

Workshop Goals

- If you are just starting:
 - You will gain familiarity with a wide range of CryoEM/ET tools and methods
 - You will NOT become an expert
- If you already have some experience:
 - Hopefully we will increase your depth of knowledge, and introduce new tools, some of which are being presented for the first time

The Week

- Day 1 - Tomography and Subtomogram Averaging
- Day 2 - Single Particle Analysis
- Day 3 - Single Particle Analysis
- Day 4 - A little Python, Model Building and Map Interpretation



Baylor
College of
Medicine

CRYO-EM CORE

Tomography

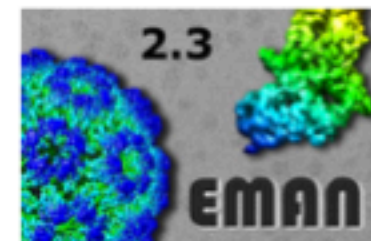
Overview of EMAN2.3

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

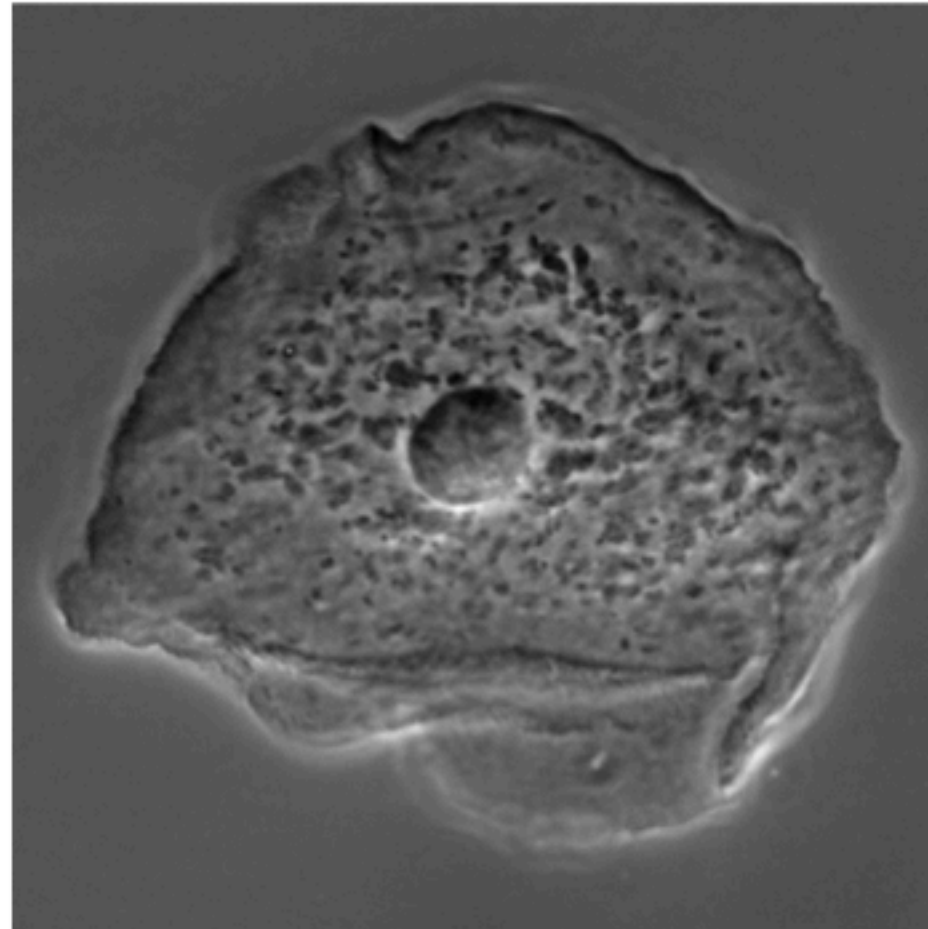
VERNA & MARRS MCLEAN
DEPARTMENT OF
BIOCHEMISTRY AND
MOLECULAR BIOLOGY



5/2019, Ludtke, UTMB

Epithelial Cell (cheek)

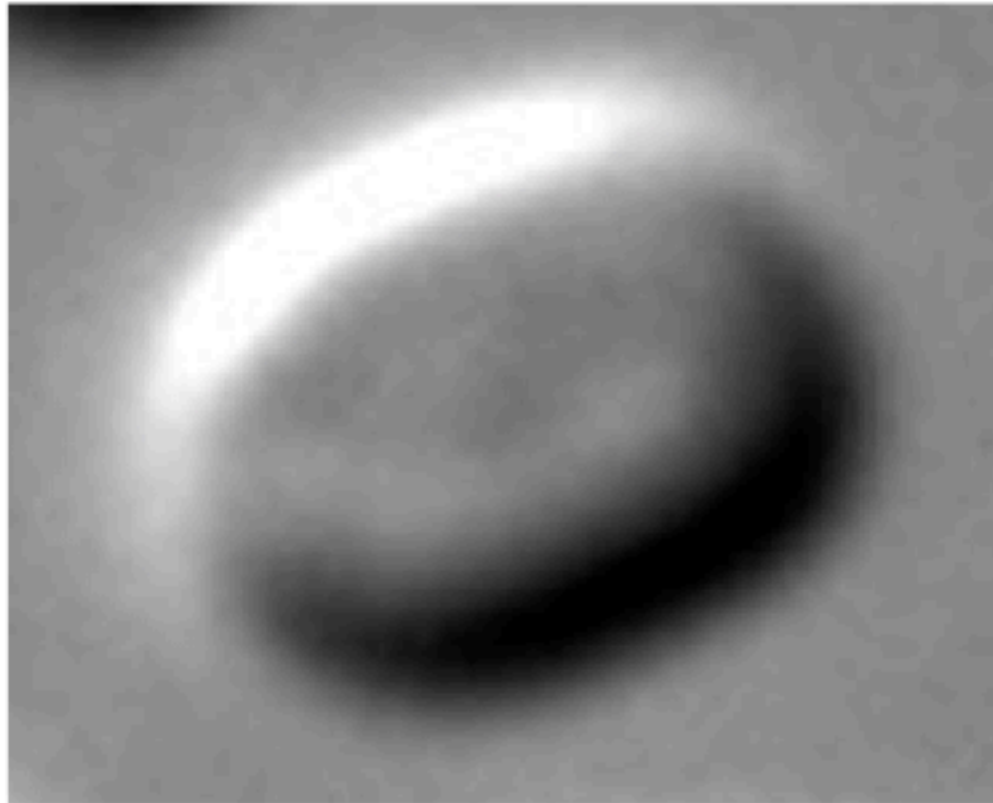
(Optical Phase Contrast Microscopy)



~10 μm

Cyanobacteria

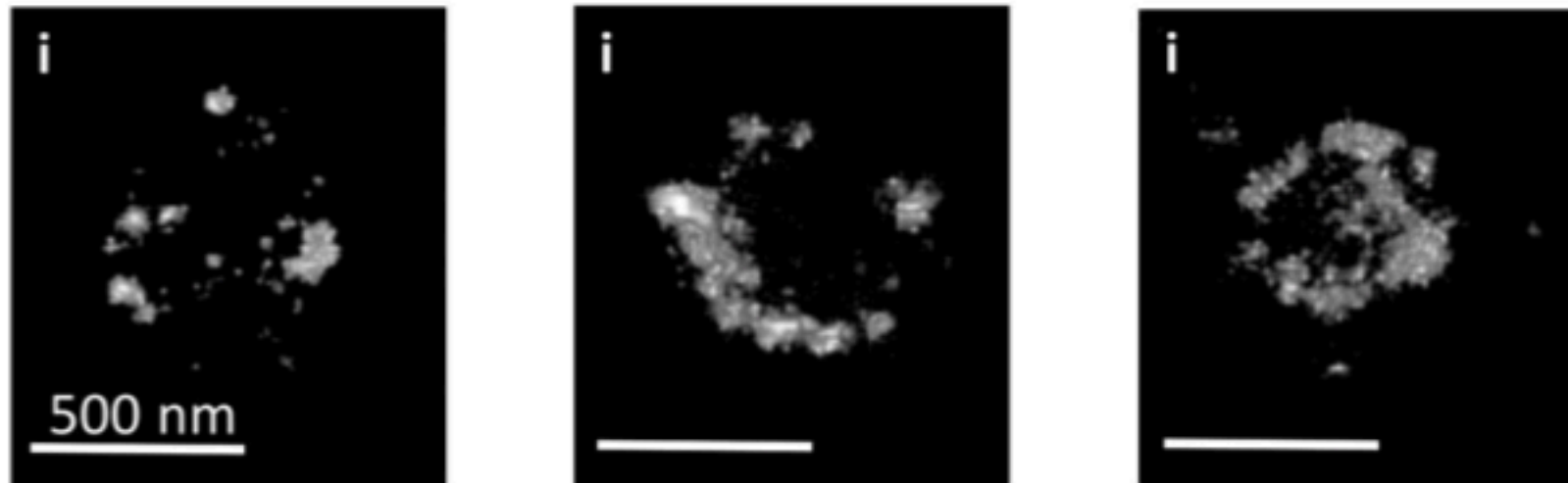
(Optical Microscope)



~0.5 μm

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

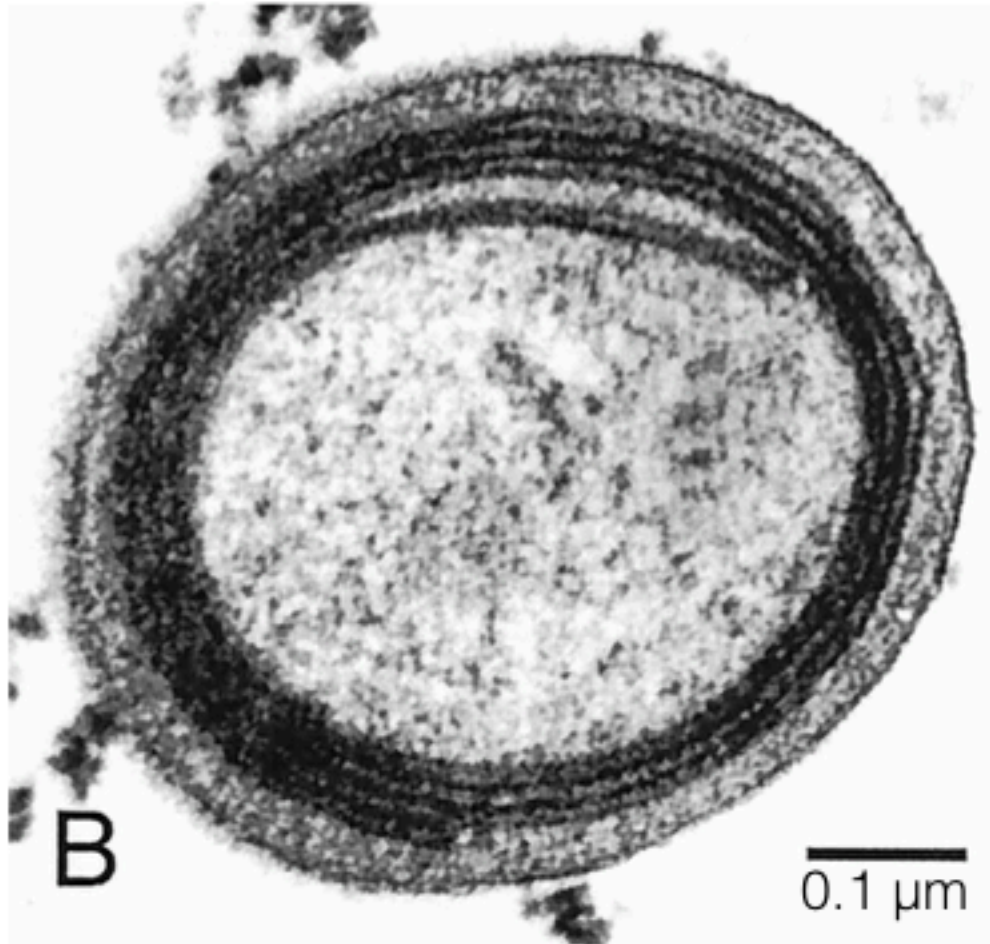
STORM Imaging of Prochlorococcus FtsZ Ring



