Published in final edited form as:

J Struct Biol. 2014 July ; 187(1): 84–92. doi:10.1016/j.jsb.2014.05.002.

Spherical Deconvolution Improves Quality of Single Particle Reconstruction

Gregory P. Kishchenko and Ardean Leith

New York State Department of Health, Wadsworth Center, Empire State Plaza, Albany, NY 12201 USA

Abstract

One single-particle reconstruction technique is the reconstruction of macromolecules from projection images of randomly oriented particles (SPRR). In SPRR the reliability and consequent interpretation of the final reconstruction is affected by errors arising from incorrect assignment of projection angles to individual particles. In order to improve the resolution of SPRR we studied the influence of imperfect assignment on 3D blurring. We find that this blurring can be described as a Point Spread Function (PSF) that depends on the distance from geometrical center of the reconstructed volume and that blurring is higher at the periphery. This particular PSF can be described by an almost pure tangential angular function with a negligible radial component. We have developed a reliable algorithm for spherical deconvolution of the 3D reconstruction. This spherical deconvolution operation was tested on reconstructions of GroEL and mitochondrial ribosomes. We show that spherical deconvolution improves the quality of SPRR by reducing blurring and enhancing high frequency components, particularly near the periphery of the reconstruction.

Keywords

Single-particle reconstruction; incorrect angular assignment; spherical deconvolution; GroEL; mitochondrial ribosome

I. Introduction

In Single Particle Reconstruction of macromolecules from projection images of randomly oriented particles (SPRR), various methods such as reference based reconstruction are used to determine a presumed projection direction for particle images. The presence of noise and low contrast in electron micrographs leads to uncertainty in angular assignment (Fig. 1). This uncertainty, in turn, leads to blurring of the resulting three-dimensional (3D) reconstruction. This research studies the quantitative effect of incorrect angular assignment

© 2014 Elsevier Inc. All rights reserved.

Corresponding author: Gregory P. Kishchenko, Wadsworth Center, P.O. Box 509, Empire State Plaza, Albany, NY 12201-0509, USA, gpk04@wadsworth.org, Tel +1 518-473-6327, Fax: none.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

on the reconstructed volume and develops an algorithm for correcting the resulting structural artifacts.

Two-dimensional averaging of EM data from multiple images has recently been used for deblurring class-averaged EM images (Park et al., 2010). It gave limited improvement in spatial resolution of the images. Two-dimensional angular deconvolution has also been applied to fiber X-ray diffraction from partially oriented specimens, where it achieves 3 Å resolution for some plant viruses and filamentous bacteriophages (Tibbitts and Caspar, 1993; Stubbs, 2001).

Deblurring deconvolution in 3D space should be more effective because the reconstruction may be produced by averaging of hundreds of thousands projections. One version of 3D deconvolution, B-factor sharpening, has been used in SPRR to increase resolution and produce more detailed maps. This procedure involves the application of a negative temperature factor to the 3D map of the SPRR (Bass et al., 2002; Rosenthal and Henderson, 2003). In their approach the degradation of image contrast due specimen movement, radiation damage, partial microscope coherence, and inaccurate determination of the orientation, is modeled by a simple uniform-shaped Gaussian kernel applied to the overall 3D map (Fernandez et al., 2008).

In our approach to 3D deconvolution we consider only the degradation associated with incorrect angular assignment. This is significantly different from B-factor sharpening because, as it will be shown later, incorrect angular assignment leads to a non-uniform, anisotropic blurring kernel. Blur depends of distance from the geometrical center of reconstructed volume, and the blurring function is different in tangential and radial directions.

For testing the proposed deconvolution technique we used three objects: a computer generated volume containing a set of single points at different distances from center, a SPRR volume of GroEL molecular complex, and a SPRR volume of a mitochondrial ribosome. The computer generated volume was used as a model for simulation of incorrect angular assignment blurring and determining the Point Spread Function (PSF). Knowledge of the PSF allows us to design an appropriate Wiener filter and perform a 3D deconvolution procedure inverting the effect of incorrect angular assignment. The advantage of using GroEL is that cryo-EM density maps are available from the Electron Microscopy Data Bank (EMDB) at both low 11.5 Å resolution (EMDB: 1080) and high 4.2 Å resolutions (EMDB: 5002). A corresponding atomic model (PDB: 3C9V) is available from the Protein Data Bank (PDB) (see Ludtke et al., 2001, 2008). Thus GroEL can serve as a test standard for deconvolution efficiency. We also applied 3D deconvolution to a low resolution (10.3 Å) SPRR of a mitochondrial ribosome for which there is no existing atomic model.

