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Salk CNL – Prof. Sejnowski
With Dr. Spencer,
Dr. Bartol, and Mr. Kuczewski

HTHNC Internship Project Goals

◇ Conduct a four-week, full-time internship in a field of interest.

◇ Create a project that benefits the organization.

◇ Learn about the workplace and careers.

Project Focus Areas

◇   Neurobiology

◇   Computer Programming

◇   *Image Segmentation Project*

My Tasks

Create traces of each membrane in an image stack.

Work with Dr. Tom Bartol to create a 3D model.

Objective: Create a Three-Dimensional Mitochondria Model from a Stack of SEM Images

The Data Set

- 299 Images, 1.5 microns by 0.7 microns
    - Area of 1 iPhone pixel, 2.7 Million per Screen
- 200 Nanometer z Resolution
    - Too Coarse
- Need 2 nm xyz Resolution for 3D solid model
    - Created with Tomogram Images
- Note: Stack→Series→Object→Section→Traces

Virtual Slices Made with Computerised Tomography

| Physical slices of a rat brain are cut 200 nanometers thick. | CT Scans create 2-nanometer thick virtual slices | Images are optimized with a Python program. |

Trouble with Tomograms

◇ Transparency of Virtual Slices
  ▪ A New Type of "Grey Wall" (Kirk, 2018)
◇ Thickness Disparity → Gaps in the Data Set

Where does the data come from?

Rat Brain

Cerebellum

Granule Cell attaching to Purkinje Cells

Parallel Fiber Axon
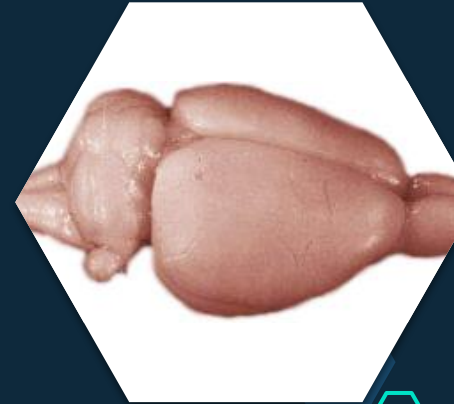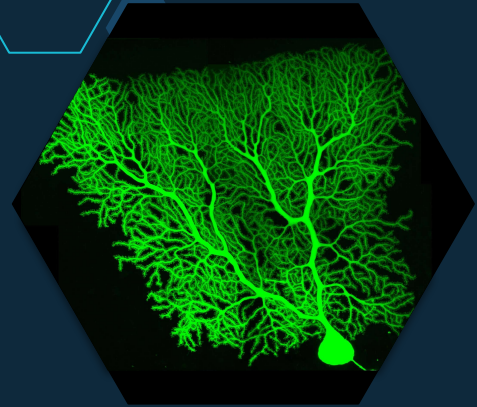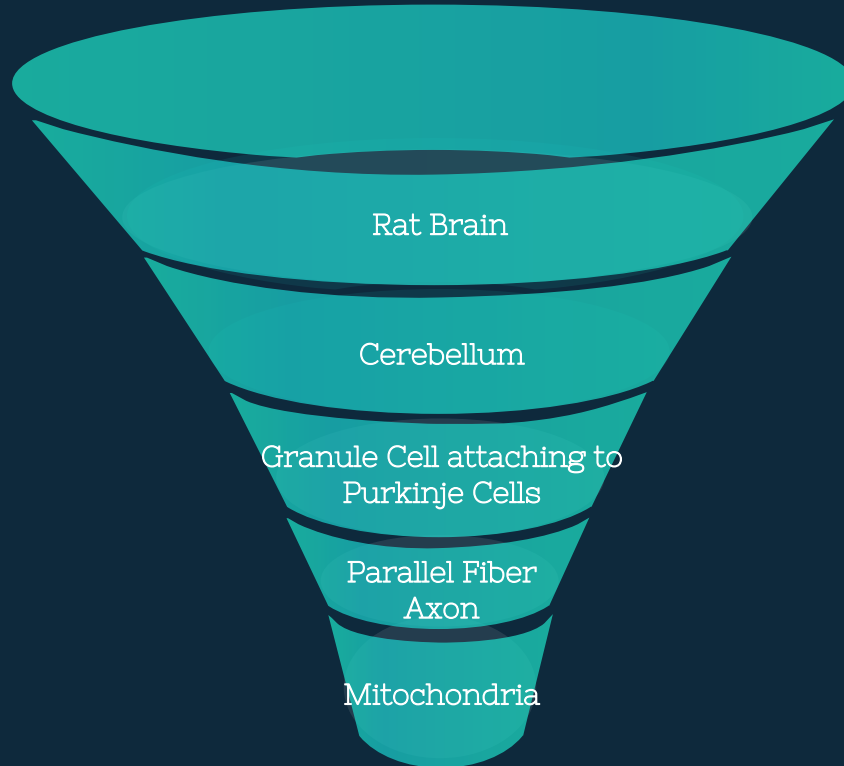
Mitochondria

