

The Rockefeller University Scientific Resource Centers
Descriptions of facilities/resources (for use in grant applications)

Please feel free to contact the director(s) of the scientific resource center(s) if you need more specific information about how your work can be supported by the scientific resource center, including information about the appropriateness and/or availability of collaborative endeavors and/or letters of support.

ANTIBODY AND BIORESOURCE CORE FACILITY

The Bi-Institutional Antibody and Bioresource Center of Memorial Sloan Kettering and The Rockefeller University generates custom monoclonal antibodies (MAbs), distributes cell lines created by both institutions and tests research samples for mycoplasma on a fee for service basis. Grounded in a thorough understanding of MAb technology, the Core's staff provides comprehensive oversight and is available for consultation throughout the custom MAb generation process. Custom MAb services include, but are not limited to, animal immunizations, B cell immortalization, isolation of IgG secreting hybridomas to maximize screening efforts, screening for MAbs that work in a myriad of applications and establishment of stable antibody producing cell lines.

The burden of distributing healthy and authenticated cell lines to researchers around the world can be significant but is essential to reproducible studies. The Antibody and Bioresource Core Facility is uniquely positioned to distribute cell lines developed at Rockefeller University and Memorial Sloan Kettering Cancer Center; shipments include well detailed information sheets.

To support the maintenance and use of contamination free cell cultures, the Core Facility offers a weekly mycoplasma testing service, complemented by consultation on how best to address this common contaminant should it be detected.

The Core Facility is headed by Frances Weis-Garcia, Ph.D., who has over 25 years' experience in immunology and monoclonal antibody biology and is supported by three additional full time staff members. The 598 sq. ft. center has facilities located on both the Memorial Sloan Kettering and Rockefeller University campuses to ensure easy access to all researchers. Based on capacity and workload, researchers from other not-for-profit research institutions can access the Core Facility services; Rockefeller and Memorial Sloan Kettering research laboratories have priority. The Antibody and Bioresource Core Facility currently supports over 100 research groups at Rockefeller and Memorial Sloan Kettering spanning basic and translational research area.

BIO-IMAGING RESOURCE CENTER

The Frits and Rita Markus Bio-Imaging Resource Center provides researchers with training, advice and access to instrumentation and image analysis for state-of-the-art optical imaging using widefield, confocal, multiphoton, light-sheet and super-resolution microscopy. Consultation on sample preparation and labeling procedures is also provided. Researchers are trained to use the microscopes and image acquisition software themselves, with staff assistance when necessary to ensure collection of high quality images. More complex work can also be performed on a collaborative basis with the staff of the center.

The 3454 sq. ft. center currently houses several widefield fluorescence/transmitted light microscopes (Zeiss and Olympus) including two DeltaVision Image Restoration microscopes (API/Cytiva) and a CellDiscoverer 7 automated live cell imaging system (Zeiss); four laser scanning confocal microscopes (a Zeiss LSM 980, Zeiss LSM 780 and a Zeiss LSM 880 NLO system, plus a Caliber ID RS-G4 resonant scanning system); three super-resolution instruments, namely an Abberior Facility Line STED microscope, a VisiTech instantSIM (iSIM) and an OMX 3D-SIM system (API/Cytiva); an FV1000MPE upright multiphoton system (Olympus); a Yokagawa spinning disk confocal microscope (Zeiss/Spectral Applied) and a Yokagawa CV1000 “CellVoyager” environmental spinning disk system (Olympus); a multi-line TIRF system (Nikon); and an Ultramicroscope light sheet system (LaVision/Miltenyi). Most of the systems are fitted with environmental chambers for live cell imaging and several with FRAP/ablation modules. In addition, several high-end workstations are available for advanced image analysis using open-source softwares and commercial packages such as Huygens, Imaris, Arivis and AIVIA.

The center has seven full-time research support staff including the senior director, Alison North, Ph.D., who has led the center since 2000. Dr. North received her Ph.D. in cell biology from Oxford University and has over 20 years of experience in light and electron microscopy. The center is staffed during business hours and under an open-access model, trained investigators can use the facility 24/7. In addition, many of the systems can be operated remotely via the center’s on-line facility management software. Priority access is given to researchers from The Rockefeller University, which provides significant financial subsidy for the center’s operations, but the center is also open to researchers from external institutions. The BIRC is located in the Bronk Building on The Rockefeller University campus.

