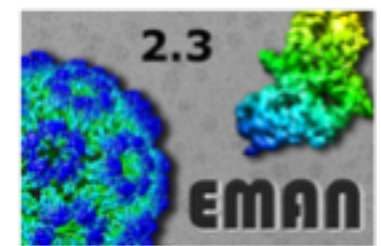




High Resolution Sub-tomogram Averaging

Steve Ludtke

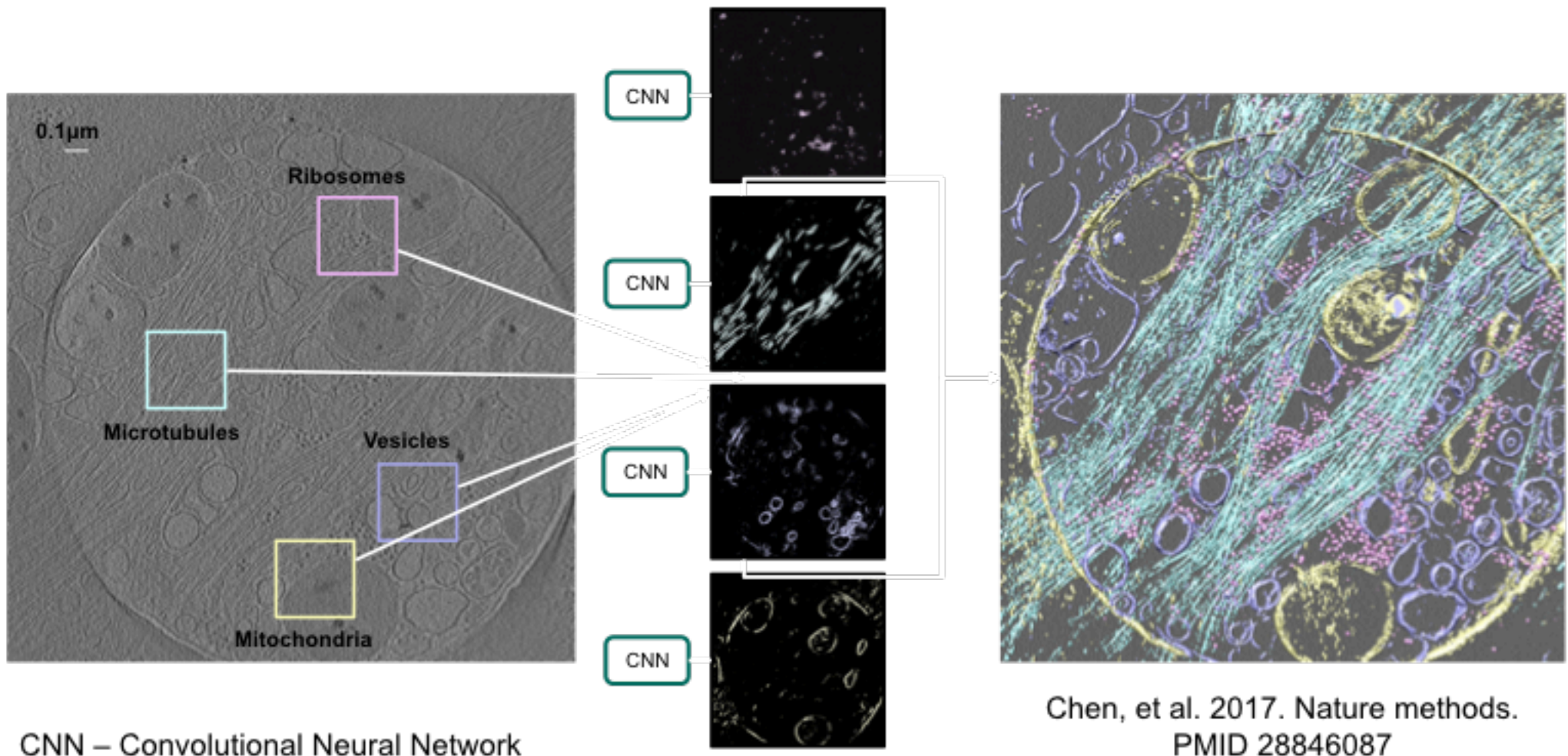
Charles C. Bell Professor
Biochemistry and Molecular Biology
Director, CryoEM/CryoET Core
Co-director CIBR Center
Baylor College of Medicine



EMAN2 Tomography Tools

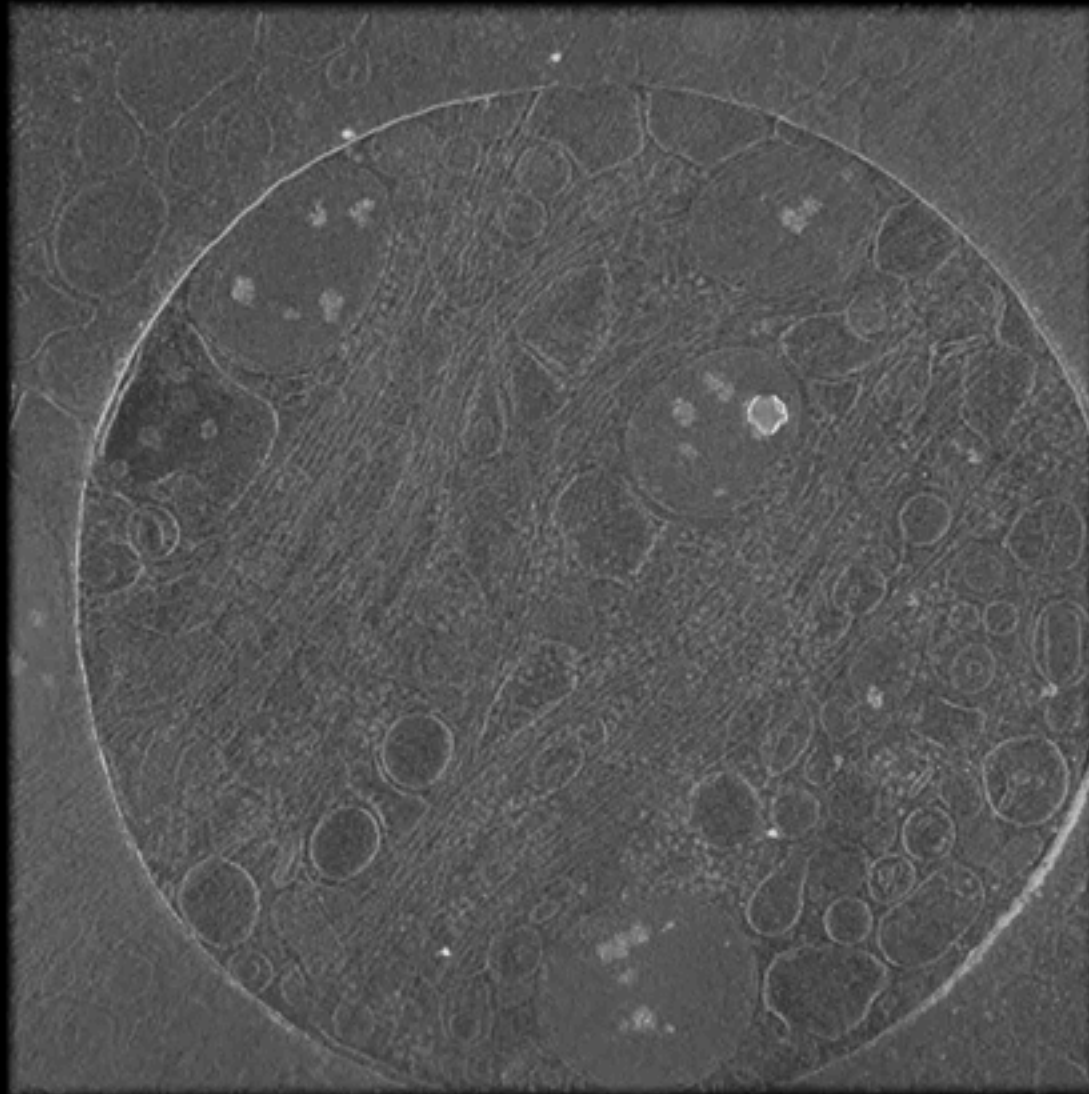
- ➔ Fiducial-free tilt series alignment
- ➔ Tiled Fourier Reconstruction
 - Tilt series CTF estimation and correction
- ➔ Manual, reference-based and deep-learning 3-D particle picking
 - Deep learning based tomogram annotation
- ➔ SGD initial average generation
 - Iterative subtomogram averaging
- ➔ High resolution subtomogram averaging with Per-particle per-tilt (PPPT) alignment and CTF refinement

