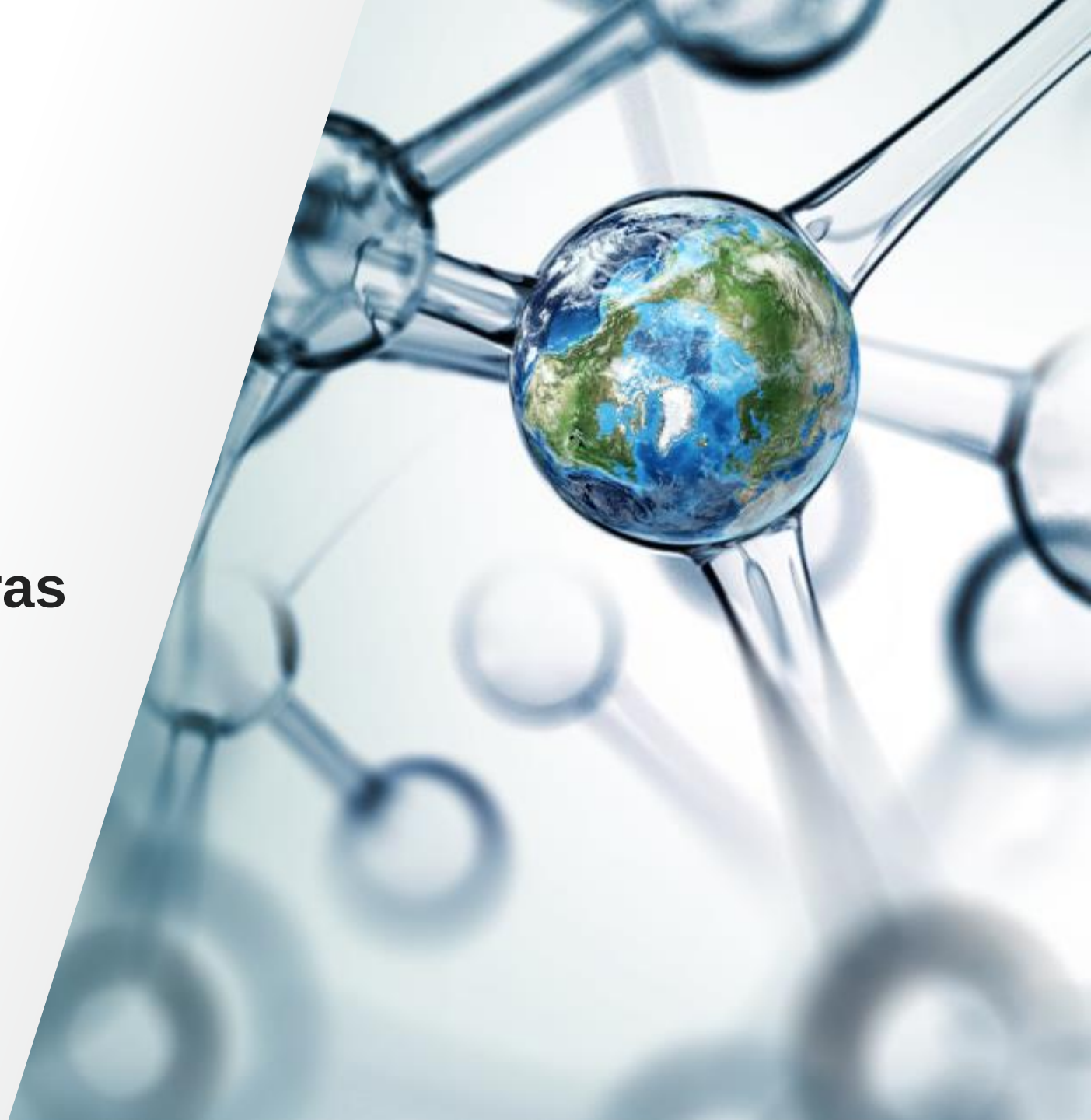
Electron microscopes & cameras

Wim Hagen



The world leader in serving science

Outline

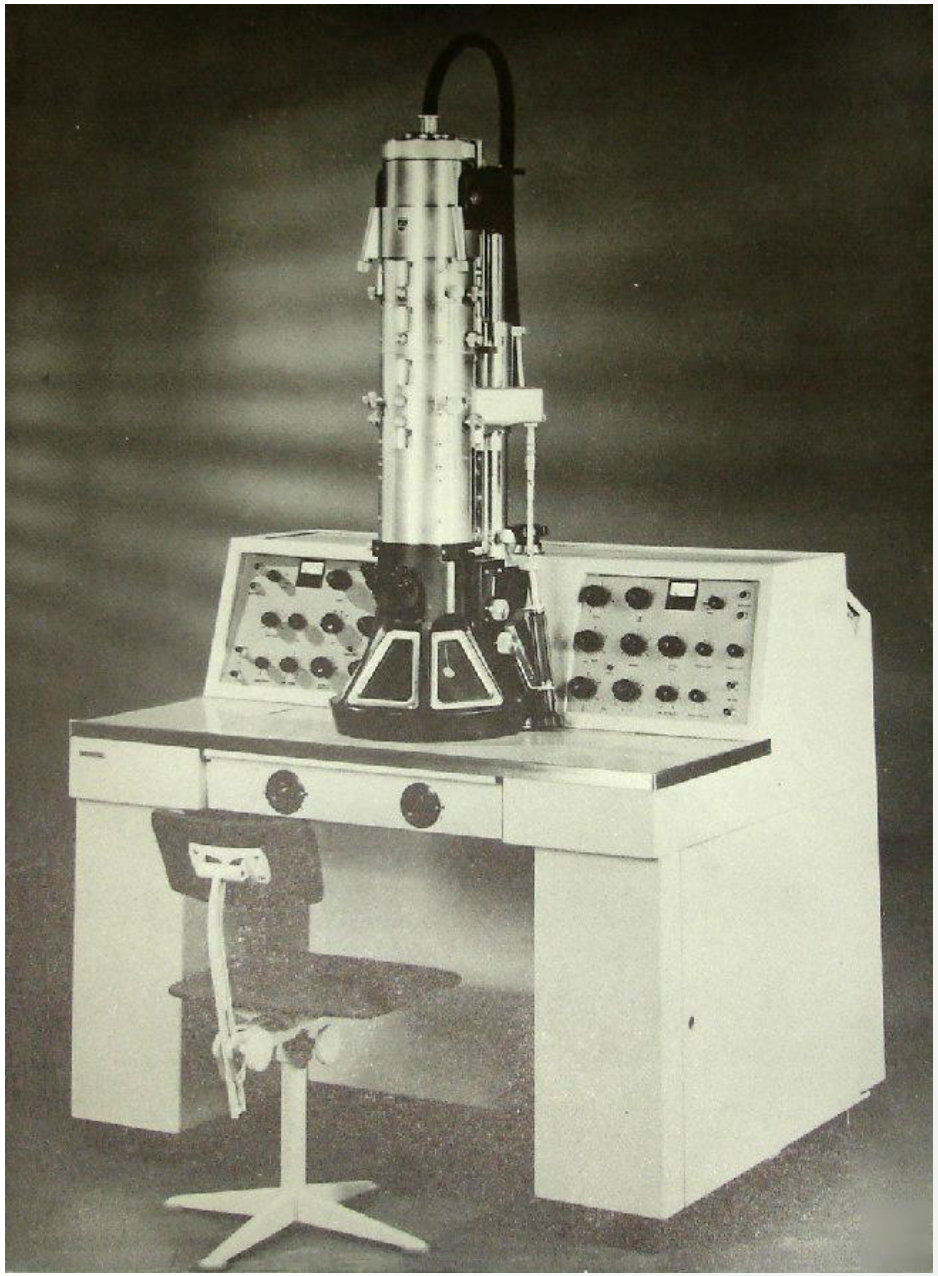
- Microscope basics.
- Optics.
- Cameras/detectors.
- What’s next?

Microscopes

Philips EM100
1949

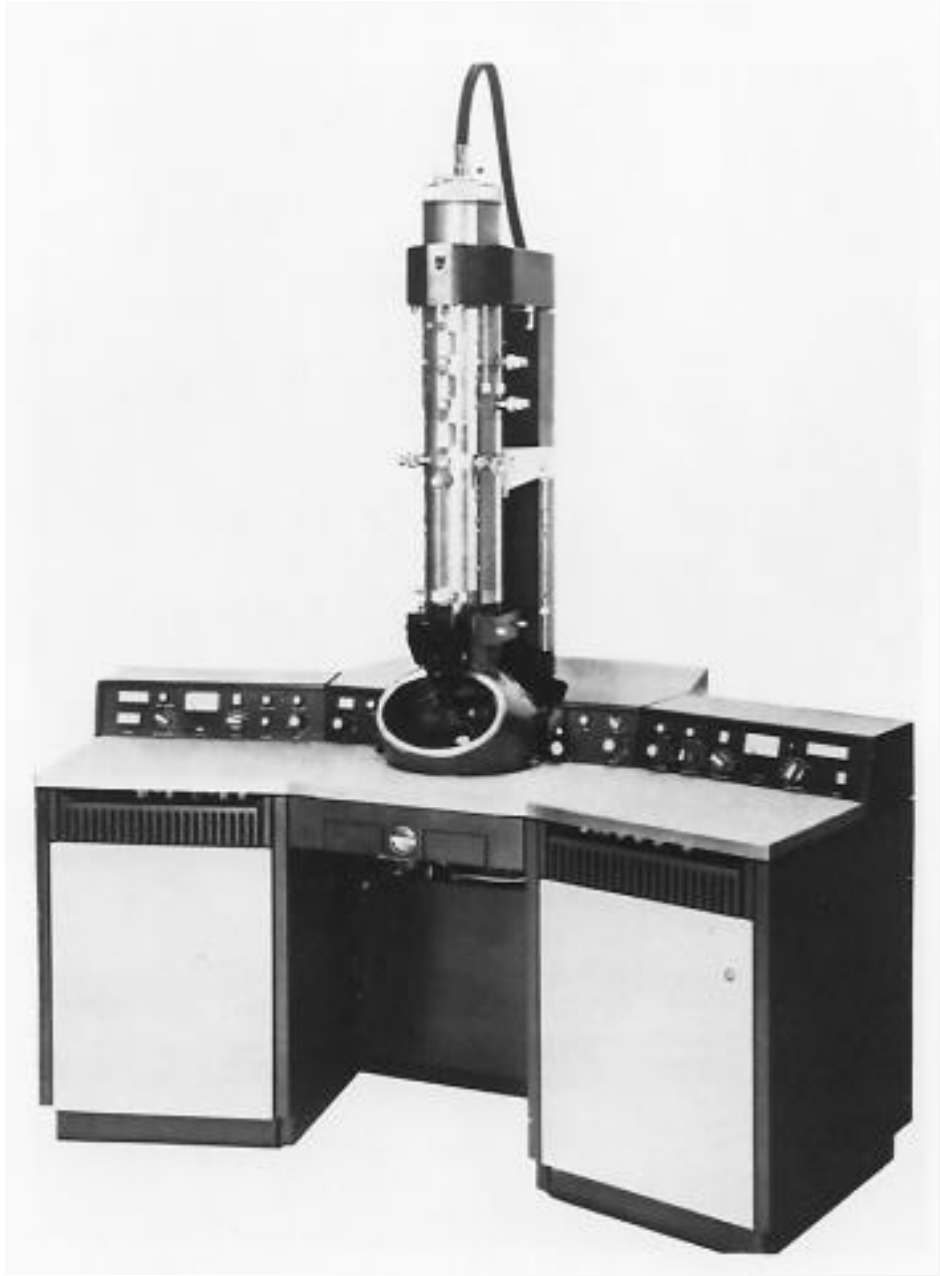
Microscopes

Philips EM200
60’s



Microscopes

Philips EM300,
70’s



Microscopes

Philips CM series
80’s, 90’s

Microscopes

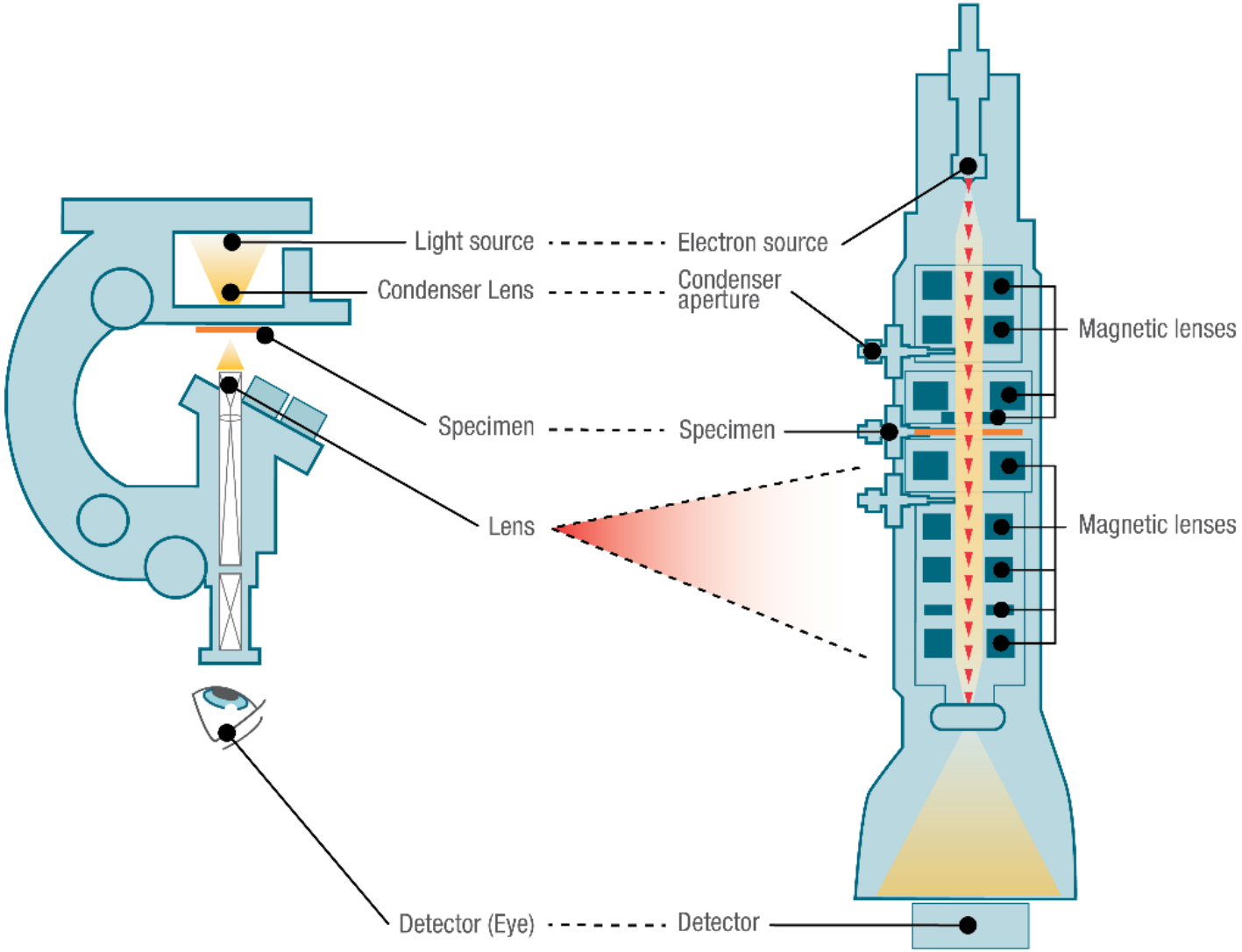
Philips Tecnai series
Late 90’s

Microscopes

FEI Titan series,
Since 2004.

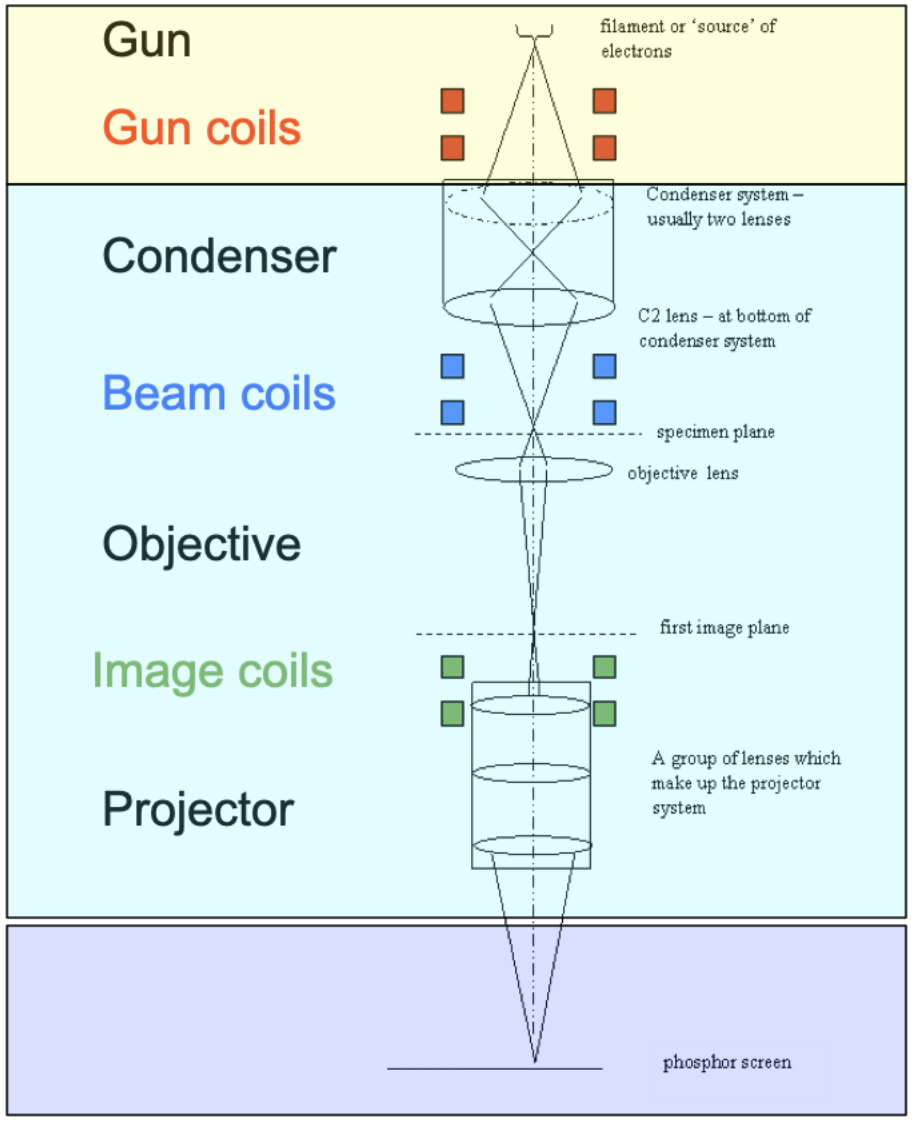
Easy usage for all skill levels!

Microscope basics



- Source.
- Condenser.
- Objective (→ specimen).
- Projector.
- Energy filter.
- Detector.

Microscope basics vacuum

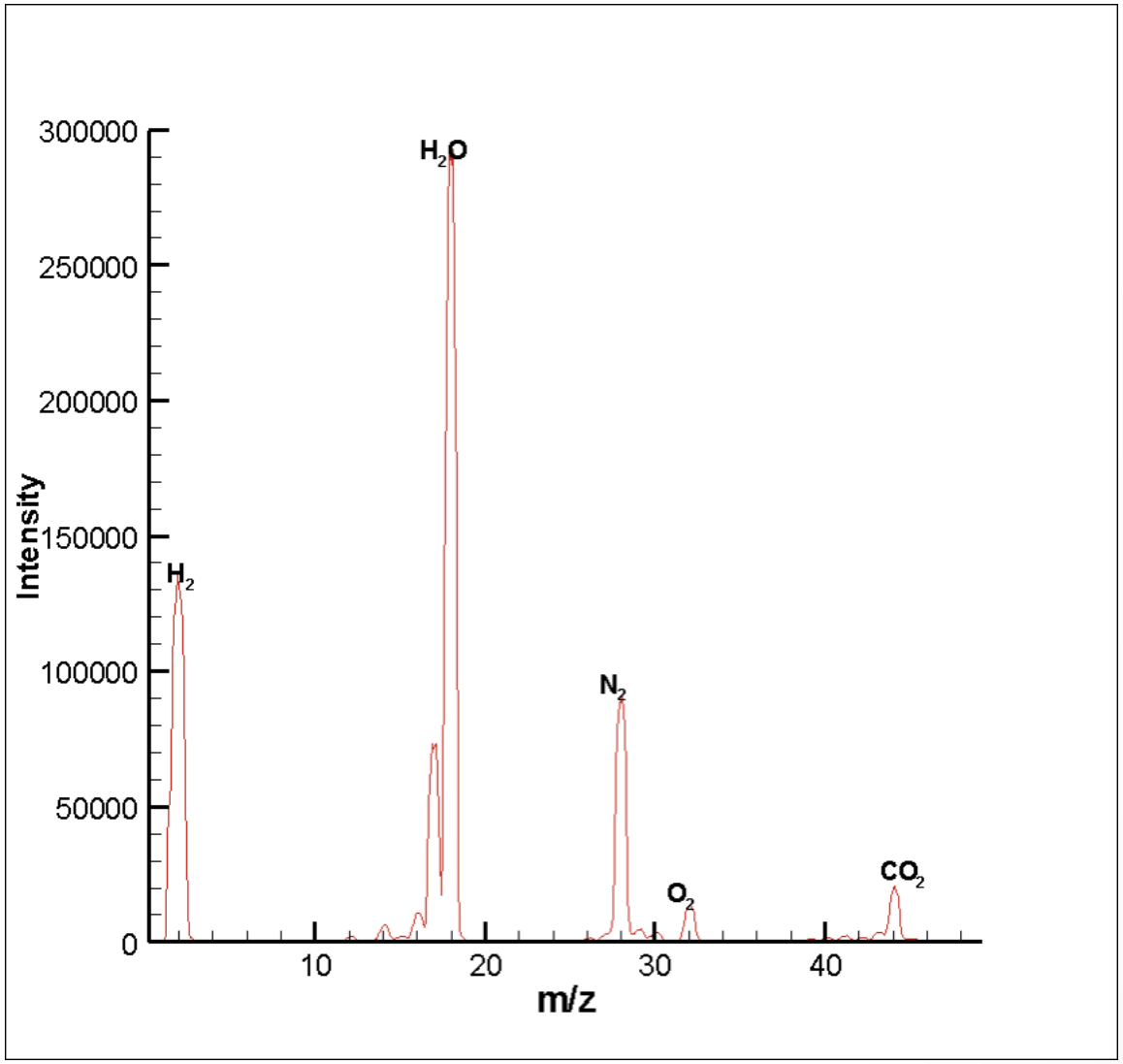


Field **E**mission **G**un < 10e-6 Pa
Thermionic gun ~10e-5 Pa

Column
~10e-5 Pa

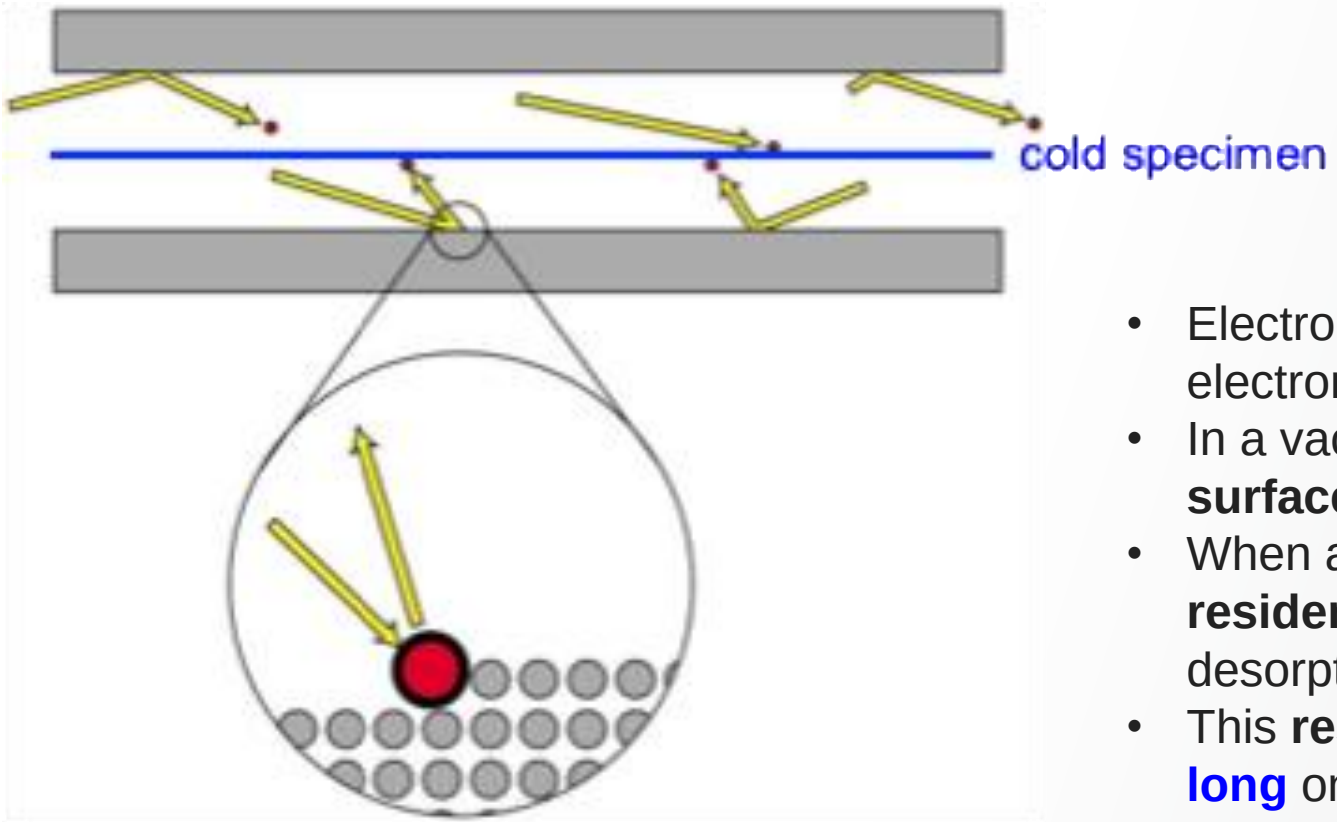
Projection chamber
~10e-3 Pa

Microscope basics vacuum



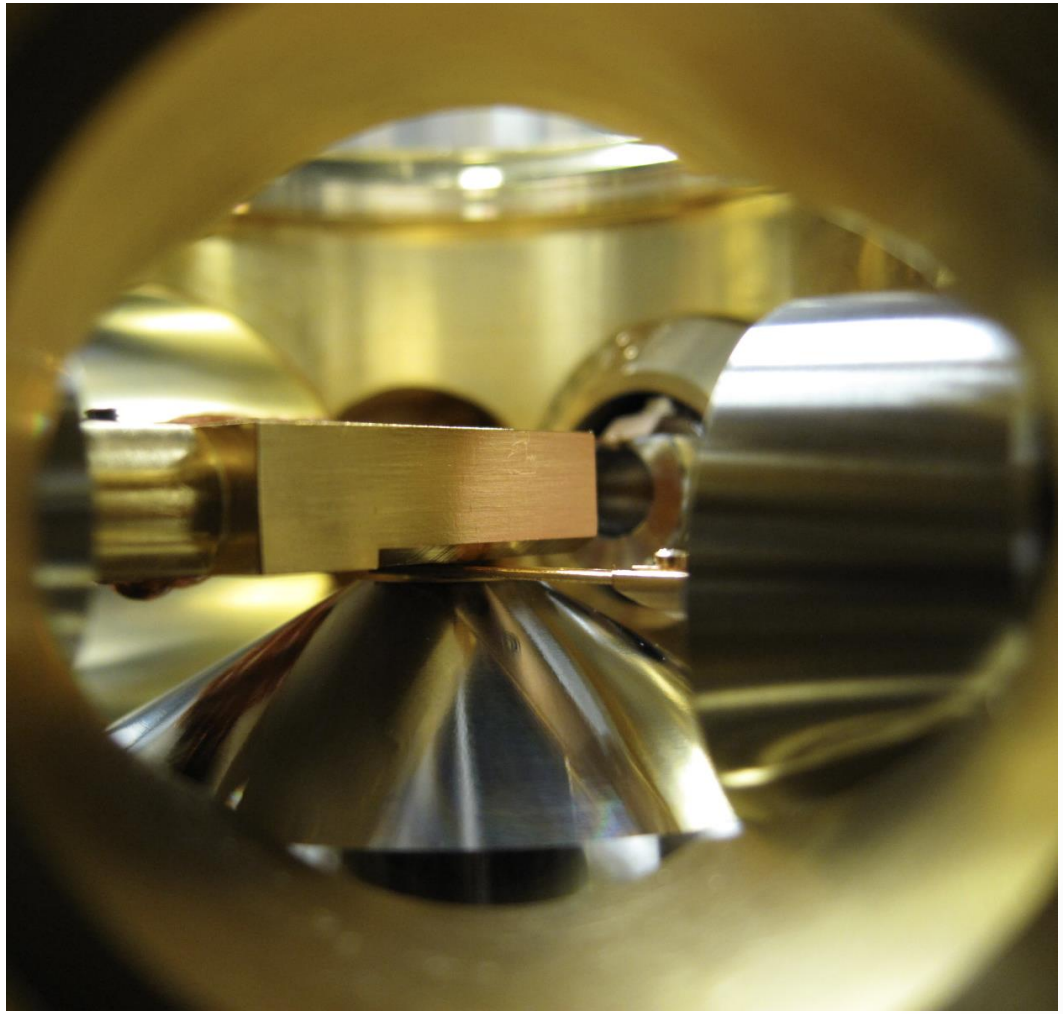
Microscope basics cryo shields

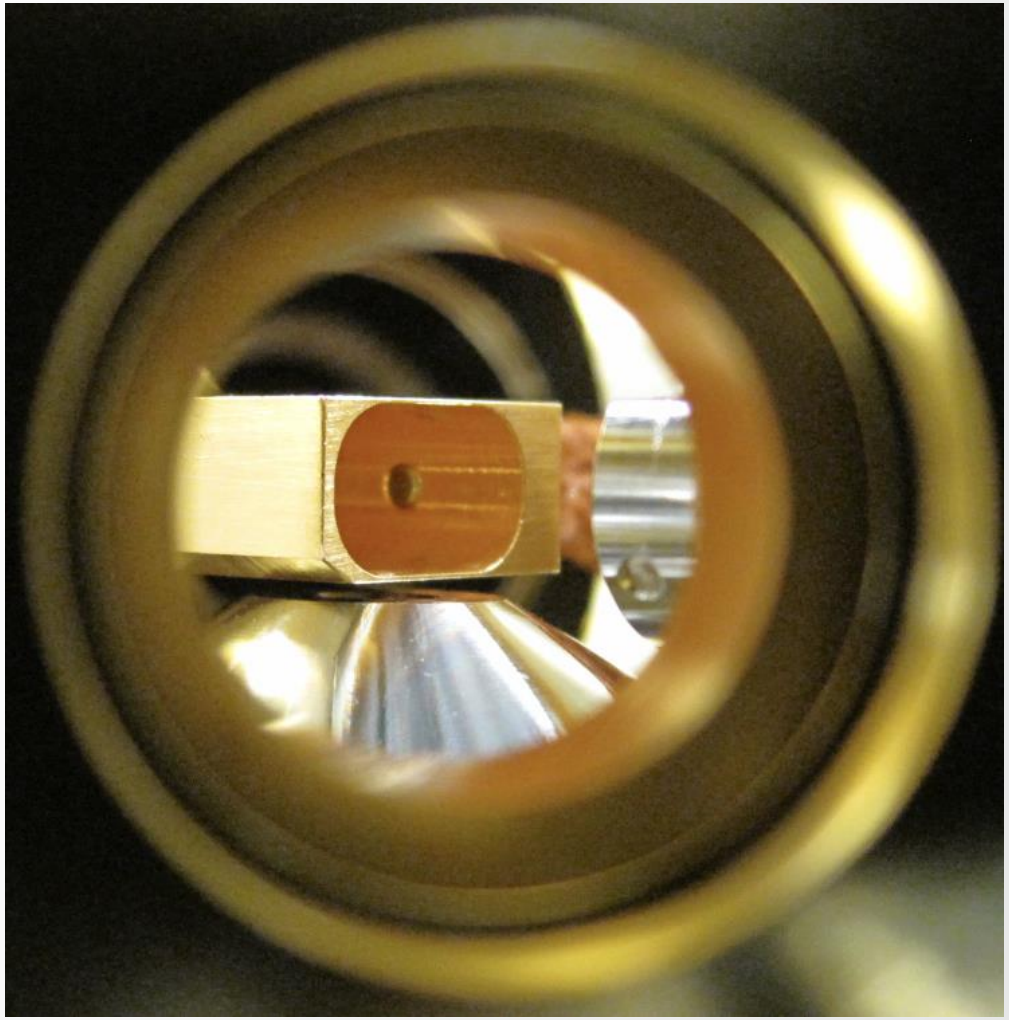
- Cryo-shielding improves vacuum quality around the specimen by at least a factor 10:
- Protects a cryo-specimen. From ice contamination build-up.
- Improves local thermal equilibrium which lowers specimen drift.



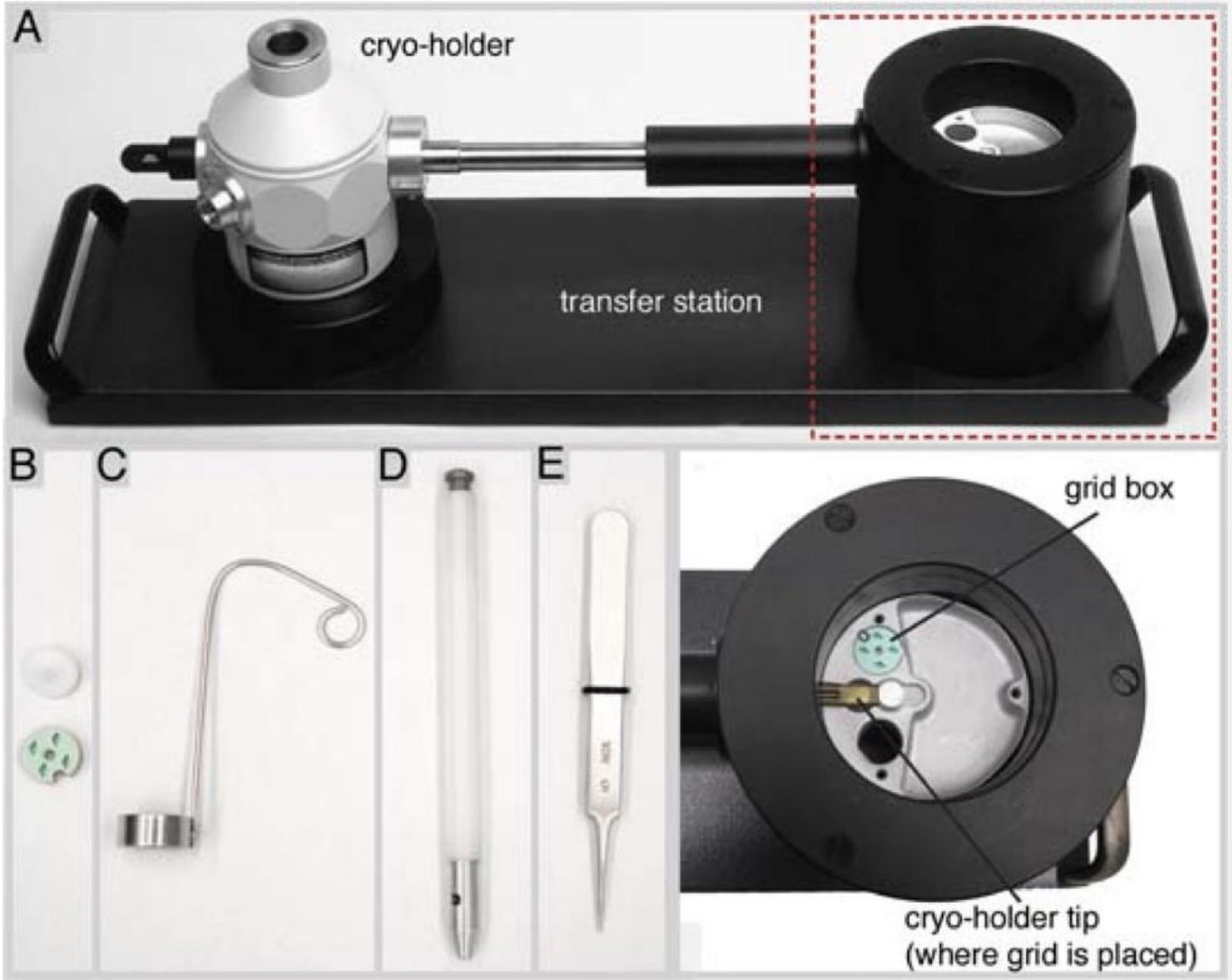
- Electron microscopes need vacuum to minimize electron interaction other then with the sample.
- In a vacuum we have **molecules colliding with surfaces** (molecular flow).
- When adsorption on a surface occurs, there is a **residence time** (time on the surface) and an eventual desorption of the molecules.
- This **residence time** is short on **heated surfaces** and **long** on **cold surfaces**.

