CRYO-FLUORESCENCE MAPPING FOR CORRELATIVE MICROSCOPY OF BIOLOGICAL SAMPLES

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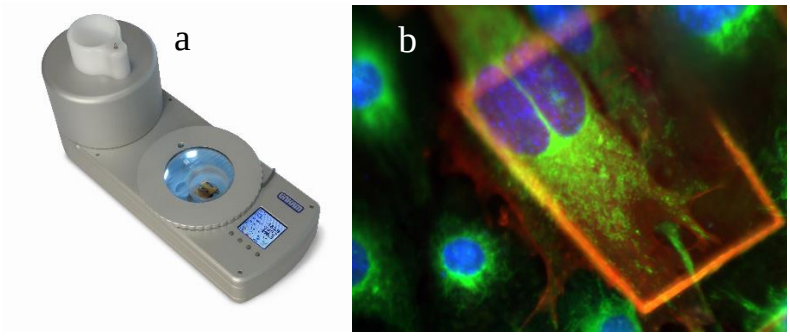
Correlative microscopy [1] fuses data from complementing imaging methods. Here fluorescence is typically used to pinpoint regions or processes of biological interest. Identified regions are subsequently studied with a high-resolution technique such as Electron Microscopy (EM), X-ray microscopy or a derived tomography technique.

In this talk we focus on the evolving method of cryo-fluorescence [2] of vitrified samples for the use in cryo-CLEM (Correlative Light and Electron Microscopy) and cryo-super-resolution. A vitrified biological sample remains in its near-native and fully hydrated state, providing fluorescence imaging with very low photo-bleaching and natural compatibility with vacuum and outstanding ultra-structural preservation. We will discuss workflow options as well as the main challenges of this method: keeping vitrified samples free of contamination, mapping coordinate systems from different imaging instruments and handling, transferring and mounting vitrified biological samples.



Cryo-fluorescence stage Linkam CMS196 (a) and cryo-widefield fluorescence of mouse embryonic fibroblast cells on quantifoil gold finder grids, imaged during the 2015 cryo-workshop at the Crick Institute, London.

Recent progress in the design of cryo-fluoescence stages enables the automated acquisition of a high-resolution large-area fluorescence maps of a whole EM grid, which is then used to navigate the sample and correlate with EM or an X-ray microscope. Cryo-fluorescence can be performed in widefield configuration, however, confocal cryo-imaging with increased resolution has also been demonstrated lately with the ZEISS LSM 880 Airyscan.

The same workflows for sample preparation and cryo-fluorescence are also very attractive for optical super-resolution, in particular the localisation microscopy techniques PALM or STORM. The vastly improved bleaching properties under cryo-conditions can contribute to improved SNR and resolution, further bridging the gap between optical and Electron or X-Ray techniques.

[1] Methods in Cell Biology, Volume 111, Pages 1-404 (2012), Correlative Light and Electron Microscopy, Ed.Thomas Müller-Reichert & Paul Verkade, ISBN: 978-0-12-416026-2, Academic Press
[2] Kaufmann R, Hagen C, Grunewald K. Fluorescence cryo-microscopy: current challenges and prospects. Current opinion in chemical biology. 2014;20:86-91. DOI: 10.1016/j.cbpa.2014.05.007