

What can be seen with a transmission electron microscope

Ottilie von Loeffelholz

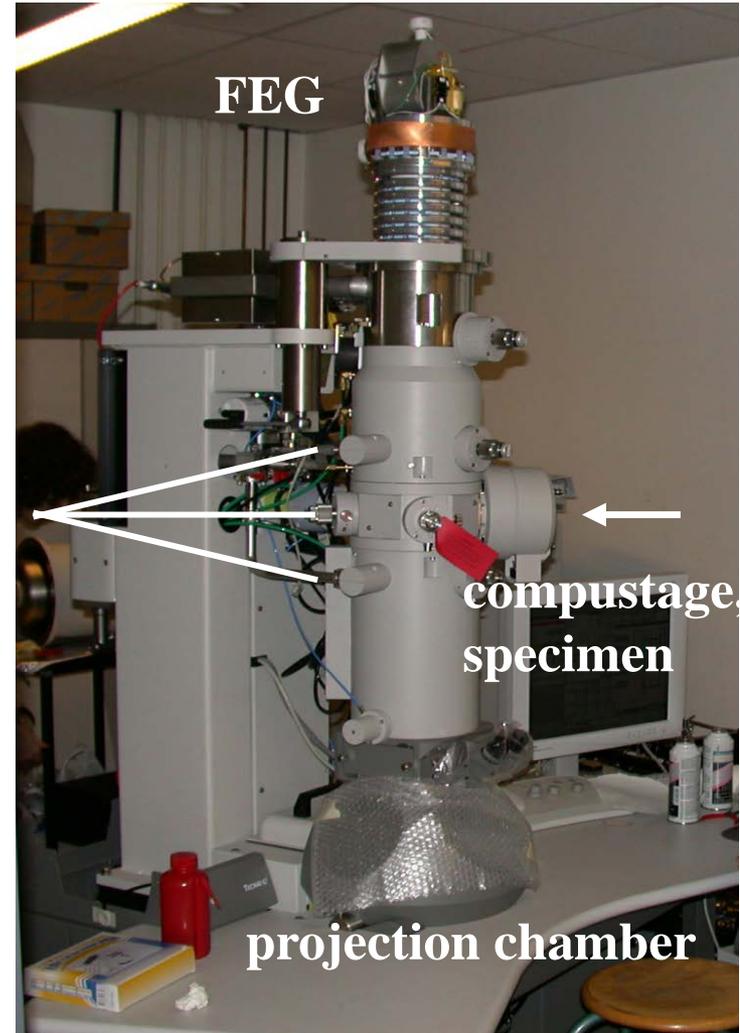
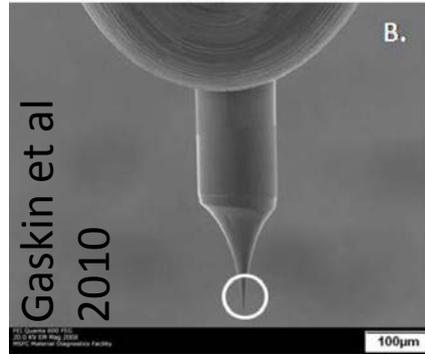
Klaholz Group, IGBMC Strasbourg

The transmission electron microscope

Thungsten filament



Field emission gun



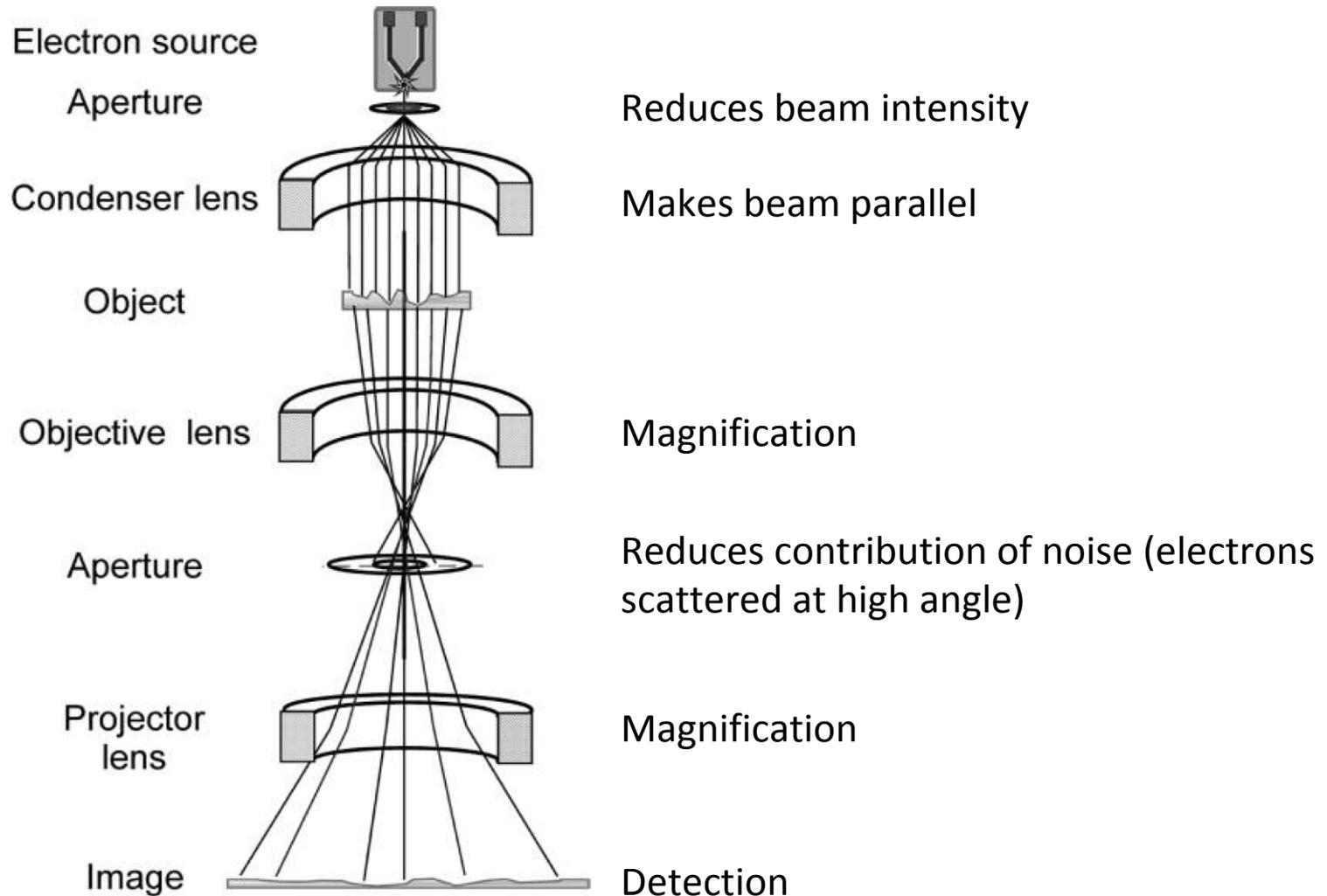
- vacuum: $\sim 10^{-6}$ Pa
- potentially high electron dose
- potentially high resolution ($\lambda \approx 0.027 \text{ \AA}$ at 200kV)
i.e. not limited by the wavelength or the optical system

apertures

compustage
specimen

projection chamber

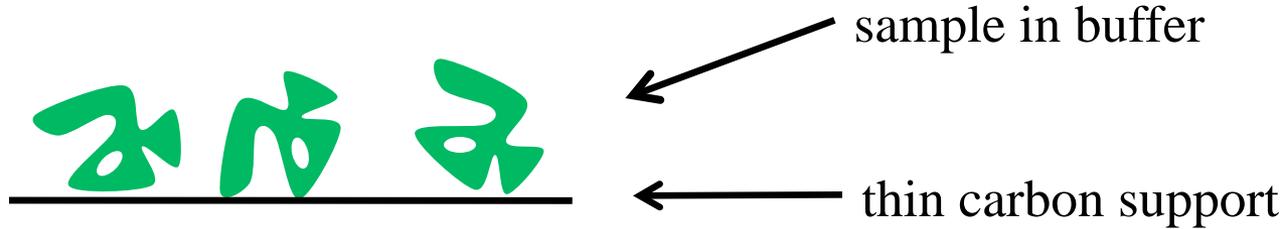
Image formation with an electron microscope



