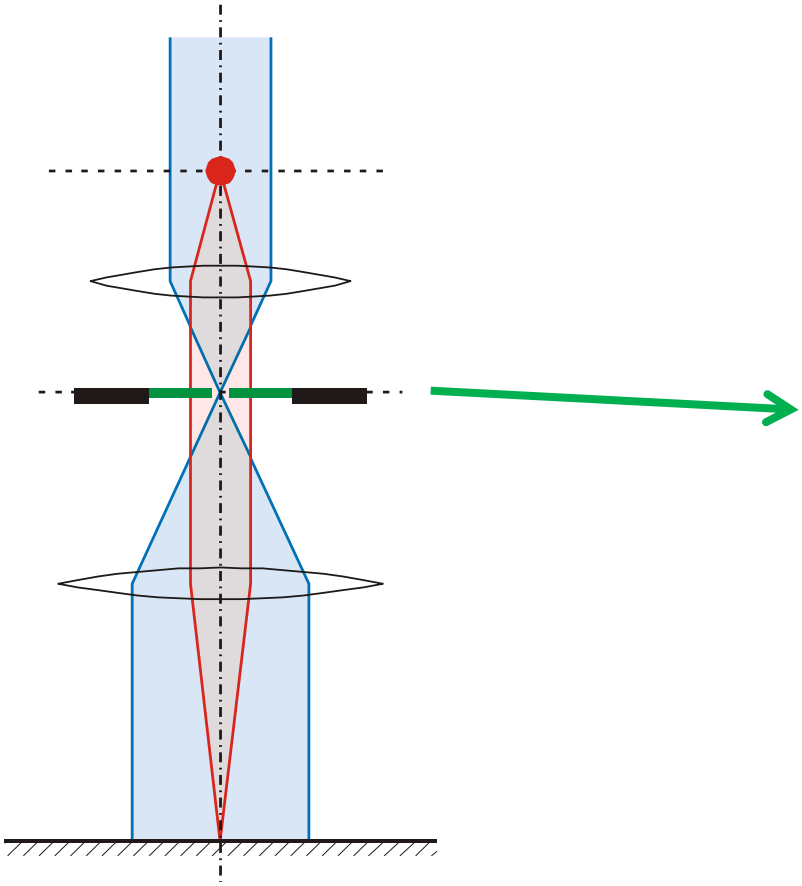
Phase Plates for Single Particles

Radostin Danev

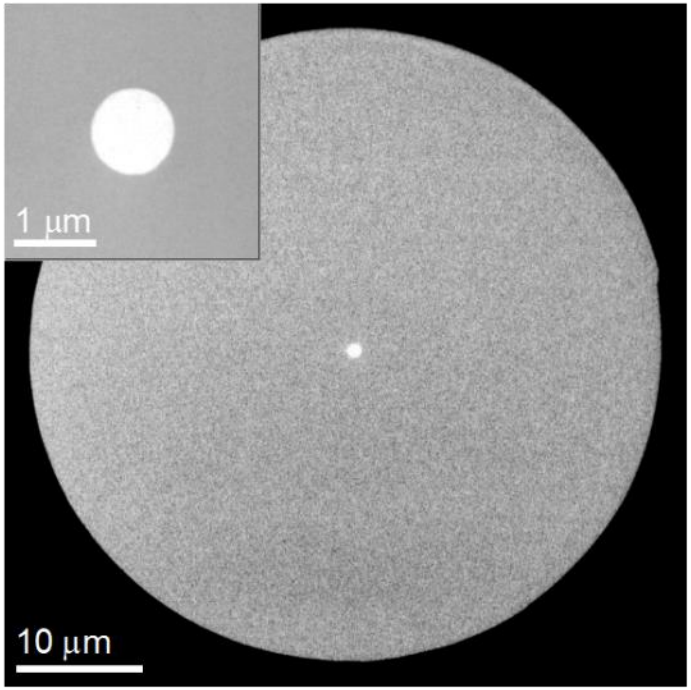
Max Planck Institute of Biochemistry, Martinsried, 82152, Germany

What is a phase plate?

ZPC-TEM



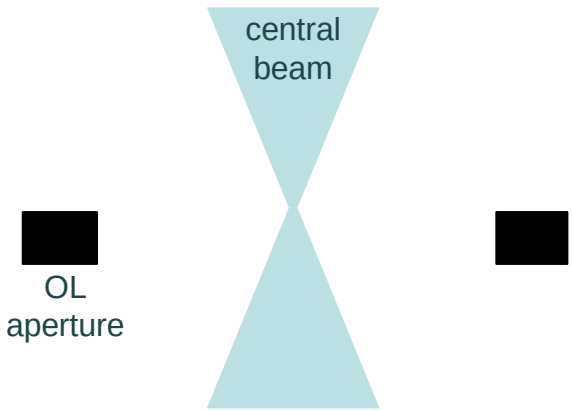
Zernike phase plate



Phase plate types

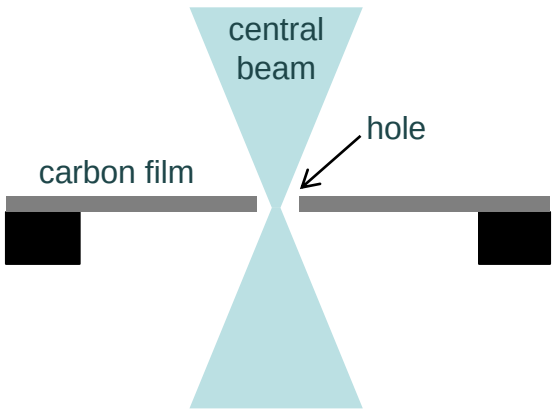
Conventional TEM

CTEM



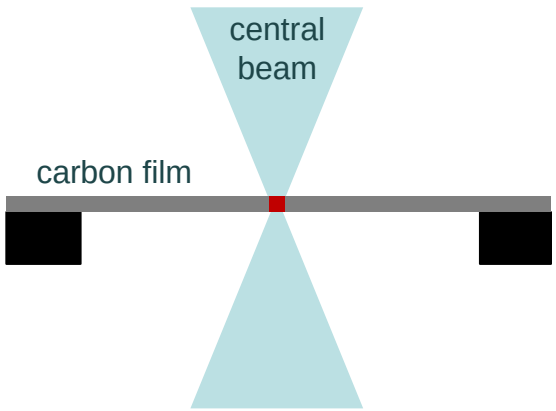
Zernike Phase Plate

ZPP

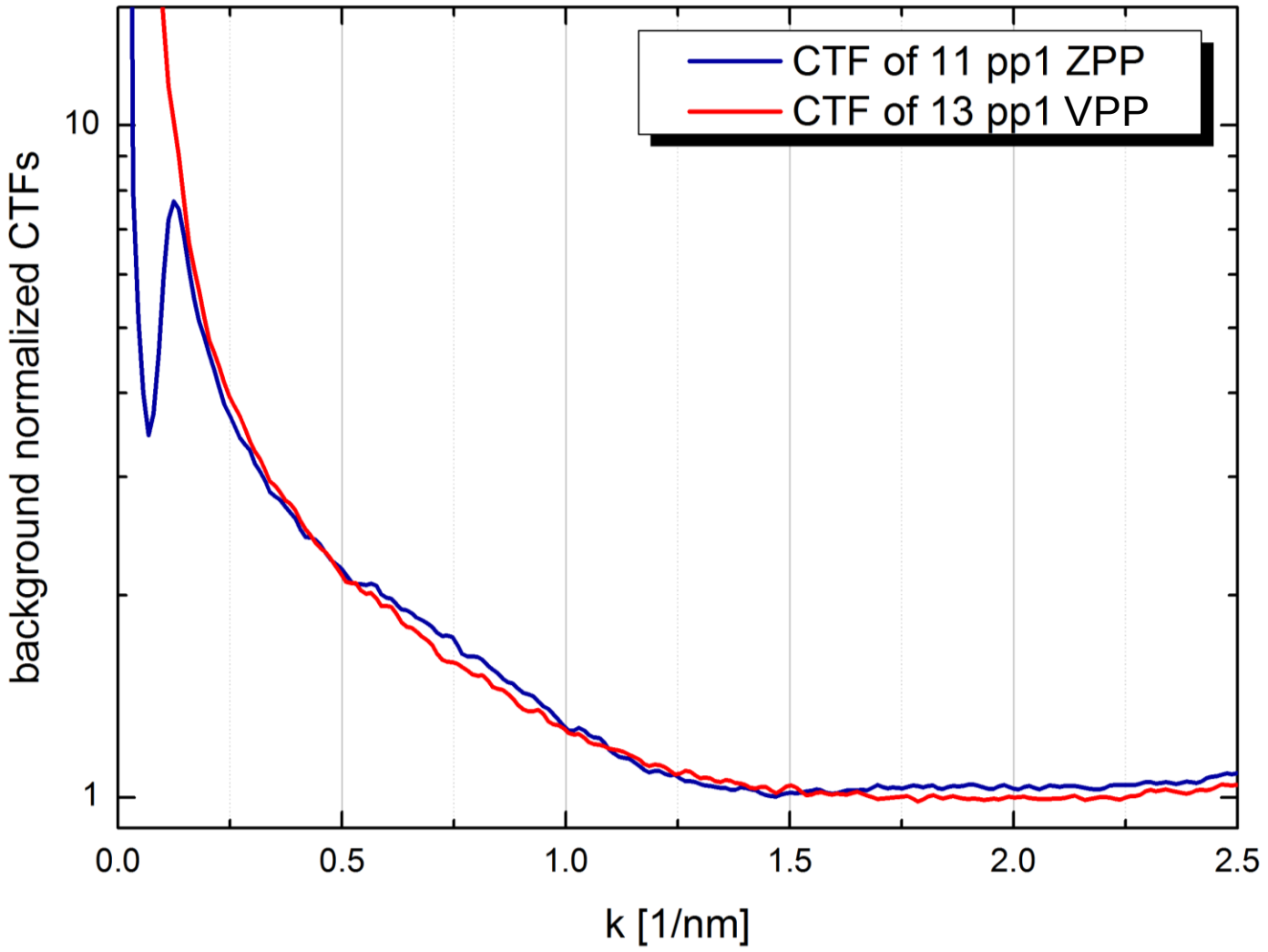


Volta Phase Plate

VPP

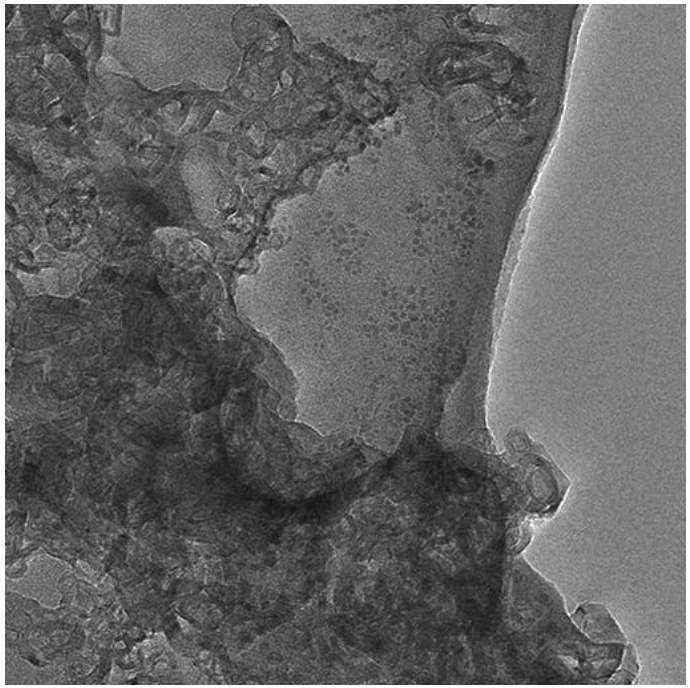


ZPP vs. VPP, in-focus CTF example

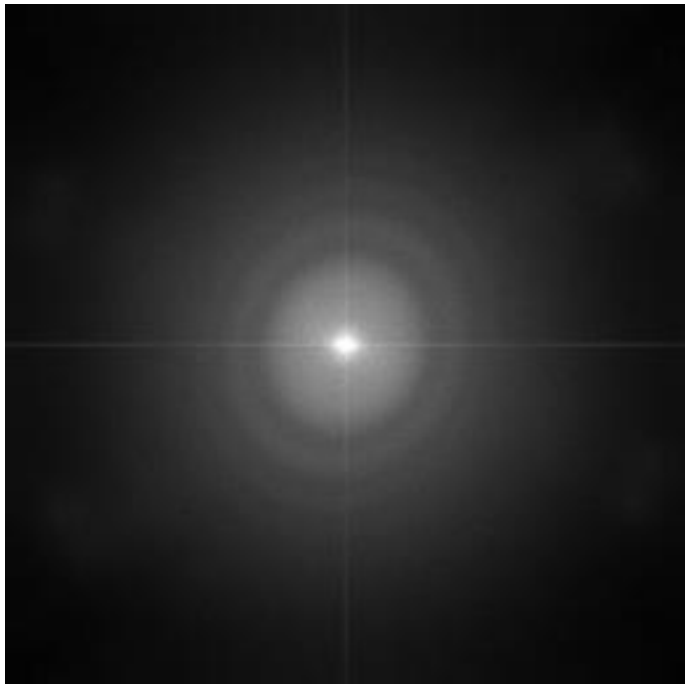


VPP evolution

VPP image series

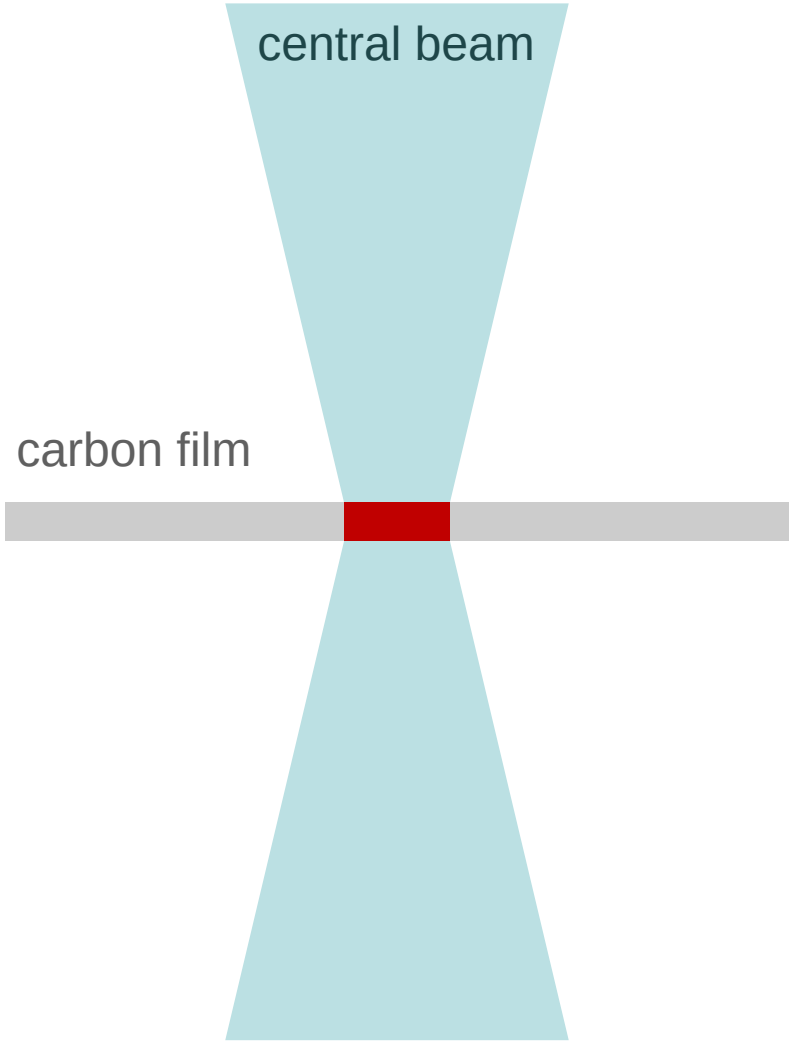


FFT



Niquist frequency ~ 4.3A

The Volta potential hypothesis

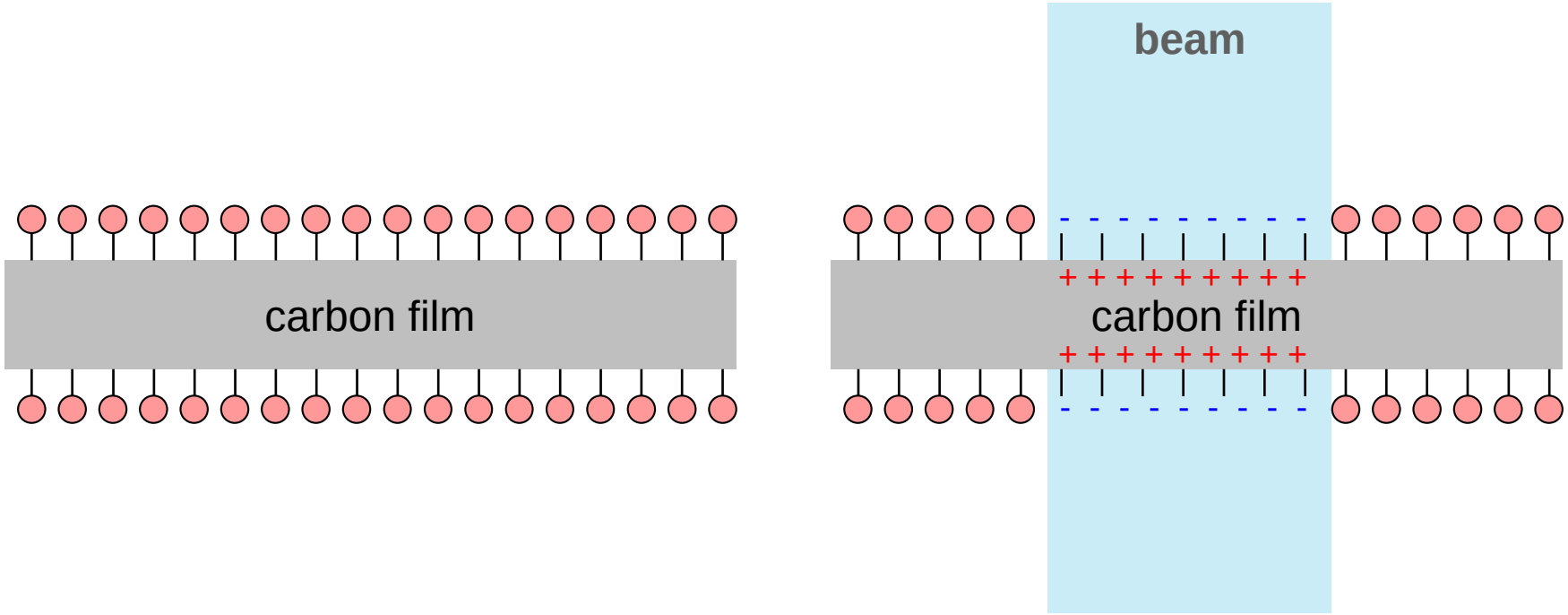


- The effect is not due to beam-induced contamination or etching – confirmed experimentally.
- The effective “charge” of the irradiated area is negative (it produces less phase shift than the surrounding film)!
- The properties of the area recover to normal in ~ 1 day (depends on the temperature).

