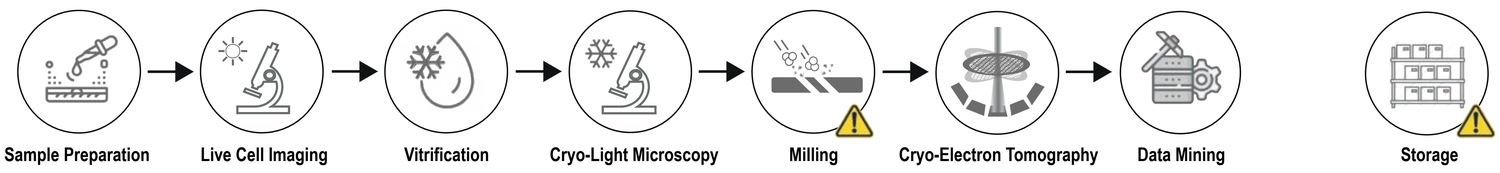
An improved cryo-FIB-ET workflow towards quantitative cryo-electron tomography

MAX PLANCK INSTITUTE OF MOLECULAR PHYSIOLOGY

Elisa Lisicki[1], Tatjana Taubitz[1], Mingjun Xu[1], Stefan Raunser[1], Sebastian Tacke[1]

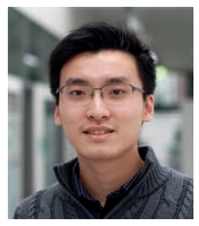
[1] Department of Structural Biochemistry, Max Planck Institute of Molecular Physiology, 44227 Dortmund, Germany

