

Automated Microtubule Detection for Subtomogram Averaging in Cryo-Electron Tomography

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Cryo-electron tomography enables three-dimensional visualization of macromolecular structures within intact cells. Filamentous structures such as microtubules are essential for intracellular transport, structural stability, and cell division. Structural analysis of microtubules often relies on subtomogram averaging, a process that requires manual or semi-manual filament picking and alignment. These steps are time-consuming, computationally demanding, and can introduce user bias, making it difficult to handle large datasets efficiently. We developed an automated pipeline for microtubule detection and particle orientation prediction to improve efficiency and consistency in subtomogram averaging workflows. The pipeline integrates filament tracing, line connection analysis, and geometric modeling to detect microtubules and predict particle angles prior to alignment. Quality control measures were incorporated to identify elliptical distortions, misaligned particles, and outliers in dense datasets. Performance was evaluated by comparing pipeline outputs with manually processed datasets, examining particle ordering, angular consistency, alignment behavior, resolution, and processing time. Automated picking preserved expected structural organization and produced angle predictions consistent with refined alignment parameters while reducing analysis time. However, performance may vary depending on tomogram quality and filament density. These findings demonstrate that automated filament detection can reduce manual effort and support efficient structural analysis in cryo-electron tomography. Further improvements may increase reliability across different cellular contexts.

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