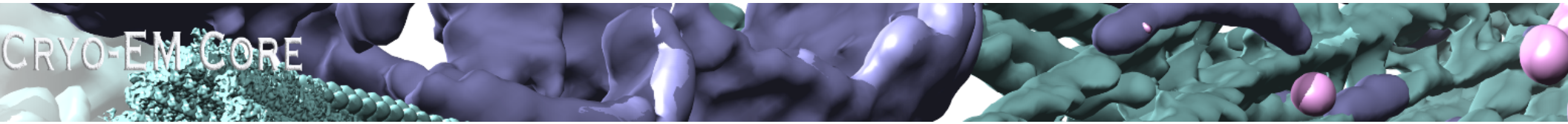


Tomography Pipeline in EMAN2

Steve Ludtke

Charles C. Bell Professor
Biochemistry and Molecular Biology
Director, CryoEM/CryoET Core
Co-director CIBR Center
Baylor College of Medicine

Baylor College of Medicine

ADVANCED TECHNOLOGY CORES

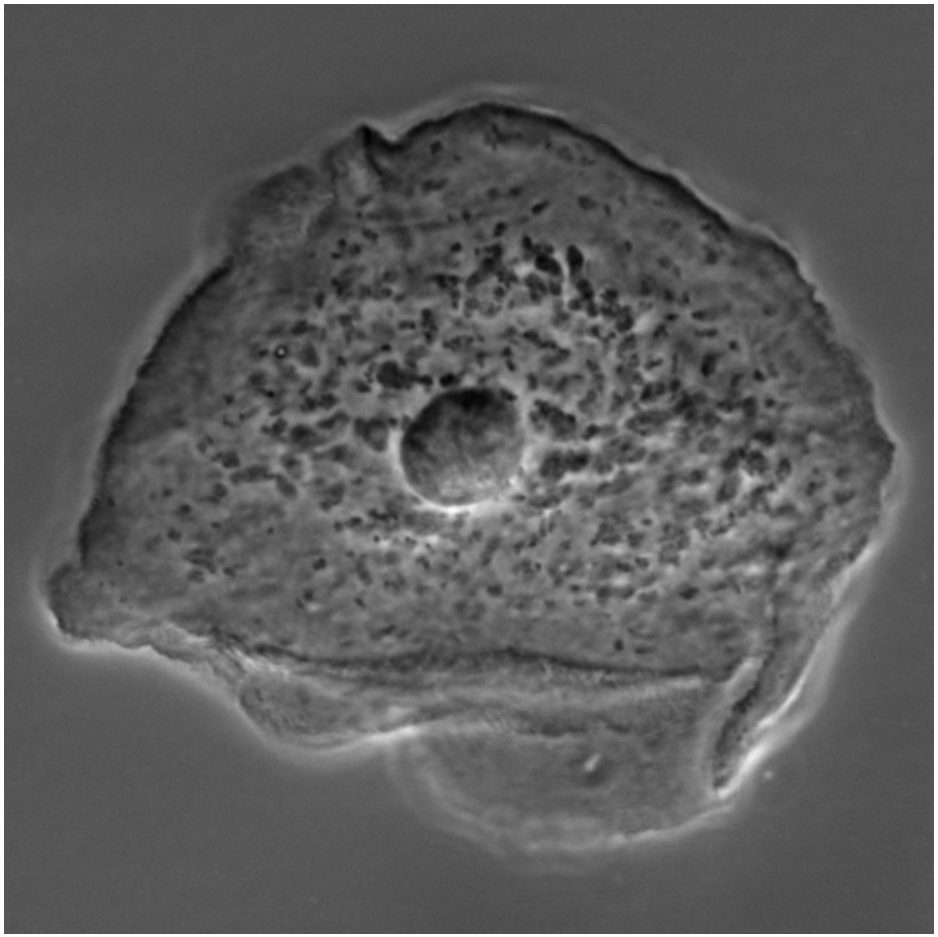
2.3 EMAN

Baylor College of Medicine

VERNA & MARRS McLEAN DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

Epithelial Cell (cheek)

(Optical Phase Contrast Microscopy)



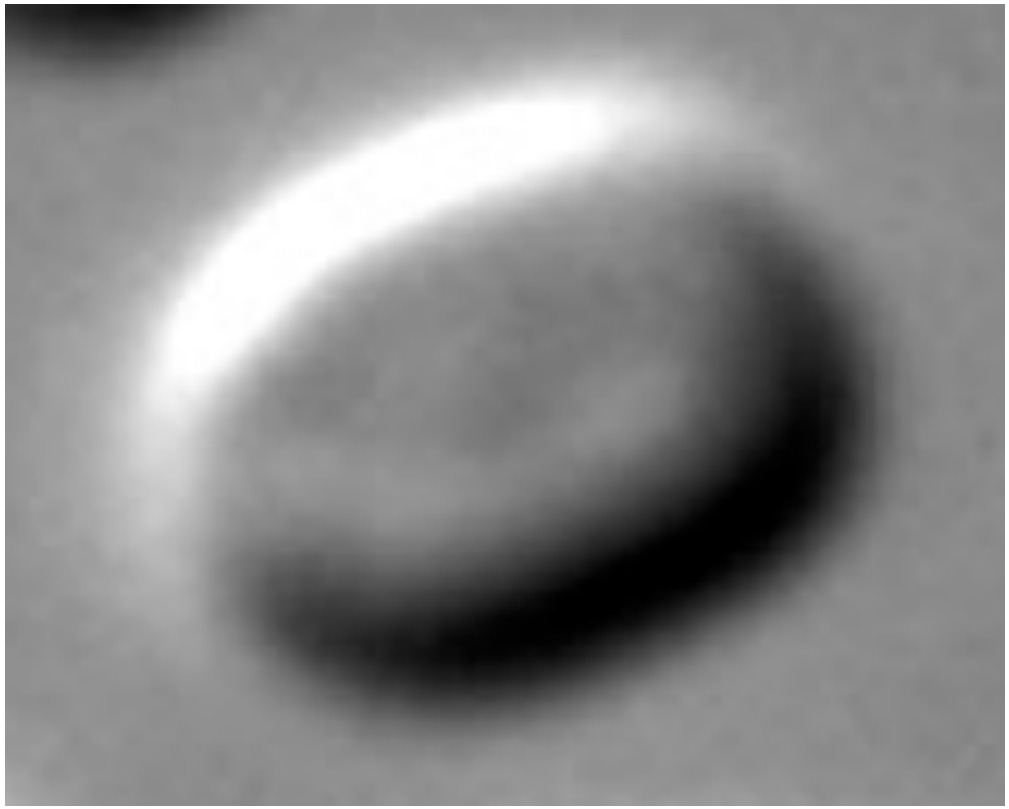
At nm resolution:
100,000 x 100,000 x 100,000
= 1 peta-voxels
(if the technology existed to do it)

~10 μm

http://calculatedimages.blogspot.com/2014_04_01_archive.html

Cyanobacteria

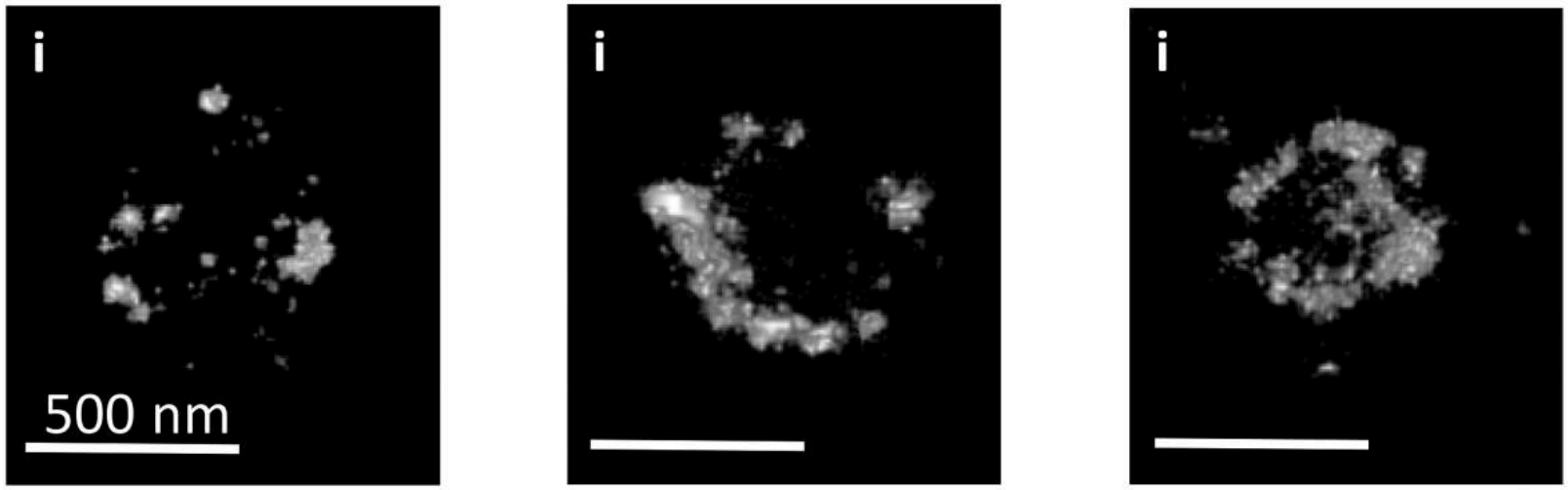
(Optical Microscope)



~0.5 μm

https://en.wikipedia.org/wiki/Synechococcus#/media/File:Synechococcus_PCC_7002_DIC.jpg

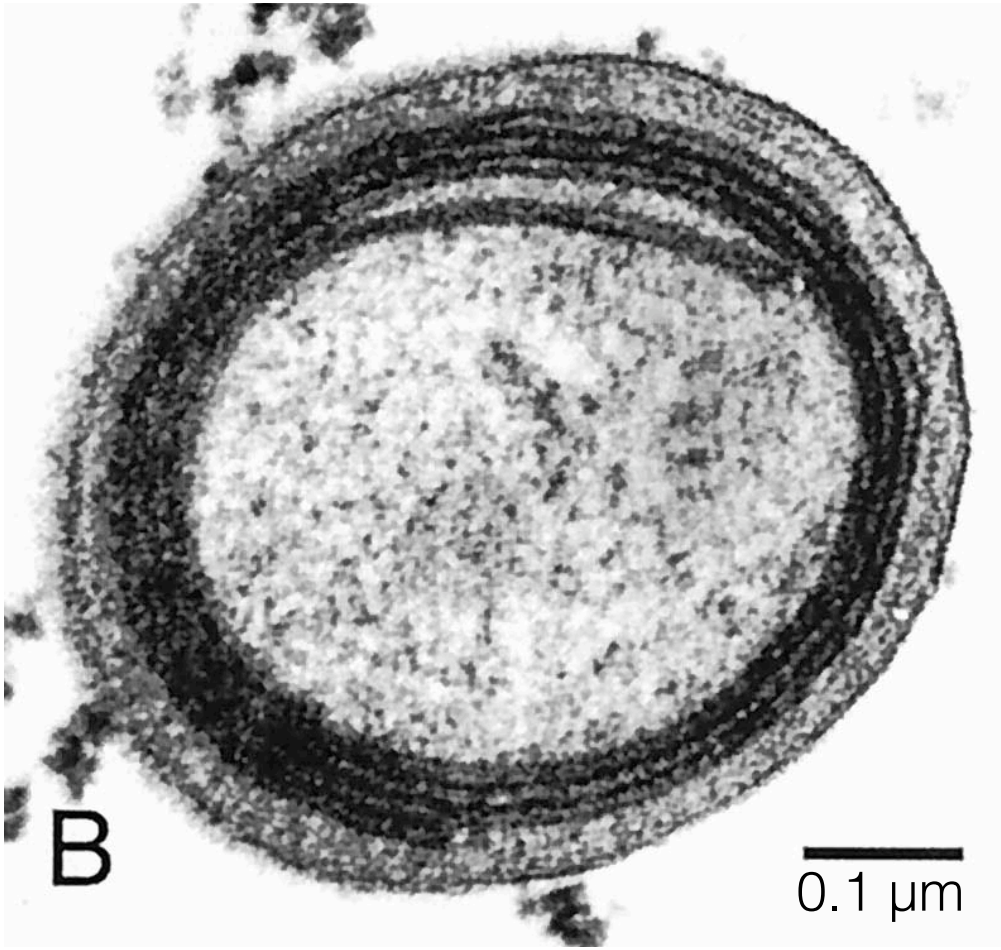
STORM Imaging of Prochlorococcus FtsZ Ring



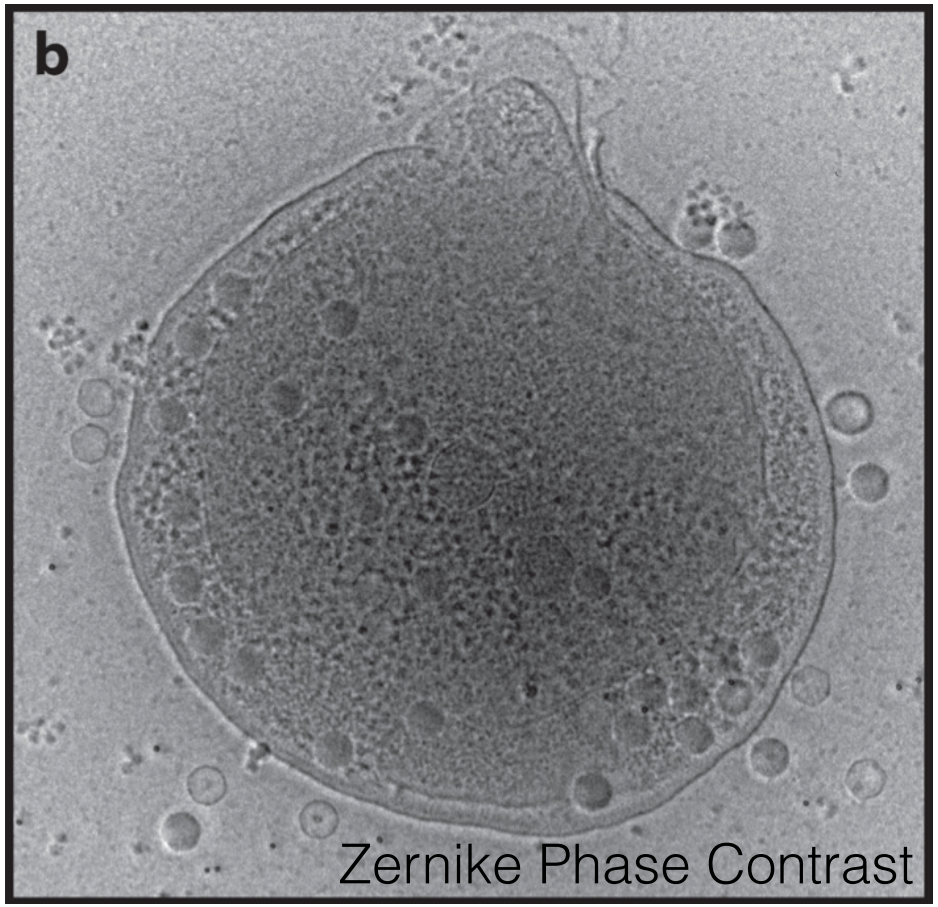
Liu R, Liu Y, Liu S, Wang Y, Li K, Li N, et al. Three-Dimensional Superresolution Imaging of the FtsZ Ring during Cell Division of the Cyanobacterium Prochlorococcus. Giovannoni SJ, editor. MBio; 2017 Nov 21;8(6):834. PMCID: PMC5698547

