

Automated cryo electron tomography and sub-tomogram averaging with the FEI Volta phase plate

Wim J. H. Hagen¹, William Wan¹, John A. G. Briggs¹

¹ Structural and Computational Biology Unit, European Molecular Biology Laboratory, 69117 Heidelberg, Germany.

Optimized automated cryo-electron tomography combined with sub-tomogram averaging currently can achieve sub-nanometer resolution using a post-column energy filter with a standard charged coupled device camera [1]. Since low frequency information is crucial for sub-tomogram alignment, we explored the use of a prototype FEI Volta phase plate [2] with our automated data collection setup to further enhance low frequency contrast.

Cryo-EM data is typically collected with defocus, to be CTF-corrected post-acquisition. At sub-nanometer resolution, CTF-correction becomes crucial for any data-averaging scheme [3]. Post-acquisition contrast transfer function correction relies on defocus determination for each individual raw image. This is challenging for close-to-focus tomographic data, and the need to also determine the phase shift of the Volta phase plate complicates things further.

We explore optimization of microscope alignments and calibrations to meet the increased stability demands needed for in-focus phase plate data acquisition. Absolute focus and beam tilt calibrations on the microscope are crucial. Autofocus routines have been optimized to establish stable absolute focus, rather than stable relative focus, during automated data acquisition, as well as for determining and logging phase-shift. These routines facilitate collecting data at a predetermined focus and phase shift, as well as determining and correcting the resulting CTF for structure determination by sub-tomogram averaging.

[1] Schur, F. K. M. et al, *Nature*, doi:10.1038/nature13838

[2] Danev, R. et al, *Proceedings of the National Academy of Sciences*. doi:10.1073/pnas.1418377111

[3] Schur, F. K. M. et al, *Journal of Structural Biology*, doi:10.1016/j.jsb.2013.10.015

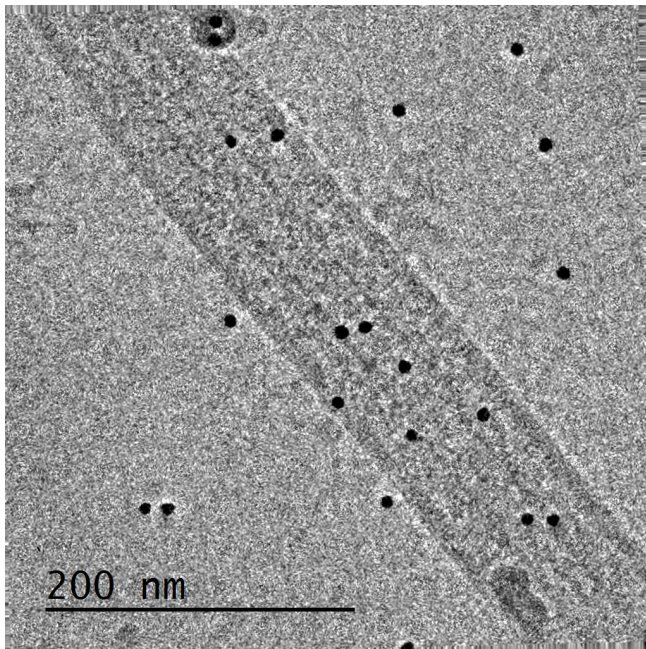


Figure 1. In-focus zero-tilt energy-filtered image of Marburg virus at 200KV, 1.1 electrons per square Angstrom dose using the Volta phase plate.

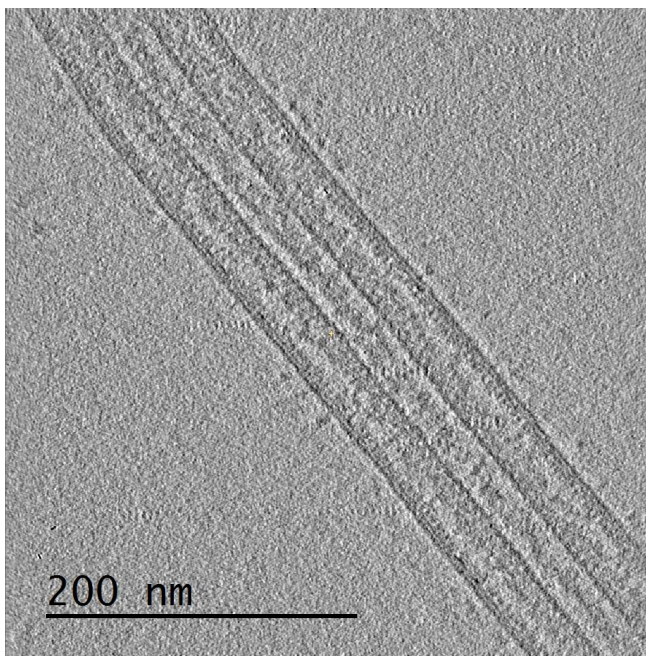


Figure 2. Ten slices through an in-focus tomogram of Marburg virus at 200KV, tilt range +/- 60 degrees, tilt step 3 degrees, total dose 70 electrons per square Angstrom dose using the Volta phase plate.