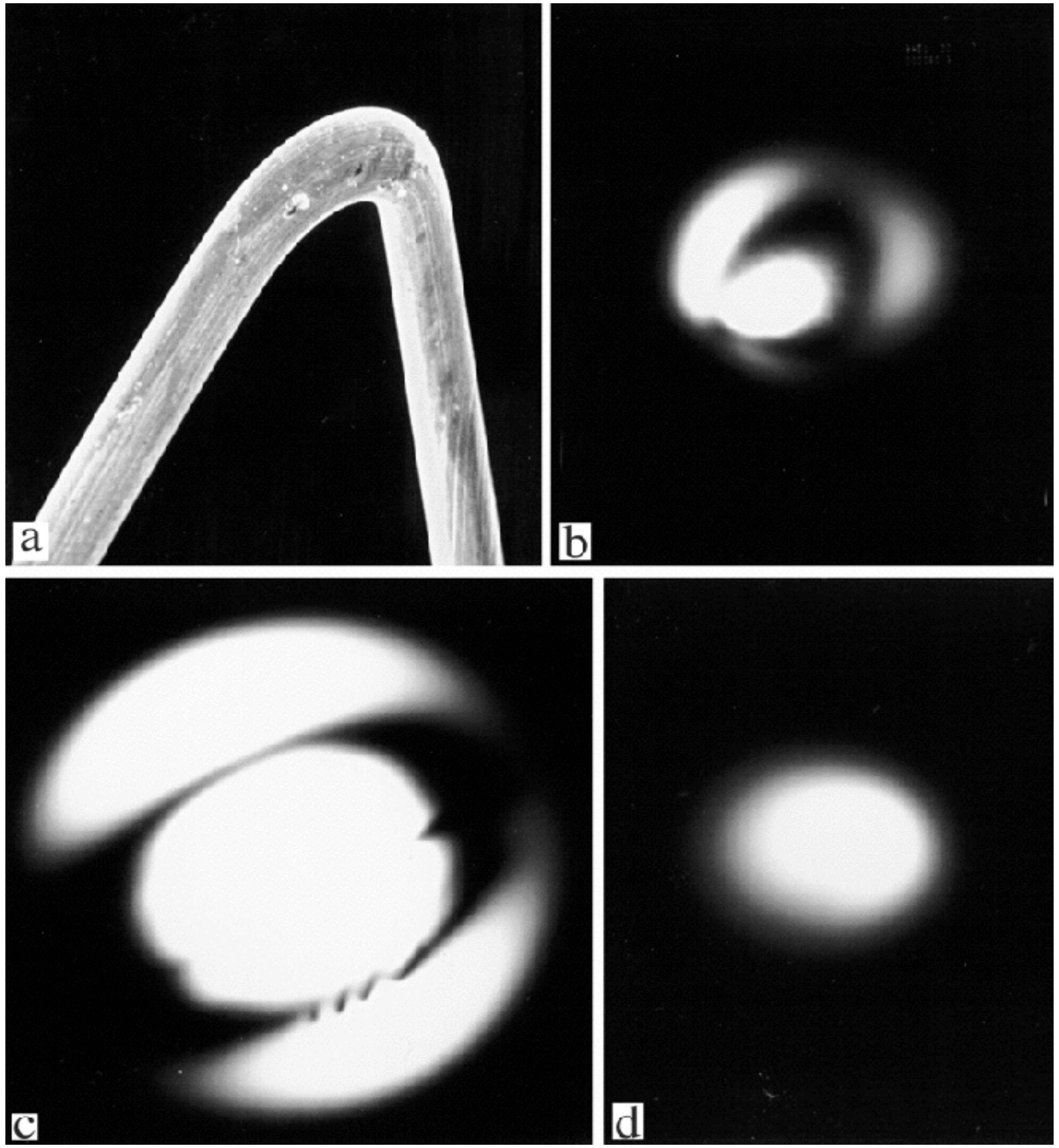
Dimensions

Cross-over



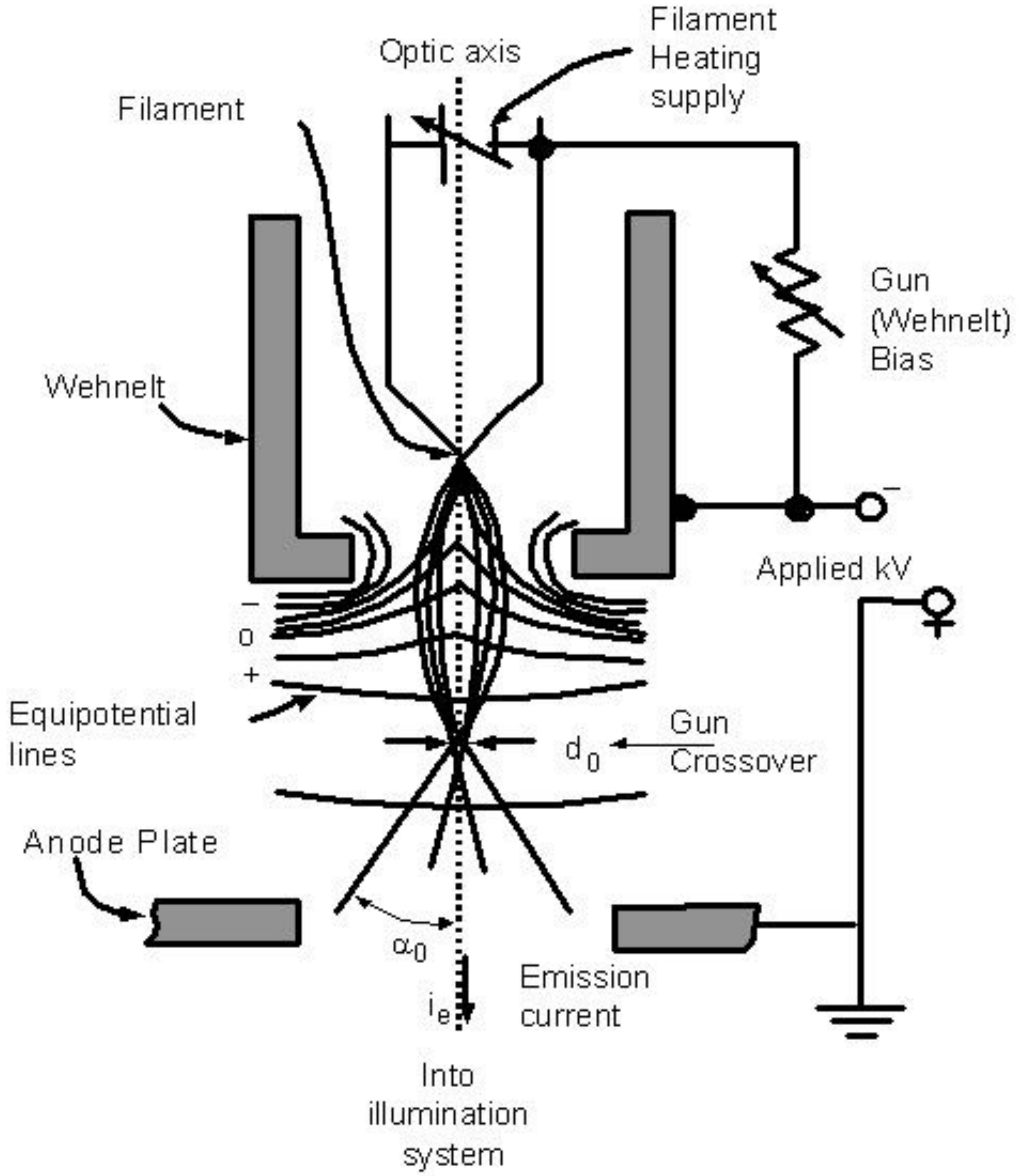
Hairpin filament

Underfocused
& off center



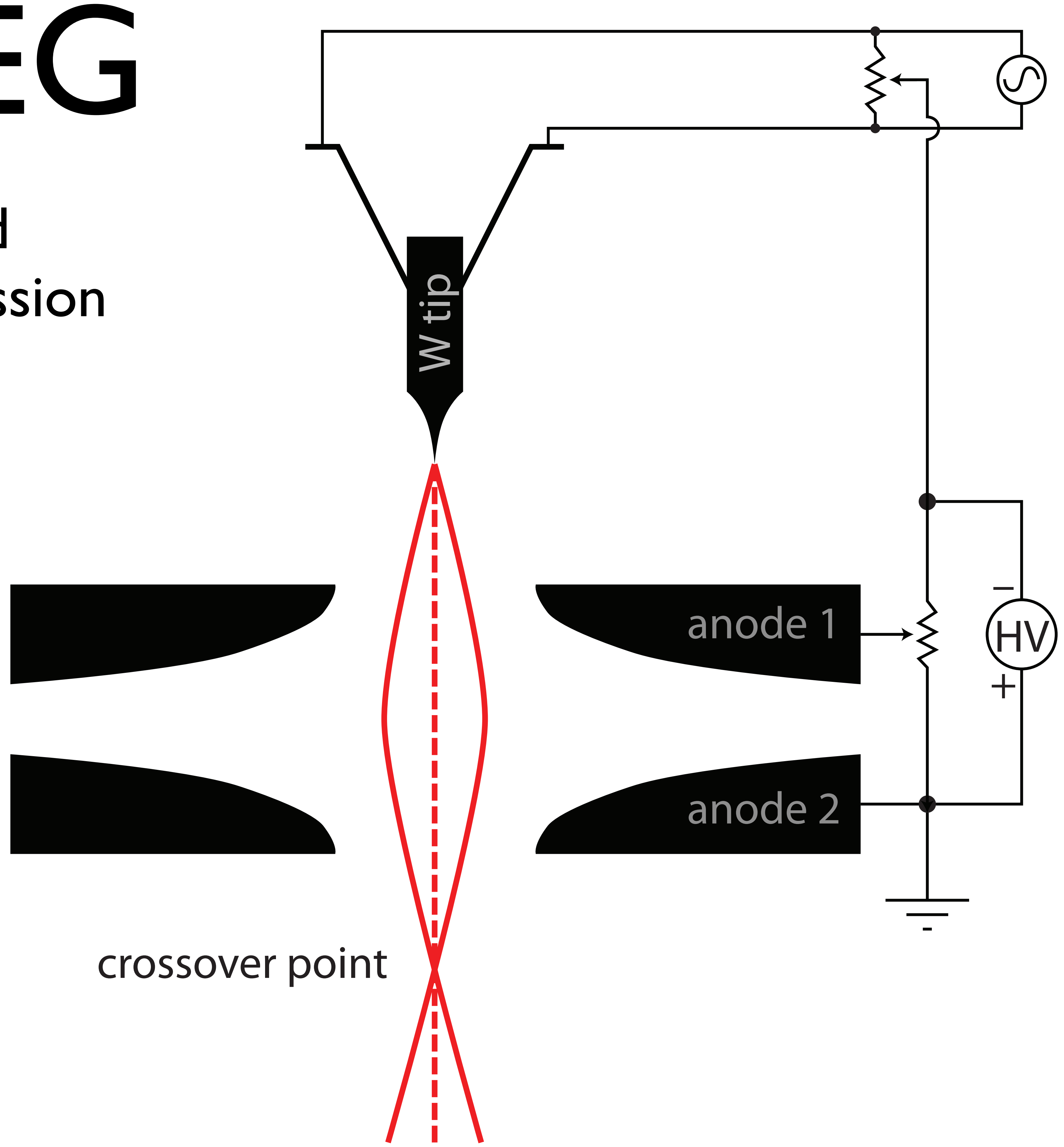
Underfocused but
centered

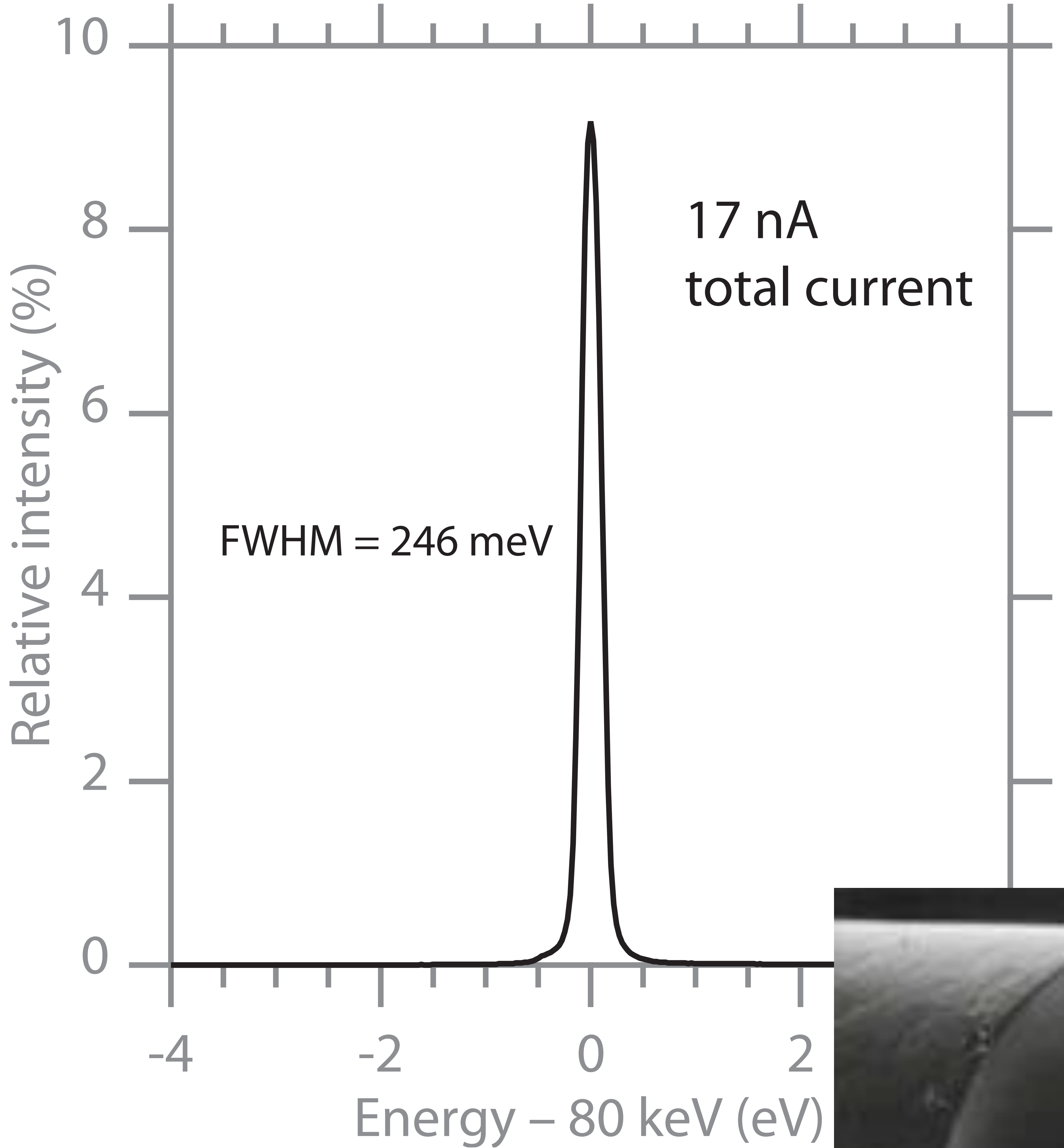
Centered &
focused

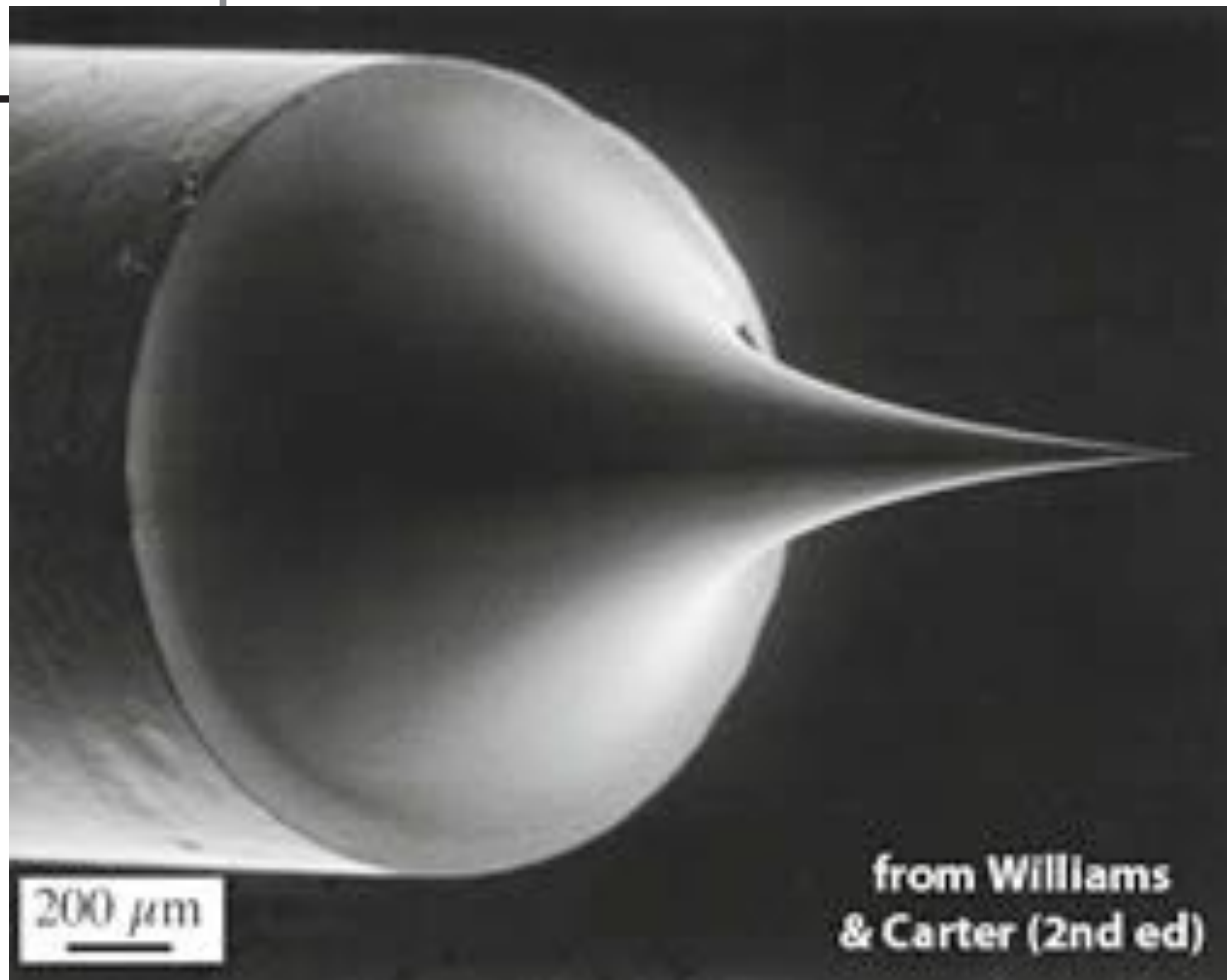


FEG

Field
Emission
Gun







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

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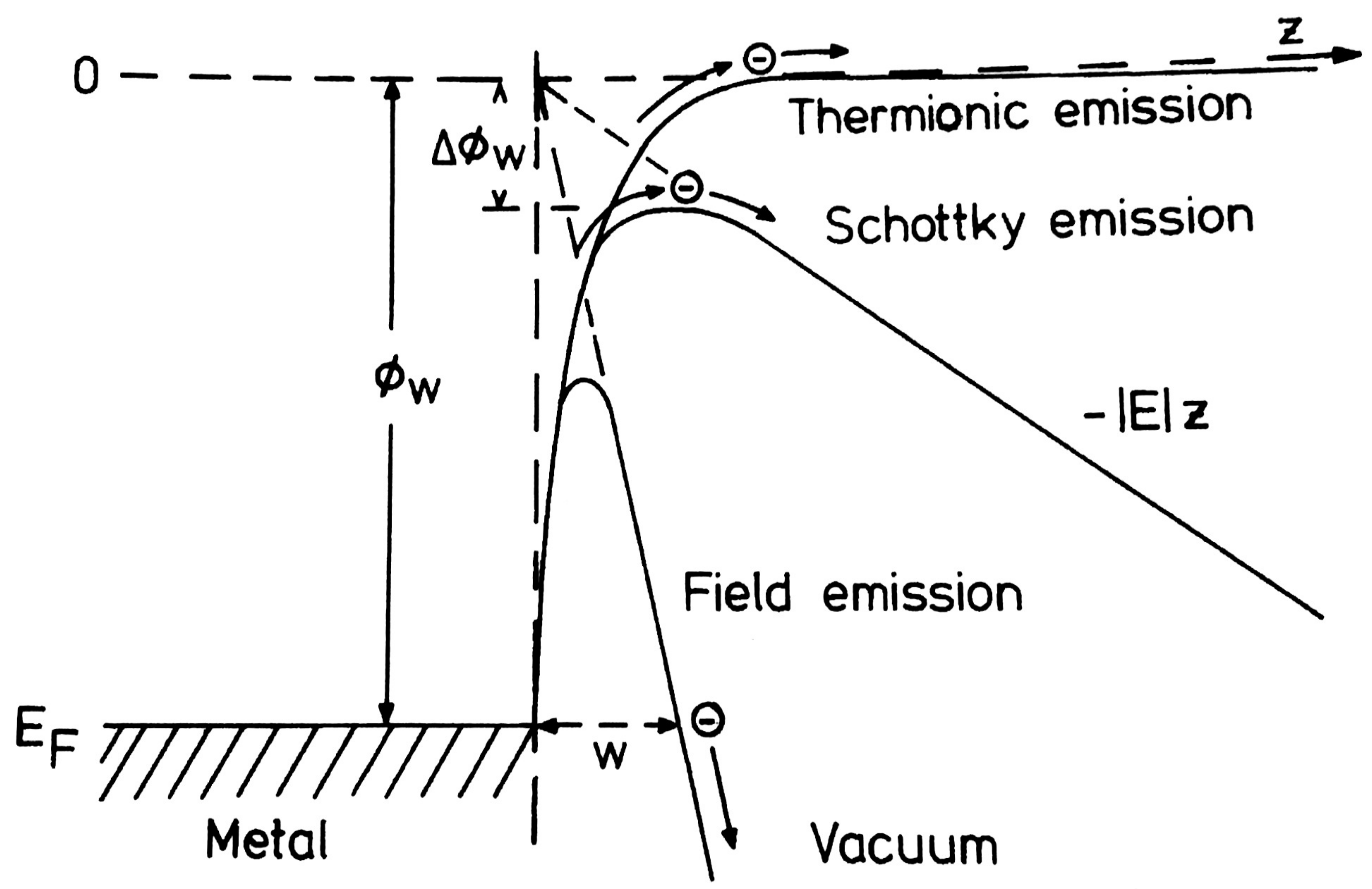
Characteristics of Electron Sources



	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

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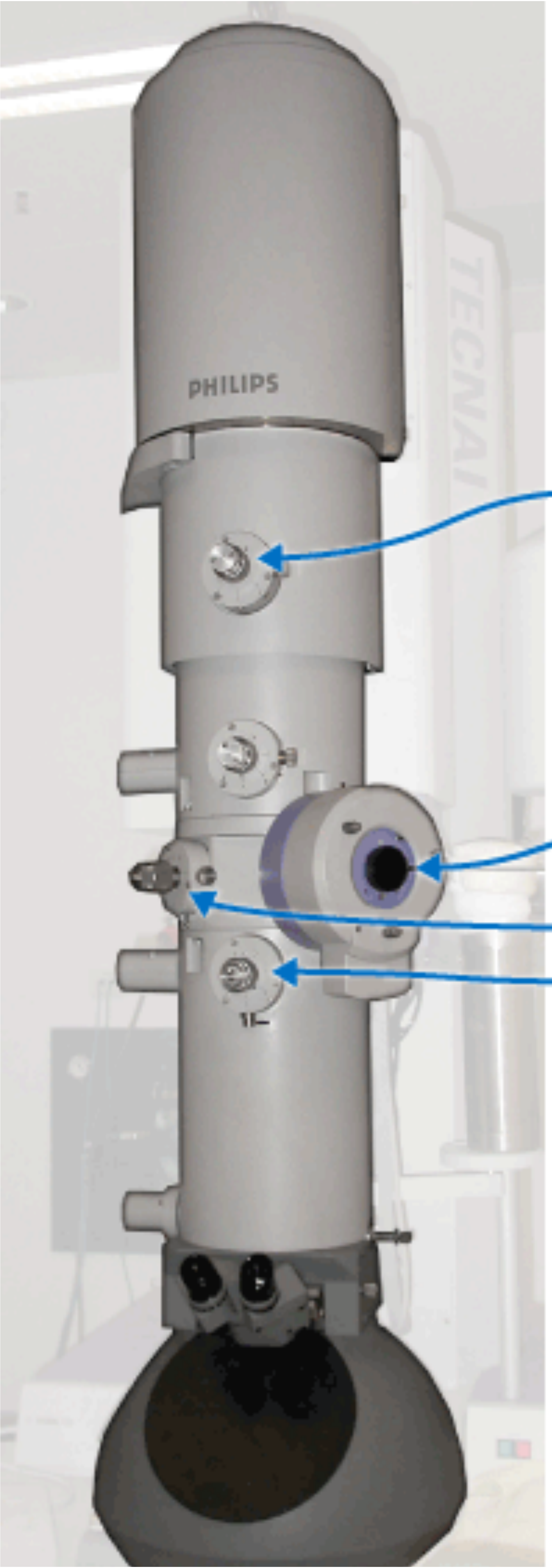
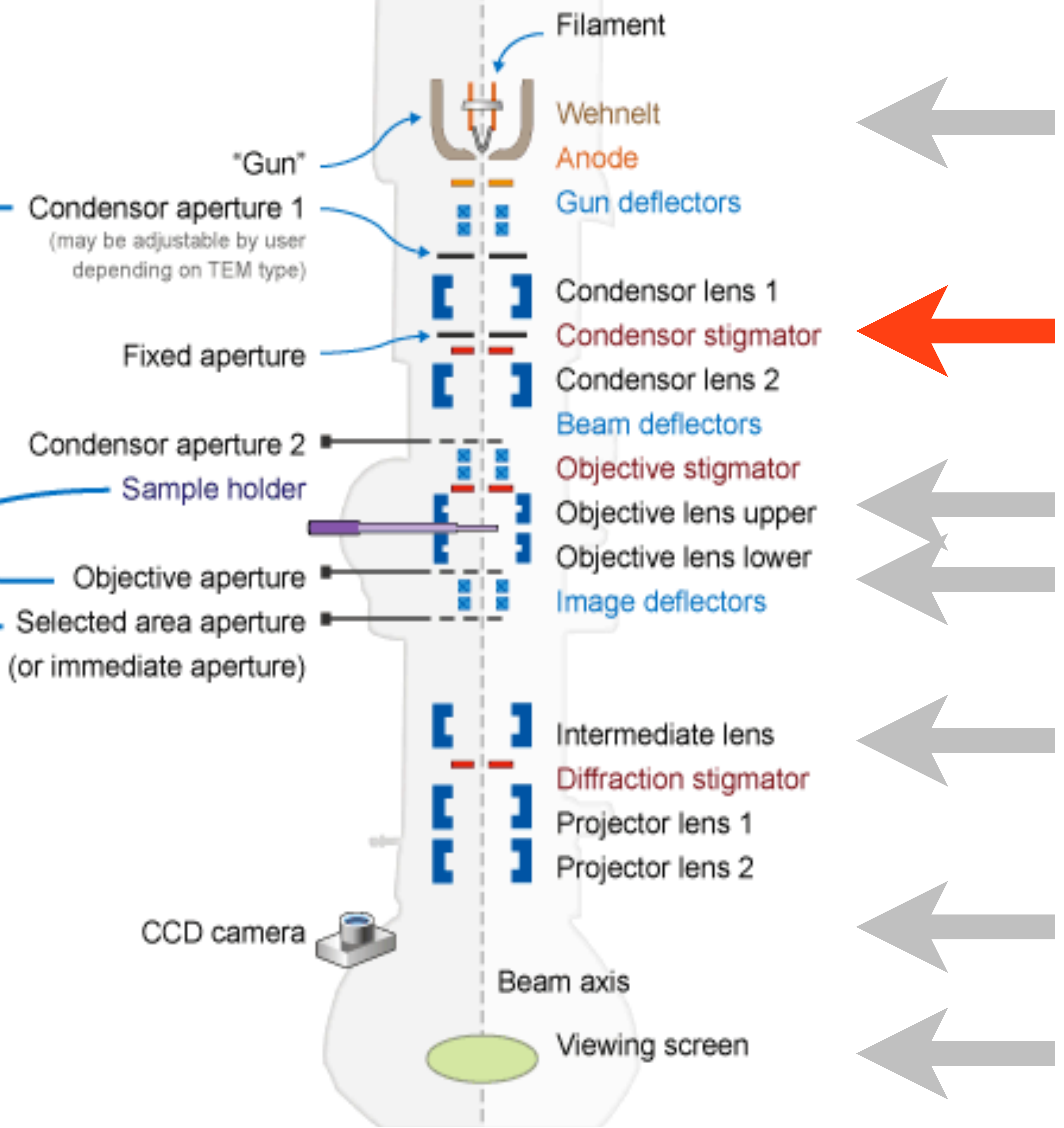
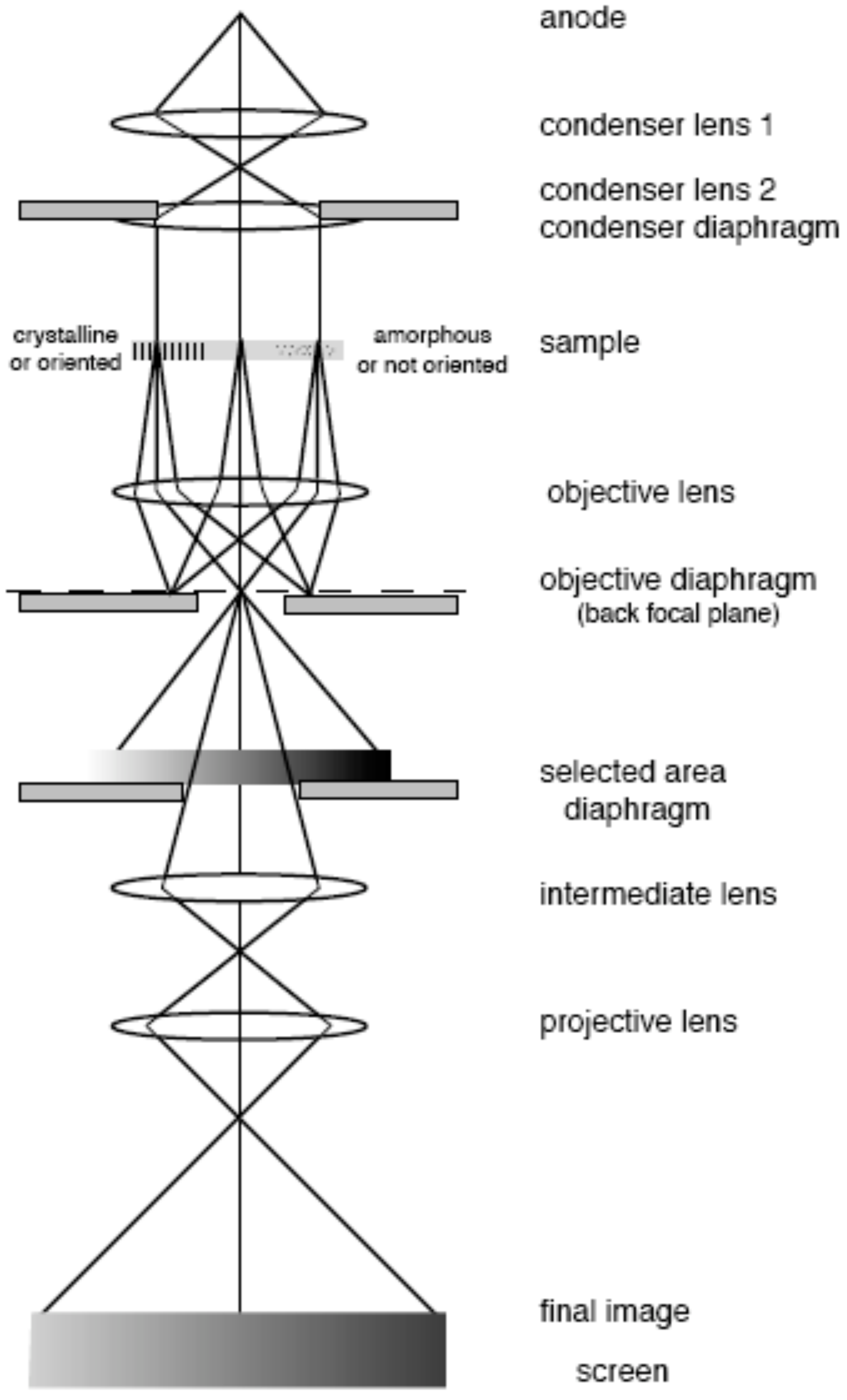
Example TEM schematic

One of many types of TEMs

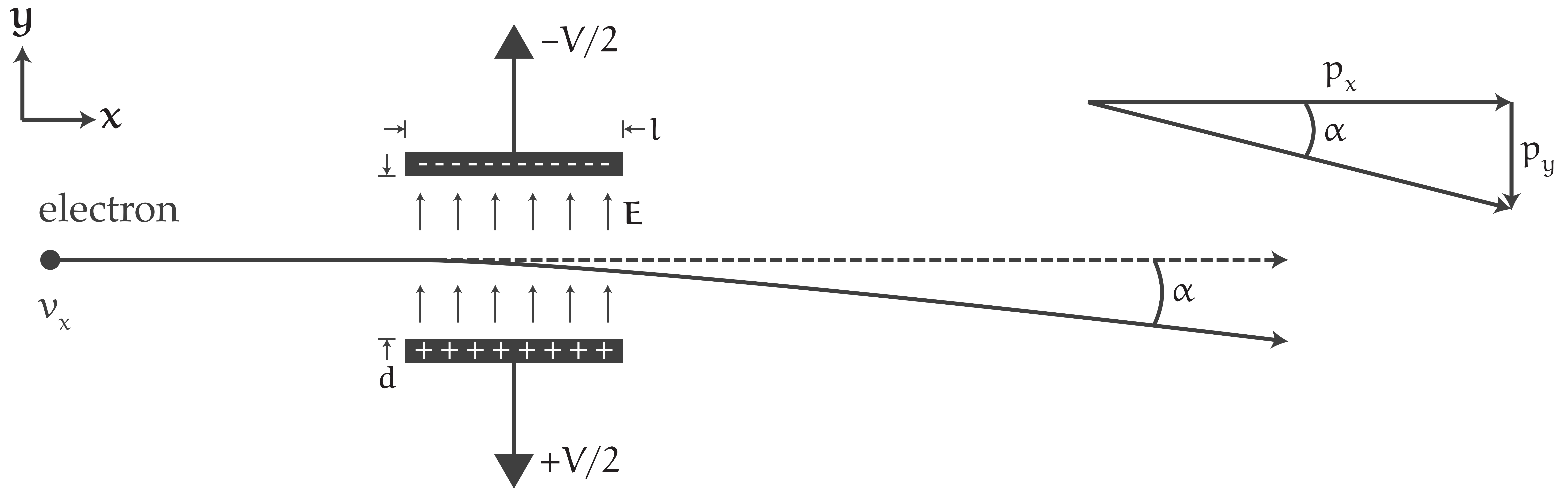


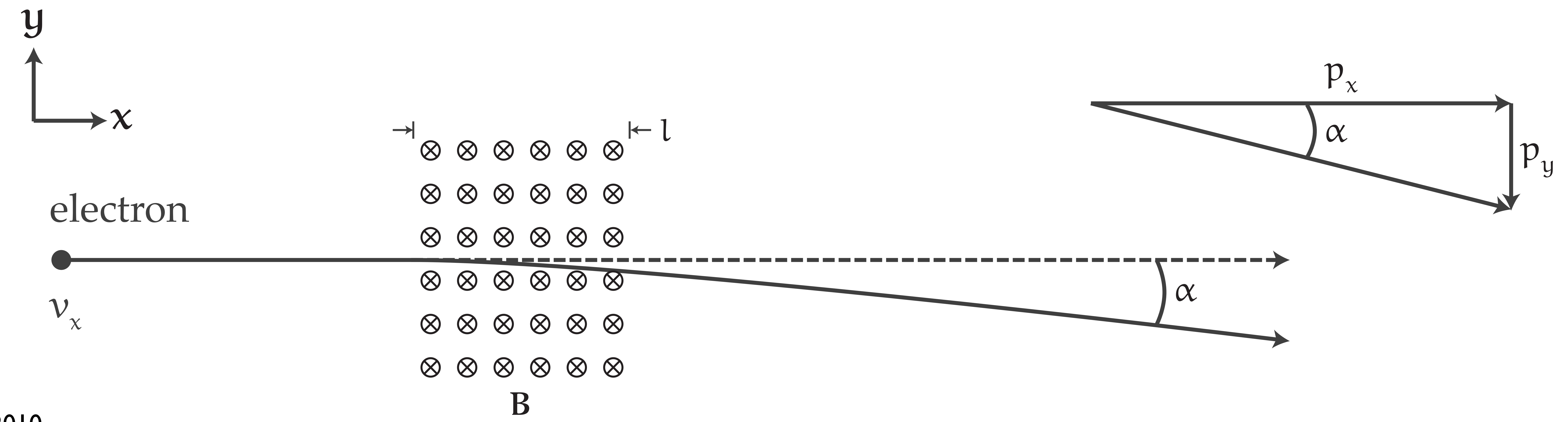


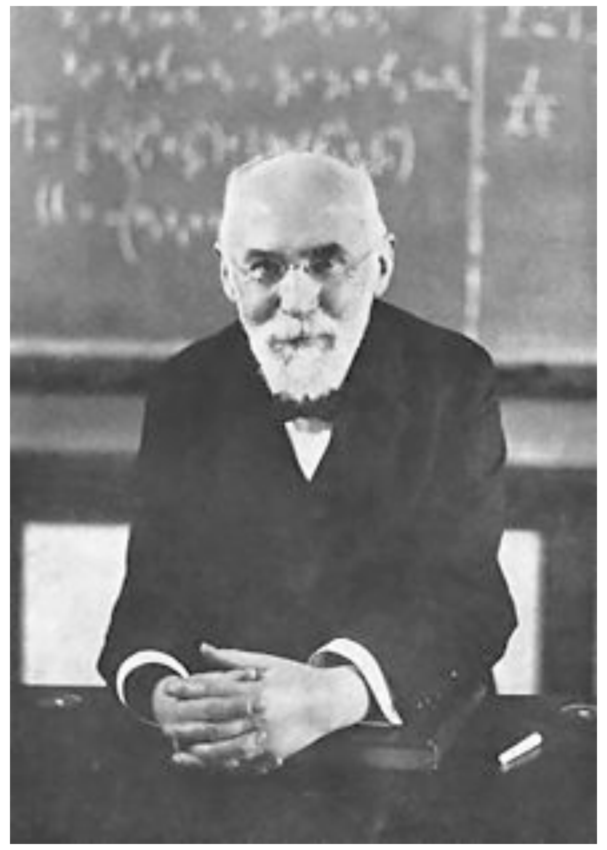


The Lorentz force

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







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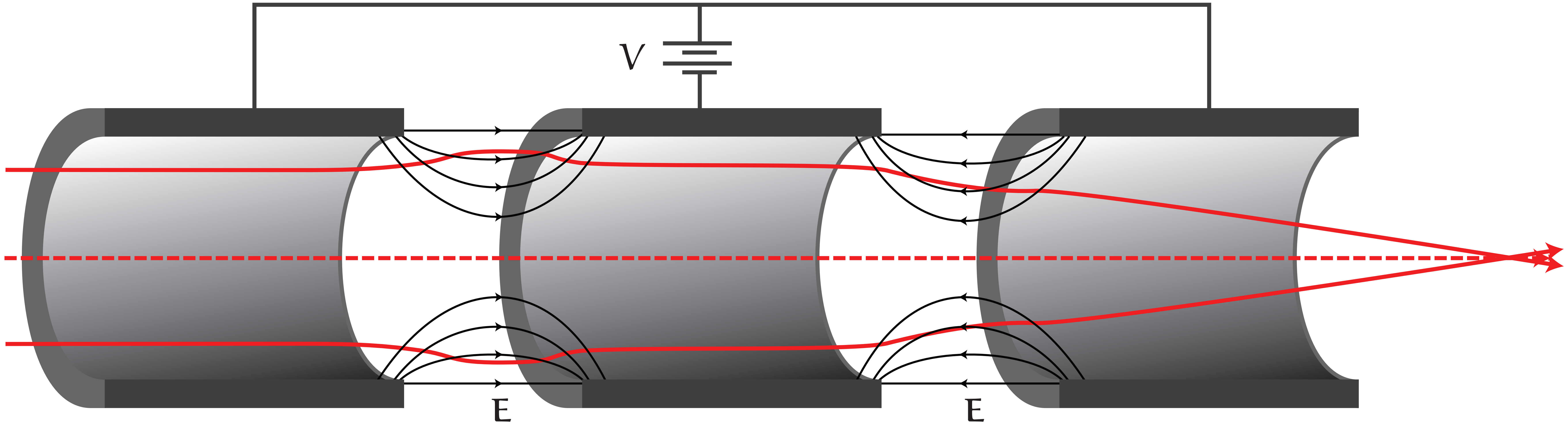

