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SUPPLEMENTARY INFORMATION

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# A complete data processing workflow for cryo-ET and subtomogram averaging

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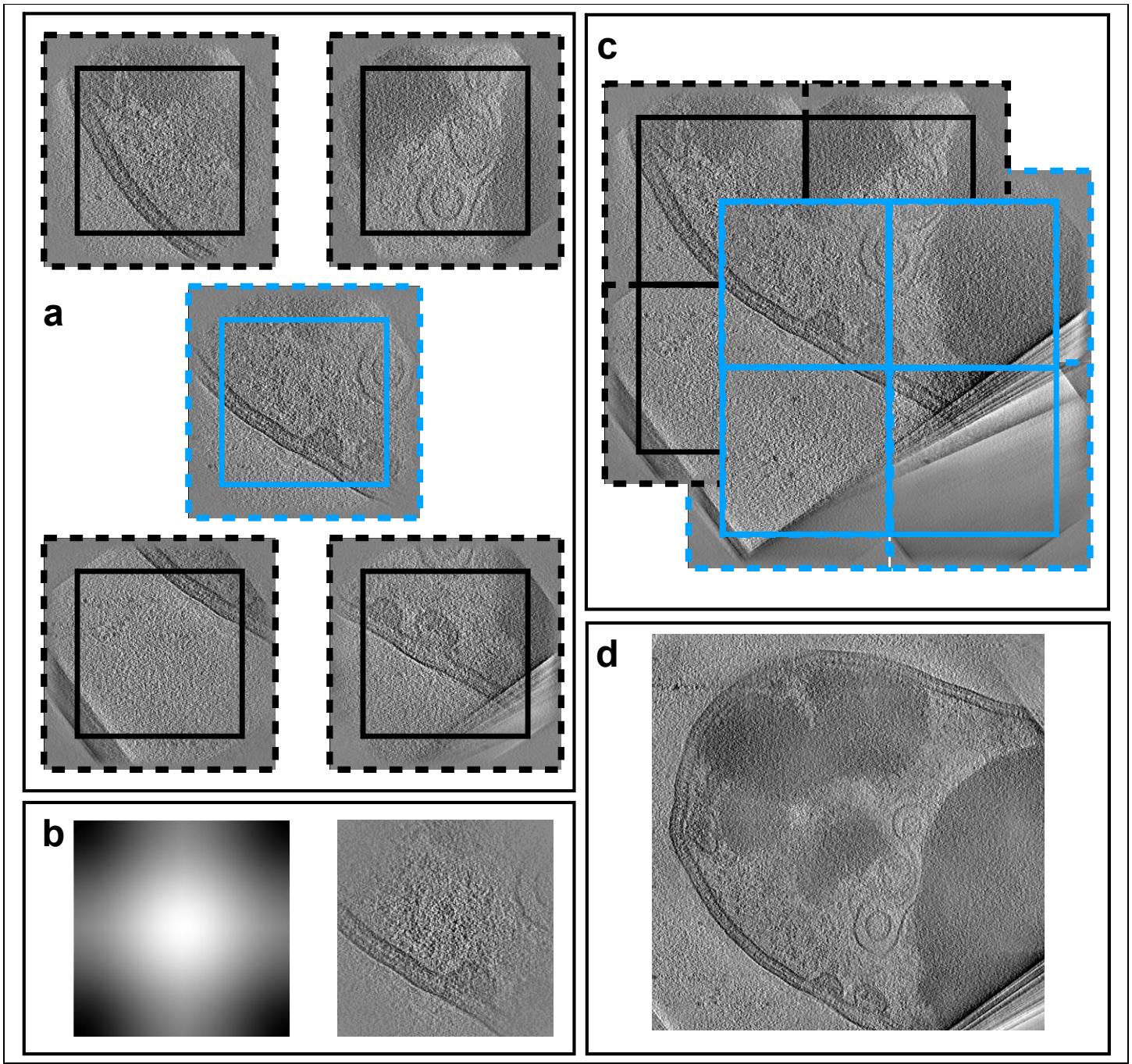
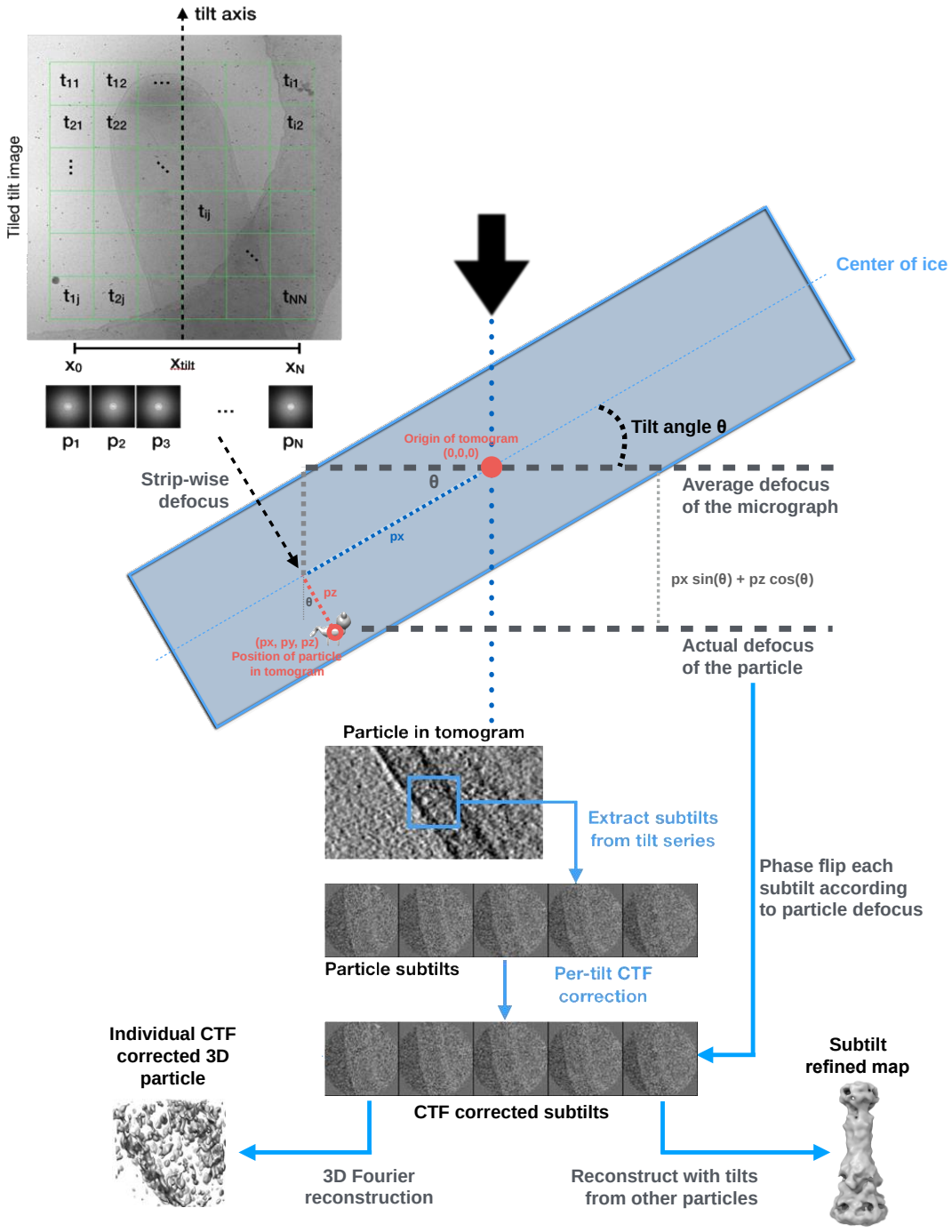



