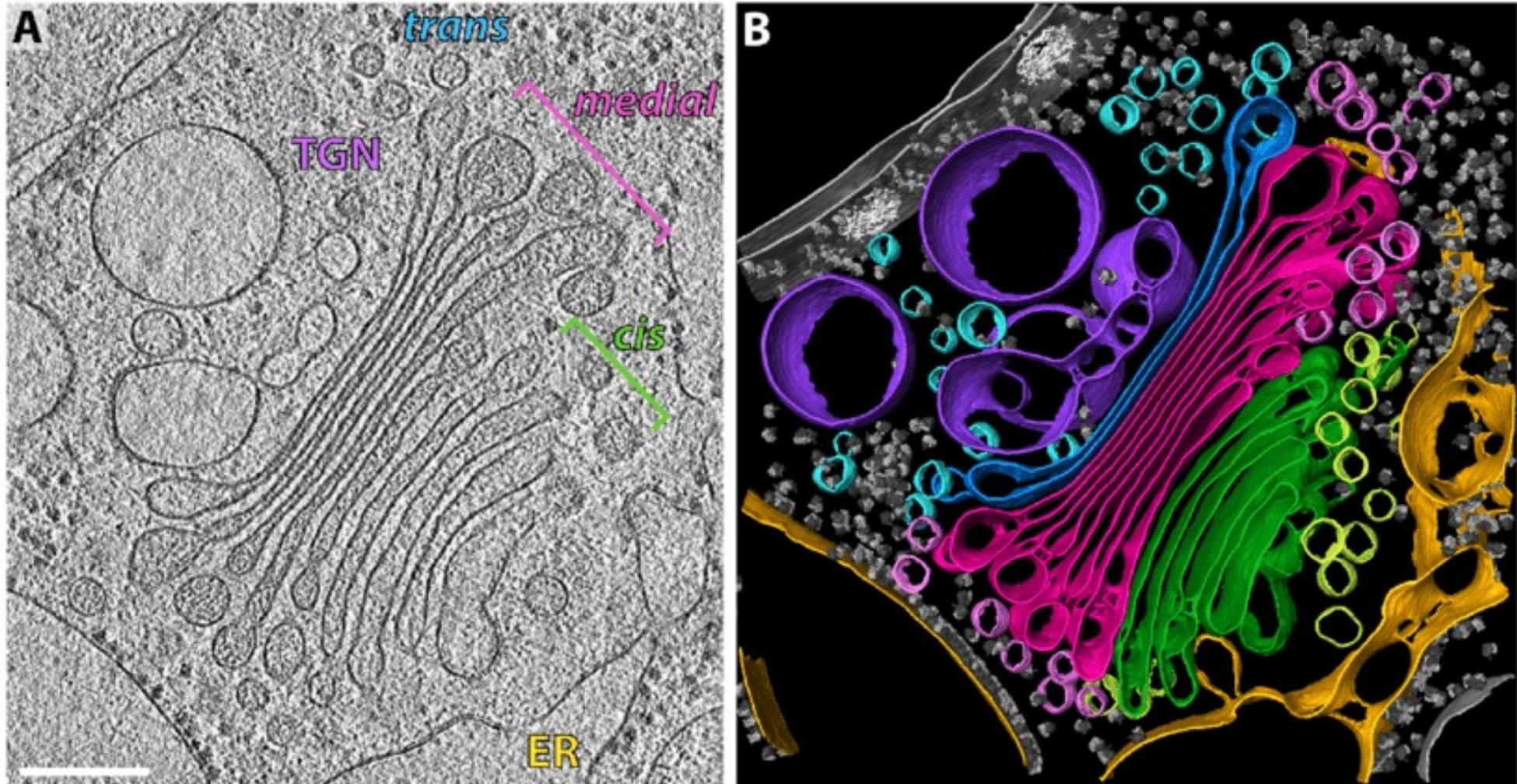


Segmenting CryoET Data

Making models out of cellular maps

Benjamin Barad – Grotjahn Lab @ Scripps Research

Turning Grayscale into Color



Bykov et al 2017

What is a segmentation?

- **Literal definition:** A semantic segmentation is a per-voxel classification of which voxels in a volume belong to each of a set of given classes (membrane, filament, particle, etc).
- **Practical meaning:** A segmentation is the modeling of the raw data of the tomogram, akin to the atomic model that is built into a cryoEM map.

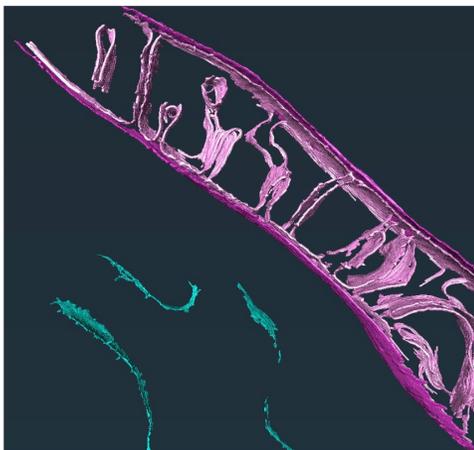
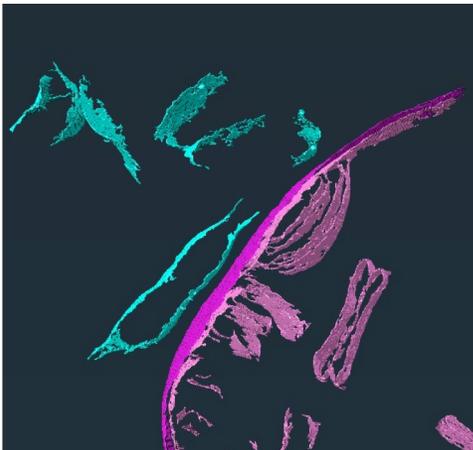
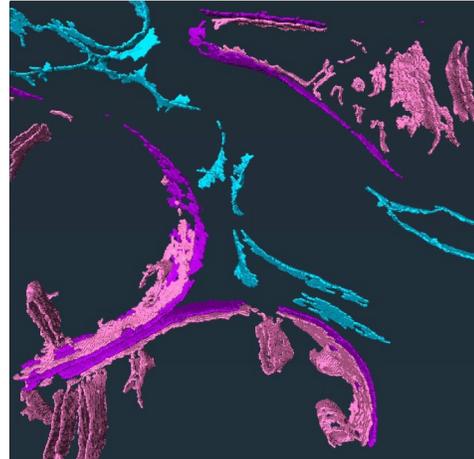
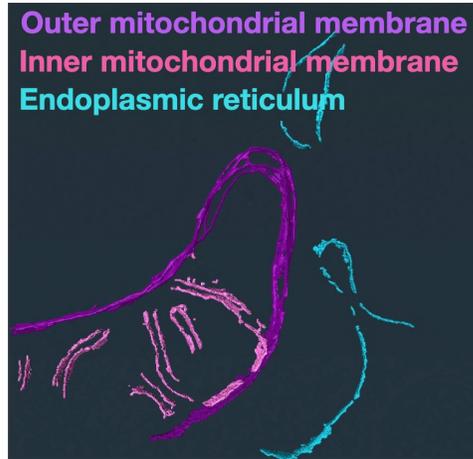
Why Segmentation?

- Visualizing spatial relationships in 3D
- Identifying specific biological features
- “Hiding” the noise through modeling
- Quantifying ultrastructure within cells
- Improving particle picking with guided search
- Improving subtomogram averaging with guided angle assignment

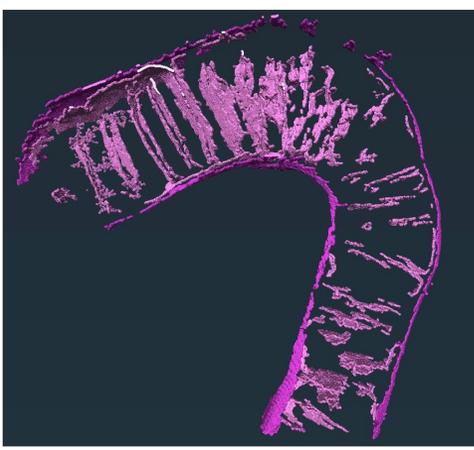
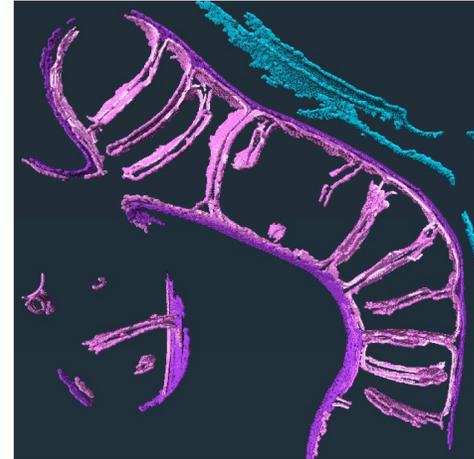


Segmentation for Visualization and Hypothesis Driving

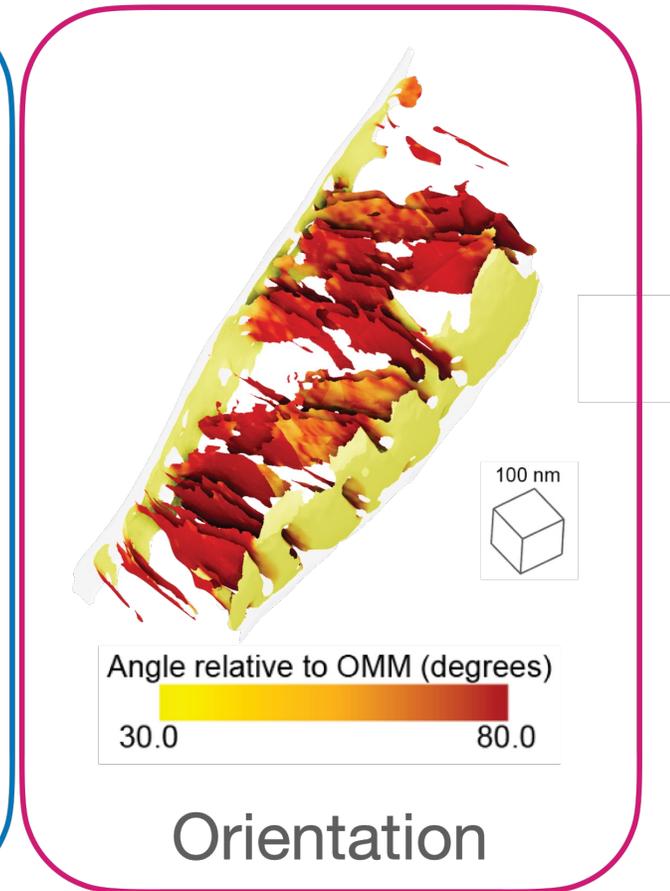
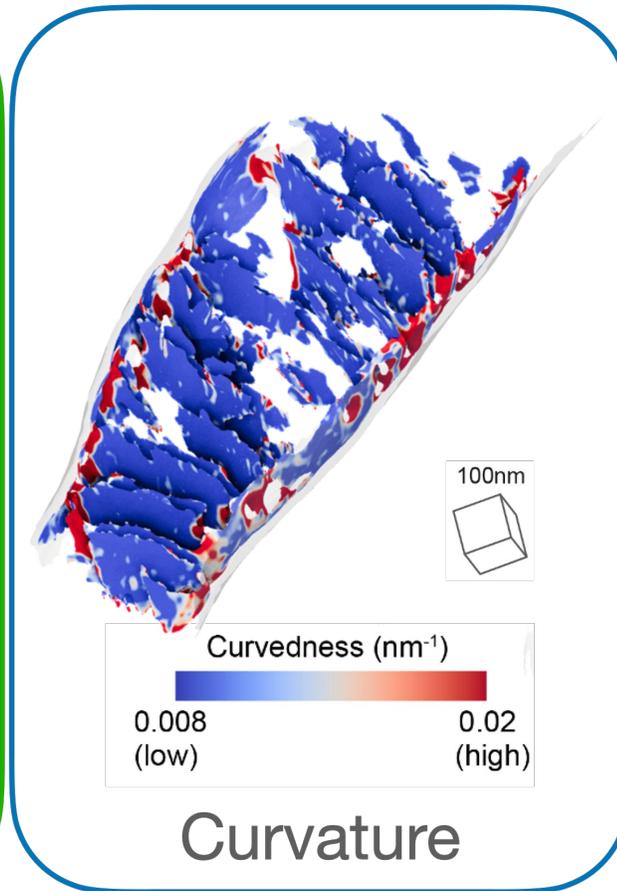
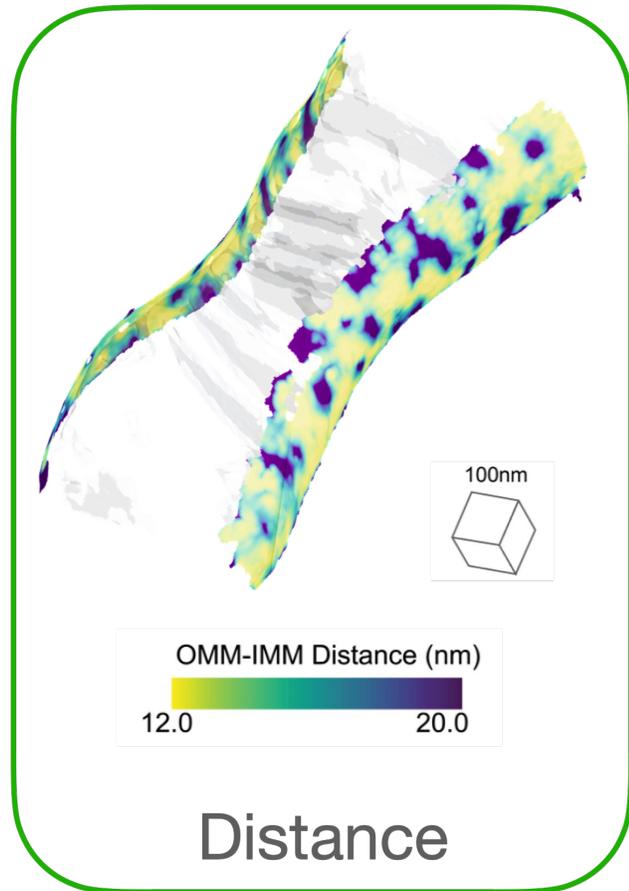
Vehicle (- ER Stress)



