

TomoJ: new tools for electron tomography

Messaoudi C.^{a**}, Halary S.^{a,b}, Sorzano C.O.S.^{c,d}, Marco S.^a and Boudier T.^a

^aImagerie Intégrative Institut Curie - INSERM U759, Centre Universitaire d'Orsay. Bât 112, 91405 Orsay CEDEX, France;

^bAdaptation et Evolution en milieux extrêmes UMR 7138 CNRS Université Pierre et Marie Curie, 7 Quai Saint Bernard, 75005 Paris, France;

^cUnidad de Biocomputacion, Centro Nacional de Biotecnologia (CSIC), Campus Universidad Autonoma s/n, 28049 Cantoblanco, Madrid, Spain;

^dDept. Ingenieria de Sistemas Electronicos y de Telecomunicacion, Univ. San Pablo-CEU, Campus Urb. Montepincipe s/n, 28668 Boadilla del Monte, Madrid, Spain

ABSTRACT

Cellular electron tomography allows the computation of three-dimensional reconstructions of objects from a series of projections recorded at various tilt angles. Electron tomography has highly contributed to the understanding of the structures of organelles and other sub-cellular components. To compensate the fact that it is not possible to record projection at all possible tilt angles, some alternative schemes for acquisition have been developed. We have created a software, called *TomoJ*, composed of three different plug-ins for ImageJ to perform 3D reconstructions from 2D projections and to align reconstructed tomograms. In addition, a plug-in was also developed for Energy Filtered Transmission Electron Tomography (EFTET) opening the way to the 3D localization of chemical elements on samples.

Keywords: Electron tomography, electron microscopy, 3D reconstruction, ImageJ.

1. INTRODUCTION

Determination of cellular structures is important for the comprehension of cellular processes. At the cellular level the method of choice for imaging is the Transmission Electron Microscopy (TEM). The problem of TEM is that the information is a 2D projection of the sample. This is of course very valuable for many studies, however the 3D visualization of cellular structures permits the gain of important biological information. For this purpose, Transmission Electron Tomography (TET) has been developed in the last decade^{1,2}. This technique consists in tilting the sample around one axis using a goniometer to obtain many views of the sample of interest at different orientations. TET has been successfully applied to the study of Golgi^{3,4}, syncytial-type cell plates⁵, post-meiotic cytokinesis⁶, higher-plant chloroplast thylakoid membranes⁷ and *Chlamydomonas reinhardtii* basal bodies⁸.

The first step in TET is the acquisition of a tilted serie. Indeed this acquisition step is not perfect; due to mechanical problems, shifts of the specimen are observed. These shifts can be partly corrected during the acquisition process, but there is still need to align the tilt series before performing the reconstruction. For technical reasons it is not possible to acquire views at all angles, thus some information will not be recovered for very high tilt angle. To lower the quantity of missing information, an improvement has been proposed by acquiring a second series of views by tilting the sample of interest around another axis perpendicular to the first one. The two tilted series are reconstructed separately and then combined. This technique is called dual-axis tomography⁹. We have extended this approach to multiple-axis tomography¹⁰, which consist of multiplying the number of tilt axis.

The second step will be to align the raw tilt series before reconstruction. Currently, software for TET^{11,12} are performing 2 types of alignment, automatic by cross-correlation and manual using fiducial markers (the most often gold

*Send correspondence to C. Messaoudi

email : cedric.messaoudi@curie.fr Tél : +33 1 69 86 31 72

beads). They usually propose only one reconstruction algorithm, the weighted back-projection. Some other iterative 3D reconstruction algorithm used in CAT scan such as algebraic reconstruction technique¹³ (ART) and simultaneous iterative reconstruction technique¹⁴ (SIRT) are more robust to the missing information but not always proposed. In addition, new techniques such as Energy Filtered Transmission Electron Tomography¹⁵ (EFTET) presently emerging and opening the way to the 3D localization of chemical elements on samples are not yet supported in software for electron tomography.

We propose the *TomoJ* package as a set of plug-ins for ImageJ^{16,17} for transmission electron tomography studies. The first plug-in allows the alignment of tilt series, computation of the tilt axis and the three reconstruction algorithms. The second plug-in performs the alignment of reconstructed tomograms for dual and multiple-axis tomography. The third plug-in permits the reconstruction of 3D chemical maps for EFTET.

MATERIAL AND METHODS

Alignment of tilt series

The first step is to align the raw tilted serie. Translations between each two consecutive images are determined by cross-correlation using an adaptation of ImageJ's *FHT3D*. The average of all computed translations is subtracted from all individual translations to better dispatch the loss of parts of the images. Rotations eventually existing between consecutive images are also determined by correlation. Rotations are applied to the second image in a range of +/- 15° and the rotation obtaining the best correlation score is kept. As for translation the average rotation is computed and subtracted to all individual rotations.

