

CTF correction for “high-resolution” subtomogram averaging

Acknowledgements

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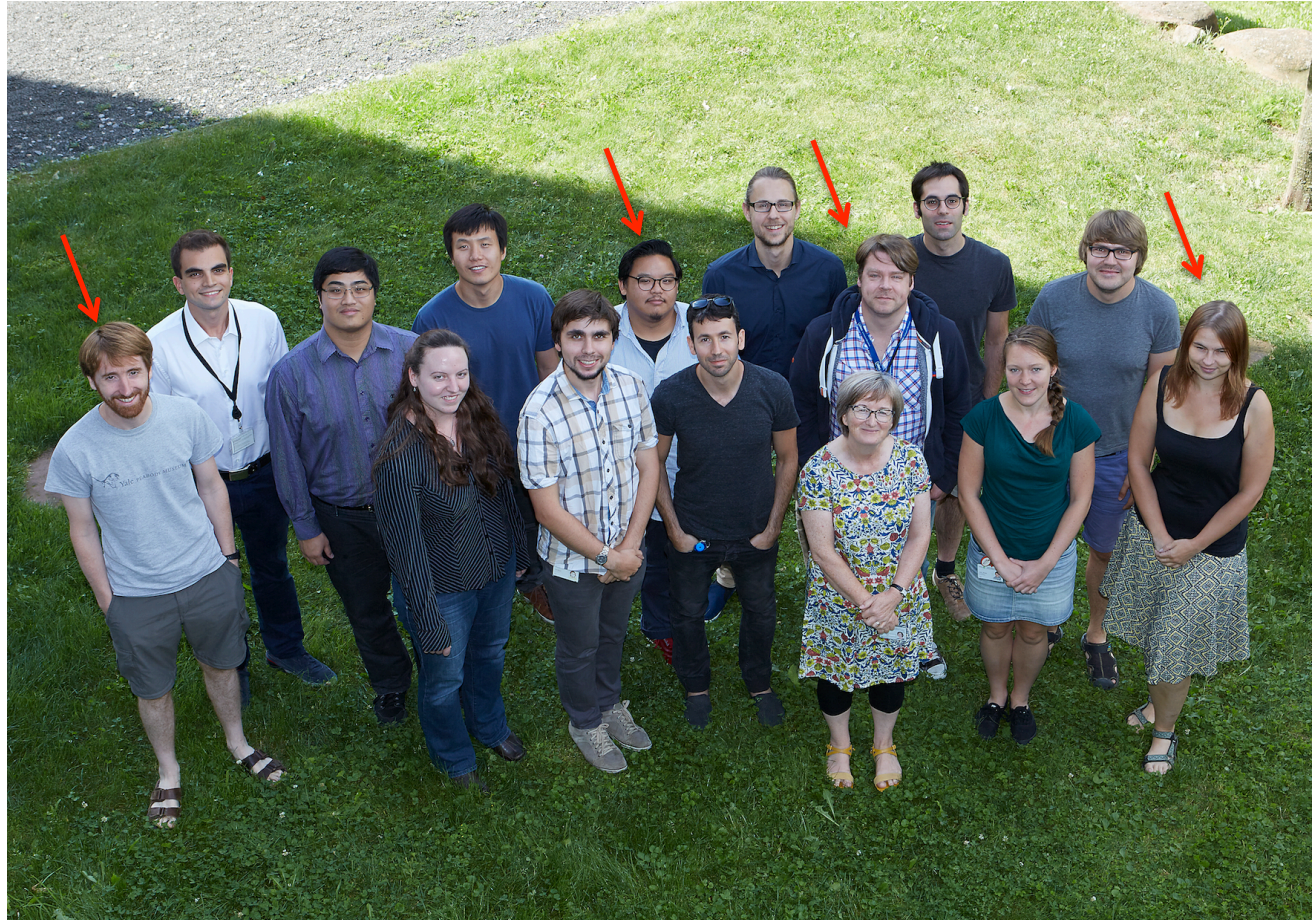
EMBL IT-services

Frank Thommen

Serial EM

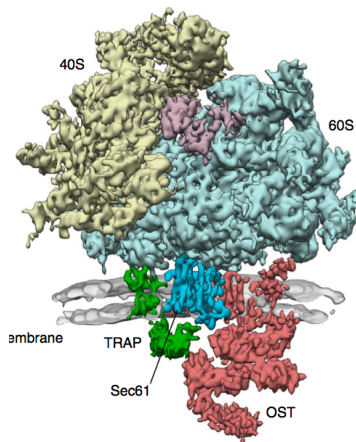
Boulder, University of Colorado

David Mastronarde



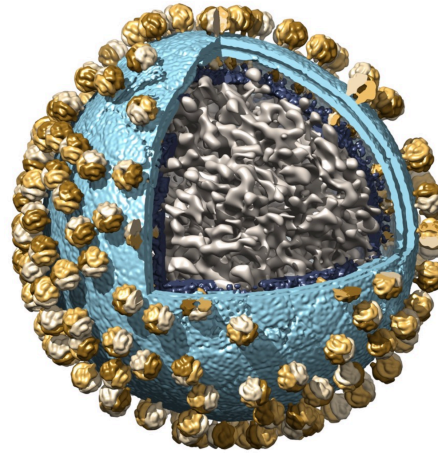
The power of cryo-ET and subtomogram averaging

