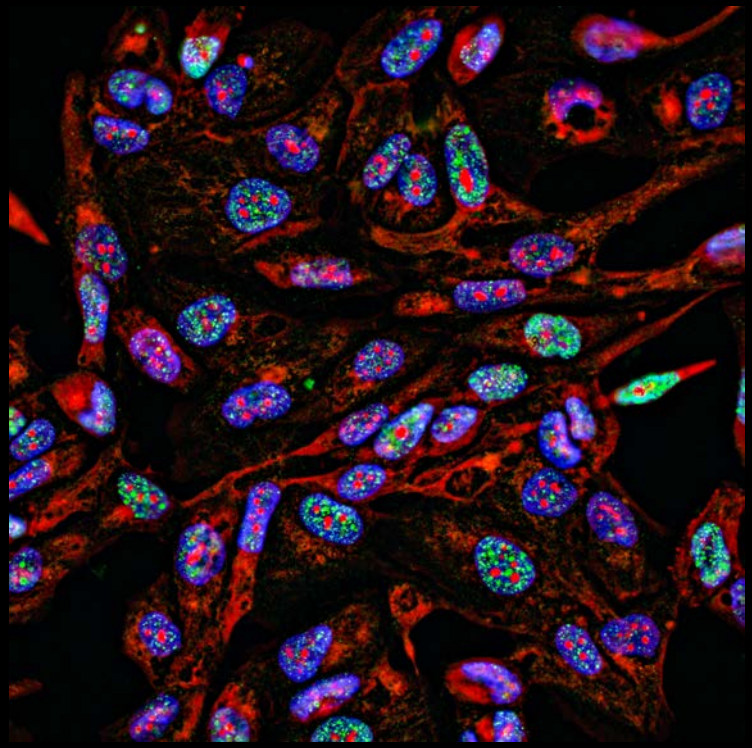
SD-NSC HETEROGENEITY OF AGING CORE

AGE-RELATED CHANGES TO COCHLEAR HAIR CELL ORGANELLES



Uri Manor, Ph.D.
Director, Waitt Advanced Biophotonics Core
Assistant Research Professor
Chan-Zuckerberg Initiative Imaging Scientist

SAN DIEGO NATHAN SHOCK CENTER
2022 WORKSHOP

salk Where cures begin. | Sanford Burnham Prebys | UC San Diego School of Medicine

The Amazing Salk Biophotonics Manor Lab Team

Rebecca Gilson

Colbie Chinowsky

Cayla Miller

Cara Schiavon

Aaditya Prasad

Yuning Wang

Zack Deane

Leo Andrade

Sammy Weiser Novak

Arlo Sheridan

Yuzuru Ninoyu

Elena Chrysostomou

Pilot Grant Applicants' services and rates

Access to **HIGH-END LIGHT MICROSCOPY IMAGING MICROSCOPES** and **IMAGING SERVICES** are available. Generally, LM scope time is ~$35/hour for unassisted usage, whereas imaging by SD-NSC staff is performed at a rate of $75/hour. Image analysis is similarly $75/hour when performed by SD-NSC staff, but workstations are available for $8/hour. Training is available at a rate of $75/hour.

More involved projects including live-to-EM CLEM imaging, and/or deep learning-based model training and prediction are also available for a set rate, depending on the scope of the project.

ELECTRON MICROSCOPY

EM offerings	Sample preparation	Imaging	Analysis	Time Frame	Notes
TEM	$200 / sample. Includes ultramicrotomy and 2 hours of imaging.	$50 / hour (autonomous - please inquire: special training required) / $100 / hour (assisted)	$50 / hour (2 hours) + $75 / hour	6 weeks / 4 samples, respective to queue	For quantitative approaches, demand references and detailed approach, and charging $75
SEM	$100 / 5 samples. $50 / stub (mounting and sputtering) (first 2 included)	$50 / hour (autonomous - please inquire: special training required) / $100 / hour (assisted)	$50 / hour (2 hours) + $75 / hour	3 weeks / 5 samples, respective to queue	For quantitative approaches, demand references and detailed approach, and charging $75
Negative staining	$100 / up to 4 grids	$50 / hour (autonomous - please inquire: special training required) / $100 / hour (assisted)	$50 / hour (2 hours) + $75 / hour	1 week / 4 grids, respective to queue	
EELS	Sample dependent	$100 / hour	Sample dependent	Sample dependent	
Chip mapping	$150 / up to 4 samples. Ultramicrotomy: $50 / block sectioned (first 2 included)	$50 / hour (autonomous - please inquire: special training required) / $100 / hour (assisted)	$50 / hour (2 hours) + $75 / hour	6 weeks / 4 samples, respective to queue	
VP hydrated samples	Included in imaging time	$100 / hour	$50 / hour (2 hours) + $75 / hour	Same day, respective to queue	

SD-NSC HETEROGENEITY OF AGING CORE - **IMAGING**

salk Where cures begin. | Sanford Burnham Prebys | UC San Diego School of Medicine

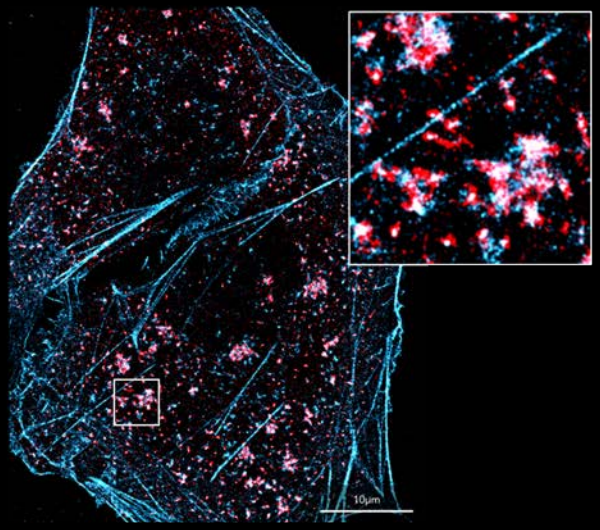
3D-EM offerings	Sample preparation	Imaging	Analysis	Time Frame	Notes
3View	$400 / up to 4 samples + $100 / pin	$500 / 50GB of aligned data	Free training (2 hours) + $50 / hour first 20 hours, $75 / hour	8 weeks / aligned volume	Encourage quantitative approaches with discounts on analysis
S3EM (Serial Sections in the SEM)	$200 / up to 4 samples, $150 / ribbon (up to 100 sections)	$35 / hour (2 hours assisted), $75 / hour (assisted) + $35 / hour (overnight)	Free training (2 hours) + $50 / hour first 20 hours, $75 / hour	8 weeks / aligned volume	
Tomography	$100 / up to 4 samples. Ultramicrotomy: $50 / block sectioned (first 2 included)	$125 / hour	$75 / hour	2 weeks / aligned volume	

Immuno-EM techniques	Sample preparation	Imaging	Analysis	Time Frame	Notes
Array Tomography	$500 / up to 4 samples	Optimizing IF: $30 / block; Ribbon: $50; SEM rates	Free training (2 hours) + $50 / hour first 20 hours, $75 / hour	10 weeks / aligned volume	
Pre-embedding labeling (room temp)	$200 / 4 samples	$35 / hour (2 hours assisted), $50 / hour (autonomous)			
Pre-embedding labeling (AFS)	$400 / 4 samples	$35 / hour (2 hours assisted), $50 / hour (autonomous)			
Post-embedding labeling (AFS)	$400 / 4 samples	$35 / hour (2 hours assisted), $50 / hour (autonomous)			
Immuno-negative staining	$200 / 4 grids	$35 / hour (2 hours assisted), $50 / hour (autonomous)			

Light Microscopy - Super resolution

Nanoimager

- **Applications** map localization of individual proteins and molecules
- **Resolution** 5-20 nm
- **Techniques** dSTORM, PALM, DNA-PAINT

