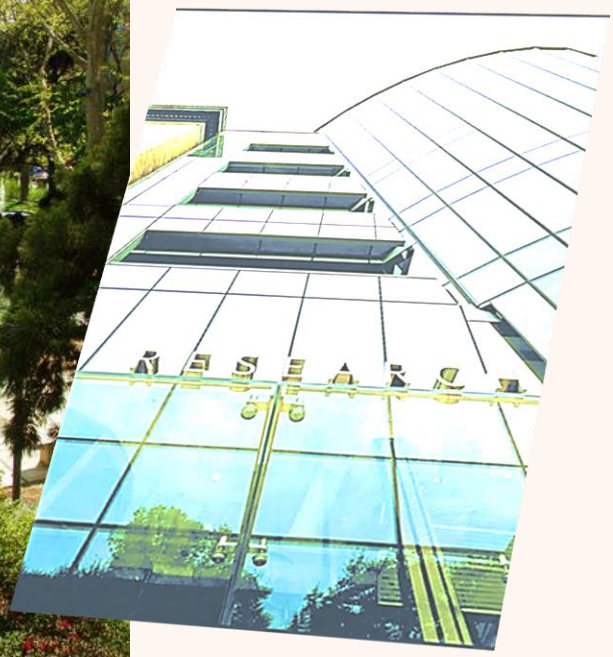
Introduction to The Rockefeller University Scientific Resource Centers



Overview for New Graduate Fellows

September 8, 2022

The Role of Core Facilities

- Ensuring and encouraging access to expertise, equipment, services and products that are beyond the financial or technical means of most individual laboratories
- Improving competitiveness for researchers and the University
- Training and education in specialized methods and technologies
- Supporting research in a cost effective and scientifically effective manner
- Providing continuity of staffed expertise
- Serving as scientific brokers both internally and externally

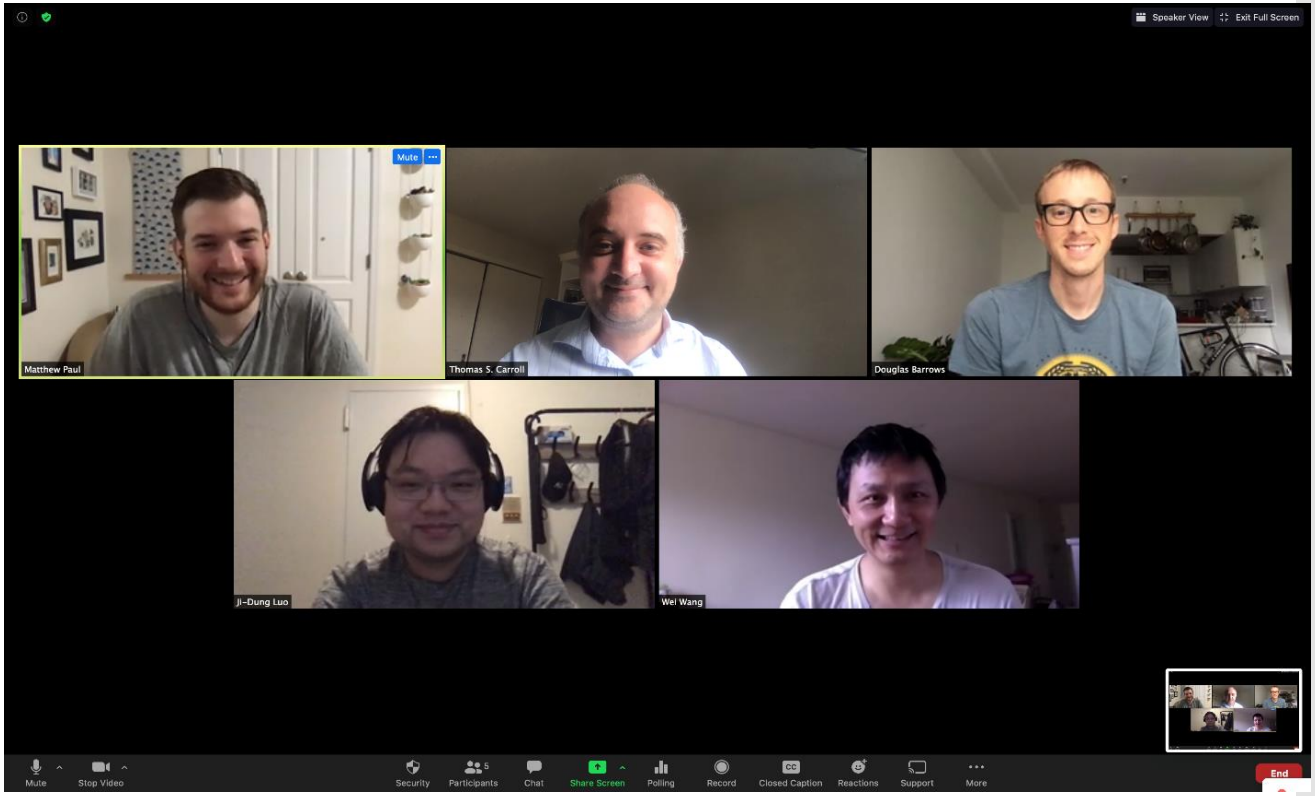
Bioinformatics Resource Center

Who are we?

- Thomas Carroll
- Ji-Dung Luo
- Matt Paul
- Wei Wang
- Doug Barrows

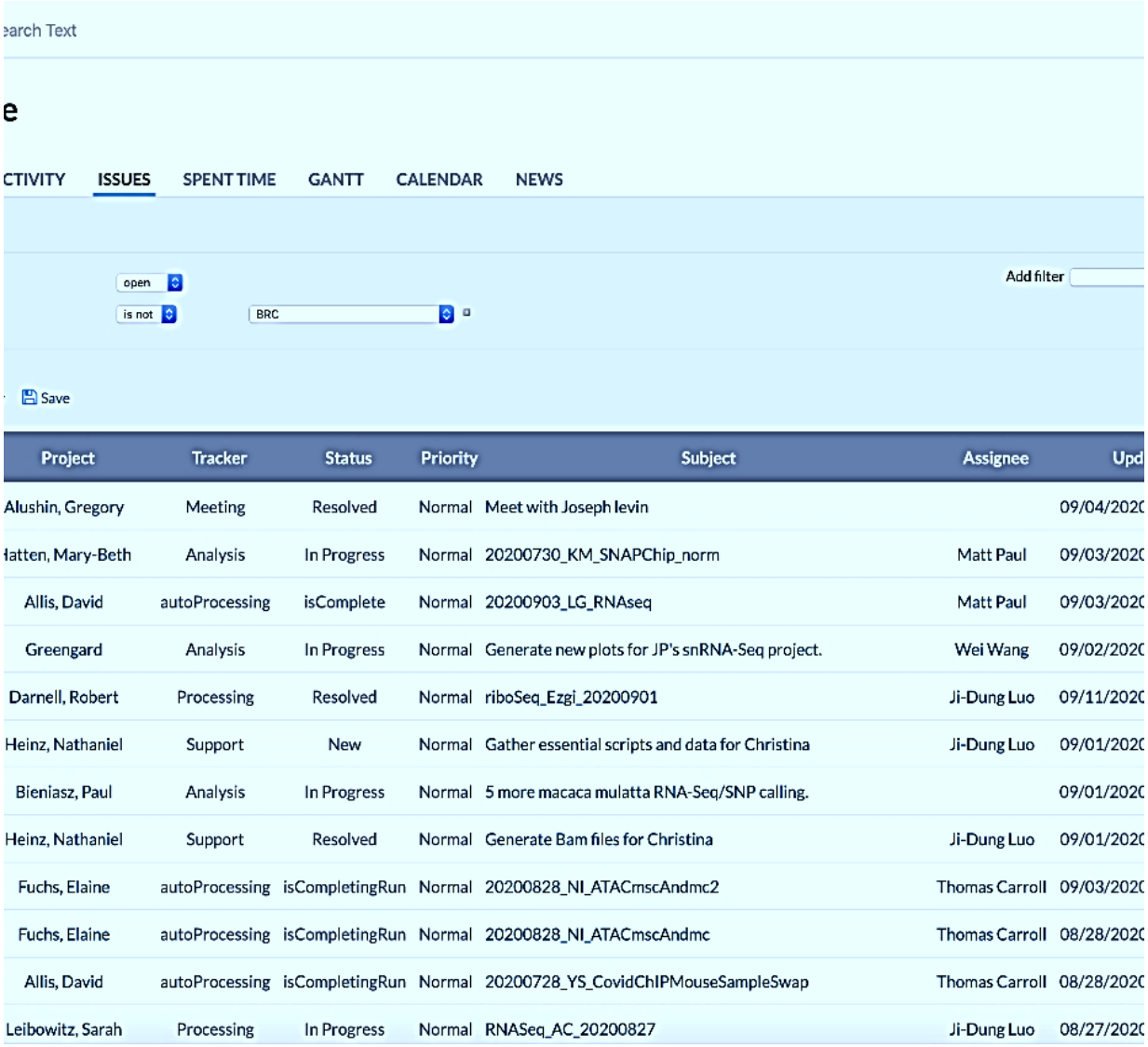
What do we do?

- Analysis.
- Training
- Software



Bioinformatics Resource Center

- Bioinformatics Analysis
- Types of analysis
- High-throughout sequencing (RNA-seq, ChIP-seq, ATAC-seq, Ribo-seq, scRNA-seq, CLIP-seq, WGS)
- Image analysis.
- ~ 38 groups, 1000 bioinformatics projects in 3 years.
- 1 to 1 analysis/training.



Project	Tracker	Status	Priority	Subject	Assignee	Upd
Alushin, Gregory	Meeting	Resolved	Normal	Meet with Joseph levin		09/04/2020
Hatten, Mary-Beth	Analysis	In Progress	Normal	20200730_KM_SNAPChip_norm	Matt Paul	09/03/2020
Allis, David	autoProcessing	isComplete	Normal	20200903_LG_RNAseq	Matt Paul	09/03/2020
Greengard	Analysis	In Progress	Normal	Generate new plots for JP's snRNA-Seq project.	Wei Wang	09/02/2020
Darnell, Robert	Processing	Resolved	Normal	riboSeq_Ezgi_20200901	Ji-Dung Luo	09/11/2020
Heinz, Nathaniel	Support	New	Normal	Gather essential scripts and data for Christina	Ji-Dung Luo	09/01/2020
Bieniasz, Paul	Analysis	In Progress	Normal	5 more macaca mulatta RNA-Seq/SNP calling.		09/01/2020
Heinz, Nathaniel	Support	Resolved	Normal	Generate Bam files for Christina	Ji-Dung Luo	09/01/2020
Fuchs, Elaine	autoProcessing	isCompletingRun	Normal	20200828_NI_ATACmscAndmc2	Thomas Carroll	09/03/2020
Fuchs, Elaine	autoProcessing	isCompletingRun	Normal	20200828_NI_ATACmscAndmc	Thomas Carroll	08/28/2020
Allis, David	autoProcessing	isCompletingRun	Normal	20200728_YS_CovidChIPMouseSampleSwap	Thomas Carroll	08/28/2020
Leibowitz, Sarah	Processing	In Progress	Normal	RNASeq_AC_20200827	Ji-Dung Luo	08/27/2020

Bioinformatics Resource Center

https://rockefelleruniversity.github.io

Training

- Publicly available site
- Automatically tested on current R and latest software versions.
- Autocompilation of material covering 1000 slides, 200 pages.
- Training clinics alongside courses.
- > 150 people over last two parallel zoom sessions.