Cyanobacteria

Conventional Stained/Fixed Electron Microscopy



Phage Infection of Cyanobacteria

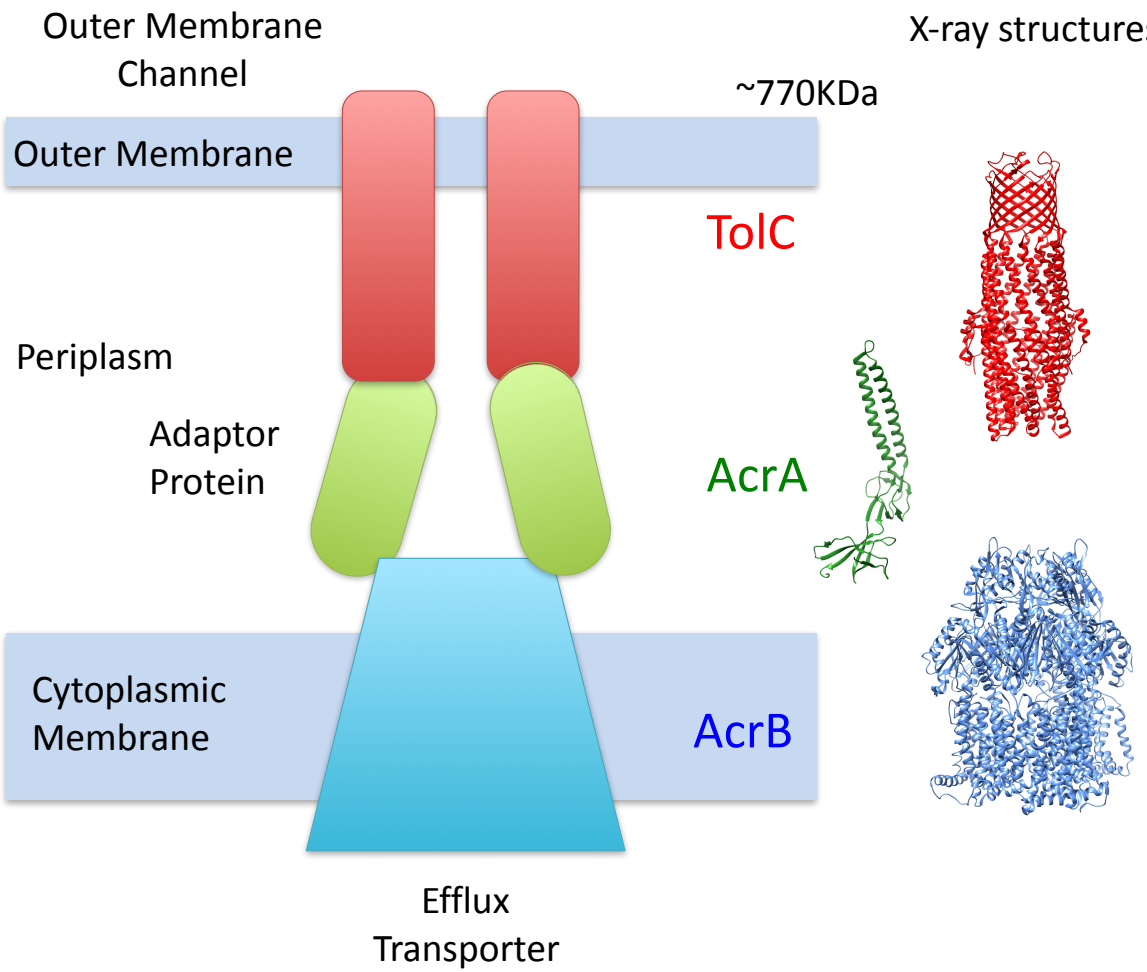


100 nm

Dai, W., et. al. (2013). Visualizing virus assembly intermediates inside marine cyanobacteria. *Nature*. 502:707-710.

5/2019, Ludtke, UTMB

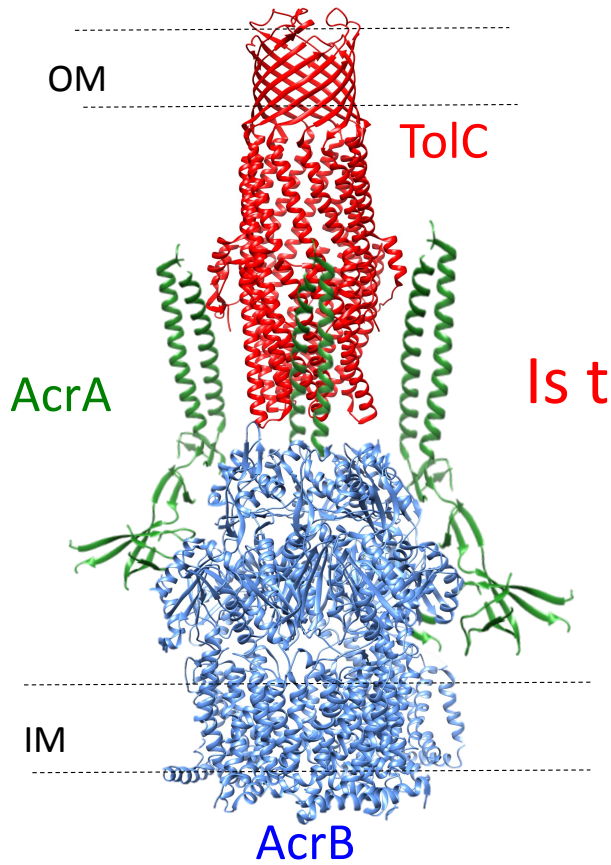
Efflux Pumps in Gram-Negative Bacteria:
Tripartite Complex



X-ray structures:

Proposed Model for Efflux Pump

Putative Ratio of TolC :AcrA :AcrB is 3:3:3



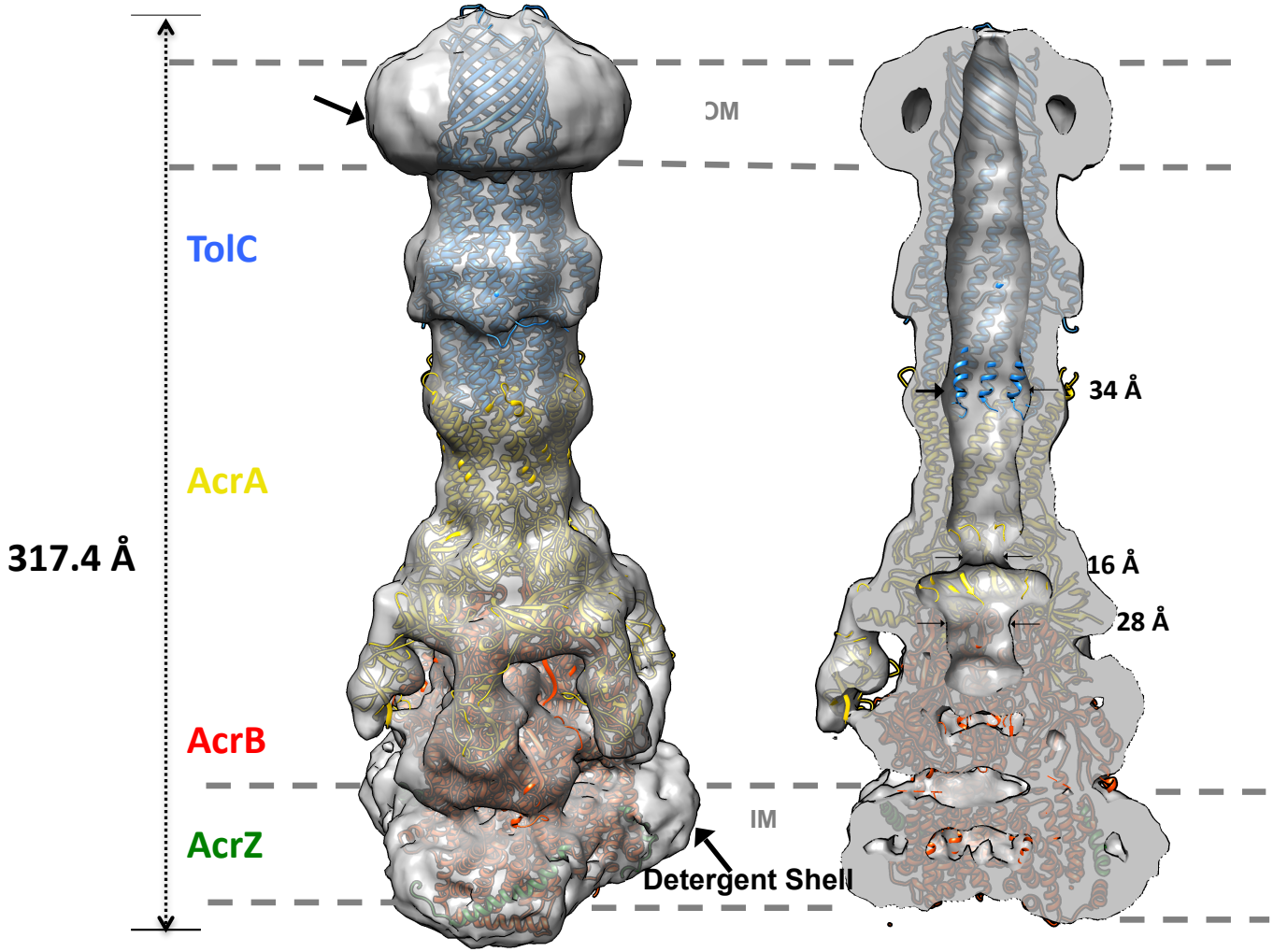
Is this model correct?

AcrAB-TolC

- Collaboration between Wang, Luisi and Ludtke Labs (currently)
- Structure of the AcrAB–TolC multidrug efflux pump. D Du, Z Wang, NR James, JE Voss, E Klimont, T Ohene-Agyei, H Venter, W Chiu, BF Luisi. *Nature*, 509, 512–515 (2014).
- An allosteric transport mechanism for the AcrAB-TolC Multidrug Efflux Pump. Z Wang, G Fan, CF Hryc, JN Blaza, II Serysheva, MF Schmid, W Chiu, BF Luisi, D Du. *eLIFE*, 6 (2017).
- In situ structure and assembly of the multidrug efflux pump AcrAB-TolC. X Shi, M Chen, Z Yu, JM Bell, H Wang, I Forrester, H Villarreal, J Jakana, D Du, BF Luisi, SJ Ludtke & Z Wang. *Nature Comm.,* 10, 2635 (2019).
- A complete data processing workflow for CryoET and subtomogram averaging. M Chen, JM Bell, X Shi, SY Sun, Z Wang & SJ Ludtke. *Nature Meth.*, 16, 1161–1168 (2019).