**Supplementary Figure 1**

Tiling strategy for tomogram reconstruction.

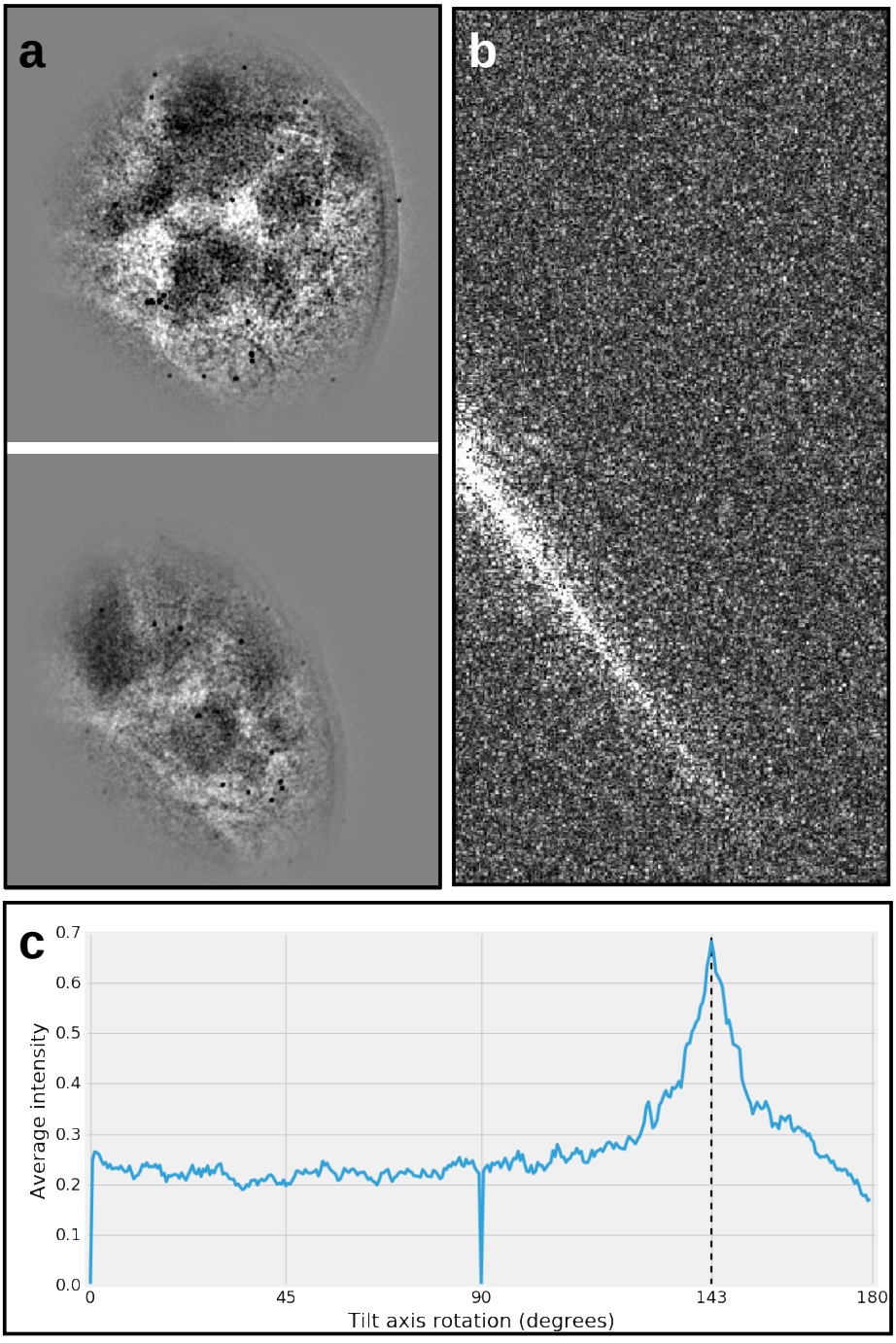
(a) Reconstruction of individual tiles. Each tile is padded to the size of the dashed box during the reconstruction, and clipped to the size of the solid box. (b) The per-tile weighting function and slice view of a masked tile. (c) Overlapping tiles to reduce edge effects. (d) Resulting by-tile reconstruction.




**Supplementary Figure 2**

Subtilt CTF determination.

