



## **Recommended coverslip thickness:**

Most microscope lenses have the designation 0.17 somewhere on the lens. This number indicates the expected coverslip thickness (mm) that was used to calculate the optical corrections in the lens. Using coverslips of the wrong thickness, particularly with lenses that have numerical apertures higher than 0.65, can add optical aberrations to your images. For example, at a numerical aperture of 0.95 (typically a 40X dry lens), a 0.01 mm difference in thickness reduces image formation by 45% from the ideal image (G.S. Benham, in *Cell Biological Applications of Confocal Microscopy*, Matsumoto, Academic Press, 2002). A quick check of the Fisher Scientific web site shows that the thicknesses of their coverslips are: #1 (0.13-0.17mm), #1.5 (0.16-0.19mm) and #2 (0.17-0.25mm). This is why we recommend that labs always use #1.5 thickness coverslips.

## **Immersion oil problems:**

Be careful when reviewing your samples at high magnification on two different microscopes. If you find that you need immersion oil to see fine details in your sample, please do not "mix" two different brands of immersion oil. Vendors tell us that they have seen instances where the two different oils combine on the lens surface and form a sticky substance that's very hard to clean off the lens. If you find you need to use two different microscopes, try to first clean the original oil off the slide using lens cleaner, followed by a wash with a gentle stream of 100% ethanol.

## **Image Analysis updates:**

The Compix image analysis system in AHSC 4233 has been upgraded to the newest version (5.2) of the SimplePCI software. This system is capable of analyzing any digital image for a large number of parameters including lengths, areas, shape factors, object count, intensity, and color intensities. The system is fast and the data (including statistics and graphs) can be exported to Microsoft Excel format for further analysis. The Compix in AHSC is attached to an inverted microscope with a motorized XY stage and a high resolution greyscale CCD camera. The microscope can image in brightfield, phase contrast, DIC and fluorescence imaging modes. The system has been used to acquire time-lapse movies of cultured cells, create montage images of large histological sections, and capture images for the analysis of DAB stained sections.

The Arizona Cancer Center also has a Compix image analysis system (software ver 3.7) that we can use. This system was recently moved to room 3964. The AZCC system is attached to an upright microscope and has a color CCD camera.

## **InstantImager update:**

In our last newsletter we mentioned that the Packard InstantImager was in need of a very expensive repair. Due to the lack of financial resources and the low number of users on this instrument, we have asked the College of Medicine's research council for permission to surplus this instrument.

## **Science Fair:**

Just a reminder, the SWEHSC Science Fair is 3-5pm on May 20, 2003 at the University Marriot in the Madera room. After the science fair there will be get-together to say farewell to Dr. Liebler and enjoy refreshments in Canyon Room B&C.

## **Advanced Fluorescence Techniques:**

We've prepared a 30 minute overview of the types of advanced fluorescent techniques that can be performed on the Zeiss multiphoton microscope. We would be happy to come make a presentation at a lab meeting to introduce your group to these powerful techniques.