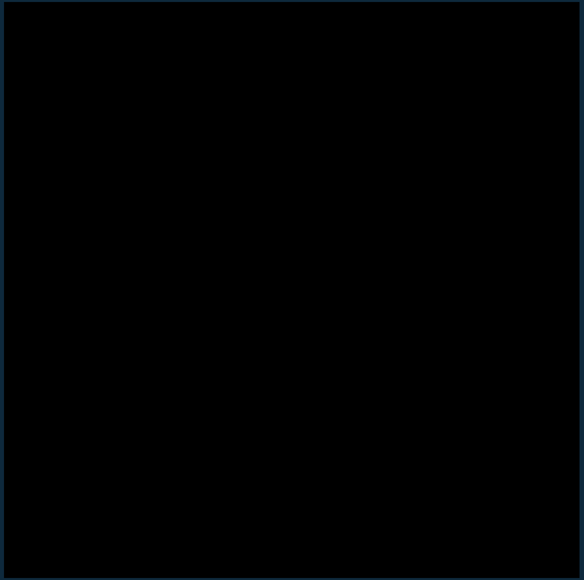
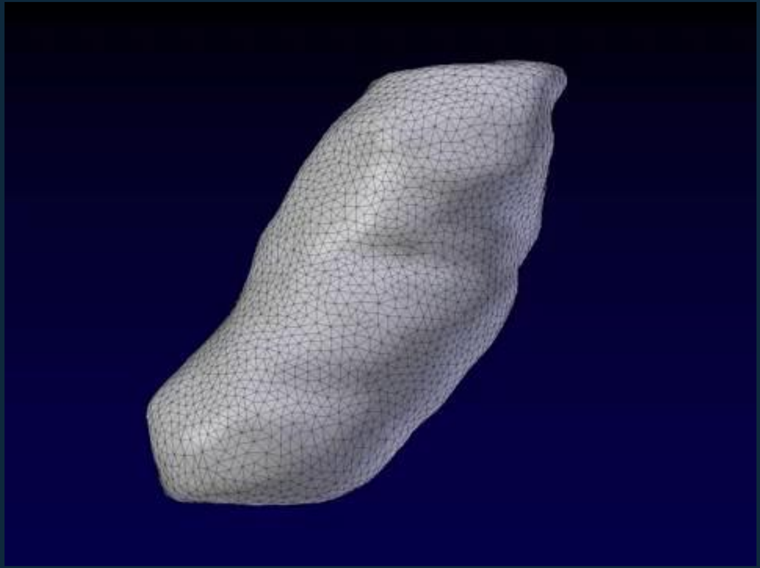
Design principles of mitochondrial architecture in neurons

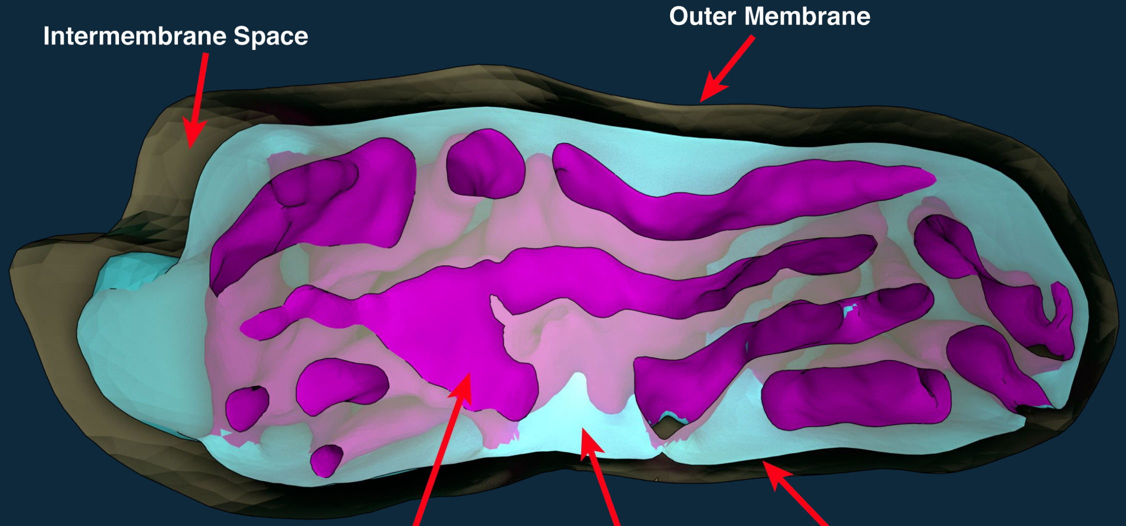




Rachel Mendelsohn
Sejnowski Lab
Salk Institute

Christopher T. Lee
Rangamani and Holst Labs
University of California San Diego

Dual membranes are key for mitochondrial function

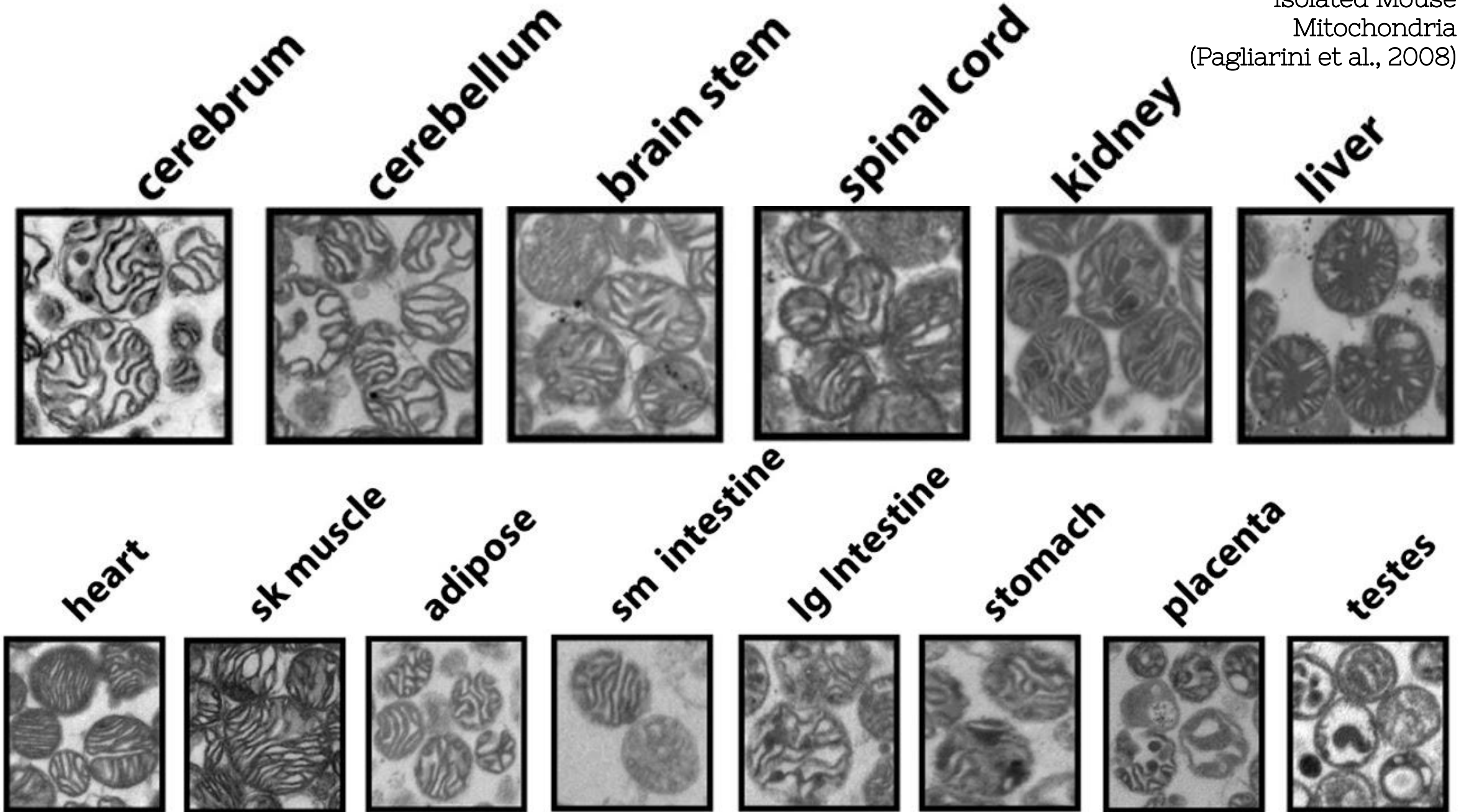


Four Steps for ATP:

Glycolysis → Pyruvate Oxidation → Citric Acid/ Krebs Cycle → Oxidative Phosphory-lation

Cytoplasm | Matrix | Matrix | Inner Membrane

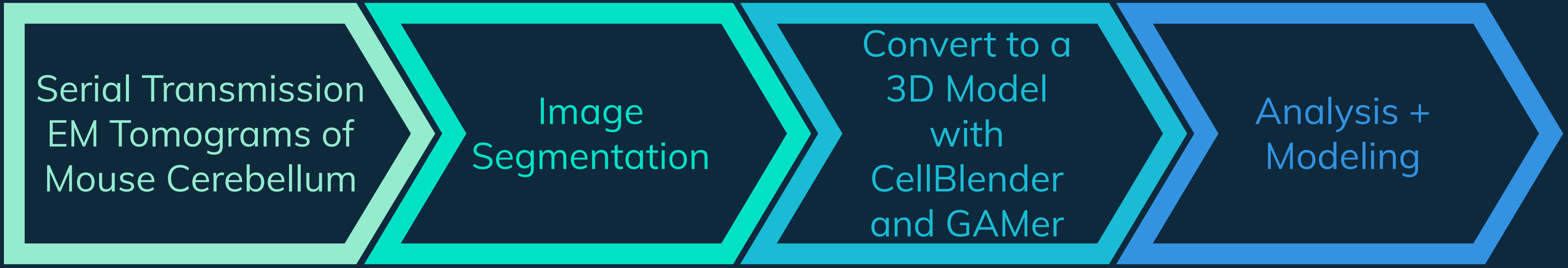
Isolated Mouse Mitochondria (Pagliarini et al., 2008)



Specificity Suggests Specialization

- Unique mitochondrial structures reflect unique bioenergetic needs.
- Neurons have, arguably, the most specialized mitochondria of any cell type.
- Information about neuronal energy dynamics is contained in mitochondrial structure.

Enabling morphological and modeling analysis with real geometries constrained by EM tomography



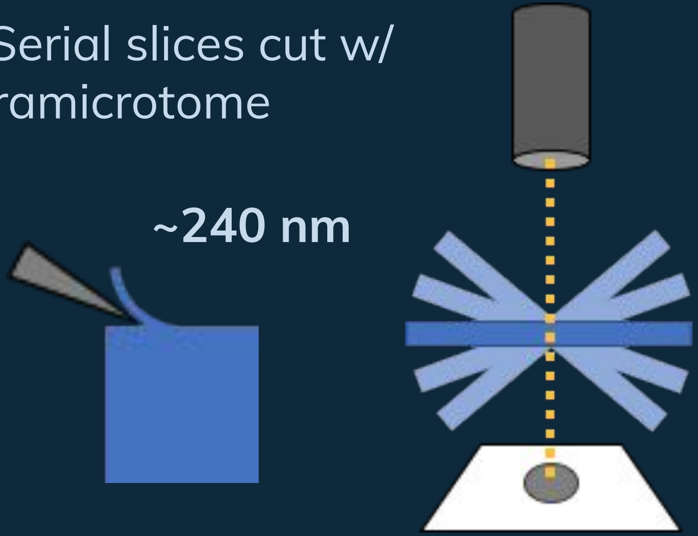
Serial Transmission EM Tomograms of Mouse Cerebellum

Image Segmentation

Convert to a 3D Model with CellBlender and GAMer

Analysis + Modeling

1. Serial slices cut w/ ultramicrotome



2. Slices imaged using multi-tilt electron tomography

2x Datasets:

~2.4x2.5x1 um^3

~2.4x2.4x1.6 um^3

1.64 nm isotropic virtual voxel size

~35 Total Mitochondria

18 Full

17 Partial

Eric Bushong, Sebastian Phan, Ellisman Lab

Serial Transmission EM Tomograms of Mouse Cerebellum

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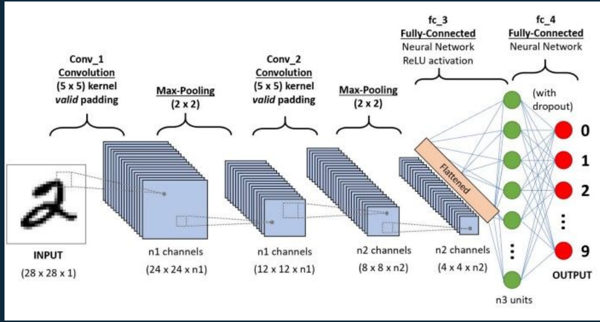
Analysis + Modeling

Semi-automated Machine Learning:

- iLastik (https://ilastik.org)
- CDeep3m (NCMIR)
- Other tools

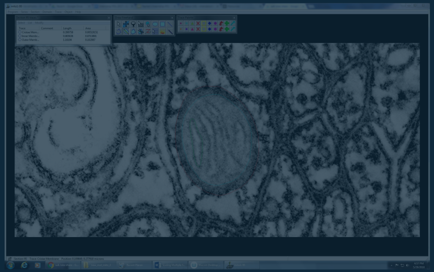


https://towardsdatascience.com/

Manual Tracing:

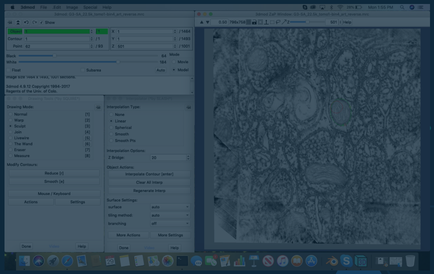
Reconstruct

- Developed by Kristen Harris Lab (UT Austin)

