CrossMark

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never reacted with the plastic of the dishes. Here is how I do the actual embedding of the cell monolayers in the dishes:

1) After the last 100% ethanol, remove the alcohol and cover the bottom of the well with a layer of resin mixture that is about 2 mm deep.
2) Insert embedding tubes that are made by cutting the pyramidal bottoms off of BEEM capsules (just slice them with a fresh razor blade and be sure to insert them so that the manufactured end rather than the cut one is sitting against the dish).
3) After inserting labels into the tubes, put them into the oven at 60º overnight.
4) In the morning, fill just the embedding tubes and return everything to the oven again to finish polymerizing.
5) When the resin is cured, grab the tubes with a pair of needle-nosed pliers and snap them out. Sometimes a bit of the bottom of the dish comes away with the block, but often a very smooth block face results. If some of the dish comes up, it is easy to see under a dissecting microscope, and the dish portion comes away easily when trimming the block face.

I often cut away part of the block face with a jeweler's saw, either to keep it in reserve or to re-embed it in order to get cross sections, and then trim the rest into a narrow rectangle. When sectioning the resulting block *en face*, start at 0.25 µm (no thick sections), pick up and stain the sections as they are cut. Smooth thin sections should be obtained within a micron. I usually trim a very long rectangle and then start to section in such a way that I am a degree or two off of being perfectly *en face* from top to bottom, so that I first get sections from one edge of the rectangle and then have a lot of "acreage" to work through if I need more sections later on. ■

Disclaimer: I have no financial interest in either Ladd or EMS -– I'm just a happy customer who believes in using what works.

Flies in a Box

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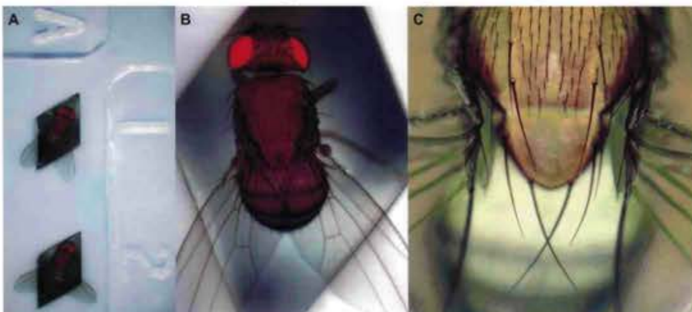
A challenge was presented to us to find a way to support *Drosophila melanogaster* so that both the anterior and posterior scutellars could be easily visualized under a dissecting microscope and photographed for purpose of measurement. The resulting solution was to utilize a standard grid box with the lid removed. Several holes were filled with *Gary's Magic Mountant* [1] to just above the rim of the hole. Freshly dead fruitflies were gently lifted with forceps. Then each was placed into a filled hole and both the head and abdomen were pushed into the mountant. The wings were arranged on the plastic on either side of the hole as seen in the micrographs.



The best results were obtained if the body of the fly was evenly covered with Gary's Magic Mountant and if the photography was accomplished within a few hours of the death of the fly.

As experiments were being done, it was realized that this technique is also useful to visualize wing form, wing hair information and to study the length and formation of the head and thorax bristles. ■

Acknowledgements

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Gary's Magic Mountant [2]

Dissolve 1.75g Canada balsam in 1.0ml Methylsalicylate (oil of wintergreen)

Stir without heat for about 2 days in a metal container

Pour into glass jar (scintillation vial) and store at 4ºC

[1] Lawrence, P.A., P. Johnson, G. Morata. Methods of marking cells, In Drosophila: A practical approach (ed. D.B. Roberts), pp. 229-242. IRL Press, Oxford, 1986.

[2] Ashburner, M. Drosophila A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989.

A Comment on using FLIM with FRET

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Depending on the nature of the study and what sort of information one is trying to gather through the use of FRET, FLIM has some compelling advantages in certain situations, and can provide a quantitative evaluation of the donor, acceptor and FRET pair stoichiometry. It does require access to specialized equipment and software. Different approaches to FLIM data acquisition have different strengths and weaknesses. For dynamic studies requiring high time resolution, FLIM acquisition times can fall well short of ideal.

If a yes/no answer to whether FRET is occurring is all that is required, then the polarization anisotropy of the acceptor can be used to determine FRET between fluorescent proteins (Rizzo and Piston, 2005). This is a relatively simple and robust method for confirming the presence/absence of FRET.

A more complex method, also based on polarization anisotropy, is provided by Mattheyses *et al.* (2004). This polarized FRET technique has the advantage that the pixel-by-pixel concentrations of donor, acceptor and FRET pairs in a manner analogous to FLIM. The advantages to this approach are that it is ostensibly less expensive to implement, and acquisition requires only a single exposure per time point.

When either of the above two approaches to FRET are implemented using widefield illumination in conjunction with a beam-splitting device to permit simultaneous capture of multiple channels using a CCD camera, very high quantum efficiency for detection is possible. All of the pixels are captured in parallel, so noise due to molecules drifting from pixel to pixel during the course of acquisition is minimized if exposures are kept short.

Spectral detection provides advantages as well. Because spectral unmixing can provide correction of the donor bleedthrough into

the acceptor channel, post-acquisition data manipulation can be streamlined. Quantitative methods of spectral FRET detection and analysis have been developed and compared to data acquired using FLIM instrumentation (Thaler *et al.* 2005). Also, spectral imaging and unmixing technology provides the ability to successfully use FRET pairs with greater overlap, such as GFP-YFP (Zimmermann *et al.*, 2002). Pairs with greater overlap can yield increased FRET efficiency. ■

Mattheyses, Hoppe, and Axelrod (2004). 'Polarized Fluorescence Resonance Energy Transfer Microscopy.' Biophysical Journal 87:2787-2797.

