

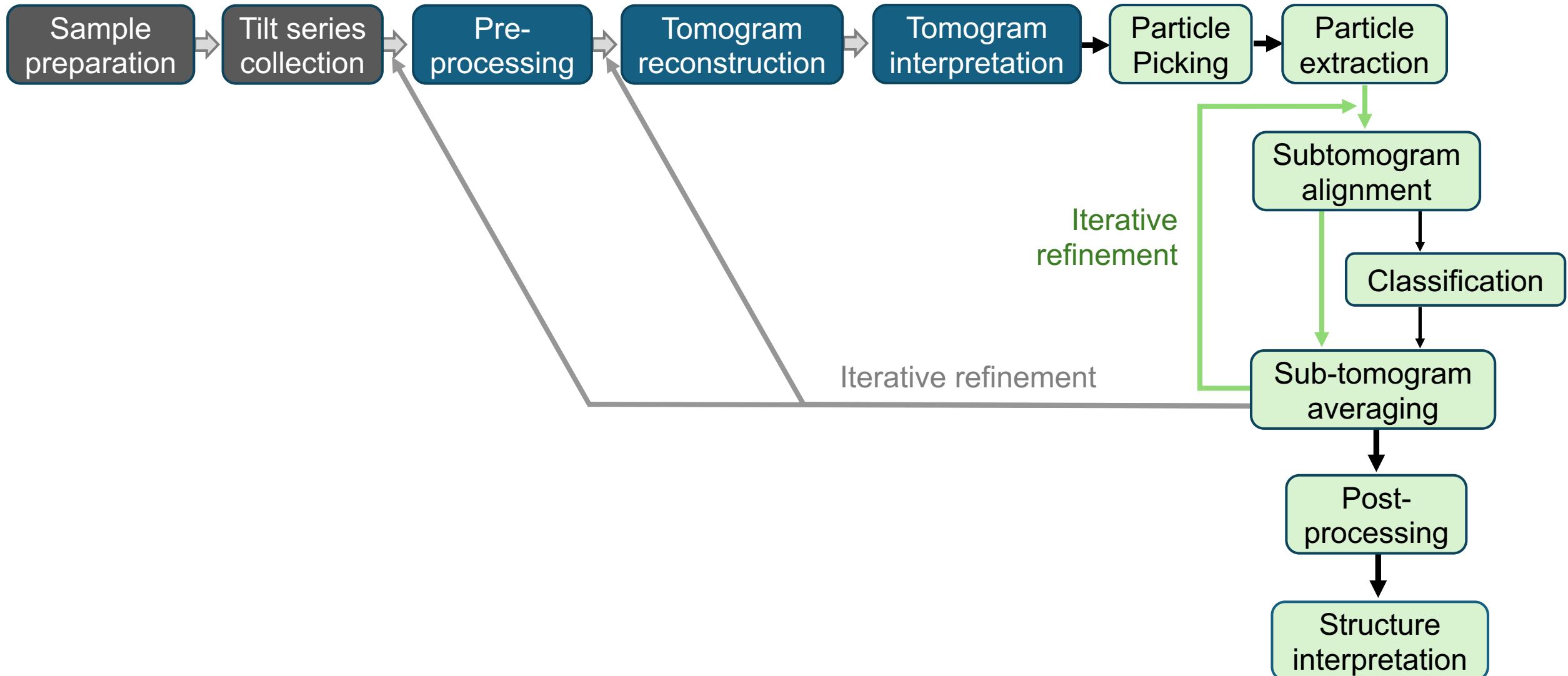
# **CryoET Workflow and Summary of the**

## **Michigan CryoET Workshop**

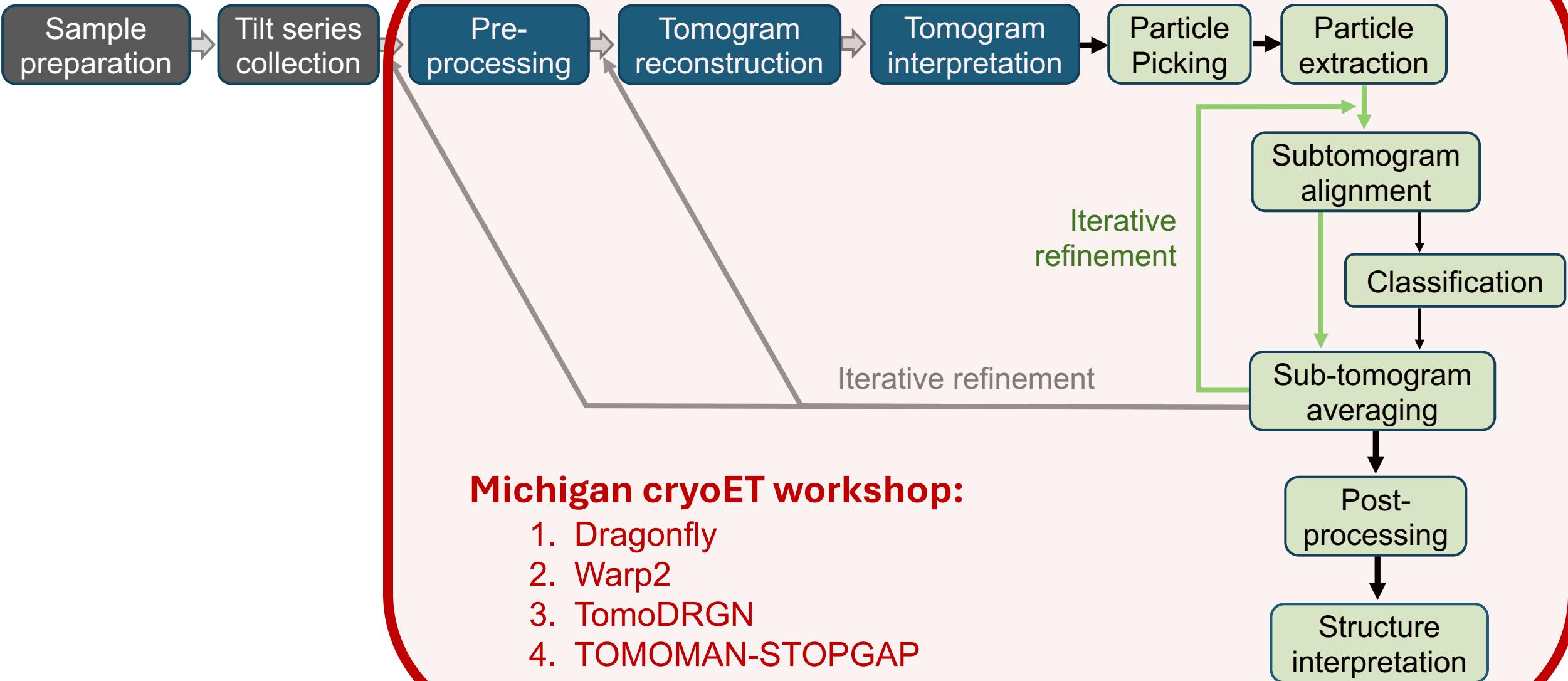
Kangkang Song

08/06/2024

# CryoET workflow

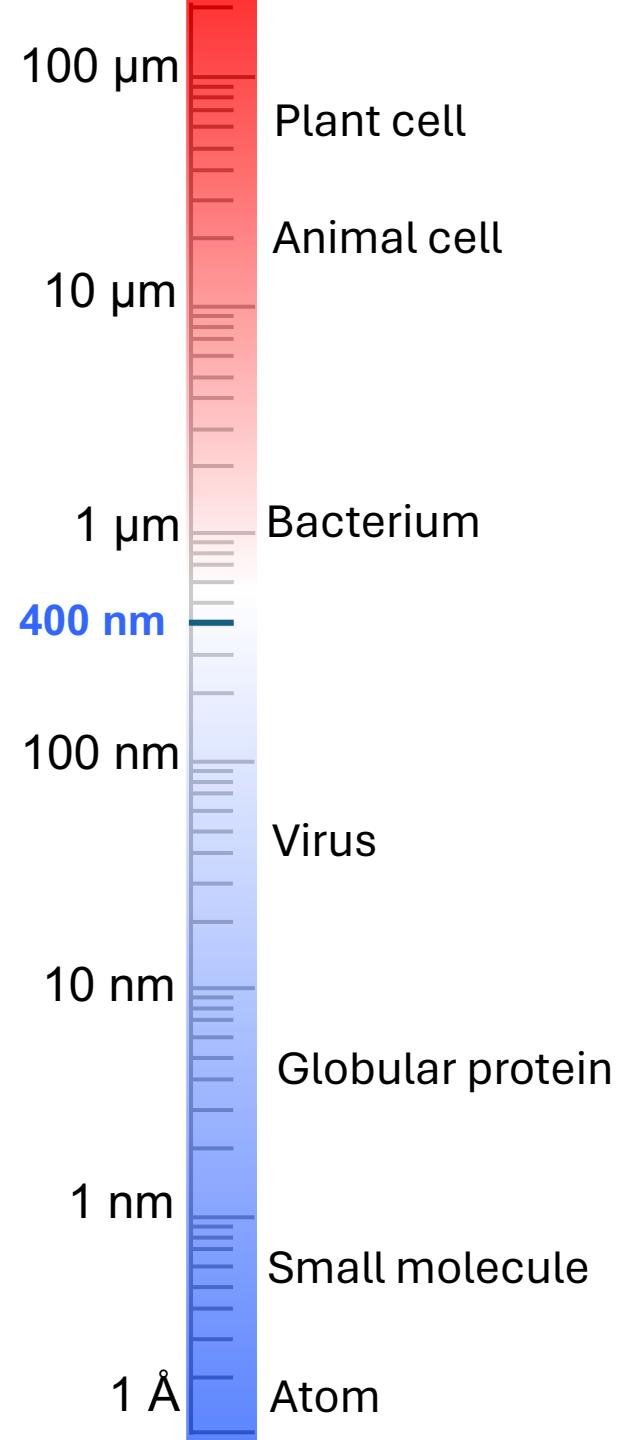
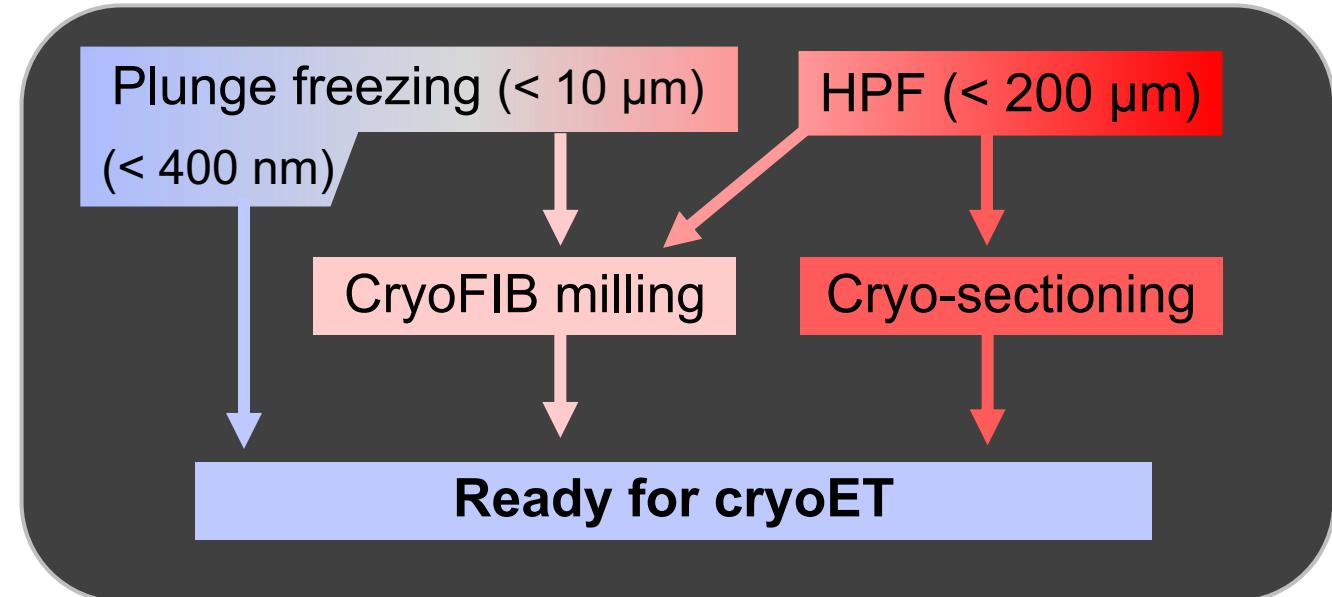
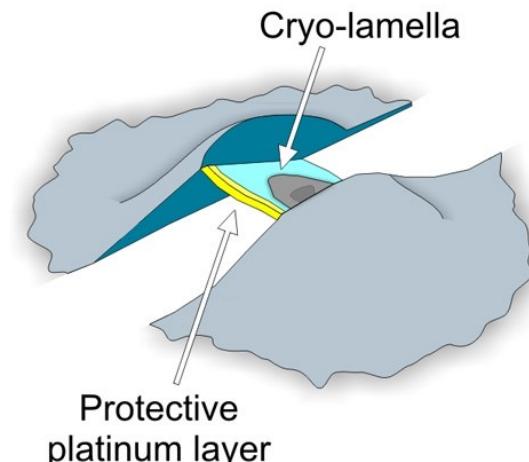
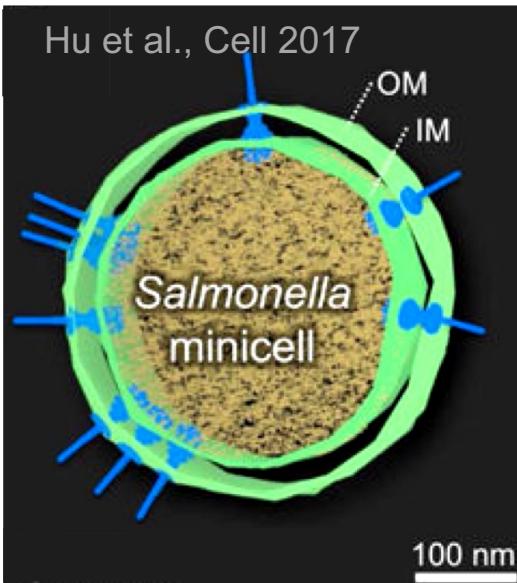


# CryoET workflow



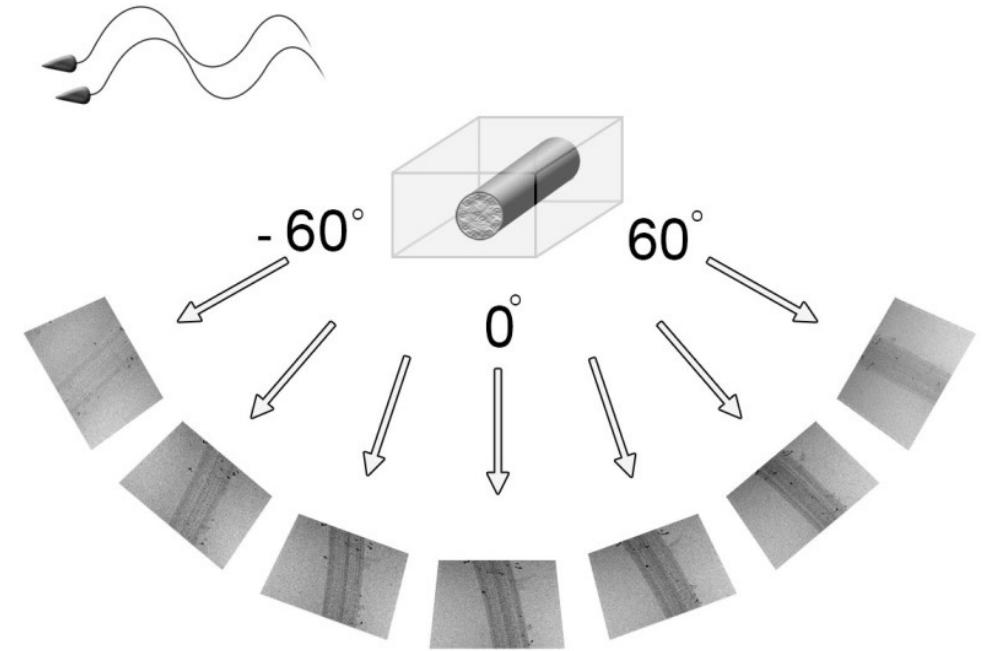
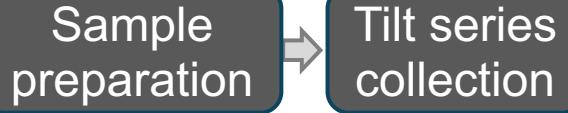
# CryoET workflow

