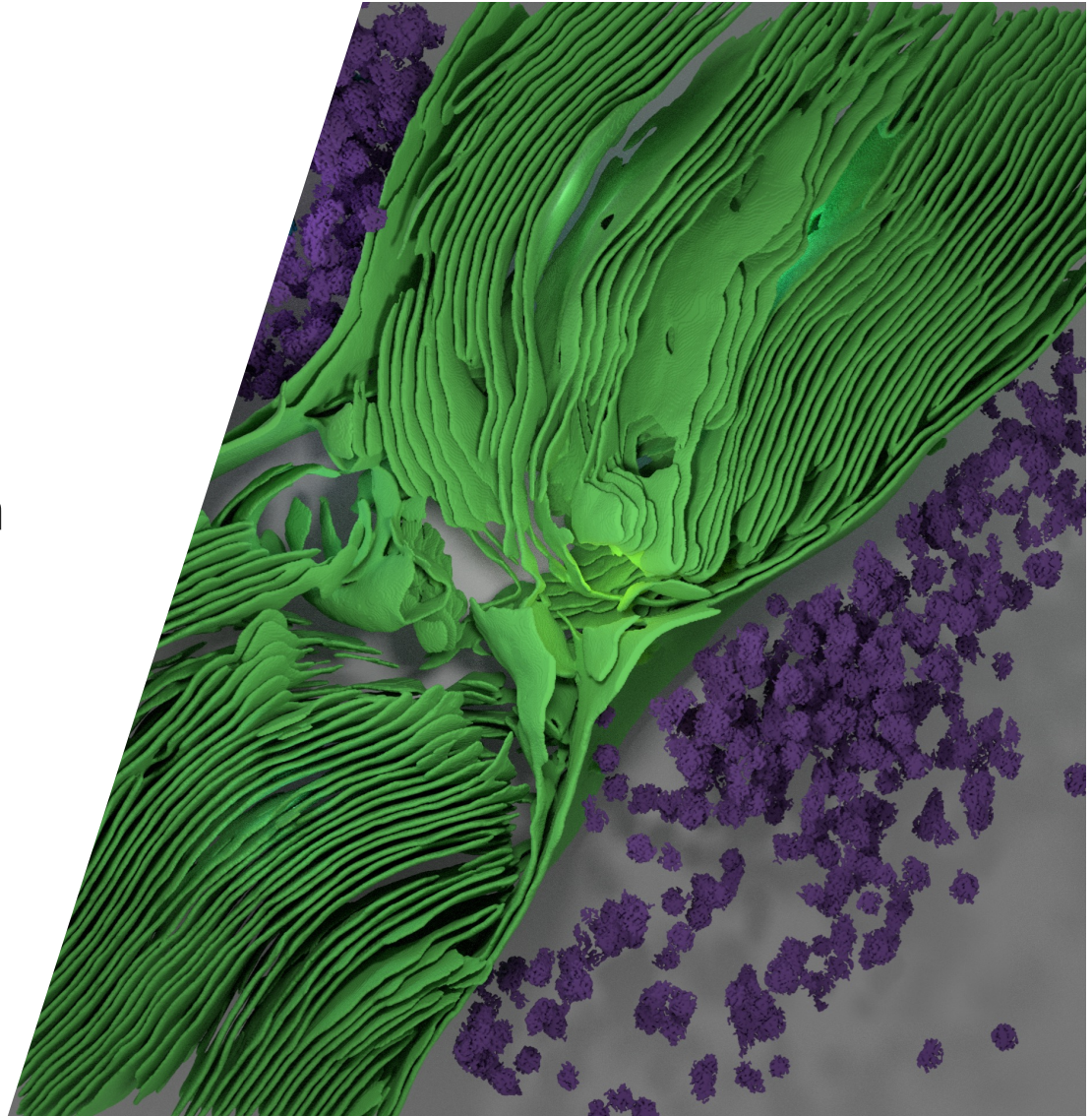


Segmentation of Cryo-Electron Tomograms

Jessica Heebner

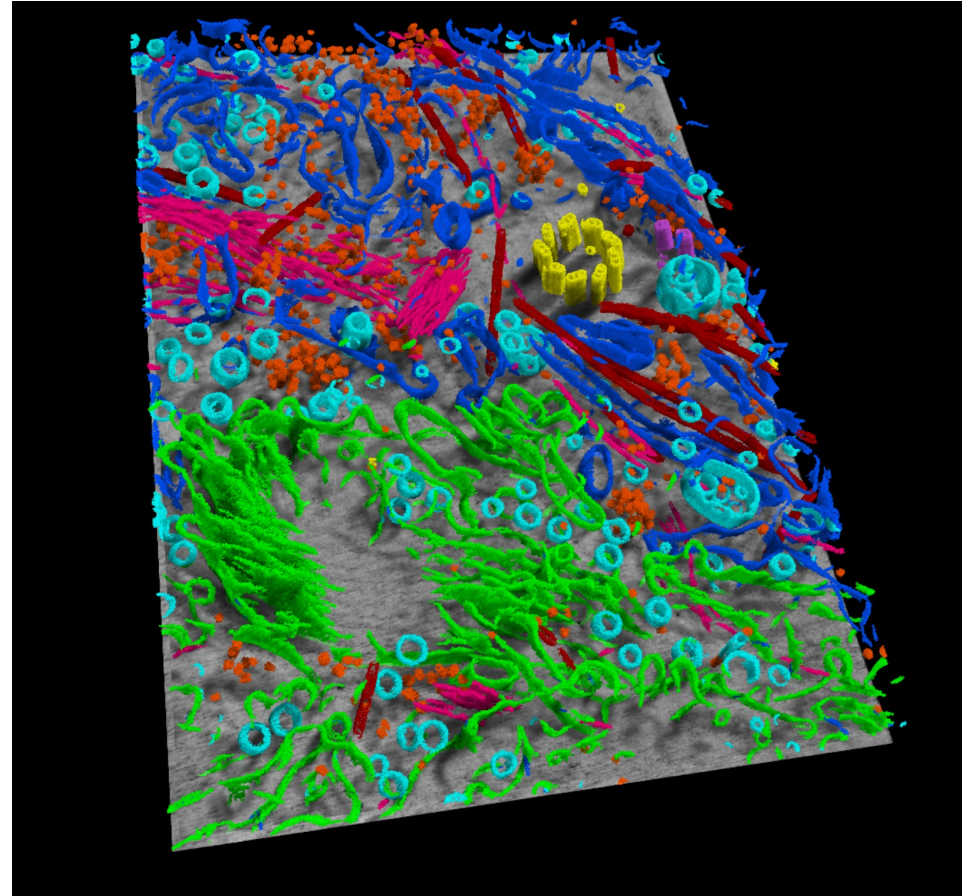
June 6, 2025

 The world leader in serving science



The Plan

- 1 Introduction
- 2 The Project That Started It All
- 3 Segmentation Fundamentals
- 4 Why Segment?
- 5 Useful Resources



Data courtesy of Petr Chlanda, University of Heidelberg

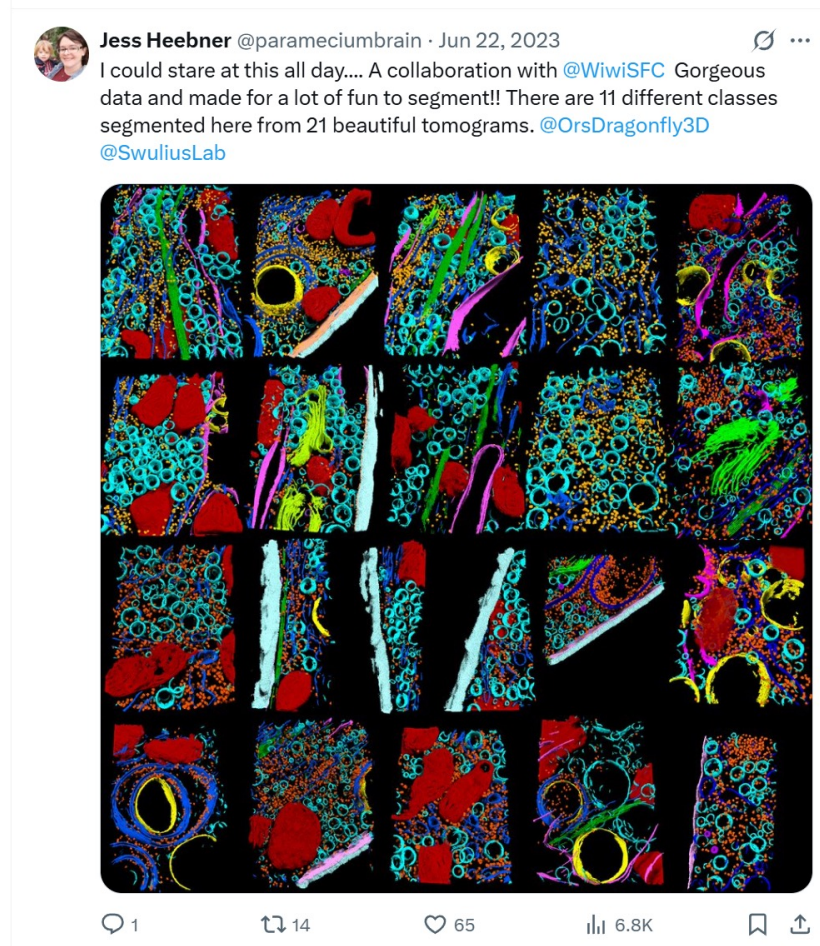
Who am I?

