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Angle determination for side views in single particle electron microscopy

Lindsay A. Baker, John L. Rubinstein *

Molecular Structure and Function Program, The Hospital for Sick Children Research Institute, and Department of Biochemistry, University of Toronto, 555 University Avenue, Toronto, Ont., Canada M5G 1X8

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Abstract

In order to build a first model in single particle electron microscopy the relative angular orientation of each image of a protein complex must be determined. These orientations can be described by three Eulerian angles. Images of complexes that present the same view can be aligned in two-dimensions and averaged in order to increase their signal-to-noise ratio. Based on these averaged images, several standard approaches exist for determining Euler angles for randomly oriented projection images. The common lines and angular reconstitution methods work well for particles with symmetry while the random conical tilting and related orthogonal tilt reconstruction methods work in most cases but require the acquisition of tilt pairs of images. For the situation where views of particles can be identified that are rotations about a single axis parallel to the grid, an alternative algorithm to determine the orientations of class averages without the need to acquire tilt pairs can be applied. This type of view of a complex is usually called a side view. This paper describes the detailed workings and characterization of an algorithm, named rotational analysis, which uses real-space fiducial markers derived from the averages themselves to determine the Euler angles for side views. We demonstrate how this algorithm works in practice by applying it to a data set of images of affinity-purified bovine mitochondrial ATP synthase.
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1. Introduction

Single particle electron cryomicroscopy (cryo-EM) is a rapidly developing technique in structural biology for obtaining information about the structure of macromolecular assemblies at resolutions between 5 and 50 Å. Images are obtained of randomly or semi-randomly oriented complexes embedded in a layer of vitreous ice. To a good approximation, the images correspond to projections of the three-dimensional (3D) structure of the complex convoluted with the point-spread function of the electron microscope and degraded by noise. The signal-to-noise ratio in images can be increased by averaging images that present the same view of the complex and, with an appropriate set of images, the effects of the point-spread function can be corrected. The Fourier transforms of these projection images are approximately central slices through the 3D Fourier transform of the intact assembly (DeRosier and Klug, 1968). Before an initial 3D model can be constructed from cryo-EM images, missing information about the relative angular orientation of the images must be determined. This information is usually presented in the form of three Eulerian angles, ψ, θ and ϕ (Frank, 2006). Once obtained, an initial model can be refined to high-resolution by projection matching, either in real space or Fourier space, to extract the high-resolution information that is present in low contrast molecular images (Grigorieff, 2007; Harauz and Ottensmeyer, 1984).

Several methods have been described to generate an initial reference volume. The common lines approach (Crowther et al., 1970) and its real-space equivalent, angular reconstitution (van Heel, 1987), can accurately determine the relative orientations of particles that have a high degree of internal symmetry, but tend to be unreliable for asymmetric complexes due to the low signal-to-noise ratio in

* Corresponding author. Fax: +1 416 813 5022.
E-mail address: john.rubinstein@utoronto.ca (J.L. Rubinstein).

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the images. Random conical tilting (Radermacher, 1988) and the closely related orthogonal tilt reconstruction technique (Leschziner and Nogales, 2006) are robust methods for determining initial models for the structure of asymmetric single particles. Random conical tilting can work both in situations where the particle is randomly oriented or has a preferred orientation on the grid but suffers from the "missing cone" problem. The orthogonal tilt reconstruction approach does not suffer from a missing cone, but requires that the particles do not have a preferred orientation on the EM grid. Both approaches can require the collection of a large data set of tilt pairs of images, a step that may be technically difficult. In most situations where an initial model of an asymmetric particle must be generated, the random conical tilting or orthogonal tilt reconstruction approaches are currently the best solution.

