Cryo-electron tomography in EMAN2

Steve Ludtke

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Charles C. Bell Professor
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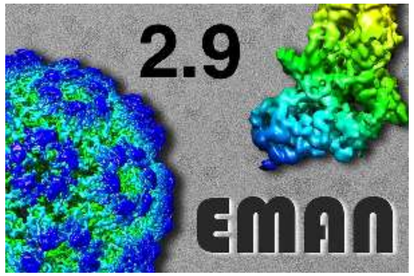
Biochemistry and Molecular Biology
Director, CryoEM/CryoET Core

Baylor College of Medicine

ADVANCED TECHNOLOGY CORES



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VERNA & MARRS McLEAN DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

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ADVANCED TECHNOLOGY CORES

Acknowledgements

TolC

Baylor College of Medicine

VERNA & MARRS MCLEAN DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

Zhao Wang (BCM)
Xiaodong Shi
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Mary Chang

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Tomography Pipeline

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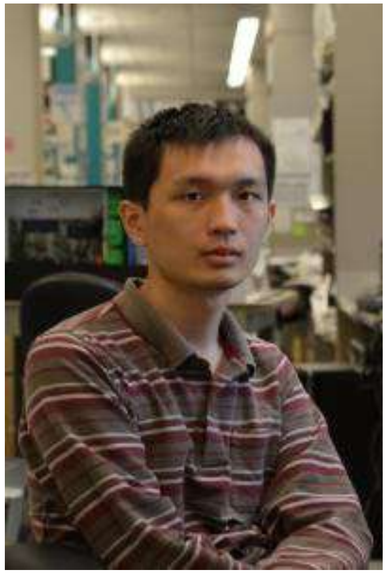


Muyuan Chen

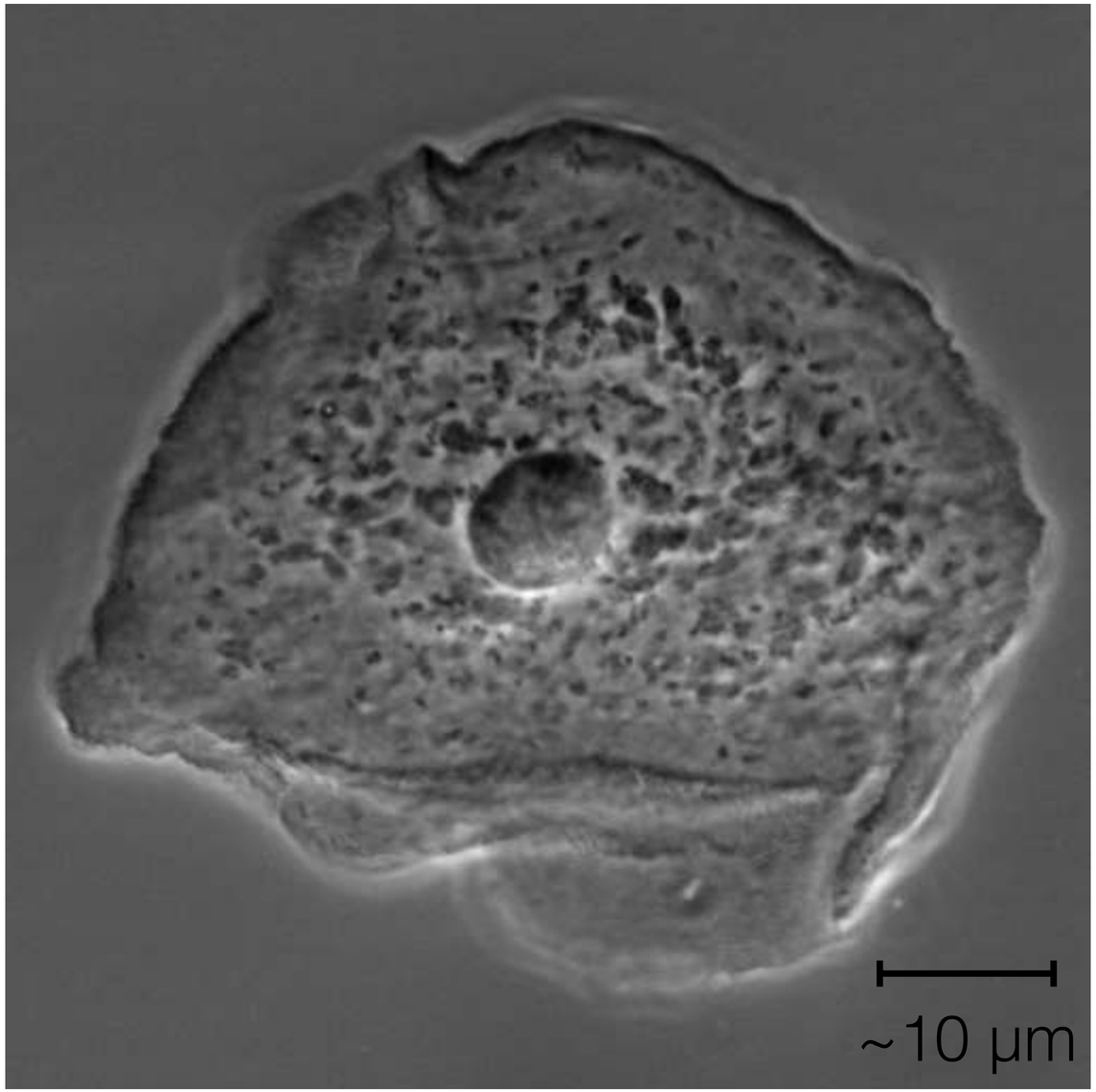
James M. Bell

Chen M, Bell JM, Shi X, Sun SY, Wang Z, Ludtke SJ. A complete data processing workflow for cryo-ET and subtomogram averaging. Nat Methods. 2019; 16(11) 1161-1168. PMID: 31611690. PMC6858567.

Chen M, Dai W, Sun SY et al. Convolutional neural networks for automated annotation of cellular cryo-electron tomograms. Nat Methods. 2017; 14(10) 983-985. PMID: 28846087. PMC5623144.

Epithelial Cell (cheek)

(Optical Phase Contrast Microscopy)



Target: 0.2 nm (2 Å) resolution
0.1 nm/pixel -> 400 nm FOV

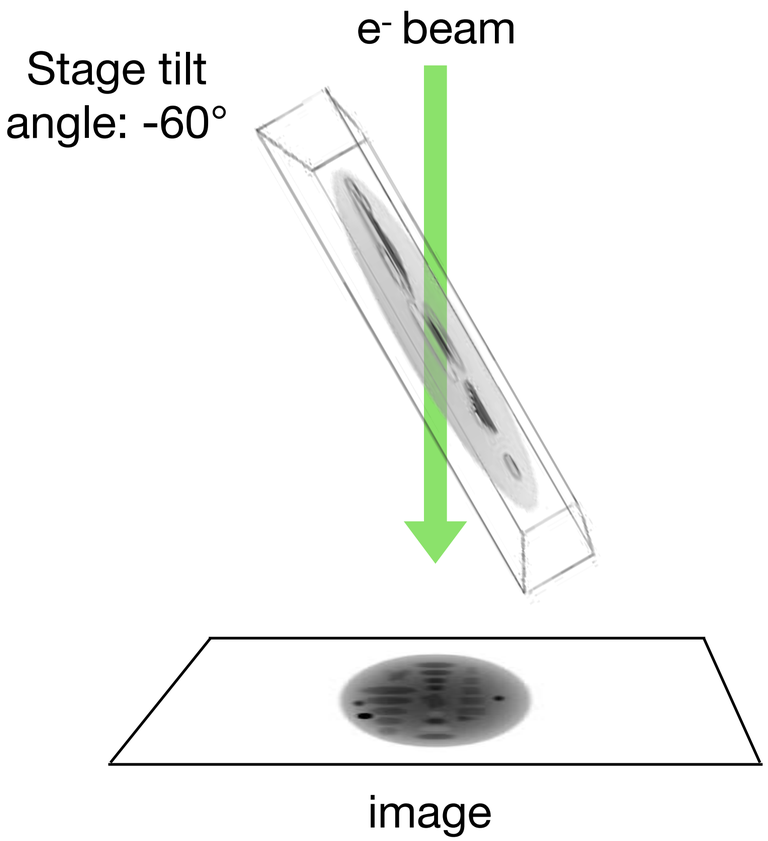
40 um cell -> $3x10^{16}$ voxels
(30 PB, 1M tomograms)

Target: 2 nm (20 Å) resolution
1 nm/pixel -> 4 um FOV
(400 nm thick)

40 um cell -> $3x10^{13}$ voxels
(30 TB, 10k tomograms)

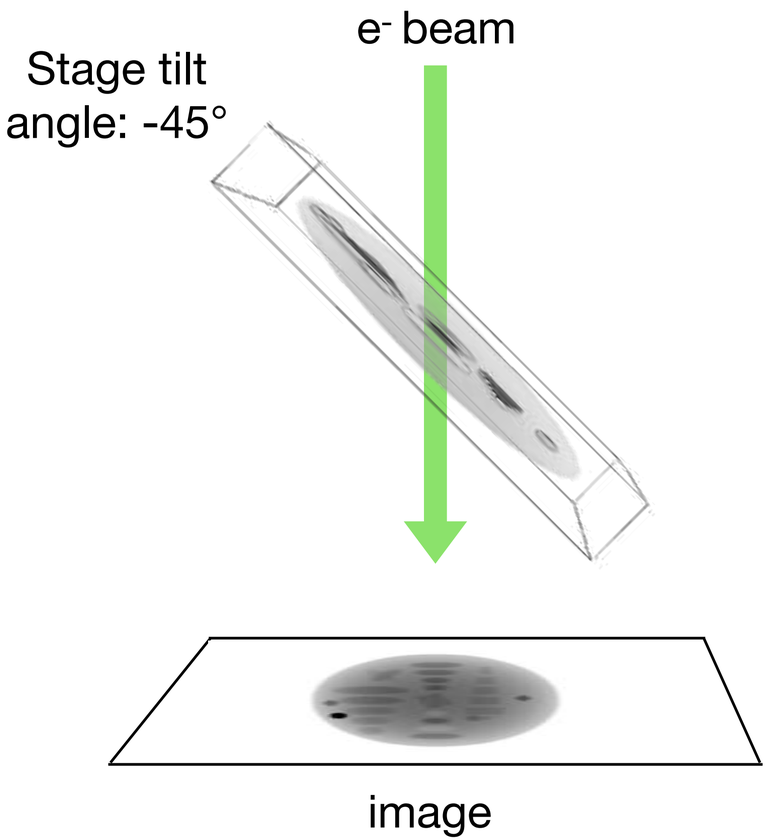
http://calculatedimages.blogspot.com/2014_04_01_archive.html

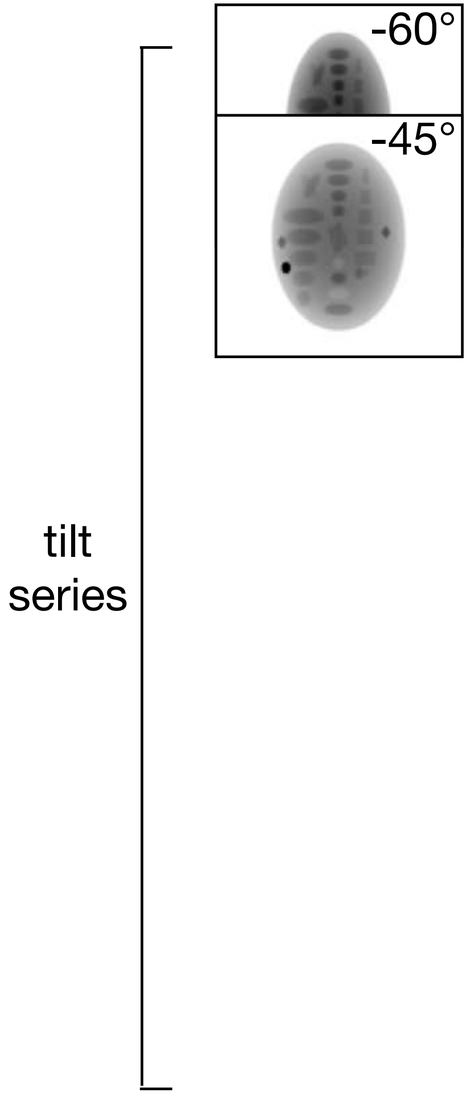
Electron cryo-tomography (cryoET)



Courtesy J.M. Bell

Electron cryo-tomography (cryoET)





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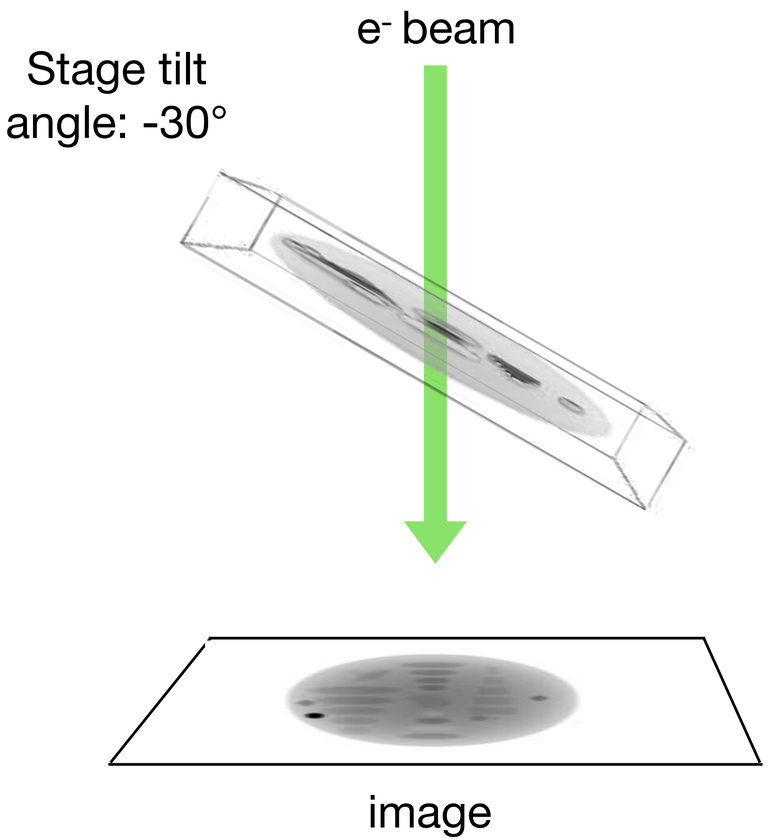
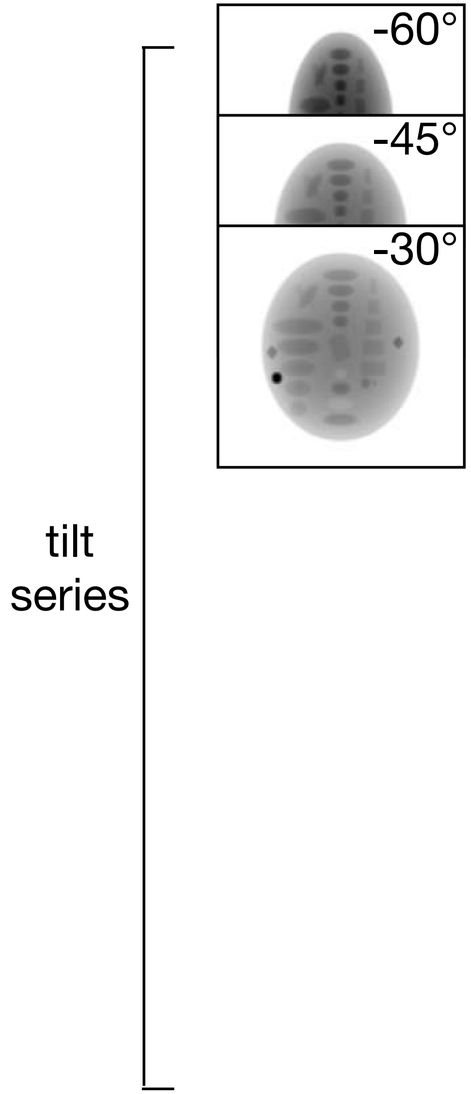
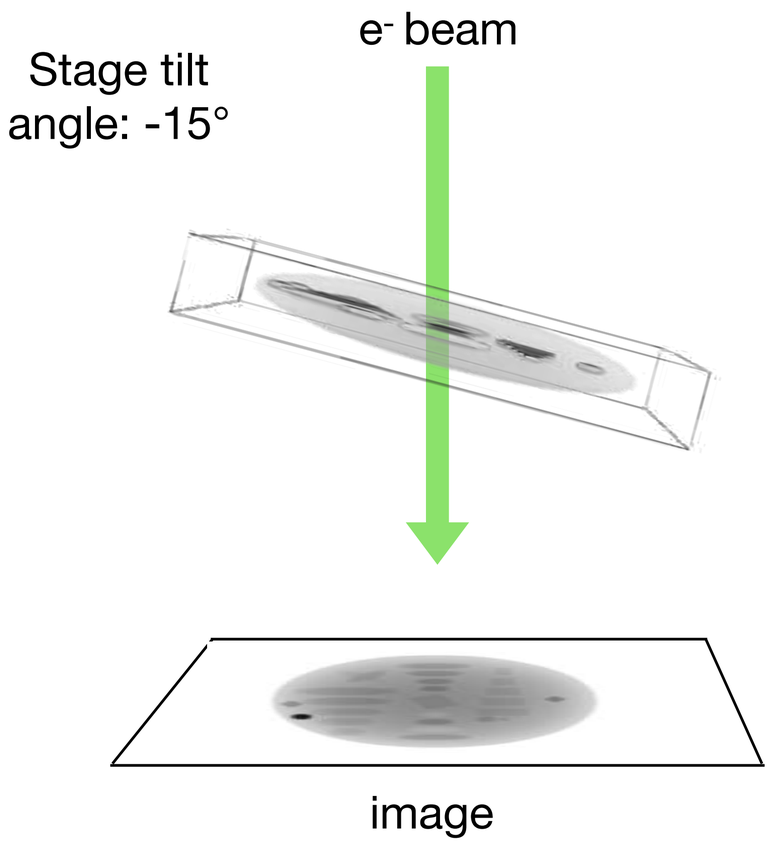
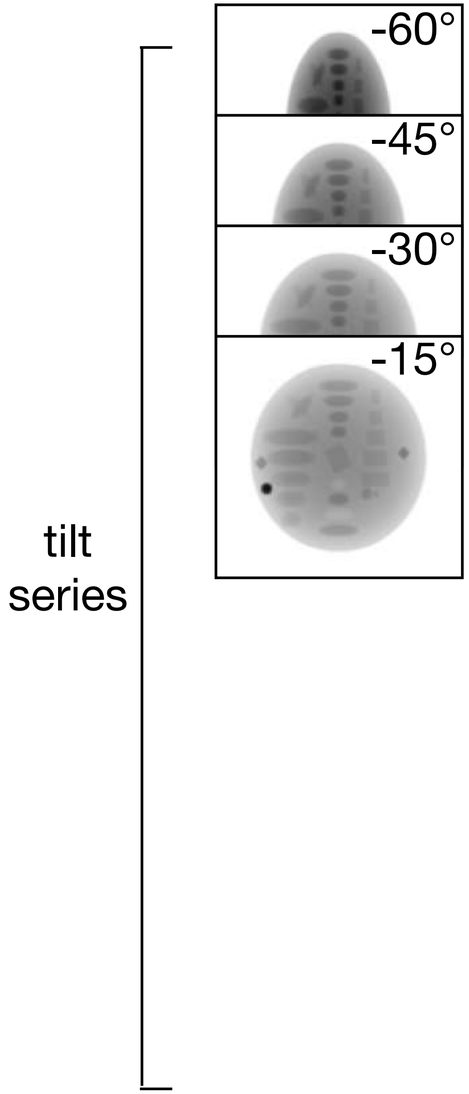




Courtesy J.M. Bell

Electron cryo-tomography (cryoET)





