Major Equipment

The High Resolution Imaging Facility (HRIF) provides state-of-the-art microscopy and image analysis platforms including confocal and live cell imaging, transmission electron microscopy, OPTIR, Lightsheet, multi-photon animal and tissue imaging, widefield, super-resolution, and image analysis. We provide sample processing for electron microscopy and consultations, expert training, and support for all our systems.

Confocal

Nikon A1R A confocal laser scanning microscope optimized for fixed cell/tissue or live cell imaging of cellular dynamics and molecular events at high speed. The Nikon Ti microscope is on an anti-vibration table and is fully automated with an automated *x-y-z* stage and the Perfect Focus System to allow for time lapse and multi-point live cell imaging without the specimen drifting out of focus. Z-stacks allow 3D investigation of samples. The hybrid dual scanning confocal system has both a galvanometric and resonant scanner, which can be easily alternated or used together for simultaneous stimulation and imaging. Images can be acquired at 512x512 up to 30 frames per second, or with a reduced size up to 420 fps. Photomanipulation (FRAP, FRET) experiments can be conducted in multiple user-defined regions of interest (ROIs) simultaneously. The system has 404, 457, 476, 488, 514, 568, and 638 nm laser lines and 4 PMT detectors (plus a transmitted light channel for DIC). The system is run by NIS Elements software.

Nikon A1R HD This confocal laser scanning microscope is on a Nikon Ti2 inverted microscope body (shared with SIM system) on an anti-vibration table and is fully automated with an automated x-y-z stage and the Perfect Focus System to allow for time lapse and multi-point live cell imaging while maintaining focus. Z-stacks allow 3D investigation of samples. The hybrid dual scanning confocal system has both a galvanometric and resonant scanner, which can be easily alternated or used together for simultaneous stimulation and imaging. System is equipped with the following objective lenses: Plan Apo lambda 10x, Plan Apo VC 20x 0.75 NA DIC, Plan Fluor 40x oil 1.2 NA DIC, Apo 60x oil lambdaS 1.4NA DIC, and SR Apo TIRF 100x 1.49NA. Images can be stitched together to achieve high resolution of large areas. Images can be acquired at 512x512 up to 30 frames per second (fps); or up to 420 fps with a reduced scan size. Photomanipulation (FRAP, FRET) experiments can be conducted in multiple user-defined regions of interest (ROIs) simultaneously. The system has 405, 488, 561, and 638 nm laser lines and 4 detectors, 2 high sensitivity PMT and 2 GaAsP, (plus a transmitted light channel for DIC). The system is run by NIS Elements software. There is a Tokai Hit live cell imaging chamber that can maintain cells on the stage at 37 degrees and 5% CO_2.

Nikon C2 A confocal laser scanning microscope optimized for fixed cell or tissue imaging. This microscope has 10x, 20x, 40x, 60x, and 100x and four lasers, 405nm, 488nm, 561nm and a 647nm. This system is on a Nikon Ti2 inverted microscope body (shared with STORM system) on an anti-vibration table and is fully automated with an automated *x-y* stage, piezo *z* and Perfect Focus System. The system has 2 GaAsP detectors, which are used as up to 4 virtual fluorescence channels (plus a transmitted light channel for DIC). The system is run by NIS Elements software.

Light Sheet Fluorescence Microscopy(LSFM)

The Zeiss Lightsheet 7 microscope provides fast and gentle imaging of living model organisms, tissues, and organoids as well as fixed optically cleared specimens. Specimens can be up to 2 cm in size and at any refractive index between 1.33 and 1.58 compatible with most clearing solutions. It's capable to acquire lower resolution overview images or whole volume at subcellular resolution. Two high quantum efficiency PCO Edge 4.2 cameras enable observations of the fastest processes at the low illumination light levels. The light sheet geometry illuminates only the parts of the sample you are imaging, greatly reducing photobleaching and phototoxicity. You'll get a real-life view of your samples without the adverse effects of excitation light. The microscope is equipped with 10 and 20x water dipping objectives, two 20x clear dipping objectives for different refractive index, dry objectives 2.5x and 5x, and two sets of illumination objectives with 5x and 10x magnification. The Zeiss Lightsheet 7 offers environmental module with CO_2, temperature, and humidity control, four imaging chambers for imaging live, fixed, and cleared samples in broad range of sizes, four lasers at 405, 488, 561, and 647nm, and advanced processing and analysis software (Zeiss Zen Blue, Zeiss Arivis, and Imaris 10) operating on separate high efficiency workstation computers.