Thapsigargin (+ ER Stress)

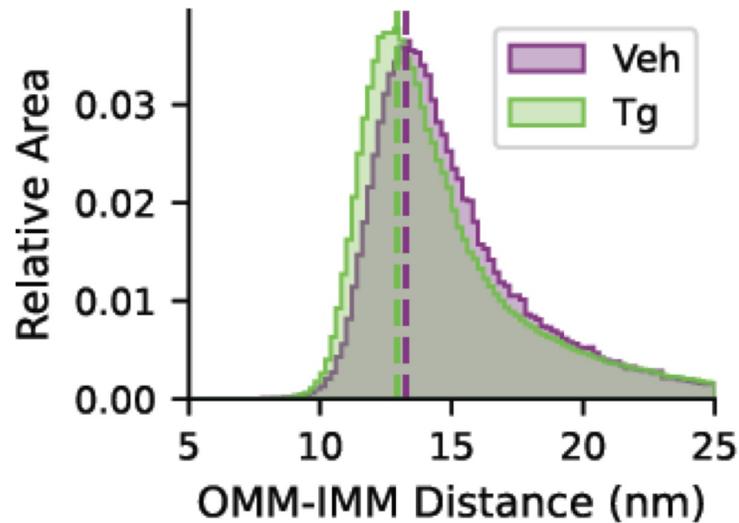


Segmentation for Quantification

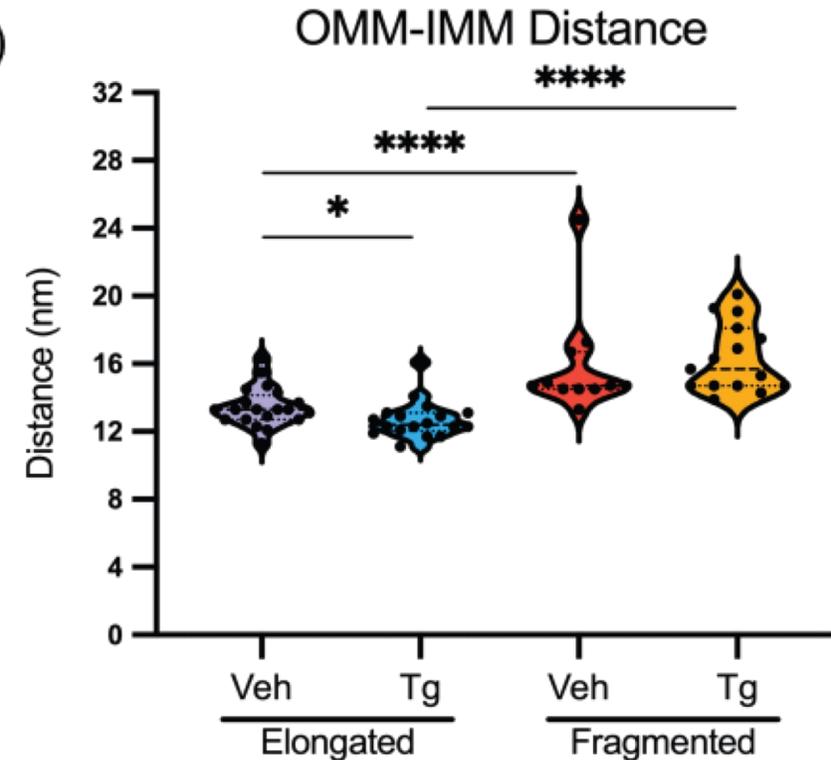
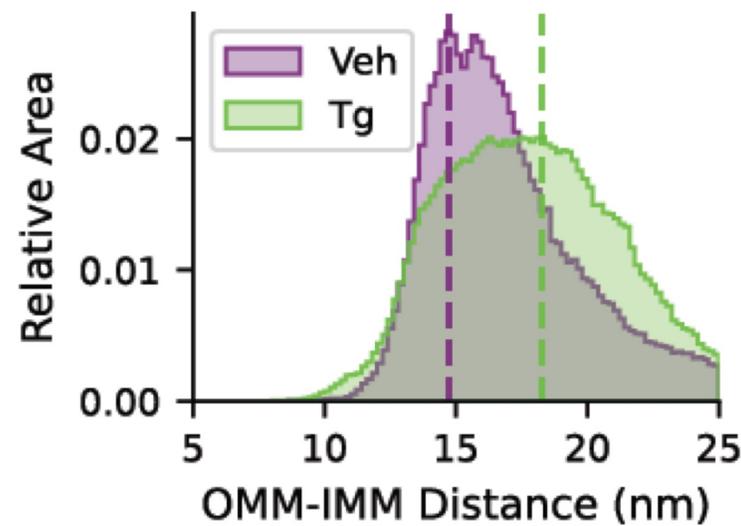


Segmentation for Quantification

OMM-IMM Distance (Elongated)



OMM-IMM Distance (Fragmented)