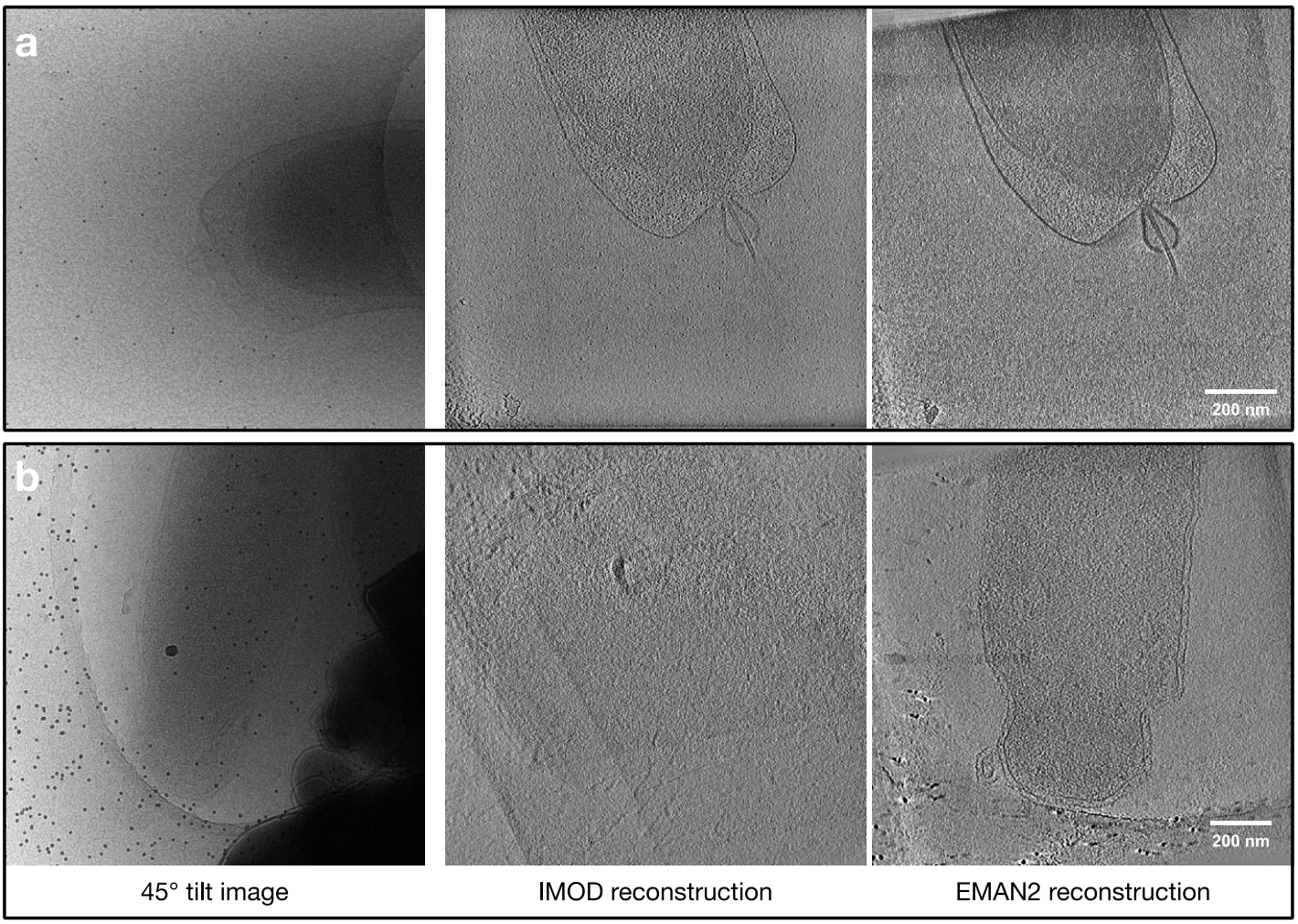
We measure CTF in each tilt image by tiling the tilt images and calculating coherent power spectra along strips parallel to the tilt axis. These power spectra, geometric information from the tilt angle, and the 3D position of each extracted particle are used to determine per-particle defoci. Once CTF curves have been fit to the data, the parameters are used to phase flip individual particle subtilt images for subsequent processing.




**Supplementary Figure 3**

Determination of tilt axis rotation angle using Fourier sum approach.