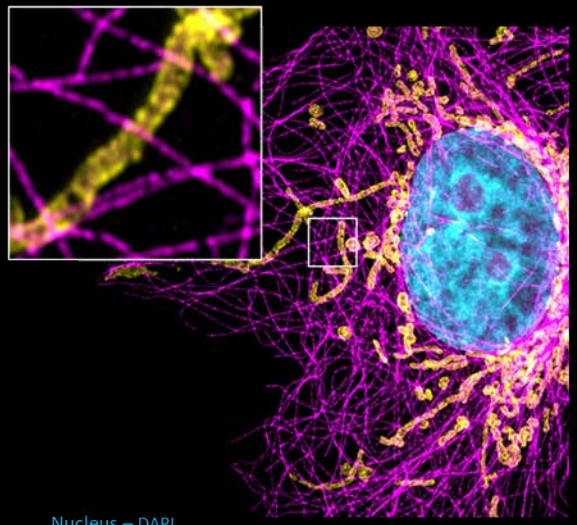


Actin – Phalloidin AF647
EBOV nucleoprotein – AF555

Prof. Dr. Stephan Becker, German Centre for Infection Research, Marburg

Zeiss 880

- **Applications** organelle interactions, dendritic spine morphology
- **Resolution** 120 nm
- **Techniques** Airyscan and Airyscan FAST

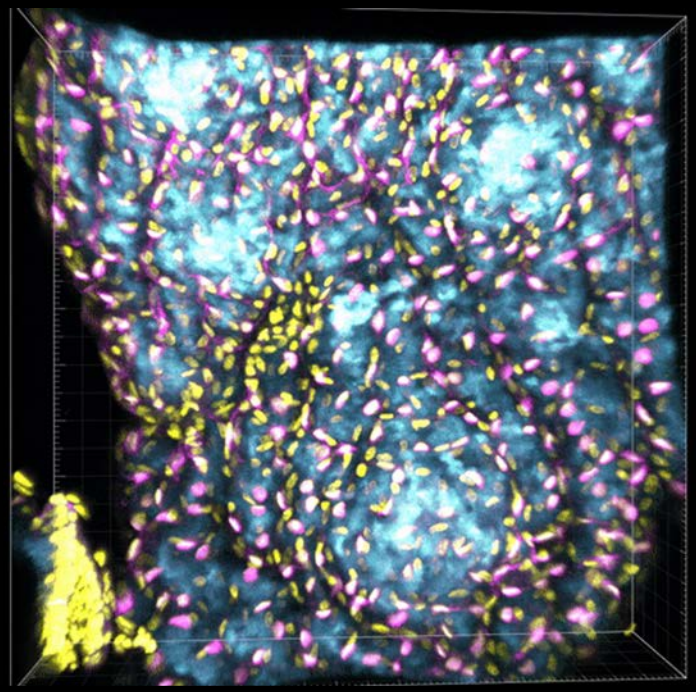


Nucleus – DAPI
Microtubules – α-Tubulin with Alexa Fluor 555
Mitochondria – Tom20 with Alexa Fluor 488

Light Microscopy - Confocal

Zeiss 880 and 710

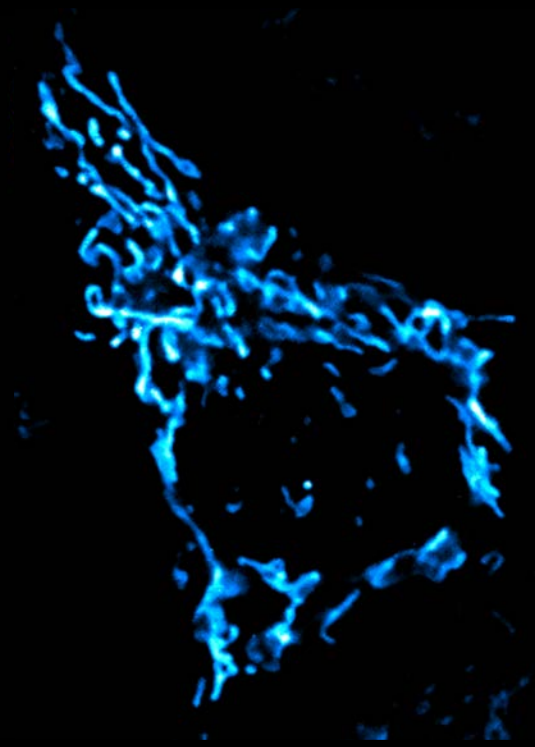
- Thick tissue imaging, subcellular localization



Mammary pad from Wahl Lab

Spinning Disk

- Fast live cell imaging and long time-lapses

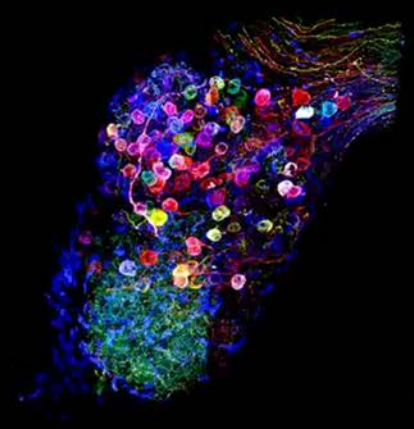


Mitochondria - MTDR

Light Microscopy - Tissue and large specimen imaging

Olympus FV3000

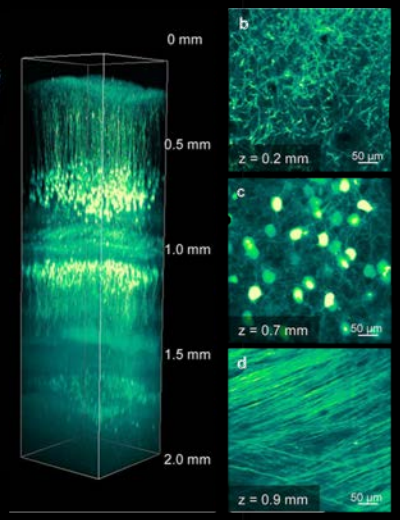
- Deep tissue imaging with silicon objectives



Cleared chick ciliary ganglion.
Dr. Ryo Egawa. Tohoku University Graduate School of Life Science.

Olympus 2-photon

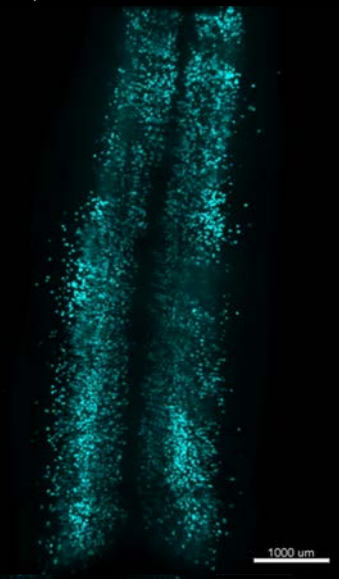
- Deep tissue imaging with label-free SHG (collagen) and CARS imaging



EYFP expressing neurons
Aoyagi (2015) Plos One.

Zeiss Light sheet

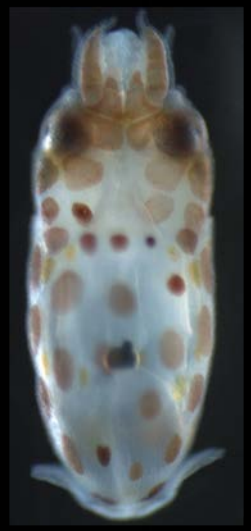
- Large 3D and 4D imaging with CO2 and temperature control



Mouse Spinal Cord
Sofia Pimpinella, Goulding Lab

Zeiss Stereoscope

- Dissection and large sample fluorescence imaging

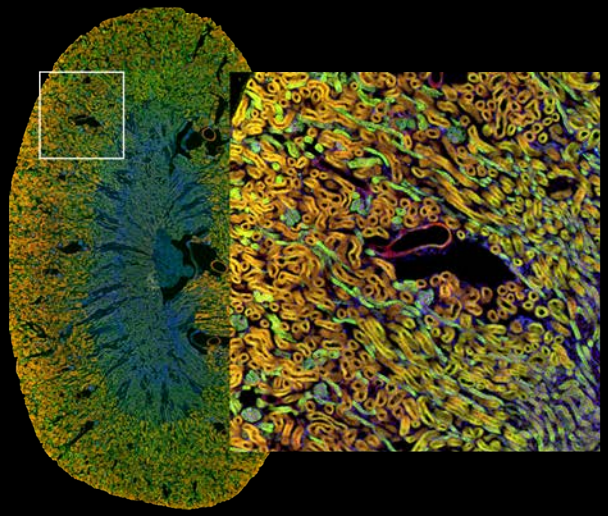


Embryonic squid
Cassandra Extravour
Cambridge, Massachusetts

Light Microscopy - Widefield and automated

Slide Scanner

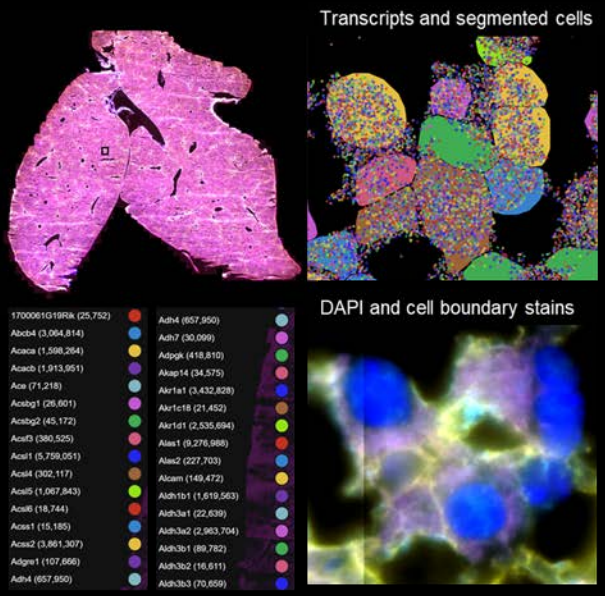
- Automated whole slide imaging
- Fluorescence and bright field



