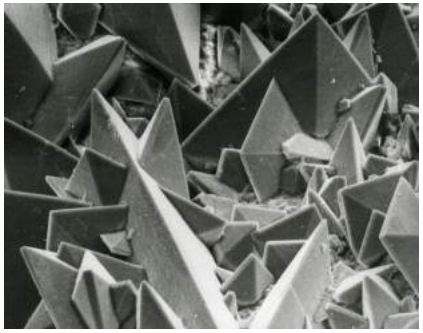
Introduction to image formation and contrast transfer function (CTF)

Marta Carroni, Swedish Cryo-EM Facility, Stockholm
Midlands, Cryo-EM workshop, 16th March 2021

Stockholm University

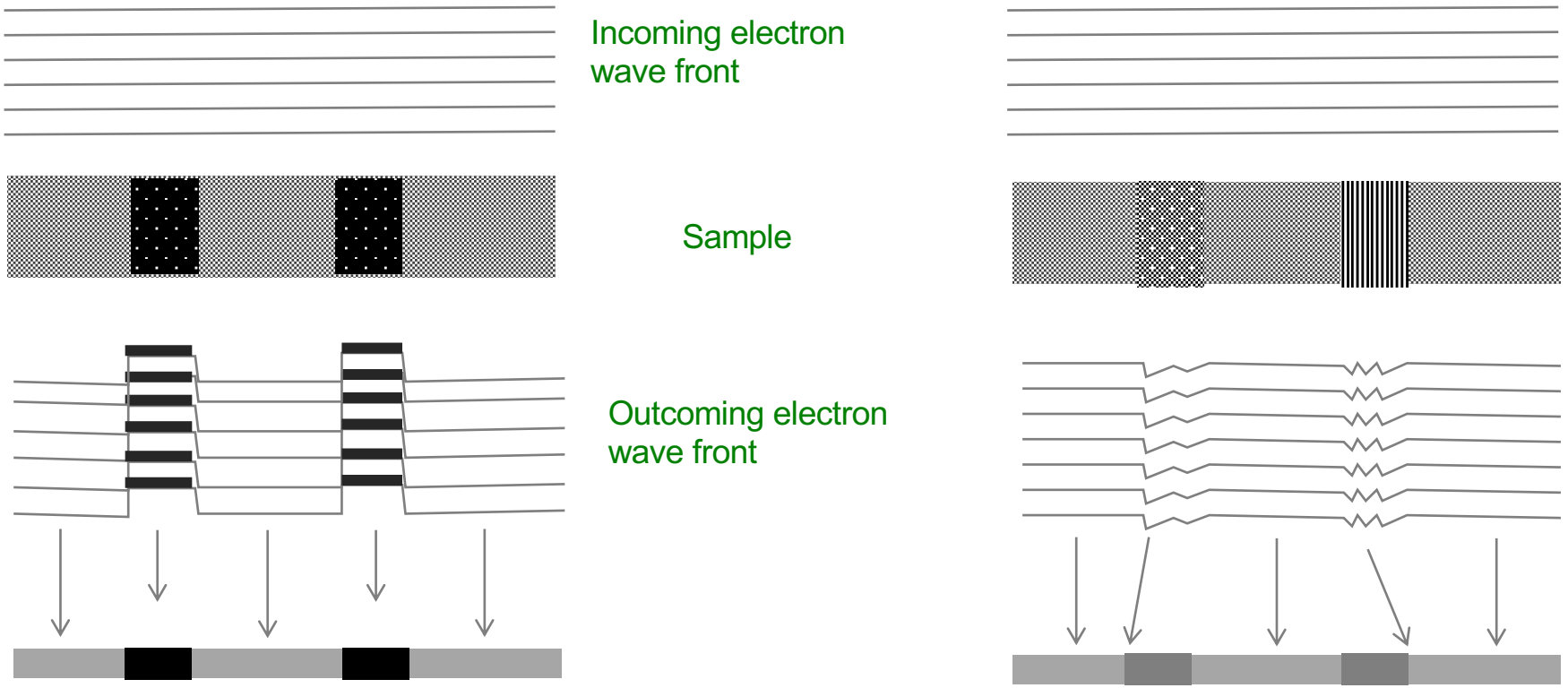
SciLifeLab

Electron-sample interaction



Back scattered
used for SEM

Image formation: amplitude and phase contrast

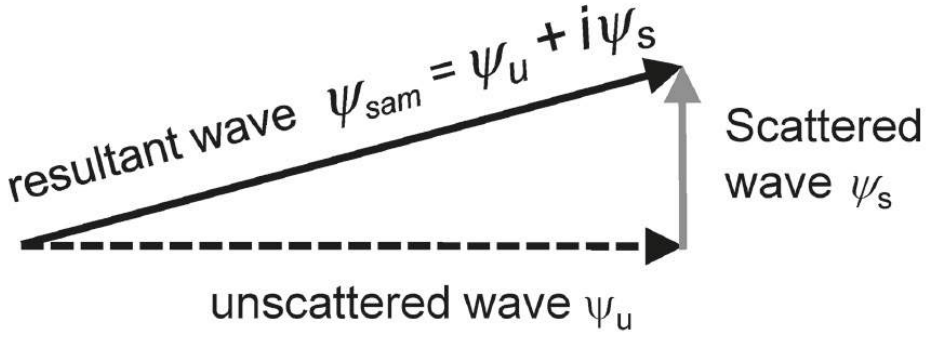


Amplitude contrast
resulting from the absorption of the incident beam

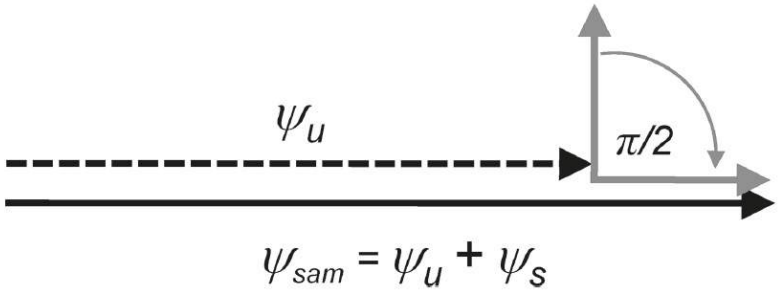
Phase contrast
resulting from phase-shift of the incident beam

The amplitude contrast in cryo-EM biological sample is minimal (~10%). The contrast is achieved by "transforming" phase shift into amplitude contrast

Increasing the contrast



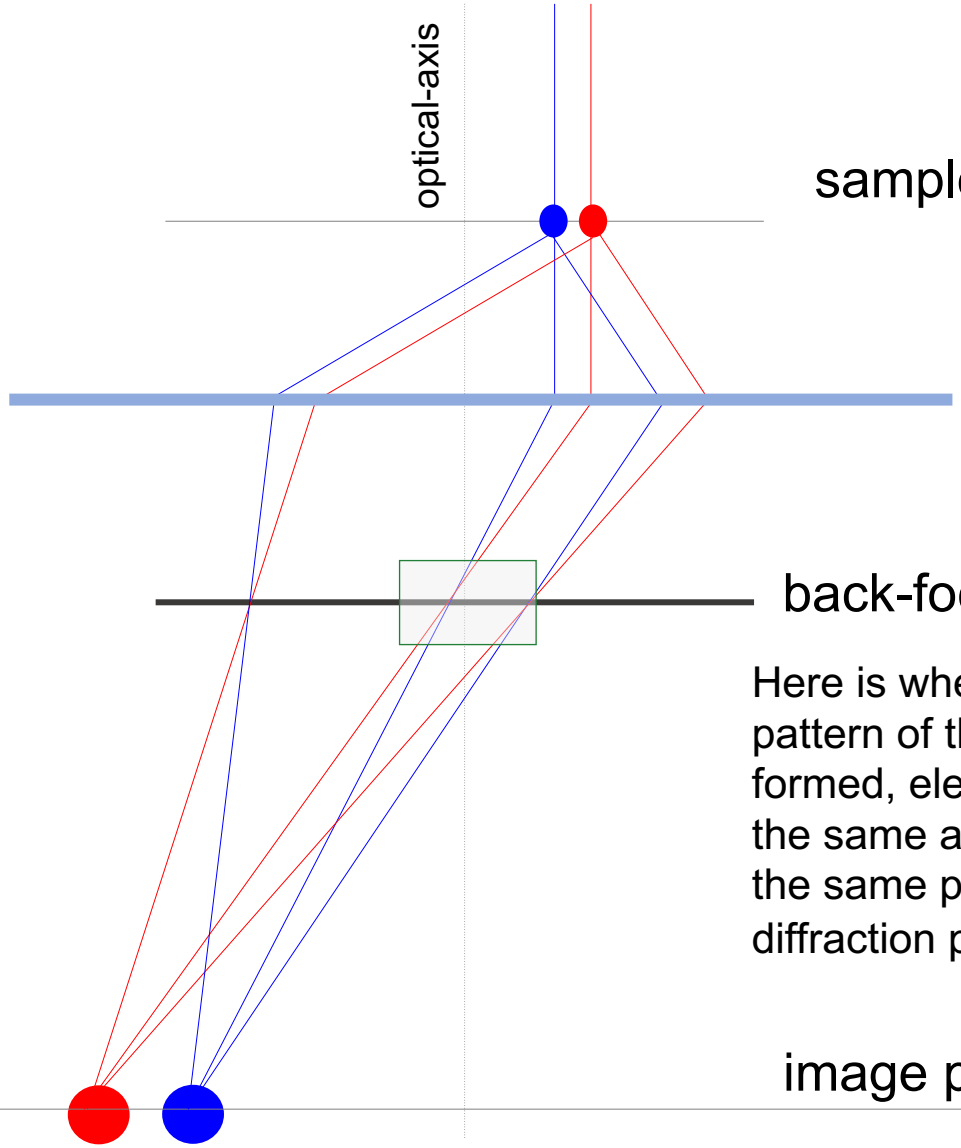
- The image is formed by the interference between the unscattered beam and the scattered one. The modules of the two vectors are not very different.



