

Let Our State-of-the-Art Rangos Cores Ratchet Up Your Research

August 11, 2020

12:00 PM – 1:00 PM

Animal Imaging Core



Dr. Yijen Wu

Histology Core



Dr. Sunder Sims-Lucas

Cell Imaging Core



Dr. Krishna Prasad

Flow Cytometry Core



Dr. Abbe de Vallejo

Metabolic Core



Dr. Clinton Van't Land

Genetic Sequencing Core



Dr. Amanda Poholek

Gnotobiotic Core



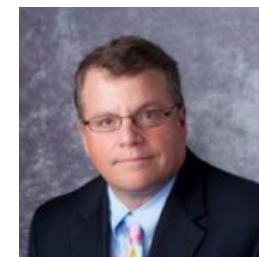
Dr. Tim Hand

Bioinformatics Core



Dr. Dhivyaa Rajasundaram

AAV Production



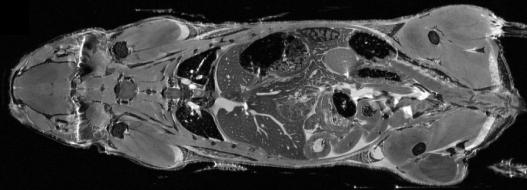
Dr. George Gittes

Animal Imaging Core

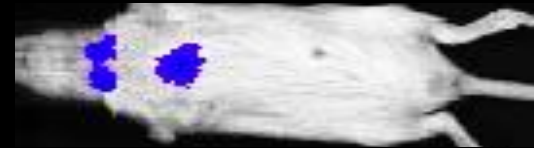
Systems, Tissue
in vivo, ex vivo

IVIS, Ultrasound, PET/SPECT/CT, MRI

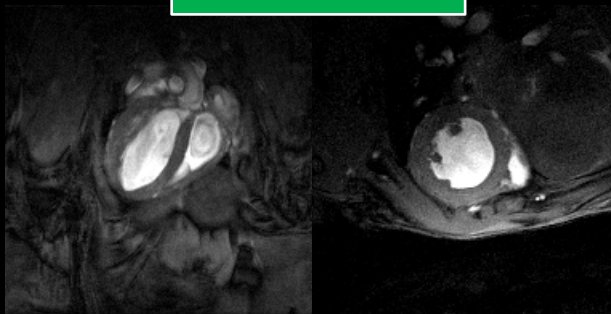
Anatomy



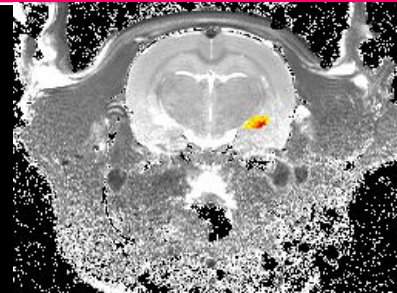
Cellular/Molecular



Function

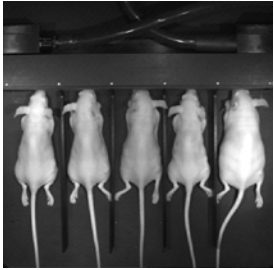


Tissue Characterization

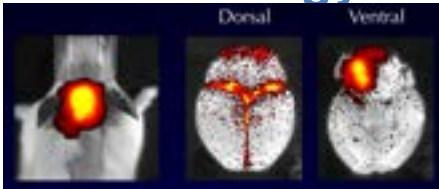


Optical *In Vivo* Imaging System (IVIS)

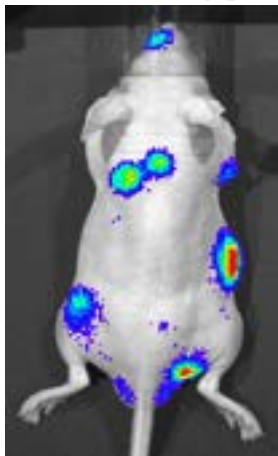
5 Mice



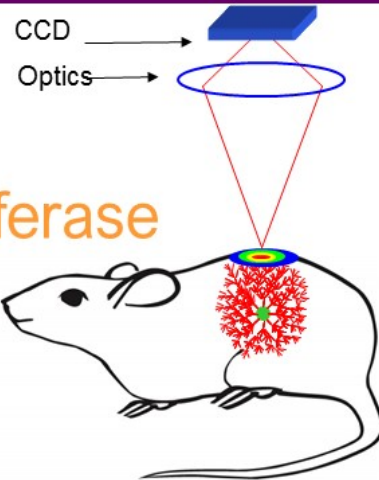
Neurology



Oncology

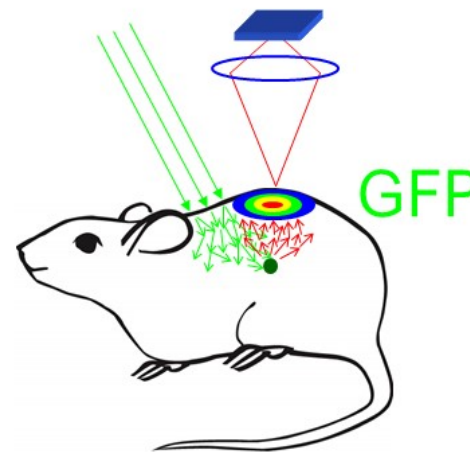


Bioluminescence



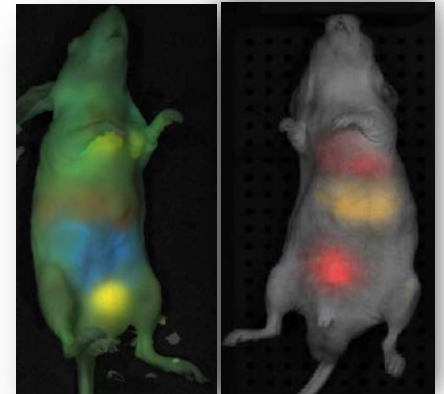
Luciferase

Fluorescence

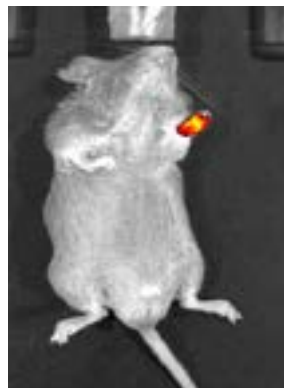


GFP

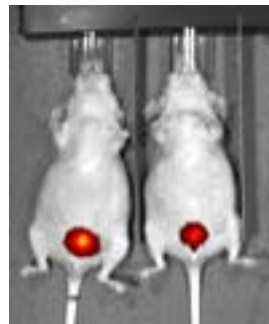
Spectral Unmixing



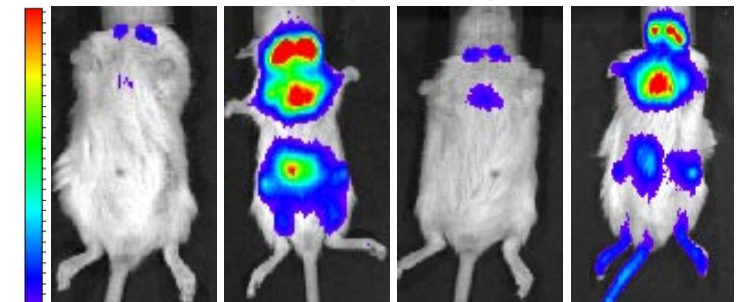
Inflammation



Infectious disease



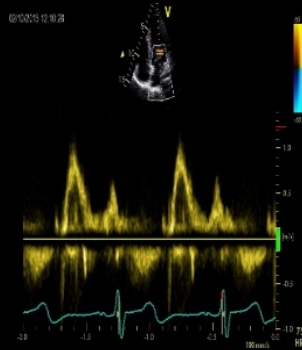
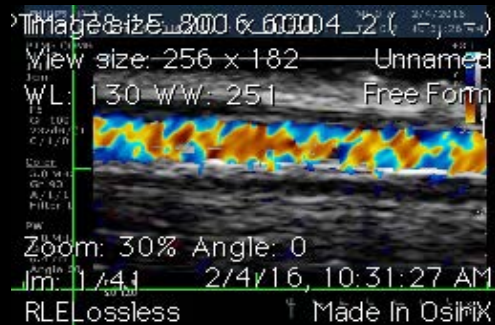
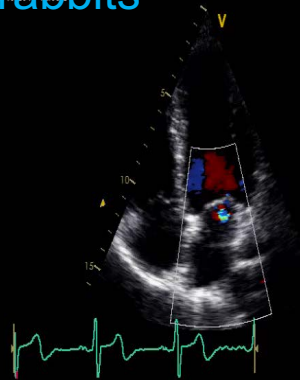
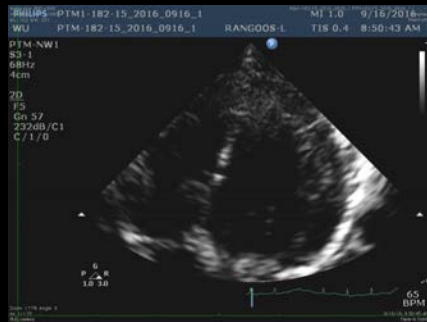
Signaling



Good, et. al. *Mucosal Immunol.* 2015;8:1166-79

Ultrasound & Ultrasound-guided Microinjection

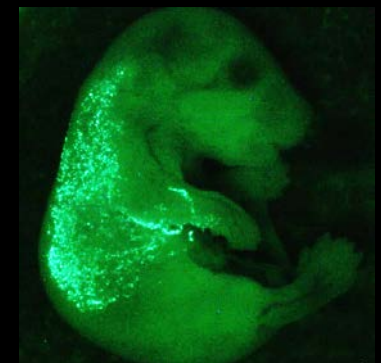
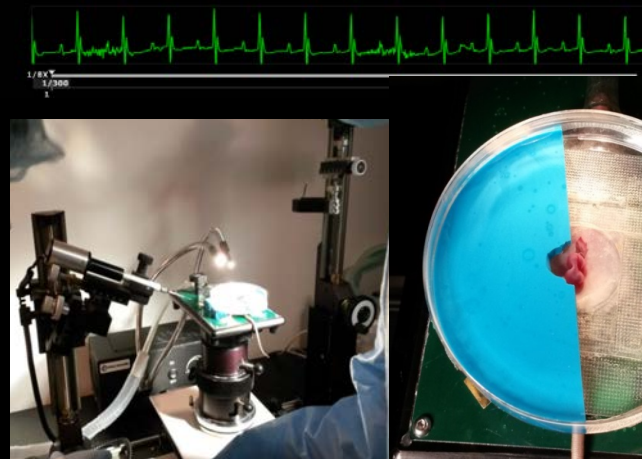
Clinical Grade For monkeys and rabbits



High-frequency Mice

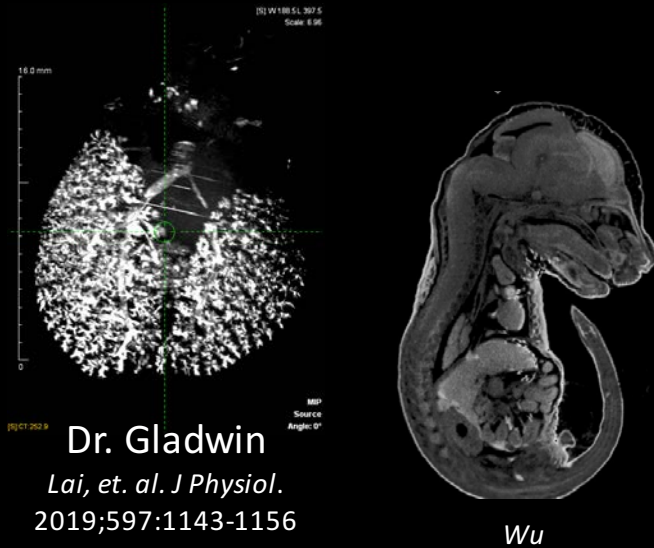


U/S guided microinjection

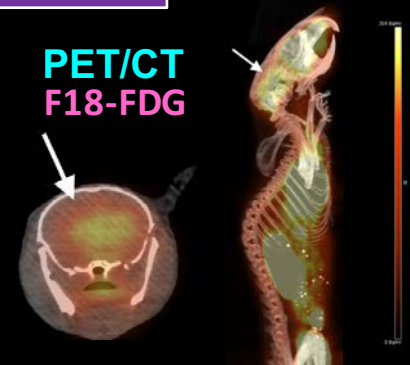
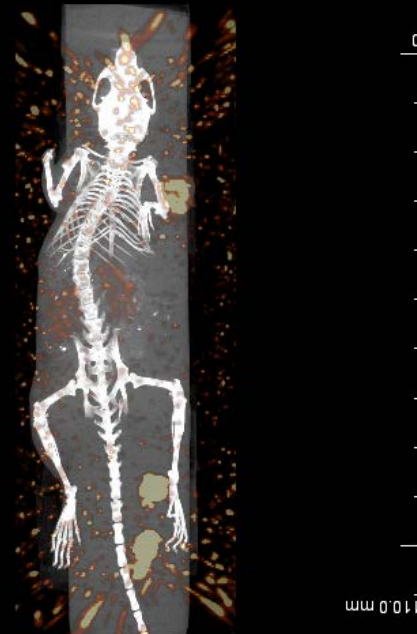


PET/SPECT/CT

Molecular Imaging



Theranostics SPECT/CT Lu-177-PSMA-targeted CTT1403



SPECT/CT mTc99-Sestamibi



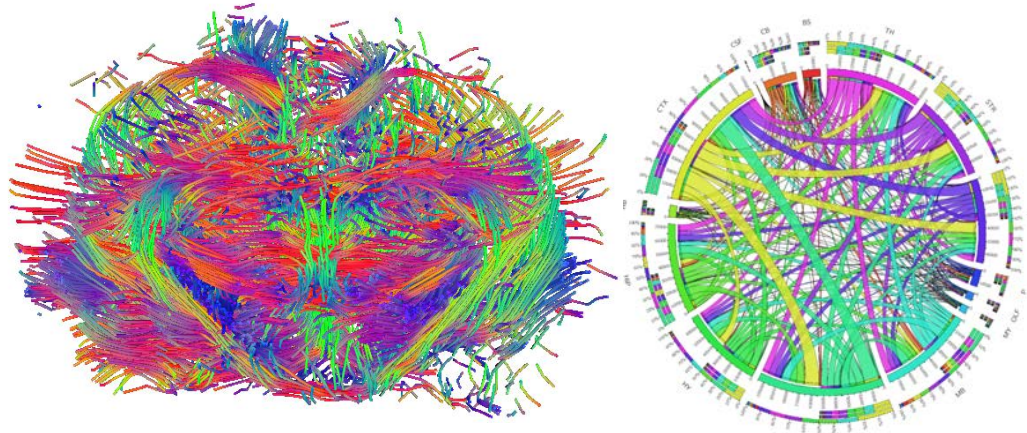
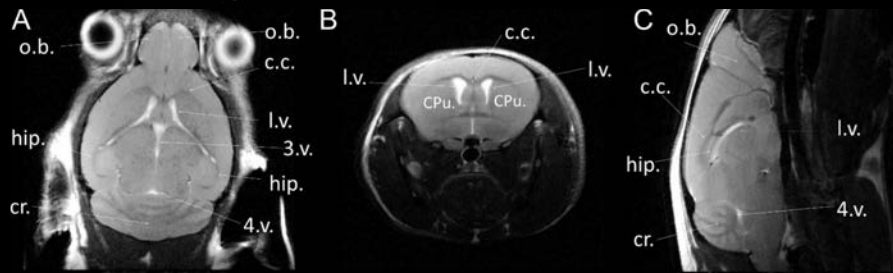
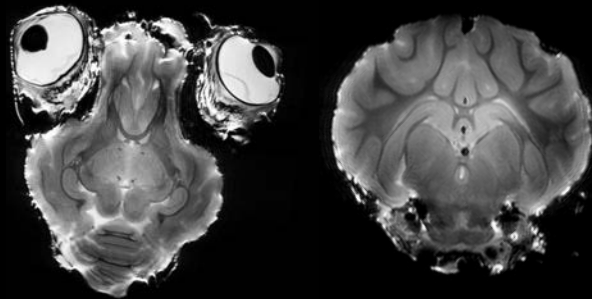
Dr. Campfield
Henkel, et. al. *Am J Respir Crit Care Med.* 2020;201:934-945.

