CRYO-FLUORESCENCE STAGE DESIGN WITH HIGH STABILITY FOR CRYO-SUPER RESOLUTION AND CRYO-CLEM

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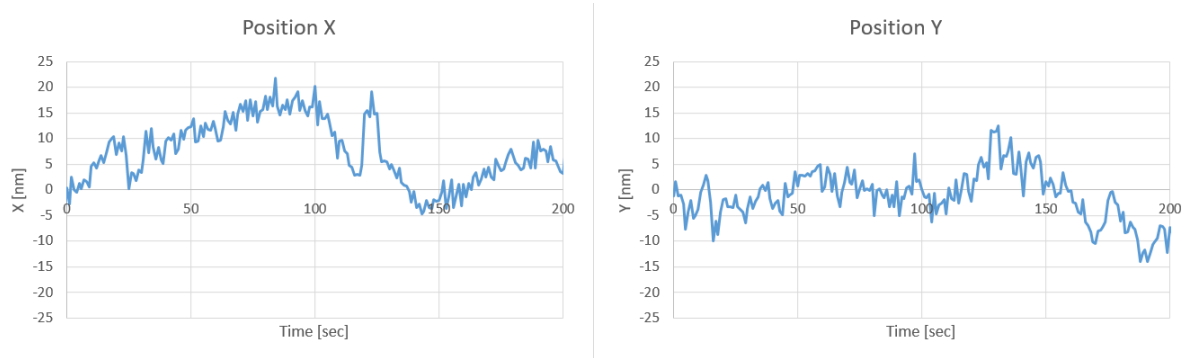
Cryo-imaging of biological samples embedded in vitrified ice is of great interest recently - driven by applications such as particle cryo-EM (a technique used to investigate the molecular structure of smaller bio-molecules [1]) and CLEM (Correlative Light and Electron Microscopy [2][3]). Cryo-fluorescence is used during such workflows to identify events or regions with well-established labelling techniques complementing EM. The biological cryo-sample remains in a fully hydrated state with superior preservation down to ultra-structural level.

The cryo-FM stages developed at Linkam deliver contamination-free imaging for hours because they use liquid nitrogen in the sample chamber acting as a cold trap. With the emergence of super resolution (SR) imaging methods the requirements for positional stability have increased, also because SR methods are attractive under cryo conditions due to reduced bleaching [4].

We have combined a number of new cryo-design features to achieve both low contamination and high stability at the same time. In this paper we present quantitative results for stability measurements obtained by tracking fluorescent beads. Typical values for position stability over times typical for image acquisition were better than 25 nm (peak to peak) in X and Y over 2 minutes.



[1] https://www.nature.com/news/cryo-electron-microscopy-wins-chemistry-nobel-1.22738
[2] Celler et al., *NATURE COMM.* (2016), 7:11836 | DOI: 10.1038/ncomms11836
[3] 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
[4] M. Nahmani et al.,*PNAS* 114 (15) 3832-3836 (2017) https://doi.org/10.1073/pnas.1618206114