- We want to shift the phase of the scattered wave by 90º to rise the module of the resultant wave. This is for example achieved by using a phase plate.

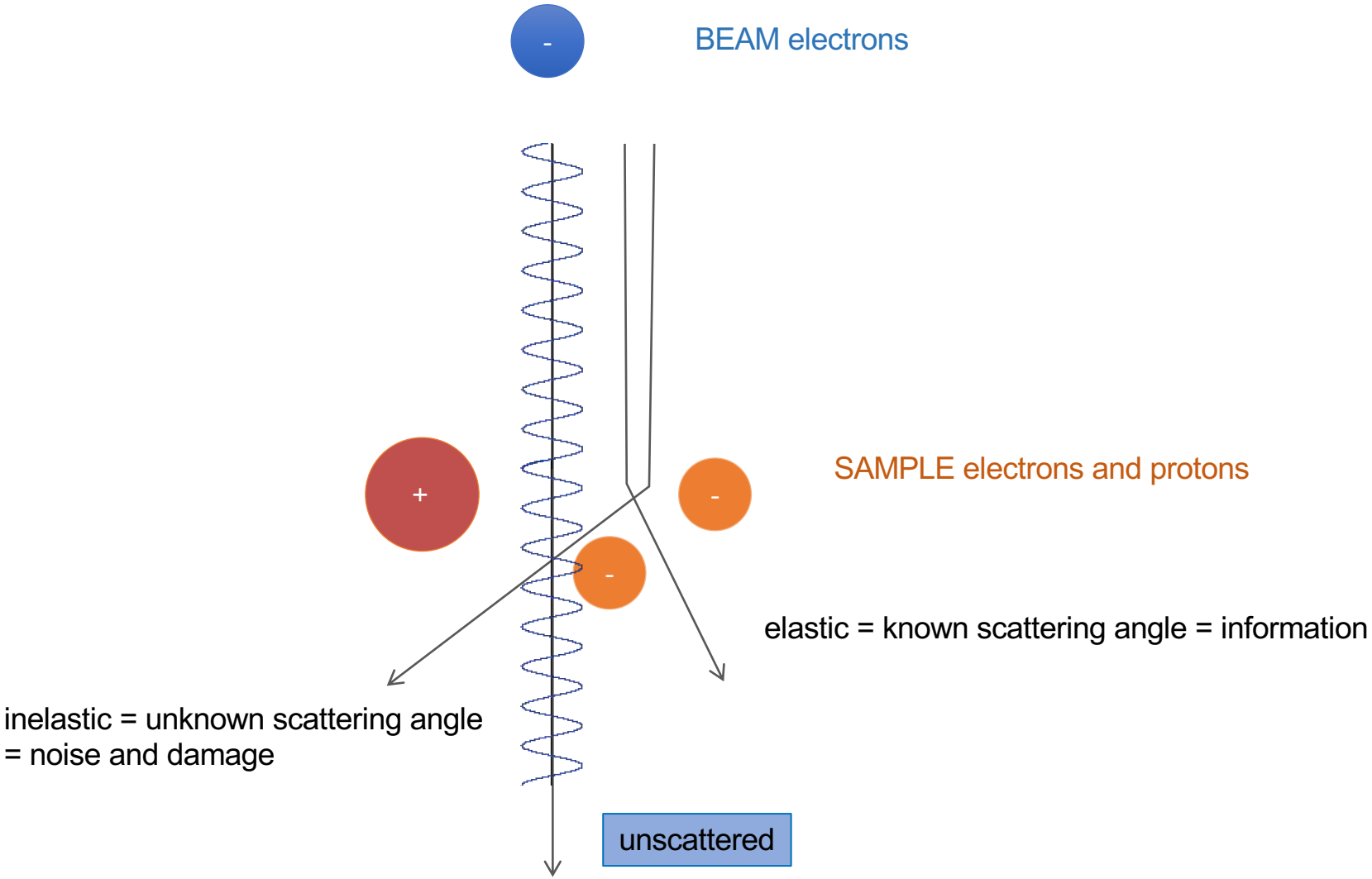
images from Orlova and Saibil, 2011

Image formation

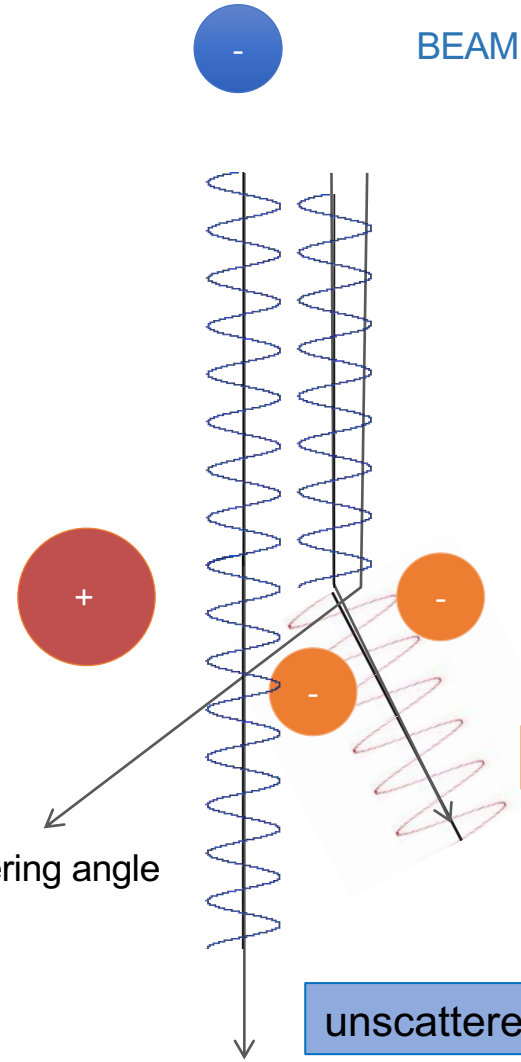


Here is where a diffraction pattern of the object is formed, electron scattered at the same angle converge in the same point in the diffraction plane.

Electron-sample interaction, wave paths



Electron-sample interaction, wave paths



BEAM electrons

SAMPLE electrons and protons

elastic = known scattering angle = information

inelastic = unknown scattering angle
= noise and damage

Waves scattered at a certain angle will experience a certain phase shift.

unscattered

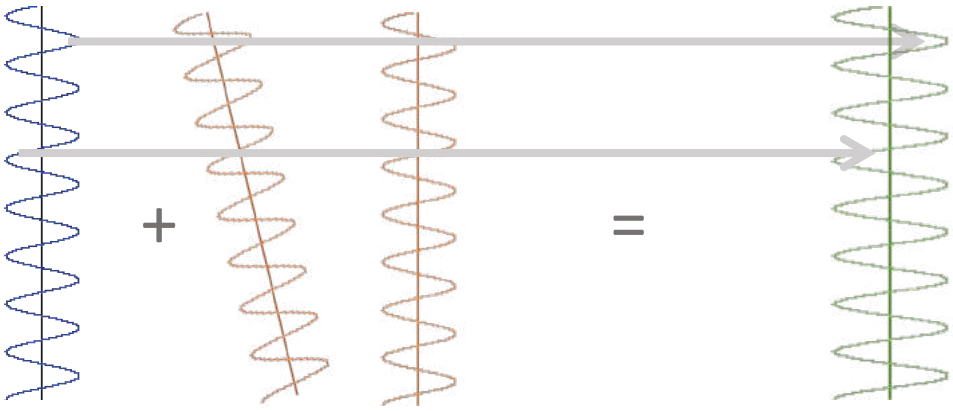
Electron-sample interaction, wave paths

The final image is a combination of the unscattered and the scattered beam.

For each angle of scattering we have a certain phase shift.

When this phase shifted wave is combined with the unscattered wave a certain type of interference occurs.

When the interference is constructive the contrast will increase when destructive will decrease or will be equal to zero.



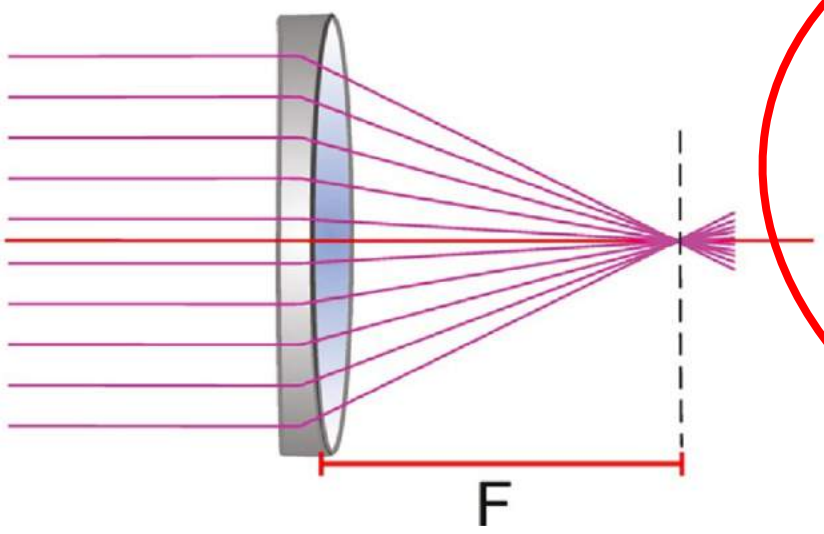
unscattered

Scattered at angle theta

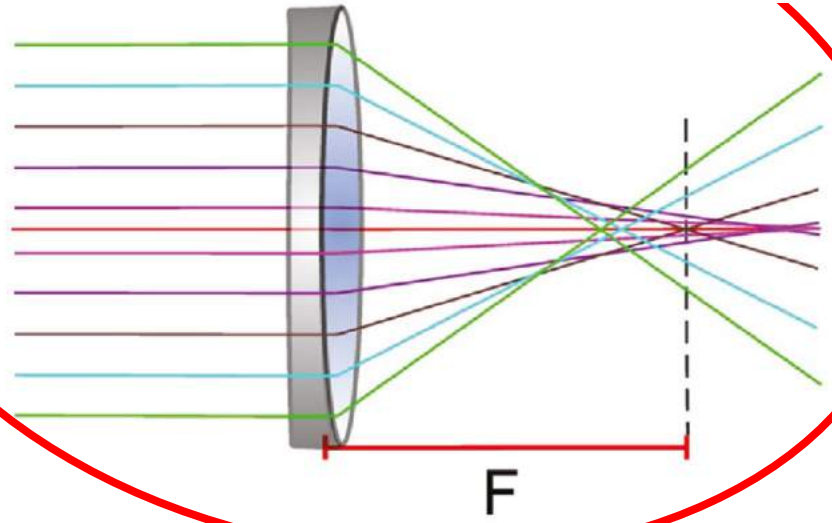
Constructive interference > more amplitude > more constrast

