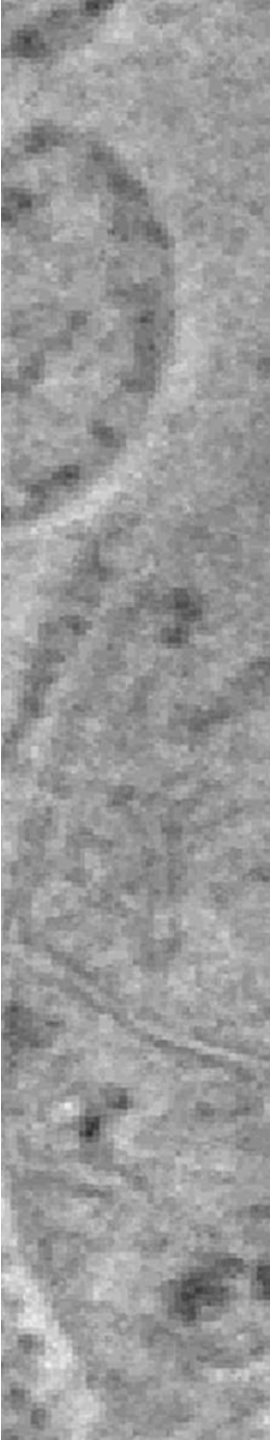
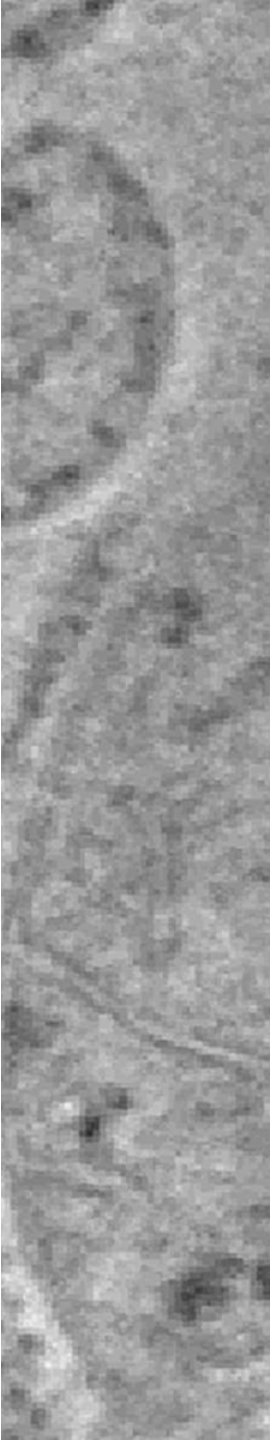


IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

Basics of Image Formation in Transmission EM

Online workshop: Introduction to Cryo-Tomography

Michael Elbaum
Dept of Chemical and Biological Physics
Weizmann Institute of Science



IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

Basics of Image Formation in Transmission EM

Demystifying the Transmission Electron Microscope

An Impractical Guide to TEM

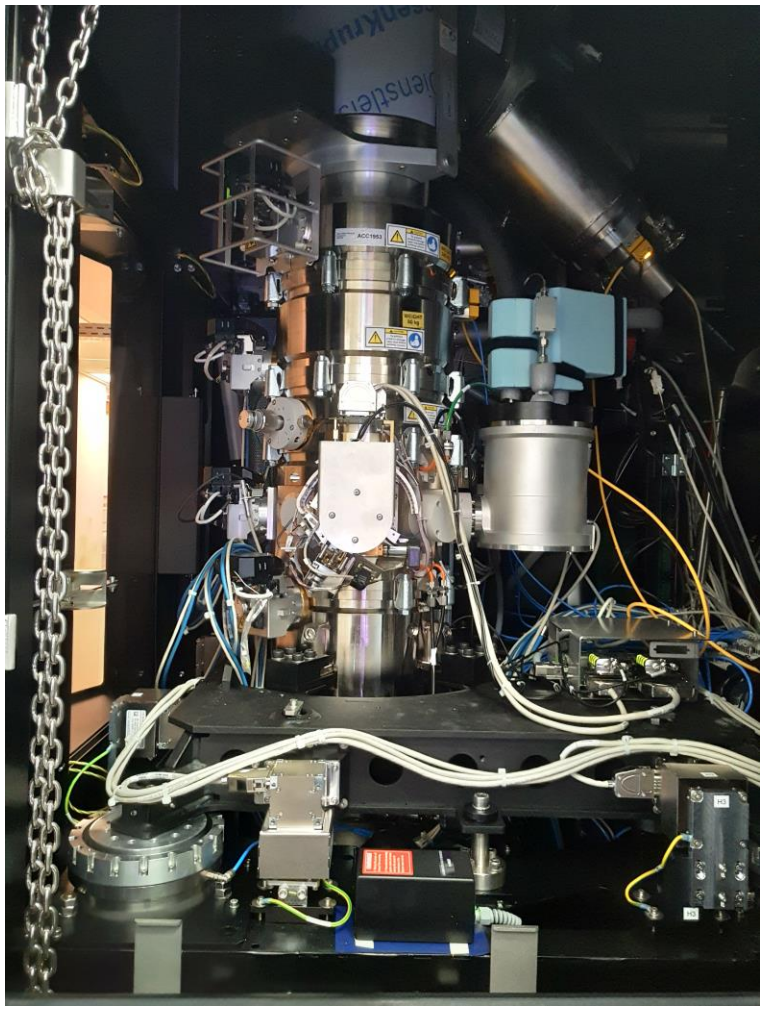
Demystifying the TEM

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

Attractive boxes. What's inside?

Demystifying the TEM

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS



Ouch! Now we understand why they keep covered up!

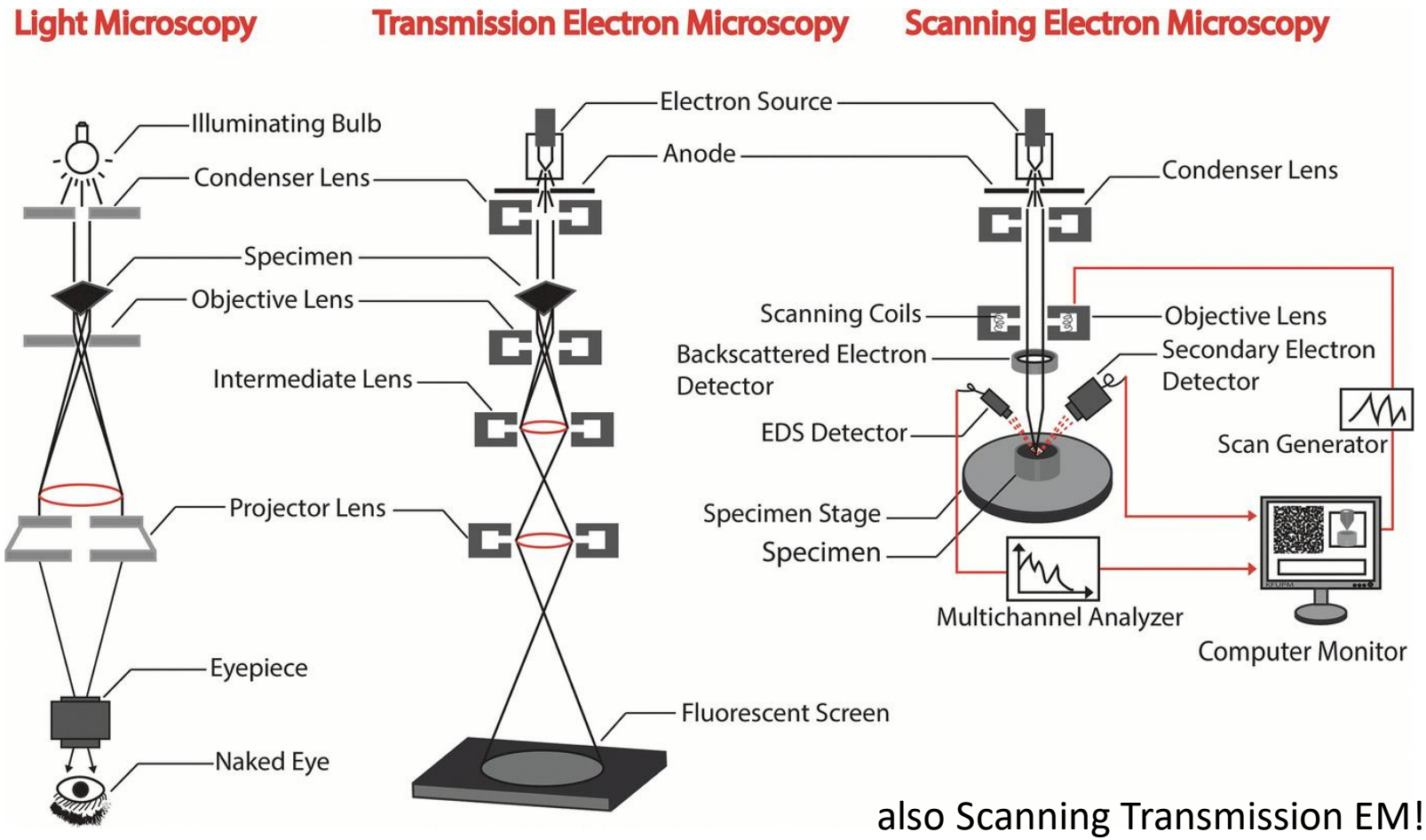
Components of a microscope

IMAGING LIFE FROM MOLECULES TO CELLS

A view of the classical TEM is more informative. Similarities to the familiar light microscope.

Components of a microscope

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS



also Scanning Transmission EM!