- Veterinary Oncology Nurse

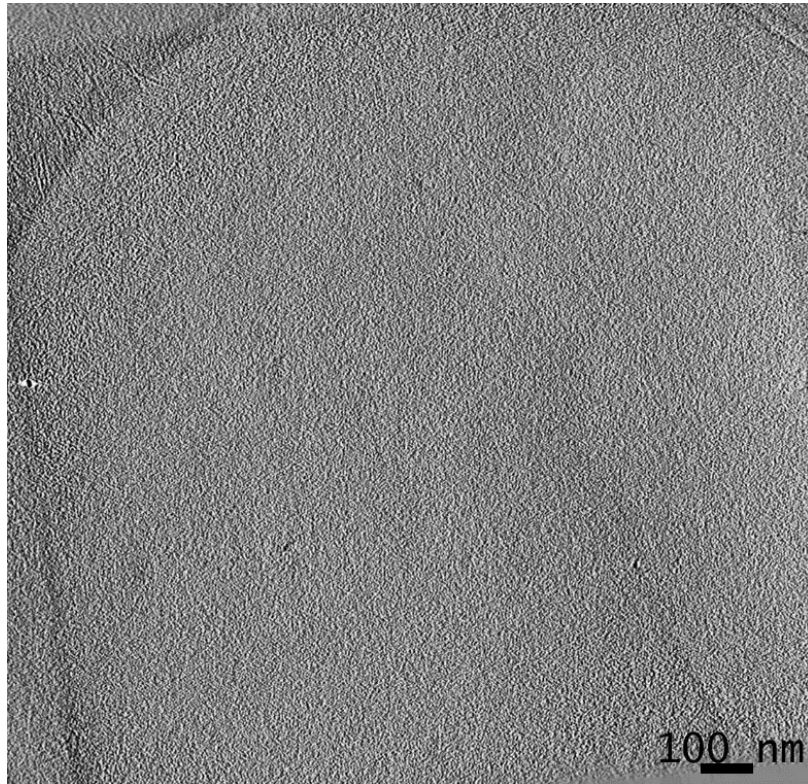
- Graduated July 2023



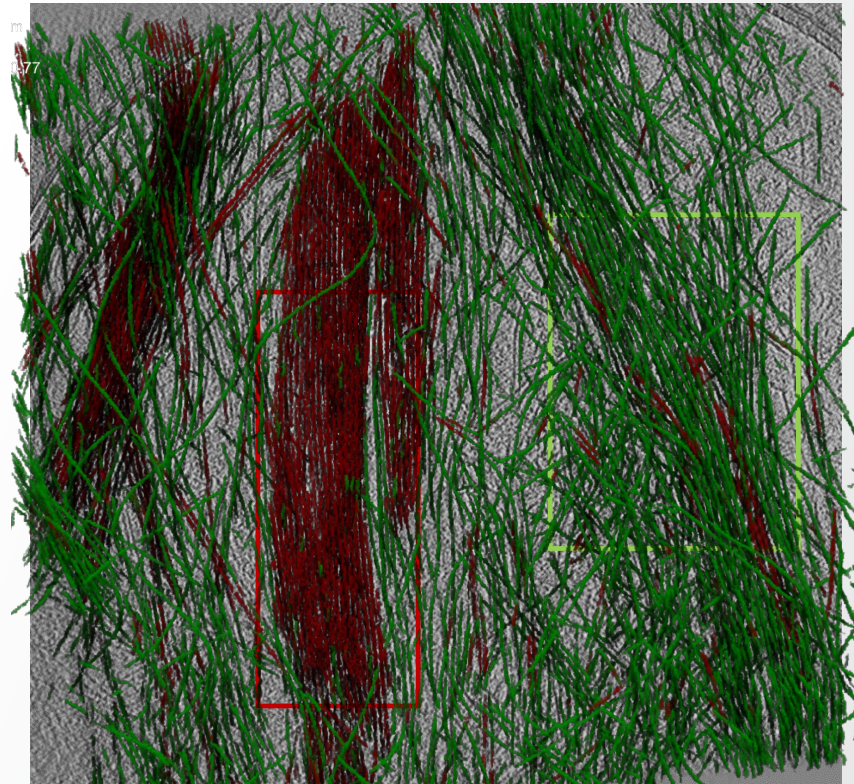
- Joined the Apps Team Sept 2023

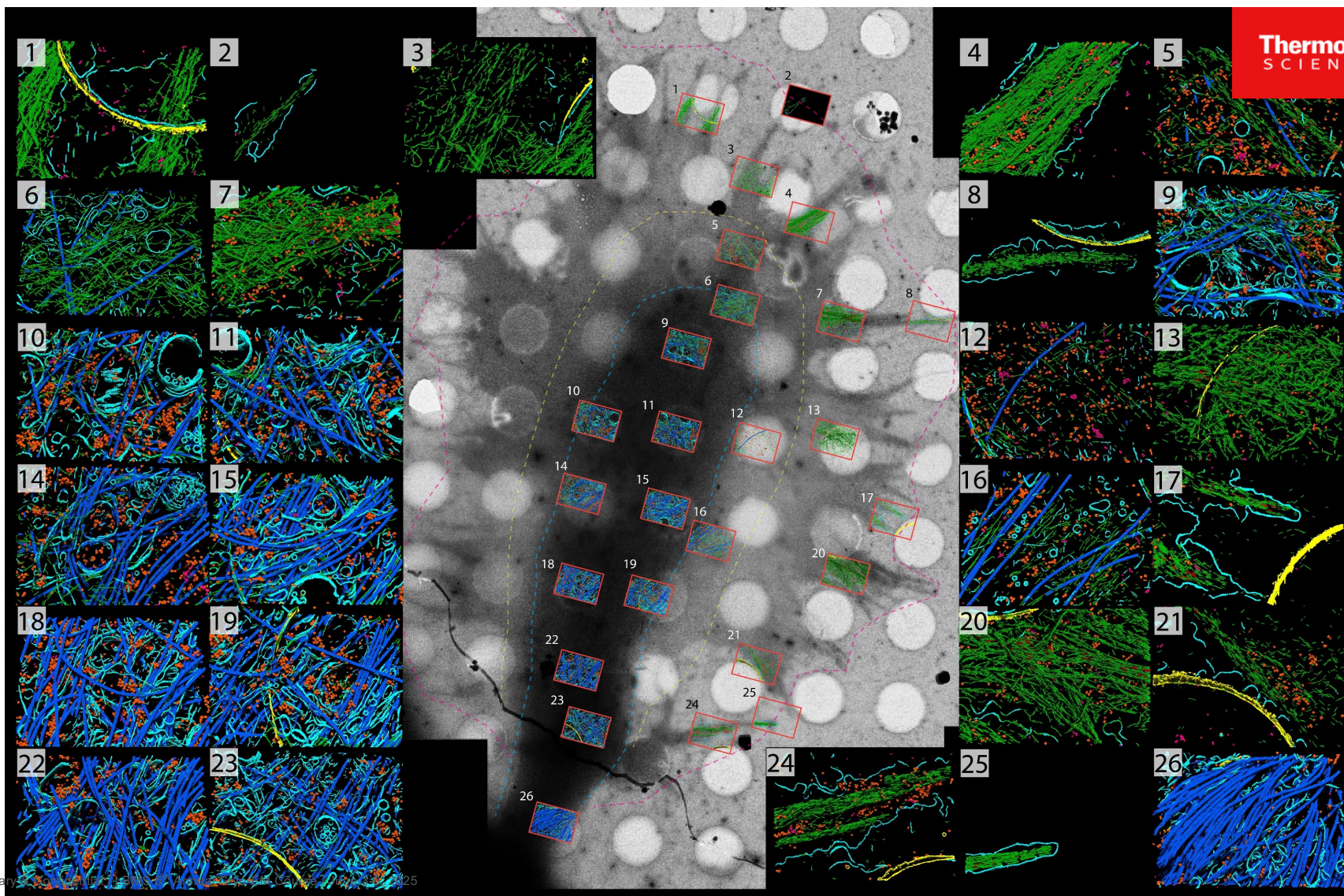


How did I get started?



Data courtesy of Matt Swulius, Penn State College of Medicine





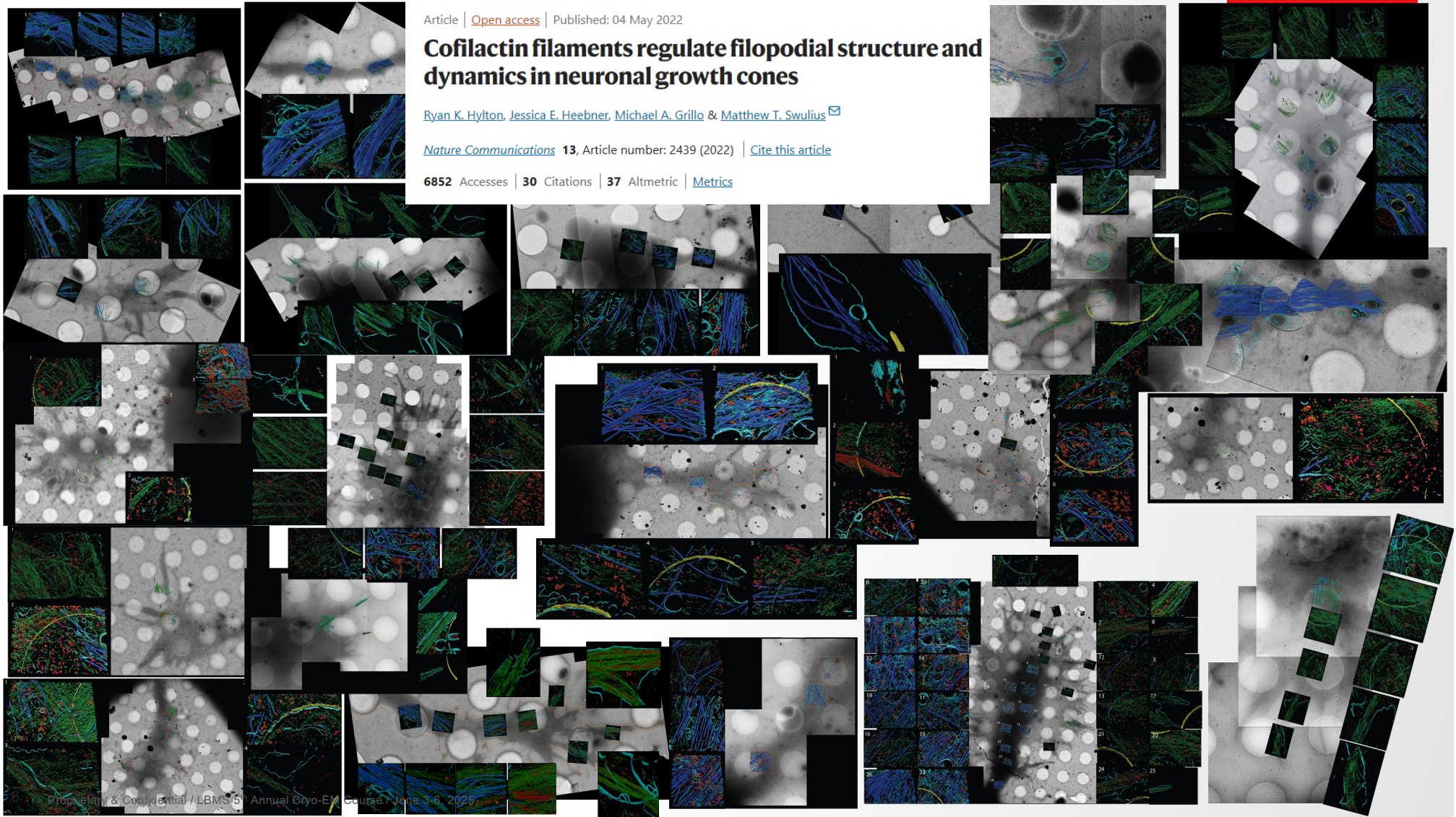
Article | [Open access](#) | Published: 04 May 2022

Cofilactin filaments regulate filopodial structure and dynamics in neuronal growth cones

Ryan K. Hylton, Jessica E. Heebner, Michael A. Grillo & Matthew T. Swulius 

Nature Communications **13**, Article number: 2439 (2022) | [Cite this article](#)

6852 Accesses | 30 Citations | 37 Altmetric | [Metrics](#)



Where should you start?

Terminology

What exactly is segmentation?

