Correlative Microscopy in Life and Materials Science

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Preservation of Alexa Fluor® fluorescence in tissue for correlative light and electron microscopy

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It is a demanding task to locate single, rare events or structures in big cellular volumes or, even worse, within tissue samples prepared for electron microscopic investigations. Therefore correlative light and electron microscopy is a useful method to not only find the event or structure of interest again much faster but also to gain valuable information about the ultrastructural context of the labelled feature. We established two protocols to maintain Alexa Fluor® fluorescence during the embedding of mouse muscle tissue in Lowicryl HM20 resin. The structure of our interest is the neuromuscular junction (NMJ), the synapse between the axon of a motoneuron and a skeletal muscle fibre which is responsible for voluntary movement [1]. Both approaches start with the excision of diaphragm muscle. In both cases the acetylcholine receptors (AChR) of the NMJs are labelled using α-bungarotoxin (BGT), a snake venom, conjugated to Alexa Fluor® 555 (AF555, Invitrogen) or 647 (AF647, Invitrogen). The first method is a high pressure freezing approach. After excision of the diaphragm we dissect it into pieces small enough to put them into planchettes suitable for high pressure freezing. During this time the muscle is labelled with BGT-AF555/-AF647. Subsequently the appropriate pieces are high pressure frozen, freeze substituted and embedded in Lowicryl HM20 resin [protocol modified on the basis of 2]. The second method is a chemical fixation approach starting with excision and chemical fixation of muscle tissue. Afterwards the muscle is labelled and dissected into pieces for further processing steps. A progressive lowering of temperature method is applied to embed the tissue at low temperature [protocol modified on the basis of 3]. We obtained preservation of the Alexa Fluor® fluorescence during embedding of the mouse muscle tissue in resin with both methods.

In the first instance the whole block can be investigated using confocal laser scanning microscopy providing the possibility to relocate predefined regions. The fluorescence is further detectable in ultrathin sections by either common fluorescence light microscopic techniques or by more sophisticated methods such as high numerical aperture (NA) fluorescence microscopy (Figure 1), confocal laser scanning or localization microscopy (Figure 2). Following light microscopic investigations the same sections can be imaged with transmission or scanning electron microscopy and images from all modalities can be correlated (Figure 2). An intermediate step of immunogold on section labelling can be performed to specifically label molecules of interest within the tissue section. This allows complementing the fluorescence signal detectable in light microscopy with an electron-dense marker (Figure 3). Using our approaches it is possible to use a commercially available fluorescence dye to label molecules or structures of interest and to monitor the fluorescence in electron microscopic preparations. With this tool rare events can be relocated within bigger volumes and be further investigated in their ultrastructural context enabling the researcher to “see” not only the labelled entities but to explore much more about their actual cellular environment.

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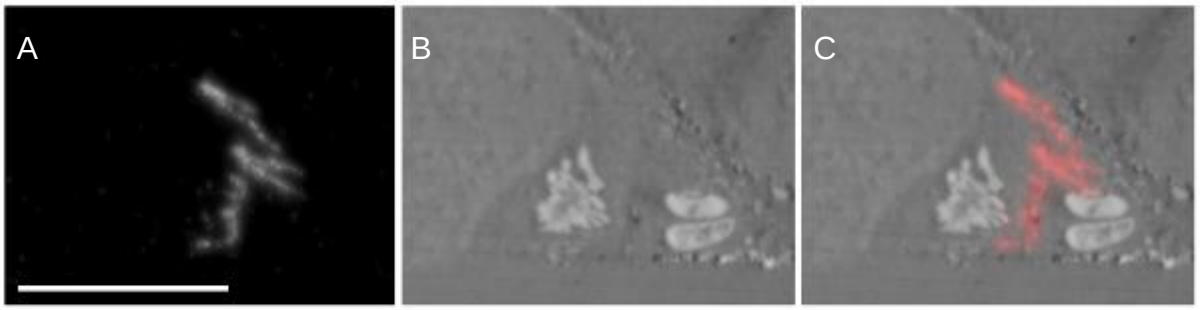

Figure 1: Light micrograph of a 70 nm HM20 section on an indium tin oxide (ITO)-coated cover slip recorded with an oil immersion objective lens (100x/1.4 Plan Apochromat, Carls Zeiss). A: fluorescence image of AChR labelled with BGT-AF555; B: bright field image; C: Overlay of fluorescence and bright field image. Scale bar: 10 µm.

