Accepted Manuscript

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PII: S1047-8477(17)30128-4

DOI: <http://dx.doi.org/10.1016/j.jsb.2017.07.008>

Reference: YJSBI 7083

To appear in: *Journal of Structural Biology*

Please cite this article as: Han, R., Wan, X., Wang, Z., Hao, Y., Zhang, J., Chen, Y., Gao, X., Liu, Z., Ren, F., Sun, F., Zhang, F., AuTom: a novel automatic platform for electron tomography reconstruction, *Journal of Structural Biology* (2017), doi: <http://dx.doi.org/10.1016/j.jsb.2017.07.008>

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AuTom: a novel automatic platform for electron tomography reconstruction

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Abstract

We have developed a software package towards automatic electron tomography (ET): Automatic Tomography (AuTom). The presented package has the following characteristics: accurate alignment modules for marker-free datasets containing substantial biological structures; fully automatic alignment modules for datasets with fiducial markers; wide coverage of reconstruction methods including a new iterative method based on the compressed-sensing theory that suppresses the “missing wedge” effect; and multi-platform acceleration solutions that support faster iterative algebraic reconstruction. AuTom aims to achieve fully automatic alignment and reconstruction for electron tomography and has already been successful for a variety of datasets. AuTom also offers user-friendly interface and auxiliary designs for file management and workflow management, in which fiducial marker-based datasets and marker-free datasets are addressed with totally different subprocesses. With all of these features, AuTom can serve as a convenient and effective tool for processing in electron tomography.

Keywords: Electron tomography, Alignment, Reconstruction, Image processing workflow

1. Introduction

Electron Tomography (ET) has become an indispensable tool for biological scientists. In particular, ET fills the wide gap between high-resolution methods (X-ray crystallography or single particle analysis) and light microscopy. In ET, the three-dimensional (3D) structure is reconstructed from a series of micrographs (tilt series). These micrographs are collected under a range of different tilt angles. The process of tomographic reconstruction consists primarily of tilt series alignment and reconstruction. Both alignment and reconstruction have a deterministic effect on the 3D structure quality.

Currently, there are a variety of software packages for tomographic reconstruction (Fernández, 2012), such as IMOD (Kremer et al., 1996), TOM (Nickell et al., 2005), Bsoft (Heymann and Belnap, 2007), Protomo (Winkler and Taylor, 2006), and UCSF tomography (Zheng et al., 2007). All of these packages have well-defined workflows and can achieve good alignment and reconstruction results. At the same time, the technique of electron microscopy has significantly advanced over the past several decades, and the collection of tilt-series is becoming more effective. There is a data explosion in ET: first, a remarkable number of groups have begun to use subtomogram averaging to analyze high-resolution structure in-situ (Wan and Briggs, 2016), which requires multiple tilt series; second, the size of micrographs grows rapidly during the past years, which requires a relatively faster process speed. These new challenges increase the demand for batch processing in ET and require a software package to have more automatic features.

Noble and Stagg (2015) proposed an automated batch marker-free alignment workflow named Appion-Protomo by combining the Protomo (Winkler and Taylor, 2006) and Appion framework (Lander et al., 2009). Because Appion-Protomo achieves tilt series alignment without the requirement of fiducial markers, it is a good choice for the datasets which have no fiducial markers or low-quality fiducial markers. Morado et al. (2016) proposed a protocol named Tomoauto for high-throughput automated cryo-electron tomography. Tomoauto is an application for batch processing of cryo-ET datasets. Because Tomoauto is a batch processing script based on the modules from several popular softwares (such as tilt series alignment module from IMOD and CTF module from CTFFIND4 (Rohou and Grigorieff, 2015)), its ability depends on the implementation of the original modules. IMOD has recently published its automatic solution for the alignment and reconstruction of tilt series (Mastronarde and Held, 2017). The latest IMOD realized its automatic tilt series alignment based on fiducial marker's 3D prereconstruction and reprojection, and proposed a set of auxiliary tools. The new property in IMOD has greatly improved its automation, though there may be some unexpected potential failure points because of its long workflow.

In this paper, we propose AuTom, a software package that attempts the following: AuTom provides an accurate alignment module for datasets that contain substantial biological structures; AuTom provides a fully automatic alignment module for datasets embedded with fiducial markers; AuTom has a wide coverage of reconstruction methods with a new iterative algebraic reconstruction version as well as GPU acceleration that support faster algebraic reconstruction. AuTom also has a user-friendly interface and an easy-to-use support system for process management. AuTom aims to present computationally efficient and highly automated electron tomography.

2. Overview

2.1. Workflow and design of AuTom

The workflow of AuTom is illustrated in Fig.1. The computational process of tomographic reconstruction consists of the following steps: 1) preprocessing, 2) alignment, 3) geometry calculation, and 4) reconstruction. AuTom processes the datasets in different ways based on whether the tilt series have fiducial marker embedded in or not. AuTom's marker-free subprocess mainly consists of coarse alignment, feature-based alignment (Han et al., 2014) and geometry calculation. AuTom's marker-based subprocess mainly consists of marker-based alignment (Han et al., 2015), marker erasing and geometry calculation. Compared with marker-free subprocess, marker-based subprocess does not require coarse alignment. AuTom provides several popular algorithms for tomographic reconstruction as well as these algorithms' accelerated versions.

