THE MICRO TOME

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MICROSCOPY IMAGING CENTER
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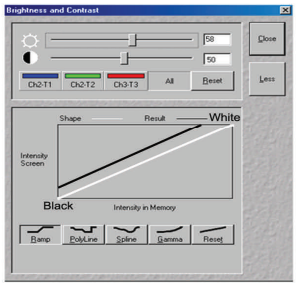
Brightness/Contrast – The Light and Dark of the Matter

Brightness/Contrast adjustments are tools that are readily available and frequently used in digital imaging. These adjustments are considered imaging manipulation, but where does image manipulation cross the line and become unintentionally unethical? Is anyone checking? The short answer is, yes. Publications such as the Journal of Cell Biology and the Office of Research Integrity are closely watching this issue and have led efforts to support scientific imaging integrity. As the ease and prevalence of image manipulation increases, many more journals are checking for image manipulation and, if found, the image will be rejected. What is acceptable and how much is too much? Our position in this discussion is that, if you are unwilling to list in your figure legend what image manipulation you have done post-capture, you probably should not be doing it. If you think of your digital image as a grid of intensities, any change in those intensities can drastically alter the image data in terms of quantitation and perception. Follow a short set of guidelines to ensure the best imaging techniques.

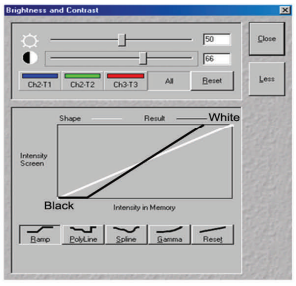
1. Always run controls with your incubations and image them at the same settings.
2. Always keep an unaltered copy of the original captured image.
3. Know the limitations of your sample and the instrument's capabilities.
4. Small changes in brightness and contrast are usually acceptable as long as you have applied the same changes to your controls. These changes should be to the entire image, not a region within an image.
5. If you do make brightness or contrast changes, be aware that all programs are not the same. Programs such as Photoshop vary version to version as to how the brightness contrast controls operate – note in the following pictures…

Brightness is a linear correction to all pixel intensities, either brighter or darker. Contrast, takes a midpoint of the intensities and rotates on that axis, causing pixels that were not black to become black and pixels that were not white (saturated) to become white. Low level fluorescence is reduced and intensity changes in upper level pixels are eliminated. In Photoshop version CS3 or newer there is a check in the brightness/contrast dialog box that says "use legacy". If this is selected, then the brightness and contrast control will operate as described above. However, if this is not checked then the controls will anchor the black intensities and proportionally change the remaining intensities similar to "levels" or "curves"; in other words, the change to the image is no longer "linear". Photoshop uses algorithms that are not explained within the dialog box. Be sure you are aware of what digital changes are taking place before you use these controls. For any questions concerning the manipulation of digital images, please contact Marilyn or Doug in the MIC.

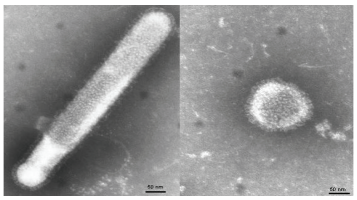
Brightness



Contrast



MIC H1N1 Preparedness



Negatively stained virus particles imaged with a JEOL 1210 TEM.

Along with the UVM campus and College of Medicine, the Microscopy Imaging Center is preparing for a possible pandemic of the H1N1 influenza virus. The facility has created an action plan that would allow us to maintain all services in the event of a pandemic at the University. To air on the side of caution we are also placing Lysol wipes and hand sanitizers in all of the imaging rooms and the main laboratory. Feel free to use these products on keyboards, countertops, focus knobs and stage controls of microscopes.

Increasing Volocity's Velocity

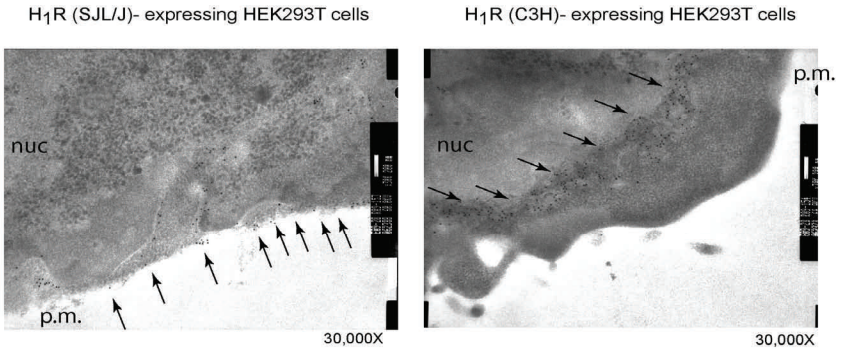
The computer running Improvision Volocity 3D rendering and analysis software has been replaced with a state-of-the-art high-end system. The new computer is a Dell Precision T7400n Mini-tower, containing two Quad Core Xenon processors and running Microsoft Vista. The system has 8 GB of memory and an nVidia video card, greatly enhancing the processing speed of large data sets, and exceeding the requirements of the software as recommended by the manufacturer. At the same time, we have upgraded to Volocity Version 5, which has a completely redesigned, more intuitive user interface. A new manual to accompany the Volocity system is being compiled, and in the meantime check with an MIC staff member for further details or training on the system. If you have confocal images requiring 3D processing or analysis, Volocity may be the right choice for you!

