The Rocking Phase Plate – another step towards improved stability

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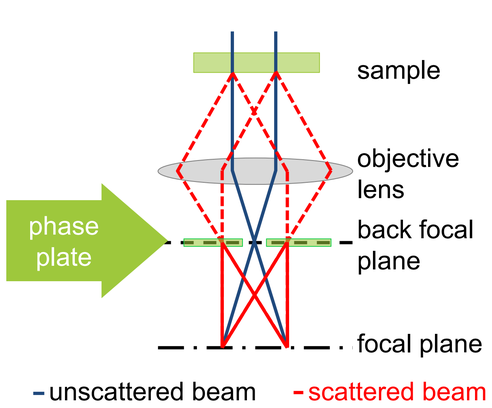
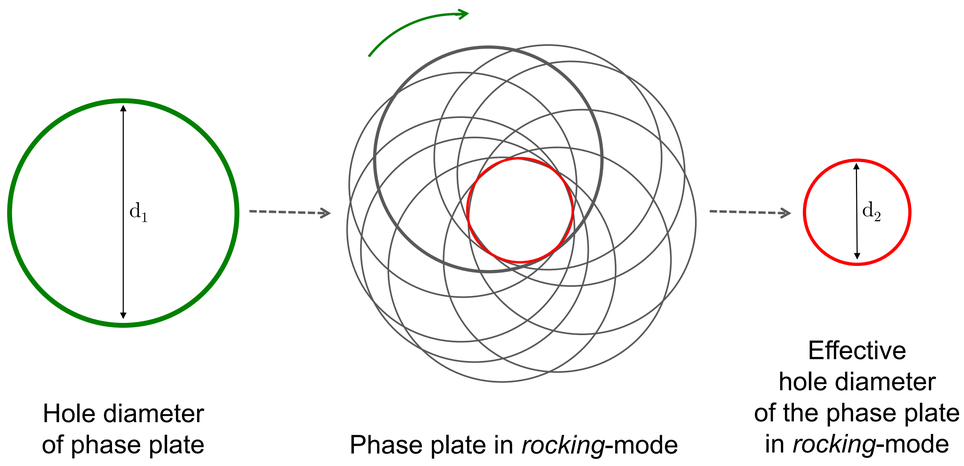
Introduction

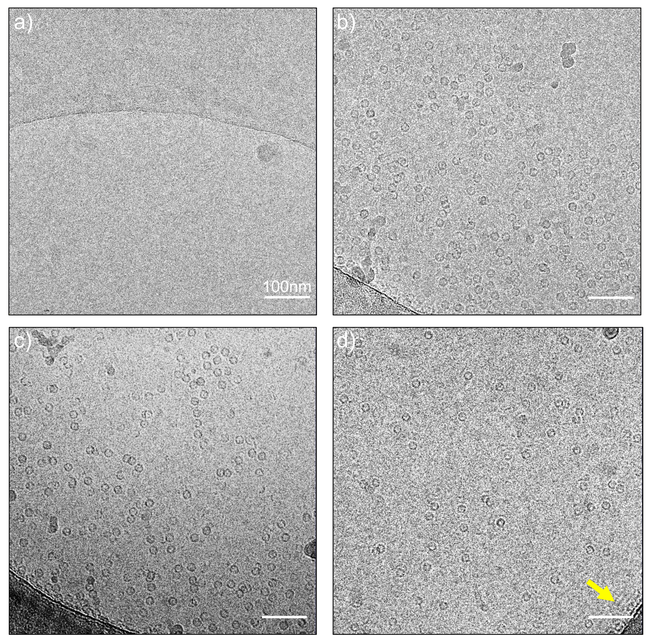
We optimized the properties of a Zernike phase plate to achieve sufficient stability for single-particle acquisition. Therefore, we modified and investigated the following parameters:

- Material choice → 6.5 nm iridium film instead of carbon
- Optimization of the hole diameter
- *Rocking*-mode
- Modification of single-particle acquisition workflow