FluoCells™ Prepared Slide #3 (mouse kidney section with Alexa Fluor™ 488 WGA, Alexa Fluor™ 568 Phalloidin, and DAPI)

Merscope

- Spatial transcriptomics using MERFISH

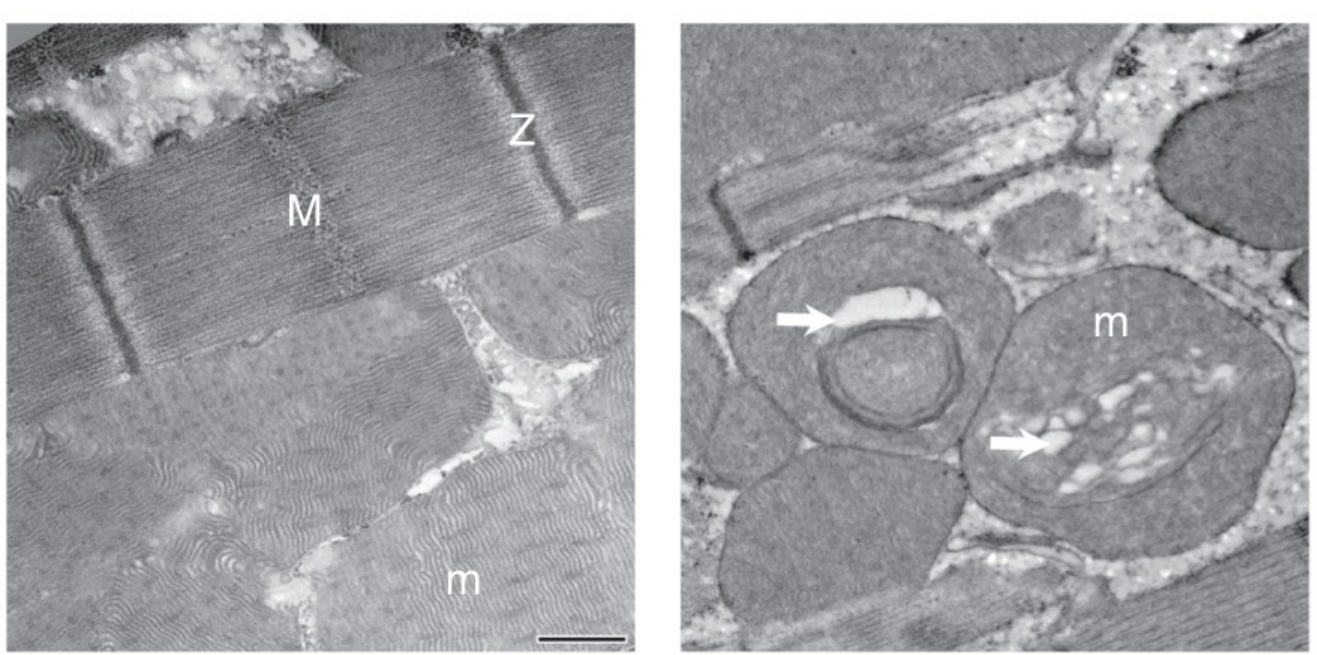


Vizgen MERFISH Mouse Liver Map January 2022

Transmission Electron Microscopy

Control

High-fat diet

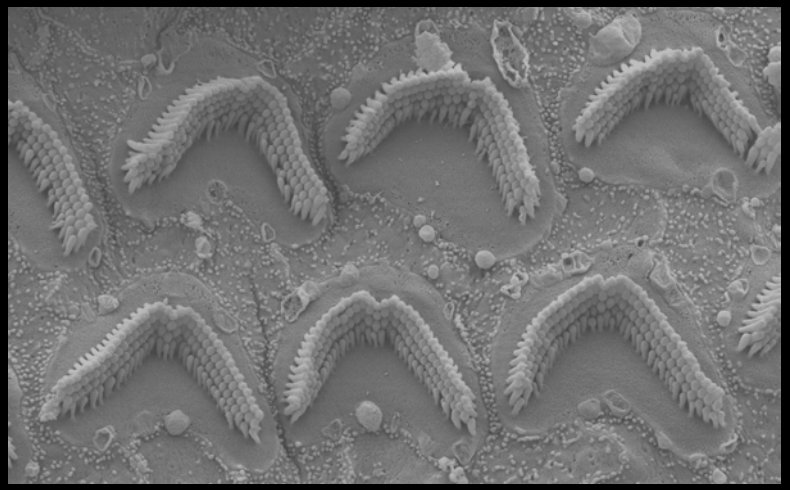


Villaneuva et al. Nature Communications, 2019

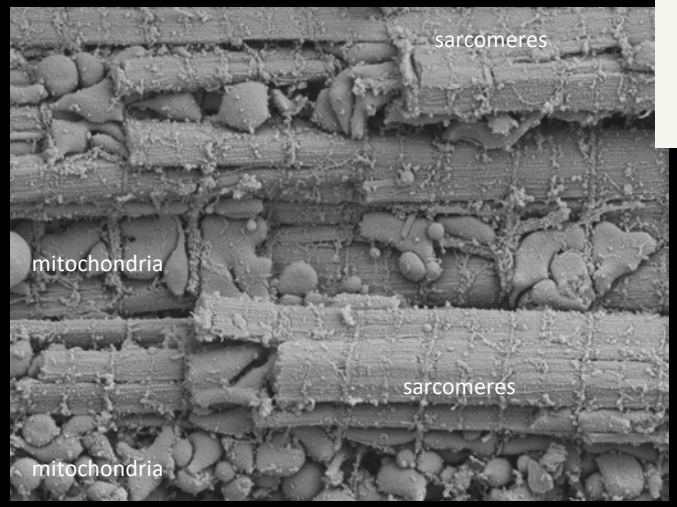
Scanning Electron Microscopy

Goal: To observe the sample topography, overall shape, and fine features of Cells surface. Samples can be chemically fixed or rapid frozen. Dehydrated, CDP, metal coated.

Marmoset outer hair cells stereocilia

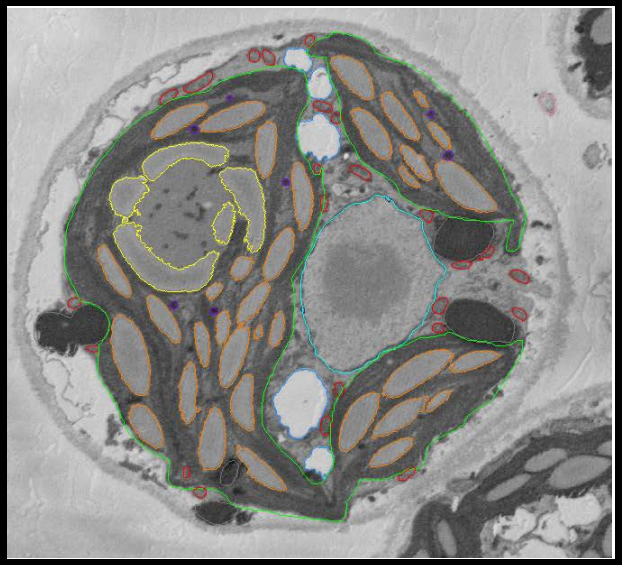


Marmoset heart



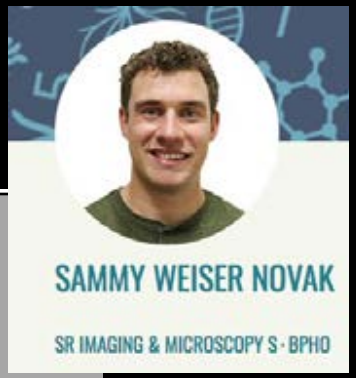
2D EM is misleading, especially for mitochondrial imaging!

