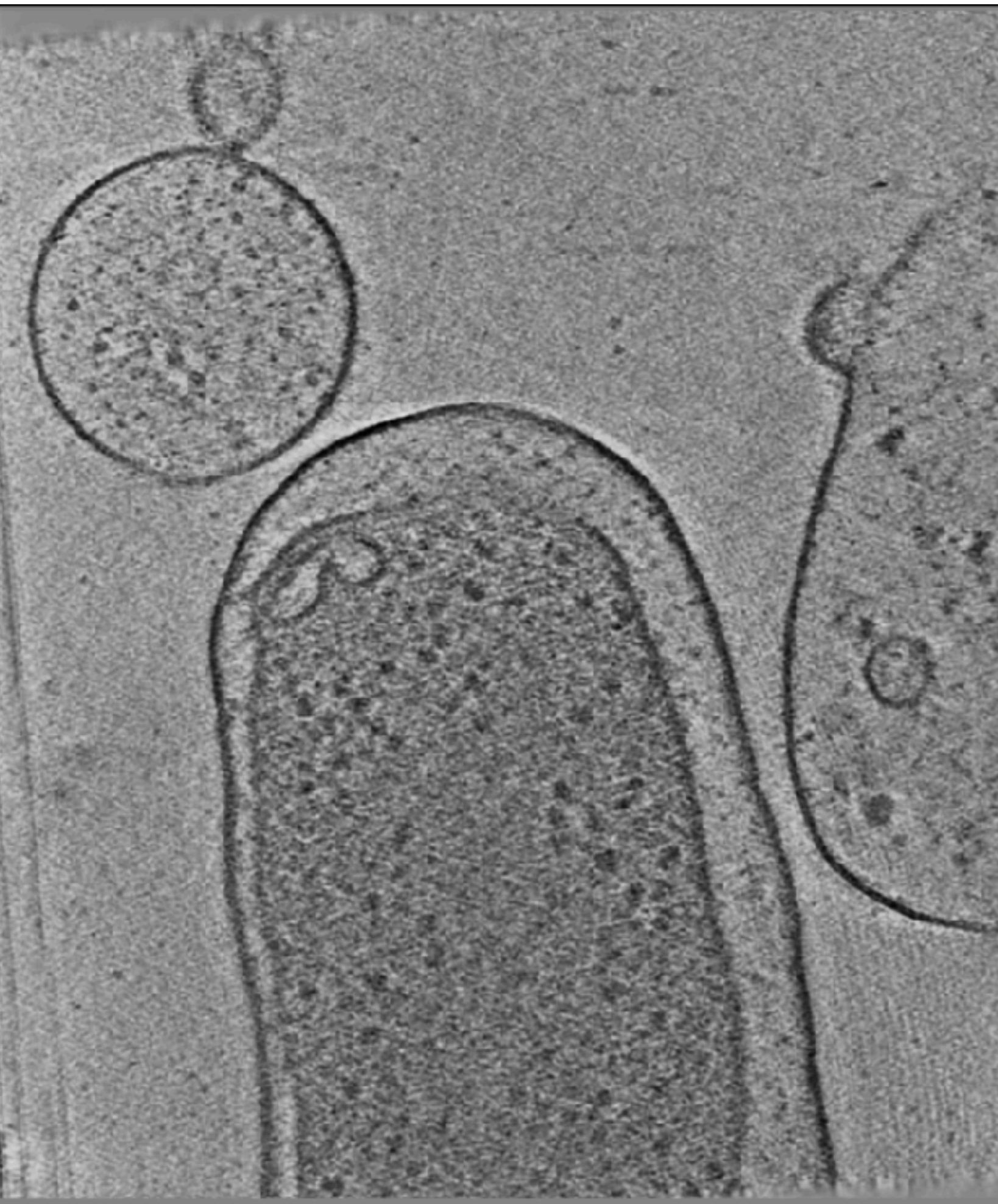
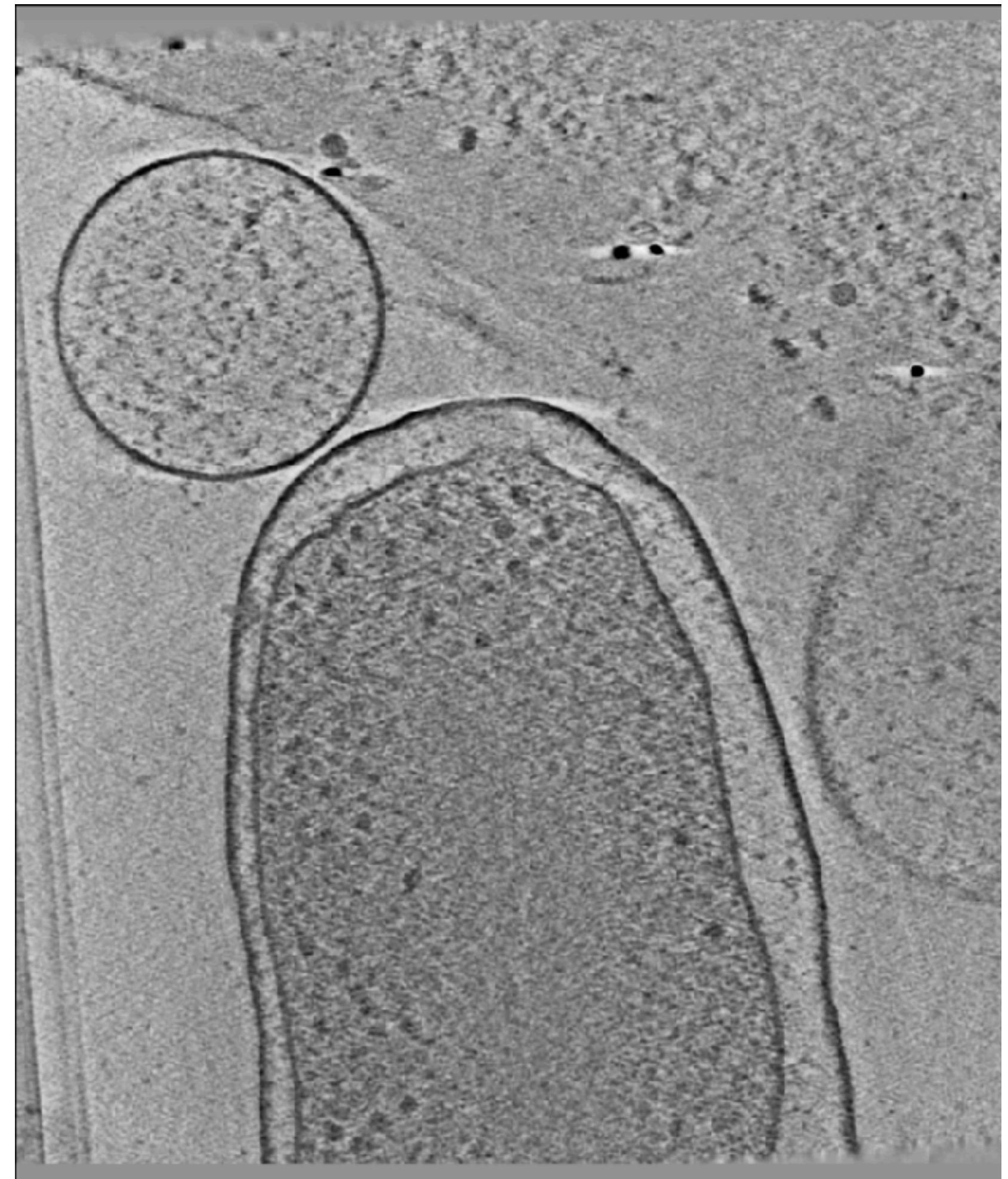


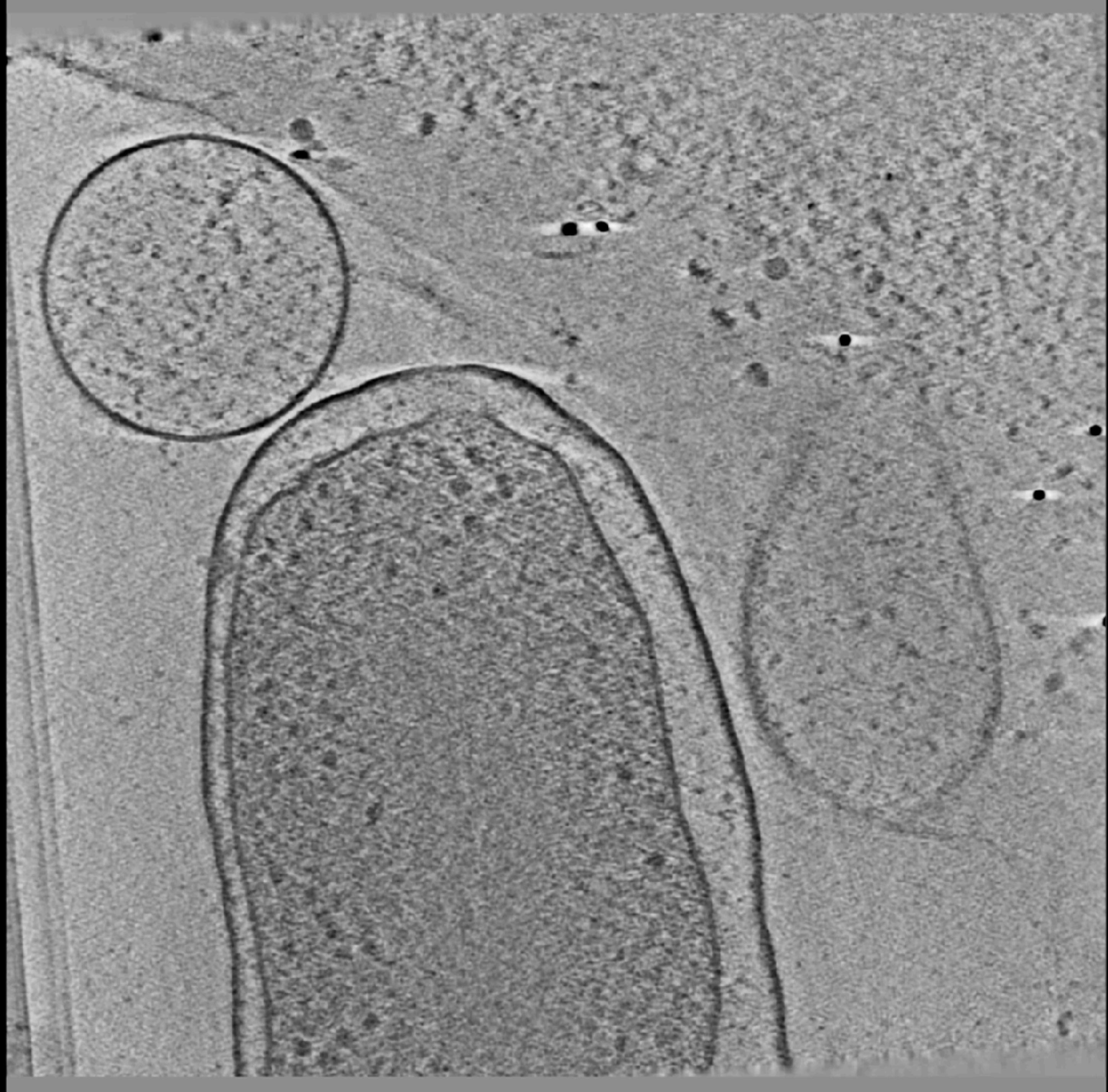
LBMS cryo-EM lecture: Tomographic Data Segmentation

