NCI Core Resources Guide

December 2020

There are many resources available within CCR to aid investigators in their research. Typically, a Core Facility Open House is held each year to acquaint CCR researchers with technologies and resources. We have found this event to be tremendously beneficial for helping scientists connect with facility managers and for fostering new research ideas within the community. Unfortunately, due to the distancing restrictions imposed by COVID-19, we are not able to hold this event in the same way in 2020. However, we did not want to lose the opportunity to update the CCR community on core offerings.

For that reason, we, like so many others, have gone virtual this year. Each facility has put together a one page summary of the technologies offered and included contact information for those responsible. Please feel free to reach out to those contacts with questions you may have. We hope that this resource will spark new ideas for experiments and initiate new research.

For ease of browsing, we have included two lists of resources at the beginning of this guide. The first is organized by technology and the second by location. Clicking on the name of the facility in either list will take you directly to their summary page.

We hope to be able to return to the live Open House format in 2021.

Sincerely,

Liz Conner and Lisa Jenkins

NCI Core Resources – By Technology

Click on Core name and it will take you directly to Core summary page

NCI Core Resources – By Location

Click on Core name and it will take you directly to Core summary page

Electron Microscopy	Frederick	baxau@mail.nih.gov	24
Advanced Biomedical Computing	Frederick	collinja@mail.nih.gov	26
OSTR			1
CRex		lakshmi.darbha@nih.gov	28

OSTR

Facilitating CCR Science through Advanced Technologies and Scientific Resources

https://ostr.ccr.cancer.gov/

Our Mission

The Office of Science and Technology Resources (OSTR) catalyzes the advancement of cancer research through making innovative technologies and scientific resources accessible to scientists at the Center for Cancer Research (CCR). OSTR develops partnerships, collaborations, and agreements with outside organizations with the primary goal of furthering the scientific mission of the CCR.

Advanced Technology Corner

The Advanced Technology Corner (ATC) is a unique educational and informational resource offered by the Office of Science and Technology Resources (OSTR) designed to introduce users to various scientific methodologies, i.e. protein mass spectrometry and molecular microscopy, with the use of video tutorials. In addition, the ATC also provides information on current advancements in scientific methods and instrumentation in the Emerging Technologies section.

Cores - CCR Cores and collaborative resources make services and expertise available to CCR investigators. Technologies and expertise offered through collaborative resources (CCR Research Labs and Lab/Branch Facilities) are at the discretion of the Principal Investigator or Lab/Branch Chief.

Collaborative - Centralized research resource facilities that provide access to advanced instrumentation, cutting-edge technologies, and expert consultation. Cores provide opportunities to be hubs of innovation and connect scientists with tools and expertise that can advance their research to the next level.

CREx - The Collaborative Research Exchange (CREx) is an online NIH marketplace for investigators to search, browse, and request information for research services, technologies, or products offered by NCI/NIH Cores, Collaborative Resources, and commercial vendors.

Flow Cytometry Core (LGI) offers established technologies to support studies using flow cytometry and cell sorting. The Flow Cytometry Core is open to CCR investigators who do not have their own flow cytometry facilities, with preference given to Bldg. 37 scientists.

Instrumentation

- 2 FACSCaliburs – 2 lasers (488, 635)
- LSRII – 5 lasers (355, 405, 488, 561, 635)
- LSR Fortessa – 4 lasers (355, 405, 488, 639)
- FACS Aria (standard) – 3 lasers (405, 488, 639)
- MoFlo Astrios – 5 lasers (355, 405, 488, 561, 640)

Established Technologies

Applications that run on FACS Caliburs include:

- Immunophenotyping (up to 4-color)
- Intracellular markers, including cytokines and phosphoproteins
- Cell cycle analysis using propidium iodide for mammalian cells and cytox green for yeast
- Cell cycle-associated BRDU labelling of S-phase cells and phosphohistone H3 labeling of mitotic cells
- Apoptosis by annexin V binding, TUNEL, caspase, or FLICA
- Detection of fluorescent reporters GFP, YFP, and dsRed
- Membrane potential with oxonol and cyanine dyes
- Mitochondrial membrane potential with JC-1, Mito Tracker Dyes, oxidative activity using DCFH-DH and DHR
- Lipid probes
- Aldefluor

Applications used on the LSRs include:

- Immunophenotyping using up to 10 simultaneous probes (including CFSE)
- SP cells (side population)
- RFP (red fluorescent proteins, including cherry and tomato)
- Qdots
- Cell cycle analysis with the UV-excitable dyes Hoechst 33342 and DAPI on both mammalian cells and bacteria
- Simultaneous analysis of cell cycle (HO3342) and GFP in viable cells
- HO/Pyronin for DNA/RNA
- Intracellular calcium ratiometric measurements using indo-1

Cell Sorting:

- Subsets of murine T, B, and stem cells (including SP cells) for in vitro culturing and functional assays, re-injection into the mouse, or preparation of RNA, DNA, or protein
- Primary murine keratinocytes, melanocytes, thryroid, lung, liver, prostate, and mammary cells – sorted by expression of specific antigens
- Sorting of tumor xenografts
- Sorting of potential cancer stem cells from cell lines and primary tumors based on surface antigen expression, SP (side population), or aldeyde dehydrogenase
- Cell lines transfected with GFP, YFP, or RFP reporters
- Preparation of high-luciferase cell lines for imaging based on co-expression with GFP or RFP
- Cell cycle compartment sorting using viable cells stained with Hoechst 33342 for characterization of cell cycle-related proteins
- Plate-sorting for single cell PCR
- Slide-sorting for single cell PCR (Advalytix)

Following successful individualized training on the bench-top flow cytometers, a user will run his/her own experiments and may access the flow cytometers on a 24/7 basis.After individual consultation, cell sorting is done by the core facility staff on a scheduled basis.

Contact Details Address: Building 37, Rooms 6008 and 6011, NCI-Bethesda
Facility Manager: Ferenc Livak, MD
Email: ferenc.livak@nih.gov
Facility Head: Subhadra Banerjee, PhD
Email: banersub@mail.nih.gov

NCI Vaccine Branch Flow Cytometry Core Facility

Building 41, Room C310, D702A

FACS Core Instruments

FACS Analyzers

- **LSRIIB (Monty)**
  - 4 Lasers (488nm, 532nm, 640nm, 405nm)
  - 18 Colors
- **FACSymphony A5 (Pegasus)**
  - 5 Lasers (488nm, 532nm, 640nm, 405nm, 355nm)
  - 28 colors
- **FACSymphony A5 (Apollo)**
  - 5 Lasers (488nm, 532nm, 640nm, 405nm, 355nm)
  - 28 colors
- **FACSymphony A5 (Andromeda)**
  - 5 Lasers (488nm, 532nm, 640nm, 405nm, 355nm)
  - 28 colors

Cell Sorters

- **FACSAriaIII (Athena)**
  - 4 Lasers (488nm, 561 nm, 640nm, 405nm)
  - 18 Colors
- **Astrios EQ (Poseidon)**
  - 5 Lasers (488nm, 561nm, 405nm, 640nm, 355nm)
  - 19 Colors
- **Sony MA900**
  - 4 Lasers (488nm, 561nm, 405nm, 628nm)
  - 12 Colors
  - Installation in February 2021
- **Miltenyi autoMACS**
  - Magnetic Bead Sorter

Applications Supported

- **Multicolor Immunophenotyping**
  - Lineage markers
  - Activation and memory markers
  - Intracellular cytokine production
- **Cell sorting**
  - Infectious cell sorting
  - Fluorescent protein sorting
  - 96 well cell sorting
- **Stem cell analysis and sorting**
- **RNA transcript + Phenotypic Analysis**
  - PrimeFlow
  - PCR + FACS
- **Phosphorylated Protein Analysis**
- **Proliferation and Cell Cycle Analysis**
  - Cell Cycle
  - BrdU
  - Ki67
  - CFSE
- **Apoptosis**
  - Active Caspase 3
  - Annexin V
  - JC1
  - TUNEL
- **Quantitative Flow Cytometry**

And anything else that can be measured by flow cytometry

FACS Analyzers

FACSymphony A5 (Apollo)

FACSymphony A5 (Pegasus)

Cell Sorters

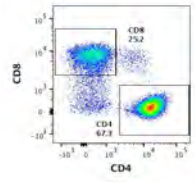
FACSAria III

Astrios EQ

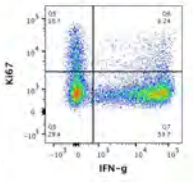
Sony MA900 will be installed in February 2021

FACS Applications

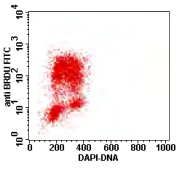
Immunophenotyping



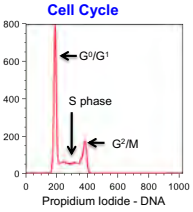
Intracellular Analysis



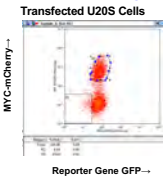
Proliferation



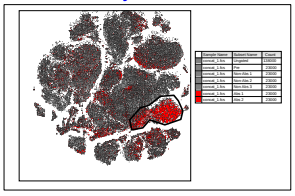
Cell Cycle



Cell Sorting



tSNE Analysis of 26 color FACS



Personnel and Location

Sophia Brown
41/D804
240-760-6584
sophia.brown@nih.gov

Kathy McKinnon
41/B715
240-760-6659
mckinnonkm@nih.gov

Center for Cancer Research
Laboratory of Cancer Immunometabolism

Flow Cytometry Core Laboratory

Fort Detrick, Building 560 Rooms 31-53, 31-28

The LCIM flow cytometry core lab provides an array of instruments and technical support for CCR* investigators' cell analysis and separation needs, including:

- High-parameter tools, keeping pace with evolving methods and fluorophores
- Dedicated staff for cell sorting, maintenance, troubleshooting and scientific consulting
- Materials for training and planning

** Non-CCR investigators may access the lab with a usage fee*

Benchtop analyzers:

BD Symphony A5 (5B, 5G, 8V, 3R, 7UV) Lab 31-54

BD LSRII-Fortessa (2B, 5G, 6V, 3R) Lab 31-54

BD LSRII-SORP (2B, 5G, 6V, 3R) Lab 31-28

BD LSRII (5B, 3V, 3R) Lab 31-54

BD FACS Canto II (4B, 2V, 2R) Lab 31-28

Miltenyi MACSQuant-16 (6B, 5V, 3R) Lab 31-54

Cytek Aurora spectral analyzer (5 lasers, up to 40 colors) Lab 31-28

Cell sorters:

BD FACS Aria II (2B, 5G, 6V, 3R, 2UV) Lab 31-52

BD FACS Aria II (2B, 5G, 6V, 3R) Lab 31-26

BD Symphony S6 (5B, 8V, 8UV, 5YG, 3R) Lab 31-26

Questions? Need Help?

Please call or text the flow team any time:

Flow Lab: 301-846-5811
Sort Room: 301-846-6126

Jeff:
jeff.carrell@nih.gov
240-285-5166 mobile

Megan:
megan.karwan@nih.gov
703-625-9454 mobile

leidos
Leidos Biomedical Research, Inc.

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

NIH NATIONAL CANCER INSTITUTE
Center for Cancer Research

CCR Genomics Core

The NCI CCR Genomics Core is in Building 37 on the NIH Bethesda campus. The facility has been in operation since 1997 and operates on a cost-recovery basis. The primary goal of the Core is to provide investigators from NCI and other institutes within NIH with access to genomic technologies and Next-Generation Sequencing (NGS) with rapid turnaround on smaller-scale projects or projects that are not ready for production. The Core is unique in granting user-accessible instrumentation. Additional resources include training, consultation services, bioinformatics support, and secure data delivery/management. We also provide DNA and RNA quality control (QC) services.

Current Technologies

Sanger Sequencing

- 2-3500xL and 1-3730xL Applied Biosystems Genetic Analyzers
- Rapid, accurate and affordable sequencing of DNA samples

Digital Gene Expression Analysis

- NanoString nCounter MAX Analysis system
- Pathway expression profiling
- miRNA profiling
- Performed directly on total RNA or lysates

Next-Generation Sequencing

- Illumina MiSeq & NextSeq550, 2000
- Viral and bacterial genomes sequencing
- T-Cell & B-Cell receptor sequencing
- RNA-seq
- Amplicon sequencing
- Custom projects

Droplet Digital PCR

- BioRad QX200 ddPCR System
- Absolute quantification of target genomic DNA or cDNA
- Detection of copy number variations
- Rare mutation detection
- TruSeq RNA Library Preparation

Digital Spatial Profiling

- Offered in collaboration with the Collaborative Protein Technology Resource (CPTR)
- nCounter® GeoMx Digital Spatial Profiling
- FFPE or fresh frozen
- nCouner: 20-plex protein and 84-plex RNA panels
- NGS: 1,833 genes across 55 pathways

Nanopore Sequencing

- Oxford Nanopre MinION
- Long-read sequencing for both DNA and RNA

Analytic & Preparative Electrophoresis

- Sage Science PippinHT- high throughput sizing
- Agilent 4200 & 4150 Tapestation – QC platform

Additional equipment:

- Agilent BRAVO Liquid handler
- Formulatrix Mantis Liquid Handler
- Qubit Fluorometric Quantification

Contact us:
Bldg 37, 2135
Group e-mail: ncilecdnacore@mail.nih.gov
Website: https://genomics.ccr.cancer.gov/
iLab website:
https://nci.corefacilities.org/account/login

The Core has a dedicated bioinformatics consultant who advises customers on experimental design, interpretation of QC data and helps to direct users to the existing bioinformatics tools under CCBR and other available bioinformatic entities.