Tyler Wittkkop (Noel lab) and Sammy Weiser Novak (WABC)



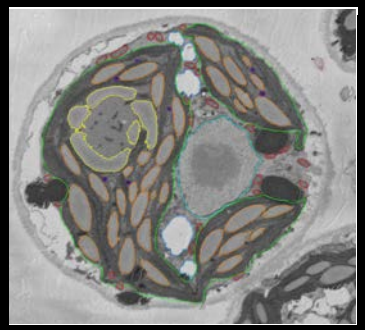
Mitochondrial length ranges from 1-3 μm

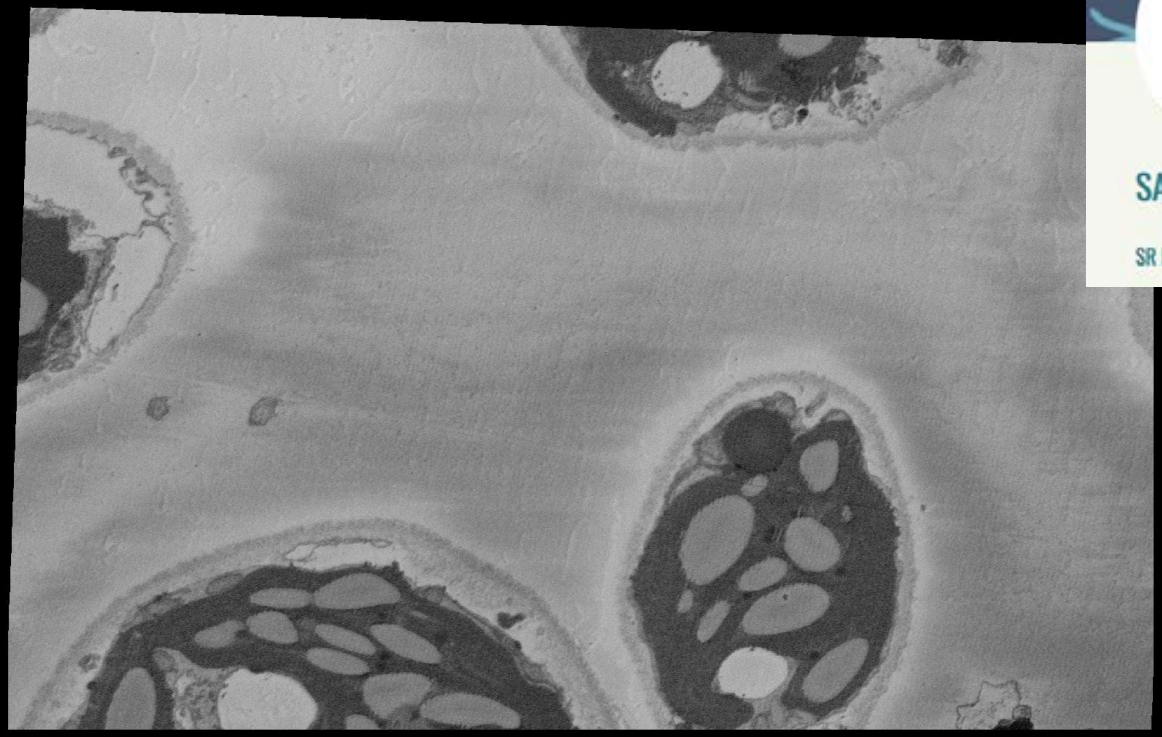
chloroplast
nucleus
mitochondria
lipid droplets
starch granules
starch sheath around pyrenoid
plastoglobules
vacuoles

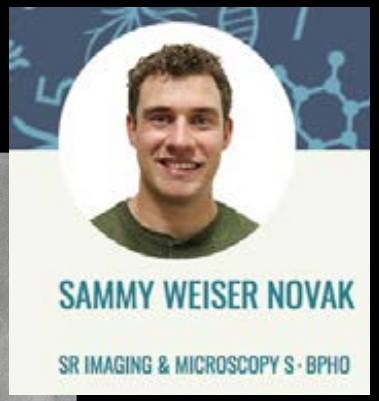


3View Serial Blockface EM

Tyler Wittkkop (Noel lab) and Sammy Weiser Novak (WABC)

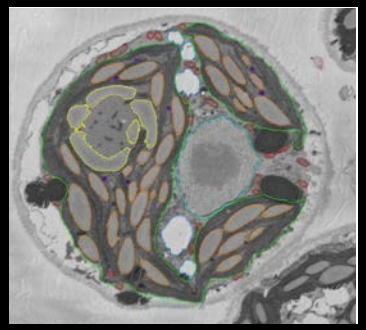


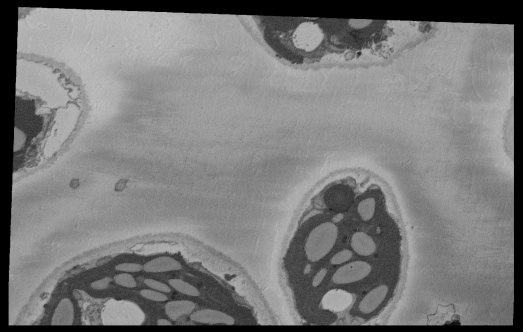


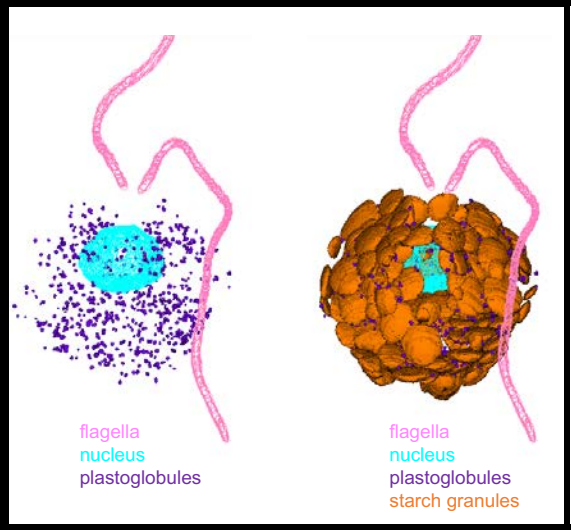


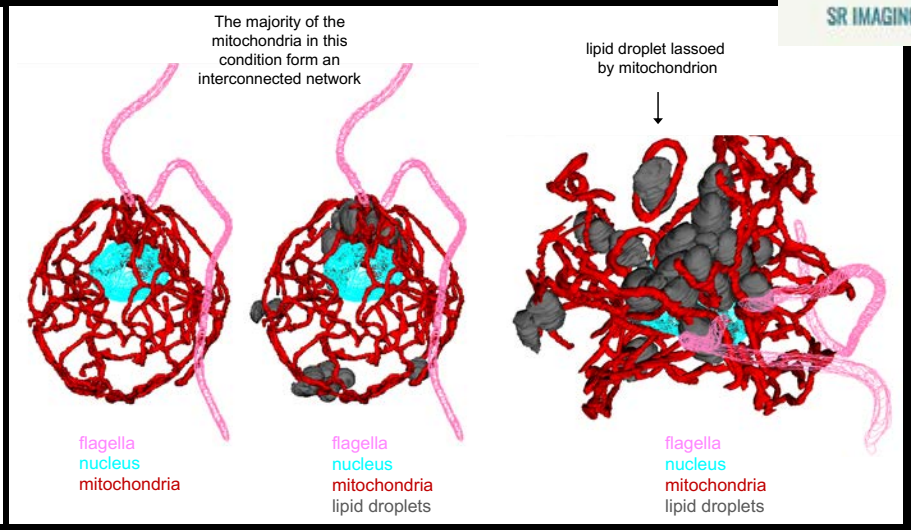
3View Serial Blockface EM

Tyler Wittkkop (Noel lab) and Sammy Weiser Novak (WABC)