The software architecture is illustrated in Fig.2. The architecture of AuTom is based on Model-View-Controller (MVC)¹ and contains three levels. The basic level in AuTom is "function module". "function module" is the minimum functional unit in the workflow. A module provides the basic function that focuses on a single operation or a set of algorithms. Consequently, a function module is designed as a plugin of AuTom and compatible with many other classic packages. The middle level is "process-control". "process-control" primarily maintains the initialization, status tracking and destruction of function modules. This level also provides the file management and workflow management, which allows the user to manage the data much more easily. The top level is "interface". The visualization of micrographs is realized in "interface" level. This level also provides views of the data system.

¹Model-View-Controller is a software architectural pattern. It divides a given application into three interconnected parts in order to separate internal representations of information from the ways that information is presented to and accepted from the user. The MVC design pattern decouples these major components allowing for efficient code reuse and parallel development.

2.2. Projection and geometry model in AuTom

For electron tomographic reconstruction, two kind of parameters (projection parameters and geometry parameters) should be solved. Projection parameters describe the proceed of projection, which is a 3D to 2D mapping; geometry parameters describe the specimen position in the 3D space, which is also called “tomogram positioning”. In AuTom, the projection is modeled as a classic orthogonal projection. The detailed model is described as follows:

$$\begin{pmatrix} u \\ v \end{pmatrix} = s\mathbf{R}_\gamma\mathbf{P}\mathbf{R}_\beta\mathbf{R}_\alpha\begin{pmatrix} X \\ Y \\ Z \end{pmatrix} + \mathbf{t} \quad (1)$$

where $(X, Y, Z)^T$ is the spatial location of the ultrastructure or fiducial markers, s is the image scale change, γ is the inplane rotation angle, α is the pitch angle of the tilt axis of the projection, β is the tilt angle of the specimen, $\mathbf{t} = (t_0, t_1)^T$ is the translation of view, $(u, v)^T$ is the measured projection point and $\mathbf{P}$ denotes the orthogonal projection matrix. The details of $\mathbf{R}_\alpha$, $\mathbf{R}_\beta$, $\mathbf{P}$ and $\mathbf{R}_\gamma$ are noted as

$$\begin{aligned} \mathbf{R}_\alpha &= \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos \alpha & \sin \alpha \\ 0 & -\sin \alpha & \cos \alpha \end{pmatrix}, \\ \mathbf{R}_\beta &= \begin{pmatrix} \cos \beta & 0 & -\sin \beta \\ 0 & 1 & 0 \\ \sin \beta & 0 & \cos \beta \end{pmatrix}, \\ \mathbf{P} &= \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \end{pmatrix}, \\ \mathbf{R}_\gamma &= \begin{pmatrix} \cos \gamma & \sin \gamma \\ -\sin \gamma & \cos \gamma \end{pmatrix} \end{aligned}$$

The tilt series alignment will solve the projection parameters $s, \alpha, \beta, \gamma, t_0, t_1$ to secure parameters coincident with the tilt series. If the pitch angle of every projection is sufficiently small, it can be omitted to simplify the process of reconstruction.

Fig.3 demonstrates the geometry model used in AuTom. Appropriate geometry parameters can well pose the tomogram and make it convenient for further analysis. As shown in Fig.3, the position of a tomogram is defined by the following parameters: thickness h (the thickness of the specimen), pitch angle φ (the tilt angle around the x -axis), offset θ_0 (the starting tilt angle around the y -axis), and z -shift m (the distance from the tilt axis to the center of the specimen in the z -direction).

2.3. Interface and Interaction of AuTom

For a scientific package, a user-friendly design is also very important. AuTom introduces the concept of “process” to manage the process of the entire workflow. All the data generated during user operation are included and managed by “process”. Fig.4 illustrates several main user interfaces of AuTom. AuTom uses project management to direct the user operation. Fig.4(a) shows the project management interface. If a user wants to start a new process, he just needs to input the required initial files into area 1 of the interface. Users can also choose an already existing process to continue the operation. After the initialization of a process, users are asked to choose the workflow for alignment, as shown in Fig.4(b) (area 2). All of the user’s operations are recorded in the project configuration file. Fig.4(c) illustrates a classic project configuration file in which a marker-based alignment workflow is defined and the input and output files are recorded. Then, users are navigated to the main interface of a “process”. Fig.4(d) and (e) illustrate the main interfaces for alignment and reconstruction, respectively. As shown in Fig.4(d), the process navigation bar is presented on the left (area 3) of the main interface. The center area (area 4) presents user interaction choices for each different stage of a process. Users can access the generated files from the file management (area 6). With the project configuration file, users can interrupt the workflow in any status and recover their operation whenever they want. Users can view and re-operate the dataset by using the navigation button (the selected button in area 5). AuTom also provides an execution log (area 9) and an auxiliary tool (area 8) to help users analyze the result. AuTom includes several customized widgets to assist data display, such as the sketch button (selected by area 8). Apart from the UI, users can access the functions from the terminal. Some predefined scripts based on our alignment and reconstruction modules are provided for unsupervised tomography reconstruction.

3. Materials

Two datasets are selected to illustrate the performance of AuTom.