Inquiries? Contact Liz Conner, Core Manager, liz_conner@nih.gov

Single Cell Analysis Facility (SCAF):

Shared Resource & Single Cell Innovation Laboratory

Allison Ruchinskas, Kimia Dadkhah, Ian Taukulis, Maria Hernandez, Michael C. Kelly

Cancer Research Technology Program, Leidos Biomed, Inc. CCR, NCI

NIH NATIONAL CANCER INSTITUTE

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Introduction

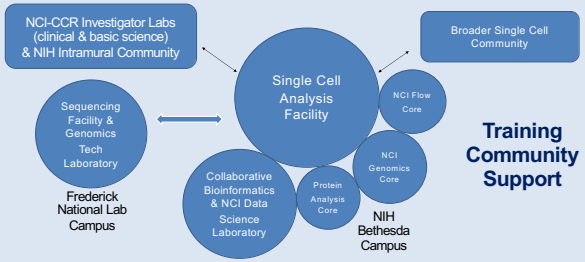
The rapid advancement of single cell technology has provided new powerful tools to answer many biological questions, such as identifying new or rare cell populations and characterizing the complex heterogeneity involved in cancer biology. Realizing the great potential of single cell technology in cancer research, the CCR has established the Single Cell Analysis Facility (SCAF). SCAF provides the most advanced single cell genomics technologies to CCR investigators, including many established platforms, as well as emerging technologies.

These technologies give CCR-investigators the options for single cells studies, ranging from few cells to hundreds of thousands of cells, and from whole transcriptome single cell RNA-seq to innovative multi-modal profiling at single cell resolution.

CCR Resources for Integrated Single Cell Analysis

Project Consultation	Capture & Library Prep	Sequencing	Primary Informatics	Secondary Informatics
Project Consultation with group performing Capture & Library Prep (*ideally include individuals who will be involved in Secondary Informatics*)	Capture & Library Prep — usually performed by Single Cell Analysis Facility (Bethesda) or Sequencing Facility (Frederick)	Sequencing typically coordinated by facility performing Capture & Library Prep. Sequencing usually performed at Sequencing Facility or Bld 37 Genomics Core	Primary Informatics (*raw sequence data processing and initial project qc*) performed by facility performing Capture & Library Prep	Secondary analysis support through CCBR, hand-off to Investigator Lab, or Informatics collaborator. Some support via Capture & Library Prep facility.

Collaborative Integration with Existing Resources & Expertise



Initiating single cell projects:

Bethesda – Single Cell Analysis Facility
Mike Kelly
michael.kelly3@nih.gov

Frederick – Sequencing Facility
Monika Mehta
monika.mehta@nih.gov

Informatics Support:

Maggie Cam's team (CCBR)
maggie.cam@nih.gov

Yongmei Zhao's team(SF)
yongmei.zhao@nih.gov

Mike Kelly's team (SCAF)
michael.kelly3@nih.gov

Sequencing cores:
Bethesda – Liz Conner's team(GC)
Frederick – Bao Tran's team (SF)

Office of Science & Technology Resource
Dave Goldstein & Mariam Malik

Bioinformatics Training & Education Program
Amy Stonelake

What does SCAF Support?

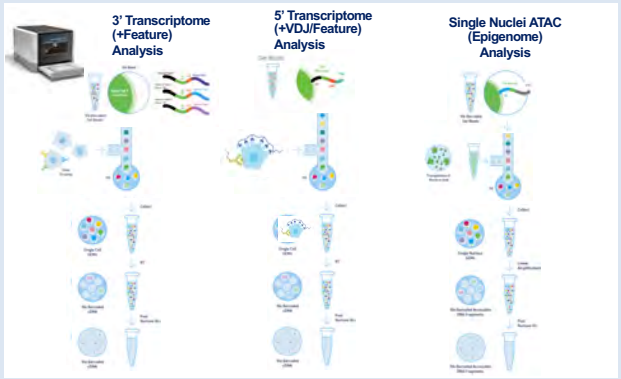
Sample Preparation & Isolation

- Advice and guidance on cell isolation & dissociation
- Experience on effects of sample quality in final data

Gene Expression Profiling & Add-on modalities (scRNA-Seq+)

- VDJ sequencing for TCR or BCR expression
- Cell surface protein measurements with barcode-conjugated antibodies
- Antigen-binding specificity assay with barcode-conjugated epitopes
- Expressed barcodes to track clonal relationships
- Functional genomics with CRISPR-based perturbations

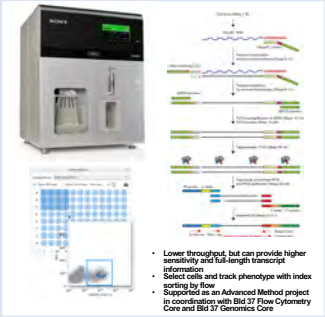
10X Genomics Chromium Platform



Modified from 10X Genomics Promotional Material

Plate-Based (Smart-Seq2, etc.)

Advanced Methods

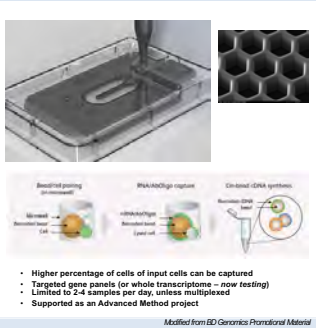


- Lower throughput, but can provide higher sensitivity and full-length transcript information
- Select cells and track phenotype with index sorting by flow
- Supported as an Advanced Method project in coordination with Bld 37 Flow Cytometry Core and Bld 37 Genomics Core

Modified from Sony Promotional Materials and Picelli et al 2014

Microwell (BD Rhapsody)

Advanced Methods



- Higher percentage of cells of input cells can be captured
- Targeted gene panels (or whole transcriptome – *now testing*)
- Limited to 2-4 samples per day, unless multiplexed
- Supported as an Advanced Method project

Modified from BD Genomics Promotional Material

Bioinformatics Analysis Support

Generalized Workflow

(Project Design, Capture Details, & Informatics all inform each other)

Data Quality Control Assessment → Primary Analysis Including Alignment, Counts Matrix Generation → Access & Support to Secondary Bioinformatics Analysis Packages

What's next for SCAF?

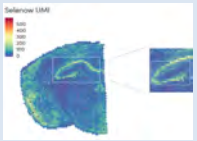
Supporting walk-up access to core single cell technologies

- Training and scheduling access on 10x Genomics and BD Rhapsody platforms
- Increased flexibility for performing single cell captures (after hours / weekend) after training
- Good for advanced users who need greater schedule flexibility or want to set their own pace

Improved reproducibility and improved timelines with support on automation platform

- Up to 8 samples per day
- Currently limited to 3' gene expression profiling
- 5' gene expression with VDJ (2021)
- Sequencing ready libraries next day

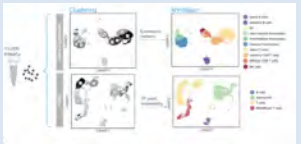
Spatial Transcriptomics: Understanding Gene Expression Within a Tissue Microenvironment



- Tissue section (currently fresh-frozen only) placed on spatially barcoded slide
- Imaging to capture tissue phenotype information
- Whole transcriptome (or target panel) across entire tissue with 50-micron spatial resolution

Droplet-based multi-ome (10x Genomics): Gene Expression with ATAC-Seq on same cell

- Profile gene expression and chromatin accessibility on same single nuclei
- Similar sensitivity to single nuclei RNA-Seq or single nuclei ATAC-Seq alone
- Improve resolution of cell types and gain insight into gene regulation patterns along with direct expression read-outs



How Do I Access SCAF Resources & Support

Contact SCAF

CCR-SCAF@nih.gov
or
Mike Kelly, PhD
SCAF Team Lead
michael.kelly3@nih.gov
Bld 37 / Rm 1042

→ Project Cost Estimate & Approvals via NCI at Frederick Accessioning System (NAS)

→ Project Initiation with Support from Sample Handling, Capture, Sequencing & Informatics

- Project requests must be submitted, estimated and approved in NAS before work can begin
- Scheduling for capture support requests should be made in as much in advance as possible
- Dissociation and quality control of sample is the responsibility of the Investigator
- Single cell suspensions need to arrive before 4pm the day of the capture
- Advanced method platform support may be limited by size and frequency of support request

The Single Cell Analysis Facility (SCAF) is funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Frederick National Laboratory is a Federally Funded Research and Development Center operated by Leidos Biomedical Research, Inc., for the National Cancer Institute

The LICI Microbiome and Genetics Core

Wuxing Yuan, Shah Rashed, Isabelle Kwan, Jonathan Badger, John McCulloch, Richard Rodrigues and Colm O'hUigin

Center for Cancer Research, Cancer Inflammation Program, Building 37, Room 4137, National Institutes of Health, Bethesda and Leidos Biomedical Research Inc.

Facilities and Personnel

The NCI/CCR Laboratory of Integrative Cancer Immunology (LICI) Microbiome and Genetics Core (MGC), located in Building 37 of the NCI in Bethesda, enables NCI and NIH investigators to query any aspect of microbiome involvement in clinical and healthcare research

Members of the MGC team specialize in different aspects of microbiome science - sample handling, purification and sequencing (Yuan, Rashed, Kwan), bioinformatics and computational analysis (Rodrigues, Badger, McCulloch) and additional support in genetics and statistical epidemiology (O'hUigin). Our workflows include both amplicon-based 16S rDNA sequencing or fungal ITS protocols as well as comprehensive metagenome and metatranscriptome sequencing and analysis.

The MGC facilities consist of wet-bench areas in Rooms 4137 and 4141, with dedicated pre-PCR clean rooms for nucleic acid extraction, along with a sequencing room and a central lab space for storage and quantification. Computational facilities are located in Room 4146.

Equipment

The Core uses a high-throughput, robotics-based approach designed to minimize sample processing times and operator-introduced variability.

1. Eppendorf epMotion 5073 liquid handling robot for nucleic acid isolation.
2. QIAxcel Advance, QuantStudio 6 Flex Real-Time PCR and Agilent TapeStation 4200 systems for nucleic acid quantification and quality control
3. Two Eppendorf epMotion 5075 robots for sequencing library preparation and normalization of sample concentrations
4. Illumina MiSeq/NextSeq platforms for sequencing, with large projects outsourced to Novaseq/HiSeq.

Workflows, Sample Output and Usage

Bioinformatics support

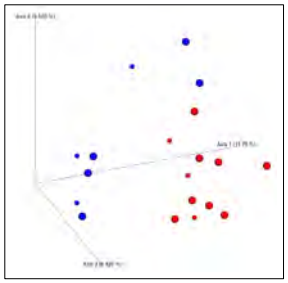
The NIH Biowulf2 cluster is used for most major analyses. A RAID with 128Gb memory and 24TB storage provide additional archival capacity for the core. The Core provides free bioinformatics analysis with all sequencing projects, along with consulting for experimental design.

Microbiome sequencing protocols offered through the core

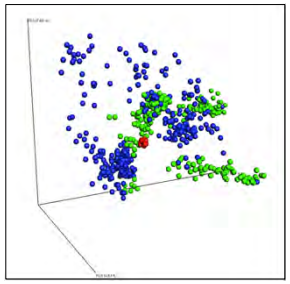
We have designed and tested processing pipelines to move source biological samples (fecal pellet, swabs or tissue) through DNA isolation, target amplification, normalization and sequencing to bioinformatics analyses and phylogenetic quantification.

16S ribosomal RNA amplicon sequencing

Various targeted rRNA amplification products (V1-V3, V3-V4, V4, ITS etc.) can be processed using several standard tools and workflows such as QIIME2, DADA2 and USEARCH.



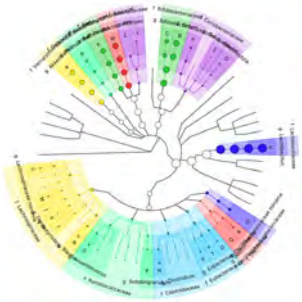
Oral microbiome differences differentiating wild-type and mutant mice in a periodontitis study



PCA showing highly reproducibility of 16S sequencing protocols in the Core

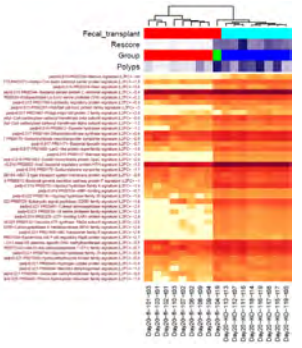
Shotgun metagenome sequencing

With rapid advances in next-generation DNA sequencing, shotgun metagenomics (i.e. sequencing of complete genomes of all bacteria in a sample) is rapidly becoming the gold standard in microbiome studies. The Core offers this protocol as a high-throughput service using a fully robotized pipeline with in-house bioinformatics support using custom software. Currently, we are in the middle of a paradigm shift, with increasing numbers of intramural investigators moving from 16S sequencing to large-scale (n > 100) shotgun metagenome projects. The core is prepared to support these initiatives by having standardized both experimental solutions (for low per-sample cost) and informatics solutions (to manage large amounts of data produced in such studies).

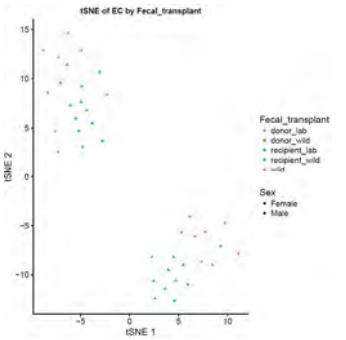


GraPhlAn output demonstrating high-resolution species tree and abundances in shotgun metagenome dataset

Abundance of unclassifiable species even after shotgun metagenome sequencing



Functional differences in the microbiome pre and post fecal transplant as uncovered by YAMS software suite



Improved resolution for functional differences though tSNE analysis of EC numbers

Metatranscriptomics

The Core is developing sequencing of bacterial mRNA with selective depletion of 16S and 23S transcripts and purified from host nucleic acid contamination. This protocol allows for microbiome-based projects to involve a functional component linking species composition changes to causal mechanisms.

Access to core services

The Core's services are available to CIP and other NCI/NIH scientists for all projects related to microbiome characterization. NCI/CIP users are charged for consumables only. To inquire about new projects and pricing, please contact Colm (ohuiginc@mail.nih.gov)

Core Technologies at the Genomics Laboratory

Xiaolin Wu

Genomics Laboratory, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, National Cancer Institute

Overview

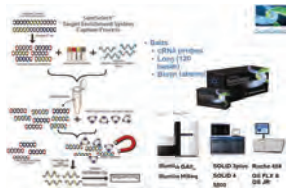
At the Genomics Laboratory, we have broad genetic and genomic analysis capabilities. Our services cover both DNA application and RNA application, from whole genome to focused panel.