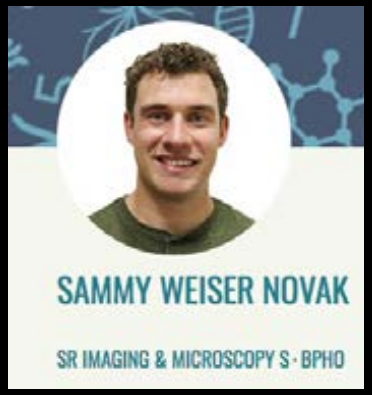


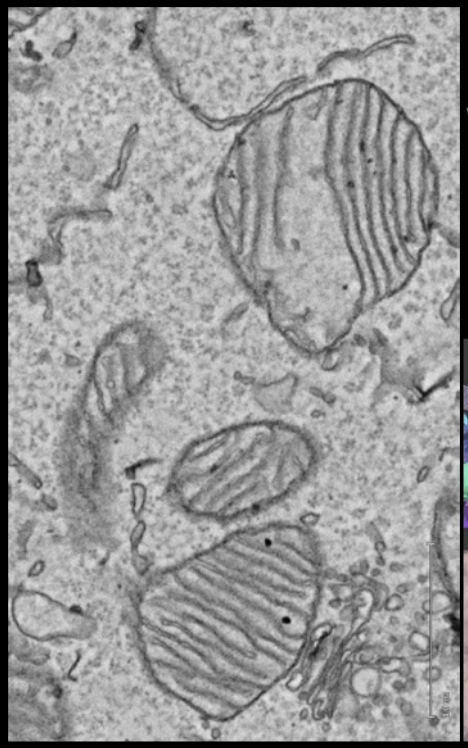


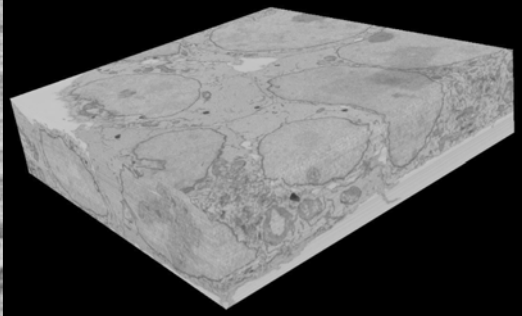


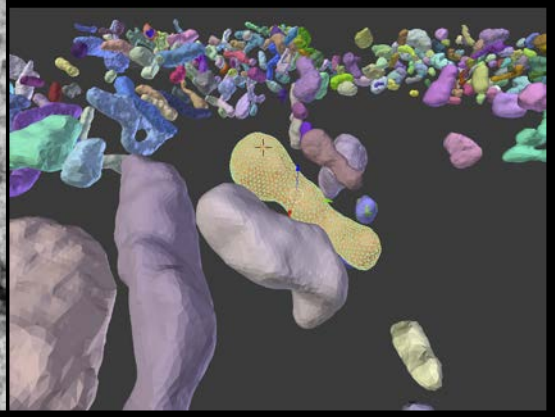


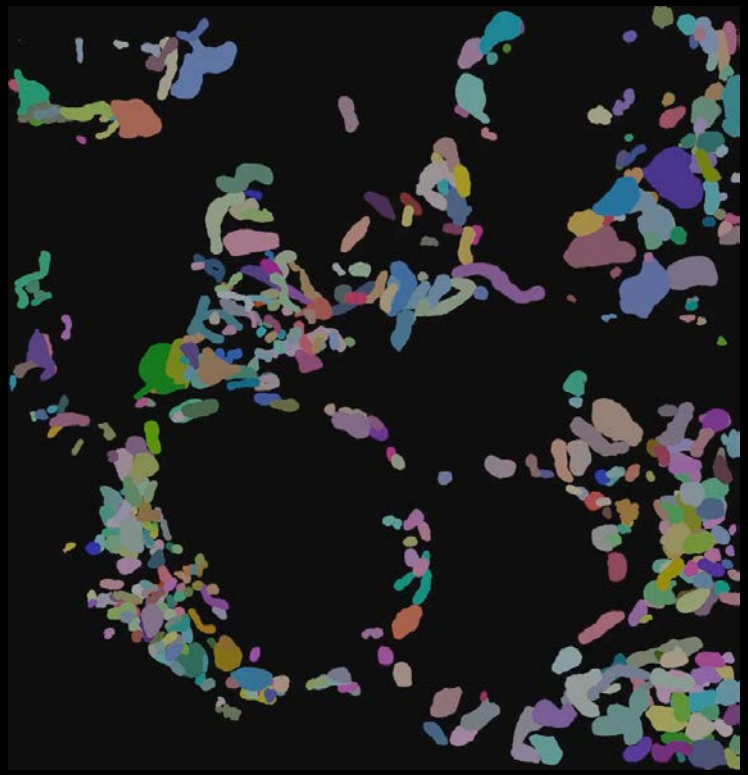
S3EM: Serial Section Scanning Electron Microscopy











In collaboration with Nazma Malik, Shaw Lab

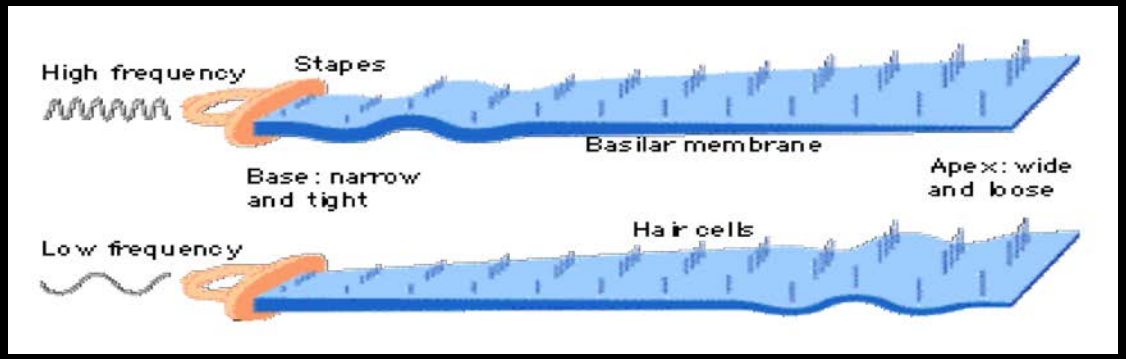
Blindness separates us from things but deafness separates us from people.
-Helen Keller

Hearing loss is a global health crisis

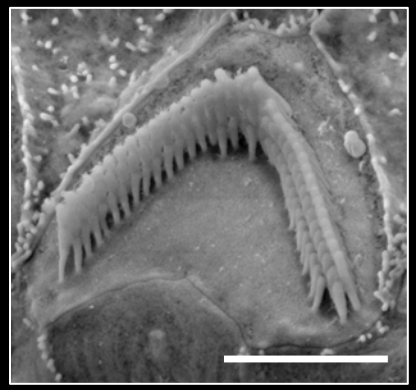
- ~1.5 billion people affected by hearing loss
- ~2.5 billion projected to be affected by 2050
- Age-related hearing loss (ARHL) is associated with a 200-300% increased risk for Alzheimer's disease (AD)
- ARHL is the major modifiable risk factor for AD

AJ Hudspeth, Rockefeller University

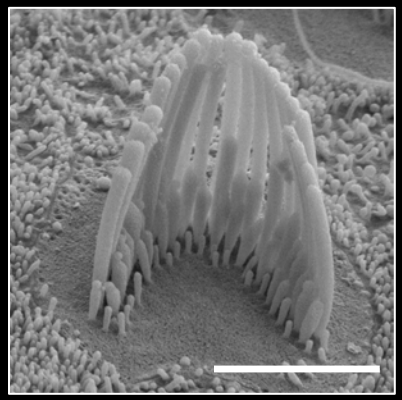
Hair cell lengths are tuned for frequency sensitivity