The first dataset is a tilt series of a plastic-embedded cell section around a centriole region (Fig.5(a)), which was taken on an FEI TF30 microscope (operated at 300 kV) with a Gatan Camera. This dataset was obtained from the IMOD tutorial². The tilt angles of

²<http://bio3d.colorado.edu/imod/files/tutorialData-1K.tar.gz>

the projection images range from $+65.0^\circ$ to -61.0° at 2° intervals. In total, there are 64 images in the tilt series. The size of each tilt image is 1024×1024 , with a pixel size of 1.01 nm. The initial orientation of the tilt azimuth with respect to the vertical direction of the image is -12.5° .

The second dataset is a tilt series of adhesion belt structure (Fig.5(b)). The adhesion belt dataset was provided by the National Institute of Biological Sciences of China. The data were collected by an FEI Titan Krios (operated at 300 kV) with a Gatan US4000 camera (model 895). There are 113 images, with tilt angles ranging from $+54.0^\circ$ to 58.0° at $1^\circ \sim 2^\circ$ intervals. The size of each tilt image is 2048×2048 , with a pixel size of 2.03 nm (2 magnitude-binned). The initial orientation of the tilt azimuth with respect to the vertical direction of the image is 2.4° .

4. Implementation Details and Results

4.1. Preprocess of tilt series

The preprocess stage contains the following optional operations: hotpixel erasing, azimuth correction, and normalization. 1) Hotpixel erasing truncates the pixels with extremely high or low values. 2) Azimuth correction creates a new tilt series with the rotation axis vertical to the x -axis. 3) Normalization reproduces a tilt series with pixel values normalized to 0-1 by default. Table 1 illustrates the function modules used in the preprocess stage.

Table 1: Function modules used in preprocess of tilt series.

Program	Use
hotpixel	Erase the hot pixels in the tilt series
mrcstack	Create new stack of micrographs
mrcheader	Scan the header of micrograph stack
norm2d	Normalize the pixel values of micrographs

4.2. Alignment of tilt series

For alignment, two traditional paradigms exist: marker-free alignment and marker-based alignment. Cross-correlation (Guckenberger, 1982), common lines (Liu et al., 1995), iterative alignment method combining cross-correlation with reconstruction and reprojection (Winkler and Taylor, 2006, 2013), and feature-based alignment (Brandt et al., 2001; Brandt and Ziese, 2006; Castaño-Díez et al., 2007a, 2010; Phan et al., 2009; Sorzano et al., 2009; Han et al., 2014) are commonly used marker-free alignment. Fiducial marker-based alignment requires fiducial markers to be embedded in the specimen (Lawrence, 1992; Kremer et al., 1996; Mastronarde, 1997; Frank, 2006; Mastronarde, 2007; Amat et al., 2010). Both kinds of alignment strategies have their limitations and applications.

The proposed AuTom package integrates marker-free and marker-based alignment. For marker-free alignment, AuTom integrates the cross-correlation method and the feature-based method based on scale invariant features (Han et al., 2014). AuTom also integrates a fully automatic alignment scheme for datasets with fiducial markers (Han et al., 2015), which can identify and track the fiducial markers without any manual intervention.

Marker-free alignment subprocess: AuTom realizes coarse alignment by cross-correlation (Guckenberger, 1982) and common lines (Liu et al., 1995) to correct large shifts and in-plane rotation of the micrographs. The coarse alignment can also approximately estimate the rotation azimuth and make the rotation axis as vertical as possible. In the next step, AuTom adopts feature-based alignment proposed by Han et al. (2014) to carry out fine alignment, in which the stable features are used and tracked instead of fiducial markers. The projection model in Eq.1 is solved in parameter optimization by Levenberg-Marquardt (L-M) algorithm (Kanzow et al., 2004). For specimens that contain substantial ultrastructures, the feature-based module can achieve alignment results comparable to that of the marker-based alignment.

Fig.6 illustrates the result of centriole processed by our fiducial marker-free alignment. The dataset was first aligned by the coarse alignment and then finely aligned by the scale invariant features (Han et al., 2014). As shown in Fig.6(a) and (b), about a thousand features were detected and matched; all of the matched features in Fig.6(a) and (b) were marked by $\times$. The matched features were then used as “fiducial markers” and tracked across the tilt series. Based on the tracks of features, the projection parameters were calculated. Fig.6 (c) and (d) illustrate the reconstructed volume of the centriole. The reconstruction is by ICON (Deng et al., 2016), which is our most recently reconstruction method. We can notice the contrasting fiducial markers on the volume in Fig.6(c) and the clear ultrastructure of centriole in Fig.6(d). The result benefits from the accurate marker-free alignment and ICON’s compensation of “missing wedge” effect (the effect of ICON will be introduced later).

AuTom provides a visual interface for geometry determination, which is based on a pre-reconstruction of the tilt-series. ICON is adopted for the pre-reconstruction to secure a clear boundary of the reconstructed specimen, as shown in Fig.7. The geometry determination in AuTom is semi-automatic and should be supervised by the user. The marker-free alignment subprocess is adaptable for those datasets that are negatively stained or with substantial ultrastructures. The target resolution is at the cell organelle level.

Because the location of feature detected from ultrastructure details is not so robust as fiducial markers under the impact of noise, the accuracy of marker-free alignment will be limited if a tilt series has very low signal-to-noise (SNR).