CRYO-ELECTRON MICROSCOPY RESOURCE CENTER

The Evelyn Gruss Lipper Cryo-Electron Microscopy Resource Center (CEMRC) provides its users with a world class environment to make advances in structural biology. The 3438 sq. ft. center is equipped with the three high end dedicated cryo-electron transmission electron microscopes and a dual-beam SEM. The microscopy suite was meticulously and specially designed to allow these instruments to perform beyond their specified resolutions. This center provides users with some of the world’s most stable dedicated cryo-electron microscopes, optimized for high resolution single particle analysis of proteins and protein complexes, as well as high resolution cellular tomography. Users will work alongside CEMRC staff until they have mastered electron microscope operation, at which point they will be able to work autonomously.

The center is home to the FEI Talos Arctica, a 200kV transmission electron microscope (TEM), and two FEI Titan Krios units, which are 300kV TEMs. All systems are equipped with an Autoloader, allowing storage and automated transfer for up to 12 frozen hydrated samples at once. These systems can be run completely autonomously and remotely, providing the ability for 24/7 operation. The systems are equipped with high brightness field emission guns (X-FEGs). These FEGs produce coherent electrons, aiding in boosting contrast for data acquisition. The Center is also equipped with an Aquilos dual beam Cryo-FIB SEM which will has enhanced and supported cellular tomography studies on campus. The Aquilos is outfitted with a long operation run time upgrade that allows for greater than 3 days of run time on a single dewar fill.

The Arctica TEM is fitted with Gatan K2 Summit direct electron detectors (DED) and used predominantly for screening although it may be also used for collection of small data sets. “Krios 1” is out fitted with a K3 direct electron camera and a cutting edge BioQuantum HD energy filter from Gatan. Krios 1 is also

outfitted with a fringe free imaging setup which drastically speeds up data collection and efficiency. The second Krios is equipped with the Gatan K3 camera and BioQuantum energy filter. These energy filters enable the visualization of smaller samples for single particle work and also enhance contrast in thick, cellular samples. These tools are tailored for high throughput data collection of 2D and 3D images. 2D images (micrograph movies) are taken from the 3 microscope computers (Arctica, Krios1 and Krios2). The sizes of these movie files vary from 300-800MB each, depending on their acquisition conditions and the microscope used for collection. Users are typically able to collect an average of 1300 movies (650 GB) per day on the Arctica, 6000-8000 movies (~6TB GB) per day on Krios1, and 6000-8000 movies (~6TB) per day on the Krios2. These files are subsequently copied to 1 of 2 GPU computers (one for Arctica and Krios1 data, and one dedicated to Krios2 data) and further processed to produce single micrograph images (90 MB each, 2 for each movie). These images are used for identifying issues with the collection in real-time and can be utilized for further data processing tasks by users outside the CEMRC. These movie and micrograph files are then synced to the HPC archive directories for each lab group and can be accessed by members of their respective labs for further processing of the acquired data.

The CEMRC suite is located in the Collaborative Research Center. Priority access to the CEMRC is given to Rockefeller scientists and the University generously subsidizes the cost of operation. The center has a staff of three, including Mark Ebrahim who heads the group. Mark has over 15 years of combined multi-disciplinary research and industry experience in the fields of materials science, physical biology, and electron optics. Mark received his master's degree in physics, concentrating in optics and ultrafast laser spectroscopy, at CUNY Hunter College.

ELECTRON MICROSCOPY RESOURCE CENTER

The Electron Microscopy Resource Center offers state-of-the-art instruments and competent expertise to support scientists with a broad range of electron microscopy (EM) studies, such as sample preparation, transmission and scanning electron microscopy image acquisition and interpretation. Our staff is very experienced in assisting with the design of EM experiments, in choosing the most appropriate approach for each project and in the interpretation of results. Basic services include sample preparation for a variety of experimental models which includes but is not limited to bacteria, yeast, cells in culture/suspension, ants, *C. elegans*, *Drosophila*, zebrafish, and mice, using conventional chemical fixation, microwave techniques, high-pressure freezing, and freeze-substitution. We offer semi-thin and ultra-thin sectioning, staining and EM imaging. Advanced services include immuno-labeling at the EM level, correlative light and electron microscopy techniques and the development of protocols for special research needs. If required, scientists can be trained in the operation of instruments and methodologies or alternatively, EMRC staff can perform the experiments upon request.