Software

- Develop internal and on demand software
- Internal
  - NgsPipeR – Custom pipeline for analysis of HTS data. (> 5000 samples)
  - Profileplyr, Rfastp, Herper* released(*ing) in Bioconductor.
- On demand
  - Shiny/R tools ranging from simple lab calculations to complex visualisations.
  - R/C++ package for high throughput processing of custom sequencing types.

High Performance Computing Resource Center

Contact us when your laptop isn't keeping up

- Computing resource for data intensive science and scale-out workloads
  - Compute: 335 TFLOP/sec: CPUs (5,012 cores) and GPUs (64 nVidia)
    - Min 256 GB RAM/node; some specialty configurations (3 TB RAM, large-ish NVMe)
    - Additional 1,280 cores and 24 GPUs (4xA100, 20xA10) on order for this year's R&R
  - Data Storage: 8.9 PB high performance storage and 3.5 PB cold storage (Data Park)
  - Networking: 56 Gbit/sec low-latency internal network
  - Batch (slurm) and interactive (OOD) use
- Combination of shared and lab-dedicated resources

OPEN OnDemand

R jupyter python Wolfram Mathematica SCHRÖDINGER. PyMOL RELION

How to access HPC

Shared

- No cost (this year) for computing
- Pay for (annual) storage allocation
  - Scratch: $16/TB/mo
  - Backed up: $28/TB/mo

Dedicated

- Host your lab's node for a one-time setup fee + monthly hosting ($625 + $36+/mo.)

Data Park

- $6/TB/mo

it_hpc@rockefeller.edu


Jason

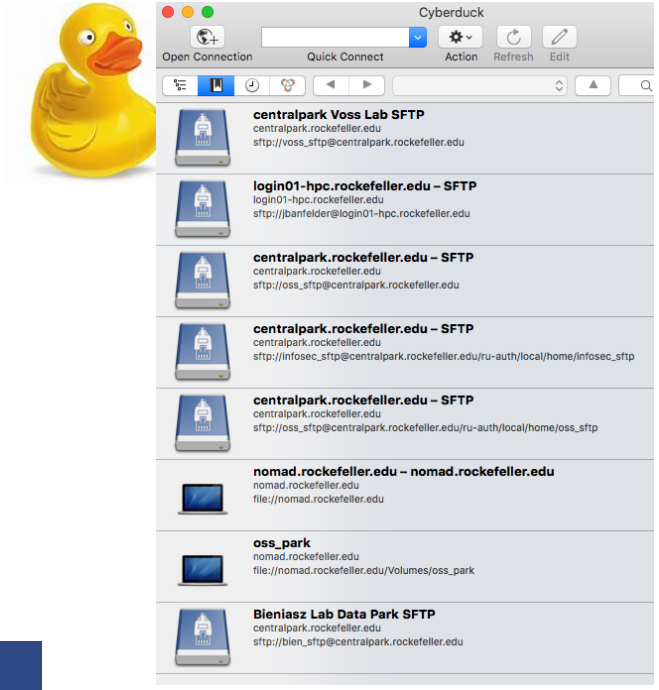

Rebecca


Bala


Logan

Other Data Storage and Transfer Considerations

- RUFS
  - Windows/Mac (SMB) or NFS
  - Includes snapshots and replication
- Data Park
  - Available via sftp (cyberduck, lftp)
  - “cheap, deep, and simple”
    - ca. 1/3 the cost of other options
- Other Cloud (e.g. Amazon Glacier, Wasabi)
  - Be aware of bandwidth, file size limitations, and especially egress fees.
- Extramural (and intramural) collaboration
  - Campus DropBox subscription for “small” data volume
  - Globus for large data volumes
  - Good command-line tools: `rclone` and `lftp`
- Data curator accounts
- Protected or regulated data (e.g. HIPAA)?



Services at the Genomics Resource Center

- Next-generation sequencing
  - Whole genome and whole exome sequencing
  - Targeted sequencing
  - RNA-Seq: Transcriptome analysis
  - Small RNA seq: small RNA discovery and quantitation
  - ChIP-Seq/CUT&Run/CUT&Tag: Protein-DNA binding
  - Methyl-Seq: epigenetic analysis of DNA methylation
  - Ribo-Seq: Ribosome profiling for active translation
  - TRAP-Seq: cell type specific mRNA profiling
  - ATAC-Seq: chromosomal accessibility
  - CROP-Seq: pooled CRISPR screening with single-cell transcriptome readout

NovaSeq 6000

NextSeq 500

MiSeq

- 10 x Genomics Chromium Single Cell System
  - Single cell gene expression
  - Single cell full-length V(D)J profiling
  - Single cell ATAC-Seq
  - Single cell RNA Seq with CRISPR Screening

10X Genomics Chromium

Coutess 1 and 3

- Realtime PCR systems
  - Individual gene expression analysis
  - Pathway analysis
  - Individual SNP genotyping

QuantStudio 12K-flex

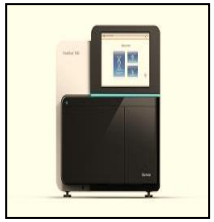
- Others
  - Covaris Ultrasonicator
  - Agilent Bioanalyzer
  - Agilent TapeStation
  - Qubit fluorometr
  - NanoDrops
  - PCR machines 96/384-well

Sequencer Selection

NovaSeq

- ATAC-Seq
- Whole genome and Whole exome sequencing
- Paired-end RNA-Seq
- Large scale RNA-Seq
- Single cell RNA-Seq

800 million to 10 Billion reads



NextSeq

- Small scale RNA-Seq up to 16 samples
- ChIP-Seq
- Small RNA Seq

400 million reads

MiSeq

- PCR Amplicon Sequencing
- 16S rRNA Metagenomic Sequencing as300bp x 2
- Library prep method development
- Library QC

1 – 20 million reads

Transcriptome Sequencing (RNA-Seq)

- mRNA sequencing
  Start with 100 ng total RNA
  Gene expression and splicing analysis of coding RNA
- Total RNA sequencing (with rRNA depletion)
  Start with 100 ng Total RNA
  Gene expression analysis of coding and non-coding RNA
- Low-input mRNA sequencing
  Start with as low as 100 pg – 1 ng total RNA
- Ribo-profiling
  Which mRNA are actively translated
- TRAP-Seq
  Cell type specific mRNA expression
- Single Cell RNA sequencing
  mRNA profiling at single cell level

Genomics Resource Center

WRB 723 24/7 access

www.rockefeller.edu/genomics

genomics@rockefeller.edu

Bin Zhang
Research Support Specialist

Christine Lai
Research Support Specialist

Connie Zhao, Ph.D.
Director

Hong Duan, PhD
Research Support Specialist

Jackie Woodruff
Research Support Assistant

Bioinformatics Resource Center

- Tom Carroll
- Wei Wang

Proteomics Resource Center

Analytical Mass Spectrometry applied many different questions, but mainly metabolomics and proteomics. 600+ projects yearly from ~200 users.

How we work:

- We share our expertise with you and your project.
- 'Full Service' model - you drop-off samples*.
- If needed, we help you understand the data.

https://www.rockefeller.edu/proteomics/

Inbox (13,629) - henrik.molina@... | The Rockefeller University » Sam...

rockefeller.edu/proteomics/sample-preparation/

Apps | Thermo Fisher Scie... | plims | Web Content Mana... | Inside The Rockefell... | New Tab | Forms and Policies... | https://formspolici... | Other bookmarks

THE ROCKEFELLER UNIVERSITY
Science for the benefit of humanity

INSIDE ROCKEFELLER | CALENDAR | DIRECTORY | CAREERS | GIVE

Our Scientists | Research | Education & Training | News | Events & Lectures | About | Support Our Science

Phase III Operations: The University is open for expanded research operations; only authorized personnel will be admitted on campus. More info here.

Sample Submission

For most sample submissions it is beneficial to discuss the experiment, goals and expectations and we therefor encourage users to arrange, ahead of time, an appointment. For peptide synthesis we suggest to fill out our Custom Peptide and Peptide Library Submission Form.

For a user opting to ship/mail samples, please use our mailing address and share tracking number if available.

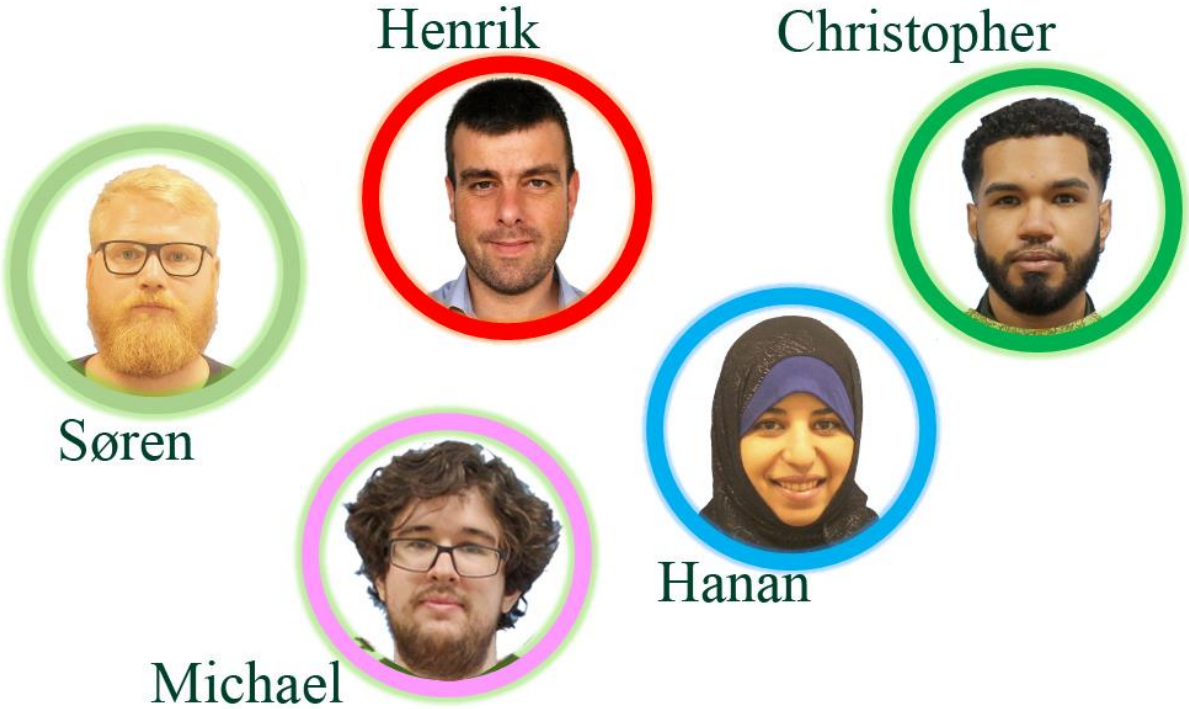
All samples are logged and labelled with a tracking number which typically begins with P (for peptides) or MS (for mass spec experiments) followed by a number where the two first digits indicate the Fiscal Year. Internal users can track the progress/status for submitted requests here. When contacting the PRC re. past analyses, please refer to the P or MS number.

