

THE MICRO TOME

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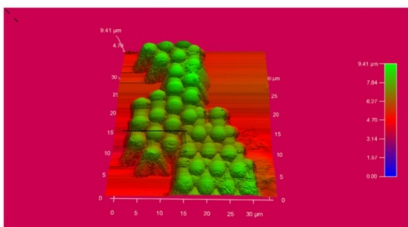


MICROSCOPY IMAGING CENTER
HTTP://WWW.UVM.EDU/MICROSCOPYIMAGING

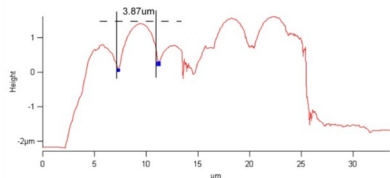
Spring 2011

Atomic Force Microscopy goes to the Asylum.....Research that is!

Microscopy Imaging Center Acquires the State-of-the-Art MFP-3D BIO™ Atomic Force Microscope by Asylum Research



The Microscopy Imaging Center received a National Center for Research Resources Grant for the acquisition of a high-resolution atomic force microscope from Asylum Research of Santa Barbara, California. The more advanced MFP-3D Bio AFM replaces the 15 year old Veeco Bioscope AFM.

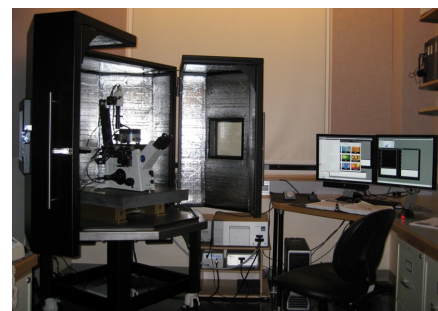


The MFP-3D-BIO™ AFM consists of a head assembly that is mounted on an Olympus IX71 inverted microscope

with combined optical integrations to include bright field, phase contrast and epi-fluorescence. The IX71 and attached accessories reside on a Herzan AVI 350-S Active Vibration Isolation Table which dynamically dampens the most extreme vibrational disturbances. The system is totally enclosed in an air tight, state-of-the-art, BCH-45 Large Acoustic Isolation Hood with air temperature control

capabilities. The closed looped system allows investigation of dry or hydrated biological surface features at very high resolution, to include dynamic Real3D™ visual analysis, force spectroscopy, and/or optical (fluorescent or phase) acquisitions. AC mode (tapping mode) with complete control of attractive (non-contact) and repulsive (intermittent contact) modes while imaging soft biological specimens, and available fluorescence of the same area of interest allows one to overlay fluorescent structures in precise registration with AFM acquired topographical information. In addition, there are more advanced modes such as nano-manipulation (lithography), nano-indentation and dual AC mode, to mention a few. The AFM system software modules are written in Igor Pro: an integrated software program by Wavemetrics that provides advanced features for visualizing, analyzing, transforming and presenting experimental data.

Several University of Vermont investigators have already earmarked projects to utilize force spectroscopy with this new equipment to study cancer via cellular membrane elasticity and movement, venous thrombosis, HIV, induced stress on tracheal cells and osteoarthritis.

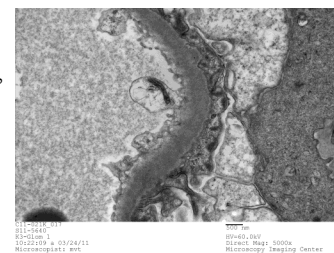


I Dream of JENIE.....*(blink, nod, poof)*.....new JEOL 1400 TEM



The JEOL 1400 Transmission Electron Microscope is a high-contrast, high-resolution instrument utilizing a LaB6 filament, and capable of operating at accelerating voltages up to 120 kV. It incorporates a newly designed electron optics system which is optimized for integrated use with a digital ccd camera imaging device. The instrument is controlled via the "TEM Center" graphical user interface operating environment,

with redundant manual operation afforded by two conveniently located control panels. The image orientation system allows image rotation at multiple angles, and specimen stage movement is achieved with a precision trackball device. Software modules (JEOL Electron Microscope Navigation Interactive Engine: "JENIE") provide step-by-step video tutorials covering instrument operation and alignment, especially useful for novice operators. Image acquisition is provided by an AMT XR611 high resolution 11 megapixel mid-mount ccd camera. This camera is positioned near the normal film plane of the TEM affording a wide field of view for image capture. Moreover, the mid-mount configuration displays virtually no TEM distortion. Digital images will now contain important information, including magnification, scale bar, date and time and a place for user defined



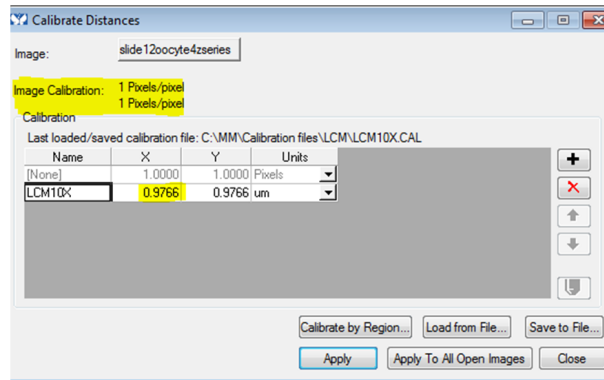


Appearances can be deceiving!

