Correlative Microscopy in Life and Materials Science

MIM.4.P058
Correlative light and electron microscopy of ultrathin sections

S. Kretschmar[1], D. Eberle[2], M. Ader[2], H. Gandhi[3], C. Bökel[2], T. Weidemann[3], T. Kurth[1]

[1]TU Dresden, CRTD, EM-Facility, Dresden, Germany
[2]TU Dresden, CRTD, Dresden, Germany
[3]TU Dresden, BIOTEC, Dresden, Germany

thomas.kurth@crt-dresden.de

Correlative microscopy combines the versatility of the light microscope with the high spatial resolution of the electron microscope. However, many CLEM approaches, in particular those combining *in vivo* fluorescence and TEM-imaging, are used preferentially in cell culture systems [1,2]. For the correlative analysis of tissues an alternative approach is the on-section labeling of resin or cryo-sections using fluorochrome-coupled antibodies and gold probes [3-7].

Here, we describe fast and simple protocols for correlative immunofluorescence and immunogold labeling on the very same section. The protocols are demonstrated on sections of tissue samples embedded in the methacrylate Lowicryl K4M (Figure 1) and on Tokuyasu cryo-sections through transfected cells expressing a GFP-labeled protein. Ultrathin 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). Labeled structures selected at the fluorescence microscope can be identified in the TEM and analyzed at high resolution. This way, fluorescent signals can be directly correlated to the corresponding subcellular structures and a corresponding immunogold signal in the area of interest. Alternatively, the samples are processed completely, and the dried grids are analyzed in the FLM and the TEM. The latter approach is mandatory for the correlative imaging of sections in integrated systems such as the Tecnai™ with iCorr™, and to achieve a reasonable balance between fluorescence and EM contrast in these samples is a challenging task [8].

1. RS Polishchuk, EV Polishchuk, P Marra, S Alberti, R Buccione, A Luini, and A Mironov, J. Cell Biol. 148 (2000), p. 45-58.
2. P Verkade, J. Microsc. 230 (2008), 317-328.
3. H Schwarz and BM Humbel, Methods Mol. Biol. **369** (2007), p. 229-256.
4. T Takizawa and JM Robinson, Methods Mol. Med. 121 (2006), p. 351-369.
5. G Vicidomini, MC Gagliani, M Canfora, K Cortese, F Frosi, C Santangelo, PP Di Fiore, P Boccacci, A Diaspro, and C Tacchetti, Traffic 9 (2008), p. 1828-1838.
6. D Eberle, T Kurth, T Santos-Ferreira, J Wilson, D Corbeil, and M Ader, PLOS One 7, e46305.
7. G Fabig, S Kretschmar, S Weiche, D Eberle, M Ader, and T Kurth, Methods Cell Biol. 111 (2012), p. 75 93.
8. MA Karreman, EG Van Donselaar, AV Agronskaia, CT Verrips, and HC Gerritsen, J Histochem Cytochem 61 (2013), p. 236-247.
9. The authors gratefully acknowledge funding from the European Fund for Regional Development (EFRE).

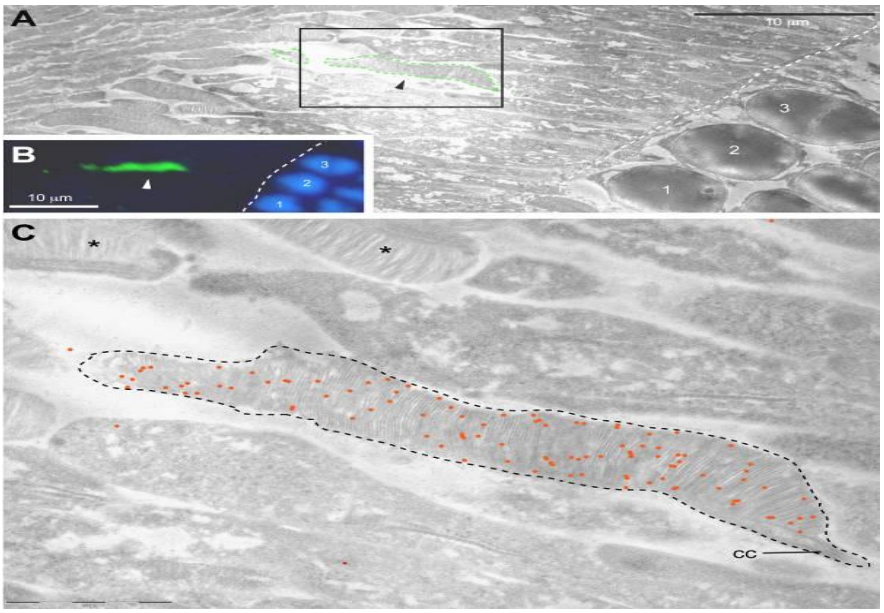

Figure 1: CLEM of a K4M section through mouse retina after transplantation of GFP-labeled photoreceptor precursor cells (PPCs with Rhodopsin-GFP) into a wt-mouse. **A**,**B** An integrated PPC (A, fluorescence: rabbit anti-GFP, goat anti-rabbit Alexa488, B, TEM: rabbit anti-GFP, protein A 10 nm Gold) **C** Higher magnification of the area highlighted in A. Gold-labeling in the outer segment of the PPC is highlighted in red. The unlabeled reference space with inner segments and unlabeled outer segments of the host (*) is visible; cc, connecting cilium of the transplanted cell (A and B from [6]).