

MODULE 1

INTRODUCTION TO HETEROGENEITY OF AGING CORE

Peter Adams
(SBP)



Martin Hetzer
(Salk)



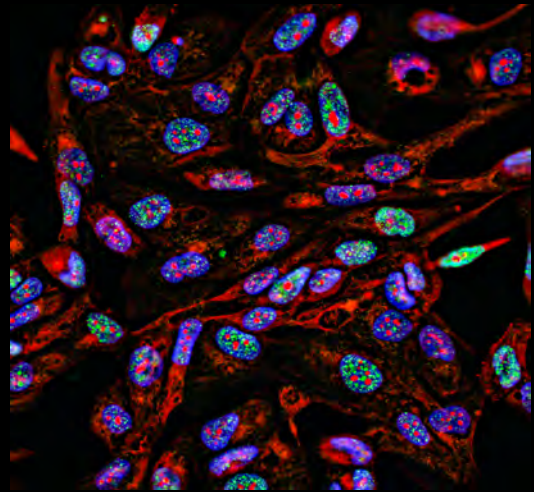
Jolene
Diedrich
(Salk)



Nasun
Hah
(Salk)



Uri
Manor
(Salk)



Technologies to address

- inter-individual heterogeneity
- cell – cell heterogeneity within tissues

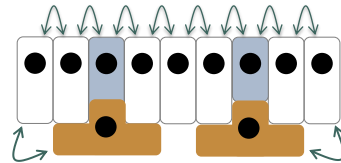
WWW.SALK.EDU

1

INTRODUCTION TO HETEROGENEITY OF AGING CORE

salk
Where curio begins

Function of young, healthy tissues depends on precise tissue organization and cell – cell interactions of multiple cell types.



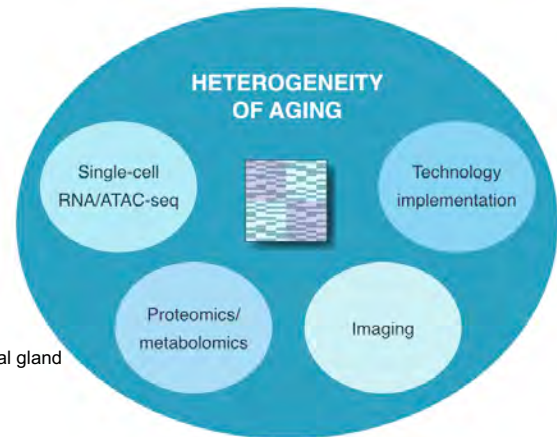
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2

INTRODUCTION TO HETEROGENEITY OF AGING CORE

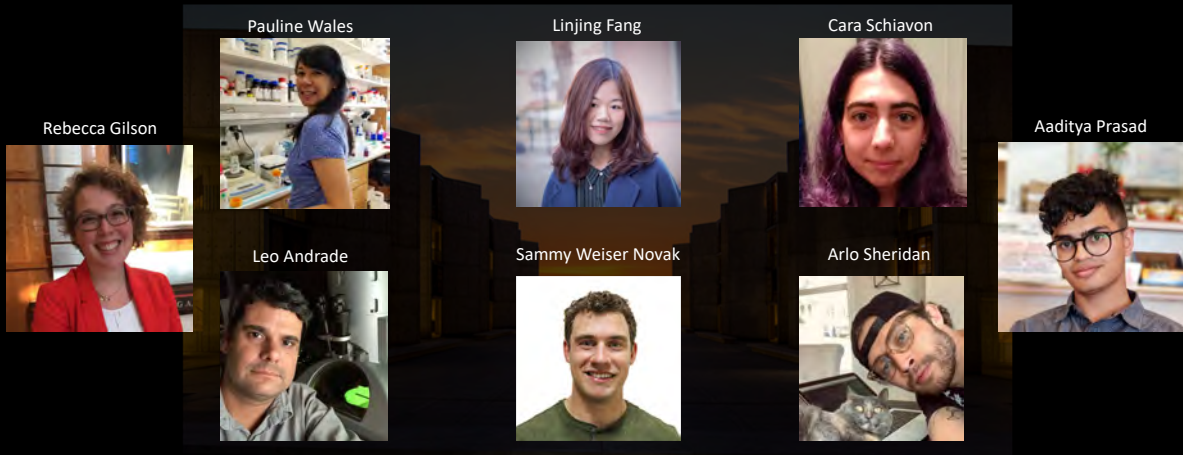


- **URI MANOR:** OVERVIEW OF **IMAGING FACILITIES**
- **JOLENE DIEDRICH:** OVERVIEW OF **METABOLOMICS/PROTEOMICS FACILITIES**
- **NASUN HAH:** OVERVIEW OF **SINGLE-CELL RNA/ATAC SEQ FACILITIES**
- **EXAMPLES**
 Adam Konopka, Univ of Wisconsin – Metformin and Exercise
 Vanessa Delcroix, Scripps Research - A single-cell atlas of aging lacrimal gland
- **NEW TECHNOLOGIES:** E.G., SPATIAL *IN SITU* –OMICS (MERFISH, CODEX, NANOSTRING DSP, VISIUM etc)



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SD-NSC-Heterogeneity of Aging Core - Imaging



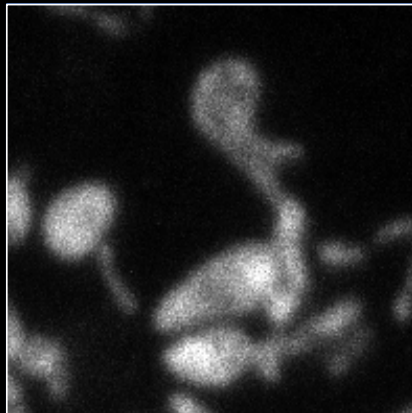
WAITT ADVANCED BIOPHOTONICS CENTER

salk
Where cures begin.

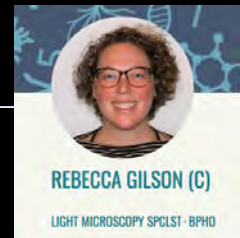
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SD-NSC-Heterogeneity of Aging Core - Imaging Airyscan Microscopy - $\sim 1.7\times$ resolution + 8x sensitivity