Ul-Hamid A. (2018) Introduction. In: A Beginners' Guide to Scanning Electron Microscopy. Springer, Cham

Components of a microscope

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

source

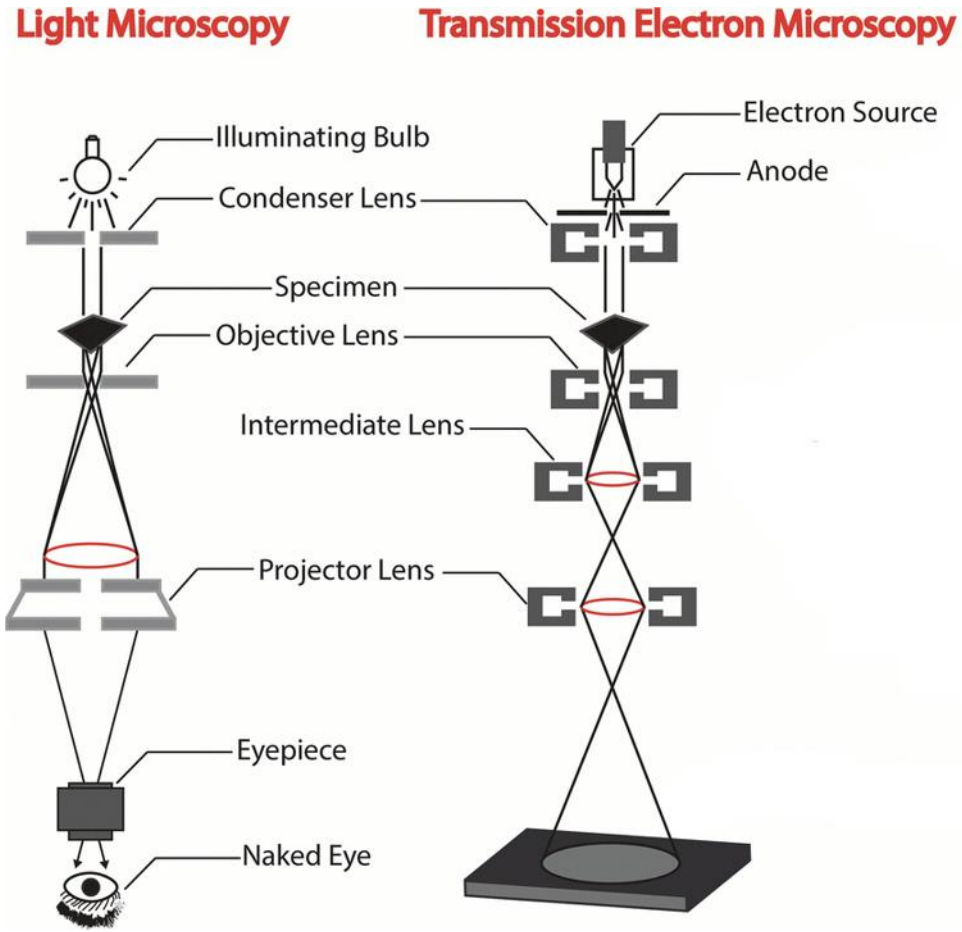
condenser

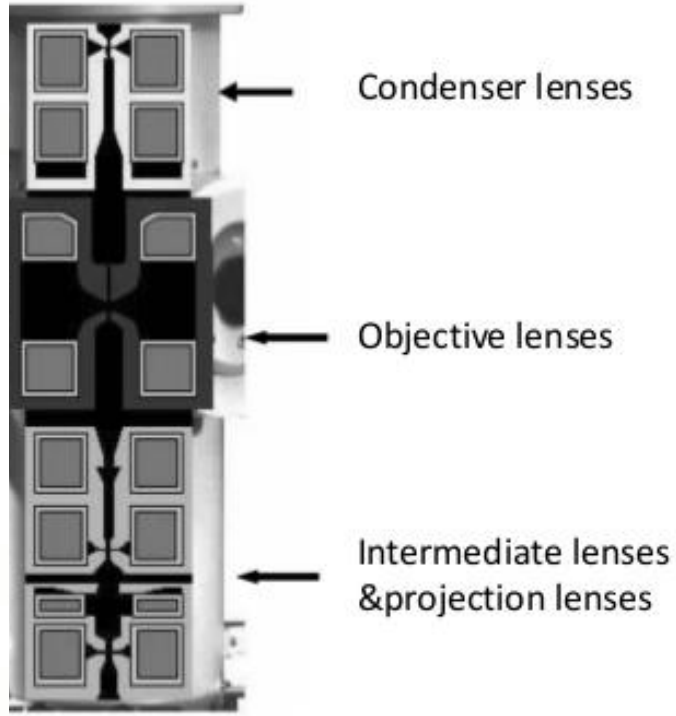
specimen

objective

projector

camera





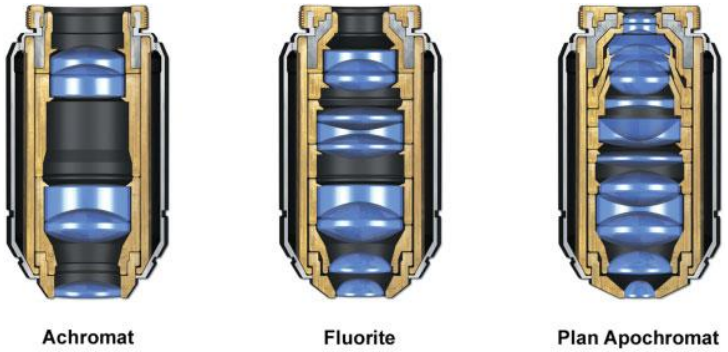
Components of a microscope

IMAGING LIFE FROM MOLECULES TO CELLS

Light microscope:

- operates in air or any transparent medium
- almost perfect lenses
- very high numerical apertures (0.25 ~ 1.4)
- fixed focal length but movable focus
- light travels in straight paths
- wavelength 400-700 nm (>1000 x atomic radius)

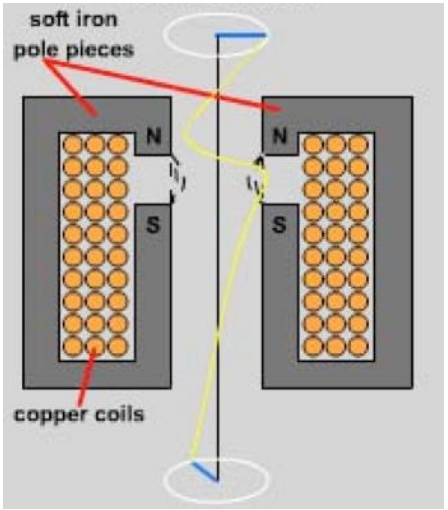
Murphy & Davidson 2013 DOI:10.1002/9781118382905

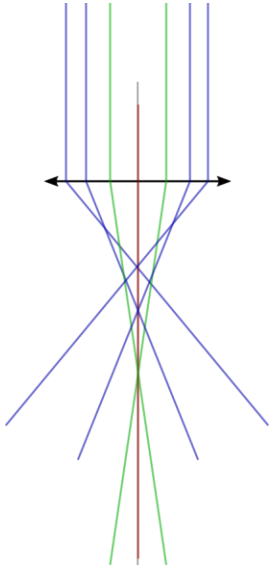


Electron microscope:

- operates in vacuum
- extremely poor lenses (aberrations)
- very small numerical aperture < 0.01
- adjustable focal length but fixed in position
- helical paths in magnetic field
- wavelength 2-4 pm (~1/100 atomic radius)

Williams & Carter 2009 DOI: 10.1007/978-0-387-76501-3_1



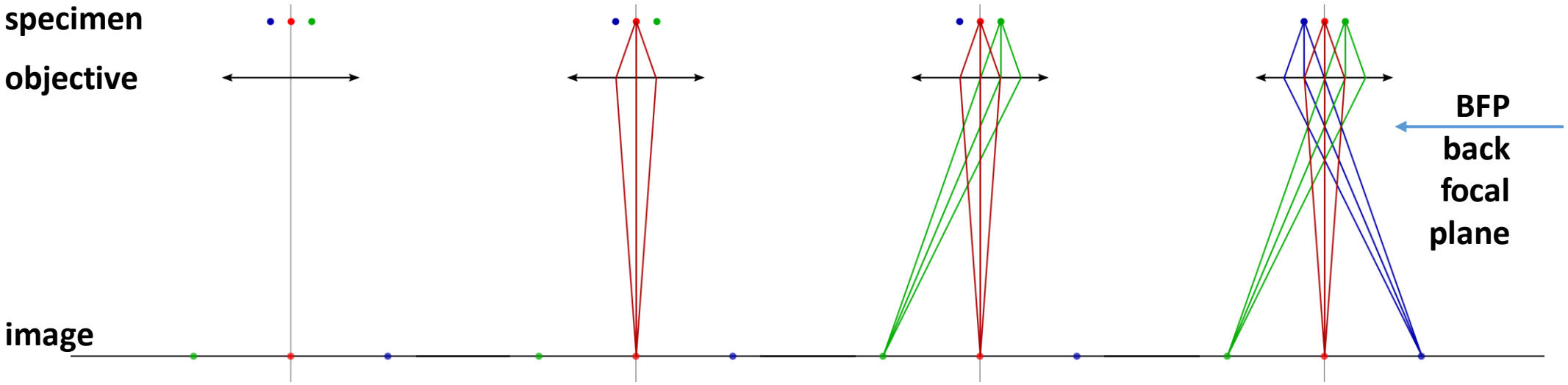


spherical aberration Cs

The Objective

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

The task of the objective lens is to map the emission from any point on the specimen to its corresponding point in the image.

