CRYO-FLUORESCENCE FOR CLEM WITH IMPROVED STABILITY AND PLATFORM INDEPENDENT TOOL FOR COORDINATE TRANSFORMATION

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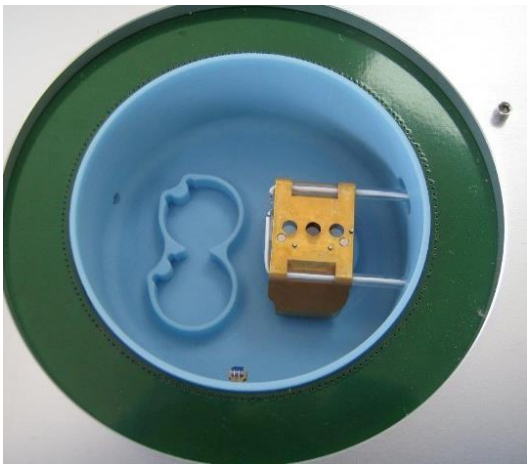
KEY WORDS: fluorescence imaging of vitrified biological samples, cryo-fluorescence (cryo-FM), cryo-EM, CLEM (Correlative Light and Electron Microscopy), imaging stability improvement due to non-bubbling LN2 environment, prevention of sample contamination

Imaging of biological samples embedded in vitrified ice has become of great interest in recent years as it provides several advantages: the biological sample is in a fully hydrated state with superior preservation down to ultra-structural level, a vitrified sample is naturally compatible with the vacuum required for EM / CLEM (Correlative Light and Electron Microscopy) [1,2] and fluorescence can pinpoint biological or genetic events. The recent nobel prize for cryo-EM [3], a requirement for cryo-CLEM, did further fuel interest in this technique.



The cryo-FM stages developed at Linkam over several years delivers contamination-free imaging for hours, which is a key feature of any cryo-FM stage. However, a further feature that is particularly relevant for high resolution imaging is the mechanical stability of the cryo-stage during imaging, which can be affected by the boiling of liquid nitrogen in the vicinity of the sample. In this talk we will discuss a novel design, naturally preventing the effects of boiling LN2 in the vicinity of the sample, therefore improving imaging stability of the system. We will also share details of the setup we have implemented to measure system stability in a cryo-FM setup in the XY plane down to nm resolution.

In a second part of the presentation we discuss the coordinate transform topic in CLEM, which is required to use the cryo-fluorescence image as a navigation map to locate regions of interest for subsequent imaging during cryo-EM. Due to request from researchers, Linkam has developed a coordinate transformation tool that can be used with existing cryo-EM and cryo-FM platforms to simplify the workflow.

[1] Celler et al., NATURE COMM. (2016), 7:11836 | DOI: 10.1038/ncomms11836
[2] A.Sartori et al., “Correlative microscopy: Bridging the gap between fluorescence light microscopy and cryo-electron tomography”, Journal of Struct. Biology 160 (2007) 135–145
[3] https://www.nature.com/news/cryo-electron-microscopy-wins-chemistry-nobel-1.22738