Correlative Light and Electron Microscopy Methods for SEM and Dual-Beam applications.

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Fluorescence Microscopy (FM) screening approaches allow one to capture multichannel fluorescence images on a large cell population, so that different cellular events can be monitored with high statistical accuracy. However, this technique lacks the ability to resolve fine subcellular details and therefore, when higher resolution is needed electron microscopy (EM) becomes the method of choice. The downside of the EM technique is that it lacks the ability to observe the dynamic processes in real time, as it is restricted to only taking a series of high-resolution images of key stages in biological processes. We have evidently reached a point where neither of these two microscopy techniques alone can offer all the information needed to fully understand how complex biological structures function. The objective of this work is to establish and develop a cell biology correlative FM and EM workflow (CLEM) that will provide us with information regarding cellular interactions and cellular ultrastructural details.

FEI’s specially designed environmental tissue culture compatible chambers provide an excellent mechanism for imaging living cells on the CorrSight™ fluorescent microscope. These chambers are supported on a microscope stage in which the cellular culture can be imaged at the optimum growth conditions (temperature and CO₂ levels) for extended periods of time. Conditions are absolutely critical to consider when designing a live-cell imaging experiment in which the environment components play a key role in cellular organization, physiology and drug response. The environmental chambers contain an imprinted grid system that facilitates the orientation of the sample during scanning electron microscopy imaging (SEM). The CorrSight™ can be utilized to acquire widefield images both in fluorescent and transmitted light modes to be used as a reference for the CLEM workflow. This system is equipped with a specifically designed microfluidics system that ensures a state of the art post imaging processing integrated setup in which the entire EM preparation takes place. The EM fixative, as well as post-fixation solutions, heavy metal stains and dehydration solutions are flushed under controlled conditions, such as volume and flow rate, through the samples. Importantly, and based on the design of the slides, the resulting plastic blocks contain the embedded cells as well as the surface imprint of the grid pattern, that is straightforwardly detected by secondary electrons (SE) SEM imaging. Stained cells can also be identified by backscattered electrons (BSE) using a dedicated solid state back scatter detector (DBS). The SE SEM image can then be correlated using the FEI MAPS™
software on the Helios 660 DualBeam™ and using the low-resolution widefield image of the Corrsight™ instrument the sample can be positioned to localize the region of interest and set up a serial sectioning experiment for 3D volume data acquisition using FEI Auto Slice and View™ software.

Cellular topology and labeling of extracellular markers can also be studied using the same tools. *In vitro* cultured cells grown on coverslips can be imaged using the Corrsight™ as mentioned above and then submitted to conventional SEM protocols, which include fixation, postfixation and dehydration procedures. Detailed surface information can be acquired using the Helios 660, equipped with an Elstar XHR (extreme high resolution) immersion lens FESEM (field emission SEM) column with a unique triple detector technology. In addition, a combination of SE and BSE detection in immersion mode unveils the cellular topology and chemical contrast needed to precisely locate gold particles or quantum dots used to label extracellular components.

**Figure 1:** Superimposed images of breast cancer cells, fluorescently labeled for two specific molecular components and with DAPI for nuclear counterstain, captured on the FEI CorrSight and Helios 660 DualBeam.

**Figure 2:** Superimposed BSE-SEM and FM images of breast cancer cells. The clean block face shows a BSE-SEM image of a cell revealing mitochondria (M), vesicular system (V), plasma membrane (P), macromolecules (glycogen storage, Gly), and cytoskeleton component (intermediate filaments, IF).