Choosing Between Confocal Microscopes: A Fly-Eye's View

Mark. H.C. Lam* and David A. Jans
John Curtin School of Medical Research,
Canberra City, Australia
*mark.lam@anu.edu.au

We have recently been shopping for a confocal microscope and have found a simple way to test the capabilities of the various units that have been demonstrated to us. Some of the important aspects that we were looking for in our "ideal" multi-user system were:

1) Ease of use.

2) The ability to obtain good X-Y, X-Z and hence X-Y-Z resolution, and the accurate display of these images.

3) The depth within the sample at which the system can resolve structures (this of course depends mostly on the lenses used), but is still an important criteria as it reflects on the system as a whole.

4) "extras" such as a transmission detector and reconstruction/analysis/image manipulation software.

The Sample

We found a simple way to prepare a good biological sample to test for these capabilities.

To prepare the sample, simply mount a common housefly (*Musca domestica*) by adhering its wings using some nail polish onto a glass slide, and place a glass coverslip on top (as close to the fly's eye as possible). We also found that fixation with ethanol was harmful to the specimen whilst the unfixed (dry) sample stored at 4^{O} C could be kept for up to six months without losing any of its quality. The glass coverslip can then be attached to the slide using some adhesive tape.

The best part of this procedure is that there is no staining of the sample involved. Simply focus onto the fly's eye. The fly's compound eye contains endogenous chromophores that fluoresce green, red and far red (see Lee *et al.,* 1996) upon excitation at various laser wavelengths (ranging from UV to visible light) so that it can be used to test systems equipped with a wide variety of lasers. The sample does not photobleach easily so that a single sample can be used to test numerous microscopes. The structural detail within the compound eye enables the resolution of the microscope to be pushed to respectable limits.

Fig.1: Confocal slice of a fly eye captured using a Zeiss LSM-510 attached to a Zeiss Axiophot and a 20X plan lens. Dual-laser excitation.

Testing the Confocal Microscope

Once you are allowed to "play" with the microscope yourself, simply go through the following steps:

1) Work through the range of lenses and obtain as sharp an image as possible (usually using slow scan mode, with the appropriate filtering *e.g.,* Kalman filtering) and at as high pixel density as possible. Make sure that the sample is not too bright (some manufacturers have a color-coded "look up table" to help with this) as the resolution of the sample will be lost. You can use one, two or three excitation wavelengths (488 or 488/530) depending on the specifications of the system tested. The data set can be used to compare the resolution between systems, as well as the built-in distance/size measuring accuracy of the systems. An example of a slice through a fly eye using a 20X lens is shown in Figure 1.

2) To test the optical sectioning abilities, we found that using the Z-sectioning mode of the microscope with the 20X lens worked well. Collect images (5-6 passes with the Kalman filter per slice) by stepping through the sample 2-5 microns at a time.

The dataset generated provides an opportunity to test the file management systems within the operating software and the exportability of the generated files. Some manufacturers have their own file systems so that they can include some information of the image hence, the requirement for a conversion algorithm so that the images to be read by other imaging software (We use NIH Image routinely, for example). Generally, these files can be converted to formats such as GIF, BMP, TIF, etc. without any problems.

The data set (from 2) can be used to test three-dimensional (3-D) reconstruction software (see Figure 2). Things to look out for in the reconstruction software include the faithfulness of reconstruction of the structure, the resolution of the image and the ease of operation of the software.

We found that the most suitable system for a multi-user facility manned by biologists should be easy to operate but at the same time include state of the art technology that provides images of the highest quality. We hope this has helped you choose such a system from a fly-eye point of view! ■

For examples of movies visit us at: http://jcsmr.anu.edu.au/dbmb/lam/flyeye.html

Lee RD, Thomas CF, Marietta RG and Stark WS. Vitamin A, visual pigments, and visual receptors in Drosophila. Microsc Res Tech. 15, 35(6), 418-30. (1996).