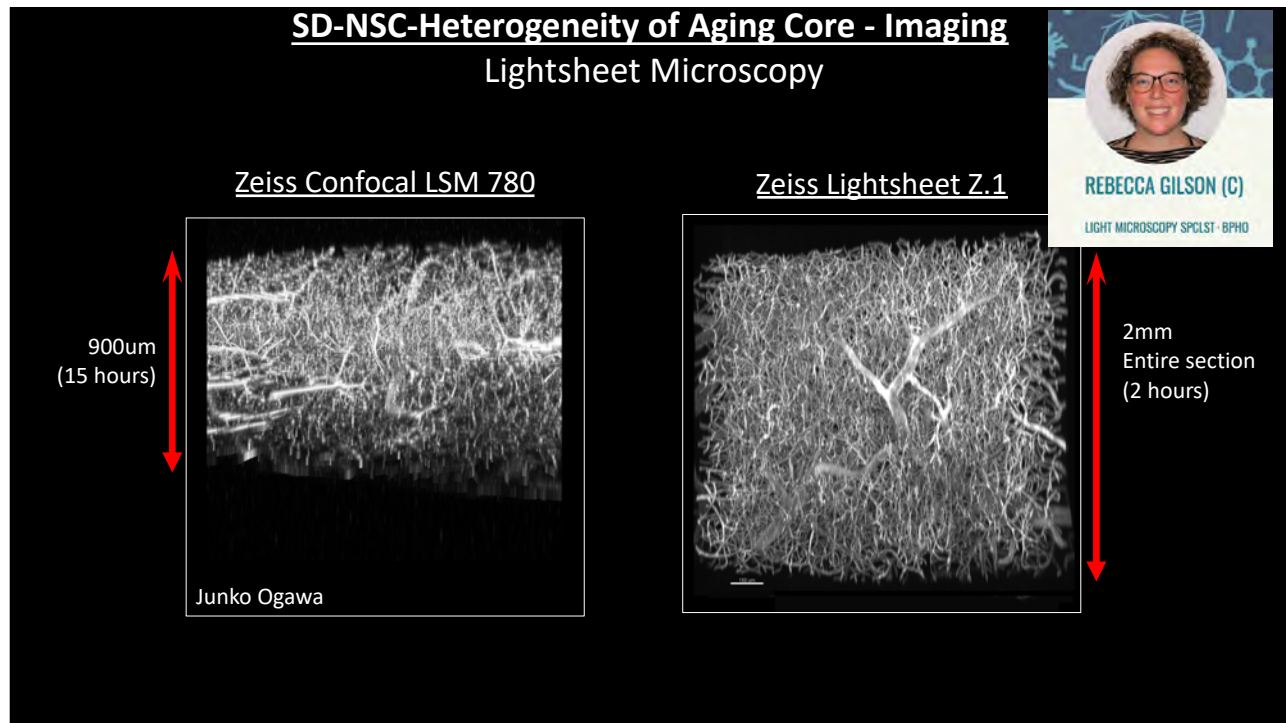
Confocal



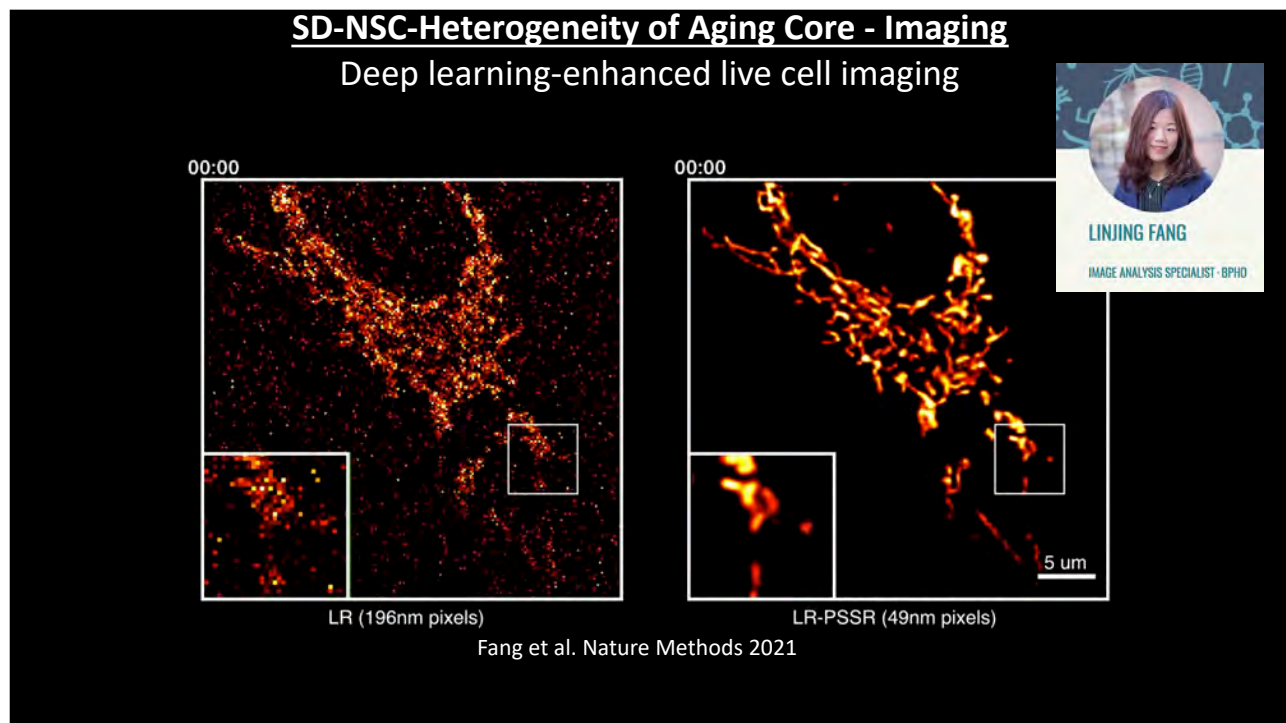
Airyscan



2



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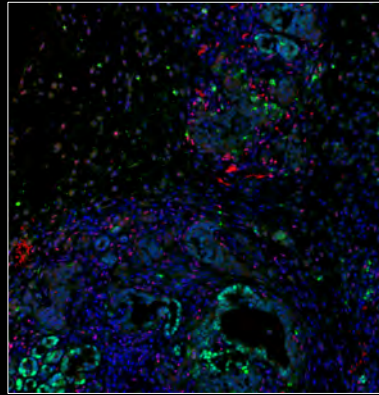
SD-NSC-Heterogeneity of Aging Core - Imaging

Automated High Throughput Image Analysis

(collaboration with Hunter, Shaw, Evans, and Wahl labs)

Yu Shi (Hunter lab)+
Linjing Fang (WABC)

aSMA: stromal cells
Ki67: proliferating cells
Pdx1: tumor cells
DAPI: all cells



Shi et al. Nature 2019



5

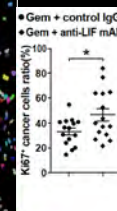
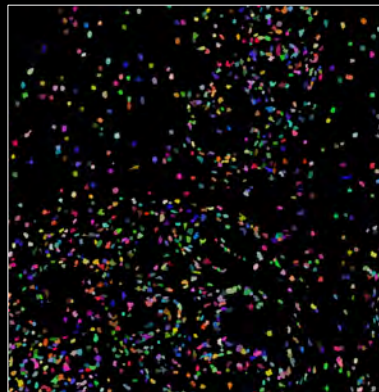
SD-NSC-Heterogeneity of Aging Core - Imaging

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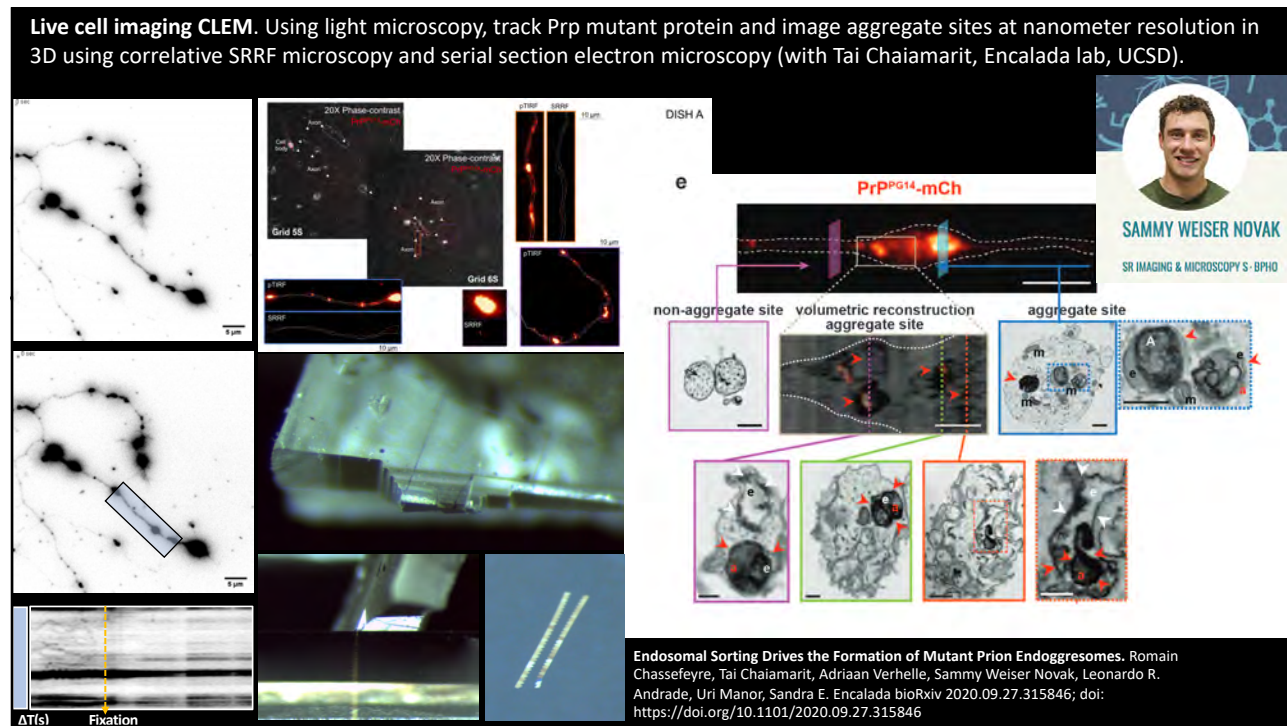
aSMA: stromal cells
Ki67: proliferating cells
Pdx1: tumor cells
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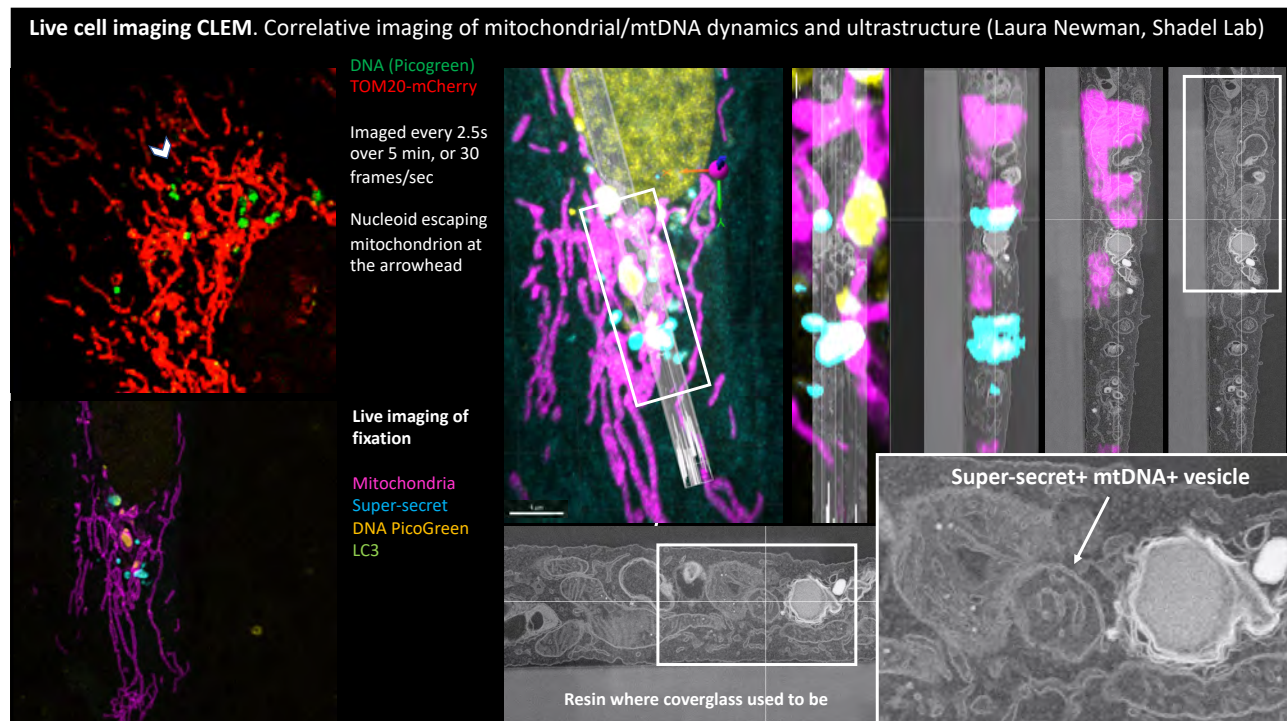
Shi et al. Nature 2019



