# Improvements to high resolution refinement in EMAN2.12

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## Abstract

EMAN2.12 is an extensible software suite designed primarily for performing high resolution single particle analysis (SPA) of electron cryomicroscopy (cryoEM) data [1,3]. While canonical refinement in EMAN2 has undergone extensive development since its inception, recent technological advances in image acquisition via direct detection devices have thoroughly changed the nature of image processing in the field of cryoEM. Furthermore, community-wide efforts to validate published maps through the Electron Microscopy Data Bank (EMDB) Map Challenge have presented an opportunity to improve our software. As a result, we have developed novel 2D classification-based schemes for "bad" particle removal, enhanced our 3D masking and radial correction routines, and improved our iterative amplitude correction strategy for high resolution refinements. These combined changes enable EMAN2 users to refine protein structures to resolutions that compete and even surpass the current state of the art.

## Classification of "Bad" Particles

We have devised a strategy for removing outlying particles that disagree significantly with a preliminary 3-D reconstruction. First the Fourier ring correlation is computed between each particle and its corresponding projection from a preliminary 3-D map and integrated over four resolution bands from low to high resolution. We have observed a characteristic shape in this 4-dimensional distribution which is readily classified using K-means and visualized in 2-D (Figure 1). By excluding the low quality "arm" of this distribution and performing additional refinements using the remaining particles, we improve resolution as measured by gold-standard FSC and enhance the interpretability of the resulting map. In the case of TRPV1, we eliminated 18006 particles using this method, improving our resolution from 4.5Å to 3.9Å angstroms according to our even/odd FSC with corresponding improvements to real space features (Figure 4).

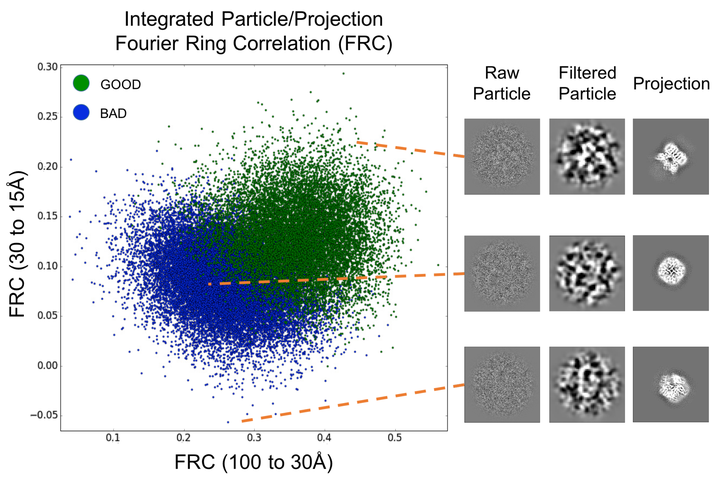


**Figure 1:** Left: K-means clustering (K=2) of "good" and "bad" TRPV1 particles on the basis of multi-resolution integrated FRC with respect to map projections. Right: Representative raw and filtered particles and corresponding projections with rotations and translations for visual comparison.

## "Flattening" Amplitude Correction

To compensate for the "B-factor" falloff of high resolution due to experimental and computational factors, EMAN2's canonical approach has been to impose a 1-D canonical structure factor at high resolution. However, at resolutions beyond 6Å it becomes possible to use a more pure data-based strategy instead [2]. We accomplish this by forcing the structure factor to be essentially flat beyond 15Å. To avoid over-amplification of noise during this process, we apply a cutoff as well as a Wiener filter based on the FSC between even and odd maps produced during gold-standard refinements in EMAN2.

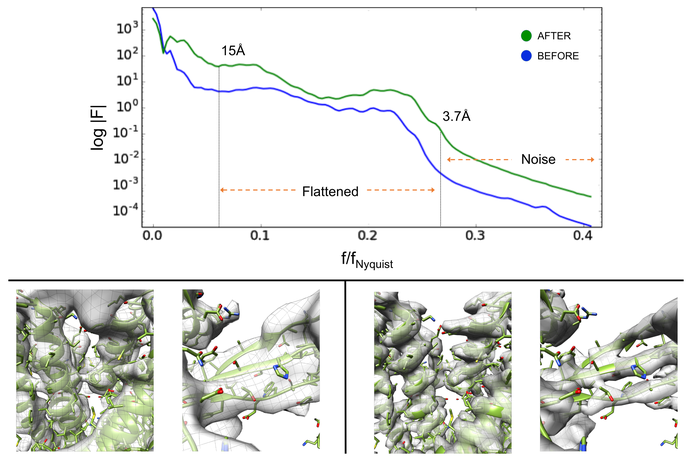


**Figure 2:** (Top) A Guinier plot of the spherically averaged Fourier space amplitude computed using "structure factor" (blue) and "flattening" (green) amplitude correction. (Bottom) TRPV1 features (Left) before and (Right) after iterative amplitude correction.