16Å resolution Cryo-EM (single particle) AcrABZ-TolC

l drift corrected

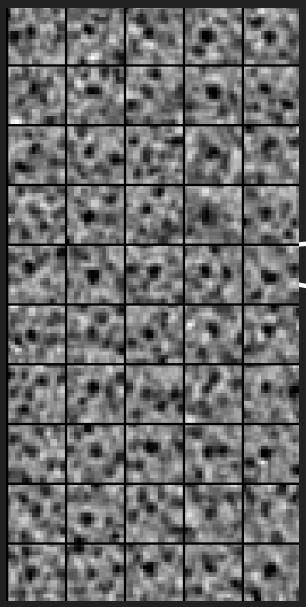


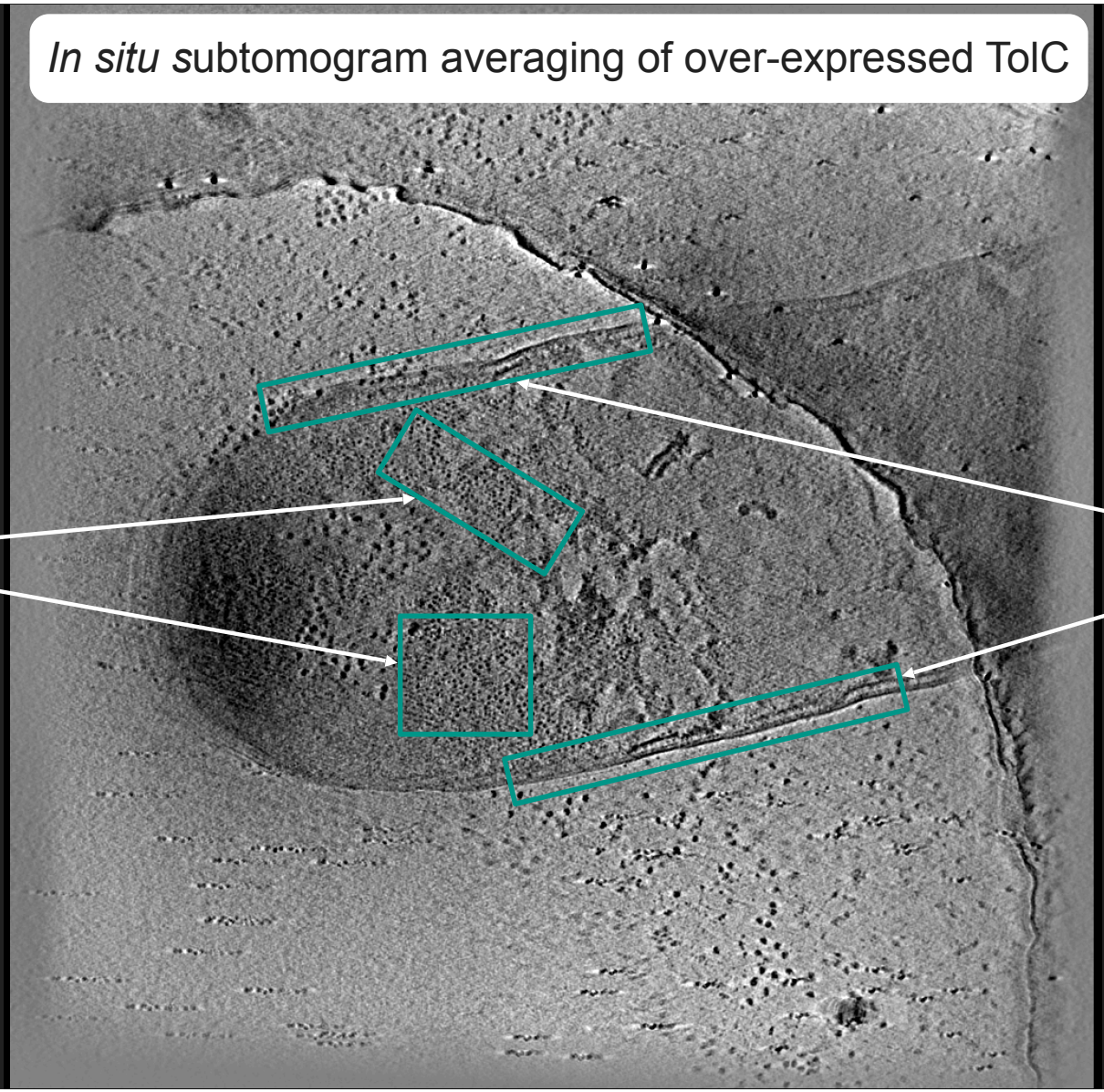
TolC :AcrA: AcrB = 3:6:3

D Du, Z Wang et al Nature 2014

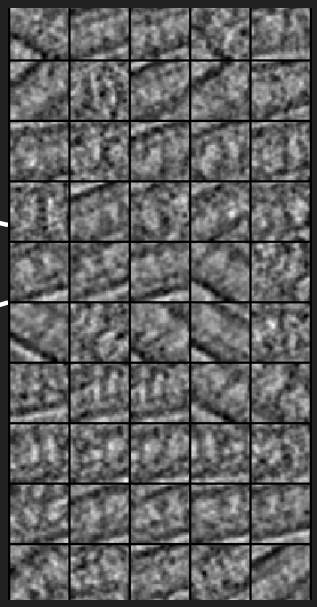
In situ subtomogram averaging of over-expressed TolC

~ top views



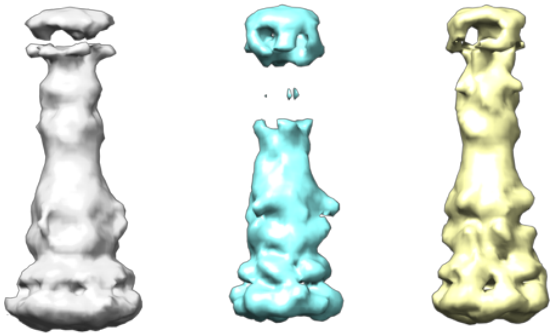


~ side views



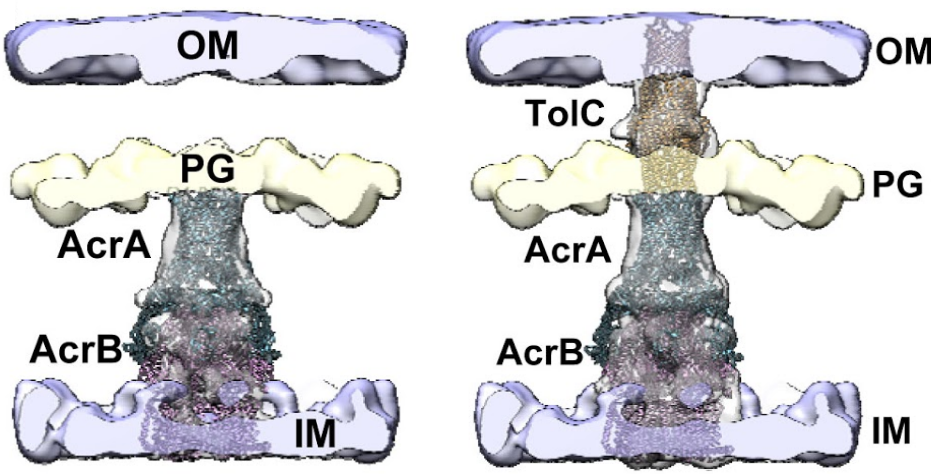
Unpublished,
X Shi, Z Wang, BCM

Outer Membrane



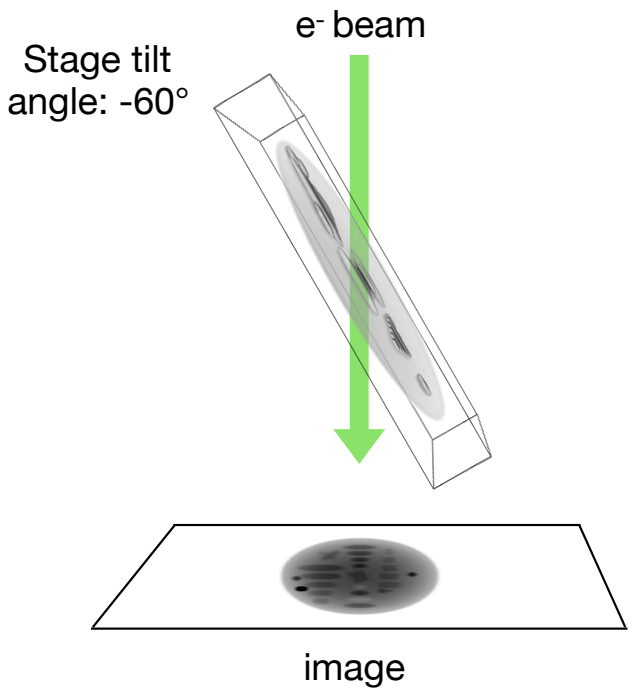
Focused classification results revealed a subset of partially assembled pumps

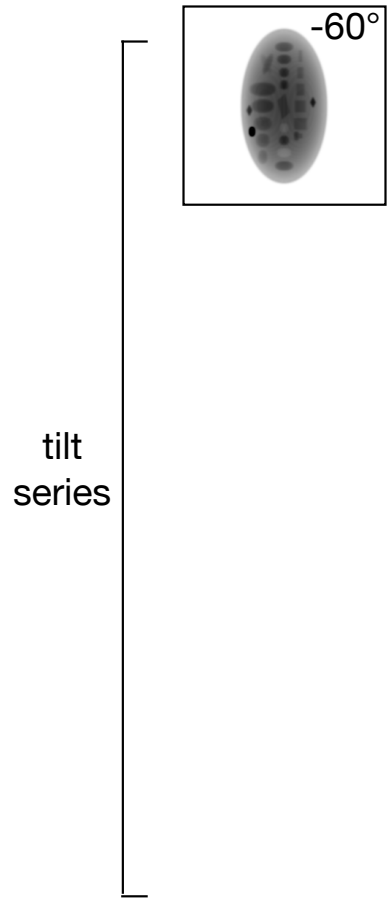
Mechanistic insights into the *in vivo* assembly of AcrAB-TolC



Paper in review
Xiaodong Shi, et al.

Electron cryo-tomography (cryoET)





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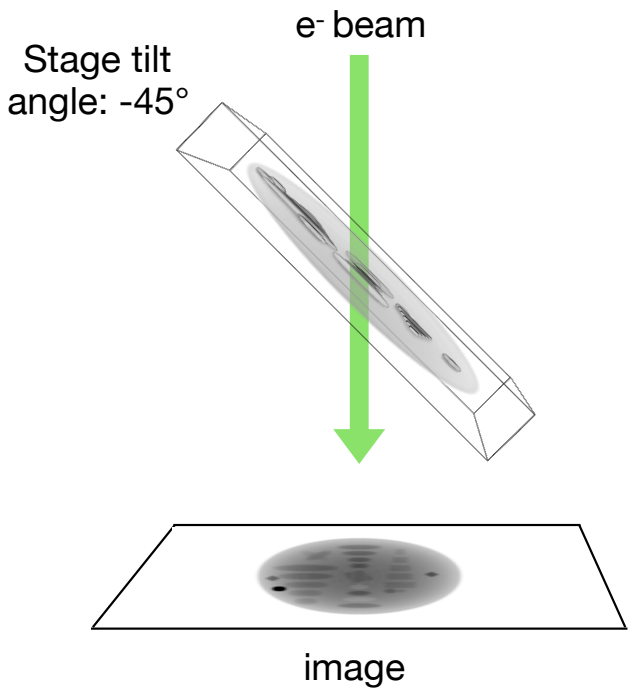
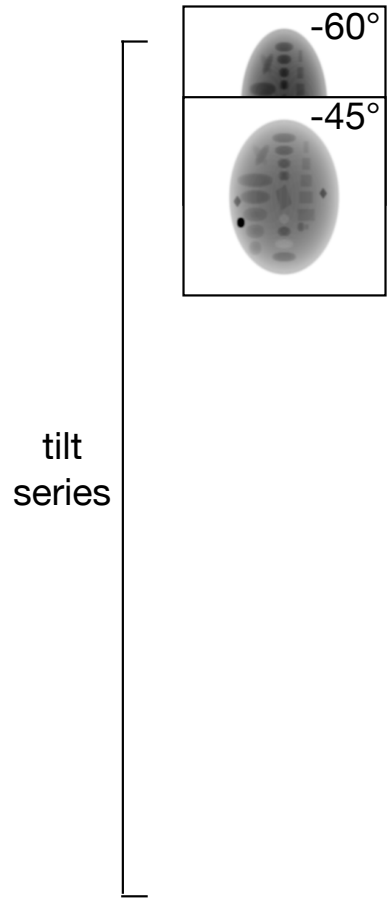
Electron cryo-tomography (cryoET)





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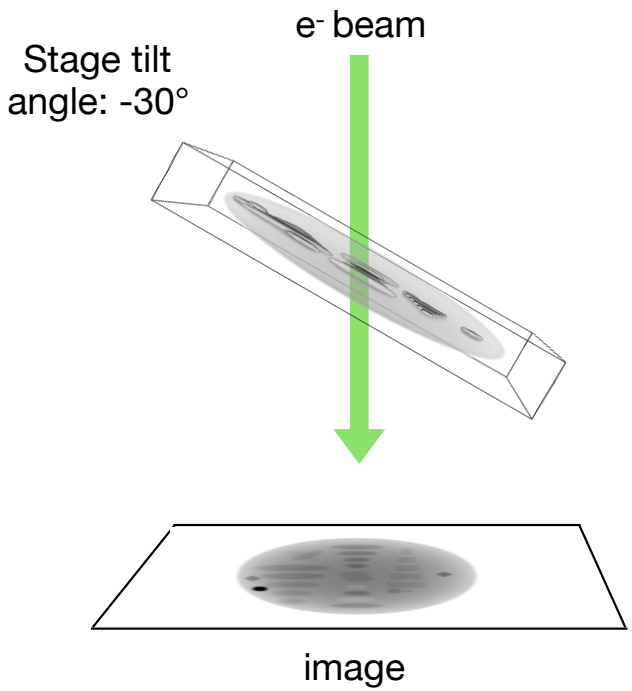
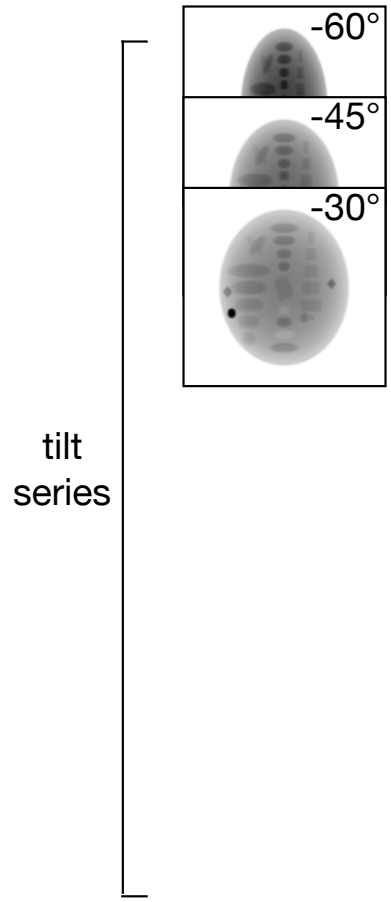
Electron cryo-tomography (cryoET)





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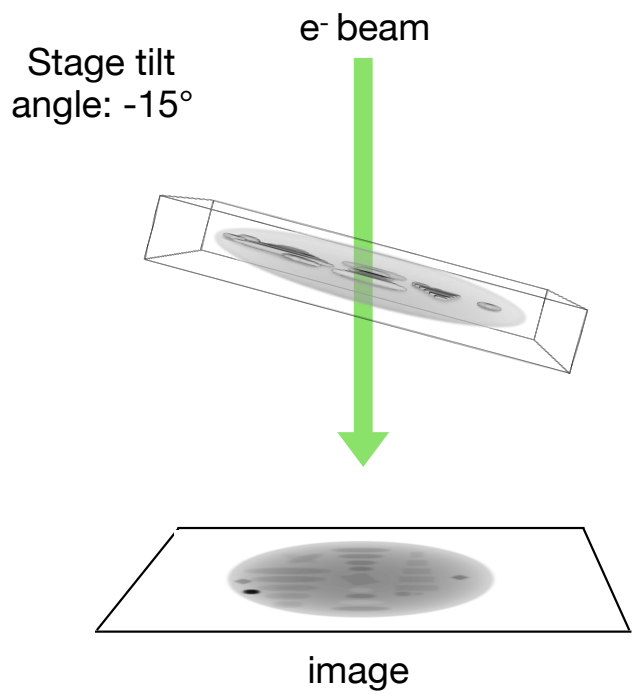
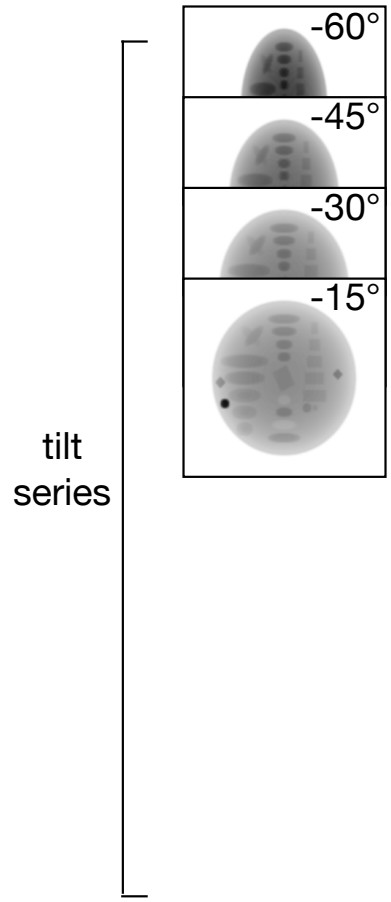
Electron cryo-tomography (cryoET)