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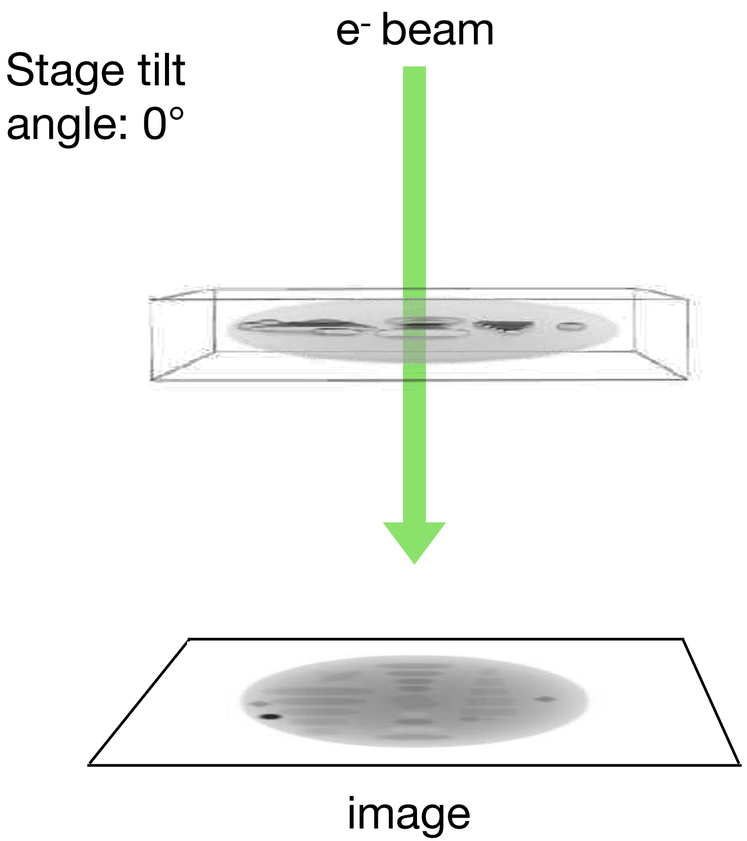
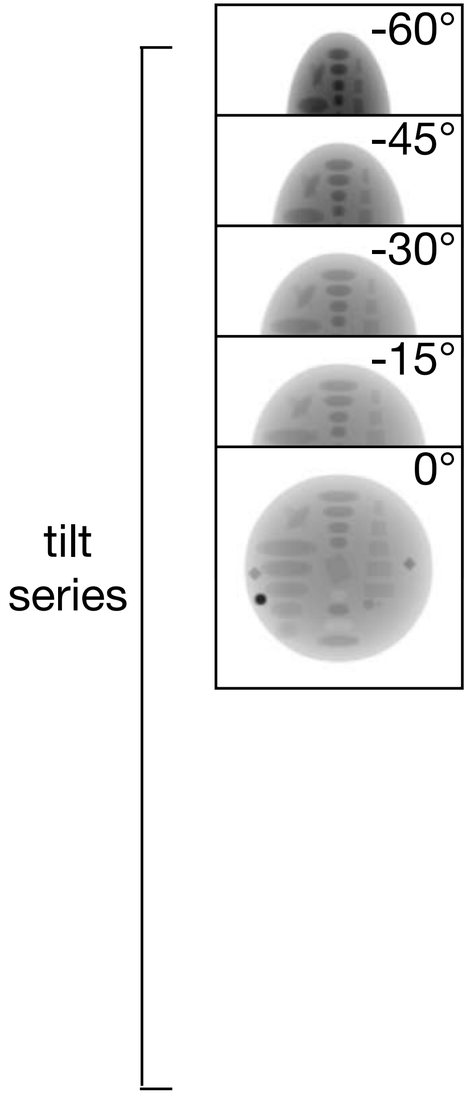
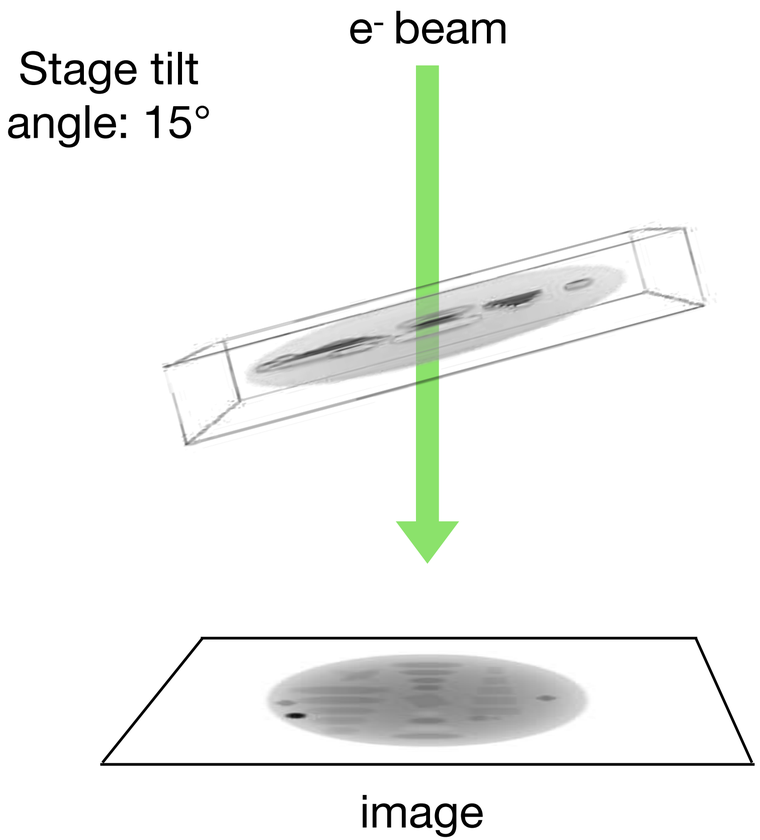
Electron cryo-tomography (cryoET)

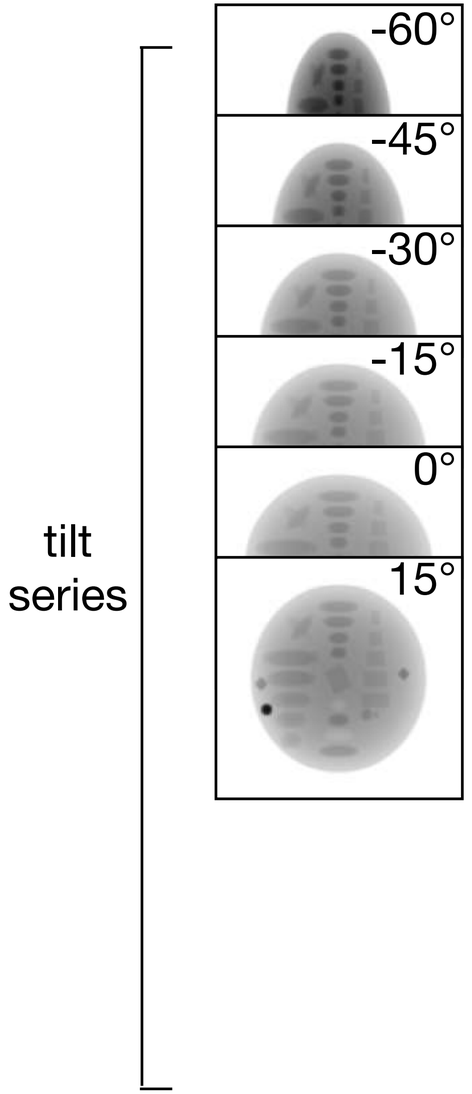




Courtesy J.M. Bell

Electron cryo-tomography (cryoET)





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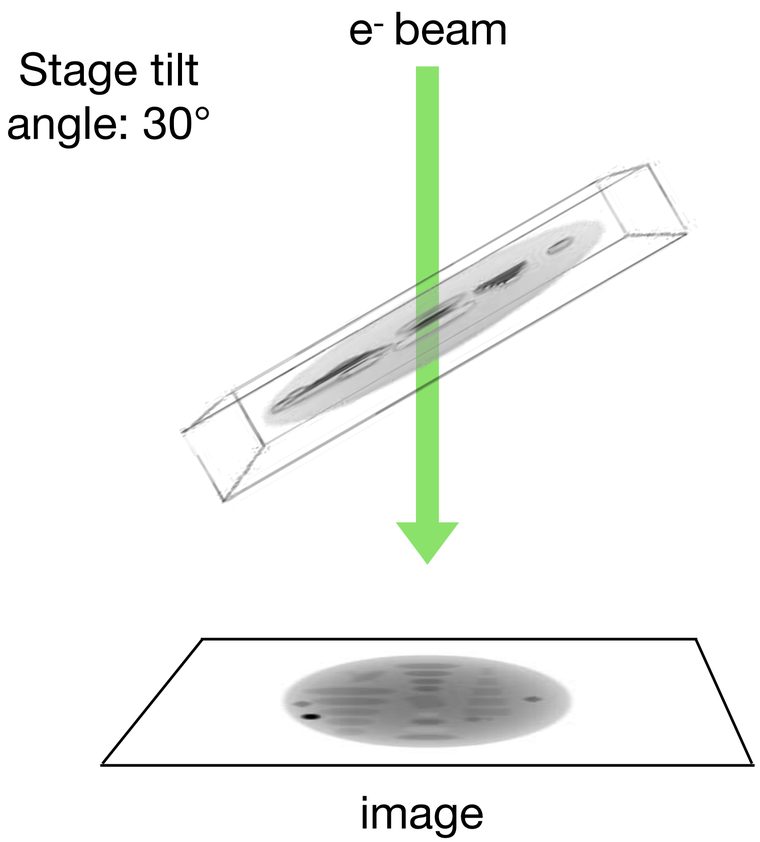
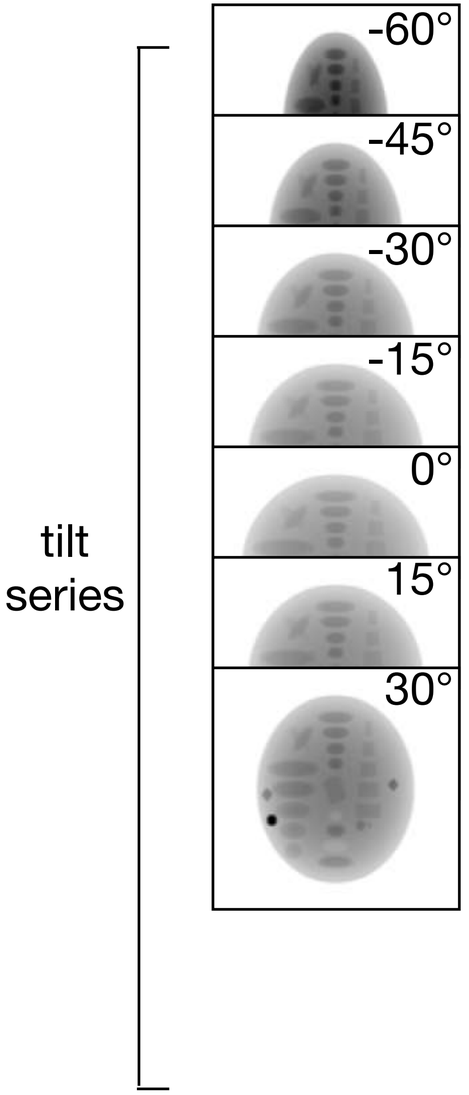
Electron cryo-tomography (cryoET)





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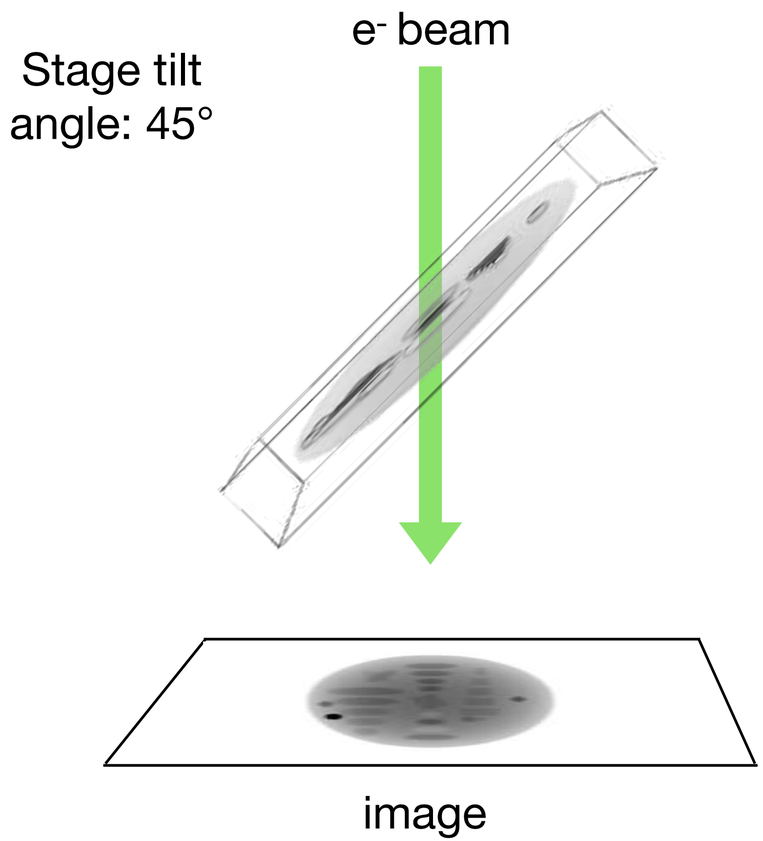

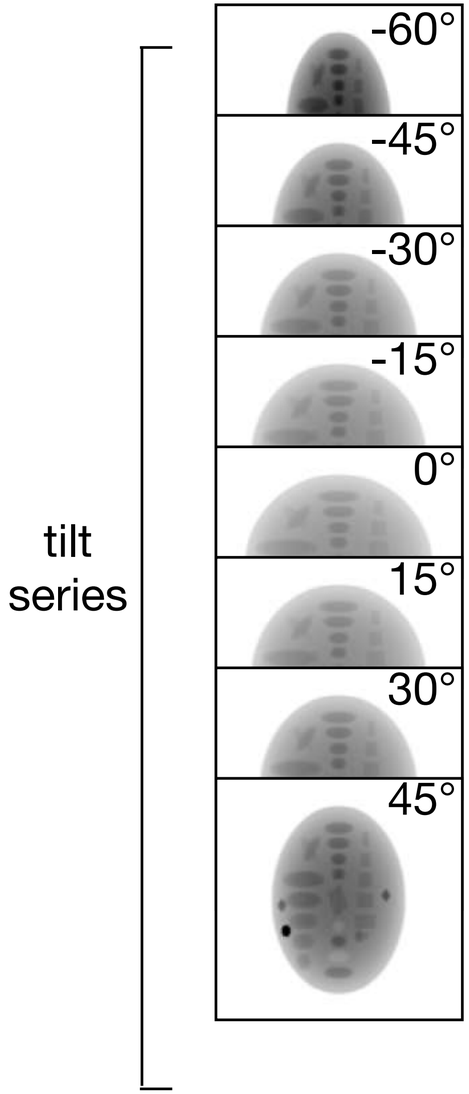

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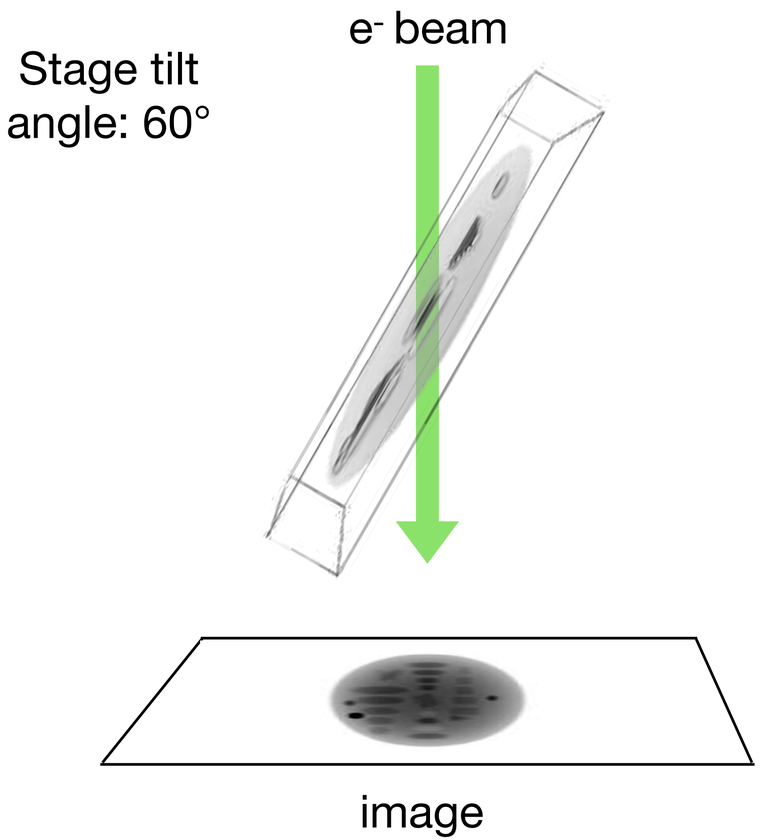
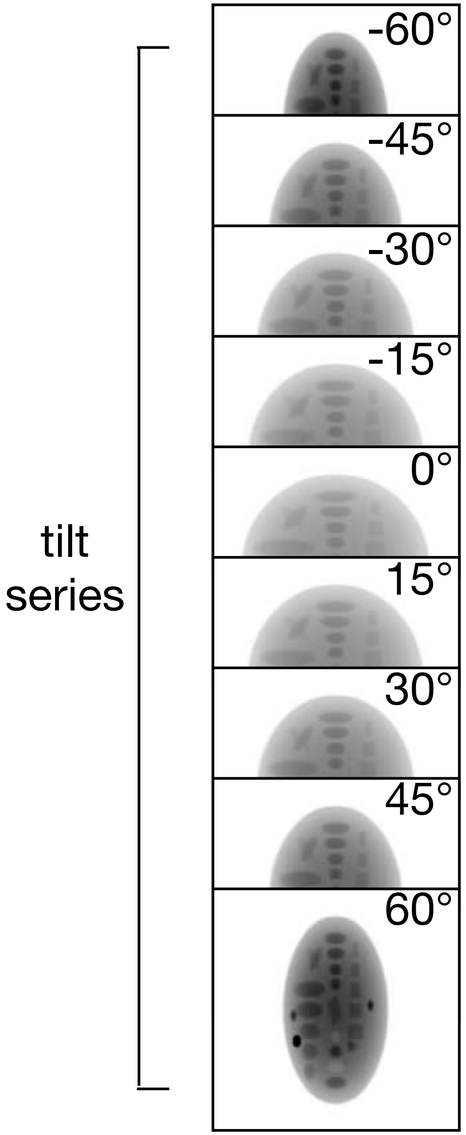
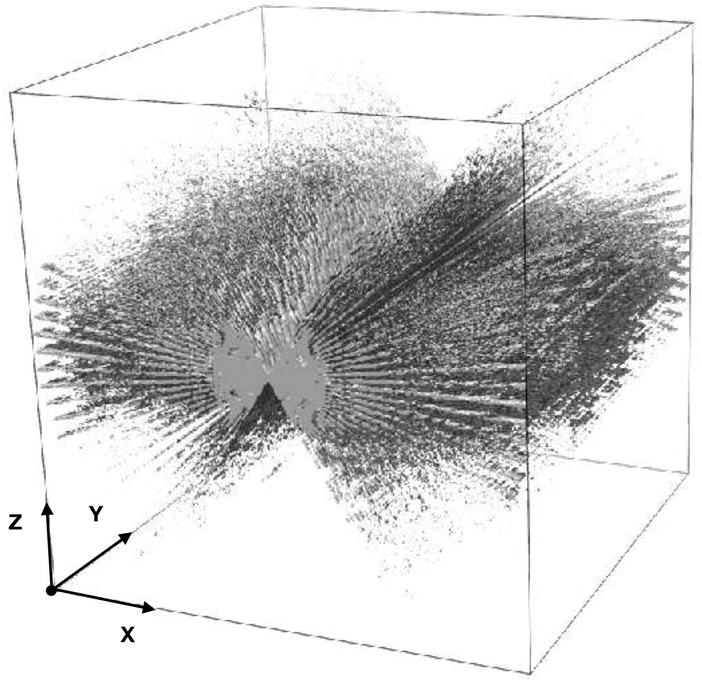