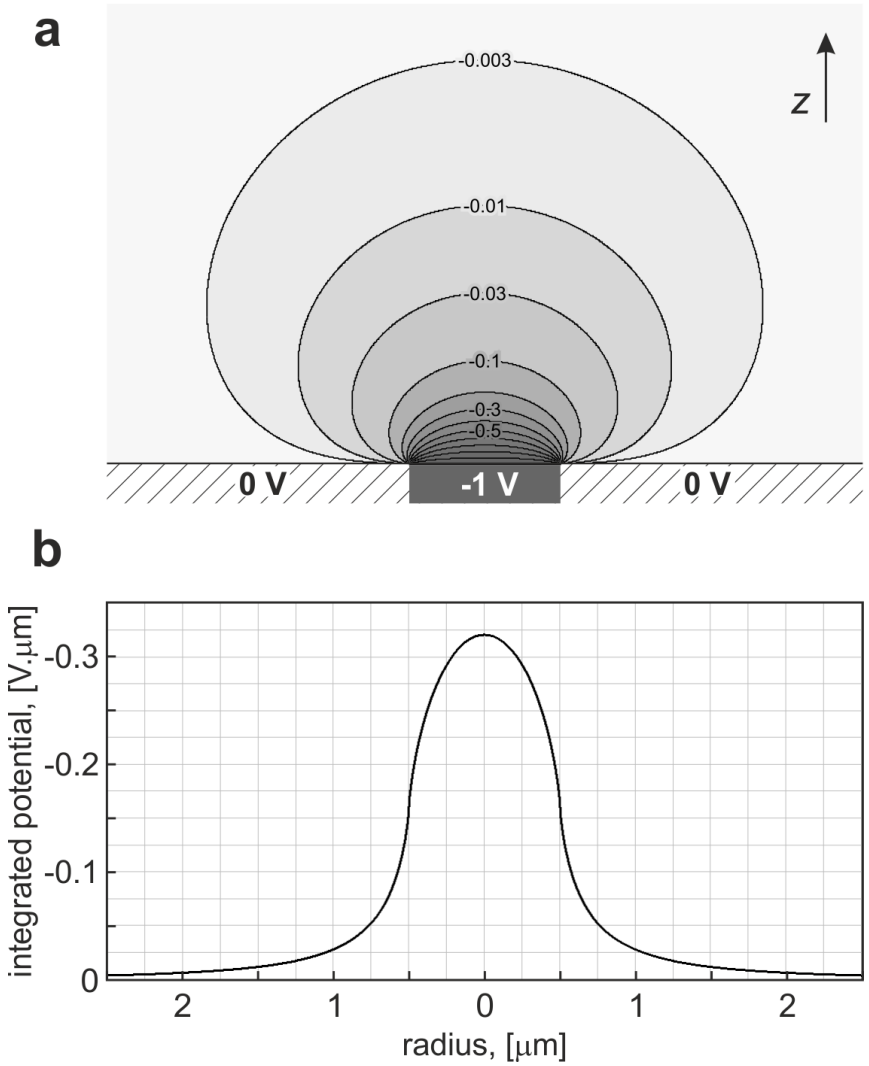
-> The central beam changes the electronic properties of the irradiated carbon film area.

Volta potential - surface chemistry hypothesis

- The carbon surface is in a chemical equilibrium with the residual gases in the vacuum.
- Electron irradiation breaks the bonds and causes local de-termination of the surface.
- Unsaturated dangling bonds cause excess of electrons on the surface.



A constant potential disk on a surface



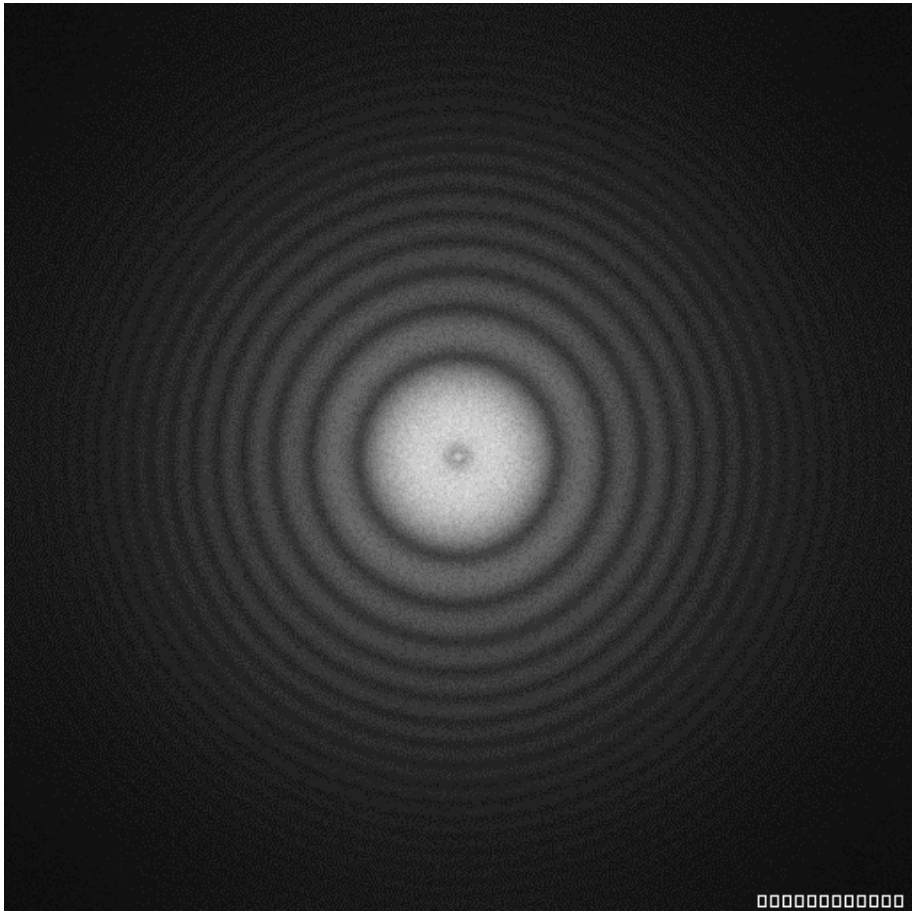
- -1 V potential
- 1 um diameter disk

- 215 V.nm -> $\pi/2$

  phase shift @ 200 kV.

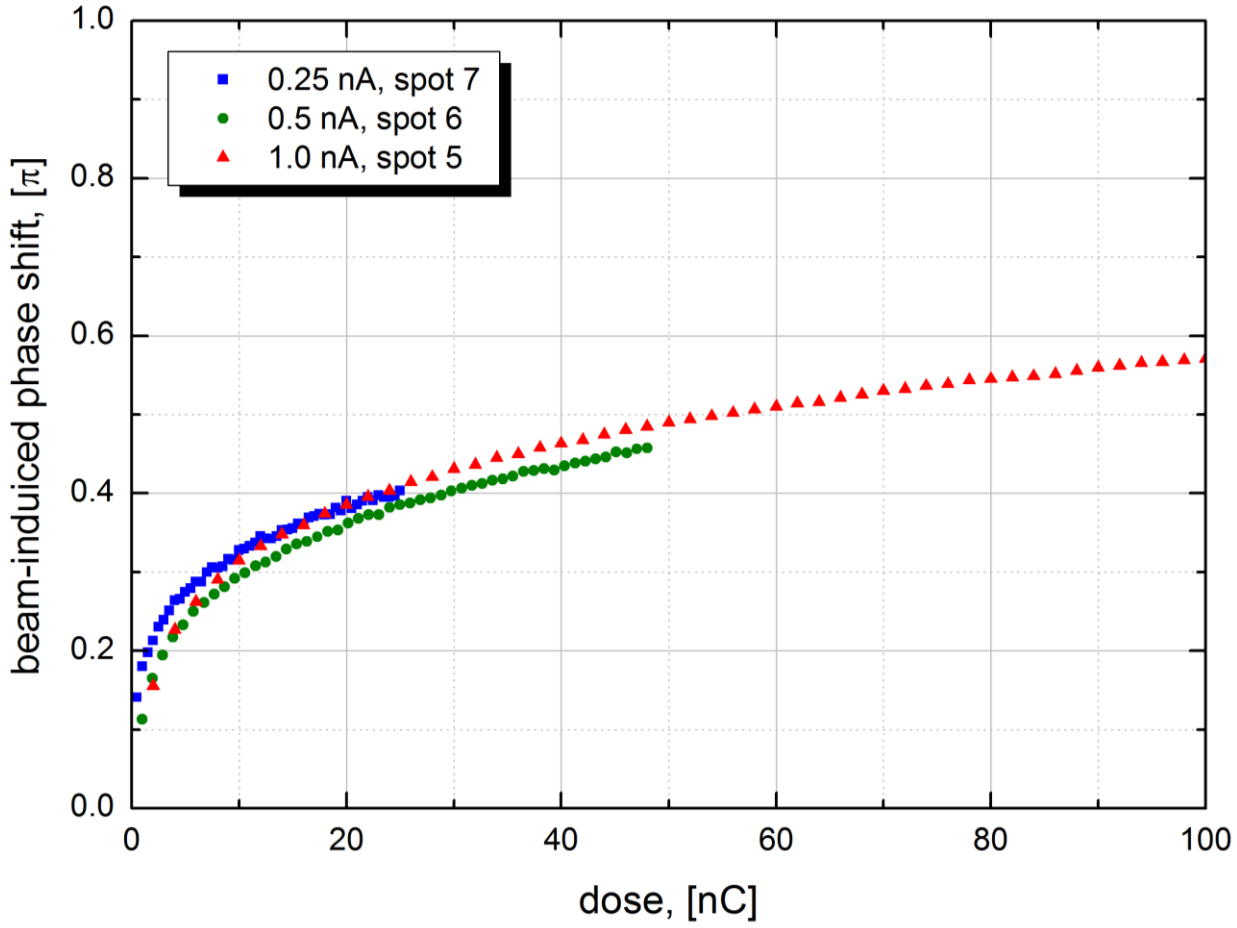
Beam-induced phase shift series

- 12 nm thick carbon film; beam current: 1.0 nA; beam diameter: 1 μm



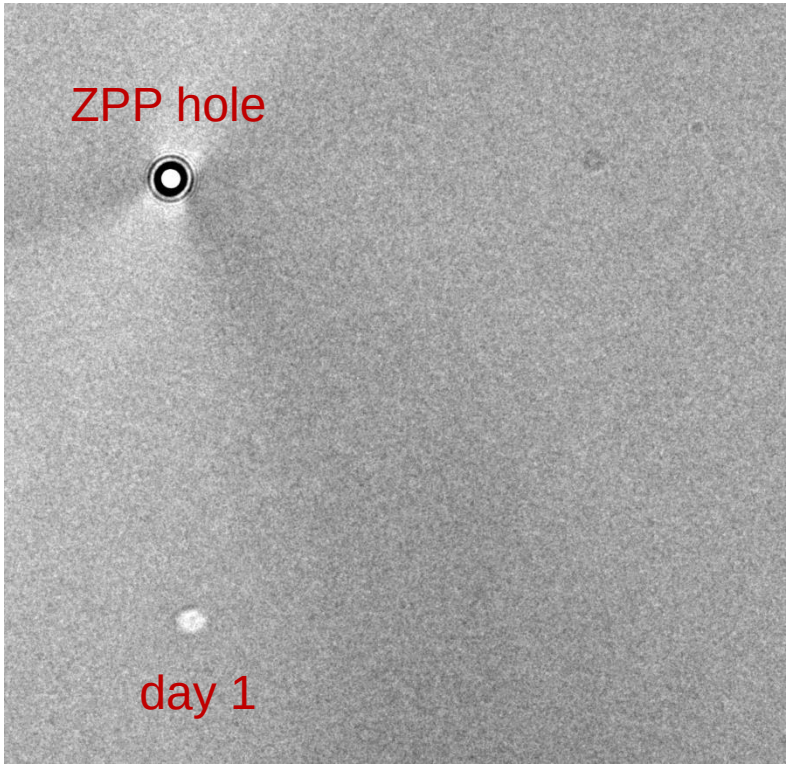
VPP – phase shift vs. beam current

- The phase shift depends on the total dose and not on the dose rate.

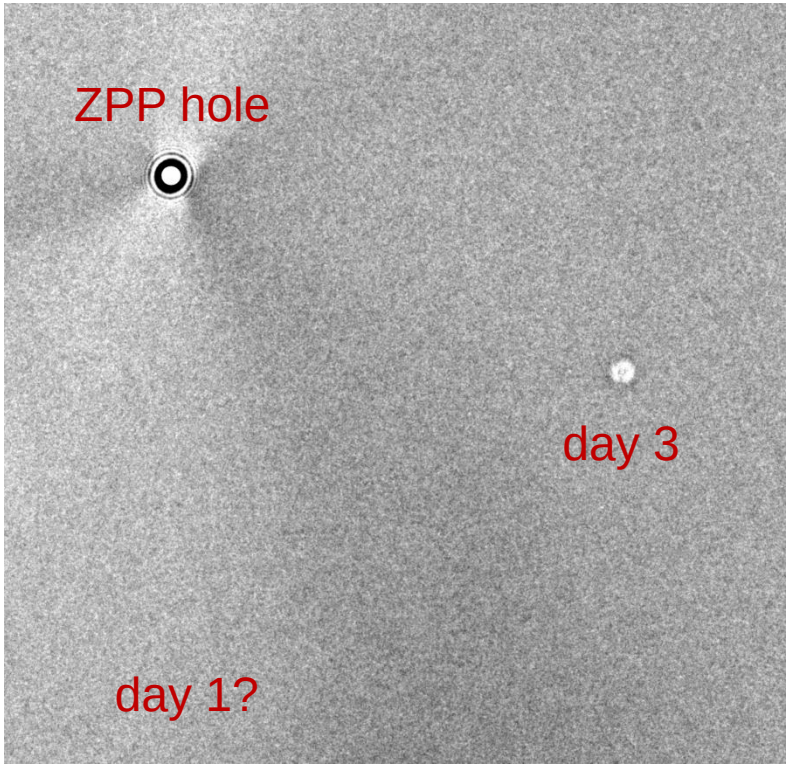


VPP beam spots

day 1

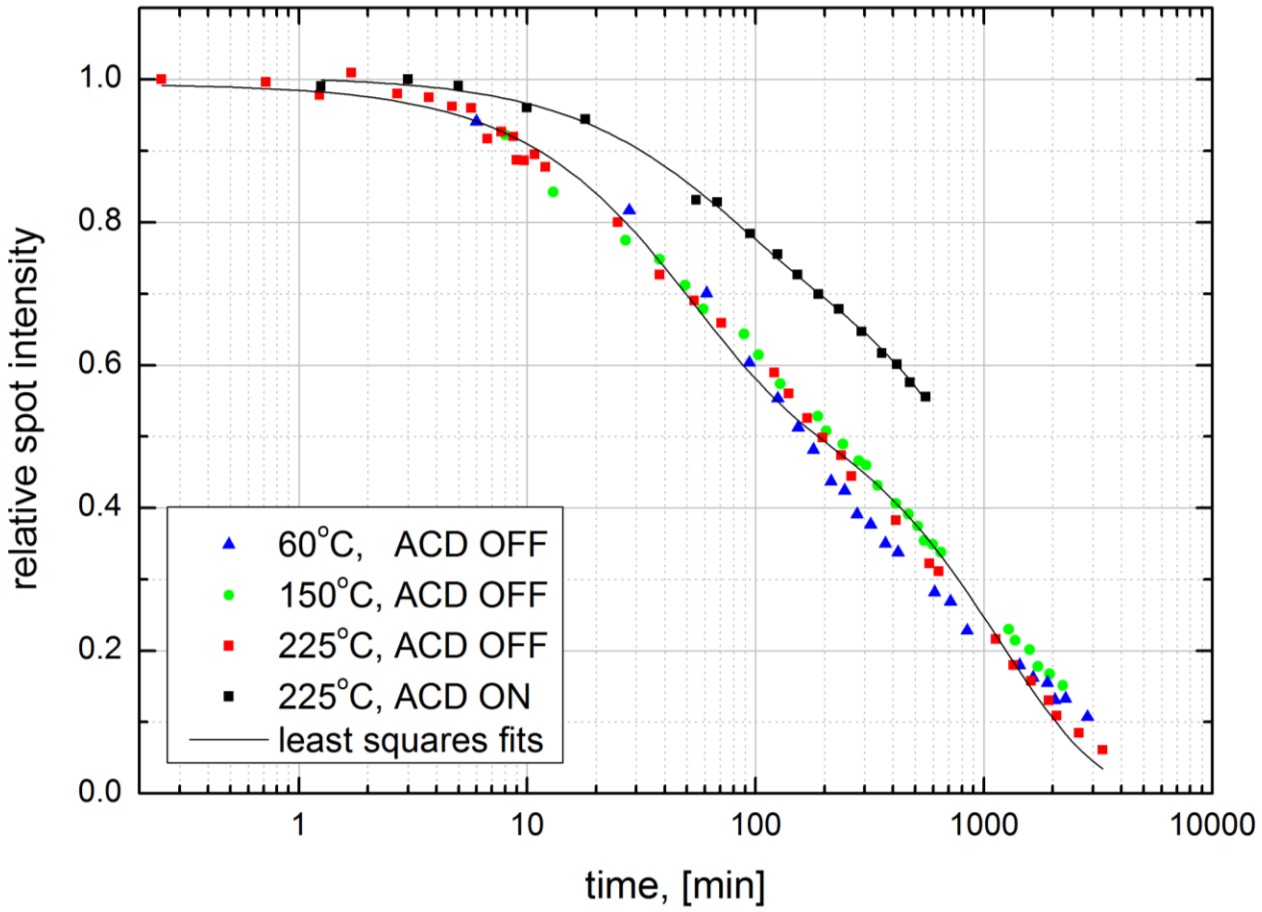


day 3,
day 1 spot has disappeared!

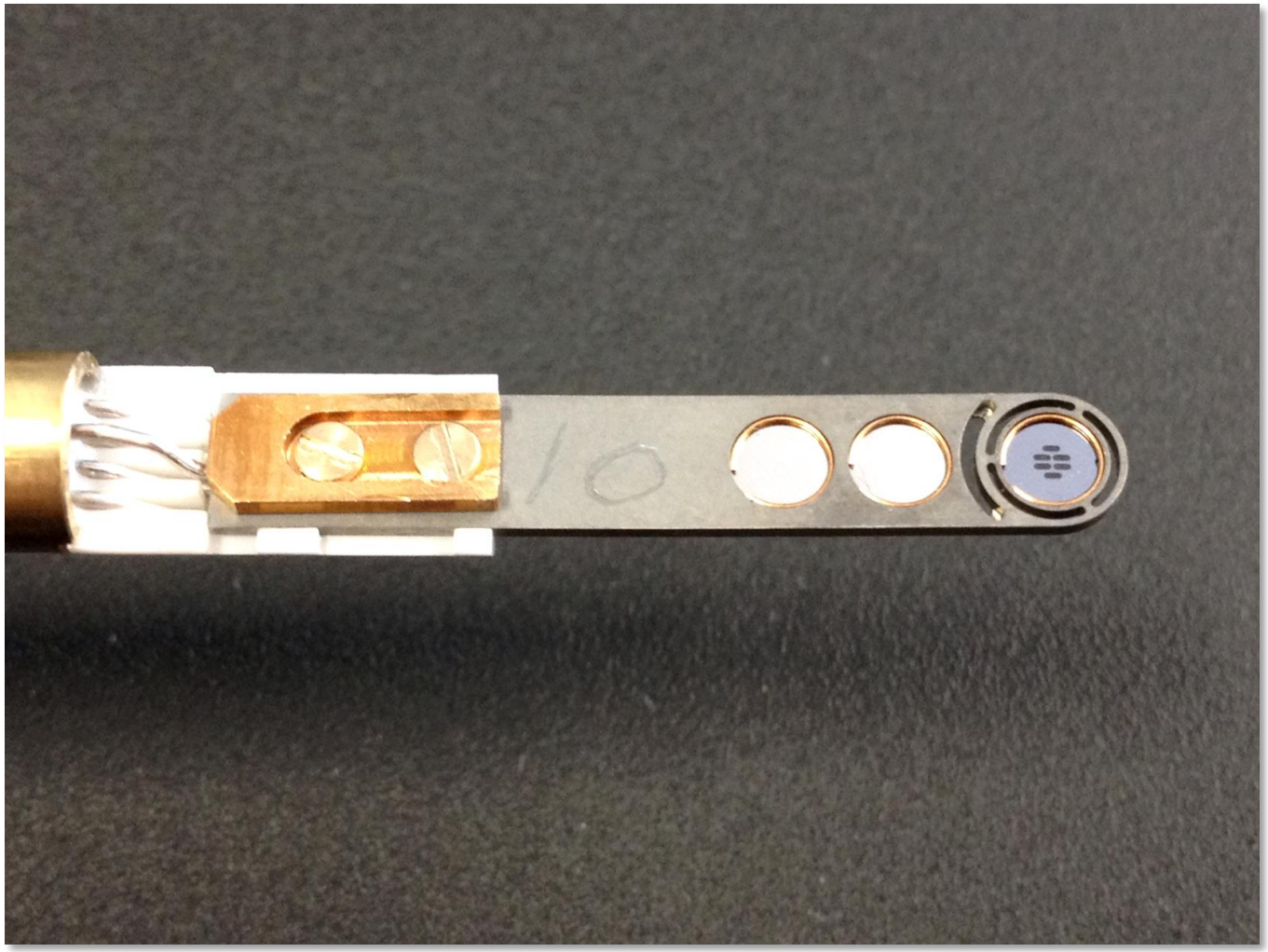


VPP beam spot recovery

- The recovery speed of beam spots does not depend on the temperature.

