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An adaptation of the Wiener filter suitable for analyzing images of isolated single particles

Charles V. Sindelar [1], Nikolaus Grigorieff *

Howard Hughes Medical Institute and Department of Biochemistry, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, 415 South Street, Waltham, MA 02454, USA

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ABSTRACT

The Wiener filter is a standard means of optimizing the signal in sums of aligned, noisy images obtained by electron cryo-microscopy (cryo-EM). However, estimation of the resolution-dependent ("spectral") signal-to-noise ratio (SSNR) from the input data has remained problematic, and error reduction due to specific application of the SSNR term within a Wiener filter has not been reported. Here we describe an adjustment to the Wiener filter for optimal summation of images of isolated particles surrounded by large regions of featureless background, as is typically the case in single-particle cryo-EM applications. We show that the density within the particle area can be optimized, in the least-squares sense, by scaling the SSNR term found in the conventional Wiener filter by a factor that reflects the fraction of the image field occupied by the particle. We also give related expressions that allow the SSNR to be computed for application in this new filter, by incorporating a masking step into a Fourier Ring Correlation (FRC), a standard resolution measure. Furthermore, we show that this masked FRC estimation scheme substantially improves on the accuracy of conventional SSNR estimation methods. We demonstrate the validity of our new approach in numeric tests with simulated data corresponding to realistic cryo-EM imaging conditions. This variation of the Wiener filter and accompanying derivation should prove useful for a variety of single-particle cryo-EM applications, including 3D reconstruction.

1. Introduction

Single-particle cryo-EM is increasingly used to produce high-resolution 2D and 3D maps of biological macromolecules. The raw data obtained by cryo-EM pose numerous technical challenges for the image processing done to obtain useful descriptions of the target molecules. Individual particle images exhibit extremely high levels of noise, owing to the extreme radiation sensitivity of biological specimens which in turn requires minimizing electron exposure in order to limit radiolysis. In addition, the image signal is itself scrambled by the microscope optics, as characterized by the Contrast Transfer Function (CTF) of the microscope, leading to partial or complete loss of the particle signal at regular intervals throughout Fourier space. Numerous techniques have been developed to address these challenges, but nevertheless the processing of cryo-EM images remains a topic of considerable research interest.

One of the early advances in single-particle cryo-EM was the application of digital signal processing theory, in order to improve estimates of the reconstructed particle density as well as to assess the quality of the reconstructions themselves (Frank, 2006). Frank and Ali described a connection between image correlation and signal-to-noise ratio (Frank and Al-Ali, 1975) that was subsequently extended to yield various resolution assessment techniques, including the Fourier Ring Correlation (FRC) for 2D projection averages, and the analogous Fourier Shell Correlation for 3D reconstructions (Harauz and van Heel, 1986). Numerous approaches have been used to compensate for CTF effects and high noise levels, including phase-flipping and iterative reconstruction.

One of the methods more commonly applied in reconstruction algorithms is the Wiener filter (Wiener, 1949; Kolmogorov, 1941), which is designed to produce estimates of signal measurements having the least possible mean-squared error, given some level of prior knowledge about the system such as the signal-to-noise ratio (SNR) of the images. The benefits of the Wiener filter are widely acknowledged, and numerous applications to various

Abbreviations: CCC, cross-correlation coefficient; CTF, contrast transfer function; FRC, Fourier Ring Correlation; PSSNR, single-particle SSNR; SNR, signal-to-noise ratio; SSNR, spectral signal-to-noise ratio.

* Corresponding author. Fax: +1 781 736 2419.
E-mail address: niko@brandeis.edu (N. Grigorieff).
[1] Present address: Department of Molecular Biophysics and Biochemistry, Yale University, 333 Cedar St., New Haven, CT 06520-8024, USA.

