Optimizing Phase Contrast in TEM by the use of an electrostatic Boersch Phase Plate

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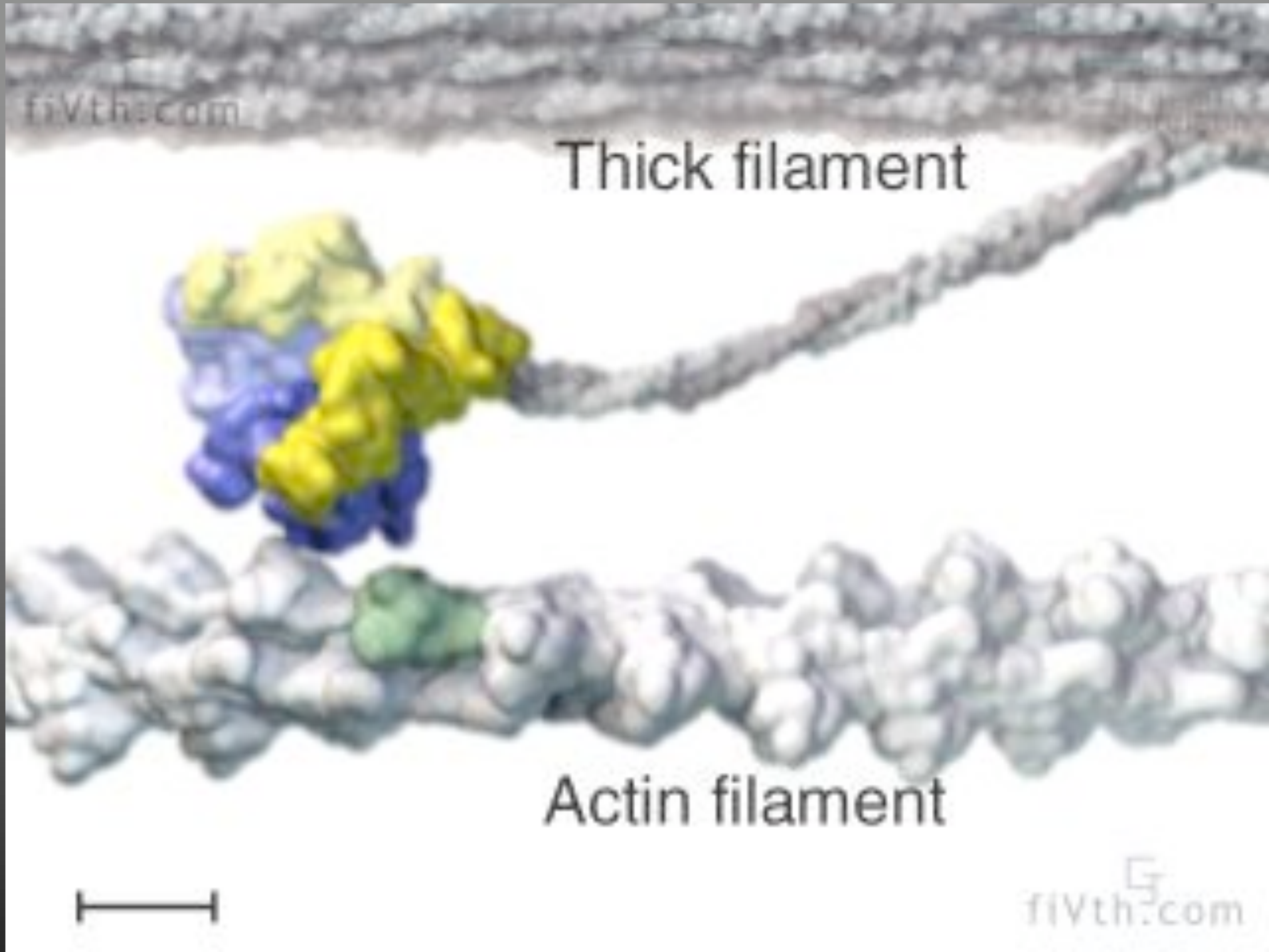
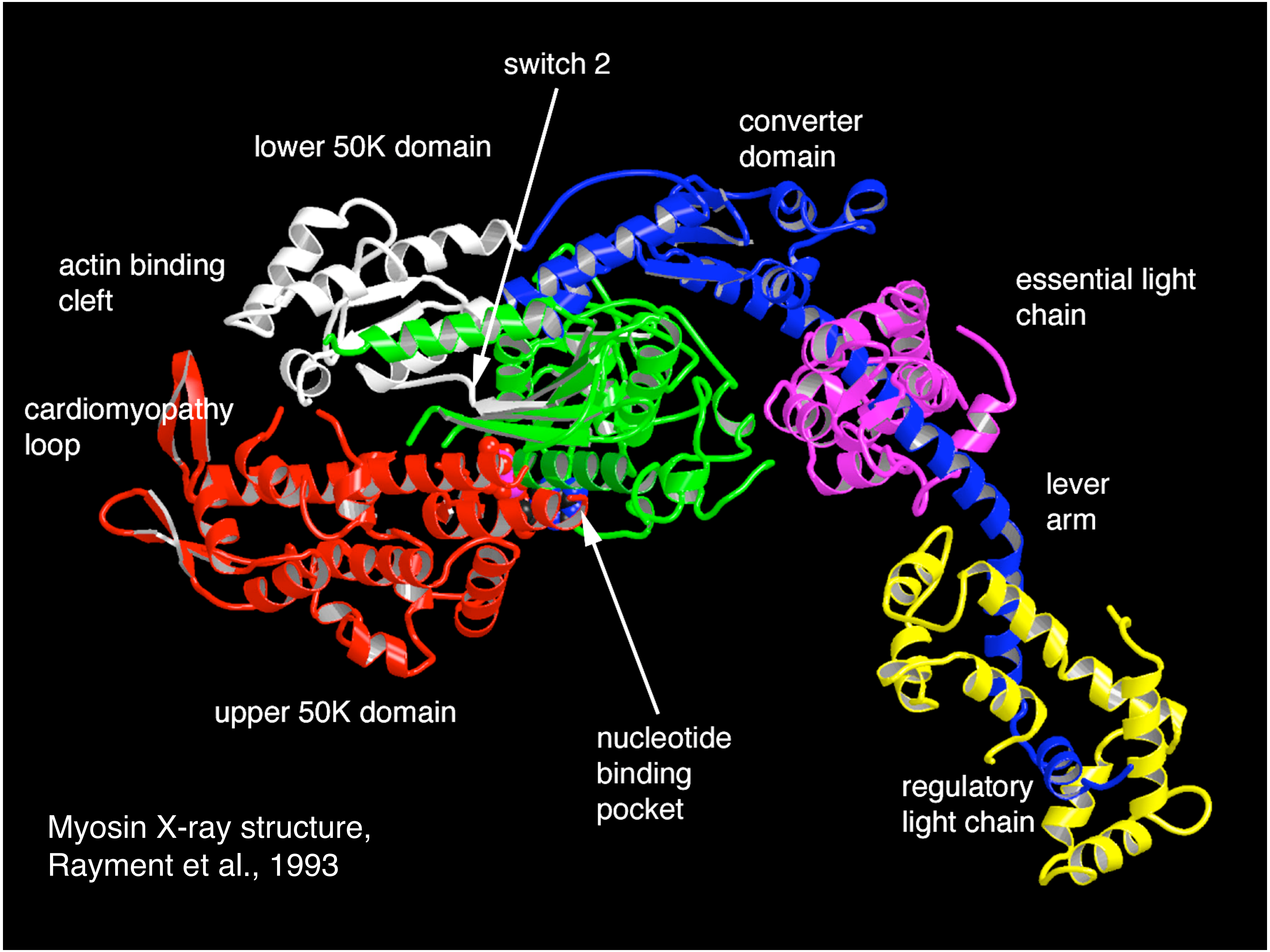
Endre Majorovits	MPI Med. Research / University Oxford
Radostin Danev Kuniaki Nagayama	NIPS, Okazaki University
Werner Kühlbrandt	MPI Biophysics, Frankfurt/Main

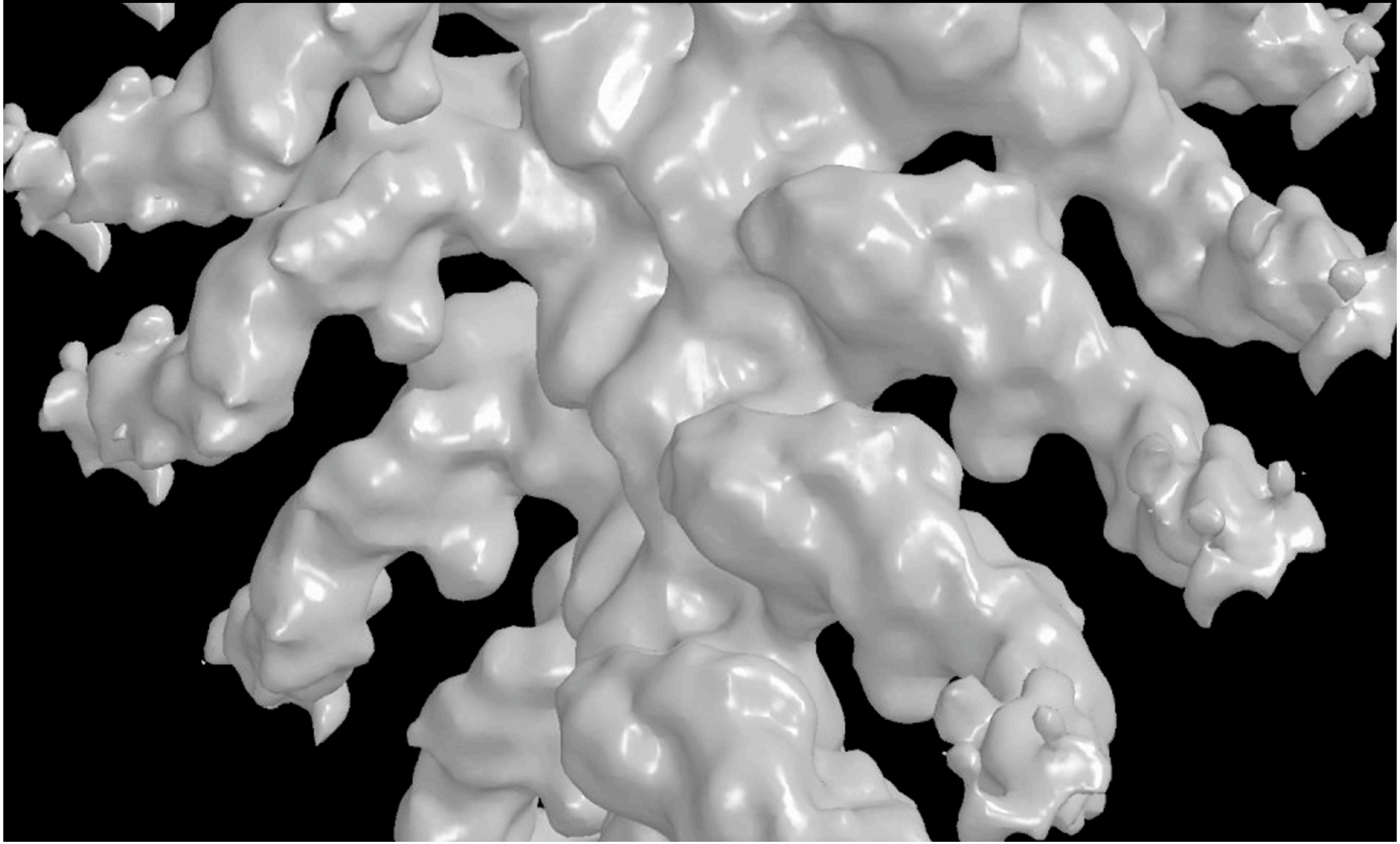


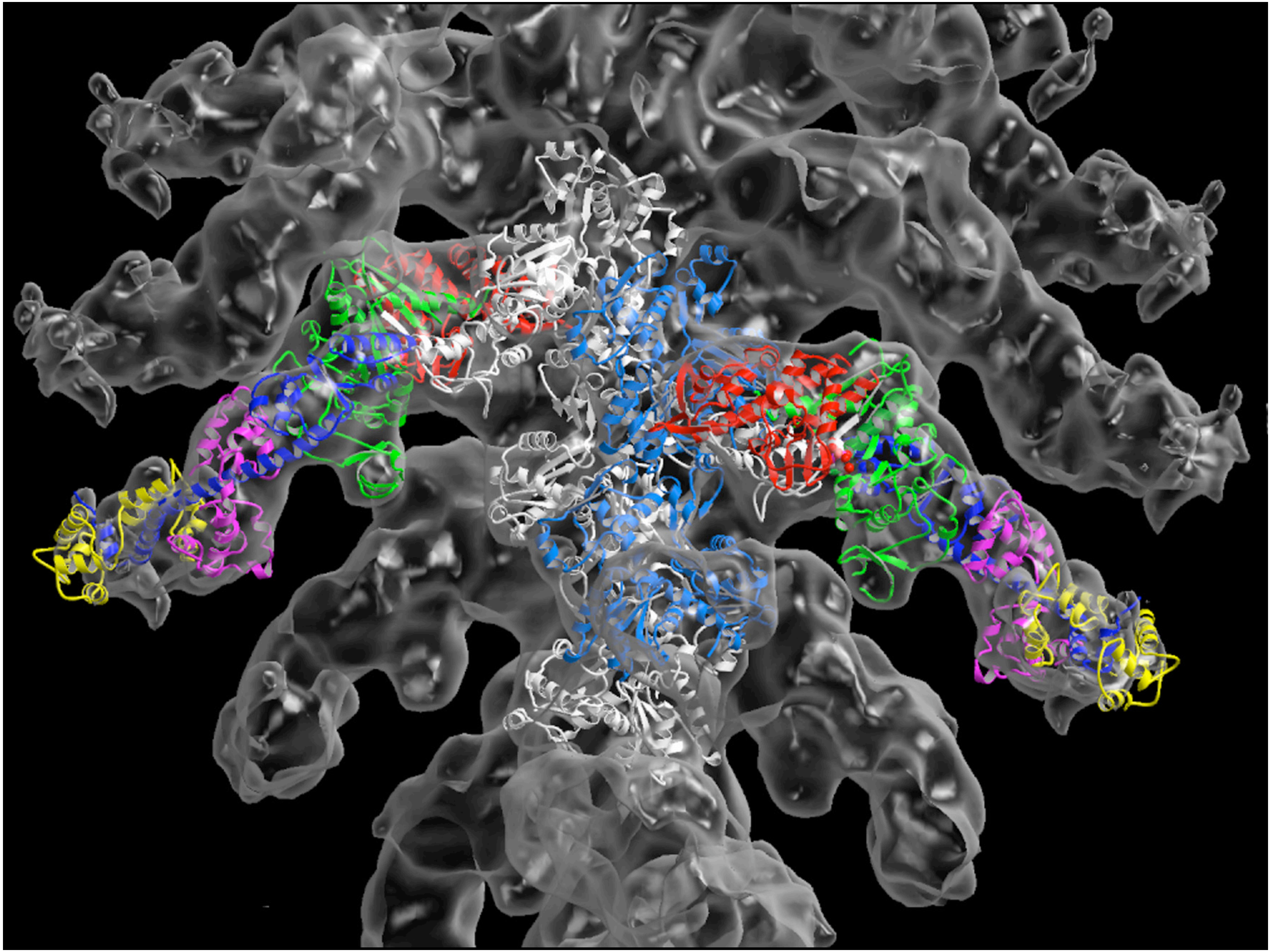
“How muscle works …”, from Ron Vales’s web page


switch 2
lower 50K domain
converter
domain
actin binding
cleft
essential light
chain
cardiomyopathy
loop
lever
arm
upper 50K domain
nucleotide
binding
pocket
regulatory
light chain
Myosin X-ray structure,
Rayment et al., 1993