Marker-based alignment subprocess: AuTom provides a fully automatic alignment scheme for specimens that have fiducial markers embedded in. The technique proposed in Han et al. (2015) is adopted for marker-based alignment, in which the positions of fiducial markers are exhaustively detected and tracked in a globally consistent way. Therefore, the marker-based alignment in AuTom can cope with datasets in any initial state. Another significant improvement is that the marker-based alignment in AuTom requires minimal input of parameters, which generally only requires the tilt series and tilting angle file as input. An initial value of fiducial marker diameter is optional in the case that the initial diameter estimation fails. Similar with marker-free alignment, the projection model in Eq.1 is solved by L-M algorithm (Kanzow et al., 2004). However, with the help of high-quality fiducial marker tracks, the optimization here is fast enough and able to solve the projection parameters in several seconds to minutes.

Fig.8 illustrates the result of the adhesion belt dataset processed by our marker-based alignment. First, the locations of fiducial markers were extracted from the projections, which is very robust and exhaustive. Here, we selected a projection with high tilt angles to illustrate the effectiveness of our fiducial marker detection. As shown in Fig.8(a), the marker detection was very successful even though the fiducial markers are very blurry and the background is highly misleading (for the entire tilt series, there were approximate 150-180 fiducial markers detected on each projection). Then, the detected fiducial markers were matched to compose tracks. For adhesion belt dataset, we totally obtained 103 tracks that cover more than 90 micrographs (approximately 80% of the entire tilt series). In AuTom, only the long tracks are used for projection parameter estimation. Fig.8(b) shows the locations of tracks before alignment, composed of a mass of disordered lines. Fig.8 (c) and (d) show the aligned tracks of the adhesion belt dataset from different directions. The mean alignment residual is 0.37 pixel and we can notice that the alignment is successful. Here, we also draw the tracks that were generated but not used for projection parameter optimization. We can find the tracks (the tracks drawn in the four corners of Fig.8 (d)) that are cut by the change of view. These tracks can be used as supplemental data for the alignment of larger datasets to correct deformation (Lawrence et al., 2006).

Table 2: Function modules used in the alignment of tilt series.

Program	Use
cr_xyshift	Correct the shift in x and y
cr_rotation	Correct the azimuth or in-plane rotation of micrographs
atomalign	Align the tilt series by scale invariant features
markerauto	Align the tilt series based on the information of fiducial markers
markererase	Erase the fiducial markers in the micrographs
mrcstack	Create new stack of micrographs

AuTom also provides a marker erasing module as an alignment option. The visual interface for geometry determination is also accessible in the marker-based alignment subprocess. AuTom has been challenged by several open datasets and has achieved good results for most of these datasets³. For users who want to achieve high alignment quality under low-SNR conditions, marker-based alignment could be a good choice.

Table 2 illustrates the function modules used in the alignment stage.

4.3. Reconstruction

4.3.1. Reconstruction methods in AuTom

The tomographic reconstruction in ET is to obtain the 3D volume of the specimen from the projections. For tomographic reconstruction, there are two kinds of reconstruction paradigms: central-section-theorem-based technique and algebraic technique. Weighted back-projection (WBP) and convolution back-projection are commonly used reconstruction algorithms based on the central section theorem, which treats the Fourier transform of a projection as a central section of specimen's 3D Fourier transform (De Rosier and Klug, 1968; Frank, 2006). The 3D volume of the specimen can be obtained from the inverse Fourier transform of an assembly of the projections' 2D Fourier transforms. The algebraic technique treats the 3D reconstruction problem as a large system of linear equations and solves the optimization by iterative algorithms, which is usually called real-space reconstruction because

³AuTom has been tested by freely available tilt-series datasets on EMPIAR: <https://www.ebi.ac.uk/pdbe/emdb/empiar/entry/10045/>, <https://www.ebi.ac.uk/pdbe/emdb/empiar/entry/10046/>, <https://www.ebi.ac.uk/pdbe/emdb/empiar/entry/10047/> and <https://www.ebi.ac.uk/pdbe/emdb/empiar/entry/10048/>. AuTom successfully aligned the datasets in EMPIAR 10045 (7 datasets total), EMPIAR 10046 (73 datasets total) and EMPIAR 10047 (1 datasets total). Among these, AuTom achieved parameter-free alignment for EMPIAR 10045 and 10046. AuTom failed in EMPIAR 10048 (1 dataset total) because of its extremely dark spots.

of its direct operation on real space. Algebraic Reconstruction Technique (ART) (Gordon et al., 1970; Marabini et al., 1998), the Simultaneous Iterative Reconstruction Technique (SIRT) (Gilbert, 1972; Sorzano et al., 2001), Component Averaging Methods (CAV) (Censor et al., 2001; Fernández et al., 2002) and the Simultaneous Algebraic Reconstruction Technique (SART) (Andersen and Kak, 1984; Castaño-Díez et al., 2007b) are well-known algebraic algorithms for electron tomographic reconstruction.

The two paradigms have their advantages and disadvantages. WBP and convolution back-projection are computationally efficient but can be easily affected by limited-angle data and noise. Real-space reconstruction methods can compensate for the effects of the limited-angle data but require much more computational resources.