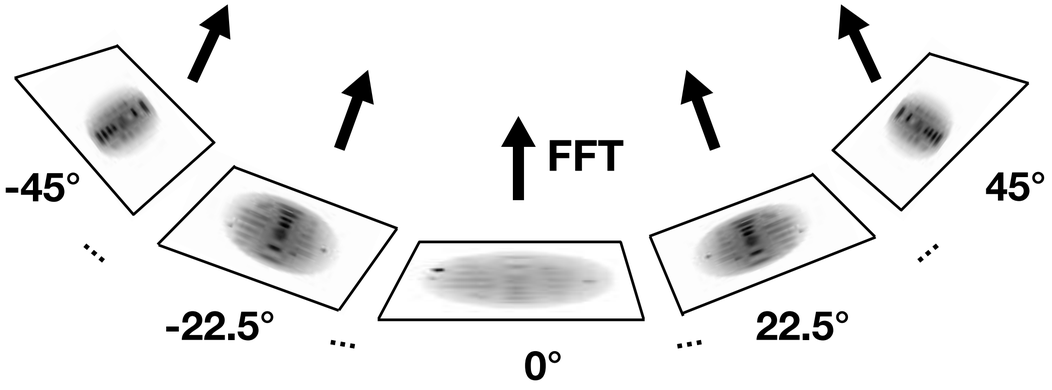


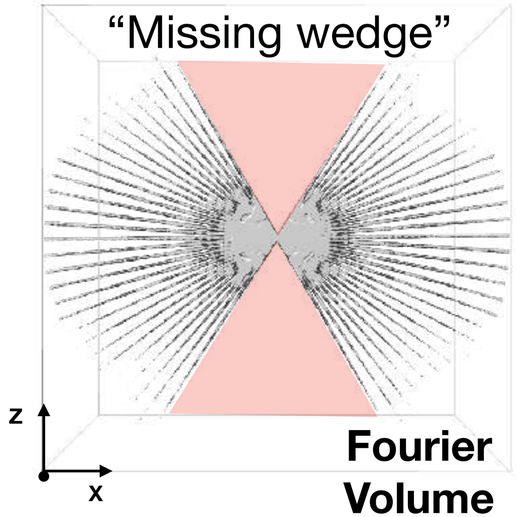
Courtesy J.M. Bell

3D Fourier reconstruction

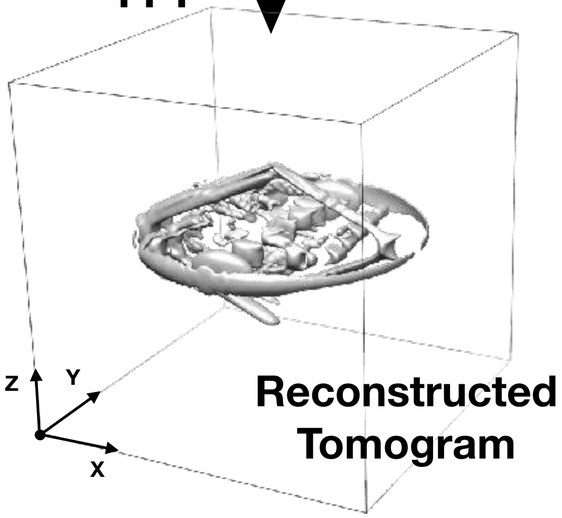


Fourier Volume



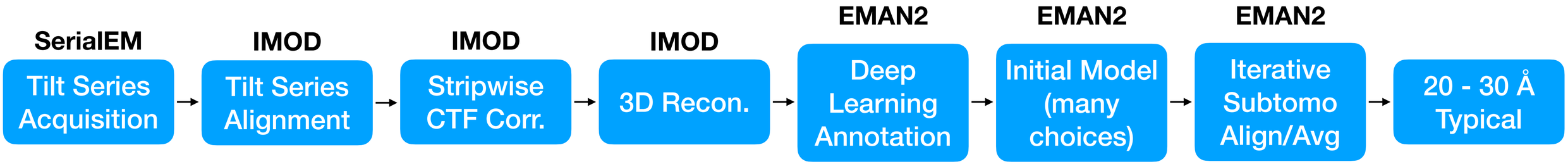


Inverse FFT



Courtesy J.M. Bell

~3 Years Ago


SerialEM
Tilt Series Acquisition
IMOD
Tilt Series Alignment
IMOD
Stripwise CTF Corr.
IMOD
3D Recon.
EMAN2
Deep Learning Annotation
EMAN2
Initial Model (many choices)
EMAN2
Iterative Subtomo Align/Avg
20 - 30 Å Typical

Works, but not ideal..

- Tilt series alignment: best results require human, need for fiducials
- 3-D reconstruction of 4k+ volumes slow and storage intensive
- Identifying specific molecules/complexes in the cell
- Limited resolution. Traditional approach gets stuck even with CTF.
- Better way to do tilt-aware CTF correction?
- Good global alignment ≠ good local alignment
- Multiple software packages
- Files are extremely large

Now

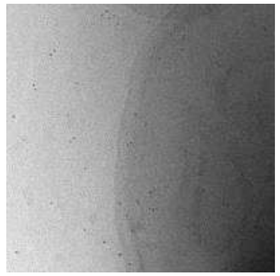
EMAN2
One Automated Step

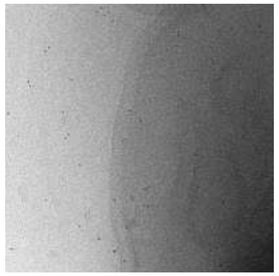
EMAN2

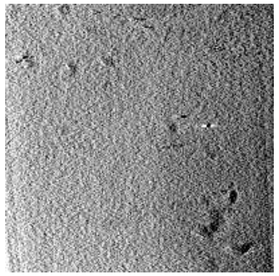
EMAN2

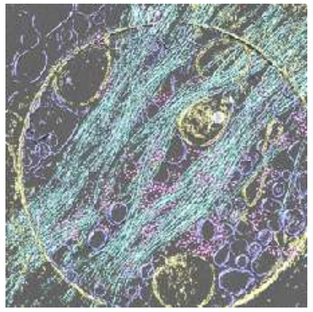
EMAN2

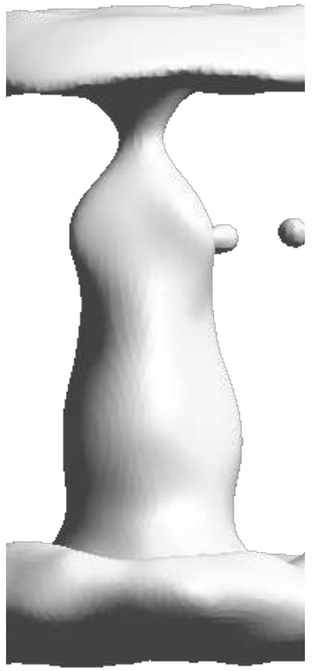
Tilt Series Acquisition → CTF → Tilt Series Alignment ⇄ 3D Recontstruct → Deep Learning Annotation → Initial Model (SGD) → PPPT Refinement → up to ~3 Å Resolution

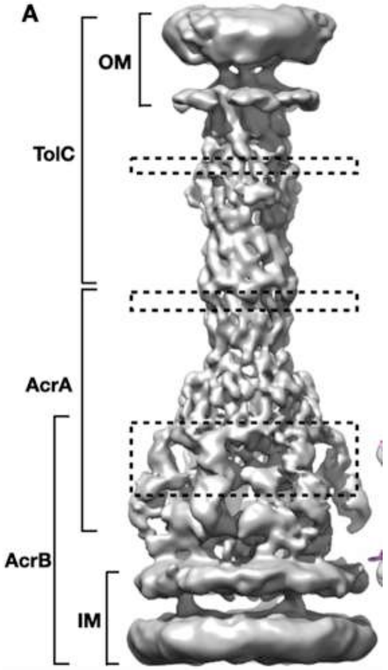












- **Compression throughout, 5-20x typ**
  - **1k tomogram: ~2 gb -> ~400 mb**
  - **mask: 36 mb -> 0.5 mb**

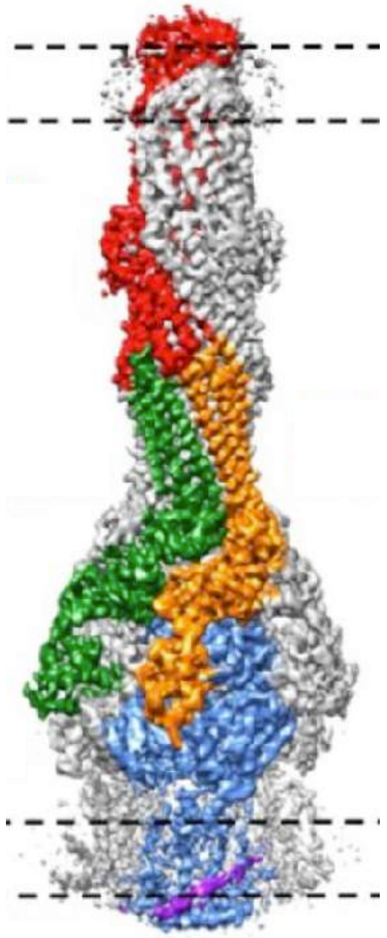
Described in:
Chen, M. et al. (2019) *Nat Methods*, **16**, 1161-1168. PMC6858567

AcrAB-TolC

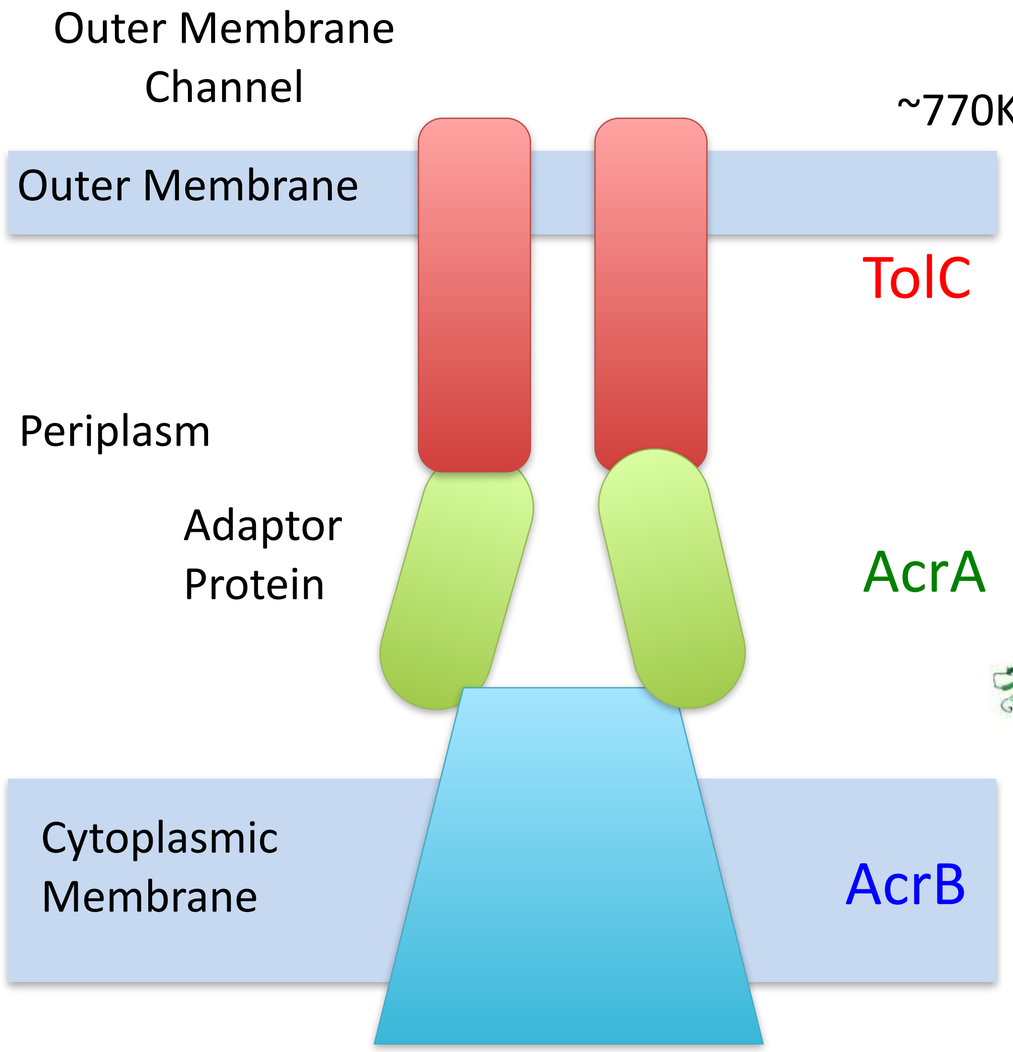
- Early single particle work Chiu & Luisi, in-situ collaboration Wang, Luisi, Serysheva and Ludtke Labs
- Structure of the AcrAB–TolC multidrug efflux pump. D Du, Z Wang, NR James, JE Voss, E Klimont, T Ohene-Agyei, H Venter, W Chiu, BF Luisi. *Nature*, 509, 512–515 (2014).
- An allosteric transport 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).
- In situ structure of the AcrAB-TolC efflux pump at subnanometer resolution. M Chen, X. Shi, Z Yu. et al. (2020) bioRxiv. https://www.biorxiv.org/content/10.1101/2020.06.10.144618v1

Efflux Pumps in Gram-Negative Bacteria:

Tripartite Complex



2017
CryoEM
3.6 Å



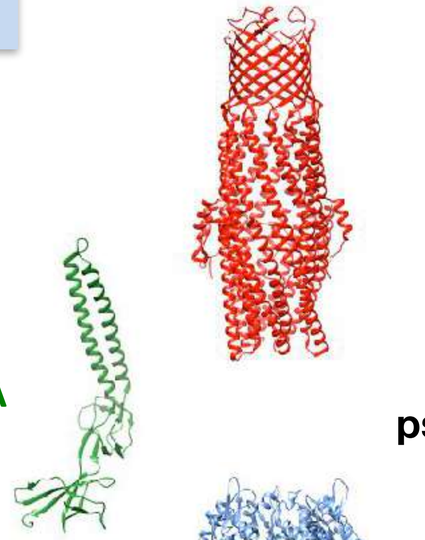
~770KDa

TolC

AcrA

AcrB

X-ray structures:



3:6:3
pseudo C6

Now

SerialEM

Tilt Series Acquisition

EMAN2
One Automated Step

CTF → Tilt Series Alignment ⇄ 3D Recontstruct

EMAN2

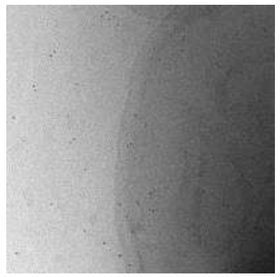
Deep Learning Annotation

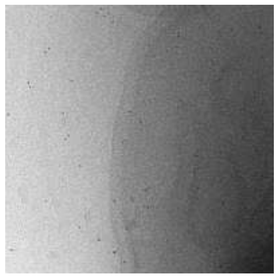
EMAN2

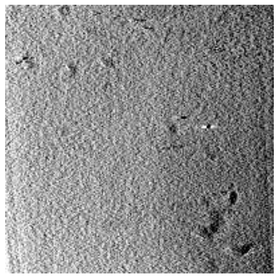
Initial Model (SGD)

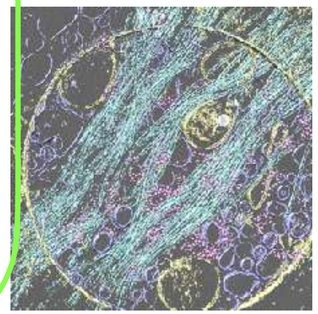
EMAN2

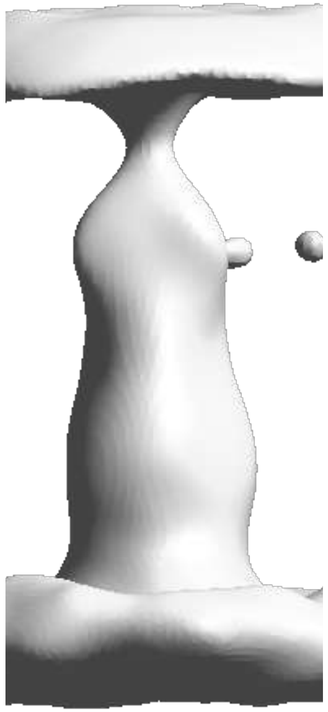
PPPT Refinement → up to ~3 Å Resolution

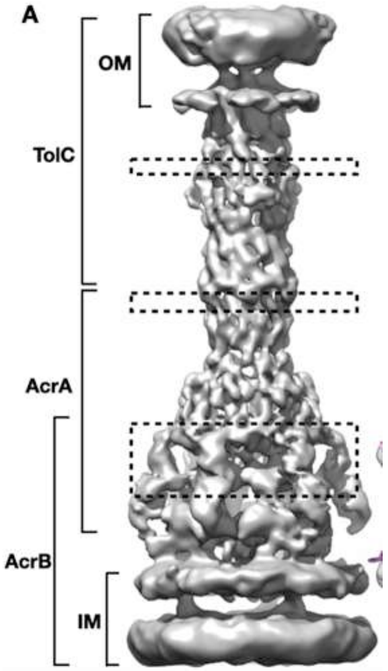








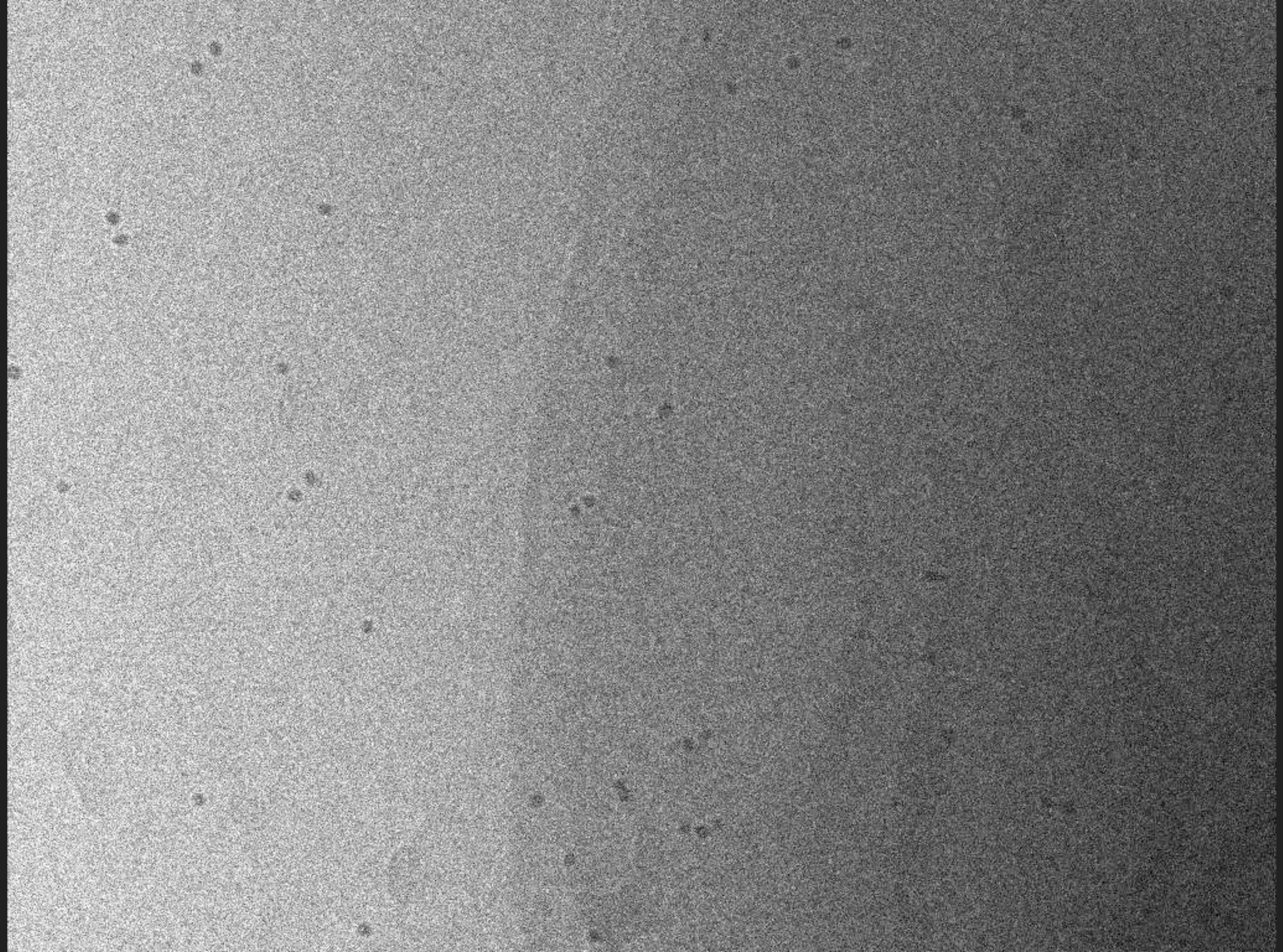




- **With/without fiducials**
- **Completely automated**
- **Works on almost any tilt series**
- **Fast, typ. ~3 min including 3-D**