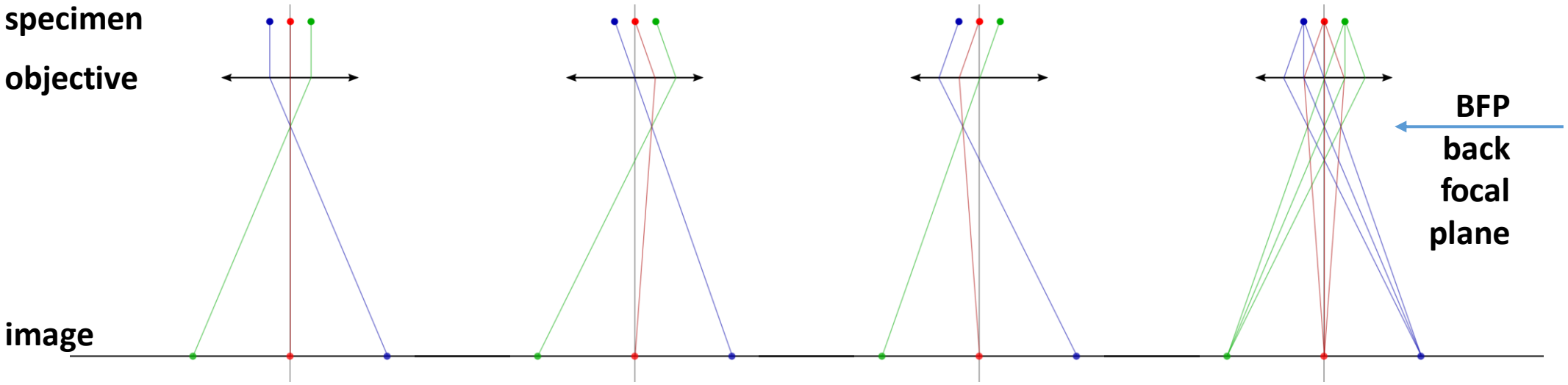


- magnification is the ratio of distance image-lens:lens-sample
- image is inverted
- optical path lengths from pt to pt are equal for all angles
- waves travel along ray directions & interfere at convergence

The Objective

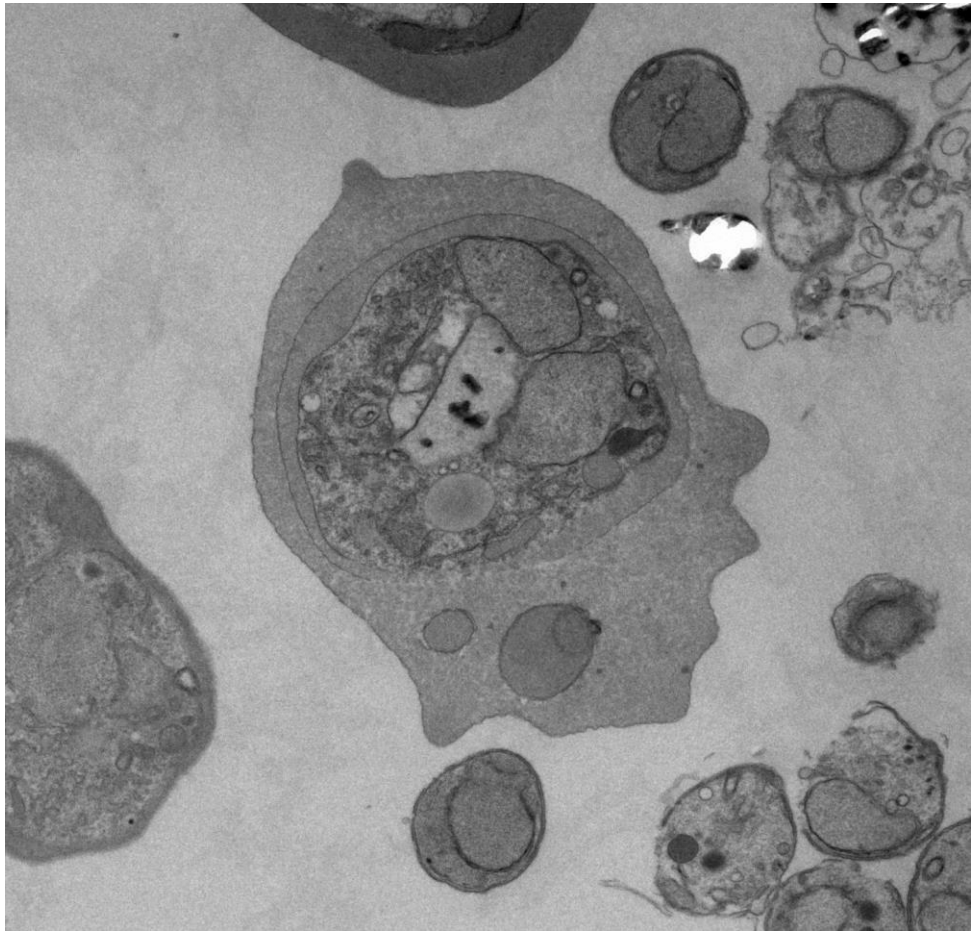
IMAGING LIFE FROM MOLECULES TO CELLS

The task of the objective lens is to map the emission from any point on the specimen to its corresponding point in the image.



- all rays of same angle pass through the same point in the BFP
- Heavy elements cause scattering to large angles – hence use as stains
- an aperture or stop in the BFP limits scattering contribution – contrast

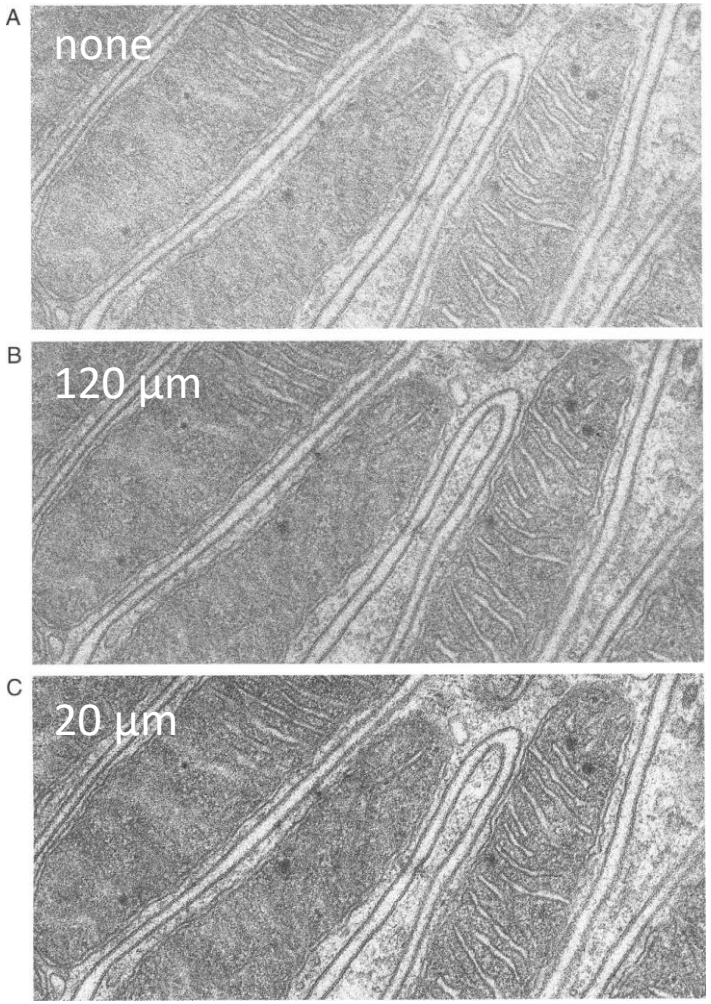
Scattering contrast



A sample stained with heavy metals (e.g., Os, U, Pb) scatters electrons in proportion to the local stain concentration. An objective aperture removes their contribution to the image – dark on bright background: Bright Field.

Scattering contrast

objective



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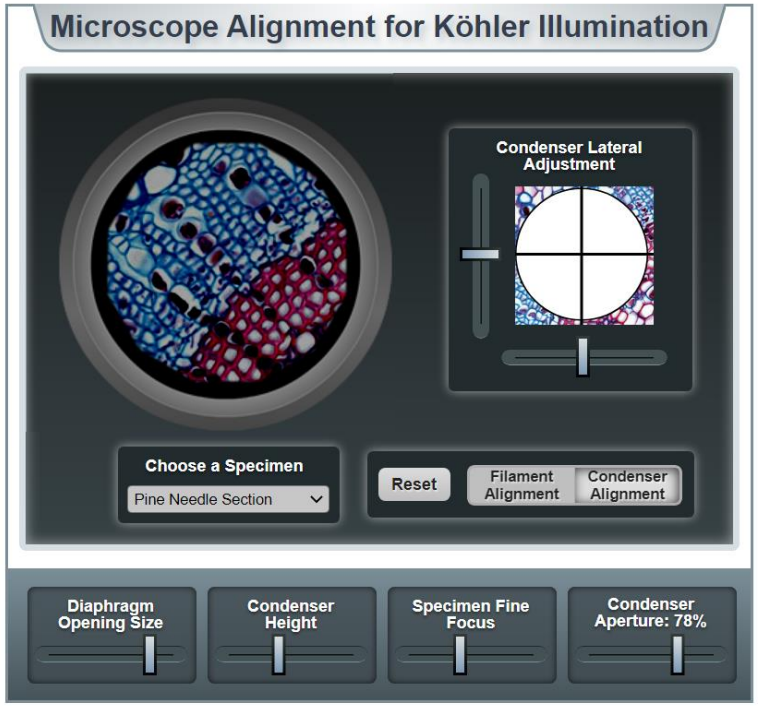
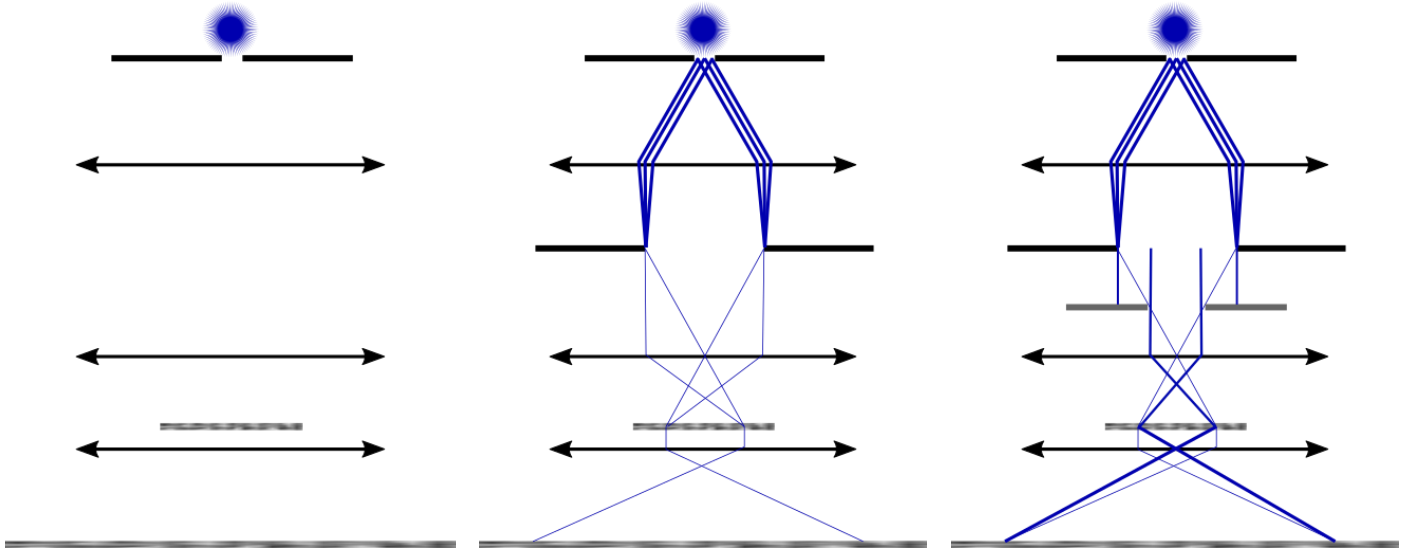
Maunsbach and Afzelius 1999 DOI:10.1016/B978-0-12-480610-8.X5000-8

