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 60° 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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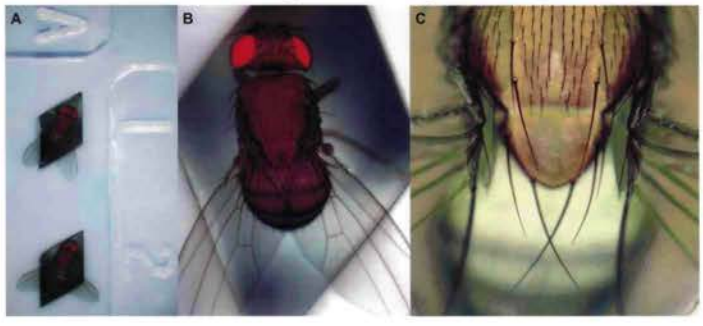
A challenge was presented to us to find a way to support *Drosophila melanogaster* so that both the anterior and posterior scutellars could be easily visualized under a dissecting microscope and photographed for purpose of measurement. The resulting solution was to utilize a standard grid box with the 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. ■

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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 4°C

[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