ROBOTIC CRYO-VITRIFICATION DEVICE AND CRYO-FLUORESCENCE WORKFLOW FOR CRYO-EM AND SPA

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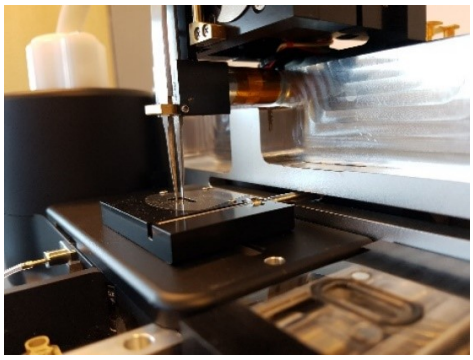
Cryo-imaging of biological samples embedded in vitrified ice has unique benefits and the development and implementation of cryo-EM and SPA has led to the Nobel Prize in 2017 [1]. One benefit is that the vitrification preserves proteins, cellular assemblies and cells in a fully hydrated and near native state. Vitrified samples are compatible with the vacuum conditions required for EM and ultra-structural preservation is considered the gold standard. More and more protein structures are solved with SPA, which complements or even partly replaces the established XRC and crucially, does not require the step of crystallization.

One challenge for cryo-ET and SPA is, however, the consistent and repeatable preparation of vitrified samples and mastering cryo-specific sample handling protocols. To make such workflows for SPA, thin cells or lamella cutting in FIB-SEM more widely accessible, Linkam and LUMC have together developed an automated robotic plunge freezer, which is now in beta-testing phase. Here the paper-blotting step, a typical feature of current cryo-plunger devices, has been replaced with a programmable controlled suction method which allows real-time optically monitoring by a built-in microscope and control of the liquid film thickness prior to plunging. Because the grid handling, glow discharge, sample application, plunging and storage are all automated in the robotic system (image below) the preparation of cryo-samples is simplified and more repeatable.



A benefit of cryo-preparation and imaging techniques in LM is the significantly reduced photo-bleaching in cryo-fluorescence: CLEM allows to map and target regions of interest without EM beam damage and by using complementing labelling techniques. Reduced bleaching and improved photon statistics aids the localization precision for super-resolution techniques.

In this poster we give an overview on the workflows and capabilities of the plunger system under development and show how the new plunger system interfaces with the cryo-fluorescence stage CMS196.

[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