In a manual version, translations are computed thanks to markers clicked by the user. These markers are often present in the form of gold beads (5-20 nm of diameter) which are added to the preparation. But the markers can be any recognizable feature. *TomoJ* provides a very user friendly interactive tool for assigning manually the markers position; it uses the *PointPicker* plug-in for ImageJ in a transparent way for the user. In a well aligned tilt series a point will follow a circle arc around the tilt axis in the 3D space. As the images are projections, this trajectory becomes linear and perpendicular to the tilt axis. If the markers given by the user are not along lines, the images are translated in order to obtain a better linear trajectory of markers. The average of all computed translations is subtracted from all translations to better dispatch the loss of parts of the images.

Tilt axis determination

For the reconstruction algorithms, the axis around which the specimen was tilted is to be known. If the user knows it by any means (acquisition software, microscope calibration ...) he can enter it directly in the software. A manual procedure is also available to help the user compute the tilt axis. The tilt series first needs to be well aligned. The user clicks on some markers. If these markers were already selected for alignment there is no need to go to click new ones. As for manual alignment, each marker should follow an axis perpendicular to the tilt axis. With a simple linear regression, the lines followed by each marker are determined. A statistical test on regression is then performed to eliminate markers that do not follow a linear trajectory. The tilt axis is then computed as the axis perpendicular to the general axis of displacement of the markers. The tilt axis is given as an angle (in degrees) between the tilt axis and the vertical axis; it is also the angle by which rotating the tilt series to put the tilt axis vertical.

Reconstruction

Following successful alignment, the three dimensional reconstructed volume can be generated using different algorithms. The first one is the back-projection which is actually the most used algorithm for reconstruction in TET. However the overemphasis of low frequencies in Fourier space can be avoided by using a weighting scheme prior to the reconstruction. In *TomoJ* an analytical, frequency-dependent weighting is used in Fourier space¹. Subsequently, the aligned and optionally weighted projections are back-projected into a 3D volume using tri-linear interpolation.

More robust algorithms, based on the comparison between the experimental projections images and the projections computed from the reconstructed volume, are also implemented. This is done in an iterative way so that the projections of the reconstructed volume computed through an image formation model resemble the experimental

projections obtained by the microscope. *TomoJ* implements two different variants of iterative 3D reconstruction algorithm: algebraic reconstruction technique¹³ (ART) and simultaneous iterative reconstruction technique¹⁴ (SIRT).

3D alignment

The alignment of multiple tomograms is performed as described in Messaoudi *et al.*¹⁰. Basically it is the adaptation to 3D of well known algorithms to align 2D images in molecular tomography. Volumes are aligned two by two, using cross-correlation coefficients. The order of these two by two alignments follows three different schemes: one is the alignment of all volumes against the first one taken as a reference. This is quite efficient but if the first volume is different from the others it can produce biased results. To prevent this, two reference-free alignments have been proposed in Penczek *et al.*¹⁸ and Marco *et al.*¹⁹. The first consists of aligning a volume against the average of all other already aligned volume. The second consist of aligning two by two randomly chosen number of volumes, and then aligning two by two the averages of this first alignment and so on until there is only one final average. This average is then used as the reference to align all the volumes. With the three schemes a refinement of alignment is possible by aligning again each volume against the computed average¹⁸.

EFTET

When performing acquisition in EFTET¹⁵, several series must be recorded, each one at a particular energy. More precisely a series must be recorded at the characteristic energy of the studied chemical element, and other series must be recorded before and after this energy in order to estimate the background signal. The first step is then to align the different series, as in classical tomography, then to align all the series to a common reference. The background level is then computed for each point of each projection using the different values coming from the different energies before or after the characteristic signal. Once this background is subtracted from the characteristic signal, the reconstruction can be performed as in classical electron tomography.

RESULTS

The *TomoJ* package for ImageJ provides plug-ins to perform reconstruction in electron tomography. The first plug-in, *TomoJ interface*, proposes to perform alignment of tilt series, tilt axis determination and reconstruction using different algorithms. This will change the normal toolbar of ImageJ to the one from *PointPicker* which is useful for manual alignment and tilt axis determination. The interface (fig 1) is divided into 4 panels and one menu. The panel on top permits some actions such as removing an image from the tilt series, undoing the last modification of alignment or resetting every modification, saving or loading the transformations for alignment. In the case of modification of the tilt series by any processing in ImageJ, the panel permits to update the images. The button *switch toolbar* allows to switch between the toolbar of ImageJ and the toolbar of *PointPicker*. The second panel contains functions for filtering the images and automatic alignment of the tilt series. The third panel provides functions for manual alignment and tilt axis determination. The last panel permits the computation of the 3D volume from the tilt series. The menu proposes some of the actions allowed in the top panel plus some more such as modifying the tilt angles of each images of the tilt series or the possibility to load an already computed tomogram in order to refine it.

