1330
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Thermo Scientific™ Tundra Cryo-TEM: 100kV Cryo-TEM dedicated for Single Particle Analysis

Zuzana Hlavenková[1], Dimple Karia[2], Miloš Malínský[3], Daniel Němeček[4], Fanis Grollios[5], Vojtěch Doležal[1], Ondřej Sháněl[1], Abhay Kotecha[6], Markéta Červinková[7], Lingbo Yu[1] and Anke Mulder[8]

[1]Thermo Fisher Scientific, United States, [2]Thermo Fisher Scientific, Eindhoven, Noord-Brabant, Netherlands, [3]Thermo Fisher Scientific, Brno, Jihomoravsky kraj, Czech Republic, [4]Thermo Fisher Scientific, Czech Republic, [5]Thermo Fisher Scientific, Eindhoven, Netherlands, [6]Thermo Fisher Scientific, Noord-Brabant, Netherlands, [7]Thermo Fisher Scientific, Brno-Cernovice, Czech Republic, [8]Thermo Fisher Scientific, Oregon, United States

Single Particle Analysis (SPA) application of cryo-electron microscopy (cryo-EM) has become a well-established method for determination of the 3D structure of wide variety of proteins and their complexes, revealing the mechanism of their function and showing their interactions with known and novel drugs[1]. However, as the popularity of this technique increases, so does the need for greater efficiency and accessibility from not only microscopy experts but also from scientists of different scientific backgrounds and with little to no cryo-EM experience.

For the most part, the operation of an electron microscope for high-resolution cryo-EM data collection still requires an experienced person. Training of a skilled EM operator can take months and is perceived as a significant bottleneck in broader adoption of cryo-EM.

The Thermo Scientific Tundra Cryo-TEM is a new transmission electron microscope operating at 100kV high tension dedicated to SPA[2] which has been specifically developed for new users from biochemistry and biology labs. The Tundra Cryo-TEM brings a new level of automation and user guidance for microscope operation and SPA data collection; moreover, the Tundra Cryo-TEM fits into a standard laboratory room, thereby reducing costs associated with room renovation.

Benchmark cryo-EM measurements using apo-ferritin demonstrates that Tundra Cryo-TEM can achieve 2.6Å resolution of a reconstructed 3D map (Figure 1). At this resolution, *de novo* protein structures can be determined, and important biological questions answered. Data was collected with pixel size of 0.75Å, for about 17 hours and processed using Relion 3.1[3]. The data was collected using Thermo Scientific™ EPU™ software with pre-defined settings. We used a new functionality in EPU that automatically checks and refines optical alignments and provides system status for high-quality data acquisition. Furthermore, data quality was monitored on-the-fly using EPU quality monitor.

Data was collected on a new scintillator-based camera CETA-F with speed enhancement dedicated for operation at 100kV high tension. It has 4 times better sensitivity with respect to CETA™ 16M. CETA-F also brings the possibility of dose fractionation mode as implemented on the Falcon camera. Dose fractionation allows storage of image frames for correction of beam induced motion. in a post processing pipeline.

Additionally, a new objective lens was developed for Tundra Cryo-EM to decrease the spherical and chromatic aberrations at 100-kV acceleration voltage and boost signal at high resolution frequencies.

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To load the sample into the microscope, we have introduced a novel semi-automated loading technology (SAL) on Tundra. Samples can be loaded into the microscope within minutes with minimum ice contamination on the grid (Figure 2). SAL allows a new, iterative way of working to optimize of sample concentration and vitrification conditions that can quickly qualify samples for high resolution data collection. SAL also has a fully guided workflow on the on-screen display (OSD), to guide users with different levels of expertise.

All these new features that are introduced within the Tundra Cryo-TEM allows novice as well as expert users to achieve relevant resolution of their biological samples while keeping an accessible price point. This would make cryo-TEM accessible to many scientists across all life science branches.

