Characterization and correction of specimen motion in movie-mode images collected by direct detection devices

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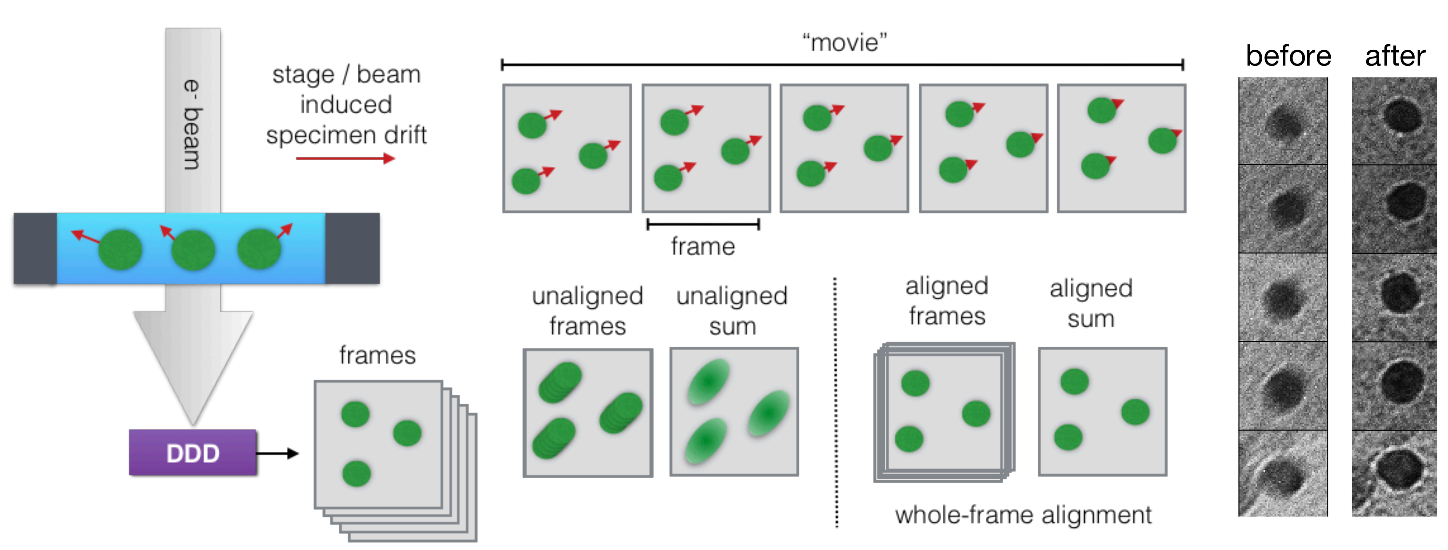
Abstract

CryoEM has made a dramatic leap forward over the last decade, thanks largely to the development of direct electron detection devices. Transmission electron microscopes image an entire field at once, meaning they are subject to any motion of the specimen during imaging. Even motions as small as fractions of a nm can degrade image quality and hence the final 3-D structures. In addition to counting individual electrons, the new direct detectors can provide uncompressed 8k x 8k image data at a rate as high as 30 frames per second. This permits corrections for specimen motion to be made, dramatically improving high resolution structures. However the motions occurring within the specimen are still poorly understood, and the existing algorithms to compensate for this motion do not provide identical answers when characterizing these motions. We are developing an approach for use of high-contrast gold fiducials to establish the ground-truth for specimen motion, and better understand the strengths and weaknesses of each algorithm as well as develop new insights into the motion itself. In turn these insights may lead to improved corrections, and improved structures, or at the very least more productive use of collected image data.

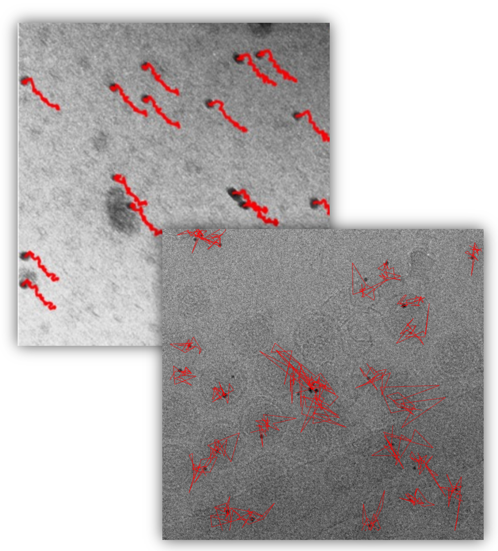
Whole-frame motion correction



DDD movies consist of a stack of electron micrographs recorded in rapid succession with short exposure times. The resulting frames are aligned computationally to compensate for stage and beam induced motions of the imaged specimen. In the absence of such motion correction, high resolution information is blurred.