## Sample preparation



- Virus: e.g., Covid-19
- Isolated or reconstituted systems: e.g., ciliary axoneme
- Small/thin cells: e.g., minicells
- Peripheral regions of cells: e.g., mammalian cells
- Cryo-sections
- Cryo-lamellae

# CryoET workflow

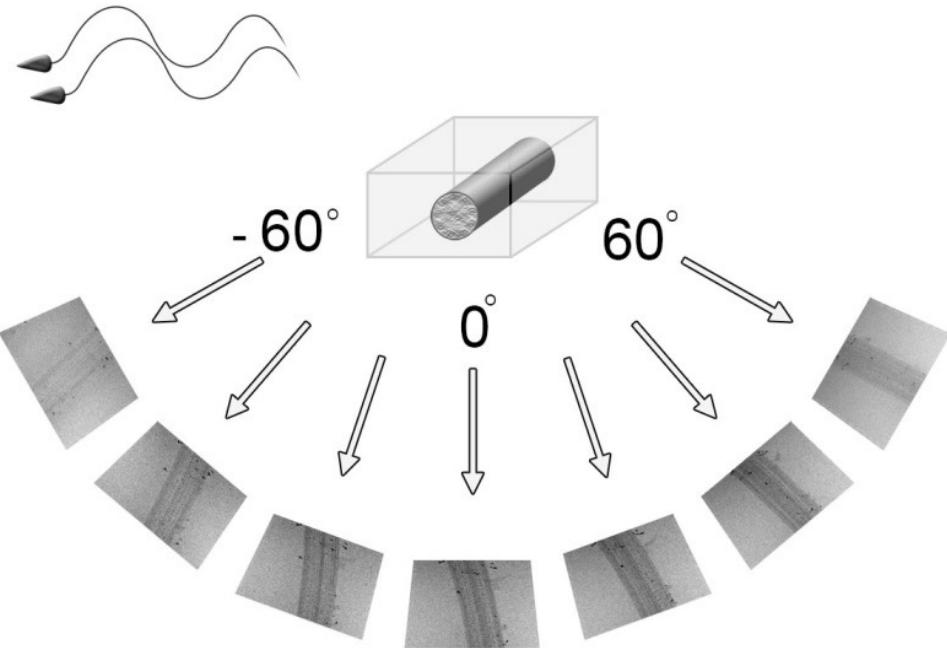


e.g., ~90 e/ $\text{\AA}^2$  total  
dose for the 61 images

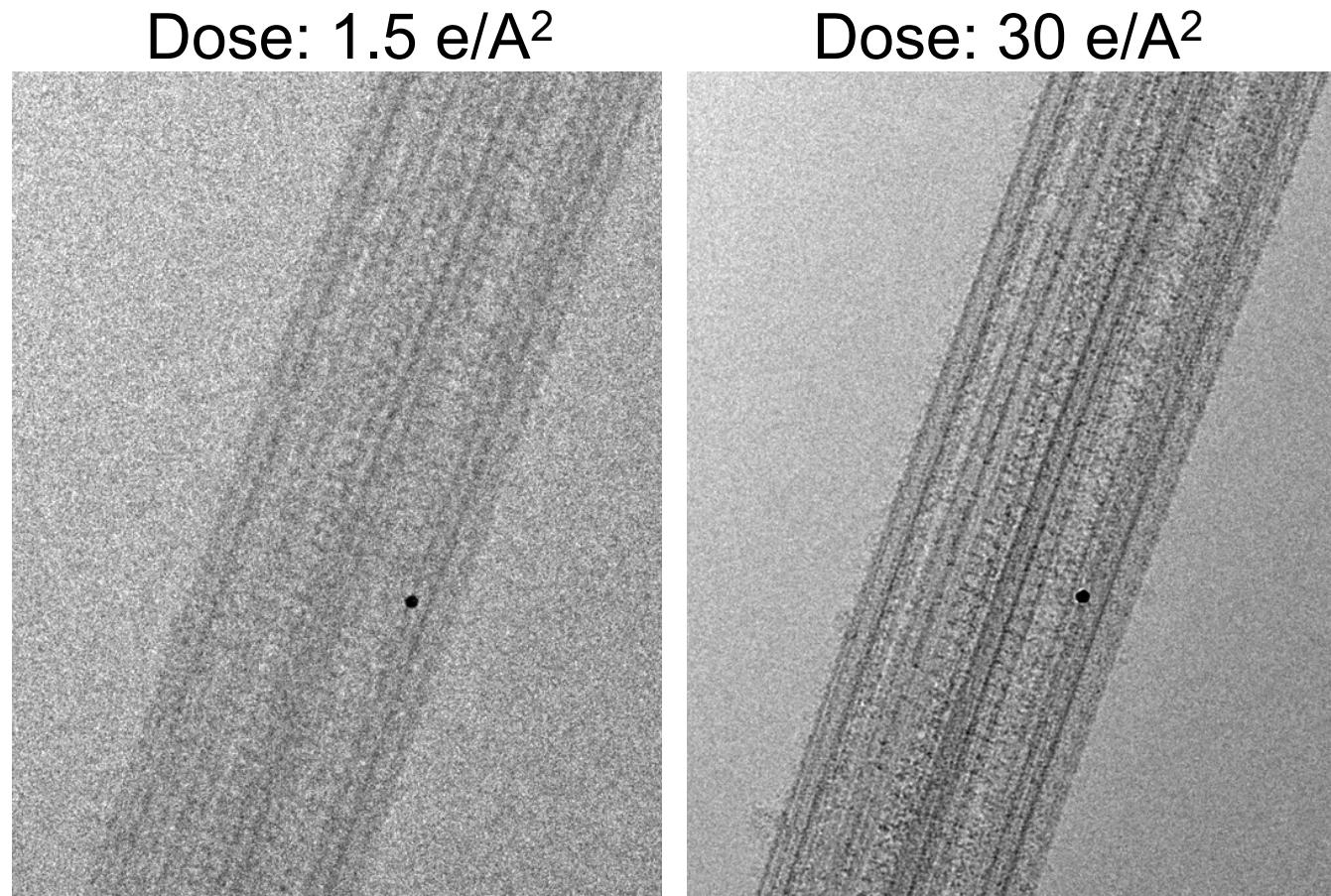
# CryoET image processing for high resolution is challenging



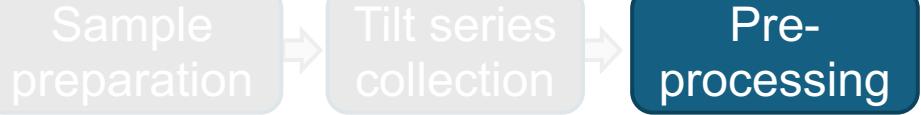
- Low SNR
- Defocus depth and defocus gradient
- Significant beam-induced motion & radiation damage



e.g., ~90 e/A<sup>2</sup> total dose for the 61 images

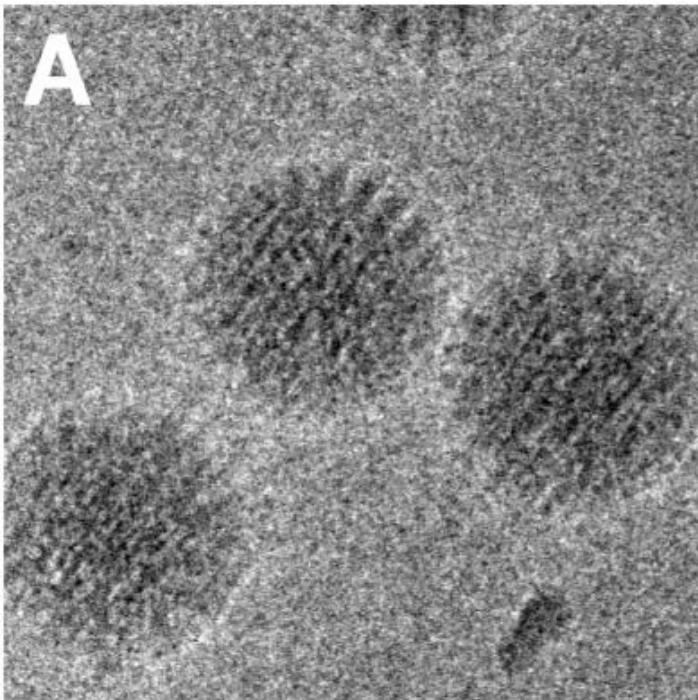


# CryoET workflow

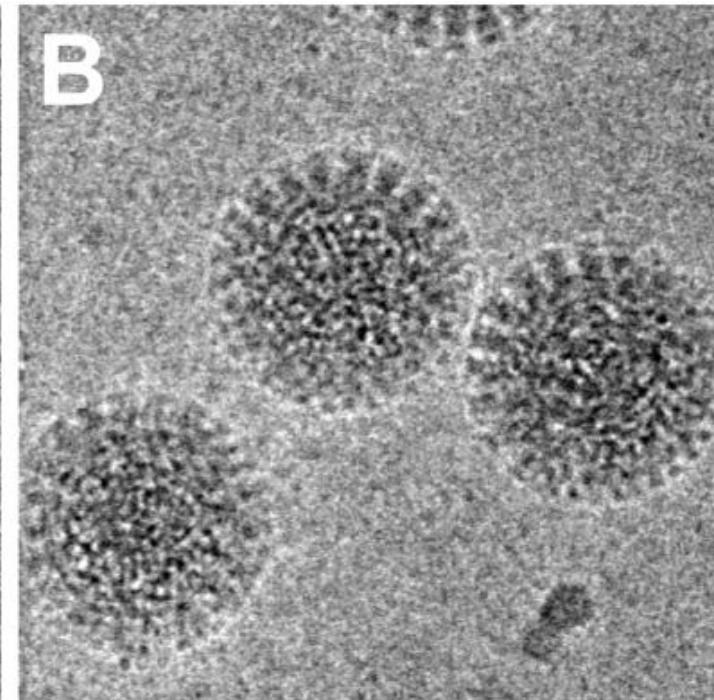


- Frame alignment & Dose weighting (exposure filtering).  
e.g., ***Unblur***, ***MotionCor2***.

From 60-frames: without alignment



with alignment

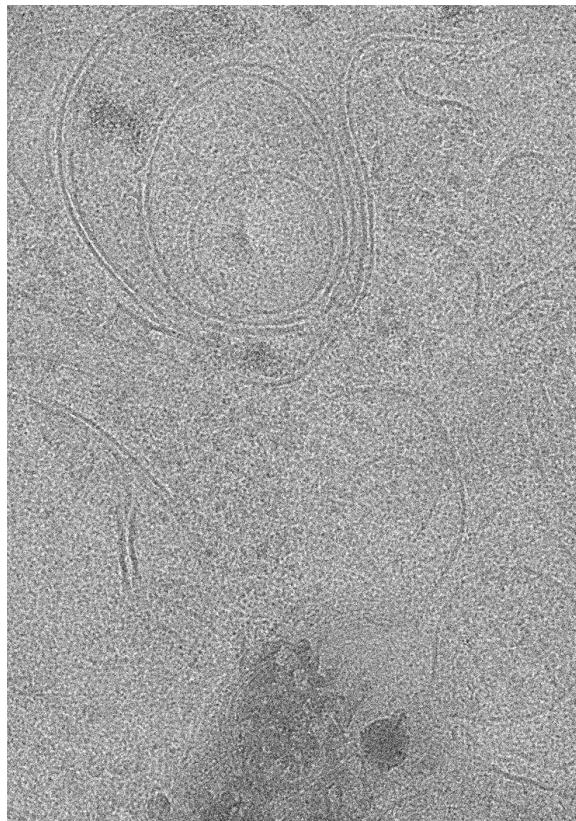


# CryoET workflow

Unpublished data.



➤ **Tilt series alignment:** e.g., IMOD, AreTomo.



Aligned tilt series

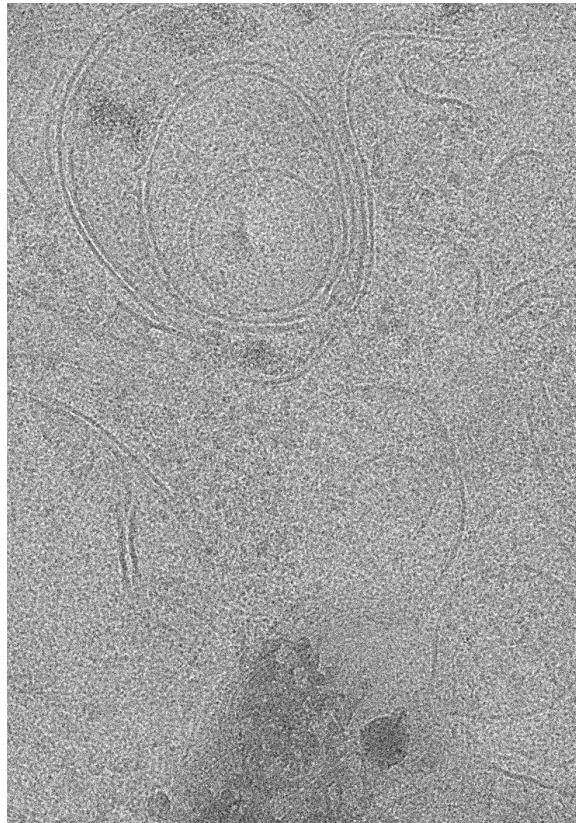
Unpublished data

# CryoET workflow

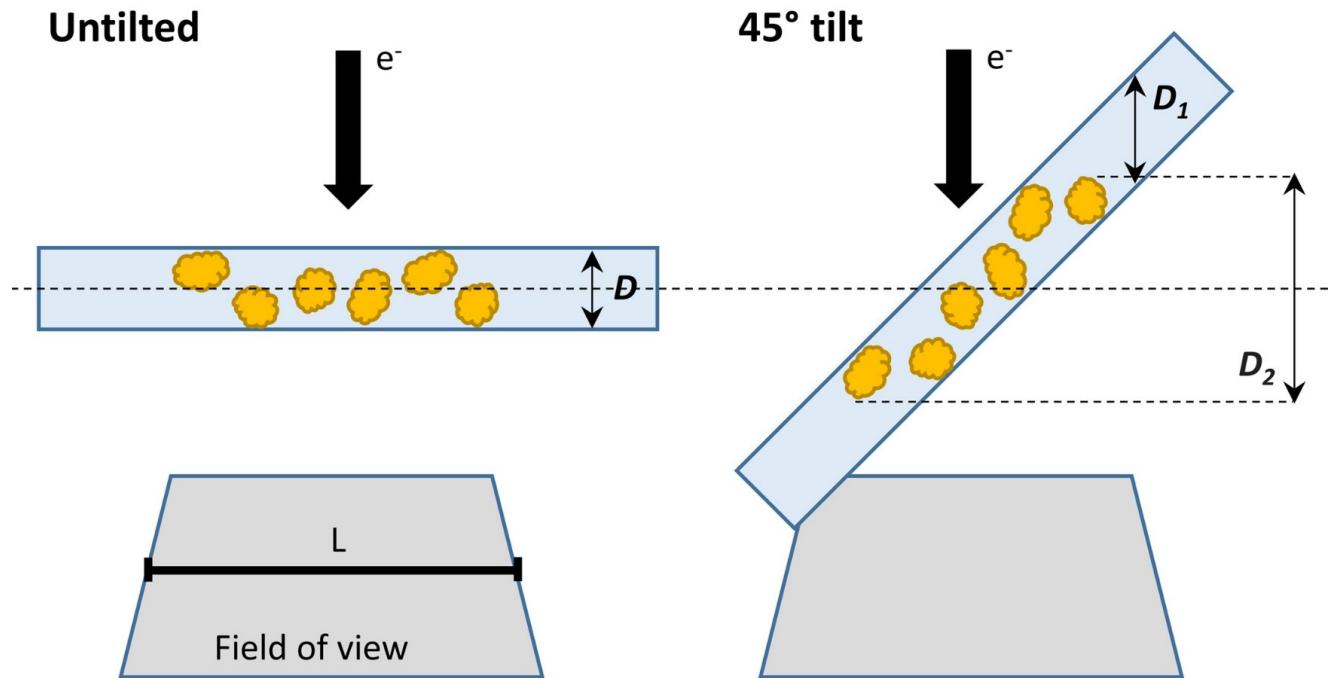
Unpublished data.



