Sample Preparation Methods

IM.7.141
CLEM Workflow in an Electron Microscopy Facility

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Robust sample preparation is key to any multiuser, high-end Electron Microscopy Facility. At the EMBL our facility is permanently hosting 10 to 15 projects in parallel, and provides service to approximately 50 users per year. Each of these users face a number of different challenges when it comes to sample preparation and have different levels of experience. Our goal is to assist by standardizing methods and developing a toolbox to help projects become feasible and more efficient.

In recent years correlative light and electron microscopy (CLEM) has become a highly fashionable method using fluorescent markers to locate the region of interest (ROI) by light microscopy (LM) and combining it with the high-resolution data from achieved from the electron microscope. Our Facility is using a number of different CLEM methods and has played an active role in developing strategies to capture and study dynamic events at high-resolution.

Catching a rare event in a living cell or organism to later perform ultrastructural analysis at the exact moment of interest is not trivial. One method we are using is live-cell imaging followed by fixation. On cultured cells, the fixation can be performed with either chemical fixatives or by high-pressure freezing. The correlation is performed by recording the position of the cell of interest relative to a system of coordinates pre-existent onto the cultured surface [1], or by adding landmarks while performing the LM imaging [2]. For correlative studies on larger specimens such as starfish oocytes or *C.elegans* embryos, we have also implemented specific techniques where the sample is processed inside micro-capillary tubes [3].

Not only can the fluorescence be imaged on living specimens but also after fixation and sample preparation for EM, as demonstrated in 2011 by Kukulski et al. [4]. This is second method that has been developed at the EMBL and allows one to directly visualize fluorescence labels on a thick section. Imaging the section on the LM allows for a more rapid screening of the ROI in several cells. By using fluorescent fluorospheres as bi-functional fiducials, one can relocate the points of interest in the LM and then in the EM with high precision. This technique has become extremely popular with increasing demand and is an excellent example of transfer from scientific group to core facility.

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