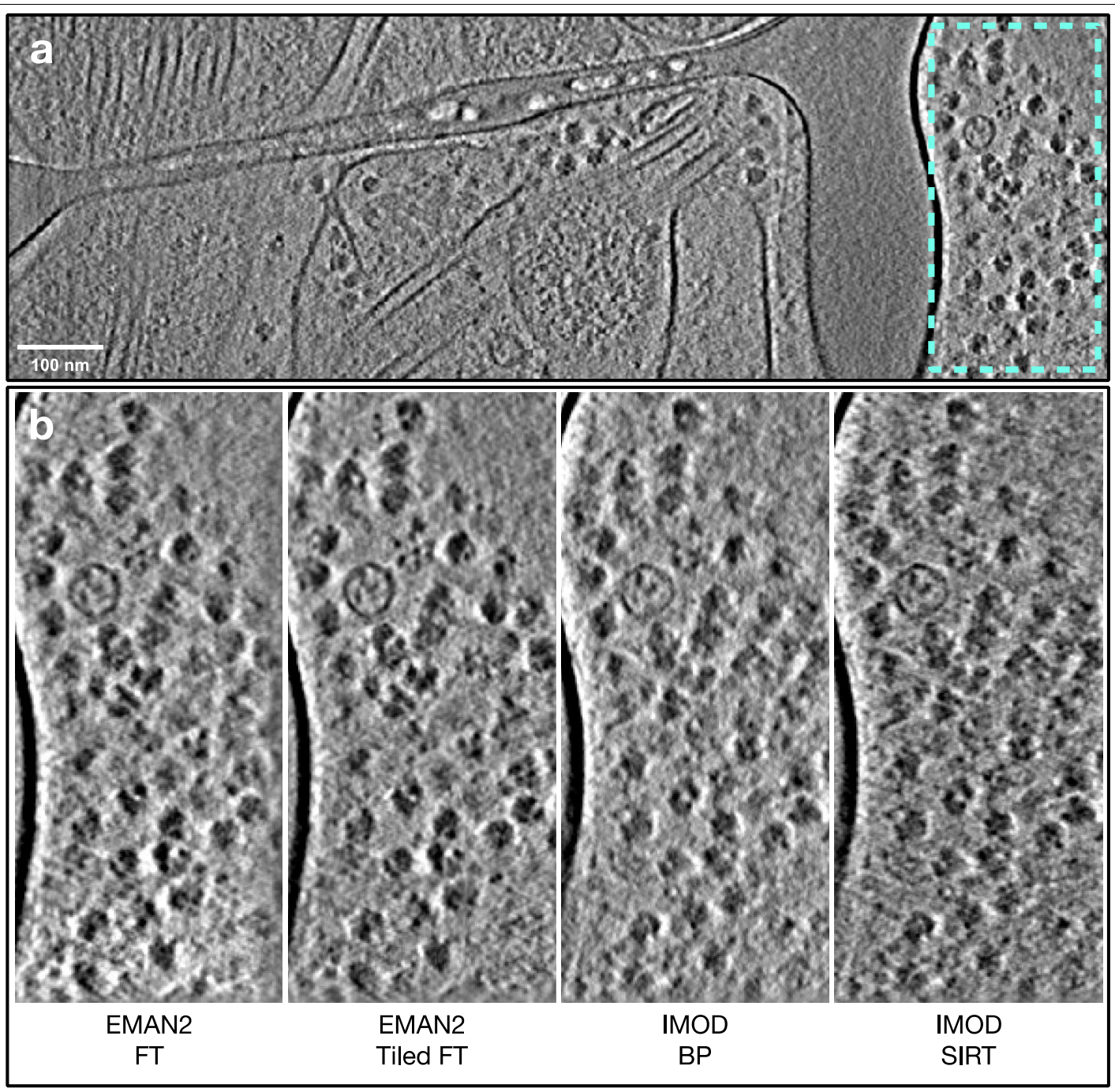
(a) Filtered and masked images from the center tilt and a high angle tilt in a tilt series. (b) Coherent average of Fourier transform intensity of all translationally aligned and masked images in the tilt series. (c) Plot of the average intensity of (b) along a ray at different orientation. The peak annotated by the dashed line is the tilt axis orientation. Note the points at 0 and 90 degrees are set to zero to avoid the impact from background pattern.




**Supplementary Figure 4**

Comparison of automatic tilt series alignment protocol.