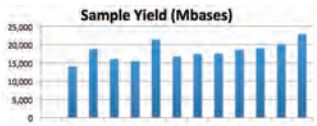
Whole Exome Sequencing

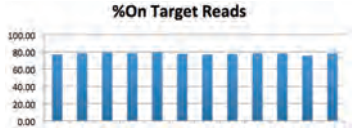
In conjunction with SF*

Agilent SureSelect: Exome capture

- Hybrid-based enrichment strategy
- 120 mer cRNA bait
- High % on target reads
- Fresh or FFPE samples
- V5/7 all exon +UTR 50-70Mb target regions
- Human and mouse
- Required amount of DNA: > 200 ng for reg. input
- Required amount of DNA: > 10 ng for low input



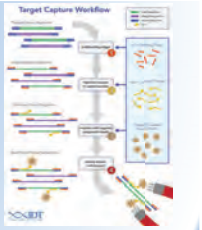




Targeted Exome Sequencing

Custom Gene Panels

- Why go targeted panel?
- Much better sensitivity
- Much lower cost, often less than $100 per sample
- High-throughput, hundreds of samples
- $100/gene setup fee from IDT
- Very flexible, pick and choose almost any gene
- OncoVar Cancer Gene Panel



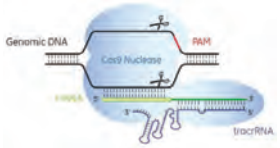
Special Sequencing Projects

ImmunoSeq

- Analysis of clonality based on TCR sequencing
- Commercial kit from Adaptive Biotech
- Data analysis pipeline using Adaptive webserver
- $240 per sample before OSTR subsidy
- $120 per sample after OSTR subsidy

CRISPR-Cas9 screening

- We have designed numerous assays and developed bioinformatics pipelines to check the efficiency of specific CRISPR sgRNA for knockout% or knockin%
- CRISRP single cell cloning/verification
- sgRNA screening for enrichment



16S microbiome analysis

- Automated high-throughput DNA extraction from fecal samples in 96 well format
- PCR library preparation of 16s V4 region
- Multiplex 96-384 samples per MiSeq run

Retroviral integration site analysis

- Each retroviral integration site can serve as a unique barcode for the cell
- We perform high-throughput retroviral integration site analysis, which can be use to track cell clonality

DNA Methylation Pipeline

Genome-wide Methylation Analysis

- Illumina Infinium 850k EPIC Methylation array
- Illumina Methyl Capture EPIC library/NGS
- New: mouse genome-wide Methylation array

Targeted Methylation Analysis

- Pyromark DNA sequencing for specific CpG
- Low cost, highly quantitative

Microarray Core Service

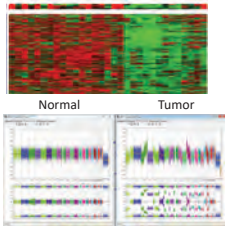
Low cost global expression array

- $160 per sample on Affymetrix Clariom S array.

OncoScan FFPE CNV array

- Designed and optimized for FFPE samples.
- Excellent CNV and LOH detection for clinical samples

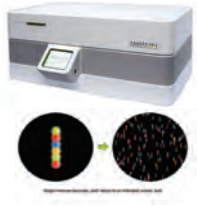
CLIA DMET for pharmacogenetics test



Focused Gene Panel Analysis

Nanostring

- Direct digital counting
- No amplification or enzymatic reaction
- Expression analysis for up to 800 genes
- 12+ sample multiplexing
- Off the shelf gene panels
  - Inflammation
  - Cancer
  - miRNA



HTG EdgeSeq

- RNase protection based assay
- Probes for targeted gene panel up to a few thousand genes
- miRNA or Oncology panel
- Readout is Illumina Seq count
- Can work directly with FFPE and cell lysate

Digital Droplet PCR

- Single gene or small panel of genes
- Can go up to 96 well format
- Absolute count, no standard needed
- Highly accurate
- Copy number, allelic discrimination

Contact: CCRgenomicsTechnologyLab@mail.nih.gov

- Xiaolin Wu, PhD, forestwu@mail.nih.gov, 301-846-7677

Frederick National Laboratory for Cancer Research

CCR Sequencing Facility

operated by Leidos Biomedical Research, Inc.

The CCR-SF mission is to utilize high-throughput sequencing technologies in order to enrich cancer research and ensure that the NCI community can leverage the leading-edge of Next-Generation Sequencing technology.

Technology, Expertise, and Collaboration

The recent introduction of DNA sequencing instruments capable of producing billions of DNA sequence reads in a single run is rapidly changing the landscape of genetics and cancer biology. This technology is providing the ability to answer complex questions with unimaginable speed. The Sequencing Facility (SF) is a second and third generation high throughput sequencing core laboratory located at the Advanced Technology Research Facility (ATRF) in Frederick, MD, and offers sequencing services on both the Illumina and Pacific Biosciences platforms.

These two platforms have complementary strengths and can be used separately or in a combined approach to answer many genomics questions. The established Illumina platform, with access to two state-of-the-art Novaseq 6000, two Nextseq 2000, two Nextseq 500 and two Miseq sequencers, has been in production at the SF since 2009. The newer Pacific Biosciences platform, the PacBio Sequel II, with polymerase read lengths averaging greater than 120,000 bases per molecule, with maximum read lengths > 200,000 bases, facilitate genome assembly and mapping of repetitive regions with high fidelity circular consensus reads. Both offer unique advantages for different sequencing applications, including whole genome sequencing, exome and transcriptome sequencing, targeted amplicon resequencing, ChIP-seq, base modification detection, and sequencing complex repeats, secondary structures, and AT and GC-rich sections of DNA.

Furthermore, a Chromium 10X Genomics Platform and a Mission Bio Tapestri system for Single-Cell analysis and Next-Generation Optical Mapping using BioNano Saphyr for structural variation analysis are available for production at the SF.

SF scientists provide consulting throughout the design and execution of your project to ensure the most effective experiments are being conducted to help you efficiently address your research needs.

Second-Generation Sequencing Applications Using Illumina Platform

De Novo Sequencing

- **Paired-end reads – Generate long scaffolds and contigs using multiple insert lengths**
- **Read length – Use paired-end reads in excess of 108 base pairs (bp) for mammalian-scale *de novo* assembly**

Resequencing

- **SNP discovery and confirmation**
- **Insertions and deletions (indels) and copy number variations (CNVs)**
- **Structural variant discovery**

Transcript Profiling and Discovery

- **Characterizing splice variants, coding SNPs, and relative expression of transcripts**
- **mRNA-Seq, tag profiling, and small RNA analysis**
- **Measuring alternative isoforms, discovering novel structures and coding SNPs**

Bisulfite Sequencing and DNA–Protein Interactions

- **Detecting variations in methylation signatures at single-base resolution**
- **Sequencing repetitive bisulfite-converted genomes using proprietary reversible-terminator chemistry**
- **CpG methylation, histone modifications, chromatin structure, or DNA–protein interactions**
- **Chromatin immunoprecipitation-paired sequencing approach (ChIP-Seq)**

Third-Generation Sequencing Applications Using Pacific Biosciences Platform

De Novo Sequencing

- **Long read lengths, averaging more than 25,000bp with some reads as long as 75,000bp, can be used to scaffold bacterial and viral genomes and enable the discovery of structural variation**
- **A hybrid strategy using PacBio data and any short-read sequence data can be used to assemble larger genomes**

Resequencing

- **Plasmids, BACs, and other clones can be easily sequenced on the PacBio at a more reasonable cost than other platforms, and without needing to be highly multiplexed.**
- **Multiplexed amplicons allow for targeted areas of the genome to be efficiently examined, and SNPs to be discovered and confirmed**

Full-Length Transcript Sequencing

- **One read, one transcript, no assembly required**
- **Complete information on alternatively spliced exons, transcriptional start sites, polyandenylation sites and strand orientation**
- **Greater gene coverage for both 5' and 3' ends compared to other sequencing platforms**

Base Modification Detection (In Development)

- **Direct detection of several modified bases from native (unamplified or treated) DNA**
- **Polymerase speeds up or slows down in predictable patterns at these base modifications; these patterns can be analyzed to determine the locations of modifications**

Reasons for allowing the Sequencing Facility to assist you in your genomic research needs:

Flexibility

SF offers a tremendous range of genomic applications to fit every research need and budget. This centralized service promises to maximize cost effectiveness and the economies of scale.

Service

SF provides industry-leading practices, technologies, and expertise with a consistent focus on quality, efficiency, and cost.

Collaboration

SF scientists are available to work with you on the design and execution of your research project. We focus on technology development so we can ensure that the NCI community is able to stay on the leading edge of next-generation sequencing technology.

If you are interested in learning more or working with SF, contact:

Mr. Bao Tran, Director of Operations
301-360-3460
tranb2@mail.nih.gov

Dr. Dwight Nissley, Director, Cancer Research Technology Program
301-846-1181
nissleyd@mail.nih.gov

https://ostr.cancer.gov/resources/fnl-cores/sequencing-facility

NCI-Frederick

leidos
Leidos Biomedical Research, Inc.

Operations and Technical Support Contractor at NCI-Frederick

203211

CPTR Mass Spectrometry Resource

The mass spectrometry resource, located in Building 37 on the Bethesda campus, brings cutting-edge protein-based technologies to the NCI CCR community to facilitate basic and translational research. We seek to help researchers with their proteomics experiments, beginning with initial experimental design and sample preparation and continuing through data interpretation and design of follow-up experiments. The resource operates collaboratively with partial cost recovery.

Current Instrumentation

Thermo Orbitrap Fusion Tribrid

- Used for sensitive peptide analysis
- Configured with ETD capability for expanded analysis of post-translational modifications

Thermo Exploris 480

- Ultrafast speed and sensitivity for peptide/proteome analyses
- Optimized methods for quantitative proteomics

SCIEX Q-TOF X500B

- Built for biologics characterization and can perform intact mass, subunit analysis, and peptide mapping characterization

Thermo Q Exactive Plus Orbitrap

- Configured for HDX-MS analyses

Agilent 6495 Triple Quadrupole

- Triple quadrupole instrument for targeted quantitation of molecules, including clinical samples

Thermo iCAP-q

- Elemental analysis of biological samples
- Can achieve ppt-ppb quantitation of elements

Inquiries

Lisa Jenkins, *Facility Manager*
Lisa.Jenkins@nih.gov
Xu Zhang
Xu.Zhang@nih.gov

Examples of Current Experiments

Intact Molecule Analysis

- Intact protein mass measurement
- Analysis of secreted small molecules in media

Interactome Analysis

- Protein-Protein interactions
- Protein-nucleic acid interactions

PTM Analysis

- Phosphorylation
- Acetylation

Global Quantitation

- Organelle/cell body proteomes
- Secreted proteomes
- SILAC, isobaric (TMT) tags, label-free methods

Targeted Quantitation

- Quantitation of proteins in clinical samples
- Quantitation of specific biomolecules

Structural Mass Spectrometry

- Chemical crosslinking
- Limited proteolysis

Metal Ion Quantitation

- Pt, Cu, Fe levels in cells after treatment
- Gold nanoparticle uptake

Please contact us to discuss your unique biological questions and how mass spectrometry can be used to address them.

CPTR Nanoscale Protein Analysis Section

The Nanoscale Protein Analysis Section, located in Bulding 37 Room 1044, is a CCR resource specializing in evaluation, development and implementation of cutting - edge antibody-based analysis technologies to facilitate discovery and translational research. Provided services include consultation on platform selection and experimental design, sample preparation and performing experiments, as well as primary or complete data analysis depending on the platform. We operate collaboratively on a cost recovery basis.

Current Technologies

Automated Capillary Immunoassay system: the Simple Western technology

- Fully automated high-throughput Western analysis of multiple targets in low amount of sample
- Employs MW (size-based) or IEF (charge-based) separation, followed by target-specific immunoprobing to profile proteins and respective post-translational modifications
- Well established assays for about 300 targets, including ~120 assays in PBMC samples

Highly multiplex immunofluorescence imaging: CODEX technology

- Detects single cell level expression of up to 40 different proteins from a single tissue section preserving spatial information
- Preconfigured and customizable antibody panel for human FFPE, human fresh frozen and mouse fresh frozen samples

Digital Spatial Profiling with GeoMx

- Offered in collaboration with Genomics core
- works on FFPE (for protein and RNA) or fresh frozen (RNA) tissues
- enables ROI based detection of mouse and human protein and RNA targets:

HUMAN:

- **Protein:** up to **84 targets** with nCounter® readout
- **RNA: 96 target** IO panel with nCounter readout
  CTA panel: 1,833 genes across 55 pathways using NGS readout

coming soon **WTA:** *30 million reads/sample, 18,000 plus genes*

MOUSE:

- **Protein:** up to n=53 IO targets
  coming soon: neurooncology and cell death panels
- **RNA:** *coming soon* **CTA** panel

In-solution multiplex sandwich Elisa: Luminex XMAP Technology

- Quantitative multiplex analysis of cytokines, chemokines, growth factors, metabolites, etc. in low amount of sample (serum, plasma, cell culture supernatants) using preconfigured and custom assay panels

Single Cell Western system

- Quantitative detection of multiple proteins in individual cells in parallel for 1000-2000 cells

Contact us:
Bldg 37, Room 1044

Website: https://cptr.ccr.cancer.gov

Inquiries:
Noemi Kedei, *Facility Manager*
kedein@mail.nih.gov
240-760-6922

Protein Characterization laboratory (PCL) CCR dedicated mass spectrometry service, focus on protein, proteomics and metabolite analysis

The laboratory is located at the ATRF in Frederick MD, and is a dedicated CCR mass spectrometry facility focusing on protein and metabolite characterization and molecular interaction/kinetic measurement by SPR. In addition, we have expertise in chromatographic separation of a variety of biological molecules as well as assay development. We engage in both short and long-term projects, all depending on the needs of individual NCI laboratories.