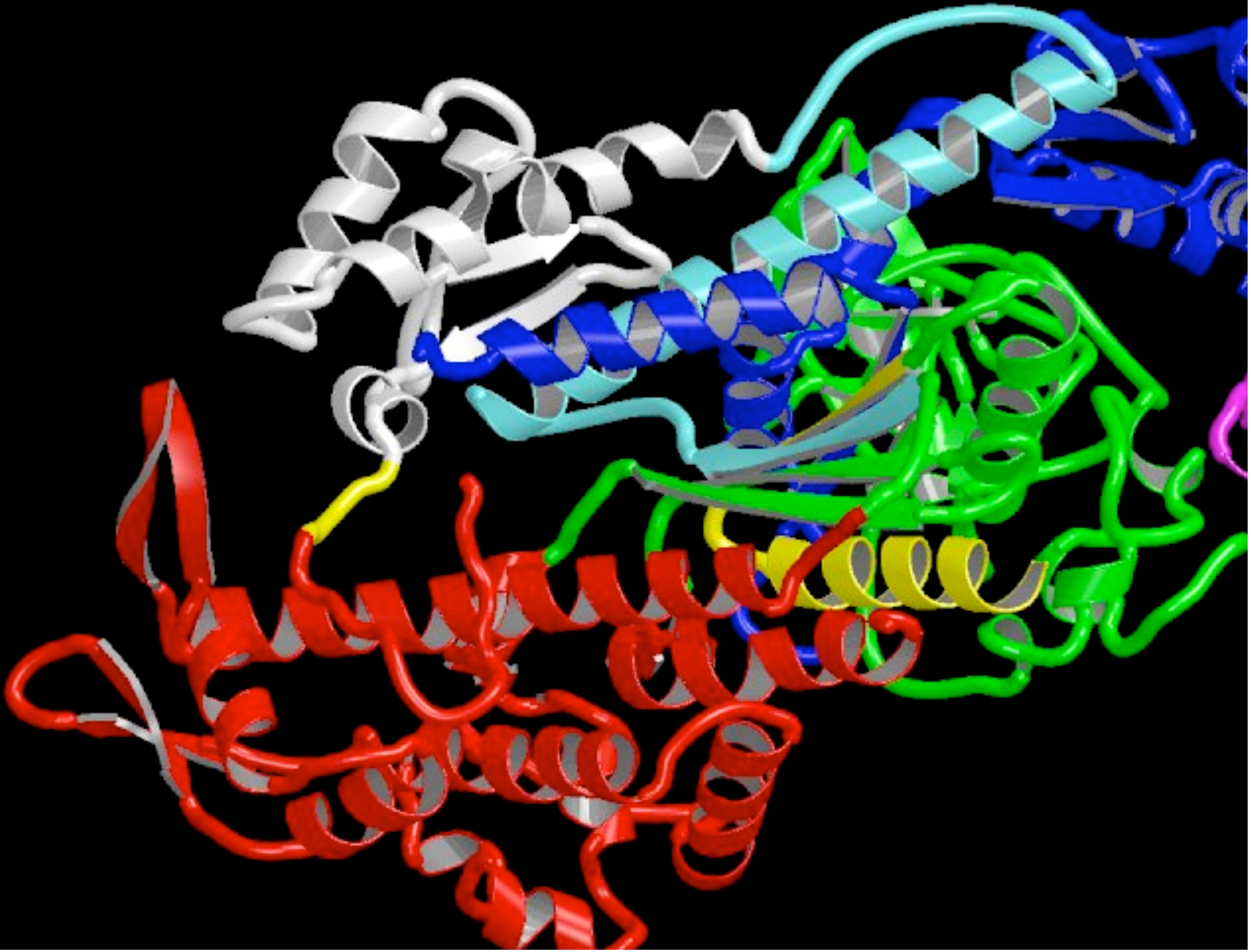
Reconstruction of the rigor actin-myosin complex (chicken skeletal myosin II papain S1, Holmes et al., 2003)

Resolution 13-15Å (Fourier shell coefficients)

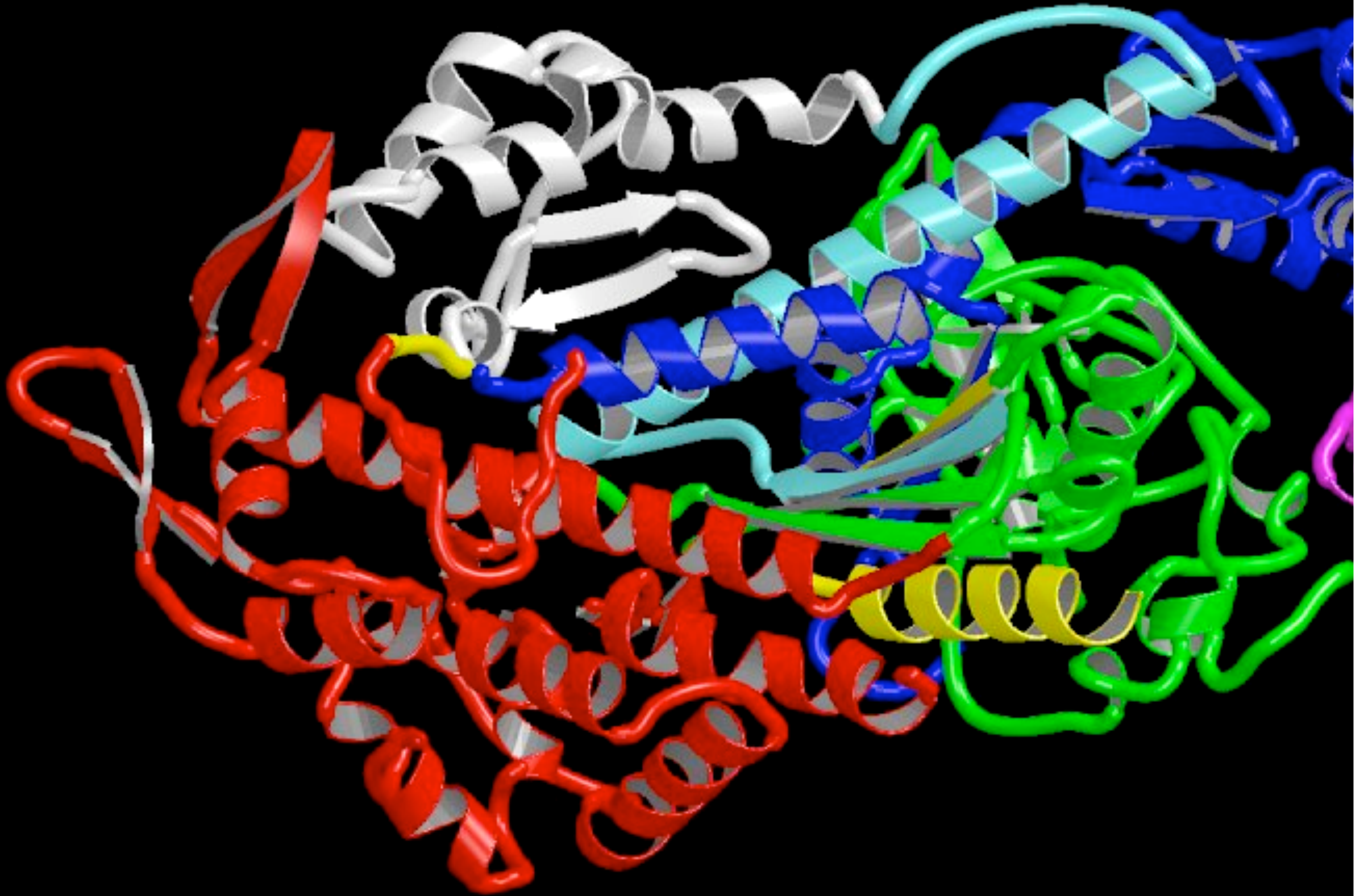


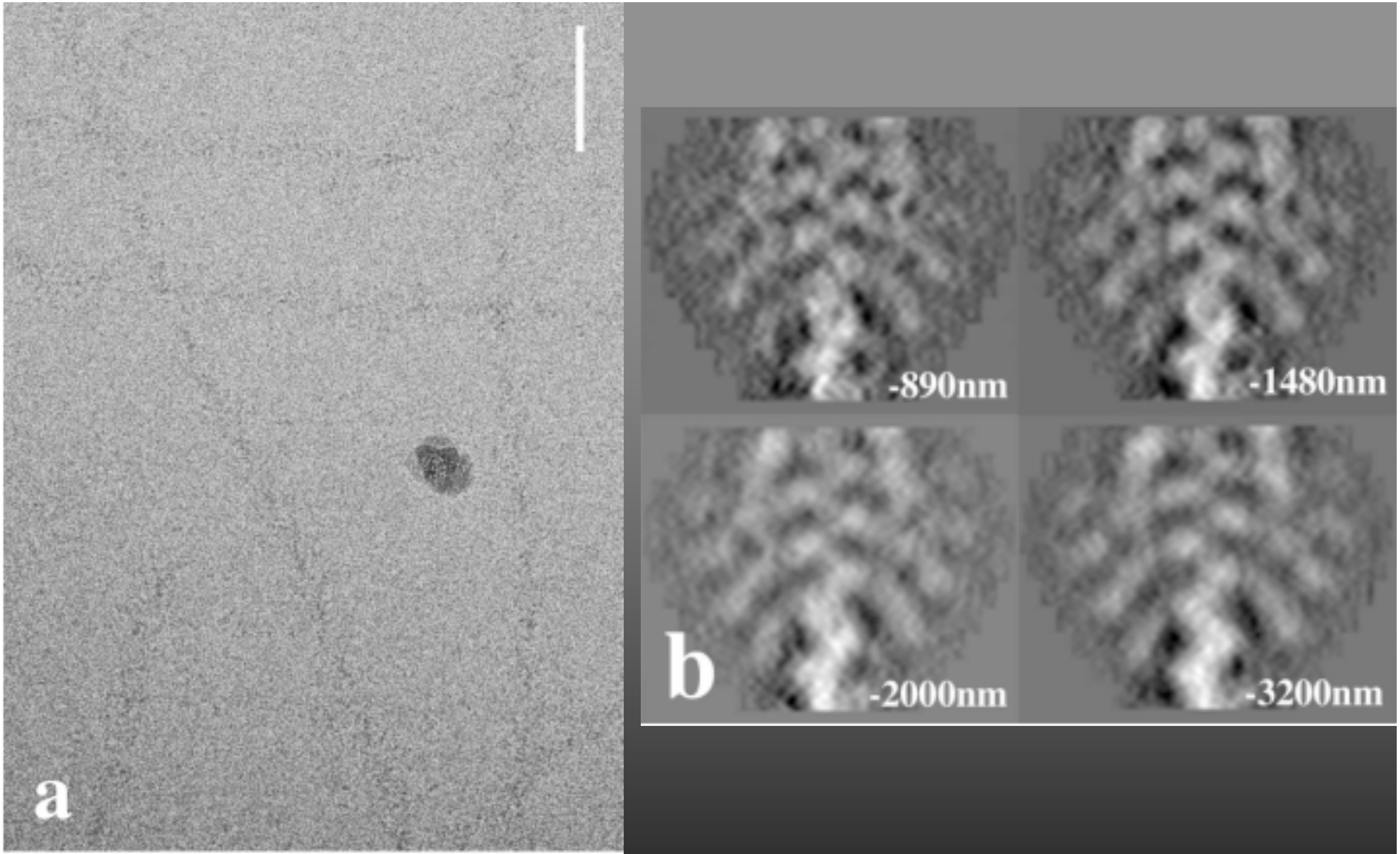


chicken skeletal



upper/lower 50K free





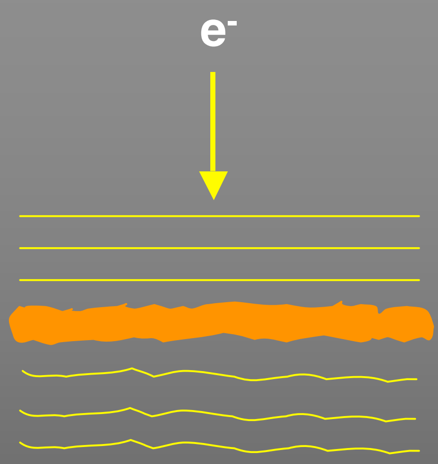
a - a typical sample of decorated actin at an underfocus of 1.48 µm. (scale bar 77 nm)

b – averages of 104 identical object areas selected from the four exposures in one defocus series. Half a 38.5 nm repeat is shown for each underfocus exposure

Outline

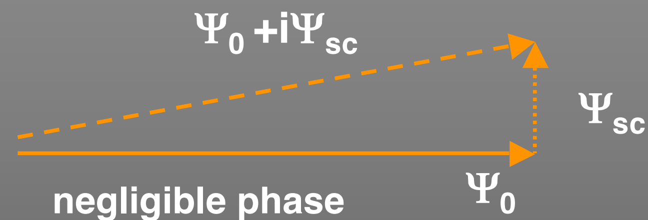
- Phase contrast in transmission electron microscopy
- Why an electrostatic phase plate?
- The design of a Boersch-type electrostatic phase plate
- First experimental results
- The future

Phase Contrast in Electron Microscopy

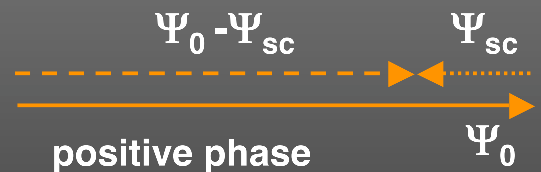


biological sample: weak phase object

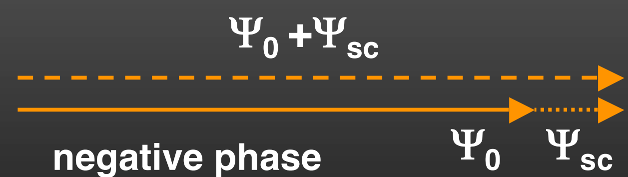
(H, N, C, O, S ...)



negligible phase contrast



positive phase contrast



negative phase contrast

<u>weak phase object</u>

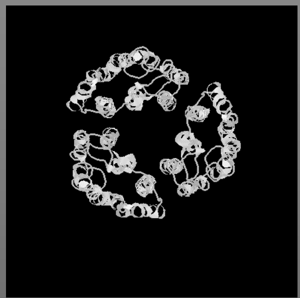
→ π/2-phase shift due to elastic scattering + small scattering amplitude (1st order Born approximation)

