Semi-automatically aligned tilt images in electron tomography

doi: info:doi/10.1109/ICIIBMS.2017.8279738

Semi-Automatically Aligned Tilt Images in Electron Tomography

Qingyuan Tian, Lars-Göran Öfverstedt, Ulf Skoglund
Structural Cellular Biology Unit
The Okinawa Institute of Science and Technology Graduate University
1919-1 Tancha, Onna-son, Okinawa, Japan 904-0495
qingyuan.tian@oist.jp

***Abstract*—In electron microscope tomography, alignment of tilt series images is a major determinant of resolution in 3D reconstructions. One alignment method uses gold beads deposited on or in the specimen as fiducial markers. We have developed software to semi-automatically align tilt series images. It runs two processes iteratively: (1) Marker picking. In this process, it uses a cross-correlation function to determine the shift between tilt images and predicts marker coordinates. Subsequently it refines them in a local search area, and detects and corrects erroneously picked markers automatically. The coordinates of the picked markers are used to align the images. (2) Image alignment. In this process, it uses a least squares method to estimate image rotation, image shift, and image scale factor.**

Keywords—image processing; image alignment; electron tomography; least sqaure method; automation

I. INTRODUCTION

Using 2D electron microscope images taken at different tilt angles, electron tomography (ET) obtains z-axis information and uses back-projection to provide 3D reconstructions of objects. ET is used to characterize molecular complexes in structural and cellular biology research. Beside the number of projections acquired, a major determinant of resolution in 3D reconstructions is the accuracy of alignment of the projections, which are a series of tilted images (abbreviated as "tilts" hereafter). One alignment method utilizes fiducial markers, which appear as high-contrast projections of gold beads deposited on, or in, the specimen prior to data collection. Measured coordinates of these markers are used to align the tilts [1][2][3]. Other methods make use of the cross-correlation function to determine the shifts, rotation- and skewing-angles between tilts [4]. The fiducial marker method is usually used in low-dose cryo electron tomography because it normally enables consistent alignment across the full range of tilts. Fig.1 illustrates the electron tomographic scheme.

In the fiducial marker method, markers should be measured on every tilt. We call this process "marker picking". Marker picking can be done manually by an operator, but it is time consuming. Sometimes manually picked markers are not accurate, especially when the operator is inexperienced, when tilts are noisy and there are hundreds of images to process. Agard and colleagues developed and reported an automated fiducial-based alignment scheme [5], but their description is too brief for other researchers to implement. Ress and his colleagues also developed an automated system to acquire fiducial markers and to align tilts [6]. They start by using an image processing method to identify candidate markers. Then they compare permutations of candidate markers in the new tilt to those in the previous tilt to index the markers. However, their implementation can fail when tilts are noisy and the time complexity of their algorithm is high.

We developed software called "xpix" to pick markers and we have continued to add features to it [7][8][9]. In the current version, when using xpix, the operator manually selects markers only on the 0° tilt, enabling the software to automatically pick all other tilts and finally to compute the alignment of the tilts using these markers.

In this article, Section 2 introduces the application background. Section 3 describes our electron tomography geometry model, and Section 4 describes features in xpix. In Section 5, we discuss the performance of xpix. In section 6, we draw conclusions and introduce subsequent work.

II. BACKGROUND

In our research unit, the in-house software, xpix, has been used to pick markers. This software aids the operator by locating a circular object with the highest density in a specified area near the curser, which it selects as a marker when the mouse is clicked. When picking markers at a new tilt, it can predict the position of the marker in the new tilt. When all the markers in all tilts have been picked, their coordinates and radii are saved in a text file in XML format [10], which is read as input by our software "Refine," which aligns the tilts using the least square method [11].

In order to make "xpix" more efficient, we have added a feature called "AutoPick" to enable marker-picking semi-automatically. In AutoPick, markers at 0° tilt are selected manually, and they are used as initial information for all subsequent processes. AutoPick uses a cross correlation function to predict the shift between the previous tilt and the new tilt. It accesses a new tilt with an increased tilt angle, and markers identified in the previous tilt are used to predict markers in the new tilt. The predicted marker is refined into the picked marker by searching within a circular shaped envelope

978-1-5090-6664-3/17/$31.00 ©2017 IEEE

to find the highest integrated density value. The picked marker might be erroneously picked to a position where there is no marker, or where there is another marker. AutoPick tests these errors automatically, and adjusts the size of the search envelope automatically to correct these errors. Once the markers in a tilt are picked, this tilt becomes the "previous" tilt, and AutoPick moves to another new tilt. This process is repeated until all tilts are picked [8].