Microscope basics cryo-shields



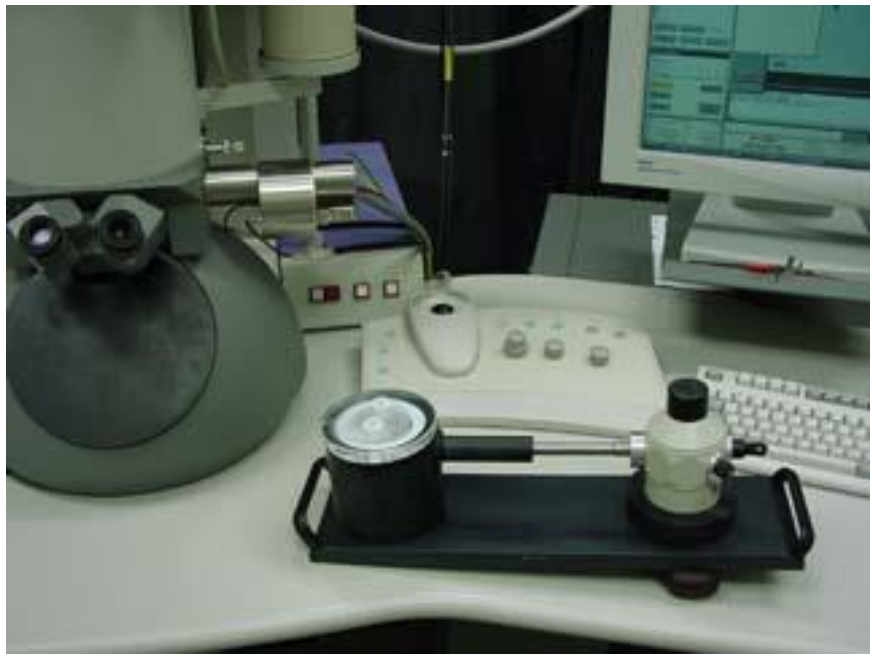


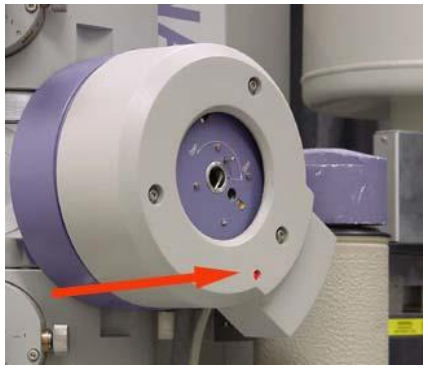
Microscope basics side-entry cryo-holder

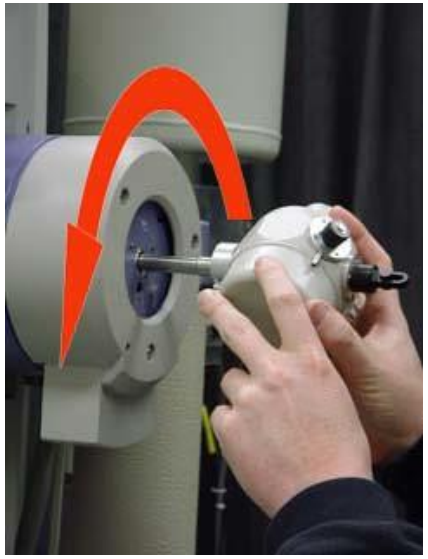


Cryo-Electron Tomography for Structural Characterization of Macromolecular Complexes, ulia Cope, John Heumann, Andreas Hoenger, Current Protocols in Protein Science, 2011

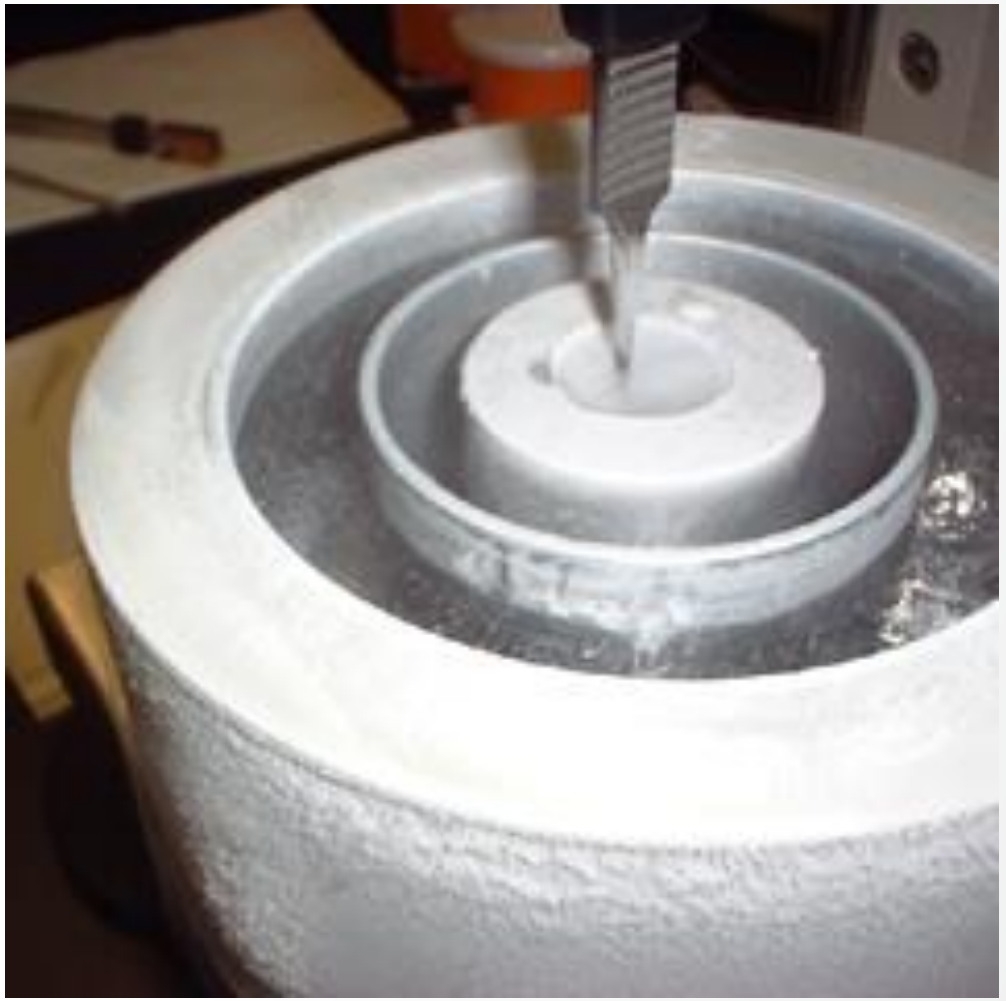
Microscope basics side-entry cryo-holder





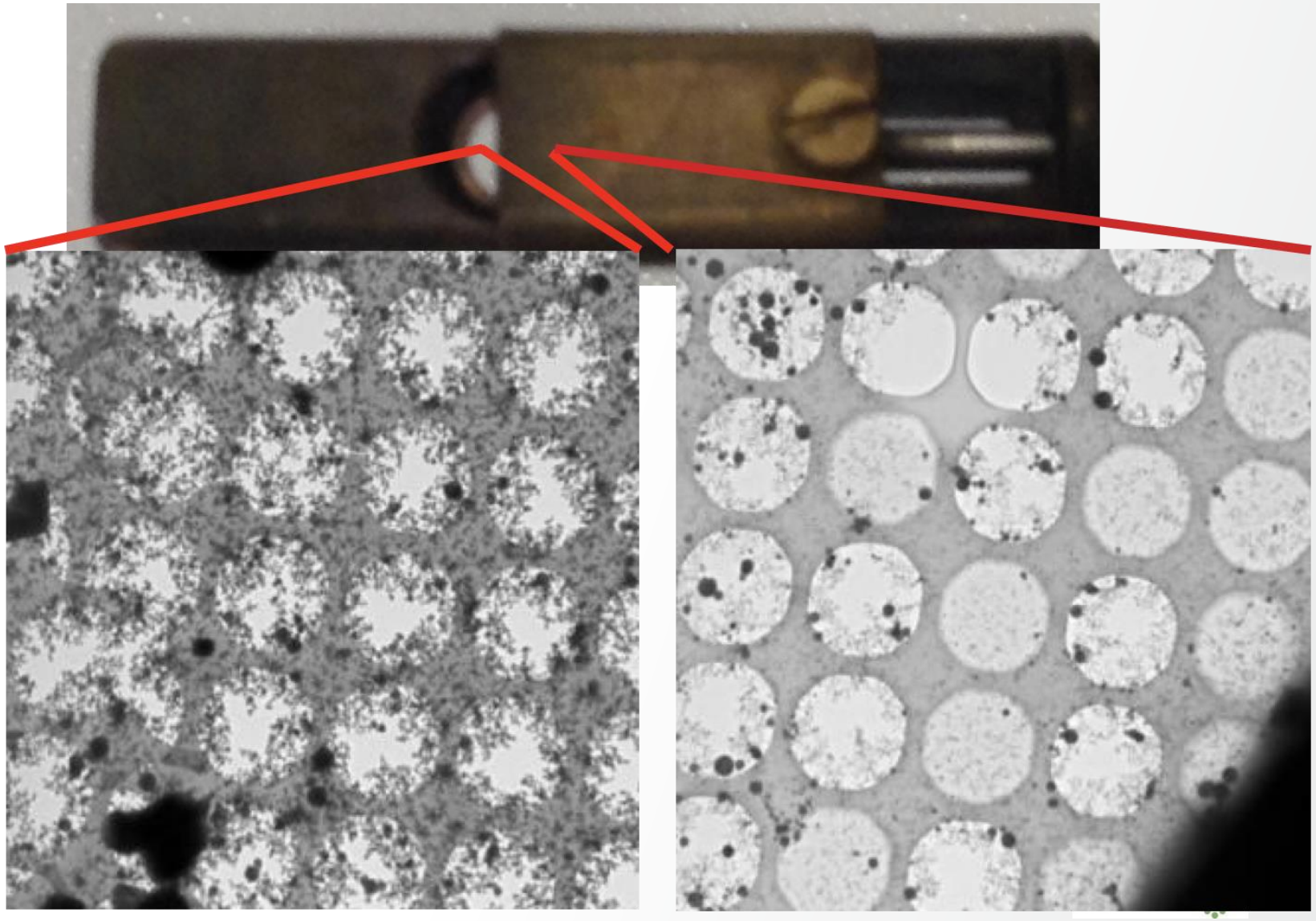


Microscope basics side-entry cryo-holder ethane

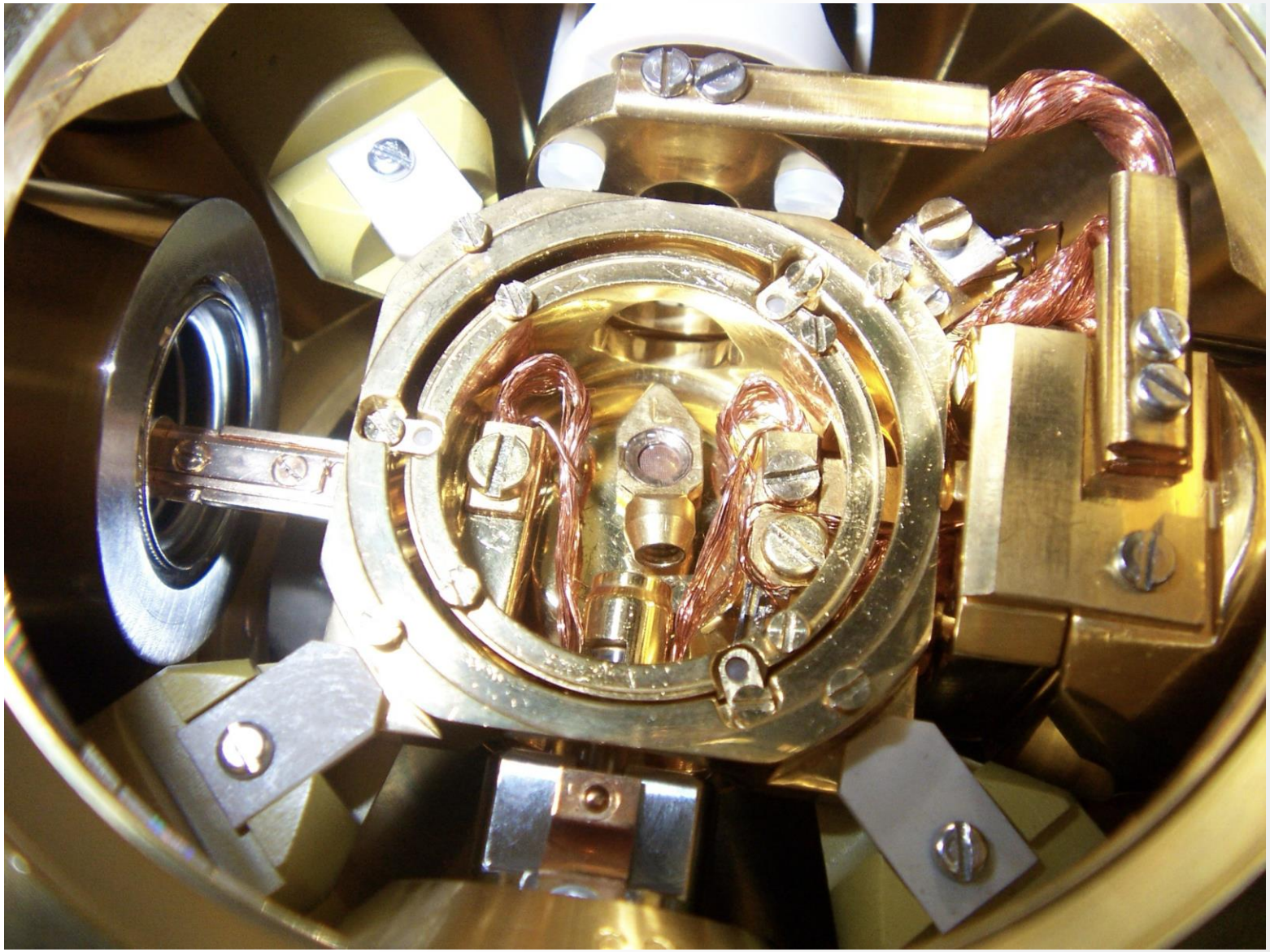


http://www.snaggledworks.com/em_for_dummies/freeze.html

Microscope basics side-entry cryo-holder



Microscope basics cryo-shields



Microscope basics column

Gun

Gun coils

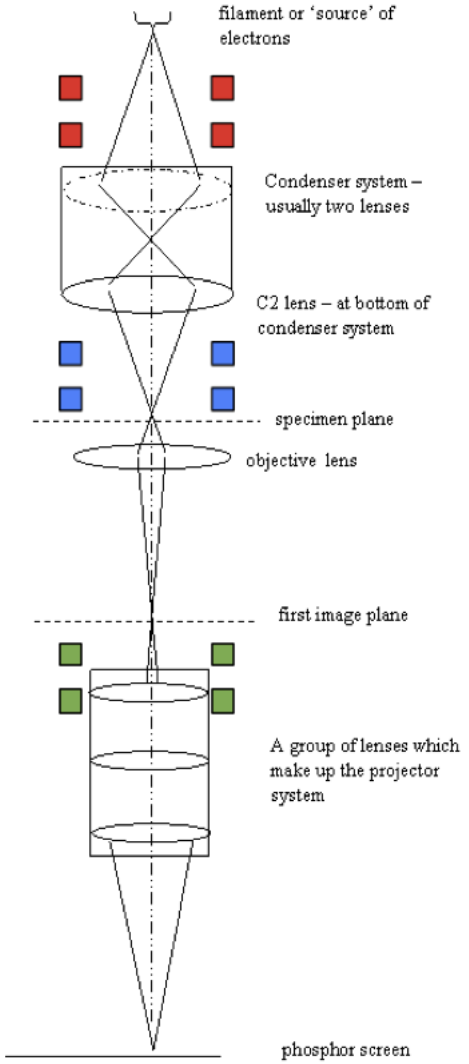
Condenser

Beam coils

Objective

Image coils

Projector



Accelerator and Condenser 1 lens (spot size):
The electron point source (0.1 -10 microns) is magnified to several hundreds of microns, illuminating condenser 2 aperture which can be ~10 to ~150 microns in size.

Condenser 2 aperture/condenser 2 lens:
De-magnify image of the condenser aperture to a beam size of a few microns.

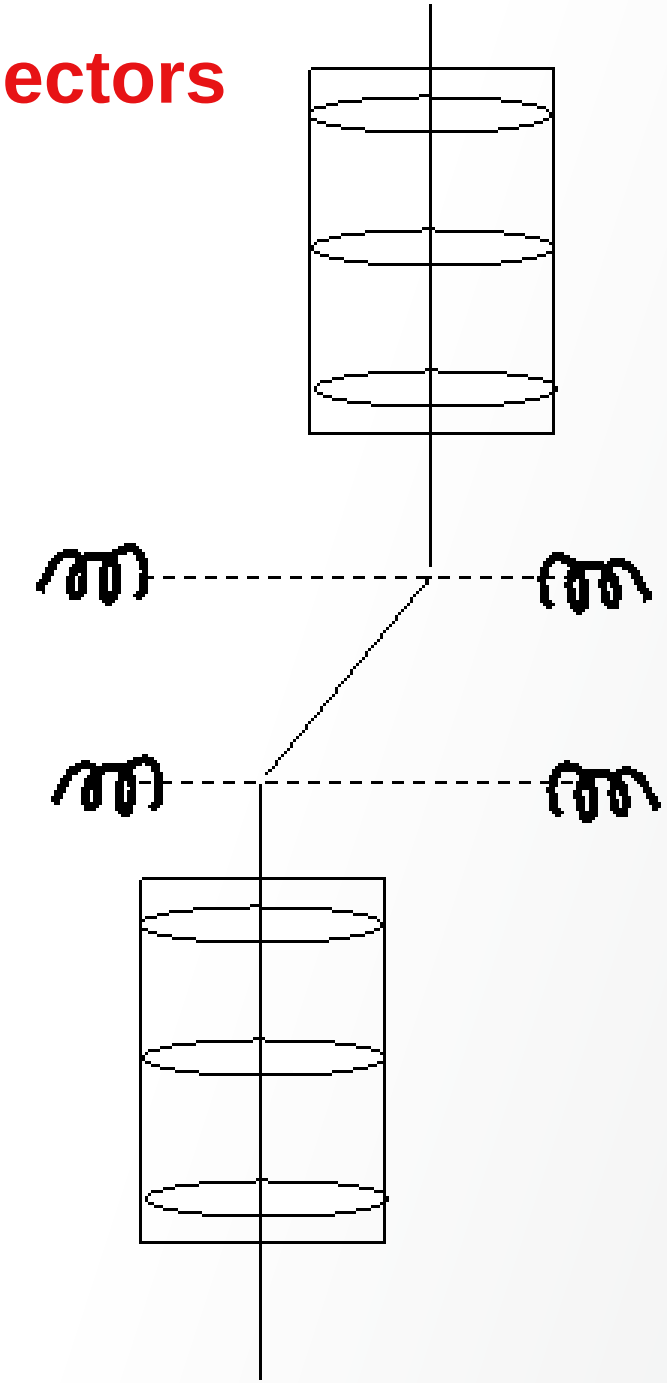
Objective Twin lens:
Illuminate & magnify specimen.

Projection system (4 lenses):
Project/magnify image or diffraction pattern.

Detect image/diffraction pattern.

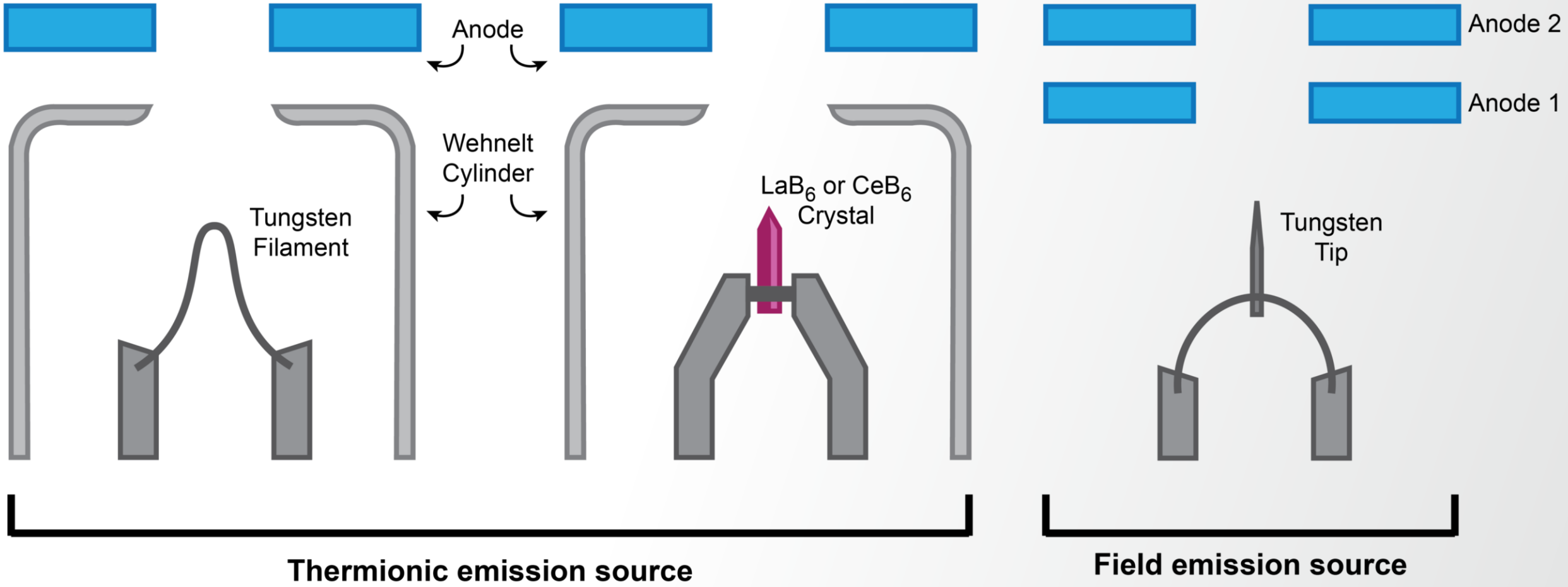
http://www.rodenburg.org/

Microscope basics deflectors

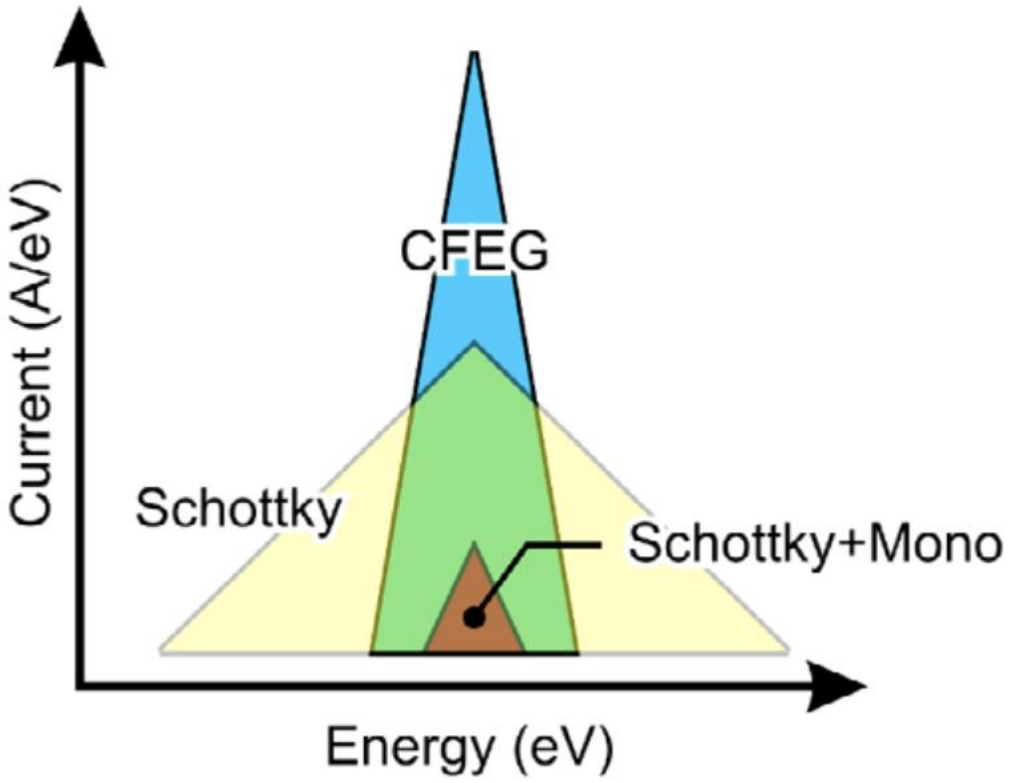


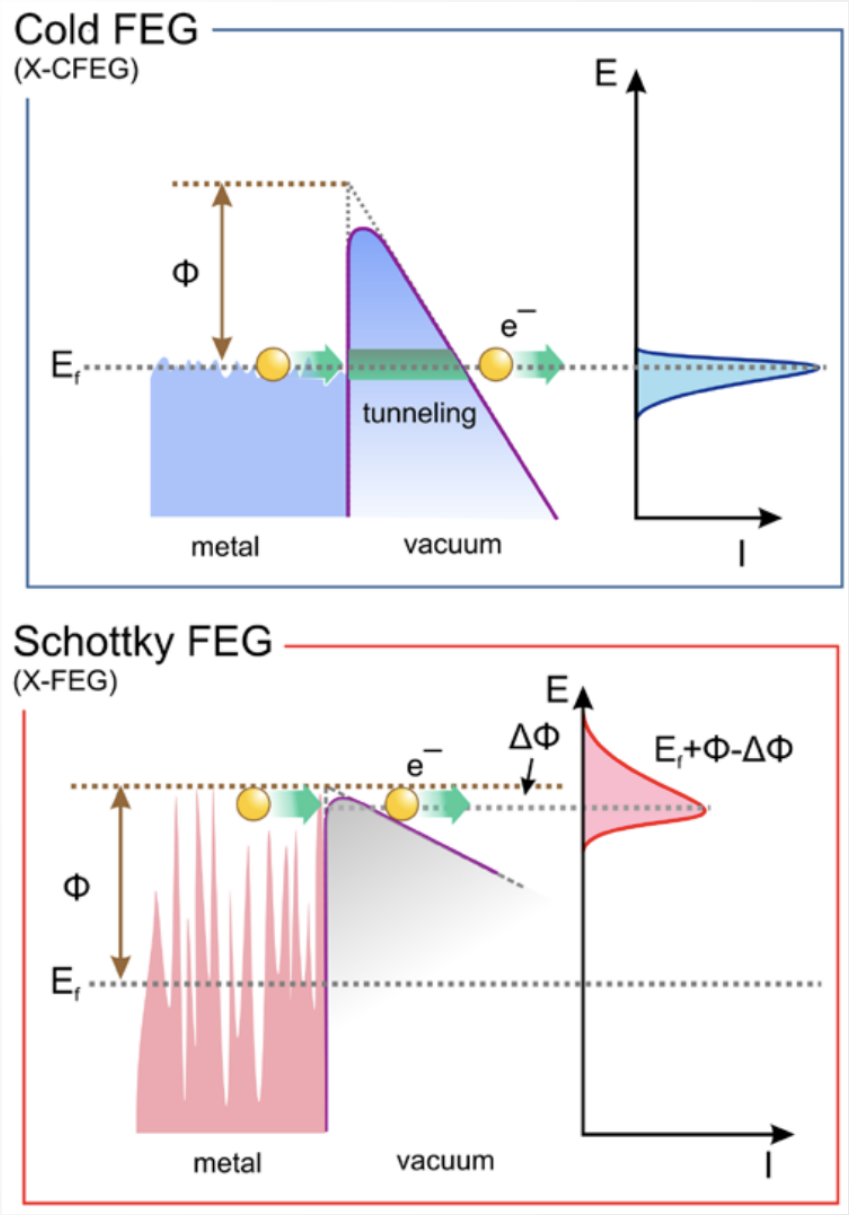
http://www.rodenburg.org/

Microscope basics column source



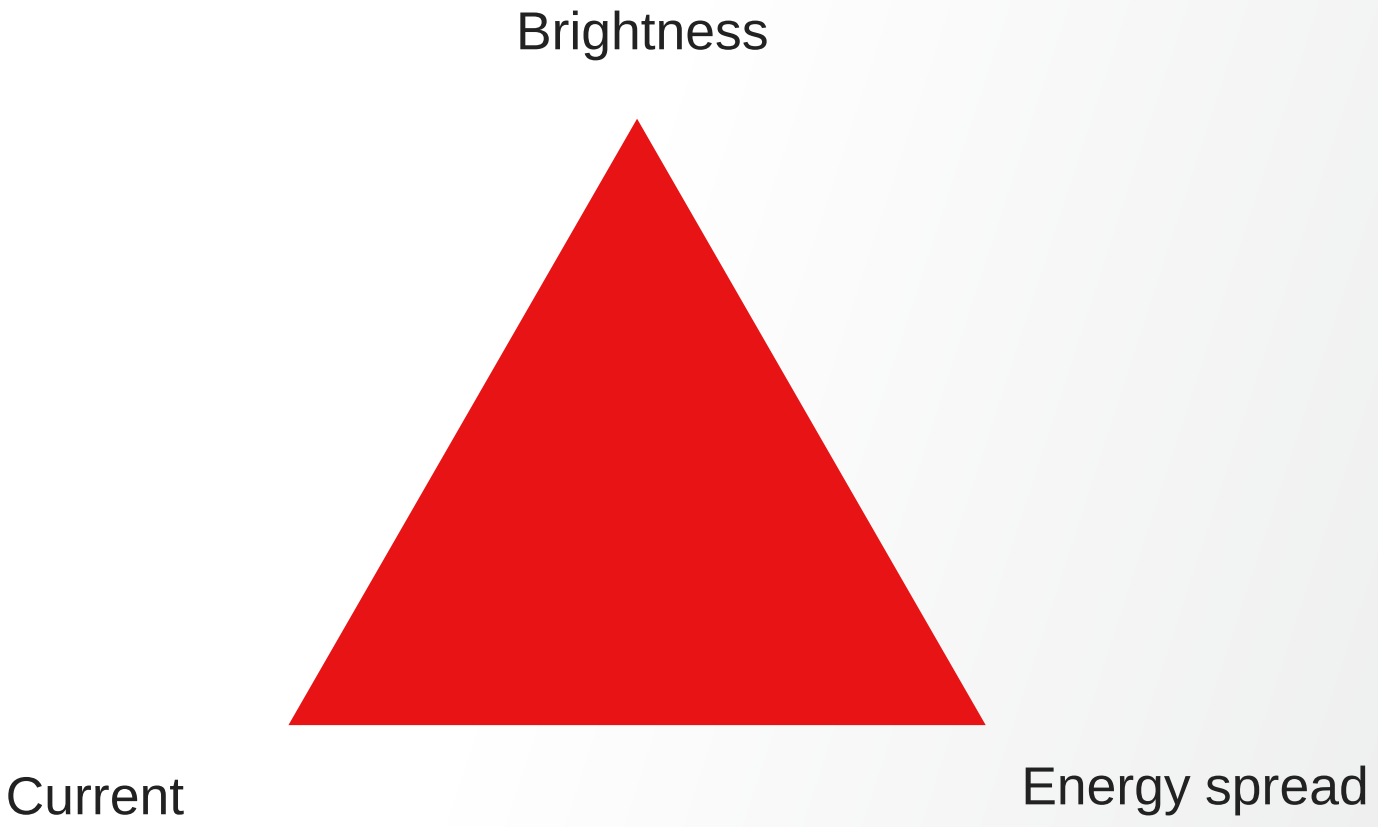
Microscope basics column source





https://assets.thermofisher.com/TFS-Assets/MSD/Application-Notes/xcfeg-application-note-an0171.pdf

Microscope basics column source



Microscope basics column source

The coherence angle can be tuned by changing the magnification from the source to the source image in the front focal plane of the upper objective lens. A higher gun lens or a higher spot number gives a smaller source image and a better coherence. However, it also gives less beam current. This balance between coherence and beam current is described by the **law of conservation of brightness**:

$$I = B \cdot \pi \alpha_2 \cdot (\pi/4) D_2 \cdot V_{rel}$$

Where:

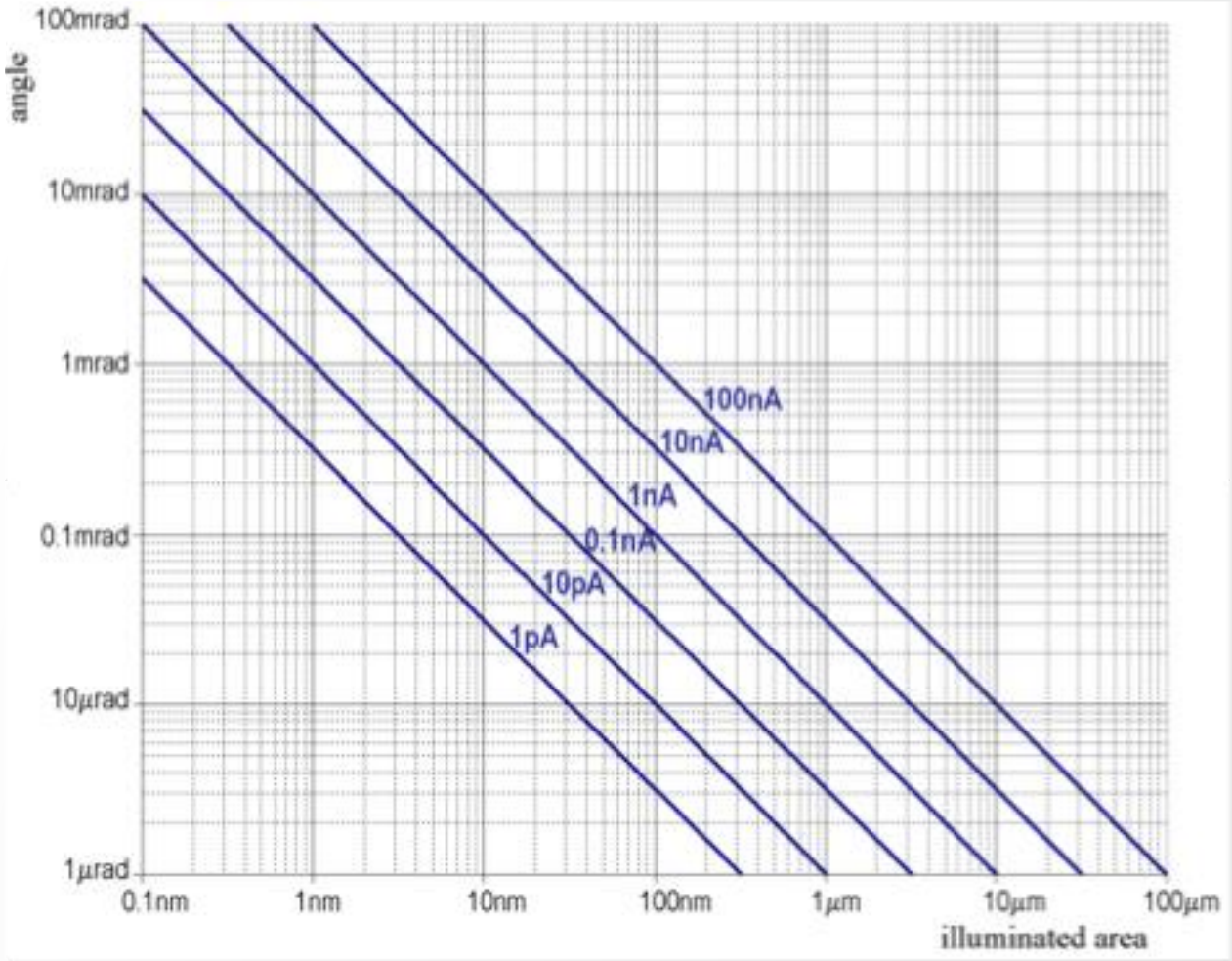
I = beam current on the specimen

B = brightness of the FEG, typically between $5 \cdot 10_6$ and $2 \cdot 10_7$ $A/m_2/sr/V$

α = half-coherence angle

D = diameter of the illuminated area on the specimen

$V_{rel} = V \cdot (1+V/V_0)$, the relativized high tension ($V_0 = 2m_{electron}c_2/e = 1022kV$)



Beam current as a function of illuminated area and coherence angle for a FEG operated at brightness $B=10^7$ $A/m^2/sr/V$.

