Integrated Laser and Electron Microscopy: Applications and Sample Preparation

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With Transmission Electron Microscopy, biological samples can be investigated with a higher resolution (0.5-1 nm) compared to light microscopy (±200 nm). On the other hand, with a fluorescence microscope (FM) a few labeled cells can easily be traced within a large area. Moreover, immunofluorescence labeling is much more efficient compared to immunogold labeling, used in the TEM. To combine the advantages of both techniques, they are often used together: correlative microscopy. Various approaches towards correlative microscopy exist and are commonly used in biological research.

To improve the success rate of correlative microscopy and speed up the acquisition procedure we developed an Integrated Laser and Electron Microscope (ILEM) [1]. Here, a specially designed laser scanning microscope is mounted on a side port of a conventional TEM. Imaging in FM and TEM mode is done sequentially, using the original sample stage and specimen holder of the TEM. To image samples with the ILEM, resin or Tokuyasu sections of cells are mounted on an electron microscopy grid and subsequently fluorescently labeled for the antigen of interest. The grids are then imaged and mapped for the location of positively labeled cells using the laser scanning microscope followed by high resolution imaging with the TEM. One of the main advantages of ILEM compared to the other approaches for correlative microscopy is that searching the cell of interest in the fluorescence mode and analysis by TEM can be done in the same microscope. The locations found by FM can be stored and easily recovered in the TEM mode.

Using this method, we managed to identify a novel structure in the nucleus of UVC stressed cells. Treatment of cells with UVC radiation leads to the formation of DNA cross-links which, if not repaired, can lead to apoptosis. γ-H2AX and cleaved caspase-3 are proteins formed during UVC-induced DNA damage and apoptosis, respectively. Following the treatment of human umbilical vein endothelial cells (HUVECs) with UVC radiation in the majority of cells γ-H2AX is formed, whereas only in a subset caspase-3 is activated. Using ILEM, in severely damaged cells with high levels of γ-H2AX a round, electron dense nuclear structure is found, which was hitherto not identified in UV-stressed cells. This structure is only found in the nuclei of cells containing cleaved caspase-3 and is present during all stages of the apoptotic process (Figure 1) [2].

The ILEM demands samples suitable for both immunofluorescence microscopy and transmission electron microscopy. Biological specimens are typically composed of atoms with low mass, which yield only low contrast in the TEM. To visualize the ultrastructure of biological samples, sections can therefore be stained with heavy metals, e.g., uranyl salts. However, uranyl ions, like all heavy metals, quench the fluorescence signal of the fluorophore. This has to be taken into account when contrasting the samples for ILEM, since

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these need to have both a fluorescent signal and sufficient contrast for TEM. At present, the technical development of the ILEM is in its final stages. However, as described above, the next challenge lays in the sample preparation techniques.

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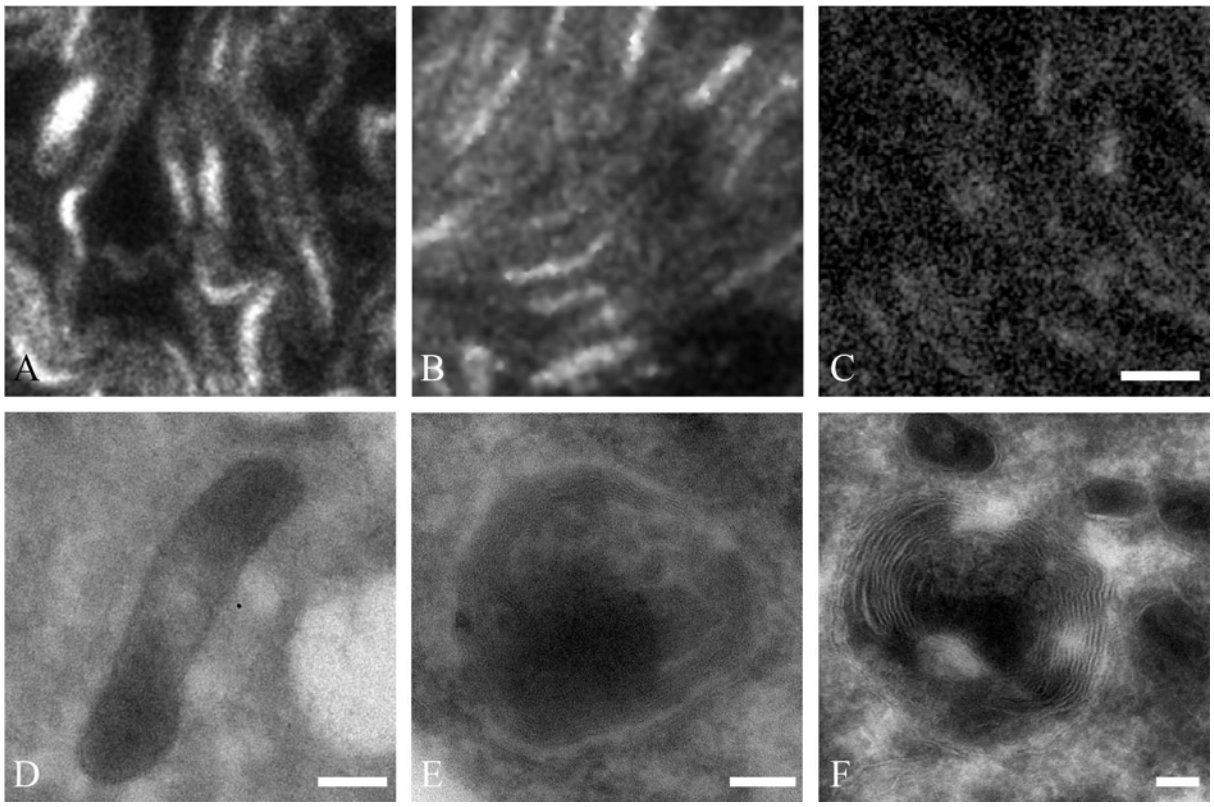

Figure 1. The effect of different post-staining methods on the fluorescent signal of γ-H2AX labeling and the contrast in TEM. Cryo-sections of UVC stressed HUVECs were labelled for γ-H2AX and detected with Alexa 488 (A-C), contrasted with uranyl oxalate (A,D) only, or uranyl oxalate and then uranyl acetate (B,E) or contrasted with uranyl oxalate and then embedded in uranyl acetate/methyl cellulose (C,F). The fluorescence images were scaled for optimal contrast and signal. Note the absence of specific signal when the sections were embedded in uranyl acetate/methyl cellulose. No membranes could be seen around the mitochondrion after post-staining with uranyl oxalate only (D). In contrast, membranes in lysosomes post-stained with uranyl oxalate and uranyl acetate (E) or post-stained with uranyl oxalate embedded with uranyl acetate in methyl cellulose (F) were clearly visible. Scale bars: 10 μm (A-C), 200 nm (D), 100 nm (E, F) [2].

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