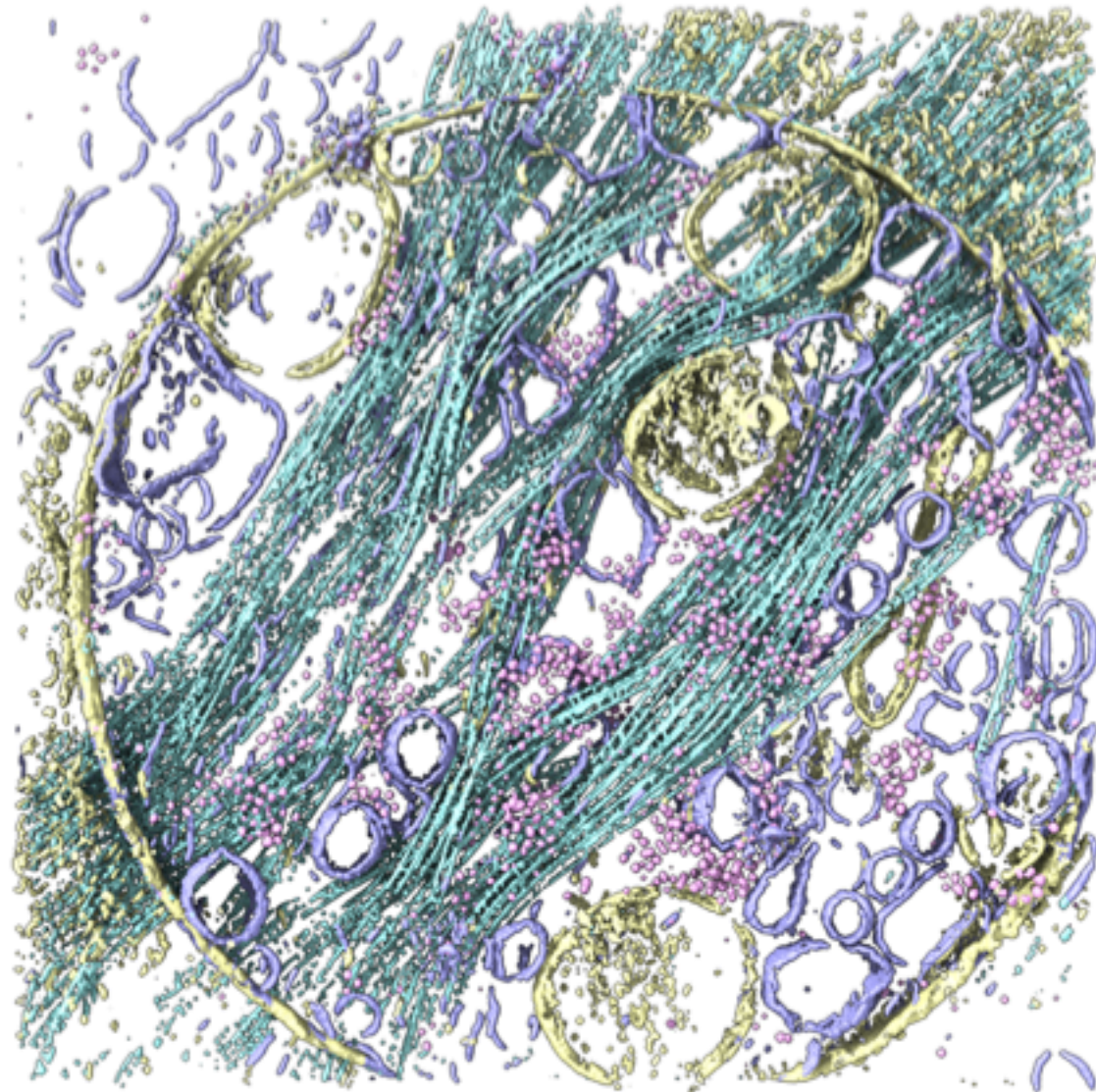
Deep learning-based cellular annotation



CNN – Convolutional Neural Network

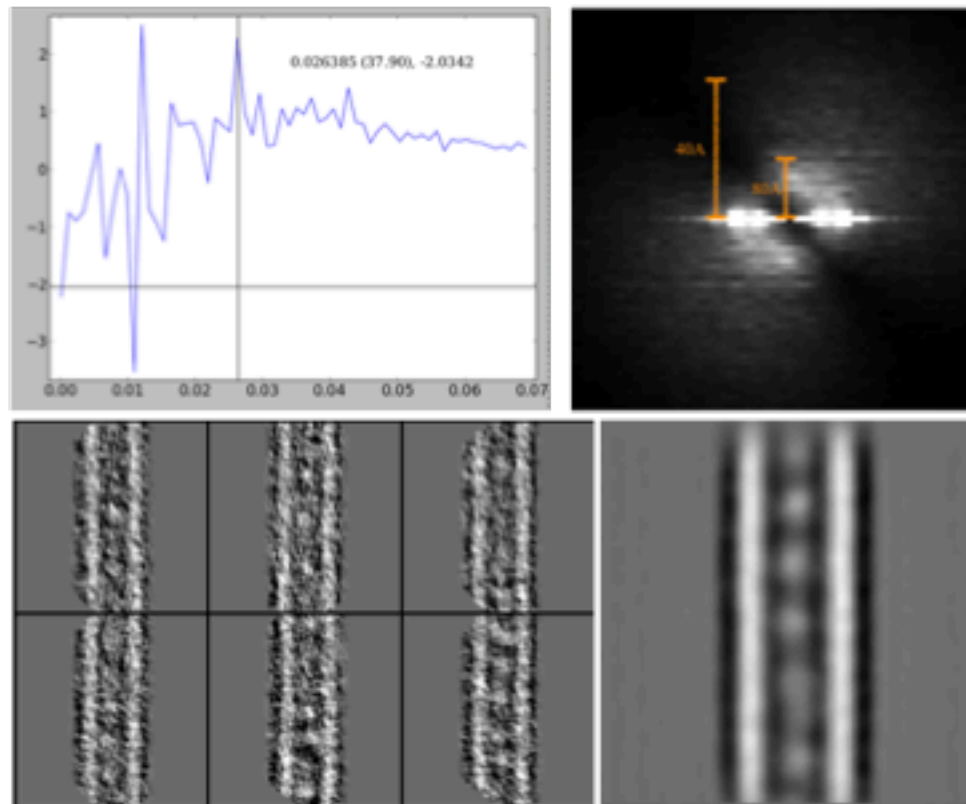
PC12 JEM2100 CCD





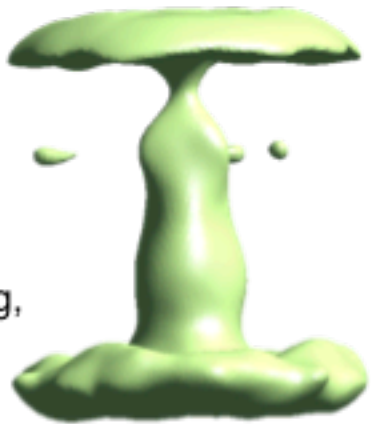
Microtubule

PC12, JEM2100, Apix=14.3