Titan condenser manual

Microscope basics column source

Physics of Schottky electron sources

Title
Physics of Schottky electron sources

Author
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Contributor
Kruit, P. (promotor)

Faculty
Applied Sciences

Department
Imaging Science & Technology

Date
2009-12-21

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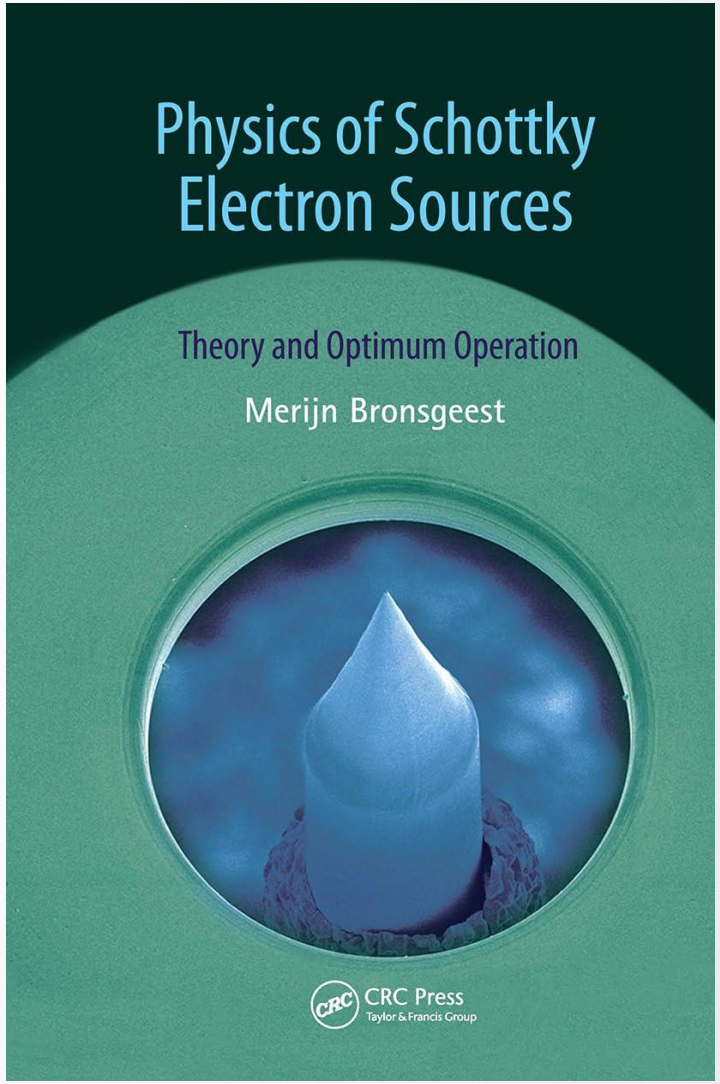

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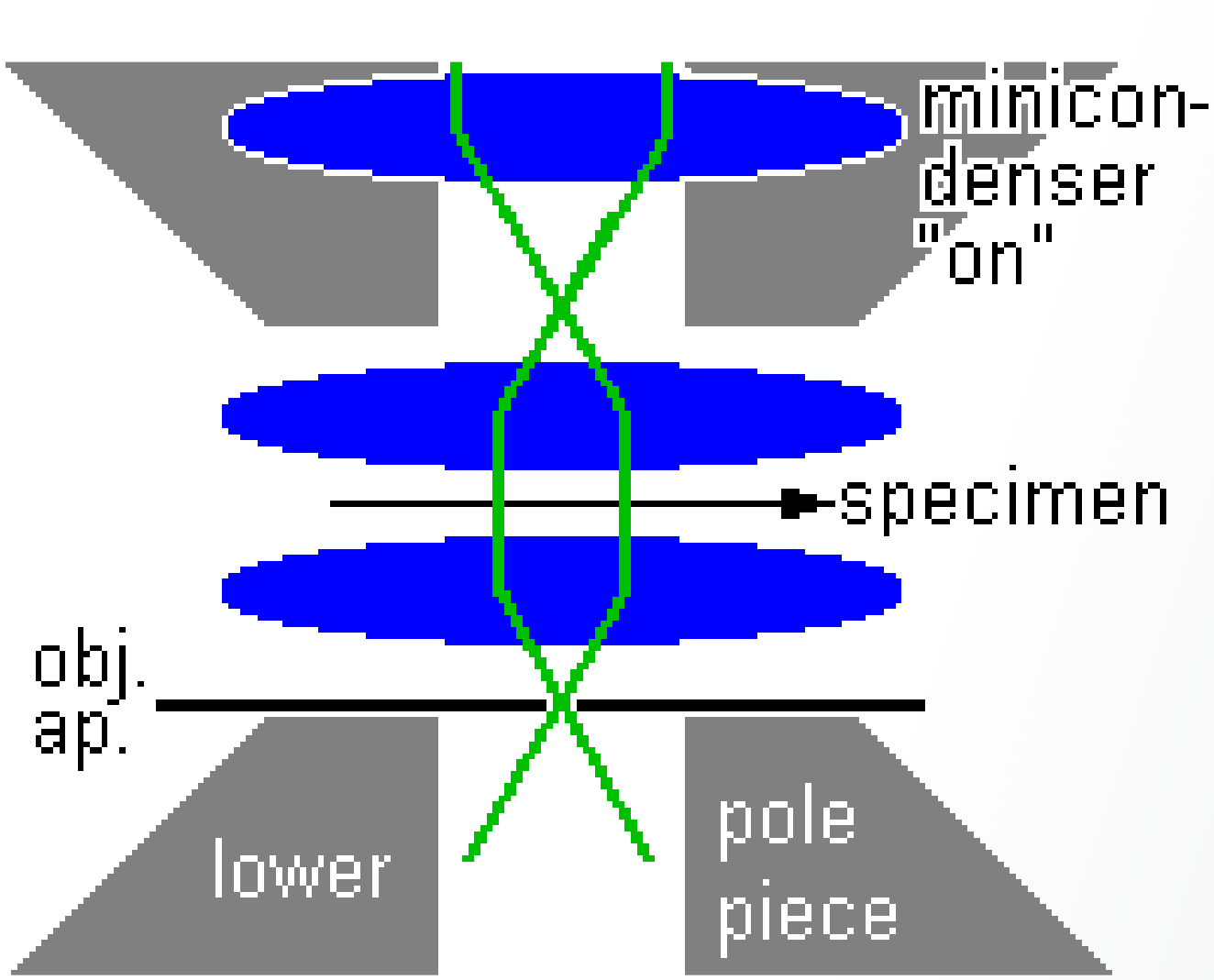
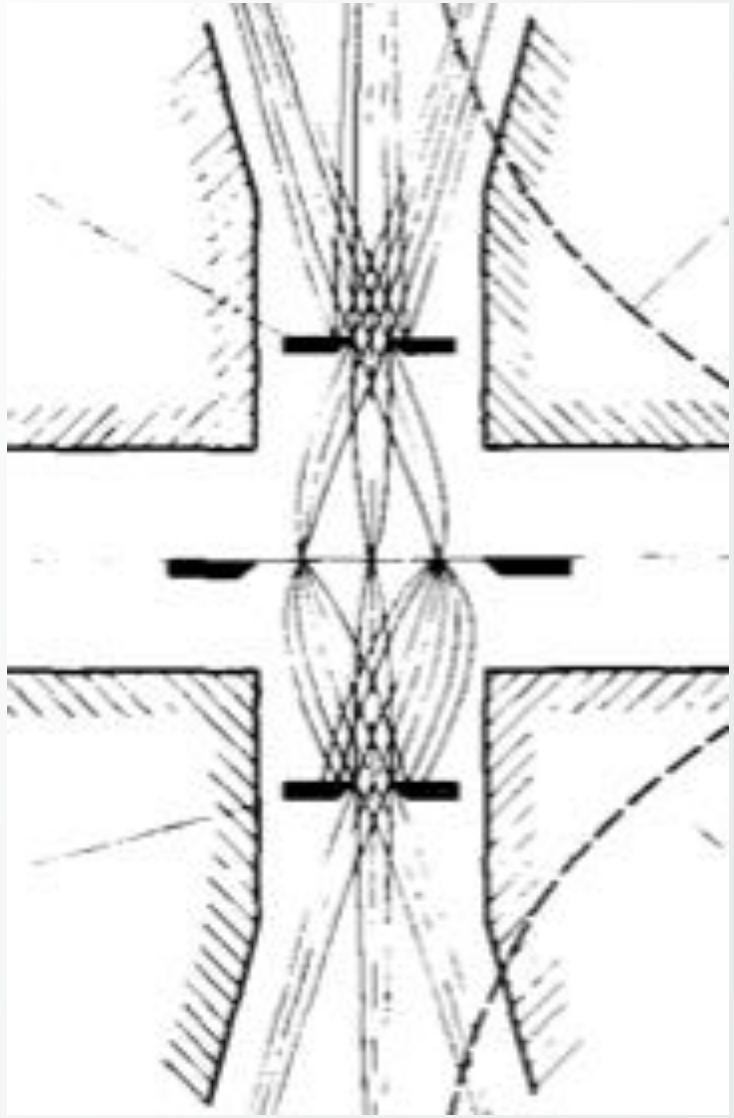
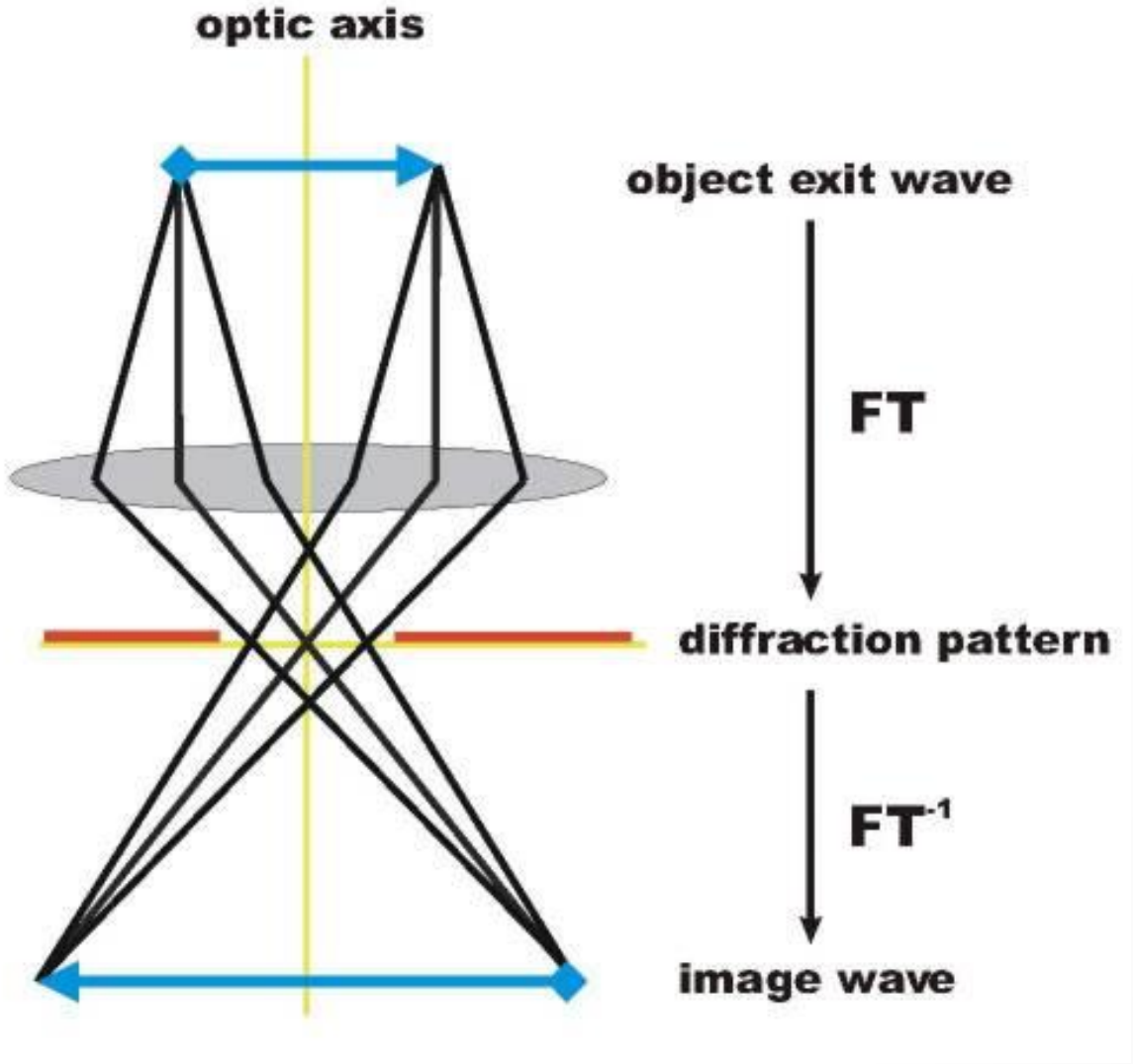
Microscope basics column lenses





Microscope basics objective aperture



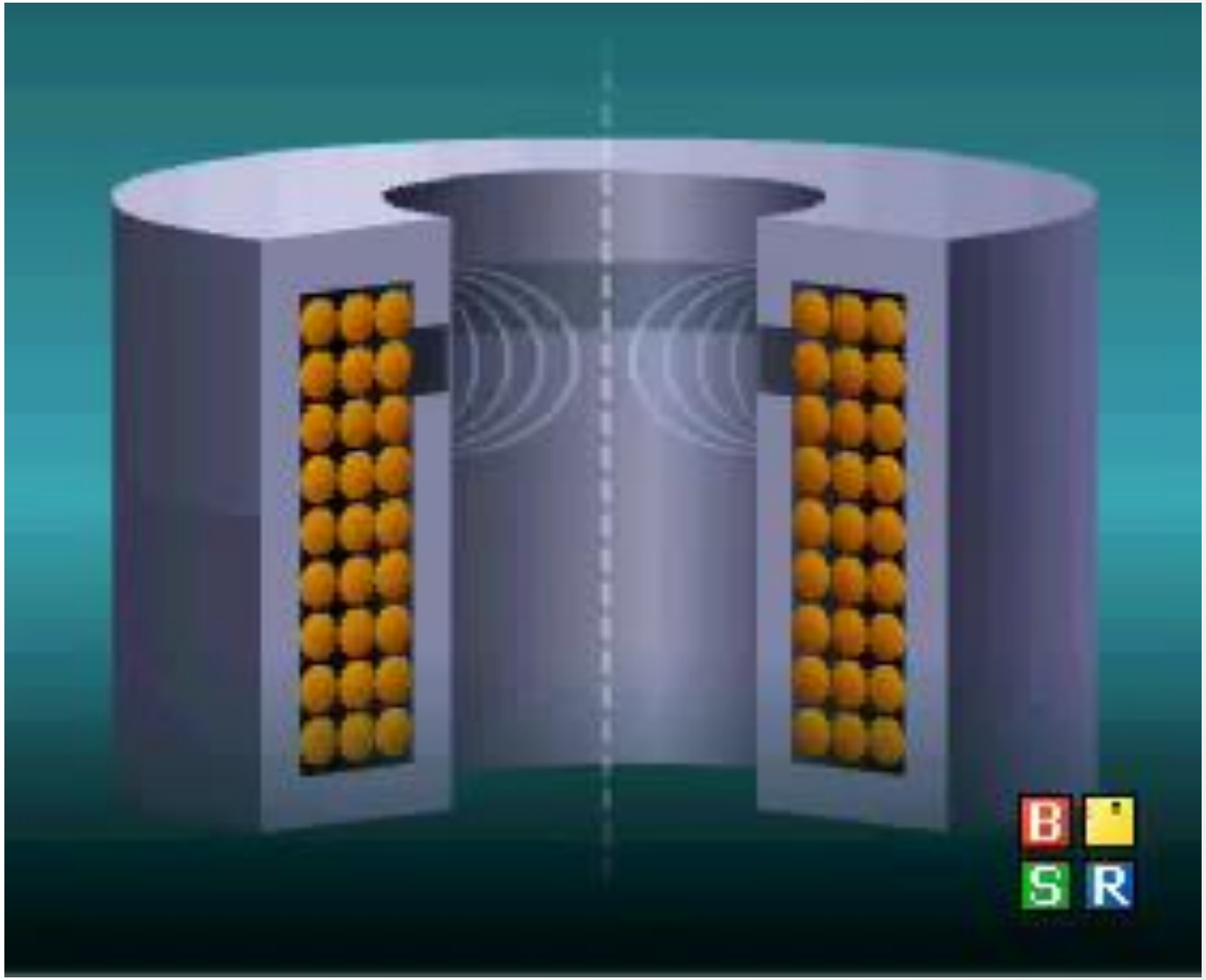
Angle = Lambda (wavelength) / d (resolution)
Maximum angle = R (radius aperture) / F (focal length)
Cut off = F x Lambda / R

Focal length:
Titan CTWIN = 3.4 mm
Tecnai TWIN = 2.9 mm
Tundra = 2.5 mm
JEOL cryo = 2.8 mm

Thermo Scientific CTWIN systems	Cut-off @300 kV	Cut-off @200 kV
100 µm	1.3 Å	1.7 Å
70 µm	1.9 Å	2.4 Å
50 µm	2.7 Å	3.4 Å
30 µm	4.5 Å	5.7 Å

https://www.microscopy.ethz.ch/TEM_mathe.htm

Microscope basics column lenses



Microscope basics column lenses

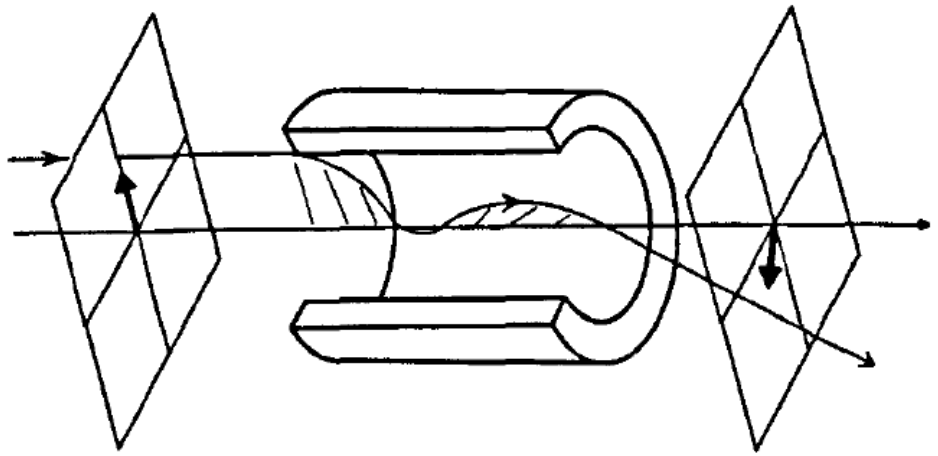


Fig. 10. Schematic diagram showing the trajectory of an electron through a magnetic lens. After Loretto [12], p. 9.

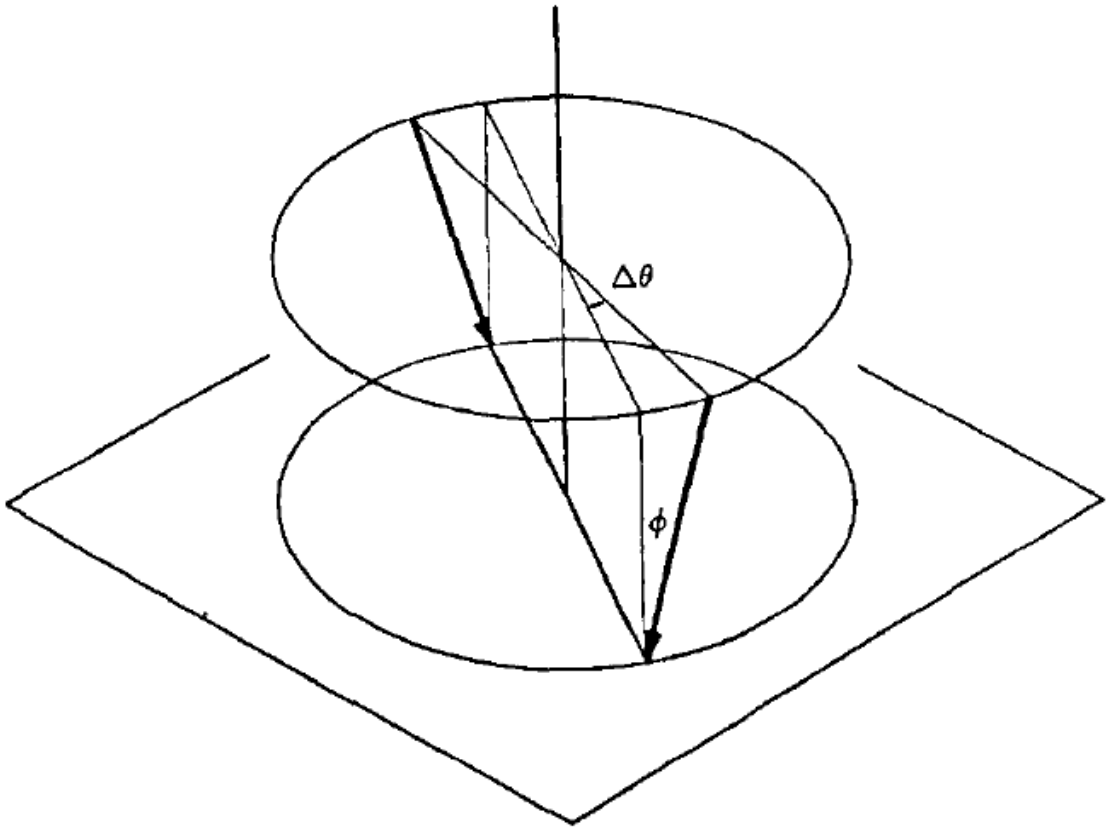


Fig. 11. Diagram to show the variation in the angle of incidence of an electron beam at the specimen in an immersion lens. After ref. [1].

Ultramicroscopy 26 (1988) 113–132
North-Holland, Amsterdam

Microscope basics column lenses

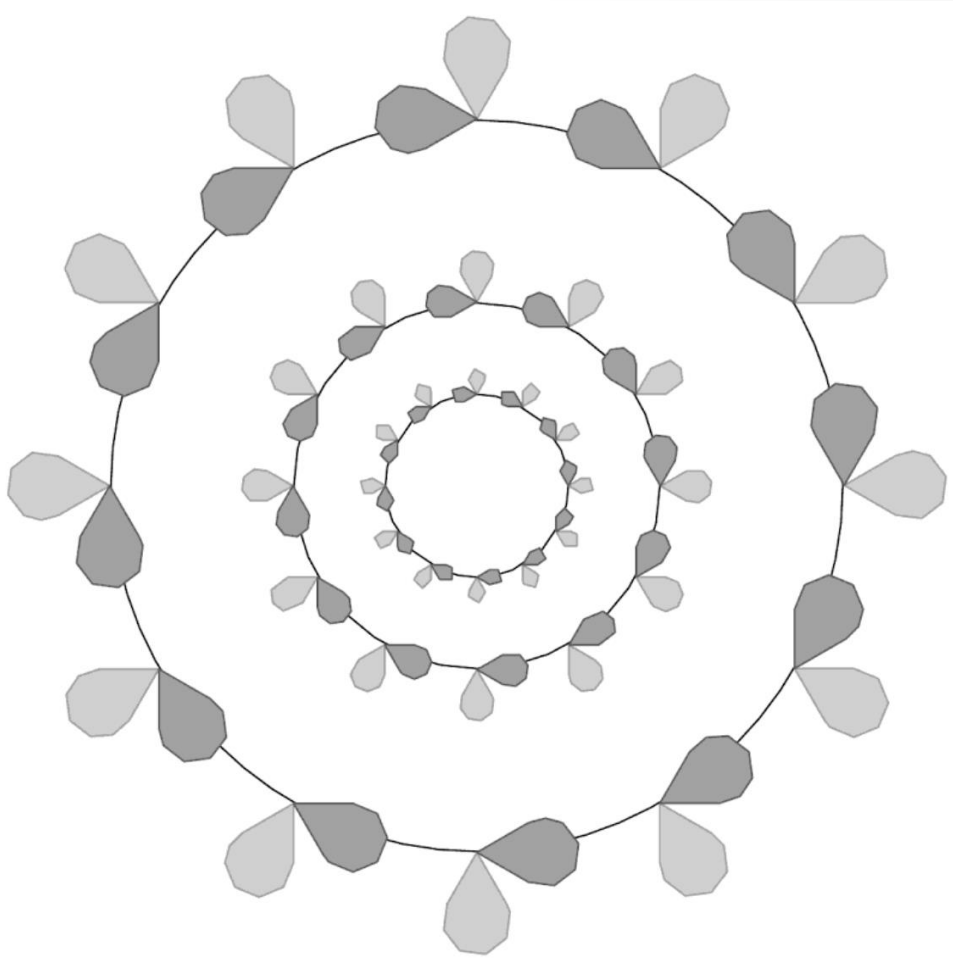
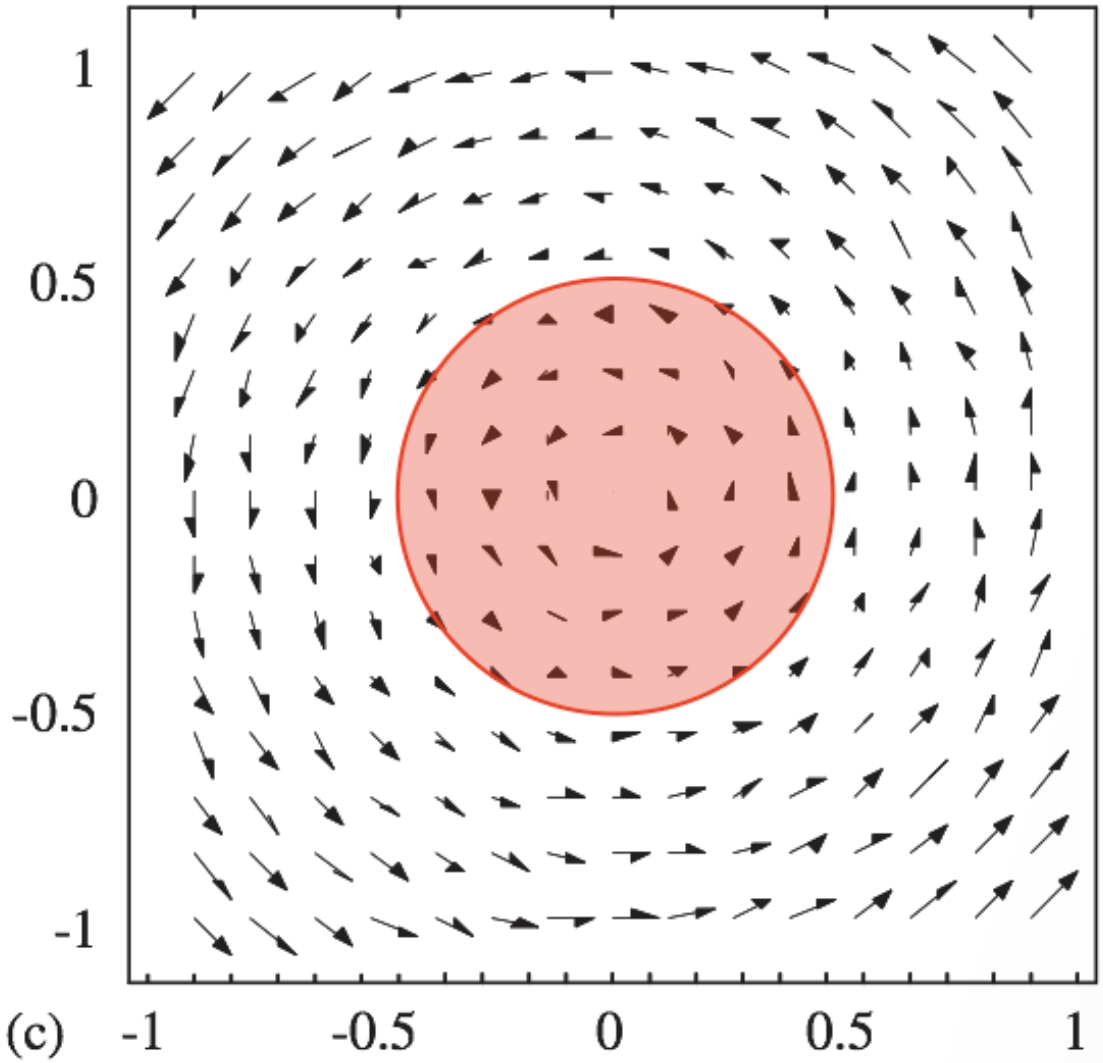
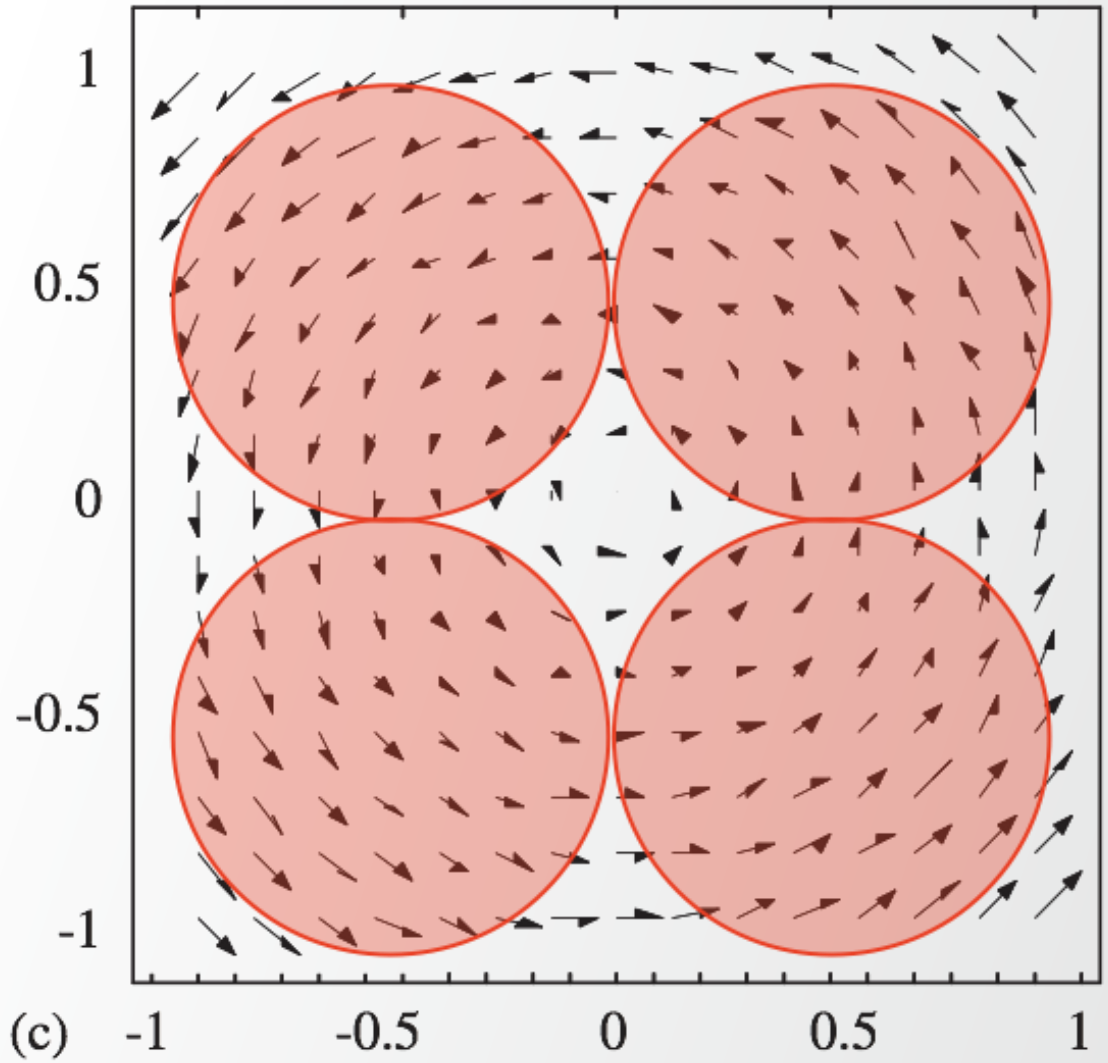


FIGURE 41 The delocalization area of both the isotropic/radial (light grey) and the anisotropic/azimuthal (dark grey) off-axial coma. For increasing circles in the image plane, the shape of the comet-tail–like delocalization figure is depicted. The diameter of the shape increases linearly with the distance to the aberration-free axial point. If a *semi-aplanat* is perfectly aligned, only the azimuthal component remains.

