

PENN VET IMAGING CORE FACILITY

LEICA SP5 II CONFOCAL/FLUORESCENCE LIFETIME IMAGING MICROSCOPE (FLIM)

Location: Old Vet building, room 394E

Applications:

- FLIM-based Förster resonance energy transfer (FRET)
- Routine confocal microscopy and spectral scans

Microscope Stand: Leica DMI 6000B inverted microscope with motorized stage equipped with Tokai Hit stage-top environmental chamber (accommodates 35 mm coverglass-bottom dishes or standard microscope slides)

Software: Leica Application Suite Advanced Fluorescence (LAS AF) with FLIM wizard, FRAP wizard, Live Data Mode; PicoQuant SymphoTime software for FLIM; Globals for Images-SimFCS (software developed by Enrico Gratton for FLIM analysis); access to Volocity software is available via the imaging core license server for 3D rendering and analysis of confocal images

Lasers:

- Argon laser (458, 476, 488, 514 nm)
- HeNe lasers (543 and 633 nm)
- PicoQuant Sepia II multichannel (405, 470, and 640 nm) picosecond pulsed diode laser

Detection: 3 internal detectors (2 APDs for imaging or FLIM, 1 HyD for imaging), 1 trans detector (PMT); resonant scanner available for rapid (8000 kHz) scanning

Objective Lenses:

- 63x water immersion HCX PL APO (1.20 NA; correction collar)
- 10x dry HCX PL FLUOTAR (0.30 NA)
- 20x* dry HC PLAN APO (0.70 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)
- 100x* oil immersion HCX PL APO (1.46 NA; correction collar)

** The 20x dry, 40x oil, 63x oil and 100x oil lenses are shared among the Leica microscopes in the core, but are normally available upon request*



FLIM Equipment:

- PicoQuant Sepia II multichannel picosecond diode laser
- PicoQuant fiber coupling unit with manual control of laser output power
- PicoQuant PicoHarp 300 TCSPC (time-correlated single photon counting) module and picosecond event timer