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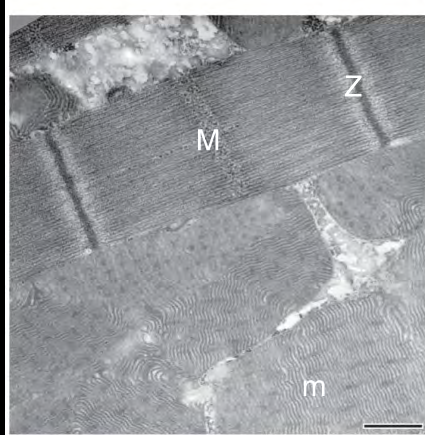
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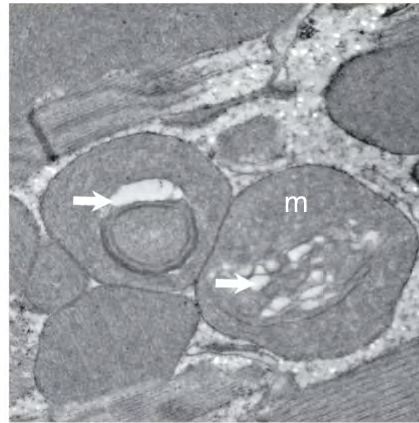
8

Conventional TEM

Control



High-fat diet



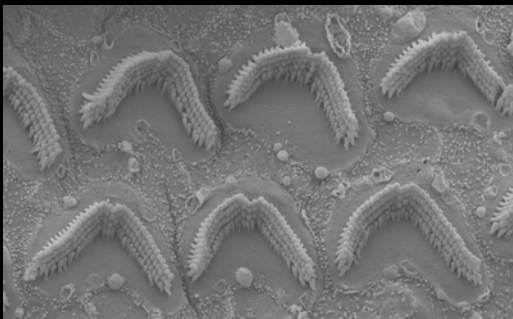
Villaneuva et al. Nature Communications, 2019

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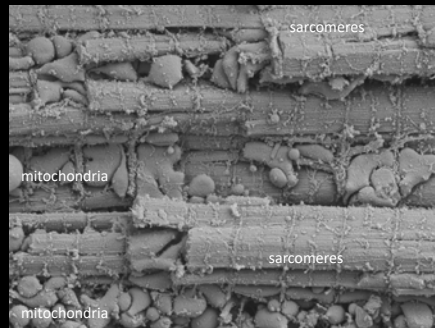
Conventional SEM

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

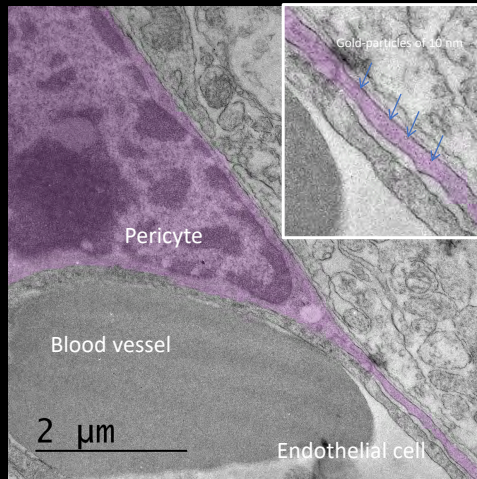


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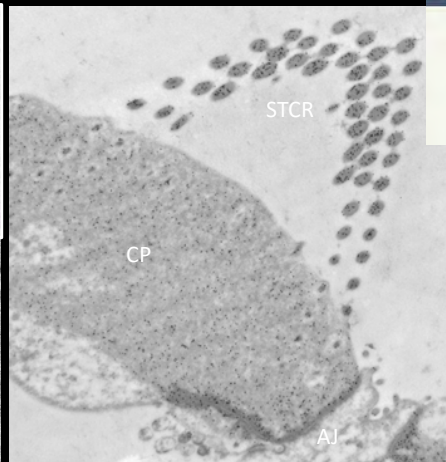
Immuno-gold labeling

Goal: To label specific proteins, organelles or cells of interest. It can be pre-embedding or post-embedding labeled. Samples are usually chemical fixed for pre-embedding and freeze-substituted for post-embedding.

Pre-embedding: rat cell labeled in a chimera mouse

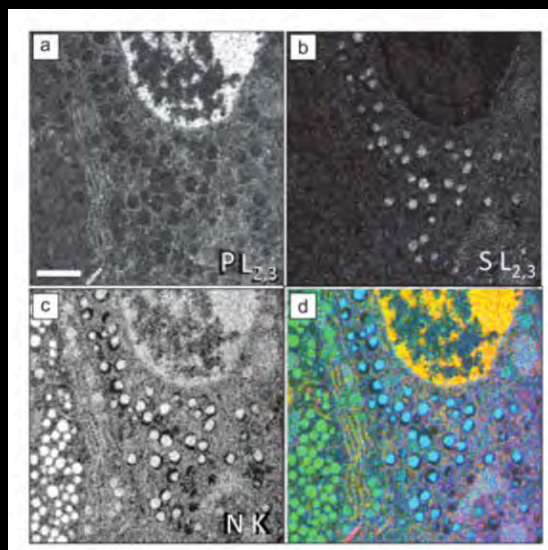


Post-embedding: Gamma-actin localization in an OHC.



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Electron Energy Loss Spectroscopy (mapping elements in a sample)



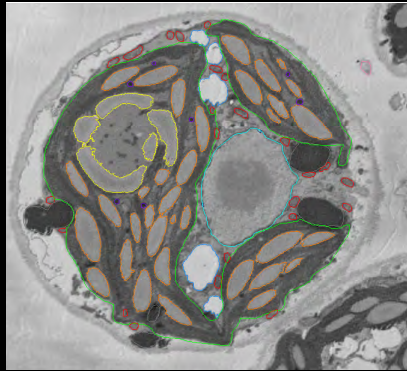
Phosphorous
Sulfur
Nitrogen

Aronova and Leapman 2012

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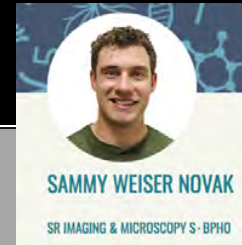
2D EM is misleading, especially for mitochondrial imaging!

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



Mitochondrial length ranges from 1-3 μm

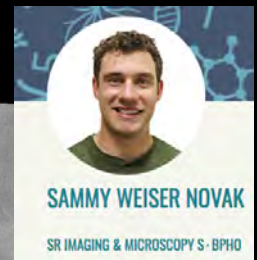
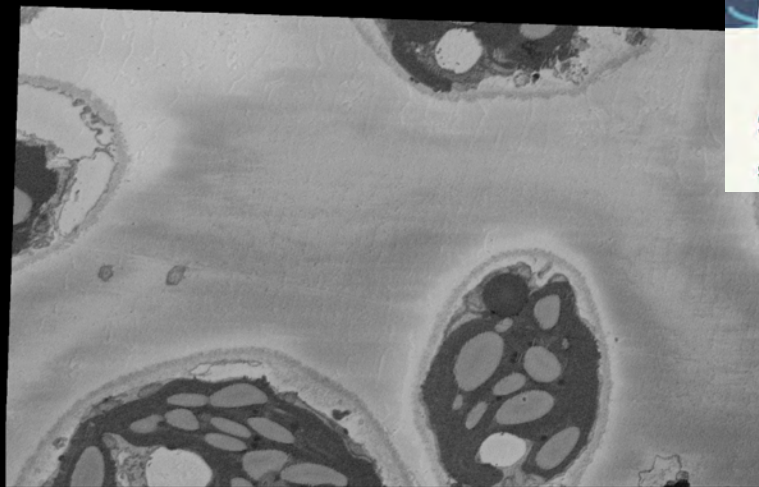
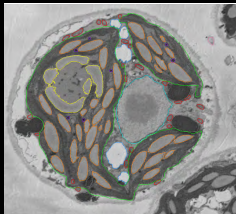
chloroplast
nucleus
mitochondria
lipid droplets
starch granules
starch sheath around pyrenoid
plastoglobules
vacuoles



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3View Serial Blockface EM

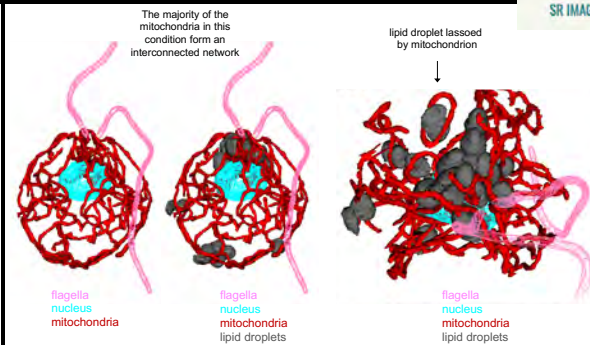
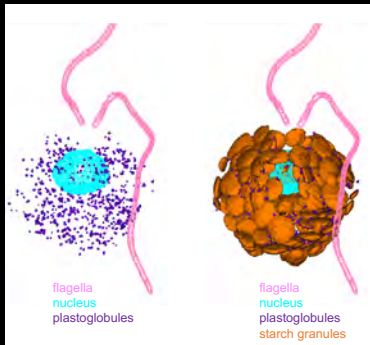
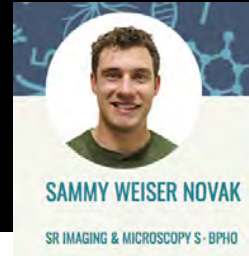
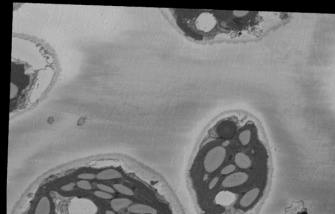
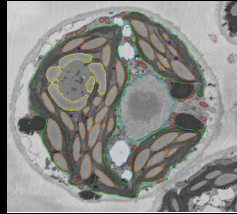
Tyler Wittkkop (Noel lab) and Sammy Weiser Novak (WABC)



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3View Serial Blockface EM

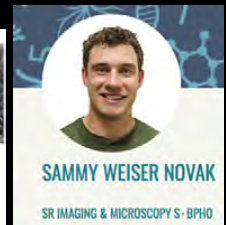
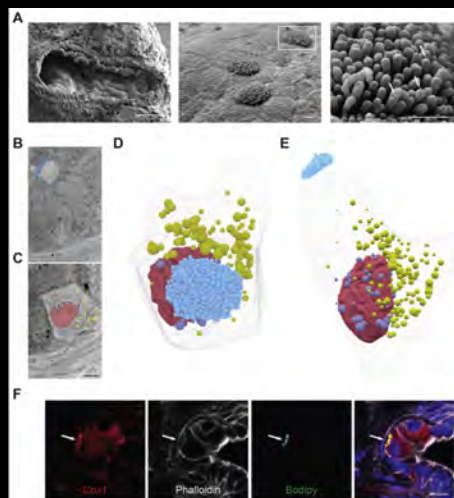
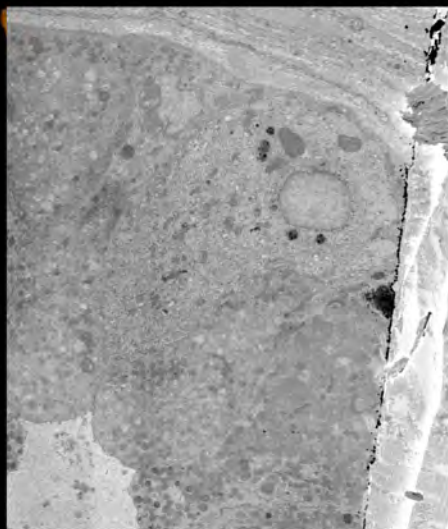
Tyler Wittkop (Noel lab) and Sammy Weiser Novak (WABC)



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3View. A diamond knife is mounted above a sample block in the SEM and the blockface is imaged and cut iteratively. Large volumes can be collected at high spatial resolution, constrained in balancing electron dose and signal to noise.