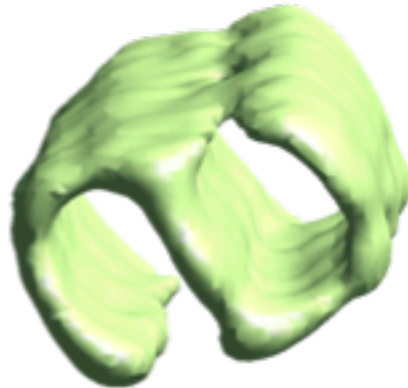


Reference-free initial model generation

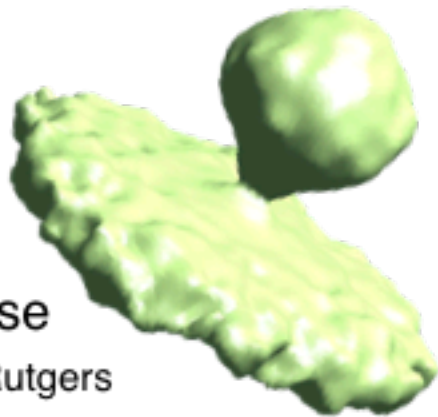
ToIC
X Shi,
Z Wang,
BCM



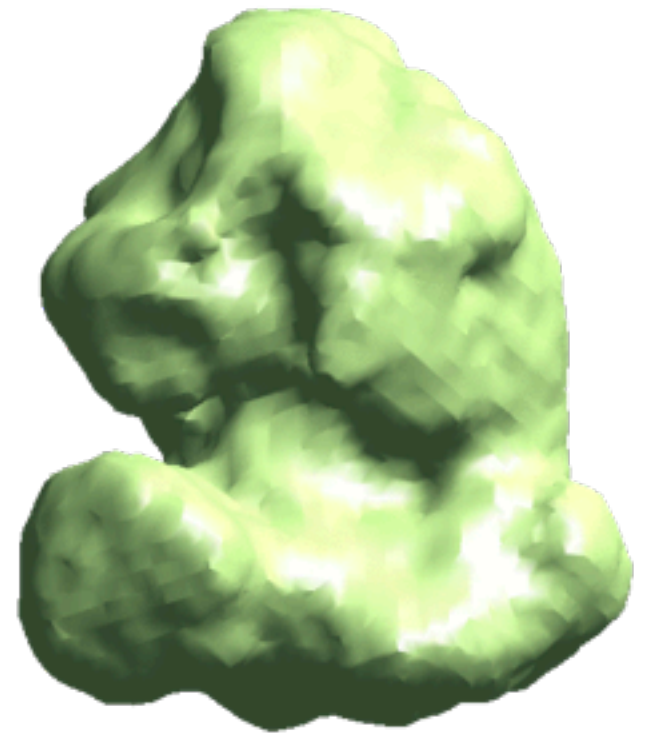
microtubule
doublet
SY Sun, Stanford



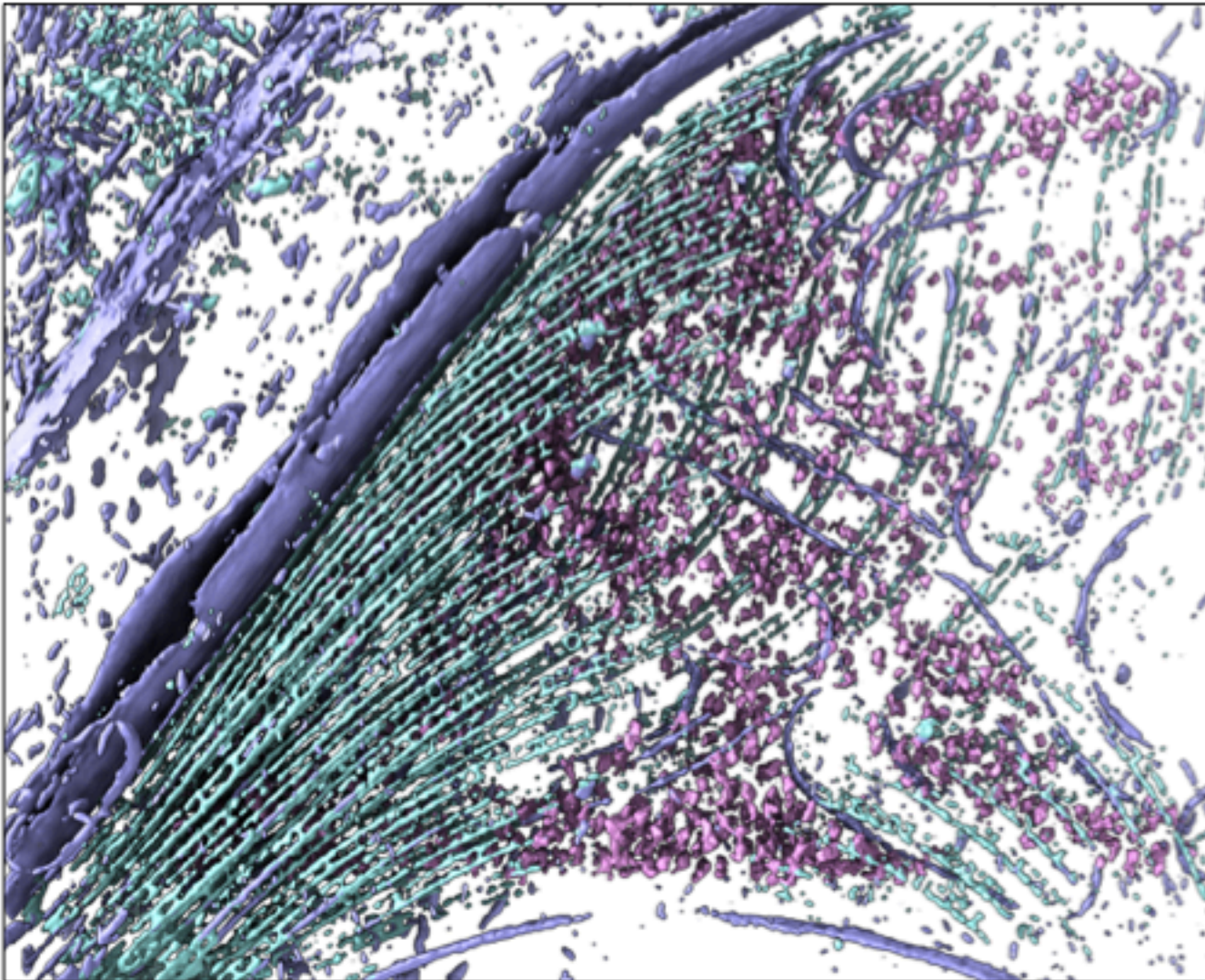
