UT Health San Antonio Institutional Optical Imaging Core Facility

The Optical Imaging Core Facility (OIF) provides high quality services to researchers at UT Health San Antonio (SA), other academic research institutions, and business corporations. The core houses a wide spectrum of state-of-the-art optical imaging systems to meet the demands for various biomedical *in vitro* and *in vivo* applications, from single molecules to whole animals and from cultured cells to deep tissue, including high temporal resolution for live cell imaging of rapid events. The OIF is located in two campus sites, one situated in the Dental School Building on the main (Long) campus and the other in the South Texas Research Facility Building on the Greehey campus, together totaling 24,00 sq. ft.. The core is managed jointly by the Institution and the Cancer Center.

Personnel:
Dr. James Lechleiter, Professor of Cell Systems and Anatomy, has served as Director of the OIF since 2004. He has extensive experience with imaging technology and its application towards current problems in cell biology. He lectures and co-directs a graduate level course entitled "Practical Optical Microscopy (CSAL5083)" and shares patents on a confocal microscope for simultaneous imaging with visible and ultraviolet light as well as a multi-photon laser scanning microscope using an acoustic optical detector. Dr. Lechleiter has served as a member of the National Science Foundation (NSF) Study Panels on Instrumentation Development for Biomedical Research and has served on Signal Transduction and Regulation.

Dr. Exing Wang joined UT Health SA in October 2011 and has served as Associate Director since. Previously, he managed the Indiana Center for Biological Microscopy and was the director of the Microscopy and Imaging Core Facility at Rensselaer Polytechnic Institute. He has an extensive background in quantitative optical microscopy and applied optics. He has built and modified several different types of advanced microscopes, including confocal, multiphoton, and light sheet. He has developed many image analytical methods through diverse biomedical studies. Since joining UT Health SA, he has been teaching a graduate level course entitled "Practical Optical Microscopy (CSAT 5083)", and serves as the course co-Director.

Mr. Jimmy Wewer is the core facility imaging technologist who has a background in both engineering and biology. He has worked for the OIF since 2006 and is familiar with advanced optical microscopy techniques. He provides user training and assistance services as well as facility maintenance.

Major Services and Technologies:
The OIF offers imaging services that can be divided into five major areas:

A. Optical Imaging

Confocal and Multiphoton Microscopy:

Zeiss LSM 710 Confocal (STRF 252)
This confocal is equipped with an Axioimager Z1 upright microscope, three spectral channels offering flexible optical configurations, motorized stage for tiling, and 5 lasers: Violet diode (405 nm), Argon (458, 488, and 514 nm), laser diode 561 nm, HeNe 594 nm, and HeNe 633 nm. This confocal is ideal for 3-dimensional imaging of fixed tissue slices and cultured cells.

Olympus FV1000 Confocal/Multiphoton (Dental Building 2.518U)

This confocal and multiphoton system is equipped with an Olympus IX 81 inverted microscope, three internal channels for confocal, including two spectral channels for flexible optical configurations, and three external non-descanned channels for multiphoton imaging. This system is ideal for 3-dimentioanl imaging of fixed cultured cells, tissue slices as well as in vivo imaging. Multiphoton enhances depth of penetration and contrast for imaging of thick specimens. LSM Technology objective inverter device is available for converting this inverted microscope to an upright style for conducting intravital microscopy. Visible lasers for confocal microscopy: vilet diode (405 nm), Argon (458, 488, 514 nm), and HeNe (543 nm), Diode (635 nm); femtosecond pulse laser for multiphoton microscopy: Coherent Chameleon Ti:Sapphire, tunable range 715 – 900 nm.

Prairie Confocal/Multiphoton System (STRF 252)
This laser scanning confocal and multiphoton imaging system is mounted on a Nikon FN1 upright microscope. There are three internal detectors for confocal imaging and three external non-descanned detectors for multiphoton imaging. The system is equipped with five visible lasers for confocal imaging and a Coherent Chameleon Ultra II broad range tunable femtosecond Ti:Sapphire laser for multiphoton imaging. The system also includes a high performance motorized Prior Zdeck stage platform with holes for accessory mounting. The height of the stage platform is adjustable, providing flexibility for setting up experiments requiring various accessories. The upright configuration along with a Nikon 25X long working distance and high resolution water dipping/immersion objective makes it a powerful imaging system for intravital microscopy. Visible lasers for confocal microscopy: Individual diode lasers: 405, 488, 542, 561, and 640 nm; femtosecond pulse laser for multiphoton microscopy: Coherent Chameleon Ultra II Ti:Sapphire, tunable range 680 – 1080 nm.