Electron microscopy techniques

Negative staining

1) adsorption



2) wash with 2% uranyl acetate



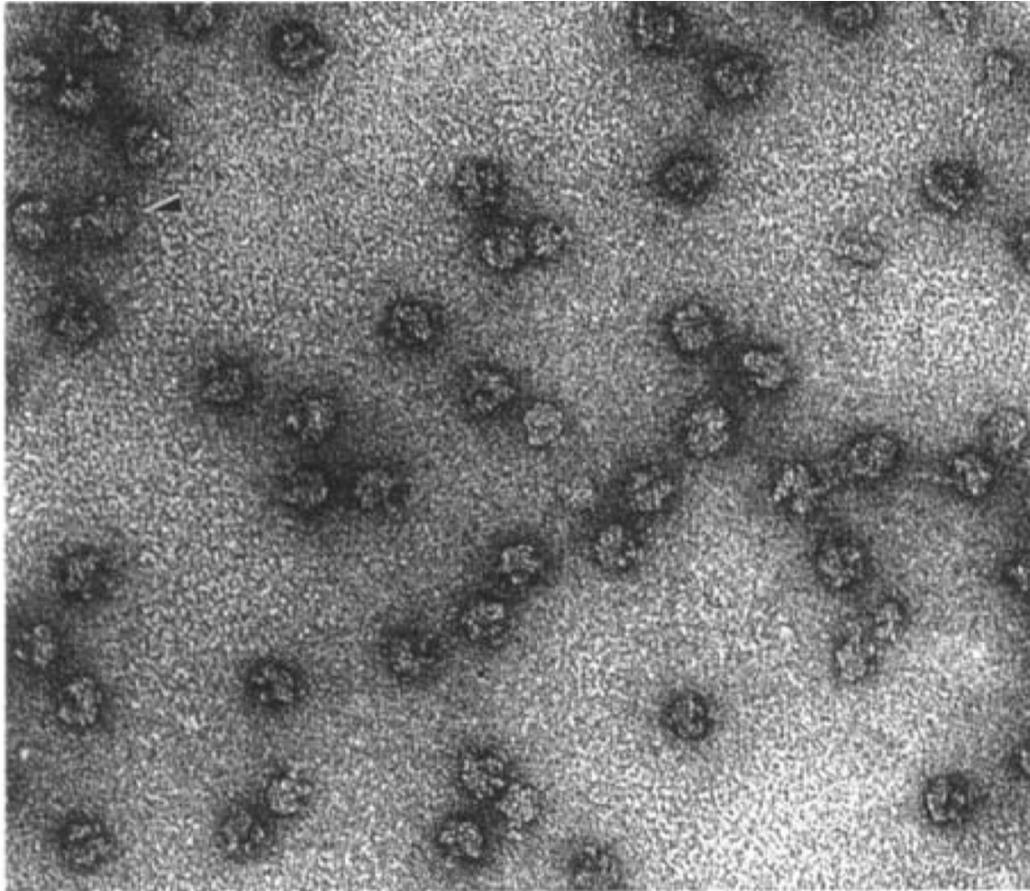
3) air-dry



Heavy metal stains:
ammonium molybdate, uranyl acetate,
uranyl formate, phosphotungstic acid
(PTA), aurogluconate and others ...

Electron microscopy techniques

Negative staining



- Fast
- Technique for low resolution information
- 3D reconstruction is a “shell”
- Good for screening