ATP
synthase
W Dai, Rutgers



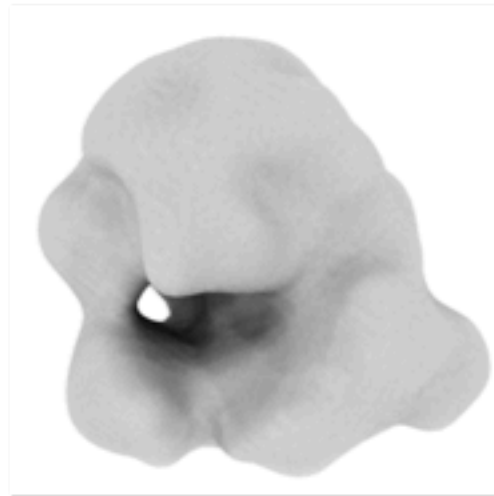
80S Ribosome
EMPIAR 10064



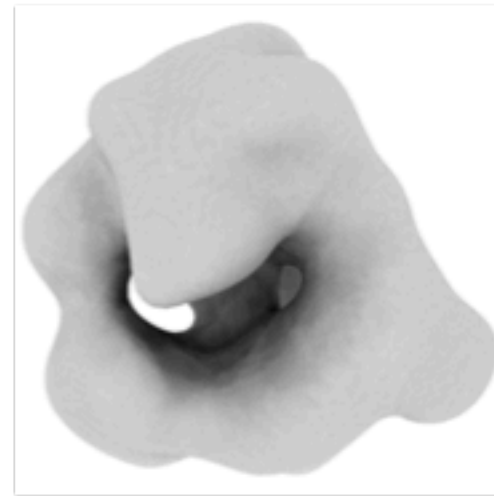
Trypanosoma Brucei



Ribosome

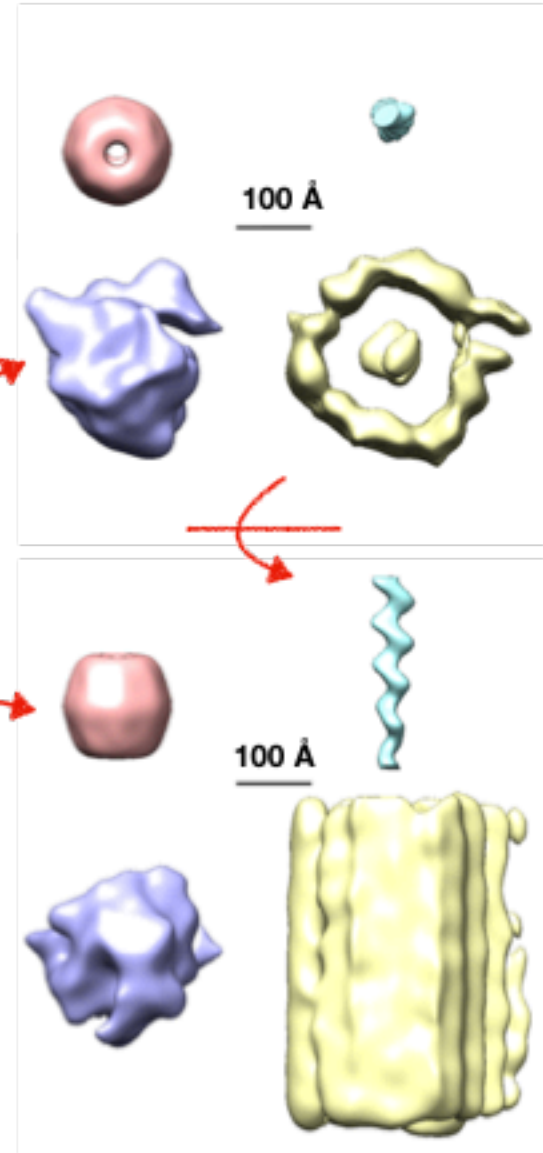
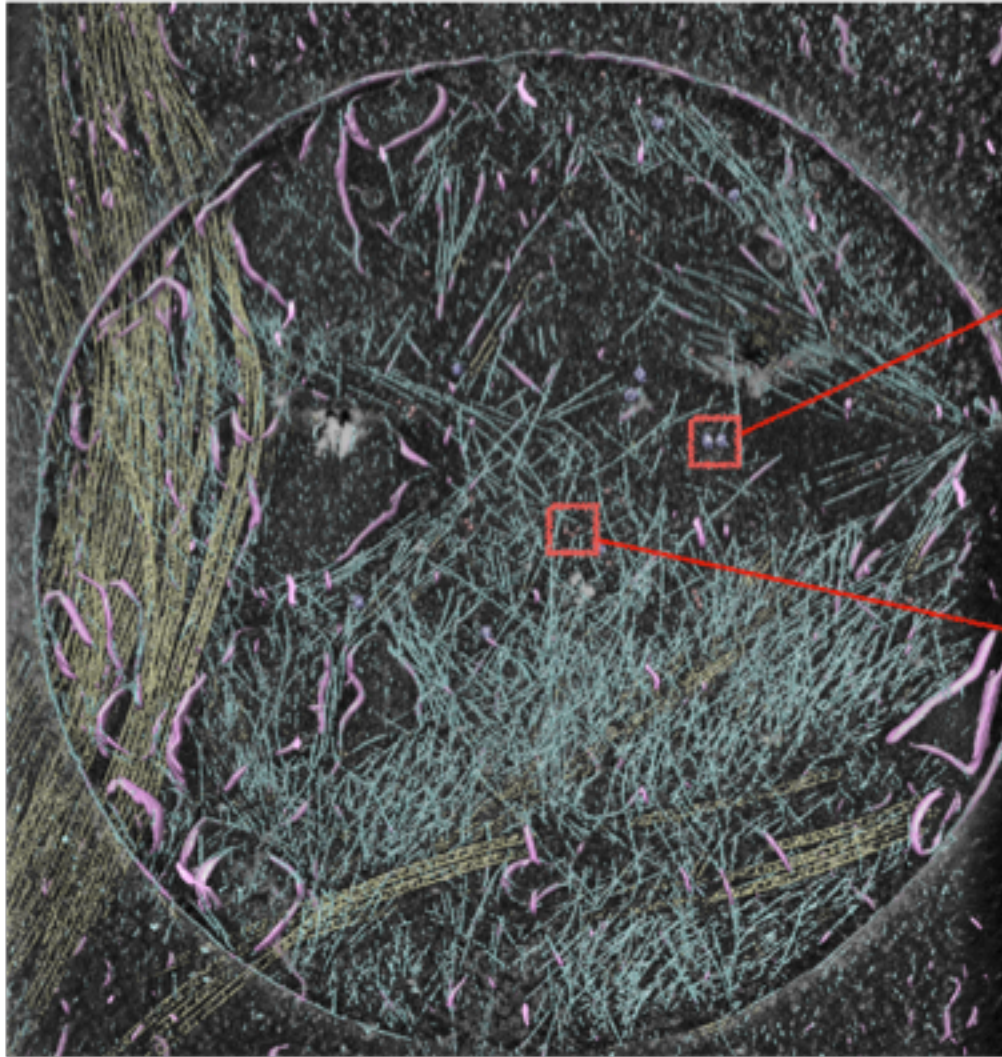