→ negligible phase contrast

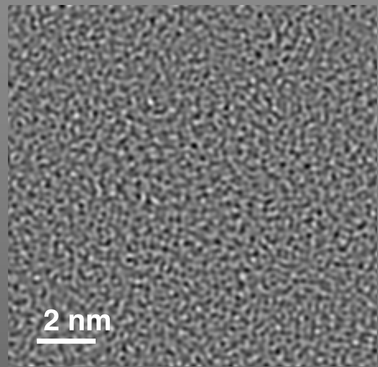
→ low object signal

Simulation of bacterio rhodopson (bR) without/with Phase Plate

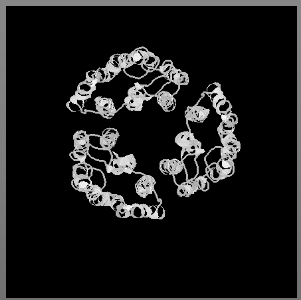
without phase plate



200 keV
C_s=2.7 mm
focus

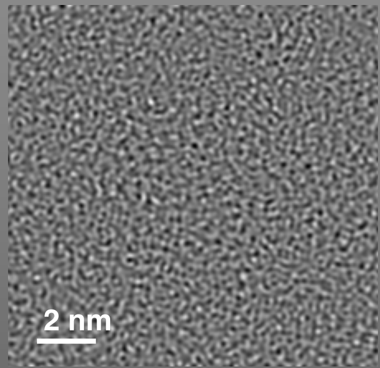


Simulation of bacterio rhodopson (bR) without/with Phase Plate



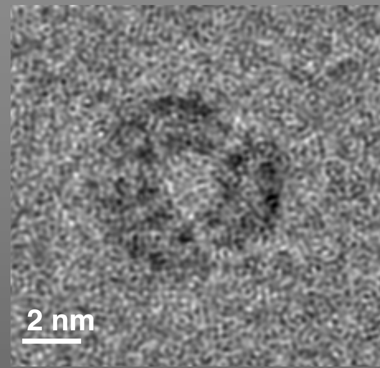
200 keV
C_s=2.7 mm
focus

without phase plate



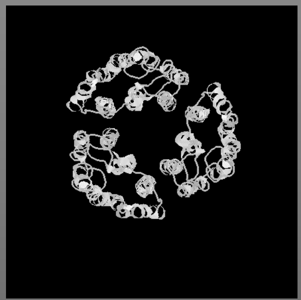
π/2-phase shift
→
phase plate

with phase plate

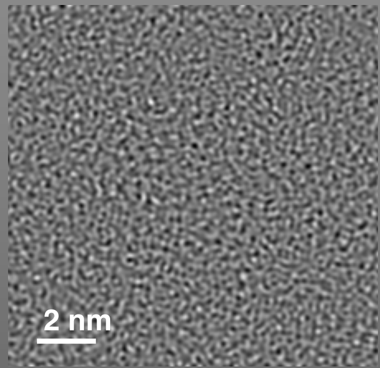


Simulation of bacterio rhodopson (bR) without/with Phase Plate

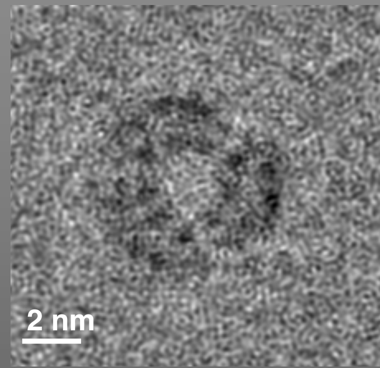
without phase plate | **with phase plate**



200 keV
C_s=2.7 mm
focus



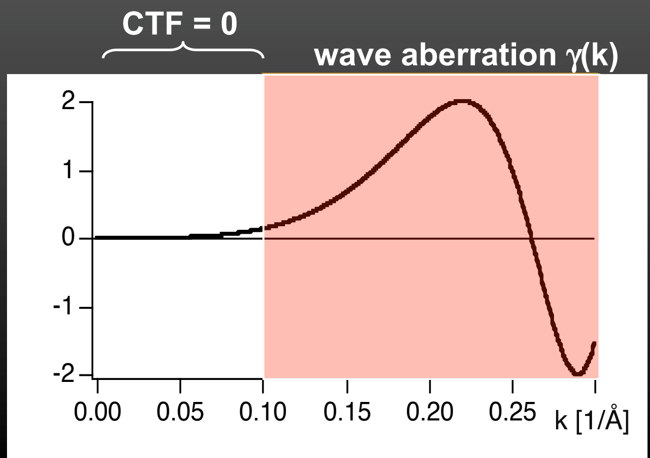
π/2-phase shift → phase plate

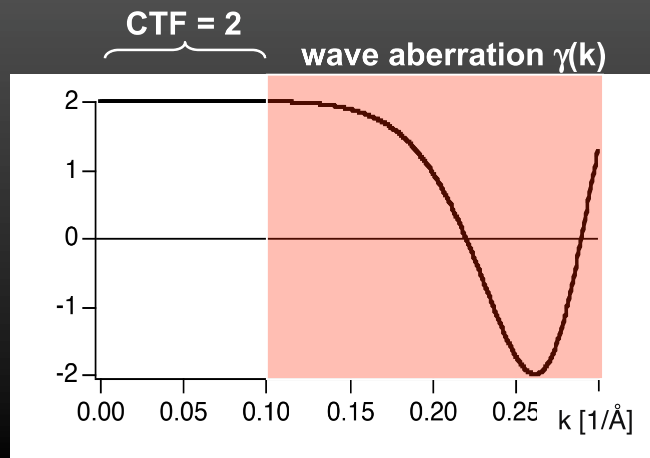


CTF(k) = Contrast Transfer Function (k = spatial frequency)

$CTF(k) = 2 \sin \gamma(k)$ → $CTF_{\pi/2}(k) = 2 \cos \gamma(k)$

Fourier space



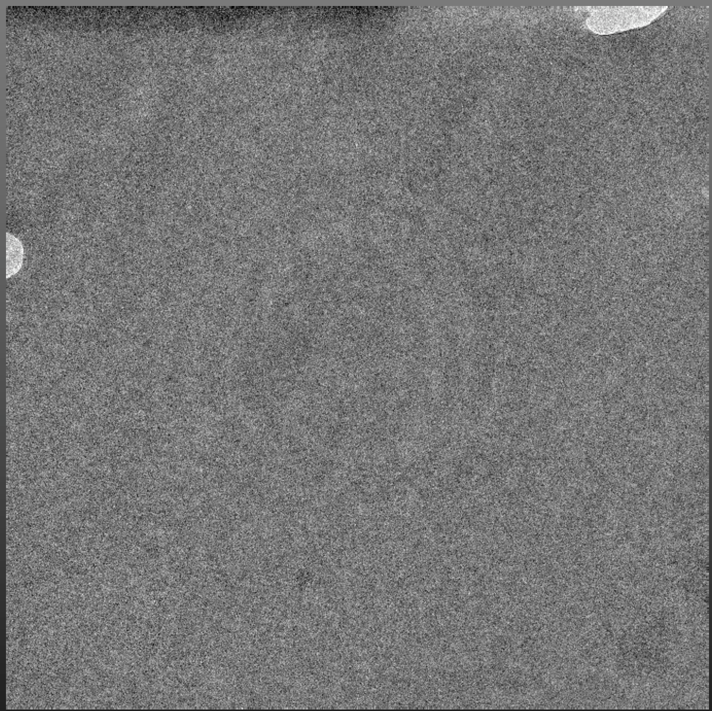


C. elegans Cell Section

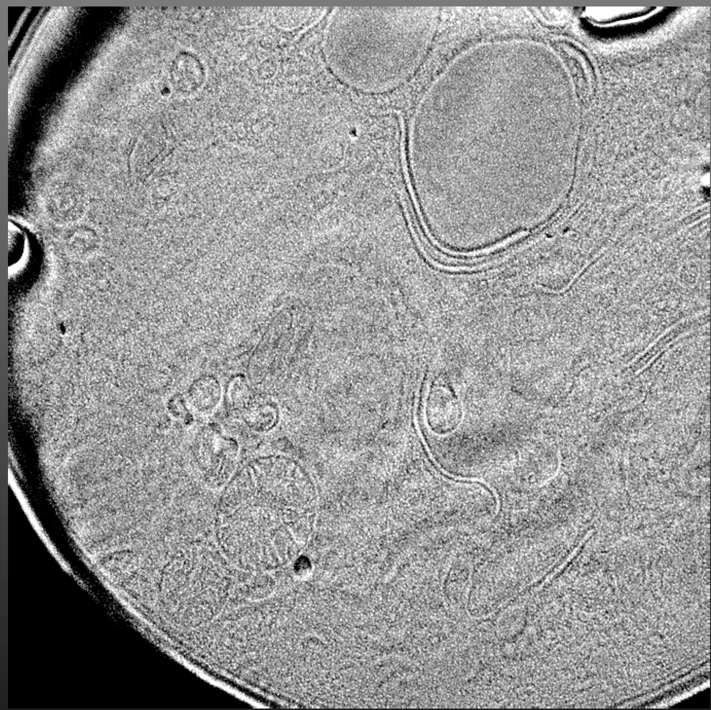
High pressure frozen, freeze substituted without stain (Martin Müller)

Images taken at 300keV, JEOL 3100 EFE at 4K specimen temperature (Kuniaki Nagayama)

conventional bright field



Zernike phase contrast (hole in C-film)

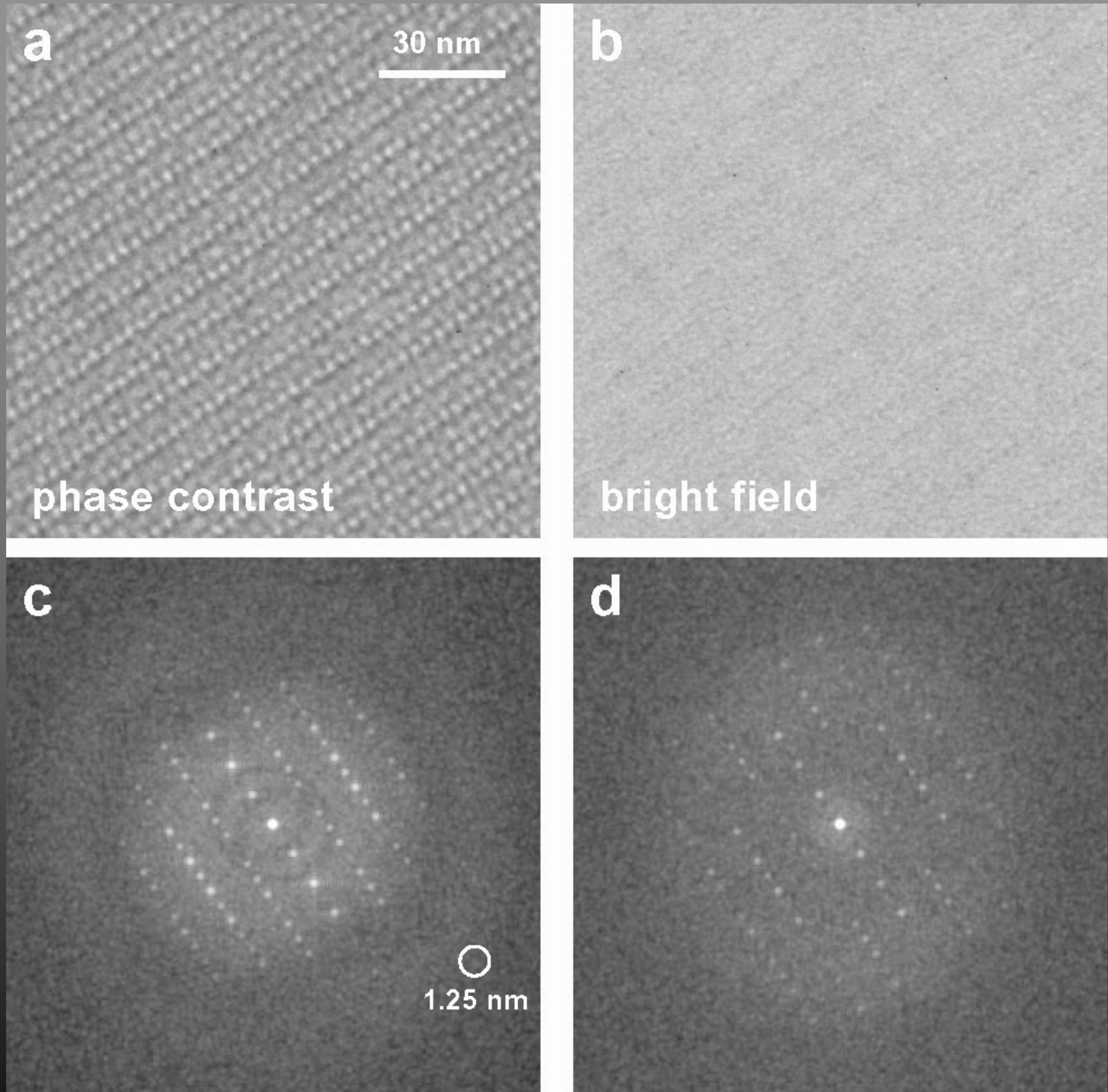


(field of view 50μmx50μm)

Catalase protein crystal
(ice embedding)

Zeiss-NTS 922
FEG EFTEM

images

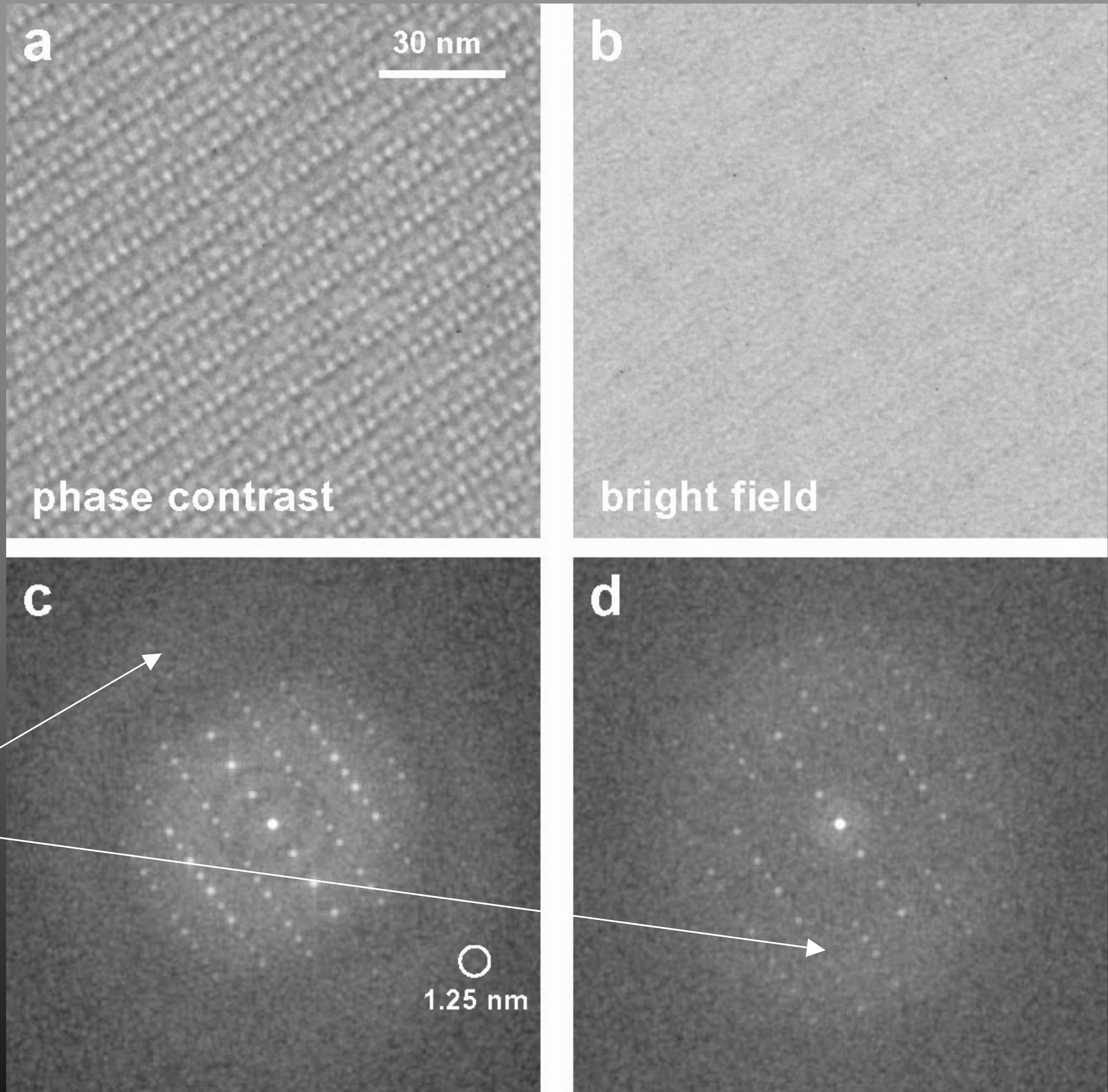


power spectra

Catalase protein crystal
(ice embedding)