Dr. Anderson
Ling, et. al. *Mol Imaging Biol.* 2020;22:274-284

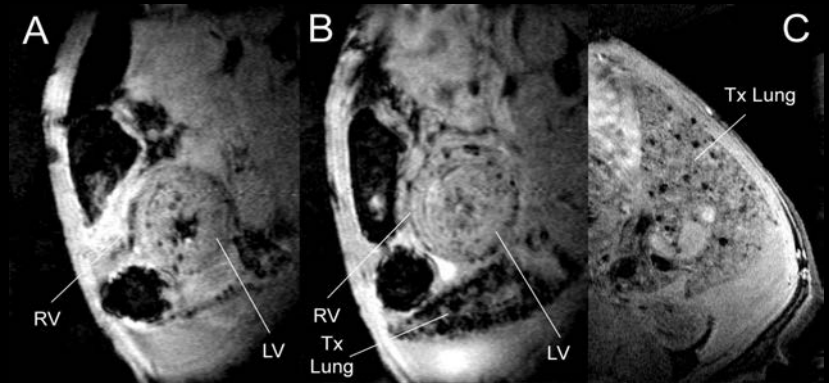
MRI

Pig

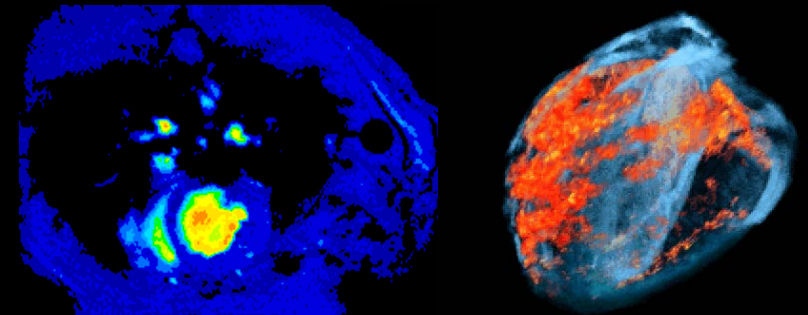
Mouse



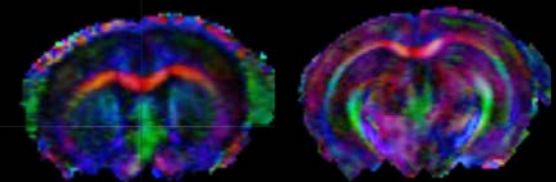
Wu & Lo, *Birth Defects Res.* 2017;109:758-770



(Wu, et. al. *Proc Natl Acad Sci*, 103(6):1852-7)



(Wu, et. al. *Circulation CV Imaging*, 6:965-973)



- ✓ **Free consultation:** project planning, data guidance, optimization, “de-bug”
- ✓ **Grant support**
- ✓ **IACUC protocol support**
- ✓ **Manuscript support**
- ✓ **Training**

Animal Imaging Core: (412) 692-6035

Sam Wyman

<SKWYMAN@pitt.edu>

Jackson Landman <JEL177@pitt.edu>

Yijen Wu <yijenwu@pitt.edu>



Histology Core

Rooms 3537

Mission: To provide high quality individualized histological services in order to facilitate productivity and success of investigators based in the Rangos Research Center and researchers in other units of Pitt / UPMC, as well as clients in other academic institutions in the Pittsburgh area.

Website:

<https://www.pediatrics.pitt.edu/research/cores-and-research-support/histology-core>

Scientific Director:

Sunder Sims-Lucas, PhD (sunder.sims-lucas@chp.edu; sus58@pitt.edu)

Ph: 412-692-9440

Manager:

Dan Bushnell (daniel.bushnell@chp.edu)

Histologist:

Michele Mulkeen (mlm159@pitt.edu)

Student worker

Sarah Su

Services offered

Routine Histological Services:

Routine Histology including:

- Tissue processing (frozen and paraffin)

- Tissue embedding

- Sectioning (blank slides and staining)

Routine histological stains

- Hematoxylin and Eosin

Special stains

- Oil red O

- Trichrome

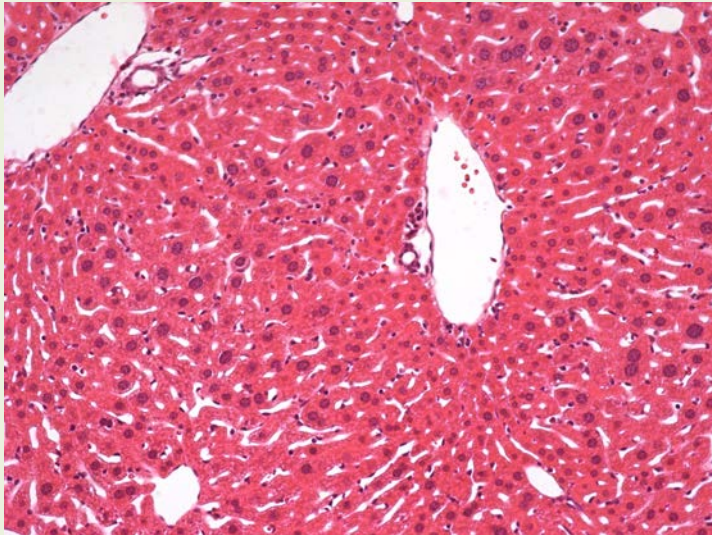
- PAS

- Toluidine blue

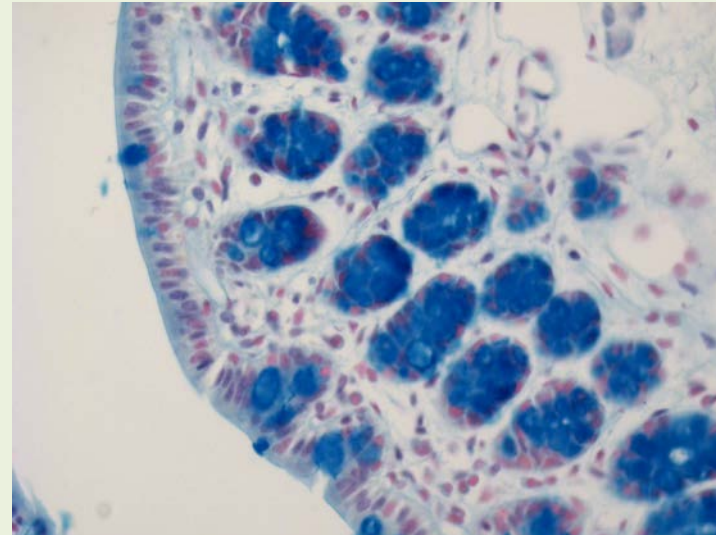
- Alcian blue

Routine/Special Histological stains

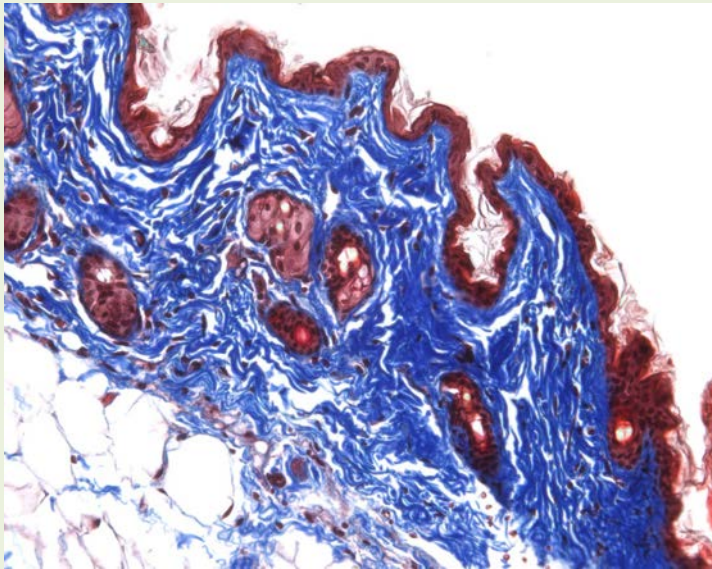
**Liver
H&E**



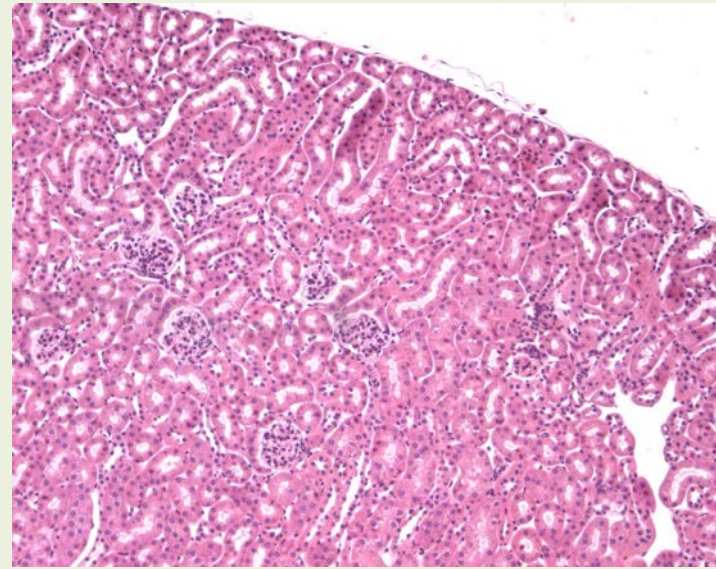
**Small
Intestine
Alcian
Blue**



**Skin
Trichrome**



**Kidney
PAS**



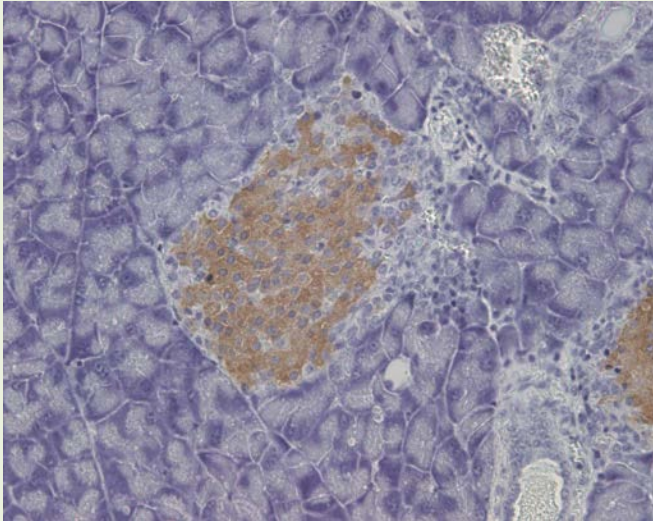
Services offered

- Immunoperoxidase staining:
IHC and IF staining with working protocols for Kim1, cd31, endomucin, f4/80, MHCII, and caspase-3, and quite a few more
- Antibody work up:
Antibody optimization for those antibodies without a current working protocol
- Other services upon request:

