Live Microscopy Core

Location: 3rd floor Medical Sciences Building
Director: Dr. Christian I. Hong
Full time manager: Chet Closson

800 sq ft facility. Access to the facility and equipment is available 24/7 via keycard swipe. All instrument host computers networked: remote access to data is available to users 24/7 via an automatic backup data server. IACUC approval to import mice for acute experimentation from sites within or outside the University of Cincinnati. The facility includes:

**Mouse surgery room.** Hood for anesthesia available, surgical instruments, fiber optic lights, lighted magnifying stands, heating pads. Ideal for animal and/or tissue preparation directly prior to imaging experiment. The facility supplies investigators with chambers for mounting isolated living tissues, procedures and instruction on animal anesthesia and surgery to expose intact organs for imaging over extended intervals.

**Zeiss LSM510 NLO confocal and two-photon microscope.** 4 lasers (Ti-Sa [700-990 nm; wavelength selection under computer control], Ar [458,477,488,514 nm], Green HeNe [543 nm] and Red HeNe [633 nm]), 6 detectors [3 confocal fluorescence/reflectance, 1 Nomarski transmitted, 2 non-descanned high sensitivity two-photon detectors]). Inverted microscope stage enclosed in light-tight, temperature-controlled environmental chamber. Useful for deep tissue imaging (better than standard confocal) while retaining subcellular resolution in living tissue. The two-photon absorption also allows fluorophore uncaging, cell damage/ablation, spectral shifting, and other photo-catalytic events to be performed with sub-micron resolution in three dimensions.

**Zeiss LSM510 META confocal microscope.** 3 lasers (Ar [458,477,488,514 nm], Green HeNe [543 nm] and Red HeNe [633 nm]), 4 detectors [2 confocal fluorescence/reflectance, 1 Nomarski transmitted, 1 META detector including 8 channels for simultaneous analysis of multiple emission wavelengths spanning 450-800 nm [spectral fingerprinting, emission ratioing at single detector, etc]). This instrument provides rapid and high resolution confocal imaging with a META spectral detector for flexible selection of one or many wavelengths (10 nm resolution).

**Zeiss LSM710 LIVE Duo Confocal Microscope.** Equipped with two separate scanning units, the fast-speed line (LIVE) scanner (up to 120 frames/sec at 512x512) and the new LSM710 high-resolution point scanner, integrated into the same microscope. Point Scanner: 6 laser lines (405, 458, 488, 514, 560, 633nm) and 4 detectors (3 spectral confocal fluorescence plus 1 transmitted light detector). LIVE fast line scanner: 3 laser lines (405, 488, 560nm) and 2 confocal fluorescence detectors. The Duo system allows the two scanners to be used in combination for complex, high-speed bleaching or photomanipulation experiments. Temperature-controlled environmental chamber encasing entire stage.

**Microinjection and Electrophysiology Imaging Station.** The confocal microscope has a custom perfusion chamber that allows high resolution live cell/organoid/tissue imaging while simultaneously allowing access by 3 stage-mounted micromanipulators that can be used in combination with (1) microinjectors (Drummond Nanject II) that allow injection of dyes, drugs or bacteria, and/or (2) microelectrodes for two-electrode voltage clamping, measurement of ion transport, and/or tissue/membrane resistance. The system is equipped with an Axon Instruments Cyber Amp 380 and AI-402 ultra low noise amplifier allowing for use of ion selective...
microelectrodes. The system includes a stage-top sample heater and objective heater for performing work at 37°C. To facilitate preliminary positioning of electrodes/needles, the system includes a swing-arm dissection microscope with video output.

**Zeiss accessories, interchangeable among LSM microscopes:**

**Objective lenses.** 10x/0.3NA dry, C-Apo 10x/0.45NA water immersion, Plan-Neofluar 10x/0.30NA, Plan-Apochromat 20x/0.8NA, Plan-Neofluar 20x/0.8NA dry, Plan-Neofluar 40x/0.8NA water immersion, LD C-Apochromat 40x/1.1NA water immersion, C-Apo 40x/1.2NA water immersion, C-Apochromat 63x/1.2NA water immersion

**Objective Inverter (LSM Tech).** An optical device which can be attached to any Zeiss inverted microscope to change the optical pathway to come from above, as in an upright microscope.

**Environmental chamber** for precise temperature, CO₂, and humidity control for live cell or tissue experiments.

**Leica MZ16FA Stereo Zoom Fluorescence microscope.** Continuous zoom from 7x – 230x total magnification. Host computer drives 12-bit color camera to capture digital images in bright field, dark field, or fluorescence. Filters for UV and GFP fluorescence excitation, or can accept user-defined filters. Stereo microscopy allows viewing specimens three-dimensionally, with great depth of field, in large fields of view.

**Arcturus (Life Technologies) ArcturusXT Laser Capture Microdissection microscope.** Automated collection of specific cells of interest directly from tissue sections, suitable for RNA, DNA, and/or protein analysis. Up to three slides can mounted simultaneously, and regions to be collected can be defined under transmitted light imaging and/or fluorescence imaging. Objective lenses: 2X/0.06NA, 10x/0.3NA, 20X/0.45NA, and 40X/0.6NA. Fluorescence filters: Blue (EX 455-495nm, EM >510nm), Green (EX 503-548nm, EM >565nm), Red (EX 570-630nm, EM >655nm). An IR laser melts a microscopic region (7 micron diameter) of thermoplastic film that attaches to the desired tissue region(s) and provides the mechanism to capture. Optionally, a UV laser is used for surgical cutting (submicron cutting width) of surrounding tissues or the membrane upon which tissue is placed, to more strictly isolate the central region for IR capture. In addition to tissue sections, LCM can be performed on living cells, cell smears, or plant tissue.

**Perkin-Elmer EnVision Multilabel Plate Reader.** High-speed plate reader for detection of luminescence, absorbance, fluorescence intensity, fluorescence polarization, HTS AlphaScreen, and time-resolved fluorometry. Includes plate stacker for up to 50 plates, plate shaker, and dispenser with 2 pumps for injection. Compatible with plates for 24-well up to 1536-well plates.

**Low light ratio imaging microscope.** Roper Coolsnap HQ camera on Zeiss Axiovert 200 inverted microscope with Sutter filter wheels and shutters controlling both excitation and emission light. Driven by MetaFluor (Universal Imaging) software on PC host computer. Objective lens: 10x/0.5NA dry or other Zeiss objectives.

**Li-Cor Odyssey CLx Infrared Imager.** Infrared fluorescence detection and quantitative analysis of Western blots. Two IR lasers (680nm and 780nm) for simultaneous two-color
excitation and fluorescence detection on individual blots. Can also be used to detect whole body fluorescence of anesthetized animals.

**Leica CM1900 Cryostat**. Microtome encapsulated in a cryochamber to cut frozen tissue sections up to 60 micrometers thick. Includes separate specimen cooling system, independent of chamber cooling, both adjustable from 0 to -35 degrees Celsius.