Nikon sweptfield confocal (STRF 252)
The sweptfield confocal system is equipped with a Nikon Eclipse Ti inverted microscope and a highly sensitive QuantEM camera. The scanner has 32-pinhole and slit scanning options. The live-cell imaging chamber is accessorized with a temperature controller and a CO2 regulator. The microscope system is also equipped with an anti-focus drift device, namely Perfect Focus System, which is essential to combat axial fluctuation in real time during long-term live cell imaging sessions. Its unique rapid scanning mechanism combined with a highly sensitive detector makes it a best choice for rapid live cell imaging with minimal phototoxicity. The sweptfield confocal is capable of acquiring to up to four channels. This microscope system also has a Photometrics CoolSnap HQ2 camera, mounted on the right side port, to be used for DIC and widefield Epi-fluorescence imaging.

Laser-Based Special Techniques:

Nikon N-STORM Super Resolution Microscope (STRF 252)
Super-resolution is used to localize individual proteins beyond the optical diffraction limit. By first labeling a protein target with either a photoconverting or photoactivating fluorescent probe, a subset of molecules can be isolated and their centers targeted using single-molecule localization techniques. Spatial resolution can be improved from ~200 nm to 20 nm. Available laser lines: 405, 488, 561, and 647 nm.

Zeiss Lightsheet 7 Microscope (Dental Building 2.664U)
Acquired with funding from NIH Shared instrumentation grant, this light sheet microscope is fully loaded with comprehensive features for rapid three dimensional imaging of transparent live organisms and optically cleared tissues up to 20 mm, at refractive index between 1.33-1.58. The illumination is delivered by dual opposing illumination objectives. Fluorescence signal is imaged

by a detection objective in a perpendicular direction to the illumination beam. Illumination objectives are equipped with an adjustable focusing collar to compensate focal shift due to refractive mismatch to ensure the waist of the illumination light sheet is centered in the geometry of the sample chamber. The thin horizontal illumination sheet is scanned vertically to generate homogeneous illumination across the specimen. As with illumination objectives, the detective objectives used for clearing medium are also equipped with focus adjustment to ensure in-focus signal is collected. The scanning mechanism is accompanied by pivot scan technology to eliminate the shadow effect in illumination induced by some structures in specimen to create artifacts free images. Dual cameras are available to simultaneously capture two channels, which is especially important for live specimen imaging. Specimen, loaded from the top of the chamber, can be rotated 360º automatically allowing for easy sample positioning. Black and Blue versions of Zen software are used for acquisition and analysis, respectively. The system includes two workstations, one for acquisition and one for data analysis. Acquired data can be transferred from the acquisition workstation to the offline workstation in real time during data collection.

IVIS Spectrum *in vivo* imaging system (Dental building LAR)
IVIS Spectrum is a pre-clinical imaging system for non-invasive longitudinal studies in small animals. It is capable of performing both fluorescence and bioluminescence imaging with 20 microns resolution and a detection wavelength range between 490-850 nm, which covers a broad range of available fluorescent and bioluminescent reporters.

Widefield fluorescence and transmission imaging (available on both sites)
These are conventional fluorescence imaging techniques that typically use a non-laser light source to image live or fixed thin layer sections or cultured cells, and can be combined with a contrast technique in transmission mode. Acquisition speed is usually faster than with laser scanning. The OIF has standalone microscopes with these techniques.

B. Analysis of Digital Images and Processing of Images for Publication
The OIF maintains workstations on the confocal, multi-photon excitation (MPE), and super-resolution microscopes and Windows-based offline workstations. Programs available are: Imaris (Oxford Instruments), Metamorph (Molecular Devices), Fluoview and Zen software for confocal or MPE data acquisition; Nikon NIS software, Adobe Photoshop; and ThunderStorm for super-resolution data. Macros for ImageJ were written in house to analyze co-localization of fluorescent signal, count objects, and report membrane potential changes, and image segmentation.

C. Consultation
OIF staff advise users on experimental design, probe selection, and specimen preparation.
All investigators are expected to first meet with OIF staff to discuss their projects to ensure feasibility, optimal design, and the best use of OIF equipment. Once the project has begun, facility staff continue to provide consultation and training.

D. Training and Teaching
Fully trained users are authorized to operate the imaging equipment 24/7. Users can also opt to have OIF staff perform imaging at an additional cost. Training on a given instrument is done in two steps. First, users participate in a tutorial session during which they are instructed in both the theory and basic operation of the instrument. Next, users participate in a hands-on practical session performing the basic setup and operation of the instrument supervised by OIF staff. Users then learn to optimize the instrument's operation for their projects. After successfully completing this session, users are considered independent and can reserve time on a first-come basis through web-based calendars. The OIF also offers a graduate-level optical imaging course (CSAT 5083) annually, covering both theory and practice of optical imaging and analysis.