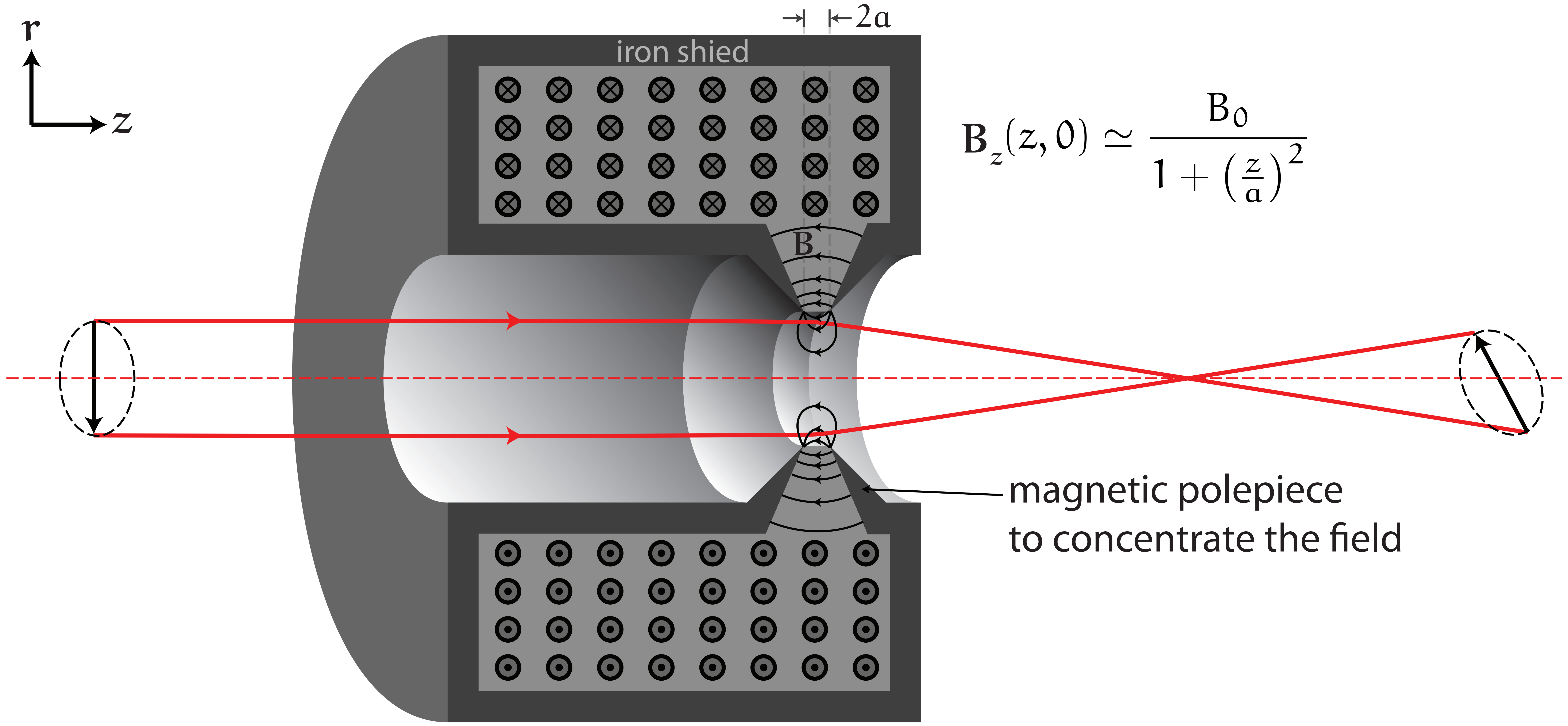

$$B_z(z,0) \simeq \frac{B_0}{1+\left(\frac{z}{a}\right)^2}$$

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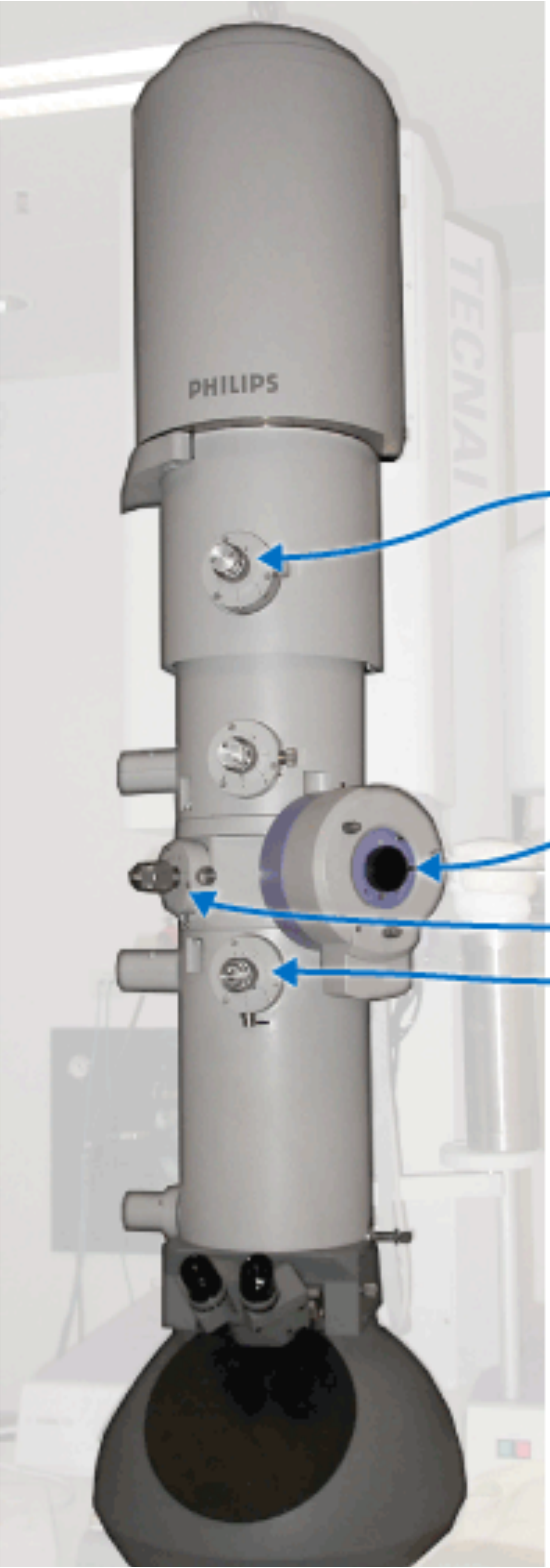
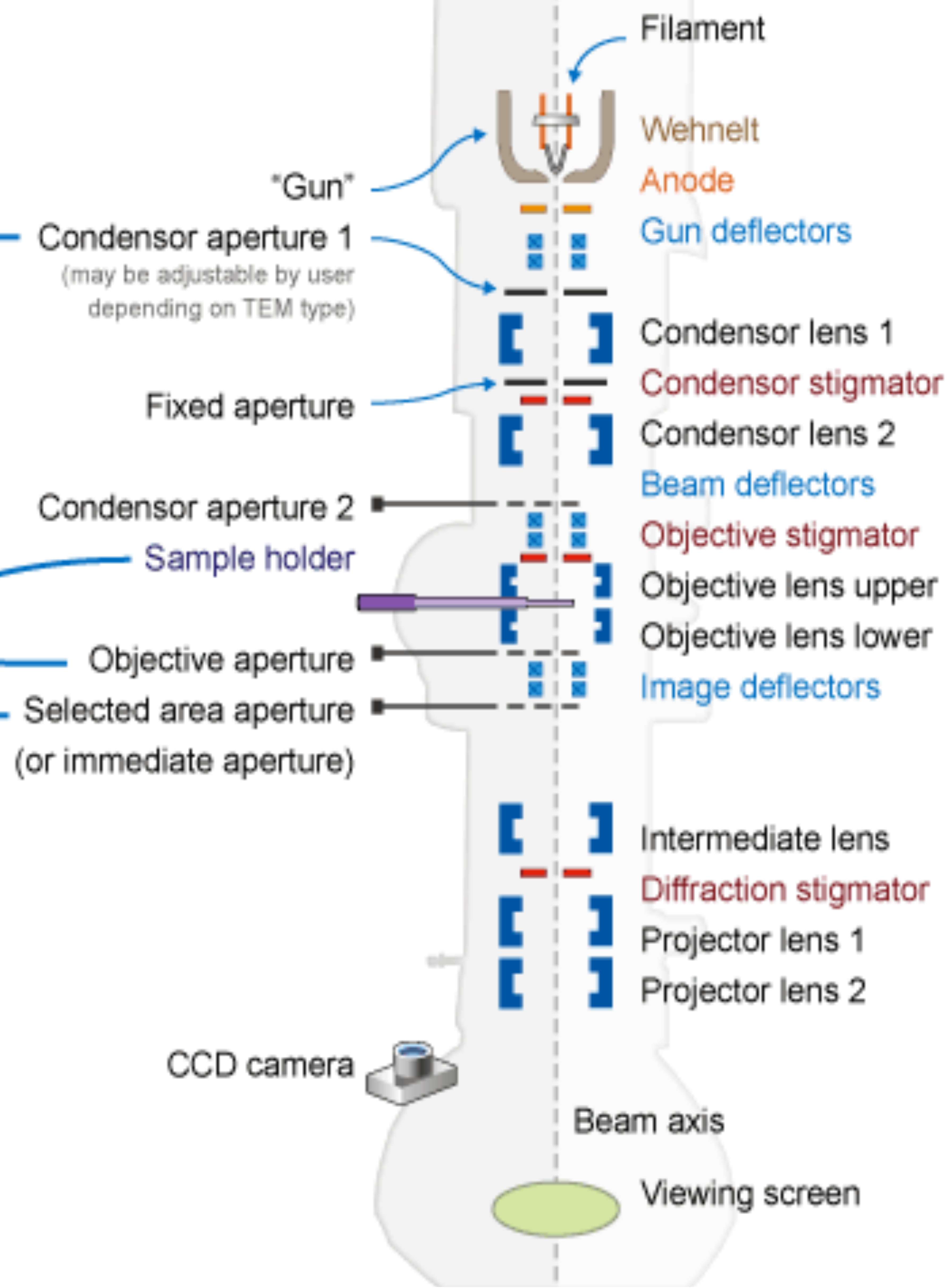
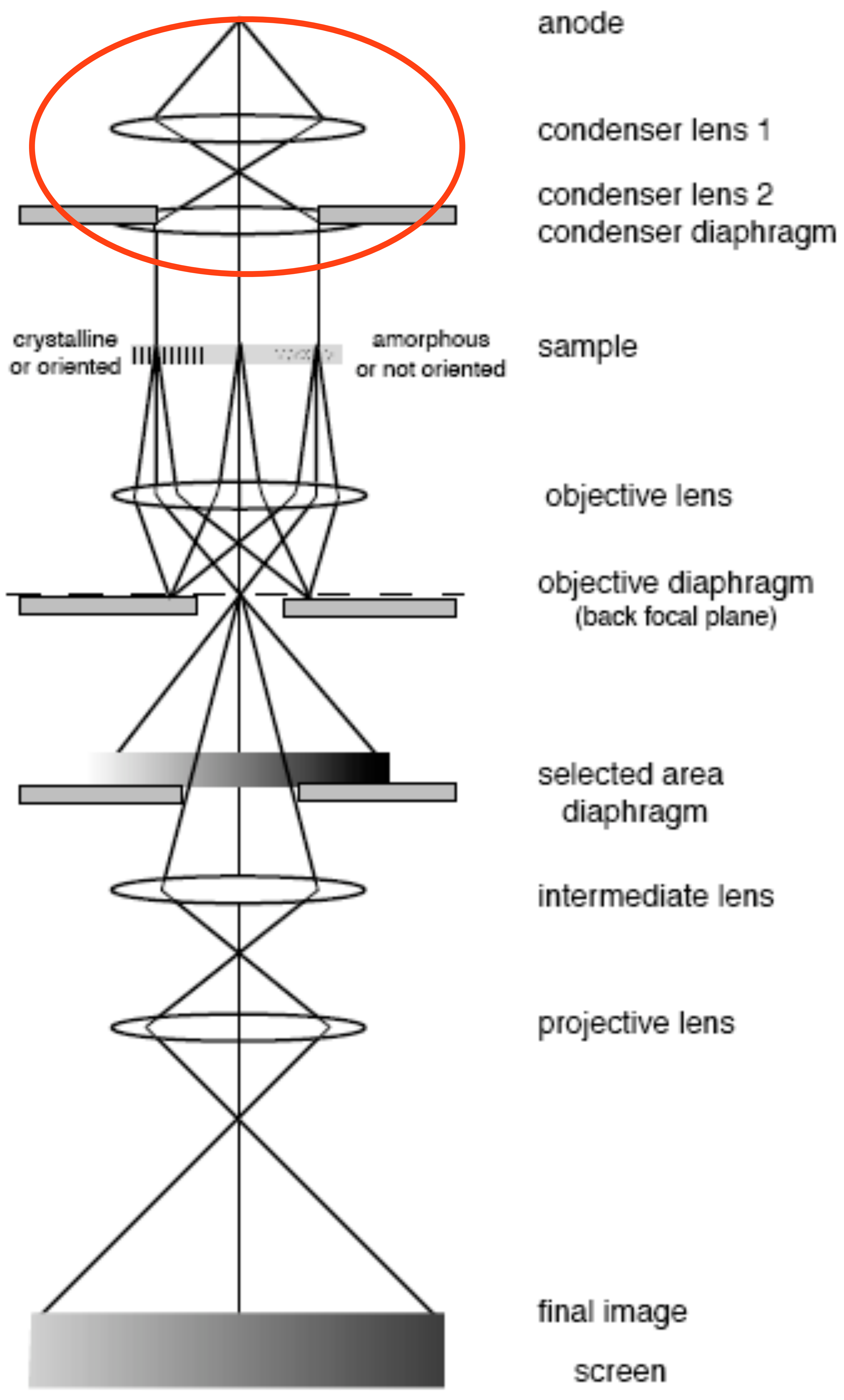
Example TEM schematic

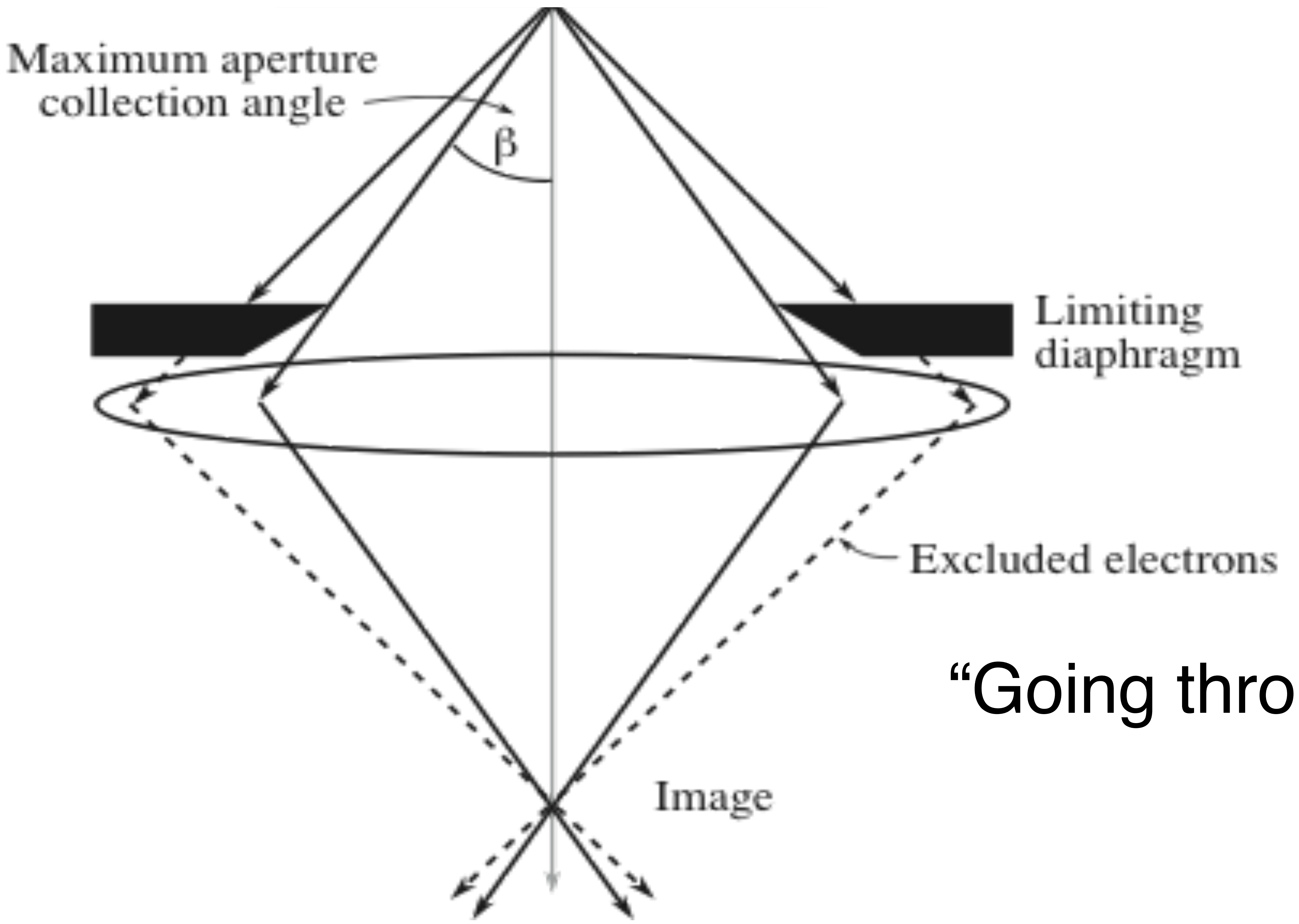
One of many types of TEMs







Diaphragms & Apertures

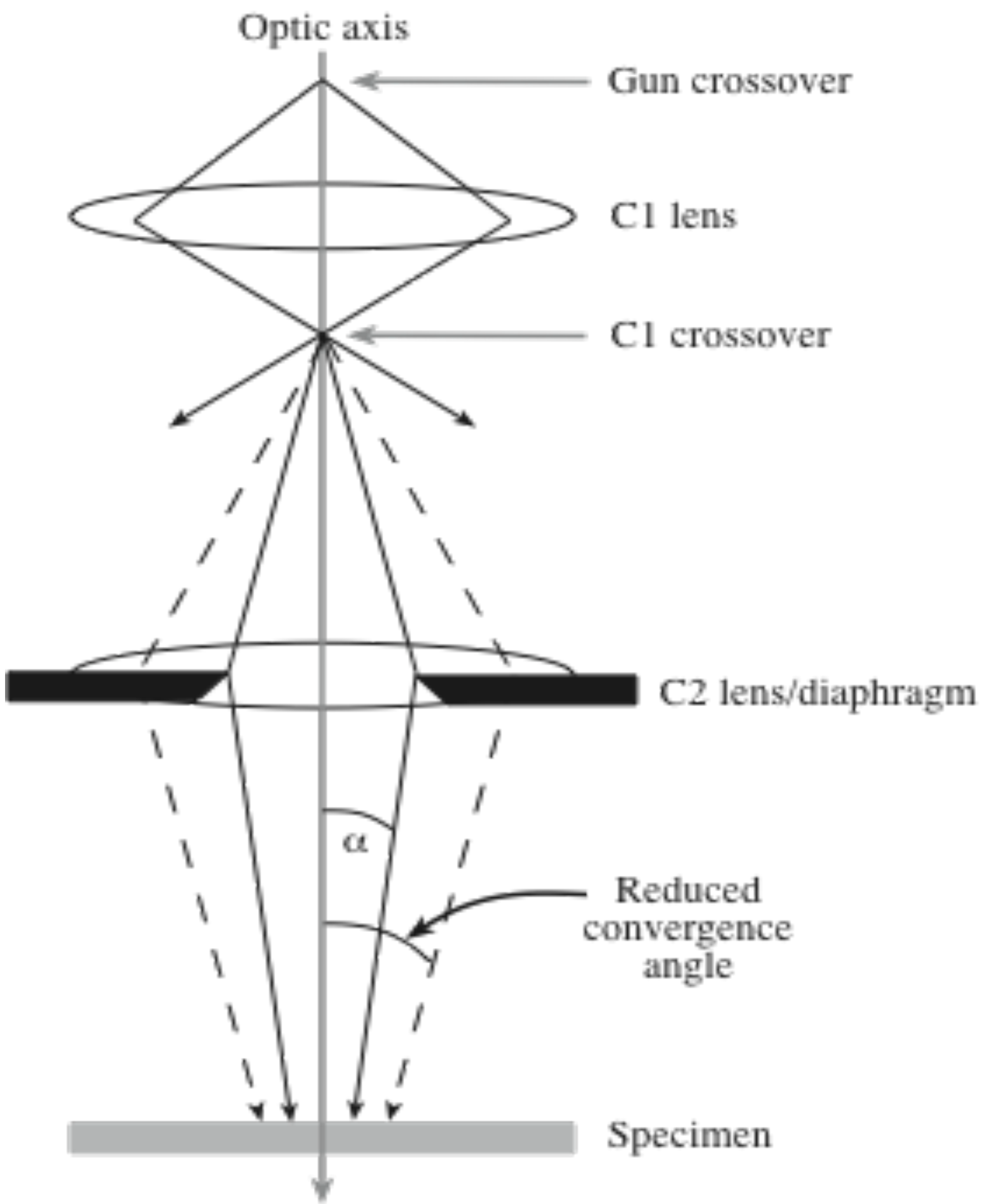


"Going through the door"