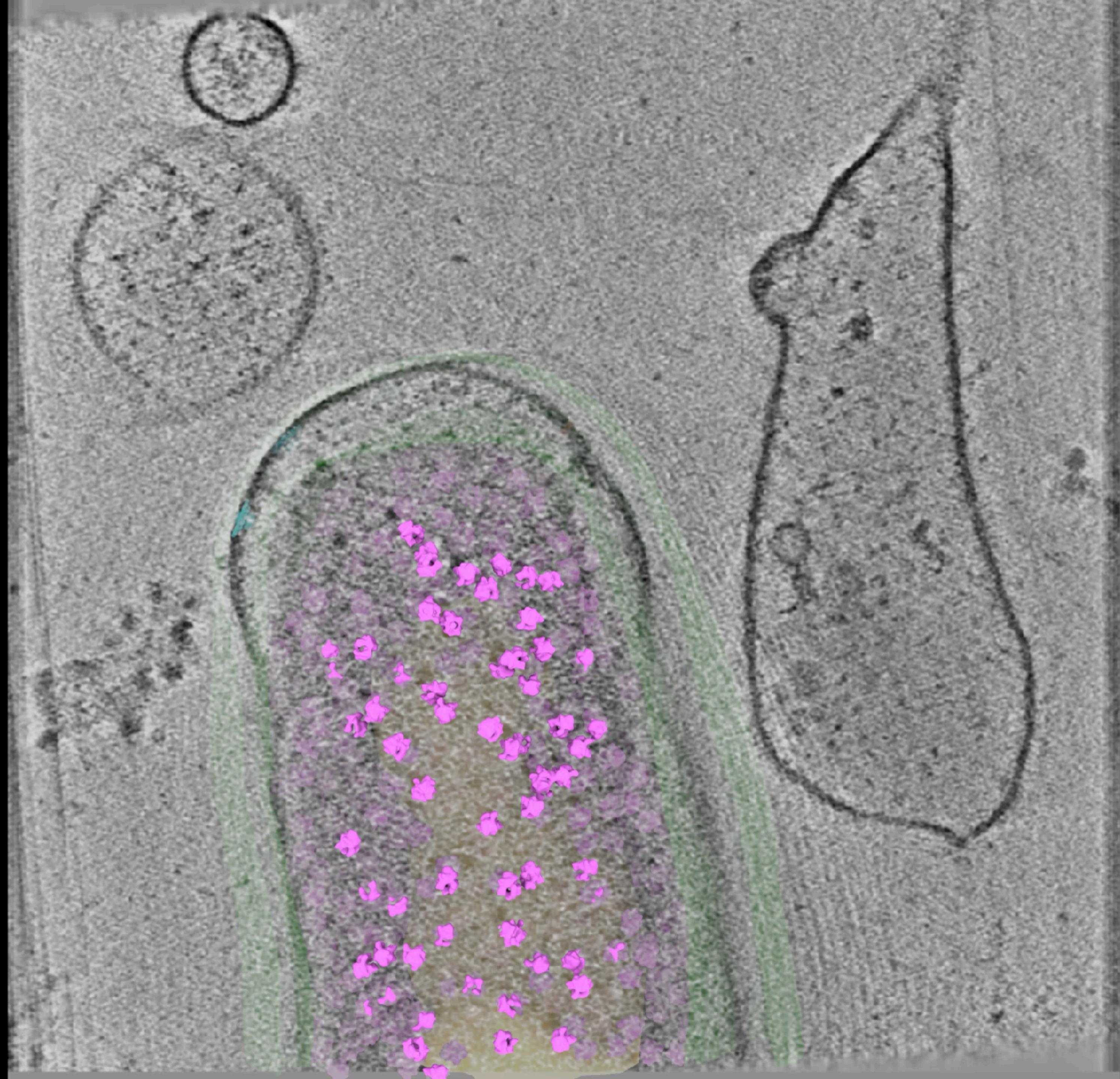
Raphael Park

Yale University, Liu Lab

Tomogram is 3-dimensional





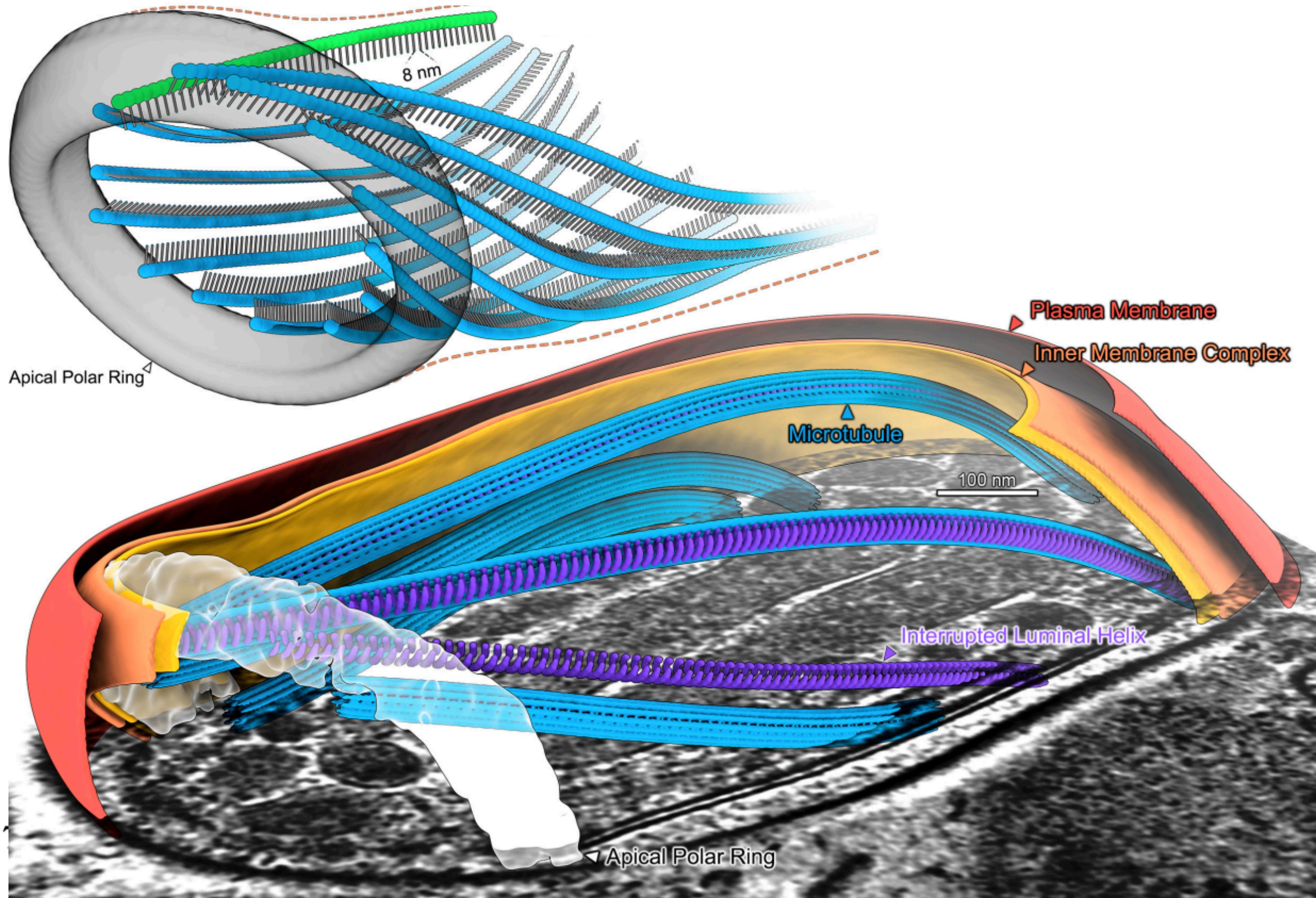




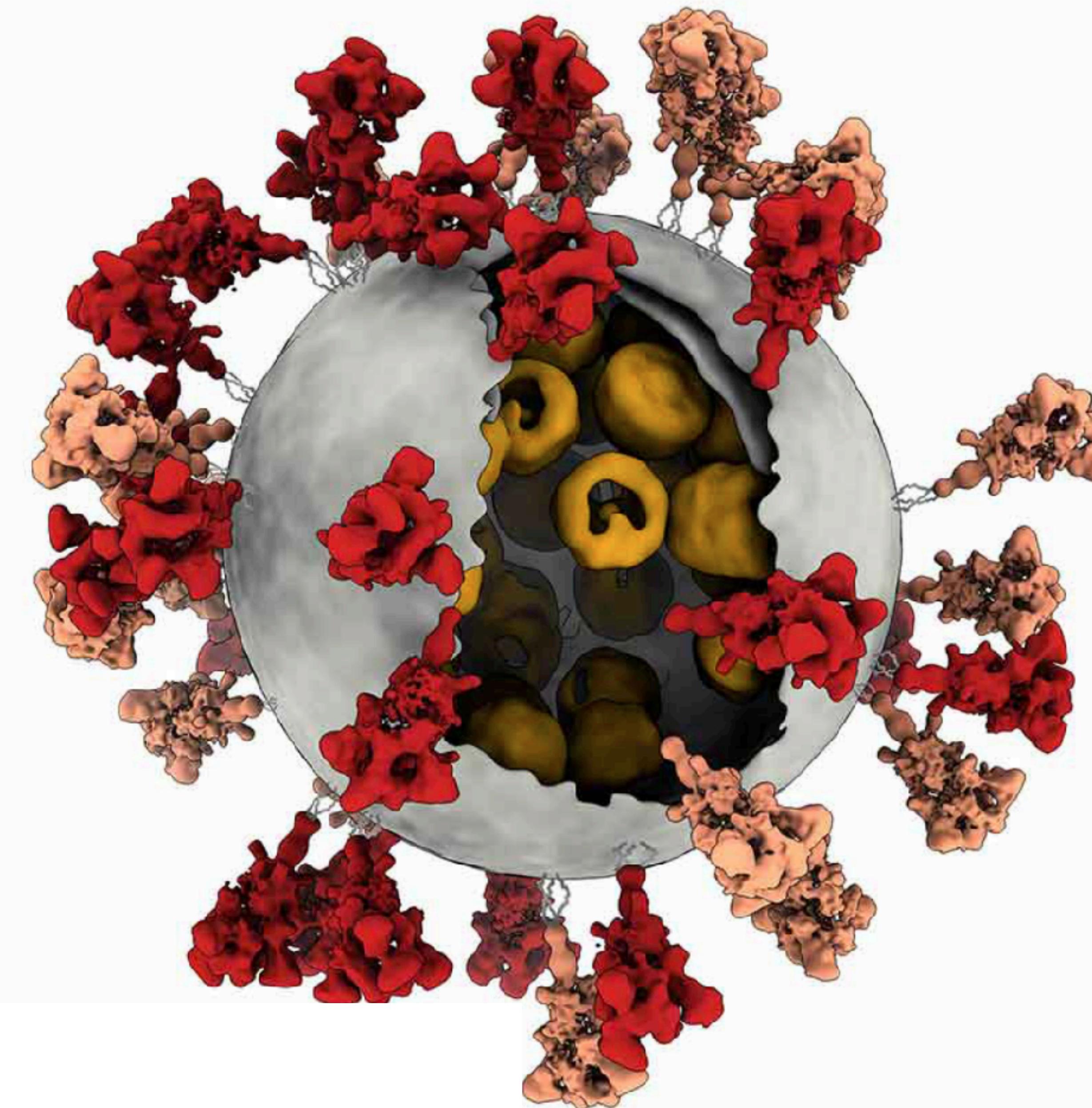
- Bacterial membranes
- Ribosomes
- Nucleoid
- Secretion machines

Cryo-ET segmentation examples

Malaria parasite

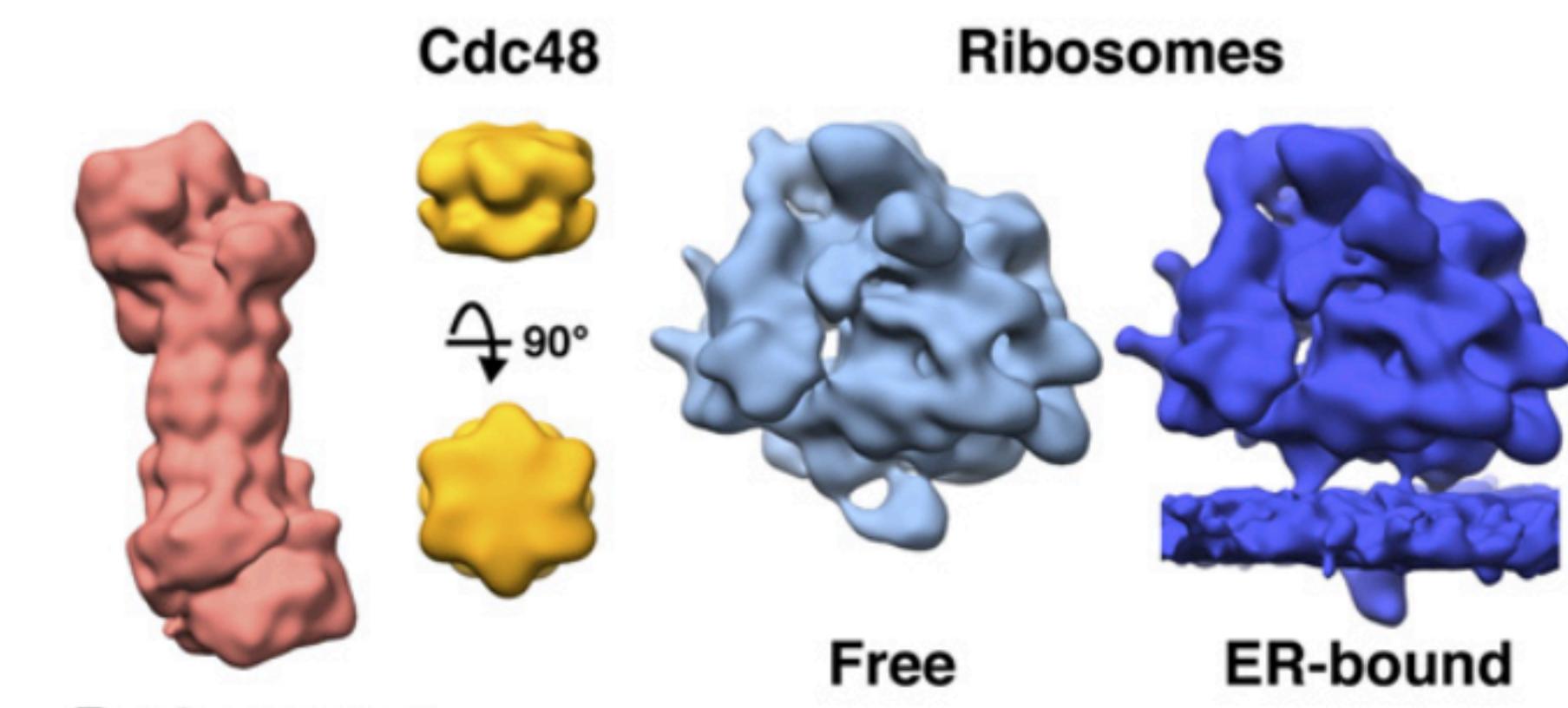
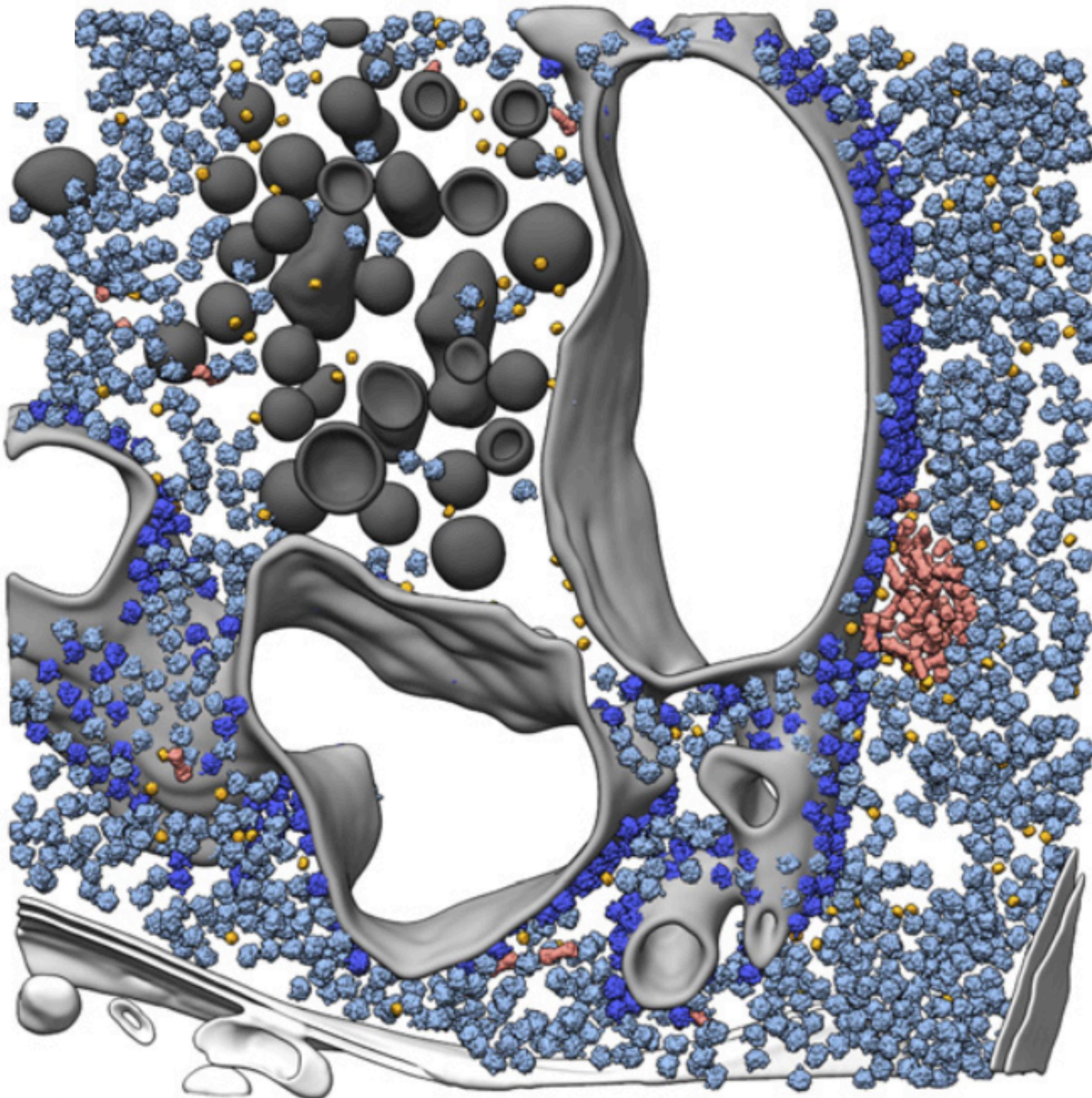


SARS-CoV-2 Virus



Yao et al., *Cell* (2020)

