Image Analysis Core Facility

1) **Nikon T1-E microscope with A1 confocal and STORM super-resolution**
This system allows for the imaging of cells and tissues labeled with fluorescent probes using live or fixed specimens to obtain 3D images using conventional confocal microscopy. In addition, using a new modality of optics (STORM) allows us to obtain images beyond the optical limit of resolution to observe single molecules fluorescently labeled. The system is also equipped to perform spectral imaging experiments and other state-of-the-art fluorescent optical approaches (TIRF, FRET, FRAP). A high temporal and spatial resolution EMCCD camera is attached to the system. The system is equipped with a Stage top Environment chamber for prolonged observation of living cells. It operates using an inverted microscope.

2) **Zeiss LSM510/Meta confocal microscope**
This system is a conventional confocal microscope fully equipped to image cells and tissues labeled with fluorescently labeled fluoroprobes. It also allows for the study of ions with high sensitivity in the inline-scanning mode. It has the capability of performing spectral imaging analysis, albeit at lower resolution that the Nikon system. The system operates using an upright microscope.

3) **Multimodality microscopes for imaging un-stained or fluorescently labeled cells and thin sections**
The CCD camera (AxioCam from Zeiss) is used to capture images. This camera can be easily attached to a small inverted microscope (Olympus CKX41) (long working distance for plates or plastic) or to our high end inverted (Zeiss Axiovert 200 M) or upright (Olympus BX51W) microscopes, which are fully equipped with phase contrast and DIC objectives and filters. The Zeiss Axiovert is fully motorized.

4) **Metafluor Imaging System for the study of ion movement**
This system allows for the study of ions using ion-fluorescent indicators in living cells. It utilizes a cooled CCD camera (Pentamax) and a rapid filter changer (Lamda DG4) to allow for the changing of the excitation wavelength in as fast as 1 msec. This allows for fast temporal and spatial resolution of images in living cells to study calcium waves, proton gradients or other ions. It is attached to an inverted microscope (Olympus IX70) equipped with DIC optics.

5) **Zeiss Axioimager M2 microscope for stereoscopic analysis**
This system is fully motorized and configured with bright field and multi-channel fluorescent work with Stereo Investigator and Neurolucida, and deconvolution software (MBF Bioscience). Images are acquired with a monochromator digital camera, low noise CMOS chip 2048 x 2048 pixels (Orca-flashpoint 4.0, Hamamatsu). The system can also obtain color images using an Axiocam (Zeiss) color CCD camera. Equipped with x, y, z motorized stage and Zeiss ApoTome system for confocal imaging.
6) **IVIS Lumina XR from Caliper Sciences**
This allows for the real time tracking of cells expressing bioluminescent or fluorescent proteins simultaneously with X-ray digital imaging technology in live mice. It can also be used for tracking bacteria engineered to express luminescent or fluorescent proteins. We have two systems. One of them is located in the vivarium to conveniently study immunocompromised mice. The system located in the imaging center can be used for non-immunocompromised mice or any other small animal. Sterile isolation chambers allow for the imaging of both immunocompromised and non-immunocompromised mice in the same imaging system.

7) **Cryostat (Bright OTF5000)**
This instrument produces thin sections for immunohistochemistry using cold cryogenic temperatures for the preparation of samples. The samples can then be imaged using any of our other imaging systems.

Additional equipment available in the Image Analysis Core Facility include CO2 incubators and O2/CO2 incubators for normoxic or hypoxic experiments as well as two Biological Safety II Cabinets to allow for the handling of biological samples under sterile conditions.
Molecular Biology Core Facility

1) Meso Scale Discover (MSD) Sector Imager 2400 This multi-array technology (96 and 384 well, small spot) enables the detection of biomarkers in single and multiplex formats. It utilizes special kits for profiling biomarkers, cell signaling pathways, cancer markers, angiogenesis, cardiovascular diseases, neurodegenerative disorders, apoptosis, metabolites, cytokines and others. It can also be customized for individual applications. It is equipped with an ultra-low noise CCD camera for high dynamic range, sensitivity and rapid read times.

2) The ImageQuant LAS 4000 Digital Imager is a multipurpose 16-bit 3.2 megapixel CCD camera system for sensitive and quantitative imaging of chemiluminescence, standard UV transillumination, or white light gel or blot documentation.

3) The Typhoon FLA 9000 Biomolecular Imager is a variable mode laser scanner for flexible, sensitive imaging and quantitation of proteins, nucleic acids, and other molecules using filmless autoradiography, fluorescence, chemifluorescence, and gel documentation.