II. Effect of incorrect angular assignment on the point spread function and modulation transfer function

To study the effect of uncertainty in angular assignment we carried out preliminary work on synthetic images. We created projections from a set of single points (Fig. 2a) at different

distances from geometrical center of volume. Uncertainty in angular assignment was modeled using vectors of projection directions distributed uniformly in a cone with angular width σ (as in Fig. 1b) instead of a single direction for each projection. We then reconstructed a volume by back-projecting the blurred projections using interpolation in Fourier space. This step was necessary to determine how the angular Point Spread Function (PSF) and Modulation Transfer Function (MTF) depend on radius and angular assignment uncertainty.

In Fig. 2b we show the results of reconstruction for the case of a 15° uncertainty of angular assignment. Blurring is mostly tangential with a negligible radial component. Blurring increases nearer the periphery, and the width of blurring depends linearly on distance from the geometrical center of reconstructed volume. This means the blur is spherical, and the process of deblurring should employ spherical angular deconvolution on spherical shells of the volume.

In Fig. 3a we show the profiles of the PSF for reconstructions with 50 pixels distance for 3° , 5° and 15° uncertainty of angular assignment. The MTF was calculated by Fourier transforms of the PSF (Fig. 3b). We used a number of functions to mimic PSF shape, such as exponential, Gaussian, and up to 6-th order polynomials. None of these functions gave an acceptable fit. The MTF is more suitable to shape estimation. Indeed, the central part of MTF has a near-perfect triangular shape (dashed lines in Fig. 3b). We have modeled the shape of the MTF by combination of a central triangular pulse and an exponential tail (dotted lines in Fig. 3b):

$$MTF(i) = \begin{cases} 1 - |i \cdot \sigma / 360|, & |i \cdot \sigma / 360| < 0.65 \\ 0.35 \cdot \exp(-|i \cdot \sigma / 360| / 0.35), & \text{otherwise} \end{cases} \quad (1)$$

where i is an integer angular frequency index, and σ is a convolution angle (uncertainty of angular assignment). The estimator (1) was employed for design of a 2D Wiener filter, which is used in the deconvolution.

III. Description of the spherical deconvolution algorithm

Deconvolution is a technique to reverse the effects of convolution, and most deconvolution schemes are implemented in the frequency domain. Such deconvolution consists of three steps: the image is Fourier transformed, multiplied with a suitable filter function, and then transformed back into the spatial domain. As mentioned above the blurring has a spherical nature. Therefore spherical angular deconvolution must be performed on concentric spherical layers of the volume, and the layers combined to recreate a volume. Deconvolution on spheres involves calculation of a harmonic expansion series on a sphere.

Currently harmonic expansion on spheres can be performed in two different ways: with an algorithm based on spherical harmonics, and an alternative algorithm based on a double Fourier expansion in spherical coordinates. The advantages and disadvantages of using spherical harmonic functions have been discussed in detail by a number of researchers (Orszag, 1974; Khairy and Howard, 2008; Shen, 1999; Driscoll and Healy, 1994). Spherical

harmonic expansion has a low computational speed due to an expensive zero padding (Orszag, 1974; Khairy and Howard, 2008; Shen, 1999). Recently the spherical harmonics transformation has been improved by implementation of faster algorithms (Driscoll and Healy, 1994; Healy et al., 2003), and even more sophisticated algorithms will likely be developed in the future. However, the spherical harmonics approach has another drawback: it has a tendency to enhance the density gradients of processed 3D data even at low expansion orders with noisy data (Khairy and Howard, 2008). Therefore processed data requires strong smoothing to yield realistic results (Khairy and Howard, 2008). We consider this smoothing to be the greatest disadvantage of the spherical harmonics approach because it leads to loss of high frequency information.

