Journal of Structural Biology 187 (2014) 66–75

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Automated particle correspondence and accurate tilt-axis detection in tilted-image pairs

Maxim Shatsky [a,*,1], Pablo Arbelaez [b,1], Bong-Gyoon Han [c], Dieter Typke [c], Steven E. Brenner [a,d],
Jitendra Malik [b], Robert M. Glaeser [c]

[a] Physical Biosciences Division, Lawrence Berkeley National Laboratory, CA 94720, USA
[b] Electrical Engineering and Computer Science Division, University of California, Berkeley, CA 94720, USA
[c] Life Sciences Division, Lawrence Berkeley National Laboratory, CA 94720, USA
[d] Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA

ARTICLE INFO

Article history:
Received 24 October 2013
Received in revised form 7 March 2014
Accepted 21 March 2014
Available online 30 March 2014

Keywords:
Particle correspondence
Tilted pairs
Tilt-axis detection

ABSTRACT

Tilted electron microscope images are routinely collected for an *ab initio* structure reconstruction as a part of the Random Conical Tilt (RCT) or Orthogonal Tilt Reconstruction (OTR) methods, as well as for various applications using the “free-hand” procedure. These procedures all require identification of particle pairs in two corresponding images as well as accurate estimation of the tilt-axis used to rotate the electron microscope (EM) grid. Here we present a computational approach, PCT (particle correspondence from tilted pairs), based on tilt-invariant context and projection matching that addresses both problems. The method benefits from treating the two problems as a single optimization task. It automatically finds corresponding particle pairs and accurately computes tilt-axis direction even in the cases when EM grid is not perfectly planar.

1. Introduction

The primary goal of single-particle cryo-electron microscopy (EM) is to obtain a three-dimensional (3D) reconstruction of a particle from its two-dimensional projections (Frank, 2006; Glaeser et al., 2006). *Ab initio* reconstruction methods from frozen samples have only shown limited success, and thus projection matching against a template volume currently is the most widely used approach. In cases when an initial model is not available, a low-to-medium resolution model can be obtained from negatively stained samples by the Random Conical Tilt (RCT) (Radermacher et al., 1986; Radermacher, 1988) or Orthogonal Tilt Reconstruction (OTR) (Leschziner and Nogales, 2006; Leschziner, 2010) procedures. RCT has also been applied to low-tilt cryo-EM samples of the 70S ribosome (Penczek et al., 1994), but this approach has not been widely used in further work.

In both the RCT and OTR approaches, two images of the same sample are collected. The computational processing steps for both methods are mostly equivalent. The geometry of the images has to be estimated, which includes tilt angle and tilt-axis direction (i.e., the direction of the goniometer relative to the recorded image). Next, particles are identified and boxed in both images, and the correspondence between the same particles is established. Then, particles from one image are used to compute class averages. Usually the largest cluster of similar projections is picked and its corresponding paired images are used to reconstruct a 3D model. To improve the quality of the model, reconstructions from several class averages can be merged (Penczek et al., 1994; Scheres et al., 2009).

A second use of tilt-pair images is to determine the absolute hand of a structure (Belnap et al., 1997; Cheng et al., 2002), improve the refinement of Euler angles, and reduce the tendency of the model to match noise. In the “free-hand” method introduced by Rosenthal and Henderson (2003) and Henderson et al. (2011), for example, projection matching is used to assign Euler angles to particle images obtained with an untilted specimen. An exhaustive search is then used to determine the amount and orientation of tilt for which corresponding projections of the current model agree best with a second image, obtained after tilting the specimen by +10°. Errors in the initial assignment of Euler angles affect how closely the search results agree with the known tilt angle, as set by the goniometer. A target function was thus constructed that used tilt-pair images to obtain improved assignments for the Euler

---

[*] Corresponding author. Address: 461 Koshland Hall, University of California, Berkeley, CA 94720-3102, USA.

E-mail address: max.shatsky@gmail.com (M. Shatsky).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jsb.2014.03.017
1047-8477/

angles in each cycle of refinement (Rosenthal and Henderson, 2003). Although initially not widely adopted, the "free-hand" approach is gaining in use, and it is now available in various single-particle software packages (SPIDER: Shaikh et al., 2008; EMAN2: Tang et al., 2007).

The RCT, ORT and "free-hand" processes rely on accurate determination of corresponding particle pairs. While there are several packages that offer automated particle selection from a single micrograph (for a review and comparison see Arbeláez et al. (2011)), particle picking from a tilted pair of images is usually done by hand with a help of graphical interface like that in the XMIPP package (Scheres et al., 2008). Recently, an automated approach has been proposed that brings into correspondence particle pairs from a pair of tilted images (Hauer et al., 2013).