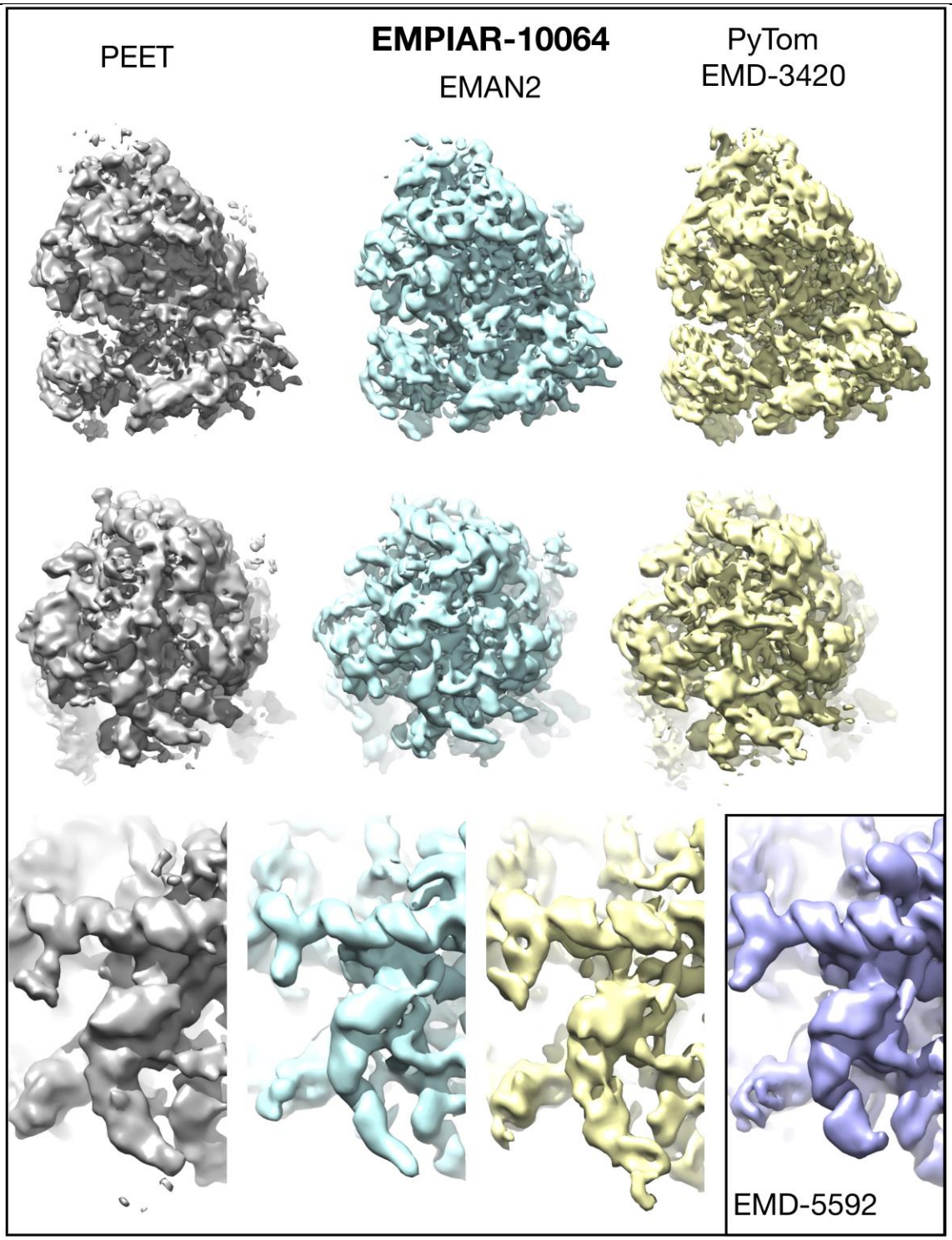
(a) An example of successful case for both IMOD and EMAN2 pipeline. From left to right: 45 degree tilt image; center slice view of the tomogram from ETDB (using IMOD); center slice view of the tomogram reconstructed in EMAN2. (b) An example of “failed” case for IMOD that is successful aligned by EMAN2. Note the large amount of ice contaimination in high angle tilt images that likely leads to the failure of original IMOD alignment.




**Supplementary Figure 5**

Comparison of different reconstruction methods.

