

PENN VET IMAGING CORE FACILITY

LEICA SP8 CONFOCAL/MULTIPHOTON MICROSCOPE SYSTEM

Location: Hill Pavilion, room 374

Applications:

- Intravital 2-Photon imaging
- Second harmonic generation (SHG) imaging (both backward and forward SHG)
- Third harmonic generation
- Fluorescence recovery after photobleaching (FRAP)
- Intensity Förster resonance energy transfer (FRET)
- Routine confocal microscopy and spectral scans

Microscope Stand: Leica DM 6000 upright microscope; Scientifica motorized stage with interchangeable variableheight platforms

Software: Leica Application Suite (LAS X) with FRAP and FRET wizards, live data mode (which allows programming of complex acquisition sequences), and 3D viewer

Huygens deconvolution and Imaris 3D rendering and analysis software are available on workstation computers in the imaging core

Volocity software is available via the imaging core license server for 3D rendering and analysis of images

Lasers:

•Solid state lasers (488, 552, 638 nm)

•Coherent Chameleon Vision II Ti:Sapphire laser (tunable, 680-1080 nm) and MPX optical parametric oscillator (tunable, 1000-1350 nm), which are capable of simultaneous dual emission (Vision II limited to 780-870 nm range during simultaneous emission)

Detection: 3 internal spectral detectors (2 PMT, 1 HyD), 1 trans detector (PMT) optimized for forward SHG, 4 external non-descanned detectors (HyD)

Filter sets for NDDs: Standard configuration is DAPI/FITC (SP680, BS495LP, BP460/50, BP525/20), mCherry/Cy5 (SP800, RSP650, BP624/40, BP685/40); also available: CFP/YFP (BS505LP, BP483/32, BP535/30) in place of DAPI/FITC and tdTomato (BP 610/75) in place of mCherry (BP624/40)

Resonant scanner available for rapid (8000 kHz) scanning



Objective Lenses:

• 20x water immersion HCX APO L (1.00 NA) [*This lens has a separate mount from the lenses listed below*]

- 25x water immersion (0.9 NA)
- 10x dry HC PL APO CS (0.40 NA)

• 40x* oil immersion HCX PL APO CS (1.25-0.75 NA; variable iris)

• 63x* oil immersion HCX PL APO CS (1.40-0.60 NA; variable iris)

Peripheral Equipment:

- Isoflurane anesthesia machine
- Temperature control

 \circ Fine Science Tools TR-200 temperature controller with small (3x4") and large (3.5x9") mouse heating pads

• Warner Instrument Corporation TC344B temperature controller, syringe heater, inline heater, and thermal control for culture chambers

<u>Perfusion system</u> with heat, oxygenation, and vacuum
Culture chambers

• Warner heated cell culture chamber Model QE-1 for 25 mm coverslips and 35 mm dishes

• Warner heated perfusion chamber (PM-1 platform with RC-22 insert) for tissue explants

* The 40x oil and 63x oil lenses are shared among the Leica microscopes in the core, but can usually be made available upon request

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