Cryo-FIB parameter optimization for cryo lamella preparation

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Cryo-FIB lamella preparation is a versatile and exciting technique allowing the thinning of large non-electron transparent biological samples preserved in a frozen hydrated state. The ability to look into complex biological systems such as cells and observe the molecular process within, whilst preserving the biological context has the potential to revolutionise structural biology [1,2]. Proteins can be for the first time be observed in their native state completely without the need for complex pre-processing of bulk samples as in the case for SPA. Simply samples can be vitrified, milled and observed. This approach when combined, with sub-tomogram averaging offers both a high resolution and contextual verification using the same tools.

In order to correctly identify and selectively mill lamella which contain the region of interest several things need to be optimised, sample substrates and cell presentation, correlation markers, cell preprocessing, milling and imaging parameters. This talk will highlight these optimisation steps and how to get the most from your samples.

For instance, sample substrates typically used are often TEM grids, biocompatibility often requires the use of softer gold TEM grids (Figure 1). When preparing the grids the amount of material left after blotting often contributes greatly to the freezing rates but also the mechanical stability. The density and position of the cells on the supports plays an important role, with cells often preferring the grid bars to the TEM foils. If optical targeting is used, creating the best correlation between the light microscope and the SDB has its challenges.

Many steps can be used to improve the stability of cells during milling. Do you need a sputtered metal layer to render the sample conductive? What is the optimum protective layer required to protect your cells during milling [3]. What is the effect of milling currents, voltages on the lamella itself and what is the impact for TEM. What is electron milling he cost to the final TEM tomogram. This talk will review the current state of the art, giving examples and explaining the impact of selection of different parameters.

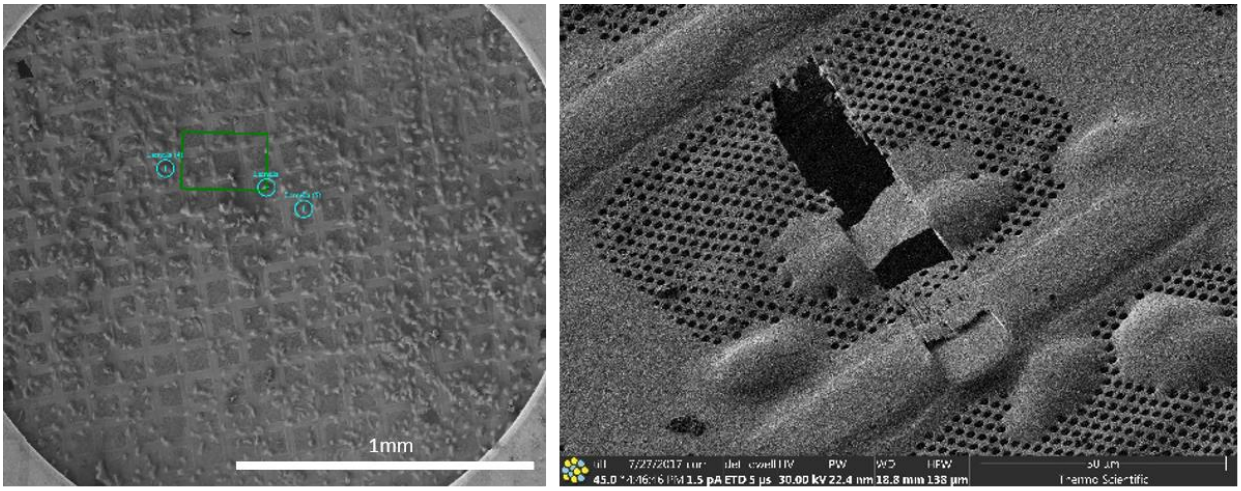

Figure 1. Left, A9 cells grown on a TEM grid, Right, FIB lamella made at one location

1. *In Situ* Structure of Neuronal *C9orf72* Poly-GA Aggregates Reveals Proteasome Recruitment

   Guo, Qiang et al. Cell , Volume 172 , Issue 4 , 696 - 705.e12
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   Mahamid, Julia et al. *Science* 26 Feb 2016 : 969-972
3. Optimized cryo-focused ion beam sample preparation aimed at in situ structural studies of membrane proteins.

   Schaffer et al. Journal of Structural Biology, Volume 197, Issue 2, 2017, Pages 73-82