ER-associated ribosome



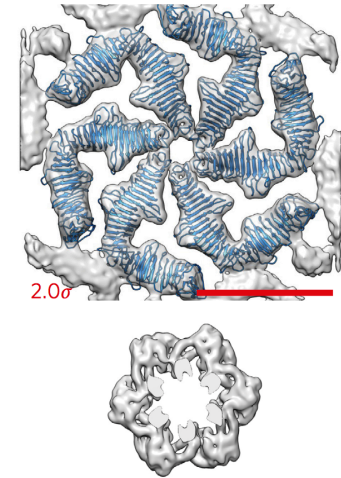
Pfeffer S et al, 2015, Nat.Comm.
PyTOM

Lassa virus glycoproteins



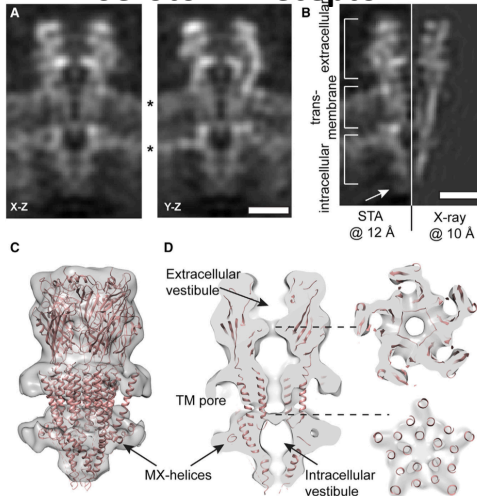
Li, S et al, 2016, PLOS Pathogens
Dynamo

S-layer on *C. crescentus*



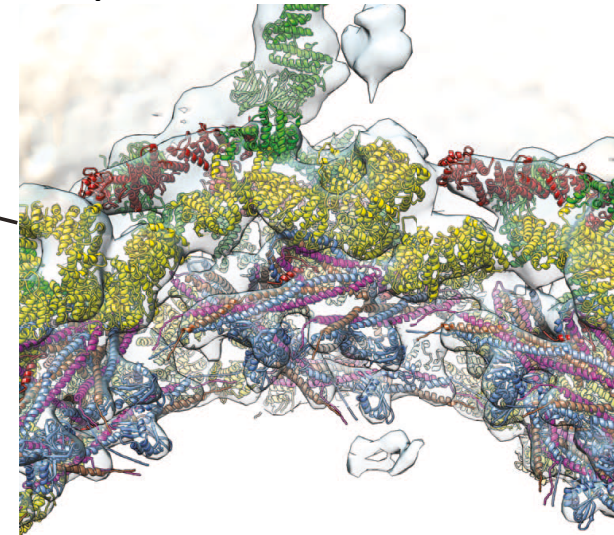
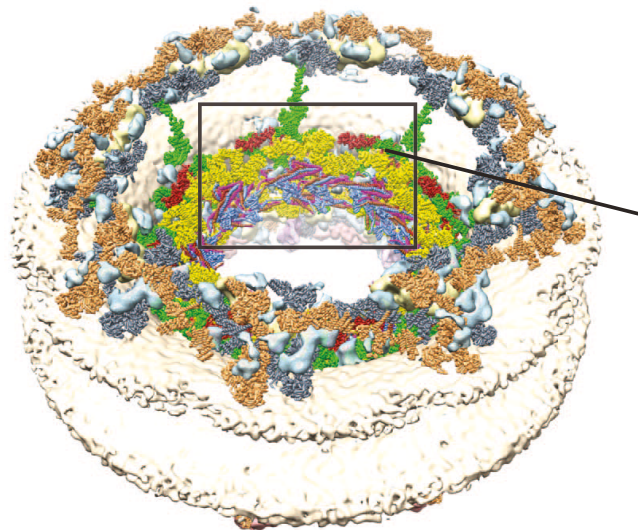
Bharat T, et al, 2017, Nature Microbiology
TOM/AV3/Relion

Serotonin-receptor



Kudryashev M, et al, 2015, Structure
Dynamo

Nuclear pore



Kosinski J, et al, 2016, Science
TOM, AV3 3

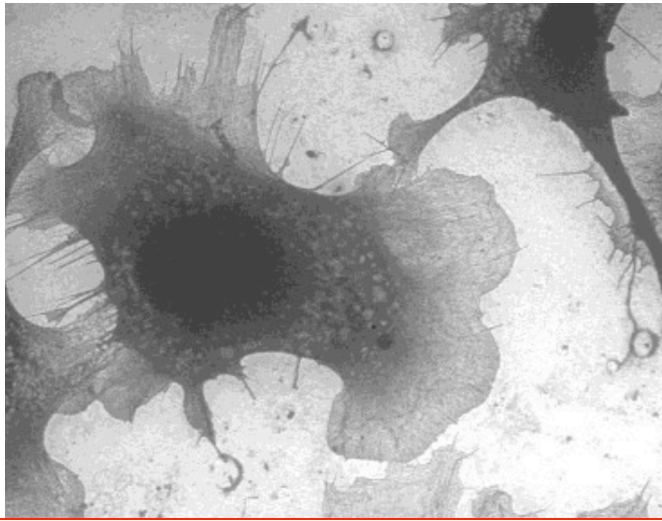
What determines the resolution in cryo-ET and subtomogram averaging?