Illumination in the Light Microscope





https://www.microscopyu.com/tutorials/kohler

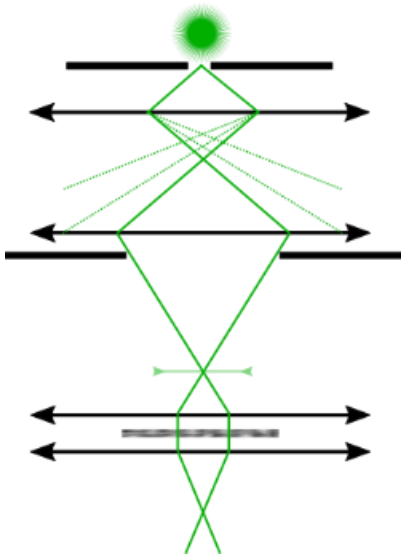
Illumination in the TEM

IMAGING LIFE FROM MOLECULES TO CELLS

Source (Gun) is intense but not collimated.
Normally **Field Emission Gun** (FEG) for Cryo-EM.

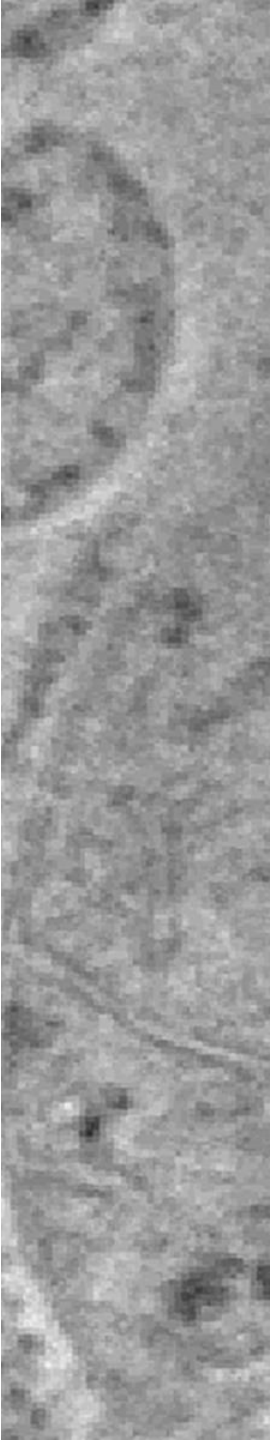
Condenser lens C1 is the Collector.
Spot Size setting controls C1 strength.

Condenser lens C2 focuses the beam toward the specimen.
Intensity dial controls the C2 lens current.
C2 Aperture limits the fraction of C1 spot that passes.



Ideal is parallel illumination. Field of view is fixed by C2 aperture and precise C2 lens setting.
Very restrictive to match camera size – adjust C2 to fill the frame: illumination is converging/diverging
Intensity knob controls size of the spot & **Spot Size** controls the intensity!
Three lens condenser provides continuous control of the illuminated field.

Illumination in the STEM



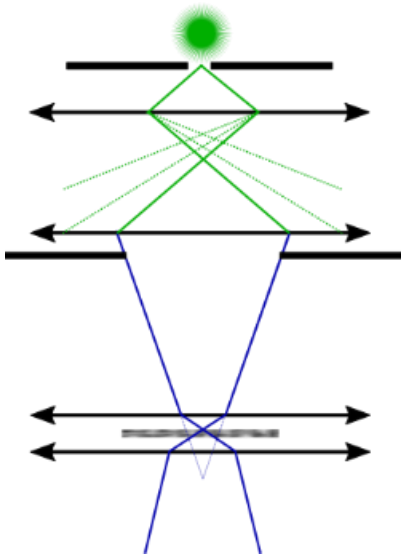
IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

Source (Gun) is intense but not collimated.
Normally **Field Emission Gun** (FEG) for Cryo-EM.

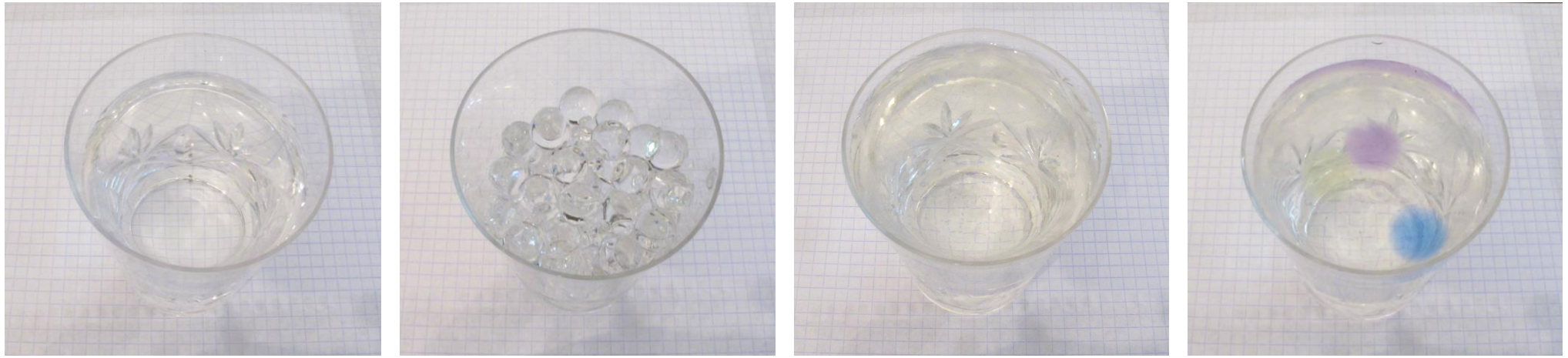
Condenser lens C1 is the Collector.
Spot Size setting controls C1 strength.

Condenser lens C2 focuses the beam onto the specimen.
Intensity dial controls the focus.
C2 Aperture controls the angular convergence.

Scanning Transmission Electron Microscopy:
scan the spot (probe) across the specimen.
record the scattered signals point by point.

