Microscopy SRF

The facility provides high-quality advanced microscope systems, image analysis software, and microscopy expertise for live cell, live animal, and fixed specimen imaging and supports studies that range in scale from tissue structure to molecular interactions. A staff of two fulltime biomedical engineers and a scientific director provide consultation services on imaging and image analysis protocols, give hands-on training, and maintain and develop the instrumentation. Advanced techniques in use include FRET, automated cell migration and process growth analysis, and second-harmonic imaging. The facility served 110 Mount Sinai laboratories in the past year, with more than 4000 user sessions and more than 7000 hours of equipment use. In 2007, in response to increasing needs for confocal microscopy (as documented by the number of user hours and user fees), the Dean provided funds to purchase a new confocal microscope and matched investigators' contribution to upgrade an existing confocal.

Major instruments for image acquisition include four confocal microscope systems: a Zeiss LSM510 Meta CLSM; Leica SP-5 CLSM upright system; a Leica high-speed SP-5 CLSM inverted system with stage environmental chamber; and a BioRad/Zeiss Radiance 2000 MP single- and multiphoton CLSM. Three widefield microscope systems, equipped with high quality digital cameras, perform fluorescence, brightfield, and DIC imaging. Structured illumination (Zeiss ApoTome®) is available for increased axial resolution Computer-controlled, automated long-term time-lapse recording of living cells is accomplished with an Olympus IX-70 based microscope system which includes motorized X, Y, Z movement, motorized shutter and motorized filters, fluorescence and brightfield optics, and a stage incubator to maintain live cells over periods as long as several days. Additional equipment supports short-term (hours) imaging of live cell preparations on any of the microscope systems in the facility, using Bioptechs FCS2 closed chamber and ΔTC3 open chamber environmental control systems and Bioptecs objective heater system. Transmission electron microscopy of tissue sections is performed with a Hitachi H7000 transmission electron microscope and scanning electron microscopy is available in the Department of Pathology.

In an adjacent SRF-associated laboratory there are to two live-cell inverted microscopes one of which has an optical-gradient laser trap (laser tweezers)and the other high-speed fluorescent microscope has been modified to include both total-internal reflection fluorescence (TIRF) microscopy and spinning disk confocal microscopy. This microscope also includes a laser for photo-bleaching and photo-activation that permits bleaching or uncaging of GFP variants and the resolution of molecular interaction on a cellular and subcellular level. Both microscopes permit climate control and routine bright-field microscopy.

Image analysis on the SRFs computers provides deconvolution for increased resolution, 2D and 3D image rendering and visualization, and multiple modes of image analysis including fluorescence colocalization. Software packages to accomplish these include ImageJ, Volocity, Metamorph, Amira, Autodeblur and Adobe Photoshop.

Spinning Disk Confocal Microscope for live cell imaging. The spinning disk system exposes cells to lower levels of excitation, reducing phototoxicity and allowing the investigator to perform time-lapse imaging over much longer intervals than is possible with the scanning laser confocal microscope. We expect this to be used for *in vitro* studies of metastatic disease in combination with information on cell motility and protein localization. For live animal imaging, we have a new multiphoton confocal CLSM system. This system has four external detectors for observation of interactions between four or more cell populations marked with different colors to be observed in living organs such as lymph nodes and spleen. It is also equipped with an electronically tunable laser, to facilitate optimization of multiprobe visualization and of other modalities that take advantage of intrinsic characteristics such as second harmonic generation and visualization of native fluorescence.

For more information:

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