Microscopy of the Drosophila facet eye: vademecum for standardized fixation, embedding, and sectioning

Michael W Hess, Kristian Pfaller, Bernhard Hampölz, Stefano Longato, David Teis, Angelika Flörl, Karin Gutleben & Lukas A Huber

We describe here a standardized method for histological processing of the Drosophila compound eye. Primary fixation with 2.5% glutaraldehyde, obligatorily supplemented with 0.1% household detergent regularly yielded the best structural preservation, as compared with that of other, more complicated fixation protocols tested. Notably, it proved indispensable not only to cut off the fly’s head to facilitate the penetration of the reagents but also to open the chitinous head capsule. For this, we locally pierced the cuticle between the eyes, leaving the head structurally almost intact, a prerequisite for precisely aligning the head for microtomy. We developed a two-step re-embedding procedure allowing for exact and reproducible orientation of the fly heads. Thus, highly comparable series of cross sections through a representative number of ommatidia were obtained. The feasibility of our embedding and sectioning approach is finally demonstrated by three-dimensional reconstructions of the middle segments of the R1, R7, and R8 photoreceptor cells. We present reconstructions from structurally modified ommatidia, as seen after RNAi-mediated depletion of the endosomal adaptor protein p14, and from normal ommatidia corresponding to the wildtype.

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