Lens aberrations

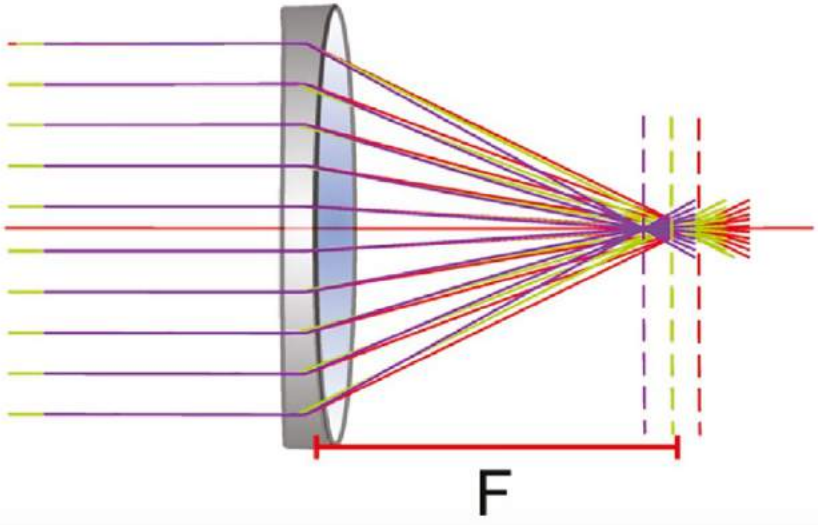
perfect lens



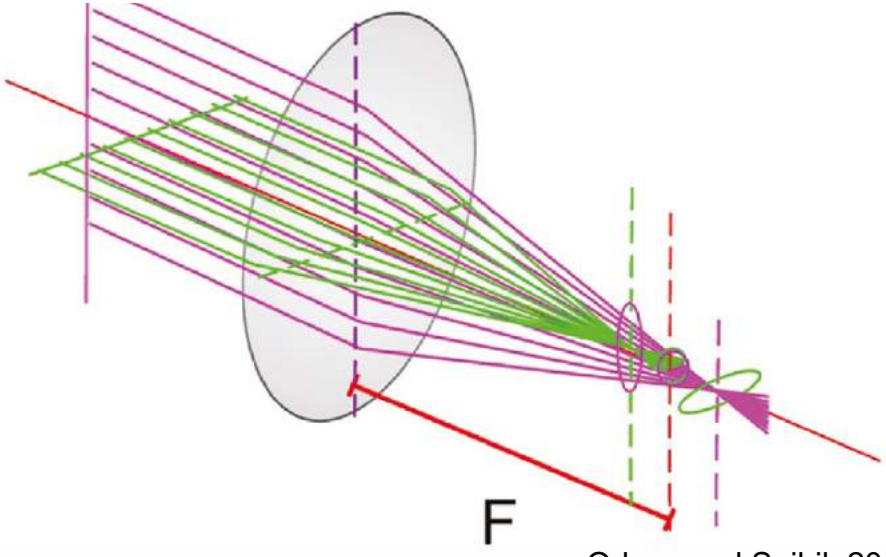
spherical aberration (Cs)



chromatic aberration (Cc)