In some cases, AutoPick can fail to calculate translation-shifts between tilts and consequently it can pick markers erroneously. We have added another feature to test this failure, and to pause AutoPick to await manual guidance in the event of such a failure. The operator can adjust the translation-shifts manually if needed, and then resume AutoPick from where it paused [9].

We have released an updated version of "xpix" with these two features, and they usually make "marker picking" faster, more efficient, and more accurate than manual operation [8][9].

When AutoPick predicts a marker in a new tilt for the first time, it normally assumes that the marker's z coordinate in the specimen is 0. This potentially introduces an error into the prediction, since most markers don't lie in one plane. We call this a "parallax error." In order to deal with the "parallax error," we integrated our tilt alignment software "Refine" into "xpix," and made it run iteratively with AutoPick. This paper mainly describes this new feature.

III. Geometry Model

This section outlines the geometric model of electron tomography used in our software for electron microscope tomography [1][2][7][8][9][11].

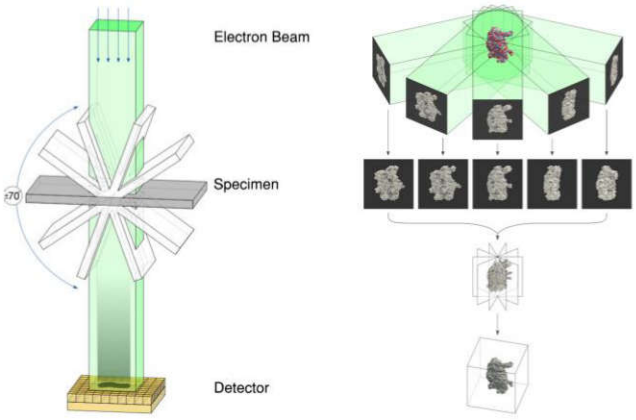

Fig. 1. Electron microscope tomography. *Fig. 1(A) presents that the specimen is tilted and tilt series images are recorded. Fig. 1(B) is a more schematic illustration of tilt projection and back-projection. After aligning all projection images, the 3D structure is computed using weighted back-projection.*

With reference to 0° tilt, let $\emptyset$ represent the microscope mechanical rotation angle, while θ represent the specimen tilt angle. Let Ω represent the rotation angle of projection (including the sample orientation in the sample holder and rotation changes upon acceleration voltage changes), and let s represent the relative scaling factor. Let x_s, y_s represent the center shift along the X and Y coordinate axes in a tilt's projection. A point in the specimen with coordinates (x, y, z) will project to the point (x_p, y_p) in the tilt projection image, according to formulae (1) ~ (4).

$$A1 = \begin{pmatrix} \cos\emptyset & -\sin\emptyset & 0 \\ \sin\emptyset & \cos\emptyset & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad (1)$$

$$A2 = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\theta & -\sin\theta \\ 0 & \sin\theta & \cos\theta \end{pmatrix} \quad (2)$$

$$A3 = \begin{pmatrix} \cos\Omega & -\sin\Omega & 0 \\ \sin\Omega & \cos\Omega & 0 \end{pmatrix} \quad (3)$$

$$\begin{pmatrix} x_p \\ y_p \end{pmatrix} = s \cdot \left(A3 \cdot A2 \cdot A1 \cdot \begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} x_s \\ y_s \end{pmatrix} \right) \quad (4)$$

When tilts are acquired in the electron microscope, their parameters $\emptyset$, θ, Ω, s, and (x_s, y_s) are set to certain values by the mechanism system of electron microscope. These are nominal values of tilt parameters; thus they have errors and need to be refined. Let (x_m, y_m) represent observed coordinates of a picked marker. Their theoretical values are (x_p, y_p), calculated in formula (4). Using these observed values, the least squares method iteratively refines tilt parameters and estimates marker coordinates (x, y, z) by minimizing the errors: the distances between (x_m, y_m) and (x_p, y_p). In our research, this process is implemented using the in-house software "Refine", written in Fortran [11].

IV. Features and Methods

In "xpix," nominal values of tilt parameters are applied to the geometry model to calculate (x_p, y_p) as the prediction of marker coordinates (x_m, y_m) in a new tilt.

In "xpix," we nominally assume that marker z coordinates in the specimen are 0. As is shown in Fig. 2, if an assumed z coordinate has an error Δz, and the tilt angle is θ, the error when using formulae (1) ~ (4) to predict the marker in the tilt image will be $(\Delta z \cdot \sin\theta)$. We call this "parallax error". It increases as the tilt angle increases.