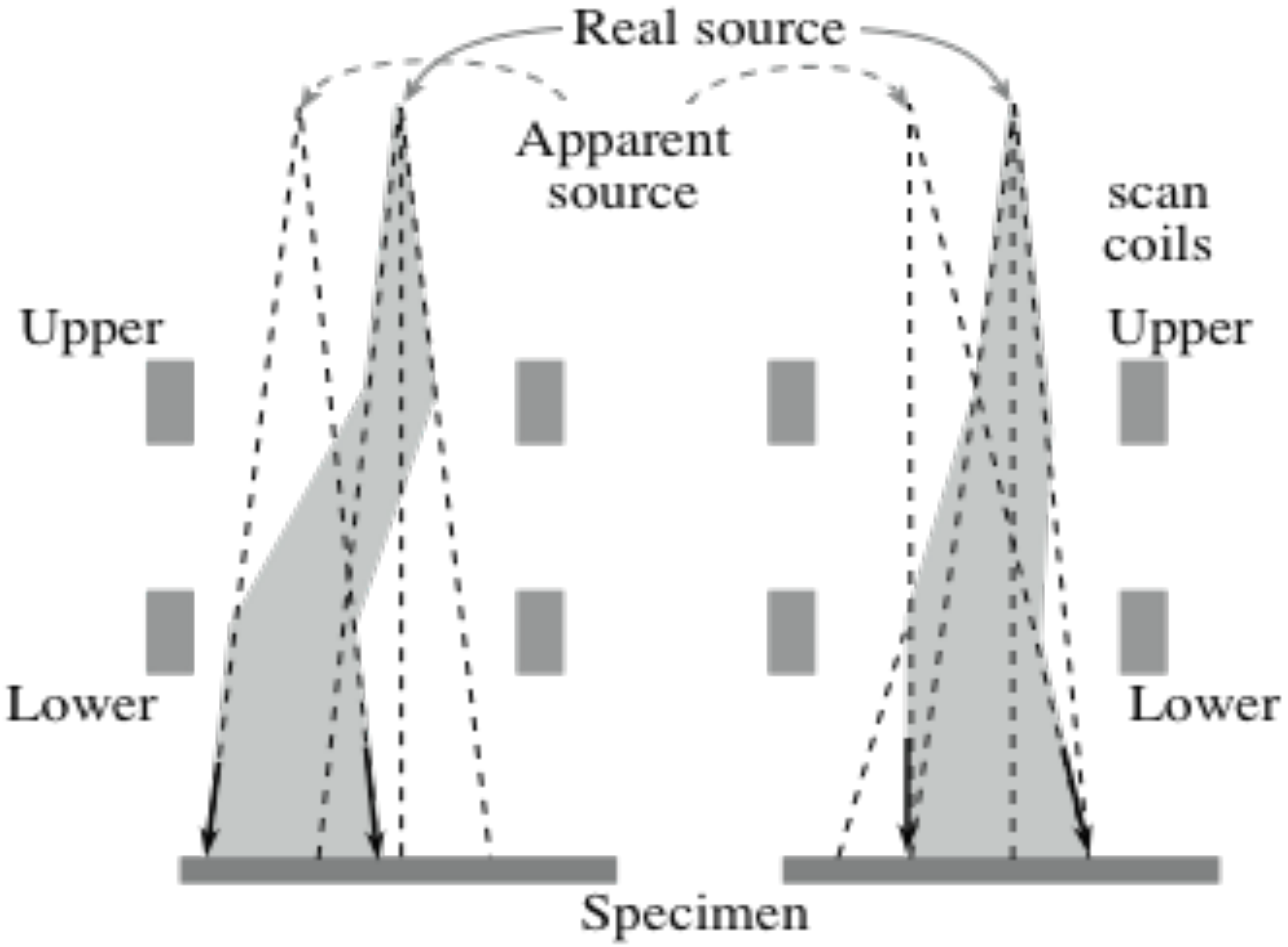
The Basic Electron Condenser System

Most TEMs 2 lenses + 1 aperture

Krios: 3 lenses + 1 aperture



Shift and tilt through a lens



Shift

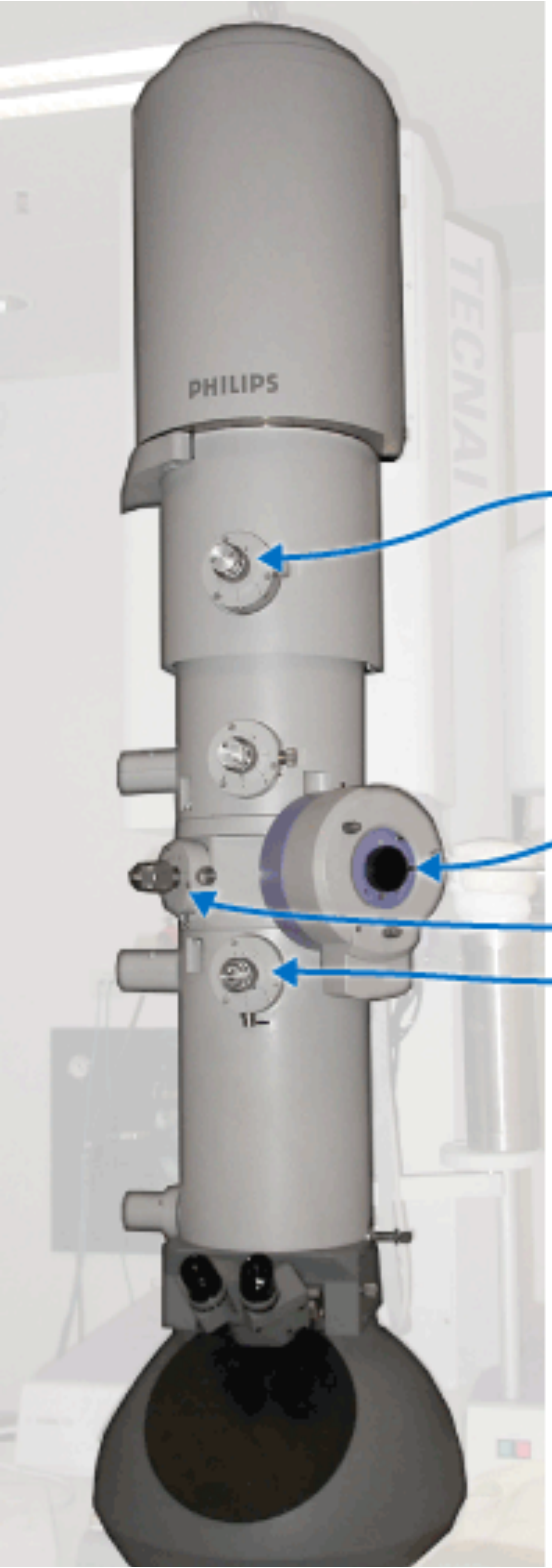
Tilt

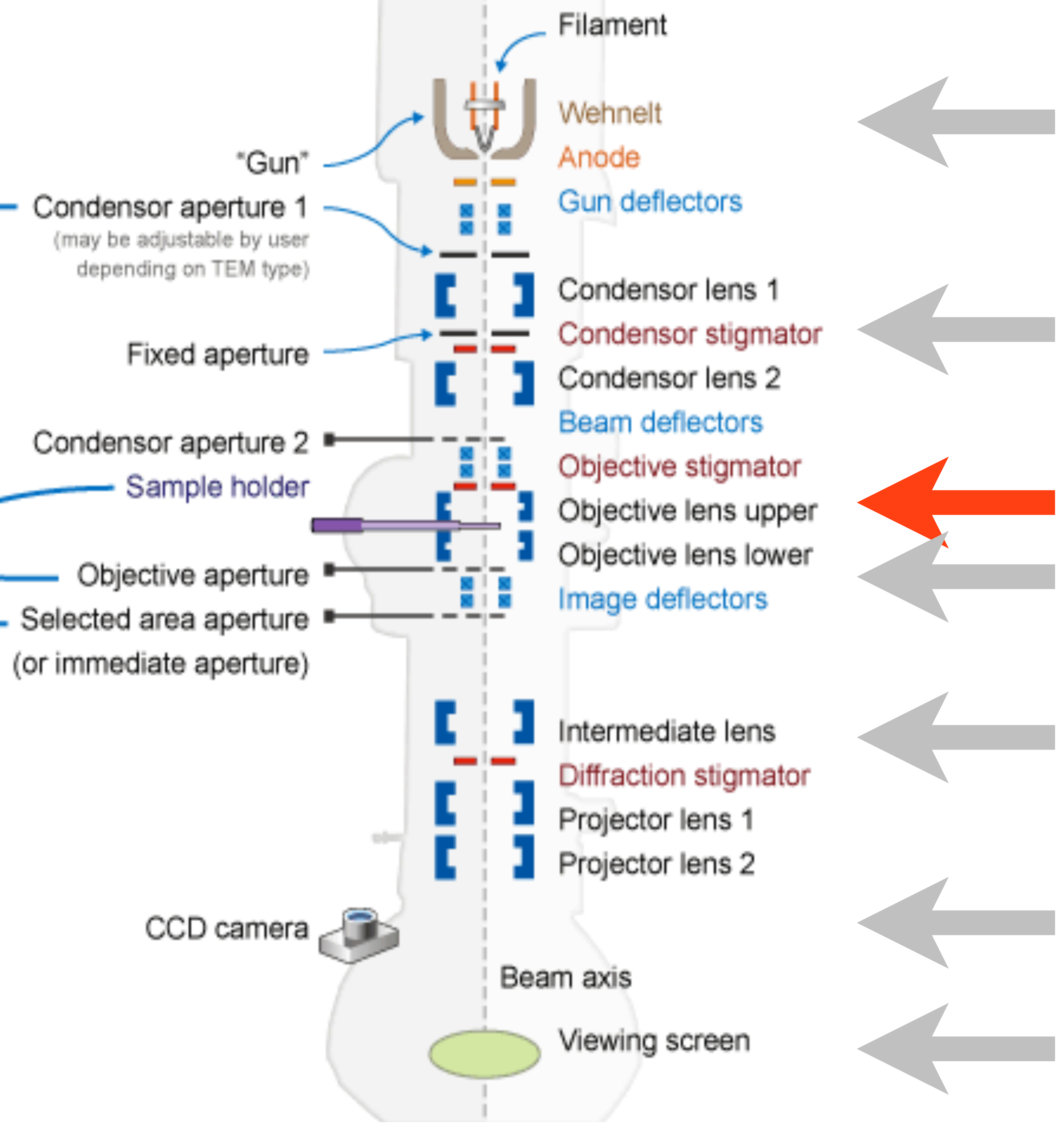
Same direction -> Different area

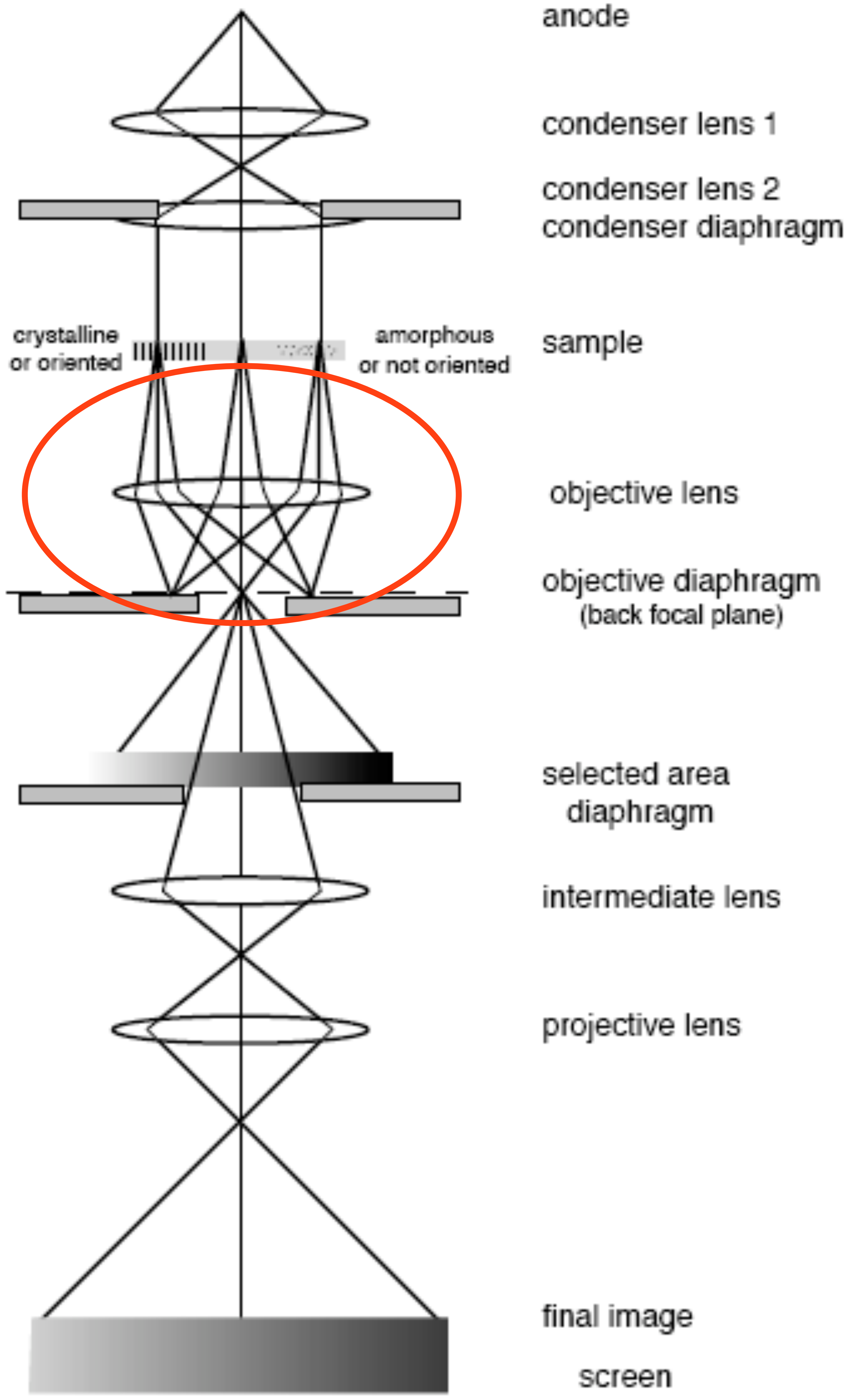
Same area -> Different direction

Example TEM schematic

One of many types of TEMs

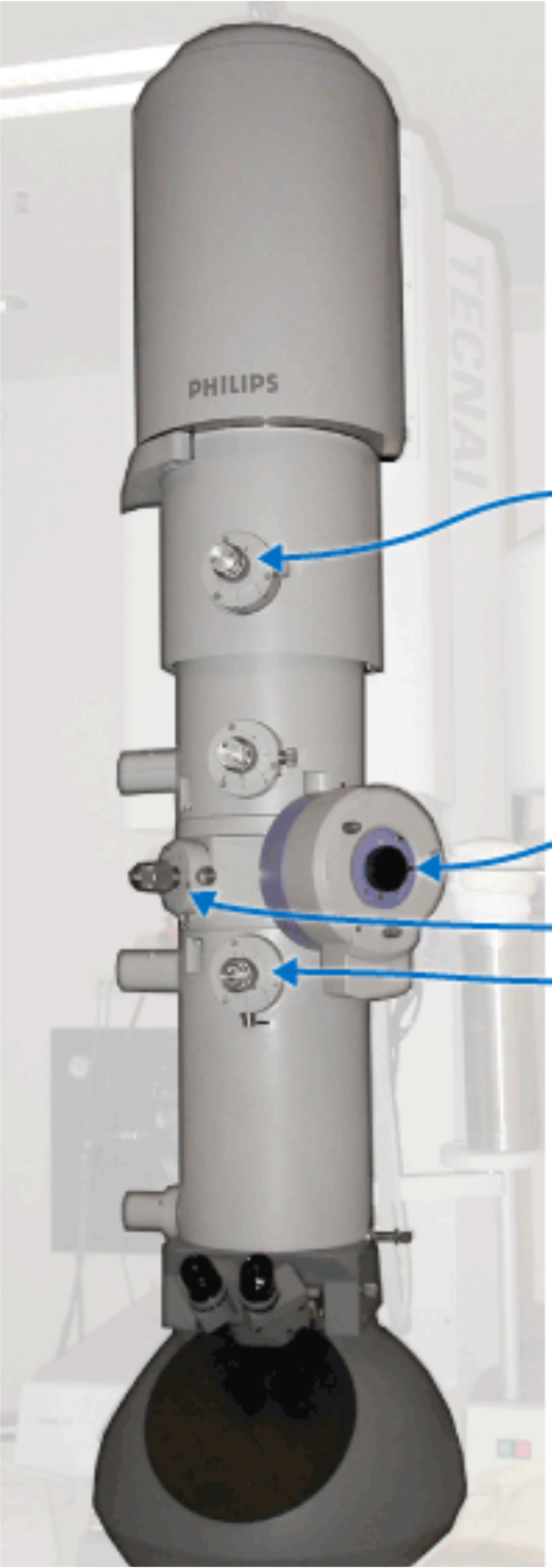


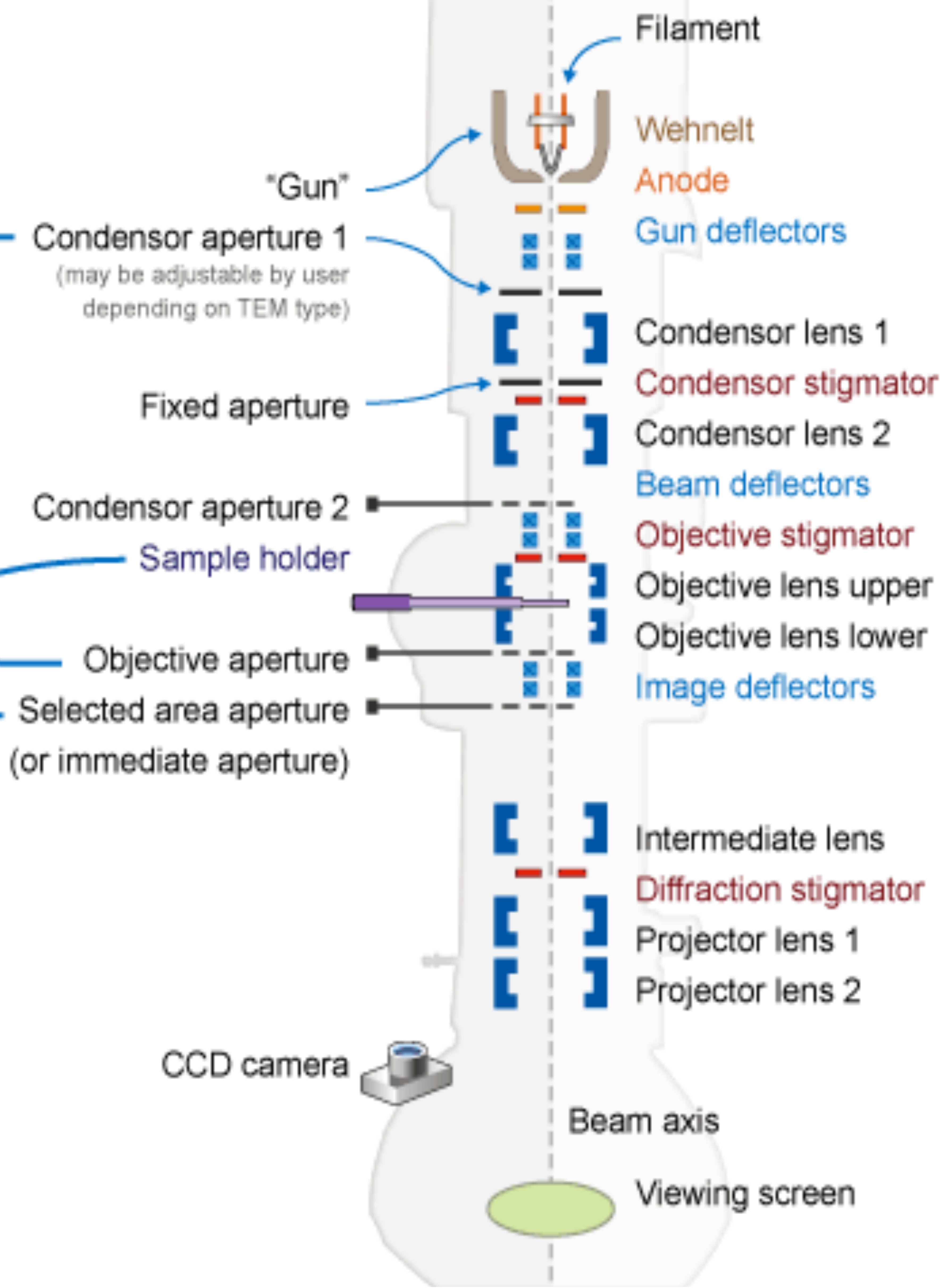


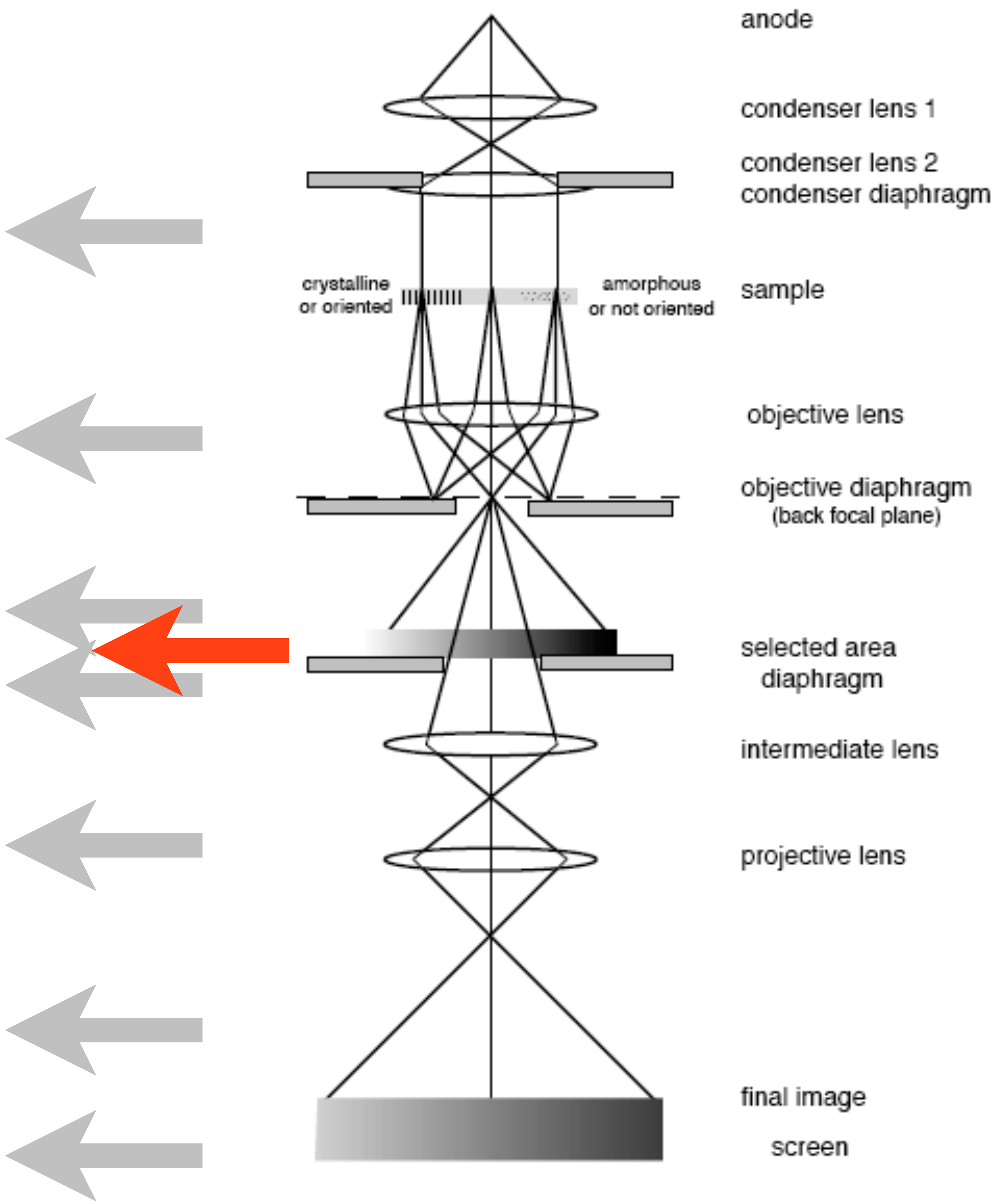


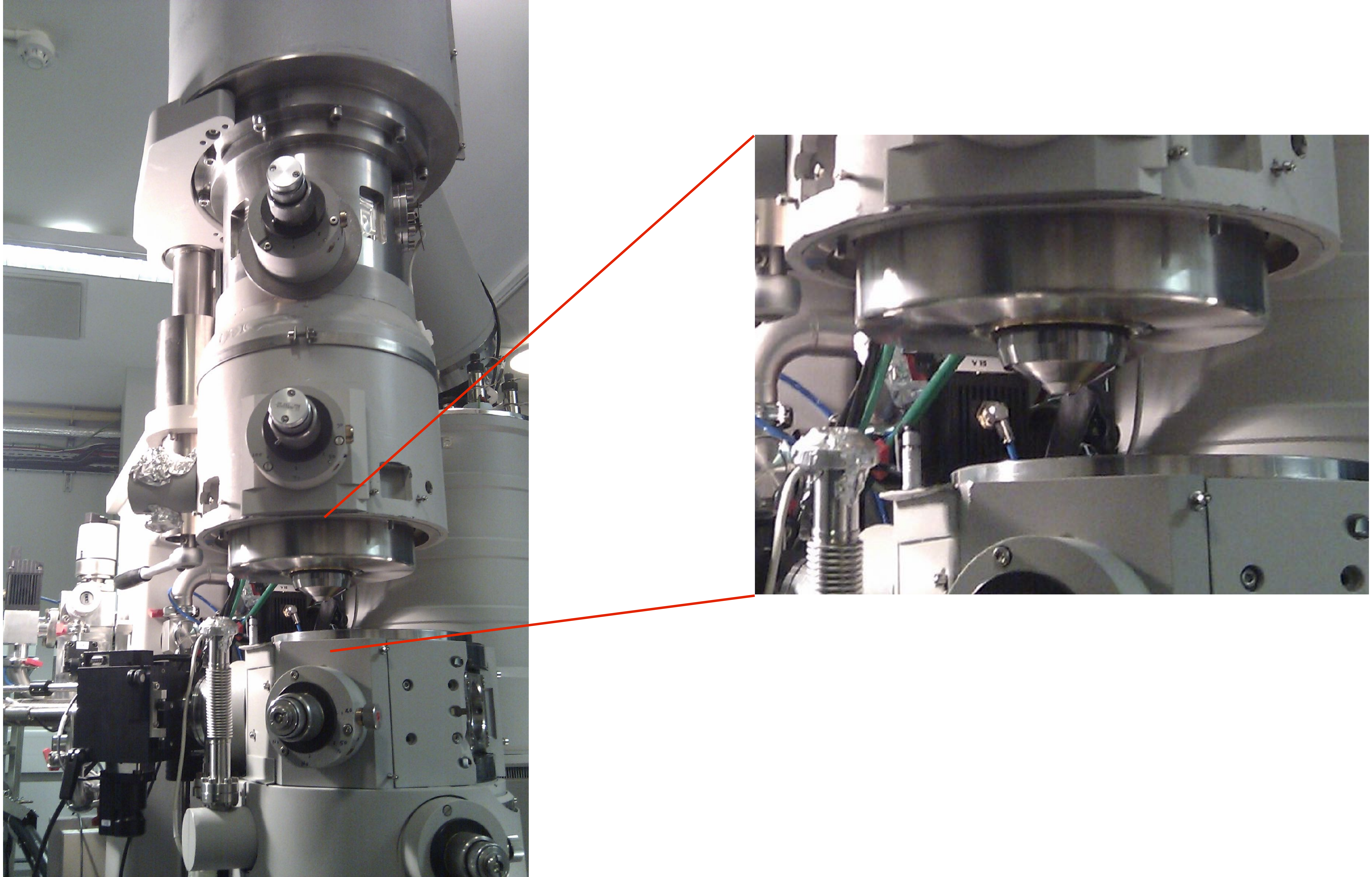
Example TEM schematic

One of many types of TEMs







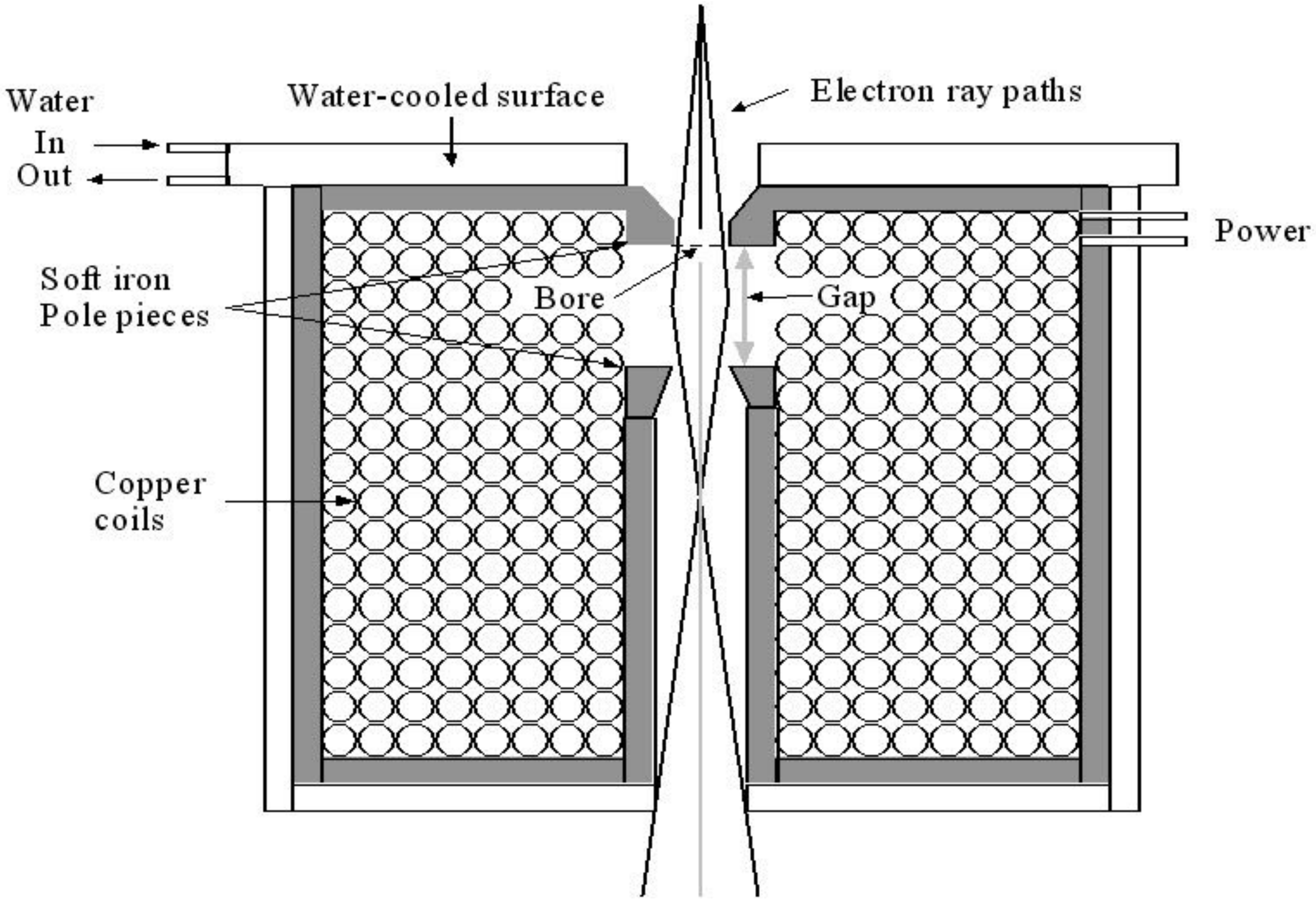


Magnetic Lens

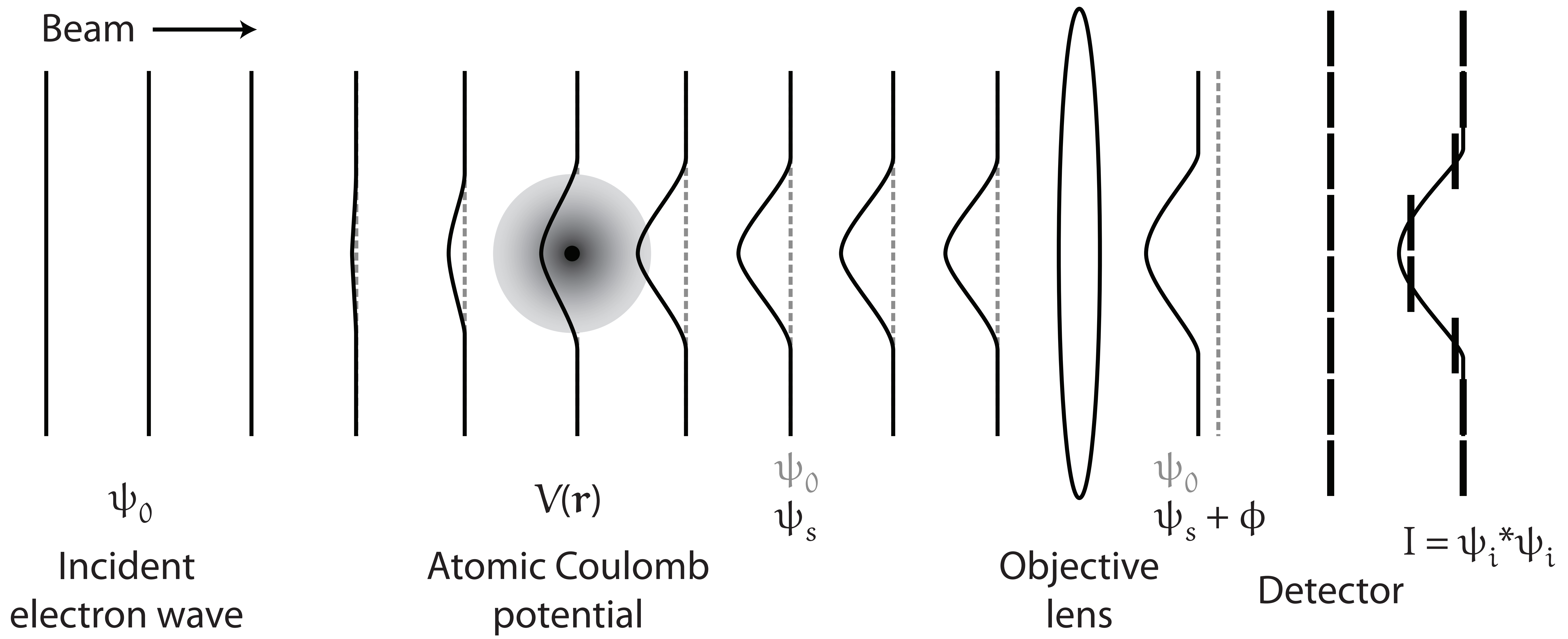
Concentrate flux

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

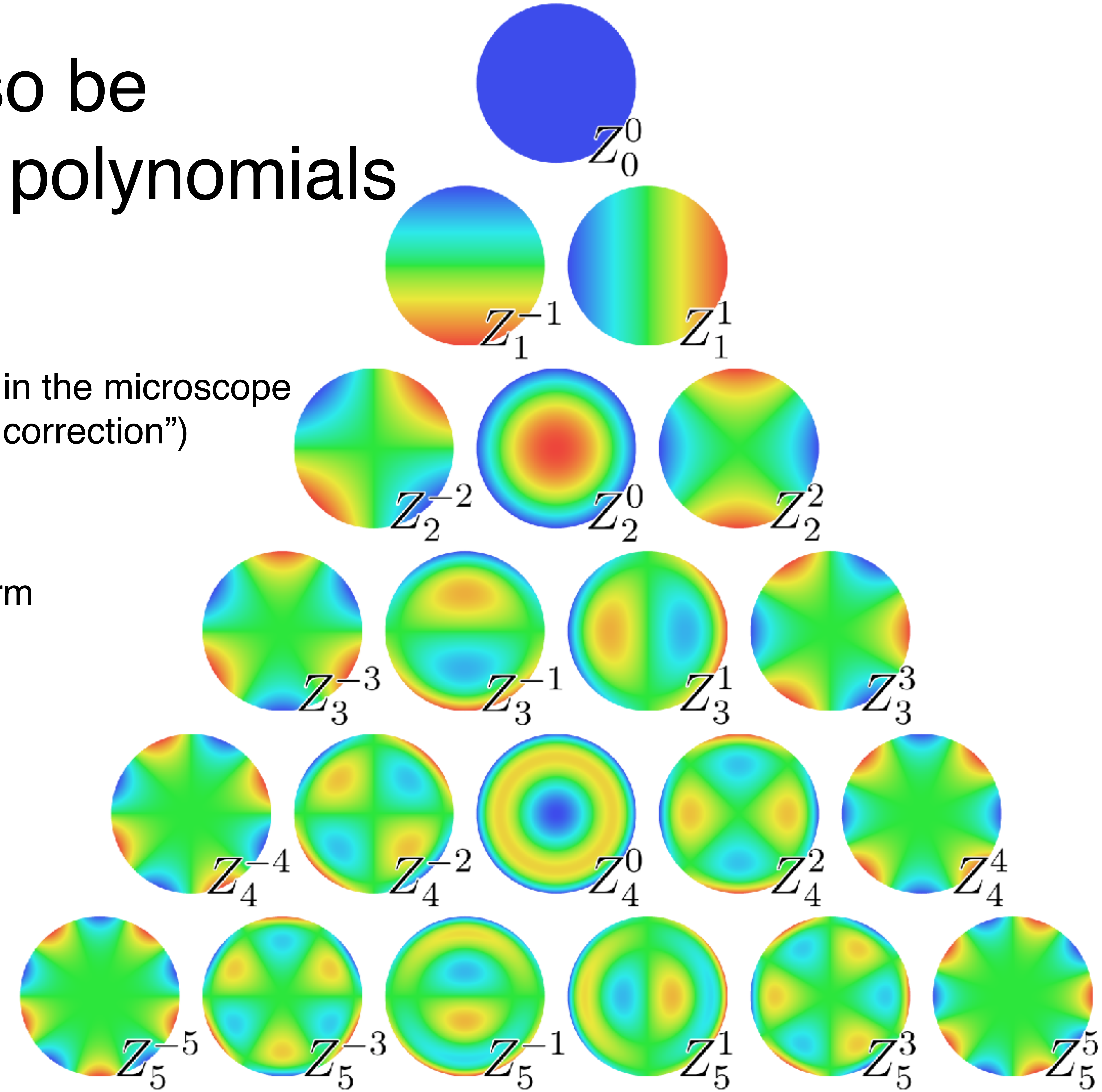
$$B(\mathbf{k}) = \exp\left[i\frac{2\pi}{\lambda}W(\mathbf{k})\right]$$

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



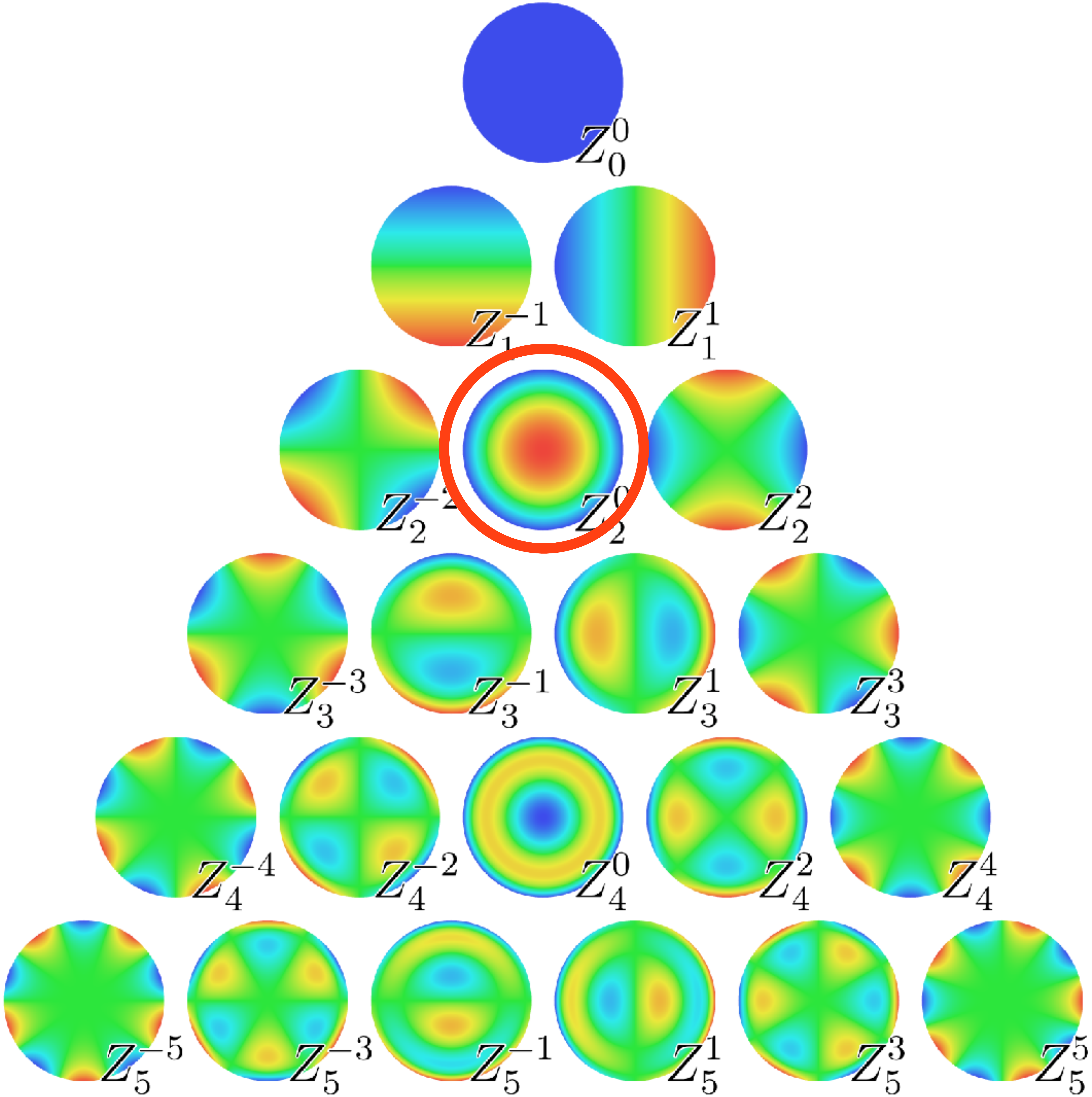
Aberrations are corrected with additional lenses in the microscope or in software after the image is collected (“CTF correction”)

Complete set of orthogonal functions

Zernike transform analogous to Fourier transform

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

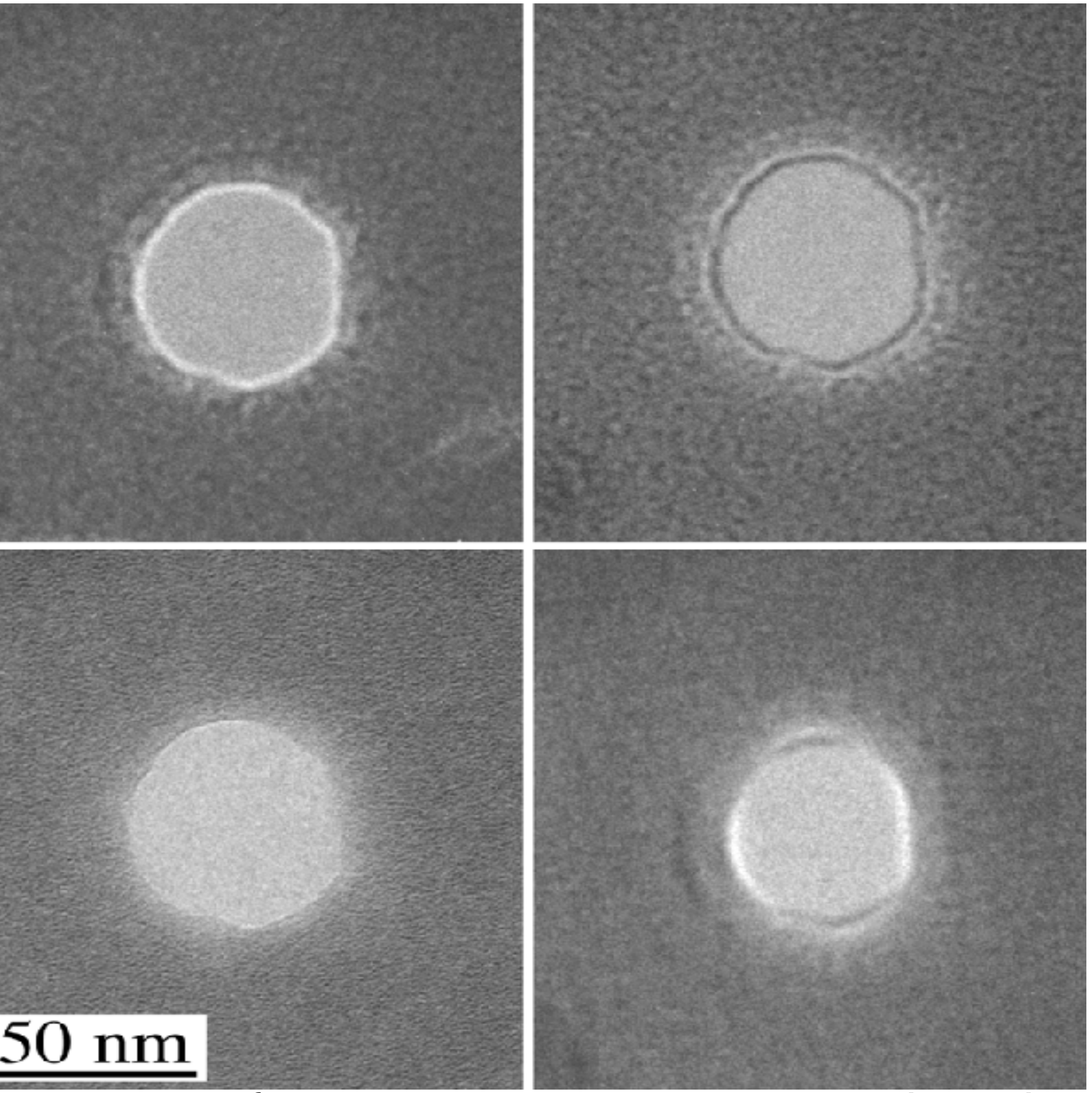
Defocus



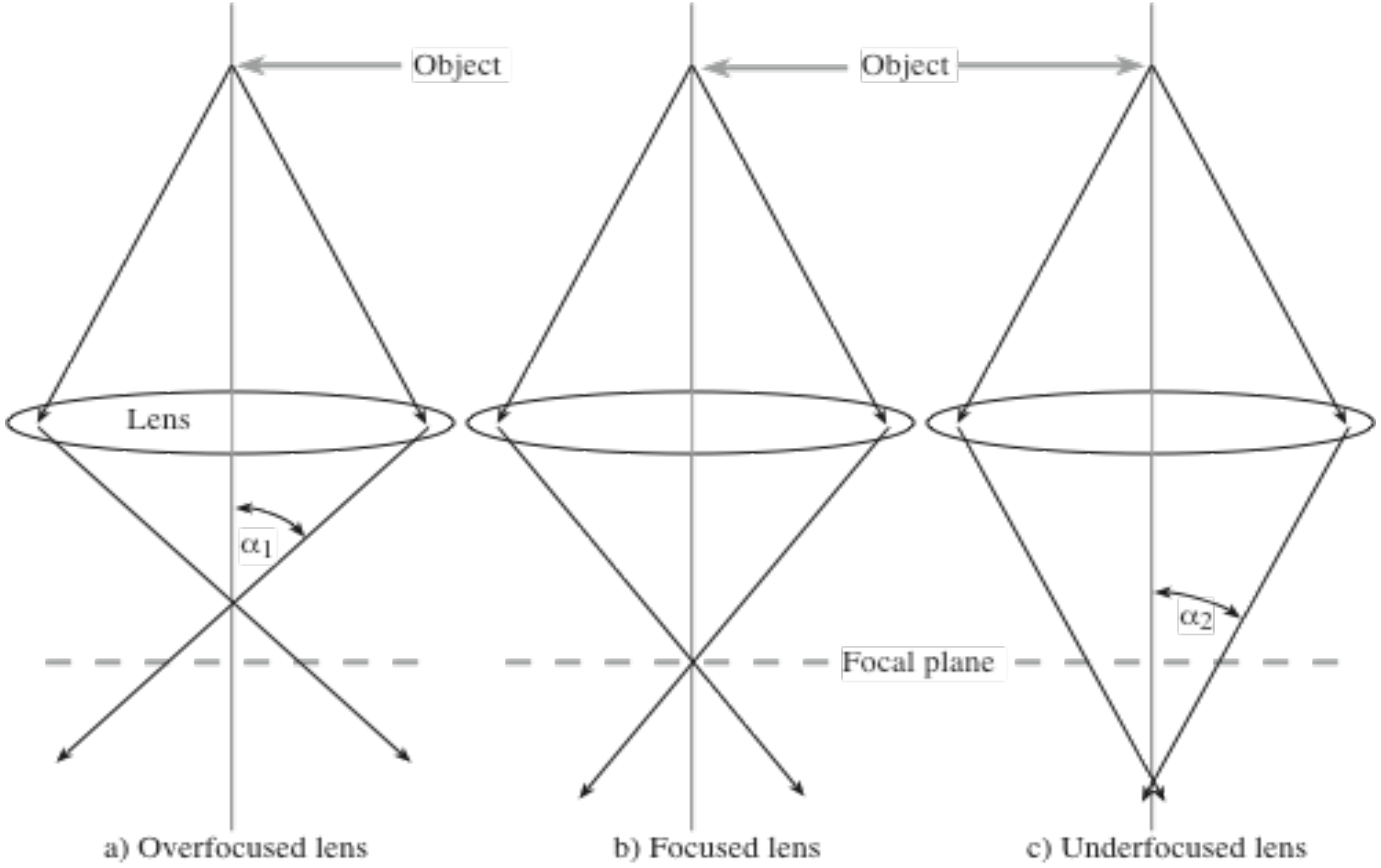
Z_0^0
Z_1^{-1}
Z_1^1
Z_2^{-2}
Z_2^0
Z_2^2
Z_3^{-3}
Z_3^{-1}
Z_3^1
Z_3^3
Z_4^{-4}
Z_4^{-2}
Z_4^0
Z_4^2
Z_4^4
Z_5^{-5}
Z_5^{-3}
Z_5^{-1}
Z_5^1
Z_5^3
Z_5^5

Focus terminology

underfocus overfocus

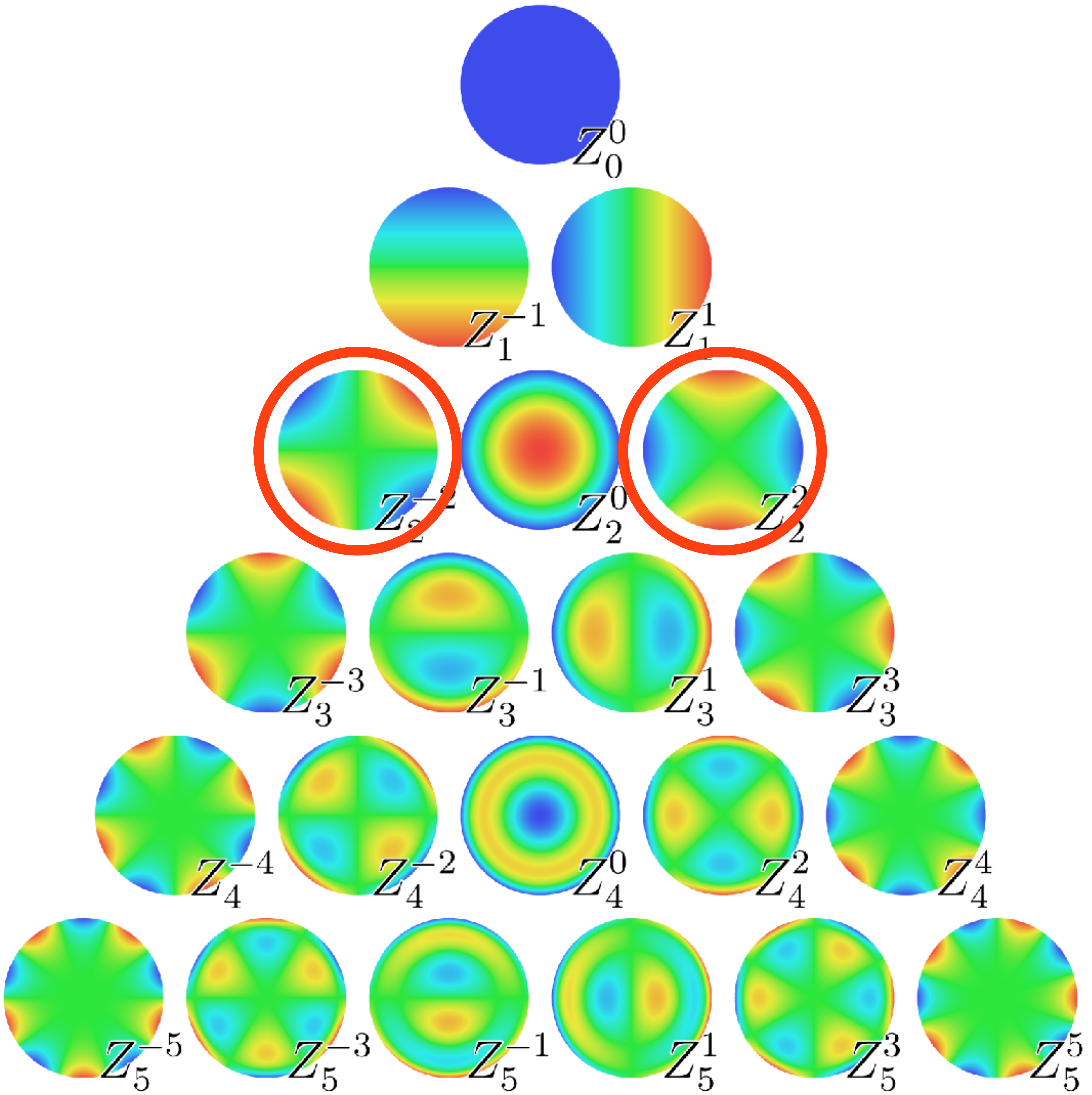


exact focus astigmatism

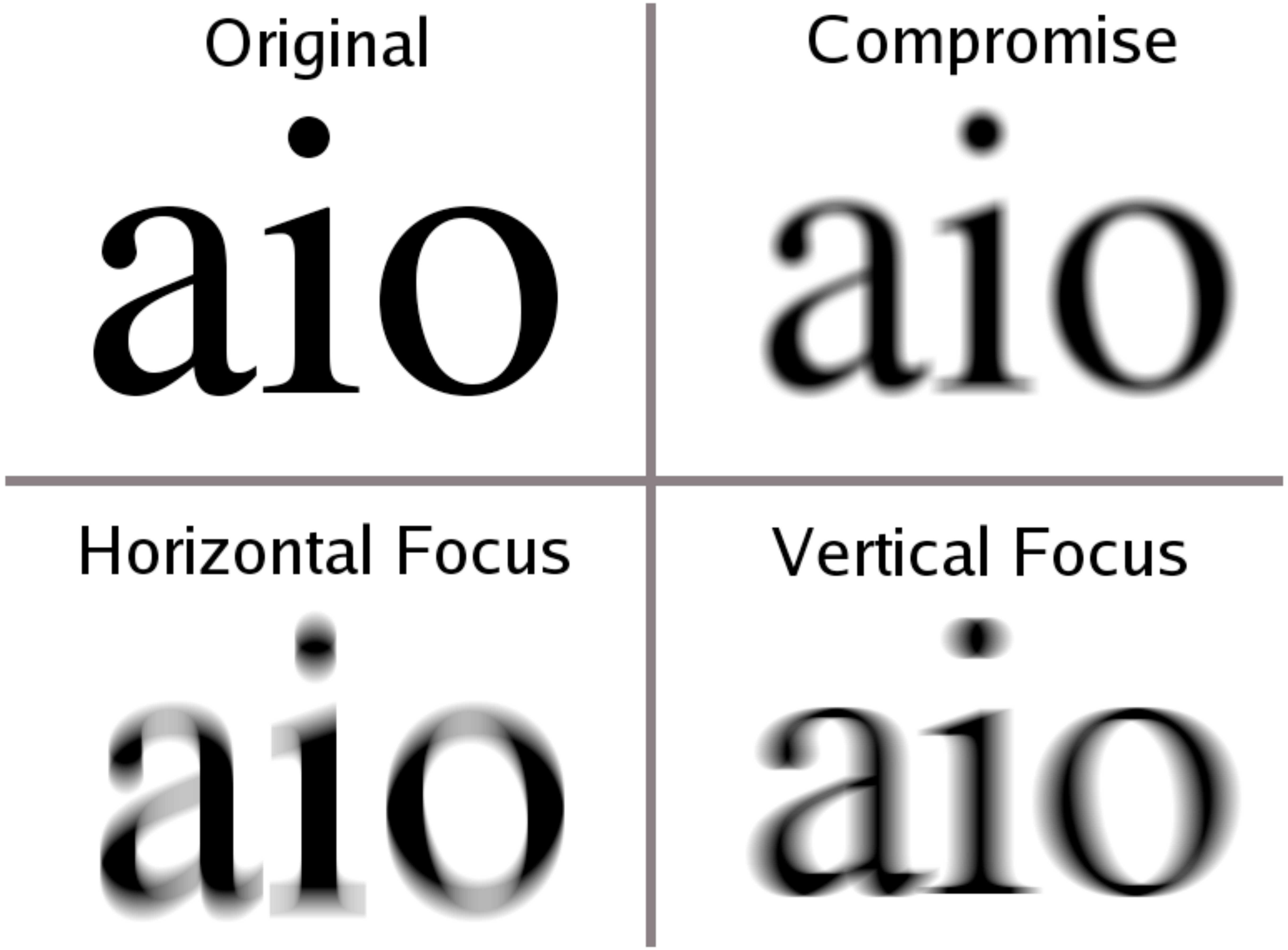


Too strong Just right Too weak

Astigmatism



Astigmatism (example)



Astigmatism Correction

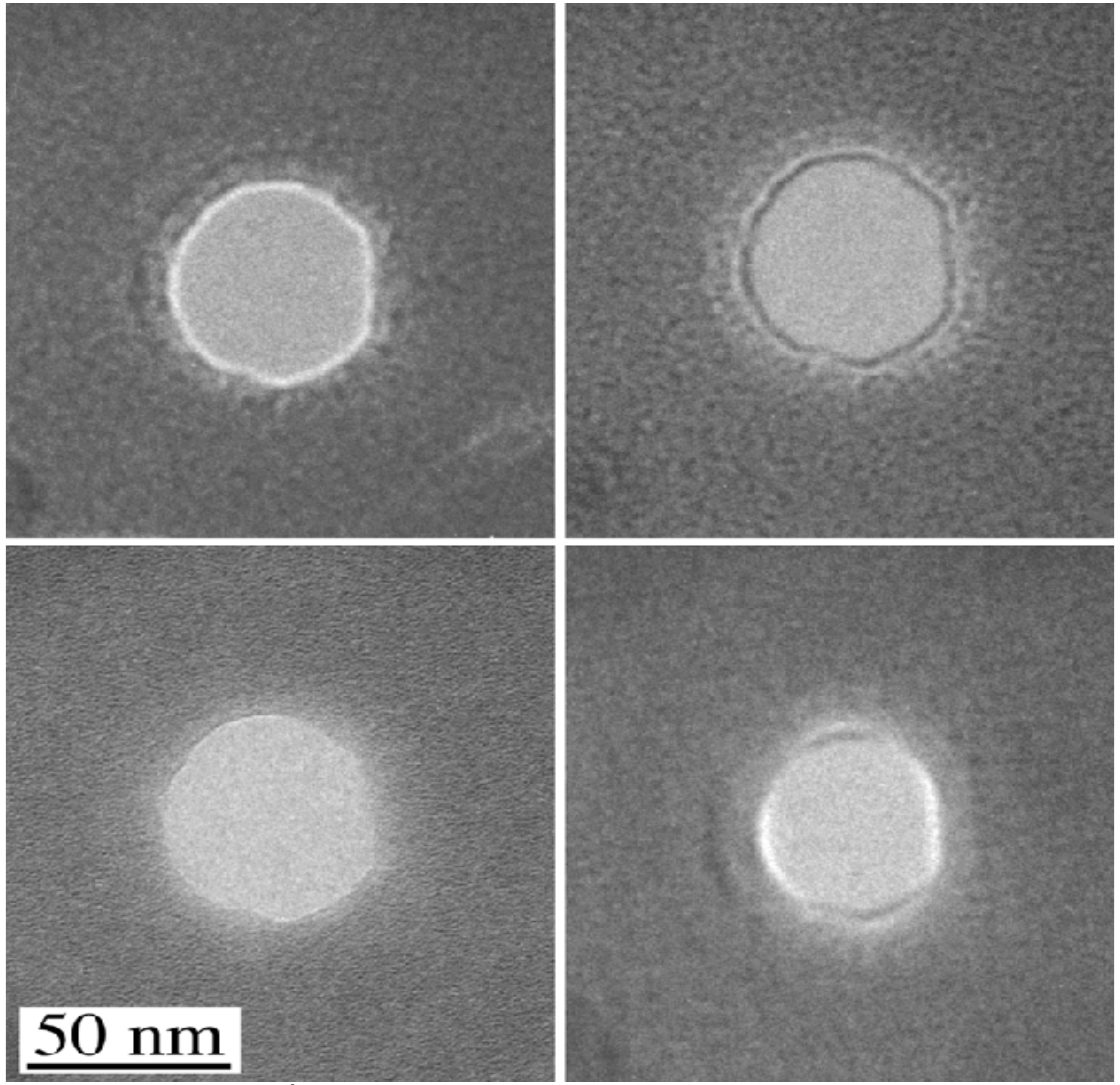
Correcting the astigmatism on the objective lens

Routine alignment using Fresnel fringe

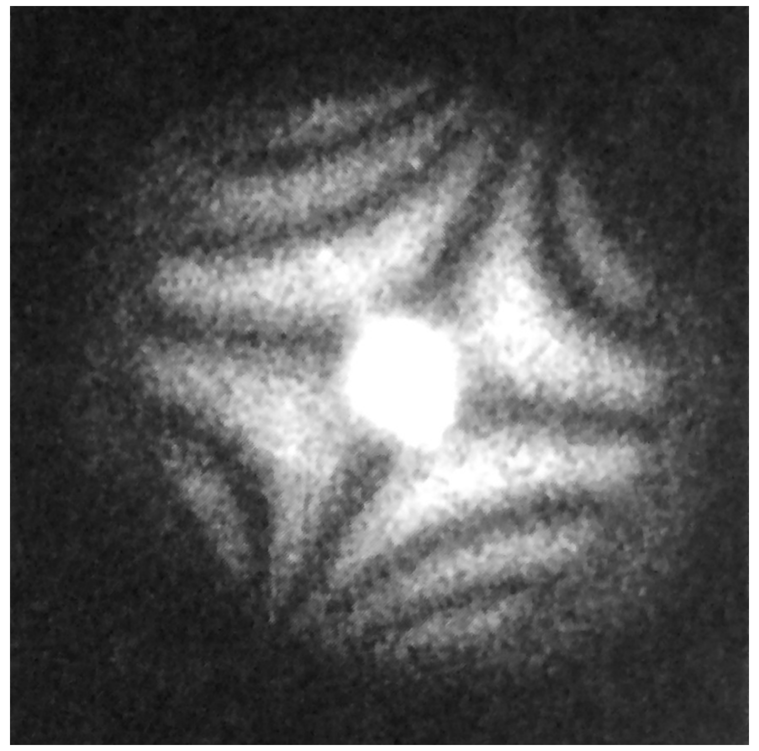
More accurate with FFT

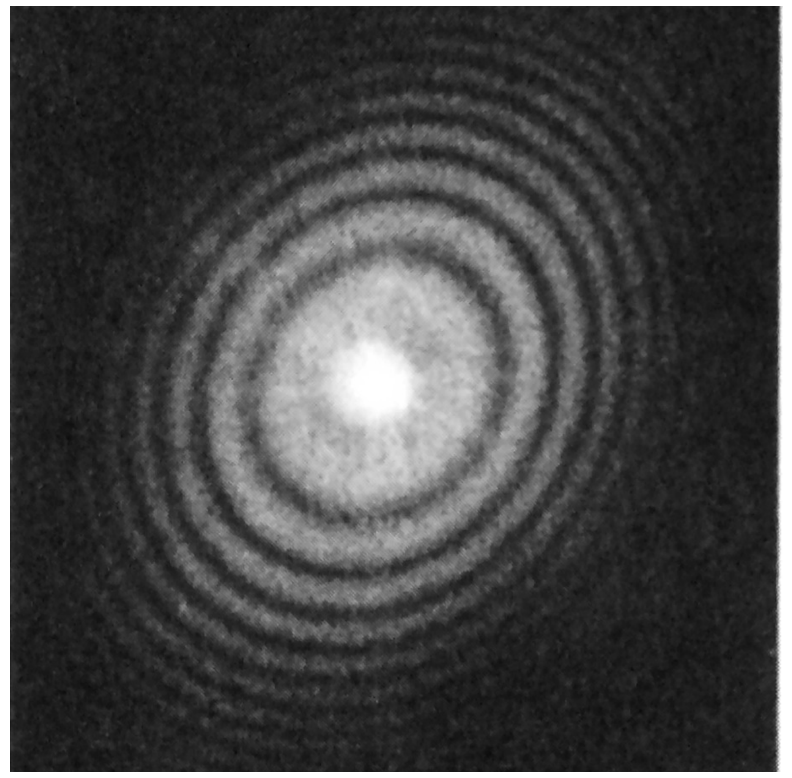
Remember to correct the condenser lens too