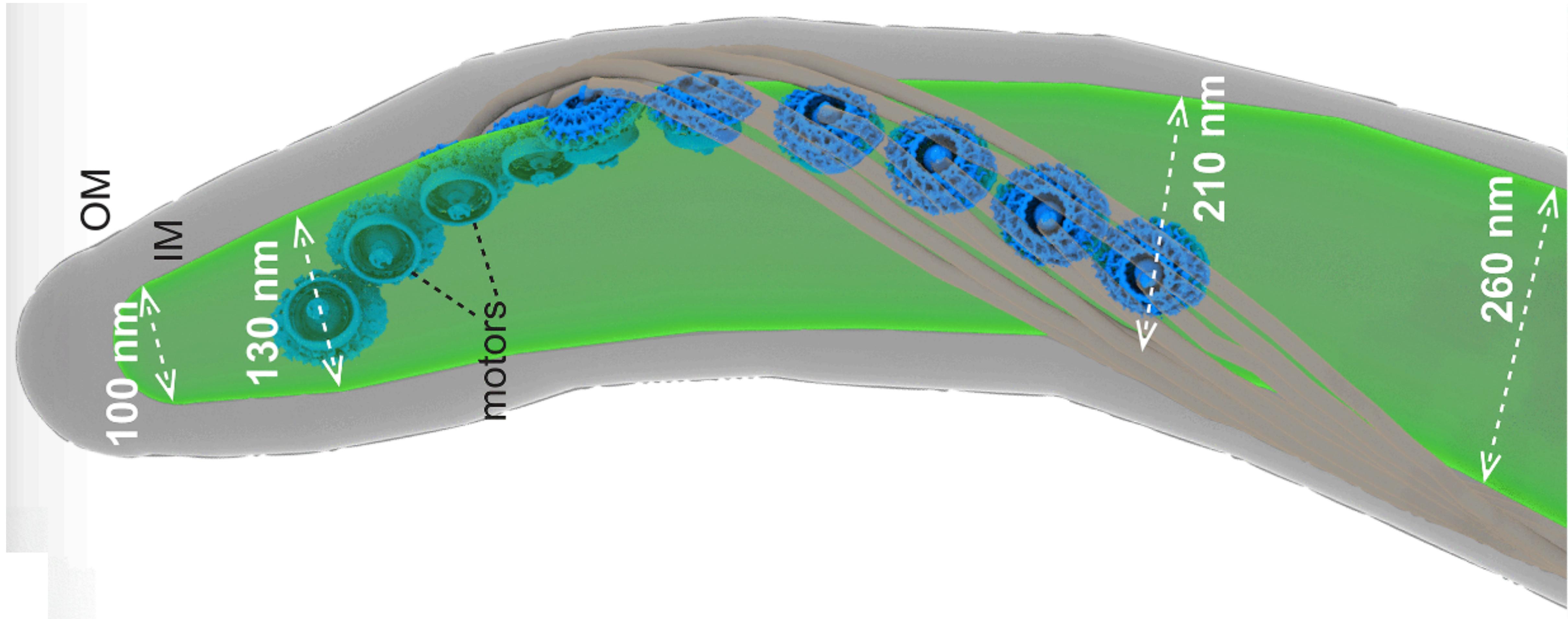
Degradation microcompartments at the ER membrane



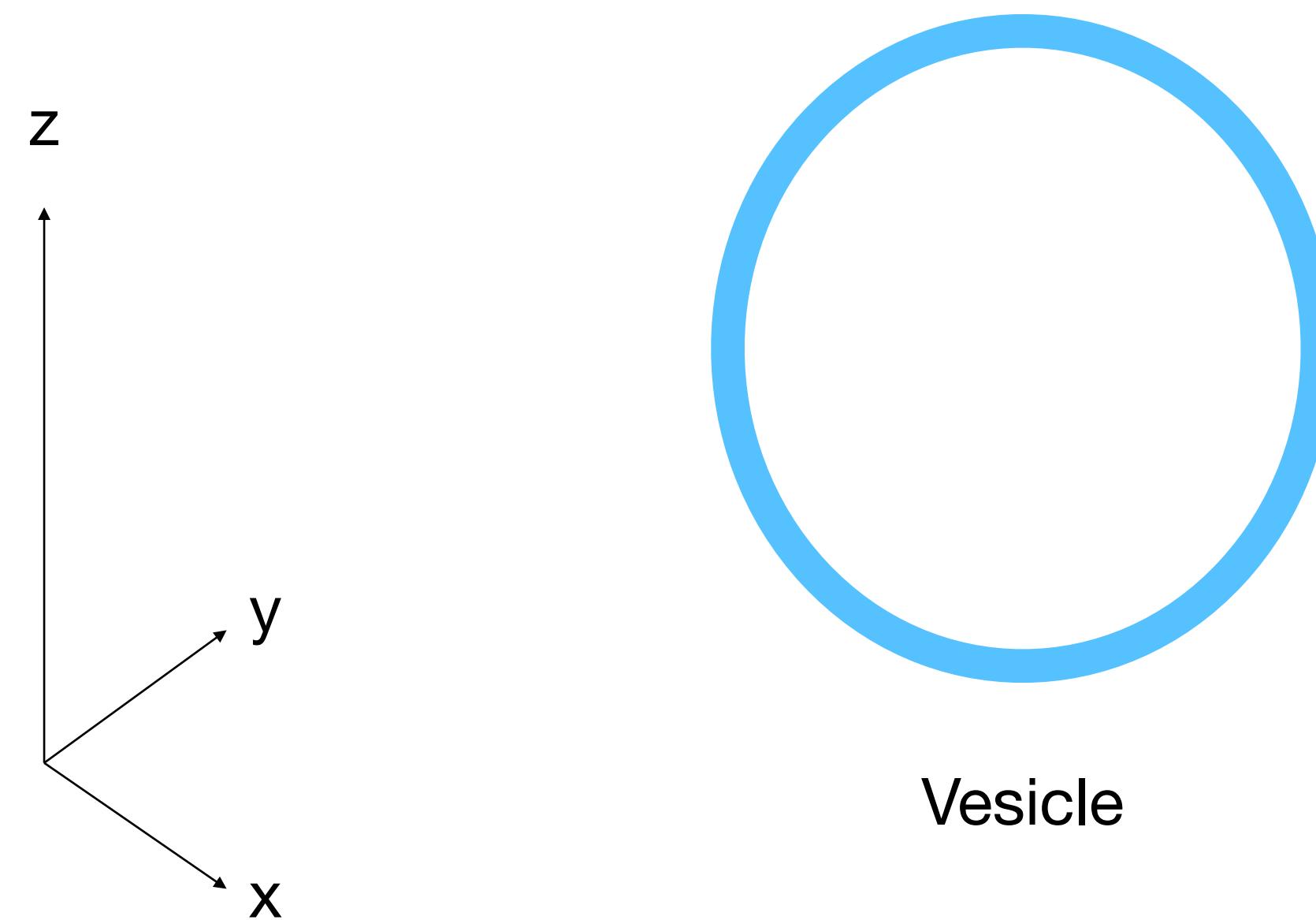
Segmentation tools

Manual segmentation (IMOD)

Bacteria

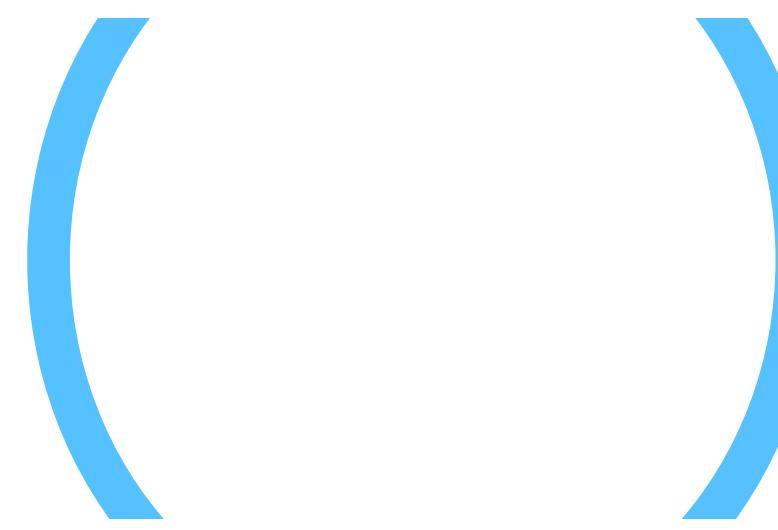


Manual segmentation (IMOD)



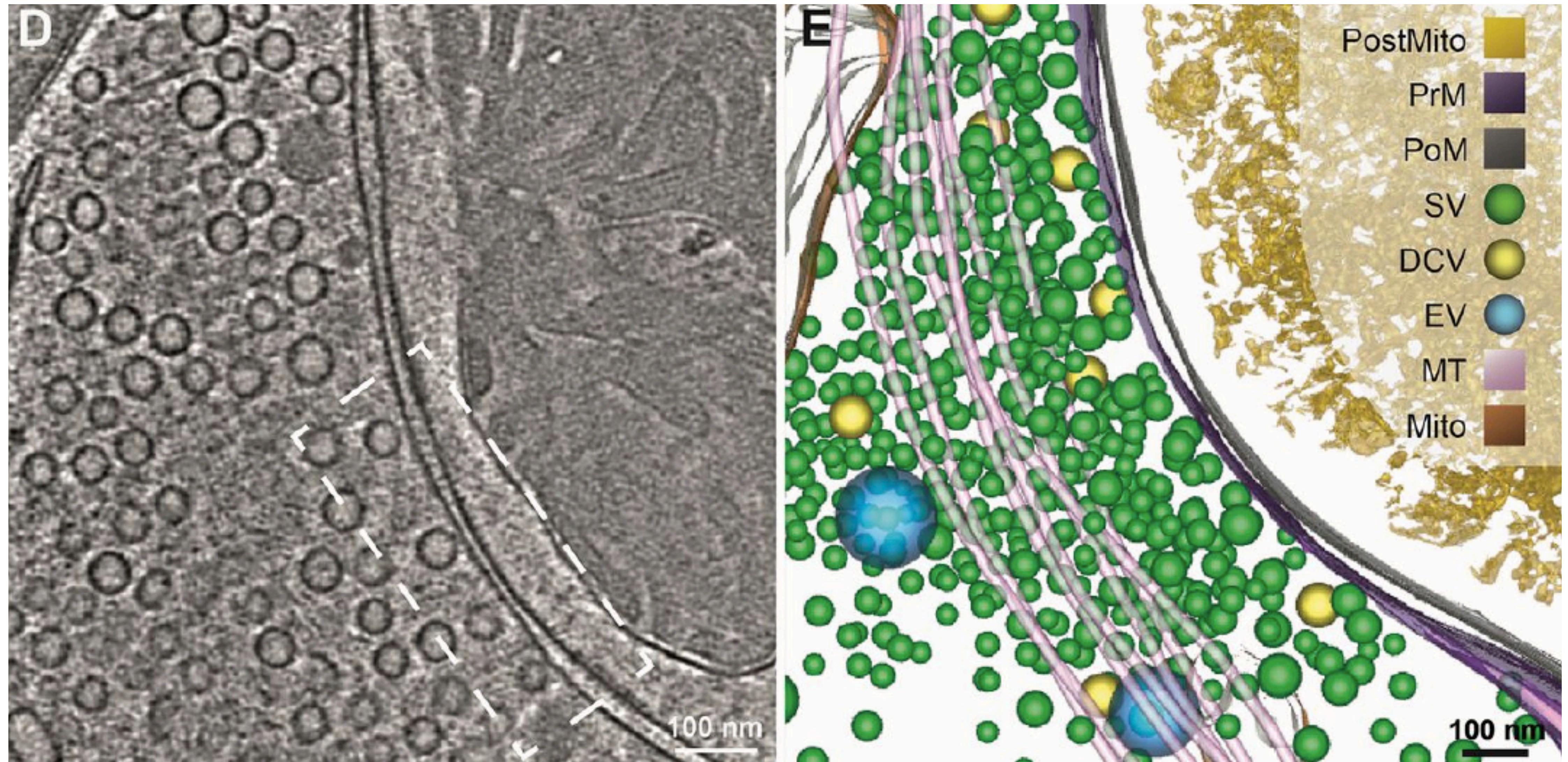
Vesicle

How vesicles appear in tomogram (Missing-wedge)