In addition to the computational efficiency, artifacts caused by the missing wedge are an everlasting problem in ET. Recently, we proposed the Filtered Iterative Reconstruction Technique (FIRT) (Chen et al., 2016) and Compressed-sensing Optimized Non-uniform fast Fourier transform reconstruction (ICON) (Deng et al., 2016) to cope with the artifacts and missing information, respectively. FIRT combines the algebra reconstruction technique (ART) and the nonlinear diffusion (ND) filter technique to get a smoother and enhanced reconstruction volume; ICON uses compressed-sensing and the assumption of sparsity of biological specimens to compensate the missing wedge information. Currently, AuTom integrates several algorithms, including FBP⁴, SART, SIRT, and ICON. AuTom also provides the Back Projection Technique (BPT) for the comparison and validation of the other methods.

Fig.9 shows the center slice of the reconstructed volume of the adhesion belt dataset, which has been processed by our marker-based procedure. The dataset was reconstructed by different methods on a Ubuntu 12.02 system with 128Gb memory, 2 E5-2620v2 (2.1 GHz) CPU and 2 Tesla K20 GPU. Fig.9 (a) shows the result of FBP in AuTom. FBP is the fastest reconstruction method offered by AuTom and does not need any auxiliary parameters. However, the result of FBP can be easily affected by limited-angle data; thus, the reconstructed volume is not very clear compared with that obtained using other methods. Fig.9 (b) shows the result of SART, which is very similar to that of FBP but is clearer and has a higher contrast. Fig.9 (c) shows the result of SIRT. The visual result of SIRT is very similar to the original projections, which is due to similarity of the pixel distribution. However, the result of SIRT seems to have more artifacts and is not as clear as the result of SART. Fig.9 (d) shows the result of ICON. The result of ICON is similar to SART in pixel distribution but has less background noise and higher structure contrast.

Although the ET technique has developed for decades, the scope still suffers from the lack of general measurements of volume resolution that can evaluate the results of different methods (Cardone et al., 2005). Here, we use the Normalized Correlation Coefficient (NCC) between the reprojections of different reconstruction methods and the original tilt series to give a coarse evaluation of the methods proposed in AuTom. Fig.10 shows the NCC curve for each method, which is mainly used as a metric for reprojection consistency. The average value of NCC is 0.87, 0.91, 0.79 and 0.98 for FBP, SART, SIRT and ICON, respectively. In the view of reprojection consistency, ICON performs much better than the other methods. SART and SIRT have different performances owing to their different iteration and convergence strategies. Fig.10 also shows that the signal contribution of projections with high tilt angles decreases rapidly in FBP, SIRT and ICON. This phenomenon is caused by the defocus and increase of thickness along the electron trajectory. SART has a good NCC value at high tilt angles because SART updates the volume value with every projection separately in each iteration (for comparison, SIRT updates the volume value with the entire tilt series in each iteration). The voxel update strategy of SART can lead to high NCC value in high tilt angles but may cause ill convergence, so AuTom uses a random choice of projection in every iteration. Though the NCC value is a good metric for reprojection consistency, it does not necessarily imply the superiority of one reconstruction method over another. The reconstruction quality analysis is a much more complex problem (Cardone et al., 2005; van Heel and Schatz, 2005): different filter effects and weights for micrographs may enhance the signal strength in different frequency and result in different reconstruction results; for reconstruction quality comparison, the analysis of signal strength and noise level in 3D volumes should be more comprehensive, and the reprojection consistency revealed by NCC is only a part of the analysis.

4.3.2. Acceleration of reconstruction

Table 3: Reconstruction time of different methods in AuTom on different HPC architectures. All the methods were executed with 2 MPI threads (40 iterations were used for the iterative methods, without OpenMP).

AuTom	FBP	SIRT	SART	ICON
CPU	268 s	24837 s	36362 s	190554 s
GPU	20 s	559 s	1117 s	8282 s
MIC	49 s	8532 s	11431 s	31021 s

The reconstruction is the most time-consuming stage in ET. Iterative reconstruction techniques have advantages in coping with noisy and limited-angle data but require massive computational resources and considerable processing time. Generally, users are suggested to use an accelerated version of the reconstruction method. AuTom provides accelerated reconstruction methods for

⁴FBP is an implementation of convolution back-projection; Carazo et al. (2007) describes the primary difference between FBP and WBP.

three high performance computing (HPC) architectures: GPU, MPI and the Xeon Phi platform (Wang et al., 2017), with multi-core and multi-GPU solutions. In AuTom, MPI provides a coarse-grained parallel strategy; GPU or the Xeon Phi-based technique provides fine-grained parallel strategies. Because the reconstruction of a volume can be divided into several slices and each slice is reconstructed in multi-core systems, it is easy to assess the acceleration rate in MPI. Table 3 shows the execution time of the reconstruction of the adhesion belt dataset ($2048 \times 2048 \times 150$) by different methods (40 iterations were used for all the iterative methods) in different architectures, where all methods were running with two MPI threads (two Tesla K20 card for GPU; Xeon Phi 31SP many-core coprocessor for MIC). As shown in Table 3, on GPU platform, we achieve acceleration rates of 44x for SIRT, 33x for SART and 23x for ICON. On the Xeon Phi platform, we achieved 3x, 3x and 6x for SIRT, SART and ICON, respectively. Judging from Table 3, for a dataset with image size of $2048 \times 2048 \times 150$, we need about 10 minutes to get a volume reconstruction by SIRT or SART with 40 iterations on a platform with two general GPU cards. For the methods on the Xeon Phi platform, we achieved about 3x acceleration. Because the parallel computing strategy on MIC is based on multi-cores, we could obtain much better acceleration for volume reconstruction if we used more MPI threads.