astigmatism



Orlova and Saibil, 2011

Image formation, putting together all aberrations

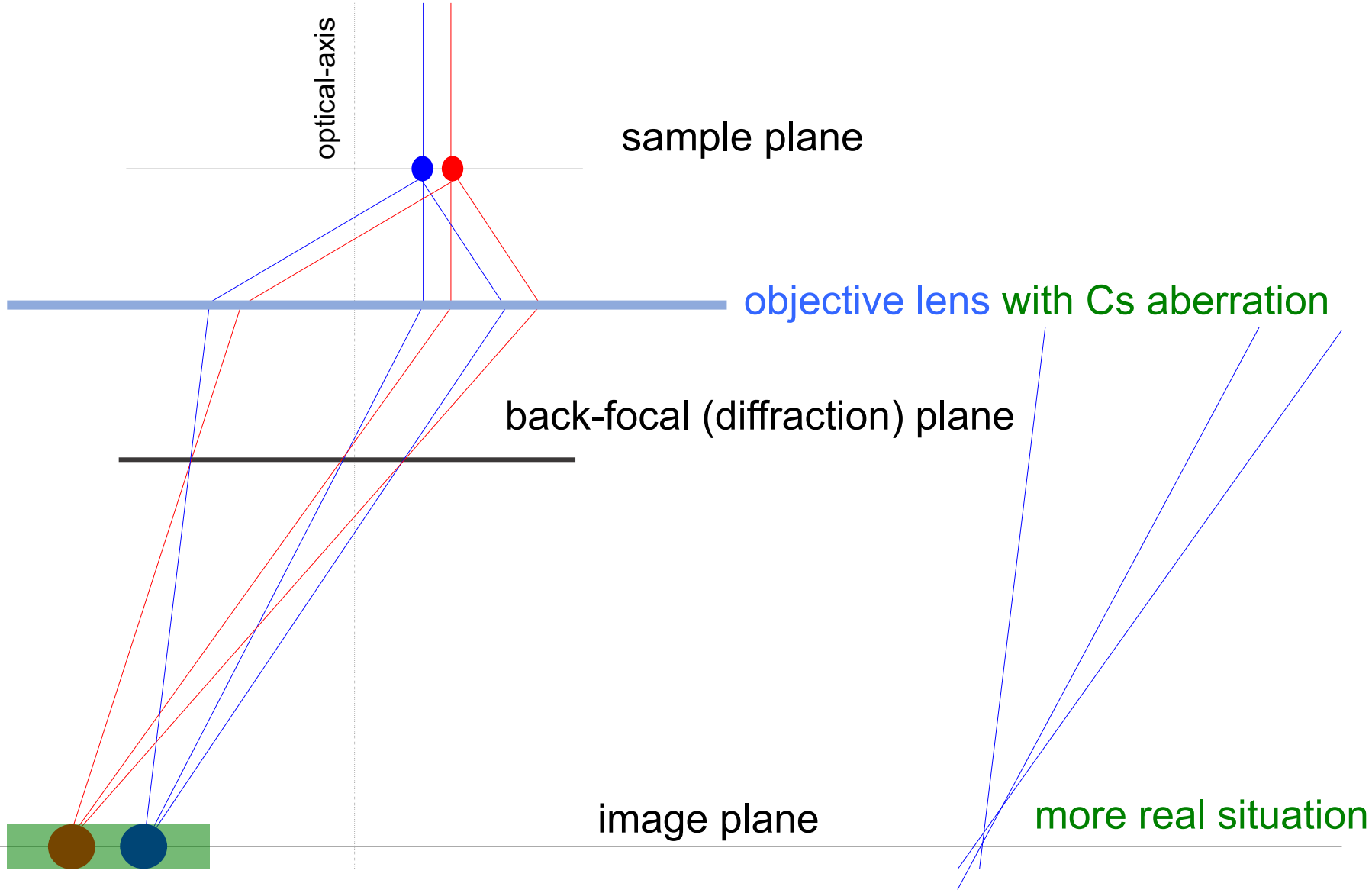


Image formation, ray paths, Cs

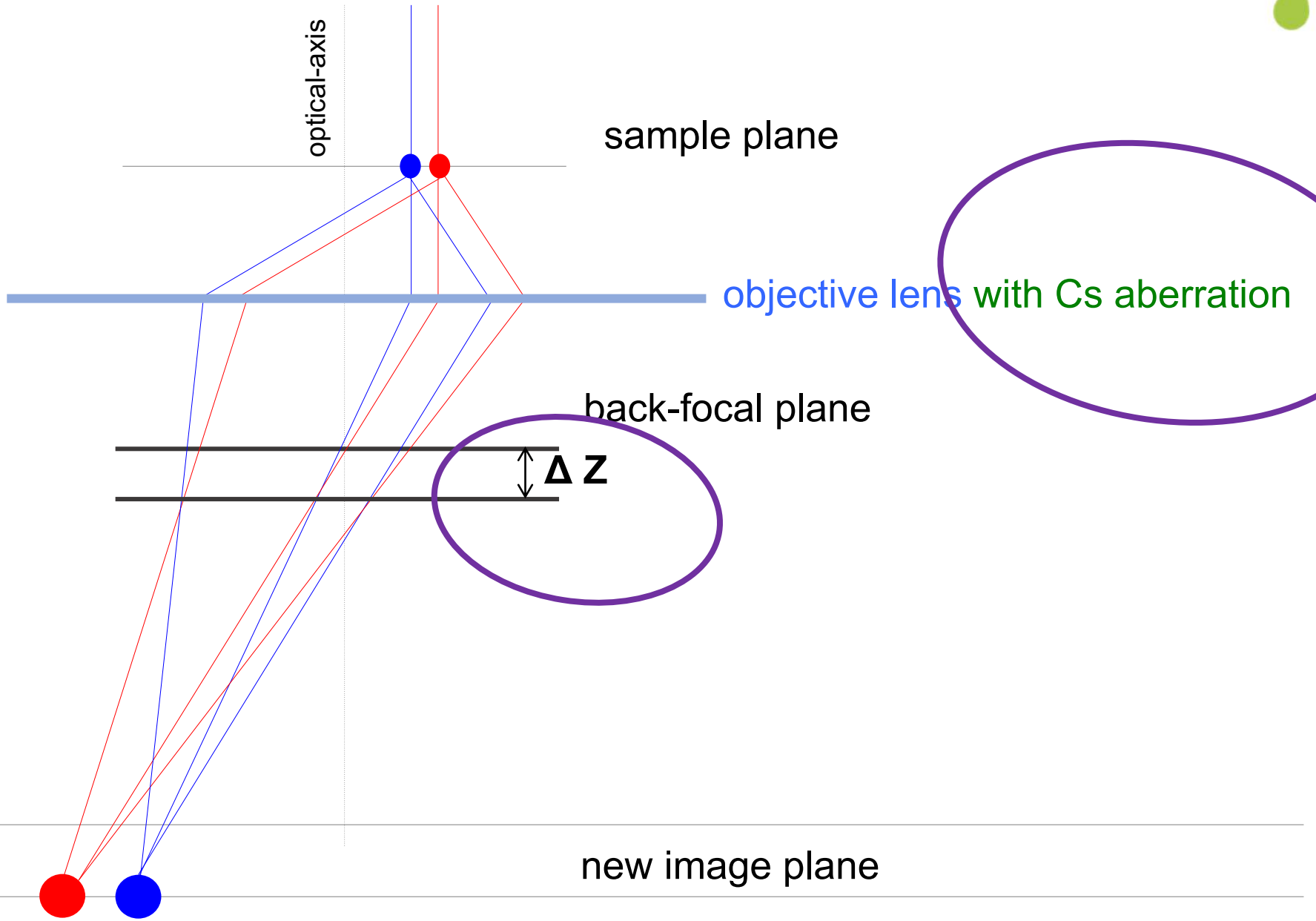
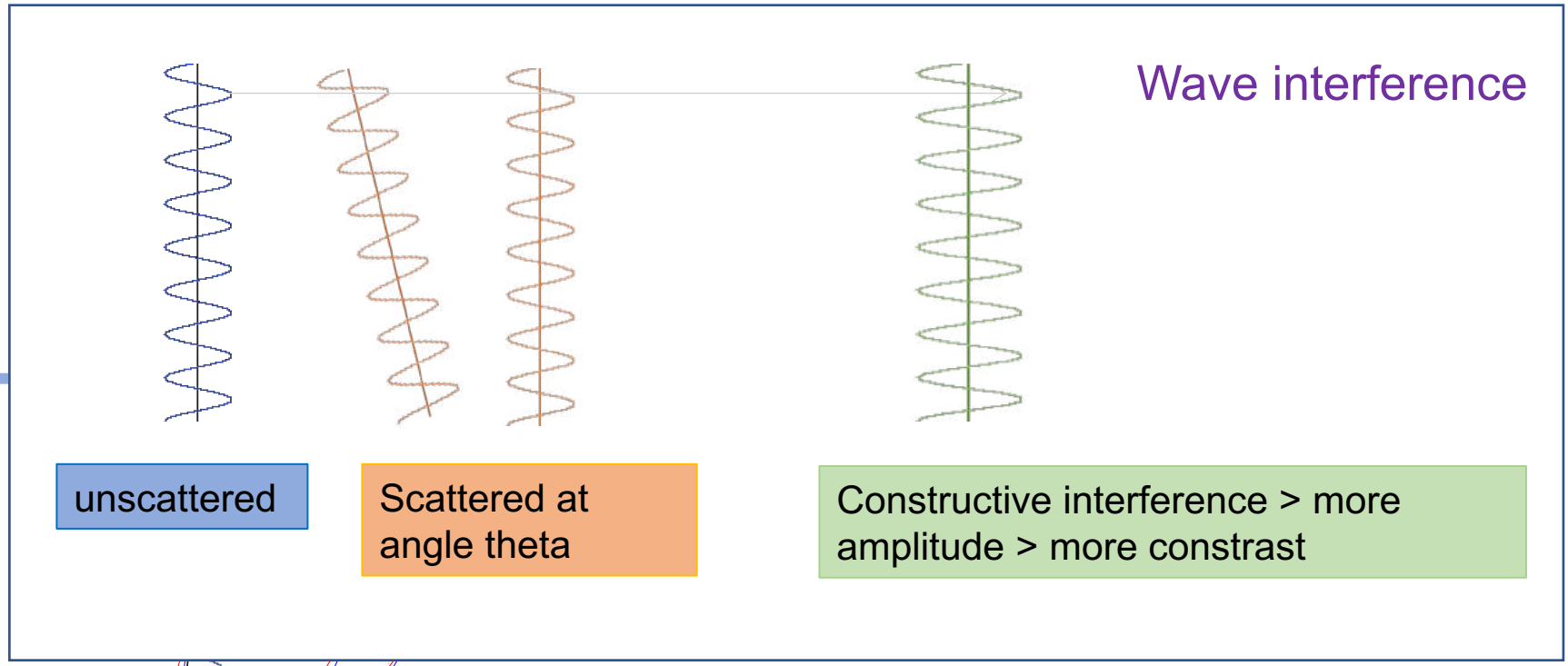


Image formation, putting together all aberrations



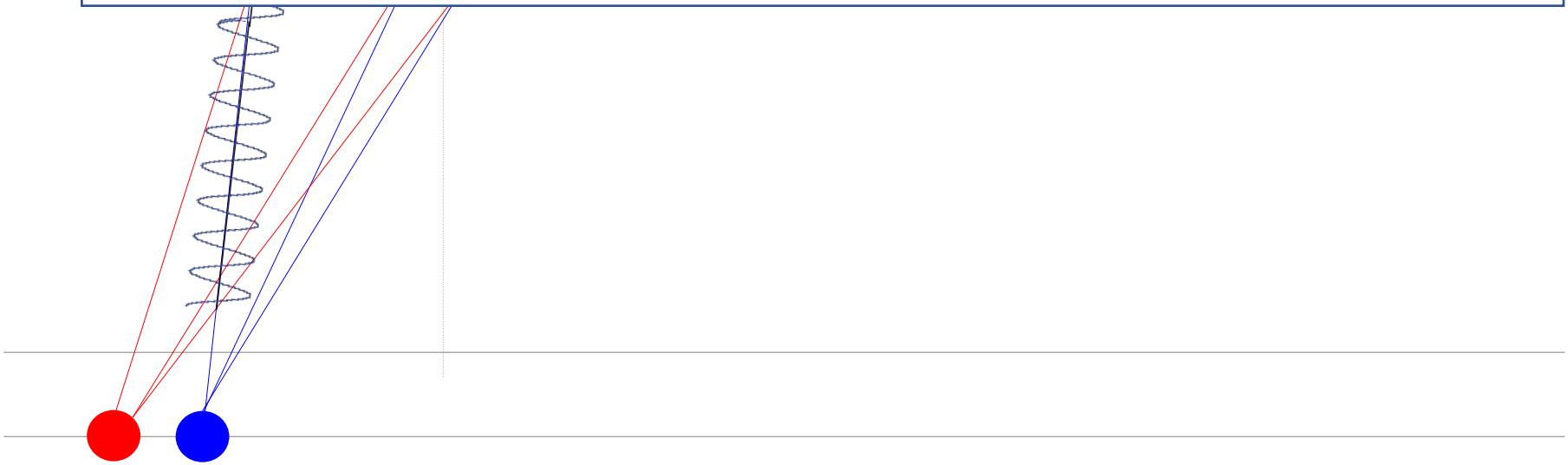
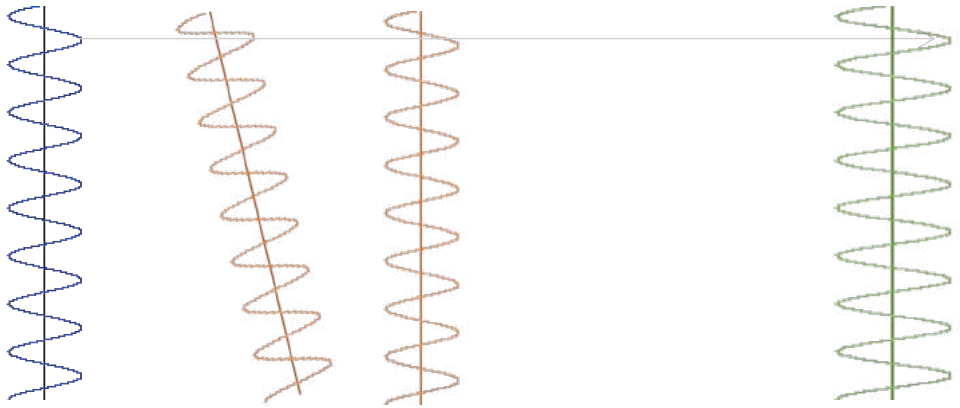


Image formation (waves paths)