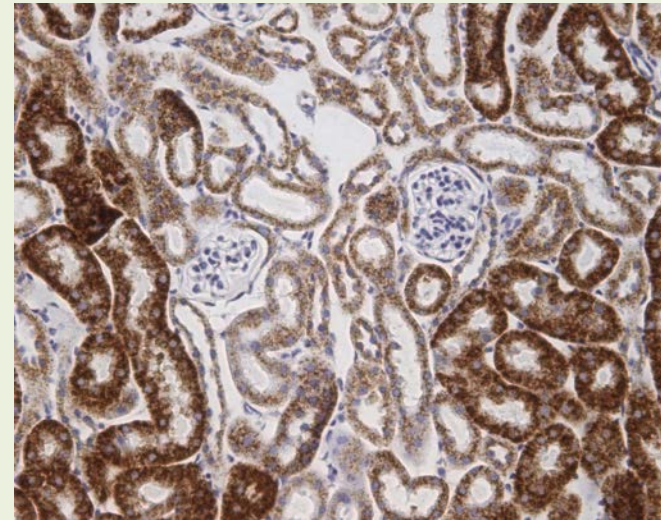
Immunoperoxidase staining

Antibody work up

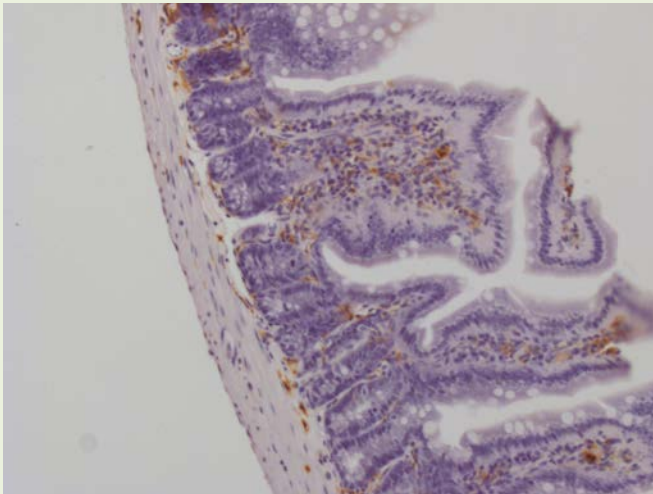
Pancreas
Insulin



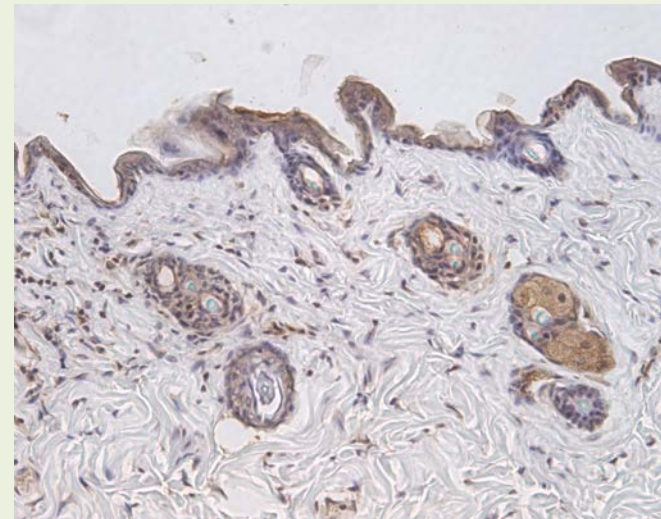
Kidney
PMP70



Small intestine
MHCII



Skin
Smad2

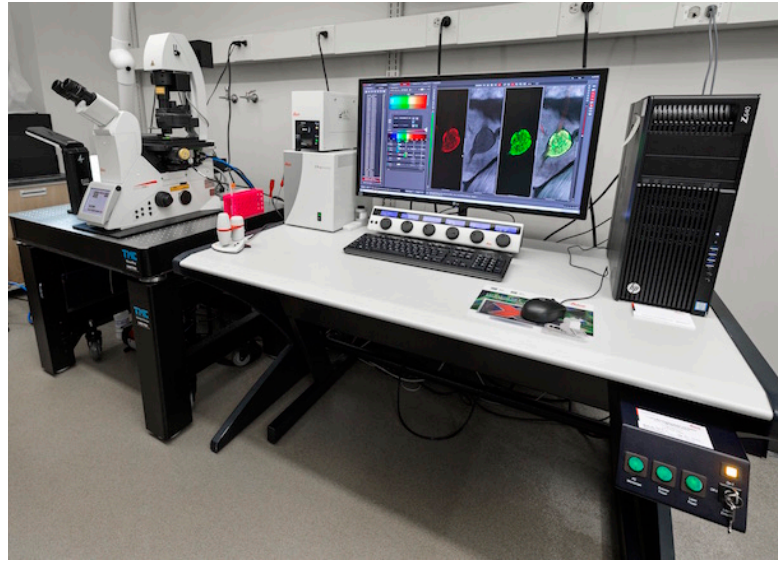


Cell Imaging Core

Zeiss LSM 710



Leica TCS SP8



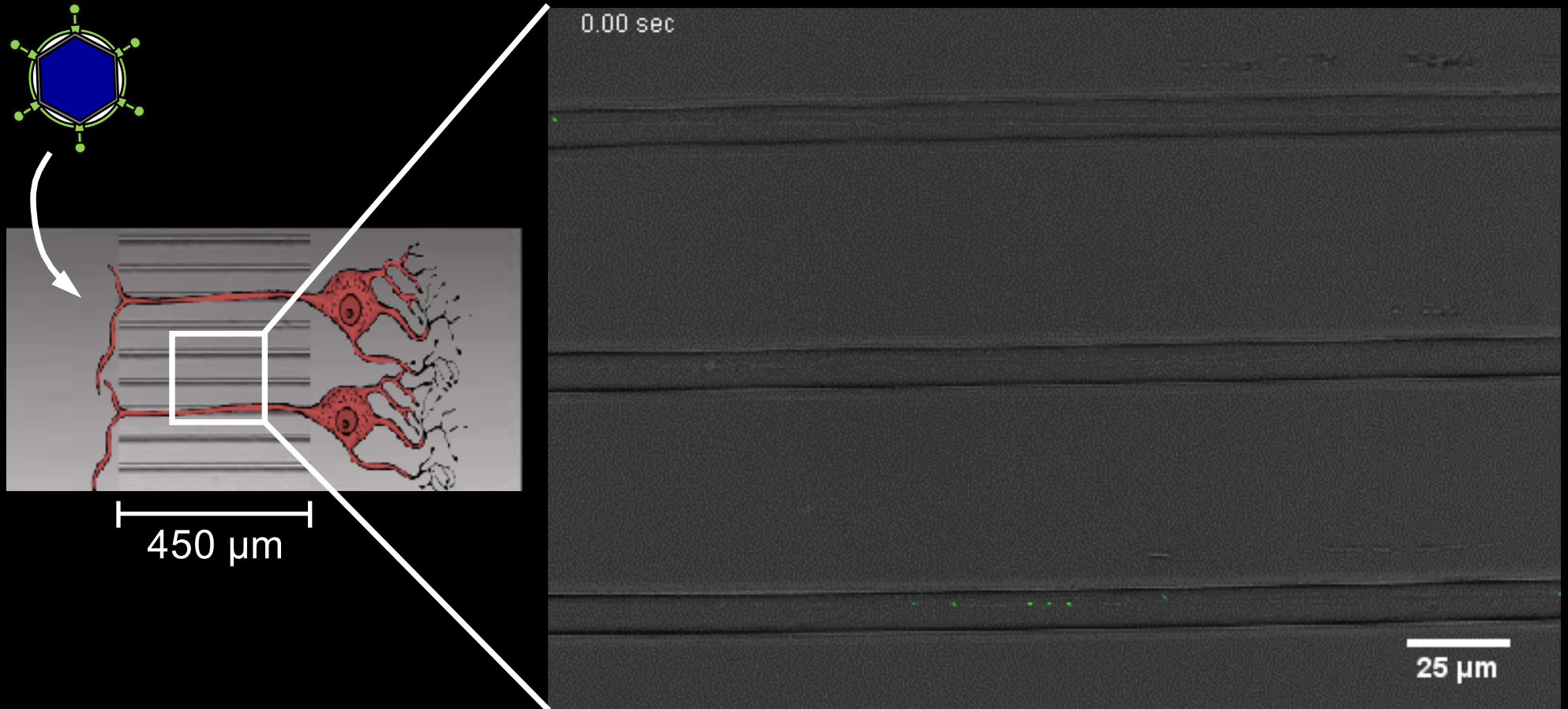
Olympus Vivaview



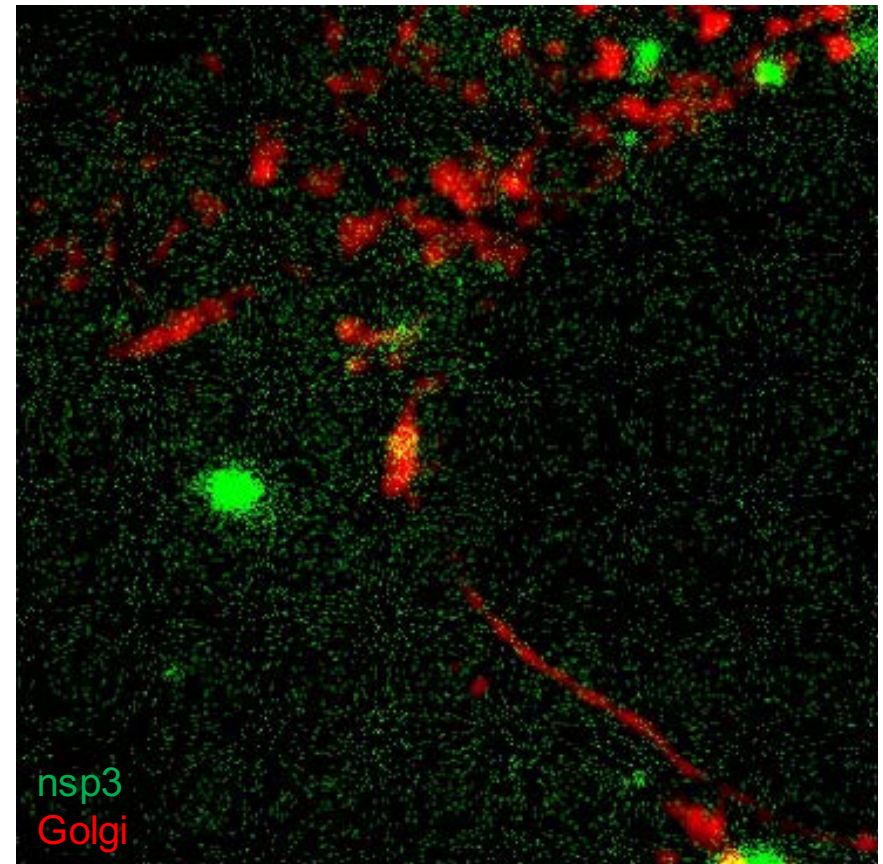
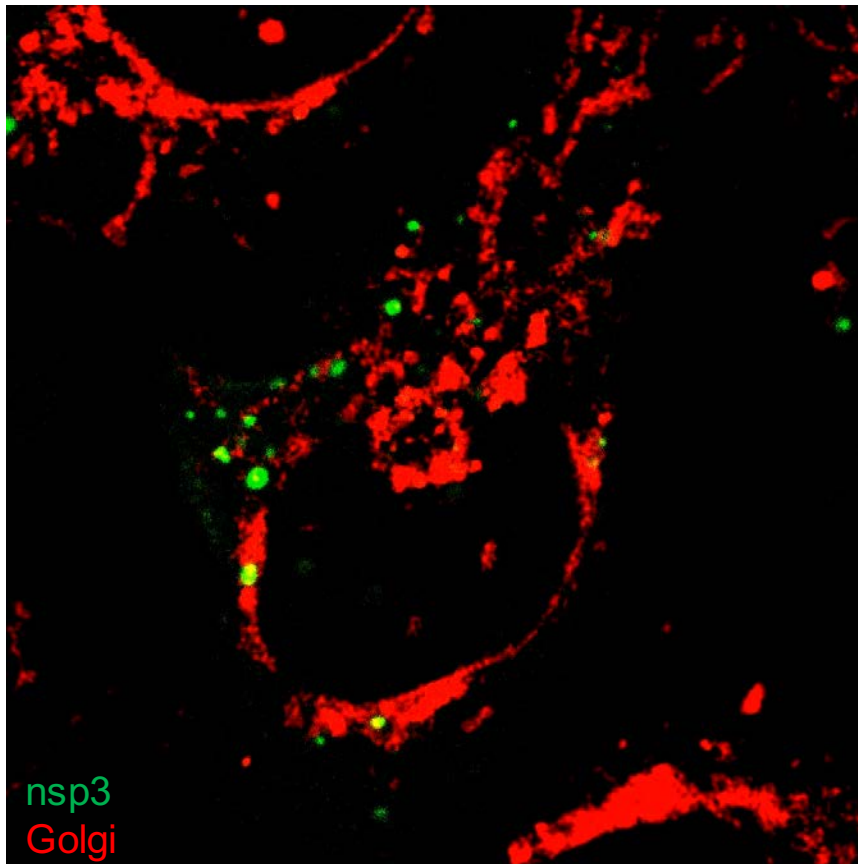
Olympus MMI CellCut laser
microdissection (LMD)



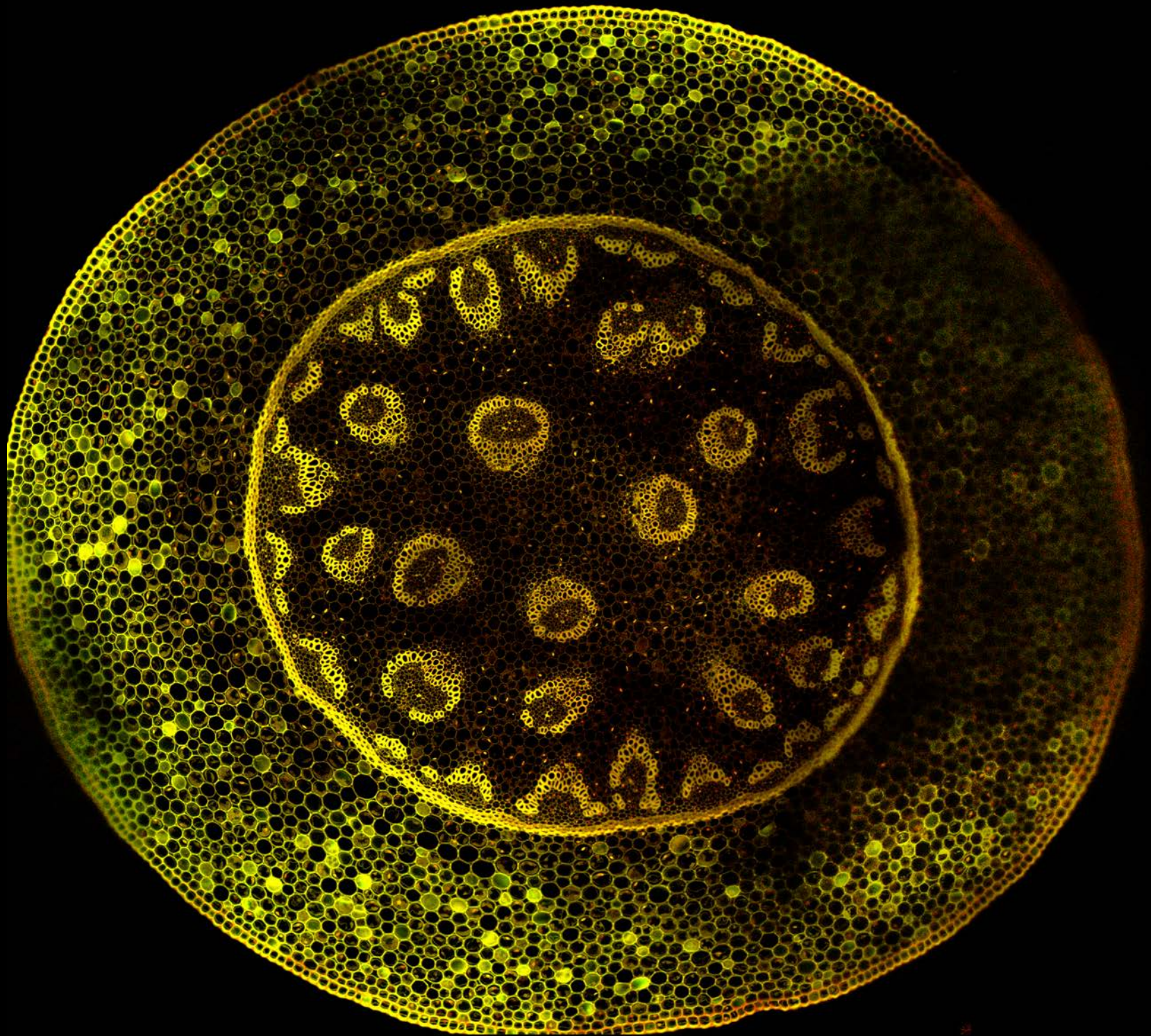
Live Imaging Shows Single Reovirus Particles Transported in Neurons Cultured in a Microfluidic Device