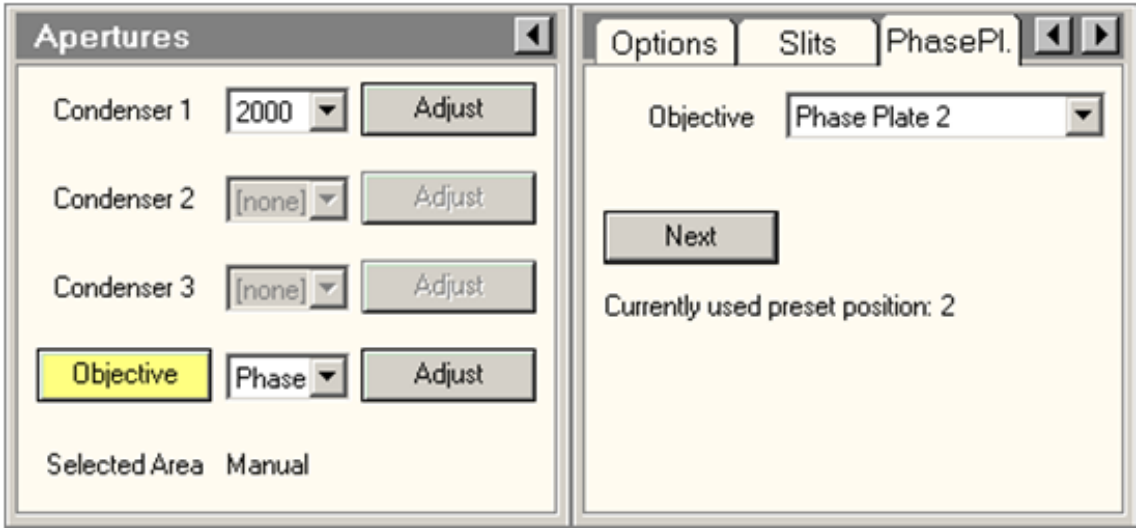


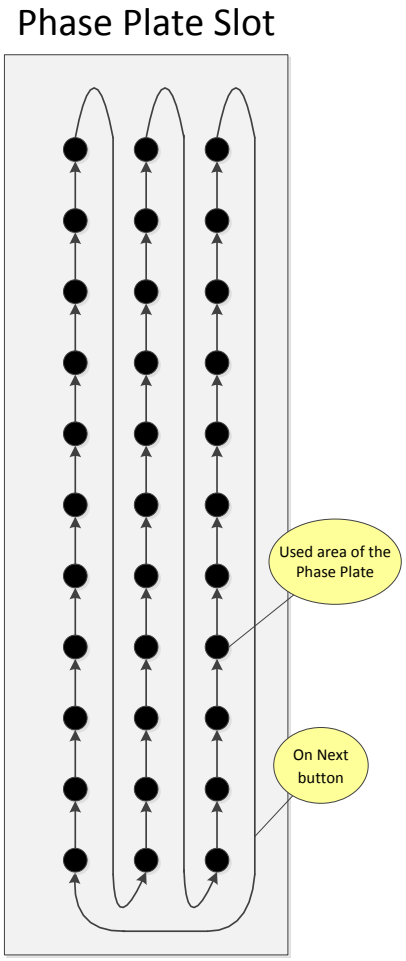
New PP holder and phase plate



FEI tools for phase plate navigation

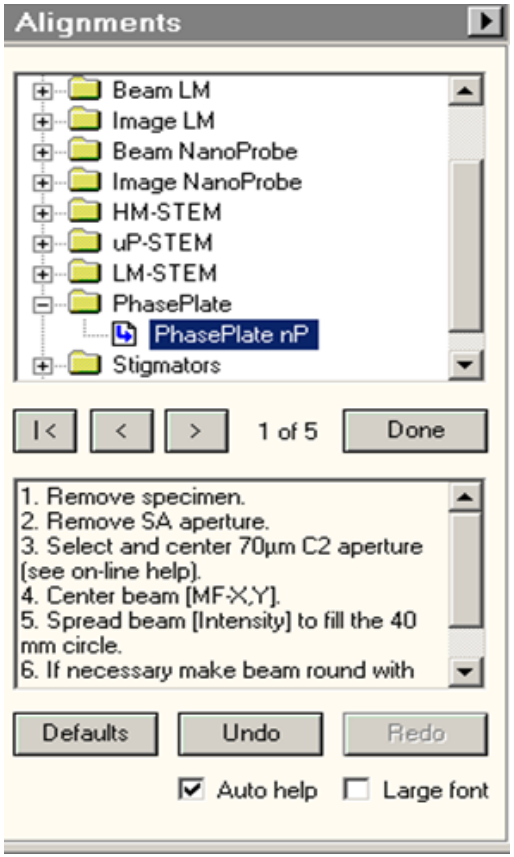
- 6 slots x 125 positions → 750 fresh areas
- Single area for ~1 hr operation

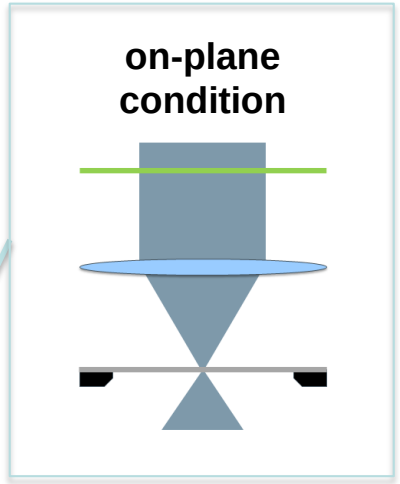


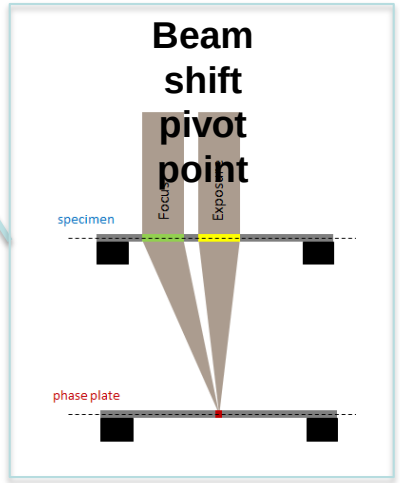


FEI tools for phase plate alignments

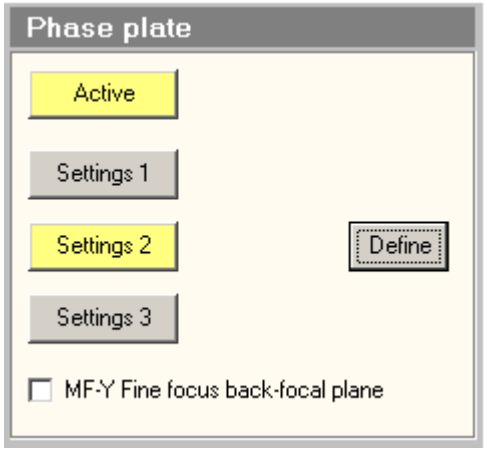
Alignments







routine use

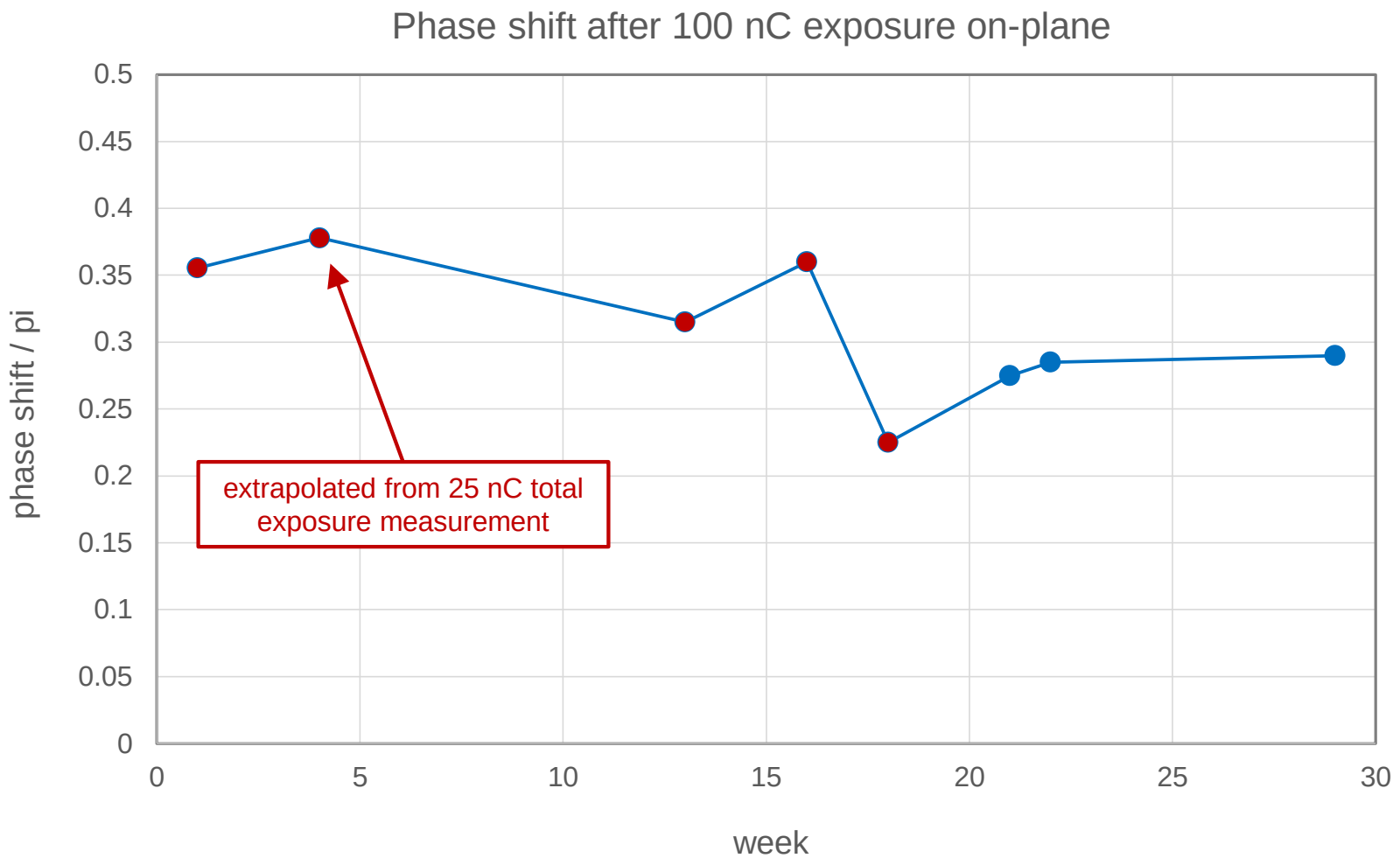


Phase plate lifetime tests

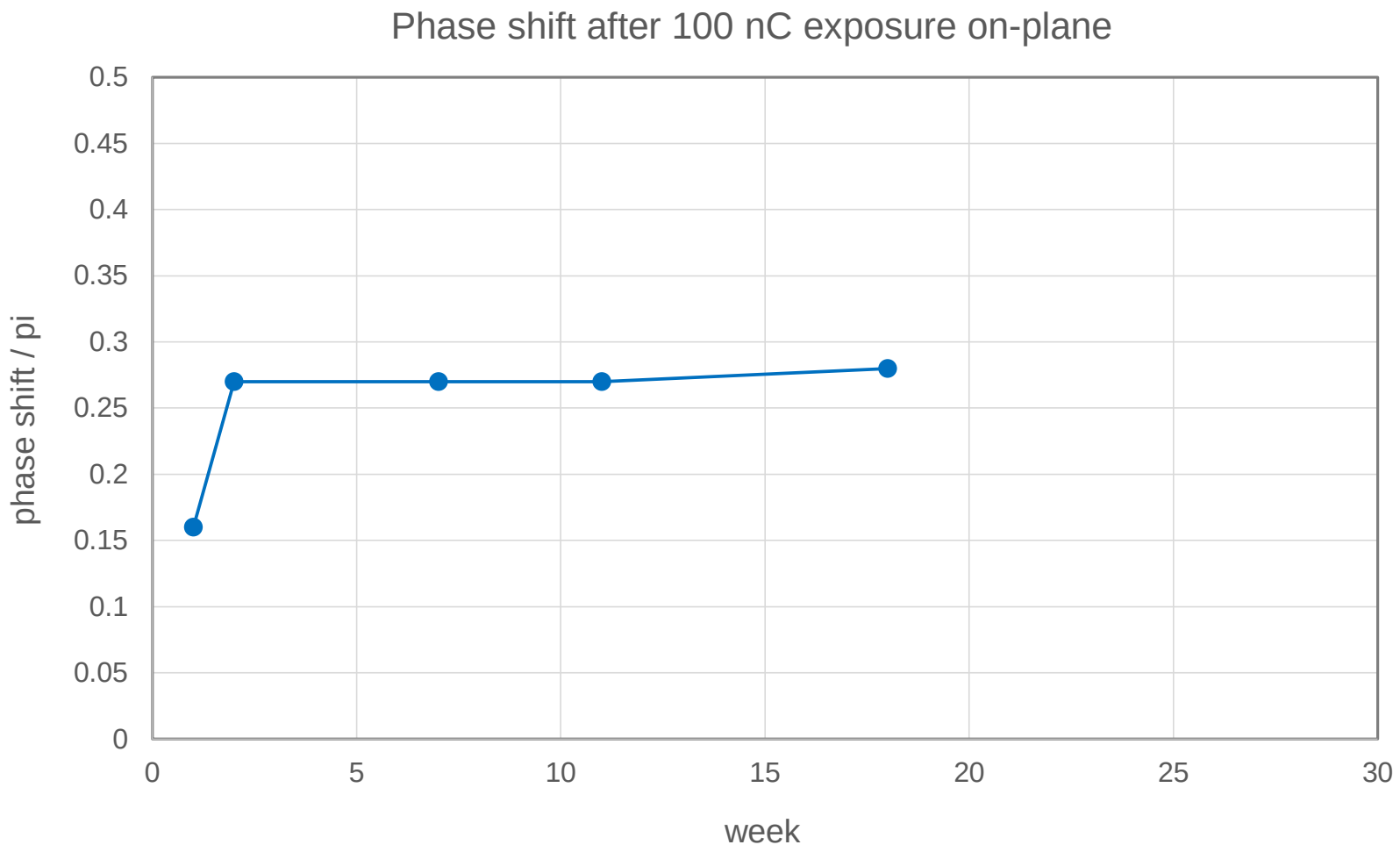
- The first phase plate of the new type installed in the Tecnai F20 was used for > 6 ½ months with no observable deterioration in performance.

- The first phase plate installed in the Titan Krios has been there for > 4 months with no performance degradation.

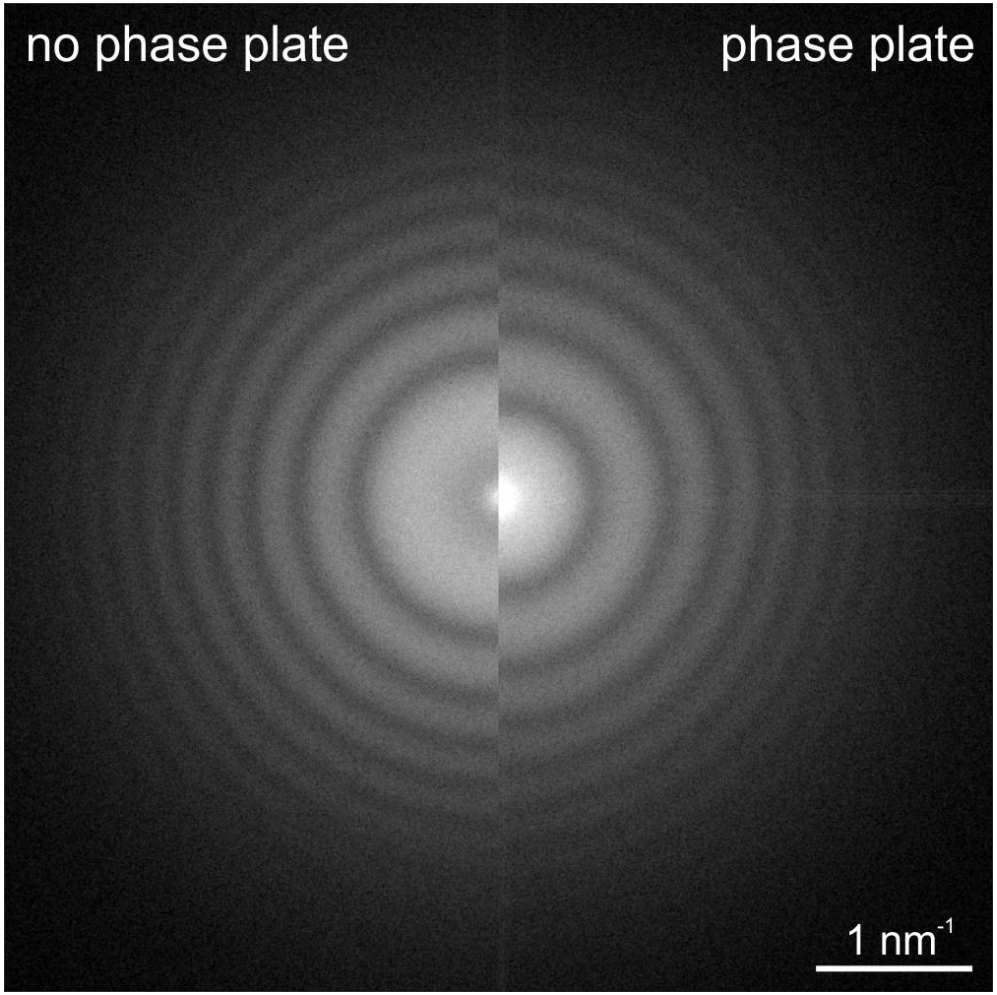
Phase plate lifetime – grid 121, Tecnai F20