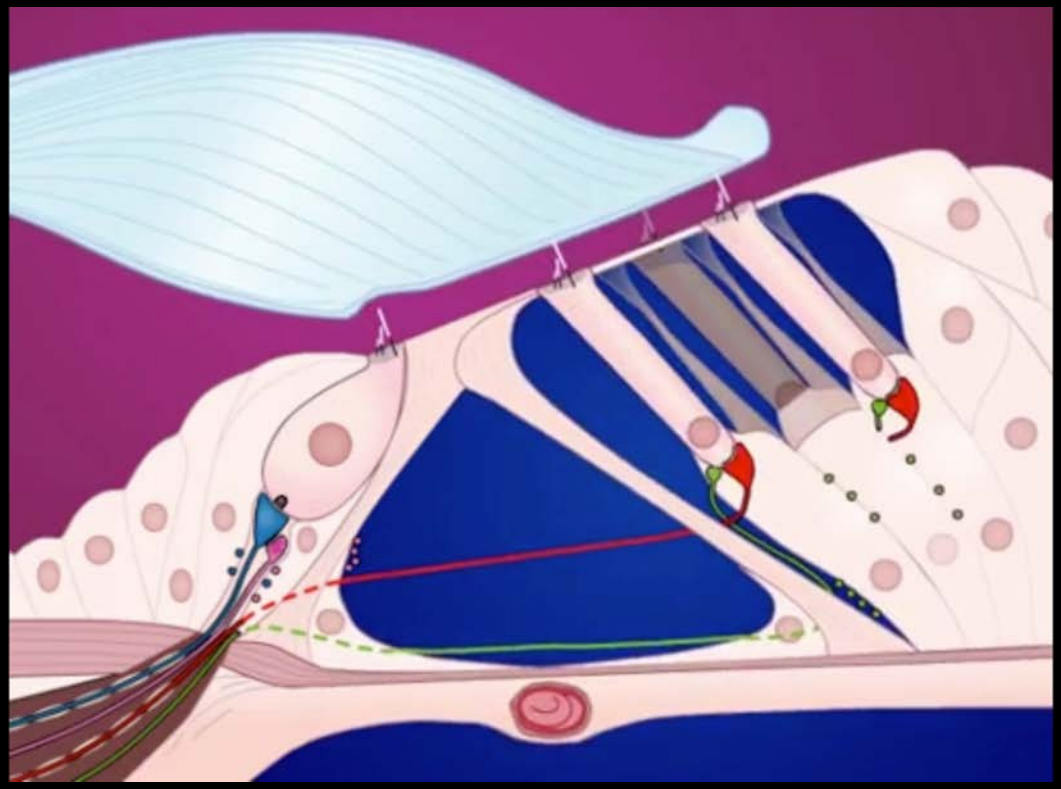


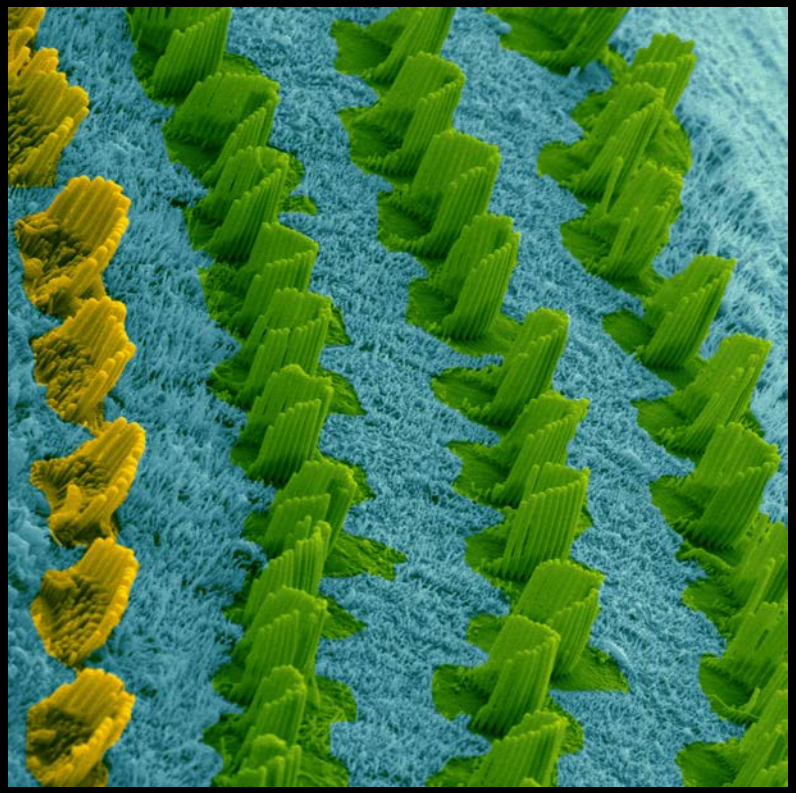
Basal Hair Cell Bundle (high frequency)



Apical Hair Cell Bundle (low frequency)

