DEVELOPMENT OF CORRELATIVE BIOMOLECULAR IMAGING METHODS

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Background: Correlative microscopy, by using combined light-, probe-, laser- and electron microscope techniques have become increasingly important for the analysis of the structure and function of cells and tissues. New concepts and progress in structural and molecular cell biology have been discovered thanks to improving correlative microscopy techniques that continues to rely predominantly on advances in new, three-dimensional (*X*, *Y* and *Z*) visualization techniques. It is clear from the literature that the development of correlative imaging methods has not come to an end yet and that soon the time dimension (*X*, *Y*, *Z* and *t*) will be added by bridging the time resolution gap by using rapid transfer systems. It is our aim to develop straightforward approaches to combine fluorescent and electron microscopic information on the same cell.

Outcomes:
- Devolping detection- and imaging methods in which a fluorescence signal of a small molecule is reconciled with a signal from the electron microscope; and,
- Advancing our understanding of the development of novel sample preparation protocols for correlative biomolecular imaging at the cellular and molecular level.

Progress to date:
- Availability of a method to cross-correlate information at the fluorescence *versus* scanning electron microscopic level

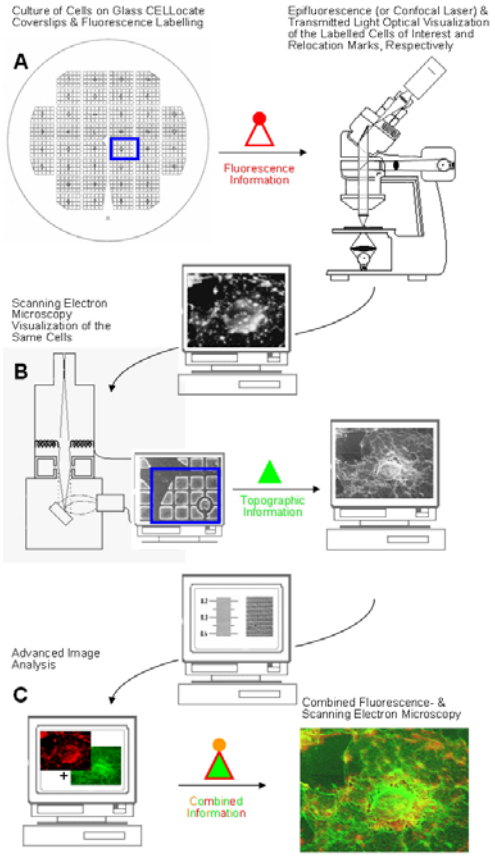

*Diagram summerizing the main steps in the combined fluorescence- and scanning electron microscopy method for retrieving simultaneous cytoplasmic and topographic information, respectively. (**A**) Cells are cultured on CELLocate-microgrid® glass cover slips and subsequently prepared for fluorescent labelling. Subsequently, photographs were taken with a confocal laser scanning microscope and cells of interest were located by using the alphanumerically marked grids on the cover slip in the light optical mode (for example, the blue rectangle denoting the Q-mark). (**B**) The cover slip is recovered and transferred to glutaraldehyde-fixative and further processed with tannic acid, osmium tetroxide, dehydrated in ethanol series and hexamethyldisilazane-dried The coverslip is then mounted on a stub and sputter coated gold. Next, the sample is examined with a scanning electron microscope and the previously visualized cell of interest in the fluorescence microscope is relocated by the aid of the alphanumerically marks on top of the cover slip (blue rectangle, Q-mark). Images of the corresponding cell are taken.* (**C**) *Digitized images of the same area of interest from both microscopies were transferred in ImageJ and processed at an identical end magnification by using light optical- and electron microscopy cross grating calibration standards.*

Things still to do:
- Developing a method to cross-correlate information at the fluorescence *versus* transmission electron microscopic level.