THE MICRO TOME

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MICROSCOPY IMAGING CENTER
HTTP://WWW.UVM.EDU/MICROSCOPYIMAGING

Summer 2004

New Equipment Purchased By MIC

We have ordered two new microscopy systems for late Summer/early Fall delivery. The Zeiss 510 META confocal microscopy system is built upon an inverted microscope platform, and with the attached full environmental incubation system, is ideal for live-cell confocal imaging. The system will be equipped with a variety of laser options (405 nm blue diode, 458/477/488/514 nm argon, 543 nm and 633 helium neon), and the attached META detector provides for multiple fluorescence labeling of 8 fluorophores! This system was purchased with funds awarded through an NIH-NCRR Shared Instrumentation Grant, and many thanks to Drs. Tom Jetton, Wolfgang Dostmann, Yvonne Janssen-Heininger, Steve Lidofsky and Karen Lounsbury for their contributions towards the success of the grant! Please note that this new confocal microscope will not replace the current BioRad MRC-1024, but will be added as a second confocal imaging system to the MIC.

Zeiss LSM 510 META Confocal

The second system we have recently ordered is a JEOL JSM-6060 scanning electron microscope to replace our current 23 year old JEOL T-300. This is the newest all-digital SEM with 3.5 nm resolution and a magnification range from 5X to 300,000X. The microscope will be equipped with a backscattered electron detector for compositional, topographic or shadow imaging. This SEM was purchased in part with funds from Vermont EPSCoR, and we would like to heartily thank Dr. Chris Allen for his continued support of the MIC!

Laser Capture Microdissection of Living Cells *In Vitro*

The Arcturus PixCell II Laser Capture Microdissector (LCM) has been a very useful system for the analysis of RNA and DNA from cells removed from paraffin-embedded or cryostat sections. A recent innovation now allows for the PixCell II system to be used with live cultured cells. Positive selection of living adherent cells followed by subsequent re-cultivation of a homogeneous clonal population is now possible! Please see an MIC staff member for details of this exciting extension of LCM capabilities!

JEOL JSM 6060 SEM

New Image Analysis Room

An additional Imaging Room has been added to the Microscopy Imaging Center. Room 206 HSRF will become our Image Analysis and Processing room. This room will contain several internet connected systems available for use 24 hours a day, 7 days a week with swipe card access after normal working hours. The dedicated systems include:

- Dell computer system with a Metamorph Image Analysis system, Adobe Photoshop for image processing and Panaview Image Assembler for image stitching, a WACOM graphics tablet for precise drawing capability, an attached Epson Stylus Printer for general printing and a Microtek ScanMaker 5950, specifically for scanning documents.
- Dell computer system, equipped with Adobe Photoshop, Panaview Image Assembler and Adobe Illustrator for image design, with an attached Fujix Pictrography 3500 Printer for photographic quality prints, an Agfa Studio Star Scanner for general scanning as well as gel scanning and a Microtek ScanMaker 8700, used for scanning negatives.
- Dell computer system with attached Epson 2450 Scanner and Epson Stylus C80 Printer, used primarily for Program Project work.

CAP Accreditation

The Microscopy Imaging Center, as a provider of diagnostic electron microscopy services for Fletcher Allen Health Care, has been awarded an accreditation by the Commission on Laboratory Accreditation of the College of American Pathologists (CAP), based on the results of a recent on-site inspection.

The College of American Pathologists is the world's largest association composed exclusively of pathologists and is widely considered the leader in laboratory quality assurance. The CAP Laboratory Accreditation Program is recognized by the federal government as being equal to or more stringent than the government's own inspection program.

During the accreditation process, inspectors examine the laboratory's records and quality control of procedures for the preceding two years. CAP inspectors also examine the entire staff's qualifications, the laboratory's equipment, facilities, safety program, quality improvement program, and the overall management of the laboratory. This stringent inspection program is designed to specifically ensure the highest standard of care for the laboratory's patients.