Widefield

Lionheart FX Automated Imaging System the Lionheart FX Automated Imaging System from Biotek is a small footprint microscope that offers a broad variety of high-quality imaging techniques. The system will accommodate imaging requirements for basic as well as advanced time-course experiments. Features include imaging of slides, dishes, or plates, fluorescent, color or black and white brightfield, and phase-contrast imaging, high speed fluorescent imaging of up to four channels with laser autofocus, z-stacks, imaging of multiple regions, image stitching, and environmental control for live imaging.

Widefield Microscopy A Nikon TE2000U inverted microscope is ideally set up for widefield and FRET experiments with an Optical Insights Dual View, a Roper CoolSnap HQ CCD camera, Universal Imaging Corporation Metamorph Premiere software, and a Sutter Lambda DG-5 light source with a piezo filter changer. The system is designed for sensitized emission live or fixed cell FRET experiments. The results may be displayed as time dependent curves for each selected ROI and as pixel-by-pixel color encoded images. The Dual View allows for simultaneous viewing of excitation and emission of both the donor and acceptor emission. This capability translates into faster image acquisition times. The Lambda DG-4 by Sutter Instruments is configured with piezo motors that provide microsecond shifts between excitation filters. With the combination of the Dual View and the DG-4, FRET can be determined with much greater speed than conventional filter wheels. The system can image the FRET pairs CFP/YFP, mTFP/Venus and GFP/mRFP1.

Super-resolution

Nikon N-SIM A super-resolution Structured Illumination Microscope (SIM) that doubles the resolution of conventional widefield and confocal microscopy (resolution capable: 120 nm x-y and 300 nm z). Sample preparation is the same as for widefield or confocal. The Nikon Ti2 microscope body has a motorized x-y stage and piezo z control and perfect focus system. The system is equipped with a Hamamatsu Orca Flash 4.2 cMOS camera with a fast frame rate, large field of view, and high sensitivity. It has 488, 561, and 647 nm laser lines for 3 color 3D-SIM imaging (XYZ super resolution up to 20μm deep). Additionally, the microscope is equipped with Tokai Hit incubation chamber for live cell imaging and an anti-vibration table. The system is run by Nikon Elements.

Nikon dSTORM/TIRF microscope dSTORM achieves fluorescence imaging at a resolution of 20nm *x-y* and 50 nm *z*. The dSTORM TIRF microscope is built on a fully automated Nikon Ti2 microscope and includes: a vibration isolation table; 20x, 40x, 60x, and a 100x 1.49 NA objectives; motorized and joystick controlled *x-y* positioning stage with linear encoder; piezo *z*- control; auto- correcting Perfect Focus System that enables focus lock during acquisition; full filter cube sets for widefield (DAPI, GFP, mCherry, Cy5) and quad-pass laser filter cube for TIRF/dSTORM; LED for EPI excitation, a Andor iXon EMCCD cameras; Tokai Hit incubation chamber to maintain 37°C and 5% CO_2 while imaging; integrated H-TIRF platform with a motorized launcher and an agelient laser launch with laser wavelengths 405, 488, 568, and 647 nm (at between 120-180 mW each) and an AOTF for rapid switching between laser lines. The system is run by Nikon Elements software with dSTORM acquisition and analysis module accommodating any combination of fluorophores, and direct or activator-reporter STORM imaging.

Multiphoton

Nikon A1R Multiphoton This microscope allows for intravital (live animal) imaging with high magnification and resolution. This upright microscope is equipped with a large platform stage and a Coherent Chameleon Vision II laser tunable from 680 to 1080nm. Multiphoton excitation penetrates farther into tissue with less scattering than traditional confocal while restricting the excitation volume in x-y-z. The system has gallium arsenide phosphide (GaAsP) non-descanned detectors (NDDs). A resonant imaging mode provides high speed scanning at up to 30 fps (512x512) or 420 fps (reduced scan region). This system is mounted on an upright Nikon Ti microscope equipped with objectives designed for multiphoton imaging and can image single cells, a live mouse, or thick

tissue sections. The system is run by Nikon Elements software. This microscope is the result of a cooperative arrangement with the Birmingham Veterans Administration Medical Center.

