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ID-1-P-2967 CLEM: the use of thawed cryosections and multiple labeling strategies

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Correlative light and electron mictoscopy is gaining attention in the last years. Besides prompt development of new hardware, preparation of the sample for reliable transfer and observation in the electron microscope requires even higher focus. We present here a detailed protocols for labelling of ultrathin cryosections according to Tokuyasu as well as resin sections using either FluoroNanogold-conjugated antibody fragments followed by silver enhancement; antibodies directed to fluorescence markers and conjugated to gold particles; and finally palladium nanoconjugates (1). Fluorescence microscopy and scanning electron microscopy were performed on the same sample. We used carbon-based imprints of finder grids for accurate and reliable localization of objects of interest within a specimen for imaging using both types of microscopes (2). After fluorescence, cryosections were postfixed with osmium tetroxide, dehydrated, dried (critical point drying using carbon dioxide, alternative t-butanol drying) and finally carbon coated. Different staining protocols have been used to improve contrast of membranes. The quality and resolution of both the secondary electron and the back-scattered electron imaging have been influenced by either carbon or gold conductive coating of glass slides. We showed that metal nanoparticles as well as silver precipitates were optimally visible from an accelerating voltage 4 kV using either the back-scattered electrons or the secondary electron signal. Different atomic numbers of metal nanoparticles and back-scattered electron imaging allow multiple labelling in SEM studies.

References:

1. Vancová, M et al. (2011). Microsc Microanal. 17, 810-816.
2. Brown, E. et al. (2009). Seminars in Developmental Biology 20, 910-919.

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