

Light Microscopy Core

The Light Microscopy Core is a multi-use core facility offering fluorescence, confocal, super resolution, laser capture and atomic force microscopy. The core contains a central preparatory laboratory and high-end computer workstations for data analysis and image processing. Technical support ranges from investigator training on instrumentation, allowing independent use, to complete processing of samples and final imaging by facility staff. The facility is GRP accredited, undergoes annual compliance checks and provides extensive standard operating procedures for all instrumentation.

Confocal Microscopy services are available in the Health Kentucky Research Building (HKRB) Suite 080-088. Instrumentation includes:

- Nikon AXR Confocal Microscope featuring a high-resolution Galvano scanner for scanning up to 8k x 8k pixels and an ultra-high speed resonant scanner for acquisitions at rates of up to 720 frames per second with a large 25mm field of view. This scope is equipped with 4 GaAsP detectors for greater sensitivity, and an enhanced resolution module using deconvolution to provide higher spatial resolution than the diffraction limit. The AI based tools allow the system to automatically determine the optimal illumination and detection settings and remove shot noise to enable clearer images with shorter exposure time. AI tools also make the post-acquisition image processing and analysis more efficient and reliable. The microscope features 10x and 20x air objectives, 40x water immersion objectives, as well as 60x and 100x oil immersion objectives. It also features a motorized stage and 405 nm, 488 nm, 561 nm, and 633 nm lasers lines. Tokai hit stage top incubator provides temperature/humidity/CO₂ control for live-cell imaging, a programmable fluidic control system allows easy perfusion, media exchange, and drug delivery and mixing without disturbing the sample, and a hypoxia system for low oxygen studies.
- Nikon A1R Confocal Microscope equipped with GaAsP detectors for greater sensitivity, and a spectral detector comprised of 32 individual PMTs allowing for linear unmixing. The microscope is equipped with both a Galvano scanner for high resolution scanning and a resonant scanner for high-speed acquisitions at rates of up to 400 frames per second. It is also equipped with an enhanced resolution module using deconvolution to provide higher spatial resolution than the diffraction limit. The microscope features 10x and 20x air objectives, 20x and 40x water immersion objectives, as well as 60x and 100x oil immersion objectives. It also features a motorized stage and 405 nm, 488 nm, 561 nm, and 633 nm lasers lines. An incubation insert that provides temperature/humidity control and injected CO₂ is also available for live-cell imaging.
- Nikon CSU-W1 SoRa Microscope is a spinning disk super-resolution

microscope. It offers a unique combination of high-speed imaging and exceptional resolution without the need for specific sample preparation processes. Unlike standard confocal, which scans a single point at a time, the spinning disk confocal scans hundreds of points simultaneously. The addition of super-resolution imaging allows researchers to surpass the diffraction limit, achieving resolution down to 120 nm. What can be imaged in confocal mode can be further visualized in super-resolution, providing a seamless transition between imaging modalities. The Hamamatsu Quest camera provides faster readout speeds in extremely low-noise scan mode and increased sensitivity in the ultraviolet region. The microscope features 10x and 20x air objectives, 40x water immersion objectives, as well as 60x and 100x oil immersion objectives. It also features a motorized stage and 405 nm, 488 nm, 561 nm, and 633 nm lasers lines. Tokai hit stage top incubator provides temperature/humidity/CO₂ control for live-cell imaging, a programmable fluidic control system allows easy perfusion, media exchange, and drug delivery and mixing without disturbing the sample, and a hypoxia system for low oxygen studies.

