NIH Equipment Description
Advanced Imaging and Microscopy Core

Microscopy and imaging systems are available for use in the AIM facilities. In room A522 of the Medical School Research Wing there are two inverted laser scanning confocal microscopes with non-linear optics for advanced imaging, one upright brightfield and epifluorescence system for stereology, and one inverted brightfield and epifluorescence system with an enclosed stage. In addition, users have access to an upright and dissecting stereomicroscope for sample viewing or preparation and three dedicated offline analysis workstations. The AIM facility also offers access to an upright brightfield and epifluorescence microscope for double stain imaging and a slide scanner which are hosted by labs outside of the core facility.

Microscopy Facility Major Equipment

Zeiss LSM 710 NLO Confocal Microscope. The core has a Zeiss Axio Observer Z1 inverted LSM 710 NLO laser scanning confocal imaging workstation that provides a wide variety of imaging technologies including multiphoton excitation and spectral analysis. The system has a Violet HeNe (405 nm); Argon Multiline laser (458, 488, 514 nm); Green DPSS laser (561 nm); Orange HeNe (594 nm); Red HeNe laser (633 nm); and Coherent Chameleon Vision II Ti:sapphire laser (680-1080 nm) for multiphoton spectroscopy as well as an X-Cite metal halide lamp for epifluorescence. The system has a 34 channel QUASAR multispectral scanning module, a transmitted-light PMT detector, and 2-non-descanned detectors. The microscope is equipped with a motorized stage suitable for generating high resolution mosaic images from large preparations and a fast piezo motorizer for rapid Z-axis acquisition. The objectives include a EC Plan NEO 5X 0.3 NA; EC Plan NEO 10X 0.3 NA; Plan-APO 20X 0.8 NA; LD A-plan 40x; Plan APO 63X 1.4 NA Oil; Plan APO 100X 1.4 NA Oil; C-Plan APO 10X 0.8 NA W; C-Plan APO 63X 1.2 NA W.

Availa*ble Upon Request*

Cell electrophysiology. The system has a patch voltage-clamp subsystem (HEKA EPC10 double) and a Patchstar double manipulator.

Vessel studies. There is a Danish Myo Technology pressure (CP120) and wire (CW120) myographs, with an AD Instruments Powerlab data logging system and a Dell Dimension workstation.

Live-cell imaging. There is a PECON heating and environmental control system and an 8 channel Automate Scientific programmable perfusion system with Teflon valves.
Multiphoton imaging ("upright" configuration and live animal studies). The Zeiss 710 has an LSM Tech Objective Inverter, stage platform, a Harvard apparatus stereotaxic unit for mice and rats, and equipment for whole-animal anesthesia and ventilation. The inverter system has an Apochromatically corrected DIC 40X W Plan-Apochromat with an NA of 1.0 - an immersion objective designed for electrophysiology recordings on an upright microscopy workstation.

This system is used routinely to generate whole-cell and Ca2+ spark recordings as well as for more routine cytochemistry and co-localization imaging. It is available as needed in A522.

Zeiss LSM 900 with Airyscan 2 & jDCV. This inverted (Axio Observer 7) confocal imaging workstation produces super-resolution, high-contrast images while minimizing light exposure, to reduce photobleaching and channel bleed-through. The system has a diode laser module allowing for light emission at 405 nm, 488 nm, 561 nm, and 640 nm. It utilizes two GaAsP PMT detectors, one transmitted light detector, and an Airyscan 2 detector with Multiplex Mode for super-resolution imaging along with adaptive focusing capabilities. The Airyscan 2 is an area detector with 32 detection elements which allows for rapid confocal imaging for spatial and temporal recordings, as well as generating mosaic tiling for multidimensional reconstruction. The microscope performs deconvolution using LSM Plus and Joint Deconvolution (jDCV), using a dedicated computer workstation. LSM Plus uses a Wiener filter to enhance signal noise, improve resolution, and provide spatial information. jDCV works alongside the Airyscan 2 detector to add additional structural information and further improve resolution.

The system uses an Axiocam 705 mono, which provides high-resolution monochrome image capture, low light fluorescence imaging, high frame rate imaging, and broad spectral sensitivity from UV to near-IR light. The microscope is equipped with a motorized stage suitable for tiling of large areas and a Z piezo stage to generate Z-stacks with precision. For multi-day, multi-position time-lapses, the system utilizes Definite Focus 3 to compensate for focus drift, resulting in sharp, high-contrast images. An opaque, integrated incubation module creates stable temperature conditions when imaging temperature-sensitive samples and eliminates the effects of potential ambient light. The objectives include EC-Plan NEO 2.5X 0.085 NA; Plan-APO 10X 0.45 NA; Plan-APO 20X 0.8 NA; Plan-APO 40X 0.95 NA; C-APO 40X 1.2 NA W; Plan-APO 63X 1.4 NA Oil.

This system is ideal for observing cell signaling, in-situ hybridization, protein expression and localization, interactions between molecules and proteins, spatial reconstruction, spatial-temporal relationships, and subcellular transport. It is available as needed in A522.

