

Multi Photon Intravital and Tissue Imaging (MITI) Core Facility

The MITI Facility, part of the Preclinical Imaging Center housed within the Fred & Pamela Buffett Cancer Center and Translational Imaging and Behavioral Assessment (TIBA) Core within the Cognitive Neuroscience of Development & Aging (CoNDA), is equipped with an Olympus FVMPE-RS Multiphoton Laser Scanning Microscope. This system is specially configured for deep tissue imaging in both living and fixed samples. Dual beam, multiphoton excitation is accomplished using a Spectra Physics Dual line InSight X3 near infrared laser. The main output from the InSight laser is a tunable line ranging from 680 to 1300 nm with a second multiphoton excitation line at 1045 nm. This extended excitation range supports both traditional fluorophores (blue to long range red) and fluorescent proteins, as well as, second and third harmonic imaging. Potential signal crosstalk during simultaneous imaging is reduced by transitioning to sequential scanning (line or frame). Likewise, this system utilizes a Quadralign 4 axis alignment module to autocorrect potential laser beam misalignment and pixel shift during multi-color excitation.

Equipped with 5, 10 and 25x objectives, Z-axis and x, y stage motors, this system can image both intravital and fixed samples. Deep Tissue imaging is facilitated using a highly specialized light path containing silver coated, high reflectance scanner mirrors equipped to increase detection of scattered and low intensity fluorescence. This system uses both a traditional Galvanometer scanner and a high-speed Resonant scanner capable of imaging 512 x 512 pixels at 30 frames per section or up to 438 frames per section when imaging 512 x 32 pixels. Two multi-alkaline and two gallium arsenide phosphide (GaAsP) are available for multichannel detection, the latter delivering optimum quantum efficiency and improved signal to noise ratios.

The Olympus FVMP-RS system is additionally equipped with 458 and 588 nm stimulation laser lines exclusively positioned for photobleaching, light stimulation and/or photoactivation of various fluorescent or caged constructs. A Neurotar Mobile Home Cage integrates real-time imaging with behavioral/locomotion tracking. Researchers can perform real-time imaging of neuronal activity concurrently with activity tracking using this accessory while imaging on the Olympus system. In addition to automating a number of imaging procedures, the Olympus software is available for collecting and interacting with various types of data including optical Z-sectioning, 3D imaging, multi-location imaging at individual and multiple time points and fluorescence quantification.