Protein and Proteomics:

- **Protein identification:**
  - Gel bands, in solution, tissue
  - Other biological matrixes
- **Global Quantitative proteomics methods:**
  - TMT (11 and 16 plex)
  - Label free quantitation
  - Dimethyl labeling
  - SILAC
- **Macromolecular interaction:**
  - Protein-protein interaction
  - Protein-DNA/RNA interaction
  - Protein-peptide interaction
- **PTM analysis:**
  - PTM mapping (phosphorylation, ubiquitination etc)
  - Global PTM analysis using quantitative methods
  - Method development for new/novel PTMs
- **Intact mass analysis:**
  - Antibody – drug conjugation
  - Accurate mass on recombinant protein
- **Off-line HPLC fractionation:**
  - Protein
  - Peptides
- **Method development:**
  - Sample preparation and liquid chromatography
  - Mass spectrometry data acquisition
  - Data analysis
- **Instruments:**
  - Orbitrap tribrid: Eclipse and Fusion
  - Orbitrap QE: Classic and HF

Metabolite analysis:

- **Targeted metabolite analysis and assay development:**
  - Quantitation of known metabolites and small molecules.
  - Targeted assay development.
  - Analysis of multiple samples using specific assay(s)
  - Targeted identification and quantitation of lipids
  - Instrument: Triple quadrupole and QE high resolution
- **Metabolomics analysis:**
  - Un-targeted identification of metabolites in different biological samples.
  - Different chromatographic separation (RP, HILIC)
  - High resolution mass accuracy with MS2 fragmentation spectra for increased identification accuracy.

Molecule interaction and kinetic measurement:

- **Surface plasmon resonance (SPR):**
  - Binding kinetics (on and off rates)
  - Binding affinities

Contact and Information:

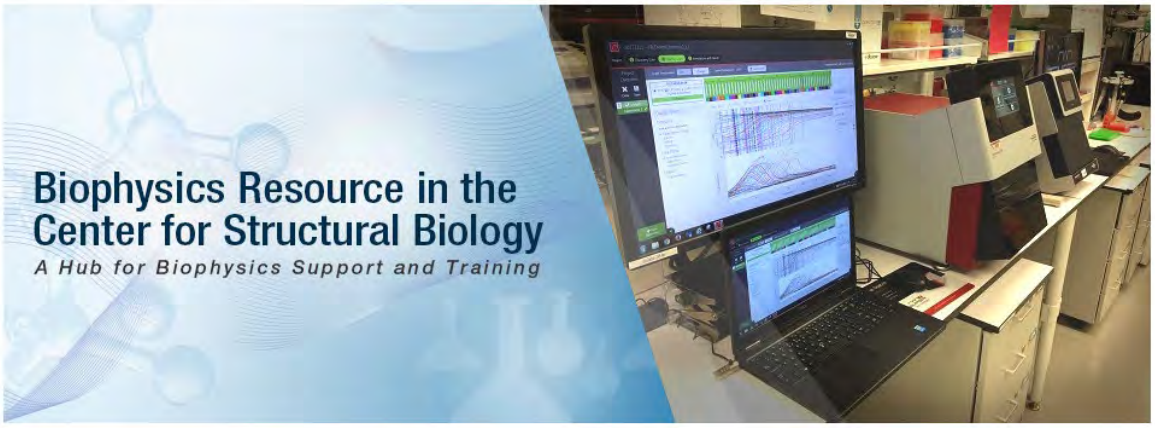
Thorkell Andresson
andressont@nih.gov

Protein and Nucleic Acid Production (CCR) – Center for Cancer Research (CCR) Dedicated Services

FNLCR scientists provide unique value in working with the biomedical industry, academic research institutions, non-profits and other organizations, consistent with the mission of the NCI. FNLCR scientists utilize their extensive knowledge and expertise in a variety of scientific areas to analyze data and provide solutions through partnerships.

The Protein Expression Laboratory (PEL) provides a full suite of protein production services with "DNA to Cells to Protein" convenience. PEL utilizes the unique technologies listed below to enable rapid, low-cost protein production.

Service Type	Contact	Service Areas & Description
Combinatorial Cloning	Carissa Grose 301-360-3427	• Custom cloning and mutagenesis for protein expression • Sequence validation of clones is included • Combinatorial Gateway recombination-based cloning platform • Consultation on clone design and downstream usage • New baculovirus genomes and production systems for higher virus titer and enhanced protein quality
Protein Production	Jane Jones 301-846-1201	• High-throughput scouting of expression/purification conditions at microscale • Supports large numbers of parallel clones varying in promoters, solubility/purification tags, and expression conditions • Expression in bacteria, insect cells, and/or mammalian cells • Large-scale purification from ugs to grams • Consultation on scale-up potential, protein quality issues, buffer conditions, and yield
Eukaryotic Expression	Jane Jones 301-846-1201	• Baculovirus production and titer, including BacMam, with advanced titering methods • Insect cell expression in cell line which has improved protein production yields • HEK293 expression using transient transfection • Bioreactor-based protein expression for reduced cost large-scale production • Experimental design consultation, data analysis and interpretation



The Biophysics Resource (BR) operates as an open, shared-use facility; in general, BR users learn to operate the instruments and conduct their own experiments. BR staff members train all first-time users and are also available to consult on experimental design/analysis or collaborate with them on more complex studies.

Some of the equipment is too delicate to permit casual use and some studies may require special expertise in experimental design, data analysis, and interpretation. In these cases, users typically collaborate with BR staff on their research projects.

The BR offers cutting-edge biophysics technology in the following areas:

- **Circular dichroism** (CD) spectroscopy to study the optical activity and conformation of biomacromolecules;
- Steady-state and time-resolved **fluorescence spectroscopy** to study the structure and environment of biomacromolecules and the mechanism(s) of these interactions;
- **Isothermal titration calorimetry** (ITC) for thermodynamic characterization of biomacromolecular interactions;
- **Differential scanning fluorimetry** (nano-DSF) for determining thermal stability and transitions in biomacromolecular systems;
- **Liquid chromatography with mass spectrometry** detection (LC-MS and LC-MS-MS) for macromolecular mass characterization;
- **Dynamic light scattering** to determine macromolecular particles size and degree of aggregation;
- **Microscale thermophoresis** (MST) for macromolecular binding studies;
- **UV-Vis spectrophotometry** with thermal scanning option;
- **switchSENSE** molecular dynamics technology for binding, kinetic and sizing studies.

Contact: Sergey.Tarasov@NIH.GOV 301-846-1223 Marzena.Dyba@NIH.GOV 301-846-6105

NCI-Frederick Bldg./Room 538/111

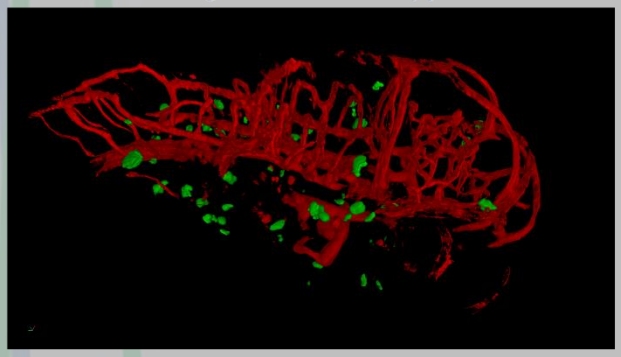
Microscopy and Digital Imaging in the CCR Microscopy Core

The CCR Microscopy Core provides NCI investigators access to state-of-the-art imaging tools and techniques, including light sheet fluorescence, high-resolution confocal, multi-photon, and super-resolution microscopy. The primary mission of the Core is to support the microscopy and digital imaging needs of investigators studying the biological structures and cellular processes involved in the cell biology of cancer. The Core is located in building 37, room B114 on the Bethesda NIH campus and operated by 3 support staff; Michael Kruhlak, PhD as Facility Manager and biologists Langston Lim, MSc and Andy Tran, PhD. The instrumentation and services of the CCR Microscopy Core are open and accessible to all NCI and NIH researchers.

Microscopes:

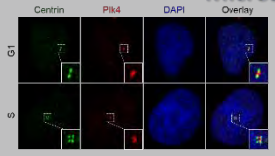
- **Zeiss ELYRA SIM Super-resolution Microscope**
- **Zeiss LSM 880 Airyscan Super-resolution Microscope**
- **Nikon SoRa Spinning Disk Microscope**
- **Zeiss LSM 780 Confocal Microscope**
- **Zeiss Z1 Lighsheet Microscope**

Lightsheet Microscopy



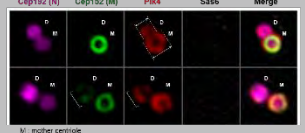
Joanna Thomas (Tanner Lab), Laboratory of Cell Biology

Super-resolution – Structured Illumination Microscopy (SIM)



Confocal Image

The ring shape of Plk4 at centrosomes can not be resolved using confocal microscopy. centrosomal Plk4 just looks like a dot.



3D-SIM image

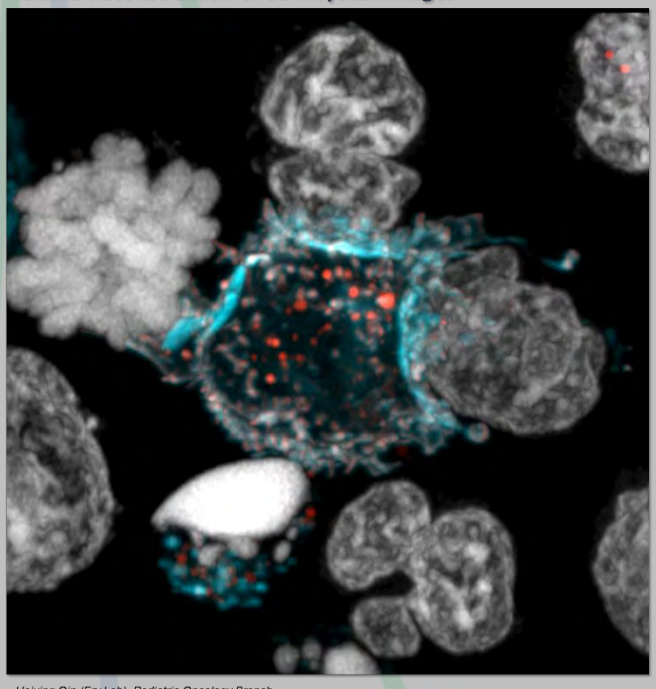
Using 3D-SIM, several centrosomal proteins such as Cep192, Cep152 and Plk4 are resolved as a ring-like structure with different sized rings, depending on location from the center.

Jung-Eun Park & Kyung Lee, Laboratory of Metabolism

Image Processing and Analysis

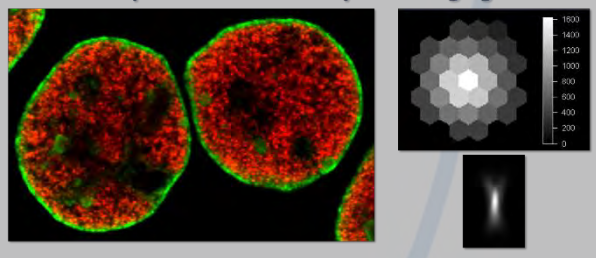
- Numerous software packages are available for processing and measuring image data, as well as developing customized analysis algorithms including: Zen (macro editor/python), Imaris (including Matlab XTensions), Arivis with virtual reality rendering and machine learning, ImagePro Plus with 3D reconstructor and deconvolution, ImageJ, R with shiny apps, and MIPAV.
- Separate high end workstations are available for image processing of SIM images, Airyscan streaming and processing, as well as image processing and analysis.

Volume Reconstruction of 3D Airyscan Images

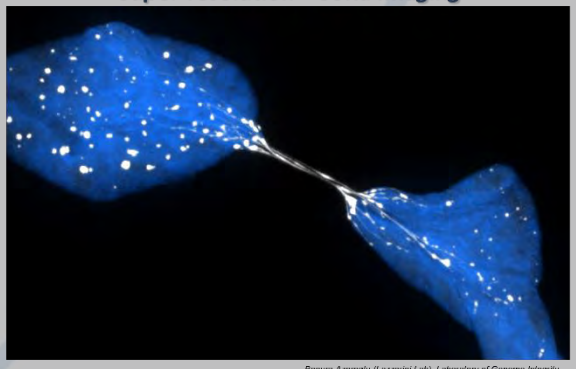


Haiying Qin (Fry Lab), Pediatric Oncology Branch

Super-resolution – Airyscan Imaging



Super-resolution – SoRa Imaging



Bennura Azeroglu (Lazzerini Lab), Laboratory of Genome Integrity

Contact Info:
Michael.Kruhlak@nih.gov
Langston.Lim@nih.gov
Andy.Tran@nih.gov

https://labshare.nih.gov/nci/CCR-Confocal/SitePages/Home.aspx

Laboratory of Genitourinary Cancer Pathogenesis Microscopy Core Facility

Center for Cancer Research, National Cancer Institute

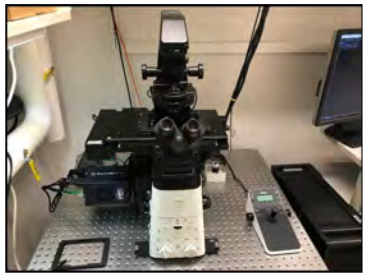
Introduction

The Laboratory of Genitourinary Cancer Pathogenesis (LGCP) Microscopy Core Facility provides state-of-the-art equipment, materials, and expertise to researchers in the LGCP. The Core is a comprehensive collaborative unit that supports the researcher from experiment design through data acquisition, image analysis, and preparation of figures for publication.

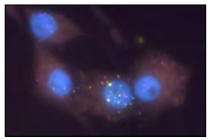
The Core is open to all NCI intramural researchers, although LGCP scientists have priority use of the equipment. Researchers from other institutes are also welcome to use the facility. Core staff provides individual training for all new users. Core personnel are also able to assist with training in the techniques of specimen preparation, immunofluorescent staining and image acquisition using the appropriate equipment.

Unless special arrangements are made, the equipment in the facility may be used Monday to Friday, 10:00am – 6:00pm

smRNA FISH Microscope



The Core has a Nikon **Eclipse Ti2-E** inverted microscope that is optimized for single molecule RNA FISH imaging. It is equipped with a Photometrics Prime BSI sCMOS camera and a Lumencore SOLA SE 365 FISH light engine. The microscope has a motorized scanning stage with Z piezo insert for fast Z and multi position imaging. Available objectives are all plan Apochromat, and include a 10x/0.45 DIC, 20x/0.75 DIC, 40x/1.3 Oil DIC, 60x/1.4 Oil DIC, and a 100x/1.45 Oil DIC objective. The microscope has a number of available filter cubes, including narrow band filters optimized for FISH imaging using DAPI, FITC, Gold, Red, and Cy5. Images are acquired using Nikon Elements software.