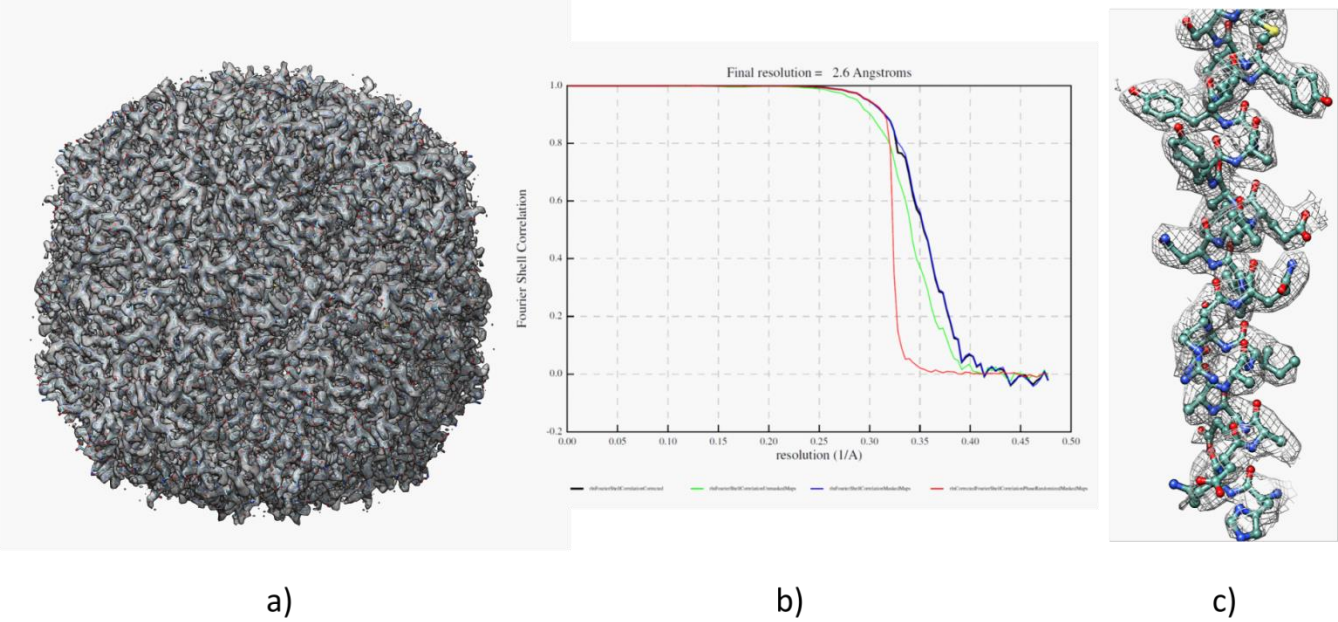

Figure 1. Fig.1 Structure of Apoferritin protein determined at 100 keV. a) 3D reconstruction of apoferritin at 2.6Å resolution, b) Gold-standard FSC plot corresponding to the calculated map, showing the correlation between the phase-randomized (red), unmasked (green) and masked (blue) half-maps, c) Electron density of the 2.6 Å resolution map showing the apoferritin α-helix

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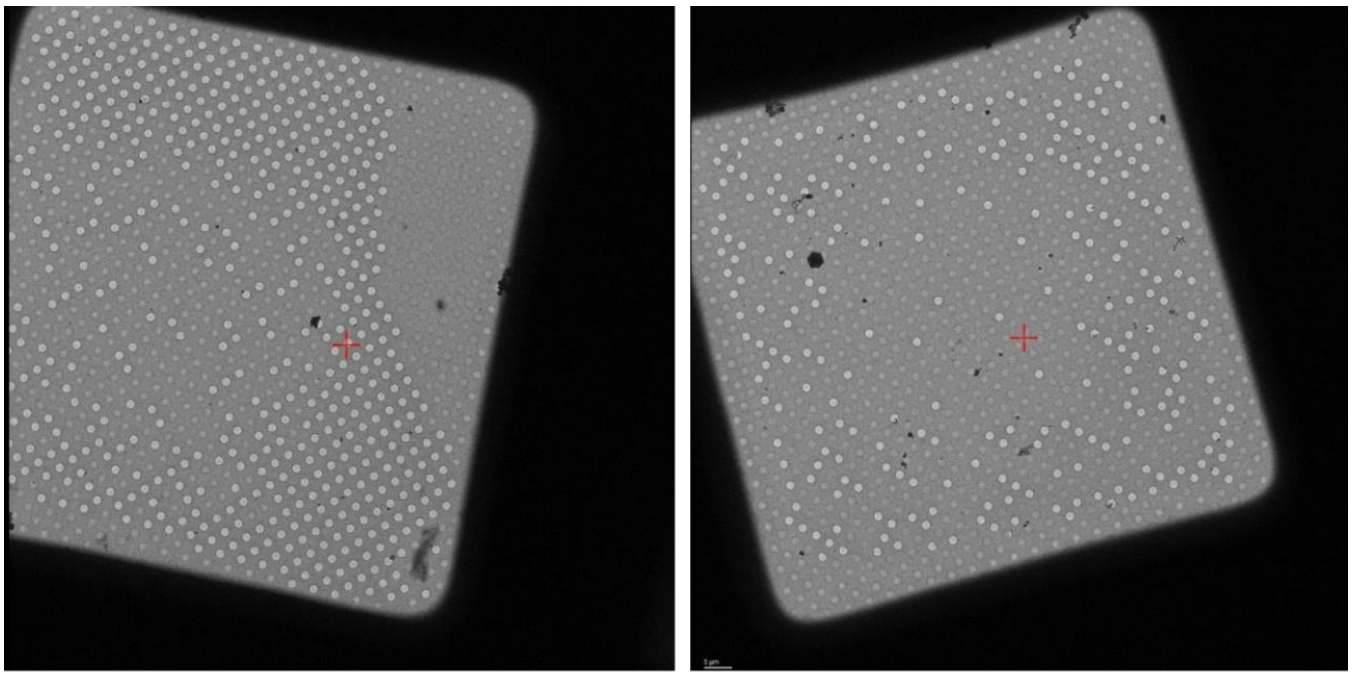

a) b)

Figure 2. Fig. 2 Grid Square images showing limited to no ice contamination upon transfer to Tundra using SAL technology.

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