# Disentangling the retinal cable mess – FIB-SEM based 3D-reconstructions of the anchovy inner retina in high resolution

Martin Heß, Petra C. Koch, Gerhard Wanner

BioZentrum der LMU München, Großhaderner Str. 2, 82152 Planegg, Germany
hess@bio.lmu.de

**Introduction** The anchovy retina is distinguished by an exceptional photoreceptor fine structure suggesting **polarization contrast vision**.
Concomitantly two cone photoreceptors (long cones LC, and short cones SC) with orthogonally oriented e-vector analyzers are arranged in a tessellate pattern sustained down to the level of their synaptic pedicles (figs. 3, 8). This implies that the impressive geometric regularity is continued through the synaptic layers of the retina representing the structural basis of bioelectric image processing handling polarization information instead of spectral information.
A morphological investigation of a suchlike neuronal network has to cope with reams of very fine, three-dimensionally interwoven dendrites (diameters only a few nanometers) on the one hand, and with cell dimensions of several dozen micrometers on the other. Therefore an electron-microscopic approach has to be followed that provides both, high resolution and contrast in 2D, and high z-resolution and perfect alignment of subsequent image planes for 3D-reconstruction.

**Methods** The latter can hardly be achieved with conventional thin-section series and TEM due to inevitable image distortion and comparatively „rough“ slicing. In order to visualize every single cell of a small piece of the inner retina and to clarify the wiring rules in the plexiform layers of the European anchovy *Engraulis encrasicolus* we used a **FIB-SEM crossbeam workstation (Zeiss Auriga ®)** as imaging device on Os/U-stained, resin embedded and carefully oriented retinal fragments in combination with **3D-rendering software Amira®** (fig. 8)**.**
To balance resolution and image acquisition speed we used voxel sizes between (9x9x10)nm³ and (14x14x50)nm³ depending on the retinal layer that was imaged. Altogether the raw data comprise about 5200 image planes and a total data volume of about 30 GB. The „final product“ of manual segmentation followed by surface rendering is a digital 3D-model of the investigated retinal fragment that can be virtually explored by free choice of perspective and zoom, as well as composition, colour and transparency of single objects (see figs. 1-7).

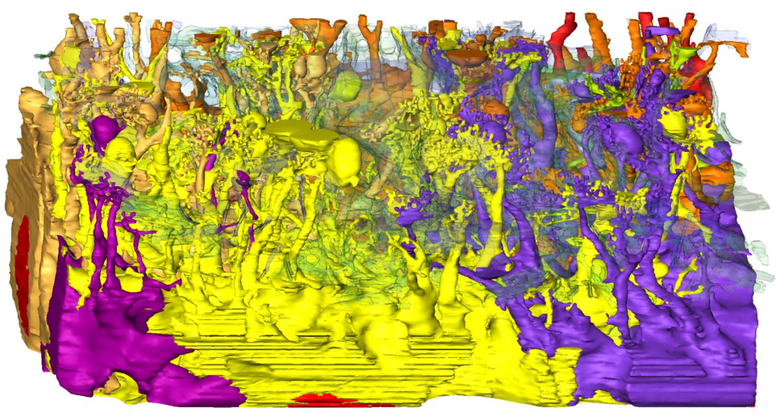

*Fig. 2* Eight different horizontal cells H1 with their bushy dendritic endings connecting to both, LC and SC (cone pedicles transparent).

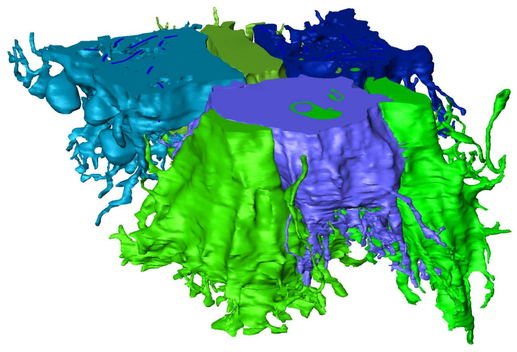

*Fig. 3* Long cone pedicles (bluish) and short cone pedicles (greenish) with their telodendrites.