Rotavirus protein NSP3 nsp3 co-traffics with golgi marker



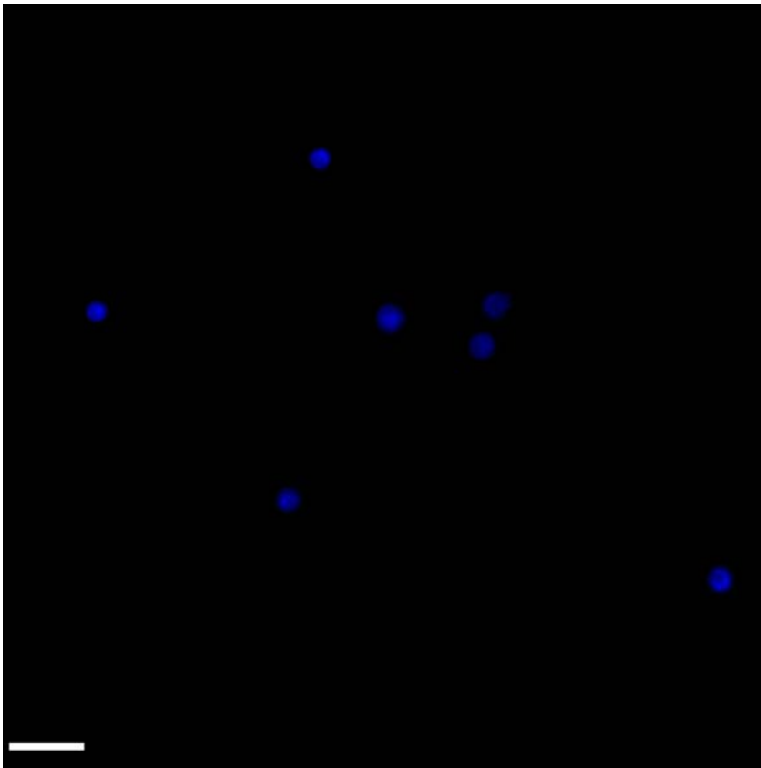
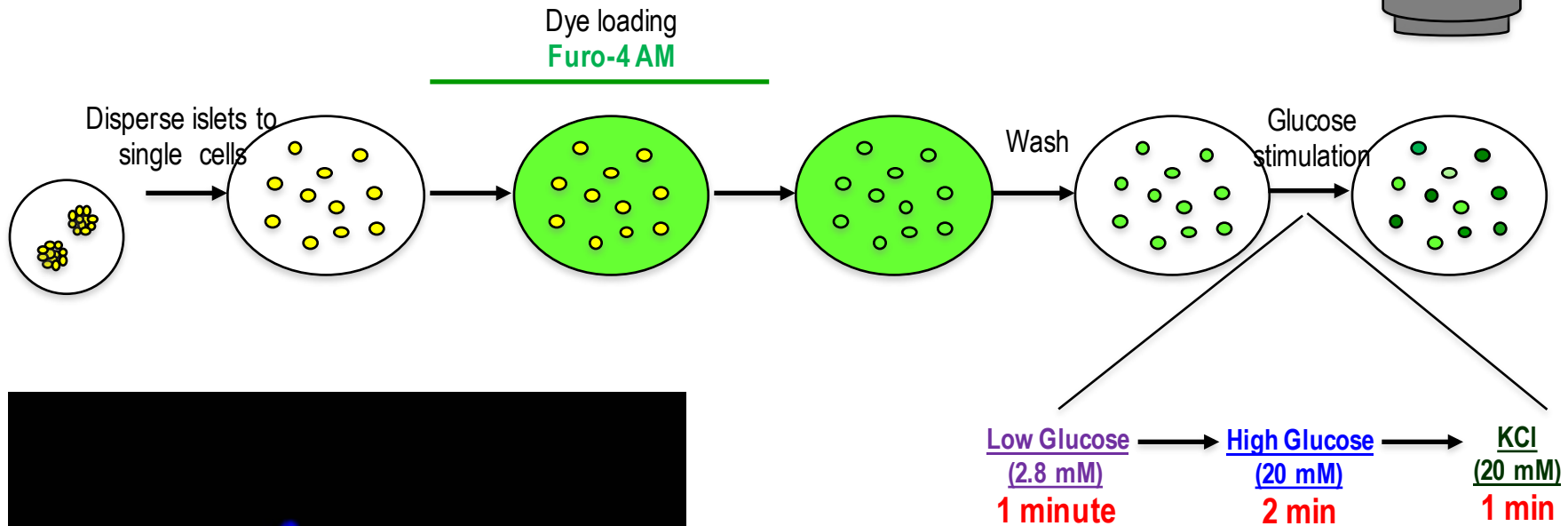
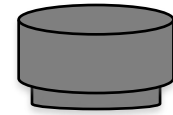
181-GFP, 5MOI U2-OS, 7.5 8.5 hpi



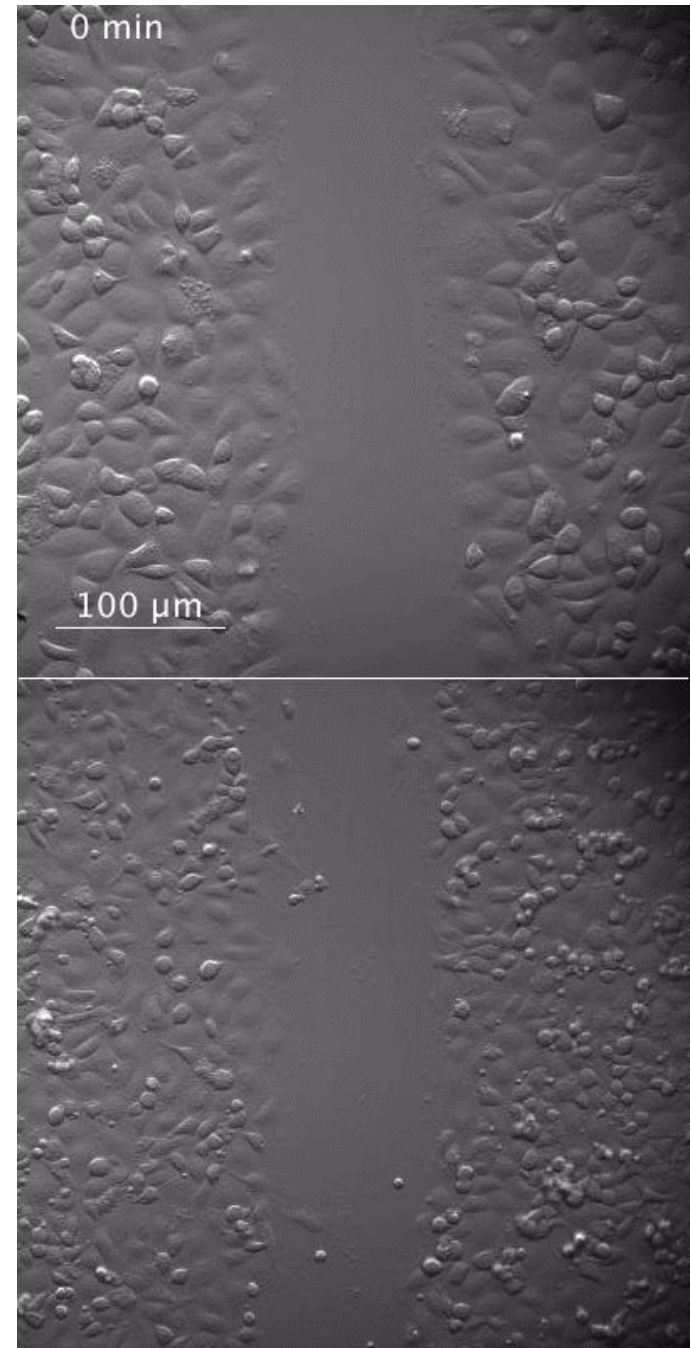
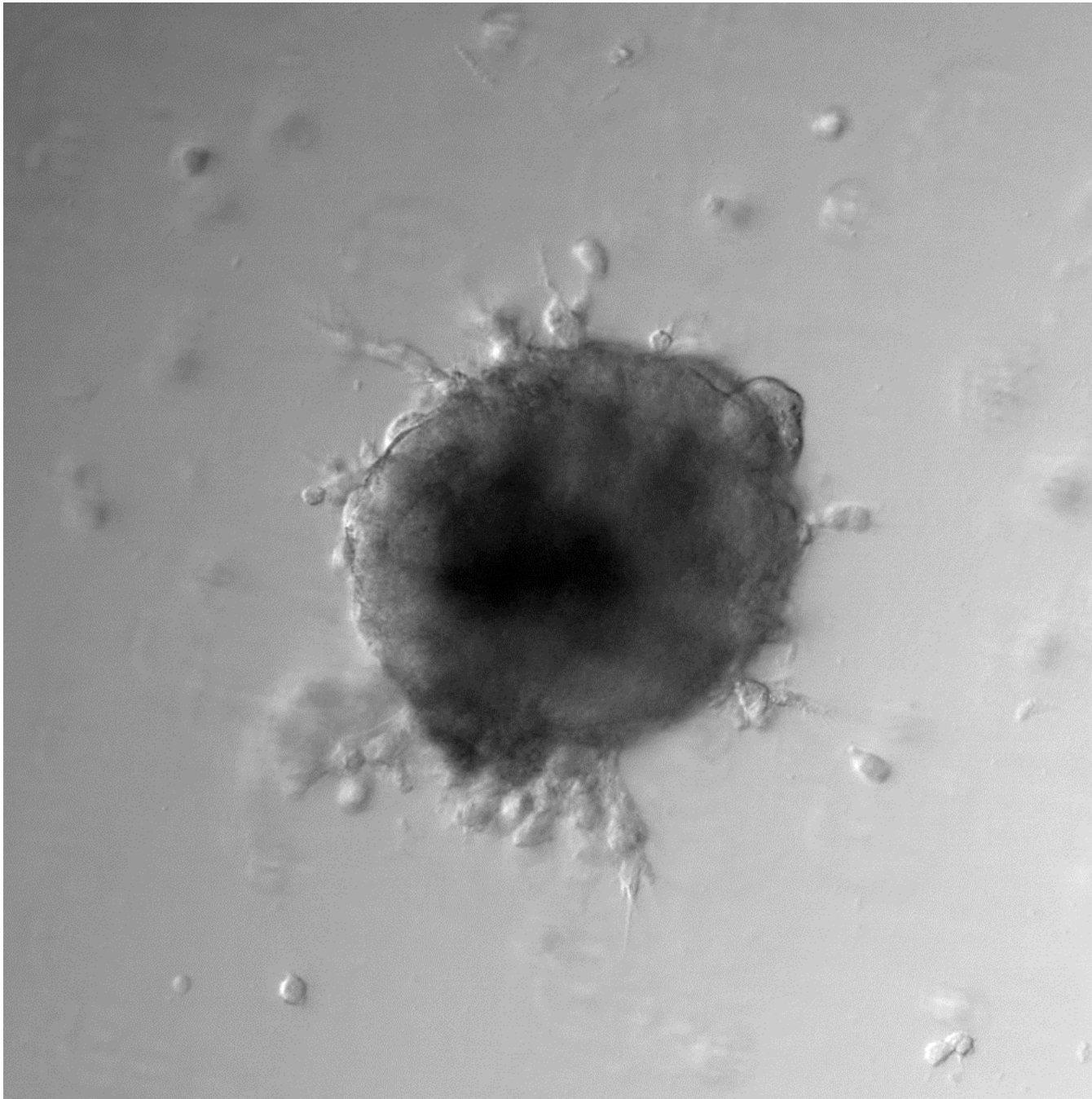
Calcium Imaging

FoxO1 Deficient Islets Have Reduced Calcium Release Response to High Glucose

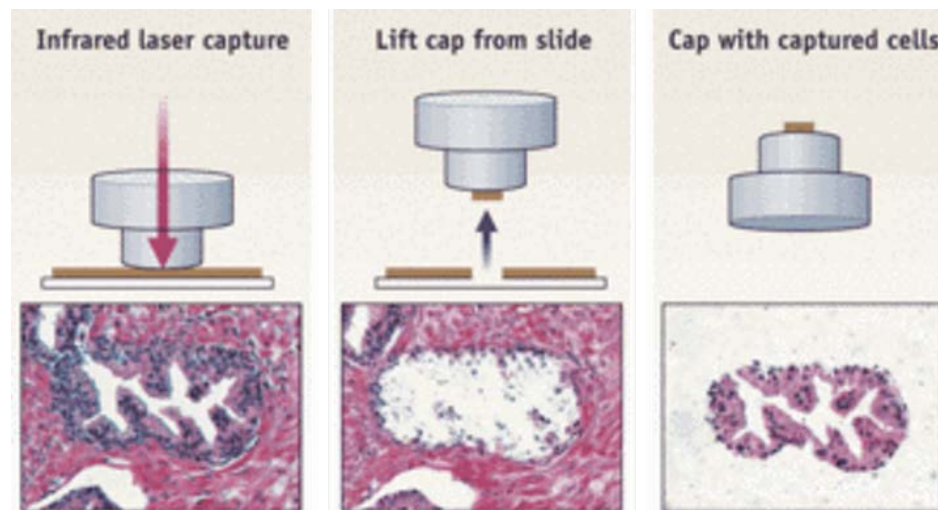
Zeiss LSM
710 confocal



Live cell imaging using Olympus Vivaview



Laser capture imaging



Cell Imaging Core
5th floor room 5148

Contact: Krishna Prasad

Krishna.prasadan@chp.edu

412-692-9211

- Training is required to get access to the imaging core. First training is free. Re-training is charged \$60/- per hour.
- The core microscopes can be reserved for up to three hours at a time during regular working hours 8 am-5 pm, and longer duration after regular hours. The facility can be accessed 24/7 throughout the year.
- The flat rate is \$30 per hour, and the minimum time that can be reserved is one hour.
- Consultation is available for experiment setup or for grant submission.

Flow Cytometry Core

John G. Rangos Sr. Research Center
Rooms 8148 and 6148

Mission: To provide congenial and individualized cytometry services in order to facilitate productivity and success of investigators that are based in the Rangos Research Center and researchers in other units of Pitt/UPMC, as well as clients in other academic institutions in the Pittsburgh area

Email contact: flow1core@chp.edu

Online scheduling: <https://rangosflowcytometrycorechp.setmore.com>

Cytometrists:

Josh Michel (Room 8148, Tel: 692-6968)

Alex Styche (Room 6148, Tel: 692-3025)

Scientific Director: Abbe N. de Vallejo PhD (andv26@pitt.edu; vallaj@upmc.edu)

Flow cytometry

Technology platform employed to study cells, organelles, or molecules by taking advantage of cell-intrinsic refractive property and/or fluorochrome signals of molecular probes when they are exposed to light.