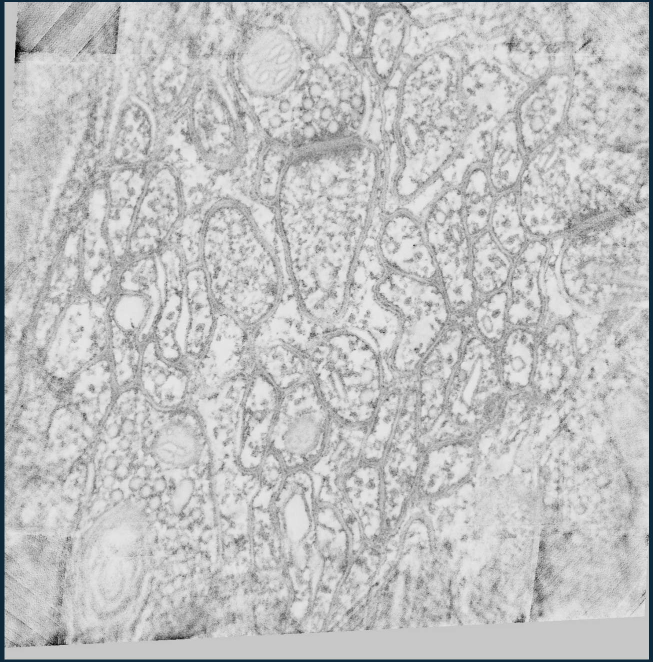


IMOD

- Developed by Boulder laboratory for 3-D EM of cells

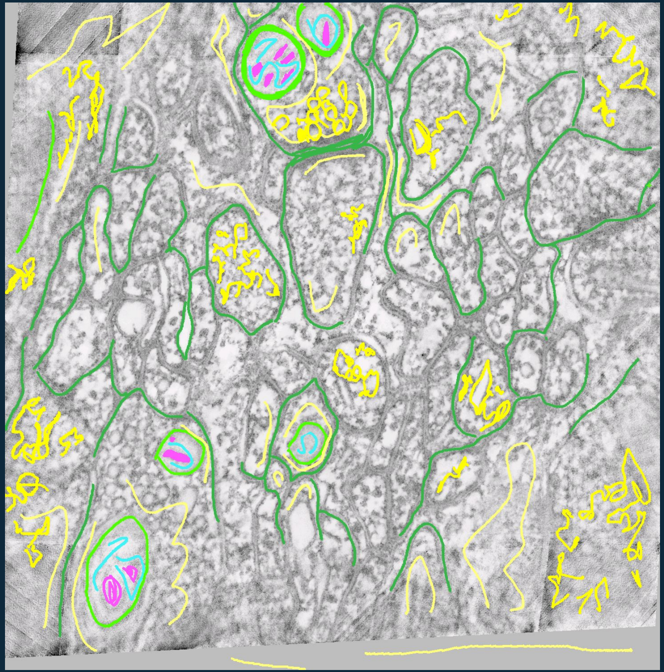


Object Classification Using Random Forest NN in iLastik



Step 1:

Manually label components to train Random Forest Classifier

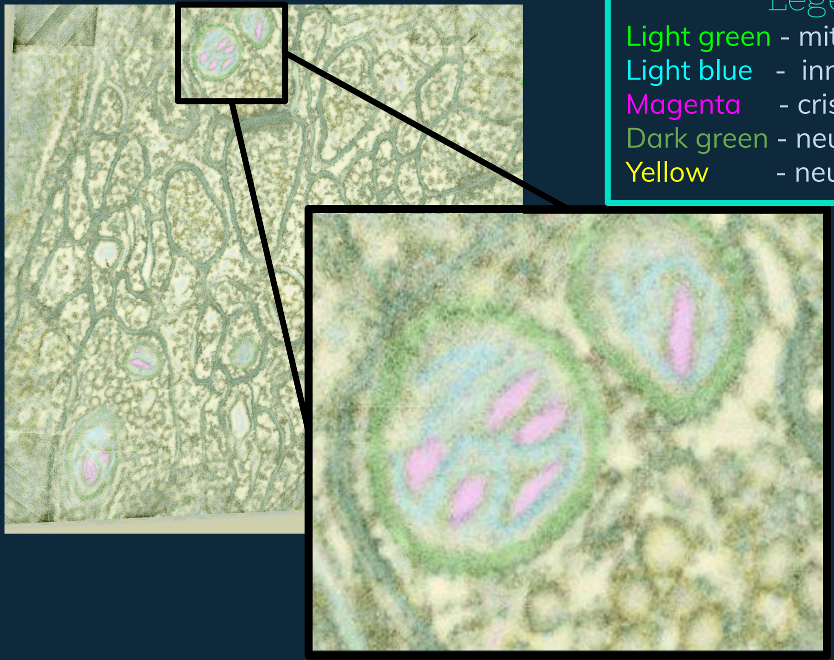


Legend

Light green - mito outer mem
Light blue - inner mem space
Magenta - cristae mem
Dark green - neuron mem
Yellow - neuron cytosol

Meagan Rowan

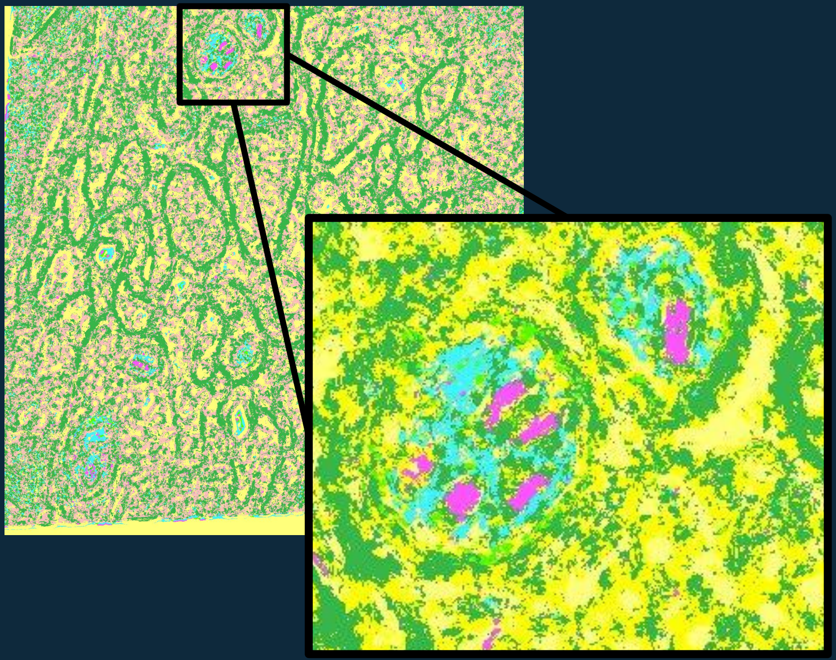
Segmentations from iLastik are unsuitable for mesh generation



Overlaid Fuzzy Predictions

Legend

Light green - mito outer mem
Light blue - inner mem space
Magenta - cristae mem
Dark green - neuron mem
Yellow - neuron cytosol



Crisp Labels Segmentation

Meagan Rowan

Serial Transmission EM Tomograms of Mouse Cerebellum

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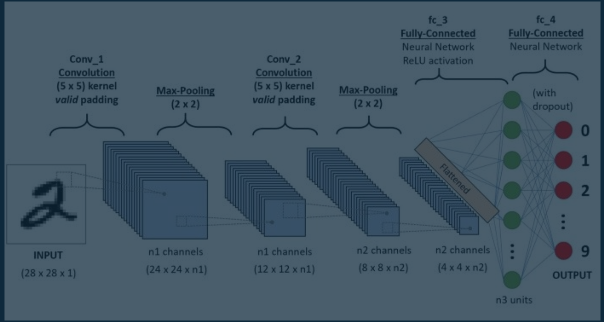
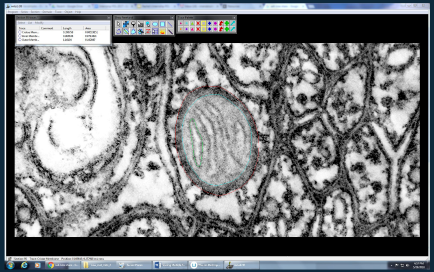


https://towardsdatascience.com/

Manual Tracing:

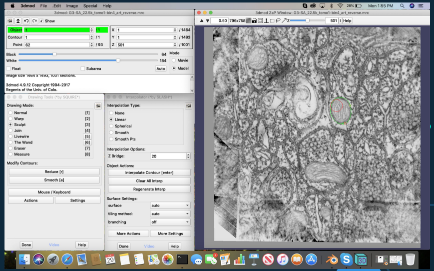
Reconstruct

- Developed by Kristen Harris Lab (UT Austin)
- Designed for image segmentation



IMOD

- Developed by Boulder laboratory for 3-D EM of cells
- Cross platform modeling suite focused on EM reconstructions



Serial Transmission EM Tomograms of Mouse Cerebellum → **Image Segmentation** → Convert to a 3D Model with CellBlender and GAMer → Analysis + Modeling

IMOD and Reconstruct
Manual tracing

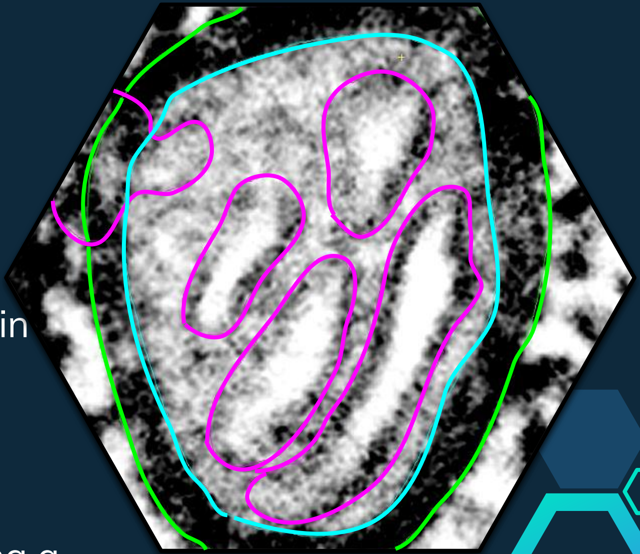
General Guidelines for Tracing:

1) Trace Outer, Inner, Cristae separately.

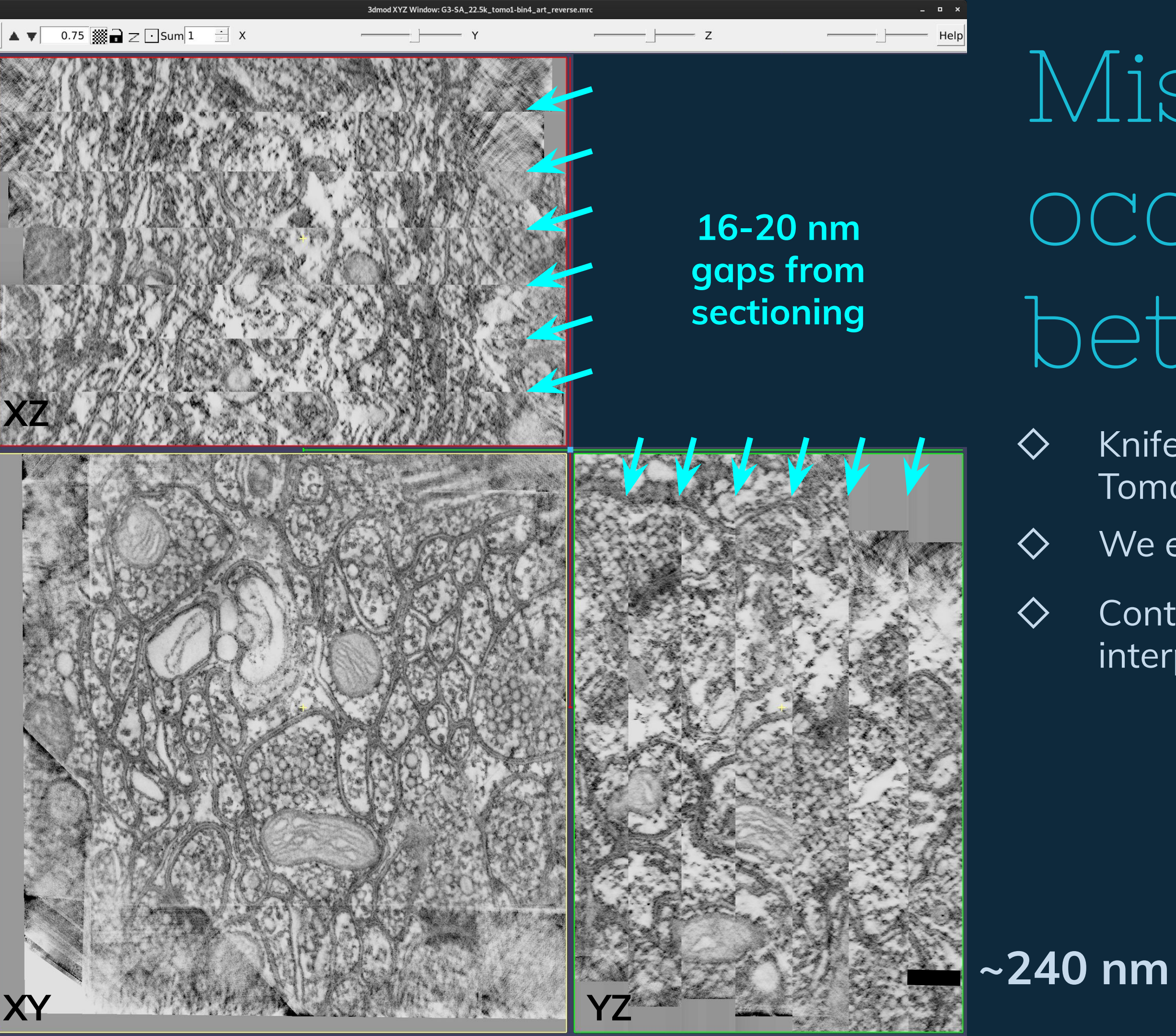
2) Create a smooth outer membrane, excluding potential protein structures on the surface