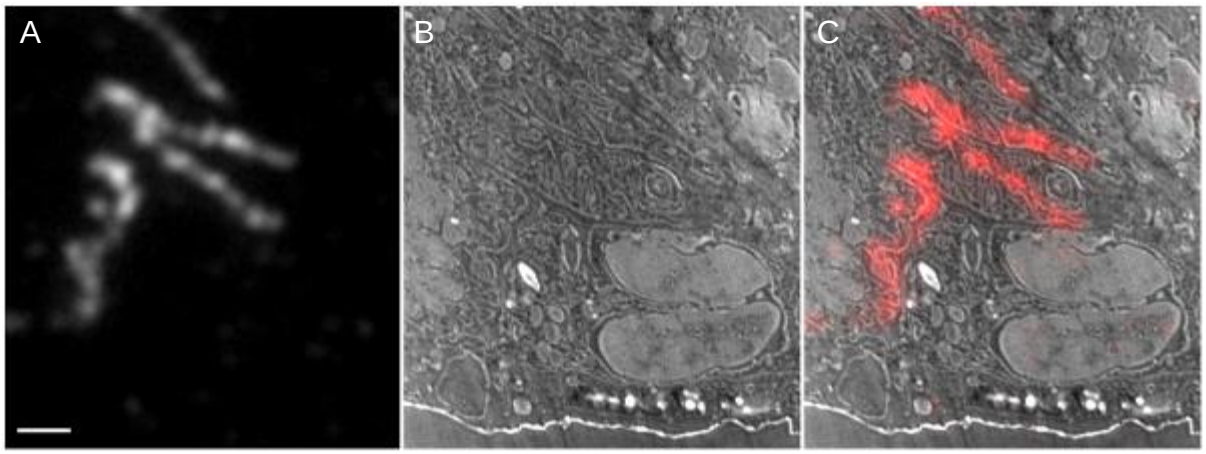

Figure 2: Correlation of high resolution light and scanning electron micrograph of a 70 nm HM20 section on an ITO-coated cover slip. AChR are labelled with BGT-AF555. A: Fluorescence light micrograph recorded with an oil immersion objective lens (100x/1.4 Plan Apochromat, Carl Zeiss); B: scanning electron micrograph recorded with a Supra 40 (Carl Zeiss); C: Overlay of fluorescence light and scanning electron micrograph. Scale bar: 1 µm. Please note also abstract at this conference: „*Comparison of localization algorithms for correlative 3D reconstruction of super-resolution fluorescence images of ultrathin serial sections*" by J. Fuchs et al.

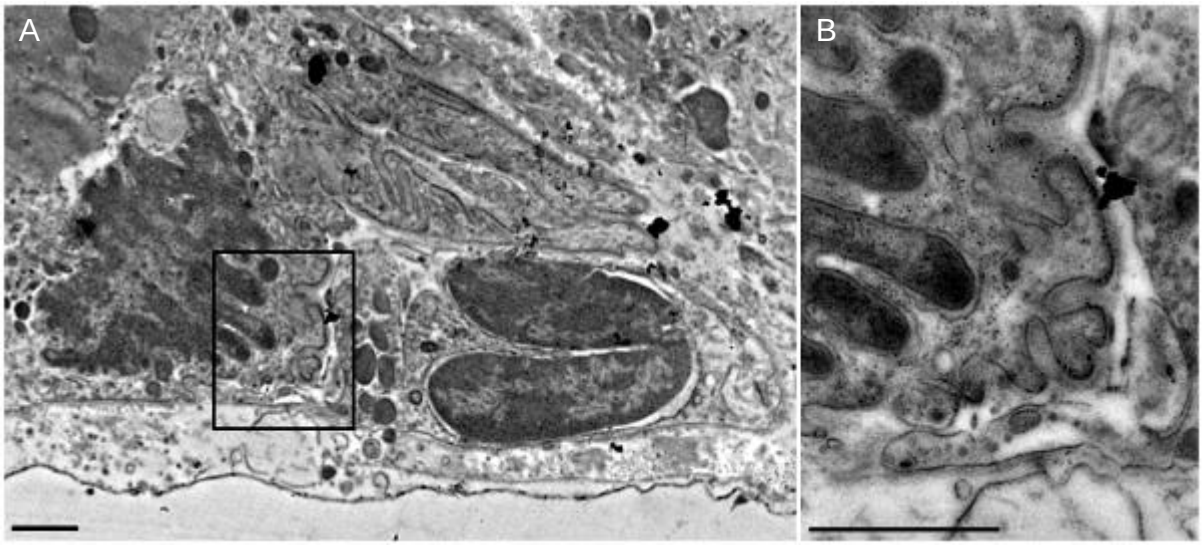

Figure 3: Transmission electron micrograph of a 70 nm HM20 section on a grid. AChR are labelled with anti-AChRα-antibody (BD Biosciences) and Protein A Gold (cmc). A: Overview; B: Magnification of the boxed region in A. Scale bars: 1 µm.