

INTEGRATED FLUORESCENCE AND TRANSMISSION ELECTRON MICROSCOPY

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Fluorescence microscopy (FM) is one of the most commonly used imaging techniques in biological and biomedical studies. It is well suited for fast searching for regions of interest in large fields of view. The spatial resolution of standard fluorescence microscopes is limited to $\sim 0.3 \mu\text{m}$. Electron microscopy (EM), on the other hand, has the power to visualize cellular ultra structure at high resolution ($\sim 2 \text{ nm}$). However, in order to detect standard EM label (10-15 nm gold particles) high magnifications (15.000 - 20.000 x) should be used. Such magnifications result in small fields of view, not even sufficient to image a complete cell.

Results of FM and EM on the same specimen can be combined, this combination is known as correlative microscopy. At present correlative microscopy is carried out in two separate set-ups. Such an approach is slow and prone to errors. To improve the success rate of correlative microscopy and speed up the acquisition procedure, we have developed an integrated fluorescence and electron microscope (ILEM, Integrated Laser Electron Microscope). The ILEM consists of a laser scanning fluorescence microscope that is mounted on a side port of a standard transmission electron microscope (TEM). Imaging in FM and TEM mode is done sequentially, using the original sample stage and specimen holder of the TEM. Inter-modal coordinate retrieval is fully automated using the custom software.

The ILEM is at present equipped with a 473 nm solid-state laser and a single photon counting detection system. Its optical resolution is $0.55 \pm 0.03 \mu\text{m}$; the maximum (aberration free) field of view is $>300 \times 300 \mu\text{m}^2$ and positions in the fluorescence and TEM images can be correlated with an accuracy of $\pm 0.5 \mu\text{m}$.

The potential of the integrated approach is demonstrated in a study on UV-C stressed human umbilical vein endothelial cells (HUVEC). UV-C irradiated cells have been labelled with a fluorescent (Alexa488) secondary antibody either against the phosphorylated histone γ -H2AX (DNA damage marker) or against cleaved caspase 3 (apoptosis marker). Fluorescence images show that all HUVEC have been damaged by UV-C, but only a small fraction of the cells go into apoptosis. Moreover, a round unexpected structure is found in the nucleus of several cells. The appearance of this structure is accompanied by a cleaved caspase 3 signal. Moreover, the structure turned out to mainly contain RNA. To the best of our knowledge this structure has never been observed in UV-C stressed cells.