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Boosting Contrast of Cryo-EM Images Without a Phase Plate

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Transmission electron microscopy (TEM) imaging of frozen-hydrated biological requires careful selection of exposure in order to balance the goal of maximizing image contrast with the necessity of minimizing radiation damage [1]. In general, increasing the total exposure of each image improves low-frequency contrast at the expense of high-resolution information due to radiation damage.

One of the paradigm-shifting features of several new CMOS-based direct detection cameras is the ability to collect movies—a continuous stream with negligible dead time between frames [2,3]. Several studies have already shown how movies can be exploited to correct stage drift and beam-induced specimen motion. However, movies also provide intrinsic dose fractionation, allowing microscopists to choose their image exposure *ex post facto* by using subsets of frames from each movie.

We have implemented a new image processing method for using these direct detection movies to generate images with both high contrast and high resolution. This method does not require additional instrumentation such as a phase plate. The method applies a low-pass filter to each movie frame based on the expected radiation damage at the corresponding cumulative specimen dose. Briefly, a movie is acquired of a specimen at 2-3× the normal total electron exposure. To correct specimen drift (which is consistent across the entire image), the frames from the movie are iteratively aligned, and to correct beam-induced specimen motion and charging (which are local effects that vary across the image), sub-regions for each frame are iteratively aligned [4]. To ameliorate the effects of radiation damage, low-pass filters are applied to each frame based on expected damage rate of the specimen. For biological specimens, this damage rate can be estimated from previous radiation damage studies [5,6].

We have demonstrated the benefits of this method by using images of frozen-hydrated Brome mosaic virus (BMV). Images generated based on our method show improved isotropic high-frequency SNR along with significantly improved low-frequency contrast compared to conventional imaging (Fig. 1). We processed a data set using both the conventional method and our new “damage compensation” method to generate *de novo* three-dimensional reconstructions of BMV. Resolution was determined by the gold-standard Fourier Shell Correlation (FSC). Our new method improved the resolution significantly from 4.4 Å to 4.1 Å resolution, thus demonstrating the power of damage compensation with a direct detection camera for high-resolution structural studies.

We have applied this method to both large macromolecular complexes (such as viruses) and much smaller complexes (including GroEL and small RNA particles). Compared to conventional imaging techniques, the visual contrast of the particles is noticeably higher using our damage compensation method.

Additionally, in cases where the exposure rate is low enough, individual electron events can be isolated and counted to further improve image quality. Since electron counting occurs in software as an image processing operation, this technique can be applied to images from any camera with sufficient single electron signal-to-noise ratio. We have implemented our own counting algorithm, which further boosts the contrast of images primarily by eliminating Landau noise.

References:

[1] RM Glaeser, and RJ Hall, Biophys J 100 (2011), pp. 2331-2337.
[2] L Jin and R Bilhorn, Microsc Microanal 16 (2010), pp. 854-855.
[3] BE Bammes et al, Microscopy & Microanalysis Conference Proceedings (2012), LB-36.
[4] AF Brilot et al, J Struct Biol 177 (2012), pp. 630-637.
[5] BE Bammes et al, J Struct Biol 169 (2010), pp. 331-341.
[6] LA Baker et al, J Struct Biol 169 (2010), pp. 431-437.
[7] The authors acknowledge funding from the National Institutes of Health, grant number 8R44GM103417-03.

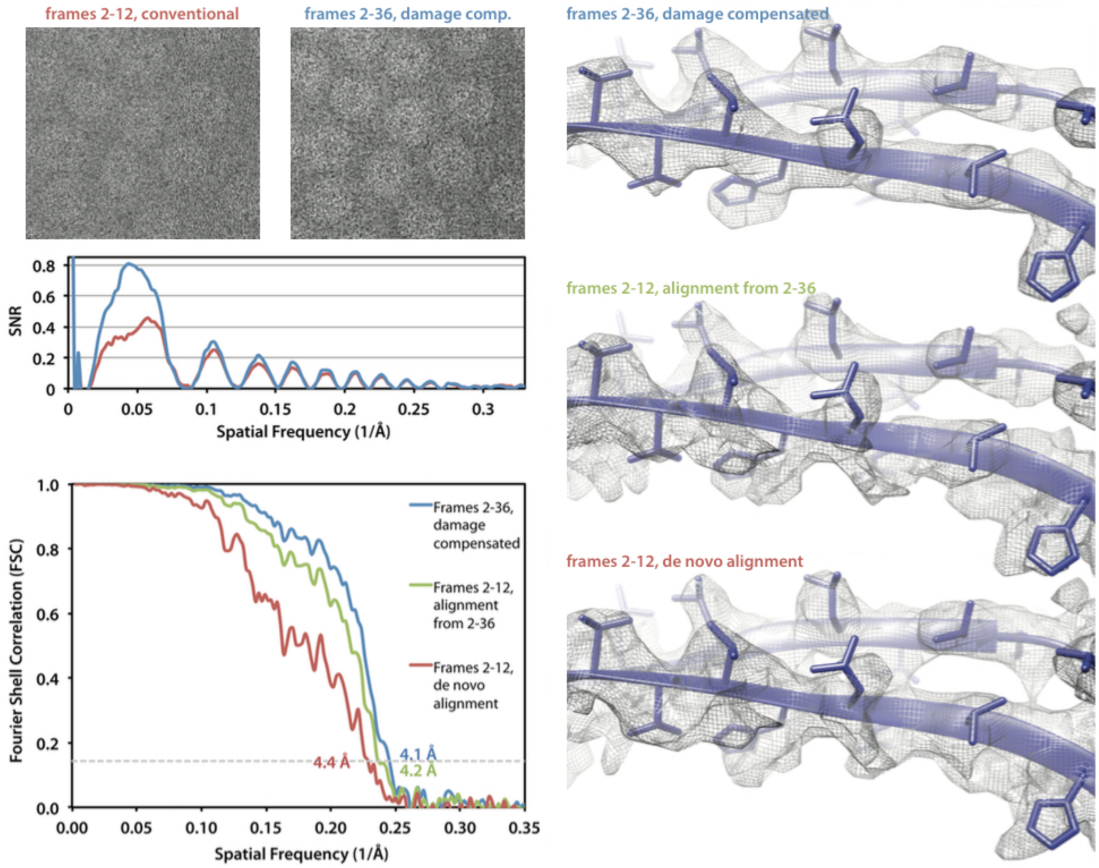

Figure 1. Damage compensation applied to Brome mosaic virus (BMV). Data was collected with a DE-12 Camera System on a JEOL 3200FSC with in-column energy filter, at 61,000× magnification. Images were collected over 1.5 seconds at 25 fps and a total exposure of 53 $e^-/Å^2$. Left-Top: An example cropped region and SNR plot (from the particle stack) of typical image at 0.68 µm defocus, processed conventionally (frames 2-12, ~17 $e^-/Å^2$ exposure) and with damage compensation (frames 2-36, ~53 $e^-/Å^2$ exposure). In each case, the first two frames were discarded. Left-Bottom: Gold-standard FSC curves for reconstructions using damage compensated data, conventional data, and conventional images using alignments from damage compensated data. Right: Comparison of beta sheets for the final density map in each case. Data, 3D reconstructions, and atomic model courtesy of Wah Chiu, Zhao Wang, and Corey Hyrc (Baylor College of Medicine).