3) Trace the matrix side of the inner and cristae membrane (Boundary later moved in Blender)

4) Include cristae junction curvature in the cristae trace, creating a smooth intersection with the inner membrane trace



Mito 4



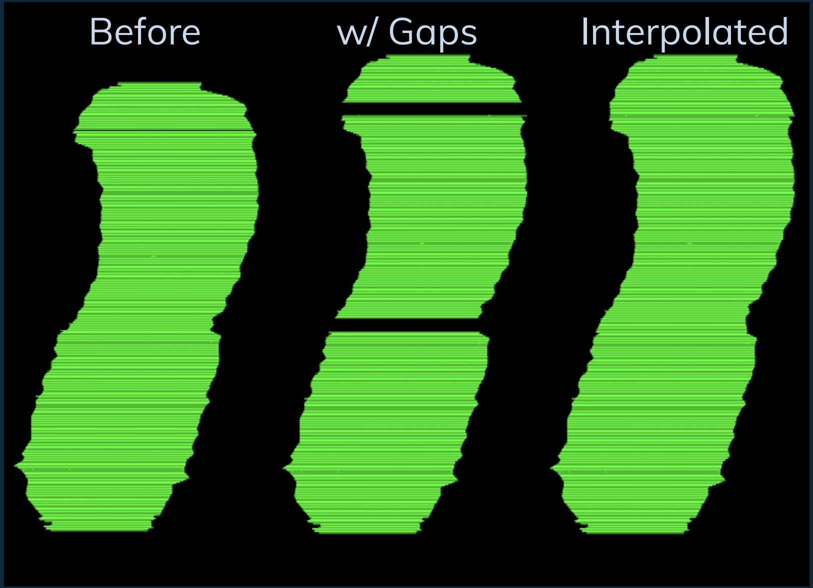
Missing data occurs between slices

- Knife Removes Material Between Tomograms
- We estimate 16-20 nm of material lost
- Contours across gaps can be manually interpolated in IMOD

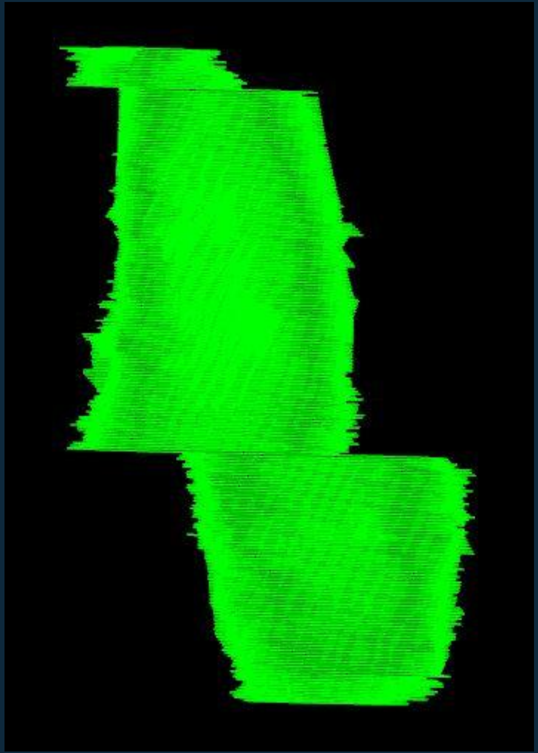
How did we do gap interpolation?

Our Approach...

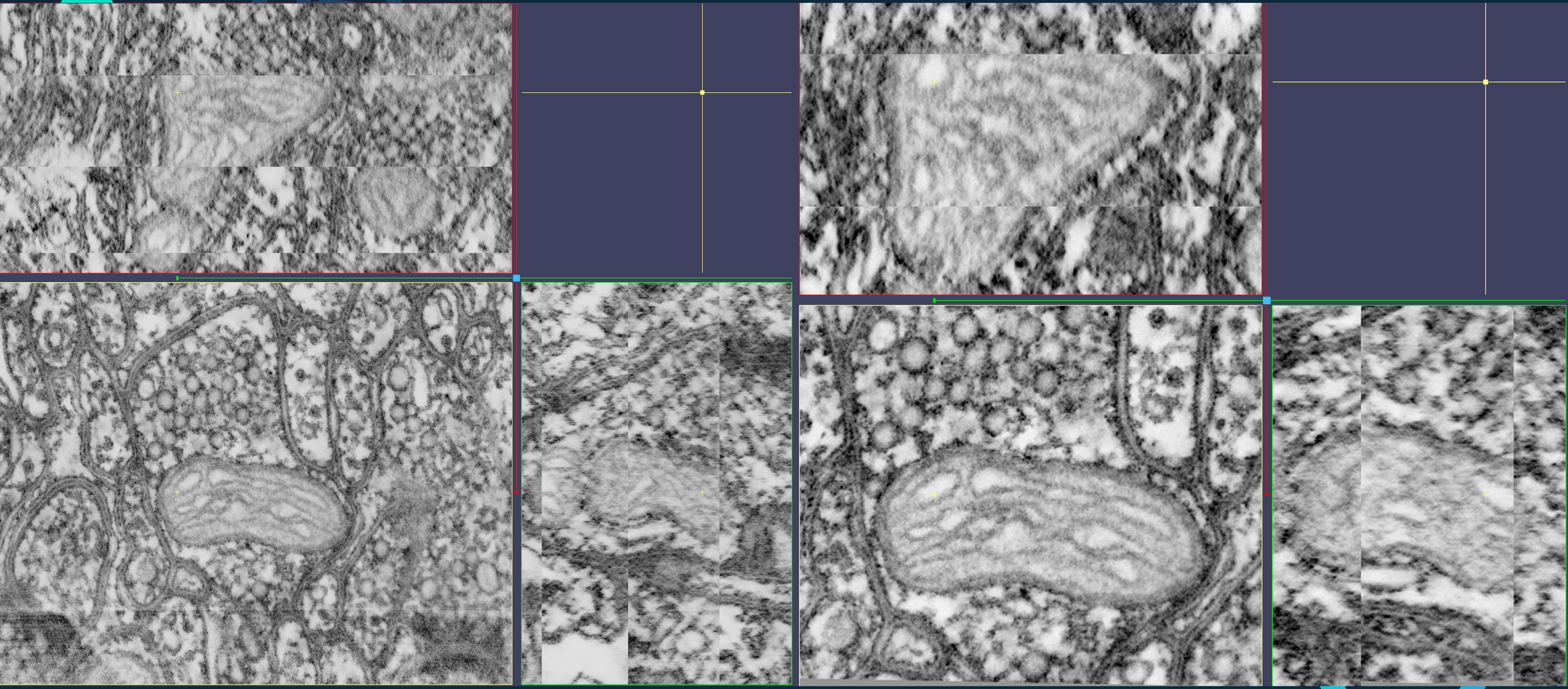
- Must be Manually Interpolated with the Help of IMOD's Interpolator