Uses

- Cell phenotypes or changes thereof (surface, cytoplasmic, nuclear)
- Cellular activities/responses (e.g. cell cycle, mitochondrial function, DNA damage)
- Cell sorting (isolate live cells for further experimentation)
- Bacteria, mycoplasma, viral particles
- Identify/quantify molecules in solution
- (Pharmaco)Kinetics of receptor-ligand interactions
- Dying (apoptotic) cells
- Membrane bound vesicles, cell organelles (mitochondria), isolated chromosomes
- Flow FISH

Flow cytometry

SERVICES

Electronic transfer of cytometry data to PI through the PI-only access subdirectory
(*courtesy service requiring IMS authorization)

Services offered (BSL1, BSL2, BSL2+ specimens; NO BSL3 specimens)

- Staff-assisted analysis and cell sorting
- Training for self-users on the analyzers, and “super-users” on the cell sorters
- Training on the use of Flow-Jo software
(access to Flow-Jo require annual user-license fee; contact Dewayne Falkner falkner@pitt.edu in the Dept Immunology)
- Off-line data analysis
- Design / troubleshooting of cytometry protocols

BSL2+ cytometry work requires PI-authorized research personnel to submit IRB(PittPRO) [or IACUC] plus IBC approval information

Flow Cytometry Core

John G. Rangos Sr. Research Center
Rooms 8148 and 6148

Analyzers



Cytometer	Configuration set up		OS
Fortessa	5 lasers	18 PMTs	DiVa 8.0.1 Windows 7
	355nm (UV)	2	
	405nm (violet)	6	
	488nm (green)	2	
	561nm (yellow)	5	
	640nm (red)	3	
LSRII	3 lasers	8 PMTs	DiVa 8.0.1 Windows 7
	405nm (violet)	2	
	488nm (green)	4	
	640nm (red)	2	

Cell Sorters



8th Floor and 6th Floor

Cytometer	Configuration set up		OS
Aria IIu	3 lasers	16 PMTs	DiVa 8.0.1 Windows 7
	405nm (violet)	8	
	488nm (green)	5	
	640nm (red)	3	
Aria II SORP	5 lasers	16 PMTs	DiVa 8.0.1 Windows 7
	355nm (UV)	2	
	405nm (violet)	5	
	488nm (green)	5	
	561nm (yellow)	2	
	640nm (red)	2	

Flow Cytometry Core

John G. Rangos Sr. Research Center
Rooms 8148 and 6148

GENERAL GUIDELINES

- a) User-training is required regardless of background
- b) Access to cytometry rooms by badge swipes (*sharing of badges is prohibited*)
- c) Enforcement of Biosafety as required by EH&S and by the COVID-19 committee
 - *PPE: Face mask, gloves, lab coats
 - *Physical distancing; Disinfectant wiping of work areas including keyboards/mic
 - *Entry logbook: Sign in/out
- c) Prior on-line scheduling required
- d) Submission of job request and safety disclosure form
- e) Penalty charges for no-shows, overtime, or undertime – *Schedule appropriately; call the core staff for schedule changes 24 hours BEFORE anticipated change*
(*penalty fee CANNOT be charged to federal grants)
- f) Machine clogs, software failures, and other emergent problems must be reported to the core staff immediately

Metabolic Core

<https://www.pediatrics.pitt.edu/research/cores-and-research-support/metabolic-core>

Rangos Rooms 5157, 5156 & 5151

Clint Van't Land 412-692-7652

clv19@pitt.edu / clinton.vantland@chp.edu

Kaitlyn Bloom 412-692-9133

knb52@pitt.edu / kaitlyn.bloom@chp.edu

The Metabolic Core supports investigators involved in the study of energy metabolism, metabolic pathways, and cellular metabolic function in cells from biological fluids, cell cultures and tissues.

Staff-assisted Service(s)

- Seahorse extracellular flux analyzer

Staff-conducted Services

- LC/MS/MS (tandem mass spectrometry)
- Amino Acid analyzer
- HPLC with UV(PDA)/fluorescence/electrochemical detectors

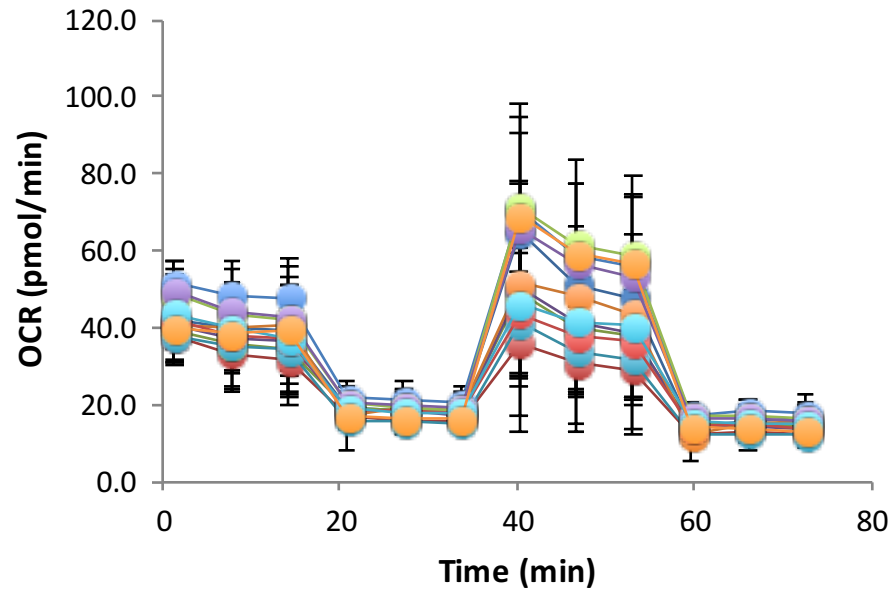
Self-conducted Services

- Spectrofluorometer
- Spectrophotometer

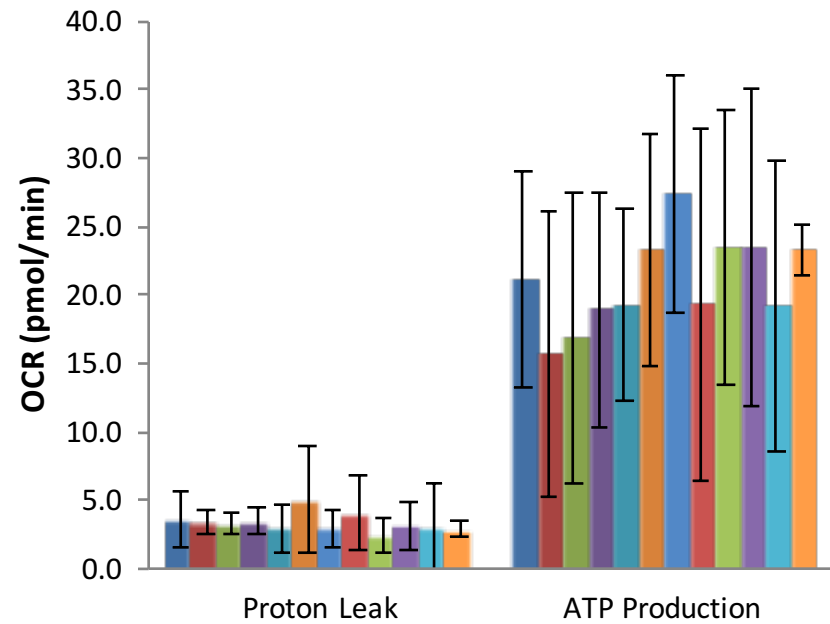
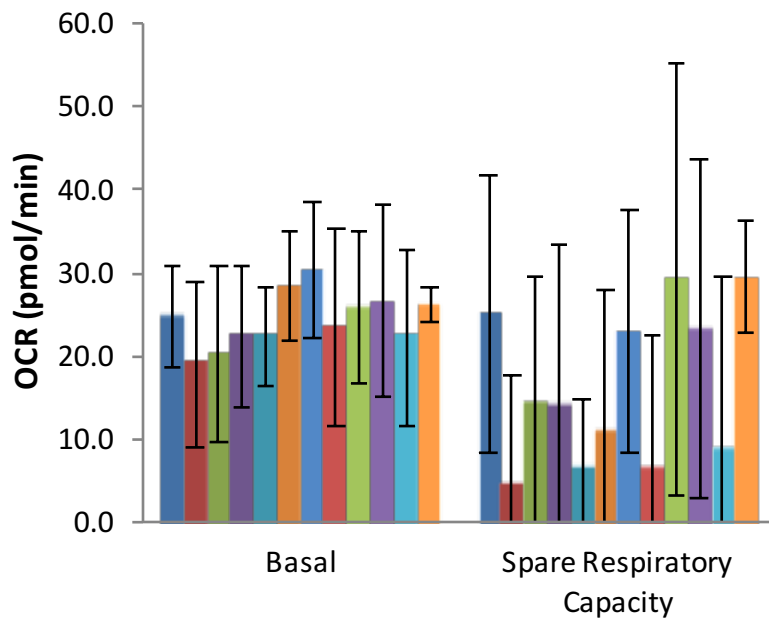
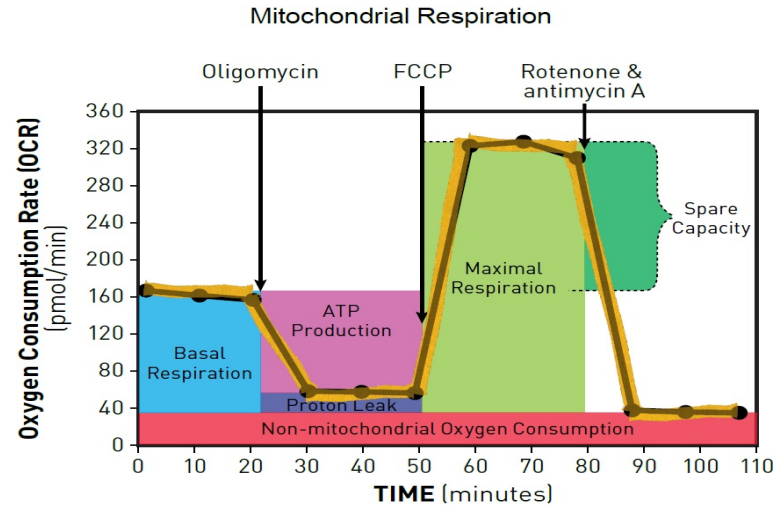
Project Consultation

Technical consultation will be required prior to the initial (first) sample submission.

Mitochondrial Respiration



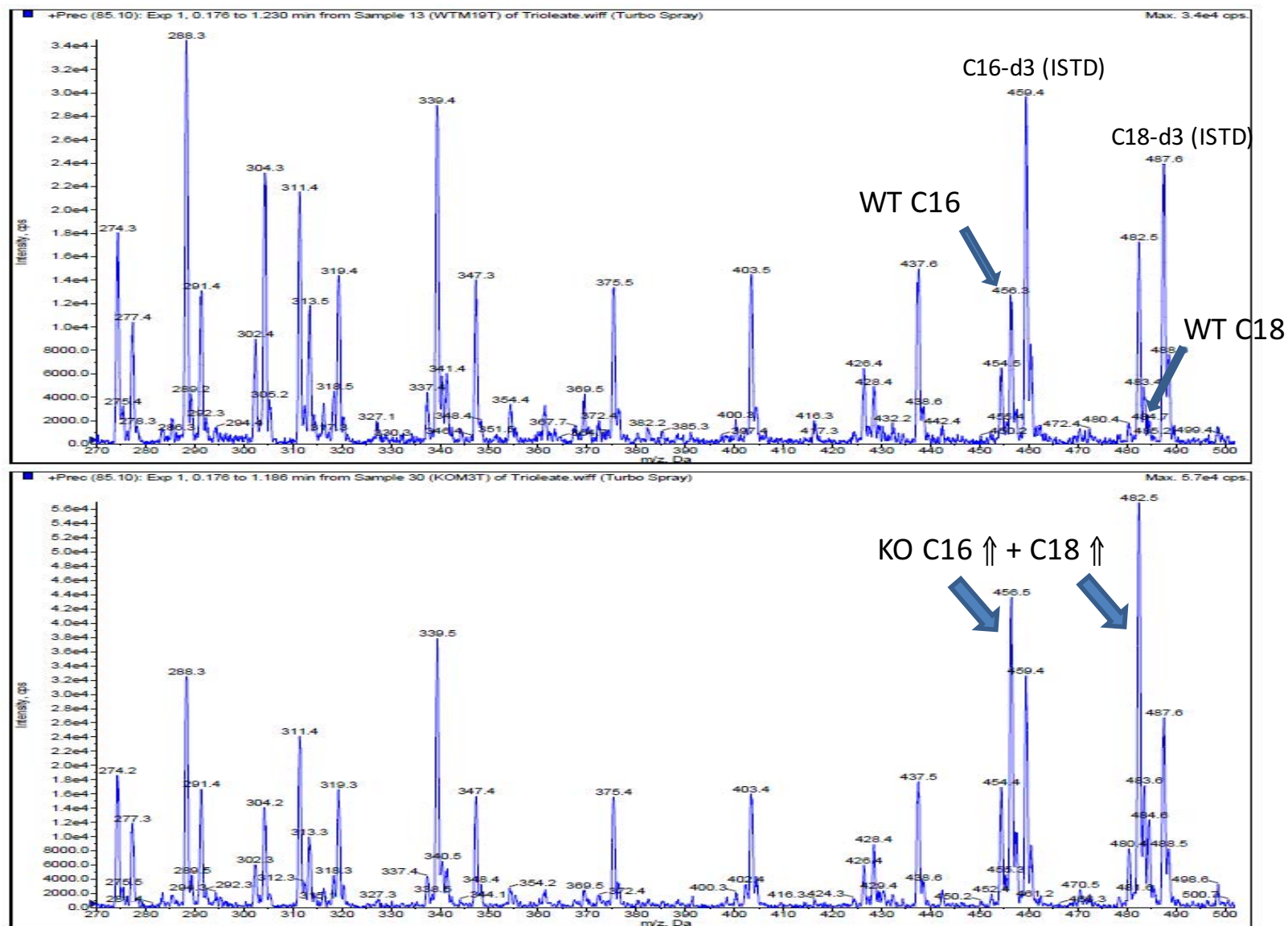
Seahorse XF Cell Mito Stress Test Profile