- Sample
- Data acquisition
- Image processing

They all should be considered when talking about defocus estimation and CTF-correction

SIZ

+

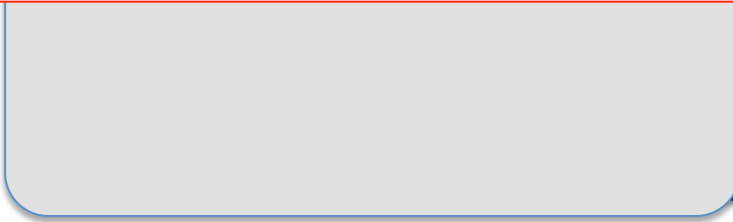


Eukaryotic cells

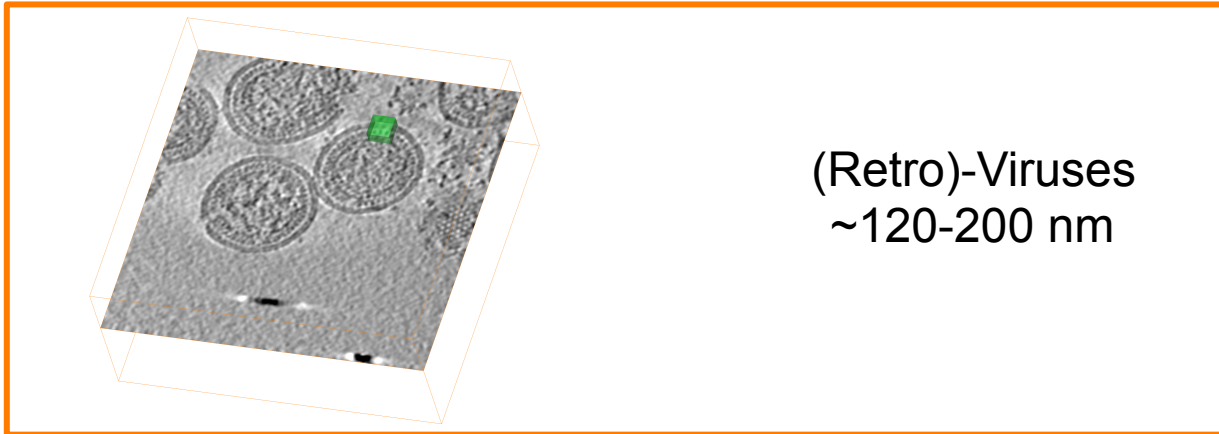
+

Defocus gradient in thick specimens

CHALLENGE



Prokaryotes



(Retro)-Viruses
~120-200 nm

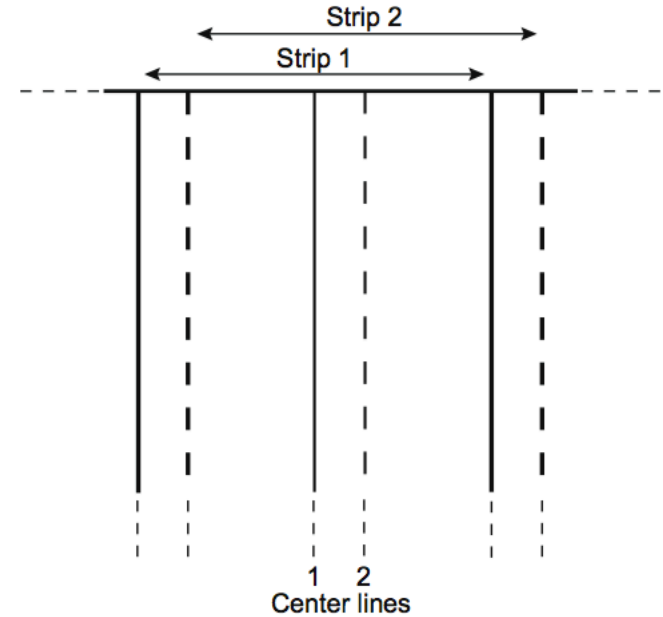
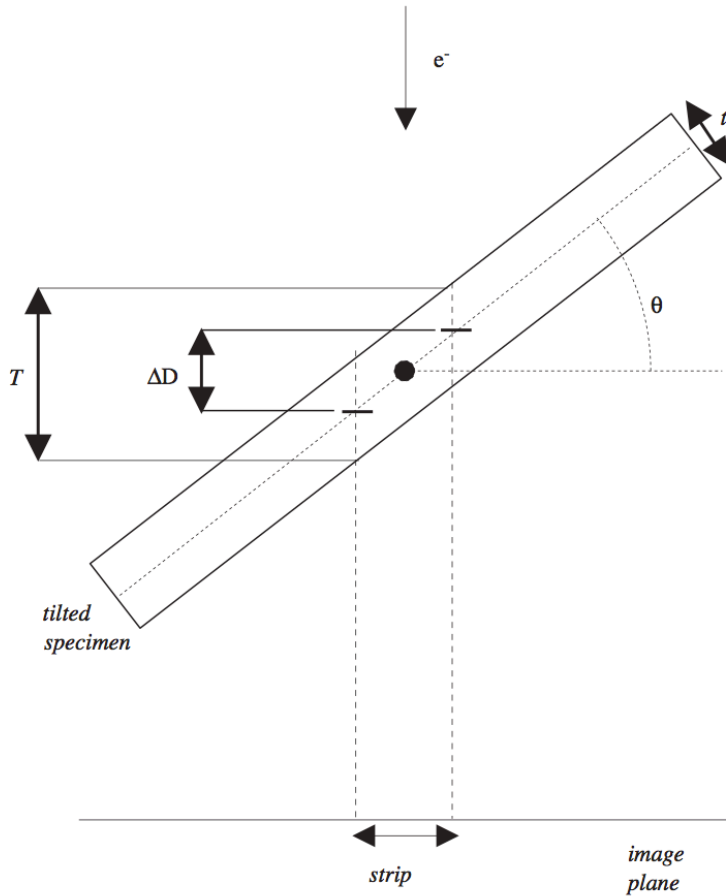
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Nothing is shown to scale

