

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

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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 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. European anchovies (*Engraulis encrasicolus* L.) were obtained from fishermen in Rovinj (Croatia), the eyes were enucleated and fixed in 4% formaldehyde. DAPI stained retinal whole mounts 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 μm^2 . Classification of different cell nuclei was effected considering their position, size, shape and staining pattern. Topographic and correlative isocontour maps were generated with IDL 7.0.

Our data reveal an area of highest visual acuity in the ventro-temporal quadrant involving all cell types of the cone pathway (cones, horizontal, cone bipolar and cone ganglion cells). The viewing direction of this area is ahead-upward to focus on prey and predators. The light sensitive major rod area is located ventrally, as also dim light comes from above. The correlative analysis of density values and layer thicknesses is an appealing option, especially at the level of map-computations. Correlation-maps provide

information about convergence, dimensions of receptive fields and complexity of retinal processing power in different sectors of visual space.

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