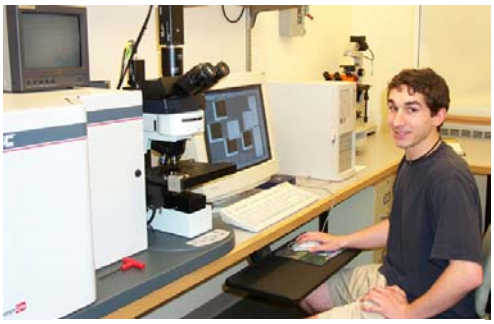
Lab Tours

The Microscopy Imaging Center guided 102 students and others through the facility this Spring. Laboratory personnel demonstrated the imaging technologies available and discussed some of their current applications. The students ranged from high school level, through undergraduate and graduate level. A group of researchers from the AMES Research Laboratory and from NASA, Palo Alto, California also toured the facility.

New Use for Laser Scanning Cytometer

In Dr. Gary Ward's lab (Department of Microbiology and Molecular Genetics) we study how the Apicomplexan parasite *Toxoplasma gondii* invades host cells. One area of interest is defining the function of Apical Membrane Antigen-1 (AMA-1), a protein conserved in many Apicomplexans including Plasmodium, the causative agent of malaria. AMA-1 is one of the leading malaria vaccine candidates and is thought to be involved in the attachment/invasion process. Therefore, studying this protein's function necessitates use of accurate attachment and invasion assays.

Our field traditionally uses microscope-based differential fluorescence assays to measure the invasiveness of parasites. Similar techniques are used to determine the ability of parasites to attach to a host cell. These methodologies are hindered by field-to-field variability and procedural inconsistencies, and require manual sample counts. We have successfully adapted existing invasion and attachment assays to be scored by the MIC's Laser Scanning Cytometer (LSC). This advancement has increased the area scored and number of parasites counted, which combine to overcome the limitations of the manual assays. The development of these assays has helped us study the ability of parasites lacking AMA-1 to attach to and invade host cells and has advanced the field's overall ability to accurately measure attachment and invasion. *(description provided by J. Mital)*



Jeff Mital,Graduate Student in Dr. Ward's Laboratory , at LSC

Laser Capture Microdissection

Jan Schwarz recently attended two conferences highlighting Genomic and Proteomic Sample Preparation and Laser Capture Microdissection. Various speakers presented their research with the Human Genome Project, microarrays, optimization of tissue collection and handling, gene expression profiling, nucleic acid extraction, quantitative gene expression, RNA amplification and gene chip technologies. Many of the projects utilized formalin-fixed, paraffin-embedded tissue (FF PET) which has now opened up new opportunities for the use of vast amounts of archived tissue blocks – both human samples and animal models. The MIC is currently developing a protocol for the use of FF PET Laser Capture Dissection which will be available on our website in the near future. Please see Jan Schwarz if you have any questions or for training on the Arcturus Pixcell II Laser Capture Microdissector.

Equipment Available:

- JEOL 1210 STEM
- JEOL JSM 6060 SEM (Fall 2004)
- BioRad MRC 1024 Confocal LSM
- Zeiss LSM 510 META Confocal (Fall 2004)
- Olympus IX 70 Inverted Microscope for fluorescence and phase contrast
- Eppendorf Microinjector System
- DI Atomic Force Microscope
- Arcturus PixCell II LCM
- Zeiss Axioplan 2 Microscope
- CompuCyte Laser Scanning Cytometer
- Olympus BX50 Microscope
- Universal Imaging MetaMorph Workstation
- Dell NT Image Processing Workstation
- Fujix PictroGraphy

MIC Services Provided:

- Morphologic services and consultation at the light and electron microscopy level.
- Morphometry (semi-quantitative morphology).
- Light and electron microscopic immunocytochemistry.
- Confocal scanning laser microscopy.
- Laser scanning cytometry.
- Atomic Force microscopy.
- Scanning and transmission electron microscopy.
- Laser capture microdissection.
- Preparation of paraffin sections and frozen sections.
- Training for use of the above equipment.
- Special histological stainings.
- Testing of new antibodies, developing new staining techniques.
- Photo quality printing for publications and posters, computer-assisted digital imaging and analysis.