- **Tilt series alignment:** e.g., IMOD, AreTomo.
- **Correct CTF:** e.g., CTFFIND4, Gctf, novaCTF.



Aligned tilt series



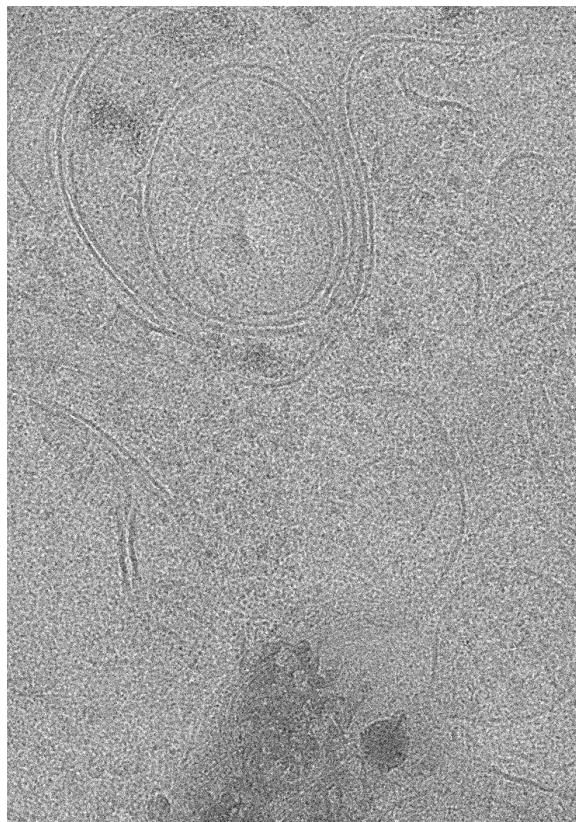
Leigh et al., 2019

# CryoET workflow

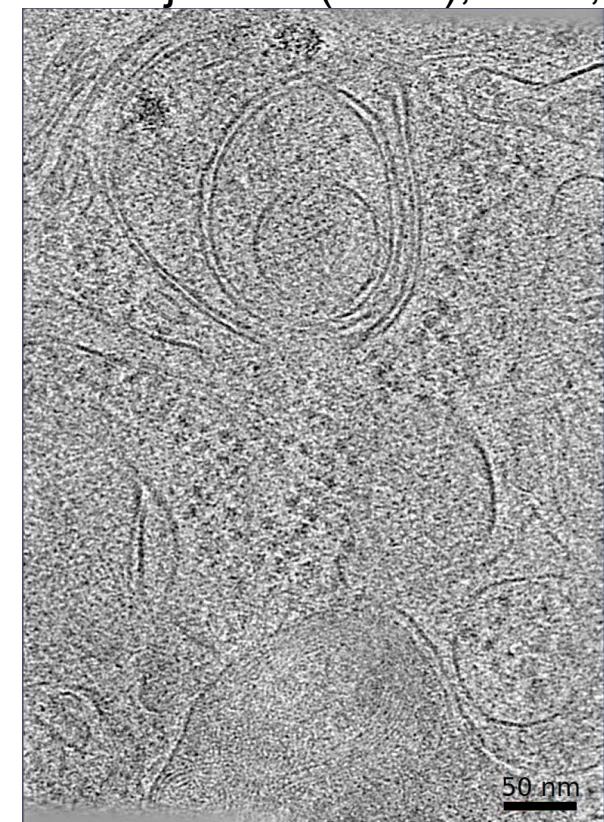
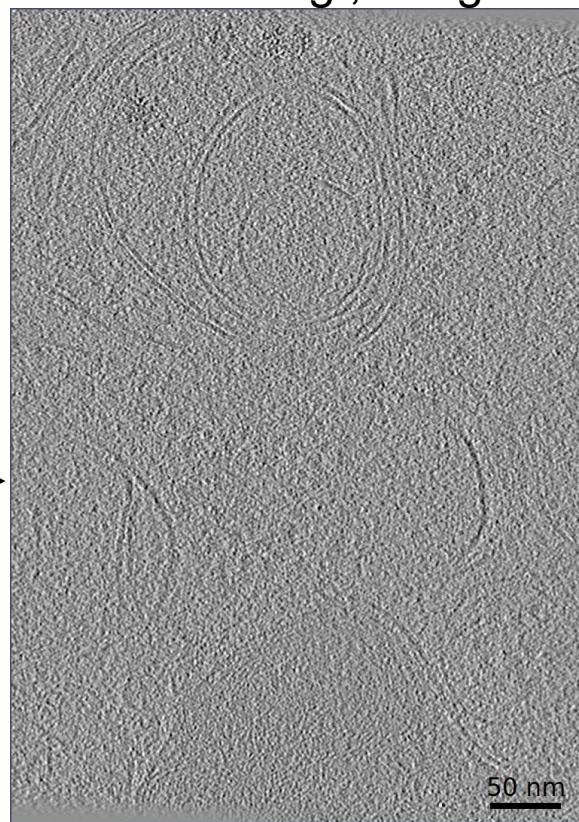
Unpublished data.



- **Tilt series alignment:** e.g., IMOD, AreTomo.
- **Correct CTF:** e.g., CTFFIND4, Gctf, novaCTF.
- **3D reconstruction:** e.g., Weighted Back Projection (WBP), SIRT, SART



Tomogram  
reconstruction  
→



3D tomogram

# CryoET workflow

Sample preparation

Tilt series collection

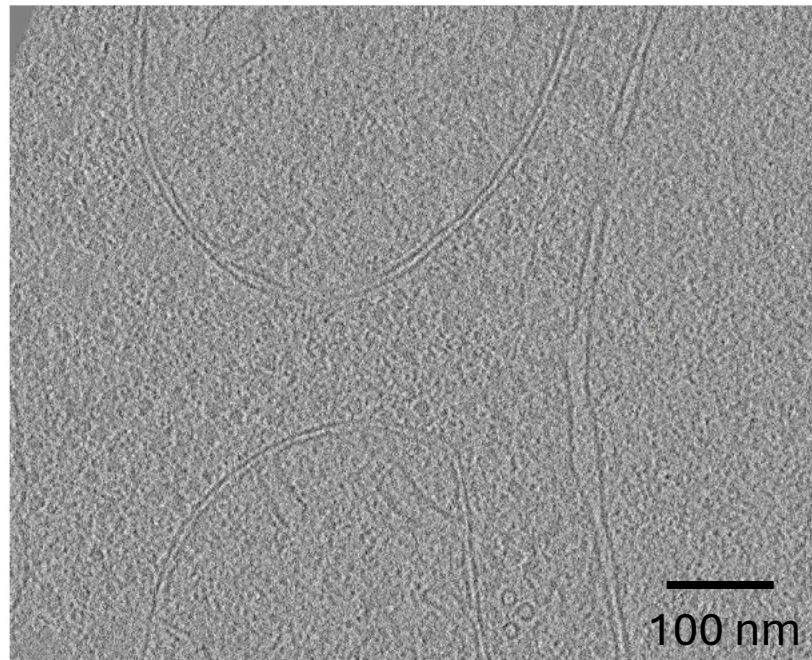
Pre-processing

Tomogram reconstruction

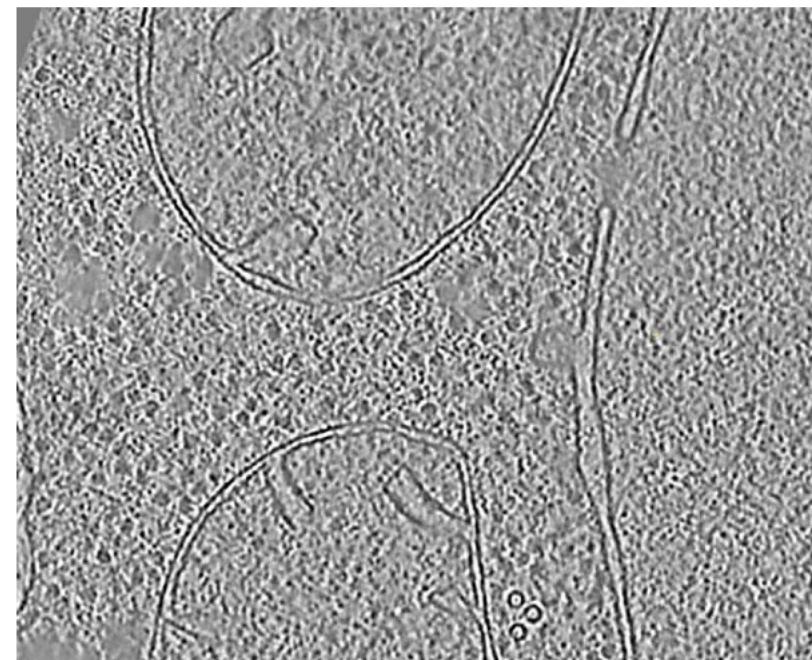
Tomogram interpretation

➤ Denoising (e.g., cryoCARE, Topaz-Denoise.)

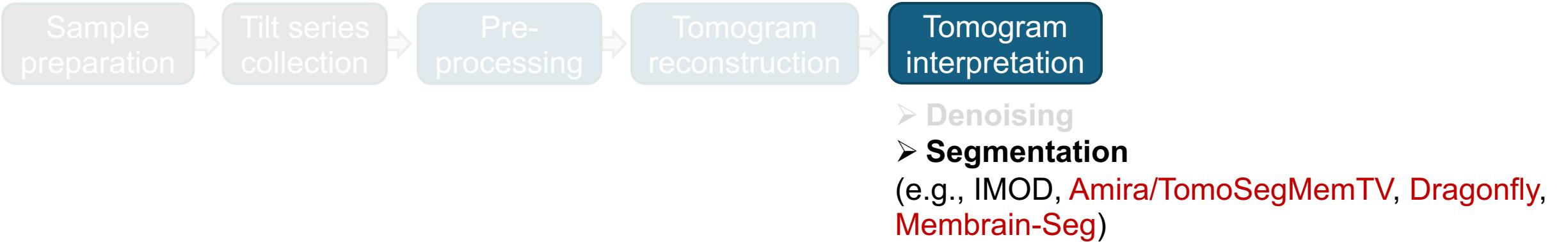
Original



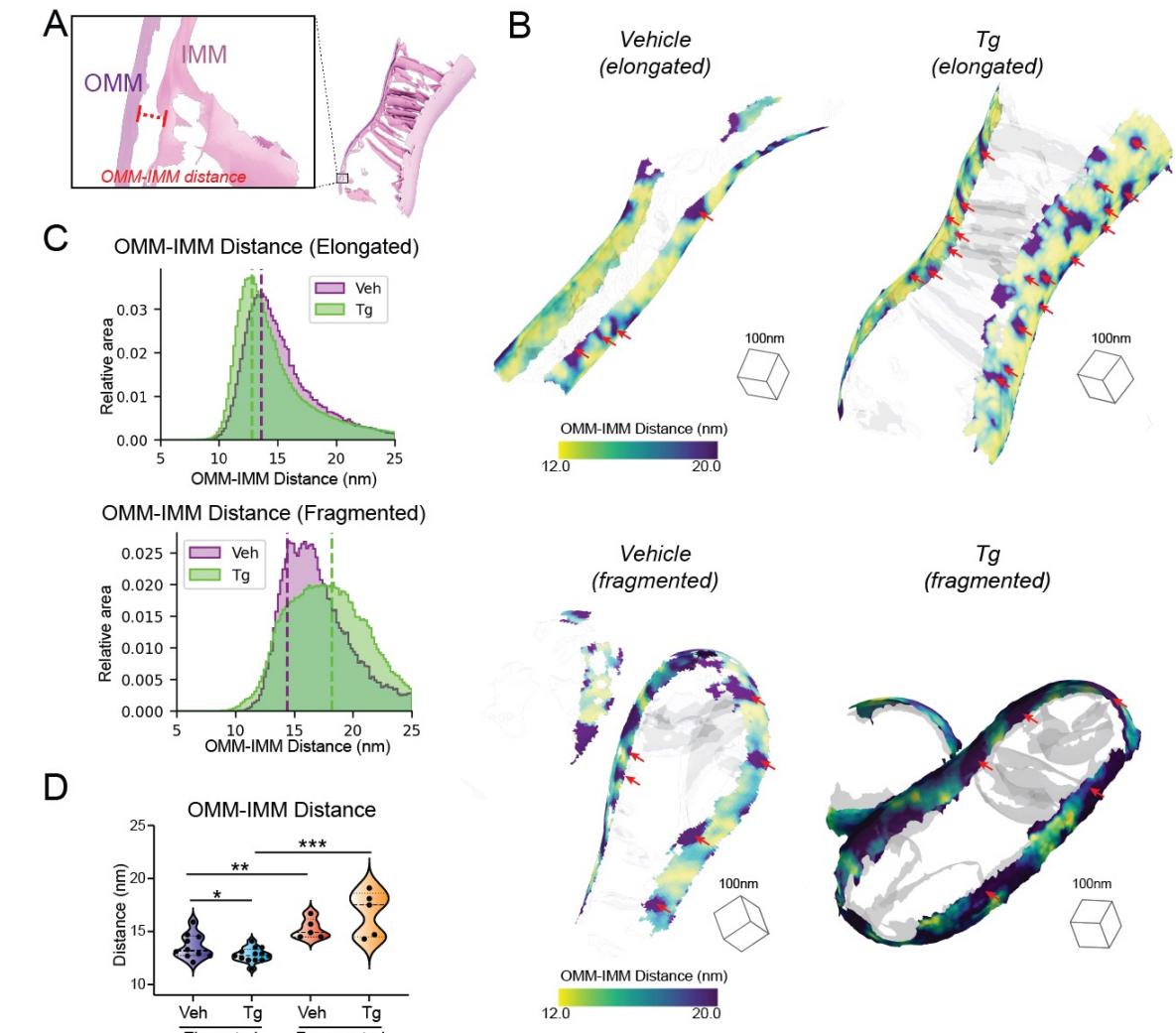
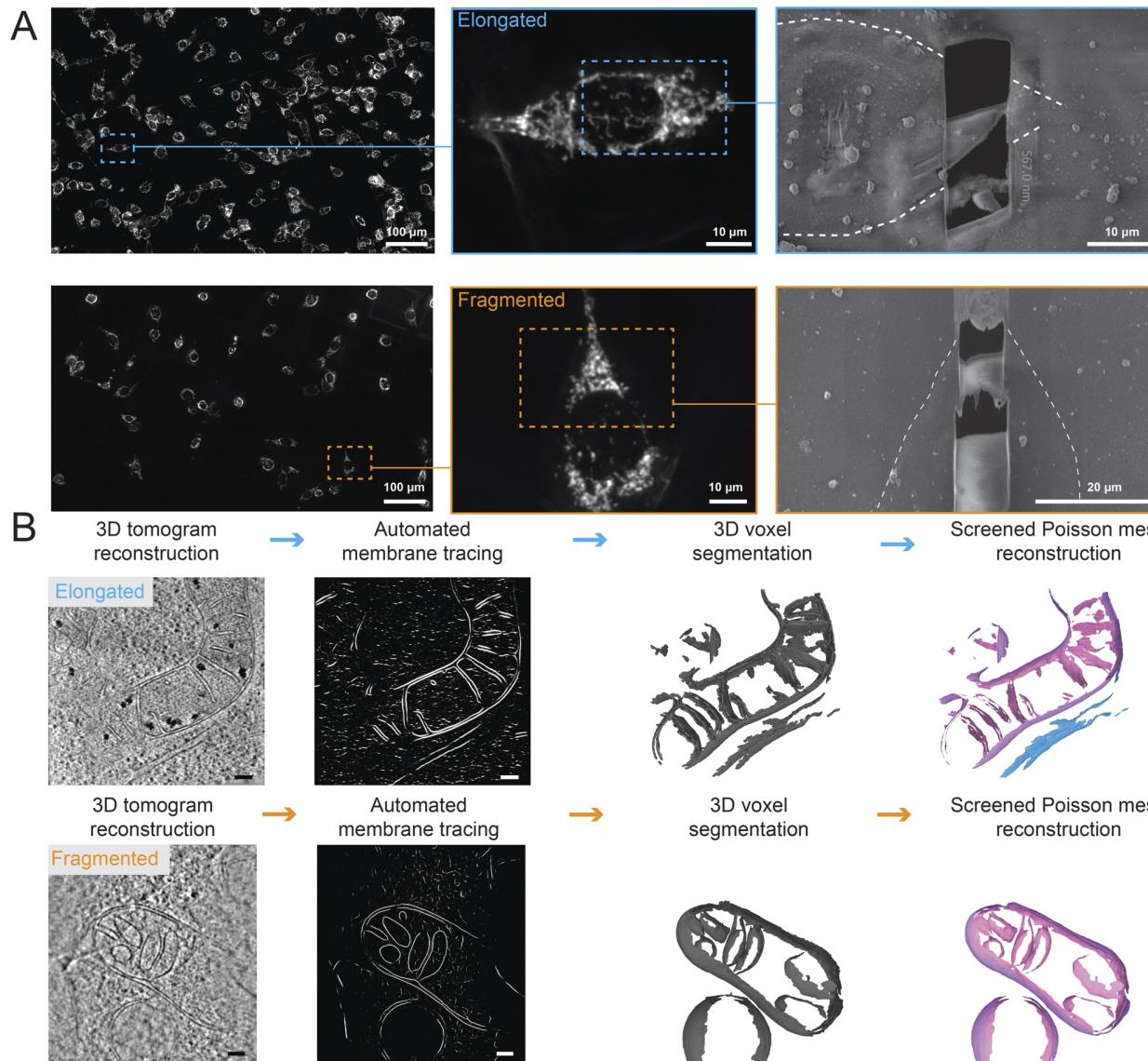
Topaz-Denoise