Advances in imaging and electron physics: aberration-corrected electron microscopy, Volume 153

Microscope basics column lenses





Microscope basics column lenses

Journal of Structural Biology 174 (2011) 1–10

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Review

Precise beam-tilt alignment and collimation are required to minimize the phase error associated with coma in high-resolution cryo-EM

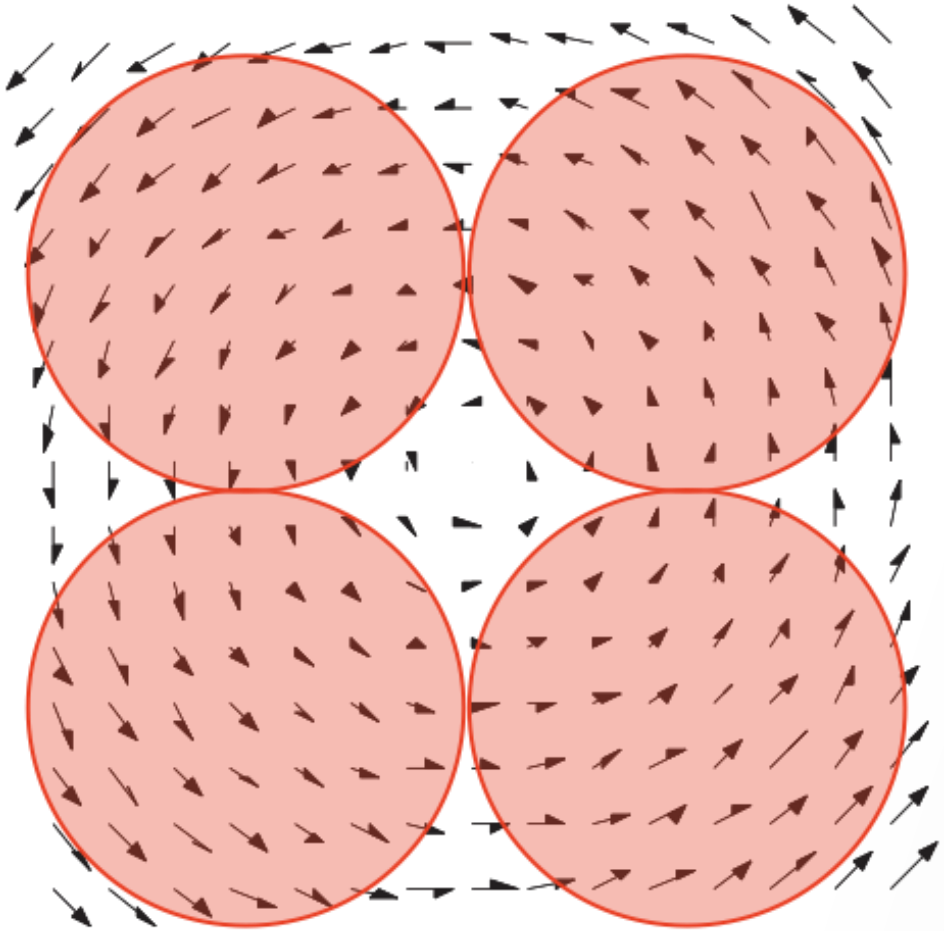
Robert M. Glaeser [a,*], Dieter Typke [a], Peter C. Tiemeijer [b], James Pulokas [c], Anchi Cheng [c]

[a] *Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720, USA*
[b] *FEI Company, P.O. Box 80066, 5600 KA Eindhoven, The Netherlands*
[c] *National Resource for Automated Molecular Microscopy, The Scripps Research Institute, La Jolla, CA 92037, USA*

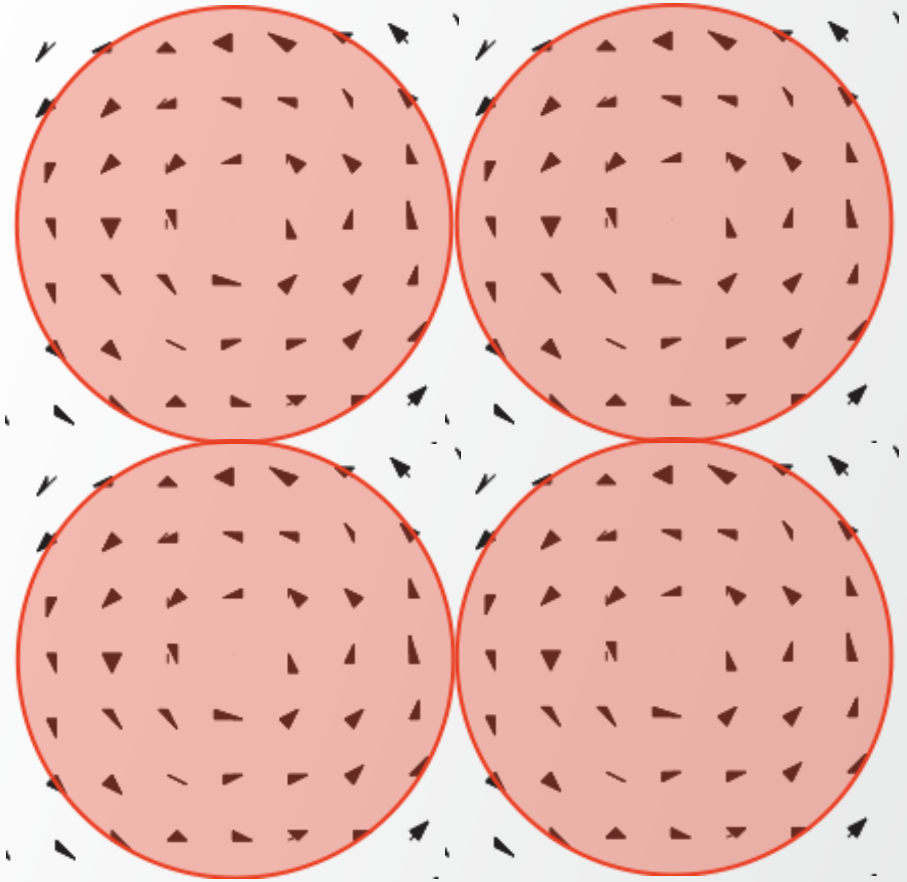
Microscope basics column lenses

Off-axis coma can, in principle, be avoided by combining parallel illumination of a relatively small area, deflection of the beam to successive positions, and dynamic adjustment of the tilt angle at each new position. This same approach was used previously by Eades to maintain a fixed orientation of the beam relative to the specimen over extended areas of a thin crystal (Eades, 2006). The concept in our case is to ensure that both the isotropic and anisotropic coma can be ignored within any one spot, regardless of its position. We emphasize that dynamic compensation for the position-dependence of coma should not be required if data are to be collected from positions that are only ~2 μm or less from the coma-free axis, but that it is likely to be useful for automated data collection covering much larger areas.

Microscope basics column lenses



“Beam tilt compensation”



Microscope optics

Spatial coherence

$$E_s(u) = \exp\left[-\left(\frac{\pi\alpha}{\lambda}\right)^2\left(\frac{\delta X(u)}{\delta u}\right)^2\right] = \exp\left[-\left(\frac{\pi\alpha}{\lambda}\right)^2(C_s\lambda^3u^3 + \Delta f\lambda u)^2\right],$$

Temporal coherence

$$E_c(u) = \exp\left[-\frac{1}{2}(\pi\lambda\delta)^2u^4\right],$$

Focal spread

$$\delta = C_c\sqrt{4\left(\frac{\Delta I_{\text{obj}}}{I_{\text{obj}}}\right)^2 + \left(\frac{\Delta E}{V_{\text{acc}}}\right)^2 + \left(\frac{\Delta V_{\text{acc}}}{V_{\text{acc}}}\right)^2},$$

https://en.wikipedia.org/wiki/High-resolution_transmission_electron_microscopy

Microscope basics column source

The coherence angle can be tuned by changing the magnification from the source to the source image in the front focal plane of the upper objective lens. A higher gun lens or a higher spot number gives a smaller source image and a better coherence. However, it also gives less beam current. This balance between coherence and beam current is described by the **law of conservation of brightness**:

$$I = B \cdot \pi \alpha_2 \cdot (\pi/4) D_2 \cdot V_{rel}$$

Where:

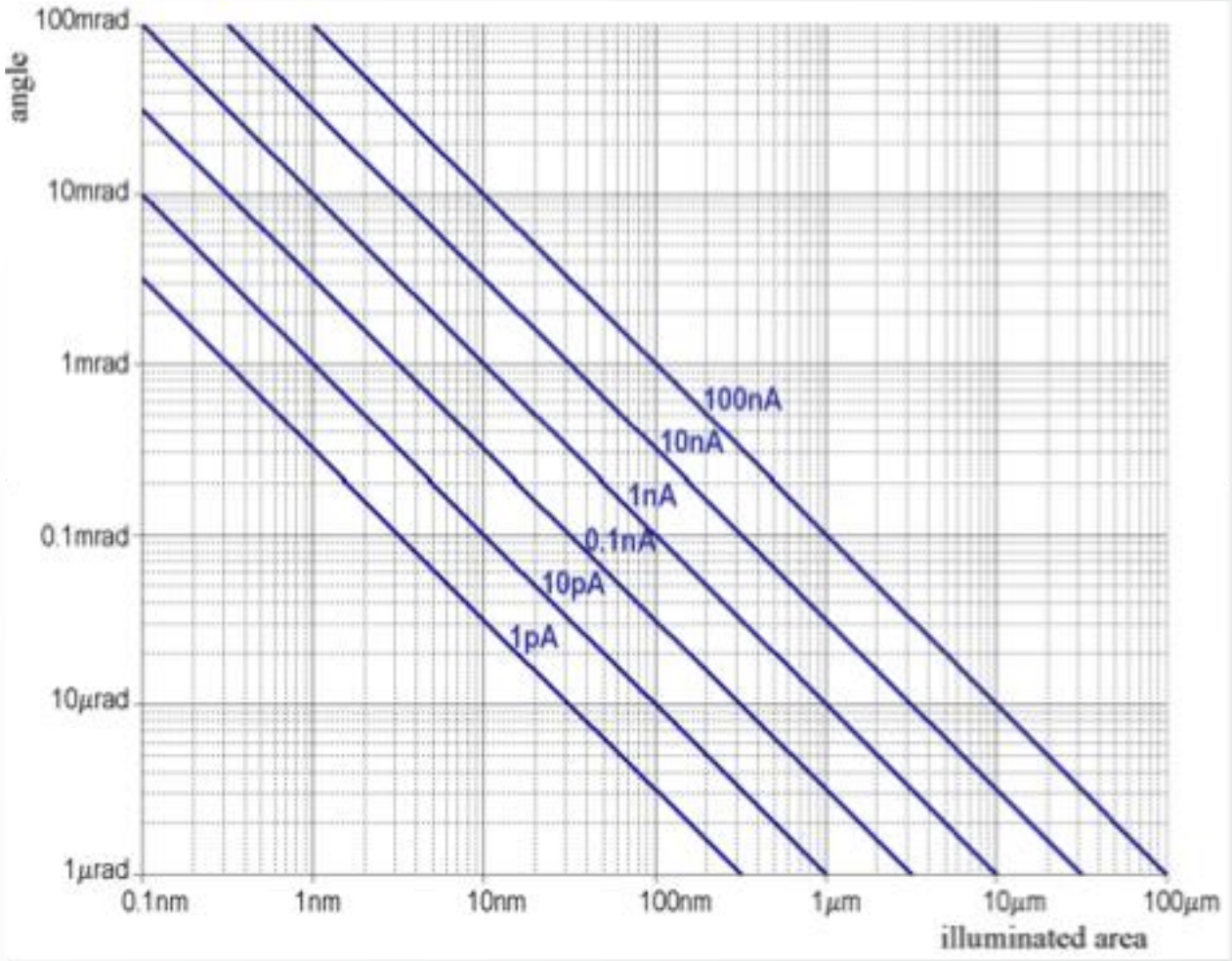
I = beam current on the specimen

B = brightness of the FEG, typically between $5 \cdot 10_6$ and $2 \cdot 10_7$ $A/m_2/sr/V$

α = half-coherence angle

D = diameter of the illuminated area on the specimen

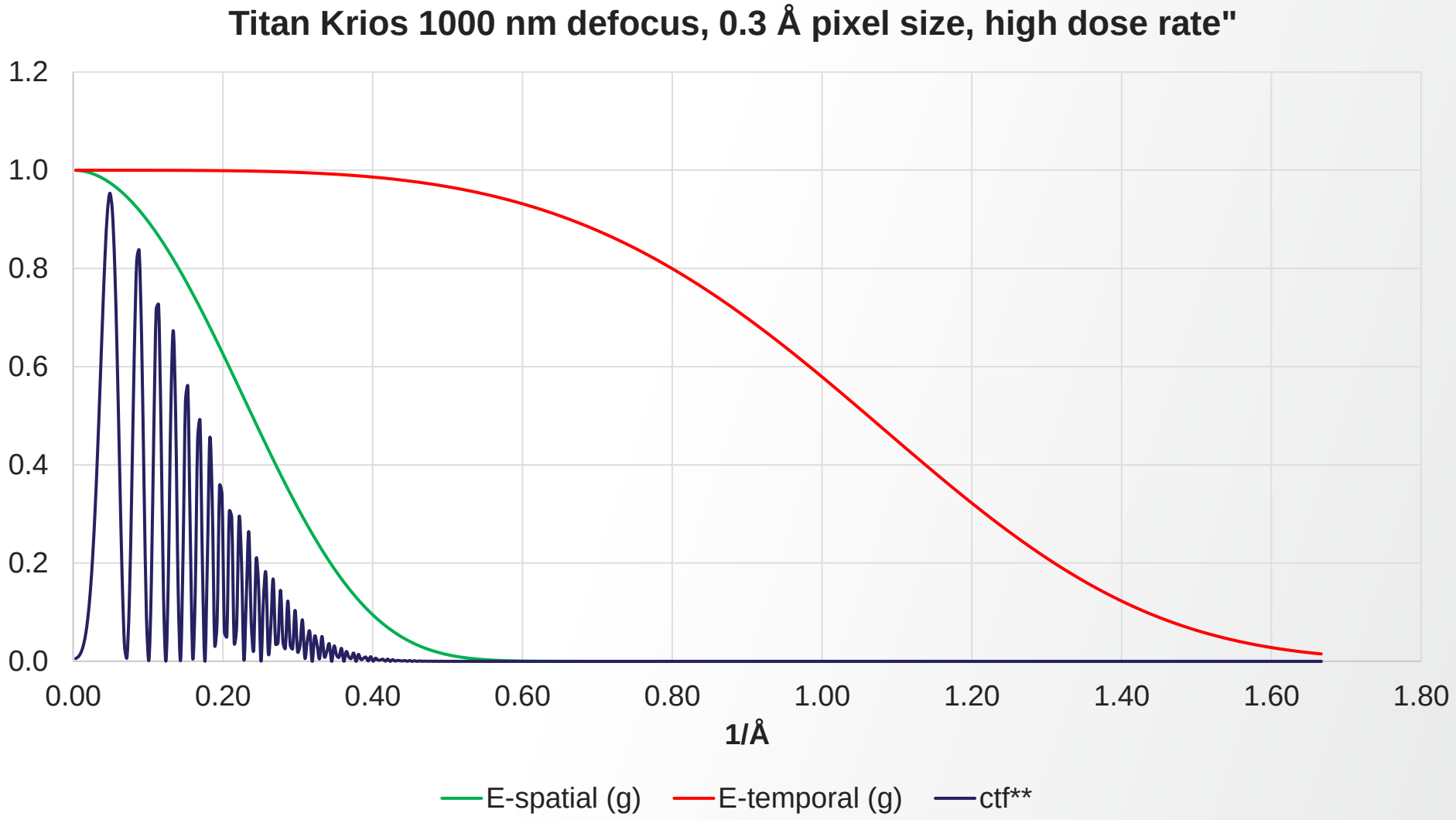
$V_{rel} = V \cdot (1+V/V_0)$, the relativized high tension ($V_0 = 2m_{electron}c_2/e = 1022kV$)



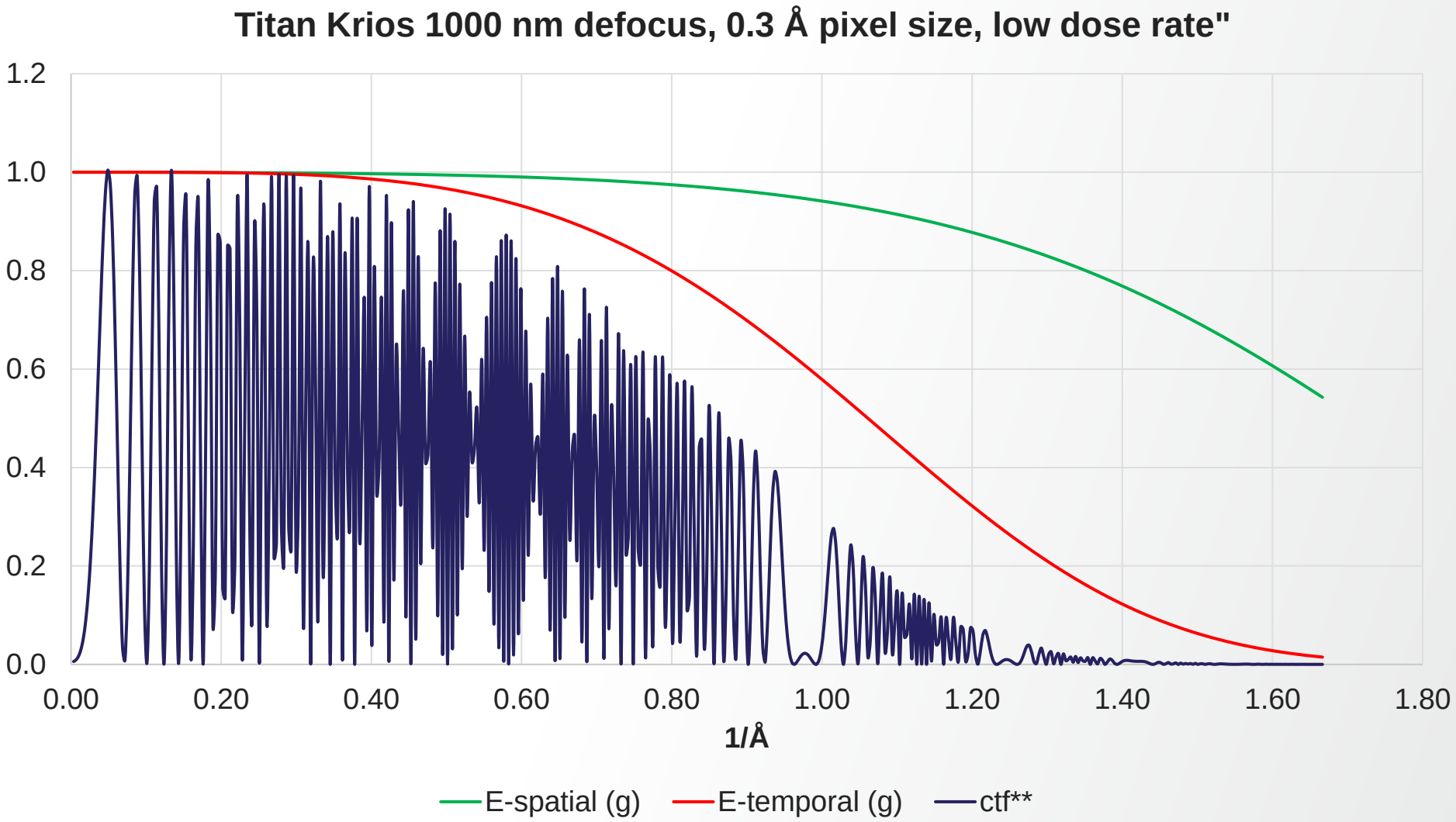
Beam current as a function of illuminated area and coherence angle for a FEG operated at brightness $B=10^7$ $A/m^2/sr/V$.

Titan condenser manual

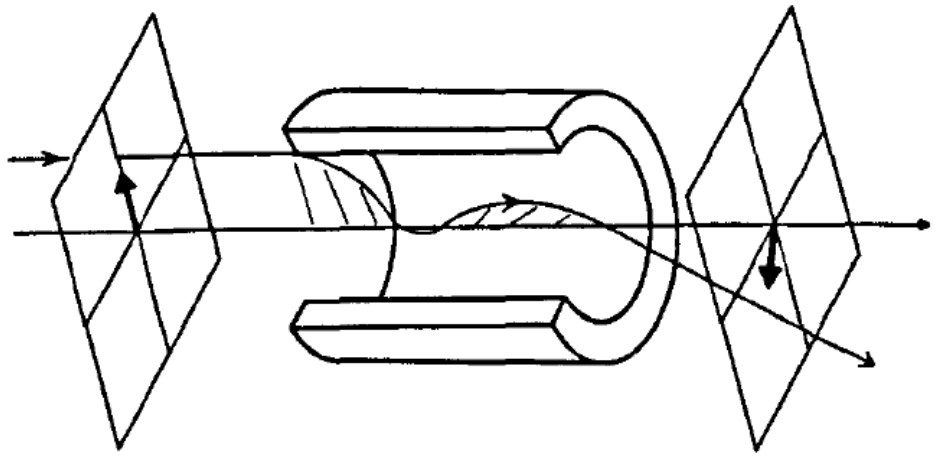
Microscope basics coherence



Microscope basics coherence



Microscope basics column lenses



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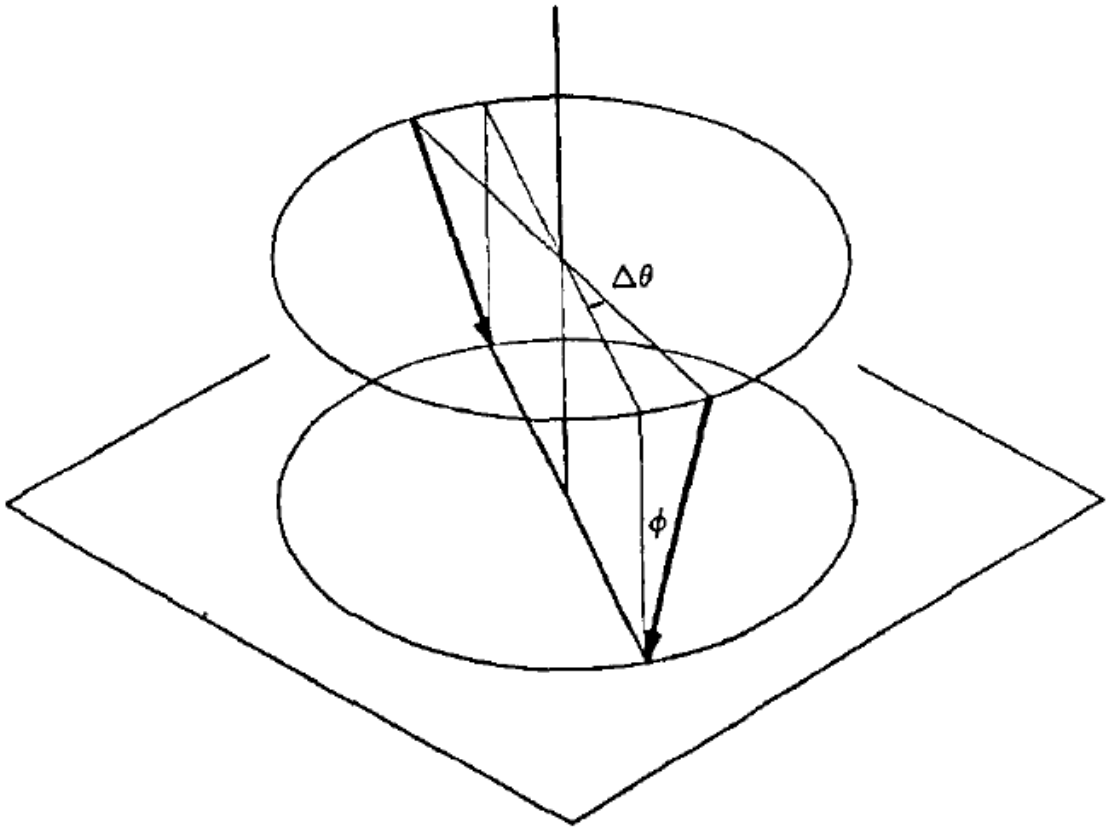


Fig. 11. Diagram to show the variation in the angle of incidence of an electron beam at the specimen in an immersion lens. After ref. [1].

Ultramicroscopy 26 (1988) 113–132
North-Holland, Amsterdam

Microscope basics BCOR

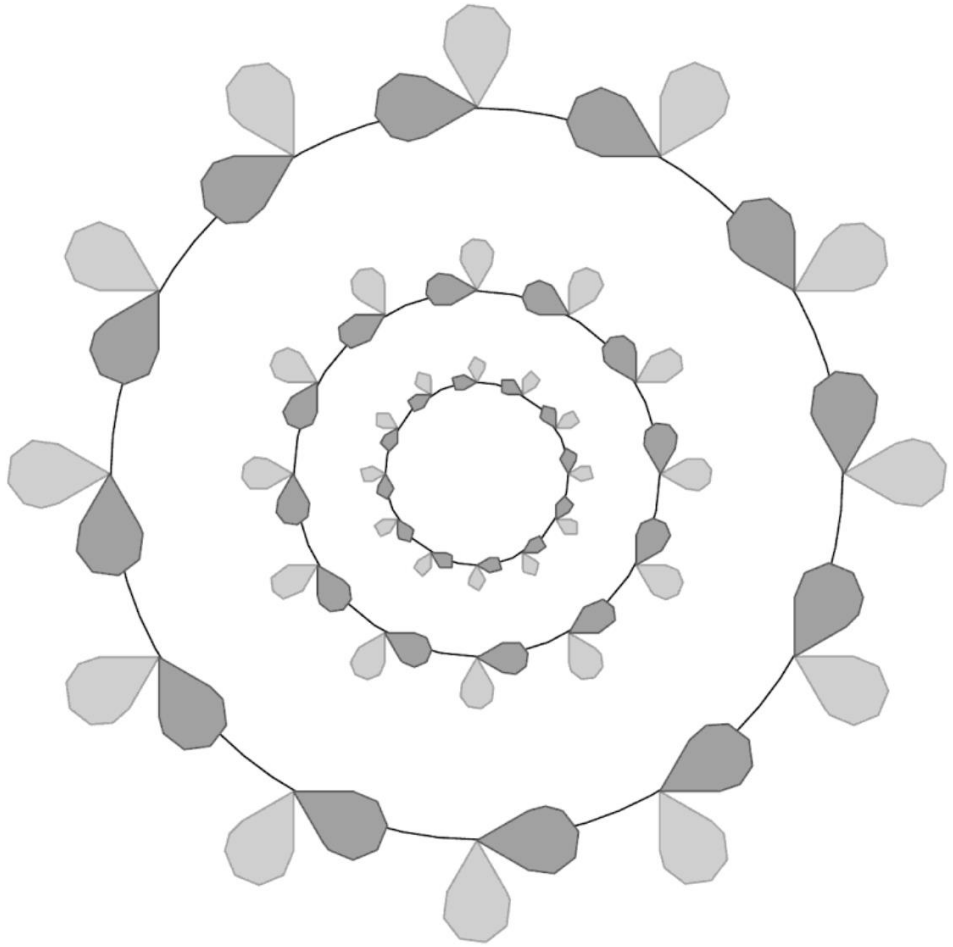
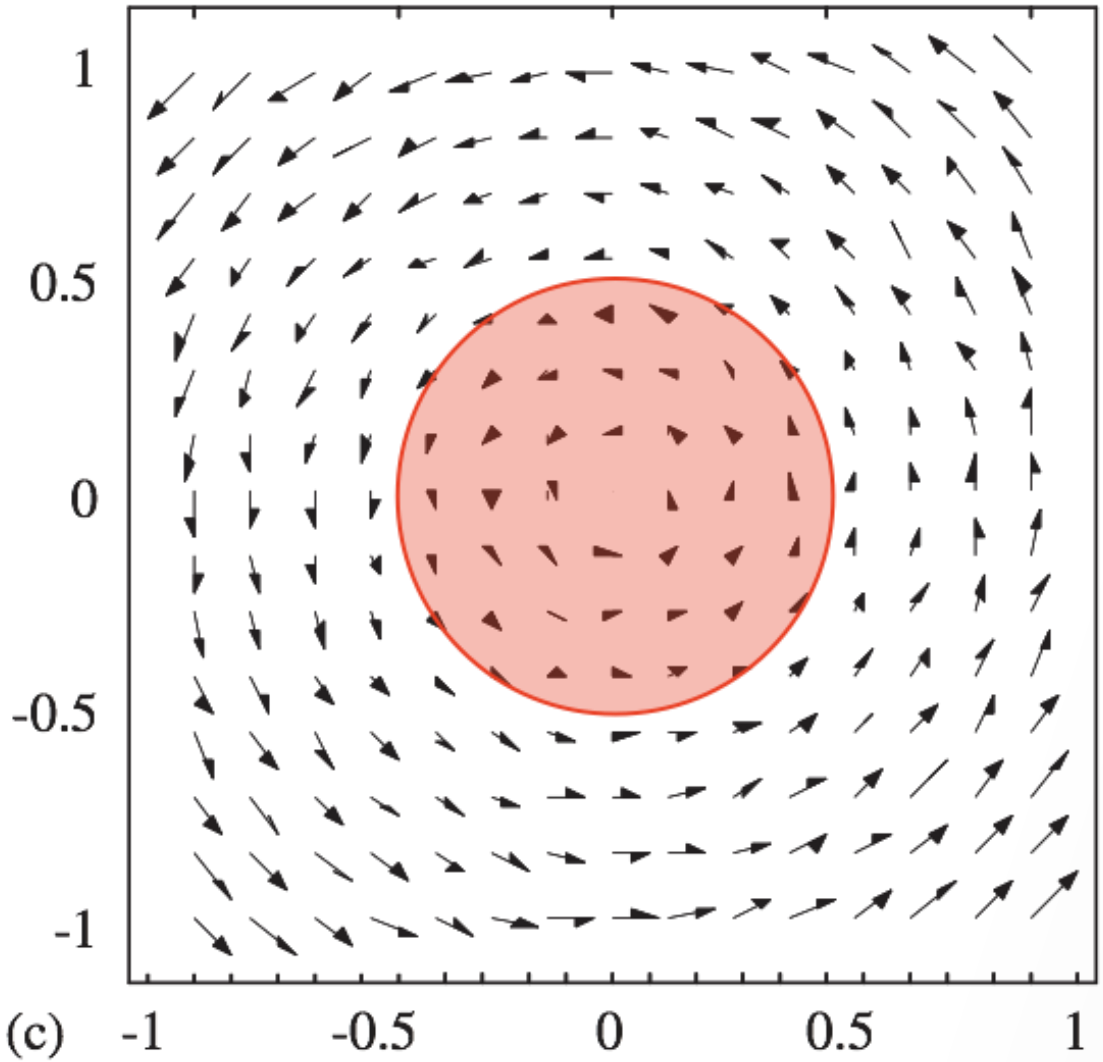
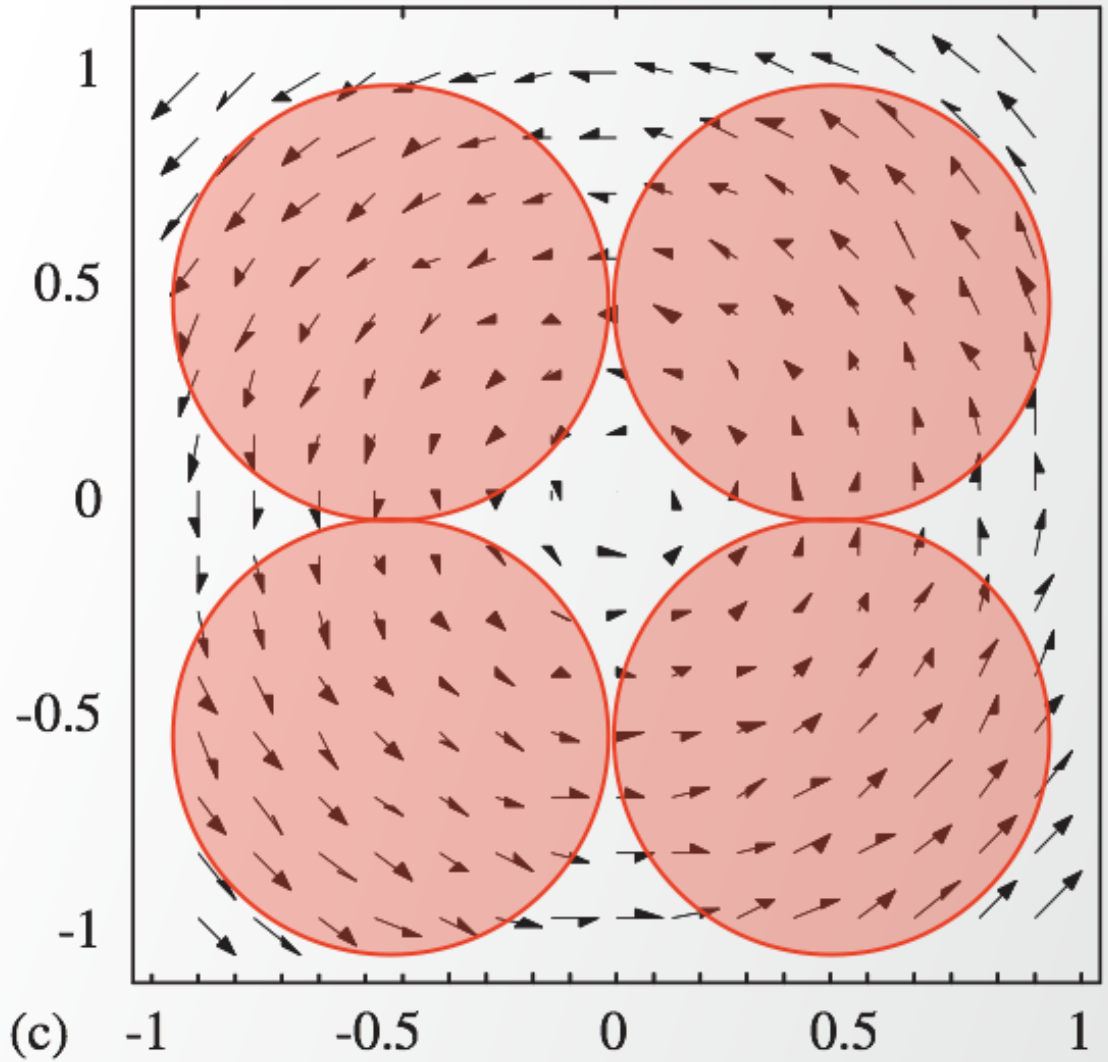


FIGURE 41 The delocalization area of both the isotropic/radial (light grey) and the anisotropic/azimuthal (dark grey) off-axial coma. For increasing circles in the image plane, the shape of the comet-tail–like delocalization figure is depicted. The diameter of the shape increases linearly with the distance to the aberration-free axial point. If a *semi-aplanat* is perfectly aligned, only the azimuthal component remains.

Advances in imaging and electron physics: aberration-corrected electron microscopy, Volume 153

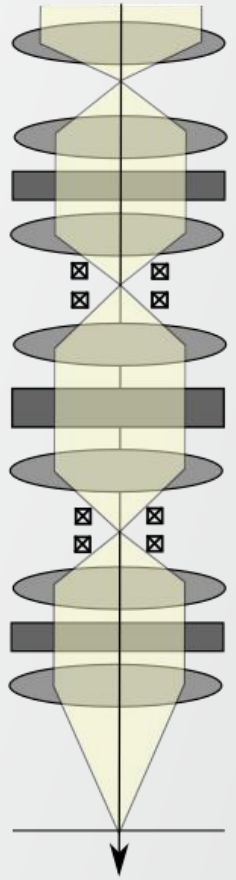
Microscope basics BCOR





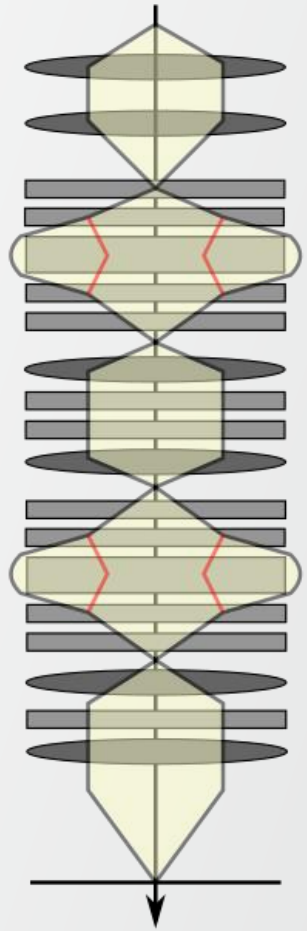
Microscope BCOR

- BCOR adds Cc.
- Focal spread from increased Cc can be somewhat compensated by using source with smaller energy spread.
  - E-CFEG.
  - Monochromator.
- Difficult to automate so far.