The EMRC has two transmission electron microscopes: JEOL JEM1400 Plus, FEI TECNAI 12 BioTwin, and one scanning electron microscope: JEOL JSM IT500HR. The JEM1400 Plus and Tecnai 12 have automated tomography and montage capabilities. In addition, the JEM1400 Plus is equipped for Scanning Transmission Electron Microscopy (STEM). The EMRC also offers a collection of accessory devices; including Leica Sputter Coater ACE600, Critical Point dryers, a Wohlwend Compact 3 High Pressure Freezer, a Leica EM FSP Freeze Substitution unit, microwave system (PELCO BioWave), Vitrobot Mark IV and ambient Ultramicrotomes.

The EMRC is generously supported by The Rockefeller University and is open to all University researchers and to non-Rockefeller University researchers, depending on capacity. The Center is under

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the direction of Hilda Amalia Pasolli, Ph.D. Dr. Pasolli, who obtained her Ph.D. in Chemical Sciences at Cordoba National University in Argentina, has more than 25 years of experience in biochemistry, cell and developmental biology and her research focuses on applying EM to answer scientific questions. The EMRC encompasses over 2868 square feet of lab space on the first floor of the Rockefeller Research Building on the Rockefeller University campus.

FISHER DRUG DISCOVERY RESOURCE CENTER

[\(https://www.rockefeller.edu/ddrc/\)](https://www.rockefeller.edu/ddrc/)

The Fisher Drug Discovery Resource Center (DDRC) guides and supports our researchers in drug discovery by improving the efficiency of their bioassays, identifying compounds for drug refinement and development, understanding the targets for drugs, and in providing technologies for the measurement of drug/receptor interactions. The center has a collection of 420,000 drug-like compounds, along with semi-automated liquid pipetting devices, and bioassay instruments for supporting drug discovery programs. Importantly, using advanced instrumentation, the center serves as a training ground for biophysical studies of the interactions of drugs with their targets. ([RRID:SCR_020985](#)).

LABORATORY AND STAFF

The 3490 sq. ft. laboratory space is staffed full-time under an open-access model, whereby, once trained, investigators can use the facility 24/7. The center is directed by Dr. J. Fraser Glickman who has over three decades of experience in drug discovery including 16 years working in the pharmaceutical and biotech industries. Dr. Glickman has been directing the DDRC since 2008. Dr. Glickman is readily available for scientific and technical consultations. There are also four research staff specialists with a cumulative 30 years of biophysics, biochemistry, cell biology, and assay development expertise, who support the training, assay miniaturization, automation, data processing, liquid handling, instrument QC and maintenance.

SPECTROSCOPY AND BIOMOLECULAR INTERACTIONS

An excellent portfolio of instruments and expertise are available for high throughput screening and the analysis of binding kinetics and affinity of protein-small molecule, protein-protein, and protein-nucleic acid interactions. The center supports experimental design, training and guidance in the use of an Applied Photophysics Chirascan™ circular dichroism spectrometer (CD), a high-throughput surface plasmon resonance (SPR) instrument (Cytiva Biacore 8K™), a microvolume isothermal calorimeter (Malvern AutoITC 200™), a capillary-based thermophoresis instrument (Nanotemper Monolith™) high throughput temperature-related intensity change (TRIC) instrument (Nanotemper Dianthus NT 23 PicoDuo™), a label-free thermal melt analysis instrument (Tycho NT6™) and 2 BioRad CFX™ 384-well thermal melt analysis instruments. For measurement of compound integrity, purity and solubility, the center supports, a Wyatt Dynapro™ Dynamic Light Scattering Instrument and an Agilent HPLC-TOF mass spectrometer.