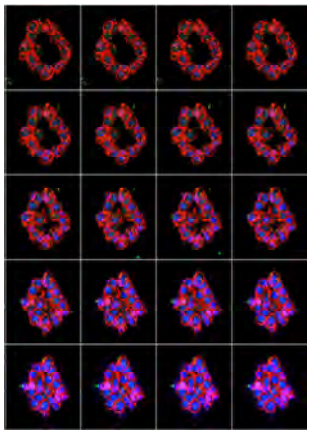


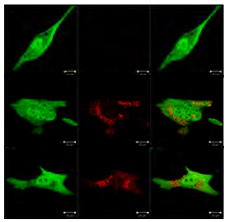
smRNA FISH staining of PC cells

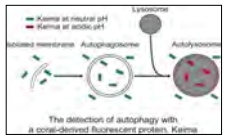
Masking for smRNA FISH Quantification

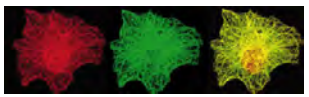
LSM780 Confocal Microscope

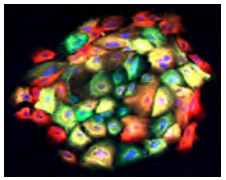
The core facility houses a **Carl Zeiss LSM780** scanning module mounted on a motorized AxioObserver Z1 inverted fluorescent microscope. This confocal microscope is equipped with two PMTs and one 32 channel GaAsP spectral detector. It has 6 laser lines (405nm, 458nm, 488nm, 514nm, 543/561nm, 594nm & 633nm), and has a fully automated stage, Definite Focus module, and stage top incubation system for long term live cell imaging. The microscope is equipped with a variety of objectives including an EC Plan Neofluar 10x/0.3 DICI, Plan Apochromat 20x/0.8 DICII, a Plan Apochromat 40x/1.4 DIC, Plan Apochromat 63x/1.4 Oil DIC, and Plan Neofluar 100x/1.3 Oil DIC.



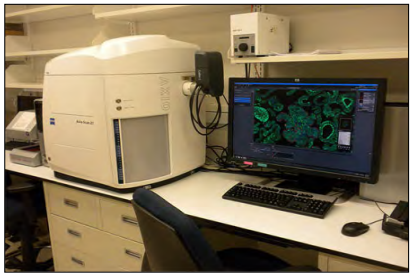




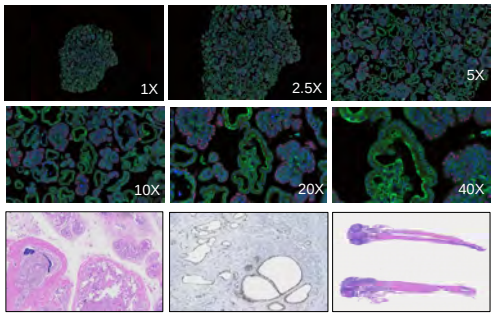




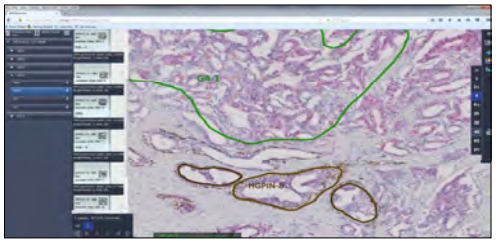
Axio Scan Z1 Slide Scanner



The Zeiss **AxioScan Z1** is a fully automated slide scanner able to create both brightfield and fluorescent virtual slides in high quality and at speed. The system is equipped with a Colibri7 LED source with 7 lines allowing multiplex imaging. Objectives available include a 5X/0.25, 10x/0.3, 20x/0.3, and a 40x/0.95 Corr. Up to 100 slides can be digitized at one time, and images are acquired and analyzed using Carl Zeiss Zen software.

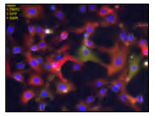


Zen Browser is server-based image data basing software accessible via any web browser. Used for storage, organization, and backup of large digital data sets. It allows for remote collaboration and digital pathology with annotation

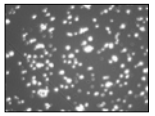


Epifluorescence Microscopes

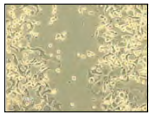
The Core has two Zeiss **AxioObserver Z1** fluorescent microscopes equipped with objectives from 5x to 63x, Axiocam 506 or Hamamatsu OrcaFlas4.0 digital camera, and ZEN software which allows acquisition of high resolution color, black and white or fluorescent images of fixed and live cells.



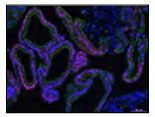
Stained cells



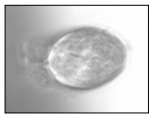
Cell adhesion



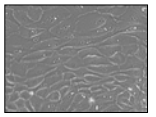
Cell migration



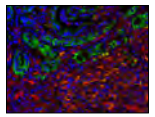
Stained sphere sections



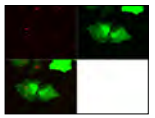
Sphere forming assays



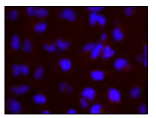
Cell morphology



Stained tissue sections



GFP tumor cells labeled with fluorescent FeO_2 beads



Duo-Link Assay for studying protein-protein interactions

Contact Information

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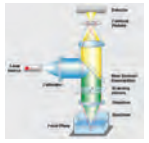
Bethesda, MD 20892
Phone: 240-760-6824
E-Mail: laker@mail.nih.gov

The Laboratory of Cellular and Molecular Biology Microscopy Core Facility: Building 37 Room 2033

Valarie A. Barr and Itoro Akpan

Use of instruments: Training is provided for all instruments. Instrument availability is shown on an Outlook-based calendar.
Trained users from outside LCMB may use the equipment during hours that the Core staff are present, 8:30am-5pm weekdays.
To begin a project at the LCMB Core, send an email to Valarie Barr barrv@mail.nih.gov

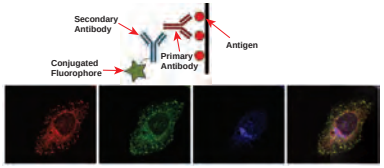
Laser Scanning Confocal Microscope

Equipment:
- Objectives-100X, 63X, 40X, 32X, 20X
- Laser Lines-405nm, 445nm, white light laser 470-740nm
- Detectors-2 PMT, 3 Hybrid high sensitivity PMT
- Emission- Prism based selection
- On-stage incubator with CO_2 capability

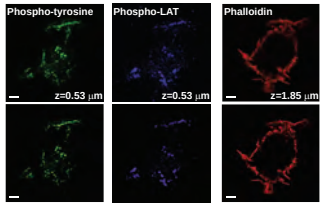
Commonly used techniques

1) Indirect immunofluorescence

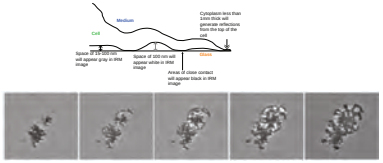


Internalized Epidermal Growth Factor in HeLa cell

2) Deconvolution after acquisition

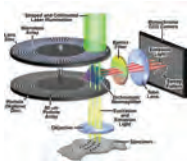


3) Interference reflection microscopy



Activation-induced spreading in Jurkat T cell

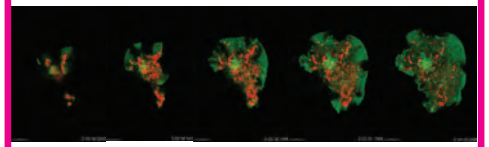
Spinning Disk Confocal Microscope

Equipment:
- Objectives-100X silicone, 100X, 60X, 40X, 20X, 10X
- Laser Lines-405nm, 445nm, 488nm, 514nm, 561nm, 594nm, 647nm
- Detector-3 sCMOS Flash4 or 1 sCMOS Prime 95B
- Emission-Band pass filters

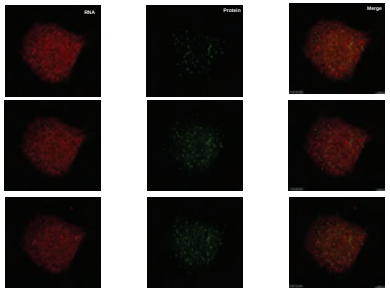
Commonly used techniques

1) Fast high-resolution (6.5 µm sensor pixels) live cell imaging with up to 3 simultaneous channels



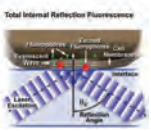
Imaging of the T cell receptor and a SNARE protein, syntaxin 3, in a Jurkat T cell

2) Fast high-sensitivity (11 µm sensor pixels) live cell imaging with up to 2 simultaneous channels using back-illuminated sCMOS



Imaging of localized RNA molecules and translated protein in a fibroblast cell.

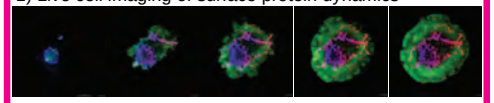
Total Internal Reflection Microscope

Equipment:
- Objectives-100X,60X,40X, 20X
- Laser Lines-440nm,488nm, 514nm, 561nm, 633nm
- Detectors-2 EM-CCD Cameras
- Emission-Band pass filters
- On-stage incubator with CO_2 capability

Commonly used techniques

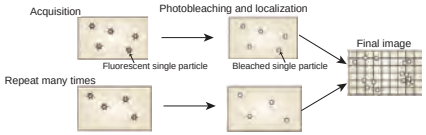
1) Live cell imaging of surface protein dynamics



Clustering of the T cell receptor, the kinase ZAP-70 and actin in a Jurkat T cell.

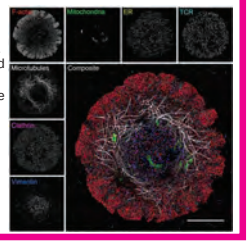
2) Single Molecule Localization Microscopy

In this technique, a few molecules put into a fluorescent state and are imaged until they are completely photobleached. The centers of diffraction limited Airy disks are determined and the process is repeated thousands of times to build up a picture with thousands of well-resolved individual molecules.

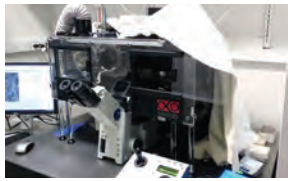


Direct Stochastic Optical Rendering Microscopy of Cellular Structures in a Jurkat T cell

The blinking of dye molecules can be used to produce the localization image. Here the dye Alexa 647 was conjugated to primary antibodies which were used for immunofluorescent staining. Multiple rounds of staining were used to image several molecules: phalloidin (red), anti-tubulin (white), anti-clathrin heavy chain (magenta), anti-Vimentin (blue), anti-HSP60 (green), anti-calnexin (yellow) and anti-T cell receptor phospho-zeta chain (cyan)



Long Term Incubation




Equipment:
- Objectives-40X,20X,10X, 4X
- Epifluorescence and phase optics
- Detector-CCD Camera
- Emission-Band pass filters
- Full incubator with CO_2 capability

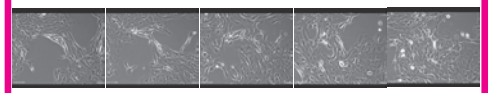
Commonly used techniques

1) Long term live cell fluorescent imaging

Fluorescent images of the cell nuclei make it easy to track cell migration.

2) Long term live cell phase imaging

The phase plate shifts the phase of the light passing through it based on where the light hits. Light passing through the sample hits the phase plate differently than light that did not go through the sample, because the sample diffracts the light. When the phase-shifted light rays recombine, they make a diffraction image where the difference in phase appears as different shades of gray.



The phase images show ruffling and changes in cell shape.

The LCMB Microscopy Core provides equipment for light microscopy, training and advice on imaging experiments. While our primary responsibility is to assist researchers in the LCMB, scientists from other laboratories and other institutes can also use our services. We support all stages of research from experimental design to preparation of figures for publication. There is no charge for use of our equipment.

NCI/CCR/LRBGE Optical Microscopy Core

T. S. Karpova, D. A. Ball

OUR MISSION

Welcome to the NCI/LRBGE Optical Imaging Core!

NCI/CCR/LRBGE Optical Microscopy Core (OMC) is open to everyone within the NCI, and we also welcome collaborations with investigators from other NIH institutes or beyond. We operate state-of-the-art fluorescence microscopes, confocal, wide-field, and super resolution, and custom-built HILO microscopes, that enable a broad spectrum of fluorescence imaging experiments. We provide expert guidance and troubleshooting in the design, execution, interpretation and publication of these experiments. We offer the conventional methods of fluorescence microscopy, including time lapse imaging of cells in 2D or 3D, with computational methods for removal of out of focus light (deconvolution), as well as sophisticated quantitative microscopy: FRAP, FRET, superresolution (SIM and PALM) and single molecule tracking (SMT). The staff participates in the development of quantification microscopy technologies.

FLUORESCENCE MICROSCOPY

Although electron microscopy provides better resolution, fluorescence microscopy permits live cell analysis. This field has grown explosively due to the development of appropriate technology.

Digital enhancement facilitates visualization of dim objects, and subsequent computerized image processing, and quantitative analysis. Fluorescence markers have been developed to observe proteins, lipids, DNA and RNA. These include fluorescent antibodies, fluorochrome-labeled oligonucleotides, GFP and other fluorescent protein fusions, fusions to ligand-bound Halo Tag, Snap Tag, CLIP tag etc., and fluorescent stains for lipids, DNA and RNA. Specific markers are suitable for live cells and for biophysical quantification of diffusion, of binding rates, and of protein dimerization by FRAP, FCS, N&B. Biosensors allow observation of protein interactions and enzymatic activity dynamics by FRET. Resolution in X, Y, and Z may be improved by deconvolution and by super resolution: SIM (Structural Illumination Microscopy), PALM (Photoactivation Localization Microscopy) or STED (Stimulated Emission Depletion Microscopy). This advanced technology is available at CCR Cores, including OMC.