CTF-correction

2D CTF-correction Tile vs. Strip



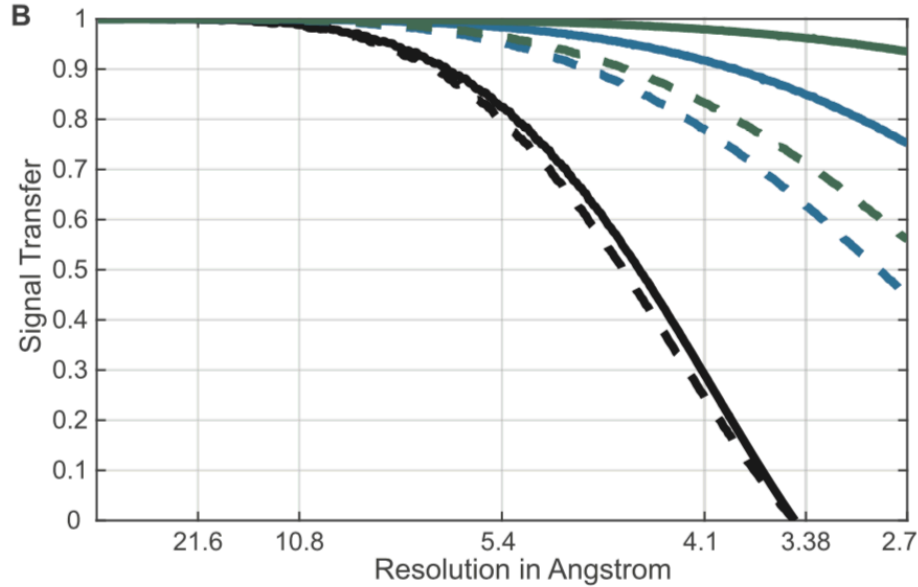
Fernández, J. J., Li, S., & Crowther, R. A. (2006). CTF determination and correction in electron cryotomography. *Ultramicroscopy*, 106(7), 587–596.

Xiong Q, Morpew MK, Schwartz CL, Hoenger AH, Mastrorade DN(2009), CTF determination and correction for low dose tomographic tilt series, *J.Struct.Biol* (168)

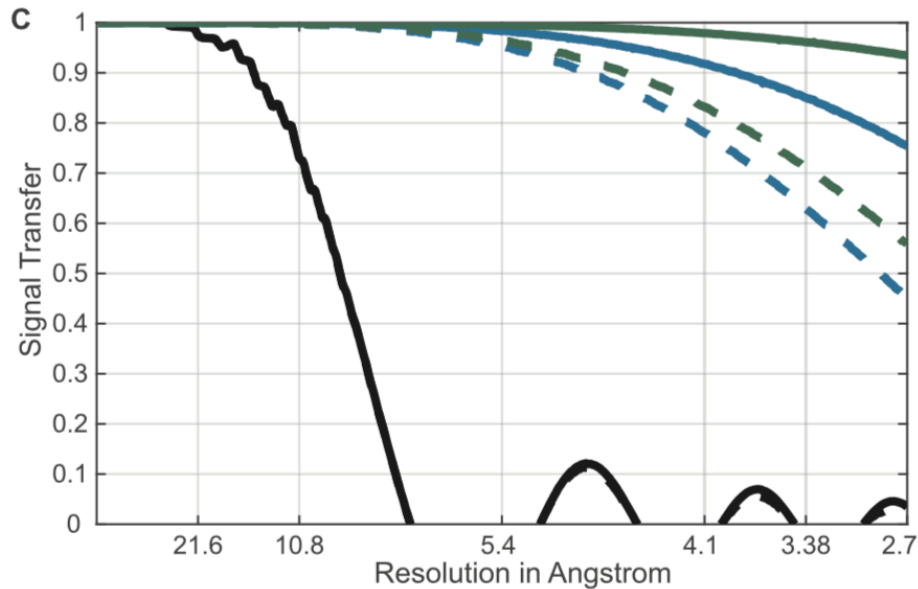
How accurate does the defocus estimation/CTF correction have to be?

