**UT Health San Antonio Institutional Optical Imaging Core Facility**

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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 Weweris 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**

*Widefield fluorescence and transmission imaging*: 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.

*Laser Scanning Optical Imaging*: Confocal microscopes create optically thin sections (~0.5 μm) to quantify probe fluorescence within subcellular compartments. Multi-photon (MPE) excitation of fluorophores occurs via simultaneous absorption of two (or more) photons, which confines excitation to the plane of focus. Photobleaching and phototoxicity are reduced, enabling longer live-cell studies. The efficiency of photon collection is further improved by mounting detectors closer to the specimen, yielding a higher signal-to-noise ratio (contrast) deeper into scattering tissue. In detail, we have an Olympus FV-1000 system, equipped with 4 visible light lasers (405, 488, 514, 543 and 633 nm) that is capable of imaging 4 fluorescent labels simultaneously as well as recording the transmitted light image. This instrument can also be used for multiphoton imaging (Chameleon, Coherent). A second instrument, the Zeiss 510 NLO microscope, is equipped with 3 visible light lasers (457, 488, 514, 543 and 633 nm) and also equipped with a second Titanium-Sapphire pulsed laser (Verdi/Mira system, Coherent, Inc) for multiphoton imaging. At the STRF facility, the OIF has a two-photon microscope that has been custom designed for *in vivo* imaging and which was also configured for 2nd harmonic imaging. This system, the Prairie Technologies ULTIMA two-photon microscope, is built around an upright Nikon microscope with a 25x, 1.1 numerical aperture, 2 mm working distance objective that permits high resolution, high sensitivity and deep *in vivo* cortical brain imaging. The OIF also has a Zeiss 710 NLO microscope equipped with 5 visible lasers (405, 458, 488, 515, 561, 594 and 633 nm).

*Other Laser-Based Special Techniques*: The OIF has a super-resolution system, the Nikon N-STORM. 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. The OIF also has a new Zeiss Lightseet 7 microscope. Light sheet microscopy is used 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. Dual cameras are available to simultaneously capture two channels, which is especially important for live specimen imaging.

**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.