Phase plate lifetime – PP1, Titan Krios

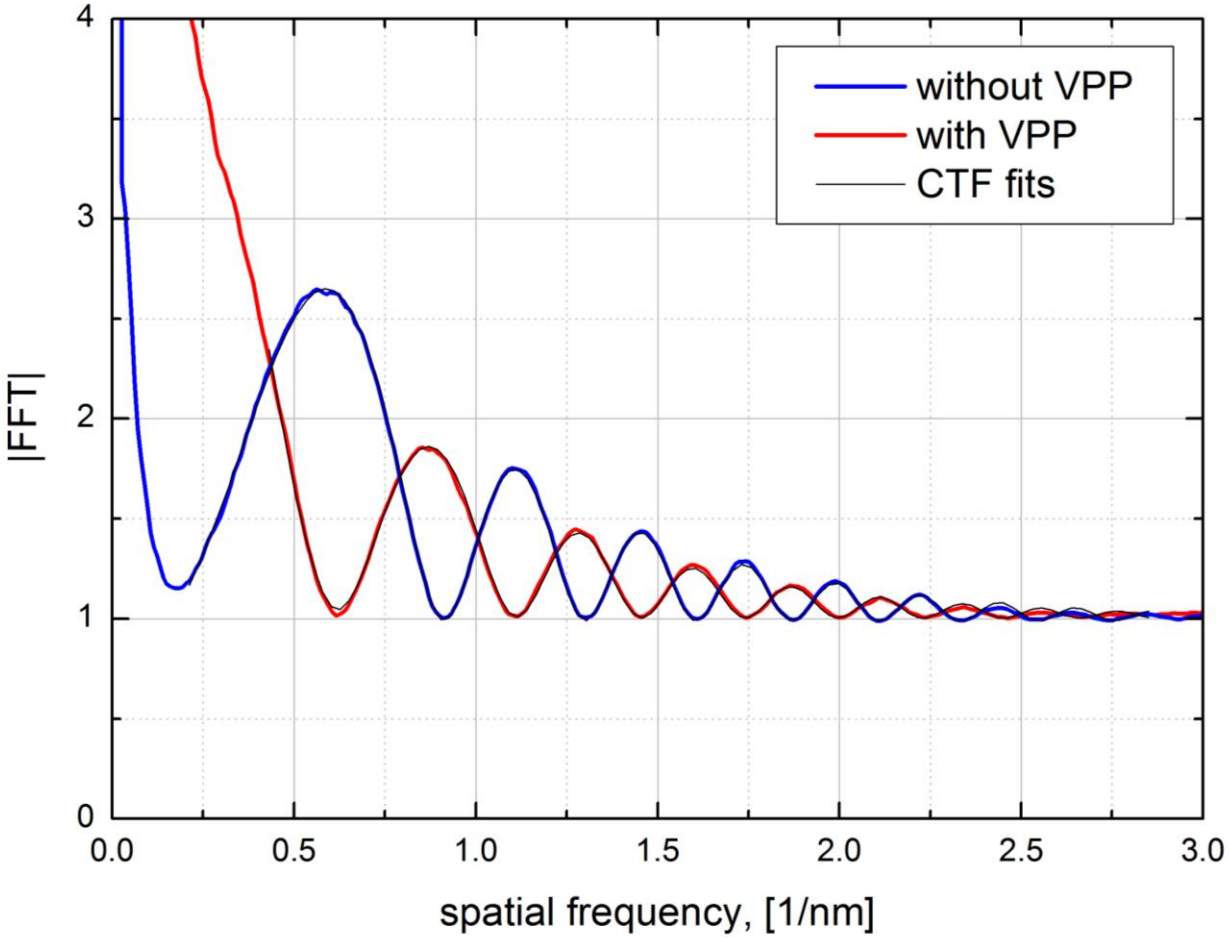


VPP CTF performance



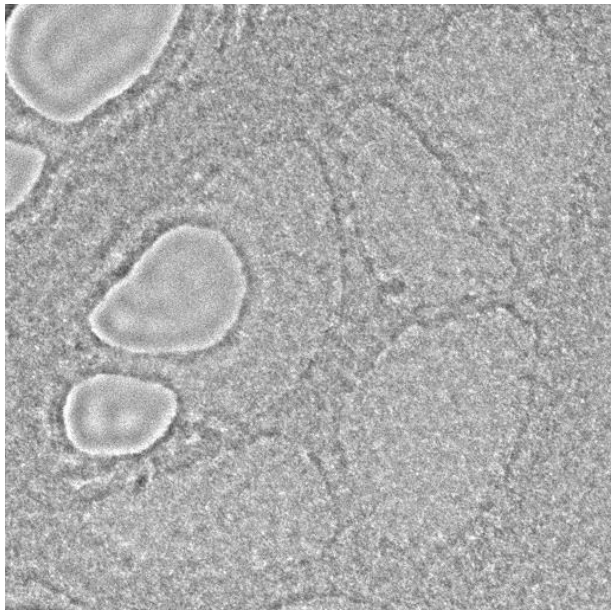
VPP CTF performance

- 500 nm defocus; ~ 18% signal loss, expected ~ 12%

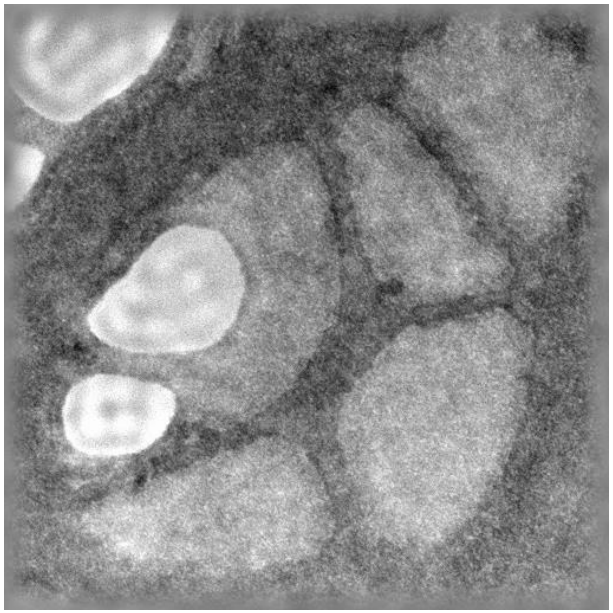


ZPP vs VPP images

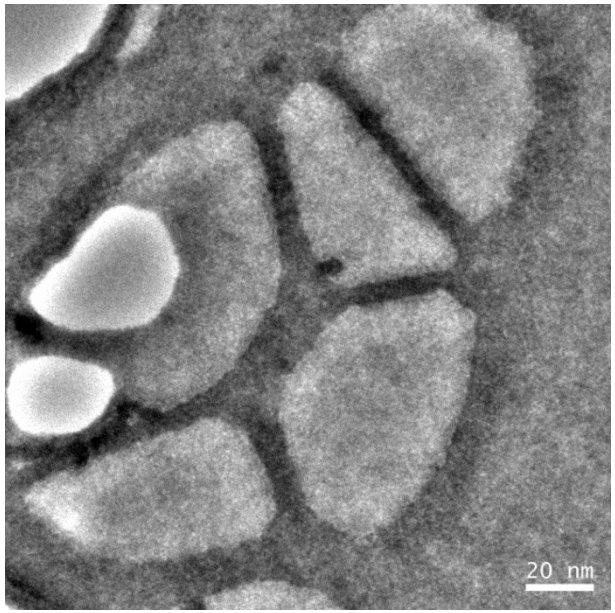
ZPP-TEM



de-fringed ZPP-TEM



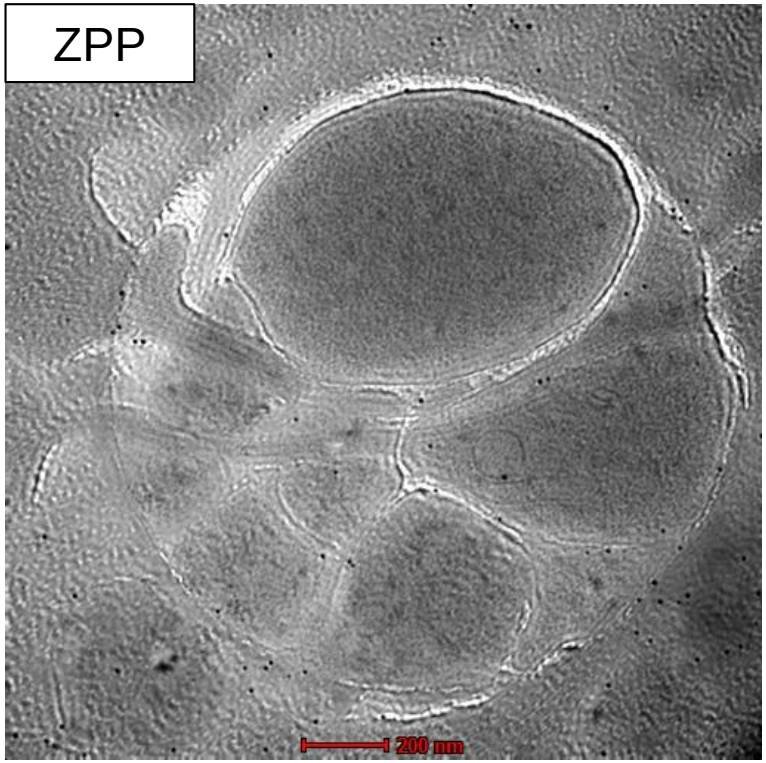
VPP-TEM



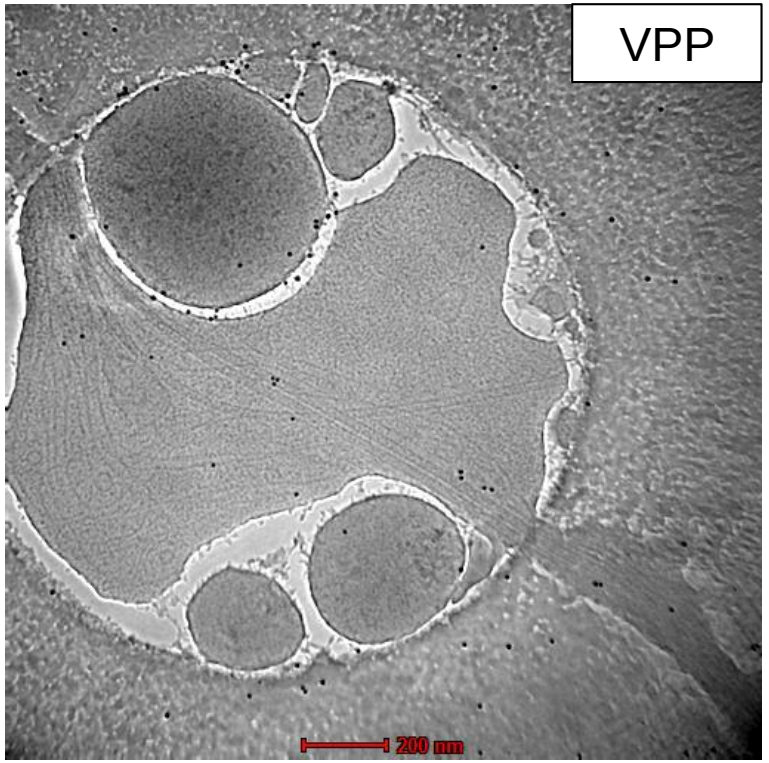
Cryo-EM, ZPP vs VPP

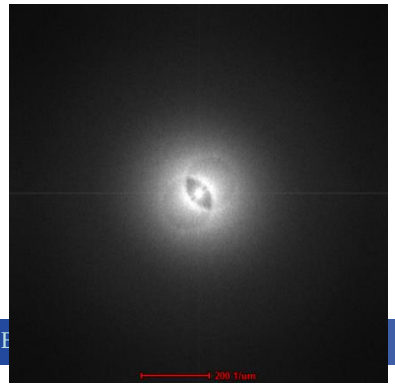
- Sample & data collection by Fuku; primary neuron culture.

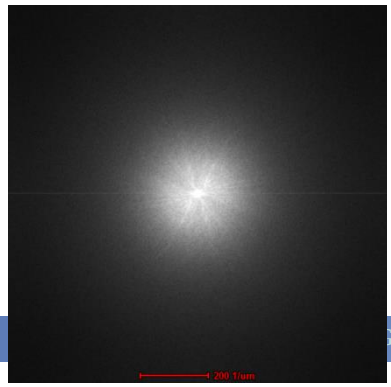
ZPP



VPP







VPP vs. ZPP

VPP	ZPP
long life	constant phase shift
less information loss?!?	
no fringes!	
user friendly	
easy to automate	

Important practical points for VPP use

- **Beam shift pivot points** - the beam must pass through the same spot on the phase plate in both Focus and Exposure low-dose modes. This allows the phase plate to be "renewed" at any time by moving to another place on the PP film followed by "conditioning" (continuous irradiation for ~ 30 sec) in Focus mode.

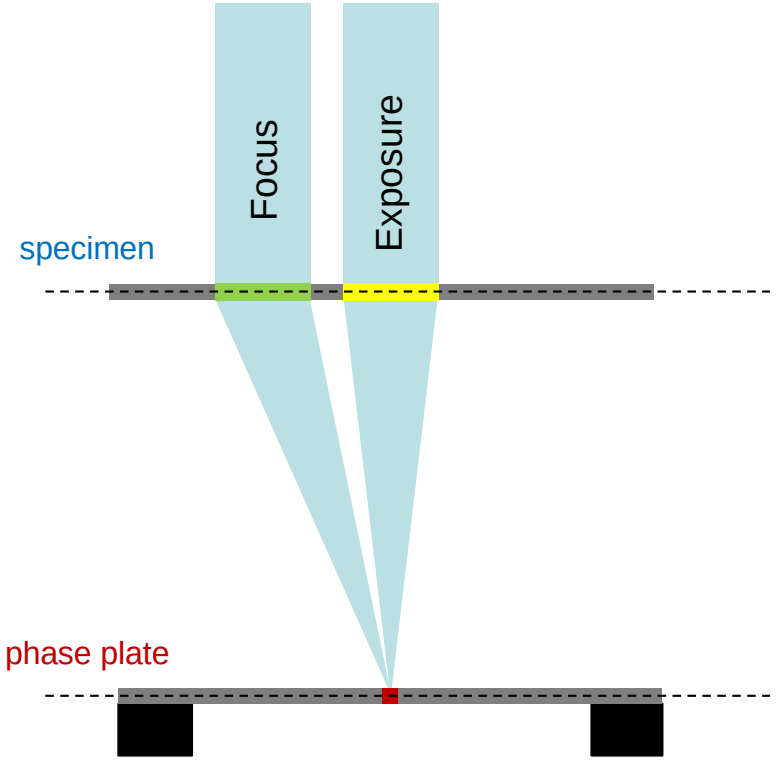
- **On-plane condition** – best contrast (lowest cuton); ~ 0.25 π phase shift; no PP film grain projected on the image (a la Ronchigram).

- **Defocus** – for tomography work VPP requires some defocus for best transfer of the high spatial frequencies. The defocus is set so that the conventional (no phase shift) CTF has its first maximum at ~ 2/3 Nyquist. Typical defocus value: ~ 500 nm underfocus.

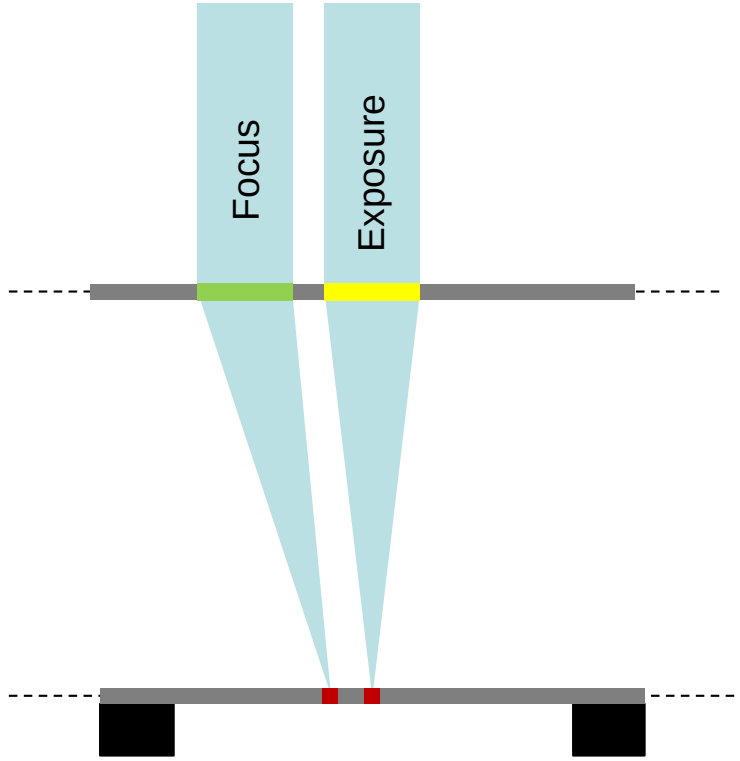
- **Heating** – to ~ 300 ^{o}C is essential for maintaining the phase plate in "Volta mode". It prevents contamination and accelerates the "healing" (~ 1 – 2 days) of the used spots on the PP.

Beam shift pivot points

Correct pivot point setting



Incorrect pivot point setting

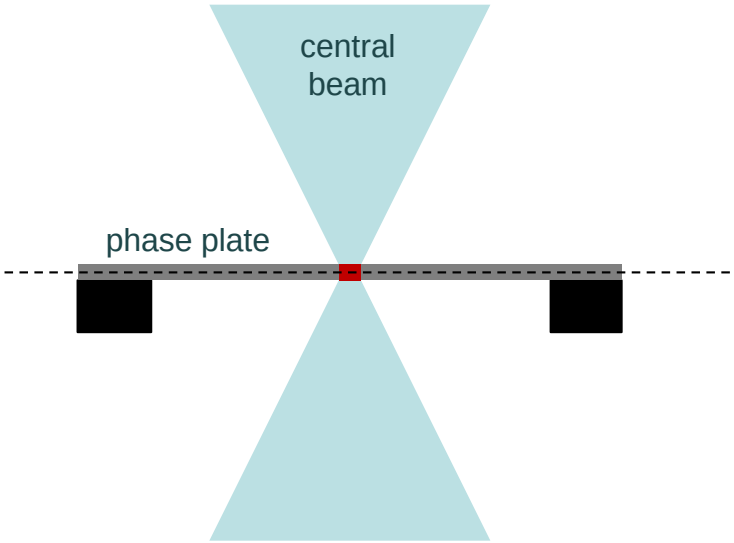


PP on-plane & off-plane

Volta Phase Plate

on-plane

(parallel illumination)

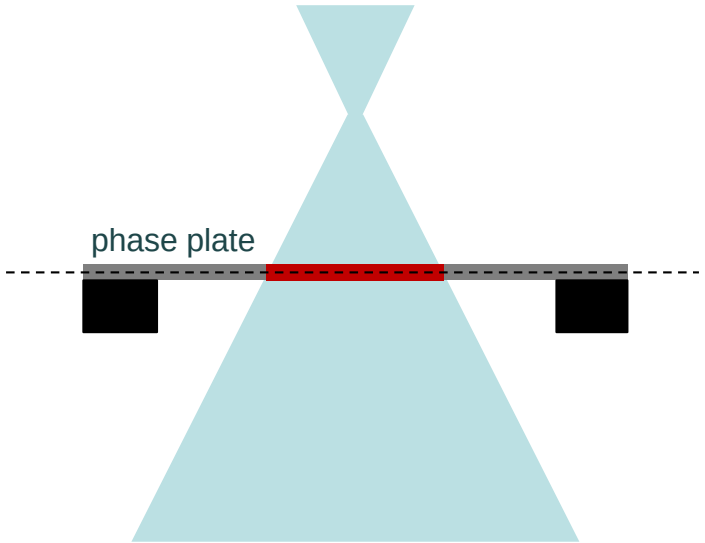


back focal plane

Volta Phase Plate

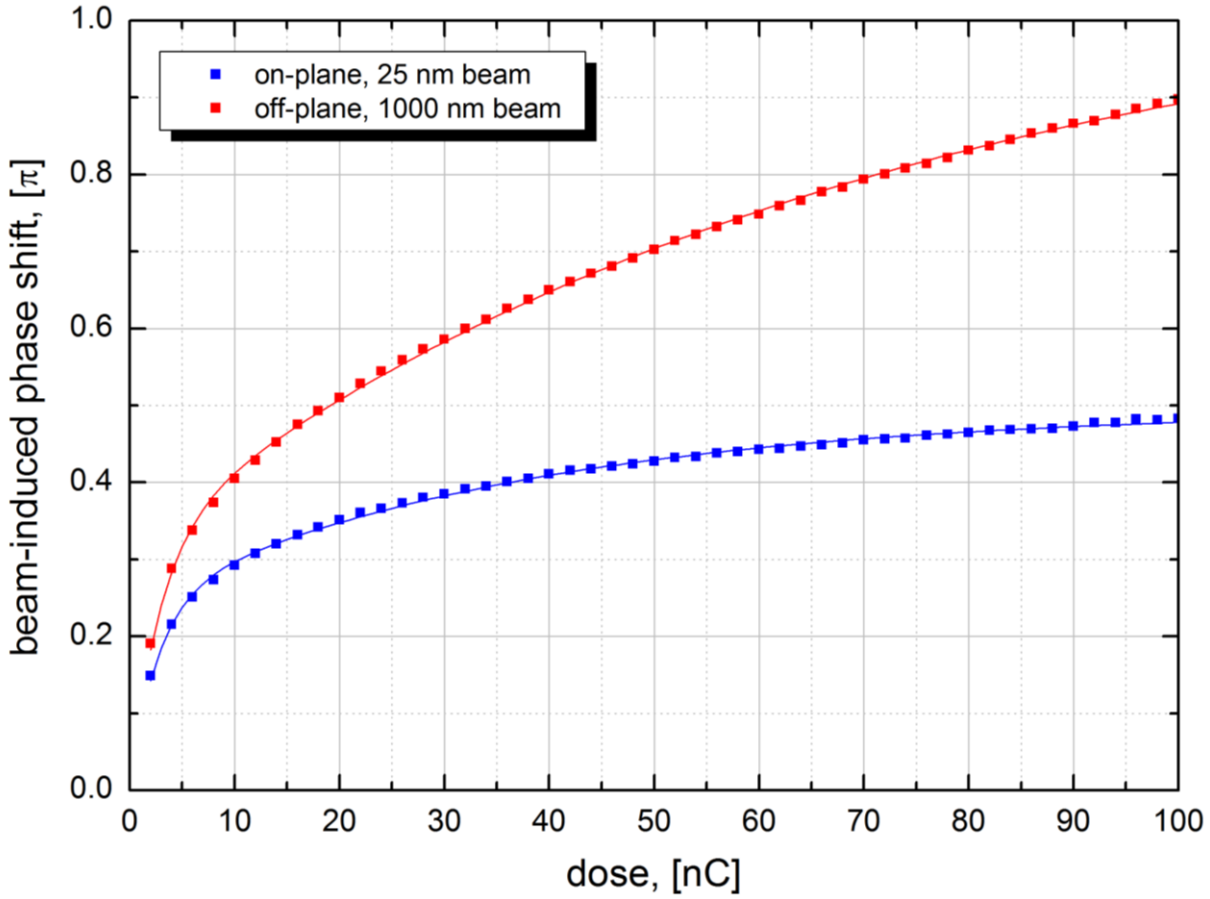
off-plane

(non-parallel illumination)