# CryoET workflow



# Quantifying organellar membrane ultrastructure using a surface morphometrics pipeline with segmentation by Amira/TomoSegMemTV

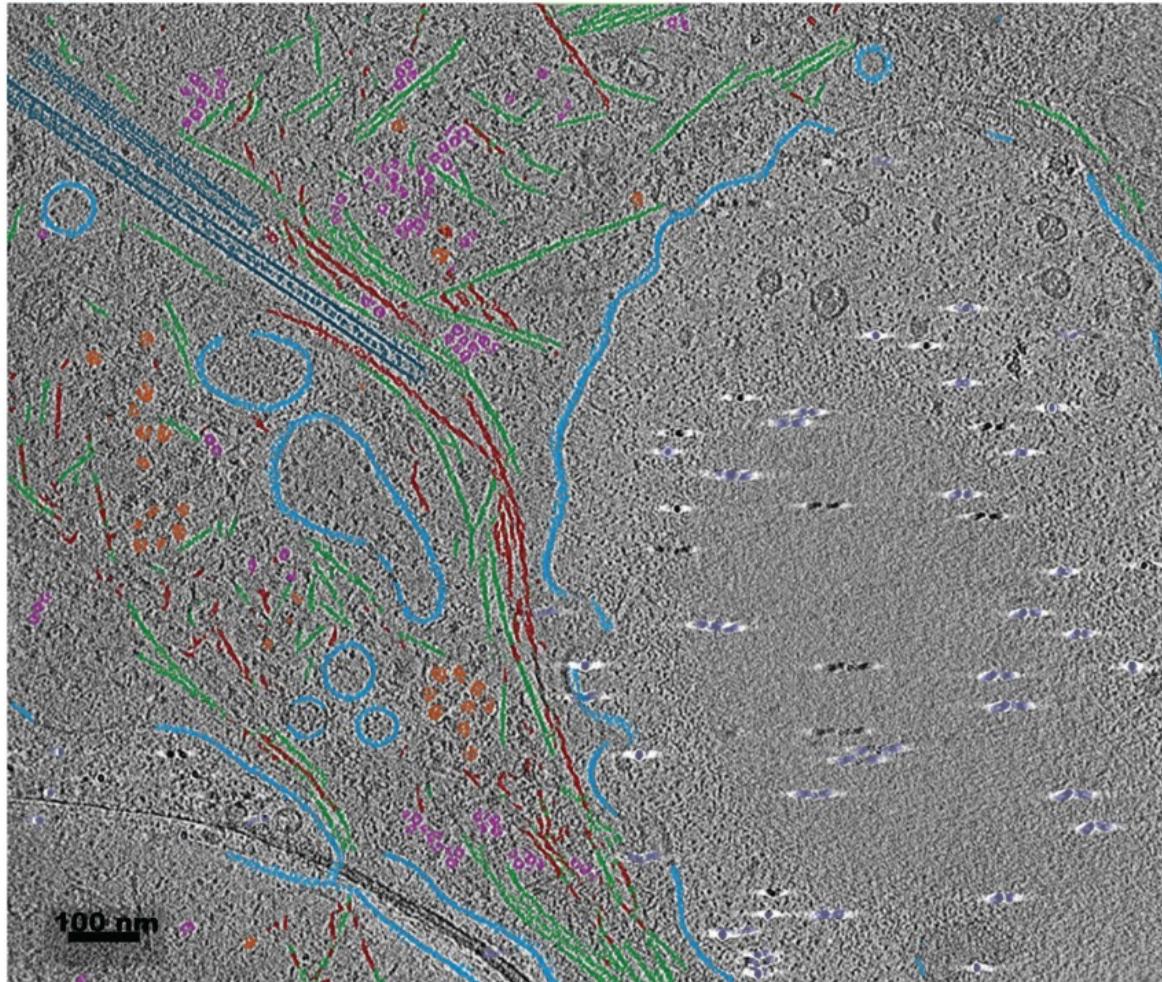


# Deep learning-based segmentation of 3D tomograms by Dragonfly

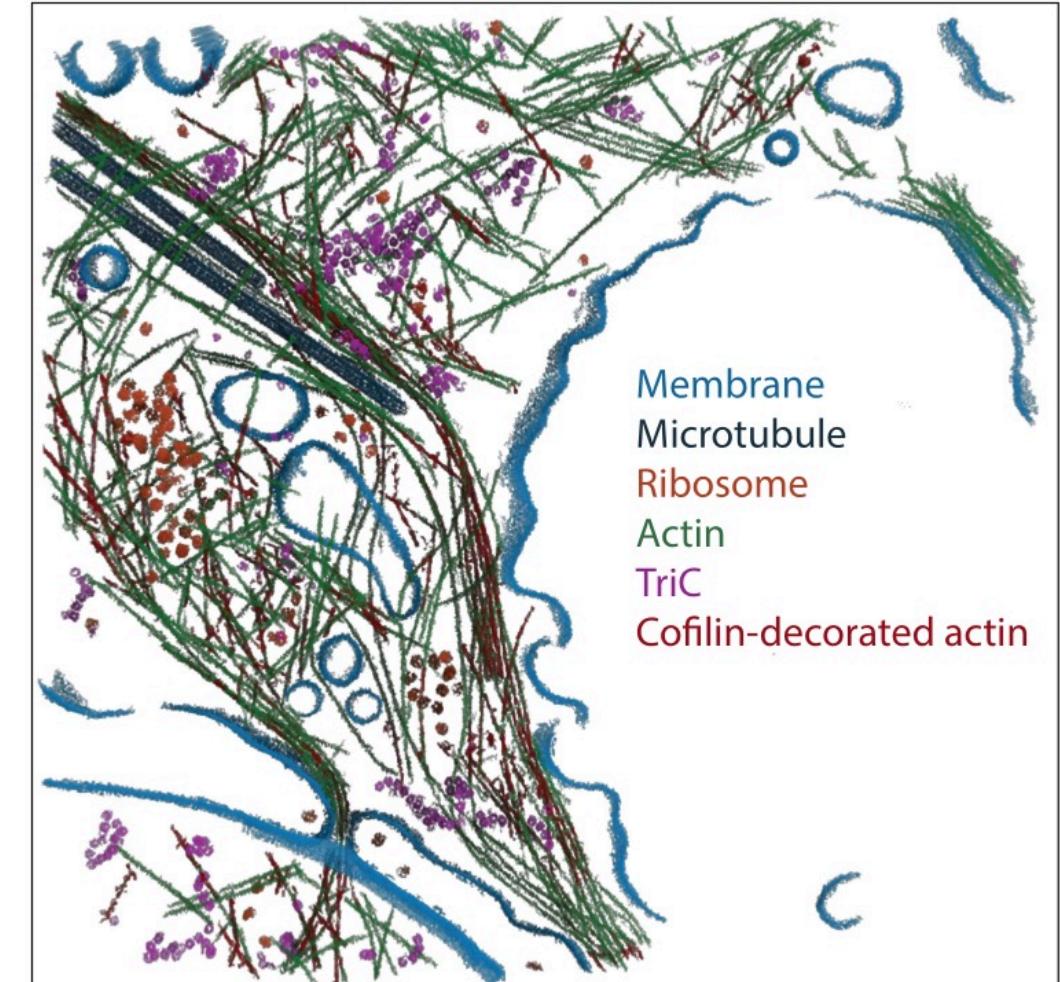
Intel® Xeon® Gold 6124 CPU 3.2GHz; NVIDIA Quadro P4000

<https://www.theobjects.com/dragonfly/tutorials.html>

UNet Segmented Tomogram



3D Rendered Segmentation



# Membrain-Seg segmentation introduced by Dr. Benjamin Barad from OHSU



Membrain-Seg  
→



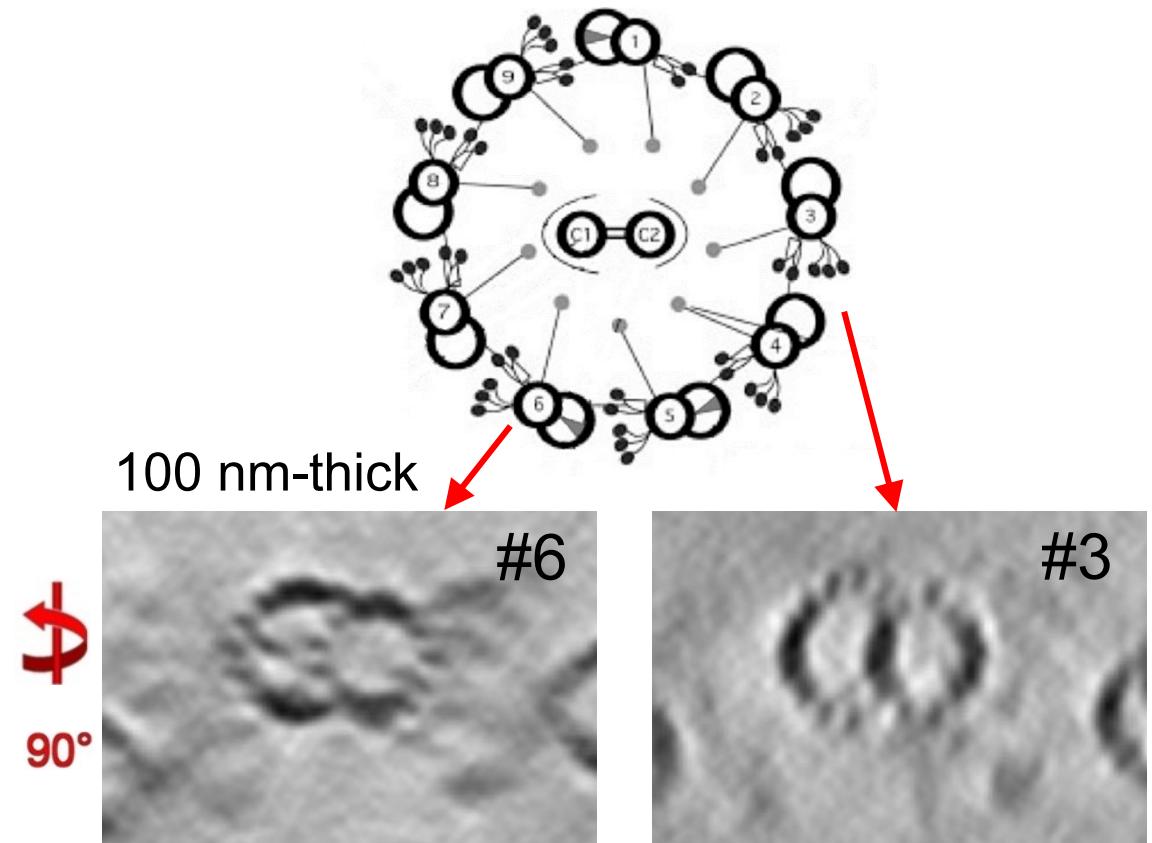
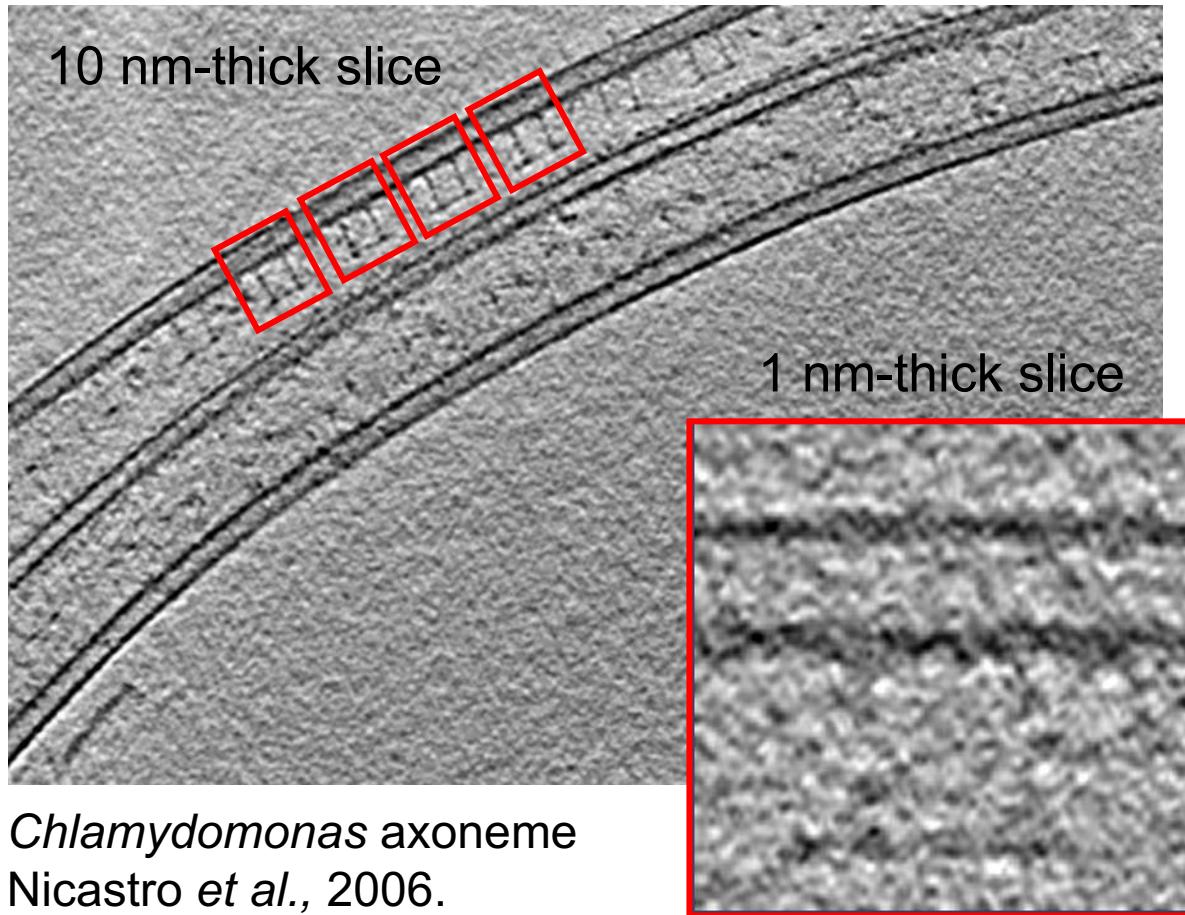
Unpublished data.