Zeiss-NTS 922
FEG EFTEM

Thon rings =
show directly
the $|CTF|^2$



Transfer function and wave aberration

without phase plate

$$CTF \sim -2\sin\left(\frac{\pi}{2}\left(C_s\lambda^3k^4 - 2\Delta z\lambda k^2\right)\right)$$

with phase plate

$$CTF \sim -2\sin\left(\frac{\pi}{2}\left(C_s\lambda^3k^4 - 2\Delta z\lambda k^2\right) + \varphi(k)\right)$$

+90° phase shift

$$CTF \sim -2\cos\left(\frac{\pi}{2}\left(C_s\lambda^3k^4 - 2\Delta z\lambda k^2\right)\right)$$

C_s correction
(spherical aberration)

$$CTF \sim -2\cos\left(\frac{\pi}{2}\left(0 - 2\Delta z\lambda k^2\right)\right)$$

in focus

$$CTF \sim -2\cos\left(\frac{\pi}{2}\left(0 - 0\right)\right) = 2\cos 0 = 2$$

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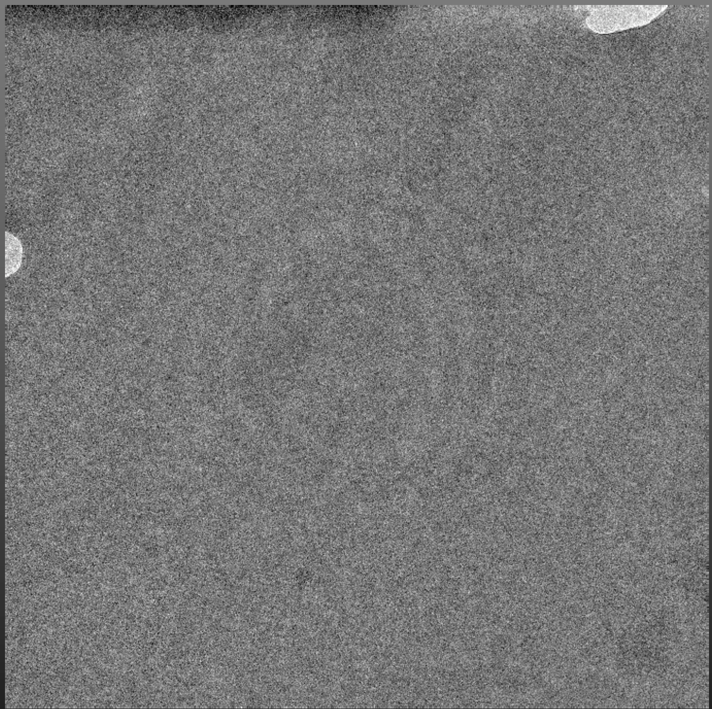
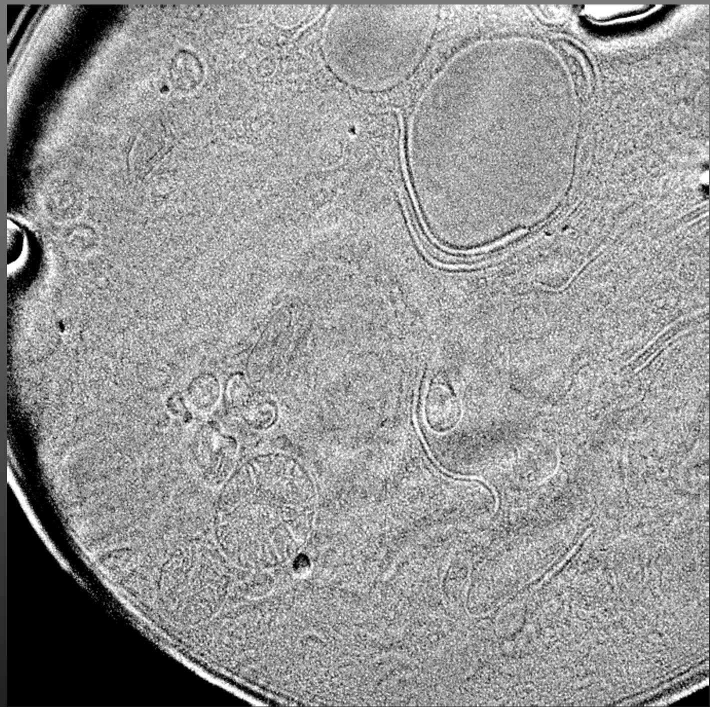


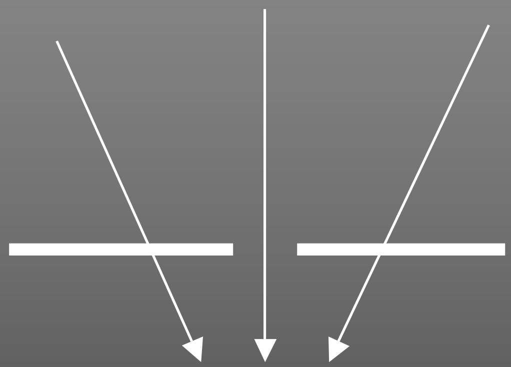
(field of view 50μmx50μm)

Zernike phase contrast (hole in C-film)



Two Types of Phase Plates

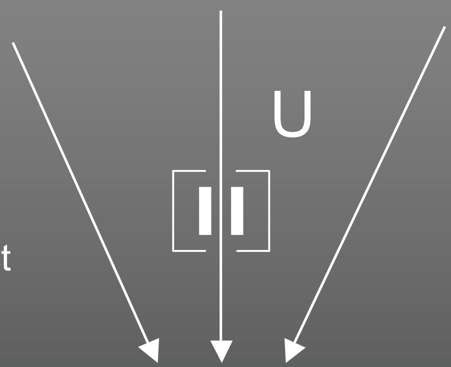
Zernike



back focal plane:

scattered and direct beam separated

Boersch

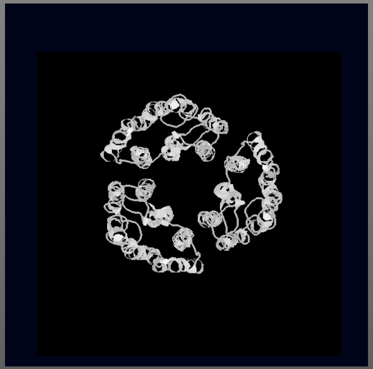


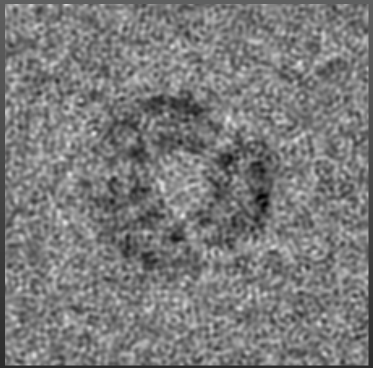
- phase shift of scattered electrons by inner potential of carbon film
- loss of coherence
- granularity ⇒ non-constant phase shift
- contamination ⇒ charging

- phase shift of central beam by electrostatic potential
- no interaction with matter
- adjustable potential / phase shift
- no contamination

Measure resolution as a function of noise and coherence loss by Zernike-type thin film phase plate

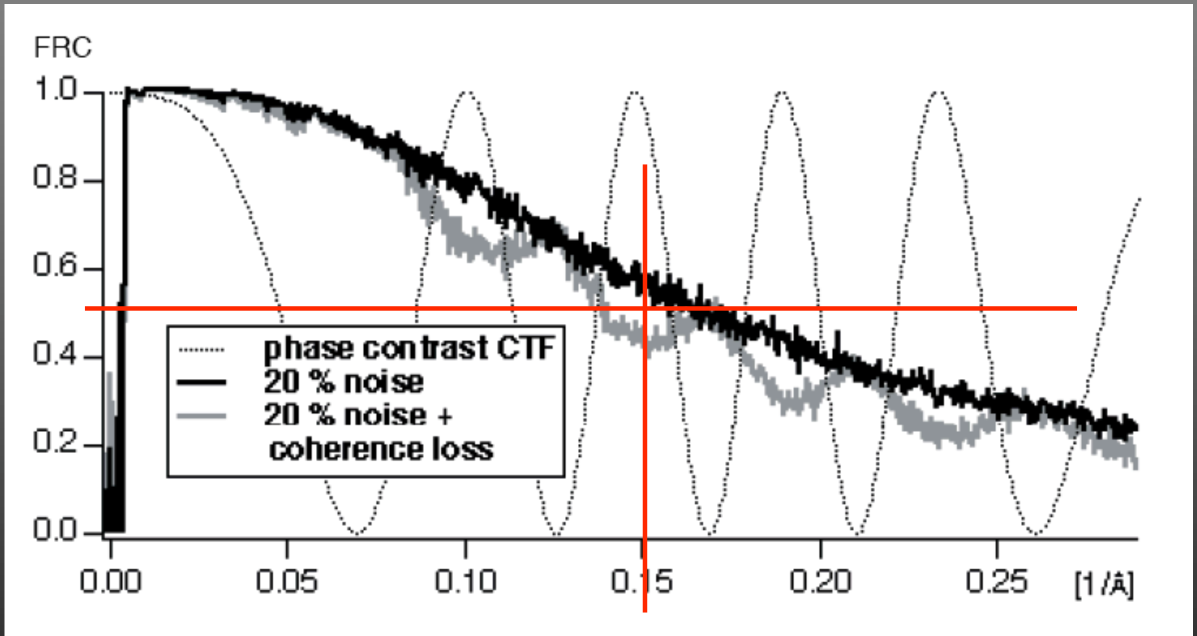
original





simulated phase contrast image

Fourier ring correlation



resolution limit 6-8 Å
cmp: 2-3 Å by electron crystallography

Majorovits and RRS, unpublished

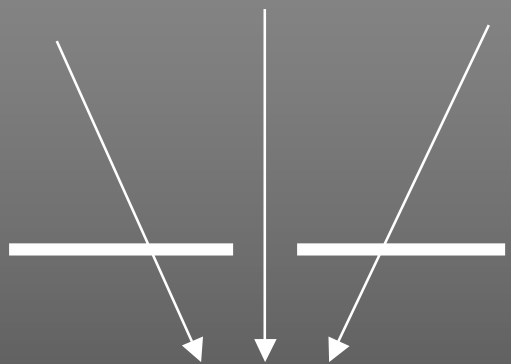
Prerequisites for ideal phase plate

- phase shift by magnetic or electric field
  -> no additional scattering

- no other effects than homogeneous phase shift
  -> non-homogeneous effects (lens)
  -> obstructions in the back focal plane

Two Types of Phase Plates

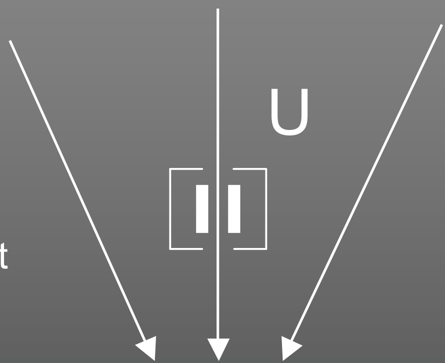
Zernike



back focal plane:

scattered and direct beam separated

Boersch



- phase shift of scattered electrons by inner potential of carbon film
- loss of coherence
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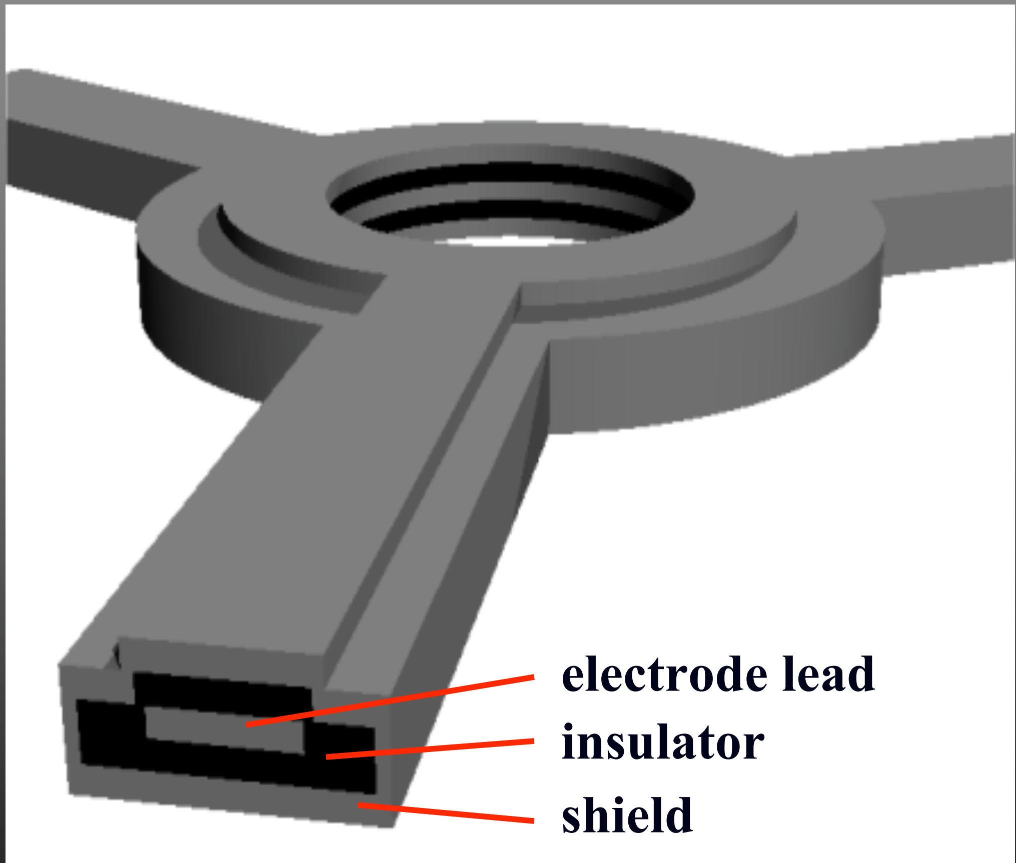
Design of the Boersch Phase Plate