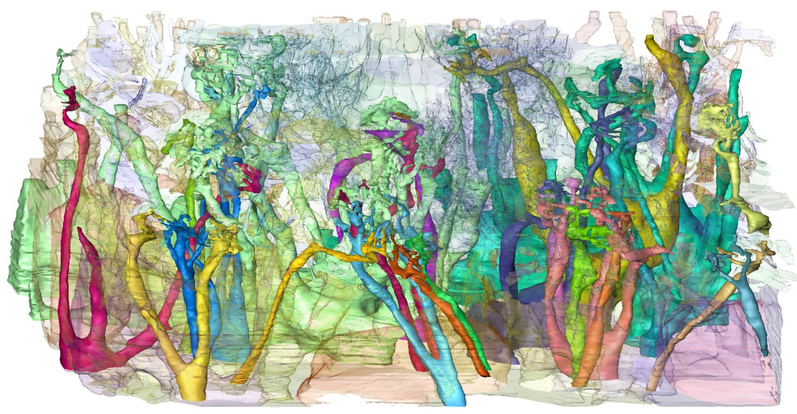

*Fig. 4* Horizontal cells H2 and dendrites of horizontal cells H3 connecting to SC (cone pedicles transparent).

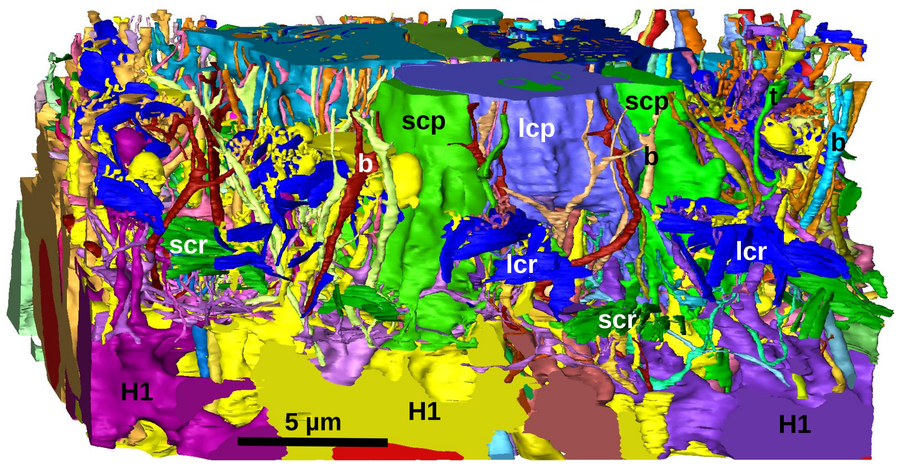


*Fig. 1* **Outer plexiform layer (OPL)**: cone pedicles and second order neurons.
**b** bipolar cell, **H1** horizontal cell 1, **lcp** long cone pedicle, **lcr** long cone ribbons, **scp** short cone pedicle, **scr** short cone ribbons, **t** telodendrite.

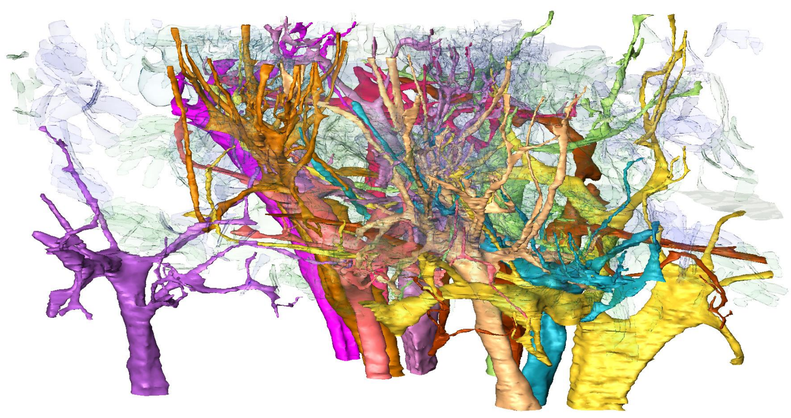

*Fig. 7* Cone specific bipolars preferentially connecting to SC or LC (cone pedicles transparent).