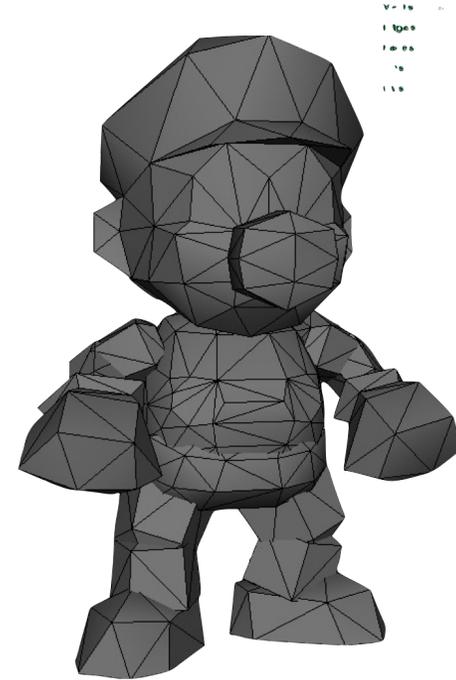


Geometric models are improvements on voxel segmentations for many purposes



Voxels

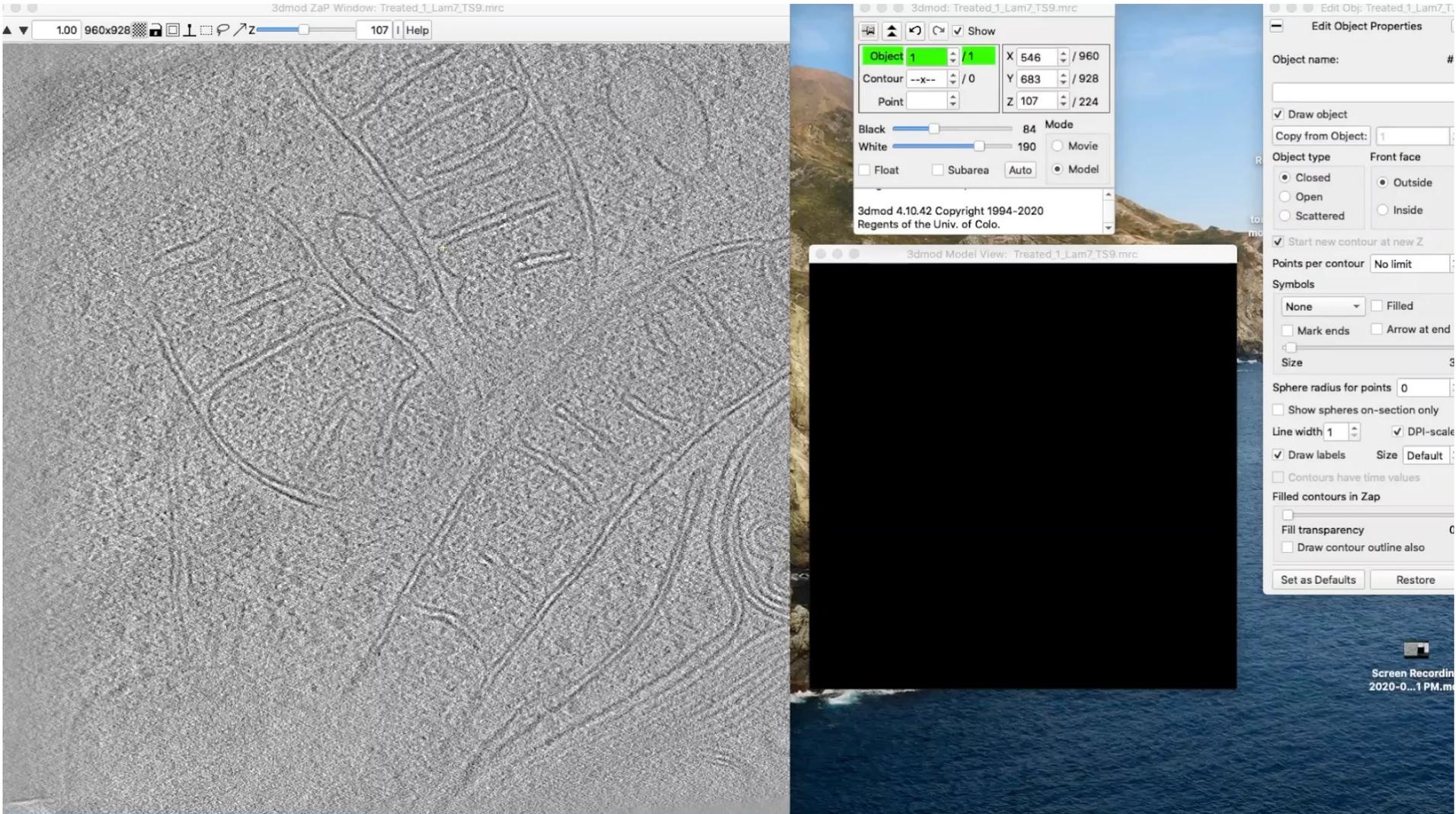
No encoding of geometry or connectivity



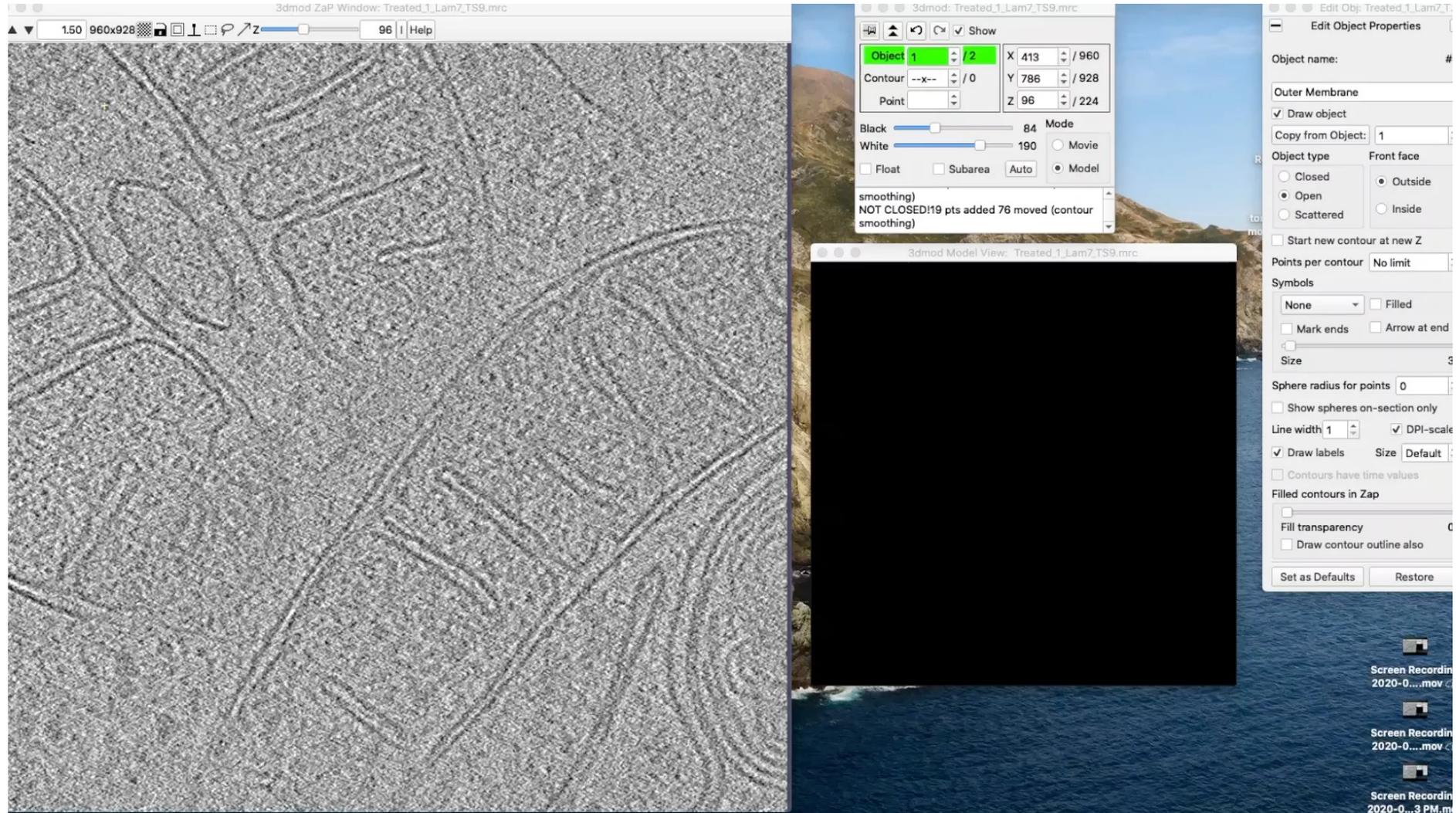
Polygon Meshes

Orientation and **Connectivity** encoded
Reconstruction of high quality meshes is complex

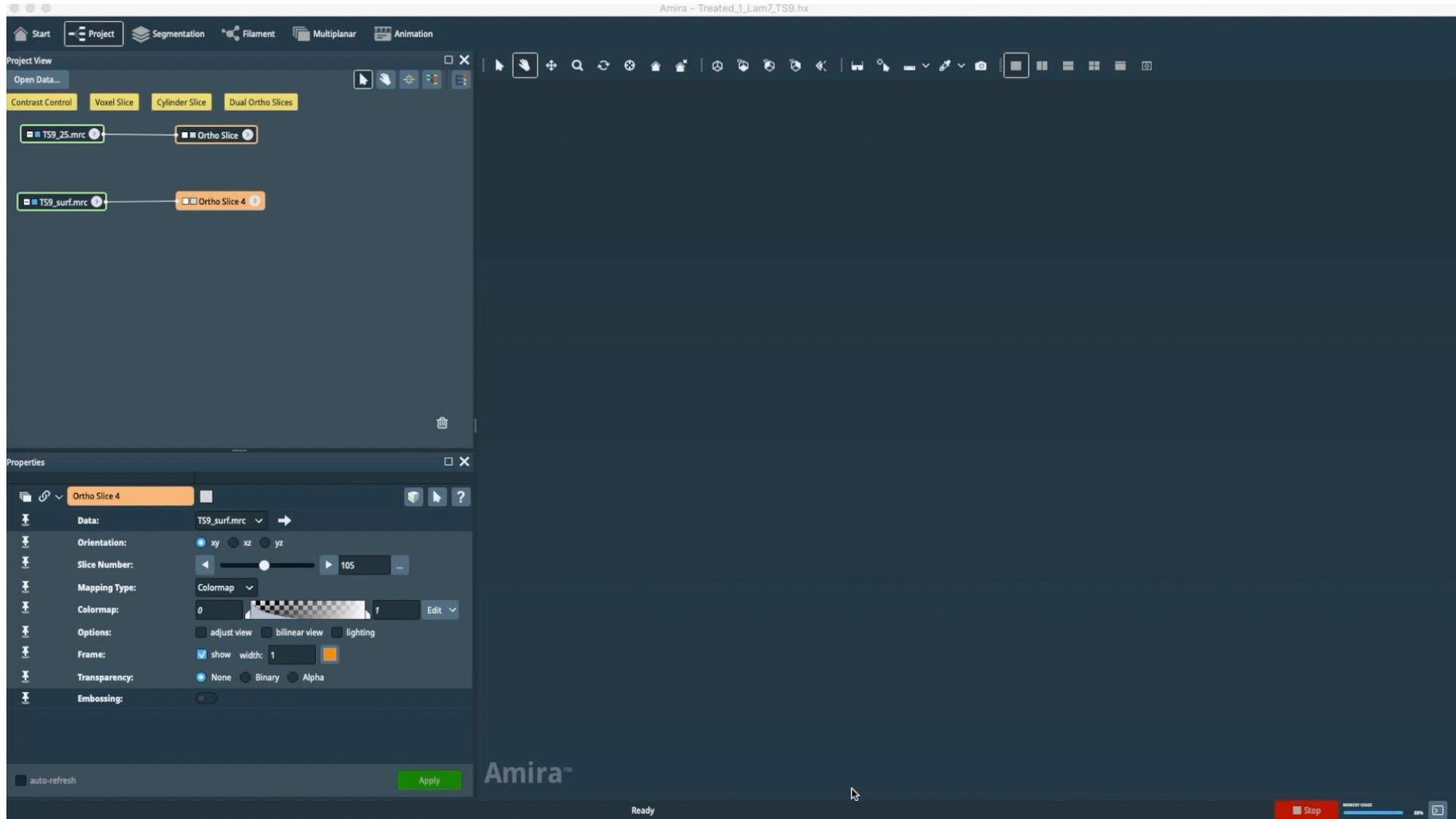
Old School Geometric Segmentation: Contouring by hand



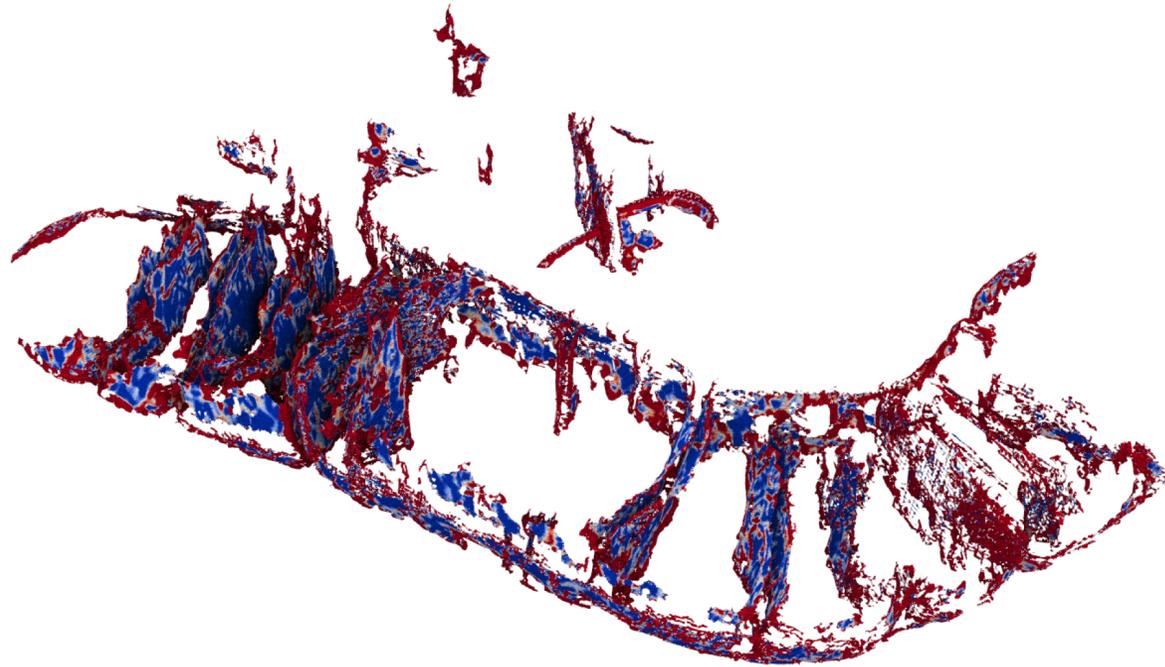
Old School Geometric Segmentation: Contouring by hand



