

AN IMPROVED ALGORITHM FOR CLASS AVERAGING OF 2D CRYO-EM IMAGES

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ABSTRACT

Single particle reconstruction (SPR) from cryo-electron microscopy (EM) is a technique in which the 3D structure of a molecule needs to be determined from its CTF-affected, 2D projection images taken at unknown viewing directions. One of the main challenges in cryo-EM is the typically low signal to noise ratio (SNR) of the acquired images. 2D classification of images, followed by class averaging, improves the SNR of the resulting averages, and allows inspection of the dataset [1]. We introduce a new metric, akin to the Mahalanobis distance, to compare cryo-EM images belonging to different defocus groups. The new metric is employed to rank nearest neighbors, thereby leading to an improved algorithm for class averaging. We evaluate the performance of the improved class averaging on synthetic datasets and note an improvement compared to [1].

Index Terms— Cryo-electron microscopy, class averaging, Mahalanobis distance, denoising, CTF.

1. INTRODUCTION

SPR from cryo-EM is a rapidly advancing technique in structural biology to determine the 3D structures of macromolecular complexes in their native state, without the need for crystallization. First, the sample, consisting of randomly oriented, nearly identical copies of a macromolecule, is frozen in a thin ice layer. An electron microscope is used to acquire top view images of the sample, in the form of a large image called a 'micrograph', from which individual particle images are picked semi-automatically. After preprocessing the acquired raw projection images, the preprocessed images are next classified, and images within each class are averaged (known in the cryo-EM community as "class averaging"), to obtain a single image per class, that enjoys a higher signal to noise ratio (SNR) than the individual images. To minimize radiation damage, cryo-EM imaging must be constrained to low electron doses, which results in a very low SNR in the acquired 2D projection images. Class averaging is thus a crucial step in the SPR pipeline; class averages are used for a preliminary inspection of the dataset, to eliminate outliers.

In [1], the authors introduced a new rotationally invariant representation to compute the distance between pairs of

cryo-EM images. The images are first expanded in a steerable basis. Next, the bispectrum is computed from this expansion. After projecting the computed bispectrum to a lower dimensional space using a randomized PCA algorithm, the distance between images is computed as the Euclidean distance between their rotationally invariant representation. This completes the initial round of 2D classification.

The nearest neighbors detected at this stage can suffer from many outliers at high levels of noise. The initial classification is next improved by checking the consistency of in-plane rotations along several paths between nearest neighbors, by employing Vector Diffusion Maps (VDM). Finally, the detected nearest neighbors are aligned in-plane and averaged. The resulting class averages enjoy a much higher SNR than the input raw images, thereby allowing inspection of the dataset and elimination of outliers. Class averages are used for subsequent stages of the SPR pipelines, such as viewing angle detection, and finally, determination of the 3D structure.

Recently [2], it was shown that this preliminary inspection can in fact be performed at an earlier stage, by better denoising the acquired images using an algorithm called Covariance Wiener Filtering (CWF). In CWF, the covariance matrix of the underlying clean projection images is estimated from their noisy observations. This estimated covariance is then used in the classical Wiener filtering framework to obtain denoised images, which can be used for a preliminary viewing of the underlying dataset, and outlier detection.

There are two main contributions of this paper. First, we introduce a new metric, which can be viewed as a Mahalanobis distance, to compute the distance between pairs of cryo-EM images. Second, we use the proposed Mahalanobis distance to improve the class averaging algorithm described in [1]. We first obtain a list of P nearest neighbors using the algorithm described above (see section 2 for details), and then rank these neighbors using the Mahalanobis distance. The top Q nearest neighbors, where $Q < P$, given by this procedure are finally aligned and averaged to produce class averages. We test the new algorithm on synthetic datasets and observe an increase in the number of nearest neighbors correctly detected.

2. BACKGROUND

2.1. Image Formation Model

Under the linear, weak phase approximation, the image formation model in cryo-EM is given by

$$y_i = a_i \star x_i + n_i \quad (1)$$

where y_i is the noisy projection image in real space, x_i is the underlying clean projection image in real space, a_i is the point spread function of the microscope, and n_i is additive Gaussian noise that corrupts the image. ~~It is more common to work with Fourier space images,~~ and \star denotes the convolution operation. In the Fourier domain, images are multiplied with the contrast transfer function (CTF), and eqn.(1) can be rewritten as

$$Y_i = A_i X_i + N_i \quad (2)$$

2.2. Class Averaging

The procedure for class averaging, described in [1], consists of 3 main steps. First, Fourier Bessel Steerable PCA is used to denoise images and estimate the 2D covariance matrix of the images and their in-plane rotations. Phase flipping is used as a CTF correction scheme. Second, the bispectrum of the expansion coefficients in the steerable basis is computed. The bispectrum is a rotationally invariant representation of images, but is typically of very high dimensionality. It is projected on to a lower dimensional subspace using a fast, randomized PCA algorithm. One way to compare the distance between images after this step is to use the normalized cross correlation. However, this method suffers from outliers in the nearest neighbor detection at very low SNR's. So, the third step uses Vector Diffusion Maps to improve the initial classification by viewing angles.