## Radial Kernel Compensation

EMAN performs 3-D reconstructions using a method called Direct Fourier Inversion, which involves local interpolation in Fourier Space. We have modified this kernel and added a new real-space compensation to reduce radial density falloff in the final map, and reduce the need for postprocessing corrections. Note how the noise in image B remains smooth at high radius, whereas it decays rapidly in A. This effect is subtle but extends into the structure.

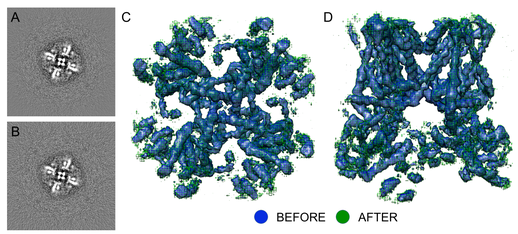


**Figure 3:** Central 2-D slices (133/256) of TRPV1 reconstructed (A) without and (B) with radial correction. (C) 3-D Bottom and (D) side views of TRPV1 reconstructed with and without radial correction.

## Combined Results

All improvements have been applied to TRPV1 as part of the 2015-2016 EMDB Map Challenge. An visual overview of our results using the aforementioned improvements is shown below.

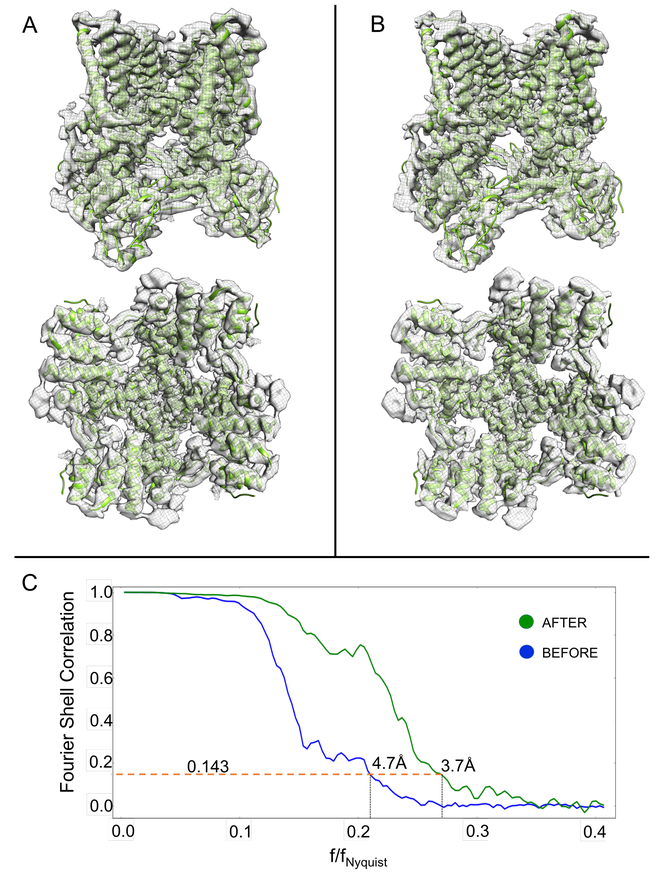


**Figure 4:** Real space features of TRPV1 refined to pseudo-convergence (A) before and (B) after improvements. (C) Gold-standard Fourier shell correlation (FSC) resolution measure of TRPV1 before and after improvements.

## References and Acknowledgments

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[3] G. Tang, L. Peng, P. R. Baldwin, D. S. Mann, W. Jiang, I. Rees, and S. J. Ludtke. EMAN2: An extensible image processing suite for electron microscopy. *Journal of Structural Biology*, 157(1):38–46, 2007.

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