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

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Anchovies feature 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 with orthogonal e-vector sensitivity. Our present investigations on the retina of *Engraulis encrasicolus* tend to uncover the meshwork structure of the outer plexiform layer (OPL) and its wiring rules. How are the two cone types synaptically linked to bipolars and/or horizontal cells and are these cells cone type specific? For structure data acquisition this task requires both, an investigation volume containing several cone pedicles to discover whole dendritic fields, and high EM resolution for the tracing of fine dendrites. Subsequent 3D-reconstruction of synaptic ribbons and dendritic arbors finally reveals wiring rules.
To achieve this, **two methods** were tested:

1 An ultrathin section series of the OPL, composed of 300 radial 60 nm sections, was investigated with a TEM (JEOL JEM-1230). Ten adjacent pedicles from each section were photographed at a resolution of 3.4 x 3.4 nm. To achieve this, 4–6 slightly overlapping images per section were necessary and had to be stitched with Photoshop® CS. During the alignment with Amira®, distortions due to sectioning compression and deformation in the electron beam became obvious, hindering the detection of finer dendrites through the retinal subvolume. Semi-automated elastic registration by Fiji-software helps to partly overcome the distortion-problems, but turned out to be too time consuming and defective, as 40–60 landmarks had to be set manually for each adjacent pair of images.

2 A focussed ion beam-SEM (Zeiss Auriga®-CrossBeam® Workstation) was used to get high resolution image stacks of the OPL without evident distortions. The ion beam stepwise milled 10 nm of the sample, followed by SEM-images of backscattered electrons of the exposed sample surfaces at a resolution of 10.8 x 10.8 nm.
In a first approach a subvolume of about 21 x 23 x 7.7 µm was investigated by 3D-reconstruction including two cone pedicles of each type, but none was captured completely, thus only partial results concerning wiring rules and receptive fields of cells are available yet.

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 (holding focus during 15 hours, slightly irregular milling) and Amira® apparently reaches its limits dealing with image stacks of about 3.2 GB. Further experiments and optimization work have to be done.