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Web server for tilt-pair validation of single particle maps from electron cryomicroscopy

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ABSTRACT

Three-dimensional structures of biological assemblies may be calculated from images of single particles obtained by electron cryomicroscopy. A key step is the correct determination of the orientation of the particle in individual image projections. A useful tool for validation of the quality of a 3D map and its consistency with images is tilt-pair analysis. In a successful tilt-pair test, the relative angle between orientations assigned to each image of a tilt-pair agrees with the known relative rotation angle of the microscope specimen holder during the experiment. To make the procedure easy to apply to the increasing number of single particle maps, we have developed software and a web server for tilt-pair analysis. The tilt-pair analysis program reports the overall agreement of the assigned orientations with the known tilt angle and axis of the experiment and the distribution of tilt transformations for individual particles recorded in a single image field. We illustrate application of the validation tool to several single particle specimens and describe how to interpret the scores.

1. Introduction

Imaging by electron microscopy of unstained, frozen-hydrated single particle specimens in vitreous ice has become a successful approach to the structure determination of large biological assemblies that are difficult to study by other high-resolution techniques such as X-ray crystallography (Baker and Henderson, 2012; Orlova and Saibil, 2011). Averaging of low contrast, noisy projection images of randomly-oriented single particles acquired with minimal electron exposure can yield electron potential maps at high enough resolution for interpretation by atomic structural models, as demonstrated by several studies of homogeneous and large complexes that scatter electrons strongly (Grigorieff and Harrison, 2011). The method requires the assignment of the relative orientation and translation of the particle in the projection image (Henderson, 1995). In addition, microscope parameters such as magnification and defocus that affect the image of the specimen must be known to extract structural information.

A low resolution, initial map, calculated by one of several available approaches (Frank, 2006), is typically improved by iterative, model-based refinement. In each refinement step, calculated projections of the current map are scored for agreement with particle images, and orientation parameters are assigned to the image for use in computing a subsequent map. For a correct starting map, and where there is sufficient signal in the images, orientation parameters for the particles will improve during refinement and the map may converge to one that matches the data to a resolution determined by the number of particle images and the noise level in the images (Henderson, 1995). Where the signal-to-noise ratio is low, as may be the case with cryomicroscopy images, map projections have the potential to match noise in the image and incorrect orientations may be erroneously assigned. In such cases iterative, model-based refinement can only yield a map that looks like the starting map (Sigworth, 1998), whether it is consistent with raw data or not. Model bias is a more significant problem when single particle analysis is applied to lower molecular weight specimens. A further complication arises when particles have structural or conformational heterogeneity, potentially requiring accurate assignment of different particle conformations and their respective orientations (Henderson et al., 2011; Lyumkis et al., 2013; Scheres, 2010).

These problems highlight the importance of validation criteria that prove the consistency of map projections with particle images (Henderson et al., 2012). In addition, the validity of a map may be demonstrated by the presence of high-resolution features typical of protein structure, or agreement at lower resolution with the fold shape or secondary structure of a known model obtained by X-ray

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crystallography or NMR spectroscopy, where such information has been rigorously excluded during structure determination and refinement. Confidence in a map is increased by the use of methods and measures of quality that are least affected by model bias such as comparison of maps that are independently calculated from separate halves of the image dataset (Scheres and Chen, 2012; Stewart and Grigorieff, 2004), an unbiased FSC calculation (Chen et al., 2013), or agreement between such maps at a higher resolution than the data used in assigning orientations ("free shells") (Rosenthal and Henderson, 2003; Shaikh et al., 2003). Unfortunately, detecting the instances of a wrong or biased model may require a detailed knowledge of the history of the calculation of the starting map and its subsequent refinement and direct examination of a large image dataset. Validation procedures that can be applied to the final map or model and show the consistency of map and data are therefore essential.

Tilt-pair analysis is a cross-validation procedure for orientation determination based on the consistency of the assignment of orientations in two images of the same particle field recorded with a known relative tilt about the goniometer axis (Rosenthal and Henderson, 2003). The procedure may be applied to the optimization of orientation determination, reducing the matching of noise in favour of true structural features of the particles. Such optimization yields more accurate orientation parameters and reduces computational errors that blur high-resolution features. (Henderson et al., 2011; Rosenthal and Henderson, 2003). Recently, tilt-pairs have been applied to a range of single particle specimens (Henderson et al., 2011; Murray et al., 2013) and they should be presented routinely to support new structure determinations. Recording a tilt-pair is straightforward on modern microscopes, and standalone tilt analysis software will make it easier to perform the analysis on the increasing number of single particle maps (Patwardhan et al., 2012). Toward this end we have created an automated validation tool (http://cryoem.nimr.mrc.ac.uk/software/) that performs the cross-validation procedure given an input map, two stacks of images corresponding to tilt-pairs, and user-determined particle orientation parameters for one of the image stacks. The test does not require further details of the map's prior history. Here we describe the theory and implementation of the tilt analysis procedure and provide a guide to interpreting results. The tool facilitates computing and reporting the validation results and may be useful during structure determination, at the time of publication, or when retrieving maps from a database.