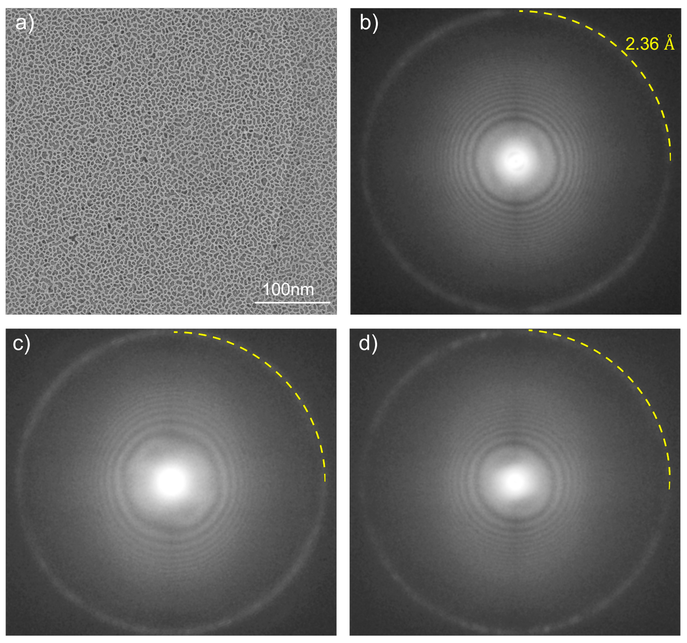


Principle of a *rocking*-mode phase plate (PP). The PP is continuously rotated using the piezo motors of the positioning system [1].



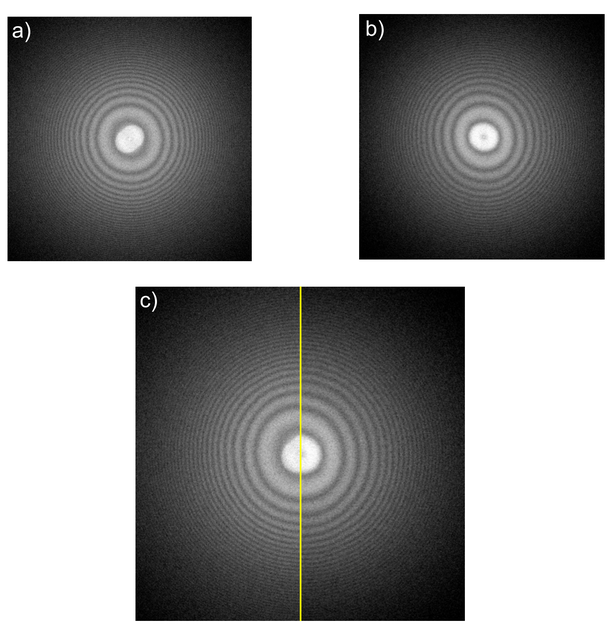
Cryo micrographs of Apoferritin:
(a & b) without phase plate near focus (a), -2 µm defocus (b).
(c & d) with PP near focus, hole diameters 4 µm (c), 2 µm (d).

Phase plate does not limit resolution



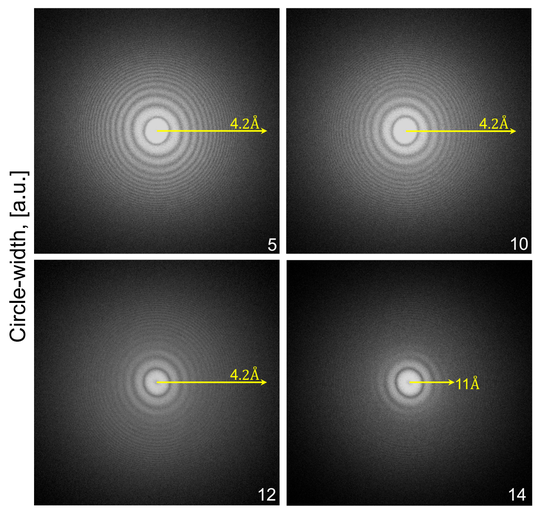
Micrograph of gold on carbon without PP (a).
(b – d) Fast Fourier Transformation (FFT) of micrographs acquired with PP. Hole diameter 4 µm (b), 3 µm (c) and 2µm (d). All micrographs acquired in static mode.

Rocking vs. static mode

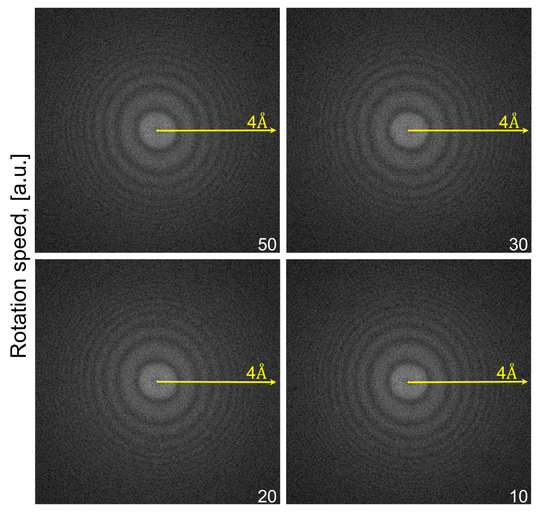


Fourier transformations of micrographs with phase plate in static mode (a) and *rocking*-mode (b).
Side-by-side comparison of a & b (c).