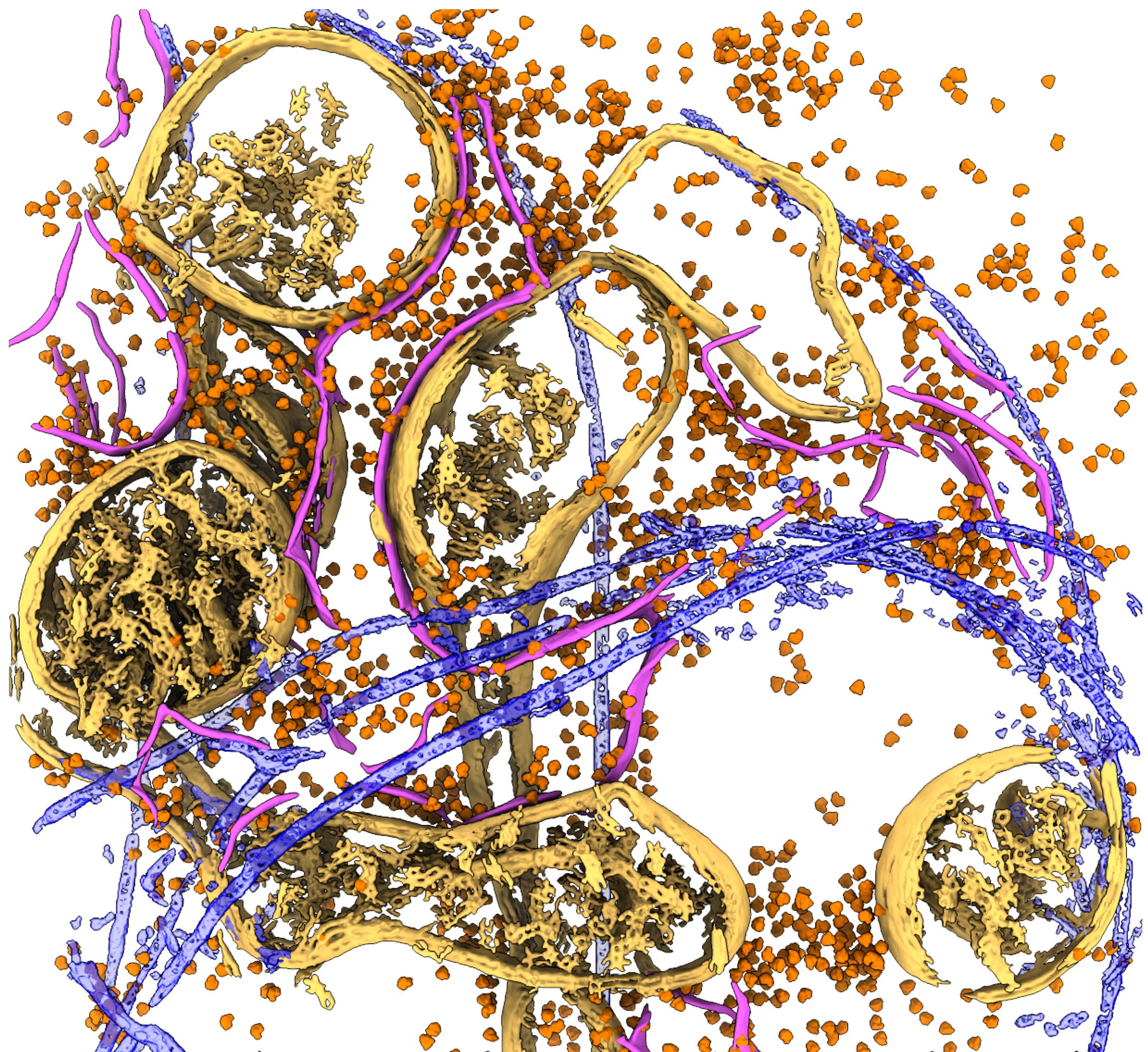
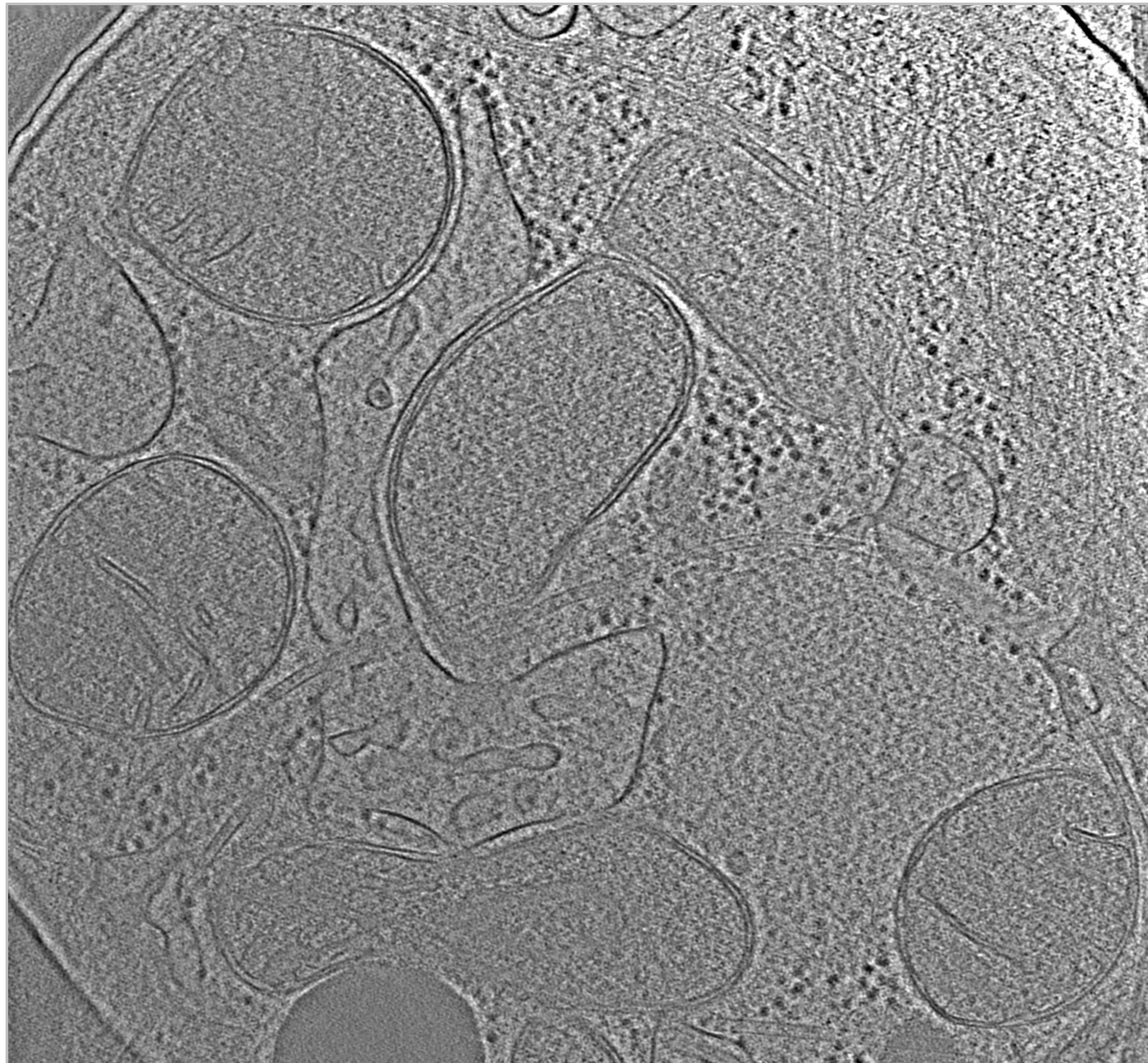
Manual segmentation (IMOD)

Synaptic vesicles



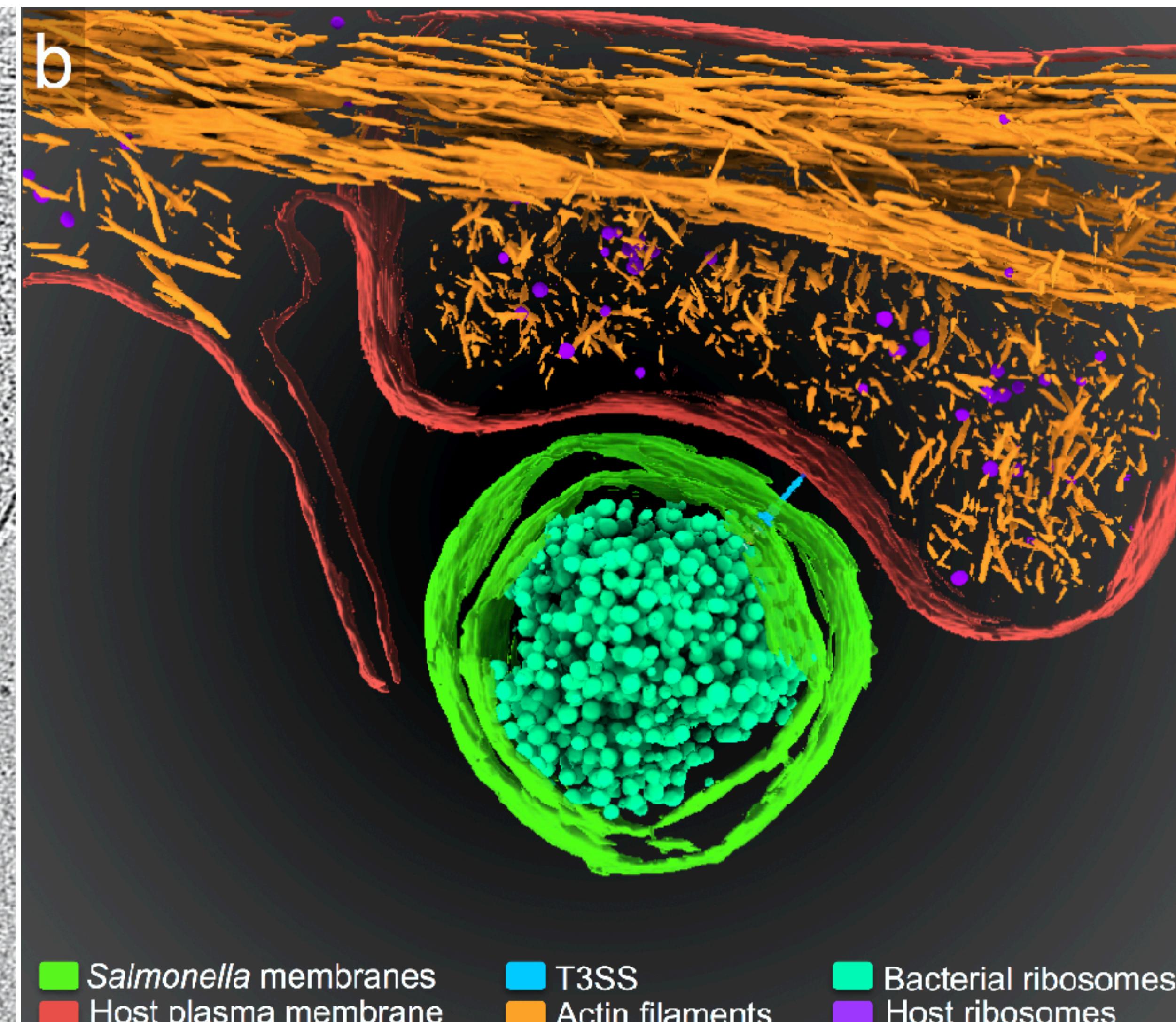
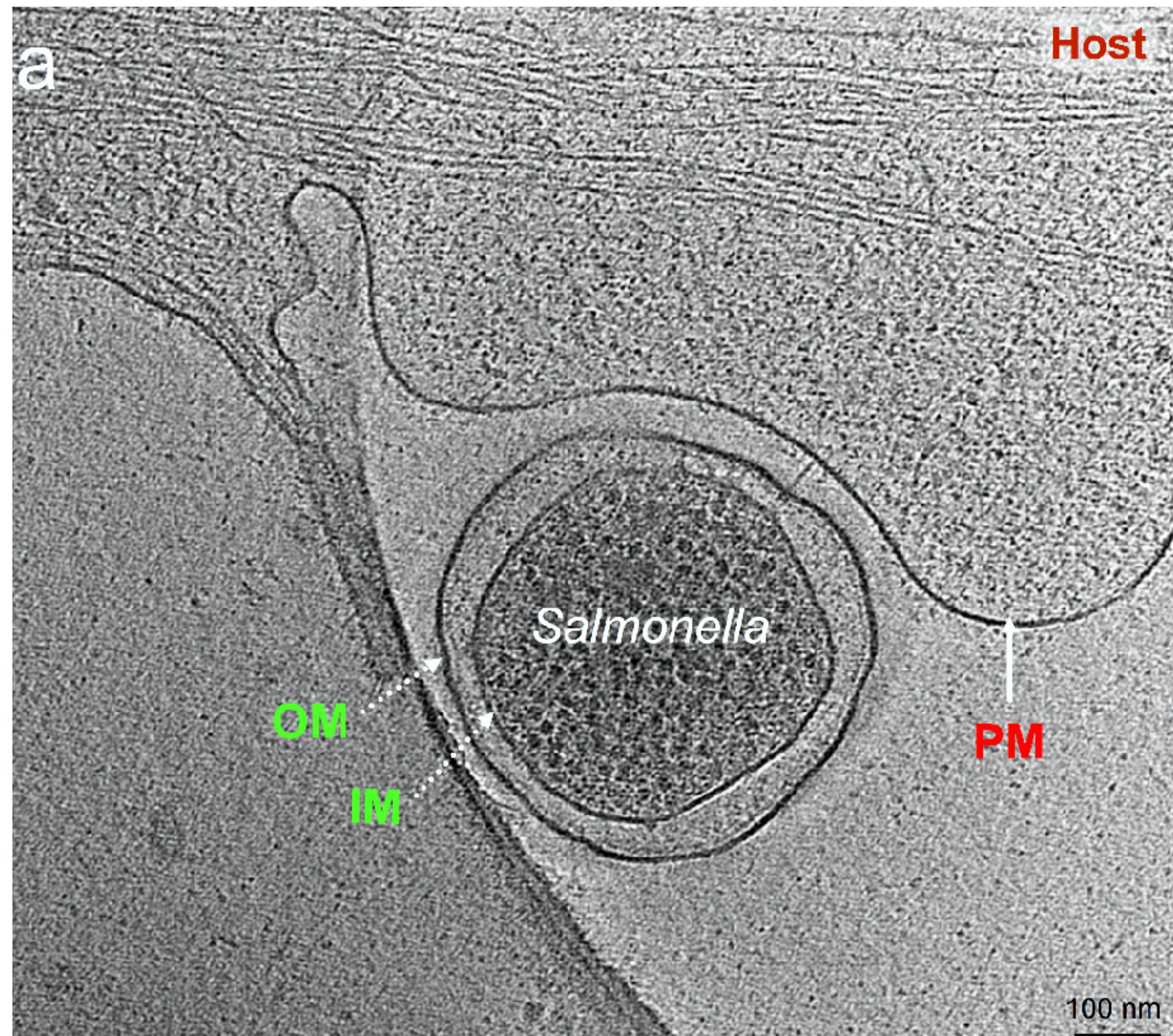
Semi-automatic segmentation (EMAN2)