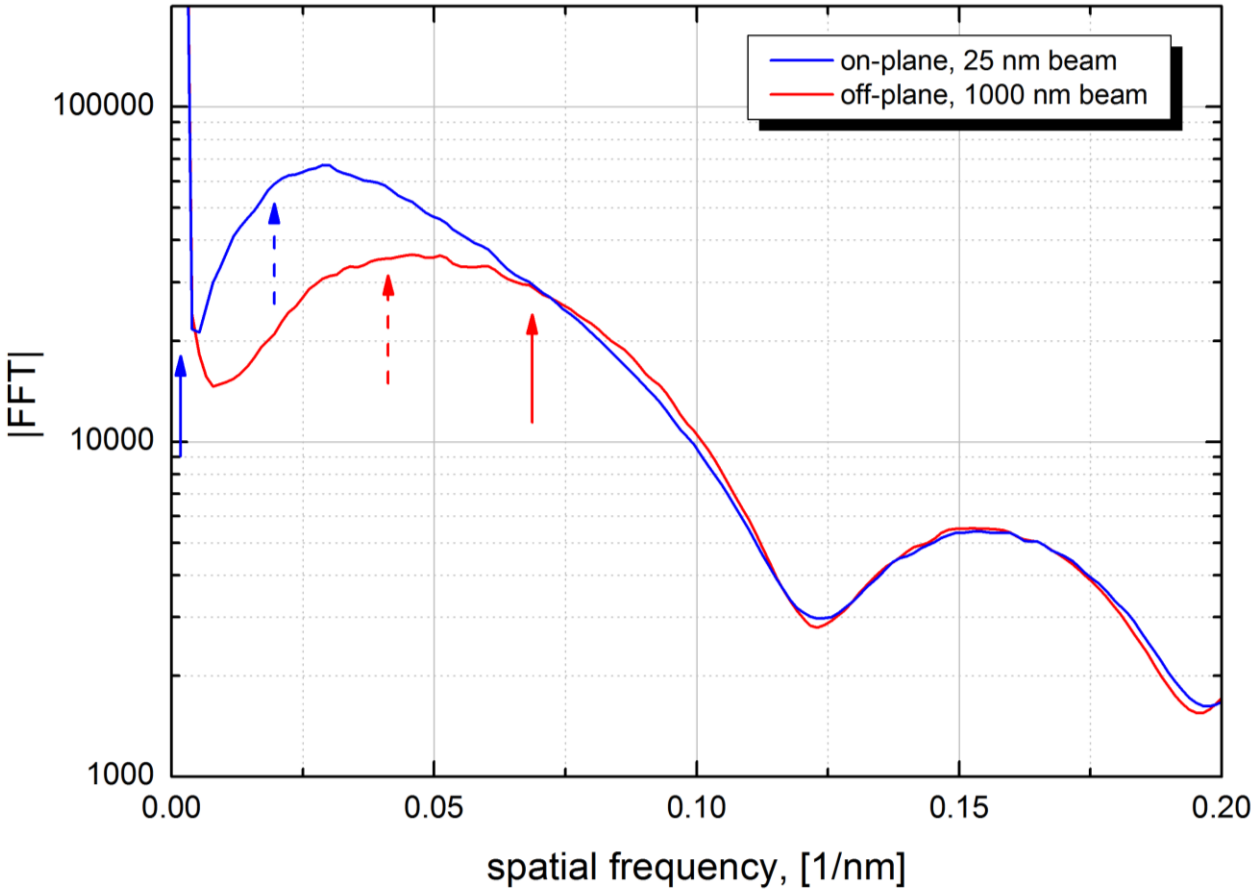
VPP – on-plane vs. off-plane

- beam current: 1.0 nA;



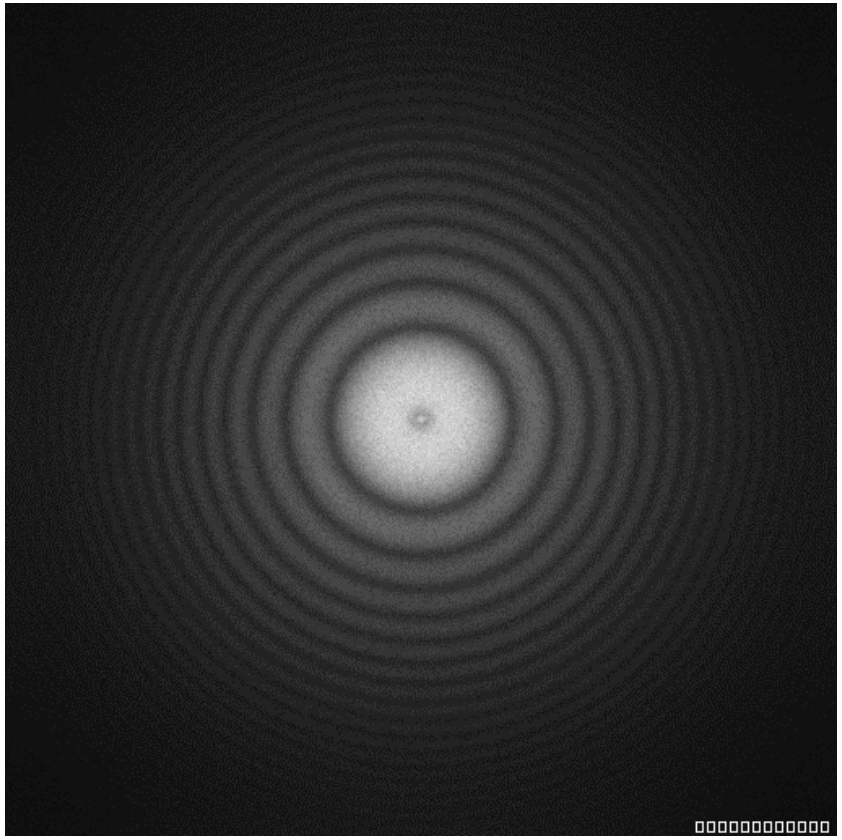
VPP – on-plane vs. off-plane

- CTFs on-plane & off-plane; 50 nm cut-on periodicity on-plane.

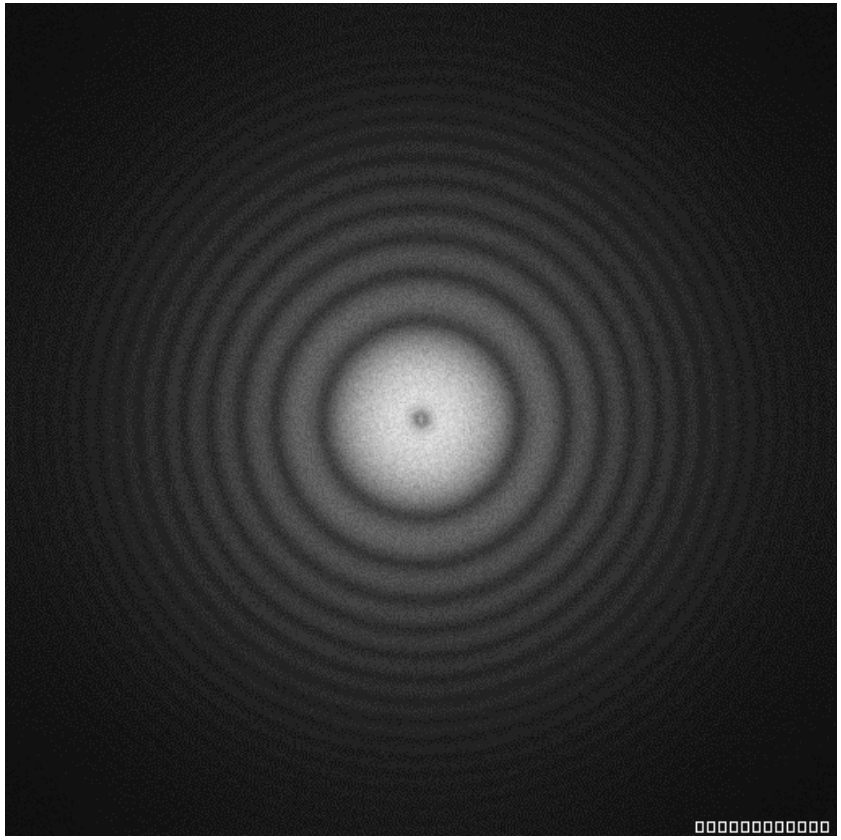


VPP – heating is essential

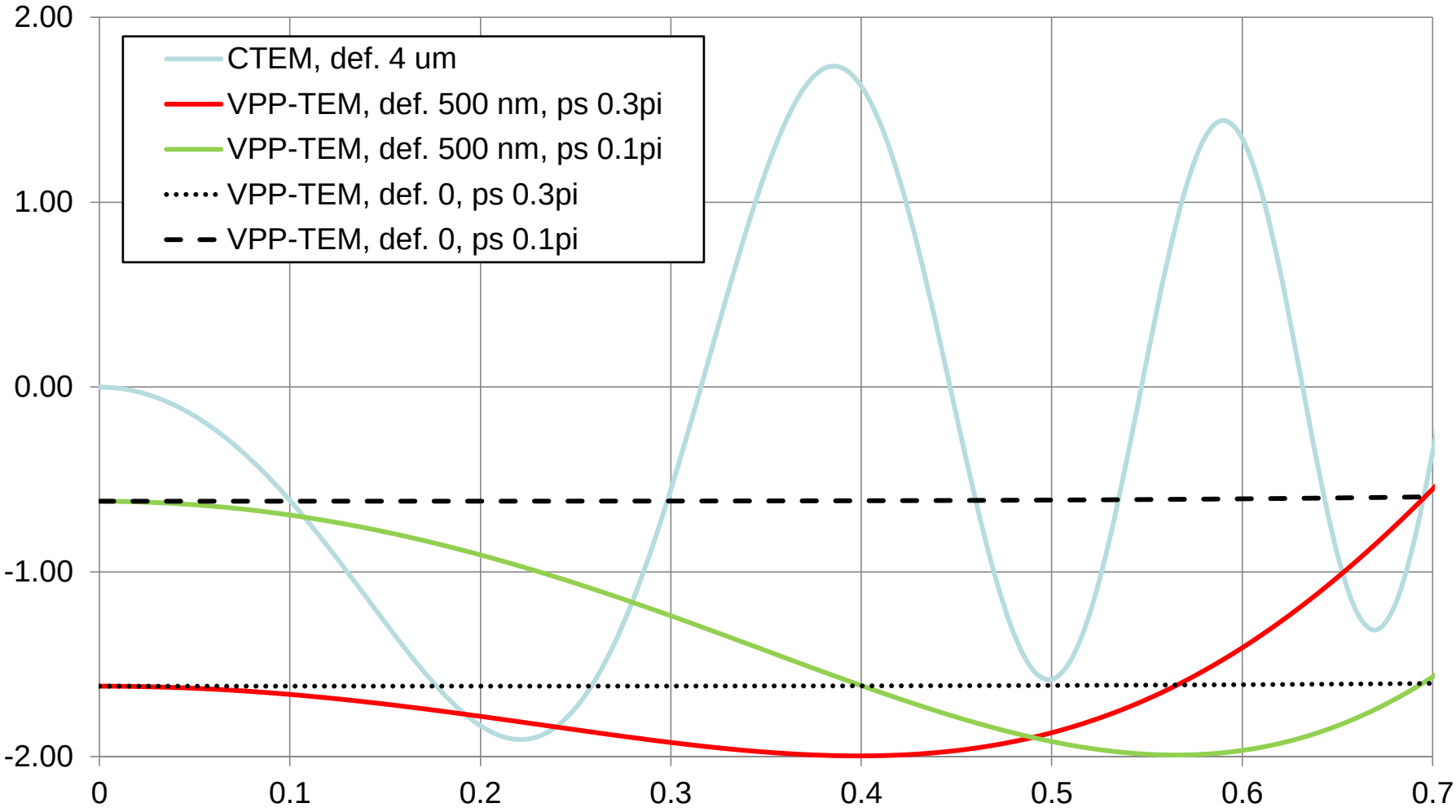
phase plate at 225°C:
Volta regime



phase plate at 60°C:
Contamination & charging



VPP for tomography - CTFs



Phase plates for Single Particle Analysis – ideal world

- Automatically collect images in-focus with the phase plate.
- Easy automatic picking of the particles.
- No CTF fitting or correction.
- Reconstruct the data and get 3 A resolution with 10,000 particles.
- Press the “Publish” button and order a Mai Tai on Waikiki beach …

The VPP for Single Particle Analysis

+ Works well with the FEI EPU software.

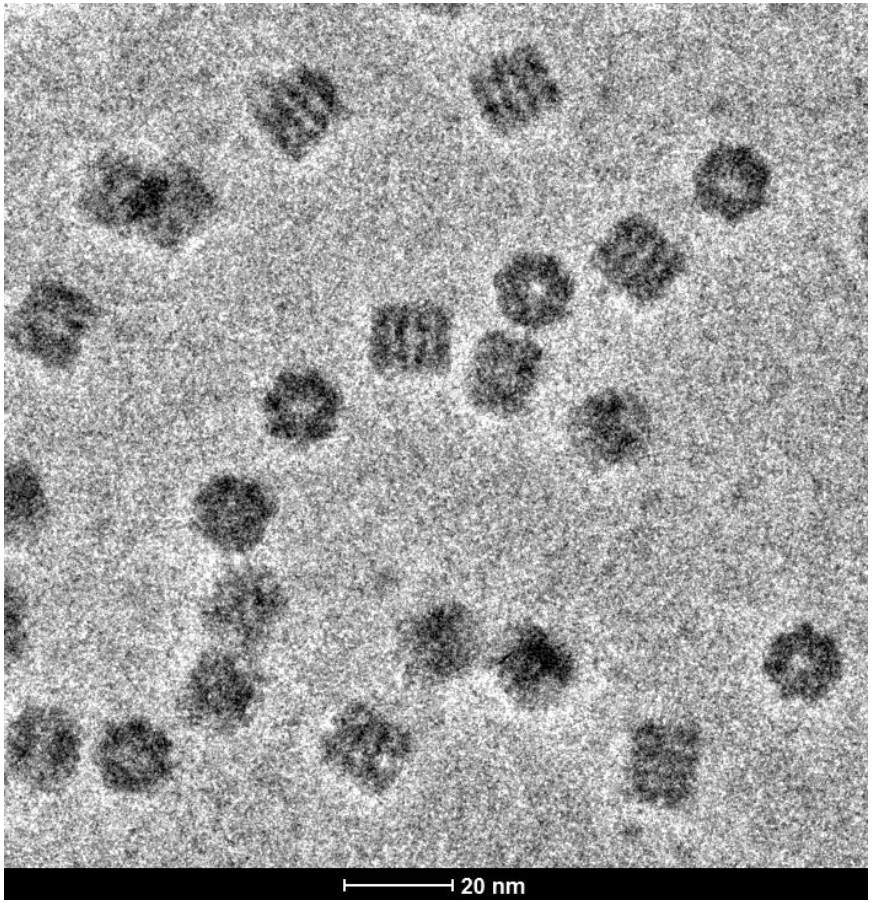
+ A couple of additional steps before starting the automated acquisition – insert a phase plate, wait ~ 5 min for the thermal drift of the PP to settle and adjust the on-plane condition.

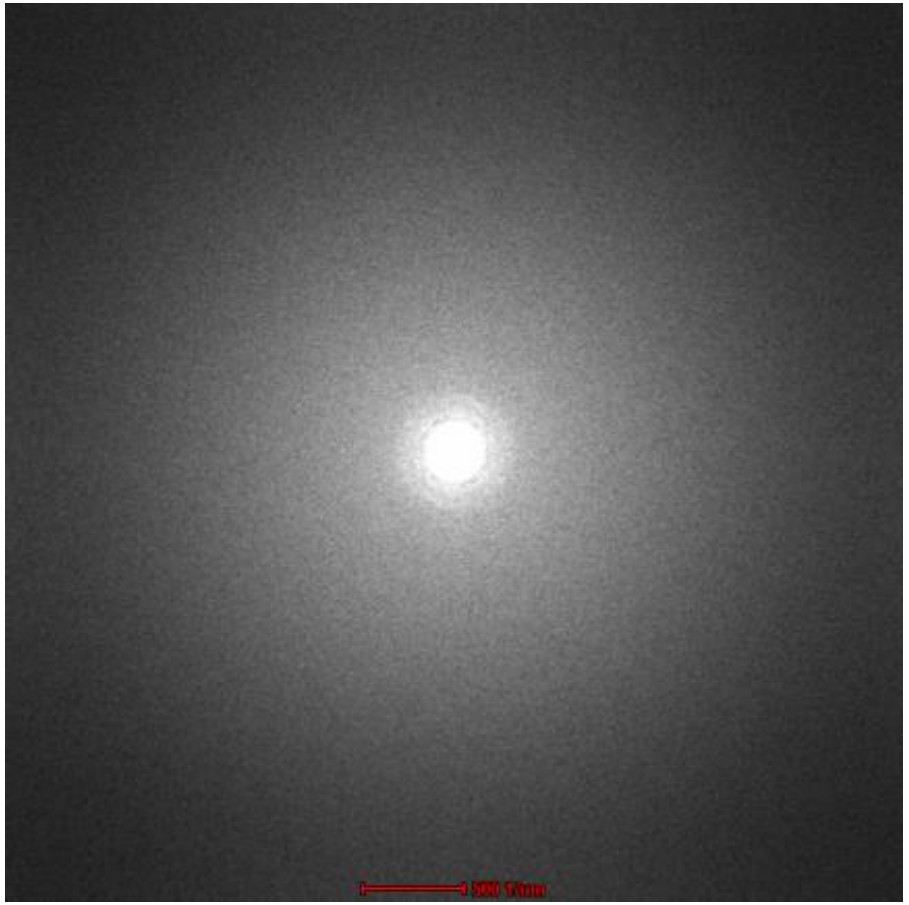
- For best results requires a switch of the phase plate every ~ 100-200 images. This will be automated in next versions of EPU.

- At present, CTF fitting and sign-flipping is necessary due to low focusing accuracy.

SPA of GroEL with the VPP, Tecnai F20

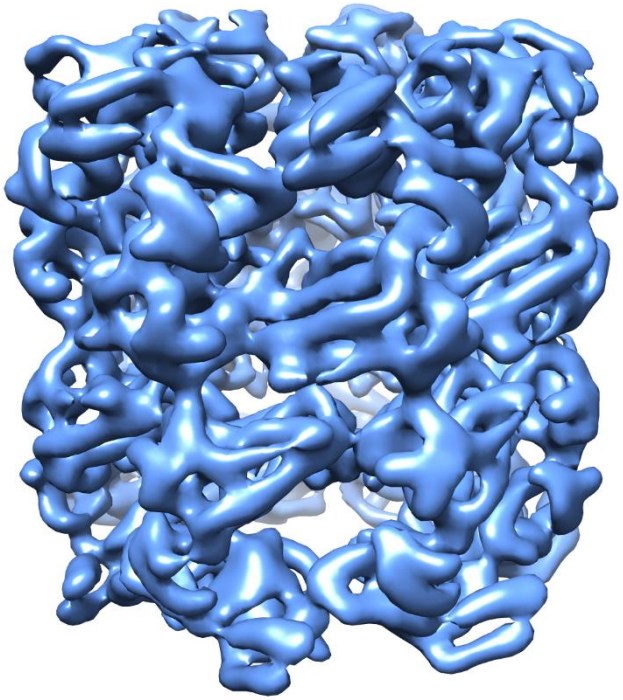
- Sample: GroEL; provided by Thomas; ~ 800 kDa; manual data collection.