If we just analyze the total execution time, the acceleration ratio illustrated here is not indicative for reconstruction speed. FBP is a one-turn reconstruction algorithm, and for FBP, the execution time is reduced from 268 s to 20 s on GPU platform. It is really exciting because the result announces that we can get a reconstruction result in half a minute for a 2048×2048 dataset. However, the acceleration ratio for FBP is only about 14x. If we peer into the execution time of FBP, we can find some clues. Within the 20 seconds, 17 seconds are used for IO operation (reading and writing of data) and only 3 seconds are used for algebraic calculation. If we excluded the 17 seconds used for IO operation, the accelerate ratio for FBP will be 83x ($251/3$). It is an amazing result that indicates IO operation has become the main bottleneck in ET reconstruction with the advance of computational hardware and algorithm (GPU or multi-cores). Therefore, AuTom provides not very high acceleration ratio but high-speed reconstruction module.

Table 4: Function modules used in tomographic reconstruction.

Program	Use
tomorec3d	Provide the tomographic reconstruction methods (compatible for GPU and Xeon Phi acceleration)
ICON	Provide the compressed-sensing based reconstruction method

Table 4 illustrates the function modules used in the reconstruction stage.

5. Conclusion and Discussion

In this paper, we presented a new software package for electron tomography reconstruction. Our package introduces several of the most recently developed methods to the ET process, which can significantly improve the automation and computational speed. Our package has the following merits:

- AuTom provides a well-developed workflow, which is easy-to-use and works well for a variety of datasets;
- AuTom provides a variety of reconstruction methods and HPC solutions across multiple architectures;
- AuTom provides the most recent reconstruction method, which has outstanding performance on missing wedge compensation;
- AuTom can achieve unsupervised volume reconstruction for most marker-embedded datasets with non-specialized parameters.

Therefore, we believe that AuTom can be a very useful package in the field of computational structural biology (the readers who are interested in the performance difference between AuTom and other classic packages can find a comparison of the key functions between AuTom and IMOD in the appendix).

As a newborn package, problems such as bugs are inevitable. We will keep improving the quality of AuTom. There are still several problems remaining in AuTom. The first one is the application of our alignment technique to large-scale datasets. We do not provide MPI or GPU versions of the alignment module. However, the increasing demand of batch processing for subtomogram averaging would extend the execution time of the alignment process. Therefore, providing an accelerated alignment module is necessary. The second problem is the acceleration of our newly proposed reconstruction method ICON. The current acceleration rate is not very satisfactory. We are trying to make it faster on GPU and MIC platforms. We also plan to integrate FIRT into AuTom. The third problem is the lack of auxiliary tools in AuTom. Although we have provided some auxiliary tools for the analysis of alignment and reconstruction, these are still not convenient enough. Currently, users may need the assistance of other classic packages such as Bsoft or IMOD.

A further development of AuTom is necessary to cope with the future demands of electron tomography (Hayashida and Malac, 2016; Wan and Briggs, 2016). We are developing new tools and methods for subtomogram averaging and large-field electron tomography, in which CTF correction and other subsequent tools will be added to AuTom. Dual-axis tomographic reconstruction will be added to AuTom in the near future. AuTom has been challenged with a variety of datasets and encountered some problems in

special cases (for example, datasets with no fiducial markers embedded or no obvious ultrastructure). How to cope with these special datasets smoothly will also be our future work. In AuTom, we provide several Linux/Unix bash scripts based on our modules for fully automatic alignment and reconstruction of tomography data. Though we are trying to solve the automatic geometry parameter determination (Chen et al., 2017), the robust automatic determination of geometry parameters still remains a problem, which will be one of our future work.

6. Availability

General versions of AuTom are available on our website ear.ict.ac.cn. On the website, there are documents and videos for our software, and we also provide several compiled versions of AuTom for Linux OS. Accelerated version of reconstruction methods for MIC platform don't have pre-compiled version, because we don't have such a standard MIC platform. It is available upon request.

Acknowledgements

Thanks Jose-Jesus Fernandez for opening the CTF module to us. Thanks Ce Liu and Shuangbo Zhang for the works to improve the quality of AuTom. This work was supported by the Strategic Priority Research Program of Chinese Academy of Sciences (Grant No.XDB08030202), the National Natural Science Foundation of China (Grant No. 61232001, 61232991, 61472397, 61502455, 61672493, U1611263, U1611261), the National Key Research and Development Program of China2017YFA0504702), the King Abdullah University of Science and Technology (KAUST) Office of Sponsored Research (OSR) under Awards No. URF/1/1976-04, URF/1/2602-01, and URF/1/3007-01, Special Program for Applied Research on Super Computation of the NSFC-Guangdong Joint Fund (the second phase), and the National Basic Research Program (973 Program) of Ministry of Science and Technology of China (2014CB910700).

