

Response to Reviewers' Comments

April 3, 2016

We thank both reviewers for their valuable feedback which was extremely helpful. We have incorporated their suggestions into the paper to improve both content and readability of our paper. Below we first comment on some overall improvements and changes that we made to the algorithm, followed by point-by-point reply to the reviewers' comments.

Improvements to the Manuscript

Details about using conjugate gradient to solve eqn. 9

While L in eqn. 9 is PSD, the new effective operator in the LHS of eqn. 11 is not necessarily PSD in general. In order to use conjugate gradient, we solve the system $S^{-1}L(S^{-1}\Sigma_S S^{-1})S^{-1} = S^{-1}MS^{-1} - I$, where $\Sigma_S = S\Sigma S$, in which the operator acting on Σ_S in the LHS is PSD. Σ is then obtained from the estimated Σ_S . We note that this change did not effect whatsoever the output of the algorithm, but we wanted the reviewers to be aware of this small change.

Outlier detection

The reviewers raised a very important question of how outliers are affected by the denoising procedure. This is a very interesting question, and we were curious to see the results ourselves. We find that outlier images such as pure noise images have relatively low contrast after denoising using CWF. So a simple linear classifier based on the image contrast can be employed for outlier detection. More complicated situations such as outliers from a different class of a heterogeneous dataset are difficult to distinguish with denoising. We have added an experiment in section 3.7 for detecting pure noise images as outliers, to demonstrate how CWF can be extended to build a classifier for outlier detection.

Response to Reviewer 1

- Page 2: "The first is a heuristic approach known as phase flipping". I would remove the word heuristic, since correcting for the sign, although not perfect, is already a well-justified correction.

As per the reviewer’s suggestion, we have dropped the word ‘heuristic’.

- In the theoretical justification of the method, one of the main assumptions is that the X_i and ξ_i coefficients are iid random variables. X_i are not independent of each other (see C.O.S. Sorzano, J. Vargas, J. Otón, V. Abrishami, J.M. de la Rosa-Trevín, S. del Riego, A. Fernández-Alderete, C. Martínez-Rey, R. Marabini, J.M. Carazo. Fast and accurate conversion of atomic models into electron density maps. AIMS Biophysics, 2: 8-20 (2015)) and they are not identically distributed (it is well-known that Fourier components of low frequency have more energy than Fourier components of high frequency). Also, the assumption of iid ξ_i variables is not well supported in EM in which we normally have noise before CTF (and consequently affected by CTF) and noise after CTF (which is not white, see C.O.S. Sorzano, S. Jonic, R. Núñez, N. Boisset, J.M. Carazo. Fast, robust and accurate determination of transmission electron microscopy contrast transfer function. Journal Structural Biology 160: 249-262 (2007)). Despite of all these assumptions, which are not fulfilled in EM, it is better to have a method with incorrect assumptions but yielding useful results, than no method at all. And this seems to be the case with this algorithm.

We agree with the reviewer, and to clarify this we have made a note about our assumptions in section 2.1. We also now cite the above mentioned references. In this context, it makes sense to recall the famous saying of George Box: “All models are wrong but some are useful.”

- In the article, it is not well remarked that the Epsilon covariance matrix is supposed to represent the covariance of the Fourier coefficients of projection images irrespective of their orientation. This assumption implies an increase of variance (due to the different orientations), in addition to the variance due to the different frequencies, which is not well explained in the article.

We agree with the reviewer that this point was not well enough explained in the paper. We have added the required clarification about covariance due to different orientations to Section 2.1, please see the sentence beginning with “Since the clean images are 2D projections of the 3D molecule in different orientations...”

- Eq. 13. Since there is an important source of noise affected by the CTF, a purely “whitening” filter would boost the noise close to the CTF zeroes, and it would not be possible to have $W\xi_i$ distributed as $\mathcal{N}(0, \sigma^2 I)$. How to construct the whitening filter should be better explained.

The whitening filter is constructed by estimating the 2D isotropic power spectral density of noise. To do this, we first estimate using correlograms the 2D autocorrelation of the corner pixels of the images which contain mostly noise and no signal. These

corner pixels are used to estimate the 1D autocorrelation, which is then extended to populate the 2D isotropic autocorrelation. We have added a description of how W is constructed in section 2.2.