Data Analysis

Analysis workstations: We offer high-end workstations for image processing and analysis. The available software includes Nikon Elements, Biotek Lioheart analysis software, Zen Blue by Zeiss, BioTek Gen5 Software for Imaging & Microscopy, and Imaris 10.1 with Machine Learning by Bitplane, Fiji/ImageJ, IPLAB Spectrum by Scanalytics, and Adobe software. In this way image data may be analyzed without hindering access to the confocal or conventional microscopy equipment.

Zeiss Arivis Pro
Arivis Pro is modular image analysis software when working with multi-channel 2D, 3D, and 4D images of virtually unlimited size. This software can process large data sets of more than a Terabyte in size. Highly scalable computing, not dependent on local system resources. Processing and quantifying any kind of multidimensional image data. Combining different operators for complex analysis. Denoising, AI-driven segmentation, filtering, thresholding and more.

Agilent BioTek Gen5 Software for Imaging & Microscopy
BioTek Gen5 Software for imaging and microscopy offers automated image capture, processing, and analysis for a broad range of samples, from whole organism imaging to high magnification subcellular imaging. These include single image capture or montaging and z-stacking to visualize greater areas and depths of a sample of both brightfield and fluorescent images. Beaconing can also be included to image independent areas across multiple wells of a microplate, or across a vessel. Images can also be captured kinetically, using these same methods, across minutes, hours, or days. Raw data and images are transformed into meaningful results with powerful built-in capture, process, and analysis tools. The automated process, known as Augmented Microscopy, enables simple and straightforward imaging and analysis workflows. This is the software used to run the Lionheart microscope in the HRIF core.

Bitplane Imaris 10.1 with Machine Learning
A state-of-the-art software package designed specifically for quantitative analysis of fluorescence microscopy data. Each image taken on a microscope has a huge array of quantitative information – protein locations, organelle morphology, Imaris is the leading visualization and analysis software for widefield, confocal, light sheet, two-photon, electron microscopy, CLEM, OPT and other imaging modalities. Imaris for Core Facilities provides smart detection and visualization of complex objects, tracing of neurons, blood vessels and other filamentous structures, 3D tracking (including cell division detection), interactive plotting and batch analysis. Additionally, Imaris can handle extremely large (terabytes) data files.

Nikon Nis Elements Analysis
Nikon's Flagship NIS-Elements Package. Optimized for advanced research applications, Nikon's flagship software package features fully automated image acquisition, advanced device control and powerful analysis and visualization tools. From Single to Multi-Dimensional imaging. Intuitive display of multi-dimensional datasets with multiple viewing options (e.g. slice, 3D volume, time, spectral, binary, etc.), easy data management, and export ready. From simple manual measurements to automatic data collection of hundreds of parameters. Full suite of binary and image processing tools in General Analysis (GA) Multi-channel binary software assay toolbox, 2D and 3D Tracking and Measurement, Realtime analysis of live cell dynamics during acquisition, Intensity Based Measurements, Morphological Based Measurements, Object Tracking, 3D Volume Measurements and counting. Easily view tiled/stitched large images, Unique Extended Depth of Focus (EDF) view for creating beautiful, high-contrast, in-focus 2D projection images from 3D data, Advanced 3D and 2D deconvolution algorithms for extending resolution, Unique visualization options such as XYT view.

Zeiss Zen Blue
ZEN is the universal user interface you will see on every imaging system from ZEISS. For simple and routine works, ZEN leads you straight to result. For complex research experiments, ZEN offers the flexibility to design multi-dimensional workflows the way you wanted. No matter what microscopy task you have, you will find intuitive tools and modules to assist you:

Acquire images using smart automation, Process images with scientifically proven algorithms, visualize big data by GPU powered 3D engine, Analyze images via Machine Learning-based tools, Quantitative Processing. More than 180 image processing tools help you transform and manage your data. ZEN will read the metadata of the input image, then display only the logical processing steps, and optimize the default parameters automatically. You can even process images from other platforms using third-party import tools. With a dedicated workspace, you can also batch process multiple images with ease for quantitative and unbiased results. Quickly generate a huge 2D tile scan with multi-channels at high magnification, and smoothly zoom in and out with a simple mouse scroll.