Example: Mouse model of precancerous pancreatic injury, describing the distribution of lipid droplets in a metaplastic tuft cell involved in eicosanoid signaling ([pancreatic-ultrastructure.github.io](https://github.com/pancreatic-ultrastructure)).

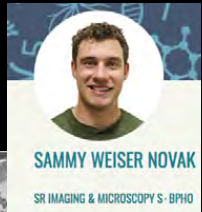
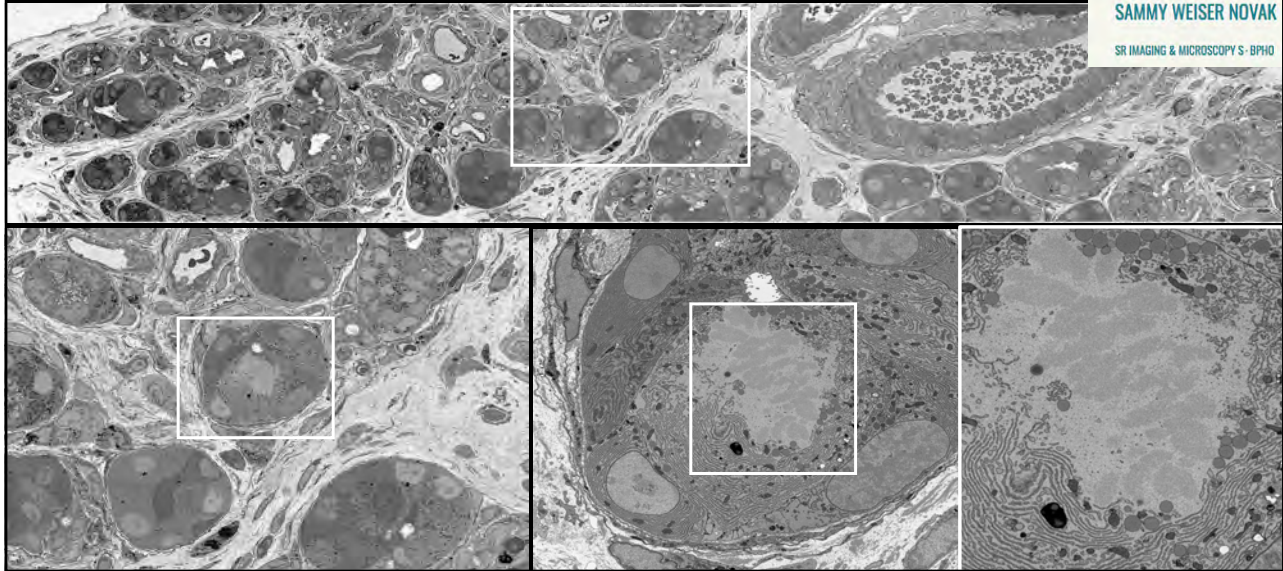


DelGiorno, Kathleen E., et al. **Tuft cells inhibit pancreatic tumorigenesis in mice by producing prostaglandin D2.** *Gastroenterology* 159.5 (2020): 1866-1881.

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Chip mapping. Ultrathin (50-100nm) sections of a resin embedded sample are collected on a silicon chip, which may then be imaged at multiple resolutions using the scanning electron microscope and backscattered electron detector.

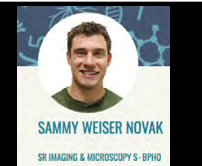
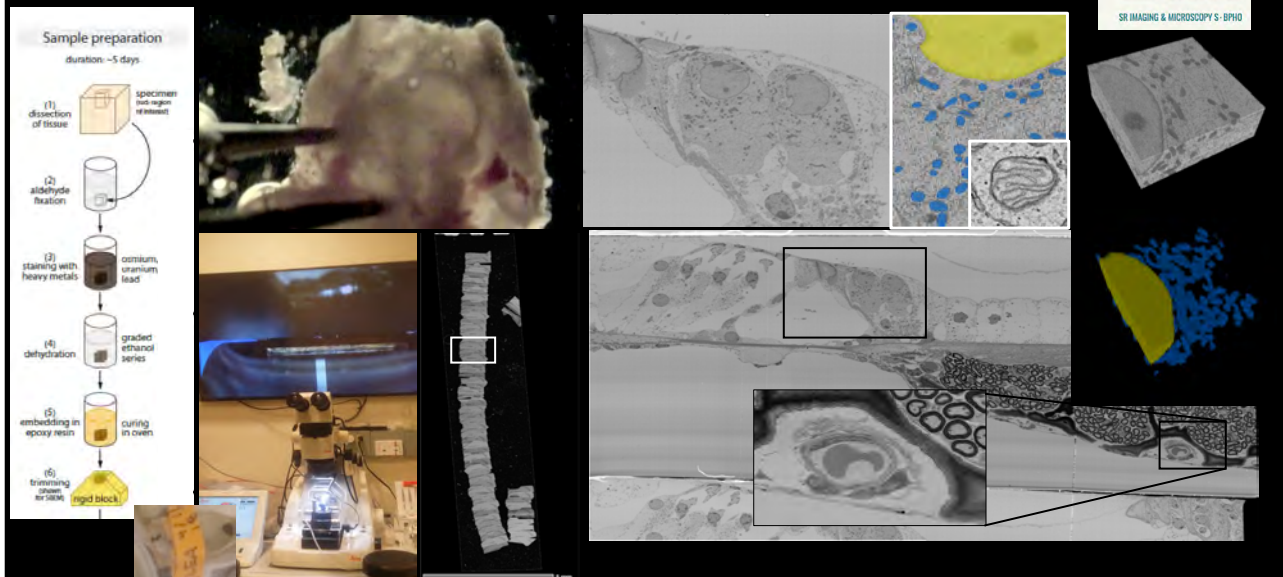
Example: Pancreatic injury, (pancreatic-ultrastructure.github.io) searching for emergent metaplastic cell types in precancerous lesions. Here, we find a rare cell division event, with a lipid droplet anchored to a chromosome (with Kathy Delgiorno, Wahi lab)



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S3EM. Serial sections in the scanning electron microscope. Similar to chip mapping, serial ultrathin (50-100nm) sections of a resin embedded sample are collected on a silicon chip. A region of interest is identified, and images are collected from the ROI in the contiguous sections, facilitating 3D reconstruction of nanoscopic structures.

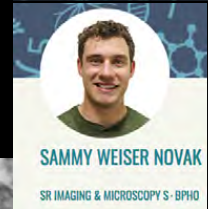
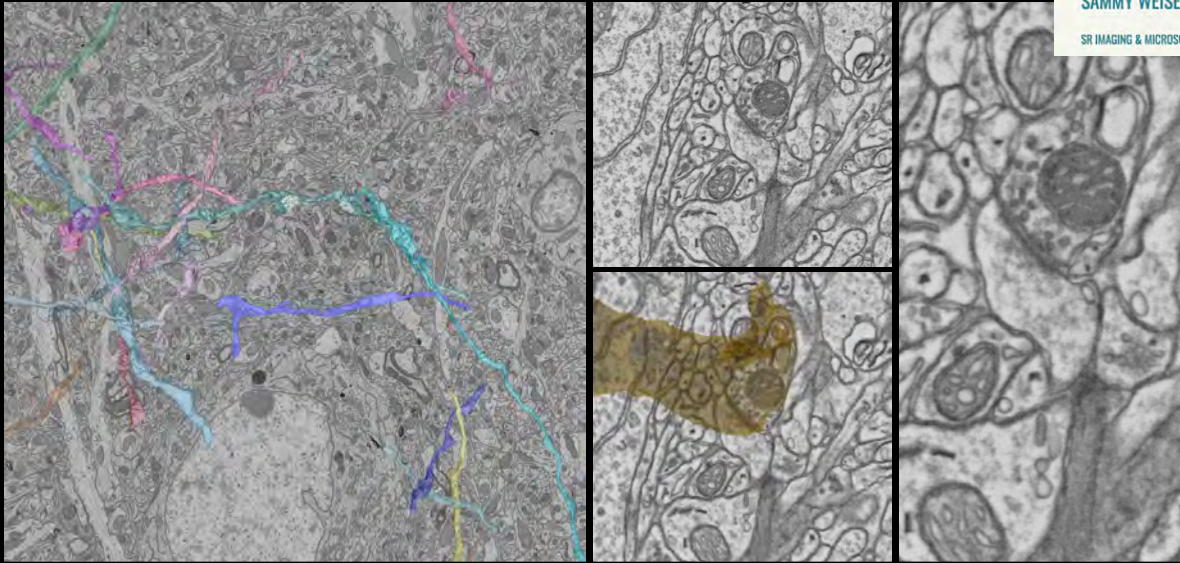
Example. Determining changes in mitochondrial organization in cochlear hair cells following aging or noise-induced hearing loss (with Rick Friedman, UCSD)



