Graduate School Event

Thesis Defense: Large-scale imaging of cellular actin networks at single-filament resolution using montage cryo-electron tomography

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Host: Rafal Klajn

Cell motility depends on the protrusive force generated by the actin cytoskeleton at the leading edge of cells. In lamellipodia, thin protrusions at the front of migrating cells, actin forms higher-order networks with versatile physical properties. Previous studies have hinted at the presence of different sub-populations of filaments and their spatial organization within actin filament networks at the leading edge of migrating cells. Due to limitations of existing experimental methods, it has not been possible to fully describe the complexity of the actin filament populations involved in protrusions at the leading edge.Cryo-electron tomography (cryo-ET) has been used to describe the actin networks in cellular protrusions at single filament resolution in 3D. However, these descriptions have been limited to 1-2 µm2 area of the protrusions, providing merely snapshots of much larger assemblies that span serval tens to hundreds of square microns area. Consequently, they have not provided a holistic overview of the existing variability within actin networks. We therefore lack a comprehensive understanding of how actin network geometries orchestrate cell migration.To address these challenges, I developed a montage cryo-electron tomography workflow to obtain three-dimensional views of lamellipodial regions covering areas of up to 100 µm2 at single-filament resolution. Seamless three-dimensional tomogram stitching is achieved by combining neural-network-based denoising strategies with novel optical-flow-based algorithms, thereby preserving filament continuity across large reconstructed volumes. Our computational analysis pipeline enabled us to vectorize all filaments within these volumes, including filaments reaching lengths of up to 4 µm. The data generated here represent a valuable resource for the actin community, as they provide one of the most detailed and extensive three-dimensional descriptions of actin filament architecture in the lamellipodium. Specifically, they reveal accurate F-actin concentrations, filament length distributions, connectivity, and the spatial organization of filament sub-populations. These datasets can be used to support and constrain biophysical and theoretical models of actin network organization, dynamics, and force generation.

Thursday, August 13, 2026 09:30am - 10:30am
Sunstone Bldg / Ground floor / Big Seminar Room B / 63 seats (I23.EG.102) and Zoom

This invitation is valid as a ticket for the ISTA Shuttle from and to Heiligenstadt Station. Please find a schedule of the ISTA Shuttle on our webpage: https://ista.ac.at/en/campus/how-to-get-here/ The ISTA Shuttle bus is marked ISTA Shuttle (#142) and has the Institute Logo printed on the side.