This slide contains unpublished data

LC/MS/MS Assay: Acylcarnitine Profiling

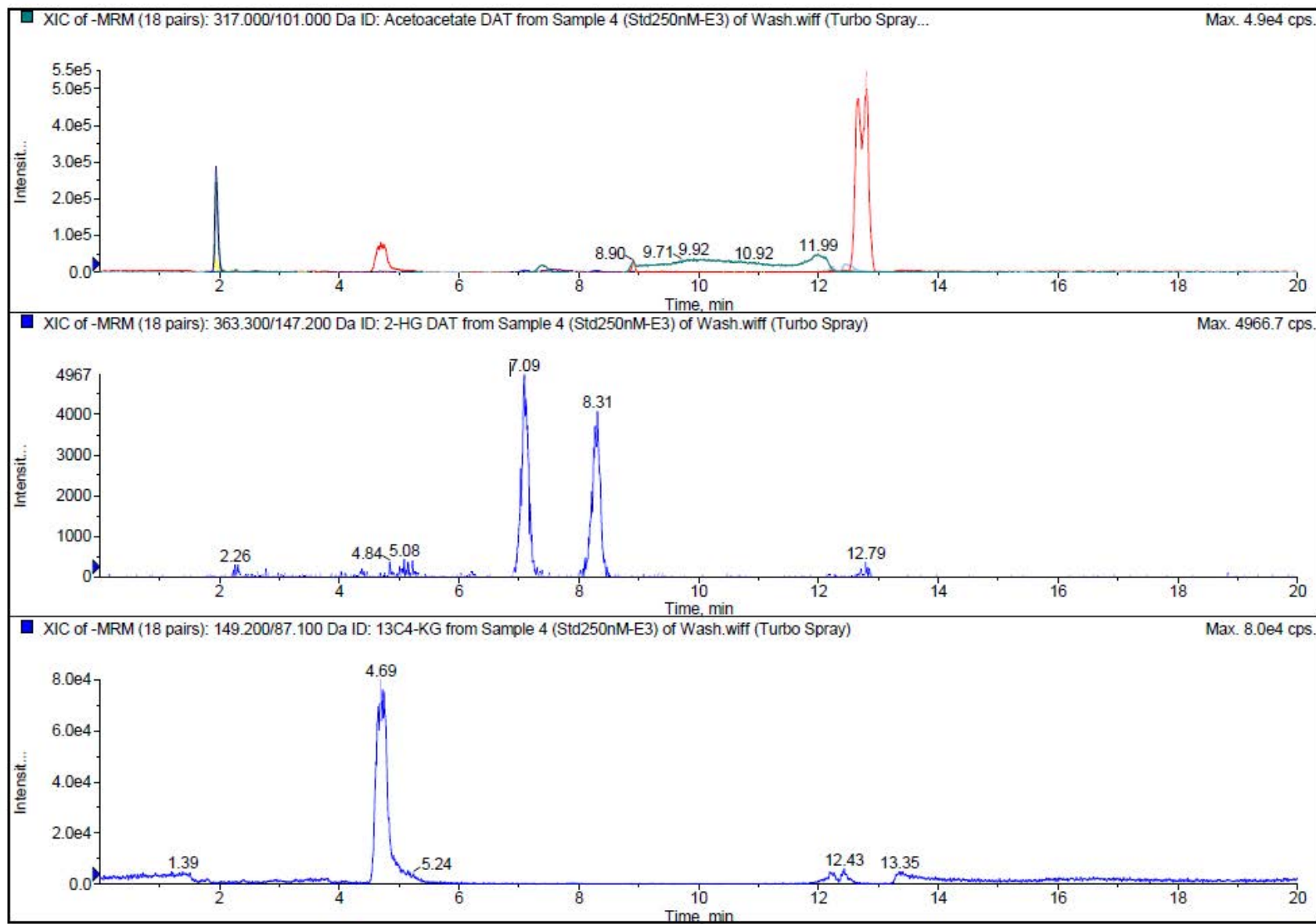
WT & KO Mouse serum after feeding fatty acid oil for {N}-days



This slide contains unpublished data

LC/MS/MS Assay: (D & L)-2-hydroxyglutarate “oncometabolite”

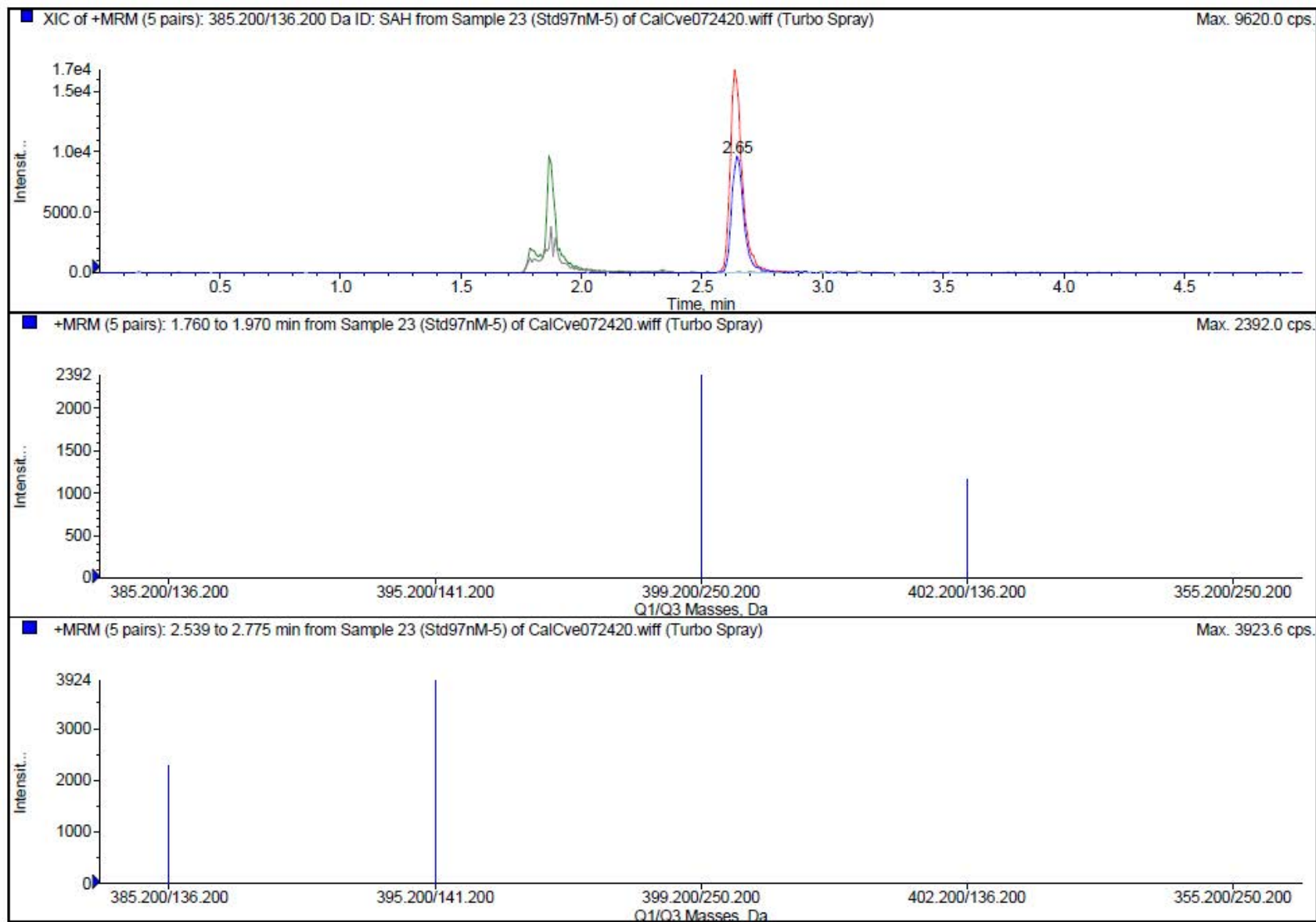
D- & L-2-Hydroxyglutarate (253 nM) / [13C4]-Ketoglutarate (505 nM) standard solution



This slide contains unpublished data

LC/MS/MS Assay: S-Adenosylmethionine (SAM \rightarrow SAH)

SAM and SAH (≈ 97 nM) / SAM-d3 (112 nM) & SAH-13C10 (218 nM) standard solution

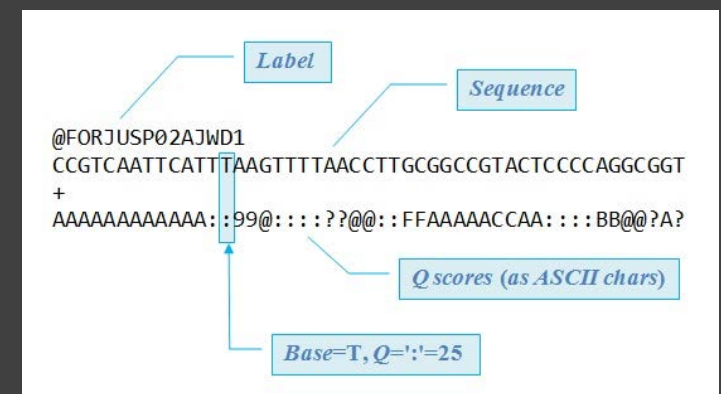


This slide contains unpublished data

Health Sciences sequencing core

@ Children's Hospital of Pittsburgh
Rangos 8th floor

[Website: http://nextgen.pitt.edu/](http://nextgen.pitt.edu/)



HSSC @ CHP

- STAFF BASED AT RANGOS AND THE GENOMICS RESEARCH CORE
- PERFORM THE SAME SERVICES.
- CONSULT WITH THE MOST CONVENIENT FACILITY

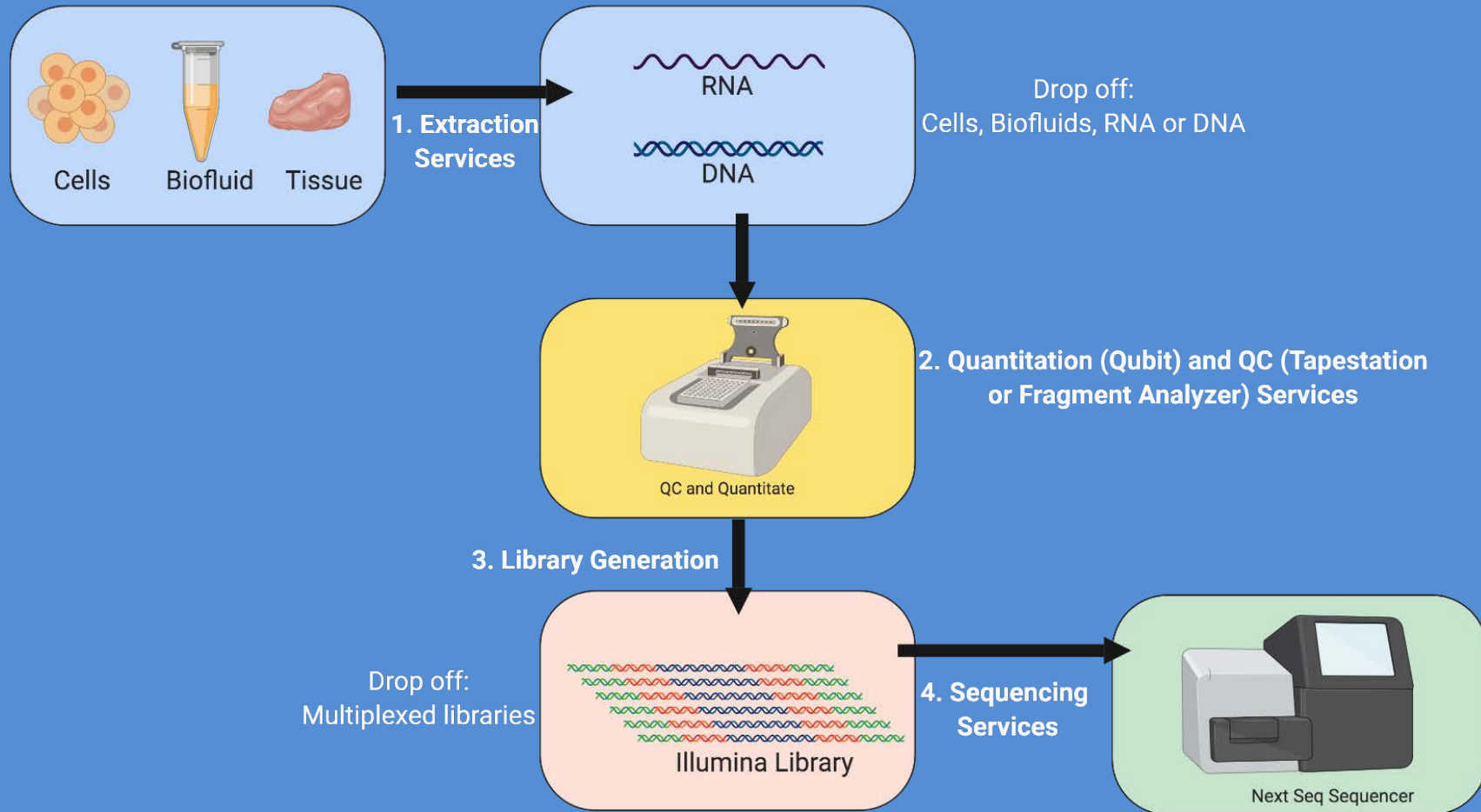
Children's Team: 8th floor Rangos, Rm 8145

- Dr. Amanda Poholek, PhD – Director poholeka@pitt.edu
- Dr. Will MacDonald, PhD - Assistant Director w.a.macdonald@pitt.edu
- Rania Elbakri – Technician Rania.Elbakri@chp.edu

Oakland Team (Genomics Research Core - Forbes ave.):

- Janette Lamb – Director, GRC
- Debby Hollingshead – Technical Director, GRC
- Bryan Thompson – Technician, GRC
- Heidi Monroe – Technician, GRC

SEQUENCING WORKFLOW



LIST OF SERVICES OFFERED

1. RNA/DNA EXTRACTION

- TISSUE, CELLS, WHOLE BLOOD, SALIVA, BIOFLUID

2. QUALITY CONTROL

- ADVANCED ANALYTICS FRAGMENT ANALYZER
- AGILENT TAPESTATION 2200
- QUBIT FLUOROMETER

3. LIBRARY PREPARATIONS

- MRNA
- TOTAL RNA
- RNA ACCESS (FFPE) TARGETED SEQUENCING
- SMARTER-SEQ V4 ULTRA-LOW INPUT RNA- CLONTECH
- SMALL RNA
- NEXTERA/NEXTERA XT DNA
- CHIP-SEQ
- ATAC-SEQ

4. SEQUENCING

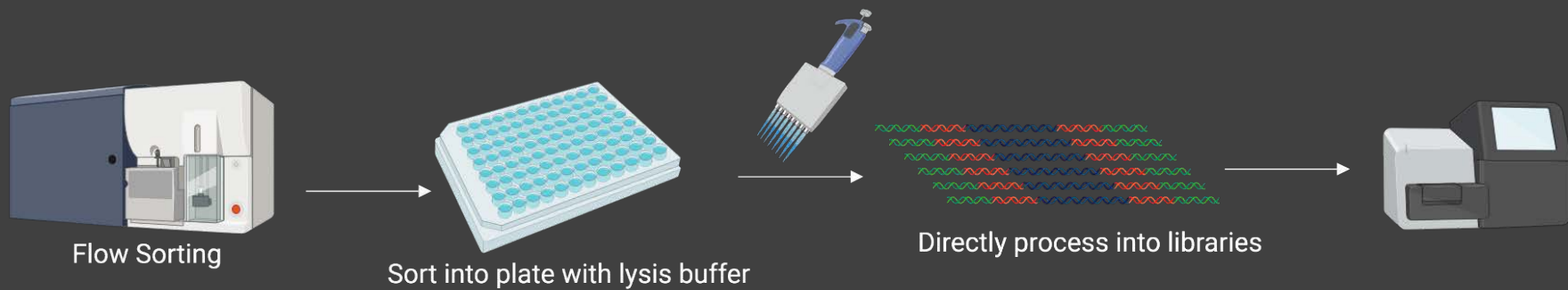
- **NEXTSEQ 500**
 - HIGH OUTPUT (400M READS)
 - MID OUTPUT (130M READS)
- **MISEQ (OAKLAND)**
 - 16S SEQUENCING
 - TCR SEQUENCING

Other Projects:

- Amplicon Sequencing
- ATAC-seq
- ChIP-seq
- Cell free DNA
- Cell free RNA
- Bulk mouse TCR sequencing
- CUT&RUN (low cell number ChIP-seq assay)
- Crispr library screens

- If you have a sequencing project, please contact us. We want to help you go from sample to sequencer

SMART-SEQ LOW-INPUT RNASEQ



Starting material:

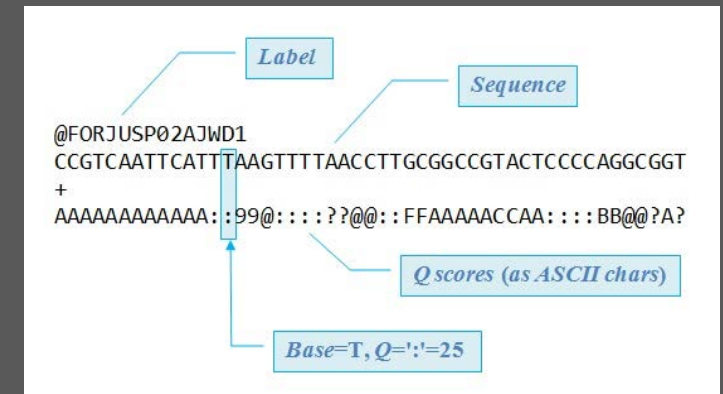
- 1-1000 cells
- 10pg-10ng RNA

- CHP Flow Core has experience working with us on this, we can also prepare plates of lysis buffer for other sort facilities

WHAT DO WE DELIVER TO YOU?