What is the target resolution?

100nm thick tomogram

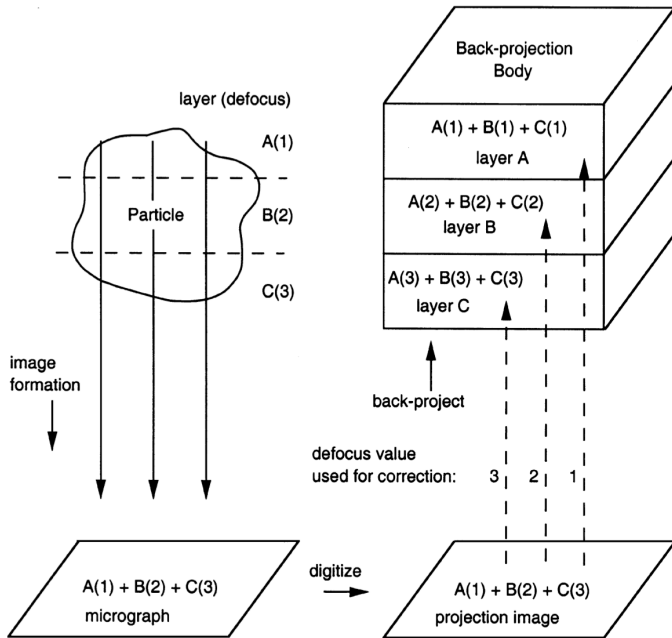


500nm thick tomogram

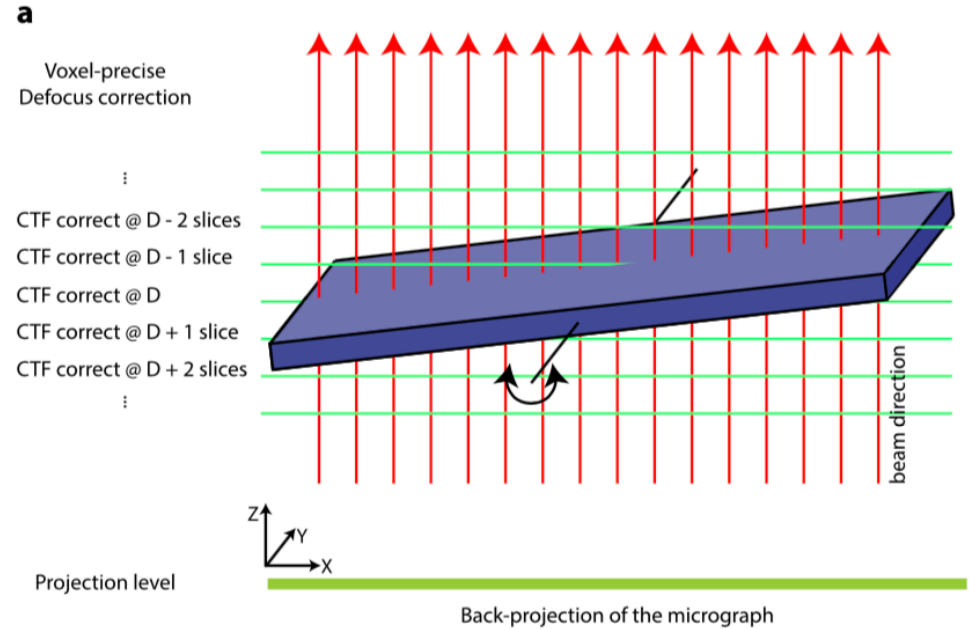


Solid line: no error in defocus determination
Dashed line: normally distributed error (0nm mean, 12 nm SD)
2D CTF-correction
3D CTF (30nm defocus step)
3D CTF (15nm defocus step)