HIGH-THROUGHPUT SCREENING INSTRUMENTATION

The center has several varied high-throughput microplate readers with automation, suitable for high throughput fluorescent and bioluminescent assays, as well as high-content screening. These allow for the performance of cell-based and biochemical assays in 384-well format, including Agilent/Biotek Synergy NEO multi-function plate readers, a Molecular Devices ImageXpress Micro high-content screening system, a Hamamatsu FDSS fluorescent kinetics microplate reader and an Agilent Rapid-Fire high throughput solid-phase extraction mass spectrometry system.

To augment these capabilities, the center has automated liquid handling including, a Perkin-Elmer Janus compound pipetting system and an Agilent/Biotek Multi-Flo reagent dispenser.

SMALL MOLECULE LIBRARIES

The compound collection currently consists of 430,000 compounds custom selected from a variety of commercial vendors. The purchasing strategy has striven to include known drugs and their scaffolds, natural products, semi-synthetic compounds, and highly diverse lead-like compounds. Compounds have been chosen from vendor catalogs based on Tanimoto-based fingerprint clustering, "relaxed" Lipinski guidelines (molecular weight is <700, except for natural products), and more recently, metrics such as Q.E.D. score (*Bickerton et. al., 2012 Nature Chemistry. 4:2, 90-98.*) and Fsp(3) score (*Lovering et. al. 2009. J. Med. Chem. 52. 6752*) to select diverse compounds from various clusters with biophysical properties consistent with drug development. We have either flagged or removed reactive substructures, dyes, and the frequent hitters, which represent less than 5% of the screening compounds. Most of the compounds can be re-ordered in larger batches for hit follow-up and secondary assays. Analytical data is required of all purchased compounds such that a minimum of 95% purity is met.

Drug Repurposing and Annotated Compounds

The HTSRC maintains a collection of approximately 1200 known drugs, clinically used compounds and approximately 4000 annotated tool compounds procured from various vendors, and includes the NIH clinical collection, the Prestwick Collection and the Selleck Collection of known drugs and annotated kinase inhibitors.

Fragment-Based Screening

The HTSRC maintains a fragment-based screening platform based on 2184 fragments purchased from LifeChemicals, with measured high solubility in aqueous media. Each fragment represents a cluster, from which analogs of hits can be purchased for initial structure-activity studies. Our basic platform includes 240 μ M- 1mM primary screening using DSF, or Temperature-Related Intensity Change (TRIC) followed by hit confirmation using either MST, SPR or ITC. Functional assay readouts are also amenable to this form of compound screening.

GENERAL PRIORITIZATION OF HITS AND FOLLOW-UP PROCEDURES

The output of our compound screening process is a series of concentration response curves selected from the primary hit list, and the associated HPLC-MS analyses of the sample composition. High-throughput secondary assays, cytotoxicity assays and/or selectivity assays can be performed in parallel to eliminate false positives, ensure mechanism of action, or choose the more selective hits.

DATA STORAGE AND ANALYSIS

All screening data can be normalized, stored, processed, and queried using the *Collaborative Drug Discovery* Database. (CDD, Burlingame California). The database can be accessed over the internet by all users and the data remains separated by project. All data can be downloaded into Microsoft Excel, or with structural information such as .sdf or comma delimited smiles formats. Thus, data can be manipulated online or offline, or uploaded into various software or databases such as Open Science Framework. The database is duplicated and hosted on one Canadian, one North American and one Western European server, to augment security.

Using this CDD database, calculated properties, frequent hitters, cytotoxic compounds and “heatmap” displays are easily viewed. Compound profiles can be determined using cross screen analysis. Statistical values such as Z and Z-prime can readily be calculated. High-throughput concentration-response curve-fitting and classification (Inglese et. al. 2006. PNAS 103:31, 11473) is also performed. Studies of the structure-activity relationships, similarity, sub-structure searches and Bayesian predictions can be accomplished using Accelrys Pipeline Pilot™ software. Licenses for Data Plotting software (Dotmatics, Vortex, GraphPad PRISM™) are also maintained for data visualization, clustering, and publication illustrations.