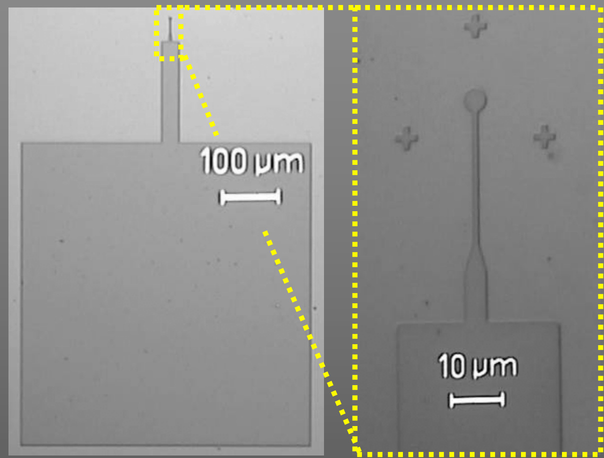
- Realization of an electrostatic microlens by a five-layered electrode structure in the center of the phase plate
- Confinement of the electrical field to the central lens opening by surrounding Au layer

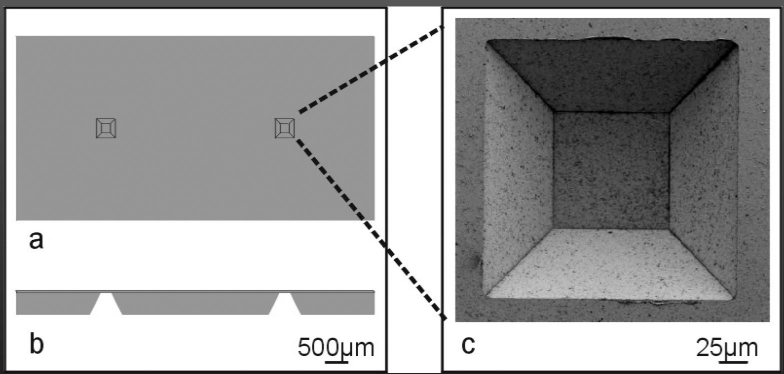
Matsumoto and Tonomura, Ultramicroscopy, 63 (1996)

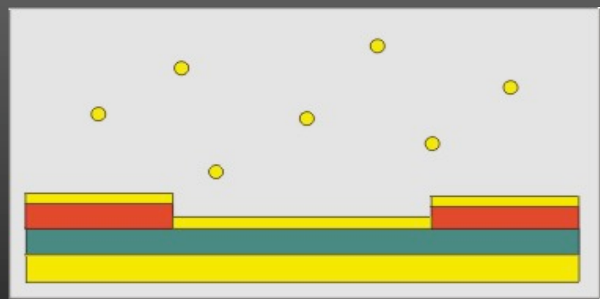
Schultheiß et al., J. Instrum. Res. 77 (2006)

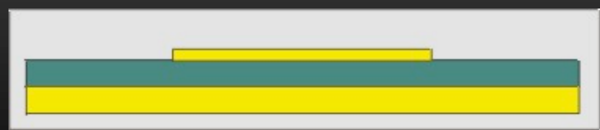
Substrate Material / Lower 3 Layers

- Commercially available low-stress $Si_{3+x}N_{4-x}$ membranes as basic material
- Electron-beam evaporation of the lower shielding Au layer
- Patterning of the electrode layer by electron-beam lithography

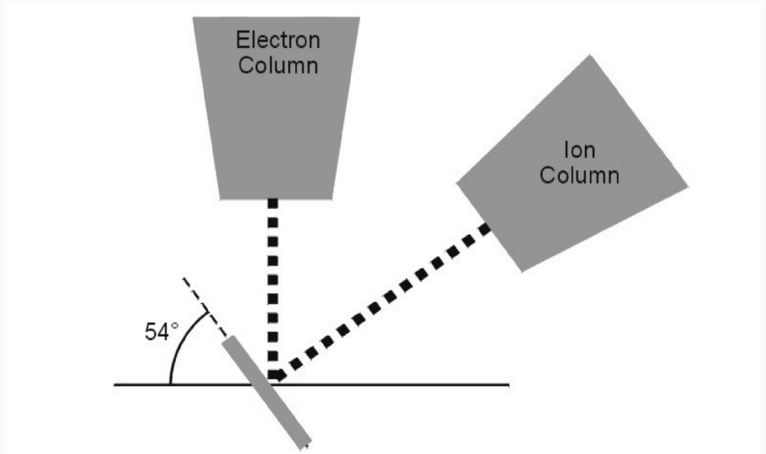




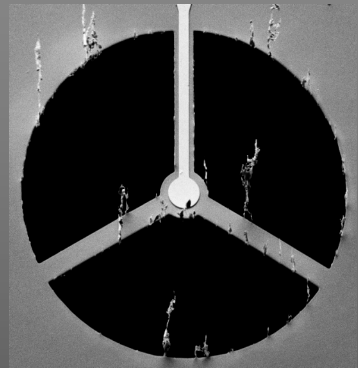




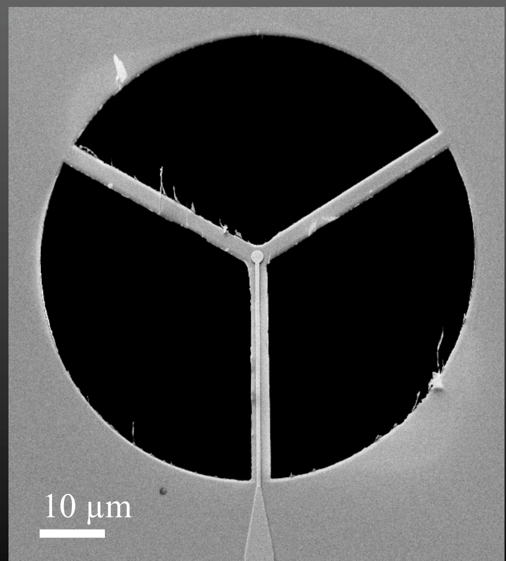
Focused Ion Beam (FIB) Lithography



- Shaping of phase plate by FIB lithography



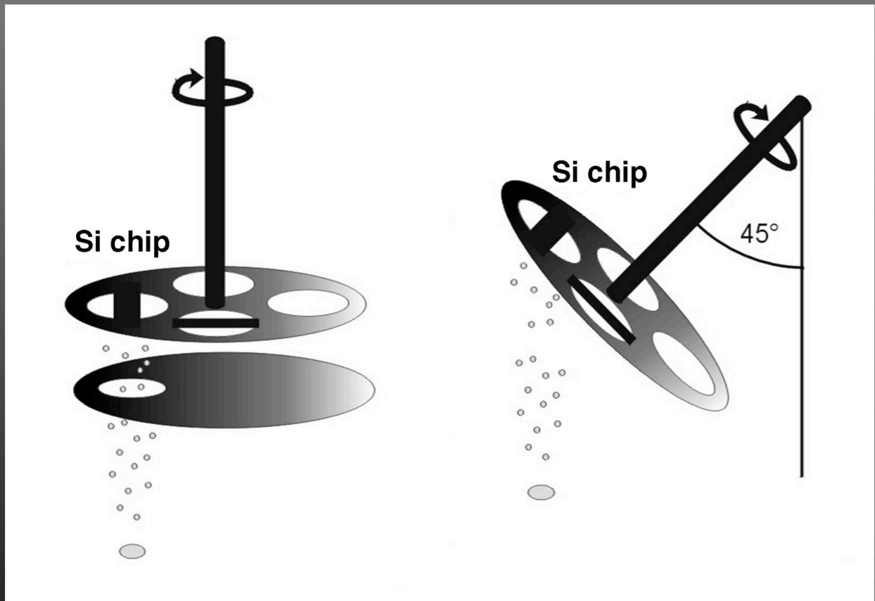
First tested design

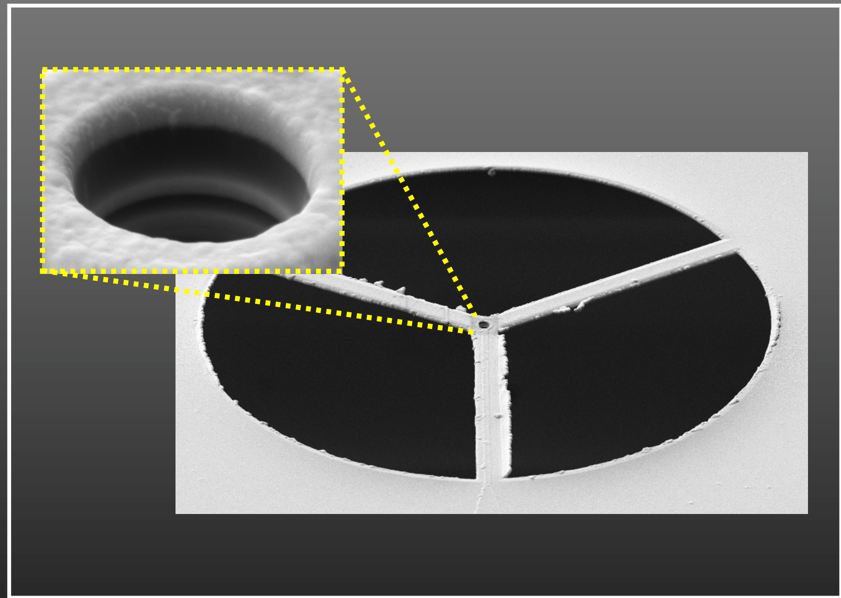


Improved design with reduced supporting bar width

Shielding Layer / Central Hole

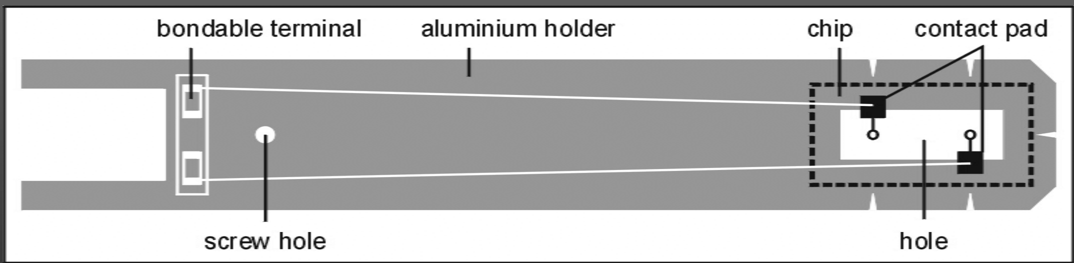
- Electron-beam evaporation of 2nd insulating layer (Al_2O_3)
- Electron-beam evaporation of shielding gold layer in tilted rotating holder
- Central lens opening by FIB milling

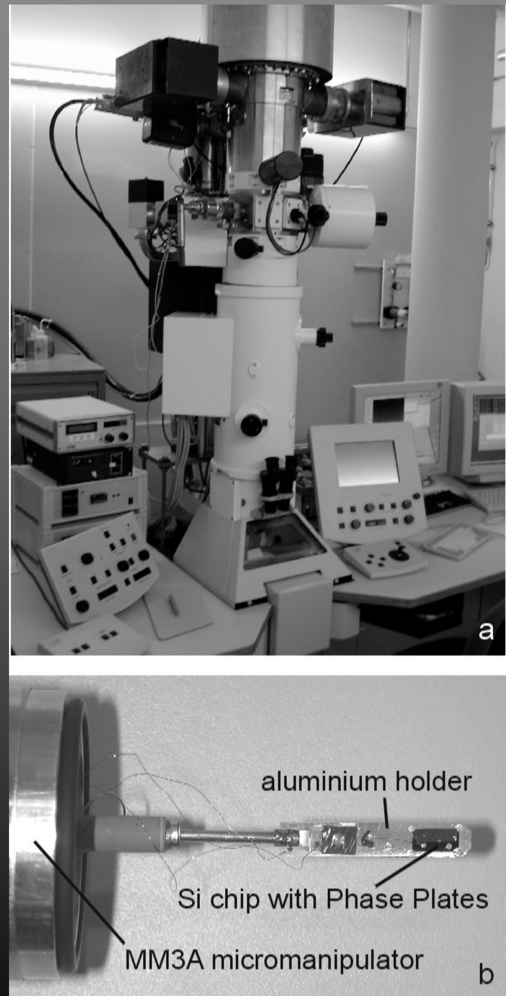




Connection and Implementation

- Chip glued on an aluminium holder
- Mounting of aluminium holder on a piezodriven micro manipulator to position the phase plate in the back focal plane
- Electrical bushing through flange





Transfer function and wave aberration

without phase plate $$CTF \sim -2\sin\left(\frac{\pi}{2}\left(C_s\lambda^3k^4 - 2\Delta z\lambda k^2\right)\right)$$

with phase plate $$CTF \sim -2\sin\left(\frac{\pi}{2}\left(C_s\lambda^3k^4 - 2\Delta z\lambda k^2\right) + \varphi(k)\right)$$

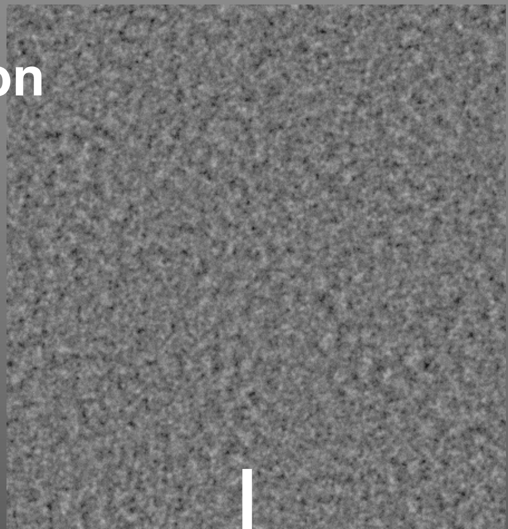
+90° phase shift $$CTF \sim -2\cos\left(\frac{\pi}{2}\left(C_s\lambda^3k^4 - 2\Delta z\lambda k^2\right)\right)$$

C_s correction
(spherical aberration) $$CTF \sim -2\cos\left(\frac{\pi}{2}\left(0 - 2\Delta z\lambda k^2\right)\right)$$

in focus $$CTF \sim -2\cos\left(\frac{\pi}{2}\left(0 - 0\right)\right) = 2\cos 0 = 2$$