Cryo-PFIB-ET pipeline



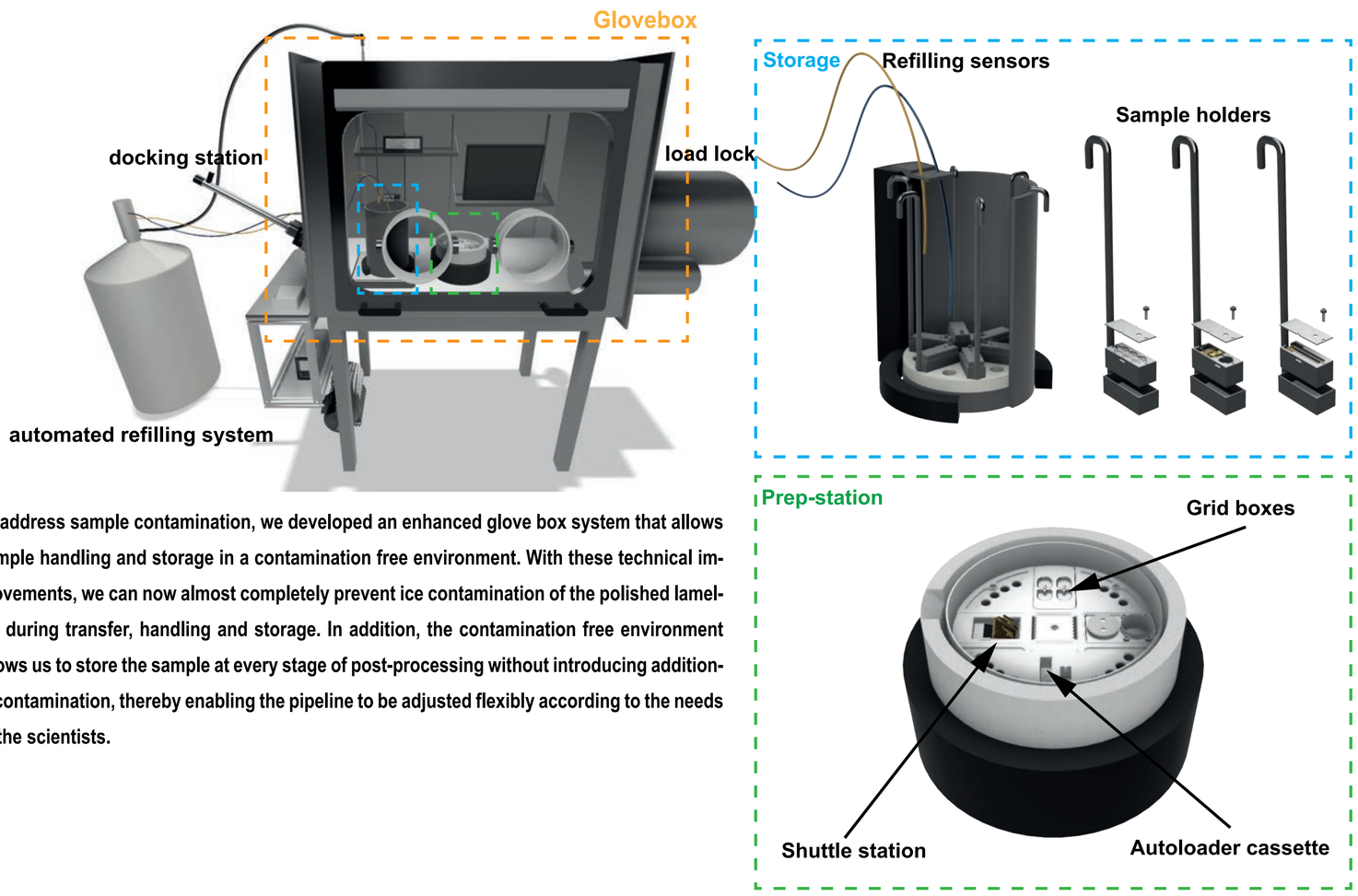

Tatjana Taubitz


Elisa Lisicki


Mingjun Xu

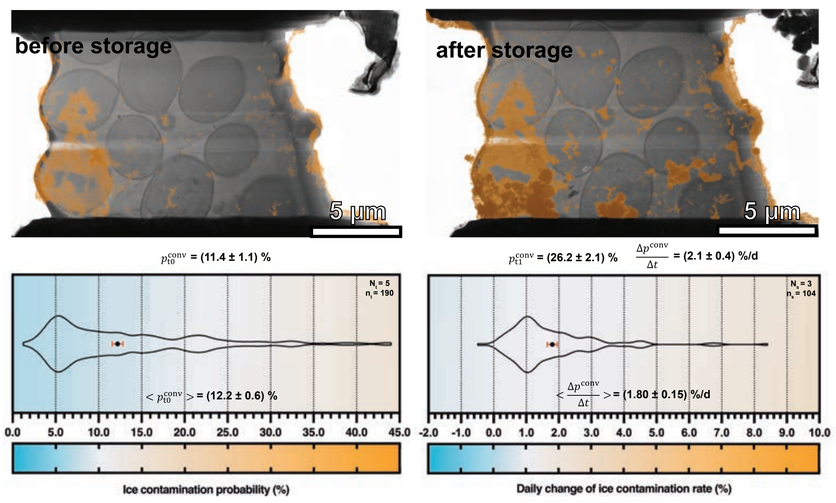
In recent years, cryo-electron tomography (cryo-ET) has become a versatile tool to qualitatively describe the ultrastructure of cellular systems [1, 2] and characterize previously elusive protein complexes in their native context [1]. The beauty of cryo-ET is the wealth of information which can be visualized within a single tomogram. In theory, cryo-ET data can be utilized to visualize and analyze the complex proteome, it can be used to study protein-protein and protein-organelle interaction, to calculate protein densities and distributions. To unveil the full complexity captured in a dataset, a quantitative analysis is essential. This necessitates a sufficiently large dataset, requiring each step of the complex cryo-ET workflow to be as efficient as possible. Despite notable improvements in sample preparation [3], post-processing [4, 5], sample handling [6], and data acquisition [7] in recent years, some challenges remain. Firstly, the complex cryo-ET workflow often demands the samples to be stored either for further processing or for final imaging. Although improvements were made, ice contamination during handling and storage still limits the overall efficiency of the preparation pipeline. Secondly, current preparation protocols do not make use of high currents, limiting the throughput, especially for thick samples like organoids or tissues. Here we present solutions to overcome the aforementioned limitations.