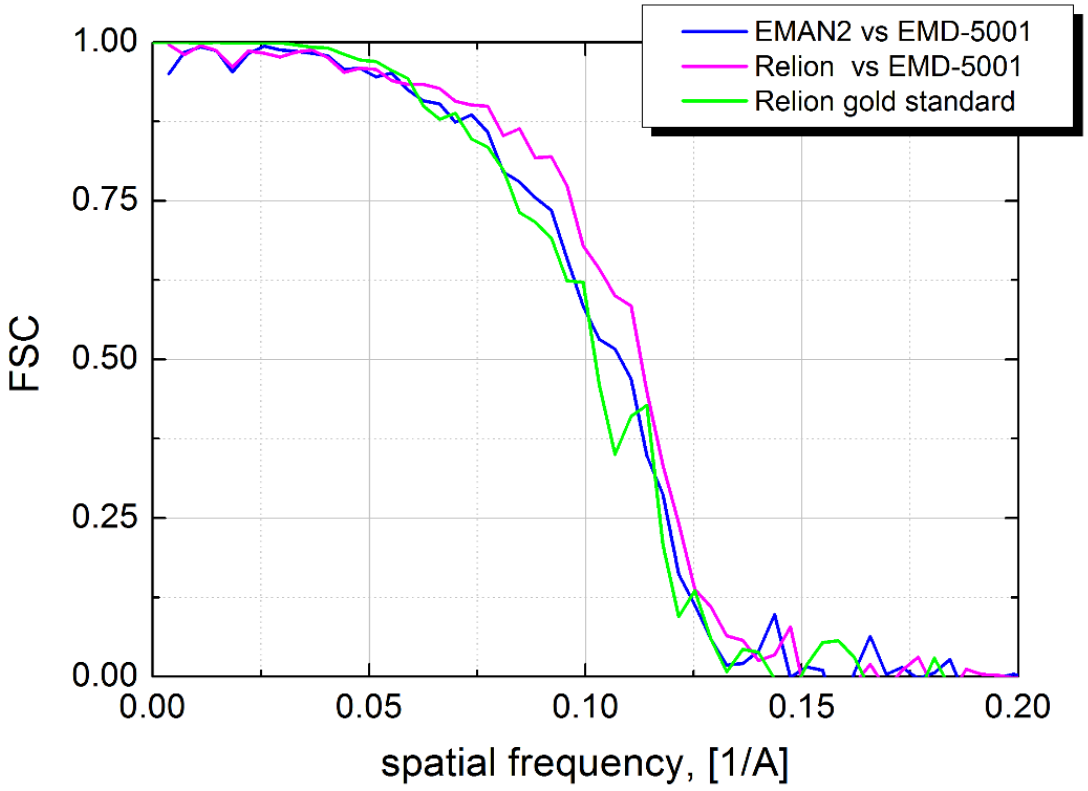




Reconstructions by EMAN and RELION

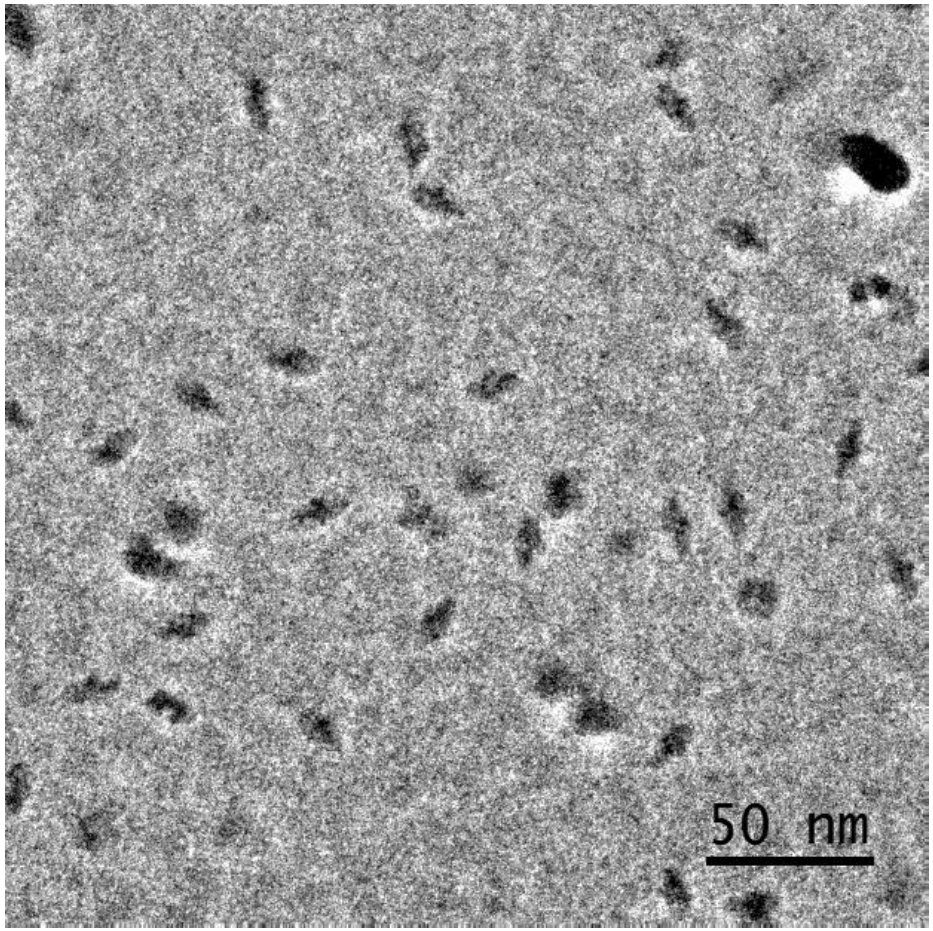
- SPA reconstruction: 50 micrographs; 3081 particles; D7 symmetry; resolution ~ 8.3 A.
- **No CTF correction**

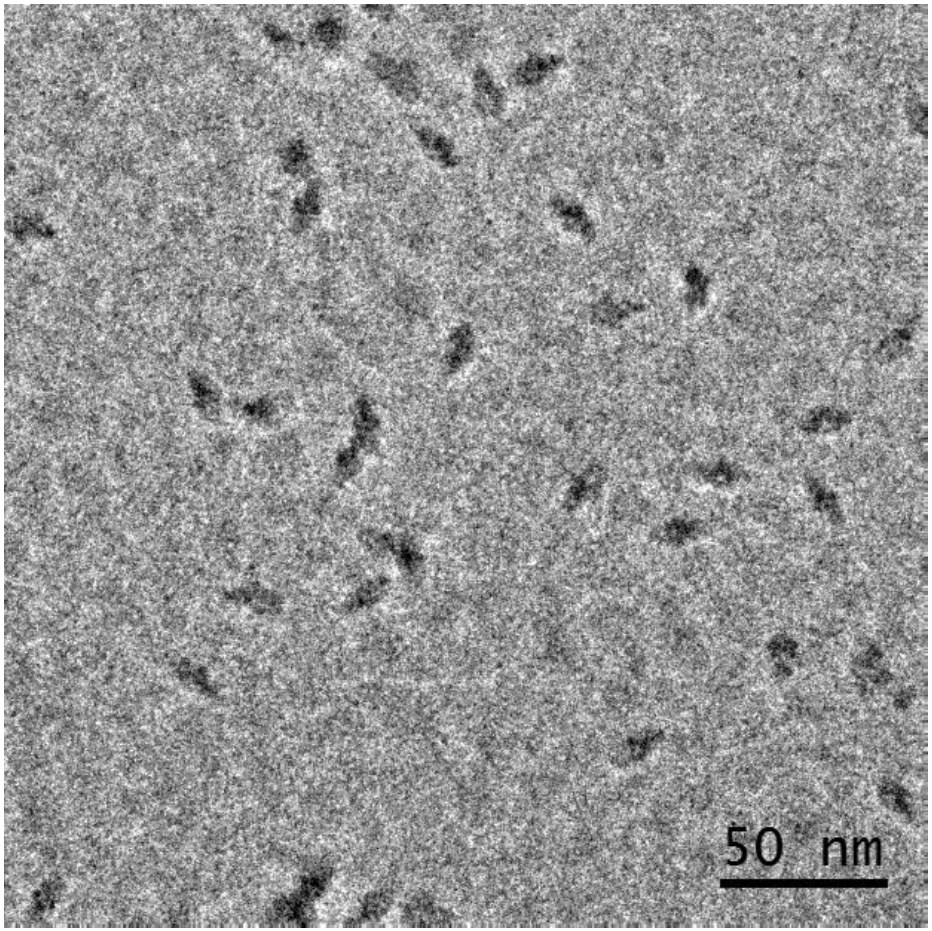




SPA of β-galactosidase with the VPP, Tecnai F20

- Sample: purchased from Sigma; ~ 450 kDa

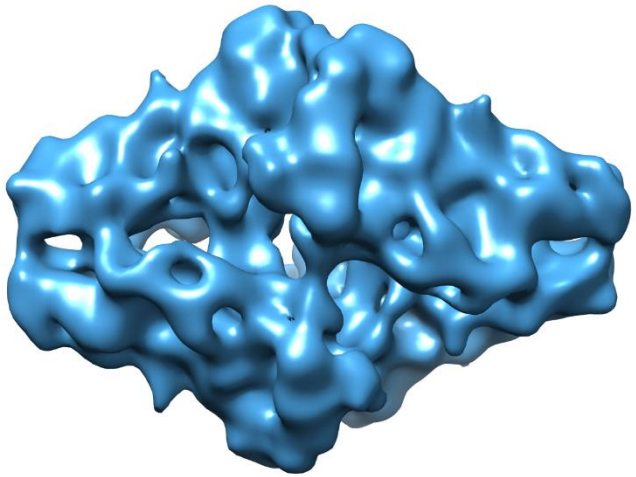


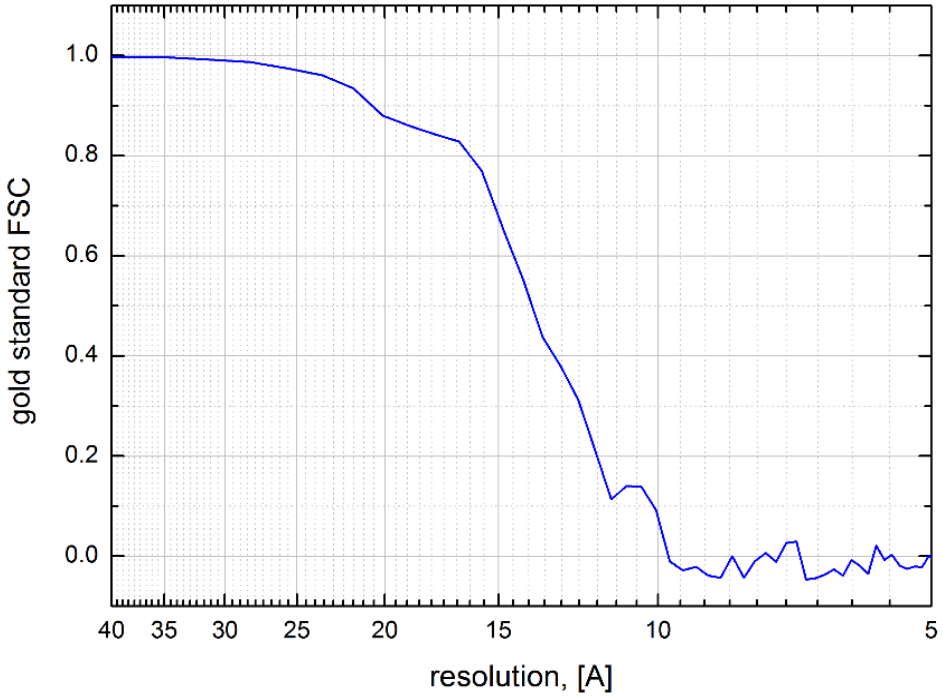


SPA of β-galactosidase with the VPP, Tecnai F20

- Sample: purchased from Sigma; ~ 450 kDa

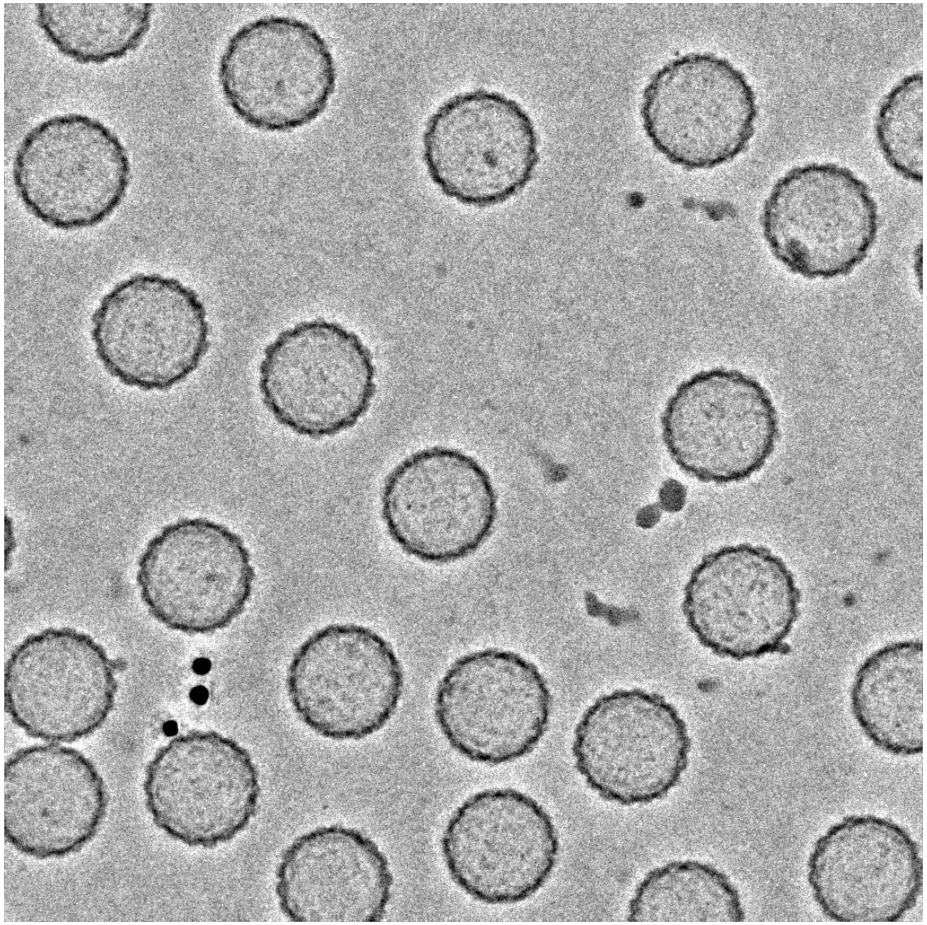
- SPA reconstruction: Eagle CCD camera, EPU data collection, 484 micrographs; 6221 good particles; D2 symmetry; resolution ~ 12A
- **CTF corrected**

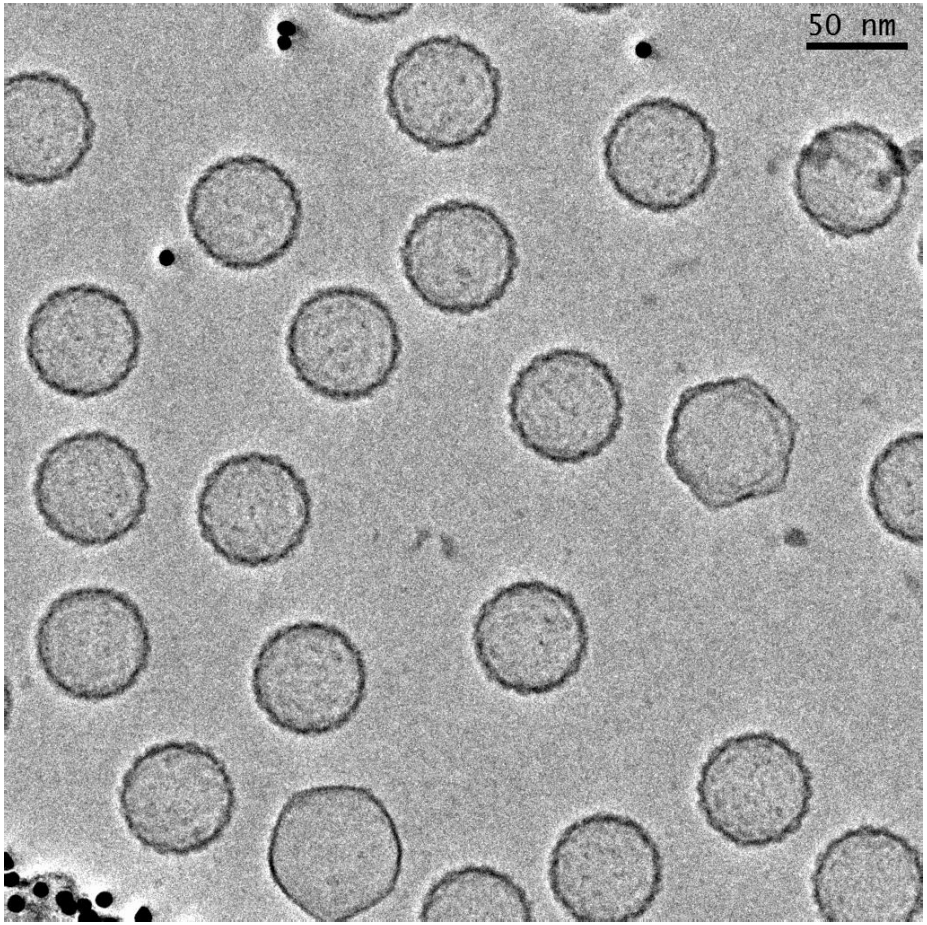




D3 virus, Tecnai F20

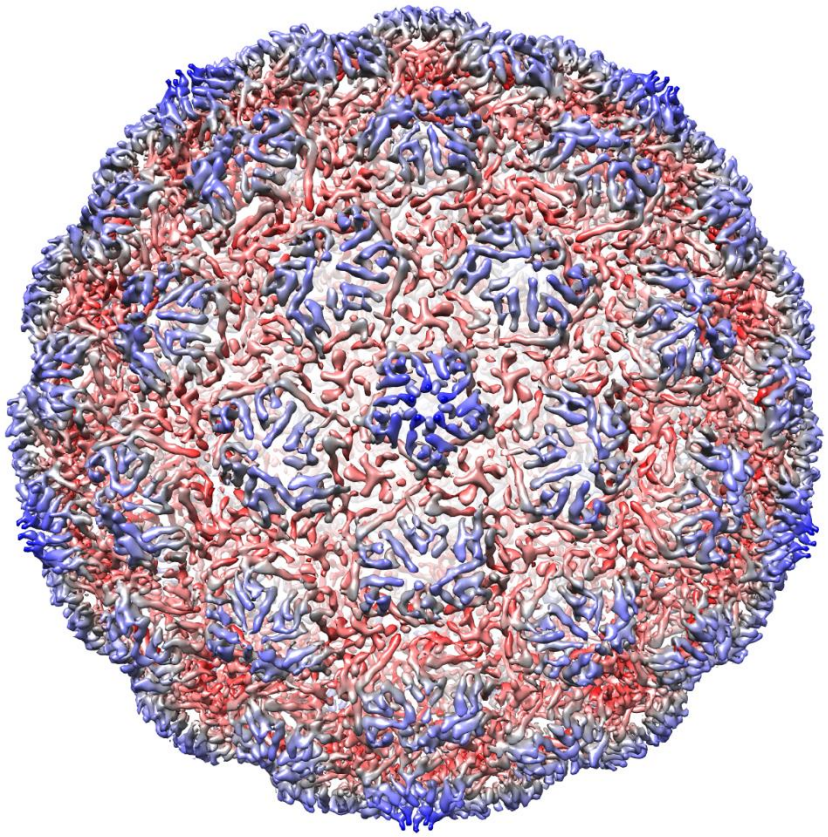
- Sample: D3 virus capsid; provided by James Conway through Matthijn Vos

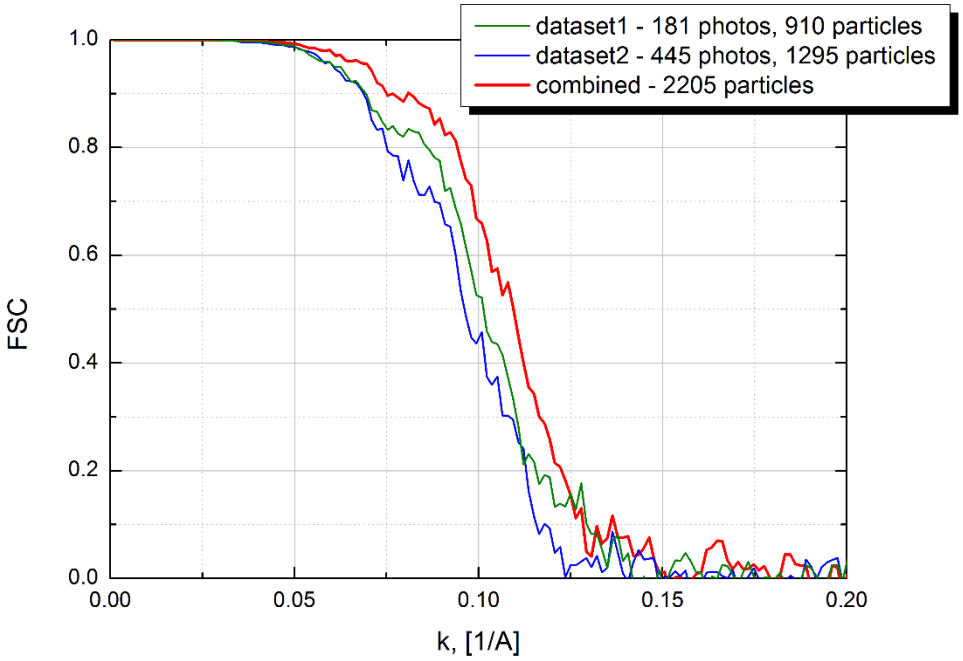




D3 virus, Tecnai F20

- Sample: D3 virus capsid; provided by James Conway through Matthijn Vos
- SPA reconstruction: 626 micrographs; 2205 particles; I1 symmetry; resolution ~ 8.1 A
- **CTF corrected**

