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ScienceDirect
Journal of Structural Biology 161 (2008) 232–242
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

Computational resources for cryo-electron tomography in Bsoft

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Received 25 April 2007; received in revised form 30 July 2007; accepted 1 August 2007
Available online 11 August 2007

Abstract

The Bsoft package [Heymann, J.B., Belnap, D.M., 2007. Bsoft: image processing and molecular modeling for electron microscopy. J. Struct. Biol. 157, 3–18] has been enhanced by adding utilities for processing electron tomographic (ET) data; in particular, cryo-ET data characterized by low contrast and high noise. To handle the high computational load efficiently, a workflow was developed, based on the database-like parameter handling in Bsoft, aimed at minimizing user interaction and facilitating automation. To the same end, scripting elements distribute the processing among multiple processors on the same or different computers. The resolution of a tomogram depends on the precision of projection alignment, which is usually based on pinpointing fiducial markers (electron-dense gold particles). Alignment requires accurate specification of the tilt axis, and our protocol includes a procedure for determining it to adequate accuracy. Refinement of projection alignment provides information that allows assessment of its precision, as well as projection quality control. We implemented a reciprocal space algorithm that affords an alternative to back-projection or real space algorithms for calculating tomograms. Resources are also included that allow resolution assessment by cross-validation (NLOO2D); denoising and interpretation; and the extraction, mutual alignment, and averaging of tomographic sub-volumes.
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Keywords: Electron microscopy; Image processing workflow; Distributed processing; Micrograph alignment; Fiducial markers

1. Introduction

Cryo-electron tomography is opening new vistas in molecular and cellular structural biology (Baumeister and Steven, 2000; Subramaniam and Milne, 2004). The requirements for producing optimal tomograms are firstly the quality of the tilt series of micrographs, and secondly the computational processing of these data. In ongoing efforts to enhance our tomographic capabilities, which hitherto have been applied primarily to pleiomorphic and asymmetric virus particles and other protein complexes (Grunewald et al., 2003; Harris et al., 2006; Heymann et al., 2006), we continue to develop relevant computational resources.

Here, we describe the implementation of tomography-specific utilities in the software package, Bsoft (Heymann, 2001; Heymann and Belnap, 2007). The primary aim is to provide the user with tools to perform the projection alignment and reconstruction in an efficient manner. A secondary aim is to make processing as easy as possible for the user, without losing control of the important parameters and still allowing detailed manipulation. Underlying both aims is a capability to handle, in the form of a database, the large number of parameters generated in a tomographic analysis. In Bsoft, this is accomplished through the use of parameter files. For some operations, e.g., micrograph alignment and reconstruction, we developed new code; for others, we simply incorporated pre-existing programs into Bsoft, making them readily accessible in a processing framework in which a user may seamlessly access the other image processing operations currently supported. While some of the same functionality is available in other packages, such as IMOD (Kremer et al., 1996), TOM (Nickell et al., 2005), and EM3D[1] (Ress et al., 1999), we attempted

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[1] *Abbreviations used:* 2D, two-dimensional; 3D, three-dimensional; EM, electron microscopy; ET, electron tomography; STAR, self-defining text archiving and retrieval.

1047-8477/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.jsb.2007.08.002

to provide a clear workflow associated with a parameter database and adhering to the 3DEM conventions (Heymann et al., 2005).

2. Materials and methods

2.1. Tilt series acquisition

To illustrate and analyze the various processing steps for tomography, tilt series of vitrified specimens of coated vesicles (Heymann and Belnap, 2007; Heymann et al., 2006) and herpes simplex virus type 1 (HSV-1) A-capsids (Cardone et al., 2007) were used. In brief, a Tecnai-12 electron microscope (FEI, Hillsboro, OR) operating at 120 keV and equipped with an energy filter (Gatan, Inc.) was used to record single-axis tilt series at 1° steps, covering ranges of ~−70° to +70°, at a defocus of ~4 μm with a cumulative dose of ~60 electrons/Å^2. Data were recorded under low-dose conditions, using SerialEM (Mastronarde, 2005).