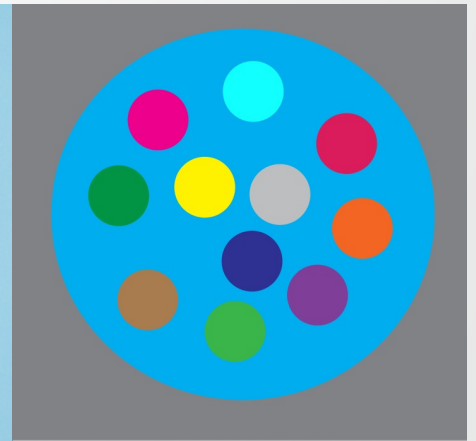
Semantic Segmentation



Instance Segmentation

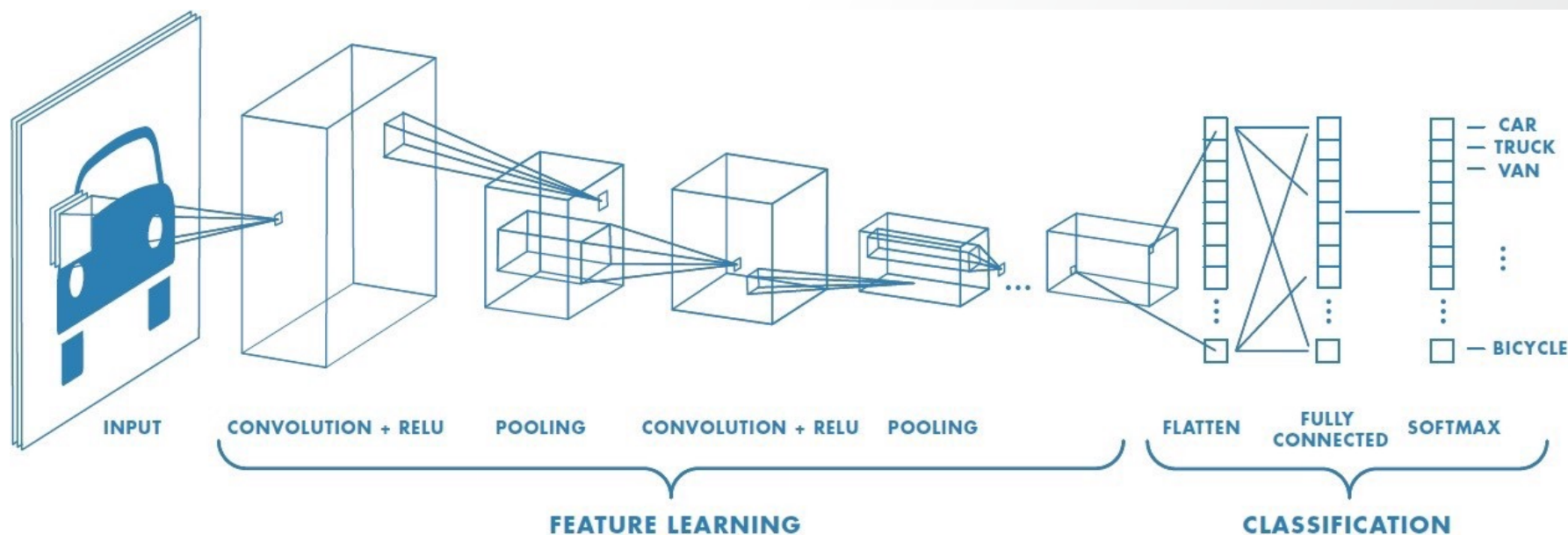


Panoptic Segmentation

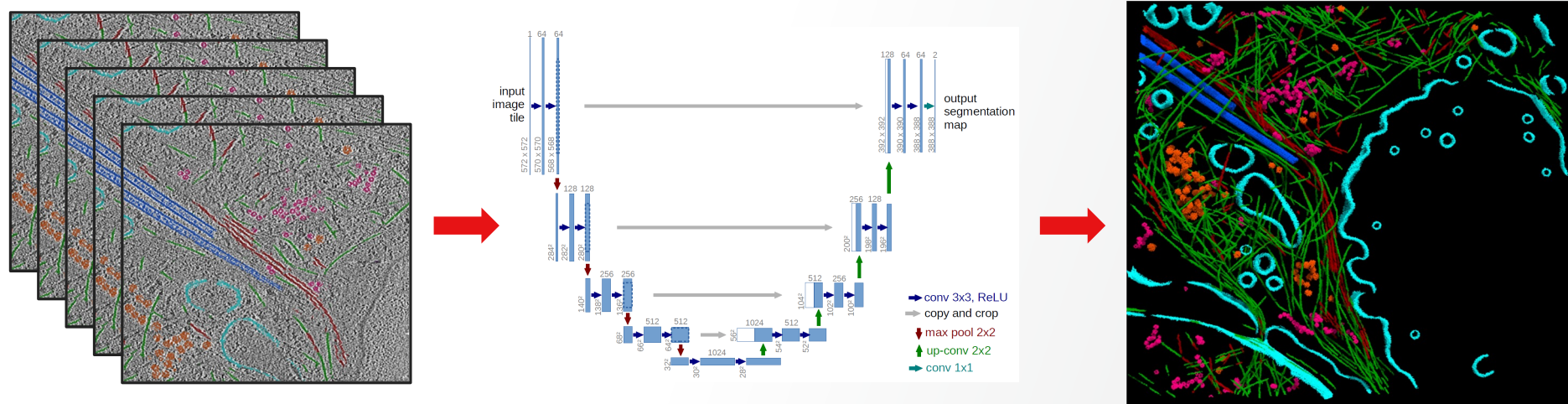


Deep Learning

Convolutional Neural Networks excel at identifying features and labeling images



Basic Training Workflow



Data courtesy of Matt Swulius, Penn State College of Medicine

Open Source Options



- Napari – [Installation guide](#)
 - Python based image viewer
 - Manual and deep learning segmentation plugins



- IMOD – [Installation guide](#)
 - A suite of software for tomogram reconstruction and processing
 - Manual segmentation tools and a custom CNN that can be trained with your data



- Fiji/ImageJ – [Download page](#)
 - Software for processing and analyzing scientific images
 - Manual and deep learning segmentation plugins

Commercial Options

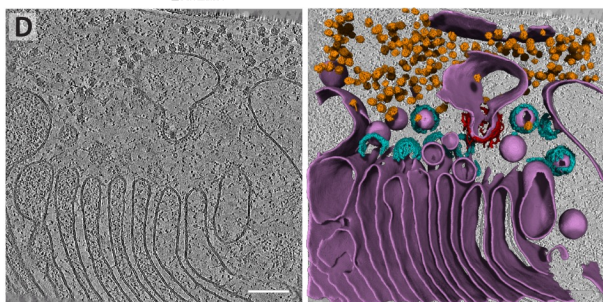
Thermo Fisher Scientific

ThermoFisher
SCIENTIFIC

Amira™ 3D

Dedicated segmentation workroom with tools for manual and AI assisted annotation, as well as the option to train your own CNNs.

- [Free Trial](#)

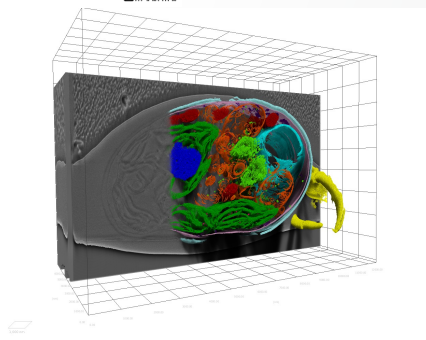


Comet Technologies

dragonfly 3D World

Built in segmentation tools for both manual annotation and a framework for training your own CNNs.

- [Free Trial](#)

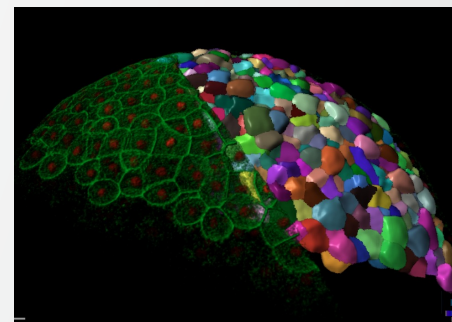


Oxford Instruments

Imaris



Offers segmentation tools and the ability to train CNNs for your own data.

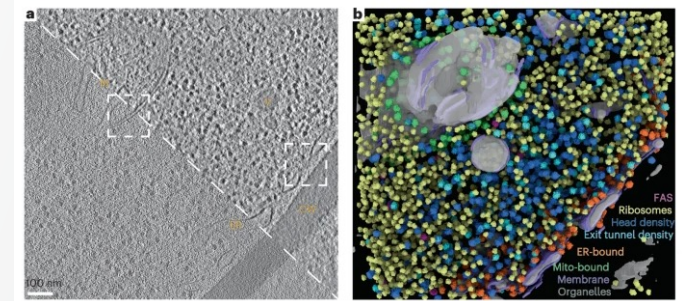
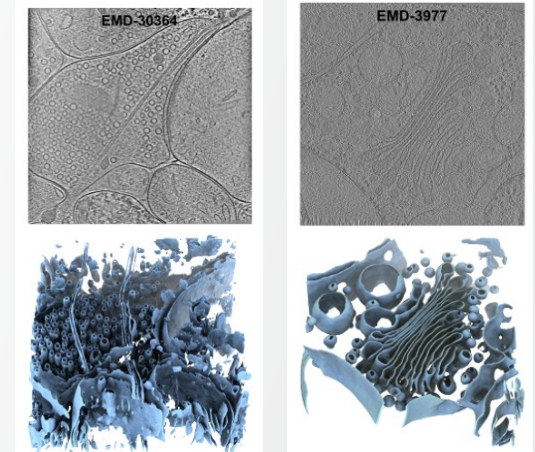
- [Free Trial](#)



Out of the box solutions?

Very few solutions exist that don't require you to provide training data

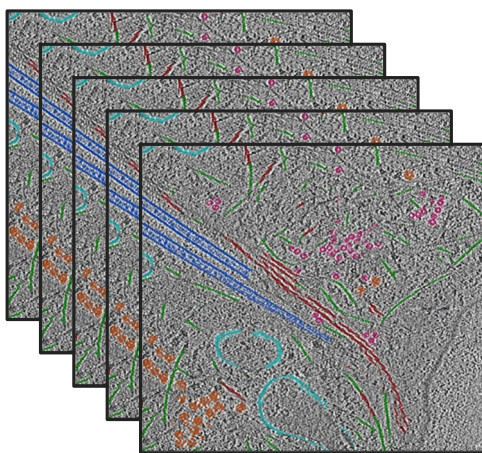
- **Membrain-Seg V2** – [Github repo](#) 
 - 3D UNet for segmenting membranes in cryoET data.
 - Pretrained network is provided that works well on many datasets.
 - Can also be trained using your own data.
- **DeePiCt** – [Github repo](#) 
 - 2D UNet for segmenting cellular compartments and 3D UNet for segmenting continuous structures (membrane and filaments) and particle localization in cryoET data.
 - Pretrained networks are provided that are shown to work on datasets the networks are naïve to in the paper
 - Can also be trained using your own data.



I've chosen a software, now what?

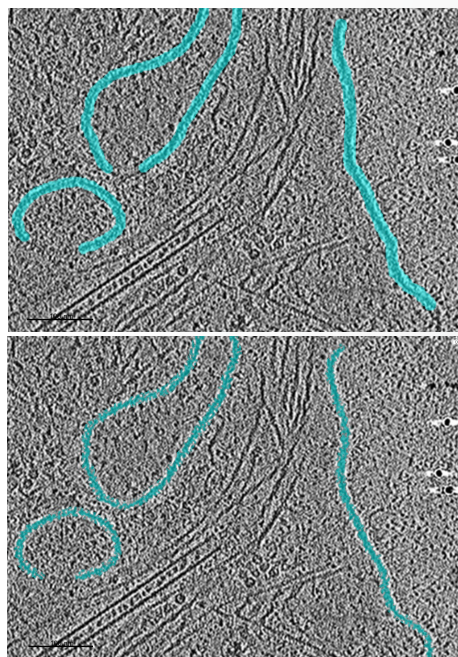
A CNN is only as good as its training data.