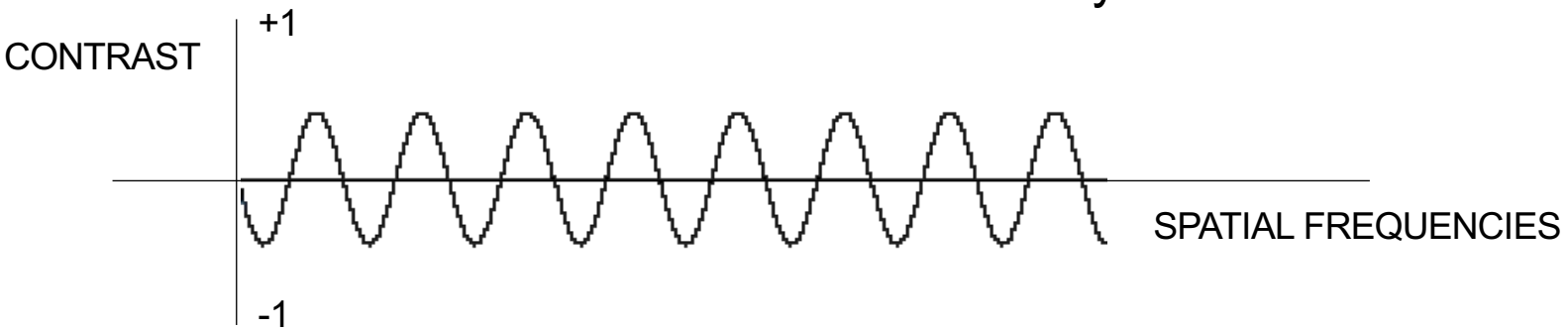
Wave interference

unscattered

Scattered at angle theta

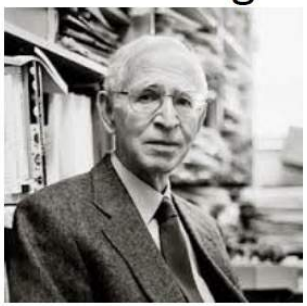
Constructive interference > more amplitude > more constrast

Oscillatory transfer of information



Signal modulation by aberrations

Aaron Klug



The modulation of amplitudes of the scattered wave by lens aberrations is easily described at the diffraction plane as a function of the scattering angle (i.e. of the spatial frequency that is represented).