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. PMID: PMC5698547

Cyanobacteria

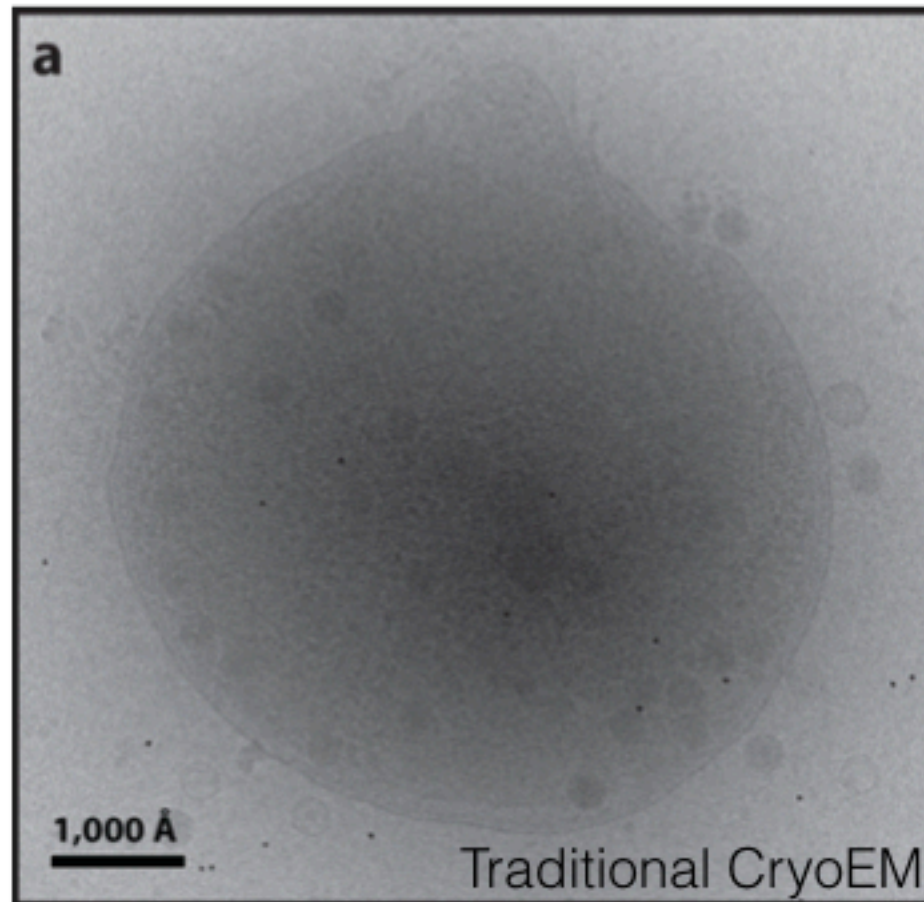
Conventional Stained/Fixed Electron Microscopy



Cyanobacteria

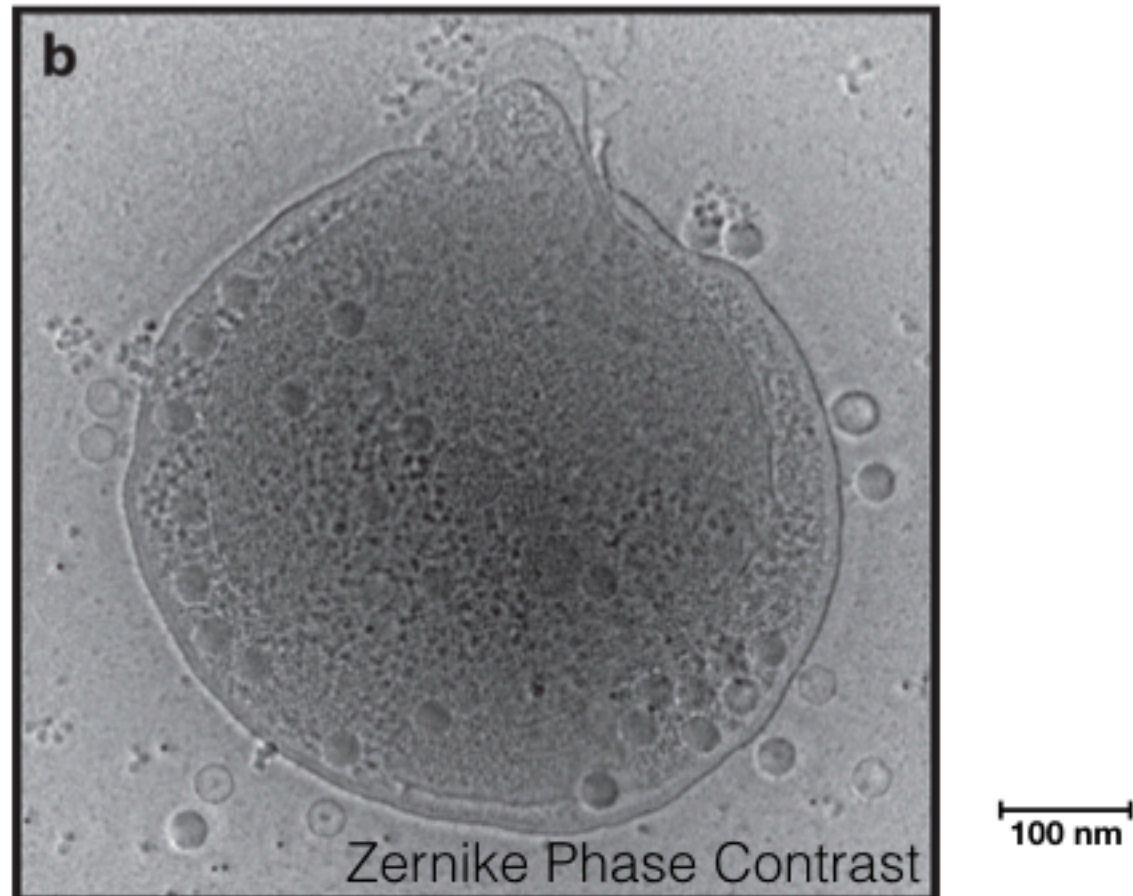
- Dai, W., et. al. (2013). Visualizing virus assembly intermediates inside marine cyanobacteria. *Nature*. 502:707-710.
- Responsible for ~25% of carbon fixation on earth
- Renewable energy production
- *Synechococcus* + Syn5 (phage)
- Phages
 - Lateral gene transfer (lysogenic)
 - Destroy cells (lytic)

Phage Infection of Cyanobacteria



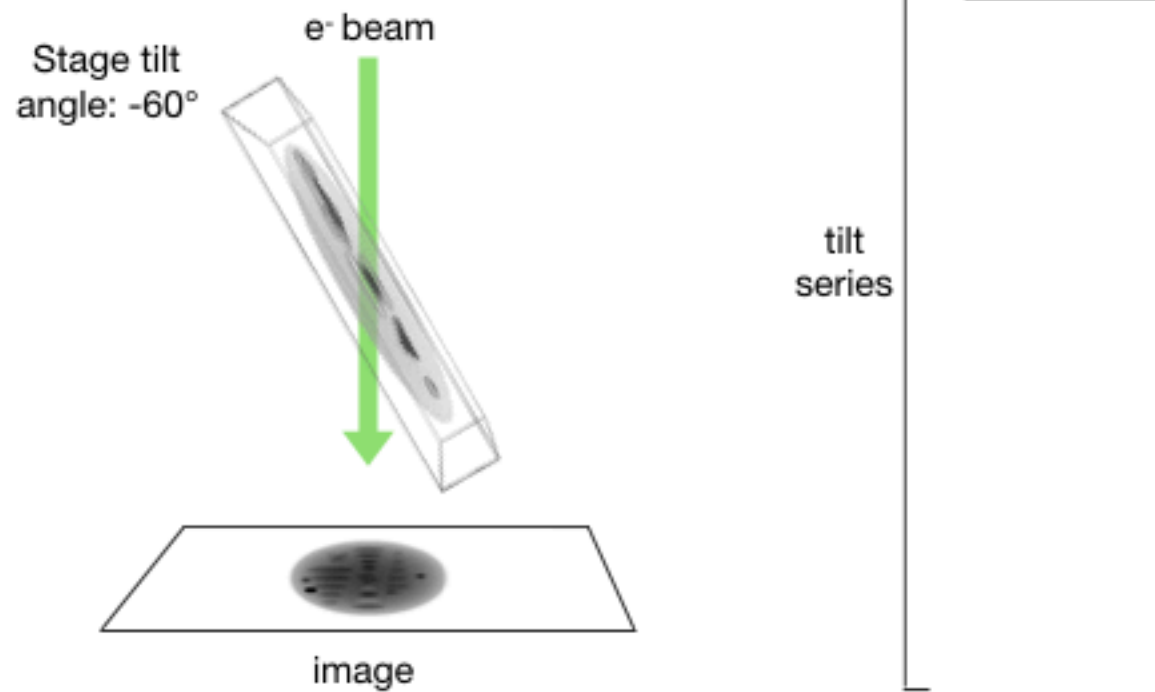
Dai, W., et. al. (2013). Visualizing virus assembly intermediates inside marine cyanobacteria. *Nature*. 502:707-710.