- How much is necessary?

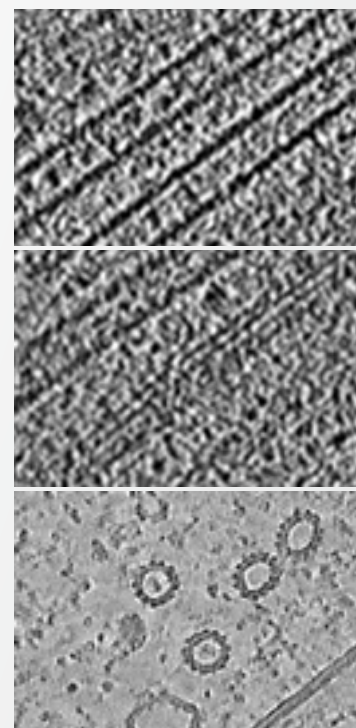


Start with less, but more is generally better. 3-5 slices usually sufficient for a 2D or 2.5D CNN.

- Accurate



- Representative



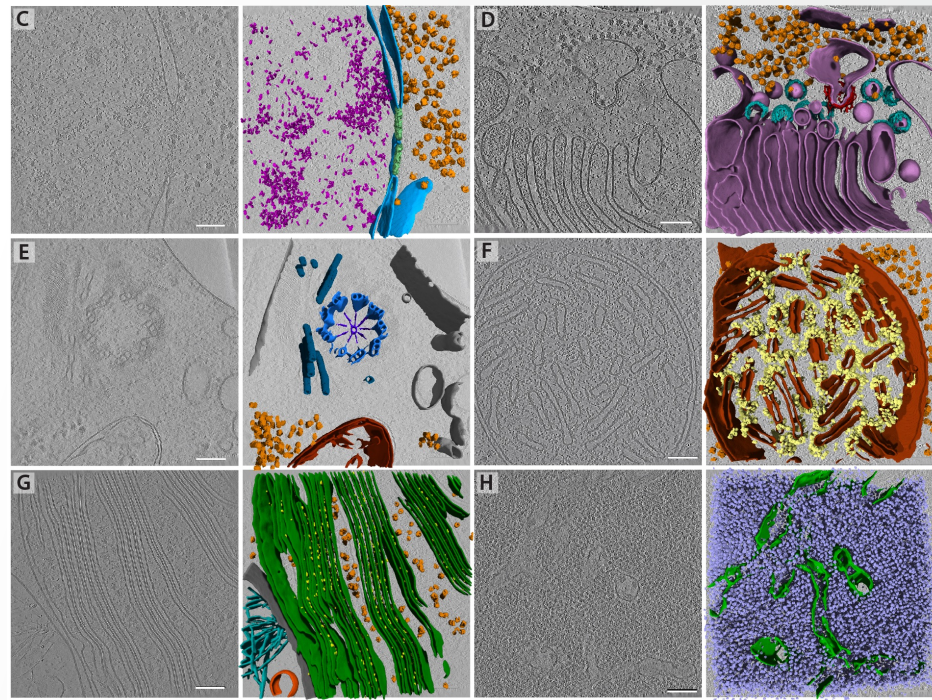
What network should I train?

You name it, a UNet exists for it...

- UNet
- 3D UNet
- Attention UNet
- Dense UNet
- Trans UNet
- UNet++
- UNet3+
- Inception UNet
- TernaUSNet
- R2-UNet
- nnUNet (“No New UNet”)
- ... you get the idea

For cryoET data, in most cases a basic UNet performs excellently.

* With one caveat

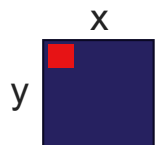


<https://www.biorxiv.org/content/10.1101/2024.12.28.630444v1>



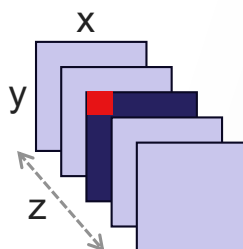
UNet Dimension is Important

2D



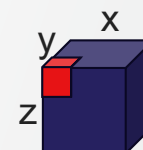
- The network sees and segments small portions of one slice of data at a time.
- **Patch size** of a network defines how big the “small portion” is in X and Y

2.5D



- The network segments one slice of data at a time but can see pixel intensity from one or two slices above and below to make a more informed segmentation decision.
- **Patch size** defines the same area as in 2D network.

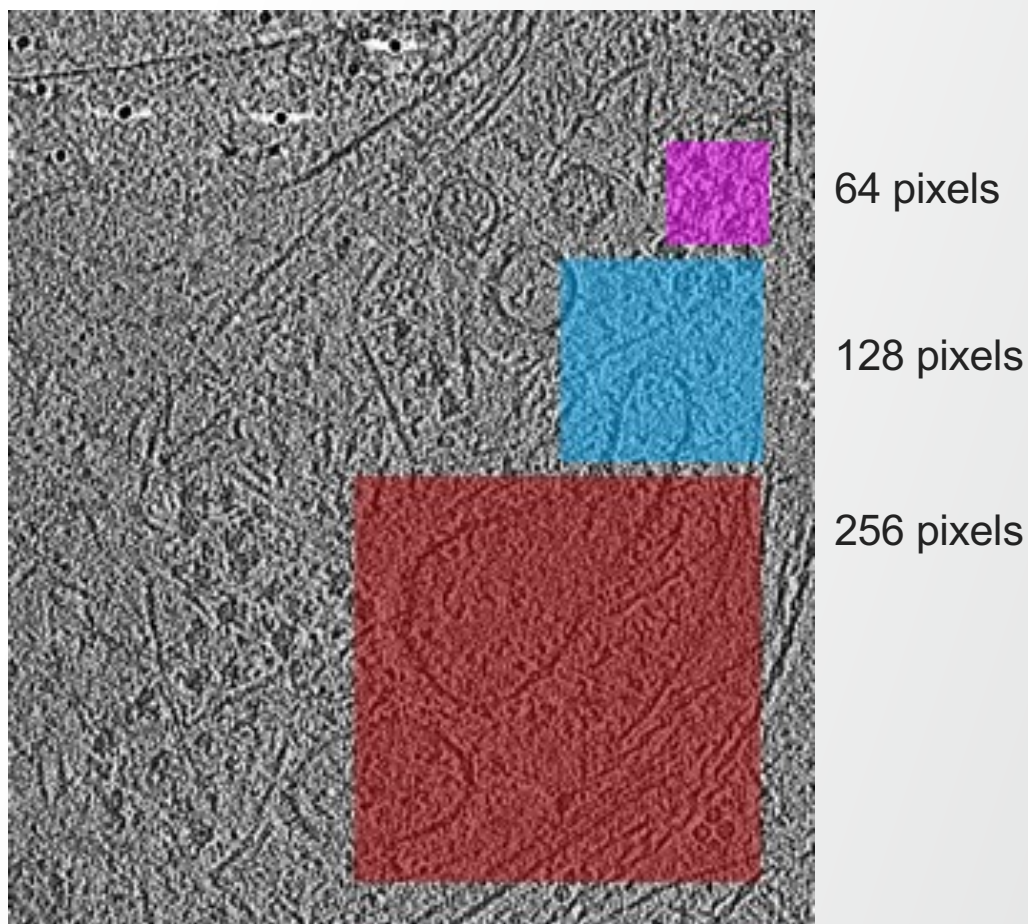
3D



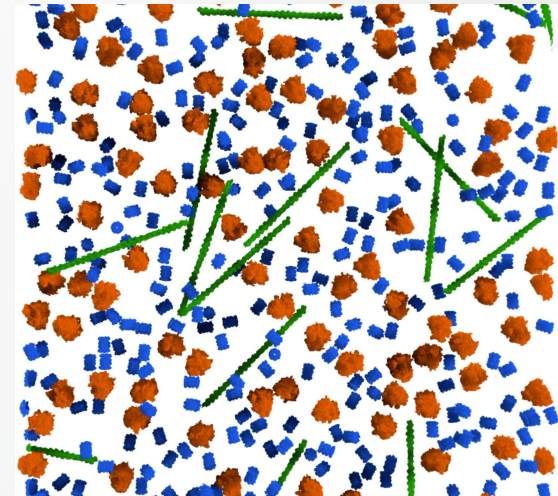
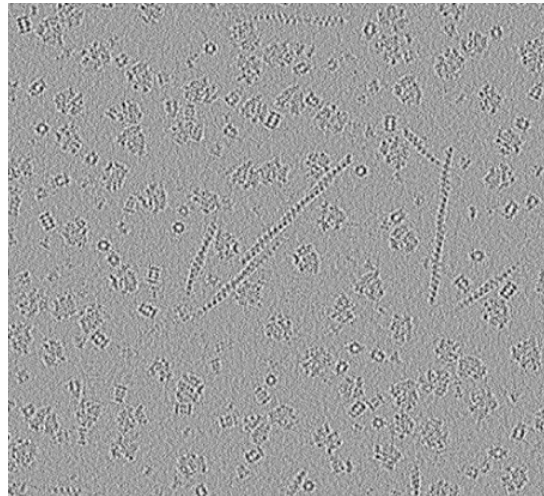
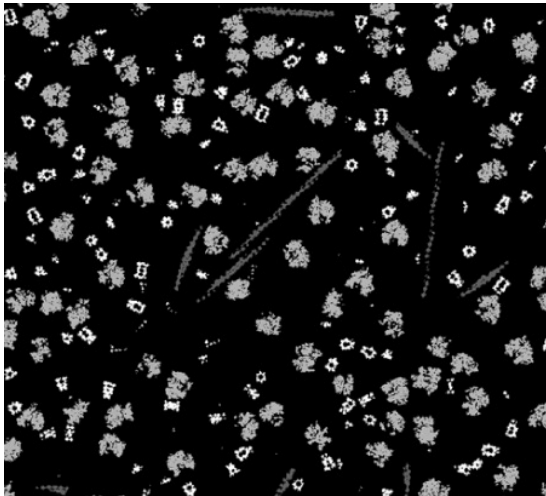
- The network sees and segments a full cube of data.
- **Patch size** defines the 3D cube.

How do I determine the right patch size?

- More context is better
- Trade off computationally
 - Fewer resources required to train a network with small patch size
 - Try to find the largest patch size that will reliably train on the computer you are using.



What about synthetic training data?