Mitochondria

- ◇ Create ATP, providing energy for the cell.
  - ■ Matrix → Pyruvate Oxidation and Krebs Cycle
  - ■ Inner Membrane → Electron Transport
 - ■ $C_6H_{12}O_6 + 6O2 \rightarrow 6CO_2 + 6H_2O + 38$ ATP
- ◇ Instigate apoptosis, the "pruning" of cells.
  - ■ Shut Down Cellular Respiration & Activate Cysteine Proteases
 - ■ Dysfunctional → Lipofuscin Granules (Moreira et al., 2010)
- ◇ Create body heat as a "waste product" of energy production.
  - ■ 50℃ or 122℉, About 30% Higher than Body Temperature (Chretien, 2017)
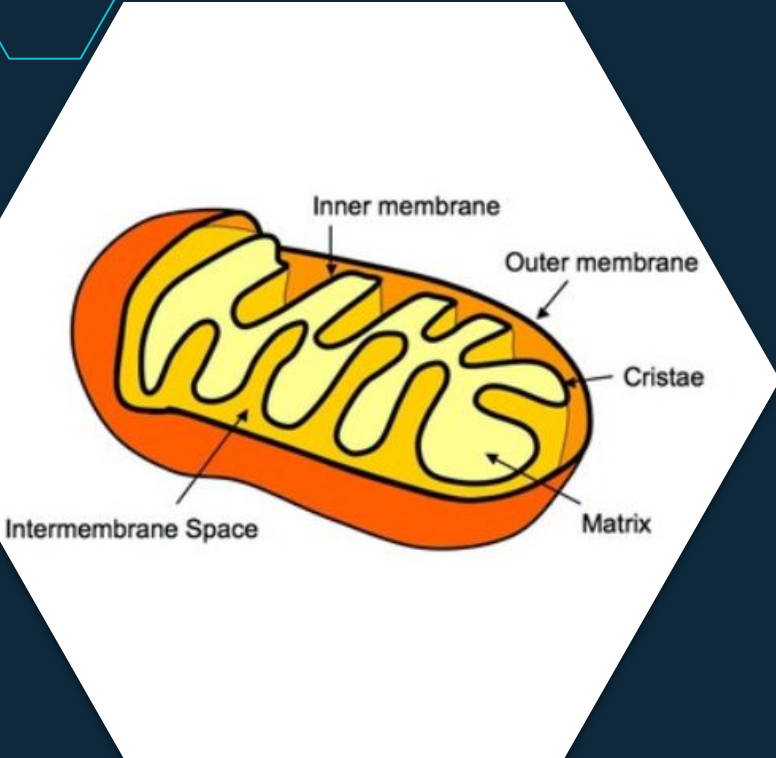
Reconstruct

◇ A Program Specially Designed by Prof. Kristen Harris for Image Segmentation

◇ Allows for traces to be compiled into a 3D model.