Phage Infection of Cyanobacteria

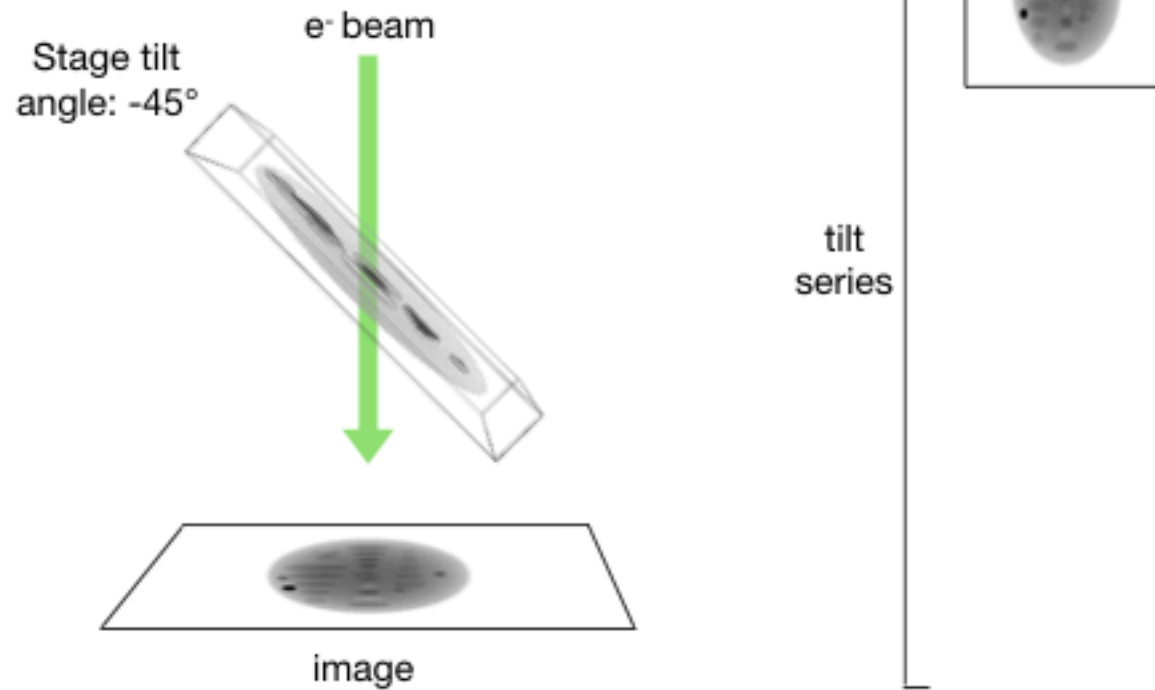


Dai, W., et. al. (2013). Visualizing virus assembly intermediates inside marine cyanobacteria. *Nature*. 502:707-710.

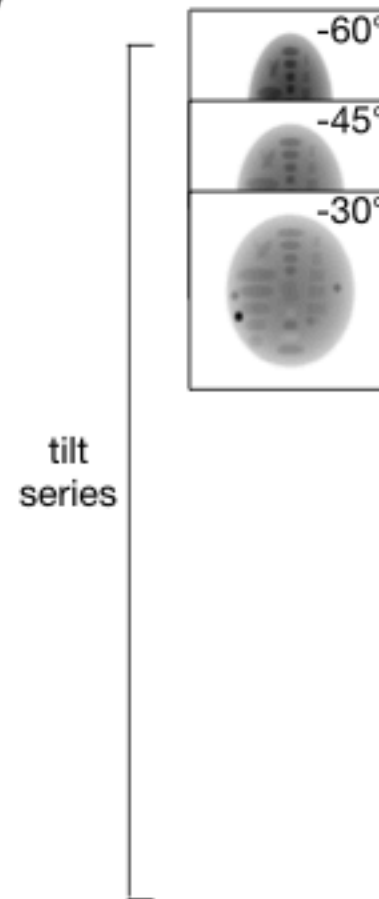
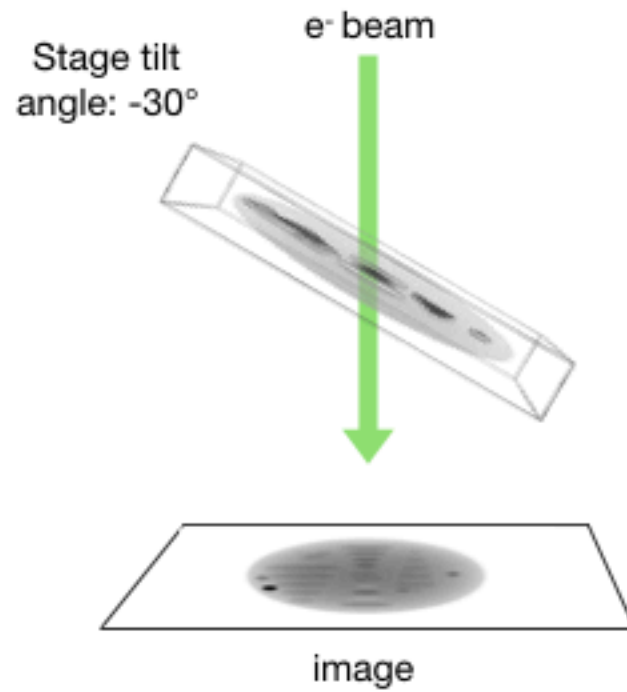
Electron cryo-tomography (cryoET)



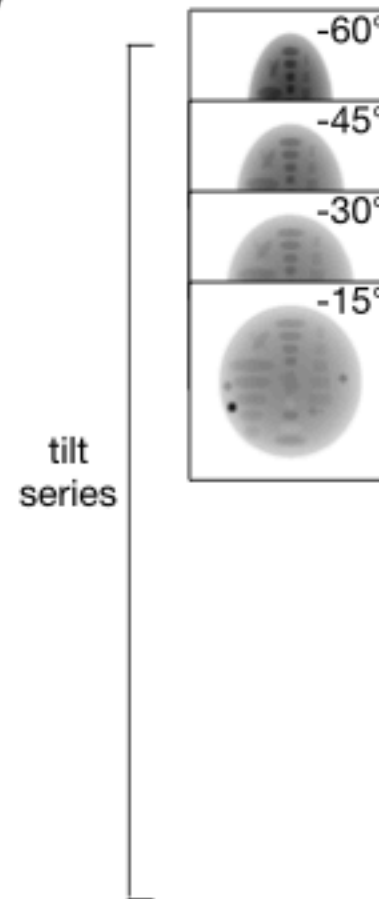
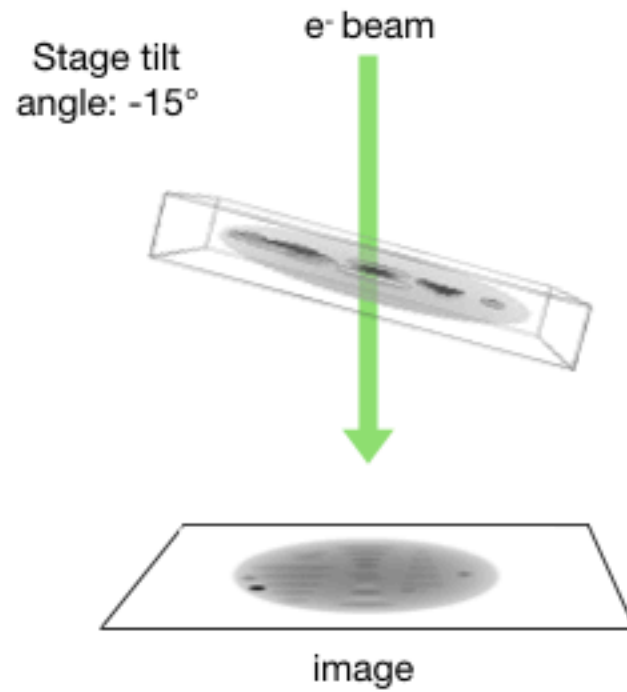
Electron cryo-tomography (cryoET)



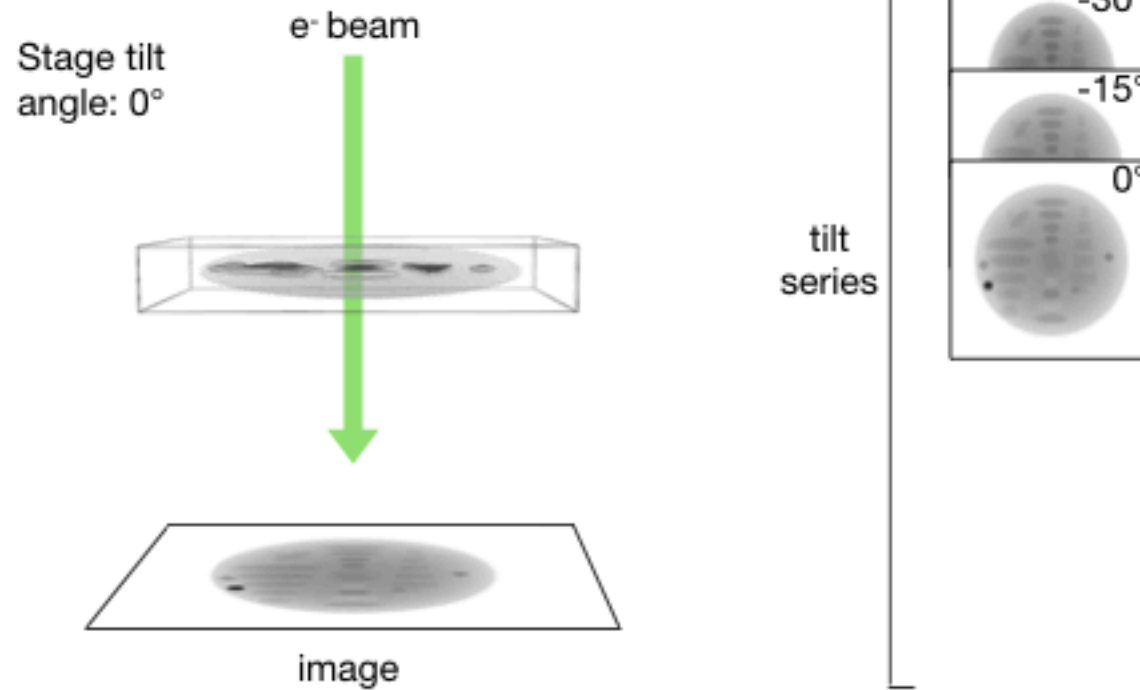
Electron cryo-tomography (cryoET)