2.2. Aligning the tilt series

We implemented a procedure for aligning tilt series based on the well known method of using gold fiducial markers. The tilt series is first preprocessed by normalizing the images with *bnorm* and generating the first parameter file with *btomo* or *bshow* (Table 1). To prepare for the alignment, parameters such as the pixel size, the tilt axis angle and the tilt angles are set, and a marker seed is picked in *bshow*. If the tilt axis angle is not well known for the microscope and the magnification used, it needs to be determined more accurately by running a set of single iterations of *btrack* with tilt axis angles close to the expected value (Table 2). This is then followed by a multi-iteration tracking of the markers through the tilt series by building a 3D marker model and determining the origins of the individual micrographs, using the nominal tilt axis angle and tilt angles. The result can be inspected in *bshow* and errant markers manually corrected. Finally, a full geometric refinement of the micrograph views, origins and scales, and the 3D marker model is done with *btrack* (see Supplementary Material for more details).

Table 1
Programs in Bsoft for use in tomography

Program	Use
btomo	General setup for a tomographic tilt series
bshow	Interactive image display with a setup for tomography
bnorm	Tomographic tilt series normalization
btrack	Fiducial marker tracking and refinement
bmgft	Generation of Fourier transforms and associated parameter files
btomrec	Tomogram reconstruction, full or in slabs
bzfft	Concatenation of slabs generated by btomrec and backtransform
btomres	Estimation of the resolution of a tomographic tilt series
bnad	Non-linear anisotropic diffusion filter
bbif	Bilateral filter
bmedian	Iterative median filter
btile	Splitting a big image into overlapping tiles
bpatch	Reassembling overlapping tiles into a single image
bpick	Extraction of particles/sub-volumes
bsubvol	Tracking sub-volumes in a tilt series
bfind	Reference-based orientation of 3D sub-volumes
badd	Weighted averaging of images

Table 2
C-shell scripts for user convenience and distributed processing

Script	Programs used	Use
tomax	btrack	Determining the tilt axis angle
tomrec	bmgft, btomrec, bzfft	Piecewise large tomogram reconstruction
tomres	bmgft, btomres	Resolution determination
tomnad	btile, bnad, bpatch	Piecewise large tomogram denoising

2.3. Reconstruction

The tomograms are reconstructed in reciprocal space using oversampling and a nearest neighbor algorithm, using *btomrec* (Tables 1 & 2). The fiducial markers used in the tilt series alignment can be removed (“painted” by replacement with the image average) from the micrographs before reconstruction (see Supplementary Material for more details).

2.4. Determining resolution

The quality of the tomogram may be assessed by the noise-compensated leave-one-out (NLOO) method of Cardone et al. (2005). Because of the inherent anisotropy of the reconstruction, the resolution is assessed in 2D for each micrograph in the series. The NLOO approach involves the calculation of the ratio of two Fourier ring correlation (FRC) curves, the first comparing each micrograph with an appropriate reprojection of a tomogram without that micrograph, and the second comparing the micrograph with a reprojection of the full tomogram. In the original implementation based on weighted backprojection, in addition to the full tomogram, another tomogram needed to be calculated for every micrograph. Here we make use of the central section theorem, also known as the Fourier slice theorem (Kak and Slaney, 1988), to simplify and accelerate the calculation, requiring only the reconstruction of two 2D central sections for comparison to each micrograph. Because the beam is not perpendicular to all but one of the micrographs, the corresponding voxels between two Fourier transforms is offset perpendicular to the tilt axis as a function of the tilt angles. This is included in calculating the central sections in addition to the basic geometric transformations (see Appendix A).

Resolution is assessed with *btomres*, which calculates the resolution for one micrograph at a time based on the