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Nomenclature

$\mathbf{r}$ real-space vector coordinates.
$\mathbf{s}$ Fourier-space vector coordinates.
$R = |\mathbf{s}|$ radius in Fourier space.
dR grid spacing used in digital Fourier space image representation.
N number of measured images.
$x^{(i)}(\mathbf{r})$ i'th image.
$m(\mathbf{r})$ noise-free particle image.
$n^{(i)}_{\text{particle}}(\mathbf{r})$ i'th specific instance of "particle" noise (i.e. signal fluctuations from the sample itself, such as embedding medium or support film); this is modulated by the CTF.
$n^{(i)}_{\text{image}}(\mathbf{r})$ i'th specific instance of "image" noise (from the measurement process); not CTF-modulated.
$n^{(i)}(\mathbf{r})$ effective noise contributed from both $n^{(i)}_{\text{particle}}(\mathbf{r})$ as well as $n^{(i)}_{\text{image}}(\mathbf{r})$.
$n_1(\mathbf{r}),\ n_2(\mathbf{r})$ summed noise from images $1 \ldots N/2$ and $N/2+1 \ldots N$, respectively.
$X^{(i)}(\mathbf{s}),\ M^{(i)}(\mathbf{s}),\ N^{(i)}_{\text{particle}}(\mathbf{s}),\ N^{(i)}_{\text{image}}(\mathbf{s}),\ N^{(i)}(\mathbf{s}),\ N_1(\mathbf{s}),\ N_1(\mathbf{s})$ refer to Fourier-space equivalents of the corresponding uncapitalized symbols.
$\text{CTF}^{(i)}(\mathbf{s})$ contrast transfer function for the i'th image.
$\hat{M}_W$ Wiener filter estimate derived from a series of N noisy images.
$k^{(i)}_W(\mathbf{r}),\ K^{(i)}_W(\mathbf{s})$ real-space and Fourier-space representations of the Wiener filter weighting function.
$\text{env}(\mathbf{r})$ real-space binary envelope function.
$\text{env}_{\text{smooth}}(\mathbf{r})$ envelope function obtained by applying a low-pass filter to $\text{env}(\mathbf{r})$.
$\text{ENV}(\mathbf{s})$ Fourier-space equivalent of $\text{env}(\mathbf{r})$.
$\text{FRC}_{\text{mask}}(\mathbf{r})$ FRC obtained when the compared images are both multiplied by $\text{env}_{\text{smooth}}(\mathbf{r})$.
SNR overall signal-to-noise ratio of an image.
$\text{SSNR}_{\text{no CTF}}(R)$ ratio of signal power (*prior* to CTF modulation) to noise power in raw data images.
$\text{SSNR}_{\text{merged}}(R)$ spectral signal-to-noise ratio in the final, averaged image.
$\hat{M}_{\text{SPW}}$ single-particle Wiener filter estimate derived from a series of N noisy images.
$k^{(i)}_{\text{SPW}}(\mathbf{r}),\ K^{(i)}_{\text{SPW}}(\mathbf{s})$ real-space and Fourier-space representations of the modified filter weighting function for the single-particle Wiener filter.
$f_{\text{particle}} = \langle \text{env}(\mathbf{r})^2 \rangle_{\text{image}}$ the fraction of a boxed image with non-zero signal corresponding to $m(\mathbf{r})$.
$f_{\text{smooth}} = \langle \text{env}_{\text{smooth}}(\mathbf{r})^2 \rangle_{\text{image}}$ the fraction of the image within $\text{env}_{\text{smooth}}(\mathbf{r})$.
$\text{PSSNR}(R) = \frac{1}{f_{\text{particle}}} \text{SSNR}_{\text{no CTF}}(R)$ "single-particle" SSNR corrected for the fractional area containing signal from the particle.
σ^2_{Rs} signal variance at Fourier radius R.
σ^2_{Rn} noise variance at Fourier radius R.
$\hat{\sigma}^2_{Rs}$ estimator of the signal variance σ^2_{Rs} (biased).
$\hat{\sigma}^2_{Rn}$ estimator of the noise variance σ^2_{Rn} (unbiased).
n_R number of Fourier pixels within a given resolution zone (R).