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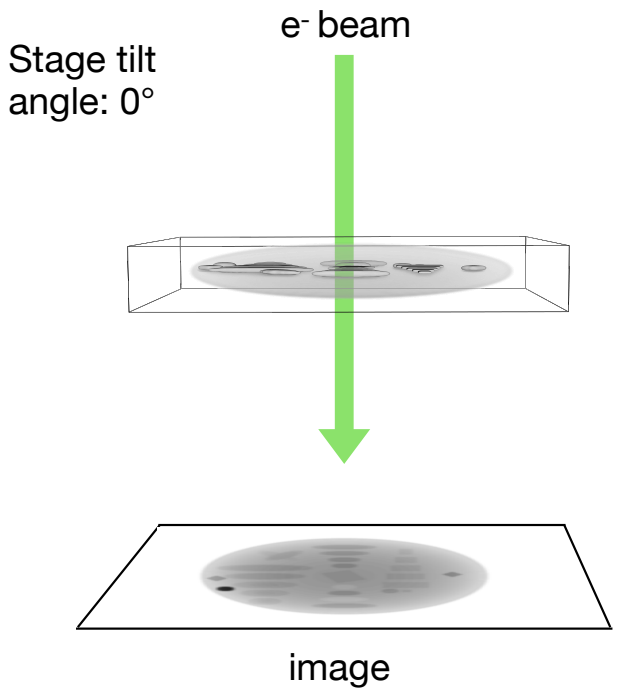
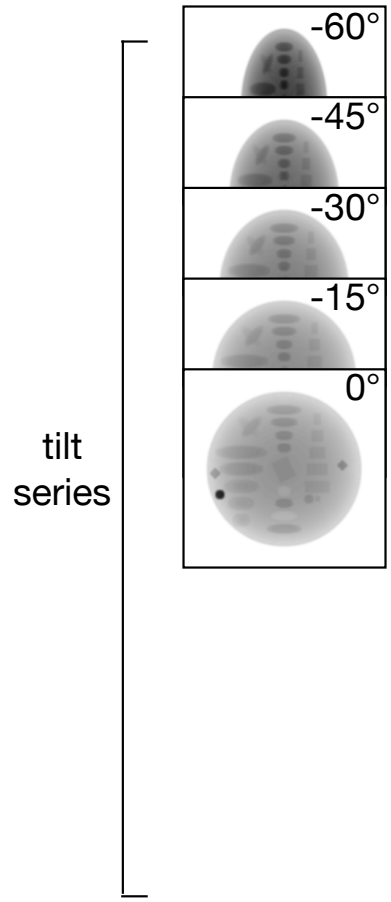
Electron cryo-tomography (cryoET)





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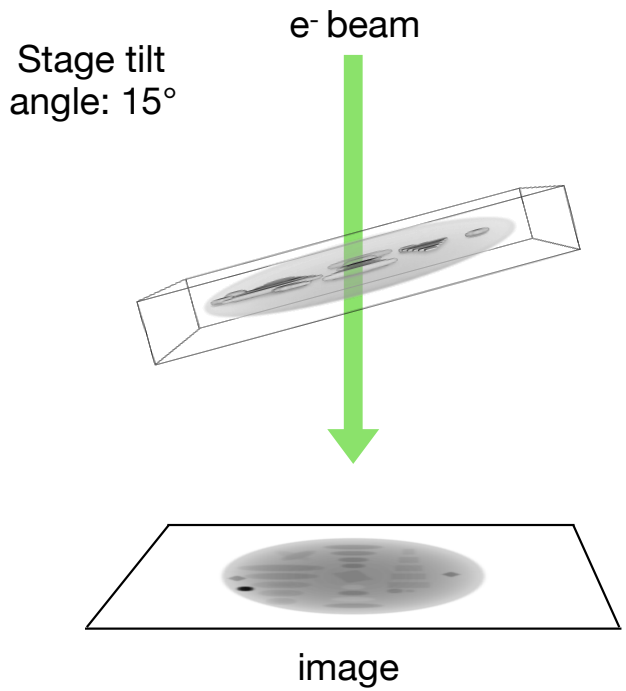
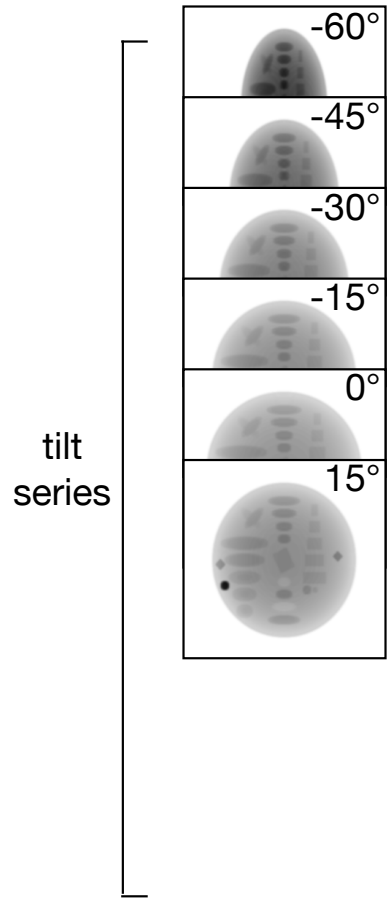
Electron cryo-tomography (cryoET)





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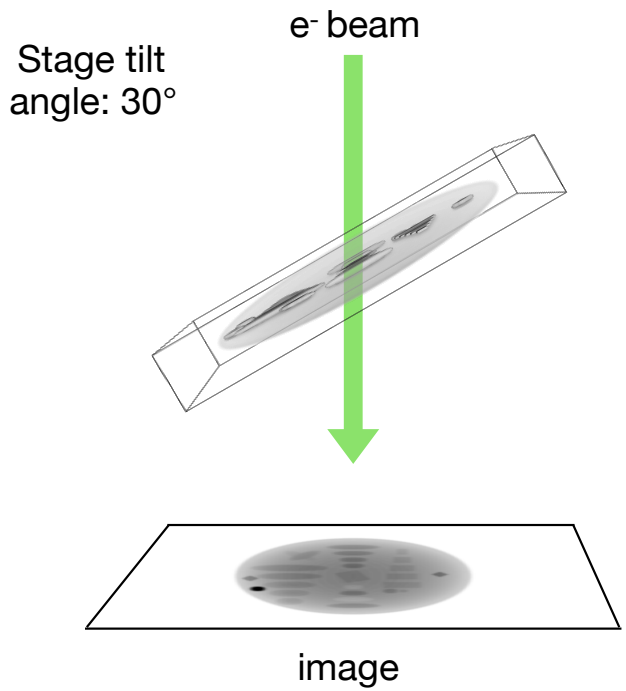
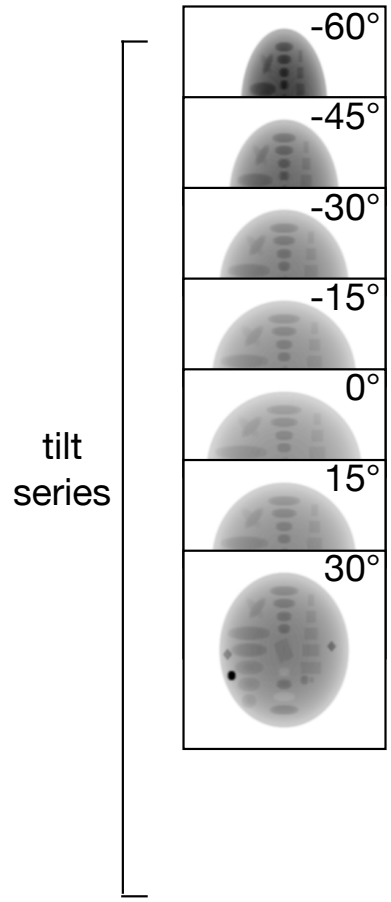
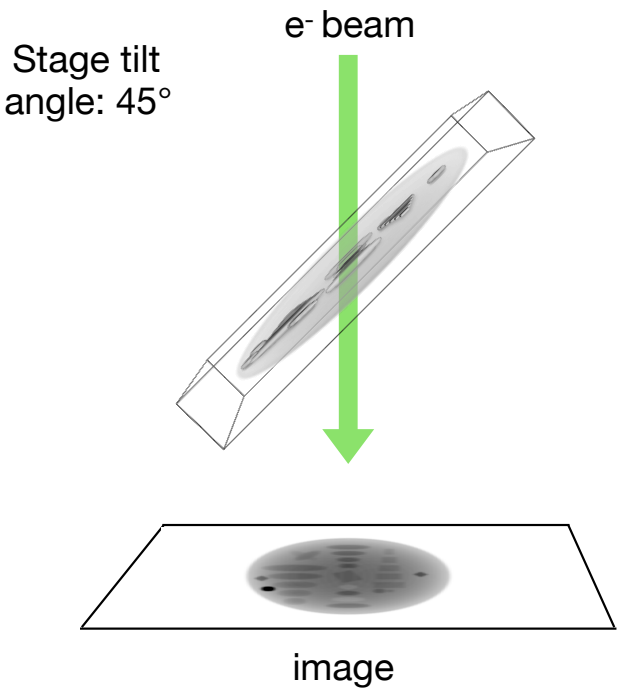
Electron cryo-tomography (cryoET)

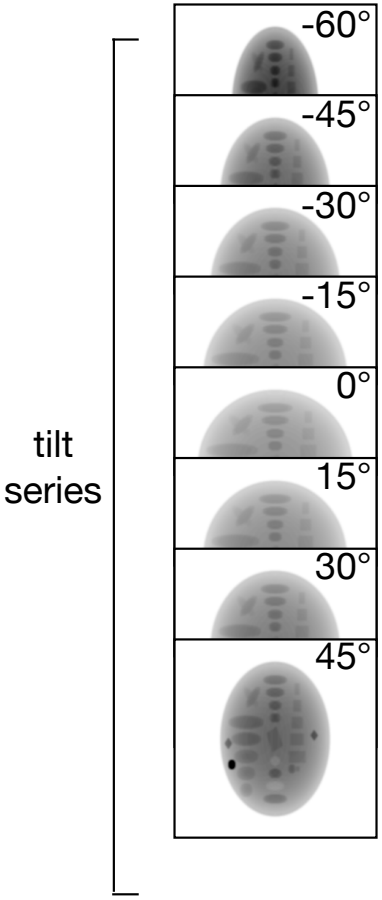




5/2019, Ludtke, UTMB

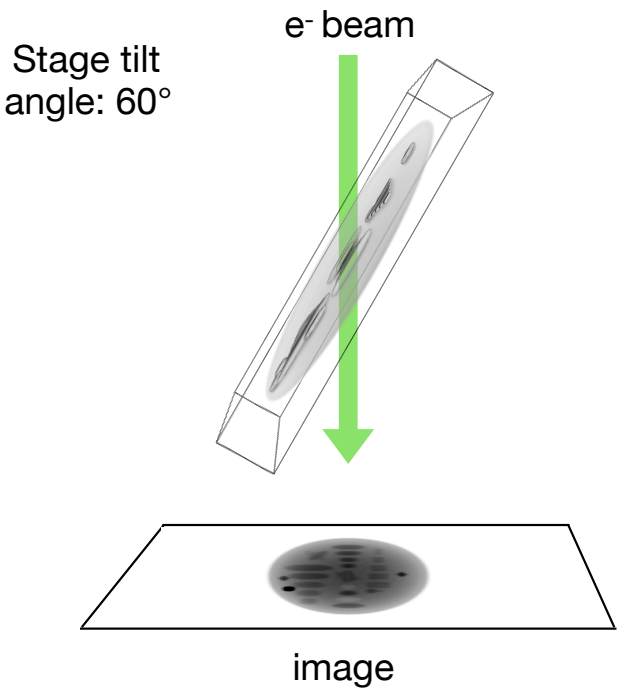
Electron cryo-tomography (cryoET)

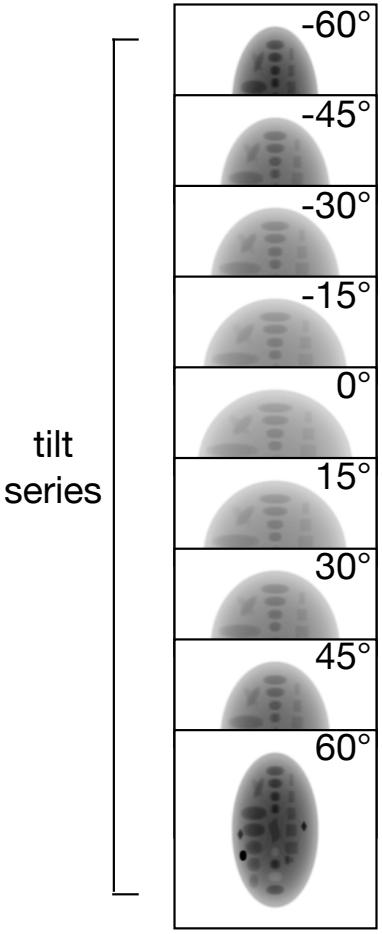




5/2019, Ludtke, UTMB

Electron cryo-tomography (cryoET)