Contamination free sample handling and storatge

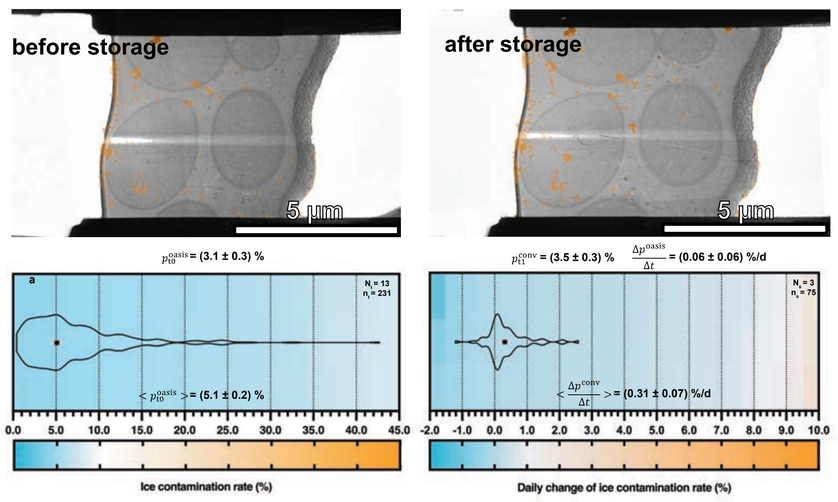


To address sample contamination, we developed an enhanced glove box system that allows sample handling and storage in a contamination free environment. With these technical improvements, we can now almost completely prevent ice contamination of the polished lamellae during transfer, handling and storage. In addition, the contamination free environment allows us to store the sample at every stage of post-processing without introducing additional contamination, thereby enabling the pipeline to be adjusted flexibly according to the needs of the scientists.