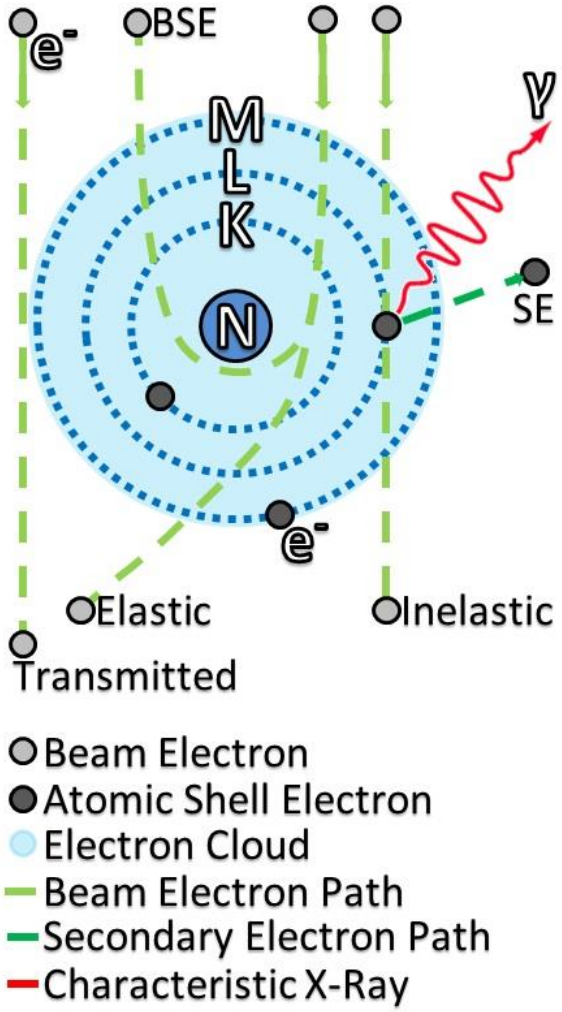


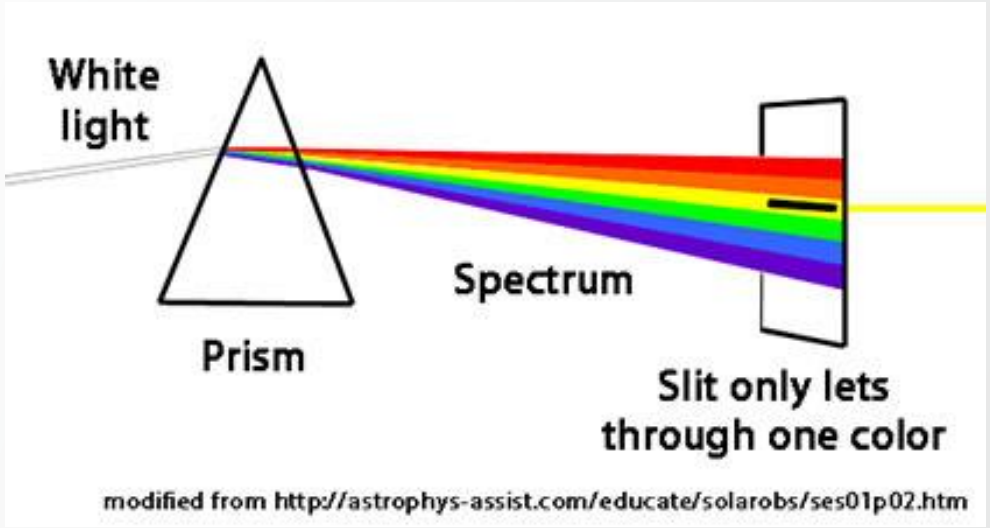
Microscope Cc-correction

- Too early to say.
- First results seem to indicate it only works in-focus.
- Working in-focus requires phase plate.



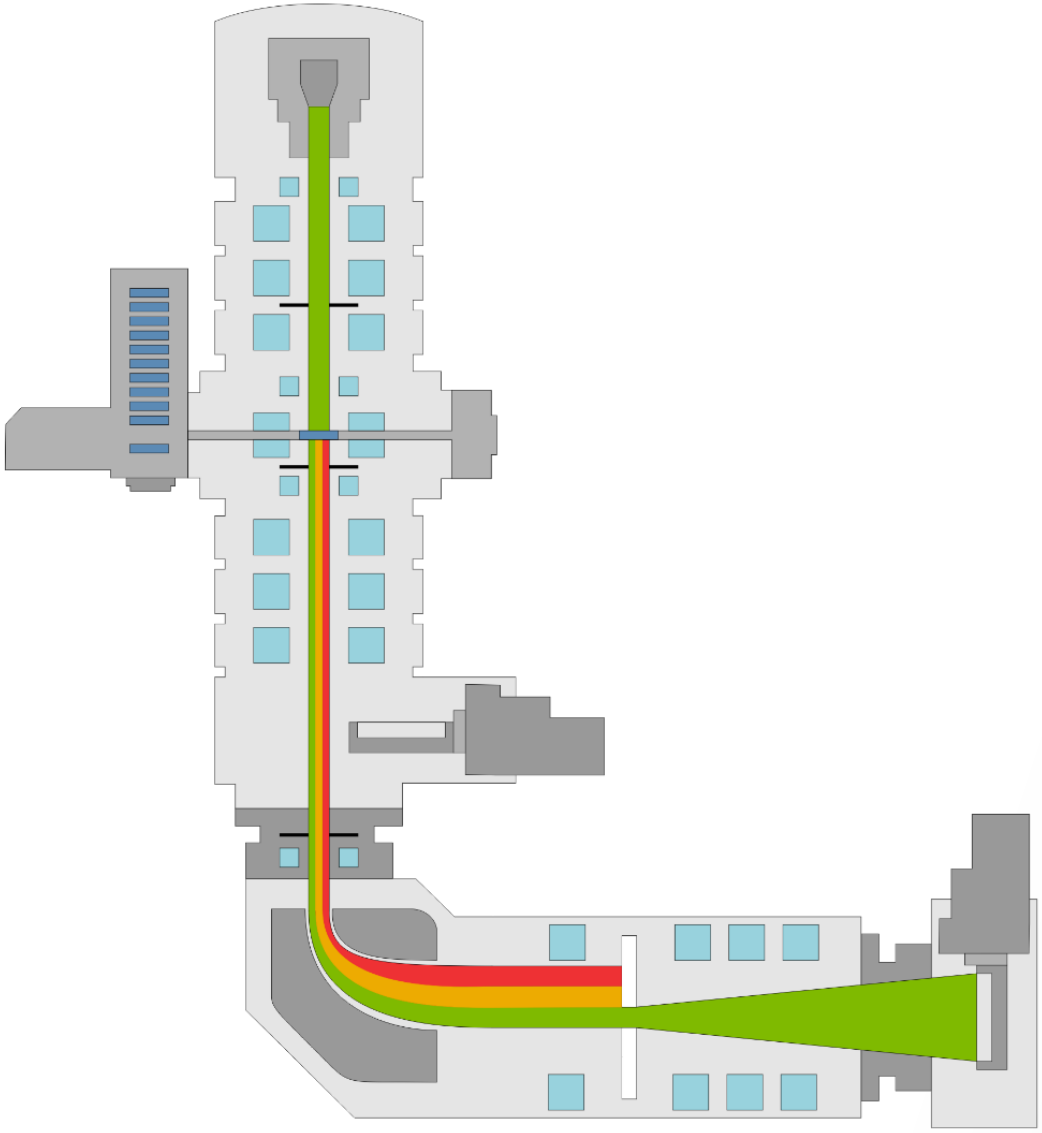
Microscope energy filtering

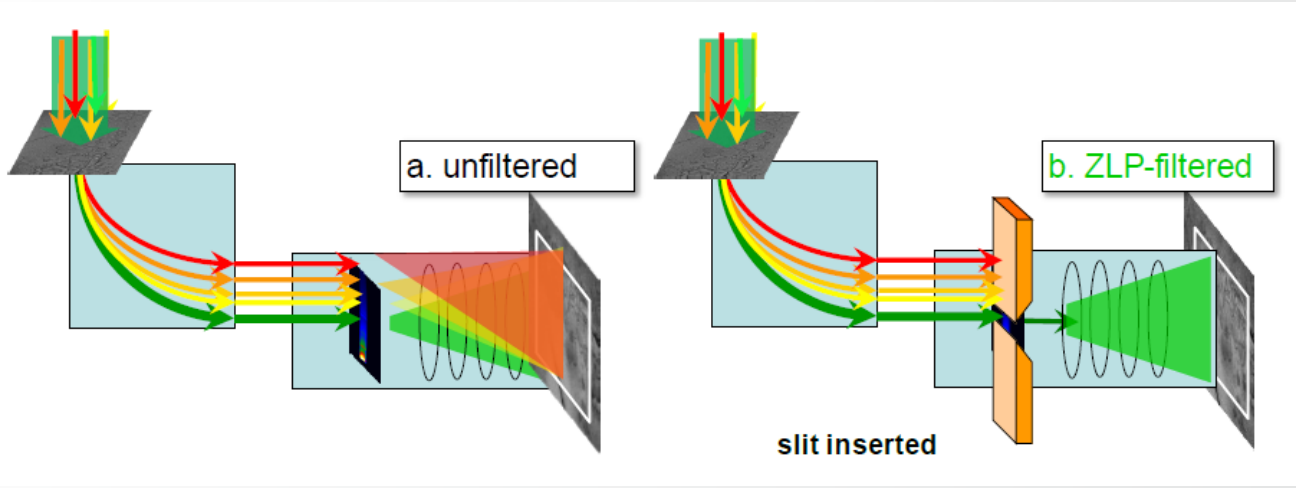




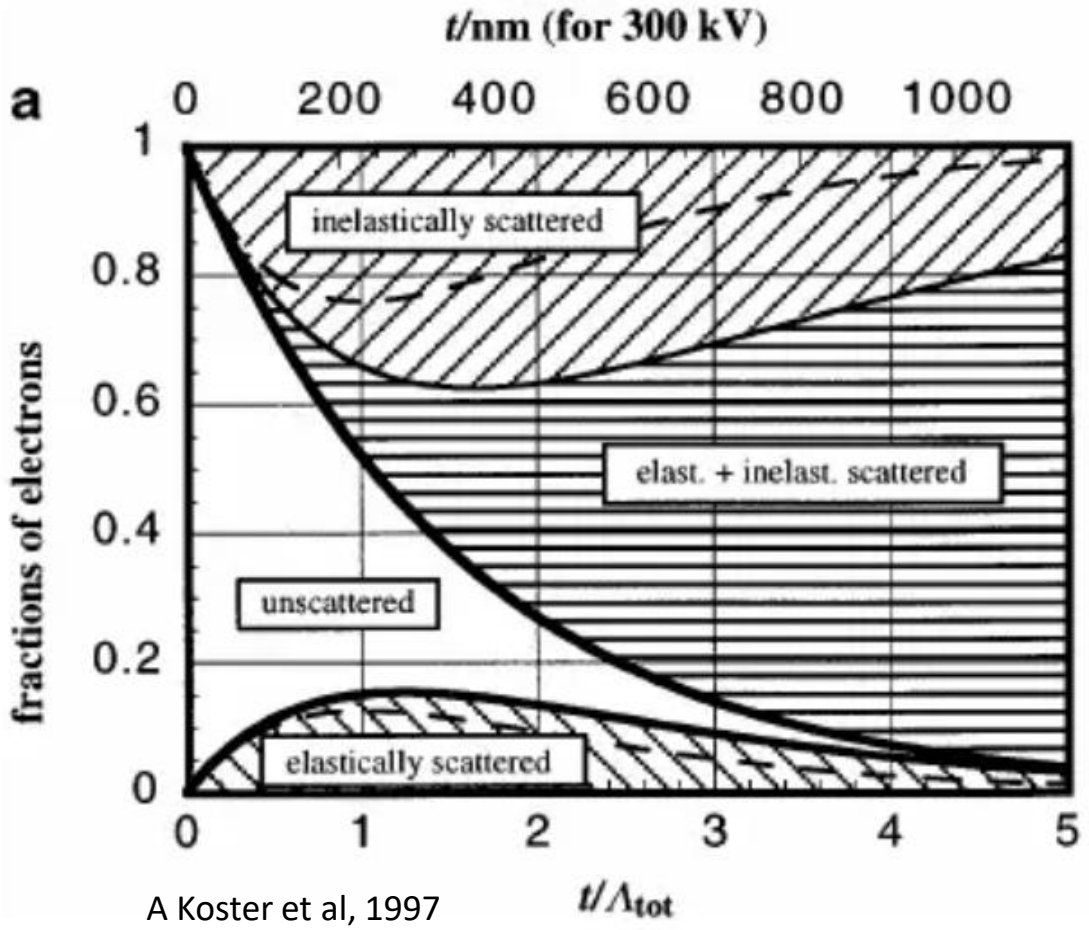
https://en.wikipedia.org/wiki/Electron_scattering

Microscope energy filtering

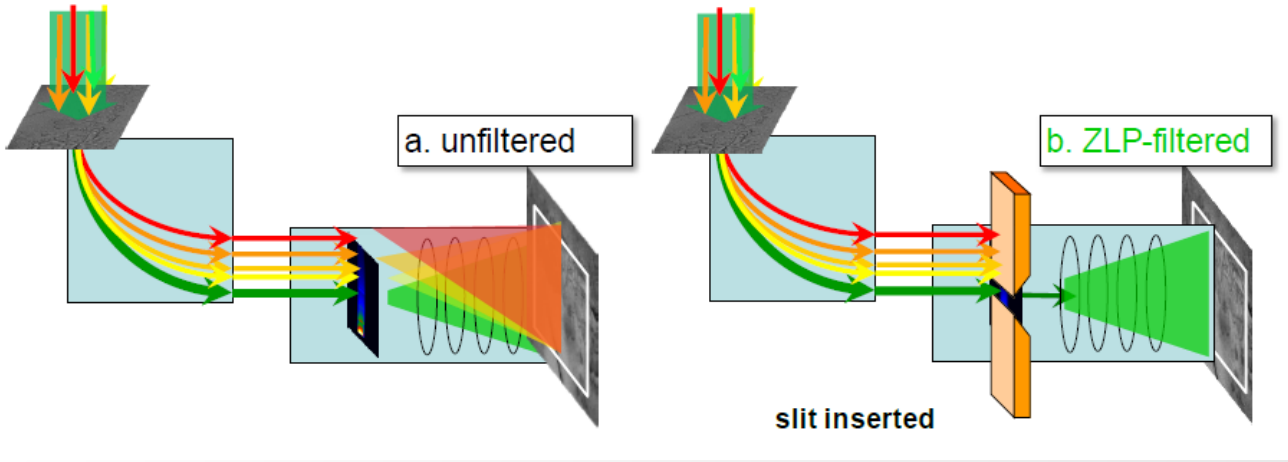


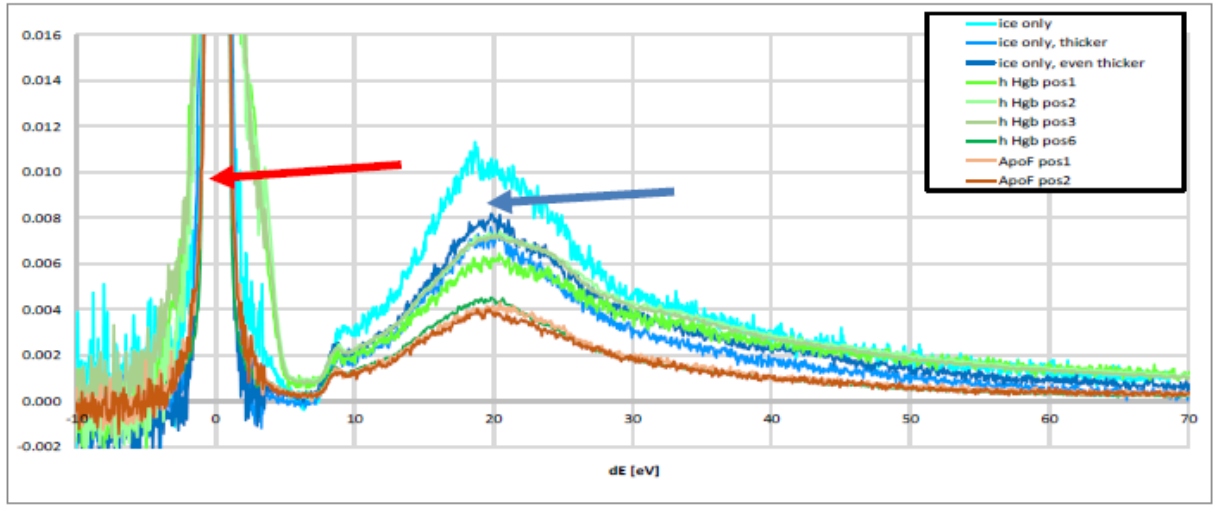


Microscope energy filtering

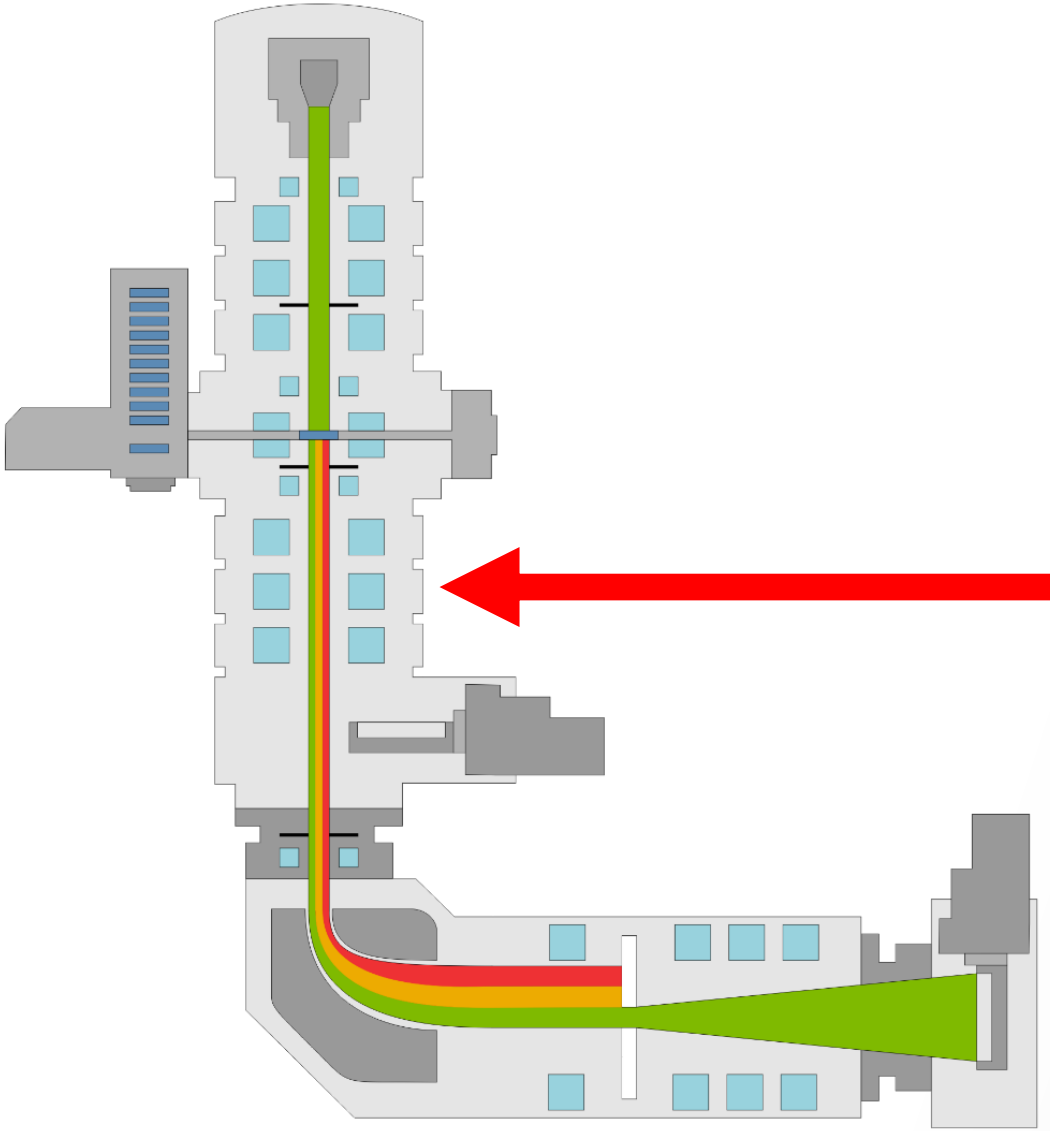


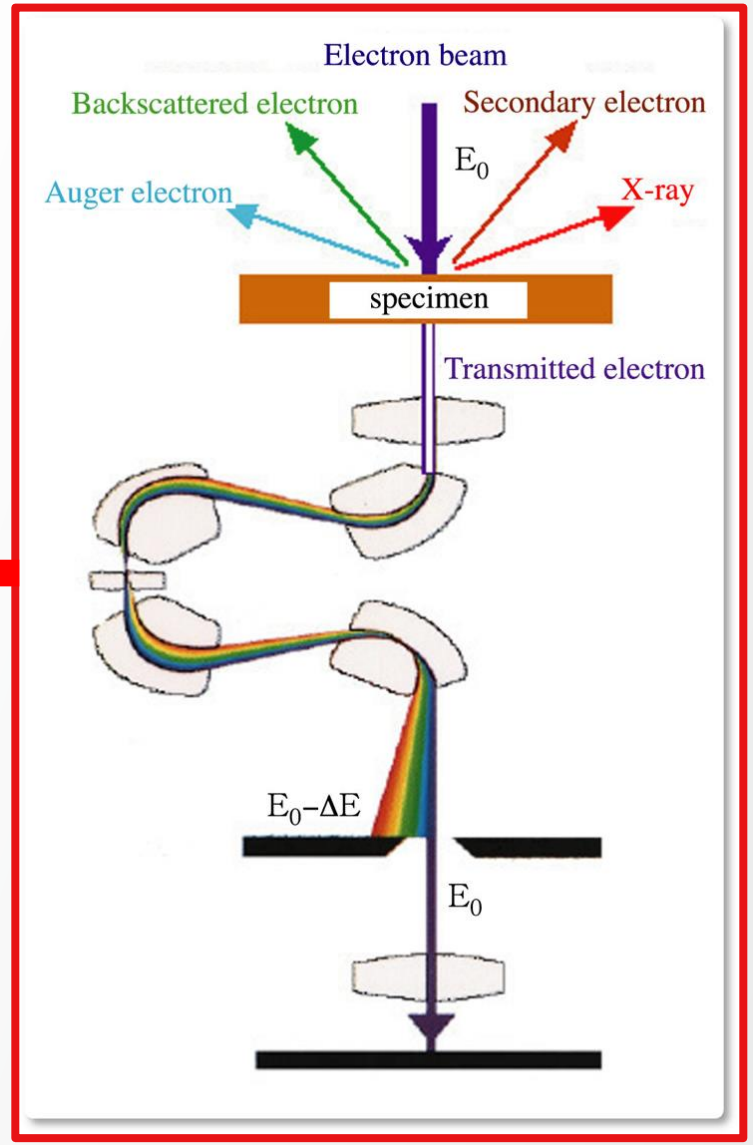
A Koster et al, 1997





Microscope energy filtering, post vs in-column





Microscope basics phase contrast

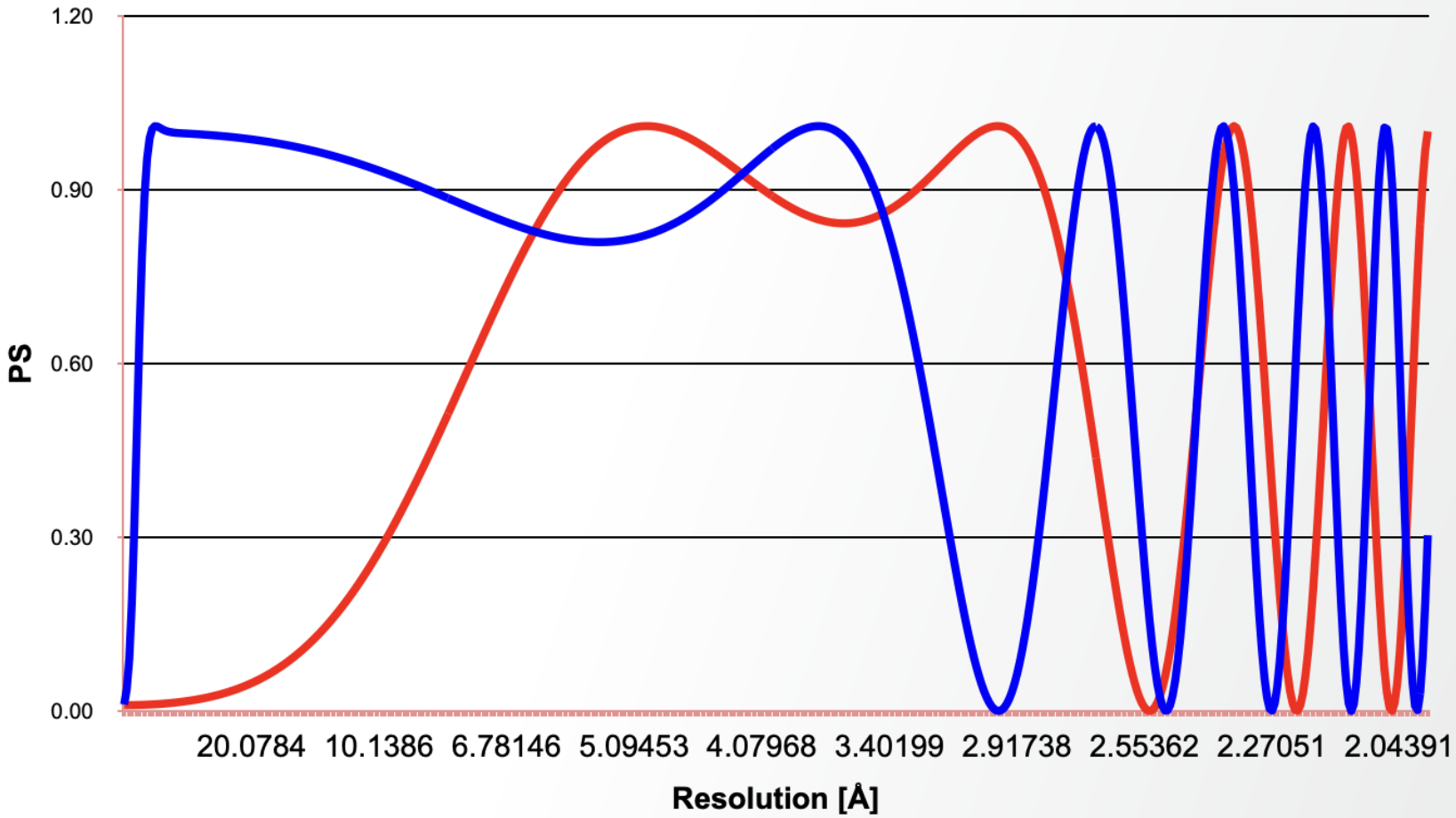
PHASE CONTRAST, A NEW METHOD FOR THE MICROSCOPIC OBSERVATION OF TRANSPARENT OBJECTS

by F. ZERNIKE, Groningen

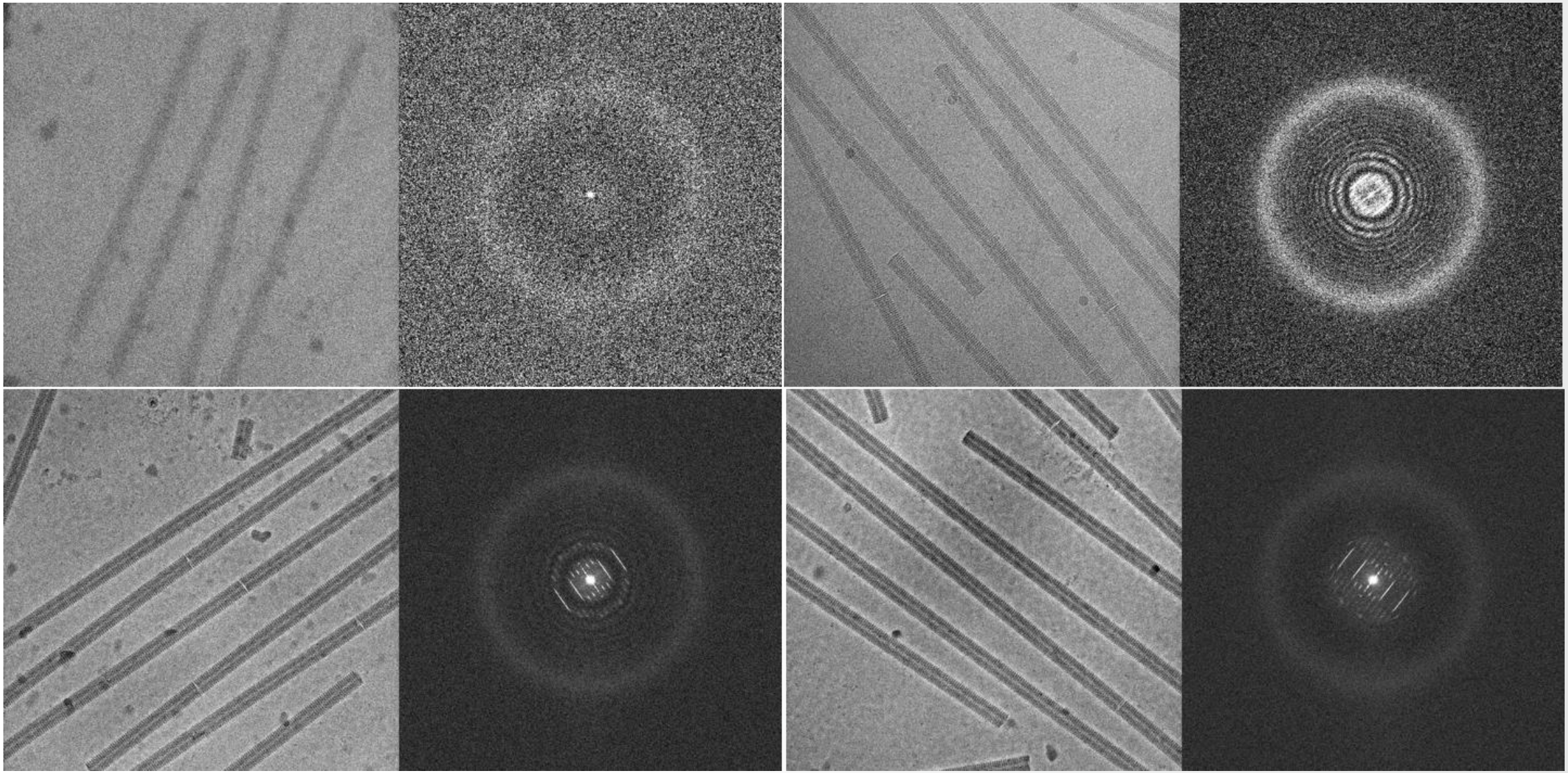
PART I

Every microscopist knows that transparent objects show light or dark contours under the microscope in different ways varying with change of focus and depending on the kind of illumination used.

