## Advanced Light Microscopy Core

More info: <https://research.missouri.edu/advanced-light-microscopy>

The Advanced Light Microscopy Core (ALMC) is a campuswide resource for all types of light microscopy, including confocal, super-resolution, widefield, and stereomicroscopy. Expert advice and training on all aspects of sample preparation, immunocytochemistry, imaging, and image analysis are available. The ALMC is a shared resource for all types of light microscopy, including widefield, confocal, super-resolution, and stereomicroscopy. The core provides access to state-of-the-art instruments, imaging technologies, services, training, and expert consultation in support of the educational and research missions of the University of Missouri. The core is open to the entire University of Missouri community as well as to researchers from other academic institutions and companies in the area.

**Laser Scanning Confocal Microscopy** is available on two Leica SP8 spectral confocal microscopes. One is equipped with three high-sensitivity hybrid detectors capable of photon counting, and with two conventional PMT detectors. Sample excitation can be accomplished by multiple lasers including a pulsed white light laser tunable from 470 to 670 nm. Fixed laser excitation lines of 405, 458, 476, 488, 496, and 514 nm are also available. For two-photon imaging, including FLIM-FRET, the system has a MaiTai DeepSee Ti-Sapphire ultrafast femtosecond laser. This SP8 is also equipped with both a conventional point scanner for work requiring the highest sensitivity and a fast resonant scanner for live-cell imaging (28 fps at 512x512). An environmental chamber for temperature and CO2 control during prolonged live-cell imaging experiments is available. The second SP8 confocal has one hybrid and two PMT detectors and is equipped with powerful diode lasers of 405, 488, 552, and 638 nm for conventional confocal imaging. The system is also equipped with a digital light sheet (DLS) module that illuminates a sample with a thin plane of light, allowing rapid acquisition of multiple images with minimal photobleaching and subsequent 3D rendering.

**Super-Resolution Microscopy** can be performed on two microscopes. One of itsour Leica SP8 confocals is equipped with a STED 3X system that can achieve a resolution of 50 nm or better. Three depletion lasers (592, 660, and 775 nm) enable multicolor detection of a wide range of fluorophores. The LightGate technology allows gated emission detection (gated STED) which further improves resolution. The Leica SR GSD 3D wide-field super-resolution microscope can achieve a resolution down to 20 nm in the lateral plane and 50 nm in the axial plane. The system is capable of precise single-molecule localization in 2D and 3D. The microscope is equipped with 488, 560, 642, and 405 nm lasers for multicolor detection of up to 3 fluorophores and with a fast, highly sensitive Andor iXon Ultra 897 EMCCD camera. The microscope also includes a fully automated TIRF system, allowing super-resolution to be combined with TIRF microscopy.

**Wide-field Microscopy** using brightfield, phase contrast, polarized light, DIC, darkfield, or fluorescence can be done on multiple microscopes. Two Zeiss Axiovert 200 inverted microscopes, each with a computer-controlled motorized xy-stage, z-motor drive, and fluorescence filter wheel for automated acquisition of multiple wavelengths and focal planes (3D imaging) are equipped with either a fast, sensitive Hamamatsu ORCA-ER CCD camera or a 3-megapixel Leica DFC290 color CMOS camera to acquire high-quality images, including those requiring automated tiling of larger fields of view. A Leica DM5500B upright microscope is equipped with a Leica DFC295 high-resolution color camera and a Leica DFC340 high sensitivity cooled monochrome camera. Cell injections and manipulations can be carried out on a Leica DMI4000B inverted microscope equipped with an Eppendorf microinjection system.

**Fluorescent and Brightfield Stereomicroscopy** is possible on a Leica Thunder Imager Model Organism stereoscope system outfitted with 0.63×, 1×, and 2× objectives, a high-sensitivity DFC9000 GT 4.2-megapixel sCMOS monochrome camera and a high-resolution DMC5400 20-megapixel CMOS color camera. The Thunder opto-digital technology removes out-of-focus blur from fluorescent images, delivering sharpened imaging data in real time. A motorized XY scanning stage controlled by the Navigator software allows large areas to be scanned at high magnification for high-throughput screening of samples in multiwell plates. A Leica M205 FA stereomicroscope equipped with a motorized filter wheel (UV, GFP, dsRed, CFP, YFP, mCherry, Cy5) and z-drive and with a highly sensitive Leica DFC7000 color camera is also available. LAS AutoMontage software allows for the acquisition of images with extended depth of focus.

Image Processing and Analysis is carried out by Huygens Professional software for deconvolving 3D data sets from STED, confocal, and wide-field microscopes. Imaris, MetaMorph, and LAS X software are available for 2D and 3D image rendering, neuron tracing, analysis, and processing.

**Preparative Equipment** includes a cryostat microtome, paraffin microtome, ultramicrotome, and Vibratome 3000 Plus for preparing fresh or fixed tissue sections. The Leica CM3050 S research cryostat is a motorized instrument that can produce sections ranging from 0.5 to 300 microns in thickness. The precise specimen positioning system in the X and Y directions ensures both accurate and repeatable orientation of frozen tissue blocks. Moreover, the cryostat offers separate cooling controls for the cryochamber and specimen, enabling optimal sectioning temperatures for a wide range of animal and plant tissues. The Leica CM3050 S cryostat is useful in preparing samples for immunohistochemistry or performing the initial steps of Visium Spatial Gene Expression profiling. The recently acquired Leica ARTOS 3D automated ultramicrotome simplifies the production of semi-thin sections for high-resolution light microscopy and immunocytochemistry. The ARTOS ultramicrotome also effectively eliminates many of the manual manipulations needed to produce consistent ribbons of serial sections for array tomographic reconstruction of 3D volumes using either immunofluorescence or electron microscopy.