Described in:
Chen, M. et al. (2019) *Nat Methods*, **16**, 1161-1168. PMC6858567

Whole Cell
E. coli



X Shi,
Z Wang,
BCM

Tilt series alignment

Start: Unaligned tiltseries

Coarse alignment

3D Reconstruction

Pick landmarks (3D)

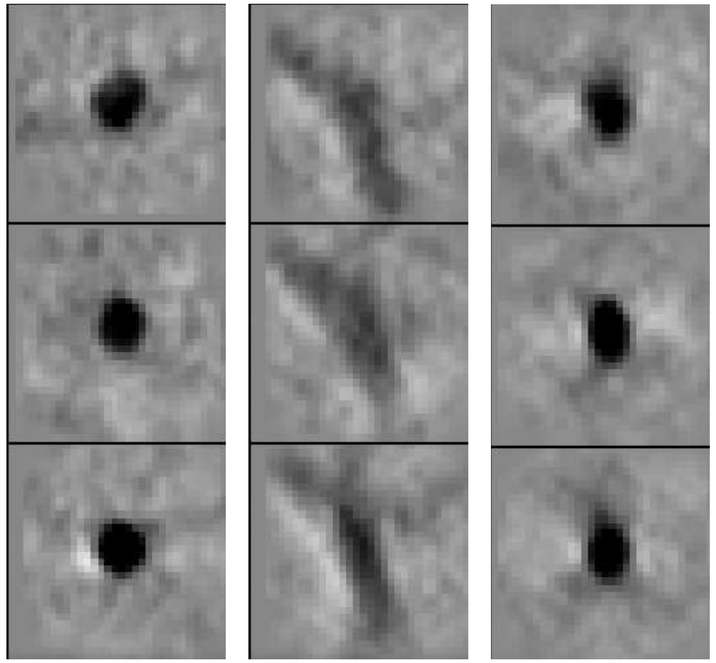
Refine landmark coordinates

Refine alignent parameters

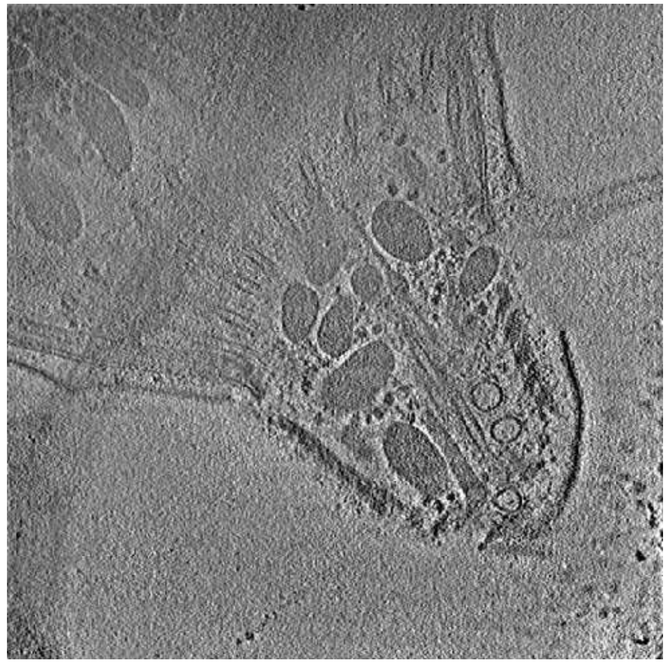
Bin by 8x 4x 2x 1x

Landmarks

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



Tomogram slice view

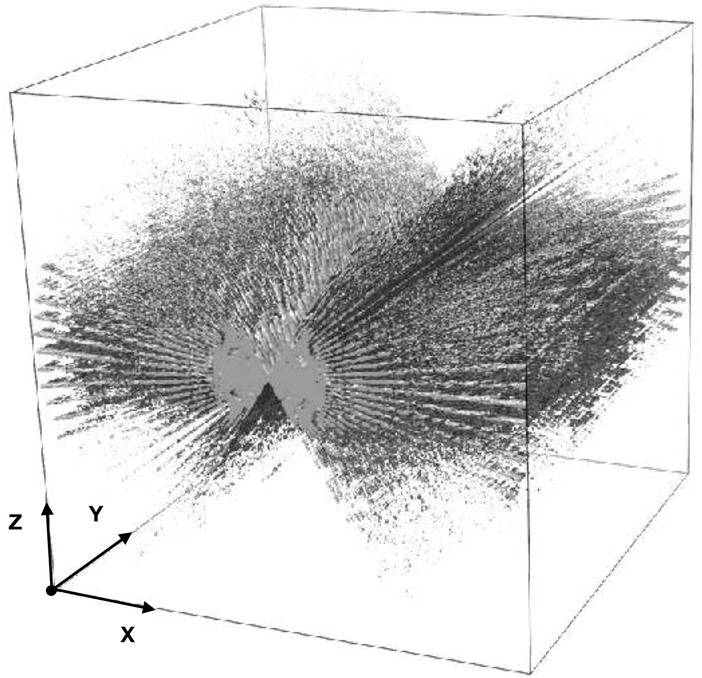


Toxoplasma gondii

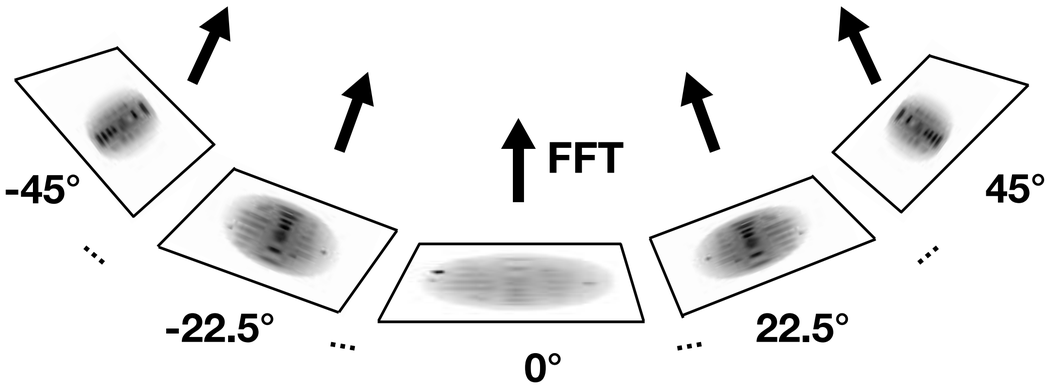
Stella Y. Sun

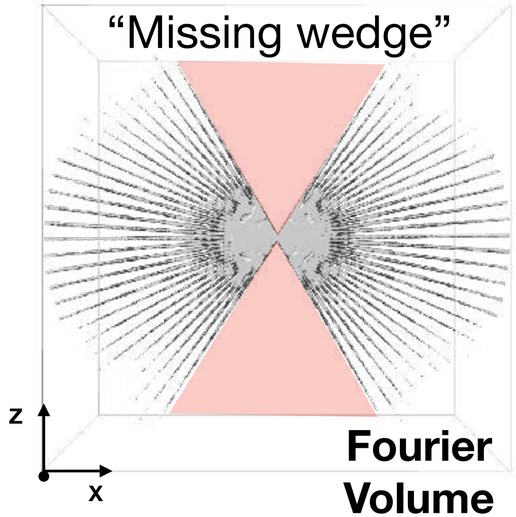
~5-10 min total per tomogram

3D Fourier reconstruction

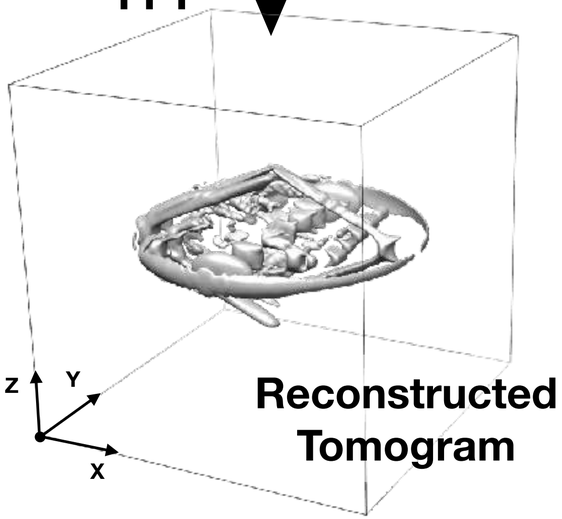


Fourier Volume



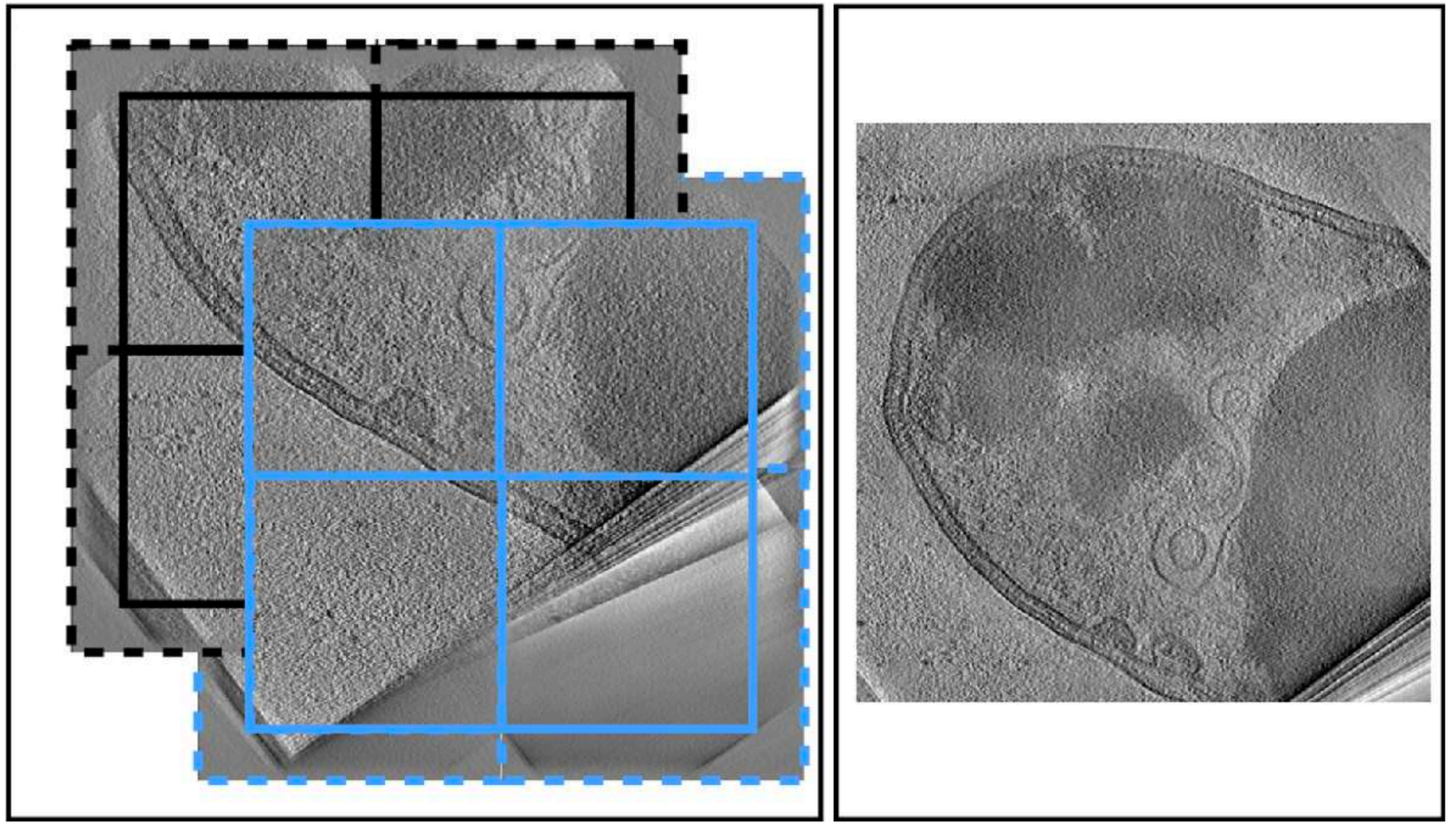


Inverse FFT



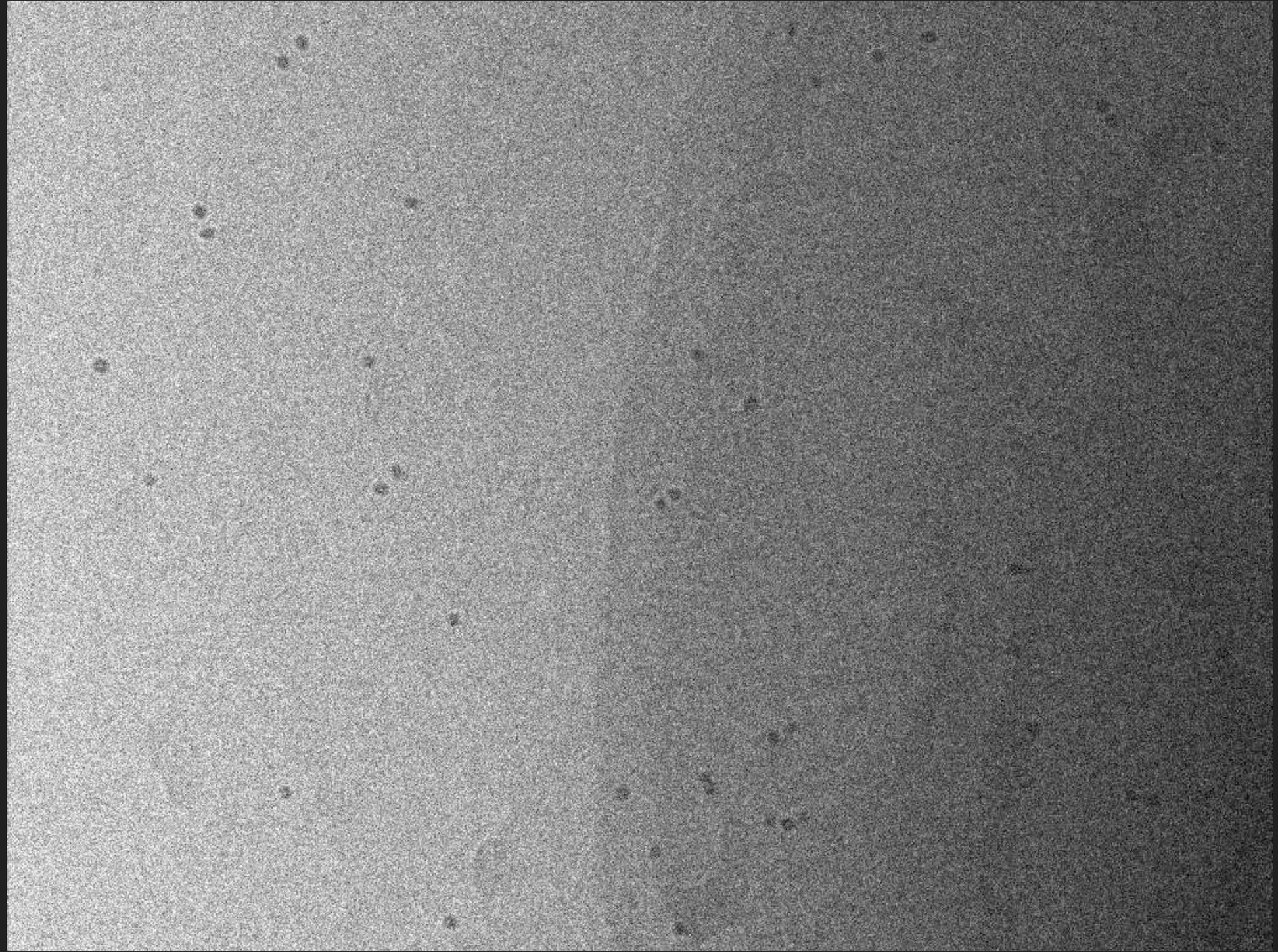
Reconstruction via tiled direct Fourier inversion