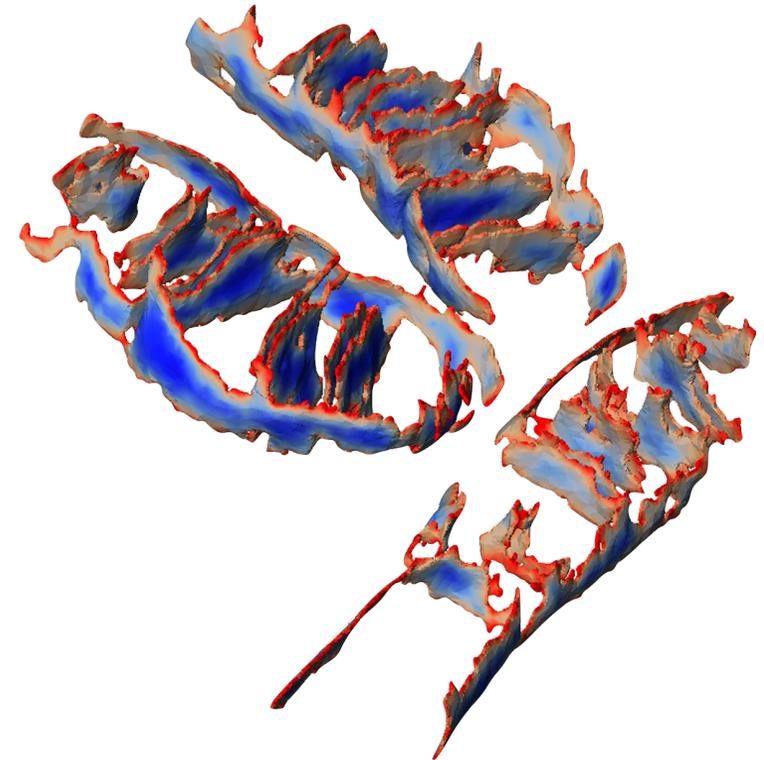
Today: Faster (Voxel) Segmentation with Amira and Tomosegmentv



Building geometric models of non-watertight membranes from voxels is surprisingly hard



Pycurv Reconstruction Algorithm

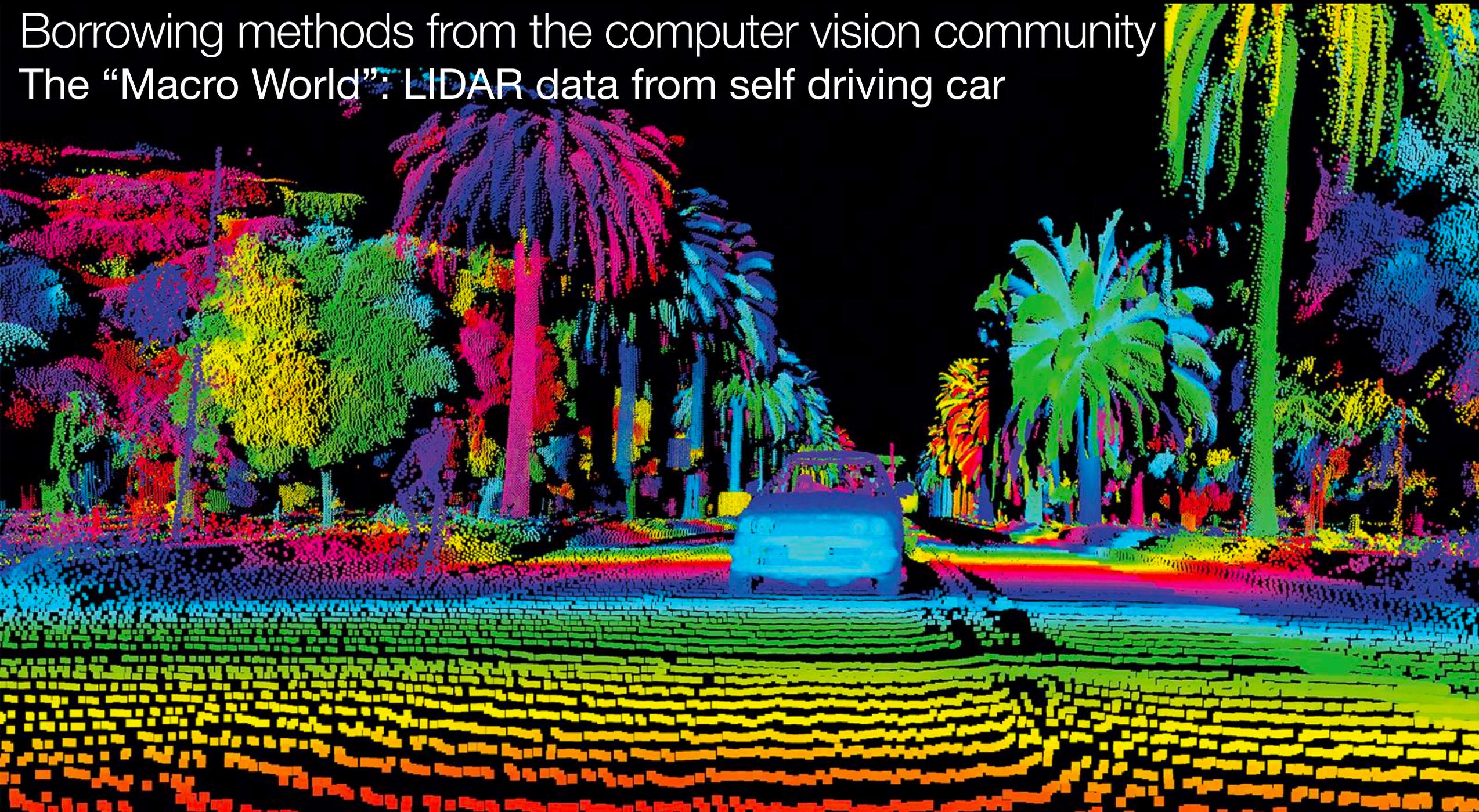


Isosurface Reconstruction

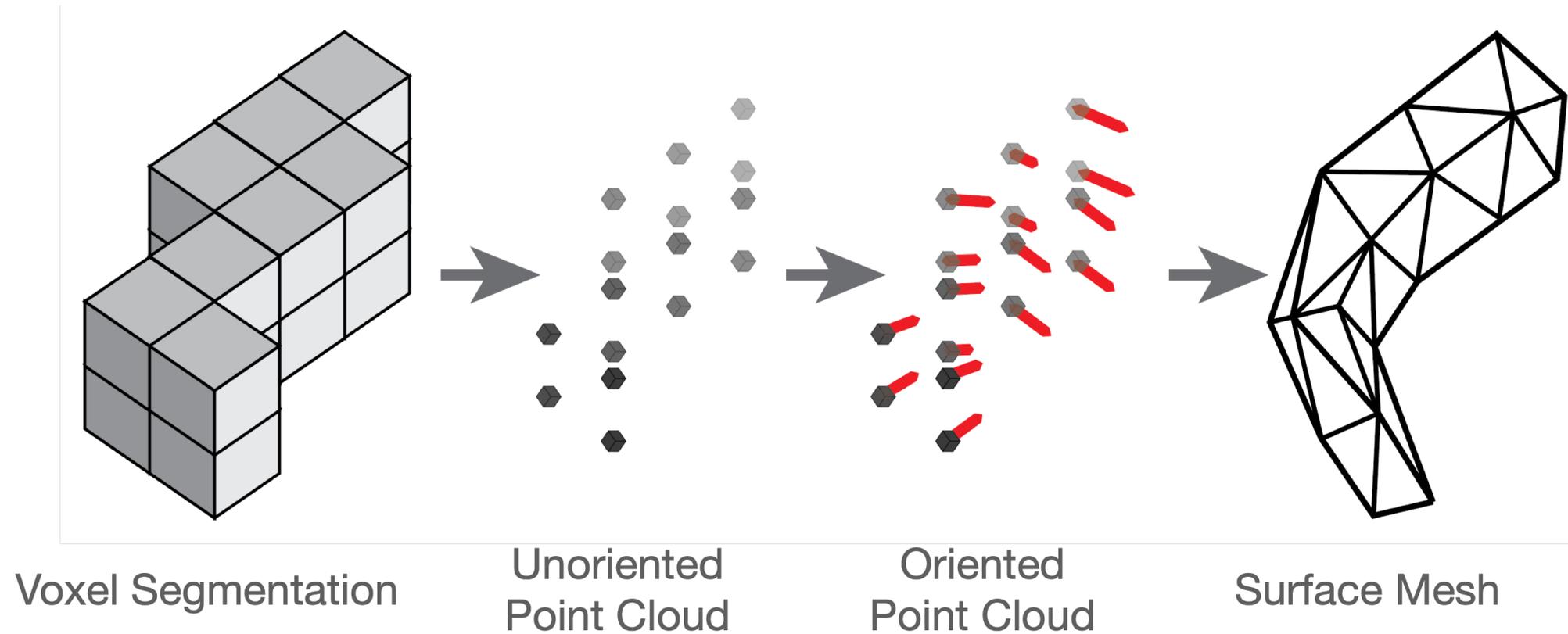


Curvedness

Borrowing methods from the computer vision community
The “Macro World”: LIDAR data from self driving car