◇ Used to create "Objects" out of each membrane.

Outer Membrane

Inner Membrane
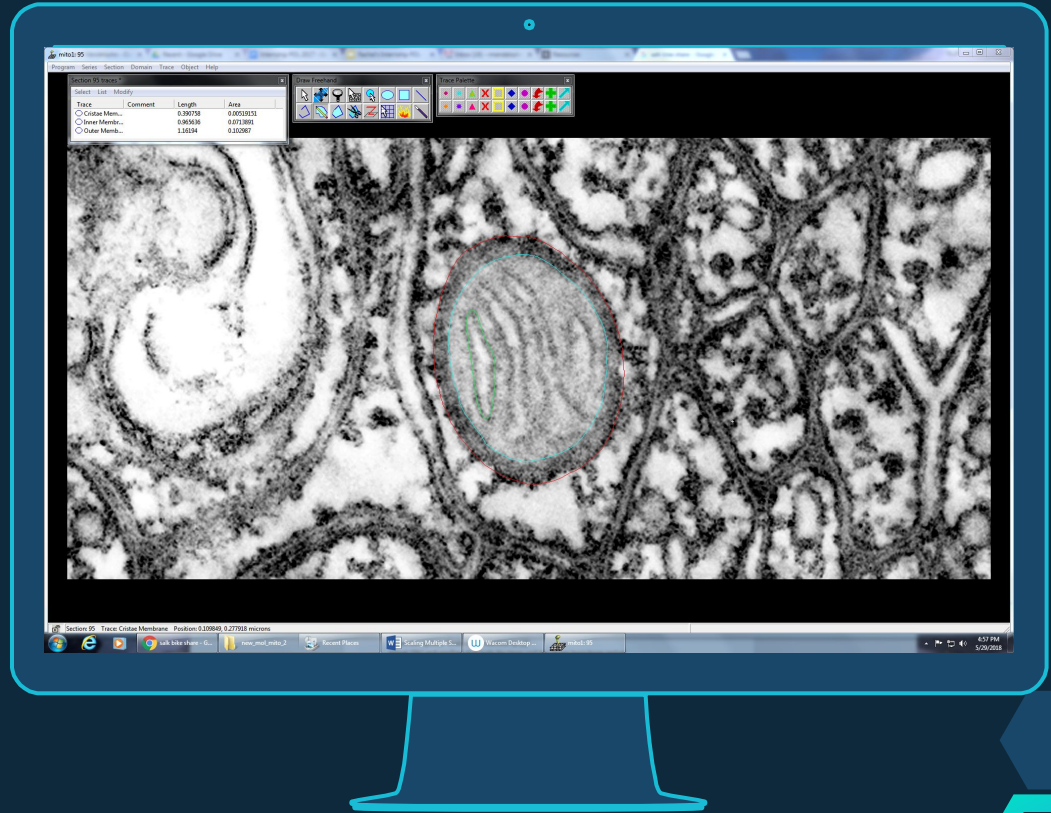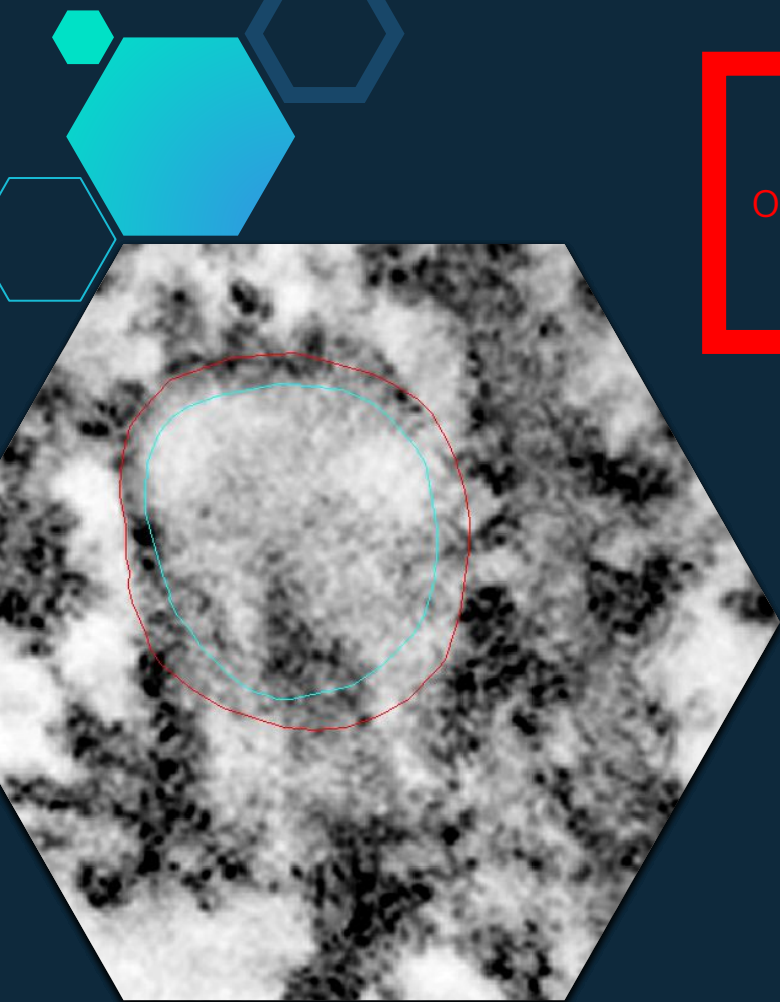
Cristae Membrane