Appendix: Comparison with classic package

Currently, there are a variety of classic software packages for tomographic reconstruction (Fernández, 2012). Every package has its merits. AuTom tries to be comprehensive and easy-to-use, but as a new software package, it still needs improvement in many aspects. Here, we choose the most widely used package IMOD (Kremer et al., 1996) as a comparison to illustrate the merits and defects of AuTom.

Workflow comparison

Current AuTom only supports ET reconstruction while IMOD is a much more comprehensive package. For tilt series alignment and tomogram building, the workflow of IMOD treats the tilt series as a marker-embedded dataset by default, whereas AuTom explicitly distinguishes these two different datasets.

IMOD provides an excellent marker-based alignment workflow, which is based on a schedule composed of pre-selection of fiducial markers, pre-reconstruction of spatial fiducial markers and motion prediction of the fiducial marker reprojection. Typically, a prealignment will be carried out for the convenience of pre-reconstruction of spatial fiducial markers and their motion prediction. For the sake of accurate motion prediction, IMOD may also need manual check to correct the positions of the fiducial markers that have large deviations. In AuTom, due to the fact that the selection of fiducial markers is based on a statistical method and the tracking is based on point set registration, prealignment of the input tilt series is not necessary, and the prediction of tracks is much more stable. Therefore, AuTom can detect and generate fiducial marker tracks exhaustively, and requires minimal tuning of input parameters. Recently, IMOD also proposed its own automatic alignment workflow based on its previous schedule (Mastrorade and Held, 2017). However, there may be some unexpected potential failure points because of its long workflow. By comparison, the marker-based alignment in AuTom is simpler and needs fewer parameters as input. In other aspects, IMOD has more auxiliary functions in its workflow. These auxiliary functions can improve the alignment and reconstruction results in some special conditions. AuTom is currently lacking of such auxiliary modules and further study is needed in the future.

In the stage of reconstruction, AuTom provides more comprehensive reconstruction methods than IMOD. Especially, AuTom provides a compressed-sensing based reconstruction method (ICON) (Deng et al., 2016) to solve artifacts and information missing. ICON is a good choice that can provide a reconstruction with much better boundaries and ultrastructural details. AuTom also has optimized version on MIC platform for classic reconstruction methods, which makes the software package accessible for special platforms.

Results comparison

Alignment and reconstruction are the most important modules for tomogram building. Here, we illustrate a comparison of the results between AuTom and IMOD.

Fig.11 shows reconstruction results for the centriole dataset with different marker-free alignment methods. In Fig.11, the left pictures are the results processed by IMOD's patch-tracking alignment and the right ones are the results processed by AuTom's marker-free alignment (both reconstructed by IMOD's WBP). IMOD's patch-tracking alignment divides the micrographs into small patches, which are then tracked by cross-correlation. For the centriole dataset, patches with different sizes were tried and the 64×64 px² patch resulted in the best alignment result. We analyzed the reconstruction result by NCC value between the reprojections and that of the original tilt series, and got the average value of NCC as 0.589 and 0.592 for IMOD and AuTom, respectively. The experiment demonstrates that the performance of these two methods are very close to each other, which is also proved by the similar ultrastructures in Fig.11.

The marker-based alignment module in AuTom is based on the work in Han et al. (2015), wherein the result of centriole dataset processed by our marker-based alignment has been compared with that in IMOD. Here, the adhesion belt dataset was also processed by IMOD for comparison. The marker diameter is set to 9 pixels and the selected fiducial marker number is set to 150 as the initial parameter value in IMOD. The entire process is based on the semi-automatic procedure in IMOD. After several rounds of marker position correction and missed marker selection, 58 tracks were generated for projection parameter determination. For the adhesion belt dataset, the mean value of the final alignment residual is 0.72 pixels. In contrast, AuTom detected and used 103 long tracks (the tracks covered > 80% tilt series); finally, AuTom obtained a mean alignment residual value of 0.37 pixels. There are two reasons underlying the better results from AuTom. The first one is the fiducial marker parameter re-estimation. In AuTom, the parameters of the fiducial markers are relocated and re-estimated for each projection, which provides a better fiducial marker detection result. Another reason is that AuTom used more fiducial marker tracks to estimate the projection parameters. Fig.12 shows an overlay of the aligned fiducial marker tracks in the adhesion belt dataset, where the left part represents the results from IMOD and the right part represents the results from AuTom. As shown in Fig.12, IMOD detected and used few tracks but the tracks covered almost the entire tilt series; AuTom used more tracks, which were long enough but did not always cover the entire tilt series. Fig.12 (a) and (b) show the difference between the marker-based alignment in IMOD and AuTom, where we can notice that the aligned tracks by AuTom are smoother and more horizontal than the ones by IMOD, for example, the selected green ellipse areas. Is it necessary for the tracks to cover all the tilt series? In our opinion, it is not necessary: if a fiducial marker cannot be located as a candidate fiducial marker by our exhaustive marker detection, it means that this fiducial marker is of low quality and the corresponding position is of low confidence; a manually relocated fiducial marker position may lack precision and may lead to low quality alignment. Because projection parameter estimation is an over-determination problem, more tracks can lead to more accurate prediction. Therefore, in AuTom, we suggest exhaustive fiducial marker extraction and tracking.

Table 5: Reconstruction time of different methods in IMOD. All the methods were executed for 40 iterations with 1 main thread (with OpenMP on 12-cores (two 6-cores GPUs)).