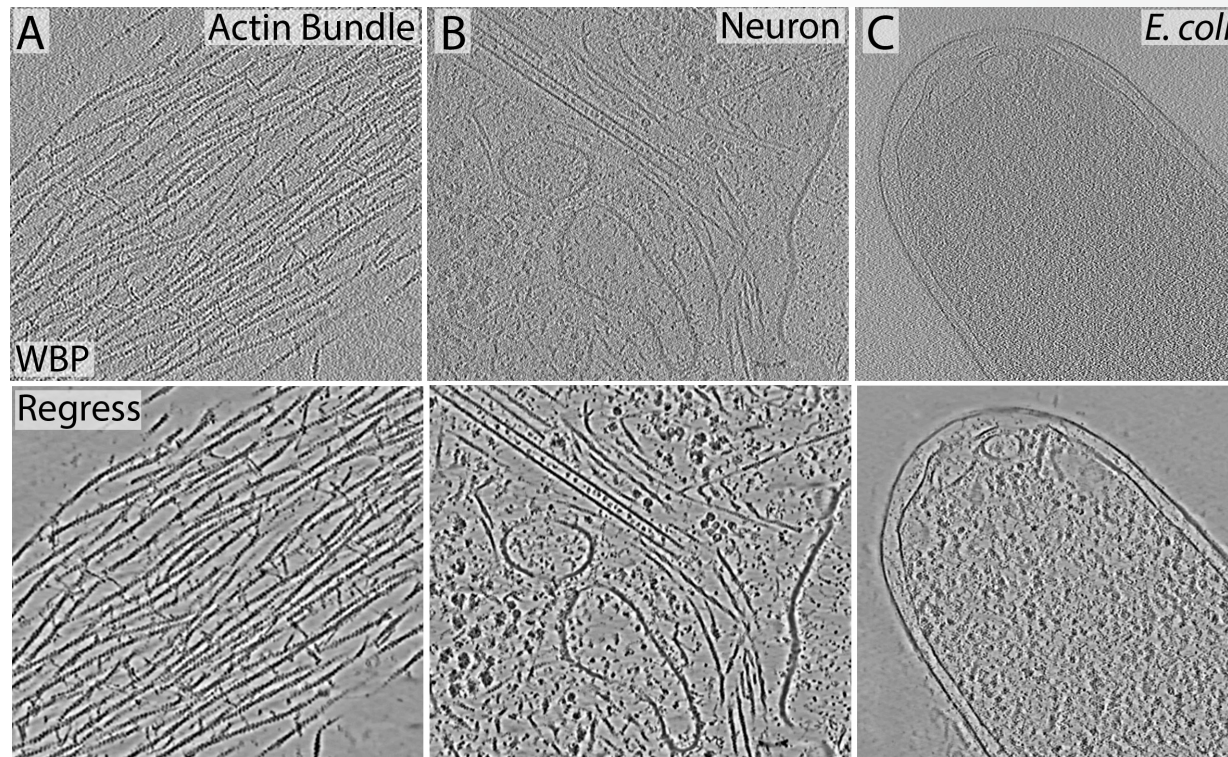
[PolNet](#)



[CryoTomoSim](#)

Denoising?

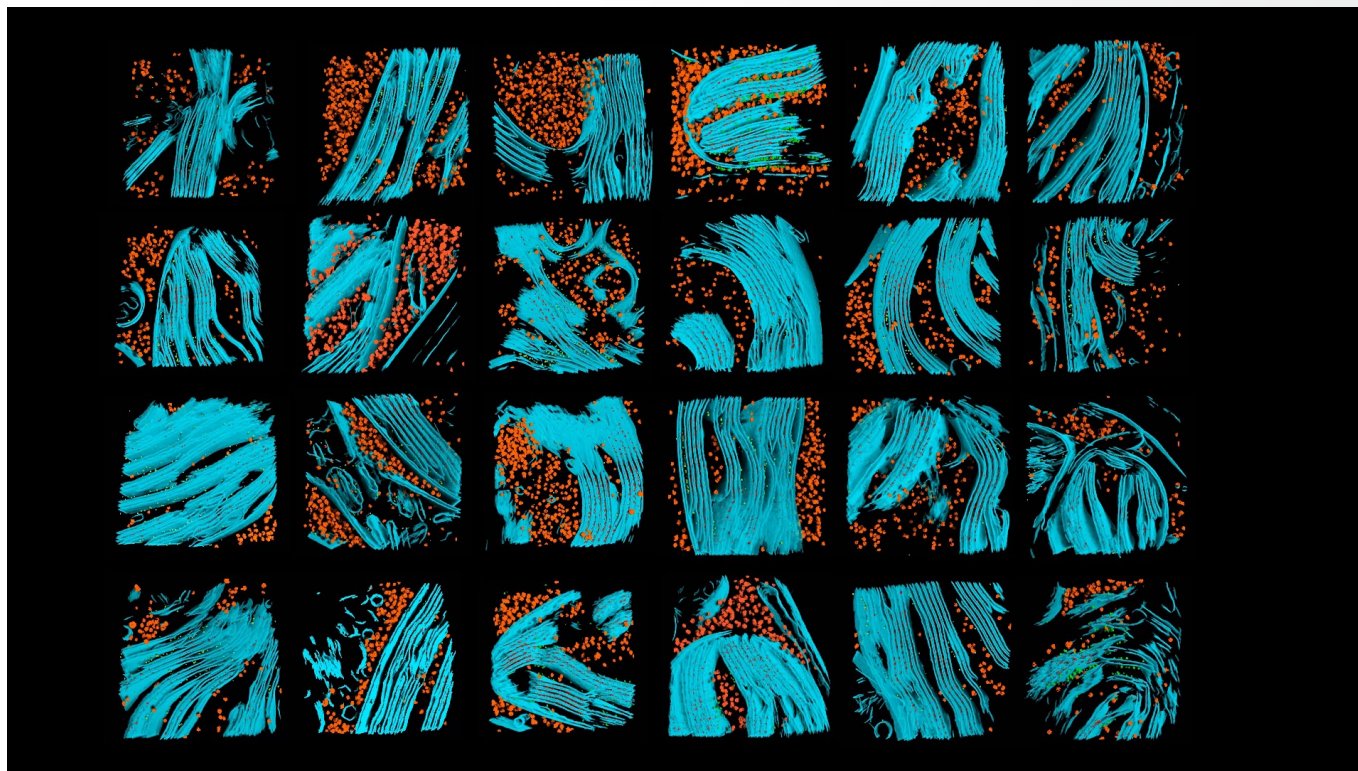
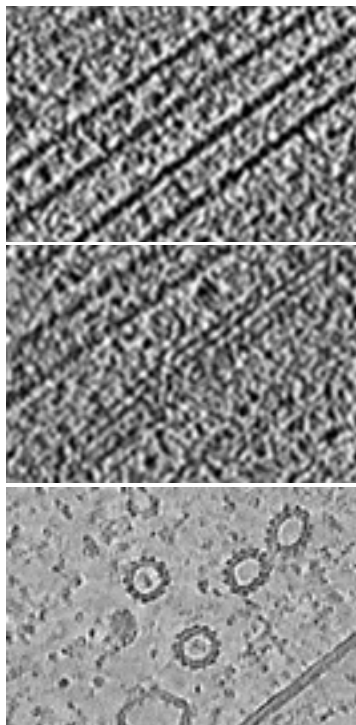
Should you denoise your data before segmentation?



- Useful for manual annotation, not necessary for accurate segmentation

How to optimize for inference/generalizability?

- Representative



- Inference relies on a representative training set, often requiring training data from multiple tomograms

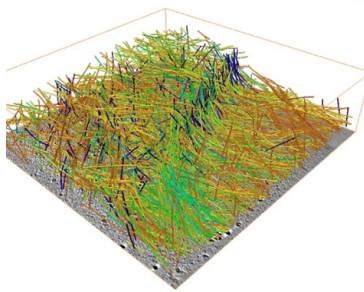
Data from EMD 11830

Instance Segmentation?

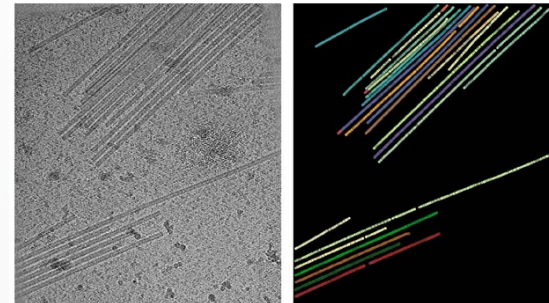
Instance Segmentation



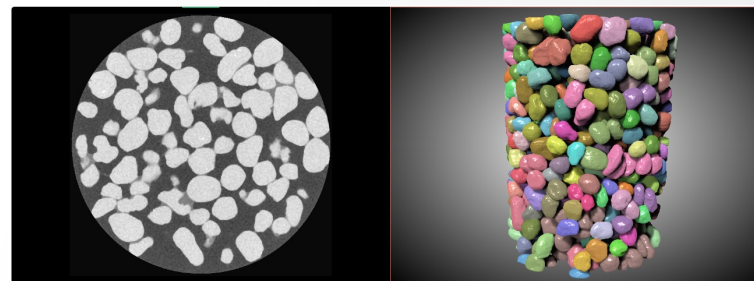
Amira 3D



TARDIS

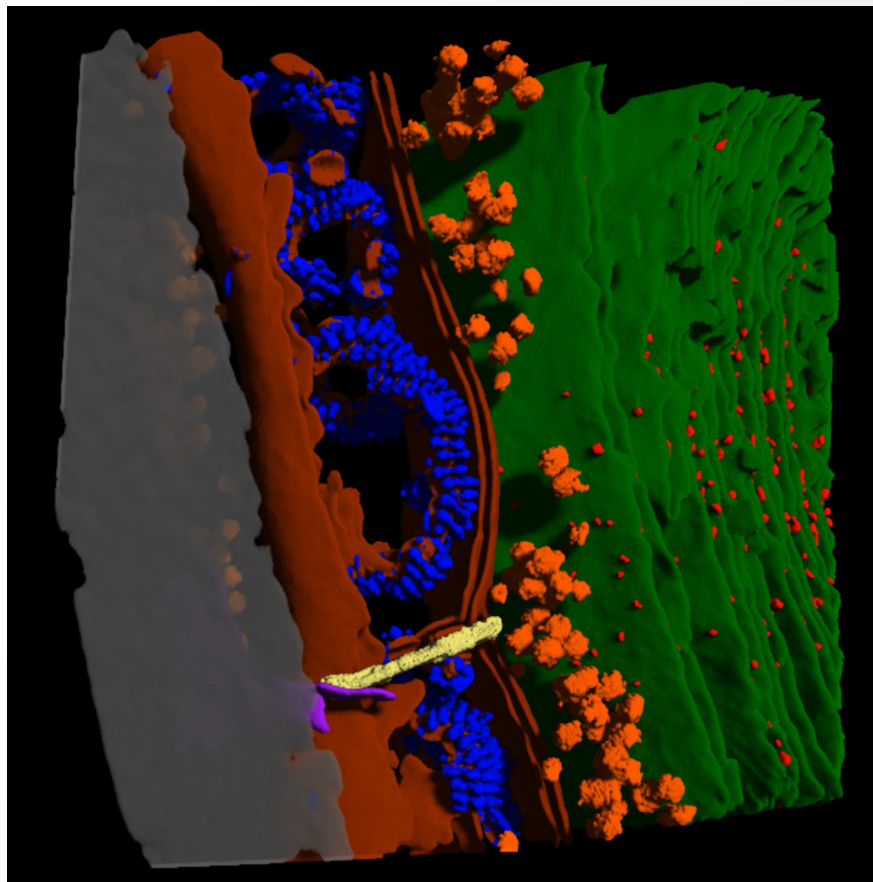


Watershed Transform



What do you do once the data is segmented?