SIM/ PALM SUPER RESOLUTION

Elyra PS.1 superresolution (SR) microscope for photoactivated localization microscopy (PALM) and structured illumination microscopy (Carl Zeiss Inc) combines two microscopes in one. The system includes 405, 488, 561 and 640 nm lasers, Andor EMCCD for PALM/dSTORM. 3D PALM is achieved by a double-phase ramp. The software includes PALM and SR-SIM processing module.

SR methods allow the up to 4-5 times increase of microscopy resolution beyond that of the conventional light optical imaging (~ 200 nm in XY and ~ 700 nm in Z). SR is beneficial (1) for the high resolution and 3D maps of the intracellular structures, neuronal synapses, intercellular contacts, large chromatin domains, cytoskeleton architecture etc., (2) for the accurate measurements of the cellular objects, (3) for the resolution between densely packed structures such as individual transcripts in the cytoplasm, (4) for quantification (molecule counting in organelles and structures).

High Resolution

WIDE-FIELD DECONVOLUTION

Deconvolution microscopy computationally reduces out-of-focus light. The Core a Delta Vision Elite (Applied Preision. Inc) deconvolution fluorescence wide-field inverted microscope: Seven LED light sources are available for imaging in regular and fast mode. Images are collected by 14/16 bit sCMOS camera. The system is based on inverted Olympus microscope. It is equipped with CO2/temperature-controlled environmental chamber.

This microscopy system can be used not only for deconvolution, but also for low-light imaging of live specimens in multiple channels. An automatically controlled stage allows imaging of several fields of view in the same time-lapse sequence. Elite is equipped with environmeental chamber controlling temperature and CO2.

Images are deconvolved by the SoftWoRx program to enhance the resolution and to reduce the out of focus light.

Deconvolution

Biophysics in vivo

Single Molecule Tracking HILO

The Core custom-built a HILO (highly inclined laminated optical) microscope suitable for single molecule tracking of individual molecules. This technique increases signal-to noise ratio and allows observation of low light signal; thus it allows observation and tracking of individual molecules (SMT).

The instrument consists of TIRF Olympus IX81 microscope, four lasers (405 nm, 472 nm, 561 nm, 647 nm) controlled by AOTF and a custom set of filters. The system is controlled by Micromanager software.

Proteins to be observed must be fused to Halo Tag protein that makes covalent links with Halo-specific fluorescent ligands. At a low density of labeling, individual molecules may be observed and tracked in 2D. Tracking and biophysical quantification are performed with custom-developed MatLab based software.

This technique is still under development.

LOCATION AND CONTACTS

The Core is located in Building 41, Rm. C615 (first and only floor).

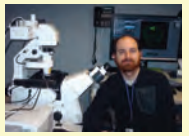
Director: Dr. Tatiana Karpova,
240-760-6637
karpovat@mail.nih.gov
https://confocal.cancer.gov

Dr. David Ball
240-760-6577
David.ball@nih.gov

First time users should contact Dr. Tatiana Karpova to discuss the proposed work and for advice on which approaches to take. Contact Dr. Tatiana Karpova to sign up for instrument or computer time.

Confocal Imaging

LSM780 CONFOCAL MICROSCOPE



Confocal imaging optically reduces out of focus light, thereby improving image contrast. The Core has the Zeiss LSM880 (above) and Zeiss LSM780 confocal microscope.

The Zeiss LSM780 confocal inverted microscope equipped with sensitive GaASP detector is used for optical sectioning in multiple channels, live imaging, FRAP and FRET, DNA damage experiments and for the biophysical Number and Brightness and HOMO FRET experiments. Laser lines available are 355, 405, 458, 488, 514, 561, 594, 633 nm. This system is equipped with a temperature and CO2 control insert.

Live Imaging with Super Resolution

LSM880 AIRYSCAN MICROSCOPE

Airyscan Zeiss LSM880 confocal microscope is capable of the conventional confocal imaging. In addition, it is equipped with Airyscan module. This module improves resolution in XYZ beyond the diffraction limit for the live cultures and small animals (zebrafish).

The Zeiss LSM880 confocal inverted microscope is equipped with three sensitive GaASP detectors and Airyscan detector. Laser lines available are 405, 458, 488, 514, 561, 594, 633 and 730 nm. This system has a temperature and CO2 control insert and environmental chamber for live imaging.

Lattice Light Sheet Imaging

LATTICE LIGHT SHEET (LLS) MICROSCOPE

Lattice-generated light sheet illuminates only a thin slice of the cell compatible by thickness with a working distance of the objective. In comparison to other imaging methods, the lattice light sheet technique (1) substantially increases a signal to noise ratio and reduces the light exposure, (2) allows ultra-thin optical sectioning (3) ensures extremely low phototoxicity, (4) substantially increases a signal to noise ratio, (5) allows very fast time lapse imaging on the scale of milliseconds (6) increases resolution (XYZ = 230 nm x 230 nm x 370 nm). The thickness of the sheet is 0.4 um, and the length of the sheet may be modulated from 20 um to 70 um.

The system is equipped with water immersion objective (62.5 X total magnification).Two 2K x 2K sCMOS Hamamatsu Orca-Fusion camera, 6.5 um pixels, 80% QE, speed up to 100 fps. Four high-power 500 mW lasers (405 nm, 488 nm, 560 nm, 642 nm). OKO lab stage insert with temperature and CO2 control.

The NCI High-Throughput Imaging Facility (HiTIF) works in a collaborative fashion with NCI/NIH Investigators by providing them with the necessary expertise, instrumentation, and software to develop and execute advanced High-Throughput Imaging (HTI) assays. These can be paired to screen libraries of RNAi or CRISPR/Cas9 reagents to discover and characterize novel cellular pathways by functional genomics. In addition to functional genomics screens, HiTIF also develops HTI assays to extract and quantify single-cell information about the molecular mechanisms underlying rare or heterogeneous biological events in cellular populations.

To initiate a project with the HiTIF, the investigator first needs to request a consultation with the Facility Head via the HiTIF iLab Webpage.

Contact Details
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Head, High-Throughput Imaging Facility (HiTIF)
Laboratory of Receptor Biology and Gene Expression
Phone: 240.760.6696
Email: gianluca.pegoraro@nih.gov
https://ccrod.cancer.gov/confluence/display/HTIF/Home
Address: 41 Library Drive, Building 41, Room B909, Bethesda, MD 20892

Established Technologies

HiTIF Core provides infrastructure and expertise to NCI intramural scientists for:

- Fully automated, high-throughput fluorescence and bright-field microscopy (Yokogawa CV7000, Yokogawa CV8000)
- High-Content Analysis (HCA) to measure up to hundreds of cellular features from microscopy images
- Assistance with miniaturization of cell-based assays in 96-well and 384-well microplates
- Robotic sample preparation, reagent addition, and plate washing
- Characterization of cellular mechanistic pathways using RNAi- and CRISPR/Cas9-based screens
- High-throughput live-cell fluorescence microscopy
- Automated tracking and image processing of subcellular objects in live cells

Developing Technologies

- Third-generation automated microscopy instrument optimization/development
- Automated RNA in situ hybridization methods for single molecule detection of endogenous genomic DNA loci, or mRNA transcripts
- 3D HTI assays
- Deep Learning methods for cellular segmentation and classification of cellular objects

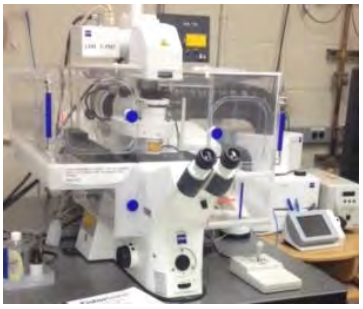
Usage Guidelines

CCR Investigators can request a STARS subsidy to partially defray costs associated with the development and execution of HTI projects at HiTIF. OSTR provides 50% subsidy towards smaller, pilot projects through the HiTIF. To request subsidies go to the OSTR Subsidy website. Subsidies for larger projects will be considered through the RRS (Resource Request System) website. When logged into the RRS select “OSTR Technology Supplement.”

Optical Microscopy and Analysis Laboratory (OMAL)

- Please discuss your science with us
- We train you to be the expert

Microscope Capabilities:

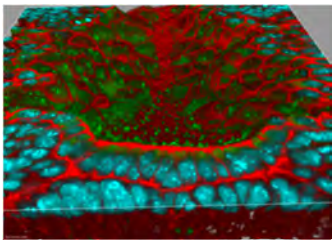


- Wide-field, fluorescence
- Confocal, 3D, live cell
- Super-resolution of fixed samples
- Plate reading microscope for high throughput
- Atomic force microscope

Sample Preparation Expertise:

- Live cell preparation and handling
- Unique restricted exchange environment chamber (REEC) for studying oxygen and nutrient gradients on live cells
- Multiplex antibody labeling
- Tissue clearing and expansion

Image Analysis Capabilities:



Confocal microscopy

- 3D / 4D visualization
- Deconvolution
- 2D / 3D cell segmentation
- Cell and molecule tracking
- Co-localization analysis
- High content image analysis

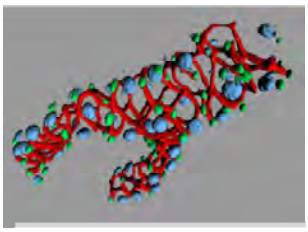
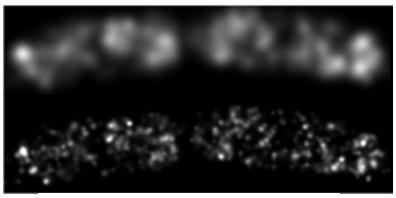


Image analysis

Personnel

Stephen Lockett	301 846 5515
Valentin Magidson	301 846 6092
William Heinz	301 846 1239
David Scheiblin	301 846 6002
Kimberly Peifley	301 846 6561
Sarah Flaherty	301 846 6452
Abigail Walke	301 846 5992
Sally Feng	301 846 5242



Super-resolution

Educational Material

- microscopyu.com
- ibiology.org/online-biology-courses/

https://confocal.ccr.cancer.gov/nci-microscopy-core-labs/frederick/omal/

High Resolution Imaging Center for Molecular Microscopy (CMM)

NIH NATIONAL CANCER INSTITUTE

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

Weimin Wu [1,2], Htet Khant [1,2], Rebecca Dillard [1,2], Tapan Kanai [1,2], Clayton Smith [1,2] and Natalia de Val [1,2,3]

[1] Center for Molecular Microscopy, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD, USA.
[2] Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research Inc., Frederick, MD, USA.
[3] Electron Microscopy Laboratory, Leidos Biomedical Research, Inc. Frederick National Laboratory for Cancer Research, Frederick, MD, USA.

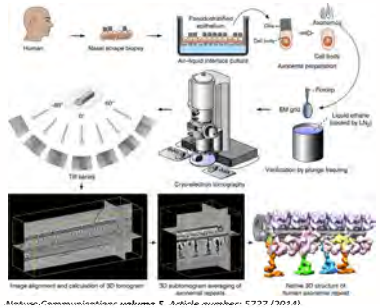
Our Goal: Technology, Expertise, and Collaboration

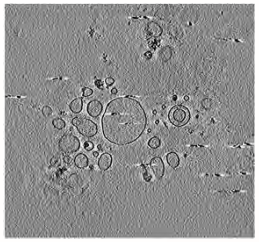
Our goal is to apply high resolution imaging technologies for 3D electron microscopy to problems of fundamental interest in cancer and HIV/AIDS biology. We are collaborating with the entire NIH community to perform structural studies of a wide portfolio of samples, from viruses to macromolecular complexes.

Our Techniques

➢Cryo Electron Tomography (Cryo-ET)

- ❖To visualize multiprotein complexes in their environment (*in-situ* analysis)
- ❖When you cannot purify the protein of interest
- ❖When you want to know the: location, interaction partners and occurrence





Cryo Tomogram of exosomes collected in our Tecnai T20

➢Micro-Electron Diffraction (Micro-ED)

- ❖When you have crystals but they are too small (micro and nanocrystals)
- ❖Determines structures at atomic resolution using electron diffraction
- ❖Electrons interact with matter stronger than X-rays and deposit less energy

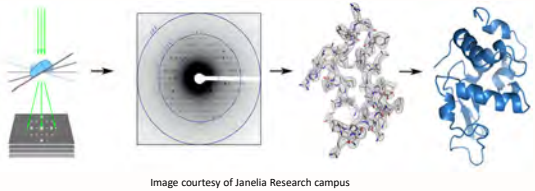
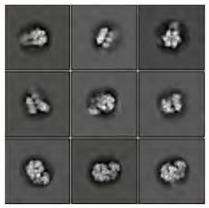
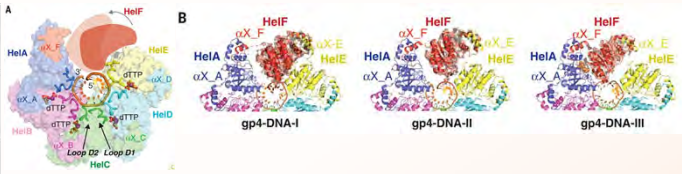


Image courtesy of Janelia Research campus

➢Single particle Cryo-Electron Microscopy

- ❖ No need for crystals
- ❖ No upper limit to size or complexity
- ❖ Near atomic resolutions (breaking the barrier of 2Å)
- ❖ Heterogeneous, dynamic or unstable samples can be analyzed
- ❖ Only micrograms are required
- ❖ Macromolecules visualized in a native state





Gao Y, Cui Y, **Fox T**, Lin S, Wang H, **de Val N**, Zhou ZH, **Yang W**. ***Science***. ***2019*** **Jan 24** pii: eaav7003. doi: 10.1126/science.aav7003.

Our Instruments

Our Successes (in the last 9 months!)

➢ **High resolution structures solved, by Single Particle Cryo-EM**

Total: 29 structures

- ❖**17 at 2.16A to 3A**
- ❖**11 at 3A to 3.84A**
- ❖1 at ~4A

➢ **Manuscripts: 5 published, 2 submitted and 5 in preparation**

Contact us to collaborate: natalia.devalalda@nih.gov

Our Team

Natalia de Val, PhD
Group Leader
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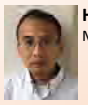
Weimin Wu, PhD
Data analysis specialist



Htet Khant
Microscopist

Clayton Smith
Microscopist

Rebecca Dillard, PhD
Microscopist

Tapan Kanai, PhD
Microscopist

Center for Molecular Microscopy
Cellular Imaging by Volume EM

What is volume EM?