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

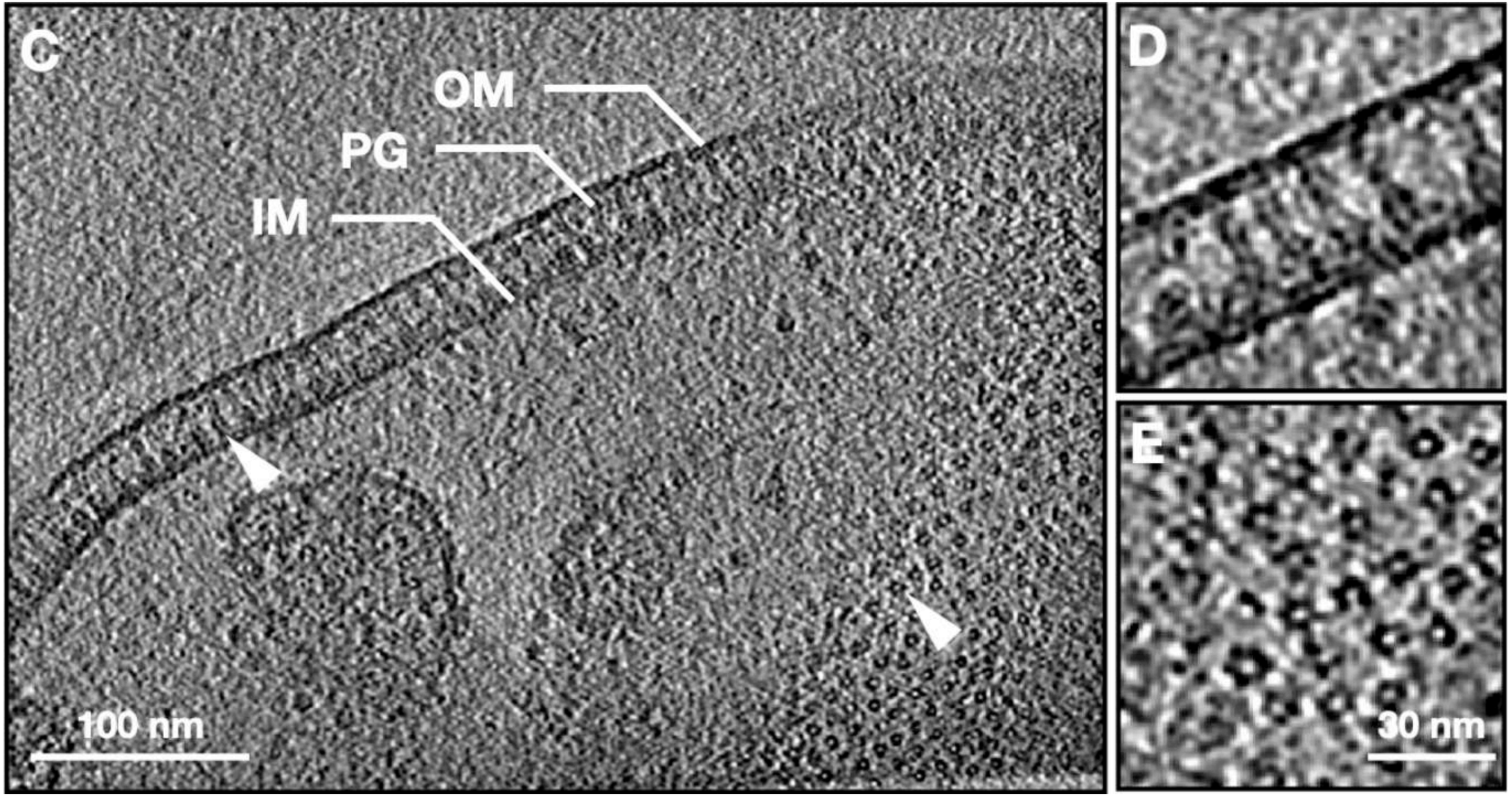


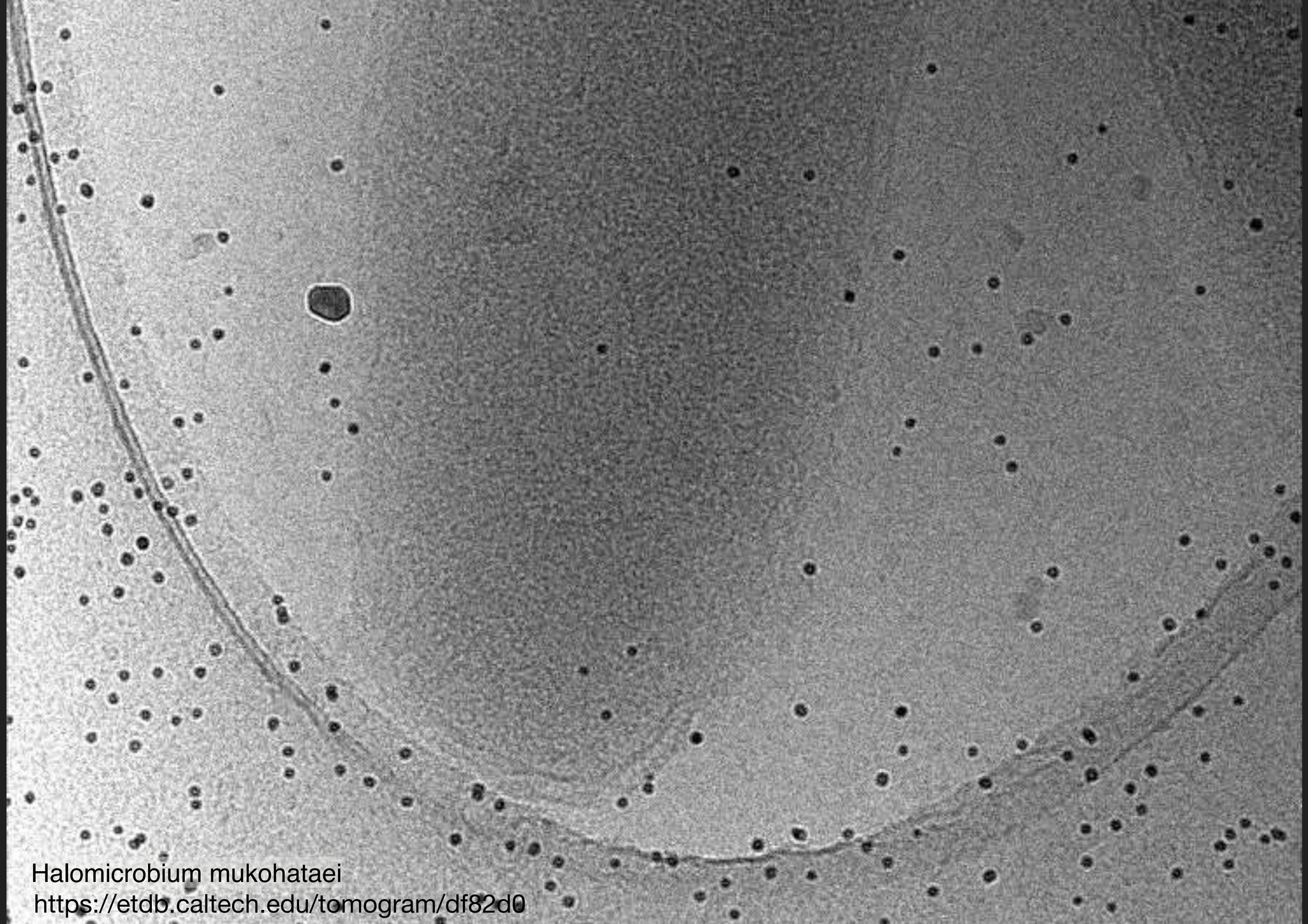
Whole Cell
E. coli

Runtime:
~5 minutes
per tomogram



X Shi,
Z Wang,
BCM


C
OM
PG
IM
100 nm
D
E
30 nm



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

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

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

Now

SerialEM

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EMAN2
One Automated Step

CTF

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3D Recontstruct

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Deep Learning Annotation

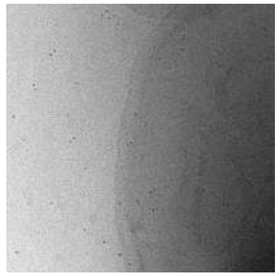
EMAN2

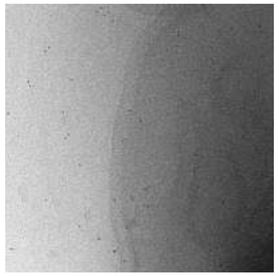
Initial Model (SGD)

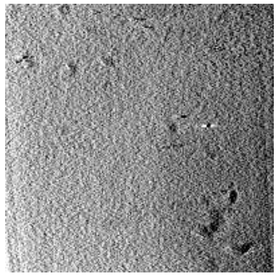
EMAN2

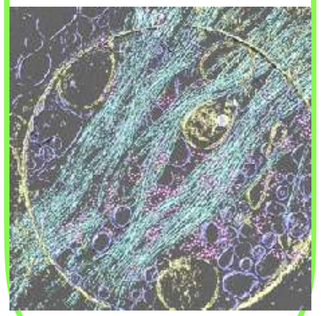
PPPT Refinement

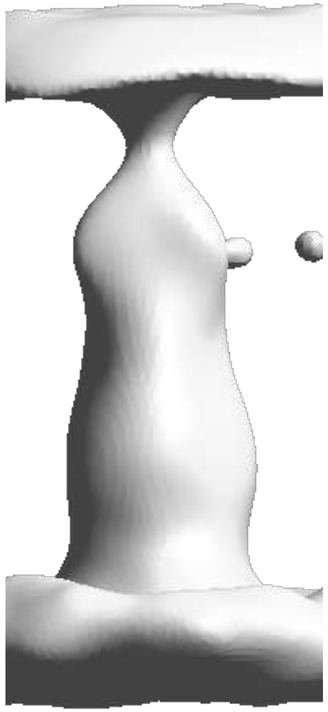
up to ~3 Å Resolution

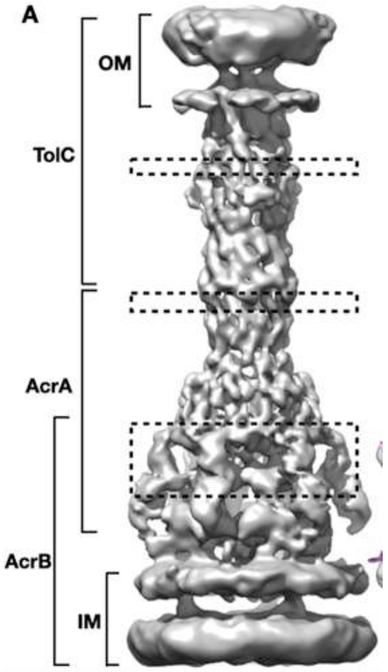






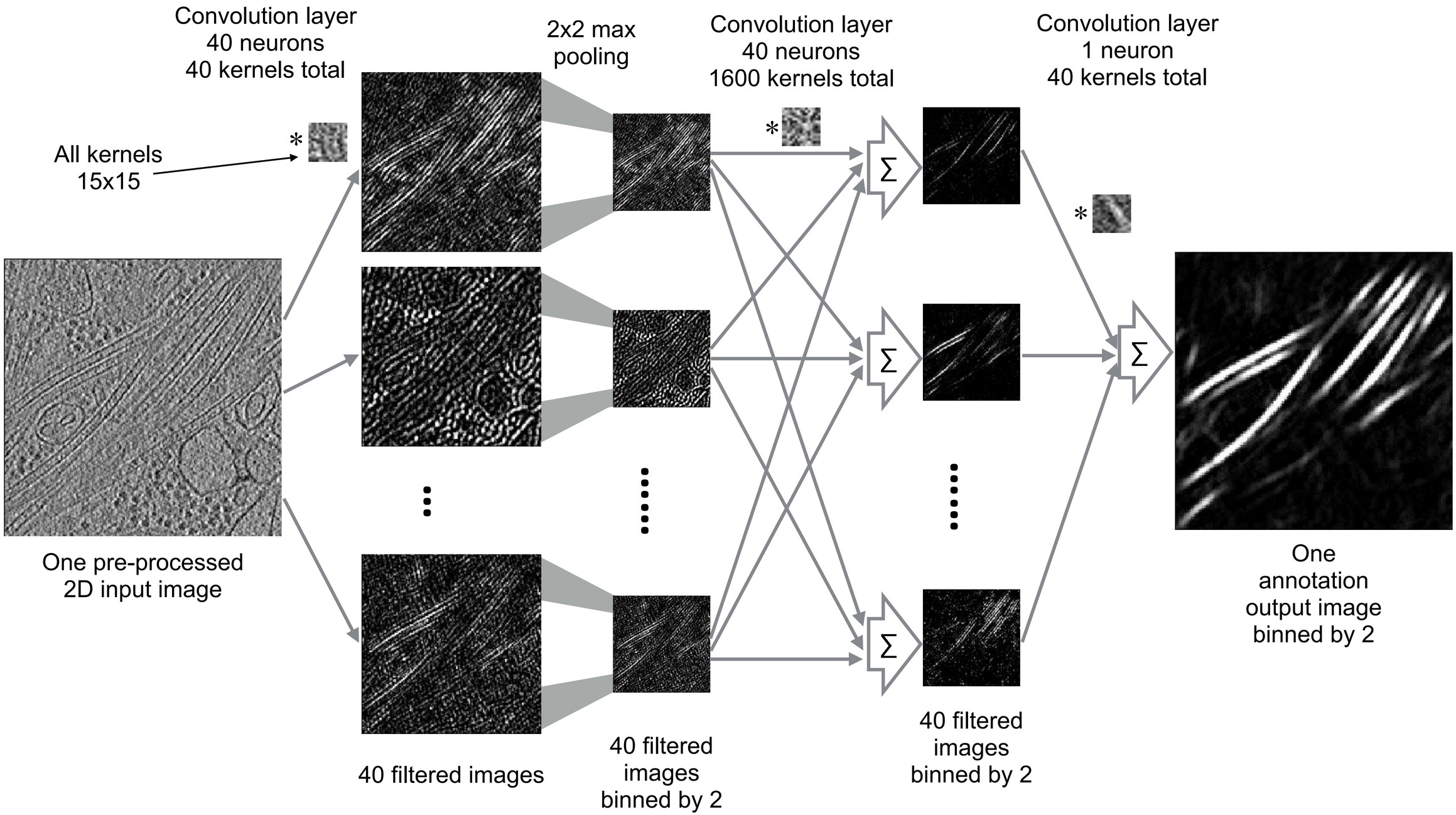




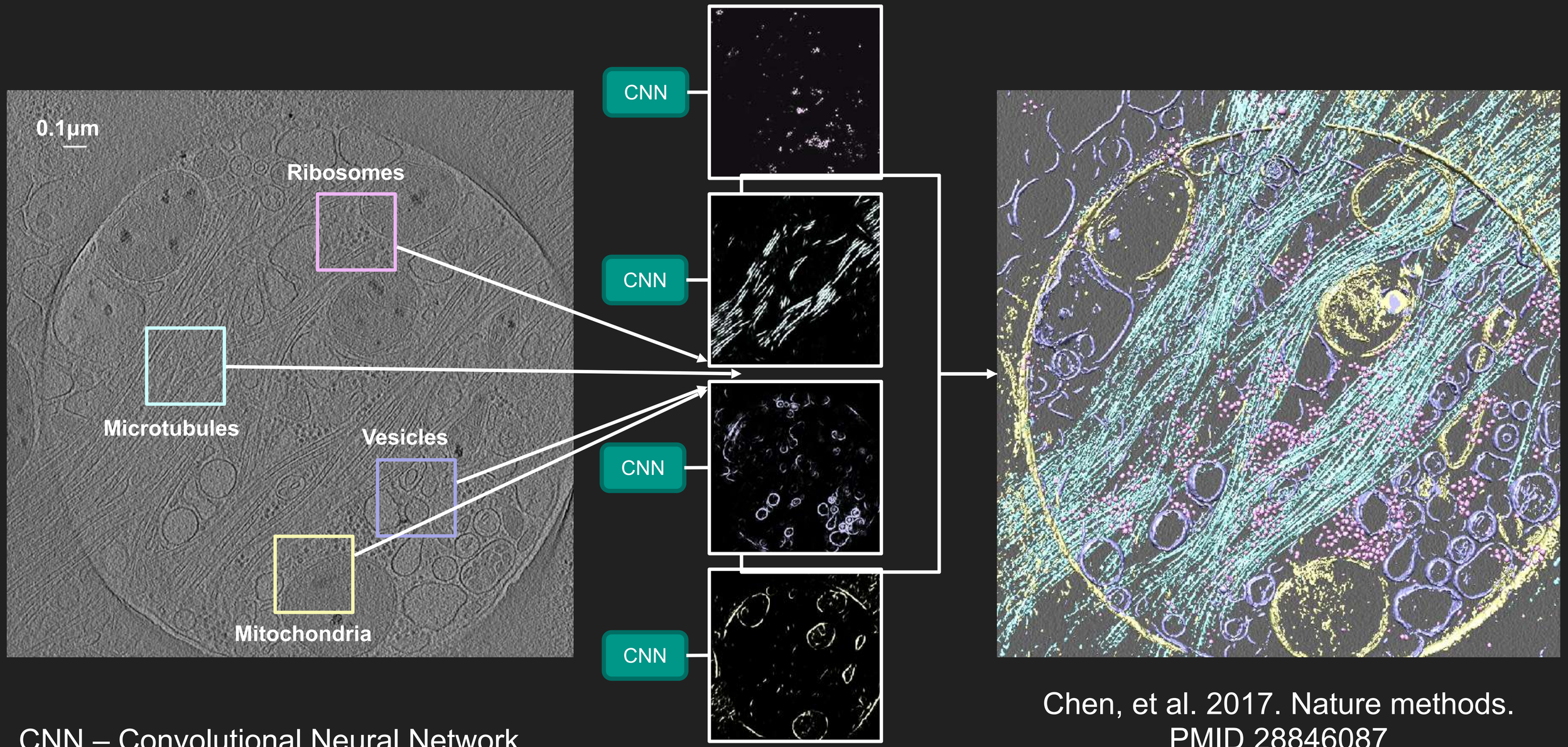


Described in:
Chen, M. et al. (2019) *Nat Methods*, **16**, 1161-1168. PMC6858567

Convolutional Neural Network (CNN)