PC12 cell



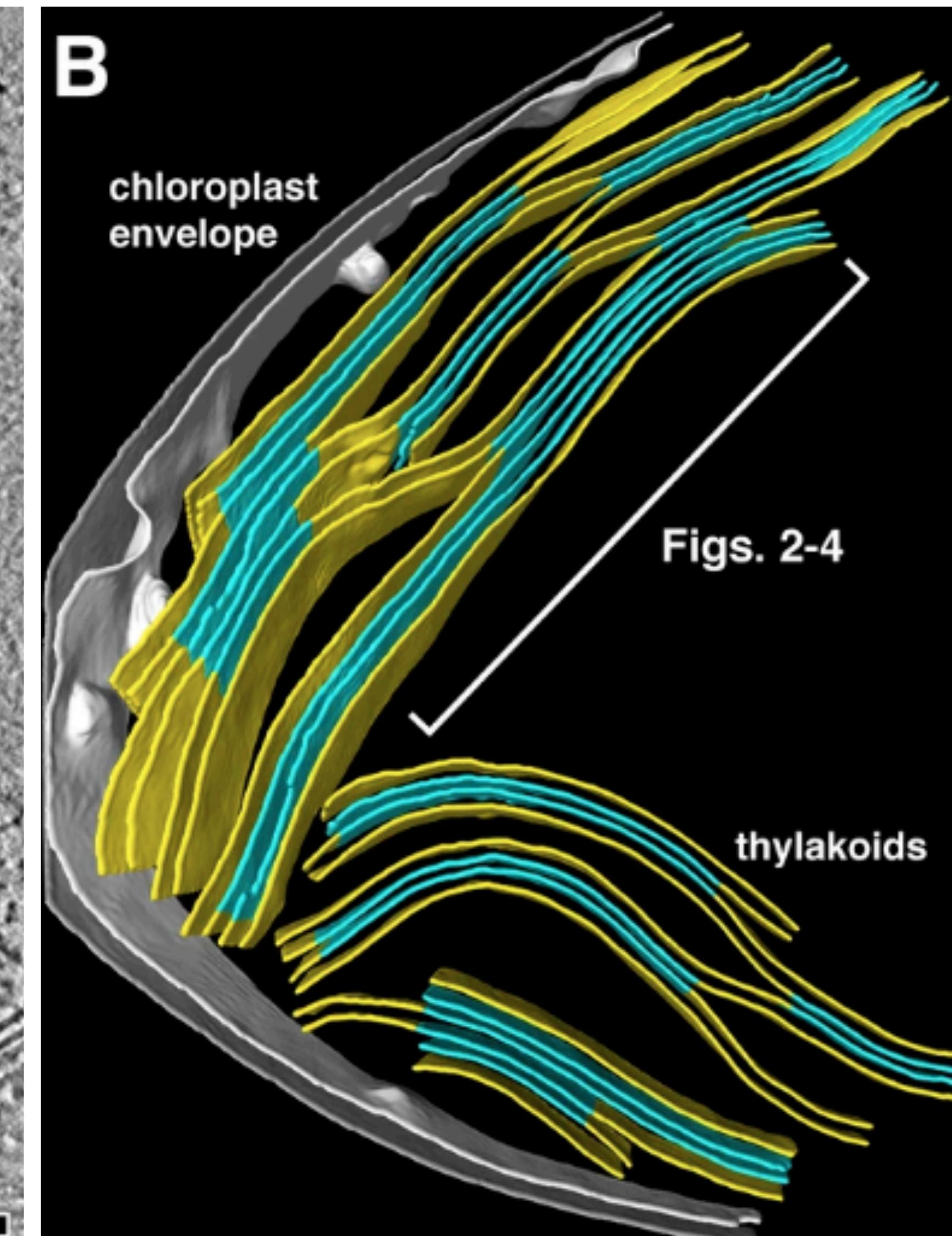
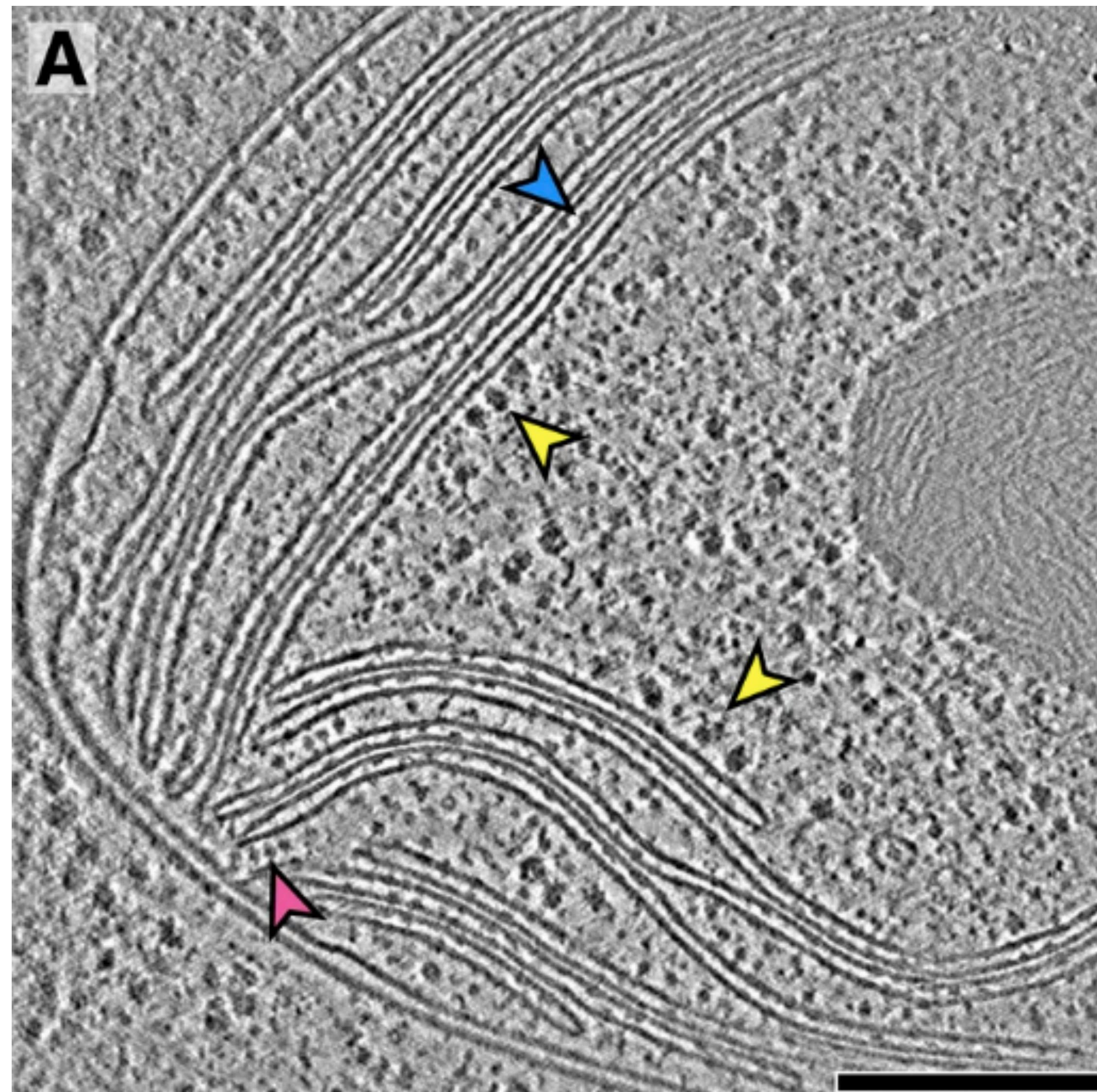
Semi-automatic segmentation (EMAN2)

Salmonella - Host



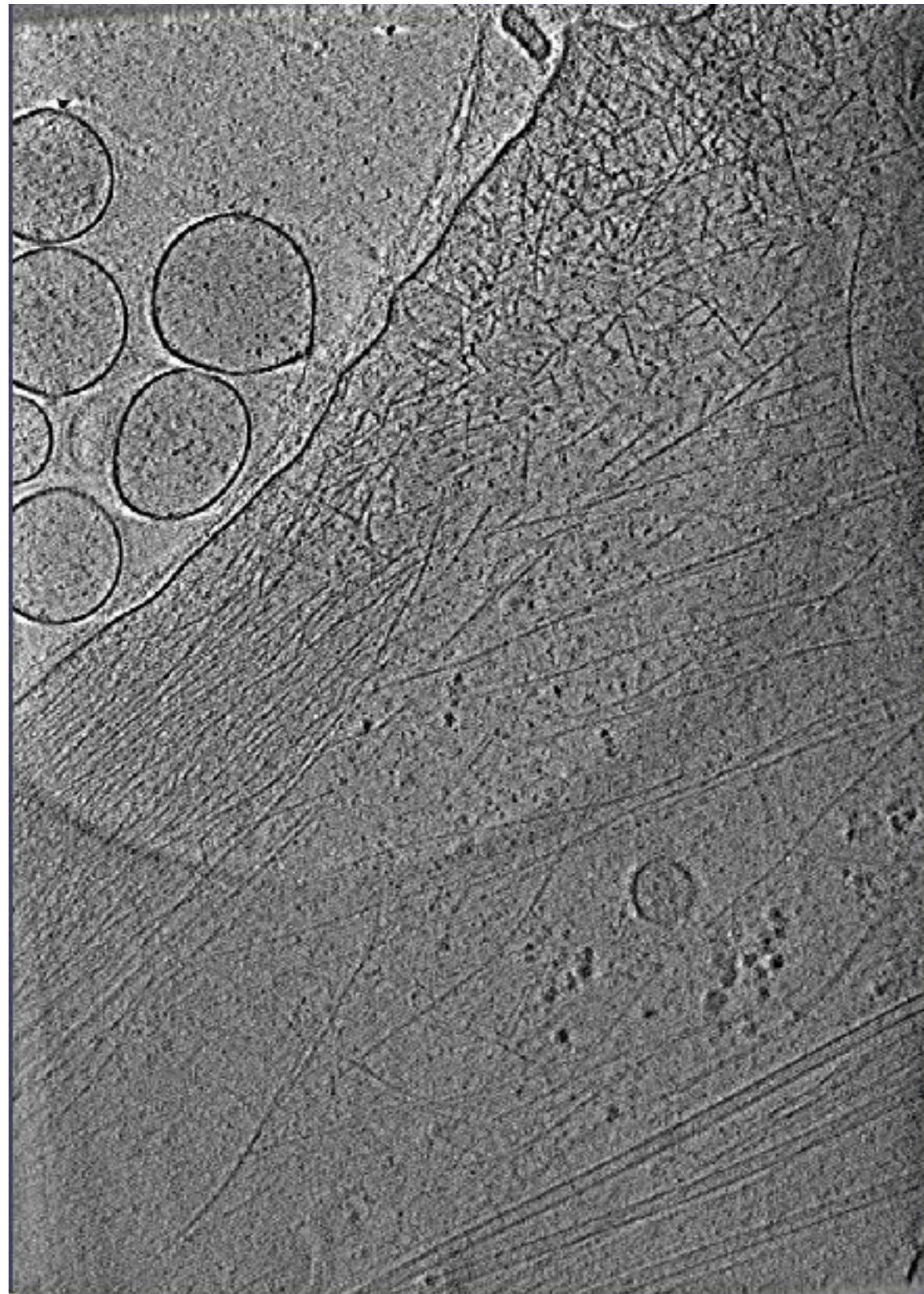
Automatic segmentation (TomoSegMemTV)

Thylakoid membranes



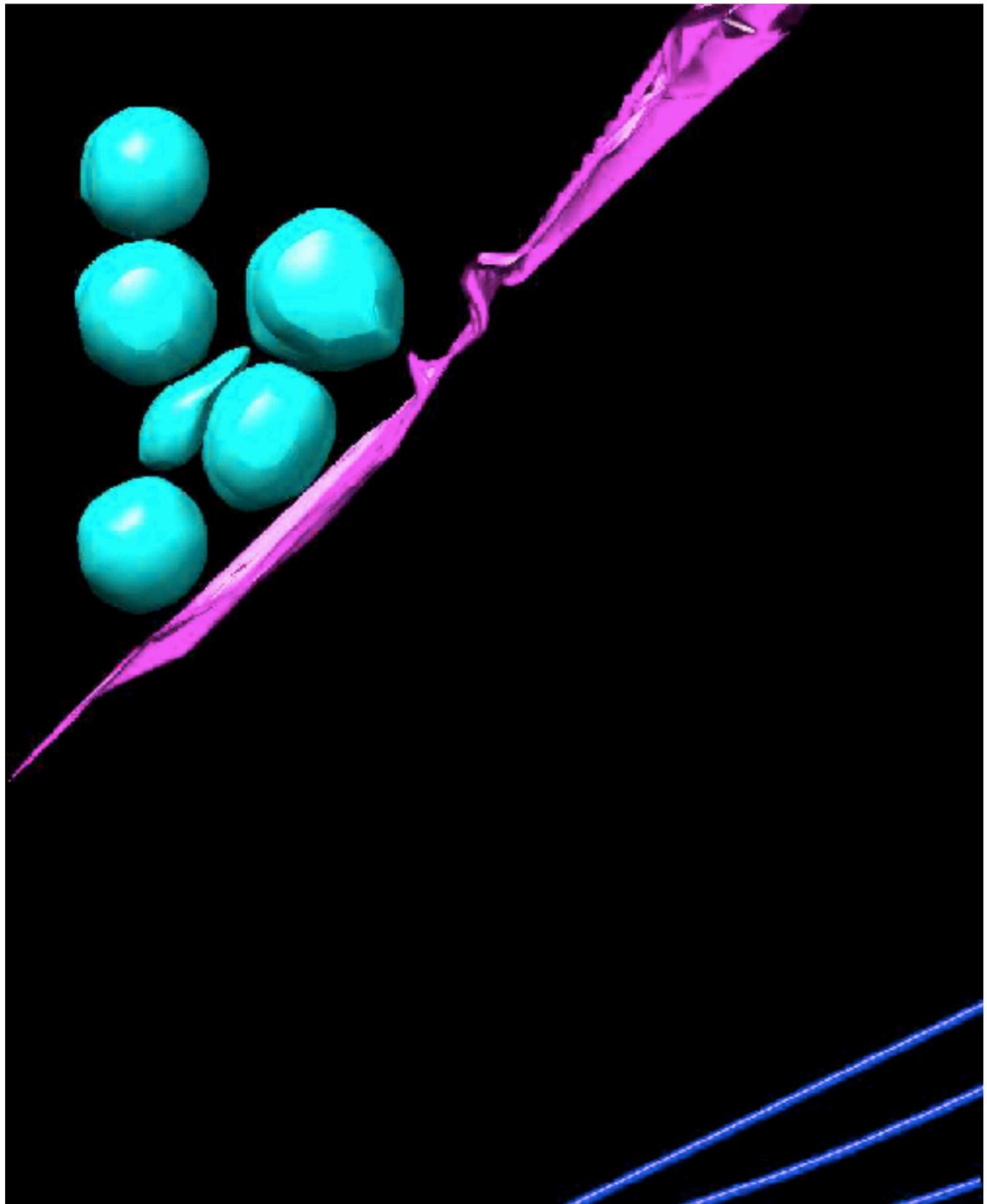
Comparison

Tomogram



IMOD

(manual segmentation)



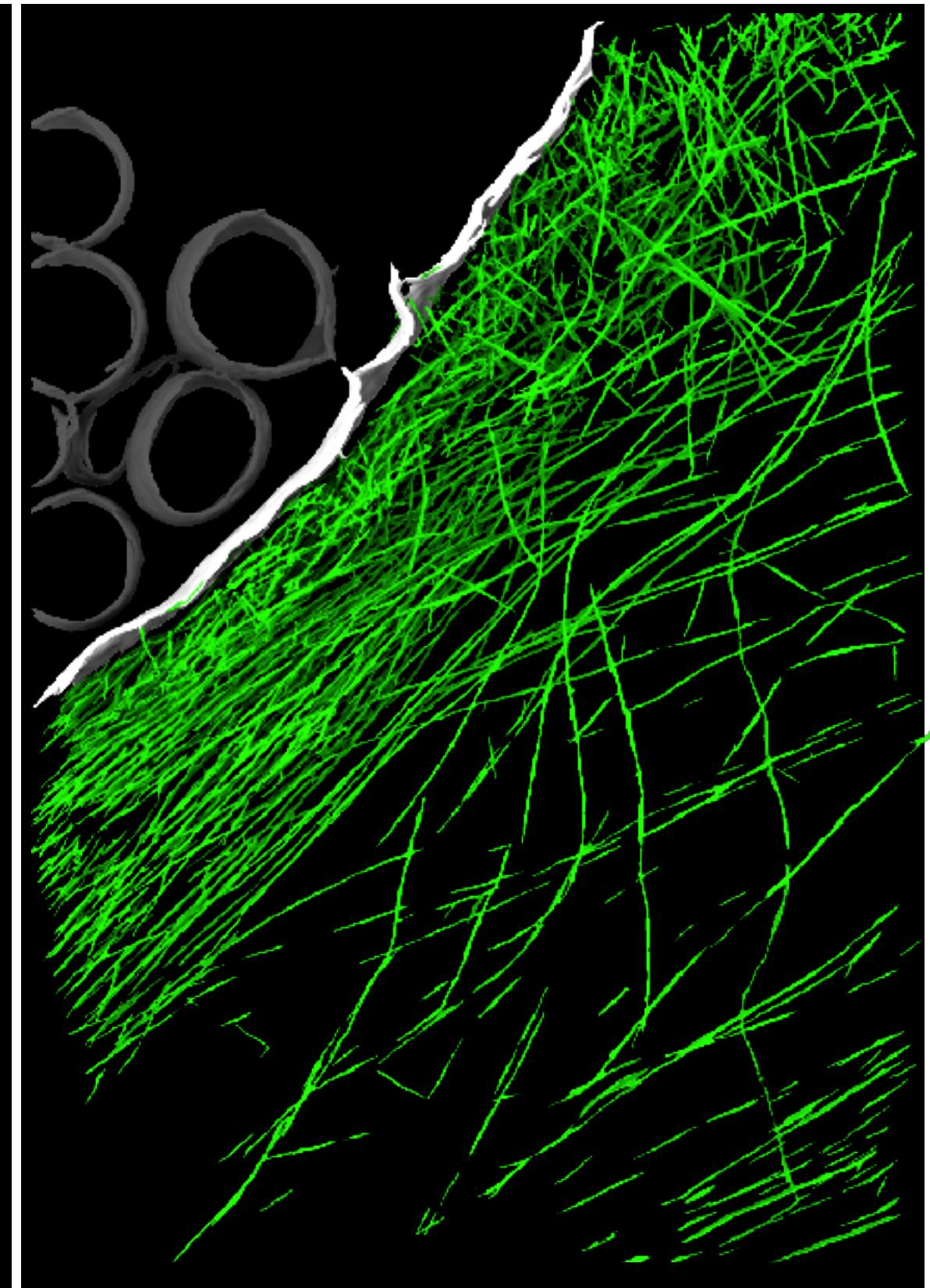
TomoSegMemTV

(automatic)