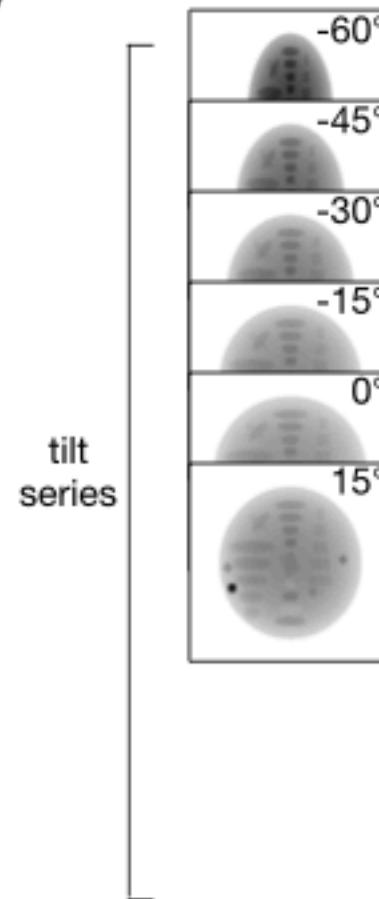
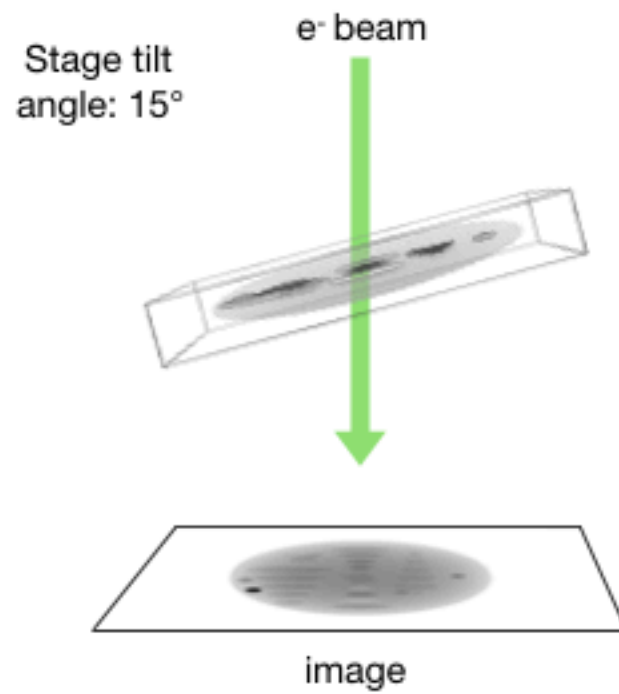
Electron cryo-tomography (cryoET)



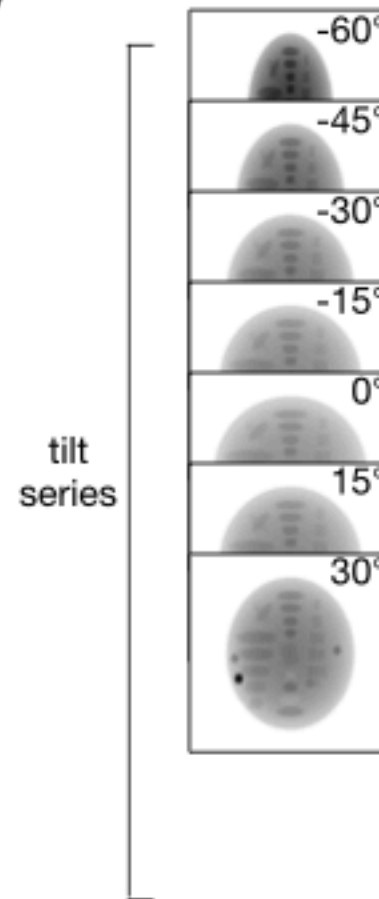
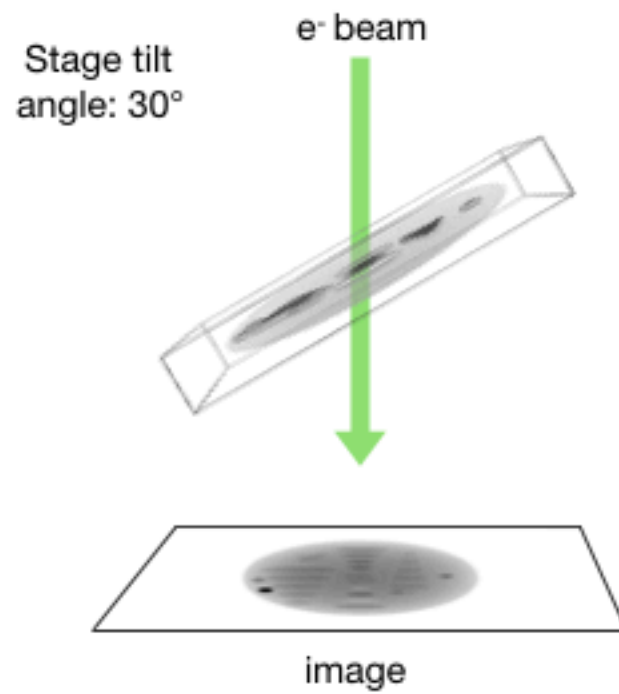
Electron cryo-tomography (cryoET)



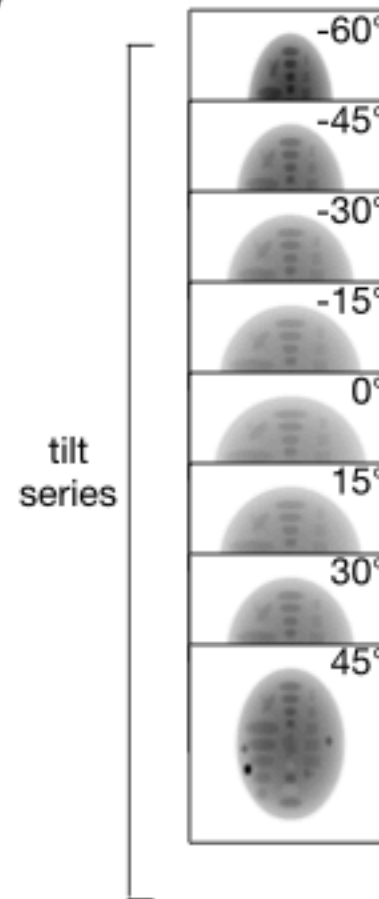
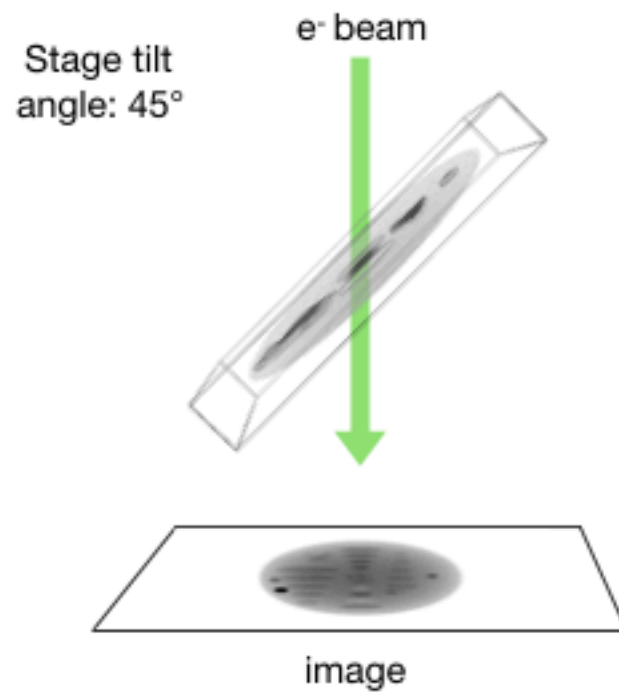
Electron cryo-tomography (cryoET)



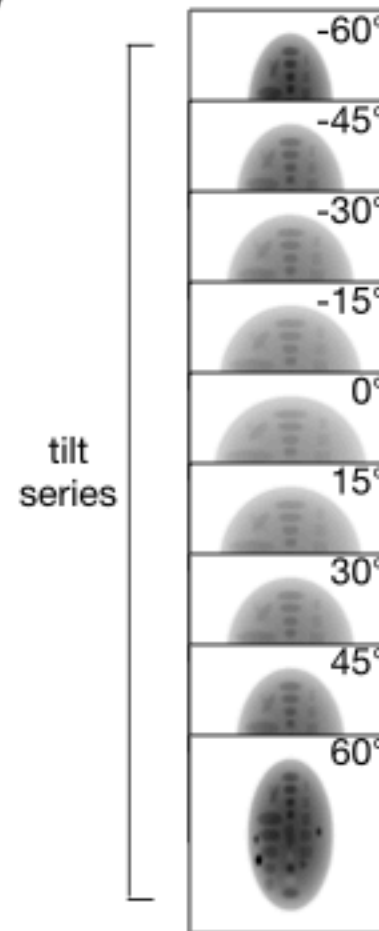
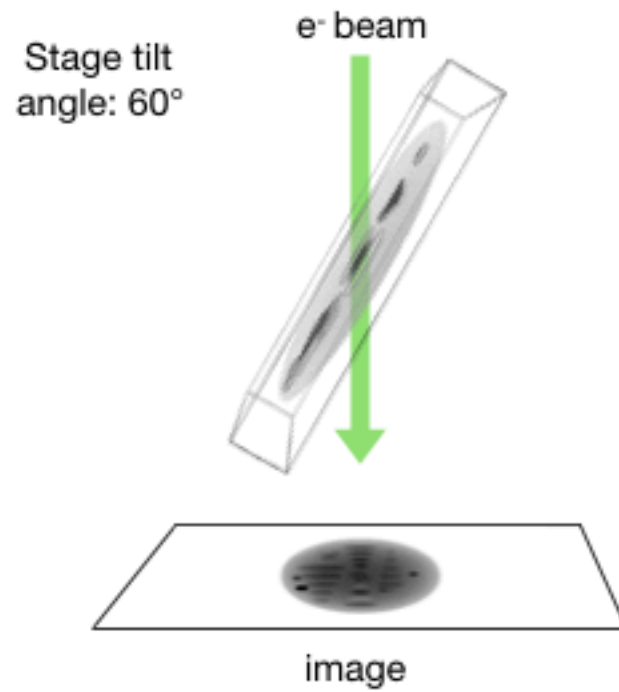
Electron cryo-tomography (cryoET)