- When we see more, we learn more
- Analysis requires localization
- CryoET data is exponentially more engaging when it is viewed in 3D

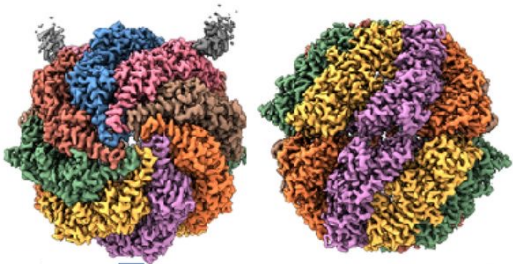


Data from EMD 11830

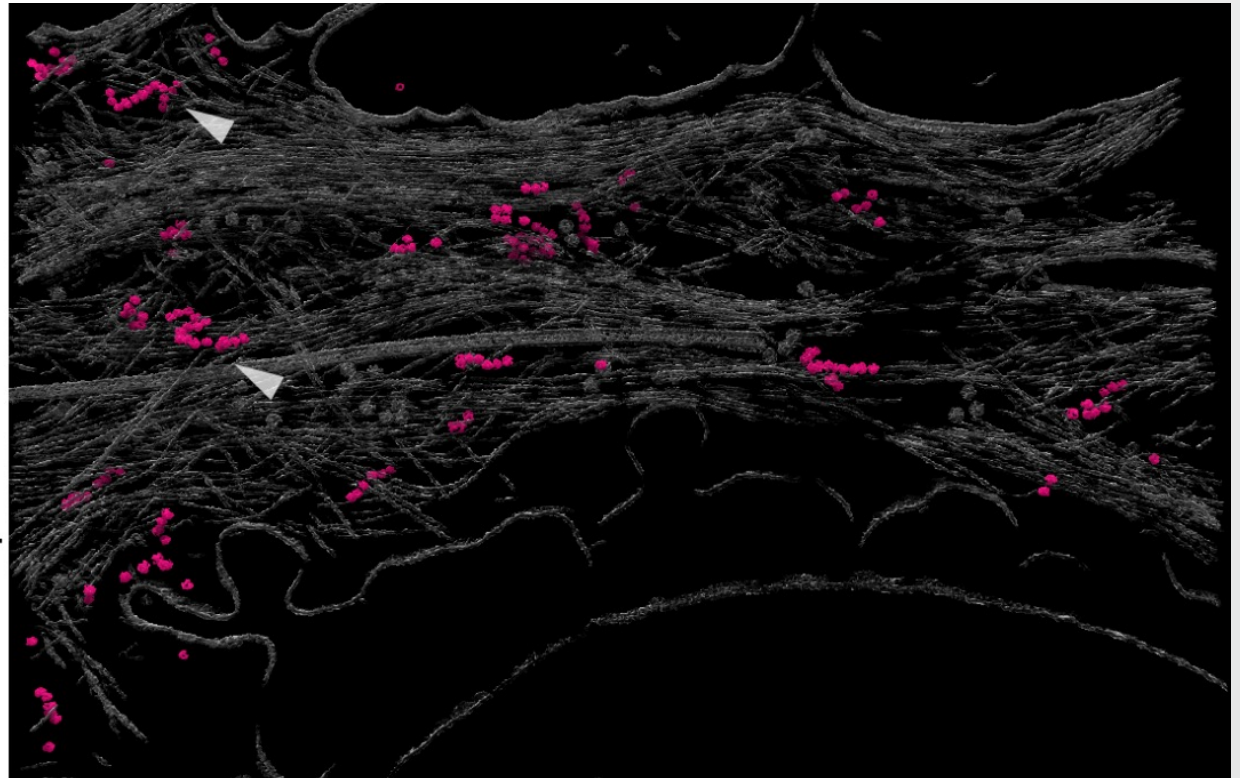
When we see more, we learn more

~~T-complex protein Ring Complex (TriC)
or Chaperonin Containing Tailless
complex polypeptide 1 (CCT)~~

TRiC/CCT



Peripheral Domain



Data courtesy of Matt Swulius, Penn State College of Medicine

Respirasomes

RESEARCH ARTICLE | CELL BIOLOGY

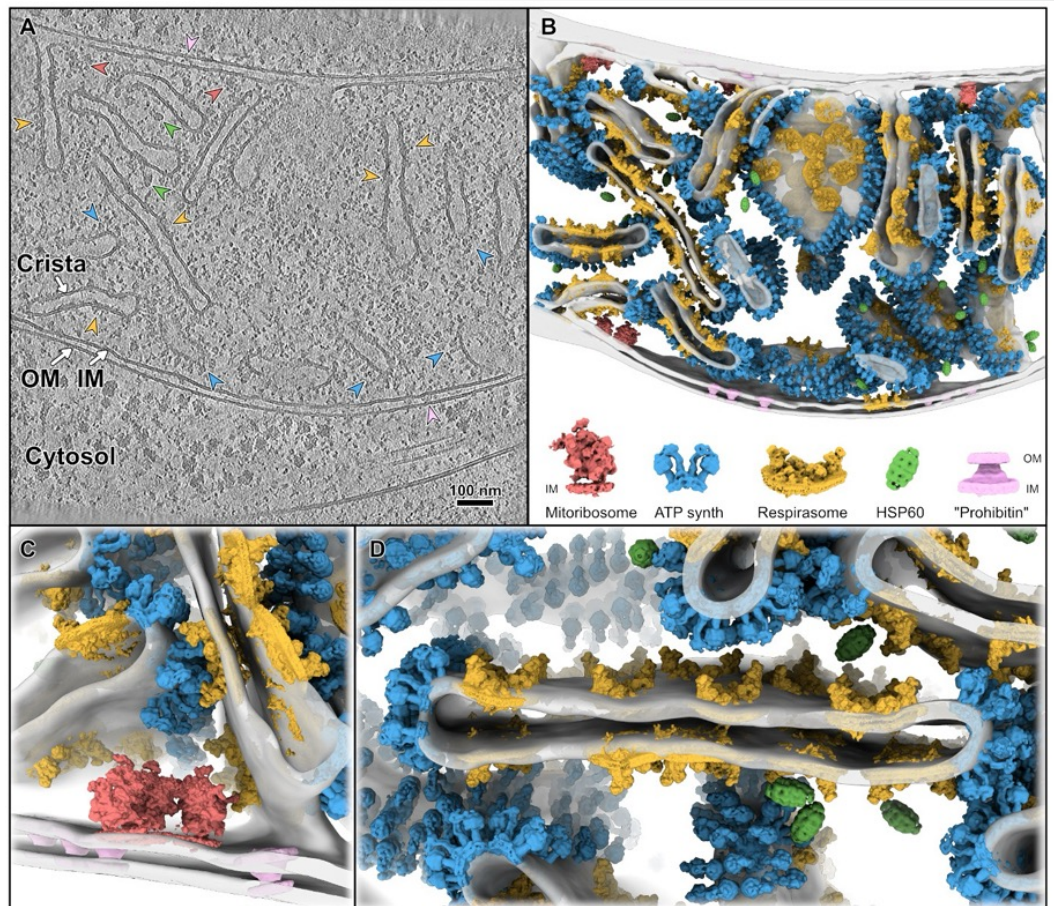
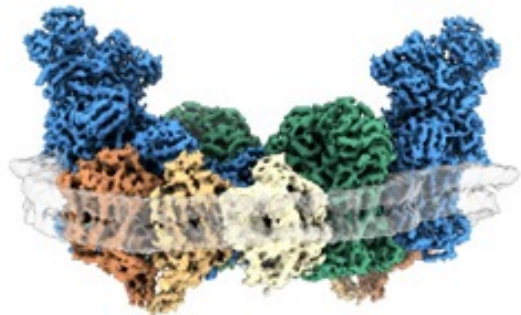
f X W in

In-cell architecture of the mitochondrial respiratory chain

FLORENT WALTZ, RICARDO D. RIGHETTO, LORENZ LAMM, THALIA SALINAS-SIEGÉ, RON KELLEY, XIANJUN ZHANG, MARTIN OBR, SAGAR KHAVNEKAR, ABHAY KOTACHA, AND BENJAMIN D. ENGEL

[Authors Info & Affiliations](#)

SCIENCE • 20 Mar 2025 • Vol 387, Issue 6740 • pp. 1296-1301 • DOI: 10.1126/science.ad9738



Segmentation for in situ structural biology

ThermoFisher
SCIENTIFIC



Towards community-driven visual proteomics with large-scale cryo-electron tomography of *Chlamydomonas reinhardtii*

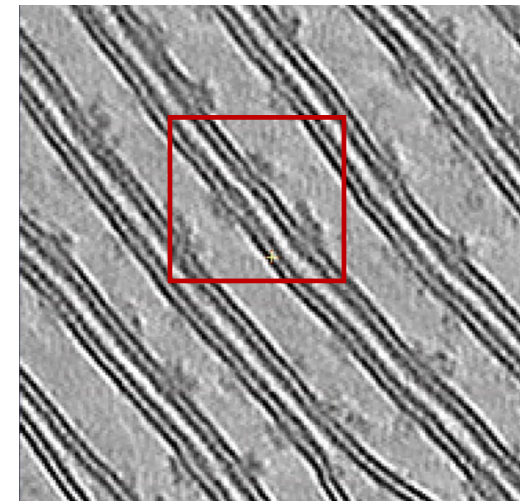
Ron Kelley, Sagar Khavnekar, Ricardo D. Righetto, Jessica Heebner, Martin Obr, Xianjun Zhang, Saikat Chakraborty, Grigory Tagiltsev, Alicia K. Michael, Sofie van Dorst, Florent Waltz, Caitlyn L. McCafferty, Lorenz Lamm, Simon Zufferey, Philippe Van der Stappen, Hugo van den Hoek, Wojciech Wietrzynski, Pavol Harar, William Wan, John A.G. Briggs, Jürgen M. Plitzko, Benjamin D. Engel, Abhay Kotecha

doi: <https://doi.org/10.1101/2024.12.28.630444>

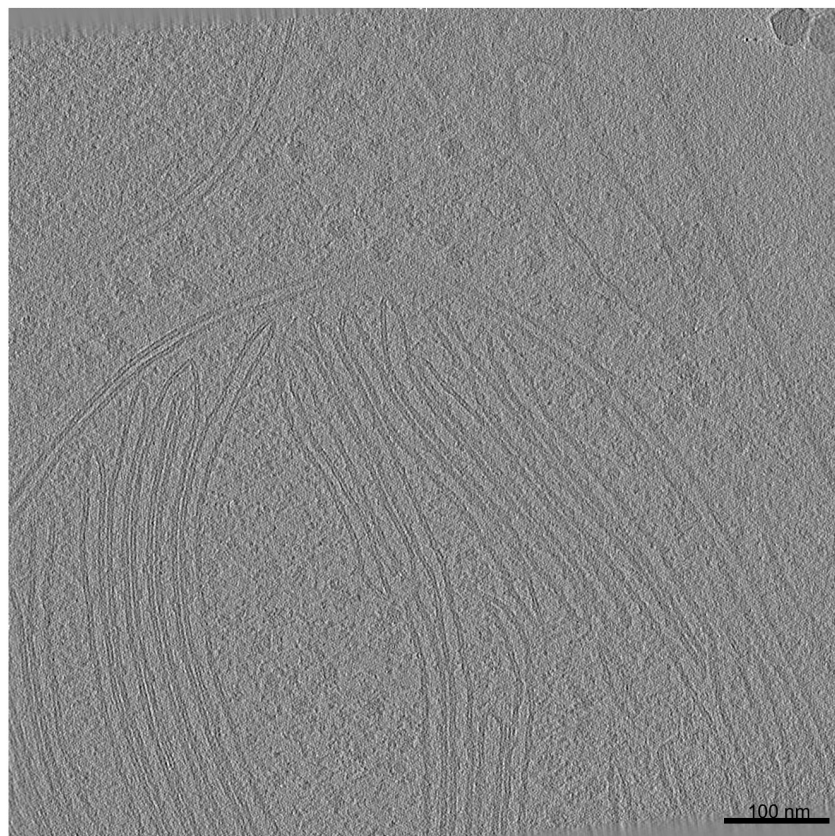
This article is a preprint and has not been certified by peer review [what does this mean?].



