We appreciate the response to this publication feature and welcome all contributions. Contributions may be sent to José Mascorro, our Technical Editor, at his e-mail address: jmascor@tulane.edu. José can also be reached at (504) 584-2747

A Farewell Note

Phil Oshel

I would like to insert a short note here, stating that as of the Microscopy and Microanalysis 2002 meeting in Quebec City, I have retired as Technical Editor. It was a good time, and I leave it with some reluctance. Ron Anderson certainly pressed me to stay on. I did want to continue with MT over the transition, and help make the change of ownership and editors smooth. Now that the new gang has Microscopy Today going, it's doing well and should continue to do so. It's something of a relief to be able to read the listservers without constantly thinking "There's an article here. Can I get permission?" and contacting people, following up, and so on. It's been an interesting and fun time. I'm very happy that Don Grimes offered me the position, but after just over five and a half years, I've done what I could and hope it helped make Microscopy Today the magazine it now is.

Ron Anderson and José Mascorro, the new Tech Editor, will continue to improve Microscopy Today, and the new look brought by Dale Anderson is a nice change that improves the magazine. I look forward to my future copies, and ask that anyone who might have articles or requests for articles outstanding with me will send their contributions to José Mascorro at jmascor@tulane.edu or Ron Anderson at Microtoday@attglobal.net

Many thanks to all the people I met and corresponded with over the past years. See you all on the listservers and in San Antonio.

...Phil

Some Uses of Fluorescent Plexiglas in Fluorescence Microscopy

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A few years ago, in response to a discussion on an Internet microscopy bulletin board touting the benefits of fluorescent uranyl glass to illustrate the variation in cone angle with numerical aperture, we purchased scraps of fluorescent Plexiglas as a surrogate. Plexiglas has benefits of being cheap, readily available, and non-fragile.

Fluorescent Plexiglas may be very useful for the microscopist. The following applications may be done by other methods, such as a solution of a fluorescent probe in a shallow dish, but Plexiglas has the benefits of being cheap, dry and quick. Routinely our lab uses fluorescent Plexiglas to:

1) check for flat field illumination

2) locate a spot for uncaging or micro CALI (chromophore assisted laser inactivation, see http://www.med.harvard.edu/publications/On_The_Brain/Volume4/Number2/Bomb.html for an explanation)

3) measure fluorescence intensity (caveats on using as a standard below)

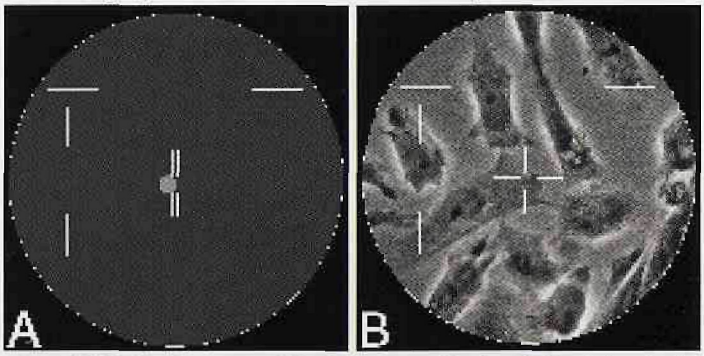
4) illustrate diffraction patterns, numerical aperture and properties of fluorescence.

However, fluorescent Plexiglas is not recommended for multiphoton microscopy. The infra-red femptosecond pulsed laser either burns a spot in the Plexiglas due to low diffusion of heat or due to other light induced chemical reactions.

Some specific uses we've found for fluorescent Plexiglas are:

1) Kohler alignment of mercury arc lamps is relatively simple, but checking for flat field illumination or response in laser scanning confocal systems can be more problematic. Direct visualization of response across a flat field is possible with the fluorescent Plexiglas.

2) Our uncaging apparatus consists of two mechanically shuttered mercury arc lamps on a T connector on the back of an Olympus IX 70. One of the lamps is aligned for Kohler illumination and filtered for imaging and the other aligned for critical illumination for uncaging. With a small machined aperture at the field



A.) Using fluorescent Plexiglas, a spot for irradiation is located at a specific location in the field. B.) Replacing the Plexiglas with a cell culture dish allows for using the reticule and phase contrast illumination to place a cell or portion of a cell in the correct location.

diaphragm of the critically aligned uncaging light path, the light can be directed at a small spot (< 5 μm diameter). To locate this spot, a fluorescent Plexiglas slide is placed on the microscope stage in place of a standard slide or culture dish. It is illuminated with the uncaging light path and the spot brought into focus. Through the eyepiece, the fluorescent spot can be moved to a reference location on the reticule. The uncaging lamp is shuttered and the Plexiglas is replaced by the sample. Using filtered (to prevent global uncaging) phase contrast, the location to be uncaged is matched with the reference location on the reticule. [Fig. 1]

3) The issue of fluorescent standards is one that plagues microscopists. Because it does bleach (in our hands up to 20% depending on he wavelength and intensity), fluorescent Plexiglas may not serve well as a fluorescent standard, but can be used for rough comparisons. For instance, we use it to compare the responses of different objectives. An objective of choice is rotated into position and focused on the Plexiglas. With the light off, the slide is moved to a new field that has not yet been illuminated. This prevents measuring a bleached area. With a cooled CCD camera or with a photometer directly against the Plexiglas, the light is switched on and a picture or light reading taken immediately. Two problems with this method are that after multiple uses, it is difficult to know which areas of the slide have already been subjected to bleaching unless marked and the slide has a relatively short lifetime of usefulness. For most microscopists though, this method would prove sufficiently accurate.

4) Place fluorescent Plexiglas on edge above an objective with the appropriate fluorescent excitation and the cone formed by the angle of illumination lights up. This is an excellent illustrative tool for explaining numerical aperture and, with a confocal, the diffraction limited spot.