Quantitation of phase shift using carbon film

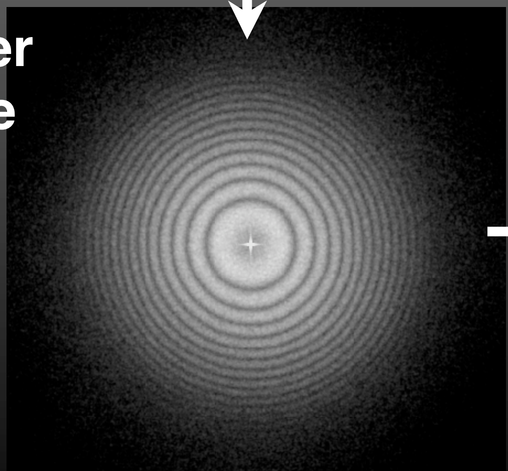
carbon film

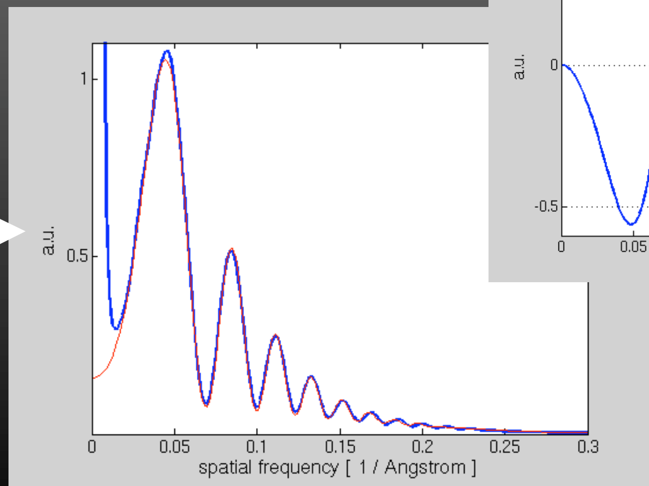


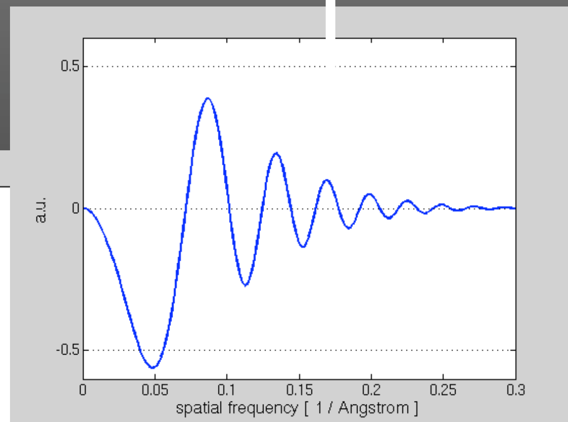
$$CTF \sim -2\sin\left(\frac{\pi}{2}\left(C_s\lambda^3k^4 - 2\Delta z\lambda k^2\right) + \varphi(k)\right)$$

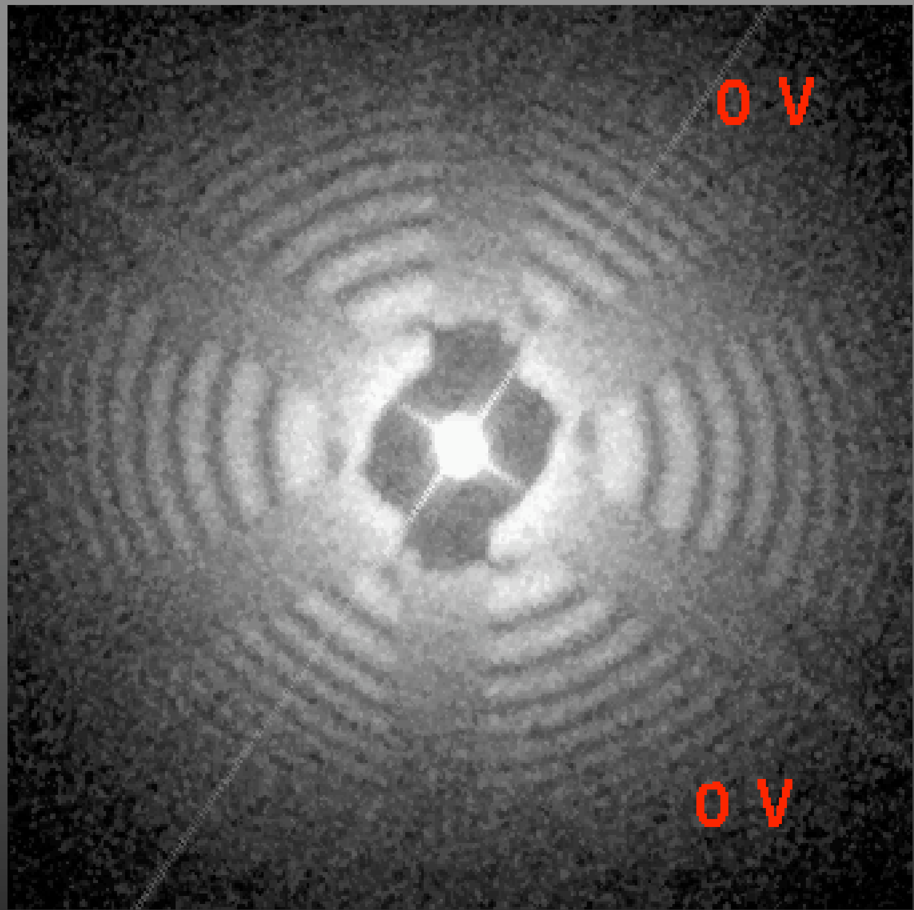
$$\varphi(k) = phase\ shift$$

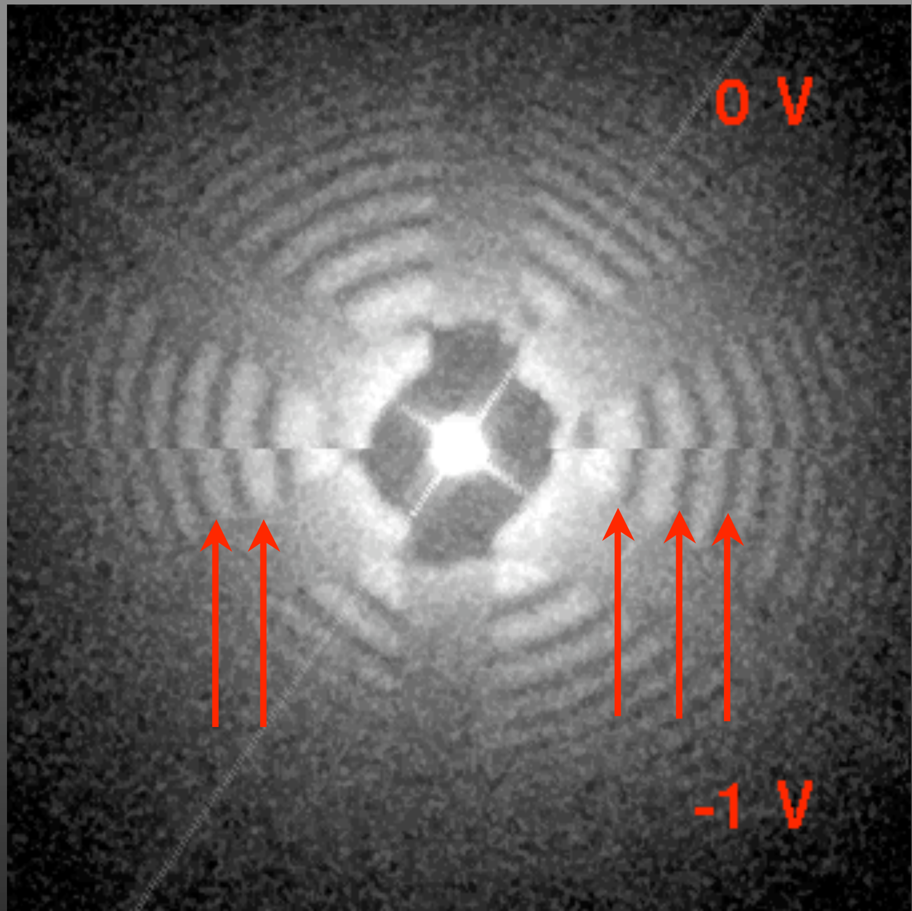
Fourier space

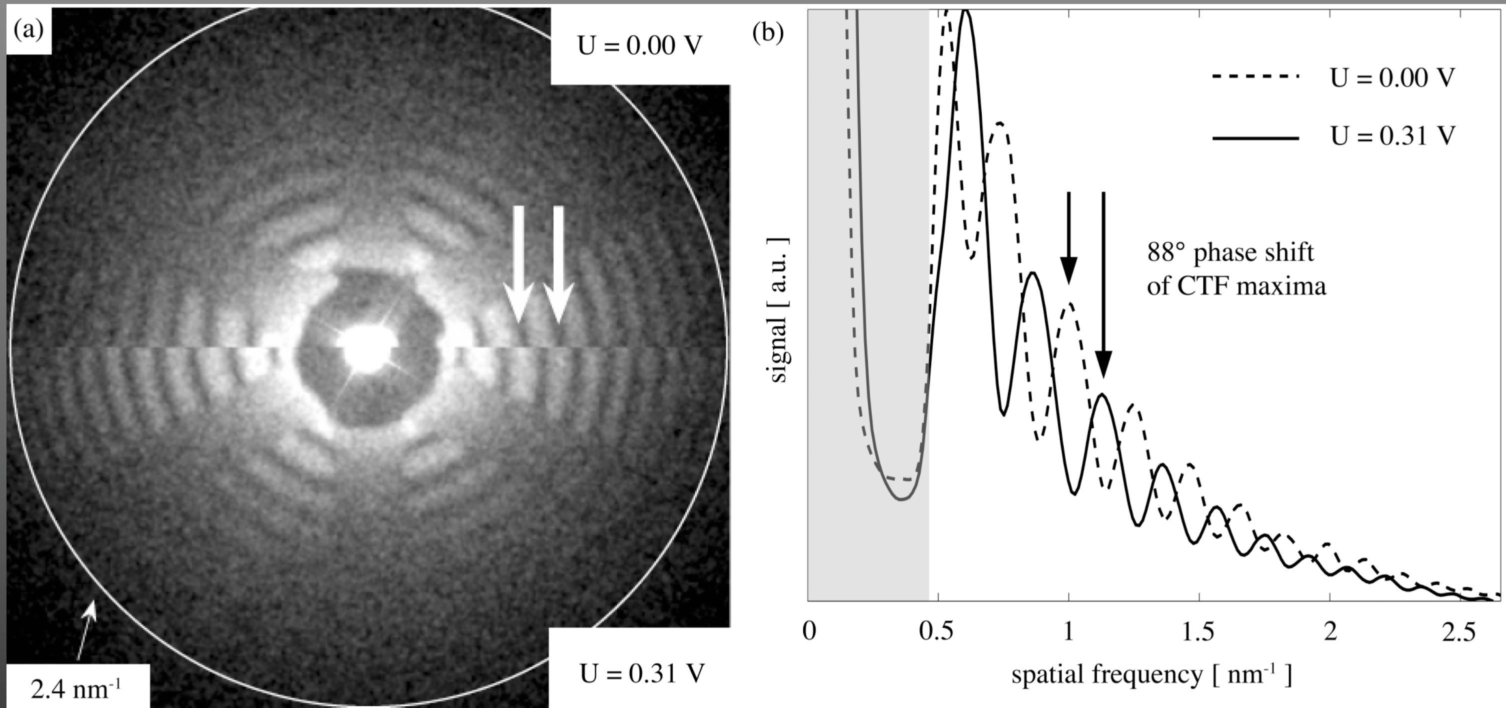






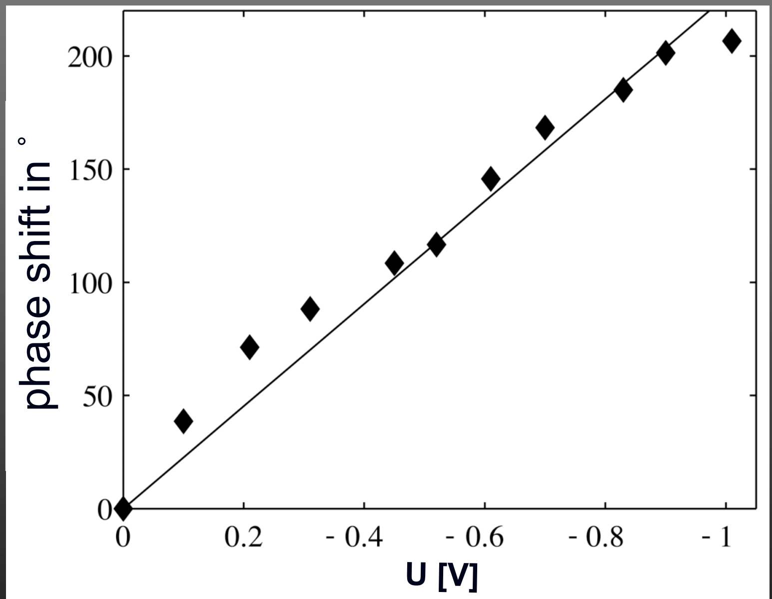

O V
O V

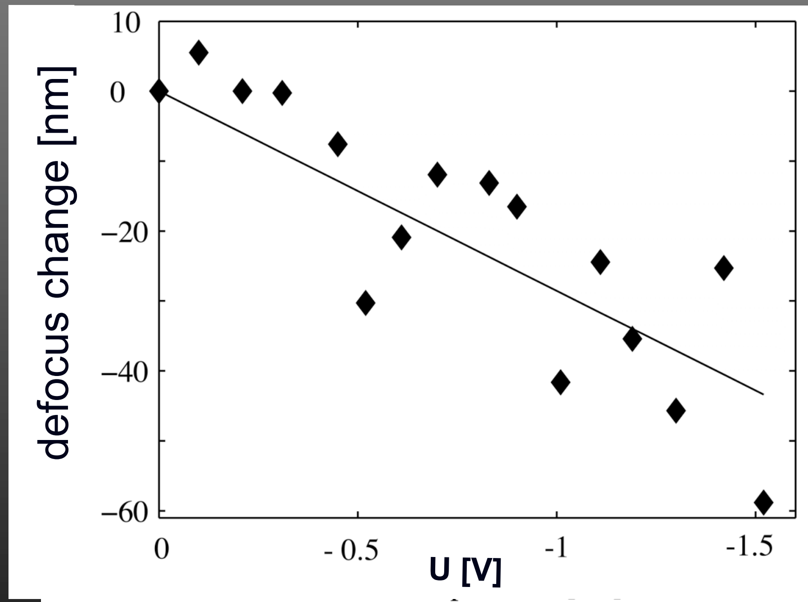

0 V
-1 V


(a)
U = 0.00 V
U = 0.31 V
2.4 nm⁻¹
(b)
U = 0.00 V
U = 0.31 V
88° phase shift
of CTF maxima
signal [a.u.]
spatial frequency [nm⁻¹]
0
0.5
1
1.5
2
2.5

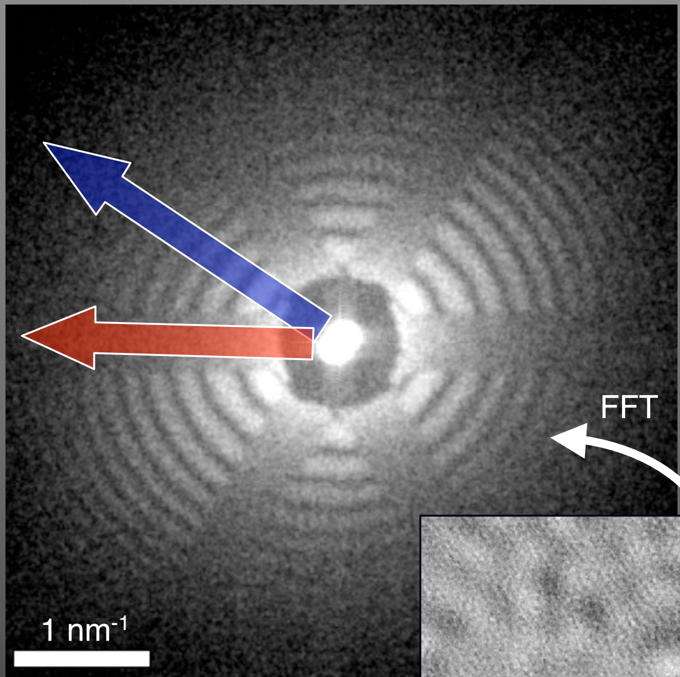
It works!