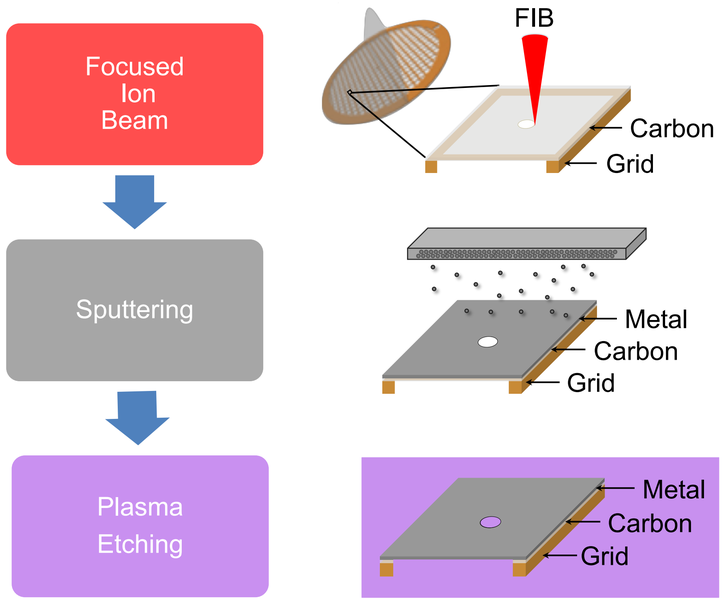
Rocking-mode does not limit resolution



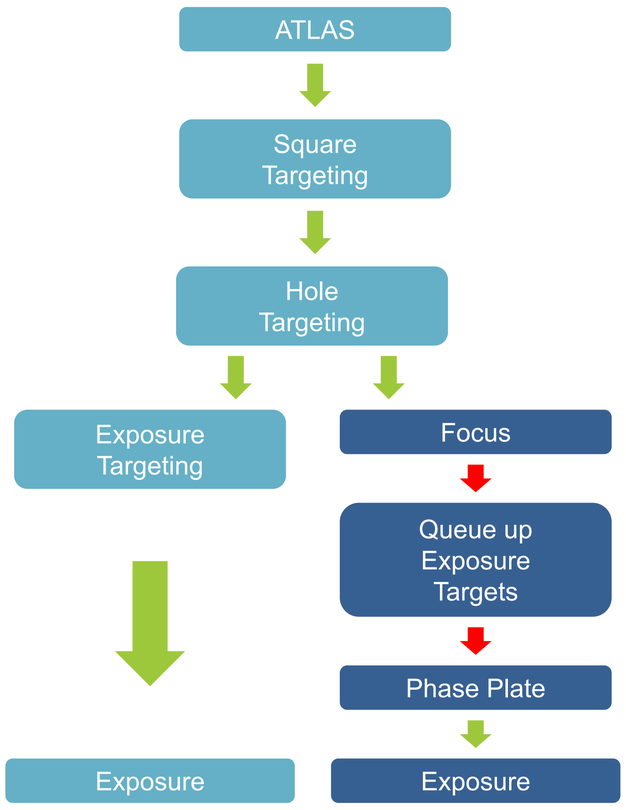
Variation of *rocking* amplitude (upper panel) and speed (lower panel).
Resolution limits are marked (yellow arrows).

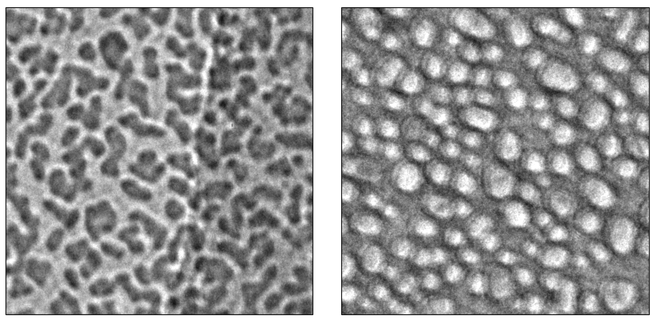


Phase plate fabrication process



Leginon workflow with phase plate





Micrographs of gold on carbon.
Left image with PP (4 µm hole) in *rocking*-mode.
Right image with PP (2 µm hole) in static mode.

Conclusion & outlook

- Metal-based Zernike PP shows sufficient resolution and stability.
- *Rocking*-mode reduces artifacts.
- Workflow modification allows automatic single particle acquisition.
- Phase shift is stable over acquisition period.
- CTF fitting is still challenging using standard software.

References

[1] Kurth, P., Pattai, S., Rudolph, D., Overbuschmann, J., Wamser, J., Irsen, S. (2014). Microscopy and Microanalysis 20, 220–221

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