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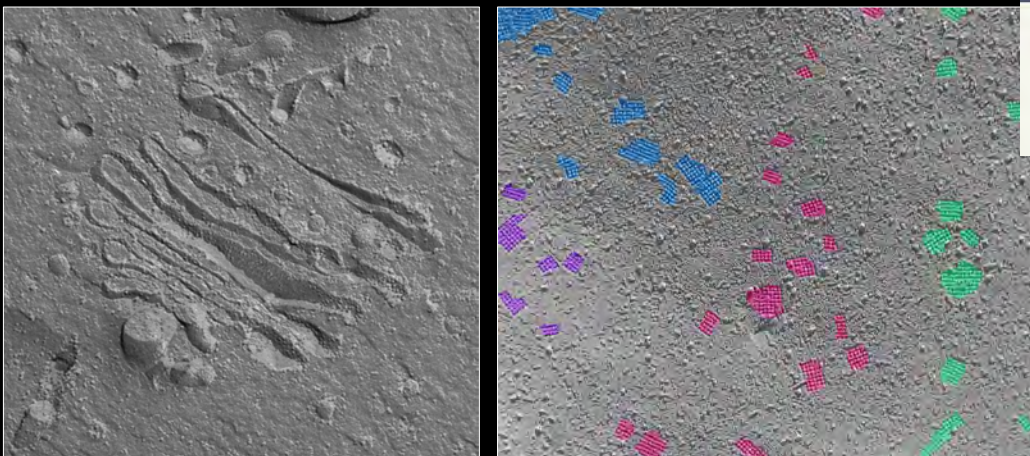
S3EM. Serial sections in the scanning electron microscope. Similar to chip mapping, serial ultrathin (50-100nm) sections of a resin embedded sample are collected on a silicon chip. A region of interest is identified and images are collected from the ROI in the contiguous sections, facilitating 3D reconstruction of nanoscopic structures.

Example: Determining axonal bouton volumes in APEX2-MLS-DAB labeled mouse synapses (with Alex Bosworth, Allen lab)



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Slam freezing and freeze etching (Leo Andrade)



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SD-NSC-Heterogeneity of Aging Core - Imaging

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.

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SD-NSC-Heterogeneity of Aging Core - Imaging


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	

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SD-NSC-Heterogeneity of Aging Core - Imaging

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)			

PROTEOMICS / METABOLOMICS CORE



Mass Spectrometry Core for Proteomics and Metabolomics


Jolene Diedrich, Antonio Michel Pinto.

Faculty Advisors: Alan Saghatelian, John R Yates III






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PROTEOMICS / METABOLOMICS CORE




Mass Spectrometers


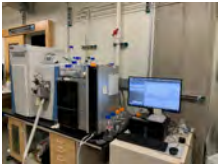

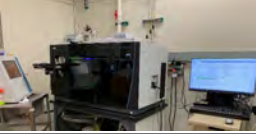

	Proteomics	Metabolomics/Lipidomics
Fusion		
Lumos		Q Exactive 
Eclipse		Quantiva 

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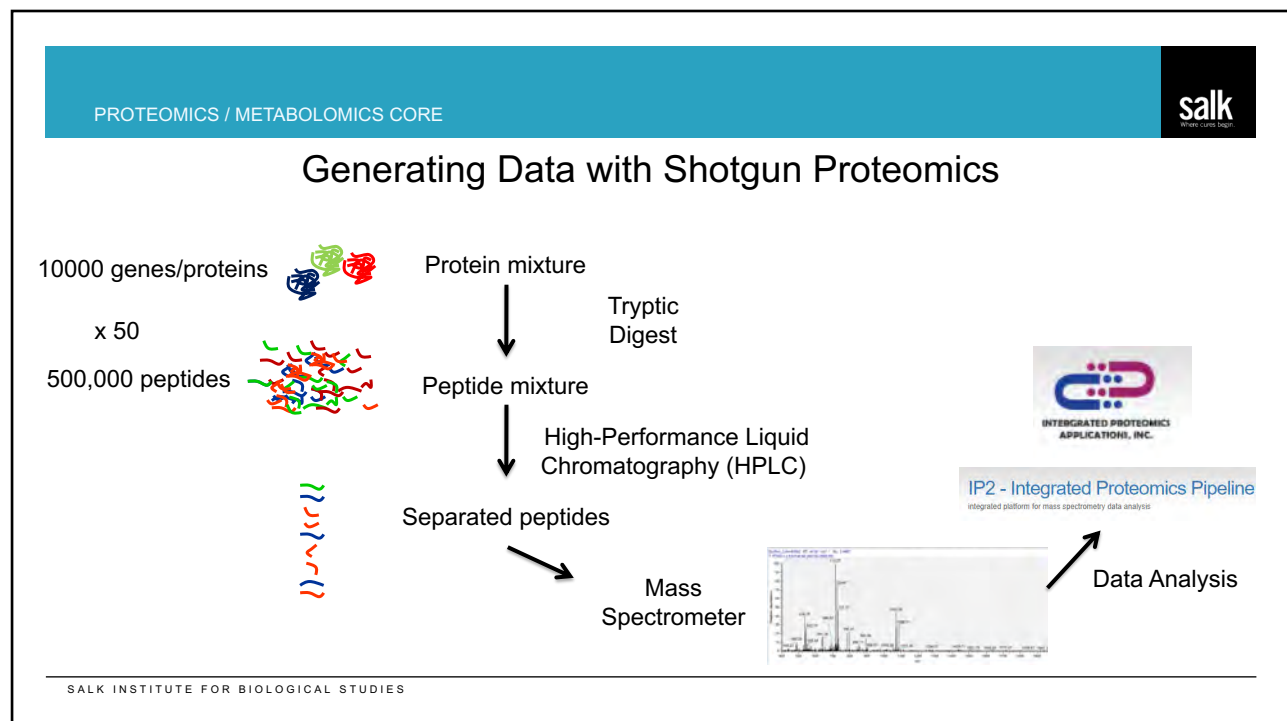
PROTEOMICS / METABOLOMICS CORE 

Mass Spectrometers

	Proteomics	Metabolomics/Lipidomics
Fusion		 Q Exactive
Lumos		
Eclipse		
		 Quantiva

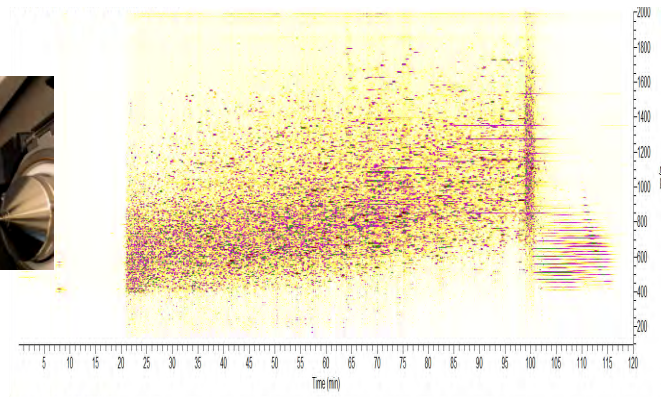
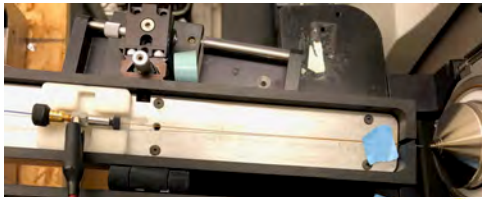
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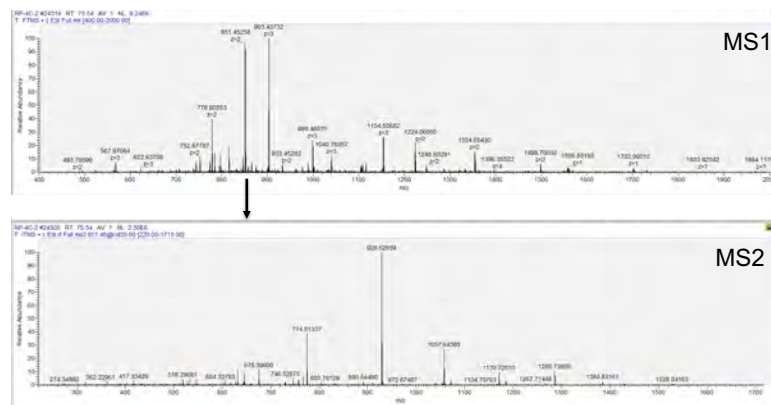
HPLC is Used to Limit the Variety of Peptides Entering the Mass Spectrometer in Time



5

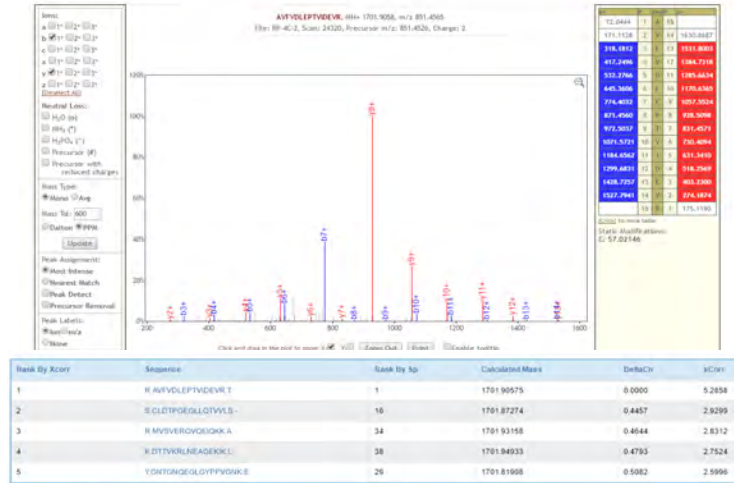
The Mass Spectrometer Measures the Mass, Charge and Relative Abundance of Peptide Ions

