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Merging Focal Pairs for Improved Particle Selection and Orientation Determination

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In single particle electron cryomicroscopy, radiation damage is virtually always the factor limiting the resolution of the final reconstruction. When attempting to achieve resolutions close to the limits of the available instrument, both dose and defocus must be carefully controlled to insure both that the specimen is not damaged at the desired resolution and that the envelope function of the microscope does not fall off too rapidly. Even on microscopes equipped with field emission guns, very far from focus micrographs do not extend to as high a resolution as those close to focus. However, the low overall contrast of close to focus micrographs often prevents accurate particle orientation determination, and in some cases even locating the particle within the micrograph or CCD frame becomes impossible.

Historically a popular approach for dealing with this issue is to collect a focal pair. The initial, low damage, micrograph is collected sufficiently close to focus as not to compromise resolution. A second micrograph of the identical field is then collected much further from focus. Generally this second micrograph is used to locate particles, and in some cases, particularly icosahedral particles, used to determine initial estimates of the 3D particle orientation.

We present the next logical step in the evolution of this technique. For projects pushing the frontiers of resolution, it is still often desirable to collect such focal pairs, since they allow recording of images with the best possible high-resolution information, and still obtaining some amount of high contrast low-resolution information. Rather than treating the micrographs separately or performing a simple average of the micrographs (which has been proposed previously), we apply the robust CTF correction procedure used by EMAN [1,2] to the process of combining the two images optimally. The combined image then replaces the far from focus image in standard reconstruction techniques.

The merging process occurs in three phases. First, the second micrograph is aligned to the first using a routine which can rapidly align two very large (sometimes over 12,000x10,000 pixels in size) images to 1 pixel accuracy. Second, both micrographs are analyzed to determine CTF, envelope function and Noise paramaters within EMAN's standard model. Finally, the images are combined with CTF correction and optional Wiener filtration.

When Wiener filtration is applied as part of the merging process, the resulting image has optimized contrast. Such images are useful for eliminating false positives often generated by single particle autoboxing techniques. The individual particles can be distinguished more easily, and in many cases, it is possible to distinguish damaged or otherwise unsuitable particles.

For orientation determination, Wiener filtration is not used, since it suppresses much of the high resolution information required for accurate orientations. While the incorporation of the damaged second image may be unsuitable for use directly in high-resolution studies, it can, at least, provide initial values for particle orientation, which can then be refined using image data from the first

micrograph alone. The merging process allows one to take advantage of both micrographs rather than just using the low resolution information from the second microgrpah.

This technique is available in the standard EMAN (http://ncmi.bcm.tmc.edu/~stevel/EMAN/doc) distribution. Future work will include more automation of the merging process, and additional testing of the reliability of the orientations determined from the merged particles.

[1] S.J. Ludtke, P.R. Baldwin, and W. Chiu, "EMAN: Semiautomated Software for High-Resolution Single-Particle Reconstructions," J. Struct. Biol. 128: 82-97 (1999).
[2] S.J. Ludtke, J. Jakana, J. Song, D.T. Chuang and W. Chiu. "An 11.5 Å Single Particle Reconstruction of GroEL Using EMAN". J. Mol. Biol., 314:241-250 (2001).
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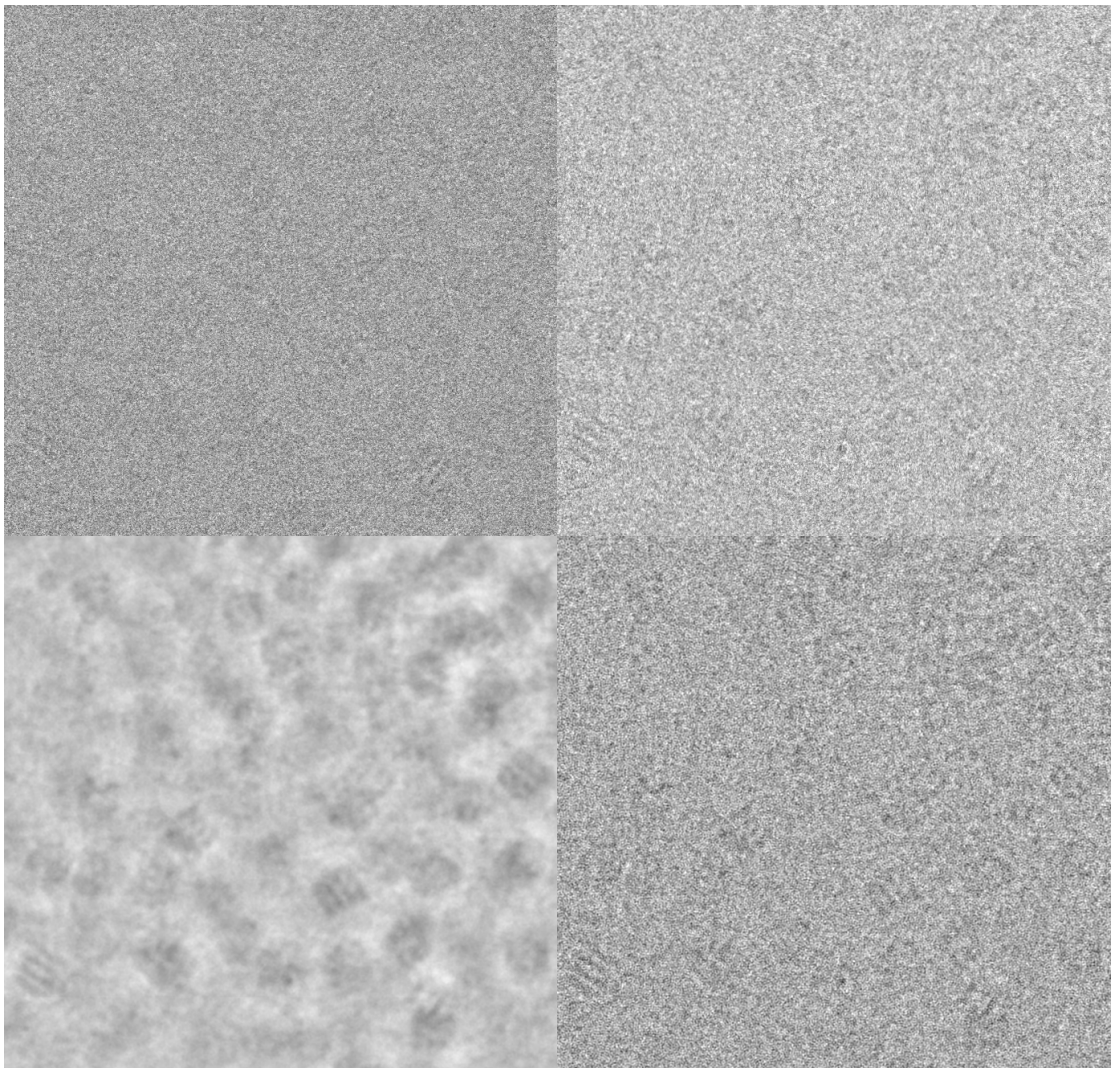

Figure 1- An example of focal pair merging. All four frames represent the same field from a pair of micrographs. In the upper left and right are the original close and far from focus images. In the lower left is the merged image with Wiener filter and in the lower right is the merged image without Wiener filter.