The magnitude of a tilt angle is a user-controlled parameter, and in practice it accurately corresponds with the resulting experimental value. What is usually left undetermined is the direction of the tilt-axis relative to micrographs of tilted and un-tilted specimens. This value can be determined from the geometry of the positions of the corresponding particles. Commonly used packages for determining the direction of tilt assume that the specimen is planar. Given this assumption and a set of identified corresponding particle-center pairs $\{v_i, u_i\}$, an affine transform can be computed that relates particle centers in both images: $v_i = s\ A\ u_i$, where A is a rotational matrix and s is a scaling vector which depends on the tilt angle. TiltPicker (Voss et al., 2009) optimizes least squares for this linear relation, and the tilt-axis directions of both images are extracted from A. TiltPicker adopts a semi-automated iterative approach to resolve the difficulty of computing the required parameters, i.e., tilt angles, shift and corresponding particle pairs. The XMIPP package (Scheres et al., 2008) assumes that one image is non-tilted. Given the corresponding particle pairs it essentially reconstructs 3D coordinates of the particles from the tilted-image. Again, the assumption is that the EM grid is flat. CTFTILT (Mindell and Grigorieff, 2003) is based on the assumption that the contrast transfer function (CTF) changes the least along the direction of the tilt-axis. Therefore, this approach also assumes that the EM grid is planar and there is no pre-tilt of the sample in a direction different from that of the goniometer. In practice, however, the deviations from these two assumptions could result in errors of several degrees for tilt-axis calculations. Consequently, errors in tilt-axis direction are expected to result (a) less accurate particle matching identification, if a method relies on tilt geometry like the presented PCT and the recently introduced MaverickTilt (Hauer et al., 2013) methods; and (b) wrong placement of images in Fourier space. Thus in both cases, the tilt-axis errors may impact the reconstruction quality. For microscopes based on film detectors the tilt-axis direction needs to be computed for every pair of tilted images, since the film can at least be slightly rotated when placed into a microscope and then when placed into a scanner. Such problems do not occur with CCD or DDD equipped detectors; however, direction of the tilt-axis changes with magnification and after a microscope is serviced.

In this work our goal is to give an accurate solution to finding both the particle correspondence and the tilt-axis estimation. For the first stage of particle picking in individual images we make use of the semi-automated TextonSVM method (Arbeláez et al., 2011), where the user selects a small set of characteristic particles that serve as exemplars, and their characteristic texture is used to train an SVM classifier. Here, we extend this approach to identify corresponding particle pairs from a pair of images of a tilted sample. The texture descriptors of TextonSVM are extended with tilt-invariant context descriptors. These new descriptors allow the identification of an initial correspondence between the particles as well as to find approximate tilt-axis angles. The correspondence and the tilt-axis angles are consequently refined using a method similar to a common-line comparison that relates two micrographs based on their 1D projections of particle centers. Using 1D projections allows us to avoid assumptions about EM grid planarity, and at the same time allows us to avoid a complex modeling of EM grid geometry. We have also developed a graphical interface that allows manual editing of selected particle pairs. In Section 5 we demonstrate the accuracy of our method, PCT (particle correspondence from tilted pairs), on three sets of experimentally produced images. In addition, using synthetic data, we show how reasonably expected geometrical deformations of EM grid affect tilt-axis estimates by the commonly used affine transform, while our approach gives robust results in the presence of these types of deformations.

2. Methods

2.1. Data acquisition

2.1.1. Tilted projections of gold particles

The aim of the experiments described in the following was to test recording of tilt pairs on the JEOL SFF 3100 TEM. A home-made carbon film was used to which 10 nm gold particles had been applied. Tilt pairs were recorded at ±15° tilt angles, at 300 kV accelerating voltage, and 60,000× EM magnification. Images were recorded with a CCD camera. To minimize the displacement between the images of the tilt pair, the eucentric height was carefully adjusted. In order to determine the direction of the tilt-axis, additional images of the same area were recorded at 0°, 30°, and 60° tilt angles. For this paper we considered one series of five images recorded at −15°, 0°, 15°, 30° and 60°.

2.1.2. Tilt pairs of inosine-5′-monophosphate dehydrogenase

Inosine-5′-monophosphate dehydrogenase (IMP dehydrogenase, YP_010265.1) with MW of 420 kDa was purified from *Desulfovibrio vulgaris* Hildenborough and negatively stained with Uranyl Acetate solution (Han et al., 2009). EM images were recorded on Kodak SO-163 film at a magnification of 40,000 with under focus values ranging from 1 to 2 μm on a JEOL 4000 microscope operated at 400 kV. Initially, the specimen stage was tilted to 45°, and images were collected by using low dose technique. Following this, the specimen stage was tilted back for the collection of images of untilted specimens from the same area. The exposure for each image was ~20 electrons/Å^2. A total of seven tilt-pair images were digitized with a resolution of 1.59 Å/pixel at the sample level.

2.1.3. Tilted projections of *Escherichia coli* 70S ribosome particles

The samples were prepared using the same protocol as in (Han et al., 2009). The purified ribosome samples, a gift from the laboratory of Prof. Jamie Cate, were applied on a glow discharged holey carbon grid (Quanti foil). The grid was frozen in liquid ethane for cryo-electron microscopy, by using a Vitrobot operated at ambient temperature and 80% humidity. The cryo-EM images were recorded on Kodak SO-163 films at a magnification of 50,000× using a Philips CM200 TEM operated at 200 kV with under-focus values of 2–3 μm. A total of 34 tilt-pair images were recorded at ±15° using low-dose conditions with an exposure of ~15 electrons/Å^2 per image. The recorded images were digitized using the Nikon Super Coolscan 8000 ED densitometer, operated by a film scanning