Mito 4

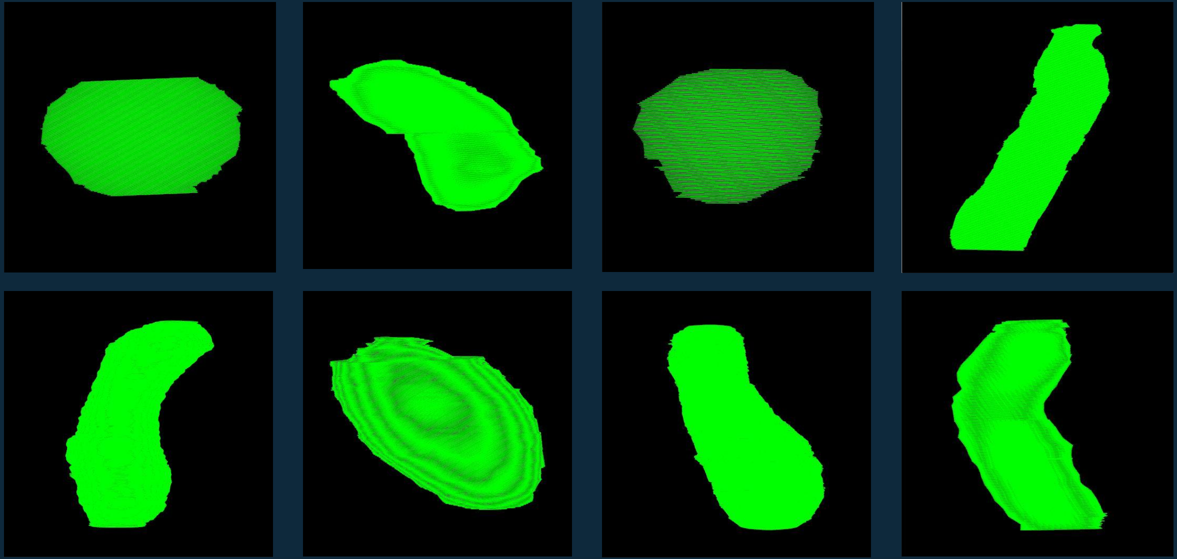


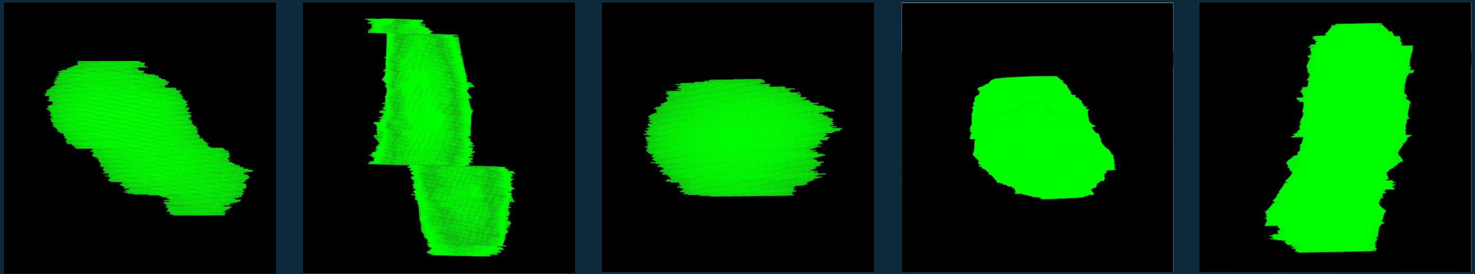
Mito 7



Alignment

Summer 2019 Mitochondria Meshathon

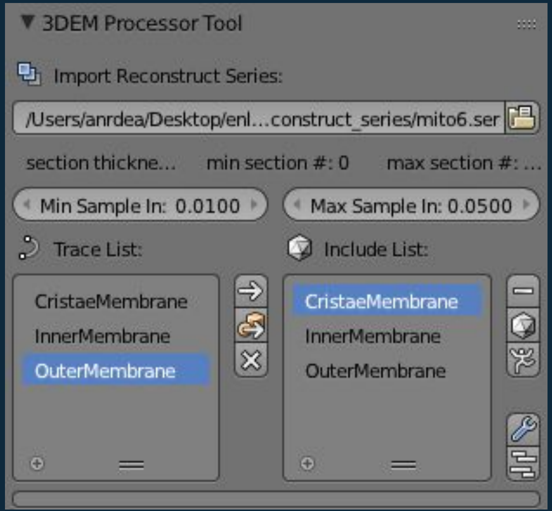


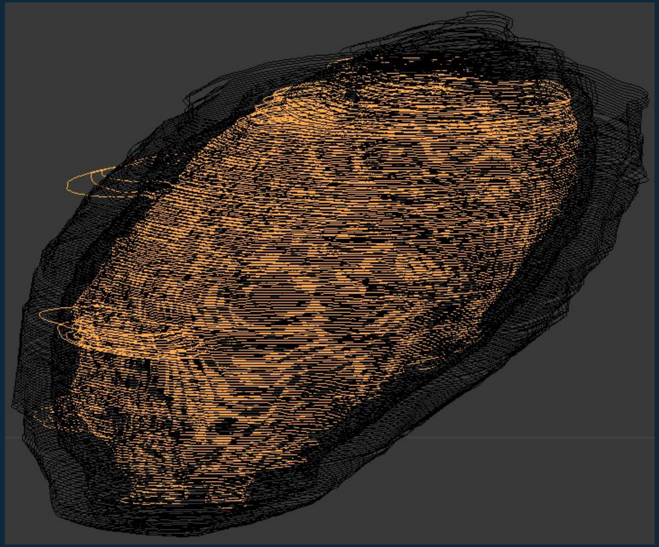


+3 Mitochondria Segmented in Reconstruct

Contour Tiling IMOD/Reconstruct to Blender Mesh

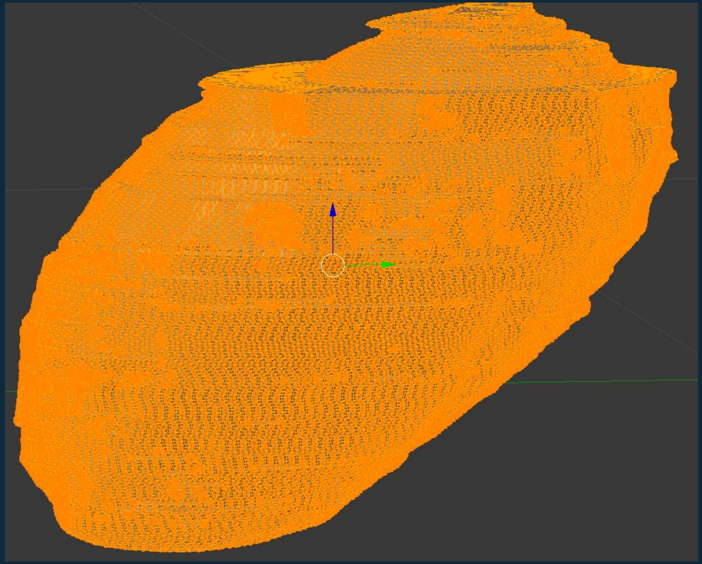
Neuropil Tools





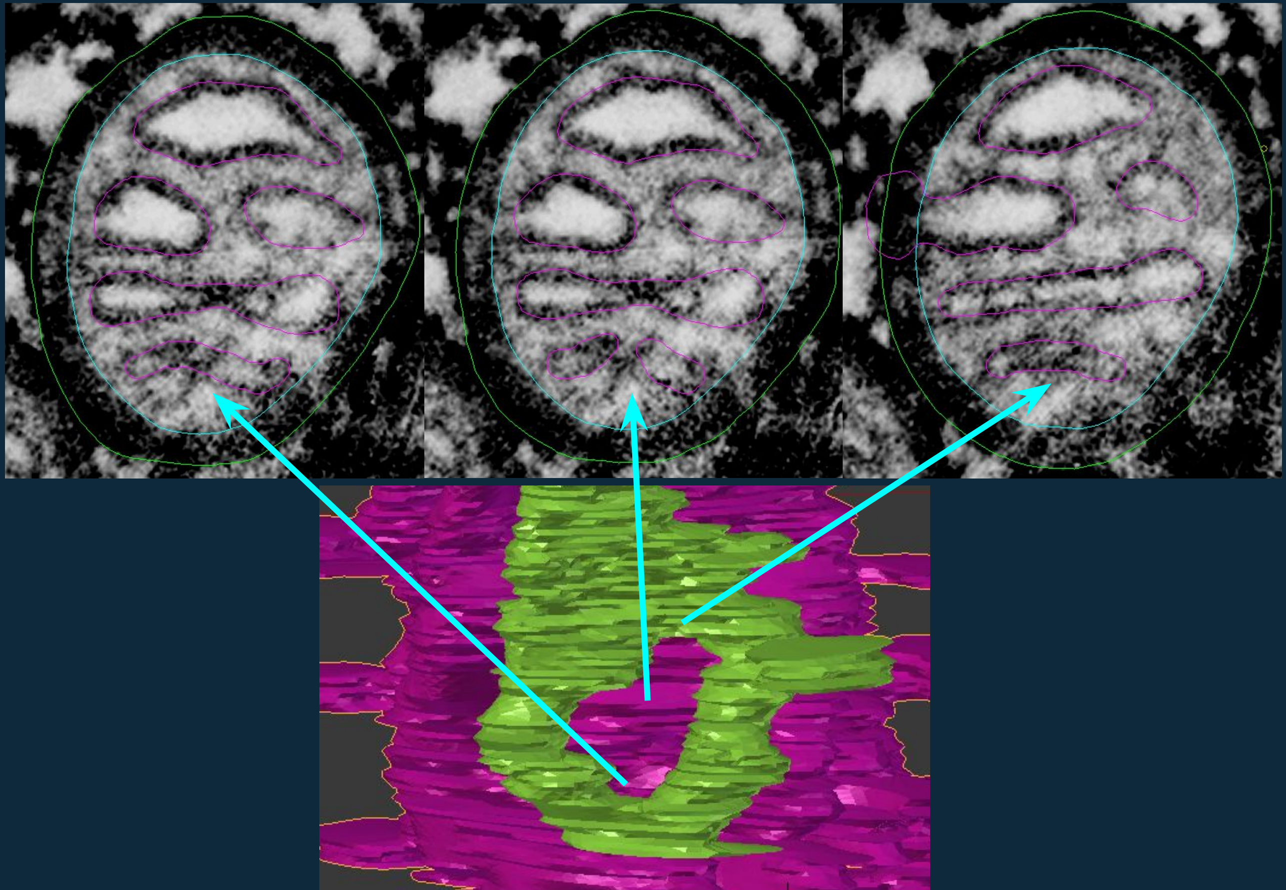
Contours

Tiled Mesh of Outer Membrane

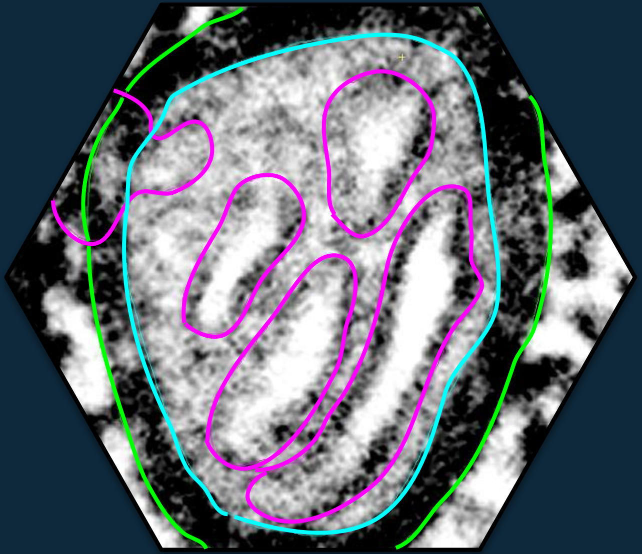


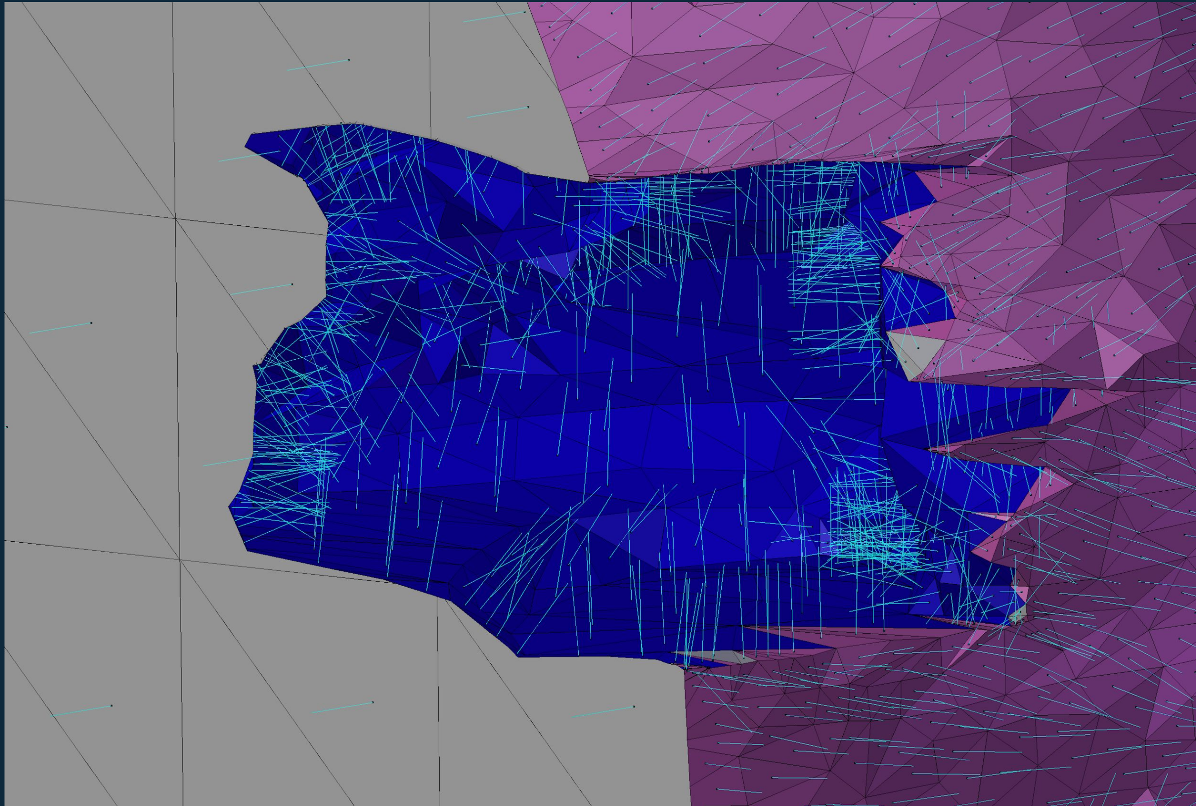
Edwards, J.; et al. Neuroinform (2014)

Disappearing EM contrast leads to ambiguity



Anisotropic smoothness of cristae junctions XY vs Z



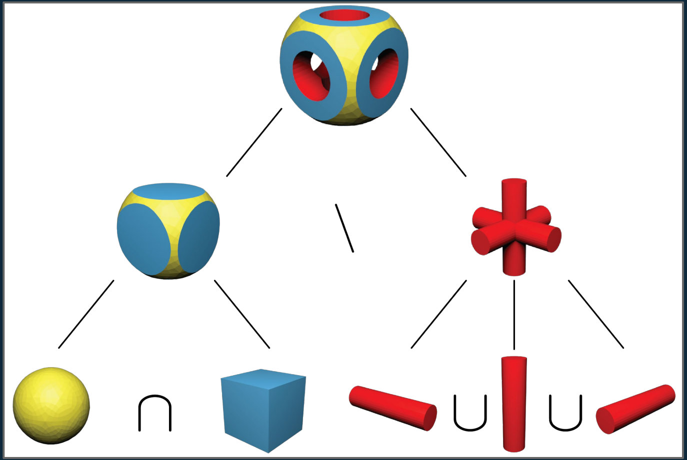


Serial Transmission EM Tomograms of Rat Cerebellum

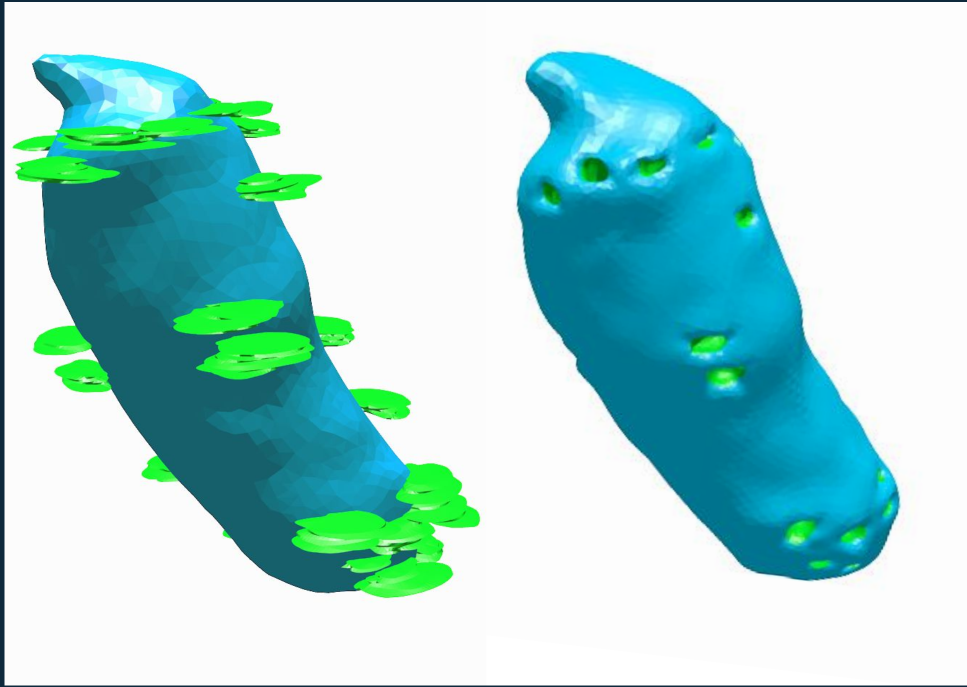
Image Segmentation

Convert to a 3D Model with CellBlender and GAMer

Analysis + Modeling



Geometric Boolean Operations



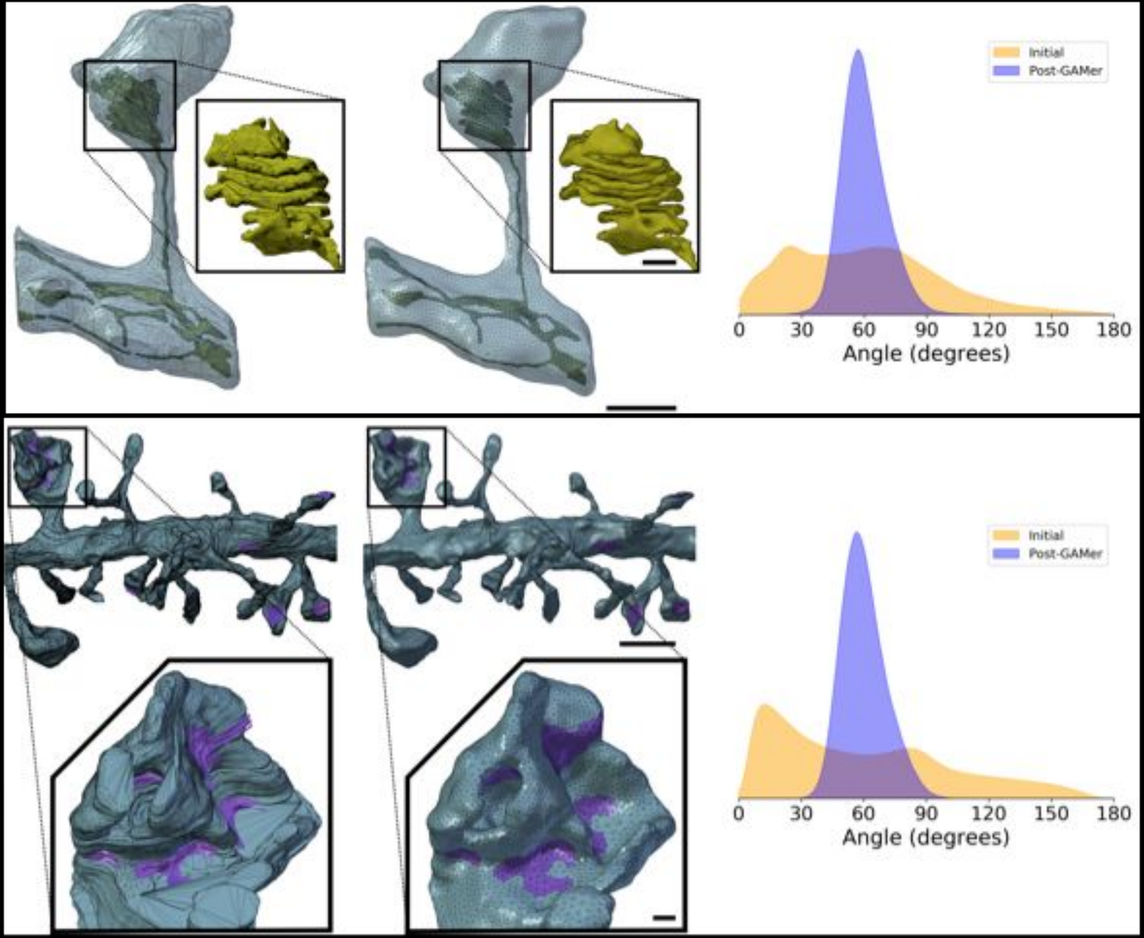
GAMer 2: Geometry-Preserving Adaptive MeshER