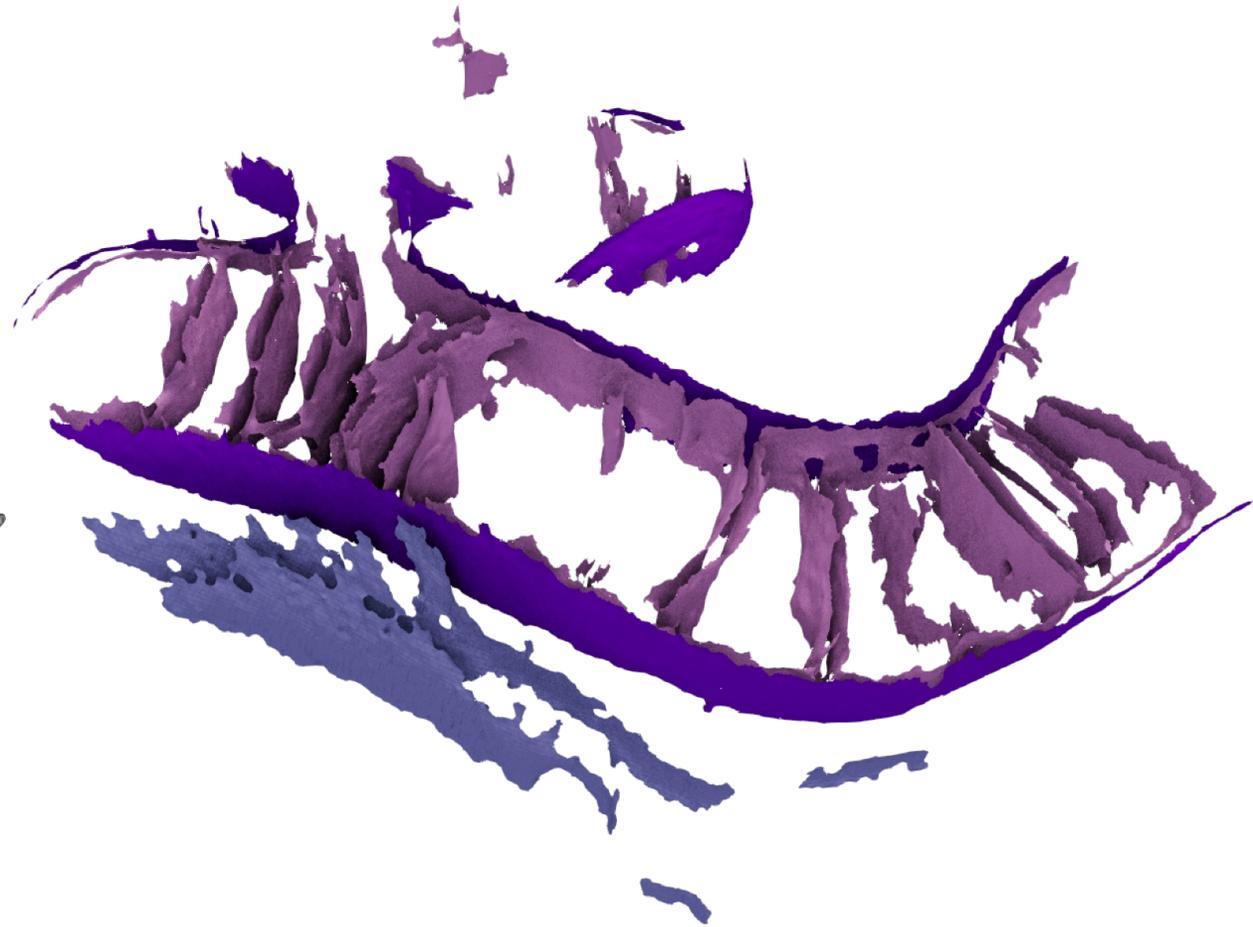
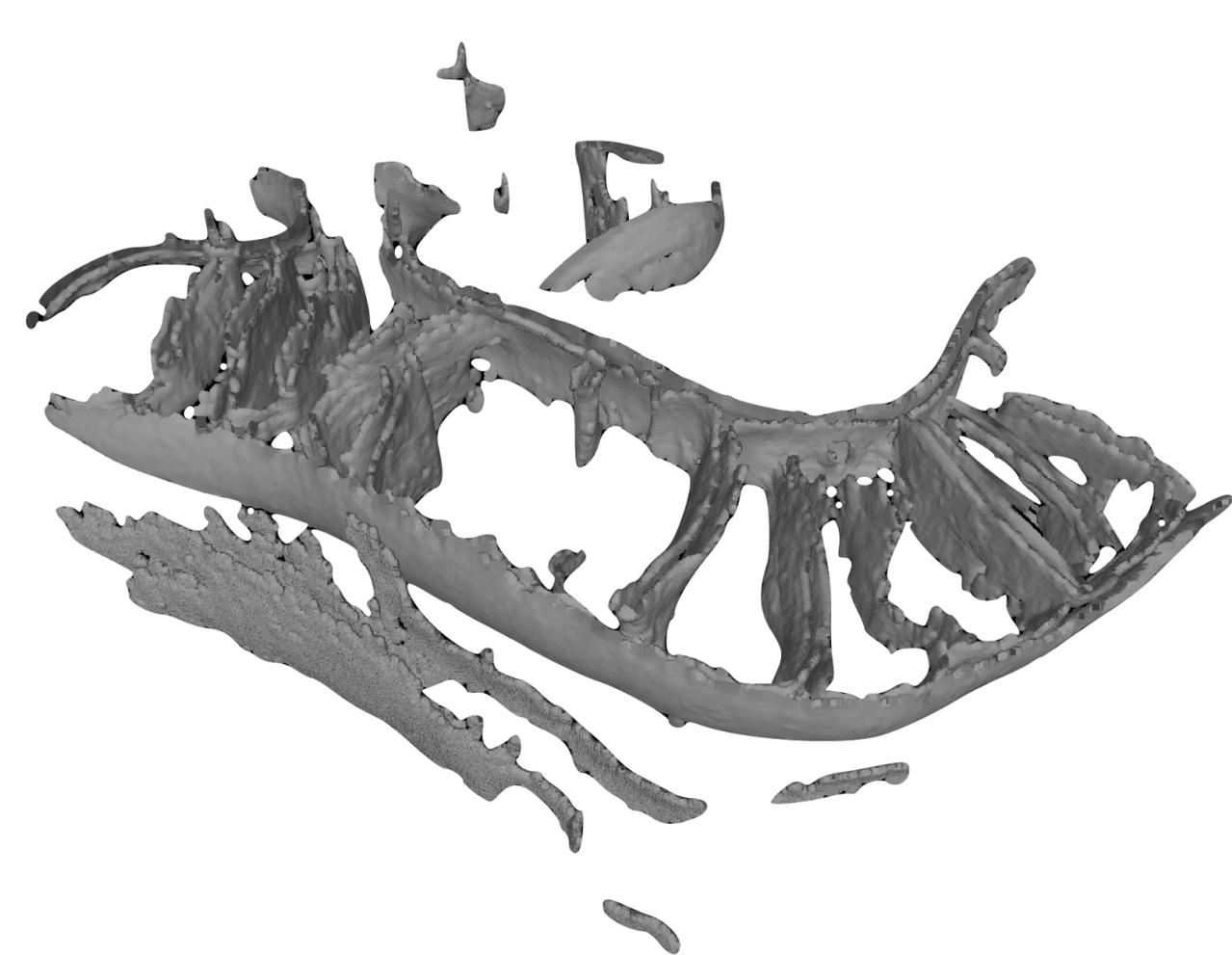


Screened poisson reconstruction efficiently converts voxel membranes into geometric surfaces

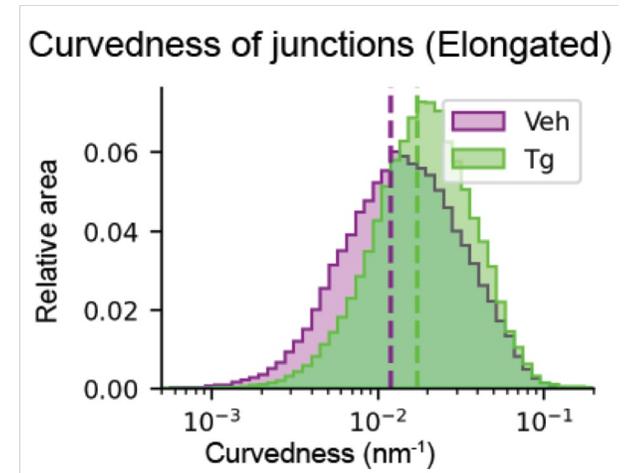
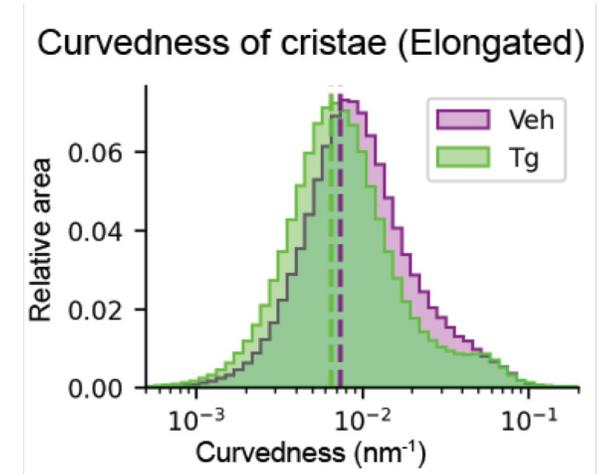
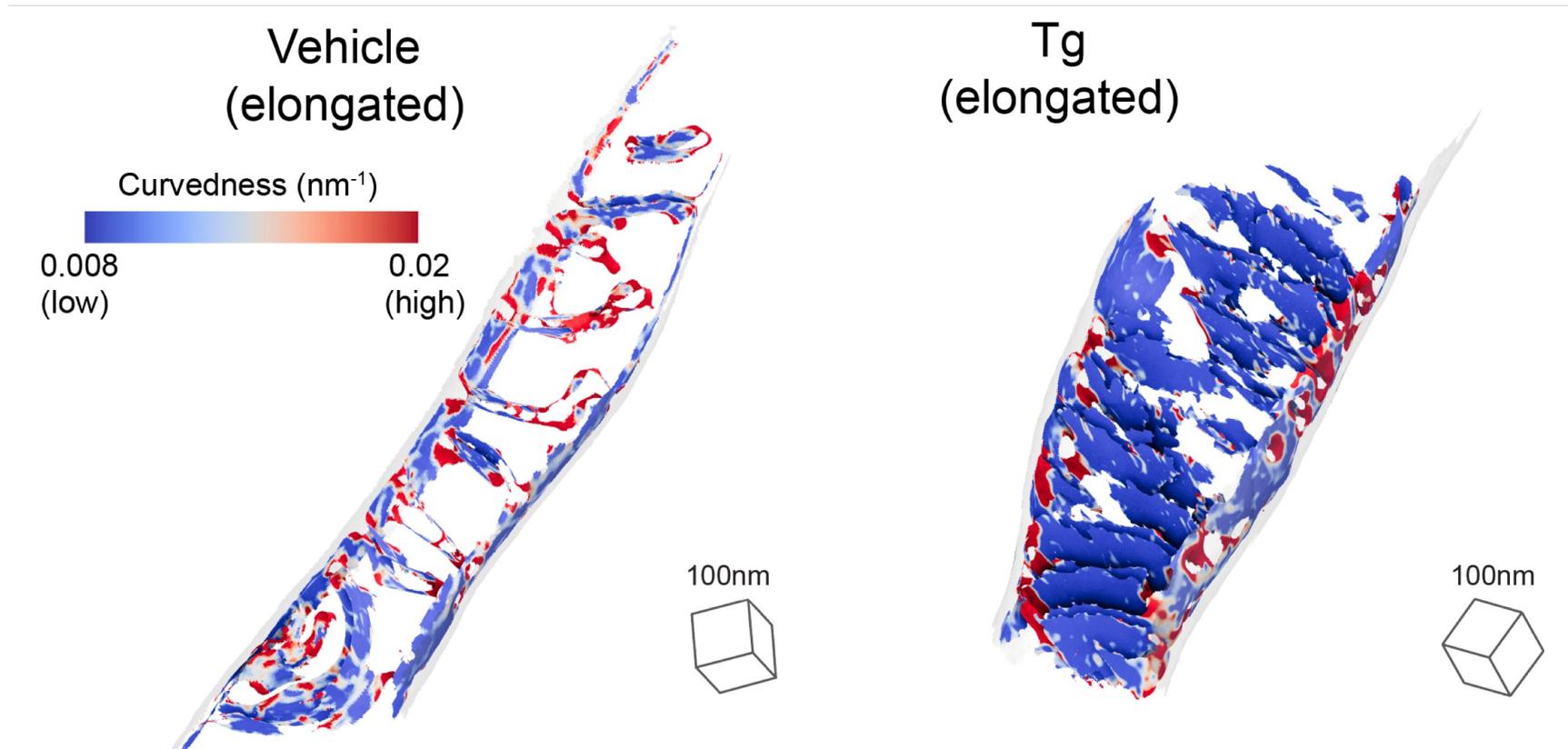


