Valleys deep, mountains high – 3D-topography of cell nuclei in the anchovy retina

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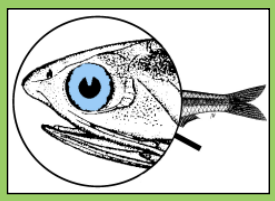
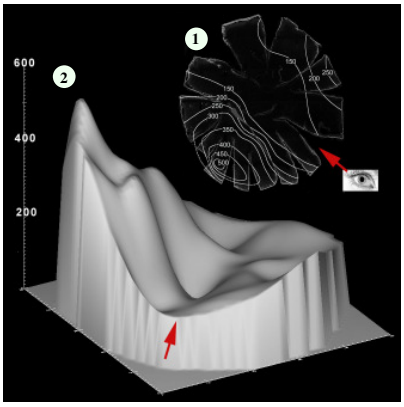
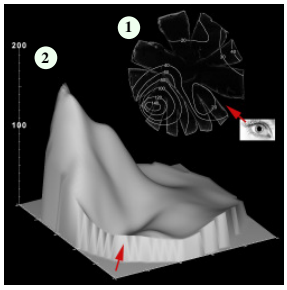
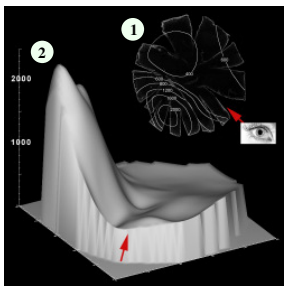




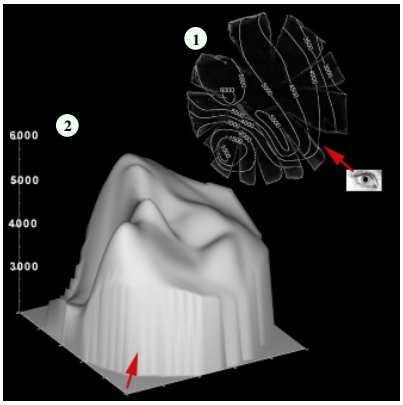
A Density distribution of **cone photoreceptors** as isodensity contour map (1) and shaded surface map (2).



B Density distribution of **ganglion cells** as isometric contour map (1) and shaded surface map (2).



C Density distribution of **bipolar + amacrine cells** as isodensity contour map (1) and shaded surface map (2).



D Density distribution of **rod photoreceptors** as isodensity contour map (1) and shaded surface map (2).

Introduction

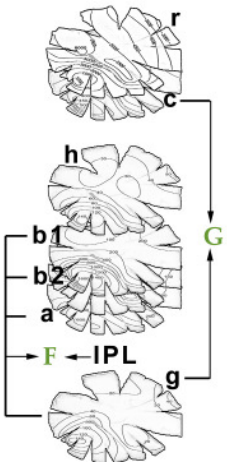
From morphological point of view retinal cell topography is a complex character that is shaped non-accidentally by evolutionary pressure. Thus topography has high significance regarding functional morphology and the visual ecology of a retina. In this study we present a feasible technique for the parallel three-dimensional examination of all retinal cell types (see fig. I) without laborious sectioning or staining techniques. The combination of fluorescent labelling of cell nuclei with two-photon-microscopy, in conjunction with computer aided evaluation of digital 3D-image data provides new possibilities of topographic and correlative examination of retinal structures.

Material & Methods

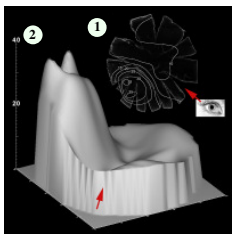
European anchovies (*Engraulis encrasicolus* L.) were obtained from fishermen in Rovinj (Croatia), and eyes were fixed in 4% formaldehyde. DAPI stained retinal wholemounts were scanned with a two-photon microscope at 34 scanning sites with increased sampling density in the ventro-temporal quadrant. Manual labelling and three-dimensional reconstruction (AMIRA®) allowed for counting cell nuclei, values thus obtained were converted to cells/10,000 µm². Classification of different cell nuclei was effected by their position, size, shape and staining pattern. Topographic and correlative contour maps were generated with IDL 7.0 (Interactive Data Language).

Results

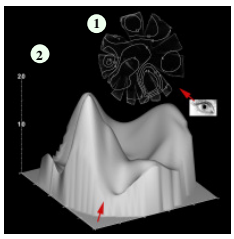
Cones, second and third order neurons show very similar distributions, with highest density in the ventro-temporal quadrant (area temporalis) and slightly increased density at the dorso-nasal periphery (figs. A–C). Both, rods and a subpopulation of bipolar cells (b2), show reverse distribution with two areae situated ventrally and dorso-temporally (fig. D). Several correlations can be calculated, two of them are shown here: Cells that account for the thickness of the IPL with their axons and dendrites (b, a, g) are more numerous in the area temporalis than the thickness of the IPL would suggest (fig. F). The ratio of cones to putative cone ganglion cells (c/g–0.001*r, i.e. convergence) is lowest in the area temporalis and highest in the rod-maxima and the retinal periphery (fig. G).



E Isodensity contour maps of the nuclei of different cell types. **r**: rods, **c**: cones, **h**: horizontal cells, **IPL**: inner plexiform layer, **b1**: bipolar cells type1, **b2**: bipolar cells type2, **a**: amacrine cells, **g**: ganglion cells. Brackets mark the correlations shown in **F** and **G**.



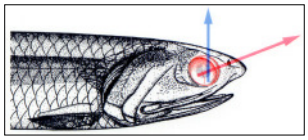
F Correlation map showing the ratio b1+b2+a+g/d(IPL) as isocontour map (1) and shaded surface map (2).



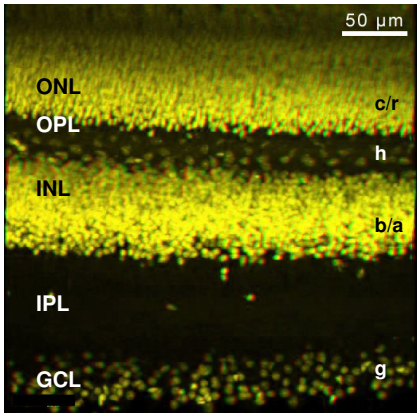
G Correlation map showing the ratio c/g–0.001*r as isocontour map (1) and shaded surface map (2).

Discussion

We established and optimized a feasible method for mapping the cell nuclei of a vertebrate retina 3-dimensionally. Our data reveal an area of highest visual acuity in the ventro-temporal quadrant of anchovy retina, involving all cell types of the cone pathway (c, h, b1, a, g). The viewing direction of this area is ahead-upward to focus on prey and predators (fig. H). The light sensitive rod area is located ventrally, as also dim light comes from above (fig. H). In the area temporalis the IPL is relatively slim, due to less horizontal branches, more vertical direct connections and thus low convergence. This conclusion is also supported by the small c/g-ratio within the area temporalis, i.e. obviously only 3–5 cones project onto a single cone ganglion cell.



H Viewing directions of the cone area (red arrow) and the major rod area (blue arrow).



I 3D-anaglyph of retinal cell nuclei (3 layers). To be viewed with red-green-glasses! **ONL** outer nuclear layer, **OPL** outer plexiform layer, **INL** inner nuclear layer, **IPL** inner plexiform layer, **GCL** ganglion cell layer.