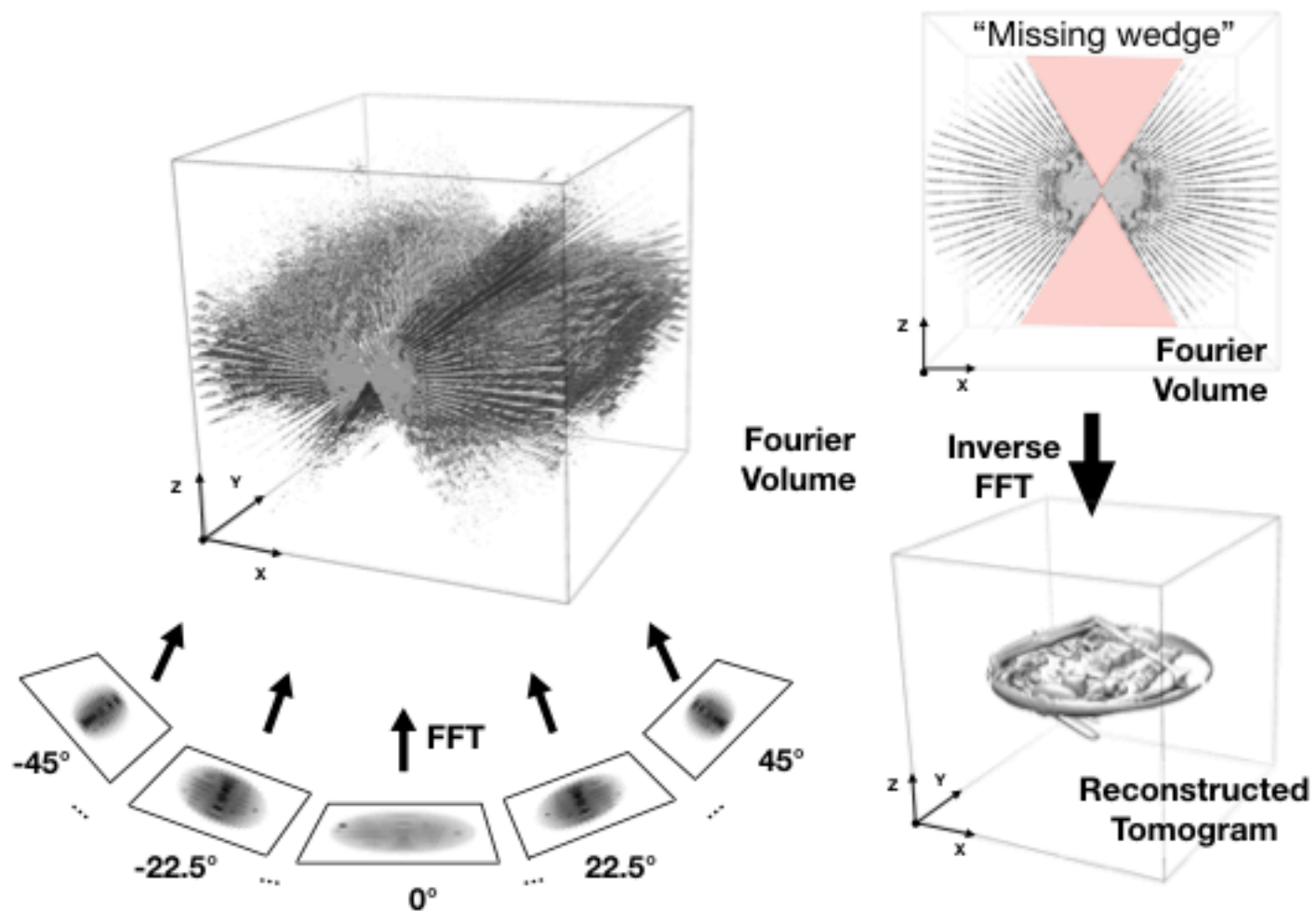
Electron cryo-tomography (cryoET)

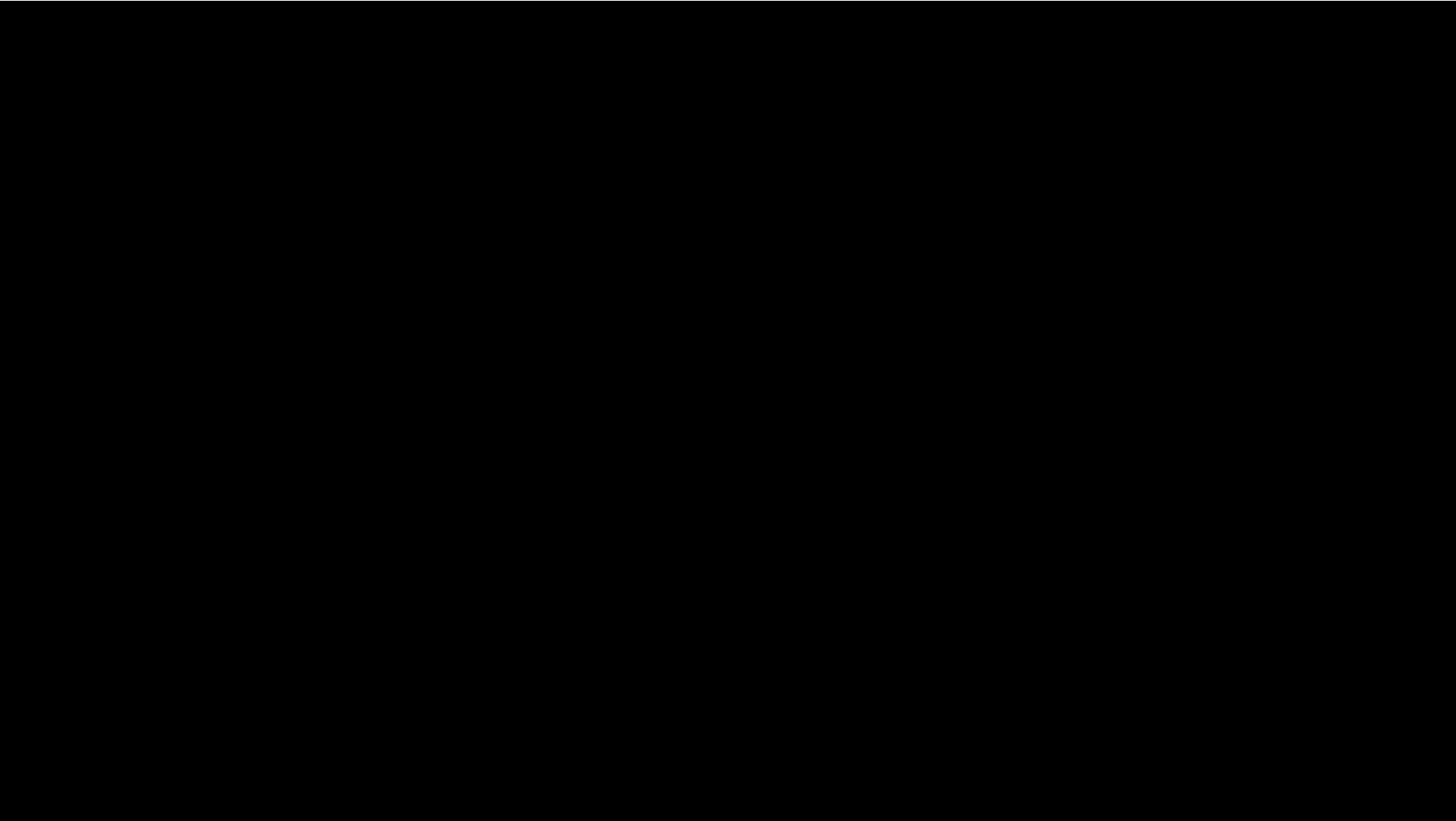


Electron cryo-tomography (cryoET)

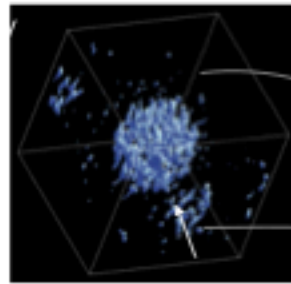


3D Fourier reconstruction





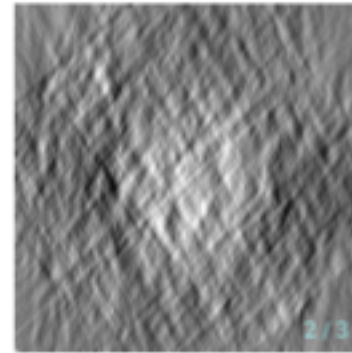
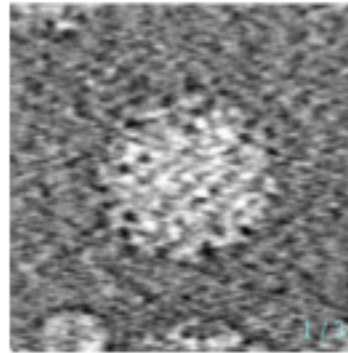
Impact of Missing Wedge



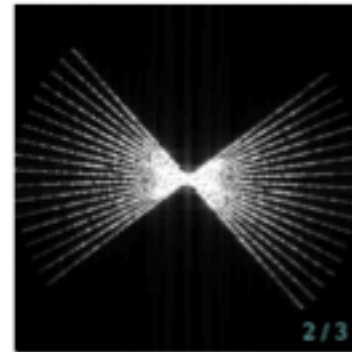
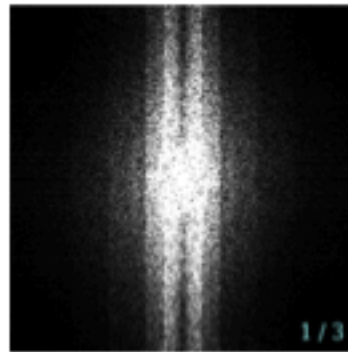
x-y