Ions are selected for fragmentation based on relative abundance



6

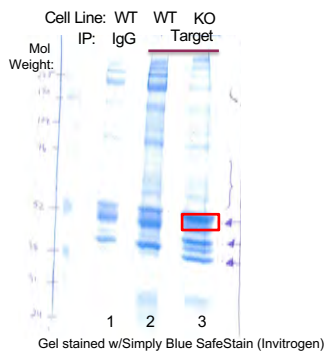
Fragmentation Pattern is Used to Identify the Peptide Sequence



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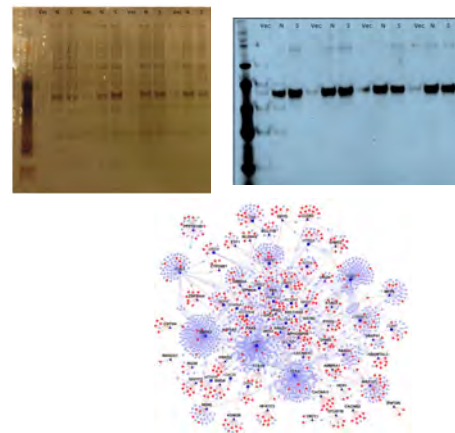
7

Protein Identification: Gel bands or IPs



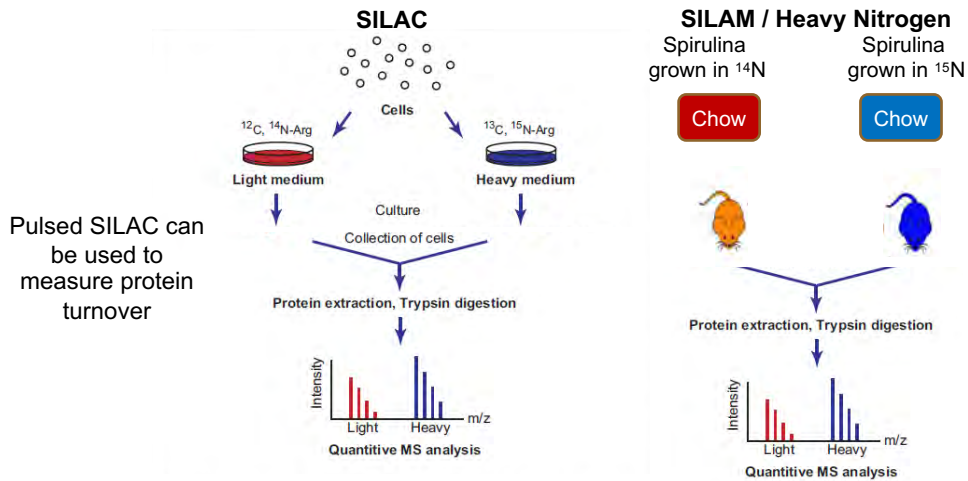
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Protein Identification: Binding Partners by Co-Immunoprecipitation



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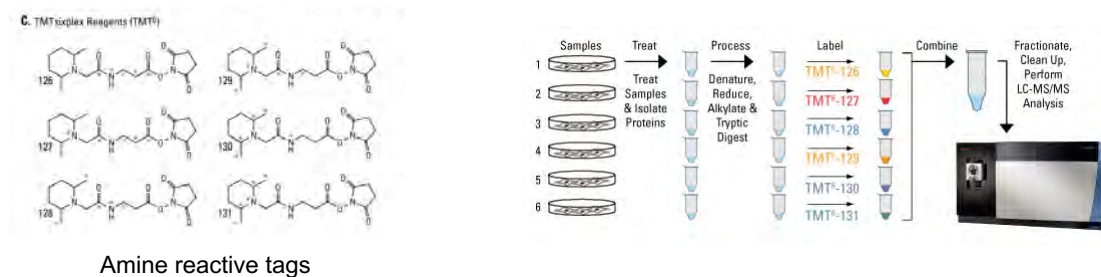
Metabolic Labeling Improves Quantitation but Increases Complexity



Front Genet. 2011 Jun 23;2:29. doi: 10.3389/fgene.2011.00029. eCollection 2011.
Systems Biology Reveals MicroRNA-Mediated Gene Regulation. Watanabe Y¹, Kanai A.

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Tandem Mass Tags (TMT) Enable Quantitation Without Increasing Complexity



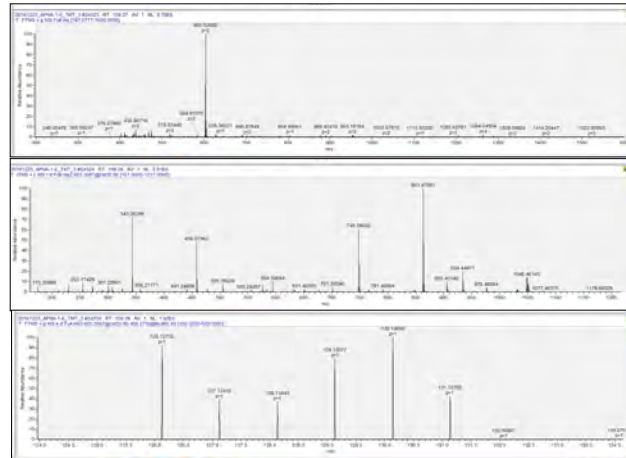
10

Tandem Mass Tags (TMT) Enable Quantitation Without Increasing Complexity

MS1 Scan-
Single Ion Enters

MS2 Scan-
Fragmentation Pattern
to Determine ID

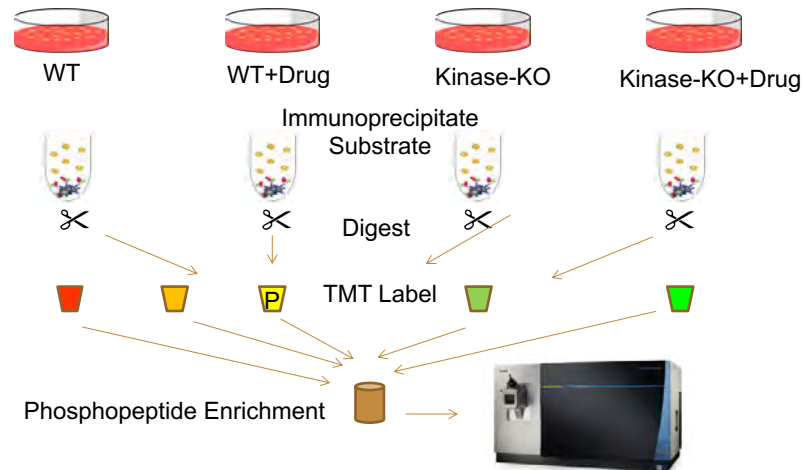
MS3 Scan-
Tags Removed and
Measured



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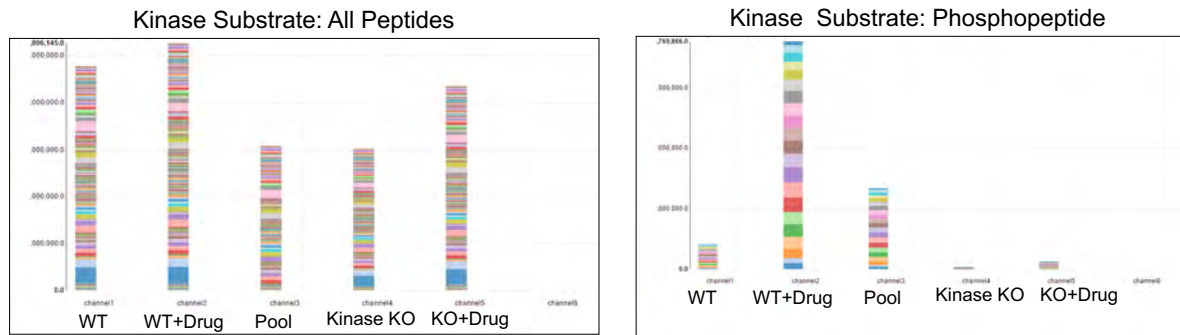
Quantifying Phosphorylation in Response to Drug



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Quantifying Phosphorylation in Response to Drug



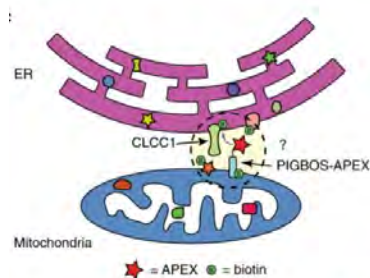
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Proximity Labeling to Identify Protein- Protein Interactors

Many types of proximity labeling

- BirA
- BioID
- BioID2
- Turbo ID
- APEX
- HRP



Nat. Commun. 2019, 10: 4883
Published online 2019 Oct 25; doi: 10.1038/s41467-019-12816-z

PMCID: PMC6814811
PMID: 31553959

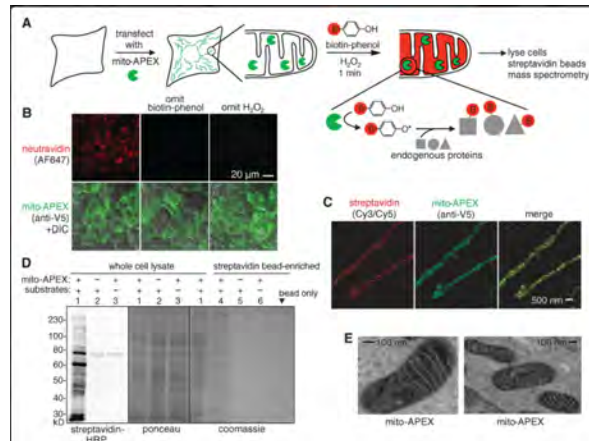
Regulation of the ER stress response by a mitochondrial microprotein

Qian, Cui,¹ Thomas, F., Martinez,¹ Samir, W., Novak,² Cynthia, J., Donohue,¹ Ben, Tan,¹ Joan, M., Vourhien,¹ Tina, Chao,¹ Jolene, K., Diederich,¹ Leo, Andrade,² Andrew, Kim,¹ Juno, Zhang,² Uri, Manor,^{2,3} and Jun, Sanghvi,^{1,2,3}

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Proximity Labeling for Mapping of Organelles



Mass spectrometry and imaging using the same constructs

REPORT
Proteomic Mapping of Mitochondria in Living Cells via Spatially Restricted Enzymatic Tagging