4) Infinite M100 PRO Quadruple monochromator microplate reader (Tecan) Automated microplate reader for UV, VIS absorption, fluorescence intensity with time resolved fluorescence, fluorescence resonance energy transfer and alpha screen technology. It can also quantify luminescence and luminescence spectra. Temperature controlled system (up to 42°C) and orbital shaking capabilities. Accommodates 6, 12, 24, 48, 96, 384, and 1536 well plate formats. The spectral resolution is 1 nm.

5) 4D-Nucleofector Core X/Y (Lonza) The X unit supports nucleofection of various cell numbers in different formats and it is used for transfection of cells in suspension. The Y unit enables transfection of adherent cells in 24 well plates. Use for transfection of cells with DNA or RNA.

6) Biolector (MP2 labs). Uses microplate wells (48 and 96) to grow bacteria, yeast, or mammalian cells in suspension to continuously monitor cell growth (biomass), fluorescence, acid production (pH) and oxygen consumption. CO2, oxygen, humidity and temperature are also fully controlled. It allows rapid optimization of growth parameters and cytotoxicity bioassays. A companion for this system is a shaker system (Multitron II; offline) that allows to perform parallelized experiments, thus freeing the time in the biolector.
7) Quant Studio 12K Flex real-time PCR system (Life Technologies) For gene expression analysis, microRNA profiling, and non-coding RNA analysis using TaqMann assays, and open array technologies using the Open Array Chips. It supports real-time PCR in 384 well plate format.

8) Access Array (Fluidigm) The access array is a high throughput target-enrichment system. It can enrich multiple unique targets (exons) from a large number of samples, all at one time. It has sample Bar coding for multiplexed systems and sequence library prep using amplicon tagging.

9) C1 Single-cell auto prep system (Fluidigm) Useful to study gene expression, single-cell gene expression, and single-cell mRNA analysis. It uses microfluidic technology that allows to rapidly and reliably isolate, process, and profile individual cells for genomic analysis. Useful for the study of cell differentiation, measure individual responses to specific stimuli, evaluation of molecular markers of disease, knockdown of genes using siRNA. It can process 96 single cells for DNA or RNA analysis.

10) Cellometer Vision Image Cytometry. The system is use for simple image cytometry in a 20 µl sample, capable of performing cell cycle analysis, apoptosis, autophagy, cell proliferation, mitochondrial membrane potential, viability assays and cell counting. Equipped to monitor fluorescence of GFP and RFP as well as other commonly used fluorprobes. It capture images using an integrated digital camera and 20 x objective.

11) The 2100 Bioanalyzer (Agilent). It uses microfluidics to run RNA, DNA, and protein samples. It is useful to determine the quality of RNA before running microarrays or real time quantitative PCR measurements. The individual samples are analyzed and a pseudogel image is created, and the bands are sized and quantified. It uses 4 µl samples. It replaces time-consuming techniques associated with agarose gels or SDS-PAGE with fast, automated, high quality digital data. Preparation time is 5 min and digital data is obtained in 30 minutes.

12) High resolution microscopy in flow (ImageStream Mark II, Amnis) This system combines the speed, statistical power, and fluorescence sensitivity of flow cytometry with the functional insights of high resolution microscopy. It is equipped with 20x, 40x and 60x magnification objectives, dual camera are used as detector devise, 4 lasers (405 nm, 488 nm, 561 nm, 642 nm, 785 nm), and 12 imaging channels. This system can be used to evaluate many different fluorprobes in non-adherent cells, i.e., cells in suspension alive or fixed.

13) BD FACSJazz cell sorter (BD Biosciences) The system is equipped with three lasers (blue, red, and yellow-green) and supports six colors. This configuration allows the use of many typical fluorprobes (FITC, GFP, PerCP-Cy, APC, Cy7, DsRed, mCherry, PE-C5). The sheath pressure and drop drive frequency and pre-selected to minimize set-up for sorting parameters. Useful for
immunophenotyping, cloning, stem cell and cancer research, among many other applications. It is housed in a biological safety cabinet with high efficiency particulate air (HEPA filters, to prevent accidental exposure of operators to biological samples).

14) **BD Accuri C6 flow cytometer (BD Biosciences)** The system is equipped with a blue and red laser, and four fluorescence detectors with optical filters optimized for the detection of most common fluoroprobes (e.g., FITC, PE, PerCP, and APC). It supports any brand of 12 x 75 mm sample tubes and microcentrifuge tubes. It is also capable of automation for 48 and 96 well plates. It can be used to study many cellular functions, including apoptosis, cell cycle, cell proliferation, mitochondrial membrane potential, immunoassays, cell phenotyping, cytosolic calcium and pH, nitric oxide.