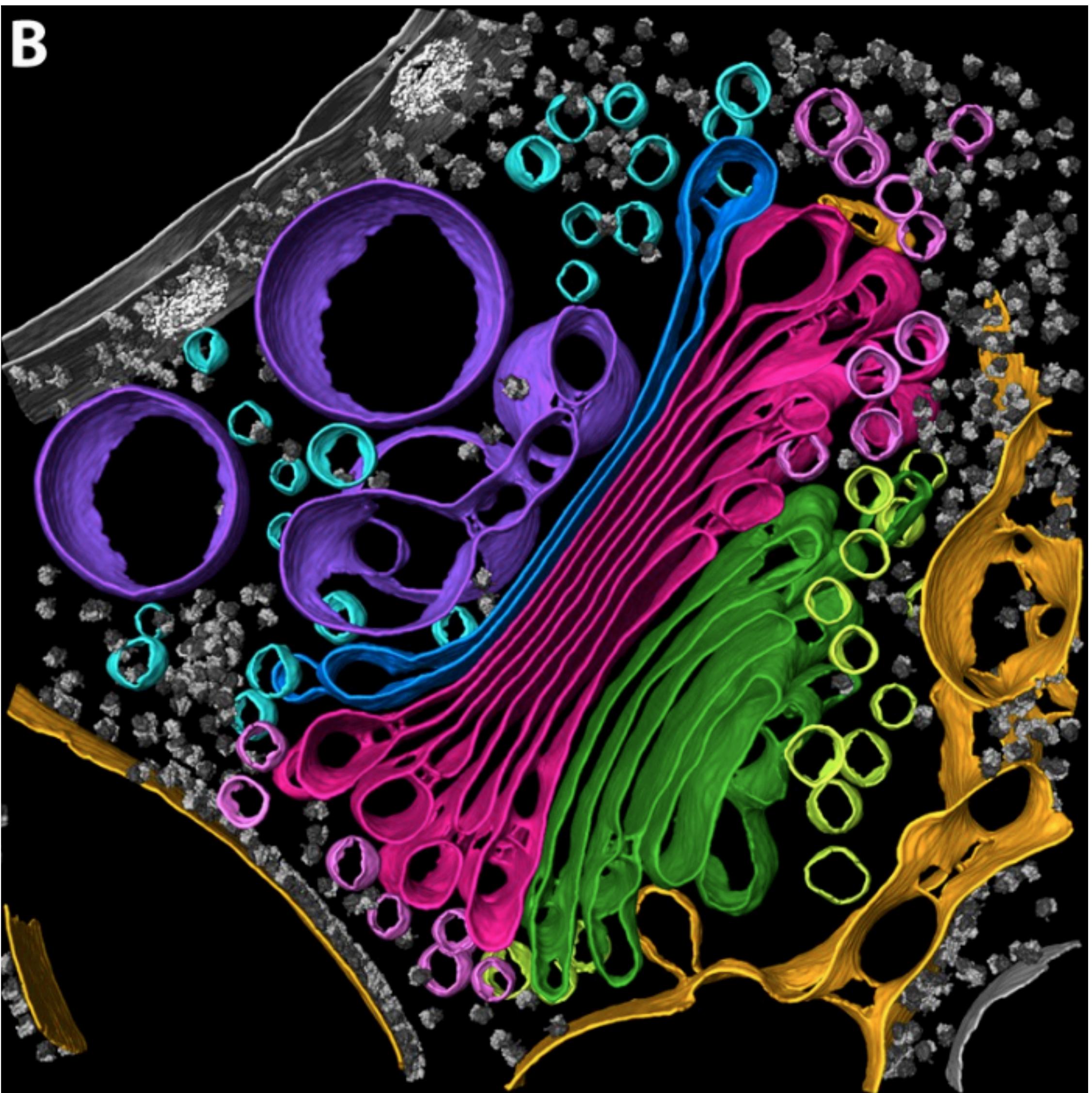
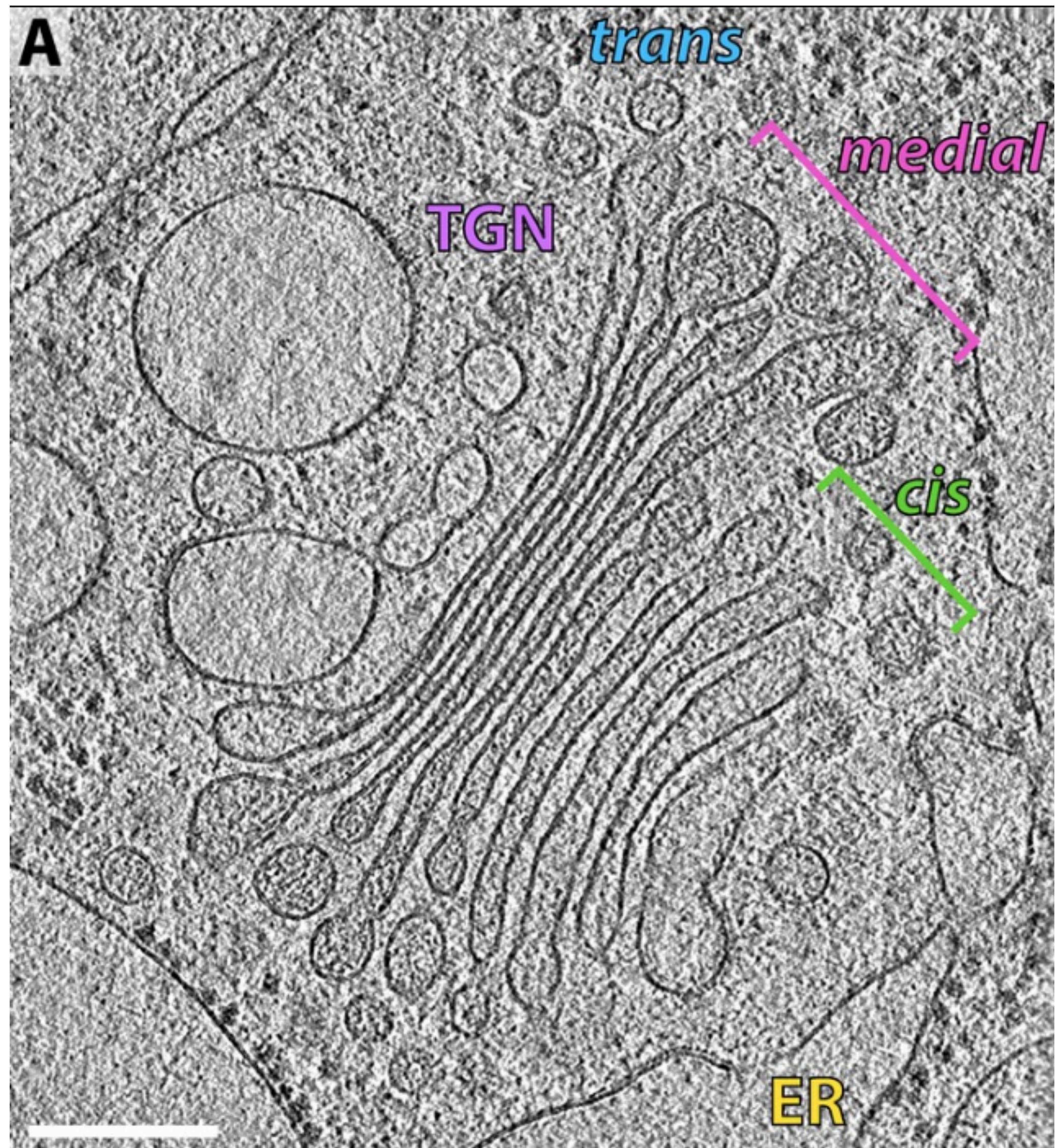
EMAN2

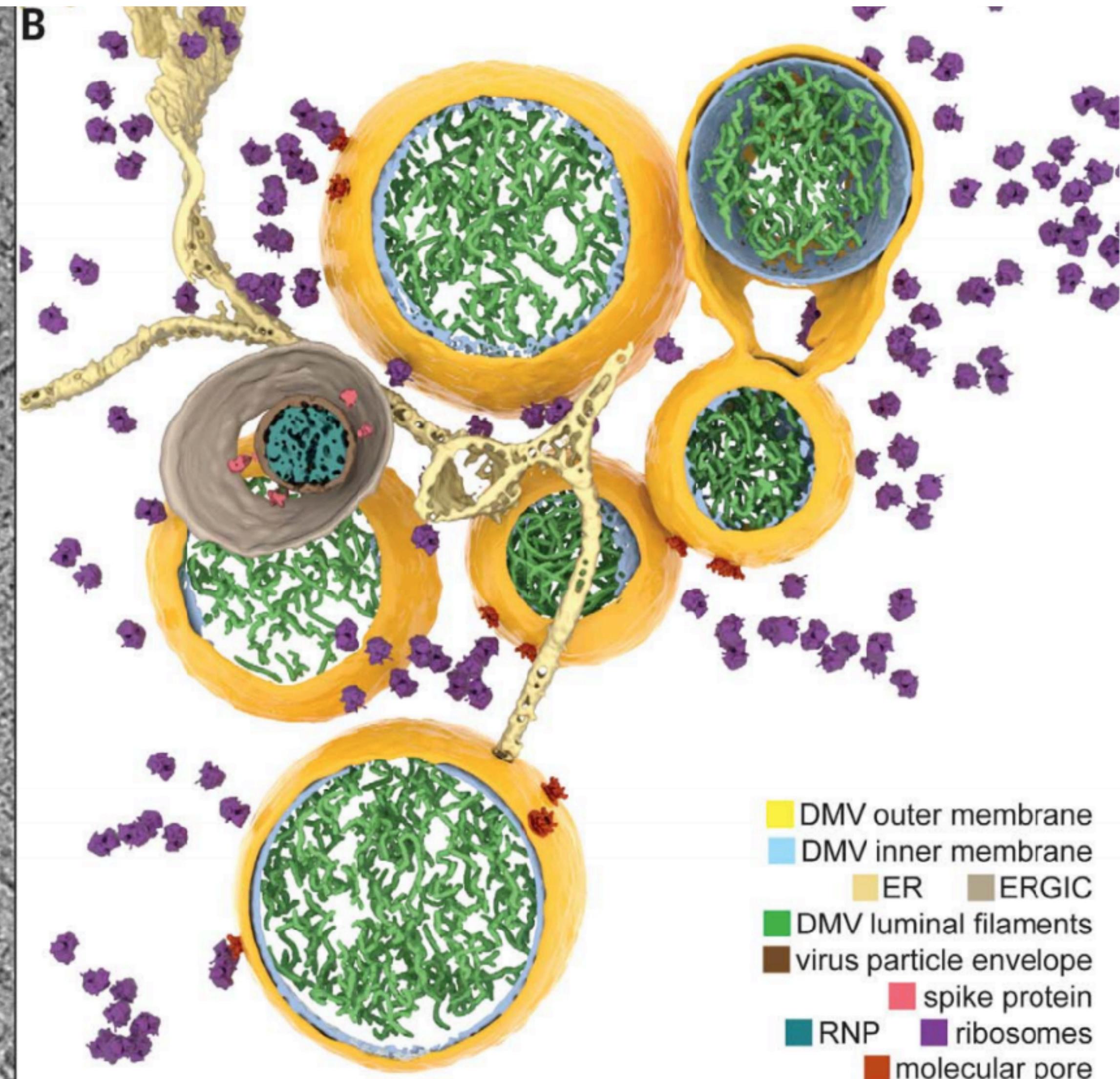
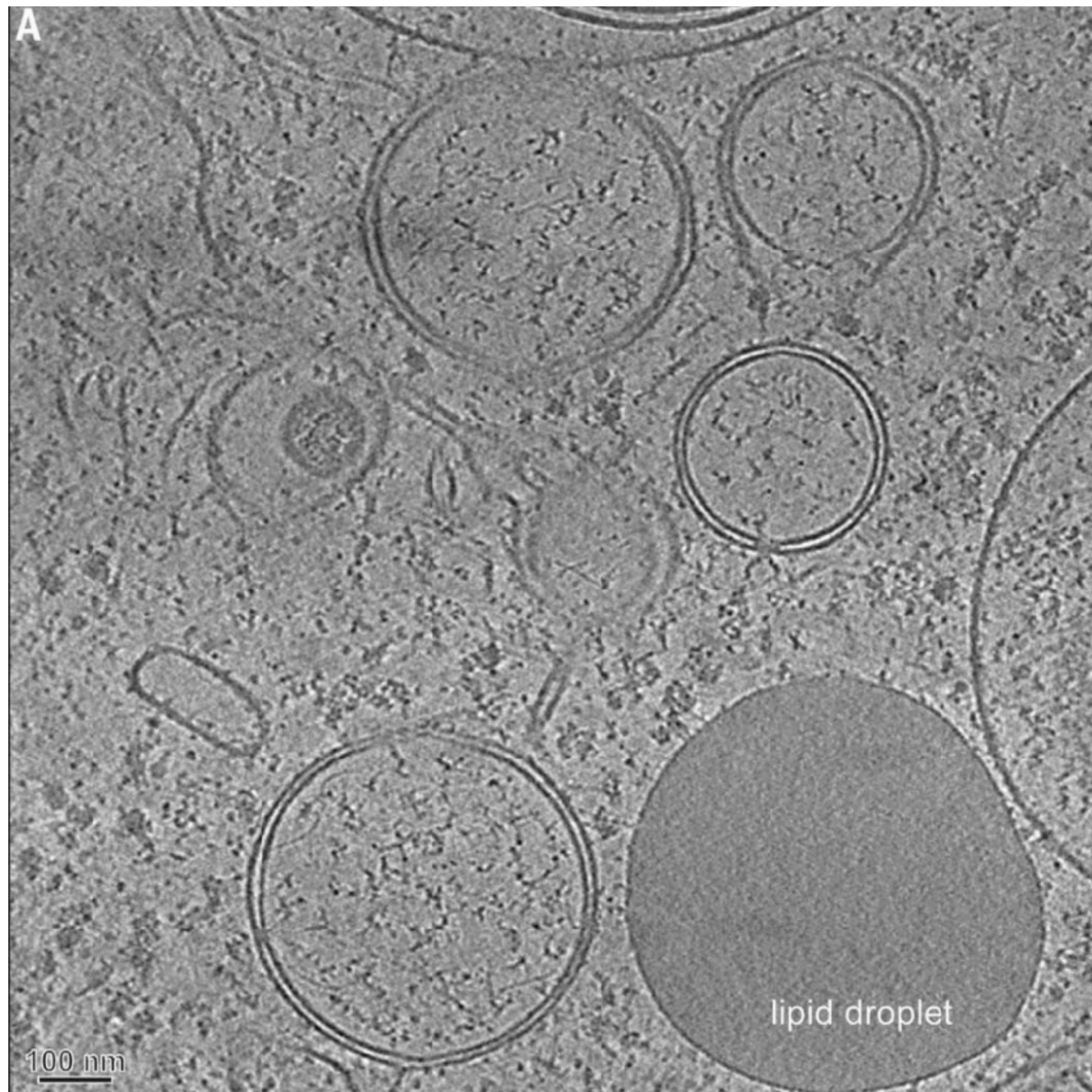
(semi-automatic)



