RESOURCE DESCRIPTIONS

HIGH RESOLUTION X-RAY CT

The BRC Imaging Facility performs full-service CT scans on small animals, insects, plants, fossils and a variety of other materials and devices. Resolution ranges from 0.5-50 microns depending on the size of the object, the time scanned and the field-of-view required. Users typically will drop off samples and discuss with the operator their research objectives. The facility will produce a 3D reconstruction of object density values that is transferred to the user. Further analysis is supported by training on and access to a variety of software packages (see Image Analysis and Visualization Software section).

Our **Xradia (now Zeiss) Versa XRM-520** creates 3D datasets with a maximum resolution of 600 nm/voxel (smallest voxels = 150nm). Using proprietary interchangeable focusing optics allows users to locate and scan small sub-regions within a specimen as large as 30 cm in height and 30 cm in diameter. This feature is unique among micro-CT devices, which typically have strict limits on the physical size of a specimen. The focusing capability is particularly useful for examining specimens that are too rare or delicate to be sectioned destructively (fossils, museum specimens, exotic materials). 4D/5D+ datasets can be created via repeated scanning of the same specimen (e.g. before/after treatments, temperature changes, pressure changes, etc). The XRM-520 is used to visualize and measure structures in plants, insects, seeds, fossils, electronic/fluidic devices, material constructs, soft or dense biological tissues, etc. For examples, biomechanics and materials science researchers use this instrument to characterize microscopic cracks within an intact specimen.

The **Skyscan 1276 (Bruker)** is used for faster and lower radiation dose scans-- acquiring data with voxels ranging from 3um-80um with a 7cm bore diameter limit. Though the instrument is designed for live mice with onboard anesthesia, dosage monitoring and cardiac gating, it can accommodate plants and other specimens as well. In this system the x-ray source and detector rotate around the bore so that the specimen can remain stationary. Data sets from this instrument can be merged with 3D bioluminescence or fluorescence datasets acquired from the IVIS Spectrum located in the same room.

OPTICAL MACROSCOPY: BIOLUMINESCENCE AND FLUORESCENCE IMAGING

A PerkinElmer IVIS-Spectrum can be used to acquire macro images of specimens with variable fields-of-view ranging from 3.9 to 22.5 cm. The instrument has 10 excitation and 18 emission filters with spectral unmixing algorithms, for flexibly accommodating all common bioluminescence and fluorescence moieties. There is onboard heating and gas anesthesia for live mice (up to 5 at a time). The system supports 2D and 3D imaging modes. 3D datasets can be aligned with CT data from the Skyscan 1276 located in the same room.

CONFOCAL AND MULTIPHOTON MICROSCOPY

The BRC Imaging Facility manages several confocal and multiphoton microscopes. The most highly utilized systems (Zeiss LSM710 and LSM780) are under full service contract to ensure that they are operating to their fullest capacity. Signal and laser power metrics are measured monthly by facility staff to ensure reproducible imaging.

The **Zeiss LSM710 confocal microscope** was acquired in 2009 through an NIH shared instrumentation grant (S10RR025502). This system, built around an inverted Axio Observer.Z1 microscope, supports many laser lines (405, 458, 488, 514, 543, 561, and 633 nm) and fully spectrally-resolvable emission channels (center wavelength, width and number of channels). It has an automated stage for monitoring multiple positions or tiling, and *Definite Focus* for focus stabilization over time. An objective heater and removable environmental chamber supports heating and gas regulation (carbon dioxide and oxygen). Overnight series are encouraged by discounted off-peak rates.

The **Zeiss LSM780 confocal/multiphoton microscope** was acquired in 2014 through a NYS health grant (C029155). This system, built around an inverted Axio Observer.Z1 microscope, supports many laser lines (405, 458, 488, 514, 543, 561, and 633 nm) and fully spectrally-resolvable emission channels (center wavelength, width and number of channels). The linear array detector in the detection unit has a GaAsP photocathode, which is twice as sensitive as the multi-alkali photocathode on the older LSM710 module. The system also has a fully integrated Spectra Physics Insight multiphoton excitation source with automated tuning and pulse dispersion control from 700nm-1300nm. Multiphoton imaging can be done with the spectrally resolvable confocal detectors or with a non-descanned filter-based unit (BiG = Bi GaAsP). The non-descanned unit only supports two imaging channels, but offers better sensitivity and depth penetration in optically tough specimens. Multiphoton excitation enables photoactivation, photobleaching and photoconversion that is restricted to the focal volume in three-dimensions. Multiphoton photomanipulation of user-defined regions-of-interest can be integrated with standard confocal or multiphoton imaging. The LSM780 has an automated stage for monitoring multiple positions or tiling large fields of view. In addition, the system has full fluorescence correlation spectroscopy (FCS) capabilities for measuring diffusion coefficients and kinetics in sparse samples. The *Definite Focus* unit provides focus stabilization over time, and a full-surround plexiglass unit provides a stable, heated, and humidified environment with carbon dioxide regulation for supporting long-term time series. The facility encourages these types of studies with discounted off-peak rates.

The **Andor Revolution spinning disk microscope** was acquired in 2012 through an NIH shared instrumentation grant (S10OD010605). This system illuminates the sample with ~1000 spots of light simultaneously. It is built around a Yokogawa CSU-X1 Nipkow disk integrated to an inverted Olympus IX-83 microscope platform. It has four laser lines (405, 488, 561 and 640nm) and filters that accommodate most fluorescent labels. This system enables fast imaging (~100 frames/sec) with two electron-multiplying cameras (Andor Ixon Ultra 897) that can be used simultaneously and a fast piezo-electric focusing unit integrated into the microscope stage (ASI MS-2000). XY motion on the microscope stage is also computer-controlled for monitoring multiple positions or automated tiling. ZDC2 focal drift compensation can be set to maintain focus stability over long periods of time. A FRAPPA unit enables targeted photo-activation or bleaching at user defined regions using any of the laser lines. A Tokai-Hit objective heater and removable environmental chamber supports heating and gas regulation (carbon dioxide and oxygen). Overnight series are encouraged by discounted off-peak rates.

