SIMULTANEOUS CORRELATIVE LIGHT
AND ELECTRON MICROSCOPY

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We have built a platform that allows for complete integration of optical and scanning electron microscopy in a single apparatus. Imaging with both techniques can thus be performed simultaneously and on the same location in a sample. The platform is designed as a retrofit for existing microscopes: an interchangeable door of a Scanning Electron Microscope (SEM) is equipped with an objective lens and a sample holder, while optics for light excitation and detection can be mounted onto the door outside SEM vacuum chamber. Thus, an existing SEM can be easily equipped with a light microscope (LM), and the overlay between both modalities is acquired in a simple, straightforward manner (see, e.g. Figure 1).

In correlative microscopy, optical microscopy is typically used to rapidly locate areas of interest and to visualize and identify cellular proteins through fluorescent labelling. The electron microscope then provides nanometer-scale spatial resolution of the cellular structure. We show several examples where information from optical microscopy is directly overlaid onto structural details measured with the SEM. In particular, we focus on the spatial organization of fluorescent labelled proteins in relation to the occurrence of membrane protrusions. We discuss the overlay accuracy and the alignment between optical and electron axis and sketch the prospects for a system where correlative microscopy can be performed in a fast and reliable way.

Figure 1 (a) Optical transmission image of gold chloride stained human cheek cells recorded in-situ in the SEM. (b) SEM image of the boxed area in (a)