Center for Toxicology, Southwest Environmental Health Sciences Center (an NIEHS Center)

Experimental Pathology Core Newsletter

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Introducing the Zeiss LSM510NLO-Meta multiphoton confocal:

Background Information - This state-of-the-art microscope was installed in the Biotechnology Imaging Facility *(Arizona Research Laboratories)* located in Life Sciences North 410B in June of 2002. Major funding for the instrument came from a Department of Defense grant written by Dr. Lantz. Additional funds came from the VP for Research, the ARL Biotechnology program and the College of Medicine Nielsen Trust.

Specimens - Typical samples would include paraffin or frozen-sectioned tissues stained with one or more fluorescent dyes and mounted on a microscope slide. It is imperative that the microscope slides have a #1.5 glass coverslip to ensure the highest resolution images. In addition to microscope slides, fluorescently-labeled cell cultures or tissues slices can be examined either fixed or using the BiopTechs live cell system at 37°C.

Confocal - This type of microscopy works with fluorescently labeled specimens. Confocal refers to two optical pinholes that are placed at conjugate planes in the light path. The pinholes discard any light that comes from regions of the sample that are not in the plane of focus. Images taken with a confocal microscope are significantly more crisp than images taken with a widefield fluorescence microscope. The disadvantage of confocal microscopy is that the intense laser light source can cause fading of fluorescent dyes, photodamage in living cells and the necessity of the pinholes means that it is difficult to image dim fluorescence.

Multiphoton - By using a tunable femtosecond pulsed infrared laser, this instrument can image living cells with significantly reduced photodamage. The infrared laser can also be used to excite dyes that are normally excited by UV wavelengths *(e.g., DAPI, Hoescht).* The infrared laser can penetrate deeper into tissues than visible light wavelengths.

DIC/Nomarski - This non-confocal imaging technique can be used to show overall cell or tissue structure and the images can be combined with the fluorescence information. Currently this technique is only available on the three highest magnification objective lenses. DIC cannot be performed with plastic dishes or coverslips.

Wavelength Scanning - The META detector on this instrument uses a diffraction grating, which allows users to capture the full emission spectra from the sample. The META can be used as a configurable band-pass filter, to acquire xyλ data and to separate fluorescent dyes with overlapping emission peaks.

Other Advanced Capabilities - The instrument can be used to perform FRET *(fluorescence resonance energy transfer)*, FRAP *(fluorescence recover after photobleaching)*, to perform ratio imaging of ion or pH sensitive dyes, and it can automatically acquire data at specific time intervals from living cells.

File Management - The manufacturer provides a very useful free utility that allows user to view, annotate and manage their images in a database. The Windows-based utility also makes it easy to add scalebars to images, to print working copies of images and to export the images into commonly used image file formats.

Training - We encourage users of the existing Leica TCS-4D confocal microscope to sign up for training on this instrument. We expect that training will involve three 3-hour sessions over the course of 2-3 weeks. Session 1 will be an introduction to the instrument and is open to a maximum of two individuals. Session 2 will be one-on-one coaching in how to use the instrument to acquire high quality images of their samples. Session 3 will be one-on-one training to maximize the user's familiarity with the instrument's capabilities and software. Contact Renee Benally to schedule a training session. The hourly rate for training sessions is $21/hr.

Administration/Billing - The instrument is administered by the Biotechnology program of Arizona Research Laboratories. Barb Carolus (626-2047, <carolus@u.arizona.edu>) is the principal operator of this instrument and she provides training to non-SWEHSC users. The hourly rates for this system *(as of 12/1/02)* will be $21/hr for unassisted use, $28/hr for use of the multiphoton laser, and $46/hr for assisted use. An improved on-line calendar will be available for scheduling time on the instrument.

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