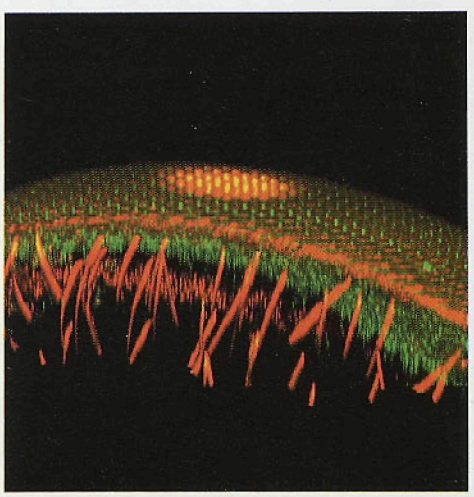
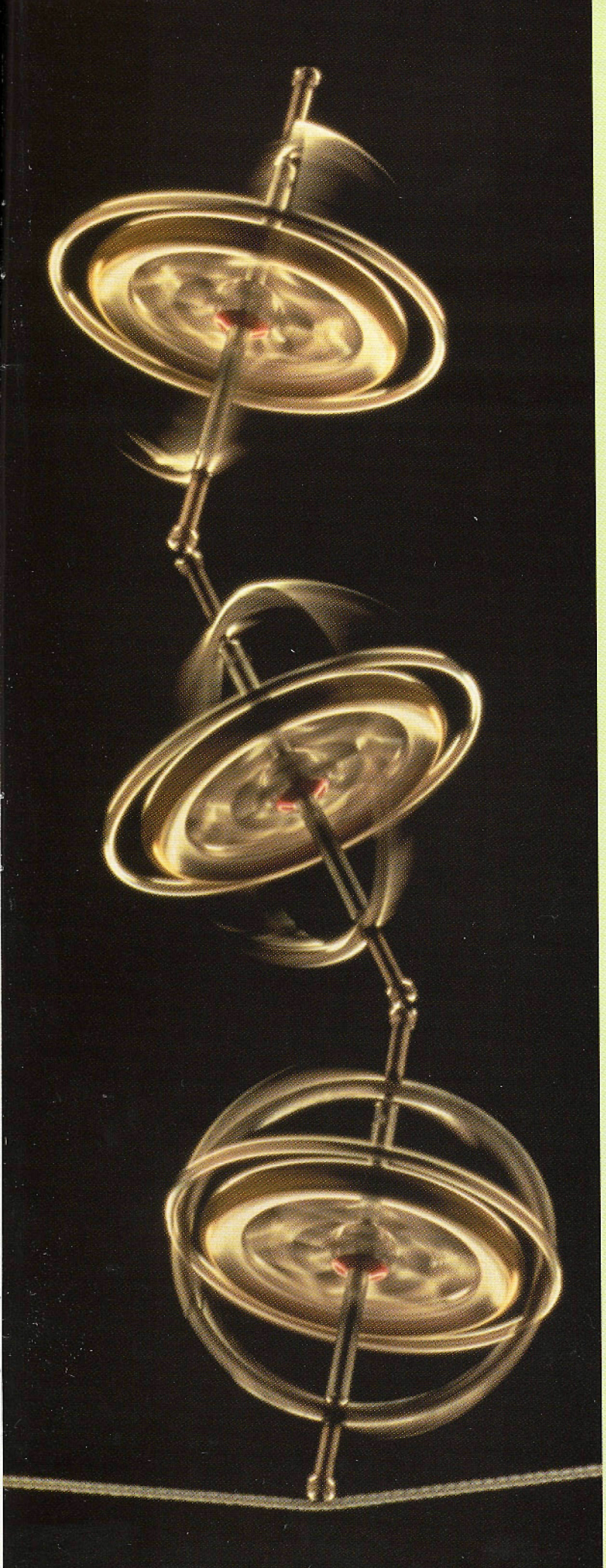


Fig. 2: 3-D reconstruction of a fly eye consisting of 36 z-slices using on-board image analysis/manipulation software. The image was initially captured using Zeiss LSM-510 with a Zeiss Axiophot, and 20X plan lens.



Getting All Your Detectors Working Together Can Be A Tough Balancing Act.

That's Where Emispec Comes In.

If balancing all components of your detectors has your head spinning, you should be talking to us.

You see, at Emispec Systems, Inc., we approach data acquisition differently. Instead of creating systems targeting one detector, we focus on integration. This concept can be applied equally to new and existing electron microscope installations. Core acquisition capabilities of our products include:

- **Digital scanning** for **STEM**.
- **Digital EDX** acquisition and analysis.
- **EELS** acquisition and analysis.
- **CCD** and **TV** imaging.

Integrated microscope control, imaging and spectroscopy allows automation of demanding experiments, such as **spectrum imaging**. Emispec enhances these capabilities with extensive on-line and off-line processing.

To find out how Emispec can help your lab keep in balance, visit our Web site today at **www.emispec.com.** See why we are fast becoming the leader in microscope detector technology solutions.

Emispec

Emispec Systems, Inc
2409 South Rural Road, Suite D
Tempe, Arizona 85282 USA
Phone: 480.894.6443 ▪ Fax: 480.894.6458
Web: www.emispec.com

thinking beyond the box™