The MetaMorph software has been recently upgraded and has some redesigned features. One of the most critical is the calibration of tif images. All LSM (confocal) images are imported with header information read by the software, which will automatically calibrate the image. Tiff images do not contain calibration information that is read by the software and calibrations must be done by the user. If an image is not calibrated, all measurements will be expressed as pixels rather than microns or other measureable units.

NOTICE*** - in the Calibrate Distances dialog box, the image calibration must match the xy calibration units, otherwise your image will not be properly calibrated (illustrated in the highlighted area below). Notice that it may appear you have the proper calibration chosen - in this case LCM10X (with a black box around it), however that calibration is still not loaded until you click the name LCM10X and click apply. The image calibration will now display your proper calibration.

Note also at the bottom of the MetaMorph screen your pixel calibration will be displayed. Region Measurements will also show your calibration in Excel when data are logged if the "log image calibration" is selected in the configure tab. The IMA (Integrated Morphometry Analysis) will not show your calibration. If your image is not correctly calibrated, you may be generating numbers based on pixels rather than square microns.



Do More, Less Well - Stereologically

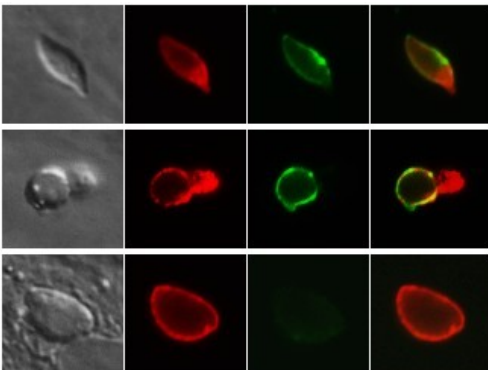
Image Analysis Update: StereoInvestigator Version 9 is available in the MIC

Through an alliance with MBF Bioscience of Williston, Vermont, the MIC has acquired the workstation version of StereoInvestigator for offline analysis. This image analysis software is designed to count cells, quantify lengths, area, and volumes in biological samples while employing stereological principles. With 13 stereological probes, it will efficiently allow you to obtain precise and unbiased estimates of cell number (Optical Fractionator probe), volume fractions (Cavalieri Estimator probe), or spatial distribution (Nearest Neighbor probe) of tissue components. Results can be exported directly into Microsoft Excel. In addition to the help menu and excellent technical support offered on MBF Biosciences website, the MIC has staff trained in stereological principles to help in project design and introduce you to this software.

LSC Monitors Toxoplasma Invasion

The Phylum Apicomplexa includes important pathogens responsible for malaria (*Plasmodium* spp.), toxoplasmosis (*Toxoplasma gondii*), and cryptosporidiosis (*Cryptosporidium parvum*) in humans, and a wide range of livestock diseases. To adapt to an obligate intracellular lifestyle, apicomplexan protozoa have evolved specialized secretory organelles, which sequentially secrete proteins that are necessary for host cell invasion and the subsequent formation of the parasitophorous vacuole (PV) around the penetrating parasite.

Dr. Gary Ward's lab (Department of Microbiology and Molecular Genetics) studies the mechanisms by which *T. gondii* gains entry into the cells of its hosts. One area of interest is defining the function of Apical Membrane Antigen-1 (AMA-1). AMA-1 is a conserved and essential transmembrane protein of apicomplexan pathogens and one of the most promising candidates for inclusion in a multi-antigen malaria vaccine. While the requirement for AMA1 in host cell invasion is clear, the precise role it plays in this rapid process is an intense area of investigation. We are currently studying the function of *T. gondii* AMA1 cleavage and "shedding" from the parasite surface during invasion by engineering non-cleavable AMA-1 mutants. To score the effect of these mutations on host cell attachment and penetration, we have developed a Laser Scanning Cytometer-based invasion assay. This methodology has dramatically increased the area scored and number of parasites counted, which combine to overcome the limitations of manual assays (description provided by Fabiola Parussini).



Equipment Available:

- JEOL 1400 TEM
- JEOL JSM 6060 SEM with Oxford INCA EDS system
- BioRad MRC 1024 Confocal LSM
- Zeiss LSM 510 META Confocal
- Olympus IX70 Inverted Microscope
- Applied BioPhysics ECIS Z0
- AR MFP-3D BIO™ Atomic Force Microscope
- Arcturus PixCell II LCM
- Zeiss Axioplan 2 Microscope
- CompuCyt Laser Scanning Cytometer
- Olympus BX50 Microscope
- Universal Imaging MetaMorph Workstation
- Velocity 3D Software
- Olympus SZX12 Dissecting Microscope
- Leica MZ16F Fluorescence Dissecting Microscope
- MicroBrightField Stereo Investigator

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 and frozen sections
- Training for use of the above equipment
- Special histological stainings
- Testing of new antibodies and developing new staining techniques
- Computer-assisted digital imaging and analysis