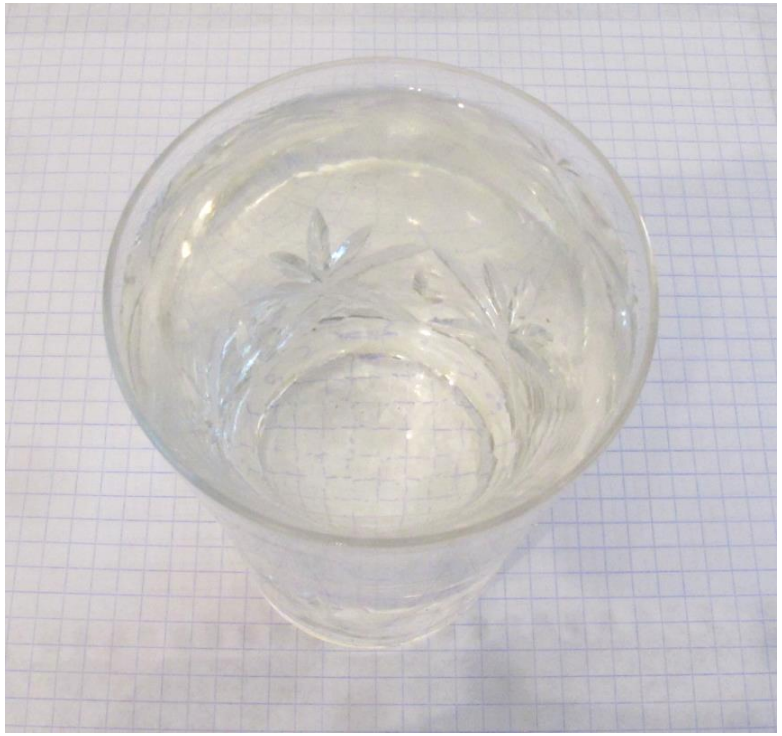


Phase Object



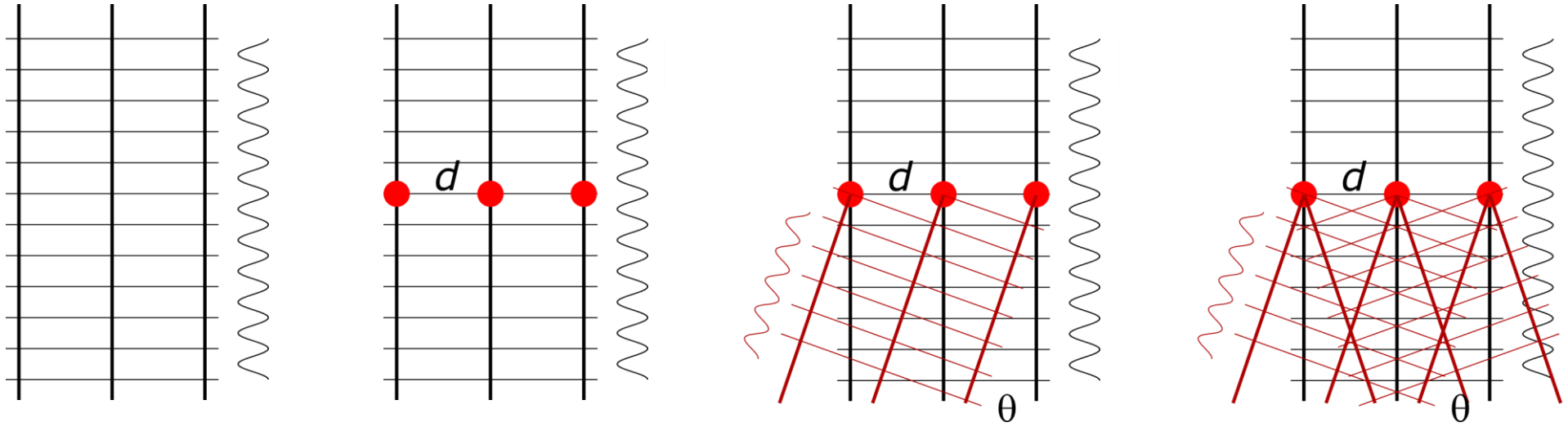
- Florists water beads “disappear” in water – minimal scattering contrast
- Still, they refract the transmitted light – potential for phase contrast
- Dyed beads absorb certain colors – absorption or fluorescence contrast

Phase Object



Note distortion of the graph paper lattice underneath.

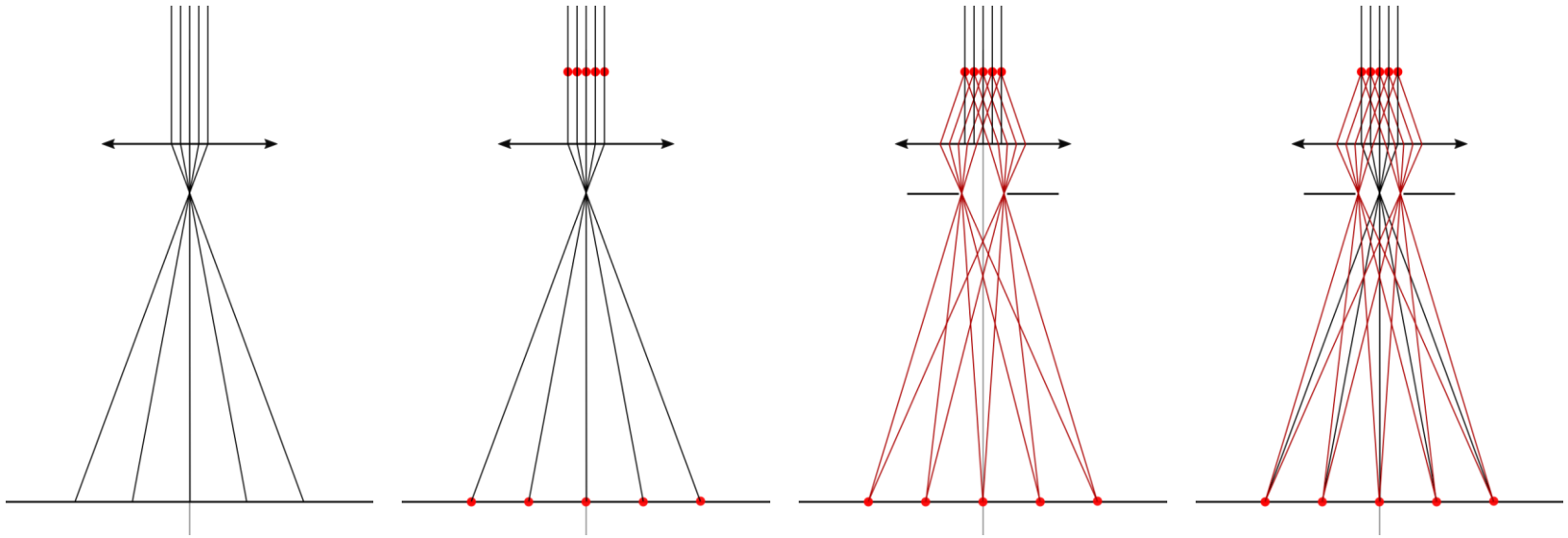
Coherent scattering



Sample as multiple slit interference $d = \lambda \sin\theta$
(essence of Fourier decomposition)

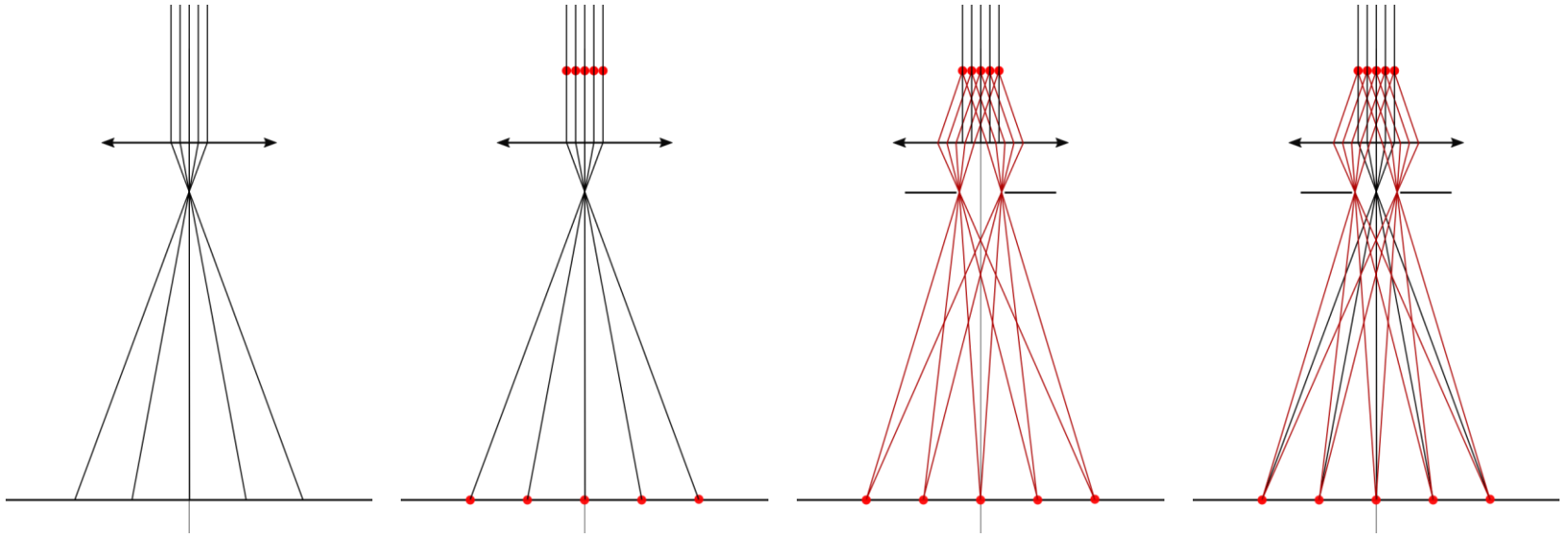
λ=0.0025nm @ 200 kV: $d = 2.5\text{Å}, \quad \sin\theta = 0.01$

Phase contrast

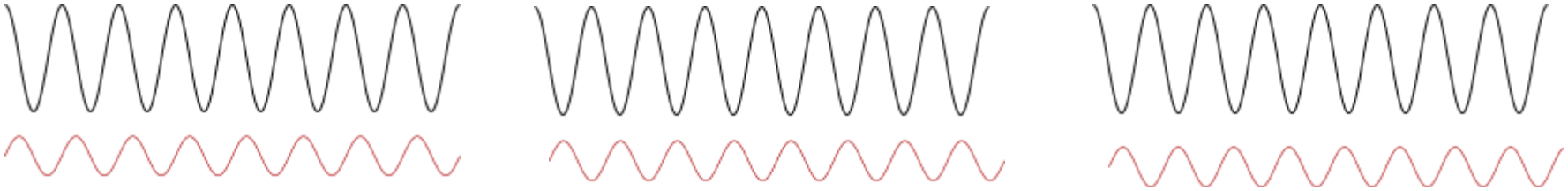


Phase contrast from a weakly scattering specimen depends on wave interference between scattered and unscattered contributions to the transmission. Have to open the objective aperture in order to retain the scattered ray directions. The challenge is to obtain interpretable contrast, so that dark image intensities represent dense areas of the specimen.
Remember: wave interference may be destructive (dark) or constructive (bright).

