ROUTINE CRYOEM SAMPLE OPTIMIZATION WITH CHAMELEON

S. DE CARLO [1], P. THAW [2]

[1]SPT Labtech ltd. - Melbourn (Switzerland), [2]SPT Labtech ltd. - Melbourn (UK)

In the cryogenic electron microscopy (cryoEM) workflow, improvements in microscope stability, direct detectors and image processing have enabled high throughput data collection strategies and shifted the remaining bottleneck to sample quality. The process of obtaining a film of vitreous ice of an appropriate thickness, with evenly distributed particles is not straightforward. To address the limits of achievable resolution due to the ubiquitous effects of the air water interface (AWI) [1] conventional sample preparation methods use iterative cycles of trial-and-error optimization experiments and screening routines to search untraCEAble data for favourable outcomes [2]. When it fails, the abundance of low-quality samples causes downstream inefficiencies in the workflow. Brute force data collection methods used to overcome poor sample quality bottleneck microscope and computing resources. This results in a failure to achieve early research milestones important for funding and resource allocations and while excessively large data sets may improve outcomes for a few samples, they do not address the root-cause of the issue – poor quality samples.

The chameleon system is a blot-free, pico-litre dispense instrument that enables high-speed plunging (< 1 sec) to robustly freeze and optimize samples for use in cryoEM [1]. The chameleon system was developed from Spotiton [2,3] and uses self-wicking copper nanowire grids to control the formation of the thin sample film [4]. This process occurs ‘on-the-fly’ as the grid passes in front of the dispenser on its way to the cryogen bowl, resulting in a viewable stripe of sample across the frozen grid. Automated procedures and intuitive workflows allow users to optimize for fast plunge times and ice thicknesses to address behaviour for the unique sample in hand.

By varying plunge times (as fast as 54ms) while controlling ice thickness on the fly, the chameleon system is commonly used to understand and then mitigate negative sample specific AWI effects in fewer traCEAble steps. Workflow examples and methods will be discussed alongside sample outcomes from chameleon users in the field.

Reproducible outcomes and walk-up usability contribute to enable a democratized sample preparation workflow accessible to non-specialists through chameleon. Researchers require the ability to freeze where and when the biochemistry is carried out, to be able to pursue cutting-edge targets consistently and confidently despite poorly behaved cryoEM samples. The requirement for change is placed squarely on the sample preparation workflow to modernize, improve quality, and reduce training burden