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ID-1-P-2832 Correlative light and electron microscopy in the classroom – simple protocols for undergraduate courses

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Correlative microscopy combines the versatility of the light microscope with the high spatial resolution of the electron microscope. It is generally regarded as state-of-the-art technology and many protocols are dependent on superhuman technical skills and/or sophisticated instrumentation [1-4]. However, on-section labeling of resin or cryo-sections with fluorochrome-coupled antibodies and gold probes is a versatile and fast method for CLEM of cells and tissues [5-8]. It is easy to perform and suitable for teaching in undergraduate student courses.

Here, we describe fast and simple protocols for correlative immunofluorescence and immunogold labeling on resin- and cryo-sections successfully used in our student practicals. Ultrathin tissue sections are mounted on EM-grids and stained simultaneously with fluorescent and gold markers. The samples are analyzed at the fluorescence microscope (FLM), demounted from the microscope slide, stained with uranyl acetate and then imaged in the transmission electron microscope (TEM). Subsequently, labeled structures selected at the FLM are identified and analyzed in the TEM. This way, fluorescent signals are correlated to the corresponding subcellular structures and corresponding immunogold signals in the area of interest (Fig. 1).

Labeling and analysis can be performed within 1-2 days, depending on the number of students attending the course. In Dresden we perform a 1-week basic EM-course for master students. The CLEM experiment is used as our routine Immunogold experiment and takes 1,5 days for 12-15 students. The procedure works in 80-100%, which hopefully encourages the students to consider even sophisticated EM-methods for their future work.

Alternatively, the samples can also be processed completely, including staining with uranyl acetate and drying before imaging. Such samples can be used for demonstrations of the method without any sample preparation (Fig. 1C-E) if time is very limited.

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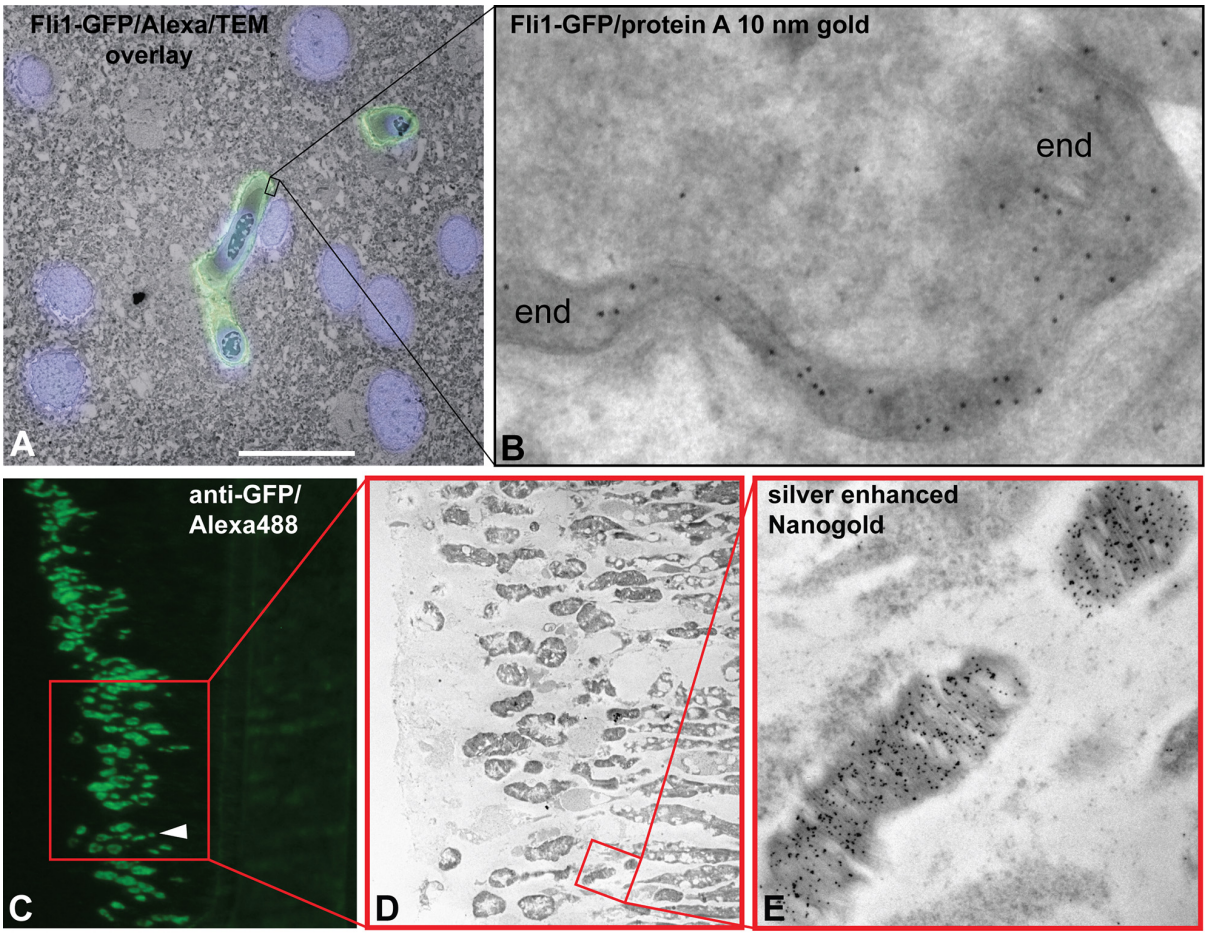

Fig. 1: A,B Cryo-section through zebrafish brain with labeled endothelial cells. A Overlay of fluorescence (anti-GFP, DAPI) and EM. B Immunogold labeling in the endothelial cells (end). C-D K4M section through the retina with GFP-labeled photoreceptors. C Fluorescence in the outer segments of rod photoreceptors. D,E corresponding EM-micrographs.