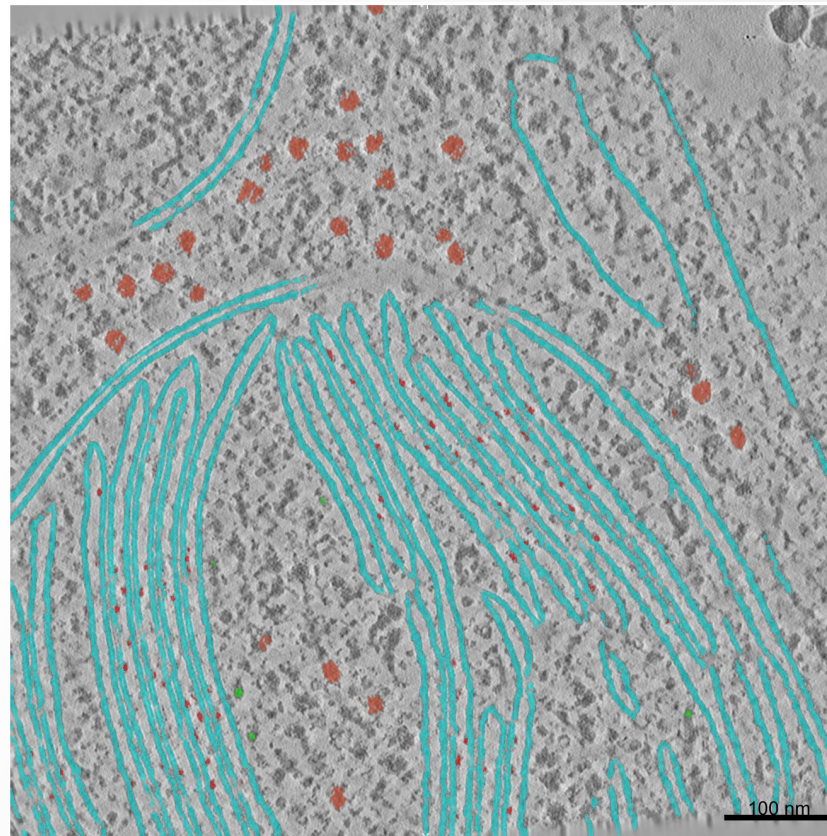
Membrane embedded complexes PSI, PSII, Cyt b₆, etc.



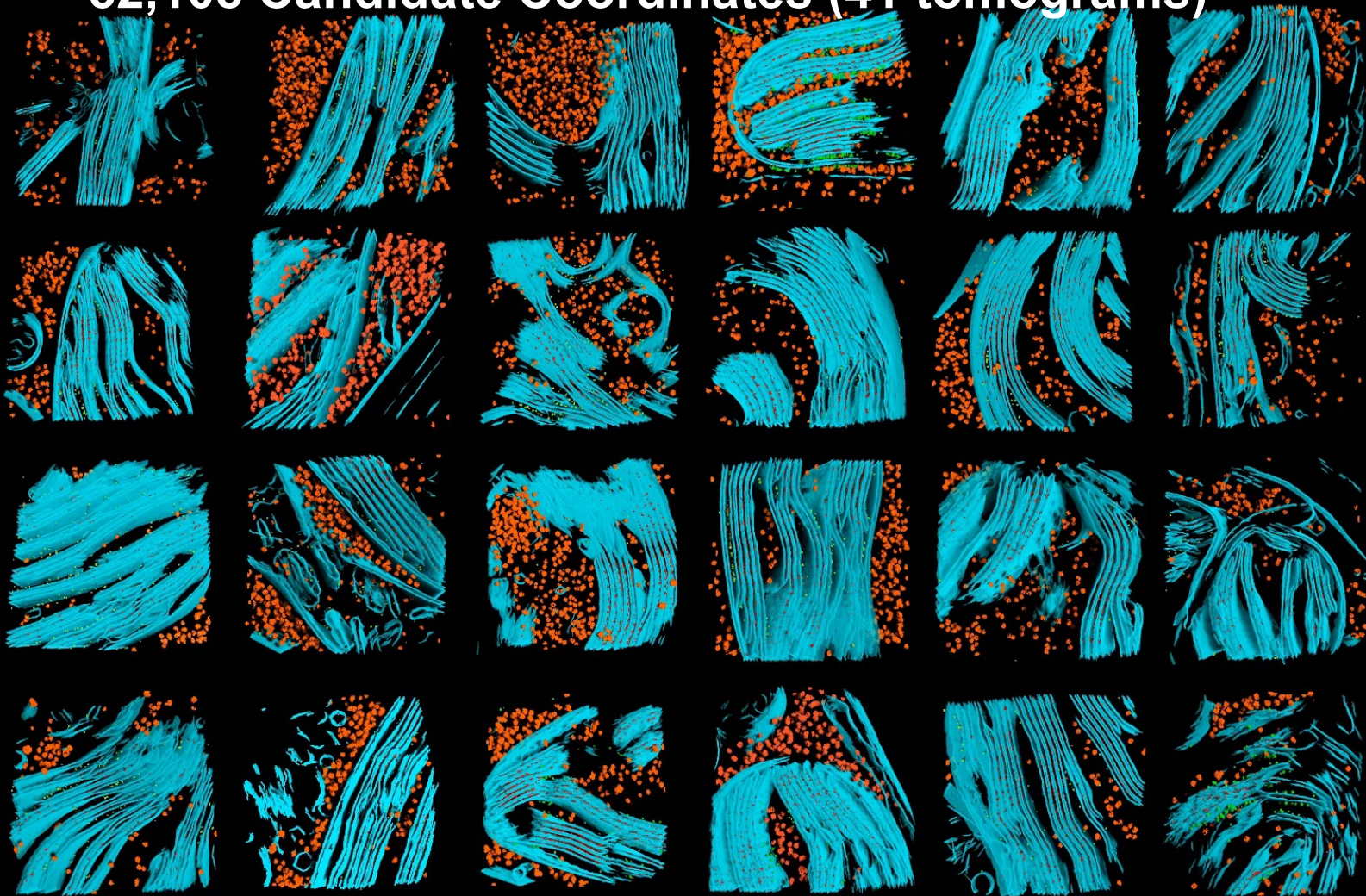
Unfiltered Tomogram



Denoised

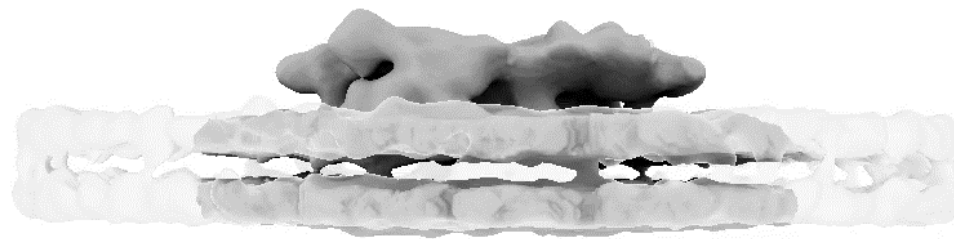


52,108 Candidate Coordinates (41 tomograms)



Photosystem II, 19 Å

ThermoFisher
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Quantifying organellar ultrastructure

ThermoFisher
SCIENTIFIC

Tools | February 14 2023

Quantifying organellar ultrastructure in cryo-electron tomography using a surface morphometrics pipeline

In Special Collection: Structural Biology 2023


Benjamin A. Barad , Michaela Medina , Daniel Fuentes , R. Luke Wiseman , Danielle A. Grotjahn 

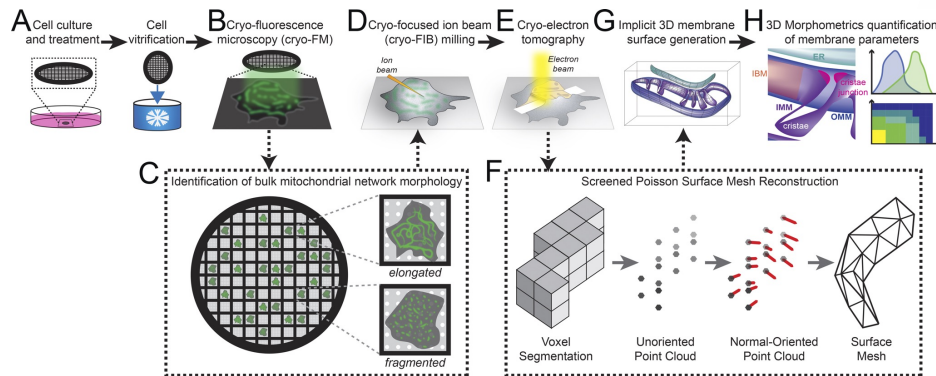
+ Author and Article Information

 Check for updates



J Cell Biol (2023) 222 (4): e202204093. | <https://doi.org/10.1083/jcb.202204093> | [Article history](#)

 Review History



Report | March 06 2025

Cytoplasmic ribosomes on mitochondria alter the local membrane environment for protein import


Ya-Ting Chang , Benjamin A. Barad , Juliette Hamid , Hamidreza Rahmani , Brian M. Zid , Danielle A. Grotjahn 