<https://github.com/teamtomo/membrain-seg?tab=readme-ov-file>

# CryoET workflow



Two major issues of tomograms: **low S/N & missing wedge artifacts**

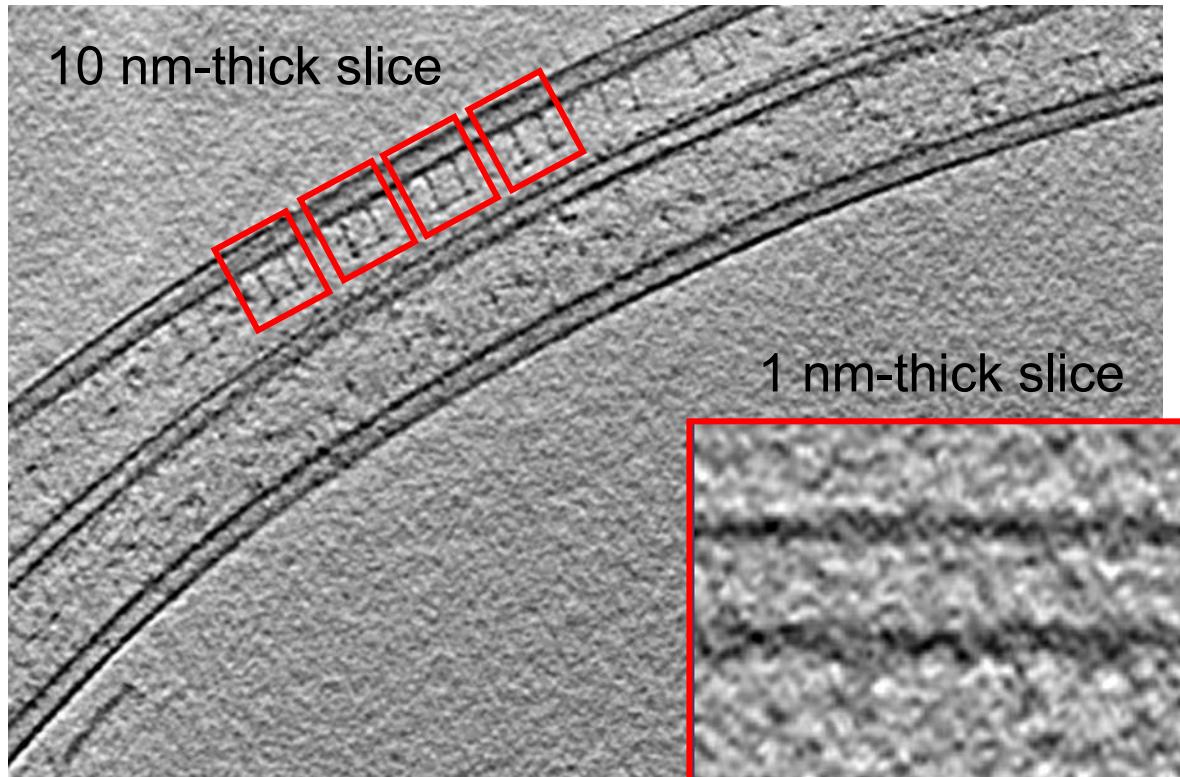


DMTs at different orientations look different.

# CryoET workflow

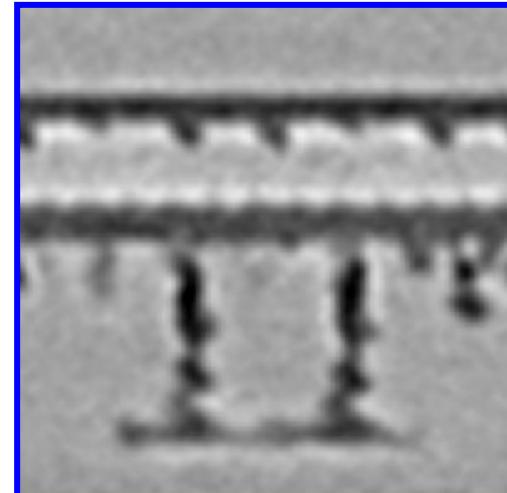


Two major issues of tomograms, **low S/N & missing wedge artifacts**, can be addressed by **Sub-tomogram averaging**



*Chlamydomonas* axoneme  
Nicastro et al., 2006.

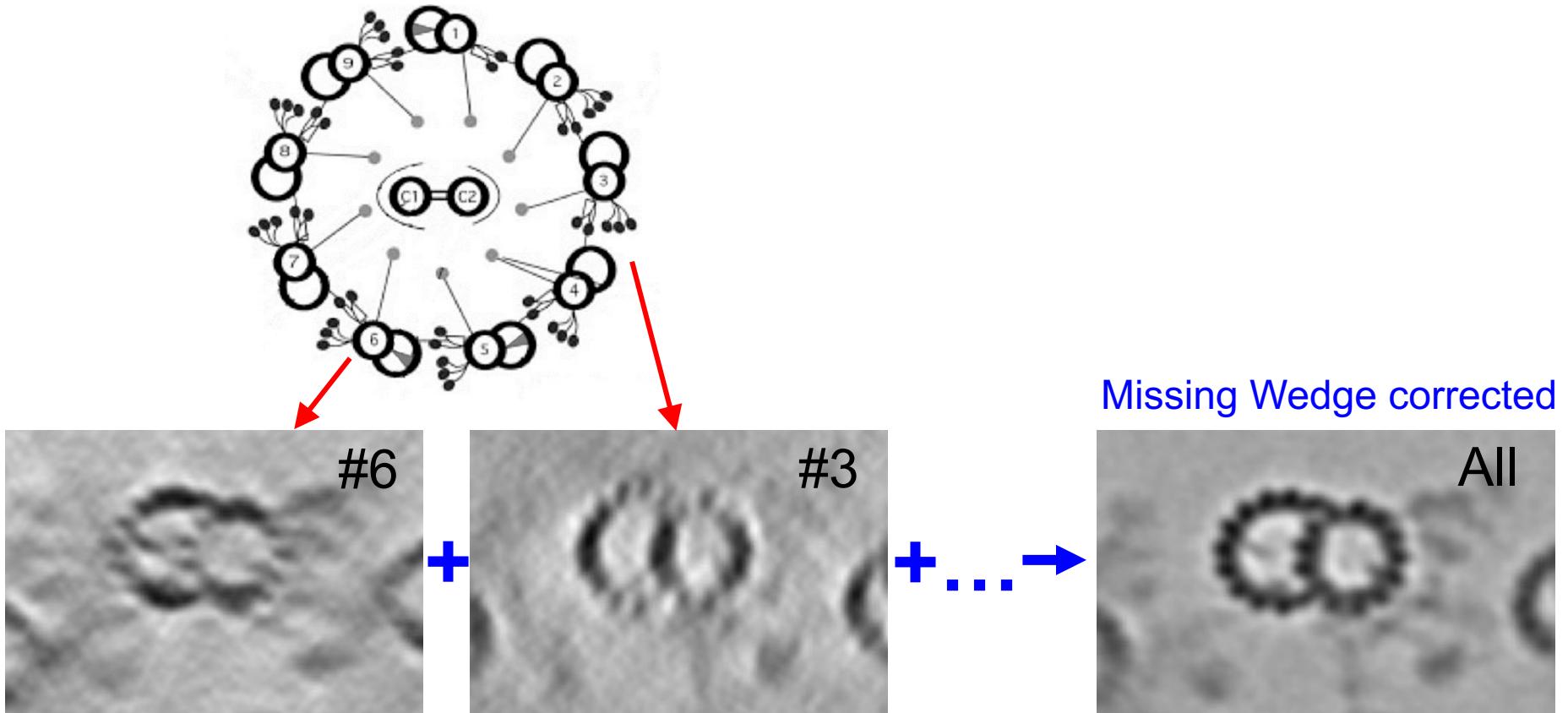
700 repeats averaged



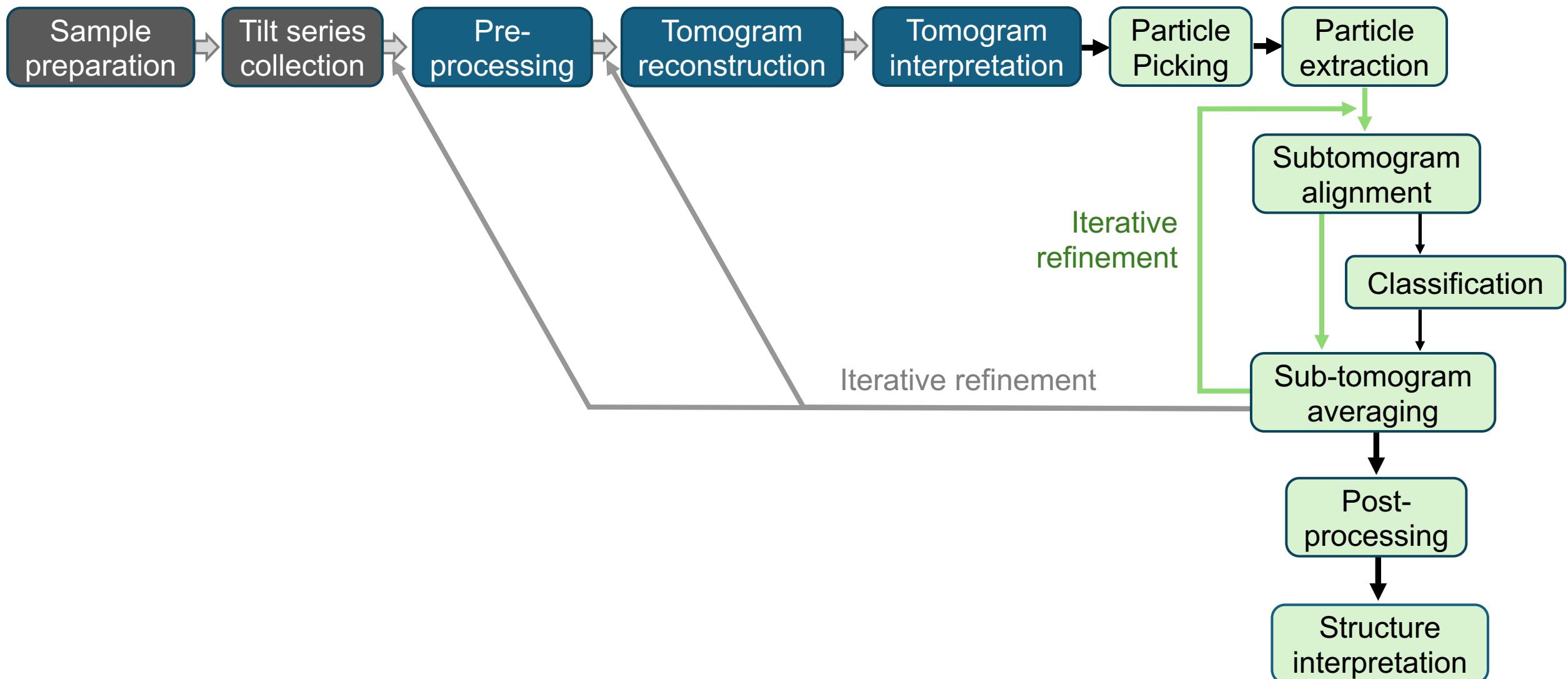
# CryoET workflow



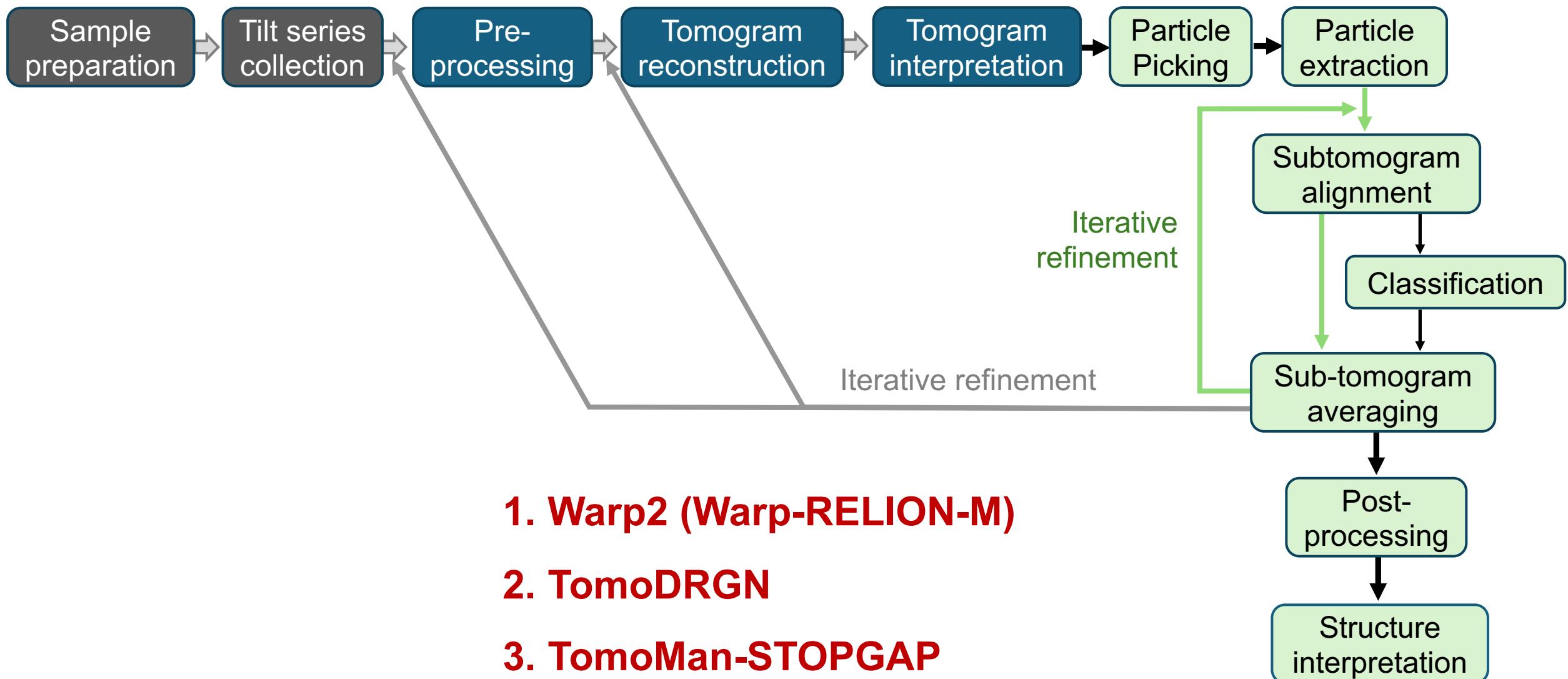
Two major issues of tomograms, **low S/N & missing wedge artifacts**, can be addressed by **Sub-tomogram averaging**