Hyon-Woo Shin^{1,2}, Peng Zou^{1,2}, Namrata D. Udeski¹, Jeffrey D. Marini¹, Vamsi K. Mootha^{1,2}, Steven A. Carr¹, Alex Y. T. Leung^{1,2}

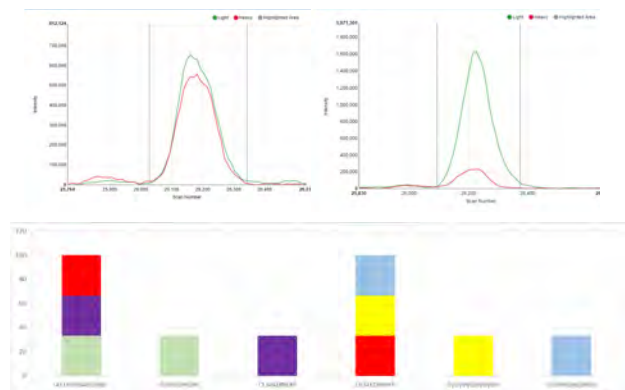
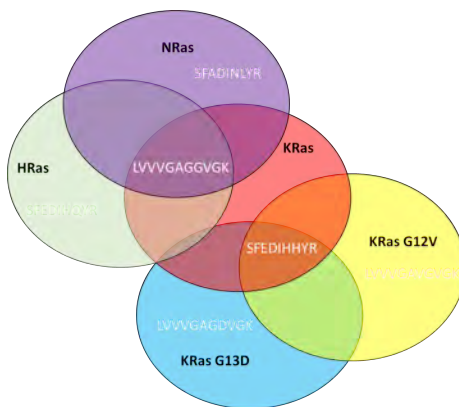
¹Sanford J. Schuster and Affiliations

Science 13 May 2018
DOI: 10.1126/science.1255029

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Targeted Proteomic Assays : Panel to Quantify Ras Variants

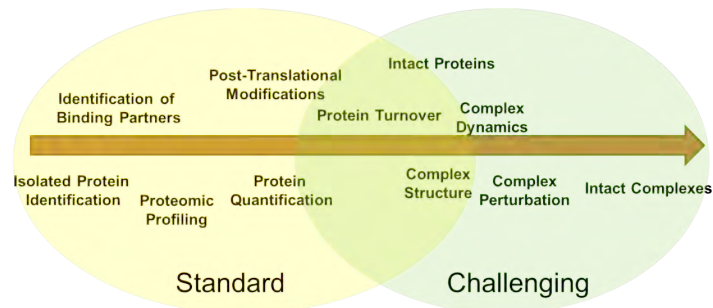


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McFall T, et al. 2019, Science Signaling, Edward Stites Laboratory

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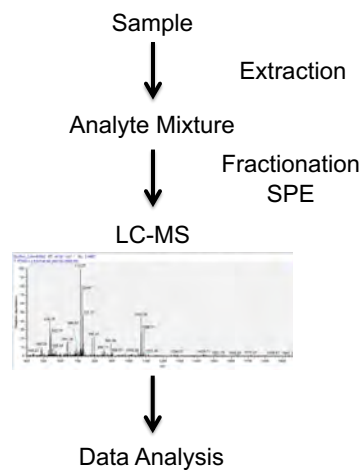
Various Proteomic Approaches Available to Investigate Aging Systems



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METABOLOMICS

- **Untargeted Lipidomics**
 - Lipid profiling
- **Targeted Metabolomics**
 - Metabolites of Interest
 - Method Development



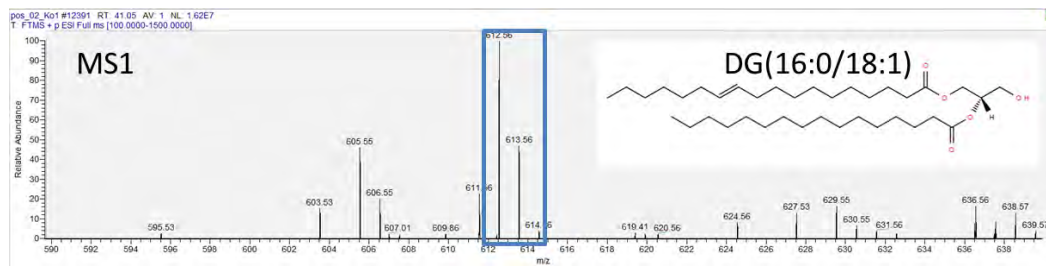
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LIPIDOMICS

- Instrument: Q-Exactive
- Lipid profile (positive/negative ionization modes)
- Database-like search (LipidSearch)
- Relative Quantitation
 - Internal Standards, Heavy-Labeled Lipids

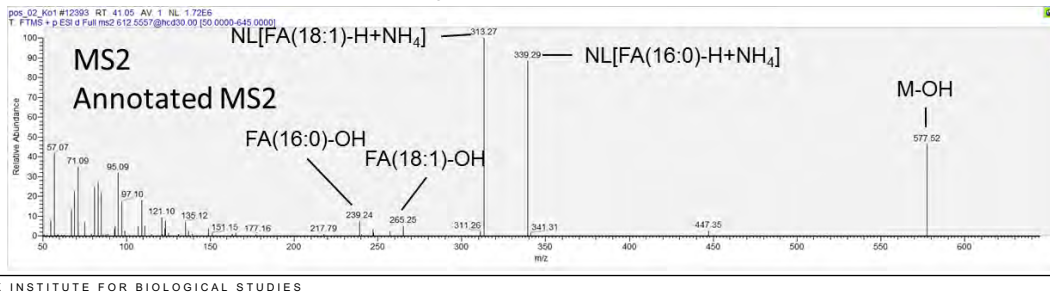
LIPID IDENTIFICATION

- LipidSearch (Thermo)
- Database search, similar to proteomics
- MS1, isolation and fragmentation



LIPID IDENTIFICATION

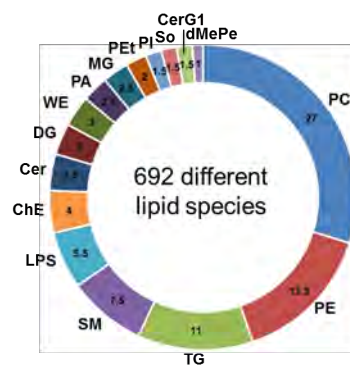
- LipidSearch (Thermo)
- Database search, similar to proteomics
- MS2, identification of major peaks, annotation



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LIPIDOMIC PROFILE OF MAMMALIAN CELLS

- Lipid Profile



Lipid class	%
PC	phosphatidylcholine 27
PE	phosphatidylethanolamine 13.5
TG	triglyceride 11
SM	sphingomyelin 7.5
LPS	lysophosphatidylcholine 5.5
ChE	cholesterol ester 4
Cer	ceramides 3.5
DG	diglyceride 3
WE	wax esters 3
PA	phosphatidic acid 2.5
MG	monoglyceride 2.5
Pet	phosphatidylethanol 2
PI	phosphatidylinositol 1.5
So	sphingosine 1.5
CerG1	simple Glc series 1.5
dMePe	dimethylphosphatidylethanolamine 1

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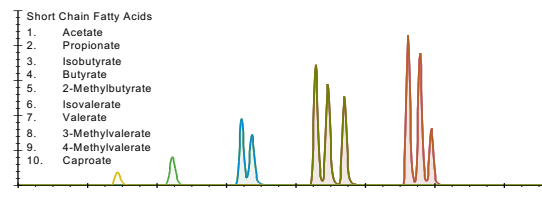
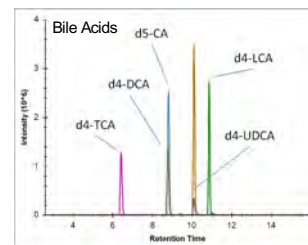
TARGETED METABOLOMICS

- Instrument: Quantiva (Triple Quadrupole)
- Multiple Reaction Monitoring (MRM)
- Extracted Ion Chromatogram (Skyline)
- Relative or Absolute Quantitation
 - Internal Standards, Heavy-Labeled Metabolites

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TARGETED METABOLOMICS

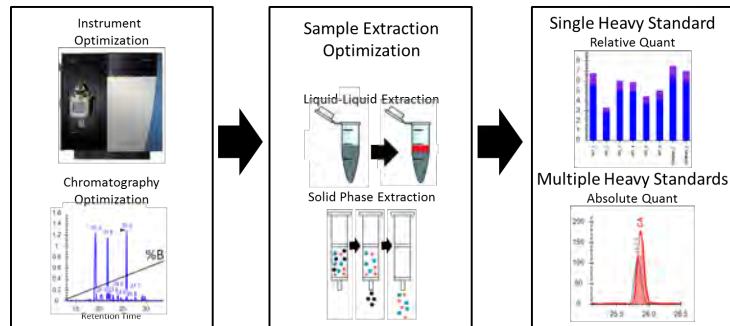
- Several Targeted Methods Available
 - Bile Acids
 - Polar Metabolites (TCA Cycle)
 - Amino Acids
 - Short Chain Fatty Acids
 - FAHFAs
- Method Development
 - Customized methods



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TARGETED METABOLOMICS

- Method Development
 - Customized methods
 - Metabolites, Chemicals and Drugs



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Thanks!