FLOW CYTOMETRY RESOURCE CENTER

The Flow Cytometry Resource Center (FCRC) provides University investigators with equipment and support for cell sorting (separation), acquisition, and analysis of flow cytometric data. The FCRC has a wide variety of state-of-art multi-laser/multi-color flow cytometry sorters and analyzers, as well as spectral flow cytometers. The FCRC five full time staff members maintain the instruments, assist with experimental design and troubleshooting, advise on sample preparation, consult on data analysis and provide individualized training.

For cell sorting, the FCRC at RU is equipped with four **Cell Sorters: three BD FACSArias** from BD Biosciences and **one Sony MA900** from Sony Biotechnology. These Cell Sorters are equipped with up to six lasers (488, 561, 640, 355, 405 and 445 nm excitation wavelengths) and 18 fluorescence detection channels and can perform high-purity sterile sorts into tubes, 96- and 384-well plates, slides or custom devices at flow rate up to 20,000 events per second. All of the Cell Sorters are placed in either BioBubble (Class I) or Baker (Class II) Safety Cabinets which incorporate built-in aerosol management systems (AMS) that operate independently to actively evacuate aerosols from the sort collection chamber. This ensures safe sorting of RG2 materials. **BD FACSAria** Cell Sorters are operated by FCRC staff only, while

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the **Sony MA900** can be operated either by FCRC Staff or by researchers after completion of the required training.

For analysis purposes, the FCRC at RU is equipped with **six benchtop analyzers**, which cover the wide range of researchers' needs. **Two Cytek Aurora** (Full Spectrum Analyzers) have five lasers (488, 561, 640, 355, and 405 nm) and 64 fluorescence channels. Cytek Auroras have a unique capability of measuring the entire emission spectra of the fluorescent dyes excited by multiple lasers installed on the instrument. The full spectrum capture enables the use of the novel un-mixing algorithms for further data analysis. Currently, up to 40 different fluorescent labels can be resolved, even when multiple markers are co-expressed on the same cell. FCRC has four benchtop alignment-free multipurpose Advanced Analyzers: **BD LSRII-1**, **BD LSRII-2**, **BD LSR-Fortessa**, and **ThermoFisher Attune NXT**. Diverse laser/detector configurations on these instruments allow for analysis of cell samples stained with 488 nm, 561 nm, 640 nm, 355 nm (UV), 405 nm, and 445 nm excited dyes. A number of applications, including the multicolor analysis of cell phenotype, gene expression, cell cycle, and others may be performed.

After orientation training (Virtual Classroom, "Beyond the Basics" Class, "Pre-Sort and Sample Preparation Overview" Session, and Instrument Hands-On Training), Cytek Auroras, BD LSRIs, BD LSR-Fortessa, and ThermoFisher Attune NXT are available for use directly by the investigators.

To help researchers to improve sample quality for the Flow Cytometry and downstream experimental approaches, FCRC has established and is growing the pipelines of the **sample preparation equipment**. Several devices have been already installed: Miltenyi Biotec gentleMACS™ Octo Dissociator, Curiox Laminar Wash™ HT2000, Mettler Toledo Liquidator™ 96, Beckman Coulter Allegra™ 6R Centrifuge, and Microplate Orbital Shaker. All these devices are available 24/7 free of charge after short introduction by FCRC Staff.

For data analysis, FCRC has **six analysis workstations**, which are loaded with the flow cytometry and office software for data analysis and preparation materials for the publications and are available 24/7 free of charge.

The 3174 sq. ft. center is located in the Bronk Building on The Rockefeller University campus, is directed by Svetlana Mazel, who received her Ph.D. in immunology from the Gabrichevsky Institute for Epidemiology and Microbiology in Moscow, has over 25 years of experience in flow cytometry, and has been directed the FCRC since 2001.

GENOMICS RESOURCE CENTER

The Genomics Resource Center offers comprehensive services and state-of-the-art instruments to support genomics research. The 3850 sq. ft. center houses an Illumina NovaSeq 6000 sequencer, two Illumina NextSeq 500 sequencers, one Illumina MiSeq sequencer, a 10X Genomics Chromium Single Cell System, and a Life Technologies QuantStudio 12K flex realtime PCR system. The center also provides several accessory instruments for sample quantity and quality validation: Agilent Bioanalyzer, Agilent TapeStation, NanoDrop spectrophotometer, and Qubit fluorometer.