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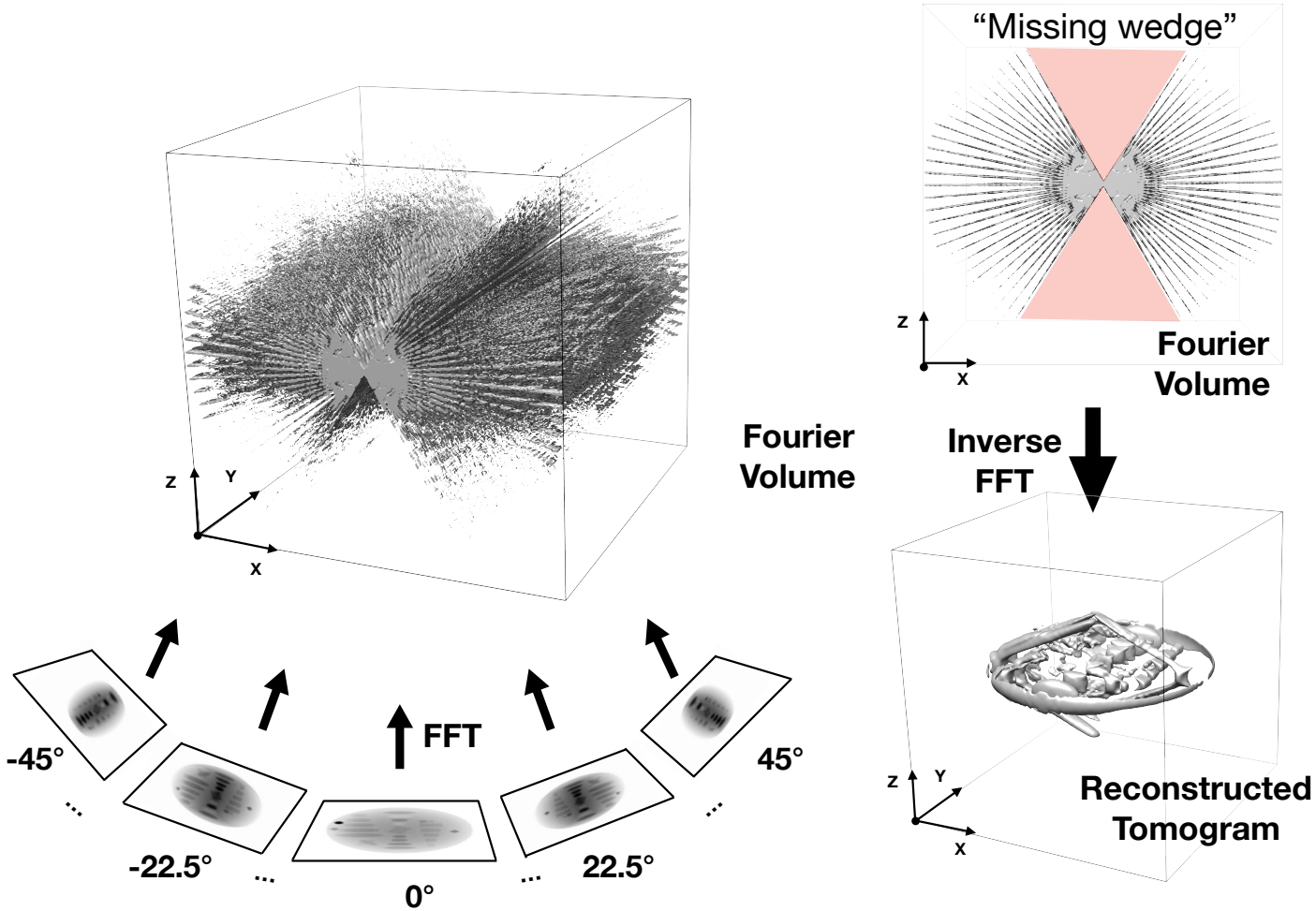
3D Fourier reconstruction



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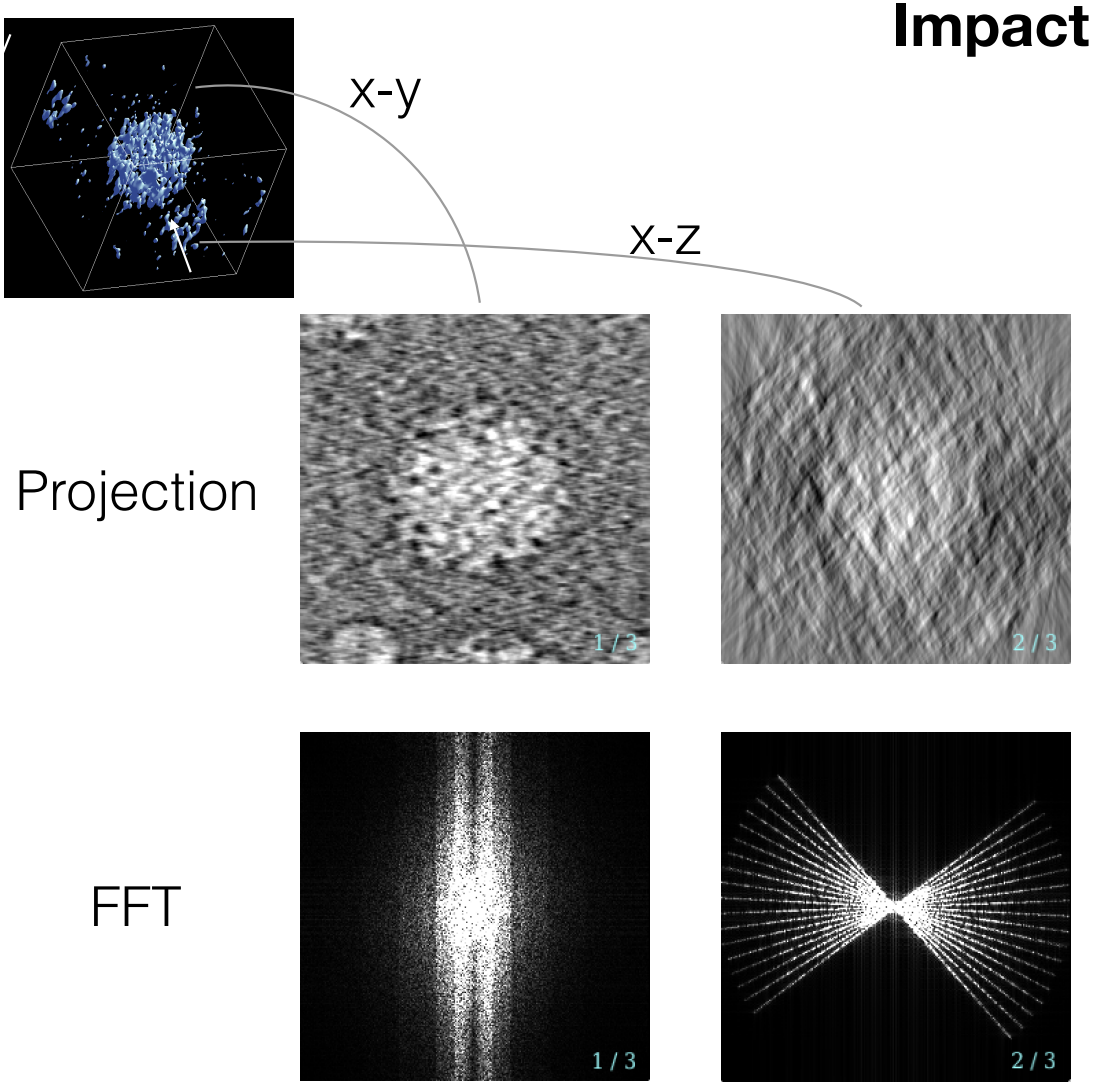
Impact of Missing Wedge



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Great, but things aren't that easy...

- Tilt series alignment, fiducials
- Specimen thickness problems (e^- beam penetration)
- 3-D reconstruction of 4k+ volumes
- Finding specific molecules in the cell
- Missing wedge impact on alignment and isotropy
- Good global alignment ≠ good local alignment
- Tilt-aware CTF correction

EMAN2 Tomography Tools

➡ Fiducial-free tilt series alignment

➡ Tiled Fourier Reconstruction

➡ Tilt series CTF estimation and correction

➡ Manual, reference-based and deep-learning 3-D particle picking

- Deep learning based tomogram annotation

➡ SGD initial average generation

- Iterative subtomogram averaging

➡ High resolution subtomogram averaging with Per-particle per-tilt (PPPT) alignment and CTF refinement (sub-nm resolution *in-situ*!)

5/2019, Ludtke, UTMB

X Shi,
Z Wang,
BCM

Tilt series alignment

Start: Unaligned tiltseries

Coarse alignment

3D Reconstruction

Pick landmarks (3D)

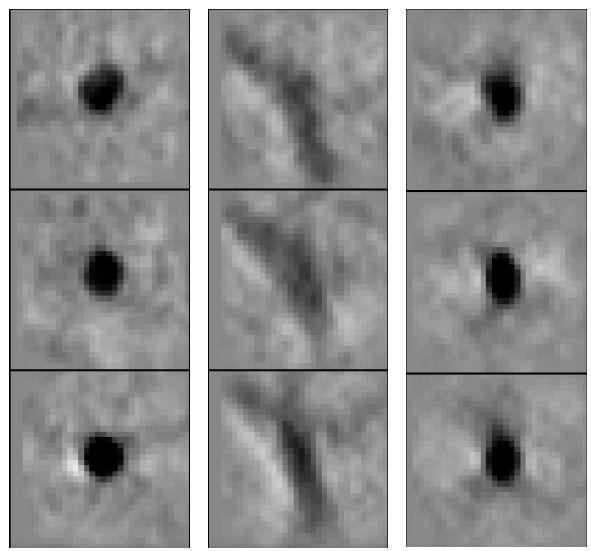
Refine landmark coordinates

Refine alignent parameters

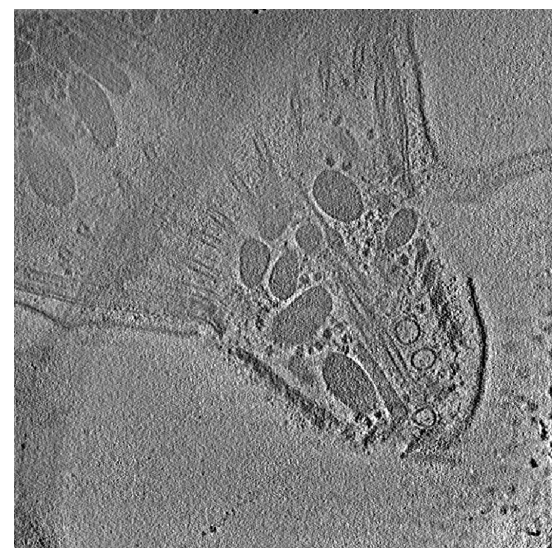
Bin by
8x
4x
2x
1x

Landmarks

Top view | **Side view coarse align** | **Side view iteration #4**



Tomogram slice view



Toxoplasma gondii

Stella Y. Sun

~5-10 min total per tomogram

Runtime:
~10 minutes on
12 threads

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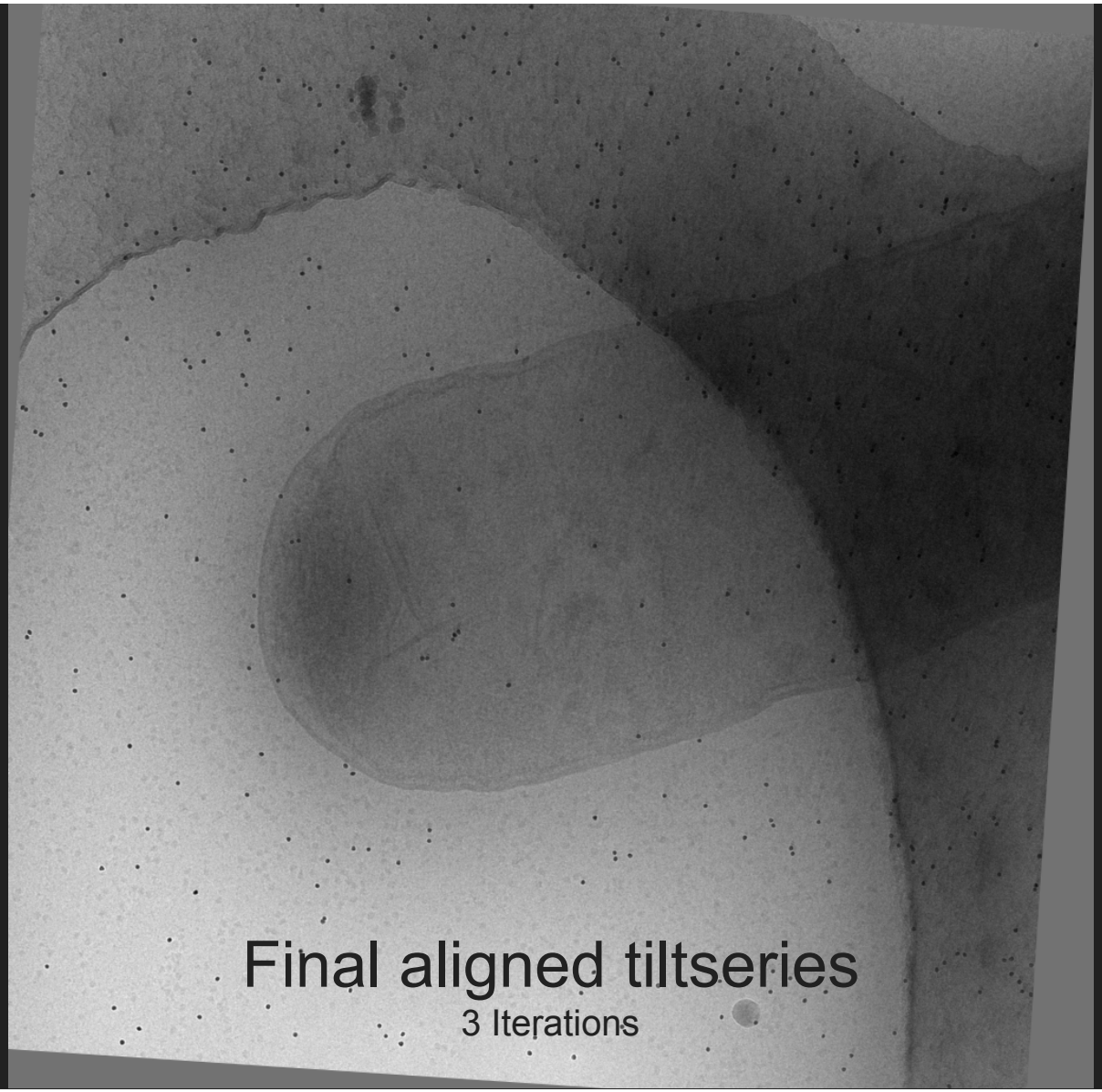
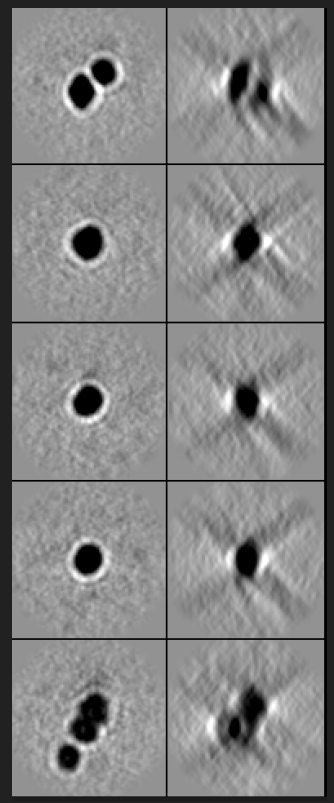
Z Wang,
BCM