Amira by Thermo Scientific

Golgi stack

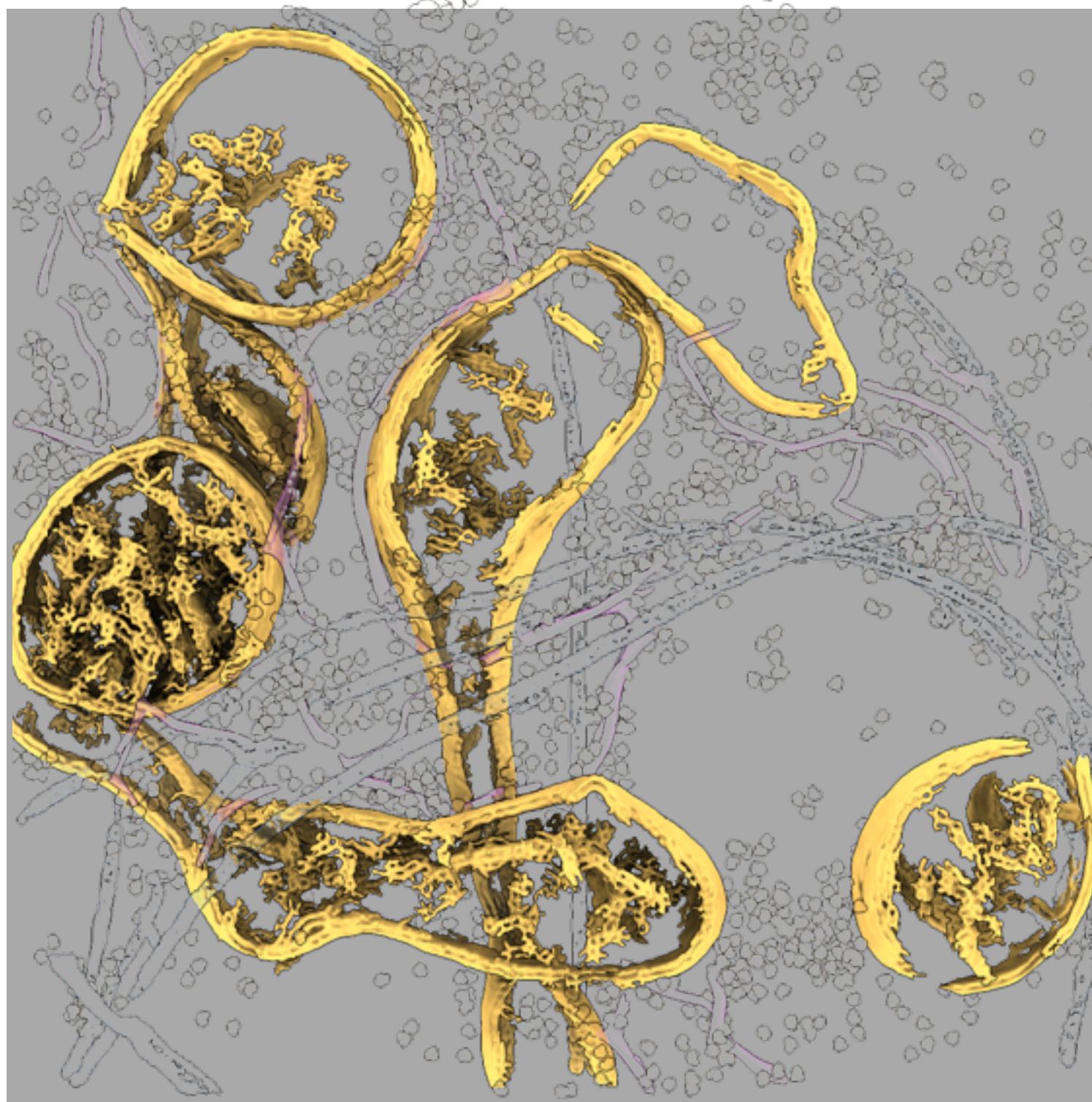




Combination of segmentation tools

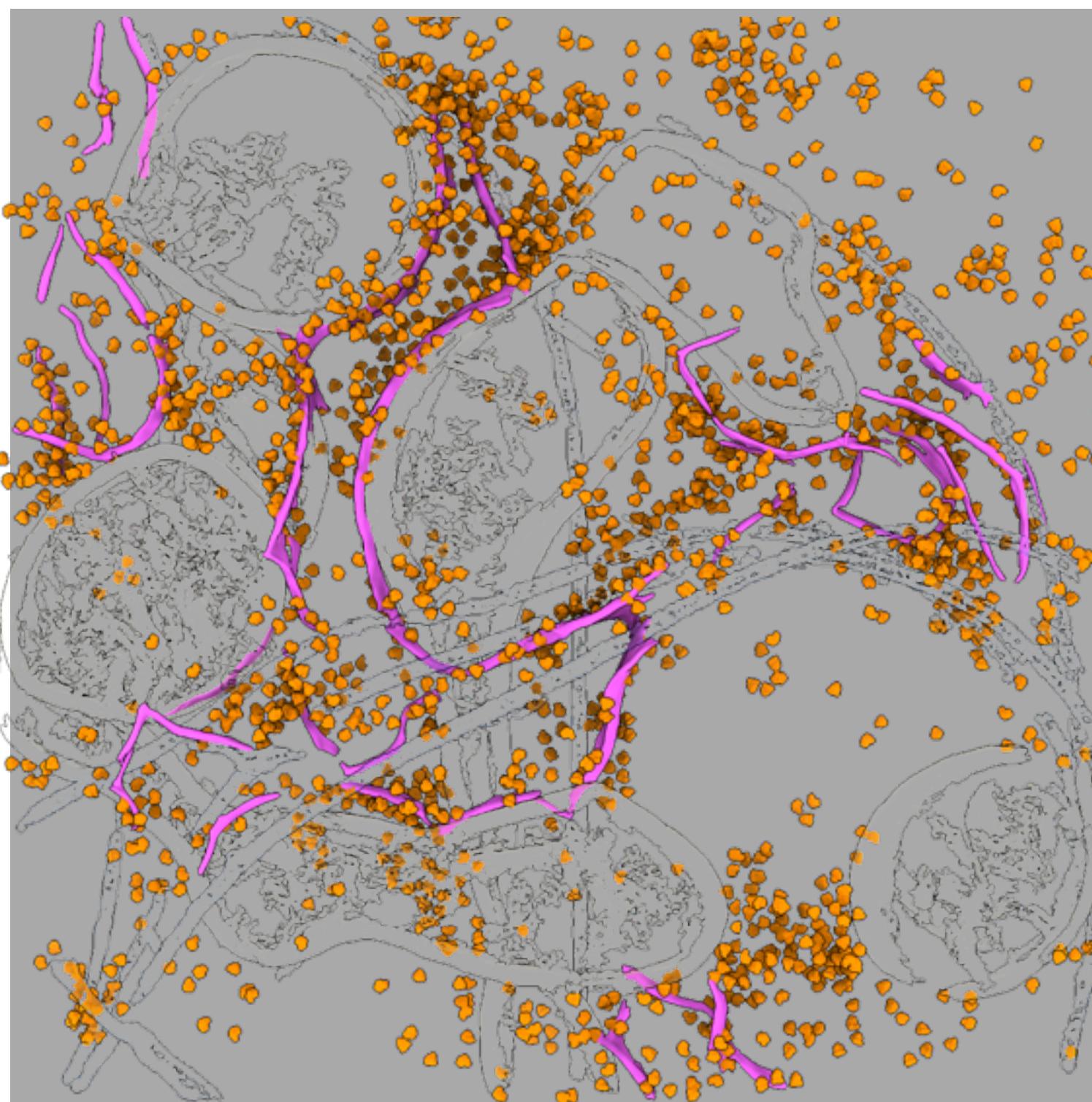
Example 1

Chimera - Segment mitochondria

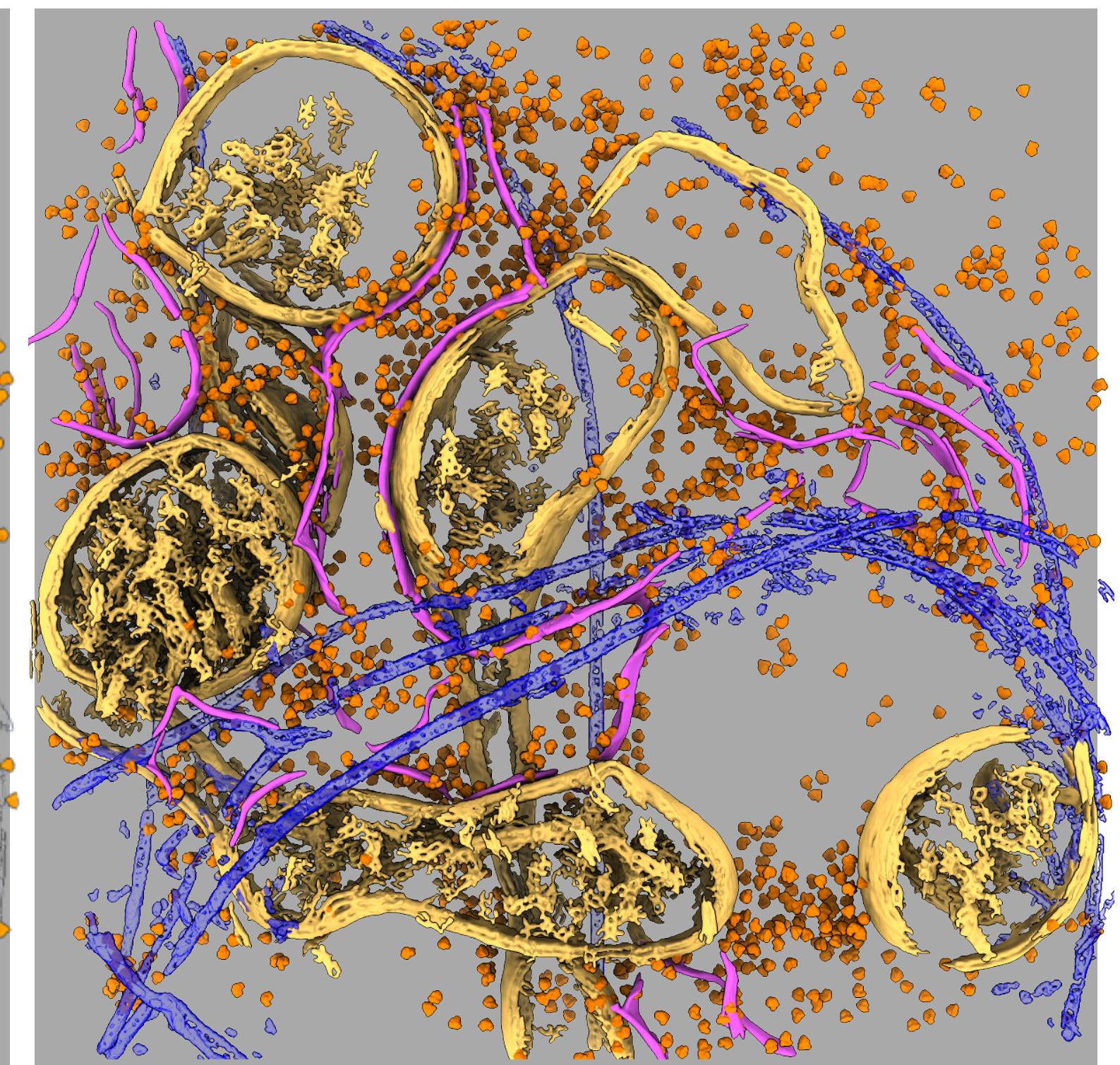


EMAN2 - Segment ER and Microtubules

EMAN2 - Segment ribosomes, particle search,
subtomogram averaging, and map-back

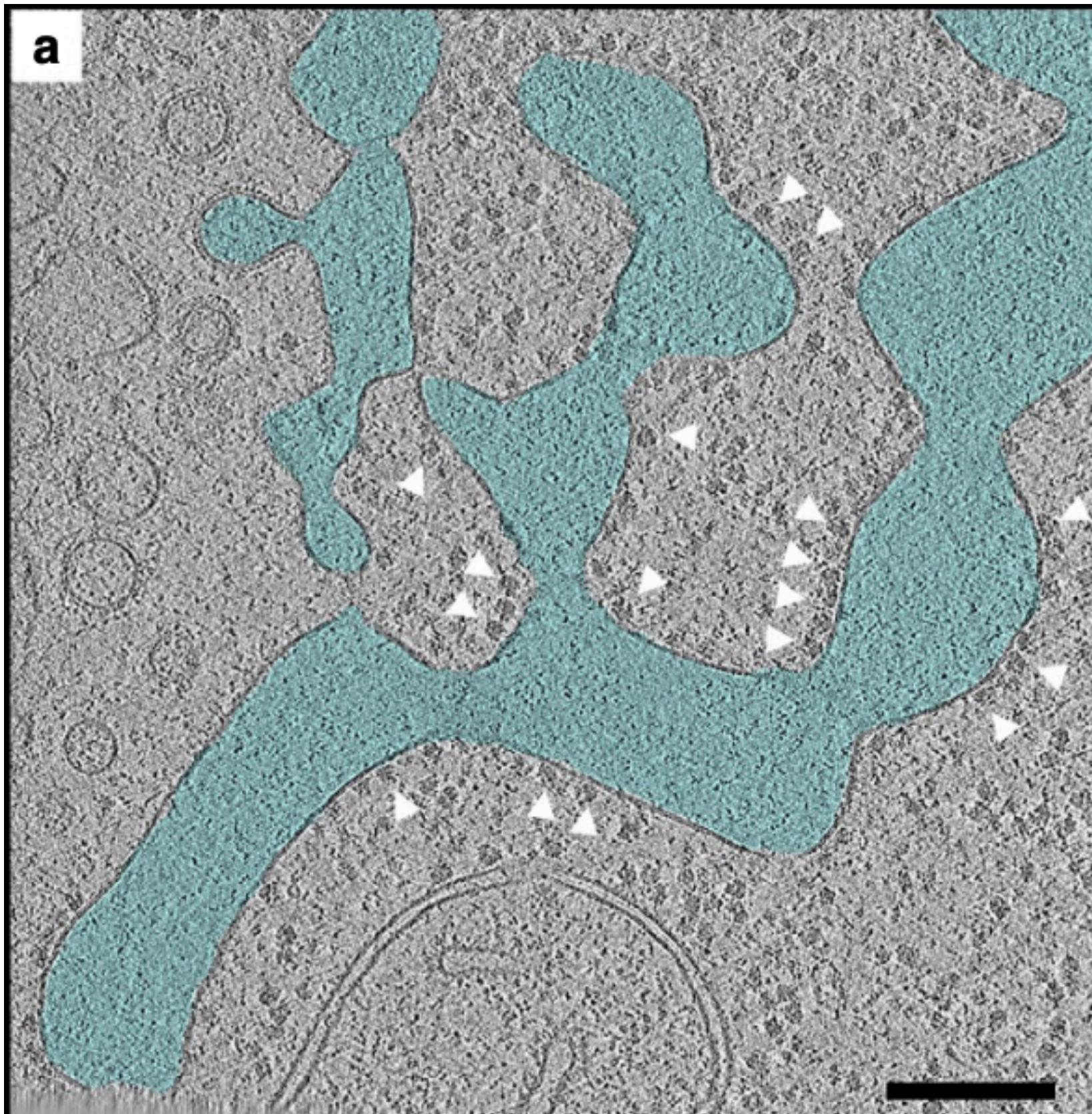


ChimeraX - Combine all segmentations and
assign colors and chose graphical style