x-z

Projection

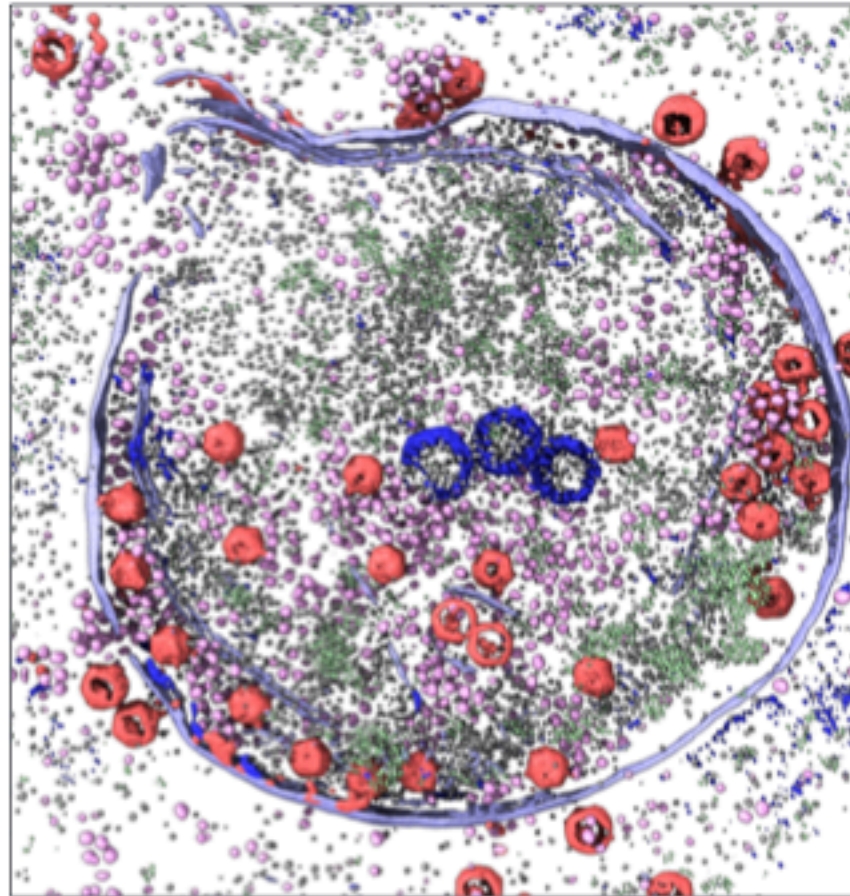


FFT

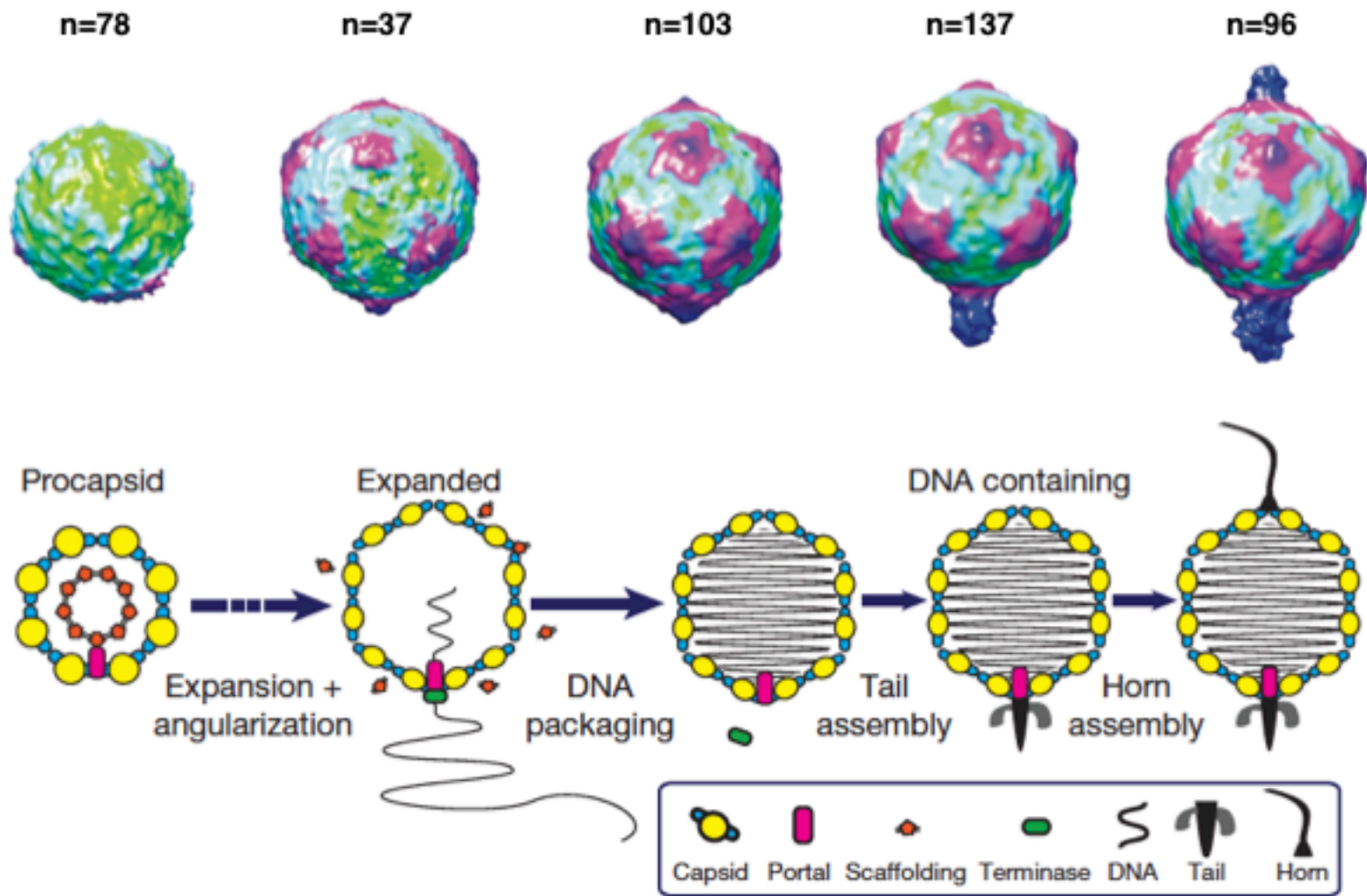


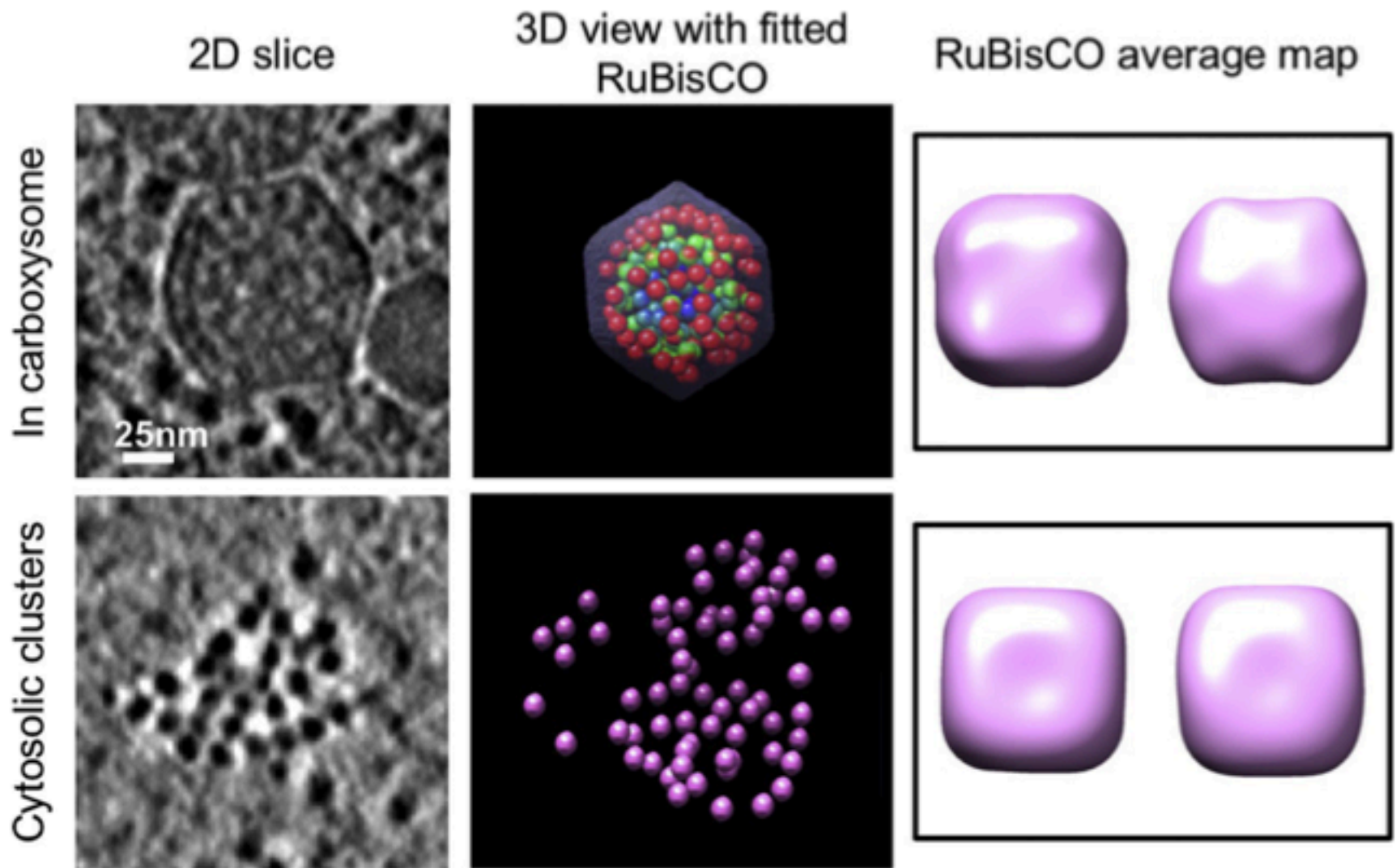
Cyanobacteria

(CryoET, annotated)

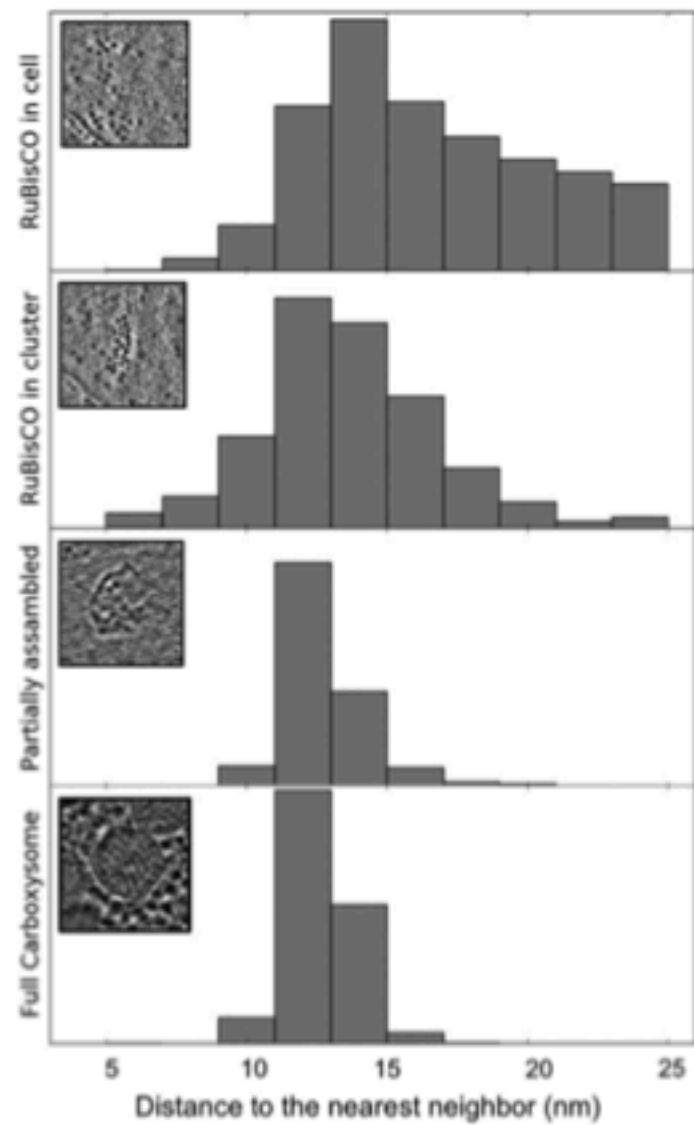


—
0.2 μm =
2000 \AA





Dai, W., et.al., (2018). Visualizing Individual RuBisCO and Its Assembly into Carboxysomes in Marine Cyanobacteria by Cryo-Electron Tomography. *JMB*. 430:4156-4167. ^{AB}



