

INTEGRATED LIGHT AND ELECTRON MICROSCOPY (ILEM): BRIDGING THE GAP BETWEEN LIGHT AND ELECTRON MICROSCOPY.

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Modern microscopy in life sciences covers a large field of applications ranging from the nano-scale to the meso-scale. Correlating the knowledge at the macro-molecular level with the overall cellular architecture leads to better understanding of the functioning of cells. Biological processes are highly dynamic and take place in a cellular context. Many events only occur in sub population of cells or scattered over a tissue. Fluorescence Microscopy methods allow to study these dynamic processes and localize fluorescently tagged proteins in cells and tissues. However, these techniques lack the fine structural information which can be provided by Electron Microscopy. Bridging the gap between LM and EM would help to pinpoint to ultra structural changes which take place at a certain time point. Correlative light electron microscopy can identify the structure of interest by fluorescence light microscopy and then allow zooming in on that same structure by electron microscopy.

In the ILEM, a laser scanning fluorescence microscope is integrated in a standard transmission electron microscope (TEM) [1]. The scanning laser fluorescence microscope module is mounted on one of the side ports of the TEM objective lens. Imaging in FM and TEM mode is done sequentially, using the original sample stage and specimen holder of the TEM. Characteristics of the ILEM are: the optical resolution is $0.55 \pm 0.03 \mu\text{m}$; the aberration free field of view is at least $300 \times 300 \mu\text{m}^2$; correlation of the positions in the fluorescence images and the TEM images is reproducible within $\pm 0.5 \mu\text{m}$ for both X and Y directions.

The potential of the integrated approach is demonstrated on studies of UV stressed human umbilical vein endothelial cells (HUVEC). UVC-irradiated cells have been labeled with Alexa488 fluorophore either against the phosphorylated histone γ -H2AX (DNA damage marker) or against cleaved caspase 3 (apoptosis marker). The fluorescence mode of ILEM shows that all HUVEC cells have been damaged with UV, but only a small fraction of cells start to go to apoptosis. TEM investigation of these same cells, with specific cleaved caspase 3 signal, showed the presence of a round structure in the nucleus. To our knowledge this structure is new and is, probably, an early morphological marker for cells determined for apoptosis [2].

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