Keyence BZ-X710. This system is an inverted fluorescence phase contrast microscope with a fully contained stage that utilizes brightfield and epifluorescence imaging. For brightfield, the system is capable of oblique illumination, phase contrast, and color imaging and is equipped with a polarized light filter. For epifluorescence imaging, the system has filter cubes for capturing GFP, DAPI, Texas Red, and Cy5. The system is equipped with a motorized x, y, and z stage and thus is capable of performing multidimensional imaging and tiling. The microscope has Plan APOλ 2X 0.10 NA; Plan APOλ 10X 0.45 NA; Plan APOλ 20X 0.75 NA; Plan APOλ 40X 0.95 NA; Plan Fluor 10X 0.25 NA Ph1; Plan Fluor 20X 0.45 NA Ph1.

This system is well-suited for cell counting and high-speed capture of wide-area images. Filter cubes are interchangeable; more filters can be added upon request. This system is available as needed in A522.

Nikon Optiphot. This upright microscope has the ability to perform brightfield and epifluorescence imaging using 10X, 20X, 40X, 100X Oil objectives and a HBO 100W lamp source. For fluorescence, the system is equipped with DM400 (UV-2A), DM430 (V-1A), DM510 (BA 580), and DM580 (G-1A, BA 580). . For image capturing, the microscope is fitted with a Spot Pursuit 4MP digital camera that is used together with Spot 5.1 Advanced digital imaging software. Image Pro Plus 6.3 software for image analysis of fluorescent-labeled samples is also available.

This microscope only has the option to perform double stain imaging. It is available in A572, hosted by Dr. Steven Yellon.

Zeiss Axio Imager A1-Stereologer. The core has a Zeiss Axio Imager A1 upright microscope with multiple objectives (4X, 10X, 20X, 40X, 100X), and an HBO lamp source. For fluorescence, the microscope is equipped with Zeiss filter set 17 (excitation: BP 485/20nm, emission: BP 515-565 nm), filter set 20 (excitation: BP 546/12nm, emission: BP 575-640 nm), filter set 49 (excitation: G 365nm, emission: BP 445/50 nm), and filter set 50 (excitation: BP 640/30 nm, emission: BP 690/50 nm). Additionally, the microscope is equipped with a motorized X-Y-Z

stage (Applied Scientific) Three-Axis Stage Controller for use with closed loop D.C. Servo motor stages, and Z-axis drive. The microscope is fitted with an Allied Vision Technologies Pike F-421C which allows for 2448(H) x 2048(V) display and 3.45 x 3.45 (um) pixel size. It also comes with *Stereologer* software for state-of-the-art unbiased cell/object counting, branching, and volume metrics. FIJI (ImageJ-based) image acquisition software with extensive plug-ins is available for analysis and post-hoc image processing.

The Stereologer software is well-suited for cell/object counting, branching, and volume metrics. It is available as needed in A522.

Olympus Slideview vs200 Slide Scanner. The Olympus vs200 Multiple Tray Loader slide scanner can accommodate up to 210 slides and is capable of brightfield and fluorescence observation. The scanner is equipped with UPLXAPO 4X 0.16 NA, UPLXAPO 10X 0.4 NA, UPLXAPO 20X 0.8 NA, UPLXAPO 40X 0.95 NA, UPLXAPO 60X Oil 1.42 NA objectives. For fluorescence imaging, the scanner has a 4-line laser combiner (405 nm, 488 nm, 561 nm, 638 nm). Fast image acquisition is achieved via automated sample detection, focus mapping, scanning, and stitching. It uses robotics to load and unload trays while simultaneously coordinating the focus and scan unit. An integrated barcode reader is used to capture and record slide information.

The slide scanner is ideal for projects requiring basic imaging of many slides and is best suited for imaging fixed, stained tissue samples. It is available in Alumni Hall Rm 308, hosted by Dr. Konrad Talbot.

Stereomicroscope Workstation. A Zeiss stereomicroscope and halogen light source is available in the imaging core facility for tissue and imaging chamber preparation.

Offline analysis systems. The core facility has three offline analysis systems with Intel Xeon-based processors. Two of these are custom-built Origin computers and the third is a Dell Precision computer. All analysis workstations have the most recent versions of the Fiji build of Image J and RStudio, 2023.12.1. The Dell workstation is additionally equipped with Zeiss Zen software and SparkLab 5.8.2, custom-made program for analysis of linescan images. One of the Origin analysis workstations has a fully functional copy of Imaris and Imaris Stitcher 10.1 by Andor. The other has Aperio ImageScape v12.4.6, OlyVIA V4.1.1, and a permanent site license

of Visiopharm. Imaris can be used for visualization, segmentation, and analysis of 3D and 4D datasets and is particularly suited for fluorescent imaging. Visiopharm utilizes Al-based image analysis and tissue mining tools to allow users to detect, classify, and interpret a variety of sample types and is well suited for performing automated analysis of large datasets.