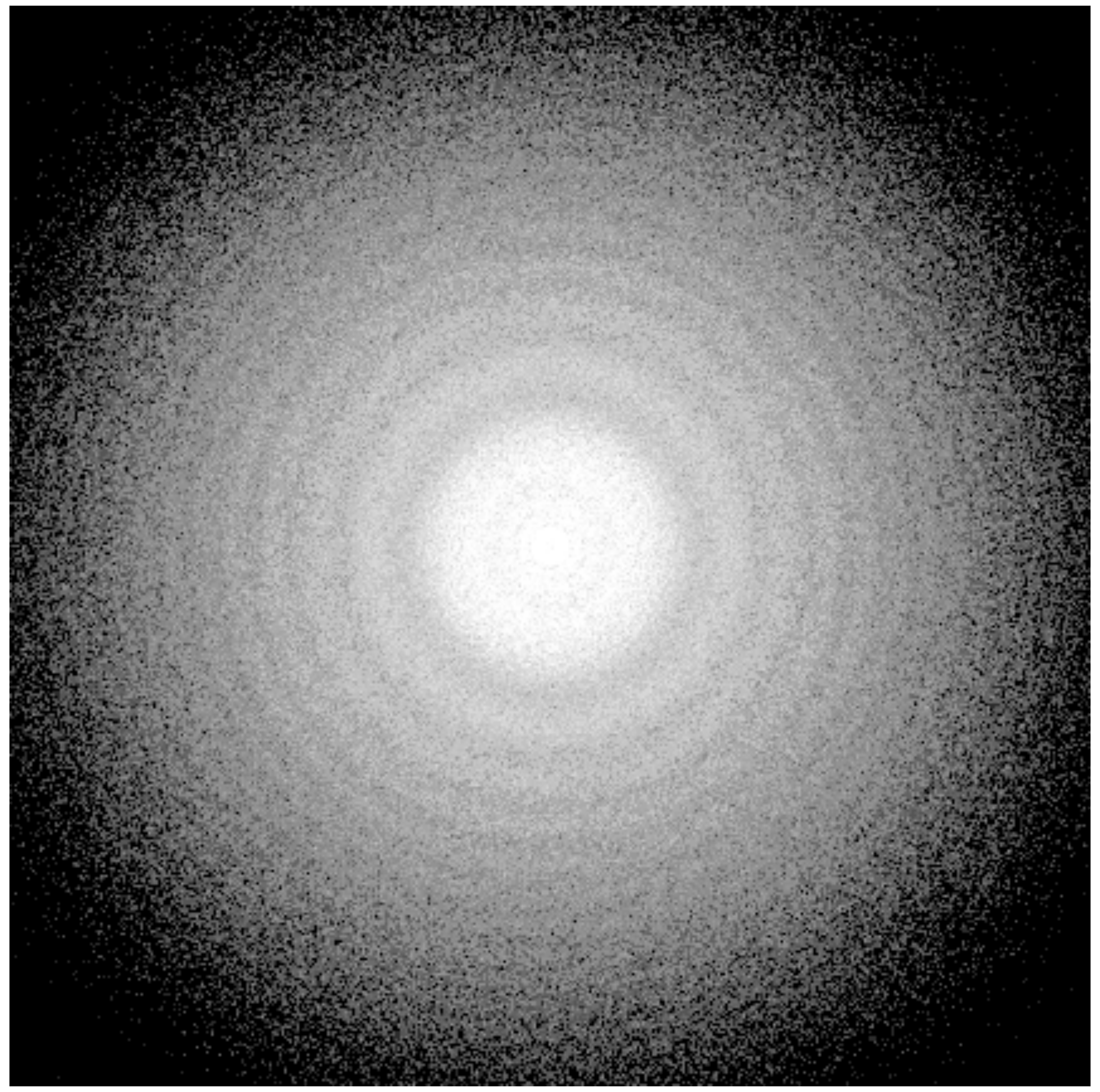
underfocus overfocus



exact focus astigmatism

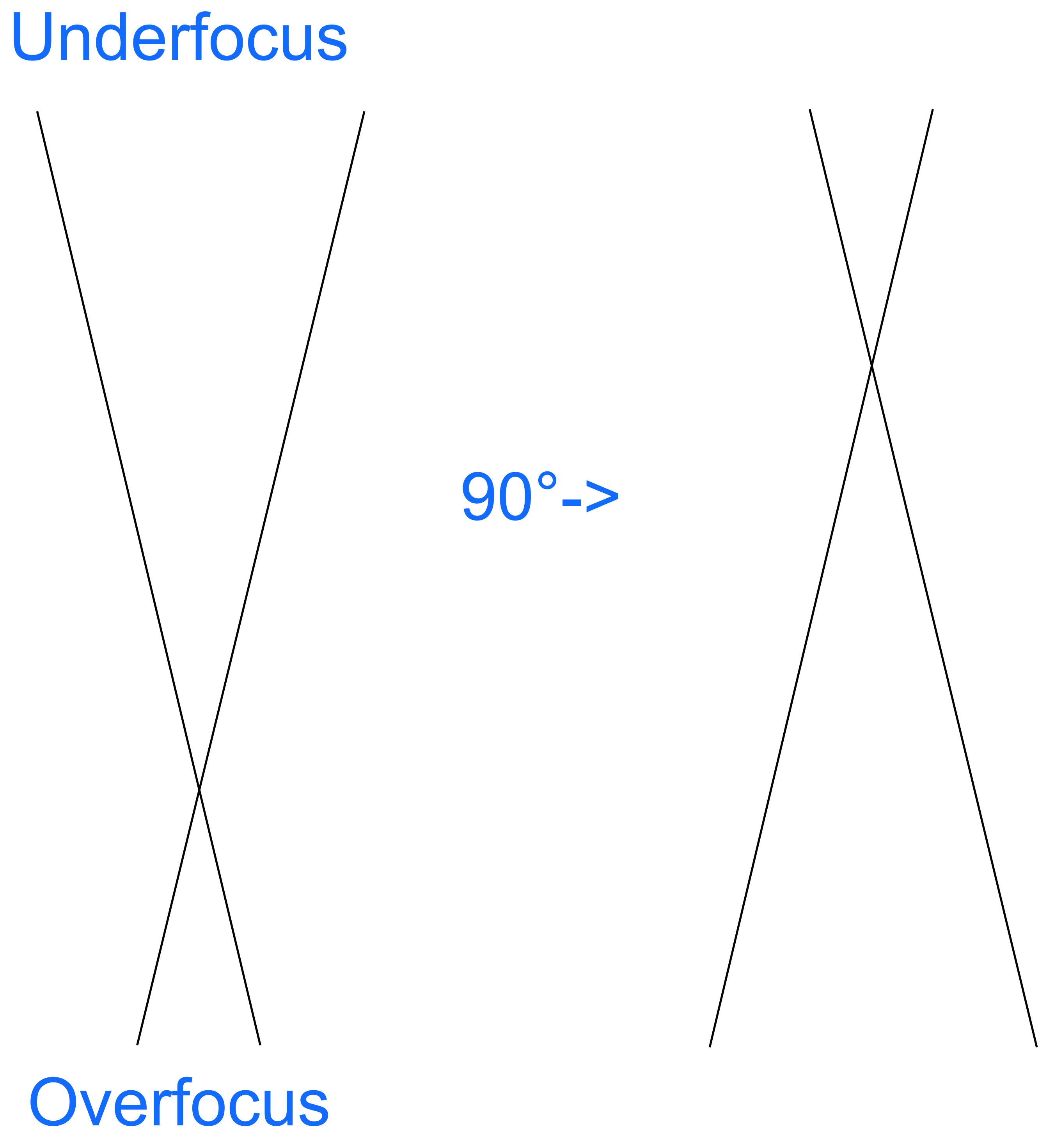


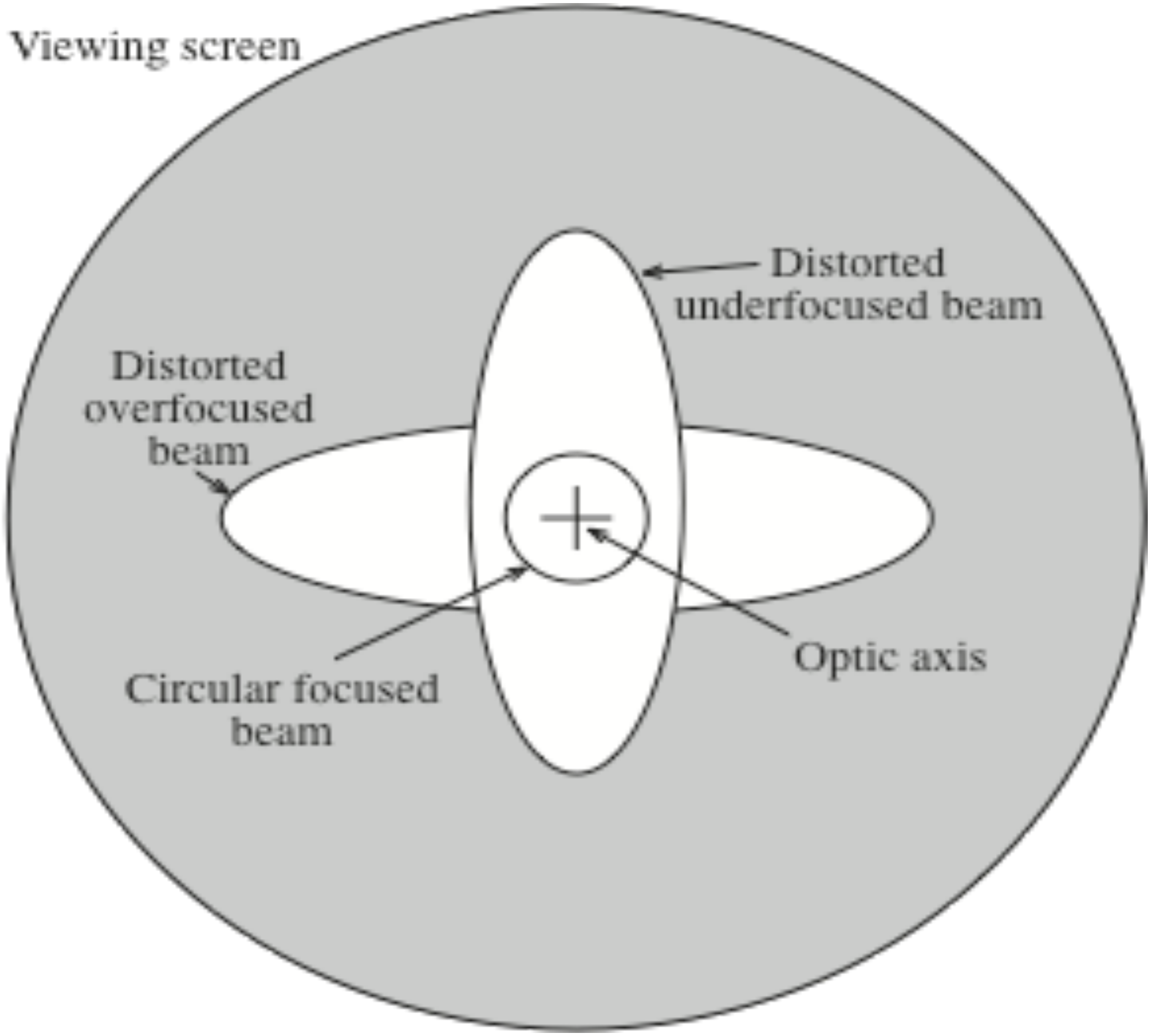




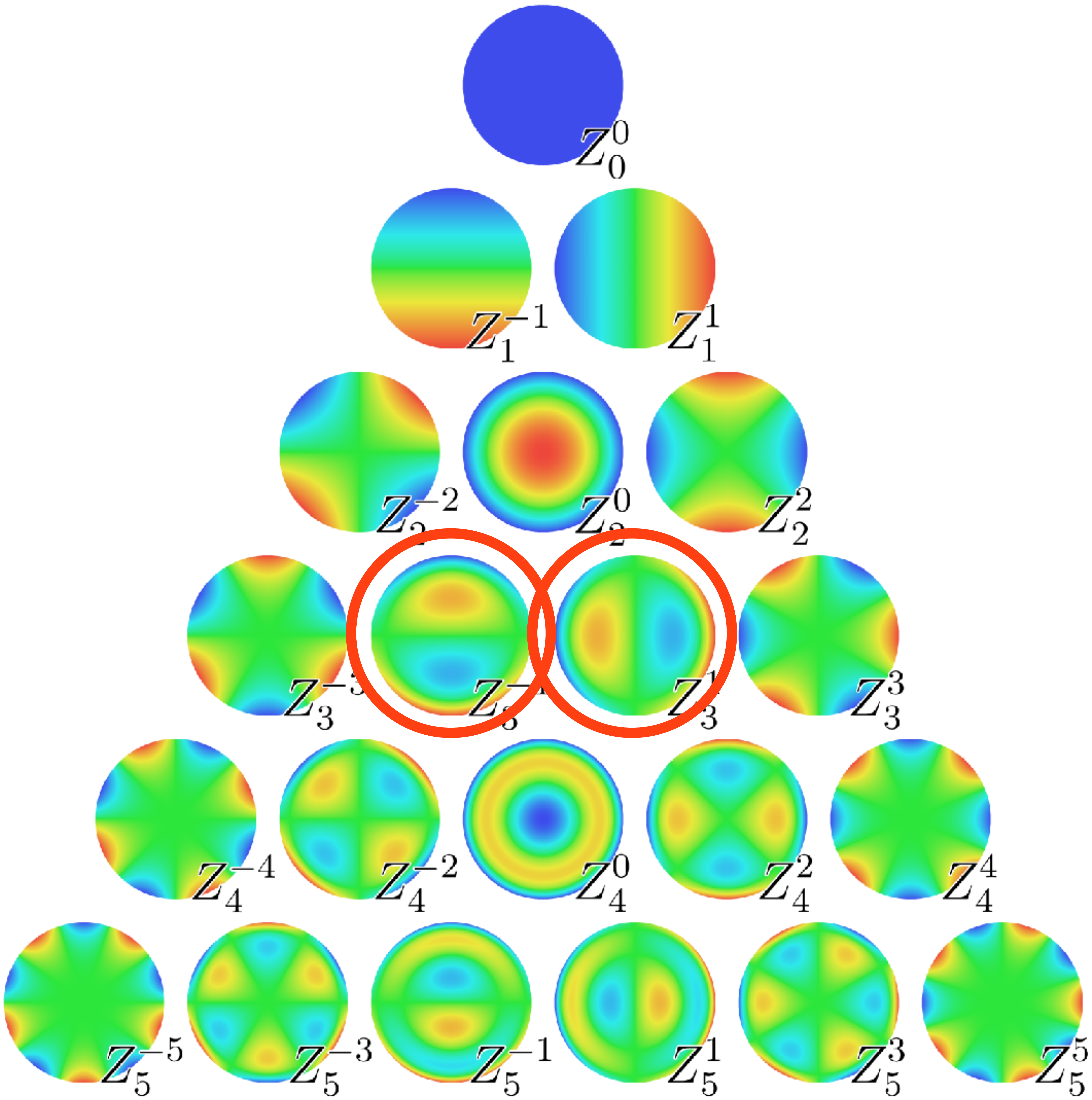
Beam Astigmatism Correction

Just change focus

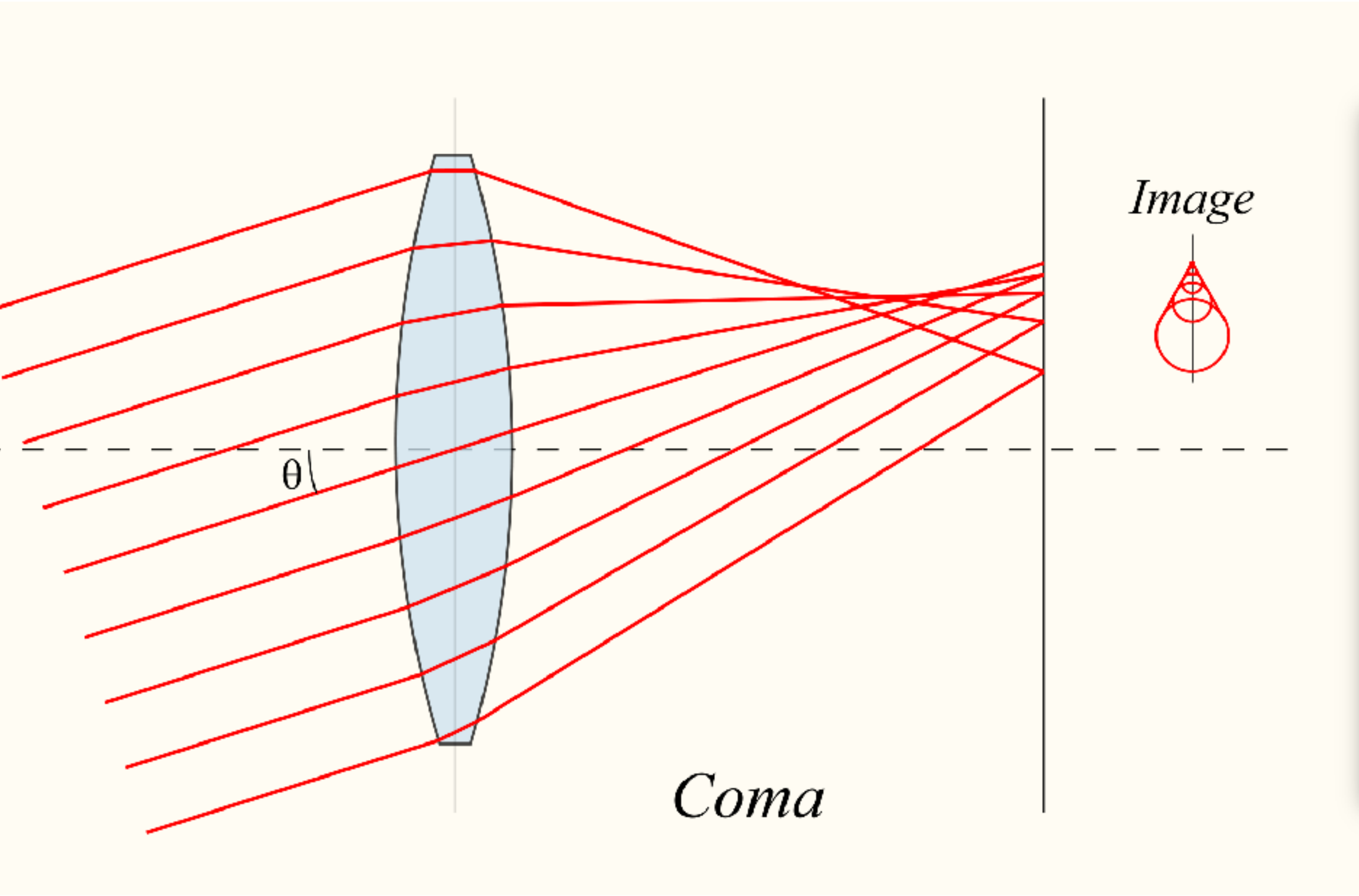


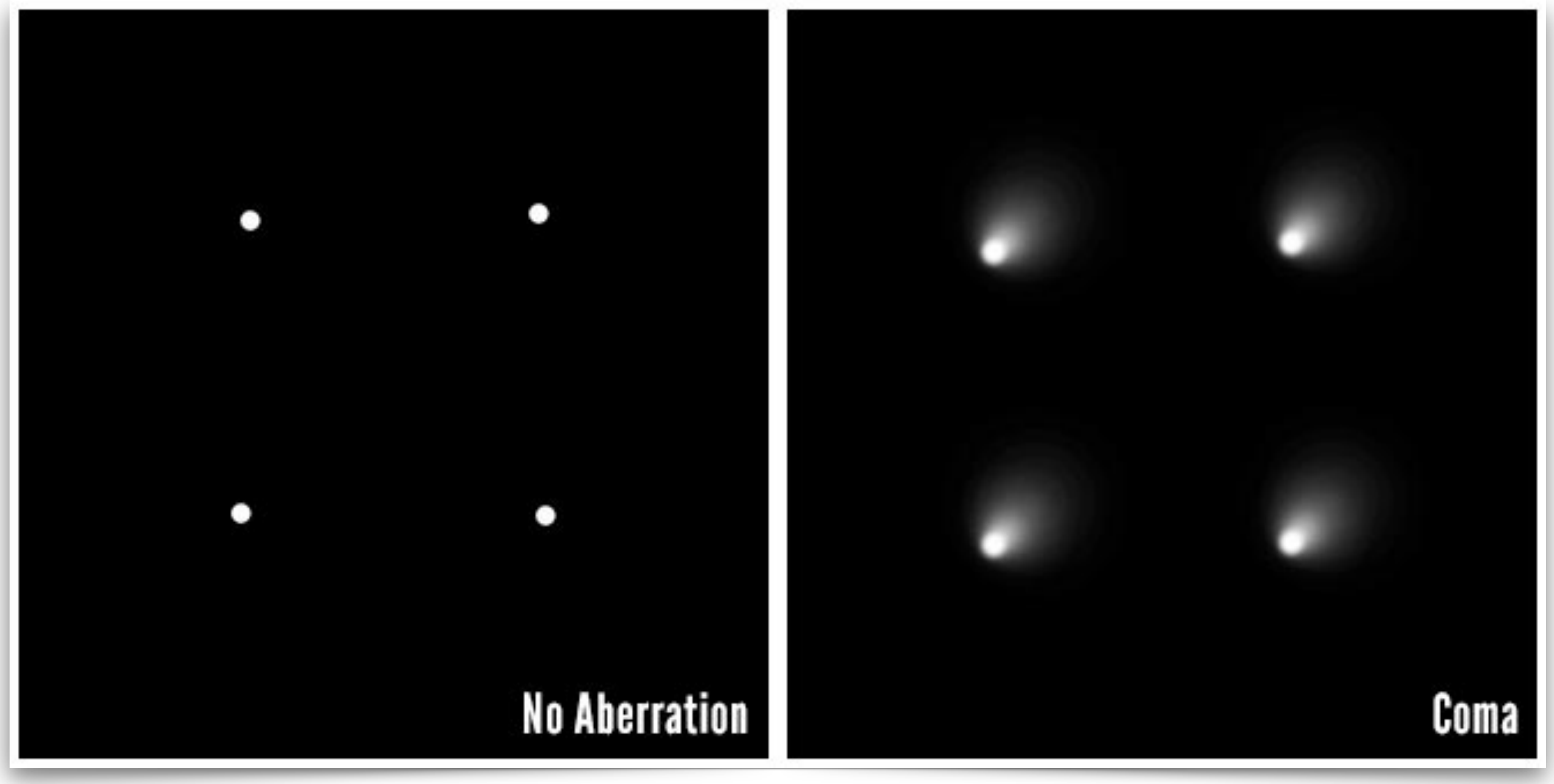


Coma



Z_0^0
Z_1^{-1}
Z_1^1
Z_2^{-2}
Z_2^0
Z_2^2
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

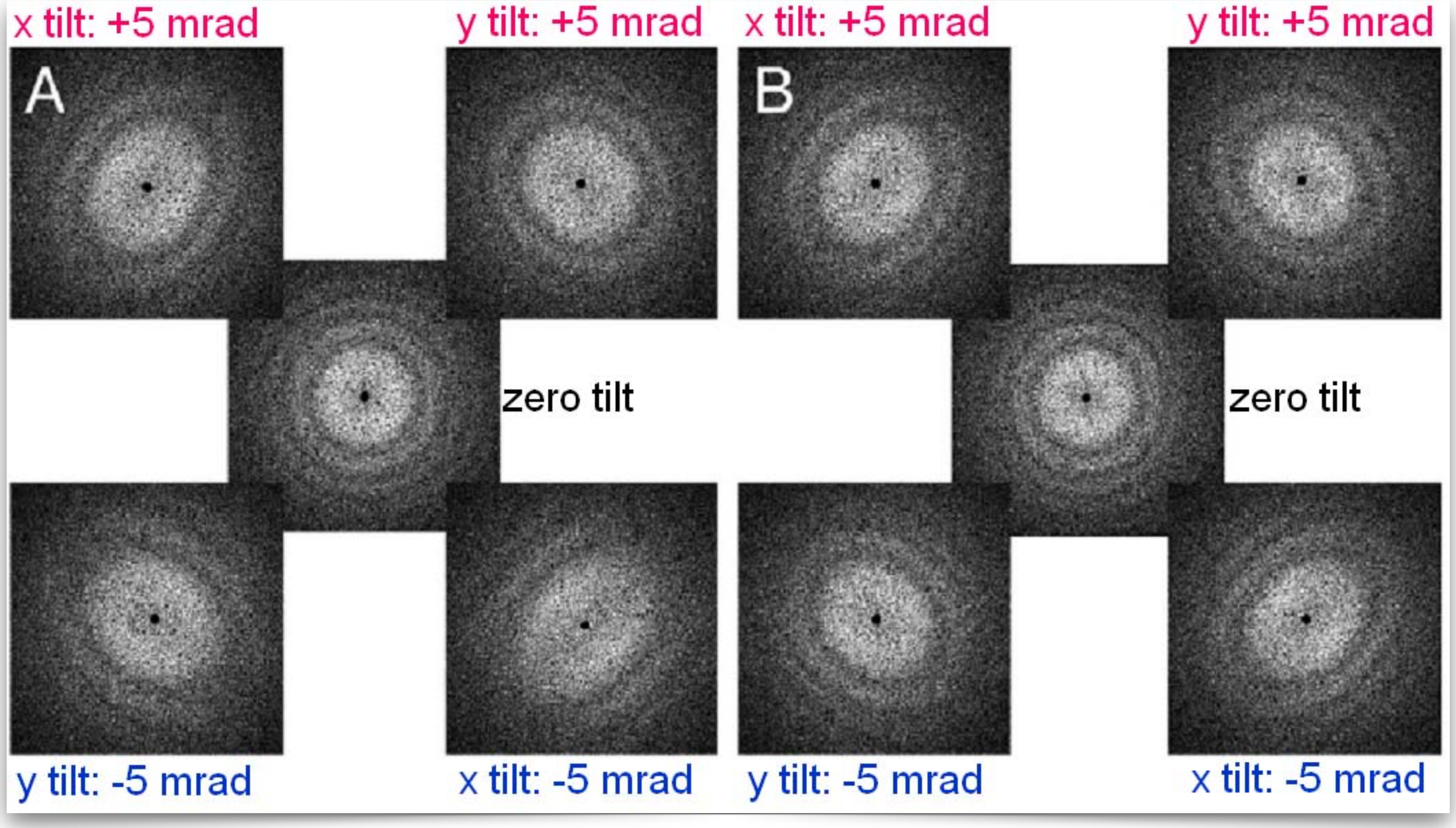




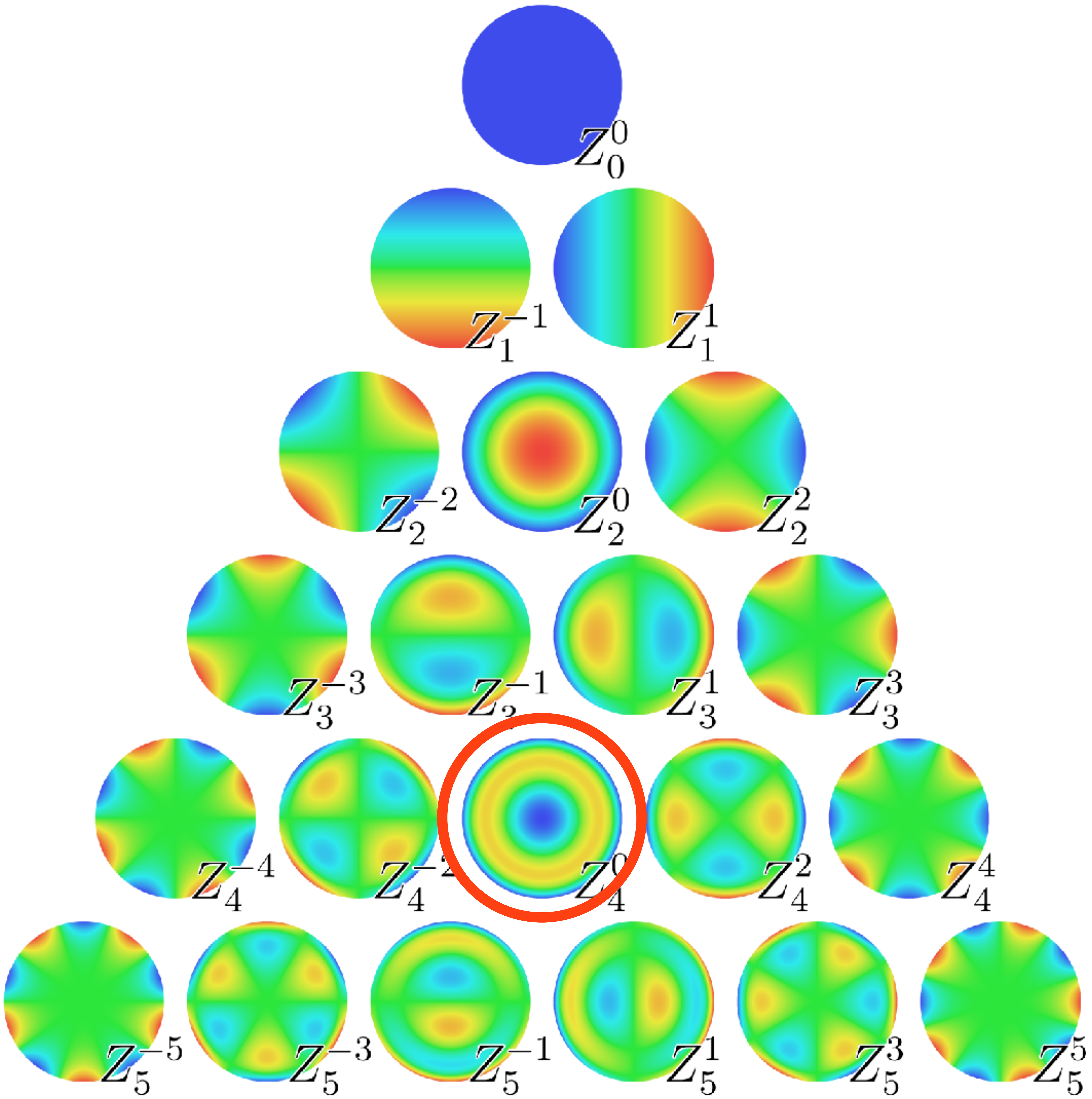
Example image from I. Norman

Reducing coma by minimising beam tilt

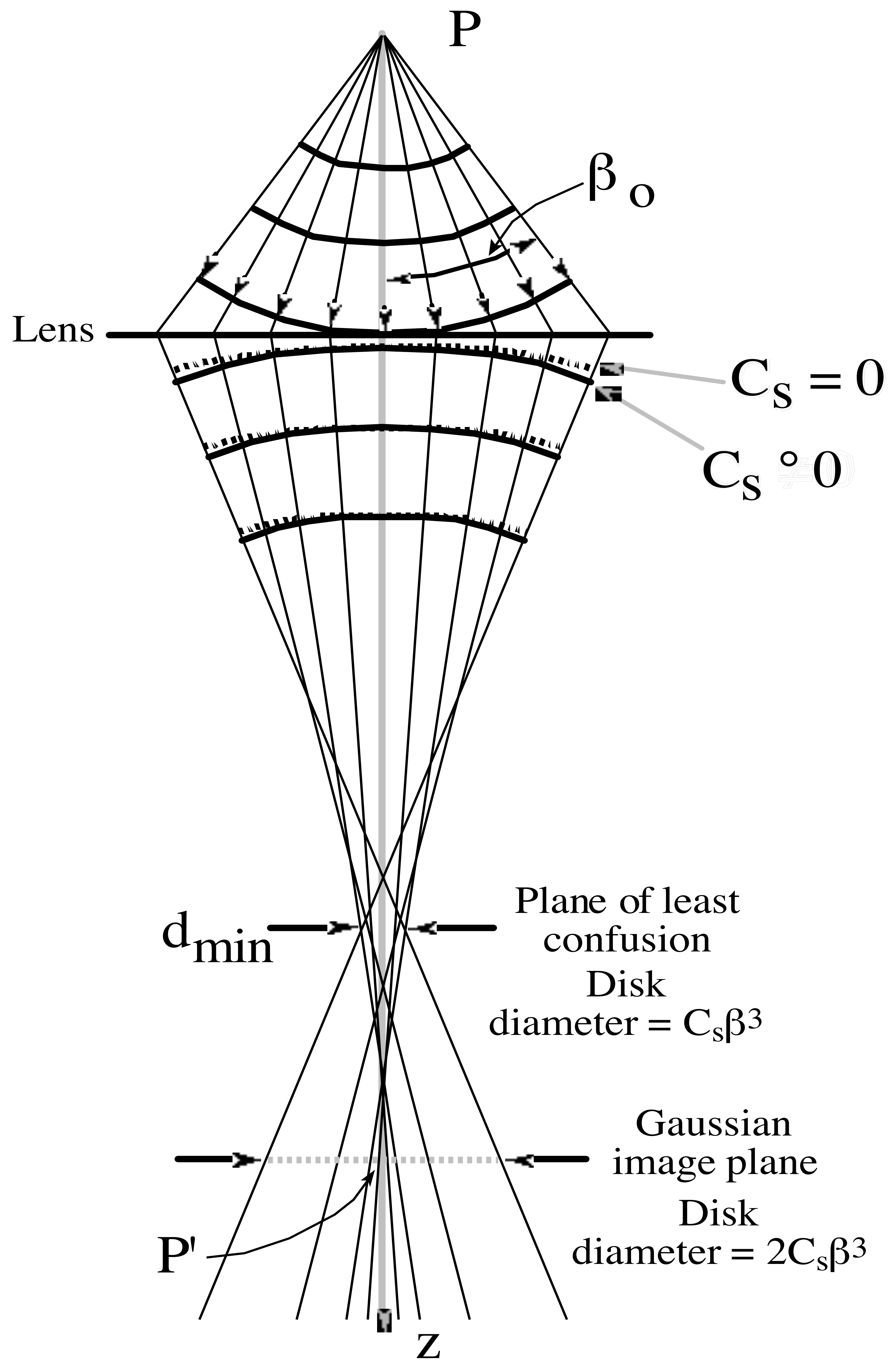
1. Voltage centring
2. Current centring
3. Zemlin Tableaux



Spherical aberration



Spherical Aberration



Lens is stronger off axis

Plane of least confusion

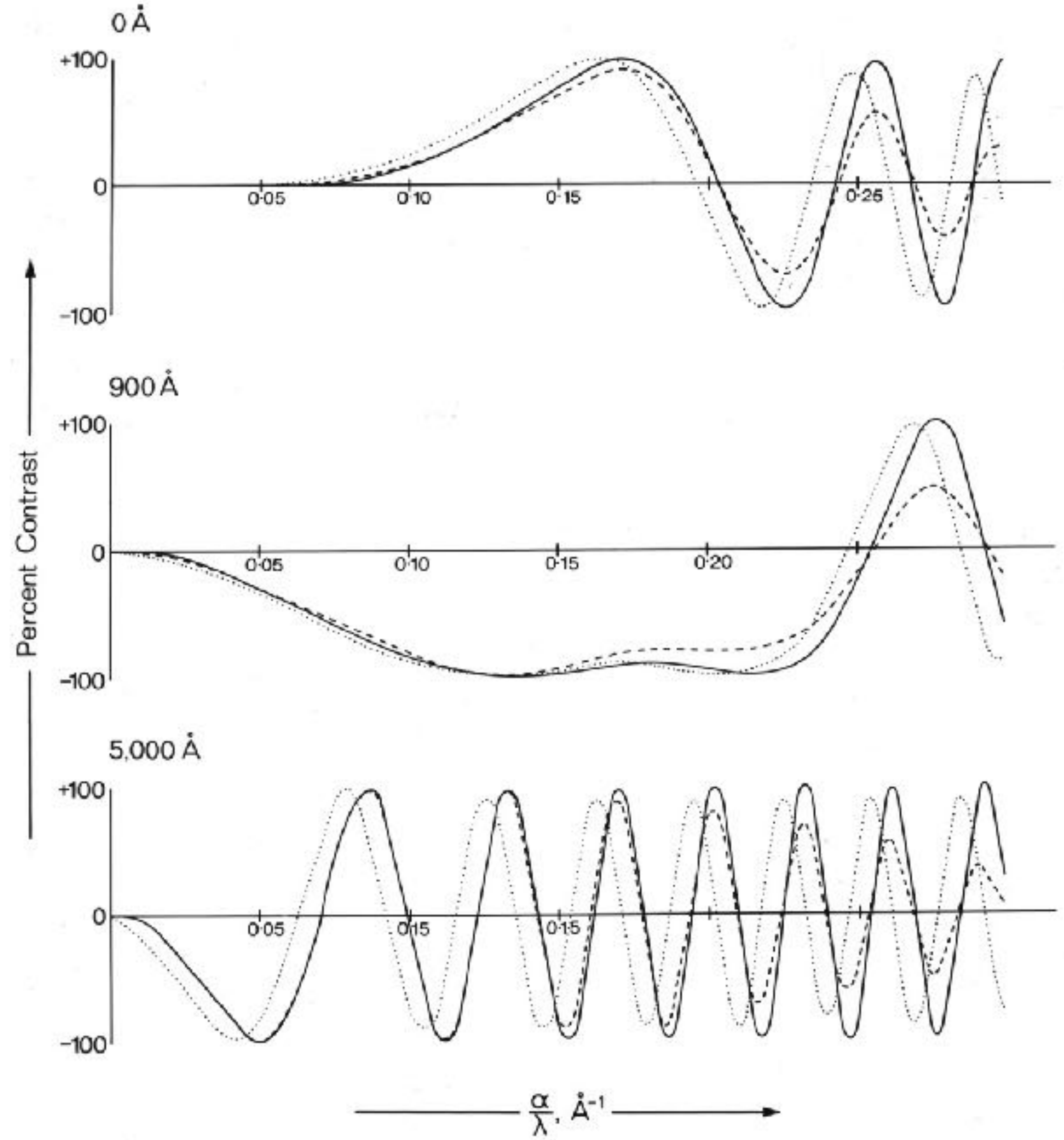
CTF

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

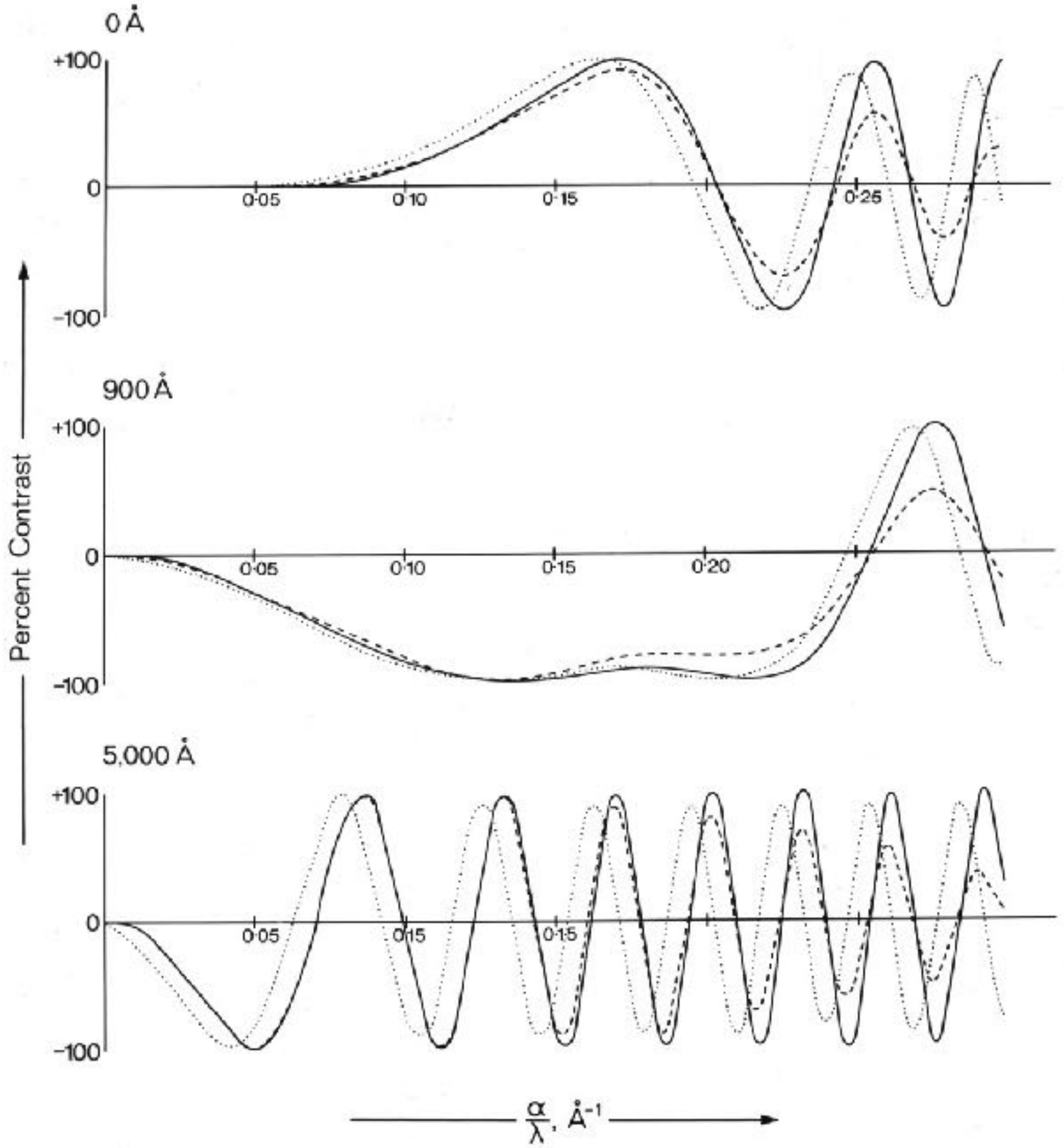
Measurement and compensation of defocusing and aberrations by Fourier processing of electron micrographs

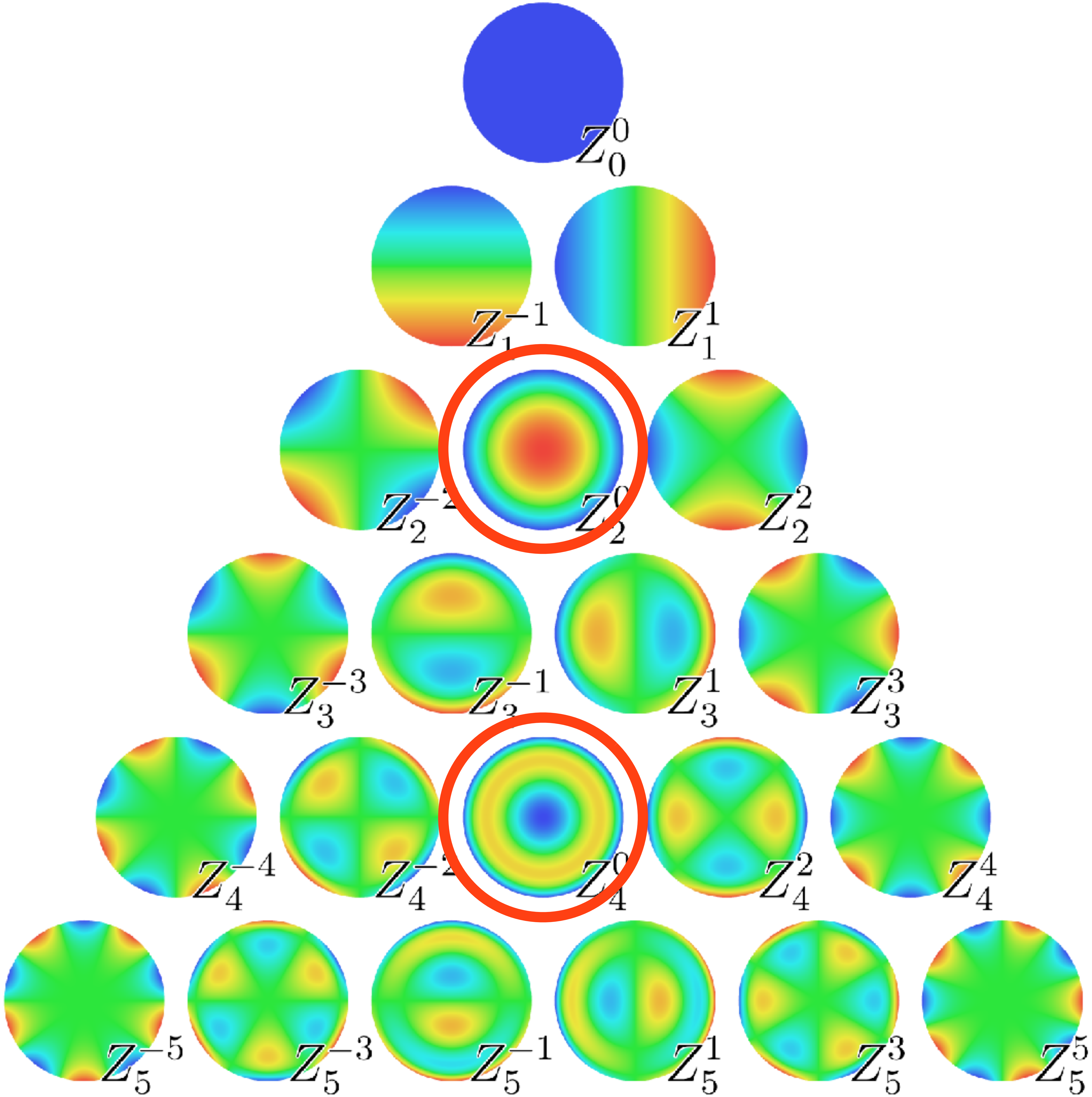
BY H. P. ERICKSON AND A. KLUG, F.R.S.
Medical Research Council Laboratory of Molecular Biology, Cambridge

Contrast
Transfer
Function



Correct with software instead: CTFFIND, GCTF or similar

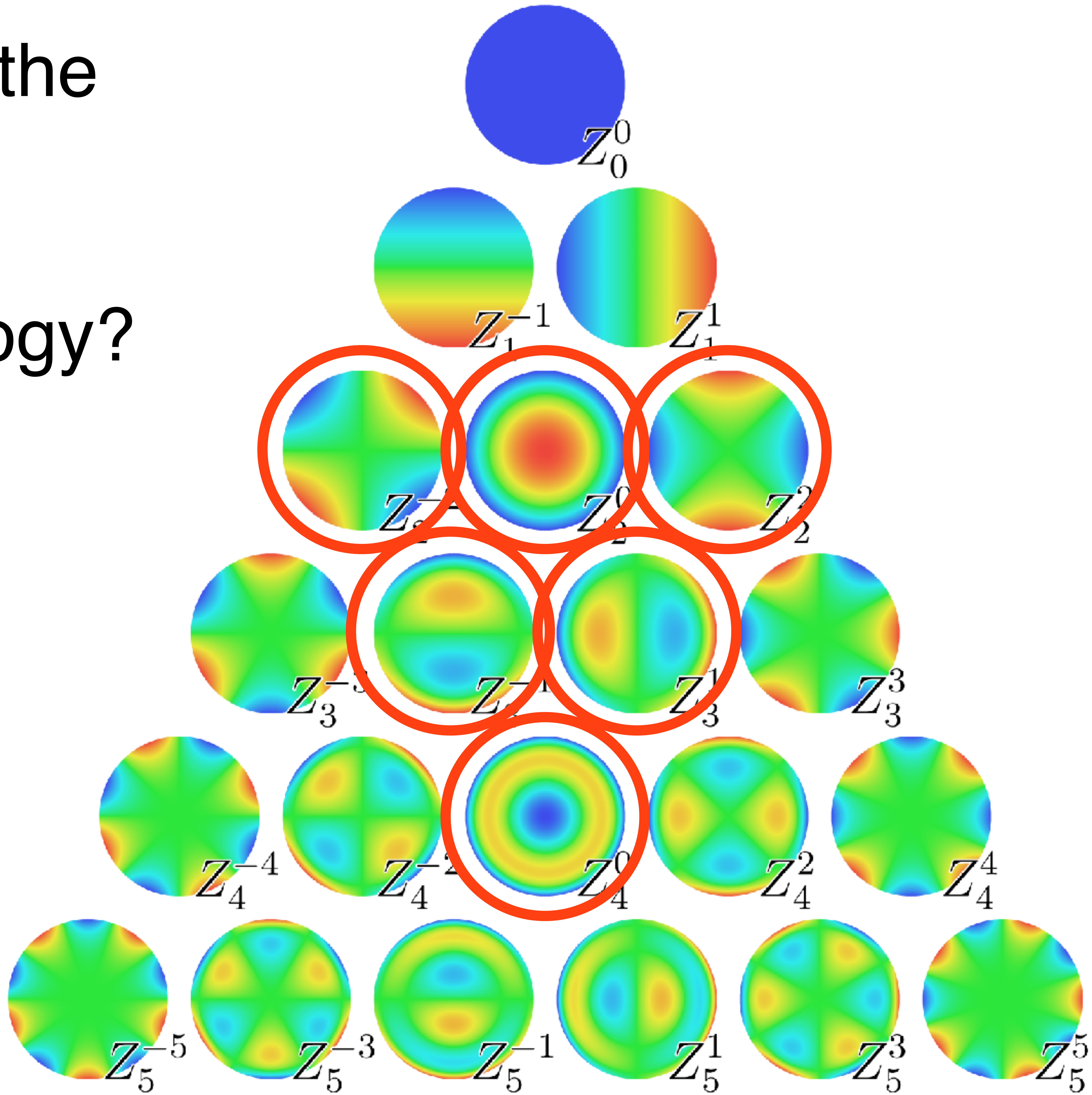




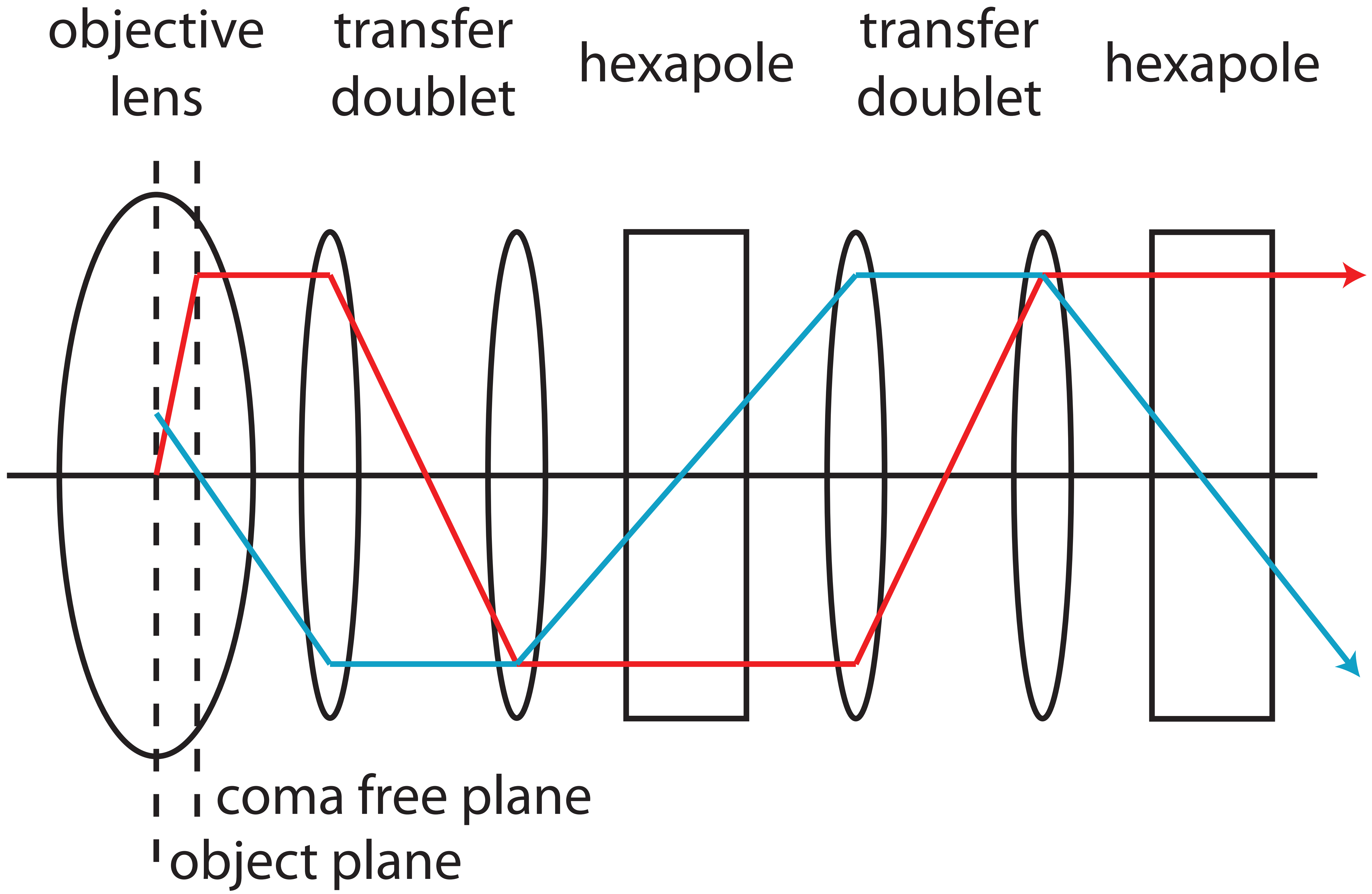
What about the rest of the Lens aberrations?