Electron microscopy techniques

Shadowing technique of supercoiled DNA



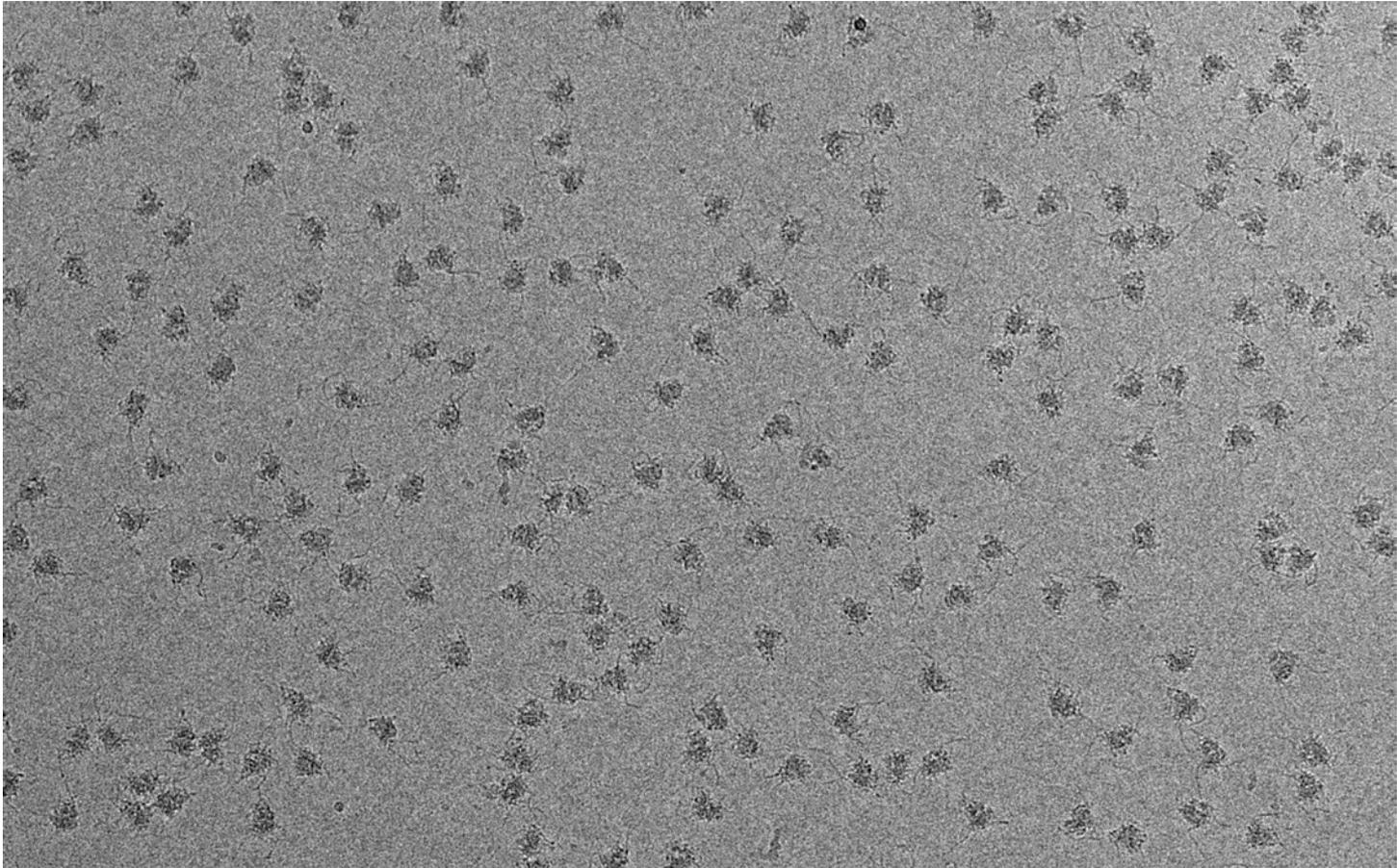
Patrick Schultz

- Heavy atoms evaporated on a sample under an angle

Electron microscopy techniques

Cryo electron microscopy

Purified human ribosomes



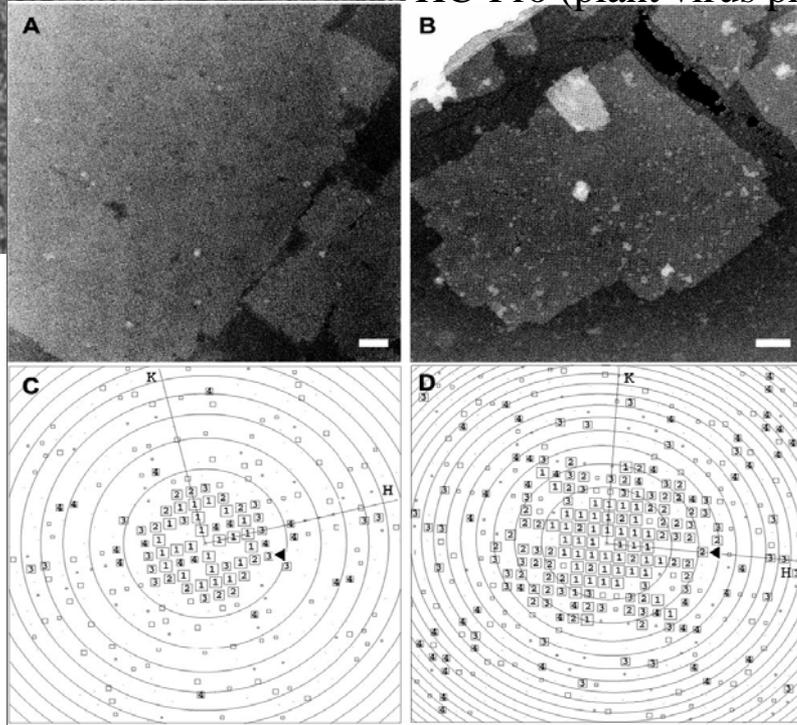
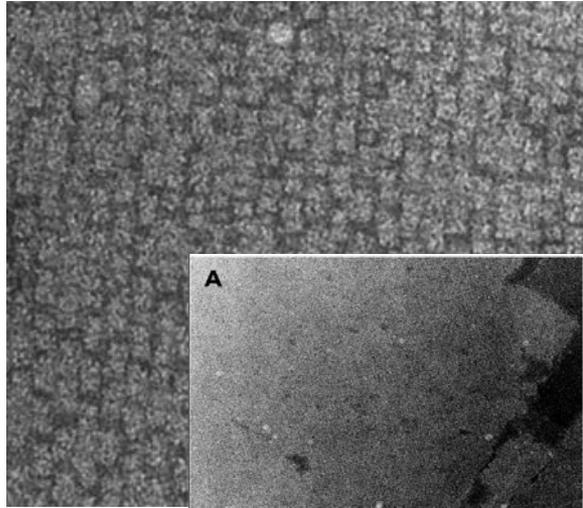
Khatter *et al.*, *Nucl. Ac. Res.*, 2014

- Full preservation of sample in vitreous ice, potential to reach high resolution

Slide from Bruno Klaholz

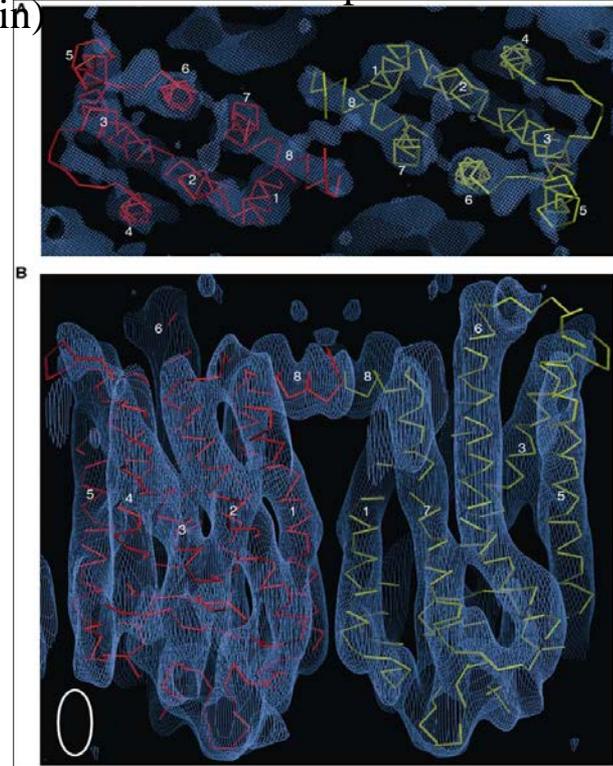
Electron microscopy techniques

electron diffraction – 2D



Plisson *et al.* (2003); *JBC*, **278**, 23753–23761.

Metarhodopsin structure



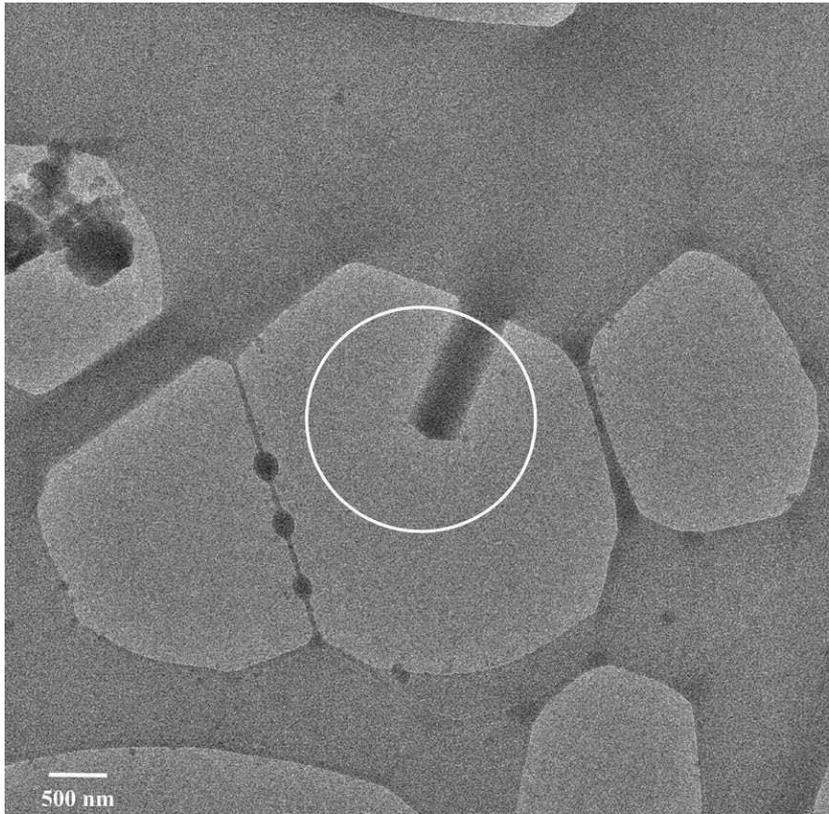
Ruprecht *et al.* (2004); *EMBO*, **23**, 3609-3620

Slide from → e.g. transmembrane helices visible

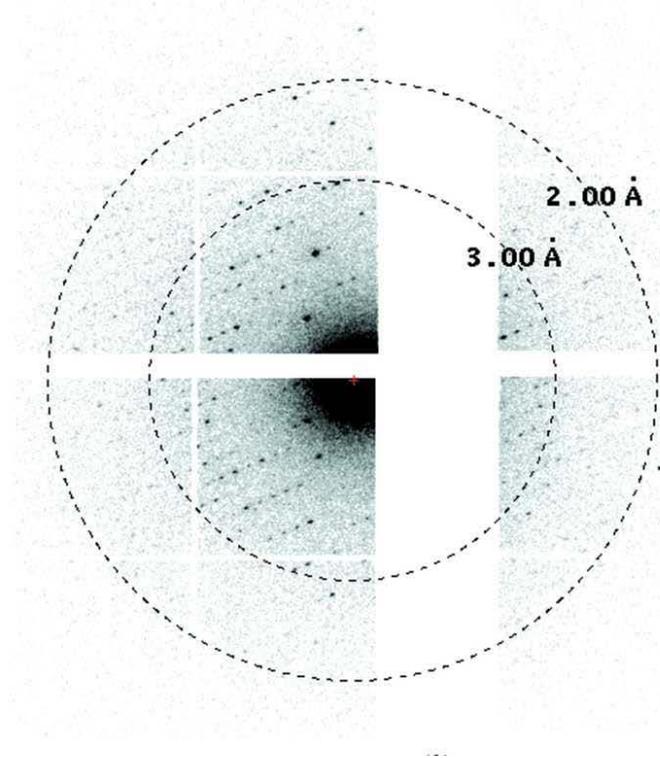
Bruno Klaholz **Limitation of 2D crystals: usually anisotropic resolution**

Electron microscopy techniques

electron diffraction – 3D



Lysozyme nanocrystal seen in TEM



Diffraction of lysozyme

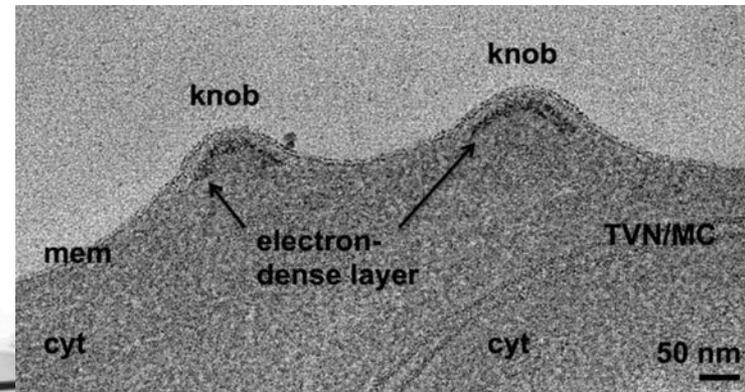
-May be alternative to X-ray crystallography