2.3. Covariance Wiener Filtering (CWF)

CWF was proposed in [2] as an algorithm to (i) estimate the CTF-corrected covariance matrix of the underlying clean 2D projection images and (ii) using the estimated covariance to solve the associated deconvolution problem in 2 to obtain denoised images, that are estimates of X_i for each i in 2. The first step involves estimating the mean image of the dataset, μ , as $\hat{\mu}$, followed by solving a least squares problem to estimate the covariance Σ as $\hat{\Sigma}$. Under the assumption of additive white Gaussian noise, the estimate of the underlying clean image X_i is given by

$$\hat{X}_i = (I - H_i A_i) \hat{\mu} + H_i Y_i \quad (3)$$

2.4. Fourier Bessel Steerable PCA

For both class averaging and CWF, the images are expressed in the Fourier Bessel basis. Since the basis elements are outer

~~products of radial functions and angular Fourier modes, the covariance matrix is block diagonal in this choice of basis. Therefore each block of the covariance, corresponding to a particular angular frequency, can be computed separately, making the computation more efficient.~~

3. IMPROVED CLASS AVERAGING

3.1. A New Metric: Mahalanobis distance

Our goal is to define a metric to compare how close any two cryo-EM images are, that is, given the CTF-affected, noisy observations for a pair of images

$$\begin{aligned} Y_1 &= H_1 X_1 + N_1 \\ Y_2 &= H_2 X_2 + N_2, \\ X_1, X_2 &\sim N(\mu, \Sigma) \\ N_1, N_2 &\sim N(0, \sigma^2 I_n) \end{aligned} \quad (4)$$

we define a metric that gives a notion of distance between X_1 and X_2 . In our statistical model, the underlying clean images X_1, X_2, \dots, X_n (where n is the total number of images) are assumed to be independent, identically distributed (i.i.d.) samples drawn from a Gaussian distribution. Further, we assume that the noise in our model is additive white Gaussian noise.

$$\begin{aligned} X_1, X_2 &\sim N(\mu, \Sigma) \\ N_1, N_2 &\sim N(0, \sigma^2 I_n) \end{aligned} \quad (5)$$

We denote the covariance of Y_1 and Y_2 by K_1 and K_2 .

$$\text{Cov}(Y_1) = H_1 \Sigma H_1^T + \sigma^2 I_n = K_1 \quad (6)$$

$$\text{Cov}(Y_2) = H_2 \Sigma H_2^T + \sigma^2 I_n = K_2 \quad (7)$$

Using the Gaussian property, we have the following probability density functions (pdf)

$$f_{X_1}(x_1) = \frac{1}{(2\pi)^{\frac{n}{2}} |\Sigma|^{\frac{1}{2}}} \exp\left\{-\frac{1}{2}(x_1 - \mu)^T \Sigma^{-1}(x_1 - \mu)\right\} \quad (8)$$

$$f_{X_2}(x_2) = \frac{1}{(2\pi)^{\frac{n}{2}} |\Sigma|^{\frac{1}{2}}} \exp\left\{-\frac{1}{2}(x_2 - \mu)^T \Sigma^{-1}(x_2 - \mu)\right\} \quad (9)$$

$$f_N(y_1 - H_1 x_1) = \frac{1}{(2\pi)^{\frac{n}{2}} \sigma^n} \exp\left\{-\frac{1}{2}(y_1 - H_1 x_1)^T \frac{1}{\sigma^2}(y_1 - H_1 x_1)\right\} \quad (10)$$

$$f_N(y_2 - H_2 x_2) = \frac{1}{(2\pi)^{\frac{n}{2}} \sigma^n} \exp\left\{-\frac{1}{2}(y_2 - H_2 x_2)^T \frac{1}{\sigma^2}(y_2 - H_2 x_2)\right\} \quad (11)$$

$$f_{Y_1}(y_1) = \frac{1}{(2\pi)^{\frac{n}{2}} |K_1|^{\frac{1}{2}}} \exp\left\{-\frac{1}{2}(y_1 - H_1 \mu)^T K_1^{-1}(y_1 - H_1 \mu)\right\} \quad (12)$$