Internal Rockefeller users can access pLIMS sample information, progress, and final invoices using the link below.

SAMPLE STATUS

Proteomics Resource Center

- OVERVIEW
- TYPICAL PROJECTS
- SAMPLE SUBMISSION
  - Pr...
  - Us... mbers
  - Quality Control & Amounts (Proteomics)
  - Reduction & Alkylation
  - Sample Pr... ration Guidelines
  - Wo... etergents
  - Data & Analysis
- USER FEES
- SELECTED TECHNOLOGIES & TOOLS
- NEWS & ANNOUNCEMENTS
- USEFUL LINKS


Henrik
Christopher
Søren
Hanan
Michael

GLASSWASHING SERVICES

CRC Room CO2E
(Greenberg Building)
7AM-3PM
http://www.rockefeller.edu/glasswashing/

PIPETTE CALIBRATION CLINIC

Clean, Repair, Replace seals, O-rings, and Calibrate. Monthly on the second Tuesday of each month.

Rainin professional technicians preform all service including hands on training on proper pipetting techniques.

Staff

- Angela Howell
- Lourdes Mathew
- Khalil Koiner
- Beverley Guthrie-Turenne
- Derek Boadie-Ansah

Lourdes

Angela

Estrella

Khalil

Beverley

glasswashing@rockefeller.edu

Fisher Drug Discovery Resource Center
DWB 216
http://inside.rockefeller.edu/htsrc

The DDRC provides scientific guidance, technical support and access to instrumentation in the following areas:

- assay cascades used in drug discovery projects
- miniaturized assay development
- high throughput liquid handling
- high-throughput screening
- Access to a large compound library (430K) for drug/tool compound discovery
- Discovery of tool compounds or compounds for drug discovery
- measuring the kinetics and thermodynamics of biomolecular interactions

How Is An HTS Project Accomplished?

1 assay/many compound classes

Chemical library

Nanoliter Compound delivery into microtiter plates using nanosyringes or pin-tools

Biological assay

Biological assays are developed using cells, cell extracts, enzymes, purified proteins, antibodies. Microtiter plates are filled with liquid-handling robots

Read assay

Assays are read using optical microplate readers or automated microscope

Database

Results entered into a database with software allowing for sub-structure /similarity searching and comparison of assay results. Hits are selected

Concentration-response

Compounds picked and re-tested for EC50 determination

Secondary assays

HPLC-MS

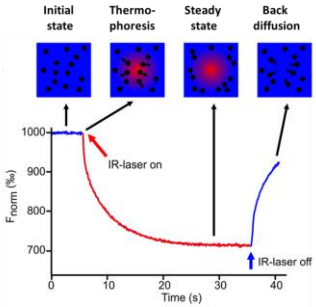
Many assays/ few compound classes

Biophysical Techniques for Binding Measurements

Microscale Thermophoresis: Nanotemper MST

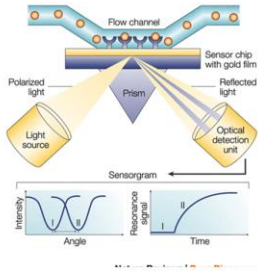
Principle

Molecules migrate along a temperature gradient at different rates depending upon size, shape (hydration shell) and charge thus allowing differentiation between unbound and bound state



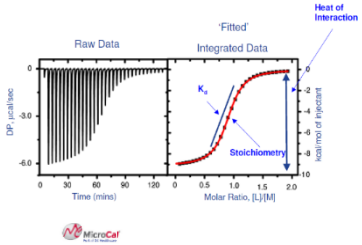
Surface Plasmon Resonance : Proteon XPR

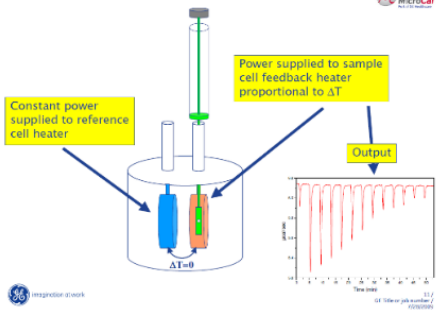
20-50ugs Protein
Immobilized to Gold Sensor Chip
Measures on-rate and off-rate
Immobilization through free-amines, antibody, biotin



Isothermal Calorimetry

- Label-free
- Autosampler
- 400uL/1 mg protein per experiment



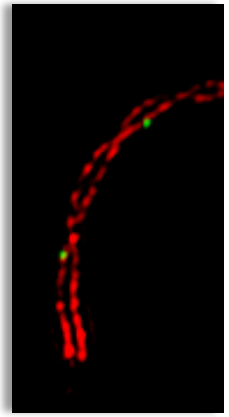


HTSRC Staff

- Fraser Glickman, Ph.D., *Director*
- Chloe Larson, B.S., *Research Support Assistant*
- Timothy McGinn, Ph.D., *Research Support Specialist*
- Ilona Nudelman, Ph.D., *Research Support Specialist*
- Victor Bustos, Ph.D., *Research Support specialist*

Bio-Imaging Resource Center

- Advice on microscopy and specimen preparation
- Training on the microscopes
- Training in image analysis
- Initial consultations and tours
- Possible collaborations



Widefield

Light Sheet

TIR
F

Super Resolution

Confocal

Spinning disk confocal

Deconvolution

Multiphoton

FRET

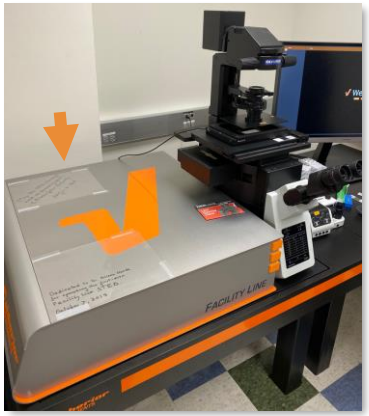
FLIM

FCS

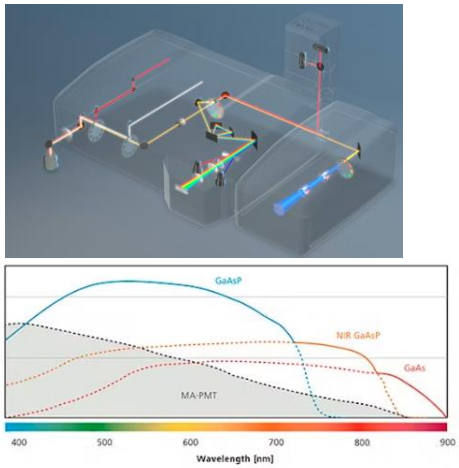
Laser microdissection

We have the most comprehensive set of super-resolution systems of any regional imaging facility

- 3D-SIM (OMX, GE)
- iSIM (VisiTech)
- STORM/PALM (Nikon)
- Airyscan (Zeiss)



The first Abberior Instruments Facility Line STED system installed worldwide, personally signed by Stefan Hell, the Nobel laureate who invented the STED technique.



One of the first LSM980 installed locally which is equipped with two PMTs, one 32CH-GaASP detectors, two NIR detectors and the Airyscan2.

Staff of the BIRC

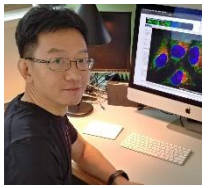
(DWB 201-203)
http://inside.rockefeller.edu/bioimaging/

Alison

- **Alison North** (Senior Director) - Ph.D. in Cell Biology - any microscope I can get my hands on in between doing the less exciting administrative stuff, plus OMX specialist;
- **Christina Pyrgaki** (Operations Manager) - Ph.D. in Molecular Biology – all types of microscopy;
- **Tao Tong** (Research Support Specialist) - Masters in Computing and in Biochemistry/Molecular Biology – systems administration, image analysis;
- **Priyam Banerjee** (Research Support Specialist) – Ph.D. in Cell Biology, all types of microscopy;
- **Bridget Biersmith** (Research Support Specialist) – Ph.D. in Cell Biology – all types of microscopy.
- **Ved Sharma** (Image Analyst) – Ph.D. in ??? – advanced image analysis
- **Behzad Khajavi** (Optical Engineer) – Ph.D. in Physics – imaging system design and fabrication

Christina

Ved



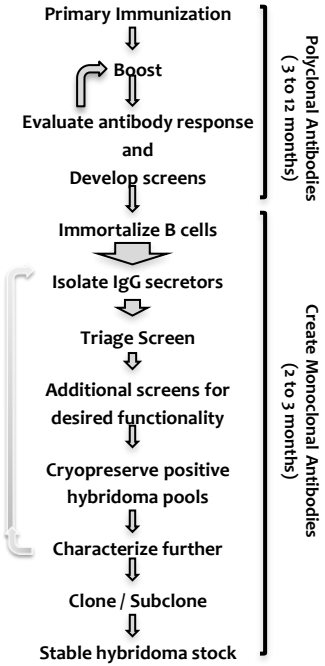
Tao

Priyam

Bridget

Behzad

Antibody and Bioresource Core Facility



Custom MAb Development

- Comprehensive project design and management
- Generate robust humoral response
  - Immunogens: Cells, protein, peptides, and other haptens
  - Hosts: Mice (WT/KO), hamsters, and rats
- B cell immortalization (10's of thousands of hybridomas)
- Isolate IgG secreting hybridomas (100's pf hybridomas)
- Screen hybridomas by ELISA, IF, Flow Cytometry, WB …
- Cryopreserve antigen specific hybridomas
- Clone hybridoma cultures to establish stable lines
- Advise on further characterization and validation

Antibody and Bioresource Core Facility

Mycoplasma Testing

- Weekly
- Cell culture media

Cell Line Distribution Services

- Alleviates the work of distributing cell lines (e.g. MTA, validation, shipping logistics)
- Promotes reproducibility of data
- Submit to Cellosaurous and obtain a new RRID
- Informational and physical repository for published cell lines
- Hybridomas & cell lines (e.g. tumor and stem cell)

Antibody and Bioresource Core Facility

Anthony Yasmann	New MAbs
Anne Mui	Administrative Assistant
Lou Mattera	New MAbs
Brendan Buehler	Cell Line Distribution
Frances Weis-Garcia	Head

Bronk 415
x 7030

ZRC 1553
646-888-2331

skiabcf@mskcc.org

FCRC Location and Staff

Detlev W. Bronk Laboratory (DWB), Rooms 205 - 211

http://www.rockefeller.edu/fcrc/ Rooms 306 - 308

- **Svetlana Mazel**
  - Director
  - mazels@rockefeller.edu
  - #7656

- **Songyan Han**
  - Senior Research Support Specialist
  - shan@rockefeller.edu
  - #7657

- **Stanka Semova**
  - Operations Manager
  - ssemova@rockefeller.edu
  - #7657

- **Samer Shalaby**
  - Research Support Specialist
  - sshalaby@rockefeller.edu
  - #7657

?