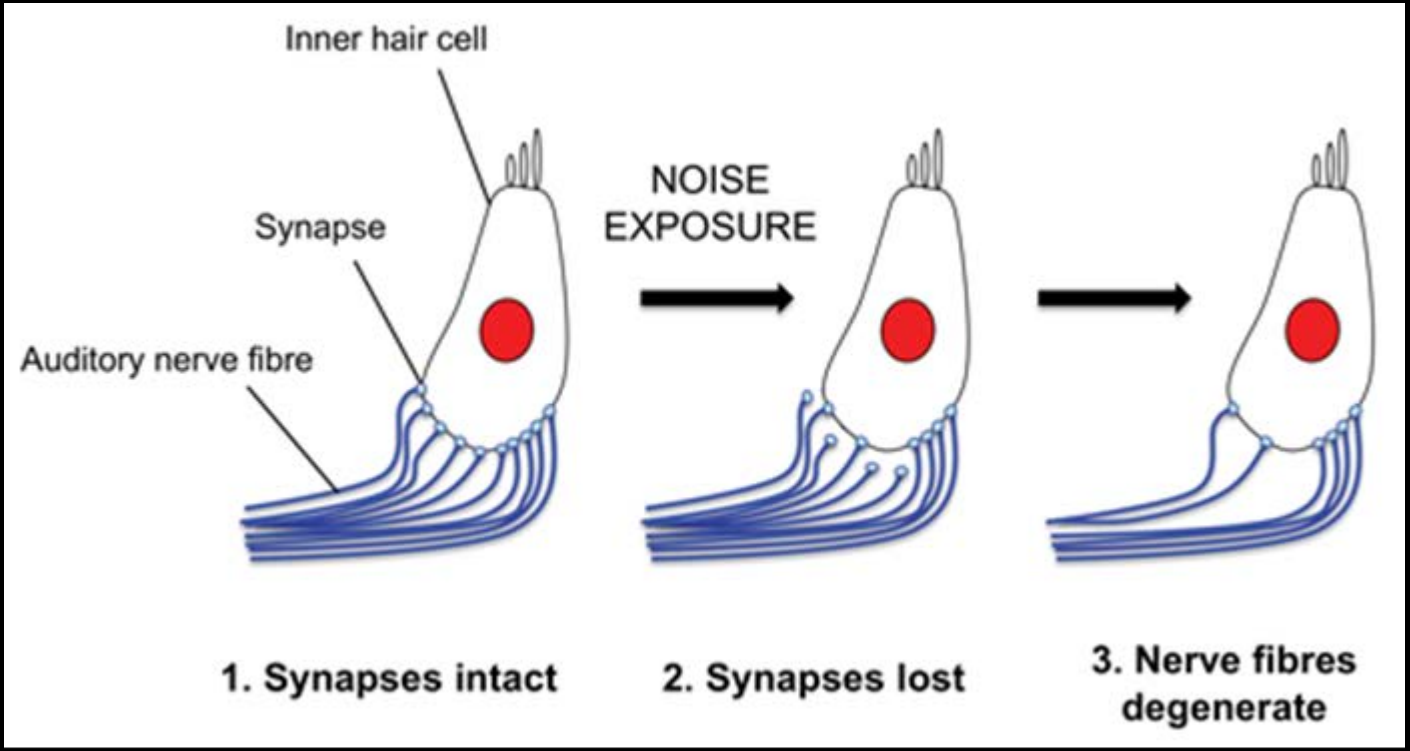




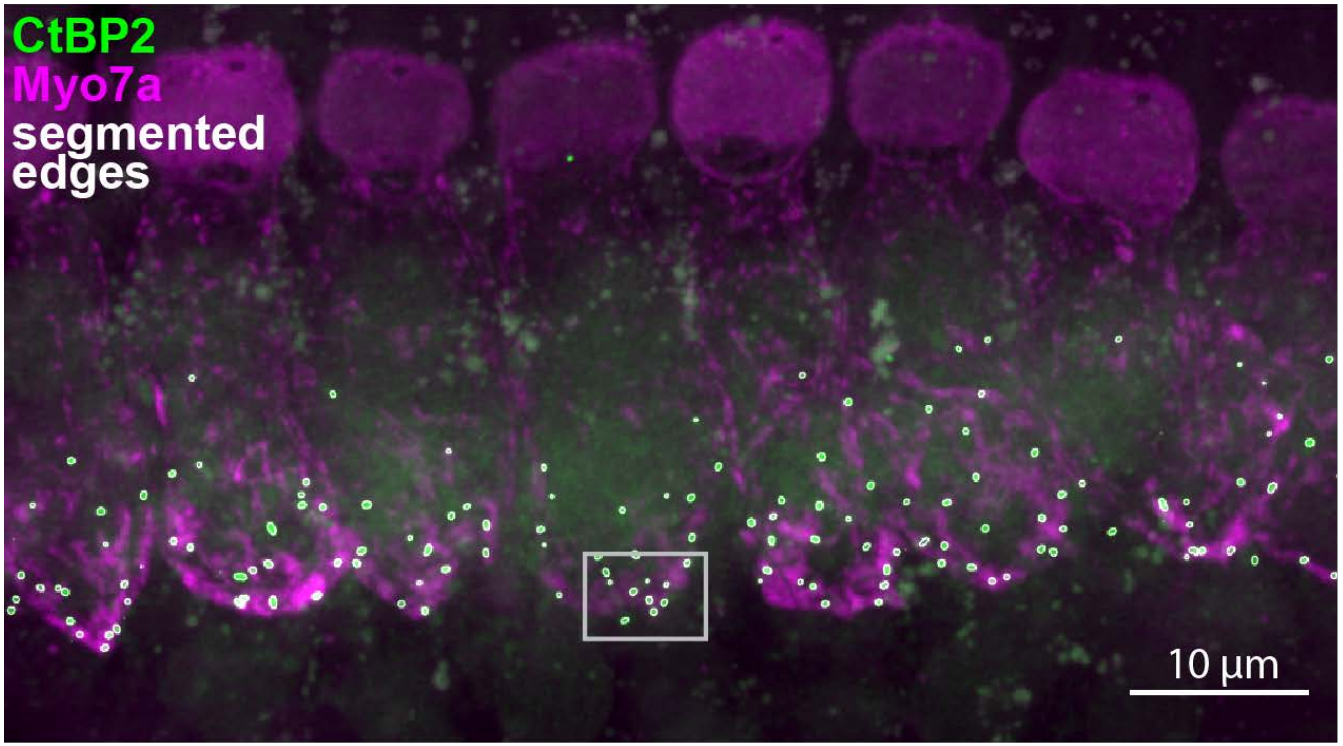


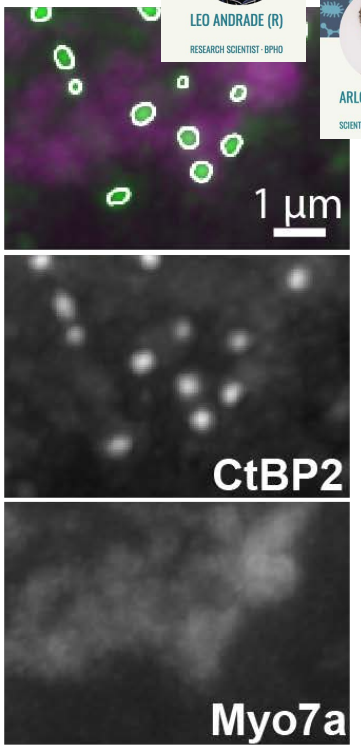
Leo Andrade

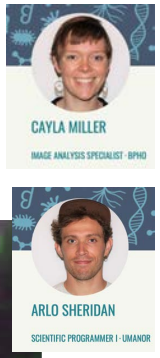
Noise- and age-induced hearing loss is associated with synaptopathy

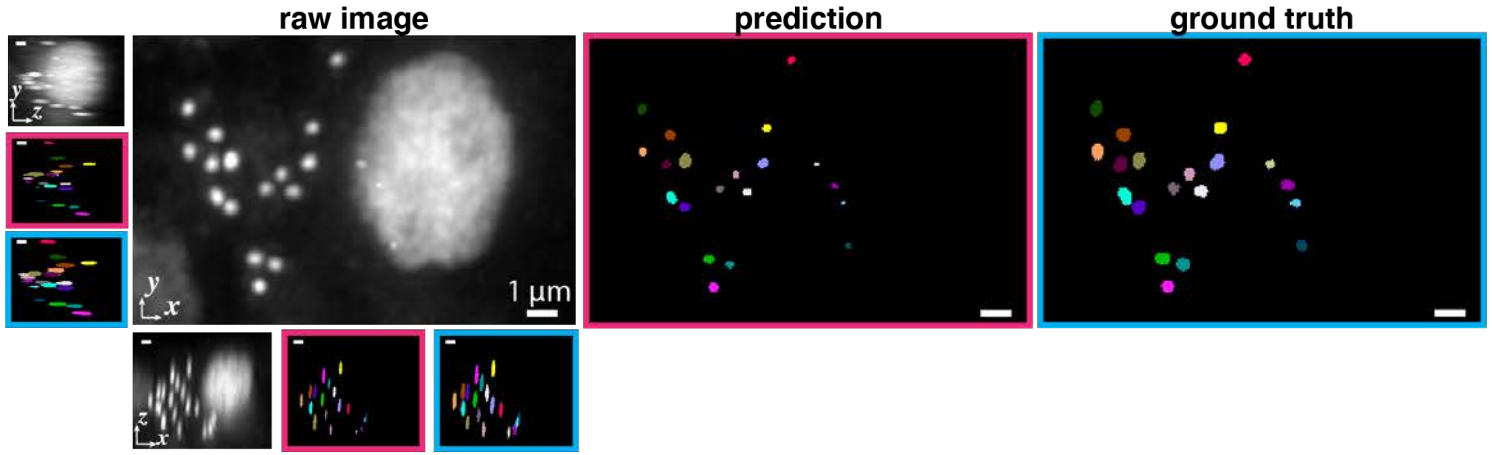


Deep learning-based automated cochlear synapse quantification









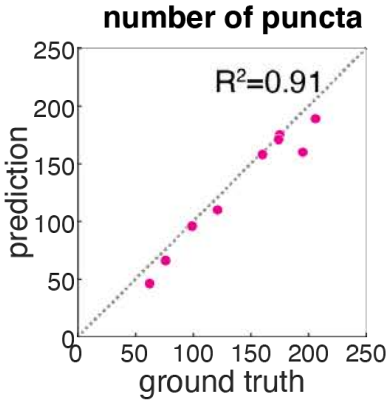
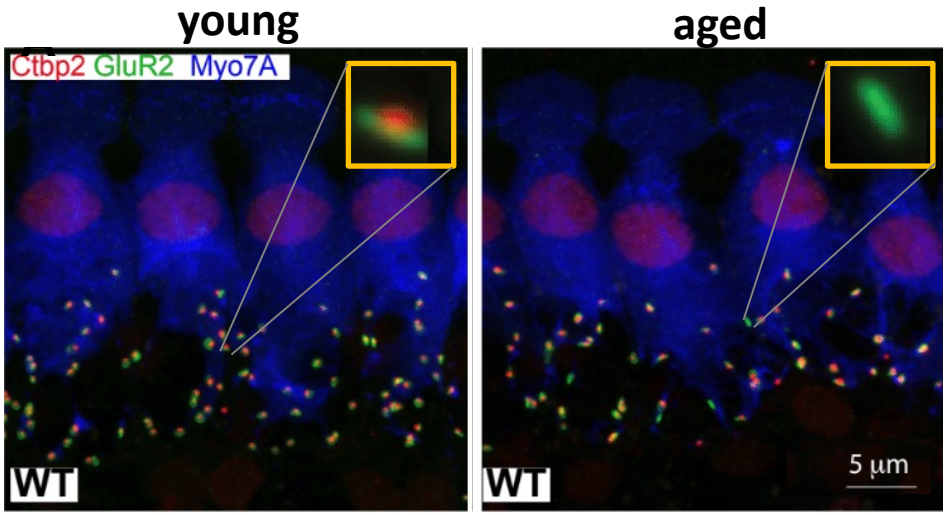
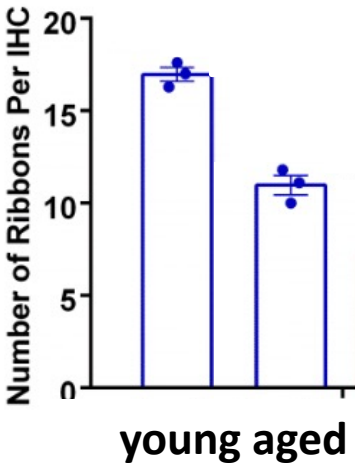
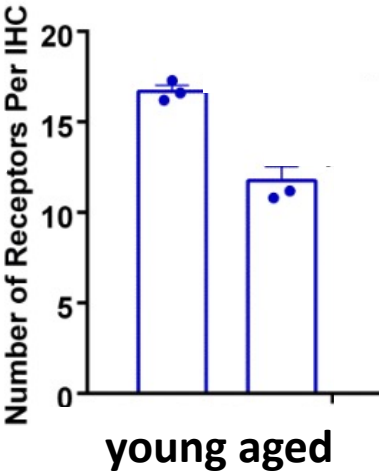