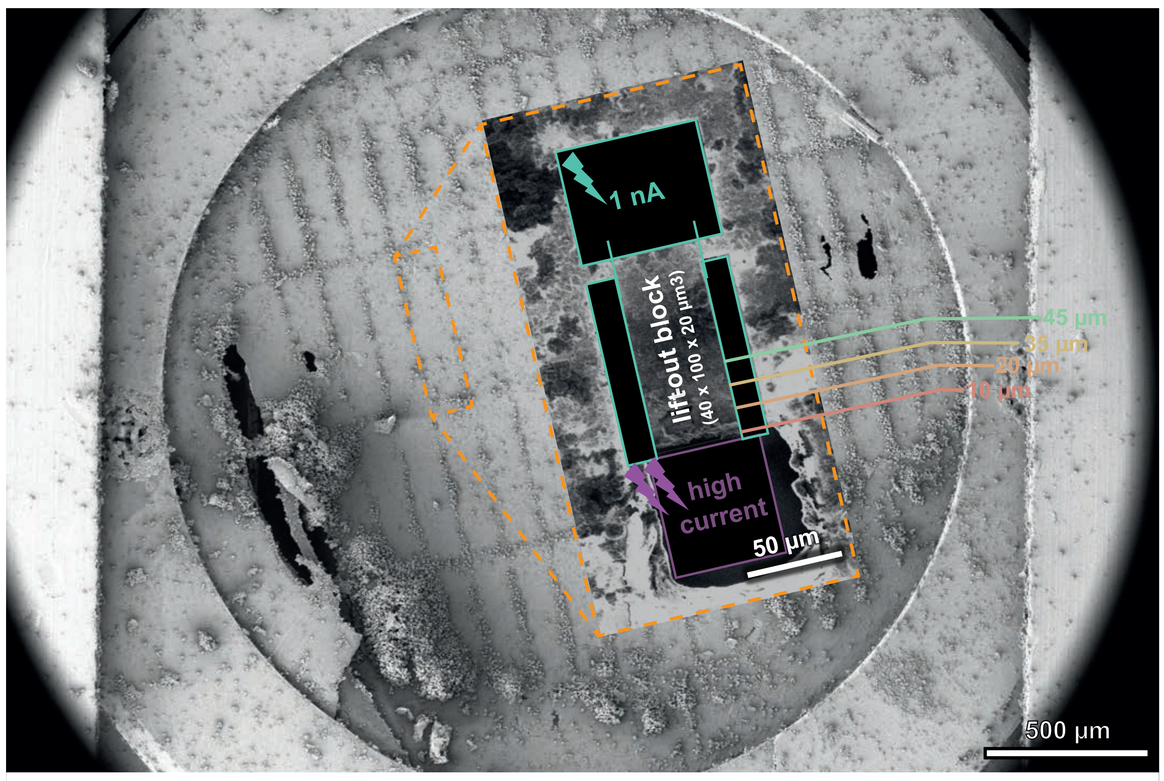
Without glovebox

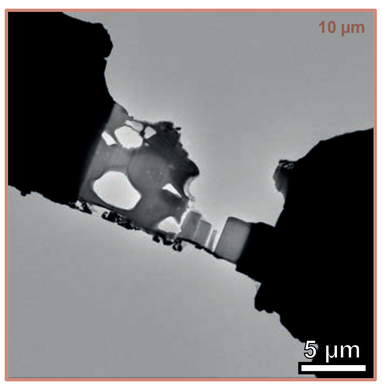


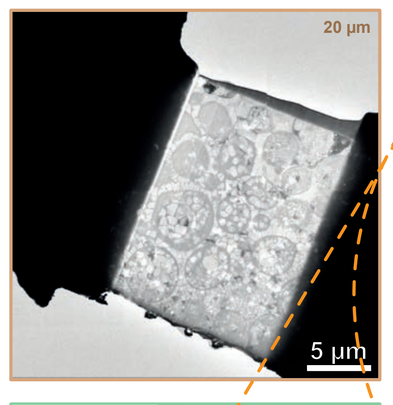
With glovebox

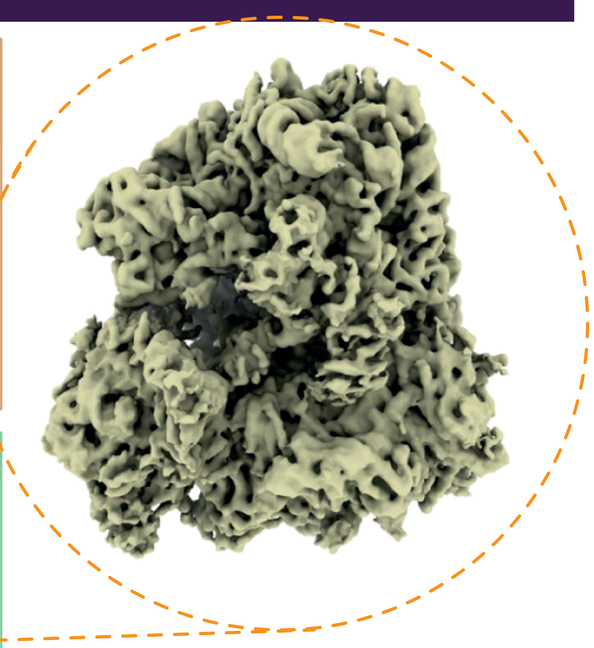


High-throughput PFIB milling









5.5 Å at 50 µm prepared by 2.5 µA Xenon

Acknowledgements

Elisa Lisicki, Tatjana Taubitz and Sebastian Tacke acknowledge the fruitfull discussions with Cveta Tomova (Thermo Fisher Scientific) and John Mitchels (Thermo Fisher Scientific). Part of the project was done in collaboration with Thermo Fisher Scientific and Tatjana Taubitz gratefully acknowledges the financial support provided by Thermo Fisher Scientific.

References

[1] Wang et al. 2021 The molecular basis for sarcomere organization in vertebrate skeletal muscle. Cell 184, 2135-2150

[2] Tamborrini et al. 2023 Structure of the native myosin filament in the relaxed cardiac sarcomere. Nature 623, 863-871

[3] Toro-Nahuelpan et al. 2020 Tailoring cryo-electron microscopy grids by photo-micropatterning for in-cell structural studies. Nature Methods 17, 50-54

[4] Zach et al. 2020 Fully automated, sequential focused ion beam milling for cryo-electron tomography. eLife 9, e52286

[5] Buckley et al. 2020 Automated cryo-lamella preparation for high-throughput in-situ structural biology. J. Struct. Biol. 210, 107488

[6] Tacke et al. 2021 A streamlined workflow for automated cryo focused ion beam milling. J. Struct. Biol. 213, 107743

[7] Eisenstein et al. 2024 Smart Parallel Automated Cryo Electron tomography. Nature Methods 21, 1612-1615