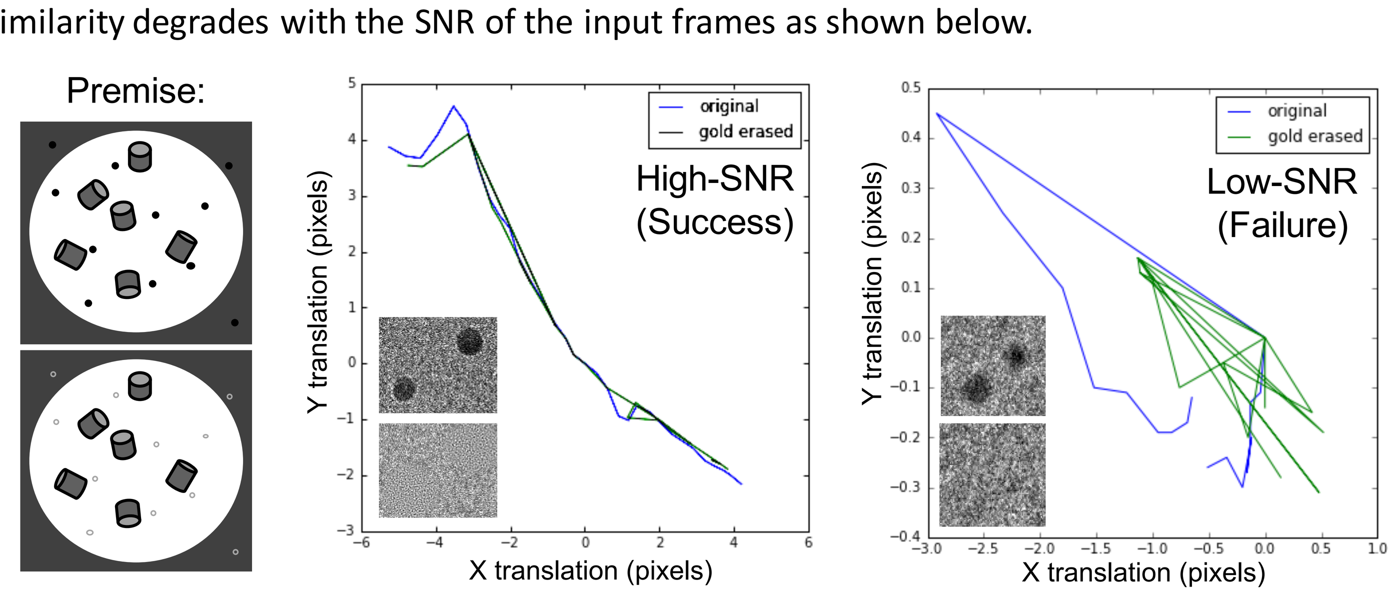
Local motion correction

Recent publications have observed locally correlated and completely uncorrelated specimen motion in DDD movies. This finding has led to a collection of local and per-particle motion correction approaches; however, while they are technically more precise, they may be less accurate. Whereas whole-frame alignment routines take advantage of the signal present in the entire field of view, local algorithms seek to find peaks in cross correlation images computed using significantly smaller image sizes. Ultimately this reduces the signal to noise ratio of computed cross correlation images, making peak detection more challenging and computed trajectories less accurate.