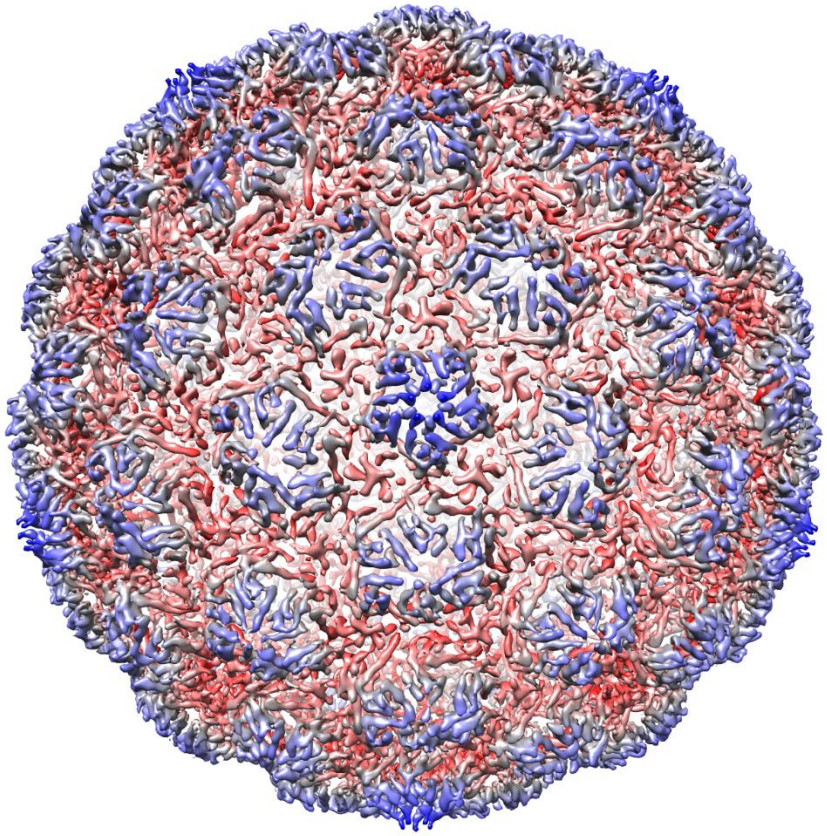




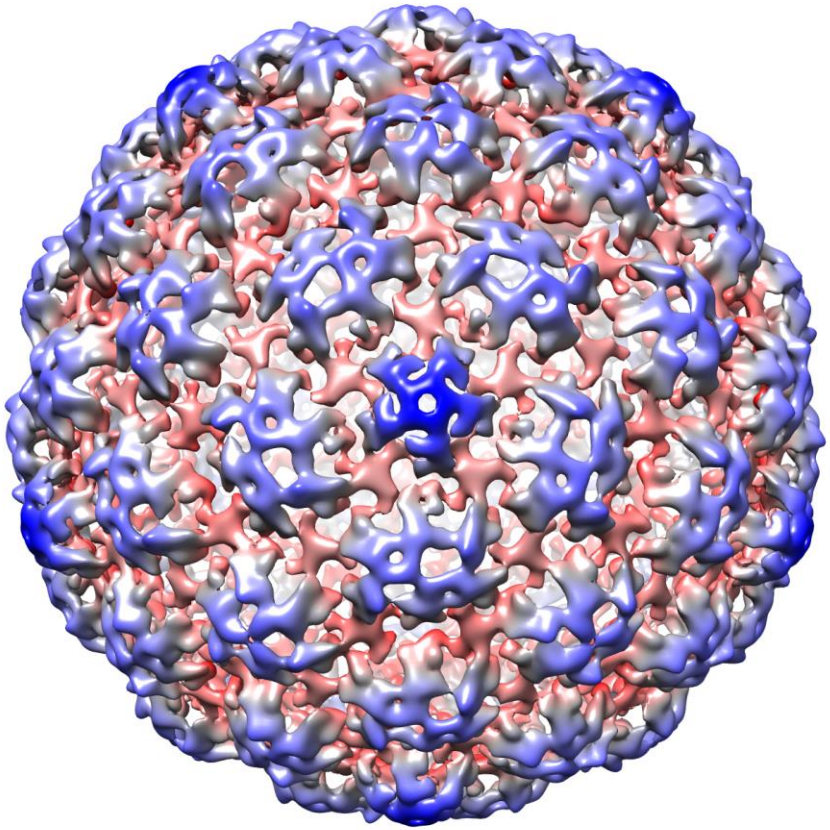
D3 virus – CTF corrected vs not corrected

- Sample: D3 virus capsid; provided by James Conway through Matthijn Vos

CTF corrected – 8.1 A



CTF uncorrected – 14.4 A

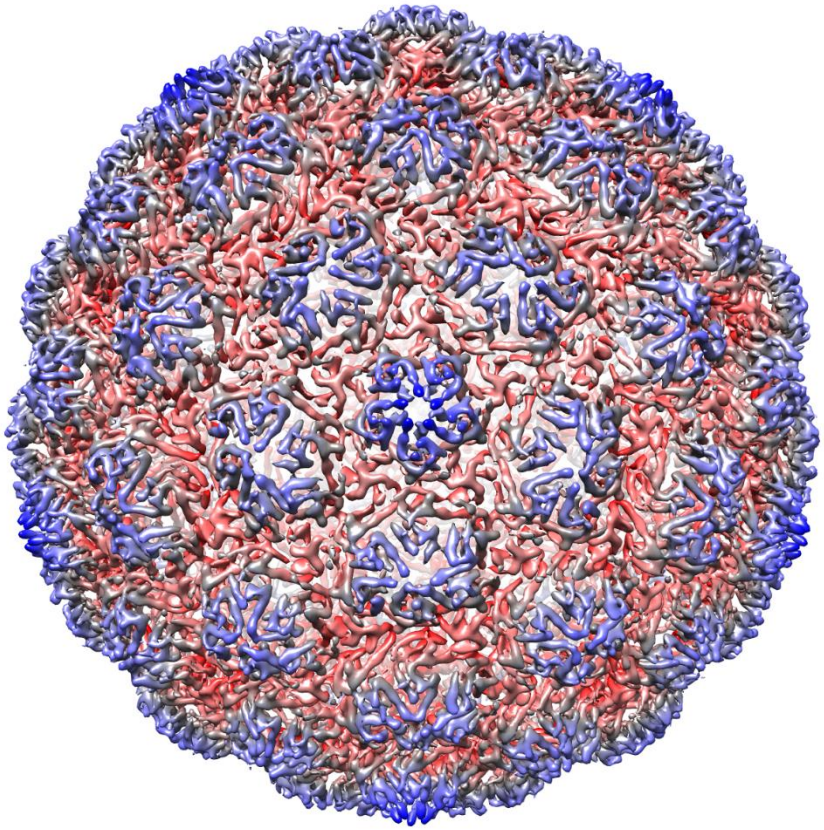


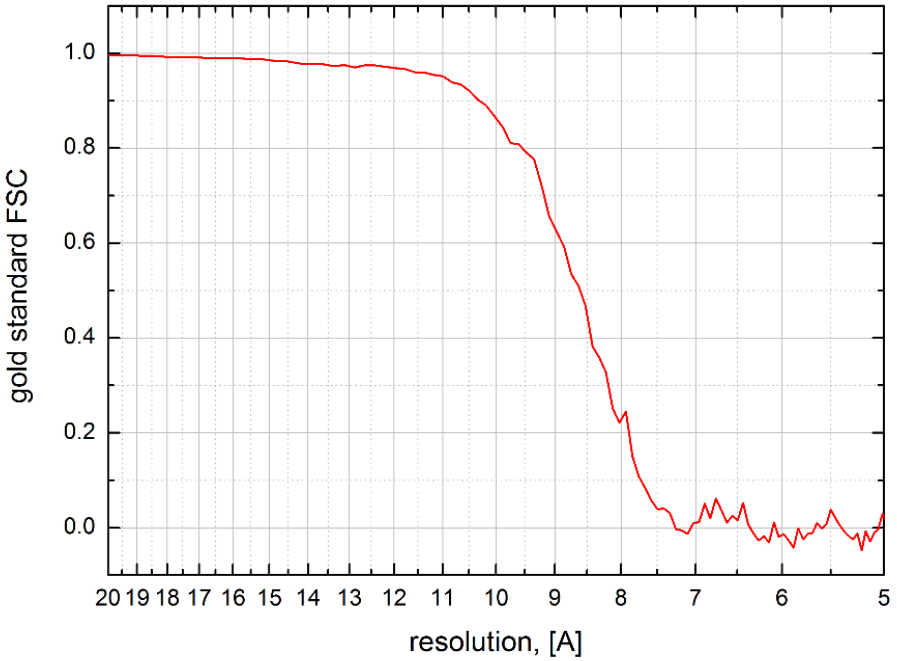
D3 virus, Titan Krios

- Sample: D3 virus capsid; provided by James Conway through Matthijn Vos

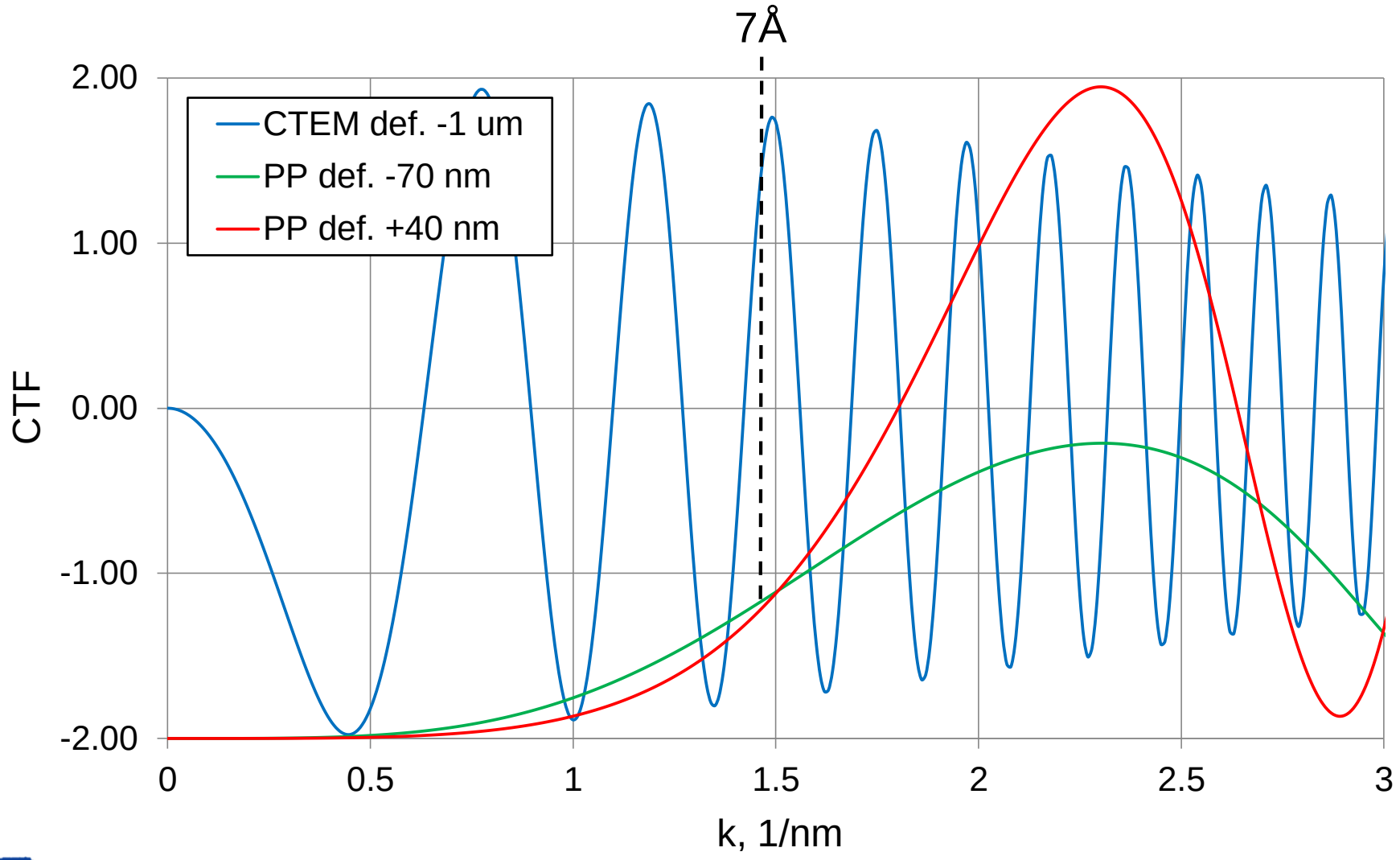
- SPA reconstruction: K2 camera, 155 manually acquired micrographs; 936 particles; I1 symmetry; resolution ~ 8 A

- **CTF uncorrected**





PP focus accuracy requirement



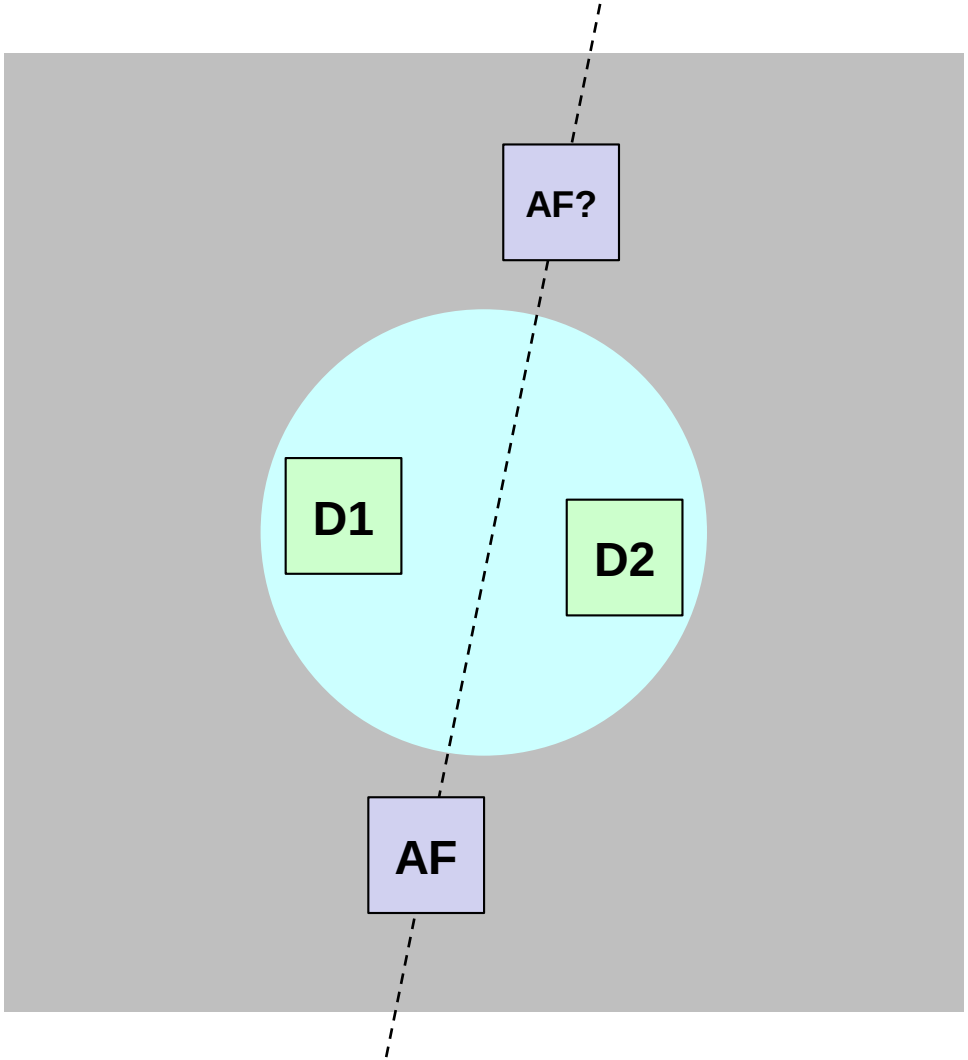
Strategies for SPA with the phase plate

- **Close to focus approach – ideal approach with a PP**
  + No CTF zeros.
  + No CTF fitting & correction steps.
  - Requires precise focusing (+40 nm to -70 nm for 7A resolution) !!!
  - No CTF zeros -> cannot fit & correct the CTF, cannot measure SNR, cannot evaluate image quality.

- **Hybrid approach – approach 1**
  + Collect focus pairs: close to focus + defocused image.
  + Use the defocused image to fit the CTF and correct the close to focus image.
  - CTF fitting and correction steps.
  - Slower data acquisition + larger data volume.

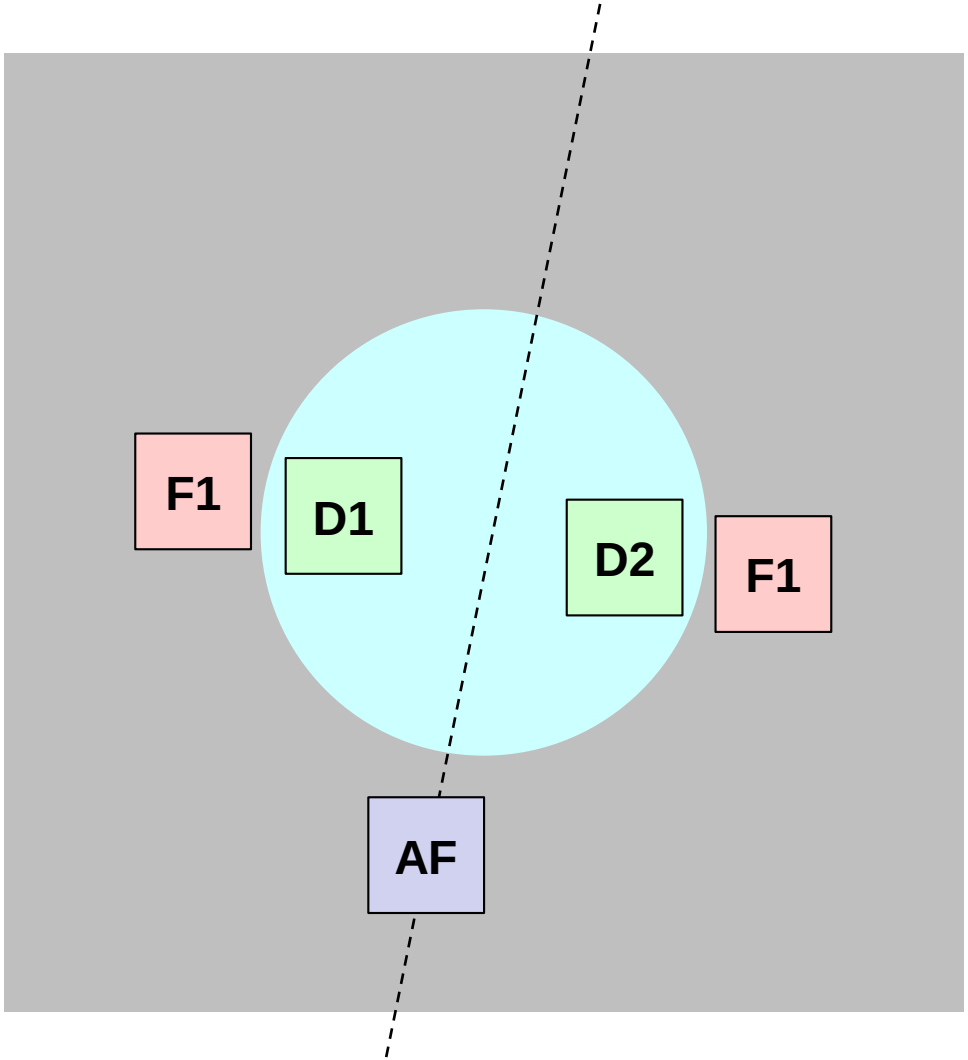
- **Defocus approach – approach 2**
  + Does not require precise focusing -> some defocus variation is actually necessary!
  + Can fit & correct the CTF -> precise defocus & phase shift determination, SNR measurement, image quality assessment.
  - CTF zeros -> information loss, half of the advantage of the PP is lost.
  - CTF fitting and correction steps.