Electron microscopy techniques

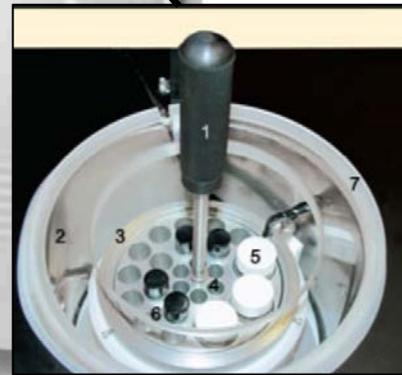
Tomography – plastic embedding

Freeze substitution

High pressure freezer



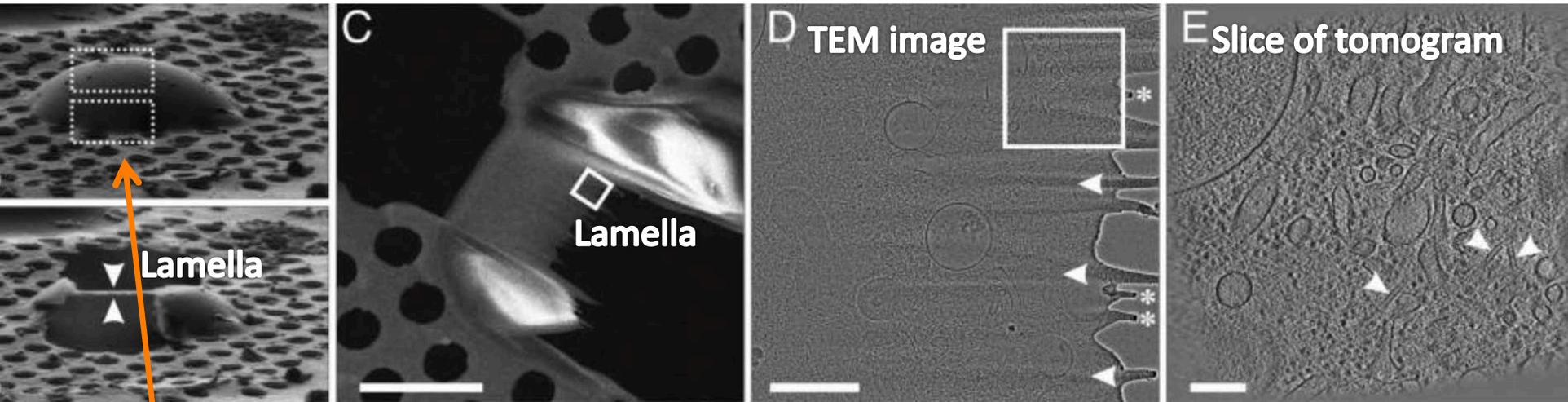
Watermeyer et al., Blood, 2016



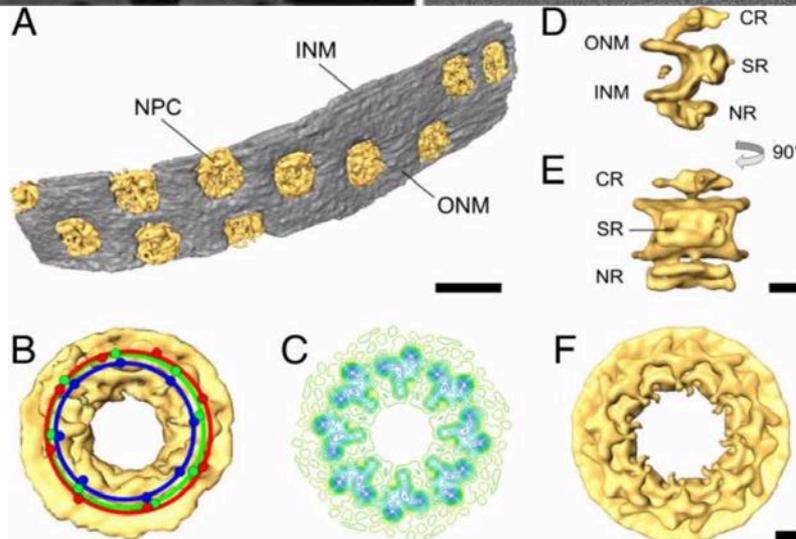
Slice through electron tomogram of Knobs in the membrane of a *P. falciparum*-infected erythrocyte

- Fast freezing that prevents ice formation in thicker samples
- Limited resolution, artifacts from sectioning?

Electron microscopy techniques cryo electron tomography in cells



Cell frozen on an EM grid in liquid ethane

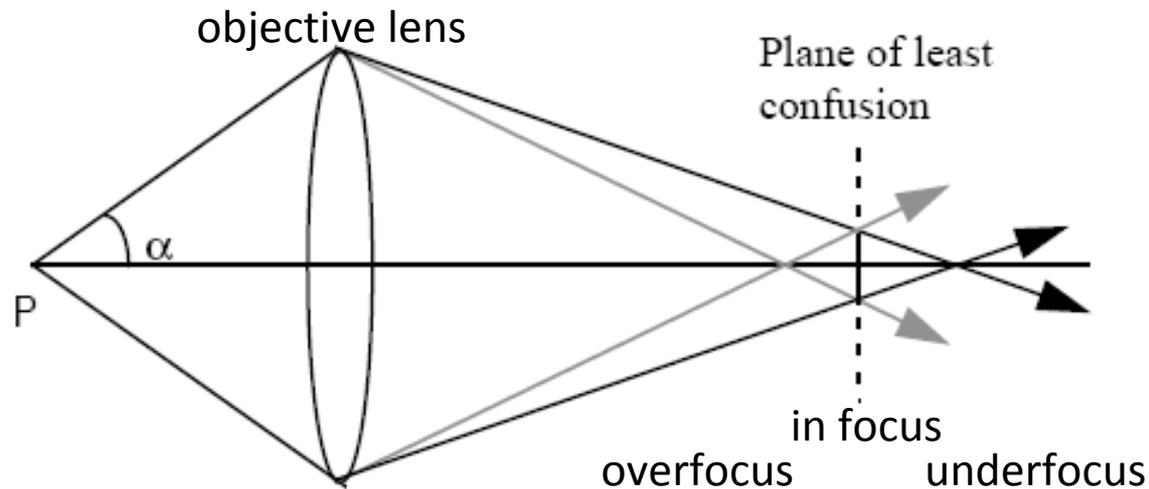


Subtomogram averages of the nuclear pore complex

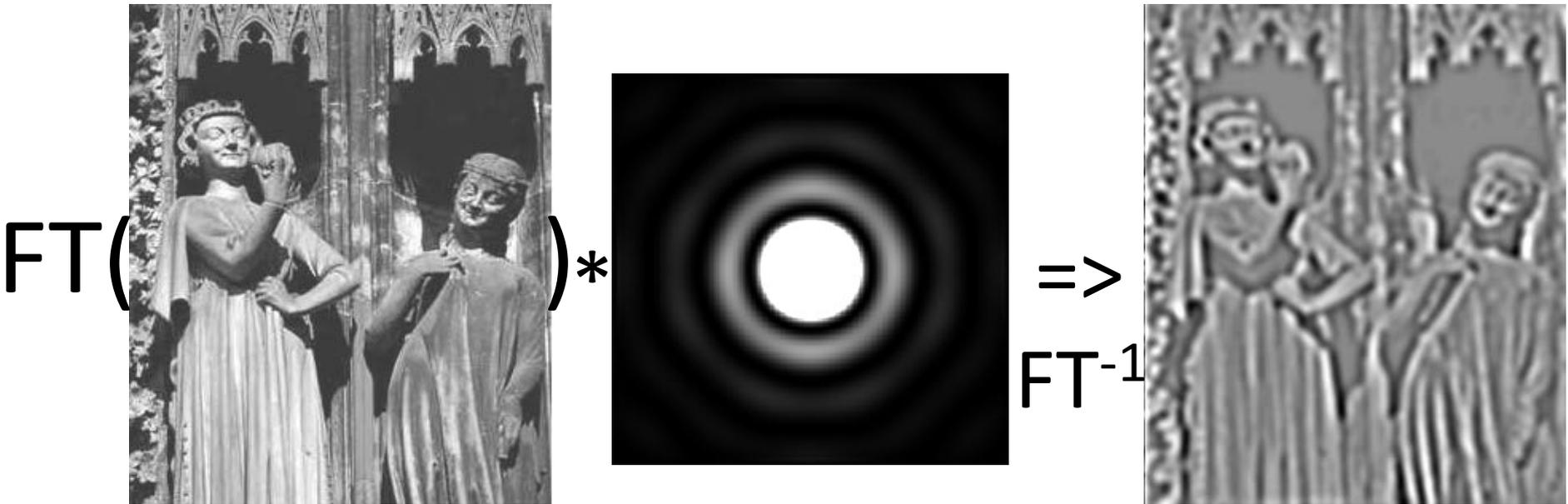
- Great potential of the technique, but very tedious