- **Brandon Yoo**
  - Information Technology
  - Senior Computer Support Specialist

FCRC Services

On Equipment

- **Staff-operated equipment at FCRC**
  - Cell sorting on three BD FACSAria (cell sorters)
  - Data acquisition on the ImageStream-X (imaging

- **All the Flow Cytometry equipment at FCRC**
  - Maintenance
  - Quality control testing
  - Troubleshooting and minor repairs
  - Communication with the vendors for appropriate service
    - Service request
    - Post-service follow-up
  - Data management
- **Equipment and computer/programs upgrades and acquisitions**
  - Proposals
  - Installations with sufficient quality controls

Education and Training

- **"Beyond the Basics" Flow Cytometry Class**
  - In groups of 5-15 people
  - Once in 8-10 weeks
- **Pre-Sort and Sample Preparation Overview Session**
  - In groups of 3-5 people
  - Once in 4-6 weeks

- **Instrumental Training "Hands-on"**
  - Personal sessions scheduled based on request
  - Hands-on trainings
  - Help with instrument setup and troubleshooting
- **Software Troubleshooting**

Consultation and Help

- **Consultations**
  - Experimental Design
  - Pre-Sort
  - Pre-ImageStream
  - Pre-Hands-On

- **Troubleshooting**
  - On experimental design
  - On the instruments

- **Help and Assistance with:**
  - Data analysis
  - Data preparation for the publication and scientific presentations
  - Experiments to produce preliminary data for proposals and grant applications
  - Letters of support for proposals and grant applications

Instruments and Analysis Stations (09/2022)

		Instrument	Year of Manufacture	Location	Hours of operation	Fee $/hour	Purpose of use: Cell Sort	Purpose of use: Data Acquisition	Purpose of use: Data Analysis	Purpose of use: Data Storage	Lasers (excitation beam); Pre-set configuration (nm): I Laser	II Laser	III Laser	IV Laser	V Laser	VI Laser	Number of fluorescent detectors (by laser)
CELL SORTERS		BD FACSAria-1	2005/ 2010	DWB207	extended business hours	144	√	√	√	√	405	488	561	640			13 (3/3/4/3)
CELL SORTERS		BD FACSAria-2	2008	DWB207A	extended business hours	144	√	√	√	√	355	405	488	561	640		16 (2/4/3/4/3)
CELL SORTERS		BD FACSAria-3	2010	DWB207	extended business hours	144	√	√	√	√	355	405	445	488	561	640	18 (2/4/2/3/4/3)
CELL SORTERS		Sony MA900	2019	DWB211	extended business hours	144 / 72	√	√	√		405	488	561	640			12
ANALYZERS	SPECTRAL	Cytek Aurora-1	2018/ 2019	DWB308	24/7/365	55		√	√		355	405	488	561	640		64 (16/16/14/10/8)
ANALYZERS	SPECTRAL	Cytek Aurora-2	2020	DWB308	24/7/365	55		√	√		355	405	488	561	640		64 (16/16/14/10/8)
ANALYZERS	ADVANCED	BD LSR II-1	2004/ 2009	DWB211	24/7/365	55		√	√		355	405	488	561	640		16 (2/4/3/4/3)
ANALYZERS	ADVANCED	BD LSR II-2	2007/ 2011	DWB211	24/7/365	55		√	√		405	445	488	561	640		16 (4/2/3/4/3)
ANALYZERS	ADVANCED	BD LSR-Fortessa	2014	DWB211	24/7/365	55		√	√		355	405	488	561	640		18 (3/5/3/4/3)
ANALYZERS	ADVANCED	ThermoFisher Attune NxT	2018/ 2020	DWB211	24/7/365	39		√	√		405	488	561	640			14 (4/3/4/3)

	Analysis Workstation	Location	Hours of operation	Software
PC	AnalysisPC1	DWB306	24/7/365	FACSDiVa 8.0.1, FCAP Array 1.0.2
PC	AnalysisPC2	DWB306	24/7/365	FCS Express 4, FlowJo 7.6.5/10.0.5, Legendplex 8, IDEAS 6.2, CFlow Sampler 1.0, FCAP Array 1.0.2, ModFit LT 3.3.11
PC	AnalysisPC3	DWB211	24/7/365	IDEAS 6.2 (Analysis software for ImageStream-X files), FCS Express 7
PC	AnalysisPC5	DWB211	24/7/365	Sony Cell Sorter Software
PC	Aurora-Client1	DWB306	24/7/365	SpectroFlo 3.0.3, FlowJo 7.6.5/10.4, FCS Express 6/7, Sony Cell Sorter Software
PC	Aurora-Client2	DWB306	24/7/365	SpectroFlo 3.0.3, FlowJo 7.6.5/10.4, FCS Express 7, Sony Cell Sorter Software

Small Tools which Help to Improve the Sample Preparation

- **Rebel Hybrid Microscope**
  by Echo a Bico Company – FY19
  with Cell Counting module

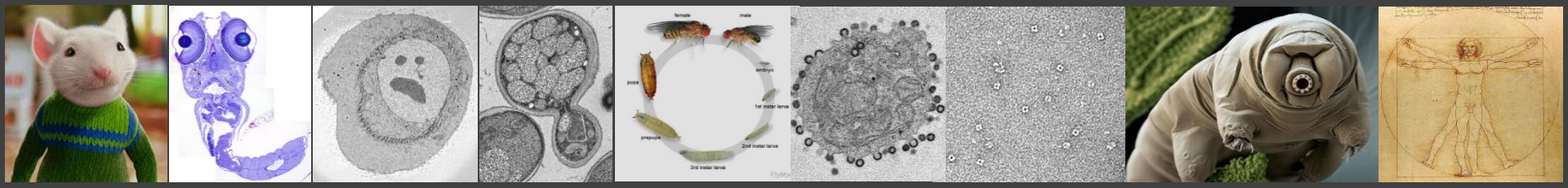
- **Revolve Hybrid Microscope (with fluorescence capabilities)**
  by Echo a Bico Company – FY22
  with Cell Counting module

- **The gentleMACS™ Octo Dissociator with Heaters**
  (by Miltenyi Biotech) - FY21

Benchtop instrument for the fully automated and standardized tissue dissociation or homogenization of up to eight samples

- **Laminar Wash HT2000 System** (by Curiox) – FY22
  - Better cell recovery than centrifugation
  - Greater reproducibility
  - Higher stain index for cleaner resolution of populations
  - Less cell clumping and the subsequent clogging of FCM
  - Faster workflows that can completely wash 96 samples in just 5 minutes

Electron Microscopy Resource Center



We can do EM for a variety of experimental models!!

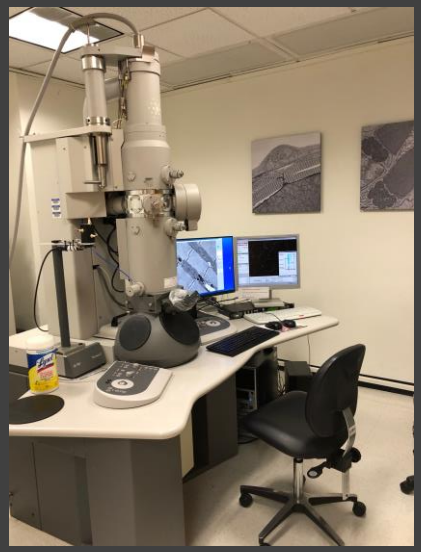
Hilda Amalia Pasolli, Ph. D
Director-Research Associate Professor
apasolli@rockefeller.edu

Anurag Sharma Ph.D.
Senior Research Support Specialist
asharma02@Rockefeller.edu

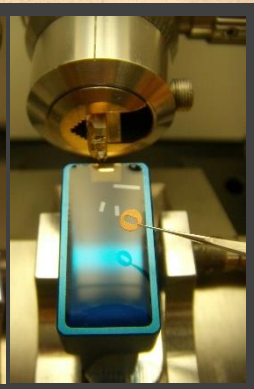
RRB 120-130
http://inside.rockefeller.edu/emrc/

How do we do EM?

NEW!!

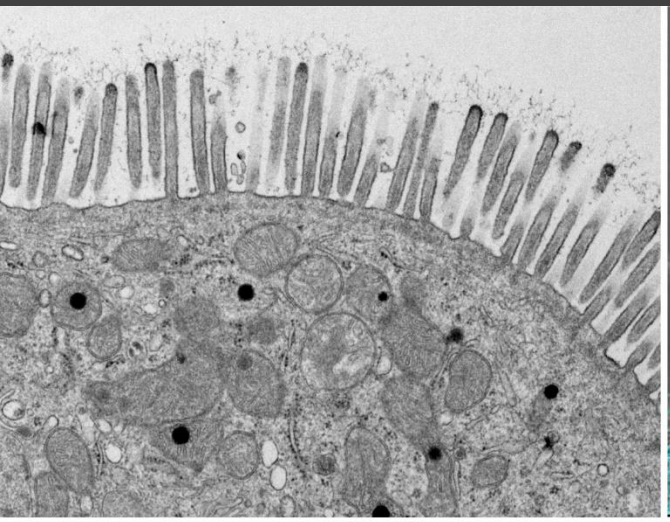





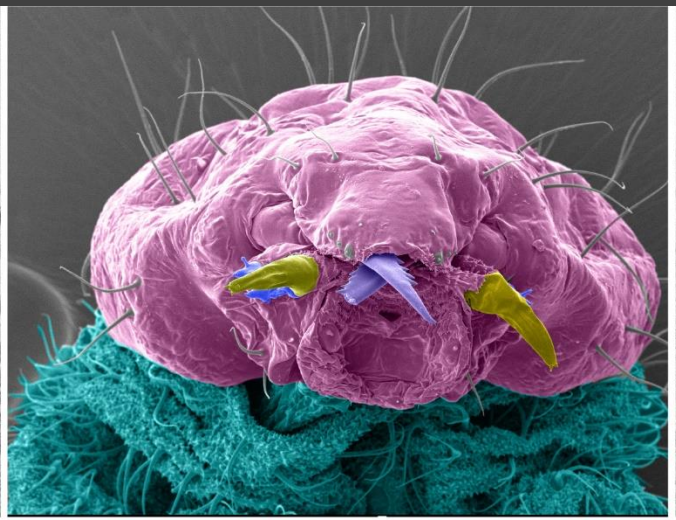


- Sample preparation for Transmission and Scanning Electron Microscopy.
- Development of EM protocols according to the scientist needs.
- Immuno-labeling (colloidal gold, HRP, APEX)
- Correlative light-electron microscopy (CLEM)
- Training in the use of equipment and techniques
- Interpretation of images