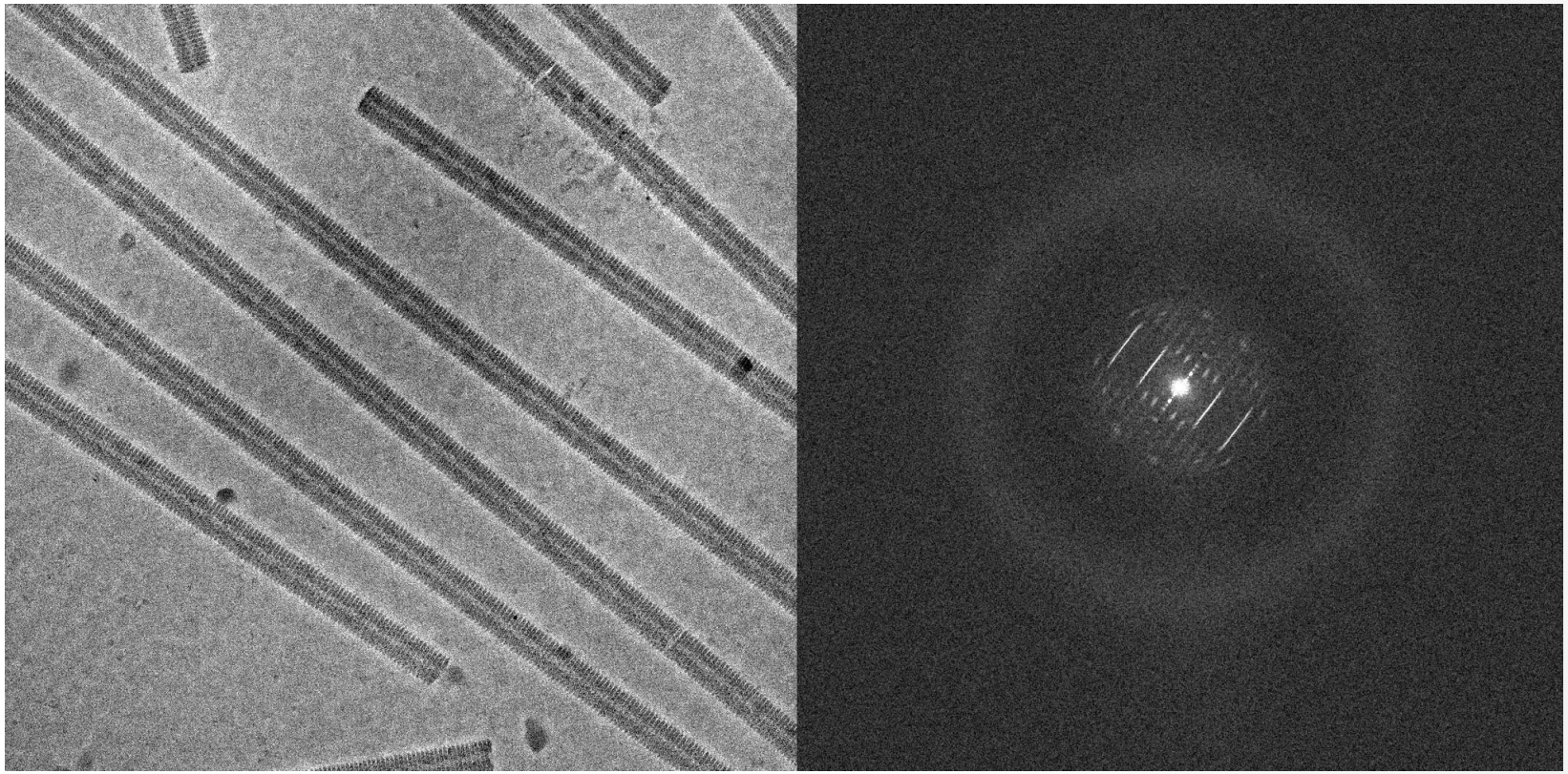
Microscope basics phase contrast

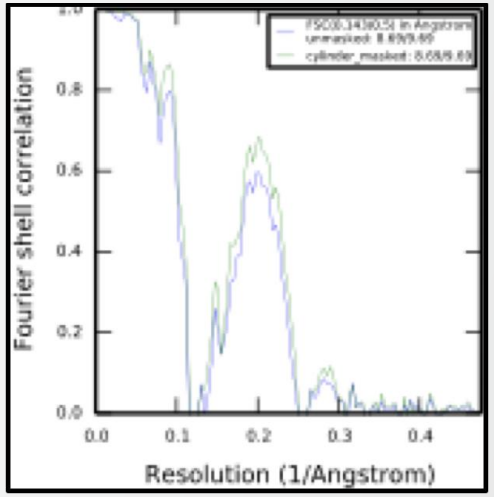


Microscope basics phase contrast

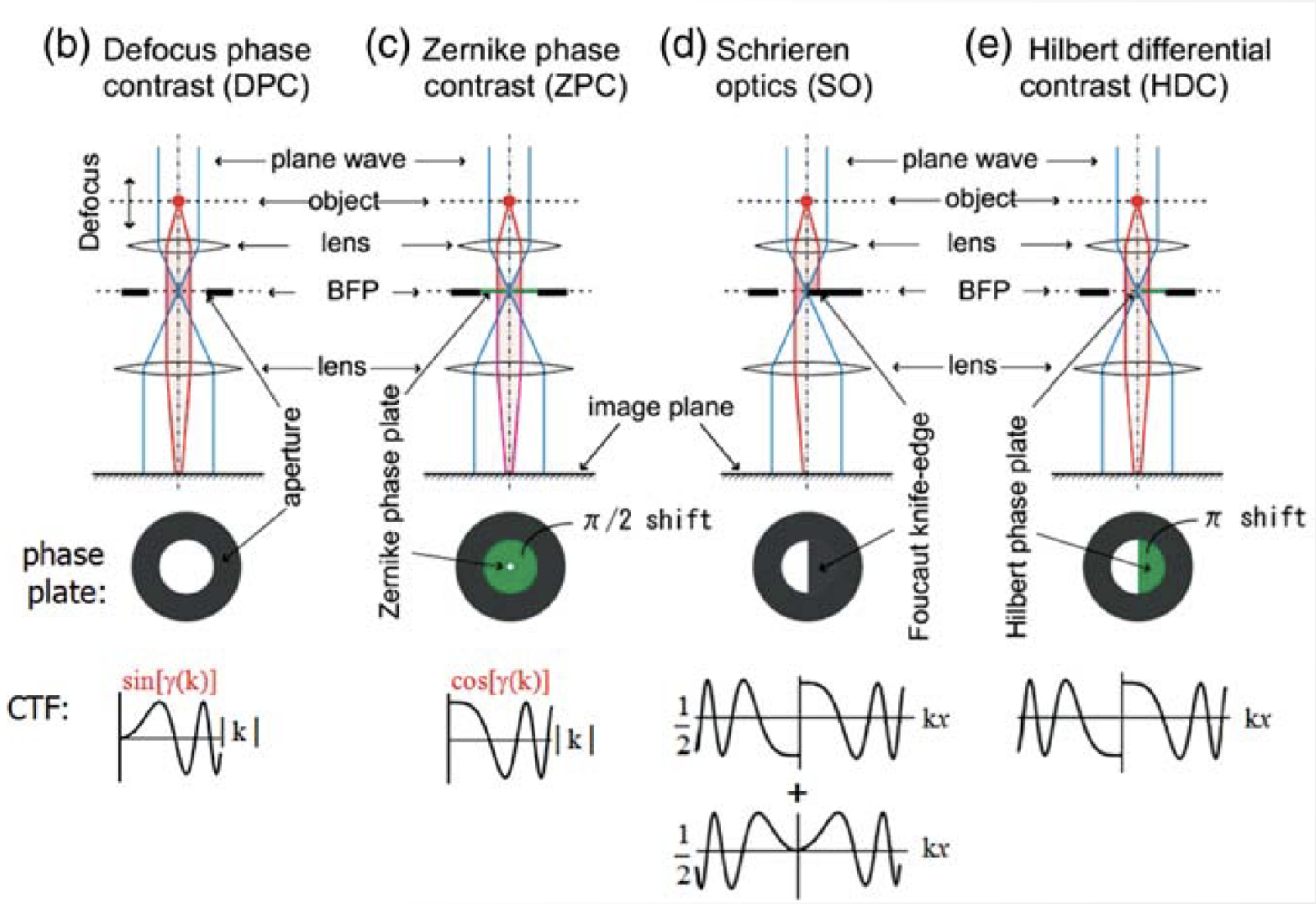


Microscope basics phase contrast



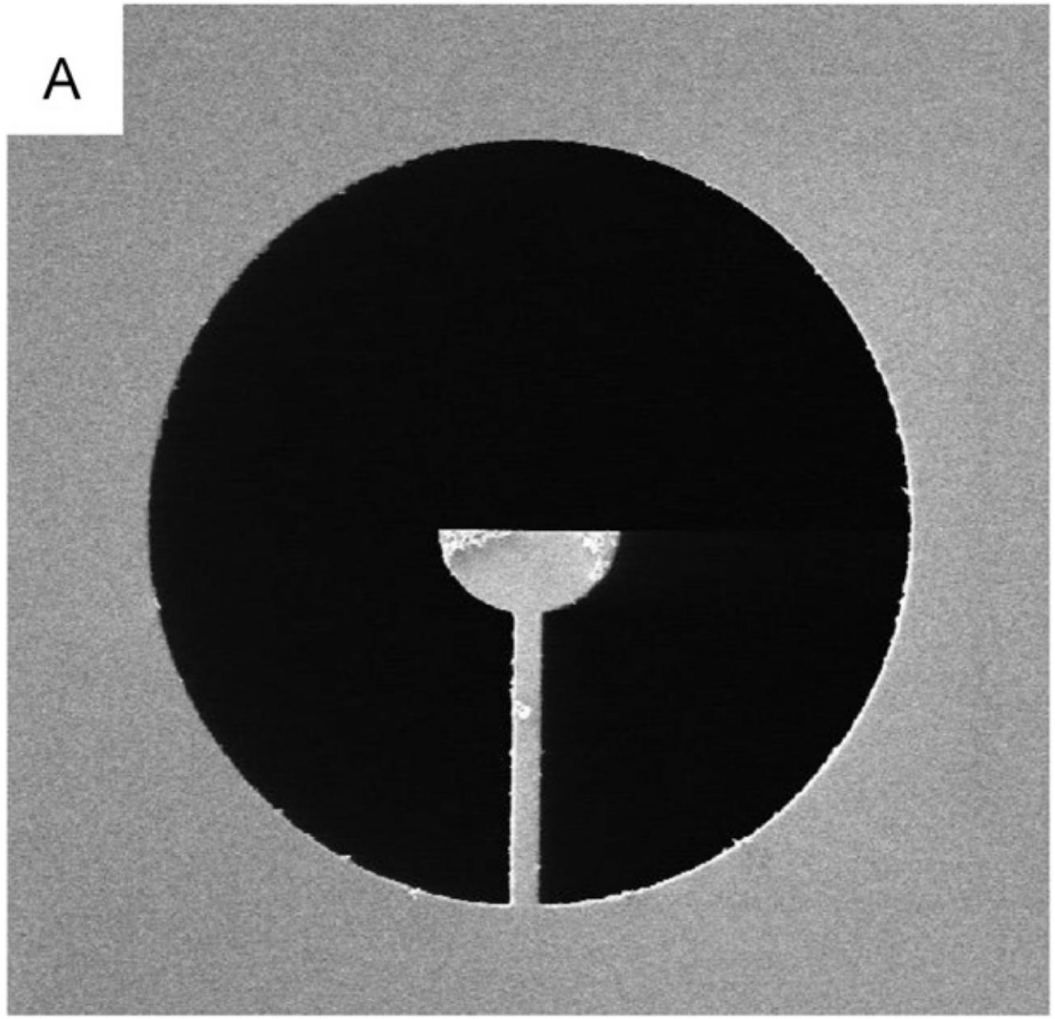


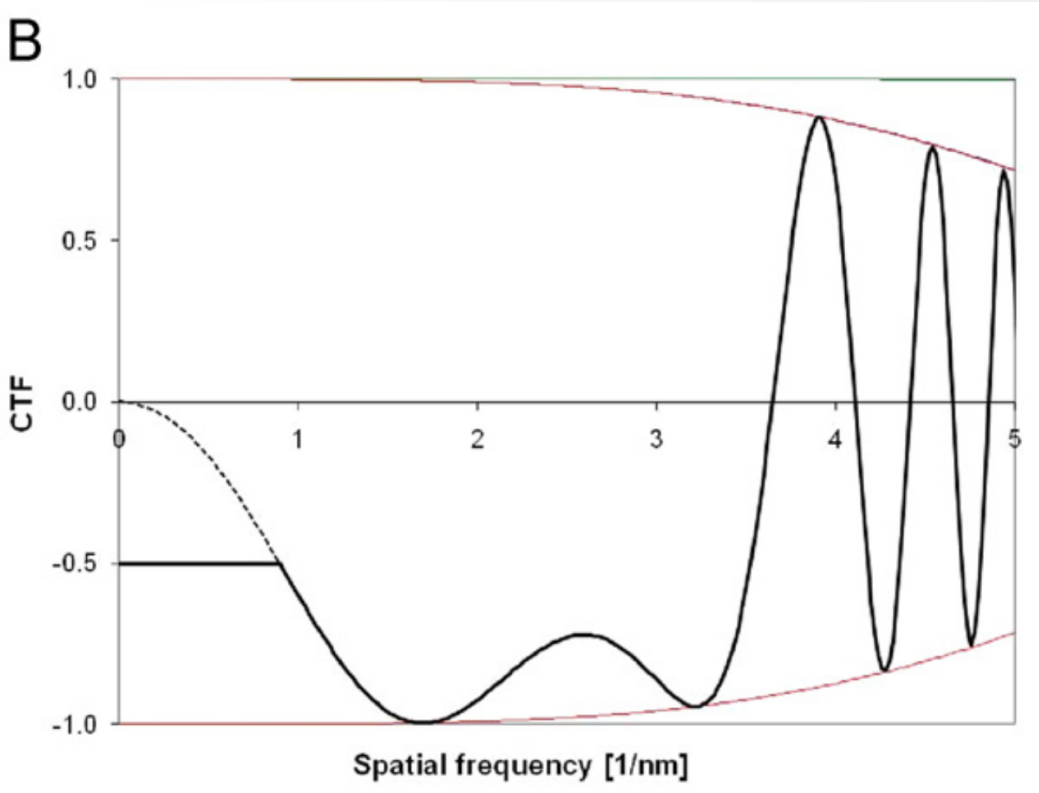
Microscope basics phase contrast



Nagayama, K. (2011).
Another 60 years in electron microscopy: development of phase-plate electron microscopy and biological applications.
Journal of Electron Microscopy, 60(supplement 1), S43–S62. http://doi.org/10.1093/jmicro/dfr037

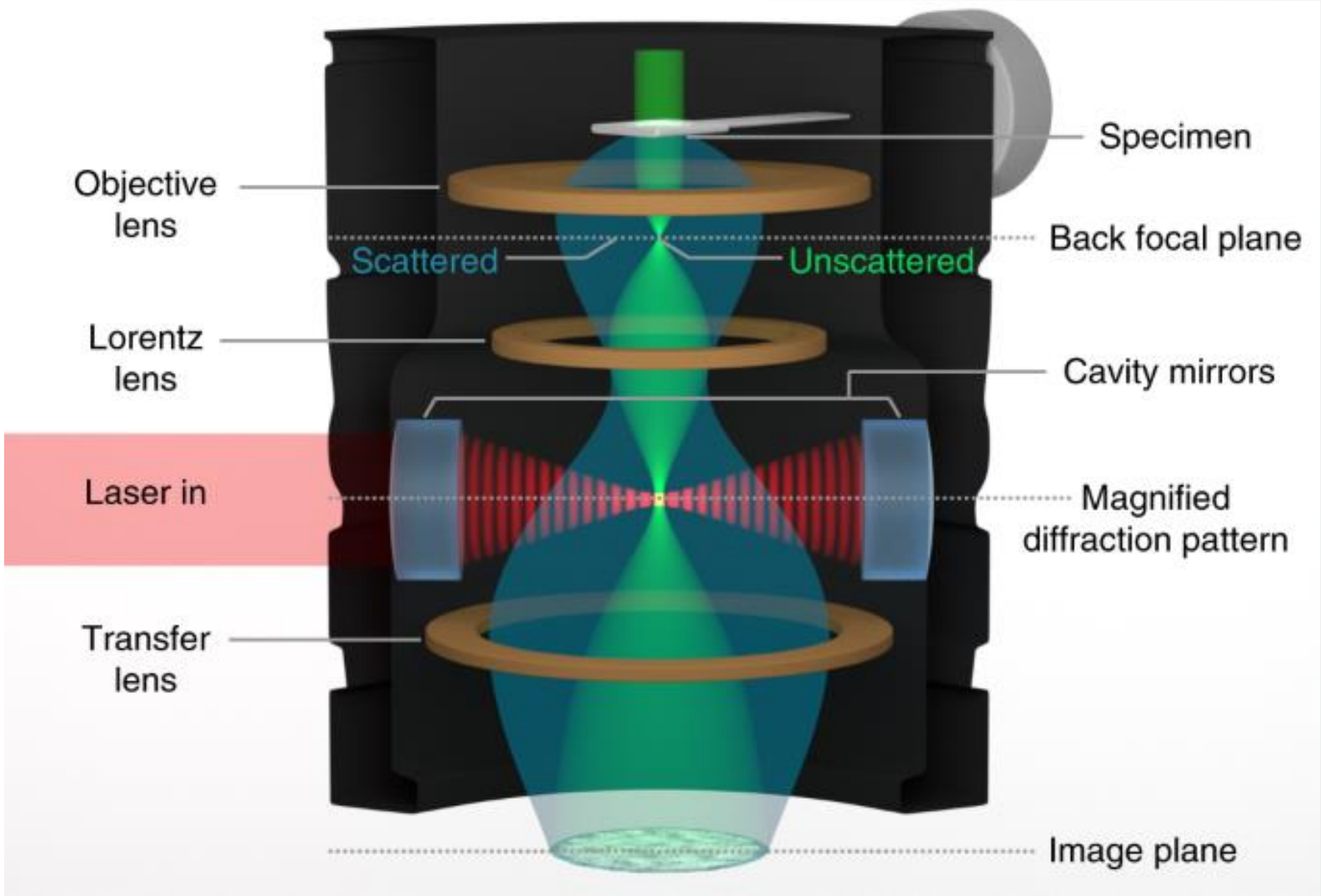
Microscope basics phase contrast





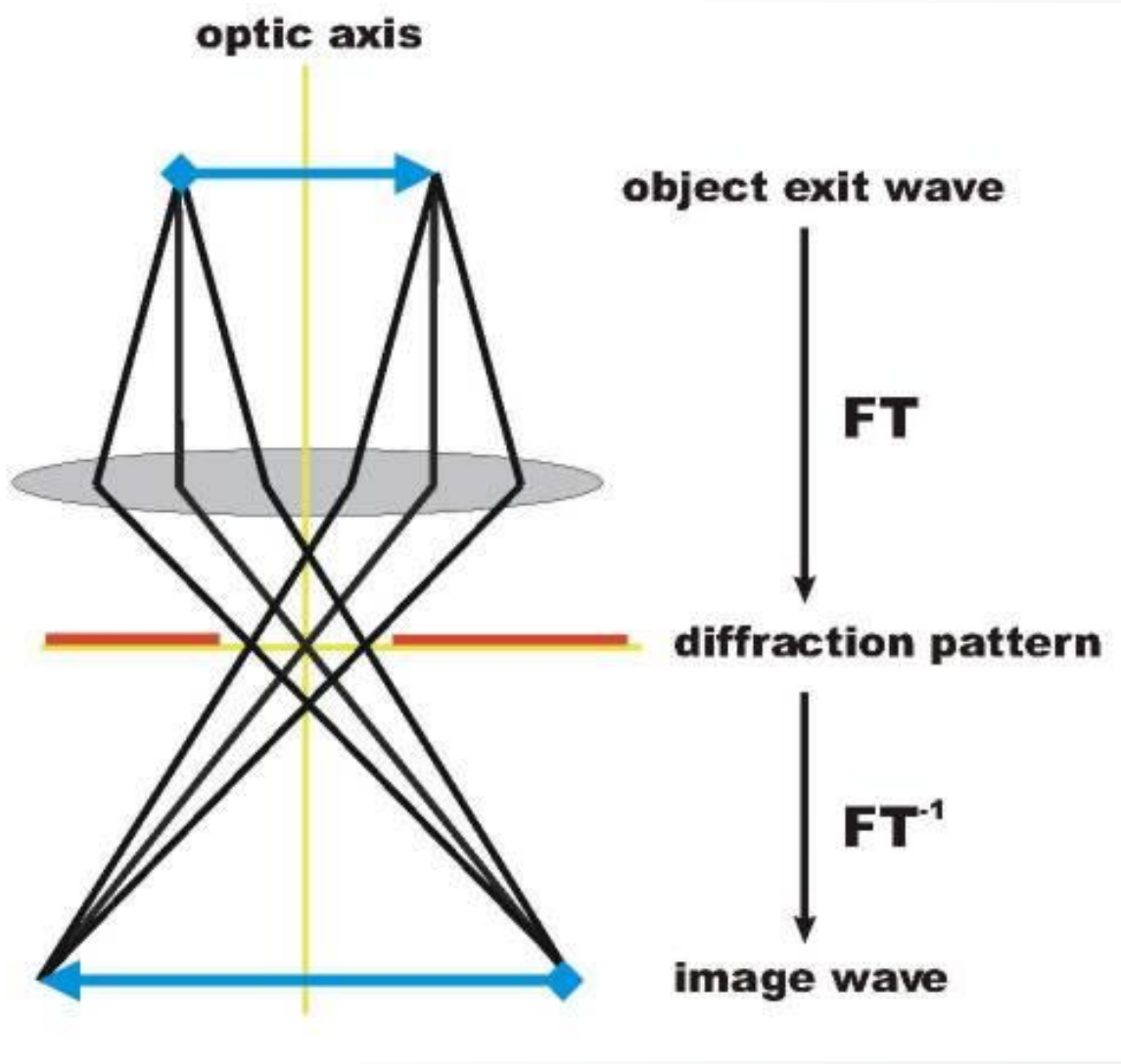
Buijsse, B., van Laarhoven, F. M. H. M., Schmid, A. K., Cambie, R., Cabrini, S., Jin, J., & Glaeser, R. M. (2011). Design of a hybrid double-sideband/single-sideband (schlieren) objective aperture suitable for electron microscopy. Ultramicroscopy, 111(12), 1688–1695. http://doi.org/10.1016/j.ultramic.2011.09.015

Microscope basics phase contrast



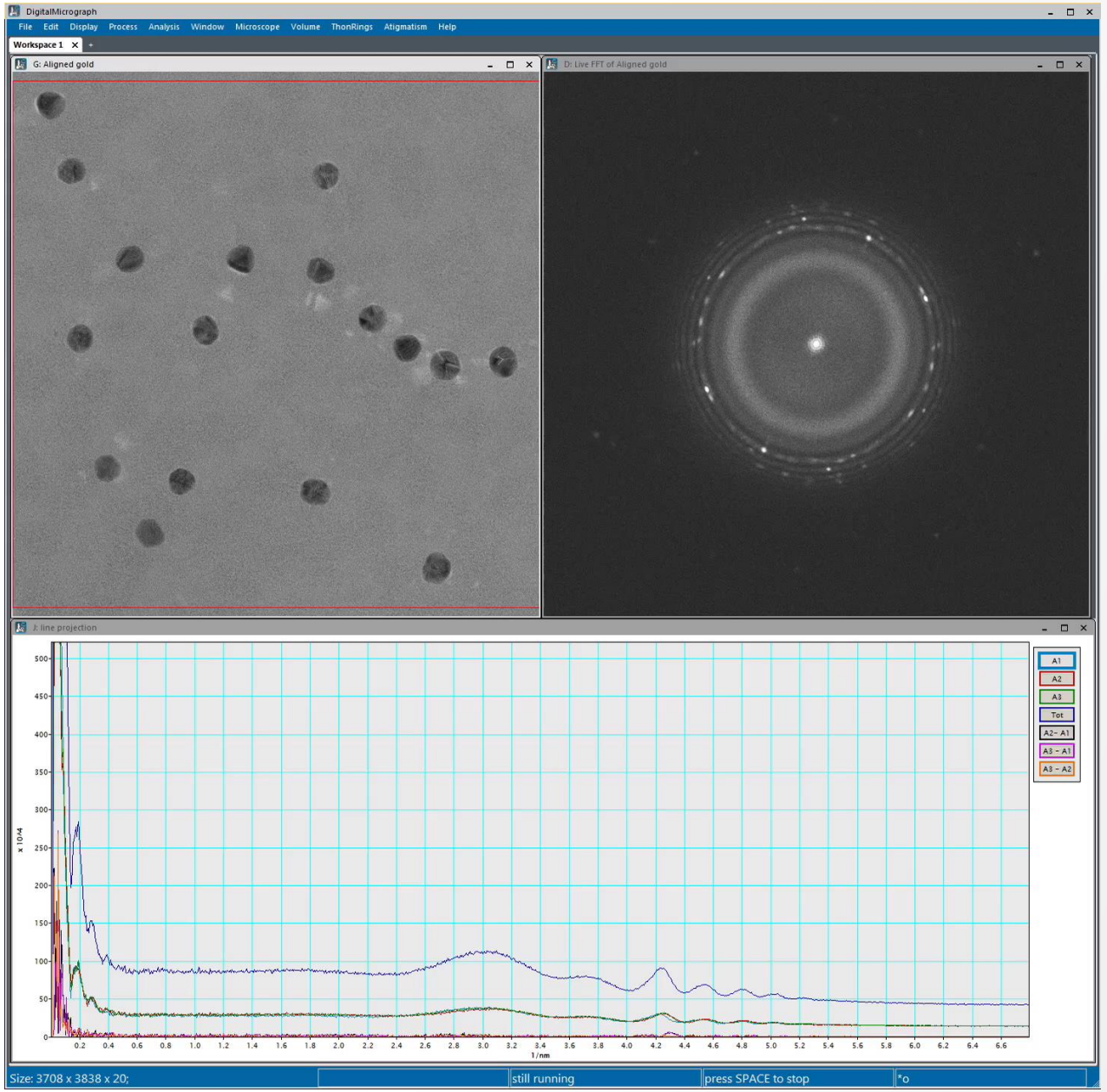
Schwartz, Osip, Jeremy J. Axelrod, Sara L. Campbell, Carter Turnbaugh, Robert M. Glaeser, and Holger Müller.
"Laser Phase Plate for Transmission Electron Microscopy."
Nature Methods 16, no. 10 (October 1, 2019): 1016–20. https://doi.org/10.1038/s41592-019-0552-2.

Microscope basics



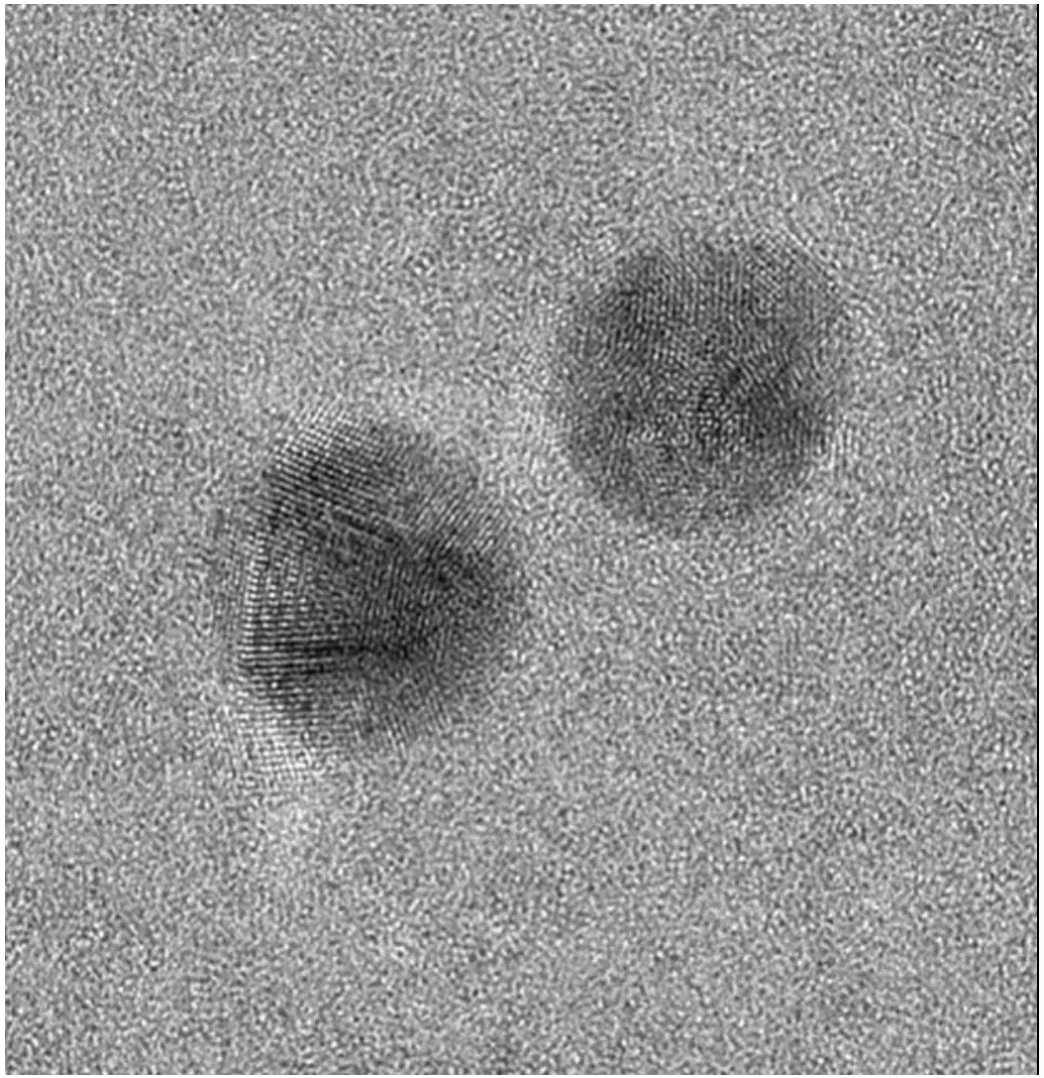
https://www.microscopy.ethz.ch/TEM_mathe.htm

Delocalization

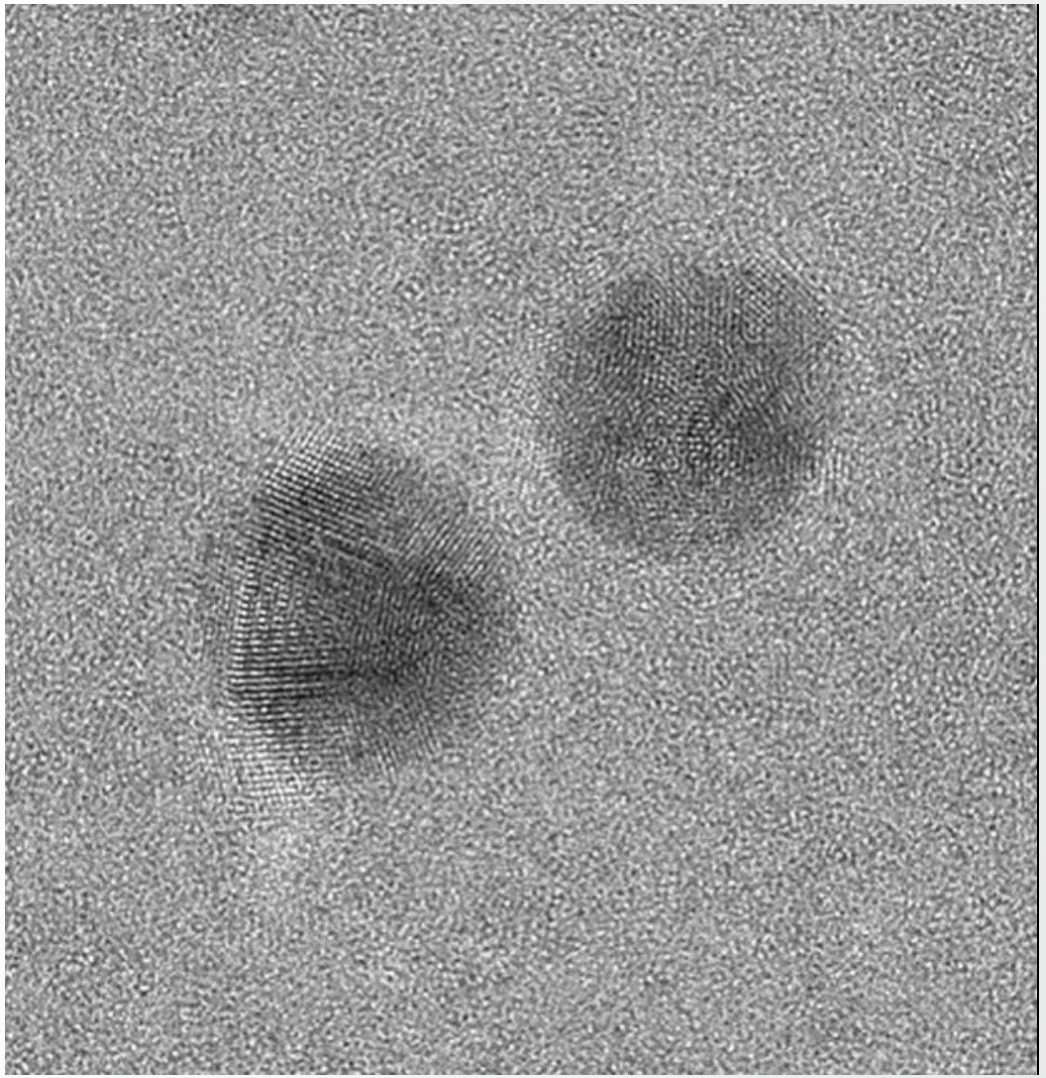


Delocalization

Raw



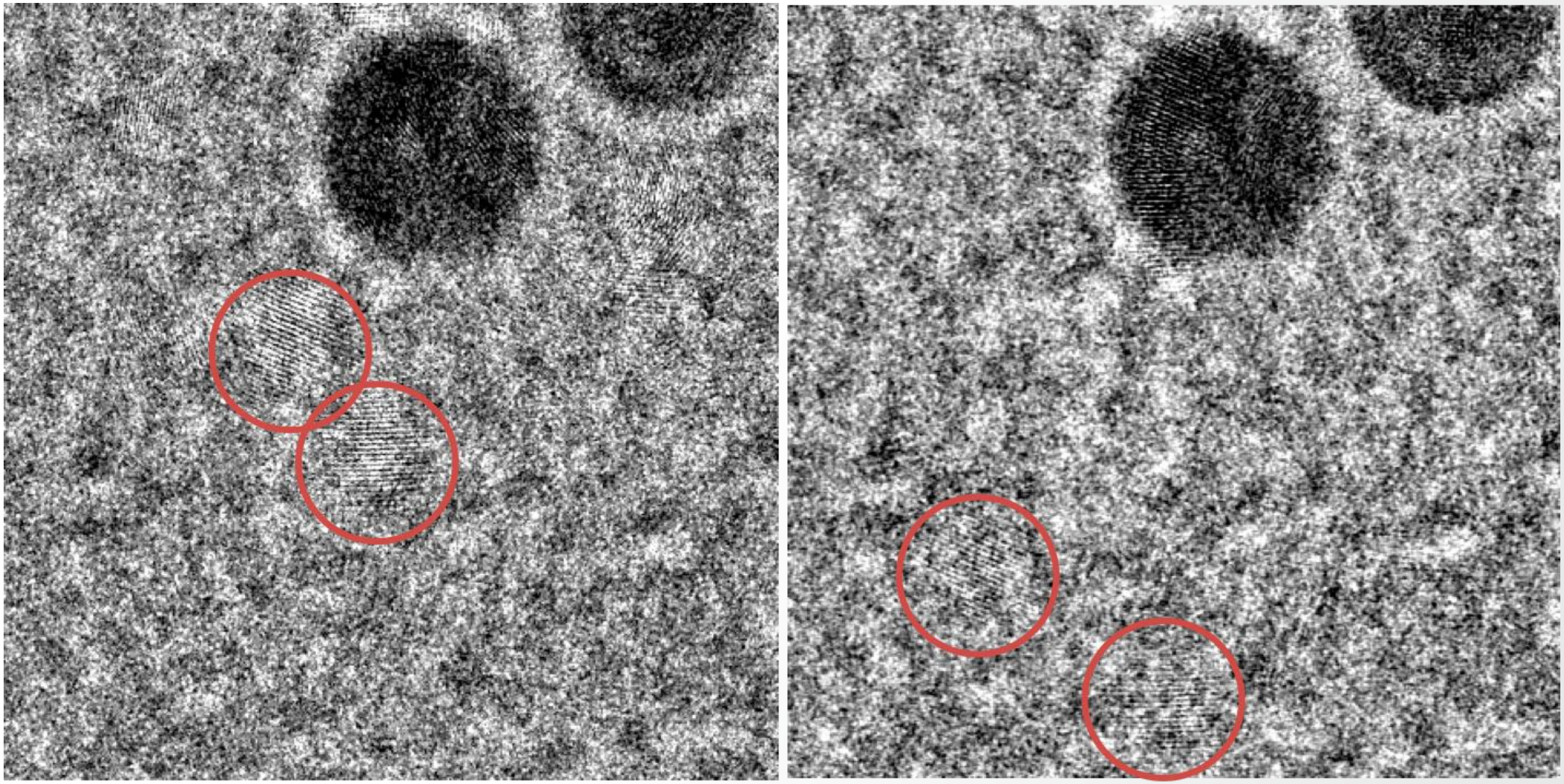
Phase-flipped



Delocalization

Raw

Phase-flipped

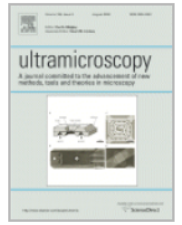


Delocalization

ELSEVIER

Ultramicroscopy

Volume 108, Issue 9, August 2008, Pages 921-928



Restoration of weak phase-contrast images recorded with a high degree of defocus: The “twin image” problem associated with CTF correction

Kenneth H. Downing, Robert M. Glaeser

Microscope

- Optics are good but can still be made better (BCOR, monochromator, Cc-correction?).
- Should we fix certain aberrations in hardware or software (e.g. Relion, cryoSPARC)
- Camera is the biggest limitation instrument-wise.

Sample prep is the biggest problem in cryo-EM!

Cameras

- *Film*
- *Imaging plates*
- Electronic image converters
- CCD
- Direct detection
- Electron counting

Cameras, electronic image converters

Cameras, electronic image converters

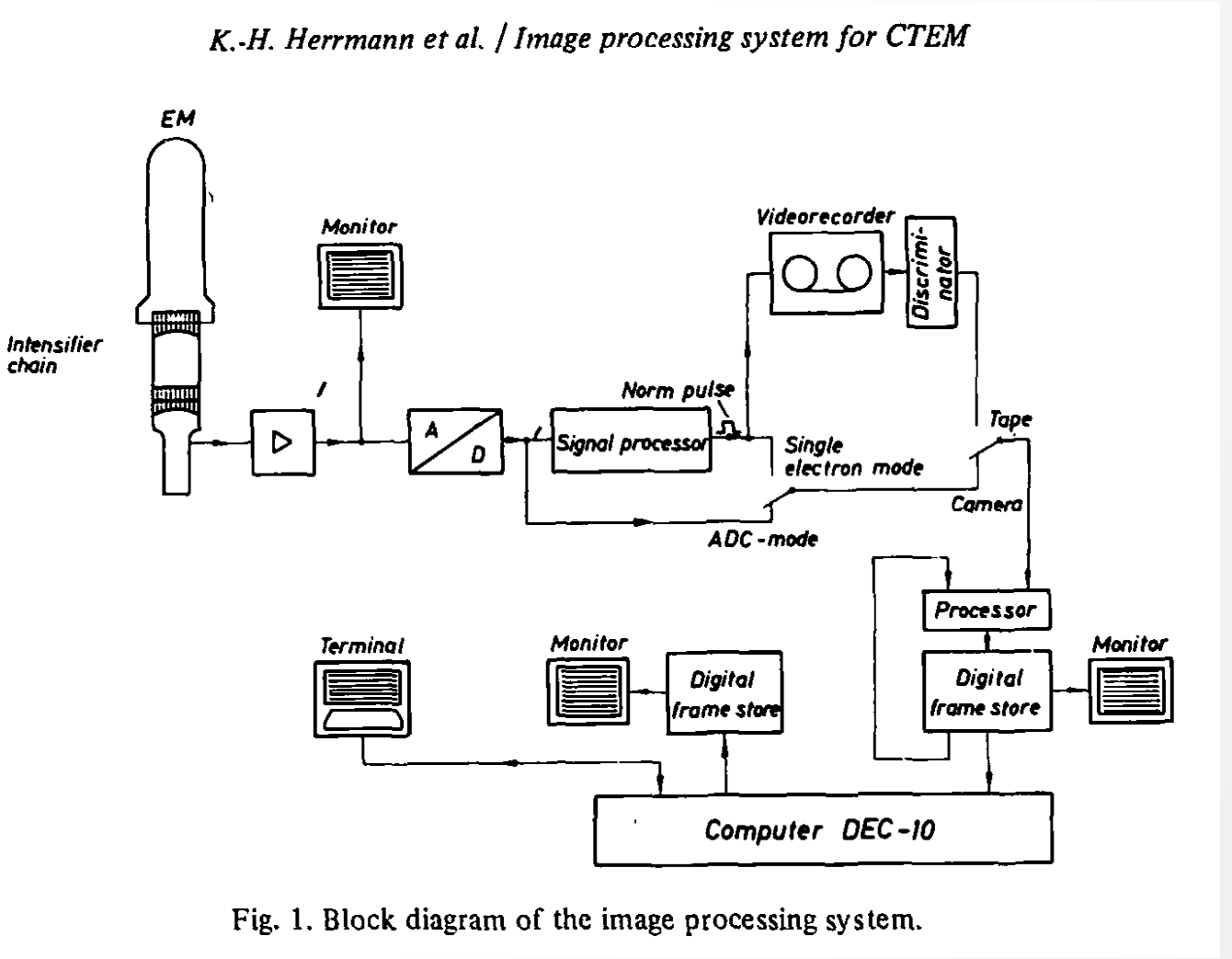
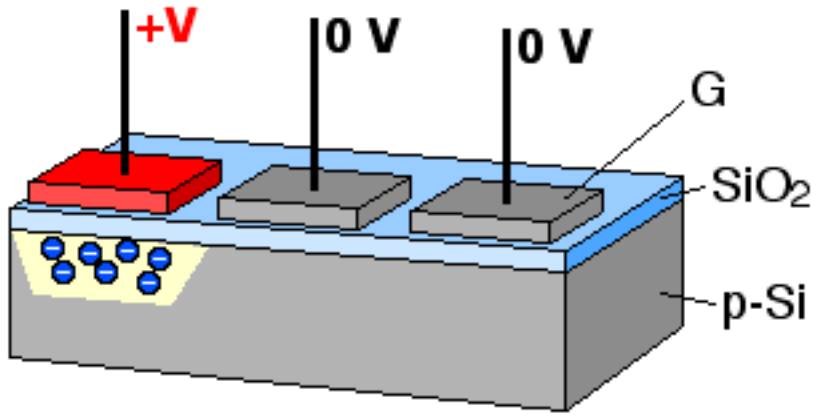


Fig. 1. Block diagram of the image processing system.

