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Alignment of cryo-EM movies of individual particles by optimization of image translations

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ABSTRACT

Direct detector device (DDD) cameras have revolutionized single particle electron cryomicroscopy (cryo-EM). In addition to an improved camera detective quantum efficiency, acquisition of DDD movies allows for correction of movement of the specimen, due to both instabilities in the microscope specimen stage and electron beam-induced movement. Unlike specimen stage drift, beam-induced movement is not always homogeneous within an image. Local correlation in the trajectories of nearby particles suggests that beam-induced motion is due to deformation of the ice layer. Algorithms have already been described that can correct movement for large regions of frames and for >1 MDa protein particles. Another algorithm allows individual <1 MDa protein particle trajectories to be estimated, but requires rolling averages to be calculated from frames and fits linear trajectories for particles. Here we describe an algorithm that allows for individual <1 MDa particle images to be aligned without frame averaging or linear trajectories. The algorithm maximizes the overall correlation of the shifted frames with the sum of the shifted frames. The optimum in this single objective function is found efficiently by making use of analytically calculated derivatives of the function. To smooth estimates of particle trajectories, rapid changes in particle positions between frames are penalized in the objective function and weighted averaging of nearby trajectories ensures local correlation in trajectories. This individual particle motion correction, in combination with weighting of Fourier components to account for increasing radiation damage in later frames, can be used to improve 3-D maps from single particle cryo-EM.

1. Introduction

The use of CMOS technology in direct detector device (DDD) cameras for electron cryomicroscopy (cryo-EM) has enabled the acquisition of exposure series ‘movies’. Movies of radiation sensitive specimens revealed that beam-induced motion blurs images (McMullan and Faruqi, 2008; Glaeser et al., 2011; Brilot et al., 2012). DDD movies are typically acquired with exposures of 1 to 3 $e^-/\text{Å}^2$/frame on the specimen, which corresponds to 2 to 5 e^-/pixel/frame on the detector, depending on microscope magnification. These low exposures result in low signal-to-noise ratios (SNRs) in individual movie frames. Optimal extraction of high-resolution information from images of single particles requires alignment of movie frames, a process that is complicated by the low SNR. Fig. 1A shows the average of a 30 frame movie acquired with 2.5 e^-/pixel/frame, corresponding to 1.2 $e^-/\text{Å}^2$/frame on the specimen at 200 kV with a K2 Summit DDD (Gatan Inc). A few representative particles are circled in red. Fig. 1B shows a single frame from the movie, illustrating the low SNR of the frames. Frame alignment is complicated further by the presence of fixed pattern noise in images from errors in sensor gain normalization. Significant progress in image analysis has already been enabled by programs to perform rigid body translational alignment of entire field-of-view movie frames (currently 4000 × 4000 pixels for most cameras). A method introduced by Li and colleagues (Li et al., 2013) decreases the weight of high spatial frequencies in images to suppress artifacts from fixed pattern noise before calculating pairwise cross-correlation functions between movie frames. The optimal frame displacement values from the cross-correlation functions are used to create a system of over-determined linear equations. Matrix algebra is then used to determined the frame-to-frame translations that best fit the data in a least squares sense. This least

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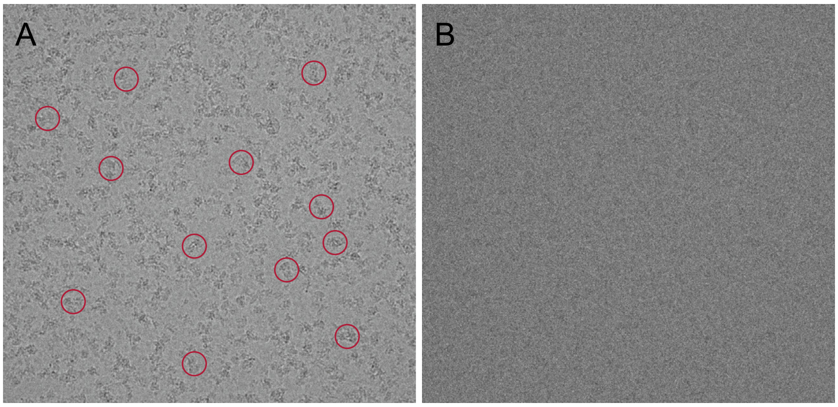

Fig.1. Example micrograph. A, The average of 30 frames after least squares alignment of whole frames for an exposure series movie of the *Saccharomyces cerevisiae* V-ATPase in ice. The exposure used 2.5 e$^-$/pixel/frame and each pixel corresponds to 1.45 × 1.45 Å. The *S. cerevisiae* V-ATPase has a molecular weight of 900 kDa. Several example complexes are circled in red. B, An individual frame from the movie shows the low SNR in frames.

squares whole frame alignment method has allowed high-resolution structures to be determined for important biological macromolecules (Li et al., 2013; Cao et al., 2013; Liao et al., 2013).