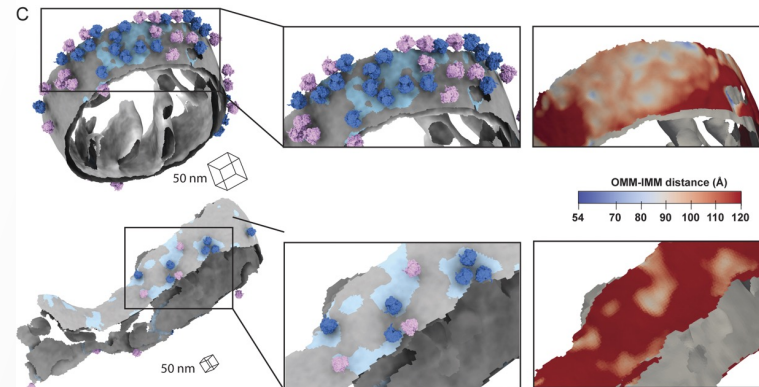
+ Author and Article Information

 Check for updates

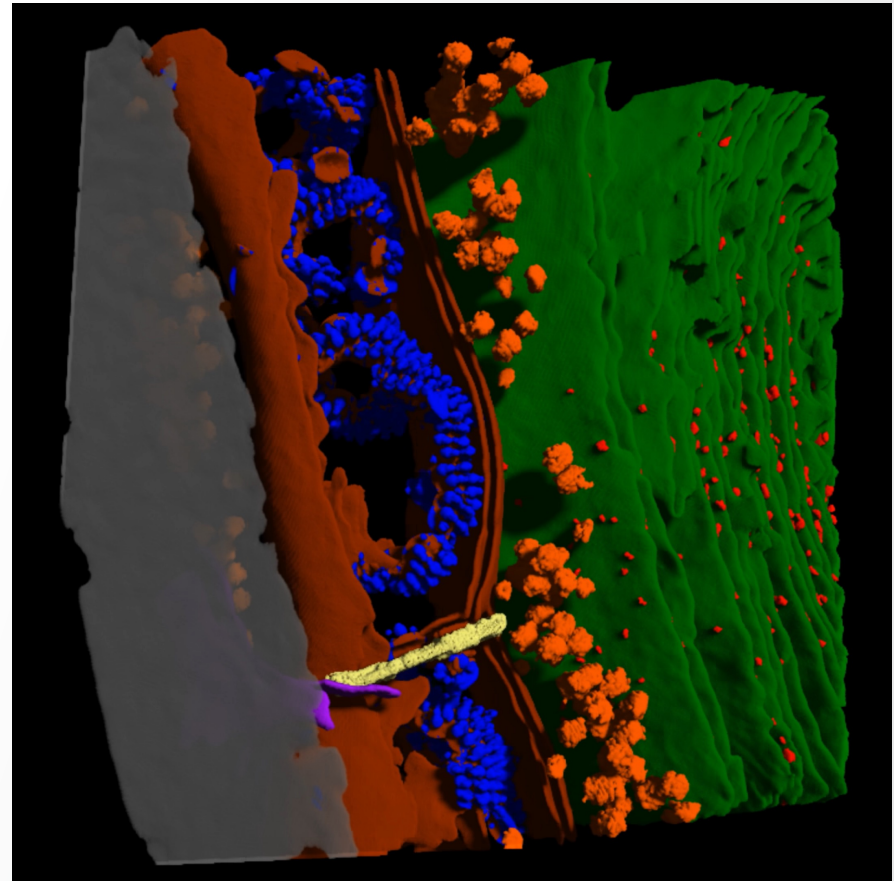
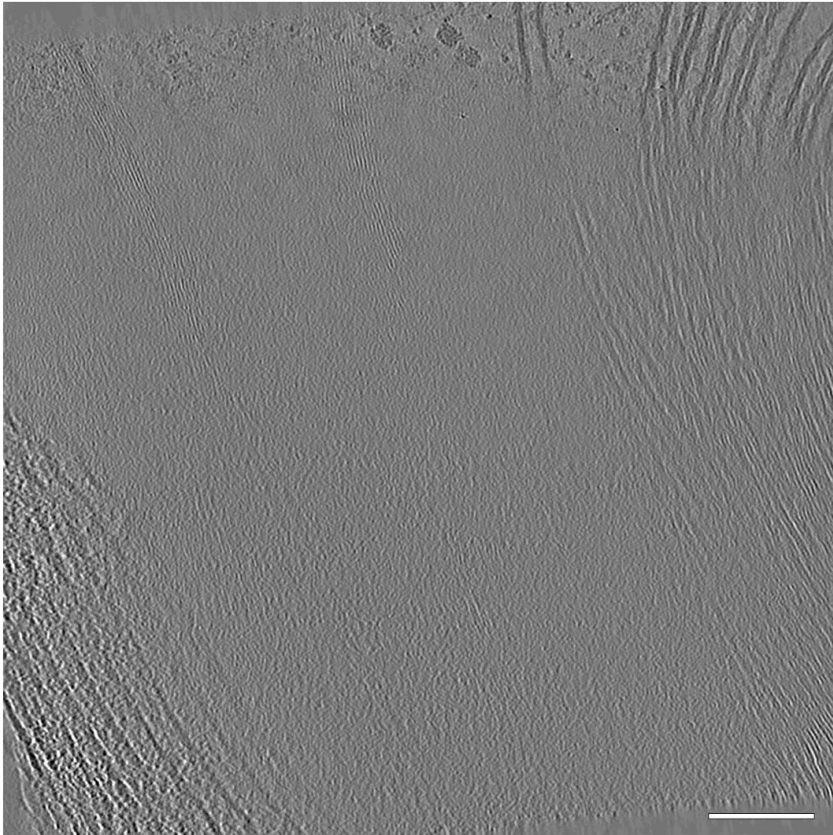


J Cell Biol (2025) 224 (4): e202407110. | <https://doi.org/10.1083/jcb.202407110> | [Article history](#)

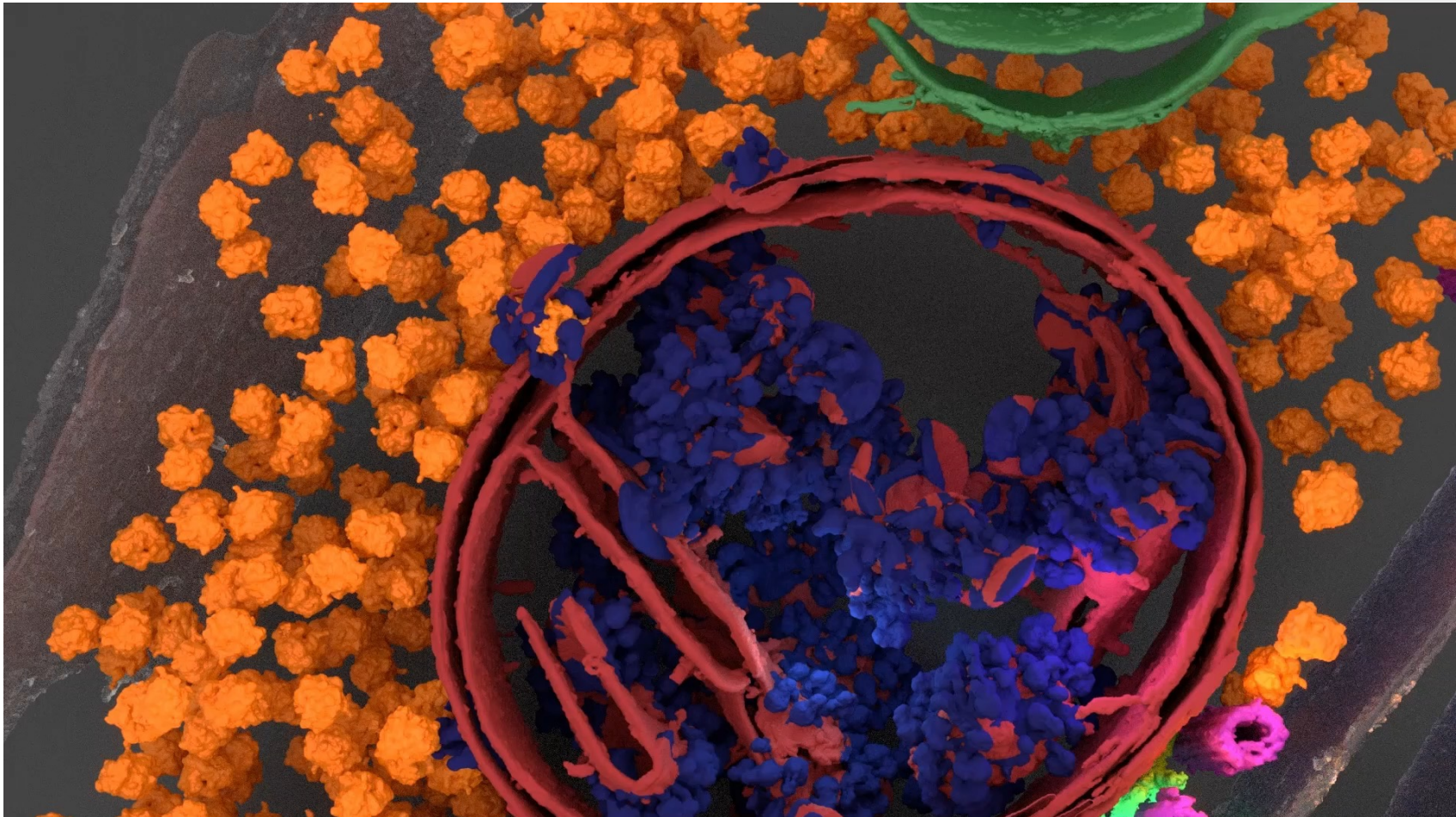
 Review History



Mitochondrial Fission



Mitochondria Transport

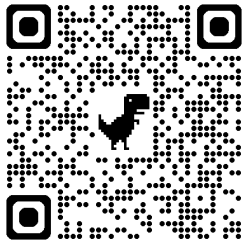


Useful Resources

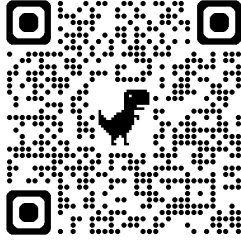
[MemBrain-seg
Installation Guide
and Tutorials](#)



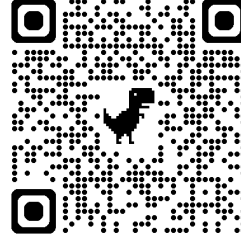
[Napari Installation
Guide and
Tutorials](#)



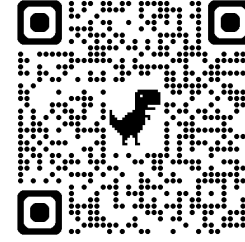
[Image.sc Forum](#)



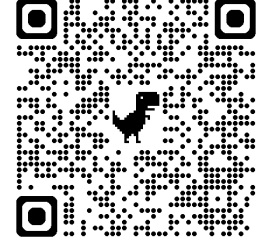
[Amira Tutorials](#)

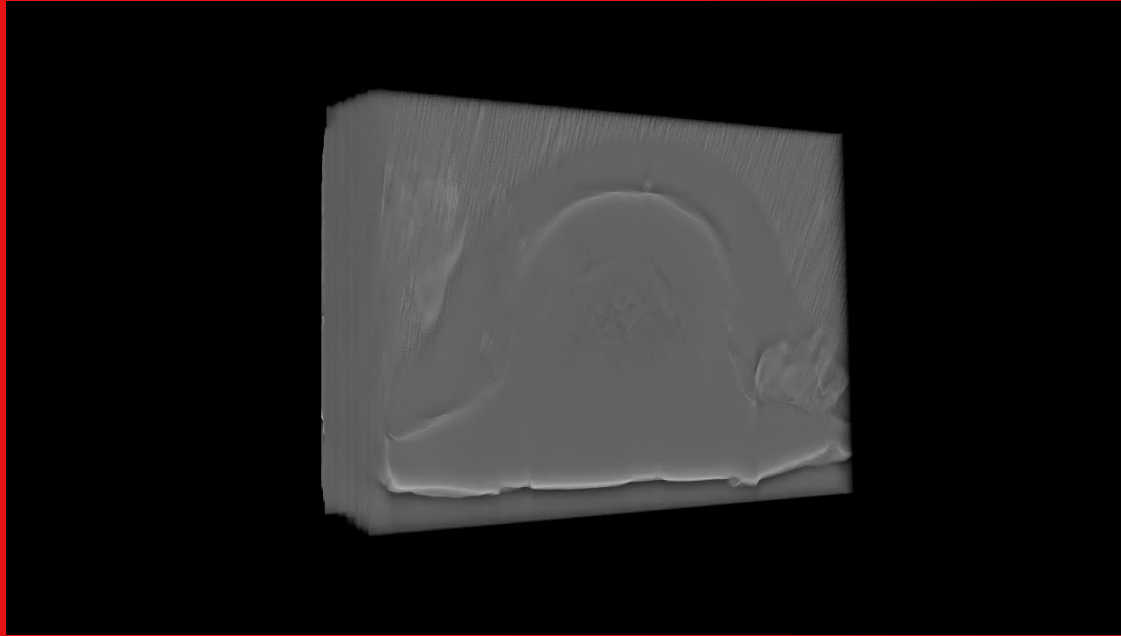


[Dragonfly Tutorials](#)



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Segmentation
Tutorial](#)





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Questions