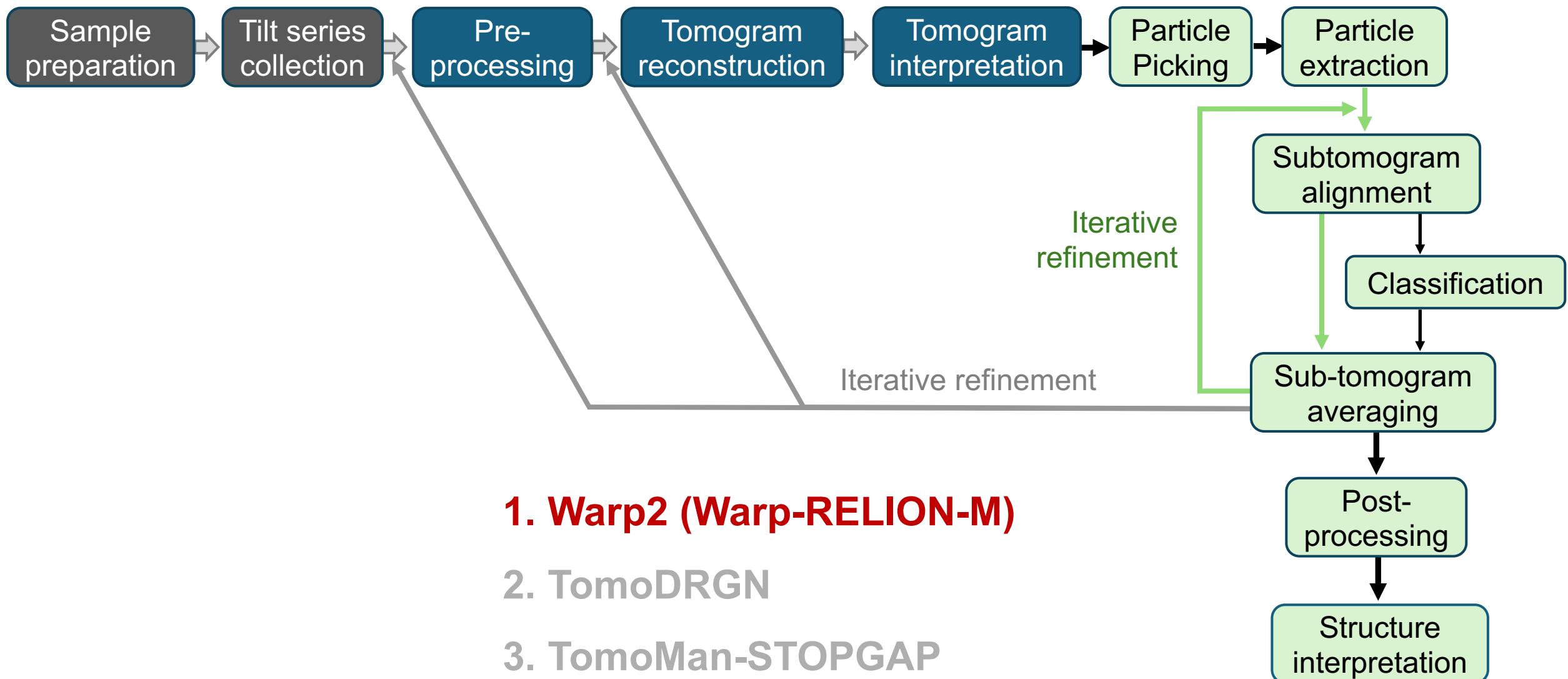
# CryoET workflow



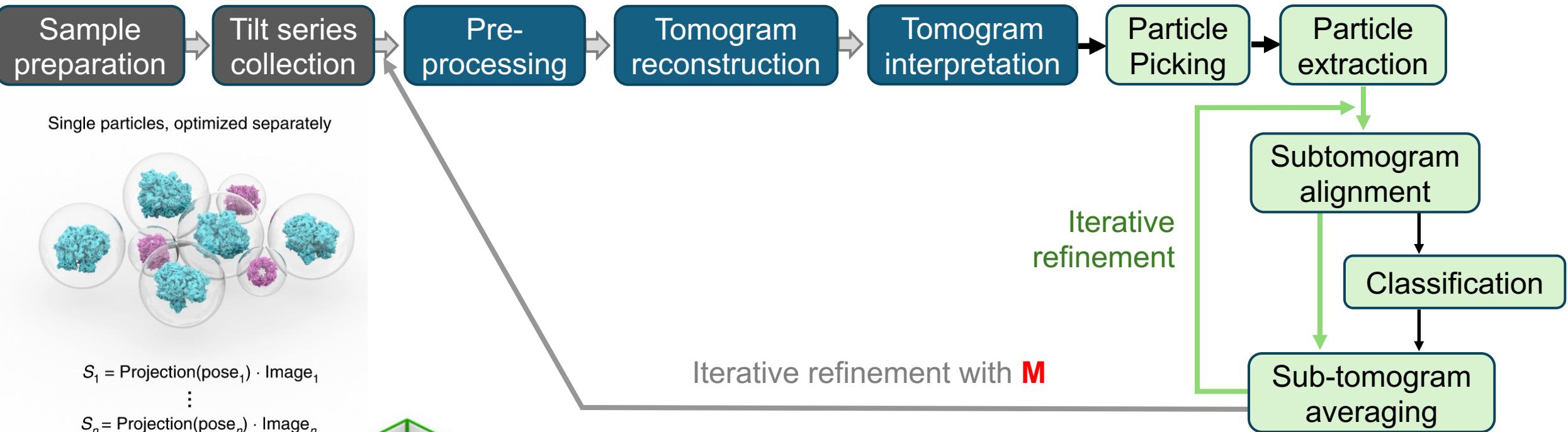
# CryoET workflow



# CryoET workflow



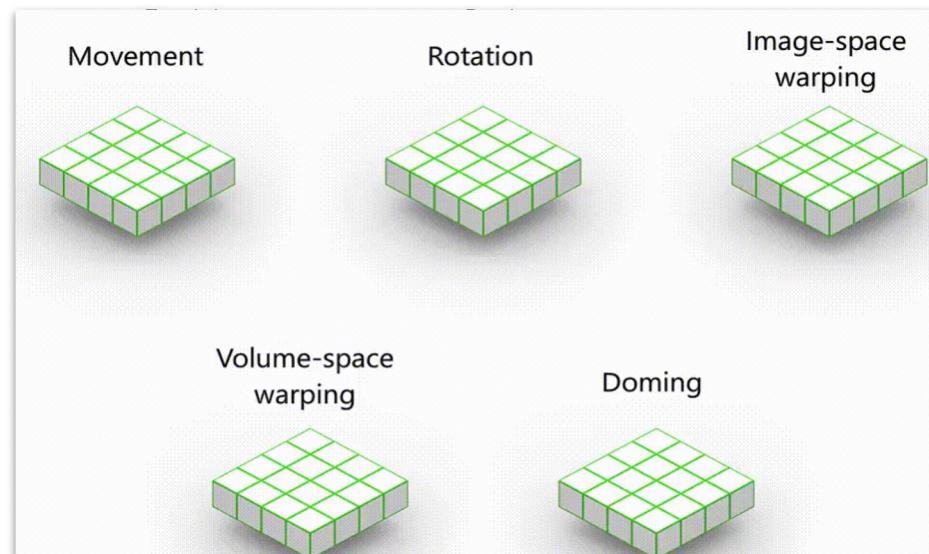
# CryoET workflow



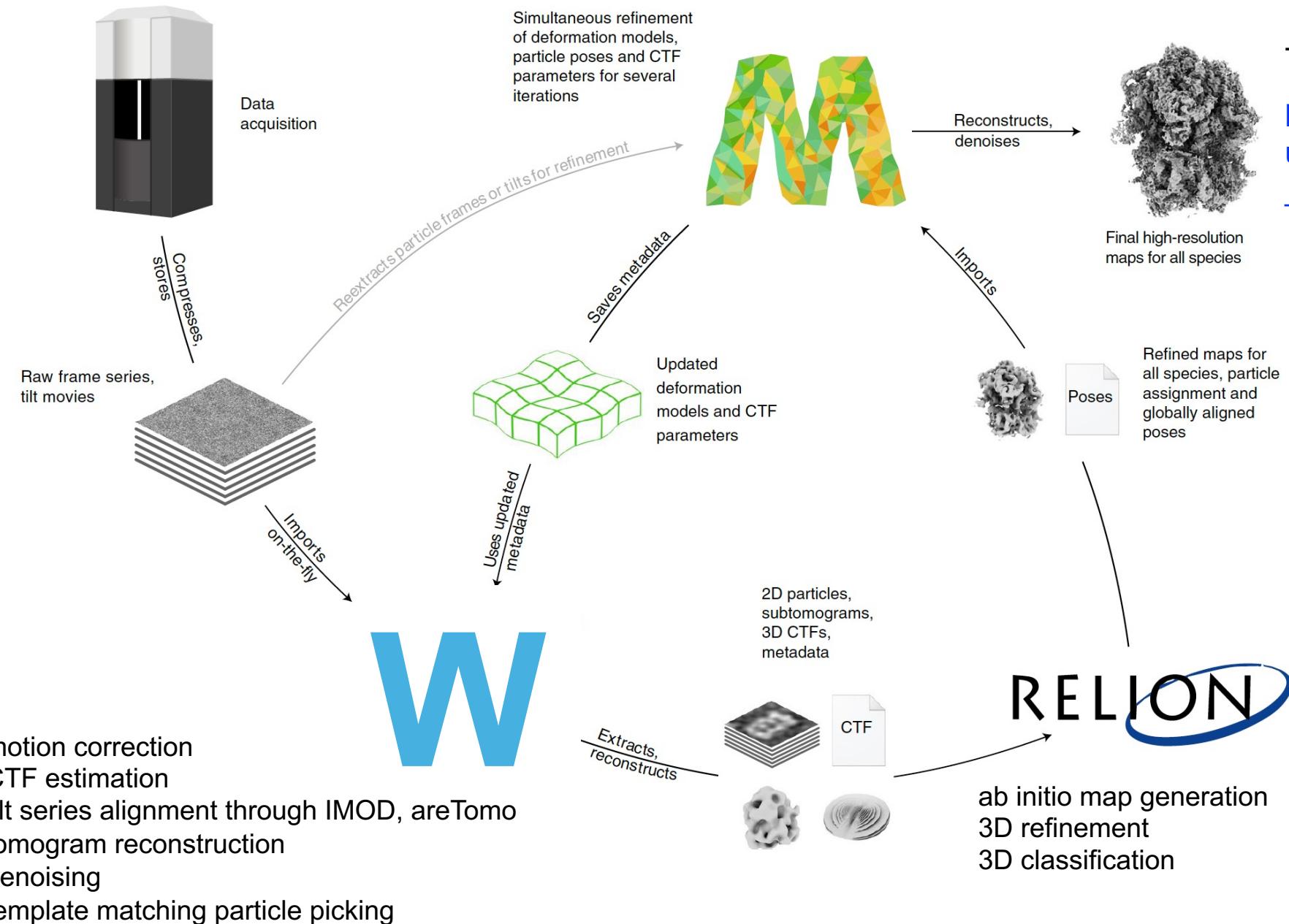
## Warp-RELION-M

$$M = \sum_s^{N_{\text{species}}} \sum_p^{N_{\text{particles}}} \sum_f^{N_{\text{frames}}} \text{Projection}(\text{pose}_{s,p,f} + \text{correction}_{s,p,f}) * \text{Image}_{s,p,f}$$

Tegunov et al., Nature Methods, 2021; Burt et al., PLOS Biology, 2021

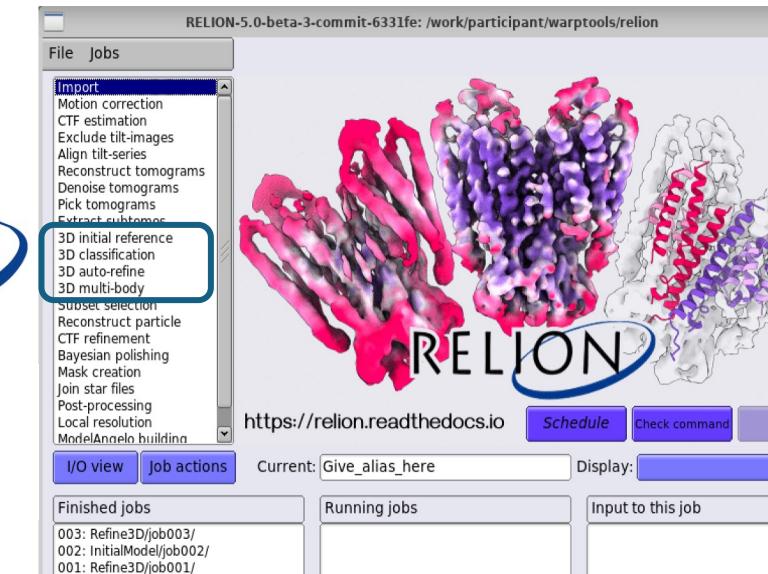


# The Warp-RELION-M workflow

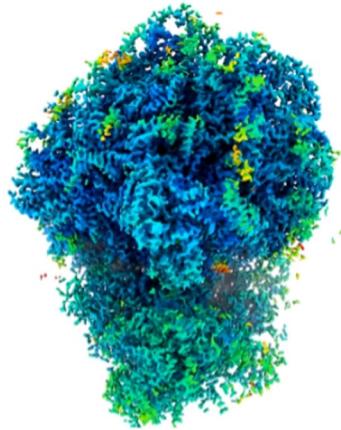


Tegunov et al., Nature Methods, 2021

[https://war pem.github.io/warp/user\\_guide/warptools/quick\\_start\\_warptools\\_tilt\\_series/#running-m-with-mcore](https://war pem.github.io/warp/user_guide/warptools/quick_start_warptools_tilt_series/#running-m-with-mcore)

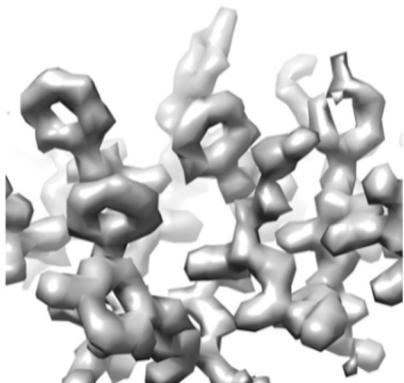


# Warp-M holds resolution records in Tomo and SPA to date

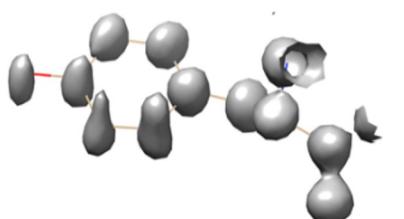


Examples from Dmitry & Alister

*In situ* tilt series, 80S ribosome: 2.5 Å  
Huaipeng Xing & Martin Beck's team



*In vitro* apo ferritin tilt series: 1.5 Å  
Martin Obr & Abhay Kotecha's team



*In vitro* apo ferritin frame series: 0.99 Å  
My reprocessing of Holger Stark's EMPIAR-10591