Refined
landmarks
(bin x2)

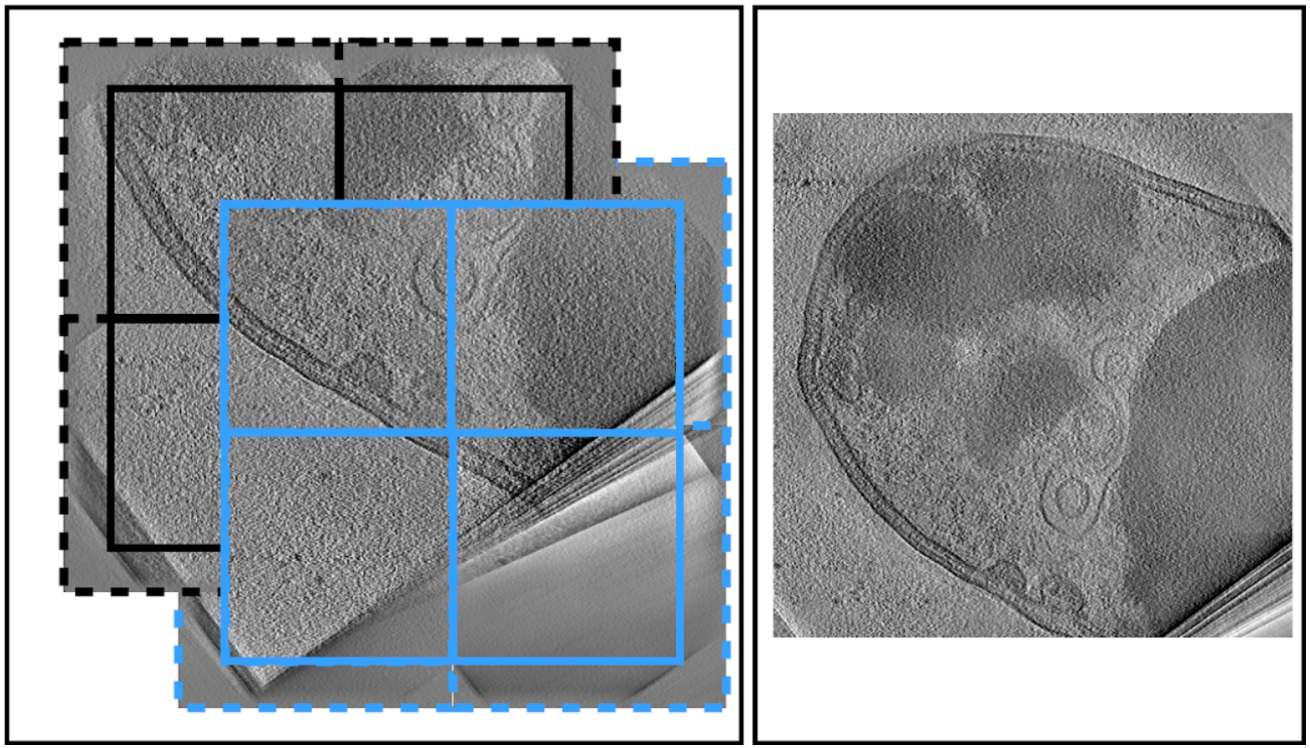


Y Z

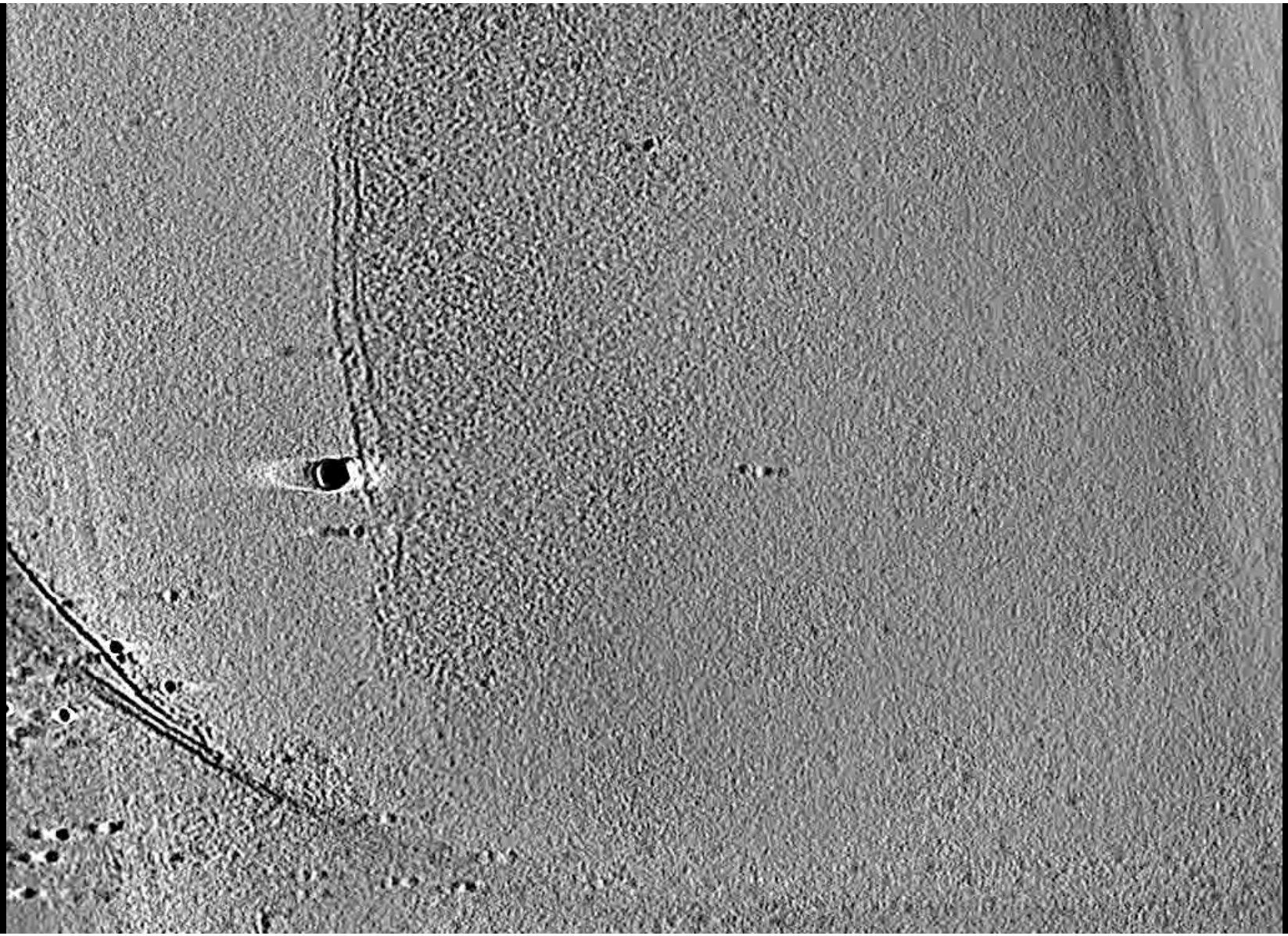
X X

Reconstruction via tiled direct Fourier inversion

- Reconstruction via tiled direct Fourier inversion
- Normally only generate 1K or 2K reconstructions
  -> <u>for visualization and annotation</u>



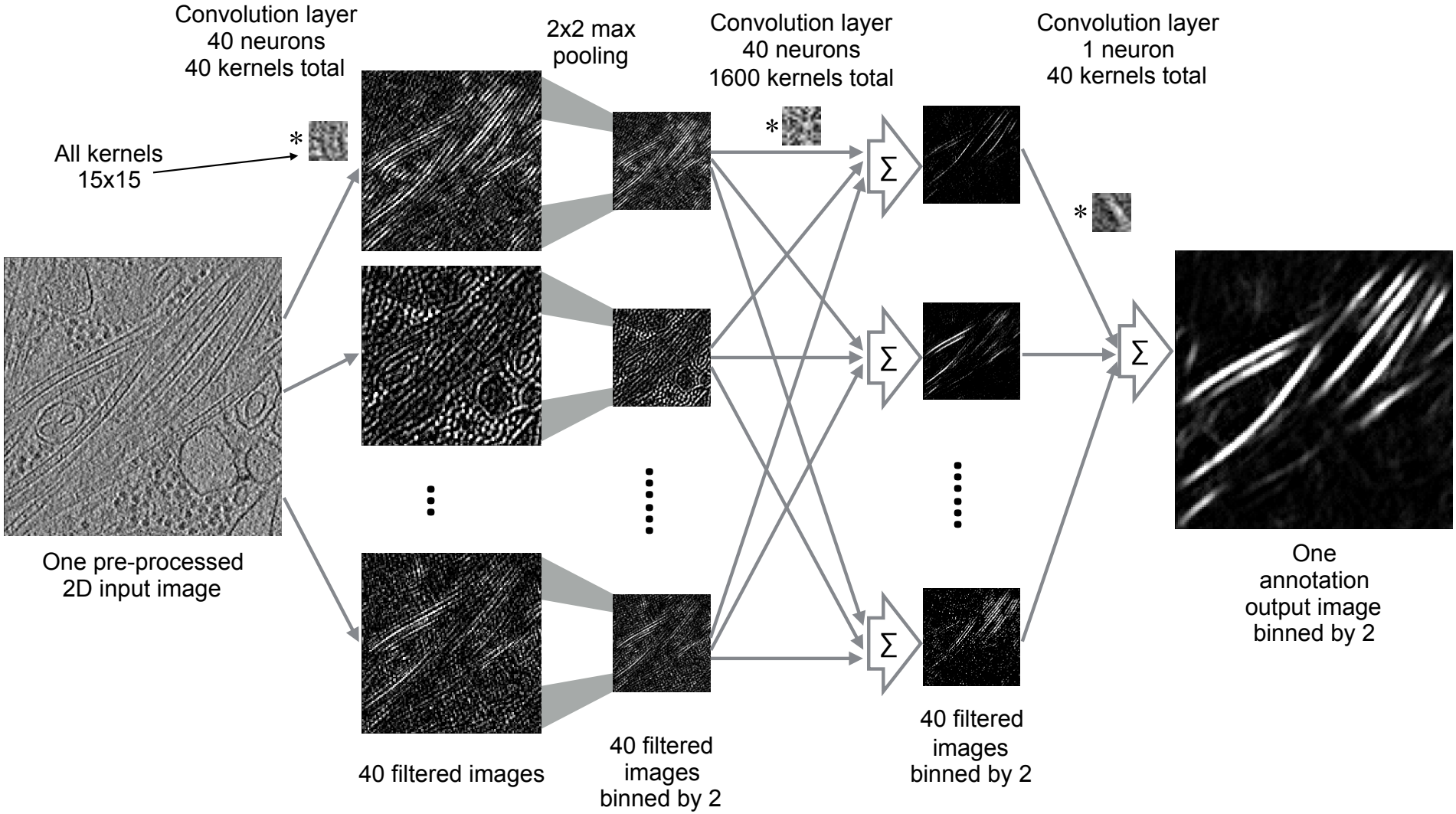

Halomicrobium mukohataei
https://etdb.caltech.edu/tomogram/df82d0



CryoET of PC12 Cells

UTMB

Convolutional Neural Network (CNN)