The **Leica TSP SP2 confocal microscope** was acquired in 2002 through an NSF major research instrumentation grant. This system is integrated to an upright Leica DMRE microscope. It has a variety of laser lines (458, 476, 488, 514, 543, and 633 nm) and fully spectrally-resolvable emission channels (center wavelength and width) with up to three detection channels.

BASIC MICROSCOPY AND SPECTROSCOPY

An upright **Olympus BX-50 microscope** running Metamorph software can be used for acquiring fluorescence, DIC, phase and polarization images. A **stereo microscope** can be used for dissections and for basic imaging on a more macro scale. A **PTI QM4 spectrofluorometer** is available for excitation, emission and steady-state anisotropy measurements.

LIVE CELL FLUX ANALYSIS

Agilent Seahorse extracellular flux analyzers (XFe96 and XFp models) measure oxygen consumption and pH production from live cells in 8- or 96-well formats, indicating levels of respiration and/or glycolysis. Measurements can be done simultaneously with drug or inhibitor injections of up to 4 compounds/well.

FACS

The BRC Imaging Facility operates and maintains a **BD FACSAria Fusion cell sorter** with 405, 488, 561, and 640 nm excitation laser lines and 12 emission channels. Input materials include fixed or live cells, and sorting can be accomplished

by gating regions from a broad spectrum of channels into tubes or multiwell plates in a temperature-controlled collection chamber. Sorting speed is variable depending on cell size and required purity and speed, but sorting speeds are typically set at 5000-10,000 cells/sec into tubes. Sorting into plates can be done at the single cell level for cloning experiments. All sorting is done by a facility staff member, though users can be trained for analysis-only experiments. A containment hood allows for the possibility of risk-level-2 agents to be accommodated. FlowJo is installed on a stand-alone workstation for post-acquisition analysis if necessary.

LCM

The BRC Imaging Facility manages and trains users to operate a **Zeiss PALM Microbeam laser capture microscope** on an inverted Axio Observer.Z1 platform. This system supports standard widefield and fluorescence imaging with 5x, 20x, 40x, 63x and 150x objectives. A 355nm laser cuts user-defined regions with a resolution dependent on the objective NA (can be as sharp as 100nm). A subsequent defocusing of the laser enables a photon pressure pulse to catapult the cut piece into a collection chamber. This method is ideal for overcoming electrostatic interactions sticking the tissue to the substrate without introducing contamination from contact surfaces. It is capable of collecting user-defined regions from thin <20um fixed tissue, archival material or cultured cells. The facility instrument has been used to capture material for DNA, RNA, and protein analyses as well as living cells for subsequent re-culture. Sample preparation protocols generally require cells and tissues to be placed on specialized slides and/or dishes with a thin plastic layer (PEN or PET) for aiding in the cutting process. The facility instrument can collect up to two simultaneous tissue "types" to two separate tubes in one experiment. An estimate of cell numbers required for various analyses follow (*Espina et al., 586 | Vol.1 No.2 | 2006 | Nature Protocols*):

Molecule	Methodology / Assay	Cellular yield / Area of microdissection
DNA	Loss of heterozygosity	100 – 1,000 cells
	Imprinting / DNA methylation	200 cells
	Genetic mosaic analysis of gDNA	2,000 cells
RNA	cDNA library construction	5,000 – 25,000 cells (~15 – 90 ng total RNA)
	Gene-expression arrays	100 cells from FFPE
	Real-time RT-PCR	Single cell – 22,000 cells
	qRT-PCR	100 – 5,000 cells
Protein	Western blot (optimized blotting procedure)	500 cells
	Western blot	2,500 – 10,000 cells
	2D gel electrophoresis	10,000 – 100,000 cells
	2D-DIGE	30,000 cells / 40 µl
	Molecular profiling: reverse-phase protein microarray	5,000 – 30,000 cells
	Mass spectrometry: MALDI or LC/MS-MS	10,000 – 100,000 cells
	Mass spectrometry: SELDI	1,500 – 5,000 cells

ULTRASOUND

The BRC Imaging Facility manages and trains users to operate a **VisualSonics Vevo-2100 high resolution ultrasound** that was funded through an NIH shared instrumentation grant (S10OD016191). This system generates images at 20-70 MHz with phased array, solid state probes giving a resolution in the 50-100 um range. There is a selection of application specific probes designed for mice and rats with onboard vitals statistics (breath and heart rate), heating and gas anesthesia. Peripheral devices include a station for image-guided injection, and a low-frequency probe for targeted release of sonosensitive pharmaceuticals. This instrument is particularly good for non-invasive imaging of anatomical structures and motion. A variety of Doppler modes can be used to quantify the direction and magnitude of blood flow. The Vevo-2100 is designed around the same principles as a human ultrasound, but is scaled down 100-fold for applicability to mouse cardiology, development and cancer studies. This instrument can also be used for examining plant morphology or fluid flow in appropriately designed systems.

IMAGE ANALYSIS AND VISUALIZATION SOFTWARE

Arivis Vision4D, Imaris Bitplane, Avizo and Metamoph image analysis and visualization packages are available on facility workstations. Facility staff periodically run group workshops for training on these and ImageJ, FIJI and Horos shareware packages. Custom-written image analysis protocols are sometimes developed by facility staff if appropriate tools are unavailable or require automation.