← <10%

EMAN2 Tomography Tools

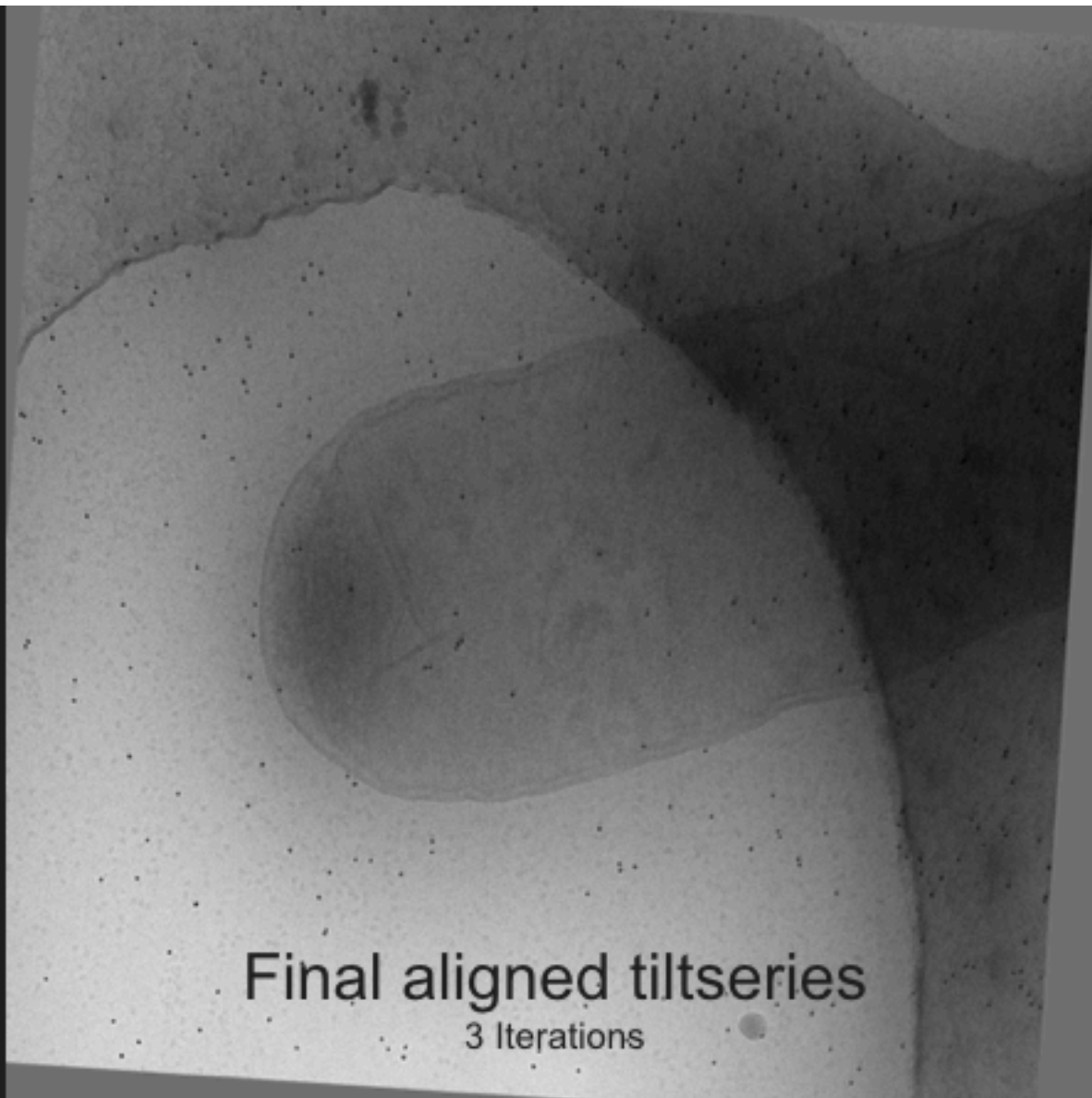
- ➔ Fiducial-free tilt series alignment
- ➔ Tiled Fourier Reconstruction
 - Tilt series CTF estimation and correction
- ➔ Manual, reference-based and deep-learning 3-D particle picking
 - Deep learning based tomogram annotation
- ➔ SGD initial average generation
 - Iterative subtomogram averaging
- ➔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

X Shi,
Z Wang,
BCM

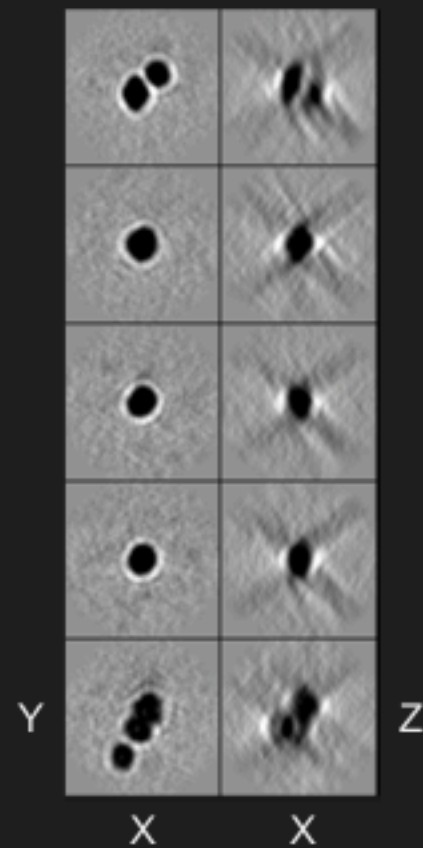
Unaligned tiltseries

8k x 8k DE-64 images

Runtime:
~10 minutes on
12 threads



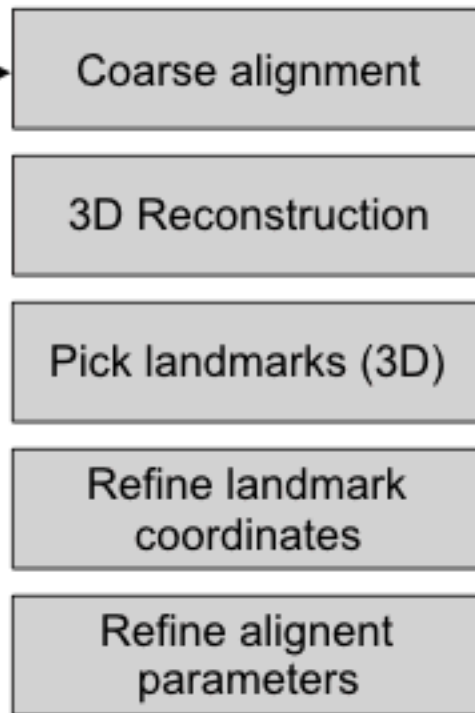
Refined
landmarks
(bin x2)



