

Major Equipment – Core D

Microscopy

B429A Life Sciences (Dr. Jackson's Laboratory)

Nikon Eclipse TE300 inverted microscope equipped with a CSU-10b Nipkow disk confocal head and a 25 mW 488 nm diode laser for high speed (120 frames/s) confocal imaging of intracellular Ca^{2+} using Fluo-4 or related dyes. Images are captured by a Stanford Photonics Mega XR/Turbo EX iCCD camera controlled by Stanford photonics Piper software for high-speed confocal imaging. This microscope also is equipped with IR-sensitive CCD camera for capturing transmitted light images and monitoring blood vessel diameter. The microscope is outfitted with long working distance UV objectives (20X, NA 0.5 and 40X, NA 0.75). The microscope is configured such that preparations can be transilluminated with red light ($> 600 \text{ nm}$) with images captured by the CCD camera, while simultaneously epi-illuminating the preparations for fluorescence measurements using the Spinning disk confocal system. Images from the CCD camera are captured by a second computer running Vasotracker automated diameter tracking software for study of isolated blood vessels. A gravity-driven, automated, heated perfusion system is available for superfusion of preparations with physiological salt solutions at 37° C . This system is configured such that either cells, isolated tissues or cannulated, pressurized blood vessels (+ or – PVAT) can be studied.

Leica DM LFSA equipped for IR-DIC and conventional epi-fluorescent microscopy, and widefield imaging of Fluo-4 and other intensimetric dyes in cannulated arteries and veins, and in mesenteric PVAT. The microscope has long working distance water immersion objectives (20X, 0.5 NA; 40X, 0.8 NA; 63X, 0.9NA). Illumination for conventional epi-fluorescence microscopy is provided by a ??????. A second 100 W Hg lamp controlled by a Solamere DX-1000 high speed optical switch provides rapid switching between 340 and 380 nm excitation light for Fura 2-based Ca^{2+} imaging. This system is equipped with Warner Instrument solution heaters, controllers, valves and a peristaltic pump for superfusion of preparations with heated (37° C) solutions. A Grass S48 electrical stimulator and a SIU5A stimulus isolation unit are present for electrical stimulation of tissues.

B429 Life Sciences (Dr. Jackson's Laboratory)

Micropipette Manufacture: Sutter P-97 computer controlled pipette puller, Narishige MF-9 microforge for pipette polishing, Stoelting microforge for perfusion pipette construction, and WPI pipette beveler.

Microsurgery: Three Wild M5A stereomicroscopes mounted on custom tables for epi- and transillumination, and two cooled-circulating temperature controllers for isolation of PVAT, arteries and veins for pressure myography or live cell/tissue imaging.

Zeiss Axovert 35 inverted microscope completely equipped for pressure myography with 10X, 20X and 40X objectives and including Warner instruments solution and bath heating units and controllers, a Masterflex pump for bath perfusion and recirculation, a pressure column for pressurization of isolated vessels from 25-350 μm internal diameter, CCD camera and system computer with DMT MyoView software for continuous recording of vessel internal and external diameter.

B428 Life Sciences (Dr. Jackson's Laboratory)

Microsurgery: Two Nikon SMZ 800 stereomicroscopes mounted on custom tables for epi- and transillumination, and one cooled-circulating temperature controller for isolation of PVAT, arteries and veins for pressure myography or live cell/tissue imaging.

Deconvolution Workstation based on a Xeon Quad Core 2.4GHz Intel processor with 32GB RAM based and a 30" IPS monitor. This workstation is equipped with AutoQuant X3 deconvolution software which offers a complete suite of 2D and 3D deconvolution algorithms.

Zeiss LSM 880 NLO microscope system with an Airyscan superresolution detector and Airyscan Fast scanning system. Components of the system include: Axio Examiner.Z1 motorized upright stand with temperature controlled chamber, scanning stage and piezo z-drive; LSM 880 confocal scanhead featuring 2 Multi-Anode-Photomultiplier Tubes (PMTs) and 1 Gallium arsenide phosphide (GaAsP) high-sensitivity detector; Airyscan superresolution detector (enabling up to 140 nm, resolution in XY and 400 nm in Z) including a GaAsP Airyscan

detector; Airyscan Fast beam-shaping optics, enabling up to 145 x 180 x 450 nm (X,Y,Z) resolution at 19 fps (for 512 x 512 pixel); 405, 458, 488, 514, 561, 633 nm laser lines for confocal imaging; IR laser (Coherent Vision II) with routing optics for multiphoton imaging; 2-channel GaAsP non-descanned detector (for reflected light and conventional multiphoton imaging); An additional transmitted light detector ("T-PMT") for visualizing un-stained tissues; Objectives - 10x/0.5, 20X/1.0 (water dipping), 40x/1.0 (water dipping), and 63x/1.0 (water dipping) with DIC optics; ZEN 2.3 software package including Z-stack, time lapse, tiles/positions, photobleaching, photoactivation, spectral unmixing, automated image analysis, measurements, colocalization, autofocus, batch exporting, etc.; 2 HP Z840 Xeon CPU-based workstations with 3.5 TB RAID arrays for image storage, and large format anti-vibration table. This system is also equipped for superfusion of cells/tissues with Warner instruments in-line solution heater and syringe heating systems, Masterflex recirculating pump, pressure column (for pressure myography) and Grass S88 stimulator for electrical stimulation of vessels. A Ventomac precision isoflurane vaporizer is also available for imaging tissues in anesthetized mice and rats.

Fabrication and Design Equipment

B436A Life Sciences (Dr. Tykocki's Laboratory)

The Fabrication lab (B436A Life Sciences) includes a Prusa i3 Mk3S 3D FDM Printer capable of printing objects in a wide array of plastics with a Z resolution $\geq 50 \mu\text{m}$. Also included is a Carbide 3D Nomad 883 Pro Desktop CNC machine that can mill objects out of a wide range of materials, from low-density polyethylene to 6061 aluminum. Additionally, the Fabrication Lab contains a wide array of manual and power tools (taps, dies, drill press, rotary tools, etc.) needed for fabrication and assembly of scientific instrumentation. The two computers in the Fabrication Lab contain all necessary software for translating 3D designs into printable code (Slic3r software) or millable code (MeshCAM software). These systems are all compatible with renderings from a variety of open-source 3D design software packages. The computers in the Fabrication Lab also have educational licenses for Fusion360, Trimble SketchUp, and Creo CAD suites.

Nikon SMZ-18 Stereo Intravital Microscope equipped for traditional epifluorescent recording of green (GCaMP, eGFP) and red (tdTomato, mCherry) fluorophores in live tissue and animals. This microscope has an SHR Plan Apo Objective (1X, 0.15 NA, w.d. 60mm) and zooming body, allowing for magnification from 7.5 – 135X. Tissues are illuminated using a SOLA SE II 365 Light Engine LED light source with electronic controller and shutter. The system is coupled to an Andor Zyla 4.2Plus cooled sCMOS 16-bit camera for quantitative, high-speed (max 56 fps at full-frame) and low-light recording. The camera is controlled by a Quad-Core imaging PC running Micro-Manager software. This system also includes in-line heating and recirculating systems to maintain tissue superfusion and temperature while recording. Also available is a custom-designed warming pad for maintaining animal core body temperature and position during recording under anesthesia.