

Marker-Free Alignment of Dual-Axis Tilt Series and Subvolume Analysis of Data from Dual-Axis Tomograms

Hanspeter Winkler and Kenneth A. Taylor

Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306 USA

In recent years, electron tomography has become a mainstream technique for 3D reconstruction of macromolecules, macromolecular assemblies, organelles, and whole cells. While the resolution that can be obtained with tomography is not as high as that obtained by alternative techniques such as single particle methods, the analysis of subvolumes extracted from tomograms provides a relatively straightforward way to characterize structural or conformational heterogeneity with well established alignment and classification techniques.

One of the problems encountered in electron tomography is incomplete sampling of the reconstruction in Fourier space, which is due to the limited range in which the specimen can be tilted. Typical angular ranges from $\pm 60^\circ$ to $\pm 75^\circ$ leave a considerable volume unsampled: a wedge shaped region for single-axis tilt series, or a pyramidal region for dual-axis tilt series, respectively. Dual-axis tomography results in a better coverage of Fourier space and a more isotropic resolution, since only the intersection of the missing wedges of the two contributing single-axis tilt series with approximately perpendicular axes remains unsampled. The more complete coverage is also advantageous for the processing of subvolumes extracted from such tomograms. Computational means to compensate missing wedge effects in the cross-correlation alignment or the classification of subvolumes then become less critical in order to produce reliable averaged structures.

Using dual-axis tilt series of a plastic embedded and sectioned insect flight muscle specimen, we have explored two approaches for tilt series alignment and 3D reconstruction: first, the separate processing of a dual-axis tilt series as two single-axis tilt series with subsequent merging of the two computed maps [7], and second, a novel simultaneous alignment technique [8]. The merging of the two tomograms in the first approach was carried out according to Mastronarde [3]; the single map in the second approach was computed with weighted back-projection from the combined simultaneously aligned data. In both approaches we used marker-free tilt series alignment [4]. This procedure is based on area matching, in which the images are not only translationally aligned, but also matched by considering rotation, shear and scale changes. In addition, it includes a refinement of geometric parameters and a reference construction scheme based on the computation of a preliminary, continually evolving 3D reconstruction. Alignment is carried out with respect to computed references which are reprojected from the preliminary reconstruction. The whole registration procedure is iterative, consisting of cycles of area matching and geometry refinement.

For the subsequent subvolume processing and resolution assessment we applied procedures that we developed for the analysis of cryo-tomograms of myosin V, SIV and HIV virions [6], that included missing wedge compensation. Subvolumes were extracted from the dual-axis tomograms at identical positions relative to the specimen structure for comparison purposes. The analysis showed that the simultaneous alignment approach produced averages with higher resolution as measured with Fourier shell correlation

than the merging approach. The resolution increase can be attributed to a more accurate geometry determination for simultaneous alignment, and partly to technical improvements of the alignment procedure, which included the compensation of the non-perpendicularity of the electron beam relative to the tilt axis.

The software package for marker-free alignment of single and dual-axis tilt series with area matching, *protomo*, is available on the website www.electrontomography.org. The automated alignment procedure and the geometry refinement are implemented as a Python extension module written in the C language, that easily integrates with other software such as SPARX/EMAN2 [1] or Appion [2]. For visualization and manual alignment a graphical tool is provided. The basic image processing operations called by the Python extension module are organized in external libraries which are shared with the software package for subvolume processing (*i3*), which is based on shell scripts and command line programs [5].

References:

- [1] M. Hohn, G. Tang, G. Goodyear, P. R. Baldwin, Z. Huang, P. A. Penczek, C. Yang, R. M. Glaeser, P. D. Adams, S. J. Ludtke, SPARX, a new environment for cryo-EM image processing, *J. Struct. Biol.* 157 (2007) 47–55.
- [2] G. C. Lander, S. M. Stagg, N. R. Voss, A. Cheng, D. Fellmann, J. Pulokas, C. Yoshioka, C. Irving, A. Mulder, P.-W. Lau, D. Lyumkis, C. S. Potter, B. Carragher, Appion: An integrated, database-driven pipeline to facilitate em image processing, *J. Struct. Biol.* 166 (2009) 95–102.
- [3] D. N. Mastronarde, Dual-axis tomography: an approach with alignment methods that preserve resolution, *J. Struct. Biol.* 120 (1997) 343–352.
- [4] H. Winkler, K. A. Taylor, Accurate marker-free alignment with simultaneous geometry determination and reconstruction of tilt series in electron tomography., *Ultramicroscopy* 106 (2006) 240–254.
- [5] H. Winkler, 3D reconstruction and processing of volumetric data in cryo-electron tomography, *J. Struct. Biol.* 157 (2007) 126–137.
- [6] H. Winkler, P. Zhu, J. Liu, F. Ye, K. H. Roux, K. A. Taylor, Tomographic subvolume alignment and subvolume classification applied to myosin V and SIV envelope spikes, *J. Struct. Biol.* 165 (2009) 64–77.
- [7] S. Wu, J. Liu, M. C. Reedy, H. Winkler, M. K. Reedy, K. A. Taylor, Methods for identifying and averaging variable molecular conformations in tomograms of actively contracting insect flight muscle, *J. Struct. Biol.* 168 (2009) 485–502.
- [8] H. Winkler, K. A. Taylor, Marker-free dual-axis tilt series alignment, *J. Struct. Biol.* in press (2013).