3D CTF correction



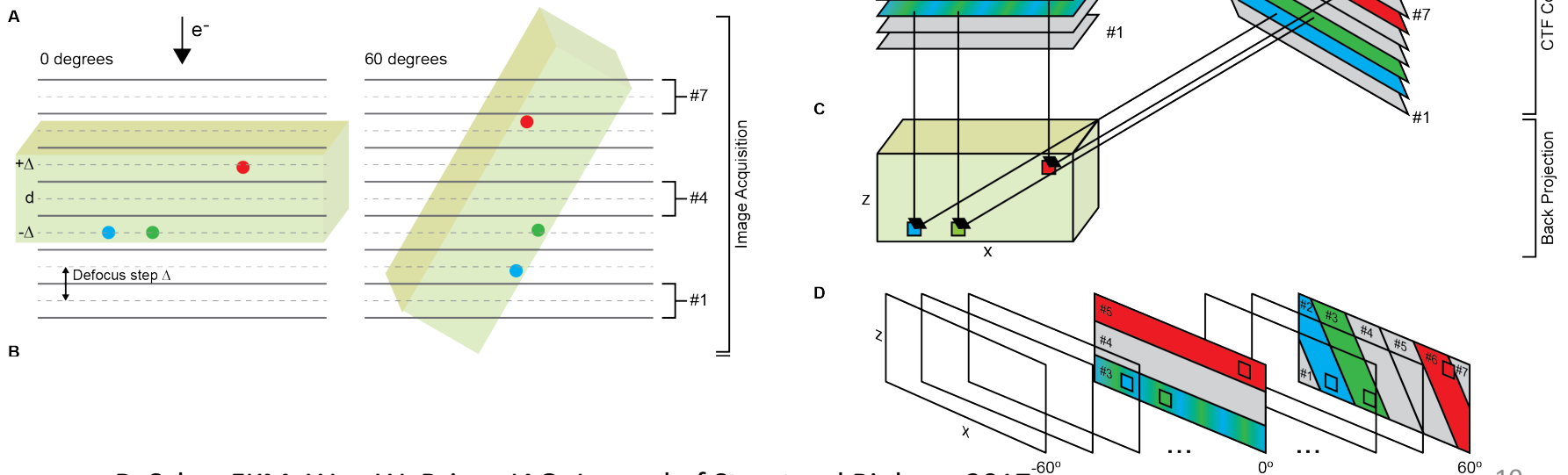
Jensen and Kornberg (2000)
Micrograph is CTF-corrected
several times with a different
defocus value



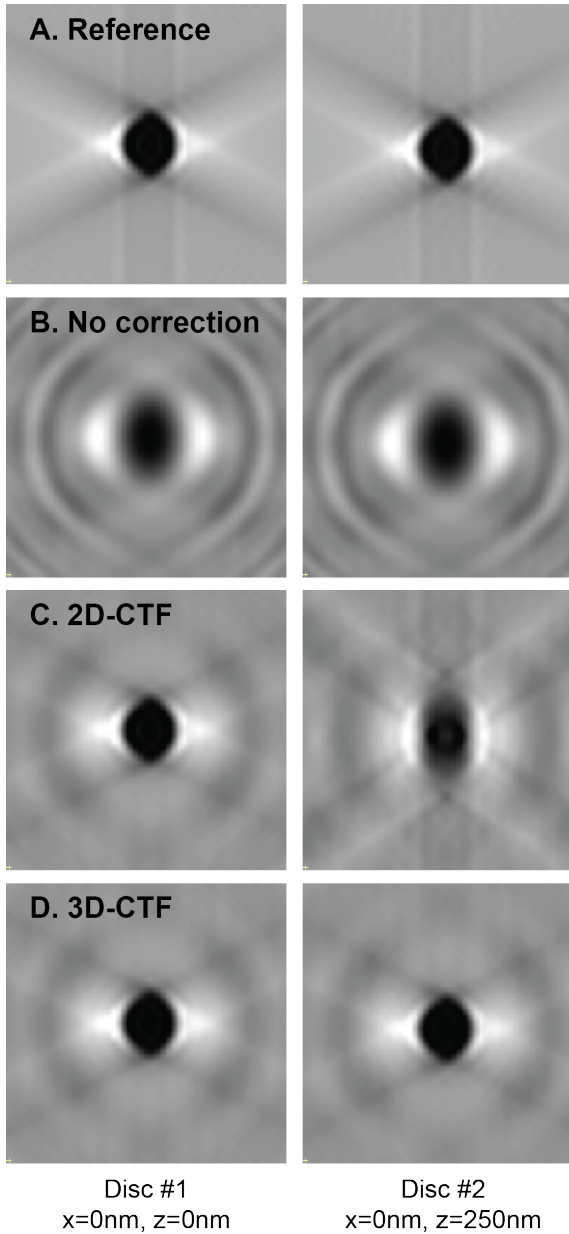
Kunz and Frangakis (2017)
WBP and SART
Tomographic slices are
corrected

3D-CTF correction NOVACTF

- NovaCTF can be easily integrated to standard IMOD tomography workflow
- Command-line based, reads IMOD input files
- Phase-flipping, multiplication, astigmatism correction
- Download from
 - <https://github.com/turonova/novaCTF>
 - With helpful wiki, how compile, run etc....
- Workflow
 1. Generate defocus files
 2. CTF-correction
 3. Reconstruction