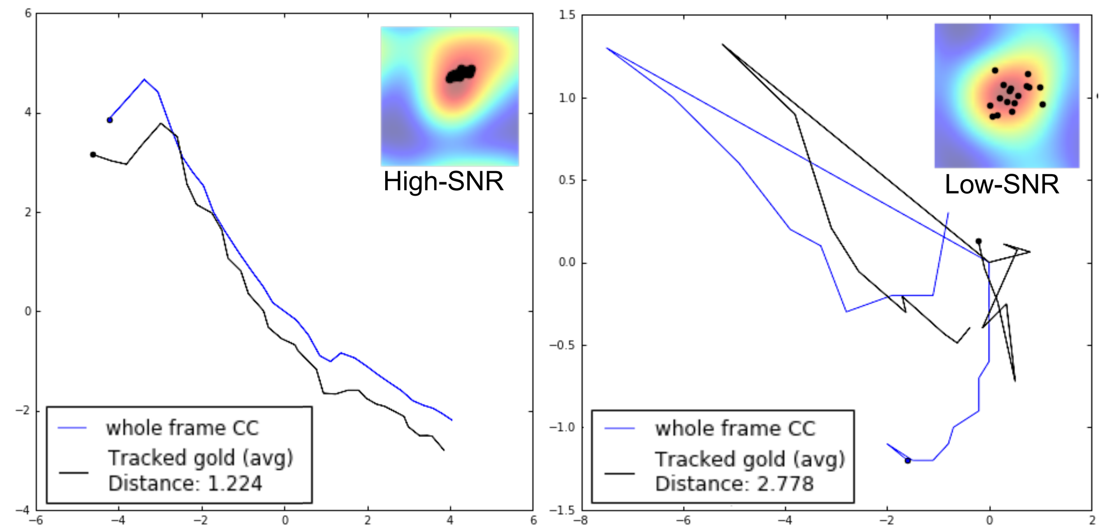
Erasing nanogold clusters

Our approach to validate whole-frame motion is to erase fiducials from frames and compare alignments between the processed and unprocessed frames. The quality of a whole frame alignment can then be measured by the similarity between computed whole frame motion and tracked gold particles. As a control, we compare motion measured in the processed (gold erased) and unprocessed frames, observing strong consistency in signal-rich data; however, similarity degrades with the SNR of the input frames as shown below.

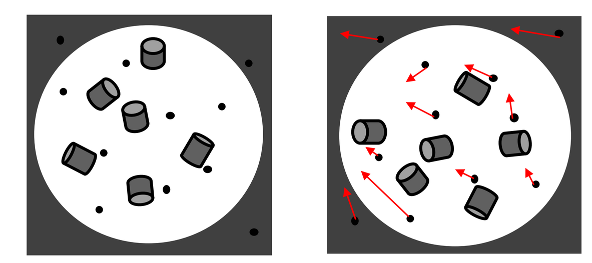


Validating whole-frame trajectories

Individual nanogold particles were measured locally across multiple micrographs using standard 2D Gaussian fitting. Calculated gold trajectories were then averaged and compared against whole-frame, cross correlation alignments. In both high and low SNR cases, individual nanogold trajectories were consistent with measured whole frame motion. Therefore, in cases where gold-erased frame trajectories agree with unprocessed frame trajectories, we can use measured gold motion as ground truth for specimen motion.

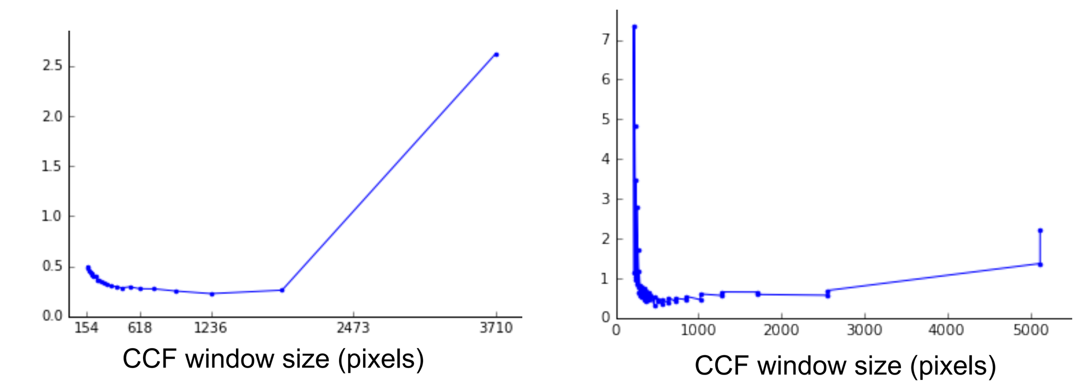


Premise:



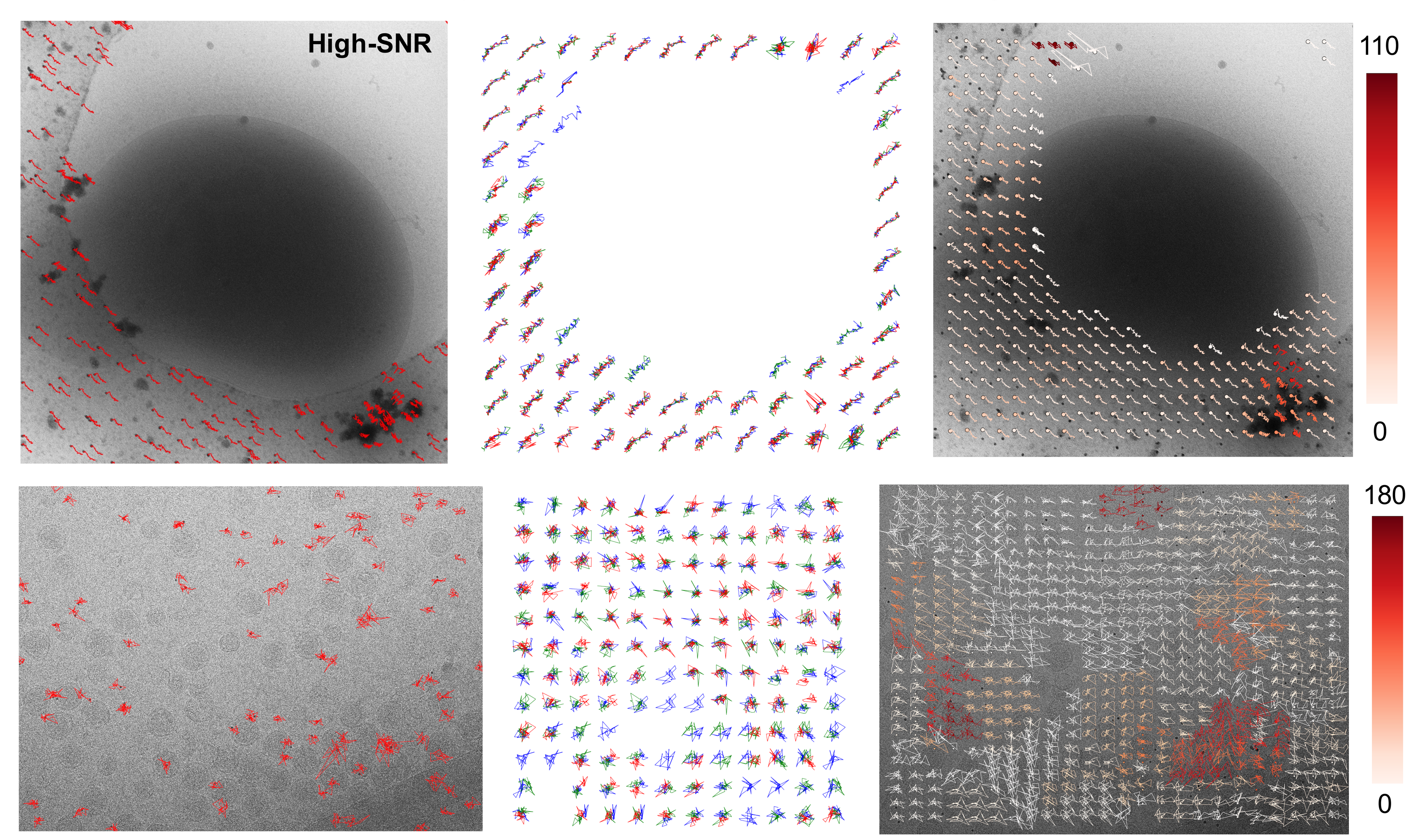
Above: *Comparison of tracked nanogold trajectories and computed whole-frame alignments.* Results indicate strong similarity, differing by a maximum of 1.224pix in a high SNR case and 2.778pix in a low SNR case. As shown in the upper right corners, as SNR decreases, the variance of the measured trajectories increase proportionate to the noise level present in the image.