Kazhdan M and Hoppe H. *Transactions on Graphics* 2013

Mesh generation works for very complex membranes

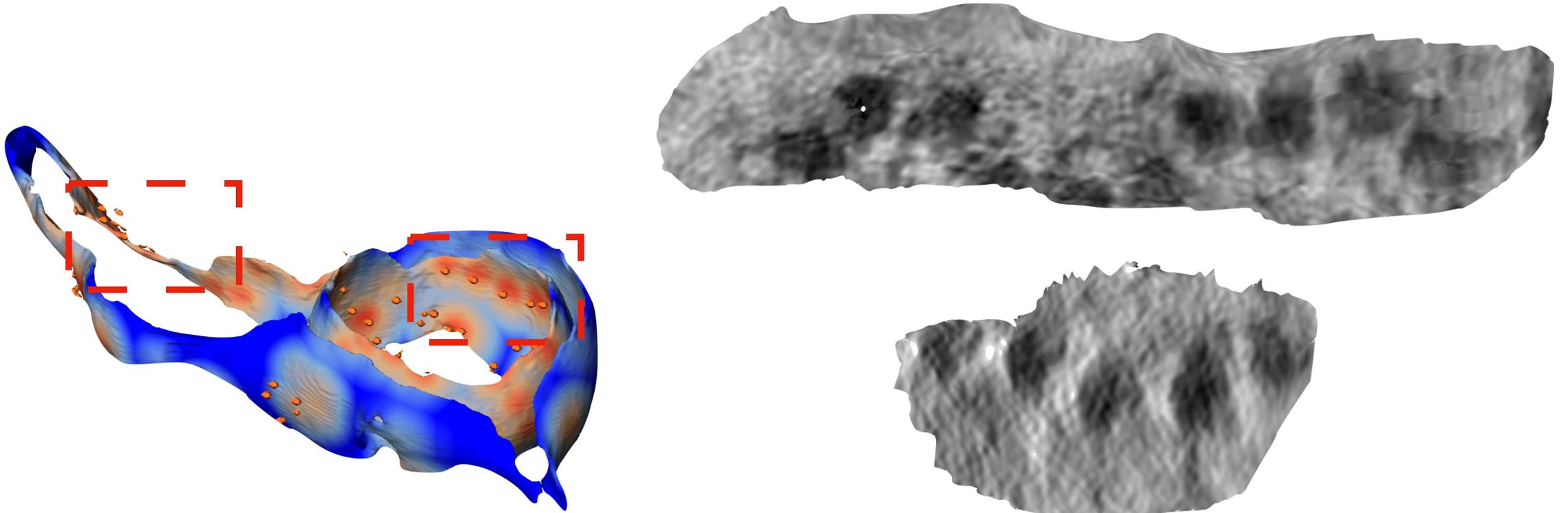


Surface modeling enables quantification of complex behaviors of membranes



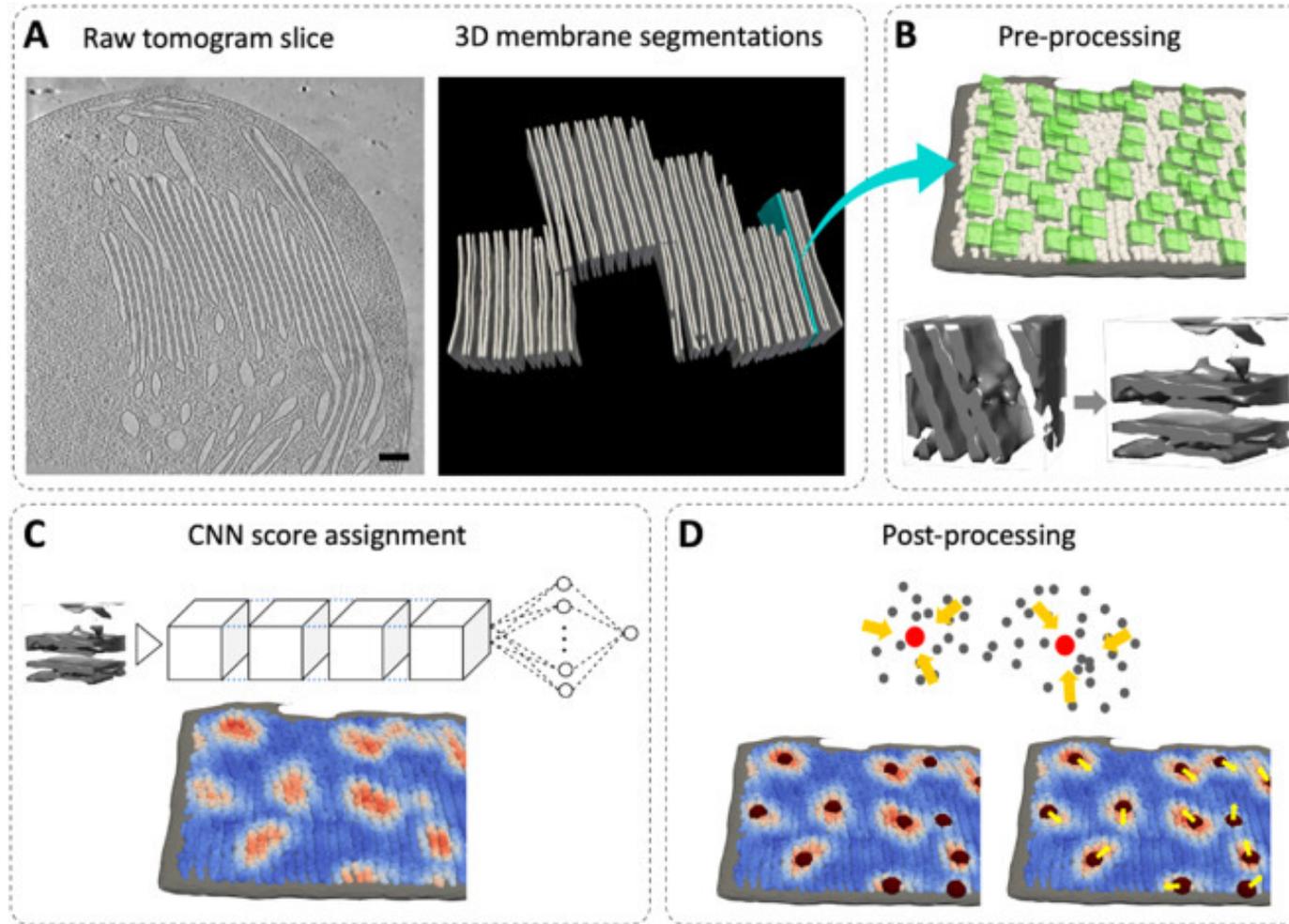
Barad, Medina et al 2023
Salfer et al 2021

Segmentation enables particle picking

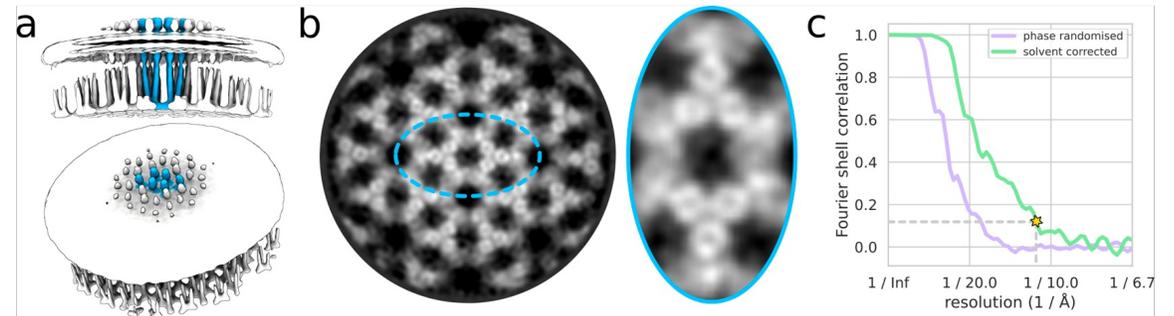
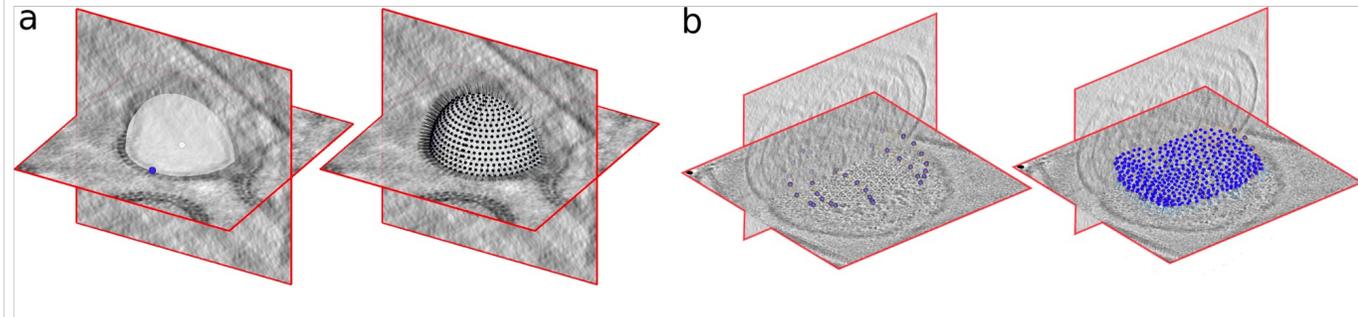
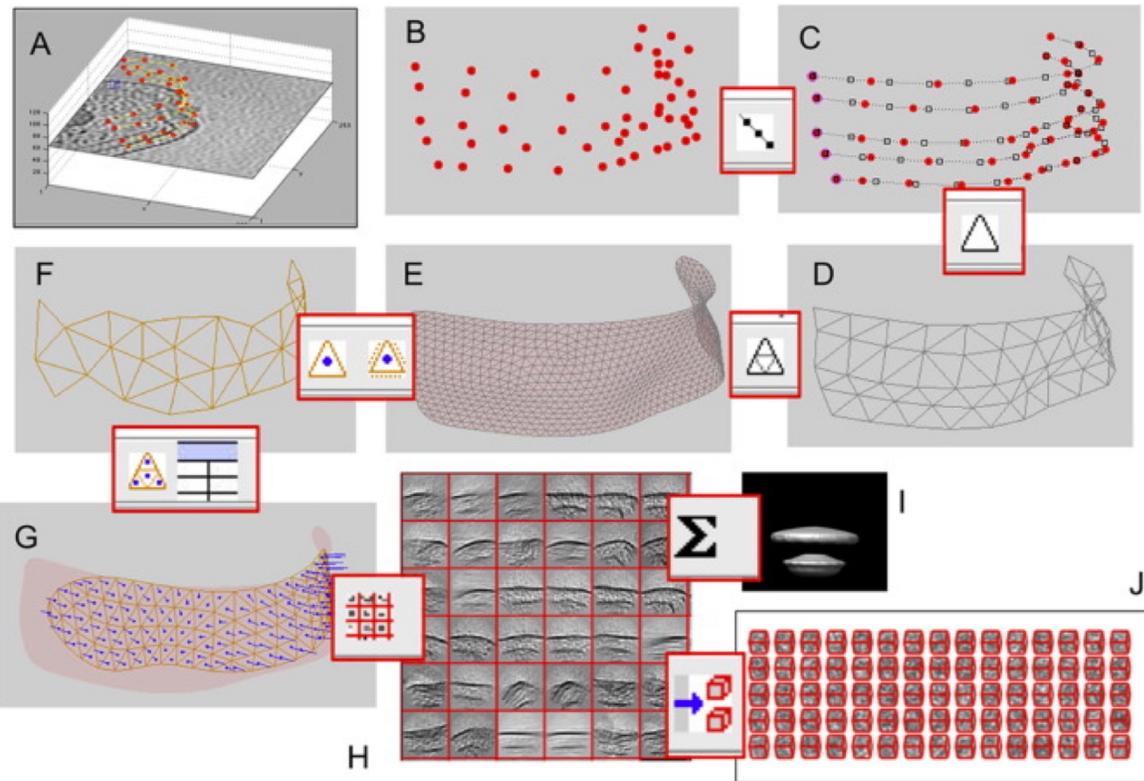