Unfortunately, the second approach, Fourier series expansion in a spherical coordinate system, faces another problem, a higher concentration of grid points near the poles compared to the equator. As a result, the sampling grid in a spherical coordinate system does not have a uniform solid-angular distribution needed for processing with a uniform convolution kernel. To deal with this problem, a method of locally defined coordinate grids made up of nearly orthogonal great circles has been proposed (Haber et al., 1995; Bogart et al., 1995). The application of this remapping technique has been beneficial for processing solar oscillation data in comparison with using a latitude-longitude grid (Zaatri et al., 2008; Haber, 2004).

This inspired us to adopt a coordinate system based on great circles. Our algorithm for deconvolution of spherical shells consists of multiple steps. First, we converted the original rectangular gridded density map to a set of concentric spherical layers (shells) represented in spherical coordinates. This conversion was carried out by trilinear interpolation in real space. At the next step we deconvolved density map of every shell by sequential one-dimensional deconvolution of signal along each “meridian”, followed by one-dimensional deconvolution of the obtained signal on the “equator” (Fig. 4a). Deconvolved signal along the “equator” was saved as a part of deconvolved shell. Then we rotated the equator-meridians grid by a small angle around the horizontal axis represented by dashed line in Fig. 4a, again converted the original density map to rotated spherical coordinates, and repeated the deconvolution procedure, thereby calculating a deconvolved signal on a second, rotated, “equator” (Fig. 4b). The rotation-deconvolution procedure was repeated until the circle closed. Thus we covered the entire surface of a spherical shell by a grid made of “equators” (Fig. 4c). Each one-dimensional deconvolution was completed by applying an inverse Wiener filter as described below (formula 2). It is important to note that each deconvolution along the meridians and equator was processed *de novo* (using original non-deconvolved data), because rotation around the above-mentioned axis generates new equators and a new set of meridians. After completion of deconvolution for all of the radial shells the data were converted back by trilinear interpolation in real space to rectangular coordinates, completely filling the entire reconstruction space.

The major steps of our spherical deconvolution implementation are shown in Fig. 5.

We chose to use pseudo-inverse Wiener filtering in the deconvolution procedure because, contrary to a straight inverse deconvolution, the Wiener filter gives an optimal balance between inverse filtering and noise smoothing in the mean square error sense.

The formula for the 1D Wiener deconvolution is:

$$H(i) = \frac{MTF(i)}{MTF^2(i) \cdot (1 - \varepsilon) + \varepsilon} \cdot G(i) \quad (2)$$

where $G(i)$ is the spatial frequency component of the original signal, $H(i)$ is the spatial frequency component of the deconvolved signal, $MTF(i)$ is the modulation transfer function (1), and ε is a small value parameter ($\varepsilon = 0.05$) which is necessary to prevent a zero value in the discriminator.

IV. Testing spherical deconvolution on a computer generated volume containing a set of single points

The effectiveness of this spherical deconvolution technique was tested first on a set of computer generated points reconstructed by back-projecting after incorrect angular assignment blurring. In Fig. 3c, 3d, 3e we compare the PSF profiles after deconvolution with blurred PSF profiles for 3°, 5° and 15° uncertainty of angular assignment. As seen in Fig. 3c, 3d, 3e our spherical deconvolution procedure leads to visible narrowing of central peaks and outer sidebands of PSF profiles.

V. Testing spherical deconvolution on GroEL reconstruction

As we mentioned above, GroEL cryo-EM density maps are available from the EMDB at both low 11.5 Å (EMDB: 1080) and high 4.2 Å resolutions (EMDB: 5002). A molecular model at atomic resolution is also available (PDB: 3C9V), and can serve as a test standard for deconvolution efficiency. We carried out spherical deconvolution at different values of angle σ , and compared the deconvolved volume with the original reconstruction and with the atomic resolution model at the same resolution.

In Fig. 6 we show one of the slices of a 11.5 Å resolution reconstructed GroEL volume deconvolved by Wiener filters at different deconvolution angles, σ , compared with the same slice of the original reconstruction, as well as the same slice of the atomic model low-pass-filtered to the same resolution.