Phase contrast

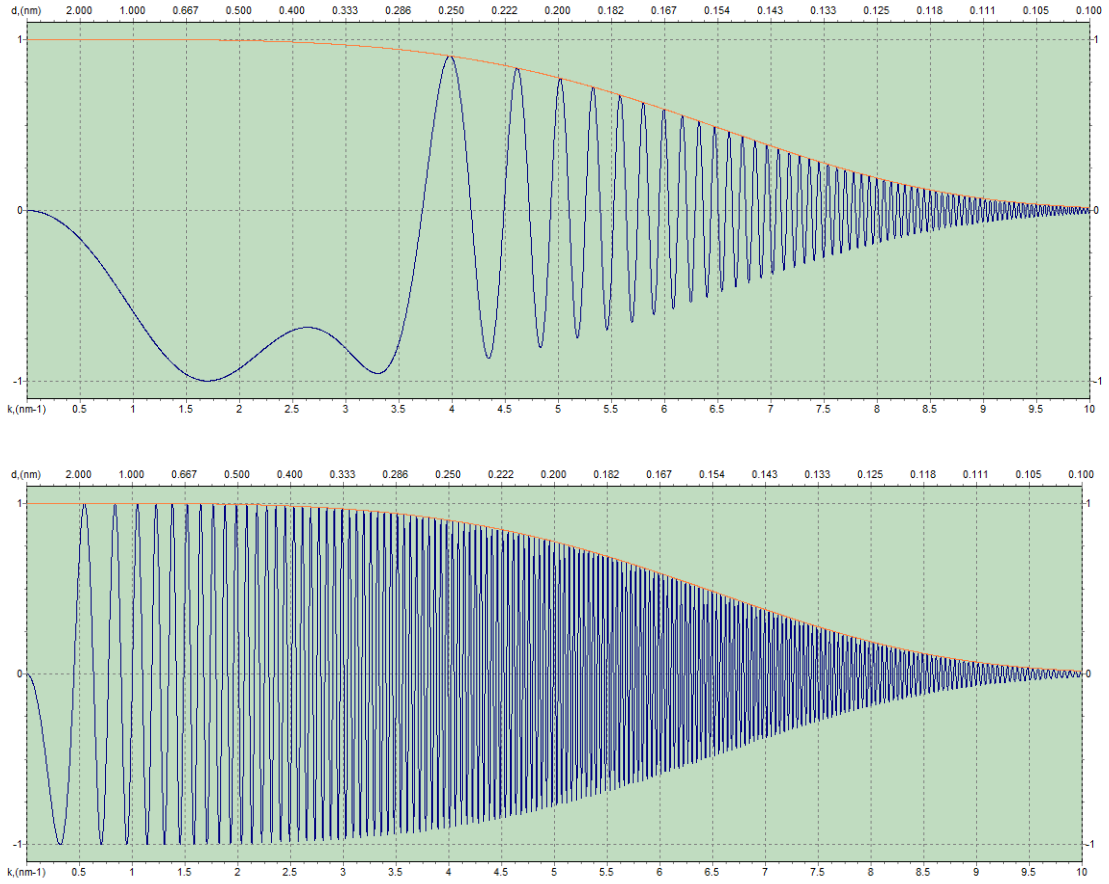


no sample | weak sample | weak scattering | contrast by wave interference

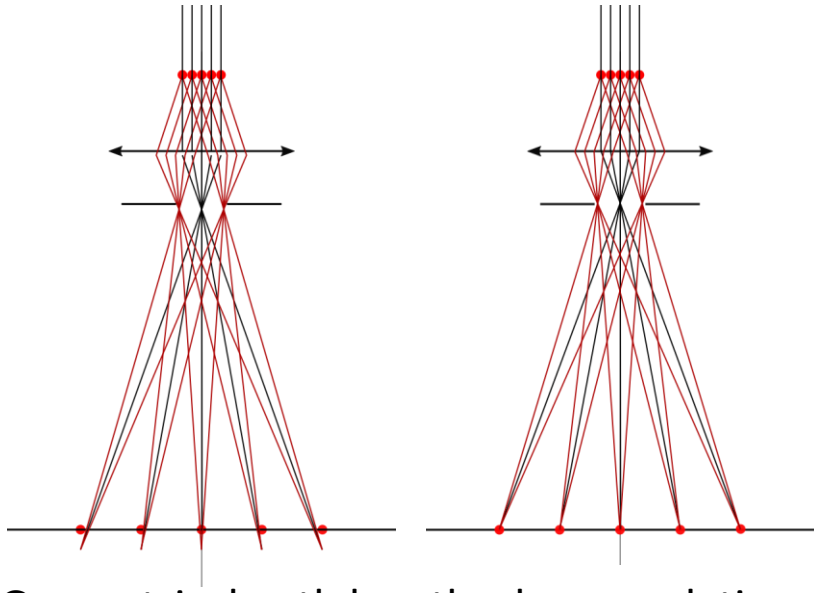


90 deg phase shift (minimal contrast) | Interference condition (destructive) | Inelastic scattering (wavelength changes)

Contrast Transfer Function



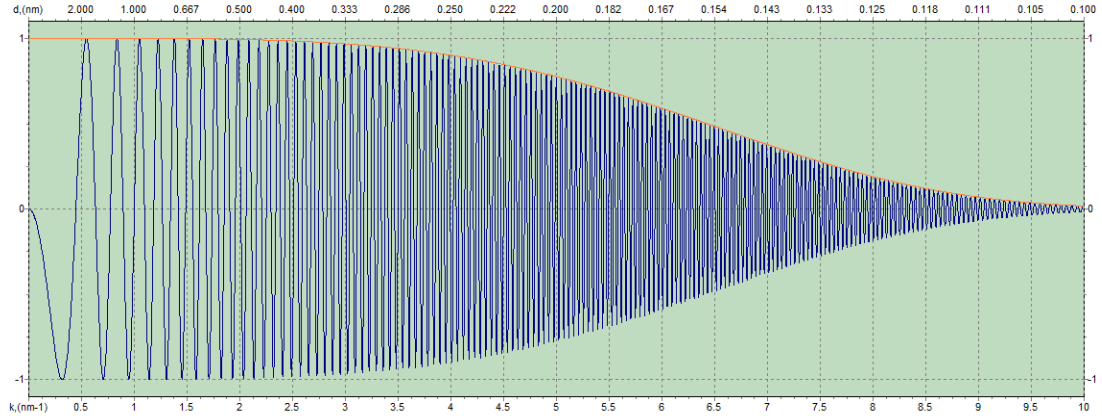
Simulation of Tecnai F20.
defocus = -87 nm (above), -2 um below

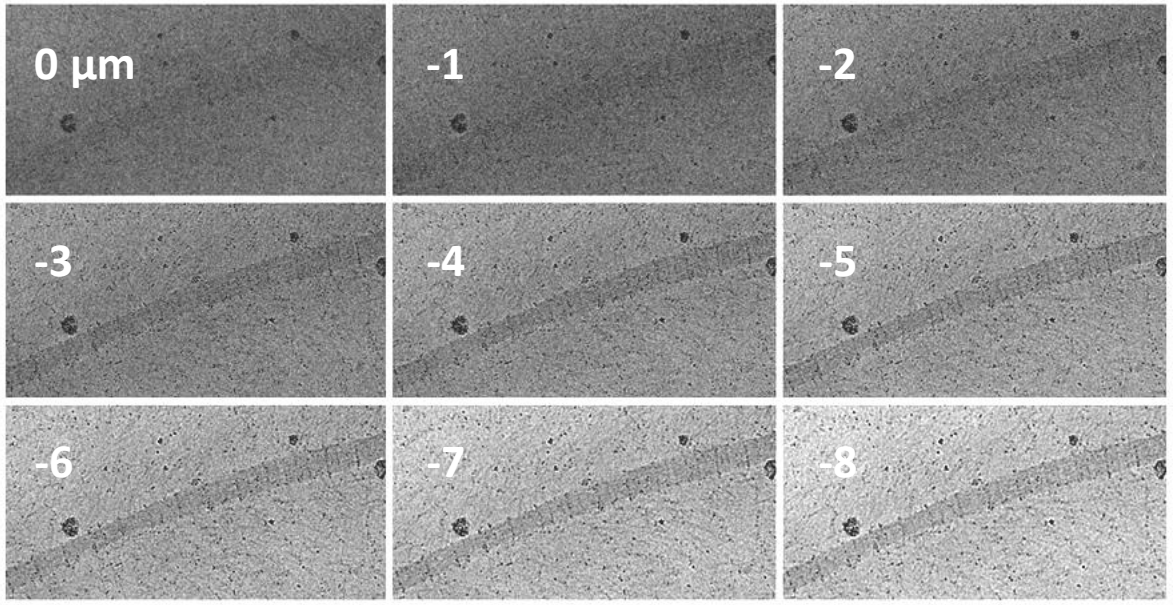


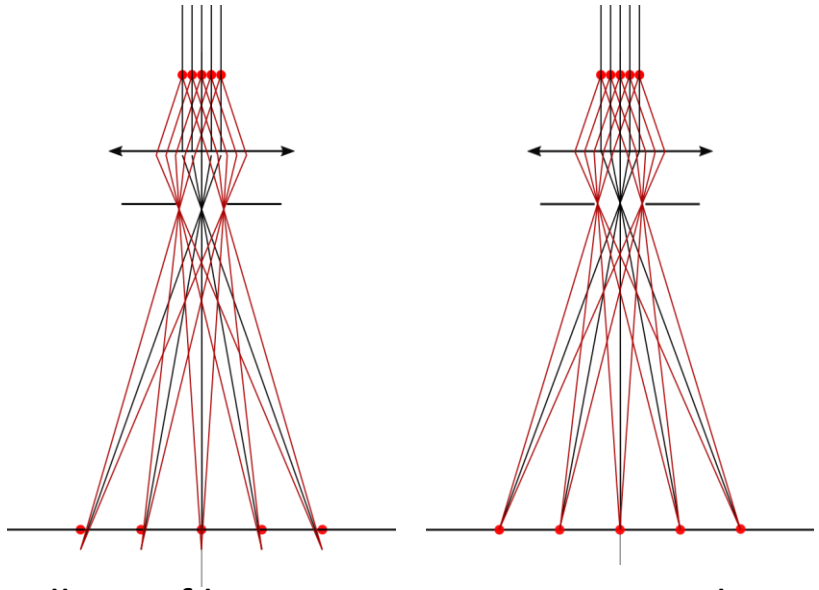
Geometrical path lengths, hence relative phases, hence interference conditions depend on diffraction angle, so different spatial frequencies (Fourier components) appear darker or brighter than background.