X Shi,
Z Wang,
BCM

Tilt series alignment

Start:
Unaligned
tiltseries



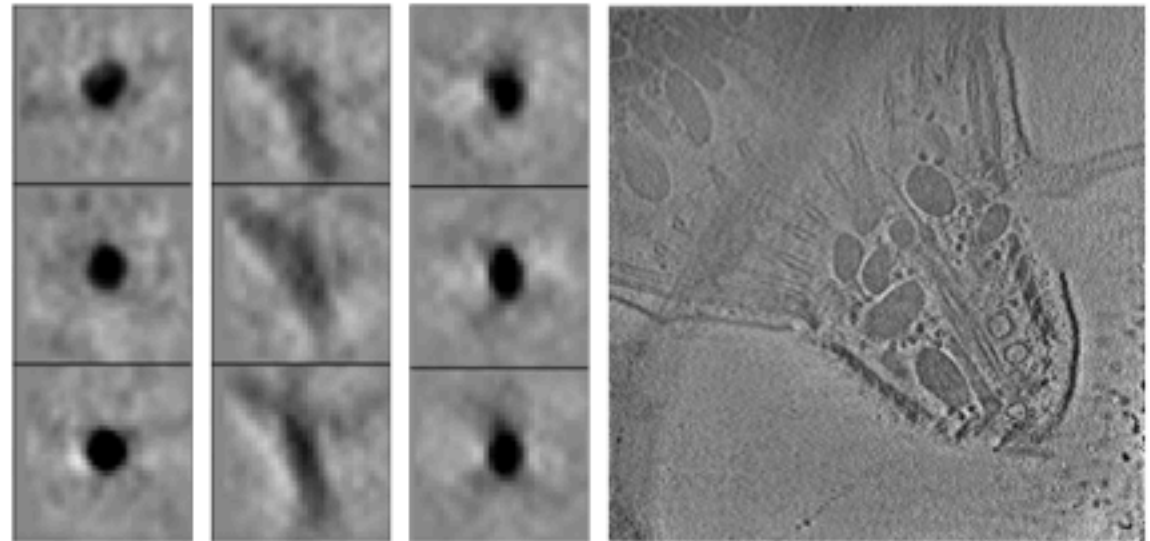
Bin by
8x
4x
2x
1x

Landmarks

Top view

Side view
coarse align
iteration #4

Side view

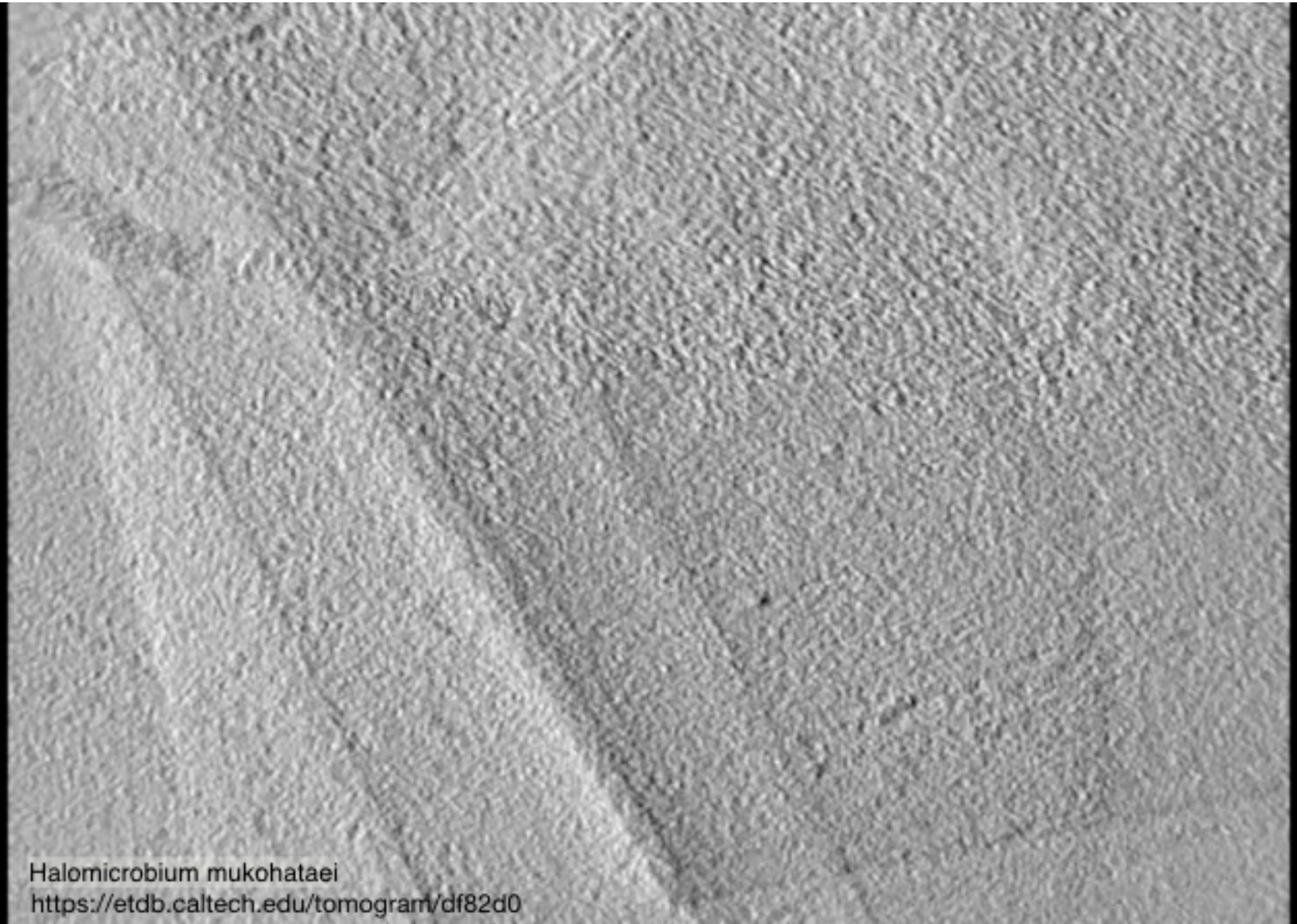


Tomogram slice view

Toxoplasma gondii

Stella Y. Sun

~5-10 min total per tomogram



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

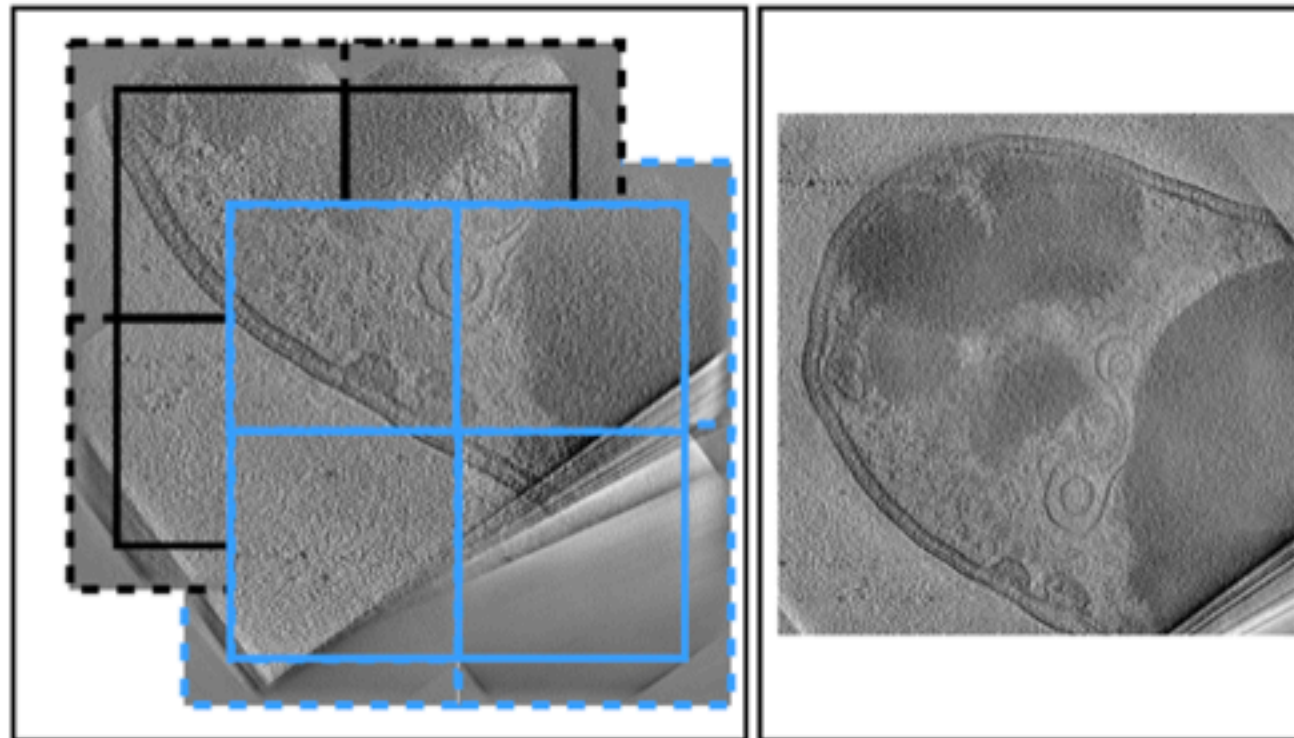


Reconstruction Algorithms

- Back Projection
- Filtered Back Projection
- Direct Fourier Inversion
- SIRT
- SART
- ...

Reconstruction via tiled direct Fourier inversion

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



EMAN2.3

<http://eman2.org>

EMAN2 and SPHIRE/SPARX share a common C++ core and are distributed together. If you have one installed, you also have the other! However, they are completely independent beyond that.

(this should also soon include Niels Volkman's PyCoan)

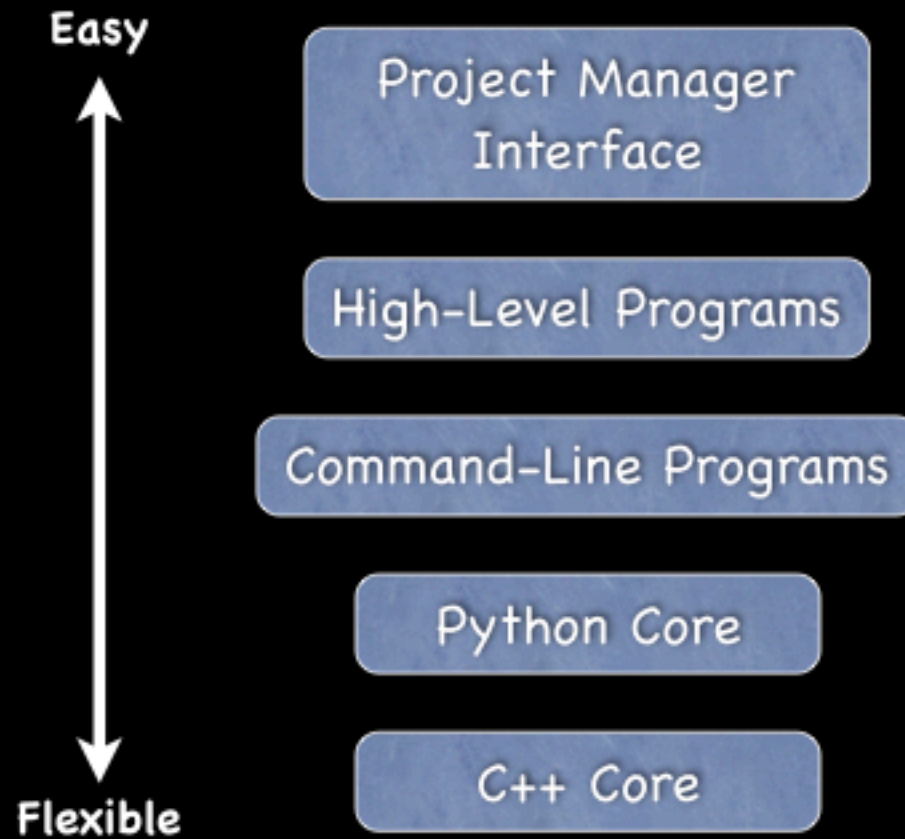
What can EMAN2 do?

- Movie alignment (work in progress, can call)
- Micrograph screening
- CTF (micrograph, particle, tilt series)
- High Resolution Single Particle Analysis
- 2-D Variability
- 3-D Variability
- Tomography alignment and reconstruction
- Tomogram Segmentation
- Subtomogram averaging (traditional and hybrid)
- Utility Functions (file conversion, image processing,...)

EMAN2 Features

- Complete graphical workflow
- Project system which organizes data and records all reconstruction info.
- PyQt/OpenGL for 2d & 3d display.
- Support for all documented cryoEM file formats (and others)
- Over 200 general purpose image processing algorithms
- Interoperability features with Frealign, Relion, ResMap, IMOD...
- Parallel processing using MPI and/or Threads
- Google Group for questions with searchable archive

EMAN2 Architecture



Extensible Core

Type	Description	#
Processor	Generic image processing algorithms, filters, masks, thresholds, etc.	220
Aligner	Algorithms used to align 2 images or volumes to each other	32
Projector	Routines to generate 2-D projections of 3-D objects	7
Reconstructor	Routines to reconstruct 3-D objects from 2-D projections	13
Cmp	Similarity metrics used to compare two images or volumes	15
Averager	Average together stacks of images in various ways	12
Analyzer	Perform various operations on sets of images, such as classification or PCA	9
Orientgen	Routines describing how projections cover the asymmetric triangle	7

File Formats

MRC	R/W	IMAGIC	R/W
SPIDER	R/W	HDF5	R/W
PIF	R/W	ICOS	R/W
VTK	R/W	PGM	R/W
Amira	R/W	Xplor	W
Gatan DM2	R	Gatan DM3	R
Gatan DM4	R	FEI SER	R
TIFF	R/W	Scans-a-lot	R
LST	R/W	PNG	R/W
Video-4-Linux	R	JPEG	W

Programs

- Command-Line Programs (EMAN2)

syntax:

```
e2<name>.py --help
```

```
e2<name>.py <file> [--option=value] [--option] [-O]
```

<> - required parameter

[] - optional parameter

- e2help.py <category>

Project Folder

- Tomography (most common folders):
 - info/ - All project metadata in human readable .json format
 - particles/ -
 - particles3d/ - Individual reconstructed 3-D particles
 - sets/ - Contains .lst files, allows for data subset selection without duplication
 - spt_XX/ - e2spt_refine, traditional subtomogram refinement
 - sptsgd_XX/ - e2spt_sgd, results of initial model generation
 - subtilt_XX/ - e2spt_tiltrefine.py, hybrid subtomogram/single particle refinement
 - tiltseries/ - Unaligned tilt series
 - tomograms/ - Full tomograms, usually downsampled to 1k or 2k
 - tomorecon_XX/ - Intermediate files from tilt-series alignment (optional)

Project Folder

- Single Particle Analysis (most common folders):
 - info/ - All project metadata in human readable .json format, 1 file per micrograph, project.json, etc.
 - m2d_XX - 2-D Motion/variability analysis results
 - micrographs/ - Raw and/or processed micrographs in HDF format, variants use __
 - movies/ - Movie stacks in any format (LZW compressed tiff best)
 - multi_XX/ - Multimodel refinement results for 3-D variability
 - particles/ - Boxed out particles in HDF format, variants use __
 - r2d_XX/ - Results of e2refine2d, traditional 2-D unsupervised classification
 - r2db_XX/ - Results of e2refine2d_bispec, new bispectrum based unsupervised classification
 - refine_XX/ - Results of e2refine_easy.py, high resolution single particle analysis
 - report/ - Complete refinement report, open in browser (index.html)
 - sets/ - Contains .lst files referencing particles/, allows for data subset selection without duplication