Do they matter for biology?

not till < 2 Å



Aberration corrector



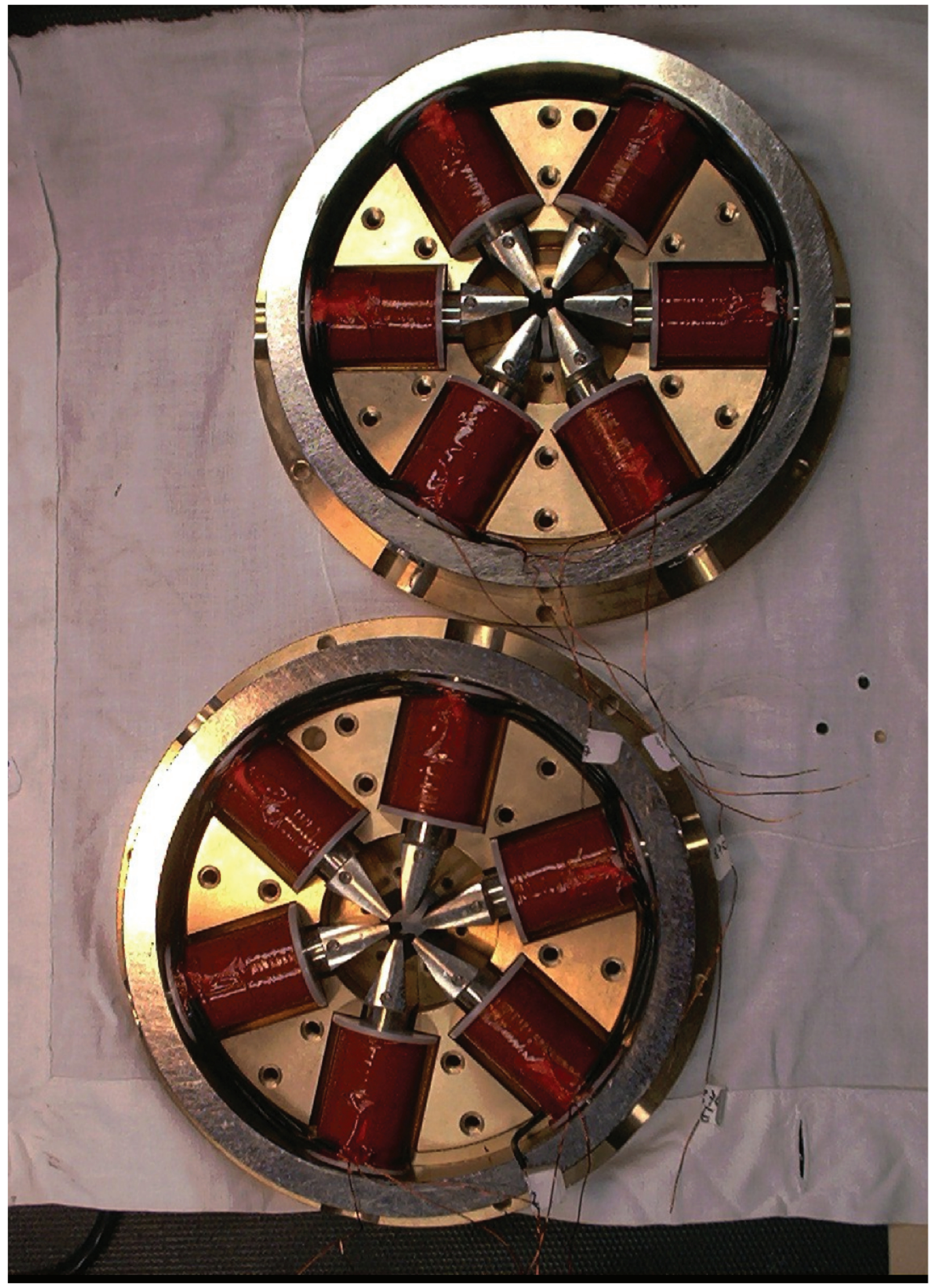
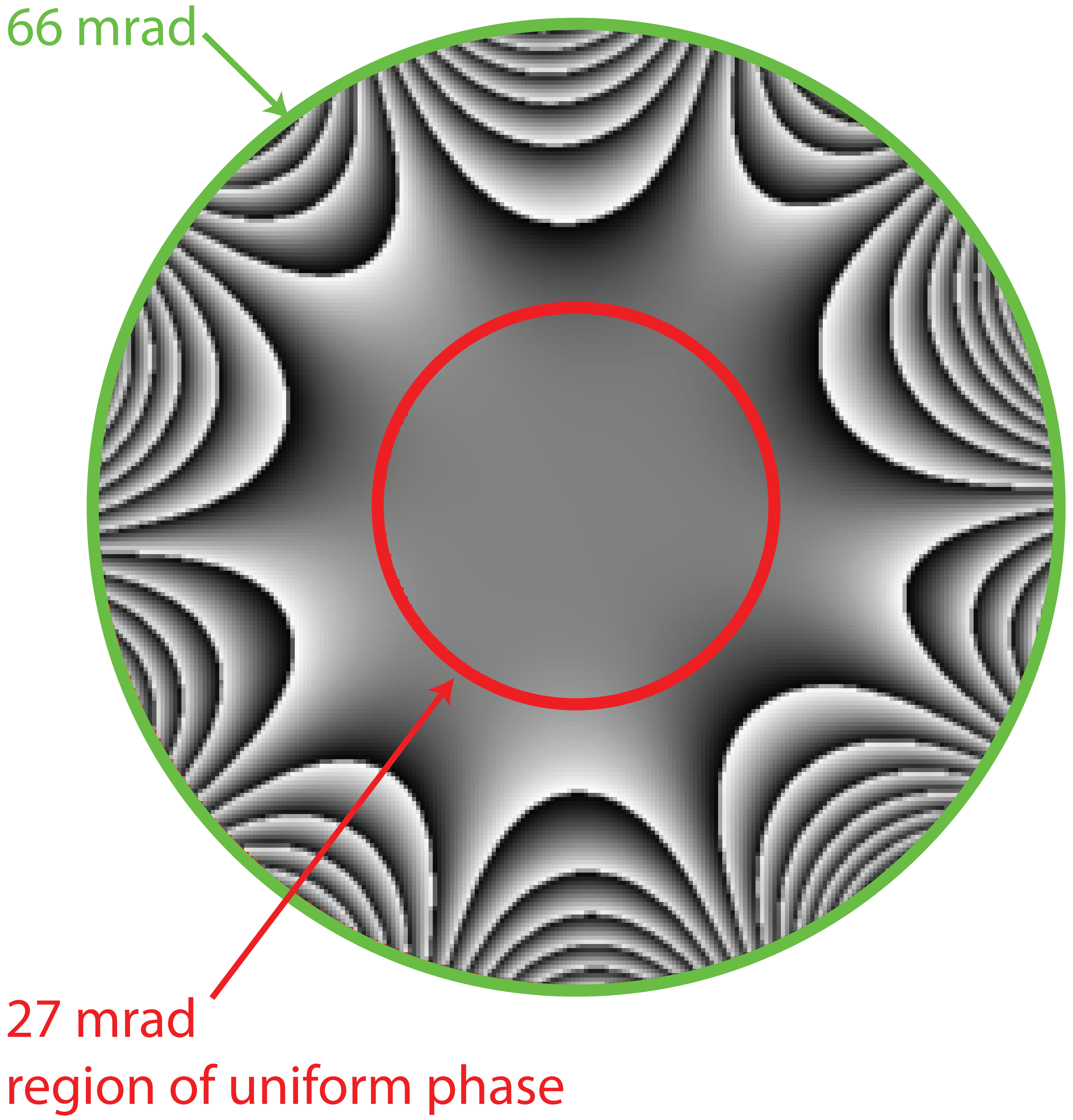


Image courtesy of M. Haider

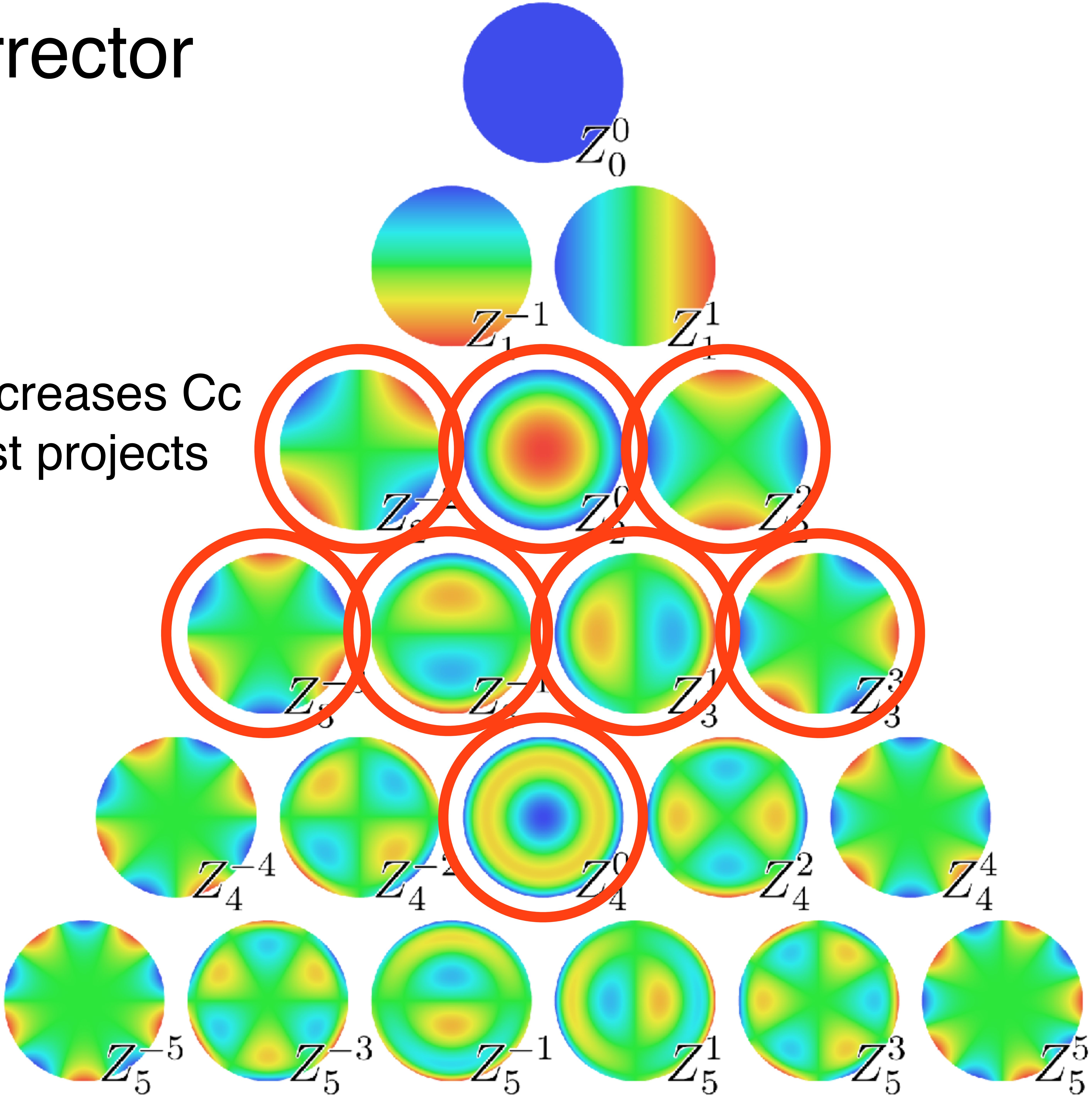
3rd order aberration corrector

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

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

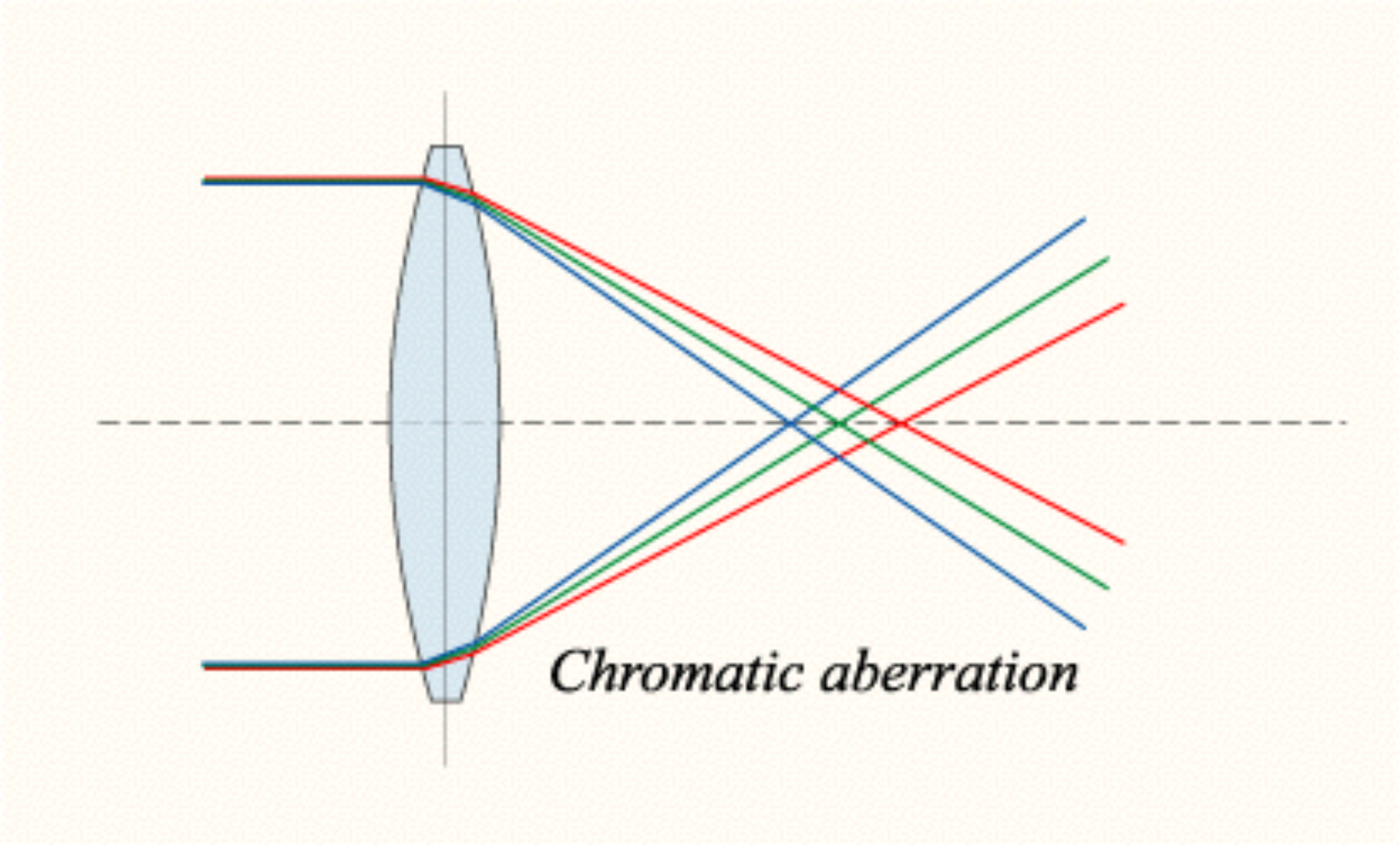


Russo 2010



Chromatic Aberration

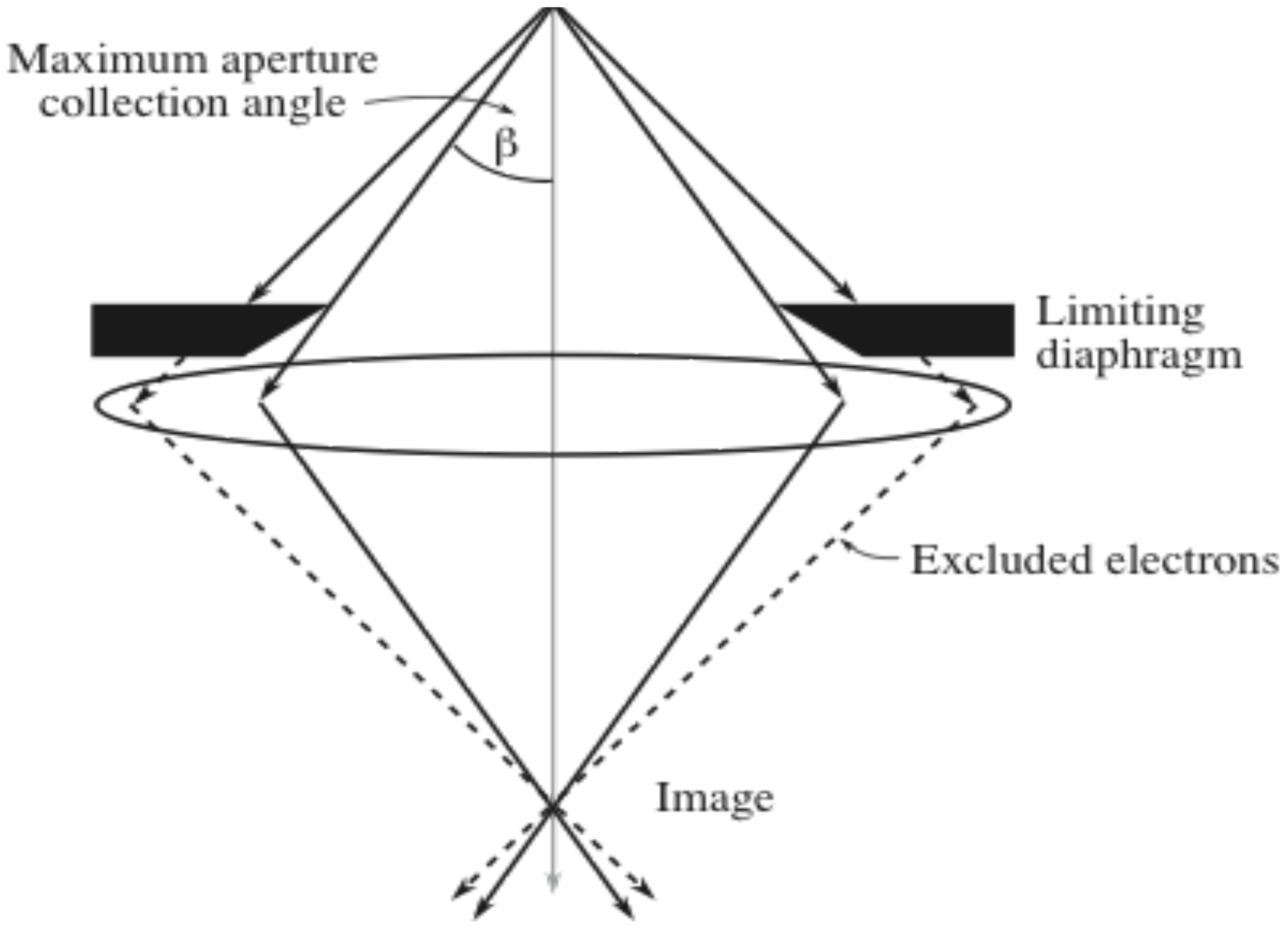
- Different wavelengths focus at different planes



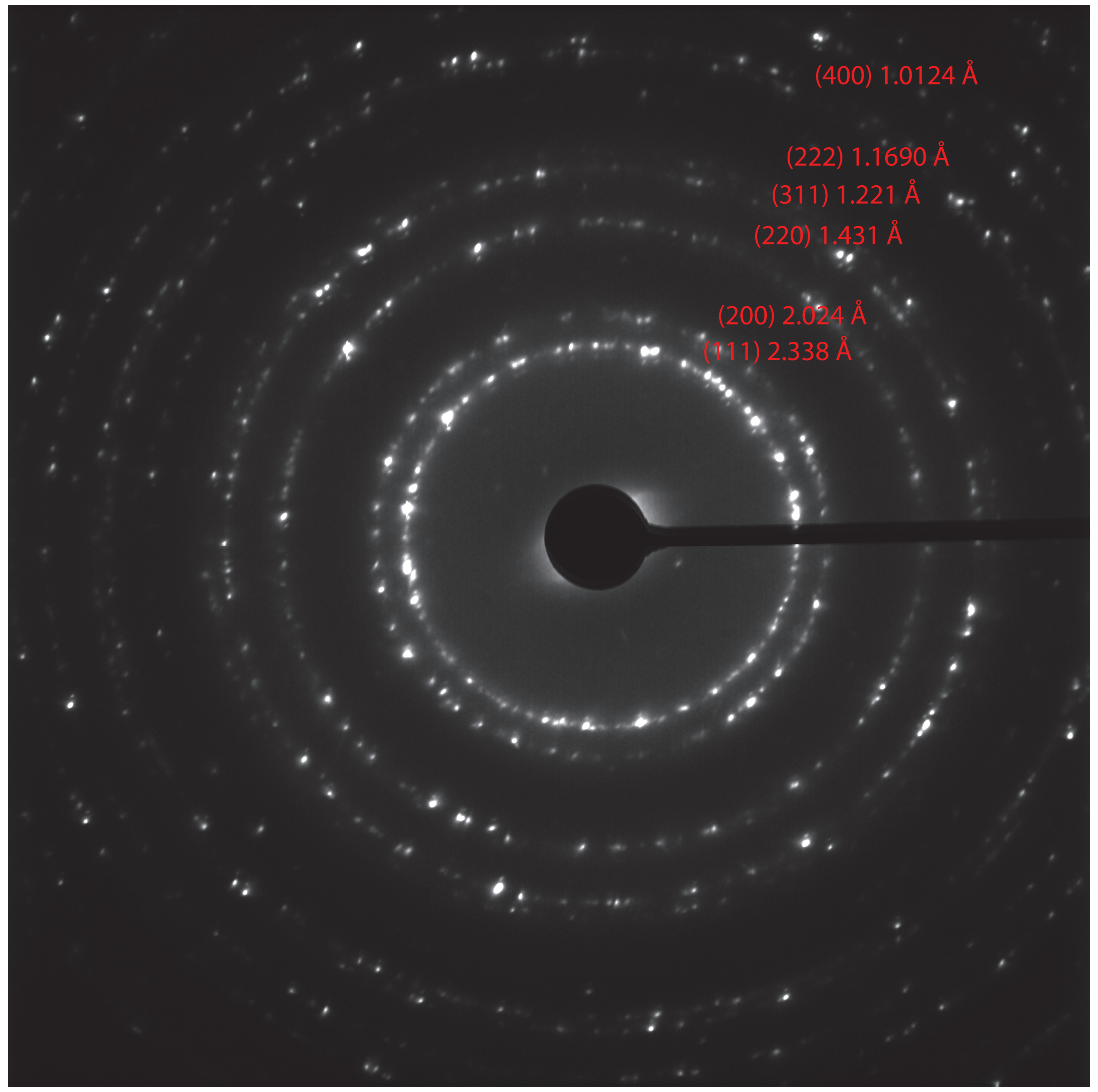
Example TEM schematic

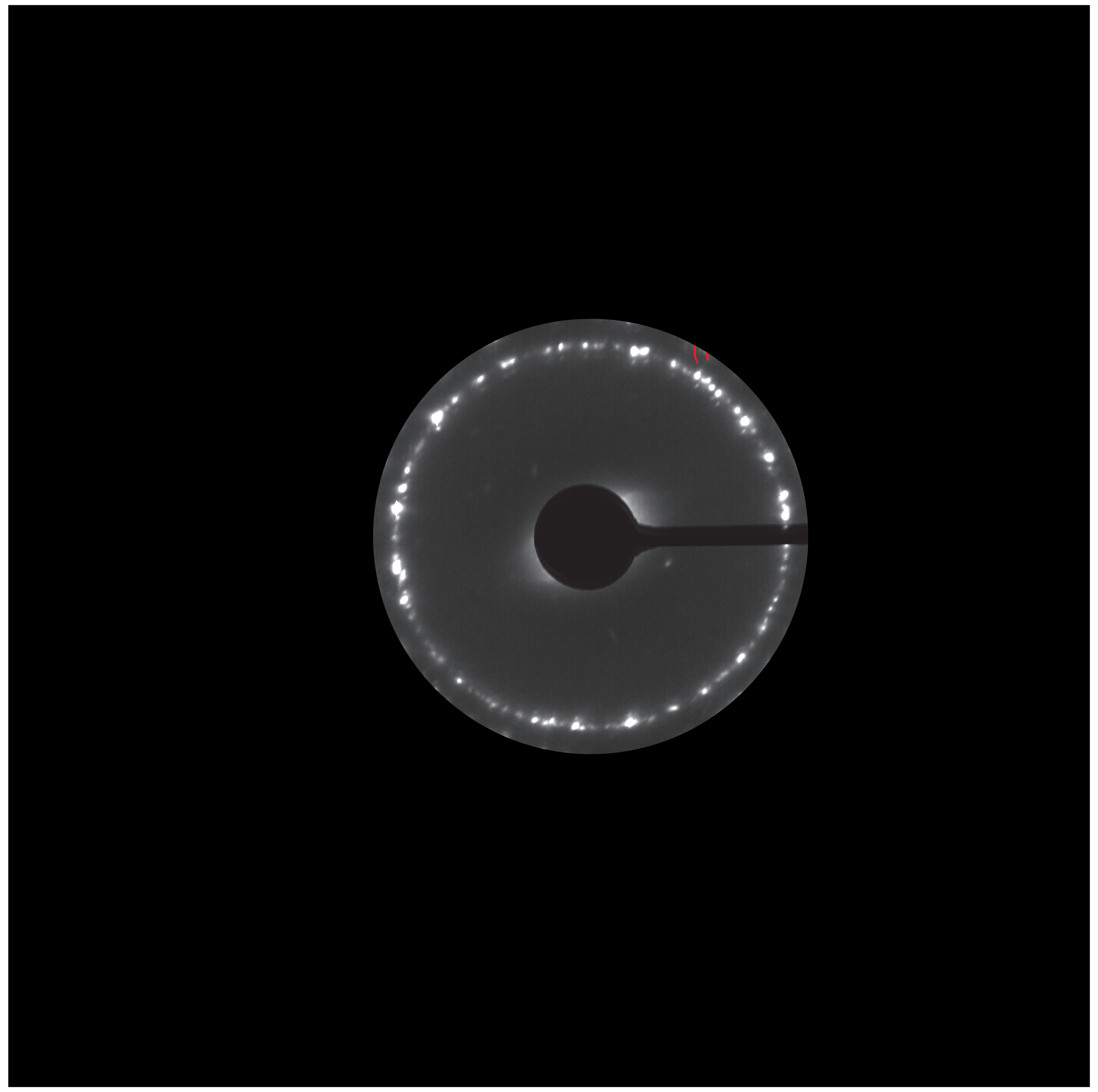
One of many types of TEMs

Objective aperture

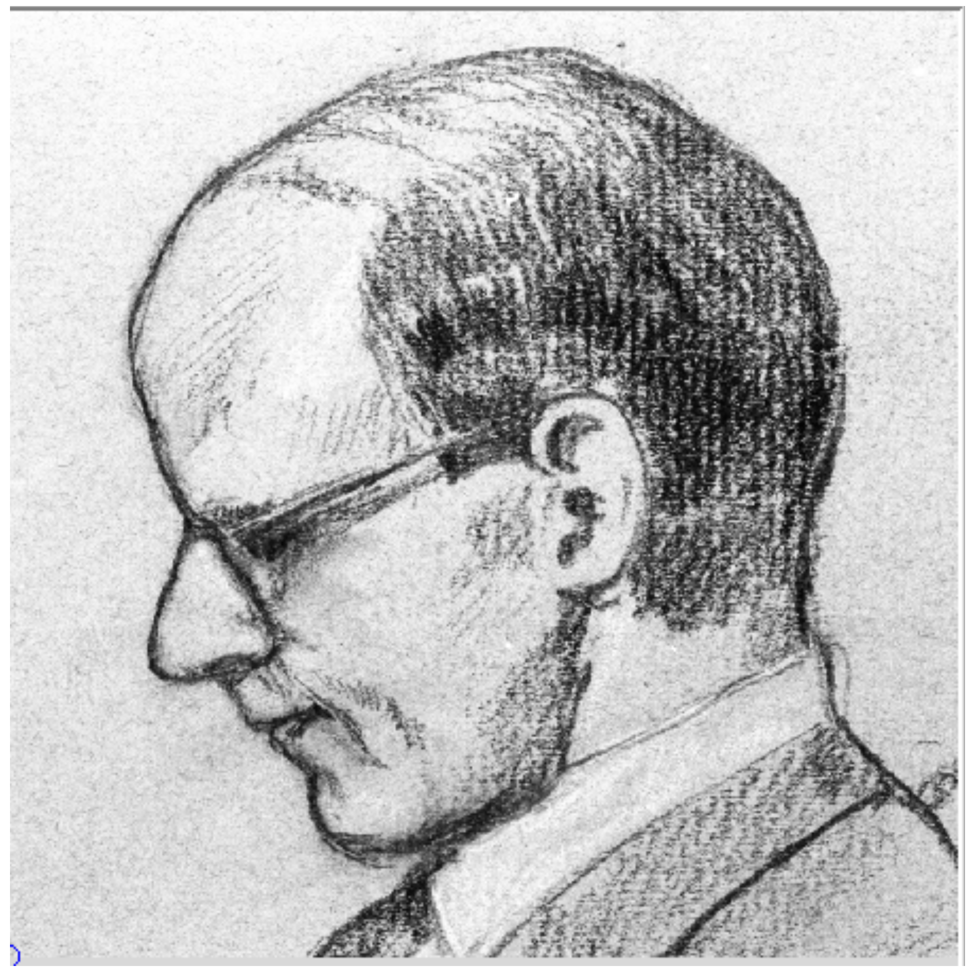


Objective aperture

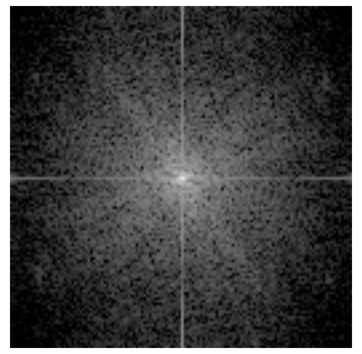




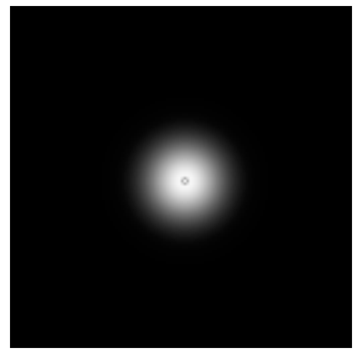
Beware...



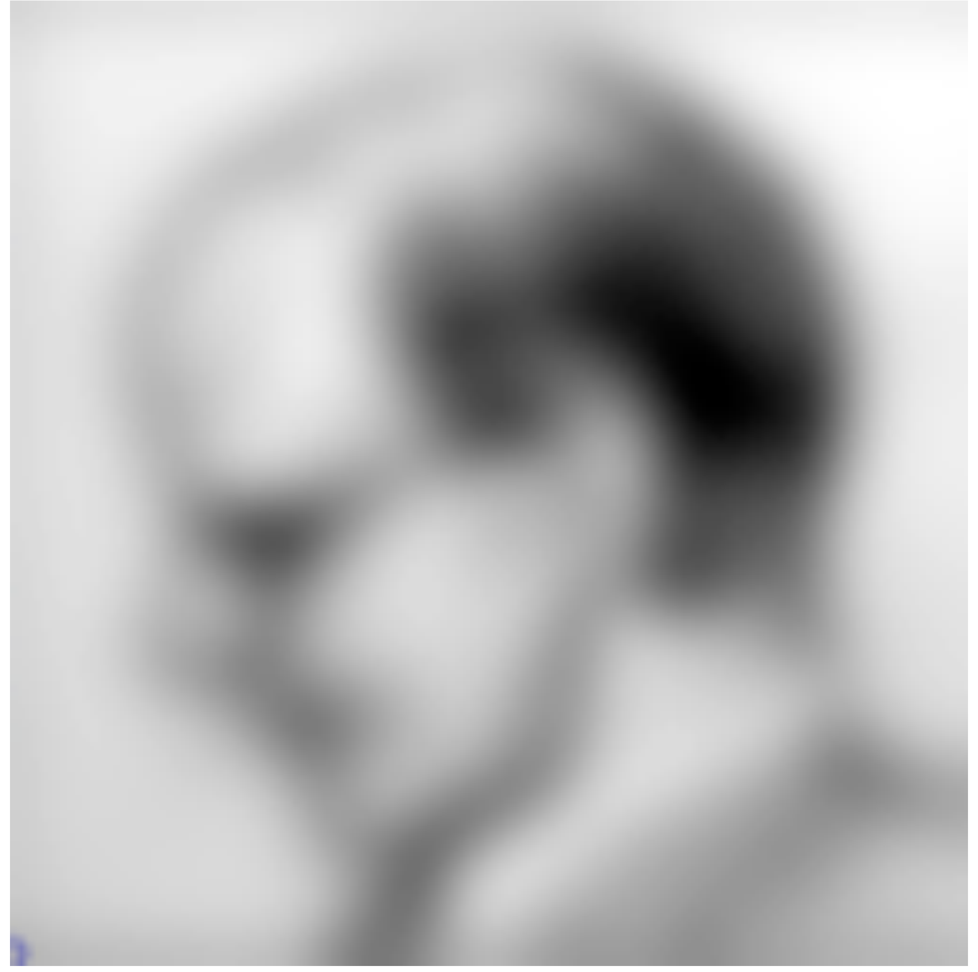
FT



x

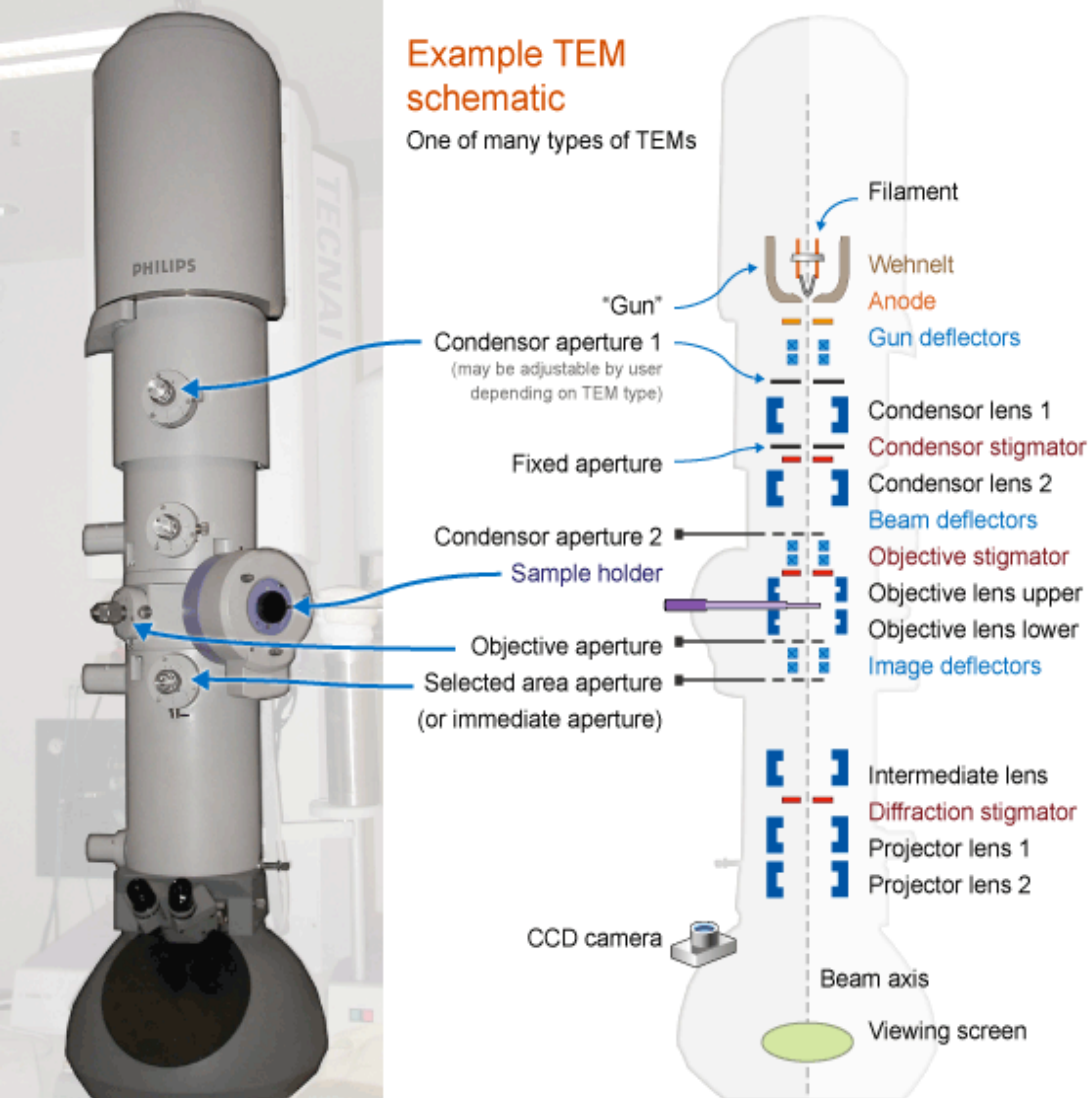


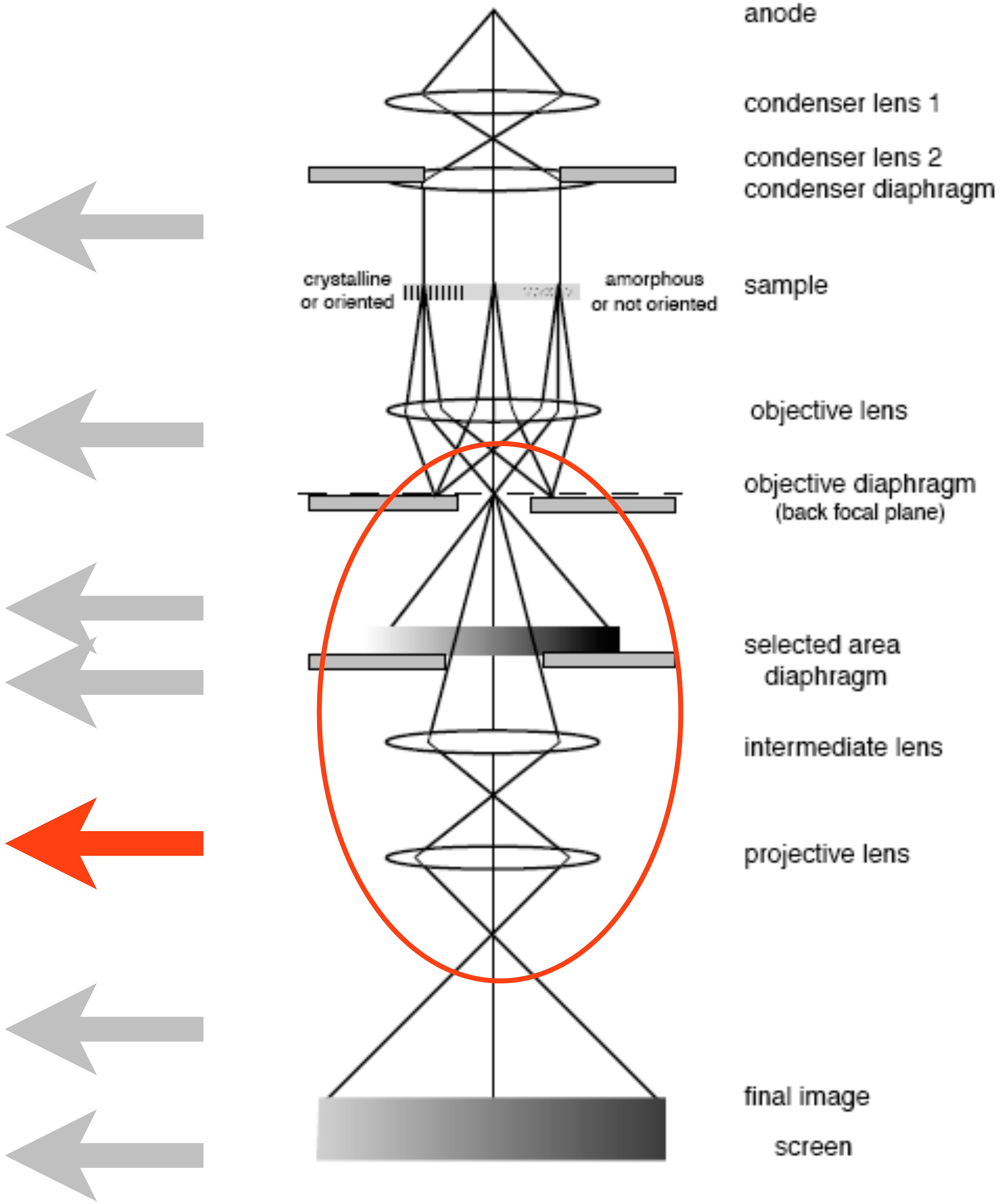
Low-pass filter



Example TEM schematic

One of many types of TEMs





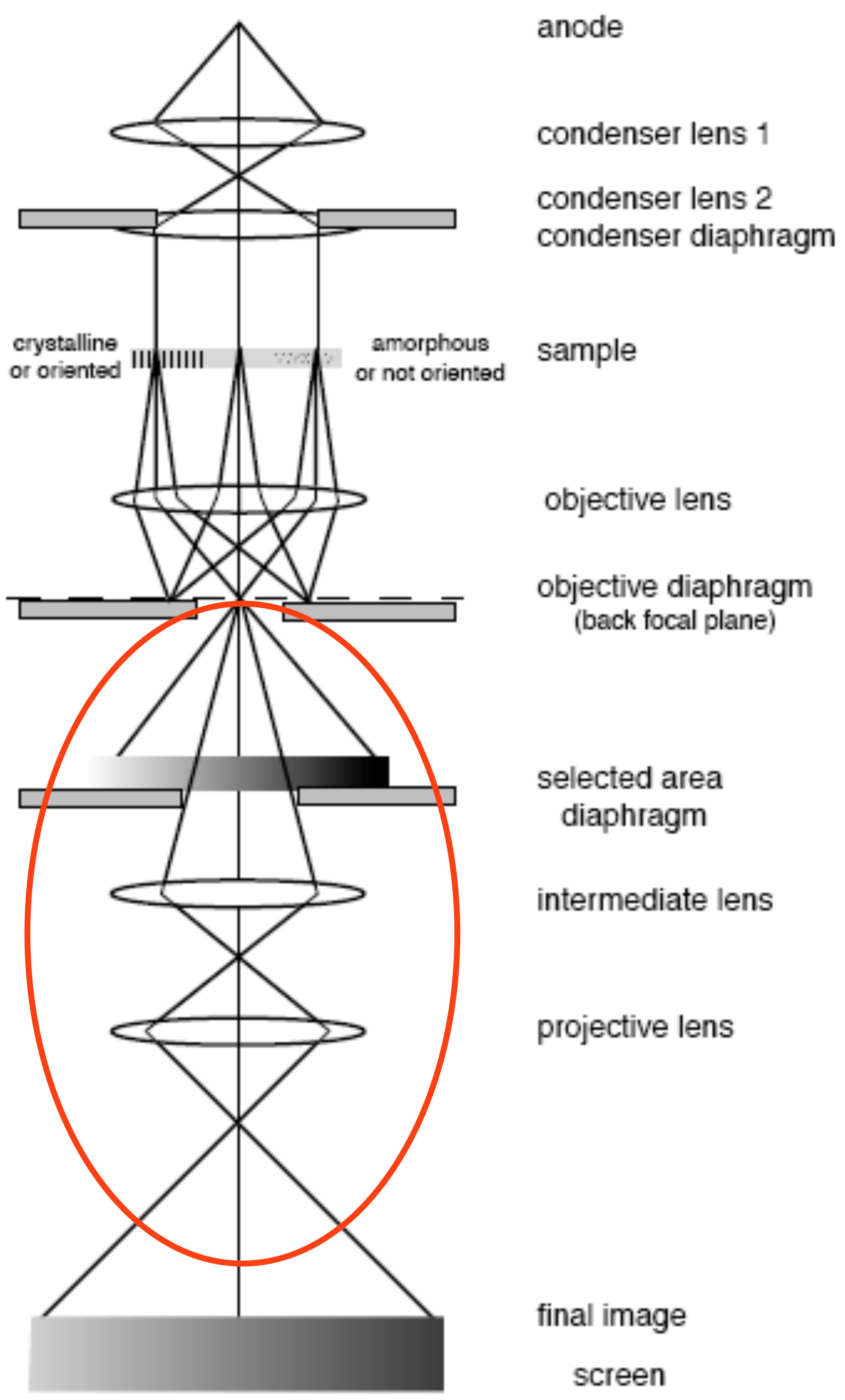
Magnify it!

