Application Note
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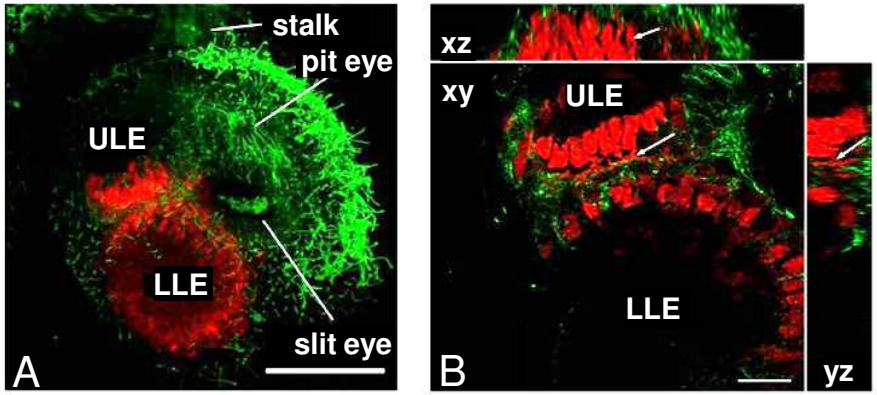
Analysis of box jellyfish photosytems using 64-bit Volocity



C. marsupialis whole-mount rhopalium of newly metamorphosed medusa. Figure A shows an extended focus projection of 194 confocal optical sections. UV-Opsin immunoreactivity (UV-IR; red) is expressed in photoreceptor cell bodies in the upper and lower lens eyes (ULE and LLE) but is absent from the pit and slit eyes (Bar: 50 µM). The surface cilia, neurites and occasional neuronal cell bodies are shown in green (labelled with anti-Glu tubulin, ID5). Figure B shows confocal xy, xz and yz optical sections (Bar: 10 µM). UV-IR is expressed in photoreceptor cell bodies and basal neurites (arrows) that intermingle with ID5-immunoreactive neurites.

The visual system of Cubomedusae (box jellyfish) is complex and intriguing, with 24 eyes distributed in four sensory structures called rhopalia. Each rhopalium has six eyes; two pairs of pigment cup eyes (the pit eye and slit eyes) and an unpaired upper and lower lens eyes. In this study, to further the understanding of visual processing in the rhopalium, researchers investigated whether multiple types of photoreceptor cells are present in the rhopalium, and whether the different eye types possess different types of photoreceptors.

The eyes of two box jellyfish species, *Carybdea marsupialis* and *Tripedalia cystophora* were examined using different vertebrate opsin antibodies to label the photoreceptors. Immunoreactivity was visualized by immunofluorescence and detailed analysis by confocal microscopy was performed on whole excised rhopalia. Images were captured at optimal Nyquist sampling frequency (pixel size 0.097 µm, sampling interval 0.38 µm) or as 1024 x 1024 pixel frames (pixel size 0.10 - 0.20 µm, sampling interval 0.38 µm). To improve depth resolution, series of confocal section stacks of rhopalia were used to create extended focus and 3D-projections of the neural elements using the high performance rendering capabilities of Volocity 64-bit software. All photoreceptor cells in the lens eyes of both species were found to be of one type since they only displayed immunoreactivity for a single antibody directed against zebrafish UV opsin, and not against any other tested rhodopsin or cone opsin antibody. The pit and slit eyes of both species were not immunoreactive for any of the opsin antibodies (Figure A). Where the upper and lower lens eyes lie very close to each other, the basal parts of their photoreceptors are separated only by a thin neuropil layer. Analysis with Volocity Visualization software clearly revealed that the basal neurites of photoreceptors in the two lens eyes were intertwined with the Glu-tubulin (ID5) immunoreactive neurites that surrounded the retinas (Figure B).

This study concluded that the lens eyes of box jellyfish are composed of a single population of photoreceptor cells, expressing a single photopigment, and are therefore color-blind. The absence of immunoreactivity for zebrafish UV opsin in the pigment cup eyes suggests that they express a different photopigment. This data supports the hypothesis that the lens and pigment cup eyes of box jellyfish are involved in different and specific visual tasks.

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