Correlative Light and Electron Microscopy

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Correlative microscopy has become *en vogue* the last few years. It is interesting to see that there are many facets of correlative microscopy; most of them correlate light microscopy with electron microscopy. The most immediate approach are those, where cellular processes are observed by light microscopy and then arrested by either chemical or better cryo-fixation [1, 2].

Most of the applications, however, use resin [3, 4] or Tokuyasu cryo-sections [5]. Here, thin or ultra-thin sections are mounted on microscope slides and on electron microscopy grids. The section on the glass slide is labelled with a fluorescent antibody the other with a gold marker. The fluorescence image serves as a map for orientation in the electron microscope. Micheva and Smith described a very neat approach for multiple labelling [6]. They mounted serial sections on a glass slide and labelled the sections with an antibody. After imaging the fluorescence pattern, the antibodies were stripped and a next round of labelling took place. This cycle could be repeated up to 10 times in total. After the different fluorescence pattern had been recorded the ultrastructure was imaged with back-scattered electrons in a scanning electron microscope.

A more advanced method has been developed by Anne Sartori [7]. GFP labelled cells were cryo-sectioned, CEMOVIS, collected on electron microscopy grids and mounted in a TEM cryo-holder. The sections were first analysed in a cryo-light microscope and the coordinates stored in a computer. In the cryo-electron microscope the labelled structures could be traced by the coordinates and cryo-tomograms of the area of interest were recorded. Last but not least there is also an integrated approach: Agronskaia et al. at Utrecht University [8] build a laser light microscope that can be attached to an electron microscope. The fluorescently labelled section is first imaged by the laser light microscope and then by the electron microscope [9]. All these approaches are driven by the need to study cells in their natural context, in the tissue or even organ. At low resolution with a large field of view the cells must be identified to be studied at high resolution.

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