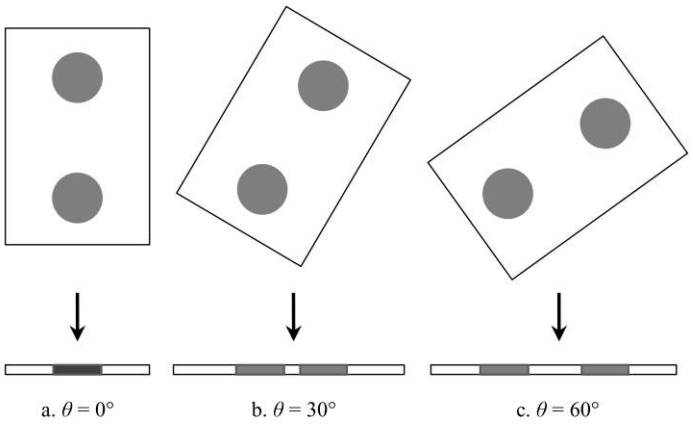

Fig. 2. Parallax errors. *The 2 grey circular shapes represent 2 markers (gold beads) in the specimen. They have the same x and y coordinates, but different z coordinates. If the difference of their z coordinates is Δz, and the tilt angle is θ, in the tilt their markers (the projections of the gold beads) will have a distance of (Δz·sinθ). Thus, the assumtion that all markers have the same z coordinates in a specimen normally causes significant "parallax errors."*

If the real value of the z coordinate of a marker is used to predict the marker's position in a tilt, the "parallax error" can be removed. But the actual value of the z coordinates of the markers are not known, and cannot be observed either. An efficient way to obtain these values is by using a least squares method to estimate them after the markers in a few tilts are picked (observed) and compared.

In order to deal with the "parallax error," we propose a new feature for xpix. In this feature, markers in the tilts with lower tilt angles ("low tilts" hereafter) are picked by assuming their z are 0. Then z values are estimated using the least squares method, and subsequently markers in the tilts with higher tilt angles ("high tilts" hereafter) are picked, using estimated z coordinates instead of 0 to predict their positions in those tilts. We implemented this feature by integrating "Refine" into "xpix," to be run in an iterative fashion with AutoPick.

To support this feature, we also implemented the following functions:

- Using an XML formatted file with stored acquisition data information to open tilts.
- Creating automation information to control automatic running of AutoPick and Refine.
- Processing arrays in column-major order in the C language to cooperate with the Fortran routine.
- Skipping bad tilts.
- Adjusting markers by manual drag-and-drop.

Fig. 3 provides an overview of the work flow using this new feature to align tilts.

A. Using XML File to Open Tilts

There are several formats used for storing image files acquired with electron microscopes. In order to process them in a consistent manner, an XML file was created for the tilts. In this XML file, meta data are saved, such as image type, image file name, and nominal Ø, θ, Ω, s, and (x_s, y_s).

When tilts are opened and accessed using the XML file, their meta data are also read into "xpix." Based on the meta data, a tilt list is created and sorted by nominal tilt angle θ.

B. Creating Automation Control Information

To run AutoPick and Refine automatically, "xpix" creates automation control information beforehand.

Table 1 is an example of automation control information for a dataset with 25 tilts, spanning -60° to +60° in 5° increments. Using this table, "xpix" will first run AutoPick to pick markers in tilts 14 to 19 (tilt angles 5° to 30°), and then tilts 12 to 7 (-5° to -30°), and subsequently it will run Refine to align tilts 7 to 19 (-30° to 30°). After that, it will run AutoPick to pick markers in tilts 20 to 25 (35° to 60°), and then in tilts 6 to 1 (-35° to -60°). Then it aligns all the tilts. This is the default process of running Refine within "xpix."

If the operator decides not to run pre-refine at low tilts, he can un-check the option "pre-refine tilts between -30°~30°." In that case, "xpix" will create automation control information as in Table 2. Using this table, "xpix" runs AutoPick to pick markers in all positive tilts, and then in all negative tilts, and subsequently it runs Refine to align all tilts.