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

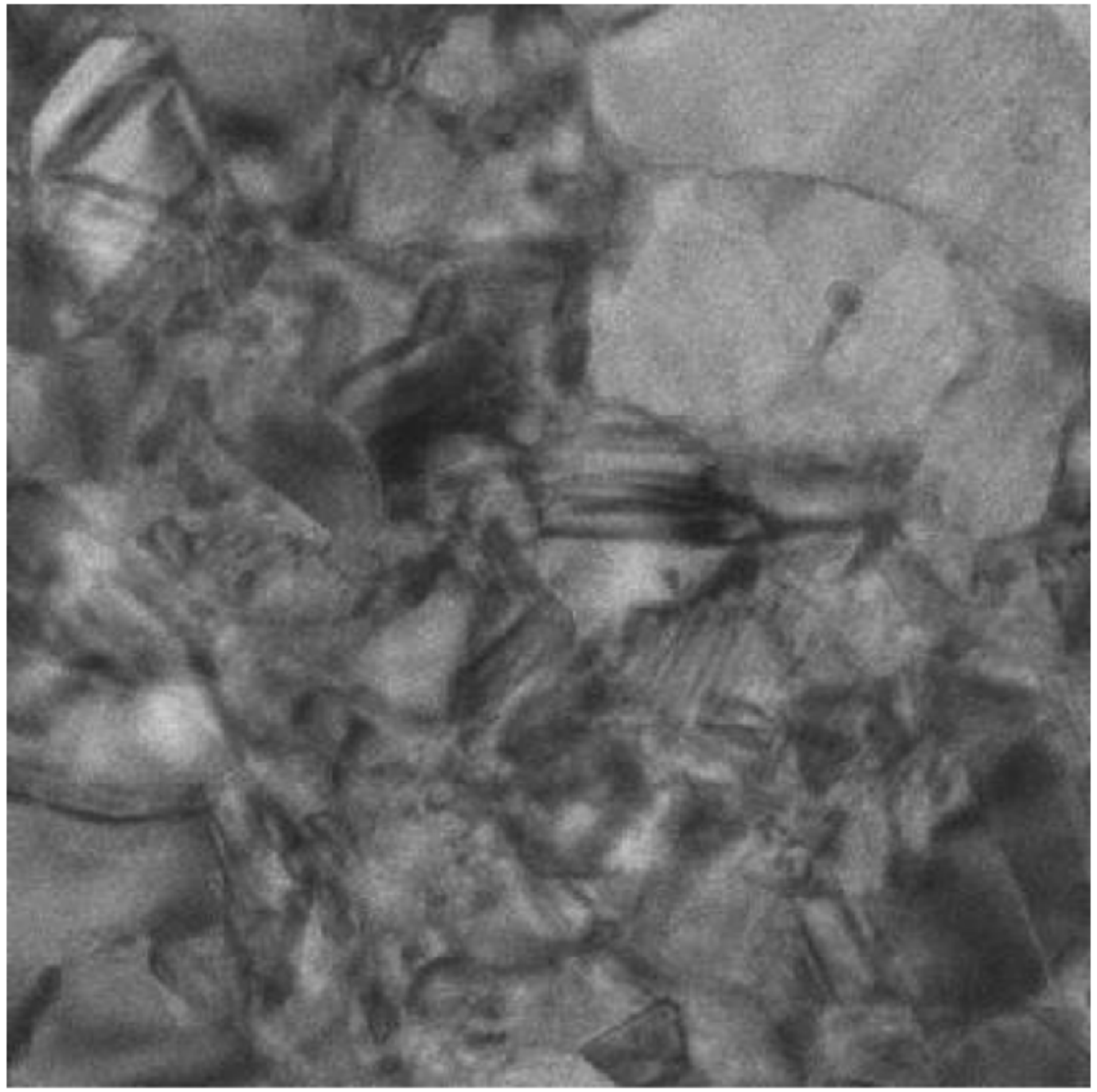
Beware: If not aligned properly, projector lenses can distort image causing differential magnification and other weird effects (barrel, etc.)

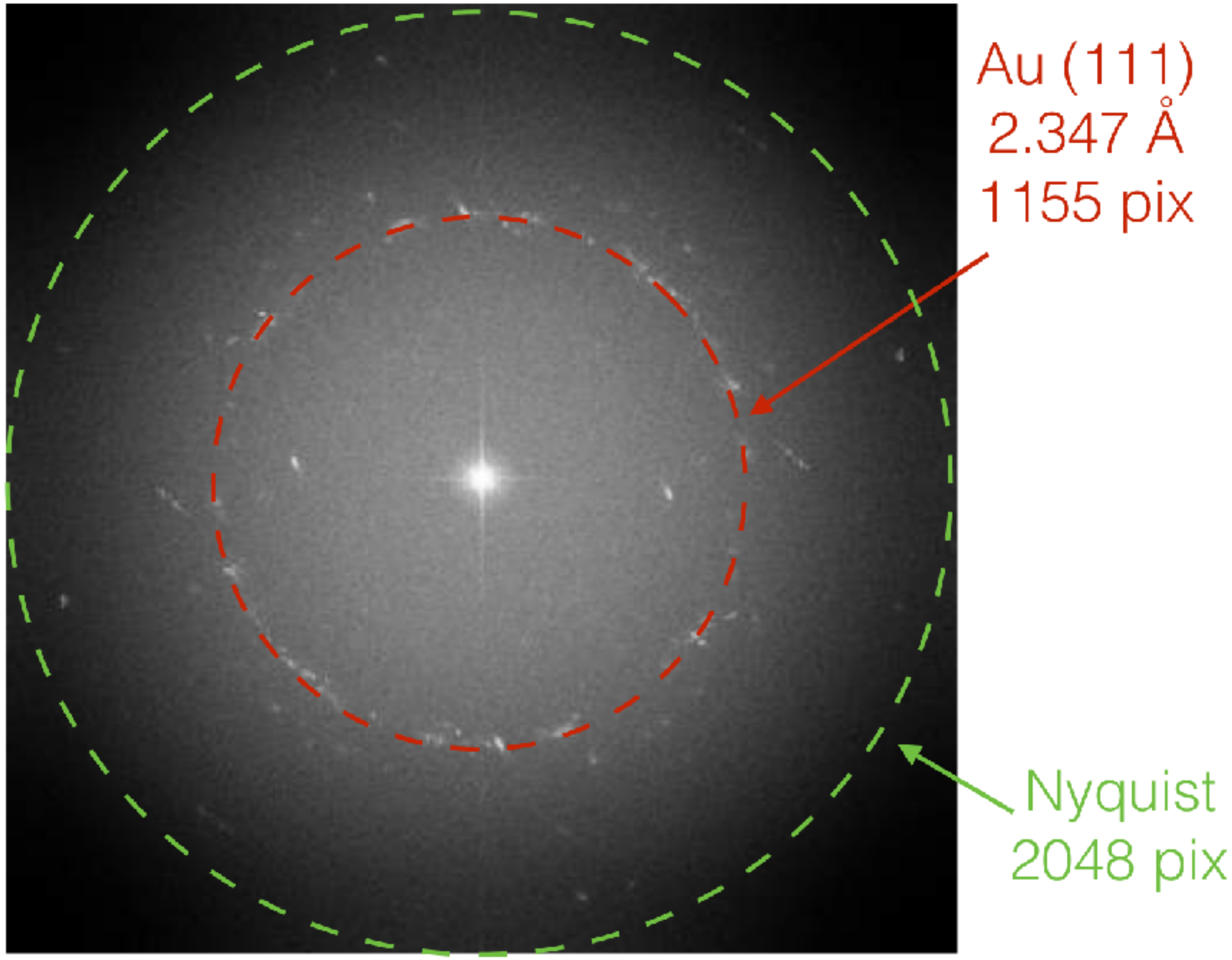


Magnification Calibration

- Pixel size is fixed - a property of the detector
- Magnification is variable from ~ 10 to 10^6 - must be calibrated
- Do it yourself for each dataset - it is easy
- Take an image of gold crystal or graphitised carbon (many other choices as well)
- Also helpful to check that the microscope is well aligned.

FT

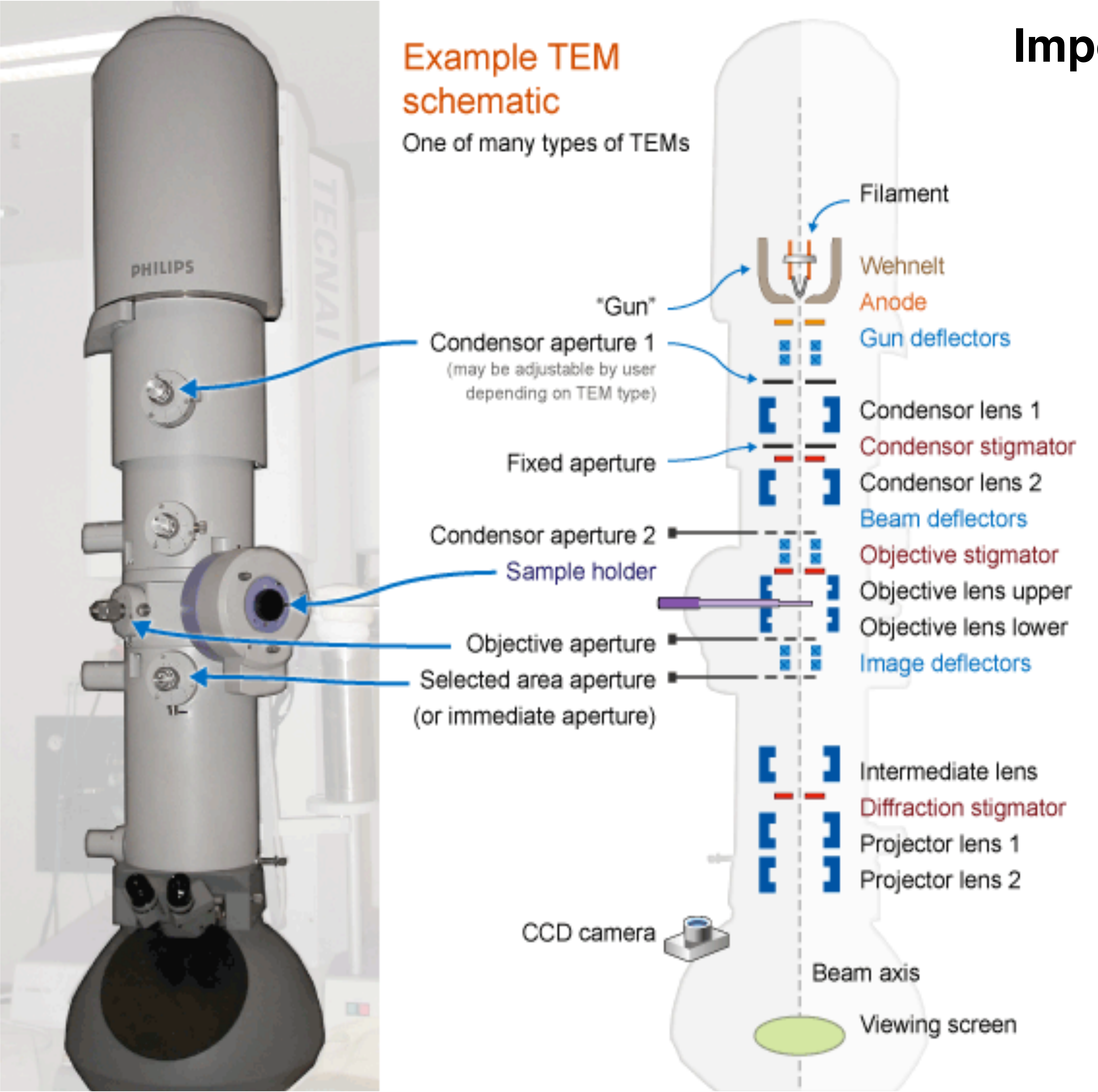




Gold foil micrograph
(120 kX nominal)
4096 x 4096

$$\frac{1155}{2048} = \frac{1/2.347\,\mathring{A}}{2/\mathrm{Pix.size}}$$

$\Rightarrow$ 0.662 Å/pix



Important hardware advances in CryoEM

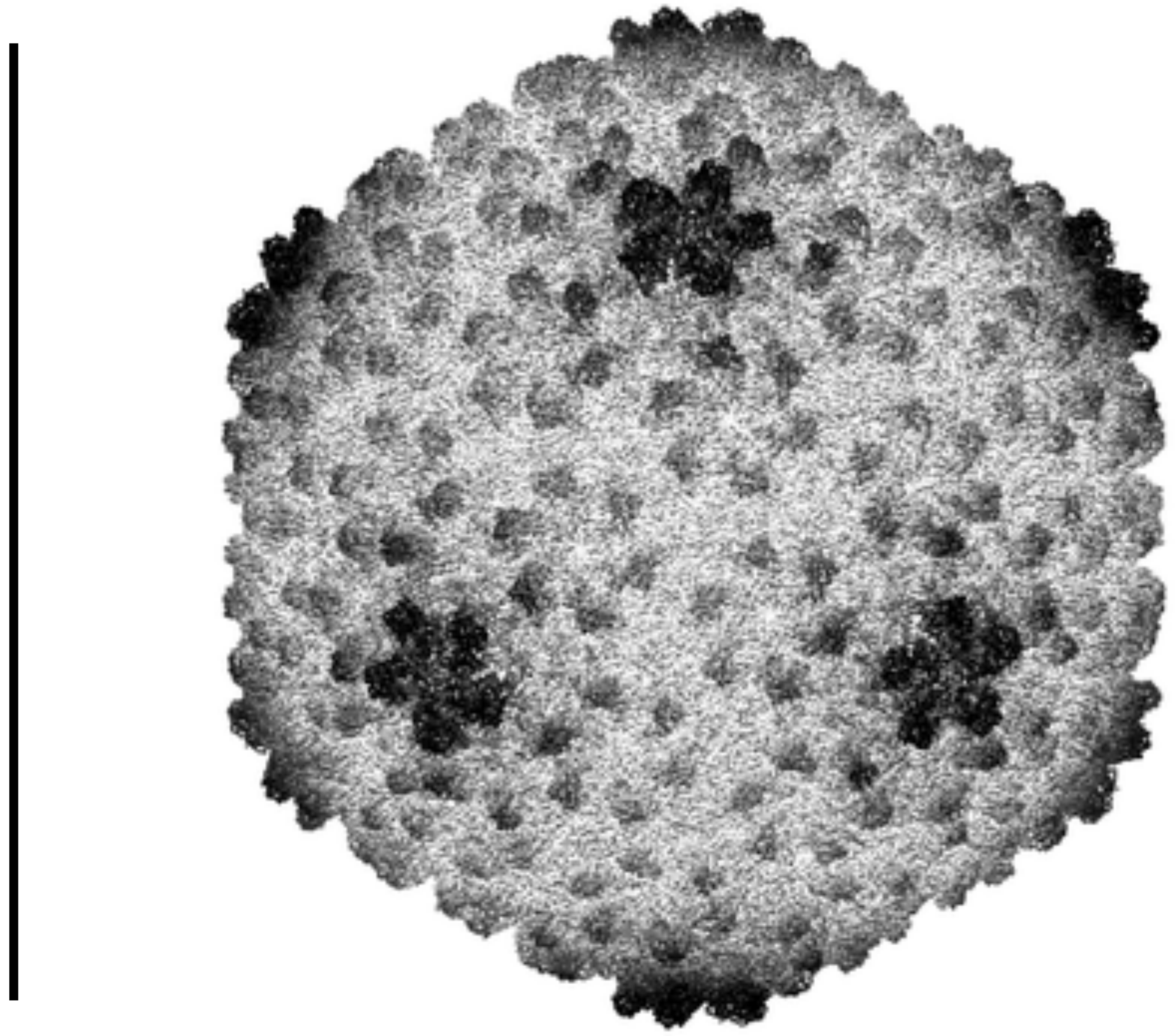
- Electron sources
- Stable lenses and power supplies
- Improved high vacuum systems w/ anti-contamination systems
- High-resolution objective lenses
- Low drift, low vibration, sample stages and cryo-specimen holders
- Stable specimen supports
- Computer control and automation of microscope lenses,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

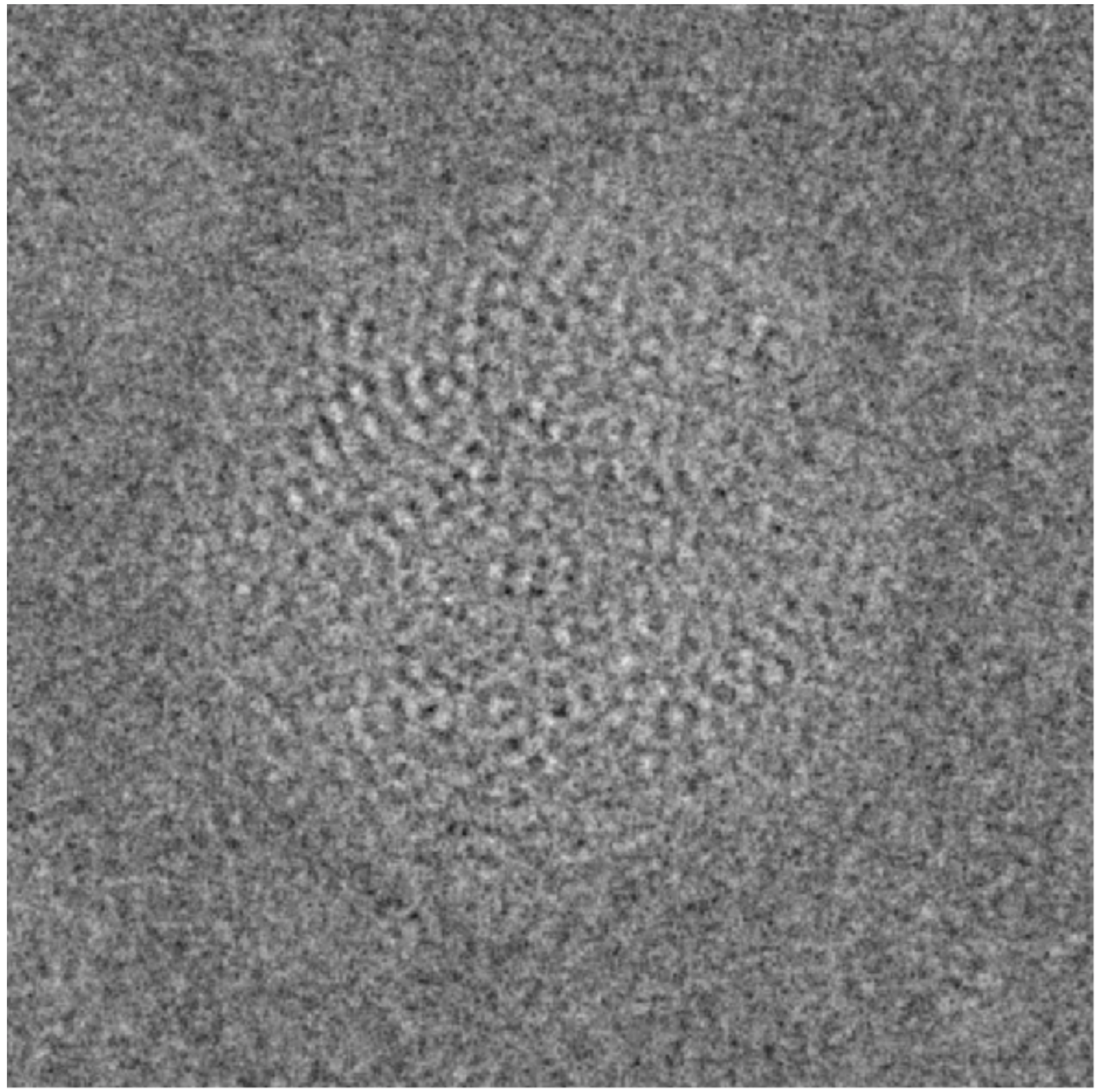
A brief aside

Ewald Sphere correction in EM

P22 virus structure
EMPIAR-10083

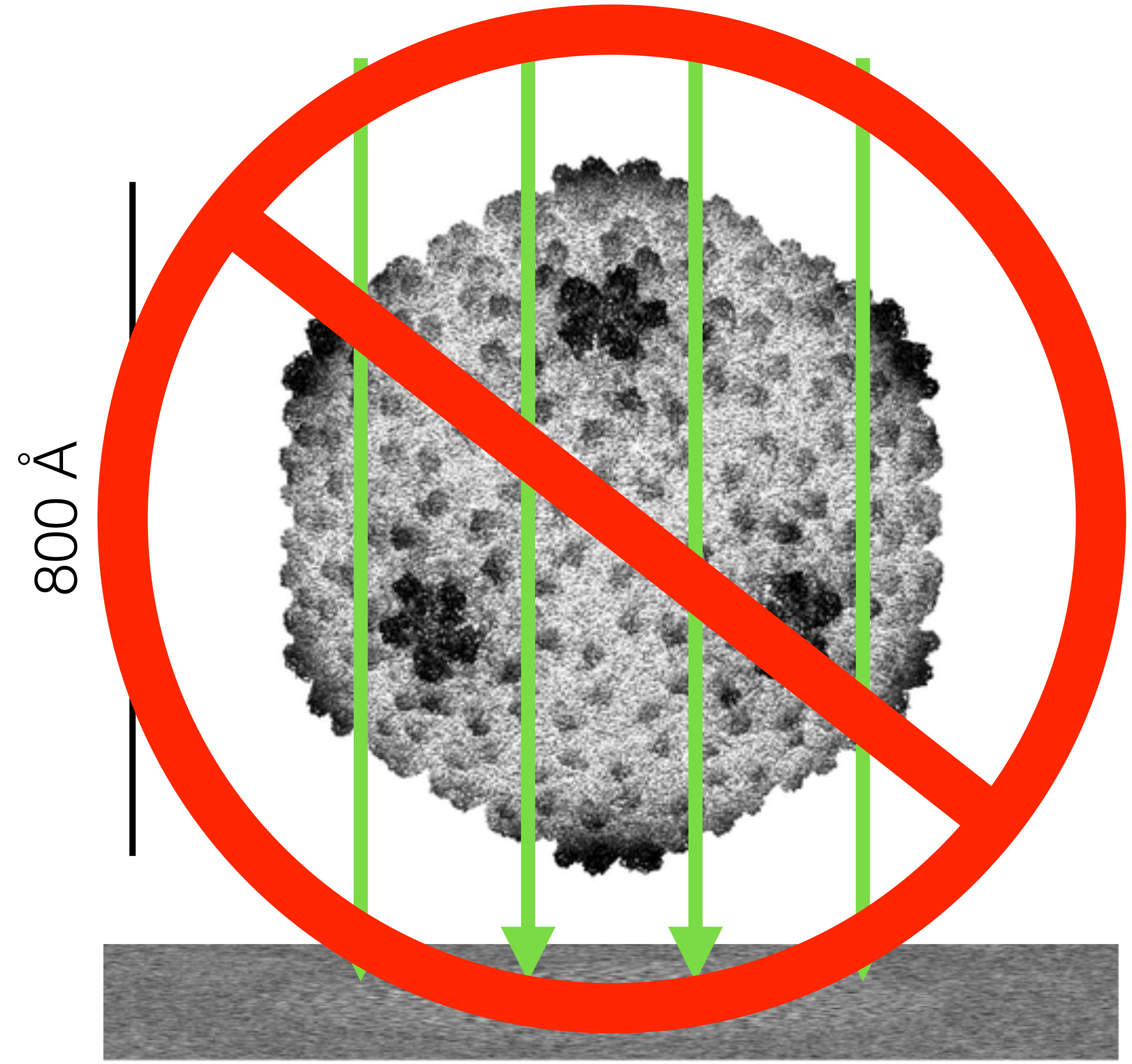
800 Å

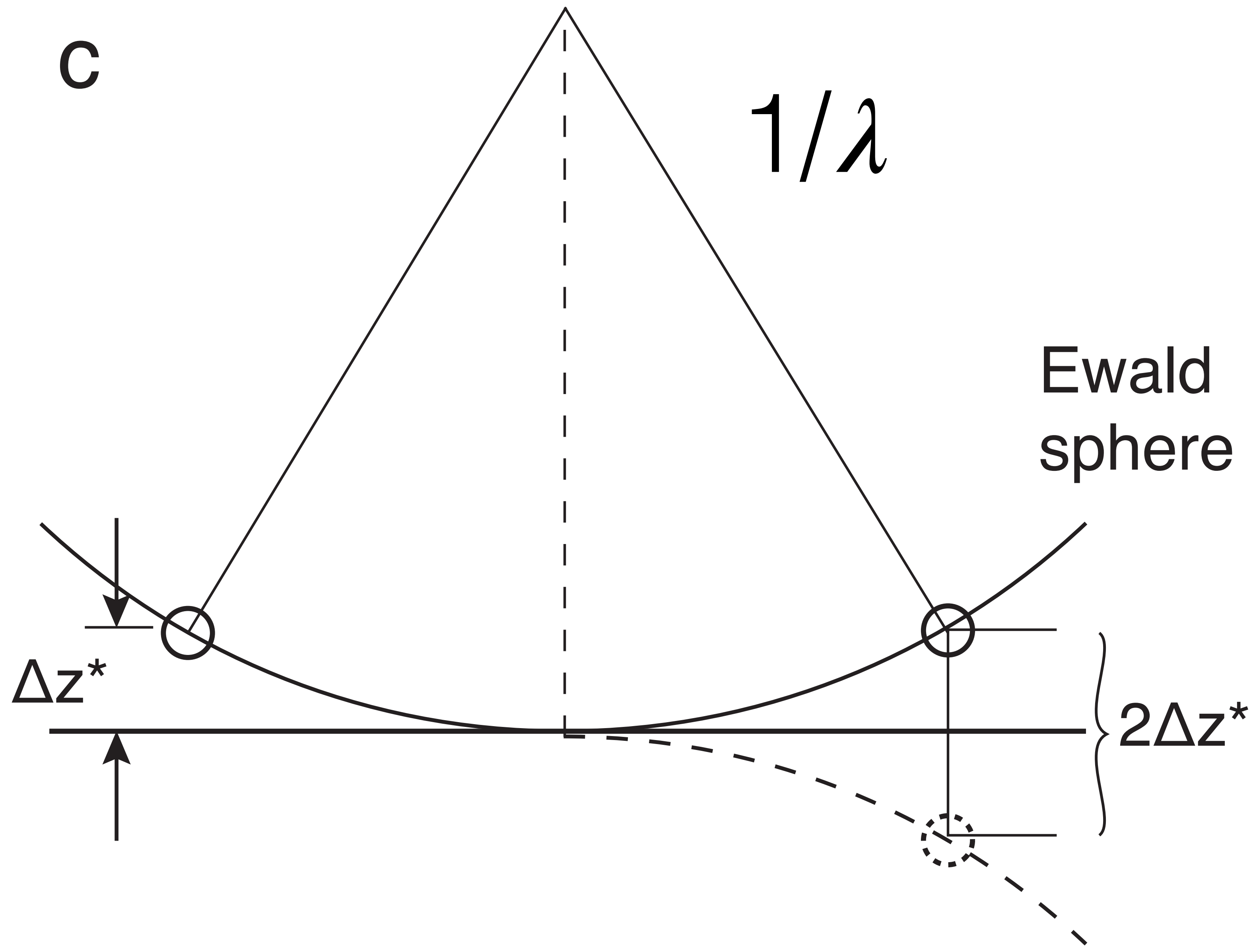




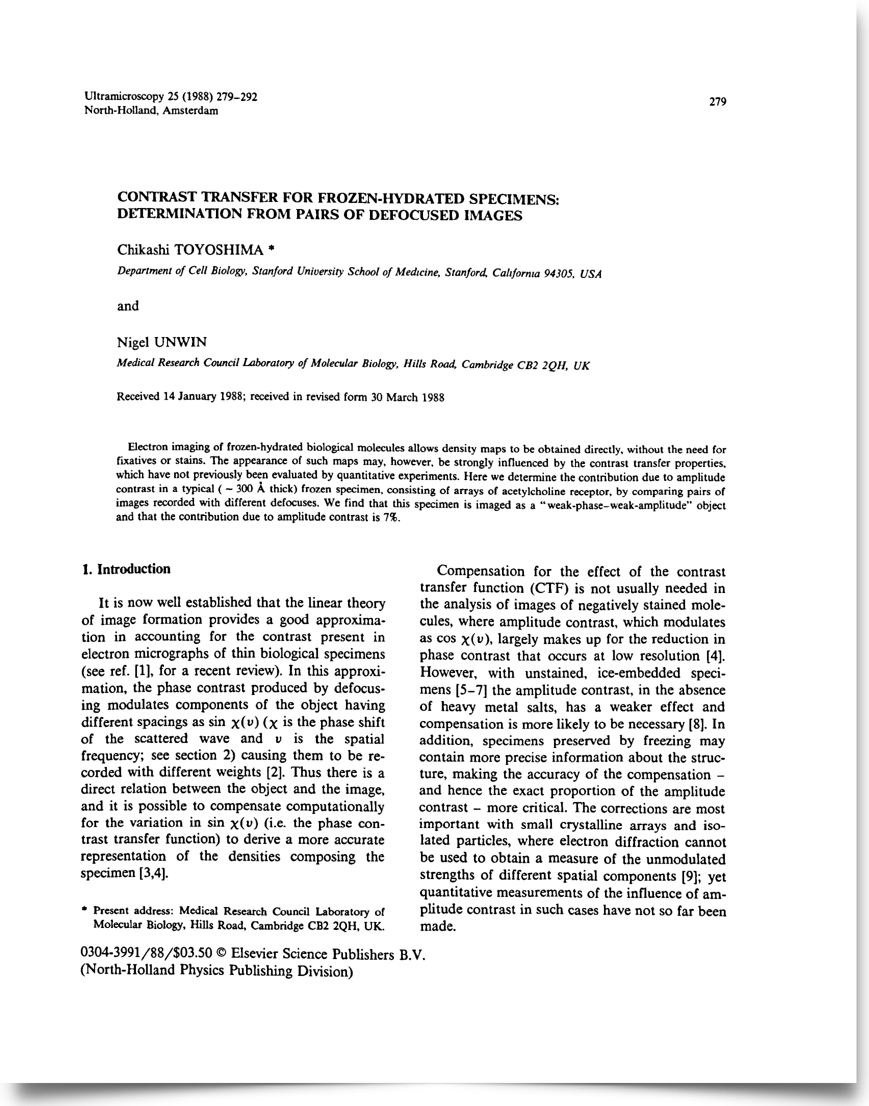
Chen et al. 2017

P22 virus structure
EMPIAR-10083





Some previous work on Ewald sphere correction


Ultramicroscopy 25 (1988) 279–292
North-Holland, Amsterdam

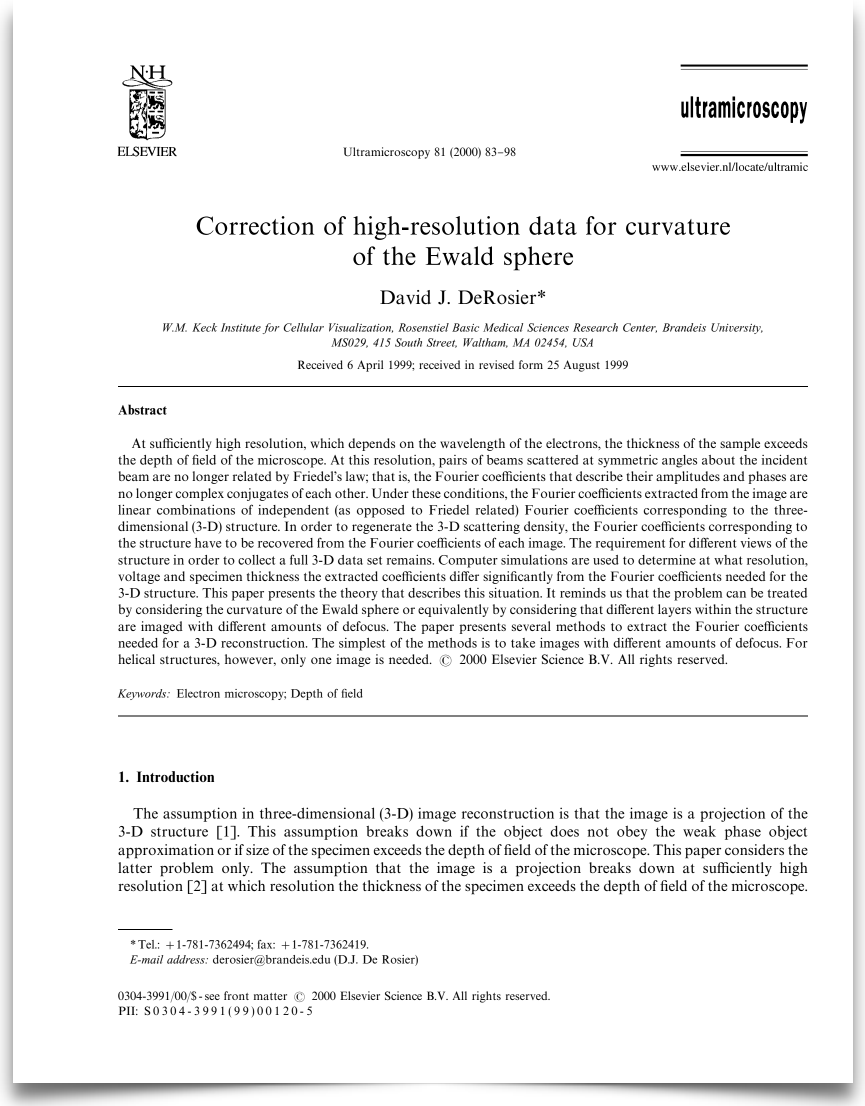
CONTRAST TRANSFER FOR FROZEN-HYDRATED SPECIMENS: DETERMINATION FROM PAIRS OF DEFOCUSED IMAGES

Chikashi TOYOSHIMA *

and

Nigel UNWIN

Toyoshima & Unwin 1988

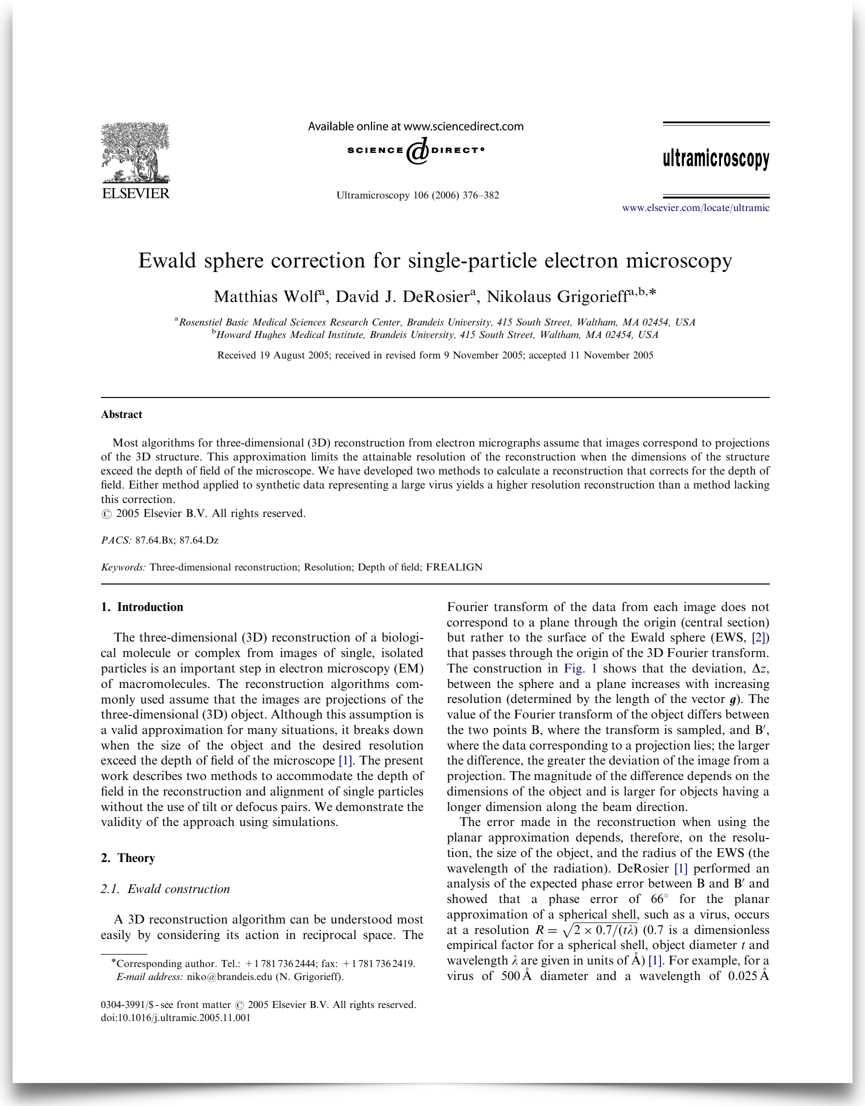

ultramicroscopy

Ultramicroscopy 81 (2000) 83–98

Correction of high-resolution data for curvature of the Ewald sphere

David J. DeRosier*

DeRosier 2000


ultramicroscopy

Ultramicroscopy 106 (2006) 376–382

Ewald sphere correction for single-particle electron microscopy

Matthias Wolf, David J. DeRosier, Nikolaus Grigorieff

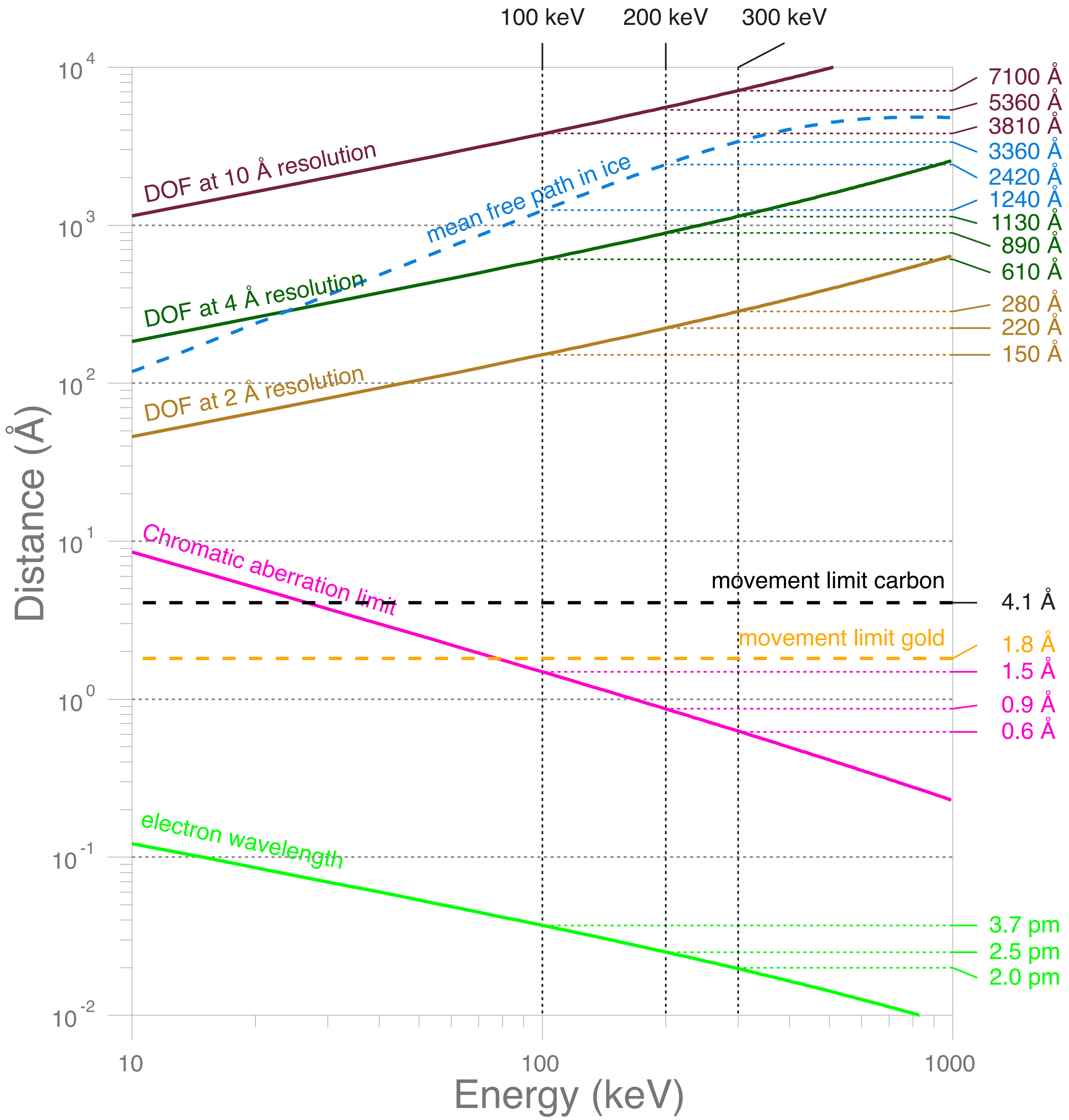
Wolf et al. 2006


Ultramicroscopy

Estimating the effect of finite depth of field in single-particle cryo-EM

Kenneth H. Downing, Robert M. Glaeser

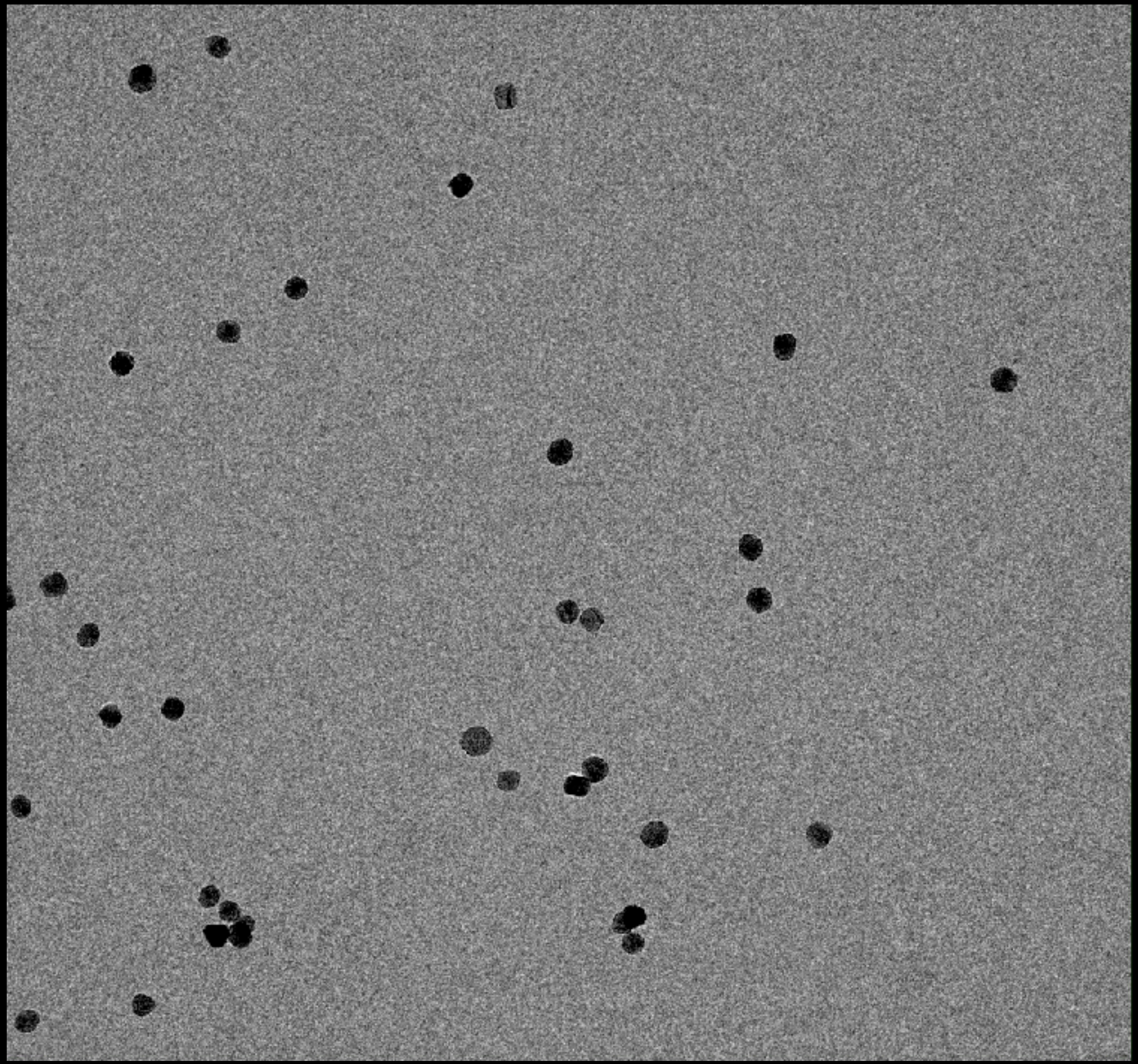
Downing & Glaeser 2017



Electron energy scaling limits relevant to cryo-EM

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

Russo & Passmore 2016b

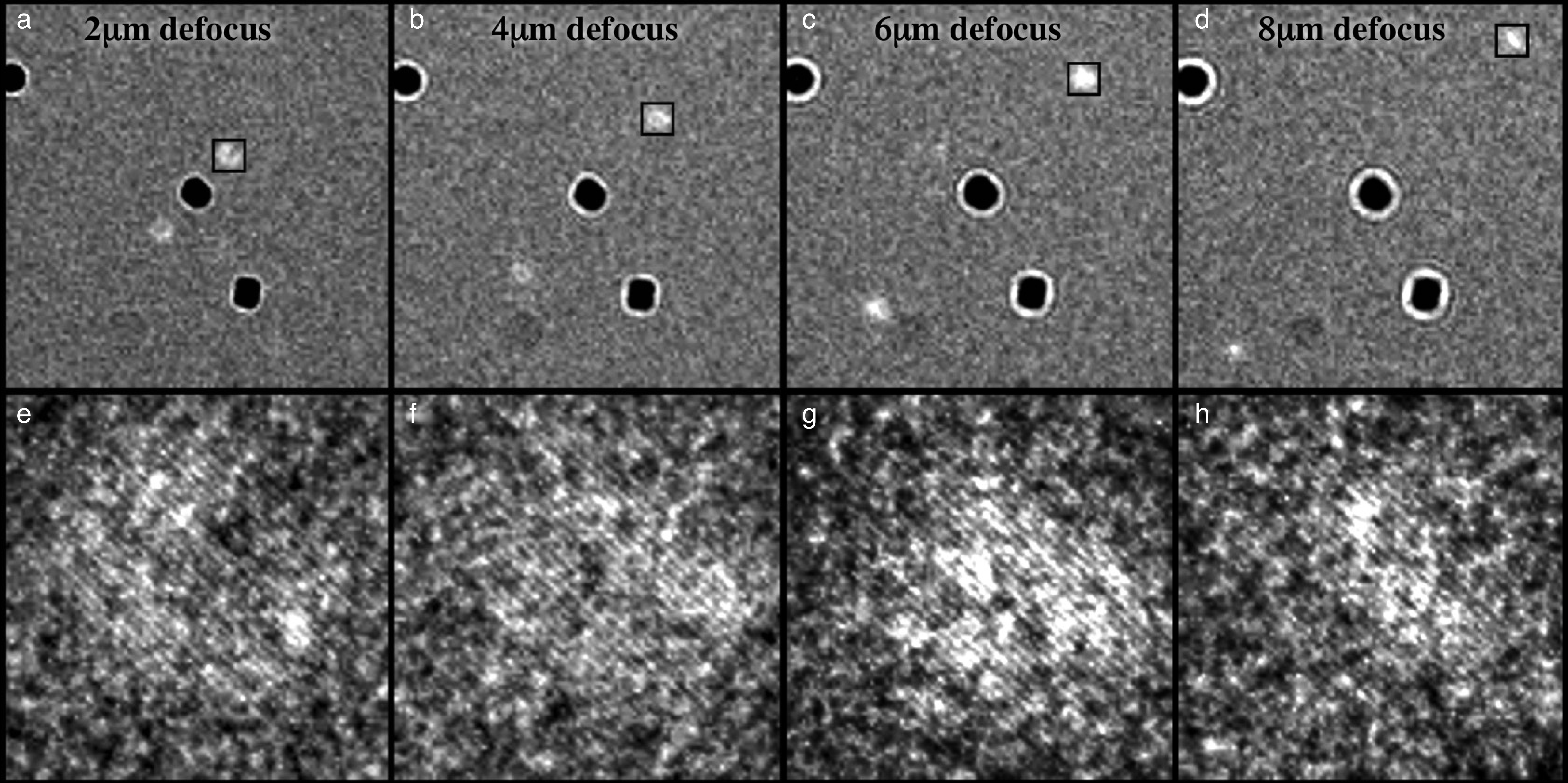


10 nm gold particles in amorphous ice

defocus series from 0 to –10 µm

300 keV
80 K

Russo & Henderson 2018b



a
2μm defocus
b
4μm defocus
c
6μm defocus
d
8μm defocus
e
f
g
h

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

[Plates 17 and 18]

The effects of defocusing and spherical aberration in the electron microscope image are most simply and directly displayed in the Fourier transform of the image. We have investigated the process of image formation by determining the changes in the transform of the image of a thin crystal of catalase, which has discrete diffraction maxima in the resolution range of 10 to 2.5 nm, as a function of defocusing. The changes in amplitude and phase of these diffraction maxima have been measured and compared with the predictions of a first-order theory of image formation. The theory is generally confirmed, and the transfer function of the microscope is completely determined by finding the relative contributions from phase and amplitude contrast. A 'true' maximum contrast image of the catalase crystal, compensated for the effects of defocusing, is reconstructed from the set of micrographs in the focal series. The relation of this compensated image to individual underfocused micrographs, and the use of underfocus contrast enhancement in conventional electron microscopy, are discussed.