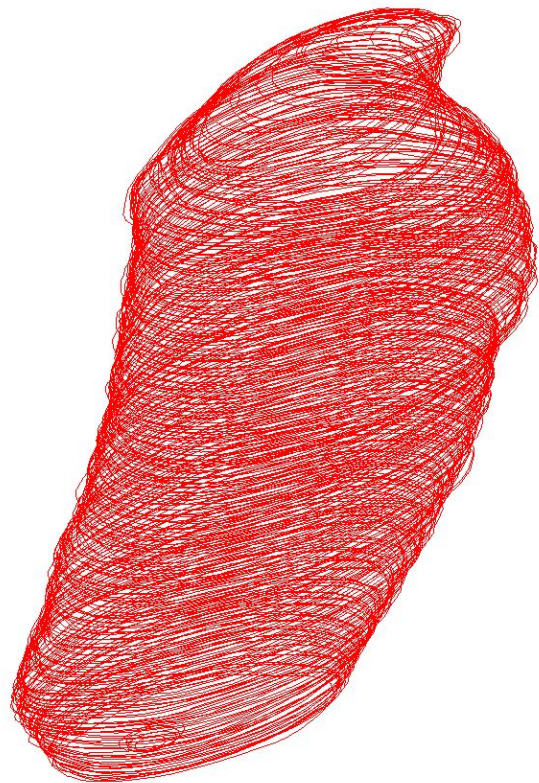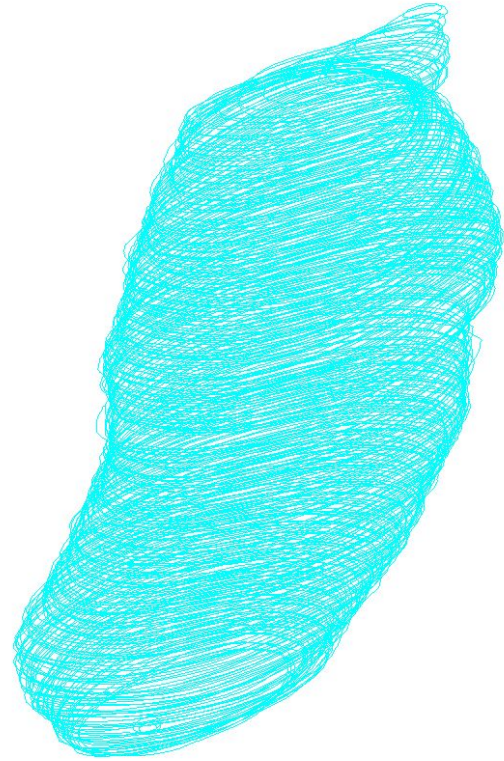# Tracing Protocol

◇ Compare with known traces.