MIC Takes Richmond

Two technicians from the MIC attended the annual Microscopy & Microanalysis meeting in Richmond, Virginia this summer. Jan Schwarz focused her attention on presentations related to microscopy educational outreach programs, innovative imaging of kidney, and microwave assisted antigen retrieval and immunolabeling techniques. She was a valued participant in the special event titled, *Microscopy in the Classroom: How to Use It and How To Teach It.* She was able to share her extensive experience bringing Project MICRO to middle school classrooms around our county and beyond. In addition, she attended demonstrations offered by several companies of the latest transmission electron microscopes and digital cameras for TEMs. Michele von Turkovich attended lectures, tutorials and demonstrations related to the latest instrumentation in the field of elemental analysis by Energy Dispersive Spectroscopy. The new, liquid nitrogen free (Peltier cooled) Silicon Drift Detectors are replacing the traditional LN_2 cooled Si(Li) detectors. We anticipate adding one to our SEM in the near future. Both attended a pre-meeting short course; *Digital Imaging 101: Scientific Imaging with Photoshop* taught by John C. Russ of North Carolina State University.

What's the Diehl? Gold, Gold and More Gold!

Multiple sclerosis (MS) is the major demyelinating disease of the central nervous system (CNS) in humans affecting about 1 in 1000 of the North American population. Susceptibility to MS involves both genetic and environmental factors. With regard to environmental triggers, the vasoactive amine histamine and its main cellular sources, mast cells and basophils, have been long implicated in the etiology of MS through effects on the blood brain barrier (BBB) and inflammatory immune cells. The Teuscher lab previously identified the histamine H_1 receptor (*Hrh1*/H_1R) as a susceptibility locus for experimental allergic encephalomyelitis (EAE), the principal autoimmune model of MS, and found that T cells expressing the H_1R are critical for disease development. These findings exemplified the notion that both environmental and genetic elements strongly influence EAE. From the genetic studies we determined that "sensitive" or



HEK293T cells stably expressing N-terminal hemagglutinin (HA)-tagged SJL or C3H H_1R alleles were analyzed by immunogold transmission electron microscopy (TEM) using an anti-HA Ab. In scanning several regions of multiple ($n \geq 5$) cells, abundant plasma membrane staining for the SJL allele was observed, while

"resistant" versions of H_1R exist among different mouse strains, SJL or C3H, for example. Sensitive (SJL) and resistant (C3H) H_1R alleles differ by three amino acids (AA) within the third intracellular loop of H_1R, which is a seven-pass transmembrane G-protein coupled receptor (GPCR). Being the largest intracellular domain of H_1R, this domain is associated with signal transduction, but may also be important in protein folding and trafficking, as are the intracellular domains of other GPCRs. Consistent with this, initial confocal studies performed by former graduate student, Raj Noubade, showed reduced surface expression and increased intracellular retention of the C3H allele compared with the SJL/J allele. Currently Sean Diehl, a Research Associate in the lab is working with Nicole Bishop of the MIC to use Immunogold transmission electron microscopy in stable HEK293T lines to confirm these findings. Future research is aimed at more closely detailing the site of intracellular retention of the C3H allele using TEM and confocal microscopy; whether the C3H or SJL alleles of H_1R have different intracellular protein binding partners using yeast two-hybrid and mass spectrometry analysis; or whether these two alleles exhibit different signaling abilities for G-protein or non-G protein pathways using biochemical approaches. Together these approaches will shed light on how polymorphisms influence the trafficking and signaling of H_1R and how this may affect disease susceptibility. Not only for EAE, but findings from these studies may also have relevance for other diseases in which the trafficking and surface expression of other GPCRs is affected.

Equipment Available:

- JEOL 1210 STEM
- JEOL JSM 6060 SEM
- BioRad MRC 1024 Confocal LSM
- Zeiss LSM 510 META Confocal
- Olympus IX 70 Inverted Microscope
- Applied BioPhysics ECIS Zθ
- DI Atomic Force Microscope
- Arcturus PixCell II LCM
- Zeiss Axioplan 2 Microscope
- CompuCyte Laser Scanning Cytometer
- Olympus BX50 Microscope
- Universal Imaging MetaMorph Workstation
- Volocity 3D Software
- Multiple Dell Image Processing Workstations
- Olympus SZX12 Dissecting Microscope
- Leica MZ16F Fluorescence Dissecting Microscope

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