defocus

scattering angle

$$CTF(f) = A\sin(\pi\lambda\Delta z f^2 - \frac{1}{2}\pi\lambda^3 c_s f^4)$$

wavelength

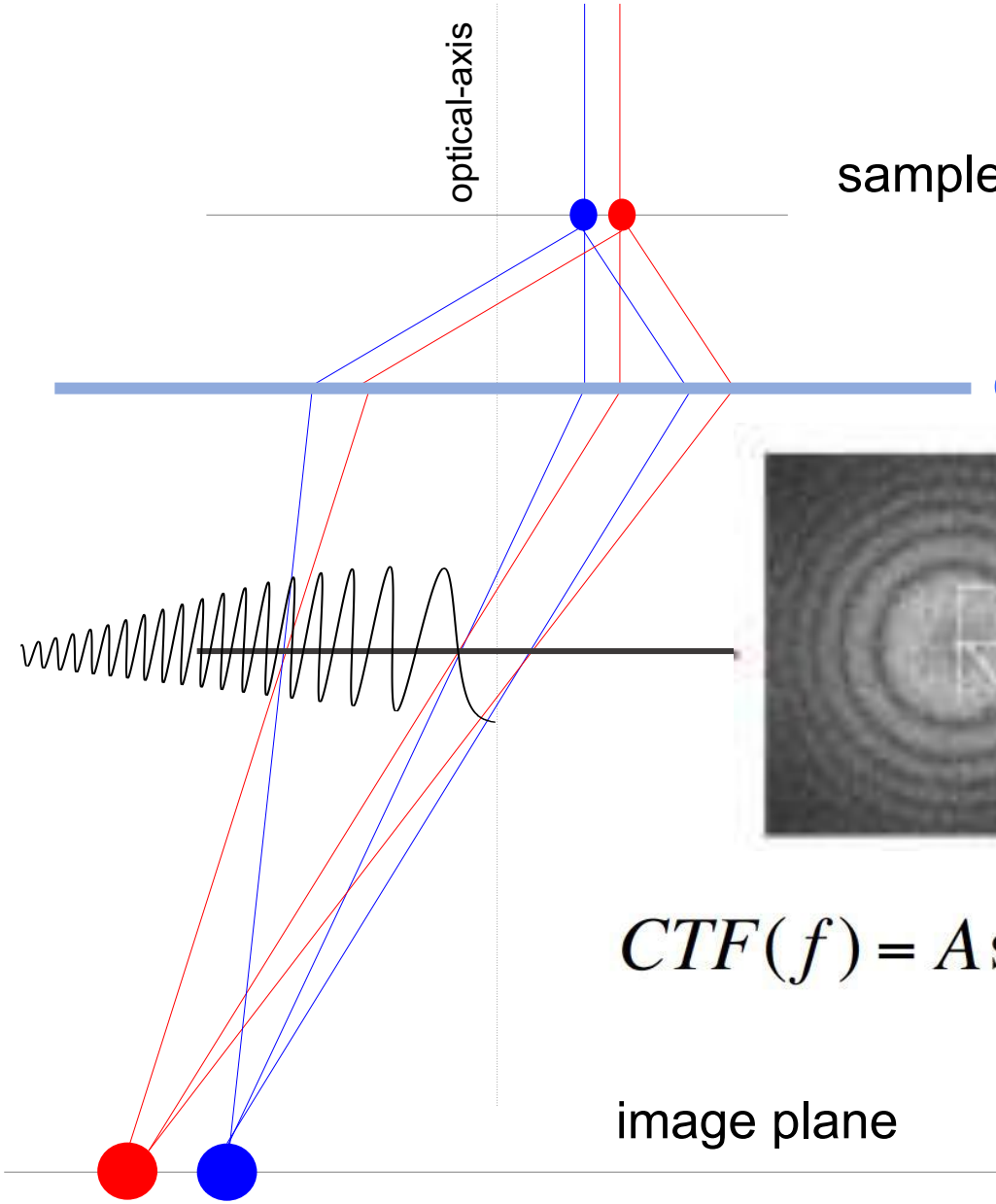
spherical aberration

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

The image formed is affected by the CTF



sample plane

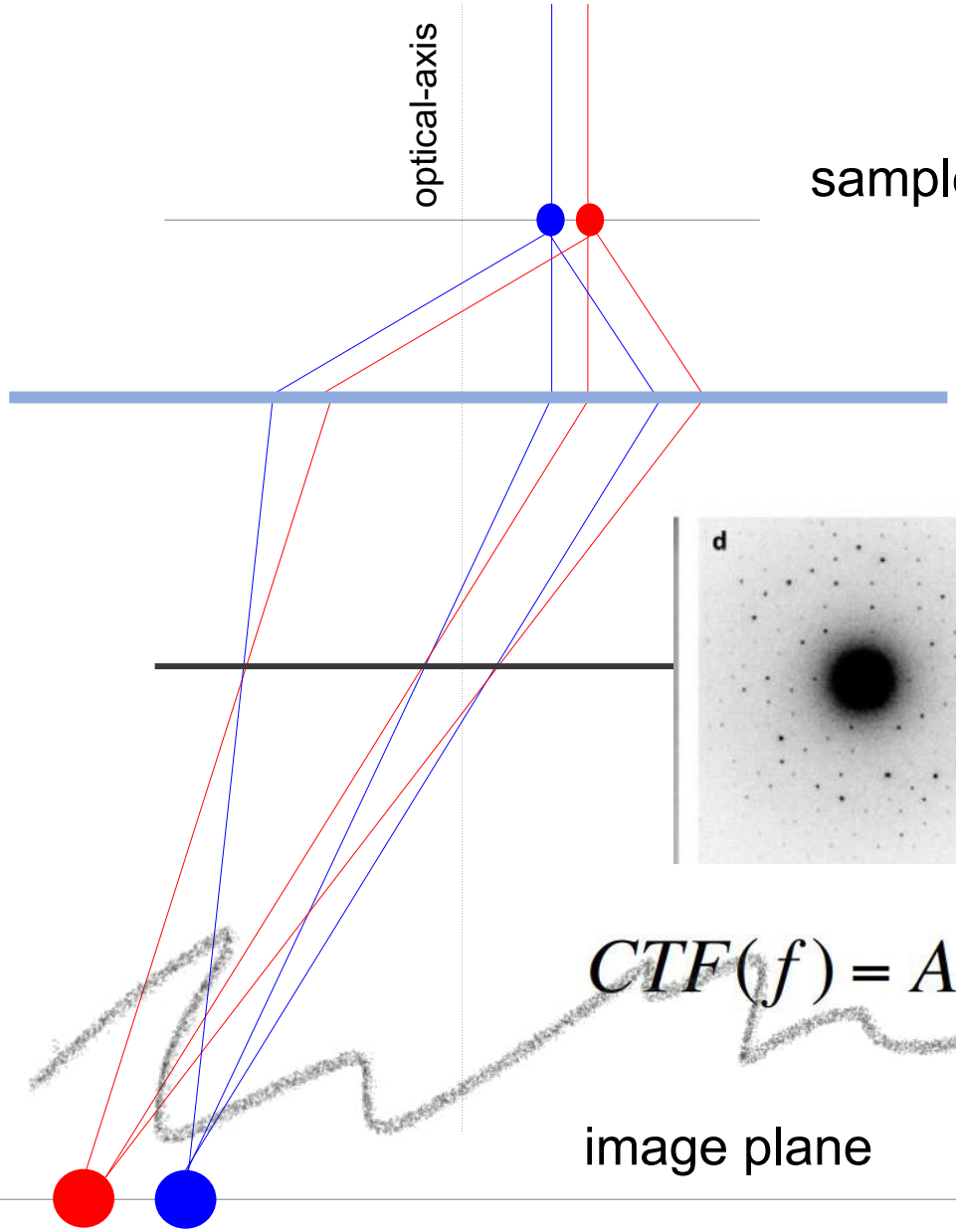
objective lens with Cs aberration

back-focal plane

$$CTF(f) = A\sin(\pi\lambda\Delta z f^2 - \frac{1}{2}\pi\lambda^3 c_s f^4)$$

image plane

The diffraction pattern is not affected by the CTF



sample plane

objective lens with Cs aberration

back-focal plane

$$CTF(f) = A\sin(\pi\lambda\Delta z f^2 - \frac{1}{2}\pi\lambda^3 C_s f^4)$$

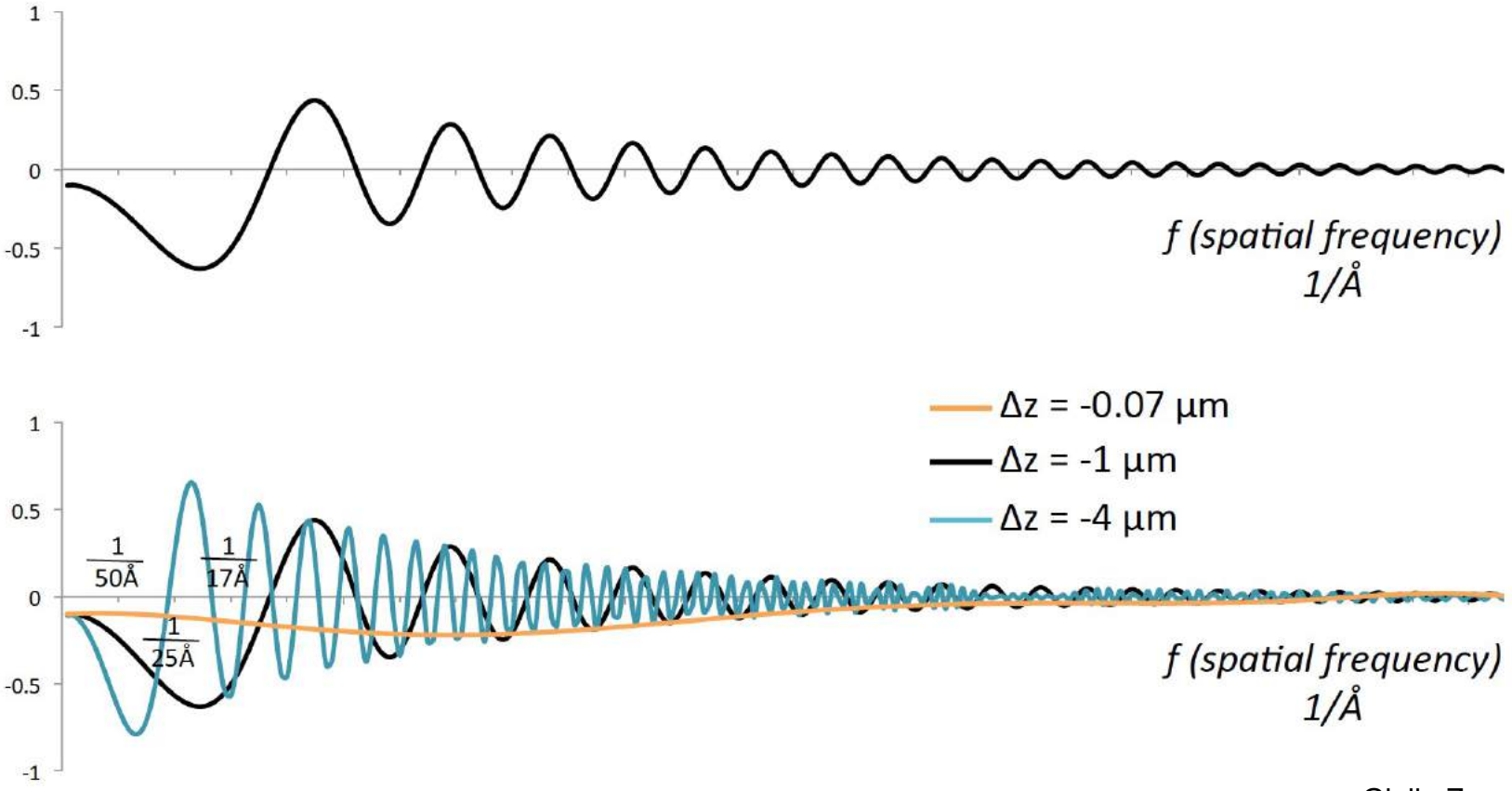
image plane

At different defoci different zeros and inversions

$$CTF(f) = A\sin(\pi\lambda\Delta z f^2 - \frac{1}{2}\pi\lambda^3 c_s f^4)$$

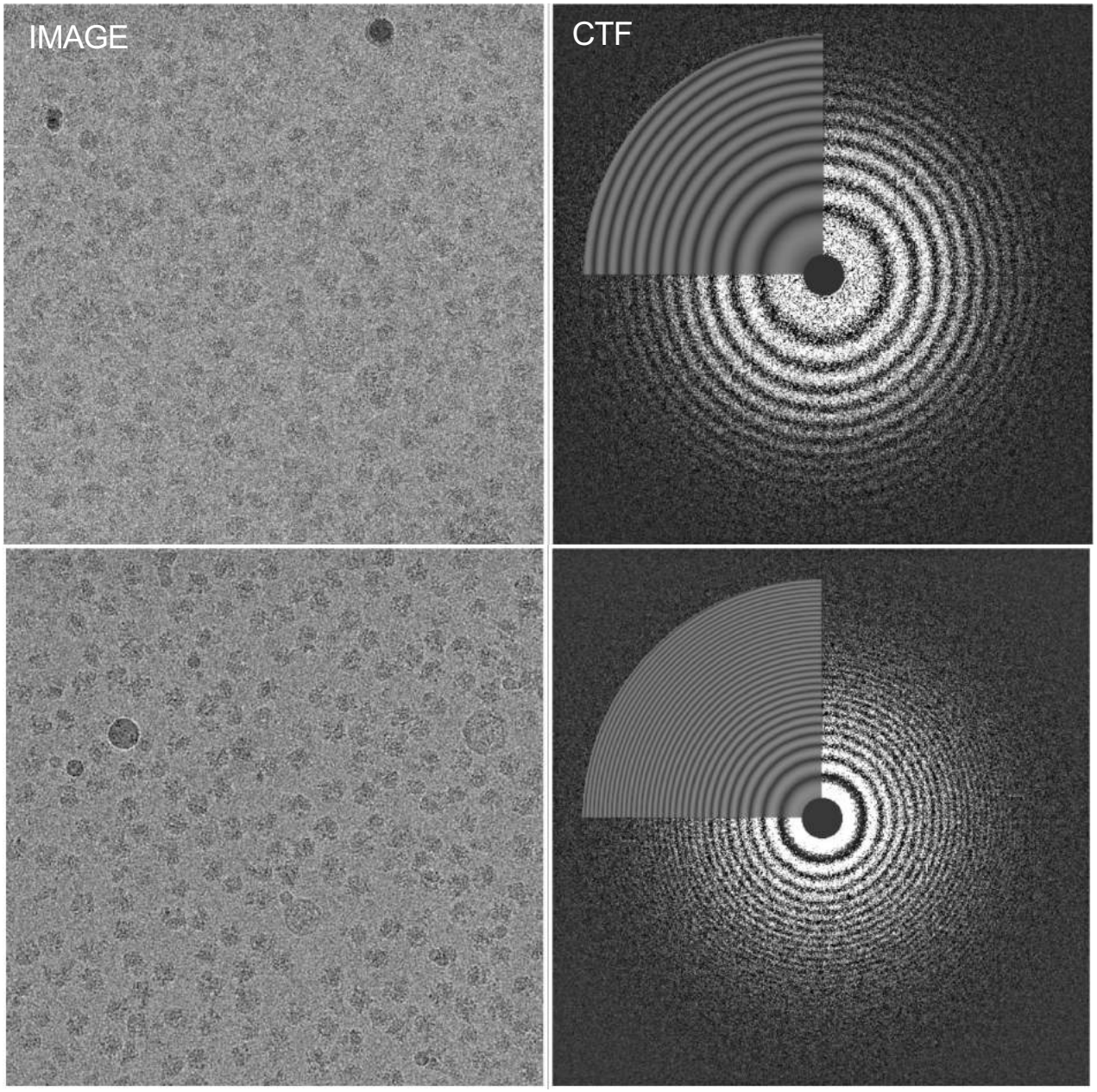