single-particle applications have been described in earlier work (Tang et al., 2007; Zhang et al., 2008). However, somewhat surprisingly, the benefits of the Wiener filter are rarely if ever quantified in comparison to other image restoration techniques, leaving it an open question how beneficial this filter is in practice. Perhaps related to this issue, it is commonly considered impractical to extract useful spectral SNR (SSNR) characteristics from data sets of aligned images alone (Downing and Glaeser, 2008); instead, earlier work has suggested that additional experimental information (X-ray scattering factors, for example) is necessary to obtain useful SSNR estimates for the purpose of applying a Wiener filter (Tang et al., 2007). In the absence of accurate SSNR estimates, an arbitrary constant term is commonly substituted for the SSNR expression within the Wiener filter (Grigorieff, 2007; Zeng et al., 2007; Frank, 2006), with the result that the filter no longer minimizes the mean-squared error of the particle estimate.

Here, we present a quantitative evaluation of the Wiener filter for combining pre-aligned cryo-EM images to produce estimates of the projected density. Our results demonstrate that for images of isolated single particles, the conventionally-defined Wiener filter fails to optimize the estimate of the particle density itself, owing to the presence of a substantial signal-free solvent region in the raw data images. We address this problem by developing a modified version of the filter, which we call the single-particle Wiener filter, which is designed to optimize the density estimate within a defined mask region when the SSNR characteristics of the raw images is available. We also present a straightforward method for obtaining accurate estimates of the average SSNR characteristics from the images themselves, with no need for additional experimental information, via a masked FRC calculation. Our new treatment of the Wiener filter thus establishes a self-contained method for defining a least-squares estimate of a single-particle density map from aligned image data sets.

2. Theory

2.1. Wiener filter expression

We begin with the derivation of the Wiener filter expression (Saxton, 1978). We consider a series of aligned images, whose signal and noise is modeled as follows:

$$\begin{aligned} x^{(i)}(\mathbf{r}) &= \text{FT}^{-1}\{\text{CTF}^{(i)}(\mathbf{s})\} * (m(\mathbf{r}) + n^{(i)}_{\text{particle}}(\mathbf{r})) + n^{(i)}_{\text{image}}(\mathbf{r}) \text{ (real space)} \\ X^{(i)}(\mathbf{s}) &= \text{CTF}^{(i)}(\mathbf{s})(M^{(i)}(\mathbf{s}) + N^{(i)}_{\text{particle}}(\mathbf{s})) + N^{(i)}_{\text{image}}(\mathbf{s}) \text{ (Fourier space)} \end{aligned} \tag{1}$$

where "$*$" represents the convolution operator, and other terms are defined as follows. For the i'th image: $x^{(i)}(\mathbf{r})$ is the recorded image; $m(\mathbf{r})$ is the corresponding noise-free particle image; $n^{(i)}_{\text{particle}}(\mathbf{r})$ and $n^{(i)}_{\text{image}}(\mathbf{r})$ are specific instances of "particle" noise (i.e. signal fluctuations from the sample itself, such as embedding medium or support film) and "image" noise (from the measurement process), respectively; and $\text{CTF}^{(i)}(\mathbf{s})$ is the contrast transfer function of the microscope. The symbols $\mathbf{r}$ and $\mathbf{s}$ denote vector coordinates in real space and Fourier space, respectively. Capitalized symbols $X^{(i)}(\mathbf{s})$, $M^{(i)}(\mathbf{s})$, $N^{(i)}_{\text{particle}}(\mathbf{s})$, $N^{(i)}_{\text{image}}(\mathbf{s})$ refer to Fourier-space equivalents of the corresponding uncapitalized symbol. Note that the CTF term here is implicitly assumed to include all transfer-function-related effects related to the imaging process, including signal attenuation due to envelope function (Glaeser, 2007).

To facilitate analysis, we will treat the noise contribution as a single term, $n^{(i)}(\mathbf{r})$. This approximation is justified by at least two aspects of cryo-EM data: (1) when a large number of images having varying CTF functions are treated, CTF modulations of the particle noise Fourier transform will effectively disappear in the summed particle estimate, resulting in a "net particle noise" in the particle estimate whose contribution can be grouped together with the