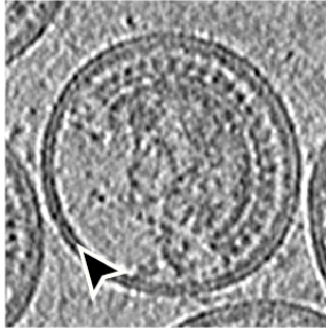


2D vs 3D CTF-correction

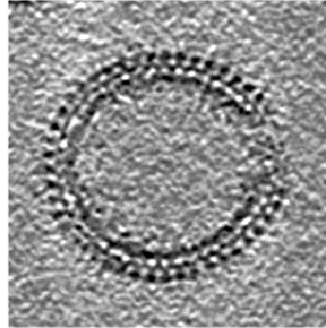


2D CTF-correction – phase-flipping

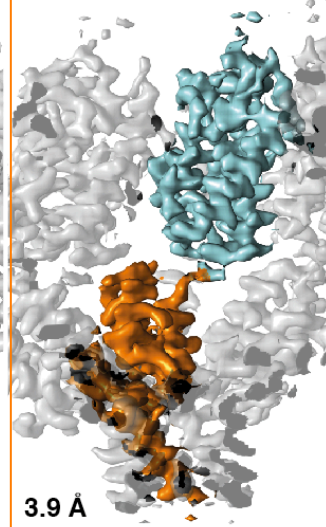
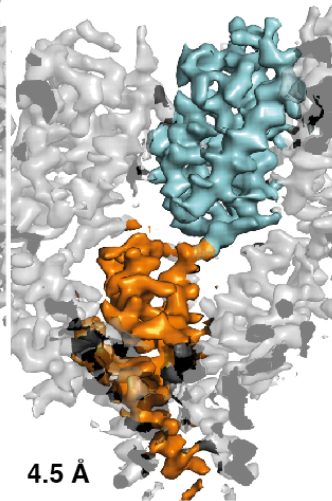
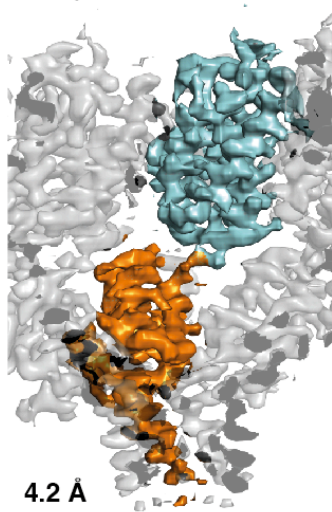
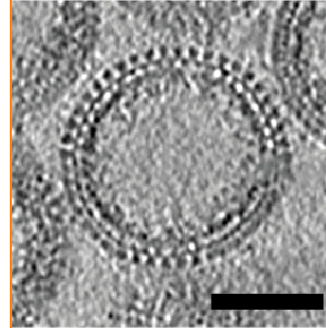
immature HIV-1 particles
(D25A)