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MODULE 1


SD-NSC-Heterogeneity of Aging Core : NGS

Single Cell Transcriptomics
Single Cell Epigenomics



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1

INTRODUCTION TO NGS CORE: SINGLE CELL TRANSCRIPTOMICS/EPIGENOMICS


Sequencing & Library Preparation
 Standard library prep & sequencing
 Custom library prep & sequencing
[Single Cell library prep & sequencing](#)

Continual workflow improvement

- Illumina Sequencers
- Pacific Bioscience Sequel II
- ONT GridION X5

Outreach
 Education: Seminars, Workshops
 Training (Hands-on)
 Consultation

- Design Experiments
- Discuss methods
- Help with grant/Manuscripts preparation
- Protocol Sharing

Innovation/Collaboration
 Protocol improvement/ development
 (i.e. low cell numbers/low cost, high throughput)
 Protocol/New Technology Assessment
 (e.g. single cell RNA-Seq comparison)

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CURRENT EQUIPMENT : ILLUMINA SHORT READ SEQUENCING PLATFORMS

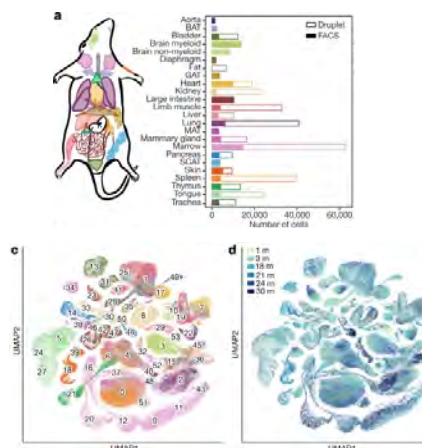
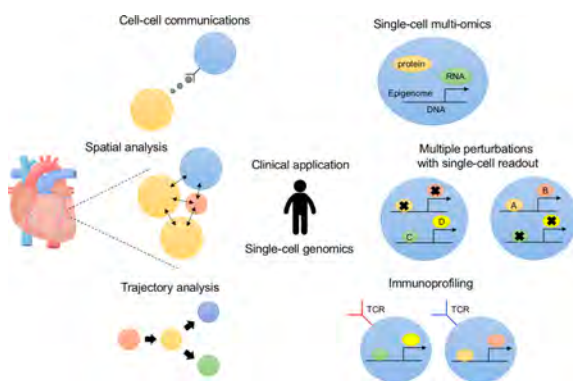
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	NovaSeq 6000	HiSeq4000	NextSeq500	MiniSeq
Output Range	134–6000 Gb	125–1500 Gb	20–120 Gb	2–7 Gb
Run Time	13–44 hr	< 1–3.5 days	11–29 hr	5–24hr
Reads per Run	Up to 20 billion	2.5–5 billion	130–400 million	7–25 million
Maximum Read Length	2 × 150 bp	2 × 150 bp	2 × 150 bp	2 × 150 bp
Relative Price per Sample	Lower Cost	Mid Cost	Higher Cost	Higher Cost
Relative Instrument Price [†]	Higher Cost	Mid Cost	Lower Cost	Lower Cost

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SINGLE CELL ANALYSIS IN AGING RESEARCH

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where cures beginNomura, *Journal of Human Genetics* (2020)The Tabula Muris Consortium, *Nature* (2020)

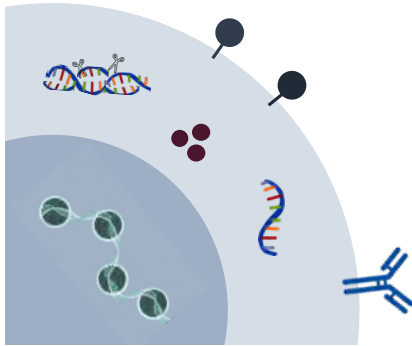
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SINGLE CELL SERVICES AT SD-NSC-HETEROGENEITY OF AGING CORE : NGS



1. SMART-Seq Approach: SMART-Seq V4 (Takara Clontech) + Nextera XT :Full-length Transcripts with poly(A)
2. 10X Genomics Chromium Single Cell System
 - scRNA-Sequencing
 - snATAC-Sequencing
 - Multiome scRNA+scATAC-Seq Sequencing
 - Featured Barcode: CITE-Seq (Total-Seq/REAP-Seq)
 - scCRISPR screening
 - Immune cell and V(D)J immune repertoire analysis
 - Spatial Transcriptomics: Visium



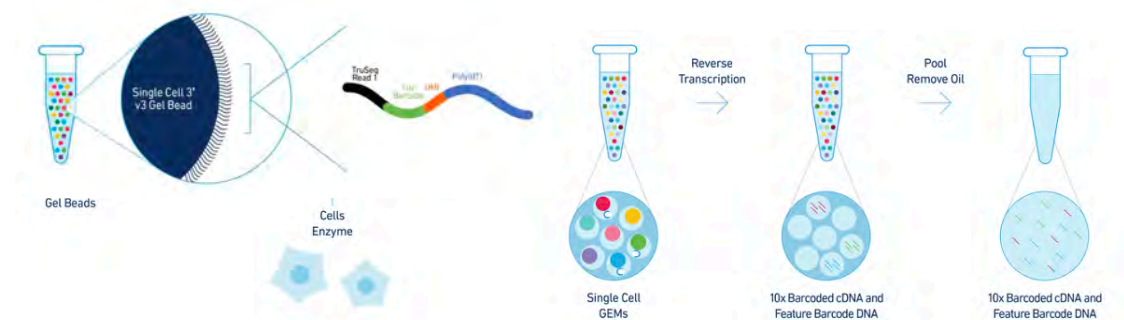
Single Cell Set-Up: Sample Preparation

- Cell Viability
- Understand cell types
- Minimizing the presence of cellular aggregates and dead cells
- Minimize the loss of cells
- Preventing non-cellular nucleic acids
- Potential inhibitors of reverse transcription

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10X GENOMICS: SINGLE CELL GENE EXPRESSION



Sample Requirements:

Cell Viability: >90%
Cell concentration: 750-1200 cells/ μ l
Total volume: 50 μ l

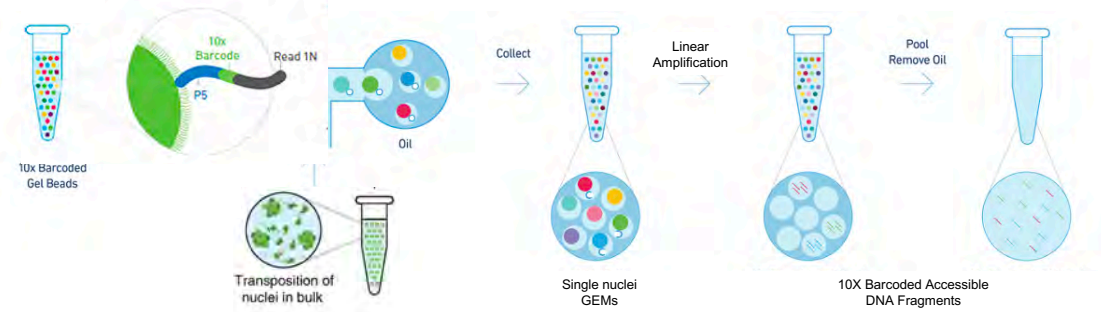
Sequencing Requirements:

- Recommended Sequencing Depth: Minimum 20,000 read pairs/cell
- Read 1 (Cell barcode & UMI): 28 bp
- Index 1 (Sample Index): 8bp for single index (10bp for dual index)
- Index 2 (Sample Index): 10bp for dual index, only for dual index
- Read 2 (Insert): 90

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10X GENOMICS: SINGLE CELL ATAC-SEQ

salk
where curiosity begins**Sample Requirements:**

Cell Viability: >90%
 Cell concentration: 750-1200 cells/ μ l
 Total volume: 50 μ l

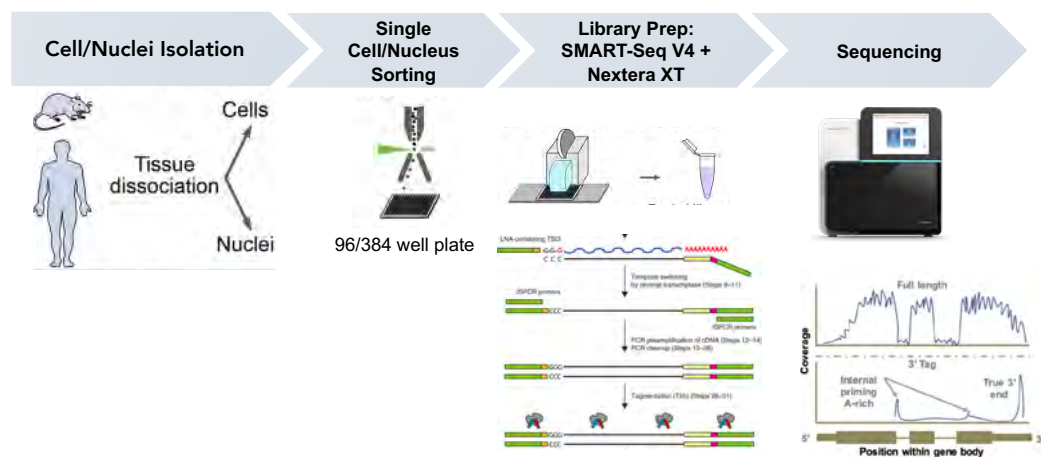
Sequencing Requirements:

- Recommended Sequencing Depth: Minimum 25,000 read pairs/nucleus
- Read 1 (Transposed DNA): 50 bp
- Index 1 (Sample Index): 8bp for single index
- Index 2 (10x Barcode): 16bp
- Read 2 (Transposed DNA): 50 bp

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SMART-SEQ2: FULL-LENGTH TRANSCRIPTS ANALYSES

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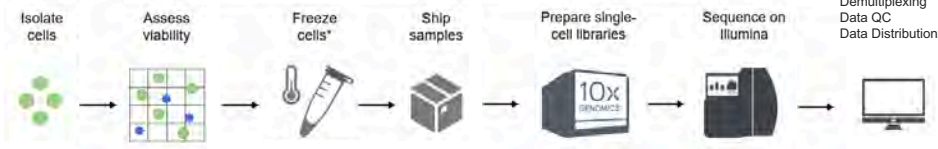
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HOW TO SUBMIT SAMPLES?


By Users

By SD-NSC-Heterogeneity of Aging Core: NGS




Plan your experiments: Please consult and discuss with us on experimental details.
Email: SampleSubmission@salk.edu

Sample Preparation
(**CRITICAL**: Optimize sample preparation)

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SD-NSC-HETEROGENEITY OF AGING CORE: NGS


Email: nhah@salk.edu

<https://www.salk.edu/science/research-centers/san-diego-nathan-shock-center/>
<https://www.salk.edu/science/core-facilities/next-generation-sequencing/>

Salk NGS Team

Nasun
Ling
Grace
Tzu-Wen
James

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