Post Staining Fixation to Preserve Fluorescent-labelled Immunodetection of DNA Damage after Embedding of Irradiated Cells into Resin for CLEM

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Introduction: Correlative light and electron microscopy (CLEM) combines the advances of light microscopy (LM) and electron microscopy (EM). With LM it is possible to gain information about dynamic processes and the sample preparation is relatively fast and simple. In addition it allows for fast screening for regions of interest, if a suitable fluorescent marker is available. In contrast, EM has a far better resolution and therefore it is possible to obtain information about the substructures of features not resolvable in standard confocal microscopy. Unfortunately, commonly used immunostaining of fixed samples (e.g. of DNA repair proteins after irradiation) is at least in part lost during the embedding into resins like LR White for ultramicrotomy. Preserving the immuno-fluorescent marker by a post-staining fixation protocol facilitates locating features of interest (like radiation induced foci) in thin sections using fluorescence microscopy, which can be revisited in the EM by means of a pattern visible in both microscopes.

Methods: NIH 3T3 cells were cultured as monolayer on 13,7mm cover slips. Cells were irradiated (1Gy, x-ray) and fixed 15 min post-irradiation. For immunocytochemical staining γH2AX antibody (AB) was used. As fluorescent marker of the secondary AB mostly Alexa488 was used. After immunostaining the Antibodies were fixed by formaldehyde. The DNA was counterstained with DAPI. After a first imaging of whole cell nuclei, the cells were dehydrated and embedded in LR White. The samples were imaged in the fluorescence microscope again both after embedding as well as slicing to obtain information about the preservation of the signal.

Results and Conclusion: The post fixation using formaldehyde causes a cross linking between the AB preventing the dissolution during the incubation with LR White. The fluorescence signal largely remains in the embedded samples compared to non-post-fixed samples. Using this protocol, it will be possible to image the immunostaining of DNA damage signals under the same conditions in LM (for screening for regions of interest) as in EM (for an improved analysis of substructure).

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