defocus

The oscillation of the CTF depends on the defocus

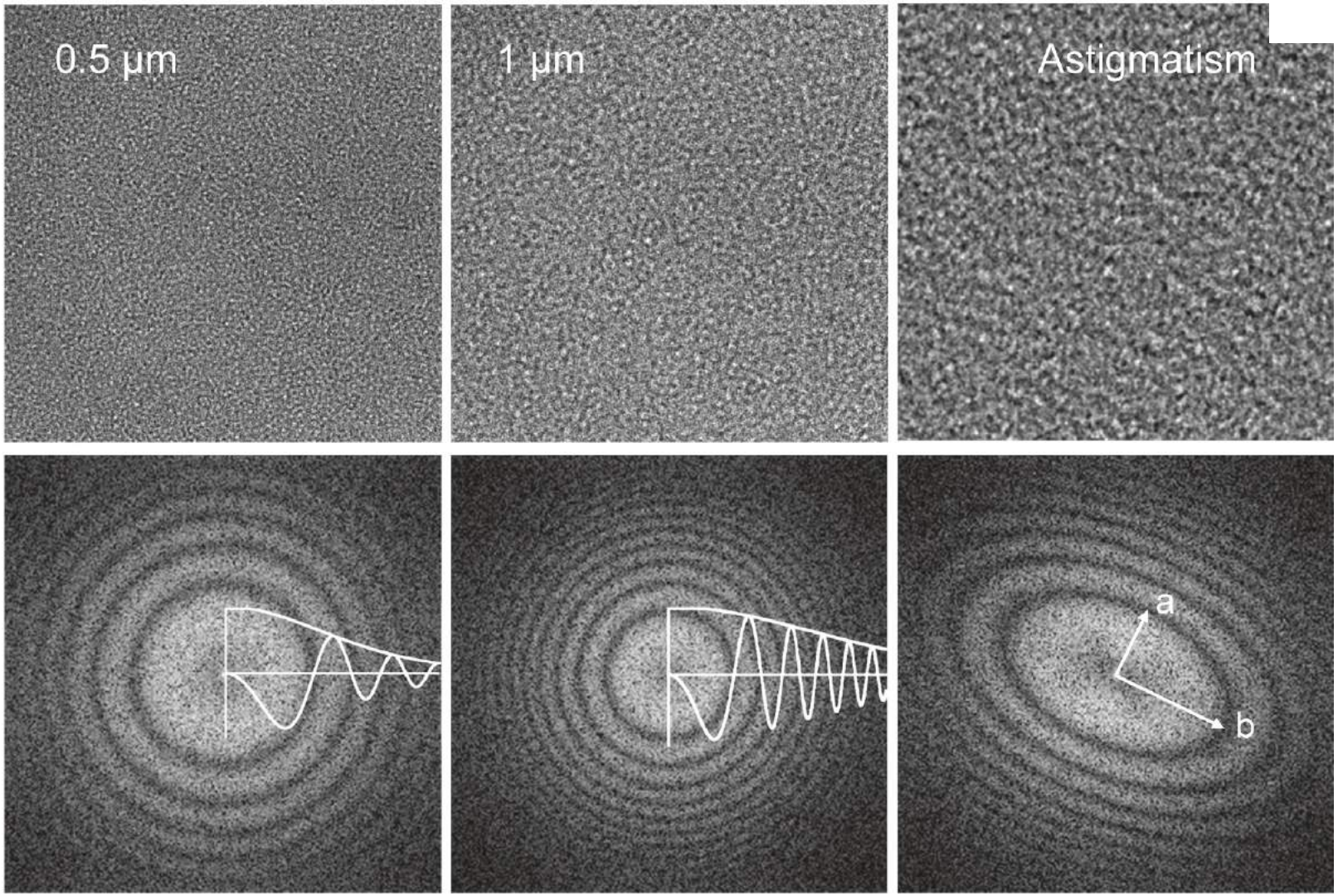


Giulia Zanetti

Defocus effects



Information from the power spectrum

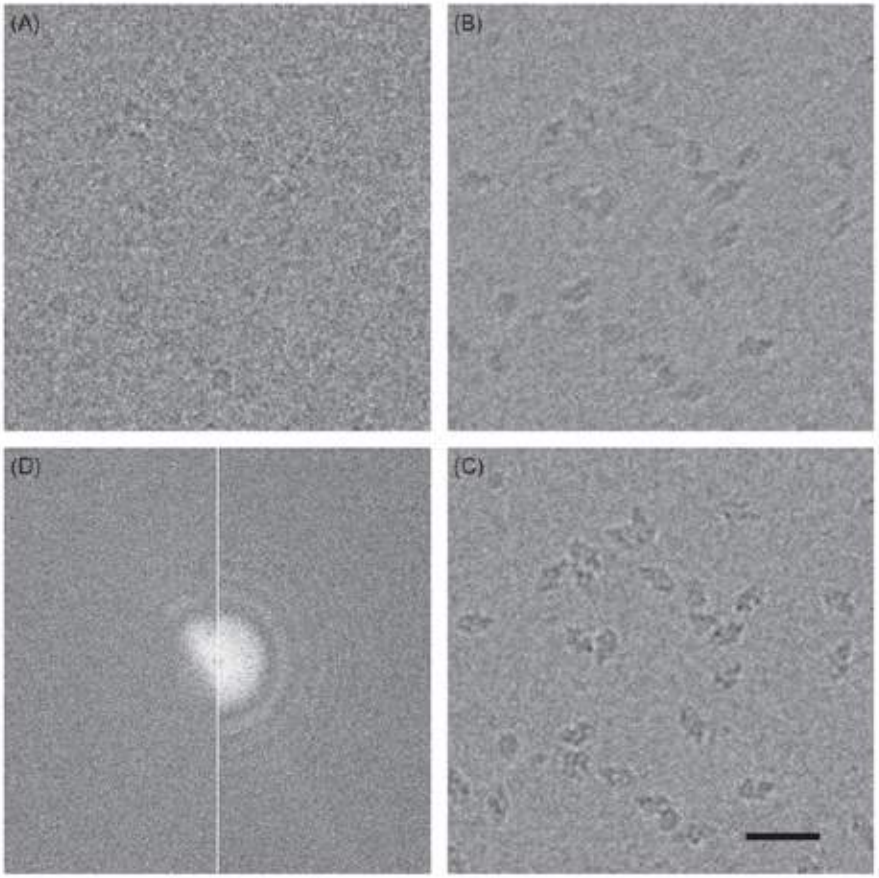


Orlova and Saibil, 2011

Information from the power spectrum, drift

single frame with an electron dose of 6 electrons/Å2, the sum of movie frames with a total electron dose of 50 electrons/Å2

the power spectra (Fourier transforms) of the unaligned (left) and aligned (right) images



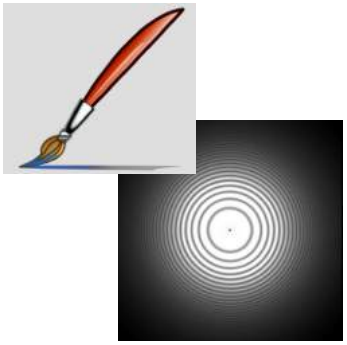
Without alignment

With alignment

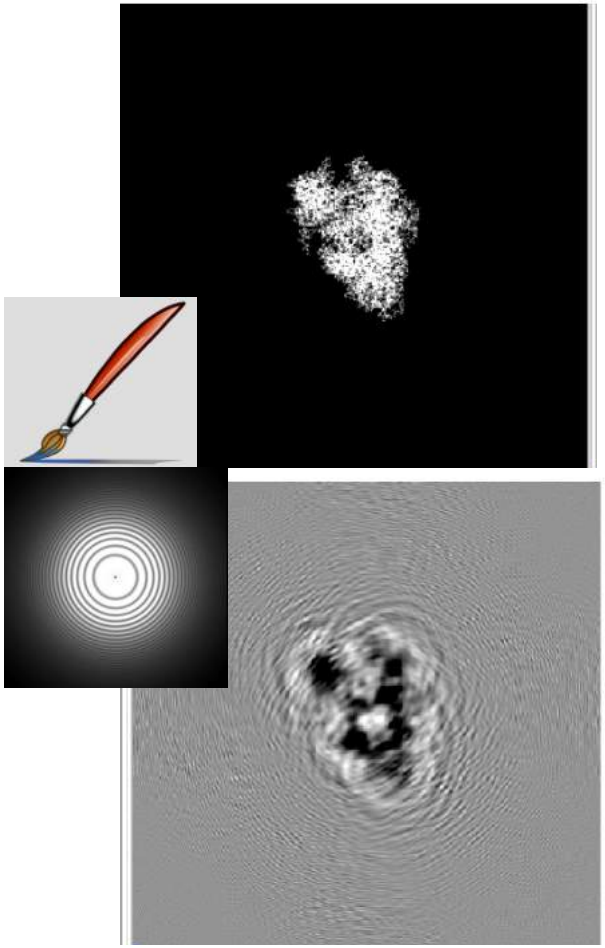
From Richard Henderson

CTF, the microscope brush

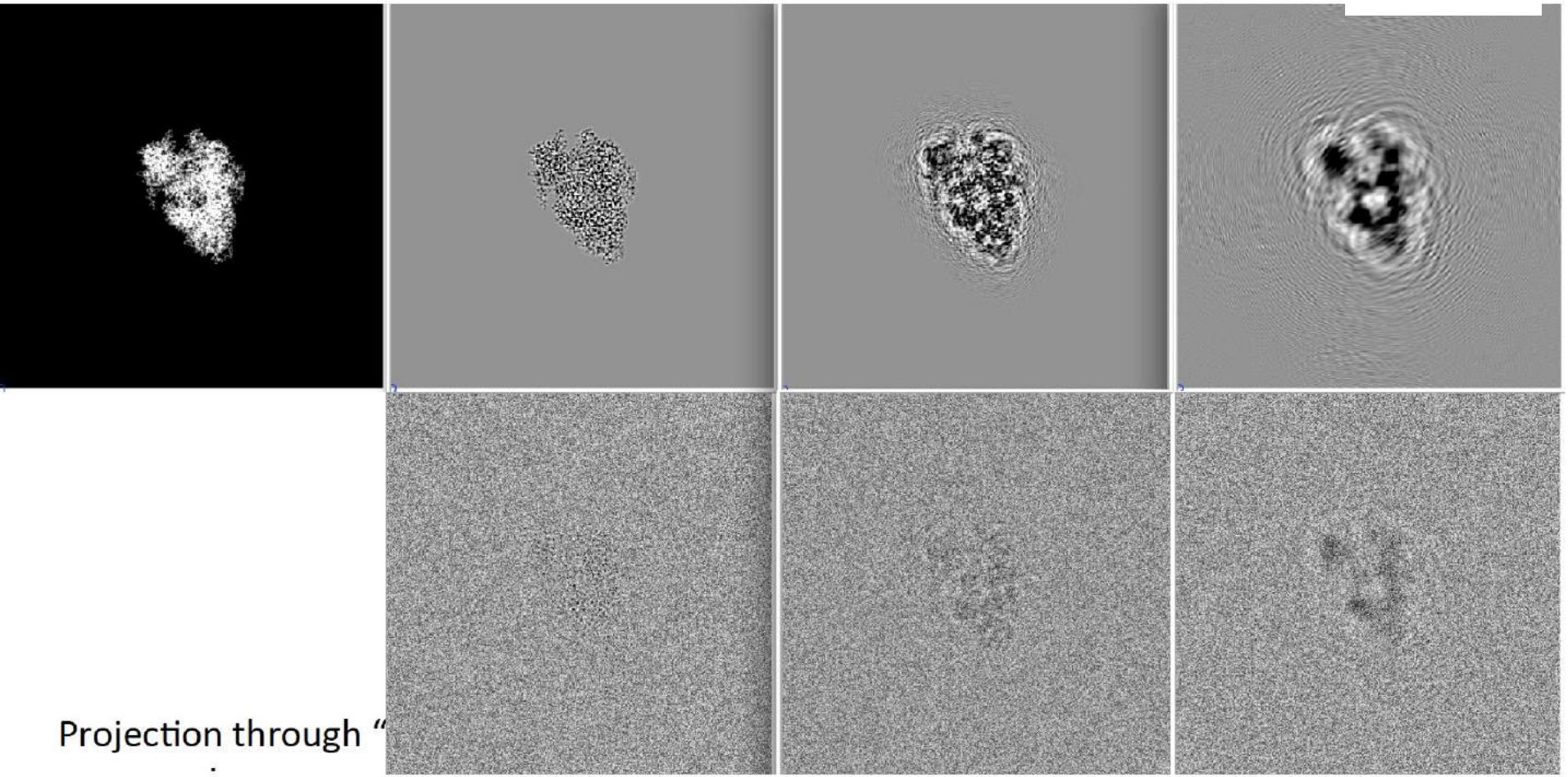
correction



Microscope aberrations and ways of improving contrast affects the resulting image by the contrast transfer function (CTF).
The CTF is the EM image point-spread function.