ctlee/gamer

LGPL v 2.1 C++ Code

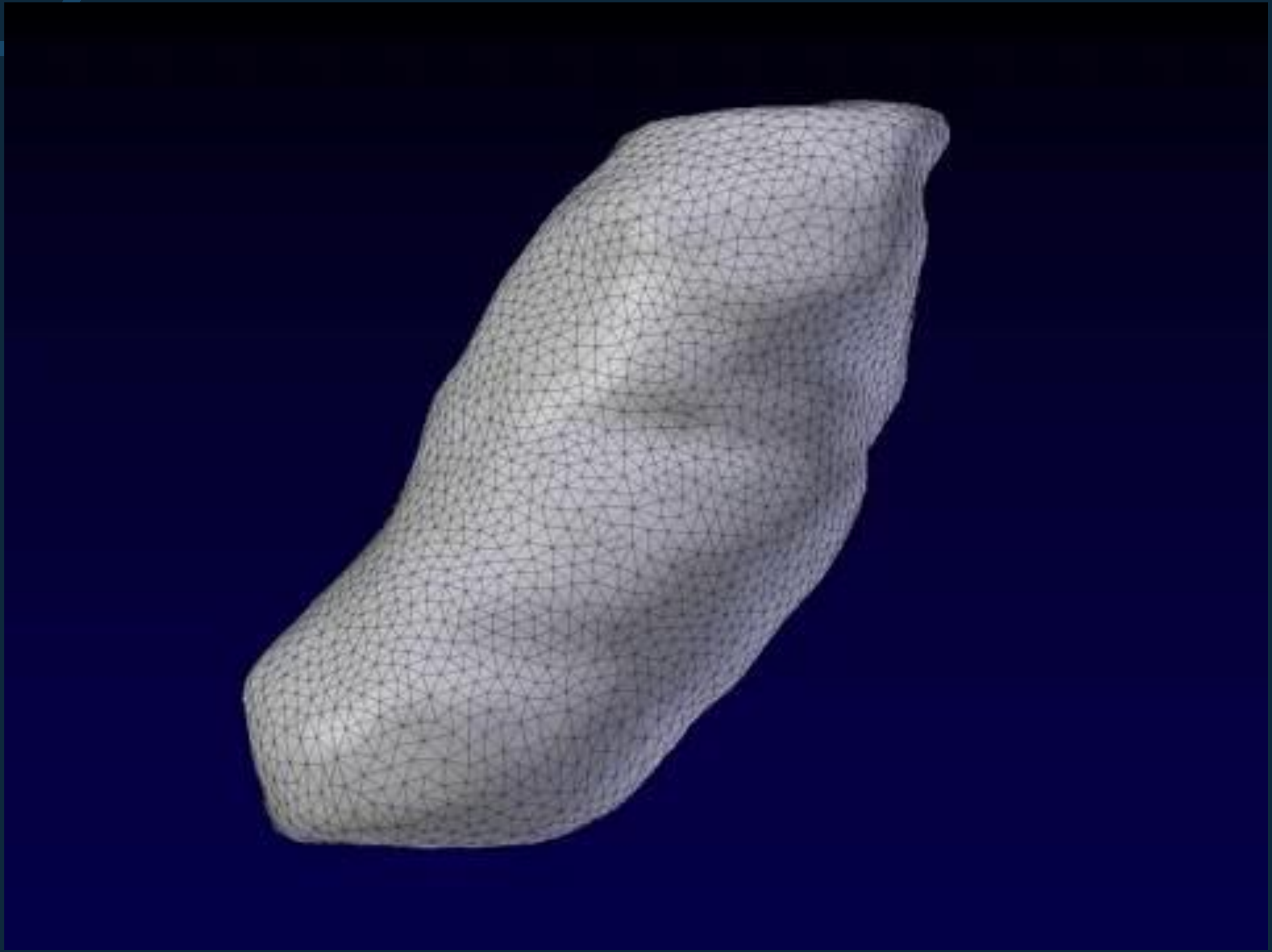
- Mesh conditioning
- Boundary marking
- Tetrahedralization (TetGen)
- Built off CASC data structure

blender



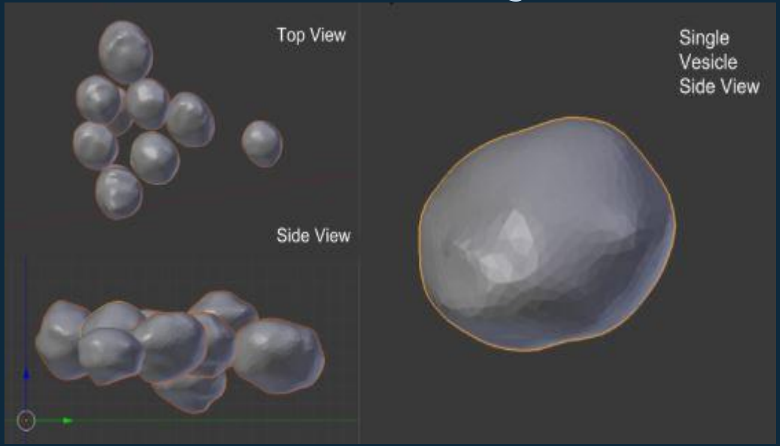
CTL*; Laughlin, JG*; et al. BioRxiv 2019.

CASC: CTL*; Moody, JB*; ACM TOMS 2019, In Press

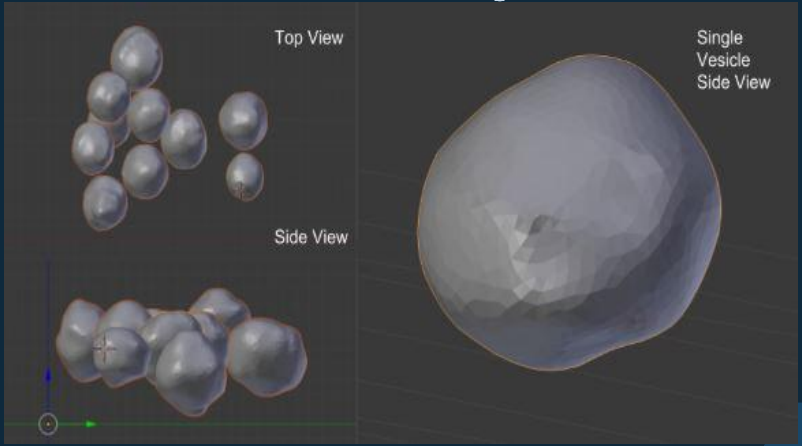


Correcting for shrinkage using sphericity of vesicles

Before Z Scaling



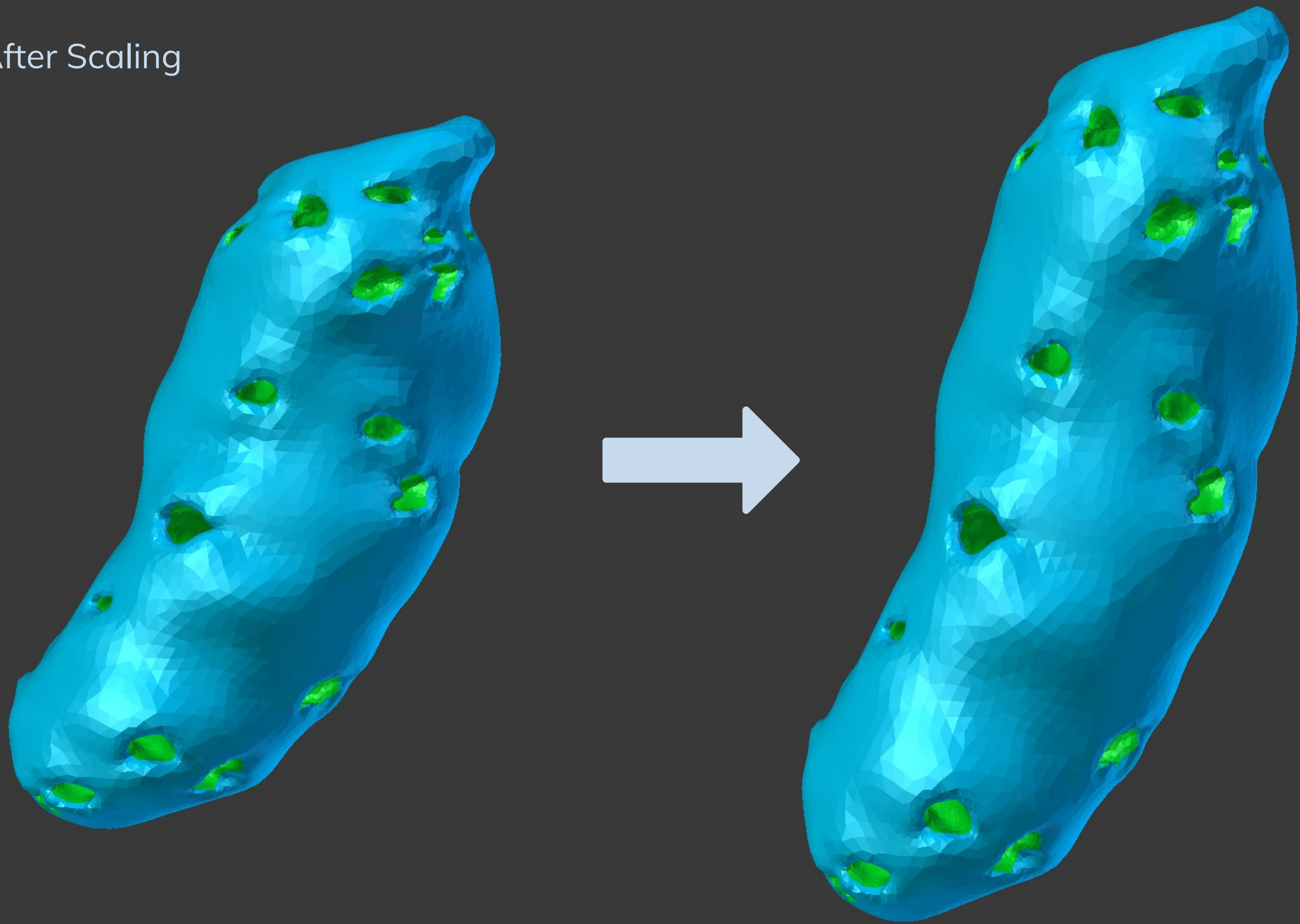
After Z Scaling



Scaling by 120% restores spherical vesicles.

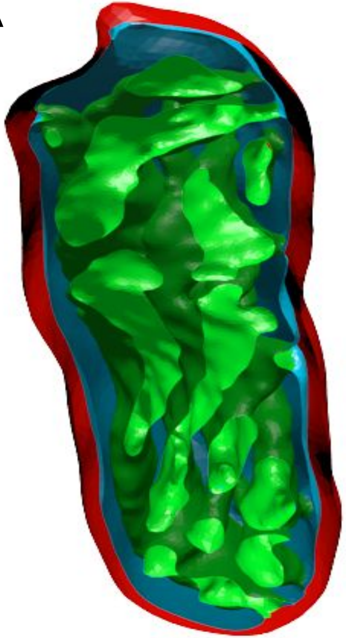
Diameters in nm	Mito 1	Mito 2	Mito 3
X	47	47	48
Y	51	50	53
Z Before Scaling	40	42	39
Z After Scaling	48	50	47

