Solving the Gordian Knot: The outer plexiform layer of the European anchovy *Engraulis encrasicolus* investigated by Focussed Ion Beam-SEM

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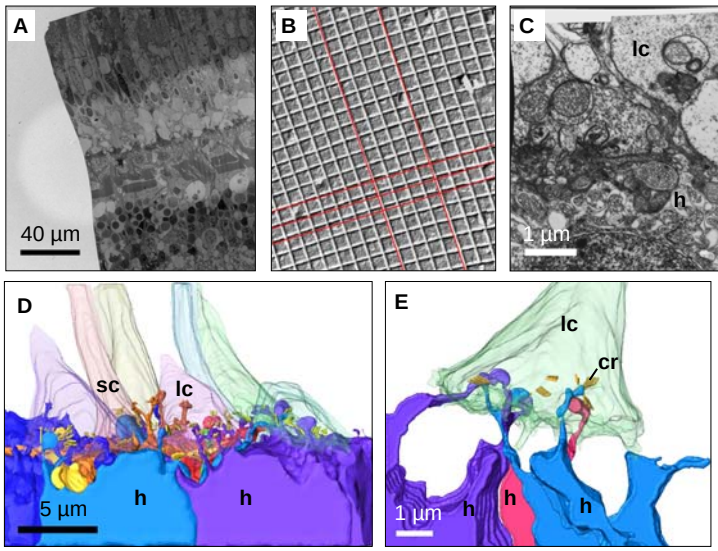

Fig. 1 Common ultrathin sections and resulting 3D-reconstructions (Amira®).
A Distortion of an ultrathin section due to the electron beam.
B *Aberration* of parallel lines of a so called „grated grid"; red lines indicate ideal parallel lines.
C Deformed TEM-image after semi-automated elastic registration (Fiji, ImageJ).
D 3D-reconstruction of mainly horizontal cells (shaded), cone pedicles (transparent) and synaptic ribbons (yellow); finer dendrites, especially that of bipolars, cannot be traced and reconstructed.
E 3D-reconstruction of a cone pedicle with synaptic ribbons and dendrites of horizontal cells.
cr cone ribbon, **h** horizontal cell, **lc** long cone pedicle, **sc** short cone pedicle

The European anchovy *Engraulis encrasicolus* features a highly specialized retinal architecture subserving polarization contrast vision. This uncommon sensory ability is based on the unique fine structure and arrangement of two cone types (short and long cones) with orthogonal e-vector sensitivity and special connectivity.

To uncover the meshwork structure of the outer plexiform layer (OPL) and its connectivity two methods were tested:

1 An ultrathin section series of the OPL, composed of 300 radial 60 nm sections, was investigated with a TEM. However, due to sectioning compression and deformation in the electron beam (fig. 1A, B) finer dendrites could not be traced through the retinal subvolume. Therefore, mainly the synaptic sites of horizontal cell dendrites and cones could be reconstructed (figs. 1D, E).
Semi-automated elastic registration by Fiji-software helps to partly overcome the distortion-problems (fig. 1C), but turned out to be too time consuming and defective.

2 A focussed ion beam-SEM (Zeiss Auriga®-CrossBeam® Workstation) was used to get high resolution image stacks of the OPL (fig. 2B) without evident distortions as images are taken from the exposed sample surface (fig. 2A). Therefore, even fine bipolar dendrites could be detected and reconstructed (fig. 2D). *E. encrasicolus*, as other teleosts, features mixed rod-cone-bipolars contacting the two cone types and several rods, as well as pure cone-bipolars apparently contacting cones selectively (fig. 2D). Thus, at least three types of bipolars exist within the retina of *E. encrasicolus*: mixed rod-cone-bipolars, short-cone-bipolars and long-cone-bipolars. As all dendritic fields are trimmed at their edges no statements about size and shape of dendritic fields can be made yet.

The bushy dendrites of horizontal cells 1 (h1) form synaptic contacts to several cone ribbons of both cone types, mostly enclosing them (fig. 2C, E). Due to the small sample volume the correlation of horizontal cell dendrites either to h2 or h3 is very difficult, but potentially h2 and h3 seem to be cone-selective and dendrites of the different horizontal cell types do not share one single cone ribbon.

The obvious advantage of FIB-SEM is the option to get undistorted, well-aligned, high resolution image stacks. However, the method has still some initial difficulties and Amira® apparently reaches its limits dealing with image stacks of more than 3 GB.

Concurrently, a second FIB-SEM-stack with even higher resolution, bigger sample volume and in tangential orientation is analysed and will give further valuable insights.

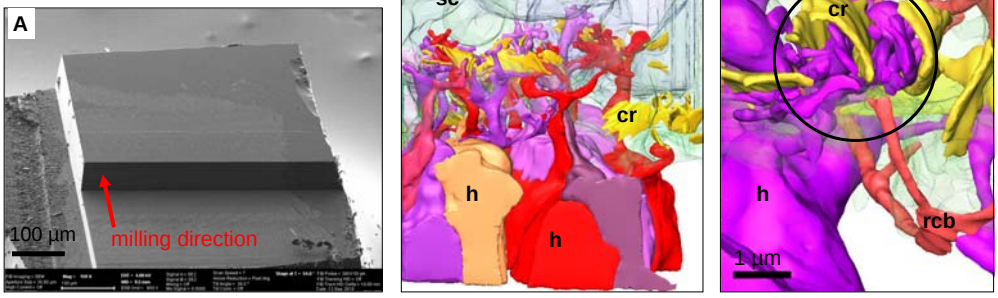

Fig. 2 FIB-SEM-Images and resulting 3D-reconstructions (Amira®).
A Embedded sample, trimmed for FIB-SEM („mesa").
B FIB-SEM-image of backscattered electrons in the range of the outer plexiform layer.
C 3D-reconstruction of horizontal cells (shaded), cone-pedicles (transparent) and synaptic ribbons (yellow).
D 3D-reconstruction of mixed rod-cone-bipolars (reddish), cone-bipolars (bluish), cone-pedicles & rod-spherules (both transparent) and synaptic ribbons (yellowish).
E Triade of h1 (violet) and rod-cone-bipolar (redish) dendrites making synaptic contact with a short cone ribbon (yellowish).
cr cone ribbon, **h** horizontal cell, **lc** long cone pedicle, **rcb** rod-cone-bipolar, **rr** rod ribbon, **sc** short cone pedicle