This approach and the experimental methods can be extended to high resolution in order to compensate for spherical aberration as well as defocusing. In as much as spherical aberration is the factor presently limiting the resolution of electron lenses, this could provide a considerable extension of the resolution of the electron microscope.

Introduction

In the analysis of structure from electron micrographs it is important to know how contrast enhancement and artefacts from defocusing and aberrations affect the image in the electron microscope. These effects are displayed much more simply and directly in the Fourier transform of the image than in the image itself, and are best analysed in terms of the transform. The analysis of these effects through the image transform is of particular interest because of the involvement of the transform in systems for the analysis of periodic structures in electron micrographs, and in the system used for three-dimensional reconstruction by electron microscopy (De Rosier & Klug 1968).

Conventional microscopy today is largely concerned with the imaging of details from 10 or 20 nm down to 2 nm resolution. Especially with the microscopy of biological specimens there is generally little preservation of meaningful structural details beyond 2 nm resolution. As modern electron lenses are essentially perfect to this resolution, the only electron optical factor affecting the image is defocusing. This is important in practical microscopy since micrographs are normally taken somewhat under focus, both because it is technically more difficult to obtain a perfectly in-focus image, and because the defocusing produces a useful enhancement of image contrast. We have analysed the effects of defocusing theoretically and experimentally, and in terms of the results of the investigation can specify conditions for the proper use of underfocus contrast enhancement and the nature of artefacts that will occur with excessive defocusing.

At higher resolution the spherical aberration of the electron lens affects the image in a manner very similar to defocusing. Under optimum conditions modern microscopes can record image details at a point to point resolution of about 0.2 nm. As discussed below, however, the

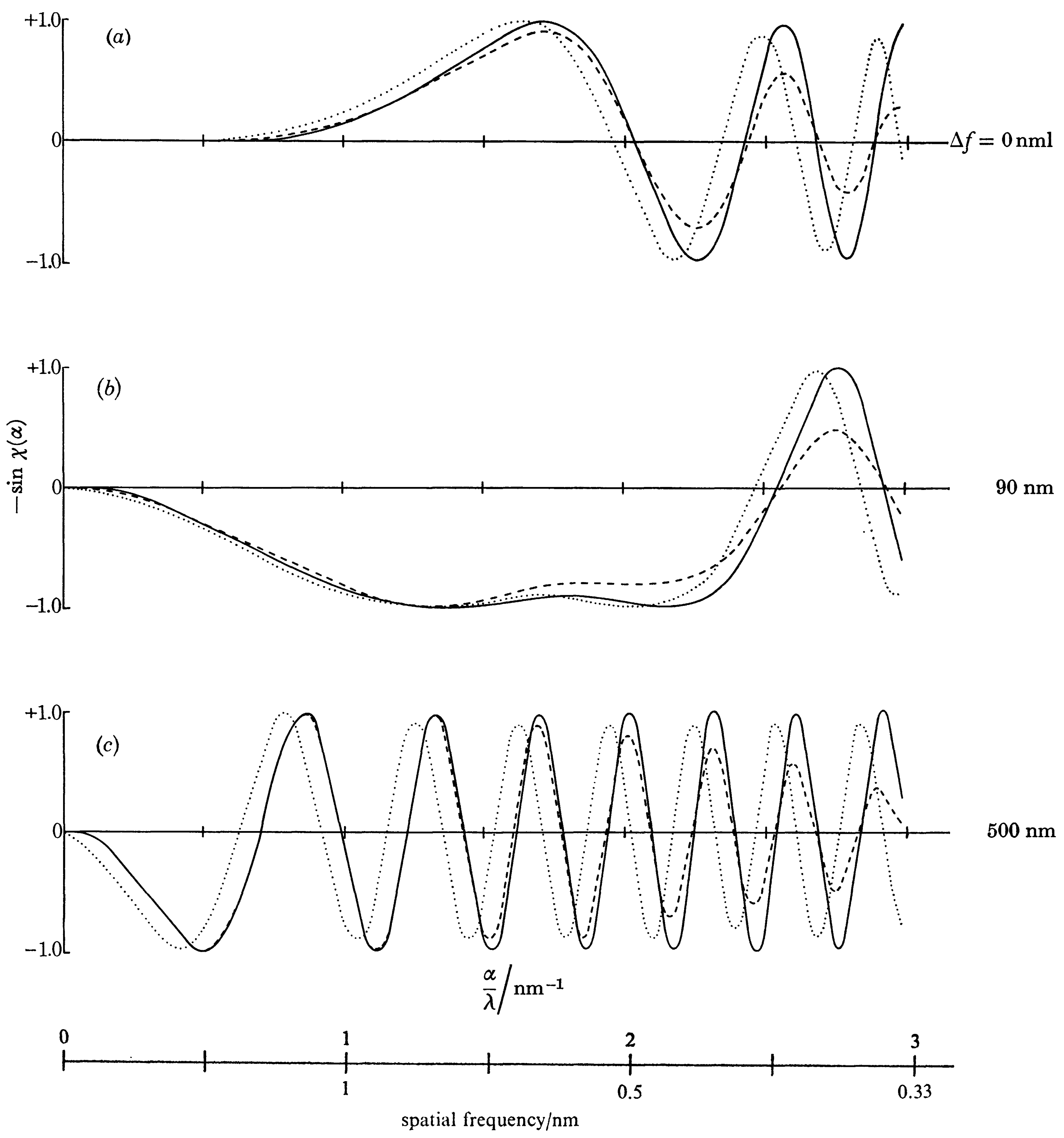
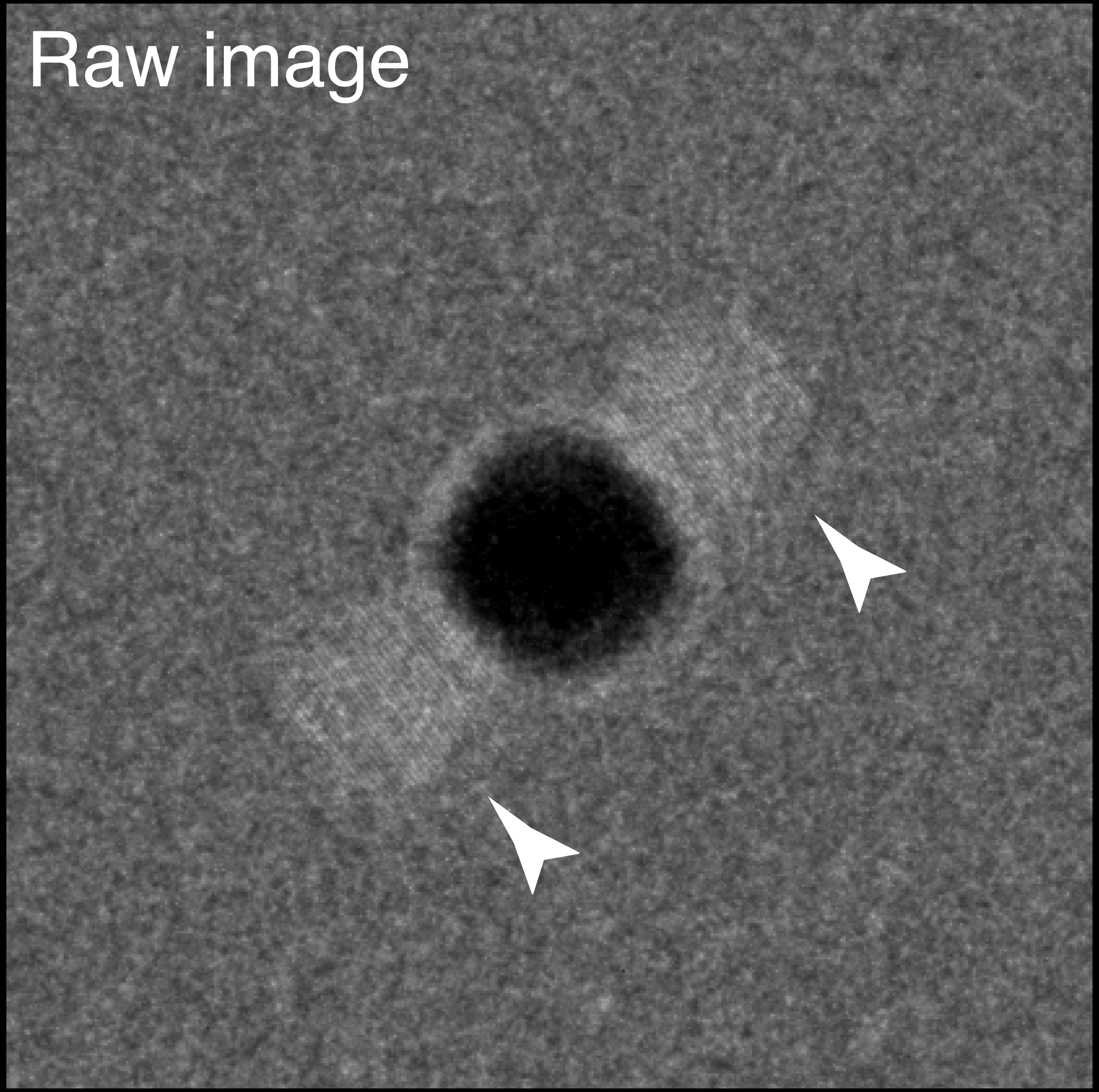
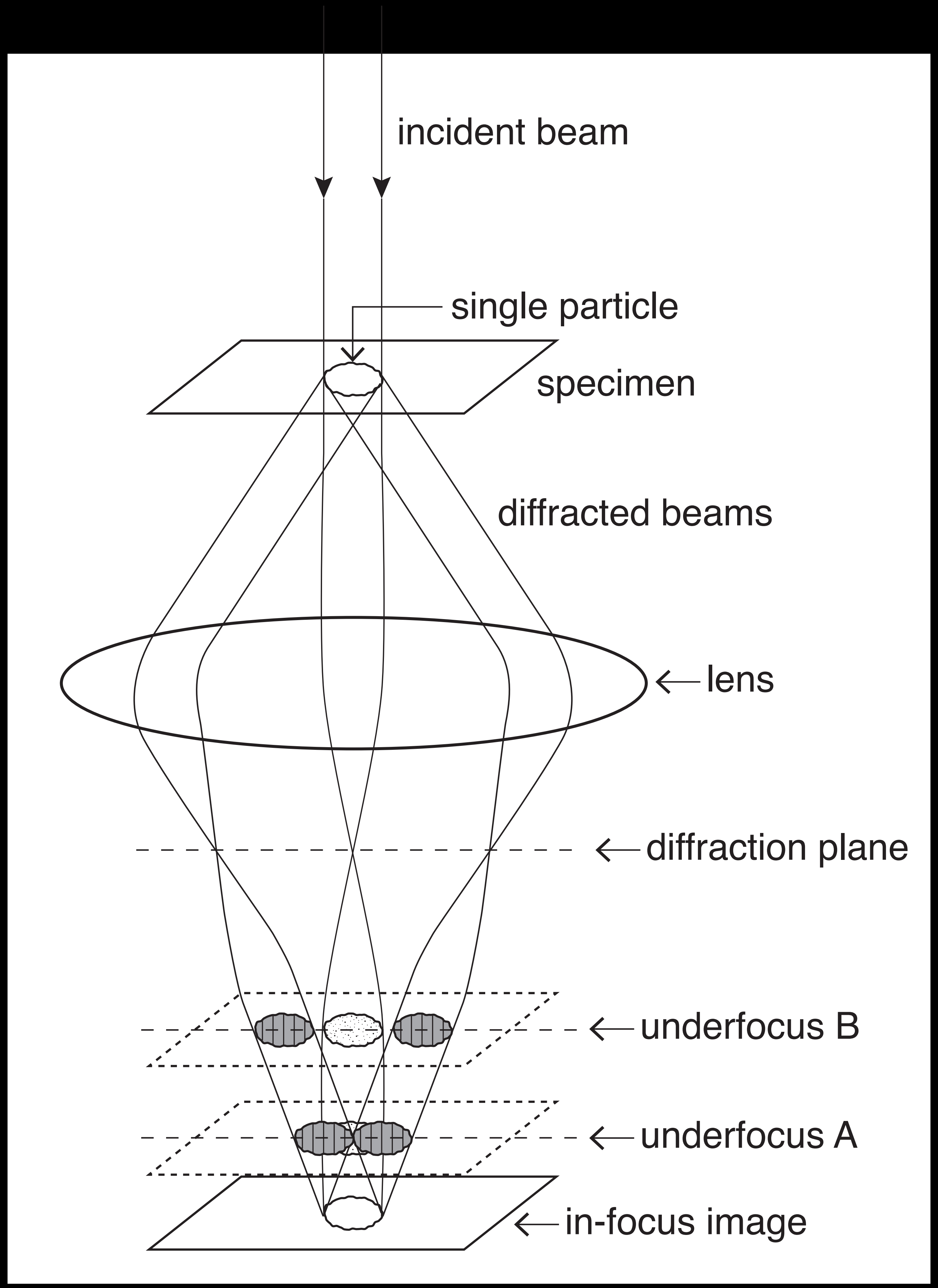


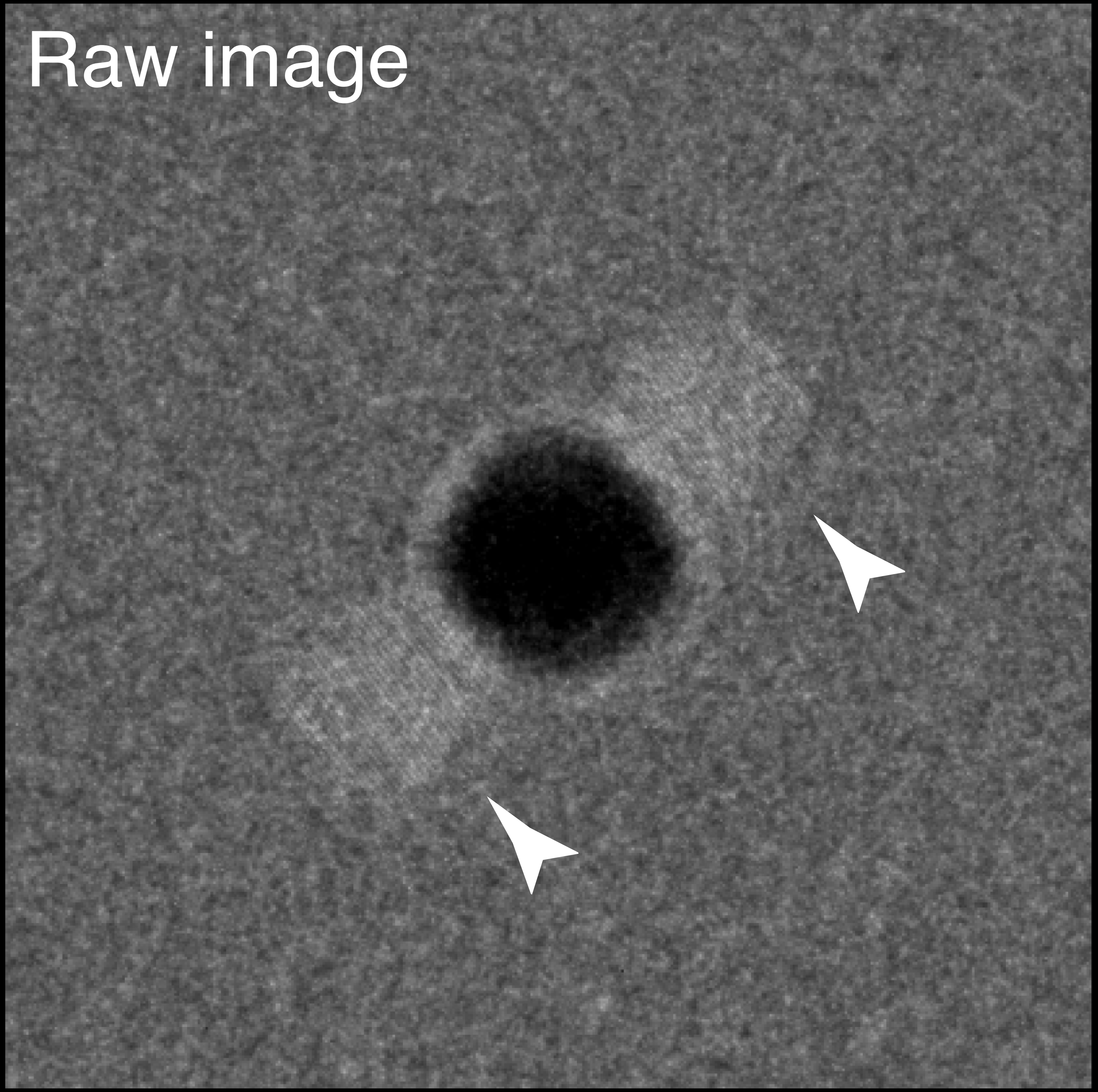
Figure 1. The phase-contrast transfer function, $-\sin\chi(\alpha)$, plotted as a function of α/λ, in nm^{-1}, for $\lambda = 0.0042\,nm$,