Rigort, PNAS, 2012

Changing focus in the microscope



The contrast transfer function (CTF)



Contrast transfer function
(CTF)

The defocus image is convoluted by the CTF

Result:

- spreading of each pixel over a bigger surface
- Inversion of contrast of some pixels

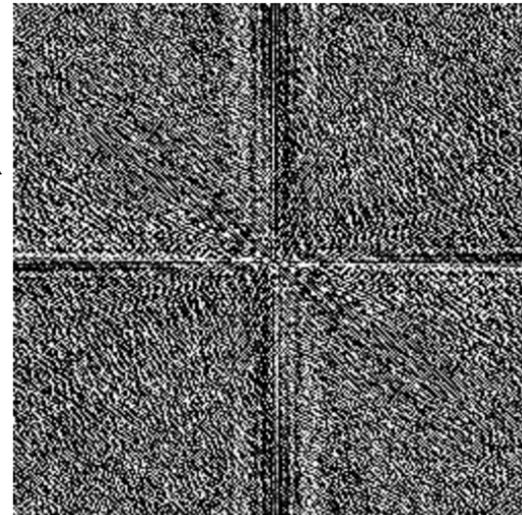
Deconvolution of the CTF: Fourier transform in image processing



Fourier transform



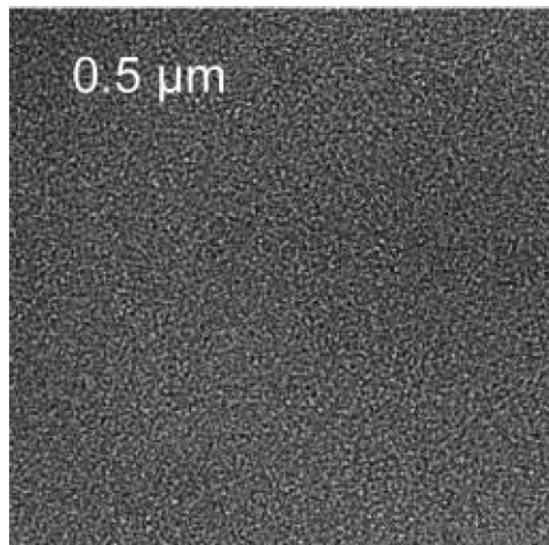
Amplitudes
= Power
spectrum



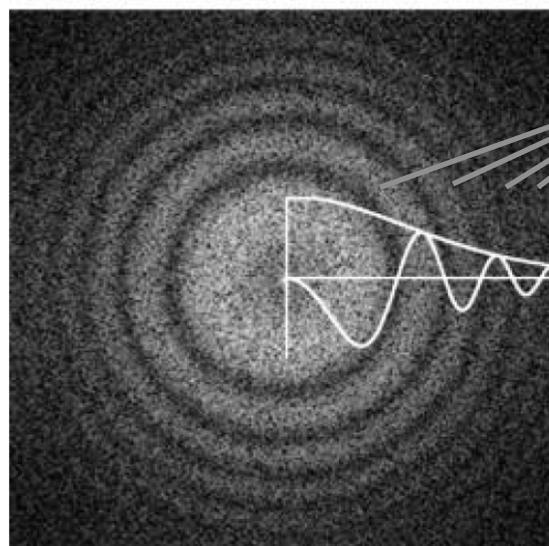
Phases

Power spectrum = Amplitude spectrum

Real image



Power spectrum

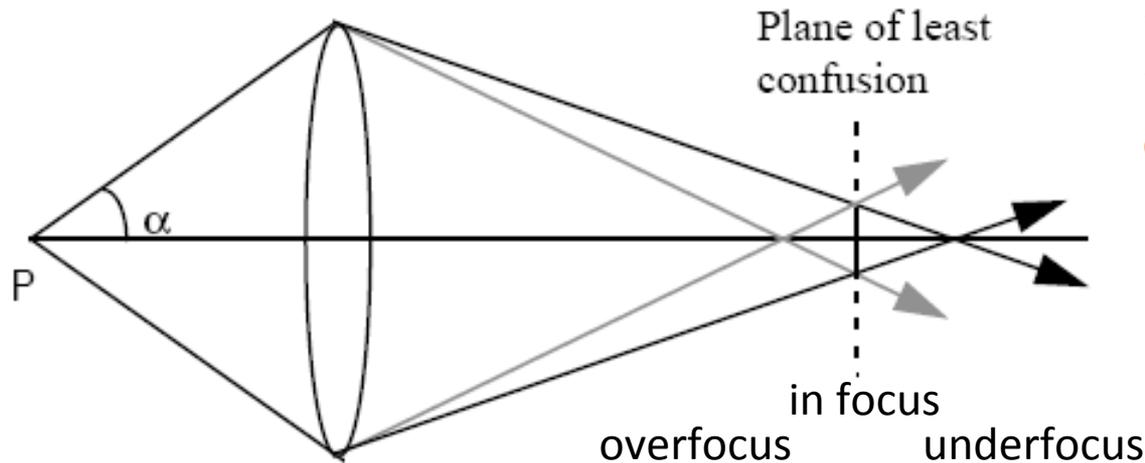


Thon rings

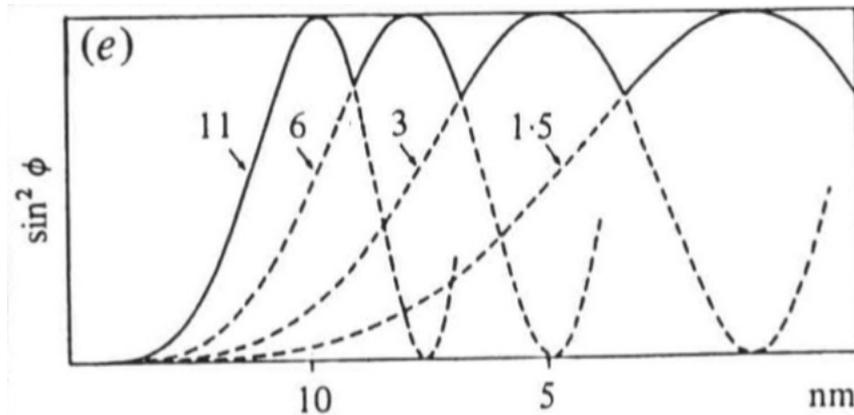
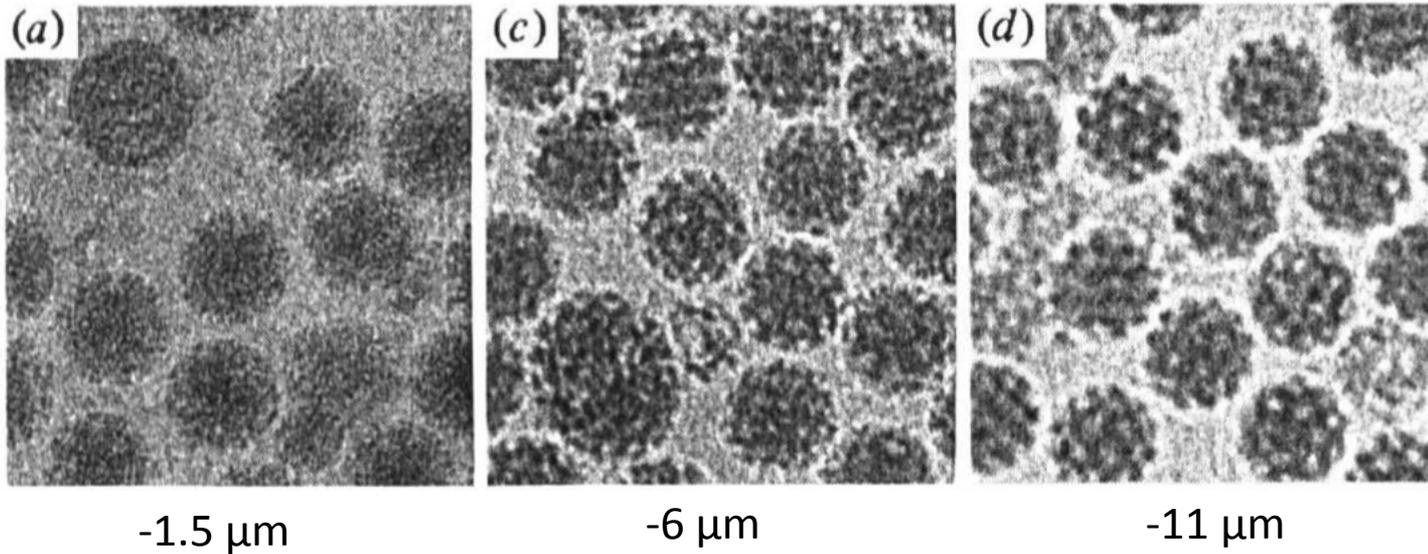
Changing focus in the microscope