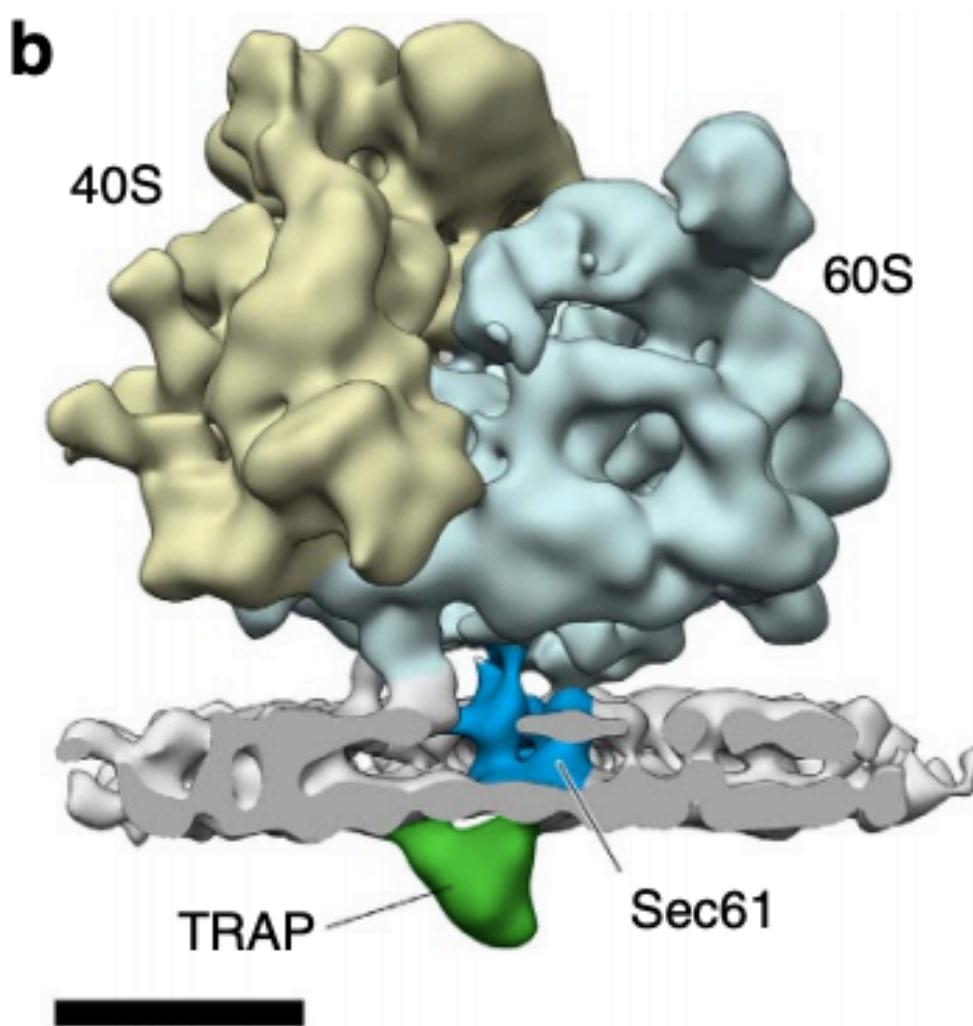


Example 1

PyTom - Template matching to pick ER bound ribosomes



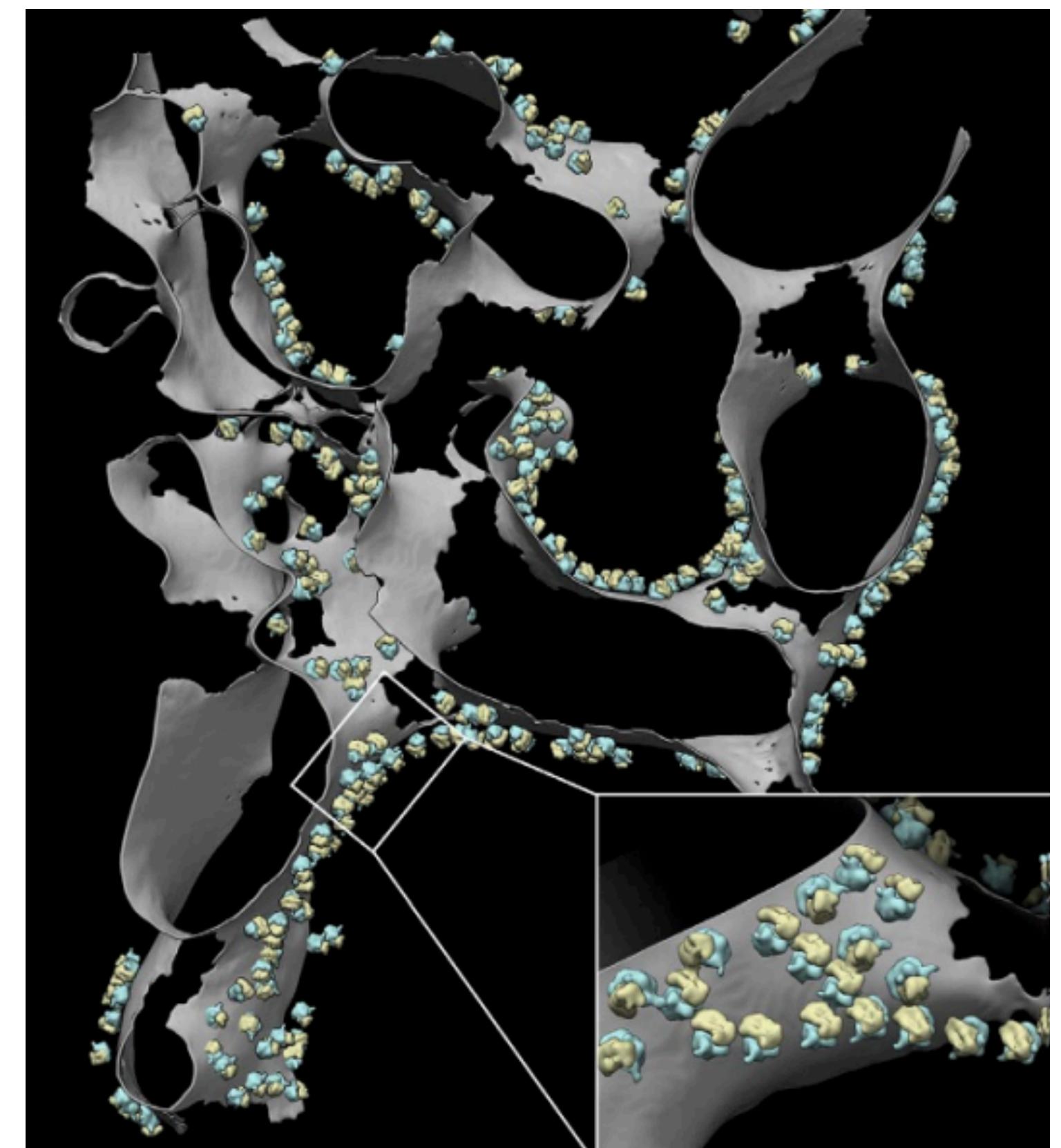
PyTom - Subtomogram averaging



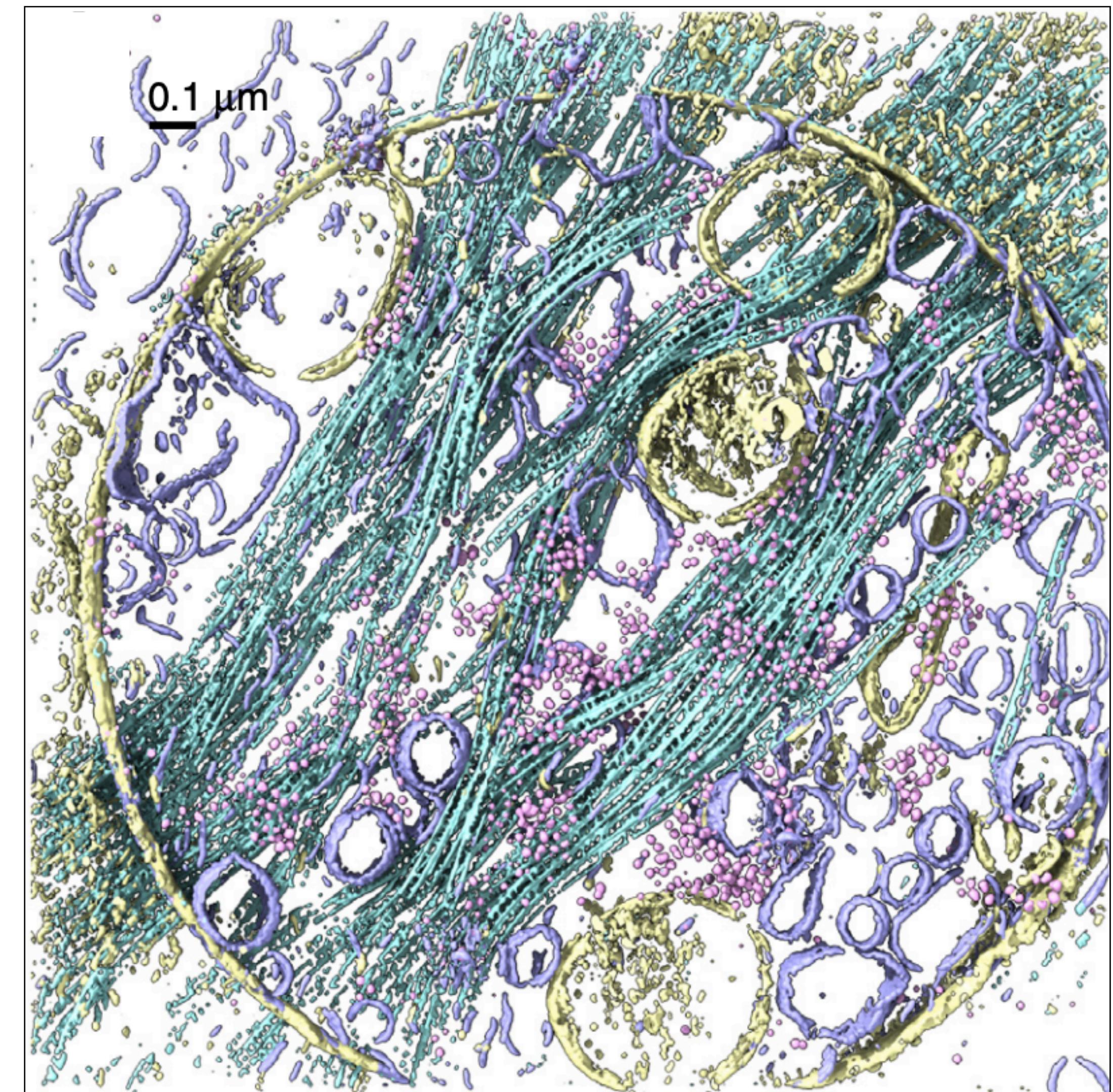
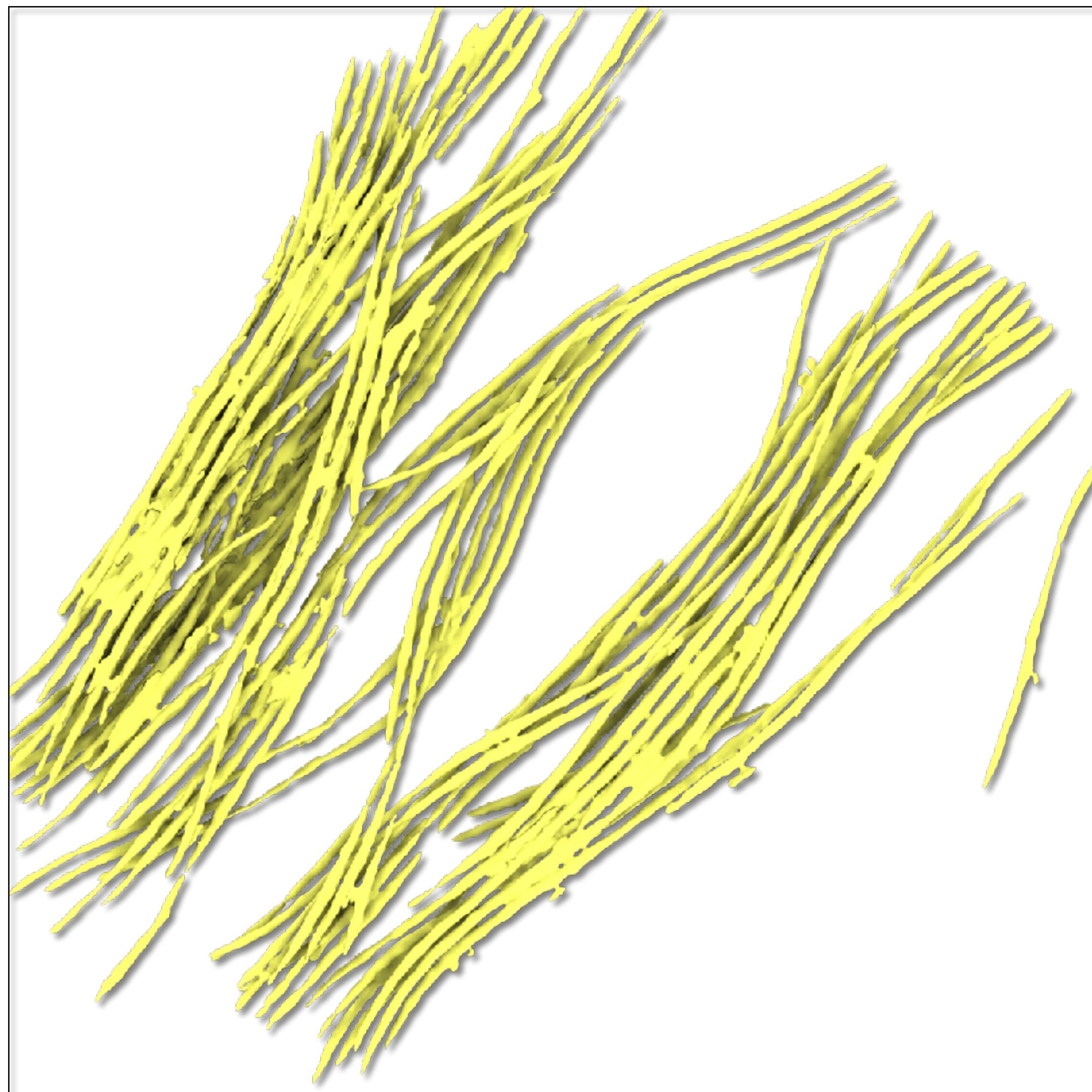
TomoSegMemTV - ER segmentation

Amira - Edit result from TomoSegMemTV
and Visualization

Averaged ribosomes are mapped back



EMAN2 Segmentation Demo



Chen et al., *Nature Method* (2017)