Data!! (Raw.....)

- Fastq files – main file output from the sequencer
 - Direct to CRC cluster path of your choice
 - Pitt Box folder
 - Hard Drive
 - Etc we will deliver to you in whatever is best option for you



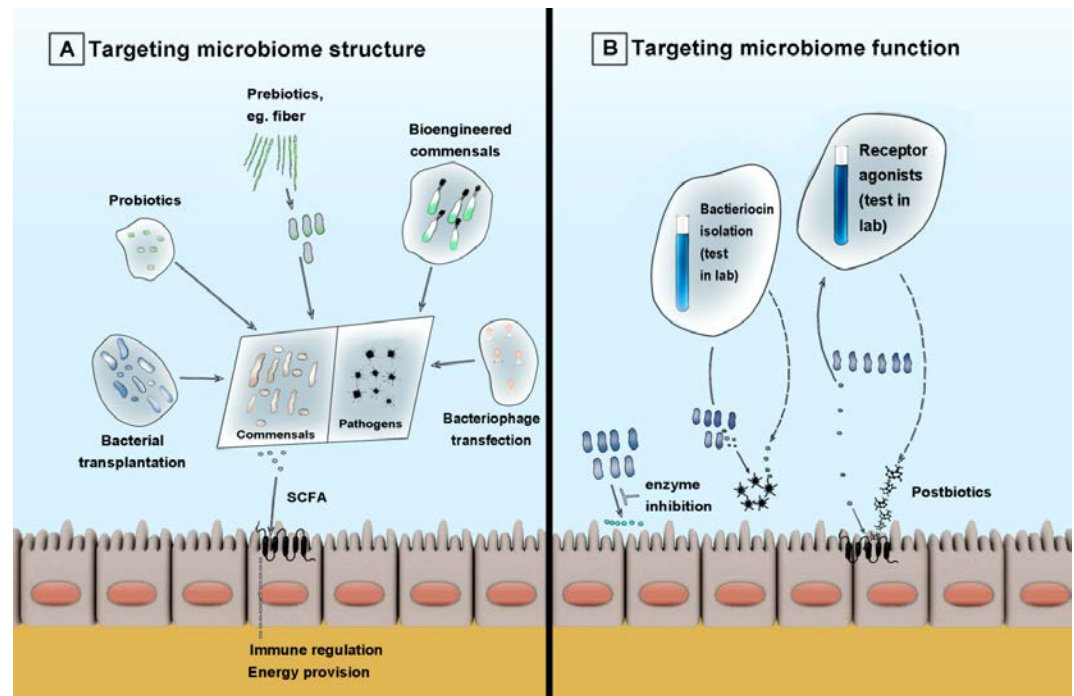
Additional resources:
Next Generation Sequencing
@ The University of Pittsburgh
<http://www.hscrf.pitt.edu/ngs>

Gnotobiotic Core

Microbiome – new paradigm for understanding biology and medicine

Therapeutics

- Pro-biotics
- Fecal transplant
- Pre-biotics
- Engineered bacteria
- Bacteriophages
- Metabolite/drug discovery



Director: timothy.hand@chp.edu

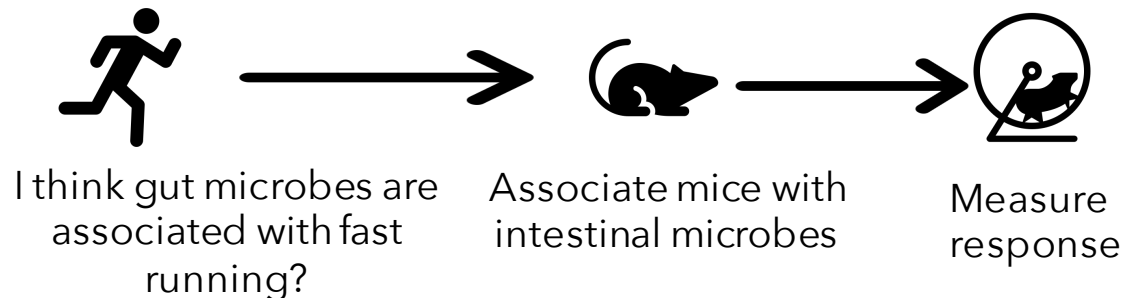
Manager: javonn.musgrove2@chp.edu

Gnotobiotic mice

- Latin for 'known life'
- What does that mean in practice?
 - Germ-free (GF) mice completely lack all culturable micro-organisms, no bacteria, archaeobacteria or fungi.
 - GF mice can be colonized with micro-organisms (single, multi, communities) to test whether a particular organism or microbiome is necessary and sufficient to produce a phenotype.
 - Germ-free or gnotobiotic mice are kept in isolators or bubbles so as to maintain their microbiological status

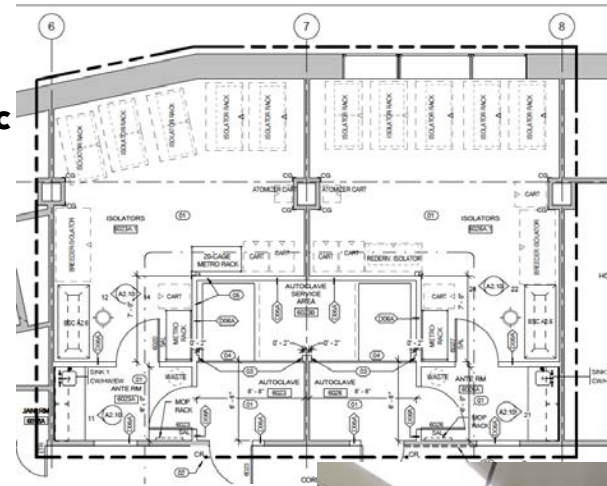


What can you do with gnotobiotic animals? Koch's postulates for the microbiome



What is the 'University of Pittsburgh Gnotobiotic facility'?

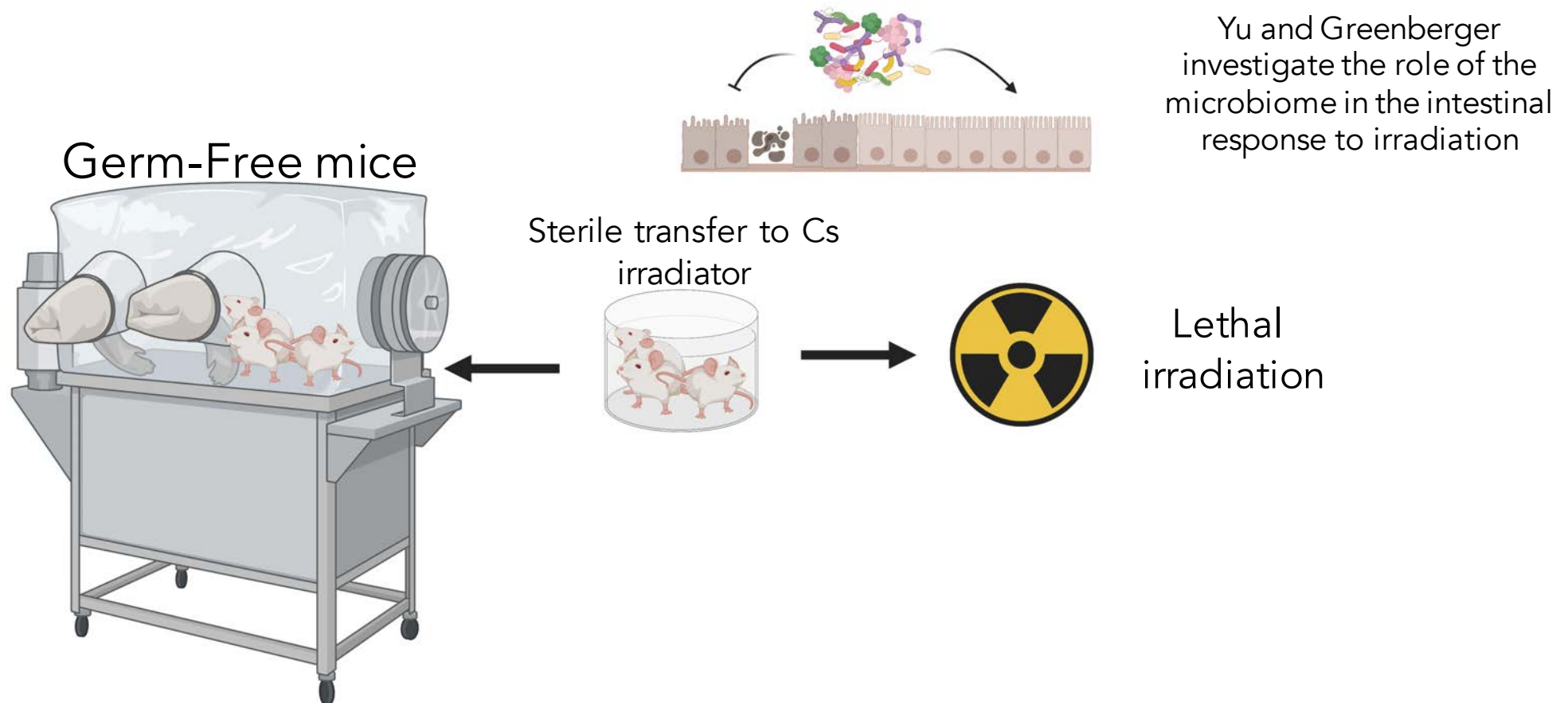
- **In BST3**
 - **Currently have established germ-free C57BL/6 and Balb/c breeding stock.**
 - Serviced by three highly trained staff:
 - Facility Manager - **Javonn Musgrove**
 - Technicians - **Eugenio Alvarez and Abby Mulhorn**
 - **Two Mirrored Facilities**
 - Limits the effect of autoclave breakdowns that cause contamination
 - **We have 10 isolator carts (each with 1-4 bubbles)**
 - 2 breeder isolators (30+ cages)
 - 1 're-derivation' isolator
 - 2 'double' carts (isolators hold 15+ cages)
 - 5 'quad' carts (isolators hold 5 cages)
- Total = 24 experimental isolators (capacity for 20 individual conditions)**



What can the gnotobiotic facility do for your research?

- **Strains in the facility:**
 - Initially, we will breed and provide C57BL/6 and Balb/c.
 - If there is interest we could also acquire Rag1^{-/-} IL-10^{-/-} and Swiss Webster.
- **Services that we will be able to provide:**
 - Javonn Musgrove is highly skilled and has 9+ years experience as technician at NIH, 5+ years in GF facility. He is happy to learn your favorite technique. **Let us say yes to your research.**
 - Facility will be certified as BSL2, **so human samples can be used**
 - We will be able to associate with any mouse microbiome you're interested in (no BSL3/4)
 - Can associate microbiome from **intestine, skin, mouth, lung, urogenital tract, eye**
 - Will be able to **use BSL2 pathogens**
 - We can treat with **drugs, biologics**
 - Can autoclave and administer **custom diets**.
 - We can **adoptively transfer cells**. We can irradiate mice for **bone marrow chimeras**.
 - Can inject **tumors** for cancer treatment studies
 - Our staff can carry out all procedures (we can draw blood/feces etc.).
 - At conclusion of experiments mice can be euthanized within biosafety cabinet and tissues removed to your own facility
 - **BONE MARROW CHIMERAS**

Technical breakthroughs in the Pitt Gnotobiotic facility – Gnotobiotic bone marrow chimeras