Contrast Transfer Function

IMAGING LIFE FROM MOLECULES TO CELLS





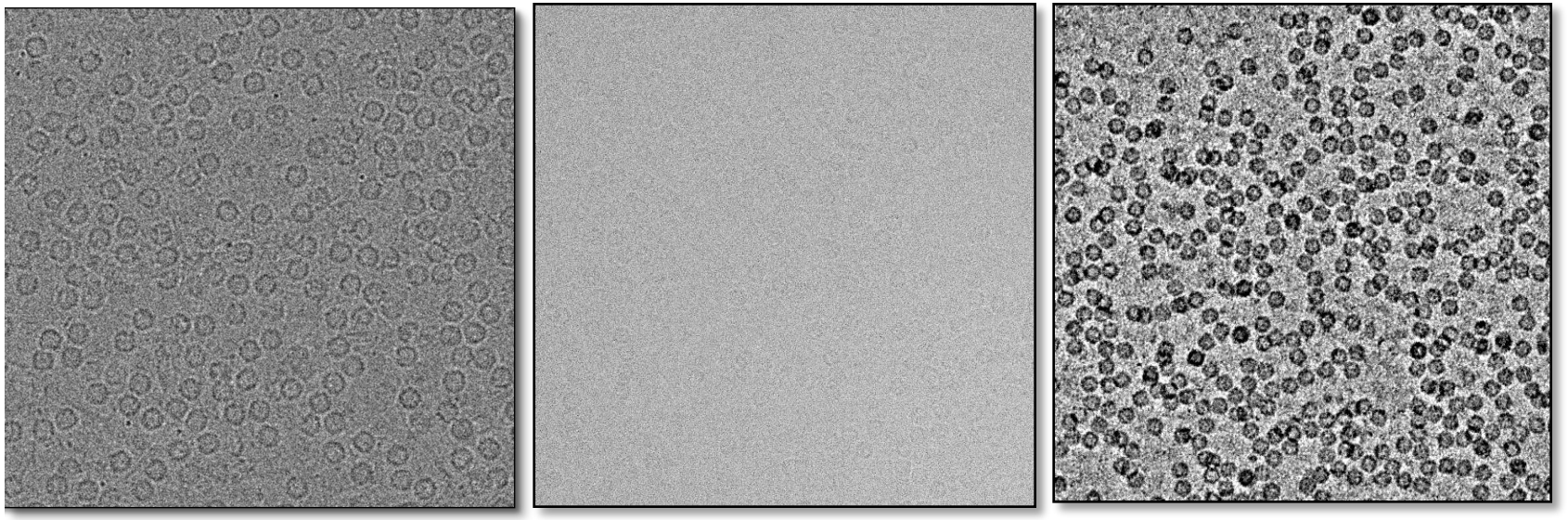


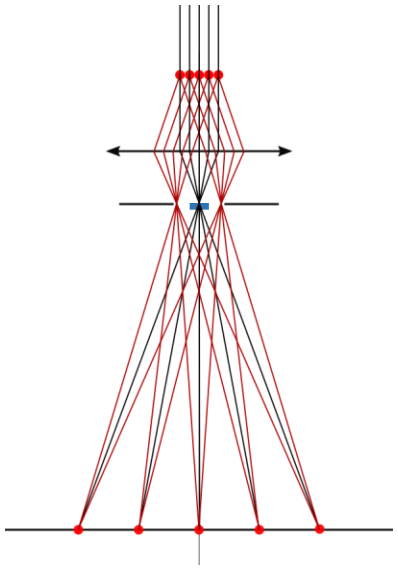
Collagen fiber. Contrast increases with defocus, but high resolution details are scrambled. Keys to high resolution are small defocus and proper correction of the CTF.

Phase plate

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

Defocus | In Focus | Phase Plate





Nadav Elad

Zernike - Volta

Phase plate generates contrast in/near focus

Coherent illumination still required (thickness)

Contrast in the STEM

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

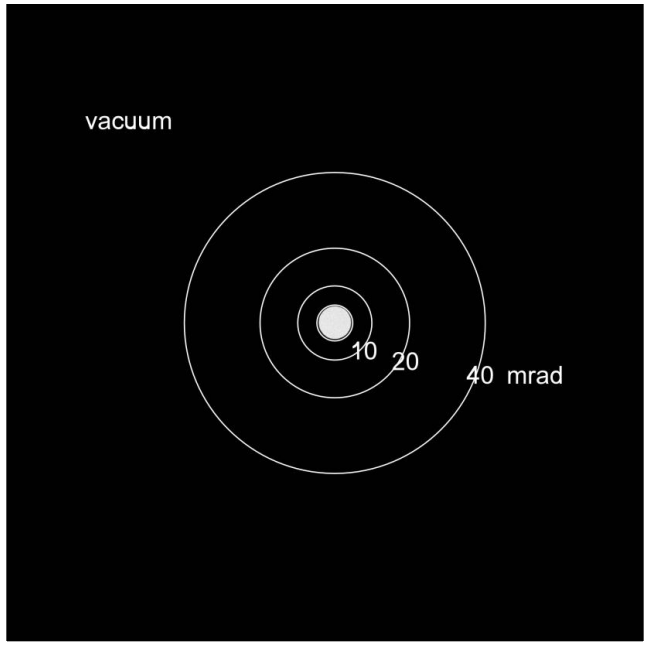
Scanning Transmission Electron Microscopy:

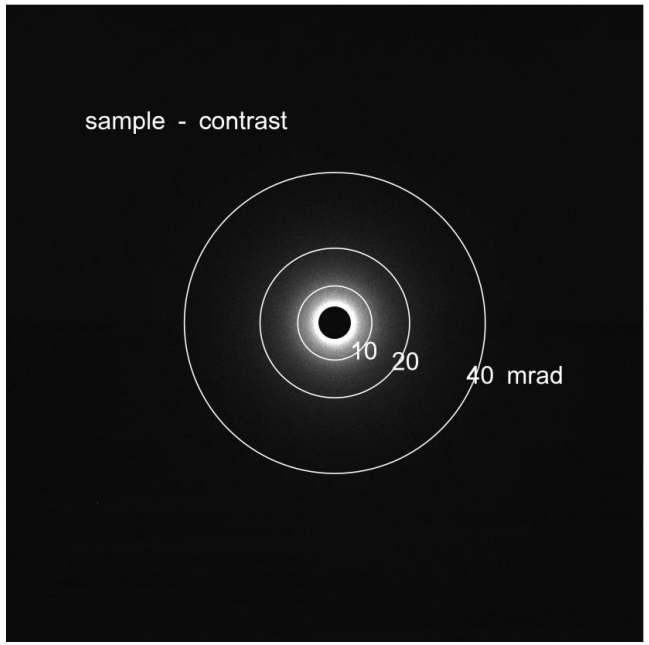
scan the spot (probe) across the specimen.

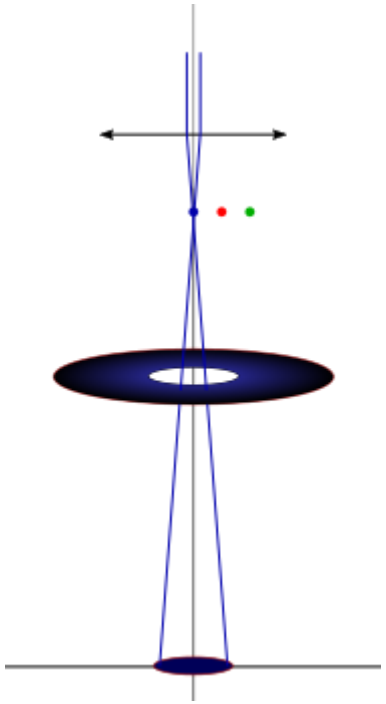
record the scattered signals point by point.

BF (Bright Field) detector collects unscattered electrons.

ADF (Annular Dark Field detector collects scattered electrons.







Probe

Specimen

ADF Detector

BF Detector

Contrast in the STEM

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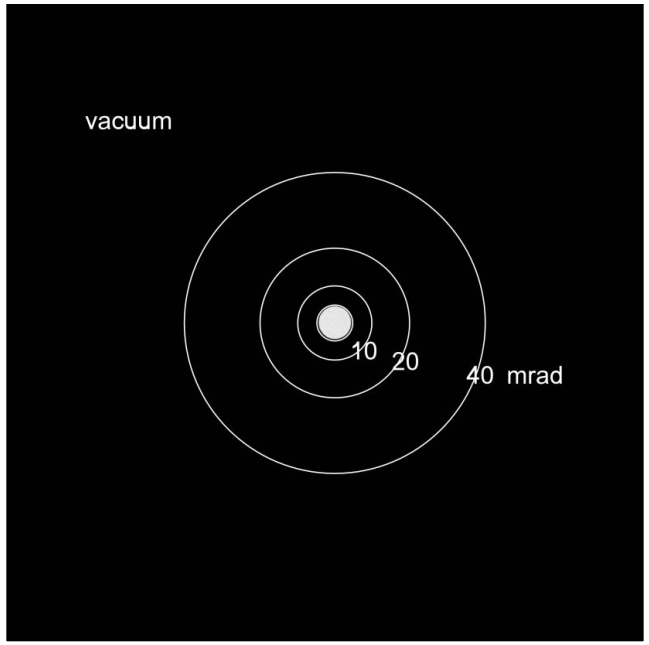