Sub Micron Particle Analysis

Malvern Panalytical Nanosight NS300, Sub-Micron Particle Imaging System (Exosomes)
The NS300 can visualize and measure particles in suspension in the size range 10-1000nm (depending on material) and addresses the needs of a wide variety of applications including protein aggregation, exosome and microvesicle research, drug delivery systems, and analysis of particles labeled with green fluorophores. The NanoSight300 provides multi-faceted analysis that includes a motorized filter wheel, polarizer for analysis of bi-fringent particles, high sensitivity CMOS camera, temperature control and 488nm laser.

OPTIR

Photothermal Spectroscopy Corp. mIRage-LS Microscope. The instrument is located in the HRIF laboratory in the Shelby Biomedical Research Building. The laboratory is central to Engineering (15-minute walk) and the Division of Pulmonology, Allergy, and Critical Care Medicine (5-minute walk). The mIRage-LS has a spectral range of 3600-2700 and 1800-800 cm^{-1} for infrared (IR) spectroscopy and a spectral range of 3900 – 200 cm^{-1} for Raman spectroscopy. X-Y spatial resolution for both methods is < 500 nm and the instrument is fitted with dual probe lasers (532 nm and 785 nm) to give greater flexibility in acquiring Raman data. Both IR and Raman spectra can be acquired by selecting specific locations. In addition, tissue can be spectroscopically mapped over large regions using single wavenumber mapping (fastest) or hyperspectral mapping where a full spectrum is acquired at each pixel. An automated IR polarizer allows assessment of signal as function of collagen orientation. The wide-field epi-fluorescence microscope has capacity for 7 fluorescence filters cubes and can be readily tailored to the needs of all histological strategies for this study. The instrument can be operated in "top-down" mode or in the new "bottom up" counter-propagating mode, which enables the use of high powered standard refractive optical microscope objectives and increased signal/noise and spatial resolution for both IR and Raman imaging. The mIRage instrument is setup for full remote operation using TeamViewer and the Banaszak Holl lab routinely uses this capacity to work remotely and/or allow full remote operation by our collaborators in both the US and overseas locations. The only operation that must be performed locally is the loading of the three-sample stage.

Electron Microscopy

JEOL 1400 HC Flash TEM This system is installed and housed in Shelby Room 130B. The JEOL JEM-1400 series 120kV Transmission Electron Microscope with an operating voltage range of 20-120 kV allows for applications including pathology, biology, quality control, nanotechnology, polymer, and materials samples. It is optimized for high contrast imaging for biological, low Z, and materials science applications with a +/- 70º tilt with support for tomography. It can be used for bright and dark field imaging, large specimen tilt, diffraction, negative stain, and routine TEM. It has a Lanthanum hexaborohydride filament and single, high tilt, and quad tilt sample holders. SerialEM software for montaging and tomography will be available in May 2024.

AMT NanoSprint43 Mk-II is installed on the TEM in a bottom-mounted configuration. It is a high-resolution CMOS camera with a 43-megapixel sampling region with a high-speed readout. The sensor size(pixels) is 7915x5436 with a phosphor pixel size(um) of 8.2x8.2.

Microscopy Innovations ASP-2000 robotic sample processor enables automated, accelerated preparation of biological samples for high-quality TEM and 3D volume EM (vEM), including serial block, focused ion beam, and array tomography SEM. The ASP-2000 allows for temperature-controlled reagents.

LEICA EM TP is an automated tissue processor built in a carousel design to easily handle light and electron-level sample protocols.

LEICA UC7 Ultramicrotomes with FC7 Cryo Kit, the HRIF houses two units to provide high-quality sections at room and cryo temperatures. Staff prepare high-quality ultra- or semi-thin sections for your transmission electron or light microscope investigation while creating perfectly smooth block face surfaces for atomic force, scanning electron, or incident light microscopy. For ultrathin cryo-sections or surfacing of cryogenic material.