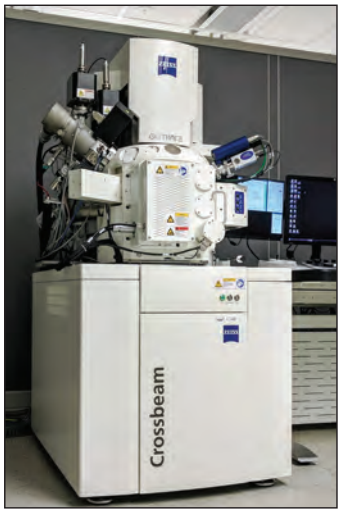
3D imaging of μm to mm-sized cell and tissue samples at nm-level resolutions, typically by emerging scanning electron microscopy (SEM) techniques, is considered "volume EM" .

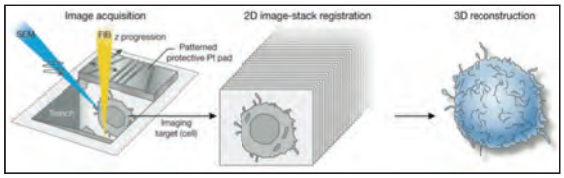
FIB-SEM & Array Tomography

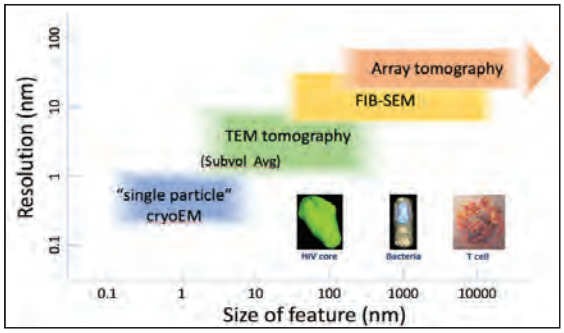
In focused ion beam scanning electron microscopy (FIB-SEM), iterative cycles of FIB milling and SEM imaging of fixed, stained and resin-embedded samples allows generation of 3-D image volumes at pixel sampling up to 3x3x3 nm. FIB-SEM imaging can capture 3-D architectural features in cells and tissues, and can be correlated with fluorescence images using appropriate protocols.

In array tomography (AT), resin-embedded samples are mechanically sectioned into ~50 nm slices, laid out on a substrate in order, and sequentially imaged by the SEM. AT resolutions are thus similar to FIB-SEM in xy, but lower in z. However, vast regions of up to ~ 1mm can be imaged by AT, albeit slowly, but this makes this approach more suitable than FIB-SEM for large tissue samples.

References:
Narayan K and Subramaniam S, *Nat Meth (2015),* Smith SJ *BMC Biol (2018),* Peddie CJ and Collison LM, *Micron (2014)*

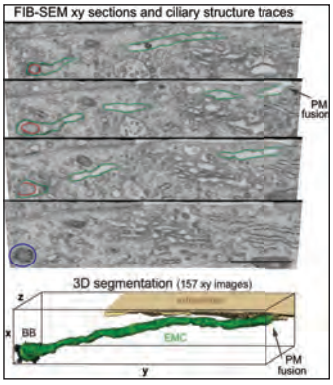


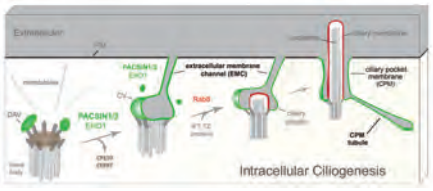




The Collaboratory

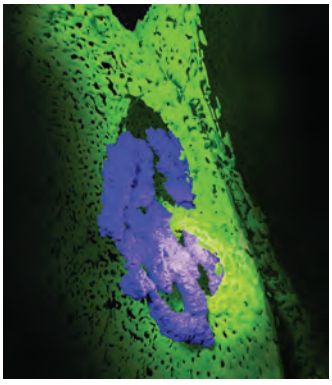
We collaborate with CCR researchers on important problems that are suited to our capabilities. We are committed to FAIR sharing (https://fairsharing.org/) of data included in publications.

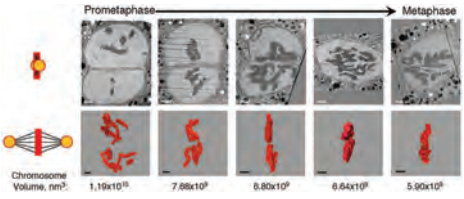




Correlative (CLEM) / FIB-SEM and 3D reconstructions of intracellular ciliation intermediates in RPE cells.

Insinna C, Lu Q et al, *Nat Commun (2019)*



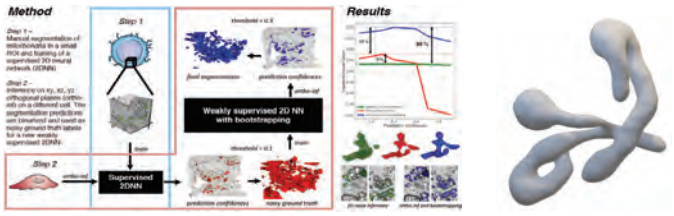


High Pressure Freezing followed by FIB-SEM imaging and 3D reconstruction of pronuclear membrane architectures in *C. elegans*.

Rahman M et al, *J Cell Biol (2020)*

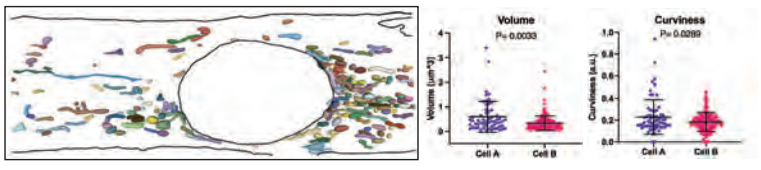
Technology Development

We actively pursue innovations in volume EM and computational approaches, expanding the technological toolkit and opening new avenues for future research.



Improved mitochondrial segmentation by a "2.5D" Neural Network that exploits isotropic resolution of FIB-SEM data.

Conrad R et al, *in review, Microsc Microanal (2020)*



Quantitative topological analyses of mitochondrial reconstructions, with 3D parameters extracted from volume meshes and skeletonization.

https://cmm.cancer.gov/volume-em


Kedar Narayan


Kunio Nagashima


Adam Harned


Irene Chang


Ryan Conrad

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

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Electron Microscopy Laboratory (EML)

NIH NATIONAL CANCER INSTITUTE

Frederick National Laboratory for Cancer Research

sponsored by the National Cancer Institute

Technology, Expertise, and Collaboration

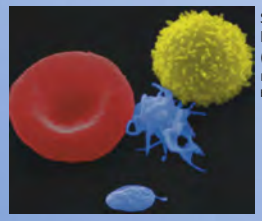
We develop protocols to meet the research needs of users and provide high-quality electron microscopy (EM) images. We offer the following techniques:

- Transmission electron microscopy (TEM)
- Scanning electron microscopy (SEM)
- Elemental analysis by energy dispersive X-ray spectroscopy (EDS)
- Cryo-electron microscopy (Cryo-TEM)
- 3D tomography
- Immuno-electron Microscopy (IEM)

We collaborate with users for specialized applications and for technology development needs.

SEM

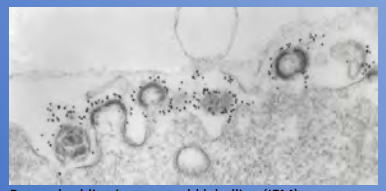
SEM analysis of cultured cells, embryos and bacteria can provide real-life images. Our SEM has variable pressure capability, an integrated gaseous SE detector, and a cooling stage that allows us to image hydrated samples.



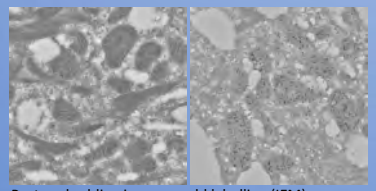
SEM image of a blood cell, lymphocyte, and platelets (red, yellow and blue respectively). False colors has been added for illustrative purposes

Immuno-electron Microscopy (IEM)

We provide with pre- and post-embedding immunolabeling to visualize and localize proteins of interest. Secondary antibodies (immunogold-conjugated) are provided by EML and tailored to primary antibodies provided by the user. IEM can simultaneously detect several proteins by using gold particles of different sizes.



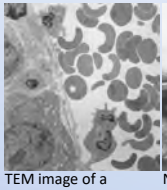
Pre-embedding immunogold labelling (IEM) of lentiviruses



Post-embedding immunogold labelling (IEM) of mitochondria

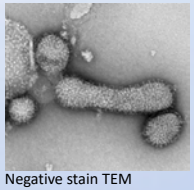
TEM

We offer traditional tissue and cell pellets processing, as well as *in situ* processing of adherent cells. Negative stain analysis can be completed with short turnaround times.



TEM image of a mouse tumor tissue

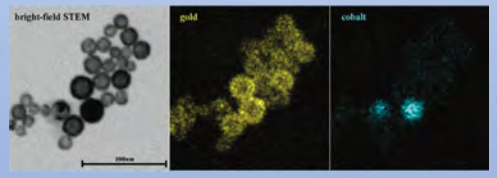
Negative stain TEM image of helicobacter bacteria



Negative stain TEM image of influenza virus

STEM/EDS

Our FEI T12 microscope with SEM capability and EDS detector allows for elemental analysis of small nanoparticles either drop-cast on carbon film or embedded in cells or tissue after TEM processing. This approach identifies the elemental make-up of nanoparticles in cells or tissues with a resolution of less than 5 nm.



STEM/EDS of gold-cobalt nanoshells: STEM image (left) and EDS maps of gold (middle) and cobalt (right).

Why to use EML?

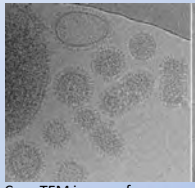
Quality: Our customers can always expect the highest quality images from EML.

Electron Microscopy: EM is the only technique that visualizes virus and subcellular/macromolecular structures.

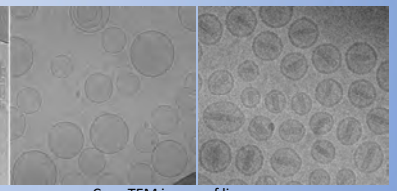
Most current Technology: EML offers the most current technologies, from cryo-TEM to 3D tomography.

Cryo-TEM

Our FEI T20 microscope in combination with a Vitrobot and a cryo-holder allows us to visualize samples in a native-vitrified state.



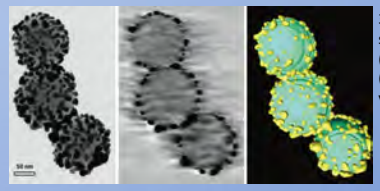
Cryo-TEM image of influenza virus



Cryo-TEM image of liposomes

3D Electron Tomography

At the EML, we offer electron tomography of plastic sections, nanoparticles, and cryo-EM specimens. In combination with serial sectioning, tomography can result in the complete 3D structure of large organelles or whole cells. This technique provides a 3D reconstructed volume that can be further segmented and analyzed.



3D tomography of partially coated gold-silica nanoshells: TEM projection image (left), slice of the reconstructed volume (middle), and segmented reconstructed volume (right). Yellow=gold, green=silica

Meet our team

Director

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Christina Burks, MS
ziqiu.wang@nih.gov

Visit us: https://ncifrederick.cancer.gov/services/accessioning/services/labservices/area/5

NCI CCR Collaborative Bioinformatics Resource

The CCBR offers microarray analysis, next generation data analysis, data mining, pathway mapping, and other bioinformatics expertise to CCR investigators.

Contact Details
Head: Maggie Cam, Ph.D.
Phone: 240.760.7179
Email: maggie.cam@nih.gov
Address: Building 37, Room 3041C, Bethesda, MD 20892

Established Technologies

- Microarray analysis across a variety of platforms and custom arrays
- Next Generation Sequence (NGS) data analysis
- Data mining, statistical and mathematical analysis using multiple approaches
- Pathway mapping and biological interpretation
- Multi-experiment data integration and correlation
- miRNA and array CGH analysis
- SNP and base calling

User Guidelines
CCR Investigators seeking CCBR services should visit the CCBR website to access the Project Submission Form, or contact Maggie Cam.