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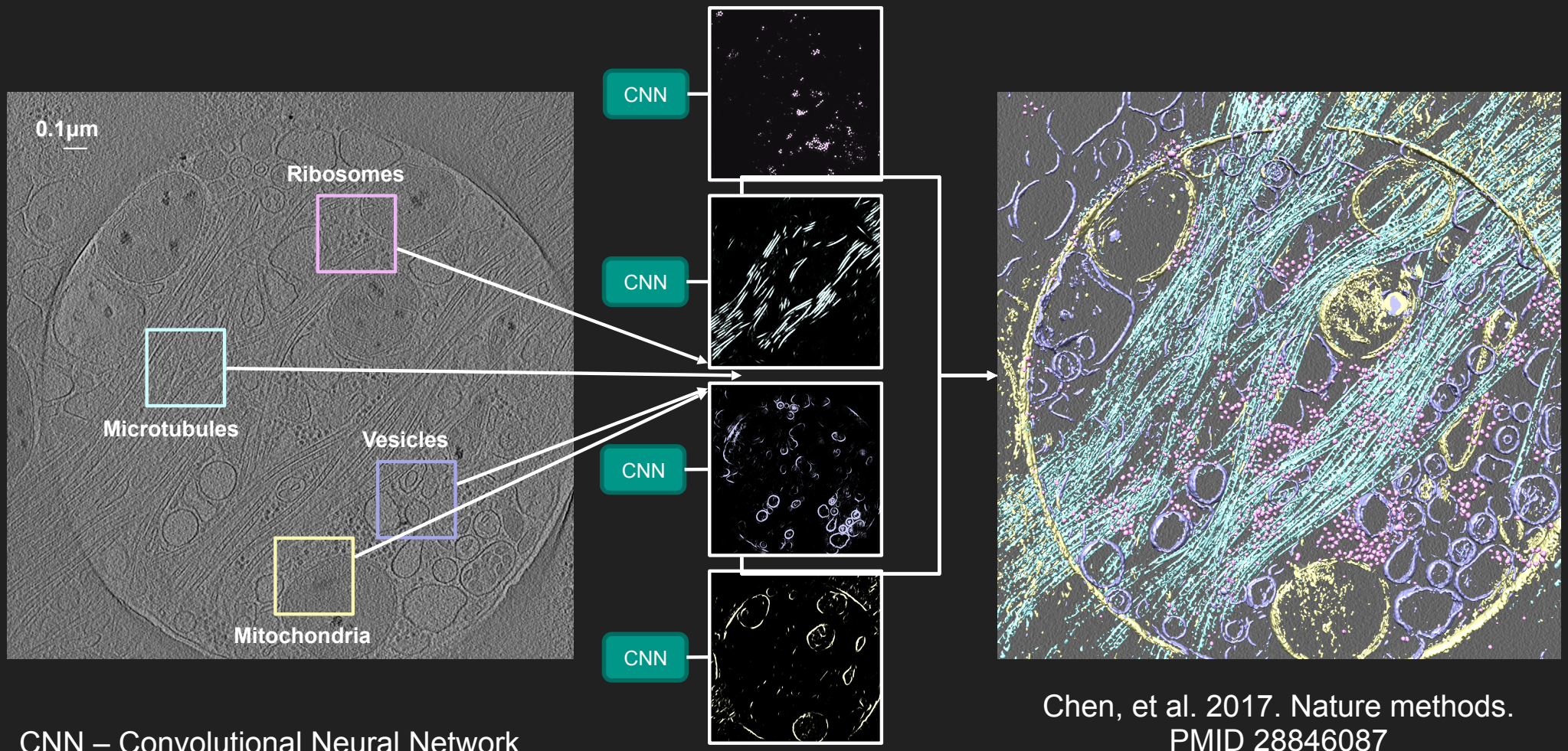
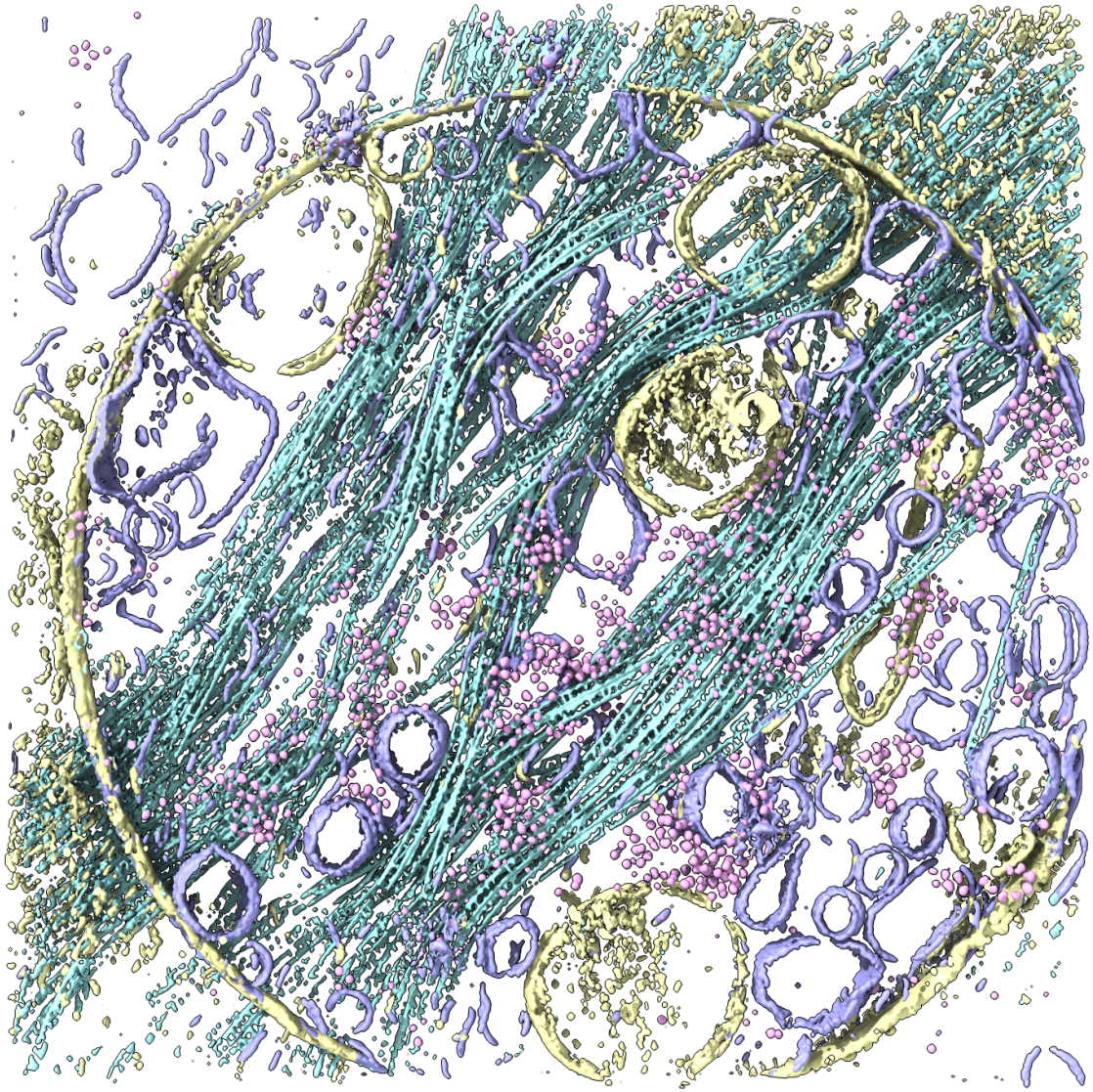
Deep learning-based cellular annotation



CNN – Convolutional Neural Network

Chen, et al. 2017. Nature methods.
PMID 28846087



5/2019, Ludtke, UTMB

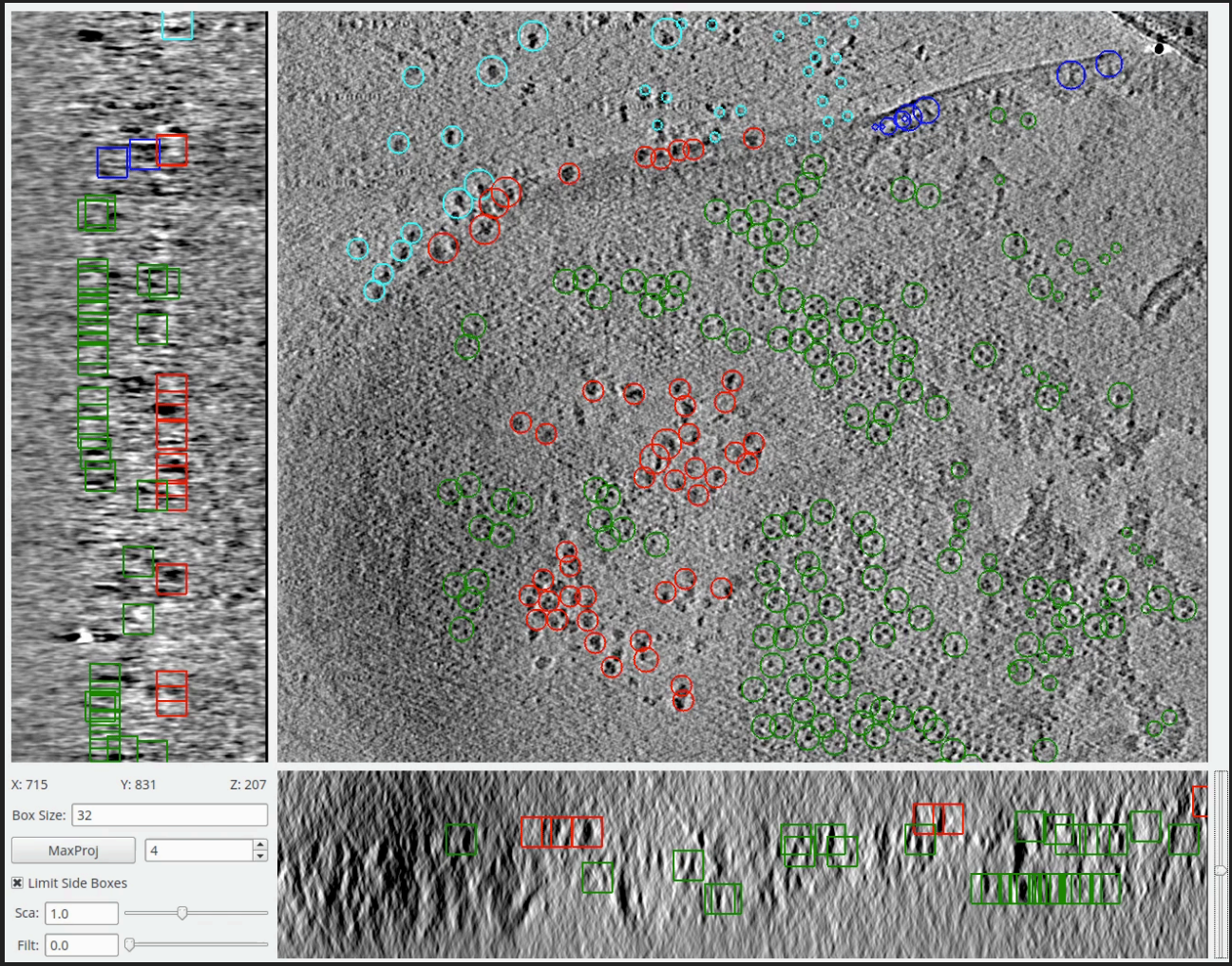
Multi-specimen subtomogram boxing

TolC - side

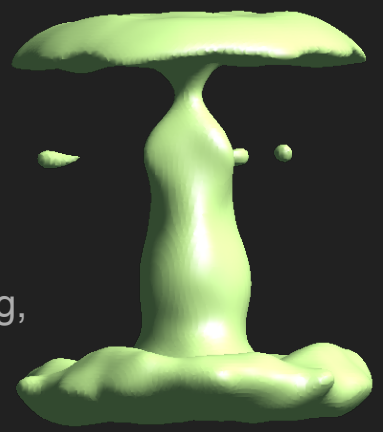
TolC - top

Ribo - in

Ribo - out

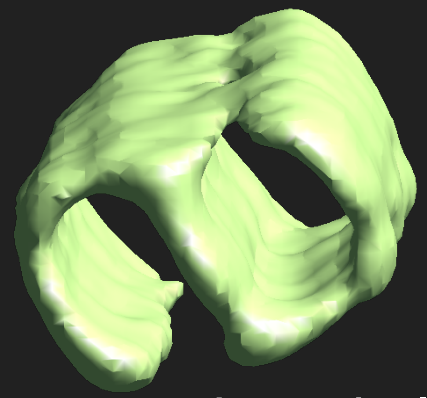


Reference-free initial model generation



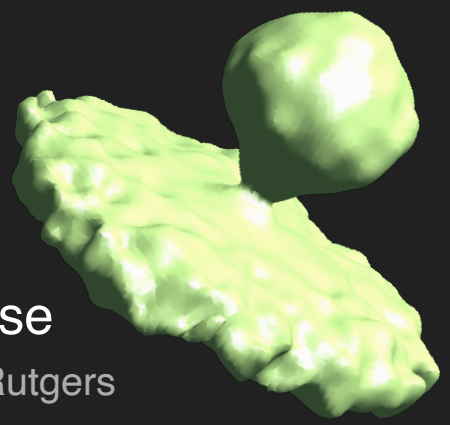
ToIC

X Shi,
Z Wang,
BCM



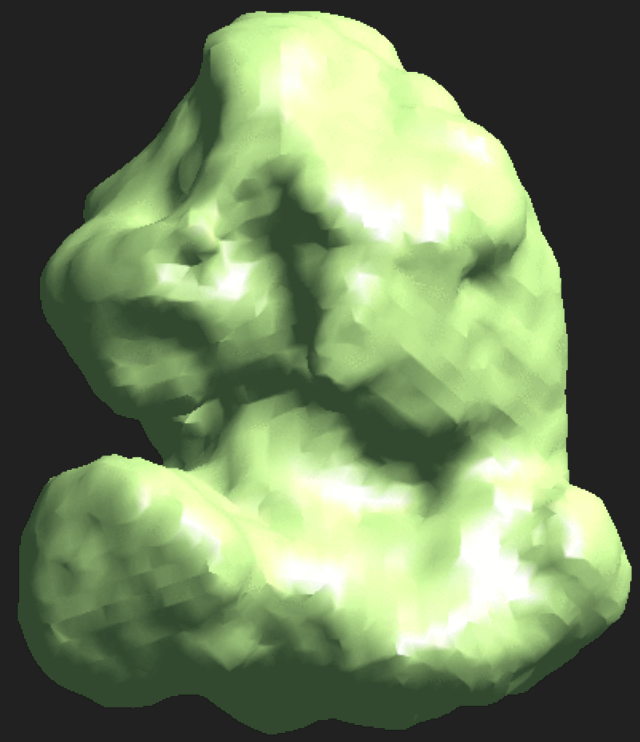
microtubule doublet

SY Sun, Stanford



ATP synthase

W Dai, Rutgers



80S Ribosome

EMPIAR 10064

Reconstruction Algorithms

- Back Projection
- Filtered Back Projection
- <u>Direct Fourier Inversion</u>
- SIRT
- SART
- ...

