Array Tomography enables multimodal visualization in neuropathology

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Volume Electron Microscopy (vEM) provides high-resolution data of target structures and resolves three-dimensional representations of otherwise ambiguous biological geometries. While EM provides high spatial resolution, search processes in volumes of several hundreds of square microns is tedious. Multimodal methods like correlated light and electron microscopy (CLEM) allow to bridge these scales. Among the available vEM techniques, array tomography methods like automated tape collecting ultramicrotomy (ATUM) have proven particularly powerful for targeting specific or rare biological structures as needed for correlation as they enable repetitive and large field of view imaging.
Here we show how to apply multimodal ATUM approaches to reveal ultrastructural correlates of neurodegenerative pathologies. While classic correlation techniques annotate ultrastructural data with one or a few molecular targets, we have developed STcEM, a method that links spatially-resolved gene expression of single cells with their ultrastructural morphology. Our results offer a comprehensive view of the spatial, ultrastructural, and transcriptional reorganization of single cells after brain injury. Moreover, we have developed ATUM-Tomo, a hybrid method that, bridges scales from scanning to transmission EM. As a proof-of-principle, we applied correlative ATUM-Tomo to study ultrastructural features of blood brain barrier (BBB) leakiness around microthrombi in a mouse model of traumatic brain injury. Overall, our new ATUM-Tomo approach will substantially advance ultrastructural analysis of biological phenomena that require cell- and tissue-level contextualization of the finest subcellular textures.

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Fluid Dynamics of Cryo-EM Sample Vitrification

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Plunge freezing has been highly successful for producing vitreous ice specimens for cryogenic electron microscopy (cryo-EM) of biological macromolecules and remains the most widely used vitrification method in the field. Despite this success, a complete physical description of the fluid dynamics and heat transfer mechanisms governing vitrification is still lacking. Previous observations of a transient surface cavity forming beneath grids during cryogen entry suggested that conductive heat transfer through the grid and support film could dominate cooling before extensive liquid contact is established [1]. While this picture is broadly consistent with successful vitrification on conventional thin grids, it leaves open how robust this mechanism is to changes in substrate geometry and surface properties. The recent emergence of thicker and microfabricated support substrates, including silicon-based chips [2] and pre-clipped cartridge assemblies, has revealed increased sensitivity of vitrification outcomes to substrate geometry and surface properties, motivating a renewed examination of the physical processes governing plunge freezing.

Here, we investigate these processes using subsurface high-speed videography to directly visualise the interaction between cryo-EM substrates and liquid ethane during cryogen entry and immersion For conventional TEM grids, we observe the formation of a shallow, quasi-static air cavity pinned near the liquid surface, while the grid below the pinned contact line rapidly establishes and maintains direct contact with the cryogen. Combined with heat-transfer simulations, these observations show that conductive cooling through edge-only contact is insufficient to vitrify supported thin films at relevant rates, and that effective vitrification requires direct liquid contact and high interfacial heat flux. In contrast, microfabricated substrates and pre-clipped assemblies exhibit deeper, persistent air cavities with delayed closure, substantially reducing the extent and duration of cryogen contact and thereby inhibiting heat transfer. By quantifying cavity collapse dynamics across substrate geometries, we propose a plausible mechanism in which impact-induced inertia competes with curvature-driven capillary forces and wettability to control cavity persistence. These findings clarify why plunge freezing is robust for conventional thin grids yet becomes less reliable for thicker or microfabricated supports, and they suggest design and experimental strategies to improve vitrification reliability for emerging cryo-EM substrates. They further show that cavity-mediated delays in cryogen contact introduce uncertainty in the onset of rapid cooling, with direct implications for time-resolved cryo-EM, where cooling dynamics define the kinetic quench. Our results therefore identify key determinants of both vitrification reliability and temporal precision in time-resolved cryo-EM experiments.

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ADVANCING IN SITU CRYO ELECTRON TOMOGRAPHY FROM PROTEIN LOCALIZATION TO QUANTITATIVE CELLULAR ANALYSIS

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Cryo-electron tomography (cryo-ET) offers three-dimensional structural information from vitrified cells at nanometer resolution, but its broader application is limited by challenges in protein localization, sample preparation, and quantitative data analysis. We present recent methodological advances that jointly address these limitations and extend the analytical power of in situ cryo-ET.