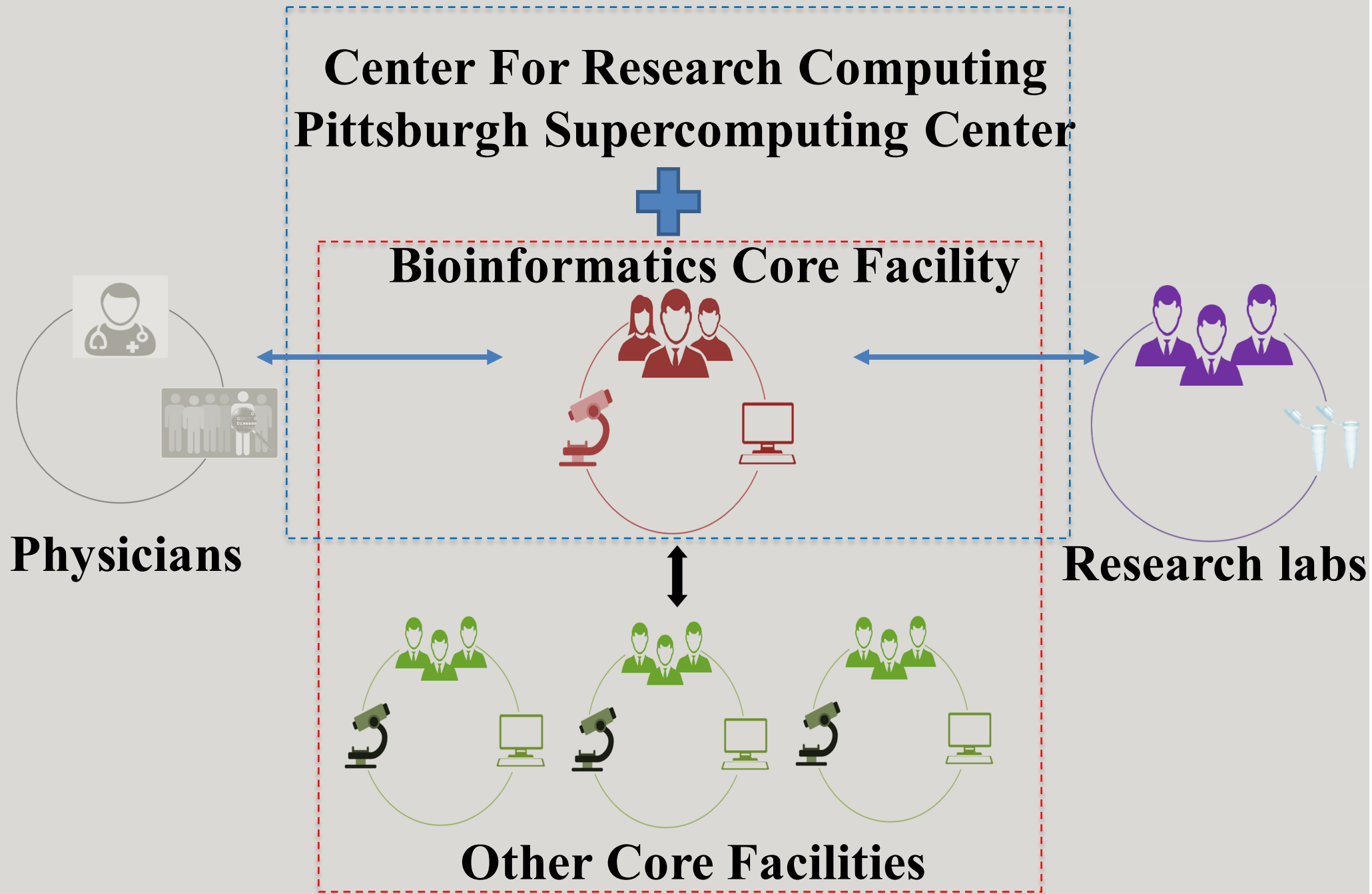
Director: timothy.hand@chp.edu

Manager: javonn.musgrove2@chp.edu

Bioinformatics Core

Director: Dhivyaa Rajasundaram, PhD

Cross-facility workflow



Services offered

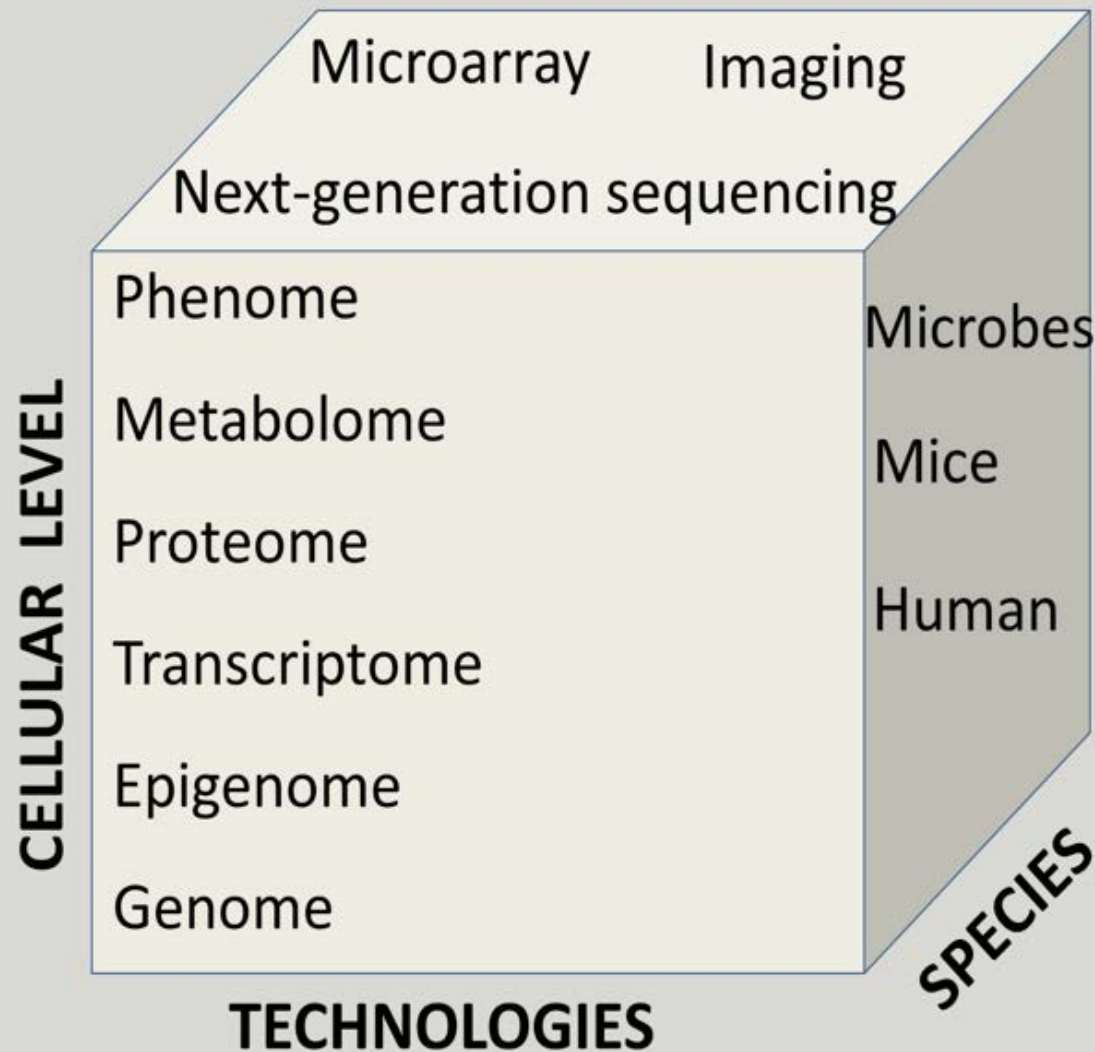


- ☐ Study design implementation
- ☐ High-throughput data analysis
- ☐ Statistical support
- ☐ Proposal writing
- ☐ Faculty collaborations

High-throughput data analysis



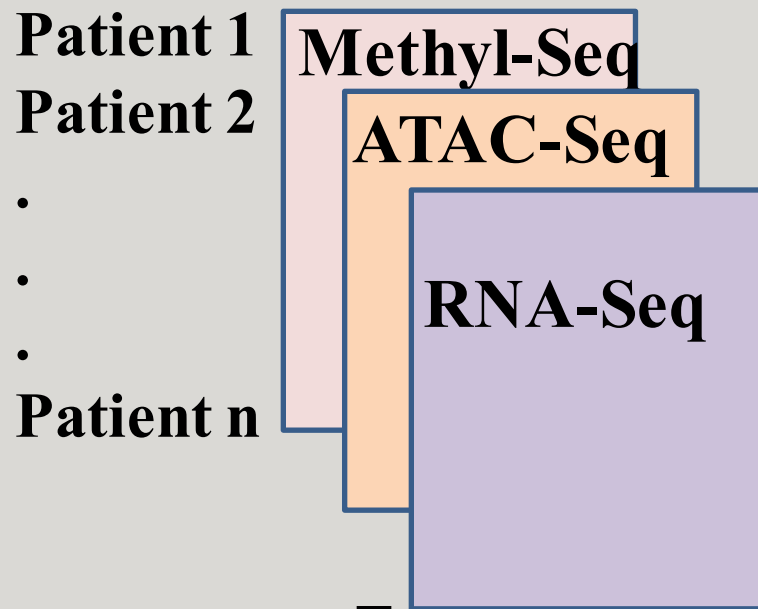
Transform high-throughput data from clinical, translational, and basic research into biologically meaningful information.



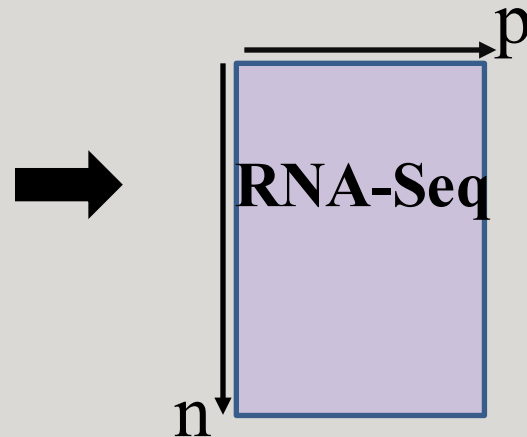
Statistical support



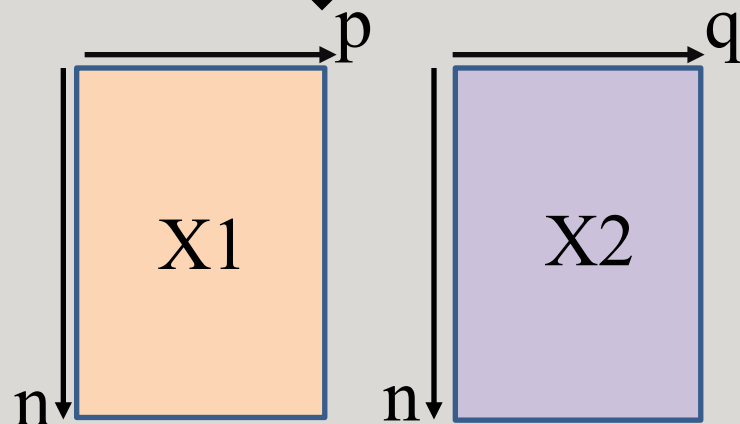
Omics data space



Exploratory data analysis



- ☐ Multivariate-, multiblock-, network-based approaches
- ☐ Machine learning methods for classification and prediction problems
- ☐ Regression approaches for causal modelling



Integrative omics analysis

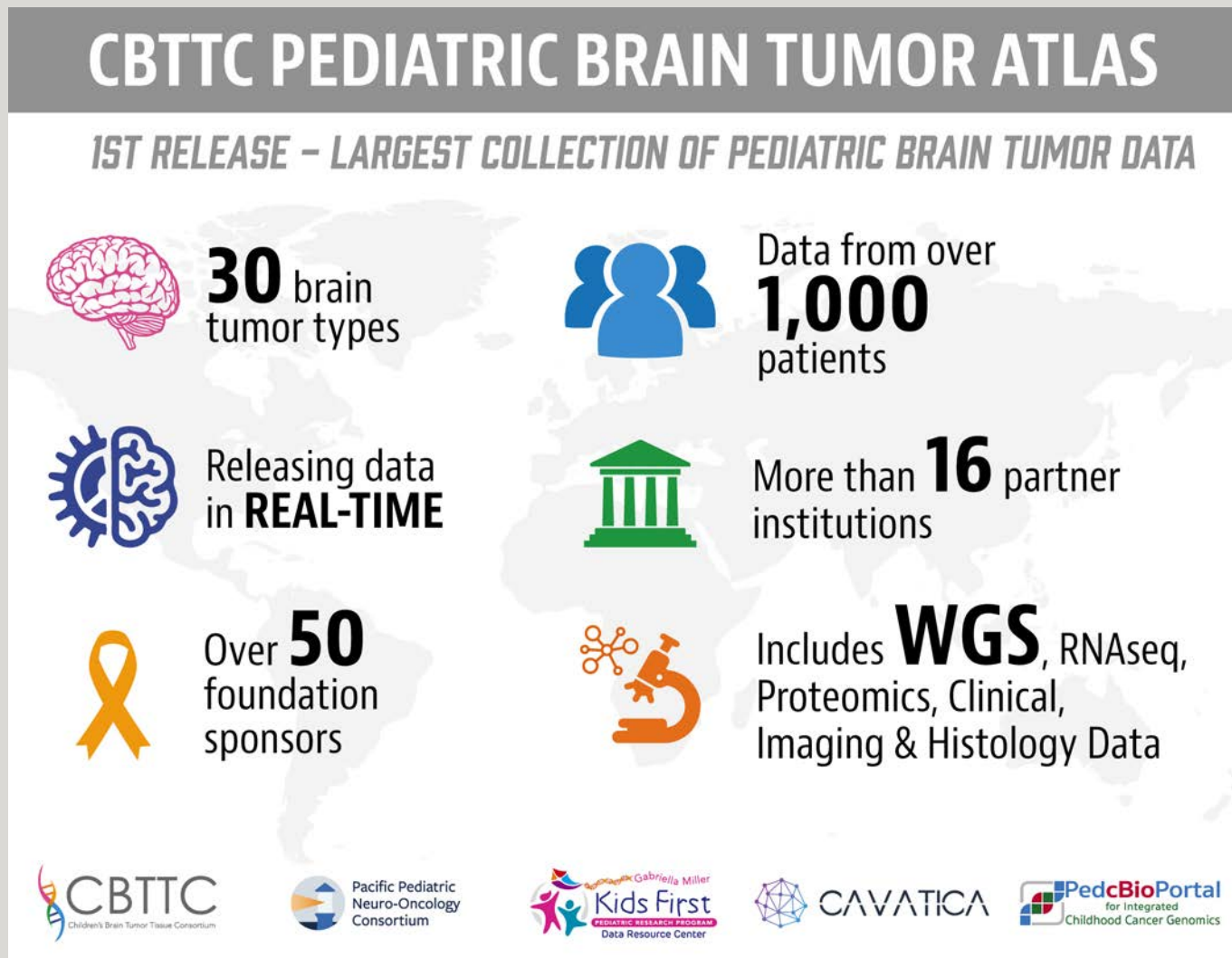
Core users



- ❑ 15 unique users
- ❑ 27 projects completed in fiscal year 2019 - 2020
- ❑ Data submission forms
<https://www.pediatrics.pitt.edu/research/cores-and-research-support/bioinformatics-core>
- ❑ Data transfer – Pittbox/Teams or hard disk

Leveraging publicly available data

- Landscape of tumor infiltrating T-cell repertoire of pediatric brain tumors (using bulk RNA-Seq) - **Dr. Gary Kohanbash**



Training



- ❑ Pitt CRC Fall 2020 workshops
- ❑ Differential expression analysis - Dhivyaa
September 24, 2020 (tentative) (1-4p)
- ❑ Single cell RNA-Seq analysis (beginner) - Dhivyaa
October 20, 2020 (1-4p)
- ❑ Single cell RNA-Seq analysis (Advanced) - Dhivyaa
October 22, 2020 (1-4p)

Cost model



❑ Project based pricing, and percent effort on grants

Project size	Examples	CHP users	Non-CHP users
Small	RNA-Seq analysis	\$1500	\$2000
Medium	Exome-Seq data analysis, CHIP-Seq analysis, ATAC-Seq analysis, Time-course RNA-Seq analysis	\$2500	\$3000
Large	Single-cell sequencing data analysis, T cell repertoire analysis, integrative omics using multivariate statistics	\$4500	\$5000

Practical aspects



- ☐ Do we offer software based (Partek, CLC,..) services?
- ☐ Do we mentor students who are interested to learn their own analysis?
- ☐ Do we have to pay for the analysis even if we do not get the expected result?
- ☐ Could two projects be charged for the price of one because the analysis pipeline is the same?
- ☐ Why do we require authorship?

Rangos Virus Production

Dr. George Gittes

- AAV serotypes 1-10 and DJ. Custom serotypes available as well.
- Adenovirus also available
- Expected titer 10^{13} - 10^{16} vg/ml, volume of 0.5-1.0ml
- Turnaround time 2-3 weeks
- Investigator must have IBC approval prior to virus production
- Fees: Reagents billed to the investigator (~\$1000/virus). Brief subcontract will be arranged to pay salary of the virus producer (~\$700/virus). Commercial virus typically ~\$3000.