The second plug-in, *TomoJ average*, allows the alignment of multiple tomograms. Some parameters are asked : the first parameter is the algorithm to use for the alignment, the next parameters are the range of search for rotation, the minimal increment of rotation and the number of refinement procedure. The third plug-in *EFTETJ* proposes reconstruction of tilt series acquired using EFTET.

CONCLUSIONS

The *TomoJ* package provides different plug-ins for electron tomography. The interfaces are to be user-friendly and easy of use.

ACKNOWLEDGMENTS

CM is recipient of a fellowship of the 6FP European network "New Electron Microscopy Approaches for Studying Protein Complexes and Cellular Supramolecular Architecture". This work was financed by the Curie Institute (PIC "Physico-Chimie du Vivant").

REFERENCES

1. Frank, J., *Electron tomography. Three-dimensional imaging with the transmission electron microscope*, Plenum Press, New York, 1992.
2. Marco S, Boudier T, Messaoudi C, Rigaud JL. "Electron tomography of biological samples," *Biochemistry (Mosc)*, **69(11)**, pp.1219-25, 2004.
3. Ladinsky MS, Mastronarde DN, McIntosh JR, Howell KE, Staehelin LA, "Golgi structure in three dimensions: functional insights from the normal rat kidney cell," *J Cell Biol.* **144(6)**, pp 1135-49, 1999.
4. Marsh BJ, Volkmann N, McIntosh JR, Howell KE, "Direct continuities between cisternae at different levels of the Golgi complex in glucose-stimulated mouse islet beta cells," *Proc Natl Acad Sci U S A*, **101(15)**, pp 5565-70, 2004.
5. Otegui MS, Mastronarde DN, Kang BH, Bednarek SY, Staehelin LA, "Three-dimensional analysis of syncytial-type cell plates during endosperm cellularization visualized by high resolution electron tomography," *Plant Cell.* **13(9)**, pp 2033-51, 2001.
6. Otegui MS, Staehelin LA, "Electron tomographic analysis of post-meiotic cytokinesis during pollen development in *Arabidopsis thaliana*," *Planta.* **218(4)**, pp 501-15, 2004.
7. Shimoni E, Rav-Hon O, Ohad I, Brumfeld V, Reich Z, "Three-dimensional organization of higher-plant chloroplast thylakoid membranes revealed by electron tomography," *Plant Cell.* **17(9)**, pp 2580-6, 2005.
8. O'Toole ET, Giddings TH, McIntosh JR, Dutcher SK, "Three-dimensional organization of basal bodies from wild-type and delta-tubulin deletion strains of *Chlamydomonas reinhardtii*," *Mol Biol Cell.* **14(7)**, pp 2999-3012, 2003.
9. Mastronarde DN, "Dual-axis tomography: an approach with alignment methods that preserve resolution," *J Struct Biol.* **120(3)**, pp 343-52, 1997.
10. Messaoudi C, Garreau de Loubresse N, Boudier T, Dupuis-Williams P, Marco S, "Multiple-axis tomography: applications to basal bodies from *Paramecium tetraurelia*," *Biol Cell.* (In press) 2006.
11. Kremer JR, Mastronarde DN, McIntosh JR, "Computer visualization of three-dimensional image data using IMOD," *J Struct Biol.* **116(1)**, pp 71-6, 1996.
12. Nickell S, Forster F, Linaroudis A, Net WD, Beck F, Hegerl R, Baumeister W, Plitzko JM, "TOM software toolbox: acquisition and analysis for electron tomography," *J Struct Biol.* **149(3)**, pp 227-34, 2005.
13. Herman GT, Lent A, Rowland SW, "ART: mathematics and applications. A report on the mathematical foundations and on the applicability to real data of the algebraic reconstruction techniques," *J Theor Biol.* **42(1)**, pp 1-32, 1973.

14. Gilbert P, "Iterative methods for the three-dimensional reconstruction of an object from projections," *J Theor Biol.* **36(1)**, pp 105-17, 1972.
15. Boudier T, Lechaire JP, Frebourg G, Messaoudi C, Mory C, Colliex C, Gaill F, Marco S, "A public software for energy filtering transmission electron tomography (EFTET-J): application to the study of granular inclusions in bacteria from *Riftia pachyptila*," *J Struct Biol.* **151(2)**, pp 151-9, 2005.
16. Abramoff, M.D., Magelhaes, P.J., Ram, S.J. "Image Processing with ImageJ," *Biophotonics International*, **volume 11, issue 7**, pp. 36-42, 2004.
- 17 Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2005
18. Penczek PA, Grassucci RA, Frank J, "The ribosome at improved resolution: new techniques for merging and orientation refinement in 3D cryo-electron microscopy of biological particles," *Ultramicroscopy*, **53(3)**, pp 251-70, 1994.
19. Marco S., Chagoyen M., Fraga L.G., Carazo J.M., Carrascosa J.L, "A variant to the "random approximation" of the reference-free alignment algorithm," *Ultramicroscopy*, **66**, pp5-10, 1996.