- Upright Zeiss LSM 880 multiphoton microscope equipped with Airyscan and Airyscan Fast super resolution modules. This microscope is equipped with an InSight X3 dual beam multiphoton laser, with one laser line being broadly tunable between 680 nm to 1300 nm and one being fixed at 1045 nm, allowing multicolor MP excitation imaging. Visible laser lines are: 405 nm, 454nm, 488 nm, 515 nm, 561 nm, and 633 nm. The Airyscan and Airyscan Fast modules, afford resolution gains between 2 and 1.5 times over that in conventional microscopes, resulting in up to 120 nm lateral resolutions. The Airyscan Fast module combines advances in resolution and scanning speeds, with acquisition rates of up to 96 frames per second. The microscope is configured for intravital imaging of small animals, with the auxiliary tools for animal anesthesia and surgery being provided in the Core. A variety of special objectives, designed for imaging cleared tissue over large working distances, are available.
- Nikon Super Resolution Microscope equipped with Stochastic Optical Reconstruction Microscopy (STORM) and Structured Illumination Microscopy (SIM) modalities. STORM is a localization technique, using the isolation of individual molecules to reconstruct images, and can provide a spatial resolution of up to 30 nm. The SIM module is using interference patterns to produce reconstructed images with 2-fold higher resolution than conventional microscopy. The microscope is equipped with an environmental chamber for live cell imaging; 100x 1.49 NA objectives; 405 nm, 488 nm, 561 nm, and 633 nm lasers; and a high speed sCMOS camera.
- Zeiss AxioScan.Z1 and Z7 are two slide scanners that allow automated scanning and digitization of up to 100 slides at a time for either traditional three-color histology stains (brightfield) or fluorescence labelled slides in 10x, 20x or 40x magnification with plan-apochromatic objectives with numerical apertures up to 0.95. The unit is equipped with 7 fluorescent channels, a huge

dye database and polarization options for brightfield. It can perform Z-stacks and extended focus, as well as a full range of image analysis and processing functions available through Zeiss software.

- JPK Nanowizard 4 Atomic Force Microscope mounted on a Nikon A1 Confocal Microscope. The hybrid scope allows for the simultaneous and complementary investigation of samples in optical and nanomechanical/topographical ways. The Nanowizard combines fast tip scanning with high resolution scan sizes of up to 100 μm^2 . The unique architecture of the instrument, together with an acoustic enclosure and a stage top Petri dish heater, provides the mechanical and thermal stability to afford time laps imaging of living cells and observation of tissue dynamics in real time, while simultaneously covering the same optical and nanomechanical field of view. A comprehensive software suite facilitates many modes of data acquisition and analysis, including imaging force spectroscopy, direct overlay and nanolithography. Further, complementary and additional software for the analysis of AFM data, Gwyddion, is available on a separate workstation. The Nikon A1 is equipped with four channels for confocal laser scanning and a high-resolution water cooled sCMOS camera for fast and sensitive epi-widefield fluorescence and brightfield/phase/DIC imaging.
- Zeiss PALM Laser Microbeam Microscope, using Laser Microdissection and Pressure Catapulting (LMPC) technology, it is possible to obtain contamination-free samples from various sources, e.g., histological specimens, living cells and cell cultures, plant material, chromosome spreads, and forensic preps. The instrument allows for analysis of DNA, RNA, and proteins and hybridization to microarrays, PCR, RT-PCR, 2-D PAGE, or immunoblot, as well as re-cultivation of live cells. The instrument is based on an AxioObserver inverted research microscope with a newly designed fluorescence beam path, featuring AxioCam MRc and MRm image acquisition for both brightfield and fluorescence high-resolution digital imaging. The AxioVision System Software provides additional functionalities, including extended focus and multichannel fluorescence.
- X-Clarity Tissue Clearing System. The facility maintains a commercial electrophoretic clearing system for rendering tissue optically transparent in a few days. The system can clear a variety of tissues including whole brains.
- Bitplane's Imaris is a powerful analysis software for data visualization, analysis, segmentation and interpretation of 3D and 4D microscopy data sets. It offers a set of high-performance tools to analyze multidimensional image data including interactive filtering, sorting, classifying, selecting and grouping objects based on statistical parameters and very advanced object tracking functionality. The high-end workstation running Imaris has 1TB of RAM to accommodate even the largest datasets.
- Spectral Instrument Ami HT Small Animal Imager is an advantaged, high-throughput system designed for precise and efficient *in vivo* imaging of small

animals. It features a 5-mouse capacity for bioluminescence and fluorescence imaging with a 25cm x 17cm field of view. The high sensitivity system using a -90°C absolute CCD camera that is thermoelectrically air cooled coupled with a high power and narrow band LED light sources provides unmatched sensitivity which enables the detection faint signals.

- NeuroStar Automated Craniotomy is a unique stereotaxic instrument designed for automated drilling and ultraprecision injections for small animals. The system features atlas integration, intuitive computer control of the stereotaxic frame, and alignment correction. The autostop drilling function can prevent damage of underlying brain tissue. Key features include multisite microinjections, precise depth control, and capabilities for brain windowing, contour drilling, and skull thinning.