Rizzo and Piston (2005). 'High-Contrast Imaging of Fluorescent Protein FRET by Fluorescence Polarization Microscopy.' Biophysical Journal: Biophysical Letters L14-L16.

Thaler, Koushik, Blank and Vogel (2005). 'Quantitative Multiphoton Spectral Imaging and its use for Measuring Resonance Energy Transfer.' Biophysical Journal 89:2736-2749.

Zimmermann, Rietdorf, Girod, Georget, and Pepperkok (2002). 'Spectral imaging and linear un-mixing enables improved FRET efficiency with a novel GFP2-YFP FRET pair.' FEBS Letters 531: 245-249.

Microscopy for Children

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Precollege science education in the United States is not what it could, and should, be. Major changes are being made in the way science is taught, but delivering those changes to thousands of schools is an enormous task. Scientific societies are a major resource; they can organize and train member-volunteers to help teachers bring "real" science to the classroom. The **Microscopy Society of America** has become part of the effort with Project MICRO (Microscopy In Curriculum - Research Outreach). MICRO is putting MSA members, teaching materials, and microscopes in middle school classrooms nationwide. The idea began in 1993, but it has taken a lot of time and effort to implement.

MSA's early decision to collaborate with experienced science educators at the Lawrence Hall of Science of the University of California at Berkeley was a wise one; their educational materials have a well-earned national reputation for excellence. The first phase of MICRO was completed in July 1998 with the publication of a teacher's manual, **Microscopic Explorations** in the LHS GEMS (Great Explorations in Math and Science [**www.lhs.berkeley.edu/GEMS/GEMS.html**] series, which is a leading resource for the advancement of activity-based science and mathematics nationally and worldwide. GEMS activities are originated at the Lawrence Hall of Science, the public science and curriculum development center of the University of California at Berkeley, and thoroughly tested by teachers nationwide before publication. GEMS units use accessible everyday materials and are designed for successful presentation by teachers who may or may not have special background in math or science. Featuring the "guided discovery" inquiry-based approach, GEMS units can stand alone as stimulating ways to involve all students in science and math, or can be combined to construct creative and effective year-long curricula. Among the many facets of the LHS GEMS program are:

- An acclaimed series of more than 70 teacher' guides and handbooks, including the MSA/GEMS guide, **Microscopic Explorations**
- Specialized teacher's workshops offered locally and nationally
- A growing national and international network of GEMS Sites and Centers
- Thousands of trained GEMS Leaders and GEMS Associates who support the MSA/MICRO effort to get microscopist-volunteers into classrooms nationwide.

A description of the collaboration between MSA and the LHS that produced **Microscopic Explorations** appears in vol. 2, #1 (Spring '03) of **Cell Biology Education**, a new online journal [**http://www.cellbioed.org/articles/vol2no1/toc.cfm**].

Where do we go from here? MICRO isn't just a manual. Publishing a GEMS guide brings the excitement of the microworld into thousands of classrooms. In the years since then, **Microscopic Explorations** has become an educational best- seller, with three printings and over 11,000 copies sold. During the same years, the general success of the GEMS program has led to the development of a national network of training centers that introduce teachers to the methods and concepts used in the series. This is important to you as a microscopist, because YOU can get help from the centers too. You can help a center present a **Microscopic Explorations** workshop for teachers, or the center can introduce you to a teacher who wants help. You'll find a list of centers on the GEMS website. And when it's possible to get a microscopist-volunteer into the classroom to help present the material, much can be accomplished. The availability of volunteers encourages hesitant teachers to use microscopy. Microscopist-volunteers take the enthusiasm generated by the manual content down many lines of inquiry, to be determined by the needs of the classes and the skills of the volunteers.

MICRO has gained an unexpected major benefit from its association with the LHS. The main problem faced by other scientific societies that have begun outreach programs has been national support of training, both for teachers and volunteers. The LHS/GEMS program is so successful that they've outgrown their ability to provide enough in-house trainers to meet the demand. So in the period since MICRO's inception, a highly trained category of teacher-trainer, "GEMS Associates" has been developed. There now are hundreds of them, all over the country. Geographic coverage is still spotty, but the numbers are growing rapidly. GEMS Associates can help MSA's local societies organize programs, and in areas that aren't served by participating local societies, they can invite individual microscopist-volunteers to attend GEMS workshops and then help them find a teacher to work with. ■

Endnotes

Great Explorations in Math and Science (GEMS) [**http://www.lhs.berkeley.edu/GEMS/GEMS.html**]

Sites and Centers launched recently, with date of launch:
Willimantic, Connecticut Site 10/03
Atlanta, Georgia Site 3/04
Carson City, Nevada Site 6/05
Sacramento, California Site 8/05
Mt. Pleasant, Michigan GEMS Center 9/05
Keene, New Hampshire GEMS Site 10/05
Gainesville, Florida GEMS Site 10/05
Pierre, South Dakota GEMS Site (Summer, 2006)

Contact information for all of these new GEMS sites is posted at http://lhsgems.org/sitescenters.html

If you're an overseas reader of **Microscopy Today**, please note that there are also GEMS centers in Europe, the Middle East, and Asia!

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