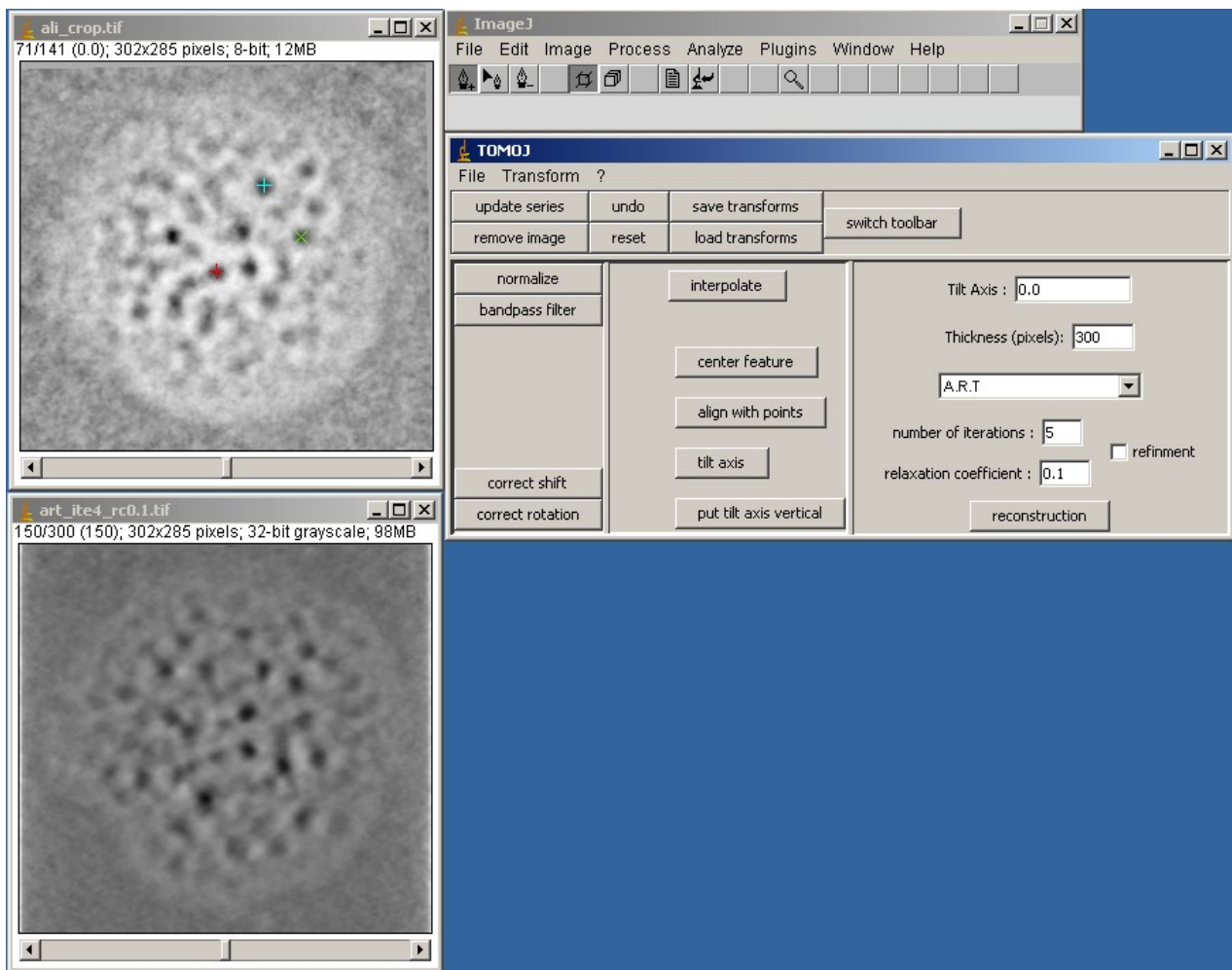


Figure 1. Interface of *TomoJ* for reconstruction of electron tomograms. The toolbar of ImageJ is changed to the one from the plug-in *PointPicker*. The stack of image on top is the 0° tilt series with some features visualized with color crosses used for alignment or tilt axis determination. The stack at bottom is the central plane of the reconstructed volume using ART.