◇ View each section in the context of surrounding sections.

◇ Start in the center and work towards the ends.

Outer Membrane
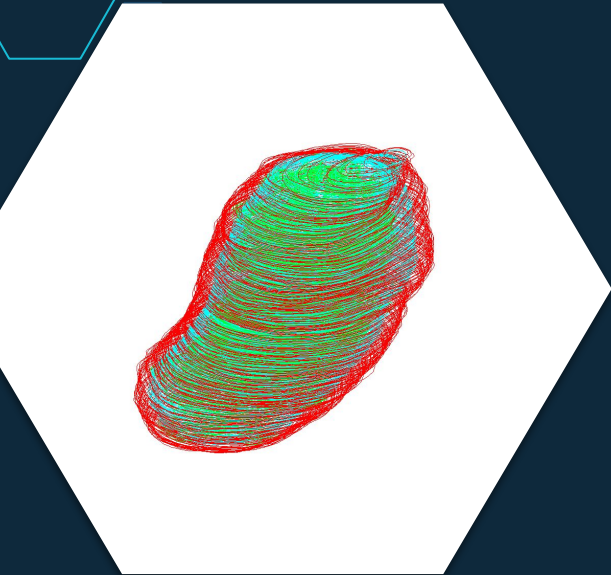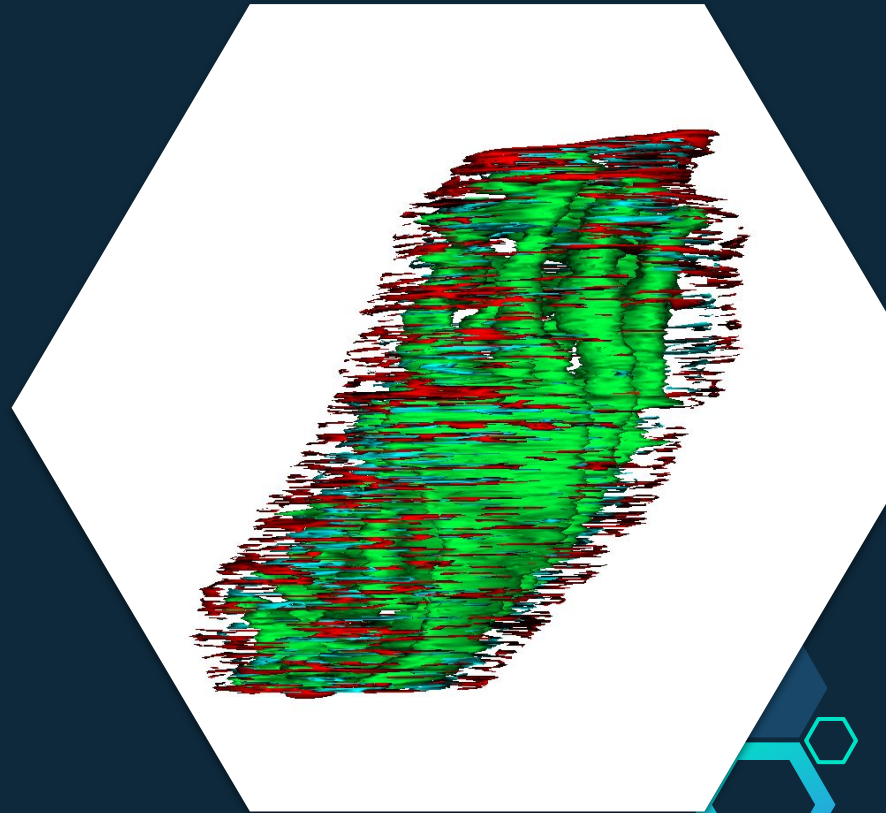
Inner Membrane