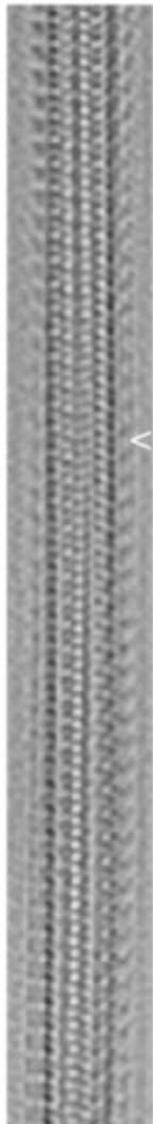
Commonly used
in EM to
increase
contrast



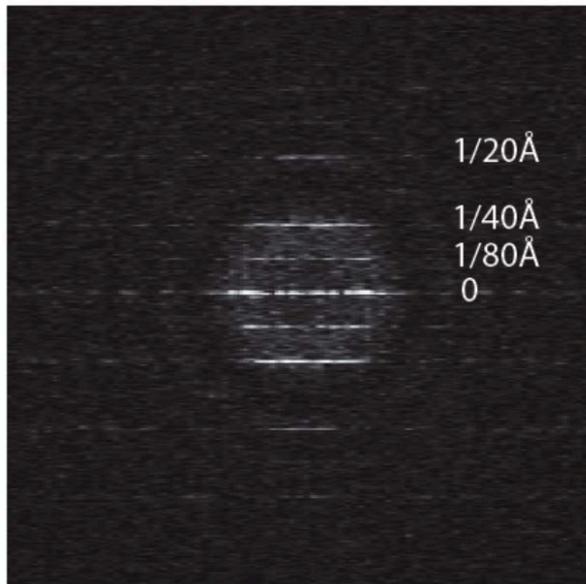
Contrast generation by Defocussing



Objects seen in Fourier space



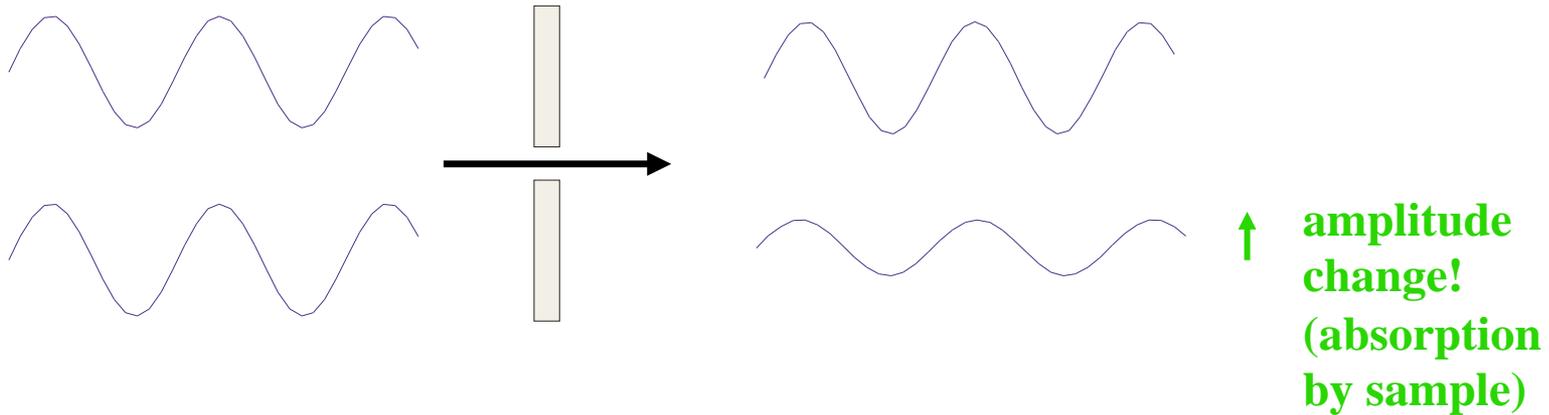
FFT



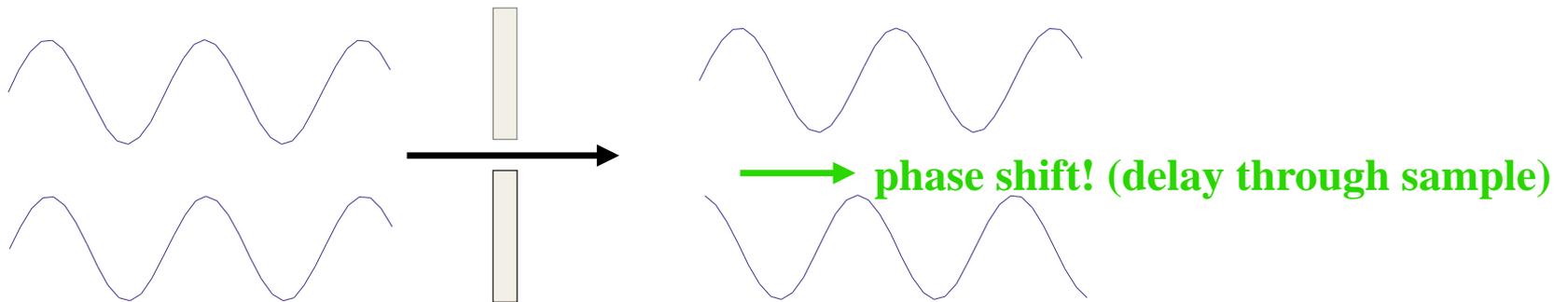
Layer lines:
Tubulin is 40 Å big and
Kinesin binds every 80 Å
on the MT lattice

Image contrast in cryo-EM

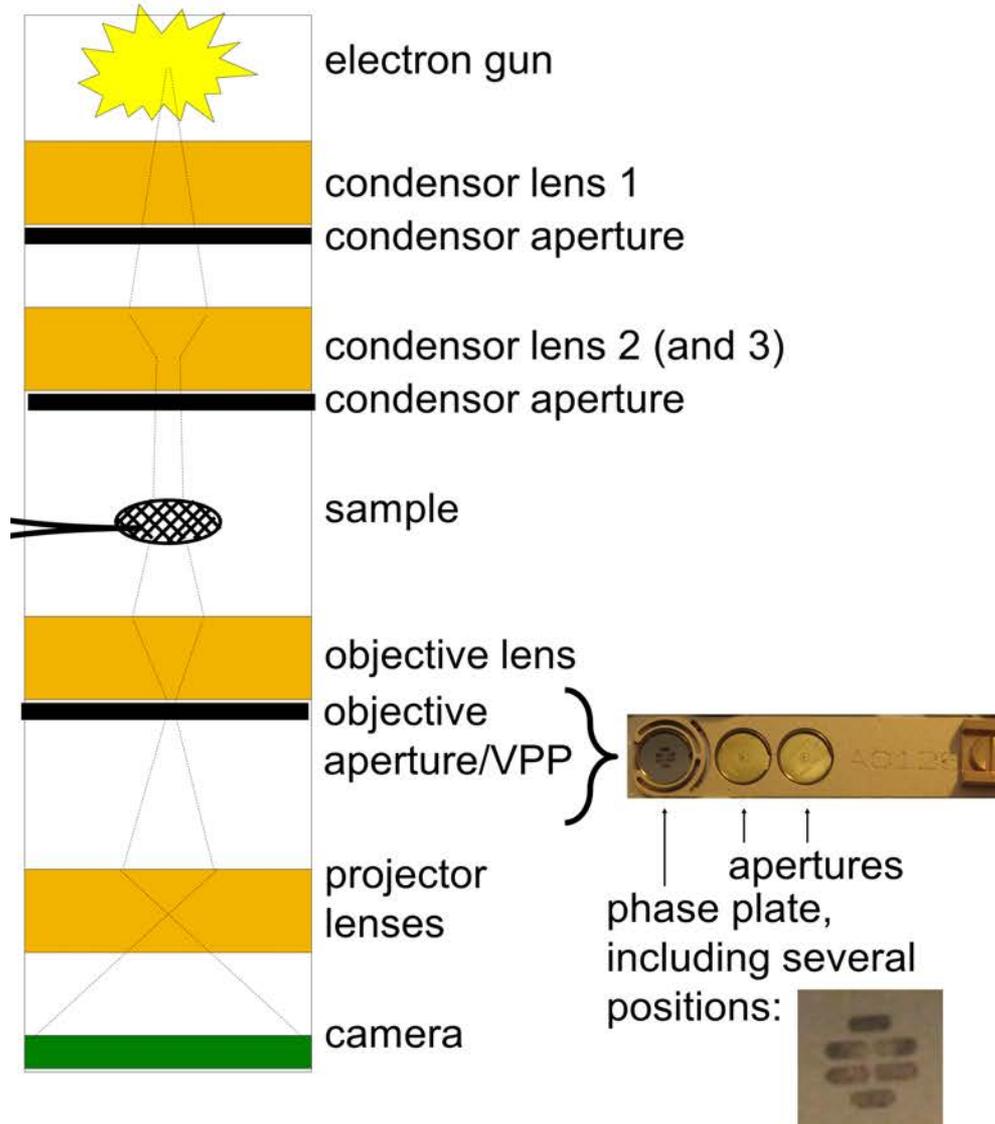
Amplitude contrast (inelastic scattering, absorption)