Wietrzynski, Schaffer, Tegunov et al. 2020

Segmentation enables particle picking

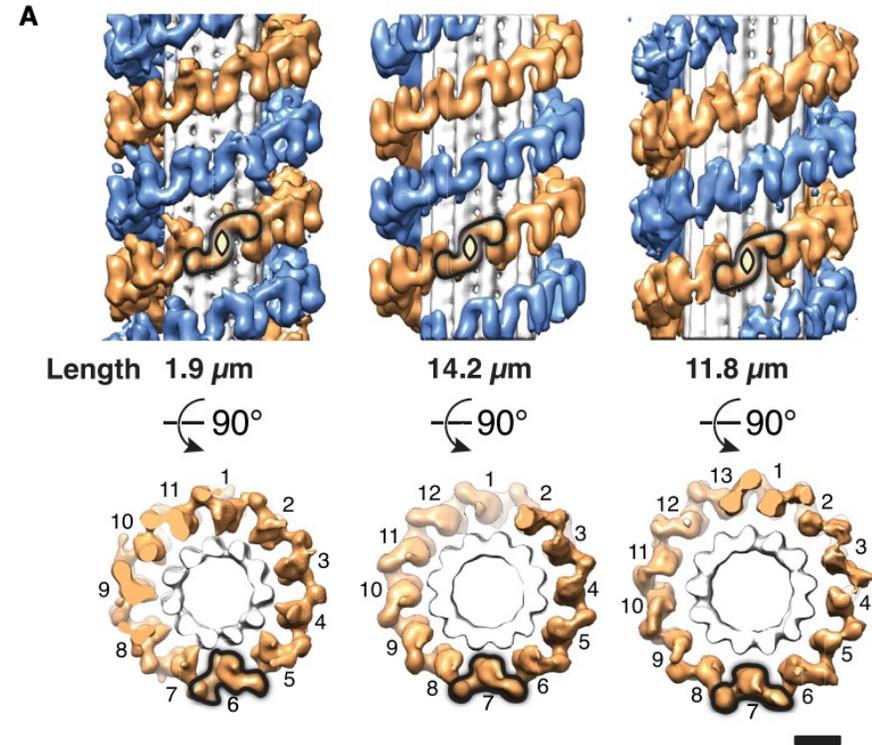
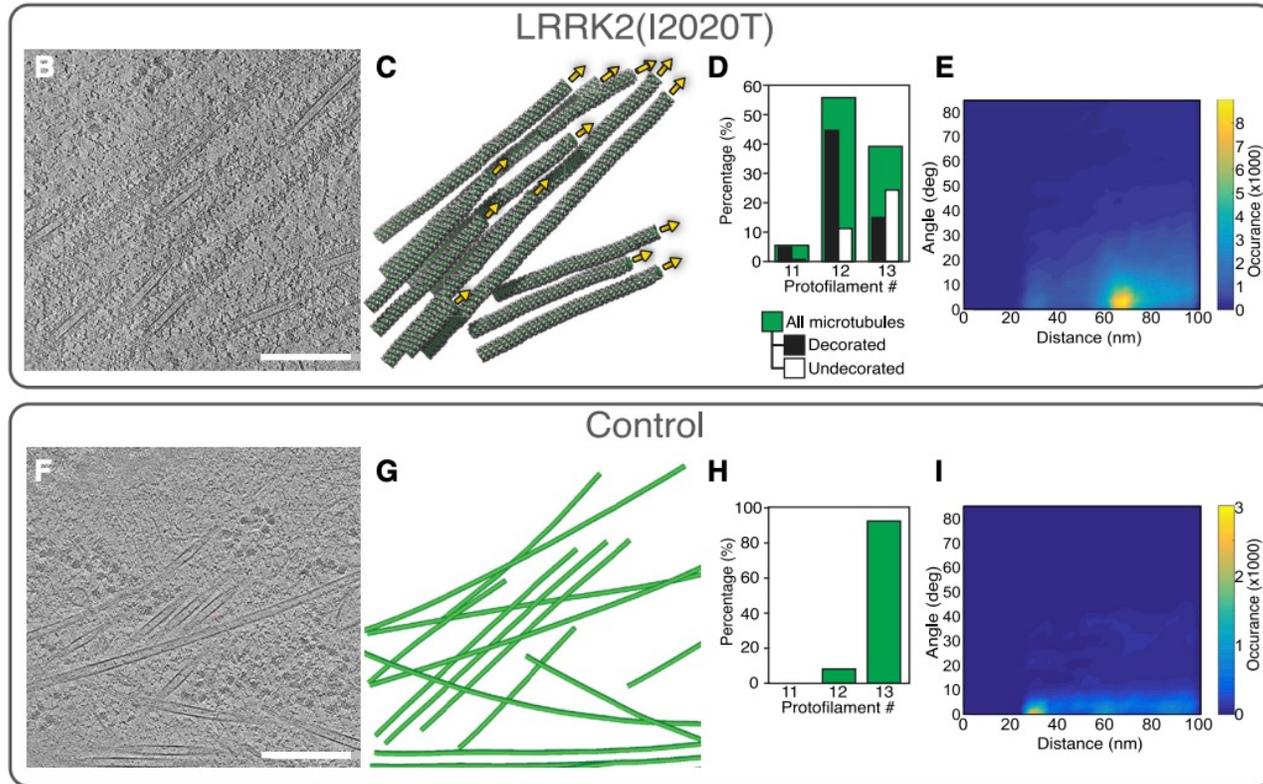


Segmentation for subtomogram averaging

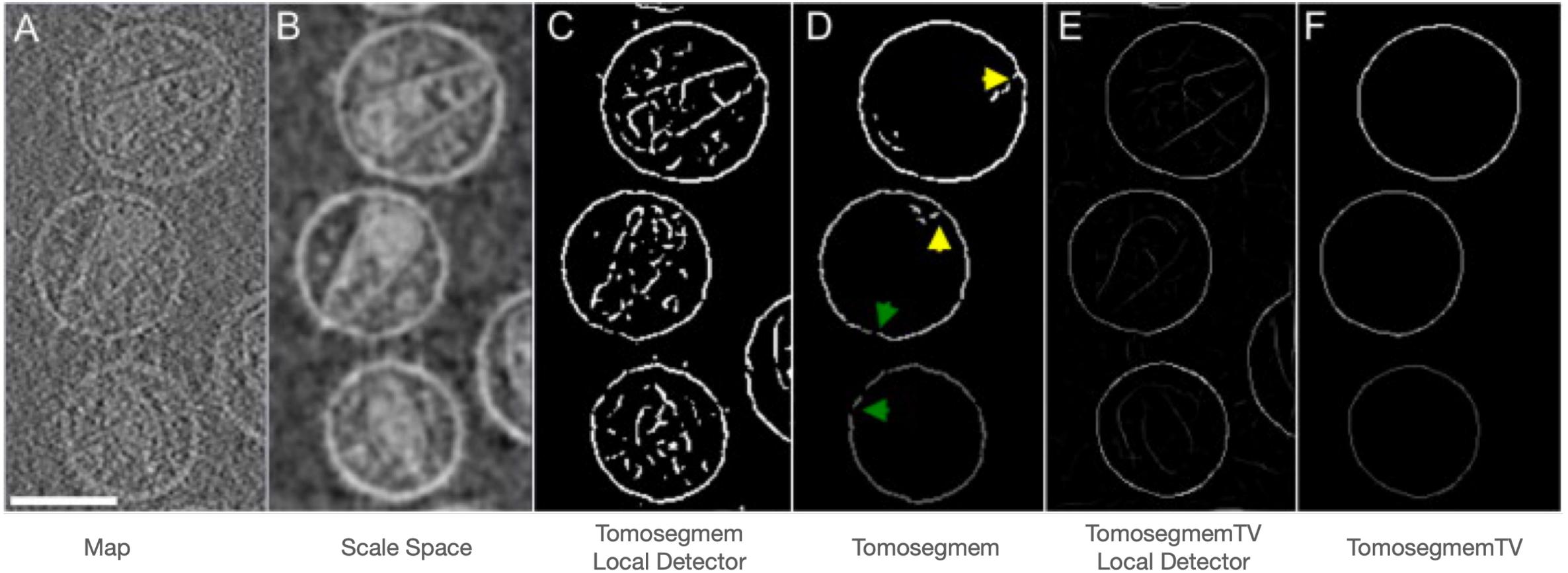


Castaño-Díaz et al, 2017
Burt et al. 2021

Segmentation for subtomogram averaging

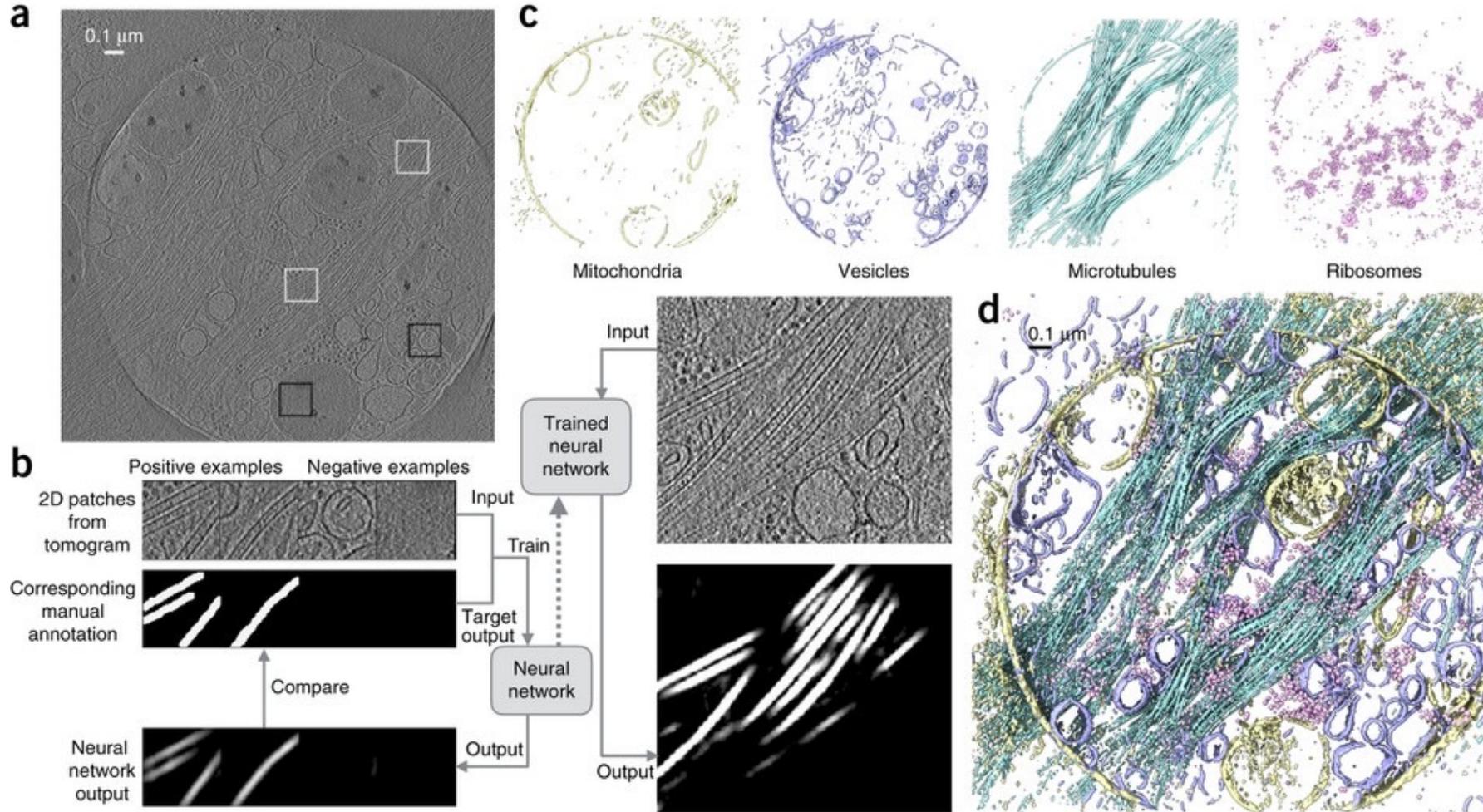


Today's Tools for Segmentation



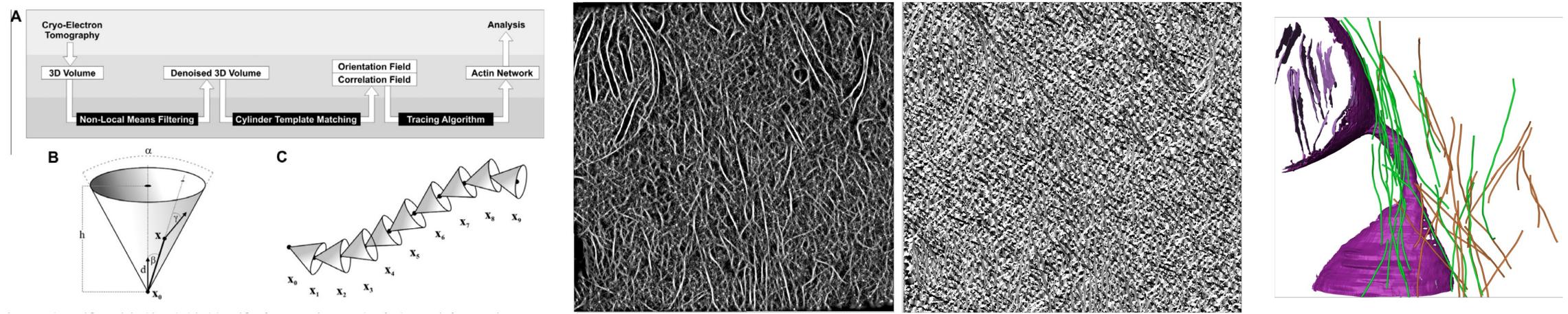
Tomosegmemtv
Martinez-Sanchez et al 2013