Advanced Biomedical Computational Science
Accelerating Scientific Insight

Consulting and Collaboration in Scientific Computing, Informatics, and Computational and Data Sciences, Machine Learning and AI

[How to Collaborate and Submit a Project](https://ncifrederick.cancer.gov/bids/abcs/project-request/)
https://ncifrederick.cancer.gov/bids/abcs/project-request/

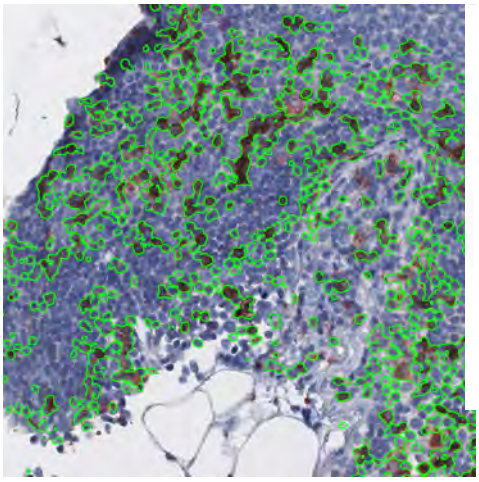
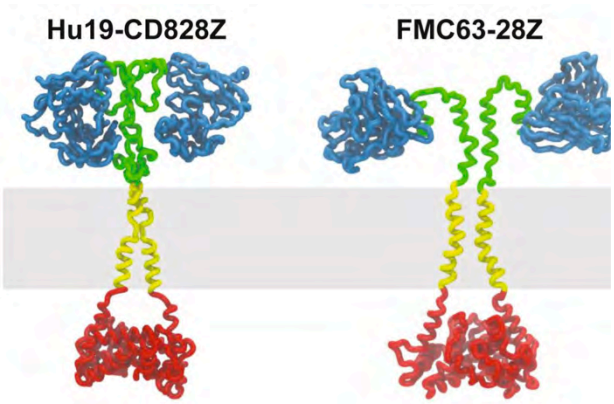


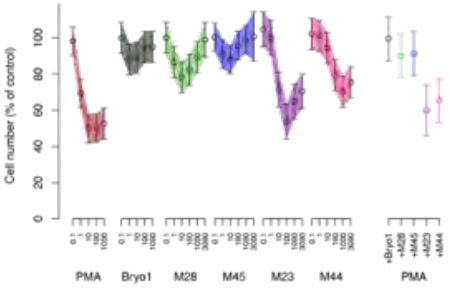
Image Analysis and detection of CD4+ T-cells (green outline) from the HIV/SIV Tissue Microenvironment section using Machine Learning/AI-Based Digital Pathology methods (see image above)

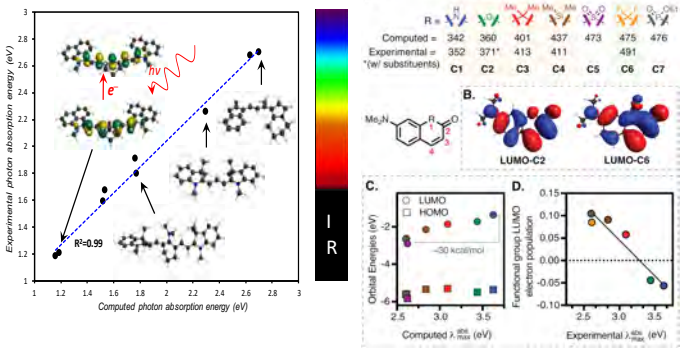


Molecular dynamics simulations CARs suggest differences between Hu19-CD828Z and FMC63-28Z. Integrating structure and molecular simulations may help design more potent, less toxic treatments for cancer. *see Brudno et al., Nat Med, 2020 (see image left)*

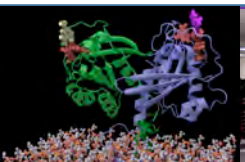
Integrate, filter, browse, and search data and metadata through customizable and interactive dashboards to gain insights from scientific and clinical data for multi-omics systems biology projects – including reports for samples and assays performed. (see image above)

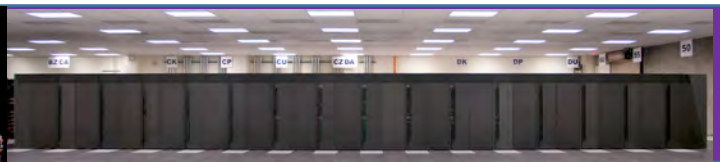
A linear regression analysis of leukemia cell lines treated with varying concentrations of protein kinase C with and without phorbol12-myristate 13-acetate was used to evaluate cellular distribution of synthetic fluorescent bryostatin analogues. (see image below)





Quantum simulations enabled engineering of molecular properties to tune the absorption spectra for better penetration in photoimmunotherapy applications. (see images above)





Frederick National Laboratory
for Cancer Research

Advanced Biomedical Computational Sciences
Capabilities and Contact Information

Advanced Biomedical Computational Science (contact Dr. Jack Collins – email: Jack.Collins@nih.gov)

The Advanced Biomedical Computational Science (ABCS) is part of the Biomedical Informatics and Data Science (BIDS) program and supports scientific research at the Frederick National Laboratory for Cancer Research (FNLCR), NCI at Frederick, NCI in Bethesda, NIH, and other federal agencies. The ABCS provides bioinformatics, mathematical simulation and modeling, image analysis and visualization, machine learning and AI, chemoinformatics, proteomic analysis, data integration support for scientific projects through database maintenance and development, and scientific web application development.

Image Analysis, ML/AI and Visualization (contact Dr. Yanling Liu – email: Yanling.Liu@nih.gov)

The Imaging and Visualization Group (IVG) supports and accelerates basic research by developing and implementing AI solutions in image analysis; scientific visualization; IT and software infrastructure; and services to facilitate data access, collaboration, and reuse; reduce duplicate efforts; and automate labor intensive workflows. Specific examples include AI solutions for biological feature such as lymphocyte detection, segmentation, and quantification on histopathological slides.

ChemoInformatics, Structural Biology and Modeling (contact Dr. Raul Cachau – email: Raul.Cachau@nih.gov)

The ABCS provides innovative solutions over a wide range of structure analysis tools and support to help accelerate the engineering and structural characterization of advanced materials, and macromolecules including modeling, implementation of hybrid methods (xray, EM, SAXS/SANS), tool development and custom workflows and services.

Data Solutions and Systems Biology (contact Uma Mudunuri – email: Uma.Mudunuri@nih.gov)

DSSB group strives to streamline and provide integrative and innovative solutions for the NCI/NIH community to access and use biological information collected across different sources and formats. The group focuses on integrating diverse data sources to enable disease agnostic access and analysis, variant impact annotation, identifier conversions across species, and merging clinical and research data. DSSB also provides scientific infrastructure, web programming and informatics management support.

Scientific Web Programming Group (contact Michael Loss – email: Michael.Loss@nih.gov)

The ABCS' scientific web programming group (SWPG) enables and supports NCI science by providing innovative web application and tool development to assist groups and researchers with managing and tracking data and interacting with data and scientific applications through web interfaces**.**

Scientific Informatics Management Group (Contact Chris Wolcott – email: Chris.Wolcott@nih.gov)

The scientific informatics management (SIM) group provides support for streamlining scientific workflows. The group provides data management, analysis and automation support for NCI/NIH researchers. In addition, the group works on streamlining application development and maintenance.

Mathematical and Statistical, Biomarker, and Proteomic Analysis (contact Dr. Brian Luke – email: Brian.Luke@nih.gov and Dr. Randy Johnson – email: Randall.Johnson@nih.gov)

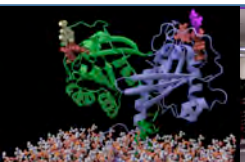
The mathematical and statistical analysis group provides mathematical and statistical analysis and modeling of cancer and HIV/AIDS, including: biomarker discovery, proteomics analysis, computational simulations, regression analysis, survival analysis, study design consultation, and parallelization/optimization of analysis scripts.

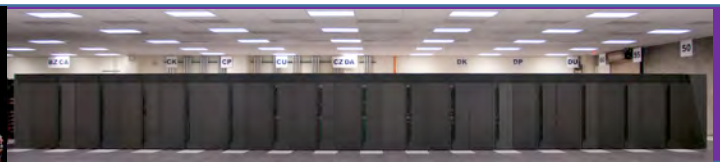
CCR and NIAID Collaborative Bioinformatics Resources and CCR-Sequencing Facility Bioinformatics (contact Parthav Jailwala (CCR) – email: Parthav.Jailwala@nih.gov, Justin Lack (NIAID) – email: Justin.Lack@nih.gov, Yongmei Zhao – email: Yongmei.Zhao@nih.gov (CCR-SF))

The ABCS provides a number of core resources to NCI and NIAID investigators. CCBR/NCBR are resources to provide a broad range of bioinformatics expertise to CCR/NIAID PIs and scientists. The analysis capabilities cover a wide spectrum of questions in biomedical research, ranging from basic biology to clinical applications. Typical requests involve the processing, analysis, and interpretation of high-dimensional data sets generated by microarray, Exome-seq, RNA-Seq, ChIP-seq, metagenomics, and mass spectrometry platforms, as well as publicly available data. The CCR-SF specializes in NGS data analysis and quality control, sequencing technology consultation, exploration/assessment of new technologies, and data analysis and management. These core groups work closely together and projects often involve multiple groups.

Computational Chemistry and Quantum Calculations (contact Joe Ivanic – email: Joseph.Ivanic@nih.gov)

The ABCS maintains expertise in computational chemistry. Specifically, expertise in quantum chemistry and drug design, small molecule properties obtained from high-level quantum chemical calculations, and algorithm and software development for GAMESS.



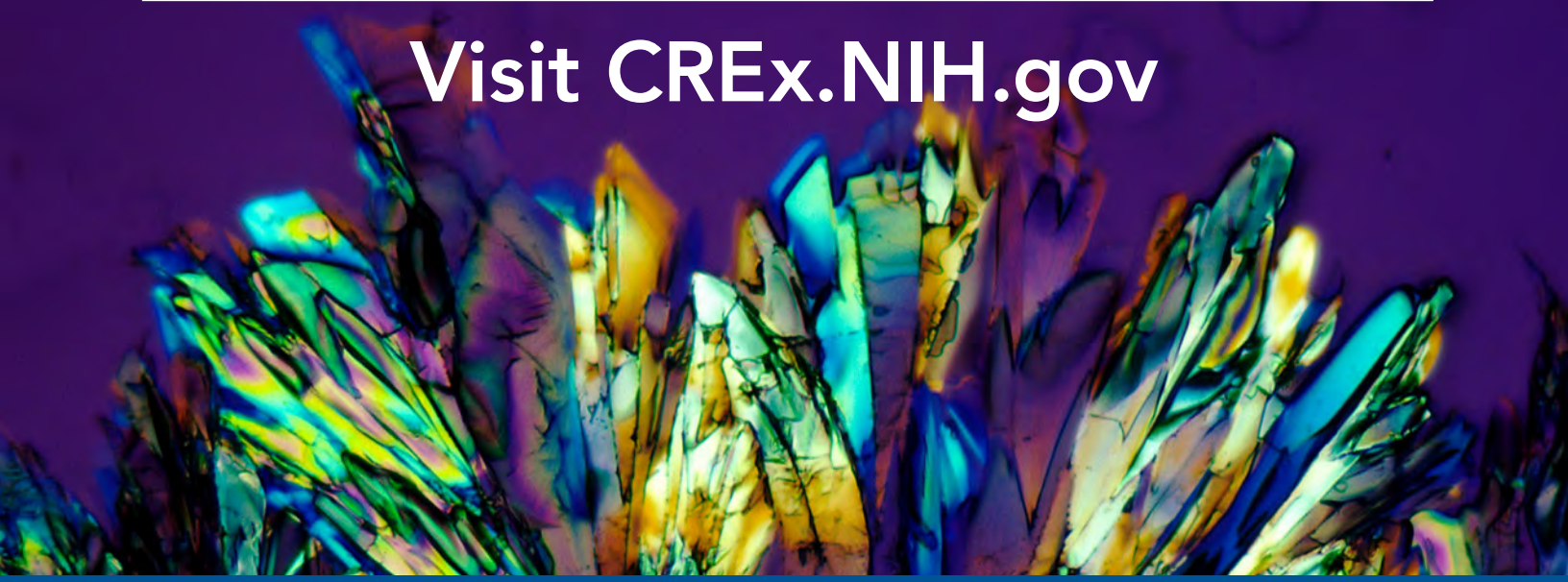


NIH Intramural Research Program

Collaborative Research Exchange (CREx)

Looking for Research Resources within NIH or External Vendors?

Visit CREx.NIH.gov



18,000 Research Vendors | **170+** IRP Cores | **30+** Trans NIH-Cores

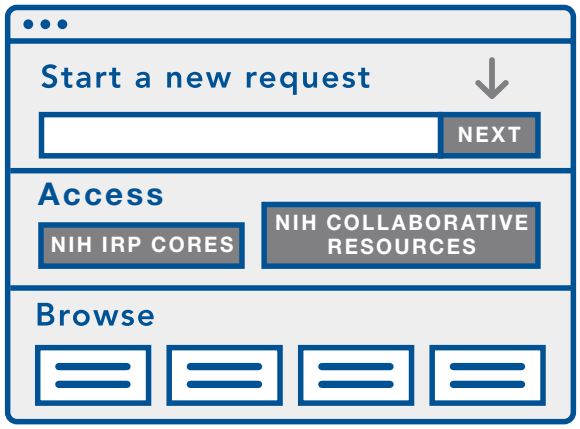
Are you new to the NIH and have questions like:
What is the right technology to use for my project?
How do I find Innovative Technologies at the NIH?

The NIH Collaborative Research Exchange (CREx), an online Custom Pre-Market Research place, developed EXCLUSIVELY for investigators of the NIH Intramural Research Program. Using CREx, you can rapidly filter search results and communicate securely and simultaneously with multiple NIH Core Lab and external research partners.

For more information about the NIH IRP CREx, contact Lakshmi Darbha at Lakshmi.Darbha@nih.gov

Quick Guide to CREx.NIH.gov

1. Search



Enter keywords, find a match then click **Go**. Access Cores and Collaborative Resources or browse by research area.

2. Enter Your Request

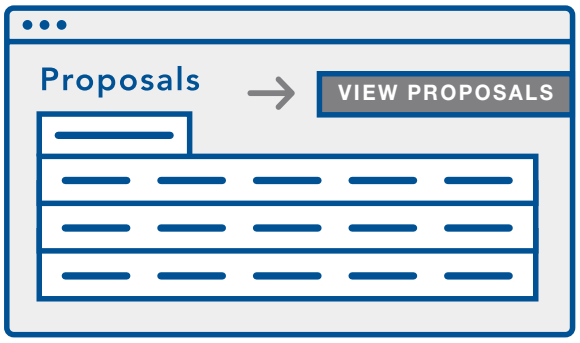
Enter a service description, attach a file, add or remove suppliers, and then click **Initiate Request**.

3. Communicate

From any open request, click **"Send A Message"** to send a note to one (or more) suppliers.

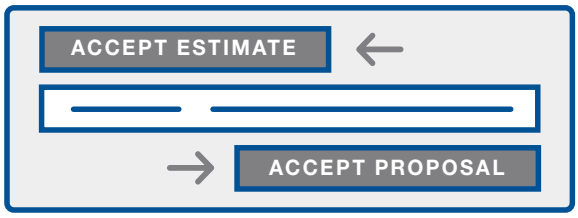
Download the PDF of the Proposal for review and approval.

4. Review Proposals



In the **Proposals** tab, click **View Proposal** to view the supplier's submission.

5. Initiate Purchase / Close Request



Once the supplier has been selected, **initiate a purchase requisition through your internal procurement process.** You can upload proposals gathered through CREx. Once the purchase order has been approved, the supplier will work directly with you to complete the work.

Click the **Accept Estimate** button, select the appropriate supplier proposal, and click **Accept Proposal** to cancel the request with the non-selected vendors.

6. Submit Rating & Review

Rate & Review the supplier to share your experience with other NIH colleagues.