Deep learning-based cellular annotation



CNN – Convolutional Neural Network

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

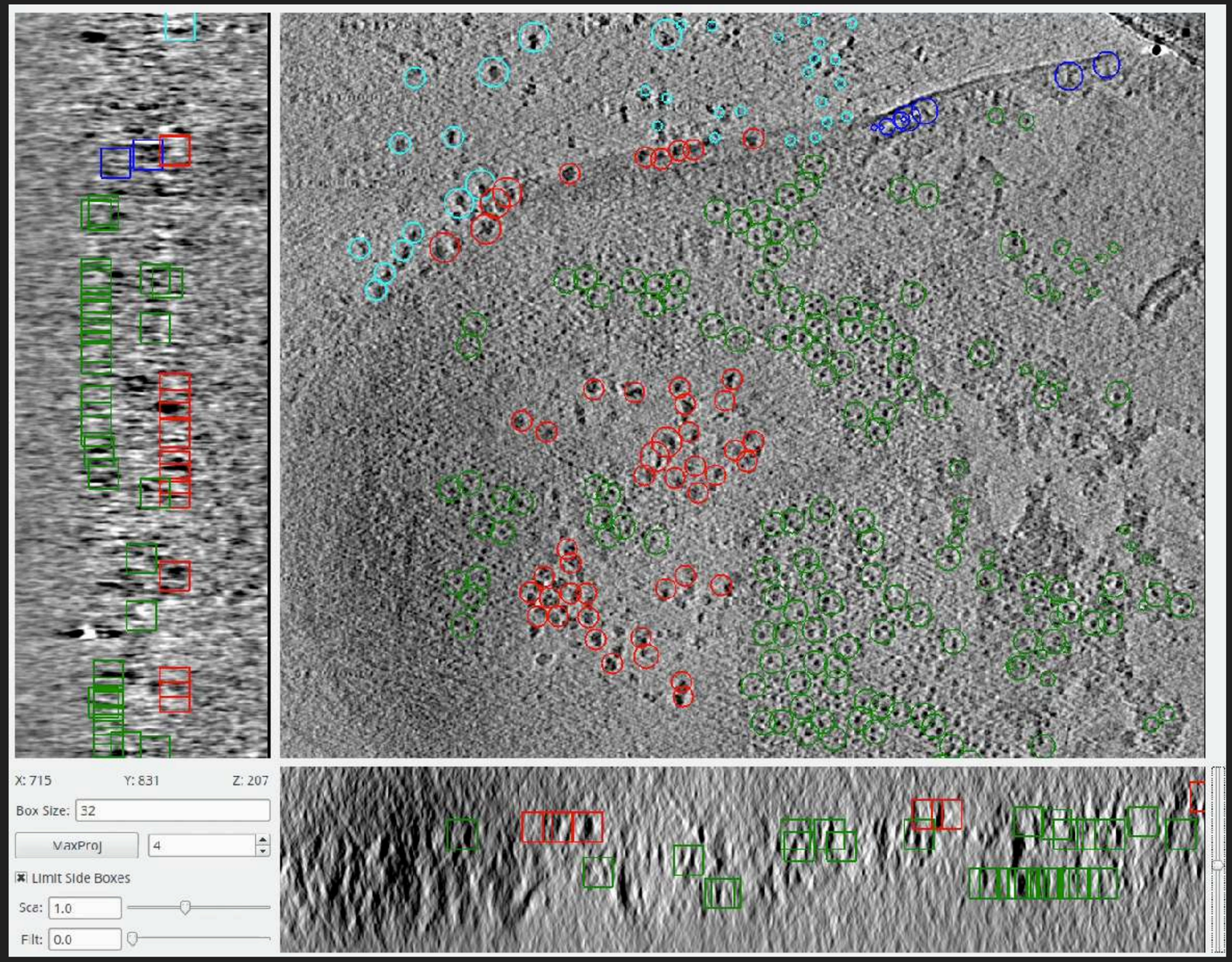
Multi-specimen subtomogram boxing

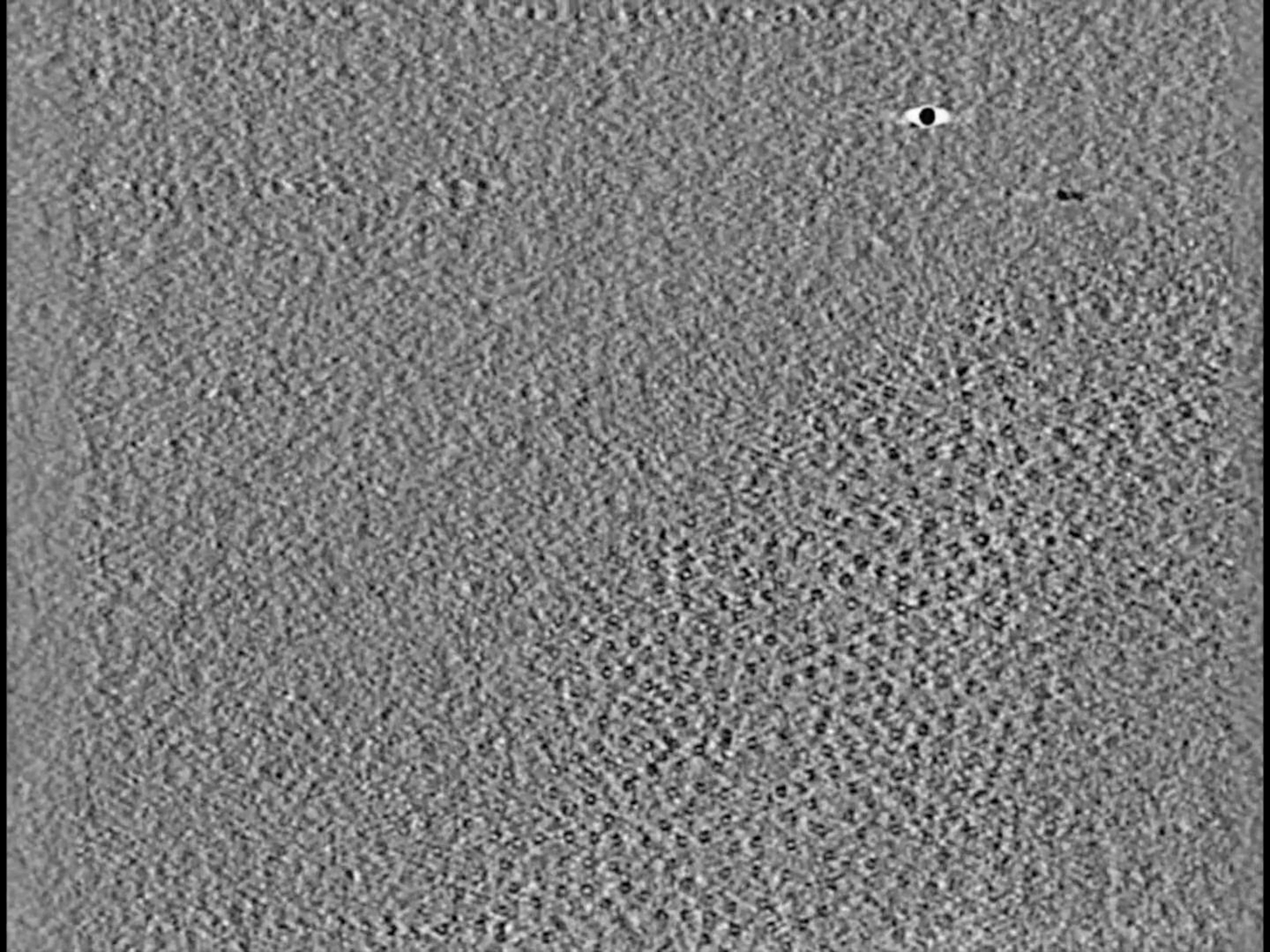
TolC - side

TolC - top

Ribo - in

Ribo - out





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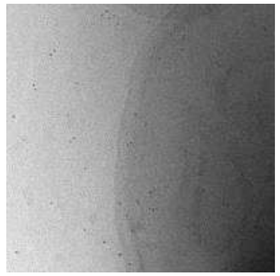
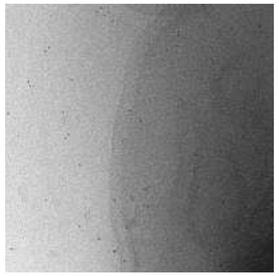
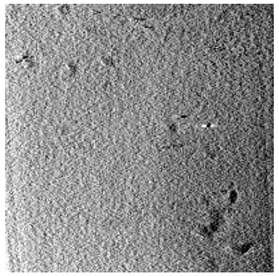
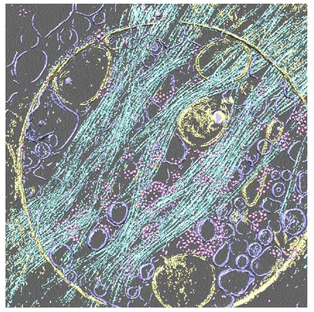
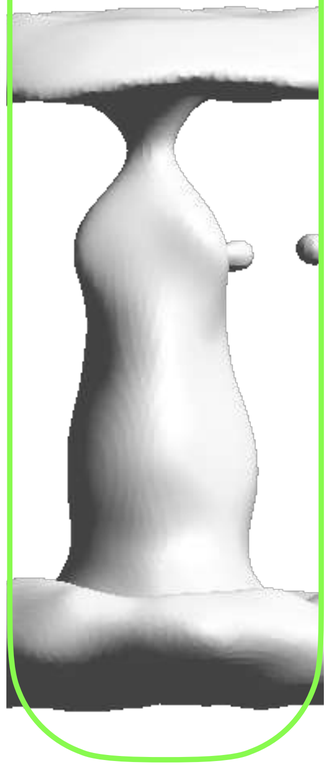
up to ~3 Å Resolution

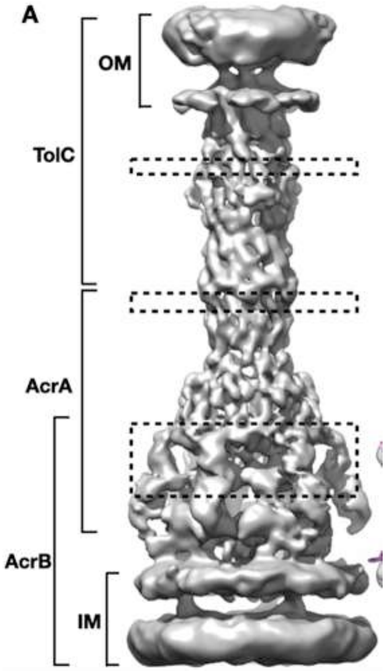












Described in:
Chen, M. et al. (2019) *Nat Methods*, **16**, 1161-1168. PMC6858567

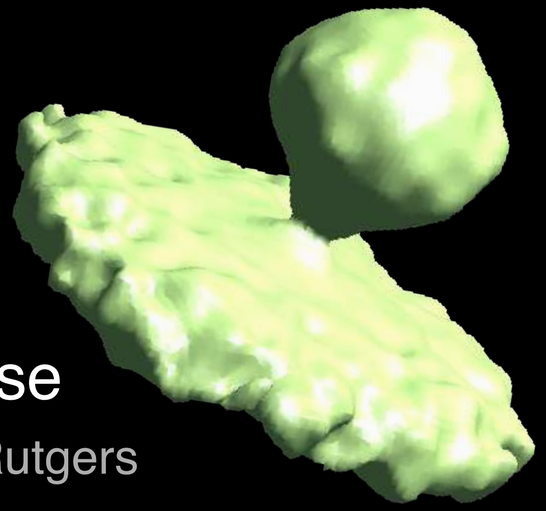
Reference-free initial model generation

TolC

X Shi,
Z Wang,
BCM

microtubule doublet

SY Sun, Stanford



ATP synthase

W Dai, Rutgers

80S Ribosome

EMPIAR 10064

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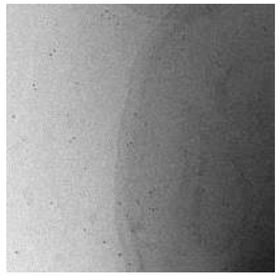
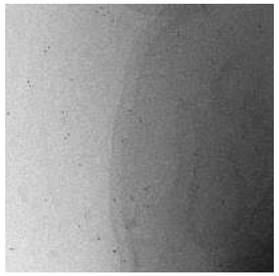
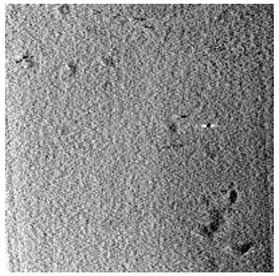
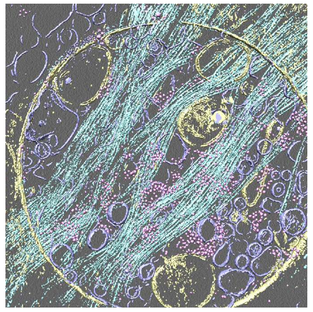
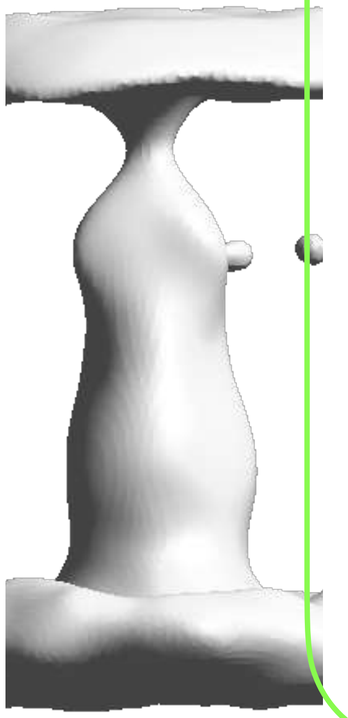
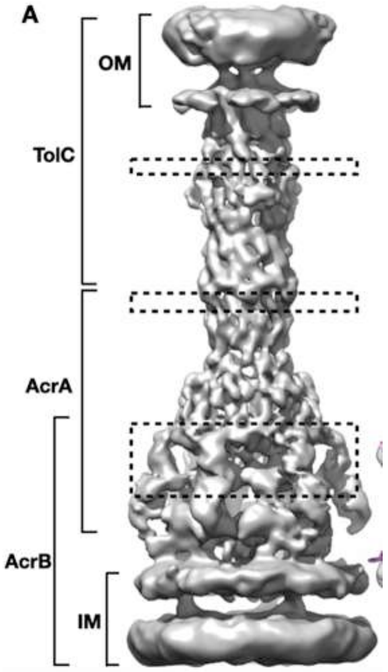












Described in:
Chen, M. et al. (2019) *Nat Methods*, **16**, 1161-1168. PMC6858567

New EMAN2 PPPT Tomographic Refinement

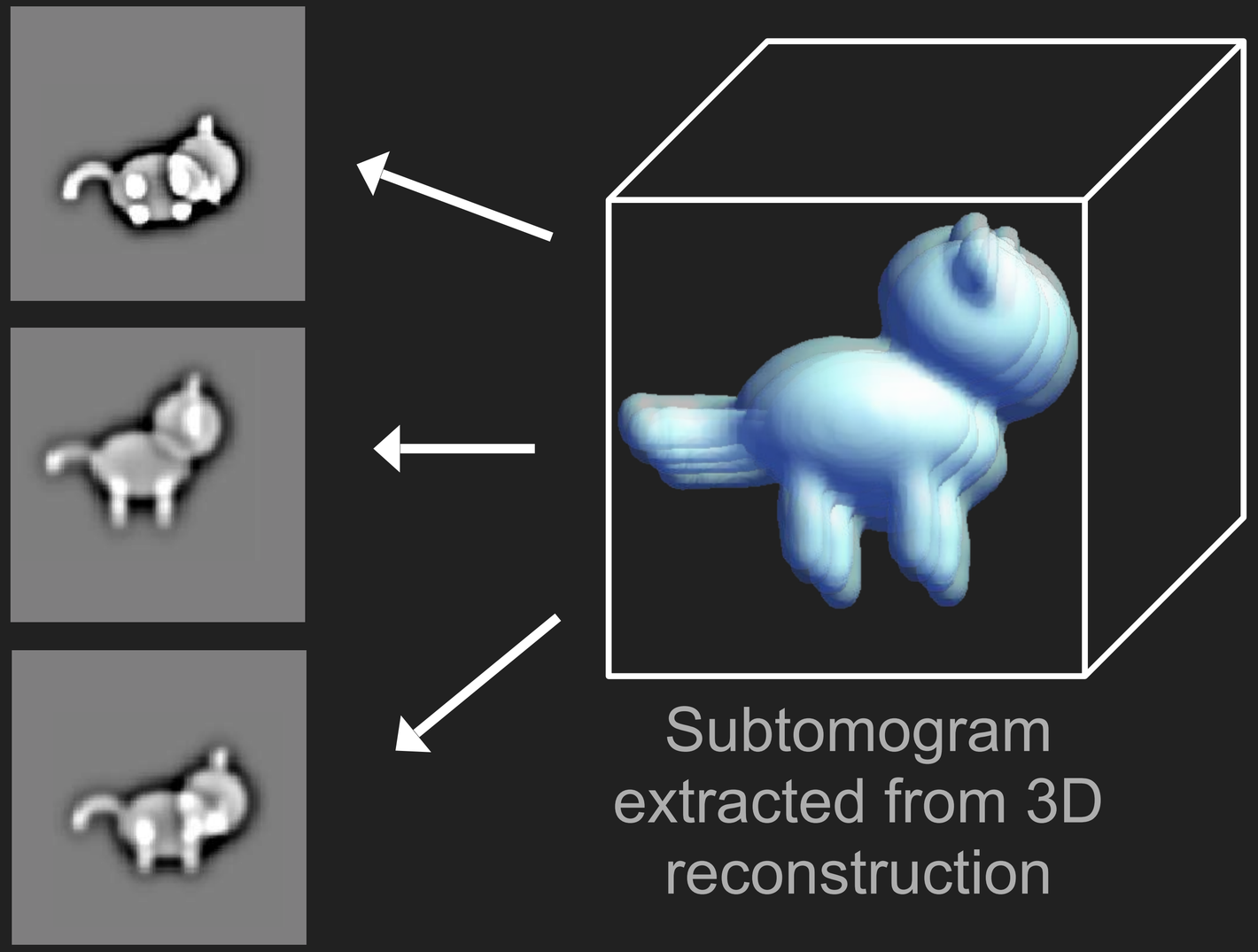
Per-particle per-tilt refinement strategy:

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

→ subnanometer to near-atomic resolution (with sufficiently good data)

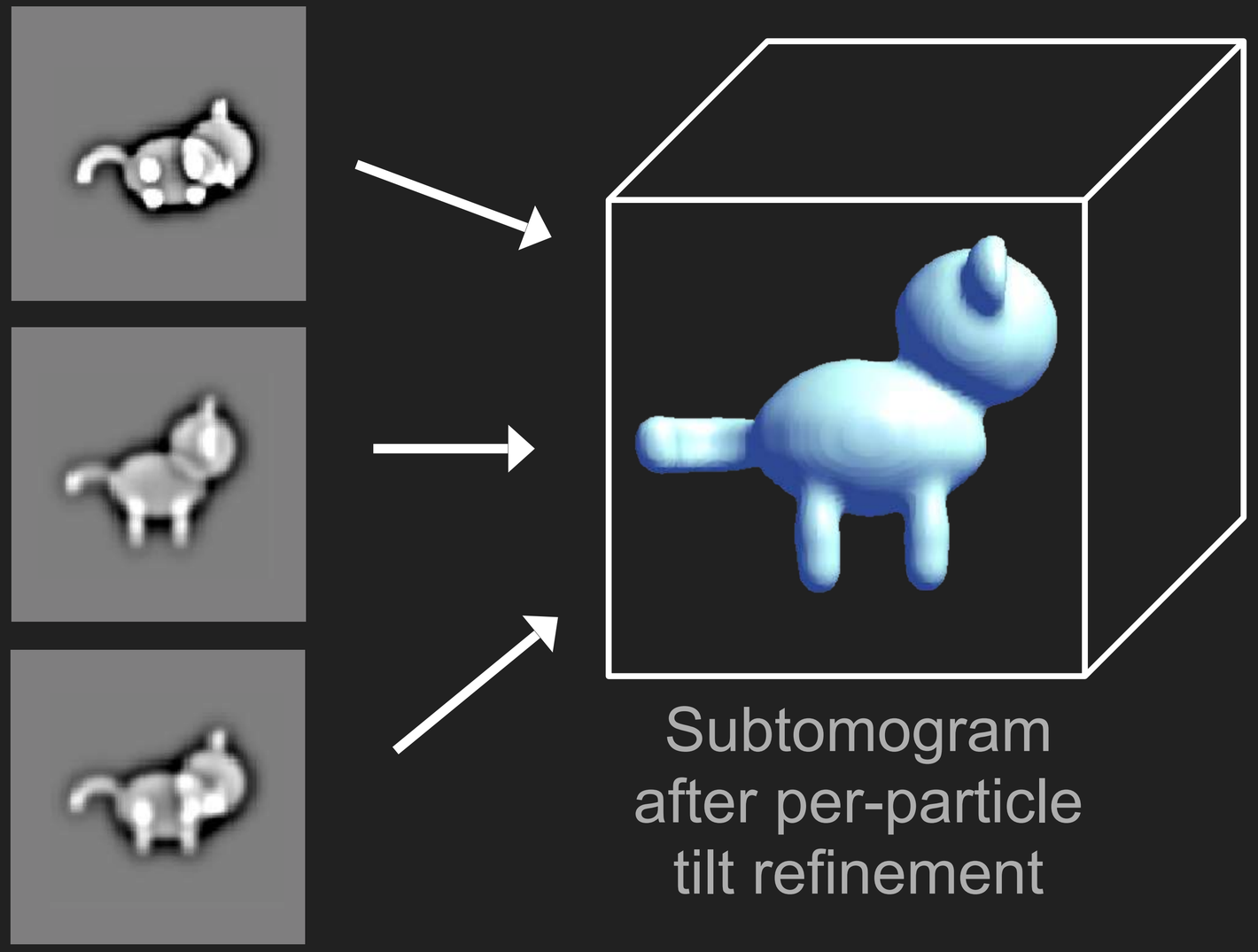
Particle tilt images
extracted from tiltseries