SPA – ideal data collection approach



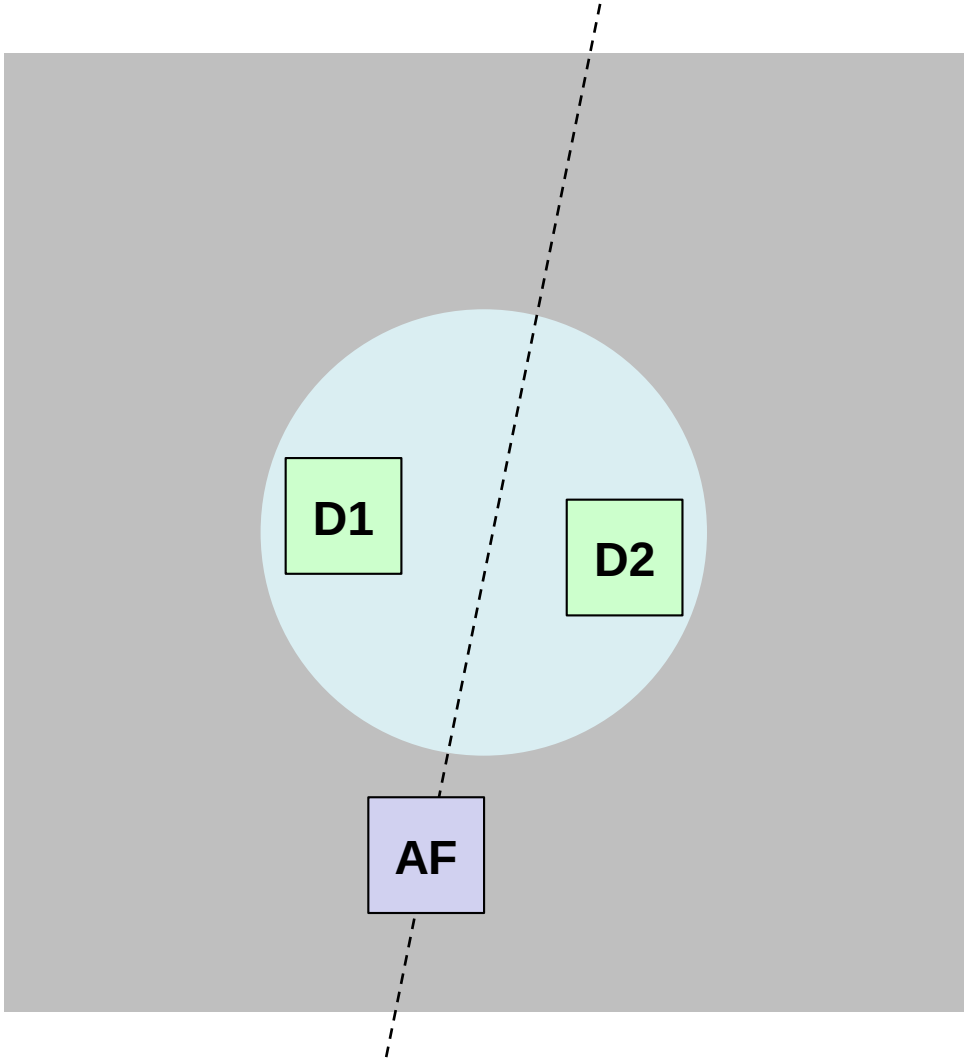
- **AF** – autofocus position
- **D1, D2** – data images taken close to focus

SPA data collection approach 1 - free ice



- **AF** – autofocus position
- **D1, D2** – data images taken close to focus.
- **F1, F2** – focus & phase shift measurement images taken at ~ 1 um defocus.

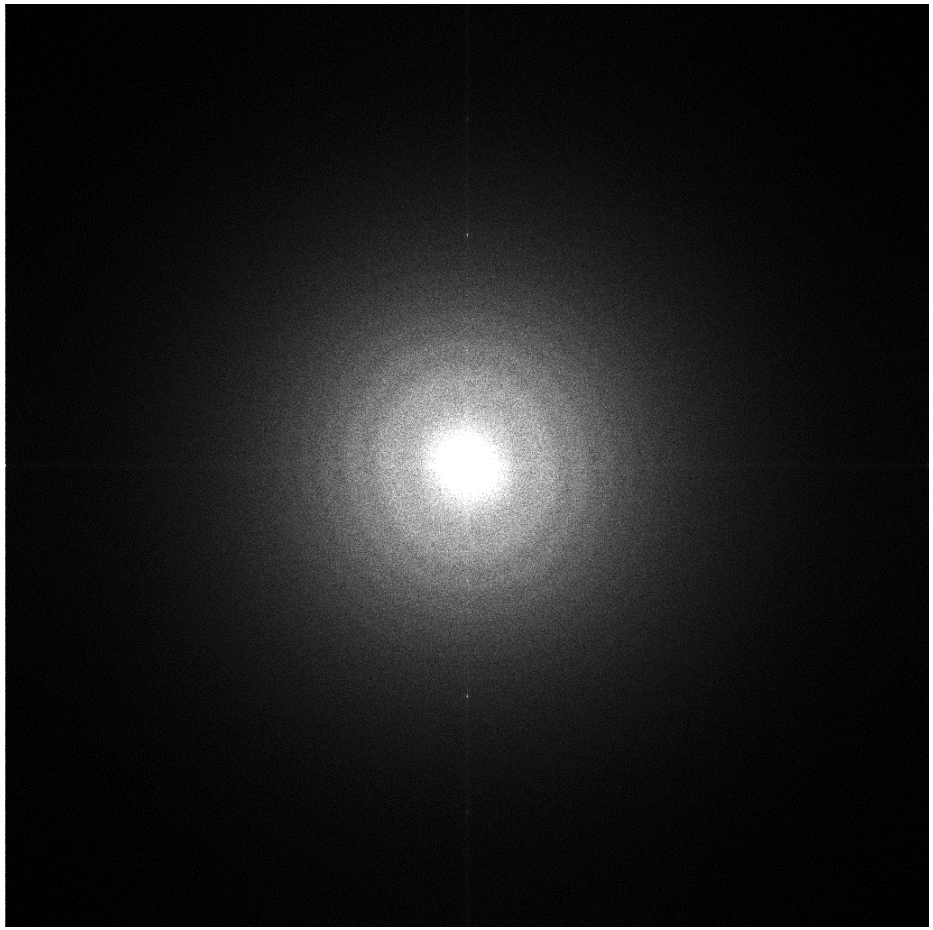
SPA data collection approach 2 – thin carbon

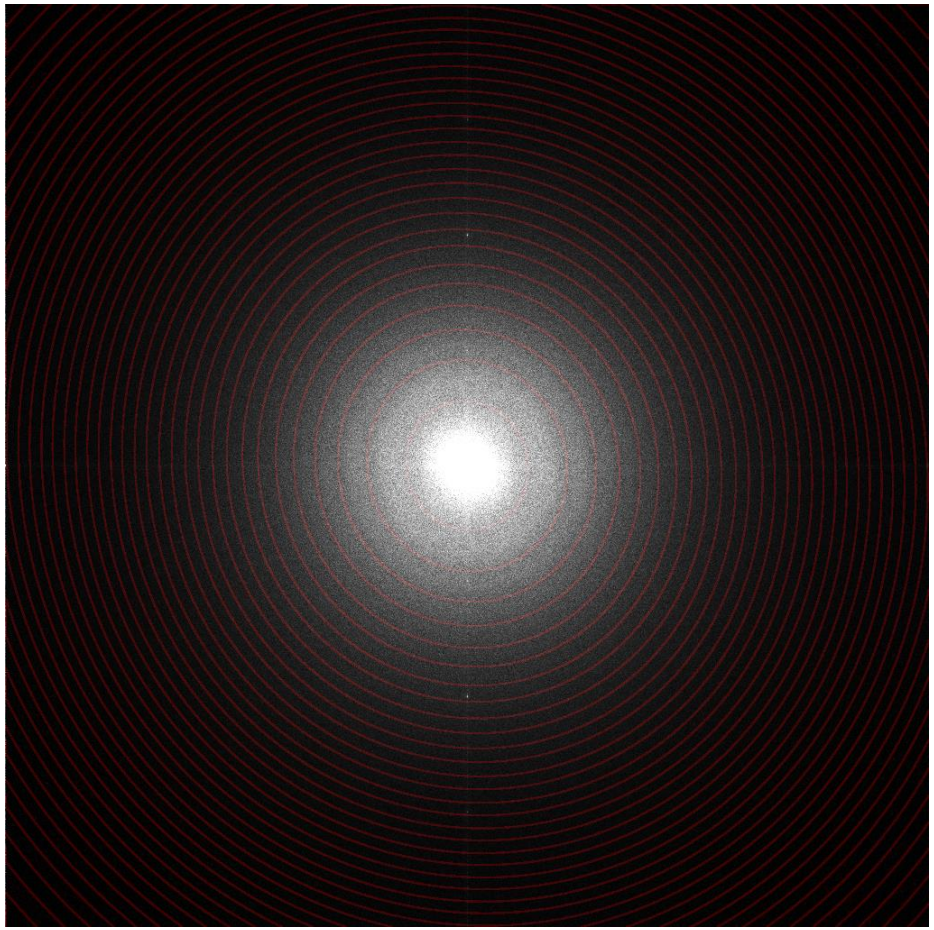


- **AF** – autofocus position
- **D1, D2** – data images taken at ~ 1 um defocus. The defocus in necessary for CTF fitting.

PP CTF fitting

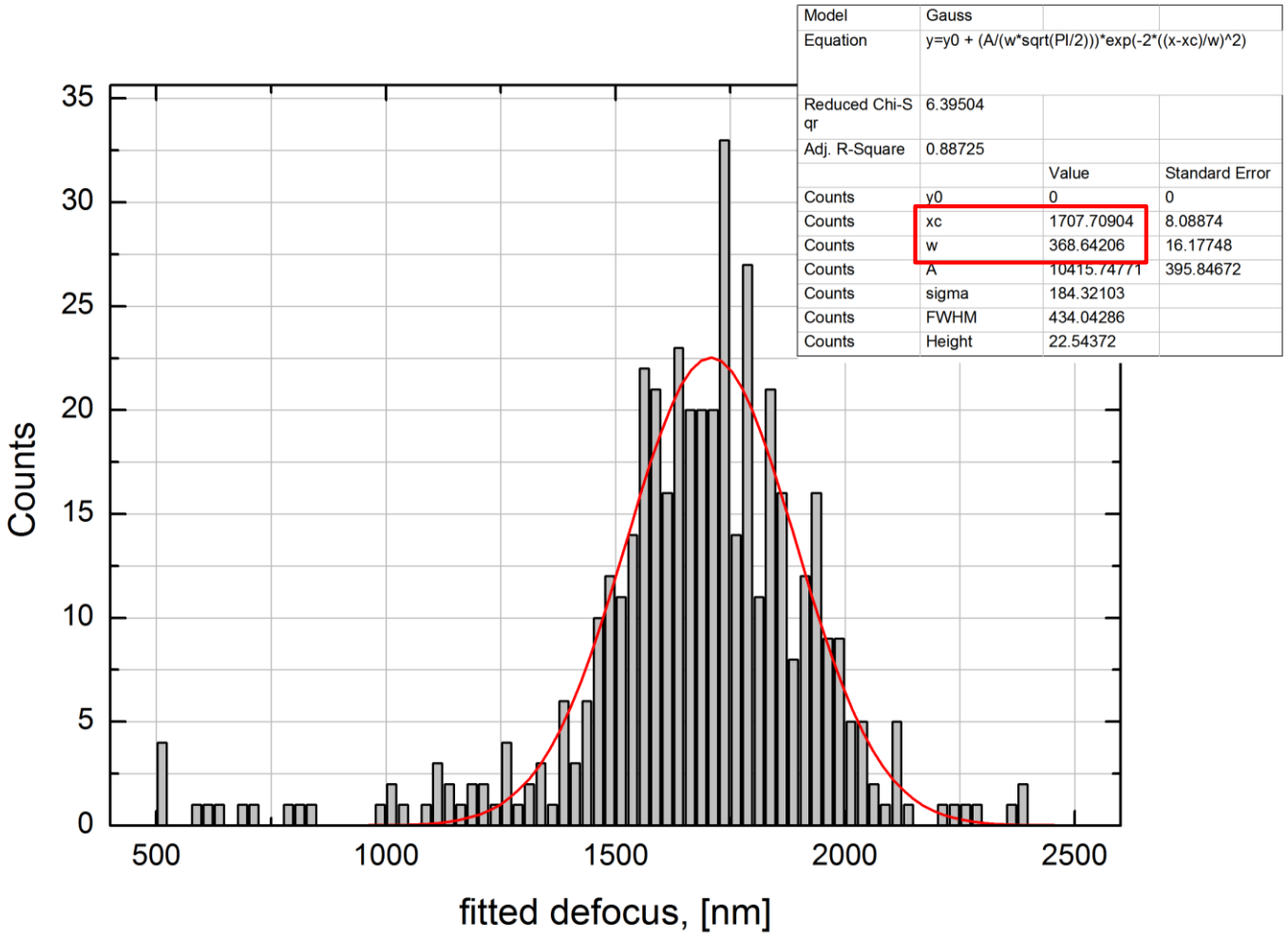
- MATLAB code; fits astigmatism, defocus and phase shift.





EPU focusing accuracy, Tecnai F20

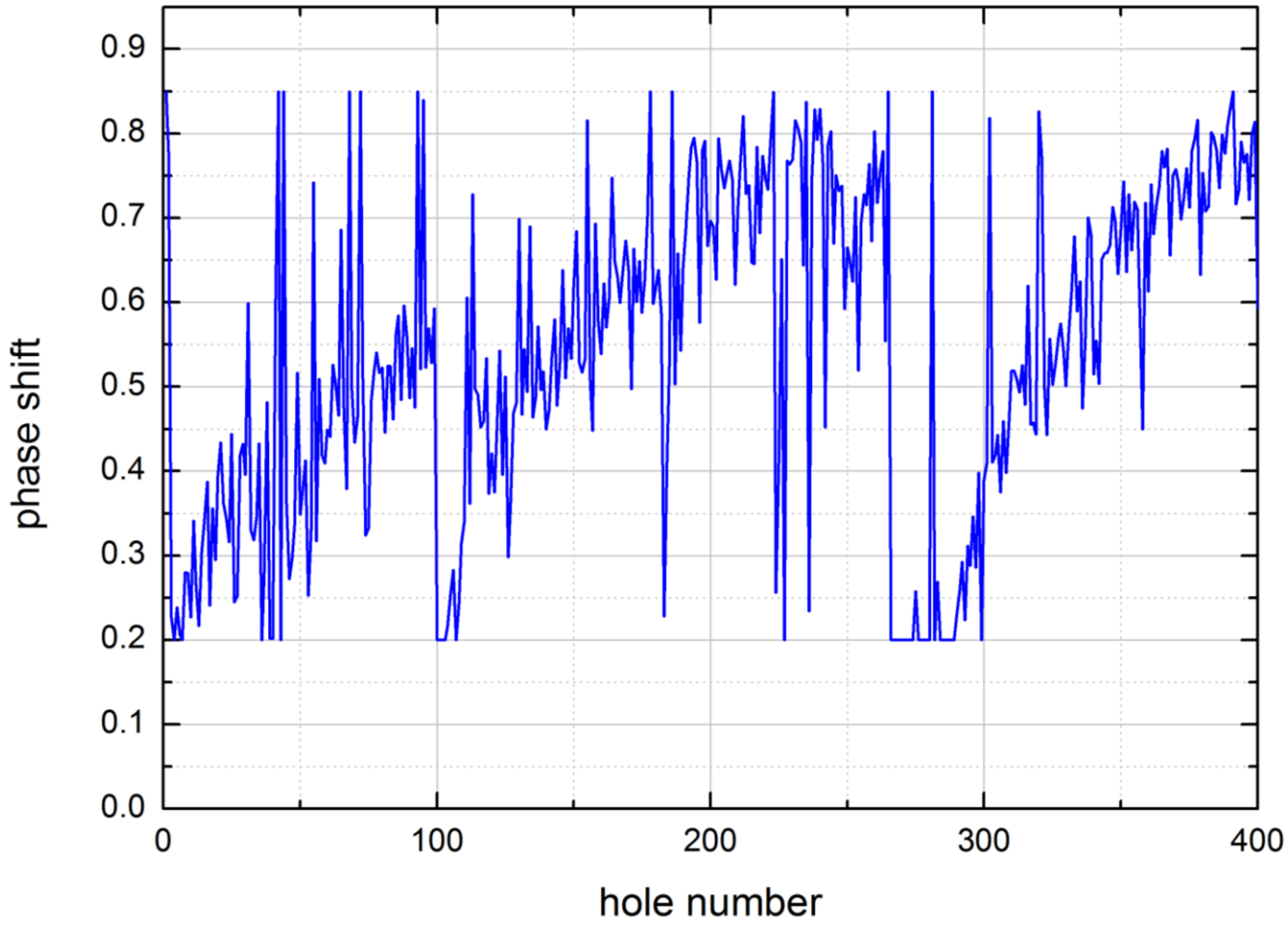
- Tecnai F20, D3 dataset.



Asked defocus: 1500 nm

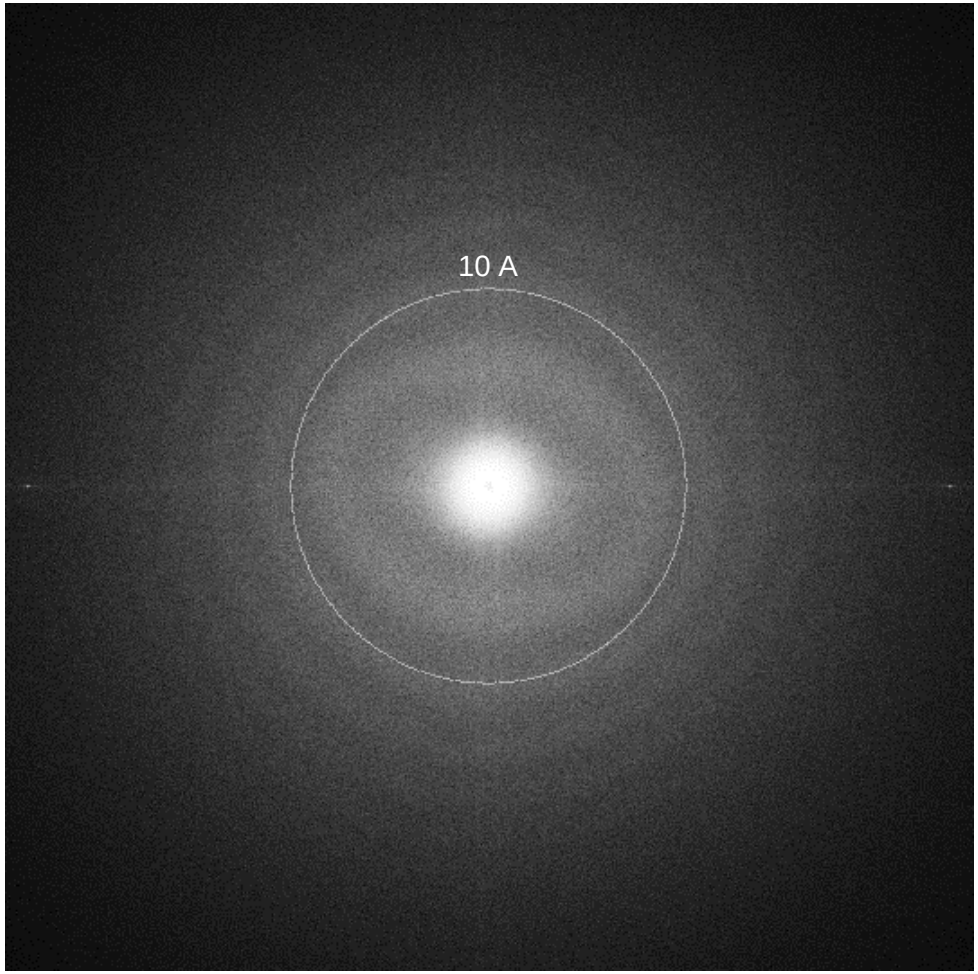
EPU data collection with the VPP – phase shift evolution

- Tecnai F20, D3 virus dataset.



CTF deformation due to focus beam spots on the VPP

- Titan Krios, EPU, Falcon camera



Usability of Phase plates for Single Particle Analysis

- Quickly build a low-resolution (~ 10 A) initial model for new samples.
- Evaluate sample quality – particle distribution, purity, state of assembly, orientation etc.
- A “native state” higher-resolution alternative to negative stain.
- Tackle heterogeneous, small or flexible samples.

Main issues and limitations

- Focusing accuracy:
  - 3 image method.
  - combine beam tilt with CTF fitting for more accurate focusing?
  - OL hysteresis!
  - Ice thickness!
- ~ 20% information loss – even thinner films for VPP (currently ~ 10 nm), graphene?
- CTF deformation due to focus beam tilt spots – increase beam tilt angle, use CTF fitting for focusing.
- Phase shift increase – switch phase plates automatically every N exposures, will be in next versions of EPU.

Acknowledgements

Max Planck Institute
of Biochemistry
Martinsried, Germany

Wolfgang Baumeister
Jürgen Plitzko
Maryam Khoshouei
Yoshiyuki Fukuda
Alex Rigort
Günter Pfeifer
Inga Wolf
Shoh Asano
Yuxiang Chen
Thomas Hoffmann

FEI™

Bart Buijsse
Raymond Wagner
Marc Storms
Petr Herzig
Kasim Sader
Gijs van Duinen
Matthijn Vos
Bob IJtsma