

## Publication

## Assessing the benefits of focal pair cryo-electron tomography

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Cryo electron tomography provides nanometer-scale information on biological matter preserved in a close-to native state. The resolution of tomograms and structures resolved by sub-tomogram averaging is typically limited by the contrast transfer function of the electron microscope, which is especially critical for thick samples. Here, we report a method to increase the attainable resolution by recording tomographic 'focal pairs', which are pairs of tilt series of the same object acquired in complementary defocus conditions. Low defocus imaging provides high resolution at low contrast, while high defocus imaging yields high contrast at the price of limited resolution. Quantitative assessment of the quality of lipid bilayer reconstructions in the resulting tomograms demonstrates stable resolution preservation beyond 3 nm for cells thicker than 500 nm. Further, in computational simulations on synthetic datasets we show the applicability of the method to sub-tomogram averaging, demonstrating its potential for achieving higher resolution.

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