**EMDB-2239
(filtered)**



**Trypanosome
Subtomogram
Average**

Cultured Mouse Neuron, 6.6 Å/pix



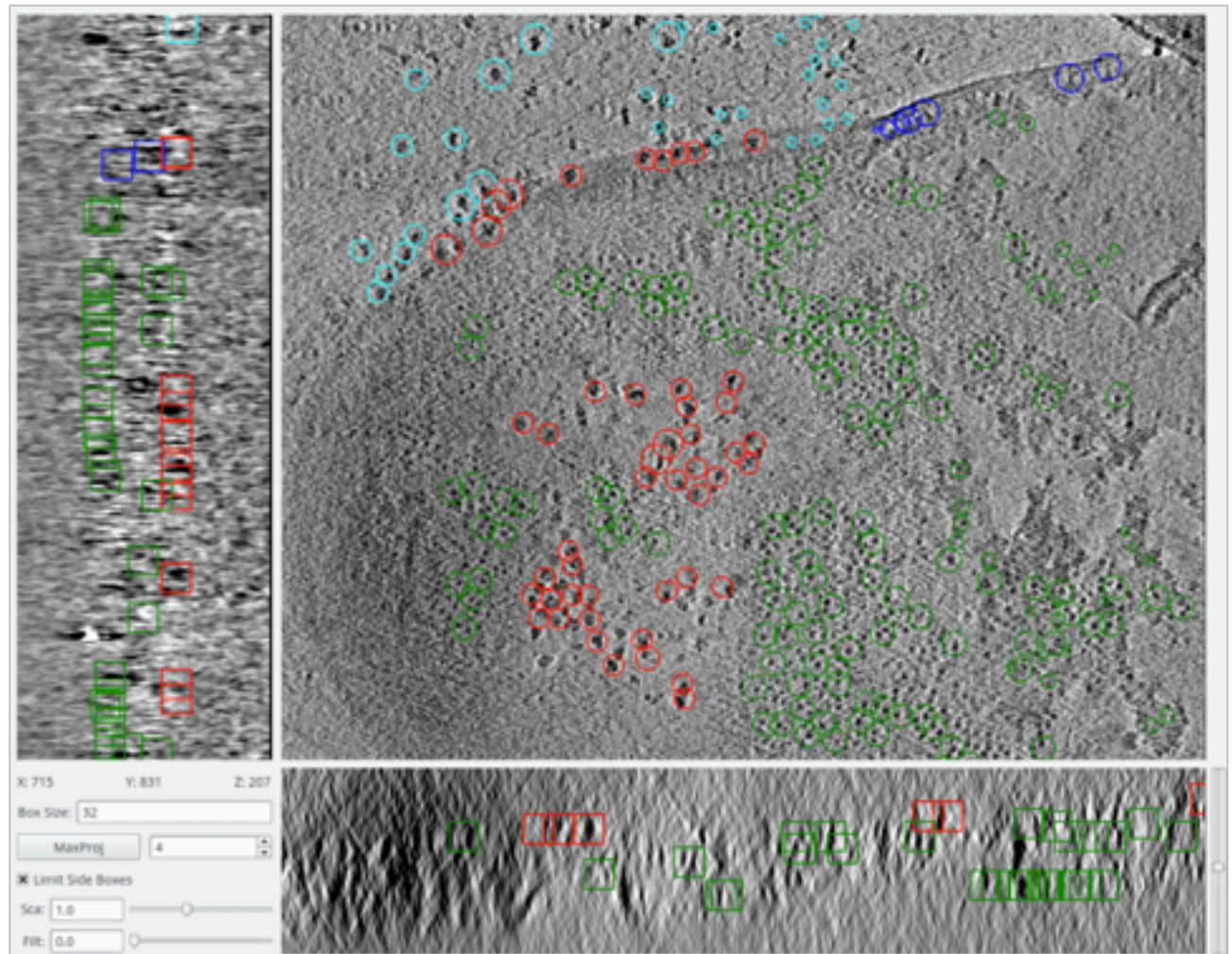
Multi-specimen
subtomogram
boxing

ToIC - side

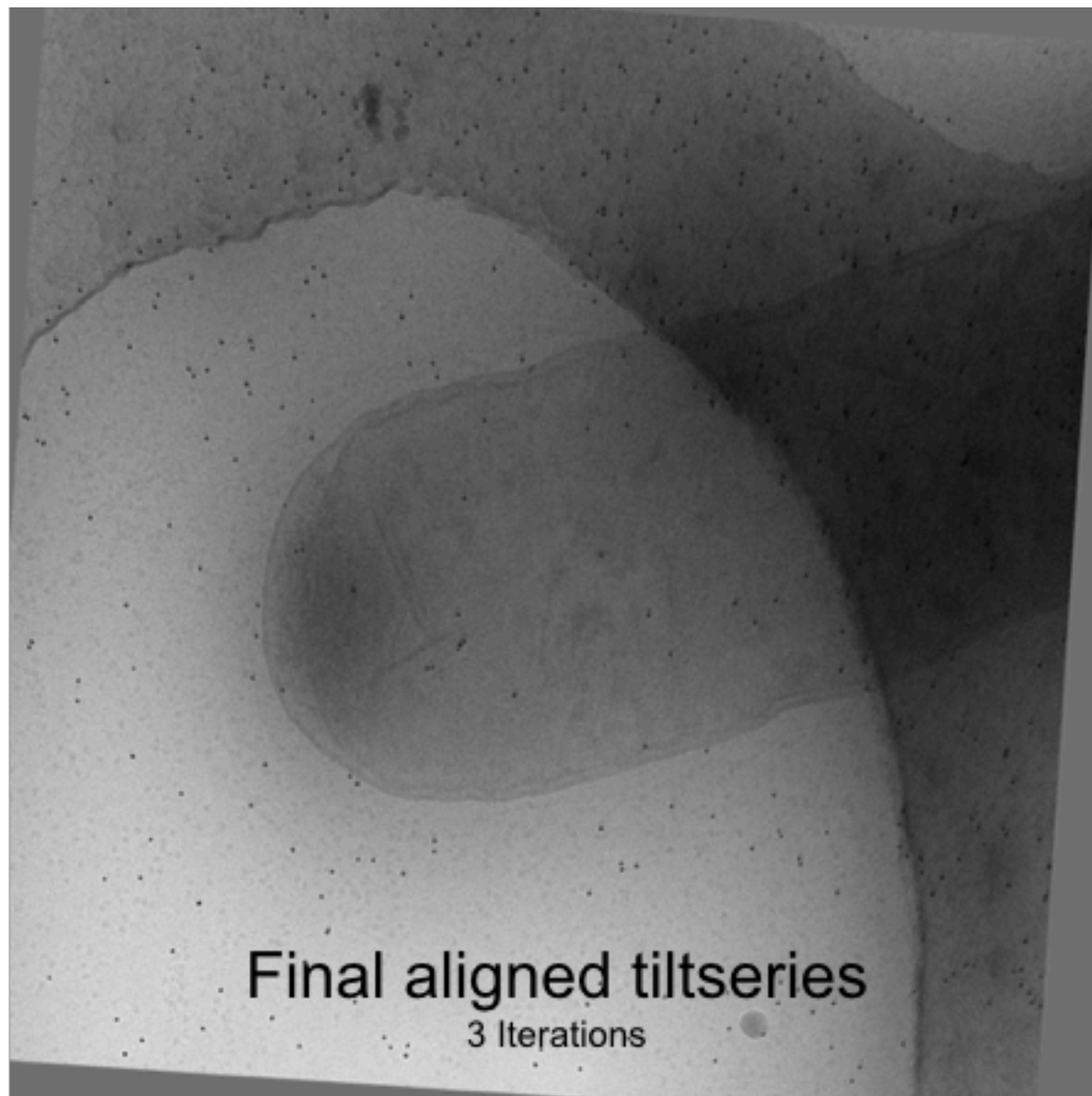
ToIC - top

Ribo - in

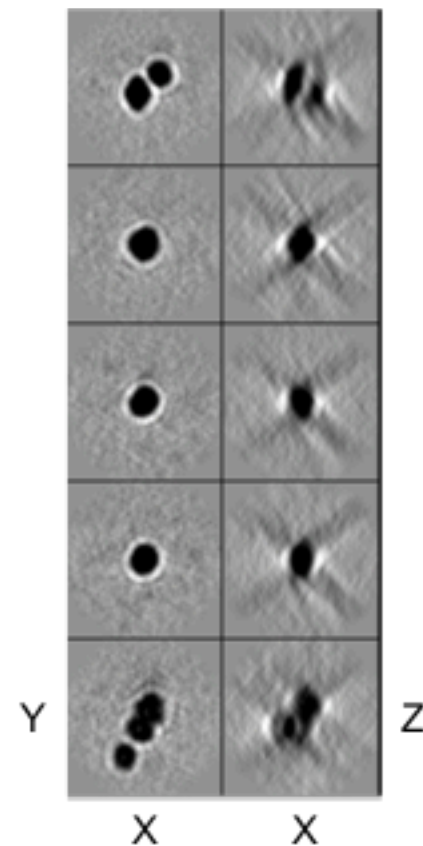
Ribo - out



Runtime:
~10 minutes on
12 threads



Refined
landmarks
(bin x2)



X Shi,
Z Wang,
BCM

Individual particle tilt series:

- Bad tilt exclusion
 - refine 3D alignment
 - Per-tilt CTF correction
- subnanometer resolution!

