

Dear Dr. Lakhotia,

Hopefully by now you have received the antibodies from Bhagwati. Here are some details on pseudopupil and deep-pseudopupil. I'm basically lifting these from Utpal's 1987 Cell paper on *sevenless*; volume 49, 281-91 with a few additional details. The original source for this technique was documented in Francheschini and Kirschfeld; *Kybernetik* 9, 159-182 (1971). The paper is mainly in french, but it does have some diagrams. If you would like me to fax you a copy I can certainly do that.

pseudopupil

A 1X3 cm plexiglass piece, 0.16 cm thick was bevelled and polished at a 40° (ed: actually, these days we use 45° you might want to try both) incline along the 1cm edge, and glued to a glass microscope slide. Using clear nail polish, fly heads were attached, so that they lay on the incline, eyes upward. A drop of immersion oil (ed: we use type B non-drying immersion oil from Cargille labs, inc., Cedar Grove, N.J. 07009, USA) was placed on the eye, thus optically neutralizing the cornea. The pseudopupil was observed in a microscope by passing light antidromically from below, using a condenser with a small aperture, and viewing with a 40X Zeiss oil-immersion objective. (ed: raise the condenser up so it almost touches the slide, open the iris diaphragm about 3/4 way and adjust the light intensity so it is not blinding. We usually look at 5 or so eyes sequentially. You should be able to count at least 100 ommatidia per eye. If you can't do this, the eye is probably not mounted at the correct angle, or the microscope needs further adjustment or the slide is dirty (clean gently with ethanol and a razor blade after each round). Note that the samples will degrade so that you cannot see a pseudopupil after maybe 5 minutes after guillotining them).

deep pseudopupil

An inverted microscope was used. (ed: we use a pad with a clear plastic bottom with CO₂ piped in from the side and we roll the fly around until we can see the dpp) or (the old fashioned way)...use a strip of double-stick tape attached to a glass slide and stick the etherized fly with its ventral side up, wings down and proboscis at an angle of about 40° upward. A condenser focused a bright light source, from above, through a 1mm aperture and a heat filter, onto the front of the head. With this antidromic illumination, the deep pseudopupil was observed from below by focusing a 10X objective at the center of curvature of the eye.

If you have any questions, please don't hesitate to e-mail me, or you can call at (310) 825-2980.

Good luck with your experiments,

Amanda Pickup