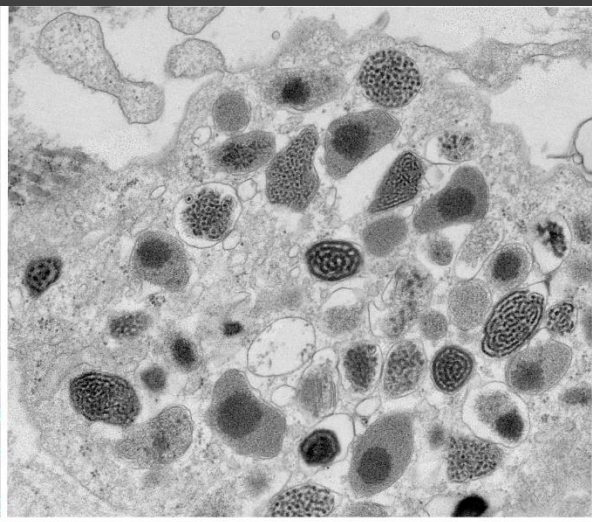
Examples of our work



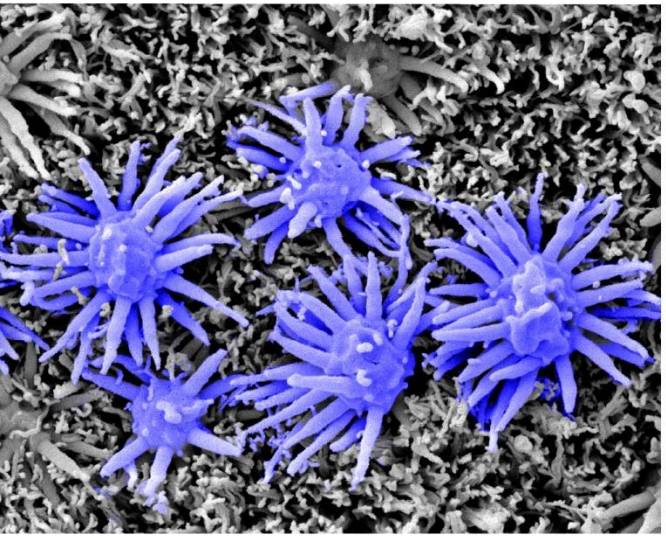
Microvilli with glycocalix-Colon



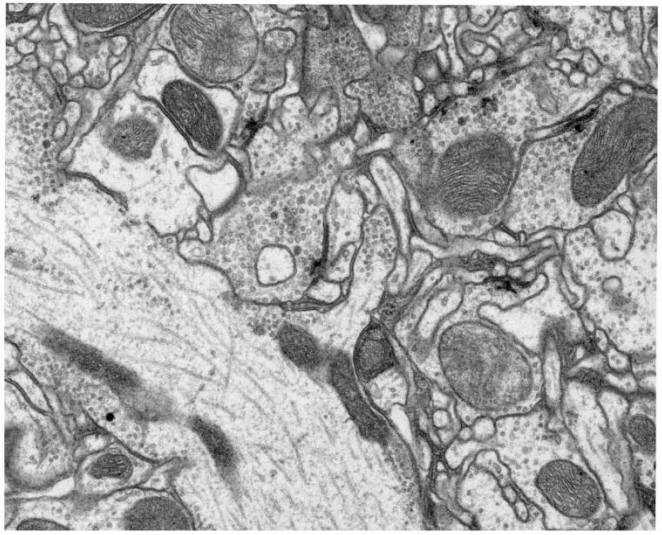
Clonal raider ant head



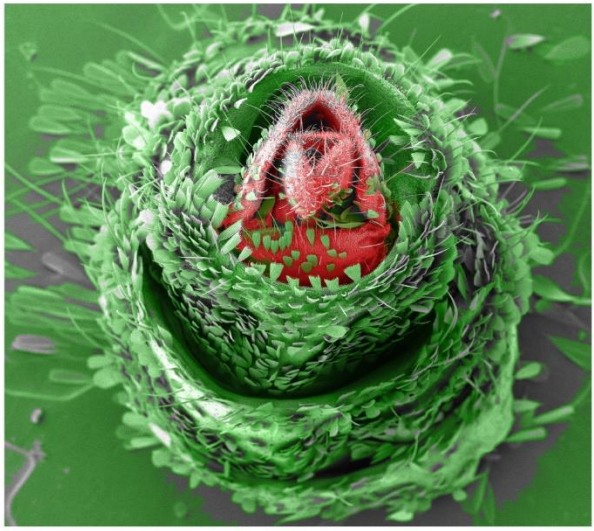
Mast cell granules (human)



Olfactory epithelium (mouse)