Particle tilt images
extracted from tiltseries



Per-particle
tilt refinement



Subtomogram
extracted from 3D
reconstruction



Refined particle
tilt images

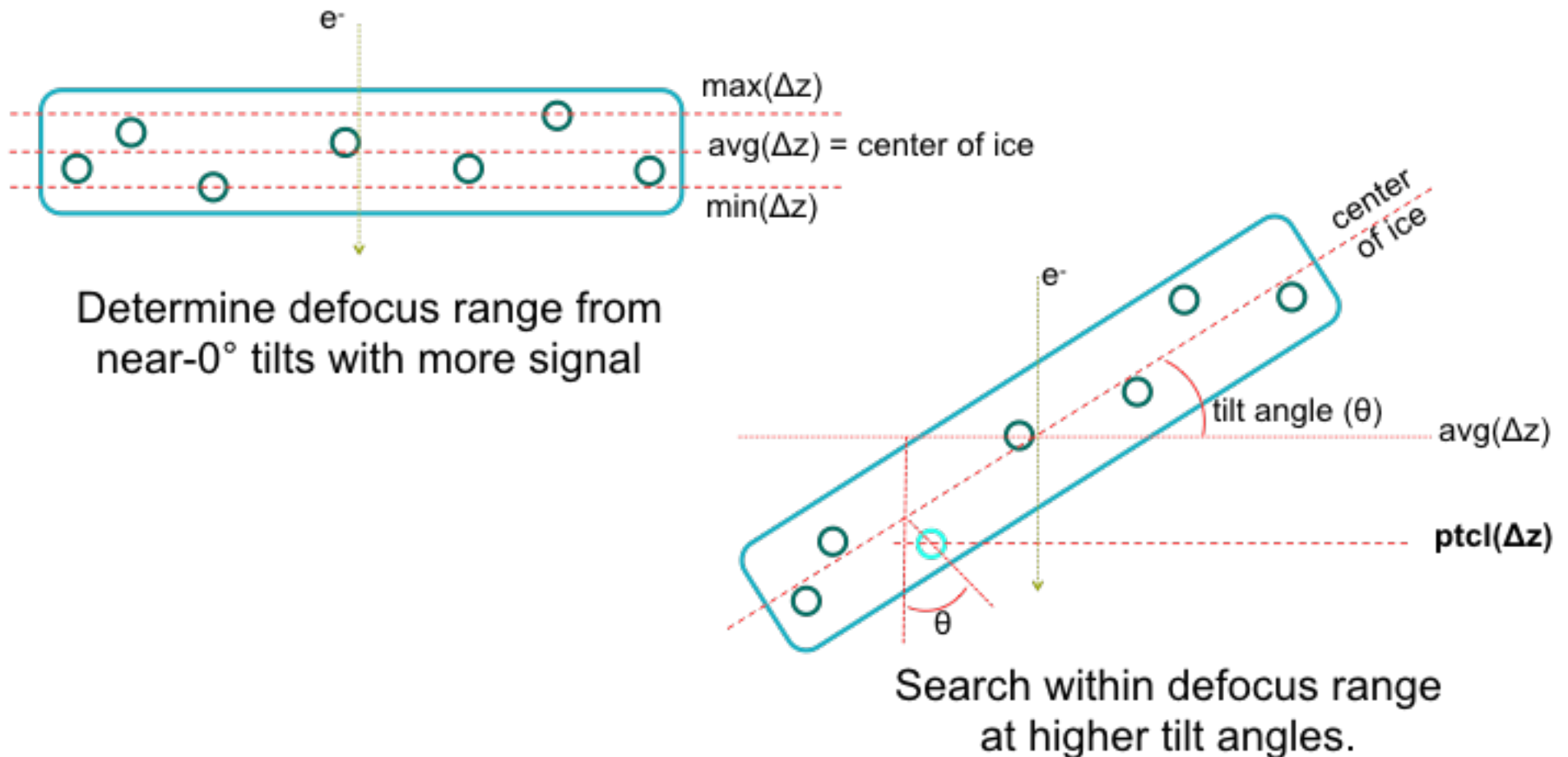


Per-particle
tilt refinement

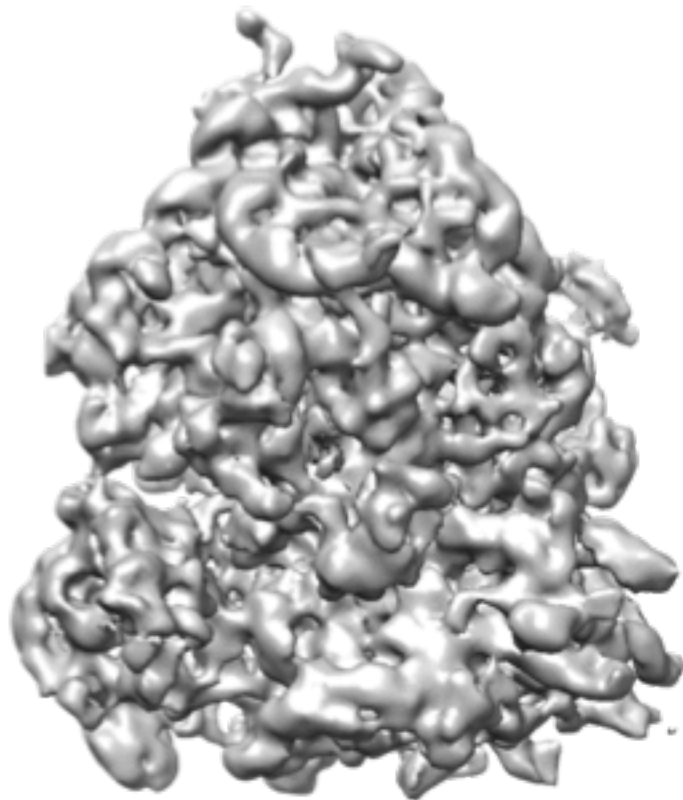


Subtomogram
after per-particle
tilt refinement

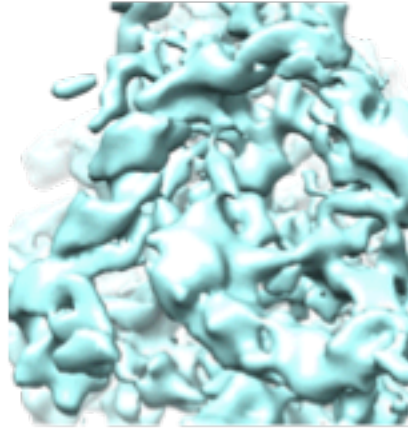
Per-particle, per-tilt CTF correction



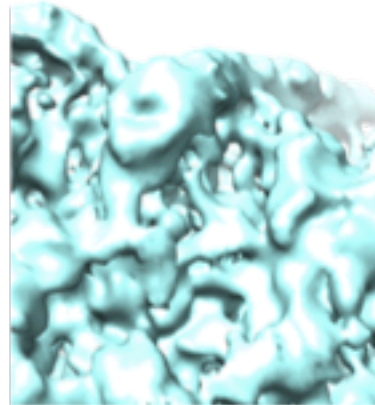
High-resolution subtomogram averaging in EMAN2