We introduce a genetically encoded FerriTag labeling strategy that enables specific localization of target proteins in vitrified cells without chemical fixation or permeabilization. The method relies on rapamycin-induced dimerization between FKBP-tagged target proteins and iron-loaded ferritin fused to FRB, producing a compact, electron-dense marker readily detectable in cryo-ET. We demonstrate robust labeling in both focused-ion-beam-milled lamellae and vitreous sections prepared by cryo-electron microscopy of vitreous sections (CEMOVIS), allowing flexible integration with different cryo-sample preparation workflows.

To enable quantitative analysis of complex tomograms, we present CryoVesNet, a deep-learning–based pipeline for automatic segmentation of synaptic vesicles. CryoVesNet combines a three-dimensional convolutional neural network with post-processing steps that enforce vesicle geometry through spherical refinement and radial intensity profiling. This approach achieves accuracy comparable to expert manual segmentation while reducing analysis time by orders of magnitude, enabling large-scale and statistically robust studies of vesicle organization.

Finally, we demonstrate that CEMOVIS sections preserve molecular structure sufficiently for high-resolution in situ cryo-EM. Using two-dimensional template matching and subtomogram averaging, we obtain near-atomic reconstructions of ribosomal subunits from vitreous yeast sections, showing that large regions of CEMOVIS samples remain structurally intact despite sectioning artifacts.

Together, these methods provide complementary solutions for labeling, sample preparation, and analysis, advancing cryo-ET toward routine, quantitative in situ structural biology.

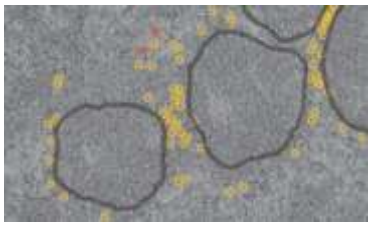

Fig. 1: Ferritag-labelled mitochondria

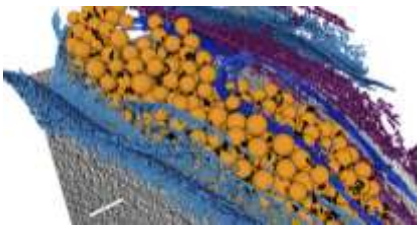

Fig. 2: CryoVesNet-segmented vesicles

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Twist and Scout: Analysis and Curation of Particles in Cryo-Electron Tomography Using TANGO