Drosophila brain



Female mosquito terminalia

Cryo-Electron Microscopy Resource Center

Microscopy Suite: CRC B13 Phone: 212-327-7282

Mark Ebrahim

Senior Staff Scientist

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Hongkit Ng

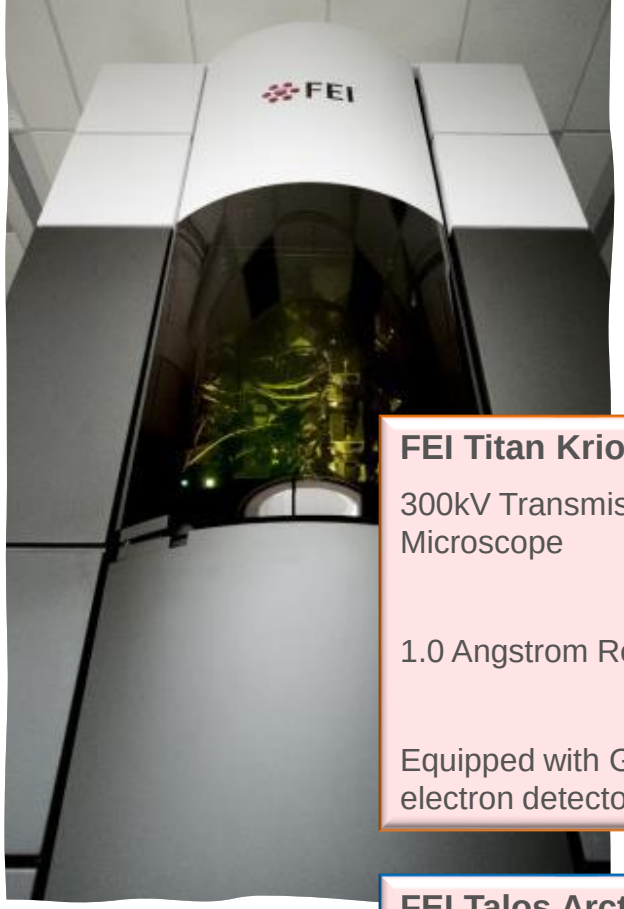
Research Support Associate

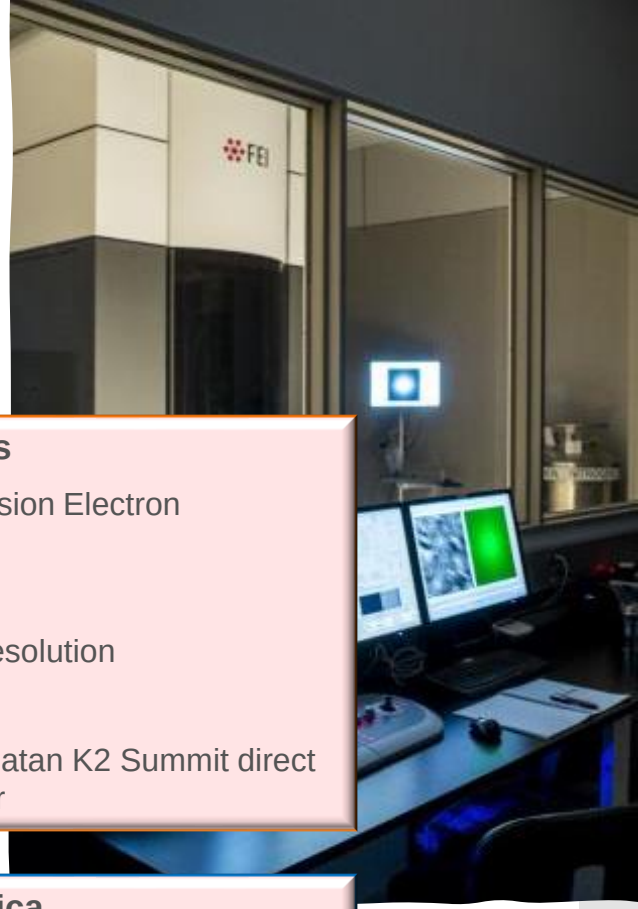
hng@rockefeller.edu

Johanna Sotiris

Senior Research Support Specialist

jsotiris@rockefeller.edu





Cryo-Electron Microscopy Resource Center

Instruments

FEI Titan Krios

300kV Transmission Electron Microscope

1.0 Angstrom Resolution

Equipped with Gatan K2 Summit direct electron detector

FEI Talos Arctica

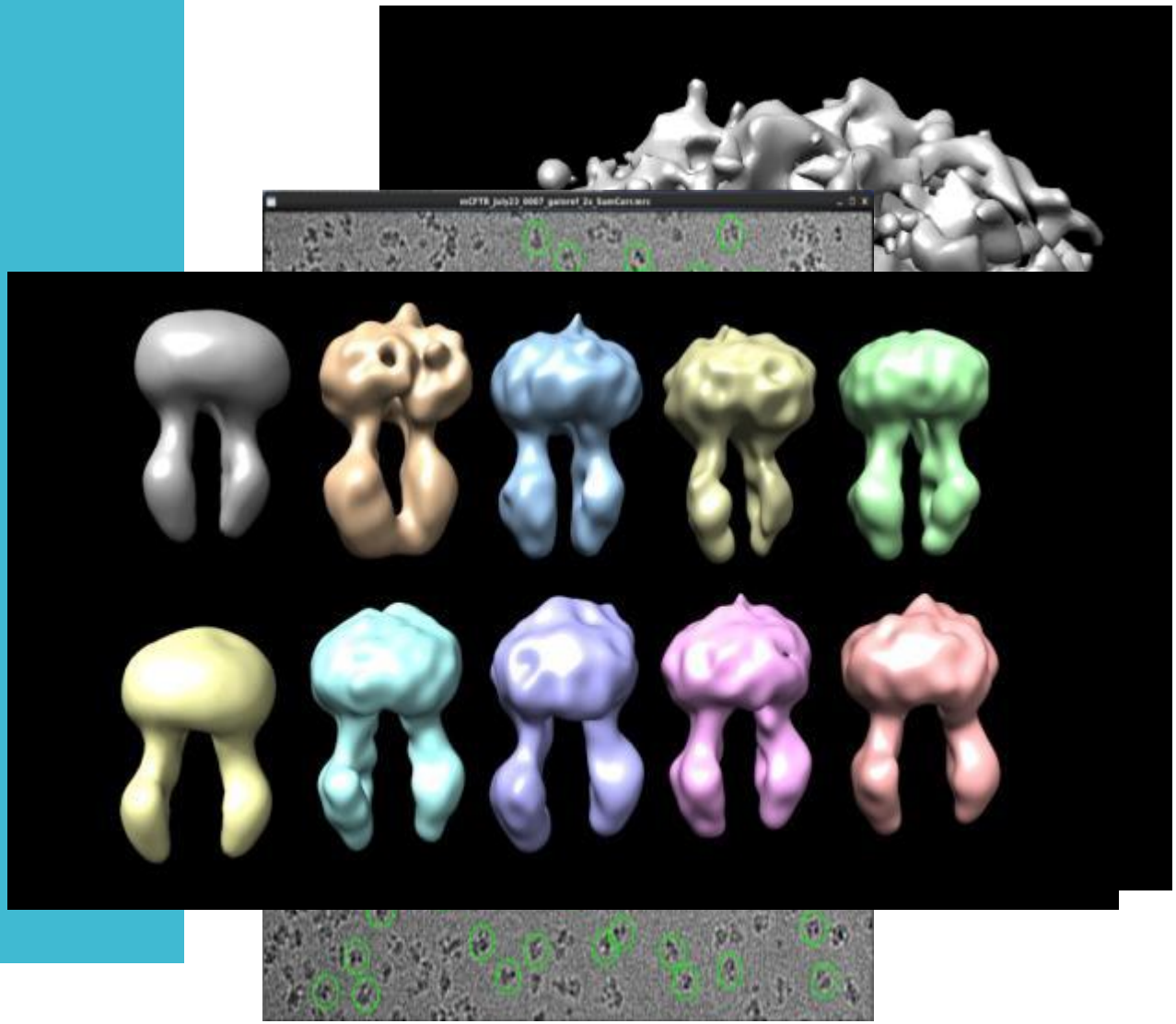
200kV Transmission Electron Microscope

1.4 Angstrom Resolution

Equipped with Gatan K2 Summit direct electron detector

Cryo-Electron Microscopy Resource Center

Applications



PRECISION INSTRUMENTATION TECHNOLOGIES

"Welcome to the PIT"

Peer Strogies

Director, PIT

Jim Petrillo

Instrumentation Engineer

Nicholas Belenko

Instrumentation Engineer

Michelle Zhang

Instrumentation Associate

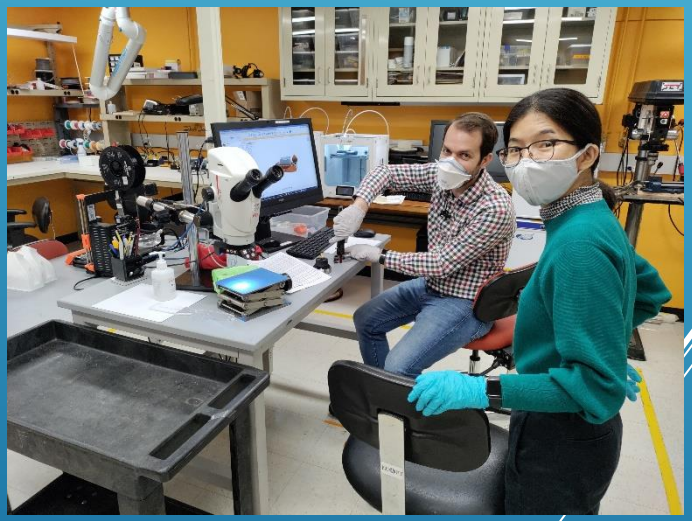
Location: Peggy Rockefeller Plaza, A level

inside.rockefeller.edu/fabrication/

Hossein @ Laser Cutter

Laser cutting

More DLP prints

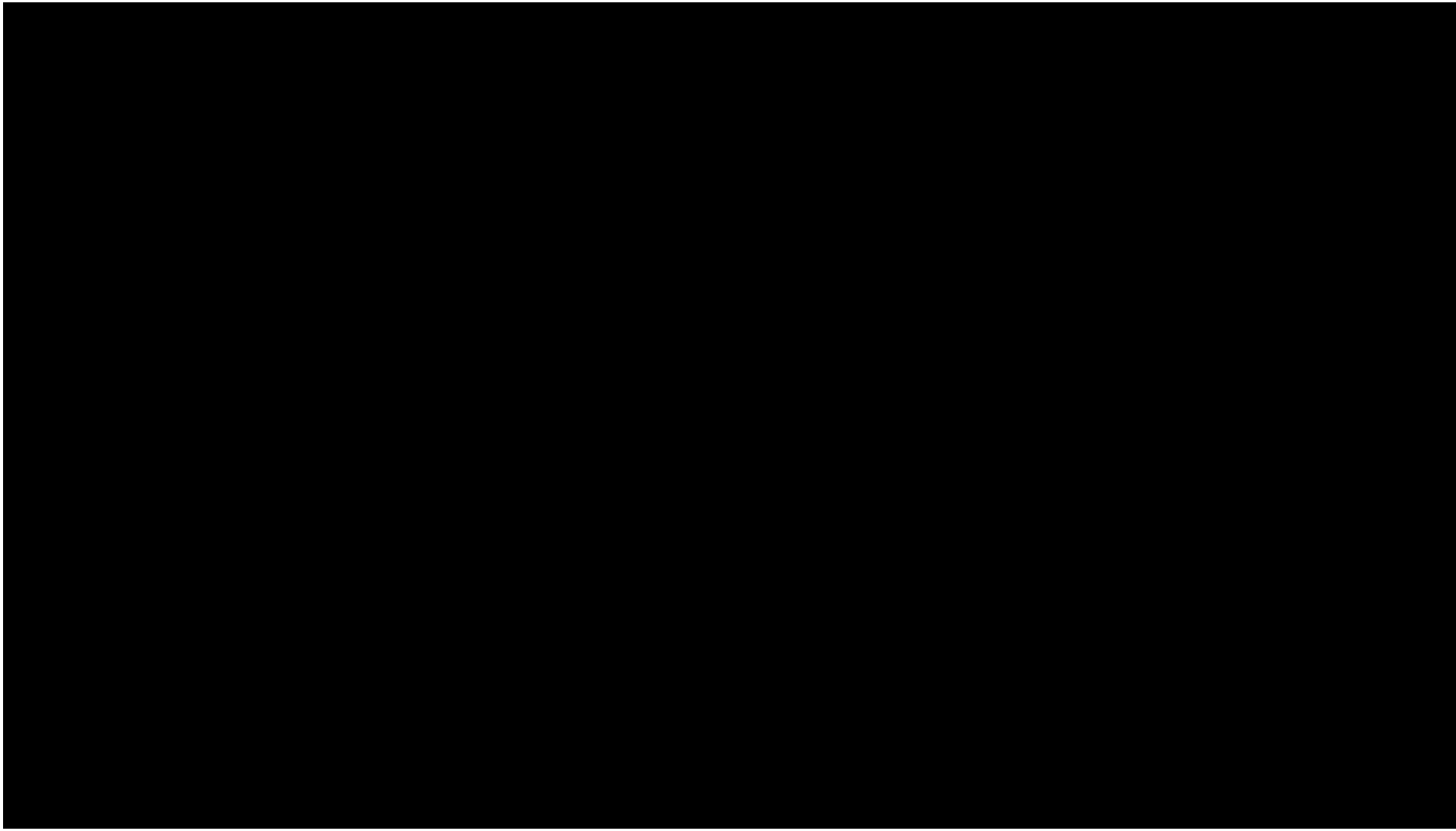


Jim and Qian @ Makerspace Island

Lathe

Bridgeport Mill

Grinders and Miter Saw



Comparative Bioscience Center (CBC)

REGULATORY SUPPORT:

Institutional Animal Care and Use Committee

IACUC Protocol Preparation

Veterinary Services:

Animal Model Development, Selection, and Experimental Design

Extensive health surveillance programs

Operations/ Husbandry:

Optimal health and high standards of care

Maintained disease free

Investigator

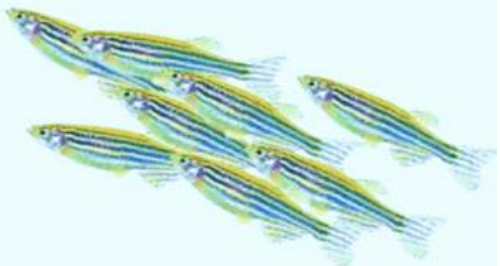
Finance and Business:

Animal Ordering

Billing

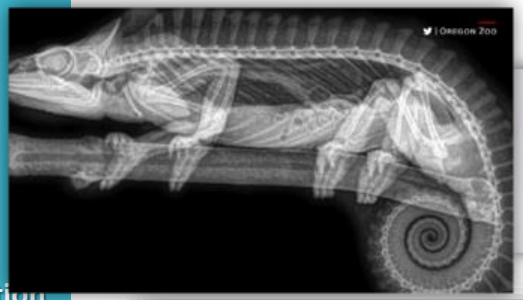
Census

Administrative Assistance



Educational/ Research Support

1. Surgery Training
   a. Surgical Procedure Space
2. Safe use of Hazards
   a. Chemical Fume Hoods
   b. Biological Safety Cabinets
3. Dosing: SC, IM, IV, PO
4. Blood/ Tissue Collections
5. Equipment Design and Installation
6. Research Diets
7. Transnetyx – TAG Center
   a. Automated Genotyping Services
8. Vevo 2100 Ultrasound
9. IVIS Spectrum
   a. In vivo fluorescence and bioluminescence imaging
10. RadPRO® OMNERA® 50 Veterinary Digital Radiographic System
11. Rad Source 2000 X-Ray Biologic Irradiator