Cryo-EM of large particles with DDDs has shown that beam-induced motion cannot be described completely by rigid body translation of entire movie frames (Brilot et al., 2012). Instead, these experiments suggested that the beam-induced movement of ice-embedded protein is better described by a translation of each particle in each frame. Examination of tilt pairs of images demonstrated that rotation of specimens, probably due to movement of the ice layer, does occur (Henderson et al., 2011). However, the magnitudes of these rotations were small and will have the most significant effect on particles with large radii, like viruses. The consequences of specimen rotation can be neglected at present without limiting map resolution for particles smaller than 1 MDa. The translation of particles in frames can be written as $-\vec{t}_z = -(x_z, y_z)$ for each particle in frame z, where $-x_z$ and $-y_z$ are the difference in particle position between frame z and frame 1 in the x and y directions, respectively. If these translations are known, their inverse ($\vec{t}_z$) can be applied to the particle images before averaging of frames to optimize the extraction of high-resolution information from the image. It is likely that accurate individual particle motion correction could extract information from images that is neglected by whole frame alignment. Despite the success of the least squares method for whole frame alignment, it was pointed out by the authors of the method that it is not reliably able to align image regions smaller than 2000 × 2000 pixels for movies acquired using typical conditions. As such, the least squares method is not capable of aligning regions of frames that contain individual particles in order to correct for deformation of the ice layer during imaging. A method to align individual particle images was introduced that is tightly integrated into the single particle orientation estimation framework of the program *Relion* and has resulted in several high-resolution structures (Scheres, 2012; Scheres, 2014; Campbell et al., 2015). For small particles this approach requires rolling averages of frames, which increases the SNR over individual frames but loses information about true trajectories. Also, the individual particle trajectories for small particles from this method include errors, and it is necessary to fit linear trajectories with constant velocities for particles, which are not necessarily a good approximation for their true trajectories. Furthermore, the approach cannot readily be used outside of the *Relion* software package.

Here we aim to identify the translations $\vec{t}_z$ for movies of individual ice-embedded particles that best bring the frames into alignment for each particle, without the use of rolling frame averages or fitted linear trajectories. In order to produce a robust and computationally efficient method for correcting the effects of beam-induced movement in small regions in images, or on individual small (<1 MDa) particles, we pose the problem in terms of optimization. We propose an objective function based on the correlation of the Fourier transforms of individual frames with the sum of all frames. A well-established iterative optimization algorithm that makes use of partial derivatives of the objective function is then used to find the desired translation values. Once optimized, this objective function gives frame-to-frame trajectories for images of individual particles that show strong local correlation. We show that smoothing of trajectories for individual particles can be used to identify and correct beam-induced particle movement. This approach, in combination with compensation for the fading of Fourier components due to radiation damage, was implemented in a new program that we call *alignparts_lmbfgs*.

2. Methods and results

2.1. Choice of objective function

Based on the observation that averages of unaligned particle frames appear blurred, a reasonable alignment for each region of the frame that contains a particle is the alignment that makes the sum of all of the frames best agree with each of the frames. Accordingly, we propose an objective function that maximizes the sum of the correlations of the Fourier transform of each shifted frame with the sum of the Fourier transforms of the shifted frames. Prior to analysis, we apply a temperature factor in Fourier space with the form $exp\left(\frac{-B}{4d^2}\right)$ to prevent fixed pattern noise from dominating the analysis (Li et al., 2013). The effect of translation on the Fourier transform of a movie frame is a phase change, ϕ_{jz}, in each Fourier component of the frame, written F_{jz} for the jth Fourier component of frame z. The phase shifted Fourier component is given by $F_{jz}(\cos\phi_{jz} + i\sin\phi_{jz})$ or $F_{jz}S_{jz}$ where $S_{jz} = (\cos\phi_{jz} + i\sin\phi_{jz})$. The amount of phase change is given by

$$\phi_{jz} = k_x(j) \cdot x_z \frac{2\pi}{N} + k_y(j) \cdot y_z \frac{2\pi}{N}, \tag{1}$$

where N is the extent in pixels in both the x and y direction of the $N \times N$ image, and $k_x(j)$ and $k_y(j)$ are the distance of the jth Fourier component from the origin in the k_x and k_y directions, respectively. As described above, $-x_z$ and $-y_z$ are the difference in particle position between frame z and frame 1 in the x and y directions, respectively. The Fourier transform of a sum is equal to the sum of Fourier