# My processing results with Warp 2.0: 5 TS of Apoferritin from EMPIAR-10491

Titan Krios, K3, Energy filter (Slit width: 20 eV), No fiducial Au

## Tilt series parameters:

Scheme: dose-symmetric

Range: -40° to +40°

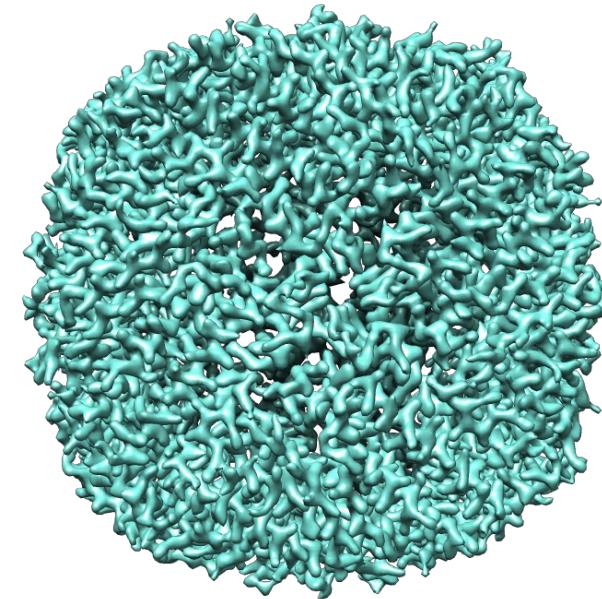
Step size: 2°

Pixel size: 0.834 Å/pixel

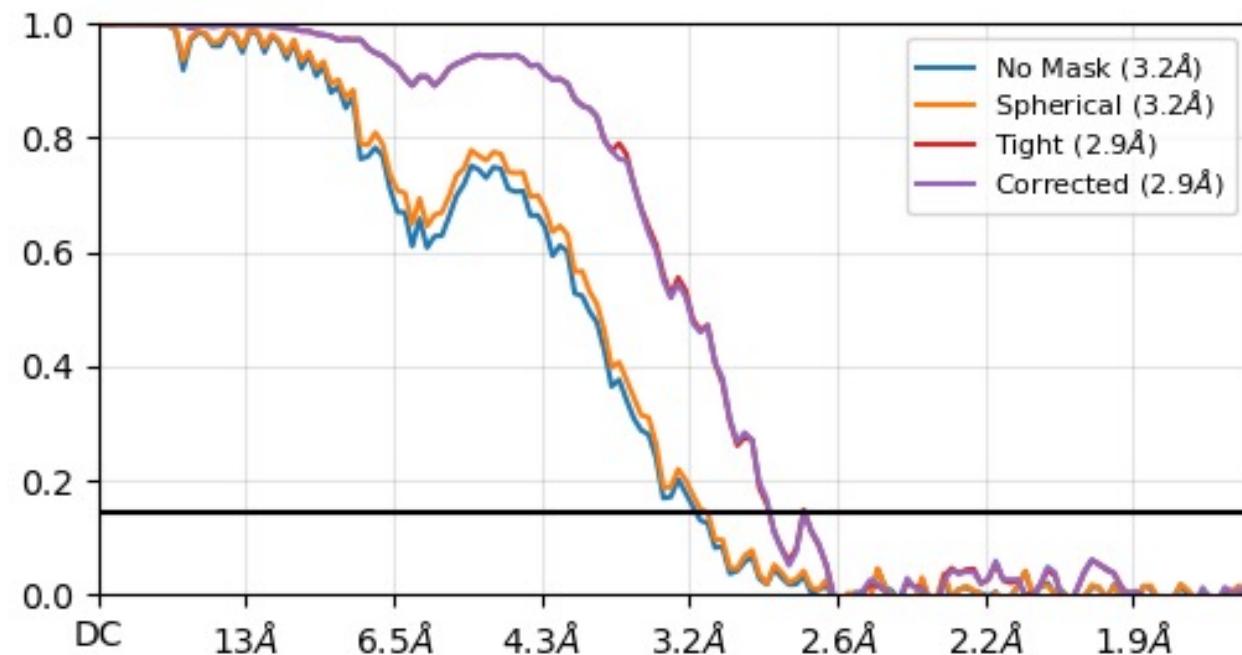
Defocus: -1.5 to -3.5 μm

Dose at each tilt: 2.7 e/Å<sup>2</sup>

1700 particles



GSFSC Resolution: 2.86 Å



# My results with Warp 2.0: 5 TS of AAV

Glacios, Falcon 4, Selectris energy filter (Slit width: 10 eV), No fiducial Au

## Tilt series parameters:

Scheme: dose-symmetric

Range: -60° to +60°

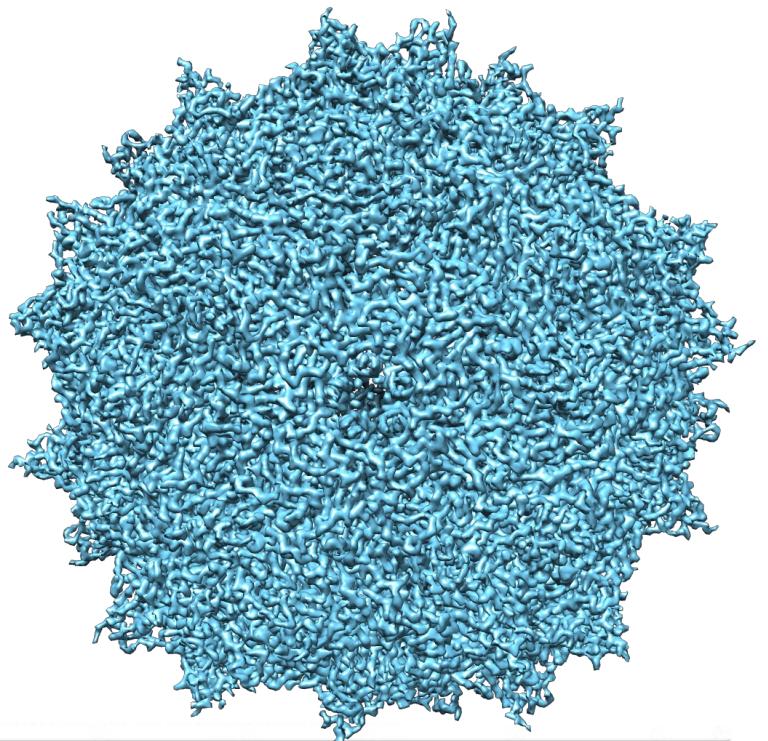
Step size: 3°

Pixel size: 1.53 Å/pixel

Defocus: -3 to -5 μm

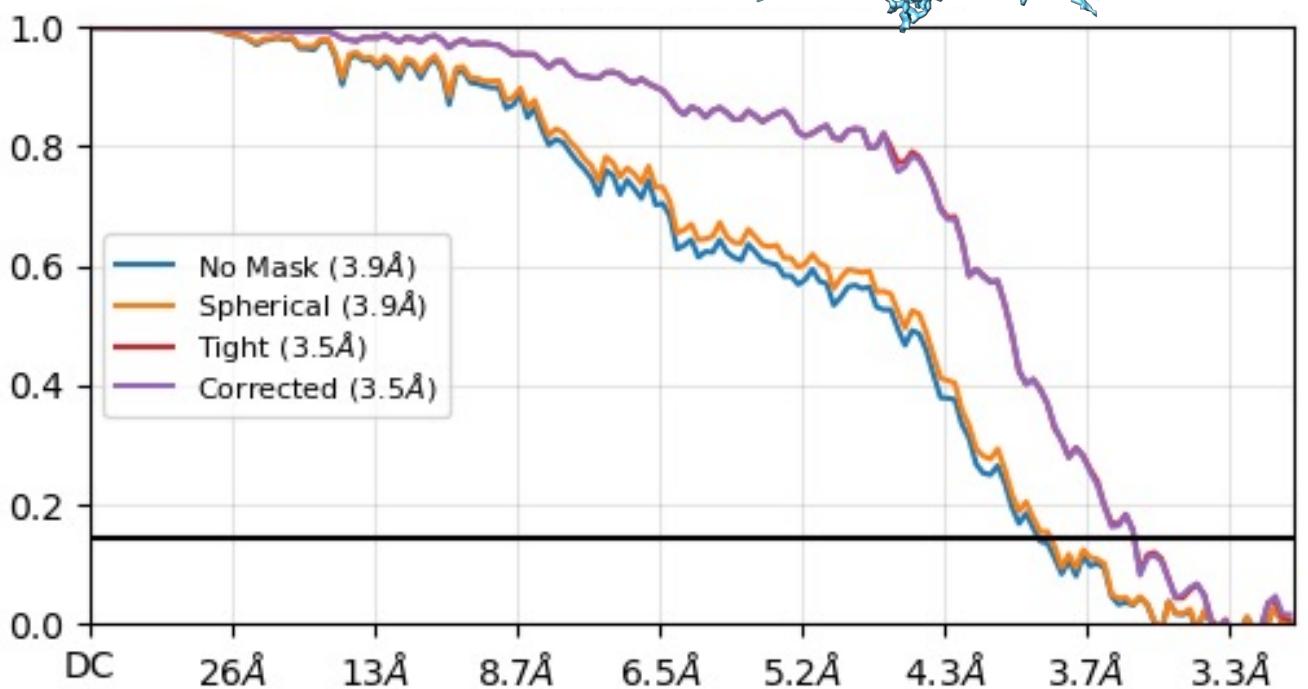
Dose at each tilt: 2.4 e/Å<sup>2</sup>

1700 particles

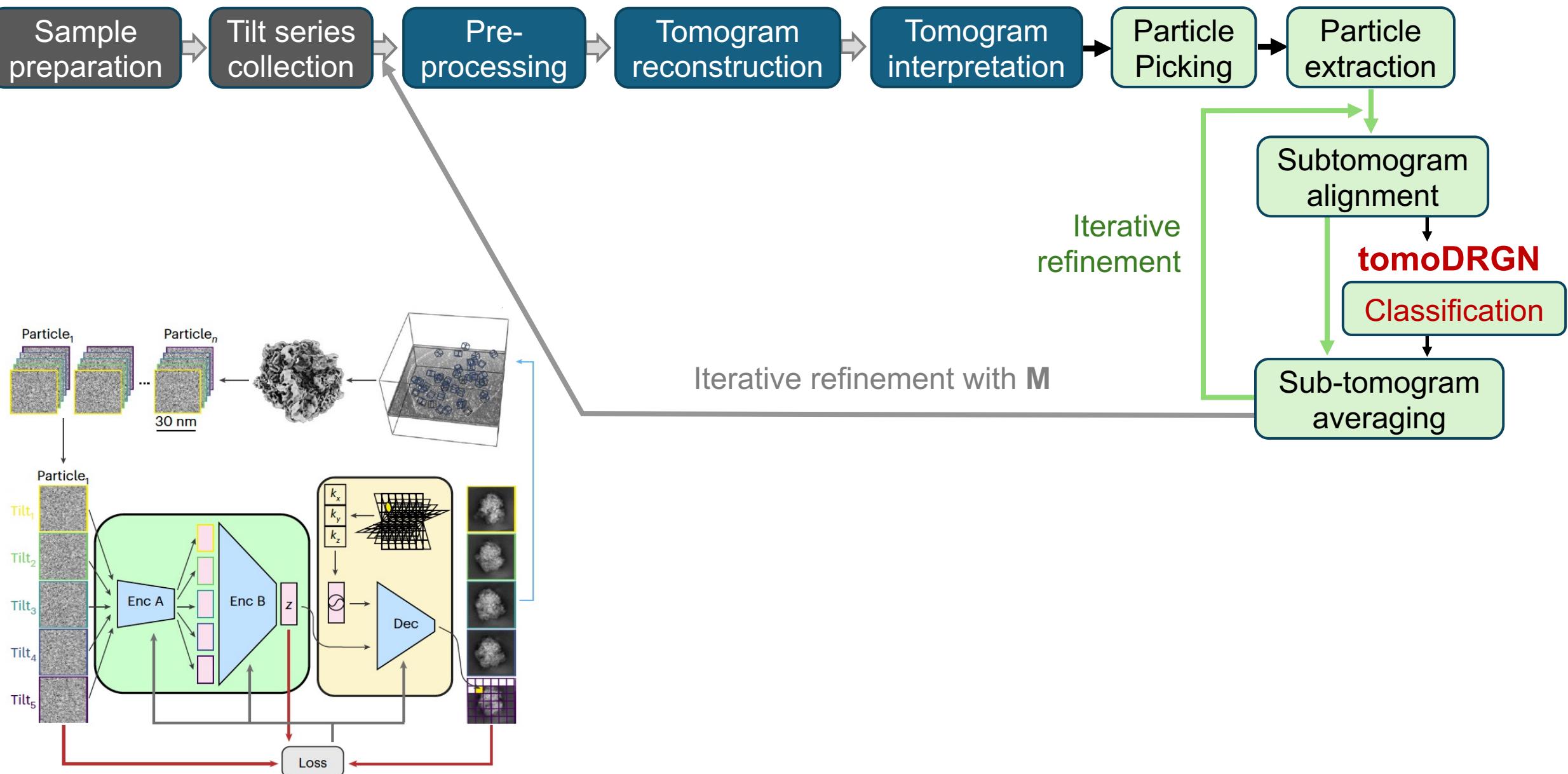


GSFSC Resolution: 3.55 Å

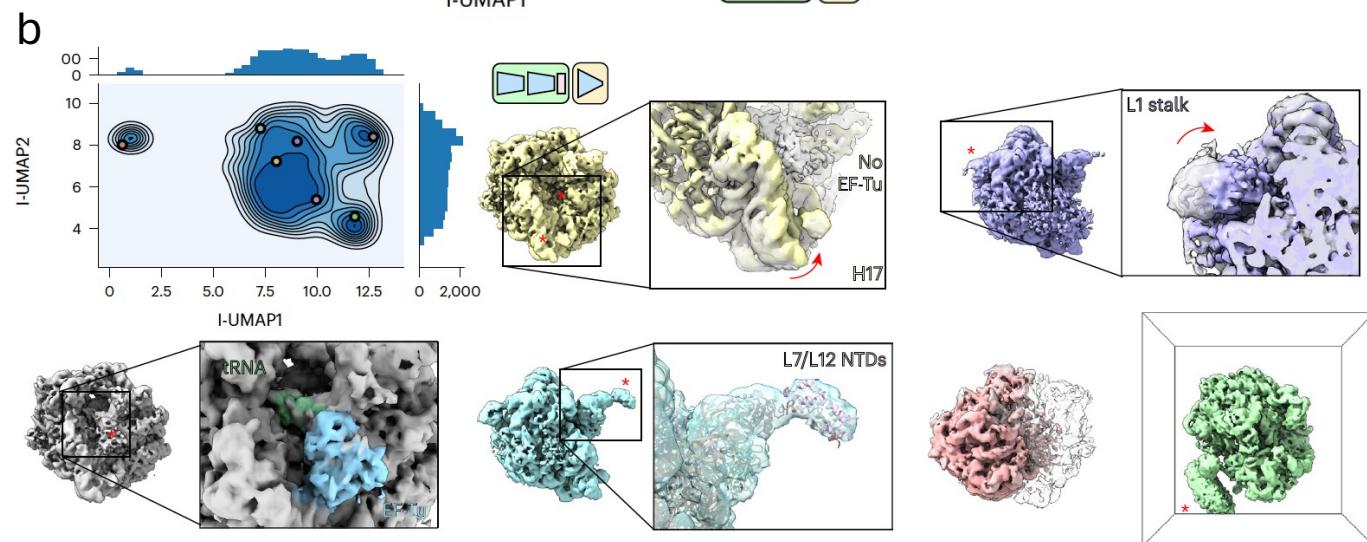
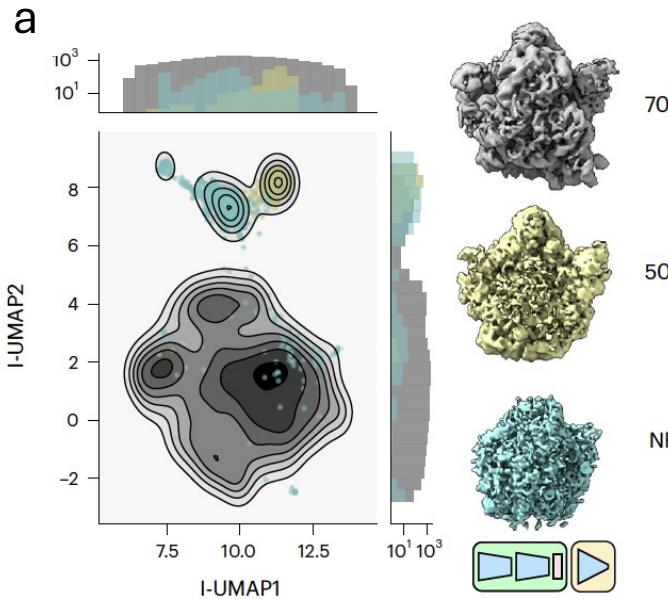
4.6 Å by RELION 5,  
though may be better  
after more optimization