We also calculated the R-factor between the deconvolved GroEL volume and its atomic model volume (Fig. 7). The R-factor is a measure of the agreement between atomic model and experimental data routinely used in crystallography (see for instance Morris et al., 1992). We adapted the R-factor to EM reconstruction by substitution of the structure factor used in crystallography by the amplitude of the 3D Fourier transform. The scaling factor for data comparison was calculated by general least-squares minimization (Segerman, 1958). In Fig. 7 we show the R-factor of a 11.5 Å deconvolved GroEL volume versus its atomic model volume as a function of angle σ . As seen in Fig. 7 the R-factor between the

deconvolved volume and the atomic model volume is minimal at $\sigma = 6^\circ$. The visual impression from Fig. 6 is consistent with R-factor plot behavior, and also points to a value of $\sigma = 6^\circ$ where the deconvolved slice has the best sharpness and contrast. An 11.5 Å GroEL volume (Fig. 8f) deconvolved at the optimal $\sigma = 6^\circ$ reveals closer similarity to the atomic model volume than the original 11.5 Å reconstruction (Fig. 8d), and demonstrates the usefulness of spherical deconvolution.

As an additional quantitative measurement of the quality of reconstruction we computed the radial distribution of amplitude averaged over spherical shells in the 3D Fourier spectrum (Fig. 9, Fig. 10). In Fig. 9 we present the radial distribution function of averaged Fourier amplitudes for GroEL volumes with 11.5 Å resolution, 4.2 Å resolution, and a GroEL atomic model volume (generated from atomic model). A 11.5 Å resolution SPRR has very low amplitude at high spatial frequency. The amplitude is suppressed beginning approximately at 17Å-22Å and it is approximately six times lower than the amplitude of 4.2 Å resolution SPRR and atomic model volume at 11.5 Å resolution. This indicates that high frequency components are underrepresented in the SPRR reconstruction.

In Fig. 10a,b we present the amplitude spectrum of the deconvolved volume as a function of σ . As seen in Fig. 10 the high frequency component increases with value of σ , and reaches a maximum at $\sigma = 6^\circ$, at the same angle at which the R-factor is minimal. This means that we are able to determine the an optimal σ value by examining the amplitude spectra of a test series of deconvolved volumes.

Enhancement of high frequency signal generated by spherical deconvolution raises the question whether the effect of this procedure is superior to a simple high-pass filtering such as sharpening by a negative temperature factor as discussed earlier. In order to compare these two different approaches we applied negative temperature factor deconvolution, high-pass filter with intrinsic spacial frequency 1/17Å, to 11.5 Å resolution SPRR volume of the GroEL molecular complex. The intrinsic frequency 1/17Å was chosen because it corresponds to approximate starting point of high frequency suppression in original GroEL reconstruction. Fig. 8 shows original and deconvolved maps fitted with atomic model. The resulting volumes reveal almost no change in the central core of reconstruction but considerable improvement at the periphery (Fig. 8b,c,e,f) after both deconvolution procedures. However, as seen in Fig. 8 negative temperature factor sharpening (Fig. 8b) gives less detail near the outer surface of the reconstruction than spherical deconvolution (Fig. 8c). The spherically deconvolved volume shows a better fit to the atomic model (Fig. 8f) than the volume deconvolved by negative temperature factor (Fig. 8e). This can be explained by the fact that the negative temperature factor's high-pass filter deconvolution is sharpening the 3D map in both tangential and radial directions whereas spherical deconvolution is only tangentially sharpening the map. In negative temperature factor deconvolution an over-sharpening of the density map in the radial direction cuts off protrusions on the surface of reconstruction.

VI. Testing spherical deconvolution on a mitochondrial ribosome reconstruction

A SPRR volume of mitochondrial ribosome at 10.3 Å resolution was provided by Dr. Rajendra Agrawal and Tim Booth (private communication). We were interested in applying spherical deconvolution to this object because there is neither an existing high resolution SPRR nor a corresponding atomic model.

In Fig. 11 we present the radial distribution of the average amplitude in 3D Fourier transform spectrum of 10.3 Å resolution mitochondrial ribosome reconstruction deconvolved by Wiener filters with different deconvolution angles σ . The optimal value of σ with the highest high-frequency component occurs at 6°. The highest high-frequency component occurs at 6°, and, therefore, $\sigma = 6^\circ$ should be considered as the optimal.