A tv system.or image recording and processing in conventional transmission electron microscopy
K.H. Herrmann, D. KRAHL and H.P. Rust
Institut fiir Elektronenmikroskopie, Fritz-Haber-Institut 1978

Cameras, slow scan charged coupled device

- Direct electrons damage the chip.
- Direct electrons saturate the pixel fast.
- Compromise:
  electrons to light, light to CCD.
- Incident photons build up charge.
- Charged cells are readout.
- All cells are reset for next image.
- Performance decreases when increasing accelerating voltage.



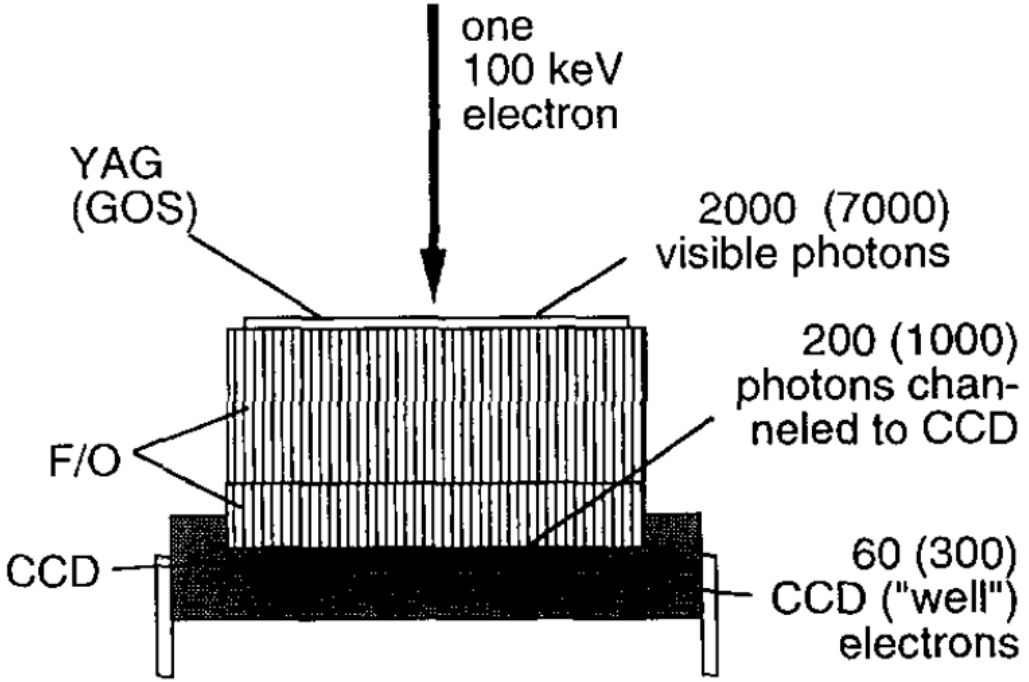


Fig. 1. Schematic diagram illustrating the detection of one 100 keV electron by a slow-scan CCD camera using a YAG scintillator (or GOS phosphor) and a fiber-optic coupling. The numbers appropriate to the GOS phosphor are shown in brackets.

Applications of slow-scan CCD cameras in transmission electron microscopy
O.L. Krivanek and P.E. Mooney
Gatan Research and Development, 6678 Owens Driue, Pleasanton, CA 94588, USA 1992

Cameras, slow scan charged coupled device

Scintillator:
- yttrium-aluminum garnet single-crystal scintillator (YAG).
- gadolinium oxysulphide powder phosphor (GOS).

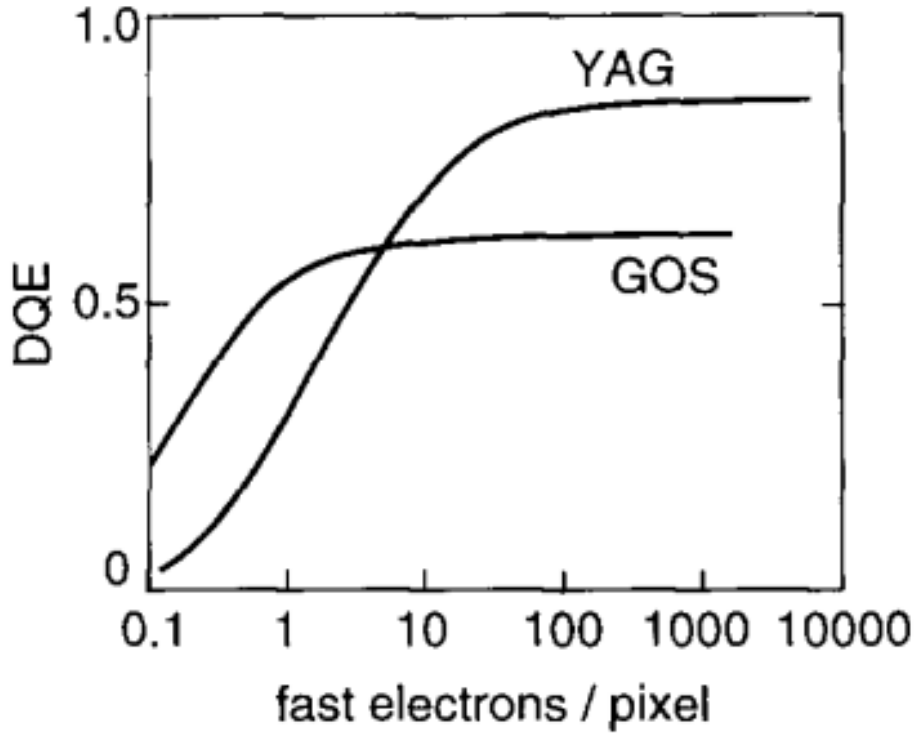


Fig. 4. Detective quantum efficiency (DQE) in gain-normalized 100 keV images recorded by slow-scan CCD cameras utilizing YAG and GOS scintillators.

Applications of slow-scan CCD cameras in transmission electron microscopy
O.L. Krivanek and P.E. Mooney
Gatan Research and Development, 6678 Owens Driue, Pleasanton, CA 94588, USA 1992

Cameras, slow scan charged coupled device

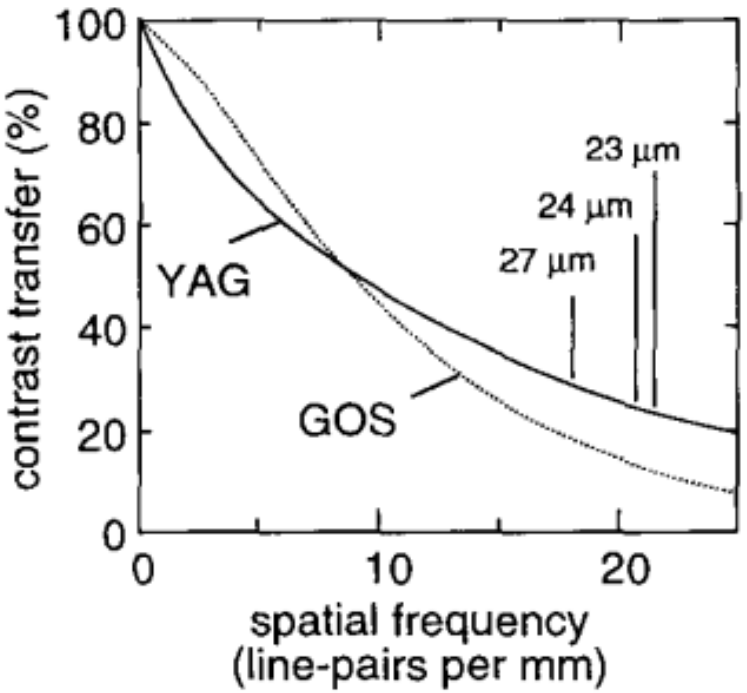
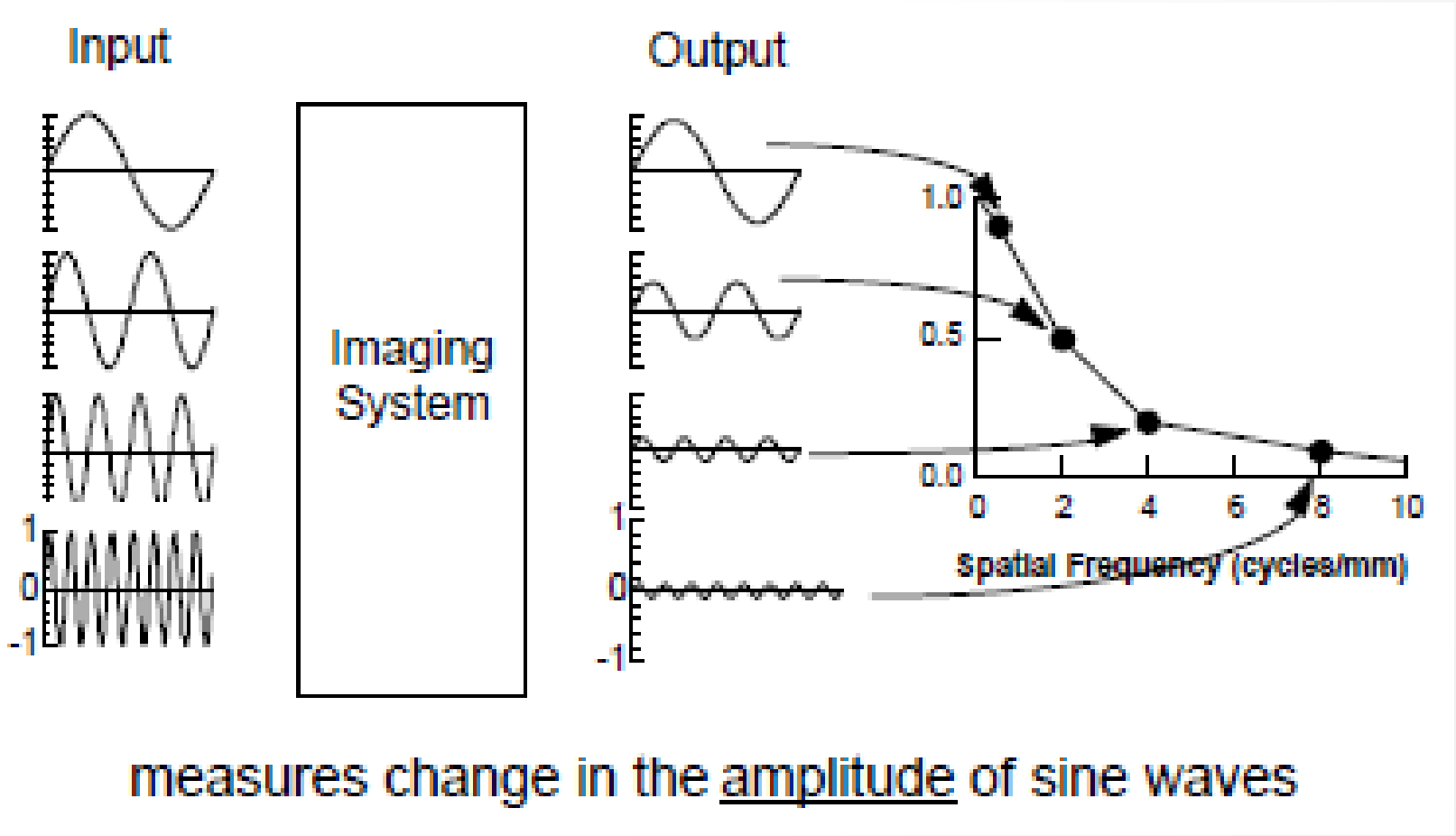


Fig. 3. Modulation transfer function (MTF) of slow-scan CCD cameras using YAG and GOS scintillators. The Nyquist limits corresponding to the physical pixel size of the various currently available CCD chips suitable for slow-scan operation are indicated by the vertical lines.

Applications of slow-scan CCD cameras in transmission electron microscopy
O.L. Krivanek and P.E. Mooney
Gatan Research and Development, 6678 Owens Driue, Pleasanton, CA 94588, USA 1992

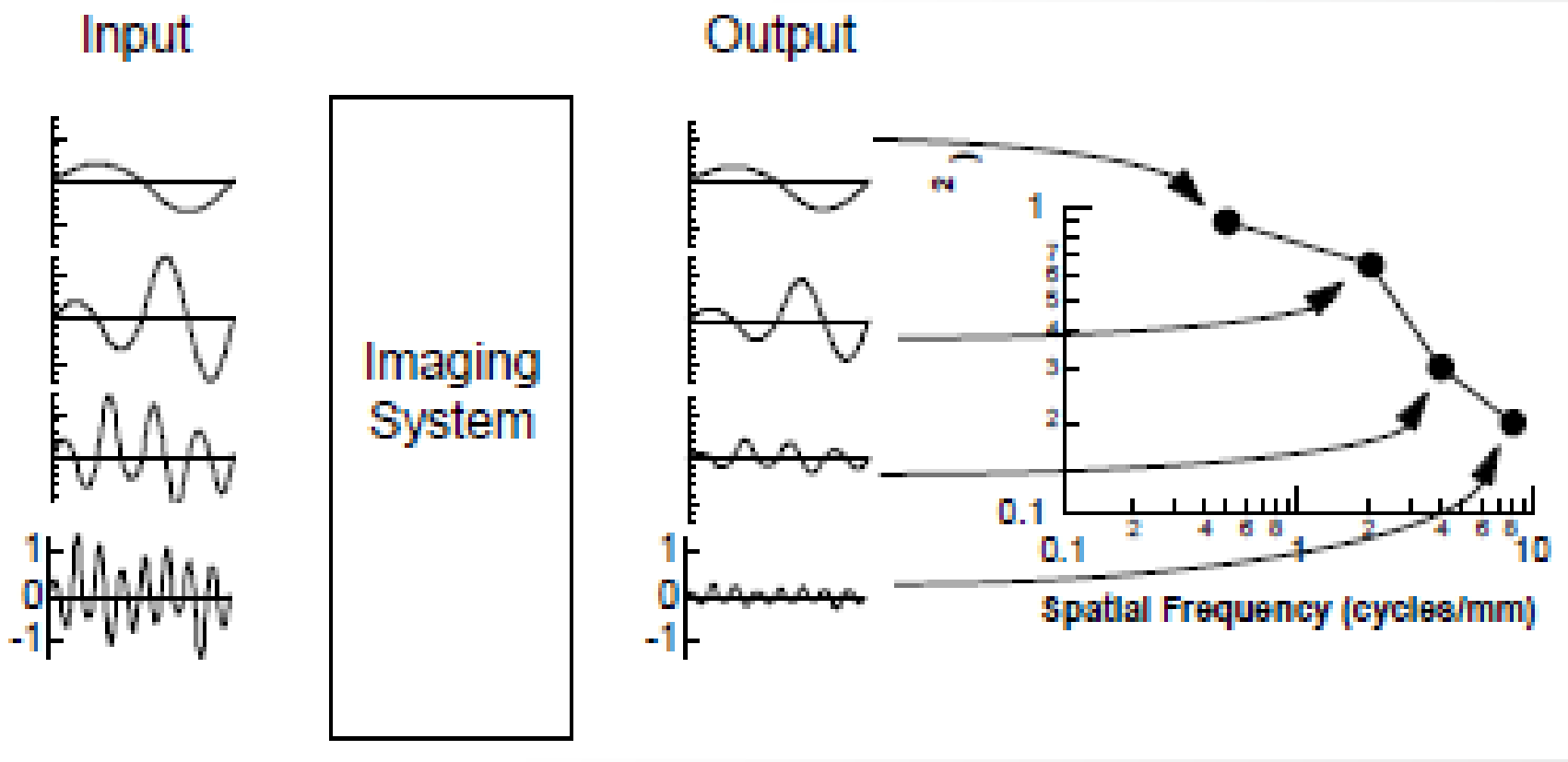
Cameras, MTF

Modulation **T**ransfer **F**unction, ratio of output to input modulation as a function of spatial frequency.



Cameras, NPS

Noise **P**ower **S**pectrum, change in variation in the amplitude of sine waves



Cameras, DQE

$$DQE(\omega) = \frac{SNR_{out}^2(\omega)}{SNR_{in}^2(\omega)}$$

$SNR_{out}(\omega)$ = SNR in the output image

$SNR_{in}(\omega)$ = SNR incident on the detector

$$DQE(\omega) = \frac{Q\ MTF^2(\omega)}{W(\omega)} \left(\frac{dO}{dQ}\right)^2$$

where $MTF(\omega)$ = MTF of detector

$W(\omega)$ = noise power spectrum of image

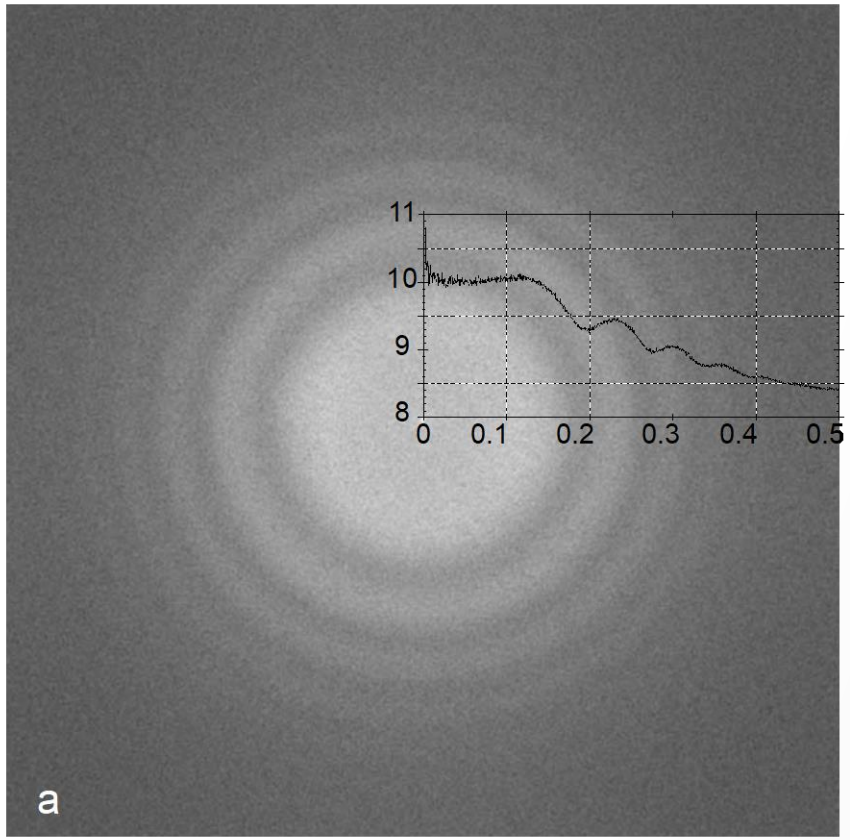
$\frac{dO}{dQ}$ = gain of the system

Cameras, decelerator

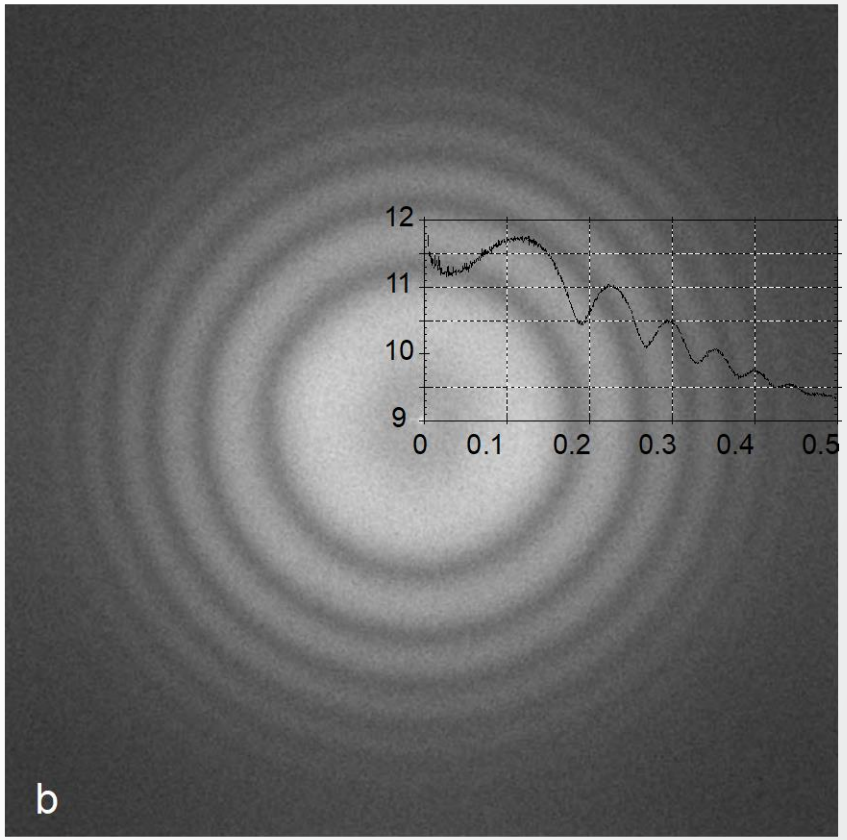
A charge coupled device camera with electron decelerator for intermediate voltage electron microscopy
Kenneth H. Downing and Paul E. Mooney 2008

Cameras, decelerator

0 kV



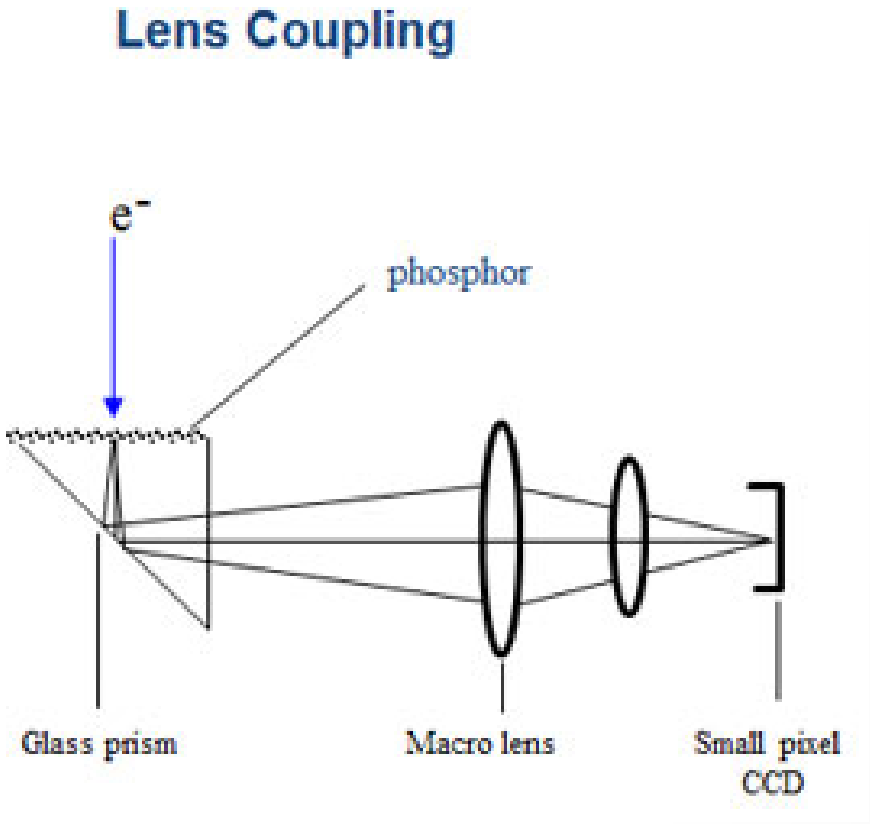
250 kV



A charge coupled device camera with electron decelerator for intermediate voltage electron microscopy
Kenneth H. Downing and Paul E. Mooney 2008

Cameras, UltraCam

- A transmission- scintillator lens-coupled back-illuminated 4Kx4K CCD camera-equipped post-column energy filter



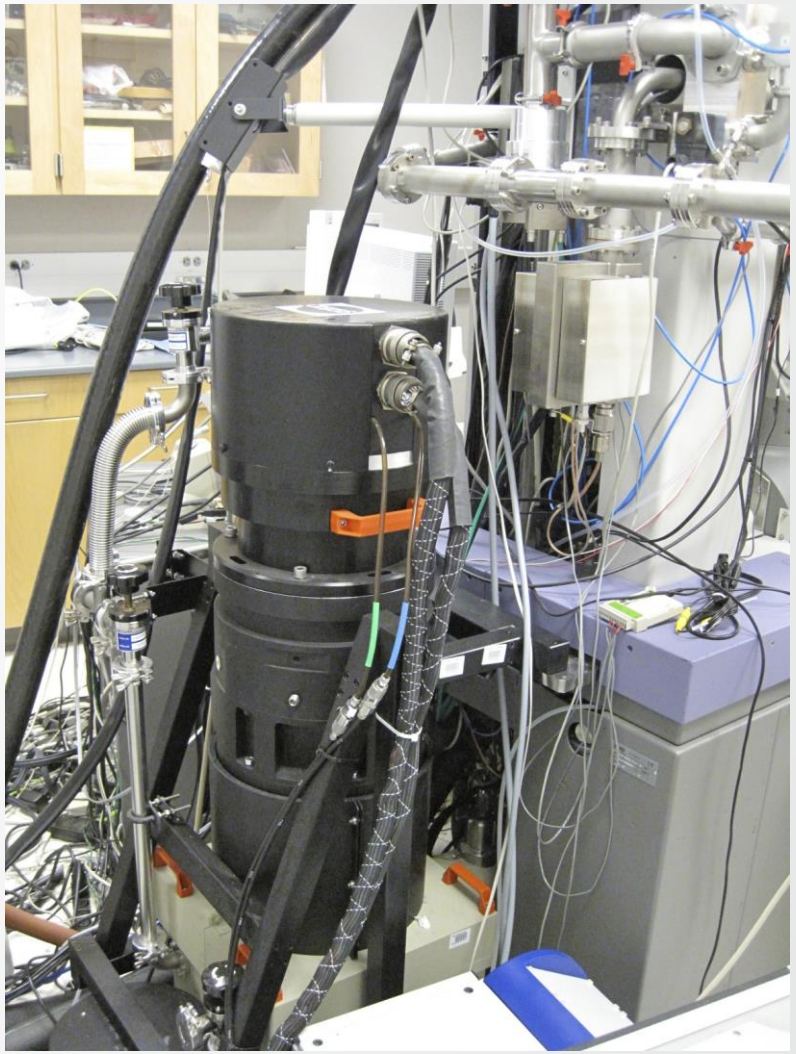
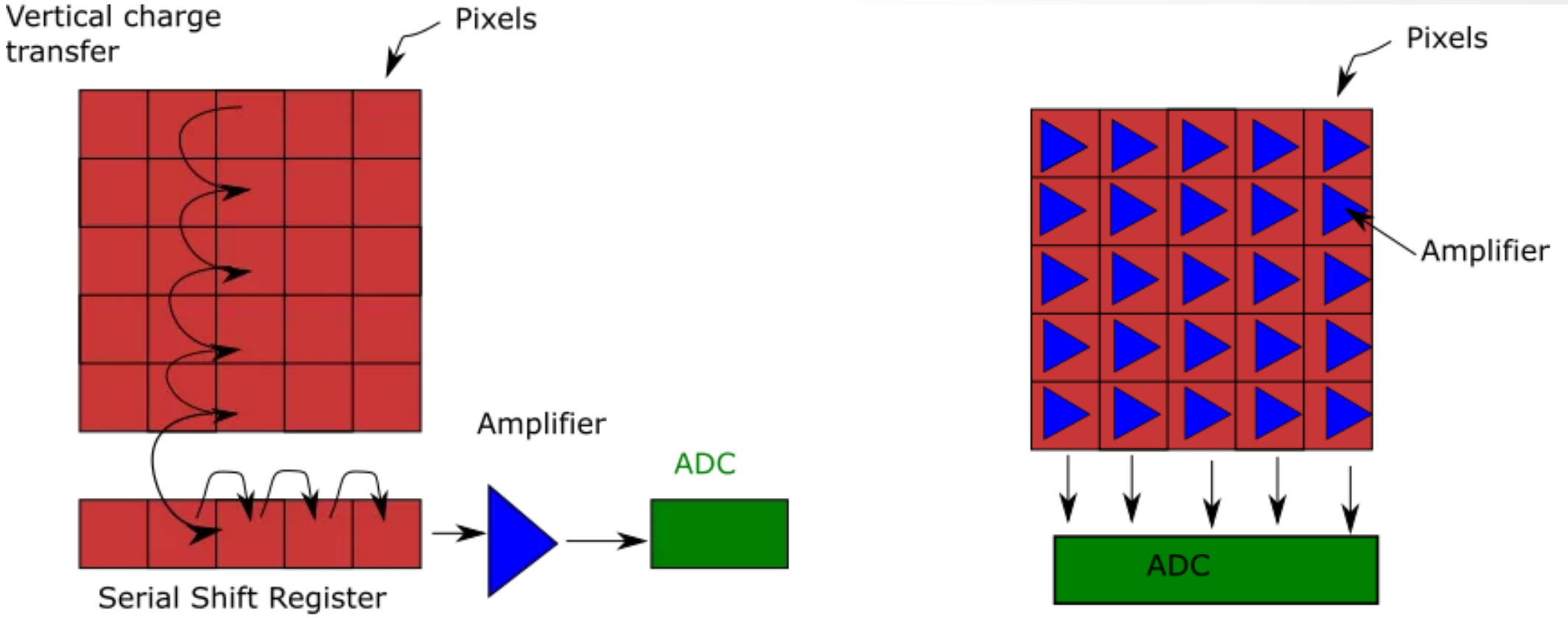


Image Information Transfer through a Post-Column Energy Filter with Detection by a Lens-Coupled Transmission-Scintillator CCD Camera
U. Luecken*, P. Tiemeijer*, M. Barfels**, P. E. Mooney**, B. Bailey** and D. A. Agard***

Cameras, CCD vs CMOS

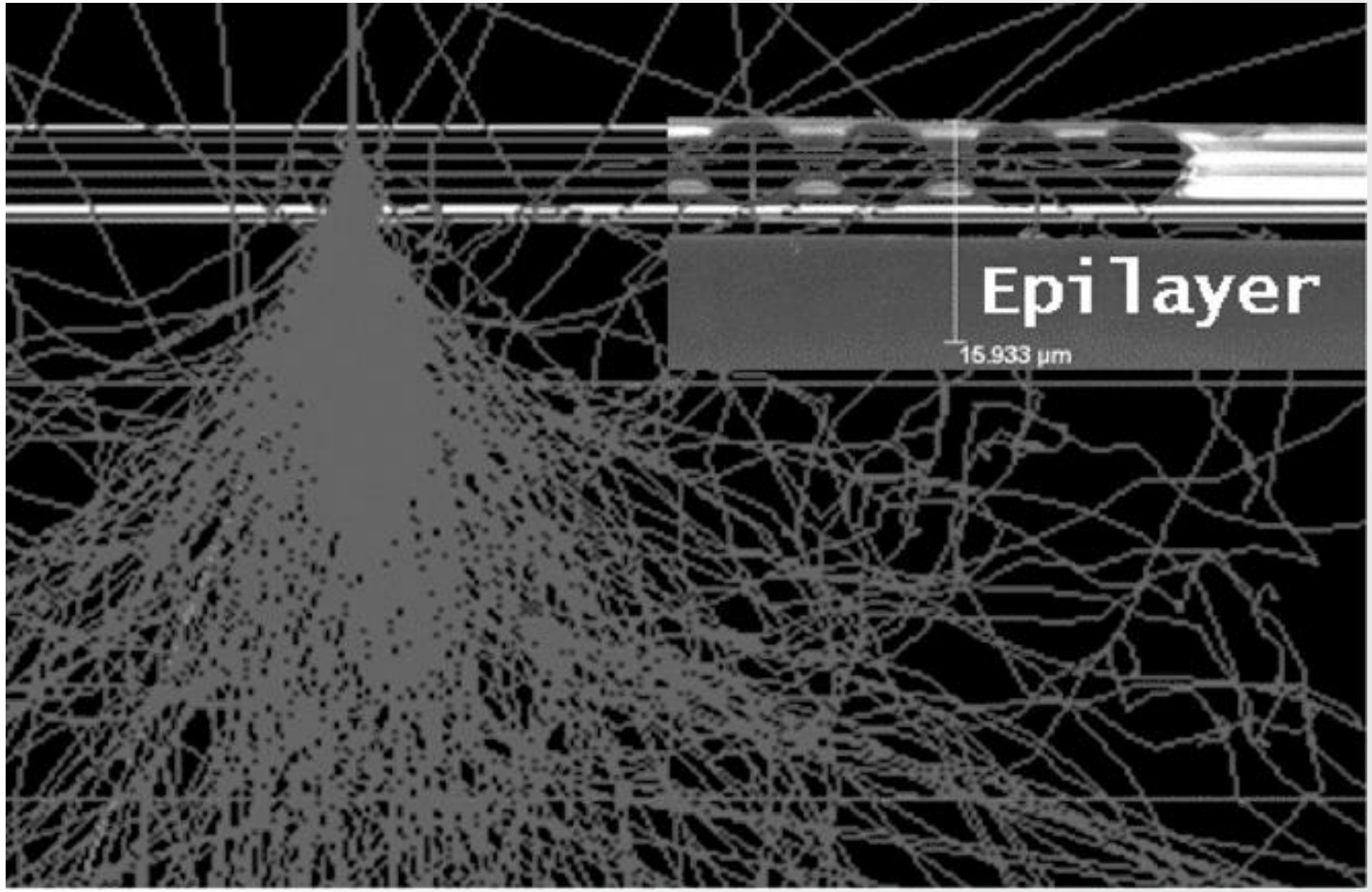


CCD Carrier Transport Process vs. CMOS Reading Process

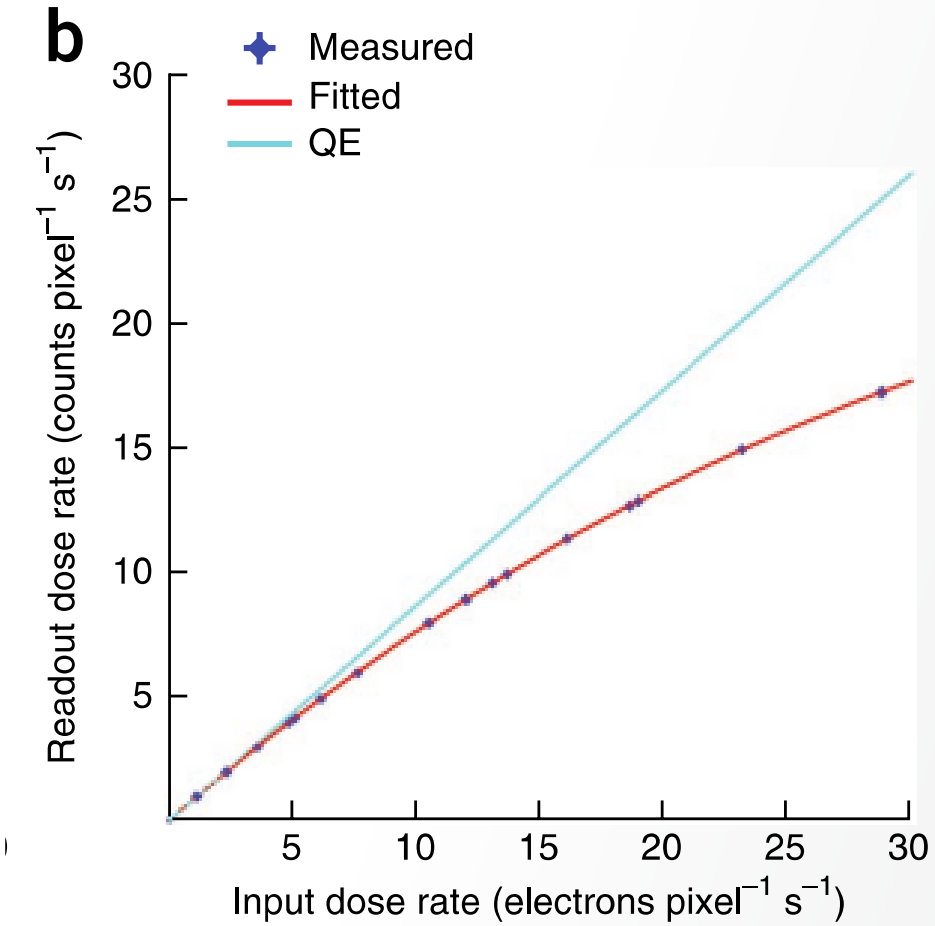
https://www.opticsforhire.com/blog/ccd-vs-cmos-image-sensor-selection/

Cameras, CMOS & direct detection

- Finally, sensors can be made which can withstand high energy electron bombardments!
- Old problem, backscatter: back thinning.
- Noise: counting



Cameras, coincidence loss



electron counting and beam-induced motion correction enable near-atomic-resolution single-particle cryo-em

Xueming Li1, Paul Mooney2, Shawn Zheng1,3, Christopher R Booth2, Michael B Braunfeld1,3, Sander Gubbens2, David A Agard1,3 & Yifan Cheng1

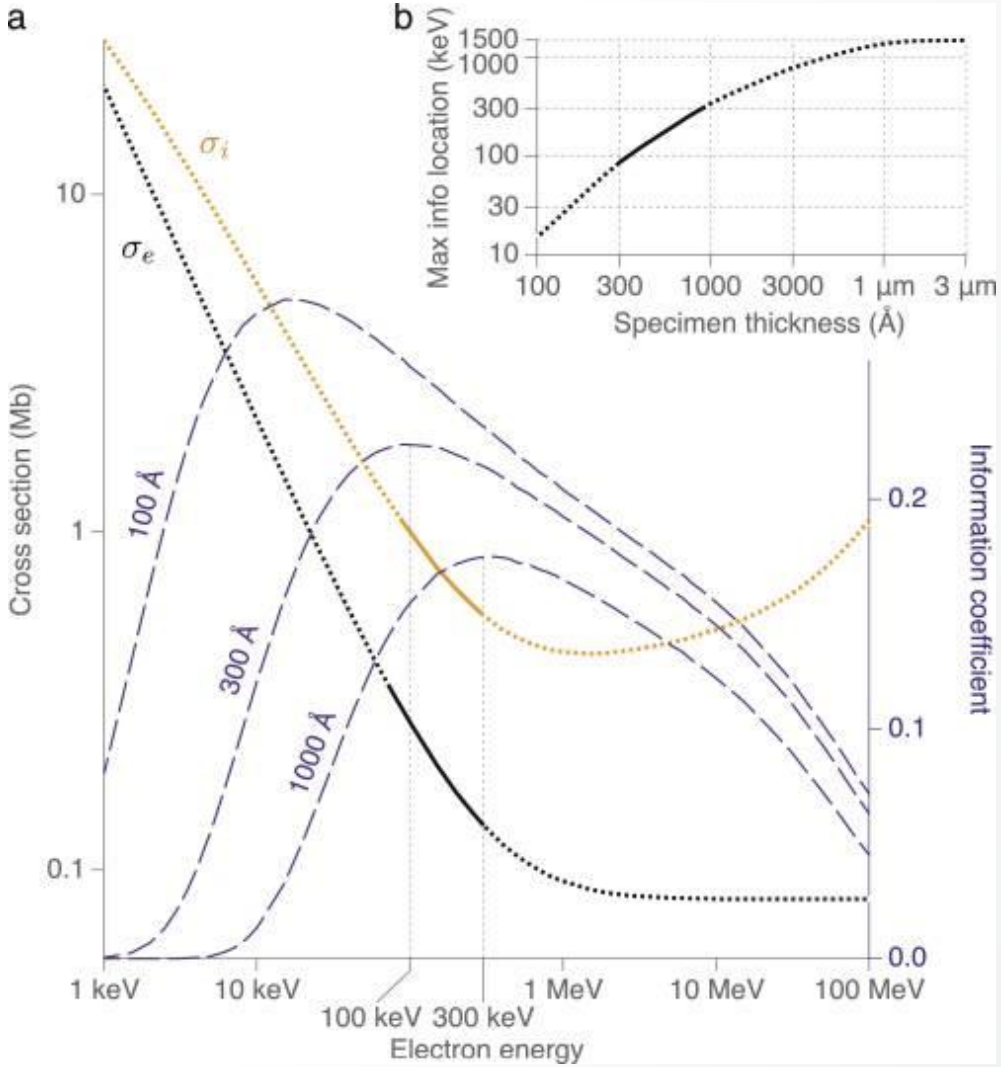
Cameras, coincidence loss

- What we need: large physical pixels, high speed, event based.
- What we have:
  - Small physical pixels, high speed counting (Gatan, Direct Electron).
  - Large physical pixels, lower speed counting, (Thermo Fisher Scientific).
  - Small physical pixels, high speed counting, event based (Direct Electron).