Before and After Scaling



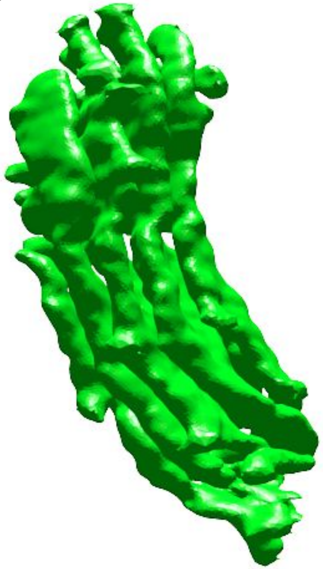
Structure

A



Cutaway of All Membranes

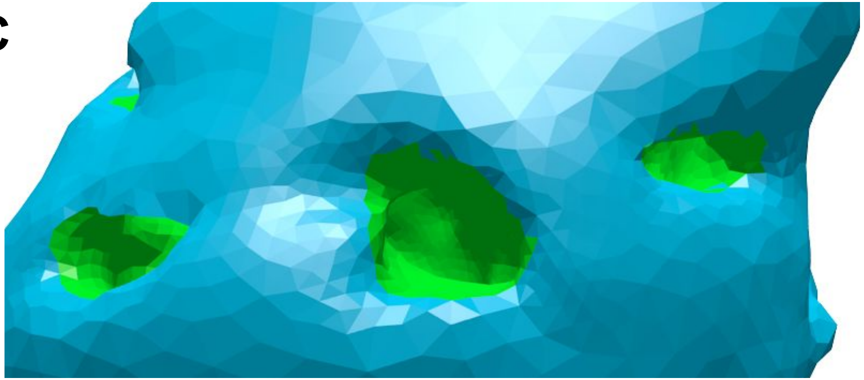
B



Cristae Displaying Lamellar Sheets

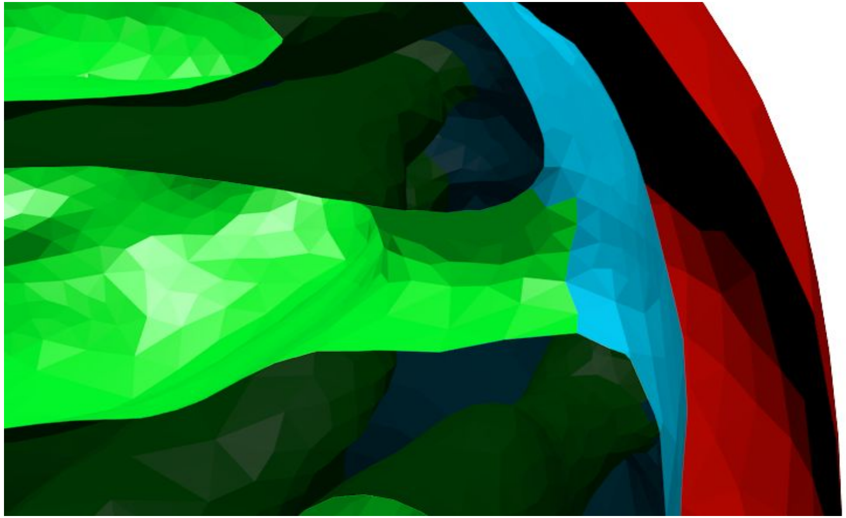
Cristae Junctions in the Inner Membrane

C



D

Cutaway of a Cristae Junction



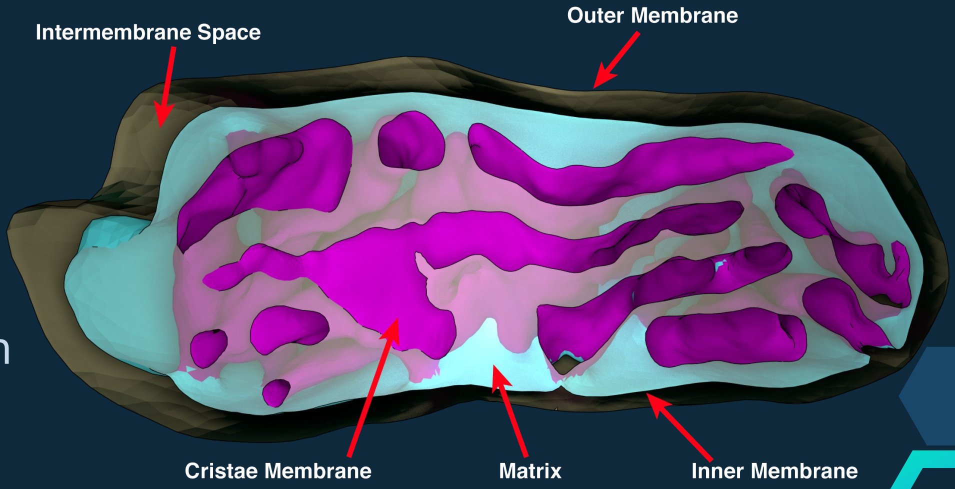
Serial Transmission EM Tomograms of Rat Cerebellum

Image Segmentation

Convert to a 3D Model with CellBlender and GAMer

Analysis + Modeling

- Compartment Volumes
  - Matrix, Intercristal spaces
- Surface Area
- Membrane curvature estimation
  - Mean, Gaussian, k1, k2



Cristae Connectivity

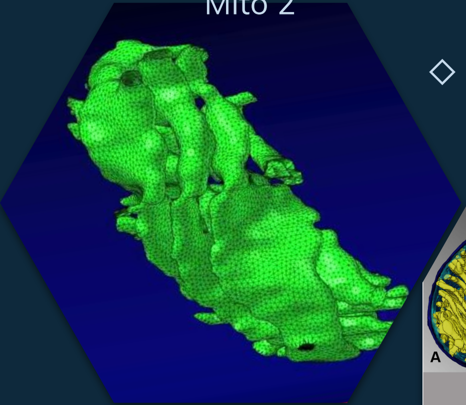
Connectivity for Adaptability

- There are no intracristal spaces which close before connecting to the rest of the network,
- There are no intracristal compartments without crista junctions.
- Free diffusion throughout the cristae compartment allows for efficient response to metabolic conditions.

Fragmentation as a Sign of Reorganization

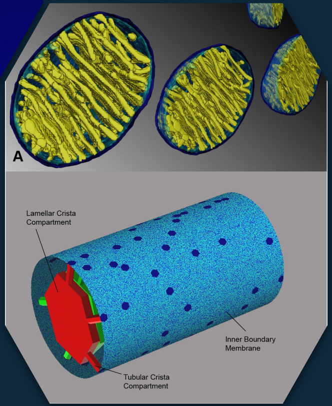
- Fragmentation is likely coupled to fission and fusion events.

Mito 2

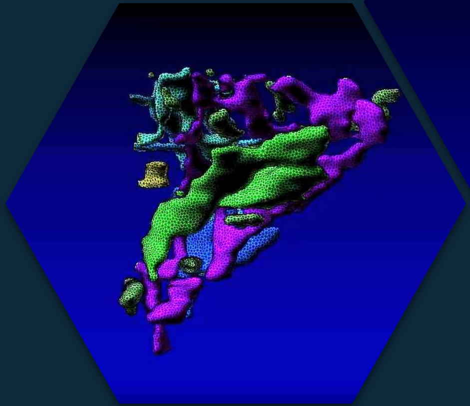


→

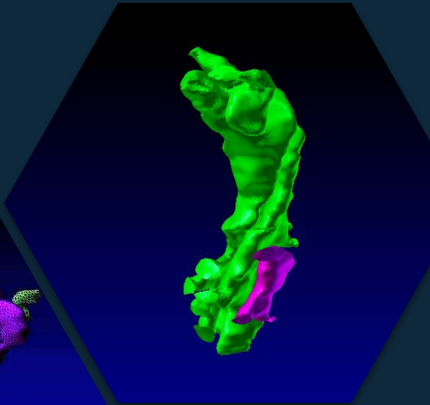
(Sukhorukov and Bereiter-Hahn, 2009)



Mito 3



Mito 4

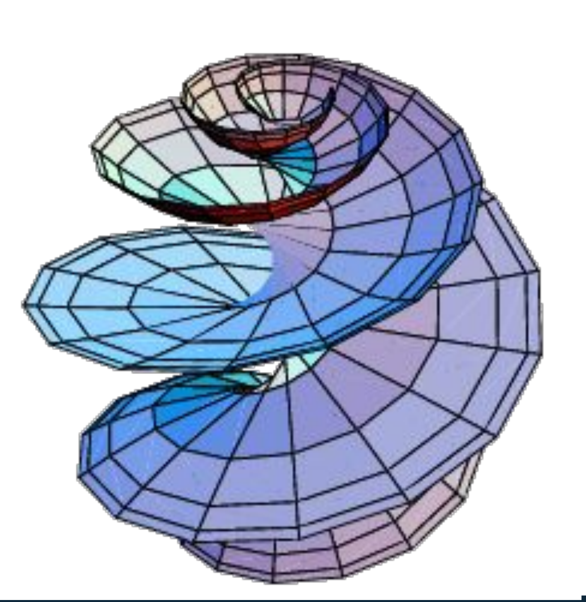


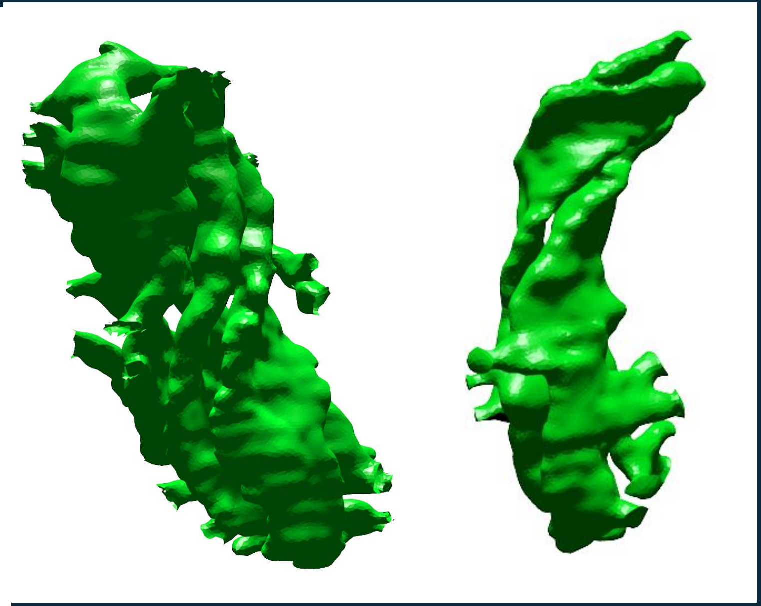
Membrane Motifs: Helicoidal Twist

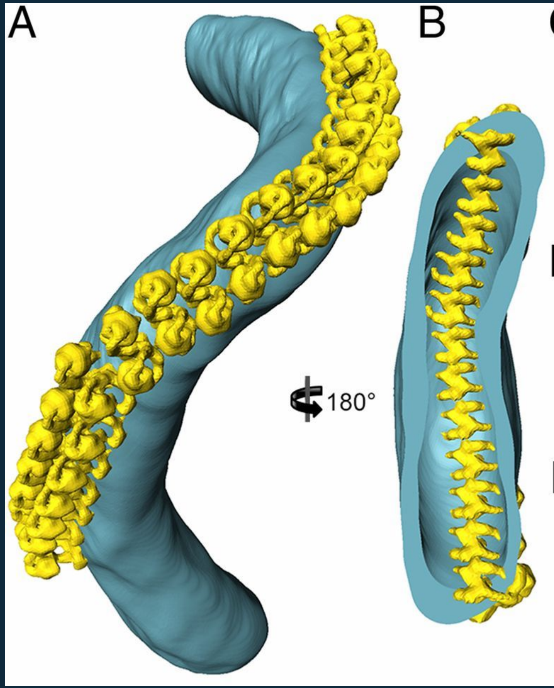
- Maximization of Space with Little Energy Expense
- Same Minimal Surface Motif Found in ER Sheets (Terasaki et al, 2017)
- All 4 Currently Meshed Mitochondria Exhibit Right-Handed Curves

ATP-Synthase suggested to be responsible for RH helix in ciliate mitochondria

Muhleip, AW; et al; PNAS 2016

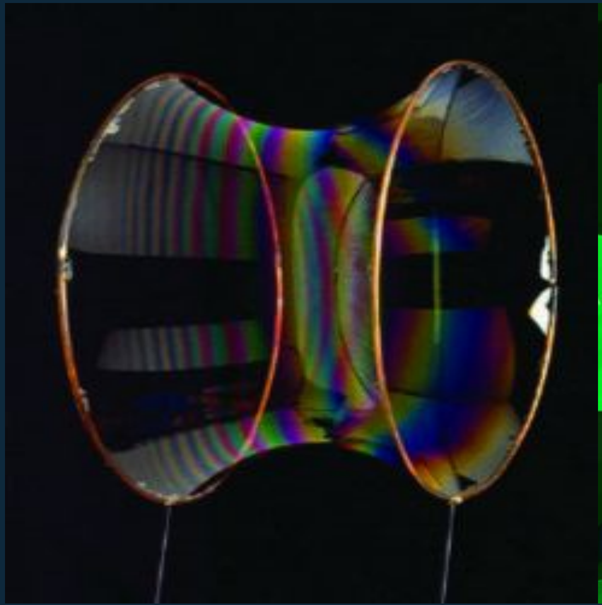
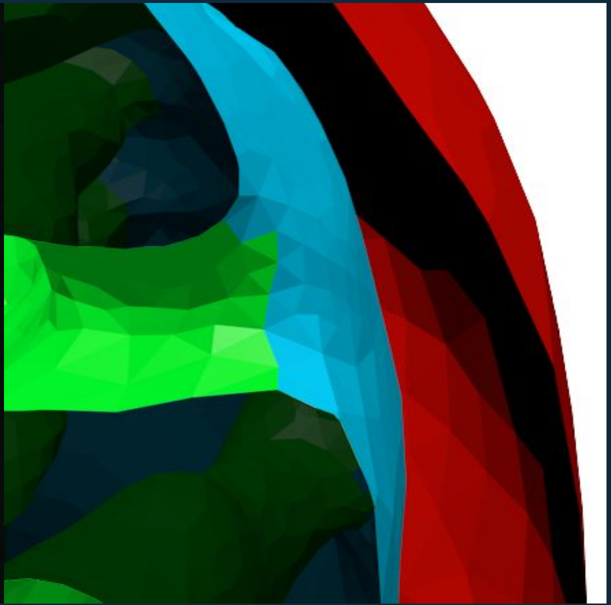




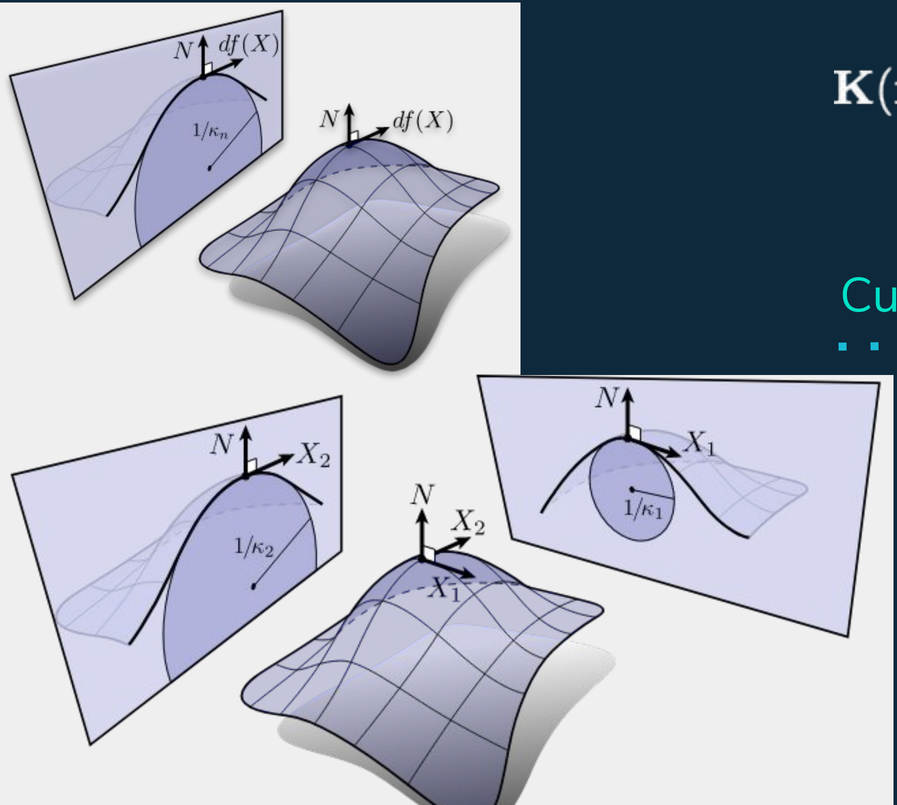


Membrane Motifs: Catenoid Cristae Junctions

- Catenoids are minimal surfaces also seen in endo- and exocytosis throughout the cell. (Chabanon and Rangamani, 2018)
- Present at cristae junctions.