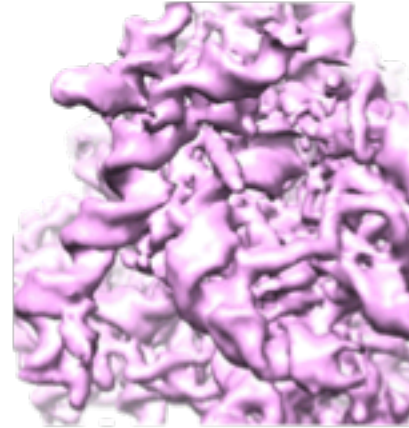
EMPIAR – 10064
80S Ribosome



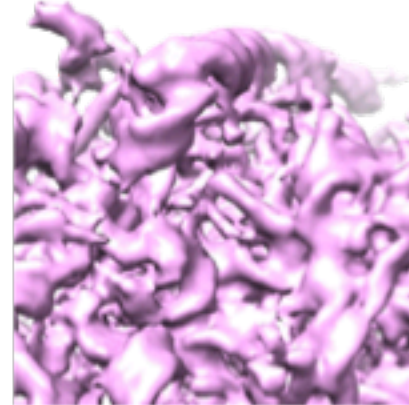
EMD-3420 - 11Å



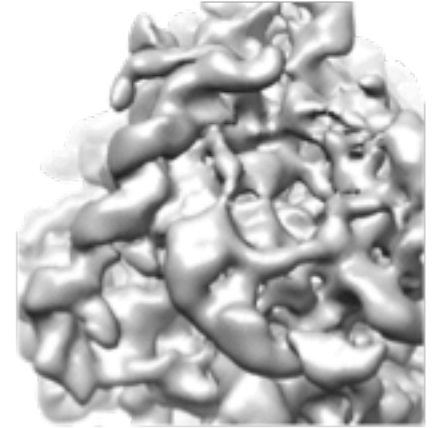
3D SPT - PyTom



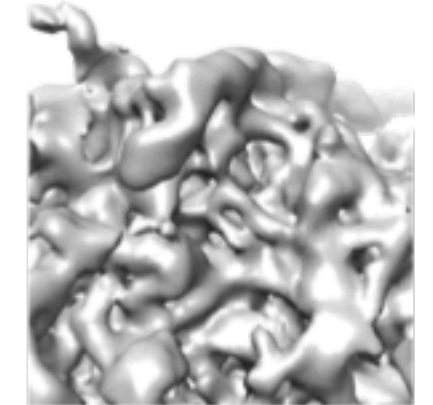
EMD-5592 - 6Å



Hi-res Single Particle



Unpublished - 9Å



3D SPT – EMAN2

Per-particle, per-tilt use cases:

in vitro

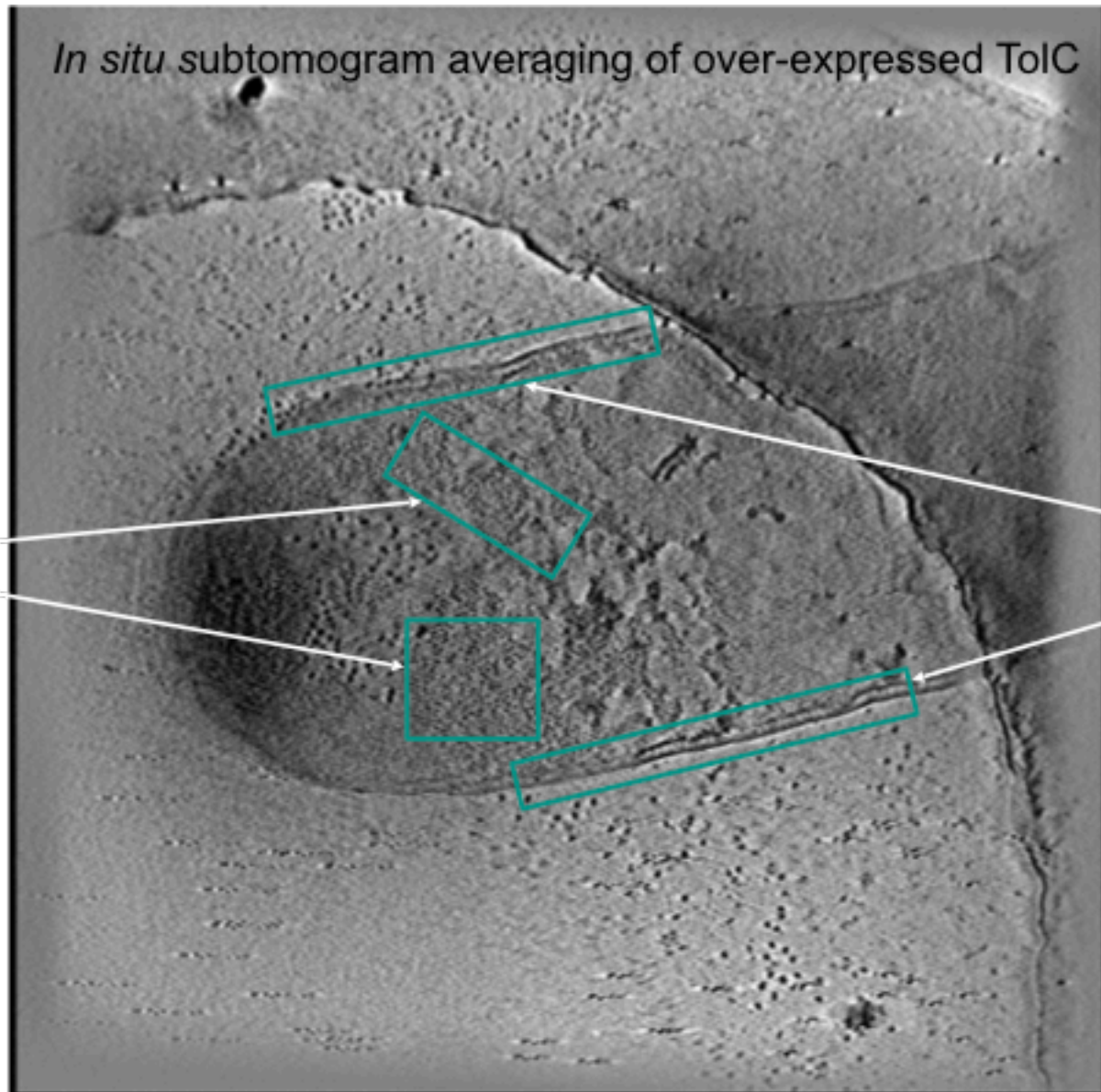
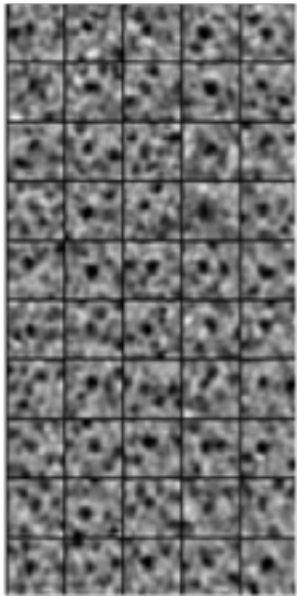
- Thin, purified samples
- Higher resolution ($<10\text{\AA}$)

in situ?

