Arctis WebUI redefines lamella preparation for cryo-electron tomography workflow

Veronika Vrbovská[1], Radovan Spurný[1], Radim Kříž[1], Radek Tichý[1], Pavel Tomášek[1], Ladislav Chvíla[1], Michaela Müllerová[1], Anna Kasáková[1], John Mitchels[1], Miloš Hovorka[1]
[1]Thermo Fisher Scientific, Brno, Czech Republic

Background
Cryo-electron tomography has emerged as a valuable technique for visualizing proteins and protein complexes in their natural cellular environment. To achieve high-resolution imaging, the preparation of thin cryo-lamellae containing the region of interest is a critical step. However, the usual methods for lamella preparation are often time-consuming and require the use of several applications, presenting challenges for inexperienced users in the cryo-tomo workflow.

Methods
The Arctis cryo-Plasma Focused Ion Beam system plays a pivotal role in the automated production of cryo-lamellae from cells. The system is supported by the Arctis WebUI, with a web-based user interface that streamlines the creation of cryo-lamellae with minimal user inputs. WebUI is a unified platform where users can map and navigate through the sample using electron, ion, or optical tilesets. Additionally, the integration of a fluorescent light microscope enables in-situ targeting of specific cellular structures. Prepared lamellae are transferred to a Transmission Electron Microscope for cryo-electron tomography. The Thermo Fisher Scientific Selectris Imaging Filter is utilized to significantly enhance contrast during data acquisition through zero-loss filtering. Data collection is facilitated by Tomography 5 software, enabling unattended data acquisition, seamlessly connected with Tomo Live software, allowing automatic on-the-fly data reconstruction.

Results
We will provide a comprehensive overview of the application workflows for lamella preparation facilitated by the Arctis WebUI. The key advancement is the use of the fully automated optical tileset, which allows the selection of cells directly based on their fluorescently labelled features that are not visible through standard electron or ion imaging. With the combination of the 3D targeting solution, users gain valuable insights into the cellular content and can precisely identify target areas for lamella preparation. The automatic determination of ROI depth in the optical stack significantly accelerates and simplifies the 3D targeting process, eliminating the requirement for manual browsing through the entire optical stack. Once the lamellae are milled, they are transferred to a cryo-Transmission Electron Microscope through the common sample loading interface and utilized for cryo-electron tomography data collection.

Conclusion
With the capability to seamlessly process multiple (up to 12) grids with cells, Arctis WebUI streamlines the preparation of cryo-lamellae for tomography, enhancing efficiency and productivity in the field of cryo-electron microscopy. The integration of optical tileset and 3D targeting enables more precise lamella placement, guarantees data collection from specific sites in cryo-TEM, and ensures the presence of accurate structures in the resulting tomography data. These workflows significantly reduce the need for extensive user input, allowing researchers to concentrate on solving biological problems while the WebUI automates the sample preparation process for cryo-tomography.

Keywords:

Arctis WebUI, cryo-electron tomography, cryo-lamellae