For the next-generation sequencing service, the center offers full services for genomic DNA-Seq and RNA-Seq, specializing in preparation of libraries from a very small amount of starting total RNA. Users can also prepare their own libraries and use the center's sequencing-only services. The center offers free

consultations on experimental designs, library preparation options, sequencer choice, sequencing depth and coverage, and biological replicates. The center works closely with the Rockefeller Bioinformatics Resource Center for data analysis.

The center is staffed by five personnel and is directed by Connie Zhao, Ph.D. Dr. Zhao received her Ph.D. in molecular genetics from Albert Einstein College of Medicine and did postdoctoral studies with Jeff Friedman at The Rockefeller University. She has led the center since 2003. In her role as the Director of the GRC, Dr. Zhao has been very successful in implementing new technologies, adding SNP genotyping, next-generation DNA sequencing, and single cell analysis platforms. The center is staffed during regular business hours and is accessible 24/7 with valid RU key cards. The center offers several instruments for use by trained users at no charge.

PRECISION INSTRUMENTATION TECHNOLOGIES (PIT)

The Precision Instrumentation Technologies facility provides services in multiple disciplines of research engineering. It combines a Makerspace with access to various fabrication and rapid prototyping equipment like different 3D-printing technologies, laser cutting, electronics bench equipment and hand tools. Members of the RU community can sign up for training and subsequently operate the equipment and interfacing software on their own with 24/7-access. In addition, the center hosts heavy, conventional manufacturing equipment like lathes and mills as well as a variety of wood/metal saws to help achieve research goals. The backbone of the 2,607 sq. ft. center is a state-of-the-art Hermle 5-axis CNC mill. The PIT provides a full service of consultation, design and fabrication handled by expert engineers, mechanics and machinists.

PROTEOMICS RESOURCE CENTER (PRC)

The Proteomics Resource Center is directed by Henrik Molina, Ph.D. who oversees a staff of five scientists. Dr. Molina's experience is based on more than two decades working in most aspects of mass spectrometry-based proteomics, 100+ publications, five years in the biotech industry, six years at The Johns Hopkins University and three years as the Director of the Proteomics Unit at the Center for Genomic Regulation in Barcelona, Spain, prior to his arrival at the University in 2011.

The Proteomics Resource Center at The Rockefeller University masters most aspects of analytical mass spectrometry which includes, *de novo* sequencing (1), targeted studies (2), quantitative proteomics profiling based on label free quantitation (3) as well as metabolic labelled samples (SILAC) (4), chemical labeling (5), tandem-mass tag technology (6) (7), absolute quantitation (8) and global post translational analysis (9, 10). The Center's Metabolomics Platform offers LC-MS based analysis of small molecules (11), polar metabolites (12) and lipids (13). Very importantly, the Center is a source for help with planning of mass spectrometry-based experiments and the Center have the capability to offer in-depth collaborative analysis. Also, the PRC is operated in a *boutique* style; encouraging the scientists of the Center to work closely with users to create a tailored approach to fit the many unique questions that can be answered by analytical mass spectrometry.

The PRC are equipped with Orbitrap type mass spectrometers (high resolution/high mass accuracy) and can separate analytes using both nano and high flow liquid chromatography. The Center is equipped with multiple high-performance servers and software for data analysis.

The Center occupies 4021 sq. ft. of lab space located on the Rockefeller campus on the Upper East Side of Manhattan, in proximity to Weill-Cornell Medical College and Memorial-Sloan Kettering Cancer Center. The Center works with around 200 users yearly spread over 500+ projects.

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REFERENCE GENOME RESOURCE CENTER (RGRC)

The Reference Genome Resource Center (RGRC) specializes in high-molecular weight DNA and long-read genomic technologies. The 1740 sq. ft. center offers both library preparation and sequencing services, including library preparation for high molecular weight gDNA, long amplicons, and full-length transcriptome sequencing (Iso-Seq method), utilizing PacBio, Bionano and 10X Chromium technologies.