2. Methods

2.1. Processing of image data

Images in the input stacks are first corrected for effects of the microscope contrast transfer function (CTF) by phase flipping using defocus values given as input parameters. The images are band-pass filtered with a cosine-softened edge at the minimum ($R_{\min}$) and maximum ($R_{\max}$) resolution limits in Fourier space according to

$$f(r)\begin{cases} 0 & r < R_1 - R', r > R_2 + R' \\ \frac{1}{2}(\cos(\pi\frac{R_1 - r}{R'} + \pi) + 1) & R_1 - R' < r < R_1 \\ \frac{1}{2}(\cos(\pi\frac{r - R_2}{R'}) + 1) & R_2 < r < R_2 + R' \\ 1 & R_1 < r < R_2 \end{cases}$$

where $R_1 = \frac{1}{R_{min}}$, $R_2 = \frac{1}{R_{max}}$ and R' is a 10 pixel boundary region. Band-pass filtering improves centering accuracy and ensures that cross-correlation alignment is calculated using the same structural information as the subsequent calculation of the phase residual similarity score. Particle images are centered using cross-correlation against the total sum of the images with integer pixel shift and no interpolation. The images are then normalized to zero mean and sigma of 1. A mask 1.5 times the particle radius (cosine-softened according to

$$f(r) = \begin{cases} 1 & r < R \\ \frac{1}{2}(\cos(\pi\frac{r-R}{R'}) + 1) & R < r < R + R' \\ 0 & r > R + R' \end{cases}$$

where R' is 1/15 box size) is applied to both stacks to reduce noise in the Fourier domain and the images padded with zeros to a final box size that is twice the mask diameter. The same padding and mask radius are applied to the map.

2.2. Image and map down-sampling

To reduce computation time, the map and images are down-sampled by an integer binning factor N that places the maximum resolution of the Fourier phase residual calculation (R_{max}) such that $\frac{f_N}{4} < \frac{1}{R_{Max}} < \frac{f_N}{2}$, where f_N is the Nyquist frequency. The minimal box size after binning is 32×32 px.

2.3. Calculation of map projections

Map projections are computed using a Fourier space projector. The map is over-sampled twice prior to the Fourier transform. An oblique slice is extracted from the 3D transform using trilinear interpolation based on 8 complex samples. The two dimensional inverse Fourier transform is then calculated to obtain the particle projection.

2.4. Tilt-pair phase residual plot

The phase residual plot is calculated over a square grid of predicted tilt operators that are applied to the orientation parameters of the untilted particle images. For each particle, the untilted orientation parameters are read as Euler angles (ψ,θ,φ) describing successive rotations of the map about the z, y, and z axes as follows:

$$R_{\psi\theta\phi} = R_\psi R_\theta R_\phi = \begin{bmatrix} \cos\psi & \sin\psi & 0 \\ -\sin\psi & \cos\psi & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \cos\theta & 0 & -\sin\theta \\ 0 & 1 & 0 \\ \sin\theta & 0 & \cos\theta \end{bmatrix} \times \begin{bmatrix} \cos\phi & \sin\phi & 0 \\ -\sin\phi & \cos\phi & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

These correspond to conventions described by Heymann (Heymann et al., 2005) and several common programs for single particle analysis (e.g. SPIDER (Frank et al., 1996), Frealign (Grigorieff, 2007), Xmipp (Sorzano et al., 2004)).

The untilted orientations are represented as quaternions according to

$$q_{\psi\theta\phi} = q_\psi q_\theta q_\phi = \left(\cos\frac{\psi}{2} - \vec{k}\sin\frac{\psi}{2}\right)\left(\cos\frac{\theta}{2} - \vec{j}\sin\frac{\theta}{2}\right)\left(\cos\frac{\phi}{2} - \vec{k}\sin\frac{\phi}{2}\right)$$

The predicted orientation parameter for the tilted particle is calculated by the quaternion product $q_{tilt} = q_y(\alpha_y) * q_x(\alpha_x) * q_{untilt}$ with two tilt operators, $q_x(\alpha_x) = (\cos\frac{\alpha_x}{2} + \vec{i}\sin\frac{\alpha_x}{2})$ and $q_y(\alpha_y) = (\cos\frac{\alpha_y}{2} + \vec{j}\sin\frac{\alpha_y}{2})$, giving rotations of an angle α about the x or y axis, the composition of which gives all in-plane rotation axes (at an angle with respect to the x-axis in the xy plane given by $\tan^{-1}\frac{\alpha_y}{\alpha_x}$, and goniometer rotation angle, $\alpha = \sqrt{\alpha_x^2 + \alpha_y^2}$. The range of rotation angles is specified on input and is sampled every 1°. For each particle, the