Per-particle
tilt refinement

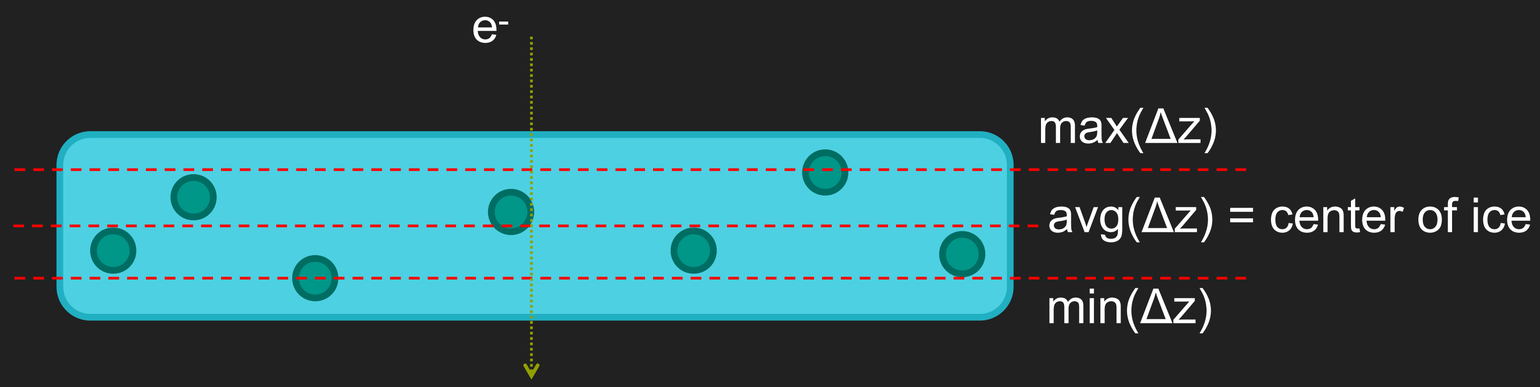


Subtomogram
extracted from 3D
reconstruction

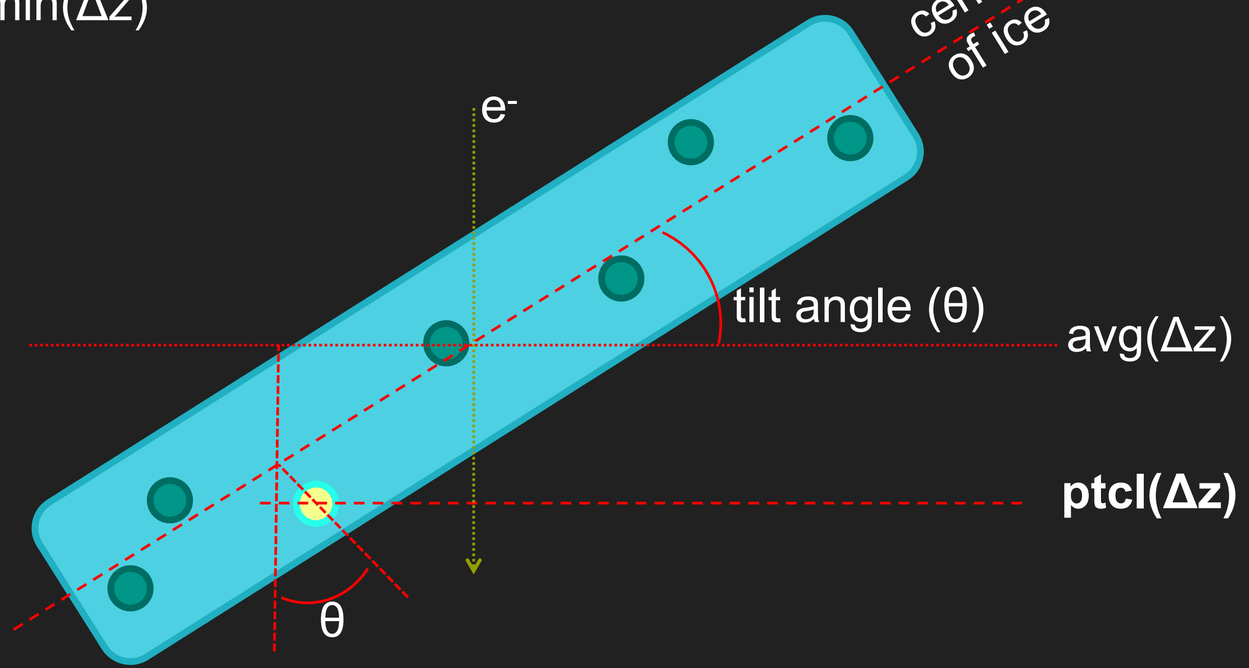
Per-particle tilt refinement



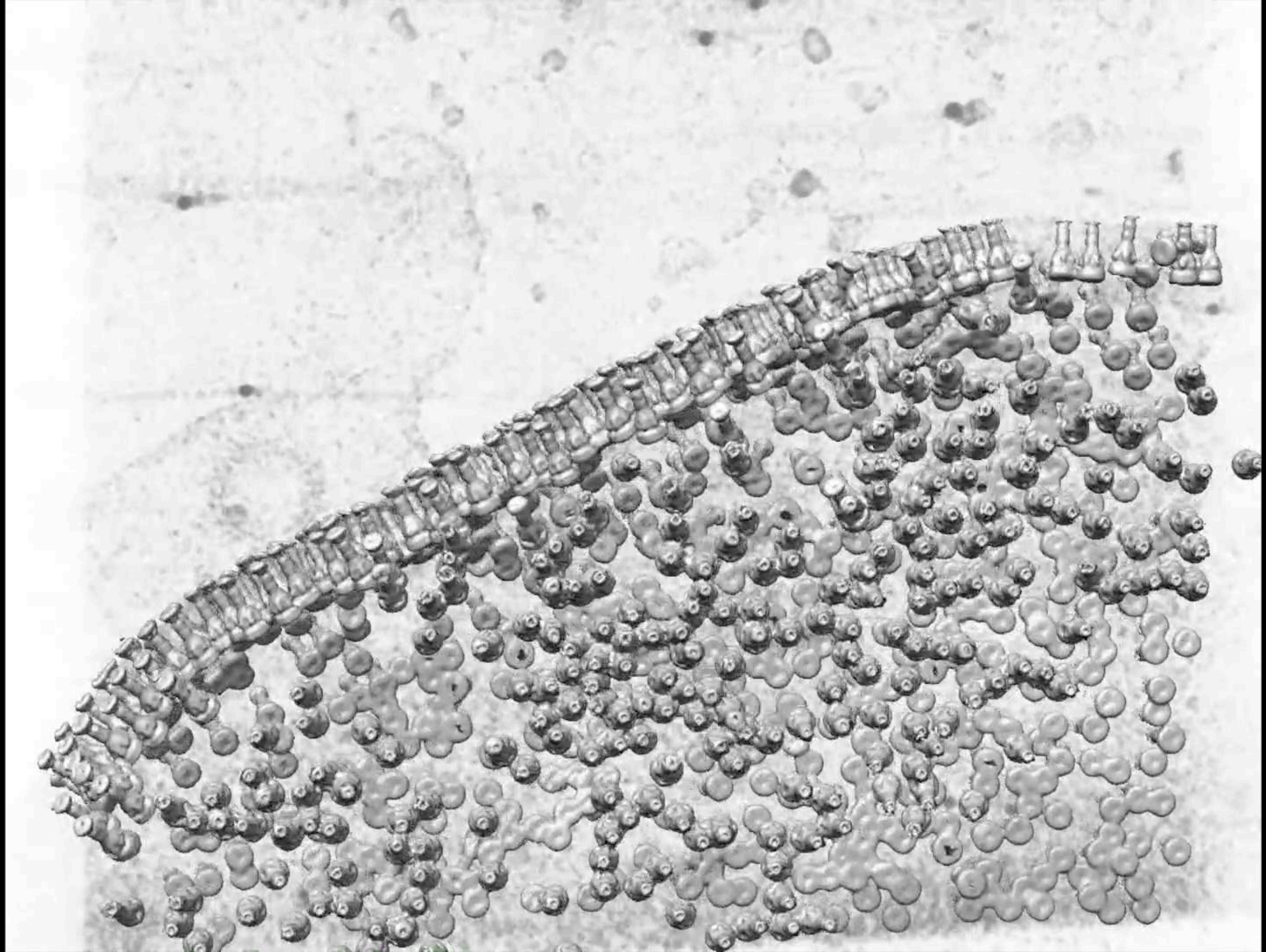
Per-particle, per-tilt CTF correction



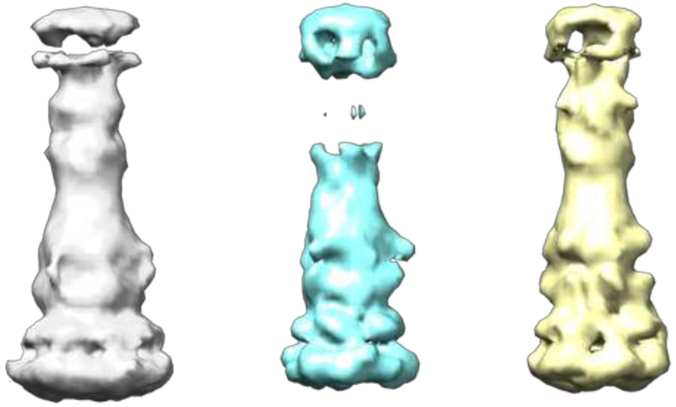
Determine defocus range from near-0° tilts with more signal



Search within defocus range at higher tilt angles.



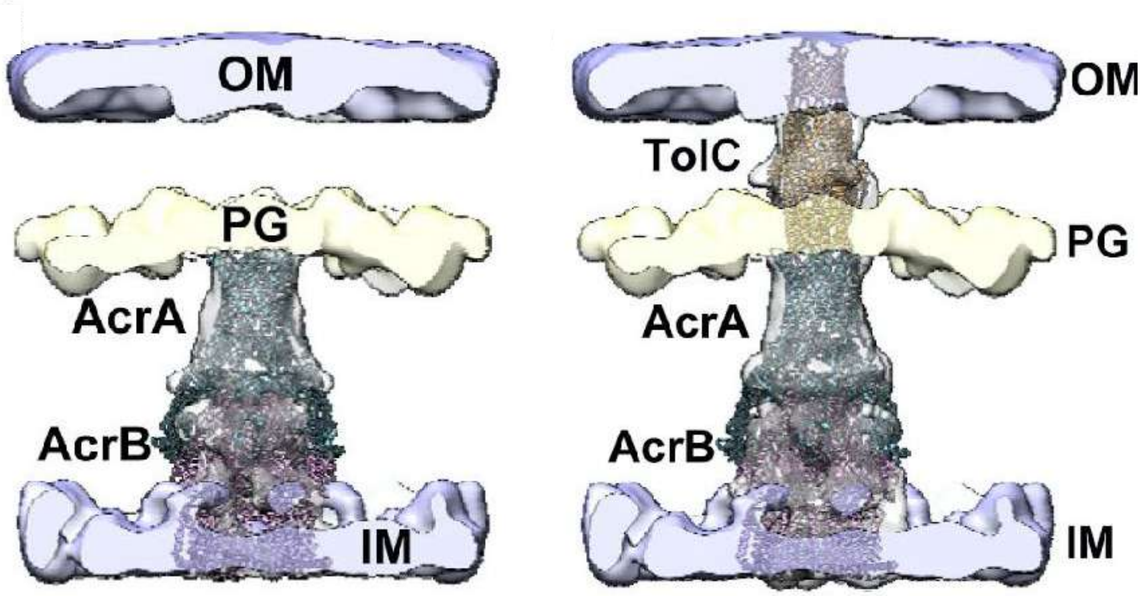
Outer Membrane



ALL particles **500 particles** **800 particles**

Focused classification results revealed a subset of partially assembled pumps

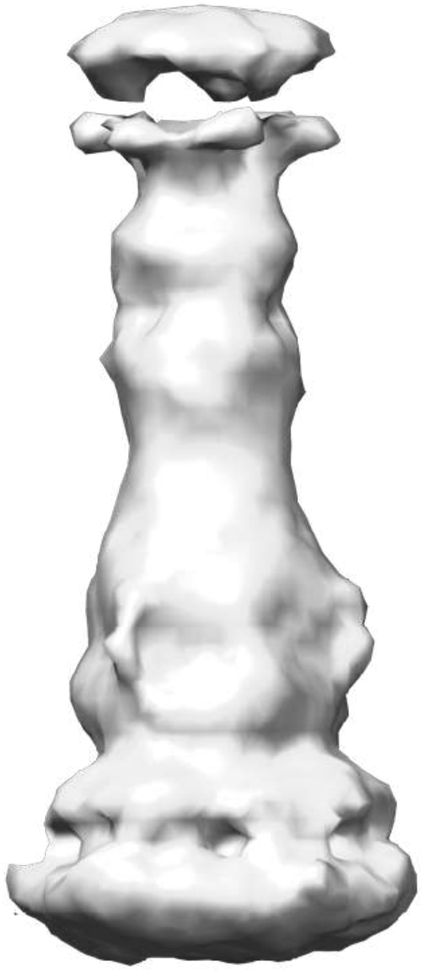
Mechanistic insights into the *in vivo* assembly of AcrAB-TolC



X Shi, et.al. Nature Comm., 10, 2635 (2019).

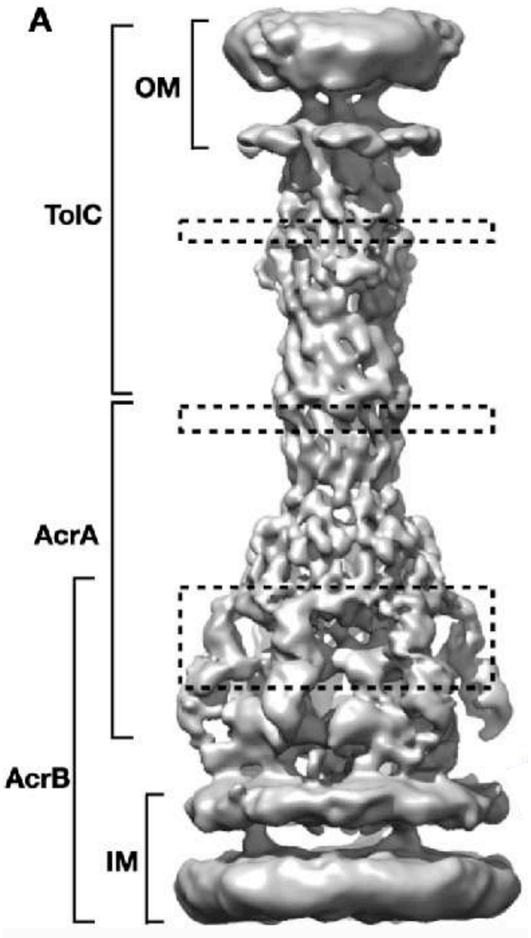
Subnanometer resolution *in-situ*

2018-19



1300 Particles

2019-2020



28,000 Particles
(102 tomograms)

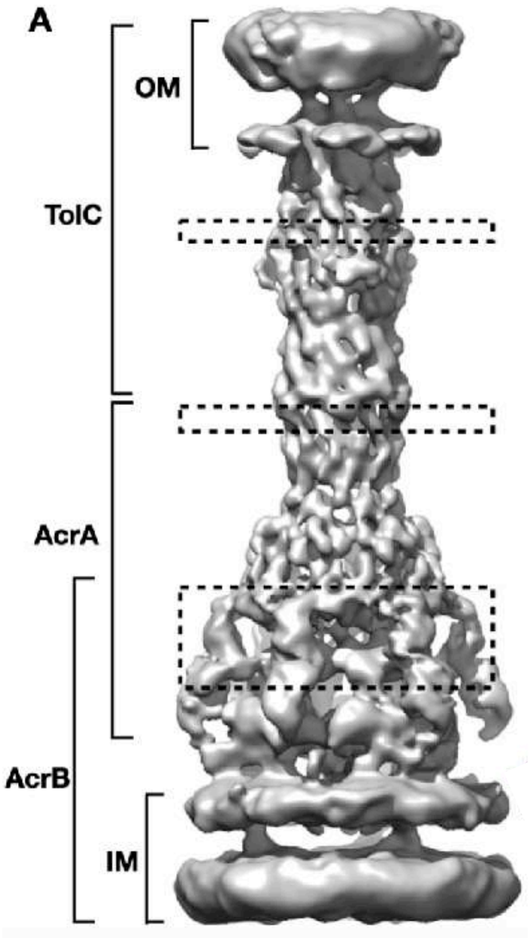
M Chen, X. Shi, Z Yu. et al. (2020) bioRxiv. https://www.biorxiv.org/content/10.1101/2020.06.10.144618v1

Subnanometer resolution *in-situ*

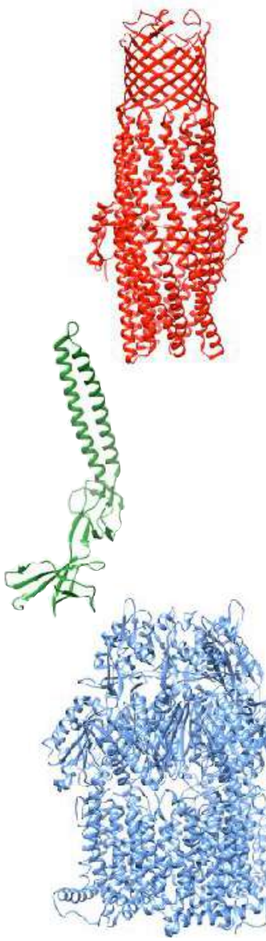
2018-19

1300 Particles

2019-2020



28,000 Particles

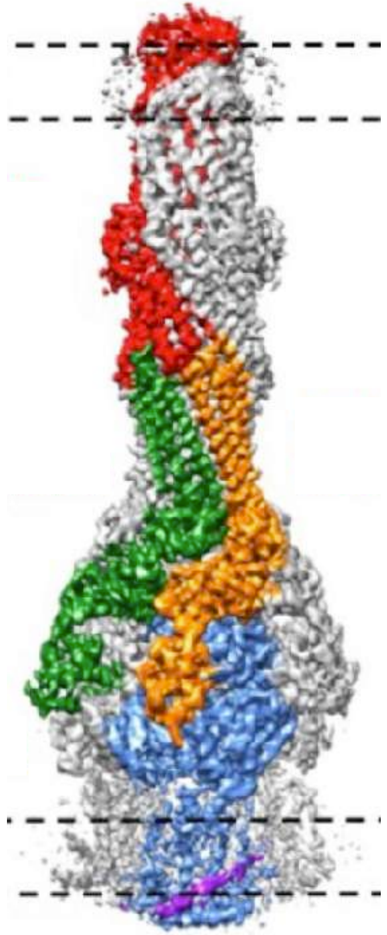


3:6:3
pseudo C6

M Chen, X. Shi, Z Yu. et al. (2020) bioRxiv. https://www.biorxiv.org/content/10.1101/2020.06.10.144618v1

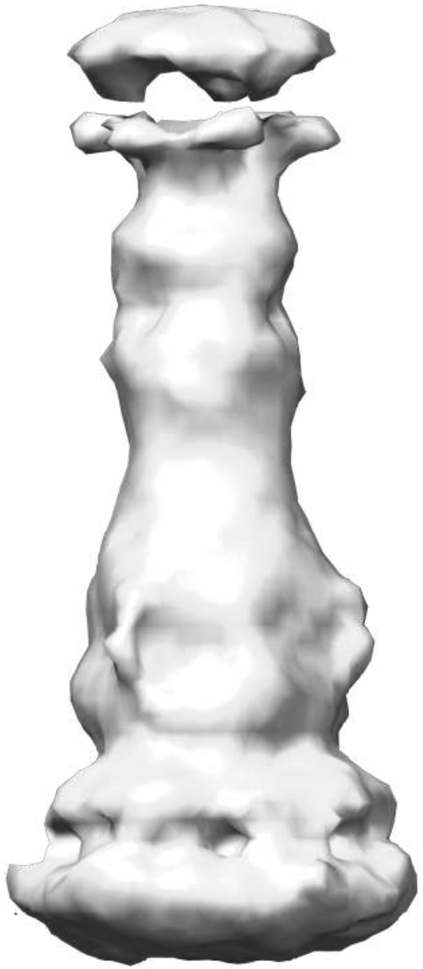
Subnanometer resolution *in-situ*

2017



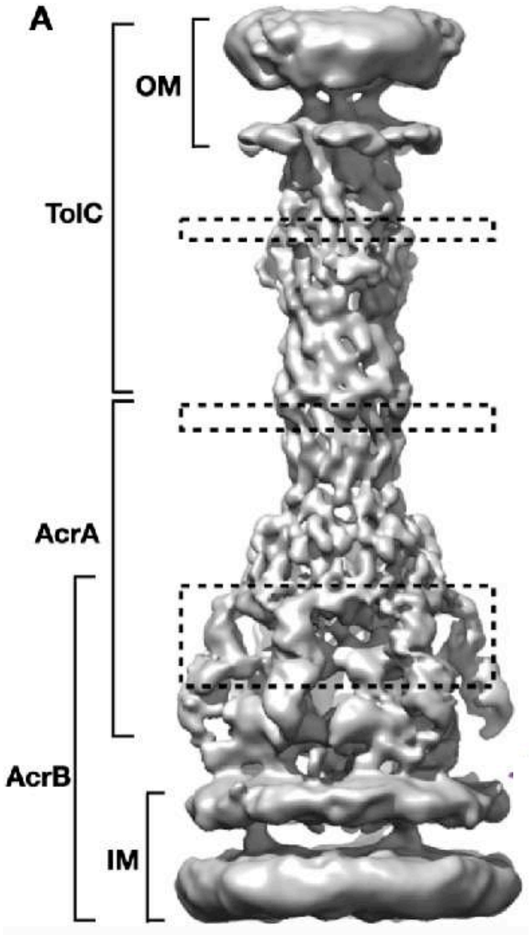
25,000 Particles
disulphide mod.
cross-linked + amphipol

2018-19

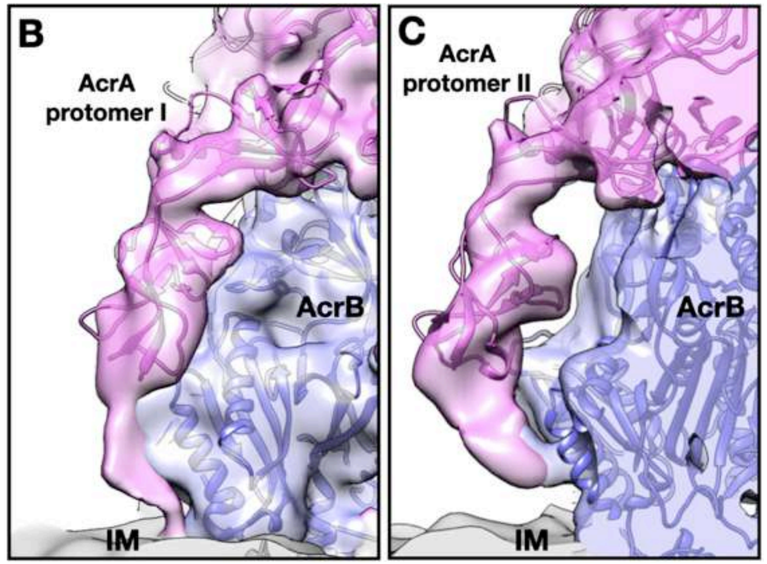


1300 Particles

2019-2020



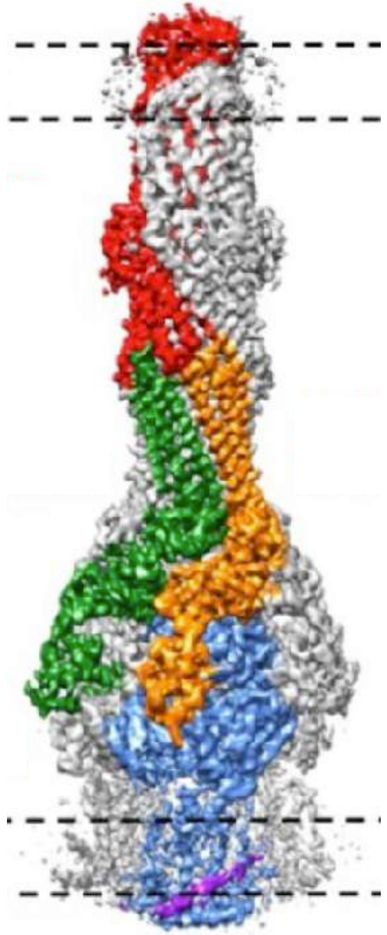
28,000 Particles



M Chen, X. Shi, Z Yu. et al. (2020) bioRxiv. https://www.biorxiv.org/content/10.1101/2020.06.10.144618v1