New EMAN2 PPPT Tomographic Refinement

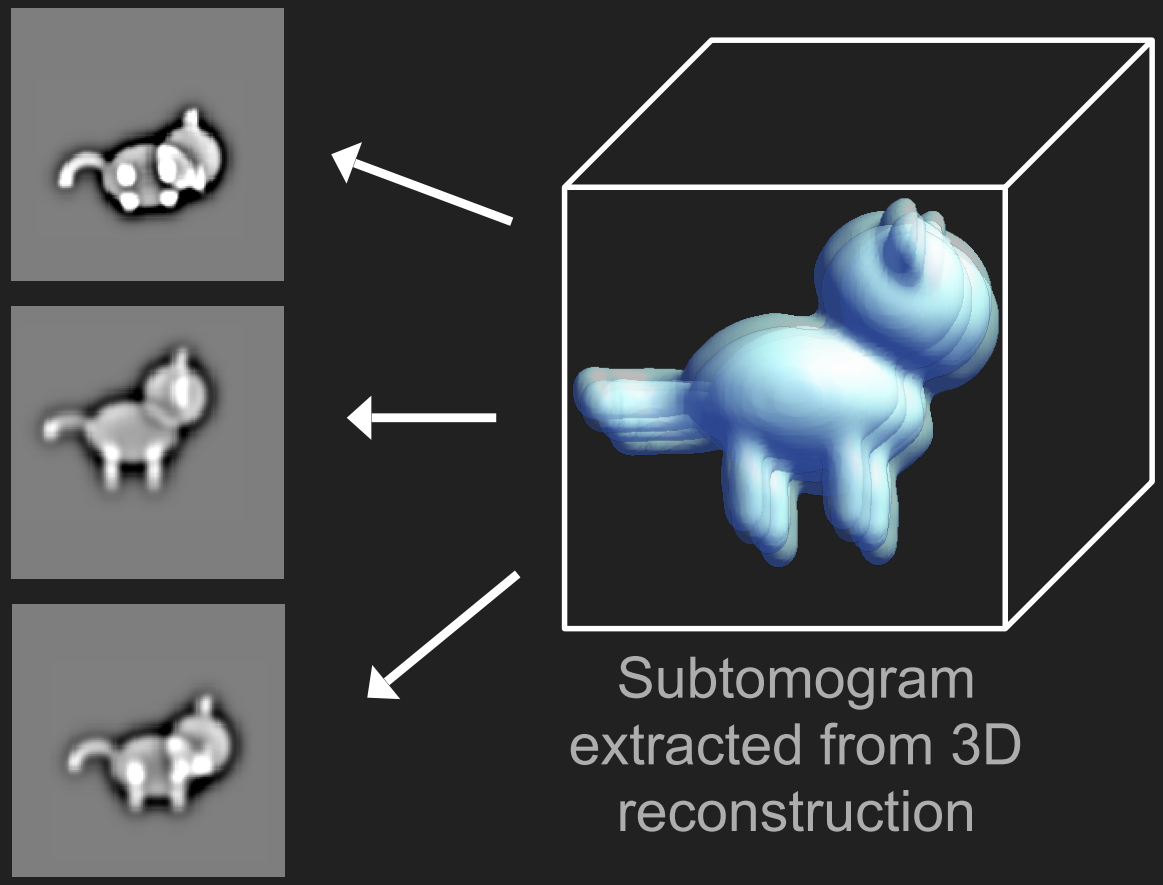
Individual particle tilt series:

- Bad tilt exclusion
- refine 3D alignment
- Per-tilt CTF correction

→ subnanometer resolution (with good data)

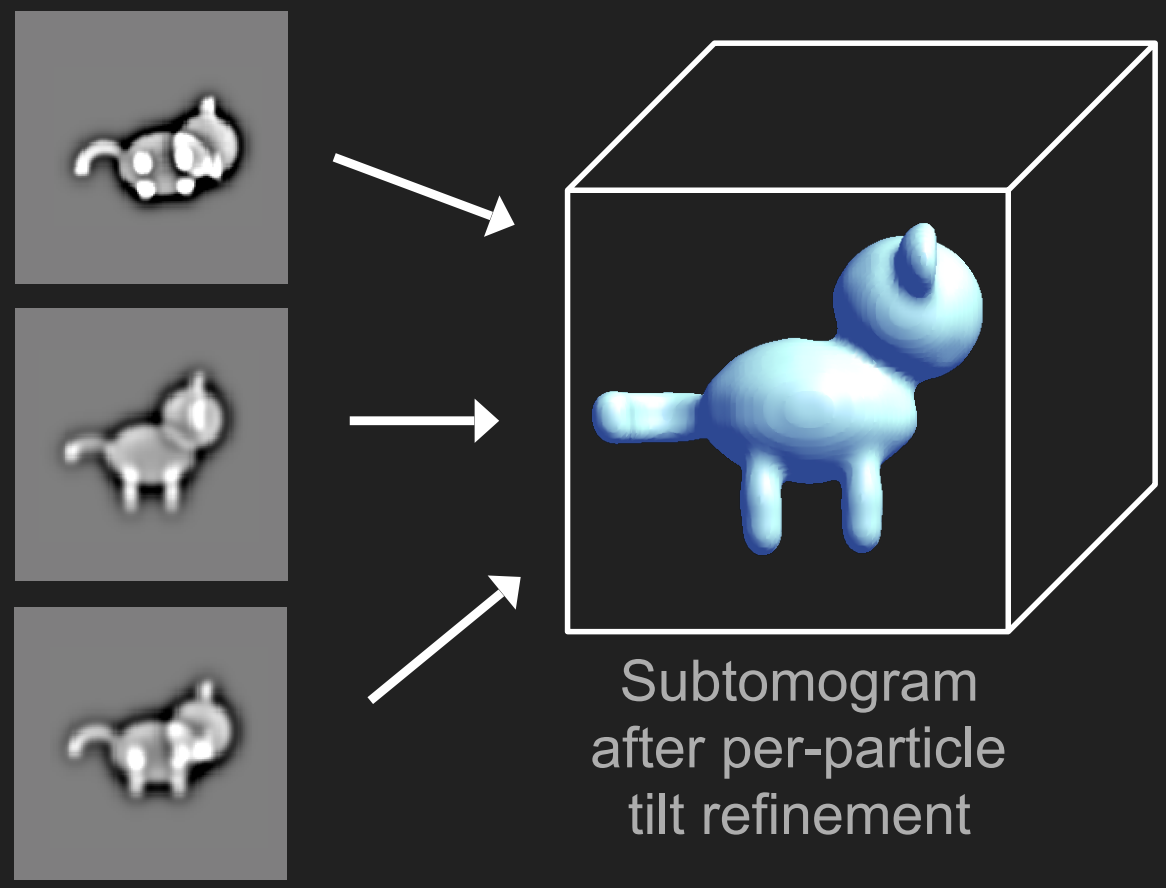
Particle tilt images
extracted from tiltseries

Per-particle
tilt refinement

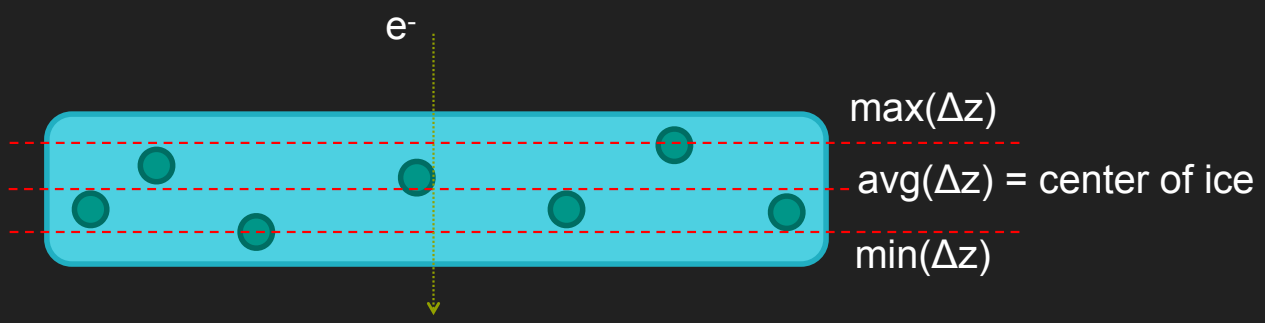


Subtomogram
extracted from 3D
reconstruction

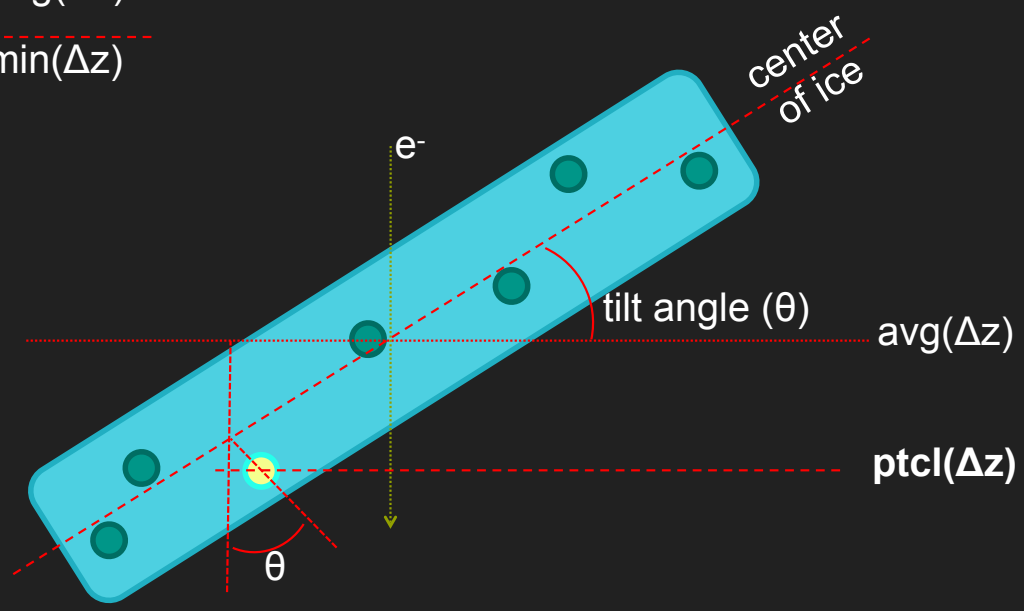
Per-particle tilt refinement



Per-particle, per-tilt CTF correction

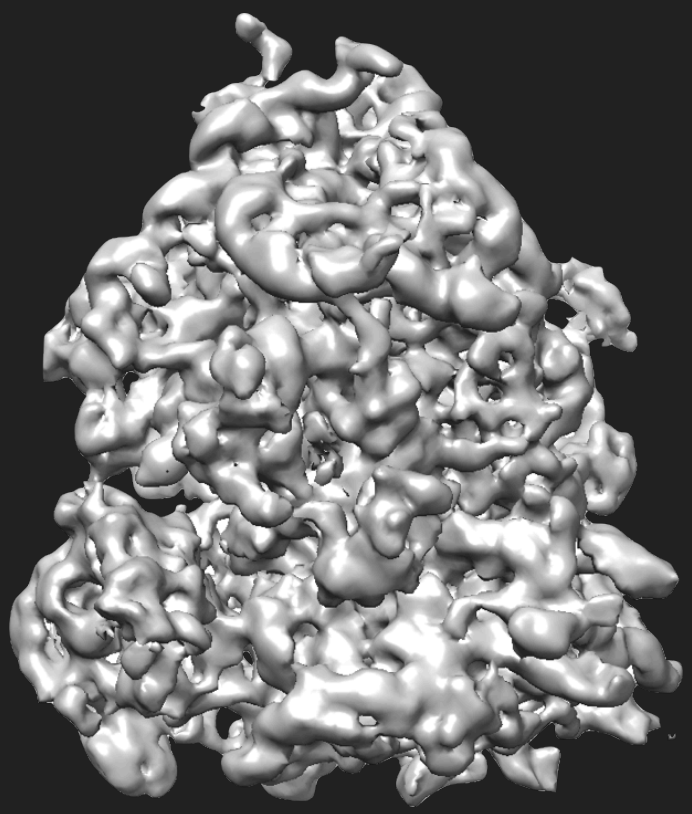


Determine defocus range from near-0° tilts with more signal

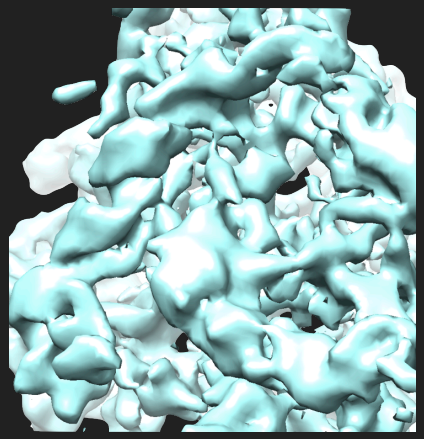


Search within defocus range at higher tilt angles.

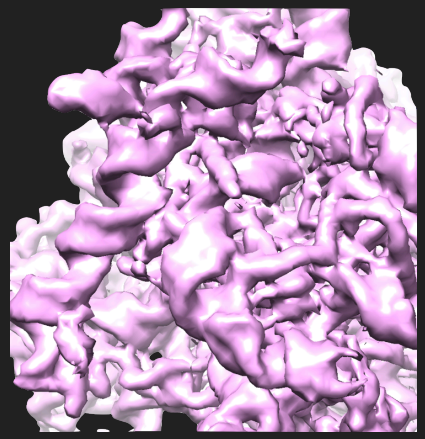
High-resolution subtomogram averaging in EMAN2



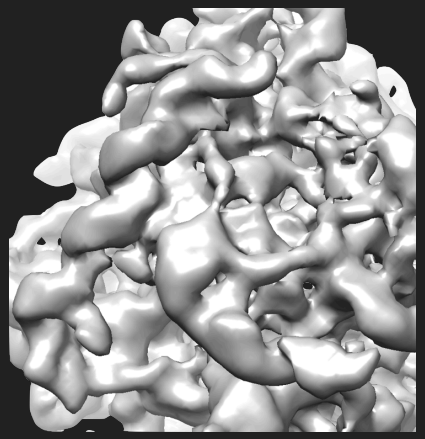
EMPIAR – 10064
80S Ribosome



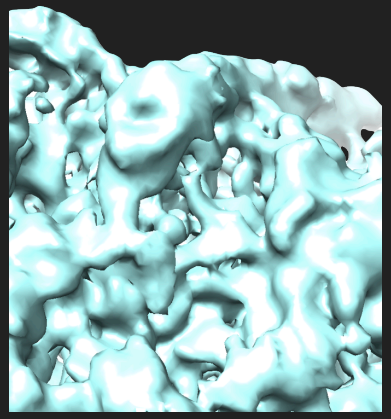
EMD-3420 - 11Å



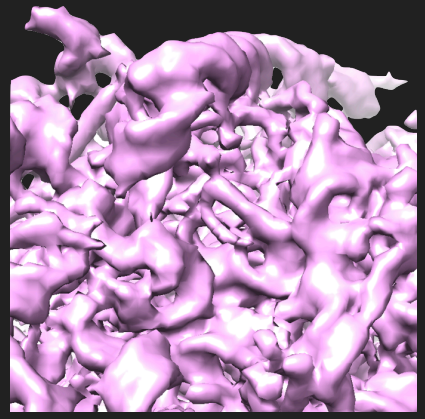
EMD-5592 - 6Å



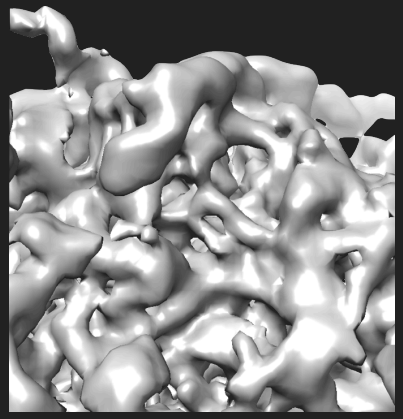
Nature Meth - 9Å



3D SPT - PyTom



Hi-res Single Particle



3D SPT – EMAN2

Future Directions *in-situ*

- Data collection protocols need to be optimized!
  - larger tilt step?
  - narrower tilt range?
  - Play with dose distribution?
- Movie-mode imaging -> ~1 $e^-/Å^2$ split into 10 or 20 frames!
- How much interference do we get from the cell with per-particle tilt series?
- Particle variability in cells (compositional and conformational)
- Dual-beam Cryo-FIB to address thickness issues

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