- It would be very interesting to see how the method deals in practice with contaminating images, further violating the method assumptions (for instance, images with just noise and no particles, particles with other particles in their surroundings, images with objects not being particles; this kind of images are easily obtained by automatic picking articles and they normally represent between 10 – 30% of the collected data).
Please see ‘Outlier Detection’ in the ‘Improvements to the Manuscript’ section.
- Although maybe out of the scope of this paper, it would be very interesting to see if the restored particles can be used for 2D classification and even 3D reconstruction without a significant loss of structural information. At least, the authors could comment on this issues as future work.
Indeed, this is a very important direction to pursue, and it is included in our plan for immediate future work. We have added a few directions for future work in section 4.
- In any case, the results shown by the authors are quite promising and the fact that EM images do not fulfill the theoretical assumptions of the method does not invalidate its results. The restored images are certainly closer to the ideal images than the acquired projections.
Thanks, we completely agree.

Response to Reviewer 2

- People spend time looking at their particle images to discover outliers. It would be great if denoising helps this. It would be useful in your simulations to show what outliers look like when denoised. For example, what do pure-noise images look like when denoised? Will they just look like μ ? What if rare (a few % of total) images are just blobs, could one distinguish them?
Please see ‘Outlier Detection’ in the ‘Improvements to the Manuscript’ section.
- Similarly, in the heterogeneous 70S dataset, do the denoised images demonstrate the heterogeneity? That is, could one classify the denoised images correctly if one knew what the different underlying structures looked like? Apart from this issue, there seems little need to add this fourth dataset.
The current CWF algorithm is not expected to be able to distinguish heterogeneous particles based on their denoised images. We have added a comment to that effect in section 3.7. We show results with 2 datasets each for new and old cameras simply to

convince the readers of the applicability of CWF to experimental datasets.

- As ice thickness varies, the contrast in the particle images varies by as much as a factor of 2. What would that do to the denoising? I guess this is related to the question of what pure-noise images look like.

The contrast factor due to variability in ice thickness can be modeled as an additional scalar parameter and absorbed into the random variable X in the simulation. CWF estimates the denoised image with its contrast included. To demonstrate this, we include varying contrast in the outlier detection experiment in section 3.7.

- In a sense you are doing something similar to class averaging. Class-averaging is nice because it allows the user to discover classes of "bad" particles and delete all the underlying images from the dataset before reconstruction. I'm trying to imagine how the use of the denoising procedure would fit into the workflow for SPR. For example, would there be a way to look at the "neighborhood" of bad particles, say in the space of eigenvectors of Σ , to be able to exclude them? Also, have you through this work discovered a better way to do class averaging in the first place?

Indeed, this is a very important direction to pursue, and it is included in our plan for immediate future work. Including CWF in the class averaging procedure is certainly at the top of our list for the next steps, as we remark in section 4.

- I'm left a little nervous by the eigenvalue thresholding procedures, not knowing what they are doing to the results. Maybe you could give an example of the effect on the results of taking different sample sizes n , to show that the shrinkage procedure isn't introducing artifacts.

Shrinkage was first shown to improve covariance estimation by Stein [1, 2], and is especially crucial in the high dimensional setting [3] such as in this paper. We demonstrate the usefulness of the eigenvalue thresholding procedure in Figure 3, by showing the improvement in accuracy of the covariance estimation when eigenvalue shrinkage is included.

- Maybe you should discuss briefly that you are not considering translations, just in-plane rotations in modeling the images.

The algorithm does not assume centered images; however non-centered images would lead to further variability and therefore an additional blurring effect. We have added a comment to that effect at the beginning of section 3.

- Page 9, middle. I'm confused by the statement " p_k decreases with increasing k ." I'd expect the opposite.

k is the angular frequency, and p_k is the maximum radial frequency for a given k . Because the images are bandlimited, in

order to avoid aliasing, for large angular frequencies we cannot allow large radial frequencies. The exact relation is given in eqn. 13 of [4].

- Page 12, middle. Please explain more what you mean by MSE. Is it the norm of the raw image minus the CTF-filtered denoised version?
Indeed, the MSE is the norm of the difference between the true, clean image and its denoised version. We have added a sentence explicitly defining the MSE in the Results section.

References

- [1] C. Stein, Lectures on the theory of estimation of many parameters, Journal of Soviet Mathematics 34 (1) (1986) 1373–1403. doi:10.1007/BF01085007. URL <http://dx.doi.org/10.1007/BF01085007>
- [2] C. Stein, Some problems in multivariate analysis, Technical report, Department of Statistics, Stanford University, 1956.
- [3] D. Donoho, M. Gavish, I. M. Johnstone, Optimal shrinkage of eigenvalues in the spiked covariance model, CoRR abs/1311.0851. URL arxiv.org/abs/1311.0851
- [4] Z. Zhao, Y. Shkolnisky, A. Singer, Fast steerable principal component analysis, IEEE Transactions on Computational Imaging 2 (1) (2016) 1–12. doi:10.1109/TCI.2016.2514700.