Curvatures estimated using discrete differential geometry on the smooth mesh



DDG Lectures, Keenan Crane

$$\mathbf{K}(\mathbf{x}_i) = \frac{1}{2\mathcal{A}_{\mathrm{Mixed}}} \sum_{j \in N_1(i)} (\cot \alpha_{ij} + \cot \beta_{ij})(\mathbf{x}_i - \mathbf{x}_j)$$

Mean Curvature

$$\kappa_H = \frac{1}{2}|\mathbf{K}(\mathbf{x}_i)| = \frac{1}{2}(\kappa_1 + \kappa_2)$$

Gaussian Curvature

$$\kappa_G(\mathbf{x}_i) = (2\pi - \sum_{j=1}^{\#f} \theta_j)/\mathcal{A}_{\mathrm{Mixed}}$$

$$= \kappa_1 \kappa_2$$

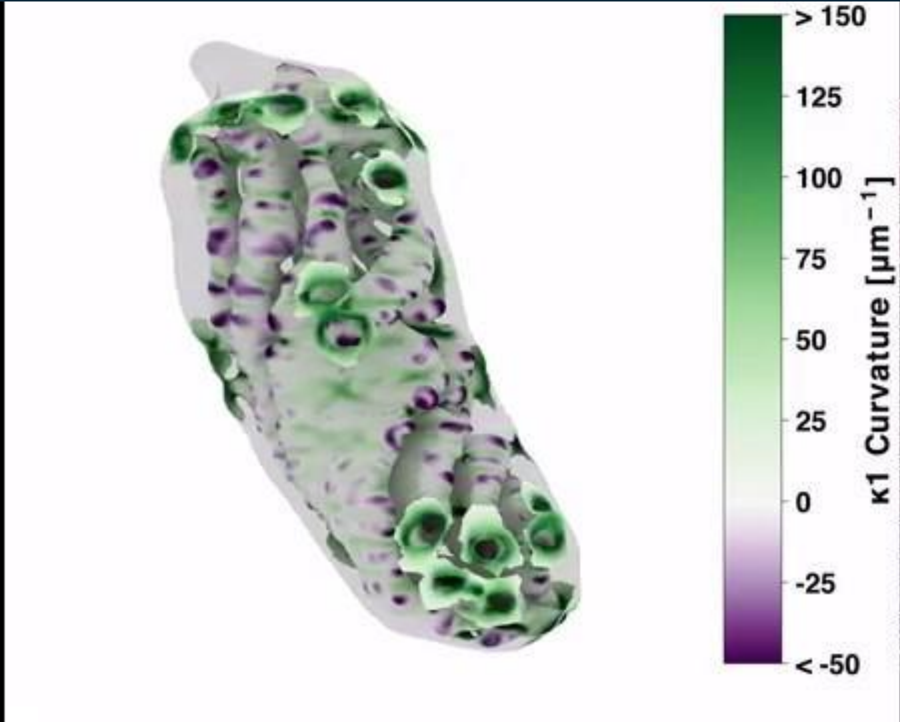
First and Second Principal Curvatures

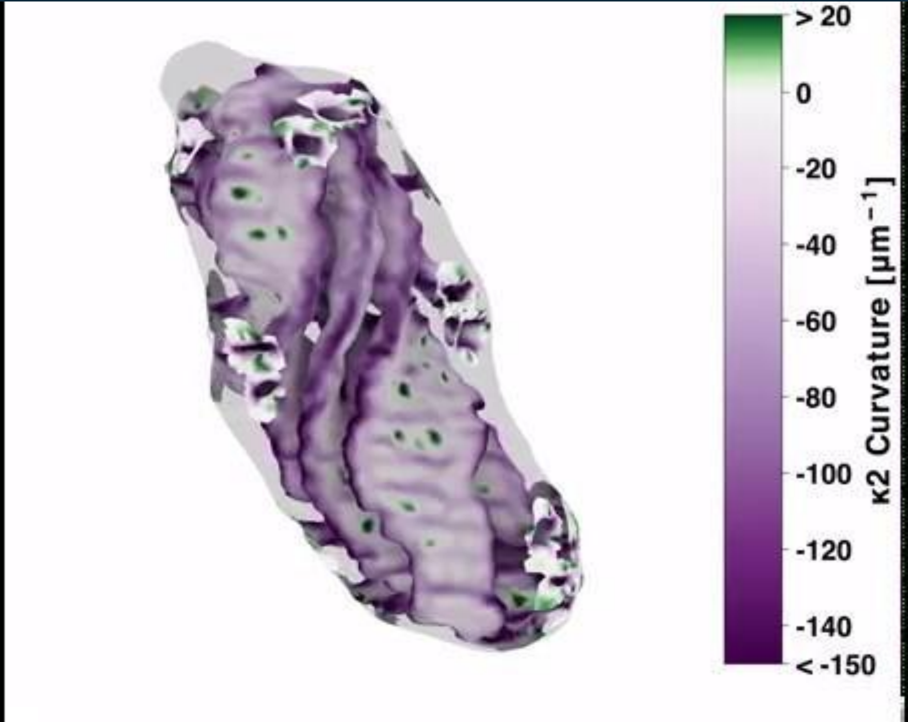
$$\kappa_1(\mathbf{x}_i) = \kappa_H(\mathbf{x}_i) + \sqrt{\Delta(\mathbf{x}_i)}$$

$$\kappa_2(\mathbf{x}_i) = \kappa_H(\mathbf{x}_i) - \sqrt{\Delta(\mathbf{x}_i)}$$

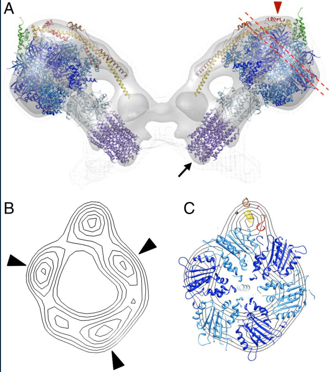
Meyer et al., (2003) Visualization and Mathematics III

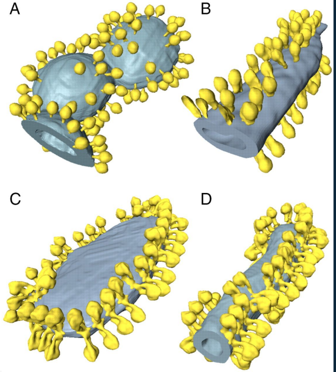
Curvatures estimated using discrete differential geometry on the smooth mesh

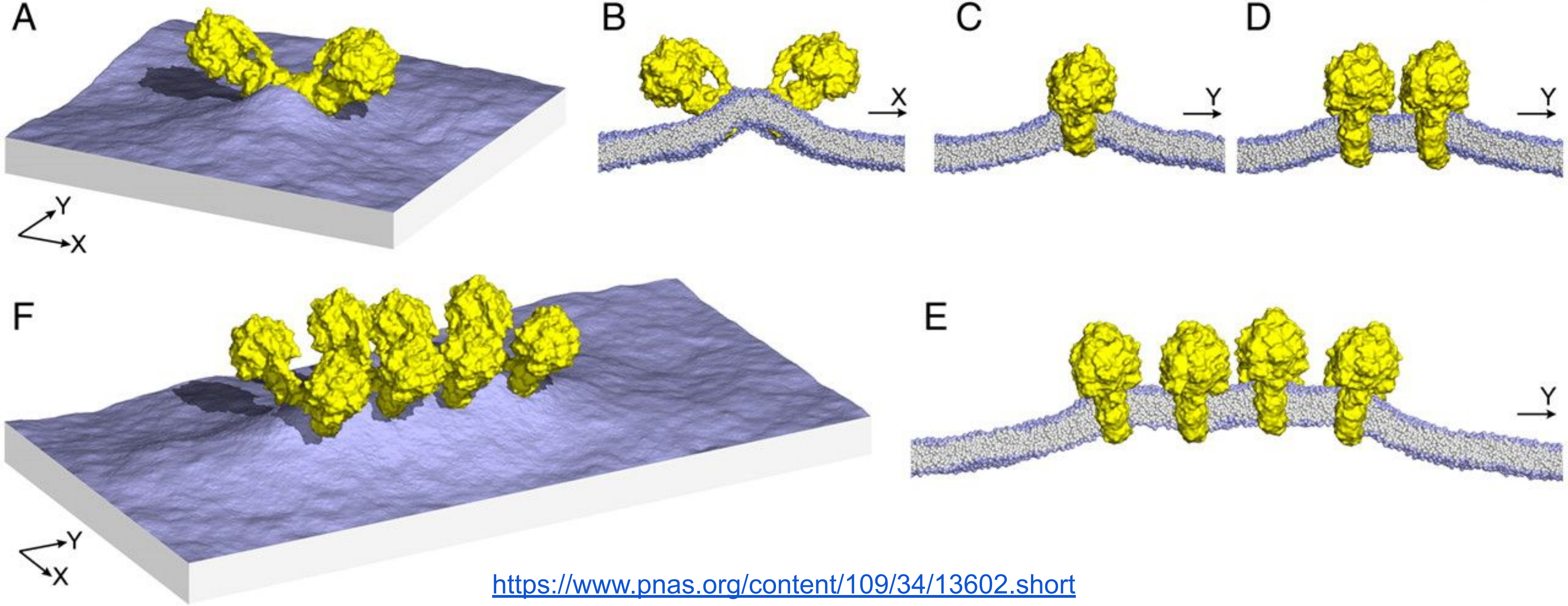




Cristae curvatures are often induced by ATP Synthase







https://www.pnas.org/content/109/34/13602.short

Dimers of mitochondrial ATP synthase induce membrane curvature and self-assemble into rows

PLOS | BIOLOGY

January 25, 2018

SHORT REPORTS

Mitochondria are physiologically maintained at close to 50 °C

Dominique Chrétien[1,2], Paule Bénit[1,2], Hyung-Ho Ha[3], Susanne Keipert[4], Riyad El-Khoury[5], Young-Tae Chang[6], Martin Jastroch[4], Howard T. Jacobs[7,8], Pierre Rustin[1,2,9]*, Malgorzata Rak[1,2,9]

PLOS | BIOLOGY

January 25, 2018

PRIMER

Hot mitochondria?

Nick Lane*

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A critique of methods for temperature imaging in single cells

Guillaume Baffou, Hervé Rigneault, Didier Marguet & Ludovic Jullien

Using 3D Models to Study Thermal Properties of Mitochondria

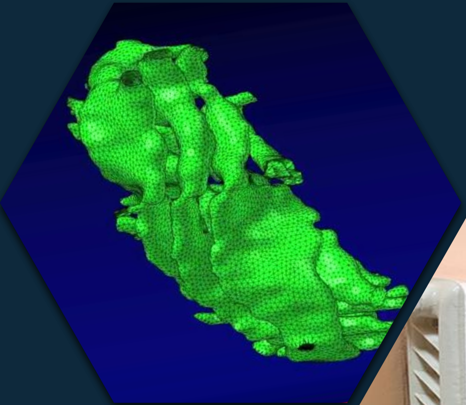
Thermodynamics & Cristae

Prevalence of Negative Gaussian Curvature

- Saddle points and areas of negative gaussian curvature facilitate large thermal fluctuations. (Evans et al., 2017)

High Surface Area to Volume Ratio

- Cristae channels maximize surface area to catalyse reactions.
- Maximum surface area leads to maximum heat distribution from the mitochondria outwards.



$$\frac{\partial \phi(\mathbf{r},t)}{\partial t} = D\nabla^2 \phi(\mathbf{r},t),$$

Summary

- 3D Mitochondria Models Useful for Morphological Analysis + Simulations
- Workflow produces meshes amenable to basic morphological analysis

Moving Forward

- Improving workflow throughput
- Priya + Meagan working on ML approaches to segmentation
- Optimizing curvature calculations
- Creating a Mechanical Model of Mitochondrial Curvature

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Padmini Rangamani (UCSD)

Electron Tomography

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Alexander Skupin (LCSB)

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General Discussion

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Harris Lab (UT Austin)

AFOSR, NSF DMS, NIH NIGMS, Hartwell Foundation

Segmentation and Meshing

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Meshathon Participants

UC San Diego

JACOBS SCHOOL OF ENGINEERING

Thank You!