In Fig. 12 we show a slice of the 10.3 Å resolution mitochondrial ribosome reconstruction deconvolved by Wiener filters at different deconvolution angles (σ) compared with the original 10.3 Å reconstruction. The slice deconvolved with $\sigma = 6^\circ$ has the highest sharpness and contrast. The highest sharpness and contrast appear at $\sigma = 6^\circ$ also. A mitochondrial ribosome volume deconvolved at the optimal $\sigma = 6^\circ$ reveals details that are absent in the original 10.3 Å reconstruction (Fig. 13), and thus demonstrates the value of the spherical deconvolution procedure.

VII. Conclusion

A reliable algorithm for spherical deconvolution of SPRR volumes has been presented. We have shown that spherical deconvolution improves the quality of final SPRR volume by reducing blurring and enhancing the high frequency components. The improvement in quality is greatest at the periphery of the reconstructed volume. This makes spherical deconvolution especially promising for improving molecular docking, because binding regions are often located near the surface of molecule rather than buried.

We would like to draw attention to an interesting aspect of spherical deconvolution. The Fourier Shell Correlation (FSC) method commonly accepted for resolution measurement (see for instance Frank, 2006) does not reveal under-representation of high frequency components in EM reconstruction, and does not show any increase of resolution after spherical deconvolution. This occurs because the FSC is based on a correlation approach which estimates “internal consistency” of the results, and ignores the magnitude of signal. In other words, the original and low-pass filtered objects (with lower sharpness and contrast) have the same FSC plot.

In SPRR the micrographs-to-reference alignment and the 3D reconstruction itself are usually repeated iteratively during the refinement steps. At each step a SPRR volume from the previous step serves as a new reference (Frank, 2006). We believe that applying spherical deconvolution to intermediate reference volumes will accelerate this process and increase final SPRR resolution. However this needs further verification. We are incorporating this algorithm in the latest release of our open source software “SPIDER” (Leith et al., 2012).

Acknowledgments

Sponsor and financial support acknowledgment: This work was supported by Grant Number 5RO1GM093681 from the National Institute of General Medical Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