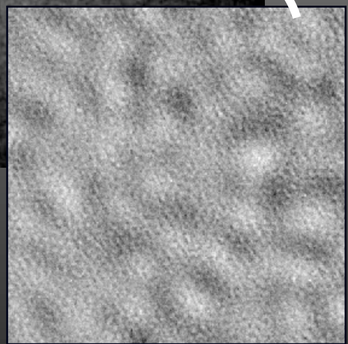
- realisation of an electrostatic Boersch phase plate
- proof of principle of high resolution PCEM



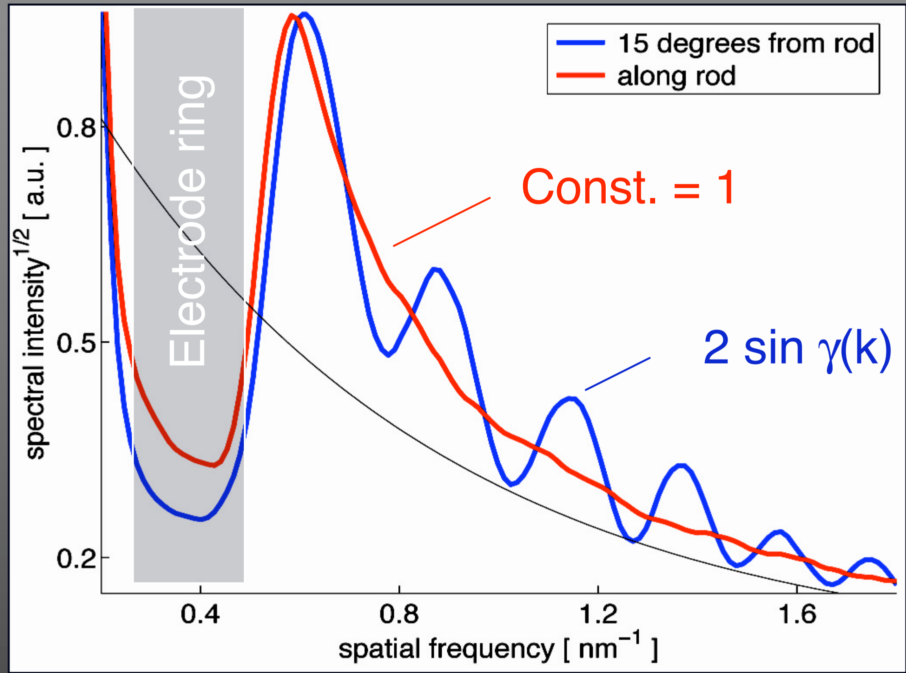


Measurement of Signal Transfer „Along“ Support Rod





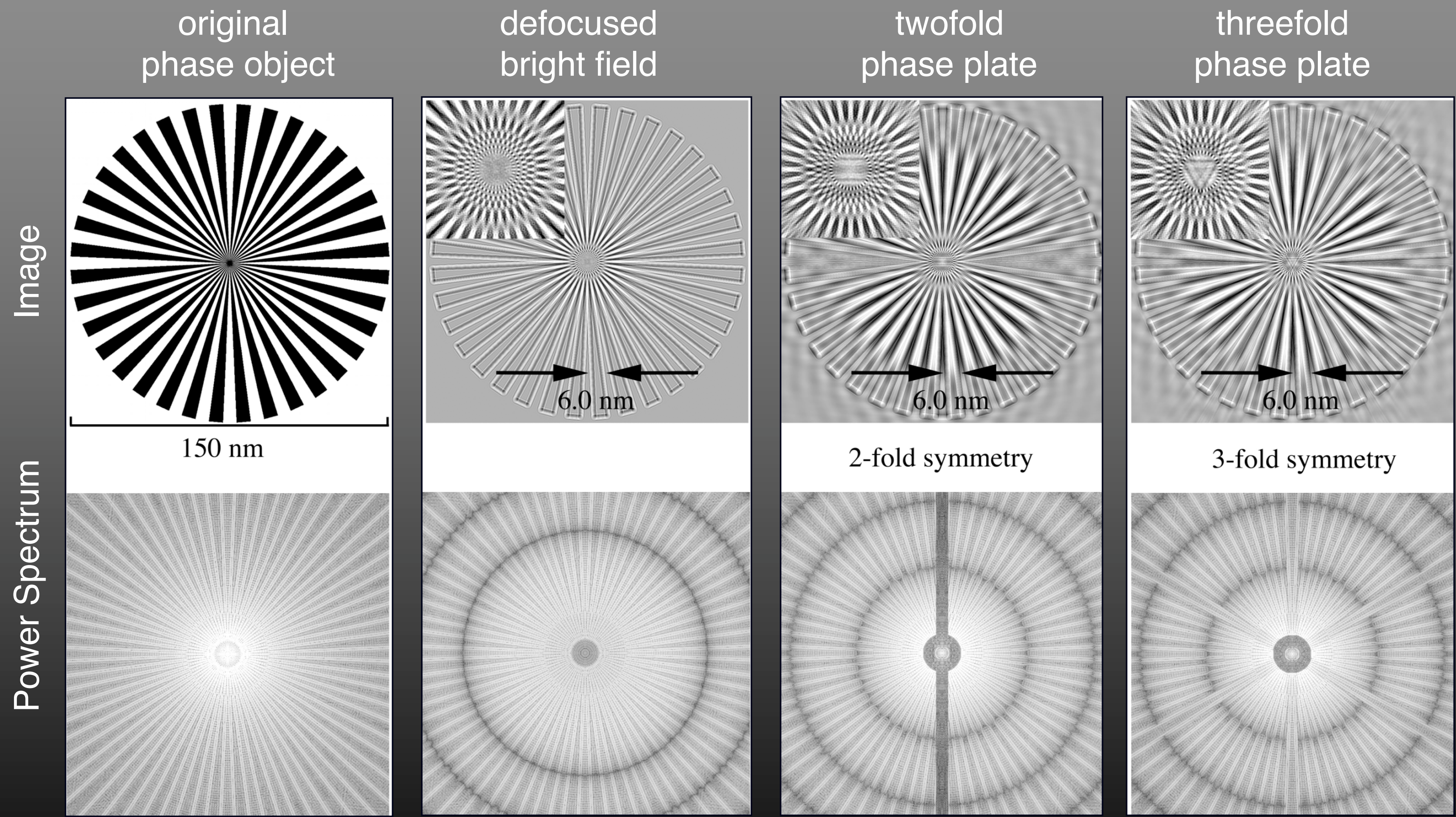
Experimantal Power Spectrum
from images of UAC-stained
carbon film



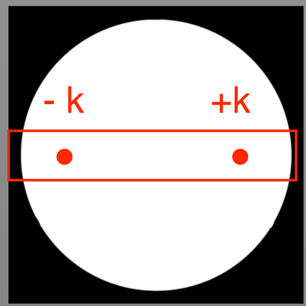
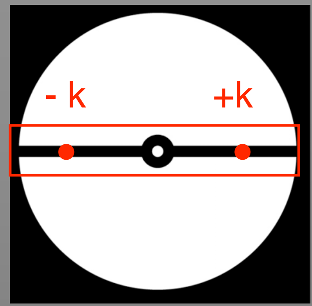
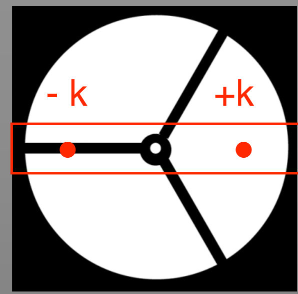
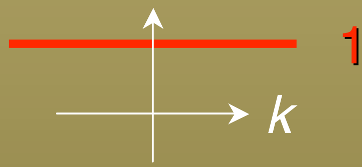
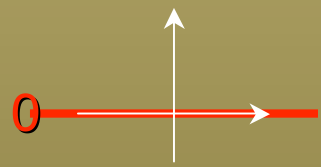
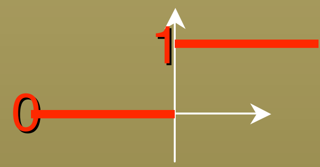
Radial intensity of power spectrum shown on right side
= Single-sideband signal vs. Bright field signal

➔ Non-oscillating single-sideband signal transfer by non-centrosymmetric support rods

Threefold Support Symmetry Preserves Object Information and Reduces Imaging Artifacts

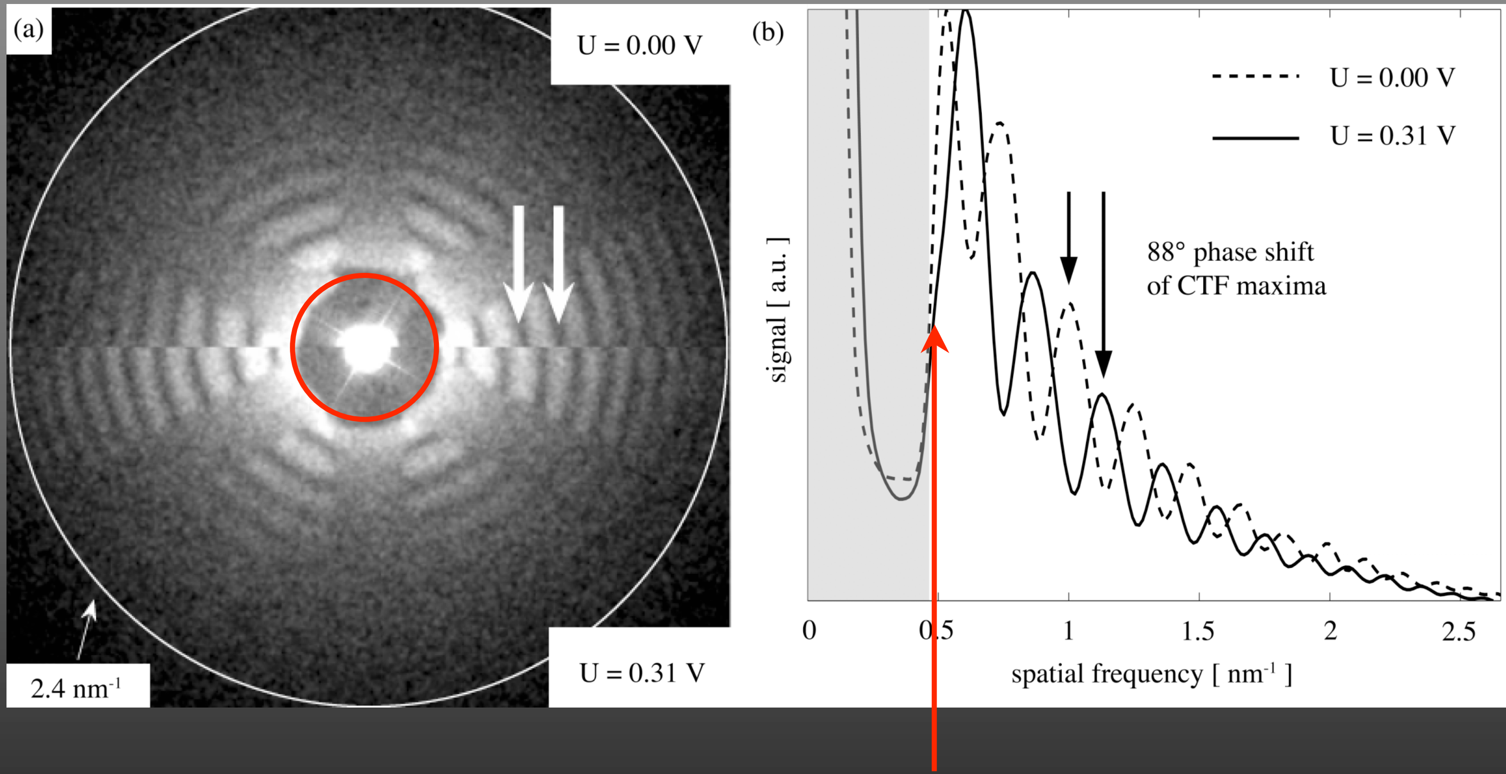


Simulation: Imaging of a weak phase object (Siemens star) with 120 kV TEM

	no phase plate	twofold PP	threefold PP
Support geometry			
Phase Plate aperture function A(k) in back focal plane (in rod direction)	 One $A_0(k) = 1$	 Zero $A_2(k) = 0$	 Heaviside step function $A_3(k) = 1/2 \{ 1 + sign(k) \}$
Wave amplitude in BFP	$\delta(k) + i\,\varphi(k) \bullet e^{-i\,W(k)}$	$\delta(k)$	$\delta(k) + i/2\ \varphi(k) \bullet e^{-i\,W(k)} \{ 1 + sign(k) \}$
Image frequency spectrum (Fourier transform)	$\delta(k) + \varphi(k) \bullet 2\,sin\,W(k)$	$\delta(k)$	$\delta(k) + \varphi(k) \bullet \underbrace{i\,sign(k)\,e^{-i\,sign(k)\,W(k)}}$ **PCTF = phase factor !**
Phase contrast transfer I PCTF (k) I	$2\,\lvert sin\,W(k)\rvert$	0	1

Another Problem:

Loss of Low Resolution Structure Factors

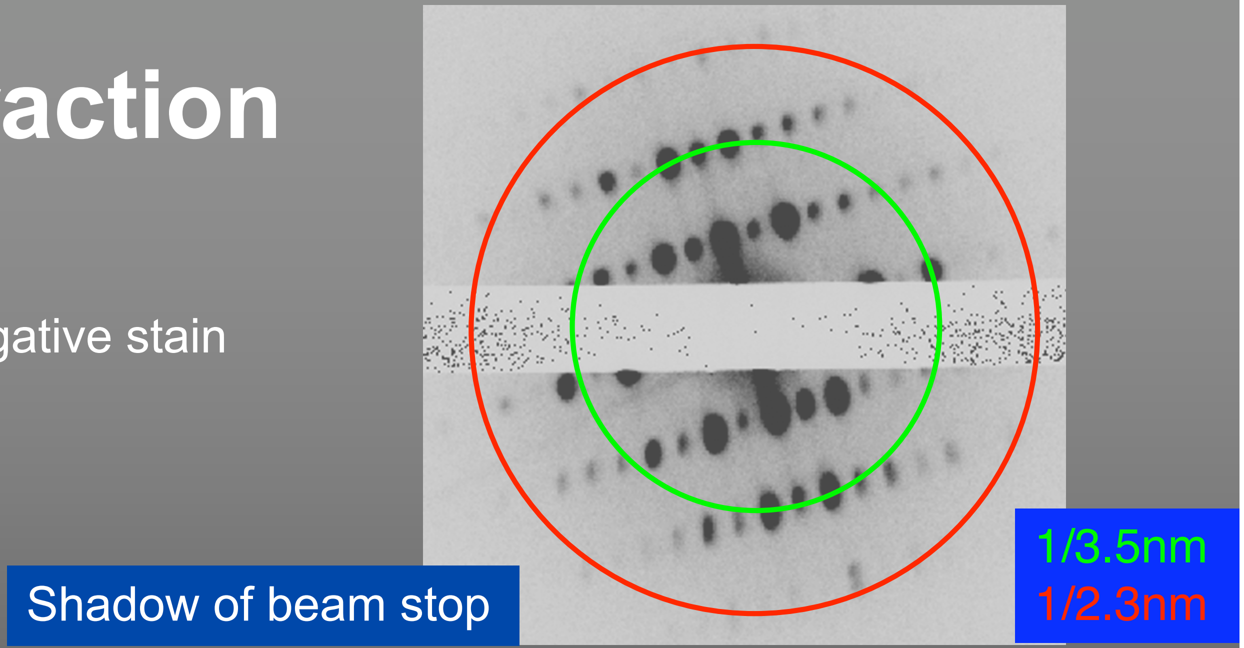


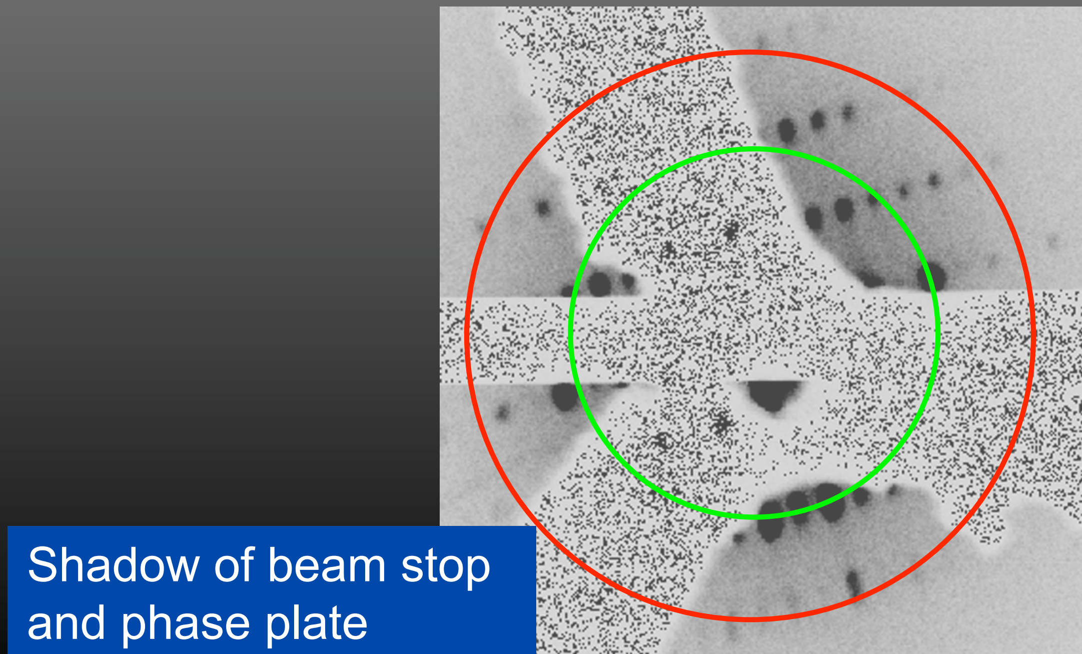
2 nm resolution ring

2 nm resolution cut-off

Electron diffraction patterns

(catalase micro crystals, negative stain 100keV, 3mm focal length)

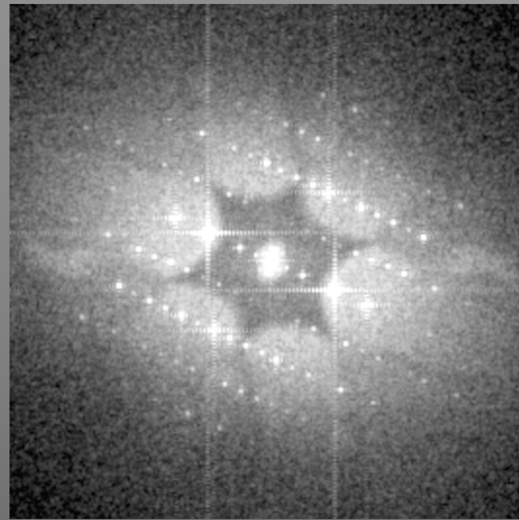




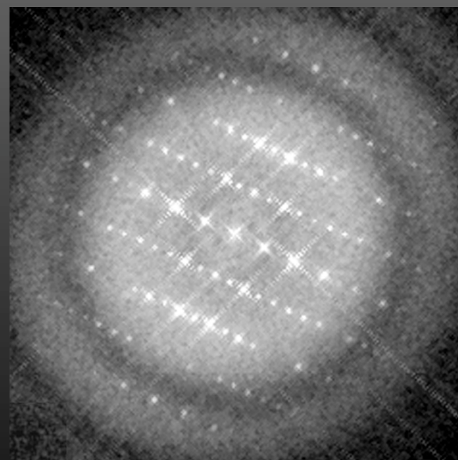
Imaging

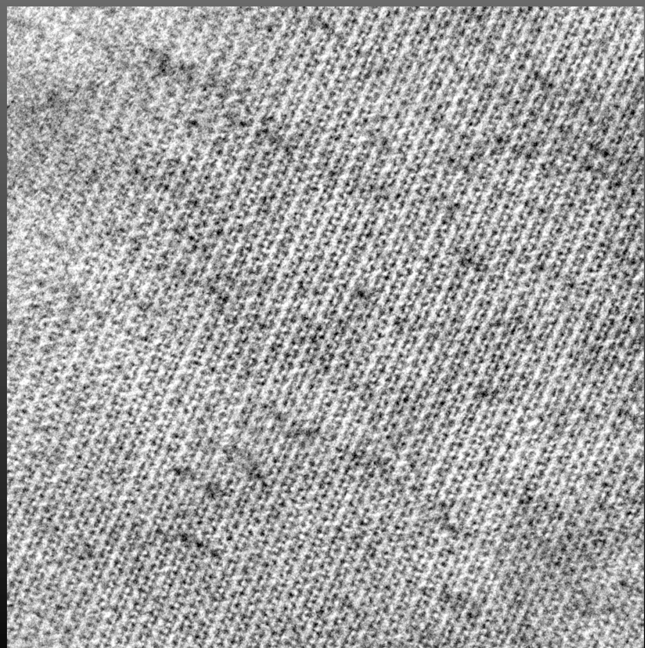
(catalase, negative stain, 100keV, 65kx on CCD 6.9nm,17.3nm)

with phase plate in diff. plane



power spectra





conventional bright field

The Roadmap to High Resolution Phase Contrast EM

Proof of principle (structure factors 2nm - 4Å)

↓

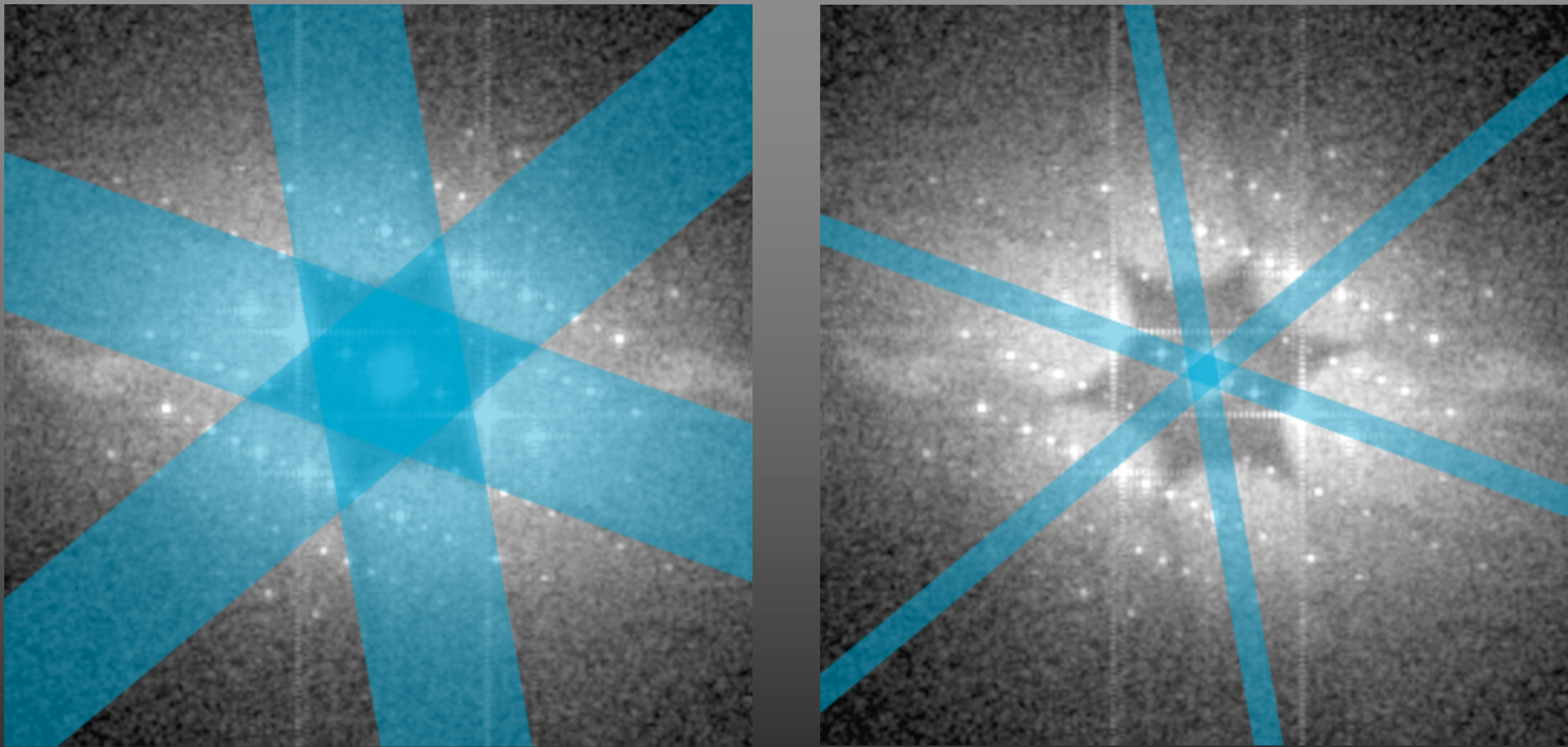
Smaller lens diameter (structure factors 4nm - 2Å) and thinner support rods

↓

Increase effective size of diffraction pattern (structure factors 40nm - 2Å)

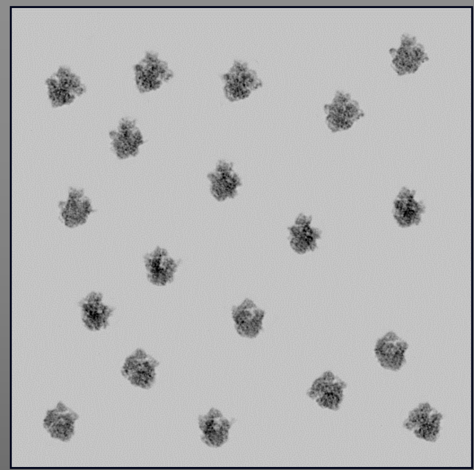
↓

Artefact-free imaging of objects of typical size of 20-30nm



Reducing Signal Obstruction by the Phase Plate

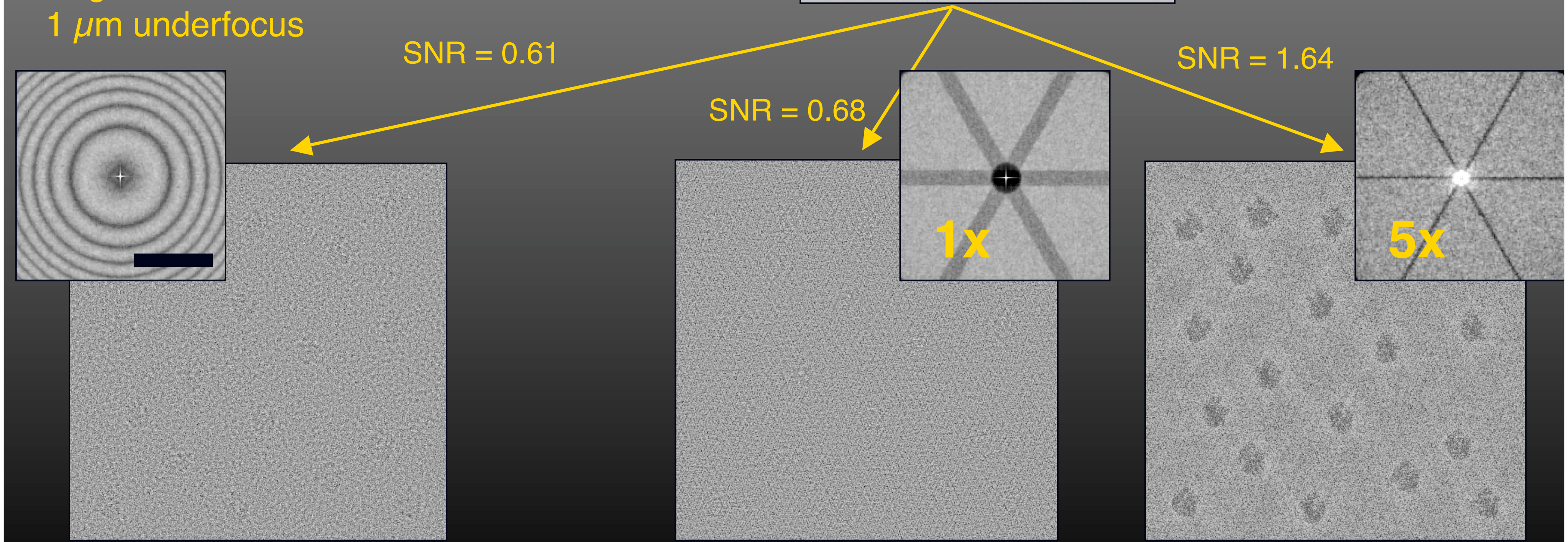
Simulate the optical magnification of back focal plane
(300kV, focaL length 2.2mm)



Test object: field of different ribosome projections (from PDB) with simulated ice embedding

100 nm

Bright Field
1 μm underfocus



3-fold Boersch Phase Plate, in gaussian focus

Conclusion and future work

• phase shift by electric field shown (proof of principle)
-> no additional scattering

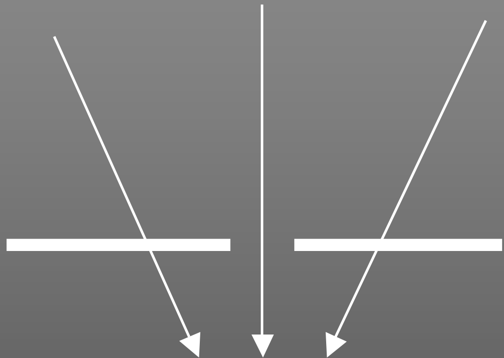
• no other effects than homogeneous phase shift
-> non-homogeneous effects (lens)
-> obstructions in the back focal plane

-> Design and construct a lens doublet to magnify
the back focal plane
(problems with spherical aberration, resolution ...)

Majorovits et al., Ultramicroscopy, in press (2006 or 2007)
on the web under: doi 10.1016/j.ultramic.2006.7.006

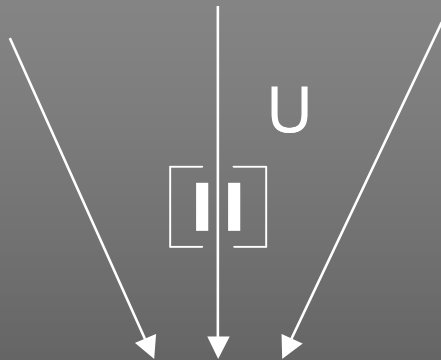
Two Types of Phase Plates

Thin-film Zernike



Fritz Zernike

Boersch



Hans Boersch