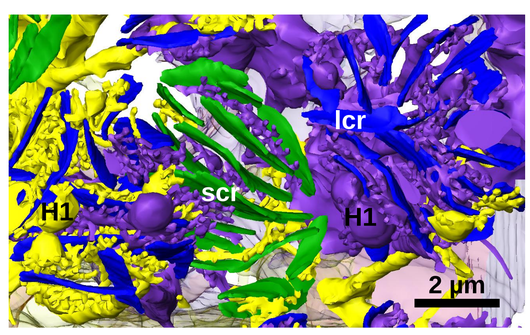


*Fig. 6* H1-cell dendritic endings (yellow, violet) embracing synaptic ribbons (green, blue) laterally.

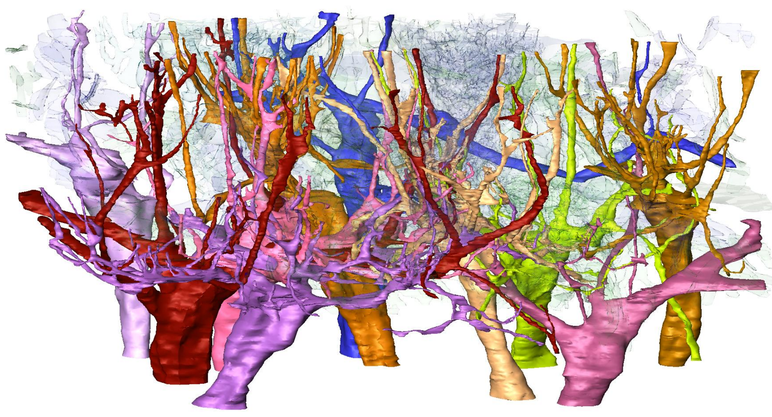

*Fig. 5* Mixed rod-cone-bipolars connecting to rods, SC and LC (cone pedicles transparent).

**Results** The first results of the ongoing evaluation show 3 types of horizontal cells (H1-3) arranged in distinct layers disclosing constant wiring rules (figs. 2, 4: H1 contact SC and LC, H2 only SC, H3 contact SC) and at least 3 types of bipolar cells concerning their connectivity in the OPL (fig. 5: mixed rod-cone-bipolars, fig. 7: specific SC- or LC-bipolars). The contact pattern of H-cell dendritic endings embracing all synaptic ribbons in their synaptic fields laterally can be determined (fig. 6). The same is true for cone-to-bipolar synapses that are found at central positions of the synaptic triades or at the pedicle bases. The dendritic fields of different bipolar types interdigitate, those of the same type do not.
**Discussion** Generally the findings indicate separation of (LC- and SC-dependent) information channels and parallel processing in the inner anchovy retina. The convergence sites/rules essential for contrast generation or encoding e-vector orientation are expected in the inner plexiform layer – its reconstruction will give further insights while approaching an understanding of the mechanisms of polarization vision in vertebrates.

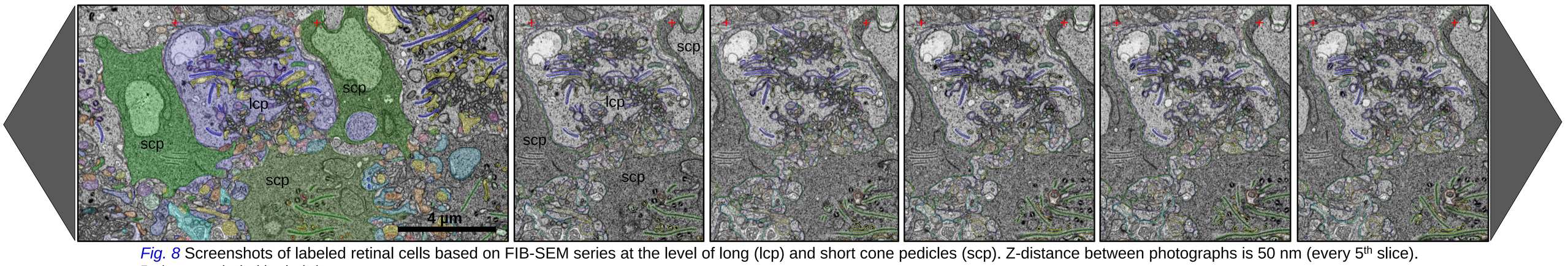


*Fig. 8* Screenshots of labeled retinal cells based on FIB-SEM series at the level of long (lcp) and short cone pedicles (scp). Z-distance between photographs is 50 nm (every 5th slice).
Red crosses depict identical sites.