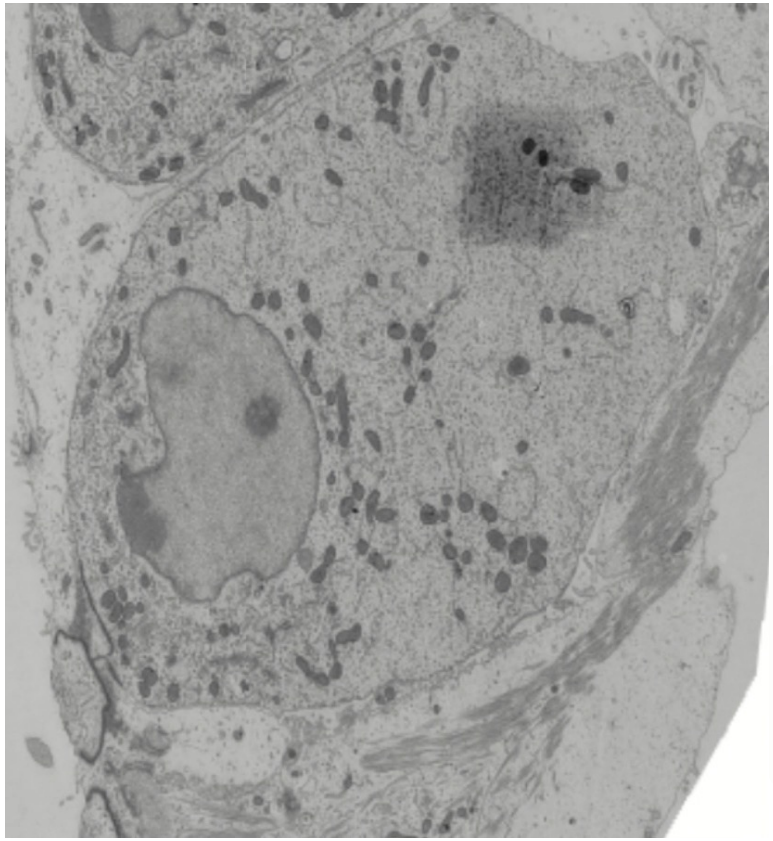
Illustration: Synaptopathy in mouse cochlea tissue

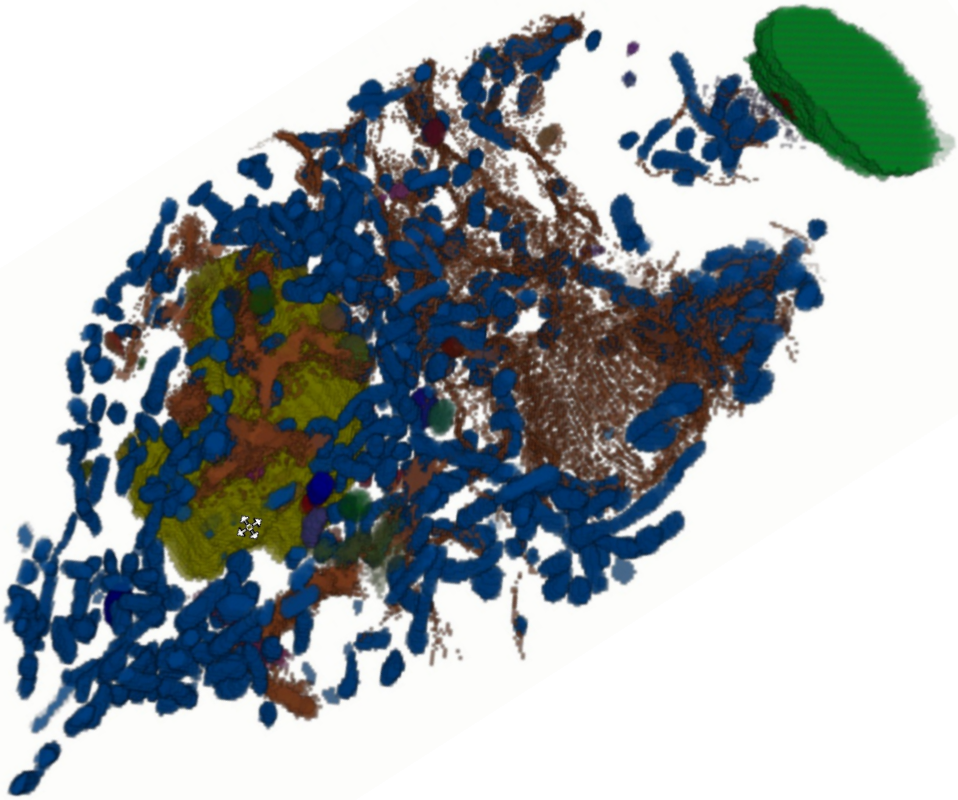


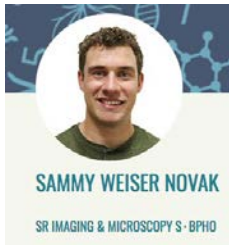




3D electron microscopy of cochlear hair cells reveals inter-organelle contacts

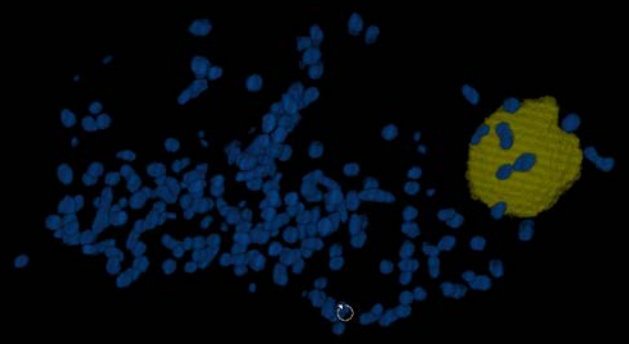




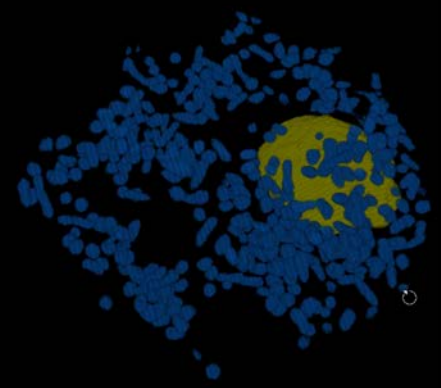


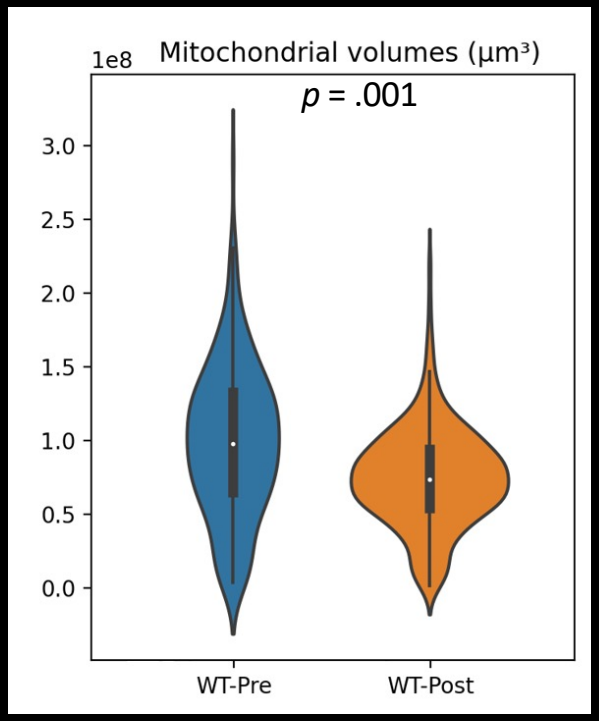
3D electron microscopy of cochlear hair cells reveals changes to mitochondria after noise

control



noise damage





	# Mito	# Mito /Cytoplasm Vol^10	Avg Mito Vol^7	Mito Vol /Cytoplasm
WT-Pre	231	4.57	9.96	4.55%
WT-Post	473	7.13	7.52	5.36%

Questions?


Oded Rechavi 🦉
@OdedRechavi
It's more of a comment than a question

Thank you!!!

Early microscope