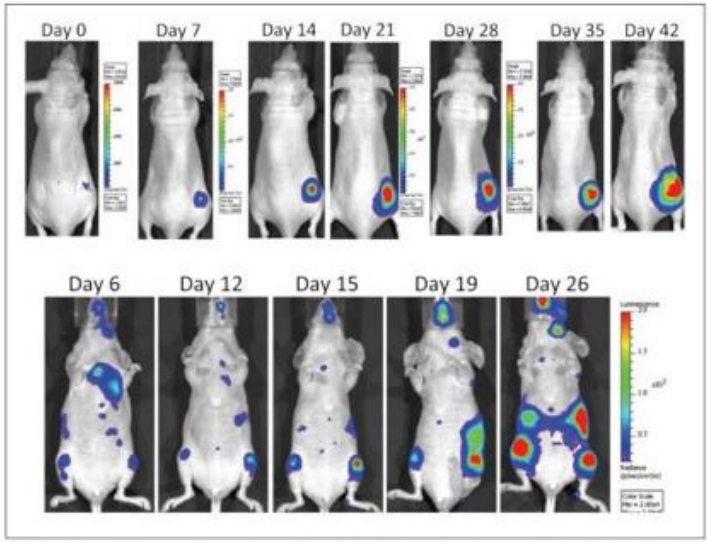
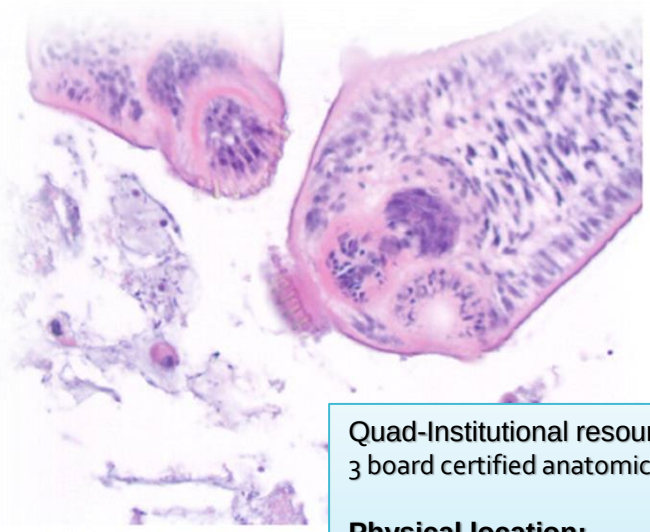


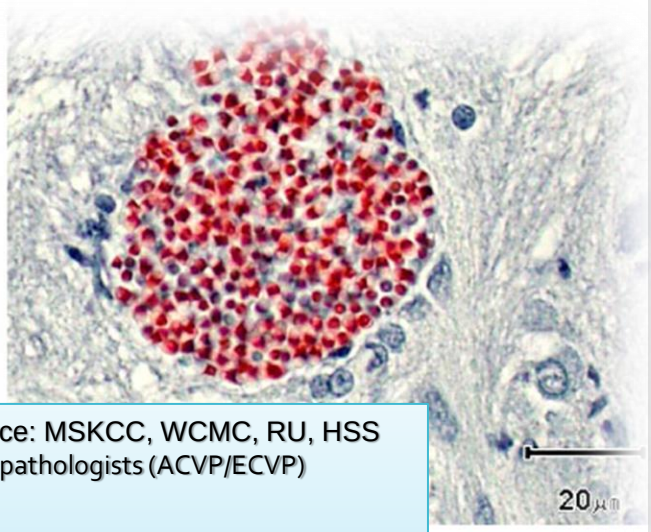
Figure 4. Detection of five 4T1-luc2 cells injected subcutaneously in nude mice (top) and monitoring metastasis post intracardiac injection of MDA MA-231-luc2 cells (bottom) longitudinally.

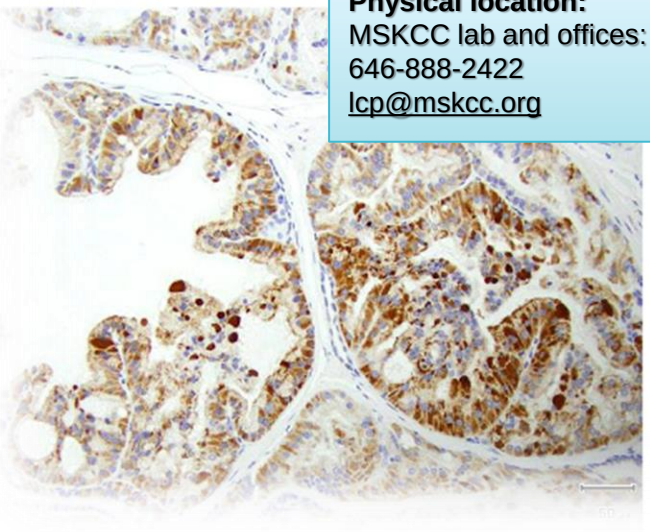
Leslie Diaz, Associate Director, CBC
ldiaz@rockefeller.edu

Laboratory of Comparative Pathology

- Genetically Modified Animal Phenotyping
- Hematology and Clinical chemistry
- Complete Necropsy
- Histology
- Radiology
- Bone marrow evaluation
- Organ-specific research studies: mammary gland, prostate, heart





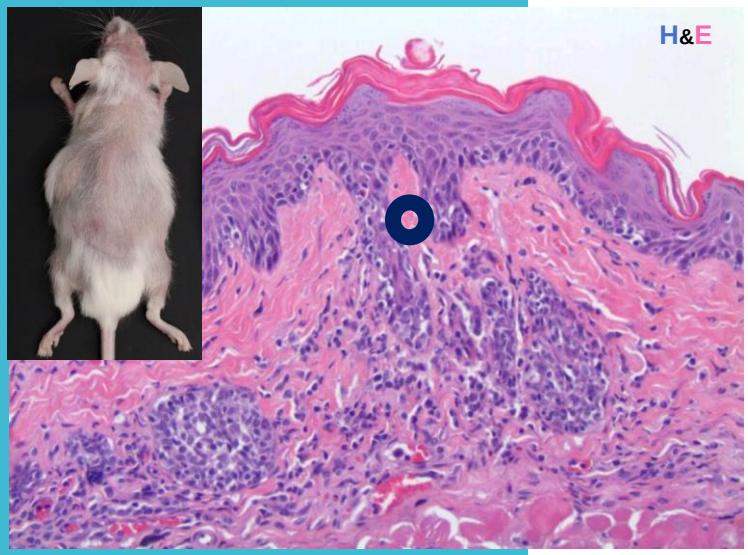


Quad-Institutional resource: MSKCC, WCMC, RU, HSS
3 board certified anatomic pathologists (ACVP/ECVP)

Physical location:
MSKCC lab and offices: Z-940 (Zuckerman Building)
646-888-2422
lcp@mskcc.org

Anatomic Pathology

Qualitative examination: Morphologic diagnosis

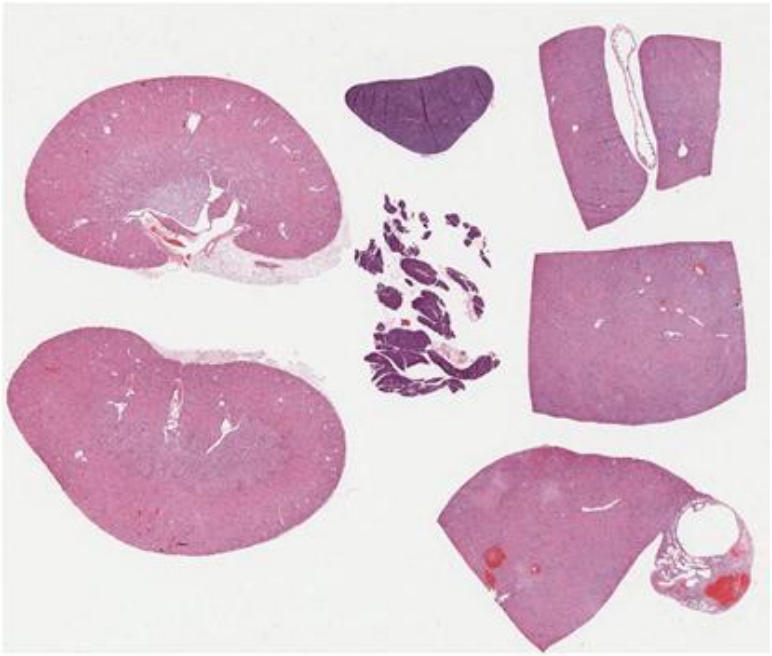


Skin: Lymphocytic interface dermatitis.

CLINICAL PATHOLOGY

Clinical chemistry
Cytology
Hematology
Urinalysis
Microbiology
Parasitology
Serology

Digital Slide Scanning



MOUSE PHENOTYPING / STUDY PLANNING AND INTERPRETATION

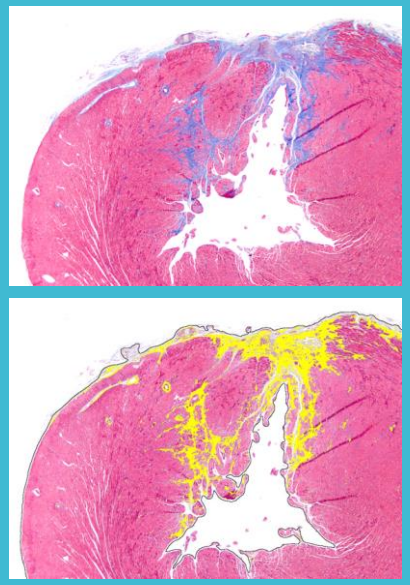
- Validation of new GMAs as animal models of human diseases
- Investigation of gene function

PATHOLOGY OF PRECLINICAL EFFICACY AND SAFETY MODELS

UNEXPECTED OUTCOME INVESTIGATIONS

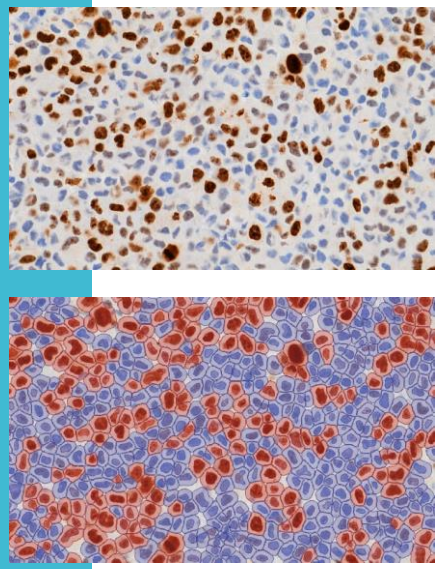
Anatomic Pathology
Quantitative image analysis

