IBEST Optical Imaging Core Facility

The IBEST Optical Imaging Core (OIC) is dedicated to creating opportunities for high-resolution imaging and high-throughput characterization of biological processes. The OIC consolidates 4 fluorescent-based microscopes, a Fluorescent Activated Cell Sorter and analysis software programs into one core facility available to all UI investigators. An experienced microscopist, Ann Norton, provides expertise in experimental design, image capture, cytometric acquisition and data analysis. With this infrastructure and staffing, the OIC is able to support a diverse range of research projects, including but not limited to, monitoring protein translocation, visualizing viral movement and isolating of stem cells.

Core Facility Infrastructure

The IBEST Optical Imaging Core has instruments for imaging biological samples such as individual cells, biofilms, tissue samples, and small organisms. There are 4 microscopes available which provide stereoscopic, epifluorescent, confocal and multiphoton imaging. A Flow Cytometer is available for high-throughput counting and sorting of cell populations. Two separate computers are available for data analysis. A small refrigerator and small incubator are also located in the laboratory.

Fluorescent Stereo Microscopy

The Leica MZ16F Stereo Fluorescence microscope with DAPI, GFP and DsRed filter sets provides color imaging of surface structure of larger samples, such as nematodes, seeds and flowering plants.

Epifluorescent and transmitted light microscopy

The Nikon Eclipse upright microscope has filter sets for standard DAPI, Violet, FITC and TRITC fluorescence. MetaMorph software (Molecular Devices) controls the Hamamatsu C4742-95 12-bit monochrome camera and filter set movements.

A Leica upright Laborlux microscope with a color digital camera is available for true color imaging of traditional histological samples.

Confocal and Multiphoton microscopy

The Olympus Fluoview upright microscope platform provides laser scanning confocal imaging of cells and tissue. The high energy of laser excitation, increased excitation options with multiple laser lines (405, 440, 468, 515, 559, 635nm) and the pinhole aperture that reduces out of focus fluorescence, aid in producing extremely high resolution images.

The Olympus Fluoview also has a Coherent Ultra 2 multiphoton tunable laser ranging from 690-1040nm. The longer wavelengths of the multiphoton laser allow for deeper penetration into samples and reduced damage to live tissue.

Spinning Disk Confocal microscopy

The Nikon TiE inverted microscope with a Yokogawa X1 Spinning Disk and perfect focus system is ideal for high speed live imaging. The Andor DU888U3 EMCCD camera provides improved sensitivity and the Andor Zyla sCMOS camera provides speeds of up to 100 frames per second to capture events on a true biological time scale.
Laser options on the Spinning Disk are 405, 445, 488, 515, 561 and 640nm. Widefield illumination includes LEDs for DAPI, CFP, GFP, YFP, mCherry and Cy5 illumination. Nikon’s NIS Elements software controls acquisition in 6 dimensions: X, Y, Z, time, multiple wavelengths and multiple points. A Galvo miniscanner photoactivation system is available using UV activation in FRAP and optogenetic studies.

**Flow Cytometry and Sorting**

The BD FACS Aria Flow Cytometer has a blue and red laser and characterizes samples for Side Scatter, Forward Scatter and multiple fluorescent markers. Sorting can be done into up to 4 tubes or multiple-well plates.

**Data Analysis Software**

Multidimensional image analysis is available using NIS Elements AR, Imaris F1 and MetaMorph packages. The NIS Elements AR package also includes deconvolution, image stitching, ratio/fret and JOBS environment for simplification of routine analysis. Imaris F1 includes a filament tracer module for automated analysis of tree-like volumetric results.

FlowJo software is available for analysis of fcs files of flow cytometry data.