What’s next?

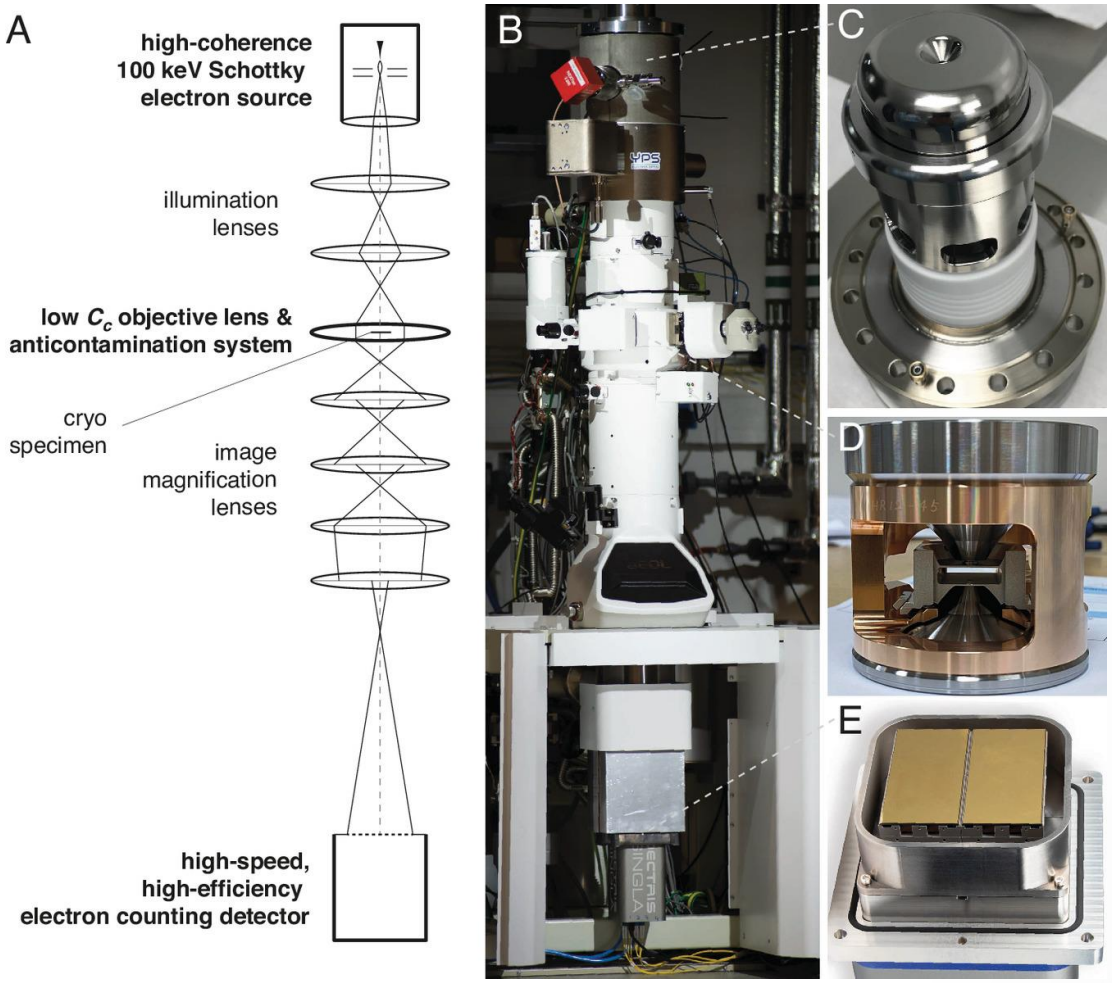
- 100 kV TEM.
- Laser phase plate & aberration correction.
- STEM.

100 kV



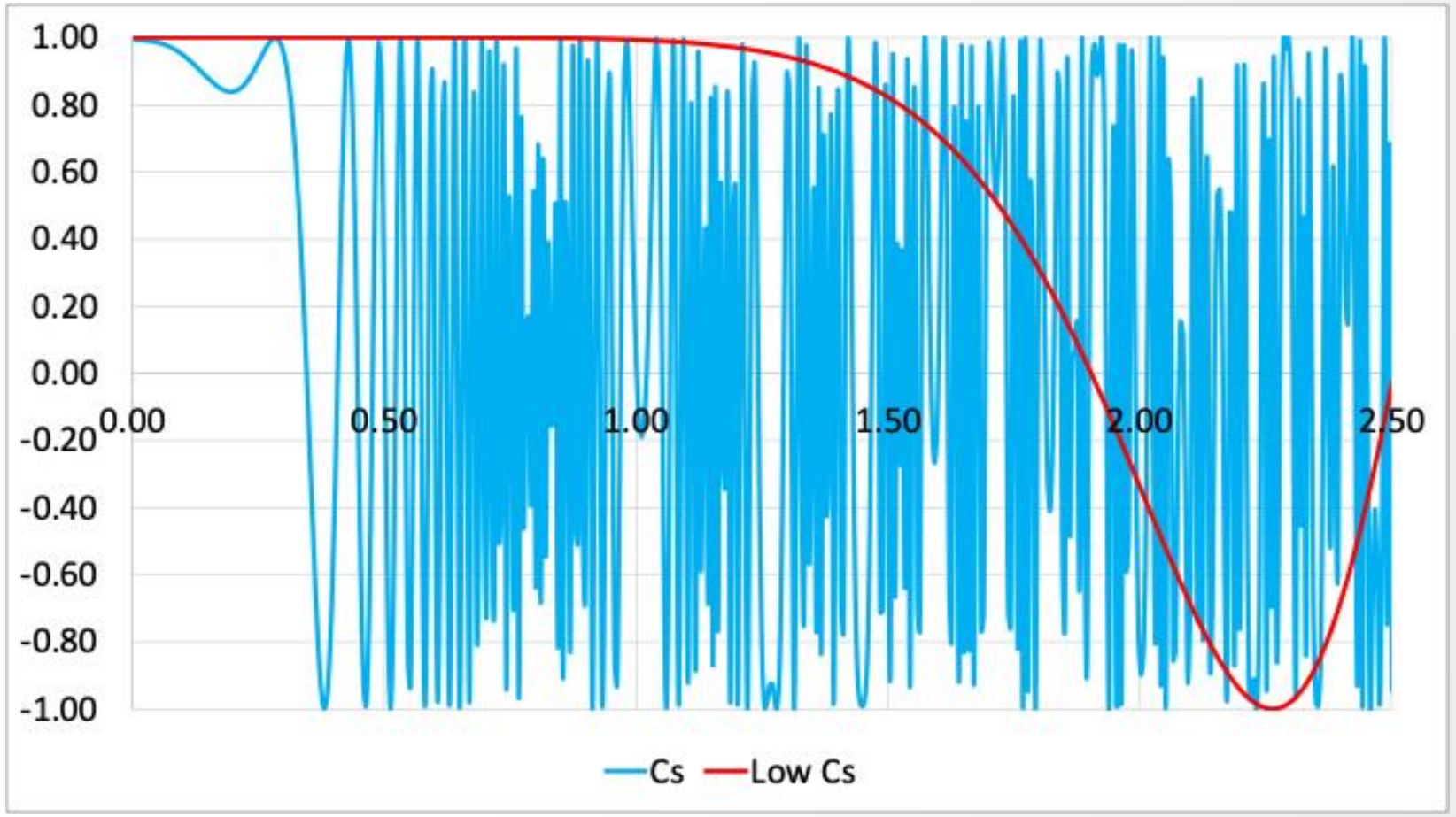
Peet, Mathew J., Richard Henderson, and Christopher J. Russo. “The Energy Dependence of Contrast and Damage in Electron Cryomicroscopy of Biological Molecules.” *Ultramicroscopy* 203 (August 2019): 125–31. https://doi.org/10.1016/j.ultramic.2019.02.007.

100 kV


A
high-coherence
100 keV Schottky
electron source
illumination
lenses
low C_c objective lens &
anticontamination system
cryo
specimen
image
magnification
lenses
high-speed,
high-efficiency
electron counting detector
B
C
D
E

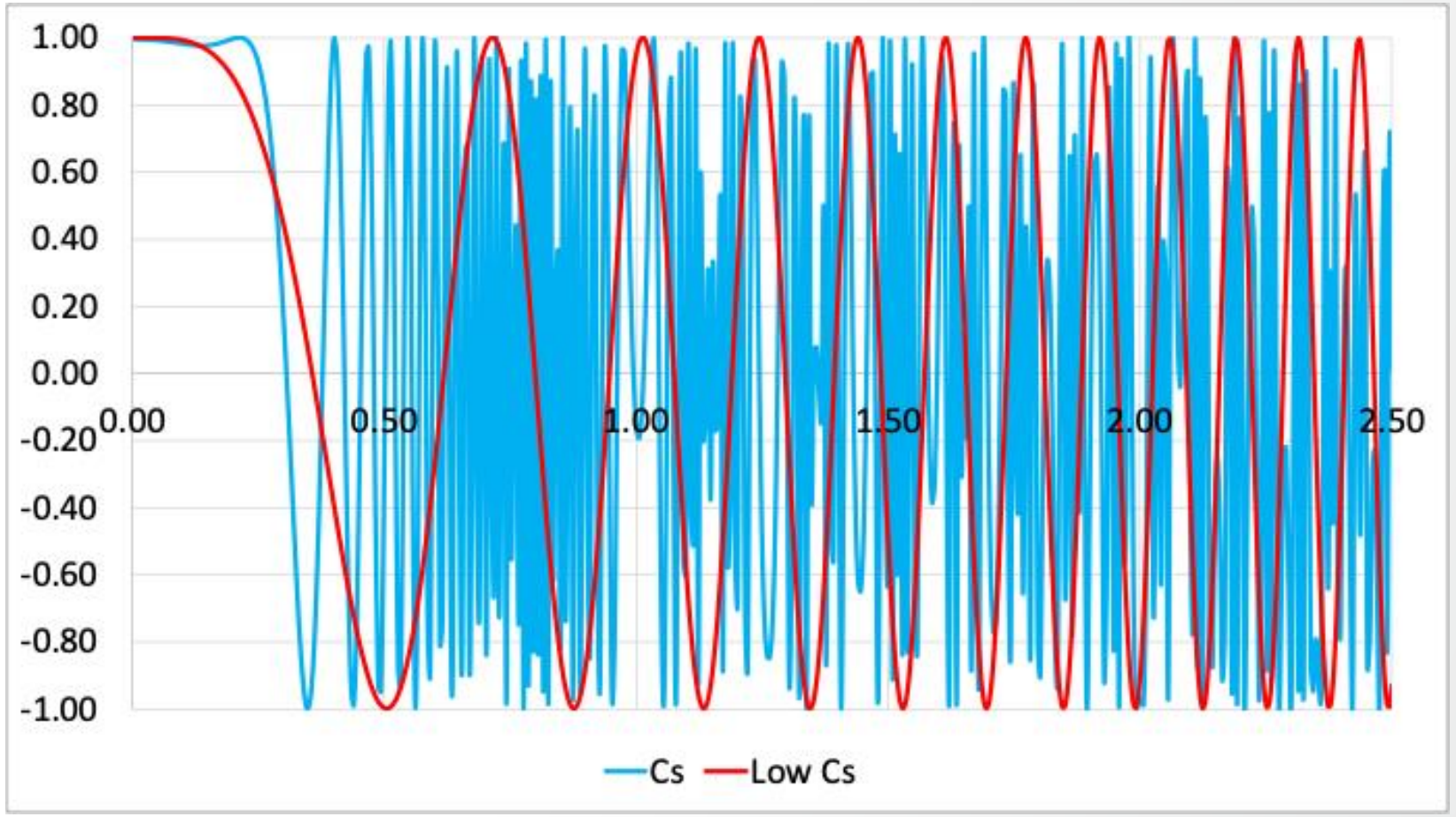

thermoscientific
Tundra

Aberration correction



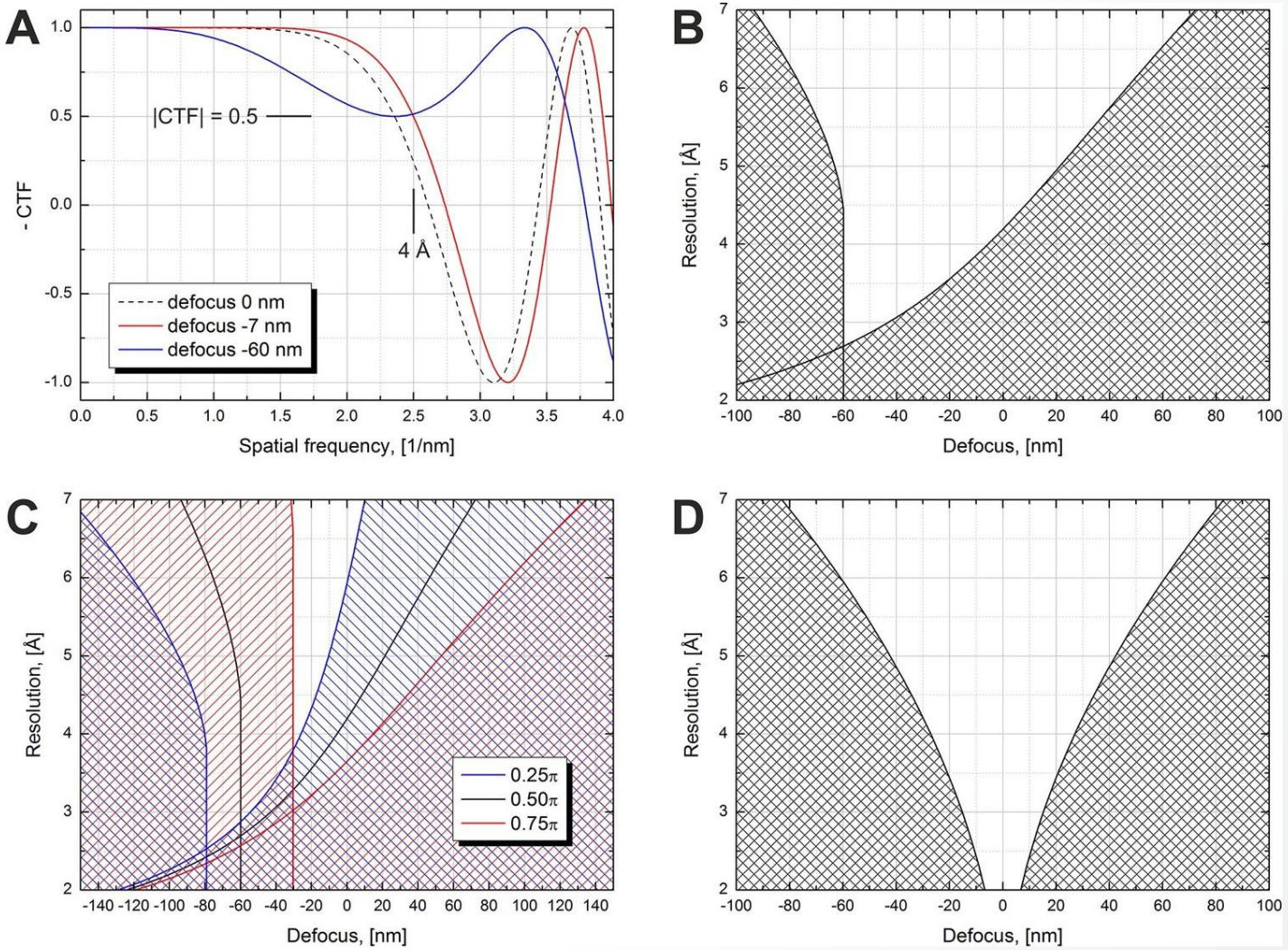
Cs 40 nm defocus, low Cs 0 nm defocus.

Aberration correction



Cs 20 nm defocus, low Cs 20 nm defocus.

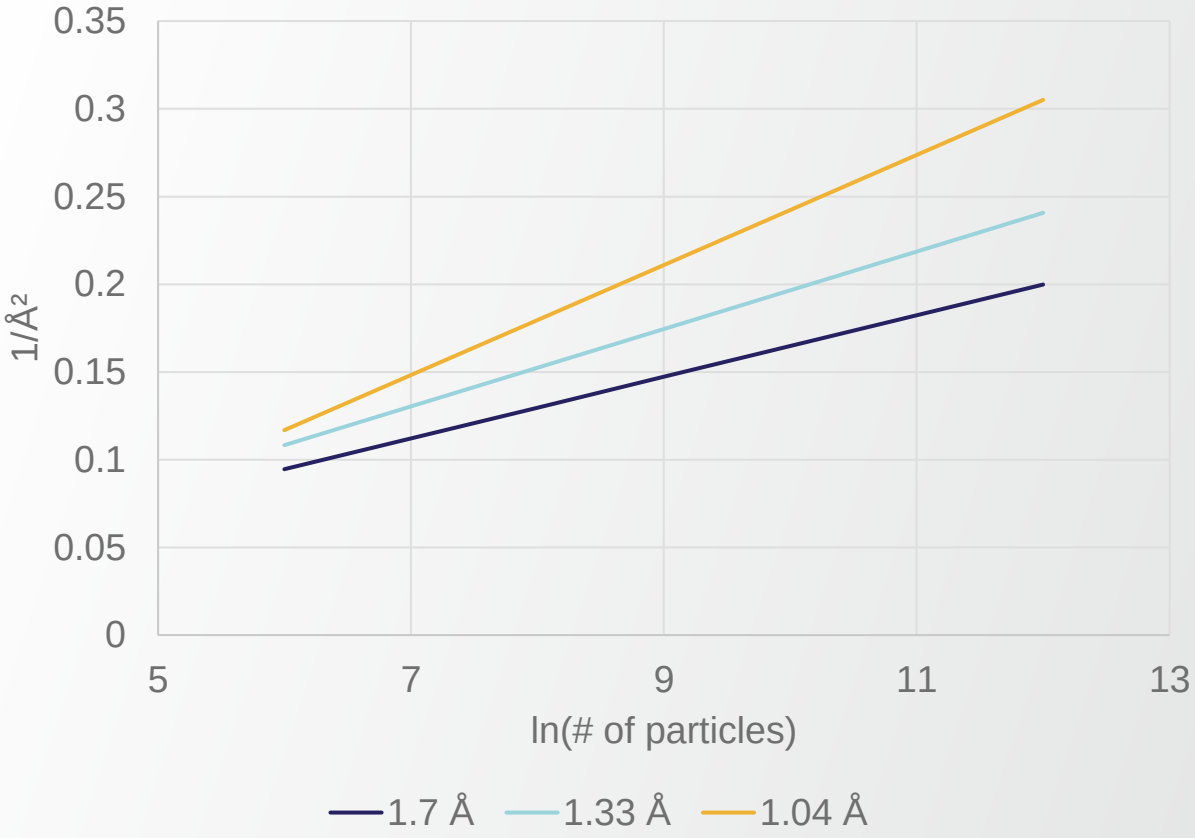
Aberration correction



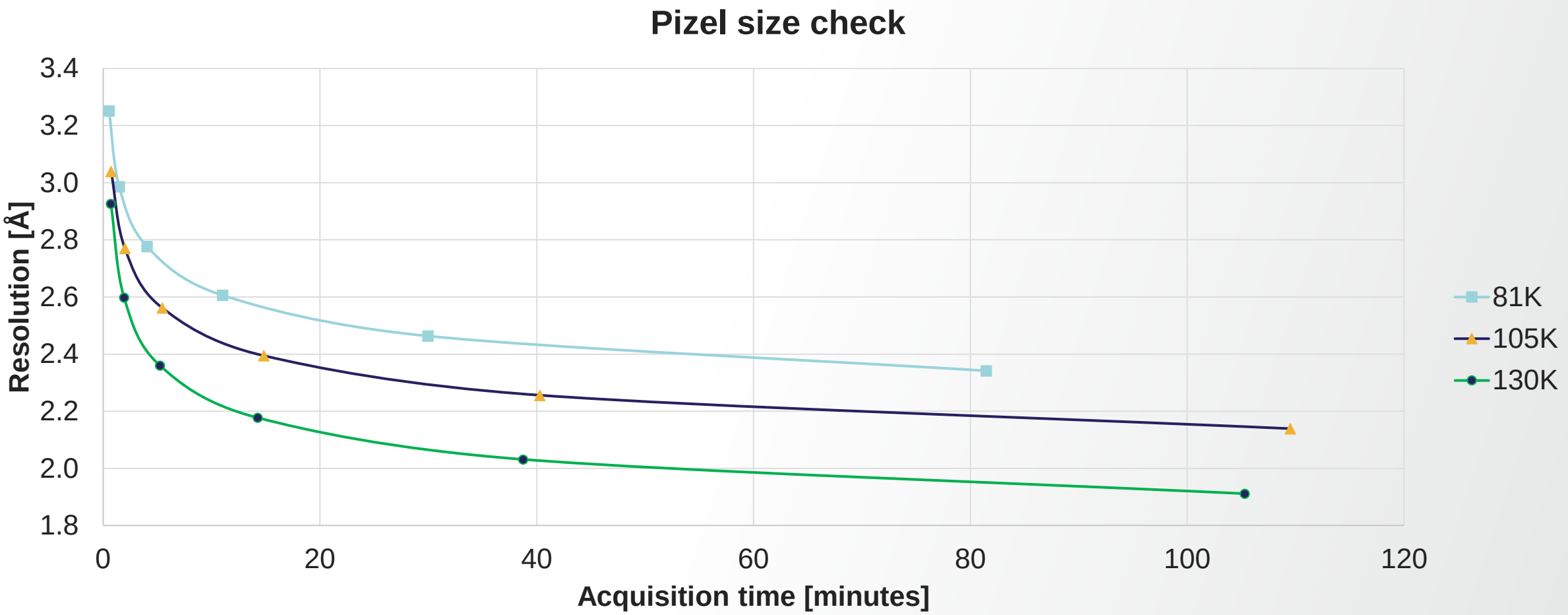
Danev, Radostin, and Wolfgang Baumeister. “Cryo-EM Single Particle Analysis with the Volta Phase Plate.” Edited by Sjors HW Scheres. *eLife* 5 (March 7, 2016): e13046. https://doi.org/10.7554/eLife.13046.

Magnification

81K	105K	130K
1.7 Å physical pixel size	1.34 Å physical pixel size	1.04 Å physical pixel size
1.27 um beam	1.38 um beam	0.6 um beam
nanoprobe spot 9	nanoprobe spot 8	nanoprobe spot 9
70 um c2	70 um c2	50 um c2
100 um objective	100 um objective	100 um objective
40 e/Å^2	40 e/Å^2	40 e/Å^2
25.4 sec total	14.8 sec total	10 sec total
0.635 sec frame	0.37 sec frame	0.25 sec frame
4.74 e/p/sec without sample	4.8 e/p/sec without sample	4.42 e/p/sec without sample
2 Å/sec drift	3 Å/sec drift	4 Å/sec drift
Single image per hole	Single image per hole	Three images per hole



Data collection: magnification

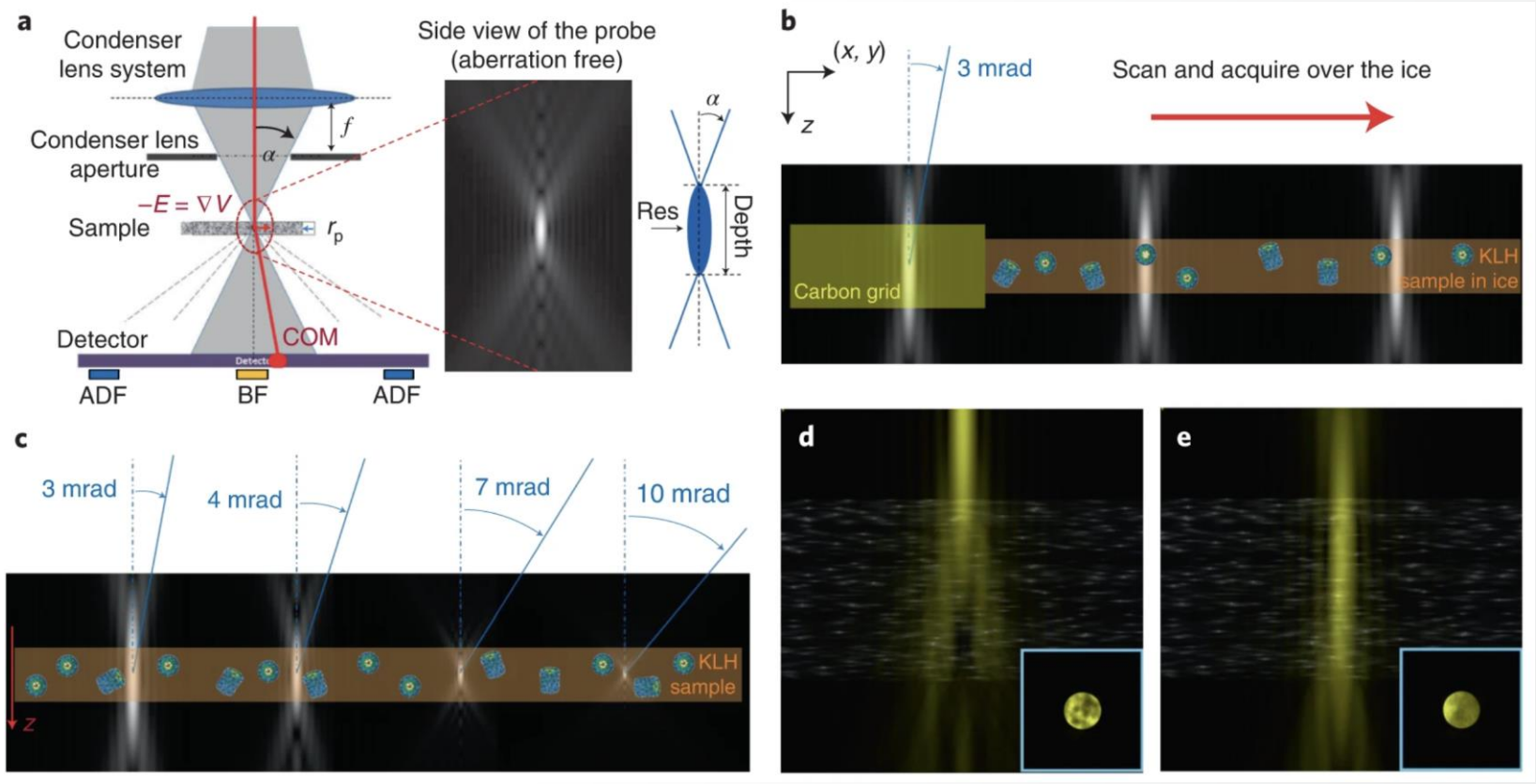


Number of particles for 2.39 Å best resolution for 81K	34546	8331	2580
Time to 2.39 Å	00:47	00:15	00:04

Defocus or in-focus?

- Defocus → CTF correction needed, higher magnifications limited by delocalization.
- In-focus → difficult to acquire images at the right defocus.

STEM



Lazić, Ivan, Maarten Wirix, Max Leo Leidl, Felix de Haas, Daniel Mann, Maximilian Beckers, Evgeniya V. Pechnikova, et al.
e"Single-Particle Cryo-EM Structures from iDPC–STEM at near-Atomic Resolution."
Nature Methods 19, no. 9 (September 1, 2022): 1126–36. https://doi.org/10.1038/s41592-022-01586-0.

STEM

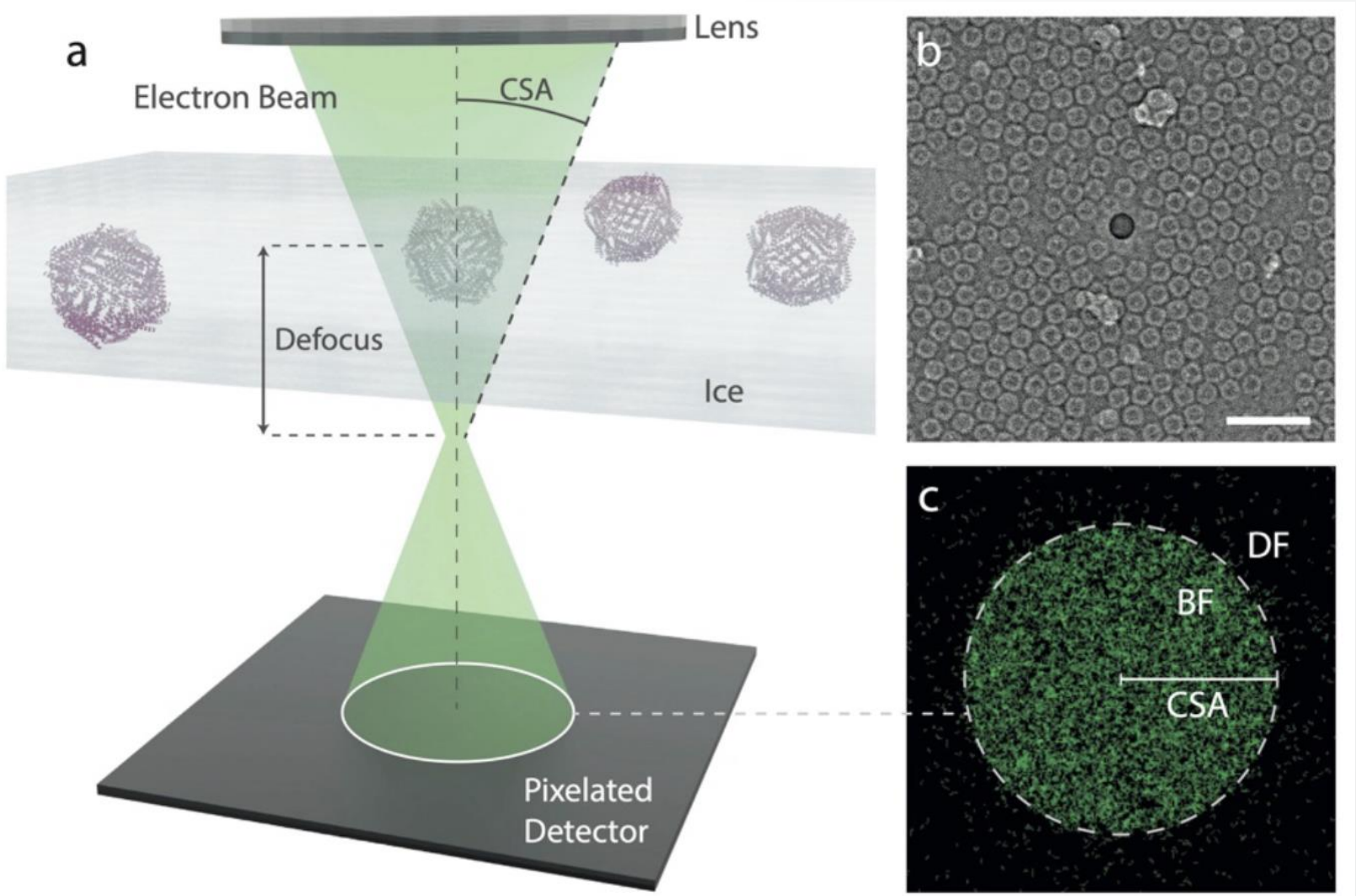


Fig. 1: Experimental setup of 4D-STEM.

Küçükoğlu, Berk, Inayathulla Mohammed, Ricardo C. Guerrero-Ferreira, Massimo Kube, Julika Radecke, Stephanie M. Ribet, Georgios Varnavides, et al.
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bioRxiv, January 1, 2024, 2024.02.12.579607. https://doi.org/10.1101/2024.02.12.579607.

Discussion/questions