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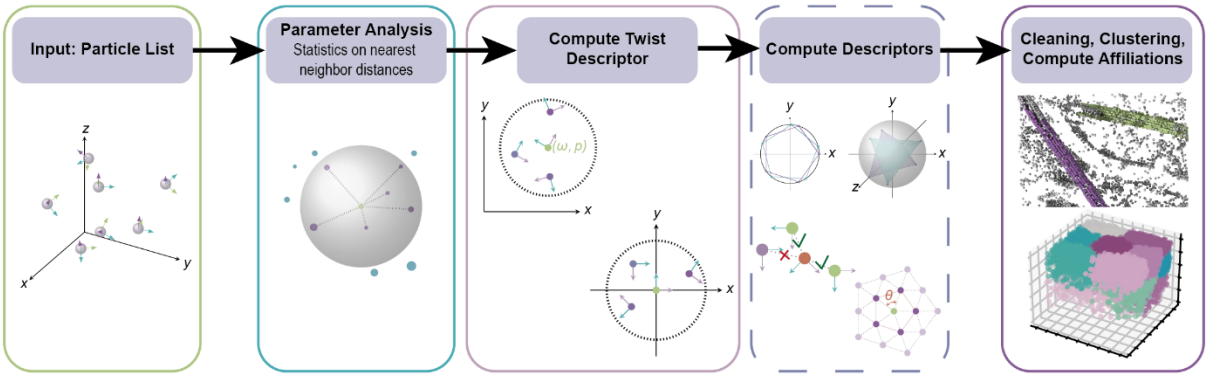
Cryo-electron tomography (cryo-ET) enables the visualization of macromolecular structures directly inside cells, providing access to the molecular organization of biological systems in their near-native context. Beyond high-resolution structures obtained through subtomogram averaging (STA), cryo-ET workflows also produce particle lists that contain spatial coordinates and orientations of macromolecular complexes. These data provide an opportunity to investigate higher-order organization and functional relationships between molecular assemblies within the cellular environment. However, tools for systematically analyzing such spatial information remain limited, and most existing approaches rely on case-specific scripts or hypothesis-driven analyses tailored to individual biological systems. Here we present TANGO (Twist-Aware Neighborhoods for Geometric Organization), a framework designed for the holistic analysis and curation of particle data derived from cryo-ET experiments. TANGO treats particle lists as orientation-aware point clouds and encodes spatial relationships between neighboring particles through twist vectors, which simultaneously capture relative positions and orientations in a rotationally invariant manner. These descriptors form the basis for extracting higher-level features describing particle neighborhoods, including local occupancy patterns, lattice topology, and deviations from expected geometric arrangements. As illustrated schematically in the figure below, the framework analyzes the spatial context of each particle by considering neighbors within a defined support radius and converting their relative spatial relationships into twist-based descriptors suitable for downstream analysis.
The resulting feature representations enable several types of analyses within a unified pipeline. TANGO can be used to quantify spatial patterns, identify structural symmetries, cluster particles according to their affiliations with larger assemblies, and detect outliers that may correspond to structural variants or false positives. The approach is applicable across a wide range of biological systems, including assemblies such as nuclear pore complexes, microtubules, and viral capsids. By providing a modular architecture with customizable features and analysis workflows, TANGO supports exploratory investigation of spatial organization in diverse cryo-ET datasets and contributes toward a deeper understanding of how molecular assemblies interact and organize within complex cellular environments.



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New developments in cryo-EM: Pushing boundaries for in situ structural determination and contextual imaging

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Cryo-electron microscopy has revolutionised structural biology and our ability to explain with molecular precision how many macromolecules function. However, it is being increasingly recognised that studying these structures in isolation—away from the context of their native cell, tissue, and organism—leaves significant gaps in our understanding. This has reinforced the need to develop techniques that enable in situ structural characterization and volume imaging, bridging cellular biology and structural biology.

The introduction of the latest generation of plasma FIBs to biological sciences has opened the door to the imaging of cells and tissues through Slice & View (serial FIB SEM) both at room and cryo temperatures in a way that achieves optimal quality and resolution. Further combining volumetric imaging approaches with cryo-ET allows insight to be gained across orders of magnitude of scale.

This presentation will highlight the integrated workflow from plasma FIB lamella preparation and serial FIB-SEM volume imaging through to high-resolution data acquisition on the 300 kV TEM Krios 5. Advances in plasma FIB technology that enable precise lamella preparation and large-scale 3D imaging will be showcased, including how new AI-driven adaptive scanning and dynamic auto functions accelerate and expand volume data collection. Finally, we will discuss the latest developments in tomography and single particle analysis data acquisition, demonstrating how these innovations together open new avenues to study biological structures across scales, from cellular landscapes to molecular detail.

Nucleocytoviricota in brown algae and their impact on cell architecture

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Giant viruses of the phylum Nucleocytoviricota with large double-stranded DNA genomes infect diverse eukaryotic hosts. Genomes of these giant viruses have increasingly been found integrated into the genomes of their hosts. The evolution and ecological impact of endogenous viral elements remain largely unknown. In the multicellular marine brown alga Ectocarpus, the endogenous viral elements can be reactivated to initiate productive viral infections. Discoveries of the molecular and cellular mechanisms underlying the virus' reactivation, replication and encapsidation processes are still in its infancy.
To contribute to the understanding of viral processes and virus-host interactions we have used electron microscopy and fluorescence microscopy to analyse the impact of the activated virus on the cell architecture. Virus activation, that is regulated by developmental and environmental signals, leads to abnormal nuclear division, and in the progress of the viral infection eventually to a nuclear breakdown. In the late stages of viral infection, the cell is largely occupied by virus particles.