Area based analysis



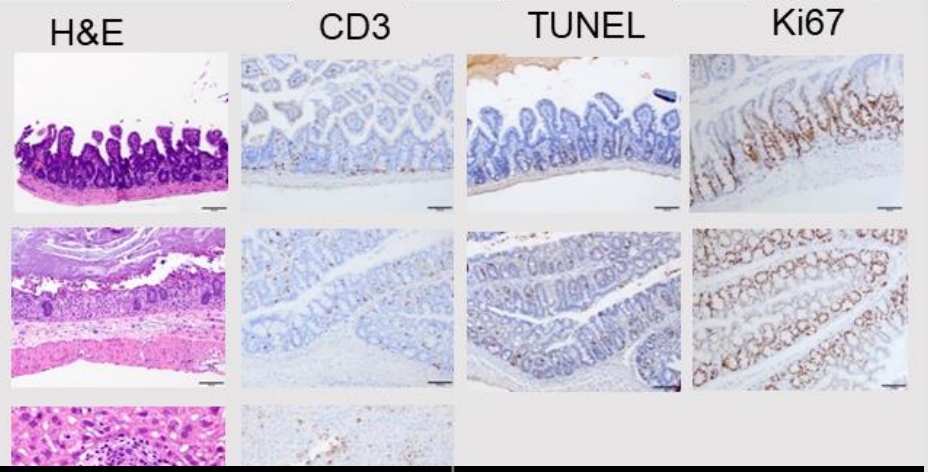
Masson trichrome, myocardial fibrosis
Result: positive pixel area / total ROI area (%)

Cell based analysis



Ki67 IHC, Urothelial carcinoma
Result: % positive cells

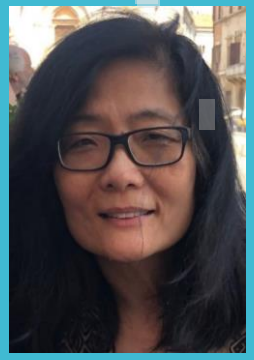
Anatomic Pathology
Manual Semi-Quantitative Scoring



Signalment		
LCP Accession #	18-3593	18-3593
LCP Animal #	1	2
Histologic examination		
Small intestine GVHD scoring	6	8
SI Villus blunting	0	0
SI crypt hyperplasia (Ki67)	1	2
SI Crypt apoptosis (TUNEL)	3	2
SI Crypt loss	0	2
SI LP fibrosis	0	0
SI CD3 positive cells	2	2
SI Mucosal ulceration	0	0

CRISPR & Genome Editing Resource Center (DWB703)

Chingwen Yang, Ph. D.
Director
Tel:8649
yangc@rockefeller.edu



Chia-Yun Han, M. S.
Research support specialist

Jing Gao, M. D.
Manager

Pradip Kar, M. S.
Research support Specialist

Vhy-Shelta Kewalder, M. S.
Research support specialist

What do we do?
CRISPR & Genome Editing Resource Center

Mutant alleles

Knockout

Knock-in: point mutations, small tags, reporter genes

Conditional Alleles: knockout, knock-in

Humannized genes in mice

Gene editing Service

Mouse embryos

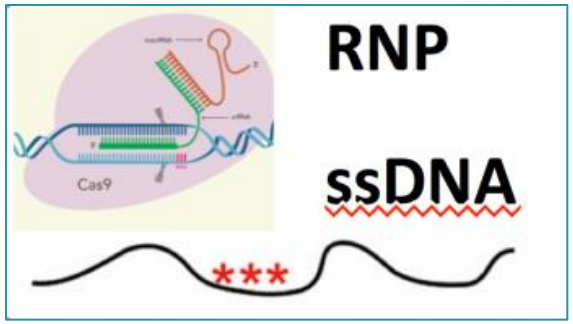
Adult mice

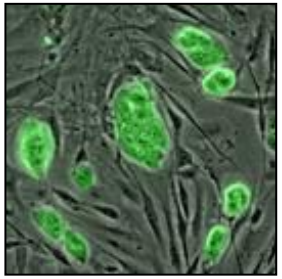
mES cells

Mouse lymphoma cell lines

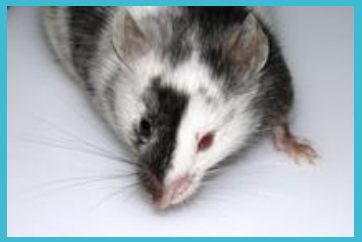
Human iPSC

Primary human cancer cells





Transgenic and Reproductive Technology Center



CBC 542-546 x 7783, x 7738

Research Support Specialists:

Jahnney Torres, Eunyong Kim , William Ramirez, Roxana Cubias

Director:

Rada Norinsky : Rada.Norinsky@rockefeller.edu

Production of Genetically Modified Mice

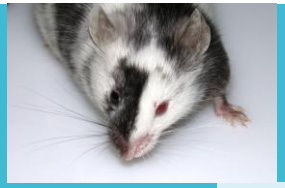
CRISPR/Cas9 mediated genome editing

- Gene KO (whole gene, small deletions)
- Gene KI (point mutations, small tags KI)
- Targeted KI (GFP, Cre)
- Conditional KO (floxed lines)
- *Method 1*
  If partial insertion (one LoxP site)
  2 methods to resolve
- Additional pronuclear injection till both LoxP sites are in
- *Method 2*
- Breeding founder to homozygocity
- IVF to create heterozygous zygotes
- Pronuclear injection of second LoxP site

Transgenic animals production

- BAC transgene microinjection
- Plasmid microinjection
- Chimera Production
- ES cells injections into host blastocyst
- ES cells injection into 8 cell morulae
- Completely ES cells derived animals
- ES cell injections into tetraploid blastocysts

Assisted Reproductive Technology



Rapid Colony Expansion

- Cohorts matched by sex and age
- Custom made with complex genotypes

Triple transgenics and/or KO, single, double mutation, Cre lines

- Cohort size 10</=300 pups (according to request)

Assisted reproduction

- Hard to breed lines
- Aged/last/never able to breed founder

Mutant zygotes generation

CRISPR/Cas9 pronuclear injection

BAC injection

Rapid embryo cryopreservation

- Custom made with complex genotypes

Triple transgenics and/or KO, single, double mutation, Cre lines

- **Sperm Cryopreservation**
- **Rederivation** of mouse lines from live animals, live embryos
- **Resuscitation** of strains from frozen embryos/ frozen sperm

All generated animals are SPF facility compliant

The Reference Genome Center

Olivier Fedrigo, Director

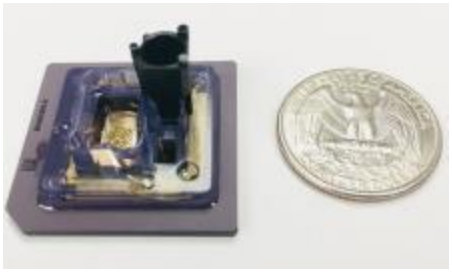
Weiss 7th floor
Email: VGL@rockefeller.edu

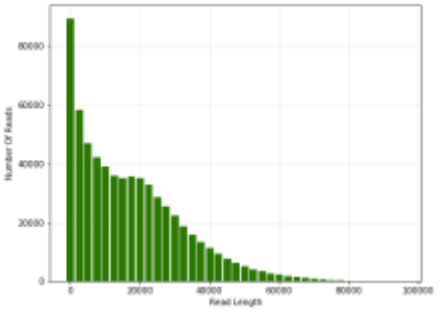
PACBIO®

BIONANO GENOMICS

PacBio Sequels

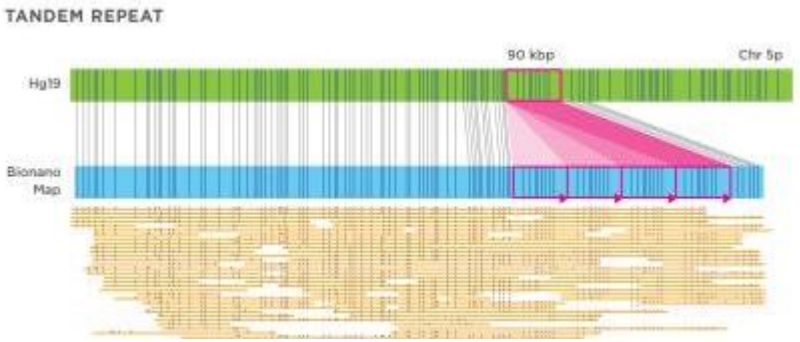
- Single molecule sequencing
- 15-20 kilobases sequences (average)
- 5-10 gigabases per run
- ~10 hours per run





Bionano Saphyr

- Optical mapping
- >150kb fragments (N50 >220kb)
- 24+ hours run; >300Gb per run



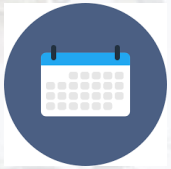
- Whole genome sequencing
- Targeted sequencing (e.g. HLA)
- Complex populations (e.g. microbial communities)
- RNA sequencing (full length transcriptomics)

- Epigenetic
- Structural variants
- Genome scaffolding

The Reference Genome Center

(212) 327-8216

VGL@rockefeller.edu



https://vertebrategenomelaboratory.youcanbook.me

http://inside.rockefeller.edu/vgl/

Our team:

Jennifer Balacco
Olivier Fedrigo
Nivesh Jain
Brian O'Toole

Weiss 7th floor
Room 735

If you are interested in **long reads**, come visit us at the VGL to discuss your ideas and projects

Scientific Resource Center Locations

1) Bio-Imaging (DWB 201-203)
2) Cryo-Electron Microscopy (CRC B13)
3) Electron Microscopy (RRB 120)
4) Flow Cytometry (DWB 205-211)
5) CRISPR & Genome Editing Center (DWB 703)
6) Genomics (WRB 725)
7) Glasswashing (CRC-C level)
8) High Throughput and Spectroscopy (DWB 219)
9) Antibody and Bioresource Core Facility (DWB 315)
10) Precision Instrumentation Technologies (Plaza Building– A level))
11) Proteomics (RRB 157)
12) Structural Biology (RRB 1st floor)
13) Transgenics (CBC 542-546)
14) Reference Genome Center (WRB 7th floor)
15) Comparative Bioscience Center (CBC)
16) Bioinformatics (TSHA, A33)
17) High Performance Computing (TSHA, C27)

Scientific Resource Center Locations

1) Bio-Imaging (DWB 201-203)
2) Cryo-Electron Microscopy (CRC B13)
3) Electron Microscopy (RRB 120)
4) Flow Cytometry (DWB 205-211)
5) CRISPR & Genome Editing Center (DWB 703)
6) Genomics (WRB 725)
7) Glasswashing (CRC-C level)
8) High Throughput and Spectroscopy (DWB 219)
9) Antibody and Bioresource Core Facility (DWB 315)
10) Precision Instrumentation Technologies (Plaza Building– A level))
11) Proteomics (RRB 157)
12) Structural Biology (RRB 1st floor)
13) Transgenics (CBC 542-546)
14) Reference Genome Center (WRB 7th floor)
15) Comparative Bioscience Center (CBC)
16) Bioinformatics (TSHA, A33)
17) High Performance Computing (TSHA, C27)