Phase contrast (elastic scattering)



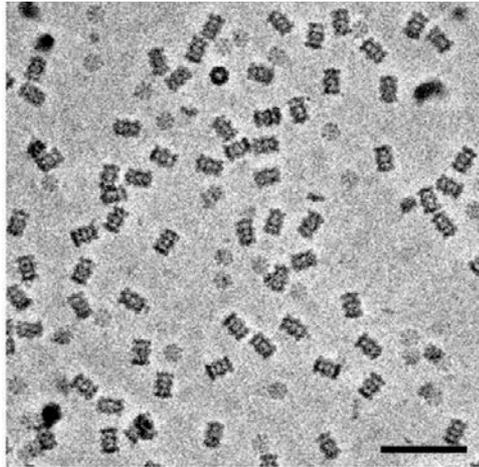
Contrast generation with phase plates



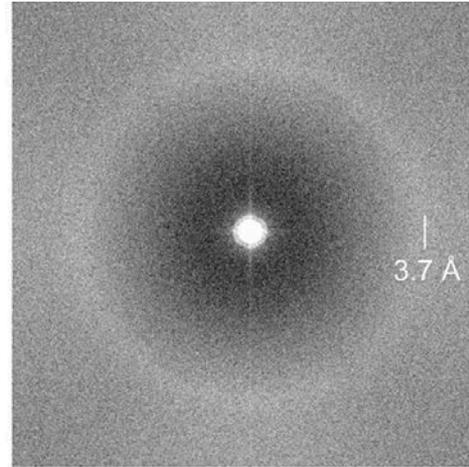
von Loeffelholz and Klaholz, 3rd Edition Meth. in Mol Biol. –Structural Proteomics, submitted; von Loeffelholz, JSB, 2017

Contrast generation with phase plates

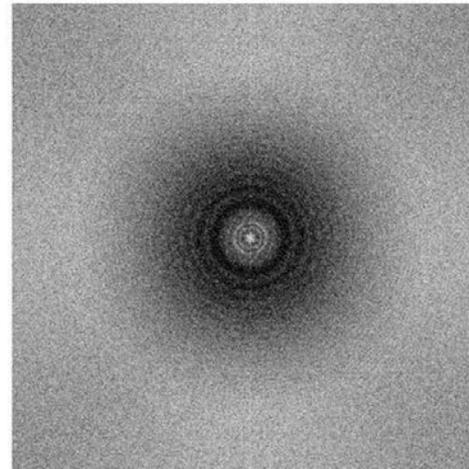
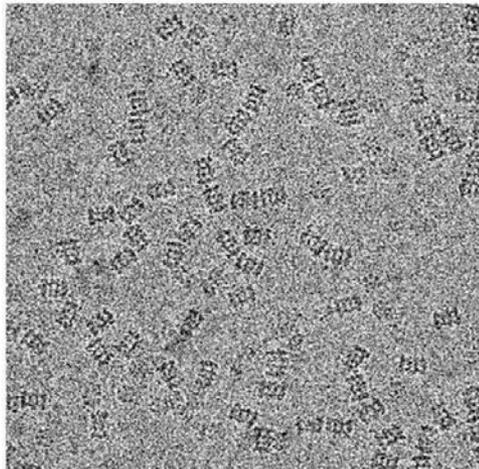
Micrograph