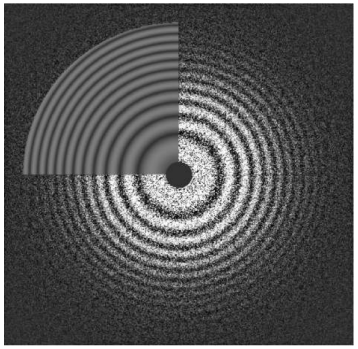


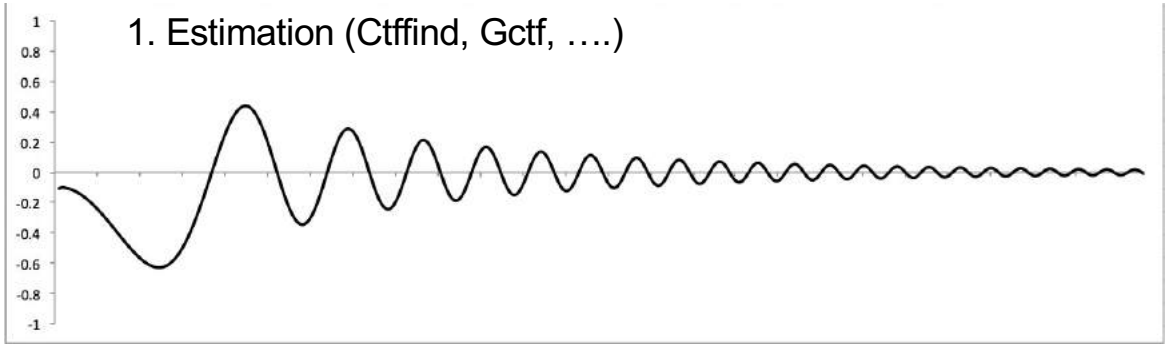
CTF, useful at low signal to noise

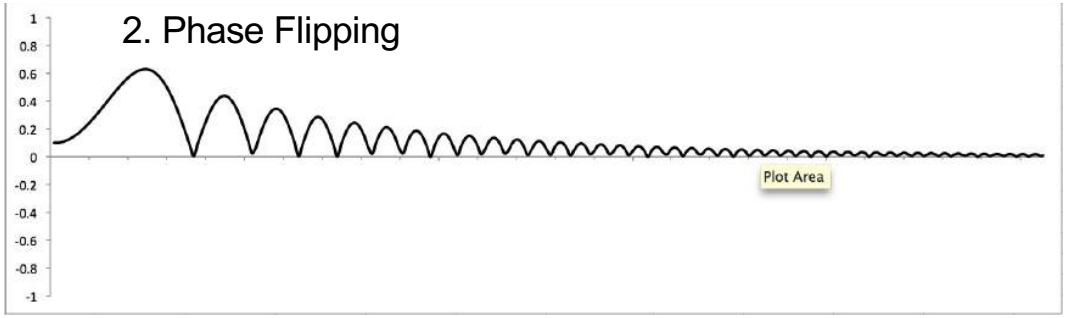


Giulia Zanetti

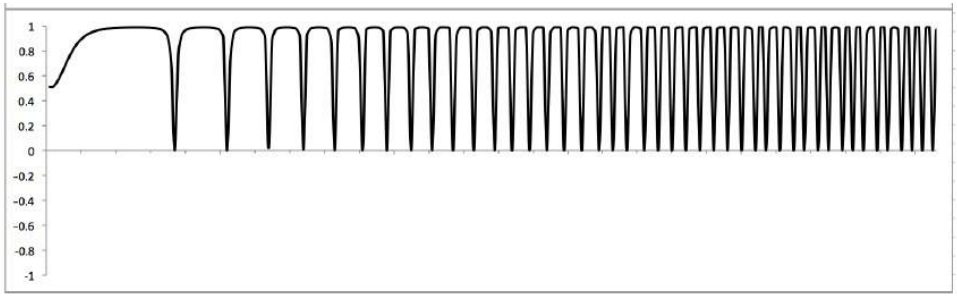
CTF correction







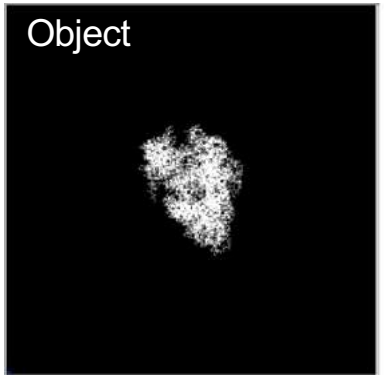
3. Full correction with Wiener filtering

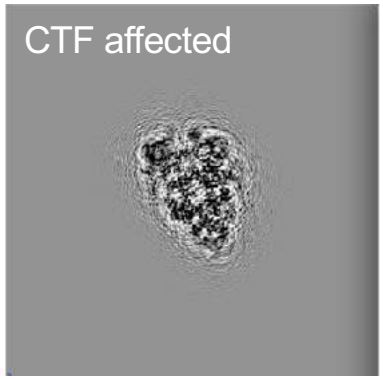


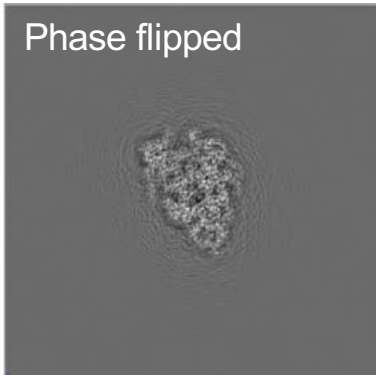
$$FT_{corr} = FT\,\frac{CTF(f)}{CTF(f)^2 + \sigma_n}$$

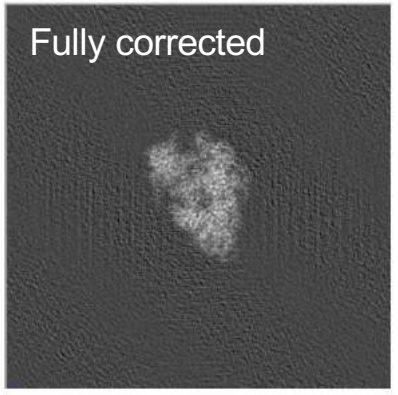
noise variance

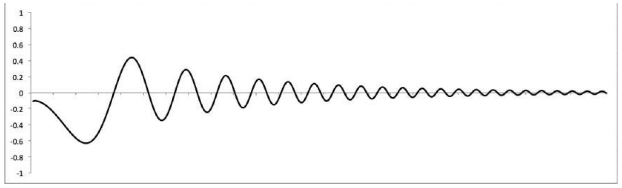
CTF correction

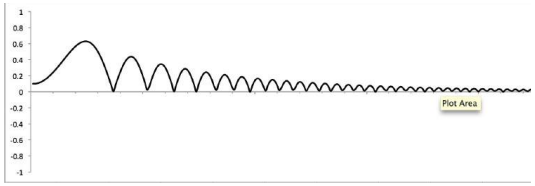


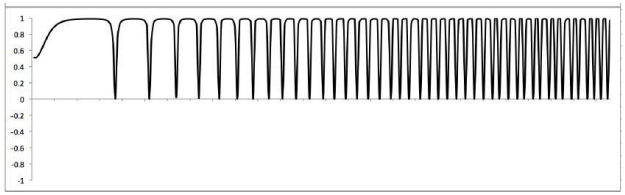






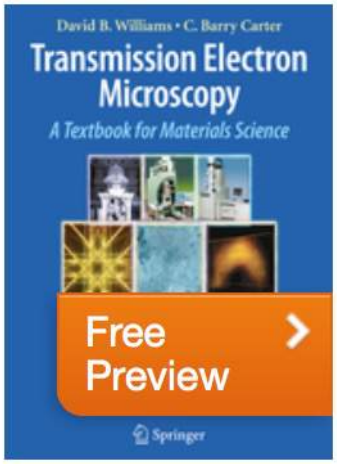






Giulia Zanetti

Some literature



© 2009

Transmission Electron Microscopy

A Textbook for Materials Science

Authors: **Williams**, David B., **Carter**, C. Barry

Henderson. The potential and limitations of neutrons, electrons and X-rays for atomic resolution microscopy of unstained biological samples (1995) Quarterly Reviews in Biophysics 28(2): 171-193.

Grant and Grigorieff. Measuring the optimal exposure for single particle cryo-EM using a 2.6 Å reconstruction of rotavirus (2015) Elife 4:e06980.

Baker and Rubinstein. Cryo-EM Part A: Chapter 15 -Radiation damage in electron cryomicroscopy (2010) Methods in Enzymology, 481:371-388.