- Bass RB, Strop P, Barclay M, Rees DC. Crystal structure of Escherichia coli MscS, a voltage-modulated and mechanosensitive channel. *Science*. 2002; 298:1582–1587. [PubMed: 12446901]
- Bogart RS, Sa LAD, Duvall TL, Haber DA, Toomre J, Hill F. Plane-wave analysis of solar acoustic-gravity waves: a (slightly) new approach. *Proc 4th SOHO Workshop - Helioseismology*. ESA SP-376. 1995; 2:147.
- Driscoll JR, Healy DM. Computing Fourier transforms and convolutions on the 2-sphere. *Adv Appl Math*. 1994; 15:202–250.
- Fernandez JJ, Luque D, Caston JR, Carrascosa JL. Sharpening high resolution information in single particle electron microscopy. *J Struct Biol*. 2008; 164:170–175. [PubMed: 18614378]
- Frank, J. Three dimensional electron microscopy of macromolecular assemblies: visualization of biological molecules in their native state. second. University Press; Oxford: 2006.
- Haber D, Toomre J, Hill F, Gough D. Solar Oscillation Ring Diagrams: Benefits of Great Circle Remapping. *Helio- and Astero-Seismology*. ASP Conference Series. 1995; 76:272–275.
- Haber DA. Subphotospheric structure and dynamics deduced from ring analysis. *Proc SOHO*. 2004; 14:676–687.
- Healy D, Rockmore D, Kostelec P, Moore S. FFTs for the 2-sphere - improvements and variations. *J Fourier Anal Appl*. 2003; 9(4):341–385.
- Khairy K, Howard J. Spherical harmonics-based parametric deconvolution of 3D surface images using bending energy minimization. *Med Image Anal*. 2008; 212(2):217–27. [PubMed: 18055248]
- Leith, A.; Baxter, W.; Frank, J. Use of SPIDER and SPIRE in Image Reconstruction. In: Arnold, E.; Himmel, D.; Rossmann, M., editors. *International Tables for Crystallography* 2012. Crystallography of Biological Macromolecules Vol F. John Wiley; New York: 2012. p. 620–623.
- Ludtke SJ, Jakana J, Song JL, Chuang DT, Chiu W. A 11.5 Å single particle reconstruction of GroEL using EMAN. *J Mol Biol*. 2001; 314:253–262. [PubMed: 11718559]
- Ludtke SJ, Baker ML, Chen DH, Song JL, Chuang DT, Chiu W. *De novo* backbone trace of GroEL from single particle electron cryomicroscopy. *Structure*. 2008; 16:441–448. [PubMed: 18334219]
- Morris AL, MacArthur MW, Hutchinson EG, Thornton JM. Stereochemical quality of protein structure coordinates. *Proteins*. 1992; 12(4):345–364. [PubMed: 1579569]
- Orszag SA. Fourier series on spheres. *Mon Weather Rev*. 1974; 102:56–75.
- Park W, Madden D, Rockmore DN, Chirikjian GS. Deblurring of Class-Averaged Images in Single-Particle Electron Microscopy. *Inverse Probl*. 2010; 26(3):3500521–35005229. [PubMed: 20221416]
- Segerman E. Scale-factor adjustment for optimum comparison of observed and calculated structure factors. *Acta Crystallogr*. 1958; 11:374–375.
- Shen J. Efficient Spectral-Galerkin Methods iv. Spherical Geometries. *Siam. J Sci Comput*. 1999; 20(4):1438–1455.
- Rosenthal PB, Henderson R. Optimal determination of particle orientation, absolute hand and contrast loss in single particle electron cryomicroscopy. *J Mol Biol*. 2003; 333:721–745. [PubMed: 14568533]
- Stubbs G. Fibre diffraction studies of filamentous viruses. *Rep Prog Phys*. 2001; 64:1389–1425.
- Tibbitts T, Caspar D. Deconvolution of disoriented fiber diffraction data using iterative convolution and local regression. *Acta Crystallogr A*. 1993; 49(3):532–545. [PubMed: 8347308]
- Zaatri A, Corbard T, Roth M, Hernandez IG, Luhe O. Comparison of geometrical mapping for ring diagram analysis. *J Phys Conf Ser*. 2008; 118(1) id. 012090.

Fig. 1.
(a) — Quasi-evenly spaced projection directions on the surface of a sphere. (b) —
Uncertainty of angular assignment for projections.

Fig. 2.

(a) — Side view of a set of points at different distances from geometrical center of reconstructed volume. (b) — Side view of reconstruction of points with 15° uncertainty of angular assignment showing tangential blurring. Blurring increases with increasing distance from the center.

Fig. 3.

(a) — Profile of the point spread function (PSF) for reconstructions at 50 pixels distance for 3° , 5° and 15° uncertainty of angular assignment. The width of the PSF peak increases with value of σ . This indicates that increased uncertainty in angular assignment leads to increased blurring of a reconstruction. (b) — One-dimensional Fourier transforms of the PSF (Modulation Transfer Function, MTF) for reconstructions at 50 pixels distance for 3° , 5° and 15° uncertainty of angular assignment. The central portion of the MTF has an almost perfect triangular shape (dashed line). The MTF peaks can be approximated by the combination of a

central triangular pulse and an exponential tail as given by formula 1 (dotted line). (c, d, e)
— Comparison of PSF profiles of deconvolved reconstructions with original PSF profiles for 3° (c), 5° (d) and 15° (e) uncertainty of angular assignment.

Fig. 4.

Deconvolution of a spherical shell based on Fourier transformation of the signal occurring on “great circles”. The spherical two-dimensional deconvolution operation is broken into two one-dimensional operations: one along all “meridians” and a second along the “equator”. (a) — Deconvolving signal on first “equator”. (b) — Deconvolving signal on second “equator” following rotation around axis (dashed horizontal line); the image of first “equator” was retained for comparison. (c) — Final deconvolution on a set of “equators” covering the entire spherical shell. Each one-dimensional deconvolution was completed by one-dimensional Fourier transformation, inverse filtration as described by Formula 2, and then inverse transformation back to rectangular coordinates.