Power spectrum

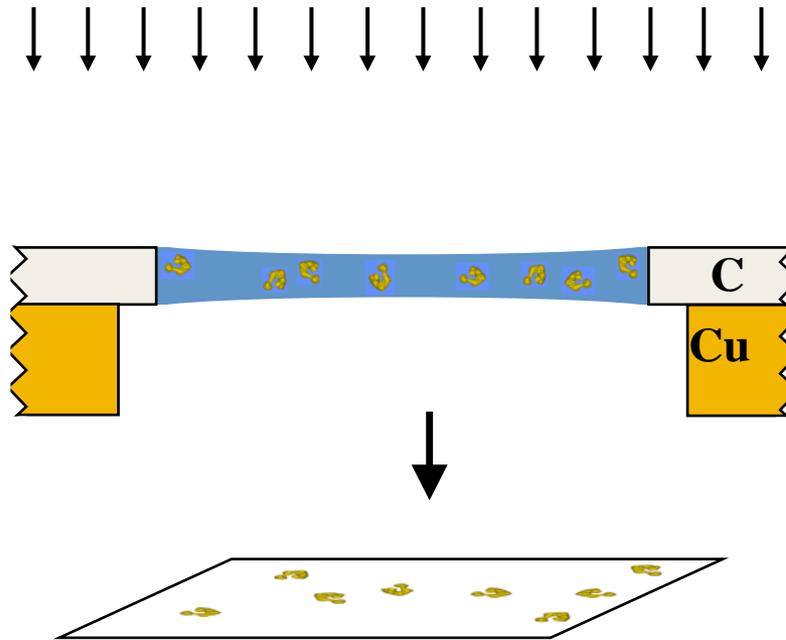


With phase plate
in focus

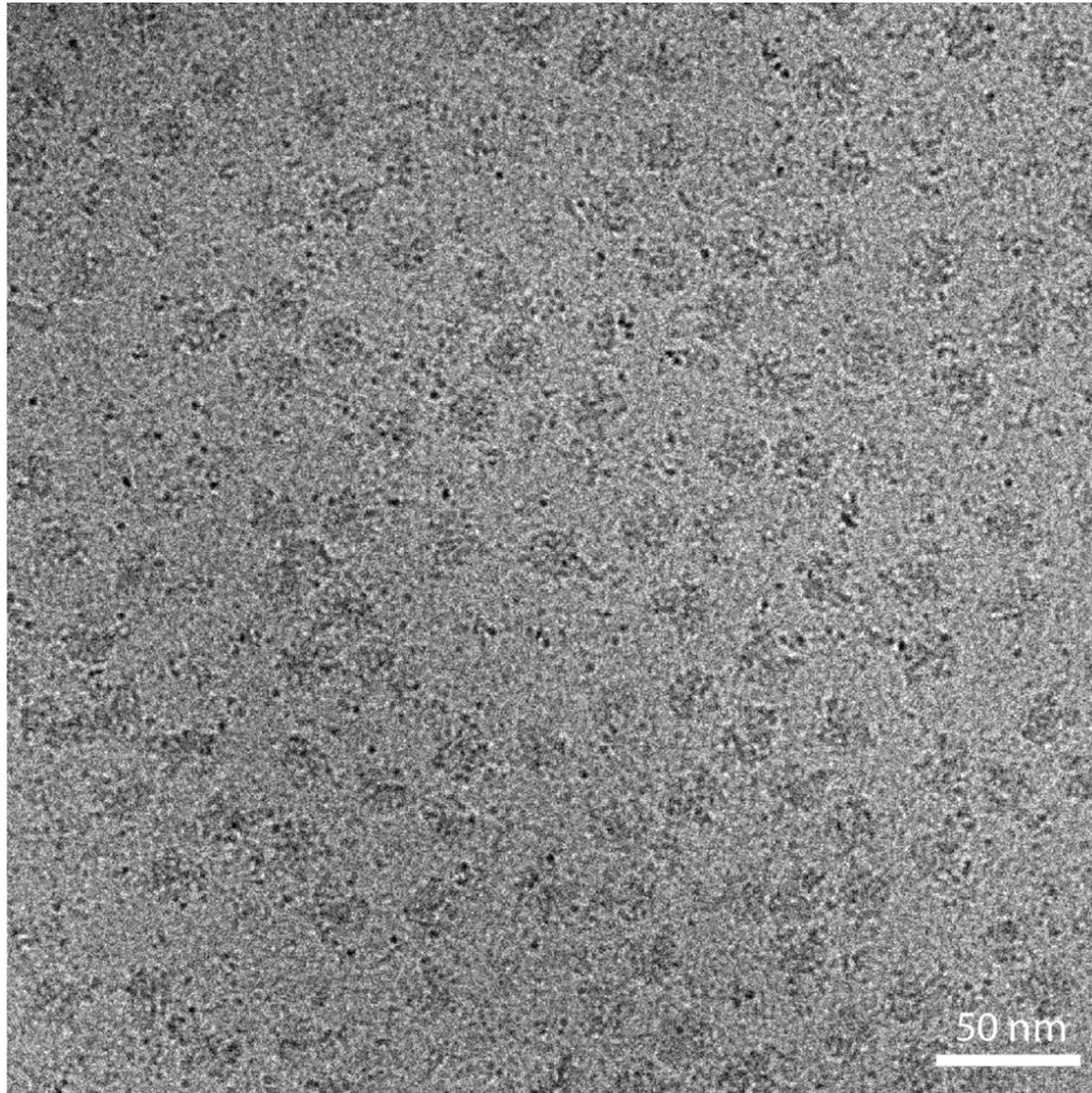


Without phase plate
-1.6 μm defocus

Cryo electron microscopy

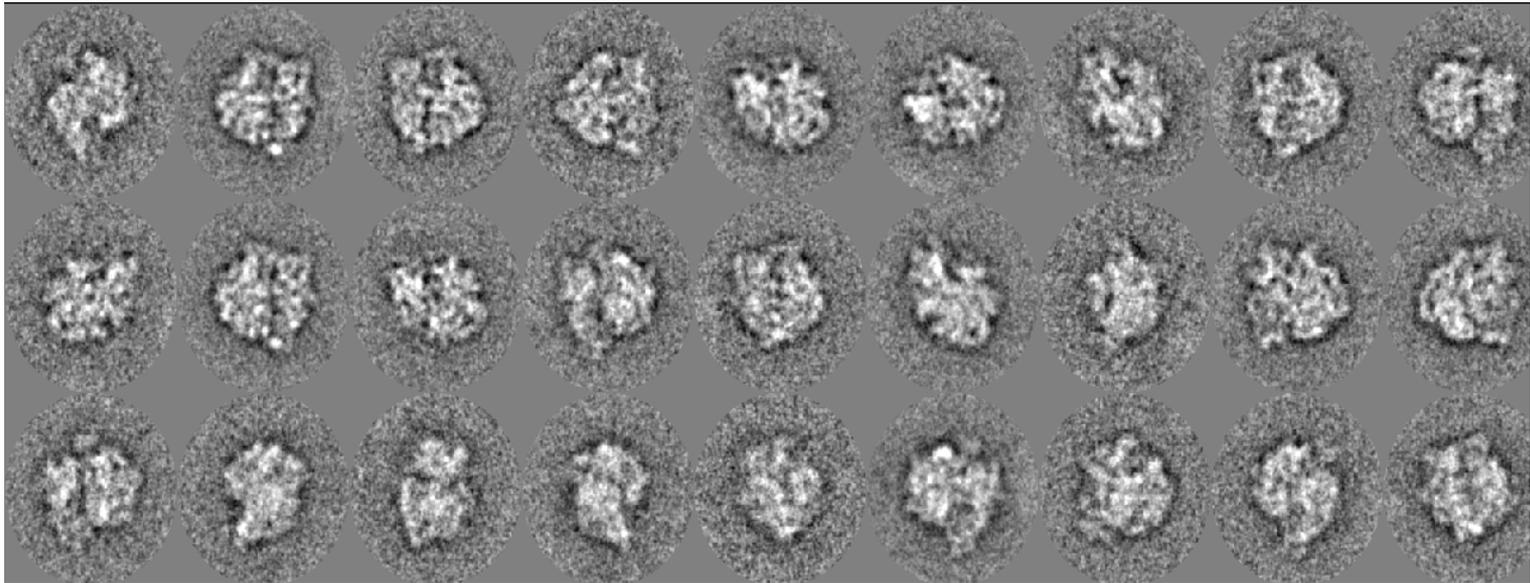


Electron micrograph of particles



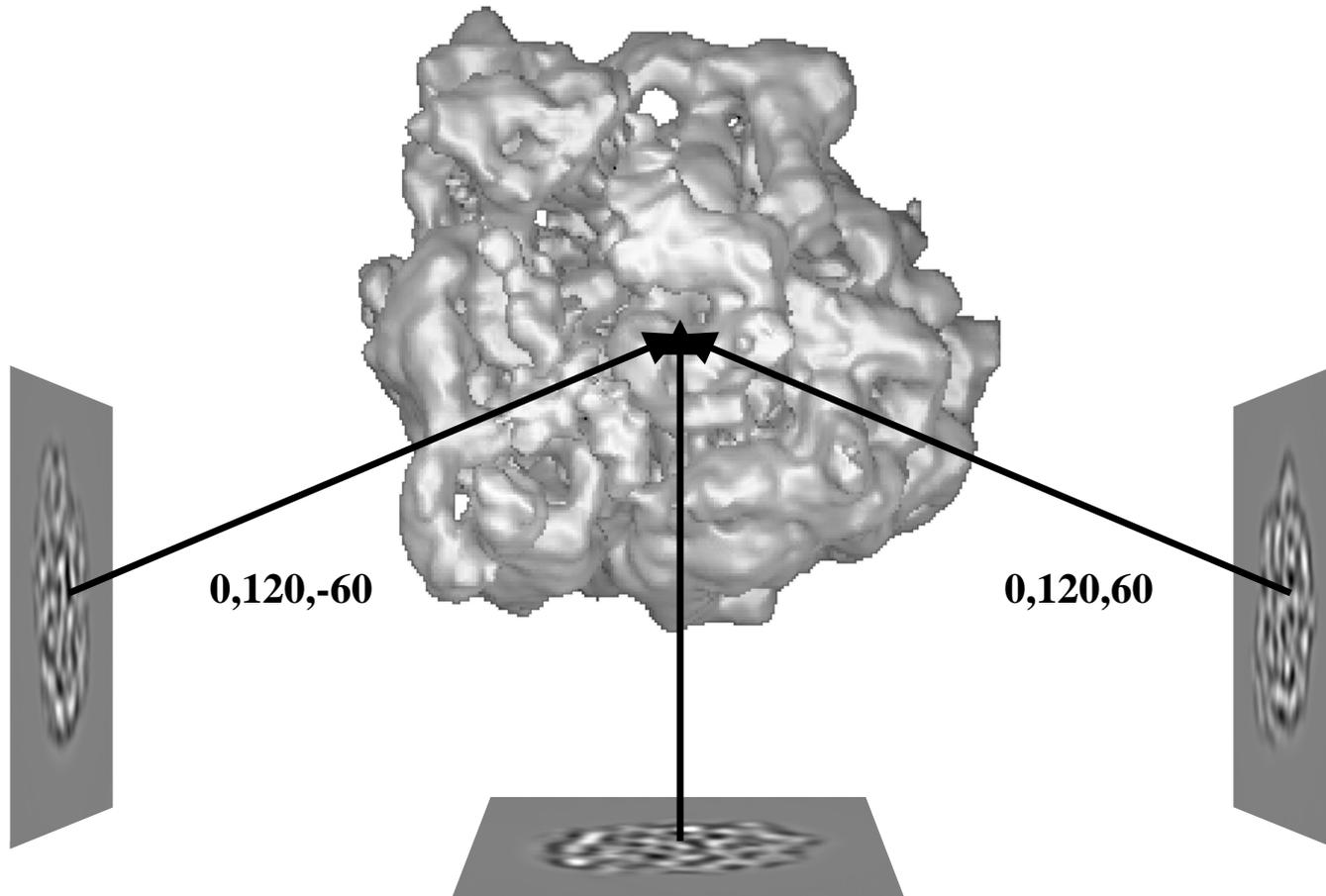
von Loeffelholz et al, PNAS, 2015

Class averages of particles



representative views of the 70S / RF2 complex; Klaholz et al., Nature 2003.

Angle assignment to the class averages or individual particles



Backprojection – 3D Reconstruction

