Cryoem Single Particle Reconstruction with A Complex-Valued Particle Stack

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Cryogenic electron microscopy single particle reconstruction (cryoEM SPR) is a complex and hierarchical process that begins with numerous very noisy multi-frame images. One of the intermediary structures generated during data processing is called a particle stack, which contains cut-out images of particles in boxes of predefined size. The micrograph that serves as the source of the boxed images is typically corrected for motion between frames before the particle stack is generated. However, at this stage, the contrast transfer function (CTF) or its Fourier Transform point spread function (PSF) is not taken into account. Historically, the particle stack was intended for larger particles with a tighter PSF, which is characteristic of lower resolution data. Nowadays, the field performs analyses of smaller particles and at higher resolutions, which results in a broader PSF that necessitates cutouts in significantly larger boxes. This, in turn, leads to an increase in the number of pixels, causing the steps relying on pixel-to-pixel comparisons to slow down significantly. Therefore, the approach to handling structures such as the particle stack needs to be re-examined to optimize data processing.

We propose using a complex-valued image as the source image for the particle stack cutouts, in which CTF correction is implicitly applied as a real component of the image. We can achieve this by applying an initial CTF correction to the entire micrograph before performing box cutouts as a subsequent step. The final CTF correction that we refine and apply later has a very narrow PSF, so cutting out particles from micrographs that were approximately corrected for CTF does not require extended buffering and the boxes during the analysis only need to be large enough to encompass the particle. This extension of the micrograph concept provides multiple advantages because the particle box size can be small, and crucial calculations for high-resolution reconstruction, such as Ewald sphere correction, aberration refinement, and particle-specific defocus refinement, can be performed on the small box data.

We will present results extending the initial concepts described in Bromberg et al.'s (2023) paper "CryoEM single particle reconstruction with a complex-valued particle stack" in the Journal of Structural Biology (Mar 6;215(2):107945. doi: 10.1016/j.jsb.2023.107945).

Our proposed approach allows for more efficient data processing, especially when analyzing smaller particles and at higher resolutions, where the PSF is broader and the use of larger boxes becomes more prevalen