Cristae Membrane

→ **Surfacing** →

Blender / M-Cell

◇ Designed by Tom for Cell Modeling

◇ Subtracts Cristae from Inner Membrane to form Invaginations

◇ Translates the Traces into a Smooth Surface Model
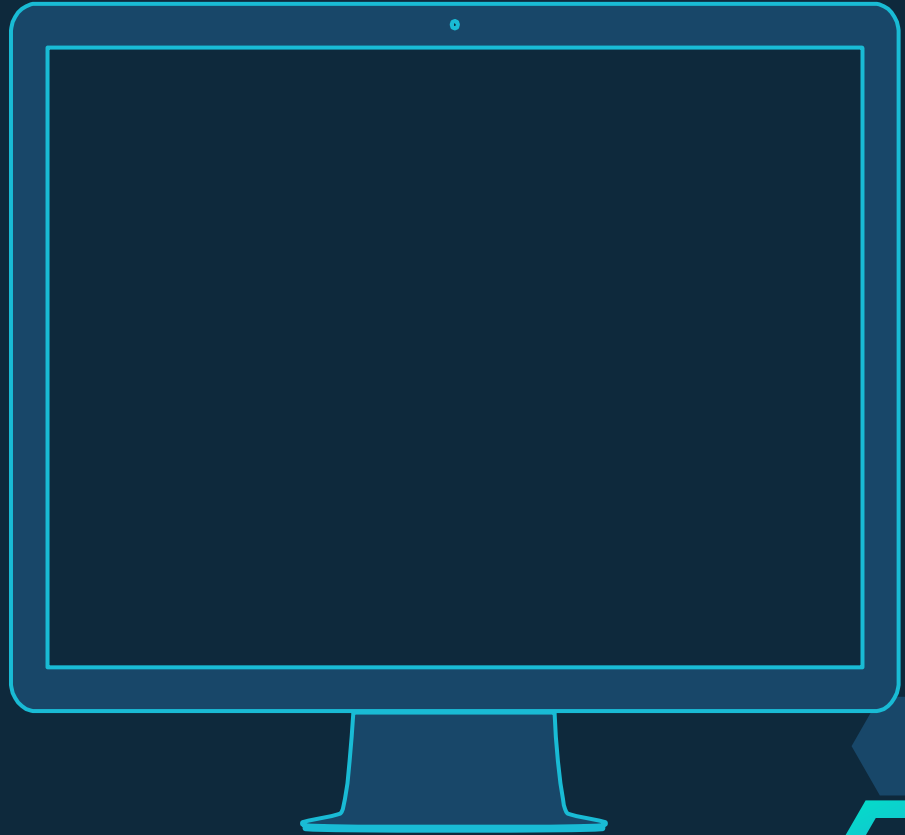
Cristae Membrane

The Diffusion Equation

◇ Describes Density of a Diffusing Material over Time

◇ Cristae Channels Maximaize Surface Area to Catalyse Reactions

The Heat Equation

◇ Describes Heat Distribution over Time

◇ Maximum Surface Area Leads to Maximum Heat Distribution from Mitochondria Outwards