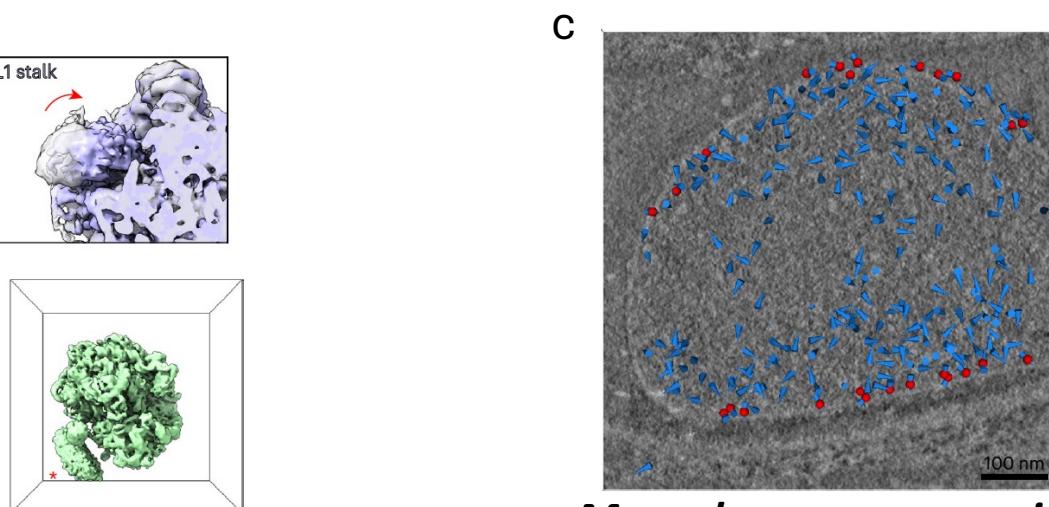
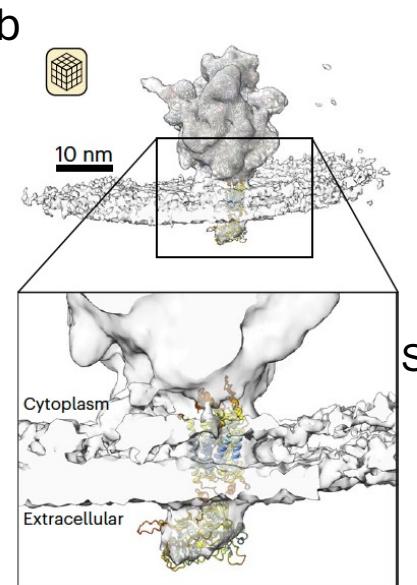
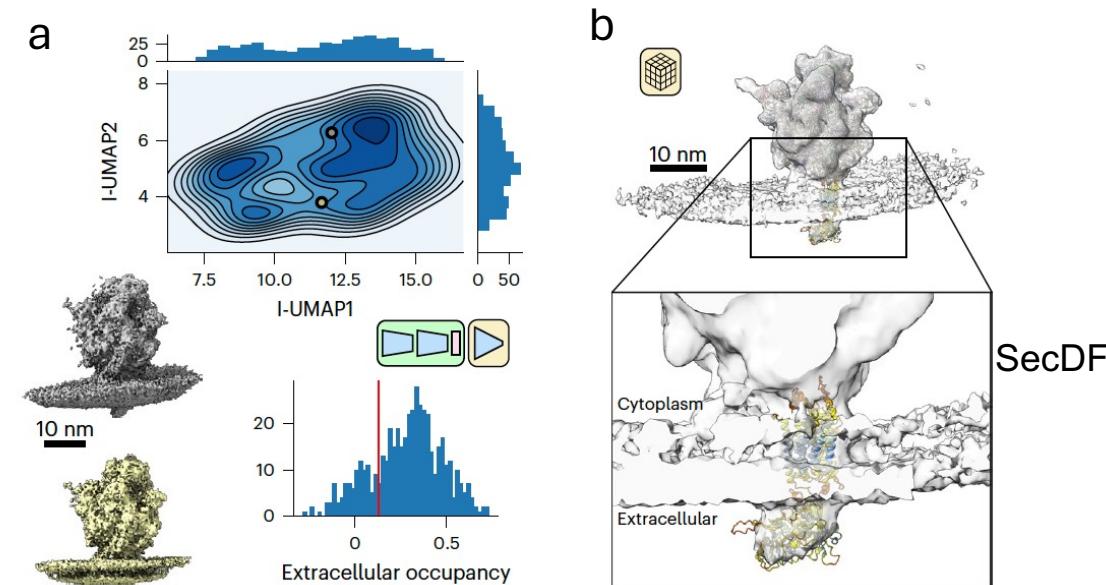
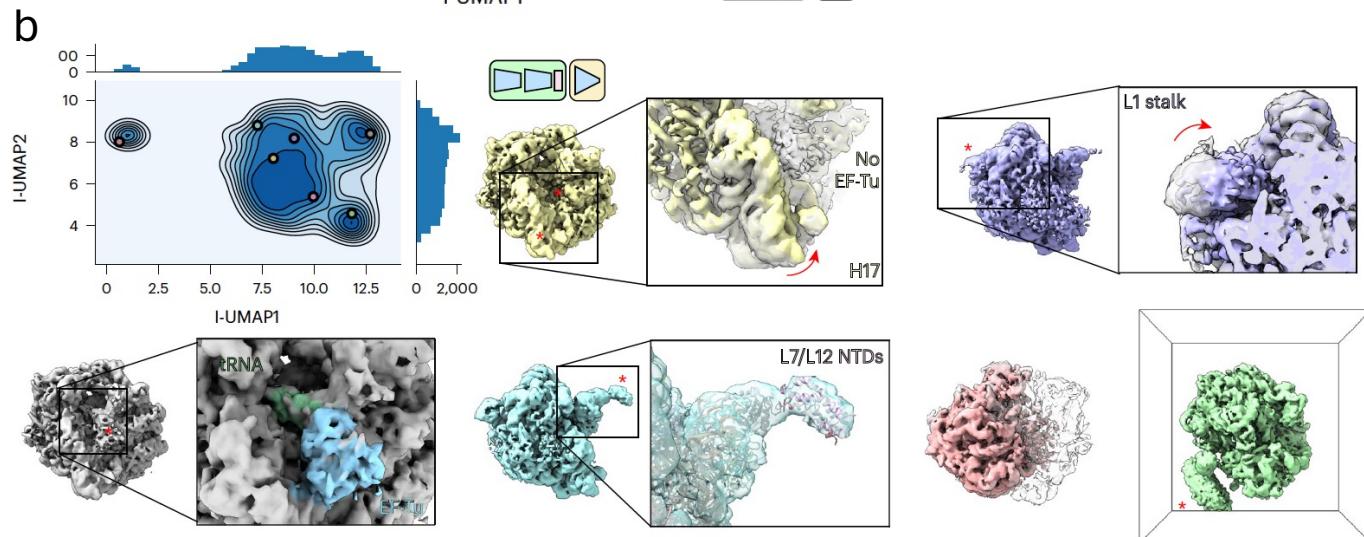
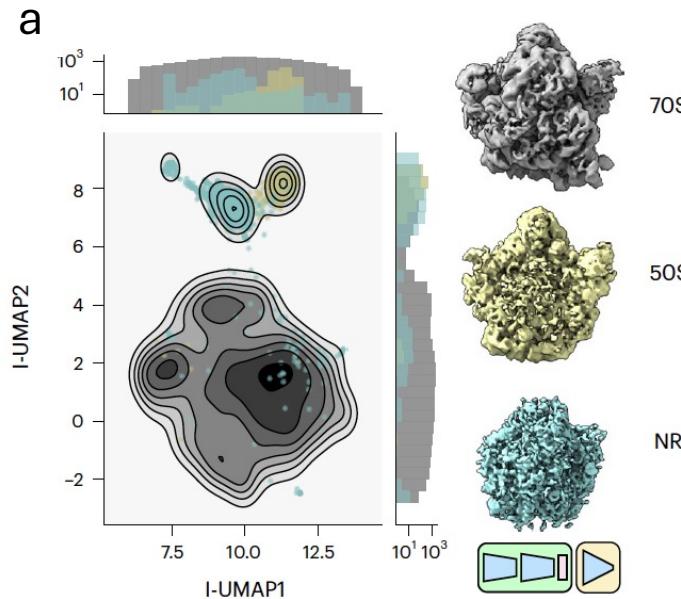
# CryoET workflow



# Structural heterogeneity in ribosomes *in situ* by tomoDRGN

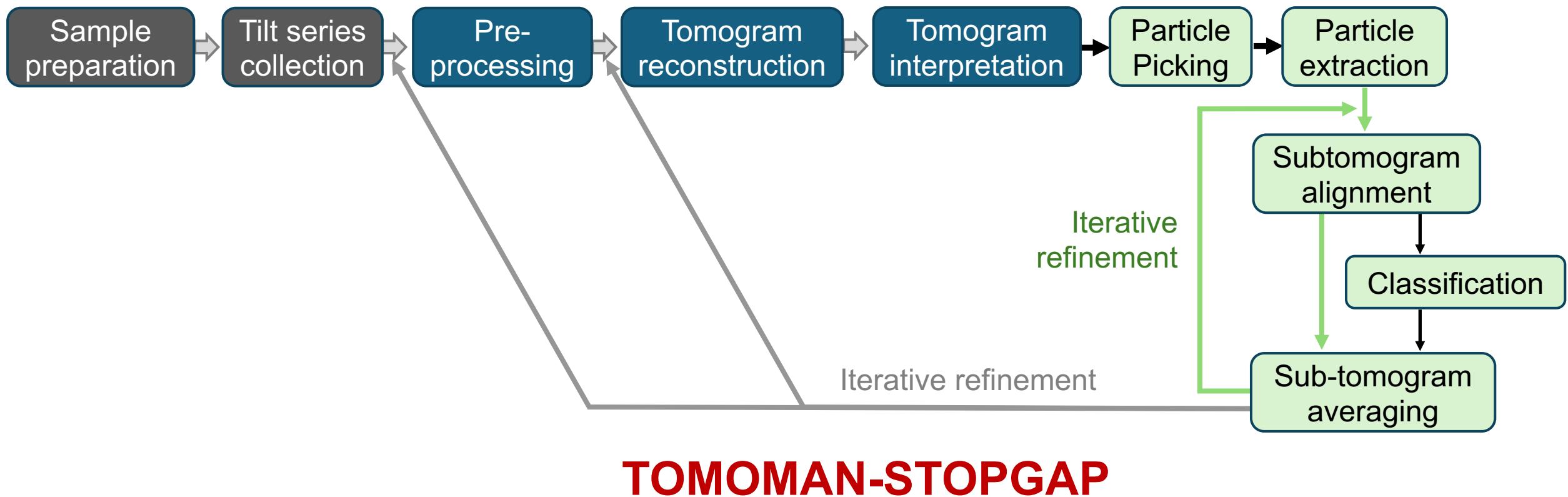


# Structural heterogeneity in ribosomes *in situ* by tomoDRGN



*Mycoplasma pneumoniae*

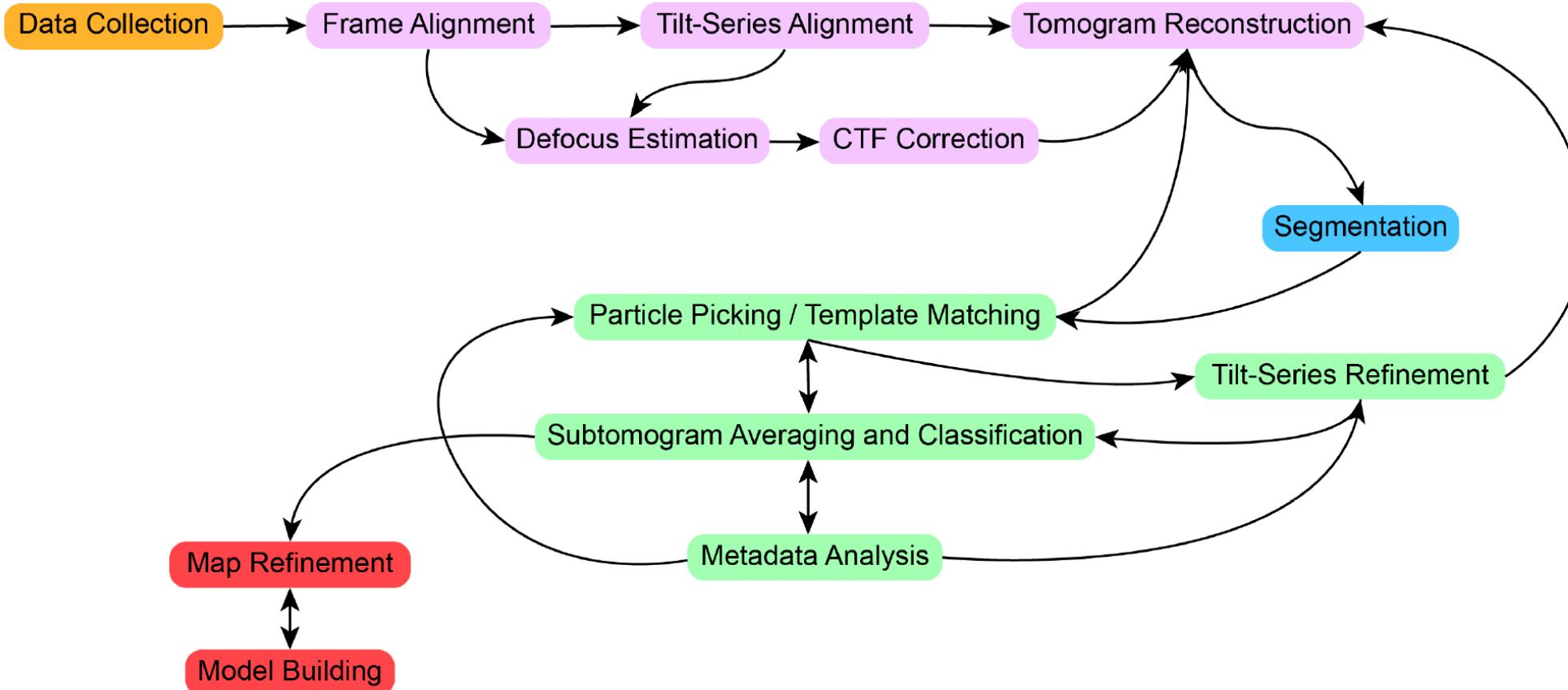
# CryoET workflow



**TOMOMAN-STOPGAP**

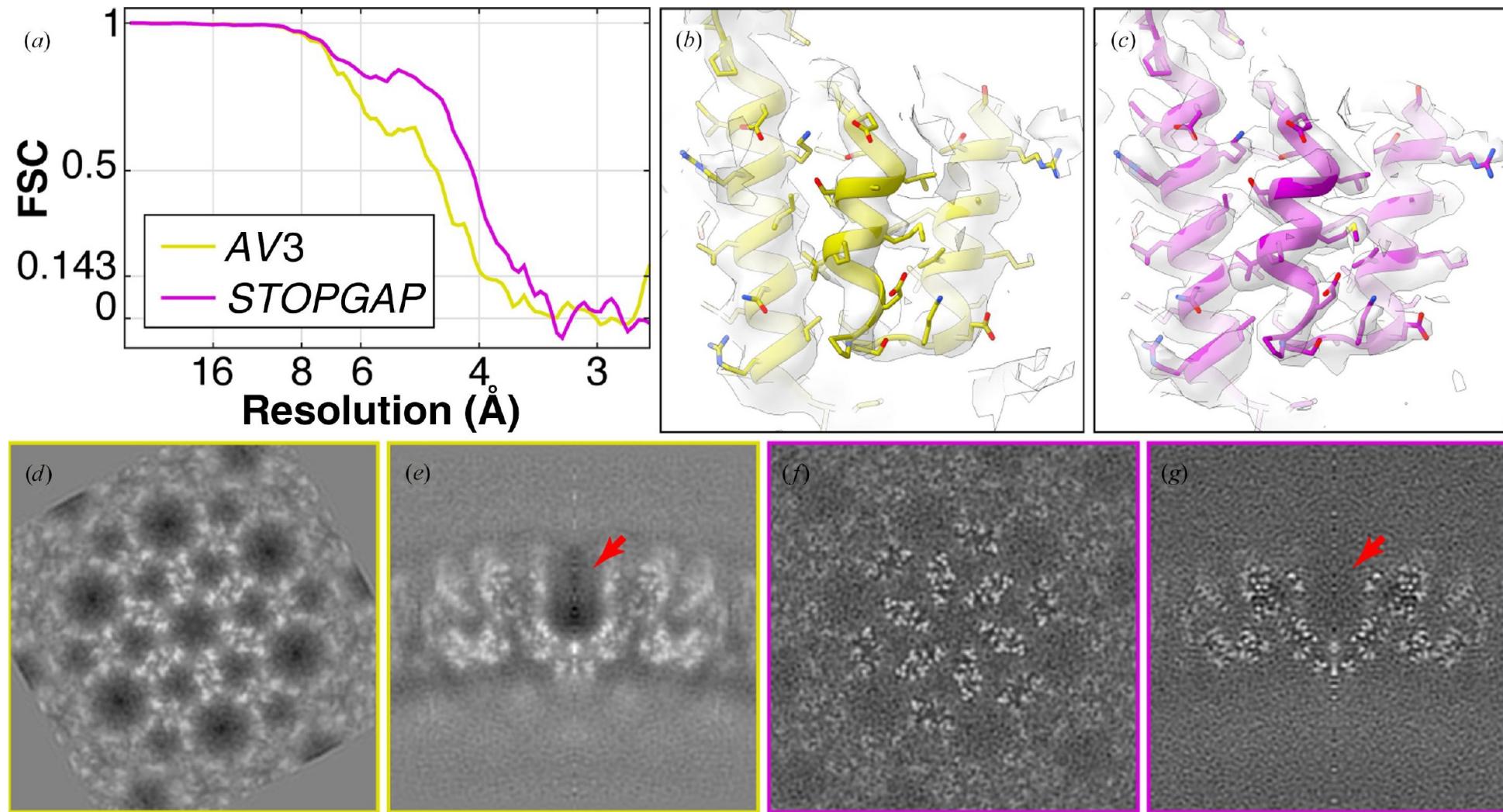
# Cryo-ET and STA Workflow

Slide from William Wan



- Workflow includes data collection, preprocessing, averaging, and postprocessing
- **TOMOMAN** is a package to manage the preprocessing phase
- **STOPGAP** is correlation-based subtomogram averaging (STA) package

# Comparison of immature HIV-1 dMACANC VLPs structures determined using different softwares



# **Summary**

**1. Dragonfly**

**2. Warp2**

**3. TomoDRGN**

**4. TOMOMAN-STOPGAP**

**RELION 5, nextPYP, Scipion...**

# Thank you!