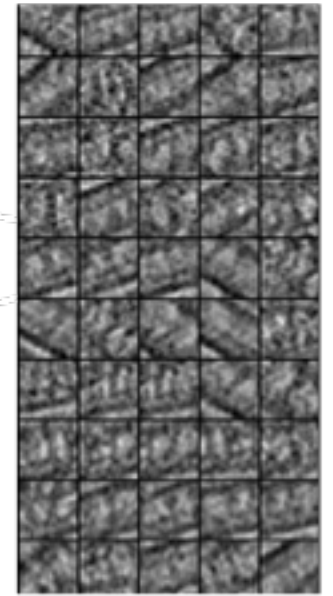
- Lower resolution ($\sim 20\text{\AA}$)
- Content above & below sample

In situ subtomogram averaging of over-expressed TolC

~ top views



~ side views



Unpublished,
X Shi, Z Wang, BCM

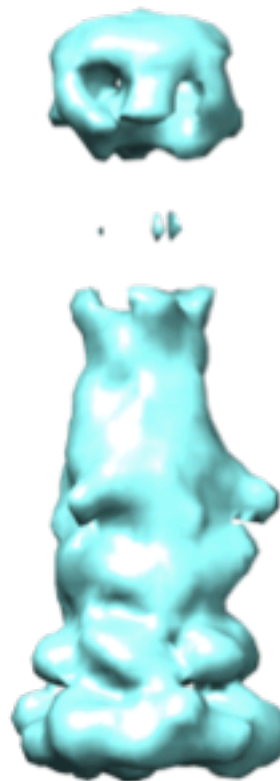
Addressing heterogeneity: Multi-reference refinement & focused classification



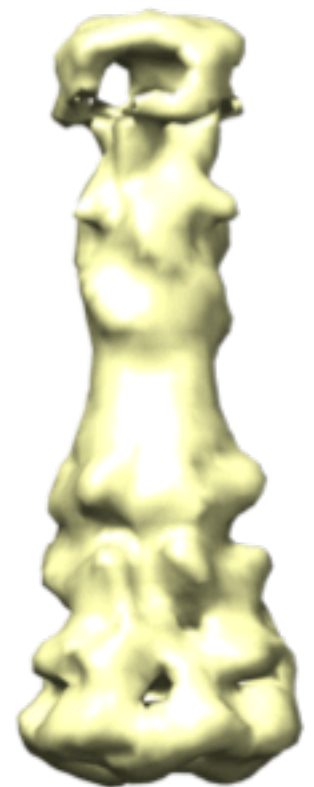
TolC (PDB: 5v5s)



All particles (1300)

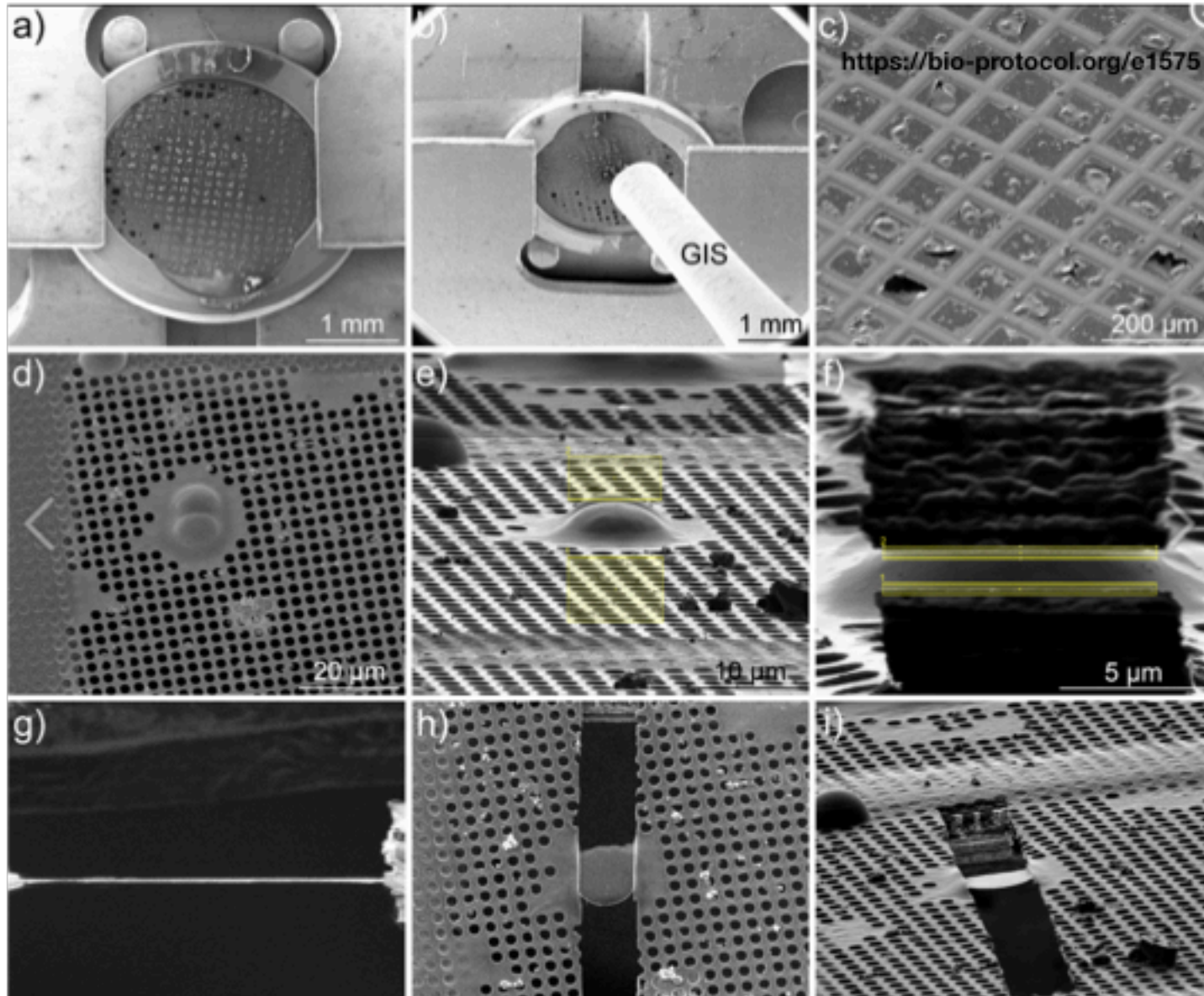


Class 1 (500)



Class 2 (800)

**Cryo
FIB
Milling**



Future Directions

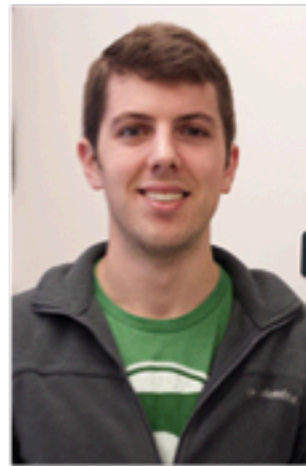
- Software now permits high resolution refinement, data collection protocols need to be optimized!
 - larger tilt step?
 - narrower tilt range?
 - Play with dose distribution?
- Movie-mode imaging $\rightarrow \sim 1 \text{ e}^-/\text{\AA}^2$ split into 10 or 20 frames!
- How much interference do we get from the cell with per-particle tilt series?
- Particle variability in cells (compositional and conformational)
- I want a Dual-beam Cryo-FIB!

Acknowledgements

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Muyuan Chen



James M. Bell

E.coli ToIC
X. Shi (BCM)
Zhao Wang (BCM)

Trypanosome
Stella Sun (SLAC)
Cynthia He (NUS)

PC12 Neurite
Wei Dai (NCMI→Rutgers)

Mouse Neurite
Patrick Mitchell (SLAC)
Gong-her Wu (SLAC)

Wah Chiu, Mike Schmid (SLAC)

Chen, M., Dai, W., Sun, S.Y., Jonasch, D., He, C.Y., Schmid, M.F., Chiu, W. & Ludtke, S.J., 2017, Convolutional neural networks for automated annotation of cellular cryo-electron tomograms, *Nature methods*.