Today's Tools for Segmentation



EMAN2 Neural Net Annotation
Chen M et al 2017

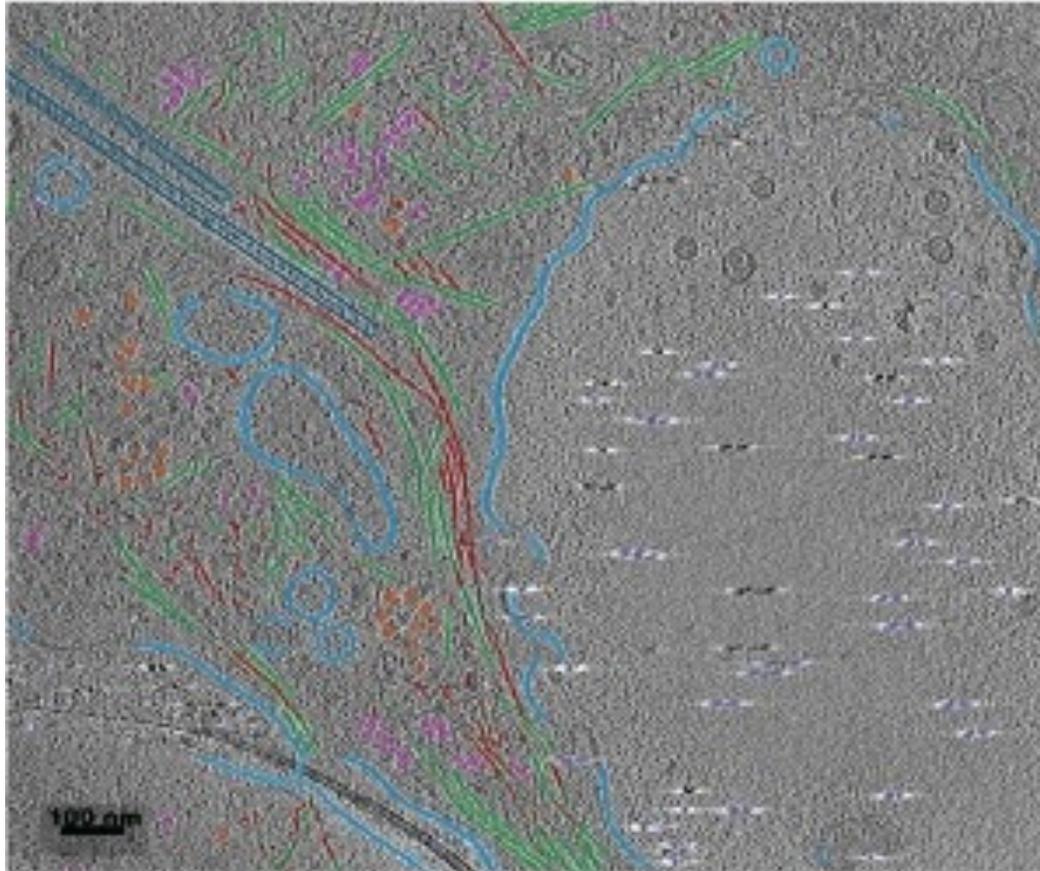
Today's Tools for Segmentation



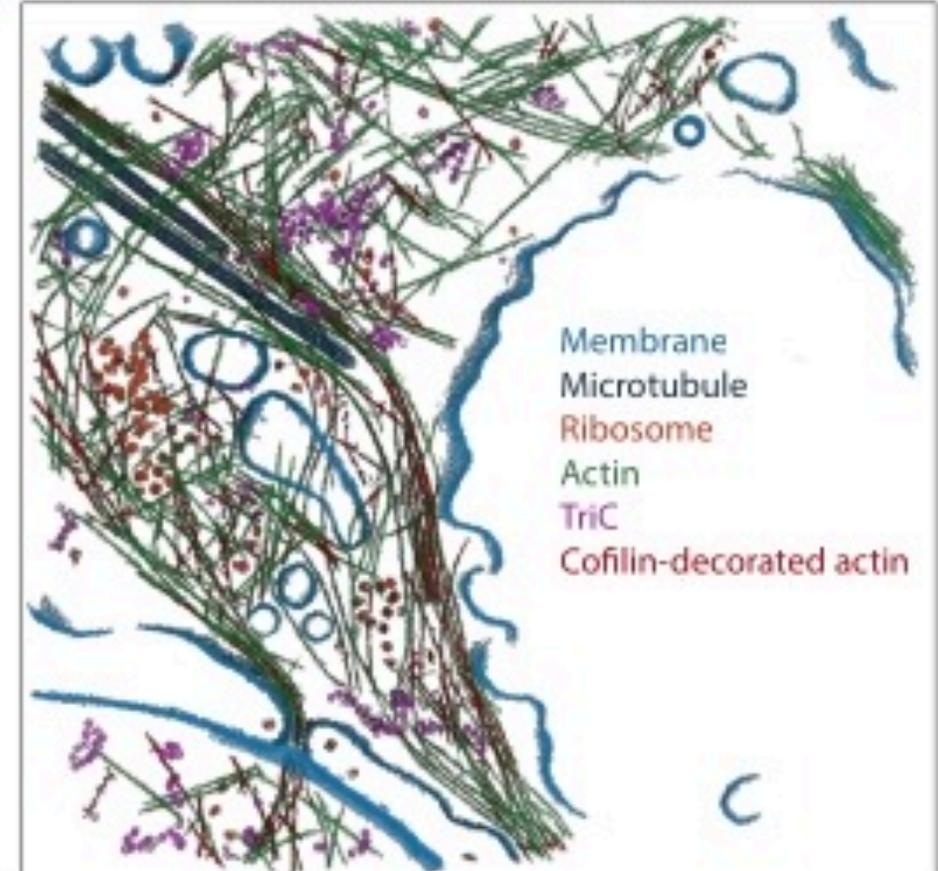
Xfiber Workflow
Rigort A et al 2012

Segmentation tomorrow and beyond

UNet Segmented Tomogram

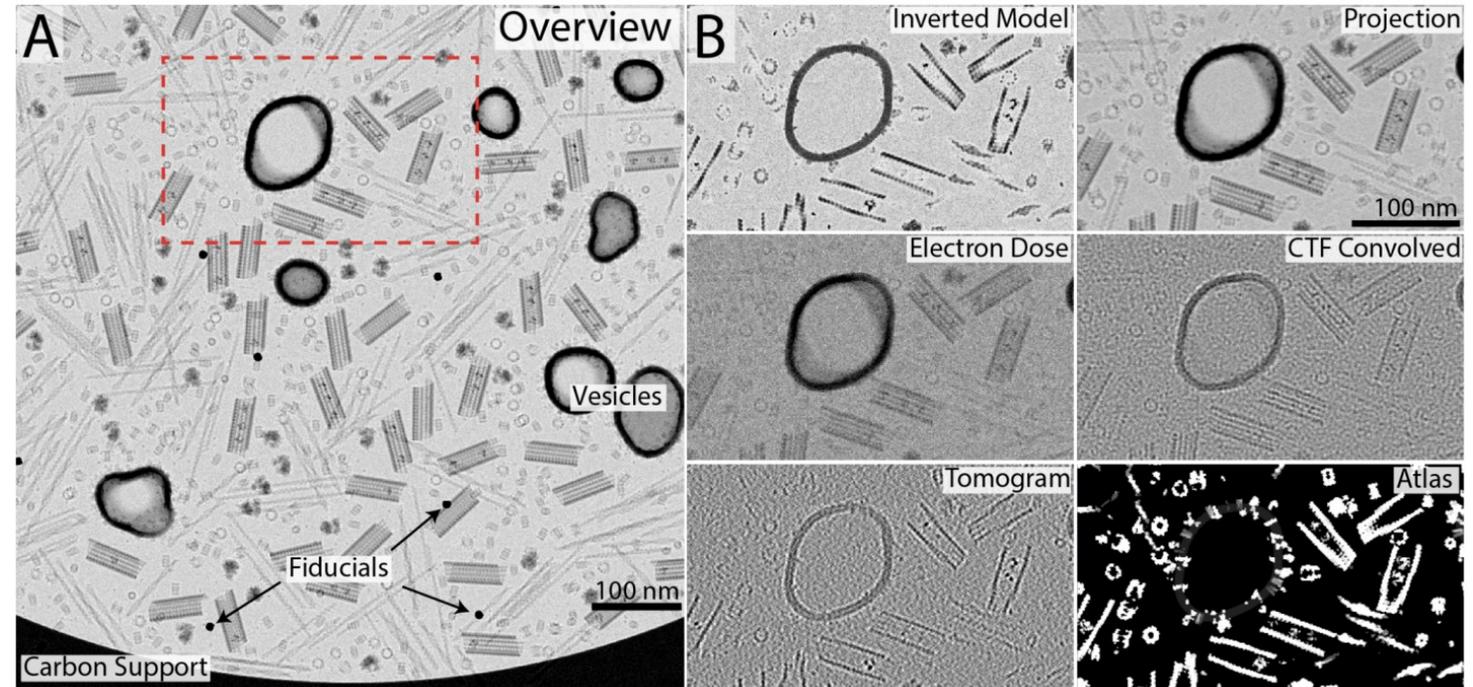
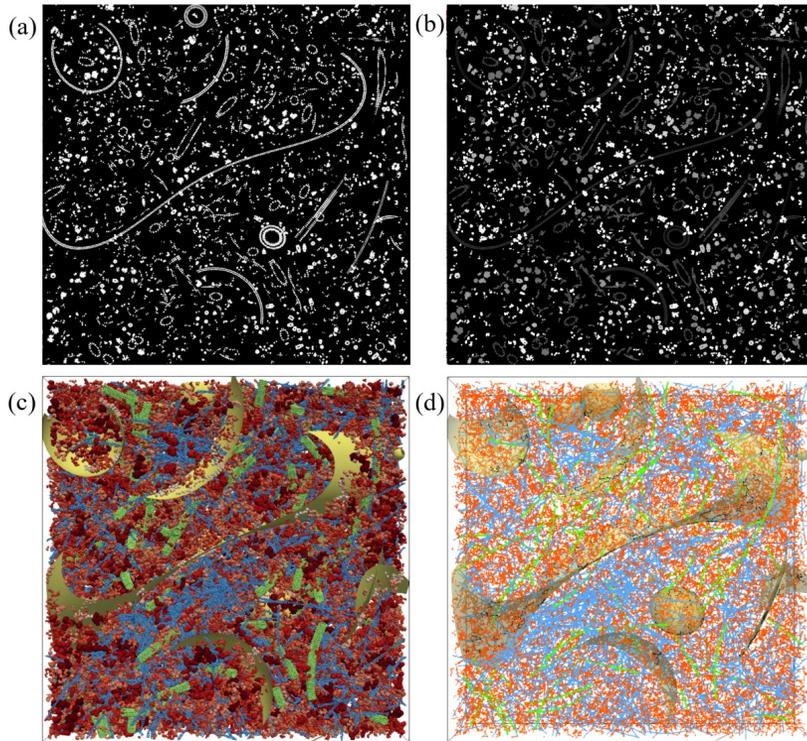


3D Rendered Segmentation



Dragonfly Workflow
Heebner et al 2022

Segmentation tomorrow and beyond



Simulation Tools
Martinez-Sanchez et al 2023
Purnell et al 2023

Questions?



Thanks to the Grotjahn Lab and especially Michaela Medina, Rachel Lian, and Misha Le Claire



Shameless Advertisement!



Barad Lab opening January 2024 at OHSU in Portland, OR
Hiring technicians, graduate students, and postdocs