Disentangling the neuronal network of the retina in 10 nm isotropic resolution - a closer look into the anchovy retina

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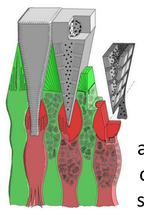
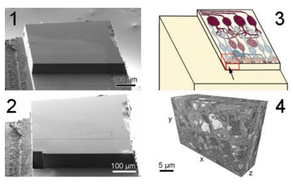
Introduction



Anchovies feature a highly specialized outer retinal architecture, suggesting polarization contrast vision. Two cone types with orthogonal e-vector sensitivity are alternately arranged, a corresponding tessellated pattern is retained at the level of their pedicles. The geometric regularity of the cone arrangement is expected to be continued through the synaptic layers, especially at the level of bipolar cells, as both, the separation of signaling pathways and cell type selective wiring represent the basis of (e.g. polarization) contrast vision. As implied by the name retina, the interweavement of neurons in two structurally most complex (plexiform) layers is the structural basis of the retinal "imaging computer". To understand some of its species-specific performance features one has to figure out the morphology of all neuron-types involved, their receptive fields, ratios and stratification patterns, as well as the selectivity and geometry of neuronal connectivity in the OPL and IPL. Difficulties arise from the huge dimension-span between cell extend (several µm) and axon/dendrite diameter (few nm), the sheer number of neurite cuts converging in a given retinal volume from different directions, and maybe from the claim to reconstruct all cell-types in a volume without any specific staining. Due to the required resolution this can be only achieved with 3D electron microscopy and computer aided 3D-reconstruction.

Methods

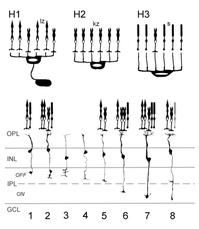


For structure data acquisition we used block-face scanning electron microscopy with the Zeiss Auriga® Crossbeam Workstation (FIB-FESEM) on Os / U- stained tissue embedded in epoxy resin. XY-resolutions of <10 nm/px with good membrane contrast, and a Z-resolution of 10 nm (milling rate) with virtually no image distortion makes perfectly aligned 3D structure data available in 10 nm isotropic resolution. Since no horizontal image stitching is possible one has to compromise about acceptable resolution and the resulting field-of-view with max. 3072 x 2304px. In addition special care has to be taken for the orientation of long radial structures to avoid drifting of e.g. bipolar cells out of the milling volume. Manual segmentation (using Amira® Software) of single cell profiles across huge stacks of EM-images is time-consuming (e.g. 9 months, several hours per day for a 3000 x 2300 x 5375px volume performed by an experienced person), but not automatable at present with suitable results. Segmentation is followed by surface rendering, resulting in digital 3D-surface models of all neurons that lie (entirely or partially) within the imaged tissue volume.

Results

The compound model can be manipulated interactively and analyzed step-by-step. Indeed the complete model of the neuronal network is not less complex and obscure than the single EM images, but now there is the option of free choice of perspective (angles, zoom) and free combination of selected cells/cell groups, as well as pleasing definition of colors and transparency of every model component. The two **cones** types build up a distinct connecting pattern via telodendrites to their neighboring photoreceptors. The contact pattern of the **horizontal cells** H1-3, embracing the synaptic ribbons of connected cones laterally, emerges as follows: the sclerally positioned H1 contact both cone types, with an apparent bias to long cones, whereas the more vitreal H2 exclusively contact short cones. Farthest vitreal, the H3 are connected to short cones and rods. Several morphotypes of ON- and OFF-**bipolars** could be identified based on their contact pattern, receptive field, shape and sublayer of axon terminals. The axon of one OFF-type contacting both cone types (and possibly rods) terminates in several stratified varicosities with fine lateral branches. Another OFF-type contacting long cones (and possibly few rods) shows a more diffuse axon terminal. An ON-type population, building up a regular tessellated pattern of knob-shaped terminals, connects to both cone types and rods, whereas another mixed rod-cone-bipolar with a larger receptive field descends into the lower IPL and terminates in a fat varicosity. Another group of bipolars contacts both cone types (and possibly rods), shows fine lateral branches midway the IPL and terminates in a small knobby varicosity in the lower IPL. The widely ramified **Müller cells** can also be displayed in detail. The reconstruction of **amacrines** and **ganglion cells** is subject of ongoing work.

Discussion



To elucidate the structural basis of bioelectric image processing handling polarization information instead of spectral information, a first step has to be the uncovering of morphologies and connectivities in a certain retinal area. To meet the requirements of this challenge we applied FIB-FESEM combined with 3D-reconstruction to a fragment of a vertebrate retina for the first time, i.e. the European anchovy, with its unique structural adaptations in the service of polarization contrast vision. Our first results show a separation of information pathways in horizontal cells and bipolars, and first signs of cell type specific interconnections in the IPL. The applied method opens up undreamed-of possibilities to elucidate complete neuronal networks, albeit further reconstruction work has to be done with the anchovy retina.

Figures

For vewing the anaglyphes (B, C, E, F) use red/cyan or red/green glasses.

A: 3D-Reconstruction of a 30 x 23 x 144 µm subvolume of the *E. encrasicolus* retina from rod spherules (top) to the scleal border of the ganglion cell layer (bottom). **OPL** outer plexiform layer, **H1-3** horizontal cell layers, **INL** inner nuclear layer, **IPL** inner plexiform layer, **GCL** ganglion cell layer.
B: 3D-view of H1 cells with dendrites and cone ribbons (long cone: greenish; short cone: red)
C: Skew 3D-view on bipolar dendritic trees in the OPL with cone ribbons.
D: Neuroanatomy of 8 bipolar cell morphotypes differing in dendritic arborization, nuclear position and IPL termination depth.
E: 3D-view on the axon terminal varicosities of 8 neighboring type 2 bipolars.
F: 3D-view on the co-stratification of amacrines (yellow, beige) with type 3 bipolar synapses (pink, blue).
G: Selected planes from the FIB-FESEM raw data. Left panel: 5x OPL ΔZ= 10 nm; right panel: H2, INL, 2x IPL, GCL.

Introduction: Anchovy polycone with long (green) and short cones (red), and pigment epithelium wedges containing an interference mirror from guanine.
Methods: Epon-mesa before (1) and after ion beam milling (2). Maximally invasive structure data aqcuisition results in perfectly aligned EM image stacks (4) of the VOI (3).
Discussion: Photoreceptor connectivity of 3 types of horizontal cells and 8 types of bipolars.