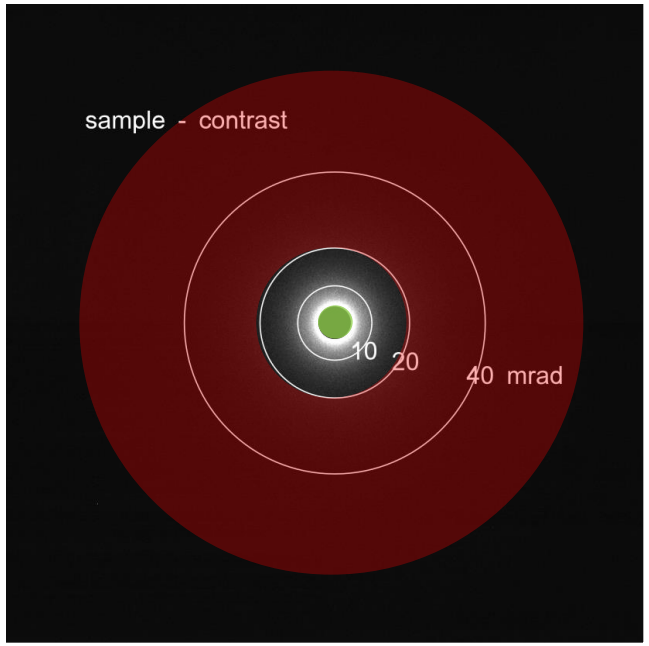

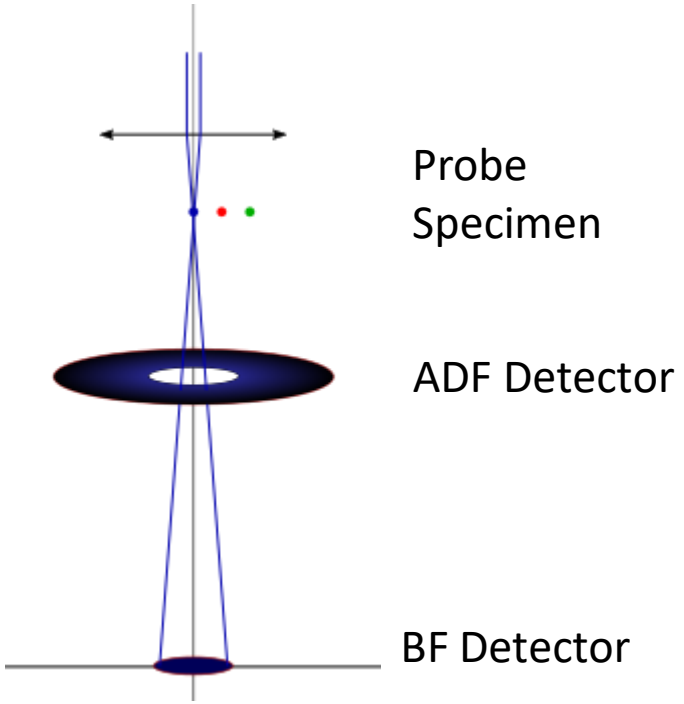

Contrast in the STEM

IMPACT IMAGING LIFE FROM MOLECULES TO CELLS

Scanning Transmission Electron Microscopy:

scan the spot (probe) across the specimen.

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BF (Bright Field) detector collects unscattered electrons.

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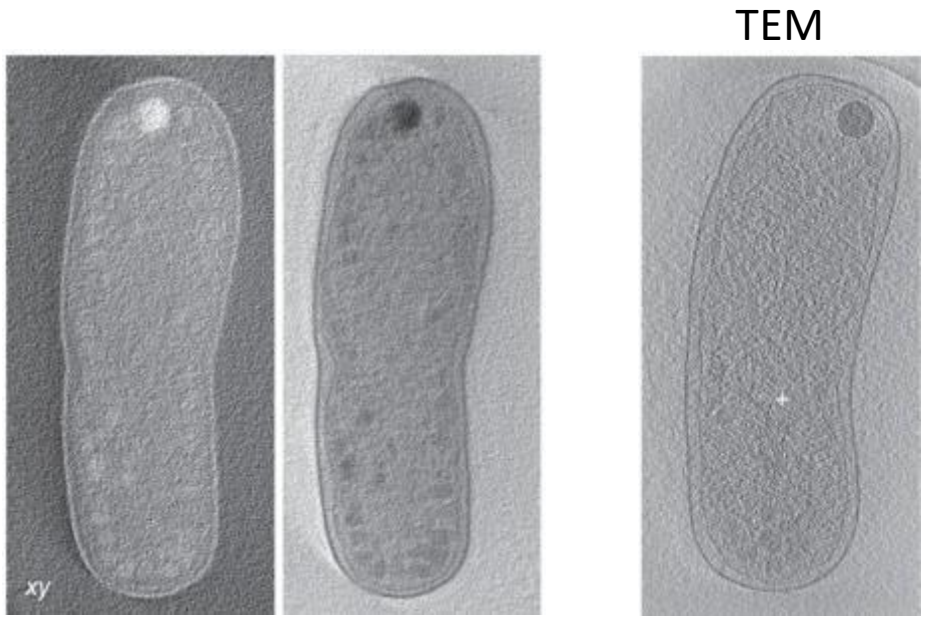
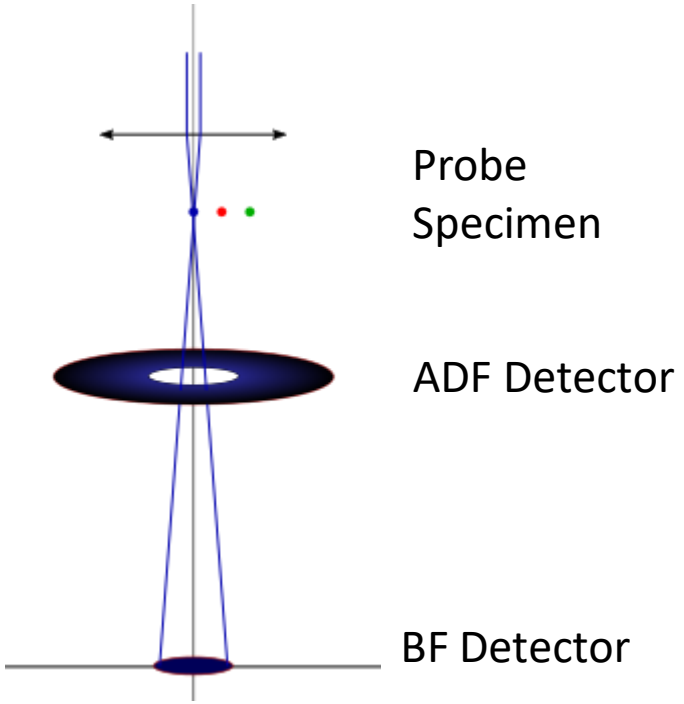
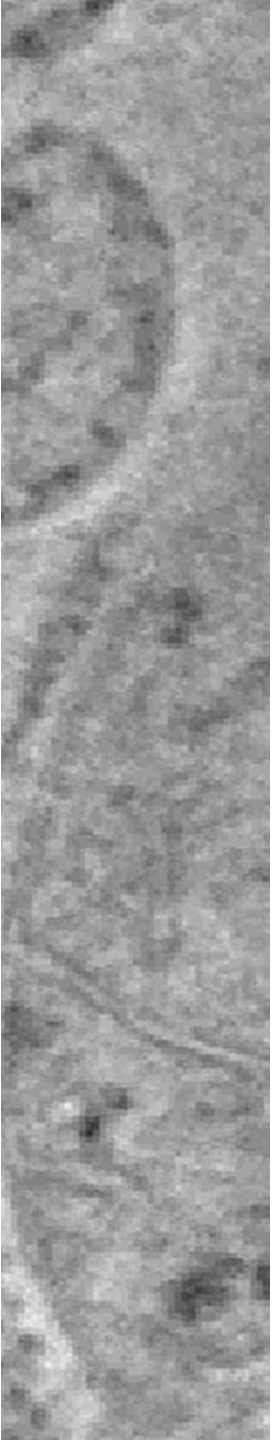
Incoherent contrast suitable for thick specimens.





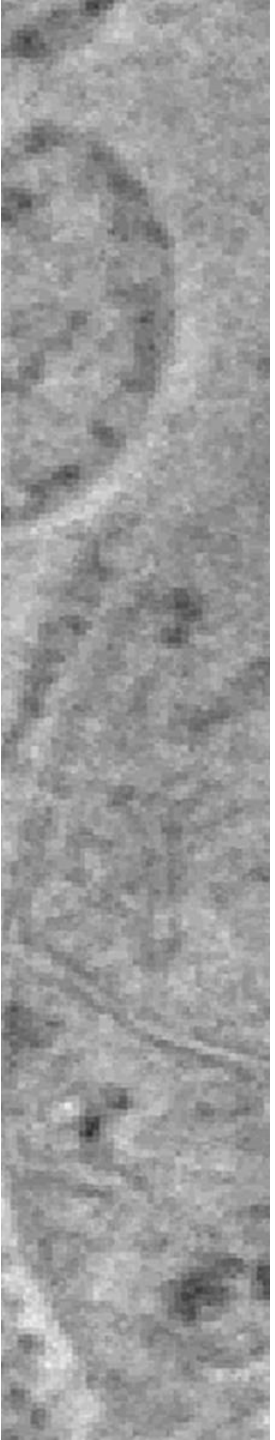
Wolf, S., Houben, L. & Elbaum, M. Cryo-scanning transmission electron tomography of vitrified cells. *Nat Methods* **11,** 423–428 (2014).



Summary

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

- The TEM is structurally similar to the light microscope
- Simple principles apply
- Configuring the illumination really is important
  it actually is confusing!
- Phase contrast is powerful but delicate
  depends on phase coherence!
- STEM is most useful when phase contrast is weak
  STEM phase contrast in the works!



Resources

IMpaCT IMAGING LIFE FROM MOLECULES TO CELLS

Ul-Hamid A (2018) Introduction. In: A Beginners' Guide to Scanning Electron Microscopy. Springer, Cham. doi:10.1007/978-3-319-98482-7_1

Maunsbach AB and Afzelius BA 1999 Biomedical Electron Microscopy: Illustrated Methods and Interpretations. Academic Press. DOI:10.1016/B978-0-12-480610-8.X5000-8

Murphy DB and Davidson MW 2012 Fundamentals of Light Microscopy and Electronic Imaging, Second Edition. Wiley-Blackwell DOI:10.1002/9781118382905

https://www.microscopyu.com/tutorials/kohler

https://www.slideshare.net/YinaGuo/a-look-inside-the-tem-em-forum-yina-guomar2016