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

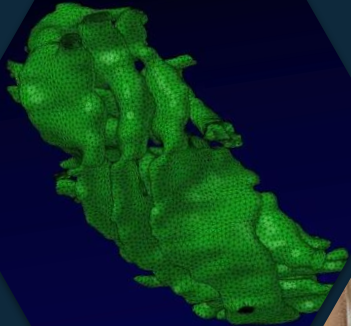# Filling in the Blanks – Interpolation
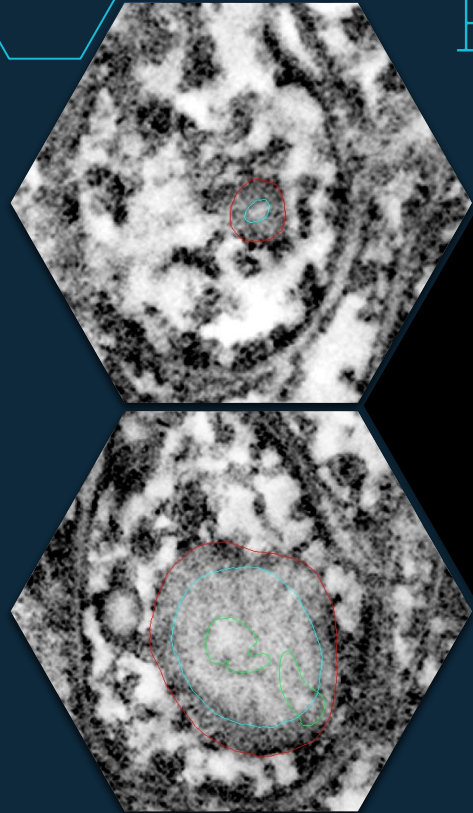
◇ Interpolation → Estimates for the Space between Known Information

◇ Skipped Areas in the Stack

◇ Put in Blender to Determine Space of Gaps → Traces Estimated in Reconstruct

◇ Must Still Be Done on the New Model

"What is the impact of mitochondrial morphology on function?"

University of Luxembourg, Guadalupe Garcia

◇ Structure → Level of ATP Production

◇ ATP/ADP Translocator

 ▪ A Protein within the Inner Membrane

 ▪ Exchange of High-Energy ATP Inside with Low-Energy ADP Outside
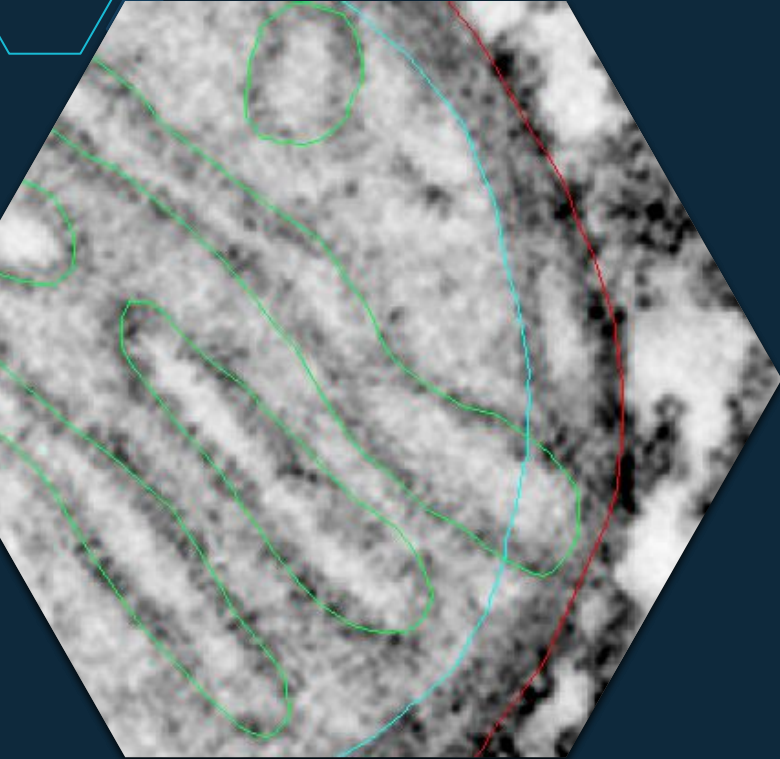
Our Questions

◇ What is the "spillage" from open cristae membrane?

◇ What are the small circles between membranes?

◇ Is the missing data a result of the knife tearing material away, or the tomogram algorithm not going up far enough?

◇ Does the structure of the mitochondrion change with age or neurodegenerative disease?