$$f_{Y_2}(y_2) = \frac{1}{(2\pi)^{\frac{n}{2}} |K_2|^{\frac{1}{2}}} \exp\left\{-\frac{1}{2}(y_2 - H_2 \mu)^T K_2^{-1}(y_2 - H_2 \mu)\right\} \quad (13)$$

$$\begin{bmatrix} X_1 \\ Y_1 \end{bmatrix} = \begin{bmatrix} I & 0 \\ H_1 & I \end{bmatrix} \times \begin{bmatrix} X_1 \\ N_1 \end{bmatrix} \quad (14)$$

$$\sim N \left[\begin{bmatrix} \mu \\ H_1 \mu \end{bmatrix}, \begin{bmatrix} \Sigma & \Sigma H_1^T \\ H_1 \Sigma & H_1 \Sigma H_1^T + \sigma^2 I \end{bmatrix} \right] \quad (15)$$

Using conditional distributions

$$f_{X_1|Y_1}(x_1|y_1) \sim N(\alpha, L) \quad (16)$$

$$f_{X_2|Y_2}(x_2|y_2) \sim N(\beta, M) \quad (17)$$

where

$$\begin{aligned} \alpha &= \mu + \Sigma H_1^T (H_1 \Sigma H_1^T + \sigma^2 I)^{-1} (y_1 - H_1 \mu) \\ L &= \Sigma - \Sigma H_1^T (H_1 \Sigma H_1^T + \sigma^2 I)^{-1} H_1 \Sigma \\ \beta &= \mu + \Sigma H_2^T (H_2 \Sigma H_2^T + \sigma^2 I)^{-1} (y_2 - H_2 \mu) \\ M &= \Sigma - \Sigma H_2^T (H_2 \Sigma H_2^T + \sigma^2 I)^{-1} H_2 \Sigma \end{aligned} \quad \text{So}$$

$$P(x_1 - x_2 | y_1, y_2) \sim N(\alpha - \beta, L + M) \quad (18)$$

Let $X_1 - X_2 = X_3$. Then

$$\begin{aligned} P(\|x_3\|_\infty < \epsilon | y_1, y_2) &= P(\|x_3\|_\infty < \epsilon | y_1, y_2) \\ &= \frac{1}{(2\pi)^{\frac{n}{2}} |L + M|^{\frac{1}{2}}} * \\ &= \int_{-\epsilon}^{\epsilon} \exp\left\{-\frac{1}{2}(x_3 - (\alpha - \beta))^T (L + M)^{-1} (x_3 - (\alpha - \beta))\right\} dx_3 \end{aligned} \quad (19)$$

For small ϵ this is

$$= \frac{(2\epsilon)^n}{(2\pi)^{\frac{n}{2}} |L + M|^{\frac{1}{2}}} \exp\left\{-\frac{1}{2}(\alpha - \beta)^T (L + M)^{-1} (\alpha - \beta)\right\} \quad (20)$$

So we can define our metric after taking the logarithm on both sides of eqn.(20) and dropping out the constant term

$$-\frac{1}{2} \log(|L + M|) - \frac{1}{2} (\alpha - \beta)^T (L + M)^{-1} (\alpha - \beta) \quad (21)$$

4. ALGORITHM FOR CLASS AVERAGING

Algorithm 1 My algorithm

1: **procedure** MYPROCEDURE

5. NUMERICAL EXPERIMENTS

Note that α and β defined above are denoised images obtained from CWF after deconvolution. In practice, we use α, β, L, M projected onto the subspace spanned by the principal components (of the respective angular frequency block). We obtain an initial list of nearest neighbors for each image using the Initial Alignment procedure in ASPIRE (steerable PCA + bispectrum). This list is then refined using the metric defined above. We test this method on simulated projection images and observe an improvement in the nearest neighbors detected.

Table 1: Number of nearest neighbors with correlation > 0.9 , $N=5000$, $nnbor=10$, $nnbor \text{ large} = 50$

SNR	VDM		No VDM	
	New	Old	New	Old
1/40	26046	26585	26685	23192
1/60	15886	14045	15830	13520
1/100	6913	5619	7758	6901

Table 2: Number of nearest neighbors with correlation > 0.9 , $N=10000$, $nnbor=10$, $nnbor \text{ large} = 50$

SNR	VDM		No VDM	
	New	Old	New	Old
1/40	58361	56758	56831	49347
1/60	34965	3213	34537	29219
1/100	17262	14431	16057	13706

5.1. Synthetic data

We perform experiments on a synthetic dataset consisting of projection images generated

6. FUTURE WORK

7. CONCLUSION

8. REFERENCES

- [1] Z. Zhao and A. Singer, "Rotationally invariant image representation for viewing direction classification in cryo-EM," *Journal of Structural Biology*, vol. 186, no. 1, pp. 153 – 166, 2014.
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