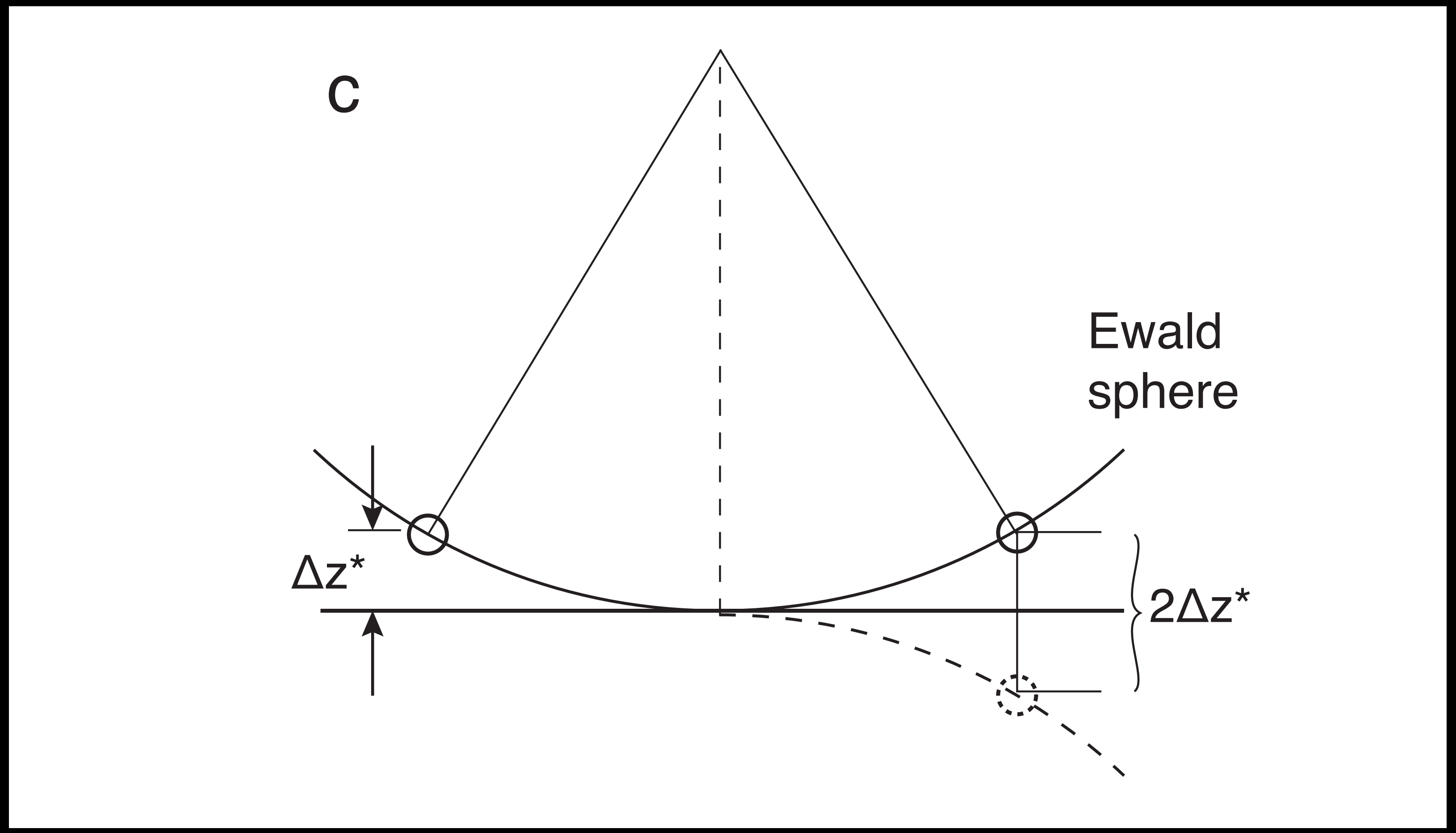


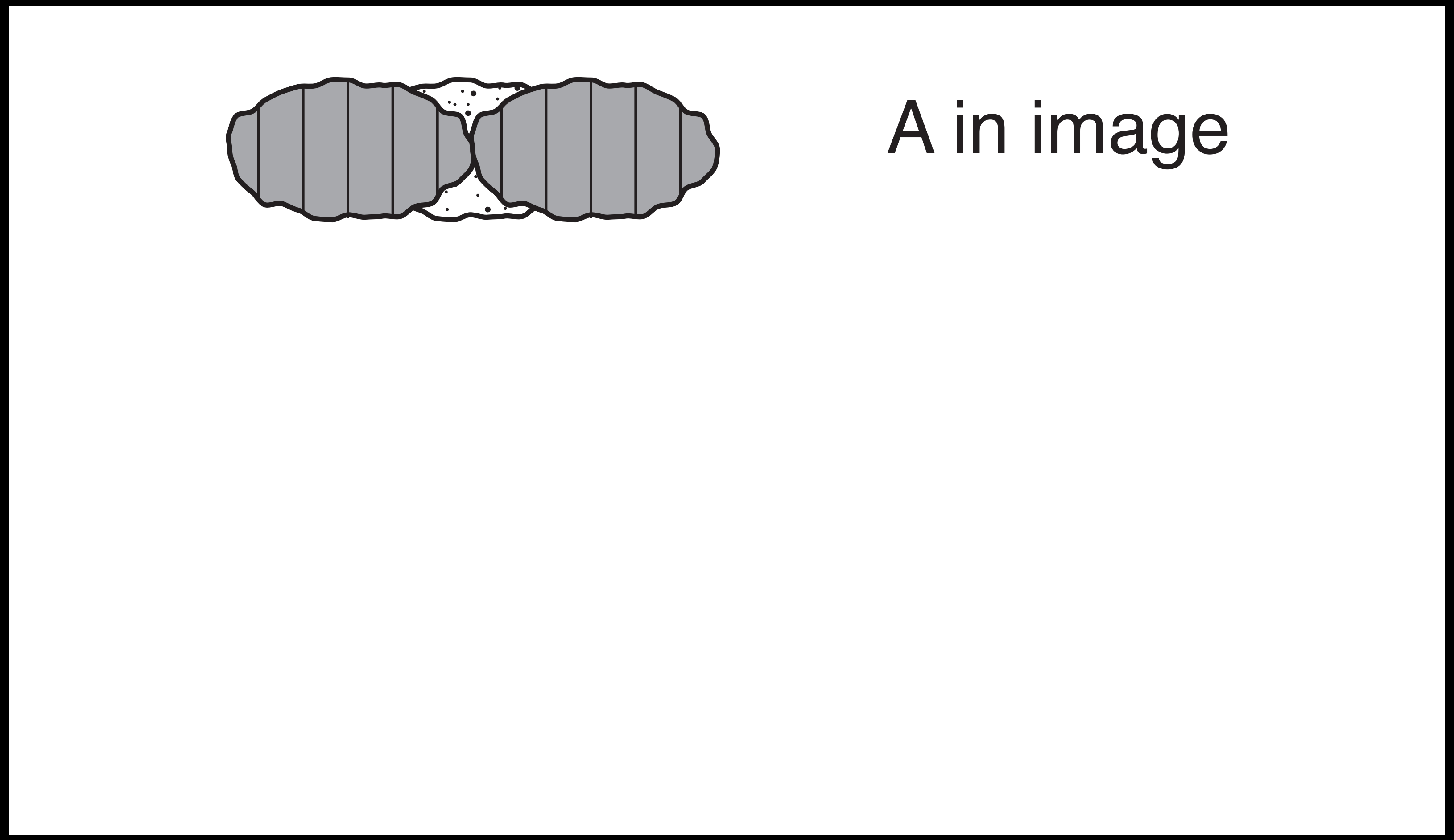
100 Å gold nanopartice in ice
300 keV



Russo & Henderson 2018c

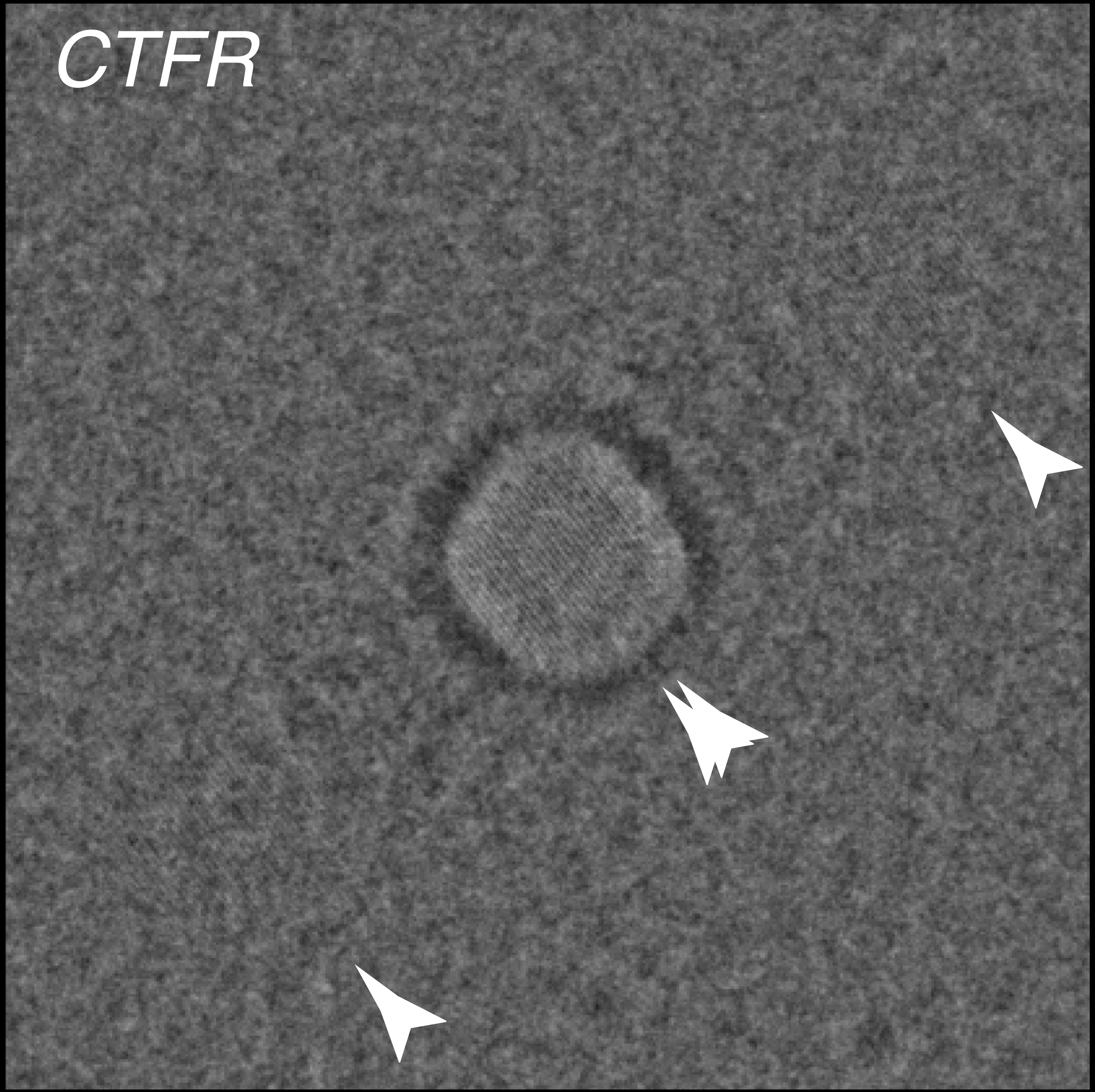


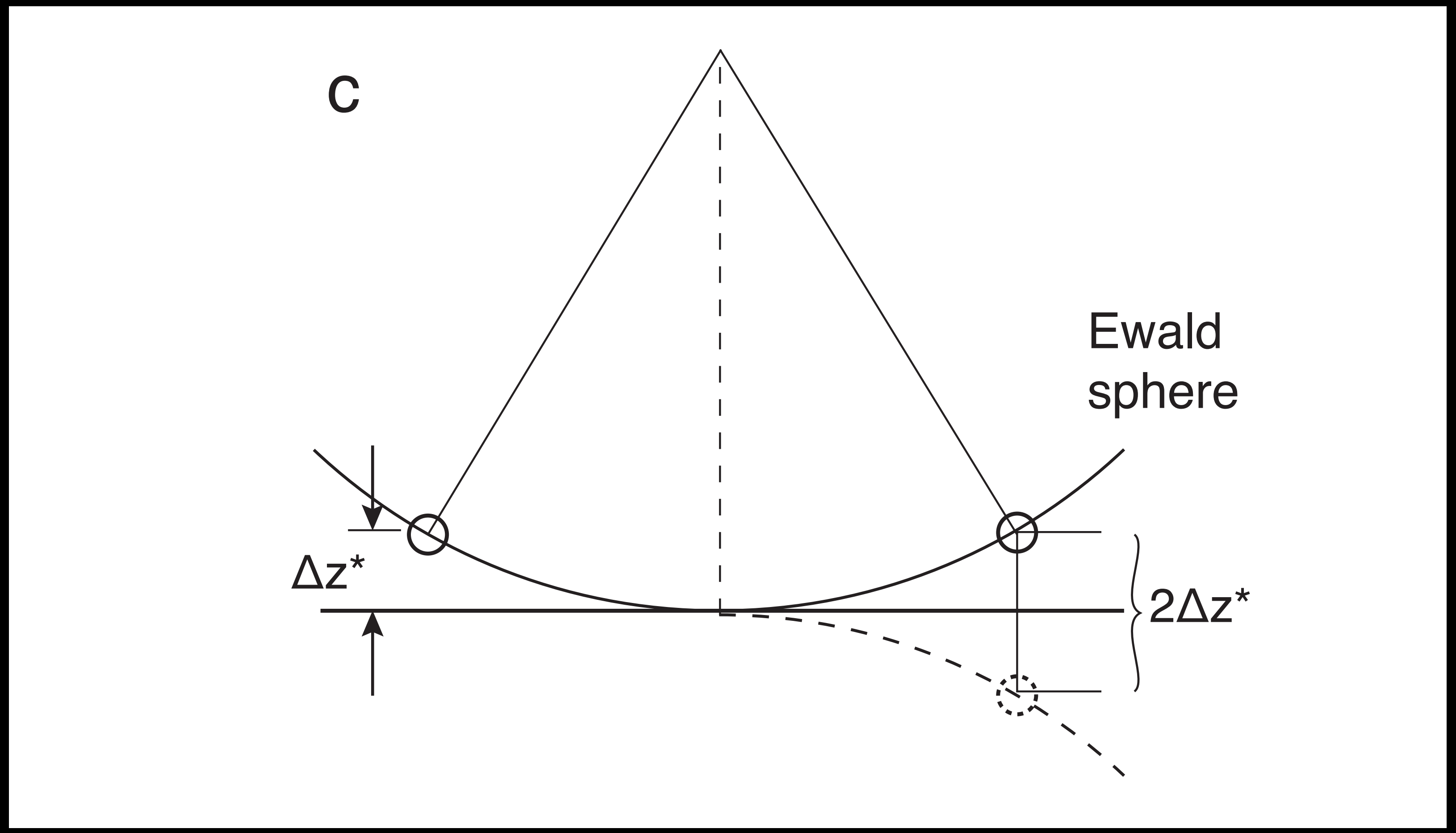


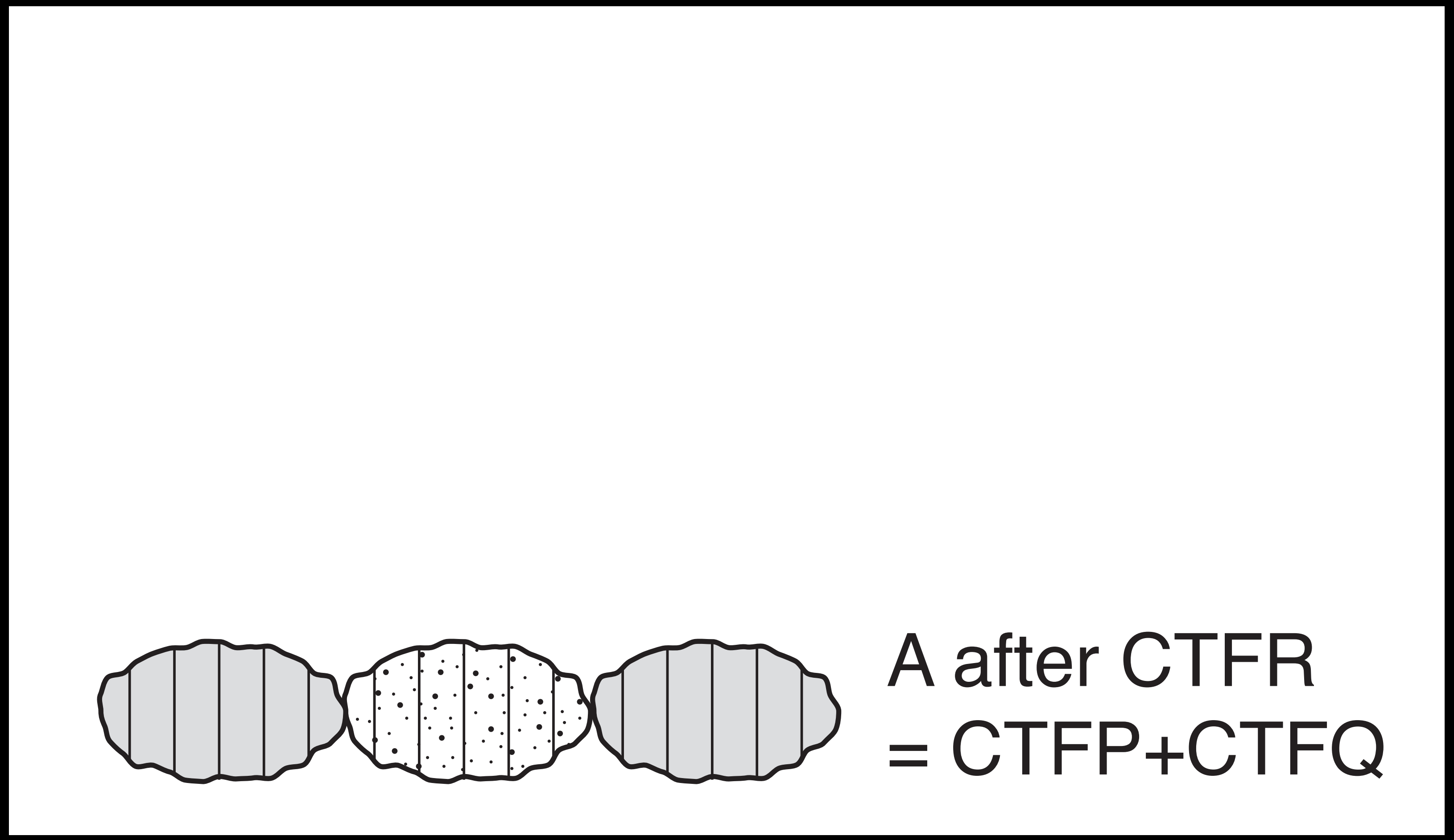


100 Å gold nanopartice in ice
300 keV

Russo & Henderson 2018c

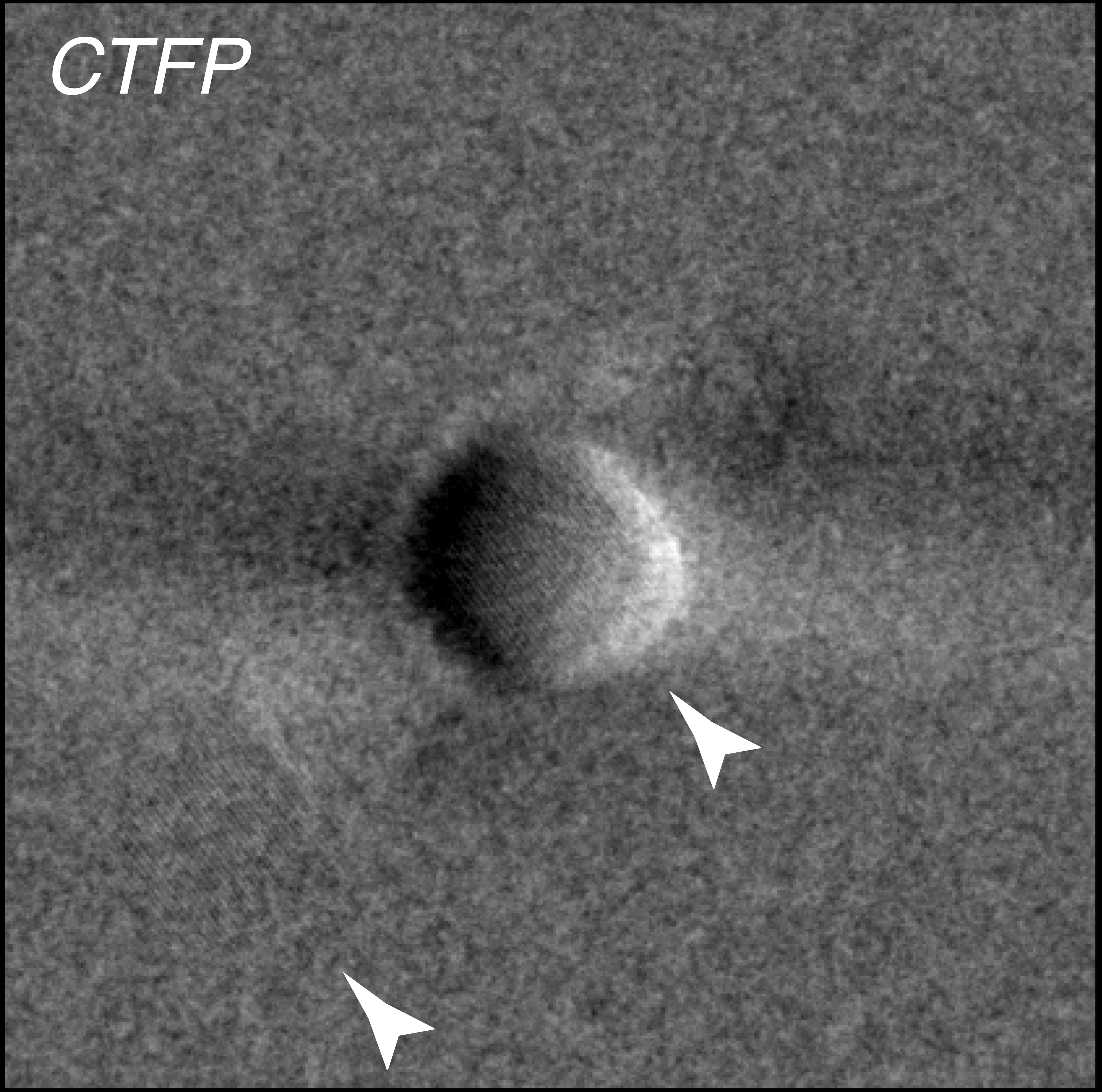


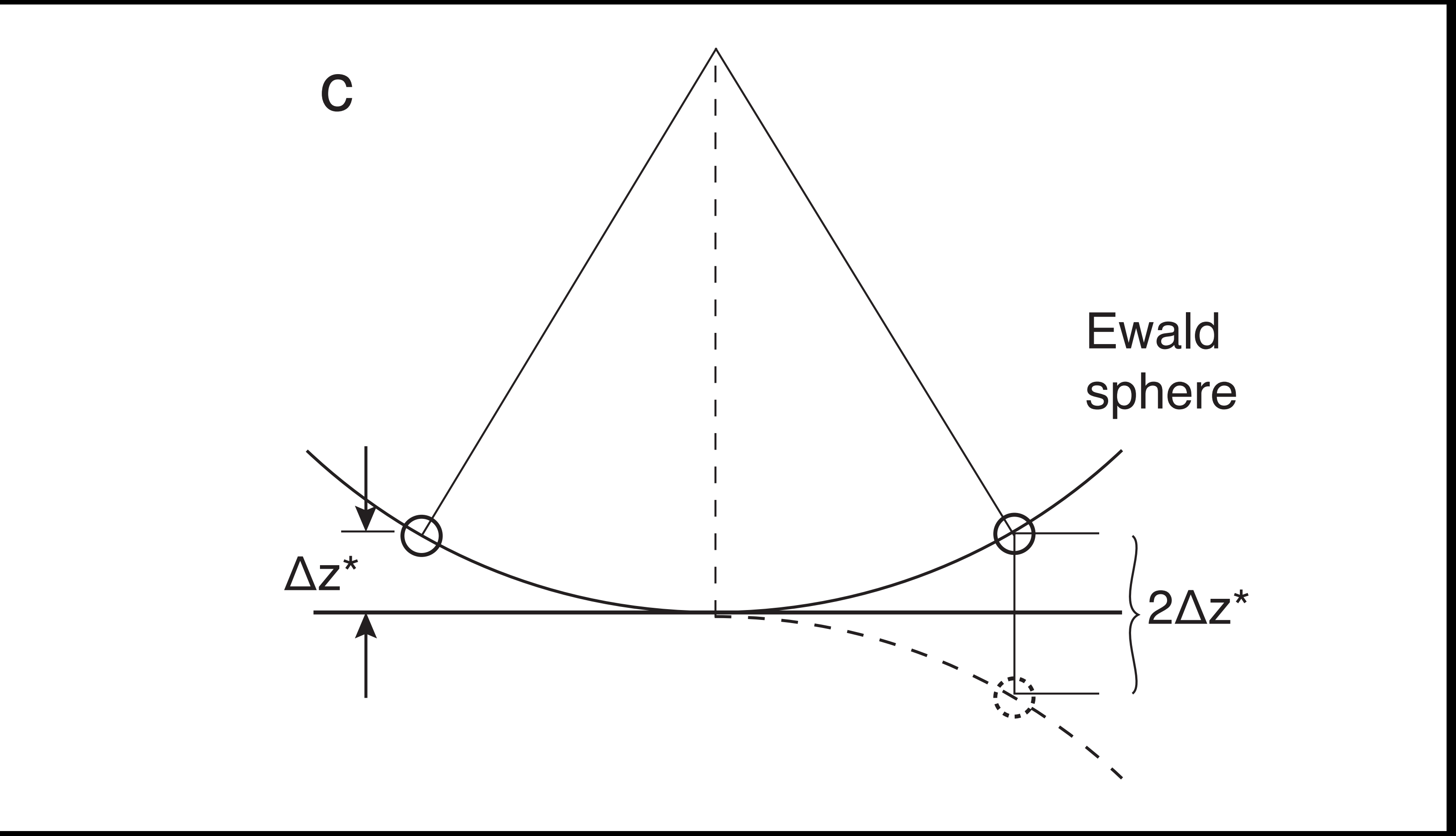


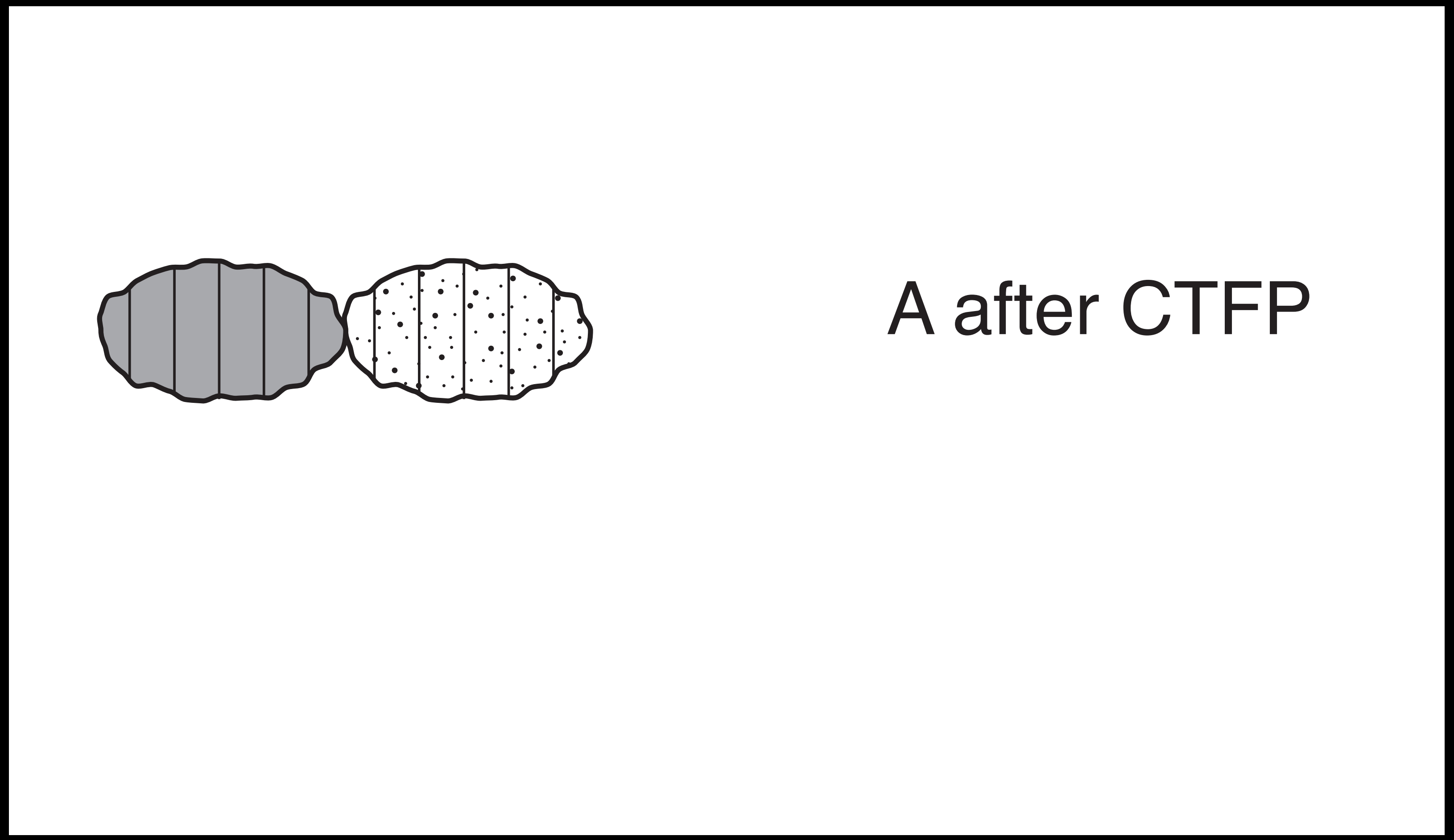


100 Å gold nanopartice in ice
300 keV

Russo & Henderson 2018c

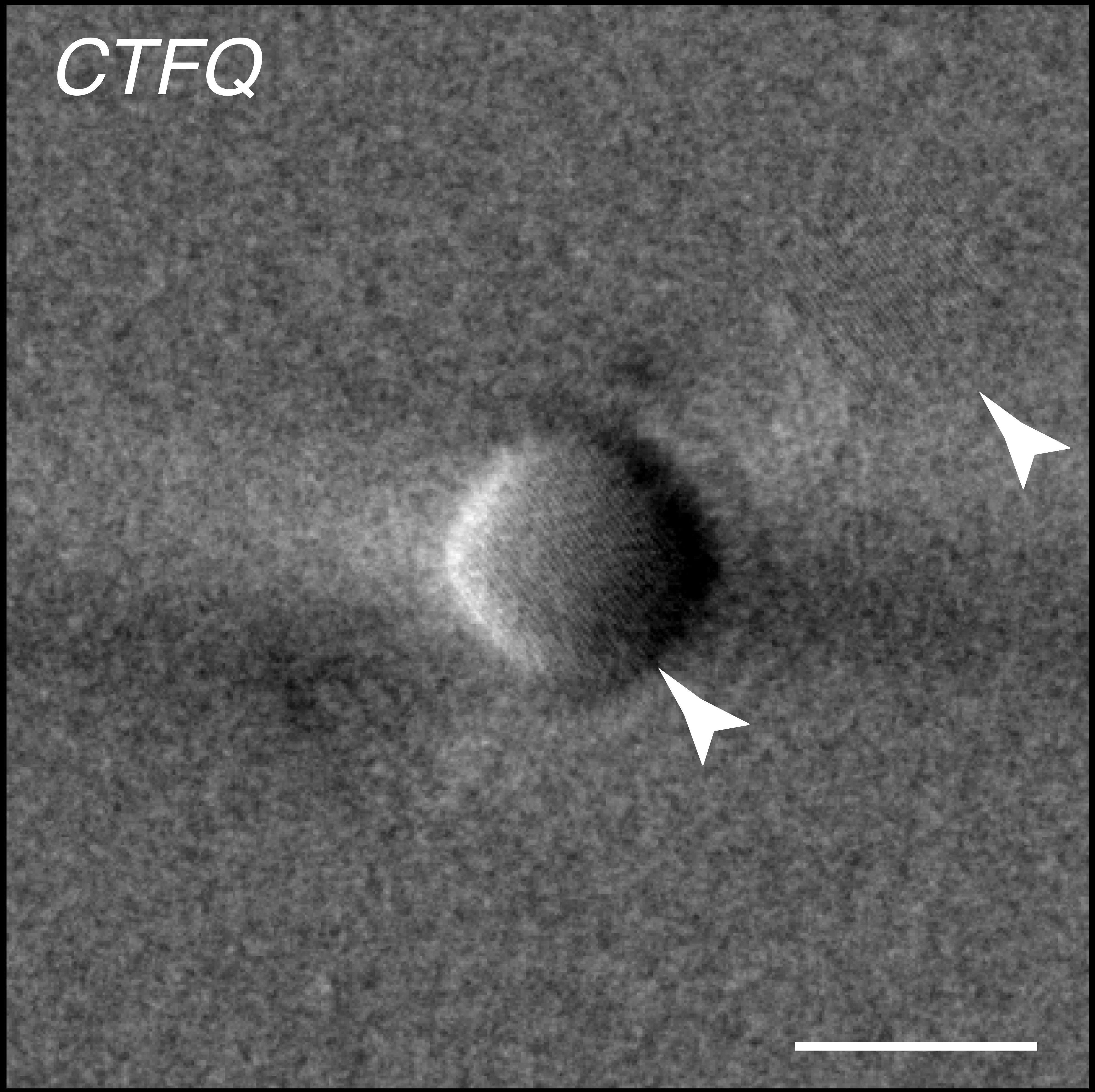


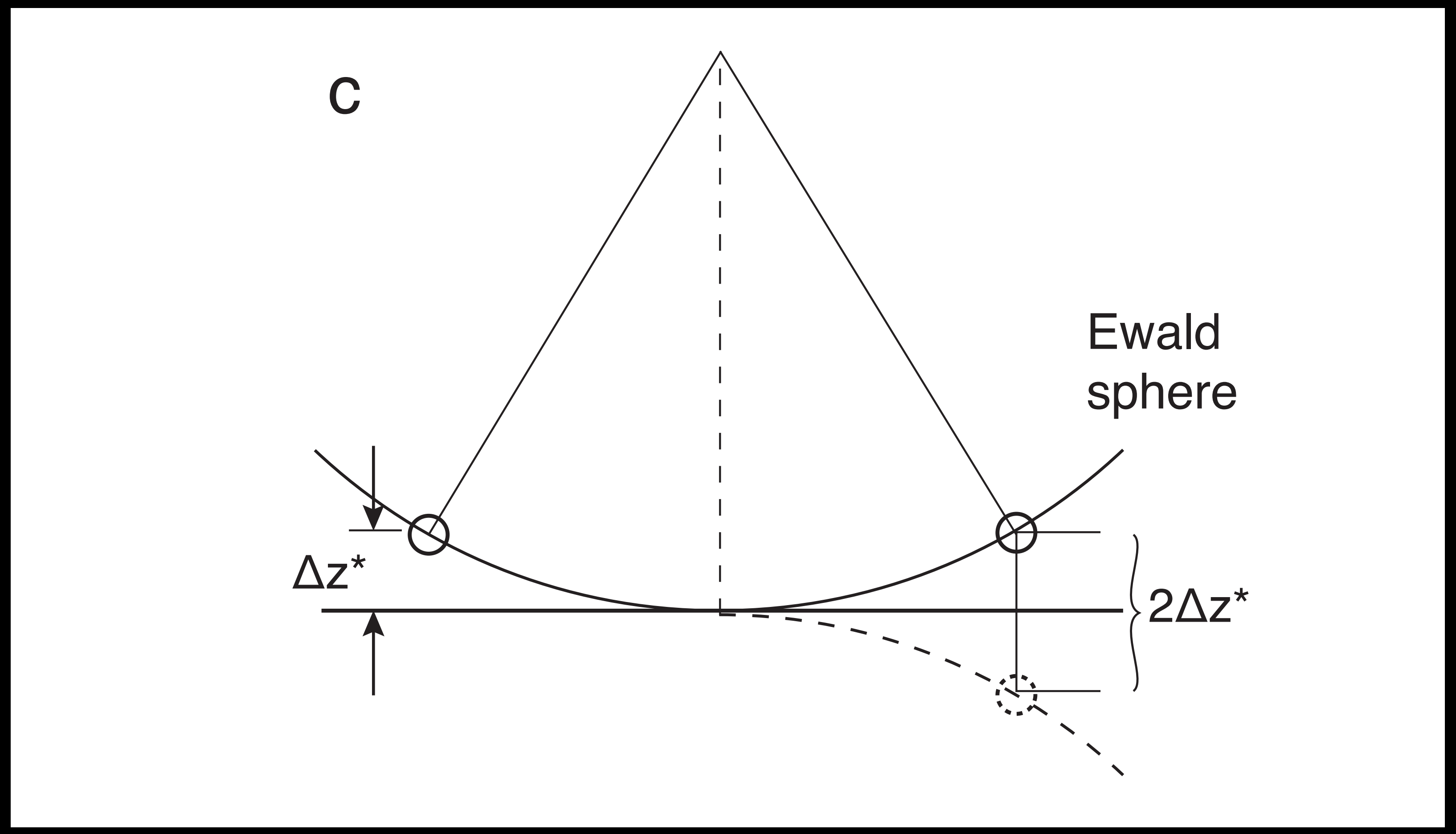


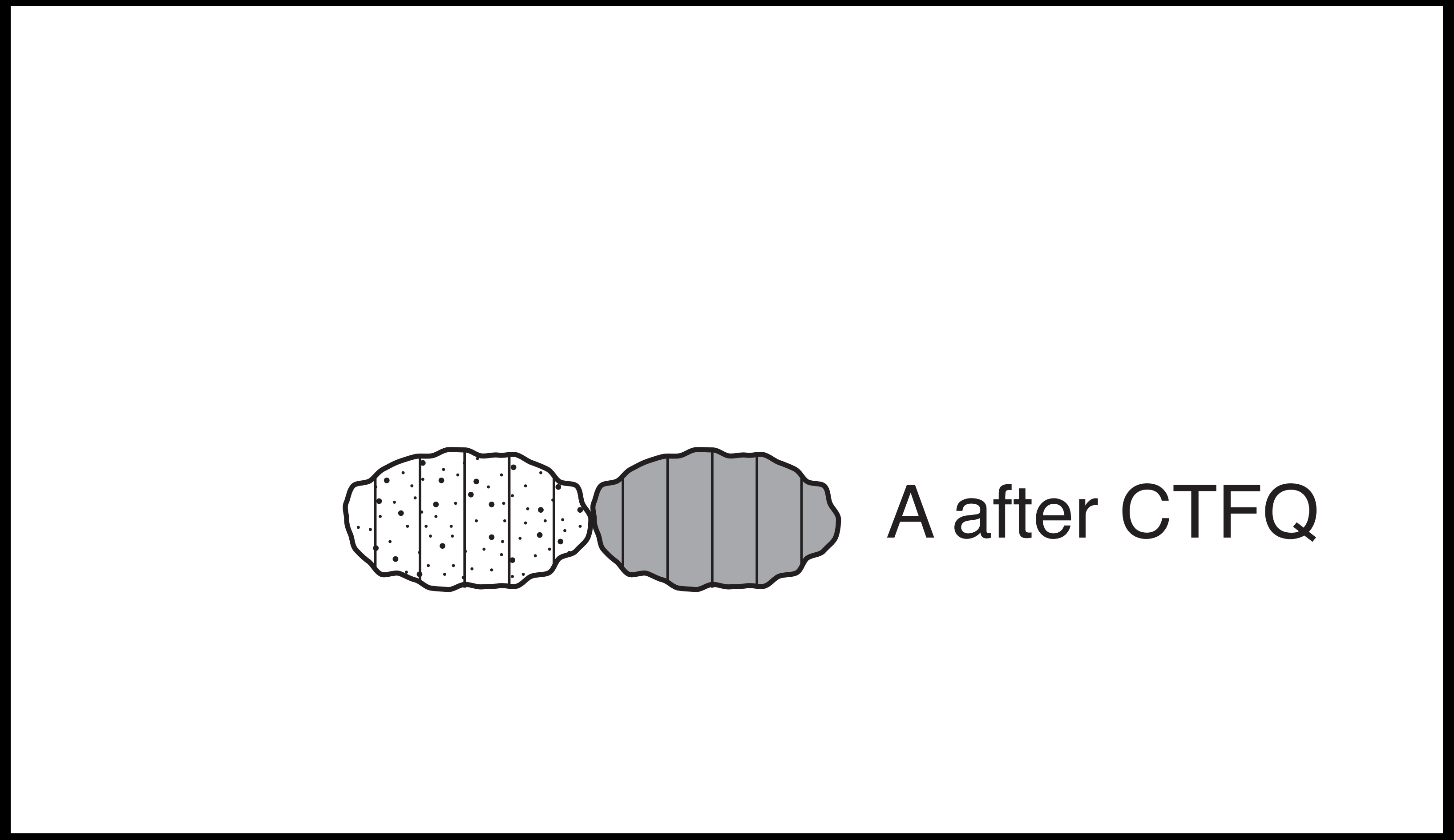


100 Å gold nanopartice in ice
300 keV

Russo & Henderson 2018c

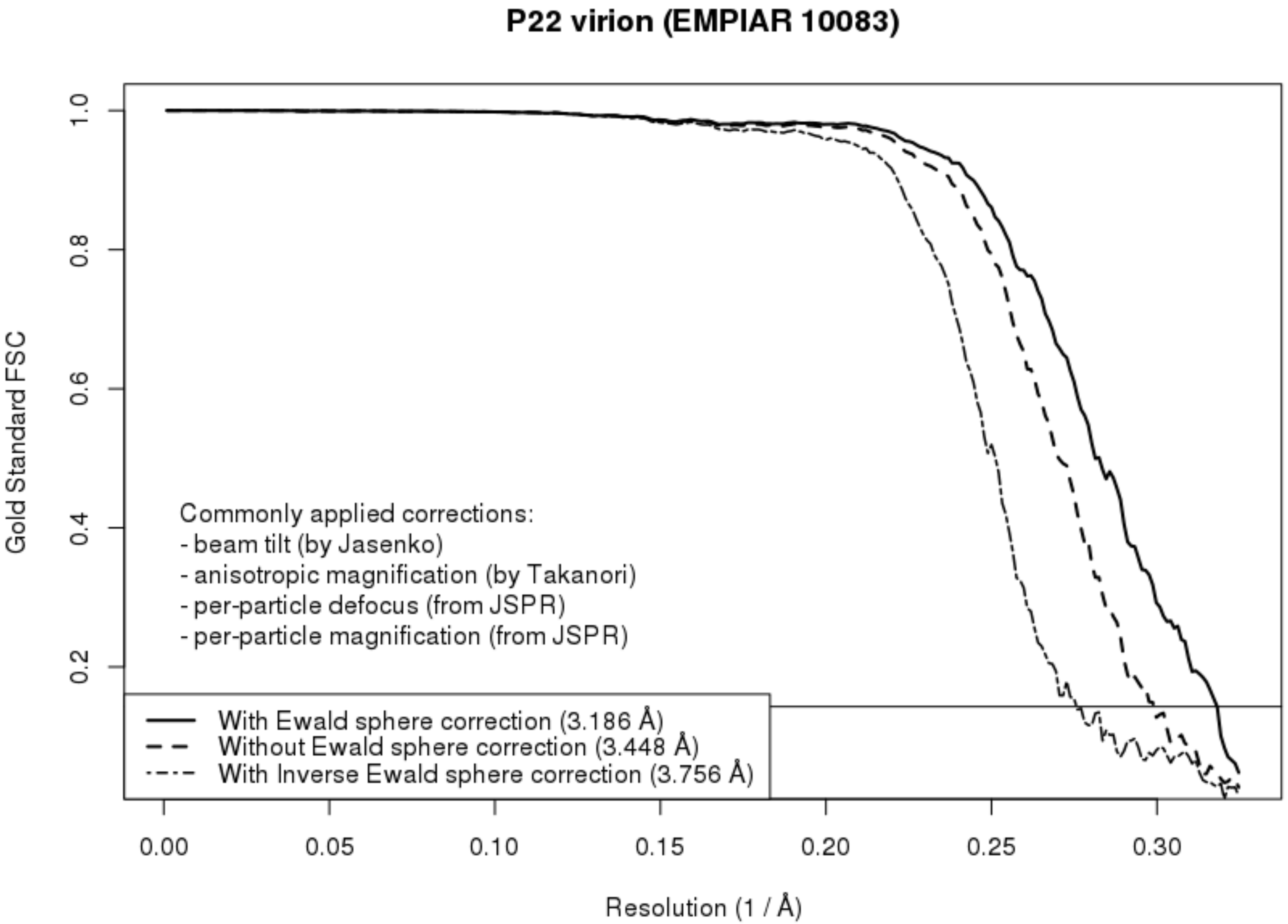


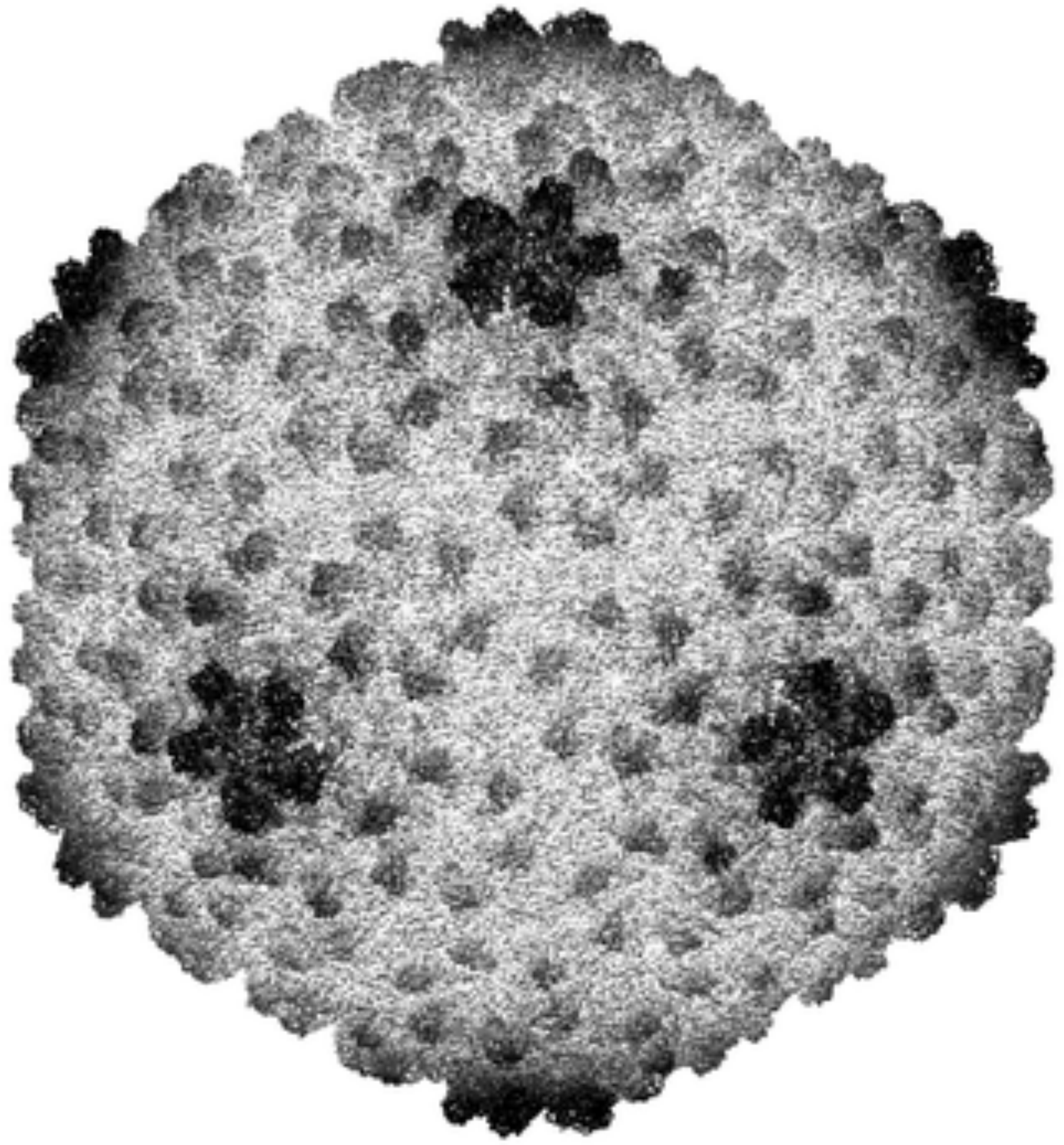




100 Å gold nanopartice in ice
300 keV

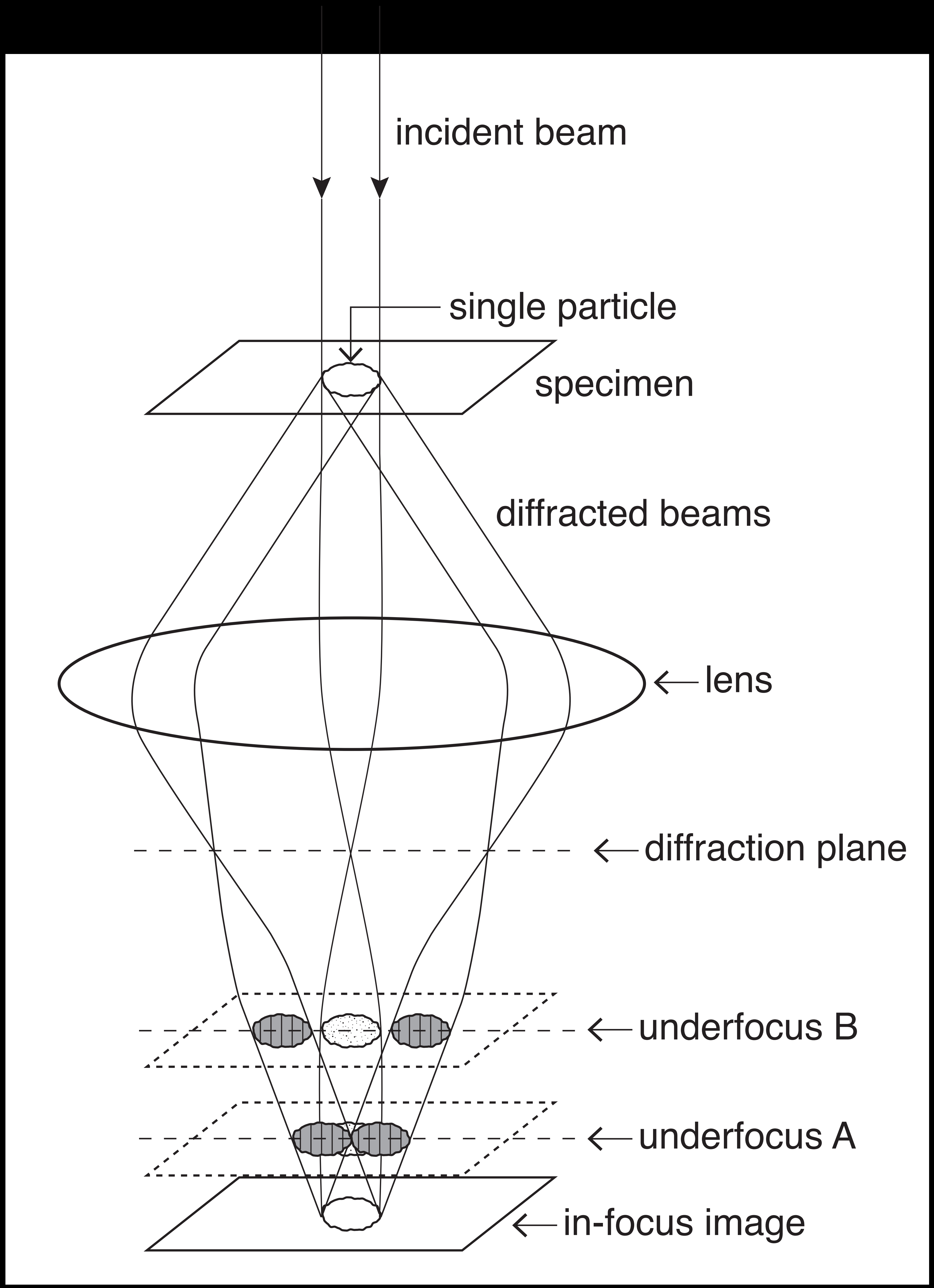
Russo & Henderson 2018c





P22 virus structure
EMPIAR-10083

Takanori Nakane
Sjors Scheres

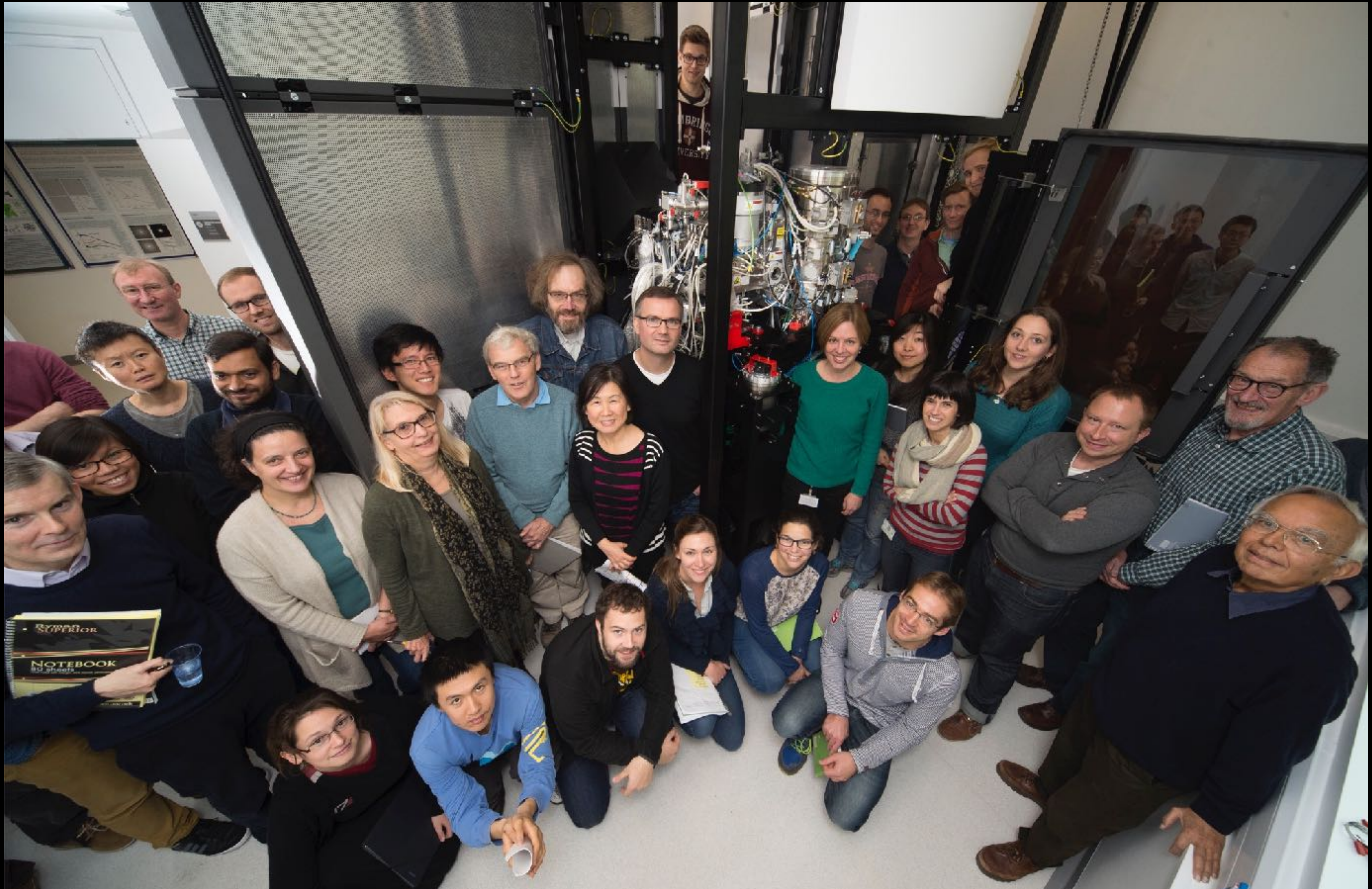


This correction method allows exact correction for Ewald sphere curvature in every micrograph

Collecting and processing images in this way will potentially improve the resolution of all cryoEM structures, particularly of those of large particles at high resolution

Perfect Ewald sphere correction will now allow the use of lower electron energies where the sphere is more curved without loss of high resolution information due to the incorrect assumption of a 2D projection

Thanks!



Greg McMullan
Wasi Faruqi
Shaoxia Chen
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Nigel Unwin
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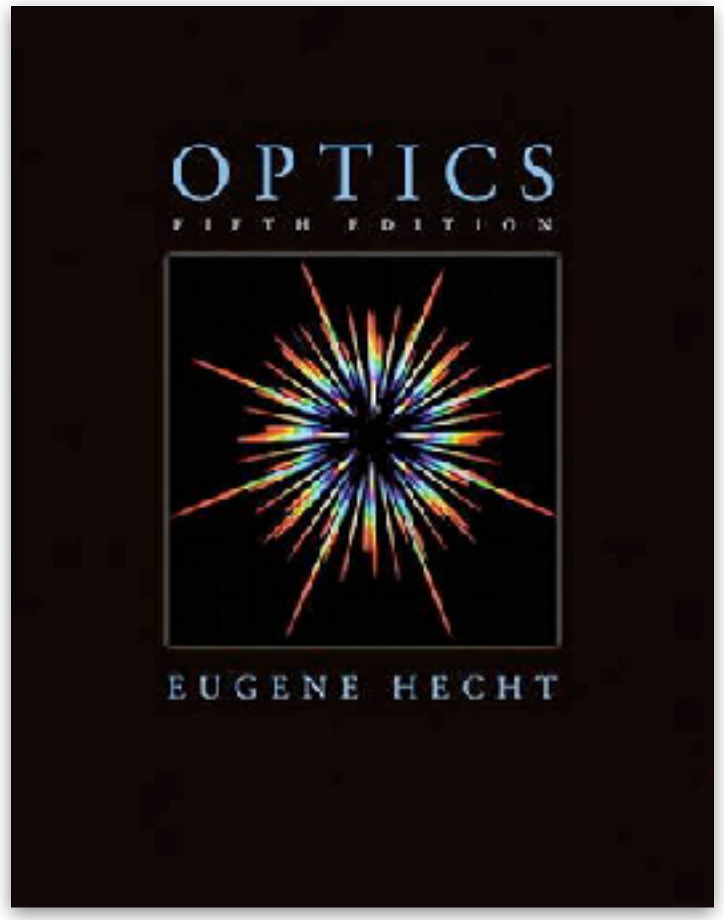
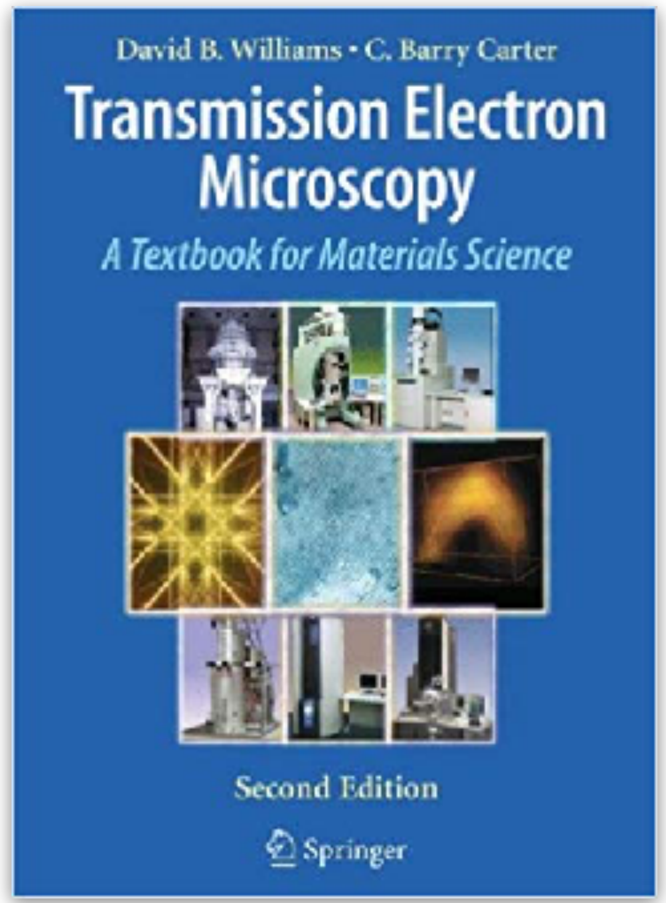
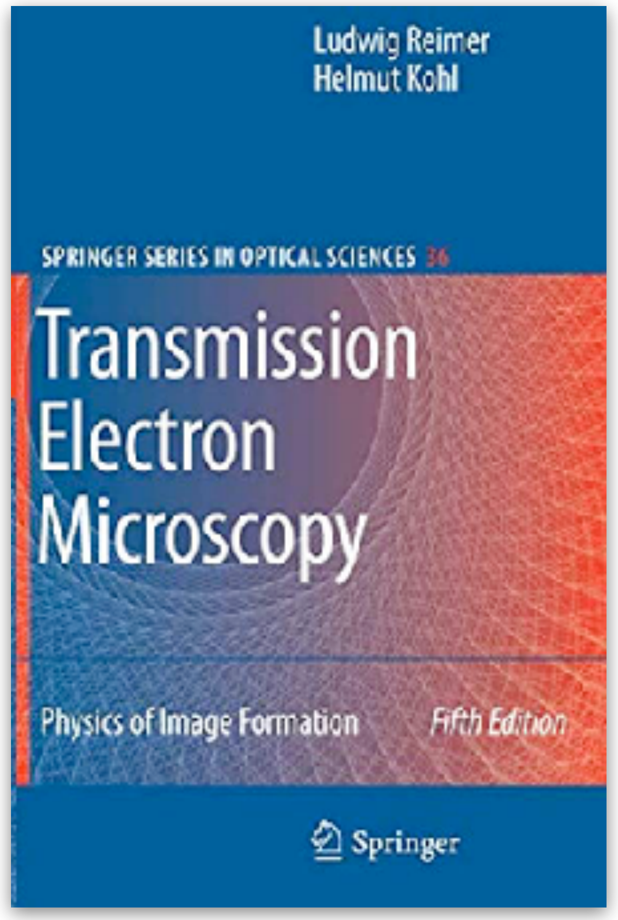
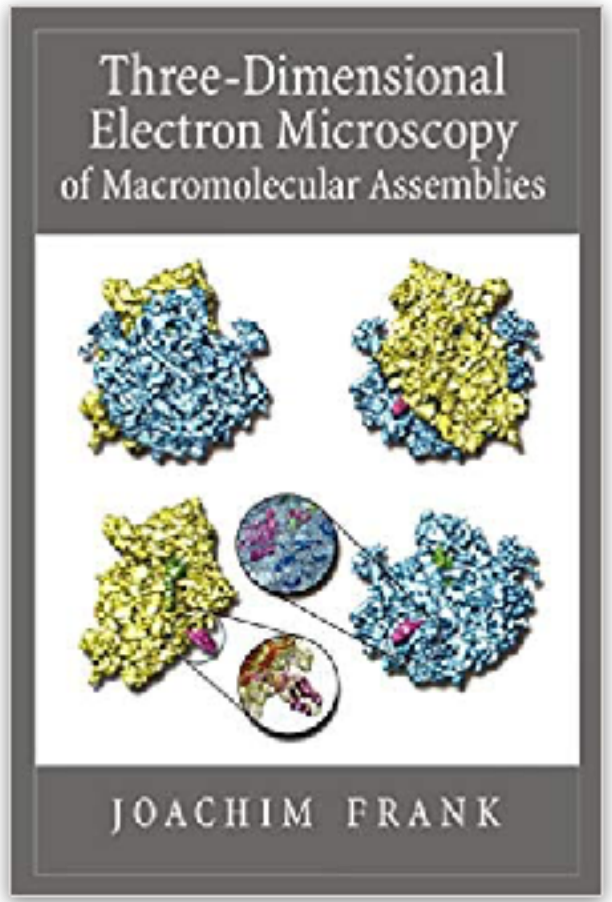
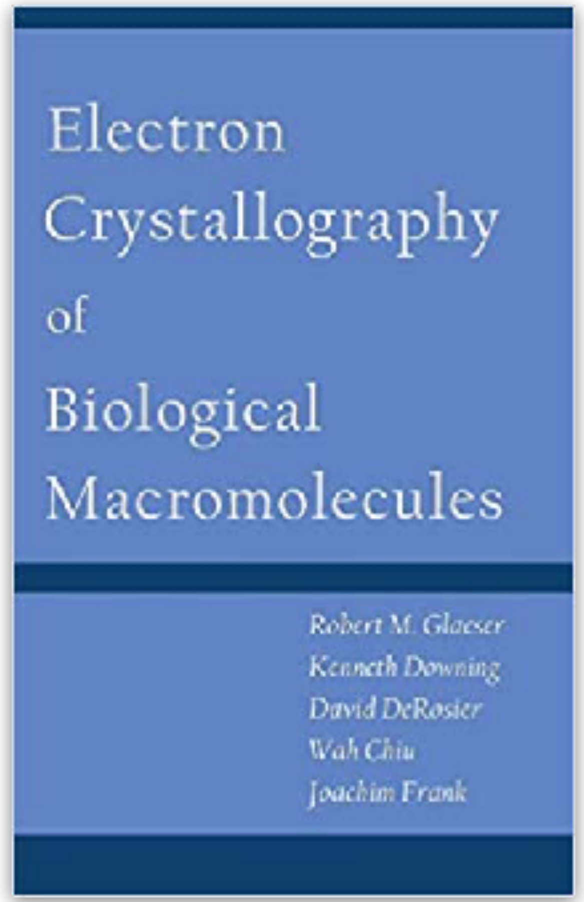
Some books you might want on your shelf

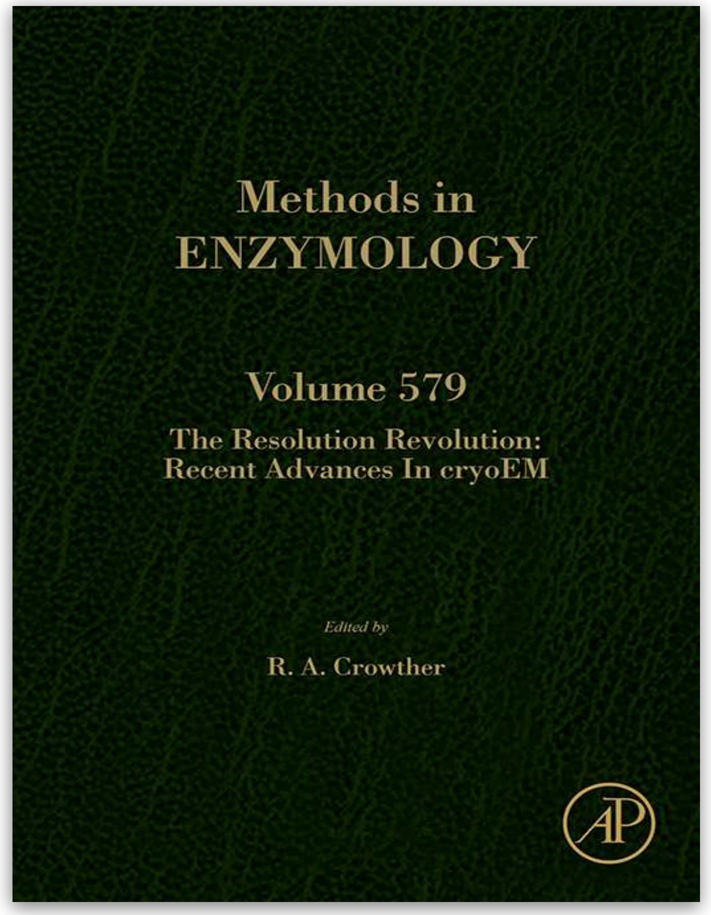












Hecht 3 - 5th ed.

Williams and Carter 2nd or 3rd ed.

Reimer and Kohl 2008

Frank 2006

Glaser et al. 2007

MIE Volume 579 - Ed. RA Crowther