In cryo-EM, the orientation of complexes is not always random. For some specimens, a situation may arise where views of the complex are related by a random rotation about an axis that lies in the plane of the EM grid. This situation usually occurs with elongated molecules so that their long axis lies along the plane of the grid and these views are commonly known as side views. Protein complexes that present an identifiable set of side views in cryo-EM include, but are not limited to, ATP synthase (Rubinstein et al., 2003), Vacuolar ATPase (Gregorini et al., 2007) and the 26S proteasome (Nickell et al., 2007). This paper describes a method for determining the relative orientations of images of complexes that present only side views or where a subset of views can be identified as side views. Once these orientations have been determined, an initial model of the complex may be constructed and refined by established projection matching algorithms. When applicable, the advantage of this approach is that an initial model may be calculated directly from a data set of untilted images without the need for collecting image tilt pairs. A different method for determining the relative orientations of side views, based on the statistical correlation of two projections at different rotations about an axis, has been developed elsewhere and applied to helical filaments (Patwardhan et al., 2004).

Beyond concerns about the signal-to-noise ratio in images, the common lines approach cannot be used in the situation were all of the views of a complex are side views. The common lines and angular reconstitution approaches require at least three common lines between any three projections. In the case where all views are side views, there is only a single common line, the rotation axis, shared by all views. Consequently there is no information with which to determine the relative rotation of each image about that axis (Fig. 1).

The method described in this manuscript has been applied to the determination of the structure of the bovine mitochondrial ATP synthase (Rubinstein et al., 2003) isolated by conventional chromatography and, more recently, the ATP synthase from *Saccharomyces cerevisiae* (Lau and Rubinstein, in preparation) isolated by metal affinity chromatography. Presented here are the detailed workings of the algorithm and characterization of the procedure's ability to correctly determine angles. The maps that result from the analysis are tested for their appropriateness as first-references for single particle EM model refinement and the procedure is demonstrated through application to a data set of cryo-EM images of affinity-purified bovine mitochondrial ATP synthase. A test is described for assessing the success of the algorithm in treating the input class averages as side views.

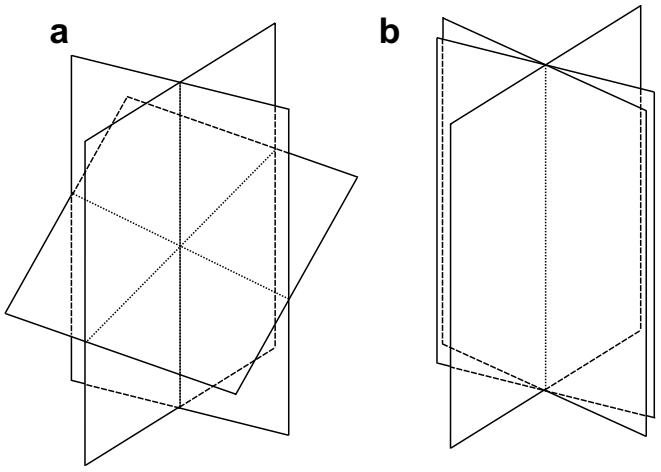

Fig. 1. Failure of the common lines method for side views. Fourier transforms of projections of an object are central planes through the 3D Fourier transform of the object. In the figure, finely dotted lines indicate the common lines shared by central sections of the 3D Fourier transform and dashed lines indicate edges of planes obscured by other planes. (a) In most situations, three arbitrary planes in Fourier space each share one common line with every other plane. By identifying the common lines the 3D arrangement of the planes is established. For the common lines or angular reconstitution methods, these multiple common lines must be present. (b) In the situation where all of the views are side views, all projections share a single common line and there is no information with which to determine relative orientations of the planes about the common line. From this diagram, it is clear that a common lines approach cannot be used to determine the relative angular orientation of side views.

2. Theoretical background and methods

2.1. Preprocessing of data

All images of complexes in single particle EM have random x- and y-positions on the EM grid and a random rotation about an axis normal to the grid. Side views differ from each other by an additional rotation about an axis in the plane of the grid and fixed in its orientation relative to the complex (e.g. the long axis of the complex). In the following description, this second axis is referred to as the tilt axis. 2D alignment techniques are used to translationally align particle images in the x- and y-directions and determine the rotation about an axis normal to the grid that lines up the tilt axis of each complex. Multivariate statistical analysis is then used to group together similar side views so that they can be averaged to increase the sig-

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