Right: *Mean distance between trajectories of original data and frames with gold erased as a function of cross correlation window size.* Window sizes larger than 500 generally produce better agreement with local gold motion. Smaller windows likely provide insufficient signal for alignment, and larger windows approaching the size of the whole frame likely include information from locations where there are no gold particles. (See **Optimal CCF Window**)



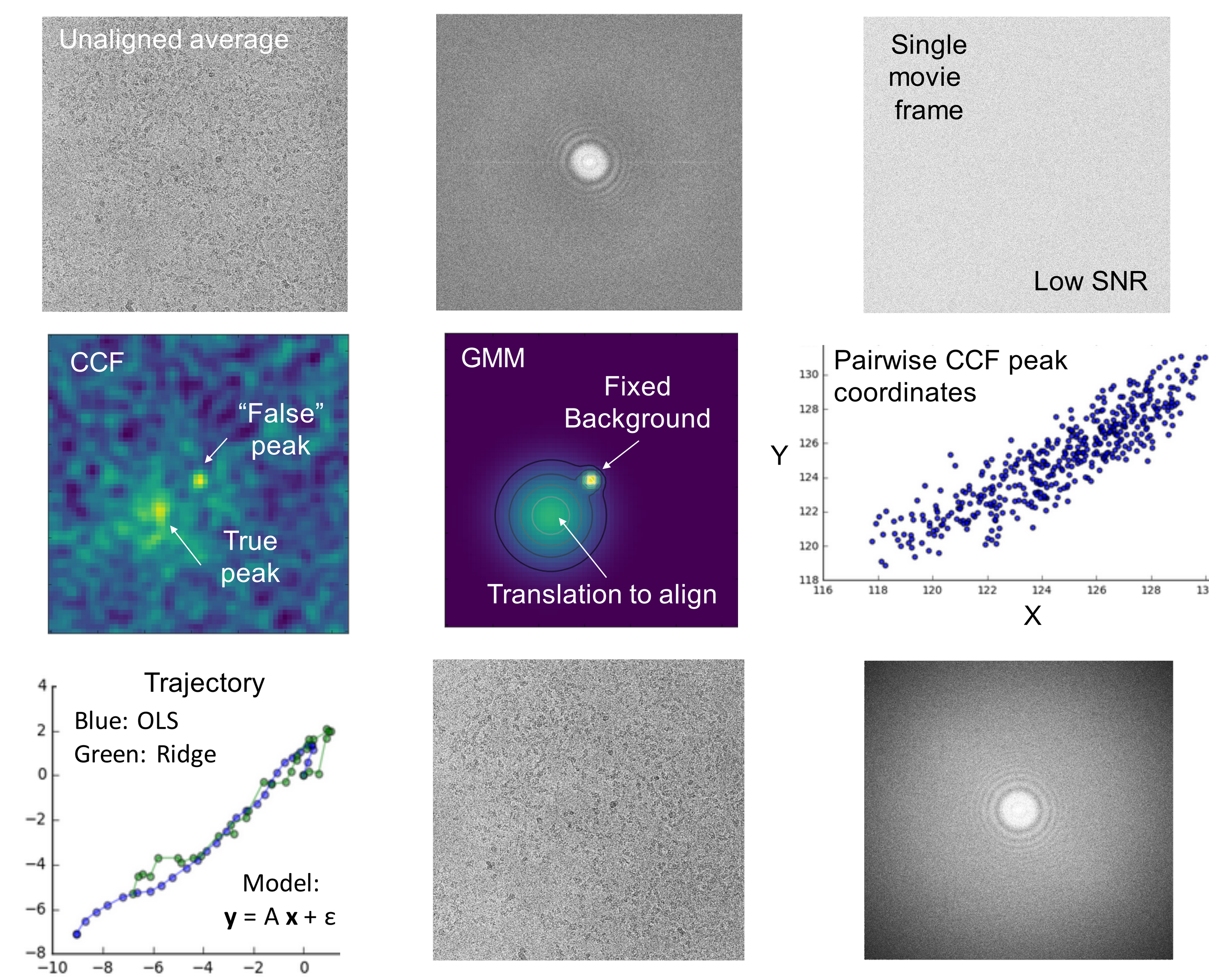
Analyzing variations in local motion

We measured the similarity of the 3 nearest nanogold cluster trajectories falling within a specified radius of points on a grid. Computing and summing the pairwise distance between these neighboring trajectories, we obtained a similarity score for each set of 3 neighboring trajectories corresponding to the amount of variability among gold trajectories at near each point in the grid. High-SNR results computed where gold is positioned largely on an amorphous carbon substrate shows little local variability except in regions with contaminants. Conversely, the low SNR example below shows significant variability and region-specific differences between gold trajectories. Further analysis of higher SNR data is required to understand the extent of local variability and the influence of noise on its observed magnitude.



Above: *Analysis of variations in local trajectories in high and low-SNR datasets.* The leftmost column depicts individual gold trajectories from two datasets (exaggerated by 10x). The central column shows a 12x12 grid of points and the 3 nearest gold trajectories within 512 pixels of each point. Note that empty space means that no gold particles were present within 512 pixels of that grid point. The right column shows the similarity score plotted in red on top of local trajectories. Local variations are distinguished both by differences in shape and color.

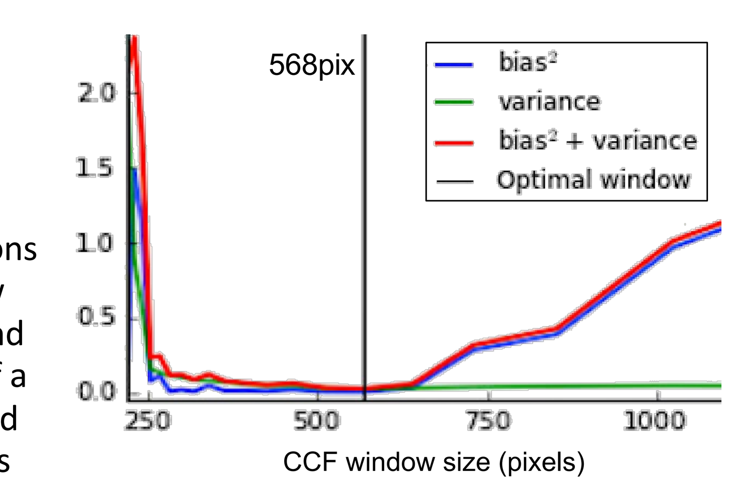
Robust whole-frame alignment