untreated Δ MACANCSP2
VLPs



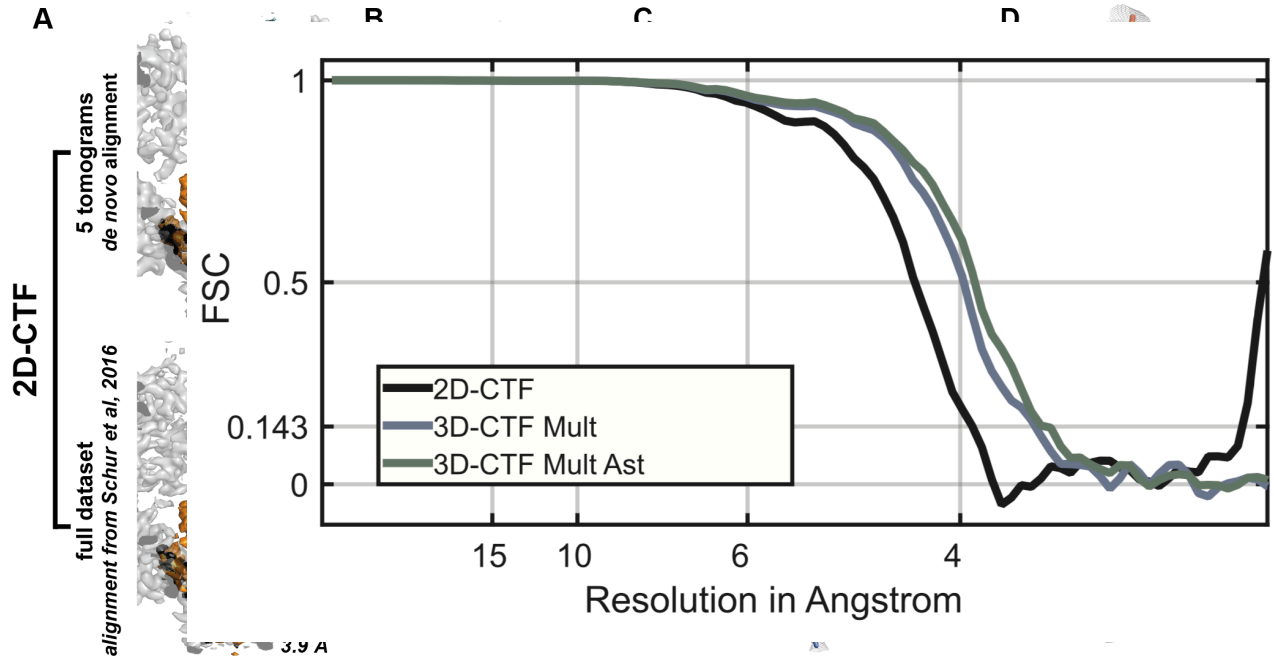
Δ MACANCSP2
VLPs + BVM



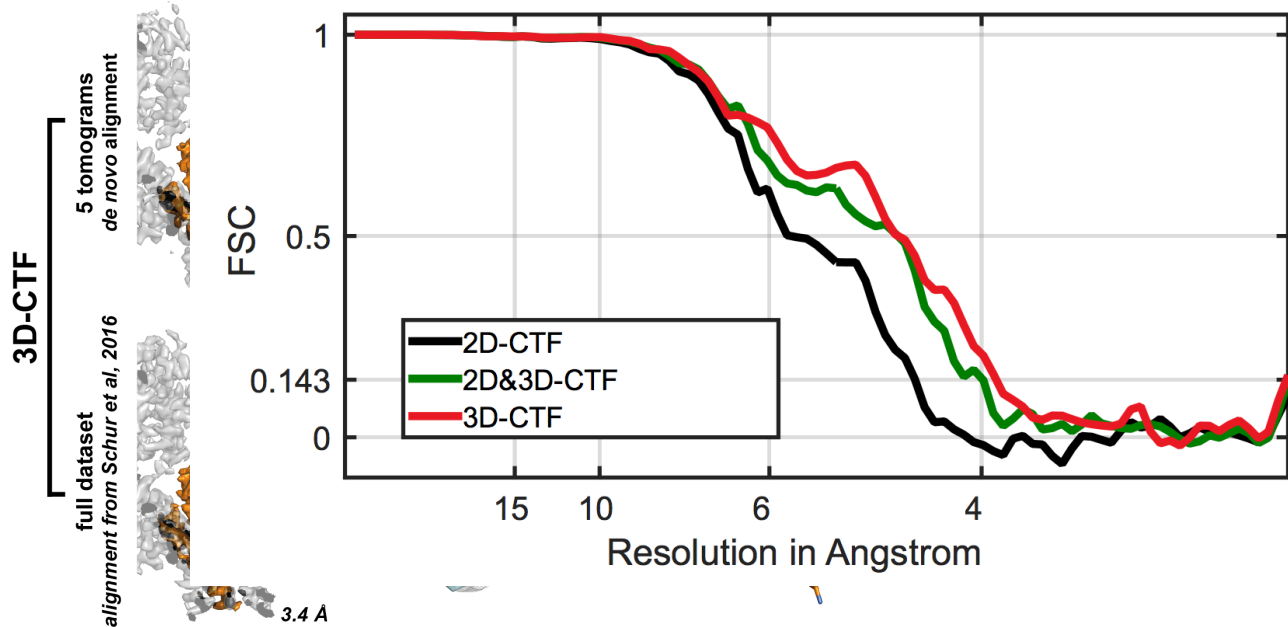
VLPs/Viruses	484	285	383
Asymmetric units Set A	301,302	265,506	386,040
Asymmetric units Set B	301,920	263,910	386,598
Final resolution (0.143 FSC) in Å	4.2	4.5	3.9

NOVA-CTF

Full dataset
3.4 Å



Aligning 5
tomograms
from scratch
3.9 Å



NovaCTF

Method	CTF Correction	Reconstruction	Total time
IMOD	19 m 36 s	31 m 57 s	51 m 33 s
NovaCTF – 1 correction per tilt image	01 m 56 s	1 h 0 m 03 s	1 h 1 m 59 s
NovaCTF – 11 corrections per tilt image	21 m 16 s	1 h 0 m 11 s	1 h 21 m 27 s
NovaCTF – 33 corrections per tilt image	1 h 03 m 48 s	1 h 0 m 43 s	2 h 04 m 31 s

	Total memory (RAM)	Temporary storage
NovaCTF – 1 correction per tilt image	734 MB	68.2 GB
NovaCTF – 11 correction per tilt image	740 MB	90.5 GB
NovaCTF – 33 correction per tilt image	753 MB	139.6 GB

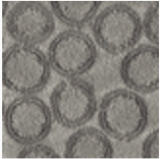
Times were measured on a Xeon E3-123V3, 3.5 GHz, 32GB RAM (single CPU). Tomogram size 3710x3710x1200 voxels

- Main limitation is I/O when running on cluster

Want something to play? EMPIAR-10164

- Full immature HIV-1 VLP dataset deposited
- 43 tilt series
 - Schur FKM, Obr M, Hagen WJH, Wan W, Jakobi AJ, Kirkpatrick JM, Sachse C, Kräusslich H-G, Briggs JAG, (2016), An atomic model of HIV-1 capsid-SP1 reveals structures regulating assembly and maturation, Science, 353(6298): 506-508. doi: 10.1126/science.aaf9620
- Raw frames including mdoc files
- Released this week

EMPIAR-10164 	Cryo-electron tomography of immature HIV-1 dMACANC VLPs [43 multi-frame micrographs composed of 8 frames each in MRC format]	Schur FK, Obr M, Hagen WJ, Wan W, Jakobi AJ, Kirkpatrick JM, Sachse C, Krausslich HG, Briggs JA, Turoňová B [Pubmed: 27417497] [DOI: 10.1126/science.aaf9620]	EMD-3782 , EMD-4015 , 5193	865.0 GB	3.4-3.9
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Thanks for your attention