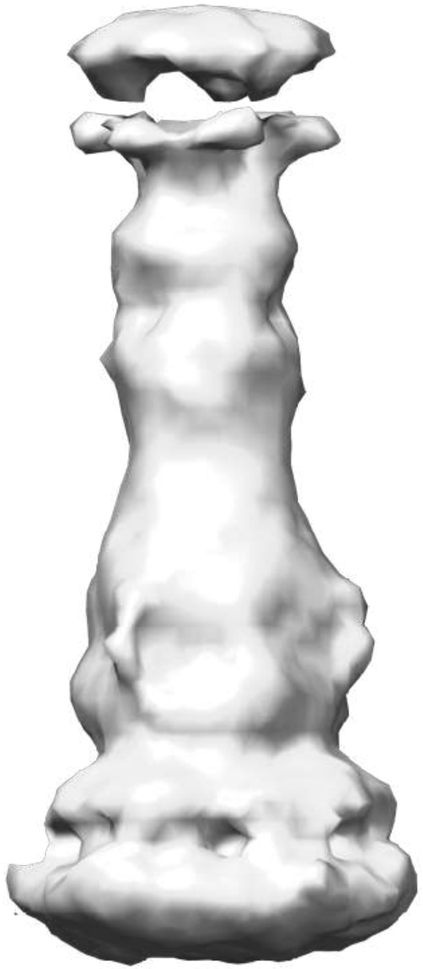
Subnanometer resolution *in-situ*

2017



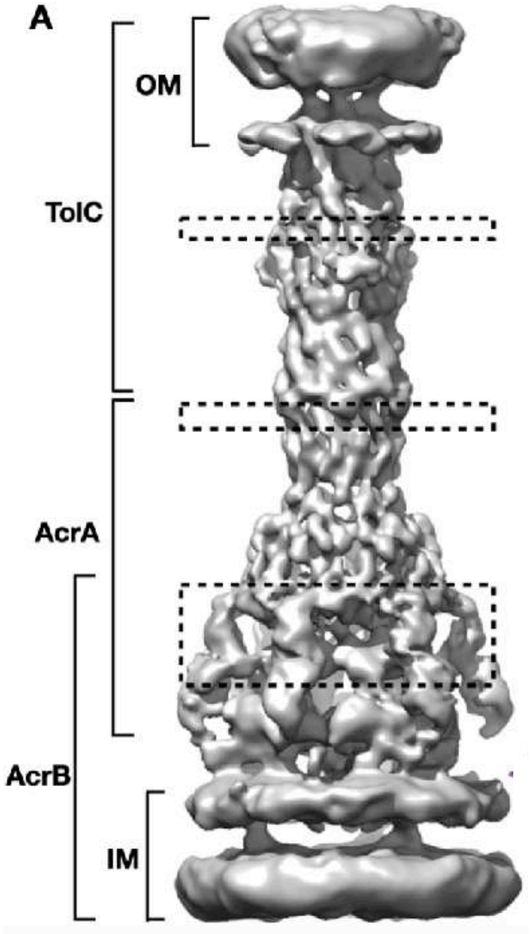
25,000 Particles
amphipol

2018-19

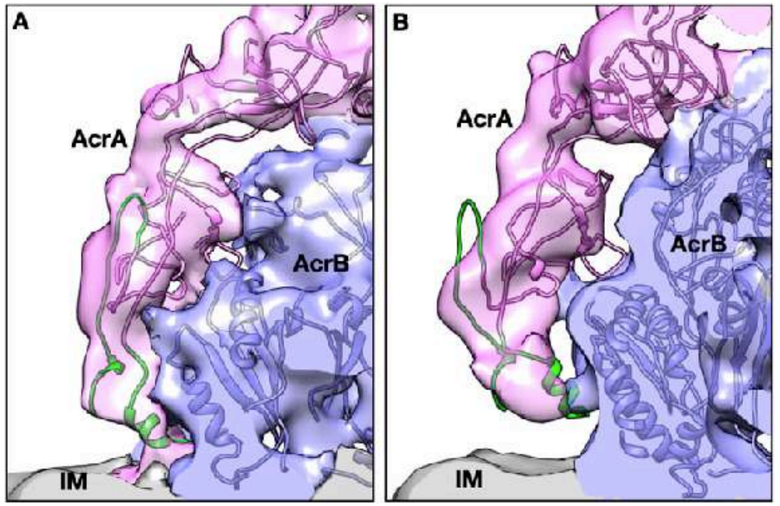


1300 Particles

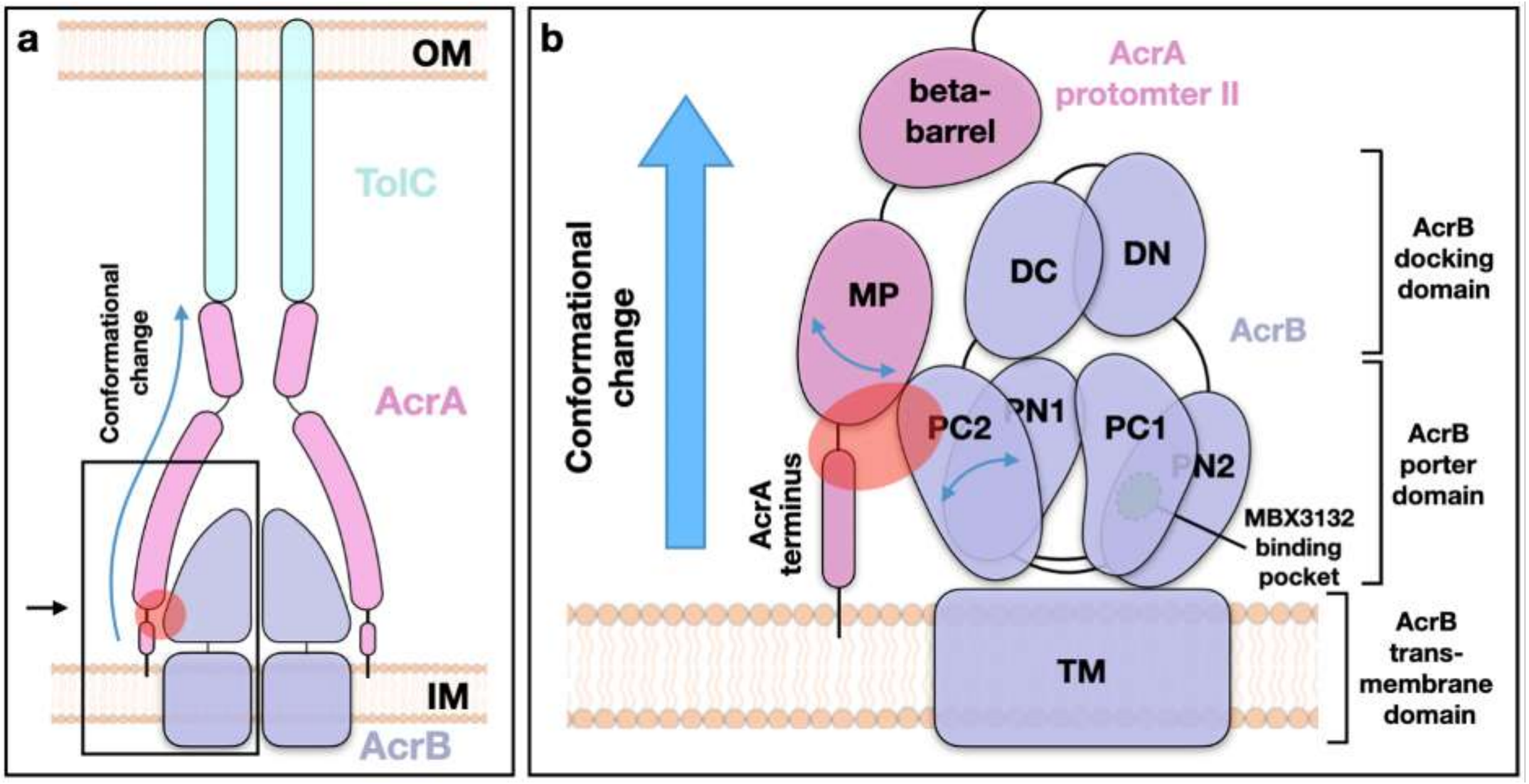
2019-2020



28,000 Particles



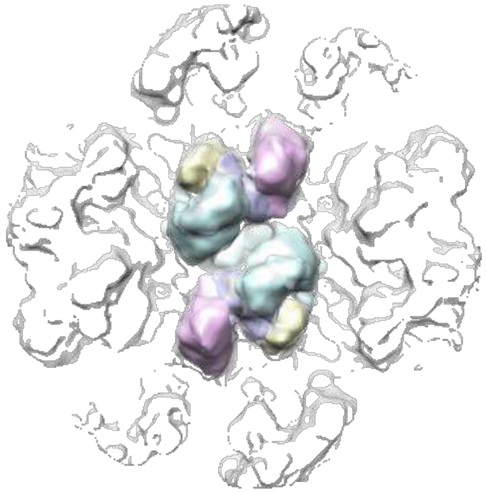
M Chen, X. Shi, Z Yu. et al. (2020) bioRxiv. https://www.biorxiv.org/content/10.1101/2020.06.10.144618v1


a
OM
TolC
Conformational change
AcrA
IM
AcrB
b
AcrA protomter II
beta-barrel
Conformational change
MP
DC
DN
AcrB
AcrB docking domain
PC2
PN1
PC1
PN2
AcrB porter domain
AcrA terminus
MBX3132 binding pocket
TM
AcrB trans-membrane domain

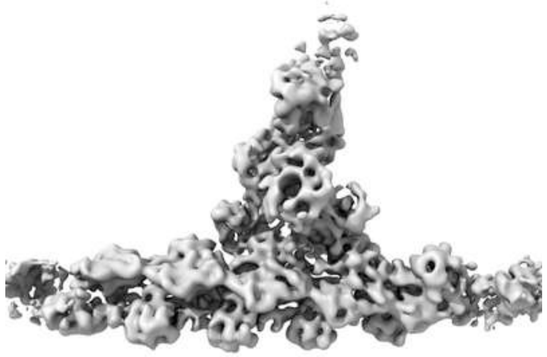
Other Subnanometer in-situ Structures

19 deposited 'cellular component' structures at <12 Å resolution
11 of these are ribosomes or ribosome-associated

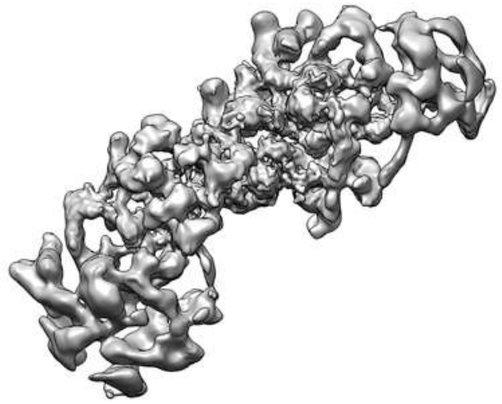
RNA polymerase-NusA, 9.7 Å
Mycoplasma pneumoniae
Rappsilber group, EMD-10680



Photosys II 2D array
10.2 Å, diatom
Dai group*, EMD-0540

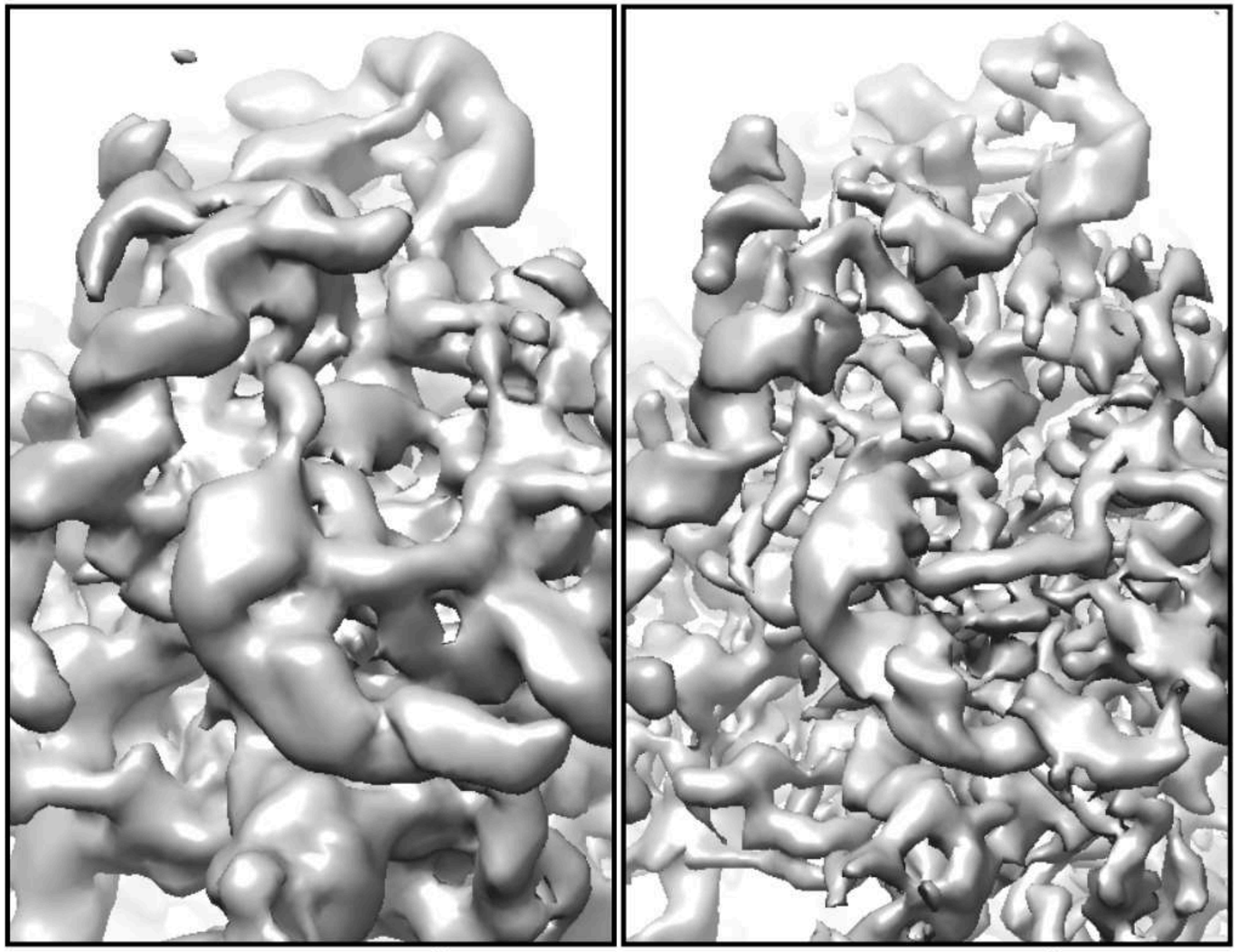


Arp2/3 complex, 9 Å
fixed mouse lamellipodia
unpub, Schur group, EMD-11869



NPC Cytoplasmic Ring
9.1 Å, xenopus l.
Shi Group, EMD-0986

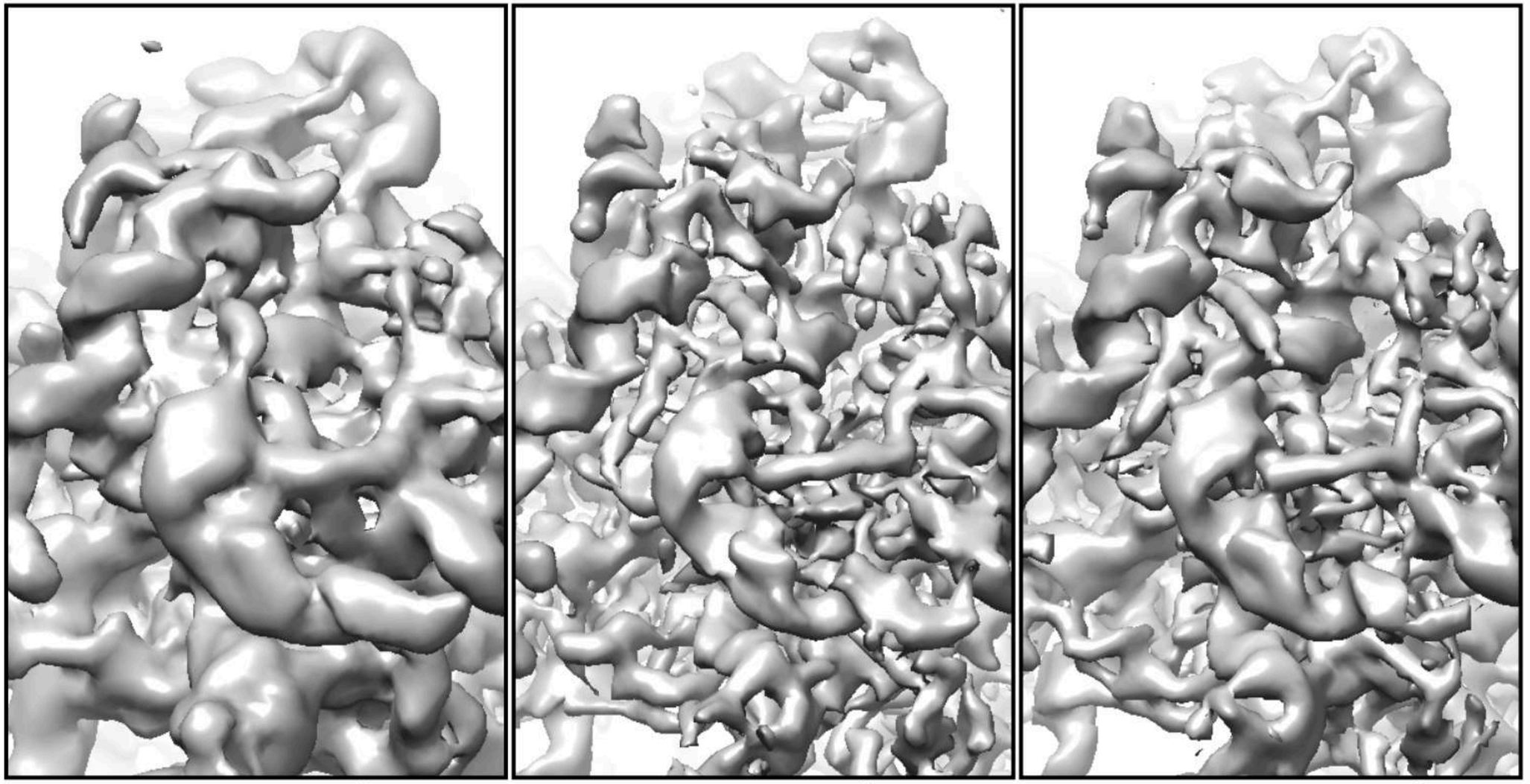
80S Ribosome
EMPIAR-10064



EMAN2.9 : 8.5Å

EMAN3.0 : 7.5Å
(devel)

80S Ribosome
EMPIAR-10064



EMAN2.9 : 8.5Å

EMAN3.0 : 7.5Å
(devel)

EMPIAR-11654 : 5.7Å
(!?)

Tegunov D, Xue L, Dienemann C, Cramer P, Mahamid J.
Nature Methods. 2021; 18(2) 186-193.

Summary

- Data compression throughout pipeline

Future Directions *in-situ*

- Data collection protocols need to be optimized
  - larger tilt step?
  - narrower tilt range?
  - Play with dose distribution?
  - FIB-milling parameters
- Particle variability in cells (compositional and conformational)
- Iterative Real/Fourier wedge filling to improve annotation
- Labelling assemblies inside the cell
- Additional improvements to PPPT refinement

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