IMOD	WBP	SIRT
CPU	609 s	178060 s
GPU	97 s	1380 s

For the assessment of AuTom's reconstruction module, we also run IMOD's WBP and SIRT. IMOD's reconstruction module is based on OpenMP but not MPI. For IMOD's reconstruction module, one main process is used and OpenMP is turned on (12 cores of threads are used). The machine and OS environment is the same with AuTom: Ubuntu 12.02 system with 128Gb memory, 2 E5-2620v2 (2.1 GHz) CPU and 2 Tesla K20 GPU. Here, IMOD's reconstruction module only used one card of GPU. Both of IMOD and AuTom used all the computational resources on the workstation. Table 5 shows the execution time of the reconstruction of the adhesion belt dataset ($2048 \times 2048 \times 150$) by IMOD's WBP and SIRT (40 iterations). We compare AuTom's FBP with IMOD's WBP and AuTom's SIRT with IMOD's SIRT. At first sight, AuTom's module run much faster than IMOD's module. However, we should multiple the execution time of AuTom's module by 2 for fair (Because AuTom used 2 MPI thread). Therefore, AuTom would cost 536 seconds for FBP and 49674 seconds for SIRT (CPU version) if 1 MPI thread used; IMOD costed 609 seconds for WBP and 178060 seconds for SIRT (CPU version). About the speed of CPU version's FBP and WBP, IMOD and AuTom is on the same level; about the speed of CPU version's SIRT, IMOD cost about 3.5x times compared with AuTom. AuTom would cost 40 seconds for FBP and 1118 seconds for SIRT (GPU version) if 1 MPI thread used; IMOD costed 97 seconds for WBP and 1380 seconds for SIRT (GPU version). About the speed of GPU version's FBP and WBP, AuTom is 2 times faster; about the speed of GPU version's SIRT, IMOD and AuTom is on the same level. By comparison, the acceleration technique is quite different between AuTom and IMOD. AuTom is realized on MPI but IMOD is realized on OpenMP. The acceleration technique of AuTom is more extendible in system with multi-cores and multi-GPUs. On our workstation, the speed of AuTom's reconstruction module seems a little faster than IMOD. However, we also find that the IO operation costs a lot time in reconstruction; and different workstation's IO is also different. Judging from the result of GPU version's SIRT, IMOD may do IO optimization based on OpenMP. So it is hard

to conclude whose implementation is better, which maybe depend on data and hardware.

Finally, we would like to make a complete comparison between IMOD and AuTom. Fig.13 shows the final reconstruction of adhesion belt dataset as processed by IMOD (aligned by IMOD and reconstructed by WBP) and AuTom (aligned by AuTom and reconstructed by FBP). There are no obvious visual differences between the central slice as processed by IMOD versus AuTom. From Fig.13(c), we can find that the NCC value between the reprojections and that of the original tilt series is higher for AuTom than that for IMOD. The difference in NCC curve is caused by two reasons. One reason is the alignment difference between IMOD and AuTom (if the tilt series aligned by IMOD and AuTom are reconstructed by the same method, there will only exists not very big difference between their NCC curves). The mainly reason is the different implementation between AuTom's FBP and IMOD's WBP. We find that AuTom's FBP has a better performance under the measurement of reprojection consistency. However, the comparison carried out by NCC is only a small part of the reconstruction quality. Much more data and more comprehensive comparison is needed for a further conclusion. Nevertheless, the result announces that the reconstruction result carried out by AuTom is comparable with the one by IMOD.

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Figure 1: Workflow of AuTom.

Figure 2: Architecture of AuTom; based on the classic Model-View-Controller (MVC).

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Figure 5: Example images from the test sets. (a) Centriole. (b) Adhesion belt.

Figure 6: Illustration of the marker-free alignment, where the used dataset is the centriole (1024×1024). (a) Detected features on the 32th projection (marked by $\times$); all the marked features have corresponding peers on the 33th projection. (b) Detected features on the 33th projection (marked by $\times$); all the marked features have corresponding peers on the 32th projection. (c) An x-y slice from the top of the reconstructed volume (reconstructed by ICON). (d) An x-y slice from the middle of the reconstructed volume (reconstructed by ICON).

Figure 7: The geometry calibration interface.

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Figure 9: Illustration of the reconstruction methods in AuTom, where the used dataset is the adhesion belt. (a) An x-y slice from the middle of the reconstructed volume (reconstructed by FBP). (b) An x-y slice from the middle of the reconstructed volume (reconstructed by SART with relaxation factor $\lambda = 0.2$ and 40 iterations). (c) An x-y slice from the middle of the reconstructed volume (reconstructed by SIRT with relaxation factor $\lambda = 0.2$ and 40 iterations). (d) An x-y slice from the middle of the reconstructed volume (reconstructed by ICON with 40 iterations).

Figure 10: The Normalized Correlation Coefficient value between the reprojections of different reconstruction methods and the original tilt series.

Figure 11: Comparison of the reconstruction of the centriole after patch-tracking alignment in IMOD (left) and marker-free alignment in AuTom (right), both are reconstructed by WBP in IMOD. (a) and (b) are similar top x-y sections. (c) and (d) are similar middle x-y sections. (e) and (f) are similar y-z section. Note that the results of the two methods are very similar to each other.

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