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 Prasadan

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

\textit{in vivo, ex vivo}

IVIS, Ultrasound, PET/SPECT/CT, MRI

\begin{itemize}
  \item \textbf{Anatomy} \hspace{2cm} \textbf{Cellular/Molecular}
  \item \textbf{Function} \hspace{2cm} \textbf{Tissue Characterization}
\end{itemize}
Optical \textit{In Vivo} Imaging System (IVIS)

5 Mice

Neurology

Oncology

Inflammation Signaling

Infectious disease

Spectral Unmixing

Good, et. al. \textit{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

PET/CT
F18-FDG

SPECT/CT
mTc99-Sestamibi

Dr. Gladwin

Dr. Campfield
Wu

Dr. Anderson
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>
**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
- Toludine 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:**
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

Dye loading
Furo-4 AM

Disperse islets to single cells

Wash

Glucose stimulation

Low Glucose (2.8 mM) 1 minute
High Glucose (20 mM) 2 min
KCl (20 mM) 1 min

Zeiss LSM 710 confocal
Live cell imaging using Olympus Vivaview
Laser capture imaging
Cell Imaging Core
5th floor room 5148
Contact: Krishna Prasadan
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.
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
## Analyzers

<table>
<thead>
<tr>
<th>Cytometer</th>
<th>Configuration set up</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fortessa</td>
<td>5 lasers</td>
<td>DiVa 8.0.1</td>
</tr>
<tr>
<td></td>
<td>355nm (UV)</td>
<td>Windows 7</td>
</tr>
<tr>
<td></td>
<td>405nm (violet)</td>
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<td>488nm (green)</td>
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<tr>
<td></td>
<td>640nm (red)</td>
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</tr>
<tr>
<td>LSR II</td>
<td>3 lasers</td>
<td>DiVa 8.0.1</td>
</tr>
<tr>
<td></td>
<td>405nm (violet)</td>
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<td></td>
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</table>

## Cell Sorters

<table>
<thead>
<tr>
<th>Cell Sorter</th>
<th>Configuration set up</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aria II SORP</td>
<td>3 lasers</td>
<td>DiVa 8.0.1</td>
</tr>
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## Locations

*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

d) Prior on-line scheduling required

e) Submission of job request and safety disclosure form

f) 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
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.*
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 → 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/
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

1. Extraction Services
   - Cells
   - Biofluid
   - Tissue

2. Quantitation (Qubit) and QC (Tapestation or Fragment Analyzer) Services
   - Drop off: Cells, Biofluids, RNA or DNA

3. Library Generation
   - Drop off: Multiplexed libraries

4. Sequencing Services
   - Illumina Library
   - Next Seq Sequencer
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.hscrp.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, archaeabacteria 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

I think gut microbes are associated with fast running? Associate mice with intestinal microbes Measure response
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

Germ-Free mice

Sterile transfer to Cs irradiator

Lethal irradiation

Yu and Greenberger investigate the role of the microbiome in the intestinal response to irradiation

Director: timothy.hand@chp.edu
Manager: javonn.musgrove2@chp.edu
Cross-facility workflow

Center For Research Computing
Pittsburgh Supercomputing Center

Bioinformatics Core Facility

Physicians

Other Core Facilities

Research labs
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

Omens data space

Patient 1
Patient 2
Patient n

Methyl-Seq
ATAC-Seq
RNA-Seq

Exploratory data analysis

RNA-Seq

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

CBTTC PEDIATRIC BRAIN TUMOR ATLAS

1ST RELEASE – LARGEST COLLECTION OF PEDIATRIC BRAIN TUMOR DATA

- 30 brain tumor types
- Data from over 1,000 patients
- Releasing data in REAL-TIME
- More than 16 partner institutions
- Over 50 foundation sponsors
- Includes WGS, RNAseq, Proteomics, Clinical, Imaging & Histology Data
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

<table>
<thead>
<tr>
<th>Project size</th>
<th>Examples</th>
<th>CHP users</th>
<th>Non-CHP users</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>RNA-Seq analysis</td>
<td>$1500</td>
<td>$2000</td>
</tr>
<tr>
<td>Medium</td>
<td>Exome-Seq data analysis, CHIP-Seq analysis, ATAC-Seq analysis, Time-course RNA-Seq analysis</td>
<td>$2500</td>
<td>$3000</td>
</tr>
<tr>
<td>Large</td>
<td>Single-cell sequencing data analysis, T cell repertoire analysis, integrative omics using multivariate statistics</td>
<td>$4500</td>
<td>$5000</td>
</tr>
</tbody>
</table>
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.