The power spectra of unaligned movies reveal degraded/missing intensity and frame alignment is required to recover it. Because individual DDD movie frames are incredibly noisy, we rely on image tiling to obtain improved sampling of the power spectrum. Using the average of these tiles, we can compute the pairwise cross correlation (CCF) between movie frames. Because these CCF lack strong signal and possess a strong "false" peak corresponding to a fixed background, we have opted to model each CCF image as a mixture of two Gaussians, which simultaneously improves peak finding and prevents mistaking the fixed background peak for the true CCF peak. Once all pairwise peaks are obtained, an ordinary or robust least squares algorithm can be used to calculate the frame trajectory assuming a simple linear model. Measured translations are ultimately applied to the frames and averaged in preparation for further processing. Future work will extend this approach to perform local frame alignment.

Optimal CCF window

Alignments based on cross correlation depend heavily on the window size. As the window increases toward the dimensions of an entire frame, CCF peak detection improves, but local variation is no longer captured. On the other hand, as the window shrinks, peak detection becomes more difficult, but more local variations are captured. To determine an optimal window size, we minimize the tradeoff between bias and variance, where bias describes the similarity of a local trajectory to the whole-frame solution and variance measures the variability of trajectories computed patch-wise across all frames.



Conclusions

We have shown that nanogold particles can be used as a pseudo-ground truth for frame alignment. We have also explored their utility as a means of understanding locally correlated motions in DDD movies. Nonetheless, we still need to identify methods to correct for local variations in locally correlated motion, which are not accounted for by current algorithms. In the coming months, we will be collecting higher SNR frames to test these validation and analysis methods toward a better understanding of cryoEM specimen motion.

Acknowledgements

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