Benefits of Staring at a Mitochondrion

◇ Better Understanding the "Powerhouse of the Cell"
◇ Strengthening Visual Perception
◇ Learning to Do Image Segmentation with Reconstruct
◇ Contributing to Ongoing Research

Most importantly, it's a great excuse to spend time at the Salk!

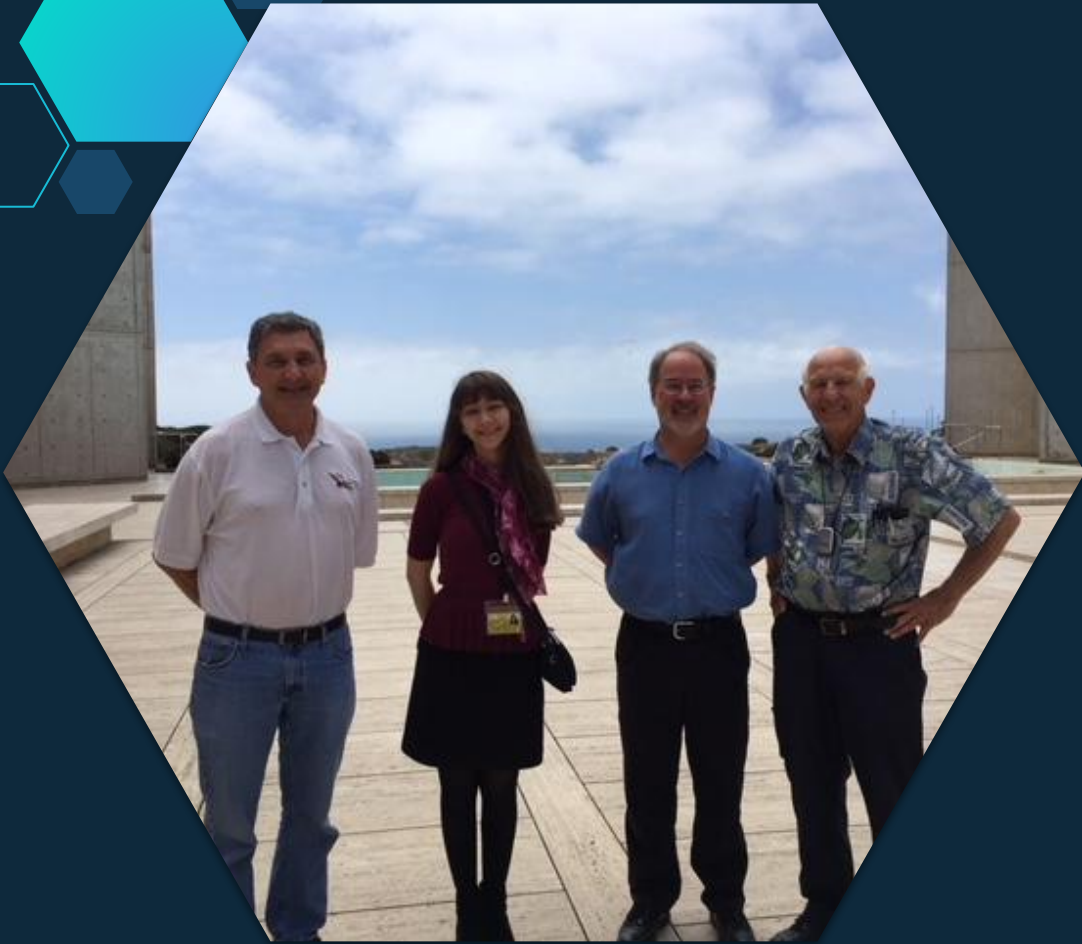# Thank you!

- ◇ Terry
- ◇ Brad
- ◇ Ramona
- ◇ Dona
- ◇ Cailey
- ◇ Sophie
- ◇ Iris
- ◇ Roger

◇ **And Everyone!**

Don, Tom, and Bob - Thank you for making it all happen!

Questions?

It's been a pleasure working with you!

Citations

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