Salk Institute

Terry Sejnowski

Tom Bartol

Don Spencer

Bob Kuczewski

Priya Khandelwal

Emily Liu

Kelly Brockmeyer

UC San Diego

Padmini Rangamani

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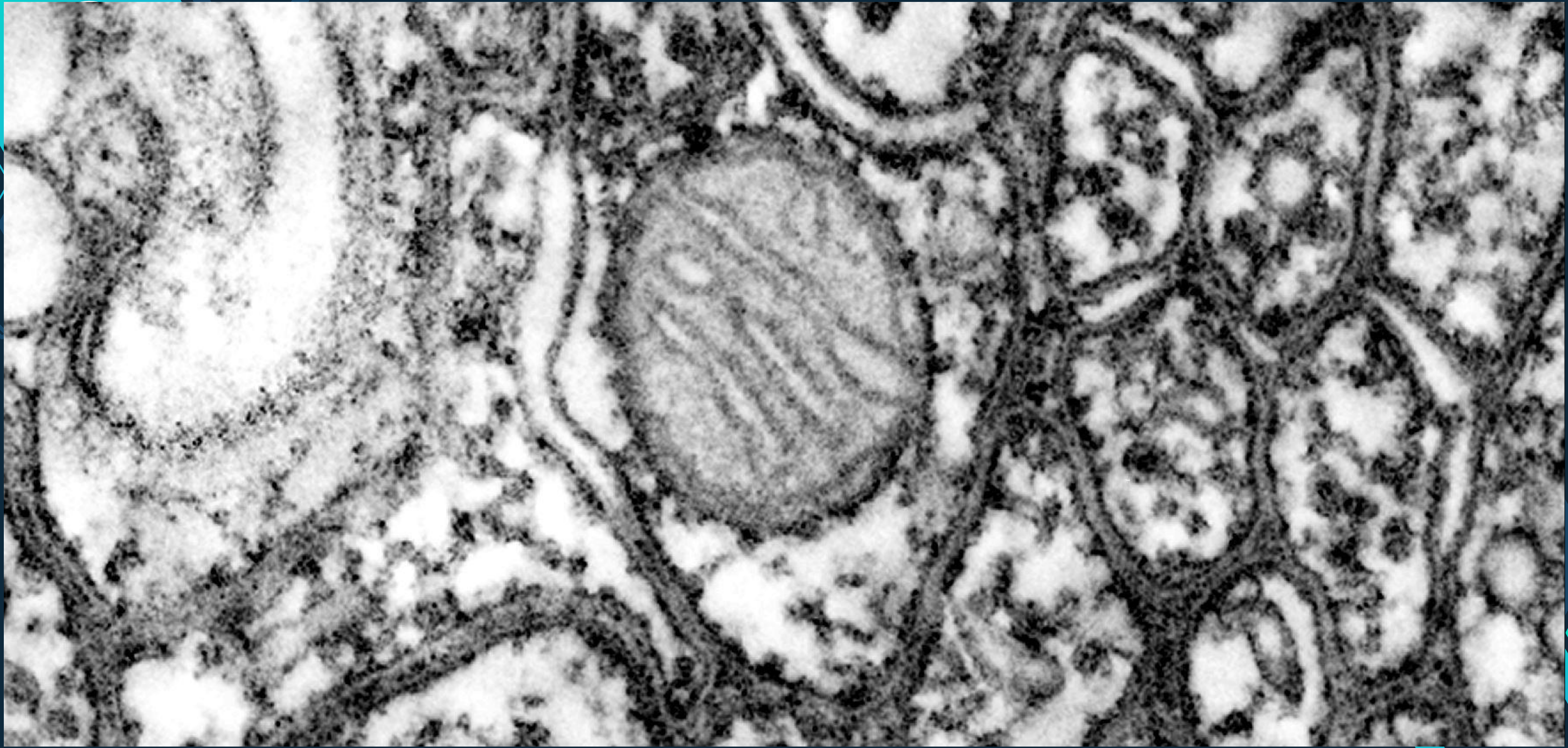
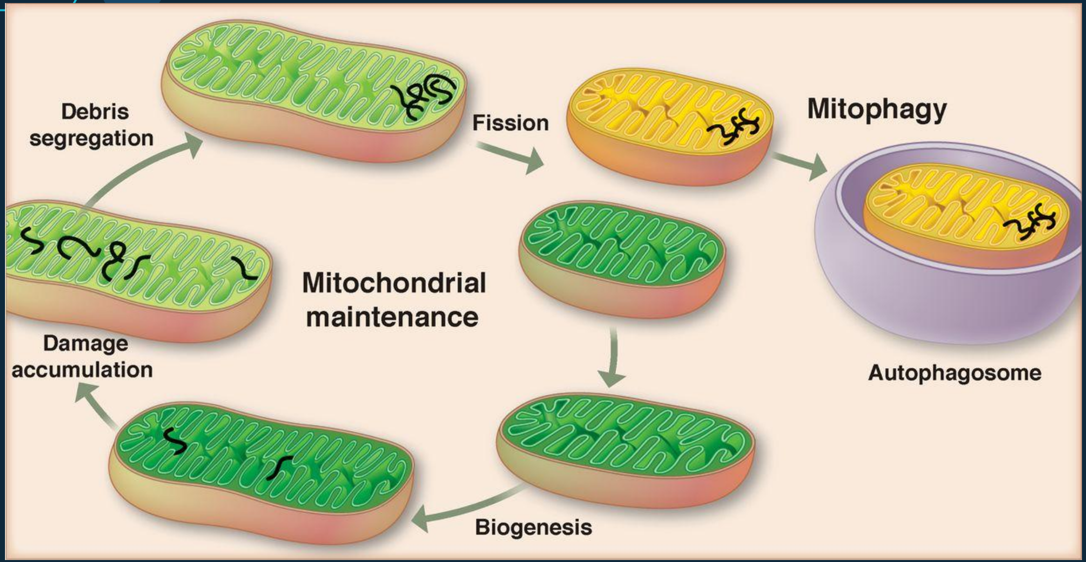


A Slice from a Mouse Cerebellum

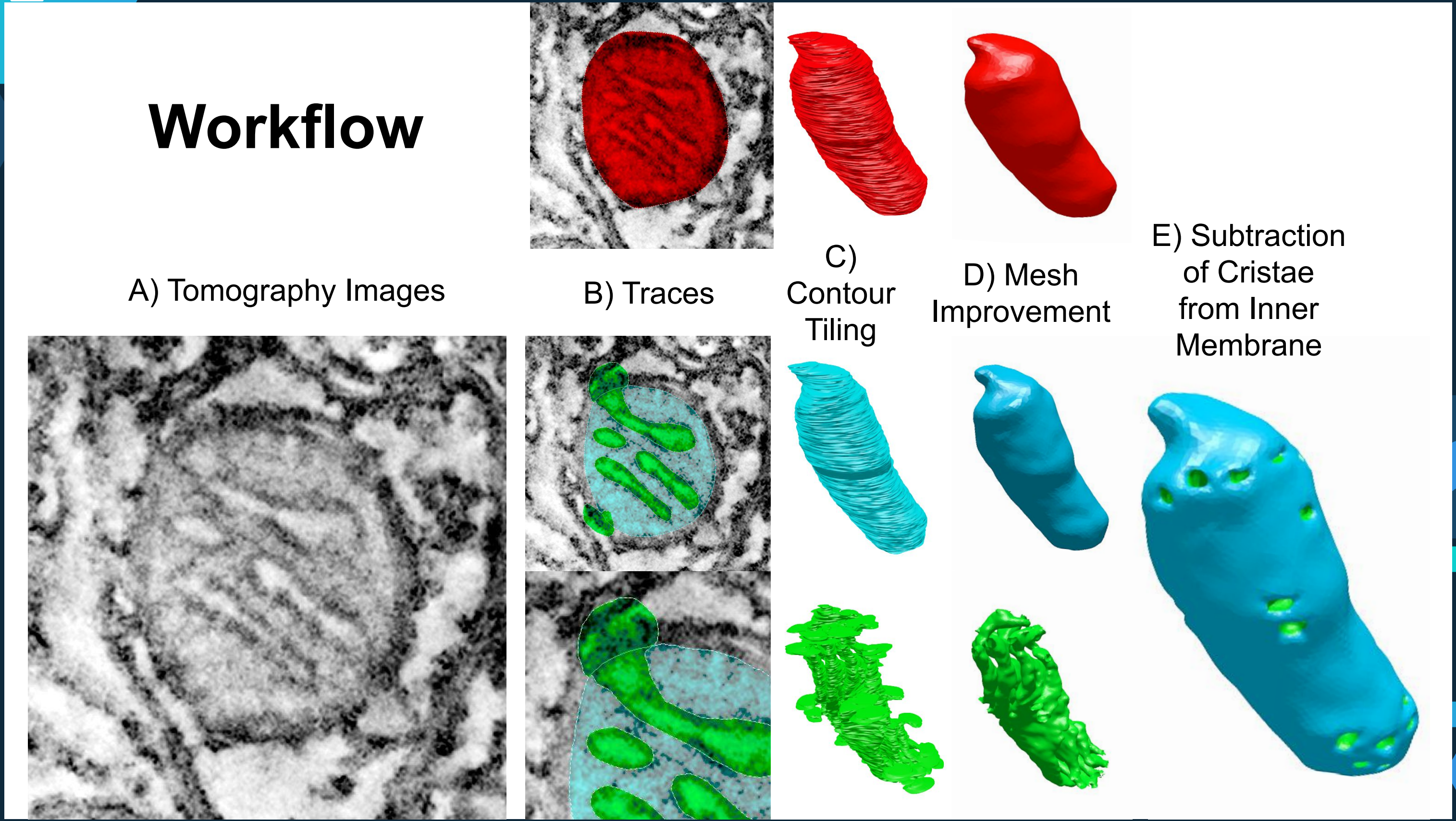
A Mitochondrial Network

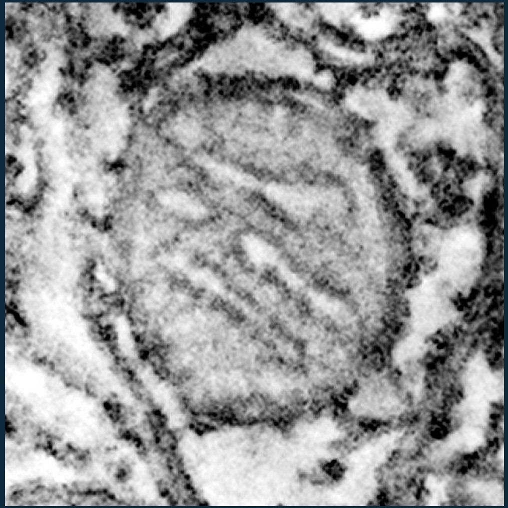
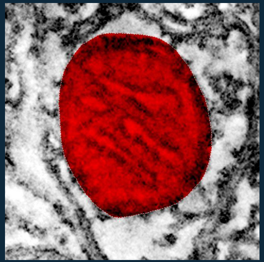
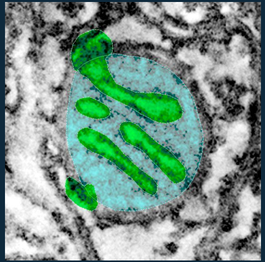
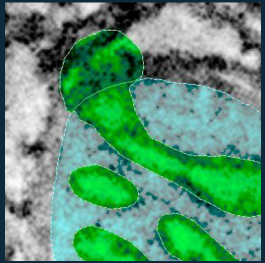


(Youle, 2012)

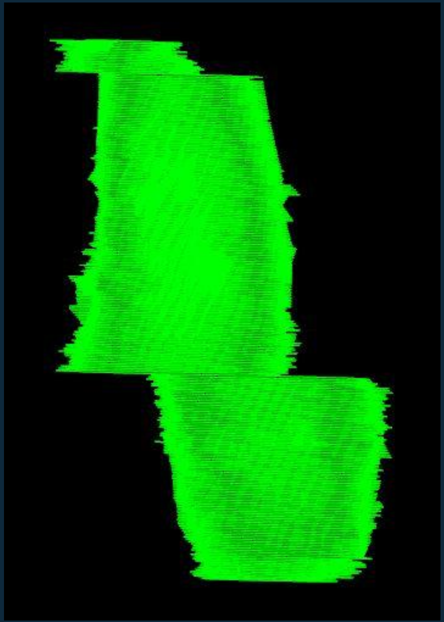
- Mitochondria can Combine (Fusion) and Divide (Fission)
- Fusion
  - Strengthens ATP Production
  - Reduces Impact of mtDNA Mutation
- Fission
  - Creates of New Mitochondria
  - “Pushes Out” Damage and Debris

Creating a 3D Reconstruction



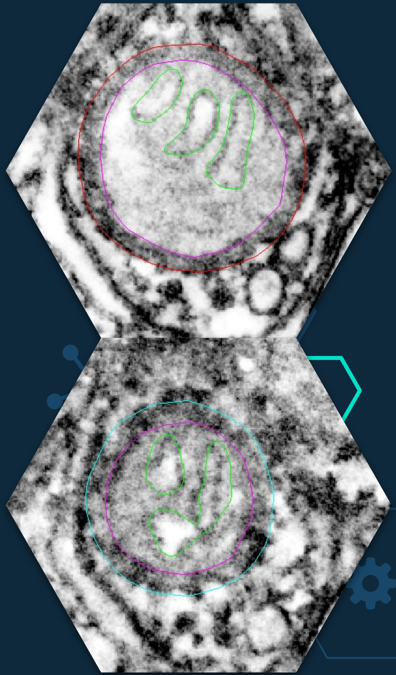





Serial Transmission EM Tomograms of Mouse Cerebellum → **Image Segmentation** → Convert to a 3D Model with CellBlender and GAMer → Analysis + Modeling



Gap Interpolation

- Physical slices of a rat brain are cut 250 nanometers thick. → CT Scans create 1.6-nanometer thick virtual slices
- Knife Removes Material Between Tomograms
- Thickness Disparity → Gaps in the Data Set
- 1.6 nm Slices, 16-20 nm Gaps
- Must be Manually Interpolated with the Help of IMOD’s Interpolator



GAMer

- Designed by Zeyun Zu with the Holst Research Group
- Translates Reconstruct Traces into a Smooth Surface Model

CellBlender

- Designed by Tom Bartol for Cell Modeling
- Subtracts Cristae from Inner Membrane to form Invaginations