High-Throughput Correlative Light and Cryo-Electron Microscopy Pipeline Using PRIMO Micropatterning, CERES Ice Shield and the METEOR In-Chamber Fluorescence Light Microscope

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High-Throughput Correlative Light and Cryo-Electron Microscopy Pipeline Using PRIMO Micropatterning, CERES Ice Shield and the METEOR In-Chamber Fluorescence Light Microscope

Sabrina Berkamp[1], Marit Smeets[2], Alexane Caignard[3], Riddhi Jani[3], Deniz Daviran[2], and Carsten Sachse[1,4,*]

[1]*Ernst-Ruska Centre for Microscopy and Spectroscopy with Electrons, ER-C-3/Structural Biology, Forschungszentrum Jülich, Jülich, Germany*
[2]*Delmic BV, Delft, the Netherlands*
[3]*Alvéole, Paris, France*
[4]*Department of Biology, Heinrich Heine University, Düsseldorf, Germany*
*Corresponding author: c.sachse@fz-juelich.de

Correlative light and cryo-electron microscopy (CLEM) can be used to study macromolecular structures in vitreous state of the complex environment of the cell. The associated workflow, however, is still cumbersome and low throughput. It includes many steps and is therefore prone to ice contamination, cracked lamellae or devitrification all of which can result in sample loss. We have implemented several technological improvements to the workflow resulting in a much simpler and higher throughput method, yielding more lamellae with reduced ice contamination.

During a traditional CLEM workflow mammalian cells are grown on electron microscopy grids, vitrified using a plunge freezer and transferred to a cryo-confocal microscope for localization of the region of interest. After cryo-transfer, samples are thinned to ~150 nm lamella in a cryo-FIB-SEM instrument to make them amenable to cryo-electron tomography. To confirm that lamellae contain the region of interest, the sample is often transferred back to a cryo-confocal microscope before being placed in a transmission electron microscope. Multiple cryo-transfer steps lead to samples being lost due to mishandling, devitrification, cracking and a build-up of ice contamination on the surface of the lamellae. Additionally, this pipeline can suffer from a lack of sites suitable for FIB-milling due to mammalian cells locating preferably to the grid bars instead of the preferred grid squares, cells flattening or clustering together, or cellular regions of interest not being located in area of the grid accessible to thinning. When taking all of these challenges together, this cumbersome combination often results in only 2-4 usable lamellae per grid when imaged in the transmission electron microscope, and some of these lamellae devoid of regions of interest.

We have updated this CLEM workflow with several technological improvements to enhance the number of usable lamellae obtained up to roughly 24; cell shape and positioning is improved via a micropatterning approach, ice contaminant build-up is reduced using an ice shield and the number of cryo-transfer steps is drastically cut down by use of a cryo-fluorescence microscope incorporated in the FIB-SEM chamber.

We found that the PRIMO micropatterning system using customized patterns can be used to direct cells to the center of the grid squares as well as to modify cell shape and thereby increasing FIB-milling access. Additionally, the ability to control cell shape can be used to direct certain cellular regions to the center of the grid square to improve the milling areas that contain the region of interest which makes some intracellular organelles/compartments more accessible. Overall, this greatly increases the number of milleable sites per grid containing the region of interest thus simplifies the milling target selection [1]. While thinning the cells in the FIB-SEM, contaminations slowly accumulate on the surface of the sample. The CERES ice shield was installed in an Aquilos2 to reduce this ice buildup contamination rate and allow longer, overnight, milling sessions and thereby increase the number of lamellae that are made in a single experiment [2]. To reduce the number of cryo-transfer steps and thereby greatly reduced surface ice contamination, the METEOR system was installed on the Aquilos2. With this in-chamber fluorescence microscope the sample can be imaged before, during and after thinning, all without transferring it out of the high vacuum cryo-chamber. This microscope allows for thinning only those cells that have the region of interest and confirm its continued presence during the milling process before further reducing lamella thickness. When the lamellae have reached the final thickness of 150-200nm, the location and presence of the regions of interest are confirmed before transferring the sample directly to a transmission electron microscope [3].

In conclusion; with the combination of the PRIMO micropatterning approach, the CERES ice shield and the METEOR in-chamber fluorescence microscopy we were able to enhance the number of accessible milling sites per grid from 2 – 4 to 24. We were able to increase the time the sample could spend in the FIB-SEM before surface contamination build-up to unacceptable levels, reduce the number of transfer steps in the pipeline and, most importantly, increase the number of lamellae while decreasing the surface ice contamination on each lamella. Together, these steps make the time spent on each instrument more efficient, resulting in many more tomograms per session and a more cost-effective workflow

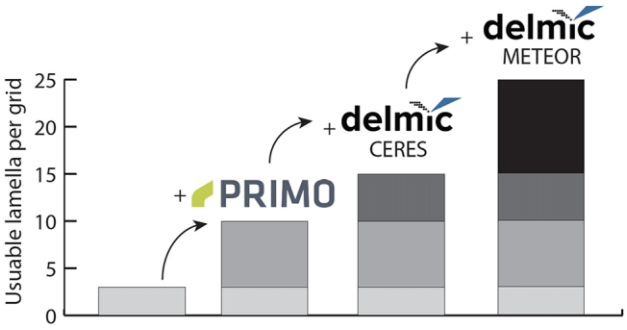

Fig. 1. Estimation of number of usable cryo-lamellae per grid, observed in the transmission electron microscope after the CLEM workflow, with and without technological improvements.

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