Interpolate the set of deconvolved shells with different radii back into cartesian coordinates for volume.

Repeat whole above process on two orthogonal axes to avoid oversampling terminal horizontal regions of equatorial circles.

Merge the two orthogonal reconstructions.

Fig. 5.
Outline of spherical deconvolution implementation.

Fig. 6. Slice of a 11.5 Å resolution reconstructed GroEL volume deconvolved with a Wiener filter at different deconvolution angles σ compared with slice of atomic model and the original reconstruction at the same resolution. The deconvolved slice has the best sharpness and contrast at value of $\sigma = 6^\circ$, and reveals increased similarity to the corresponding slice of the model density map.

Fig. 7.

R-factor of a 11.5 Å deconvolved GroEL volume versus its atomic model volume as a function of angle σ . The R-factor is minimal at $\sigma = 6^\circ$, and, therefore, a reconstruction deconvolved at $\sigma = 6^\circ$ has the highest similarity to the molecular model.

Fig. 8.

Comparison of original and deconvolved 3D maps of GroEL.

(a, d) — The original GroEL reconstruction with 11.5 Å resolution.

(b, e) — GroEL reconstruction deconvolved with a negative temperature factor.

(c, f) — The optimal spherical deconvolution ($\sigma = 6^\circ$) of GroEL reconstruction.

(a, b, c) — Solid representation of GroEL volumes;

(d, e, f) — Transparent surface representation of GroEL volumes fitted with GroEL atomic model. Few regions of the reconstruction surfaces are outlined by circles and ovals. The

consistencies and inconsistencies between the density maps and the atomic model are clearly seen within the outlined areas.

Fig. 9.

Radial distribution functions of the 3D amplitude spectrum of an 11.5 Å resolution GroEL reconstruction, a 4.2 Å resolution GroEL reconstruction, and the GroEL atomic model volume. A 11.5 Å resolution SPRR has lower amplitude at spatial frequency range between 11.5 Å and 22 Å. Therefore, the high frequency components of EM reconstruction are underrepresented, and enhancement of high frequency components can serve as a criterion of the efficiency of spherical deconvolution.

Fig. 10.

(a) — Radial distribution function of the 3D amplitude spectrum of 11.5 Å resolution GroEL reconstruction deconvolved by Wiener filters with deconvolution angles: $\sigma = 2^\circ, 4^\circ$, and 6° . The high frequency component increases with increased deconvolution angle. (b) — Radial distribution function of the 3D amplitude spectrum of 11.5 Å resolution GroEL reconstruction deconvolved by Wiener filters with deconvolution angles: $\sigma = 6^\circ, 8^\circ, 10^\circ, 12^\circ$. Over this range of deconvolution angles, the high frequency component decreases with increasing deconvolution angle. The high frequency component initially increases with σ , reaches the highest level at $\sigma = 6^\circ$, and decreases with a further increase in σ .

Fig. 11.

(a) — Radial distribution function of the 3D amplitude spectrum of 10.3 Å resolution mitochondrial ribosome reconstruction deconvolved by Wiener filters with deconvolution angles: $\sigma = 2^\circ, 4^\circ, 6^\circ$. The high frequency component increases with the deconvolution angle. (b) — Radial distribution function of the 3D amplitude spectrum of the 10.3 Å resolution mitochondrial ribosome reconstruction deconvolved by Wiener filters with deconvolution angles: $\sigma = 6^\circ, 8^\circ, 12^\circ, 20^\circ, 30^\circ$. In this range of deconvolution angles, the high frequency component decreases with an increase of deconvolution angle. The high frequency component initially increases with value of σ , reaches the highest level at $\sigma = 6^\circ$, and decreases with a further increase of σ .

Fig. 12.

Slice from a 10.3 Å resolution mitochondrial ribosome reconstruction deconvolved by Wiener filters at different deconvolution angles σ compared with the original 10.3 Å reconstruction. The slice from a reconstruction deconvolved at $\sigma = 6^\circ$ has the highest sharpness and contrast.

Fig. 13.

(a) — Original 10.3 Å reconstruction of mitochondrial ribosome reconstruction. (b) — The optimally deconvolved ($\sigma = 6^\circ$) mitochondrial ribosome reconstruction.