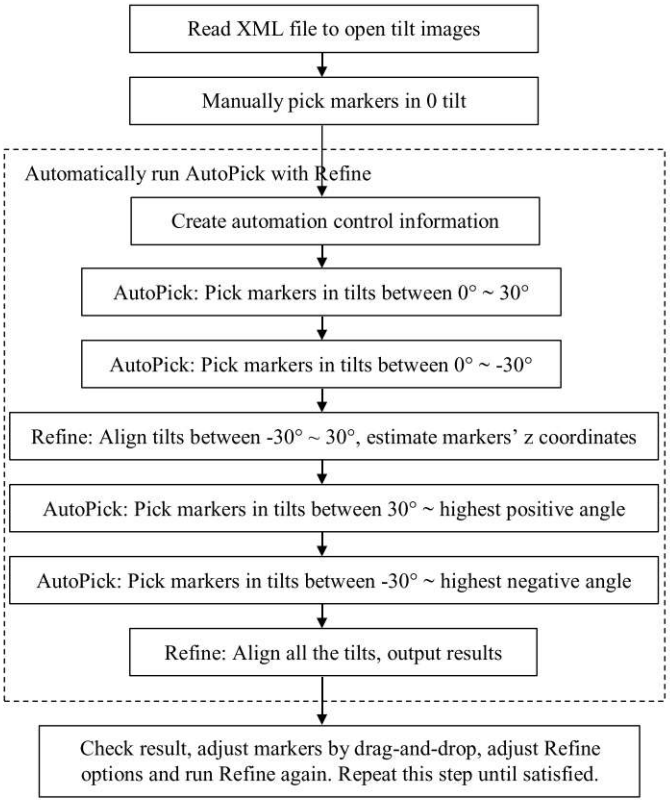

Fig. 3. Work flow of "xpix." *Processes inside the box "Automatically run AutoPick with Refine" are automatically executed. Other processes require manual operation.*

TABLE I. AUTOMATION CONTROL INFORMATION (DEFAULT)

Stage Number	Control Information		
	Action	*Start Tilt Number (Tilt Angle)*	*End Tilt Number(Tilt Angle)*
1	1[a]	14 (5°)	19 (30°)
2	1	12 (-5°)	7 (-30°)
3	2[b]	7 (-30°)	19 (30°)
4	1	20 (35°)	25 (60°)
5	1	6 (-35°)	1 (-60°)
6	2	1 (-60°)	25 (60°)
7	0[c]		

[a] Action code 1 represents AutoPick

[b] Action code 2 represents Refine

[c] Action code 0 represents Stop

TABLE II. AUTOMATION CONTROL INFORMATION (NO PRE-REFINE)

Stage Number	Control Information		
	Action	*Start Tilt Number(Tilt Angle)*	*End Tilt Number(Tilt Angle)*
1	1	14 (5°)	25 (60°)
2	1	12 (-5°)	1 (-60°)
3	2	1 (-60°)	25 (60°)
4	0		

C. Processing Array in Column-Major Order in C Language

Our "xpix" is implemented in the C language, and we have an optimized and tested "Refine" routine implemented in Fortran language. We decided to use C "xpix" to call that Fortran "Refine" routine instead of re-writing the "Refine" algorithm in C. It is necessary to pass data as arrays from "xpix" to "Refine" because C processes arrays in row-major order whereas Fortran processes arrays in column-major order. Direct passing of arrays from C to Fortran is not possible. We created structures and macros in C to processes arrays in column-major order, which enabled us to integrate the "Refine" Fortran routine into C "xpix" smoothly.

D. Skipping Bad Tilts

Some tilts in a series might be of low quality and cannot be used to construct a 3D volume. We just call these bad tilts. AutoPick is bound to fail picking markers automatically on bad tilts too. In "xpix," the operator can input a list of tilt IDs for bad tilts, and AutoPick and Refine automatically skip those.

E. Adjusting Markers by Drag-and-Drop

The operator uses this function to manually correct erroneously picked markers.

V. RESULT AND DISCUSSION

We tested the "xpix" Refine-feature on 2 data sets. One had 25 tilts, the other had 281 tilts. We ran 2 processes on each dataset. These 2 processes were:

Process 1, the process with pre-refine. As shown in Table 1, "xpix" first uses AutoPick to automatically pick markers in the tilts between -30° ~ 30°. Then it pre-refines these tilts and estimates marker z coordinates, and subsequently uses AutoPick to pick markers in all the remaining tilts. This is the process for using the Refine sub-program to deal with "parallax errors" in order to improve accuracy.

Process 2, the process without pre-refine. As shown in Table 2, "xpix" just uses AutoPick to pick markers in all the tilts. This process disables the new feature to deal with "parallax errors." It is used as a benchmark to test the new feature.