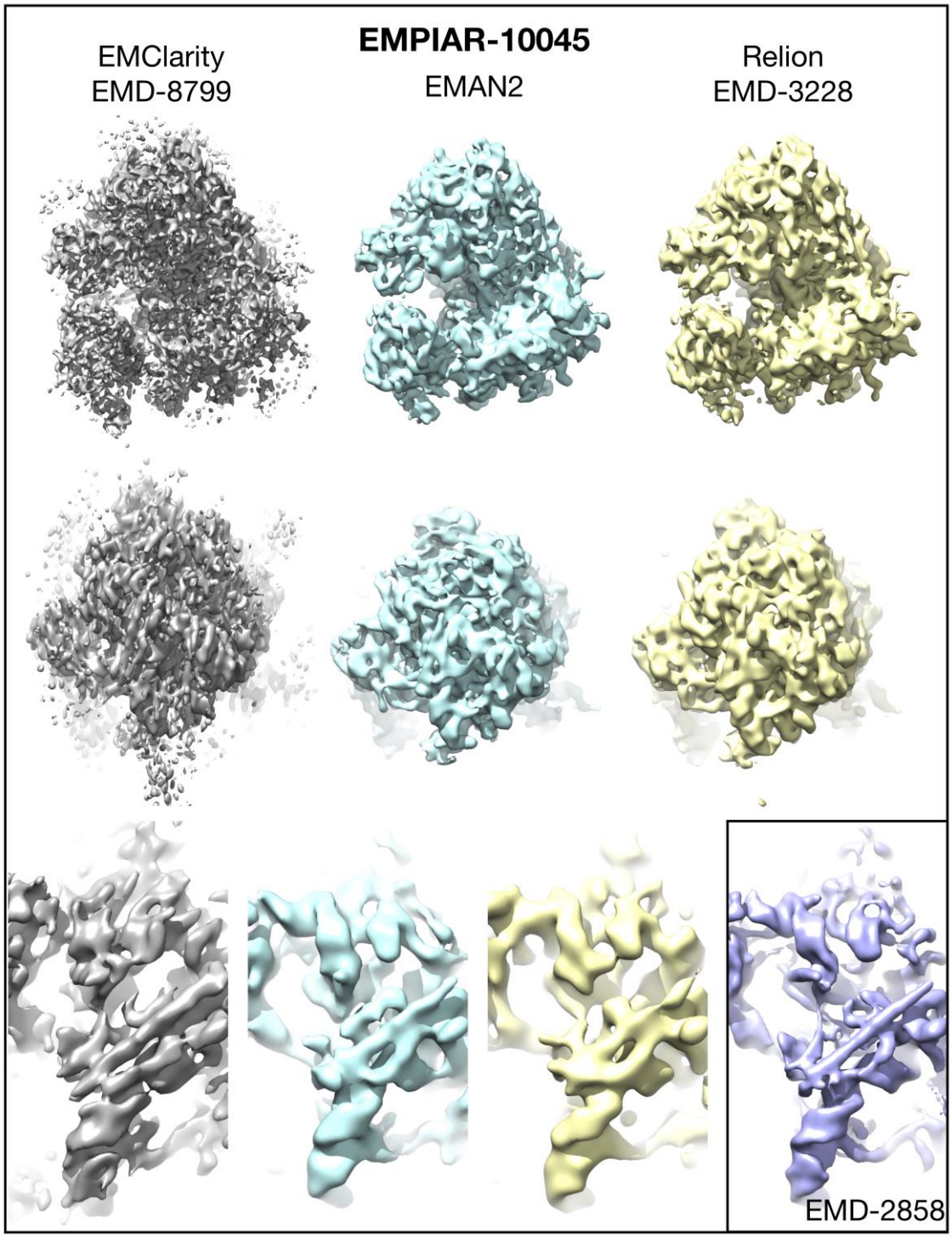
(a) A tomogram slice view of the flagellum of an anucleated *Trypanosoma brucei* cell, recontructed with tiled direct Fourier transform. Cyan box shows the tile to zoom in for comparison. (b) Slice view of tomogram reconstructed by direct Fourier transform (FT), tiled direct Fourier transform, back projection (BP) and SIRT, from left to right. The tomograms are filtered identically to be comparable.




**Supplementary Figure 6**

Comparison of structures solved by different software packages using the ribosome dataset from EMPIAR-10064.

Averaged structures solved by PEET (grey, 13Å), EMAN2 (cyan, 8.5Å) and PyTom (yellow, 11.2Å) are viewed from three orientations. A high resolution structrue (blue, EMD-5592) filtered to 10Å is shown for comparison.




**Supplementary Figure 7**

Comparison of structures solved by different software packages using the ribosome dataset from EMPIAR-10045.

Averaged structures solved by EMClarity (grey, 7.8Å), EMAN2 (cyan, 9.3Å) and Relion (yellow, 13Å) are viewed from three orientations. A high resolution structrue (blue, EMD-2858) filtered to 10Å is shown for comparison.