Table 3 compares the average errors. In the process with pre-refine, markers picked in low tilts are used to align low tilts and estimate z coordinates of markers. These estimated z coordinates are then used to predict markers in high tilts. "Parallax errors" in this prediction are smaller than just assuming that z coordinates of markers are 0. For this reason, results of the process with pre-refine are more accurate.

In "xpix," we also implemented multiple features in AutoPick to automatically test and correct erroneously picked markers[8][9]. These are necessary to manage very low-dose data sets. These features test and correct all kinds of errors in "marker picking," including "parallax errors" caused by assuming that nominal z coordinates of markers are 0. That's why in some data set (e.g., data set 2) the improvement of this Refine feature seem less eye-catching. However, in higher tilts, many gold markers tend to overlap or at least be near each other. The Refine feature of predicting a reasonable parallax is quite important to disentangle the packed image of gold markers.

VI. CONCLUSIONS

We have developed a software system "xpix." Using its AutoPick process, it can select fiducial markers semi-automatically in electron tomography low-dose tilts. We have integrated the tilt alignment software "Refine" into xpix and allowing it to run together with AutoPick iteratively. By testing "xpix" on 2 data sets, we have shown that pre-refinement on low tilts can be used to decrease "parallax errors" on high tilts; hence markers are picked and tilts are aligned more accurately.

Beside the improvement in accuracy, the new feature of "xpix" integrates processes from "marker picking" to "tilt alignment" into one interface. The most time-consuming part in these processes can be run automatically. It is user-friendly and has improved users' experience.

TABLE III. ERRORS OF MARKER PICKING

Data Set	Pixel Size (Å)	Number of Tilt	Markers Per Tilt	Average Error of Markers		
				Pre-refine[e]	*Error (Pixels)*	*Number of the markers used in Refine (Error threshold in pixels)*[d]
1	3.787	25	13	**yes**	**0.786**	**301 (3.000)**
				no	0.842	301 (4.600)
2	2.267	281	16	**yes**	**5.206**	**3937 (14.503)**
				no	5.227	3937 (14.700)

[d] Marker with error above the threshold is considered erroneously picked, and will be skipped by Refine.

[e] In pre-refine, error threshold is set to 18.935Å for each data set.

ACKNOWLEDGMENT

We are grateful for data visualization help provided by Mr. Pavel Puchenkov from the Scientific Computing and Data Analysis Section, Okinawa Institute of Science and Technology Graduate University. He helped us to visualize the electron tomographic scheme into Fig. 1.

REFERENCES

[1] U. Skoglund, K. Andersson, B. Strandberg, B. Daneholt, "Three-dimensional structure of a specific pre-messenger RNP particle established by electron microscope tomography," Nature. Vol. 319, pp. 560-564, 1986.

[2] L.-G. Öfverstedt, K. Zhang, L. A. Isaksson, G. Bricogne, U. Skoglund, "Automated correlation and averaging of three-dimensional reconstructions obtained by electron tomography," J. Struct Biol. Vol. 120(3), pp. 329-342, 1997 Dec.

[3] D. N. Mastronarde, "Fiducial marker and hybrid alingment methods for single-and double-axis tommography," In: Electron Tomography (Second Edition), J. Frank (Ed.), pp. 163-185. Springer, New York. 2005.

[4] J. Frank, B. F. McEwen, "Alignment by Cross-Correlation," In: Electron Tomography, J. Frank (Ed.), pp. 205-213. Plenum, New York. 1992.

[5] J. Fung, et al., "Toward fully automated high-resolution electron tomography," J. Struct. Biol. Vol. 116, pp. 181-189, 1996.

[6] D. Ress, M. L. Harlow, M. Schwarz, R. M. Marshall, U. J. McMahan, "Automatic aquisition of fiducial markers and alignment of images in tilt series for electron tomography," J. Electron Microsc. Vol. 48(3), pp. 277–287, 1999.

[7] Xpix, in-house softwear, the SCB unit, OIST.

[8] Q. Tian, L. G. Öfverstedt, U. Skoglund, "Automatically pick fiducial markers in electron tomography tilt images," Proceedings of 2015 International Conference on Intelligent Informatics and Biomedical Sciences. IEEE. November, 2015, pp. 33-38.

[9] Q. Tian, L. G. Öfverstedt, U. Skoglund, "Xpix2: Software to pick fiducial markers semi-automatically in electron tomography tilt images," Proceedings of The 4th International Conference on Intelligent Systems and Image Processing. IIAE. September, 2016, pp. 29-36.

[10] XML, Wikipedia. https://en.wikipedia.org/wiki/XML.

[11] Refine, in-house software, the SCB unit, OIST.